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# Metabolic Engineering Summit 2026

# Abstract book

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# Plenary Lecture

# Accelerating Metabolic Engineering with AI and Robotics

Huimin Zhao

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## Abstract

Metabolic engineering aims to design microbial cell factories for production of chemicals, fuels, and materials. However, due to the complexity of biological systems, performing metabolic engineering in a quantitative and predictive manner remains a challenge. In this talk, I will highlight our recent work on the development of artificial intelligence (AI) tools and an AI-powered self-driving biofoundry to address this challenge. Examples include but are not limited to: (a) CLEAN: an AI tool for enzyme function prediction; (b) EZSpecificity: an AI tool for enzyme substrate specificity prediction; (c) EZKinetics: an AI tool for enzyme kinetic parameter prediction; and (d) iCloudBiofoundry: an AI-powered self-driving cloud biofoundry for protein engineering, pathway engineering, and metabolic engineering. Particularly, I will discuss the challenges and opportunities in the emerging AI for Science (AI4S) field and how it will impact the metabolic engineering and synthetic biology.

**Key words:** metabolic engineering, synthetic biology, biofoundry, artificial intelligence, autonomous experimentation

**Representative publication:**

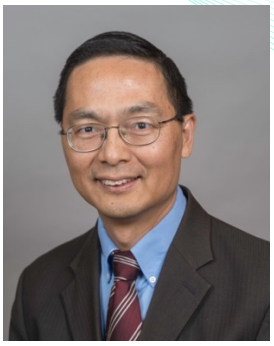
1. HamediRad, M., Chao, R., Weisberg, S., Lian, J., Sinha, S., and Zhao, H. Towards a Fully-Automated Algorithm-Driven Platform for Biosystems Design. *Nature Communications*, 10:5150 (2019).

2. Yu, T., Cui, H., Li, J. C., Luo, Y., Jiang, G., & Zhao, H. Enzyme function prediction using contrastive learning. *Science*, 379, 1358-1363 (2023).

3. Cui, H., Su, Y., Dean, T. J., Yu, T., Zhang, Z., Peng, J., Shukla, D., & Zhao, H. Enzyme specificity prediction using cross-attention graph neural networks. *Nature*, 647, 639-647 (2025).

4. Singh, N., Lane, S., Yu, T., Lu, J., Ramos, A., Cui, H., & Zhao, H. A Generalized Platform for Artificial Intelligence-powered Autonomous Enzyme Engineering. *Nature Communications*, 16, 5648 (2025).

## Biography



Dr. Huimin Zhao is the Steven L. Miller Chair of chemical and biomolecular engineering at the University of Illinois Urbana-Champaign (UIUC), director of NSF AI Institute for Molecule Synthesis (moleculemaker.org), NSF iBioFoundry (ibiofoundry.illinois.edu), and NSF Global Center for Biofoundry Applications (gcba.illinois.edu), and Editor in Chief of *ACS Synthetic Biology*. He received his B.S. degree in Biology from the University of Science and Technology of China in 1992 and his Ph.D. degree in Chemistry from the California Institute of Technology in 1998. Prior to joining UIUC in 2000, he was a project leader at the Dow Chemical Company. Dr. Zhao has authored and co-authored over 490 research articles and over 30 issued and pending patent applications. In addition, he has given over 570 plenary, keynote, or invited lectures. Forty (40) of his former graduate students and postdocs became professors or principal investigators around the world. Dr. Zhao received numerous research and teaching awards and honors such as ECI Enzyme Engineering Award and NSF CAREER Award. His primary research interests are in the development and applications of synthetic biology, artificial intelligence, and laboratory automation tools to address society's most daunting challenges in health, energy, and sustainability.

# Systems metabolic engineering of microorganisms for the biomanufacturing of chemicals and materials

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## Abstract

Microbial biomanufacturing is emerging as a key technological foundation for a sustainable bioeconomy, offering environmentally friendly routes to produce chemicals, fuels, materials, pharmaceuticals, and nutraceuticals from renewable resources. However, naturally occurring microorganisms rarely possess the metabolic efficiency, productivity, or robustness required for economically viable industrial-scale production. Overcoming these limitations requires a systematic engineering framework capable of redesigning cellular metabolism at multiple levels. Systems metabolic engineering has evolved as such a framework by integrating metabolic engineering with systems biology, synthetic biology, computational modeling, and laboratory evolution. This multidisciplinary approach enables comprehensive analysis of cellular networks, rational optimization of metabolic pathways, dynamic control of gene expression, and accelerated adaptation of microbial hosts to industrial conditions. The increasing availability of multi-omics technologies, genome-scale models, machine learning tools, and precision genome engineering methods has further expanded our ability to construct highly efficient microbial production platforms. This lecture will highlight recent developments in systems metabolic engineering and demonstrate how these advances have enabled the creation of next-generation microbial cell factories. Case studies will showcase the production of commodity and specialty chemicals, biofuels, polymers, and high-value natural products from a variety of carbon sources. Particular emphasis will be placed on strategies for improving pathway performance, balancing cellular resource allocation, and enhancing strain robustness during scale-up. The lecture will also discuss future opportunities for integrating artificial intelligence, automated biofoundries, and digital biological twins to accelerate strain development and industrial deployment. Together, these advances are transforming microbial biomanufacturing into a powerful platform for sustainable chemical production, circular resource utilization, and improved human well-being.

# Keynote Lecture

# Transforming food production for sustainability: microbes to eat CO2; humans to eat microbes

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**Abstract**

Transforming food production is essential to help with food security, land and water stress, climatic and ecological stability. I will present our integrative analysis on the status of humanity's impact on global wild biomass and proceed to describe how we evolved a microbe to change its diet from sugar to CO2. This is a milestone towards the vision of transforming CO2 into valuable products via microbial production, but can it become realistic economically? I will portray how we analyze the limitations and potential using a simplified techno-economic analysis. Going forward, I will discuss what can galvanize this essential innovative transformation by driving key catalytic elements: lower production prices, better taste and improved health outcomes.

**Key words:** microbial food production lab evolution

**Representative publication:**

Electro-microbial production techno-economic viability and environmental implications
SJ Lovat, et al. (2025), **Nature Biotechnology**
Conversion of Escherichia coli to generate all biomass carbon from CO2
Gleizer et al. (2019), **Cell**

**Biography**



Prof. Milo brings the tools of systems biology to bear on the challenges of sustainability. His research aims to understand the cellular highways of energy and carbon transformations in quantitative terms. To this end, he and his team employ a combination of computational and experimental synthetic biology tools to bear on carbon fixation, the biochemical process in which carbon dioxide (CO2) gets incorporated into organic compounds. He is working to reveal the most fundamental design principles of carbon fixation and photosynthesis, with the goal of improving the efficiency of food and fuel production. In addition, Prof. Milo brings the tools of big data analysis and systems biology to work on understanding the biosphere and human impacts on the living world, including, for example, the ecological costs of food production

# Biomanufacturing of Aromatic Compounds

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## Abstract

Aromatic compounds are an important pillar of modern material civilization and are widely used in fields like materials, food, medicine, and cosmetics. Traditional production methods have issues like unsustainable resource use and heavy pollution during production. Green biomanufacturing of aromatic compounds is an important way to achieve sustainable development for human society. This report introduces the current state of synthetic biology, as well as the scientific and technical problems and challenges in building efficient cell factories. It analyzes the logic behind selecting targets in synthetic biology and points out the key elements for building efficient cell factories. Taking the biomanufacturing of aromatic compounds as an example, high-performance aromatic compound synthesis platforms were built, surpassing the natural theoretical yield. Cell factories for over 20 important aromatic compounds, including acetaminophen, were designed and constructed. Strategies to boost the synthetic capacity of cell factories were proposed, significantly improving the production of compounds like arbutin and gallic acid. By integrating systems, industrial production lines for compounds like arbutin and 5-hydroxytryptophan were established, showcasing the huge potential of synthetic biology and providing a reference for green biomanufacturing of aromatic compounds.

**Key words:** Cell factories; Aromatic compounds; Drive enhancement; Advanced chassis

**Representative publication:**

1. Liangyu Lu, Xiaolei Wang, Tong Wang, Xiaolin Shen, Xinxiao Sun，Pingfang Tian, Yajun Yan, Jens Nielsen, Jia Wang*, Qipeng Yuan*, A bacterial platform for producing aromatic esters from glycerol, Nature Chemical Engineering, 2024, 1: 751-754

2. Li Cheng，Fengrong Zhang，Shunhao Wang，Xueting Pan，Sichong Han，Shuang Liu，Junjie Ma，Hongyu Wang，Heyun Shen，Huiyu Liuhy*，QipengYuan*，NIR‐Triggered Exogenous Enzymes to Convert Prodrugs for Locoregional Chemo‐Photothermal Therapy，Angewandte Chemie International Edition，2019，58：23-28

3. Wenna Li, Lin Ma, Xiaolin Shen, Jia Wang, Qi Feng, Lexuan Liu, Guojun Zheng, Yajun Yan, Xinxiao Sun*, Qipeng Yuan*, Targeting metabolic driving and intermediate influx in lysine catabolism for high-level glutarate production. Nature Communications, 2019,10:3337

4. Xianglai Li, Zhao Zhou, Wenna Li, Yajun Yan, Xiaolin Shen, Jia Wang, Xinxiao Sun* & Qipeng Yuan*，Design of stable and self-regulated microbial consortia for chemical synthesis，Nature Communications，2022, 13(1), 1554

5. Chang Ge, Zheng Yu, Huakang Sheng, Xiaolin Shen, Xinxiao Sun, Yifei Zhang, Yajun Yan, Jia Wang*& Qipeng Yuan*，Redesigning regulatory components of quorumsensing system for diverse metabolic control，Nature Communications，2022, 13(1), 2182

## Biography



Yuan Qipeng is a Changjiang Scholar Distinguished Professor of Education, a recipient of the 11th China Youth Science and Technology Award, one of Beijing's Top 100 Leading Talents, and Deputy Director of the National Key Laboratory for Efficient Utilization of Chemical Resources. His main research areas are synthetic biology and metabolic engineering, large-scale preparation and activity study of high-purity natural products. In recent years, he has taken on key R&D projects from the Ministry of Science and Technology, major and general projects funded by the National Natural Science Foundation, as well as provincial, ministerial, and industry cooperation projects. He has published over 300 SCI papers as first or corresponding author (cited over 20,000 times, H-index 77), and holds more than 90 authorized PCT and Chinese invention patents. Many of his achievements have been industrialized, generating significant economic benefits. As the primary contributor, he has won the National Science and Technology Progress Award Second Class once, and provincial/ministerial-level top awards including 1 Special Award, 2 First-class Awards, and 4 Second-class Awards.

# Engineering fatty acid synthase to control chain length and its application in yeast

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## Abstract

Fatty acids are important platform molecules for the sustainable production of fuels and oleochemicals. Their biological synthesis is catalyzed by fatty acid synthases (FASs), which are – in eukaryotes – large multienzyme assemblies that iteratively elongate acyl chains to defined lengths. A long-standing challenge has been to understand how chain-length control is naturally achieved by FASs, and whether this logic can be harnessed to program alternative chain lengths. In recent years, we have focused on establishing FASs as programmable platforms for tailored fatty acid biosynthesis. Starting with FAS from *Saccharomyces cerevisiae*, we demonstrated that minimally invasive, structure-guided mutations can redirect native yeast FAS toward selective production of short-chain fatty acids, enabling baker's yeast to produce C6 and C8 fatty acids. Building on these principles, we recently extended chain-length engineering to metazoan FASs. We generated a hybrid FAS composed of mouse FAS and *Escherichia coli* TesA, then tuned chain length by single amino acid substitutions in the C–C bond-forming ketosynthase domain. By balancing elongation and product release, we enable targeted production of C8 to C14 fatty acids. An *Ogataea polymorpha* strain carrying the engineered hybrid FAS produces C12 fatty acid. Both FAS systems were expanded in product spectrum by auxiliary domains.

**Key words:** fatty acid synthase, chain-length control, protein engineering, yeast cell factories, oleochemicals

**Representative publications:**

1. Ludig, D. L. et al. Engineering metazoan fatty acid synthase to control chain length applied in yeast. Nat. Chem. Biol. 2, 1078–1086 (2026).

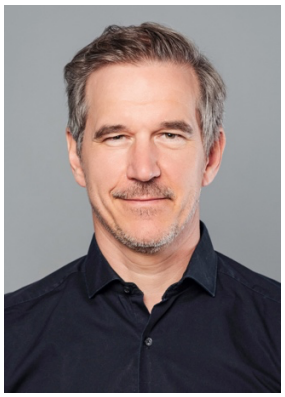
2. Gajewski, J., Pavlovic, R., Fischer, M., Boles, E. & Grininger, M. Engineering fungal de novo fatty acid synthesis for short chain fatty acid production. Nat. Commun. 8, 14650 (2017).

3. Gajewski, J. et al. Engineering fatty acid synthases for directed polyketide production. Nat. Chem. Biol. 13, 363–365 (2017).

4. Gusenda, C., Calixto, A. R., Da Silva, J. R., Fernandes, P. A. & Grininger, M. The kinetics of carbon–carbon bond formation in metazoan fatty acid synthase and its impact on product fidelity. Angew. Chem. Int. Ed. e202412195 (2024).

5. Heil, C. S., Wehrheim, S. S., Paithankar, K. S. & Grininger, M. Fatty acid biosynthesis: chain-length regulation and control. ChemBioChem 20, 2298–2321 (2019).

## Biography



Martin Grininger studied Technical Chemistry at Graz University of Technology, Austria. He carried out his doctoral research at the Max Planck Institute of Biochemistry, Martinsried, and received his doctorate from University of Munich in 2006. He was Project Group Leader at the Max Planck Institute of Biochemistry (2006–2011) and Visiting Professor at the University of Vienna (2010–2011). From 2012 to 2020 he held a Lichtenberg Professorship funded by the Volkswagen Foundation. Since 2020 he has been Professor of Biomolecular Chemistry at Goethe University Frankfurt, and since 2025 a Principal Investigator in the SCALE Cluster of Excellence (SynBio), Frankfurt.

# Microbial Cell Factories Manufacture Plant Natural Products

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## Abstract

Plant natural products have diverse structures and various biological and physiological activities including antiviral, anti-inflammatory, anticancer, antibacterial and liver protection, which are widely applied for medicine, food, cosmetics and chemical industry. However, all most of them are normally obtained by plant cultivation and chemical extraction, seriously limiting their applications because of lacking of sources, long production cycle and high production cost. Here we designed and built the microbial cell factories to synthesize the rare plant triterpenoids and flavonoids, which focused on glycyrrhetinic acid, oleanolic acid, ursolic acid, glycyrrhizin, ginsenosides and icariin. First of all, we mined and elucidated their synthesis pathway by analyzing the genome database and characterizing the key enzymes, and then modulated these pathways into yeast via three or more modules such as cyclization, oxidation and glycosylation. For improving the productivities of biomanufacturing, several strategies were used to optimize the metabolic flux of target compounds by engineering the activities of key enzymes, regulating their transcription, accelerating the transportation, organelles engineering, optimization of media and fermentation. The titers of these plant natural products reached the higher concentrations, showing the promising approaches and the potential industrial application.

**Key words:** microbial cell factories, pathway design, plant natural products, licorice

**Representative publication:**

1. Qin Lei#, Yang Bo#, Huang Rui#, Zhang Haocheng, Li Qiuyang, Li Xinyi, Tan Yingqi, Sun Wentao, Jia Haiyang, Hu Bing, Lv Bo*, **Li Chun***. Design of the biosensor-dependent coupling system stabilizes the high-synthesis phenotype of cell factory. ***Nature Communications*** 2026, 17, 5081.
2. Zhang Zhen, Li Wenqiang, Meng Fanze, Jin Yanming, Sun Wentao, Kuang Qina, Ren Shichao, Liu Wei, Zhang Liang, Qin Lei*, Lv Bo*, Jia Haiyang*, **Li Chun***. Discover the Maze-like Pathway for Glabridin Biosynthesis. ***Nature Communications*** 2026, 17:2215.
3. Li Yongxing, Du Bingjie, **Li Chun*,** Feng Xudong*. Engineering yeast peroxisome assembly enables the increased production of acetyl-CoA and its derived 5-deoxyflavonoids. ***Nature Communications*** 2025, 16(1):10468.
4. Jian Xing, Sun Qiuyan, Xu Wentao, Qu Haobo, Feng Xudong*, **Li Chun***. Engineering the Substrate Specificity of UDP-Glycosyltransferases for Synthesizing Triterpenoid Glycosides with a Linear Trisaccharide as Aided by Ancestral Sequence Reconstruction. ***Angew Chem Int Ed Engl***. 2024, 63(48): e202409867.

5. An Ting, Lin Guangyuan, Liu Yang, Qin Lei, Xu Yuquan, Feng Xudong*, **Li Chun***. De novo biosynthesis of icariin in engineered yeast. ***Metabolic Engineering***. 2023, 80:207-215.

## Biography



Chun LI is a distinguished Professor at Department of Chemical Engineering and a Director of institute of biochemical engineering, Tsinghua University. His research is interested in Biocatalysis and Synthetic Biology for biomanufacturing of plant natural products and design of industrial microorganisms with intelligent and multiple-stress resistance. He has obtained 58 authorized invention patents and has published above 480 journal papers with H factor of 61. He has compiled 11 books as chief editor or associate editor. Among them, Synthetic Biology has been widely used as textbooks in many universities. Prof. Chun LI is an associate director of Branch of Biochemical Engineering, The Chemical Industry and Engineering Society of China, and an associate director of Branch of Synthetic Biology, Chinese Society of Biotechnology. He also is a Chief-in-Editor of China Biotechnology and an Executive Chief-in-Editor of Synthetic Biology Journal.

# Glucose-driven evolution of *Pseudomonas putida* awakens catabolism of lignocellulose-derived pentoses

Prof. Pablo I. Nikel

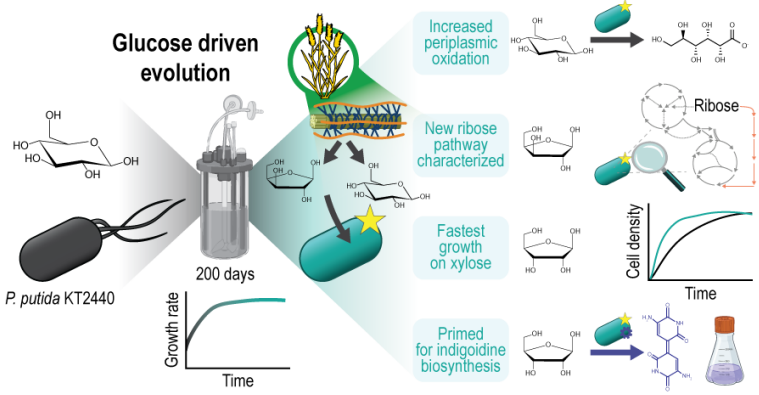
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## Abstract

The transition toward sustainable biomanufacturing requires microbial cell factories able to convert heterogeneous renewable feedstocks into valuable products. Lignocellulosic biomass is particularly attractive because of its abundance and limited competition with food production, yet its hydrolysates contain complex mixtures of hexoses, pentoses, organic acids, and inhibitory compounds that many industrial microbes cannot efficiently process. This keynote will examine how systems biotechnology can expand the metabolic capabilities of *Pseudomonas putida*, a robust environmental bacterium distinguished by broad catabolic diversity, flexible redox metabolism, and high tolerance to chemical stress. Adaptive laboratory evolution, genome sequencing, transcriptomics, and targeted pathway dissection will be discussed through the example of a glucose-evolved *P. putida* strain with improved carbon-use efficiency and enhanced growth on ribose. The analysis uncovered network-wide rewiring of periplasmic sugar oxidation and revealed a latent Weimberg-like route for ribonate assimilation. These findings illustrate how selection on one substrate can expose previously unrecognized metabolic functions relevant to pentose-rich lignocellulosic hydrolysates. Their application to indigoidine production from xylose further demonstrates how evolutionary and metabolic engineering can connect fundamental discoveries with bioproduction. More broadly, the work highlights the untapped biochemical potential of environmental bacteria and their emerging role in circular biomanufacturing.



**Key words:** *Pseudomonas putida* · Synthetic Biology · Metabolic Engineering · Non-canonical microbial hosts · Sustainability

**Relevant publications:**

1. Luppino F., Volke D.C., Gosling S., Skiadas I.V., Cho Avina Y.S., Nikel P.I., Glucose-driven evolution of *Pseudomonas putida* unlocks utilization of lignocellulose-derived pentoses for bioproduction, *Green Chemistry*, 2026. In press, https://doi.org/10.1039/D6GC01414C.

2. Volke D.C., Calero P., Nikel P.I., *Pseudomonas putida*, *Trends in Microbiology*, 2020, 28, 512–513.

3. Woo S., Lim H.G., Norton-Baker B., Lind T.M., Gladden N.E., Chen Y., de Venecia C., Eng T., Johnson C.W., Mukhopadhyay A., Petzold C.J., Guss A.M., Beckham G.T., Feist A.M., Simultaneous optimization of lignocellulosic sugar catabolism *via* systematic laboratory evolution under complex selection pressure, *Nature Communications*, 2026, 17, 9194.

4. Dvořák P., Burýšková B., Popelářová B., Ebert B.E., Botka T., Bujdoš D., Sánchez-Pascuala A., Schöttler H., Hayen H., de Lorenzo V., Blank L.M., Benešík M., Synthetically-primed adaptation of *Pseudomonas putida* to a non-native substrate D-xylose, *Nature Communications*, 2024, 15, 2666.

## Biography



Pablo I. Nikel earned a Ph.D. in Molecular Biology and Biotechnology in Buenos Aires, Argentina. After research in the United States at Rice University, supported by the American Society for Microbiology, he moved to Europe as a postdoctoral fellow in Madrid, funded by EMBO and the Marie Skłodowska-Curie Actions of the European Commission. He discovered the extraordinary potential of environmental bacteria, particularly *Pseudomonas putida*, as programmable platforms for biological engineering. Pablo is now Full Professor and Chair of Synthetic Bacterial Metabolism at DTU, where he leads the Systems Environmental Microbiology Group at DTU and serves as Scientific Director at BRIGHT, the Biotechnology Research Institute for the Green Transition. His team rewrites the core biochemistry of *P. putida* and related environmental bacteria through synthetic metabolism, systems biotechnology, and molecular engineering. Their work enables growth on unconventional substrates, synthetic $C_1$ assimilation, biological access to fluorinated chemistry, and biosynthesis of new-to-nature molecules that are difficult or impossible to obtain through biology alone. The long-term ambition of his research program is to expand the biochemical limits of microbial life and create controllable cell factories for sustainable production of chemicals, materials, and biopolymers (www.sem-cfb.com). Pablo has published more than 200 scientific articles and has received several distinctions in Europe and the Americas, including the Ascending Investigator, Pioneer Innovator, and Distinguished Innovator awards from the Novo Nordisk Foundation. He is an elected member of the American Academy of Microbiology and the European Academy of Microbiology, Editor-in-Chief of *Current Opinion in Biotechnology*, Senior Editor for *Metabolic Engineering*, *Microbial Biotechnology* and *mSystems*, and co-founder of four companies, including BioHalo, a spin-out venture from his laboratory pursuing biological production of fluorochemicals.

# Engineering Microbes for Dynamic Host-Microbiome Modulation

Abstract:

Microbes residing at epithelial interfaces continuously exchange metabolites, signals, and biomolecules with their hosts, influencing physiology across multiple scales. Advances in synthetic biology have enabled the programming of commensal microbes with defined sensing, metabolic, and regulatory functions. While much of the field has focused on using these capabilities for therapeutic intervention, an emerging opportunity is to use programmable microbes as experimental systems for understanding how host physiology is organized. In this talk, I will discuss our efforts to engineer commensal microbes as biological instruments for probing host–microbe interactions. Using examples spanning the gut–liver–brain axis, host-state sensing, and specialized epithelial niches, I will illustrate how programmable microbes can be used to investigate three fundamental questions: How far can microbial activities influence physiology? What physiological signals can microbes perceive? And how does the epithelial niche shape biological opportunity? These studies suggest that programmable microbial systems can serve not only as therapeutic agents, but also as powerful tools for uncovering the principles governing host physiology across epithelial interfaces.

Bio:



Matthew Chang is Provost's Chair Professor of Biochemistry at the Yong Loo Lin School of Medicine, National University of Singapore, and Executive Director of Singapore's National Centre for Engineering Biology. He directs the Singapore Consortium for Synthetic Biology, the Wilmar–NUS Corporate Laboratory, and NUS Synthetic Biology for Clinical and Technological Innovation. His research focuses on engineering programmable biological systems to understand how cells and communities process information and to create applications in biomedicine and biomanufacturing. He helped establish the Global Biofoundry Alliance and the Asian Synthetic Biology Association and advises international synthetic biology initiatives. He also co-chairs the World Economic Forum's Global Future Council on Generative Biology.

# Extremophile Biomanufacturing

Guo-Qiang CHEN

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## Abstract

Extremophiles able to grow on high or low pH, temperatures and osmotic pressures are found increasingly important for industrial biotechnology. As synthetic biology tools are becoming increasingly accessible and adaptable for uses in extremophiles, engineered extremophiles able to grow under open unsterile and continuous conditions have been exploited to obtain large amount of bioproducts to replace conventional and contamination prone *E. coli, Pseudomonas* spp., Bacillus spp. and Yeast et al, as observed with the arise of biotech companies based on *Halomonas* combining the advantages of tolerances to high and low pH, temperatures, osmotic pressures, growth in seawater and convenience of scale-up. Extremophiles, especially *Halomonas* spp., have rapidly become the focus of next generation industrial biotechnology (NGIB) operated under open unsterile and continuous conditions with large scale to change the conventional industrial biotechnology. NGIB will revolutionize the biomanufacturing with fresh water and energy saving advantages combined with reduced process complexity, ease of process scale-up and non-food substrates.

**Key words:** Extremophiles, *Halomonas*, Polyhydroxyalkanoates, PHB, Next generation industrial biotechnology

**Representative publication:**

1. Ye J.W., Lin Y., Yi X.Q., Yu Z.X., Liu X., Chen G.Q.* Synthetic Biology of Extremophiles, A New Wave of Biomanufacturing. Trends in Biotechnology 41 (2023) 342-357

## Biography



Prof Chen has been focusing his research on microbial materials polyhydroxyalkanoates (PHA) and 3HB metabolic engineering, synthetic biology and PHA biomaterial application since 1986. After joining Tsinghua University in 1994, he has developed the Next Generation Industrial Biotechnology (NGIB) based on *Halomonas* spp. for open unsterile industrial productions of PHA and Chemicals, has published over 400 international peer reviewed papers with over 52,000 citations (H-Index 118) (https://scholar.google.com/citations?user=qG7UQVcAAAAJ&hl=zh-CN) as reported in Google Scholar. With over 80 issued patents and 50 pending patents, Prof. Chen's technologies have been provided to several companies that succeeded in mass production of microbial polyhydroxyalkanoates (PHA). He is the most cited scientist in Synthetic Biology and Biomanufacturing in China, the most cited PHA biomaterial scientist globally. Beginning from 2015, he becomes the Funding Director of the Center for Synthetic and Systems Biology in Tsinghua University.

# From Marginal Gains to Major Improvements in Yeast Cell Factories

Kevin J. Verstrepen[1,2,3]

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[2]*CMPG Laboratory of Genetics and Genomics, KU Leuven, Gaston Geenslaan 1, Leuven, Belgium.*

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## Abstract

Our research focuses on characterizing, comparing and understanding industrial yeasts, and using these insights to generate superior yeasts with increased product yields for various fermentation processes, from traditional fermented beverages to bio-ethanol, lipids, proteins and chemical compounds.

Over the past years, we collected and characterized thousands of yeast strains from various niches. DNA analysis revealed the history and domestication of today's yeasts and opened the door to selecting better chassis strains. In addition, we use a combination of high-throughput breeding and gene editing to further improve production efficiencies.

In addition to giving an overview of the strategies we use to generate better industrial microbes, the talk will also cover a few examples of novel molecular tools such as CoMuTER and GEMbLeR, as well as novel intron-based methods to control gene expression that we developed to optimize genetic engineering and directed evolution approaches.

**Key words:** Mutagenesis, Directed evolution, Gene regulation, Biofuel, Precision Fermentation

**Representative publication:**

1. Q. Qi, P. Tomaz da Silva, V. Vangalis, S. Dockx, J. Steensels, K. Voordeckers, J. Gagneur, K.J. Verstrepen (2026). Intron location and sequence modulate gene expression in Yarrowia lipolytica. ***Nuc Acids Res.*** https://doi.org/10.1093/nar/gkag650 (IF 15)

2. B. Gallone, J. Steensels, T. Prahl, L. Soriaga, V. Saels, B. Herrera, A. Merlevede, M. Roncoroni, K. Voordeckers, L. Miraglia, C. Teiling, B. Steffy, M. Taylor, A. Schwartz, T. Richardson, C. White, G. Baele, S. Maere, and K.J. Verstrepen (2016). Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts. ***Cell***. 166(6):1397-1410.e16. doi: 10.1016/j.cell.2016.08.020 (IF 45.1)

3. C. Cautereels, J. Smets, J. De Saeger, L. Cool, Y. Zhu, A. Zimmermann, J. Steensels, A. Gorkovskiy, T. Jacobs and K.J. Verstrepen (2024). A set of 16 novel orthogonal LoxPsym sites allows multiplexed

site-specific recombination in prokaryotic and eukaryotic hosts. ***Nature Communications***. https://doi.org/10.1038/s41467-024-44996-8 (IF 18.1).

4. C. Cautereels, J. Smets, J. De Saeger, L. Cool, Y. Zhu, A. Zimmermann, J. Steensels, A. Gorkovskiy, T. Jacobs and K.J. Verstrepen (2024). Combinatorial optimization of gene expression through recombinase-mediated promoter and terminator shuffling in yeast. ***Nature Communications***. https://doi.org/10.1038/s41467-024-44997-7 (IF 18.1).

5. A. Zimmermann, J.E. Prieto-Vivas, C. Cautereels, A. Gorkovskiy, J. Steensels, Y. Van de Peer, K. J. Verstrepen (2023). A Cas3-base editing tool for targetable in vivo mutagenesis. ***Nature Communications***. doi: 10.1038/s41467-023-39087-z (IF 18.1)

## Biography



Kevin Verstrepen studies yeast genetics, genomics and various applications of yeast cell factories. He serves as endowed professor in Genetics and Genomics at Leuven University, director of the VIB Center for Microbiology, director of the Leuven Institute for Beer Research, Guest Professor at Tianjin Institute for Industrial Biotechnology and Honorary Professor at Nottingham University.

Kevin studied bio-engineering at KU Leuven. He subsequently joined the lab of Gerry Fink at MIT in Boston before starting his lab as a Bauer Fellow at Harvard University. In 2009, Kevin moved the lab back to his home country Belgium with support from Leuven University (KU Leuven), the Odysseus Project of the Flemish government and VIB (Flanders Institute for Biotechnology).

# Engineering Mixed Cultures for Gas Fermentation: Converting Biogas into Sustainable Protein

**Esteban Marcellinn**

Biogas contains methane and carbon dioxide, yet conventional microbial processes typically utilise only one of these carbon sources. Combining methane-oxidising and hydrogen-oxidising bacteria offers a route to convert both components into single-cell protein. Developing this approach requires an integrated understanding of cellular metabolism, microbial interactions and reactor performance.

This talk will examine the cocultivation of a thermotolerant Cupriavidus necator strain and Methylococcus capsulatus Bath. Methane supports methanotrophic growth, while hydrogen supplies the energy required for carbon dioxide fixation by C. necator. Continuous monoculture and coculture experiments are combined with multi-omics and mechanistic modelling to investigate gas consumption, biomass yields and community composition.

Integrated proteomics and metabolomics provide a framework for resolving species-specific metabolic responses, resource allocation, and potential metabolite exchange. Linking these molecular measurements with physiological data helps identify constraints on carbon assimilation, energy metabolism and protein production. These analyses examine whether coculture behaviour can be predicted from monoculture physiology and reveal targets for metabolic engineering.

The talk will connect these insights with strategies to balance microbial growth, optimise gas supply and manage hydrogen and oxygen competition. Integrating multi-omics with reactor models will guide the assessment of operating conditions that support community stability, carbon utilisation and protein productivity. This approach links molecular mechanisms with process design to advance mixed-culture biogas conversion and scale-up.

# Making peptides the non-ribosomal way: possibilities & limitations

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## Abstract

Several peptides derived from non-ribosomal peptide synthetases (NRPS) are used as clinical drugs to treat infections, cancer or other diseases. The architecture of NRPS, often composed of multiple modules for the incorporation and modification of specific building blocks like amino acids with carboxylic acids as starting units, makes these enzyme classes an ideal tool for synthetic biology approaches. In the past 10 years, we and others developed several NRPS engineering approaches that allow efficient modifications of natural non-ribosomal peptides (NRPs) or even the generation of completely new peptide scaffolds. Here, we show that, (i) natural NRPS can accept non-canonical amino acids (ncAAs) as building blocks, (ii) these ncAAs can be produced in situ from cheap precursors, (iii) starting units, responsible for the incorporation of amino acids, fatty acids and amino-, hydroxy- and epoxy-fatty acids can be varied efficiently and furthermore combined with new termination units allowing the simple generation of ring-sizes and -types between amino acid side chains or functional groups in the acyl starter units, and (iv) NRPS can also work in cell-free systems.

**Key words:** NRPS, peptides, macrocycles, non-canonical amino acids

**Representative publication:**

1. Podolski A, Lindeboom TA, Präve L, Dehghan M, Minas HA, Kranz J, Schindler D, Bode HB* (2025) High-Throughput Engineering and Modification of Non-Ribosomal Peptide Synthetases Based on Golden Gate Assembly. *Angew Chem Int Ed* 64(49): e202508967. doi: 10.1002/anie.202508967.
2. Bozhüyük KAJ*, Präve L, Kegler C, Schenk L, Kaiser S, Schelhas C, Shi YN, Kuttenlochner W, Schreiber M, Kandler J, Alanjary M, Mohiuddin TM, Groll M, Hochberg GKA, Bode HB* (2024) Evolution-inspired engineering of nonribosomal peptide synthetases. Science 383(6689). doi: 10.1126/science.adg4320.

3. Präve L, Kuttenlochner W, Tabak WWA, Langer C, Kaiser M, Groll M, Bode HB* (2024) Bioengineering of syrbactin megasynthetases for immunoproteasome inhibitor production. *Chem* 10 (1-12). doi:10.1016/j.chempr.2024.07.013.
4. Kaiser S, Shahmohammadi N, Goel TSM, Buchanan CJ, Garg SG, Vidaurre Barahona D, Qian Z, Lefèvre C, Schindler D, Kim Y, Bode HB*, Hochberg GKA* (2025) Drift, selection and convergence in the evolution of a nonribosomal peptide. doi: https://doi.org/10.64898/2025.12.15.694437 (in revision).
5. Gonschorek P*, Wilson CS, Schelhas C, Bozhüyük KAJ, Grün P, Bode HB* (2025) Split inteins for generating combinatorial non-ribosomal peptide libraries. bioRxiv 2025.10.02.680031; doi: https://doi.org/10.1101/2025.10.02.680031 (in revision).

## Biography



**1997**, Diploma in Chemistry, University of Göttingen (Germany)
**2000**, Dr. rer. nat., Organic Chemistry, University of Göttingen (Germany)
**2001**, Diploma in Biology, University of Göttingen (Germany)
**2000-2001**, Postdoc, University of Göttingen (Germany)
**2001-2002**, Postdoc, German Research Center for Biotechnology (Germany)
**2002-2003**, Postdoc, Department of Biochemistry, Stanford University (USA)
**2004-2005**, Juniorprofessor, Natural Product Biotechnology, Pharmaceutical Biotechnology, Saarland University, Germany
**2006-2010**, Emmy Noether Fellow (DFG), Saarland University and Goethe University Frankfurt
**2008-2017**, Merck-endowed chair (W3) for Molecular Biotechnology, Goethe University Frankfurt, Germany
**2018-2022**, Professor for Molecular Biotechnology, Goethe University Frankfurt
**since 2020**, Director and Head of the Department of Natural Products in Organismic Interactions at the MPI Marburg, Germany
**since 2022**, Professor for Chemical Biology, Department of Chemistry, Philipps-Universität Marburg, Germany
**since 2024**, Managing Director at the MPI for terrestrial Microbiology, Marburg

# Biomanufacturing of future food ingredients: From lab to industry

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## Abstract

The food industry constitutes a critical pillar of the global economy. However, conventional food production and processing paradigms are increasingly constrained by resource depletion, environmental pressures, population growth, climate change, and escalating public health demands. The emerging concept of future foods aims to systematically address challenges associated with food security, quality and safety, nutritional functionality, as well as consumer-centric sensory attributes. In this context, food biomanufacturing—defined by the utilization of renewable feedstocks and engineered microbial cell factories for the biosynthesis of food ingredients and functional components—has emerged as a sustainable and low-carbon alternative to traditional manufacturing routes. However, food biomanufacturing still faces several major challenges, including the lack of clearly defined key regulatory targets, the limited availability of regulatory elements, and inefficient metabolic pathways. To address these challenges, this report systematically explores strategies for rewiring cellular metabolic networks and improving product biosynthesis through the development of key target prediction tools, the mining and engineering of regulatory elements, and the establishment of metabolic pathway reconstruction technologies. Finally, the report highlights the construction of microbial cell factories for glucosamine and human milk oligosaccharide production, together with their industrial applications.

**Key words:** Biomanufacturing, Future food, Synthetic biology, Microbial cell factory.

### Representative publication:

1. Wu Y. K., Li Y., Jin K., Zhang L. P., Li J. H., Liu Y. F, Du G. C., Lv X. Q., Chen J., Ledesma-Amaro R., Liu L. CRISPR–dCas12a-mediated genetic circuit cascades for multiplexed pathway optimization, Nature Chemical Biology, 2023, 19, 367-377.
2. Xu X. H., Li X. L., Liu Y. F., Zhu Y. L., Li J. H., Du G. C., Chen J., Ledesma-Amaro R., Liu L. Pyruvate-responsive genetic circuits for dynamic control of central metabolism, Nature Chemical Biology, 2020, 16, 1261-1268.
3. Zhou X., Hou J. H., Wang Z. K., Li Z. D., Li Y., Li X. T., Xu X. H., Liu Y. F., Li J. H., Du G. C., Ma D. C., Tang J., Chen J., Lv X. Q., Liu L. De novo L-(+)-tartaric acid biosynthesis in multi-modular engineered yeasts. Nature Communications, 2026, 17, 9301.
4. Yu W. W., Jin K., Wang D. D., Wang N. K. Li Y. Y., Liu Y. F., Li J. H., Du G. C., Lv X. Q., Chen J., Ledesma-Amaro R., Liu L. De novo engineering of programmable and multi-functional biomolecular condensates for controlled biosynthesis, Nature Communications, 2024, 15, 7989.
5. Xu Y. M., Wang X. L., Zhang C. Y., Zhou X., Xu X. H., Han L. Y., Lv X. Q., Liu Y. F., Liu S., Li J. H., Du G. C., Chen J., Ledesma-Amaro R., Liu L. *De novo* biosynthesis of rubusoside and rebaudiosides in engineered yeasts, Nature Communications, 2022, 13, 3040.

## Biography



Long Liu, Ph.D., Professor and Doctoral Supervisor, serves as Vice President of Jiangnan University, with research expertise in synthetic biology and food biomanufacturing. Liu has led 13 national and provincial-level research projects, including key programs funded by the National Key Research and Development Program of China and the National Natural Science Foundation of China. Liu is first or corresponding author of over 200 publications in top journals, including *Nature Chemical Biology*, *Nature Synthesis*, and *Nature Communications*, and has served as editor of six books. He holds 120 granted patents, including 19 in the United States, and has received more than 10 prestigious scientific awards, including the Second Prize of the National Science and Technology Progress Award of China.

# Engineering microbes for a multidimensional circular economy – plastic monomers to OH-fatty acids as an example

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The global challenge of end-of-life management of plastics is well documented and increasingly prominent in our daily lives. Current approaches for plastic recycling include thermochemical processes such as pyrolysis, chemical depolymerization (chemolysis), and emerging biocatalytic strategies based on enzymes and microorganisms. While pyrolysis and chemolysis enable the recovery of valuable chemical building blocks, biocatalysis offers the potential for selective depolymerization under mild conditions. The use of plastic monomers as carbon and energy sources for microbes opens up new opportunities to integrate biotechnology into the plastic recycling industry and to develop circular approaches for plastic waste valorization (1).

The research is included in the Werner Siemens Foundation (WSS) research center **catalaix** (https://catalaix.com/en/). In **catalaix**, we research how to keep the plastic carbon in the loop. At MES, I will present examples how we use with partners chemo- and biocatalysis for plastic degradation and engineer microbes, mainly *Pseudomonas*, for utilizing the plastic monomers as carbon and energy source (2,3). Our newest results on synthesizing defined OH-fatty acids will also be presented (4).

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(2) Welsing G, Wolter B, Kleinert GEK, Göttsch F, Besenmatter W, Xue R, Mauri A, Steffens D, Köbbing S, Dong W, Jiang M, Bornscheuer UT, Wei R, Tiso T, Blank LM. Two-step biocatalytic conversion of post-consumer polyethylene terephthalate into value-added products facilitated by genetic and bioprocess engineering. Bioresource Technol. 2025; 417:131837
(3) Meng H, Karmainski T, Ben Ammar A, Sieberichs A, Branson Y, Vossen P, Schwanemann T, Ballerstedt H, Bornscheuer UT, Wei R, Blank LM. Engineering *Pseudomonas putida* KT2440 for open-loop upcycling of mixed plastics. Trends Biotechnol. 2026:S0167-7799(26)00370-7
(4) Meng H, Schwanemann T, Lipa MK, Michel CV, Xia J, Blank LM. Engineering of *Pseudomonas putida* KT2440 for broad-chain-length 3-hydroxy fatty acid biosynthesis. bioRxiv, 2025.11. 04.686531

# Engineering red yeast-based cell factories

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## Abstract

The red yeast *Rhodotorula toruloides* has been emerged as a powerful host for the production of lipids and oleochemicals. Over the past 10 years or so, we and others developed a series of tools for gene targeting, co-expression, down-regulation, and homologous recombination in this yeast. By using these tools, we created strains with designated features of industrial concerns and improved production of valuable lipids such as diacylglycerols and terpenoids. For example, overexpression of *Vitreoscilla* hemoglobin led to improved sugar consumption and lipid yield under oxygen-limited conditions and expression of manganese peroxidase led to improved resistance to phenolic inhibitors of lignocellulosic biomass origin. Moreover, secretory expression of Man5C facilitated lipid recovery directly from the culture broth with greener solvents. Finally, systems engineering of *R. toruloides* led to overproducing high value products such as resveratrol and isoflavones, as well as long-chain hydrocarbons for sustainable aviation fuel applications. Overall, advanced cell factories based on *R. toruloides* will be summarized and future directions be discussed.

**Key words:** *Rhodotorula toruloides*; Microbial lipids; Metabolic engineering; Synthetic biology

**Representative publication:**

1. Lyu L. T., Chen Q. Q., Xue H. Z., Mustafa S., Shah A. M., Huang Q. T., Zhang Y., Wang S., Zhao Z. K. Front. Bioeng. Biotechnol. 2024, 12, 1310069
2. Liang S. Y., Zhang Y., Lyu L. T., Wang S., Zhao Z. K. Bioresour. Bioprocess. 2023, 10, 16.
3. Zhang Y., Zhang S. F., Chu Y. D., Zhang Q., Zhou R. H., Yu D., Wang S., Lyu L. T., Xu G. W., Zhao Z. K. Front. Bioeng. Biotechnol. 2022, 10, 1034972.
4. Lyu L. T., Chu Y. D., Zhang S. F., Zhang Y., Huang Q. T., Wang S., Zhao Z. K. Front. Bioeng. Biotechnol. 2021, 9, 768934.

## Biography



Professor Zhao completed his PhD study at Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences and postdoc training at University of Minnesota and University of Texas at Austin, USA. His research interests mainly lie in energy biotechnology and synthetic biology, with special focuses on microbial lipid technology, systems biology of oleaginous yeasts, and application of non-natural coenzymes. He received the National Science Fund for Distinguished Young Scholars from NSFC. He has published more than 300 papers with over 17,000 citations. He serves as the editor of *Bioresources and Bioprocessing* and *FEMS Yeast Research*.

# Synthetic biology and process engineering facilitate the high efficient production of rhamnolipid

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## Abstract

Efficient and safe rhamnolipids production requires integrated advances in strain discovery, chassis engineering, and process design. Here, we first developed a transcription-associated FADS platform to rapidly screen high di-rhamnolipid producers. Based on this, a high-producing *Pseudomonas aeruginosa* strain was further engineered into a low-virulence chassis, reducing pathogenicity while maintaining production capacity. In parallel, we established rhamnolipid biosynthesis in *Pseudomonas putida* KT2440 as a safe and industrially relevant host. Mechanistic analysis revealed a key role of nitrate metabolism in supporting rhamnolipid production, which guided subsequent metabolic and process optimization. Overall, our work integrates high-throughput screening, virulence attenuation, and heterologous chassis development with fermentation engineering, providing a coherent and scalable strategy for rhamnolipids production.

**Key words:** Thamnolipids; *Pseudomonas putida* KT2440; Cell factory; Metabolic engineering

**Representative publication:**

Xu A.M., Zhang X.X., Cao S.X., Zhou X.L., Yu Z.Y., Qian X.J., Zhou J., Dong W.L., Jiang M., Transcription-Associated Fluorescence-Activated Droplet Sorting for Di-rhamnolipid Hyperproducers, ACS Synthetic Biology, 2022,11 (6),1992-2000.

Liu, S., Xu, A.M., Xie, B., Xin, F.X., Dong, W.L., Zhou, J., Jiang, M. (2022). Priority changes between biofilm exopolysaccharides synthesis and rhamnolipids production are mediated by a c-di-GMP-specific phosphodiesterase NbdA in Pseudomonas aeruginosa, Iscience, 2022, 25(12).

Xue, R., Qiu, C.H, Zhou, X.L., Cheng, Y., Zhang, Z., Zhang, Y., Uwe Schröder, Uwe T. Bornscheuer, Dong W.L., Wei R., Jiang, M., Enzymatic upcycling of PET waste to calcium terephthalate for battery anodes, Angewandte Chemie International Edition, 2024, 63(1): e202313633.

Wang J.N., Guo F., Xin F.X., Zhang W.M., Jiang M., Biohybrid materials enable efficient biosynthesis of chemicals, Trends in Chemistry, 2026.

## Biography



Prof. Min Jiang currently serves as Dean of the College of Biotechnology and Pharmaceutical Engineering of Nanjing Tech University. He got his Ph.D degree from Nanjing Tech University under the supervision of Ouyang Pingkai and completed his postdoctoral training at KAIST from 2000 to 2002. His research focus on the biorefinery of waste carbon resources into high-value products. He has led more than 40 research projects, including the National Key R&D Program project and the NSFC-EU collaborative project "MIX-UP".

# Engineering Microbial Cell Factories for Precision Biomanufacturing of Chondroitin Sulfate

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## Abstract

Chondroitin sulfate (CS) is a bioactive glycosaminoglycan widely used in pharmaceuticals and nutraceuticals. Conventional animal-derived production faces challenges in sustainability and structural consistency, highlighting the need for microbial biomanufacturing. Here, we developed an integrated synthetic biology strategy coupling three essential modules: chondroitin backbone biosynthesis, energy-efficient supply of the sulfate donor PAPS, and regioselective sulfation. First, high-producing chondroitin cell factories were constructed through genomic integration of biosynthetic pathways in *Escherichia coli*, *Bacillus subtilis*, and *Corynebacterium glutamicum*, respectively. Second, a novel adenine sulfotransferase was identified and engineered to establish an artificial PAPS biosynthetic pathway with reduced ATP consumption, alleviating a key bottleneck in sulfate donor supply. Third, AI-guided protein engineering generated sulfotransferase variants with enhanced stability, catalytic efficiency, and regioselectivity, enabling precise control over CS sulfation patterns. Integration of these modules enabled whole-cell biocatalytic production of CS-A, CS-C, and CS-E with degrees of sulfation exceeding 90%, as well as the development of engineered strains capable of producing structurally defined CS. This work establishes a modular and scalable platform for precision biomanufacturing of CS, offering a sustainable alternative to animal-derived production.

**Key words:** Chondroitin sulfate (CS), microbial biomanufacturing, PAPS, sulfotransferase, degrees of sulfation

**Representative publication:**

1. Liu H. Y., Wei W. Q., Pang Z. X., Gu S. M., Song W., Gao C., Chen X. L., Liu J., Guo L., Wu J., Liu L. M.*, Protein engineering, cofactor engineering and surface display engineering to achieve whole-cell catalytic production of chondroitin sulfate A, Biotechnology and Bioengineering, 2023, 120(7), 1784-1796.

2. Wang Z. H., Song W., Wei W. Q., Qi H. J., Meng W. W., Liu J., Li X. M., Gao C., Liu L. M., Hu G. P., Zhou Y. W., Wu J.*, Structural and mechanism-based engineering of sulfotransferase CHST15 for the efficient synthesis of chondroitin sulfate E, Applied and Environmental Microbiology, 2025, 91, e01573-24.

3. Gu S. M., Zhang F., Li Z. Y., Qi H. J., Huang L. X., Liu K. F., Song W., Wei W. Q., Gao C., Hu G. P., Li X. M.*, Liu L. M.*, Engineering a novel adenine-sulfotransferase for efficient synthesis of PAPS and chondroitin sulfate in microbial cells. Trends in Biotechnology, 2025, 43 (10), 2609-2626.

4. Meng W. W., Gao M. J., Li L. L., Liu L. M., Li X. M., Wei W. Q.*, Wu J.*, Redesigning chondroitin 6-O-sulfotransferase for efficient whole-cell biosynthesis of chondroitin sulfate C. Bioresource Technology, 2026, 449, 134397.

5. Qi H. J., Shi L., Huang L. X., Song X. T., Zhang L. H., Meng W. W., Gao C., Hu G. P., Liu J., Wu Z. M., Wu J.*, Li X. M.*, Coordinated optimization of 3'-phosphoadenosine-5'-phosphosulfate (PAPS) supply and sulfotransferase expression in *Escherichia coli* for production of chondroitin sulfate A with high sulfation degree. Carbohydrate Polymers, 2026, 383, 125280.

## Biography



Liming Liu is a Professor, PhD Supervisor, and Distinguished Professor of the Ministry of Education’s Changjiang Scholars Program. His research focuses on synthetic biology, microbial cell factory engineering, and industrial biotechnology. He has served as principal investigator on more than 20 national and provincial research projects and published over 250 scientific papers. He holds 125 granted invention patents and has authored six academic monographs. His research has enabled the industrial application of engineered microbial strains and preparations in leading biotechnology companies, contributing to a cumulative industrial output value exceeding RMB 100 billion over the past five years. His contributions have been recognized with prestigious awards, including the Second Prize of the State Technological Invention Award, the First Prize of the Ministry of Education Science and Technology Progress Award, and the Second Prize of the Jiangsu Provincial Natural Science Award.

# Bioconversion of lignocellulosic biomass to fuels and chemicals via DLC sugar platform

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## Abstract

Bioconversion of lignocellulosic biomass to fuels and chemicals has attracted much attention due to its benefits to environmental and social sustainability. However, the adverse properties of lignocellulosic biomass (e.g. low bulk density, easily contaminated by microbes), severe conditions required for biomass pretreatment, toxic degradation products generated during pretreatment are remaining barriers for commercialization. To address these issues, we developed a novel pretreatment method: Densifying Lignocellulosic biomass with Chemicals (DLC), which is of low-cost and easy to implement. The DLC pretreated biomass is of high density and high durability with biomass contamination prevented, which greatly facilitates biomass logistics. DLC pretreated biomass showed high enzymatic digestibility and high fermentability even at high solid loadings. Based on DLC sugar platform, we developed various microbial strains that can ferment xylose efficiently and can tolerate the toxicity of lignocellulosic hydrolysate. As a result, an ethanol titer over 100 g/L, a lactic acid titer over 200 g/L, and a succinic acid titer over 105 g/L were achieved in DLC lignocellulosic hydrolysate, without washing or detoxifying pretreated biomass. DLC pretreatment has been industrially demonstrated and the cellulosic ethanol production has also been demonstrated at 1000 ton/year scale.

**Key words:** Lignocellulosic sugar platform, Cellulosic ethanol, Biomass conversion, Metabolic engineering

**Representative publication:**

1. Chen S.[#], Xu Z.[#], Ding B., Zhang Y., Liu S., Cai C., Li M., Dale B., Jin M.*, Big data mining, rational modification, and ancestral sequence reconstruction inferred multiple xylose isomerases for biorefinery, Science Advances, 2023, 9(5), eadd8835.

2. Cai C.[#], Xu Z.[#], Zhou H., Chen S., Jin M.*, Valorization of lignin components into gallate by integrated biological hydroxylation, O-demethylation, and aryl side-chain oxidation, Science Advances, 2021, 7(36), eabg4585.

3. Chen X., Xu Z.*, Zhai R., Sha Y., Yang S., Qin M., Yuan X., Jin M.*, A versatile lignocellulosic sugar platform for green bio-manufacturing featuring high fermentability and diverse lignin valorization, Chemical Engineering Journal, 2025, 520:166263.

4. Ge M.[#], Sha Y.[#], Lu M., Zhang Y., Xu Z., Chen S., Ding Y., Jin M.*, Construction and optimization of efficient glucose–xylose co-fermenting yeast *Yarrowia lipolytica* for green and sustainable succinic acid production from lignocellulosic biomass, Green Chemistry, 2025, 27(2): 450-463.

5. Lu M.[#], Sha Y.[#], Zhang Y., Ge M., Xu Z., Jin M.*, Efficient production of citric acid from lignocellulose hydrolysate by metabolically engineered *Yarrowia lipolytica*, Green Chemistry, 2025, 27(2): 1113-1124.

## Biography



Dr. Mingjie Jin is a full professor at Nanjing University of Science and Technology, China. He obtained his Ph.D. in Chemical Engineering from Michigan State University (MSU), and then worked as a postdoc., assistant professor and R&D scientist at MSU and Edeniq Inc., respectively. In 2015, he joined Nanjing University of Science and Technology and worked there since then. He is a renowned researcher in the field of lignocellulosic biomass conversion for production of fuel ethanol, microbial lipids, lactic acid, and various other bio-chemical products. Prof. Jin's research is supported by national programs, such as National Key R&D Program, National Natural Science Foundation of China, etc. He has authored over 170 peer-reviewed articles in prestigious journals, such as Energy & Environmental Science, and Science Advances. He has obtained over 20 patents and serves as the editorial board member of several journals.

# Discovery and engineering of plant chemistry for plant and human health

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## Abstract

Plant-derived natural products and their derivatives have long been used as medicinal agents, and they still constitute a significant fraction of clinically approved drugs. The combination of the tools and principles of chemistry, together with the tools of modern biology, allows us to create complex synthetic and natural molecules, comprising processes with novel biological, chemical, and physical properties. This lecture will illustrate the opportunities at the interface between natural product chemistry and chemical biology by describing a series of examples that we are actively pursuing in our laboratory at Peking University. We take inspiration from Mother Nature to study the biosynthesis of plant-derived natural products to elucidate new enzymatic mechanisms and apply the chemoenzymatic approach to prepare complex natural products and their derivatives. Moreover, we further use bioactive natural products to explore new biology and develop novel drug candidates for human diseases, such as cancers, metabolic, and autoimmune diseases.

**Key words:** plant natural product, biosynthesis, drug development, enzymology

**Representative publication:**

1. Runze Tian, Feifan Lin, Nianxin Guo, Chendi Liu, Kaiqi Chen, Yunxi Han, Ruiyun Lan, Qiang Li, Jianbin Yan*, Xiaoguang Lei* “Complete biosynthesis of the anticancer cephalotaxinone and homoerythratine” Cell 2026, 189, 5673-5687

2. Zhanli Wang, Lu Han, Lei Gao*, Liyun Zhang, Yan Xie, Hanwu Liu, Ming Wu, Ning Yue, Yan Wang, Meng Han, Qi Ding, Xiyin Zheng, Jidong Cao, Xueqi Shen, Haijun Wang, Tuxunaili Aizitili, Chunyan Wu, Xuehong Wu, Zhenhua Liu, Yiguo Hong, Xiaoguang Lei*, Yule Liu* “Genetic basis of phytoalexin-mediated chemical defense against cross-kingdom pathogens” Cell 2026, 189, 4059-4074

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4. Xiaojiao Guo, Lei Gao, Shiwei Li, Jing Gao, Yuanyuan Wang, Jing Lv, Jiayi Wei, Jing Yang, Han Ke, Qi Ding, Jun Yang, Fusheng Guo, Haowen Zhang, Xiaoguang Lei*, Le Kang* “Decoding 4-vinylanisole biosynthesis and pivotal enzymes in locusts” Nature 2025, 644, 420–429

5. Bin Jiang, Lei Gao, Haijun Wang, Yaping Sun, Xiaolin Zhang, Han Ke, Shengchao Liu, Pengchen Ma, Qinggang Liao, Yue Wang, Huan Wang, Yugeng Liu, Ran Du, Torben Rogge, Wei Li, Yi Shang, K. N. Houk, Xingyao Xiong, Daoxin Xie, Sanwen Huang, Xiaoguang Lei*, Jianbin Yan* “Characterization and heterologous reconstitution of Taxus biosynthetic enzymes leading to baccatin III” Science 2024, 383, 622-629

## Biography



Prof. Xiaoguang Lei is the Boya Distinguished Professor of Chemistry and Chemical Biology and a senior PI of the Peking-Tsinghua Center for Life Sciences. His primary research areas include chemical biology, natural product synthesis, biosynthesis/biocatalysis, and drug discovery. He has published more than 200 original research papers, including those in Science (5), Cell (4), and Nature (2), among others, and has obtained 30 granted patents for new drug discoveries. He has developed four “first-in-class” drug candidates, one of which is currently in Phase II clinical trials and the other in Phase I. He has received many prestigious awards, including the 2025 New Cornerstone Investigator Award, 2023 Sackler Chair Award in Israel, the 2022 Molecules Tu Youyou Award, the 2022 Xplore Prize, the 2021 Boehringer-Ingelheim Senior Investigator Award, the 2020 Bayer Investigator Award, the 2018 David Ginsburg Award in Israel, the 2017 Tetrahedron Young Investigator Award, the 2017 Swiss Chemical Society Distinguished Lectureship Award, the 2015 Chemical Society of Japan Distinguished Lectureship Award, the 2014 Roche Young Investigator Award, the 2013 International Chemical Biology Society (ICBS) Young Chemical Biologist Award, and the 2010 IUPAC Young Chemist Award, etc. He is currently an Associate Editor for JACS Au.

# Spatiotemporal Control of Metabolism *via* Engineered Membraneless Organelles

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## Abstract

Metabolic engineering in prokaryotes has traditionally relied on pathway overexpression, knockout strategies, and static optimization—yet persistent bottlenecks such as toxic intermediate accumulation, pathway imbalance, and lack of dynamic regulation remain unresolved because the cytoplasm is treated as a homogeneous solution. We have developed a radically different approach: building *de novo* membraneless organelles (MLOs) in *Escherichia coli* using phase-separating protein scaffolds. These synthetic condensates serve as catalytic hubs, light-responsive switches, and even communicating networks that coordinate multi-step reactions. I will describe our journey from creating the first synthetic MLOs in bacteria, through functional diversification (enzyme recruitment, in situ nanomaterial synthesis, optogenetic control), to our latest achievement—programmable inter-organelle crosstalk in a cell-free system. This work transforms *E. coli* into a spatially organized, responsive, and networked chassis, offering a new paradigm for metabolic engineering that moves beyond static pathway design toward dynamic, compartmentalized, and intelligent bioproduction.

**Key words:** Synthetic biology, protein condensates, phase separation, biocatalysis, metabolic engineering

**Representative publication:**

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2. Qian ZG, Huang SC, Xia XX*. Synthetic protein condensates for cellular and metabolic engineering. *Nat. Chem. Biol*., 2022, 18(12): 1330-1340.

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4. Pan F, Zu H, Zhu YJ, Qian ZG*, Xia XX*. Spatiotemporal organization of functional cargoes by light-switchable condensation in *Escherichia coli* cells. *JACS Au*, 2024, 4(4): 1480-1488.

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## Biography



Dr. Xiaoxia Xia is a Distinguished Professor at Shanghai Jiao Tong University, where she heads a research group dedicated to the synthetic biology of protein materials. She received her Ph.D. in Chemical and Biological Engineering from KAIST in 2009 and completed postdoctoral training at KAIST and Tufts University before joining SJTU in September 2012. Her work centers on designing protein-based functional materials, particularly synthetic protein condensates. She has developed artificial membraneless organelles in prokaryotes that enable spatial organization of metabolic pathways within bacterial cells. This approach addresses common challenges in metabolic engineering, such as toxic intermediate accumulation and pathway imbalance, by creating catalytic hotspots and allowing dynamic control of enzyme localization. Her work has contributed to advances in biocatalysis, cellular engineering, and biomanufacturing, and has led to functional materials with various potential applications. Dr. Xia has published over 80 papers in high-impact journals such as Nature Chemical Biology, Nature Communications, and JACS, with over 6,600 Google Scholar citations and an h-index of 41. She also holds more than 20 patents, underscoring the translational relevance of her work. An active member of the international scientific community, she serves on the editorial boards of ACS Biomaterials Science & Engineering and Biotechnology Journal.

# Deciphering the Pyrethrins Biosynthetic Pathway and Heterologous Reconstitution in *Nicotiana benthamiana* and *Saccharomyces cerevisiae*

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## Abstract

Pyrethrins are a class of natural insecticides derived from pyrethrum (*Tanacetum cinerariifolium*), characterized by outstanding properties including high efficacy, broad-spectrum activity, and environmental friendliness. Their overall performance far surpasses that of chemically synthesized pyrethroids, indicating broad application prospects. However, the complete biosynthetic pathway of natural pyrethrins has long remained elusive, severely hindering their sustainable, large-scale production. In this study, the pyrethrins biosynthetic pathway was systematically reconstructed. Several previously uncharacterized key steps were identified, including a specialized *β*-oxidation process, an essential glycosylation modification step, and a terpene synthase with dephosphorylation activity that enhances pathway efficiency. Ultimately, the heterologous biosynthesis of pyrethrins were successfully achieved in both *Nicotiana benthamiana* and *Saccharomyces cerevisiae*. This study reveals the pyrethrin biosynthetic pathway and lays a crucial foundation for its sustainable microbial manufacturing.

**Key words:** Pyrethrins, Biosynthetic pathway, Heterologous biosynthesis, *Nicotiana benthamiana*, *Saccharomyces cerevisiae*

**Representative publication:**

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2. Yuan Y., Cheng S., ian G., Yan P., Ma Z., Dai W., Chen R., Fu S., Huang H., Chi H., Cai Y., Deng Z., Liu T.*, Efficient exploration of terpenoid biosynthetic gene dlusters in filamentous fungi. Nature Catalysis, 2022, 5, 277-287.

3. Zhi Y., Dai C., Fang X., Xiao X., Lu H., Chen F., Chen R., Ma W., Deng Z., Lu L.*, Liu T.*, Gene-Directed In Vitro Mining Uncovers the Insect-Repellent Constituent from Mugwort (*Artemisia argyi*), Journal American Chemical Society, 2024, 146 (45), 30883–30892.

4. Zou Y., Wang R., Liu Z., Zou Y., Zeng S., Fang M., Chen H., Wang P., Xie C., Deng Z., Zhang F., Liu R.*, Liu T.*, Development of a versatile chassis for the efficient production of diverse type II polyketides, Nature Communications, 2025, 16(1), 7321.

5. Ye Z., Shi B., Huang Y., Ma T., Xiang Z., Hu B., Kuang Z., Huang M., Lin X., Tian Z., Deng Z., Shen K., Liu T.*, Revolution of vitamin E production by starting from microbial fermented farnesene to isophytol, Innovation (Camb), 2022, 3(3), 100228.

## Biography



Tiangang Liu is a Distinguished Professor at Shanghai Jiao Tong University and serves as the principal investigator of a project funded by the National Key Research and Development Program of China under the “Synthetic Biology” special initiative. As the first-ranked contributor, he was selected for the “Top Ten Scientific and Technological Events in Hubei in 2018” and received the First Prize of the Hubei Provincial Science and Technology Progress Award in 2019. He currently serves as Executive Editor-in-Chief of Vita Microbiology and Associate Editor of Biotechnology Advances and Metabolic Engineering Communications. His research interests include efficient mining of natural products, quantitative synthetic biology rationally guides overproduction of natural products, and microbial metabolism–human interaction mechanisms.

# Yarrowia lipolytica as a chassis for synthetic biology: from genome editing to metabolic engineering

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## Abstract

*Yarrowia lipolytica* is a non-conventional oleaginous yeast with great potential in industry. Many synthetic biology tools and techniques, including promoters, terminators, DNA assembly techniques, and subcellular organelle engineering tools have been developed. Genome-scale mutagenesis, phenotypic screening, and tracking the causal mutations was also employed. Recently, enhancement of single-stranded template annealing activity by Rad52 enables precise and efficient genome editing, including small-fragment insertions, deletions, and replacements, as well as large-fragment deletions exceeding 20 kb was developed, which promoted the application of this yeast.

With these tools in hand, We constructed many metabolic pathways in *Yarrowia lipolytica*. For example, reconfiguration of the reductive succinic acid biosynthesis pathway in the mitochondria through coupling the oxidative and reductive TCA cycle for NADH regeneration results in improved SA production without pH control. In pilot-scale fermentation, the engineered strain produces 111.9 g/L SA with a yield of 0.79 g/g glucose.

**Key words:** Yarrowia lipolytica, metabolic pathways,

**References**

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2. Enhancement of Single-stranded Template Annealing activity by Rad52 during Repair of CRISPR-induced dsDNA breaks, Nucleic Acids Research, 2026, doi: 10.1093/nar/gkag288.

## Biography

Prof. Dr. Qingsheng Qi, received his Ph.D in University of Muenster, Germany. Then he became a staff member in University of Chemnitz, Germany. Since 2004, he is full professor in State Key Laboratory of Microbial Technology in Shandong University, People's Republic of China. Meanwhile, he is joint professor of National Glycoengineering Research Center.

He published approximately 200 papers in international and national journals, such as Metabolic Engineering, Appl. Environ. Microbiol., and Nature communication. He also served as editor board member of Appl. Environ. Microbial.. etc.



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Metabolic Engineering Summit 2026

# Rethinking Fermentation: Freeing Production from Redox Constraints

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## Abstract

The development of next-generation bioprocesses relies on genetically stable biocatalysts which can maintain high production yields. Here, metabolic rewiring offers a powerful strategy to couple cellular fitness to product formation. Such self-optimizing synthetic growth-coupled bioproduction (GCBP) systems ensure robust product yields and long-term genetic stability.

Conventional anaerobic fermentations, which are the key to the efficient production of ethanol or lactate, depend on ATP generation via glycolytic substrate-level phosphorylation. However, this mode of bioproduction requires a canonical glycolytic architecture and a strict balancing of reduction equivalents and thereby restricts feasible substrate–product combinations.

To break these boundaries, we engineered an obligatory fermenting *E. coli* strain by removing all enzymes catalyzing the transfer of electrons from NADH to the electron transport chain. In this background, respiratory modules can be integrated into fermentative metabolism to channel excess electrons to oxygen. Such unbalanced fermentations allow fundamentally new and previously inaccessible substrate–product combinations while maintaining high yields. When directly linked to the respiratory chain, these modules enable the construction of GCBP designs with a strong intrinsic drive for ATP generation, thereby reinforcing flux through the desired production pathway.

By strategically integrating respiratory modules, this platform expands fermentation for the synthesis of oxidized products and opens new design opportunities.

**Key words:** Synthetic metabolism, respiro-fermentation, NADH,

**Representative publications:**

1. Schulz-Mirbach, H., Krüsemann, J. L., Andreadaki, T., Nerlich, J. N., Mavrothalassiti, E., Boecker, S., ... & Lindner, S. N. (2024). Engineering new-to-nature biochemical conversions by combining fermentative metabolism with respiratory modules. *Nature Communications*, *15*(1), 6725.

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## Biography

I **studied Biology** in Düsseldorf, Germany and completed my diploma thesis at Forschungszentrum Jülich, focusing on microbial biotechnology and metabolic engineering.
During my **doctoral and postdoctoral research** in the Wendisch lab at Münster and Bielefeld, I deepened this expertise with an emphasis on second generation feedstocks.
In 2013, I joined **Algenol Biofuels** in Berlin, developing cyanobacterial strains and gaining industrial experience.
In 2015, I joined **Arren Bar-Even's group** at the Max Planck Institute in Potsdam-Golm, where rewiring metabolism shaped my approach to use growth-coupling for bioengineering.
Since May 2021, I have led the **Lindlich Lab** at Charité Berlin, developing synthetic metabolism and engineering growth-coupling platform strains to solve bottlenecks in bioeconomy.



# Manufacturing Plant Natural Products by Microorganisms

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## Abstract

Development of synthetic biotechnology has enabled microorganisms to produce a variety of value-added plant natural products that could be widely used in food and pharmaceutical components. Based on the principles of synthetic biology, the design and creation of artificial synthetic cells to produce important plant natural products can not only control the supply of raw materials, reduce production costs, but also protect natural resources. To achieve the efficient production of plant natural products through microorganisms, it is necessary to address issues such as the exploration and identification of the native anabolic pathways of these products, the rational modification and efficient expression of key enzymes derived from plants, the balanced regulation of heterologous metabolic pathways and the microbial's own metabolic pathways, as well as the selective secretion of target products and product tolerance. Combining protein engineering, pathway engineering, along with the application of artificial intelligence, efficient production of many useful plant natural products, such as chlorogenic acid, rosmarinic acid, glabridin, andrographolide, have been achieved.

**Key words:** Synthetic biology, terpenes, phenolic acids, flavonoids, enzyme engineering

**Representative publication:**

1. Xu KJ, Chen QM, Fu HR, Chen QH, Gu JH, Wang XL, Zhou JW*. Simultaneous engineering of the thermostability and activity of a novel aldehyde dehydrogenase. ACS Catalysis, 2025. 15(3): 1841-1853.

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## Biography

Prof. Jingwen Zhou is a Professor of School of Biotechnology, and the Vice Dean of Science Center for Future Foods at Jiangnan University. His research areas include metabolic engineering of microorganisms to produce natural products and vitamins, development of strategies related to fine-tuning of metabolic pathway, high-throughput screening, and AI-dependent protein/pathway design. He has over 400 peer reviewed publications and invited reviews with a H-index 56 (Web of Science). He has been awarded with National Award for Technological Invention 2nd Prize, WIPO-SIPO Award for Chinese Outstanding Patented Invention, and ACS Membership Award. He has been serving as the Editor-in-Chief of 3 Biotech (2023-) and Advisory Borad Member of Trends in Biotechnology (2025-).



# Metabolic engineering of microorganisms toward sustainable biomanufacturing of chemicals and polymers

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## Abstract

Microbial cells have long served as workhorses for converting renewable feedstocks into value-added products. Their innate biosynthetic capabilities, however, are frequently inadequate to reach the productivity and yield levels demanded for economically viable industrial processes. Metabolic engineering has therefore been developed as a powerful means of reprogramming host strains so that target compounds can be produced efficiently across a range of cultivation conditions. More recently, the integration of synthetic biology has substantially expanded these capabilities, allowing microbial cells to be designed at the systems level to push production performance to its limits.

Among the wide array of compounds obtainable from wild-type and recombinant microorganisms, industrially relevant materials—biopolymers, biofuels, and biochemicals—have drawn particular interest as sustainable alternatives that address both the depletion of fossil resources and pressing environmental concerns such as climate change and the buildup of non-biodegradable plastic waste. Biorefineries that produce chemicals, fuels, and plastics from renewable feedstocks depend fundamentally on microbial fermentation, and the productivity of the entire process is highly dependent on the microbial biocatalysts at its core. For this reason, host strains have been intensively tailored through metabolic engineering—reinforced by synthetic biology tools—to maximize their efficiency in synthesizing the desired products.

In this talk, I present the central metabolic engineering strategies to construct microbial host strains for biorefinery applications.

**Key words:** Biorefinery, Synthetic Biology, *Corynebacterium glutamicum*, C5 chemicals

**Representative publication:**

1. Jeong Y, Kim Y, Kim KA, Sohn YJ, Park KY, Song E, Park K, Park SH, Yang YH, Choi JH, Shin S, Won W, Park SJ*, Cha HG*, Kim HT*, Development of scalable precision fermentation and chemical-grade purification of 5-aminovaleric acid for high-value valerization, Chem Eng J, 2025, 524, 168950.

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5. Hur DH, Lee J, Park SJ*, Jeong KJ*, Engineering of *Pseudomonas putida* to produce medium-chain-length polyhydroxyalkanoate from crude glycerol, Int J Biol Macromol, 2024, 281, 136411.

## Biography



Dr. Park is an Ewha Fellow Professor at Department of Chemical Engineering and Materials Science, Ewha Womans University since 2017, Republic of Korea. Prior to this appointment, he served as Assistant and Associate Professor in the Department of Environmental Engineering and Energy at Myongji University (2012–2017), Senior Research Engineer at the Korea Research Institute of Chemical Technology (KRICT, 2009–2012), and Principal Investigator of the Biomaterial Research Team in Corporate R&D at LG Chem Research Park (2003–2009). He received his B.S. in Chemical Engineering from Seoul National University in 1997, and his M.S. and Ph.D. in Chemical Engineering from KAIST in 1999 and 2003, respectively. His research group focuses on the metabolic engineering of microorganisms for the production of novel biopolymers, biochemicals, and biofuels in biorefinery applications. As of April 2026, he has authored over 160 peer-reviewed articles in journals including Nature Biotechnology, Nature Communications, Metabolic Engineering, Chemical Engineering Journal, and Advanced Science, accumulating approximately 10,000 citations with an H-index of 56. He has been recognized as a World Top 2% Scientist in Biotechnology by Scopus for six consecutive years (2020–2025). Dr. Park currently serves as Editor-in-Chief of Biotechnology and Bioprocess Engineering (Springer, the flagship journal of The Korean Society for Biotechnology and Bioengineering).

# Regulation and Engineering of Monoterpenoid Indole Alkaloid Biosynthesis

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## Abstract

Monoterpenoid indole alkaloids (MIAs) comprise approximately 3,000 plant-derived natural products with diverse pharmacological activities, including anticancer, antimalarial, and antiarrhythmic effects. Their low natural abundance constrains conventional plant extraction, motivating synthetic biology approaches for sustainable production. This presentation integrates recent advances from our research group in understanding the organization of MIA biosynthesis and engineering yeast cell factories. A central biosynthetic bottleneck involves the highly unstable intermediate strictosidine aglycone, whose formation by strictosidine β-glucosidase (SGD) and subsequent conversion by geissoschizine synthase (GS) occur in spatially separated compartments. We identify VinBLAST, a cinnamyl alcohol dehydrogenase-like protein repurposed as a molecular scaffold, as a key mediator of efficient processing of this labile intermediate. VinBLAST promotes physical interaction between SGD and GS in the nucleus and allosterically enhances GS catalytic efficiency, revealing a previously missing link in MIA pathway organization. Homologues from diverse plant families enhance the biosynthesis of representative MIAs, enabling catharanthine production of approximately 160 mg $L^{-1}$ in engineered yeast—nearly 1,000-fold higher than previously reported. Together, these findings connect mechanistic insights into biosynthetic regulation with pathway engineering and provide a foundation for scalable, cost-effective biomanufacturing of geissoschizine-derived MIAs.

**Key words:** Monoterpenoid indole alkaloids, Vinblastine, Cinnamyl alcohol dehydrogenase, Protein scaffold, Yeast cell factories

**Representative publications:**

1. Gao D#,Galeung Alexander Mann S#, Chen B#, Gou Y#, Chen C#, Qiao C#, Garza-Garcia J, Shahsavarani M, Jiang X, Tran H, Bao J, Richardson M, Li J, Perley J, Hwang J, Dong F, Dong C, Huang L, De Luca V, Wang Y*, Qu Y*, Lian J*, A cinnamyl alcohol dehydrogenase-like scaffold organizes monoterpenoid indole alkaloid biosynthesis, Science, 2026, 393 (6813), 788-794.

2. Gao J, Zuo Y, Xiao F, Wang Y, Li D, Xu J, Ye C, Feng L, Jiang L, Liu T, Gao D, Ma B, Huang L, Xu Z, Lian J* (2023) Biosynthesis of catharanthine in engineered Pichia pastoris. Nature Synthesis, 2(3), 231-242

## Biography



Prof. Jiazhang Lian is a Principle Investigator in the College of Chemical and Biological Engineering at Zhejiang University. His group focuses on the construction and optimization of yeast cell factories for the production of plant natural products. He has published more than 100 peer-reviewed papers on high impact journals such as Science, Nature Synthesis, Nature Chemical Biology, and Nature Communications. He also serves as the editorial board member for BioDesign Research, Frontiers in Microbiology, Synthetic Biology and Engineering, and BMC Biotechnology.

# Toward precise kinetic models of acetogen metabolism

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Gas fermentation processes are typically designed to operate in continuous, chemostat mode. Nonetheless, slow and fast dynamics play an important role in the successful operation and optimization of gas fermentation. Both the startup process and operation near the optimal point can be challenging due to undesired dynamics, while any process at scale exposes cells to significant dynamics. Thus, understanding the dynamic responses of cells is important.

In the short term, transcriptome and protein allocation appear surprisingly stable even under significant dynamic changes. Here, posttranslational modifications, allosteric regulation and thermokinetics play the dominant role. On longer terms, protein allocation through transcriptional responses is expected to adjust as the organism adapts to the environment.

The mapping of transcriptomic responses to a lower dimension using ICA has proven effective for bacteria and a so-called iModulon model was developed for *Clostridia autoethanogenum*. The model confirmed the sparsity of the participation matrix facilitating interpretation of transcriptomics data. For the purpose of kinetic modelling, quantitative proteomics is particularly valuable, and we have developed a label free, quantitative proteomics pipeline for the full proteome, including the membrane proteome.

For thermokinetic kinetic modelling, exo- and endo-metabolome measurements are required. We have developed a robust ion-pairing method for quantifying metabolites via double isotope dilution with a new, low-cost method to generate isotope labelled standards. For many gas fermenting microbes, key metabolites are difficult to measure, including ferrodoxin and the tetrahydrofolates in acetogens, and we will discuss approaches to address these challenges.

Parameter inference in precise kinetic models using MCMC continues to be present challenges due to the need to solve the stiff non-linear, pseudo-steady state equation repeatedly. Our most recent iteration using Hamiltonian Monto Carlo with autodiff in JAX combines flexible problem formulation for accelerated solution with efficient parameter sampling.

# AI and systems approaches for multiscale optimization in biomanufacturing

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## Abstract

Successful biomanufacturing requires optimization across multiple scales, including molecular components, regulatory and metabolic networks, and fermentation processes. In this talk, I will present artificial intelligence (AI) and systems approaches for multiscale optimization of biological systems. Specifically, AI models have been developed to optimize enzymes, signal peptides, and regulatory molecules (e.g., riboswitches). Genome-scale metabolic modeling approaches have been used to characterize and optimize metabolic networks of industrially important microorganisms and mammalian cells. Finally, AI models have been developed to forecast target production trajectories and guide real-time agitation optimization in fermentation. This multiscale application of AI and mechanistic modeling will facilitate efficient engineering and improve our understanding of biological systems across these scales. These efforts should also be accompanied by the generation of high-quality, meaningful big data.

**Key words:** Biomanufacturing, Multiscale optimization, Artificial intelligence (AI), Genome-scale metabolic model (GEM)

**Representative publication:**

1. Lee B. T., Lee B., Kwon J. Y., Weber T.*, Kim H. U.*, AI and mechanistic modeling for characterizing biosynthetic pathways of natural products, Natural Product Reports, 2026, 43, 787-802.

2. Lee S. S., Kim Y. J., Kang S., Cha H. J.*, Kim H. U.*, Multi-omics analysis and metabolic modeling reveal BCL2L1- and CPT1A-driven fatty acid oxidation in culture-adapted human embryonic stem cells with implications for cell therapy safety, Chemical Engineering Journal, 2026, 531, 174074.

3. Lee B. T., Mohite O. S., Kwon M. S., Kang H. S., Weber T.*, Lee S. Y.*, Kim H. U.*, Pan-reactome analysis of *Streptomyces* strains reveals association and disconnection between primary and secondary metabolism, Metabolic Engineering, 2025, 92, 241-251.

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## Biography



Prof. Hyun Uk Kim is an Associate Professor at the Korea Advanced Institute of Science and Technology (KAIST), affiliated with the Department of Chemical and Biomolecular Engineering, the Graduate School of Engineering Biology, and the Department of AX (College of AI), and is currently a Visiting Professor at the National University of Singapore (NUS). His research centers on AI-driven systems biology and bio-big data, with applications in synthetic biology, metabolic engineering, and systems medicine. Current topics include computational optimization of microbial metabolism for producing medicinal natural products and prediction of drug targets for cancer and dementia. Prof. Kim has published over 100 peer-reviewed papers, including articles in *PNAS*, *Genome Biology*, *Nature Communications*, and *Metabolic Engineering*, and was named a 2025 Clarivate Highly Cited Researcher. His awards include the KAIST College of Engineering Technology Innovation Award, multiple Impact Research Awards, the Young Scientist Award from the Korean Society for Biotechnology and Bioengineering, and the 2026 LINKGENESIS Best Teacher Award; he was appointed the Kwon Oh-Hyun Endowed Chair Professor. He serves as an editor or editorial board member for several journals, including *Chemical Engineering Journal* and *Biotechnology and Bioengineering*, and has given keynote and invited talks at Metabolic Engineering 15 and ISBA2025.

# Designing Gene Regulatory Programs with AI

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## Abstract

DNA sequences constitute a molecular language that encodes functional information, governed by rules that can be deciphered, learned, and redesigned. Recent advances in artificial intelligence are driving a shift from sequence-based functional prediction toward the inverse design and programmable construction of functional DNA. In this talk, I will discuss recent advances in AI-driven design and optimization of gene regulatory sequences, including the generation of promoters and enhancers, cell-type-specific regulation, generalization across biological contexts, and the design of dynamic regulatory responses. I will also explore the opportunities and challenges of advancing toward generalizable and programmable gene regulation.

**Key words:** AI, gene regulation, generative model, promoter

**Representative publication:**

1. Yixuan Zhi#, Qixiu Du1#, Han Yu, Lei Wei, Xiaowo Wang*, Deciphering key factors of active learning performance in biomolecular design, Bioinformatics, 2026, 42, btag248

2. Pengcheng Zhang#, Qixiu Du#, Ye Wang#, Lei Wei, Xiaowo Wang*, Systematic representation and optimization enable the inverse design of cross-species regulatory sequences in bacteria, Nature Communications. 2025;16(1):1763

3. Jiaqi Li#, Pengcheng Zhang#, Xi Xi, Liyang Liu, Lei Wei, Xiaowo Wang*, Modeling and designing enhancers by introducing and harnessing transcription factor binding units, Nature Communications. 2025;16(1):1469

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## Biography



Dr. Xiaowo Wang is an Professor of Bioinformatics at the Department of Automation Tsinghua University. He received his Bachelor's degree of Engineering and Ph.D. in Bioinformatics from Tsinghua University. He joined the faculty of Tsinghua University since 2008. He was a visiting student in Cold Spring Harbor laboratory in 2007-2008, and a Tang Distinguished Scholar in Synthetic Biology Institute of UC Berkeley in 2012-2013. His lab aims to bring AI and biology approaches together to understand gene regulation networks quantitatively and systematically, and guide the design of synthetic biological systems.

# The Development of Ultraviolet Laser Dissociation-Structural Mass Spectrometry

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## Abstract

Ultraviolet photodissociation (UVPD) has emerged as a promising dissociation strategy in mass spectrometry (MS)-based protein sequence and dynamic structure analysis. Rich types of fragment ions including a/x, b/y, and c/z types can be generated by the most popular 193 nm UVPD, resulting in high protein sequence coverages. We develop a 60-150 nm extreme ultraviolet-free electron laser (XUV-FEL)-MS system in the Dalian Coherent Light Source (DCLS) facility. We introduce both the XUV-FEL and 193/157 nm excimer laser into the MS dissociation chamber via vacuum tube from the DCLS beamline. Then, we utilize native mass spectrometry (nMS) and ultraviolet photodissociation to systematically interrogate the structural details in activation and interactions of acyl carrier protein (ACP) with C4-C18 acyl chains. The unstable acyl-ACP intermediates can be isolated and subjected to high-sensitivity UVPD analysis individually without matrix interference. We find that the acyl chains mainly insert into ACP subpocket I until the chain length surpasses the cavity's maximum capacity by 10 carbons. Then, the hydrophobic part of long acyl chains (>C10) bends into subpocket II. Notably, Phe50 and Ile62 play a critical role in regulating the size of the hydrophobic pocket, while Loop I and Thr64-Gln66 are essential for stabilizing long-chain acyl-ACPs. Our findings might contribute to the ACP rational evolution to promote the biosynthesis of target fatty acids.

**Key words:** Ultraviolet photodissociation, free electron laser, mass spectrometry, functional conformational dynamics, acyl carrier protein

**Representative publication:**

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## Biography



Prof. Dr. Fangjun Wang obtained his B.S. degree at Zhejiang University in 2005 and PhD degree in analytical chemistry at Dalian Institute of Chemical Physics (DICP) in 2011 with the supervision of Prof. Hanfa Zou. He was selected into the "Hundred Talent Young Scientist Program" by DICP in 2011 and promoted as Professor in 2017. His current research interest is focused on structural proteomics and cutting-edge mass spectrometry (MS) instruments and methods, including intact protein and protein complex native top-down MS characterization by using self-developed extreme ultraviolet (EUV, 50-200 nm) laser dissociation-MS/MS in Dalian Coherent Light Source (DCLS). He has 150 original papers published in international journals, including Nat. Protoc., J. Am. Chem. Soc., Cell Chem. Biol., Chem. Sci., and Anal. Chem., with SCI citation over 6000 times.

# Engineering Microbial Cells and Communities for Sustainable Food Biomanufacturing

**Prof Rodrigo Ledesma-Amaro**
Bezos Centre for Sustainable Protein, Microbial Food Hub, Department of Bioengineering and Centre for Engineering Biology, Imperial College London, London,
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Abstract
Synthetic biology and metabolic engineering offer powerful approaches for developing sustainable microbial platforms capable of producing the next generation of food and foods ingredients. However, conventional strategies commonly focus on engineering individual microbial strains, placing the full metabolic burden on a single cell and limiting the efficiency, robustness and scalability of the resulting bioprocess.
Our research combines the development of advanced synthetic biology tools with the engineering of non-conventional microorganisms and synthetic microbial communities. We use these approaches to precisely control metabolic pathways, expand substrate utilisation and improve the production and recovery of valuable compounds. Particular attention is given to oleaginous yeasts, including Yarrowia lipolytica, as versatile platforms for converting low-cost and alternative feedstocks into lipids, lipid-derived chemicals, food ingredients and other high-value products.
We are also exploring how division of labour and metabolic cooperation between microorganisms can overcome the limitations of single-strain engineering. By distributing complex pathways across specialised populations, synthetic communities can reduce cellular burden, use mixed substrates and valorise fermentation byproducts that might otherwise be lost. These systems offer new opportunities to improve resource efficiency while creating more resilient and adaptable biomanufacturing processes.
This presentation will discuss how engineering at the level of both individual cells and microbial communities can support the transition from conventional fermentation towards more sustainable and circular food biomanufacturing. I will also provide an update on the progress of the Bezos Centre for Sustainable Protein and the Microbial Food Hub.

Keywords: synthetic biology; metabolic engineering; microbial communities; Yarrowia lipolytica; biomanufacturing, food, alternative proteins, precision fermentation

# Engineering Methanotrophs for Value-added Bioproduct Synthesis from C1 Gases

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## Abstract

Methane- and $CO_2$-rich low-grade gases, including biogas, are promising renewable C1 feedstocks for sustainable biomanufacturing. Methanotrophs can directly utilize methane/$CO_2$ and convert C1-derived carbon into metabolic intermediates for the production of value-added products. However, their broader application as microbial cell factories is limited by gas-transfer constraints, cultivation challenges, and restricted product diversity. Here, we present integrated bioprocess and metabolic engineering strategies to enhance the utilization of low-grade C1 gases. As a representative example of value-added bioproduct synthesis, methanotrophs were engineered to produce polyhydroxyalkanoate (PHA) from methane and carbon dioxide. Other applications based on the advantages of methanotroph's physiology and metabolism will also be presented.

**Key words:** Methanotrophs, low-grade C1 gases, value-added products

**Representative publication:**

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2. Nguyen D. T. N., Lee O. K., Na J. G., Lee E. Y.*, Biosynthesis of biodegradable plastic, poly(3HB-co-3HP), using methane as sole carbon source in metabolically engineered type II methanotroph, *Methylosinus trichosporium* OB3b, New Biotechnol., 2025, 87, 12–19.
3. Hyun S. W., Krishna S., Chau T. H. T., Lee E. Y.*, Methanotrophs mediated biogas valorization: Sustainable route to polyhydroxybutyrate production, Bioresource Technol., 2024, 402, 130759.

4. Lee O. K., Kang S. G., Choi T. R., Yang Y. H., Lee E. Y.*, Production and characterization of a biodegradable polymer, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), using the type II methanotroph, *Methylocystis* sp. MJC1, Bioresource Technol., 2023, 389, 129853.

## Biography



Eun yeol Lee is a Professor of Chemical Engineering at Kyung Hee University, Republic of Korea. He received his B.S. in Industrial Chemistry and Ph.D. from Seoul National University. He focus on the research and development of Bioinformatics, Enzyme Nanobiocatalysis, Synthetic Biology and Metabolic Engineering-inspired Biomanufacturing of pharmaceutical & cosmetic biomaterials, biochemicals, biodegradable bioplastics and biofuels from next-generation carbon feedstocks including lignocellulosic biomass, macro-/microalgae, C1 carbons such as methane, methanol, formaldehyde, formate and carbon dioxide, to contribute to carbon-neutral society.

# Convert lignocellulose into bulk chemicals through consolidated bioprocessing using filamentous fungi

Chaoguang Tian

L-malic acid is an important C4 platform compound widely used in fields such as food, pharmaceuticals, and materials. Using non-grain biomass such as agricultural and forestry waste as raw material to synthesize L-malic acid through microbial fermentation can expand the raw material sources for biomanufacturing. Consolidated BioProcessing (CBP) integrates biomass-degrading enzyme production, raw material enzymatic hydrolysis, and product fermentation into the same microbial system and reaction process, thereby reducing the need for exogenous enzymes and separate enzymatic hydrolysis steps. The Fungal Synthetic Biology Research Group led by Professor Tian Chaoguang at the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, used Myceliophthora thermophila as the chassis and conducted systematic research on fungal CBP technology for production L-malic acid using plant biomass as feedstock. The engineered strain can directly produce L-malic acid over 150 g/L and 0.5 g/g using raw plant biomass (corn-cob) feedstock.

# Data-driven Engineering Biology for Biomanufacturing: The Power of Accurate and Scalable Data Acquisition

Tomohisa Hasunuma

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## Abstract

Engineering biology is transforming biomanufacturing through the integration of metabolic engineering, automation, and artificial intelligence. As biological systems become increasingly complex, the success of Design–Build–Test–Learn (DBTL) cycles depends on the acquisition of accurate, reproducible, and scalable experimental data. High-quality datasets are the foundation for reliable metabolic pathway design, enzyme discovery, and predictive AI models. At the Kobe University Engineering Biology Research Center (EGBRC), we are developing a Smart Cell Development Platform that integrates computational design with automated experimental workflows for microbial strain engineering. We have established high-throughput platforms for metabolomics and enzyme engineering that generate standardized, AI-ready datasets for metabolic and protein engineering. These datasets have enabled the development of MUSASHI, a data-driven strategy for discovering highly active and promiscuous enzymes from large protein sequence databases, as well as an Autonomous Lab that combines robotics, Bayesian optimization, and modular laboratory automation to autonomously optimize experimental conditions through closed-loop DBTL cycles. In this presentation, I will discuss how accurate and scalable data acquisition will enable autonomous engineering biology, where AI continuously learns from experiments to accelerate sustainable biomanufacturing.

**Key words:** metabolic engineering, enzyme engineering, metabolomics, autonomous lab

**Representative publication:**

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## Biography



Tomohisa Hasunuma is the Director of Engineering Biology Research Center, and concurrently serves as a Professor at the Graduate School of Science, Technology and Innovation at Kobe University, Japan. He earned his B.Sc. (1998), M.Sc. (2000) and PhD (2004) in the Department of Biotechnology at Osaka University, Japan. In 2004, Dr. Hasunuma joined Research Institute of Innovative Technology for the Earth (RITE), before transitioning to Kobe University in 2008. From 2011 to 2014, he held the position of the Researcher for Precursory Research in Embryonic Science and Technology (PRESTO) under the Japan Science and Technology Agency (JST). Dr Hasunuma has led major biotech-related projects in Japan, including those funded by NEDO and JST. Currently, as the director of the Bio-Manufacturing Collaborative Research Center at Kobe University, he is dedicated to forming an ecosystem for industry-government-academia collaboration related to bio-manufacturing. Dr. Hasunuma has authored over 250 peer-reviewed journal papers and 90 reviews and book chapters, and has filed more than 170 patents.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Single-cell metabolic engineering (SME) and single-cell process engineering (SPE) by the Ramanomics Platform

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## Abstract

A single cell is the atomic unit of function and evolution for life on Earth and its bioprocesses. Dissecting, sorting, and designing cellular metabolism and bioprocess at single-cell resolution is therefore fundamentally important. We invented the Ramanomics Platform, comprising RamanEye (a high-throughput Raman flow cytometer with automated sample pretreatment), FlowRACS (a high-throughput Raman flow sorter), RAMS (a multi-modal Raman microfluidic sorter), DCP (a digital colony picker that uses laser for phenome-guided, contact-free picking of microdroplet clones), and RamanAI (an A.I. system that translates Raman/brightfield /fluorescent features into live single-cell metabolic/morphological/developmental phenotypes). By metabolically profiling, sorting, and cultivating/sequencing a mutant library, microbiome or animal-cell sample in a label-free, live-cell, and high-info-content manner, the Ramanomics Platform rapidly produces diagnosis/value/mechanism reports at single-cell resolution, plus a strain-bank of in-situ functional strains. For “Single-cell Metabolic Engineering (SME)”, it generates single-cell muli-omics, rapidly discovering metabolic pathways [1], and yielding superior mutants from random mutagenesis libraries within hours [2]. For “Single-cell Process Engineering (SPE)”, it enables single-cell-precision process monitoring at unprecedented speed [3] and rational process control based on inter-cellular metabolic dynamics [4]. Therefore, we proposed cellular and process ramanomes as new biological big data, and initiated the iMAPS Consortium to advance SME and SPE (http://iMAPS.info/solutions).

**Key words:** metabolic engineering, process engineering, ramanomics, RACS

**Reference:**

1. Qintao Wang#, Yanhai Gong#, et al., High-throughput Raman-activated cell sorting of microalgal genome-wide edited library revealed a regulatory pathway for carotenoid synthesis. *Nat Commun*, 2026, 17(1):8352.

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4. Juline Savigny, et al., Single cell profiling framework reveals metabolic subpopulations as drivers of bioproduction heterogeneity. *Nat Commun*, 2025, 17(1):645.

## Biography



Jian XU (xujian@qibebt.ac.cn) obtained B.S in Biotechnology from Peking University in 1997, and M.S. in Computer Science and PhD in Biochemistry in Washington University in St. Louis in 2003. He serves on Scientific Advisory Councils in the Life Science Division of Natural Science Foundation of China (NSFC), and in the Applied and Environmental Division of American Society of Microbiology. He is also founding senior editors of *mSystems*, *mLife*, etc. Single-Cell Center, which he founded in 2012, introduced new concepts for single-cell metabolic phenomics such as ramanome and metaramanome, and developed core technologies including high-throughput single-cell Raman-activated flow cytometry and sorting, as well as digital colony picking. By collaboration with Single-Cell Biotech and eCyte, they invented the Ramanomics Instrument Series (RamanEye, FlowRACS, RAMS, Digital Colony Picker, etc.). These first-in-class instruments are supporting the iMAPS Consortium (http://iMAPS.info), a global network of single-cell “metabolic sensor, strain miner and ecological restorer” platforms for microbiome, to advance the vision of “metabolic monitoring and mining of microbiota @ single-cell, anytime & anywhere”. Jian has published over 200 papers with over 22,000 citations (H-index 71). His professional contributions were recognized by National Distinguished Young Scholars Award, National Young-Scientist Award for Science and Technology, etc.

# AI-empowered Function Mining and Synthetic Bioanufacturing for Pharmaceuticals and Nutraceuticals Aiming at Translation

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## Abstract

The biological functions discovery and evidence-based evaluation of medical and health-care products, along with technological innovation in synthetic biology-empowered biomanufacturing, are of importance for development of the bio-industry. The key challenges lie in the intelligent mining and assessment of medical and health efficacy, the creation of biocatalysts by synthetic biology, biomanufacturing process engineering, and bioproduct engineering. Our research team has long conducted to multidisciplinary integration of reverse-engineering approaches, AI and systems biology methodologies, including the discovery and evdidence-based evaluation of medical- and health-centered functions of natural compounds, high-throughput technologies and equipment for construction and testing of cell factories, and industrial translation. Our team has developed serial methods for mining active polysaccharides and peptides by discovering their efficacy, elucidating their acting mechanisms, and establishing bioproduction technologies. Combined with our invented high-throughput automated biobreeding systems, we have achieved synthetic biology-based manufacturing of a variety of pharmaceuticals and nutraceuticals, providing new methods, new equipment, and new products for advencements of green biomanufacturing industry.

**Key words:** Synthetic biology; Biomanufacturing; Pharmaceuticals; Nutraceuticals; High-throughput technology

**Representative publication:**

1. Changge Guan#,*, Jiawei Luo#, Shucheng Li#, Zheng Lin Tan#, Yi Wang, Haihong Chen, Naoyuki Yamamoto, Chong Zhang, Yuan Lu, Junjie Chen and **Xin-Hui Xing***, Exploration of DPP-IV Inhibitory Peptide Design Rules Assisted by the Deep Learning Pipeline That Identifies the Restriction Enzyme Cutting Site. *ACS Omega*, **8**, 39662–39672 (2023)

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**5.** Haihong Chen, Wei Li, Wei Hu, Junyu Liu, Canyang Zhang, Yi Wang, Chong Zhang, Xizhen Zhang, Shuo Chen, Qixing Nie*, and **Xin-Hui Xing***, Discovery of a novel tetrapeptide as glucose homeostasis modulator with bifunctionalities of targeting DPP-IV and microbiota. *iMeta*, e70072 (2025)

## Biography



Prof. Xin-Hui XING received his Ph.D. from Tokyo Institute of Technology in 1992. He was selected as a full professor by the 100-Telent Scholar Program of Tsinghua University in 2000 and joined Department of Chemical Engineering since then. From 2019, he has being served the Institute of Biopharmaceutical and Health Engineering, Tsinghua Shenzhen International Graduate School as the Deputy Dean. His research field covers biochemical engineering, biobreeding technology and instrumentation, synthetic biology and bio anufacturing, glycodrug and bioactive peptide mining and biomanufacturing. He has published more thank 350 papers, co-authored 8 books, translated 2 books, and published 1 English monograph. He has been listed in the "Career-Long Impact" and "Single-Year Impact" ranking (World's Top 2% Scientists) by Stanford University & Elsevier (2025). He serves as the editor-in-chief of Health Engineering, co-editor of Biochemical Engineering Journal, and editorial board of more than 10 domestic and international journals. He has cofounded 3 venture companies for transforming research. In recent years, he has received 10 research awards and honors.

# From Proteins to Central Metabolism: Multi-Scale Engineering Redefining Biomanufacturing

Yong Tao

Green biomanufacturing offers a promising, sustainable paradigm for chemical production; however, key bottlenecks persist in reducing production costs and establishing commercially competitive industrial pathways. Recent breakthroughs in artificial intelligence (AI), high-throughput screening, and omics analysis have greatly advanced the field, providing powerful tools to engineer enzymes with higher catalytic efficiency or novel activities, and to develop innovative synthetic chassis cells to tackle core biomanufacturing challenges.

This presentation focuses on developing superior biosynthesis routes through multi-scale engineering—ranging from protein design to central metabolism reconstruction. Specifically, we address multiple levels: (1) Protein level: designing more efficient biochemical reactions to resolve catalytic bottlenecks and improve bioconversion efficiency; (2) Pathway level: developing precise precursor-supply strategies to maximize target-product yield; and (3) Chassis cell level: engineering novel chassis, such as TCA-cycle-deficient synthetic cells, to comprehensively address energy and carbon-flux efficiency issues. By integrating multi-scale engineering strategies, we establish a pioneering framework for high-efficiency biomanufacturing, redefining the boundaries of modern industrial biotechnology.

# Synthetic Biology Empowers the 5M Paradigm for Intelligent Biomanufacturing™

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Abstract: (Main text of Abstract, less than 300 words)

Streptomyces species are eminent producers of bioactive secondary metabolites (SMs, microbial drugs) with clinical, agricultural, and biotechnological applications. Industrial development of these strains necessitates an autoregulated reprogramming paradigm to orchestrate multi-target for maximal cellular contribution to SM biosynthesis; however, no such dynamic synchronicity technology is currently available. By leveraging the discovery that distinct quorum sensing (QS) receptors in *Streptomyces* genus can recognize an identical DNA binding site, we have built a QS-triggered promoter applicable across various *Streptomyces* species. Integrating this promoter with a stabilizer module and a multiplexer module, we further developed a *Streptomyces* multiplexed artificial control system (SMARTS) that can convert the transient signals of diverse QS into stable and multiplexed ON or OFF outputs with varying strengths. Using SMARTS, we established a multi-objective optimization framework that synergistically fine-tunes targets synchronously to enhance SM production. We demonstrated the applications of this SMARTS-based reprogramming by developing two *Streptomyces* strains: a re-designed native *Streptomyces avermitilis* for specialized production of the nematicide baiweimectin, and a *de novo* programmed *Streptomyces venezuelae* for heterologous production of the currently semi-synthesized antitumor drug epidoxorubicin. Notably, the baiweimectin-producing strain was scaled up to 120-$m^3$ industrial-scale fermentation with the titer of 8.4 $gl^{-1}$, underscoring the robustness of the SMARTS-based program. Our work provides a dynamic synchronicity paradigm for overproduction of *Streptomyces* microbial drugs.

**References**

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[5] Zhu et al. 2026. Journal of the American Chemical Society. 148(9):10257-10265.
[6] Wang et al. 2026. Nature Chemical Engineering. Accepted.

**Orthogonal biomanufacturing (OrthBio) using formyl-CoA elongation (FORCE) pathways**

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MojiaBio-XiangHu Laboratory

While one-carbon (C1) compounds have emerged as cost-effective and potentially carbon-negative feedstocks for biomanufacturing, their conversion to value-added products remains a challenge. This is in part due to the approaches used for strain engineering, which rely on the canonical architecture of metabolism and entail concurrent engineering of pathways for substrate utilization, central metabolism, and product synthesis. The resulting crosstalk between product-forming and growth-sustaining functions that compete for the same carbon and energy carriers lead to inefficient microbes and bioprocesses that suffer from unreliable scale-up and deployment, expensive downstream processing, and high capital expenses.

We have addressed these shortcomings by pioneering a new approach herein referred to as Orthogonal manufacturing (OrthBio™). Our OrthBio™ platform relies on the engineering of an iterative C1 elongation pathway (C1+Bio™) based on formyl-CoA elongation (FORCE) reactions that are orthogonal to the host metabolism thus allowing product synthesis independent from cell growth. The FORCE reactions, catalyzed by 2-hydroxyacyl-CoA synthases (HACS), are acyloin condensations between carbonyl compounds, such as aldehydes and ketones, and the C1 moiety formyl-CoA. In this talk I will discuss the conceptualization, design and implementation of C1+Bio™ and its deployment for the synthesis of multicarbon products at industrially relevant titers, rates, and yields and with unprecedented scalability across production platforms and scales.

- References: *Commun Chem* 7: 160, 2024; *ACS Catal* 13: 12007-12020, 2023; *Nature Metabolism* 3:1385-1399, 2021; *Nature Chemical Biology* 15:900-906, 2019.

# An intramolecular energy metabolism of *Streptococcus equisimilis* hyaluronan synthase

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## Abstract

This presentation summarizes our recent observation and understanding of *Streptococcus equisimilis* hyaluronan synthase (SeHAS), a Class I HAS with a single active site pocket, which catalyzes the synthesis of hyaluronic acid (HA), a polysaccharide characterized by its alternating β-1,3-N-acetylglucosamine and β-1,4-glucuronic acid units. We conducted *in vitro* synthesis of HA using SeHAS-liposomes and obtained HA with alternating sequence irrespective of the substrate ratios and concentrations. The molecular dynamics simulations and mutagenesis experiments reveal that the alternating uptake of substrates is jointly determined by HA-substrate interactions and distal enzyme dynamics of the transmembrane and substrate tunnels. We found that UDP hydrolysis of nascent HA triggers translocation by regulating C-loop conformation of SeHAS, an unreported ATP-hydrolysis activity that serves as the driving force for the processive synthesis of HA. Moreover, we confirmed such kind of intramolecular metabolism featured by nucleotide energization in GT family enzymes such as cellulose-, chitin-, and β-1,3-glucan synthases. These findings shed new light on the design and application of polysaccharide synthases.

**Key words:** hyaluronic acid, hyaluonran synthase, *Streptococcus equisimilis* hyaluronan synthase, intramolecular energy metabolism

**Representative publication:**

1. Zhu, K.Y., et al., .Hyaluronan synthase encodes alternating substrate specificity by allosteric gating and electrostatic Selection, ACS Catalysis, 2025, 15, 15950−15962

## Biography



Dr. Zheng Liu a Professor of biochemical engineering at the department of chemical engineering, Tsinghua University and honored as Chang Jiang Scholars. He obtained his bachelor's, master's and doctoral degrees in chemical engineering from Tsinghua University. Dr. Zheng Liu’s current research focuses on the molecular and process kinetics of enzymatic reactions with special interest on AI assisted enzyme for novel enzymatic catalysis in non-natural environment. He has published over 200 peer-reviewed journal papers and supervised over 60 graduate students.

# Catalytic Promiscuity and Protein Engineering of Bacterial CYP152 Peroxygenases for Green Biomanufacturing

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## Abstract

Enzyme catalytic promiscuity and protein engineering offer powerful strategies for expanding biocatalytic capabilities and advancing green biomanufacturing. Bacterial CYP152 peroxygenases utilize $H_2O_2$ to catalyze fatty acid decarboxylation and selective hydroxylation under mild conditions, yet their catalytic versatility remains underexplored.

Using unsaturated fatty acids as mechanistic probes, we revealed a critical role of active-site water generated during $H_2O_2$ activation and elucidated a novel Compound I-mediated aldehyde-forming pathway for C–C bond cleavage. Furthermore, we discovered that several CYP152 peroxygenases catalyze aromatic hydroxylation and dehalogenation of non-carboxyl pollutants, challenging the conventional concept of carboxylate-dependent catalysis. Guided by substrate carboxylate-anchoring interactions, we further identified and engineered CYP152 peroxygenases with expanded herbicide-degrading activities, uncovered an unusual C–O bond cleavage mechanism, and demonstrated the potential of an engineered P450 system to confer herbicide resistance in rice.

Together, these findings reveal the remarkable catalytic promiscuity of bacterial CYP152 peroxygenases and demonstrate the potential of mechanism-guided protein engineering to expand their substrate scope and reaction repertoire. This work provides new insights into enzymatic C–H functionalization and C–C, C–O, and carbon–halogen bond cleavage, paving the way for applications in green biomanufacturing, environmental remediation, and sustainable agriculture.

**Key words:** CYP152 peroxygenases; Catalytic promiscuity; Protein engineering; Reaction mechanism; Green biomanufacturing

**Representative publication:**

1. Jiang Y., Peng W., Li Z., You C., Zhao Y., Tang D., Wang B.*, Li S.*, Unexpected reactions of *α,β*-unsaturated fatty acids provide insight into the mechanisms of CYP152 peroxygenases, Angew. Chem. Int. Ed. 2021, 60, 24694-24701.

2. Jiang Y., Gong P., Li Z., Li Z., Li Y., Wang B., Huang H.*, Peng W.*, Gao X.*, Li S.*, Unexpected activities of CYP152 peroxygenases towards non-carboxylic substrates reveal novel substrate recognition mechanism and catalytic versatility, Angew. Chem. Int. Ed. 2025, 64, e202506614.

3. Jiang Y., Zhang J., Li Z., Wang L., Guo Y., Li Y., Feng X., Gao W., Li Y., Li Z., Du J., Zhang G., Gong P., Fang W., Gao X., Bai M., Hollmann F., Zhang M., Huang H., Wang B., Mo S.*, Li H., Peng W.*, Li, S.*, Anchoring mechanism-inspired discovery of a bacterial P450 gene conferring resistance to auxin herbicides, Nat. Commun. 2026, 17, 9044.

## Biography



Professor Shengying Li is one of the Vice Presidents of Shandong University (Qingdao) and Executive Deputy Director of the State Key Laboratory of Microbial Technology of Shandong University. He obtained his bachelor's degree in Biology and master's degree in Microbiology from School of Life Sciences, Xiamen University in 2000 and 2003, respectively. He earned his Ph.D. degree in Department of Medicinal Chemistry, College of Pharmacy at University of Michigan, Ann Arbor, USA in 2009. From 2010-2012, he performed his postdoctoral research in Prof. David H. Sherman's laboratory in Life Sciences Institute, University of Michigan. From 2012-2018, he served as the professor and the founding Director of Shandong Provincial Key Laboratory of Synthetic Biology at Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences. He has published more than 150 peer-reviewed papers on a number of prestigious scientific journals such as Nature, Nat. Catal., Nat. Chem., Nat. Commun., PNAS, and JACS. He serves as the Executive Deputy Editor for Engineering Microbiology, Associate Editor for BMC Biotechnology and the Editorial Board Member for Journal of Industrial Microbiology & Biotechnology, Bioresources and Bioprocessing, Green Synthesis and Catalysis, Synthetic Biology.

# Bio-upcycling of plastic waste through enzymatic and biological conversion

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## Abstract

Over 400 million tons of plastic waste was produced worldwide every year. The environmental impact of these primarily fossil-based plastics has been broadly discussed, although their strength and light weight also often provide comparative advantages. We see great potential in the bio-upcycling of plastic waste, especially when combined with the development of new polymers and composites tailored for end-of-life functionalities such as biodegradability. Such polymers often contain biosimilar C-O and C-N heteroatom linkages, including polyesters, polyamides, and polyurethanes (1). Upon hydrolysis of such polymers, a mixture of monomers is released containing alcohol, acid, or amine groups. We propose to use these plastic hydrolysates as carbon source for biotechnology (2). To enable this, we have developed Pseudomonas into a plastic monomer-metabolizing biotechnological workhorse, capable of metabolizing a wide range of aliphatic diols, dicarboxylates, and amines (3,4). These engineered strains enable the conversion of plastic hydrolysates into value-added chemicals and biopolymers.

**Key words:** Plastic upcycling, Pseudomonas,

**Representative publication:**

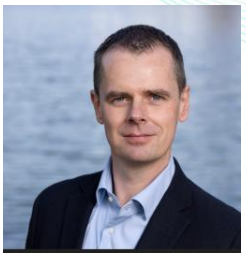
1. Ellis et al. (2021) Chemical and biological catalysis for plastics recycling and upcycling. Nature Catalysis 4:539-556

2. Tiso et al. (2022) The metabolic potential of plastics as biotechnological carbon sources – Review and targets for the future. Metabolic Engineering 71:77-98

3. Ackermann et al. (2021) Bio-upcycling of even and uneven medium-chain-length diols and dicarboxylates to polyhydroxyalkanoates using engineered Pseudomonas putida. Microb. Cell Fact. Fact. 23:54.

4. de Witt et al. (2025) Upcycling of polyamides through chemical hydrolysis and engineered Pseudomonas putida. Nature Microbiology. 10:667–680

## Biography



Nick Wierckx is Department Leader of the Microbial Catalysis group at Forschungszentrum Jülich (Germany) with an associated professorship at the Heinrich Heine University Düsseldorf. His research combines metabolic engineering, synthetic biology, systems biology, and fermentation to develop robust microbial catalysts for renewable chemical production and the biological upcycling of plastic waste. He previously led a junior research group at RWTH Aachen University in Germany. Before that he was active in the Netherlands as microbial strain engineer at the company BIRD Engineering and postdoctoral research at TNO in Delft, where he also completed his PhD. Wierckx has received an ERC Consolidator Grant and a DFG Emmy Noether fellowship.

# Discovery, modification and application of carbohydrate-active enzymes

**Heng Yin**[1],*

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CAZymes serve as core components in utilizing polysaccharide resources, performing synthesis of glycoconjugates, and analyzing glycosylated proteins. They can be regarded as the "chips" of glycoscience. Therefore, my lab focuses on the discovery, modification, and application of CAZymes. For polysaccharide degradation, over 30 highly efficient polysaccharide degrading enzymes were created using microbial mining, genomic mining, and de novo design approaches.

Particularly for LPMOs, we not only succeed enzyme discovery and structure-based catalytic mechanism elucidation and also engineered LPMOs using CBM engineering. CBM significantly influenced substrate binding affinity, enzymatic activity, and $H_2O_2$ tolerance to promote LPMO activity, this is due to the reduced auto-oxidative damage to the copper active site. We present the first comprehensive analysis of N- and O-glycosylation of LPMO, delving into their complex action mechanisms. By constructing 38 glycosylation mutants, we deeply explored the effects of O-glycosylation on the catalytic activity, structural stability, and overall performance of LPMO.

Recently, we identified and characterized a natural mutant of UGT76G1, designated UGT76G4 in *Stevia rebaudiana*. UGT76G4 exhibits unique C19-position selectivity for stevioside substrates both in vivo and in vitro, making it a key enzyme in the biosynthesis of Reb D to Reb M. Through site-directed/saturation mutagenesis, X-ray crystallography, and MD simulations, we elucidated the molecular mechanism underlying UGT76G4's substrate region selectivity, identifying the pivotal role of G200 in determining its C19 selectivity. Based on these findings, we performed semi-rational engineering of UGT76G4, yielding multiple highly efficient mutants.

# Computational Design of Functional Enzymes

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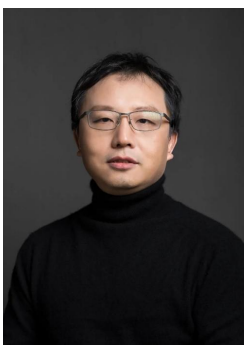
## Abstract

Designing novel and efficient enzymes is a key driving force behind the advancement of modern biotechnology. In recent years, with the deepening understanding of protein structure and catalytic mechanisms, alongside the deep integration of biological big data and deep learning technologies, artificial intelligence (AI) has brought about a profound paradigm shift in the field of enzyme design. This report will systematically introduce a series of innovative advances made by our research team in this direction, including new methods for computational enzyme design, AI-assisted novel technologies for enzyme function mining and engineering, as well as practical application cases of enzyme design in plastic biodegradation and green peptide synthesis. Our efforts aim to bridge the gap from theoretical computation to industrial application in enzyme design, thereby providing key technological support for green biomanufacturing and sustainable development.

**Key words:** Enzyme Design; Artificial Intelligence; Computational Methods; Biodegradation; Green Biomanufacturing

**Representative publication:**

1. Glycolysis-compatible urethanases for polyurethane recycling. Chen Y, Sun J, Shi K, Zhu T, Li R, Li R, Liu X, Xie X, Ding C, Geng W, Ren J, Shi W, Cui Y*, Wu B*. *Science*, 2025, 390(6772):503-509.
2. The β-d-manno-heptoses are immune agonists across kingdoms. Tang Y, Tian X, Wang M, Cui Y, She Y, Shi Z, Liu J, Mao H, Liu L, Li C, Zhang Y, Li P, Ma Y, Sun J, Du Q, Li J, Wang J, Li DF, Wu B*, Shao F*, Chen Y*. *Science*, 2024, 385(6709):678-684.
3. Development of a versatile and efficient C–N lyase platform for asymmetric hydroamination via computational enzyme redesign. Cui Y, Wang Y, Tian W, Bu Y, Li T, Cui X, Zhu T, Li R, Wu B*. *Nature Catalysis*, 2021, 4(5):364-373.
4. Structure-based self-supervised learning enables ultrafast protein stability prediction upon mutation. Sun J, Zhu T, Cui Y*, Wu B*. *The Innovation*, 2025, 6(1):100750.
5. Computational redesign of a hydrolase for nearly complete PET depolymerization at industrially relevant high-solids loading. Cui Y, Chen Y, Sun J, Zhu T, Pang H, Li C, Geng WC, Wu B*. *Nature Communications*, 2024, 15(1):1417.



## Biography

Prof. Bian Wu, a recipient of the National Science Fund for Distinguished Young Scholars, is currently a professor of Beijing University of Chemical

Technology. He earned a degree in Pharmacy from Peking University in 2004 and a Ph.D. from the University of Groningen in the Netherlands in 2010. His research primarily centers on microbial enzyme catalysis. In recent years, he has incorporated computational protein design into enzyme engineering, leading to elucidation of the detailed mechanisms by which several types of microbial enzymes catalyze carbon–nitrogen bond formation. These insights have been applied to the precise synthesis of biomolecules. Professor Wu has published dozens of papers in leading international journals such as ***Science***、***Nature Catalysis and Nature Chemical Biology***. Building on this research, he has developed a series of biosynthetic pathways that have been successfully applied at the industrial level. His accomplishments have earned him several awards, including the Hebei Province Science and Technology Progress First Prize and the Outstanding Young Enzyme Engineer Award from the Chinese Society for Microbiology.

# Engineering enzymes and cascade biotransformations for green and sustainable final chemical synthesis

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## Abstract

Enzyme catalysis is a powerful tool for green, selective, and efficient chemical synthesis, either through single-step reactions or one-pot cascade transformations. Despite significant advances in this field, developing new and improved enzymes and cascade reactions remains essential for practical chemical synthesis, particularly for the efficient production of high-value fine chemicals from readily available starting materials. In this presentation, representative examples from our recent work on enzyme engineering and cascade biocatalysis for the high-yield synthesis of several important classes of fine chemicals will be presented. These include: (a) engineering epoxide isomerases and developing cascade reactions to convert simple alkenes into chiral 2-arylpropionic acids, alcohols, and amines; (b) engineering alcohol dehydrogenases and oxidases and developing cascade reactions to convert racemic alcohols into chiral amines and amino acids; and (c) engineering decarboxylases for carboxylation reactions and developing cascade processes to convert substituted phenols and $CO_2$ into the corresponding aldehydes. These cascade biotransformations were carried out using recombinant *Escherich coli* cells expressing the required enzymes and engineered mutants, enabling simple, one-pot, and high-yielding synthesis and providing easy access to important classes of high-value fine chemicals—including chiral amines, amino acids, carboxylic acids, alcohols, and aldehydes—from readily available starting materials.

**Key words:** Biocatalysis, Cascade reactions, Enzyme engineering, Sustainable synthesis,

**Representative publication:**

1. Choo, J.P.S., Sirota, F. L., See, W.W.L, Eisenhaber, B., Li, Z.* Engineering of styrene oxide isomerase for enhanced production of 2-arylpropionaldehydes: chemoenzymatic synthesis of (*S*)-Profens. *ACS Catal.*, 2023, 13, 11268-11276.
2. See, W.W.L., Li, Z.* Enantiodivergent functionalization of aryl alkenes into diverse biarylalkanoic acids by integrating biocatalytic cascades with chemocatalysis. *ACS Catal.*, 2023, 13, 13215–13224.

3. Khanppnavar, B., Choo, J., Hagedoorn, P.-L., Smolentsev, D., Štefanić, S., Kumaran, S., Tischler, D., Winkler, F., Korkhov, V.*, Li, Z.*, Kammerer, R.*, Li, X.* Structural basis of the Meinwald rearrangement catalysed by styrene oxide isomerase. *Nat. Chem*., 2024, 16, 1496–1504.

4. Tian, K. Li, Z.* A simple biosystem for the high-yielding cascade conversion of racemic alcohols to enantiopure amines. *Angew. Chem.*, 2020, 59: 21745-21751.

5. Jung, D., Li, X., Li, Z.* Engineering of hydroxymandelate oxidase and cascade reactions for high-yielding conversion of racemic mandelic acids to phenylglyoxylic acids and (*R*)- and (*S*)-phenylglycines. *ACS Cat.*, 2023, 13, 1522-1532.

## Biography



Prof. Zhi Li joined the Department of Chemical and Biomolecular Engineering at National University of Singapore in 2006. He worked before at the Swiss Federal Institute of Technology (ETH) Zurich as a group leader in the Institute of biotechnology and as a postdoctoral fellow in the laboratory of organic chemistry. He obtained Ph.D. in organic chemistry from the University of Vienna in 1991, M. Eng. in chemical engineering from Chinese Academy of Forestry in 1985, and B.Sc. in chemistry from Nanjing University in 1982. His recent research focuses on biocatalysis for chemical synthesis, especially for enantioselective synthesis of fine chemicals and green pharmaceutical manufacturing; bio-based chemical and fuel production; and enzymatic $CO_2$ utilization for chemical production. His expertise in biocatalysis includes enzyme engineering, directed evolution, cascade reactions, enzyme immobilization, biotransformations *in vivo* and *in vitro*, and bioprocess development. He was the lead PI of the NRF CRP program on “Green and Sustainable Pharmaceutical Manufacturing via Biocatalysis”, the biocatalysis workstream leader of the Pharmaceutical Innovation Program Singapore, and the deputy president of Singapore Catalysis Society. He received Singapore Award for Sustainable Technology from IChemE (2013) and Achievement Award from Bioenergy Society of Singapore (2018).

# Novel Enzymatic Transformation Enabled by Redox Driving Forces

Han Li

Abstract:

More than 15,000 NAD(P)-dependent enzymes discovered in Nature represent a rich resource for catalyst development, with many outperforming man-made catalysts. However, their reliance on the native cofactors NAD(P) constrains our ability to control electron delivery specificity and reaction thermodynamics. Furthermore, NAD(P) adds substantial costs in enzymatic biotransformation. We demonstrate that noncanonical redox cofactors (NRCs) can support diverse redox chemistries and operate orthogonally to native metabolism. To obtain NRC-utilizing enzymes, we take two parallel approaches: mining natural diversity, and growth-based high-throughput directed evolution. Strikingly, natural and laboratory evolution arrived at the same design mechanism, i.e. pre-organization of enzyme conformation, to enable NRC activity. This principle is applied to developing NRC-active phosphite dehydrogenase (PTDH) and aldehyde dehydrogenase (ALDH). These engineered enzymes may open new routes toward a sustainable carbon economy via utilization of cheap and abundant alcohol and carboxylic acid feedstocks.

# Repurposing Thiamine-Dependent Enzymes with Radicals

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## Abstract

Thiamine diphosphate (ThDP)-dependent enzymes are versatile biocatalysts that naturally mediate asymmetric C–C bond formation and cleavage through the enzymatic Breslow intermediate via a two-electron umpolung polar mechanism. Inspired by these natural systems, chemists have made significant advances in developing biomimetic N-heterocyclic carbenes (NHCs) as organocatalysts. By integrating chemomimetic and biomimetic principles, our group has repurposed ThDP-dependent enzymes for non-natural asymmetric radical transformations triggered by photoredox and electrochemical methods. Mechanistically, an active-site ketyl radical is generated through oxidation of the resting enzymatic Breslow intermediate. Concurrently, a prochiral radical is formed via single-electron transfer from a suitable precursor. These two radical species then undergo enantioselective cross-coupling within the engineered active site, enabling the asymmetric synthesis of chiral ketones. Specifically, we have successfully employed N-(acyloxy)phthalimides as radical precursors and generated prochiral radicals in situ through the addition of electron-deficient carbon-centered radicals to olefins, thereby enabling $C(sp^2)$–$C(sp^3)$ bond formation. More recently, we engineered a new class of ThDP-dependent enzymes to realize the more challenging stereodivergent formation of $C(sp^3)$–$C(sp^3)$ bonds. In addition, we have demonstrated that such enzymatic ketyl radicals can be generated by anodic oxidation, unlocking new-to-nature biocatalysis driven by electricity.

**Key words:** Thiamine-dependent enzymes; radical biocatalysis; asymmetric catalysis

**Representative publication:**

1. Y. Xu, H. Chen, L. Yu, X. Peng, J. Zhang, Z. Xing, Y. Bao, A. Liu, Y. Zhao, C. Tian, Y. Liang, X. Huang, Nature 2024, 625, 74-78.

2. Z. Xing, F. Liu, J. Feng, L. Yu, Z. Wu, B. Zhao, B. Chen, H. Ping, Y. Xu, A. Liu, Y. Zhao, C. Wang, B. Wang, X. Huang, Nature 2025, 637, 1118-1123.

3. Chun, J.; Bao, Y.; Zhang, Q.; Hou, X.; Wu, Z.; Sun, H.; Xing, Z.; Chen, B.; Zhang, Z.; Zhao, Y.; Zhou, J.; Wang, B.; Huang, X., Nat. Catal. 2026, 9, 414-423.

4. B. Zhao, Y. Xu, Q. Zhu, A. Liu, X. Peng, T. Zhang, L. Yu, Y. Zhang, X. Huang, Nature 2025, 643, 699-704.

## Biography



Xiaoqiang Huang is a Zhicheng Distinguished Professor (tenured) at Nanjing University. He earned his B.S. (2013) and M.S. (2015) under Prof. Ning Jiao at Peking University, and his Ph.D. (2019) with Prof. Eric Meggers at the University of Marburg. Following postdoctoral work with Prof. Huimin Zhao at UIUC, he began his independent career at Nanjing University in 2021 as a tenure-track PI, receiving tenure in 2025. His research group focuses on integrating chemocatalysis and biocatalysis for asymmetric synthesis, including photobiocatalysis, and electroenzymatic synthesis. His honors include the Tan Kah Kee Young Scientist Award (2025) and the XPLORER Prize (2025).

# Invited Lecture

# The BRIGHT Biofoundry: Translating Biological Data into Biosolutions — from Pest Control to Human Nutrition and further

Jochen Förster

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## Abstract

The Novo Nordisk Foundation Biotechnology Research Institute for the Green Transition (BRIGHT) is a newly established centre at the Technical University of Denmark. Within BRIGHT, the BRIGHT Biofoundry is a technology-driven research hub for the design and development of microbial cell factories and novel food systems.

The Biofoundry integrates key enabling technologies in a multidisciplinary ecosystem, combining computational approaches with molecular biology and high-throughput experimentation to turn large-scale biological data into actionable insights, with all data management following FAIR principles.

Operating across TRL 1–6, from early-stage concept to spin-out, and supporting bioprocess development from nanolitre to 100-litre scale, the Biofoundry works closely with the Sustainable Innovation Office and the Commercialization and IP Support teams to keep projects aligned with economic feasibility and Biosustainability goals. It is one stop shop that brings ideas to sample production.

This presentation introduces BRIGHT and its Biofoundry through projects carried out with BRIGHT researchers on bird control, pest control and beyond. The presentation also covers the ontologies needed to translate data into solutions including PREFER, a new ontology for precision fermentation data.

**Key words:** Biomanufacturing, Biosolutions, Bioeconomy

**Representative publications:**

1. https://bright.dtu.dk/technologies/biofoundry
2. Ghabrali, H., et al. Enzymatic Glycosylation of Anthranilates for Enhanced Functionality. bioRxiv (2025).
3. Amigó, T., et al. PREFER: An Ontology for the PREcision FERmentation Community. Sci Data (2026).
4. Li, J., et al. Microbial production of creatine using growth-coupled selection systems. Metab Eng (2025).

## Biography



Jochen Förster is Professor and Director of the BRIGHT Biofoundry at BRIGHT, the Novo Nordisk Foundation Biotechnology Research Institute for the Green Transition at the Technical University of Denmark, a role he took up in January 2026. His work at BRIGHT supports research missions in sustainable materials, microbial food and net-zero agriculture.

He trained as a chemical engineer at Dortmund University and completed his PhD at DTU with Jens Nielsen, working on pathway analysis of the metabolic network of *Saccharomyces cerevisiae*. That project produced the first genome-scale metabolic model of a eukaryotic organism.

His career has alternated between academia and industry. He co-founded Fluxome A/S in 2002 and spent nine years as its COO, where he was involved in taking fermentation-derived resveratrol from an idea to the market. He later served as CTO at DTU Biosustain (2011–2016, and again 2023–2025), led the Yeast and Fermentation research department at Carlsberg A/S, and was CTO at Biosyntia ApS before returning to DTU.

He has co-authored 56 scientific publications, is named on 22 patent applications, and is a member of the Danish Academy of Science and Technology.

# An RNA-Tethering Platform for Target-Specific Translation Enhancement

Ji-Sook Hahn

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Improving protein expression is important for the production of recombinant proteins and metabolites in microbial cell factories. Conventional strategies have primarily focused on increasing transcription or gene dosage, whereas direct approaches to enhance translation remain relatively underexplored. In this study, we developed an RNA-tethering platform that selectively enhances the translation of target mRNAs without altering their transcript levels. Application of this platform to yeast cell factories increased proinsulin production by 1.36-fold and β-carotene production by 12.6-fold in *Saccharomyces cerevisiae*. The platform was also effective in the non-conventional yeasts *Komagataella phaffii* and *Yarrowia lipolytica*, as well as in the bacterium *Escherichia coli*, demonstrating its broad applicability across diverse microbial hosts. The potential mechanisms underlying the observed translational enhancement will also be discussed.

# Customizing micro-grain for nutrient production through low-carbon microbial manufacturing

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## Abstract

Using food-safe microorganisms to convert carbon dioxide and other bulk non-food, low-carbon feedstock into important nutrients is an effective pathway for addressing the bottleneck in arable land resources and sustainably ensuring the secure supply of food/feed ingredients. This talk will introduce research on the yeast-based synthesis of important nutrients from low-carbon substrate, acetate. Focusing on the key challenges of low acetate utilization efficiency and poor efficiency in synthesizing important nutrients in native yeast, we established and applied a technology platform for directed evolution of the yeast genome; explored the underlying principles and determinants of carbon conversion and allocation into cellular macromolecules and the on-demand synthesis of macronutrients; overcame the rigid constraints imposed by natural complex biological systems on the synthesis of nutrient molecules; and significantly improved product synthesis efficiency and rate. We created multiple low-carbon synthetic engineered yeast cells with high-performance metrics and nutrient fermentation production technologies, achieving high-level synthesis of nutrients such as artificial starch and high-quality protein.

**Key words:** Low-carbon microbial manufacturing, nutrient, yeast metabolic engineering, synthetic microorganism

**Representative publication:**

Shi Z[#], Xu Z[#], Rong W, Sun H, Zhou H, Yuan Q, Xiao A, Ma H, Cai T*, **Wang G***, Ma Y*. (2025) Reprogramming yeast metabolism for customized starch-rich micro-grain through low-carbon microbial manufacturing, Nature Communications, 6(1), 2784.

Pan Z, Guo Y, Rong W, Cui K, Cai W, Shi Z, **Wang G***, Guo K* (2025) Single-cell protein production from $CO_2$ and electricity with a recirculating anaerobic-aerobic bioprocess. Environmental Science and Ecotechnology, 100525.

Wang X, Xu Z, Shi Z, Rong W, He H, Zhou H, Liu L, Liu Z, Sui W*, **Wang G***, Ma Y. (2026) Evolving natural yeast to protein-rich micro-grain adaptable for non-sterile low-carbon biomanufacturing. Bioresource Technology, 453, 134634.

Zhou H[#], Wang D[#], He H[#], Shi Z, Chen H, Qi X, Yu H, Yuan Q, Ma H, Liao X*, **Wang G***. (2026). Elucidating the metabolic landscape of *p*-coumaric acid to deepen biological implication and enhance biomanufacturing. ACS Synthetic Biology. 15(5), 1798

**Wang G***, Wu X*, Yin Y*. (2022) Synthetic biology-driven customization of functional feed resources. Trends in Biotechnology. 40 (7): 777-780.

## Biography



Dr. Guokun Wang earned his PhD from the University of Chinese Academy of Sciences in 2016. He subsequently completed postdoctoral research at Chalmers University of Technology and the Technical University of Denmark between 2016 and 2021. In 2021, he joined the Tianjin Institute of Industrial Biotechnology as an Associate Professor, and was promoted to Full Professor in 2022.

His research focuses on the synthetic biology-driven microbial production of functional nutrients. Specifically, his team develops innovative solutions for the biotransformation of carbon dioxide into high-quality food ingredients, such as starch and protein, as well as functional polyphenols.

Dr. Wang has authored over 40 peer-reviewed papers in prestigious academic journals, including Nature Communications, PNAS, and Trends in Biotechnology, and holds or has filed for 18 patents. As a Principal Investigator, his work is supported by the National Natural Science Foundation of China—including the highly competitive Excellent Young Scientists Fund (Overseas).

Dr. Wang serves as a member of the 4th National Feed Evaluation Committee of China and is a Council Member of the Chinese Association of Young Scientists and Technologists. Additionally, he acts as a Youth Editor for The Innovation and a peer reviewer for more than 30 scientific journals.

# Synthetic Biology-Enabled Lignin Valorization via Artificial Funneling Pathways and Metabolic Engineering

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## Abstract

Lignin is the most abundant renewable aromatic resource in nature and the second most abundant biopolymer after cellulose. Green biomanufacturing of lignin has emerged as a strategic frontier in biomass resource research. Through billions of years of evolution, microorganisms have developed natural “biological funneling” pathways that enable the conversion of heterogeneous lignin into chemicals and materials, representing a sustainable and low-carbon biomanufacturing paradigm. At present, the key challenges limiting lignin valorization lie in improving its bioavailability and enhancing metabolic conversion efficiency. To address these issues, synthetic biology approaches were employed to construct microbial cell factories enabling lignin-bioconversion. Multifunctional artificial bioconversion pathways were designed and developed, including biological funneling, aromatic ring-cleavage, and atom-economic functionalization pathways, enabling the efficient production of high-value products such as aromatic fine chemicals, dicarboxylic acids, and polyhydroxyalkanoates (PHA). Furthermore, key metabolic enzymes were identified, engineered, and heterologously expressed to regulate carbon flux in lignin metabolism and enhance the synthesis efficiency of target products. A lignin cascade bioconversion concept was proposed, leading to the development of key technologies for green lignin biomanufacturing, achieving product titers at the highest levels reported to date.

**Key words:** Synthetic Biology; Lignin Valorization; Artificial Funneling; Metabolic Engineering

**Representative publication:**

1. Z.H. Liu, N.J. Hao, A.J. Ragauskas et al., Transforming biorefinery designs with ‘Plug-In Processes of Lignin’ to enable economic waste valorization, Nat. Commun., 2021, 12, 1–13.

2. X. Xin, D.F. Liu, Z.H. Liu*, et al., Redesigned pathway for de novo synthesis of vanillin and co-conersion of multiple renewable substrates in *S. cerevisiae*, JACS Au, 2025, 5, 1133–1145.

3. R.Y. Liu, B.Z. Li, Y.J. Yuan, Z.H. Liu*, Multiscale metabolic engineering in biological lignin valorization, The Innovation, 2025, 6 (11), 100993

4. Y. Zhou, R.Y. Liu, N. Fu, Z.H. Liu*, et al., Artificial biological funnel design enabled valorization of aromatic derivatives into catechol, J. Agric. Food Chem., 2025, 73, 24222–24236.

5. N. Li, J.J. Zhangyang, B.Z. Li, Z.H. Liu*, Y.J. Yuan. Reimagining Lignin Valorization: Synthetic Biology-Enabled Sustainable Aromatic Carbon Biomanufacturing. Adv. Sci., 2026, e76698

## Biography



Zhihua Liu is a doctoral supervisor, Associate Professor at Tianjin University, and Chief Scientist of a National Key R&D Program project. He received his Bachelor's degree in Bioengineering from Tianjin University (2010), Master's degree in Biochemical Engineering from Tianjin University (2013), and Ph.D. in Biochemical Engineering from the University of Chinese Academy of Sciences (2016). He previously conducted postdoctoral research and served as a Research Fellow at Texas A&M University in the United States. He was selected for the Tianjin High-Level Young Talents Program (Overseas) and has been named to Stanford University's list of the World's Top 2% Scientists for five consecutive years. His research focuses on applied fundamental studies in biochemical engineering, lignocellulose bioconversion, and microbial cell factories. He has published over 120 SCI papers in journals such as Nat. Commun., The Innovation, Adv. Sci., and JACS Au, with more than 5,000 citations, and over 20 of his papers have been selected as ESI Highly Cited Papers, highlights, or cover articles. He also serves as a member of the Biochemical Process Modeling and Control Professional Committee of the Chinese Society for Microbiology, the Biochemical Engineering Youth Committee of the Chemical Industry and Engineering Society of China, and the Science Popularization and Industry Development Committee of the Chinese Society of Biotechnology.

# Synthetic Domestication of Environmental Bacteria for Efficient Biorefineries

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## Abstract

The efficient conversion of various feedstocks is central to realizing sustainable and carbon-efficient biorefineries. Natural microbial diversity represents an untapped reservoir of metabolic and physiological potential that can be harnessed toward this goal. This presentation introduces efforts to domesticate environmental isolates as emerging microbial chassis through the integration of systems and synthetic biology approaches. These efforts include the construction of efficient microbial platforms for bioprocessing of diverse feedstocks including lignocellulosic and marine biomass, plastic, monomers, and lignin-based aromatics. Together, this presentation underscores the value of microorganisms for accelerating the transition toward scalable, carbon-efficient biorefineries.

**Key words:** Pseudomonas, Synthetic Biology, Systems Biology

## Biography



Dr. Hyun Gyu Lim is an Assistant Professor in the Department of Biological Engineering at Inha University, Korea. He earned both his Bachelor's and Ph.D. degrees from Pohang University of Science and Technology (POSTECH). During his Ph.D., he applied synthetic biology to develop microbial processes, utilizing single or multi-strain systems for the efficient conversion of non-conventional feedstocks such as glycerol, acetate, and marine biomass sugars. Following his Ph.D., he completed postdoctoral training in the Department of Bioengineering at the University of California, San Diego, where he focused on multi-omics approaches, including laboratory evolution and machine learning-guided large-scale transcriptome analyses. Dr. Lim returned to Korea in early 2023 to join Inha University. His lab focuses on integrating systems biology and synthetic biology to develop intelligent microbial systems, primarily for biorefinery applications.

# Advanced methanotrophic cell factory for methane-to-multi-carbon bioconversion

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## Abstract

Biological carbon capture and valorization offer a promising route toward sustainable biomanufacturing. Methanotrophic bacteria, which use methane as their sole carbon and energy source, represent attractive microbial chassis for converting this potent greenhouse gas into value-added products. However, efficient methane-based bioproduction remains constrained by limited synthetic biology tools, incomplete understanding of metabolic regulation, and imbalances between carbon assimilation and nitrogen metabolism. Here, we systematically remodeled methane assimilation networks to enhance the biosynthesis of high-value compounds. By optimizing methane oxidation, electron and energy supply, and the accumulation of key metabolic intermediates, and integrating multi-omics analyses with targeted gene-expression control, we redirected carbon flux toward the production of fine chemicals, natural products, biofuels, and polysaccharide biomaterials. Techno-economic analysis and life-cycle assessment further demonstrated that methane-based C1 biomanufacturing can substantially reduce reliance on glucose feedstocks, greenhouse gas emissions, and associated environmental impacts. This work establishes a versatile framework for high-efficiency methane bioconversion and provides a foundation for sustainable, large-scale C1 biomanufacturing.

**Key words**: methanotrophic bacteria, metabolic engineering, carbon flux, multi-carbon product, high cell density culture

**Representative publication:**

1. ZX. Gao, Qiang Fei*, et al. Biological Valorization of Methane and Nitrogen Gas-derived Ammonia via Methanotrophic Bacteria for Gut-Beneficial Nutrients, Nat Commu, 2026, 17: 3803

2. ZY. Jiao, Qiang Fei*, et al. Bio-Photocatalytic Synergy Enables Valorization of CH4 and CO2 into Hydrogen and 4-Hydroxybenzoate in Engineered Methanotrophic Bacteria, Angew Chem Int Ed, 2026, 65 (18): e26097

3. ZY. Jiao, Qiang Fei*, et al. A De Novo Auto-Activated Solar-Driven Biohybrid System for Hydrogen Production in Methanotrophic Cells, Angew Chem Int Ed, 2025, 64(7)：e202419973

4. SQ Guo, Qiang Fei*, et al. Scalable Electro-Biosynthesis of Ectoine from Greenhouse Gases, Angew Chem Int Ed, 2025, 64(3): e202415445

5. ZX. Gao, Qiang Fei* A novel nutritional induction strategy flexibly switching the biosynthesis of food-like products from methane by a methanotrophic bacterium, Grenn Chem, 2024, 26: 7048

## Biography



Prof. Qiang Fei received his PhD in Biochemical Engineering from KAIST. After working sequentially as a postdoctoral researcher at MIT and a staff engineer at the National Renewable Energy Laboratory from 2011 to 2016, he joined Xi'an Jiaotong University as a Principal Investigator (PI). His research focuses on the bioconversion and valorization of methane and other one-carbon (C1) substrates. Leveraging synthetic biology and high-cell-density cultivation strategies, the group targets the biosynthesis of fine chemicals, biomaterials, biofuels and other value-added products. His team also develops TEA and LCA methodologies for C1 biorefinery systems. He has published more than 100 peer-reviewed articles in journals such as Nat Commun, Angew Chem Int Ed, Chem Eng J, Green Chem, Bioresour Technol, Biotech Adv etc.

# Acetoin Bioproduction from $H_2$ and $CO_2$ with *Methanothermobacter thermautotrophicus*

Bastian Molitor

Gas fermentation represents a promising technology for advancing the transition toward a sustainable and defossilized chemical industry. Using renewable hydrogen and carbon dioxide as feedstocks, methanogenic archaea are capable of converting one-carbon compounds into methane and other products, thereby supporting carbon recycling and renewable energy storage. Thermophilic methanogens belonging to the genus *Methanothermobacter* have long been established as model microbes for investigating hydrogenotrophic methanogenesis and are already employed at industrial scale in biological methanation processes. Recent progress in the development of genetic tools, including replicating vectors, DNA transfer techniques, targeted genome engineering, including CRISPR-based methods, has made these microbes increasingly accessible for metabolic engineering. These advances create new possibilities for studying cellular physiology, enhancing process performance, and broadening the biotechnological potential of methanogenic hosts. For instance, these tools facilitate the production of heterologous enzymes, which enabled us to produce acetoin through plasmid-based, inducible expression of heterologous pathway genes. We demonstrated that acetoin formation is coupled to growth and can be sustained continuously in chemostat cultures. Furthermore, we established allelic exchange methodology and a thermostable CRISPR/Cas9 system for chromosomal engineering. Together, these advances provide a foundation for implementing more sophisticated metabolic engineering strategies. Overall, we demonstrated the production of chemicals other than methane from hydrogen and carbon dioxide while considerably expanding the available genetic toolbox, thereby moving *M. thermautotrophicus* ΔH closer to becoming a robust cell factory and versatile platform host for next-generation gas fermentation processes.

# Engineering Yeast Cell Factories for Circular Biomanufacturing from Waste Feedstocks

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## Abstract

Microbes are attractive biomanufacturing platforms, enabling the sustainable conversion of renewable and waste-derived carbon into valuable products. Among microbial hosts, yeast is a particularly promising chassis due to its robustness, metabolic versatility, and genetic tractability. However, realizing this potential with unconventional feedstocks often requires targeted metabolic and protein engineering. Here, we present engineering strategies that expand the biomanufacturing potential of yeast using lignocellulosic residues and other waste-derived feedstocks. Integrating protein and metabolic engineering with bioprocess optimization markedly improved conversion of these feedstocks into produce fuels, chemicals, and biopolymers. Furthermore, we demonstrate plastic upcycling by coupling enzymatic depolymerization with microbial metabolism. Through bioprospecting and rational engineering, plastic-derived intermediates were funneled into a biosynthetic pathway for value-added compounds. This strategy illustrates how synthetic biology bridges extracellular polymer deconstruction with intracellular carbon conversion, broadening the feedstock range of yeast cell factories. Consequently, our studies highlight engineered yeast as a versatile platform for circular biomanufacturing using biomass and plastic waste.

**Key words:** Yeast, Cell factory, Circular Biomanufacturing, Lignocellulosic biomass, Plastics

**Representative publication:**

1. Phuong Hoang Nguyen Tran, Ji Hyun Jung, Giang Huong Le, Jiin Yu, Sungmin Hwang, Jung Ho Ahn, Gyeongtaek Gong, Youngsoon Um, Ja Kyong Ko, Je Hyeong Jung*, and Sun-Mi Lee*, Engineering a modular yeast platform integrating xylose metabolism and plant polyphenol biosynthesis, 2026 Journal of Biological Engineering

2. Hyeoncheol Francis Son, Sungmin Hwang, Yujin Kim, Jung Ho Ahn, Ja Kyong Ko, Gyeongtaek Gong, Youngsoon Um, Jong Hyuk Park, Hyun June Park, Sun-Mi Lee* Enzymatic depolymerization of polyethylene using a small laccase and its potential for bio-upcycling, Journal of Hazardous Materials, 2025, 495, 139021

3. Jiwon Kim, Jaerak Ko, Yoonjae Lee, Hyunjeong Lee, Sumin Jeong, Jiyun Yoo, Sang-A Lee, Gyeongtaek Gong, Young-Woong Suh, Sun-Mi Lee*, Jeehoon Han*, Soonho Hwangbo*, High-yield oleic acid production through glucose-xylose co-fermentation process from lignocellulosic biomass: Techno-economic analysis and environmental evaluation on a large-scale case study, Chemical Engineering Journal, 2025, 510, 161171

4. Phuong Hoang Nguyen Tran, Je Hyeong Jung, Ja Kyong Ko, Gyeongtaek Gong, Youngsoon Um, and Sun-Mi Lee* Co-production of ethanol and polyhydroxybutyrate from lignocellulosic biomass using an engineered *Saccaromyces cerevisiae*, Renewable Energy, 2023, 212, 601-611

5. Jiwon Kim, Sungmin Hwang, Sun-Mi Lee* Metabolic engineering for the utilization of carbohydrate portions of lignocellulosic biomass, Metabolic engineering, 2022, 71:2-12

## Biography



**Sun-Mi Lee** is an associate professor in the Division of Environmental Science and Ecological Engineering at Korea University. She received her Ph.D. in Chemical Engineering from the University of Texas at Austin. She worked as a research scientist at the Korea Institute of Science and Technology for twenty years. She serves on the expert committee of the Presidential Advisory Council on Science and Technology in Korea and holds editorial roles as an Associate Editor for *Biomass & Bioenergy* and an Editorial Board Member of *Bioresource Technology*. Her research focuses on applying synthetic biology tools to address energy and environmental challenges. Her work primarily involves engineering yeast as a cell factory for the sustainable production of fuels and chemicals from lignocellulosic biomass, as well as for the bio-upcycling of plastics. To enhance the efficiency and expand the capabilities of these cell factories, she evolves enzymes, optimizes cellular networks, and develops integrated bio-chemical processes.

# Title Writing genomes and metagenomes, and the advent of synthetic evolution

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## Abstract

Our research is focussed in developing new principles and technologies to engineer biological systems. AI is revolutionizing biodesign, and its coupling with multiplex DNA writing enables a new concept of synthetic evolution which promises to accelerate synthetic biology. First, I will present our vision on how we are leveraging bioprospecting, AI and evolution to create new biological functions. Then, I will focus on 1) how we developed a gene writer for human cells and its deployment ex vivo (T cells) and in vivo (liver targeting). 2) how we use Cutibacterium acnes as a chassis to engineer human skin properties. We have engineered circuits in skin microbes to modulate sebum secretion, modulate inflammation or thermogenesis.

**Key words:** synthetic biology, skin microbiome engineering, gene writing, AI-based biodesign

**Representative publication:**

1. Discovery and protein language model-guided design of hyperactive transposases D Ivančić, A Agudelo, J Lindstrom-Vautrin, J Jaraba-Wallace, M Gallo, ...Nature Biotechnology, 2025

## Biography

Research Professor at Pompeu Fabra University (https://synbio.upf.edu). His work includes several synthetic biology breakthroughs (radically recoded genome, PERVs-free pigs, gene writing, skin microbiome engineering) that have produced important impact (>21,000 citations, 4 companies developing therapies, patents licensed to leading companies). He is a member of Academia Joven de España. He has been recognized as 'Highly Cited Researcher' (2019); and with the National Research Award to the young talent (2018), the August Pi i Sunyer Award (2021), the National Research Award Ángela Ruiz Robles (2022), and the Banc de Sabadell award to Biomedical Research (2026).



# Synthetic genetic control systems for smart living therapeutics

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## Abstract

Sustained and controlled delivery of glucose-lowering agents using engineered designer cells is recognized as an effective strategy for diabetes therapy; however, current technologies rely on external signal control or have been programed into mammalian cells using synthetic gene networks, which pose safety concerns arising from transplantation. Here, we developed an engineered oral deliverable glucose sensing and functional response probiotic (GIFT) living drug for sense-and-respond-based control of diabetic blood glucose. We created a glucose sensor based on a synthetic gene circuit incorporating the glucose-responsive transcriptional regulator HexR, coupled with a synthetic promoter. Upon oral administration of the engineered probiotics harboring the sensor, the cells reside temporarily in the intestine and regulate the expression of therapeutic transgenes in response to glucose levels exceeding a normal threshold. We show efficacy from the engineered probiotics for glycemic control in multiple diabetic mouse and non-human primate models, uncovering that long-term oral administration drives clear improvements in lipid profiles, while also attenuating development of multiple diabetic complications. Our probiotics-based living drug enables therapeutic dosing in response to real-time blood glucose levels, providing a programmable, orally deliverable “sense-and-respond” platform for metabolic therapy without transplantation.

**Key words:** Living drugs, Synthetic biology, Sense-and-respond, Glycaemic control

**Representative publication:**

1. Guan N. Z.#, Kong D. Q.#, Gao X. Y.#, Niu L. X., Zhou Y., Yu G. L., Gao T., Liu M. Y., Ma W. B., Jin Y. Y., Yin J. L., Zhao S. G., Ye H. F.*, Glucose responsive probiotics for glycemic modulation in mice and monkeys, Nature, 2026, 657, 477-486.

2. Qiao L. L.#, Niu L. X.#, Wang Z. H.#, Deng Z. Q., Dai D., Ma X. D., Zhou Y., Kong D. Q., Wang Q. L., Yin J. L., Jin L. L., Sun J., Feng B., Lu W. Q., Cai F. F.*, Guan N. Z.*, Ye H. F.*, Engineered bacteria for NIR light-inducible expression of cancer therapeutics, Nature Cancer, 2025, 6(4), 612-628.

3. Gao X. Y.#, Jin Y. Y.#, Liu M. Y.#, Ma W. B., Kong D. Q., Zhou Y., Niu L. X., Yin J. L., Chen H. B.*, Ye H. F.*, Guan N. Z.*, Designer probiotic-based living drugs for uric acid homeostasis control in hyperuricemic mice and rats, Cell Reports Medicine, 2025, 6(10), 102379.

4. Yin J. L.#, Wan H.#, Kong D. Q., Liu X. W., Guan Y., Wu J. L., Zhou Y., Ma X. D., Lou C. B., Ye H. F.*, Guan N. Z.*, A digital CRISPR/dCas9-based gene remodeling biocomputer programmed by dietary compounds in mammals, Cell Systems, 2024, 15(10), 941-955.

5. Kong D. Q.#, Zhou Y.#, Wei Y.#, Wang X. Y., Huang Q., Gao X. Y., Wan H., Liu M. Y., Kang L. P., Yu G. L., Yin J. L., Guan N. Z.*, Ye H. F.*, Exploring plant-derived phytochrome chaperone proteins for light-switchable transcriptional regulation in mammals, Nature Communications, 2024, 15(1), 4894.

## Biography



Ningzi Guan, Ph.D, is a Professor at the School of Life Sciences, East China Normal University (ECNU). Her research focuses on medical synthetic biology, including genetic control systems, probiotic living therapeutics, and oncolytic bacterial therapy. She has published more than 20 SCI-indexed papers in journals including *Nature*, *Nature Cancer*, and *Cell Reports Medicine*. She is a recipient of the Shanghai Oriental Talent Program and the Shanghai Bioengineering Outstanding Young Scientist Award. She has led research projects funded by the National Key R&D Program of China, the National Natural Science Foundation of China (NSFC), and the Shanghai Municipal Science and Technology Commission, among others. She has filed more than 10 domestic and international patent applications. She serves on the Youth Working Committee and Synthetic Biology Branch of the Chinese Society of Biotechnology, the Youth Working Committee of the Shanghai Society of Biotechnology, and the Shanghai Preventive Medicine Association.

# RNA splicing regulators for gene network and metabolic programming

Baojun Wang

Synthetic genetic circuits program the cellular input–output relationships to execute customized functions. However, efforts to scale up these circuits have been hampered by the limited number of reliable regulatory mechanisms with high programmability, performance, predictability and orthogonality. Here we report a class of split-intron-enabled *trans*-splicing riboregulators (SENTRs) based on *de novo* designed external guide sequences. SENTR libraries provide low leakage expression, wide dynamic range, high predictability with machine learning and low crosstalk at multiple component levels. SENTRs can sense RNA targets, process signals by logic computation and transduce them into various outputs, either mRNAs or noncoding RNAs. We subsequently demonstrate that digital logic operation with up to six inputs can be implemented using multiple orthogonal SENTRs to regulate a single gene simultaneously and coupling SENTRs with split intein-mediated protein trans-splicing. Further, we engineered DRAGON (dual RNA splicing regulation), a group I intron-based platform for programmable design of multifunctional *cis*-splicing regulating RNAs which further enabled multiplexed dynamic metabolic rewiring in a compressed architecture, and thereby significantly boosted salicylic acid bioproduction for upgraded cell factories. SENTR and DRAGON represent powerful and versatile regulatory tools at the post-transcriptional level in *E. coli*, suggesting broad biotechnological applications.

# Engineering SOS-responsive DNA damage circuits for high-precision CRISPR genome editing

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## Abstract

CRISPR genome editing in microbes is often constrained by variable efficiencies, limited scalability, and the challenge of performing precise, double-strand-break-free long-range modifications. To address these limitations, we engineered SOS-responsive genome editing strategies by coupling DNA damage response circuits with CRISPR editing and endogenous repair pathways. We developed the SELECT system in *Escherichia coli* and *Saccharomyces cerevisiae*, which leverages CRISPR-induced DNA damage to activate counter-selection, effectively purging unedited cells. This system achieved editing efficiencies of up to 100% for point mutations, knockouts, and insertions, and 94.2% for multiplexed library construction, while boosting flaviolin production by 3.97-fold. Integrating genotype-phenotype tracking ($R^2 > 0.817$) with machine learning analysis further refined screening accuracy, yielding a correlation coefficient of 0.98. Furthermore, by integrating DNA damage response circuits with enhanced DNA repair pathways, we established PE-STAR, a prime editing platform that elevates editing efficiency in microbes by up to 16-fold. PE-STAR demonstrated robust performance with 80–90% efficiency for short edits (<46 bp) and enabled 100% chromosomal integration of large cassettes (3.2 kb and 8.0 kb) via recombinases. This work establishes SOS signaling as a programmable tool to enhance CRISPR precision, streamline edited cell enrichment, and expand the toolkit for synthetic biology and biomanufacturing

**Key words:** SOS response; CRISPR genome editing; SELECT; PE-STAR;

**Representative publication:**

1. Liu X.H.[#1], Tan H.P.[#1], Wang J.Y., Cao Y.M., Li P.P., Fan X.R., Wang Q.Q., Zhang H.N., Zhang J.J., Yang T.X., Zhao G.F., Zhang X.H., Duan X.Y., Zi L.H., Liu L., Ma L.M., Chen Z.Y., Liang L.L.[*], Liu R.M.[*], SELECT: high-precision genome editing strategy via integration of CRISPR–Cas and DNA damage response for cross-species applications. Nucleic Acids Research, 2025, 53, gkaf595.
2. Duan X.Y., Zhang H.N., Lv J.G., Fan X.R., Liu X.H., Wang Q.Q., Zhang F., Zhang Q., Liu L., Liang L.L.[*], Liu R.M.[*], PE-STAR: prime editing with SOS-triggered and RecJ-augmented repair enables high-efficiency editing in *Escherichia coli*. Nucleic Acids Research, 2026, 54(6), gkag285.

3. Fan X.R.[#1], Liang L.L.[#1], Wang H.L., Liu G.N., Tan H.P., Zhang F., Liu L.[*], Liu R.M.[*], A Self-iterative orthogonal Base-editing platform enables multiplex N-to-N diversification and genome-scale functional screening in *Escherichia coli*. Nucleic Acids Research, 2026, DOI: 10.1093/nar/gkag775.
4. Liang L.L., Tan H.P., Liu R.M.[*], Emerging gene editing in industrial microbiology beyond CRISPR-Cas9. Trends Biotechnology, 2025, 43(4):742-744.
5. Liu R.M.[#1], Liang L.L.[#1], Freed E., Choudhury A., Eckert C.A., Gill R.T.[*], Engineering regulatory networks for complex phenotypes in *E. coli*. Nature Communications, 2020, 11, 4050.

## Biography



Dr. Rongming Liu is a Professor at the College of Bioengineering, Dalian University of Technology (DUT). He serves as Associate Dean of the College of Bioengineering, Deputy Director of the Key Laboratory of Intelligent Biomanufacturing (Ministry of Education), and Director of the Key Laboratory of Bioengineering for Liaoning Provincial Universities. His research focuses on CRISPR-mediated precise genome editing, high-throughput genome manipulation, and synthetic biology-enabled cell factories for the biosynthesis of bio-based chemicals, biofuels, and biomedical products. He leads multiple projects including the National Key R&D Program of China and the National Natural Science Foundation of China. To date, he has published nearly 60 peer-reviewed papers in journals such as Nature Communications, Nucleic Acids Research, and Metabolic Engineering, as well as holds more than 10 domestic and international invention patents.

# Engineering Tailoring Reactions for Efficient Natural Product Biosynthesis

Xiaolin Shen

**Abstract**: Structural tailoring reactions, including hydroxylation, methylation, and glycosylation, play essential roles in determining the structural diversity and functional properties of natural products. However, their efficient implementation in microbial cell factories remains challenging due to limitations in enzyme activity and selectivity, cofactor and precursor availability, and the coordination of multiple enzymatic reactions. To address these challenges, we integrated enzyme engineering with metabolic pathway optimization to improve key tailoring reactions and their supporting metabolic networks. For hydroxylation, we identified and characterized candidate enzymes, combined structural analysis with protein engineering, and elucidated the molecular mechanisms underlying their catalytic performance. For methylation, we systematically investigated intracellular S-adenosyl-L-methionine (SAM) regeneration and utilization, and established a triple-cycle strategy to improve SAM availability and methylation efficiency. For glycosylation, we mined and engineered glycosyltransferases while enhancing UDP-glucose supply to improve glycosylation performance. By integrating these strategies, we achieved efficient microbial production of several structurally tailored natural products, including arbutin, ferulic acid, salidroside, and syringin. Collectively, this work demonstrates the value of combining enzyme-level engineering with metabolic pathway optimization to overcome bottlenecks in natural product tailoring. These strategies provide a foundation for developing microbial cell factories for the sustainable production of structurally diverse natural products.

# Synthetic yeast communities and cell factories for biosustainability

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## Abstract

Engineering biology has transformed the biotechnology industry, opening new routes to more efficient and versatile biomanufacturing. This presentation covers the development of molecular tools and metabolic engineering strategies for building synthetic yeast communities and yeast cell factories that produce high-value compounds for the agri-food, biofuel and biomedicine sectors. I will begin with synthetic yeast communities. Engineered consortia can outperform single strains in efficiency and robustness, yet few tools existed for constructing defined co-cultures of *Saccharomyces cerevisiae*, a key industrial host. Using a high-throughput automated screening platform and the yeast single-knockout library, we evaluated auxotrophic cross-feeding pairs at scale and developed a molecular toolkit to construct and validate novel two- and three-member communities. Integrating mathematical modelling with experimental validation revealed how intracellular and extracellular factors govern community growth, and we showed that division of labour across community members enhances bioproduction of the antioxidant resveratrol. More recently, we introduced programmable cell–cell adhesion to organise communities spatially and improve productivity further. I will then turn to the cell factories that supply these communities with their parts. Using modular metabolic engineering and systematic pathway optimisation, we achieved de novo biosynthesis of high-value chemicals including lipids (cyclopropane fatty acids), raspberry ketone and vanillin, establishing reference platforms for industrial-scale production. Finally, I will introduce the current focus of my group at AIBN and the UQ Biosustainability Hub: the Yeast Engineering and Synthetic Biology (YESBio) platform. We are extending these tools to non-model industrial yeasts such as *Yarrowia lipolytica* and to one-carbon and $CO_2$ feedstocks, with the aim of moving community engineering from empirical optimisation toward predictive design, delivering sustainable biosolutions for the agri-food, biofuel and medical sectors.

**Key words:** Metabolic engineering, synthetic biology, microbial communities, yeast cell factories, biomanufacturing

**Representative publication:**

1) **Peng, H**.; Darlington, A. P. S.; South, E. J.; Chen, H.-H.; Jiang, W.; Ledesma-Amaro, R*. A molecular toolkit of cross-feeding strains for engineering synthetic yeast communities. **Nature Microbiology 2024**. 9(3), 848-863. *This work highlighted by >10 news media such as SynBioBeta, Imperial news, AlphaGalileo, Phys.org., MyScience, etc.*
2) Chen, H.#, **Peng, H.#,** Ellis, T., & Ledesma-Amaro, R. Programmable cell–cell adhesion in synthetic yeast communities for improved bioproduction. **Nature Chemical Biology 2026**. https://doi.org/10.1038/s41589-025-02081-1 (**co-first author**)
3) Jiang, W., **Peng, H.*** Membranes tame methanol in yeast. **Nature Synthesis 2026**. DOI: 10.1038/s44160-026-01122-4
4) Park Y.-K., **Peng H**, Hapeta P., Sellés Vidal L. and Ledesma-Amaro R*. Engineered cross-feeding creates inter- and intra-species synthetic yeast communities with enhanced bioproduction. **Nature Communications 2024**, 15(1): 8924.
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## Biography



Dr Huadong Peng is a Group Leader and Senior Research Fellow at the UQ Biosustainability Hub, Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland, where he leads the Yeast Engineering and Synthetic Biology (YESBio) team of 15 researchers. He is a Future Academic Leader in Australia's Food and Beverage Accelerator (FaBA) and an Associate Investigator in the ARC Centre of Excellence in Synthetic Biology. He received his PhD from Monash University in 2018, followed by postdoctoral training at Imperial College London and a Marie Skłodowska-Curie Fellowship at the Technical University of Denmark. He previously worked as a research associate at Novozymes (now Novonesis). His research develops synthetic biology tools, yeast cell factories and synthetic microbial communities for sustainable biomanufacturing across the agri-food, biofuel and biomedical sectors. He has published 45 peer-reviewed papers, including first- or corresponding-author work in Nature Microbiology, Nature Chemical Biology and Nature Synthesis, and has secured $6.25M as Chief Investigator. He received the Chinese Government Award for Outstanding Self-Financed Students Abroad (top 50 globally), serves as Associate Editor for Biotechnology for Food Future and Frontiers in Bioengineering and Biotechnology and Youth Editor for The Innovation, and collaborates with partners including Noumi Operations, Cauldron Fermentation and Woodside Energy.

**Mapping the Protein Fitness Landscape via Evolution, AI, and Industrial Automation**

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Protein engineering isat the forefront of biotechnological innovation, offering the promise of designing proteinswith unprecedented functionalities for various applications. However, current methods for engineering novel protein functions are limited by the lack of understanding of the sequence-function relationship, the difficulty in designing complex properties using AI, and the labor-intensive nature of traditional directed evolution methodologies. Here, we demonstrated that the protein sequence-function space is highly compressible (with a compression ratio of 10^48) through the development of a new evolution method, EvoScan, and a corresponding AI method, EvoAI.We have further developed an industrial-grade automation platform capable of high-throughput, efficient, and reliable evolution with minimal human intervention (uninterrupted operation for ~1 month). Additionally, we created a genetic circuit-controlled andgrowth-coupled continuous directed evolution system that enables the intelligent evolution of proteins with diverse and complex functionalities. These two systems were integrated into an industrial self-driving laboratory, iAutoEvoLab, where we demonstrated that the platform could evolve proteins from non-functional precursors to fully functional entities. The evolved proteins were then directly applied to in vitromRNA transcription and mammalian systems.Our integrated methods of using evolution, AI, and industrial automation demonstrate significant potential for protein engineering and mapping the landscape, advancing both synthetic biologyand biomedical applications.

# Synthetic biology strategies for enhanced production of aromatic polyketides in *E. coli*

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## Abstract

Aromatic polyketides are a pharmaceutically diverse class of compounds, synthesised through the iterative condensation of malonyl-CoA units by type II or type III polyketide synthases (PKSs). Synthetic biology offers a sustainable alternative to natural extraction and chemical synthesis for their production. In this talk, I will discuss the development of a colorimetric biosensor for C16 aromatic polyketides and its application to high-throughput screening of overproducers. By combining the biosensor with a genome-wide CRISPR interference library, we rapidly identified knockdown targets that enhanced C16 aromatic polyketide production. Notably, these targets also improved the production of polyketides of other chain lengths. Colorimetric high-throughput screening therefore enables the discovery of non-obvious target genes that rational examination of the metabolic pathway is unlikely to reveal.

**Key words:** Aromatic polyketide; biosensor; high-throughput screening, CRISPR interference

**Representative publication:**

1. Yang, D., Jang, W.D., and Lee, S.Y.* Production of carminic acid by metabolically engineered *Escherichia coli*, J. Am. Chem. Soc., 2021, 143 (14), 5364-5377.

## Biography



Dr. Dongsoo Yang is an associate professor in the Department of Chemical and Biological Engineering at Korea University. He received his B.S. in Chemical and Biomolecular Engineering from Korea Advanced Institute of Science and Technology (KAIST) in 2015 and his Ph.D. from KAIST in 2020, where he worked on systems metabolic engineering for natural product biosynthesis under the guidance of Prof. Sang Yup Lee. After completing his postdoctoral training at KAIST, he joined Korea University as an assistant professor in Fall 2022. His current research focuses on developing new enzymes for the production of novel medicinal natural products, building synthetic biology tools for biomedical applications, and engineering probiotic bacteria for diagnostic and therapeutic purposes. Dr. Yang's contributions have been recognized with the Merck 350 Future Researcher Award (2021), the Shimadzu Future Leader Award (2022), the Miwon Young Scientist Award (2023), and the DaSilva Award (2026).

# Semi-artificial photosynthesis enables light-driven biomanufacturing in non-photosynthetic bacteria

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## Abstract

Semiconductor biohybrid systems integrate microbial biosynthesis with semiconductor light harvesting to sustainably power energy-intensive chemical production. Although widely explored in photosynthetic bacteria, extending these systems to non-photosynthetic biomanufacturing chassis, such as *Bacillus subtilis* (*B. subtilis*), is substantially more challenging owing to poor biocompatibility between inorganic materials and biological cells. Here, we propose a non-invasive enhancement strategy that leverages the endogenous $H_2S$ pathway of the *B. subtilis* host and couples it with semiconductor materials to construct a semi-artificial photosynthetic biohybrid system. This system harnesses light energy to enhance endogenous NADPH regeneration, thereby augmenting microbial production without genetic modification of the target biosynthetic pathways. Applied to engineered B. subtilis for 2'-fucosyl lactose (2'-FL) and surfactin production, the biohybrid system increases product titres by two- to threefold. This strategy, integrated with metabolic engineering, establishes a modular platform for rapid and enhanced chemical synthesis in *B. subtilis*, with potential applicability to other non-photosynthetic bacteria harboring endogenous $H_2S$ pathways.

**Key words:** *Bacillus subtilis*; Semiconductor biohybrid; Light-driven biomanufacturing; Semi-artificial photosynthesis

**Representative publication:**

1. Li Y, Qiao M, Gao L, Zhong Y, Yang Y, Zheng J, Zuo JL, Zhang X*, Yuan S*. Dynamic Bond-Driven Encapsulation of Enzymes in Metal–Organic Frameworks beyond Pore Size Constraints. *Nat Commun* 2026, 17 (1), 3642.
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3. Gao WN, Shi SY, Li Y, Liu F, Cheng Y, Zheng J, Qiao M, Yuan S, Zhang X*, DNA-Directed Assembly of Core-Satellite MOF Structures for Spatially Compartmentalized Multi-Enzyme Cascade Catalysis. *Chemical Engineering Journal* 2026, 529, 173137.
4. Wang X, Tao Q, Zhao SY, Zhang X*, You LN, Zeng YY, Zheng LJ, Zhang JJ, Qiao M, Liu SR, Li YZ*, & Yang B*, & Zhu F*. Stereoselective C(sp3)-Si/Ge bond formation via nickel-catalyzed decarboxylative couplings. *Chem*, 2025, 12: 102713.
5. Zhang XY, Fan H, Zheng J, Li Y, Qiao M*, Zhang X*, Engineering Distant Allosteric Networks To Remodel the Substrate Access Channel for Efficient β-Arbutin Biosynthesis. J. Agr*ic. Food Chem.* **2026**, *74* (12), 10430–10440.

## Biography



Dr. Zhang is a Professor and Dean of the School of Food Science and Pharmaceutical Engineering at Nanjing Normal University. He is a recipient of the National High-Level Young Talents Program (Young Changjiang Scholars) by the Ministry of Education, a Jiangsu Specially-Appointed Professor. His research focuses on the biomanufacturing and bioactivity exploration of carbohydrate-based chemicals. He has published over 100 SCI-indexed papers in prestigious journals such as *Chem*, *Angewandte Chemie*, and *Nat Commun*. He holds 22 authorized Chinese invention patents, with 2 technology transfers completed and 4 licensing agreements executed. Prof. Zhang has received numerous accolades, including the Second Prize of Science and Technology from the China Light Industry Federation, and the First Prize of Agricultural Science and Technology from Jiangsu Province. He is also listed among the World's Top 2% Scientists by Stanford University.

# Production of etoposide and teniposide in yeast to protect endangered plant

Yating Hu

Etoposide and teniposide are essential anticancer drugs whose production currently relies on podophyllotoxin extracted from the endangered medicinal plant *Sinopodophyllum hexandrum*. To establish a sustainable alternative, we reconstructed the complex biosynthetic pathway of podophyllotoxin-type lignans in *Saccharomyces cerevisiae*. Through the identification of key glycosyltransferases and extensive pathway engineering, the engineered yeast produced a more direct precursor of etoposide and teniposide. This precursor was then converted into the two drugs through a single mild chemical step. Our yeast based chemoenzymatic platform offers a sustainable route for producing these important medicines while reducing dependence on endangered plant resources.

# Engineer enzymes and cellular metabolism for the synthesis of specialty/unusual fatty acids

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## Abstract

Fatty acids of different chain lengths exhibit distinct roles in cellular physiology and industrial applications. Palmitic acid (C16:0) and stearic acid (C18:0), together with their desaturated derivatives, such as oleic acid ($18:1^{\Delta 9}$), linoleic acid ($18:2^{\Delta 9,12}$) and α-linolenic acid ($18:3^{\Delta 9,12,15}$), are common fatty acids found in membrane lipids and triacylglycerols. Many unusual fatty acids, which possess shorter or longer carbon chains or additional structural modifications other than common fatty acids, are high-value molecules with potential pharmaceutical and nutritional activities, or serve as important building blocks for diverse chemical products. However, the enzymes and metabolic pathways responsible for their biosynthesis remain relatively underexplored. In this talk, I will present our work on engineering enzymes and cellular metabolism for the biosynthesis of several specialty and unusual fatty acids. Specifically, I will discuss the selection and engineering of fatty acid synthases for the production of medium-chain fatty acids, the biosynthesis of methyl-branched-chain fatty acids through the combination of two branched-chain acyl-CoA pathways, and the use of hydroxy fatty acid intermediates for the production of dicarboxylic acids and sophorolipids.

**Key words:** unusual fatty acids, medium-chain fatty acids, branched-chain fatty acids, hydroxy fatty acids

**Representative publication:**

1. Tan Y, Wang Z, Wang W, et al. Leveraging Fungal Type I Fatty Acid Synthase Diversity for Chain-Length Control and Enhanced Fatty Acid Production. Submited

2. Hu M, Feng C, Li M, et al. Systems metabolic engineering of Escherichia coli for high-level branched-chain fatty acid production via dual precursor pathways. Metabolic Engineering, 2026: 102509

3. Zhu Z, Hu Y, Teixeira P G, et al. Multidimensional engineering of Saccharomyces cerevisiae for efficient synthesis of medium-chain fatty acids [J]. Nature Catalysis, 2020, 3(1): 64–74.

## Biography



Prof. Zhiwei Zhu is currently a professor in the School of Bioengineering at Dalian University of Technology (DUT), China. He received his PhD from the Dalian Institute of Chemical Physics in 2014 and his bachelor's degree from the East China University of Science and Technology in 2008. Before joining DUT, he worked as a postdoctoral researcher with Prof. Jens Nielsen at Chalmers University of Technology, Sweden. His research focuses on metabolic engineering and synthetic biology for the microbial production of specialty lipids, polyols, and proteins/enzymes.

# Engineering Modular Polyketide Synthases via Domain-Domain Interaction Design

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## Abstract

Modular polyketide synthases (PKSs) are multidomain assembly lines that biosynthesize diverse natural products, making them an outstanding platform for custom production of designer molecules. Yet rational reprogramming of PKS assembly lines has long been hampered by unpredictable losses in catalytic efficiency upon domain or module swapping. To address this challenge, we recently developed a plug-and-play engineering strategy that enables modular, combinatorial assembly of PKS pathways from interchangeable genetic parts. Through extensive engineering campaigns applying this strategy across diverse PKS systems, we found that domain-domain interactions at module and domain boundaries are a critical, often underappreciated, determinant of successful reprogramming, governing substrate channeling and catalytic communication between neighboring domains. In this talk, I will summarize our recent progress in domain-domain interaction-guided PKS redesign, highlighting how systematic characterization and engineering of these interaction interfaces can restore or enhance activity in engineered assembly lines. I will also discuss how this mechanistic understanding is informing more predictable, generalizable design rules for synthetic biosynthetic pathway construction.

**Key words:** polyketides; biosynthesis; modular polyketide synthases; domain-domain interactions, enzyme engineering

**Representative publication:**

1. Huang, Z.; Xie, S.; Liu, R.-Z.; Xiang, C.; Yao, S.; **Zhang, L.*** “Plug-and-play engineering of modular polyketide synthase” *Nat. Chem. Biol.* **2025**, *21*, 1361-1367.

2. Yao, S.; Xie, S.; Liu, R.-Z.; Huang, Z.; **Zhang, L.*** “Expanding catalytic versatility of modular polyketide synthases for alcohol biosynthesis” *Nat. Chem. Biol.* **2025**, *21*, 1368-1375.

3. Kudo, K.[#]; Hashimoto, T.[#]; Awakawa, T.[#]; **Zhang, L.**[#]; Nishimura, T.; Hashimoto, J.; Kozone, I.; Kagaya N.; Suenaga, H.; Keatinge-Clay A. T.; Abe, I.; Shin-ya, K.* “Skeletal editing via multi-step engineering of a modular polyketide synthase” *Nat. Commun.* **2026**, *17*, 4979.

4. Xie, S.; Xiang, C.; Shin-ya, K.; **Zhang, L.*** “Investigation of domain-domain interaction between acyl carrier protein and thioesterase in modular polyketide synthases” *JACS Au*. **2026**, *6*, 2498-2505.

## Biography

Lihan Zhang obtained his Ph.D. from The University of Tokyo in 2017, then conducted postdoctoral study at Harvard University as a JSPS Overseas Research Fellow. In 2019, he joined Westlake University as an Assistant Professor and was promoted to Associate Professor in 2025. Fascinated by the structural diversity of natural products, his research focuses on deciphering the biosynthetic mechanism and evolutionary trajectory of natural products, as well as engineering enzymes and biosynthetic pathways for various synthetic biology applications. He was awarded multiple young talent programs from China and Zhejiang government and published over 30 papers in his career.



# Enabling Fluorinated Molecule Biosynthesis: From Biocatalysis to AI-Engineered Streptomyces Cell Factories

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## Abstract

Fluorinated motifs are indispensable in modern pharmaceuticals, yet sustainable and stereoselective routes to them remain scarce. Our group is engineering a suite of enabling technologies that bridge enzyme discovery and microbial cell-factory construction for fluorinated molecules. We first built *in vitro* biocatalytic systems around mined and engineered enzymes: chemoenzymatic cascades integrating newly mined aldolases with ammonia lyases deliver fluorinated non-canonical L-α-amino acids (up to 99% yield, >99% ee), and photoenzymatic ene-reductase systems enable asymmetric synthesis of gem-difluoro amides and γ-fluoroketones with excellent stereocontrol. To move from reaction design to cellular production, we selected a fluorinated-compound-producing *Streptomyces* as the chassis and established dedicated genome-editing tools, including engineered *Streptomyces*-originating type I-E CRISPR/Cas system (>92% editing efficiency), to construct genome-minimized and fluoride-resistant strains. In parallel, deep learning generated ~10^9 artificial promoter candidates, yielding an AI-designed promoter library (92% active; up to 17.1-fold ermE*p strength) for precise, portable pathway control across *Streptomyces*. Together, these enabling technologies establish the foundation for constructing fluorinated-molecule cell factories of pharmaceutical relevance.

**Key words:** fluorinated compounds; biocatalysis; gene editing; *Streptomyces* chassis; artificial promoters

**Representative publication:**

1. Huang C., Jin X., Liu Z., Cui C., Zhang Y., Wang B., Zhang C., Feng J., Niu D., Luo Y.*, Photoenzymatic enantioselective synthesis of fluorinated amides with remote stereocenter, *Nature Communications*, 2025, 16, 5753.

2. Zhou Q., Zhao Y., Ke C., Wang H., Gao S., Li H., Zhang Y., Ye Y.*, Luo Y.*, Repurposing endogenous type I-E CRISPR-Cas systems for natural product discovery in Streptomyces, *Nature Communications*, 2024, 15, 9833.

3. Zhou Q., Wang Y., Dong J., Zhao X., Guo A., Wang X.*, Luo Y.*, Deep learning unlocks sequence-divergent synthetic promoters to empower Streptomyces natural product engineering, *Metabolic Engineering*, 2026, 96.

4. Jin X., Huang C., Cui C., Liu H., Liu Z., Niu D., Luo Y.*, Chemoenzymatic synthesis of fluorinated L-α-amino acids, *ACS Sustainable Chemistry & Engineering*, 2024.

5. Xie Y., Liu X., Wu T., Luo Y.*, Harnessing the Streptomyces-originating type I-E CRISPR/Cas system for efficient genome editing in Streptomyces, *Science China Life Sciences*, 2025, 68, 1174-1182.

## Biography



Prof. Yunzi Luo is a recipient of the National High-Level Talent Program (Distinguished Young Scholars) award and was awarded the Distinguished Young Scholar of Tianjin in 2025. She is a Professor at the State Key Laboratory of Synthetic Biology and the School of Synthetic Biology and Biomanufacturing at Tianjin University. She received her Bachelor's degree from Tianjin University (2008) and her Ph.D. from the University of Illinois at Urbana-Champaign (2014), followed by postdoctoral research at the Carl R. Woese Institute for Genomic Biology (2014-2015). She began her independent career as a Principal Investigator at the State Key Laboratory of Biotherapy, Sichuan University (2015-2019), then joined Tianjin University as a Professor (2019-2025) before moving to her current position in 2025. Her research centers on synthetic biology, with long-standing focuses on genetic engineering tools, biocatalytic element design, and green biomanufacturing of natural products. She has published over 60 papers in journals including Chemical Society Reviews, Nature Communications, Metabolic Engineering, and ACS Catalysis, and her research is supported by the National Key R&D Program and the National Natural Science Foundation of China (NSFC). She serves on several national professional committees and on the editorial board of ACS Synthetic Biology.

# Global reprogram of regulation and metabolism for secondary metabolite production

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**Abstract**

Bioactive secondary metabolites produced by actinomycetes are extensively utilized in clinical and agricultural applications; however, their industrial-scale production is hindered by low biosynthetic efficiency. This limitation primarily stems from the lengthy substrate-to-product metabolic pathways and the intrinsic complexity of regulatory networks. Here we present a transformative strategy to overcome these challenges by reprogramming synthetic precursor supply modules that operate independently of central carbon metabolism (CCM) using an orthogonal artificial regulatory system. We demonstrate the effectiveness and scalability of this strategy by developing a high-yield erythromycin-producing strain Saccharopolyspora erythraea N9. We first introduced an artificial control system (hQSCS) that operates orthogonally to the native regulatory network. Subsequently, we designed alternative supply modules for five precursors that bypass CCM. By reprogramming both the orthogonal precursor supply modules and the intrinsic metabolic pathways via hQSCS, we not only achieved temporal decoupling of cell growth and ErA production but also relieved the dependence of erythromycin biosynthesis on CCM. Consequently, we achieved unprecedented ErA titer (8.86 g/L) and carbon yield (19.3%) in a 500-$m^3$ industrial-scale fermentation process.

# Conversion of syngas-derived C2 carbon sources to C2+ biochemicals by microbial cell factories

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## Abstract

Using the non-food based raw materials to produce commodity chemicals has attracted great attentions. C2 carbon sources, acetate and ethanol, are promising non-food renewable feedstock that can be derived from syngas, is emerging as a potential alternative to sugar for the production of chemicals in third-generation biorefineries. In our research, engineered Escherichia coli strains were constructed for production of bio-chemicals, such as succinic acid, 3-hydroxypropionic acid (3-HP), 3-hydroxybutyric acid (3-HB), limonene, and L-threonine from acetate or ethanol as sole carbon source successfully. Multiple metabolic engineering strategies were used in this study, including inducing biosynthesis pathways of different products, elimination of competing pathways, dynamic regulation of the TCA oxidation branch, cofactor engineering, and adaptive laboratory evolution. This study explored the possibility of using syngas-derived acetate or ethanol as the new carbon source for biochemical production, which would inspire the research for cost-effective biorefinery.

**Key words:** Metabolic engineering, adaptive laboratory evolution, acetate, ethanol, C2+ biochemicals

**Representative publication:**

1. He J. C., Luo Y.C., Wu H.*. High-yield biosynthesis of 3-hydroxypropionic acid from acetate by engineering bicarbonate supply and malonyl-CoA partitioning in *Escherichia coli*. ACS Sustainable Chemistry & Engineering. 2026, 14, 12543-12552.
2. Zhao W. Y.[#], Shang Y. Z.[#], Pan M. Y., Fei P., Wang Y. Y., Luo Y. C., Wu H.*. Biosynthesis of limonene using acetate as the carbon source in metabolically engineered *Escherichia coli*. Journal of Agricultural and Food Chemistry. 2026, 74, 9526-9538.
3. Fei P.[#], Zhang W. R.[#], Shang Y. Z.[#], Hu P., Gu Y., Luo Y. C.*, Wu H.*. Carbon-negative bio-production of short-chain carboxylic acids (SCCAs) from syngas via the sequential two-stage bioprocess by *Moorella thermoacetica* and metabolically engineered *Escherichia coli*. Bioresource Technology. 2025, 416, 131714.

## Biography



Dr Hui Wu received his Bachelor degree of Bioengineering in Nanjing Tech University (2004). In 2009, he received Doctor degree of Fermentation Engineering in East China University of Science and Technology. Then, he joined the Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, to conduct postdoctoral research. In 2011, he moved to the Department of Bioengineering, Rice University (US) for postdoctoral research. He joined the School of Bioengineering, East China University of Science and Technology in 2014 and moved to Dalian University of Technology in 2024. He has published over 80 papers, including Biotechnology Advances, Metabolic Engineering, Bioresource Technology, ACS Sustainable Chemistry & Engineering, et al. He holds 12 authorized Chinese invention patents and 4 authorized US invention patents. He has received honors such as the Baosteel Outstanding Teacher Award, the Young Scientist Award of Hou Debang Chemical Technology Prize, and delivered more than 20 invited and oral presentations at conferences such as the AIChE Annual Meeting, Asia-Pacific Congress on Catalysis et al. He currently serves as Associate Editor of BioDesign Research, Editorial Board Member of Bioresources and Bioprocessing, as well as Youth Editorial Board Member of Green Carbon.

# Engineering *Bacillus methanolicus* for methanol-based biomanufacturing of amino acids and single cell proteins

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## Abstract

*Bacillus methanolicus* represents a thermophilic methylotroph whose methanol utilization depends on plasmid-encoded genes. It serves as a unique model for deciphering plasmid-dependent methylotrophy and an ideal chassis for low-carbon biomanufacturing using $CO_2$-derived C1 substrates. Despite its evolutionary uniqueness and industrial potential, the lack of synthetic biology tools has hindered both mechanistic understanding and strain engineering. Here, we present a comprehensive synthetic biology platform comprising a high-efficiency electroporation protocol, a CRISPR method enabling robust and multiplex genome editing, diverse neutral loci for gene integration and overexpression, a genome evolution technology based on error-prone DNA polymerase, and a cloud-based genome-scale metabolic model *i*BM822 for user-friendly biodesign. Leveraging this toolkit, we integrated the large endogenous plasmid pBM19 into the chromosome for stable and intact methylotrophic growth and engineered methanol-based producers of amino acids and single cell proteins. This study establishes a synthetic biology framework for *B. methanolicus*, promoting mechanistic exploration of methylotrophy and low-carbon biomanufacturing.

**Key words:** *Bacillus methanolicus*, methylotroph, methanol, amino acid, single cell protein

**Representative publication:**

1. Liu P, Yuan Q, Yang X, Wang Q, Chang T, Bi Y, Wu P, Zhang T, Yang J, Guo S, Xue C, Zheng Z, Xin B, Ma H, **Wang Y**. A synthetic biology toolkit for the plasmid-dependent and thermophilic methylotroph *Bacillus methanolicus*. Cell Reports, 2026, 45, 116788.

2. Xin B, Li M, Liu J, Guo S, Liu P, Fan L, Zhang T, Xu C, Li Y, Zhong C, Xia J, Wang Q, **Wang Y**. Methanol valorization into glycerol by engineering *Bacillus methanolicus* with thermostable enzymes and a tunable promoter library. Journal of Bioscience and Bioengineering, 2026, 142, 83-90.

3. Guo S, Liu P, **Wang Y**. *Bacillus methanolicus* as an emerging platform for energy-efficient biomanufacturing from one-carbon and marine resources. Current Opinion in Biotechnology, 2026, 99, 103500.

## Biography



Dr. Wang earned his Ph.D. in Biology from Shanghai Jiao Tong University in 2016 and joined Tianjin Institute of Industrial Biotechnology as a research assistant. He was promoted to a full professor in 2022 and currently holds dual leadership roles as Deputy Director of the State Key Laboratory of Engineering Biology for Low-Carbon Manufacturing and Principal Investigator of the C1 Metabolism and Bioconversion Laboratory (C1MBL). His research program bridges synthetic biology and industrial biotechnology, specializing in microbial genome editing and C1 bioconversion technologies. He has successfully led multiple nationally competitive research initiatives, including National Key R&D Program of China and National Natural Science Foundation of China for Excellent Young Scientist. He has published 50+ first/corresponding-author publications and filed 40+ patent applications. His scientific contributions have been recognized through numerous honors, including: MIT Technology Review's "35 Innovators Under 35" Asia Pacific, DaSilva Award (Society for Biotechnology, Japan), Lun Shiyi Education Fund Outstanding Young Scholar, Meiji Life Science Award, CAS Distinguished Researcher, CAS Youth Innovation Promotion Association Membership. He is now the Editorial Board Member of *Trends in Biotechnology*, *Synthetic and Systems Biotechnology*, *Engineering Microbiology*, *Microorganisms*, *Green Carbon*, etc.

# Systematic analysis of signal peptides for engineering protein secretion in yeast

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## Abstract

Recombinant protein secretion in yeast involves protein synthesis, endoplasmic reticulum targeting and translocation, protein folding, and vesicular transport. Signal peptides direct nascent proteins into the secretory pathway, but secretion efficiency varies widely among signal peptides. We performed a genome-wide analysis of signal peptides in *Saccharomyces cerevisiae* and experimentally evaluated their effects on protein secretion. The analysis identified sequence features associated with efficient secretion and showed that signal peptide function is affected by the Sec translocation machinery. In particular, deletion of *SEC72* selectively enhanced secretion mediated by strongly hydrophobic signal peptides. Transcriptomic and physiological analyses showed that *SEC72* deletion also altered iron homeostasis, cell wall organization, and protein synthesis. Further engineering of the *SEC72*-deficient strain resulted in an α-amylase titer of 6.5 g/L in fed-batch cultivation. These results demonstrate that the effects of host engineering can depend on signal peptide properties and provide a basis for improving recombinant protein secretion in yeast.

**Key words:** Signal peptide; protein secretion; *Saccharomyces cerevisiae*; metabolic engineering

**Representative publication:**

1. Xue S., Pan Y., Qin L., Yan Z., Xie J., Huang M.*, The absence of Sec72 reshapes yeast cell functions to increase protein secretion. *Research* **2026**, 9: 1119.
2. Qin L., Pan Y., Xue S., Yan Z., Xiao C., Liu X., Yuan D., Hou J., Huang M.*, Multi-omics analysis reveals impacts of LincRNA deletion on yeast protein synthesis. *Advanced Science* **2025**, 12(13): 2406873.
3. Xiao C., Liu X., Pan Y., Li Y., Qin L., Yan Z., Feng Y., Zhao M., Huang M.*, Tailored UPRE2 variants for dynamic gene regulation in yeast. *PNAS* **2024**, 121(19): e2315729121.
4. Xue S., Liu X., Pan Y., Xiao C., Feng Y., Zheng L., Zhao M., Huang M.*, Comprehensive analysis of signal peptides in *Saccharomyces cerevisiae* reveals features for efficient secretion. *Advanced Science* **2023**, 10 (2):2203433.

## Biography



Mingtao Huang is a Professor of Microbial Biotechnology at South China University of Technology, China. He obtained his B.S. degree in Biotechnology from Sun Yat-sen University in 2006 and went on to complete his Ph.D. from the same University in 2011. In 2012, he joined the division/group of Systems and Synthetic Biology at Chalmers University of Technology as a Postdoc. In 2019, he joined South China University of Technology as a professor in his current role.

His research group concentrates on systems metabolic engineering and synthetic biology, with a primary focus on engineering microorganisms for efficient protein production. He has published more than 90 peer-reviewed papers in scientific journals such as PNAS, Nat. Commun., Research, Adv. Sci., and Metab. Eng.

# Design and Optimization of Artificial Biological Carbon Fixation Systems

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## Abstract

Biomanufacturing is an important way to achieve "dual-carbon" goals. To reduce the raw materials cost, the adoption of low-cost and renewable resources, such as one-carbon (C1) feedstocks, has gained widespread attentions. Harnessing interdisciplinary expertise in synthetic biology and materials science, we established a suite of transformative platforms for C1-to-chemical bioconversion. (1) A light-driven microbial community was engineered based on "functional complementarity and metabolic cross-feeding", enabling the direct photoautotrophic synthesis of 2-phenylethanol and tyrosol from $CO_2$. (2) By implementing a reductive TCA cycle for $CO_2$ fixation and utilizing methanol as an auxiliary reducing power donor, succinic acid production efficiency was improved by 1.1-fold in artificial methylotroph ¬E. coli, achieving record-breaking succinic acid yields. (3) To minimize carbon loss, we constructed a spatially segregated dual-fixation network comprising a peroxisomal CBB module to recycle $CO_2$ and a molecular dynamics-guided mitochondrial reductive glycine pathway to salvage formate in Pichia pastoris. To bridge the resulting energetic deficit, we deployed two modular energy-reinforcement strategies: an intracellular inorganic-biological hybrid system for orthogonal electron supply and a synthetic energy system for cofactor rebalancing, resulting in a 46.9% reduction in carbon emissions. Taken together, these findings provide a theoretical and technological blueprint for establishing carbon-negative industrial biomanufacturing routes.

**Key words:** biomanufacturing; one-carbon feedstocks; metabolic engineering; synthetic biology; bio-based chemicals

**Representative publication:**

1. Guo F., Liu K., Xin F.X.*, Jiang M., Zhang W.M.*, Evolutionary engineering of *Saccharomyces cerevisiae*: Crafting a synthetic methylotroph via self-reprogramming, Science Advances, 2024, 10, eadq3484.

2. Zhou X., Wu D.*, Zhang Y.J., Zhang W.M.*, Zhang Z.H.*, Inorganic-bacterial biohybrids for efficient solar-driven nitrogen fixation, Nature Communications, 2025, 16, 5690.

3. Shi X.F., Li C., Zhao C.W.*, Zhang W.M.*, Zhang X*, Zeng J.*, A review of electrochemical–biological coupling systems for $CO_2$ valorization: catalytic fundamentals, system integration, and industrial outlook, Angewandte Chemie International Edition, 2026, e9183730.

4. Guo F., Xin F.X.*, Zhang W.M.*, Jiang M., Bioconversion of C1 feedstocks for chemical production using *Pichia pastoris*, Trends in Biotechnology, 2023, 41 (8): 1066-1079.

5. Gao H., Foo J.L.*, Zhang W.M.*, Chang M. W., Xin F.X.*, Jiang M., Sustainable production through spatial niche partitioning in engineered light-driven microbial community, Metabolic Engineering, 2025, 92, 90-101.

## Biography



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Dr. Wenming Zhang is a professor and doctoral supervisor in Nanjing Tech University. He is among the top 2% of scientists globally. His research focuses on the efficient biotransformation of one-carbon raw materials and the construction and regulation of yeast cell factories. He has hosted nearly 20 projects, applied for 56 Chinese invention patents, 24 authorized patents, co-authored 8 textbooks and monographs, and formulated 3 group standards. He has published over 80 papers as first or corresponding author in authoritative journals such as Sci Adv, Nat Commun, Angew Chem Int Edit, Met Eng and Trends Biotechnol. The achievements have won several awards, including the first prize of the Science and Technology Progress Award from the China General Chamber of Commerce, the third prize of the Jiangsu Provincial Science and Technology Award, and the Jiangsu Agricultural Science and Technology Award for Young Scientists.

# Repurposing *Ogataea polymorpha* into a superior microbial platform for bio-productions

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## Abstract

*Ogataea* (*Hansenula*) *polymorpha* represents a promising microbial host for bio-manufacturing from inexpensive feedstocks like methanol and lignocellulose owing to the advantages in wide substrate spectrum, thermal-tolerance and high-density fermentation. However, limited to the inefficient genetic manipulation and unclear metabolic characteristics, *O. polymorpha* has only been applied in productions of protein and ethanol. Here, we proposed to repurpose *O. polymorpha* into a superior microbial platform for bio-productions. Firstly, recombination machinery engineering (rME) was explored for enhancing homologous recombination (HR) activity together with establishing an efficient CRISPR/Cas9 system in *O. polymorpha*, achieving up to 90% of editing rate and 70% HR rate. On this basis, we, for the first time, achieved the efficient fatty acid production from sole methanol, an ideal feedstock that can be beneficial for global carbon neutrality. The engineered cells were rescued from necrosis by adaptive laboratory evolution, and the relationship between phospholipids homeostasis and methanol toxicity was identified for promoting methanol-based bio-manufacturing. Finally, we engineered the *O. polymorpha* for lignocellulose bio-refinery by engineering the co-utilization of glucose and xylose, which enabled the highest production of free fatty acids (38.2 g/L) and 3-hydroxypropionic acid (79.6 g/L) from lignocellulose. Consequently, our results demonstrated the great potential of *O. polymorpha* in bio-refinery for industrial biotechnology applications.

**Key words:** *Ogataea* (*Hansenula*) *polymorpha*; Bio-productions; Metabolic engineering; Methanol; Lignocellulose

**Representative publication:**

1. Gao J.Q., Yu W., Li Y.X., Jin M.J., Yao L., Zhou Y.J.*, Engineering co-utilization of glucose and xylose for chemical overproduction from lignocellulose, Nat. Chem. Biol., 2023. (doi:10.1038/s41589-023-01402-6)

2. Gao J.Q., Li Y.X., Yu W., Zhou Y.J.*, Rescuing yeast from cell death enables overproduction of fatty acids from sole methanol, Nat. Metab., 2022, 4, 932-943.

3. Gao J. Q., Gao N., Zhai X., Zhou Y.J.*, Recombination machinery engineering for precise genome editing in methylotrophic yeast *Ogataea polymorpha*, iScience, 2021, 24, 102168.

## Biography



Dr. Jiaoqi Gao is currently an Associate Professor of Dalian Institute of Chemical Physics, Chinese Academy of Sciences (DICP). He received B.S. degree in Biological Engineering from Dalian University of Technology in 2012, and Ph.D. degree in Biochemical Engineering from Dalian University of Technology in 2017. He joined DICP as an Assistant Professor in 2017, and then worked as an Associate Professor since 2022. His research interest lies in yeast cell factory construction and methanol biotransformation. During the last 5 years, he published over 20 papers in Nature Metabolism, Nature Chemical Biology, iScience, Bioresource Technology, Biotechnology for Biofuels, and applied over 10 patents.

# AI4S Empowering Biomanufacturing

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## Abstract

Artificial intelligence for science (AI4S) is reshaping biomanufacturing by turning the engineering of living cells into a predictable, data-driven discipline. Integrating synthetic biology, enzyme engineering, and machine learning, we have developed an IT+BT methodology built on a prediction–design–control framework for bioreaction engineering. In the prediction layer, the UniKP language-model framework predicts enzyme kinetic parameters directly from protein sequences and substrate structures, enabling sequence-driven enzyme mining and virtual screening; companion tools (CLAIRE, IPPF-FE, ThermoFinder) predict reaction classes and protein properties. In the design layer, organelle engineering spatially compartmentalizes biosynthesis, relocating pathways to the endoplasmic reticulum and peroxisomes to achieve de novo production of high-value compounds such as jasmonates, cannabinoids, and flavonoids. In the control layer, automated design–build–test–learn platforms couple biosensors with machine-learning-guided flux balancing and directed evolution to dynamically optimize strains, raising titers by orders of magnitude. Together, these AI4S platforms compress the enzyme-to-product cycle and translate fundamental advances into industrial biomanufacturing.

**Key words:** AI for Science; Synthetic Biology; Biomanufacturing; Enzyme Engineering; Machine Learning

**Representative publication:**

1. Wang X., Chen N., Cruz-Morales P., Zhong B., Zhang Y., Wang J., Xiao Y., Fu X., Lin Y., Acharya S., Li Z., Deng H., Sun Y., Bai L., Tang X.*, Keasling J.D.*, Luo X.* Elucidation of genes enhancing natural product biosynthesis through co-evolution analysis. Nature Metabolism, 2024, 6(5), 933-946.

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## Biography



Xiaozhou Luo is a Professor of Synthetic Biology at the Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences (SIAT), where he serves as Assistant Director of the Institute of Synthetic Biology and Executive Director of the Center for Synthetic Biochemistry. He is also Chief Technology Officer of the National Innovation Center for Biomanufacturing Industry and of Synceres Biosciences, bridging fundamental research and industrial translation. He received his B.S. from Nanyang Technological University and his Ph.D. in Chemistry from The Scripps Research Institute with Prof. Peter G. Schultz, followed by postdoctoral research at the University of California, Berkeley with Prof. Jay D. Keasling. His research integrates synthetic biology, enzyme engineering, and machine learning to build AI-driven platforms for biomanufacturing, including the UniKP enzyme kinetics predictor, protein language model-guided directed evolution, and automated design-build-test-learn systems. He has published over 60 SCI papers in Nature, Nature Synthesis, Nature Metabolism, and other leading journals, and was named a 2023 Nanshan Top Ten Outstanding Young Person.

# Biosensor-guided genetic circuits for dynamic control of sustainable biomanufacturing

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## Abstract

Achieving a competitive, global circular bioeconomy demands bio-based processes that remain robust and efficient across scales, a goal often hindered by the inefficiencies associated with metabolic engineering approaches that prioritize pathway construction over its regulation. Natural pathways, by contrast, rely on tight dynamic control to perform reliably in changing environments. Here, we present a framework for embedding such regulation into synthetic pathways using biosensor-mediated genetic circuits. We introduce BiosInt, an allosteric transcription factor-based circuit with quasi-integral adaptation capabilities and demonstrate a multi-objective optimization approach that inversely designs optimal circuit topologies for any given pathway configuration and enzyme expression ratio. The proposed approach is validated on flavonoid production pathways, where we have characterized a library of *FdeR* whole-cell biosensors, combining mechanistic modeling with machine learning to predict context-dependent dynamic behavior across varying expression elements, media, and carbon sources. Together, these efforts constitute a Design-Build-Test-Learn pipeline: synthetic datasets from experimentally-calibrated pathway simulations train predictive models capable of identifying optimal control topologies and biosensor conditions, bridging first-principles modeling with data-driven prediction. This hybrid strategy linking mechanistic understanding, allosteric biosensing, and machine learning, establishes a scalable design pipeline for biofoundries, advancing robust, adaptive, and precisely regulated cell factories for sustainable chemical and therapeutic bioproduction.

**Key words:** biosensor, dynamic regulation, context dependence, genetic circuit, scientific machine learning

**Representative publications:**

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## Biography



Pablo Carbonell is Lead Scientist at the Institute for Integrative Systems Biology (I2SysBio), CSIC-UV Spain. His research interests are in automated design for metabolic engineering and synthetic biology. Pablo has contributed to the development of several tools for pathway design, including DetSpace, Selenzyme, RetroPath 2.0, XTMS, EcoliTox and Promis. Pablo is interested in applying the principles of machine learning and control engineering to sustainable biological design. Pablo Carbonell is Chief Specialty Editor of Synthetic Biology for Frontiers in Bioengineering and Biotechnology and one of the coordinators of the Valencia Biofoundry initiative.

# Advancing Cellular Digital Modeling through Artificial Intelligence

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## Abstract

To meet the increasing demands of industrial biomanufacturing, the development of high-performance metabolic models is essential for precise strain design. Here, we present AlphaGEM and EnzymeTuning, AI-powered tools that automate the creation of high-accuracy metabolic models. These automated platforms utilize state-of-the-art deep learning to resolve persistent issues of suboptimal predictive accuracy and time-intensive model construction. Building upon these models, we have developed a series of algorithms—ecFactory, MetaStrain, and StrainOptimizer—to automate the prediction of optimal gene overexpression, knockdown, and deletion strategies for the rational design of cell factories. This talk will highlight how the latest AI technologies empower the field of cell digital modeling, enabling the high-fidelity reconstruction of metabolic models for non-conventional industrial strains and paving the way for the intelligent design of high-performance cell factories.

**Key words:** cell factories; metabolic models; artificial intelligence

**Representative publication:**

1. Han W.S., Xiao L., Sun H., Xiang G.M., Jia Q.X., Wang H.Y., Ji B.Y., Zhang C., Kerkhoven E.J., Nielsen J., Lu H.*, AlphaGEM enables precise genome-scale metabolic modelling by integrating protein structure alignment with deep-learning-based dark metabolism mining, Nature Communications, 2026, 10.1038/s41467-026-75549-w

2. Wang X.T., Wang Y.B., Zhuang Y.P., Wang G.*, Lu H.*, EnzymeTuning improves enzyme-constrained metabolic modeling and proteome abundance prediction through deep learning, Nature Communications, 2026, 10.1038/s41467-026-73744-3.

3. Wang H.Y., Nielsen J., Zhou Y.J.*, *Lu H.**, Yeast adapts to diverse ecological niches driven by genomics and metabolic reprogramming, PNAS, 2025, 122, 1-11.

## Biography



Dr. Hongzhong Lu is a Tenure-track Associate Professor at the School of Life Sciences and Biotechnology, Shanghai Jiao Tong University (SJTU), where he serves as an independent Principal Investigator (PI) and doctoral supervisor heading the Cell Digital Modeling Group. His research focuses on the digital modeling of cellular metabolic networks and the application of cutting-edge multi-dimensional metabolic models in intelligent strain design and biological big data analytics.

Dr. Lu has published extensive research as first or corresponding author in prestigious journals, including *Nature Communications (4 papers)*, *PNAS* (2 papers), *Molecular Systems Biology* (2 papers), *Metabolic Engineering*, *Briefings in Bioinformatics*, and *Trends in Biotechnology*. He is the lead investigator for two National Natural Science Foundation of China (NSFC) grants and two industrial collaborative projects, while also contributing to National Key R&D Programs and major municipal synthetic biology initiatives. Recognized as a 2022 Shanghai Pujiang Talent, Dr. Lu also serves on the Professional Committee of Biochemical Process Modeling and Control (Chinese Society for Microbiology) and the Youth Committee of the Metabolomics Branch (Biophysical Society of China).

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# AI-driven quantification of tissue metabolic fluxes in vivo

Daniel R. Weilandt, Reha Matur, Joanne Peng, Vyas Pujari, Esteban Gonzales, Micheal Neinast, Lingfan Liang, Michel R. MacArthur, Sarah Cherkaoui, Yihui Shen, Martin Wühr, Joshua D. Rabinowitz

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## Abstract

Mammalian tissues perform specialized physiological functions through differential expression of metabolic enzymes. While proteomics and metabolomics measure enzyme and metabolite abundance, they do not directly capture enzyme activity. Metabolic flux provides a direct readout of pathway activity, but tissue-specific flux measurements remain technically challenging and typically require specialized isotope-tracing experiments.

Here, we present SigmaFlux, an AI framework that infers tissue-specific metabolic fluxes from a combination of measured reference fluxes and labeling patterns generated by stationary $^{13}C$ tracer infusions. To train SigmaFlux, we built a digital twin of mammalian metabolism that predicts tissue labeling patterns across diverse in vivo isotope infusion experiments. The model incorporates organ-specific metabolic functions through minimal mass-balanced networks capable of performing known physiological tasks, including hepatic gluconeogenesis, urea production, and cardiac fatty acid oxidation. Trained on simulated tracing datasets, SigmaFlux identifies flux states most consistent with observed labeling signatures and measured fluxes and shows strong agreement with independent flux measurements from non-stationary isotope tracing.

Applying SigmaFlux across 12 mouse tissues, we quantified more than 2,000 enzyme fluxes. Integration with absolute protein and metabolite abundances revealed that tissue-specific fuel preferences are controlled by a surprisingly small number of enzymes. All flux maps are publicly available through an interactive atlas at fluxatlas.princeton.edu.

**Key words:** Metabolic Flux Analysis, Stable Isotope Tracing, Artificial Intelligence, Systems Metabolism, Tissue-Specific Fluxes

**Representative publication:**

1. Weilandt, D.R., Tahmid, MT, Singh, M., Rabinowitz, J.D. Language-model-based identification of metabolic flux, *in preparation*

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4. Bartman, C.R., Weilandt, D.R., et al. Slow TCA flux and ATP production in primary solid tumours but not metastases. Nature, 614, 349 (2023).

## Biography

Daniel R. Weilandt is an Associate Research Scholar at the Lewis-Sigler Institute for Integrative Genomics at Princeton University. He holds a B.Eng. in Mechanical Engineering, an M.Sc. in Microsystems Engineering, and a Ph.D. in Chemical Engineering. His research combines engineering, metabolism, and computational biology to uncover the principles that govern metabolic regulation across scales, from intracellular biochemical networks to whole-body physiology.

During his doctoral studies with Prof. Vassily Hatzimanikatis, he developed computational models to investigate how intracellular complexity influences biochemical function, demonstrating how weak nonspecific DNA interactions accelerate chromatin reader search kinetics and how macromolecular crowding reshapes enzyme activity. As a postdoctoral researcher with Prof. Joshua Rabinowitz, he combined in vivo isotope tracing with mechanistic modeling to discover the principle of competitive catabolism, showing how circulating nutrients compete for oxidation and providing a quantitative framework for metabolic homeostasis and its dysregulation in obesity and diabetes.

His current work integrates large-scale isotope tracing, mechanistic models, and artificial intelligence to infer intracellular metabolic fluxes across mammalian tissues. He leads the development of SigmaFlux and the Mammalian Flux Atlas, which together provide the most comprehensive quantitative map of tissue metabolism generated to date.



# AI-driven digital cell modeling for cell factory design

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## Abstract

Cell factories are the core units of biomanufacturing, yet their development remains slow and costly because it relies heavily on iterative experimentation. Although genome-scale metabolic models have been widely used for strain design over the past two decades, their effectiveness is still limited by incomplete biochemical knowledge, sparse kinetic parameters, and insufficient representation of latent metabolic processes. To address these limitations, we developed digital cell modeling frameworks that connect metabolic discovery, parameterization, and automated model construction. By combining retrobiosynthesis, deep learning, and mechanistic constraints, we built Yeast-MetaTwin to systematically reveal underground metabolism and extend metabolic models beyond curated annotations. To address the limited availability of kinetic and thermodynamic parameters, we constructed GotEnzymes2, a large-scale enzyme parameter resource covering 10,765 species and 59.6 million enzyme-substrate pairs, providing predicted kinetic and thermodynamic properties for model building and enzyme engineering. In parallel, D2Cell uses large language models to extract and integrate metabolic engineering knowledge from the literature, supporting data-driven cell factory design. Together, these studies provide a framework for uncovering hidden metabolic space, filling parameter gaps, and automating model construction, enabling more predictive and efficient design of biological systems for biomanufacturing.

**Key words:** Metabolic model, Machine learning, Retrobiosynthesis, Metabolic dark matter

**Representative publication:**

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2. Luo J., Wang H., Moyer D., et al., Li F.*, Reconstruction of human metabolic models with large language models, *Proceedings of the National Academy of Sciences of the United States of America*, 2026, 123, e2516511123.
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5. Li F.[#], Yuan L. [#], Lu H., et al., Deep learning-based *k*cat prediction enables improved enzyme-constrained model reconstruction, *Nature Catalysis*, 2022, 5, 662–672.

## Biography



Dr. Feiran Li is an Associate Professor at Tsinghua University, specializing in cell metabolism, cell factory design, constraint-based metabolic modeling, and machine learning. She was awarded the National Excellent Young Scientists Program (Overseas) and was named one of MIT Technology Review's Innovators Under 35 China. She received her Ph.D. from Chalmers University of Technology in 2021 and subsequently worked as a postdoctoral researcher in the laboratory of Prof. Jens Nielsen until 2023. Dr. Li has published first-author and corresponding-author papers in leading journals, including *Nature Catalysis*, *Nature Communications*, *PNAS*, *Nucleic Acids Research*, *and Molecular Systems Biology*. Her research focuses on developing advanced computational frameworks for metabolic systems analysis and applying them to key challenges in biotechnology and biomedicine, particularly in metabolic engineering and cell factory design.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Rational design of metabolic engineering strategies based on modulating the expression of transcription factors

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## Abstract

The integration of genome-scale metabolic networks with gene regulatory networks enables investigation of how gene regulation impacts metabolism. Yet, we lack computational approaches to enable rational design of metabolic engineering based on transcriptional regulation on gene level. Here we present OCELOT, an approach for integration of mechanistic metabolic models with machine learning gene-regulatory models to predict transcription factor-based metabolic engineering strategies to optimize a metabolic objective of choice. Our findings demonstrate that OCELOT can predict the essentiality of transcription-factor-coding genes in *Escherichia coli* and *Saccharomyces cerevisiae* across growth conditions with accuracy of 84.88% and 89.6%, respectively, significantly outperforming the contenders. In addition, we showcase the use of OCELOT in predicting transcription-factor-based engineering strategies for a range of metabolites. We found that OCELOT predicted converging strategies that target global regulation of cell growth and stress responses, revealing a systems-level rewiring of gene regulation that favors high-production phenotypes for the investigated target metabolites. OCELOT paves the way for the use of hybrid models in guiding systems and synthetic biology efforts with established and emerging cell factories.

**Key words:** multi-omics, constraint-based modelling, strain design, synthetic biology, cell factories

## Biography



Prof. Dr. Zoran Nikoloski is a tenured professor (W3), Managing Director of the Institute of Biology and Biochemistry, Chair of the Bioinformatics Department at the University of Potsdam, Germany, Cooperative Research Group Leader at the Max Planck Institute of Molecular Plant Physiology in Potsdam, Germany, and an Honorary Professor at Nanjing Agricultural University, Nanjing, China. Prof. Dr. Nikoloski has pioneered the development and use of hybrid modeling frameworks that integrate constraint-based models of metabolism, machine learning, and multi-omics data from microbes and plants to predict and modify various traits. This framework is currently used to gain understanding of the genetic and molecular basis for plasticity and robustness of traits to different environmental cues, crucial in diverse biotechnological applications. Prof. Dr. Nikoloski has published over 250 academic papers and serves as editor in PLoS Genetics, Plant Physiology, Journal of Experimental Botany, and Planta.

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Modulating gene co-expression and metabolic flux in the yeast Saccharomyces cerevisiae by harnessing the regulatory plasticity of divergent transcription

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## Abstract

Divergent transcription is a widespread promoter architecture in nature, in which two genes are transcribed in opposite directions from a shared intergenic regulatory region. We systematically characterized 749 native yeast bidirectional promoters and demonstrated central shared regulatory elements play a key role in controlling promoter strength and bidirectional output. These unbalanced BDP strengths could be utilized for fine-tuning metabolic flux in yeast, achieving yields comparable to or exceeding those of commonly used constitutive or inducible promoters for terpenoid production under the examined conditions. However, whether divergent transcription is fixed by local cis-regulatory architecture or can be dynamically reshaped by trans-regulatory perturbation for engineering next-generation yeast production platforms remains unclear. To address this, we selected a native bidirectional promoter, and screened among 352 yeast regulatory deletion strains. Interestingly, more than 60% of the deletions increased promoter activity in both directions, and most of these changes occurred symmetrically. We further examined nine additional native bidirectional promoters across eight promising TF deletion strains. Interestingly, several promoter-TF deletion combinations in the BY4741 background achieved target-metabolite titers comparable to or higher than those obtained with an established high-producing diterpene platform, with a minimal single TF deletion modification. These results demonstrate that divergent transcription is highly plastic and can be reshaped by altered regulatory states and perturbations of the underlying transcriptional network.

**Key words:** bidirectional promoters, yeast, divergent transcription

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

1. Jin Z., Dong Y., Rafi A. M., Patwary MD M., Xu C., Raadam M. H., de Boer C. G., Ignea C.*, Unraveling the regulatory dynamics of bidirectional promoters for modulating gene co-expression and metabolic flux in *Saccharomyces cerevisiae, Nucleic Acid Research*, 2925, 53, doi.org/10.1093/nar/gkaf511

## Biography



[Please enter your Biography here, limit to 200 words] (First-line indent of 2 characters, Time new roman, font size 12)

Codruta Ignea is an Associate Professor of Synthetic Biology and Metabolic Engineering in the Department of Bioengineering at McGill University, and the recipient of dual Faculty Scholar Awards in sustainability and design, and engineering education. She earned her PhD at the University of Crete, completed her postdoctoral training at the University of Copenhagen, and joined McGill University as an Assistant Professor in 2020, where she was recently promoted to tenured Associate Professor.

Dr. Ignea focuses her research on discovering and producing high-value compounds and non-natural molecules through biological synthesis. Throughout her career, Dr. Ignea has been awarded prestigious postdoctoral fellowships from the Marie Curie Actions and the Novo Nordisk Foundation and has conducted pioneering work on synthetic metabolism, constructing metabolic pathways not found in nature. Her current research aims to harness divergent transcription and membrane engineering for the development of next-generation yeast chassis for terpenoid production. In 2022, she revitalized the McGill iGEM team, which won the Grand Prize in 2023 and 2025, becoming the first Canadian team to win this competition.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Beyond Pathway Engineering: Matching Chassis to Feedstocks and Products

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## Abstract

Metabolic engineering has traditionally focused on pathway design, enzyme optimization and genome editing, often treating the microbial host as a secondary consideration. However, the increasing diversification of both feedstocks and target products is challenging this paradigm. The transition towards a circular bioeconomy is driving the use of renewable and heterogeneous feedstocks, including lignocellulosic hydrolysates, agro-industrial side streams and other underutilized biomass streams, which impose distinct physiological and metabolic constraints on production hosts.

As a result, host selection is emerging as a critical determinant of engineering success. Different microbial chassis display unique capabilities regarding substrate utilization, inhibitor tolerance, redox balancing, stress resistance and pathway compatibility, which can significantly influence the performance of engineered cell factories. These differences become even more relevant when producing diverse molecules, ranging from biofuels and bulk chemicals to furan-derived compounds, from diverse feedstocks.

This talk will discuss why host choice should be considered an explicit design variable in metabolic engineering and how aligning chassis characteristics with feedstock composition and product requirements can accelerate strain development and improve process performance. The next generation of metabolic engineering may be driven not only by better pathways, but also by smarter matching of hosts, feedstocks and products.

**Key words:** Metabolic Engineering; Microbial Chassis; Host Selection; Sustainable Feedstocks; Cell Factory Design

**Representative publication:**

1. Araújo J. I., Domingues L. *, Engineering yeast metabolism for robust bioproduction under industrial conditions, Current Opinion in Biotechnology, 2026, 100, 103513.

2. Martins C., Costa C. E., Cunha J. T., Gullón B., Romaní A., Domingues L.*, Whole yeast cell synthesis of 5-hydroxymethylfurfural-derivatives from apple waste processed by green technologies, Bioresource Technology, 2025, 430, 132570.

3. Baptista S. L., Soares P. O., Romaní A., Alonso J. L., Domingues L. * Engineering arabinose-to-arabitol conversion in industrial *Saccharomyces cerevisiae* for sugar beet pulp valorization. Industrial Crops and Products, 2024, 222, Part 2(119718).

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5. Costa C. E., Romaní A., Cunha J. T., Johansson B., Domingues L. * Integrated approach for selecting efficient *Saccharomyces cerevisiae* for industrial lignocellulosic fermentations: importance of yeast chassis linked to process conditions. Bioresource Technology, 2017, 227, 24-34.

## Biography



Lucília Domingues holds a Chemical Engineering degree from the University of Porto (1994), a Master's in Biotechnology – Bioprocess Engineering (1997) and a Ph.D. in Chemical and Biological Engineering (2001) from the University of Minho, in collaboration with VTT Technical Research Centre of Finland. She completed her Habilitation in 2018.

She is currently President of the Portuguese Society of Biotechnology and Associate Professor with Habilitation at the Department of Chemical and Biological Engineering, University of Minho, Director of the Master's in Biotechnology and a member of the Scientific Council of the School of Engineering. She leads the Molecular Biotechnology and Precision Fermentation research group.

Her research focuses on the development of microbial cell factories using molecular biotechnology tools and on the intensification of bioprocesses for the sustainable production of biofuels and bio-based chemicals from renewable resources. Her Research profile is available at https://orcid.org/00 00-0003-1089-7627]

# Rewiring energy homeostasis in *Yarrowia lipolytica* enables efficient terpenoid biosynthesis

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*Yarrowia lipolytica* has become an important cell factory for terpenoid biosynthesis. However, its tightly regulated metabolic network diverts carbon flux toward inherent metabolites overflow, thereby constraining terpenoid production efficiency. Here we rewired the global metabolism of *Y. lipolytica* to redirect carbon flux towards acetyl-CoA for β-farnesene production by introducing the non-oxidative glycolysis pathway and disrupting phosphofructokinase. The modifications resulted in a growth defect and adaptive laboratory evolution was performed. Multi-omics profiling and flux balance analysis revealed that simultaneous inactivation of the carbon repression regulator Reg1 and glucokinase Glk1 recovered cell survival through global metabolic rewiring. This recovery was achieved by activating Snf1 kinase-mediated carbon signaling and balancing the flux between hybrid glycolysis and the tricarboxylic acid cycle, thereby reestablishing cellular energy homeostasis. Further metabolic engineering combined with fermentation design enabled β-farnesene production to reach 82.1 g/L, without inherent products overflow. This represents the highest terpenoid titer reported in *Y. lipolytica*. Asides from β-farnesene, we also engineered *Y. lipolytica* for efficient production of vitamin A, β-carotene and zeaxanthin. Our study demonstrated the feasibility of the metabolic rewiring in *Y. lipolytica* for efficient terpenoid production.

**Keywords: Snf1, metabolic rewiring, fatty acid oxidation, β-farnesene, Yarrowia lipolytica**

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2. Sun L, Zhang J, Zhai H, Xu H, Liu M, Qi Q, Hou J. Metabolic, redox, and spatial engineering of Yarrowia lipolytica for high-level zeaxanthin production. Green Chem. 2026, 28, 2087-2097
3. Zhang J, Sun L, Yang W, Xu H, Sheng J, Liu M, Qi Q, Hou J. Biosynthesis of retinyl esters in Yarrowia lipolytica through metabolic engineering and fermentation condition optimization. Green Chem. 2025. 27, 2511-2521.
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## Biography

Hou Jin, Professor at Shandong University, received her doctor degree from Shandong University in 2009, studied as a guest PhD at the Technical University of Denmark from 2007 to 2008. She worked at Green Biologics Ltd in the UK from 2009 to 2010, followed by postdoctoral research at Chalmers University of Technology in Sweden from 2010 to 2012. She has been working at Shandong University since 2012. Her research focuses on yeast synthetic biology, dedicated to the green biomanufacturing of products for pharmaceutical and nutrient as well as bulk chemicals. She has established yeast editing and regulation technology systems, elucidated yeast metabolic regulation mechanisms, and constructed high-yield yeast strains for products such as carotenoids, vitamin A, and farnesene. She has published over 50 papers in journals including *Nature Communications*, *Nucleic Acids Research*, *Trends in Biotechnology*, and *Metabolic Engineering*.



# Developing continuous evolution systems for enhancing microbial protein bioproduction

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## Abstract

Microbial protein consists recombinant expressed functional proteins and biomass-based whole cell protein, which contain all essential and nonessential amino acids for human health and is recognized as high-quality protein source. Fueled by significant developments in synthetic biology, microbial cell factories are constructed for the bioproduction of microbial proteins, providing a promising approach for scalable and cost-effective production of alternative proteins. In this study, the design and construction of continuous evolution systems both for genome and genetic elements are developed. Next, continuous evolution systems were used to construct cell factories for recombinant expression of milk proteins and yeast biomass-based microbial protein bioproduction. The continuous evolution system constructed in this work provide new tools with high general applicability and robustness for the construction of efficient microbial protein cell factories.

**Key words:** continuous evolution; microbial food protein; biomass-based microbial protein; functional microbial protein; cell factories

### Representative publication:

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2. Chu Weiran; Tian Rongzhen; Guo Yaxin; Wu Yaokang; Rehm B H Fabian; Liu Long; Li Jianghua;Du Guocheng; Chen Jian; **Liu Yanfeng***; An evolved, orthogonal ssDNA generator for targeted hypermutation of multiple genomic loci, **Nucleic Acids Research**, 2025, 53(3): gkaf051
3. Cao Yanting; Wu Yaokang; Lv Xueqin; Li Jianghua; Liu Long; Du Guocheng; Chen Jian; **Liu Yanfeng***; Development of central metabolite-responsive genetic circuits for auto-dynamic and multi-layered regulation of cell metabolism in *Bacillus subtilis*, **Chemical Engineering Journal**, 2026, 529: 173088
4. Wu Xinhong; Wu Yaokang; Lv Xueqin; Li jianghua; Liu Long; Du Guocheng; Chen Jian; **Liu Yanfeng***; Synthetic biology and protein processing engineering-driven microbial protein supply by yeast and filamentous fungi, **Trends in Food Science and Technology**, 2025, 166: 105408
5. Wang Xinyi; Zhang Xuguang; Mao Yuejian; Wu Yaokang; Lv Xueqin; Liu Long; Han Weiwei; YinShenming; Wu Ruonan; Chen Jian; **Liu Yanfeng***; Ethanol inducible bioproduction of human α-Lactalbuminin *Komagataella phaffii*, **Journal of Agricultural and Food Chemistry**, 2025, 73(15):9246-9260

## Biography

Yanfeng Liu is a professor in School of Biotechnology/Science Center for Future Foods, Jiangnan University. He focuses on using synthetic biology tools to construct cell factories for biosynthesis of important food ingredients. As the first or corresponding author, his research has been published in Nature Chemical Biology, Nature Communications, and Nucleic Acids Research. Moreover, 20 invention patents were authorized for him, including 2 US patent.

He is the project leader of National Excellent Youth Fund, National Natural Science Foundation. As the first contributor, he has won the Top Ten Advances in Chinese Food Science and Technology in 2024, the Special Prize for Technological Progress of the China General Chamber of Commerce in 2025. He is currently a member of Industrial Microbiology Committee of the Chinese Society of Microbiology and an editorial board member of Frontiers in Bioengineering and Biotechnology.



# A Versatile Genetic Toolbox for Metabolic Engineering of Xylose-Utilizing *Scheffersomyces stipitis*

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## Abstract

Non-conventional yeasts possess distinct superior biological traits over the traditional brewing yeast *Saccharomyces cerevisiae*, holding great potential for industrial biomanufacturing. Nevertheless, their large-scale industrial deployment remains constrained by poorly characterized genetic backgrounds and metabolic networks, as well as the scarcity of efficient and robust genetic engineering toolkits. In this work, we target *Scheffersomyces stipitis*, a typical xylose-utilizing yeast with great promise as a chassis host for synthesizing shikimate pathway-derived aromatic compounds. We established a comprehensive genetic manipulation toolbox for *S. stipitis*, covering centromeric plasmid vectors, strength-diversified promoters and terminators, CRISPR/Cas9-based genome editing systems, and Hermes transposon-assisted pathway integration modules. Leveraging these well-characterized genetic tools combined with rational metabolic engineering strategies, we successfully engineered efficient *S. stipitis* cell factories for the *de novo* biosynthesis of (*S*)-norcoclaurine and 5-hydroxytryptamine (serotonin). Furthermore, the tool development framework and technical protocols established in this study are universally adaptable to the genetic modification and metabolic optimization of other non-conventional yeasts, including acid-tolerant *Issatchenkia orientalis* and oleaginous *Rhodotorula toruloides*. This work provides a versatile genetic platform and lays a solid foundation for the rational design and construction of high-performance novel microbial cell factories for bioproduction.

**Key words:**

Non-conventional yeast; *Scheffersomyces stipitis*; Genetic toolbox; Metabolic engineering; Microbial cell factory

**Representative publication:**

1. Huang Y., Ge R., Wu L., Sun Y., Wang Z.*, **Cao M**.*, Machine Learning for Microbial Cell Factories: Pathway Design, Enzyme Engineering, and Metabolic Regulation, ACS Synthetic Biology, 2026, 15 (7): 2749-2765

2. Wang C., Hu S., Wang S., Du J., Zhao Z., Zheng Z., Qiu H., Ma X., Li J., Liu H., **Cao M**.*, Gao W.*, Industrial-scale production of Lacto-N-tetraose in engineered *Bacillus subtilis* via modular pathway optimization, Metabolic Engineering, 2026, 93, 232-242

3. Hu L., Qiu H., Huang L., Zhang F., Tran V., Yuan J., He N.*, **Cao M**.*, Emerging nonmodel eukaryotes for biofuel production, Current Opinion in Biotechnology, 2023, 84: 103015

4. Li Y., Luo Q., Su J., Dong G., **Cao M**.*, Wang Y.*, Metabolic regulation of *Shewanella oneidensis* for microbial electrosynthesis: From extracellular to intracellular, Metabolic Engineering, 2023, 80: 1-11

5. **Cao M**., Tran V., Qin J., Olson A., Mishra S., Schultz J., Huang C., Xie D., Zhao H.*, Metabolic engineering of oleaginous yeast *Rhodotorula toruloides* for overproduction of triacetic acid lactone. Biotechnology and Bioengineering, 2022, 119:2529-2540

## Biography



Dr. Mingfeng Cao is a Minjiang Distinguished Professor, Director of the Biochemical Engineering Institute, and head of the Key Laboratory for Synthetic Biotechnology of Xiamen City. His research interests span synthetic biology, metabolic engineering, and one-carbon bioconversion. He has authored more than 80 peer-reviewed articles in journals including Nature Communications, JACS, and Metabolic Engineering. He has co-edited two Elsevier books, filed 10 patents, and led research grants from NSFC and the National Key R&D Program (sub-topic). Dr. Cao serves as Editor-in-Chief of Biotechnology Reports, and Associate Editor for Microbial Cell Factories, Biotechnology for Biofuels and Bioproducts, and BioDesign Research.

# Biological synthesis of astaxanthin by using yeasts

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## Abstract

Astaxanthin is a carotenoid with strong antioxidant activity, which exhibits promising application prospects in cosmetics, health-care products, animal feed and other fields. Nevertheless, its low temperature tolerance and low product titer in biosynthesis restrict its industrial application. In this study, key genetic elements closely associated with astaxanthin biosynthesis and high-temperature tolerance were first mined from *Xanthophyllomyces dendrorhous*. Using the recombinant astaxanthin-producing *Yarrowia lipolytica* as the chassis host, we systematically characterized the functions of these genetic elements. With these elements as the core strategy, recombinant *Y. lipolytica* cell factories with high thermotolerance and high astaxanthin yield were constructed, and their industrial applications in cosmetics, food and other fields were further explored.

**Key words:** Astaxanthin, Yeasts, Synthetic Biology, Fermentative Engineering

**Representative publication:**

1. Zhou D, Fei Z, Liu G, Jiang Y, Jiang W, Lin C.Z.K, Zhang W, Xin F.X*, Jiang M. The bioproduction of astaxanthin: A comprehensive review on the microbial synthesis and downstream extraction, *Biotechnology Advances*, 2024, 74, 108392.

2. Zhou D, Yang L, Guo F, Jiang W, Jiang Y, Zhang W, Xin F.X*, Jiang M. High astaxanthin production by *Xanthophyllomyces dendrorhous* strain DW6 from cane molasses using two-stage pH strategies, *Green Chemistry*, 2024, 26, 4582.

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4. Gao H, Tang Y, Lv R, Jiang W, Jiang Y, Zhang W, Xin F.X*, Jiang M. Transcriptomic analysis reveals the potential mechanisms for improving carotenoid production in *Rhodosporidium toruloides* Z11under light stress. *Journal of Agricultural and Food chemistry*, 2024, 72, 3793-3799.

5. Zhou D, Yang L, Guo F, Guo F, Jiang Y, Zhang W, Jiang W, Xin F.X*, Jiang M. Influence of metabolic regulators on the astaxanthin synthesis by *Xanthophyllomyces dendrorhous* and the transcriptome analysis, *Journal of Agricultural and Food chemistry*, 2026, 74, 5: 4599-4610.

## Biography



Prof. Fengxue Xin obtained his PhD from National University of Singapore. Currently, he served as Director of the Sino-German International Joint Laboratory for Biorefinery of Waste Carbon Resources in Jiangsu Province. He is a recipient of the Jiangsu Distinguished Young Scholars Fund and Taishan Industrial Innovation Leading Talent of Shandong Province. He serves as a director of the China Fermentation Industry Association, director of the Enzyme Preparations Branch of the Chinese Institute of Food Science and Technology, Section Editor of *Biotechnology for Biofuels and Bioproducts*, and Associate Editor of *BioDesign Research.* As the first/corresponding author, he has published more than 100 SCI papers in top journals of bioengineering including *Science Advances*, *Metabolic Engineering* and *ACS Synthetic Biology* et al. He has filed 39 Chinese invention patents as the first inventor, of which 17 have been granted. He has formulated 5 group standards and authored/co-authored 9 Chinese and English monographs. He has won 4 provincial and ministerial-level awards as the first inventor.

# Engineering Electroactive Microbes: Toward Direct Electricity-Driven Biosynthesis

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Electricity-driven bioconversion mitigates surplus renewable electricity by converting intermittent solar and wind power into stable chemical energy storage. It also overcomes the theoretical limitation of chemical energy supply in conventional biomanufacturing and enhances bioconversion efficiency. In ethanol fermentation, *Zymomonas mobilis* in the cathode chamber uses electrical energy to accelerate ethanol production, and electroactive engineering of this strain further improves electricity utilization and ethanol yield. For $CO_2$-fixing succinic acid production, $CO_2$ is first electrochemically converted to formic acid. A formate-assimilating *Corynebacterium glutamicum* strain was constructed for succinic acid synthesis. The engineered electroactive *C. glutamicum* directly consumes electrical energy, increases carbon fixation efficiency and succinic acid titer, and simplifies the sequential electrochemical-biological hybrid system. This work highlights the great potential of electroactive microorganisms in direct electricity-driven bioconversion.

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2. Y. Liang, K. Li*, X. Zhao, F. Bai, C. Liu*, “Enhanced glucose utilization and succinic acid production in *Corynebacterium glutamicum* by integration of metabolic engineering and electro-fermentation” Chem. Eng. J. 5(505), 159522. (2025)
3. K. Li, X. Zhang, C. Li, Y. Liang, X. Zhao, C. Liu *, et al, “Systems metabolic engineering of *Corynebacterium glutamicum* to assimilate formic acid for biomass accumulation and succinic acid production” Bioresour. Technol. 402, 130774. (2024)
4. K. Li, J.Zhang, F. Li, X. Zhao, F. Bai, H. Song*, C. Liu *, “Electrochemical control of cell metabolism improves ethanol production of *Zymomonas mobilis* in an electro-fermentation system” ACS Sustain. Chem. Eng. 11(6), 2364-2374. (2023)

# Engineering microbial consortia in time and space

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## Abstract

Microbial consortia distribute metabolic labour across species, but their behavior is governed by population dynamics that are difficult to specify in advance: faster-growing members displace slower ones, and the composition that emerges is rarely the one intended. Here we show that both the temporal and the spatial dynamics of a consortium can be programmed through its physical context rather than through genetic circuitry alone. We first demonstrate that spatial segregation sets temporal behaviour. By confining species to separate compartments and controlling the exchange between them, competition for shared resources is decoupled from cross-feeding, so that the relative abundances follow a prescribed trajectory and are maintained over successive passages, including for pairs that collapse to a single survivor when co-cultured without separation. We then couple the microbial growth to a phase transition in the surrounding material: local proliferation triggers phase separation, so that the spatial arrangement of the community is written by its own activity and consolidated as the material sets. Together, these results establish spatial organization and material state as tractable handles on consortium dynamics, and provide a route to living materials whose composition and form are determined by design.

**Engineering microbial consortia; temporal and spatial control; synthetic evolution**

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

1. Programmable assembly of honeycomb-like living materials via microbial consortia AIP
2. On-Demand Biomanufacturing via Programmed SporeBot *Cell Reports Methods* AIP
3. Engineering functional materials through bacteria-assisted living grafting, Cell Systems, 2024, 15(3): 264-274.
4. Engineering consortia by polymeric microbial swarmbots, Nature Communications, 2022, 13(1): 3879.



## Zhuojun Dai,

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Dr Zhuojun Dai received her Bachelor degree from Zhejiang University. She obtained PhD degree in chemistry from The Chinese University of Hong Kong under the supervision of Prof Chi Wu, where she investigated the polymer chain dynamics, interactions between the polycations and polyanions from the view of polymer physics. Then, she undertook postdoctoral training at the Department of Biomedical Engineering, Duke University in Prof. Lingchong You's lab, where she integrated the tools from synthetic biology and material science to build a versatile biomanufacturing platform. Since Sep, 2018, she started her lab in iSynBio, focusing on the living fabrications by the engineered bacteria and synthetic microbial consortia. Her lab’s work have been published in journals including Nature Chemical Biology, Nature Biotechnology, Cell Systems and Nature Communications.

# Metabolic engineering-enabled C1 feedstocks upgrading

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## Abstract

Towards the 2050 net-zero carbon emission goal, our team focuses on developing metabolically engineered microbes capable of utilizing C1 feedstocks. In particular, cyanobacteria, as natural photoautotrophs, were engineered to synthesize succinic acid and 1,4-butanediol directly from dilute atmospheric carbon dioxide. Through design and evaluation of individual pathways leading to the synthesis of these diols and diacids with subsequent strain optimization, we were able to achieve production of succinic acid and 1,4-butanediol in the grams per liter level of titer. For emission sources containing higher concentration of carbon dioxide, physical and chemical capture and utilization strategies are generally more effective than biological approaches. We engineered methylotrophic bacteria to upgrade methanol, which is a commercialized carbon capture & utilization product, to plastics and precursors to promote longer life cycle as solid materials without rapid re-emission back to the atmosphere. We will share our recent progress in the effort of increasing carbon utilization efficiency.

**Key words:** succinic acid; butanediol; cyanobacteria; methylotroph

## Biography



Ethan received his B.S. degree in Chemistry from the University of Maryland College Park in 2009. He then worked with Professor James C. Liao at UCLA for his PhD degree on the development of microbial cell factory for biofuel production. He started his academic career at the Department of Biological Science and Technology of National Chiao Tung University in 2014. He is currently a professor at the Department of Biological Science & Technology and has served as the Associate Dean of the College of Engineering Bioscience since 2020. He is a recipient of the International Outstanding Young Scholar Award and the Ministry of Science & Technology Young Scholar Fellowship. His research lab pioneered synthesis of C4 di-functional compounds from carbon dioxide and the first de novo synthesis of anti-catabolic agent 3-hydroxy-3-methylbutyric acid. Currently, he holds 4 active invention patents. His works received about 3,800 citations.

# Developing the marine alga *Nannochloropsis* as a light-driven eukaryotic chassis for converting $CO_2$ to value-added products

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## Abstract

Photosynthetic microorganisms offer a sustainable route for converting $CO_2$ into fuels, chemicals, and nutritional products. The marine microalga *Nannochloropsis* is particularly attractive because of its rapid photoautotrophic growth, high lipid content, natural accumulation of eicosapentaenoic acid (EPA), and increasing genetic tractability. We are developing *Nannochloropsis* as a light-driven eukaryotic chassis by integrating genetic-tool development with lipid and carotenoid engineering. Modular expression elements and targeted genome-editing workflows have enabled pathway construction and functional analysis. By resolving and rewiring fatty-acid metabolism, we enhanced carbon flux toward EPA and altered its partitioning between membrane and storage lipids. We also introduced ketocarotenoid pathways, generating strains that produce astaxanthin while retaining EPA synthesis. These strains further reveal that carotenoid engineering can reshape pigment storage and photosynthetic energy management, including non-photochemical quenching and photosystem I performance under high light. Our current work combines genome engineering, metabolic analysis, photosynthetic phenotyping, and photobioreactor cultivation to develop strains that remain productive under dynamic, process-relevant conditions. Together, these advances establish *Nannochloropsis* as a versatile photosynthetic chassis for producing omega-3 lipids, carotenoids, and other value-added products directly from $CO_2$ and light.

**Key words:** Carotenoids; Light-driven; Microalgae; PUFAs; Synthetic biology; Value-added products

**Representative publication:**

1. Li, X.; Long, Q.; Jiang, M.; Tan, W.; Ding, N.; Sun, H.; Liu, X.; Hu, X.; Liu, H.; Li, X.; **Liu, J.***; Zhou J.*; Du, X.* Engineered microbial hydrogels with confined architecture and binary microbes for efficient hydrogen production. Nature Communications 2026, 17, 4303.

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5. **Liu, J.***; Liu, M.; Pan, Y.; Shi, Y.; Hu, H. Metabolic engineering of the oleaginous alga *Nannochloropsis* for enriching eicosapentaenoic acid in triacylglycerol by combined pulling and pushing strategies. Metabolic Engineering 2022, 69, 163-174.

## Biography



Liu Jin is a professor and currently serving as the director of the Center for Algal Innovation & Engineering Research and PI of State Key Laboratory for Food Science and Resources, Nanchang University. He completed his undergraduate and master's degrees at Sun Yat-sen University and received his Ph.D. from the University of Hong Kong. He has been selected for the National High-Level Talent Program (Youth), the Peking University Boya Young Scholars program, and featured in the list of the top 2% of global scientists. He has been funded with multiple projects by the National Key R&D Program and National Natural Science Foundation. He serves as an associate editor for the journals Algal Research and Advanced Biotechnology, and is an executive committee member of the International Society for Applied Phycology and a standing committee member of the Phycology Branch of the Chinese Society of Oceanography and Limnology. He has published over 60 papers in journals such as Nature Communications, Metabolic Engineering, New Phytologist, Molecular Plant, Plant Physiology, Plant Journal, Journal of Agricultural and Food Chemistry, and Food Chemistry.

# Tailoring Precise Genomic Integrative Expression Toward Rational Strain Development for Scalable High-titer Production of PHA

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## Abstract

Halophilic chassis has emerged as a promising biomanufacturing platform for industrial polyhydroxyalkanoate (PHA) production. However, challenges still remain in improving the production capacity, scalability and robustness, thereby lowering cost to meet market demands. Here, a high-performing halophilic strain was isolated with over 38% glucose-to-PHA conversion rate and broad non-grain substrate utilization capability. Multidimensional tools, including algorithm-guided high-expression neutral integration site (HENIS) screening toolkit designated 'SiteSeek', stop codon (TAA)-dependent enhancement of gene expression and recombinase-mediated large-fragment (> 9 kb) genomic integration, were then developed to enable precise, efficient and interference-free genomic integrative expression. Using these tools, various chromosomally engineered strains were rapidly constructed to achieve high-level production of poly-3-hydroxybutyrate (PHB, 151 g $L^{-1}$) and poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P34HB, 139 g $L^{-1}$) under high cell-density fermentation in a 5-L bioreactor. Scalability was demonstrated at 2-$m^3$ and 20-$m^3$ industry-scale fermentations, yielding up to 134 g $L^{-1}$ PHB and 127 g $L^{-1}$ P34HB. Building on the proven robustness, a two-stage continuous fermentation (TCF) process was developed using a twin-bioreactor system at 5-L and 20-$m^3$ scales, where stable and sustained PHA production lasted over 260 h and 160 h, respectively. Techno-economic analysis revealed a substantial cost-reduction space of 48% compared with conventional fed-batch process.

**Key words:** High-expression neutral integration site; Genomic integrative expression; Two-stage continuous fermentation; Polyhydroxyalkanoate (PHA); Industrial biomanufacturing

**Representative publication:**

1. Liu P., Ye J.-W.*, Tailoring Precise Genomic Integrative Expression Toward Rational Strain Development for Scalable High-titer Production of PHA, under submission.

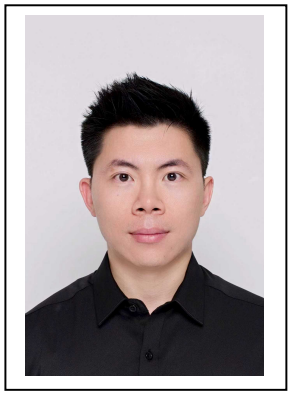
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## Biography



Jian-Wen Ye, a Professor in SCUT, dedicates to the studies focusing on the microbial metabolic engineering of diverse chassis, including *Halomonas*, *Pseudomonas*, *E. coli*, probiotic (EcN 1917), etc., for various bioproduction synthesis (PHAs, recombinant humanized proteins, value-added compounds like violacein, indigo, 1,3-PDO and so on) from lab to industry scales and precise targeted therapeutic system construction for severe IBD and skin wound.

# Drafting a guidebook on transcription factor-ome in *Yarrowia lipolytica*, and new studies beyond

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## Abstract

Transcription factors (TFs) modulate nature-evolved regulomes, frequently acting in the "backstage" of the cell's robustness. For this, they are useful in engineering complex industrially relevant traits. Then, with a limited number of genetic interventions, targeted and comprehensive modification can be executed.

In our recent project, we systematically tested over 100 TFs encoded in the *Yarrowia lipolytica* genome for their role in stress resistance and recombinant protein (rProt) production. Knowing the limitations of high-throughput *Y. lipolytica* culturing, we first developed a reliable cultivation protocol. We addressed the buffering system, medium composition, culturing volume and vessel geometry, limited the "plate-edge effects" and the coefficient of variance. The high-throughput protocol was validated as representative of bioreactor cultures.

Using this system, we run high-throughput TF-overexpression (OE) screens of *Y. lipolytica* strains across dozens of conditions. We characterized their growth and rProt synthesis, and determined the TF's responsiveness to specific environmental stimuli. We identified TFs that contribute to the desired phenotypes, like e.g., Klf1,Hsf1,Hoy1,Msn4,Dep1,Azf1, and Yas1. These were later analyzed using reverse engineering strategy (OE vs. KO) under a broad range of conditions (i.a. different rProts co-overexpressed, and exposure to toxic chemicals). Transcriptomes of the TFs driving interesting phenotypes were characterized.

Our current DICP-collaborative project focuses on yeast metabolic engineering for efficient terpenoid production.

**Key words:** YaliFunTome: https://sparrow.up.poznan.pl/tsdatabase/; *Yarrowia lipolytica*; global metabolic engineering; stress resistance; recombinant protein

**Representative publication:**

1. Gorczyca, M., Białas, W., Nicaud, J.-M., & Celińska, E. (2024). 'Mother(Nature) knows best' – hijacking nature-designed transcriptional programs for enhancing stress resistance and protein production in *Yarrowia lipolytica*; presentation of YaliFunTome database. *Microbial Cell Factories*, *23*(1), 26. https://doi.org/10.1186/s12934-023-02285-x

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5. Goczyca, M., Kubiak, P., Celińska, E. (2026) Transcriptional reprogramming for recombinant protein production in *Yarrowia lipolytica*: new insight into the role of general stress response and ribosomal-translational-vesicular transportation axis regulation. Under review

## Biography

Prof. dr hab. Ewelina Celińska is affiliated at Poznan University of Life Sciences, where she leads a small research team working in the field of yeast genetic engineering and molecular biotechnology. The team studies the biology of and applies different approaches to turn yeasts, mainly *Yarrowia lipolytica*, into efficient producers of recombinant proteins and other valuable biocompounds.
She is a member of the FEMS Yeast Research Editorial Board and a member of the International Commission on Yeasts.



# Synthetic Biomanufacturing of Amphiphilic Lipopeptide and Its Cross-disciplinary Applications

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## Abstract

Amphiphilic lipopeptides, usually produced by the genus *Bacillus*, are novel products consisting of a hydrophobic fatty acyl chain linked to a hydrophilic, often cyclized, peptide portion. Surfactin is the most popular representative molecule in the lipopeptide family. In the past two decades, we continuously engineered the *Bacillus subtilis* via synthetic biology strategies, such as promoter engineering, precursors metabolic engineering, transportation engineering, to improve its capability to produce higher and higher titer of surfactin. So far, as high as 70+ g/L of surfactin has been accomplished in the scaled-up biomanufacturing. We next highlighted diverse applications of lipopeptide. We found that it is a new-type of biostimulant in agriculture with significant growth promotion effect for diverse plants. Grass-land remediation and saline-alkali soil remediation via lipopeptide-based bio-fertilizer were also promising. Lipopeptide has also been utilized as the new molecule for enhanced oil recovery; and lipopeptide-based cosmetic products are functional and prospective as well. This work was supported by the National Technology Innovation Center for Prataculture Special Fund No. CCPTZX2024GJ05), National Key R&D Program of China (2025YFA0921800), NSFC (No. U24A6011), Key R&D Program of Shandong Province (2026CXGC010709), Beijing MSTC No. 202504841063.

**Key words:** biomanufacturing, lipopeptides, *Bacillus subtilis,* applications

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

1. Liang Y.X., Zheng Y.K., Li J.Y., Du Y., Yu H.M.*. Engineering a non-pigmented *Rhodococcus ruber* strain for enhanced bio-production of acrylamide. Chemical Engineering Journal, 2025, 508: 160950.

2. Jiang R.Z., Yuan S.T., Zhou Y.L., Wei Y.W., Yu H.M.*. Strategies to overcome the challenges of low or no expression of heterologous proteins in *Escherichia coli*. Biotechnology Advances 75 (2024) 108417.

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## Biography



Prof. Huimin YU is a tenured professor in the Department of Chemical Engineering at Tsinghua University. Her research mainly focuses on Advanced Biomanufacturing, Synthetic biology and Bio-nanotechnology. Current efforts particularly emphasize the fundamentals and scale-up applications of *Rhodococcus* whole-cell biocatalysts for valuable chemicals, *Bacillus* cell factories for lipopeptides biomanufacturing, *Corynebacterium* cell factories for polysaccharides, and bio-based metal single atoms for biomedical sensors. These works have harvested more than 80 patents and 160 journal-publications such as Trends in Biotechnology, Biotechnology Advances, Metabolic Engineering, ACS Nano, etc; meanwhile obtained the supports from multiple National Key R&D Programs, NSFC, NTICP and company collaborations, etc. She is also the founder of a new synthetic biology start-up company (Evolyzer).

Prof. YU serves as the associate editor of Biotechnology Notes, the editors of Biochemical Engineering Journal, Synthetic biology, the member of both Asian Synthetic Biology Association and European Federation of Biotechnology. She obtained Awards for Technological Invention (2nd prize, MOE), Natural Science (2nd prize, MOE), Technological Invention (2nd prize, China Petroleum and Chemical Industry Association), etc. She also obtained the honorary titles such as the Leading Talent of Beijing High Innovation Plan, New Century Excellent Talents in University (MOE), Beijing Nova (A) in Science and Technology, etc.

# Identification and Biological Effects of Gut Microbial Metabolites and Engineering Microbiology

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## Abstract

Gut microbiota-derived metabolites profoundly influence host metabolic homeostasis and the progression of metabolic syndromes. This research elucidates two distinct microbial metabolic mechanisms and establishes therapeutic interventions. First, we identified lysine phenylacetylation (Kpaa) as a novel protein post-translational modification driven by gut microbiota-derived phenylacetic acid (PAA). Pathologically elevated PAA and Kpaa directly impair hepatic mitochondrial respiration and exacerbates lipid accumulation. Conversely, the host mitochondrial enzyme SIRT3 effectively alleviates this metabolic impairment by functioning as a specific de-phenylacetylase. In parallel, we characterized a beneficial microbiota-dependent tryptophan metabolite, 5-hydroxyindoleacetic acid (5-HIAA). We demonstrated that 5-HIAA actively protects against high-fat diet-induced obesity and insulin resistance by systematically modulating the hepatic AhR-TSC2-mTORC1 signaling axis to restore metabolic balance. To translate these mechanistic insights into actionable therapies, we leveraged engineering microbiology. By designing engineered probiotic strains, utilizing chassis like *E. coli Nissle* 1917, we enabled the endogenous production of therapeutic metabolites within the host gut. Together, these findings uncover the critical pathophysiological roles of specific gut microbial metabolites and establish a translational synthetic biology framework for precision microbiome-targeted therapeutics against metabolic diseases.

**Key words:** Gut microbial metabolites, Chronic disease, Engineered microbiology, Synthetic biology

**Representative publication:**

1. Wei Du[#], Jun-Yu Xu[#]*, Yufeng Li[#], Mingya Zhang[#], Yidan Huang[#], Jingya Li*, **Bang-Ce Ye***, Minjia Tan*, *et al*. Gut microbiota-derived lysine phenylacetylation impairs mitochondrial function and is alleviated by SIRT3. ***Cell Metabolism*** 2026, 38(9):1864-1880.e8.
2. Wei Du[#], Shanshan Jiang[#], …, Nan Qi*, Ying Zhou*, and **Bang-Ce Ye***. The microbiota-dependent tryptophan metabolite alleviates high-fat diet-induced insulin resistance through the hepatic AhR/TSC2/mTORC1 axis. ***PNAS***, 2024, 121 (35) e2400385121.
3. Zhen-Ping Zou, …, Ying Zhou*, **Bang-Ce Ye***. Biomarker-responsive engineered probiotic diagnoses, records, and ameliorates inflammatory bowel disease in mice. ***Cell Host & Microbe***, 2023, 31, 199-212.
4. Zhen-Ping Zou, Ying Zhou*, **Bang-Ce Ye***, *et al*. Self-Adjusting Engineered Probiotic for Targeted Tumor Colonization and Local Therapeutics Delivery. ***Advanced Science***, 2025, 12, e06486.
5. Wei Du, **Bang-Ce Ye***, Ying Zhou*, *et al*. Metabolic Engineering of Commensal Bacteria for Producing 5-Hydroxyindoleacetic Acid as a Therapeutic Strategy for Colitis. ***ACS Synthetic Biology***, 2026, 15 (4): 1412-1425.

## Biography



Prof. Bang-Ce Ye is a Professor at East China University of Science and Technology, Director of the State Key Laboratory of Bioreactor Engineering. His research achievements have been widely recognized, with over 350 publications in leading journals, including *Cell Metabolism*, *Cell Host & Microbe*, *Nature Communications*, *PNAS*, *JACS*, and *Angewandte Chemie International Edition*. He has served as the principal investigator for numerous major research programs, including Key Projects and Instrumentation Development Projects of the National Natural Science Foundation of China, General Programs, the 863 Program, the National Key Research and Development Program, and major science and technology projects supported by the Ministry of Education.

# Intelligent Multi-Scale Biomanufacturing: Driving Sustainable Isomaltulose Production from Lab to Industrial Scale

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## Abstract

Scaling up synthetic biology solutions from the bench to commercial production remains a persistent challenge, primarily due to the highly complex, non-linear dynamics of biomanufacturing, compounded by data scarcity and poor modelling. This presentation highlights a data-driven biomanufacturing framework designed to bridge the lab-to-industry gap, validated through the industrial-scale synthesis of isomaltulose, a low-GI functional carbohydrate. To overcome carbon catabolite repression (CCR) caused by industrial glucose syrup during sucrose isomerase production, we deployed intelligent high-throughput bioreactor platforms to accelerate design and process modeling. Furthermore, multi-scale kinetic modeling of microbial and enzymatic biosynthesis guided the development of precision feeding strategies, yielding a dramatic boost in enzyme activity. By iteratively optimizing the interplay between bioreactor hydrodynamics and microbial physiology, we enhanced volumetric enzyme activity by 96%. Coupled with improvements in enzyme storage stability and biocatalytic efficiency, overall substrate conversion yield was increased by 6%, which resulted in landmark industrial batches achieving a 58.0% reduction in unit production costs, and culminating in a successful 20,000-ton production line for full-scale commercialization. This closed-loop integration of digital intelligence and scale-up engineering offers a robust, industry-proven blueprint for high-efficiency biomanufacturing.

**Key words:** Biomanufacturing, Isomaltulose, Data-driven, multi-scale scale-up

**Representative publication:**

1. Tang, M. J., Ye, Y. T., Li, Z. Z., Li, M. Z., Chen, P. P., Zuo, Q. L., Li, M., Chen, Z. X., Metformin-Induced Invertase Unfolding: Enzyme Kinetics and Activity Regulation, Journal of Agricultural and Food Chemistry, 2024, 72(32), 17977-17988.

2. Jia, D. X., Sun, C. Y., Jin, Y. T., Liu, Z. Q., Zheng, Y. G., Li, M., et al., Properties of d-allulose 3-epimerase mined from *Novibacillus thermophilus* and its application to synthesis of d-allulose, Enzyme and Microbial Technology, 2021, 148(000): 109816.

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4. Li, M., Yan, D., Wang, Y.N., et al., Recombinant engineering bacteria and application thereof in preparation of mannitol, CN121991873B [P], 2026-08-07.

5. Li, M., Yan, D., Shi, M., et al., A modified mannitol dehydrogenase and use thereof, CN121931070B [P], 2026-07-03.

## Biography



Dr. Mian Li is the Chief Scientist of Zhejiang Huakang Pharmaceutical Company and a Principal Investigator (PI) for the National Key Research and Development Program of the Ministry of Science and Technology (MOST) of China. His research focuses on the advanced biomanufacturing of functional carbohydrates and polyols. As a visionary research leader, he has successfully spearheaded numerous high-impact international and national initiatives, including the MOST National Key R&D Program, the Zhejiang Leading Innovative and Entrepreneurial Team Project, and the Zhejiang Key R&D Program. Dr. Li holds an impressive intellectual property portfolio, featuring 25 granted patents in the United States and Europe, alongside 36 granted Chinese invention patents. His pioneering work has heavily driven technology translation, successfully bridging the gap between laboratory innovation and large-scale industrial applications.

**An Integrated Synthetic Biology Platform for Sustainable Production of Structural Protein Materials from C2 Feedstocks in *Corynebacterium glutamicum***

Nature-inspired protein materials boast superior mechanical and biological properties, but efficient and sustainable production remains a major challenge. For this, we aim to develop *Corynebacterium glutamicum*, a food-grade and endotoxin-free Gram-positive bacterium, as an efficient microbial secretion platform to produce structural proteins for material applications. Leveraging the intrinsic ability of *C. glutamicum* to efficiently utilize C2 feedstocks such as ethanol and acetate, we designed a carbon source-autoinduction system in which heterologous protein expression was driven by glyoxylate cycle-associated transcriptional activation. In parallel, we developed a pH-coupled acetate-feeding fermentation strategy, providing a scalable and sustainable route for protein production from C2 feedstocks. To further enhance the engineering potential of this host, we developed a high-efficiency genetic engineering system in *C. glutamicum*. Systematic dissection of host determinants on DNA transformation and recombination identified key genetic barriers, including multiple defense systems and nucleases, whose inactivation markedly improved genetic manipulability and enabled large-scale library construction and multiplex genome editing, thereby overcoming a major constraint on genome-scale host engineering. On this basis, we proposed a high-throughput and high-resolution genotype-phenotype mapping strategy that integrates CRISPR-based screening with *in situ* deep mutational scanning. This approach establishes a continuous mapping from complex phenotypes to functional genes and further beneficial mutations at single-nucleotide resolution. Using this strategy, we identified key transcription factors and metabolic enzymes, together with nucleotide-level beneficial variants, that significantly strengthened C2-driven transcriptional autoinduction and enabled high-level secretory production of diverse structural proteins, such as collagen, supercharged elastin-like proteins, spidroins, resilin, and squid ring tooth proteins. Furthermore, through chromatography-free purification, the secreted proteins were directly processed into functional materials such as high-strength protein fibers and ultra-strong bio-glues, demonstrating their practical applicability. Overall, by integrating autoinduction system design, fermentation process development and host engineering, we established a high-performance microbial platform for structural protein production from C2 feedstocks, laying a foundation for the commercialization of protein-based materials.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Multiscale Engineering of Terpenoid Biosynthesis in *Saccharomyces cerevisiae*

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## Abstract

Terpenoid natural products comprise a structurally diverse class of isoprenoid-derived compounds with broad applications. Biological manufacturing offers particular advantages for producing stereochemically complex terpenoids that are difficult to obtain through plant extraction or chemical synthesis. However, inefficient heterologous enzymes, poorly controlled metabolic flux, and competition between cell growth and product formation constrain microbial production. Here, we present a multiscale regulation framework for terpenoid biosynthesis, spanning catalytic components, pathway architecture, and cellular resource allocation. At the component level, endogenous enzyme mining, solubility-enhancing elements, rational design, and in vivo directed evolution were applied to improve bottleneck activities. At the pathway level, biosynthetic routes were modularized around key intermediates, competing reactions, and cofactor requirements to rebalance metabolic flux. At the cellular level, dynamic circuits responsive to carbon source, temperature, dissolved oxygen, and copper were developed to decouple growth from production, while subcellular relocalization, hydrophobic-compartment engineering, transporter mining, and secretion optimization alleviated intracellular product stress. These integrated strategies enabled selective biosynthesis and extracellular secretion of retinoids, gram-per-liter-scale tocotrienol production, and efficient lutein biosynthesis in *Saccharomyces cerevisiae*. Collectively, this enzyme-pathway-cell framework provides a generalizable strategy for constructing high-performance terpenoid cell factories and advancing scalable biomanufacturing.

**Key words:** metabolic engineering; protein engineering; dynamic regulation; cell factory; lutein

**Representative publication:**

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5. Zhang Y. J., Hu Q. Y., Yu H. W.*, Ye L. D.*, Enhanced vitamin A production by engineering transporters, ATP and precursor supply, Biodesign Research, 2025, 7(2), 100023.

## Biography



Lidan Ye is an Associate Professor in the College of Chemical and Biological Engineering at Zhejiang University. Her research focuses on metabolic and protein engineering for the development of innovative biocatalysts and microbial cell factories for sustainable biomanufacturing. She has authored or co-authored over 100 peer-reviewed publications in journals including *Nature Communications*, *Metabolic Engineering*, *Biotechnology and Bioengineering*, *ACS Catalysis*, and *ACS Synthetic Biology*, and holds 20 patents. She also serves as an Associate Editor for *World Journal of Microbiology and Biotechnology* and *Engineering in Life Sciences*.

# Molecular recognition-driven biomanufacturing

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## Abstract

[Industrial enzyme catalysis is a key technological pathway for biomanufacturing to support carbon neutrality and the development of new productive forces. Effective enzymatic catalysis requires a delicate balance: enzymes must maintain conformational stability to resist environmental perturbations while preserving the dynamic flexibility necessary for catalytic activity. However, under realistic industrial conditions and complex feedstock systems, conformational stability and catalytic dynamics are difficult to coordinate and often exhibit a trade-off. Inspired by the cooperative regulation of "confined microenvironments-molecular chaperone recognition" in living cells, this study reconstructs biocatalytic systems from a chemical engineering perspective. A dynamic confinement-molecular recognition synergistic regulation mechanism is proposed and elucidated, together with a microenvironment-driven strategy for enzyme conformational regulation described as "constrained but not frozen". Based on this concept, a microenvironment-active site cooperatively driven biomimetic enzymatic catalytic system is established, enabling conformational remodeling and functional refolding of deactivated enzymes while simultaneously enhancing catalytic activity and stability. By eliminating dependence on natural protein scaffolds, the system maintains enzyme-level catalytic efficiency while achieving stable operation under harsh industrial conditions. It has been successfully applied to biocatalysis, separation and purification, and the design of abiotic-bio hybrid systems in biomanufacturing.]

**Key words:** [Biomanufacturing; Biocatalysis; Dynamic confinement; Molecular recognition]

**Representative publication:**

1. Li Y., Yin D., Lee, S. Y.*, Lv, Y. Q.* Engineered polymer nanoparticles as artificial chaperones facilitating the selective refolding of denatured enzymes, *P. Natl. Acad. Sci. USA.*, **2024**, *121*, e2403049121.

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4. Han L., Li Y., Wang X. Y., Yao Z. B., Chen Z. M., Li J. P., Tan T. W., Lee S. Y.*, Lv Y. Q.* Spatially decoupled electro-biosystem for efficient $CO_2$-to-chemicals conversion via tandem catalysis *Adv. Energy Mater.* **2025**, *15*, e03056.

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## Biography



[Prof. Dr. Yongqin Lv is the Chief Scientist of the National Key R&D Program of China and a recipient of the National Science Fund for Excellent Young Scholars. Her research is dedicated to molecular recognition-driven biomanufacturing, chemo-bio coupled catalytic carbon fixation, and biomimetic antibody engineering. Prof. Lv has published more than 80 peer-reviewed papers in SCI-indexed journals, such as PNAS, JACS, Angew. Chem. Int. Ed., and Matter, and holds 14 invention patents. She has been recognized with multiple national honors, including Distinguished Global Young Woman Scientist, the Hou Debang Chemical Science and Technology Award (Youth Award), the Second Prize of the Ministry of Education Natural Science Award, and the First Prize of the China Petroleum and Chemical Industry Federation Technical Invention Award. She has served as the principal investigator for more than ten national research projects. In addition, she is an active member of several professional societies, including the Subcommittee on One-Carbon Biotechnology of the Chinese Society of Biotechnology, the Youth Working Committee and Women Scientists Committee of the Chemical Industry and Engineering Society of China, and the Subcommittee on Microbial Metabolism and Biomanufacturing of the Chinese Society for Microbiology. Prof. Lv also serves as an associate Editor of BioDesign Research, young editorial board member of Engineering, and editorial board members of Synthetic and Systems Biotechnology, and Microchimica Acta.]

# Adenylation Domain Engineering Enables Biosynthesis of an Improved Polymyxin Antibiotic

Hajo Kries

Polymyxins are potent nonribosomal peptide antibiotics, yet their clinical use is limited by a narrow therapeutic window and nephro-/neurotoxicity. Conventional efforts to improve these agents rely on the incorporation of nonnatural building blocks that cannot be accessed biosynthetically and therefore must be introduced by total synthesis. Here we report a biosynthetic route to a next-generation polymyxin that circumvents this limitation. By engineering the substrate-binding pocket of a nonribosomal adenylation (A-)domain, we created a mutationally expanded pocket that selectively activates the fatty amino acid L-2-aminodecanoic acid (L-Ada). Incorporation of this engineered A-domain into the native polymyxin biosynthesis enabled production of Ada-polymyxin with high potential for lowered toxicity directly in *Paenibacillus polymyxa* fermentation. This work demonstrates that rational redesign of nonribosomal A-domains can redirect natural product biosynthesis toward chemically diverse, therapeutically superior antibiotics, opening new avenues for combating drug-resistant bacteria.

# Deracemization by designed photoenzymes

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## Abstract

The creation of enzymes with abiological abilities offers exciting opportunities to access new-to-nature biocatalysis beyond what is found in nature. Here, we repurpose a novel protein scaffold, CTB10, as an artificial photoenzyme through genetic code expansion. This system enables catalytic deracemization of cyclopropane and structurally diverse allenes, processes that remain inaccessible to traditional biocatalysis due to their thermodynamically unfavorable nature. Following structural optimization through directed evolution, a broad substrate scope with high enantioselectivities is achieved. Furthermore, the crystal structure of the CTB10-based photoenzyme–substrate complex clearly demonstrates how the catalytic chiral cavity is sculpted to promote efficient and selective light-enabled deracemization. Therefore, this study unlocks the potential for achieving challenging deracemization through biocatalysis.

**Key words:** Deracemization, designed photoenzyme

**Representative publication:**

1. Kai Fu, Min Li, Zhiwei Deng, Yu Qin, Zhenbo Yuan, Dejing Yin, Chao Wang, Yan Zhang, Yijian Rao*, Unlocking enzymatic deracemization of structurally diverse allenes by designed photoenzymes, Nature Commun, 2026,17 (1), 2082.

2. Min Li, Yan Zhang, Kai Fu, Zhiwei Deng, Zhenbo Yuan, Zhengshan Luo, Yijian Rao*, Light-driven deracemization by a designed photoenzyme, J Am Chem Soc, 2025, 147 (16), 13190–13199.

## Biography



Yijian Rao studied biochemistry at Freie Universität Berlin (Ph.D., 2007–2011) and conducted postdoctoral research at the Max-Planck-Institut für Biochemie (2011–2016). He then became Professor at the School of Biotechnology, Jiangnan University. Since 2026, he has moved to Fujian Agriculture and Forestry University, where he leads the Center for Biomimetic Drug Synthesis. The Rao laboratory mainly focuses on investigating the intimate connection between biocatalysts and chemical reactions by understanding the biosynthetic pathways of natural products, thereby developing novel strategies for organic synthesis and drug biosynthesis. He has published over 100 papers in journals such as Nature Communications, J. Am. Chem. Soc., and Angewandte Chemie.

# From catalytic-motif scaffolding to de novo sequence-specific proteases: computational pipelines for enzyme design

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## Abstract

Enzymes are the workhorses of metabolic engineering, yet the reactions and specificities we can access remain largely limited by what nature provides. Over the past three decades, computational enzyme design has begun to lift this constraint - from grafting idealized active sites into natural scaffolds, which gave functional but sluggish catalysts requiring extensive screening, to deep-learning pipelines that build tailor-made proteins around a defined catalytic motif. I will briefly trace this trajectory using reported catalytic efficiencies to quantify this progress.

This progress is a consequence of sophisticated design pipelines. Using the recently published Riff-Diff pipeline as an example, I will show how backbone generation by diffusion, sequence design, and in silico validation by structure prediction and energetic scoring are combined so that only a handful of designs need experimental testing to obtain efficient de novo enzymes.

Finally, I will present our unpublished application of such pipelines to a target of broad biotechnological interest: the de novo design of sequence-specific proteases, outlining our design and validation strategy.

**Key words:** computational enzyme design, de-novo enzymes, biophysical characterization

**Representative publication:**

1. Braun M., Tripp A., Chakatok M., Kaltenbrunner S., Fischer C., Stoll D., Bijelic A., Elaily W., Totaro M.G., Moser M., Hoch S.Y., Lechner H., Rossi F., Aleotti M., Hall M., Oberdorfer G., Computational enzyme design by catalytic motif scaffolding. *Nature. 2026*, 649, 237245 (2026).

## Biography



Horst Lechner leads a BioTechMed Graz Young Researcher Group at Graz University of Technology, where he both develops computational pipelines for de novo enzyme design and validates the resulting proteins experimentally. He studied chemistry and biochemistry at the University of Graz and completed his PhD there in the group of Wolfgang Kroutil. During his doctorate he spent time in the laboratory of David Baker (University of Washington). As an Erwin Schrödinger Fellow of the Austrian Science Fund (FWF), he carried out postdoctoral research with Birte Höcker (University of Bayreuth) on the design of artificial enzymes, before returning to Austria to work with Wolfgang Kroutil (University of Graz) and subsequently joining Gustav Oberdorfer's group at TU Graz.

His research interests bridge deep-learning–based protein design and biochemistry, focusing on engineering enzyme substrate specificity as well as. as well as enzyme stabilization and recombinant expression.

# Decouple Gene Transcription and Translation Regulation in Bacteria

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## Abstract

Enabling technologies for precise and efficient genetic manipulation are essential to realize the vast potential of synthetic biology. At MySynBio Lab, our efforts are dedicated to building next-generation tools specifically designed for industrially relevant microbes. To tackle the limitations of CRISPR-Cas-based genome editing, such as large protein size and off-target effects, we developed STAGE (Streptomyces-compatible TnpB-Assisted Genome Editing), a compact, high-fidelity, non-CRISPR-Cas genome engineering toolkit for Streptomyces. STAGE enables flexible gene deletion, large-fragment DNA excision, and multiplexed base editing with no detectable off-target activity. For multi-layered gene regulation, we systematically engineered a Cas13d-based RNA-targeting system named SONAR (Synthetic Orthogonal Nucleic acid Activation and Repression) that separates RNA cleavage from translational activation and repression, enabling multi-layered and reversible gene regulation. This allows us to achieve precise translational activation, gene-specific translation repression, and targeted transcript degradation within a single, highly modular platform. In addition, we developed a blue light–inducible sigma factor assembly system, which enables robust and reversible gene activation (>500-fold induction) without the need for chemical inducers, offering a new level of control and biosafety for engineered microbes. Taken together, these enabling technologies significantly expand the synthetic biology toolkit and pave the way for more programmable, reliable, and application-oriented microbial engineering.

**Key words:** Synthetic biology; gene editing; gene regulation

**Representative publication:**

1. Tong, S., Qin, Y., Sun, Y. Gao, Y., Song, M., Fang, T., **Tong, Y.***, Programmable, multiplexed and orthogonal gene control in bacteria with attenuated Cas13d systems. ***Nat. Biotechnol.*** https://doi.org/10.1038/s41587-026-03160-x (2026).
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## Biography



Prof. Yaojun Tong is a recipient of the National Natural Science Foundation of China (NSFC) Distinguished Young Scholars (Overseas) award and a selectee of the Shanghai High-Level Overseas Talent Introduction Program. In 2025, he received the Outstanding Young Scientist Award from the Shanghai Society for Bioengineering. His research centers on microbial synthetic biology, with a particular focus on developing enabling technologies of gene editing and gene regulation for bacteria. He has published 50+ peer-reviewed papers in leading journals including *Nature Biotechnology*, *PNAS*, *Nature Communications*, and *Nature Protocols*, with 4,700+ citations and an H-index of 30. In addition to his research, Prof. Tong serves as Associate Editor of Synthetic and Systems Biotechnology. He is also a member of the Early Career Committee of the International Metabolic Engineering Society. He serves as Chair of the Microbial Metabolism and Biomanufacturing Committee of the Chinese Society for Microbiology; the Standardization Working Committee of the Genetics Society of China, and the Young Professionals Working Committee of the Chinese Society of Biotechnology, among other professional service roles. Prof. Tong has served as an expert reviewer for the Dutch Research Council (NWO) and the US Chan Zuckerberg Initiative (CZI).

# From Nitrogen Fixation to Carbon Valorization: Engineering Nitrogenases for New Chemistry

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## Abstract

Nitrogenases are best known for the reduction of dinitrogen ($N_2$) to ammonia, our recent work has revealed that these metalloenzymes also catalyze the reduction of carbon dioxide ($CO_2$) to formate, carbon monoxide, and hydrocarbons. Among the three nitrogenase isozymes, the iron (Fe)-nitrogenase is best in converting $CO_2$ directly to methane ($CH_4$), making it an attractive but mechanistically poorly understood biocatalyst for $CO_2$ valorization. We performed the first directed evolution campaign on the Fe-nitrogenase to enhance its $CH_4$-forming activity and expand its hydrocarbon product spectrum. Using an *in vivo* $CH_4$ screen in *Rhodobacter capsulatus*, three rounds of site-saturation mutagenesis yielded variant V3 with an eightfold increase in $CH_4$ formation. In vitro, V3 forms $CH_4$ and CO at 2.6-fold and 6-fold higher rates than wild type, abolishes the competing formate-forming activity, and newly produces ethane. Cryo-EM structural analysis of V3 revealed substitutions that create space for ordered water molecules near the active-site cofactor, suggesting a mechanism by which altered proton and hydride transfer redirect electron flux away from formate toward $H_2$, CO, and $CH_4$, including C–C-coupled hydrocarbons.

**Key words:** $CO_2$ conversion, hydrocarbon formation, nitrogenase, metalloenzyme, enzyme engineering

**Representative publication:**

1. Oehlmann NN, Schmidt FV, Ching J, Prinz S, Zarzycki J, Claus P, Kahnt J, Erb TJ, Rebelein JG*, Directed evolution of the Fe-nitrogenase for $CO_2$ reduction to hydrocarbons. ***BioRxiv,*** https://doi.org/10.64898/2026.07.08.737278

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## Biography



Prof. Dr. Johannes Rebelein is Professor of Sustainable Biotechnology at Heinrich Heine University Düsseldorf and Director of the Institute for Molecular Enzyme Technology (IMET) at Forschungszentrum Jülich. His research focuses on metalloenzyme biochemistry and catalysis, particularly nitrogenase and nitrogenase-like enzymes. Combining biochemical, structural, synthetic microbiology and enzyme-engineering approaches, his group investigates how complex metallocofactors convert small molecules, including nitrogen and carbon dioxide, and how these principles can be harnessed to develop sustainable biotechnological solutions for greenhouse-gas valorization and artificial metalloenzyme design.

Before his appointment in Düsseldorf/Jülich, Rebelein led an Emmy Noether Research Group at the Max Planck Institute for Terrestrial Microbiology in Marburg and was a Junior Group Leader at the LOEWE Center for Synthetic Microbiology at Philipps-University Marburg. He completed postdoctoral training with Prof. Thomas Ward at the University of Basel and earned his Ph.D. in Biological Sciences at the University of California, Irvine, under Prof. Markus W. Ribbe. He holds B.Sc. and M.Sc. degrees in Biotechnology, with honors, from TU Braunschweig. Prof. Rebelein's contributions to the field have been recognized with several prestigious honors, including the European Molecular Biology Organization (EMBO) Young Investigator Award and the VAAM (Association for General and Applied Microbiology) Research Award.

# Artificial Intelligence-Driven Enzyme Sequence Optimization and Its Applications

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## Abstract

The amino acid sequence of an enzyme is the key determinant of its proper folding and catalytic function. In general, the structural conservation of enzymes in nature is greater than the conservation of their sequences. Consequently, enzyme sequences often possess considerable flexibility, allowing substantial redesign to improve their practical applicability. Recent advances in artificial intelligence tools, represented by RFdiffusion, ProteinMPNN, and Protein Hallucination, have provided powerful new approaches for large-scale sequence engineering. In this presentation, we highlight examples in which these AI tools were successfully applied to design smaller PET hydrolases and glycosyltransferases. These studies offer new paradigms for research in biocatalysis and metabolic engineering. Finally, we introduce our integrated enzyme sequence design platform, ASCEND (Autonomous Sequence Customization for ENzyme Design, ASCEND), which provides a customizable, one-stop service for enzyme sequence design. Collectively, these advances point toward an important future direction in enzyme engineering.

**Key words:** Artificial intelligence; Sequence optimization; Heterogenous expression; Enzyme design

**Representative publication:**

1. Zhang, S., Han, Y., Ding, Y. et al. Sequence redesign of glycosyltransferases for enhanced heterologous expression and glycosylation efficiency in Escherichia coli. Nature Communications, 2026, 17, 7663.

2. Ding Y., Zhang S., Hess H., Kong X., Zhang Y.* Replicating PET hydrolytic activity by positioning active sites with smaller synthetic protein scaffolds. *Advanced Science*, 2025, 12, 2500859.

3. Ding N., Jiang Y., Lee S. et al. Enzyme mminiaturization: rrevolutionizing ffuture bbiocatalysts. Biotechnology Advances. 2025, 82, 108598.

## Biography



Dr. Yifei Zhang is a Professor and Doctoral Supervisor at the Beijing Advanced Innovation Center for Soft Matter Science and Engineering Beijing University of Chemical Technology. He received his B.S. (2010) and Ph.D. (2015) in Chemical Engineering from Tsinghua University, China. He then pursued postdoctoral research at Columbia University in the city of New York (2015- 2020). His current research interests include enzyme engineering, *de novo* enzyme design, green biomanufacturing, and synthetic biology. He has published extensively in leading journals including Nature Catalysis, Nature Communications, Nature Reviews Chemistry, etc. He was recognized as the finalist for the 2020 Blavatnik Regional Awards for Young Scientists in the US and was listed among the Global Top 2% Scientists from 2022 to 2025.

# Metabolic engineering of *Escherichia coli* for methanol utilization

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## Abstract

Methanol, derivable from diverse feedstocks including natural gas, biogas, coal gasification, biomass conversion, and $CO_2$ reduction, is a promising C1 feedstock. Enhancing its utilization efficiency is key to China's "dual carbon" strategy, yet synthetic methylotrophs like *Escherichia coli* face bottlenecks due to formaldehyde cytotoxicity. This study systematically addresses the issue via cellular repair and metabolic network enhancement. For damage repair: (1) introducing phosphatidylcholine synthesis to repair membrane damage (23-fold improvement); (2) employing an exogenous ROS scavenging system (30-fold); (3) co-expressing DNA/protein repair proteins (50-fold); (4) introducing SpWss1 protease from *Schizosaccharomyces* pombe to repair DPC damage, boosting assimilation by 10-fold. For metabolic enhancement, (5) overexpressing endogenous transhydrogenases SthA and PntAB to increase NADPH and ATP supply, thereby driving methanol metabolic flux. Collectively, these strategies significantly enhance methanol utilization in *E. coli*. Our study not only deepens understanding of formaldehyde toxicity mechanisms but also offers new strategies for efficient C1 bioconversion and green sustainable manufacturing.

**Key words:** Methanol utilization; *E. coli*; formaldehyde toxicity; DPC; ROS

**Representative publication:**

1. Liu H, Zhu C, Chen Y, Tan Z*. Enhanced methanol metabolism via reinforced cellular energy and reducing power supply for sustainable carbon conversion. Metabolic Engineering. 2026 Feb 23.
2. Chen Y, Zhu C, Sun W, Gonzalez R, Tan Z*. Harnessing metalloprotease Wss1 to enhance methanol utilization. ACS Synthetic Biology. 2026 Feb 3;15(2):621-30.
3. Liu H, Chen Y, Li J, Zhu C, Peng J, Gonzalez R, Bai Y, Tan Z*. Scavenging intracellular reactive oxygen species to boost methanol assimilation. Chemical Engineering Journal. 2025 Jul 15;516:164002.
4. Zhu C, Chen Y, Sun W, Li J, Liu H, Peng J, Bai Y, Gonzalez R, Tan Z*. Repair of DNA and protein damages caused by formaldehyde improves methanol assimilation. Fundamental Research. 2025 May 17.
5. Li M, Sun W, Wang X, Chen K, Feng Y, Tan Z*. A eukaryote-featured membrane phospholipid enhances bacterial formaldehyde tolerance and assimilation of one-carbon feedstocks. ACS Synthetic Biology. 2024 Nov 20;13(12):4074-84.

## Biography



[Tan Zaigao is a professor and doctoral supervisor at the School of Life Sciences and Biotechnology, Shanghai Jiao Tong University. He is a recipient of the National Excellent Youth Science Fund and a member of the expert panel for the compilation of the National Key R&D Program guidelines in Synthetic Biology. His research has long been dedicated to the artificial construction of microbial cell factories and metabolic engineering. He has been selected for the National Science and Technology Award Review Expert Database and serves as a review expert for the National Natural Science Foundation of China's Excellent Youth and Youth programs, as well as an expert for the Ministry of Education's discipline evaluation. As the corresponding author, he has published numerous high-level papers in prestigious international journals including *Nature Catalysis*, *Biotechnology Advances*, *Fundamental Research*, *Chemical Engineering Journal*, *Biotechnology for Biofuels*, *Synthetic and Systems Biotechnology*, and *ACS Synthetic Biology*. He has led multiple national-level research projects, including the National Natural Science Foundation's Excellent Youth Science Fund and General Program. A number of his patented technologies have been granted in countries and regions including the United States, China, the European Union, Japan, and Canada.]

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Growth-coupled selection drives evolution of Rubisco promiscuity towards formate-based glycolate production

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## Abstract

Rubisco is Earth's most abundant enzyme and catalyzes $CO_2$ fixation through the Calvin cycle. Beyond carboxylation, Rubisco catalyzes a promiscuous, “wasteful” oxygenase activity producing 2-phosphoglycolate (2PG), a precursor to glycolate. This molecule is a top platform chemical for polymers currently synthesized via energy-intensive, petroleum-dependent routes. Here, using growth-coupled selection as tool, we evolved Rubisco towards enhanced oxygenase specificity and employed it for demonstrating glycolate biosynthesis from the one-carbon (C1) feedstock formate. First, we engineered *E. coli* strains auxotrophic for glycolate sensing. Second, we coupled growth of such sensor strain directly to Rubisco's oxygenase activity and downstream 2PG metabolism. This created a link between flux through the target reaction and microbial fitness. By performing adaptive laboratory evolution, we increased oxygenation-dependent flux in the sensor strain, identifying responsible mutations in Rubisco and phosphoribulokinase which impacted the enzyme’s specificity. Third, we introduced the evolved module into the bacterium *Cupriavidus necator*, a natural autotroph and formatotroph. Using a newly developed CRISPR-Cas system, we deleted glycolate dehydrogenase, leading to glycolate secretion in *C. necator*. In controlled bioreactors, the mutant strain harboring the evolved Rubisco produced glycolate at g/L titers from formate as carbon and energy source. By controlling $CO_2$ pressures in the reactior, we could control growth from production phases. Techno-economic and life-cycle analyses highlighted key constraints in the process. This work shows how growth-coupled selection can be used to evolve enzyme promiscuity toward industrial deployment for biomanufacturing.

**Key words:** Rubisco, growth-coupled selection, enzyme promiscuity, one-carbon, biomanufacturing

**Representative publication:**

1. Orsi, E., Claassens, N. J., Nikel, P. I., & Lindner, S. N. (2021). Growth-coupled selection of synthetic modules to accelerate cell factory development. *Nature Communications, 12*(1), 5295.

2. Collas, F., Dronsella, B. B., Kubis, A., Schann, K., Binder, S., Arto, N., ... & Orsi, E. (2023). Engineering the biological conversion of formate into crotonate in *Cupriavidus necator*. *Metabolic Engineering, 79*, 49-65.

3. Orsi, E., Nikel, P. I., Nielsen, L. K., & Donati, S. (2023). Synergistic investigation of natural and synthetic C1-trophic microorganisms to foster a circular carbon economy. *Nature Communications*, *14*(1), 6673.

4. Orsi, E.*, Schulz-Mirbach, H.*, Cotton, C. A., Satanowski, A., Petri, H. M., Arnold, S. L., ... & Nikel, P. I. (2025). Computation-aided designs enable developing auxotrophic metabolic sensors for wide-range glyoxylate and glycolate detection. *Nature Communications*, *16*(1), 2168.

5. Della Valle, S.*, Orsi, E.*, Creutzburg, S. C., Jansen, L. F., Pentari, E. N., Beisel, C. L., ... & Patinios, C. (2025). Streamlined and efficient genome editing in Cupriavidus necator H16 using an optimised SIBR-Cas system. *Trends in Biotechnology*, *43*(6), 1470-1491.

## Biography



Enrico Orsi is Senior Researcher and Group Leader at BRIGHT (Technical University of Denmark). He obtained his BSc in Biotechnology at the University of Bologna (Italy), and then pursued a MSc in Biotechnology, followed by a PhD in Metabolic Engineering at Wageningen University (The Netherlands), supervised by Prof. Ruud A. Weusthuis. He performed his first postdoctoral stay at the Max Planck Institute for Molecular Plant Physiology under the supervision of Dr. Arren Bar-Even. Here, he got familiar with growth-coupling and one-carbon metabolism, topics that he further developed with his second postdoctoral stay at the Systems Environmental Microbiology (SEM) group of Prof. Pablo I. Nikel (supported by the Marie Skłodowska-Curie Actions program from the European Union). The presentation in this conference is a summary of his postdoctoral period at SEM. Since 2026, Dr. Orsi leads the newly established Synthetic Microbial Biomanufacturing (SMB) group at BRIGHT, generously supported by the Emerging Investigator Grant from The Novo Nordisk Foundation. The SMB group is further exploring the concepts of domestication of C1-trophic microorganisms using growth-coupling for the development of new bioprocesses focused on emerging C1 and C2 feedstocks.

# Oral Presentation

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# A cross-feeding *Yarrowia lipolytica-Pseudomonas putida* consortium for efficient valorization of lignocellulosic oligosaccharides

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## Abstract

Lignocellulosic biomass is an abundant renewable resource for the sustainable production of fuels, chemicals, and materials. However, efficient microbial valorization of lignocellulose remains challenging because it requires the coordinated depolymerization of cellulose- and hemicellulose-derived oligosaccharides together with the assimilation of the resulting mixed sugars. Here, we developed a synthetic microbial consortium that distributes these functions between two metabolically specialized microorganisms linked through an obligate cross-feeding interaction. By assigning this reciprocal substrate processing and utilization roles to each partner, the consortium avoids direct competition for carbon sources and enables simultaneous glucose and xylose conversion through metabolic division of labour.

The consortium combines an engineered *Yarrowia lipolytica* strain secreting the fungal β-xylosidase BxTw1 to hydrolyse xylooligosaccharides while selectively consuming glucose, with a metabolically engineered *Pseudomonas putida* EM42 strain incapable of glucose utilization but equipped with a heterologous xylose assimilation pathway and a β-glucosidase for cellobiose hydrolysis and glucose release. This reciprocal exchange establishes an obligate metabolic dependency in which each microorganism relies on the other for access to its preferred carbon source. As a result, both populations remain metabolically active while minimizing substrate competition and overcoming the sequential sugar consumption patterns typically observed in monocultures.

The engineered consortium efficiently co-utilizes glucose and xylose generated *in situ* from lignocellulose-derived oligosaccharides, demonstrating the potential of cooperative microbial systems for enhanced carbohydrate conversion. Beyond substrate utilization, metabolic specialization enables parallel biosynthesis of multiple biobased products, including microbial lipids by *Y. lipolytica* and medium-chain-length polyhydroxyalkanoates (mcl-PHAs) by *P. putida*. Overall, this work establishes obligate microbial cooperation as a versatile strategy for integrating lignocellulose deconstruction, simultaneous mixed-sugar co-assimilation, and modular bioproduction within a single synthetic microbial platform.

**Key words:** *Yarrowia*, *Pseudomonas*, lignocellulose, consortia, bioproduction



# Biological upcycling of methane into multicarbon chemicals by metabolically engineered methanotrophs

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## Abstract

The growing demand for bio-based products and carbon emission constraints are driving biomanufacturing feedstock towards non-edible, low-cost, and sustainable alternatives. High abundant, renewable, and cost-effective, methane is a promising biomanufacturing feedstock. Biological fixation and transformation of dispersed methane not only reduce greenhouse gas emissions but also create new pathways for material synthesis. Methanotrophic bacteria utilize methane as their sole carbon and energy source, serving as a promising platform for developing methane-based biomanufacturing. However, limited genetic tools and conversion routes hinder methanotroph cell factory construction. To enable direct upcycling of methane into long-carbon chain products, we first developed a synthetic biology toolkit of non-model methanotrophs as chassis cells, optimizing the electroporation method and establishing cytosine base editing and sRNA-based gene knockdown systems. By integrating these tools with metabolic engineering, we achieved the biosynthesis of long-chain compounds, including triacylglycerol, taxadiene, etc., from methane. Furthermore, using an electrochemical-biocatalytic coupling system, we realized the production of ectoine and poly-γ-glutamic acid from $CO_2$-derived methane as the expanded feedstocks. Our work provides novel insights into the high-value utilization of greenhouse gas valorization, and innovative low-carbon biosynthetic routes for chemical production.

**Key words:** Methane, methanotrophic bacteria, metabolic engineering, biomanufacturing

**Representative publication:**

1. Zhang, X., Li, A., Huang, X., **Guo, S**., Zhang, C., Gonzalez, R., Fei, Q*. Metabolic engineering of methanotrophic bacteria for de novo production of taxadiene from methane. ACS Synthtic Biology, 2025,14(8): 3024-3036. ).

2. Wang, W#., Hou, Q#., **Guo, S**., Zhang, C., Gao, Z., Ma, Y., Fei, Q*. Bioupcycling methane into triacylglycerol for the production of sustainable aviation fuel by methanotrophic bacteria. Chemical Engineering Journal, 2024, 501:157639.

3. **Guo, S#**., Li, C#., Su, Y., Huang, X., Zhang, C., Dai, Y., Ji, Y., Fu, R., Zheng, T., Fei Q*., Fan, D*., Xia C*. Scalable electro-biosynthesis of ectoine from greenhouse gases. Angewandte Chemie International Edition, 2025, 64(3): e202415445.

## Biography



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Dr. Shuqi Guo is currently an associate professor at the School of Chemical Engineering and Technology of Xi'an Jiaotong University, China. He received his Ph.D. from Shanghai Jiao Tong University in 2020. His research focuses on the bioconversion of C1 greenhouse gases and metabolic engineering of methanotrophic bacteria. He currently serves as a member of the Xi'an Key Laboratory of C1 Compound Bioconversion Technology and the C1 Biotechnology Committee of the Chinese Society of Biotechnology. Dr. Guo has published 30+ peer-reviewed papers in leading journals, including Angew. Chem. Int. Ed., Green Chem., and Bioresour. Technol. His research has been supported by competitive national funding, including grants from the National Natural Science Foundation of China (NSFC) and the National Key R&D Program of the Ministry of Science and Technology of China.

# Bioproduction Valuable Acetyl-CoA Derivatives with *Rhodotorula toruloides*

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## Abstract

Microbial manufacture offers a sustainable alternative to traditional chemical production. The oleaginous red yeast *Rhodotorula toruloides*, characterized by fast growing, robustness and broad substrate spectrum, have been explored for producing value-added products such as lipids, carotenoids, enzymes, and sugar alcohols.

Metabolic engineering of *R. toruloides* is greatly facilitated by continuously improved omic information, genetic enabling toolbox, and understanding in its unique metabolic features. Previously, we have managed to rewire the red yeast toward the specific production of monoterpenes (limonene, linalool and carvone), sesquiterpenes (patchoul), tetraterpene (lycopene and β-carotene) based on its mevalonate pathway. Currently, we engineered *R. toruloides* toward efficient production of acetyl-CoA derived platform chemicals like 3-hydroxypropionic acid (3-HP) and triacetic acid lactone (TAL). Collectively, our work demonstrate the prospective future in deploying the red yeast as cell factory for valuable acetyl-CoA derivatives.

**Key words:** [Metabolic engineering, Red yeast, Cell factory, Acetyl-CoA derivatives]

**Representative publication:**

1. Gao Q.D., Dong Y.Q., Huang Y., Liu S.S., Zheng X.C., Ma Y.N., Qi Q.S., Wang X., Zhao Z.K., **Yang X.B***. Dual-regulation in peroxisome and cytoplasm toward efficient limonene biosynthesis with *Rhodotorula toruloides*, ACS Synth. Biol., 2024, 13: 2545-2554.

2. Zheng X.C., Li Y.J., Che C., Gao Q.D., Wang X., Ledesma-Amaro R., Luo Z.W., Lv X., Wang Y.N., **Yang X.B***. Metabolic engineering of *Rhodotorula toruloides* for the production of linalool, J. Agric. Food Chem., 2025, 73(9): 5395-5404.

3. Zheng X.C., Liu Y.J., Li Y.J., Wang Y.N., **Yang X.B***. *De novo* biosynthesis of 2-phenylethanol by metabolic engineering the oleaginous yeast *Rhodotorula toruloides*, J. Agric. Food Chem., 2024, 72(48): 26777-26785.

4. Zheng X.C., Li Y.J., Liu Z.H., Sun P., Dong Y.Q., Wang X., Wang Y.N., **Yang X.B***. Biosynthesis of patchoulol via metabolic engineering the oleaginous red yeast *Rhodotorula toruloides*. Syn. Syst. Biotechno., 2026,12: 293-300.

5. Dong Y.Q., Cui S.M., Du B., Zhang X.F., Hou J., Lin X.P., Qin Y., Wang X., **Yang X.B***. *De novo* production of (-)-limonene, (-)-carveol and (-)-carvone via engineering *Rhodotorula toruloides*, Syn. Syst. Biotechno., 2026,14: 536-545.

## Biography



Xiaobing Yang obtained his PhD in 2015 from Dalian University of Technology & Dalian Institute of Chemical Physics, CAS. **Interests:** Construction of efficient cell factories for high-value lipids, terpenoids, phenylpropanoids, etc. with the red oleaginous yeast *Rhodotorula toruloides* as the platform via synthetic biology and metabolic engineering. **Aim:** Providing theoretical and technical support for the high-value utilization of agricultural lignocellulosic biomass, and new options for elevating the economic efficiency of the A&F industry chain. **Work:** Characterized more than 50 native promoters, 12 mitochondrial targeting signals and 5 peroxisomal targeting signals; Established the $Cu^{2+}$ induced CRISPR-Cas9 system in *R. toruloides*. Managed to rewire the red yeast toward production of terpenes (C10, C15 and C40), aromatic amino acid derivatives (2-phenylethanol) and platform chemicals (3-HP and TAL), respectively. **Papers and Patents**: Co-authored more 40 open-published papers in *J. Agric. Food Chem.*, *ACS Synth. Biol.*, *Syn. Syst. Biotechno.*, *Bioresour Technol*, *Waste Mange.*, *Biotechnol. Adv.*, *Biotechnol. Biofuels*, *Microb. Cell Fact.*, etc. Filed 10 Chinese patents.

Dalian, China 18-22,October 2026
Metabolic Engineering Summit 2026

# Harnessing Microbial Metabolic Repertoire for Biological Upcycling of Wastes: Towards Sustainable Development

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## Abstract

The valorization of waste plastics through synthetic biology represents an emerging paradigm for addressing the global plastic pollution crisis. Centered on polyethylene terephthalate (PET), we identified a *Rhodococcus* strain capable of utilizing PET hydrolysates as the sole carbon source, which we designated *Rhodococcus jostii* PET (RPET). By developing RPET strain-specific synthetic biology toolkits, we engineered an advanced chassis strain that efficiently converts PET-derived carbon into lycopene. For polyolefin plastics such as polyethylene (PE), we first developed a transition metal-free oxidative degradation method to efficiently depolymerize PE into dicarboxylic acids using nitric acid. Then, we leveraged the intrinsic metabolic capability of the non-model bacterium *Acinetobacter* to assimilae dicarboxylic acids. Through integrated multi-scale omics analyses, we systematically elucidated the metabolic and regulatory pathways underlying dicarboxylic acid utilization in *Acinetobacter*. Guided by reverse genetics, we reconstructed a superior chassis strain to achieve high-value bioconversion of PE-derived carbon sources. Furthermore, given the chemical complexity and heterogeneity of depolymerization products from mixed plastic waste streams, conventional single-strain chassis systems are insufficient for efficient conversion. To overcome this limitation, we applied the division-of-labor (DOL) principle in microbial engineering to construct a synthetic multicellular consortium composed of RPET and *Acinetobacter*. This engineered system enables rapid and efficient utilization of mixed plastic depolymerization products to drive biomanufacturing. Collectively, this body of work highlights the substantial potential of plastic-derived carbon-driven biomanufacturing as a transformative alternative to conventional petrochemical refining for chemical production, while simultaneously offering a viable strategy to mitigate plastic pollution.

**Key words:** Plastic bio-upcycling; Microbial consortia; Metabolic engineering; Synthetic biology

**Representative publication:**

1. **Diao J.**, Hu Y., Tian Y., Carr R., Moon T.* Upcycling of poly(ethylene terephthalate) to produce high-value bio-bioproducts. ***Cell Reports***, 42 (1), 111908. (2023)

2. **Diao J.***, Tian Y., Hu Y., Moon T.* Producing multiple chemicals through biological upcycling of waste poly(ethylene terephthalate). ***Trends in Biotechnology***, 43 (3), 620-646 (Research Article, 2025)
3. **Diao J.**, Tian Y., Park S., Cho S. *; Moon T.* Engineering microbial consortia for mixed plastic upcycling. ***Nature Communications***, 17, 637. (2026).

## Biography



Dr. Jinjin Diao joined the Department of Chemical Engineering at Guangdong Technion–Israel Institute of Technology (GTIIT) as an Associate Professor and independent Principal Investigator in 2025. His research interests revolve around the understanding of intracellular metabolic and regulatory mechanisms and the development and leveraging of cutting-edge synthetic biology techniques to harness the microbial metabolic repertoire for biological upcycling of waste streams. Over the past five years, Dr. Diao has served as Principal Investigator on three competitive research grants funded by the U.S. Department of Agriculture (USDA), the U.S. National Science Foundation (NSF), and the U.S. Department of Defense (DoD). He has published 29 peer-reviewed articles in leading journals, including Nature Communications, Trends in Biotechnology (research article), Cell Reports, and Metabolic Engineering, with more than 1,300 citations to date. He holds one granted U.S. invention patent and three granted Chinese invention patents.

# Bioconversion for ursodeoxycholic acid using *β*-cyclodextrin included-chenodeoxycholic acid via immobilized cells of *Xanthomonas maltophilia*

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## Abstract

Ursodeoxycholic acid is a prevalent clinical drug widely used for hepatobiliary diseases. It offers the advantages of good efficacy, high sensitivity, low toxic side effects and multifunctionality. However, its poor water solubility and arduous biopreparation have always been obstacles. This work presents, for the first time, a continuous aerobic-biotransformation system for synthesizing ursodeoxycholic acid. The process utilizes immobilized *Xanthomonas maltophilia* cells with substrate chenodeoxycholic acid/*β*-cyclodextrin inclusion complexes. The designed inclusion complexes possessed favourable stability with solubility improved by 122 times comparing to the pure drug. Repeated batches transformation was carried out and reaction parameters like flow speed, fluid direction and filling volume were optimized. The results showed that *X. maltophilia* cells were successfully immobilized in the microspheres with typical shell-core structure and good abrasion resistance. The optimal reaction conditions were as follows: flow speed of 1.0 mL/min, fluid direction from bottom to top in a reverse orientation, and filling volume set to 1:1. The entire continuous reaction could be recycled in three consecutive batches with no decrease in the conversion of the products. This study provides a new strategy for the preparation of ursodeoxycholic acid via bioconversion, which is valuable to explore the aerobic-based strain for biotransformation.

**Key words:** Chenodeoxycholic acid/*β*-cyclodextrin inclusion complexes; Ursodeoxycholic acid; Bioconversion

**Representative publication:**

1. Yang Z. Y., Liu C. X., Jin Y., Wang J., Ma Y. Y., Zou J., Han Y. L., Guan Y. X., Zhao W. T., Wu Q. X.*, Bioconversion for ursodeoxycholic acid using *β*-cyclodextrin included-chenodeoxycholic acid via immobilized cells of *Xanthomonas maltophilia*, Journal of Biotechnology, 2026, 409, 44-56.

2. Wang# D., Wang# J., Wu Y. C., Liu C. X., Huang Y. Z., Chen Y., Ding Z. F., Guan Y. X., Wu Q. X.*, Amelioration of acute alcoholic liver injury via attenuating oxidative damage and modulating inflammation by means of ursodeoxycholic acid-zein nanoparticles, Journal of Agricultural and Food Chemistry, 2023, 71, 17080-17096.

3. Tang L., Chen Y. H., Wang Q., Wang X. H., Wu Q. X.*, Ding Z. F., Microencapsulation of functional Ovalbumin and Bovine serum albumin with Polylysine-Alginate complexfor sustained protein vehicle's development, Food Chemistry, 2022, 368, 130902.

## Biography



Wu Qingxi, Professor, Doctoral Supervisor, work in School of Life Sciences and Medical Engineering, Hefei, China. Member of the Youth Committee of the Biochemical Engineering Division of the Chinese Society of Chemical Engineering, Review experts for science and technology planning projects in Anhui/Zhejiang/Jiangsu/Guangdong/Jiangxi/Hebei provinces, and Reviewers of domestic and foreign academic journals for more than 20 magazines. Engage in fundamental research on the chemical application of polysaccharide biomass, Head of the National Natural Science Foundation of China Regular Project Fund, Anhui Province Natural Science Foundation of General Project and Anhui Provincial Key Research and Development Project, etc. Published over 50 papers in *Journal of Biotechnology*, *Journal of Agricultural and Food Chemistry*, *Food Chemistry*, *Biomaterials Advances*, *CIESC Journal*, *Acta Microbiologica Sinica*, etc., and granted 10 invention patents. Received the Third Prize for Scientific and Technological Progress in Anhui Province and First Prize in the National Undergraduate Life Science Competition, etc.

# Mechanism Elucidation of Efficient Organic Acids Production in Industrial Filamentous Fungi

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## Abstract

Filamentous fungi are excellent industrial cell factories for organic acid production, yet the molecular basis of their high productivity remains unclear, limiting further metabolic engineering and optimization. Here, the industrial *Aspergillus terreus* producing itaconate and *trans*-aconitate were employed as model to address this knowledge gap. First, we systematically digitized their physiological phenotypes across multi-dimensions throughout the industrial fermentation process. A comparative multi-omics profiling revealed pronounced reprogramming in acid resistance and transport-related modules. In terms of acid resistance, we reported a novel acid resistance system mediated by methionine-cystathionine pathway, and established a multi-layered dynamic model for acid adaptation. Regarding transport module, key transporters for mitochondrial carboxylate transport and organic acid secretion were comprehensively characterized, revealing a smart and compensatory transport system that accounts for the efficient production of target organic acids. Validation was achieved by reprogramming a lovastatin-producing strain into efficient producers of citrate, *trans*-aconitate, and itaconate, respectively. Guided by these insights, a transporter-based compartmentalization strategy was implemented, boosting *trans*-aconitate titer from 60 g/L to 106.3 g/L in a 10-ton fermenter. This work deciphers the molecular blueprint for efficient organic acid production in fungi, providing a framework for engineering robust fungal cell factories for the biomanufacturing of bulk chemicals.

**Key words:** Organic Acid, Industrial Biomanufacturing, Filamentous Fungi, Acid Resistance, Transport.

**Representative publication:**

1. Zhang X, Zhu H, Yuan J, et al. Diversity of heat shock proteins in response to various stressors in the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture. 2024, 584: 740647.

2. Zhang X, Zhang X, Yuan J, et al. ACD-containing chaperones reveal the divergent thermo-tolerance in penaeid shrimp. Science of the Total Environment. 2023, 880:163239.

3. Yuan J#, Zhang X#, Zhang X#, et al. An ancient whole-genome duplication in barnacles contributes to their diversification and intertidal sessile life adaptation. Journal of Advanced Research. 2023, 20:S2090-1232(23)00264-3.

4. Zhang X, Yuan J, Zhang X, et al. Comparative transcriptomic analysis unveils a network of energy reallocation in *Litopenaeus vannamei* responsive to heat-stress. Ecotoxicology and Environmental Safety. 2022, 238: 113600.

## Biography



Dr. Zhang received his Ph.D. and conducted his postdoctoral research at Institute of Oceanology Chinese Academy of Sciences (IOCAS), and then joined Qingdao Institute of Bioenergy and Bioprocess Technology (QIBEBT), Chinese Academy of Sciences in 2023. He is currently an associate professor affiliated with the Microbial Metabolic Engineering Group.

Dr. Zhang's main research interests include: (I) elucidation of the molecular mechanisms underlying special microbial traits; and (II) evolutionary mechanisms of microbial natural product biosynthesis. Dr. Zhang's research group focuses on microbial synthetic biology and green biomanufacturing using the chassis filamentous fungi. By constructing novel microbial cell factories and biocatalysts, his group has developed biotechnological routes for the bioproduction of various chemical and pharmaceutical molecules. Dr. Zhang's has published 10 scientific papers Journal of Advanced Research, Science of the Total Environment, Ecotoxicology and Environmental Safety journals.

# Open Fermentation Enabled by Enhanced Cellular Robustness: Mining and Design of Stress-Resistance Parts

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## Abstract

Open fermentation can substantially reduce process complexity and biomanufacturing costs, and enhancing chassis robustness is an effective strategy for its implementation. A key approach is to engineer chassis cells with stress-resistance parts. In our previous work, multi-omics analysis of thermotolerant Bacillus and salt–alkali-tolerant strains identified 15 temperature-resistance parts, including *cspD*, *ivlB*, and *gsiB*, and two salt–alkali-resistance parts, p*anC* and *sufB*. Based on these findings and literature mining, A total of 422 positive and 398 negative protein sequences were collected to construct an 820-sequence protein dataset.. We established a multimodal framework integrating ESM embeddings, physicochemical properties, secondary structures, evolutionary conservation, and AlphaFold-predicted structures for stress-resistance part prediction. The framework screened prediction model and identified 318 high-potential candidates from human gut, animal gut, and soil metagenomes. Homology analysis revealed diverse functional proteins, including molecular chaperones, chemotaxis proteins, competence proteins, penicillin-binding proteins, and histidine kinases, providing candidates for subsequent validation. Furthermore, a WGAN-based neural network was developed using heat shock proteins to generate novel thermotolerant proteins. A total of 315 sequences were generated, including 18 containing DnaJ domains, and five candidates were selected for subsequent heat-resistance validation. Overall, this study establishes an integrated framework combining multi-omics, machine learning, and generative design, providing a methodological foundation for efficient mining and generation of novel stress-resistance parts.

**Key words:**Open fermentation;stress- tolerant parts; Machine Learning and Neural Networks;

**Representative publication:**

1. KUANG Z Y, YAN X F, YUAN Y F, et al. Advances in stress-tolerance parts for microbial cell factories[J]. Synthetic and Systems Biotechnology, 2024, 9(4): 793-808.

2. XU T, HUANG D Y, HUANG T T, et al. Gene Surfing: An efficient and versatile tool for targeted enzyme mining in metagenomics[J]. Synthetic and Systems Biotechnology, 2025, 10(4): 1377-1387.

## Biography



Professor Haitao Yue is a Doctoral Supervisor. He earned his Ph.D. from Tsinghua University and currently serves as Deputy Secretary and Vice Dean of the School of Pharmacy, Xinjiang University. He also leads the Xinjiang Tianshan Innovation Team focused on synthetic biology and microbial metabolic engineering. His research focuses on advanced microbial chassis construction, stress-tolerant parts mining and design, and microbial functional proteins. Supported by the Third Xinjiang Scientific Expedition Program, he has built a library of nearly 10,000 functional microbial strains to support bio-manufacturing development.

He has received funding from the NSFC-Xinjiang Joint Fund, Xinjiang Distinguished Young Scientists Fund, and Tianshan Young Talents Program. In the past five years, he has led 5 national and 6 provincial research projects, including key R&D programs, NSFC projects, and local “Unveiling the List” projects. He has published over 70 papers and earned two First-Class Xinjiang Science and Technology Progress Awards. His students have won three national first prizes in life science competitions and three iGEM silver medals. He holds multiple academic society positions and serves as an editorial board member for *Food Macromolecules*, *Synthetic Biology Journal*, and *China Journal of Bioengineering*.

# Harnessing photoenzymatic reactions for unnatural biosynthesis in microorganisms

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## Abstract

Photobiocatalysis provides a powerful strategy for integrating light and biological catalysts to drive abiological transformations. However, its scalability is hindered by high enzyme loading, reliance on costly cofactors and instability under radical-generating conditions. Here we report the integration of light-driven enzymatic reactions into the cellular metabolism of *Escherichia coli*, bridging flavin-based photobiocatalysis with biosynthesis. Using synthetic biology strategies, we engineered microbial cells to continuously produce olefin substrates and ene-reductase while regenerating cofactors directly from glucose. By externally supplying radical precursors or introducing synthetic pathways for their in situ production, we enabled fermentation-based microbial photobiosynthesis, achieving high titres and demonstrating feasibility for scale-up in a bioreactor. This approach extends photobiocatalysis from in vitro applications to in vivo semi- and complete biosynthesis, revealing its full potential for integrating light-driven reactions into cellular metabolism.

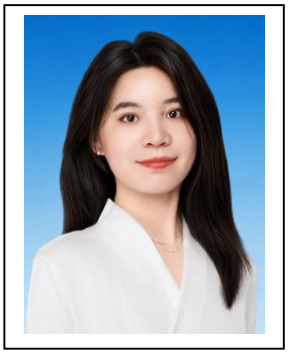
**Key words:** Photobiocatalysis, Synthetic biology, Photobiosynthesis, *Escherichia coli*

**Representative publication:**

1. Yujie Yuan#, Maolin Li#, Wesley Harrison, Zhengyi Zhang, Huimin Zhao*. Harnessing photoenzymatic reactions for unnatural biosynthesis in microorganisms. (2026) ***Nature Catalysis***, 9, 62–72.

2. Maolin Li, Yujie Yuan, Wesley Harrison, Zhengyi Zhang, Huimin Zhao*. Asymmetric photoenzymatic incorporation of fluorinated motifs into olefins. (2024) ***Science***, 385(6707), 416-421.

## Biography



Dr. Yujie Yuan joined the College of Chemistry at Nankai University as an independent Principal Investigator (PI) in April 2026, and is affiliated with the State Key Laboratory of Elemento-Organic Chemistry and the Frontier Science Center for New Organic Matter Creation. Dr. Yuan received her Ph.D. in Biochemistry and Molecular Biology from Wuhan University in 2019 under the supervision of Prof. Tiangang Liu. She subsequently carried out postdoctoral research at the Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences with Prof. Zixin Deng, and at the University of Illinois Urbana–Champaign with Prof. Huimin Zhao. Dr. Yuan's research focuses on developing innovative synthetic biology technologies through interdisciplinary approaches, including automated high-throughput platforms (BioFoundry), AI/machine learning, and novel new-to-nature enzymatic reactions, to enable the efficient discovery and intelligent creation of bioactive natural products for biopharmaceutical research and applications. As first author and/or corresponding author (including co-first/co-corresponding author), Dr. Yuan has published multiple papers in leading journals, including *Nature Catalysis*, *Nature Communications*, and *Cell Systems*, and has been granted multiple invention patents.

# Carbon- and Cost-Efficient Upcycling of Agrifood Waste into Fatty Acid Esters by Systematically Engineered *Clostridium tyrobutyricum*

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## Abstract

Microbial upcycling of agrifood waste into esters offers a sustainable alternative to petrochemical routes, yet carbon loss during glycolysis, inefficient intracellular esterification, contamination risk and high feedstock costs remain limiting. We established a carbon- and cost-efficient platform in *Clostridium tyrobutyricum* for de novo biosynthesis of butyl butyrate (BB), a C8 ester used as a flavour compound and aviation-biofuel candidate. An iterative multimodule strategy was implemented: an 84-member promoter library screened across 605 combinatorial strains balanced precursor supply; a three-enzyme colocalization system based on self-assembling peptide tags and a membrane-anchoring motif reduced precursor toxicity and accelerated ester efflux; a nonoxidative glycolysis pathway with deacylase overexpression conserved carbon by bypassing $CO_2$-emitting steps, verified by 13C tracing; and phosphite dehydrogenase expression strengthened NADH supply. The same enzyme, combined with formamide, urea and phosphite as unconventional nitrogen and phosphorus sources, further enabled antibiotic-free non-sterile fermentation with 88% population dominance. Using a medium composed exclusively of rice straw hydrolysate and shrimp shell waste, the final strain produced 31.16 g/L BB with 98.4% selectivity in 5-L fermentation, a 49.5-fold improvement, while techno-economic and life-cycle analyses indicate 53.6% lower feedstock costs and 63.4% lower carbon emissions per ton of BB.

**Key words:** Biofuels; *Clostridium tyrobutyricum*; butyl butyrate; agrifood waste; metabolic engineering

**Representative publication:**

1. Yang Z., Liu Z., Fang C., Yang Y., Ren C., Chen Y., Zhu L., Zhu Z.*, Jiang L.*, Huang H., Carbon-efficient upcycling of agrifood waste into fatty acid ester by engineered *Clostridium tyrobutyricum*, Trends in Biotechnology, 2026, doi:10.1016/j.tibtech.2026.05.004.

2. Wang Y., Ren C., Fang C., Yang Z., Wu C., Chen Y., Zhu Z.*, Jiang L.*, Non-sterile fermentation of butyl butyrate by *Clostridium tyrobutyricum* with an anti-microbial contamination system, Bioresource Technology, 2026, 460, 135319.
3. Wang H., Chen Y., Yang Z., Deng H., Liu Y., Wei P., Zhu Z.*, Jiang L.*, Metabolic and bioprocess engineering of *Clostridium tyrobutyricum* for butyl butyrate production on xylose and shrimp shell waste, Foods, 2024, 13, 1009.
4. Yang L., Yang Z., Liu J., Liu Z., Liu Y., Zhu L., Zhu Z.*, Jiang L.*, Deciphering the contribution of PerR to oxidative stress defense system in *Clostridium tyrobutyricum*, Food Frontiers, 2023, 4(1), 343-357.
5. Yang Z., Leero D.D., Yin C., Yang L., Zhu L., Zhu Z.*, Jiang L.*, Clostridium as microbial cell factory to enable the sustainable utilization of three generations of feedstocks, Bioresource Technology, 2022, 361, 127656.

## Biography



Dr. Zhengming Zhu is Professor at the School of Food Science and Light Industry, Nanjing Tech University, China. He received his PhD in Fermentation Engineering from Jiangnan University, and was a joint-training PhD student at Chalmers University of Technology and a visiting scholar at KTH Royal Institute of Technology, Sweden. His research focuses on advanced food biomanufacturing, centred on creating non-model microbial chassis and applying them to the biosynthesis of food components. His group has developed genome-editing and promoter-engineering toolkits for *Clostridium tyrobutyricum*, elucidated its stress-response and carbon-catabolite-repression regulation, and engineered strains for de novo butyl butyrate biosynthesis from rice straw hydrolysate and shrimp shell waste, integrating multienzyme spatial organization, nonoxidative glycolysis, cofactor engineering and non-sterile fermentation with techno-economic and life-cycle assessment. He has led projects funded by the National Natural Science Foundation of China and key R&D programmes, published 16 SCI papers as first or corresponding author over the past five years.

# *De novo* production of polycyclic polyprenylated acylphloroglucinols in engineered microbes

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## Abstract

Polycyclic polyprenylated acylphloroglucinols (PPAPs) are a class of structurally complex plant natural products with an extraordinarily wide range of pharmaceutical activities, including antidepressant, antibacterial, antiviral and antitumor properties. PPAPs are found primarily in species of the Hypericaceae and Clusiaceae families in very low abundance. The total chemical synthesis of PPAPs is difficult and generally uneconomical. Producing PPAPs in an engineered microbial cell factory is a promising and sustainable alternative. PPAP biosynthesis shares the polyketide pathway for the acylphloroglucinol cores and the terpenoid pathway for the $C_5$ or $C_{10}$ side chains. The isoprenoid chains are attached to the polyketide cores by prenyltransferases (PTs), which belong to the membrane-integrated UbiA superfamily. We have recently elucidated the entire biosynthetic pathways of several benzophenone- and xanthone-derived PPAPs. *De novo* benzophenone and xanthone biosynthesis has been successfully established in *Pseudomonas taiwanensis* with a titer of around 30 mg/L. The prenylation of benzophenones and xanthones has been achieved by co-expressing the relevant PTs in a specific strain of *Saccharomyces cerevisiae*. These engineered yeast cells produce benzophenone- and xanthone-derived PPAPs with a titer of up to 10 mg/L. Finally, *de novo* production of PPAPs was achieved by co-cultivating the engineered bacteria and yeast cells.

**Key words:** Benzophenone, Prenyltransferase, PPAPs, *Pseudomonas*, Yeast

**Representative publication:**

1. Ernst, L., Sayed, H.M.B., Hassanin, A., Moegenburg, R., Meents, T., Lyu, H., Kaufholdt, D., Davari, M.D., Beerhues, L., **Liu, B.**, El-Awaad, I., 2025. Reverse prenylation in plants by non-canonical aromatic prenyltransferases. **Plant Journal** 122, e70268. https://doi.org/10.1111/tpj.70268

**As co-corresponding author, I was involved in conceptualization and execution of the research, data evaluation, and manuscript writing.**

2. Ernst, L., Lyu, H., Liu, P., Paetz, C., Sayed, HMB., Meents, T., Ma, H., Beerhues, L., El-Awaad, I., **Liu, B.** (2024) Regiodivergent biosynthesis of bridged bicyclononanes. **Nat Commun.** 15: 4525. DOI: 10.1038/s41467-024-48879-w.

**I wrote the related DFG proposal which was proved, designed the research, participated in experiments, and worked on the manuscript.**

3. Sayed, H.M.B., Nassar, S., Kaufholdt, D., Beerhues, L., **Liu, B.**, El-Awaad, I. (2023) Biosynthesis of polyprenylated xanthones in *Hypericum perforatum* roots involves 4-prenyltransferase. **Plant Physiology**. kiad219. DOI: 10.1093/plphys/kiad219.

**As co-corresponding author, I was involved in conceptualization and execution of the research, data evaluation, and manuscript preparation.**

4. Tocci, N., Gaid, M., Kaftan, F., Belkheir, A.K., Belhadj, I., **Liu, B.**, Svatoš, A., Hänsch, R., Pasqua, G., Beerhues, L. (2018) Exodermis and endodermis are the sites of xanthone biosynthesis in *Hypericum perforatum* roots. **New Phytologist** 217: 1099-1112. DOI: 10.1111/nph.14929.

**I was involved in designing the research, conducting the experiments, and analyzing the data.**

5. El-Awaad, I., Bocola, M., Beuerle, T., **Liu, B.**, Beerhues, L. (2016) Bifunctional CYP81AA proteins catalyse identical hydroxylations but alternative regioselective phenol couplings in plant xanthone biosynthesis. **Nature Communications** 7: 11472. DOI: 10.1038/ncomms11472.

**I was involved in designing the research, conducting the experiments, and analyzing the data.**

## Biography



Benye Liu is a senior scientist at the Institute of Pharmaceutical Biology at the Technical University of Braunschweig in Germany. He received his PhD in agronomy from the Northeast Agricultural University in Harbin, China, in 1997. This was followed by two postdoctoral fellowships at the Institute of Botany at the Chinese Academy of Sciences in Beijing and the Institute of Pharmaceutical Biology, Bonn University in Germany, respectively.

Dr Liu's research focuses on the biosynthesis of plant natural products, particularly the Active Pharmaceutical Ingredients (APIs) in medicinal plants. His work aims to elucidate the biosynthetic pathways of APIs and produce them in microbial cell factories. He has made significant contributions to the biosynthesis of benzophenone-derived polycyclic polyprenylated acylphloroglucinols (PPAPs). Notably, he cloned the first benzophenone synthase (BPS) gene and was the first to publish studies on prenylative cyclases, which play a key role in the biosynthesis of structurally complex PPAPs.

He has published 50 peer-reviewed articles in leading journals in this field and has received the Phoenix Pharmacy Science Award twice for his publications. His current research focuses on producing PPAPs in microbial cell factories as an alternative and sustainable approach and funded by the Deutsche Forschungsgemeinschaft (DFG, German Reseach Foundation).

# The Glutathione Metabolic Regulation Is Elucidated in a New Marine Aromatic Probiotic Yeast Meyerozyma guilliermondii GXDK6 under Salt Stress

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## Abstract

High-salt environments impose significant oxidative stress on microorganisms by disrupting redox homeostasis, necessitating efficient adaptive mechanisms such as glutathione (GSH) metabolism. *Meyerozyma guilliermondii* GXDK6, a marine-derived multi-stress-tolerant yeast, exhibits robust salt tolerance; however, the molecular basis of its GSH-mediated regulatory networks under salt stress remains unexplored. In this study, a comprehensive multi-omics approach, integrating whole-genome sequencing, transcriptomics, and proteomics profiling, along with targeted physiological assays, was employed to investigate GSH metabolic regulation under salt stress. Genome-wide analysis identified 55 genes involved in the other amino acid metabolism, with transcriptomic and proteomic profiling revealing salt-induced upregulation of key GSH biosynthetic genes (*GSS*, *cysK*_2, and *glyA*) and downregulation of degradation-related gene *ggt*_2. Moreover, transcript and protein level analyses activation of biosynthetic pathway, Intracellular GSH content exhibited a biphasic response, with a 39.75% reduction at 5% NaCl, followed by a 53.01% recovery at 10% NaCl. Notably, Glutathione S-transferase (GST) enzyme activity was significantly increased under salt stress, highlighting its role in cellular detoxification. Furthermore, Exogenous application of GSH (10 mg/L) markedly improved halotolerance, resulting in a 52.7-fold increase in colony-forming units under 10% NaCl conditions. These findings demonstrate that GSH's metabolism plays a central role in redox balance. These findings provide novel insights into the *M. guilliermondii* adaptive redox strategies and lay the foundation for metabolic engineering approaches to enhance microbial performance in hypersaline and industrially challenging conditions.

**Key words:** Glutathione, Meyerozyma guilliermondii, Multi-omics analysis, Salt stress

**Representative publication:**

1. Huijie Sun, Ruihang Wei, Kailu Yin, Duotao Meng, Shanguang Wu, Huashan Bai, Zhenze Li, Muhammad Kashif, Zhongmao Liang, Shipeng Chen, Hongping Pan, and **Chengjian Jiang***. Discovery and interaction of four key biosynthetic enzymes under co-regulation for dopamine biosynthesis with marine Meyerozyma guilliermondii GXDK6 and Bacillus aryabhattai NM1-A2. **International Journal of Biological Macromolecules**. 2025. 309(Pt 2):142821

2. Ru Bu, Zhenze Li, Qiyu Qin, Huashan Bai, Can Meng, Ruihang Wei, Xinglin Chen, Shanguang Wu, Muhammad Kashif, Sheng He*, and **Chengjian Jiang***. An NRPS-like gene LYS2 contributed to the biosynthesis of cyclo(Pro-Val) in a multi-stress-tolerant aromatic probiotic, *Meyerozyma guilliermondii* GXDK6. **Journal of Agricultural and Food Chemistry**. 2025. 73(1): 507–520.
3. Xueyan Mo, Mengcheng Zhou, Yanmei Li, Lili Yu, Huashang Bai, Peihong Shen, Xing Zhou, Haojun Zhu, Huijie Sun, Ru Bu, and **Chengjian Jiang***. Safety assessment of a novel marine multi-stress-tolerant yeast *Meyerozyma guilliermondii* GXDK6 according to phenotype and whole genome-sequencing analysis. **Food Science and Human Wellness**. 2024. 13(4): 2048-2059.
4. Huijie Sun, Huashan Bai, Ruihang Wei, Duotao Meng, Qiong Jiang, Hongping Pan, Peihong Shen, Qian Ou, Sheng He, and **Chengjian Jiang***. Regulatory mechanisms of dopamine metabolism in a marine *Meyerozyma guilliermondii* GXDK6 under NaCl stress as revealed by integrative multi-omics analysis. **Synthetic and Systems Biotechnology**. 2024. 9(1): 115–126.
5. Kunmei Huang, Huashan Bai, Can Meng, Muhammad Kashif, Zhiling Wei, Zaihang Tang, Shu He, Shanguang Wu, Sheng He*, and **Chengjian Jiang***. Deciphering the ammonia transformation mechanism of a novel marine multi-stress-tolerant yeast *Pichia kudriavzevii* HJ2 as revealed by integrated omics analysis. **Applied and Environmental Microbiology**. 2025. 91(6):e0221124.

## Biography



Chengjian Jiang received his Ph.D. at 27 and is currently a full Professor and vice dean at Guangxi University of Science and Technology (GXUST). He was a Visiting Scholar at Rutgers Biomedical and Health Sciences, New Jersey, and now serves as Lead Researcher for the "Chemical Engineering and Technology" discipline under Guangxi's 15th Five-Year Plan, as well as for the "Biological Engineering" discipline at GXUST. His honors include the Guangxi Outstanding Young Scientist Award, the "National Outstanding Teacher" title, and the "Baosteel Outstanding University Teacher Award." His research focuses on the high-value utilization of subtropical characteristic biological resources. As corresponding author, he has published over 60 high-quality papers in the famous journals such as Food Science and Human Wellness, Applied and Environmental Microbiology, and Journal of Agricultural and Food Chemistry, including 5 ESI hotspot papers (top 0.1%) and 6 ESI highly cited papers.

# Construction of chassis cell for the efficient production of natural products from myxobacteria

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## Abstract

Myxobacteria represent a prolific yet underexploited source of natural products. To unlock their biosynthetic potential, we developed MxDIRECT by integrating Red/ET recombination and TnpB, establishing a novel genome editing toolkit for the model strain *Myxococcus xanthus* DK1622. Leveraging this enabling technology, we further engineered DK1622 into MxPKS, a proprietary chassis featuring streamlined metabolism, optimized precursor supply, and heterologous antibiotic efflux pumps, thereby enabling high-efficiency expression of myxobacterial polyketide gene clusters. Additionally, we introduce *E. coli* PITE, a phase-separated chassis harnessing liquid–liquid phase separation (LLPS) for terpenoid production. A 363 aa intrinsically disordered region from *M. xanthus* DK1622, discovered via machine learning, drives LLPS to enable multienzyme condensate assembly. PITE was validated through heterologous expression of a cryptic gene cluster, yielding archanqipenols—the first antibacterial halimane diterpenoids from myxobacteria. These complementary platforms collectively advance the discovery and exploitation of cryptic myxobacterial natural products for the purpose of drug development.

**Key words:** Myxobacteria; Genetic tools; Chassis cell; Liquid-liquid phase-separation; Natural products

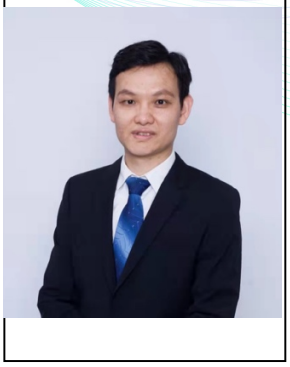
**Representative publication:**

1. Zhang, W. J.[#]; Hu, J. Q.[#]; Li, R. J.; Sheng, D. H.; Yue, X. J.; She, Q.; Zhang, Y.; Li, Y. Z.*; Zhuo, L.*; **Wu, C.*** Development of efficient genetic toolkits and heterologous chassis for bioprospecting of myxobacteria. *Nat. Commun.* **2026**, *accepted*.

2. Zhu, L. L.[#]; Yang, Q. Y.[#]; Wang, D. G.[#]; Niu, L.; Pan, Z.; Li, S. Li, Y.-Z.*; Zhang, W*; and **Wu, C.*** Deciphering The Biosynthesis and Physiological Function of 5-Methylated Pyrazinones Produced by Myxobacteria. *ACS Cent. Sci.* **2024**, 10, 555–568

3. Li, Y.[#]; Zhuo L.[#]; Li, X.; Zhu, Y.; Wu, S.; Shen, T.; Hu, W.; Li, Y.-Z.*; **Wu, C.*** Myxadazoles, Myxobacterium-Derived Isoxazole-Benzimidazole Hybrids with Cardiovascular Activities. *Angew. Chem. Int. Ed.* **2021**, *60*, 21679 –21684.

## Biography



Changsheng Wu studied pharmacy for his BSc degree (2008) and natural products chemistry for his MSc degree (2011) at Shandong University, China. He obtained his PhD in 2016 at Leiden University, The Netherlands, where he discovered new antibiotics from actinomycetes. In 2017, he conducted postdoctoral research at The Rockefeller University, USA, using the metagenomics tool to find new antibiotics. In 2019, he joined Shandong University as a full professor. His current research interests focus on the chemistry and biology of natural products from predatory myxobacteria.

# Discover and Produce Marine Natural Products Using Genome Mining and Synthetic Biology Tools

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## Abstract

Genome mining-directed heterologous expression of biosynthetic gene clusters has led to the discovery of various bioactive natural products. Here, by heterologously expressing the sesquiterpene biosynthetic gene cluster "jan" from the marine fungus *Penicillium janthinellum* H7-4, we successfully obtained a series of aromatic eremophilane-derived sesquiterpenoids. Among these structures, aza-janthinellin A features an aromatic skeleton that is formed by adding L-threonine to an eremophilane moiety. Biosynthetic studies revealed that the formation of aza-janthinellin A involves an unprecedented ring rearrangement–aromatization mechanism triggered by nucleophilic addition and accompanied by [1,2]-alkyl migrations. During this process, a FAD-dependent monooxygenase, JanF, was confirmed to oxidize the terminal hydroxyl group of the allylic alcohol to a highly reactive α,β-unsaturated aldehyde intermediate, which then serves as a strong electrophile to accept nucleophilic attack by primary amine reagents, thereby initiating skeletal rearrangement and aromatization. Based on the above mechanism, we established a biomimetic system and produced a series of novel nitrogen/sulfur-containing aromatic sesquiterpenoid derivatives. Among them, aza-janthinellin L and thio-janthinellins A–B exhibited cytotoxic activity. These findings advance our understanding of the mechanism governing the aromatization of sesquiterpene skeletons and provide a feasible method to access heterocyclic aromatic terpene derivatives.

**Key words:** Genome mining; biosynthesis; aromatization; heterocyclic sesquiterpenoid; eremophilane

**Representative publication:**

1. Xu H., Zhang X., Zhang Y., Liu G., Chen Y., Hou X., Liu X., Zhang K., Ma C., Feng R., Shen J., Pfeifer B. A., Che Q., Zhu T., Zhang G., *Nat. Commun.*, 2025, 16:7369.

2. Huang X., Xu X., Zhou L., Li J., Ma C., Wang W., Che Q., Li D., Zhu T., Zhang G*., *J. Nat. Prod.* 2025, 88, 768−776.

3. Xu X., Chang Y., Chen Y., Zhou L., Zhang F., Ma C., Che Q., Zhu T., Pfeifer B. A., Zhang G.*, Li D.*, Org. Lett. 2024, 26, 7489-7494.

4. Liu X., Li K., Yu J., Ma C., Che Q., Zhu T., Li D., Pfeifer B. A., Zhang G. *Metab. Eng*. 2024, 82, 147-156.

## Biography



Guojian Zhang received his PhD in Natural Product Chemistry and Medicinal Chemistry from Ocean University of China in 2011. For his postdoctoral work, Guojian pursued studies in the field of chemical and biological engineering, working with Prof. Blaine Pfeifer at The State University of New York at Buffalo and Prof. Robert Cichewicz at The University of Oklahoma. In July 2017, He joined the School of Medicine and Pharmacy, Ocean University of China where his research interests focus on marine natural product discovery, biosynthesis and heterologous production of bioactive compounds.

# Molecular Farming in Tobacco (*Nicotiana tabacum*): Tissue-Specific and Flux-Redirected Metabolic Engineering of Forskolin

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## Abstract

Plants synthesize diverse phytochemicals from $CO_2$, light, water, and minerals, making them promising platforms for sustainable and scalable production of valuable natural compounds. Tobacco (*Nicotiana tabacum*) is a globally cultivated crop with biomass up to 100 tons/ha, and strong capacity for diterpenoid biosynthesis, particularly α/β-cembratriene-diols (CBT-diols) in glandular trichomes (GTs). To facilitate metabolic engineering in tobacco, we optimized genetic manipulation pipelines, e.g., shortening the genetic transformation timeline from 3 months to 1 month, reducing the life cycle from 8-12 months to 3 months in the greenhouse, and establishing the Multiplex Expression Cassette Assembly (MECA) system for producing plant expression constructs. These enable rapid building and testing metabolic pathways of diverse compounds in plants.

Forskolin, a labdane-type diterpenoid that accumulates in the roots of medicinal plant *Coleus forskohlii*, activates the cAMP signaling pathway and is used for the treatment of heart complications, respiratory disorders, high blood pressure, obesity and asthma. To produce forskolin in tobacco, we reconstructed its biosynthetic pathway and generated transgenic plants. Constitutive expression of biosynthetic genes resulted in low-level production of forskolin, whereas GT-specific expression resulted in significantly higher levels of forskolin accumulation in aerial tissues, likely due to abundant geranylgeranyl diphosphate (GGPP). To redirect the GGPP flux to forskolin, we knocked out the CBT-diol synthase gene family, which boosted forskolin yield to 21.26 μg/g fresh weight (206.33 μg/g dry weight) in leaves, surpassing levels in *C. forskohlii* roots. Our work not only demonstrates a scalable and sustainable plant-based approach for forskolin production, but also represents an effective strategy that integrates tissue/organ-specific engineering with metabolic flux redirection to enhance heterologous production of high-value natural bioactive compounds in plants.

**Key words:** Tobacco, Forskolin, Terpenoid, Glandular Trichome, Metabolic Flux Redirection

**Representative publication:**

1. Sun X, Wu X. M., Jiang X., Huang H. M., Yang A. G., Li Y. T., Fan Z. J., Chang A. X., Yang C. Q.*, Heterologous Production of Forskolin in Tobacco (*Nicotiana tabacum*) via Glandular Trichome Specific Engineering and Metabolic Flux Redirection, Plant Biotechnology Journal, 2026, 24:1548-1563.

2. Jiang X., Zhang Z. X., Wu X. M., Li C. M., Sun X., Li Y. T., Chang A. X., Yang A. G., Yang C. Q.*, Multiplex Expression Cassette Assembly: A flexible and versatile method for building complex genetic circuits in conventional vectors, Plant Biotechnology Journal, 2024, 22: 3361–3379.

3. Jiang X., Zhang Z. X., Wu X. M., Li C. M., Sun X., Wu F. Y., Yang A. G., Yang C. Q.*, Heterologous biosynthesis of betanin triggers metabolic reprogramming in tobacco, Metabolic Engineering, 2024, 86: 308–325.

4. Yao L., Wu X. M., Jiang X., Shan M., Zhang Z. X., Li Y. T., Yang A. G., Li Y., Yang C. Q.*, Subcellular compartmentalization in the biosynthesis and engineering of plant natural products, Biotechnology Advances, 2023, 69: 108258.

## Biography

Professor Changqing Yang obtained his PhD from the Chinese Academy of Sciences in 2010. From 2010 to 2017, he conducted research at the Institute of Plant Physiology and Ecology, Chinese Academy of Sciences. He subsequently joined the Leibniz Institute of Plant Biochemistry in Germany, supported by the Humboldt Research Fellowship for Experienced Researchers. In 2020, he was appointed as a researcher at the Chinese Academy of Agricultural Sciences. His research focuses on the biosynthesis and regulatory mechanism of specialized metabolites in *Nicotiana* species, as well as the metabolic engineering of tobacco for the production of high-value bioactive compounds.



# A nonclassical biosynthetic route enables tocopherol synthesis in bacteria

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## Abstract

Tocopherols have been widely applied in healthcare, medicine, animal feed, and other fields. However, microbial synthesis of tocopherols with industrial potential has not been accomplished yet. Here, we report a nonclassical biosynthetic route for tocopherol synthesis in *Rhodobacter* species that bypasses the classical α-tocopherol pathway requiring the 2,3-dimethyl-5-phytyl-1,4-benzoquinone (DMPBQ) as an intermediate. In particular, a *Rhodobacter* methyltransferase with bifunctional γ/β-tocopherol methyltransferase activities is identified, which can directly synthesize α-tocopherol from δ-tocopherol, and γ/β-tocopherols. Furthermore, geranylgeranyl pyrophosphate reductase (GR) plays an important role in reducing tocotrienols into tocopherols. Through systematic metabolic engineering, the engineered *Rhodobacter sphaeroides* produced a total titer of 508.21 mg/L tocopherols in shake flasks, and 3.51 g/L under the fed-batch fermentation after process optimization. Taken together, these results offer a nonclassical biosynthetic pathway for efficient tocopherol biosynthesis, which would pave the way for future commercial production of tocopherols by microbial fermentation.

**Key words:** [tocopherols; β-tocopherol methyltransferase; geranylgeranyl pyrophosphate reductase; synthetic biology]

**Representative publication:**

1. Zhang, Y.#, Li, Z.#, Cao, L.#, Wang, Y., Wang, J., Chen, H., Li, W., Gao, X., Li, Q., Chen, Z., Xu, K., Yang, X., Wu, X., Luo, J., Liu, J., Yuan, J.*, A nonclassical biosynthetic route enables tocopherol synthesis in bacteria, Nat. Metab., 2026, DOI: 10.1038/s42255-026-01576-y.
2. Yu, D.#, Chen, H.#, Zhang, Y.#, Chen, Y., Chen, H., Lin S., Wang, J., Yang H., Ling X., He, N., Yuan J.*, Biosensor-guided indexing of genome evolution for boosting microbial chemical synthesis, Trend. Biotechnol., 2026, DOI: 10.1016/j.tibtech.2025.12.019.
3. Zhang, Y.*, Wang, X., Yuan, J.* & Guo, L.*, Multidimensional engineering of *Rhodobacter sphaeroides* for enhanced photo-fermentative hydrogen production, Chem. Eng. J., 2024, 488, 150852.
4. Zhang, Y., Meng, W., He, Y., Chen, Y., Shao, M., Yuan, J.*, Multidimensional optimization for accelerating light-powered biocatalysis in *Rhodopseudomonas palustris*, Biotechnol. Biofuels Bioprod., 2023, 16, 160.
5. Zhang, Y., Song, X., Lai, Y., Mo, Q., Yuan, J.*, High-yielding terpene-based biofuel production in *Rhodobacter capsulatus*. ACS Synth. Biol., 2021, 10, 1545-1552.

## Biography



My research primarily focuses on microbial synthetic biology, including the efficient biotransformation of non-food feedstocks such as C1/C2 substrates, plastic waste, and lignocellulose, as well as the construction of efficient cell factories using non-model microorganisms. I have been awarded more than ten research grants, including the Youth Program of the National Natural Science Foundation of China, sub-projects of the National Key Research and Development Program of China, enterprise-commissioned projects, and provincial and municipal scientific research funds, with a cumulative research funding of over 2 million RMB. I have published more than 30 peer-reviewed SCI papers in prestigious journals, such as *Nature Metabolism*, *Trends in Biotechnology*, and *Chemical Engineering Journal*. I have filed six national invention patents, among which three have been authorized. Additionally, I serve as an editorial board member for *BioDesign Research*, *Engineering in Life Sciences*, *Green Carbon*, *Model Organisms Research*, and *Discover Biotechnology*, and have been honored as Class C High-Level Talent of Fujian Province and Xiamen City.

# Fungal Metabolic Engineering for Efficient Biomanufacturing: Mining and Expanding Biodiversity

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## Abstract

Metabolic engineering of fungal strains, including yeasts and filamentous fungi, has received increasing attention owing to their importance in biomanufacturing, agriculture, medicine, and environmental protection. However, great challenges remain due to limited functional information, relatively slow growth, low genome editing efficiency, and lack of genetic elements. Therefore, integration of random mutagenesis, adaptive evolution with metabolic engineering is of great importance to mine and enlarge of biodiversity, which benefits efficient metabolic engineering. In this talk, AI-based protein design, library of artificial regulators, and development of genome editing platform for expanding genetic diversity to enhance bioproduction are discussed. In addition, the latest progress on development of high throughput screening system to obtain stress tolerance strain and cellulase overproducing mutants is presented. Finally, efficient cellulase production for cellulosic ethanol production will be summarized.

**Key words:** Yeast, *Trichoderma reesei*, cellulase, metabolic engineering, High Throughput screening

**Representative publications:**

1. Zeng D.W., Zhu Y.F., Jiang Y.H., Ding Y.H., Wang Z.Z., Li K., Liao S., Fan Y.C., Zhang L., Wang Y.G.*, Zhao X.Q.* Establishment of succinic acid production in recombinant *Kluyveromyces marxianus* enhanced by AI-guided enzyme specificity engineering and redox-controlled fermentation optimization. Chemical Engineering Journal. 2025, 523, 168210.
2. Li X.Y., Li K., Zhang F.L., Hasunuma T., Kondo A., Zhang L., Zhao X.Q*., Bai F.W. CRISPR-based synthetic biology toolkit development in *Candida viswanthii* and functional analysis of the stress responsive Ena1-like protein. Synthetic and Systems Biotechnology. 2026, 11, 298-308.
3. Wang W.B., Yuan B., Cheng C., Thongchul N., Zhao X.Q.* Advancing stress biotechnology for robust sustainable bioproduction. Current Opinion in Biotechnology, 2026, 103548.
4. Zhang F., Bai F.W., Zhao X.Q.* Enhanced cellulase production from *Trichoderma reesei* Rut-C30 by engineering with an artificial zinc finger protein library. Biotechnology Journal. 2016, 11, 1282-1290.
5. Ma C., Wei X.W., Sun C.H., Zhang F., Xu J.R., Zhao X.Q.*, Bai F.W. 2015. Improvement of acetic acid tolerance of Saccharomyces cerevisiae using a zinc-finger-based artificial transcription factor and

identification of novel genes involved in acetic acid tolerance. Applied Microbiology and Biotechnology. 2015, 99, 2441-2449.

## Biography

**Dr. Xin-Qing Zhao** is a professor at State Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University her research focuses on the bioconversion of renewable biomass with engineered microbial strains, in particular the fungal species *Trichoderma reesei* for cellulase production and various yeast species, including budding yeast and non-conventional yeasts to produce cellulosic ethanol, succinic acids, and other valuable chemicals. Up till now, her research progress has contributed 122 peer reviewed articles to reputed journals, and her technological renovations has generated 12 patents for genetically modified microbial strains and bioprocesses. She has served as principal investigator on multiple projects funded by the National Natural Science Foundation of China (NSFC) and the National Key Research and Development Program (MOST) of China, as well as by industrial partners. She is currently the commission of ICY (International Committee on Yeasts), and editorial board member of several peer-reviewed journals, including Biotechnology Advances, Biotechnology for Biofuels and Bioproducts, and BioDesign Research. Due to her achievements in microbial biotechnology, she has been awarded Young Asian Biotechnologist Prize by the Society for Biotechnology, Japan in 2015, and she is also the recipient of the Program for New Century Excellent Talents supported by Ministry of Education, China.



# Modular Engineering Strategies for the Development of Genetically Encoded Biosensors

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## Abstract

Genetically encoded biosensors convert intracellular metabolite concentrations into measurable outputs and have become powerful tools for high-throughput screening, directed enzyme evolution, and dynamic pathway regulation in metabolic engineering. However, the development of new biosensors remains limited by the lack of efficient and generalizable engineering strategies. Here, we present multiple modular strategies for biosensor development. First, we engineered a transcription factor-based glycine biosensor in *Escherichia coli* by reprogramming the native GcvA/GcvR regulatory complex using a dual-regulator design. Fine-tuning the expression balance between GcvA and GcvR significantly reduced basal fluorescence while maintaining strong glycine-responsive induction. Second, we developed a two-component biosensor for calcitriol and calcifediol detection through domain rearrangement. By fusing the vitamin D receptor (VDR) ligand-binding domain to the *Saccharomyces cerevisiae* GAL4 DNA-binding domain and RXRA to the VP16 activation domain, the biosensor enabled sensitive detection of both vitamin D metabolites in *S. cerevisiae*. Finally, we expanded the sensing repertoire of transcription factor-based biosensors using AI-driven metadata selection and machine learning, leading to the identification of a novel sclareol-responsive biosensor that was subsequently applied to improve linalyl diphosphate (LPP) synthase activity. Collectively, these studies demonstrate complementary strategies for expanding the biosensor toolbox and establish versatile, modular platforms for biosensors development.

**Key words:** Calcifediol/Calcitriol biosensor; Glycine biosensor; High-throughput screening; AI-driven signal extension

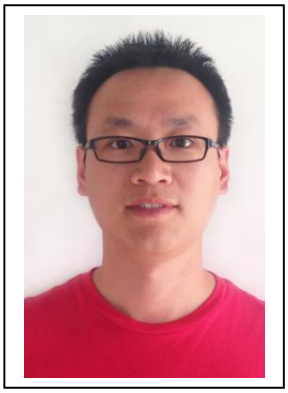
**Representative publication:** [Please list no more than 5 of your publication related to the topic]

1. Peng J., Li X., Li Y., Zhu X., Wang Y.*, Sun Z.*, Zhang X.*. Design and construction of a novel transcription factor-based glycine biosensor for directed enzyme evolution in *Escherichia coli*, Biosensors and Bioelectronics, 2026, 300, 118478.

2. Ma K., Huang Y., Li M., Li Y., Fan F., Sun Z.*, Zhang X.*. Development of highly sensitive and specific and genetically encoded biosensors for calcifediol and calcitriol detection, ACS Sensors, 2025, 10, 3725-3736.

3. Zhang K., Li M., Wang J., Huang G., Ma K., Peng J., Lin H., Zhang C., Wang H.*, Zhan T.*, Sun Z.*, Zhang X.*. Optimizing enzyme properties to enhance dihydroxyacetone production via methylglyoxal biosensor development, Microbial Cell Factories, 2024, 23:163

## Biography



Dr. Zhe Sun is a Principal Investigator and Ph.D. Supervisor at the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences. He received his B.Eng. degree from Harbin Institute of Technology in 2009 and his Ph.D. degree from the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, in 2015. From 2015 to 2021, he conducted postdoctoral research at the National Institutes of Health/National Cancer Institute, USA. He joined the Tianjin Institute of Industrial Biotechnology, CAS, in December 2021. His research focuses on the development of enabling technologies for synthetic biology and their applications in the high-throughput screening and engineering of microbial cell factories. He investigates the molecular mechanisms governing gene expression and metabolic pathway regulation, while leveraging artificial intelligence and machine learning to facilitate the design and optimization of key enzymes and biosynthetic pathways for the efficient microbial production of bulk chemicals and recombinant proteins. His research has been published in Nature Communications, Nucleic Acids Research, Biosensors and Bioelectronics, and ACS Sensors.

# Indigenous Pakistani Coal-Mine Fungi for Next-Generation Feedstock Biomanufacturing: A Green Approach to Chemical Production

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## Abstract

**Background and Aim:**
This research explores the bioconversion of low-rank subbituminous coal into valuable chemical feedstocks using indigenous fungal strains. The aim is to develop a sustainable and economically viable biomanufacturing pathway that utilizes coal as a next-generation feedstock.

**Methods:**
Fungal isolates *Neurospora crassa* (AD-3) and *Penicillium simplicissimum* (AD-5), sourced from a Pakistani coal mine, were incubated with NaOH-pretreated pulverized coal. Process optimization included the use of glucose and sodium acetate as enhancers to maximize the fungal-mediated liberation of organic compounds.

**Results:**
GC/MS analysis revealed that fungal biotransformation significantly enriched the solubilized fraction with diverse compounds, including polycyclic aromatic hydrocarbons (PAHs), ketones, aldehydes, fatty acids, and nitrogen/oxygen-containing compounds, compared to untreated controls. The synergistic chemical pretreatment enhanced solubilization rates to 50.9% (AD-3) and 68.4% (AD-5) within five days, versus 31.29% and 44.9% for untreated coal.

**Conclusion:**
This study demonstrates the high efficiency of indigenous fungi in depolymerizing pretreated subbituminous coal. This innovative bioprocess offers a promising route for valorizing coal into chemical building blocks, aligning with the goals of sustainable biomanufacturing from next-generation feedstocks.

# A Modular Chemoenzymatic Platform for Efficient Synthesis of Aporphine Alkaloids

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## Abstract

Aporphine alkaloids (APAs) possess diverse pharmacological activities, yet their chemical synthesis suffers from poor stereoselectivity and low coupling efficiency, making production largely dependent on plant extraction. We have developed a modular chemoenzymatic platform that converts inexpensive, readily available substrates into diverse APA frameworks through a sequential enzymatic cascade (engineered IRED and CNMT) followed by catalyst-free photoinduced coupling. This streamlined approach enables gram-scale synthesis of (S)-nuciferine in just four steps. Our strategy simplifies APA production, enhances stereochemical control, and provides a broadly applicable platform for the synthesis of related complex natural products.

**Key words:** Aporphine alkaloids，Chemoenzymatic synthesis；Imine reductase; Photoinduced coupling

**Representative publication:**

Lu Yang; Yuhan Dai; Lili Sun; Qiqi Wang; Yaxu Ou; Zixin Deng; Xudong Qu; Zhi Lin; A Chemoenzymatic Strategy for Efficient Synthesis of Aporphine Alkaloids, *ACS Catalysis*, 2025, 15, 19918-19925

## Biography



Dr. Zhi Lin is an associate research professor and Ph.D. Supervisor at Shanghai Jiao Tong University. She received her B.S. from Sichuan University (2012) and Ph.D. from the Shanghai Institute of Organic Chemistry, CAS (2017), followed by postdoctoral training at SJTU (2017–2020) before joining the faculty. Her research focuses on the biosynthesis of bioactive natural products, integrating enzymology, genetics, and structural biology to elucidate novel biosynthetic mechanisms of alkaloids. She also employs enzyme and metabolic engineering to optimize pathways for efficient synthesis of high-value natural compounds and drugs. She has published 25 papers in PNAS, JACS, Angew, and NPR, filed 8 patents (1 licensed), and received the Eastern Talent Program (Youth) and NSFC grants.

# Engineering a Photodecarboxylase and Enzyme Cascades for Bioproduction of Aromatic Chemicals

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## Abstract

Fatty acid photodecarboxylase (CvFAP) is a unique natural photoenzyme that catalyzes light-driven decarboxylation of long-chain fatty acids to alkanes, offering a sustainable route for chemical synthesis. However, its native substrate specificity has largely confined applications to aliphatic compounds, leaving aromatic chemicals—currently derived from unsustainable petroleum refining—largely inaccessible. To address this bottleneck, we reprogrammed CvFAP to accept non-native aromatic substrates through a series of engineering strategies. In our first study targeting ethylbenzene, semi-rational design produced a triple mutant (P460A/G462I/Y466T) with 6.3-fold enhanced productivity, achieving titers of 0.85 g/L from glycerol via coupled cascade biotransformation and fermentation. Similar approach was employed for 4-ethylphenol (0.49 g/L). Recognizing the limitation of low-throughput screening, we next established a directed evolution platform using transcription factor-based biosensors as ultrahigh-throughput screening tools—a key innovation that enabled rapid navigation of sequence space. This approach yielded two nonuple mutants with >200-fold productivity gains and total turnover numbers up to 3,500. Coupled with cascade biotransformation and fermentation, the evolved variants delivered final titers of 4.7 g/L for toluene and 20.3 g/L for p-cresol. Together, these results demonstrate that expanding CvFAP's substrate repertoire to aromatics, empowered by biosensor-enabled high-throughput evolution, opens new avenues for production of bio-based aromatic chemicals.

**Key words:** Photodecarboxylase, Enzyme Cascades, Aromatics, Protein Engineering, Ultrahigh-Throughput Screening

**Representative publication:**

1. Qin Z., Zhou Y.*, Li Z., Höhne M., Bornscheuer U.T.*, Wu S.*, Production of biobased ethylbenzene by cascade biocatalysis with an engineered photodecarboxylase, Angew. Chem. Int. Ed., 2024, 63, e202314566.

2. Zhou Y., Peng S., Bornscheuer U.T.*, Wu S.*, Recent advances in enzyme cascade reactions for the efficient synthesis of functionalized chemicals and complex pharmaceuticals, Curr. Opin. Chem. Biol., 2026, 92, 102687.

3. Xu Z., Qin Z., Wen S., Wu Y., Chen T., Zhou Y.*, Wu S.*, Biosynthesis of 4-ethylphenol via an enzyme cascade with an engineered fatty acid photodecarboxylase, J. Agri. Food Chem., 2026, 74, 22942-22951.

4. Qin Z., Zhou Y.*, Peng S., Zheng W., Xu Z., Bai Y., Höhne M., Ward T.R., Bornscheuer U.T.*, Wu S.*, Ultrahigh-Throughput Evolution of a Photoenzyme for Efficient Bioproduction of Toluene and p-Cresol. Submitted.

## Biography



Shuke Wu is currently a full professor at the College of Life Science and Technology, Huazhong Agricultural University (HZAU), China, where he established his independent research group in 2021. He received his B.S. in Biotechnology from Peking University and his Ph.D. in Chemical and Pharmaceutical Engineering from the National University of Singapore, under the joint supervision of Prof. Zhi Li and Prof. Daniel Wang (MIT). He then pursued postdoctoral training with Prof. Thomas Ward at the University of Basel (Switzerland), followed by an Alexander von Humboldt fellowship with Prof. Uwe Bornscheuer at the University of Greifswald (Germany). His research interests center on biocatalysis, enzyme engineering, (chemo-)enzymatic cascades, and synthetic biology. To date, he has secured multiple research grants from the NSFC, Hubei Province, and industrial partners. He has authored over 50 papers in high-impact journals including *Nat. Commun.*, *Angew. Chem.*, *J. Am. Chem. Soc.*, and *ACS Catal.*, with cumulative citations exceeding 4,000.

# Young Scientist Forum

Dalian, China | 18-22,October 2026
Metabolic Engineering Summit 2026

# Establishing a next-generation C1 biofoundry at BRIGHT

Rikke Rasmussen[1], Jeffrey Vieregg[2], Lachlan Munro[1], Viji Kandasamy[1], Kelly Markham[1], Fungmin (Eric) Liew[2], Shivani Garg[2], Rasmus Jensen[2], Ching Leang[2], James Clomburg[2], Michael Köpke[2], Jochen Förster[1]

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## Abstract

Gas fermentation converts C1 gases – CO, $CO_2$ and methane – into chemicals, fuels and food, offering industrial bioproduction that does not compete for arable land. Progress is limited by the pace of strain engineering rather than reactor design: automated infrastructure to screen non-model organisms and engineer strains under toxic, flammable gas conditions remains scarce, and DBTL cycles that take days in model hosts can take months for gas-fermenting microbes. In collaboration with LanzaTech, BRIGHT (Technical University of Denmark) is establishing a dedicated C1 biofoundry to shorten these cycles. The platform builds on LanzaTech's second-generation biofoundry – a validated, integrated robotics system operating under controlled anaerobic atmosphere with safe C1 gas handling, developed for engineering acetogens. As a third-generation platform it will add full gas flexibility, including aerobic and high-temperature operation, extending the engineerable palette to methanotrophs and thermophiles. Four reference organisms – *Cupriavidus necator*, *Methylococcus capsulatus*, *Thermoanaerobacter kivui* and *Clostridium autoethanogenum* – represent the metabolic-engineering challenges the platform is designed to address. Once validated, it will operate with open access for academic and industrial users. This talk presents the platform's design, the reference organisms anchoring its development, and the workflows it is designed to enable.

**Key words:** gas fermentation; C1 biofoundry; laboratory automation; non-model organisms; strain engineering

**Representative publication:**

## Biography

As the designated Head of the C1 Biofoundry at BRIGHT (Technical University of Denmark), Rikke Rasmussen serves as technical lead and primary interface between BRIGHT, LanzaTech, and DTU during the establishment of a next-generation automated platform for engineering gas-fermenting microbes. Before joining BRIGHT, she spent over a decade as an automation engineer in the U.S. biotechnology sector, including roles at Synthego, Pivot Bio, and Hexagon Bio, where she built and scaled automated workflows for microbial culturing, screening, and strain engineering. She specialises in laboratory automation for biological systems, with a focus on increasing the throughput, reliability, and reproducibility of experiments involving non-model and hard-to-handle organisms. At BRIGHT she initiated the establishment of a dedicated high-throughput laboratory which was completed in May 2025. She now leads the effort to translate research workflows into system design specifications for the C1 Biofoundry.

# Short-chain alcohols fueled orthogonal conversion of pyruvate via a non-natural cofactor

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## Abstract

As platform chemicals, short-chain alcohols can serve as energy donors for biocatalytic applications. Ubiquitous nicotinamide cofactors such as nicotinamide adenine dinucleotide mediate biological energy transfer, but lack specificity to route exogenous reducing equivalents toward target products directionally. Herein, we developed non-natural cofactor nicotinamide cytosine dinucleotide (NCD)-dependent multi-enzyme catalytic cascades that efficiently channeled the reducing power of short-chain alcohols to drive pyruvate conversion. Guided by thermodynamic analysis, NCD-preferring methanol dehydrogenase, formaldehyde dehydrogenase and formate dehydrogenase were coupled with either D-lactate dehydrogenase or malic enzyme. This design overcame the thermodynamic barrier of methanol oxidation and enabled pyruvate reduction and reductive carboxylation, with stoichiometric energy conversion efficiencies reaching 81% and 95%. We further constructed whole-cell biocatalysis for selective reduction of pyruvate to D-lactate, attaining 26% and 41% energy conversion efficiencies using methanol and formaldehyde as exogenous energy donors. Additional short-chain alcohols were then tested as energy donors. Notably, isopropanol achieved a maximum energy yield of 100% for D-lactate, indicating the orthogonal delivery of exogenous chemical energy into target product via NCD mediation. This study establishes an efficient non-natural cofactor-mediated biocatalytic strategy using short-chain alcohols as fuels for reductive biosynthesis, offering a robust platform for designing high-performance redox biotransformations.

**Key words:** Short-chain alcohols, Enzyme cascade, Nicotinamide cytosine dinucleotide, Energy transfer, Pyruvate reduction

**Representative publication:**

1. Guo X., Liu Y., Wang Q., Wang X., Li Q., Liu W., Zhao Z.K.*, Non-natural cofactor and formate-driven reductive carboxylation of pyruvate, Angewandte Chemie International Edition, 2020, 59, 3143-46.

2. Wang X., Feng Y., Guo X., Wang Q., Ning S., Li Q., Wang J., Wang L., Zhao Z.K.*, Creating enzymes and self-sufficient cells for biosynthesis of the non-natural cofactor nicotinamide cytosine dinucleotide, Nature Communications, 2021, 12, 2116.

3. Wang J., Guo X.*, Wan L., Liu Y., Xue H., Zhao Z.K.*, Engineering formaldehyde dehydrogenase from *Pseudomonas putida* to favor nicotinamide cytosine dinucleotide, ChemBioChem, 2022, 23, e202100697.

## Biography



Xiaojia Guo is an Associate Professor at the School of Bioengineering, Dalian University of Technology, Dalian, China. Her research interests include synthetic biology based on non-natural cofactors, enzyme engineering, metabolic engineering, and chemical biology.

# Engineering Schizochytrium as a Next-Generation Cell Factory for Functional Lipid Biosynthesis

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## Abstract

The growing demand for functional lipids, including omega-3 polyunsaturated fatty acids (PUFAs) and high-value lipid derivatives, has driven the development of microbial cell factories as sustainable alternatives to traditional extraction methods. *Schizochytrium* sp., a thraustochytrid marine microalga, is a promising chassis organism due to its natural capacity for high lipid accumulation and rapid heterotrophic growth. In this study, we engineered *Schizochytrium* as a next-generation cell factory for enhanced production of functional lipid compounds through advanced synthetic biology strategies. We systematically optimized the lipid biosynthesis pathway by overexpressing key enzymes, including fatty acid synthases and desaturases, while simultaneously knocking down competing pathways to redirect carbon flux toward target products. Additionally, we implemented CRISPR-based genome editing and modular pathway assembly to improve genetic stability and metabolic efficiency. The engineered strain demonstrated significantly increased titers of docosahexaenoic acid (DHA) and other functional lipids compared to the wild-type control, with improved productivity under fed-batch fermentation conditions. These results establish *Schizochytrium* as a robust platform for industrial-scale lipid manufacturing and highlight the potential of synthetic biology in constructing efficient microbial cell factories for high-value natural products.

**Key words:** Omega-3 polyunsaturated fatty acids; Microbial cell factories; Schizochytrium; lipid compounds

**Representative publication:**

1. Ma W., Li., Yan W.Q., Zhang Z.Y., Yan C.X., Huang P.W., Sun X.M.*, Efficient Biosynthesis of Odd-Chain Fatty Acids via Regulating the Supply and Consumption of Propionyl-CoA in *Schizochytrium* sp, Journal of Agricultural and Food Chemistry, 2023, 71(25), 9847-9855.

2. Ma W., Liu, M.Z., Zhang, Z.X., Xu, Y.X., Huang, P.W., Guo, D.S., Sun, X.M.*, Huang H.*, Efficient co-production of EPA and DHA by *Schizochytrium* sp. via regulation of the polyketide synthase pathway, Communications Biology, 2022, 5(1).

3. Ma W., Zhang Z.Y., Yang W.Q., Huang P.W., Gu Y., Sun X.M.*, Huang, H.*, Enhanced docosahexaenoic acid production from cane molasses by engineered and adaptively evolved *Schizochytrium* sp, Bioresource Technology, 2023, 376, 128833.

4. Ma W., Zhao L., Ma Y.D., Li Y.Q., Qin S., He B.F.*, Oriented efficient biosynthesis of rare ginsenoside Rh2 from PPD by compiling UGT-Yjic mutant with sucrose synthase, International Journal of Biological Macromolecules, 2020, 146, 853-859.

5. Wu H.X., Yang W.Q., Zhang Z.X., Lin Y.C., Ma W.*, Sun X.M.*, Huang, H., Deciphering the distinct roles of polyketide synthase subunits in polyunsaturated fatty acid and lipid accumulation in *Schizochytrium* sp., Food Bioscience, 2025, 63, 105751.

## Biography




Ma Wang, Associate Researcher at the School of Food and Pharmaceutical Engineering, Nanjing Normal University, focuses his research on the microbial synthesis of functional lipids driven by synthetic biology. He has presided over 7

national and provincial/ministerial-level projects and serves as a youth editorial board member of China Biotechnology. In recent years, he has published over 20 SCI papers in mainstream international journals, been granted 8 invention patents (with 3 transferred/licensed), and received one China Patent Award for Excellence.

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# Biomanufacturing of Omega Fatty Acids Driven by Synthetic Biology

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## Abstract

Omega fatty acids are unsaturated fatty acids classified by the position of the first double bond from the methyl (ω) end. Major types include omega-3, omega-6, omega-7, and omega-9 fatty acids, which contribute to the biosynthesis of essential endogenous molecules and support human health[1]. However, some omega fatty acids cannot be synthesized by the human body, while others cannot be produced in sufficient amounts to meet physiological demands, making dietary intake an important source. Conventional sources are mainly fish and a limited number of plant species, but their production is often constrained by low natural abundance, limited productivity, and high extraction costs. Developing alternative and sustainable routes for omega fatty acid production is therefore of considerable importance. As an oleaginous yeast, *Yarrowia lipolytica* is generally regarded as a safe microbial host and has been widely explored for the production of high-value oleochemicals because of its strong capacity for lipid biosynthesis and accumulation[2]. Through engineering of fatty acid and lipid metabolism, *Y. lipolytica* has been successfully developed as a microbial platform for the efficient biosynthesis of various omega fatty acids, including the omega-7 fatty acid palmitoleic acid[3], the omega-6 fatty acid arachidonic acid[4], and the omega-9 fatty acid nervonic acid[5].

**Key words:** Omega Fatty Acids; Synthetic Biology; Biomanufacturing; *Yarrowia lipolytica*; Metabolic Engineering

**Representative publication:**

1. Lin L., Ledesma-Amaro R.*, Ji X. J.*, Huang H.*, Harnessing oleaginous yeast to produce omega fatty acids, Trends in Biotechnology, 2024, 42(11), 1335 – 1338.
2. Wang K. F., Shi T. Q., Lin L., Wei P., Ledesma-Amaro R., Ji X. J.*, Huang H.*, Advances in synthetic biology tools paving the way for the biomanufacturing of unusual fatty acids using the *Yarrowia lipolytica* chassis, Biotechnology Advances, 2022, 59, 107984.
3. Zhou Y. F., Sun M. L., Lin L., Ledesma-Amaro R., Wang K. F.*, Ji X. J.*, Huang H., Dynamic regulation combined with systematic metabolic engineering for high-level palmitoleic acid accumulation in oleaginous yeast, Metabolic Engineering, 2025, 89, 33 – 46.

4. Wang J. P., Yu X., Wang K. F., Lin L., Liu H. H., Ledesma-Amaro R., Ji X. J.*, Reprogramming the fatty acid metabolism of *Yarrowia lipolytica* to produce the customized omega-6 polyunsaturated fatty acids, Bioresource Technology, 2023, 383, 129231.
5. Wang K. F., Lin L., Wei P., Ledesma-Amaro R., Ji X. J.*, Combining orthogonal plant and non-plant fatty acid biosynthesis pathways for efficient production of microbial oil enriched in nervonic acid in *Yarrowia lipolytica*, Bioresource Technology, 2023, 378, 129012.

## Biography



Kaifeng Wang is an Associate Professor at the College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University. His research focuses on engineering yeast chassis through synthetic biology for the efficient biosynthesis of lipophilic compounds. Using the oleaginous yeast *Yarrowia lipolytica* as a major microbial platform, his group has established biosynthetic routes for high-value omega fatty acids and their derivatives, as well as various terpenoids and aromatic compounds. He has led or participated in several research projects funded by the National Key Research and Development Program of China, the National Natural Science Foundation of China, the Natural Science Foundation of Jiangsu Province, and the China Postdoctoral Science Foundation. He has published nearly 20 SCI-indexed papers as first or corresponding author in journals including *Trends in Biotechnology*, *Biotechnology Advances*, *Metabolic Engineering*, *ACS Synthetic Biology*, and *Bioresource Technology*. He has also published three papers in Chinese core journals and holds three granted Chinese invention patents.

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Microbial synthesis of 4'-O-methylnorbelladine, the universal precursor of Amaryllidaceae alkaloids

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## Abstract

Amaryllidaceae alkaloids (AAs) are a pharmacologically valuable class of plant specialized metabolites, including galanthamine, the only FDA-approved natural drug for Alzheimer's disease. All AAs derive from the common precursor 4'-O-methylnorbelladine (4OMN), formed by condensation of p-tyramine and 3,4-dihydroxybenzaldehyde (3,4-DHBA). While p-tyramine production has been engineered in recombinant *Escherichia coli*, microbial synthesis of 3,4-DHBA had not previously been reported, limiting biotechnological access to the entire AA pathway. Here we present a method for microbial synthesis of 4OMN from L-tyrosine or glucose in recombinant *E. coli*. Two alternative routes to 3,4-DHBA were engineered: one via tyrosine ammonia lyase, the *E. coli* HpaBC complex, 4-coumarate-CoA ligase and hydroxycinnamoyl-CoA hydratase/lyase; another via 3-dihydroshikimate dehydratase and carboxylic acid reductase from 3-dihydroshikimate. Combined with p-tyramine biosynthesis and heterologous expression of norbelladine synthase, its reductase, and norbelladine 4'-O-methyltransferase from *Narcissus pseudonarcissus*, engineered strains (e.g., BABH10, BABH8) produced 3,4-DHBA and progressed toward 4OMN synthesis directly from glucose. This platform establishes the first reported microbial route to the universal AA precursor, providing a scalable, sustainable alternative to plant extraction and a foundation for producing diverse Amaryllidaceae alkaloids in engineered microbial cell factories.

**Key words:** Amaryllidaceae alkaloids; 4'-O-methylnorbelladine; microbial biosynthesis; metabolic engineering;

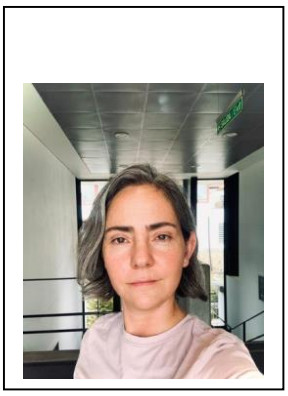
**Representative publication:**

1. **Villegas-Torres, M.F. et al.** *Síntesis microbiana de moléculas de plantas* [Microbial synthesis of plant molecules]. Colombian patent application No. NC2024/0015952, filed November 23, 2024, Universidad de los Andes.

2. Valderruten-Cajiao, M.; Salas-Nuñez, L.F.; Rodríguez-Ararat, A.C.; Caicedo, P.A.; Barrera-Ocampo, A.; Cortés, N.; Osorio, E.H.; Gamba-Sánchez, D.A.; González Barrios, A.F.; **Villegas-Torres, M.F.** Machine Learning-Assisted Discovery of Cytochromes P450 Putatively Involved in the Biosynthesis of Amaryllidaceae Alkaloids in Crinum asiaticum L. ACS Synth. Biol. 2026. DOI: 10.1021/acssynbio.5c00779.

3. Peña-Morales, M.; Vega-Paz, J.D.; Cortés, N.; Osorio, E.; Caicedo, P.A.; Barrera-Ocampo, A.; Álvarez, O.; González Barrios, A.F.; **Villegas-Torres, M.F.** Amaryllidaceae Alkaloids and Isoquinoline Alkaloids: A Perspective on Historical Approaches to Pathway Elucidation. Preprints 2025, 2025091631. DOI: 10.20944/preprints202509.1631.v1.

## Biography



María Francisca Villegas-Torres holds a PhD in Biochemical Engineering from University College London (UCL, 2014), an MSc in Biological Sciences (cum laude) and a BSc in Microbiology from Universidad de los Andes. She is currently Assistant Professor in the Department of Biological Sciences at Universidad de los Andes, where she leads a research line within the Centro de Investigaciones Microbiológicas (CIMIC) focused on microbial metabolic engineering for the biosynthesis of high-value plant natural products, including Amaryllidaceae alkaloids. Previously, as Full-Time Professor at Universidad Icesi (2016–2021), she founded and led the NATURA research group and a biomass-valorisation research centre, directing projects on carbon-fixation into fatty acids and alkaloids and on microbial consortia for biomethane production. During her doctoral and postdoctoral work at UCL (2009–2015), she developed de novo biosynthetic pathways for chiral amino-alcohol synthesis and bio-refinery protein-extraction processes. She has secured over USD 2.9 million in national and international research funding, authored more than fifteen peer-reviewed publications, and holds patent applications in microbial biosynthesis. Her research integrates synthetic biology, enzyme discovery, and machine learning approaches to engineer microbial cell factories for pharmaceutically relevant plant metabolites.

# Small RNAs Enable Programmable Metabolic Engineering at Conjugated Polymer–Microbe Interfaces

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## Abstract

Engineering microbial metabolism for electricity-driven biosynthesis has relied mainly on passive materials strategies to boost electron transfer, leaving the regulatory circuitry governing metabolic output largely untapped. Here we show that a conjugated redox-active polymer interface can actively trigger microbial regulatory reprogramming, establishing small RNAs (sRNAs) as an unrecognized control layer for metabolic engineering. Coupling *Shewanella oneidensis* MR-1 to electrodes coated with the n-type polymer PBFDO increased cathodic current density by two orders of magnitude and succinate titer up to twelve-fold versus polymer-free controls under inward extracellular electron transfer. Integrated transcriptomic, metabolomic, and electrochemical profiling attributed these gains to coordinated reprogramming, not passive conductivity, spanning the Mtr-CymA conduit, energy metabolism, and biofilm pathways, with elevated NADH/NAD+ and ATP/ADP ratios. We identified PBFDO-responsive sRNAs, delivering individual candidates into polymer-free reactors recapitulated key features of the polymer-induced phenotype, including up to threefold higher current output and shifted redox balance. This interface-sRNA-metabolism axis is a new engineering handle: microbial metabolic output can be tuned modularly and reversibly through synthetic material

interfaces that recruit native post-transcriptional regulators, without permanent genetic rewiring. This strategy offers a frontier design framework for programming electron flux and product formation in microbial electrosynthesis, bioelectronics, and biohybrid systems.

**Key words:** Extracellular electron transfer; Conjugated polymer; Small RNA (sRNA); Microbial electrosynthesis; Metabolic engineering

**Representative publication:**

1. Ohayon D.†, Liang Y.†, Wang Z., Foo J.L., Quek G., McCuskey S.R., Chen Z., Chang M.W.*, Bazan G.C.*, Conjugated polymer–microbe interactions trigger regulatory reprogramming for enhanced bioelectrocatalysis, accepted.

2. Liu Y., Song W., Zhang W., Liang Y., Saini M., et al., Mao X., Single-particle imaging uncovers reverse electron transfer efficiency between *Shewanella oneidensis* MR-1 and shaped haematite, Nature Catalysis, 2026, 9, 502–515.

## Biography



Dr. Yuanmei Liang is a Research Fellow in the Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, and a member of NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI) and the National Centre for Engineering Biology, working in Prof. Matthew Chang's laboratory. She received her Ph.D. in Environmental Engineering from Peking University in 2019, focusing on bioenergy, before moving into synthetic biology and metabolic engineering. Her research sits at the interface of synthetic biology, materials chemistry, and metabolic engineering, with a particular interest in how synthetic material interfaces can reprogram microbial regulatory networks to control cellular metabolism. Building on this, she is now expanding into one-carbon metabolism-based metabolic engineering, designing microbial chassis that convert C1 feedstocks such as $CO_2$, formate, and methanol into high-value chemicals, extending her interest in programmable microbial systems from bioelectronics toward sustainable, carbon-negative biomanufacturing.

# Ancestral reconstruction reveals a flavin-shielding design principle for photostable fatty acid photodecarboxylases

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## Abstract

Fatty acid photodecarboxylases (FAPs) convert fatty acids into hydrocarbons under blue light, but rapid photoinactivation limits sustained biocatalysis. Here, ancestral sequence reconstruction yielded photostable FAPs with activity half-lives up to 9.4 h, 27-fold longer than CvFAP, and provided an evolutionary series for dissecting this variation. Comparative photochemistry showed that flavin adenine dinucleotide (FAD) bleaching closely tracked activity loss, whereas limiting oxygen changed FAD spectral evolution and suppressed bleaching. These results support a role for oxygen in flavin photodamage during photoinactivation. Structural predictions, mutagenesis and simulations identified a remodeled lid-like loop that modulates substrate-access tunnel opening and may restrict oxygen access to FAD. Evolution-guided loop remodeling improved photostability in extant and ancestral scaffolds. An engineered ancestor sustained product accumulation over 36 h and reached near-stoichiometric conversion, whereas CvFAP plateaued after 12 h. These findings support dynamic flavin shielding as a design principle for engineering durable FAP catalysts.

**Key words:** fatty acid photodecarboxylase; ancestral sequence reconstruction; photoinactivation; flavin photodamage; flavin shielding

**Representative publication:**

1. Yang, G., Pecanac O., Wijma H.J., Rozeboom H.J., Gonzalo G., Fraaije M.W., Mascotti M.L.*, Evolution of the catalytic mechanism at the dawn of the Baeyer-Villiger monooxygenases. *Cell Reports* 43, 114130 (2024).

2. Drenth J.#, Yang G.#, Paul C.E., Fraaije M. W.*, A Tailor-Made Deazaflavin-Mediated Recycling System for Artificial Nicotinamide Cofactor Biomimetics. *ACS Catal.* 11, 11561–11569 (2021).

3. Bailleul G.#, Yang G.#, Nicoll C.R., Mattevi A., Fraaije M.W., Mascotti M.L.*, Evolution of enzyme functionality in the flavin-containing monooxygenases. *Nat. Commun.* 14, 1042 (2023).

# Biography



My name is Yang Guang, a faculty member in the College of Food Science and Pharmaceutical Engineering at Nanjing Normal University. I graduated from University of Groningen supervised by Marco W. Fraaije.

My research focuses on biocayalysis and protein engineering, with particular interests in ancestral sequence reconstruction and protein structure–function relationships. By integrating experimental approaches with computational methods, my work seeks to understand how enzymes evolve, function, and adapt, and to apply these insights to the design of proteins with improved or novel properties.

My broader interests include the application of artificial intelligence and data-driven tools in biological research. Through interdisciplinar collaboration, I aim to advance practical strategies for protein engineering and contribute to a deeper understanding of protein evolution and molecular function.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Rational engineering of cytochrome P450 catalysis overcomes biosynthetic bottlenecks for microbial manufacturing of complex natural products

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## Abstract

The efficient biomanufacturing of complex natural products is frequently limited by low-performing biosynthetic enzymes that constrain pathway flux and reduce strain productivity. Overcoming these bottlenecks remains a major challenge in developing commercially viable microbial production platforms. We addressed this challenge using doxorubicin biosynthesis as a model system by engineering the cytochrome P450 monooxygenase DoxA and its associated electron transfer machinery in *Streptomyces peucetius*. Through the integration of structural analysis with rational engineering of the natural redox partners Fdx4 and FdR3, we significantly enhanced P450 catalytic efficiency, driving a 180% increase in doxorubicin yields and optimizing the overall production profile. Mechanistic insights and density functional theory (DFT) calculations identified key determinants governing electron transfer and catalysis, establishing a rational framework for engineering P450-dependent biosynthetic pathways. Beyond improving anthracycline production, this work demonstrates a broadly applicable strategy for removing enzymatic bottlenecks in microbial cell factories. By combining mechanistic understanding with targeted enzyme engineering, our approach provides a scalable framework for accelerating the industrial biomanufacturing of high-value natural products, establishing a clear path for translating fundamental biosynthetic discoveries into robust, high-yielding industrial cell factories.

**Key words:** Metabolic engineering, Streptomyces, Cytochrome P450, Doxorubicin biosynthesis, Natural products

**Representative publication:**

1. Yamada K., Koroleva A., Artukka E., Newmister S.A. et al., Metabolic engineering of doxorubicin biosynthesis through P450-redox partner optimization and structural analysis of DoxA, *Nature Communications*, 2026, 17, 2358.

## Biography



Keith Yamada, PhD, is a research scientist and biotech entrepreneur at the University of Turku, Finland, specializing in the metabolic engineering of filamentous microorganisms for industrial and pharmaceutical applications.

His recent work resolved a 50-year enzymatic bottleneck in anthracycline biosynthesis, achieving a 180% improvement in doxorubicin yields, published in *Nature Communications* and nominated for the Falling Walls Science Breakthrough of the Year.

As co-founder and interim CEO of Metacell, Keith is building an ultra-high-throughput strain development platform to accelerate the translation of complex biosynthetic discoveries into scalable biomanufacturing processes.

He is attending MES2026 to connect with researchers and industry partners working at the frontier of sustainable bioproduction.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Design of a Novel Diamine Biosensor to Guide the Engineering of *Escherichia coli* for Cadaverine Overproduction

Jia Feng, Getao Liu, Ding Ma, Jiaxiang Yuan, Xinyi Zhang, Weihao Wang, Chen Ma, Yang Liao, Siyuan Gao, Lei Qin, Jiao Feng, Sheng Xu, Xin Wang*, and Kequan Chen*

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## Abstract

The development of *in vivo* biosensor systems represents a powerful strategy for engineering cell factories with desired production. Here, we reported a paradigm for the design, mechanistic elucidation, and optimization of the novel biosensor to detect cadaverine, an important polymer monomer. We identified a diamine-responsive promoter $P_{pyrF}$ via transcriptomic analysis for biosensor construction. Further investigation suggested that Ihfβ plays a key role in $P_{pyrF}$ activity regulation, while cadaverine and structurally related amines may activate $P_{pyrF}$ through modulation of Ihfβ-DNA interactions. Stepwise engineering was then performed to enhance biosensor performance, including mutagenesis of the promoter DNA sequence and the Ihfβ sequence, as well as RBS tuning, resulting in more than a 2.33-fold increase in fold induction. Guided by the biosensor, we implemented a multi-level engineering strategy that spanned tuning a specific exporter, mining optimized pathways from SCRaMbLE libraries, and genome-wide identification of beneficial gene targets. These iterative efforts improved cadaverine titer by 44% to a record 114.2 g/L in *Escherichia coli*, concurrently uncovering the critical roles of biofilm formation and carbon flux rewiring for overproduction. Our study establishes a biosensor-driven framework for optimizing complex pathways and uncovering novel targets in strain engineering.

**Key words:** Transcriptome, Responsive-promoters, Cadaverine biosensor, Transcription factor, High-throughput screening

**Representative publication:**

1. Feng J., Yuan J., Han Y., Liao Y., Xu S., Wang X., Chen K.*, Metabolic Engineering of *Escherichia coli* for the Production of Cadaverine from Lignocellulose, J. Agric. Food Chem., 2025, 73, 31444-31455.

2. Feng J., Han Y., Xu S., Liao Y., Wang Y., Xu S., Li H., Wang X., Chen K.*, Engineering RuBisCO-based shunt for improved cadaverine production in *Escherichia coli*, Bioresour. Technol., 2024, 398, 130529.

## Biography



Feng Jia is a postdoctoral fellow at the College of Biotechnology and Pharmaceutical Engineering, Nanjing Tech University. She received her Ph.D. in Bioengineering from Nanjing Tech university in 2026. Currently, she is conducting postdoctoral research under the supervision of Professor Wang Xin. Her primary research interests lie in synthetic biology and systems biology, with a particular focus on metabolic network rewiring in *Escherichia coli* and the biosynthesis of monomeric materials for sustainable applications. She has published three first-author papers in reputable international journals, including Journal of Agricultural and Food Chemistry, Bioresource Technology, and ACS Sustainable Chemistry & Engineering.

# Development of a Light-Inducible System in *Pichia kudriavzevii*

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## Abstract

Organic acid production in conventional hosts such as *Saccharomyces cerevisiae* and *Escherichia coli* demands costly pH neutralization that inflates environmental impact by ~30%. *Pichia kudriavzevii* sidesteps this problem through native acid and stress tolerance, yet no inducible promoter existed for this host, leaving constitutive expression as the only engineering option.

Here, we report *iss*OptoEXP, the first inducible expression system in *Pichia Kudriavzevii*, built on OptoEXP system previously applied for *S. cerevisiae*. Blue light illumination drives GFP expression ~190-fold over dark and ~4-fold above the strongest constitutive promoter available in this yeast, TDh3p. Varying light intensity and pulse duty cycle tunes expression across this range.

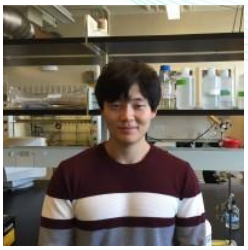
We applied *iss*OptoEXP to four metabolic targets: endogenous pyruvate decarboxylase and glycerol-3-phosphate dehydrogenase, redirecting flux toward ethanol and glycerol, pulse heterologous lactate dehydrogenase and citramalate synthase (cimA3.7) for lactic acid and citramalate. Light control further enabled two-phase fermentation, accumulating biomass in darkness before inducing production, outperforming constitutive expression while confirming that growth-to-production translates to this host. *iss*OptoEXP converts *Pichia Kudriavzevii* from a merely highly stress-tolerant chassis into one with dynamic control that has propelled metabolic engineering in other model organisms.

**Key words:** Optogenetics, *Pichia kudriavzevii*

**Representative publication:**

## Biography

Jaewan Jang is a postdoctoral fellow in the laboratory of José L. Avalos at the Omenn-Darling Bioengineering Institute, Princeton University, where his research applies optogenetics to metabolic engineering to improve existing bioprocesses.



# A hybrid system enables plasmid copy number control in yeast

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## Abstract

Synthetic biology requires plasmid systems with tunable copy numbers to balance genetic stability and expression capacity. Here, we developed a programmable plasmid copy number (PCN) platform in *Saccharomyces cerevisiae* by engineering the endogenous 2μ plasmid. First, reconstruction of the native 2μ plasmid in a 2μ-free chassis generated a stable high-copy vector with improved homogeneity and segregation stability. Building on this backbone, we introduced an inducible centromere-switching strategy that enabled reversible transitions from single-copy to multicopy states. Further integration of Leu2d-mediated metabolic selection expanded the copy number range and substantially enhanced recombinant protein expression. The platform supports precise gene dosage regulation across DNA, RNA, and protein levels and was successfully applied to tRNA functional analysis, recombinant GLP-1 precursor production, metabolic engineering of 2-phenylethanol, β-carotene, and ergothioneine, as well as improved preservation of production strains. This work establishes a versatile and quantitative plasmid toolkit for programmable gene dosage control in yeast, providing new opportunities for synthetic biology, functional genomics, and industrial biomanufacturing.

**Key words:** *Saccharomyces cerevisiae*; Plasmid copy number control; 2μ plasmid; Synthetic biology; Metabolic engineering

**Representative publication:**

1. **Li, A**., Zhao, Q., Yang, Z. et al. A hybrid system enables plasmid copy number control in yeast. *Nature Communications* (2026). https://doi.org/10.1038/s41467-026-75973-y
2. **Li, A**. et al. Expand copy number, stability and special adaptability of a selfish eukaryotic plamid. *Nature Microbiology*. In prepare.
3. **Li A**., Jiao S., et al. Syringenes A–L, bioactive dimeric eremophilane sesquiterpenoids from *Syringa pinnatifolia*. *Bioorganic Chemistry*., 2022, 125, 105879.

4. Sun J, **Li A**, et al. Dimeric eremophilane-type sesquiterpenoids from the peeled stems of *Syringa pinnatifolia*. *Phytochemistry*. 2024, 221:114048.

## Biography



Anni Li is a Postdoctoral Researcher at Xiamen University, China. She received her Ph.D. from the School of Life Sciences at Xiamen University under the supervision of Professor Zhouqing Luo. Her current research focuses on synthetic biology, with particular interest in the quantitative reconstruction and dynamic regulation of the endogenous 2μ plasmid system in *Saccharomyces cerevisiae*. Her work aims to develop programmable plasmid platforms for precise gene dosage control and advanced metabolic engineering. She previously earned her M.Sc. in Pharmaceutical Chemistry from Beijing University of Chinese Medicine, where her research focused on the isolation, purification, and structural characterization of bioactive natural products from *Syringa pinnatifolia*.

# An efficient microbial secretion platform for scalable production of protein-based materials from C2 feedstocks

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## Abstract

Nature-inspired protein materials boast superior mechanical and biological properties, but efficient and sustainable production remains a challenge. To address this, we developed a microbial secretion platform in *Corynebacterium glutamicum* for recombinant proteins overproduction using C2 feedstock as the primary carbon source, featuring a simplified purification process for the development of functional protein materials. To achieve higher protein yields, we leveraged the concept of integrating high-throughput genotype–phenotype data into cell factory design. This included CRISPRi–microfluidics screening for genome-scale target identification and *in situ* targeted mutagenesis tools for pinpointing key functional mutations that improve protein yield and secretion. Using this strategy, we achieved efficient secretion of diverse natural structural proteins in *C. glutamicum*, including collagen, supercharged elastin-like polypeptides, resilin, and squid ring teeth proteins. These recombinant proteins were further processed into advanced biomaterials such as high-strength protein fibers and ultra-strong bio-glues.

**Key words:** Microbial protein cell factory, C2 feedstock, Functional protein materials, Sustainable production.

**Representative publication:**

1. Xinyu Yu, Xiuxia Liu, Chong Zhang, Zhonghu Bai. Development of a novel platform for recombinant protein production in *Corynebacterium glutamicum* on ethanol. Synthetic and Systems Biotechnology, 2022, 7(2): 765-774.
2. Xinyu Yu, Zhonghu Bai, Xiuxia Liu, Chong Zhang. CRISPRi-microfluidics screening enables genome-scale target identification for high-titer protein production and secretion. Metabolic Engineering, 2023, 75: 192-204.
3. Jiacheng Sun[#], Xinyu Yu[#], Jiahe Li[#], Xue Z, Kai Liu, Chong Zhang. An efficient microbial secretion platform for developing supercharged polypeptides-based functional materials from C2 feedstock. Chemical Engineering Journal, 2025, 519: 165683.
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## Biography



Xinyu Yu received his B.Eng. and M.Eng. degrees from Jiangnan University and his Ph.D. from Tsinghua University, where he is currently a postdoctoral researcher in the Department of Chemical Engineering. His research has consistently focused on the sustainable biomanufacturing of recombinant proteins. Through years of research, he has developed a *Corynebacterium glutamicum*-based protein secretion platform that enables metabolic auto-induction using C2 feedstocks such as ethanol and acetate. This platform combines low production cost, environmental sustainability, and high-level protein expression, achieving protein production performance at an internationally competitive level. His related work has been published as first-author articles in journals including *Metabolic Engineering*, *Chemical Engineering Journal*, and *Synthetic and Systems Biotechnology*.

# PCR Leaping Enables High-Throughput Cloning of Biosynthetic Gene Clusters

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## Abstract

Genome mining has revealed a vast number of microbial biosynthetic gene clusters (BGCs), but experimental characterization of these clusters remains limited by the difficulty of cloning large DNA fragments at high throughput. BAC and cosmid libraries provide a robust means to preserve large genomic fragments, yet screening such libraries for specific sequences remains laborious. Here, we present leaping PCR, a simple and scalable method for high-throughput screening of BAC libraries. The approach has enabled the cloning of more than 150 BGCs and contributed to the discovery of three new bioactive natural products. PCR leaping originates from a previously unrecognized PCR phenomenon. Leaping occurs at apparently random positions, is independent of sequence repeats, and requires a single-piece template, indicating a mechanism distinct from conventional mispriming or jumping PCR. It is observed across different polymerases, template qualities, GC contents and PCR programs, suggesting that it is an intrinsic and broadly relevant feature of PCR. While PCR leaping can confound interpretation in applications such as CRISPR genome-editing validation, it also provides a simple means of pairing distant DNA regions. We harnessed this property to develop the high-throughput BAC library screening strategy described here.

**Key words:** BGC cloning; natural products; DNA library

**Representative publication:**

1. X. Jiang, T. S. Jorgensen, F. Wang, A. Lien, T. Gren, L. J. Munro, et al. A long-hidden prevalent PCR artifact and its application in DNA library screening, bioRxiv 2026 Pages 2026.07. 17.737729

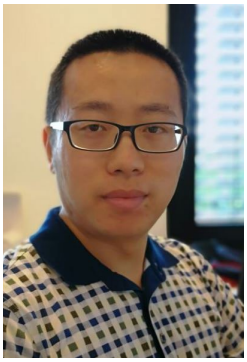
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## Biography



**Xinglin Jiang** is a researcher at DTU Bioengineering, Technical University of Denmark. His research focuses on developing new molecular and synthetic biology technologies, with particular interests in DNA amplification, recombination, genome engineering, and the discovery and engineering of microbial natural products. His work combines fundamental studies of DNA-processing mechanisms with the development of practical biotechnology platforms. He has developed high-throughput approaches for cloning and screening large biosynthetic gene clusters from microbial genomes, enabling systematic exploration of natural-product biosynthesis.

# Low-pH-Driven Succinic Acid Biomanufacturing: Chassis Optimization and Metabolic Remodeling of *Yarrowia lipolytica*

Zhiyong Cui

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## Abstract

Succinic acid is a key platform chemical, with global demand projected to reach 135,000 tons by 2032. Current bacterial fermentation requires neutral pH, increasing downstream separation and purification costs. Using the acid-tolerant, non-conventional yeast *Yarrowia lipolytica*, we developed a genetic toolkit including a non-homologous integration method and a finely regulated promoter library. By enhancing the endogenous oxidative TCA cycle and mining succinic acid transporters, we constructed high-producing strains under low pH. Then, through mitochondrial compartmentalization and cofactor regulation strategies, we established the reductive TCA pathway in this strictly aerobic yeast, achieving 111.9 g/L succinic acid with a yield of 0.79 g/g glucose — internationally leading performance. For non-food feedstock utilization, we enhanced inhibitor tolerance and perturbed xylose metabolism. The engineered strain produced 83.7 g/L succinic acid from straw hydrolysate as the sole carbon source, with a yield of 0.66 g/g mixed sugars. This study elucidates the metabolic regulatory mechanisms of succinic acid biosynthesis in *Y. lipolytica* and lays a foundation for developing China's bio-based materials industry.

**Key words:** Succinic Acid, *Yarrowia lipolytica*, Cell Factory, Low-pH Fermentation, Non-Food Feedstock

**Representative publication:**

1. Cui Z.Y., Zhong Y.T., Sun Z.J., Jiang Z.N., Deng J.Y., Wang Q., Nielsen J., Hou J.*, Qi Q.S.*, Reconfiguration of the reductive TCA cycle enables high-level succinic acid production by *Yarrowia ipolytica*. Nat Commun. 2023, 14(1): 111.
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3. Tao H.L., Hao A.M., Pan X.Y., Zhong Y.T., Cui Z.Y.*, Qi Q.S.*, Engineered non-canonical reductive TCA pathway drives high-yield succinic acid biosynthesis in *Yarrowia lipolytica*. Metab Eng. 2025, 6; 94: 36-44.
4. Zhong Y.T., Pan X.Y., Shi A.Q., Gonzalez R., Qi Q.S.*, Cui Z.Y.*, Engineering *Yarrowia lipolytica* as a next-generation biomanufacturing platform for sustainable lignocellulosic biorefineries. Biotechnol Adv. 2026, 12; 90: 108920.
5. Kang F.B., Gu F., Zhong Y.T., Cui Z.Y.*, Liang Q.F.*, Qi Q.S., Expanding the genetic toolkit of *Yarrowia lipolytica*: Dynamic promoter engineering enables high-titer biosynthesis of 3-hydroxypropionic acid. Bioresour Technol. 2025, 432: 132656.

## Biography



Cui Zhiyong is an Associate Research Fellow at the State Key Laboratory of Microbial Technology, Shandong University. His research focuses on microbial metabolic regulation and bio-based chemical synthesis. Over the past five years, he has led multiple projects including a National Natural Science Foundation Youth Program (Category C) and sub-projects under the National Key R&D Program. He has published numerous papers as first or corresponding author in prestigious journals such as Nature Communications, Advanced Science, and Metabolic Engineering, with cumulative citations exceeding 1,600. He has applied for nine national invention patents, four of which have been granted. His achievements have been highly recognized by leading enterprises including Shenghong Group, Yuanli Chemical, and Lanshan Tunhe. Notably, the low-pH succinic acid fermentation technology developed under his leadership has completed a major technology transfer valued at over ten million yuan. He was selected for the Shandong University Youth Scholar Future Plan and received the Industry Special Award at the China·Shandong (Qingdao) Postdoctoral Innovation and Entrepreneurship Achievement Competition. He serves as a Youth Editorial Board Member for Green Carbon, BioDesign Research, and the Chinese Journal of Biotechnology.

**Development of Advanced Gene Expression Tools for Bacillus licheniformis: Engineered Translation Systems and Dynamic Induction Platforms for Microbial Cell Factory Applications**

This report focuses on the development of a genetic expression toolbox for *Bacillus licheniformis*, a promising chassis cell with superior protein secretion capacity, high tolerance to extreme conditions, and a broad range of applications in industrial biotechnology. Compared to other systems, such as *Escherichia coli* and yeast, *B. licheniformis* exhibits advantages including faster growth, higher secretion capability, the absence of endotoxins, and better adaptability to harsh environments. However, the lack of precisely predictable and controllable gene expression tools remains a bottleneck.

To address this, the report introduces several advancements in gene expression systems for B. licheniformis. A wide-range and highly predictable synthetic hairpin ribosome binding site (shRBS) library was constructed, enabling improved ribosome recognition, enhanced mRNA stability, and efficient translation initiation. A prediction model was also established, capable of predicting translation rates in *B. subtilis* and improving protein expression by incorporating multiple shRBSs as translation initiation sites. Furthermore, a novel genetic tool termed Engineered Multiple Translation Initiation Sites (EMTIS) was developed. EMTIS proved effective in enhancing the production of both extracellular (e.g., keratinase and arginase) and intracellular enzymes, demonstrating broad applicability.

In addition, the report explores a multi-factor, wide-range gene induction system in B. licheniformis, including IPTG-inducible systems and other carbon-source-specific induction systems such as xylose and mannitol. These tools collectively expand the capacity for dynamic and precise regulation of gene expression, enabling high-efficiency applications in the production of various valuable biomolecules, including $\gamma$-polyglutamic acid, plant-derived natural products, industrial enzymes, amino acids, nucleic acid derivatives, and antimicrobial peptides.

This work provides a robust foundation for leveraging *B. licheniformis* as a versatile microbial cell factory and contributes to advancing the field of metabolic engineering and synthetic biology.

# Construction and Optimization of Biosynthetic Pathways for Multiple Organic Acids

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## Abstract

Green biomanufacturing from renewable resources is a crucial approach for resource substitution and carbon emission reduction. However, the complex metabolic regulation in industrial filamentous fungi and the challenge of precisely rewiring carbon flux in non-model bacteria limit efficient production. Here, we report our recent progress in engineering *Aspergillus niger* for L-malic acid production and expanding its application for green synthesis of organic acids and natural products. Concurrently, we systematically constructed a *Pseudomonas putida* cell factory for the targeted biosynthesis of various organic acids, including *cis*, *cis*-muconic acid and *β*-ketoadipic acid. Our findings reveal that precursor supply and cofactor generation are key factors for regulating carbon flux and improving productivity. Through metabolic pathway optimization and fermentation process regulation, we have established a green biomanufacturing chain from lignin to C4 organic acids, providing technical support for process upgrading in biorefineries and carbon neutrality goals.

**Key words:** *Aspergillus niger*, *Pseudomonas putida*, L-malic acid, muconic acid, metabolic engineering

**Representative publications:**

1. **Liu H**, Chang MY, Zhang C, Xu Q. Shaping *Aspergillus niger* into a next-generation cell factory for L-malic acid. Curr Opin Biotechnol. 2026; 98:103452-103461

2. **Liu H**, Xu T, Ntakirutimana S, Liu ZH*, Li BZ*, Yuan YJ. Engineering *Pseudomonas putida* for lignin bioconversion into *cis*,*cis*-muconic acid. Chem Eng J. 2024; 495:153375.

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## Biography



He Liu is an Associate Professor at Nanjing Normal University. She received her Ph.D. in Synthetic Biology from Tianjin University in 2024. Her research focuses on microbial production of organic acids and their high-value conversion. She is currently engineering *Aspergillus niger* for L-malic acid production and expanding the application of industrial filamentous fungi for green synthesis of organic acids and natural products. She has systematically constructed a *Pseudomonas putida* cell factory for the targeted biosynthesis of various organic acids, including *cis*, *cis*-muconic acid and *β*-ketoadipic acid. She has published over ten papers in peer-reviewed journals, including *Chem Eng J*, *Biotechnol Adv*, *ACS Sustainable Chem*, *Ind Crops Prod*, and *Curr Opin Biotechnol*.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Biosystem design of *Corynebacterium glutamicum* cell factory for producing biochemicals from lignocellulosic biomass

Kai Li[1], Cheng Li[2,3], Chenguang Liu[1], Xin-Qing Zhao[1], Anthony J. Sinskey[2,3], Gregory Stephanopoulos[4], Feng-Wu Bai[1]

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## Abstract

Being a Gram-positive bacterium, *Corynebacterium glutamicum* is highly diverse and has been recognized for its efficiency in producing amino acids and bulk chemicals with significant yields, thus becoming a preferable option for industrial biotechnology applications. In our first work, *C. glutamicum* was developed for efficient production of succinic acid from corn stover (CS) pretreated by concentrated-alkali under steam-assistant (CASA) conditions. Our engineered strain of *C. glutamicum* was able to produce more than 120 g/L succinate from glucose through fed-batch fermentation and could simultaneously use glucose and xylose to produce succinic acid from corn stover hydrolysate. Upon utilization of real lignocellulosic hydrolysate, the engineered strain still exhibited favorable succinic acid fermentation performance. We also engineered *C. glutamicum* to produce α-carotene from lignocellulosic hydrolysate. α-carotene is a food colorant, animal feed supplement, cosmetic, and a nutraceutical with potential health benefits, including reducing the risk of cardiovascular disease and cancer. Our work involved constructing a stable consortium of *C. glutamicum* to efficiently utilize glucose and xylose in lignocellulosic hydrolysate to produce α-carotene. Compared to single strain fermentation, the consortium demonstrated increased rates of glucose and xylose utilization and an overall higher α-carotene yield.

**Key words:** *Corynebacterium glutamicum*; lignocellulose; succinic acid; α-carotene

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

1. **Kai Li,** Cheng Li, Chen-Guang Liu, Xin-Qing Zhao, Ruiwen Ou, Charles A. Swofford, Feng-Wu Bai, Gregory Stephanopoulos, Anthony J. Sinskey*. Engineering carbon source division of labor for efficient α-carotene production in *Corynebacterium glutamicum*. Metabolic Engineering 2024, 84:117-127.
2. Chun Wan, Xue-Qing Wang, Hou-Ru Yue, Ming-Ming Zhang, Akihiko Kondo, Riaan den Haan, Tomohisa Hasunuma, **Kai Li***, and Xin-Qing Zhao*. *MDG1*-mediated transcriptional reprogramming enhances cellulase production and alters thermal activity in recombinant *Saccharomyces cerevisiae*. Metabolic Engineering 2025，93：23-24
3. Yu-Chen Liang, **Kai Li***, Xin-Qing Zhao, Feng-Wu Bai, and Chen-Guang Liu*. Enhanced glucose utilization and succinic acid production in *Corynebacterium glutamicum* by integration of metabolic engineering and electro-fermentation. Chemical Engineering Journal 2025: 159522.
4. Ming-Hou Li, Han Li, Xue Zhang, Yu-Chen Liang, Cheng Li, Meng-Lin Sun, **Kai Li***, Chen-Guang Liu, Anthony J. Sinskey. Metabolic engineering of *Corynebacterium glutamicum*: unlocking its potential as a key cell factory platform for organic acid production. Biotechnology Advances 2024: 108475.
5. **Kai Li**, Xue Zhang, Cheng Li, Yu-Cheng Liang, Xin-Qing Zhao, Chen-Guang Liu*, Anthony J Sinskey, Feng-Wu Bai. Systems metabolic engineering of *Corynebacterium glutamicum* to assimilate formic acid for biomass accumulation and succinic acid production. Bioresource Technology 2024, 402:130774.

## Biography

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Dr. Kai Li is an Assistant Research Professor at Shanghai Jiao Tong University. He has previously conducted visiting scholar and postdoctoral research at the Massachusetts Institute of Technology (MIT) and Shanghai Jiao Tong University. His primary research interests focus on the construction of high-efficiency microbial cell factories and the metabolic regulation of microbial stress tolerance. As first author and corresponding author, Dr. Li has published over 30 papers in internationally renowned journals, including Metabolic Engineering, Biotechnology Advances, Biotechnology and Bioengineering, Chemical Engineering Journal, ACS Sustainable Chemistry & Engineering, Bioresource Technology, and Chemical Engineering Science. He has filed six invention patents for innovative technological achievements, four of which have been granted. Dr. Li serves as Principal Investigator for projects funded by the National Natural Science Foundation of China (NSFC) and multiple industry-commissioned projects. He has also been invited to serve as a reviewer for international journals such as Trends in Food Science & Technology and Metabolic Engineering. He has received several national and international awards, including the YABEC Emerging Researcher Award. Additionally, Dr. Li is a member of the editorial board of Microbial Biotechnology and a Young Editorial Board member of BioDesign Research.

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Efficient genome editing for oleaginous yeast *Rhodotorula toruloides*

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## Abstract

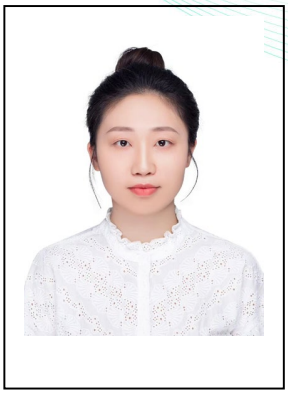
The oleaginous red yeast *Rhodotorula toruloides* is a promising microbial cell factory for lipid biosynthesis, yet its genetic manipulation heavily relies on error-prone non-homologous end-joining (NHEJ), which causes random integration and hinders rational strain engineering. Here we systematically characterized its DNA-repair machinery to boost homologous recombination (HR). Overexpressing HR-related proteins raised HR efficiency from <0.1% to 3.6%. Knocking out *RtKU70*/*RtKU80* further improved HR but impaired stress tolerance. Combining HR-protein overexpression with Ku deletion reached 5.9% HR efficiency and partially rescued stress hypersensitivity. Strikingly, CRISPR-Cas9-mediated DNA double-strand breaks, coupled with *ScRAD52* overexpression, drove HR efficiency up to 75–95% at multiple genomic loci. This work establishes powerful genome-editing tools for *R. toruloides* and offers references for other oleaginous basidiomycetous yeasts.

**Key words:** *Rhodotorula toruloides*; Basidiomycetous yeast; Homologous recombination; CRISPR-Cas9; DNA repair machinery; Genetic engineering

**Representative publication:**

1. Chen QQ, Lyu LT*, Wang S, Zhao ZK*. Systematic evaluation of the DNA repair machinery facilitated the development of efficient genome editing tools for the red yeast *Rhodotorula toruloides*. Synthetic and Systems Biotechnology 2026, 17, 96-105.

## Biography



Liting Lyu is an Assistant Professor at the School of Bioengineering, Dalian University of Technology, Dalian, China. Her research interests cover synthetic biology and metabolic engineering of oleaginous basidiomycetous yeasts.

Dalian, China 18-22,October 2026
Metabolic Engineering Summit 2026

# ESTEEM Strategy: Revolutionizing Enzymatic Catalysis through the Evolutionary Gateway of Enzyme Tunnels

Ruyue Dong, Jiaying Wang, Jian Tian, Guoshun Xu, Ziqi Liang, Xing Qin, Xiaolu Wang, Xiaoqing Liu, Huiying Luo, Bin Yao, Yaru Wang*, Tao Tu*

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## Abstract

Elucidating enzyme behavior and engineering highly efficient biocatalysts remain fundamental challenges in biocatalysis. Despite advances in enzyme engineering, significant optimization opportunities persist. Herein, we introduce the Evolutionary-Structural Tuning of Enzyme Efficiency Modules (ESTEEM) strategy, which integrates evolutionary principles with structural scaffold analysis to accelerate enzyme design. Using cytochrome P450 monooxygenase (P450s) enzymes with deeply buried active sites and intricate substrate tunnels as models, we identified key nonconserved residues (I67 and Q83) within the substrate tunnel. These distal positions critically influence tunnel architecture, and their mutation dramatically enhanced catalytic efficiency. The optimal double-variant exhibited a 106-fold increase in total turnover number without compromising stability while simultaneously broadening the substrate scope. Through adaptive steered molecular dynamics and hydrogen–deuterium exchange mass spectrometry, we uncovered a tunnel-mediated tuning mechanism: activity differences between variants and wild type were governed by a rigidity-flexibility balance in the scaffold, which modulates catalytic efficiency by stabilizing tunnel geometry. Experimental validation and retrospective analysis confirmed the robustness of the ESTEEM strategy. This study establishes how tunnel mutations stabilize substrate transport pathways to enhance catalysis, providing a framework for engineering enzymes specialized activity toward substrates requiring deeply recessed active sites advancing applications in biocatalysis and synthetic biology.

**Key words:** Evolutionary conservation; Substrate tunnels; Cytochrome P450 monooxygenase; Conformational dynamics; Protein engineering

**Representative publication:**

1. Dong R. Y., Wang J. Y., Tian J., Xu G. S., Liang Z. Q., Qin X., Wang X. L., Liu X. Q., Luo H. Y., Yao B., Wang Y. R.*, Tu T.*, ESTEEM Strategy: Revolutionizing Enzymatic Catalysis through the Evolutionary Gateway of Enzyme Tunnels, *ACS Catalysis*, 2025, 15, 20108–20122.
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4. Liao M., Dong R. Y., Li L. X., Liu X. Q., Wang Y. R., Qin X., Bai Y. G., Luo H. Y., Yao B., Huang H. Q., Tu T.*, High production of maltooligosaccharides in the starch liquefaction process: A study on the hyperthermophilic mechanism of alpha-amylase, *Journal of Agricultural and Food Chemistry*, 2023, 71(16), 6480–6489.
5. Tu T.*, Wang Q., Dong R. Y., Liu X. Q., Penttinen L., Hakulinen N., Tian J., Zhang W., Wang Y. R., Luo H. Y., Yao B., Huang H. Q., Achieving thermostability of a phytase with resistance up to 100 °C, *Journal of Biological Chemistry*, 2024, 300(12), 107876.

## Biography



Dr. Ruyue Dong is an Assistant Research Fellow at the Biotechnology Research Institute, Chinese Academy of Agricultural Sciences (CAAS). She received her Ph.D. in Biochemistry and Molecular Biology from the Graduate School of CAAS. Her research focuses on the intelligent design, directed evolution, and biological synthesis of enzymes, aiming to elucidate metabolic regulatory mechanisms and develop efficient biomanufacturing solutions using computational and synthetic biology strategies. Her research findings have been published in leading peer-reviewed journals, including *ACS Catalysis* and *Bioresource Technology*, and backed by multiple authorized patents.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Model-Driven Dynamic Circuit Design for Quorum-Sensing Activation at High Cell Densities

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## Abstract

Premature expression of engineered functions can impose metabolic burden, limiting biomass accumulation and overall process performance. Quorum sensing (QS) provides an inducer-free strategy for coupling gene expression to population density, yet many engineered QS systems activate prematurely, restricting their use in high-cell-density bioprocesses. Here, we developed a model-driven framework for engineering LuxI/LuxR QS circuits with programmable activation thresholds and independently tunable output levels. Deterministic modeling of acyl-homoserine lactone production, transport, and regulatory dynamics, combined with Sobol sensitivity analysis, identified LuxI abundance and AHL dynamics as the primary determinants of activation timing, whereas LuxR stability predominantly controlled output amplitude. These insights guided circuit redesign through feedback rewiring and coordinated transcriptional, translational, and plasmid-level tuning. The resulting circuits shifted activation from early exponential growth to late and ultra-late growth, with optimized variants exhibiting sharp induction above $OD_{600}$ of 50 in fed-batch bioreactors while maintaining robust biomass accumulation. We further demonstrated the versatility of this platform through QS-controlled membrane-vesicle production, CRISPRi-mediated population control and co-culture composition regulation, and transfer of optimized circuit architectures to *Rhodopseudomonas palustris*. These results establish a generalizable platform for programmable density-dependent regulation across high-cell-density bioprocessing, biomanufacturing, and engineered microbial communities.

**Key words:** quorum sensing; LuxI/LuxR circuits; density-dependent regulation; modeling; high-cell-density bioprocesses.

**Representative publication:**

1. Arab B., Ochs C., Chen J., Lan C., Yari A., Edghill M., Ingalls B., Chou C. P., Liu Y.*, Model-driven engineering of synthetic quorum-sensing circuits enables wide-range dynamic control, 2026, [Submitted to Nature Microbiology, NMICROBIOL-26072990-T].

2. Liu Y., Chen J., Crisante D., Jaramillo Lopez J. M., Mahadevan R.*, Dynamic cell programming with quorum sensing-controlled CRISPRi circuit, ACS Synthetic Biology, 2020, 9(6), 1284-1291.

3. Golla S. A., Abo-Hashesh M., Gupta D., Liu Y., Mahadevan R.*, Model-Based Optimization of a qCRISPRi Circuit for Dynamic Control of Metabolic Pathways, ACS Synthetic Biology, 2025, 14(10), 3890-3898.

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5. Chen J., Liu M., Chen S., Chou C. P., Liu H., Wu D., Liu Y.*, Engineered Therapeutic Bacteria with High-Yield Membrane Vesicle Production Inspired by Eukaryotic Membrane Curvature for Treating Inflammatory Bowel Disease, ACS nano, 2025, 19(2), 2405-2418.

## Biography



Bahareh Arab is a postdoctoral fellow in the Department of Chemical Engineering at the University of Waterloo. Her research lies at the intersection of metabolic engineering and synthetic biology, with a focus on engineering microbial systems for sustainable biomanufacturing. She completed her PhD in Chemical Engineering at the University of Waterloo, where she developed genome-engineering and pathway-optimization strategies for the microbial production of porphyrin-based compounds. Her current research focuses on programmable gene circuits, quorum-sensing-based regulation, and engineered microbial consortia. She combines experimental synthetic biology with computational modeling to understand and control gene expression, cellular behavior, and population-level interactions. Her work aims to develop robust and controllable microbial platforms that connect fundamental biological design principles with practical applications in biomanufacturing. Her broader research interests include dynamic bioprocess regulation, adaptive laboratory evolution, microbial production of natural pigments, and plastic biorecycling.

# Establishing *Vibrio natriegens* as a high-performance host for acetate-based poly-3-hydroxybutyrate production

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[1]*School of Molecular Sciences, The University of Western Australia, Crawley 6009, Australia*

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## Abstract

Acetate can be a sustainable and renewable carbon source which holds significant promise for biotechnological production but is underutilized industrially due to limited microbial efficiency. *Vibrio natriegens*, recognized for exceptionally fast growth rates, represents a compelling host for developing efficient acetate-based bioprocesses. In this study, adaptive laboratory evolution significantly enhanced *V. natriegens*' ability to grow on acetate as the sole carbon source, achieving an 89% increase in growth rate. Genetic and transcriptomic analyses revealed key adaptations improving acetate uptake and metabolism via increased salt tolerance, boosted Pta/AckA pathway activity, and rewired quorum sensing. Further metabolic engineering and bioprocess optimization enabled the evolved strain to reach high cell densities and efficiently convert acetate into the bioplastic poly-3-hydroxybutyrate (PHB), with productivities up to 0.27 g/L/h and PHB accumulation reaching 45.66% of cell biomass. These advances position *V. natriegens* as a highly promising microbial platform for sustainable, scalable, and cost-effective biomanufacturing using acetate as green feedstock.

**Key words:**

Acetate, Vibrio natriegens, Polyhydroxyalkanoates, Adaptive laboratory evolution

**Representative publication:**

1. Politan RJ, Della Valle S, Pineda L, Joshi J, Euler C, Flematti G, et al. Establishing *Vibrio natriegens* as a high-performance host for acetate-based poly-3-hydroxybutyrate production. Metab Eng. 2025 Nov;92:22–38. doi:10.1016/j.ymben.2025.07.003

2. Faber A, Politan RJ, Stukenberg D, Morris K, Kim R, Jeon E, et al. Expanding genetic engineering capabilities in *Vibrio natriegens* with the Vnat Collection. Nucleic Acids Res. 2025 Jun. doi:https://doi.org/10.1093/nar/gkaf580

3. Della Valle S, Faber A, Politan RJ, Lama S, Fritz G. From marsh to market: taming *Vibrio natriegens* for sustainable bioproduction. Curr Opin Biotechnol. 2025 Dec 1;96:103353. doi:10.1016/j.copbio.2025.103353

## Biography

Dr Roland Jansen Politan is a postdoctoral researcher under Associate Professor Georg Fritz in the School of Molecular Sciences at The University of Western Australia (UWA), working at the intersection of synthetic biology and sustainable bioproduction. After completing undergraduate and honours studies at Curtin University, he undertook his PhD at UWA, in collaboration with Woodside Energy, where he developed methods to engineer *Vibrio natriegens* — the fastest-growing bacterium known to science — to produce the bioplastic poly-3-hydroxybutyrate from acetate, a feedstock derivable from captured CO2. Building on this foundation, his research now centres on engineering *V. natriegens* as a broader platform for converting waste carbon into biodegradable plastic. He is currently extending this work through the Minderoo Foundation-backed VIBRANT project, applying the same organism to convert agricultural waste into biodegradable plastic.



Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Iterative dissolved-oxygen control combined with growth – production coordination enables efficient erythritol biosynthesis in *Yarrowia lipolytica* under high-temperature stress

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## Abstract

A comprehensive understanding of the growth–production trade-off under heat stress is essential for developing high-yield thermotolerant chassis. Here, the effects of elevated temperature on growth and erythritol biosynthesis were systematically investigated in YL (A high-erythritol-producing industrial *Yarrowia lipolytica* strain). Notably, a heat-induced abnormal growth-surge effect (AGSE) was observed and identified, in which excess ATP and ROS under high dissolved oxygen redirect metabolic fluxes toward stress-protective metabolites. Based on this, iterative dissolved-oxygen optimization under high-temperature conditions enabled the development of a robust erythritol-producing chassis, YL-EV-18-G5, which exhibited stable growth at 35℃ with erythritol yield of 0.526 g/g glucose. Further, a hybrid erythritol-responsive bidirectional promoter ($P_{ery\text{-}BidR\ (5AB)}$) was applied for random genomic integration, thereby coupling erythritol synthesis with the expression of growth-related genes, yielded a growth-production-coordinated strain, YL-EV-18-G5-GPcEs4-F12 (F12). Compared with parental strain YL, F12 exhibited a 179.3% increase in erythritol titer, reaching a maximum concentration of 225.50 g/L, and the yield increased by 35.34% to 0.656 g/g glucose. Mechanistically, high-productivity of F12 arises from optimized stress-protective metabolite synthesis, reduced single-

cell erythritol production heterogeneity, and adaptive stress-tolerance mutations. These findings advance the understanding of thermotolerance in oleaginous yeasts and provide a broadly applicable strategy for engineering heat-resistant microbial cell factories.

**Key words:** *Yarrowia lipolytica*, iterative dissolved-oxygen control, high-temperature stress, erythritol, growth-production coordination

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

1. **Bai W**, Huang M, Li C, Li J. The biological principles and advanced applications of DSB repair in CRISPRmediated yeast genome editing. Synthetic and systems biotechnology. 2023 Aug 30;8(4):584-96.

2. Xu K, Qin L, **Bai W**, Wang X, Li F, Ren S, Gao X, Chen B, Tong Y, Li J, Li BZ. Multilevel defense system (MDS) relieves multiple stresses for economically boosting ethanol production of industrial Saccharomyces cerevisiae. ACS Energy Letters. 2020 Jan 21;5(2):572-82.

3. Li F ‡, **Bai W** ‡, Zhang Y, Zhang Z, Zhang D, Shen N, Yuan J, Zhao G, Wang X. Construction of an economical xylose-utilizing Saccharomyces cerevisiae and its ethanol fermentation. FEMS Yeast Research. 2024;24:foae001.

4. Zhang J‡, Zhao G‡, **Bai W**‡, Chen Y‡, Zhang Y, Li F, Wang M, Shen Y, Wang Y, Wang X, Li C. A Genome-wide Evolution-Based CRISPR/Cas9 with Donor-Free (GEbCD) for Developing Robust and Productive Industrial Yeast. ACS Synthetic Biology. 2024 Jul 16.

5. Tong Y, Zhang Y, **Bai W**, Wang X, et al. Industrial brewer's yeast stress resistance modification based on brewer's yeast CRISPR library: CN 109628448A[P]. 2019-04-16.

## Biography



- 2025.09- , Tsinghua University, Biochemical Engineering, Postdoc.
- 2020.10-2025.04, University of Warwick(UK), Environment Microbiology, Ph.D.
- 2018.08-2019.06, COFCO NHRI Co., Ltd., Research Assistant.
- 2016.09-2018.06, Beijing Institute of Technology, Bioengineering, MEng.
- 2012.09-2016.06, Beijing Institute of Technology, Bioengineering, BEng.

# Development of a Sustainable Process for Manufacturing Aquaculture Feed

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## Abstract

The expansion of aquaculture is increasing demand for sustainable sources of protein, lipids and functional feed additives. Conventional aquaculture feeds remain dependent on wild-caught fish, agricultural commodities and chemically synthesized ingredients. This work develops an integrated gas-fermentation and microbial-biomanufacturing process for producing a multi-component fish feed from C1 feedstocks.

The process employs complementary microbial production modules with division of labour across protein-rich biomass, carotenoid pigments and omega-3-rich oils. Instead of isolating each product through separate purification processes, the nutrient-rich microbial biomass is designed for direct combination into a composite feed ingredient. This strategy could reduce downstream processing, improve resource efficiency and retain multiple nutritional components within the final product.

Initial studies have established key production and analytical workflows for evaluating biomass formation, lipid accumulation and carotenoid production. Ongoing development focuses on improving the productivity and nutritional composition of each module, integrating the fermentation processes and assessing the stability and suitability of the combined biomass for aquaculture applications.

The proposed platform provides a flexible route for converting low-value gaseous carbon into nutritionally complementary microbial biomass and has the potential to reduce reliance on marine resources and conventional feed-manufacturing supply chains.

**Key words:** Gas fermentation, C1 biomanufacturing, Microbial cell factory, Aquaculture feed, Labour division

**Representative publication:**

1. Wei Jiang, David Hernández Villamor, Huadong Peng, Jian Chen, Long Liu, Victoria Haritos, Rodrigo Ledesma-Amaro*. 2021. Metabolic Engineering Strategies to enable microbial utilization of C1 feedstocks. Nature Chemical Biology. 17 (8), 845-855

2. Wei Jiang, William Newell, Jingjing Liu, Lucas Coppens, Khushboo Borah Slater, Huadong Peng, David Bell, Long Liu, Victoria Haritos, Rodrigo Ledesma-Amaro*. 2025. Insights in the methanol utilization capacity of Y. lipolytica and its improvement by metabolic engineering. Metabolic Engineering. 91: 30-43.

3. Wei Jiang, Shengbao Wang, Paulo Avila, Tue Sparholt Jørgensen, Zhijie Yang, Irina Borodina*. 2024. Combinatorial iterative method for metabolic engineering of Yarrowia lipolytica: Application for betanin biosynthesis. Metabolic Engineering. 86, 78-88.

## Biography



Dr Wei Jiang is a Postdoctoral Research Fellow at the Australian Institute for Bioengineering and Nanotechnology, The University of Queensland. Her research focuses on yeast synthetic biology, metabolic engineering, genome engineering and sustainable microbial biomanufacturing. She received her PhD in Chemical Engineering from Monash University, with research conducted at Monash University and Imperial College London. Before joining The University of Queensland, she worked as a postdoctoral researcher at the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.

Her research has centred on developing genetic engineering platforms for non-conventional yeasts, particularly Yarrowia lipolytica, and applying these tools to produce food ingredients, natural products and other high-value biochemicals. She has developed modular pathway-assembly and CRISPR-based genome-engineering toolkits and engineered pathways for C1-carbon utilization. At The University of Queensland, her current work explores the integrated microbial production of protein, carotenoids and nutritional oils from sustainable feedstocks, aiming to develop scalable biomanufacturing processes for aquaculture and future food applications.

# Synthetic Biology Platform for Production of Novel Anthracycline Anticancer Agents

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## Abstract

Anthracyclines such as doxorubicin are cornerstone chemotherapeutic agents prescribed to over three million cancer patients annually. Despite high cytotoxic potency, their clinical use is limited by irreversible dose-dependent cardiotoxicity. Recent investigations indicate that generating cardiotoxicity-free anthracyclines may be possible through uncoupling DNA damage from chromatin damage. However, semi-synthetic derivatization toward this goal is complicated by the structural complexity of anthracyclines. Here, we report a modular synthetic biology platform for the complete refactoring and combinatorial biosynthesis of anthracyclines in *Streptomyces coelicolor* M1152Δ*matAB*. Using standardized synthetic DNA parts, we reconstructed three full biosynthetic gene clusters—nogalamycin (38 genes), doxorubicin (31 genes), and aclacinomycin (31 genes)—from the bottom up. The pathways were organized into four functional modules (aglycone biosynthesis, TDP-carbohydrate formation, glycosylation, and tailoring reactions), enabling efficient reconstruction. The modular design facilitated streamlined combinatorial biosynthesis, which yielded 16 derivatives, including 13 novel analogs. Bioactivity profiling across human cancer cell lines identified six active analogs. Two hit molecules harbored desired bioactivity profiles promoting histone eviction at a faster rate than doxorubicin, but without causing DNA double strand breaks. Our work highlights the power of synthetic biology in rational natural product engineering and provides a scalable strategy for exploring improved anthracycline anti-cancer agents.

**Key words:** synthetic biology, BioBricks, natural product biosynthesis, anthracycline, *Streptomyces coelicolor*

**Representative publication:**

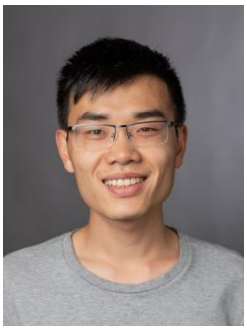
1. **Wang R.**[#], Wandi B.[#], Schwartz N.[#], Hecht J.[#], Ponomareva L., Paige K., West A., Desanti K., Nguyen J., Niemi J., Thorson J., Shaaban K.*, Metsä-Ketelä M.*, Nybo S.*, Diverse combinatorial biosynthesis strategies for C–H functionalization of anthracyclinones, ACS Synthetic Biology, 2024, 13, 5: 1523-1536.

2. **Wang R.**[#], Nguyen J.[#], Hecht J., Schwartz N., Brown K., Ponomareva L., Niemczura M., van Dissel D., van Wezel G., Thorson J., Metsä-Ketelä M.*, Shaaban K.*, Nybo S.*, A BioBricks metabolic engineering platform for the biosynthesis of anthracyclinones in *Streptomyces coelicolor*, ACS Synthetic Biology, 2022, 11, 12: 4193-4209.

3. Koroleva A.[#], Artukka E.[#], Yamada K.[#], Newmister S. A.[#], Harte R. J., Boesger H., Londen M., Sanders J., Tirkkonen H., Kannisto M., Kuin R., Hulst M., **Wang R.**, Leskinen E., Barillec M., Niemi J., van Wezel G., Neefjes J., Nybo S., Houk K., Sherman D., Kim R., Metsä-Ketelä M.*, Metabolic engineering of doxorubicin biosynthesis through P450-redox partner optimization and structural analysis of DoxA. Nature Communications, 2026, 17, 1, 2358.

4. Niemczura M., Nuutila A., **Wang R.**, Rauhanen K., Nybo S., Metsä-Ketelä M.*, Three-enzyme cascade catalyzes conversion of auramycinone to resomycin in chartreusin biosynthesis, ACS Chemical Biology, 2025, 20, 7: 1457-1463.

## Biography



Dr. Rongbin Wang is a postdoctoral research fellow at the University of Turku, Finland, funded by Novo Nordisk Foundation. He received his bachelor's and master's degrees from Jiangnan University, China, and earned his Ph.D. degree from the University of Turku in 2025 for his work on developing a synthetic biology platform for production of novel anthracycline anticancer agents. Following his Ph.D., he worked at University of Turku as a postdoctoral researcher and was awarded Novo Nordisk Foundation Postdoctoral Fellowship in 2026. His research focuses on natural product biosynthesis, synthetic biology, and metabolic engineering, with particular expertise in pathway engineering, genome mining, and biochemical characterization. His current research aims to expand the chemical diversity of microbial natural products and facilitate the discovery of next-generation therapeutics.

# Postgraduate Panel Session

# Enhancement of Pyruvate Derivative Production in Methanotrophic Bacteria via Integrated Carbon Fixation and CRISPR Base Editing

Xiaohan Huang[1]，Shuqi Guo[1]，Qiang Fei[1*]

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## Abstract

Methanotrophic bacteria hold great potential for greenhouse gas valorization and biomanufacturing due to their ability to use methane as the sole carbon and energy source. However, their industrial application is constrained by intrinsic metabolic limitations that restrict pyruvate accumulation and the production of downstream derivatives. Carbon loss through CO2 release further reduces carbon conservation and precursor availability, while conventional genetic engineering remains limited by low editing efficiency and laborious procedures.

To overcome these metabolic and genetic bottlenecks, we established an integrated strategy combining carbon fixation pathway reconstruction with precise genome editing. Introduction of the Calvin-Benson-Bassham (CBB) cycle enables reassimilation of metabolically released CO2 into central metabolism, thereby improving carbon conservation and precursor availability. Meanwhile, a CRISPR-based cytosine base editing (CBE) system enables rapid and precise in situ genome modification, facilitating rational metabolic network remodeling and alleviating constraints on pyruvate biosynthesis. Together, these strategies provide an efficient microbial engineering framework for enhancing pyruvate-derived compound production and promoting the circular utilization of C1 gas resources.

**Key words:** Methanotrophic bacteria, Pyruvate derivatives, Calvin-Benson-Bassham cycle, CRISPR base editing, Metabolic engineering

**Representative publication:**

1. Huang X.H., Song Q.Q., Guo S.Q., Fei Q.*, Transcription regulation strategies in methylotrophs: progress and challenges, Bioresources and Bioprocessing, 2022, 9, 126.

2. Guo S.Q., Li C.B., Su Y.H., Huang X.H., Zhang C.Y., Dai Y.Z., Ji Y., Fu R.Z., Zheng T.T., Fei Q.*, Fan D.D.*, Xia C. *, Scalable electro-biosynthesis of ectoine from greenhouse gases, Angewandte Chemie International Edition, 2025, 64, e202415445.

3. Zhang X.Z., Li A.P., Huang X.H., Guo S.Q., Zhang C.Y., Gonzalez R., Fei Q.*, Metabolic engineering of methanotrophic bacteria for *de novo* production of taxadiene from methane, ACS Synthetic Biology, 2025, 14, 8.

4. Guo S.Q., Jing Y.M., Huang X.H., Liu B.H., Lackner M., Fei Q.*, LexA deficiency confers enhanced thermotolerance and glycogen accumulation in *Methylomicrobium buryatense*, Bioresource Technology, 2026, 462, 135635.

## Biography



Xiaohan Huang is a Ph.D. candidate at Xi'an Jiaotong University, having earned a Bachelor's degree from Sichuan University. Her research concentrates on synthetic biology and metabolic engineering, with a primary focus on developing advanced genetic manipulation tools and molecular platforms for non-model methanotrophic bacteria.

The work aims to overcome native metabolic bottlenecks in C1-utilizing microorganisms by integrating precise genome editing technologies, such as CRISPR-based base editing and small regulatory RNA with rational metabolic network reconstruction. Through these strategies, her research facilitates the high-efficiency screening of key genetic elements, enhances carbon conservation efficiency, and drives the sustainable biosynthesis of high-value natural products from methane.

She has actively contributed to the field as an author of several peer-reviewed studies published in prestigious academic journals, including *Angewandte Chemie International Edition* and *ACS Synthetic Biology*.

# Development of a Cell-Free Enzymatic Cascade for $CO_2$-to-Formaldehyde Conversion

Tanya Ou[1], Ethan I. Lan[2*]

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## Abstract

Catalytic carbon dioxide reduction to formaldehyde is currently challenging due to unfavorable thermodynamics and the difficulty of suppressing overreduction beyond formaldehyde. While biocatalytic conversion of $CO_2$ to formaldehyde via formate dehydrogenase and formaldehyde dehydrogenase has been achieved previously, the high Km value and thermodynamic unfavourability present significant limitations. In this work, we developed a five-enzyme cascade for the conversion of carbon dioxide to formaldehyde. This cell-free synthesis approach uses pyruvate as an organic carbon acceptor that captures carbon dioxide for its subsequent reduction. We stepwise analyzed the efficiency and rate of this reaction pathway and improved its performance by recruiting higher-performing enzymes. Finally, we increased the reaction rate by 4.7-fold compared to the initial design. This pathway also has the potential to support the development of synthetic autotrophs based on existing methylotrophic organisms.

**Key words:** cell-free, synthetic pathway, $CO_2$ utilization, C1 building block

## Biography



Tanya is a M.S. student at the Institute of Molecular Medicine & Bioengineering, National Yang Ming Chiao Tung University (NYCU), where she also completed her B.S. in Biological Science and Technology. Working under the supervision of Professor Ethan I. Lan, her current research focuses on the construction of in vitro synthetic pathway.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Improved Metabolic Flux Estimations through Compositional Data Analysis

Te Chen[1*], Alberte Sloth Carlsen[1*], Nicholas Luke Cowie[1], Christian Brinch[2], Teddy Groves[1#], Lars Keld Nielsen[1,3]

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## Abstract

Isotopic Metabolic Flux Analysis (I-MFA) is a standard approach for estimating intracellular metabolic fluxes. I-MFA infers fluxes by comparing simulated and measured metabolite isotopologue distributions (MIDs) of metabolites from isotope labeling experiments. MIDs represent fractional abundances that strictly sum to one for any given metabolite, thus they are inherently compositional data. However, state-of-the-art estimation approaches rely on calculating standard Euclidean distances between MIDs in a non-compositional paradigm, introducing a systemic bias. To resolve this, our study proposes compositional I-MFA. We demonstrate how to construct a meaningful orthonormal basis for MIDs via ordered sequential binary partitioning, which can be used to perform isometric log-ratio (ILR) transformation. As a minimal change to existing I-MFA workflows, we suggest estimating fluxes by minimizing Euclidean distances between ILR-transformed MIDs. We validated this framework against traditional methods using both a toy model and a biologically realistic model, evaluating point estimates, sensitivity across varied true fluxes, and confidence intervals. In the two examples, compositional I-MFA consistently outperformed traditional approaches, reducing mean squared error of flux point estimates by an average of 42.6% and substantially narrowing confidence intervals. We conclude that compositional data analysis significantly improves I-MFA and can be implemented as a simple drop-in replacement for current pipelines.

**Key words:**

Metabolic Flux Analysis, Compositional Data Analysis, Fluxomics, Mass Spectrometry, Uncertainty Quantification

**Representative publication:**

1.
Sigrist R, **Chen T**, Montané MR, et al. Heterologous production of a plant biostimulant in *Streptomyces albidoflavus*. *Applied Microbiology and Biotechnology* Published online June 20, 2026. doi:10.1007/s00253-026-13915-w

## Biography



Te Chen is a PhD candidate at the Technical University of Denmark (DTU), based at the Novo Nordisk Foundation Biotechnology Research Institute for the Green Transition (BRIGHT) under the supervision of Prof. Lars Keld Nielsen and Teddy Groves. He holds a BSc in Computational Biology from the University of Toronto and an MSc in Biotechnology from DTU. His research focuses on metabolic engineering and machine learning, with a particular emphasis on metabolic modeling and isotopic metabolic flux analysis.

# Discover the maze-like network for glabridin biosynthesis

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[1]*Department of Chemical Engineering/ Key Laboratory for Industrial Biocatalysis of Ministry of Education, Tsinghua University, Beijing, China*

[2]*Center for Synthetic and Systems Biology, Tsinghua University, Beijing, China*

[3]*Beijing Key Laboratory of Recombinant Protein Synthetic Biomanufacturing, Beijing, China*

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## Abstract

Tailoring enzymes diversify plant metabolite scaffolds through complex, context-dependent modifications, generating maze-like biosynthetic networks that complicate metabolic pathway reconstruction. Here, we systematically deconstructed and reconstructed the biosynthetic network of glabridin, a valuable skin-whitening isoflavone from Glycyrrhiza. By integrating metabolic pathway mining with genome and 183 transcriptomes, we identified four functional routes among sixteen theoretical possibilities and uncovered a previously uncharacterized, ladder-like multi-route tailoring network. Reconstruction of such architecture in yeast revealed metabolic redundancy and interconnectivity confer unexpected robustness, enabling higher production efficiencies compared to a single-route design. Further modular engineering enabled the de novo biosynthesis of glabridin in yeast. Our work establishes a generalizable framework for reconstructing metabolic mazes and demonstrates that multi-route architectures can be harnessed to enhance the robustness and productivity of cell factories.

**Key words:**

glabridin, tailoring enzymes, synthetic biology, plant natural protucts, yeast

**Representative publication:**

1. ZHANG Z, LI W, MENG F, et al. Discover the maze-like network for glabridin biosynthesis [J]. Nature Communications, 2026, 17(1).

## Biography



Meng Fanze (2000-), doctoral candidate from Department of Chemical Engineering, Tsinghua University, major in synthetic biology.

# Construction of a multi-copy system with flexible engineering space based on rDNA autonomous regeneration

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## Abstract

Multicopy expression remains a critical bottleneck in yeast cell factory engineering, as current approaches suffer from low copy numbers, genetic instability and limited tunability. Here, we present rDE, a multicopy system that exploits the native rDNA autonomous regeneration machinery to achieve high-copy, selection-free, and multilayer regulatable gene expression. We first leveraged a yeast chassis with chromosomal rDNA deletion (*rDNAΔ*) and reintegrated a chimeric 1.5-copy rDNA cassette harboring cargo DNA into the chromosome. Through induction, we achieved homogeneous and stable regeneration of a chimeric rDNA array with 59 copies using NTS1-35S as the landing pad, and the copy number can be further elevated to >100 copies by manipulating endogenous rDNA regulators like *RTT109* or *CCR4 etc*. We demonstrate that cargo expression is tunable through nucleolar recruitment of TFIIB, tRNA supply enhancement, and diploidization. As a proof-of-concept, rDE enabled ~20-fold enhanced production of full-length human type III collagen and achieved lycopene accumulation up to ~150-fold. rDE establishes a more flexible engineering space than previously appreciated, provides insights into the regulation of rDNA and offers broad application prospects for yeast bioengineering research.

**Key words:** rDNA; multicopy; Selection-free; Multilayer regulatable; Metabolic engineering

**Representative publication:**

1. Li, A., Zhao, Q., Yang, Z., Cheng, J., Xu, D., Zhang, Y., He, T., Zhuo, B., Zhao, X., **Rao, X**., Wang, H., Chen, L & Luo, Z. A hybrid system enables plasmid copy number control in yeast. *Nature Communications* (2026). https://doi.org/10.1038/s41467-026-75973-y
2. This manuscript is currently under submission.

## Biography



Xiaolin Rao is a Ph.D. candidate at Xiamen University, China. She is currently pursuing her doctoral degree at the School of Life Sciences at Xiamen University under the supervision of Professor Zhouqing Luo. Her current research focuses on synthetic biology and yeast cell factory engineering, with particular interest in the development of multicopy expression systems. Her work centers on the rDE system, which exploits the native rDNA autonomous regeneration machinery to achieve high-copy, selection-free, and multilayer-regulatable gene expression in *Saccharomyces cerevisiae*. Her work aims to develop programmable multicopy platforms for precise gene dosage control and advanced metabolic engineering, with demonstrated applications in enhanced production of full-length human type III collagen and lycopene accumulation.

# Functional Characterization of Enone Oxidoreductase Orthologs Highlights Hidden Furaneol Biosynthetic Capacity in Apples

Chan Li, Katharina Jahr, Yujun Sun, Benedikt Johannes Thalhammer, Julia Schweppe, Xiran Wang, Timothy D. Hoffmann, Thomas Hoffmann, Wilfried Schwab*

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## Abstract

4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF, Furaneol) is an important flavor compound in many fruits. It is formed from the precursor 4-hydroxy-5-methyl-2-methylene-3(2H)-furanone (HMMF) by an enone oxidoreductase (EO), which has previously been characterized only in strawberry, tomato, and mango. Here, we heterologously produced EO orthologs from pineapple, kiwi, and grape, three species known to accumulate HDMF, as well as from apple and crab apple, which do not produce this compound. All candidates were expressed in fruit tissue and exhibited o-quinone oxidoreductase activity, reducing 9,10-phenanthrenequinone and various o-quinones. Importantly, all enzymes catalyzed HDMF formation, including those from apple species. The absence of HDMF accumulation in apples was attributed to the lack of the natural precursor HMMF and low levels of active EO, while HDMF was rapidly converted to its glucoside. The conserved o-quinone oxidoreductase activity across all EO orthologs suggests that fruit EOs involved in HDMF biosynthesis evolved from a broader quinone oxidoreductase family.

**Key words:** Enone oxidoreductase; Quinone oxidoreductase; Furaneol; Strawberry;

**Representative publication:**

1. Li, C., Jahr, K., Sun, Y., Thalhammer, B. J., Schweppe, J., Wang, X., Hoffmann T.D., Hoffmann T., Schwab, W*. Functional Characterization of Enone Oxidoreductase Orthologs Highlights Hidden Furaneol Biosynthetic Capacity in Apples. *Journal of Agricultural and Food Chemistry*, 2026, 74(17), 13898-13908.

## Biography



Chan Li is a Ph.D. candidate at Technical University of Munich (TUM), working on the Biotechnology of Natural Products, currently focusing on the enzymes catalyze the biosynthesis of key fruit aroma compound Furaneol in strawberry. She obtained her M.S. and B.S. in Enology from Northwest A&F University, China. Her research integrates molecular cloning, protein enzymology, and metabolomics to decipher flavor-related pathways. Her work aims to uncover missing biosynthetic steps of Furaneol and contribute to industrial synthesis improvement through metabolic engineering.

# Green Biosynthesis of the Cosmetic Humectant Isoprene Glycol in *Escherichia coli* via Metabolic Engineering

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## Abstract

Isoprene glycol (ISPG) is a valuable cosmetic humectant traditionally produced via energy-intensive and non-sustainable petrochemical processes. To develop a green alternative, we engineered *Escherichia coli* to produce ISPG *de novo* directly from glucose. This newly engineered pathway converts the central metabolite acetyl-CoA into a non-natural intermediate, 3-hydroxy-3-methylbutyryl-CoA (HMB-CoA). We subsequently evaluated different reduction strategies and demonstrated that a carboxylic acid reductase (CAR)-mediated route achieved the highest ISPG production. Conducting enzyme bio-prospecting and enhancing intracellular coenzyme-A (CoA) availability enabled us to optimize the metabolic flux. Our final engineered strain produced an ISPG titer of 2.2 g/L with a yield of 0.11 g ISPG/g glucose. This work establishes an efficient, environmentally benign process, providing a renewable and commercially attractive alternative for industrial ISPG production.

**Key words:** Isoprene glycol, CoA-acylating aldehyde dehydrogenase, Carboxylic acid reductase, Coenzyme A supply, metabolic engineering

**Representative publication:**

1. Chen, A. Y.; Hung, J. J.; Lan, E. I., Biosynthesis of isoprene glycol from glucose by metabolically engineered Escherichia coli. Bioresource Technology 2026, 135539.

## Biography



Jenny received her B.S. and M.S. degree of Biological Science and Technology in National Yang Ming Chiao Tung University (NYCU), Taiwan. And continued to study her PhD at NYCU Degree Program of Biomedical Science & Engineering in 2022. Since 2018, she started working with Professor Ethan I. Lan (Professor of Department of Biological Science and Technology of National Yang Ming Chiao Tung University) on the metabolic engineering and synthetic biology.

# Metabolic rewiring of oleaginous yeast for efficient production of the aromatic compound tyrosol

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## Abstract

Tyrosol is a bioactive aromatic compound with broad applications in the food, cosmetic, and pharmaceutical industries. Conventional plant extraction is constrained by low efficiency and environmental dependence, whereas chemical synthesis often involves harsh conditions and environmental burdens. Here, we developed *Yarrowia lipolytica* as a microbial platform for sustainable tyrosol production by systematically rewiring its native aromatic amino acid and Ehrlich pathways. Sequential optimization of the shikimate, tyrosine biosynthetic, and Ehrlich pathways increased tyrosol production to 1,411.86 mg/L in shake-flask cultures. To further improve precursor availability, a heterologous non-oxidative glycolysis pathway was introduced to redirect central carbon flux and balance the supply of phosphoenolpyruvate (PEP) and erythrose-4-phosphate (E4P), the key precursors for aromatic amino acid biosynthesis. This metabolic rewiring increased the tyrosol titer to 1,616.09 mg/L, representing the highest reported shake-flask titer for microbial tyrosol production. Overall, this study demonstrates the potential of *Y. lipolytica* as an efficient chassis for tyrosol biosynthesis and highlights central carbon flux redistribution as an effective strategy for enhancing aromatic compound production.

**Key words:** aromatic compound, tyrosol, *Yarrowia lipolytica*, synthetic biology

**Representative publication:**

1. L Q.L., Yu T., Li X.W., Chen Y., Campbell K., Nielsen J., Chen Y.*, Rewiring carbon metabolism in yeast for high level production of aromatic chemicals, Nature Communications, 2019, 10, 4976.
2. Liu H.Y., Xiao Q.J., Wu X.X., Ma H., Li J., Guo X.F., Liu Z.Y., Zhang Y., Lou Y.Z.*, Mechanistic investigation of a D to N mutation in DAHP synthase that dictates carbon flux into the shikimate pathway in yeast, Communications Chemistry, 2023, 6, 152.

3. Hassing E.J., Groot P.A., Marquenie V.R., Pronk J.T., Daran J.M.G.*, Connecting central carbon and aromatic amino acid metabolisms to improve *de novo* 2-phenylethanol production in *Saccharomyces cerevisiae*, Metabolic Engineering, 2019, 56, 165-180.

4. Gu Y., Ma J.B., Zhu Y.L., Xu P.*, Refactoring Ehrlich Pathway for High-Yield 2-Phenylethanol Production in *Yarrowia lipolytica*, ACS Synthetic Biology, 2020, 9, 623-633.

5. Liu H.Y., Tian Y.J., Zhou Y., Kan Y.Y., Wu T.T., Xiao W.H., Lou Y.Z.*, Multi-modular engineering of *Saccharomyces cerevisiae* for high-titre production of tyrosol and salidroside, Microbial Biotechnology, 2021, 14, 2605-2616.

## Biography



Xin Wang is currently a Ph.D. candidate at Nanjing Tech University, with research interests in synthetic biology and metabolic engineering for the sustainable biosynthesis of aromatic compounds. His research focuses on engineering the oleaginous yeast *Yarrowia lipolytica* as a microbial cell factory, with particular emphasis on pathway reconstruction, central carbon metabolism rewiring, precursor supply optimization, and metabolic flux redistribution to enhance the production of value-added aromatic compounds. Through the integration of metabolic engineering and synthetic biology strategies, his work aims to develop efficient and scalable microbial platforms for the sustainable production of bio-based chemicals. To date, Xin Wang has published one SCI Q1 article as the first author and two EI-indexed papers, and holds three publicly disclosed invention patents. His research interests include microbial cell factory development, metabolic pathway engineering, aromatic amino acid biosynthesis, and the production of high-value aromatic compounds.

# *Cardamine hupingshanensis*: an emerging plant chassis to produce Selenolanthionine and sugar-associated selenium metabolites

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## Abstract

Selenium (Se) is an essential micronutrient, yet soil Se occurs predominantly in inorganic forms, and most crop species have limited capacity to convert inorganic Se into bioavailable organic compounds. *Cardamine hupingshanensis*, the only Se hyperaccumulator reported from China and one of the few edible Se-hyperaccumulating plants, offers a distinctive platform for investigating organic Se metabolism. Here, we characterized Se metabolic profiles across Se sources, supplied concentrations, and growth stages. Water-soluble selenolanthionine (SeLan) and a sugar-associated Se fraction emerged as major Se pools with distinct developmental patterns. Under 400 mg $L^{-1}$ selenate, SeLan accounted for up to 67.1% of total Se in seedling shoots, whereas sugar-associated Se reached 58.3% in bolting shoots. Time-course analysis further revealed rapid SeLan formation within 2 h of selenite exposure, in contrast to the more gradual accumulation of sugar-associated Se. Integrated transcriptomic and metabolomic analyses highlighted coordinated changes in glutamate-associated carbon-nitrogen and redox metabolism, sulfur/selenocompound metabolism, and glycosylation-related processes. Our results provide insights into the potential metabolic basis of these emerging organic Se forms and highlight *C. hupingshanensis* as a promising chassis for engineering the biosynthesis of organic Se compounds.

**Key words:** Selenium, Selenolanthionine; Selenosugar; *Cardamine hupingshanensis*; Se hyperaccumulator.

**Representative publication:**

1. Du G.X.[#], Duan Y.H.[#], Fu Y.L.[#], Yuan L.X.*, Li T.*, et al. The mechanism and application of the selenium hyperaccumulator Cardamine hupingshanensis: status and challenges, Annals of Botany, 2026, 137, 591-607.

2. Duan Y.H., Yu Y.H., Wang Y.D., Wang Z.Q.*, et al. Highly efficient biotransformation and production of selenium nanoparticles and polysaccharides using potential probiotic *Bacillus subtilis* T5, Metabolites, 2022, 12, 1204.

3. Duan Y.H.[#], Fang F.[#], Mu X., Wang Z.Q.*, and Liu R.*, et al. Exploration of *Streptomyces fradiae* J1-021 as a potential host for the heterologous production of spinosad, Journal of Agricultural and Food Chemistry, 2024, 72, 8983-8992.

## Biography



Yuhua Duan is a fourth-year PhD candidate in Plant Biochemistry at the University of Liverpool. Her doctoral research focuses on selenium metabolism in the selenium hyperaccumulator *Cardamine hupingshanensis*, with particular emphasis on selenium transformation, speciation, metabolic regulation, and plant responses to environmental stresses. Her broader research interests include selenium biofortification, plant-microbe interactions, selenium nanoparticles, and the metabolic engineering of selenium-containing compounds.

# Construction of an efficient and universal *Yarrowia lipolytica* chassis for the biosynthesis of pterocarpans and their derivatives

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## Abstract

Pterocarpans are a class of dihydroisoflavonoids featuring a characteristic tetracyclic scaffold consisting of benzopyran-furan-benzene, which exhibit diverse bioactivities including anti-tumor, antibacterial and anti-inflammatory effects. Typical simple pterocarpans include 3,9-dihydroxypterocarpan and medicarpin, which can be biosynthesized from simple flavonoid skeletons daidzein and formononetin via a four-step catalytic pathway, respectively. Subject to modifications by pterocarpan reductase (PTR), prenyltransferase(PT), cyclase(OC) and other enzymes, the two key intermediates can be further converted into dozens of derivatives. Previous studies revealed a severe yield bottleneck during the transformation from simple flavonoid skeletons to the two key intermediates: the titer dropped from hundreds of mg/L to hundreds of μg/L (nearly 1000-fold reduction), greatly limiting high-level biosynthesis of pterocarpans and their derivatives.

In this work, enzymes responsible for the four-step conversion from simple flavonoid substrates to key pterocarpan intermediates were sequentially screened and characterized. An engineered strain capable of efficiently converting formononetin into medicarpin was constructed. Heterologous PTRs from various sources were mined and screened to achieve efficient bioconversion of formononetin to vestitol, and key amino acid residues governing PTR activity were predicted. Taking advantage of the substrate promiscuity of isoenzymes, strains that produce key intermediates 3,9-dihydroxypterocarpan and demethylvestitol using daidzein as substrate were constructed merely via substrate switching.

Different PTs were introduced into the four chassis strains capable of synthesizing pterocarpans and derivatives, leading to the production of multiple prenylated products. Distinct substrate structural selectivity was observed among different PTs, and their catalytic mechanisms were elucidated. A preparative liquid chromatography method was established and optimized for product separation and purification. Purified high-purity compounds can supply samples for pharmacological research, new drug development and functional characterization of downstream pathway enzymes. In the future, these four chassis can serve as versatile and efficient hosts for PTs

assay and discovery of novel prenylated pterocarpan derivatives.Furthermore, diverse cyclized products were generated by introducing cyclases from distinct origins into strains producing four prenylated pterocarpan derivatives.

Finally, by integrating heterologous genes responsible for simple flavonoid skeleton biosynthesis, *de novo* biosynthesis of eleven pterocarpan compounds was achieved in *Yarrowia lipolytica* for the first time.

**Key words:** *Yarrowia lipolytica*, pterocarpans, pterocarpan reductase, prenyltransferase, *De novo* biosynthesis

## Biography



Shujing Qiao received her Bachelor's degree in Viticulture and Enology from Northwest A&F University and obtained her Master's degree in Biology and Medicine under the supervision of Prof. Yongjin Zhou at the Dalian Institute of Chemical Physics, Chinese Academy of Sciences. Currently, she is a joint-program PhD candidate supervised by the Institute of Chinese Medical Sciences, University of Macau and the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences. Her main research focuses on constructing a universal synthetic microbial platform for the production of medicinal natural pterocarpan compounds. She aims to establish efficient biosynthetic routes via elucidation of natural biosynthetic pathways, enzyme engineering and metabolic engineering optimization to support pharmacological investigation, new drug discovery and commercial supply. She has published two papers as the first or co-author and filed one patent.

# Solar-driven biosynthesis of chemicals from phosphite-rich wastewater and carbon dioxide

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## Abstract

Solar-driven biosynthesis from carbon dioxide can facilitate sustainable chemical manufacturing. However, cell-density limits and contamination susceptibility have seriously hampered its practical implementation. Here we develop a solar–chemical hybrid-driven biosynthesis (SCHB) strategy integrating wastewater-based phosphorus recovery into biological photosynthetic metabolism for chemical production. Specifically, we incorporate the phosphite oxidation pathway into cyanobacteria to supplement additional electrons to stimulate bacterial growth. This strategy conferred contamination resistance and enabled the utilization of phosphite-rich wastewater. A series of chemicals including raspberry ketone, indigo and its derivatives were effectively synthesized via SCHB, and the synthesis efficiency was promoted by a factor of up to 305%. Furthermore, the scalability of SCHB was demonstrated at the 500-litre level with real wastewater, which synchronized chemical production with nutrient recovery. Life-cycle assessment and techno-economic analysis indicated notable environmental benefits and economic feasibility. This study potentially opens a viable approach for the sustainable biosynthesis of chemicals.

**Key words:** Solar–chemical hybrid-driven biosynthesis; Carbon dioxide fixation; Cyanobacteria; Phosphite; Wastewater valorization

**Representative publication:**

1. Zheng, H., Li, C., Shao, H. *et al.* Solar-driven biosynthesis of chemicals from phosphite-rich wastewater and carbon dioxide. *Nat Sustain*, 2026, 9, 866–878.

## Biography

Haotian Zheng holds his bachelor's degree in biotechonology from Shanghai Jiao Tong University. He is currently a doctoral candidate in Shanghai Jiao Tong University under the supervision of Prof. Jun Ni. He mainly focuses on developing sustainable bioprocesses for carbon neutrality and waste valorization, with his recentwork on solar-chemical hybrid-driven biosynthesis published in *Nature Sustainability*.



# Development of a novel biocatalyst for renewable formaldehyde production

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## Abstract

The demand for formaldehyde is continuously increasing due to its versatile usage in construction and semiconductor industry. To reduce the petroleum-based formaldehyde production, many approaches have been demonstrated to produce formaldehyde from renewable resources such as carbon dioxide or bio methanol. However, these methods require expensive catalysts or undergo high temperature processes. In this research, we developed a whole cell bioconversion to reduce the cost of catalysts. We designed a synthetic formaldehyde producing pathway from glycerol, a renewable resource which can be obtained from moderate production process. By rerouting the natural homoserine producing pathway in *Escherichia coli*, we successfully produced formaldehyde from glycerol and demonstrated the potential to produce formaldehyde directly from carbon dioxide.

**Key words:** Formaldehyde production, Homoserine pathway, *Escherichia coli*, Whole cell bioconversion

## Biography

Kuei-Chieh is a M.S. student at the Institute of Molecular Medicine & Bioengineering, National Yang Ming Chiao Tung University (NYCU), where he also completed his B.S. in Biological Science and Technology. Working under the supervision of Professor Ethan I. Lan, his current research focuses on engineering novel metabolic pathways for formaldehyde production.



# Improving extracellular electron transfer of *Zymomonas mobilis* to build an industrial electroactive microbial chassis

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## Abstract

Electro-fermentation can modulate intracellular redox state, regulate metabolic flux in situ, and provide energy to cells, ultimately achieving higher yields and selectivity. However, the lack of strains with both efficient extracellular electron transfer (EET) and high production capacity is a key factor hindering the industrial application. Natural electroactive microorganisms are difficult to be applied in industry due to limited substrate ranges, low production efficiency, and pathogenicity. This study systematically engineered the electroactivity of *Zymomonas mobilis*, successfully constructing chassis strains with enhanced EET. First, Mtr pathway derived from *Shewanella oneidensis* was heterologously expressed to provide conductive proteins. On this basis, heme synthesis was enhanced to provide conductive cores for these proteins. Furthermore, endogenous Rnf electron transfer complex and flavin transferase ApbE were overexpressed to enhance EET. The strain with the highest electroactivity exhibited a 32.90% increase in $Fe^{3+}$ reduction capacity and a 70.42% improvement in coulombic efficiency in a microbial fuel cell. In electro-fermentation, the glycerol production of this strain in cathode increased by 19.96% compared with the wild-type strain, while the acetic acid production decreased by 27.65%. This work provides a foundation for the further design of electroactive industrial chassis and the industrial application of electro-fermentation.

**Key words:** Electro-fermentation, Extracellular electron transfer, Electroactive chassis, *Zymomonas mobilis*

**Representative publication:**

1. W.-T. Wang, Q. Feng, T.-T. Li, M.A. Mehmood, H. Zhu, N. Wang, Y. Ma, C.-G. Liu, F.-W. Bai, Redox-sensitive genetic circuits enhance microbial stress tolerance to complex inhibitors in lignocellulose biorefinery, Chemical Engineering Journal 543 (2026).

## Biography

2021/09-Present: PhD candidate in Bioengineering, Shanghai Jiao Tong University

2017/09-2021/06: Bachelor in Biotechnology, Shanghai Jiao Tong University



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Metabolic Engineering Summit 2026

# Variation in the synthesis of specialized metabolites in Erythroxylum coca: A biotechnological approach using callus cultures

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## Abstract

*Erythroxylum coca* is a source of pharmacologically relevant specialized metabolites including tropane alkaloids, flavonoids and terpenes, yet field-derived concentration is often insufficient for industrial use, and much of its metabolic repertoire remains unexplored. *In vitro* callus culture offers a reproducible, climate-independent platform for metabolite production to test conditions modulating specialized metabolism. This study evaluated whether biotic and abiotic inducers, including precursors and elicitors, can enhance specialized metabolite accumulation in *E. coca* callus cultures.

Callus tissue was established from leaf explants collected in Caldono, Cauca, Colombia, and propagated on modified Murashige-Tucker (MMT) medium supplemented with 0.6 mg/L 2,4-D, 0.06 mg/L IBA, and 0.5mg/L BAP. Across two propagation rounds approximately 200 calluses were obtained and a validated extraction and lyophilization protocol was implemented for untargeted metabolomic profiling by orbitrap mass spectrometry. Guide by a PCA of hormonal ratios from prior *E.coca* callus studies, two hormonal assay were run: a dose-response test of 2,4-D (0,0.3,0.6 and 1.2 mg/L) and a substitution assay replacing 2,4-D with NAA and IAA at the standard 0.6 mg/L concentration. In parallel, precursor feeding with L-arginine, L-ornithine and a complementary biotic elicitor *Lysinibacillus phaericus*. All assays were conducted over 35-day culture cycles. Together, this experimental framework establishes a controlled platform for evaluating metabolic responses to hormonal modulation, precursor availability, and elicitation, supporting the identification of promising strategies for specialized metabolite production in *E. coca in vitro* systems and expanding the chemical space accessible through cultivation.

**Key words:** *Erythroxylum coca*; callus culture; specialized metabolites; elicitation; metabolomics

## Biography



Sarha Marcela García Rodríguez is a biologist and MSc candidate in Biological sciences at Universidad de Los Andes, Colombia. Her research focuses on plant biotechnology and metabolic engineering with particular interest in *in vitro* culture systems, elicitation strategies, and metabolomics for specialized metabolite production. Her current research investigates strategies to modulate metabolism in *Erythroxylum coca* callus cultures using metabolic precursors, chemical and biological elicitors. She has experience in plant tissue culture, specialized metabolite extraction, molecular biology, metabolomics data analysis, and statistical modeling.

# Metabolic Rewiring of Escherichia coli for Improved Ethylene Glycol Assimilation

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## Abstract

The transition to a low-carbon bioeconomy demands sustainable biobased processes decoupled from fossil fuels. Current bioprocesses largely depend on sugar substrates from cellulosic feedstocks that compete with food production and raise concerns about responsible land use. Ethylene glycol (EG) has emerged as a promising alternative feedstock derivable from non-food-competing sources, including polyethylene terephthalate (PET) plastic waste, $CO_2$, syngas, and lignocellulosic biomass. However, inefficiencies in EG assimilation have historically required supplementation with additional carbon sources to sustain sufficient biomass generation. To enhance *Escherichia coli*'s ability to utilize EG as a sole carbon source, we conducted multiple rounds of adaptive laboratory evolution (ALE) on an engineered strain in a continuous culture system. After more than 100 generations, we observed significant improvements in growth rate and biomass yield. The genetic and transcriptional basis of these phenotypic gains was characterized through next-generation sequencing (NGS) and reverse engineering of the ancestral strain. This work demonstrates the potential for de novo metabolic rewiring in *E. coli*, enabling efficient EG utilization and opening new avenues for biotechnological applications in the emerging bioeconomy.

**Key words:** metabolic engineering, adaptive laboratory evolution, alternative feedstocks, bioeconomy, ethylene glycol

**Representative publication:**

1. Michelle Feigis, Radhakrishnan Mahadevan, Multicriteria evaluation of ethylene glycol assimilation pathways, *Journal of Industrial Microbiology and Biotechnology*, Volume 53, 2026, kuag007.

2. Emma Harrison, Austin Zheng, Joshua Wicks, Ke Xie, Edward H. Sargent, Radhakrishnan Mahadevan; Cascading the Electrochemical Reduction of $CO_2$ with Bioprocesses for the Production of Reduced-Carbon-Intensity Chemicals. *ACS Sustainable Chem. Eng.* 24 November 2025; 13 (46): 19937–19950.

## Biography



Michelle is a PhD student in the Department of Chemical Engineering & Applied Chemistry at the University of Toronto, conducting research in the Laboratory for Metabolic Systems Engineering (LMSE) supervised by Prof. Radhakrishnan Mahadevan. She completed her undergraduate studies in Biochemistry and Environmental Science at the University of Toronto Scarborough, followed by a Master of Applied Science. Her doctoral research focuses on engineering microbial strains to efficiently utilize ethylene glycol as a sustainable, non-food-competing carbon source for bioproduction. Her work integrates computational pathway analysis, strain engineering, and adaptive laboratory evolution to advance the development of a low-carbon bioeconomy. Michelle is a recipient of the NSERC PhD scholarship.

# Ino80-mediated regulation of nitrogen metabolism and targeted epigenome engineering of stress tolerance in *Saccharomyces cerevisiae*

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## Abstract

Chromatin remodeling represents an underexplored regulatory layer for rewiring cellular metabolism and improving microbial robustness. Here, we investigated how the INO80 chromatin-remodeling complex modulates nitrogen metabolism in *Saccharomyces cerevisiae* and explored its potential for metabolic engineering. Using acetic acid stress as a perturbation model, we found that *INO80* overexpression improved cellular performance and reshaped chromatin accessibility and transcriptional programs associated with nitrogen metabolism. *GDH2*, encoding $NAD^+$-dependent glutamate dehydrogenase, emerged as a key downstream target. Ino80 enrichment at the *GDH2* promoter increased chromatin accessibility and reduced nucleosome occupancy. It also enhanced recruitment of the transcription machinery components Rpb3 and Spt15, promoting *GDH2* transcription independently of H2A.Z. Building on this mechanism, we developed a dCas9-Ino80 epigenome-editing strategy to locally remodel the *GDH2* promoter. Targeted Ino80 recruitment increased *GDH2* expression and improved stress tolerance, with editing efficiency depending on gRNA target position and Ino80 effector architecture (unpublished data). These findings establish targeted chromatin remodeling as a programmable strategy for rewiring nitrogen metabolism and engineering robust yeast cell factories.

**Key words:** *Saccharomyces cerevisiae*; INO80 chromatin remodeling complex; stress tolerance; nitrogen metabolism; epigenome editing

**Representative publication:**

1. Yuan B., Wang W.B., Wang X.Q., Liu C.G., Hasunuma T., Kondo A., Zhao X.Q.*, The chromatin remodeler Ino80 regulates yeast stress tolerance and cell metabolism through modulating nitrogen catabolite repression, International Journal of Biological Macromolecules, 2024, 258, 129041.

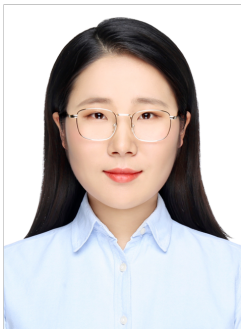
2. Yuan B., Zhu Y.F., Li K., Zhao X.Q.*, Chromatin regulation of acetic acid stress tolerance by Ino80 in budding yeast *Saccharomyces cerevisiae*, Journal of Agricultural and Food Chemistry, 2025, 73, 2951–2960.

3. Yuan B., Wang W.B., Wang Y.T., Zhao X.Q.*, Regulatory mechanisms underlying yeast chemical stress response and development of robust strains for bioproduction, Current Opinion in Biotechnology, 2024, 86, 103072.

4. Zhang M.M.[#], Yuan B.[#], Wang Y.T., Zhang F.L., Liu C.G., Zhao X.Q.*, Differential protein expression in Set5p-mediated acetic acid stress response and novel targets for engineering yeast stress tolerance, Journal of Proteome Research, 2024, 23, 2986–2998.

5. Zhang M.M.[#], Yuan B.[#], Li Y.Z., Wang X.L., Champreda V., Rangel D.E.N., Zhao X.Q.*, Differential global gene transcription of Saccharomyces cerevisiae by zinc sulfate addition under acetic acid stress and identification of novel zinc and stress-responsive genes related to cell wall function, Fungal Biology, 2025, 129, 101573.

## Biography



Bing Yuan is a Ph.D. candidate in Bioengineering at the School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, under the supervision of Prof. Xinqing Zhao. Her research focuses on chromatin remodeling, transcriptional regulation, and metabolic engineering in *Saccharomyces cerevisiae*, with particular interest in how epigenetic regulation contributes to microbial stress adaptation and metabolic robustness. Her doctoral research focuses on the role and regulatory mechanisms of the INO80 chromatin-remodeling complex in the environmental stress responses of *Saccharomyces cerevisiae*. Her work combines chromatin and transcriptomic analyses with mechanistic studies of stress-responsive pathways and targeted epigenome engineering to understand INO80-mediated stress adaptation and explore strategies for improving yeast robustness. She completed a visiting Ph.D. research stay in Prof. Kevin Verstrepen's laboratory at the VIB-KU Leuven Center for Microbiology, KU Leuven, Belgium. Her research has been published in journals including *Journal of Agricultural and Food Chemistry*, *International Journal of Biological Macromolecules*, and *Current Opinion in Biotechnology*.

# Metabolic engineering *Methylobacterium extorquens* AM1 for biosynthesis of 1,4-Butanediol and succinic acid

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## Abstract

1,4-Butanediol is a large volume commodity chemical produced at million tons scale world-wide used in synthesis of various fibers and plastics. While bio-based succinic acid and butanediol productions offer low carbon footprint alternatives to their fossil-based counterparts, current bio-production approaches rely on the use of sugar, which may compete with food resources. In this research, we engineered *Methylorubrum extorquens* AM1 to produce 1,4-Butanediol directly from methanol, which is an increasingly abundant resource derived from biomass-based syngas and captured $CO_2$. We first evolved *M. extorquens* to produce succinic acid through adaptive laboratory evolution. Subsequently, we identified the enzymes necessary for reducing succinic acid to 1,4-Butanediol. A 1,4-BDO titer of 23.6 mg/L was achieved. The results of this work offer an alternative low-carbon 1,4-Butanediol production approach.

**Key words:** Methylotroph, 1,4-BDO

## Biography

Yu Shan received her B.S. degree of Department of Bioscience and Biotechnology in National Taiwan Ocean University (NTOU). She is studying her MS at Yang Ming Chiao Tung University (NYCU) degree of Biological Science and Technology since 2024. Her research interests focus on metabolic engineering and synthetic biology, and she is currently working in the laboratory of Professor Ethan I. Lan.



# Multi-step Metabolic Engineering Optimization of *Escherichia coli* BL21 Star (DE3) for High-efficient Production of Hydroxytyrosol

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## Abstract

Hydroxytyrosol (HT), a vital natural polyphenol abundant in lipid-bound forms in olive tissues, exhibits excellent antioxidant activity and broad application prospects in food, pharmaceutical and cosmetic fields. This study established an efficient de novo HT biosynthesis system via a genome integration strategy in *Escherichia coli* BL21 Star (DE3) combined with multidimensional metabolic engineering. Key functional genes (*HpaBC*, $ARO10^{D331C}$, *ADH6*, *tyrC*, $aroG^{fbr}$) were introduced to construct the basic HT synthetic pathway; carbon flux was optimized by regulating key enzyme gene copy ratios. Competitive branch genes (*tyrR*, *pheA*) were knocked out to reduce precursor competition, and the shikimate pathway was enhanced to boost precursor supply. The *LAAD* gene was integrated to build an efficient tyrosine-to-HT catalytic module, and molecular docking confirmed the catalytic potential of cytochrome P450 enzymes and *HpaBC* for HT synthesis. In a 5 L bioreactor, the engineered strain produced 7460 mg/L HT at 43 h. This work provides a novel technical route for the green biomanufacturing of natural polyphenolic compounds.

**Key words:** de novo synthesis pathway; Synthetic biology; genome integration; The shikimate pathway; Eukaryotic cytochromes P450s

**Representative publication:**

1. **Jin W**, Wu J, Huang Z, Wang J, Qiu X, Yuan L, Chen X, Yao J. Multi-step metabolic engineering optimization of Escherichia coli BL21 Star (DE3) for high-efficient production of hydroxytyrosol. Bioresour Technol. 2026 May;447:134273. doi: 10.1016/j.biortech.2026.134273. Epub 2026 Feb 23. PMID: 41740933.

2. Liao Y, Lao C, Wu J, Yuan L, Xu Y, **Jin W**, Sun J, Zhang Q, Chen X, Yao J. High-Yield Synthesis of Lacto-N-Neotetraose from Glycerol and Glucose in Engineered Escherichia coli. J Agric Food Chem. 2024 Mar 13;72(10):5325-5338. doi: 10.1021/acs.jafc.3c08239. Epub 2024 Jan 26. PMID: 38275134.

## Biography



Weijian Jin is a PhD student in Biology at the University of Science and Technology of China, supervised by Researcher Jianming Yao from the Hefei Institutes of Physical Science, Chinese Academy of Sciences. His research mainly focuses on metabolic engineering, synthetic biology and fermentation engineering. His work involves exploring microbial potential, and regulates microbial metabolic flux through genetic engineering approaches to realize the efficient biosynthesis and yield improvement of target products, thus facilitating the application expansion and technological upgrading of the biosynthetic industry. To date, he has published 3 SCI papers as the first author or co-author in international journals including *Bioresource Technology* and *Journal of Agricultural and Food Chemistry*.

# Synthetic ATP auxotrophy enables control over cellular carbon and electron flow

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## Abstract

Current industrial bioprocesses exploit anaerobic fermentation to achieve electron and carbon conservation. To enable control over cellular carbon and electron flow in a substrate-agnostic manner, we engineered MaxKat as an E. coli platform strain that enables novel types of growth-coupled bioproduction. MaxKat can neither feed electrons into the respiratory electron transport chain nor use fermentative pathways and is thus ATP-auxotrophic. Reactions restoring either oxidative or substrate-level phosphorylation reinstate growth allowing for selective oxidative or reductive bioconversions. We experimentally validated this precise control over intracellular carbon and electron flow by switching between different ATP generation modes relying either on enzymatic NADH oxidation or quinone reduction. To showcase the potential for bioproduction, we demonstrated the growth-coupled oxidation of 10 different sugars into their respective sugar acids near the theoretical maximum. We foresee applications of MaxKat for efficient bioconversions from various feedstocks as well as a tool for the screening and evolution of enzymes to drive biotechnological innovations.

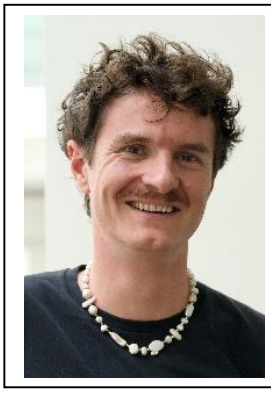
**Key words:** Synthetic Metabolism, Redox Biochemistry, New-to-Nature Bioconversion, Synthetic Auxotrophy

**Representative publication:**

1. Schulz-Mirbach, H. *et al.* Engineering new-to-nature biochemical conversions by combining fermentative metabolism with respiratory modules. *Nat. Commun.* **15**, (2024).

2. Krüsemann, J. L. *et al*. Synthetic ATP auxotrophy enables control over cellular carbon and electron flow. Under Revision at *Nature Catalysis* (2026)

## Biography



Starting with a BSc in Biology at Freie Universität Berlin and followed by a MSc in Systems Biology at ETH Zurich, I was always fascinated by bacterial metabolism and therefore learned how to harness it through genetic engineering for the fight against climate change. During my PhD, which I completed under the co-supervision of Steffen Lindner and Tobias Erb at Charité Berlin, I invented a new type of metabolism. This "Controlled Respiro-Fermentation" frees natural fermentation from the need to maintain redox-balance between substrate and product and thereby allows for efficient and otherwise impossible new-to-nature bioconversions that use waste streams. This work was recognized with the Inflection Award 2025 among 30 other young scientists working on climate technologies.

During my PhD, I went from "just" doing research via climate activism to finally conclude that I need to take the industrial implementation of my research I my own hands to accelerate its impact on the world. Currently, I am therefore working on the commercial application of new biotechnologies that help to fight climate change. My long-term career goal is to found a sustainable biotech start-up that uses the technology that I invented to take on the great challenge of carbon-negative bulk chemical production.

# Metabolic Engineering of *Methylorubrum extorquens* AM1 for P(3HB-co-4HB) Biosynthesis from Methanol

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## Abstract

Methanol is currently one of the primary products of carbon capture and utilization (CCU) processes. However, its potential is hindered by a low market value and limited applications. Furthermore, its predominant use as a fuel results in a short carbon lifecycle, which limits the overall efficiency of carbon fixation and restricts CCU development. To address these challenges, we engineered a methylotrophic bacterium *Methylorubrum extorquens* AM1, which naturally produce poly-3-hydroxybutyrate (P3HB), to synthesize the copolymer poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)) directly from methanol. As a high-value material, P(3HB-co-4HB) is distinguished by its superior mechanical flexibility and excellent biocompatibility compared to the P3HB homopolymer, making it ideal for advanced biomedical and industrial applications. In this study, we constructed a heterologous P4HB biosynthetic pathway in *M. extorquens* AM1, leveraging the strain's endogenous capacity to accumulate P3HB. The final strain reached 40%DCW P(3HB-co-16% 4HB) production using methanol as the sole carbon source.

**Key words:** Methanol utilization, Polyhydroxyalkanoates, Poly-4-hydroxybutyric acid, *Methylobacterium extorquens* AM1

**Representative publication:**

## Biography

Billy received his B.S. degree in biological science & technology from Yang Ming Chiao Tung University in 2021. He joined professor Ethan I. Lan's lab since 2019. After getting his B.S. degree, he then joined NYCU institute of molecular medicine and bioengineering in 2021 where he kept working with Professor Ethan I. Lan (Professor of Department of Biological Science and Technology of National Yang Ming Chiao Tung University) on the development of engineering methylotrophic organisms as microbial cell factory.



# Engineering a synthetic $CH_2$-THF carbon-entry module enables formate assimilation through a redesigned metabolic architecture

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## Abstract

Folate-dependent C1 metabolism is organized around a small set of carbon entry reactions, limiting how activated C1 units connect to central metabolism. Here, we reprogram folate C1 entry by creating a synthetic module that condenses $CH_2$-THF with pyruvate. Growth-coupled screening and engineering repurposed an enzyme using pyruvate as a new $CH_2$-THF acceptor, generating a non-native C4 intermediate that is subsequently converted to homoserine. Embedding this module in a THF-dependent homoserine cycle provided a route-level implementation of the new C1 connection. Cellular construction and adaptive evolution established pathway operation, while $^{13}C$-formate tracing demonstrated the transfer of formate-derived carbon through the synthetic entry module into central metabolism. Together, these results establish a new carbon acceptor connection within the folate network and show that engineering $CH_2$-THF acceptor reactions can expand folate C1 metabolic architecture, thereby providing a general strategy for redesigning how C1 carbon enters cellular metabolism.

**Key words:** synthetic metabolism; formate assimilation; one-carbon metabolism; enzyme evolution; metabolic engineering

**Representative publication:**

## Biography

Yuchen Wang is a PhD candidate in Synthetic Biology and Metabolic Engineering at Westlake University under the supervision of Professor Kechun Zhang. Her research focuses on designing synthetic metabolic pathways to expand microbial carbon utilization and enable sustainable biomanufacturing. Her doctoral work centers on engineering folate-dependent C1 metabolism, including the development of synthetic $CH_2$-THF carbon-entry reactions, enzyme evolution and pathway construction for formate assimilation. By combining protein engineering, metabolic engineering and growth-coupled selection, she aims to create new metabolic architectures that extend the metabolic capabilities of living systems.



# Genome-Guided Sugarcane Bagasse Bioconversion by *Bacillus* sp. T2 and Exometabolomic Discovery of Value-Added Product Candidates

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## Abstract

Efficient microbial valorization of lignocellulosic residues requires the coordinated integration of substrate deconstruction, carbon uptake, and product formation. In this study, we evaluated *Bacillus* sp. T2 for its ability to bioconvert sugarcane bagasse pretreated with either sulfuric acid ($H_2SO_4$) or sodium hydroxide (NaOH). Strain T2 grew well across a pH range of 5–9. Compared with uninoculated controls, it achieved approximately 7.86% and 8.11% net bagasse degradation after 5d of incubation under $H_2SO_4$ and NaOH pretreatment conditions, respectively. Scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD) analyses revealed fermentation-induced changes in surface morphology, functional-group profiles, and crystallinity. Genome annotation identified putative lignocellulose-conversion modules, including GH5_48, GH8, GH113, AA10-CBM5, AA1, phosphotransferase system transporters, and intracellular glycoside-utilizing enzymes. Exploratory LC-MS/MS-based exometabolomics detected 1,522 annotated features, among which 158 were consistently upregulated and 98 consistently downregulated across the different pretreatment and sampling conditions. Features associated with maltose and aromatic amino acids decreased, whereas those annotated as 3-phenyllactic acid, hydroxyphenyllactic acid, and phenylacetic acid increased, particularly under alkaline pretreatment. Collectively, these integrated data highlight *Bacillus* sp. T2 as a promising microbial platform for lignocellulosic biomass valorization and the production of value-added bioproducts.

**Key words:** Sugarcane bagasse; *Bacillus* sp. T2; Lignocellulose bioconversion; Exometabolomics; Biorefinery

**Representative publication: N/A**

## Biography



The first author of Tingmei Li is a graduate student in the college of Chemical Engineering and Technology at Guangxi University of Science and Technology, China. Her research focuses on microbial conversion of lignocellulosic biomass, with particular emphasis on efficient sugarcane bagasse bioconversion. Her current work investigates microbial conversion mechanisms and the discovery of value-added metabolites, aiming to advance the sustainable valorization of agricultural and forestry residues.

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Engineering yeast *Yarrowia lipolytica* for *De Novo* Biosynthesis of psychedelic compounds

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## Abstract

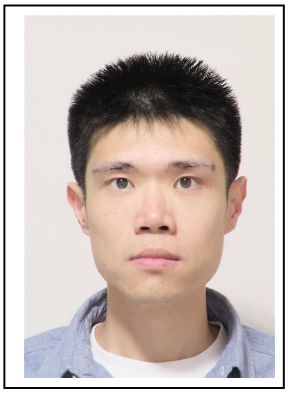
Psilocybin and its active metabolite, psilocin, are naturally occurring psychedelics found in certain mushroom species that have shown significant therapeutic potential for a range of mental health disorders. The growing interest in their clinical applications has increased the demand for psilocybin. However, its low natural abundance and the environmental and economic limitations of chemical synthesis have driven interest in microbial biosynthesis as a sustainable and scalable production alternative. In this study, a *de novo* psilocybin biosynthesis pathway with four genes was established in the non-conventional oleaginous yeast *Yarrowia lipolytica.* Efficient psilocybin production with minimal tryptamine accumulation suggests effective coupling between the heterologous P450 enzyme PcPsiH and the endogenous reductase in *Y. lipolytica*, supporting its suitability as a platform for psilocybin production. Then, modular metabolic engineering strategies were implemented to enhance psilocybin production. These included overexpressing four genes in the shikimate pathway to increase L-tryptophan (L-Trp) supply, disrupting L-Trp catabolism through targeted gene deletions, improving methylation capacity by expressing two additional genes, and screening methyltransferases to identify the most effective enzyme for psilocybin biosynthesis. The resulting strain produced 18.7 mg/L psilocybin in deep-well plate cultivation. In fed-batch fermentation using a 1.2 L bioreactor, psilocybin production reached 764.7 mg/L from glucose. These results highlight the potential of *Y. lipolytica* as a sustainable microbial platform for producing tryptophan-derived psychedelic compounds with therapeutic value.

**Key words:** Metabolic Engineering, Synthetic Biology, *Yarrowia lipolytica*, Psilocybin, psychedelic compound

**Representative publication:**

1. **Sun, B**., Xie, X., González, A., McCubbin, T., Marcellin, E., & Peng, H.*, Engineering yeast for sustainable bioproduction of tryptophan-derived aromatic compounds. FEMS Yeast Research, 26, foag012.

## Biography



Mr Baode Sun is a PhD candidate at the Biosustainability Hub, Australian Institute for Bioengineering and Nanotechnology (AIBN), The University of Queensland (UQ). He is under the supervision of Dr Huadong Peng & Prof Esteban Marcellin, focusing on Engineering *Yarrowia lipolytica* to produce valuable tryptophan-derived therapeutics. Mr Sun obtained his Bachelor of Engineering in Bioengineering from Tianjin University. During his undergraduate studies, he developed his initial interest in synthetic biology through a summer research project in Professor Yingjin Yuan's group. He earned his Master of Philosophy in nanotechnology from AIBN, UQ, in 2018. After several years of working as a bioprocess engineer in the feed additive industry, he returned to university to pursue his PhD in 2024.

Dalian, China | 18-22, October 2026
Metabolic Engineering Summit 2026

# Claim-Level Forensic Reconstruction of Metabolic-Engineering Evidence Chains

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## Abstract

Reproducibility in metabolic engineering is often treated as a publication-level property, although individual quantitative claims depend on biological state, source measurements, analytical transformations, computational artifacts, and publication versions. I developed a fail-closed forensic verification framework that evaluates reproducibility at the claim level and distinguishes numerical reproduction from evidentiary reconstruction. Across four published metabolic-engineering studies, released source data for engineered Corynebacterium glutamicum independently reproduced a reported 141.5 g L-1 1,3-propanediol titer and 2.95 g L-1 h-1 productivity. An official Author Correction subsequently changed multiple provenance-relevant experimental descriptors while stating that the experimental results and conclusions were unaffected, demonstrating that numerical reproducibility and provenance completeness can diverge. In additional cases, downstream deoxypodophyllotoxin concentration and yield calculations and a free-fatty-acid spreadsheet transformation were independently reconstructed, while unresolved upstream analytical dependencies remained explicitly unverified. A randomized controlled synthetic benchmark of 58 evidence chains further evaluated seven provenance-fault classes against artifact-presence and hash-integrity baselines. These results demonstrate that claim-level forensic reconstruction can localize evidentiary boundaries and publication-state changes without falsely invalidating otherwise supported metabolic-engineering results.

**Key words:** Metabolic engineering; reproducibility; provenance; evidence verification; digital forensics

**Representative publication:** None.

## Biography

Forest Savage is an undergraduate chemical engineering student at Oregon State University. His independent research focuses on metabolic engineering, reproducibility, provenance, and dependable forensic software, including claim-level reconstruction and fail-closed verification of scientific evidence.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# AlphaGEM Enables Precise Genome-Scale Metabolic Modelling by Integrating Protein Structure Alignment with Deep-learning-based Dark Metabolism Mining

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## Abstract

Constructing high-quality genome-scale metabolic models (GEMs) for non-model organisms remains challenging. To address this, we developed AlphaGEM, a versatile toolbox leveraging proteome-scale structural alignment, protein language models (PLMSearch), and deep-learning-based predictions for efficient genomic mining to generate GEMs ready for applications. AlphaGEM enhances homologous relationship identification compared to traditional sequence-based methods. Crucially, it employs an ensemble procedure empowered by multiple deep learning toolboxes to effectively mine dark metabolic functions encoded by nonhomologous proteins, thereby expanding species-specific networks. We validated AlphaGEM across prokaryotes (*Klebsiella pneumoniae*, *Bacillus subtilis*), eukaryotes (*Rhodosporidium toruloides*, *Pichia pastoris*), and complex mammals (*Mus musculus*, *Cricetulus griseus*), achieving predictions comparable to manually curated models while outperforming existing tools. Furthermore, we demonstrated its scalability by automatically reconstructing high-fidelity GEMs for 332 distinct yeast species. In summary, AlphaGEM enables precise, rapid GEM construction

across diverse domains, providing a solid foundation for universal functional analysis of organisms having genome sequences available.

**Key words:** Genome-scale modelling, Structure comparison, Protein function prediction, Deep learning, Dark metabolism

**Representative publication:**

1. Han, W., Xiao, L., Sun, H. *et al.* AlphaGEM enables precise genome-scale metabolic modelling by integrating protein structure alignment with deep-learning-based dark metabolism mining. *Nat Commun* 2026, 17, 8720.

## Biography



Weishang Han is a direct-entry Ph.D. student enrolled in 2025 at Shanghai Jiao Tong University. His research focuses on genome-scale metabolic modeling, artificial intelligence, and computational systems biology. He is particularly interested in integrating mechanistic models with AI approaches for high-quality metabolic model reconstruction, multi-omics analysis, and cross-species studies of cellular metabolism. He has participated in the development of AlphaGEM, an automated framework for genome-scale metabolic model reconstruction by integrating protein structure alignment and deep-learning-based functional annotation. His current research interests also include single-cell metabolic modeling and AI-assisted biological modeling.

# Precision engineering of probiotics with prime editing

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## Abstract

CRISPR-Cas-based genome editing has been used to engineer probiotics for next-generation diagnostics and therapeutics. Probiotic engineering demands versatile tools enabling non-natural functions while minimizing unintended genetic changes and interferences. Here, we present customized prime editing platforms for probiotic bacterium *Escherichia coli* Nissle 1917 (EcN) and probiotic yeast *Saccharomyces boulardii.* The optimized system enabled diverse modifications, including substitutions, insertions and deletions. In EcN, prolonged induction time would be enough for efficient prime editing. Differently, in yeast, mismatch repair genes, *PMS1* and *MSH6*, were identified as barriers to prime editing. Off-target analysis revealed reduction in unintended mutations compared with cytosine base editors with either dCas9 or dSpRY effectors.

Moreover, we uncover a non-canonical prime editing pathway in which Cas9 nickase generates a distinct cleavage at loci upstream of the classic nick, leading to editing in the 5' direction of the classic nick. This is a capability previously considered impossible through the well-established design principle of prime editing. Furthermore, we reveal a competition between the canonical and non-canonical prime editing pathways and demonstrate strategies to modulate their balance for enhanced editing efficiency. This work establishes a versatile genome editing platform and reveals a previously unrecognized mechanism for modulating gene editing.

**Key words:** CRISPR-Cas, prime editing, reverse transcription, probiotics

**Representative publication:**

1. Chen P.R., Wei Y., Li X., Yu H.Y., Wang S.G., Yuan X.Z., Xia P.F.*, Precision engineering of probiotic *Escherichia coli* Nissle 1917 with prime editing. Applied and Environmental Microbiology. 2025, 91, 2, e00031-25.

2. Chen P.R.#, Wei Y.#, Jin Y.S. *, Xia P.F.*, Rewiring metabolic pathways for biological conversion of carbon dioxide. Current Opinion in Chemical Engineering. 2026. 53, 101277.

3. Chen P.R., Xia P.F.*, Carbon recycling with synthetic $CO_2$ fixation pathways. *Current Opinion in Biotechnology,* 2024. 85, 103023.

## Biography



Peiru Chen is a Ph.D. candidate at Shandong University, where she started her doctoral research in 2022. Her research focuses on recycling $CO_2$ generated during lignocellulose hydrolysate fermentation and convert it into valuable products. She is particularly interested in developing advanced genome engineering tools, including CRISPR-Cas-based genome editing tools, and applying them to engineer microbial systems with enhanced carbon utilization and production capabilities. Her current work involves developing precision genome editing platforms and constructing engineered microbial consortia for sustainable biofuel production.

# Improving Mevalonate Yield through a Synthetic Threonine Pathway

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## Abstract

Acetyl-CoA is a key metabolic intermediate and a central precursor for mevalonate biosynthesis, which serves as a building block for terpenoid production. However, native regulation of acetyl-CoA metabolism often limits carbon flux toward mevalonate production. To overcome this limitation, we implemented a synthetic threonine pathway that integrates threonine degradation with glycine metabolism. In this pathway, carbon sources are converted to threonine, which is subsequently degraded into acetyl-CoA and glycine. Glycine is then recycled through endogenous metabolism to improve carbon utilization and increase acetyl-CoA availability for mevalonate biosynthesis. Overall, this synthetic threonine pathway provides an alternative strategy for enhancing acetyl-CoA flux and promoting the production of acetyl-CoA-derived compounds such as mevalonate.

**Key words:** Metabolic engineering, synthetic threonine pathway, Acetyl-CoA yield

## Biography

Teresa received her B.S. degree in Chemical Engineering from Feng Chia University in 2020. After getting her B.S. degree, she then joined NYCU Institute of Biomedical Engineering in 2020 where she worked with Professor Ethan I. Lan (Professor of Department of Biological Science and Technology of National Yang Ming Chiao Tung University) on the development of microbial cell factory for flavonoids production. After completing her M.S. degree, she continued her work with Professor Ethan Lan on the metabolic engineering of microbial platforms.



Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Rewiring pyruvate synthesis improves cell growth of *Clostridium ljungdahlii* during microbial electrosynthesis

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[2]*Institute for Environment and Energy, Pusan National University, 46241, Republic of Korea*

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## Abstract

In acetogens, the limited pool of reduced ferredoxin must supply both metabolites production and biosynthesis, creating a redox bottleneck that limits growth on $CO_2$ and $H_2$. In *Clostridium ljungdahlii*, reduced ferredoxin drives pyruvate synthesis from acetyl-CoA *via* pyruvate:ferredoxin oxidoreductase, tightly coupling redox balance to the supply of a cellular building block, pyruvate. We hypothesized that installing a ferredoxin-independent route to pyruvate would relax this constraint. We heterologously expressed pyruvate formate lyase from the formatotrophic acetogen *Acetobacterium woodii* in *C. ljungdahlii*, providing a formate-dependent route to pyruvate. Under $H_2$:$CO_2$ with formate, the engineered strain reached 1.5-fold higher maximum cell density than the control ($OD_{max}$ 3.07 ± 0.25 vs 2.04 ± 0.19). In formate-supplemented, $CO_2$-fed bioelectrochemical reactors, where electron supply is limiting, it doubled both biomass and acetate titer ($OD_{max}$ 0.22 ± 0.05, 21.8 ± 5.6 mM vs 0.10 ± 0.06, 10.2 ± 2.5 mM). By providing an additional route for pyruvate synthesis using formate, this design enhanced cell growth of *C. ljungdahlii* under electron-limited conditions in a bioelectrochemical system. Our results establish a metabolic engineering strategy to increase acetogenic biomass under both $H_2$:$CO_2$ gas fermentation and microbial $CO_2$ electrosynthesis, contributing to the conversion of waste gases into sustainable chemicals.

**Key words:** *Clostridium ljungdahlii*, $CO_2$, Pyruvate formate lyase, Microbial electrosynthesis, Formate

**Representative publication:**

1. Im C., Kim M., Kim J.R., Valgepea K., Modin O., Nygård Y., Franzén C.J. *, Low electric current in a bioelectrochemical system facilitates ethanol production from CO using CO-enriched mixed culture, Frontiers in Microbiology, 2024, 15, 1438758.

2. Im C., Valgepea K., Modin O., Nygård Y. *, *Clostridium ljungdahlii* as a biocatalyst in microbial electrosynthesis – Effect of culture conditions on product formation, Bioresource Technology Reports, 2022, 19, 101156.

3. Lama S., Kim Y., Nguyen D.T., Im C.H., Sankaranarayanan M., Park S.*, Production of 3-hydroxypropionic acid from acetate using metabolically-engineered and glucose-grown Escherichia coli, Bioresource Technology, 2021, 320, 124362.

4. Im C., Kim C., Song Y.E., Oh S.-E., Jeon B.-H., Kim J.R.*, Electrochemically enhanced microbial CO conversion to volatile fatty acids using neutral red as an electron mediator, Chemosphere, 2018, 191, 166–173.

5. Im C., Song Y.E., Jeon B.-H., Kim J.R.*, Biologically activated graphite fiber electrode for autotrophic acetate production from $CO_2$ in a bioelectrochemical system, Carbon Letters, 2016, 20(1), 76–80.

## Biography



Chaeho Im is a postdoctoral researcher at Pusan National University, working at the interface of metabolic engineering and $CO_2$ valorization. He completed his PhD at Chalmers University of Technology in 2025, where he engineered the acetogen *Clostridium ljungdahlii* for microbial electrosynthesis and studied how culture conditions govern $CO_2$ and CO conversion in a bioelectrochemical system. His earlier research in Korea, at Pusan National University and UNIST, addressed bioelectrochemical CO/$CO_2$ conversion using redox mediators and the metabolic engineering of *Escherichia coli* for 3-hydroxypropionic acid production. His current work focuses on the sustainable production of value-added chemicals from waste carbon using bioelectrochemical systems and metabolic engineering, with the broader goal of establishing an integrated waste-valorization process. He is particularly interested in coupling electrochemically generated C1 intermediates to engineered microbial metabolism as a route toward scalable, low-carbon chemical production.

# An AI-assisted genome-scale metabolic atlas of Y1000+ yeasts reveals dark metabolism, cross-scale diversity and mitochondrial specialization

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## Abstract

Large yeast genome collections offer an unprecedented basis for studying metabolic diversity, yet incomplete gene structures, remote protein functions and uncertain compartment assignments continue to impede systematic genotype-to-phenotype interpretation in non-model species. Here we establish an AI-assisted genome-scale metabolic atlas that harmonizes 1,150 Y1000+ standard GEMs, 594 gut-fungal standard GEMs and 1,807 *Saccharomyces cerevisiae* strain models in a common space of 6,334 reactions. Reaction features distinguished carbon generalists from specialists more accurately than KO features, while phenotype-guided correction systematically improved carbon-use consistency across 809 model pairs. ANNEVO reannotation and AlphaGEM functional integration across all 1,154 Y1000+ targets identified 35,777 model-new species–reaction links, 76.44% of which connected to pre-existing networks, with prominent signals in N-glycan precursor synthesis and thiamine salvage. Cross-scale comparisons showed continuous pan-reactome expansion and strict-core contraction from *S. cerevisiae* strains to deeper species levels; strain-level analysis further identified 78 assembly-generation-qualified panYeast-gap reactions. Gut-associated yeasts exhibited compact reaction repertoires, lipid-related module remodelling and narrower simulated nutrient use. This atlas demonstrates how AI-derived gene annotation, protein function, reaction thermodynamics and localization can be translated into comparable mechanistic networks for studying yeast dark metabolism, metabolic evolution and ecological specialization.

**Key words:** genome-scale metabolic model; artificial intelligence; dark metabolism; comparative yeast systems biology

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

1. Han W, Xiao L, Sun H, et al. AlphaGEM enables precise genome-scale metabolic modelling by integrating protein structure alignment with deep-learning-based dark metabolism mining[J]. Nature Communications, 2026.

2. Fan X, Liao W, Xiao L, et al. scYeast: a biological-knowledge-guided foundation model on yeast single-cell transcriptomics[J]. Synthetic and Systems Biotechnology, 2027, 16: 75-87.

3. Lu H, Xiao L, Liao W, et al. Cell factory design with advanced metabolic modelling empowered by artificial intelligence[J]. Metabolic Engineering, 2024, 85: 61-72.

## Biography

[Please enter your Biography here, limit to 200 words] (First-line indent of 2 characters, Time new roman, font size 12)



Luchi Xiao is a third-year Ph.D. student at the School of Life Sciences and Biotechnology, Shanghai Jiao Tong University. His research focuses on yeast cell modeling and AI-assisted biological modeling, with particular interests in integrating genome-scale mechanistic models, multi-omics data, and artificial intelligence to improve the understanding and prediction of cellular systems. He has contributed as a co-first author to studies including AlphaGEM and scYeast, which explore AI-enabled genome-scale metabolic modeling and foundation-model approaches for yeast systems biology. His current research aims to develop hybrid AI–mechanistic modeling frameworks for more interpretable and predictive virtual-cell models of yeast.

# Rapid Fire Talk

# Engineered Commensals for Metabolic Modulation of the Gut-Liver-Brain Axis

Nikhil Aggarwal[1,2,3], Matthew Chang[1,2,3,4]

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## Abstract

The gut–liver–brain axis is central to metabolic and neurological homeostasis, mediated by host–microbiota metabolic interactions. Disruption of this axis contributes to complex disorders such as hepatic encephalopathy (HE), characterized by hyperammonemia and amino acid imbalance, highlighting the need for targeted multi-metabolite interventions. Here, we engineered commensal Lactobacillus plantarum WCFS1 strains to selectively modulate key metabolites dysregulated in HE. Two strains were developed: Lp-NH3, which couples ammonia assimilation with branched-chain amino acid (BCAA) biosynthesis, and Lp-Q, designed to enhance L-glutamine utilization and suppress ammonia production. In hyperammonemic and bile duct ligation mouse models, these engineered strains significantly reduced systemic and brain ammonia levels, restored BCAA and L-glutamine balance, and improved anxiety-like and cognitive behaviors. Transcriptomic analysis of brain tissue revealed normalization of neuronal signaling pathways and reduced neuroinflammatory signatures, supporting a mechanistic link between gut metabolic modulation and central nervous system function. Importantly, these strains outperformed rifaximin, a clinically used therapy for HE, while preserving gut microbiota diversity and demonstrated favorable safety profiles without long-term colonization. Together, our findings establish engineered commensals as a modular and programmable platform for multi-metabolite intervention, offering a novel therapeutic strategy to restore metabolic homeostasis in disorders of the gut–liver–brain axis.

**Key words:** Metabolic engineering, Gut–liver–brain axis, Engineered commensals, Synthetic Biology, Metabolic Disorders

**Representative publication:**

1. Aggarwal, N.*, Shen, H., Lee, L. T., Zhou, L., Zhu, M. T., Koh, X. Q., ... & Chang, M. W. (2026). Engineered commensals for metabolic modulation of the gut-liver-brain axis. Cell, 189(13), 3950-3967.

2. Shen, H., Aggarwal, N.*, Cui, B., Foo, G. W., He, Y., Srivastava, S. K., ... & Chang, M. W. (2025). Engineered commensals for targeted nose-to-brain drug delivery. Cell, 188(6), 1545-1562.

## Biography



Nikhil Aggarwal is a Senior Research Fellow and Cluster Lead at the NUS Synthetic Biology for Clinical and Technological Innovation (SynCTI) programme and the National Centre for Engineering Biology (NCEB), National University of Singapore (NUS). His work sits at the interface of metabolic engineering and microbiome science, rewiring the metabolism of commensal bacteria to create live biotherapeutics — programmable strains that sense disease-associated host and microbial cues and actuate a therapeutic output in situ. He leads a cluster of research staff and doctoral students working across infectious disease, oncology and metabolic disorders. Their work combines metabolic engineering of Lactobacillus and other commensals with genetic circuit design and data-driven strain optimization to develop strains for targeted drug delivery, tumor-selective therapy and metabolic modulation, with validation in preclinical animal models. He received his PhD from NUS and has published in journals including *Cell*, *Nature Reviews Bioengineering, Nature Communications and Chemical Reviews*, and leads the cluster's collaborative programs with academic and industry partners alongside the mentoring of early-career scientists.

# Expanding the biosynthetic capability of *Corynebacterium glutamicum* for lutein production

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## Abstract

Lutein is a high-value xanthophyll widely used in food, nutraceutical, and feed applications, yet its sustainable microbial production remains challenging because its biosynthesis requires the coordinated operation of multiple heterologous enzymes, including plant-derived cytochrome P450 enzymes. In this study, we engineered *Corynebacterium glutamicum* for efficient de novo production of lutein by systematically expanding its capacity to synthesize and functionalize carotenoid intermediates. Metabolic flux toward the carotenoid pathway was enhanced, while the α-carotene-forming module was optimized to establish an efficient precursor supply for lutein biosynthesis. To overcome the limited activity of the heterologous P450-dependent hydroxylation step, we further engineered the P450–reductase system through optimization of reductase expression, cellular heme availability, and scaffold-mediated spatial organization of the interacting enzyme partners. Integration of these metabolic and enzyme engineering strategies enabled gram-per-liter-scale lutein production in fed-batch fermentation. These results demonstrate the potential of *C. glutamicum* as an expandable microbial chassis for the biosynthesis of structurally complex carotenoids and other plant-derived natural products.

**Key words:**

Lutein; *Corynebacterium glutamicum*; Metabolic engineering; Cytochrome P450; Carotenoid biosynthesis

**Representative publication:**

1. Eun H., Prabowo C.P.S., Lee S.Y. *, Gram-per-litre scale production of lutein in *Corynebacterium*, Nature Synthesis, 2025, 1-12.

2. Eun H., Lee S.Y.*, Metabolic engineering and fermentation of microorganisms for carotenoids production, Current Opinion in Biotechnology, 2024, 87, 103104.

3. Park S.Y., Eun H., Lee M.H., Lee S.Y.*, Metabolic engineering of *Escherichia coli* with electron channelling for the production of natural products, Nature Catalysis, 2022, 5, 726-737.

4. Prabowo C.P.S., Eun H., Yang Hucettogullari D., Jeegadeesh R., Kim S.J., Lee S.Y.*, Production of natural colorants by metabolically engineered microorganisms, Trends in Chemistry, 2022, 4(7), 608-626.

## Biography



Hyunmin Eun received his PhD in Chemical and Biomolecular Engineering from the Korea Advanced Institute of Science and Technology (KAIST) in 2025 under the supervision of Prof. Sang Yup Lee. He is currently a postdoctoral researcher in the Department of Chemical and Biomolecular Engineering at KAIST. His doctoral research focused on systems metabolic engineering of microorganisms for the sustainable production of carotenoids and other value-added natural products. In particular, he contributed to the development of engineered *Corynebacterium glutamicum* cell factories for the production of structurally diverse carotenoids, including gram-per-liter-scale production of lutein. His research interests include metabolic engineering, systems biology, synthetic biology, microbial biotechnology, and natural product biosynthesis. His current research focuses on developing high-performance microbial cell factories through systems-level pathway optimization, P450 electron-transfer engineering, enzyme engineering, and fermentation-scale bioprocess development.

# Multi-omics analysis reveals the impact of LincRNA deletion on yeast protein synthesis

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## Abstract

Non-coding RNAs (ncRNAs) significantly enhance biological complexity by regulating gene expression and cellular functions. In this study, by constructing a yeast library with long intergenic non-coding RNA (lincRNA) deletions, 21 lincRNAs affecting -amylase secretion were identified. Combined transcriptomic and metabolomic analyses of these three strains revealed that their energy metabolism and cytosolic translation capacity were enhanced, thereby promoting ATP supply and protein synthesis. Through further multi-target engineering, an engineered strain based on the SUT433 deletion mutant achieved an α-amylase secretion titer of 4.1 g/L in fed-batch fermentation[1]. Furthermore, subsequent studies also found that lincRNA deletion impacts the synthesis of cytosolic acetyl-CoA [2]. The above findings unveil the critical potential of lincRNAs in regulating cellular metabolism, providing profound insights and strategies for the development of highly efficient cell factories for protein production.

**Key words:** *Saccharomyces cerevisiae*, lincRNA, protein secretion, transcriptome, cytoplasmic translation

[1] Qin L, Pan Y, Xue S, Huang M*. Adv Sci. 2025 Apr;12(13):e2406873.
[2] Qin L, He S, Huang M*. Synth Syst Biotechnol. 2025 Nov 8;12:10-19.

## Biography

Qin Ling, Postdoctoral Research Associate, South China University of Technology



Dr. Qin has long been engaged in research on microbial synthetic biology, with a primary focus on recombinant protein expression and secretion regulation in Saccharomyces cerevisiae. She has published 7 SCI papers as (co-)first author or corresponding author, and has led 4 national and provincial/ministerial research projects, including the National Natural Science Foundation of China.

# Engineering *Penicillium rubens* as a Next-Generation Fungal Chassis for Flavonoid Biosynthesis

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## Abstract

Flavonoids are valuable plant natural products with pharmaceutical, nutraceutical, and industrial applications. Microbial cell factories provide a sustainable alternative for flavonoid production, yet filamentous fungi remain largely unexplored despite their strong biosynthetic capacity and industrial relevance.

Here, we developed the industrial fungus *Penicillium rubens* as a chassis for flavonoid biosynthesis. A secondary metabolite-deficient *P. rubens* 4xKO strain was engineered by introducing plant-derived 4-coumarate ligase (4CL) and chalcone synthase (CHS), enabling heterologous naringenin production from supplemented *p*-coumaric acid. Optimization of precursor feeding and cultivation conditions improved pathway performance, resulting in a naringenin titer of 0.88 mM with an 88% molar conversion yield.

Further LC-MS/MS analysis revealed a previously undescribed naringenin degradation pathway in *P. rubens*, involving flavonoid hydroxylation and ring-cleavage reactions that limit product accumulation. These findings identify new targets for strain engineering and establish *P. rubens* as a promising fungal platform for the sustainable production of flavonoids and other plant-derived natural products.

**Key words:** [Flavonoid biosynthesis, *Penicillium rubens*, Metabolic engineering, Filamentous fungi, Microbial cell factory]

**Representative publication:**

1. Peng, B., Wang, Z., Zhang, L., Groves, M. R., & Haslinger, K. (2026). De novo biosynthesis of alpinetin enhanced by directed evolution of 5-O-methyltransferase. Microbial Cell Factories, 25, 134.

2. Peng, B., Zhang, L., He, S., Oerlemans, R., Quax, W. J., Groves, M. R., & Haslinger, K. (2023). Engineering a plant polyketide synthase for the biosynthesis of methylated flavonoids. Journal of Agricultural and Food Chemistry, 72(1), 529–539.

3. Peng, B., Dai, L., Iacovelli, R., Driessen, A. J. M., & Haslinger, K. (2023). Heterologous naringenin production in the filamentous fungus Penicillium rubens. Journal of Agricultural and Food Chemistry, 71(51), 20782–20792.

## Biography



Bo Peng is a postdoctoral researcher at the VIB-KU Leuven Center for Microbiology, KU Leuven, Belgium. He obtained his PhD from the Department of Chemical and Pharmaceutical Biology at the University of Groningen, where his research focused on engineering microbial cell factories for the biosynthesis of flavonoids. His doctoral work involved metabolic engineering of microbial hosts, enzyme engineering, and pathway optimization to improve the production of plant-derived flavonoids. His research interests include synthetic biology and metabolic engineering approaches to expand the biosynthetic potential of microbial platforms for the sustainable production of high-value molecules.

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Metabolic Engineering Summit 2026

# Complementary Yeast Biosensors for Intracellular and Extracellular Lactate

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## Abstract

Lactic acid is a high-value platform chemical widely used in the food, pharmaceutical, and cosmetic industries and serves as a precursor to polylactic acid (PLA), a key biodegradable bioplastic. *Saccharomyces cerevisiae* offers distinct advantages over lactic acid bacteria as a production host, including phage resistance, lower nutritional requirements, and robust performance under low pH. Realizing this potential requires yeast-based lactate biosensors for real-time, *in situ* monitoring, enabling process optimization, metabolic engineering, and high-throughput strain screening.

We present two complementary whole-cell yeast lactate biosensors: a transcription factor (TF)-based sensor for intracellular detection, and a G protein-coupled receptor (GPCR)-based sensor for extracellular detection. For the TF-based sensor, lactate-responsive bacterial repressors were heterologously expressed in yeast, with cognate repressor binding sites engineered into native yeast promoters to drive repression in the absence of lactate and induction upon binding. For the GPCR-based sensor, built on a yeast chassis with a streamlined GPCR signaling pathway, we expressed human GPCR, screened chimeric G-protein variants, and replaced ergosterol with cholesterol to enhance receptor localization and function.

Together, these biosensors establish a versatile platform for real-time monitoring and screening of lactate-producing cell factories.

**Keywords:** Lactate biosensor; *Saccharomyces cerevisiae*; Transcription factor; G protein-coupled receptor; Metabolic engineering

**Representative publication:**

1. **Li, X.**, Souza, R.D.A., & Heinemann, M. (2025). Cell size is controlled by the rate of glucose uptake and metabolism in yeasts. *Current Biology*, 35(4), 788-798. e4.

2. **Li, X.** & Heinemann, M. (2023). Quantifying intracellular glucose levels when yeast is grown in glucose media. *Scientific Reports*, 13(1), 17066.

3. Yang, Y., Zheng, Y., Wang, P., **Li, X.**, Zhan, C., Linhardt, R. J., Zhang, F., Liu, X., Zhan, J., & Bai, Z. (2020). Characterization and application of a putative transcription factor (SUT2) in *Pichia pastoris*. *Molecular Genetics and Genomics*, 295, 1295-1304.

4. Nijland, J. G., **Li, X.**, Shin, H. Y., de Waal, P. P., & Driessen, A. J. (2019). Efficient, D-glucose insensitive, growth on D-xylose by an evolutionary engineered *Saccharomyces cerevisiae* strain. *FEMS Yeast Research*, 19(8), foz083.

5. **Li, X.**, Yang, Y., Zhan, C., Zhang, Z., Liu, X., Liu, H., & Bai, Z. (2018). Transcriptional analysis of impacts of glycerol transporter 1 on methanol and glycerol metabolism in *Pichia pastoris*. *FEMS Yeast Research*, 18(1), fox081.

## Biography



Xiang Li (李翔) was born in Taiyuan, the capital city of Shanxi Province, China. Enrolled as a bachelor's student in the National Life Science and Technology Talent Training program based at Jiangnan University (Wuxi, China) in 2012, he received his bachelor's degree in Biological Engineering in 2015 and obtained his master's degree in Fermentation Engineering in 2018 from the same university. During his master's studies on the transcriptional responses of methylotrophic yeasts under the supervision of Prof. Dr. Zhonghu Bai, he developed a great interest in yeast systems and synthetic biology. In 2018, he moved to the group of Molecular Systems Biology in Groningen, the Netherlands, and studied the correlation between cell size and metabolism in yeasts under the supervision of Prof. Dr. Matthias Heinemann. Following the completion of his PhD, he decided to slightly shift his focus from yeast systems biology to yeast synthetic biology and moved to Gothenburg, Sweden. Xiang is now working as a postdoctoral researcher at Chalmers University of Technology under the supervision of Prof. Dr. Verena Siewers and developing engineered yeasts for novel living materials in the scheme of a Horizon project funded by the European Union.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Global analysis of natural product diversity from extreme environments reveals vast untapped biosynthetic potential

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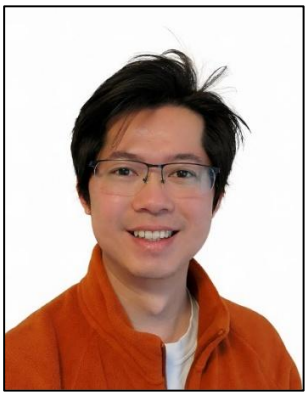
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## Abstract

Extreme environments harbor vast and largely unexplored microbial diversity. There is an increasing need to systematically investigate the biosynthetic potential of these microbiomes at a global scale. Here, we present a comprehensive analysis of natural product diversity across extreme environments, revealing extensive and previously untapped biosynthetic capacity. We further report the discovery of three ribosomally synthesized and posttranslationally modified peptides, frigopeptin, lignipeptin, and stenodin. By integrating large-scale genome mining with comparative analyses across diverse habitats, we identify distinct environmental signatures that shape biosynthetic diversity and chemical novelty. Collectively, these findings establish extreme environments as rich reservoirs of uncharacterized biosynthetic gene clusters and structurally unique natural products.

Keywords: RiPPs, lanthipeptide, thiopeptide, metagenomics, genome mining

## Biography



**Dr. Ian Yunus** is a Senior Scientist and Junior Principal Investigator at the Singapore Institute of Food and Biotechnology Innovation (SIFBI), Agency for Science, Technology and Research (A*STAR), Singapore. He received his PhD in **Metabolic Engineering** from Imperial College London and has conducted postdoctoral research at the Joint BioEnergy Institute (JBEI)/Lawrence Berkeley National Laboratory and ETH Zürich.

His research focuses on **metabolic engineering and synthetic biology**, with the goal of designing and engineering microbial systems for the production of valuable chemicals, natural products, and bioactive molecules. His work integrates pathway engineering, genome editing, systems biology, computational modelling, and high-throughput experimentation to understand and reprogram cellular metabolism.

A particular focus of his research is the engineering of **fungal and microbial cell factories**, including the discovery and reconstruction of biosynthetic pathways involving nonribosomal peptide synthetases (NRPSs), polyketide synthases (PKSs), and other biosynthetic gene clusters. He combines genome mining and computational biology with synthetic biology to expand the repertoire of biological systems available for biomanufacturing.

At SIFBI, Dr. Yunus is developing **AI-driven and automated metabolic engineering platforms**, integrating machine learning, laboratory automation, and omics technologies to accelerate design–build–test–learn cycles.

**Representative publication:**

1. **Ian S. Yunus**, David N Carruthers, Yan Chen, Jennifer W Gin, Edward EK Baidoo, Christopher J Petzold, Hector Garcia Martin, Paul D Adams, Aindrila Mukhopadhyay, Taek Soon Lee. 2026. Predictive CRISPR-mediated gene downregulation for enhanced production of sustainable aviation fuel precursor in *Pseudomonas putida*. *Metabolic Engineering* 94, 67–76. https://doi.org/10.1016/j.ymben.2025.11.007
2. David N Carruthers, Patrick C Kinnunen, Yuerong Li, Yan Chen, Jennifer W Gin, **Ian S. Yunus**, William R Galliard, Stephen Tan, Tijana Radivojevic, Paul D Adams, Anup K Singh, Jess Sustarich, Christopher J Petzold, Aindrila Mukhopadhyay, Hector Garcia Martin, Taek Soon Lee. 2025. Automation and machine learning drive rapid optimization of isoprenol production in *Pseudomonas putida*. *Nature Communications* 16, 11489. https://doi.org/10.1038/s41467-025-66304-8
3. Javier Menasalvas, Shawn Kulakowski, Yan Chen, Jennifer W Gin, Emine Akyuz Turumtay, Nawa Raj Baral, Morgan A Apolonio, Alex Rivier, **Ian S. Yunus**, Megan E Garber, Corinne D Scown, Paul D Adams, Taek Soon Lee, Ian K Blaby, Edward EK Baidoo, Christopher J Petzold, Thomas Eng, Aindrila Mukhopadhyay. 2025. Biosensor-driven strain engineering reveals key cellular processes for maximizing isoprenol production in *Pseudomonas putida*. *Science Advance*s 11, eady2677. https://doi.org/10.1126/sciadv.ady2677
4. Hyun Gyu Lim, Aparajitha Srinivasan, Russel Menchavez, **Ian S. Yunus**, Myung Hyun Noh, Megan White, Yan Chen, Jennifer W Gin, Bernhard O Palsson, Taek Soon Lee, Christopher J Petzold, Thomas Eng, Aindrila Mukhopadhyay, Adam M Feist. 2025. Evolution-guided tolerance engineering

of *Pseudomonas putida* KT2440 for production of the aviation fuel precursor isoprenol. *Metabolic Engineering* 91, 322–335. https://doi.org/10.1016/j.ymben.2025.05.007

5. **Ian S. Yunus**, Graham A Hudson, Yan Chen, Jennifer W Gin, Joonhoon Kim, Edward EK Baidoo, Christopher J Petzold, Paul D Adams, Blake A Simmons, Aindrila Mukhopadhyay, Jay D Keasling, Taek Soon Lee. 2024. Systematic engineering for production of anti-aging sunscreen compound in *Pseudomonas putida*. *Metabolic Engineering* 84, 69–82. https://doi.org/10.1016/j.ymben.2024.06.001

# Synthesis of aromatic PHAs as sustainable hot-melt adhesives

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## Abstract

Petroleum-based adhesives are non-biodegradable and cause environmental concerns. PHAs, bacterial polyesters produced from renewable carbon sources, are promising biodegradable adhesive materials due to their structural diversity. In this study, engineered *Escherichia coli* strains were developed to produce novel aromatic PHAs with hot-melt adhesive properties from glucose. Polymer biosynthesis was enhanced by optimizing heterologous gene expression, introducing additional CoA transferases, temporally regulating pathway expression, and applying in silico simulations to identify engineering targets for monomer production. The resulting polymers showed strong adhesive performance and reusability, demonstrating a sustainable microbial strategy for producing bio-based hot-melt adhesives with tunable properties. [This work is supported by the Development of next-generation biorefinery platform technologies for leading bio-based chemicals industry project 2022M3J5A1056072 and Development of platform technologies of microbial cell factories for the next-generation biorefineries project 2022M3J5A1056117 from the National Research Foundation supported by the Korean Ministry of Science and ICT.]

**Key words:** Polyhydroxyalkanoates, *Escherichia coli*, Systems metabolic engineering, Adhesive,

**Representative publication:**

**1.** Kang, M., Lee, Y., Kim, G. B. & Lee, S. Y. *, Synthesis of poly(4HB-*co*-PhLA) and poly(3HB-*co*-4HB-*co*-PhLA) as sustainable hot-melt adhesives using engineered *Escherichia coli*. *Nat. Commun.*, 2026.

## Biography

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# Rational design of self-assembling protein scaffolds for enzyme performance reconstruction and efficient cascade catalysis

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## Abstract

Self-assembling protein scaffolds offer a versatile platform for spatially organizing multi-enzyme systems, yet precise control over local microenvironments for individual enzyme optimization and seamless cascade coupling remains a grand challenge. Here, we present a rational design strategy to engineer functionalized self-assembling protein scaffolds (γ-prefoldin) for targeted enzyme performance reconstruction and high-efficiency biocatalytic cascades. By integrating structure-aware AI predictions, Rosetta energy landscapes, and high-temperature molecular dynamics (MD) sampling, we decoupled catalytic bottlenecks from thermal instability, enabling targeted structural remodeling of guest enzymes without compromising scaffold integrity.

**Key words:** protein engineering, enzyme immobilization, self-assembly, biocatalysis

**Representative publication:**

1. H. Gong, Y. Meng, Y. Sun, et al. *Design of a Programmable and Recyclable Protein Scaffolding Material with Geometrically Precise Enzyme Patterning for Improved Cascade Catalysis*. *Adv. Funct. Mater.* 36, no. 20 (2026): e14761. https://doi.org/10.1002/adfm.202514761

2.Y. Meng, L. Peplowski, T. Wu, H. Gong, R. Gu, L. Han, Y. Xia, Z. Liu, Z. Zhou, Z. Cheng, *A Versatile Protein Scaffold Engineered for the Hierarchical Assembly of Robust and Highly Active Enzymes*. *Adv. Sci.* 2025, 12, 2500405. https://doi.org/10.1002/advs.202500405

## Biography



Yiwei Meng is a Ph.D. candidate in Fermentation Engineering at Jiangnan University with a strong background in computational enzymology, synthetic biology, and protein design. His doctoral research focuses on the rational design of self-assembling protein scaffolds for enzyme performance reconstruction and efficient cascade catalysis.

Combining AI-driven predictive modeling, computational simulations, and wet-lab experiments, Yiwei excels at bridging upstream rational enzyme design with downstream industrial applications. He has published 9 SCI papers (4 as first author in top-tier journals like *Advanced Science* and *ACS Catalysis*). His expertise spans protein engineering, bioprocess scale-up, and analytical product quantification.

Yiwei has actively led and contributed to major research initiatives, including National Key R&D Programs and industry-academia projects. His accomplishments include engineering high-performance nitrile hydratases and transglycosylases for value-added chemical synthesis and optimizing bioprocesses up to 5-ton fermenter scales. Honored with the National Scholarship for Doctoral Students and the Rector's Award from Nicolaus Copernicus University, he aims to leverage his hybrid computational-experimental skills to drive industrial biotechnology innovation.

# Enhancing PgUGT activity and thermostability via mutation site screening aided by molecular docking structure and computer-aided design for efficient industrial synthesis of Rebaudioside D

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## Abstract

Rebaudioside D (Reb D) is a natural high-intensity, zero-calorie sweetener with a superior taste profile compared to stevioside and rebaudioside A (Reb A), creating high consumer demand. In this study, the glycosyltransferase $PgUGT_{M0}$ was engineered via rational design and computational strategies to enhance the biocatalytic production of Reb D. A combinatorial mutant, $PgUGT_{M2}$ (M0-M87H/I146F/H149W/Y164W/I169F/N178Y/A342L), was constructed, exhibiting a 15.4-fold increase in enzymatic activity and a 33.5-fold extension in half-life. Molecular dynamics simulations attributed this enhanced thermostability to improved cavity filling, increased hydrophobicity, and strengthened hydrogen bonding networks. Furthermore, a dual-enzyme cascade system coupling $PgUGT_{M2}$ with sucrose synthase mbSUS was established in *P. pastoris*. Under optimized fed-batch conditions at 50°C, a Reb D titer of 223.3 g/L (89.9% conversion) was achieved. Following the optimization of cell disruption and pH control, the process was scaled up to a 1.5 L enzymatic reactor, yielding 166.1 g/L Reb D with a 91.3% conversion rate within 20 h. This study presents an integrated approach—spanning enzyme engineering to process intensification—marking a critical advancement toward the industrial-scale production of Reb D.

**Key words:** PgUGT; rebaudioside D; protein engineering; thermostability; dual-enzyme cascade

**Representative publication:**

1. *Wang S., Song F., Mao Y., Hu J., Wang C., Liu P., Liang S., Lin Y.*, Enhancing PgUGT activity and thermostability via mutation site screening aided by molecular docking structure and computer-aided design for efficient industrial synthesis of rebaudioside D, Bioresource Technology, 2026, 451, 134482.*

2. *Chen M., Song F., Qin Y., Han S., Rao Y., Liang S., Lin Y.*, Improving thermostability and catalytic activity of glycosyltransferase from Panax ginseng by semi-rational design for rebaudioside D synthesis, Frontiers in Bioengineering and Biotechnology, 2022, 10, 884898.*

3. *Chen M., Zeng X., Zhu Q., Wang D., Han S., Liang S., Lin Y.*, Effective synthesis of rebaudioside A by whole-cell biocatalyst Pichia pastoris, Biochemical Engineering Journal, 2021, 175, 108117.*

## Biography



Shengding Wang is a PhD student at the School of Biology and Biological Engineering, South China University of Technology, China, under the supervision of Prof. Ying Lin. His research focuses on enzyme engineering and biocatalysis, particularly the rational design and directed evolution of glycosyltransferases, molecular-dynamics-based mechanism analysis, and bioprocess intensification for the efficient biosynthesis of high-value steviol glycosides. In this study, he constructed combinatorial mutants of the glycosyltransferase PgUGT by combining molecular docking with FoldX-aided computer-aided design, obtaining the mutant $PgUGT_{M2}$ with substantially enhanced catalytic activity and thermostability, and developed a dual-enzyme cascade system with sucrose synthase mbSUS in *P. pastoris* that achieved the highest reported rebaudioside D titer to date and successful scale-up, providing a solid foundation for the industrial biomanufacturing of natural sweeteners.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Rewiring carbon-nitrogen metabolism and remodeling methyltransferase enables high-level *de novo* melatonin biosynthesis in yeast

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## Abstract

Microbial production of melatonin, a hormone used to regulate sleep, is constrained by limited L-tryptophan supply and *O*-methyltransferase product inhibition. Here, we combined global reprogramming of carbon and nitrogen metabolism with structure-guided enzyme engineering in *Komagataella phaffii*. Tuning general amino acid control activated nitrogen assimilation and expanded the L-tryptophan pool. Remodeling the hydrophobic methoxy cavity of caffeic acid *O*-methyltransferase and introducing additional hydrogen bonds alleviated product inhibition and increased catalytic efficiency to 16.0 times that of the parental enzyme. The engineered strain produced 8.6 ± 1.1 g $L^{-1}$ melatonin from glucose in minimal medium. Evolutionary analysis revealed how the methoxy cavity structure governs substrate selectivity within the caffeic acid *O*-methyltransferase family. Together, our findings inform the engineering of yeast to produce tryptophan-derived compounds.

**Key words:** L-tryptophan derivatives, *O*-methyltransferase, *Komagataella phaffii*, metabolic engineering, enzyme engineering

**Representative publication:**

1. Ren Y.Y., Celińska E., Cai P.*, Zhou Y.J.*, Combing directed enzyme evolution with metabolic engineering to develop efficient microbial cell factories, Chem. & Bio. Engineering, 2025, 2, 449.

## Biography



Yuyao Ren, a PhD student (2024 cohort) at the Dalian Institute of Chemical Physics, Chinese Academy of Sciences, under the supervision of Prof. Yongjin Zhou. Her research focuses on metabolic engineering of *Komagataella phaffii* and directed enzyme evolution, with an emphasis on melatonin biosynthesis in yeast and engineering of key pathway enzymes. She has published two first-author papers in *Biotechnology Advances* and *Chemical & Bioengineering*.

# Engineering all human milk oligosaccharide fucosidic linkages *in planta*

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## Abstract

Human milk oligosaccharides (HMOs) are a component of breast milk which improves infant health outcomes by modulating immune responses, inhibiting pathogen adhesion, and promoting healthy gut microbiota. Although their effects are well-documented, few HMOs have been clinically or biochemically studied because only a handful of the simplest HMOs out of >200 species are currently possible to chemically synthesize or produce in microbes via metabolic engineering in microbial hosts. In contrast, plants are naturally adept at biosynthesizing a wide range of complex polysaccharides, and they have recently been established as a heterologous system for HMO production. However, much of the structural diversity of HMOs has not yet been reproduced in a plant, particularly fucosylations. Four fucosidic linkages can be found in fucosylated HMOs, but only Fucα1–2Gal has been biosynthesized in planta. Here, we screened bacterial fucosyltransferases using the model plant *Nicotiana benthamiana* to produce 3-fucosyllactose (Fucα1–3Glc), lacto-N-fucopentaose II (Fucα1–4GlcNAc), and lacto-N-fucopentaose III (Fucα1–3GlcNAc). Just twelve glycosidic linkages make up all the HMOs found in breast milk, and our findings increase the number of linkages made *in planta* from seven to ten, greatly expanding the variety of HMOs accessible in a plant.

**Key words:** glycobiology, synthetic biology, prebiotics

**Representative publication:**

1. **Tang S. N.**, Drako K., Wyatt G., Cheng L., Zerbe P., Shih P. M.*, Engineering all human milk oligosaccharide fucosidic linkages *in planta*, Metabolic Engineering, in review.

2. **Tang S. N.**, Barnum C. R., Szarzanowicz M. J., Sirirungruang S., Shih P. M.*. Harnessing Plant Sugar Metabolism for Glycoengineering, Biology, 2023, 12 (12), 1505.

## Biography



Sophia Tang is a PhD candidate in the Department of Molecular and Cell Biology at the University of California, Berkeley under the supervision of Dr. Patrick Shih and is a National Science Foundation GRFP Fellow. She received her BS in Biological Chemistry from the University of Chicago. Her work focuses on plant metabolic engineering and making plant transformation more predictable, consistent, and efficient.

# Poster Session

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Metabolic Engineering Summit 2026

# Single-cell metabolic engineering (SME) and single-cell process engineering (SPE) by the Ramanomics Platform

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## Abstract

A single cell is the atomic unit of function and evolution for life on Earth and its bioprocesses. Dissecting, sorting, and designing cellular metabolism and bioprocess at single-cell resolution is therefore fundamentally important. We invented the Ramanomics Platform, comprising RamanEye (a high-throughput Raman flow cytometer with automated sample pretreatment), FlowRACS (a high-throughput Raman flow sorter), RAMS (a multi-modal Raman microfluidic sorter), DCP (a digital colony picker that uses laser for phenome-guided, contact-free picking of microdroplet clones), and RamanAI (an A.I. system that translates Raman/brightfield /fluorescent features into live single-cell metabolic/morphological/developmental phenotypes). By metabolically profiling, sorting, and cultivating/sequencing a mutant library, microbiome or animal-cell sample in a label-free, live-cell, and high-info-content manner, the Ramanomics Platform rapidly produces diagnosis/value/mechanism reports at single-cell resolution, plus a strain-bank of in-situ functional strains. For “Single-cell Metabolic Engineering (SME)”, it generates single-cell muli-omics, rapidly discovering metabolic pathways [1], and yielding superior mutants from random mutagenesis libraries within hours [2]. For “Single-cell Process Engineering (SPE)”, it enables single-cell-precision process monitoring at unprecedented speed [3] and rational process control based on inter-cellular metabolic dynamics [4]. Therefore, we proposed cellular and process ramanomes as new biological big data, and initiated the iMAPS Consortium to advance SME and SPE (http://iMAPS.info/solutions).

**Key words:** metabolic engineering, process engineering, ramanomics, RACS

**Reference:**

1. Qintao Wang#, Yanhai Gong#, et al., High-throughput Raman-activated cell sorting of microalgal genome-wide edited library revealed a regulatory pathway for carotenoid synthesis. *Nat Commun*, 2026, 17(1):8352.

2. Xixian Wang#, Sen Wang#, Zhidian Diao#, Xibao Hou#, et al., Label-free high-throughput live-cell sorting of genome-wide random mutagenesis libraries for metabolic traits by Raman flow cytometry. *Proc Natl Acad Sci U S A*, 2025, 122(22):e2503641122.

3. Jia Zhang#, Xu Liu#, Zhihang Chen#, et al., Label-free, single-cell-precision and monomeric-unit-resolved monitoring of biopolymer fermentation by ramanomics. *Trends Biotechnol*, 2026, 44(9):2771-2795.

4. Juline Savigny, et al., Single cell profiling framework reveals metabolic subpopulations as drivers of bioproduction heterogeneity. *Nat Commun*, 2025, 17(1):645.

## Biography



Jian XU (xujian@qibebt.ac.cn) obtained B.S in Biotechnology from Peking University in 1997, and M.S. in Computer Science and PhD in Biochemistry in Washington University in St. Louis in 2003. He serves on Scientific Advisory Councils in the Life Science Division of Natural Science Foundation of China (NSFC), and in the Applied and Environmental Division of American Society of Microbiology. He is also founding senior editors of *mSystems*, *mLife*, etc. Single-Cell Center, which he founded in 2012, introduced new concepts for single-cell metabolic phenomics such as ramanome and metaramanome, and developed core technologies including high-throughput single-cell Raman-activated flow cytometry and sorting, as well as digital colony picking. By collaboration with Single-Cell Biotech and eCyte, they invented the Ramanomics Instrument Series (RamanEye, FlowRACS, RAMS, Digital Colony Picker, etc.). These first-in-class instruments are supporting the iMAPS Consortium (http://iMAPS.info), a global network of single-cell “metabolic sensor, strain miner and ecological restorer” platforms for microbiome, to advance the vision of “metabolic monitoring and mining of microbiota @ single-cell, anytime & anywhere”. Jian has published over 200 papers with over 22,000 citations (H-index 71). His professional contributions were recognized by National Distinguished Young Scholars Award, National Young-Scientist Award for Science and Technology, etc.

# Genome-wide transcriptome analyses reveal changes in pigment - overproducing *Monascus* obtained by UV mutagenesis

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## Abstract

Monascus pigments (MPs) are important secondary metabolites produced by *Monascus* species. This study focused on obtaining a pigment producing-enhanced strain, *Monascus pilosus* MU-39, through UV mutagenesis and stability screening. MU-39 exhibited a 51.07% increase in pigment color value under solid-state fermentation and an 86.48% increase in red pigment color value under submerged fermentation. Scanning electron microscopy (SEM) and comparative genomic analysis revealed morphological alterations and strain-specific protein clusters associated with transcriptional regulation, protein kinase signaling, and protein N-terminal acetylation in MU-39. Transcriptomic analysis identified 1,268 differentially expressed genes, indicating extensive transcriptional changes in MU-39. Upregulated genes were mainly associated with carboxylic acid metabolism, lipid metabolism, and oxidation-reduction processes, whereas downregulated genes were mainly involved in cell wall organization, glycosyl precursor metabolism, and carbon source utilization. Pathway analysis further revealed transcriptional alterations in carboxylic acid metabolism, fatty acid biosynthesis, carbohydrate metabolism, and Monascus pigment biosynthesis. qRT-PCR validation showed expression trends consistent with the RNA-seq results, supporting the reliability of the transcriptomic data. This article provides a paradigm for the subsequent improvement targets of *Monascus pilosus* using synthetic biology.

**Key words:** Monascus pigments, *Monascus pilosus*, Genome, transcriptome, pigment - overproducing

**Representative publication:**

1. Lin, Y., J. Lu, Z. Yang, T. Wang, H. Li, S. Sha, Z. Liu, Y. Zhao and L. Wang.*. Comparative genomics reveals key molecular targets for mutant *Pediococcus pentosaceus* C23221 producing pediocin. International journal of biological macromolecules. 2023, 242(Pt 3): 125006.
2. Qi, C., C. Wang, Y. Jiang, R. Ledesma-Amaro, Q. Wang and L. Wang.*. Genome-Based Identification and Characterization of two Bacteriocins Produced by *Lactiplantibacillus Plantarum* BL25 in Beef.

Probiotics and Antimicrobial Proteins.2026
3. Li, H. B., L. D. Guo, X. Y. Zhang, H. Q. Mu, S. F. Sha, Y. Lin, H. W. Hou and L. P. Wang.*. Whole-genome sequencing combined with mass spectrometry to identify bacteriocin and mine silent genes. LWT-Food Science and Technology. 2022, 169: 113975.
4. Gu, L., Y. Tang, J. Zhang, N. Tao, X. Wang, L. Wang.* and C. Xu.*. Synergistic Anti-*Helicobacter pylori* Effects of *Takifugu obscurus* Skin Peptides and *Lactobacillus plantarum*: A Potential Gastric Health Dietary Supplement. Foods. 2025, 14(3): 406.
5. Li, H., H. Zheng, L. Li, Y. Lin, Q. Cheng, L. Wang.*, Y. Zhao and Z. Liu.*. Different Detection Strategies of Pediocin-Like Produced by *Pediococcus pentosaceus*. Probiotics and antimicrobial proteins. 2024, 17(5): 3134-3143

## Biography



Associate professor, master's supervisor, engineer, and brewer. Studied at the School of Bioengineering, Jiangnan University from 2000 to 2004 and obtained a PhD in Fermentation Engineering. Currently employed in the Food Biotechnology Teaching and Research Office of the School of Food Science and technology at Shanghai Ocean University. Member of the Lactic Acid Bacteria Branch of Chinese Institute of Food Science and Technology, member of the Shanghai Probiotic Professional Committee, domestic visiting scholar of young backbone teachers at the School of Life Science and Technology, Shanghai Jiao Tong University (Molecular Biology major, September 2011 to July 2012), and senior research scholar at the Department of Biology, Faculty of Sciences, University of Victoria, Canada (Biochemistry and Molecular Biology major, March 2014 to March 2015). The main research directions are prebiotics (stachyose, mannan oligosaccharide, fucoidan oligosaccharide), screening and improvement of probiotics and their metabolites (antimicrobial peptides), traditional fermented foods (frozen dough starter, fermented bean products, fish sauce, fruit wine and liquor).

# Decoding Micafungin Precursor Biosynthesis for Industrial Strain Engineering

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## Abstract

Micafungin, a semisynthetic derivative of the lipohexapeptide echinocandin FR901379 produced by *Coleophoma empetr*, is widely used for the treatment of invasive fungal infections. However, the low fermentation efficiency of FR901379 increases production costs, while its incompletely understood biosynthesis limits strain engineering. Here, we characterized the complete FR901379 biosynthetic pathway through gene disruption and enzymatic analyses. Two genes located in a separate gene cluster were identified as essential for FR901379 *O*-sulfonation, encoding the cytochrome P450 McfP and sulfotransferase McfS. Heterologous introduction of this sulfonation module into a pneumocandin $B_0$-producing strain enabled *O*-sulfonation of pneumocandin $B_0$, increasing its water solubility by approximately 4,000-fold. We further demonstrated that the core biosynthetic cluster and the sulfonation cluster are coordinately regulated by the pathway-specific transcription activator McfJ. Guided by these findings, overexpression of McfJ and rate-limiting enzymes increased FR901379 production from 0.3 to 2.03 g/L in shake flasks while reducing byproducts by 85%. This strategy was also successfully applied to the rational engineering of strains producing the precursors of anidulafungin and caspofungin, resulting in titer increases of 238% and 47%, respectively. These findings provide a foundation for engineering water-soluble echinocandins and developing efficient FR901379 cell factories.

**Key words:** Antifungal agents, Echinocandin, Micafungin, *O*-sulfonation, Metabolic engineering

**Representative publication:**

1. Men P., Geng C., Zhang X., Zhang W., Xie L., Feng D.D., Du S.Y, Wang M., Huang X.N.*, Lu X.F.*, Biosynthesis mechanism, genome mining and artificial construction of echinocandin *O*-sulfonation, Metabolic Engineering, 2022, 74, 160-167.

2. Men P.[#]; Xie L.[#], Wang J.C., Zhou Y, Zhang X.X., Li Y.P, Huang X.N.[*], Lu X.F.[*], Identifying metabolic bottlenecks for micafungin precursor production via untargeted regulatory perturbation, Biotechnology for Biofuels and Bioproducts,2026,19,17.

3. Men P., Zhou Y, Xie L., Zhang X., Zhang W., Huang X.N.[*], Lu X.F.[*], Improving the production of the micafungin precursor FR901379 in an industrial production strain, Microbial Cell Factories, 2023, 22, 44.

4. Xie L.[#], Men P.[#]; Zhou Y, Li J., Zhang X., Wang M., Huang X.N.[*], Lu X.F.[*], The visualizable and marker-free gene editing platform mediated by CRISPR/Cas9 in *Coleophoma empetri*, Applied Microbiology and Biotechnology, 2026, 110, 78.

## Biography



Ping Men obtained her Ph.D. degree in Microbiology from the University of Chinese Academy of Sciences (UCAS) in 2022. She is currently an Assistant Professor in the Qingdao Institute of Bioenergy and Bioprocess Technology (QIBEBT), Chinese Academy of Sciences. Her research focuses on the rational engineering of industrial filamentous fungi for the efficient production of important antifungal drugs. Over the past five years, she has published 10 scientific papers in journals including Metabolic Engineering, Biotechnology for Biofuels and Bioproducts and Microbial Cell Factories. She has filed nine Chinese invention patent applications and one PCT patent application.

# Rational charge-modulation engineering of polyphosphate kinase for efficient ATP regeneration

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## Abstract

Adenosine triphosphate (ATP)-dependent energy-consuming enzymatic reactions are widely used in vitro biocatalysis. However, directly adding an adequate amount of ATP to the reaction system markedly raises the raw material cost, restricting its practical utility. While polyphosphate kinases (PPKs)-driven ATP regeneration systems surpass traditional ATP synthesis systems in economy, stability and efficiency, the insufficient catalytic activity and poor thermostability of PPK hinder their broad practical utilization. Through amending the electrostatic potential defect, the optimal variant Q59R/D68H/T190Y exhibited a markedly prolonged half-life (on the order of several tens of hours) at 35℃ and a several-fold higher catalytic efficiency ($k_{cat}/K_m$) than the original. Finally, the enhanced thermal stability of the mutant was verified in the one-pot in vitro synthesis of glutathione (GSH). The adoption of this ATP regeneration system can effectively increase the production of GSH. This study elucidates that charge engineering is an effective strategy for enhancing the thermal stability of industrial enzymes, providing new ideas for the rational design of industrial enzymes.

**Key words:** polyphosphate kinases, charge-modulation engineering, thermal stability, ATP regeneration

**Representative publication:**

1. Zheng R-C, Fang S-X, Lin C-P, Hua M-H, Bian Y-Z, Zheng Y-G. A temperature-controlled multi-enzyme complex and preparation method and application: CN202511877143.5 [P]. 2026-01-16.

2. Zheng R-C, Zhu M-R, Lin C-P, Hua M-H, Zheng Y-G. A polyphosphate kinase mutant, coding gene, recombinant vector, recombinant strain, preparation method and application: CN202310871912.5 [P]. 2023-11-10.

3. Zheng R-C, Hua M-H, Fang S-X, Lin C-P, Zheng Y-G. Polyphosphate kinase mutant with improved thermostability and application: CN202510092404.6 [P]. 2025-04-08.

## Biography



Miao-Hang Hua, a PhD candidate in Biochemical Engineering at the College of Biotechnology and Bioengineering, Zhejiang University of Technology, P.R. China. Her research focuses on biocatalysis and rational protein engineering, with particular emphasis on constructing robust ATP-regeneration systems for in vitro multi-enzyme biocatalysis. She integrates structural analysis, computational design (AlphaFold, APBS, Rosetta) and iterative saturation mutagenesis to reshape the surface electrostatic potential of polyphosphate kinase 2 (PPK2), aiming to overcome the long-standing trade-off between catalytic activity and thermostability. Her work addresses the cost bottleneck of ATP-dependent biomanufacturing, and the engineered PPK2 variants have been successfully applied to the one-pot biosynthesis of glutathione (GSH), demonstrating promising potential for industrial deployment. She is a named inventor of several published and granted invention patents.

# Engineering Industrial Filamentous Fungal Chassis for Efficient Recombinant Protein Production

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## Abstract

Filamentous fungi are widely used for industrial protein production, but recombinant protein production can be limited by endogenous protein secretion and inefficient strain construction and screening. Here, we engineered two fungal hosts, the mesophilic *Aspergillus niger* and the thermophilic *Myceliophthora thermophila*. In *A. niger*, an industrial glucoamylase-producing strain was engineered by deleting 13 of 20 TeGlaA copies and disrupting PepA, generating the AnN2 chassis with 61% lower extracellular protein secretion. The resulting chassis retained transcriptionally active integration sites and supported secretion of glucose oxidase, thermostable pectate lyase, bacterial triose phosphate isomerase, and Lingzhi-8 within 48–72 h. In *M. thermophila*, combinatorial engineering generated a chassis with enhanced protein secretion and a semi-automated workflow combining MtLacA laccase expression, QPix-assisted colony isolation, and ABTS-based screening. More than 500 transformants were recovered from a single transformation. The selected MtLacA-producing strain T64 showed 5,038 U/L laccase activity, while extracellular protein reached 1.1 g/L in shake flasks and 5.9 g/L in 5-L fermentation. The chassis also supported secretion of heterologous fungal enzymes and porcine growth factors. Together, these studies provide fungal chassis and strain engineering approaches for recombinant protein production in both mesophilic and thermophilic filamentous fungi.

**Key words:** Filamentous fungi; Fungal chassis; Recombinant protein production; Protein secretion; Strain engineering.

**Representative publication:**

1. Gong C., Yang X., Zhao X., Gou F., Zhou J., Sun W., Sun T., Liu W., Wang K., Wang X., Chen Y., Liu Y., Xue C., Liu Q., Tian C., Semi-automated strain engineering and screening in the thermophilic fungus

*Myceliophthora thermophila* for recombinant protein production, Applied and Environmental Microbiology, 2026, 92, e00995-26.

2. Gou F., Liu D., Gong C., Wang K., Wang X., Chen Y., Liu Q., Tian C., Development of an efficient heterologous protein expression platform in *Aspergillus niger* through genetic modification of a glucoamylase hyperproducing industrial strain, Microbial Cell Factories, 2025, 24, 160.

3. Zhang M., Dong J., Wang K., Gong C., Liu D., Gou F., Liu D., Kong Z., Hao J., Sun W., Liu Q., Tian C., Transcriptomic analysis of two ascomycete fungi on the utilization of tobacco biomass and identification of key pectinases in polysaccharides deconstruction, Environmental Technology & Innovation, 2025, 38, 104191.

4. Zhu Z., Zhang M., Liu D., Liu D., Sun T., Yang Y., Dong J., Zhai H., Sun W., Liu Q., Tian C., Development of the thermophilic fungus *Myceliophthora thermophila* into glucoamylase hyperproduction system via the metabolic engineering using improved AsCas12a variants, Microbial Cell Factories, 2023, 22, 150.

## Biography



Qian Liu, Ph.D., is an Associate Investigator at the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences. She received her Ph.D. in Microbiology from the College of Life Sciences, Zhejiang University, in 2010, followed by postdoctoral research at the same university. She joined the Tianjin Institute of Industrial Biotechnology in 2011 and became a member of the Youth Innovation Promotion Association of the Chinese Academy of Sciences in 2019. Her research focuses on fungal synthetic biology, including genetic engineering tools, filamentous fungal chassis, regulation of gene expression and protein secretion, and recombinant protein production. She has served as principal investigator for projects funded by the National Natural Science Foundation of China (NSFC) and a synthetic biology sub-project of the National Key R&D Program of China. She has published more than 20 research papers in journals including Appl. Environ. Microbiol. and Biotechnol. Biofuels, and has filed or been granted over 30 invention patents.

# Residue Engineering for Esterase Optimization through Evidence Integration and Structural Stratification

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## Abstract

Biocatalysis has become an important approach to the synthesis of chiral compounds. Engineering enzymes to act on nonnatural substrates, however, requires a targeted strategy for selecting residues for mutagenesis. Here, we integrated ConSurf conservation analysis, FoldX stability prediction, residue–substrate proximity, CAVER channel mapping, and structural stratification to evaluate all residues of an esterase for methyl chrysanthemate hydrolysis. This framework yielded 100 candidate sites spanning the active pocket, second shell, loops, buried core, and surface. In addition, we established a high-throughput method for the quantitative detection of chrysanthemic acid. Based on the aforementioned work, we constructed a saturation mutagenesis library from the 100 selected residues to be tested, and screened dozens of advantageous mutants from it. Through iterative mutations, we obtained the superior mutant m4. showed 4.6-fold higher catalytic activity and 5.3-fold greater product accumulation than the wild type in (+)-trans-chrysanthemic acid synthesis. Structural and dynamics analyses revealed the mechanistic basis of these gains. This work provides a generalizable, computation-guided strategy for targeted enzyme engineering.

**Key words**: Enzyme engineering; Residue selection; Structural stratification; Evolutionary inference; High-throughput

**Representative publications**

1. Wang L. Y., Tang H., Zhao J. Q., Wang M. N., Xue Y. P.*, Zheng Y. G., Correlation analysis of key residue sites between computational-aided design thermostability D-amino acid oxidase and ancestral enzymes, Journal of Agricultural and Food Chemistry, 2023, 71, 20177–20186.

2. Tang H., Zhu H. L., Zhong J. X., Wang M. N., Xue Y. P.*, Zheng Y. G., Efficient production of 3-amino-2-hydroxy acetophenone by multi-enzyme biosynthesis, ChemBioChem, 2023, 24, e202300165.

## Biography



Meng-Nan Wang is a doctoral student in Biochemical Engineering at the College of Biotechnology and Bioengineering, Zhejiang University of Technology, China, and began doctoral studies in 2021. His research focuses on enzyme engineering and biocatalysis, with particular emphasis on integrating computational analysis with experimental approaches to improve enzyme stability and catalytic performance. This work combines evolutionary and structural evidence, molecular simulations and experimental screening to guide residue selection and investigate the effects of enzyme substitutions. Current research explores esterase engineering for the synthesis of (+)-trans-chrysanthemic acid, building on contributions to D-amino acid oxidase thermostability engineering and multienzyme cascade biosynthesis. Wang has co-authored two research articles, published in Journal of Agricultural and Food Chemistry and ChemBioChem.

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# Synergistic enhancement of activity and stability for haloalcohol dehalogenase in aqueous-organic biphasic systems

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## Abstract

The haloalcohol dehalogenase-catalyzed conversion of 1,3-dichloro-2-propanol to epichlorohydrin is a promising biosynthetic route. However, poor stability of sub strate and product in aqueous media requires an aqueous-organic biphasic system, which challenges the enzyme's catalytic performance and conformational robustness. To address these bottlenecks, this study developed a synergistic protein engineering strategy via structural analysis and computer-aided rational design. Long-range con formational modulation and direct substrate–enzyme interaction optimization were combined to improve the catalytic efficiency, and a holistic stabilization strategy that coordinates rational reshaping of organic solvent channels and optimization of pro tein binding free energy was proposed to enhance stability in the biphasic system. The obtained variant ($HheC_{4M}^{R123A+A159T+L143N+V206A+F244W}$) exhibited a 16.1-fold prolonged half-life (138.3 h) and 3.5-fold higher catalytic efficiency ($k_{cat}/K_m$) than the parental enzyme. The engineering strategy established in this work not only broadens the application prospects of haloalcohol dehalogenases, but also provides new insights for protein engineering in organic or biphasic systems.

**Key words:** activity and stability, biphasic system, haloalcohol dehalogenase, rational engineering

**Representative publication:**

1. Zhu XY, Zhao SY, Song XY, et al. Synergistic enhancement of activity and stability for haloalcohol dehalogenase in aqueous‐organic biphasic systems. AIChE Journal. 2026

2. Tang X-L, Wen P-F, Zheng W, Zhu X-Y. Bidirectional regulation of nitrilase reaction specificity by tuning the characteristic distances between key residues and substrate. ACS Catalysis. 2023;13(15):10282-10294.

## Biography



Xuan-Ye Zhu, a doctoral student in Biochemical Engineering at the College of Biotechnology and Bioengineering, Zhejiang University of Technology, China, started his PhD research in 2022. His research focuses on biocatalysis and rational protein engineering, mainly devoted to improving enzyme performance in aqueous-organic biphasic systems. He combines structural analysis, computer-aided design and site-directed mutagenesis to optimize active sites and solvent channels of haloalcohol dehalogenase, so as to synergistically enhance enzyme catalytic efficiency and organic-solvent stability. His work addresses key bottlenecks for biocatalytic synthesis of high-value chemicals, offering new insights for developing robust industrial biocatalysts. He has first-authored one paper in *AIChE Journal* and is a named inventor of several published invention patents.

# Expression and Catalytic Performance Optimization of 2-OST and 3-OST for Enhanced Enzymatic Heparin Synthesis

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## Abstract

Heparin, a naturally occurring glycosaminoglycan, is widely used in medical applications owing to its potent anticoagulant activity. The degree and pattern of sulfation are key determinants of its anticoagulant properties. The enzymatic modification of heparin involves five enzymes: N-deacetylase/N-sulfotransferase (NDST), C5-epimerase (C5-epi), 2-O-sulfotransferase (2-OST), 6-O-sulfotransferase (6-OST), and 3-O-sulfotransferase (3-OST). In this study, we selected human 2-OST and 3-OST and achieved their soluble expression. Using molecular docking with heparin disaccharides, we characterized the substrate-binding pockets of 2-OST and 3-OST, providing a structural basis for subsequent rational enzyme engineering. We then engineered both enzymes through rational design using a computational enzyme design platform.

**Key words:** 3-O-sulfotransferase; 2-O-sulfotransferase; rational design; heparin

**Representative publication:**

1.Tiansu Tan, Jing Yu, Jianyu Long, Xiaojing Li, Zheng-Jun Li, Yang Zhang, Mingjia Yu, Tianwei Tan; Rational Design and Engineering of 3-*O*-Sulfotransferase 1 Based on Enzyme Affinity for Improved Enzymatic Heparin Preparation. *J. Agric. Food Chem.* 7 May 2025; 73 (18): 11373–11385.
2.Sodhi, H.; Panitch, A. Glycosaminoglycans in Tissue Engineering: A Review. Biomolecules； 2021, 11 (1), 29.
3.Rabenstein, D. L. J. N. P. R. Heparin and heparan sulfate: structure and function. NaturalProduct Reports 2002, 19 (3), 312-331.

## Biography

Shuwei Zhang. I am a Ph.D. student at the College of Life Science and Technology, Beijing University of Chemical Technology. My current research focuses on the efficient expression and application of enzymes.



# Application of the modular strategy to the production of ergothioneine by engineered *Saccharomyces cerevisiae*

Ming Ma

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## Abstract

Ergothioneine (EGT), an amino-acid derivative with multiple bioactivities, shows promising prospects in pharmaceutical, food, nutraceutical and cosmetic industries. Existing microbial EGT biosynthesis generally relies on externally supplemented histidine, methionine and cysteine, raising the industrial production cost. Herein, to realize high-level EGT biosynthesis without exogenous precursor addition, eight heterologous EGT biosynthetic gene combinations from distinct sources were constructed and screened in *Saccharomyces cerevisiae*. The endogenous precursor metabolism was modularized into histidine biosynthesis, S-adenosylmethionine cycle and sulfur assimilation pathways, whose key genes were further screened and combinatorially optimized. Via multi-copy integration together with knockout-complementation of histidine-metabolic genes, both heterologous EGT synthetic pathway and native precursor supply were reinforced. The engineered strain yielded 161.98 mg/L EGT in shake-flask culture. In 5-L fed-batch fermentation without precursor supplementation, the maximum EGT titer reached 3053.3 mg/L after 240 h fermentation, with a productivity of 12.72 mg/L/h. To date, these represent the highest reported EGT titers for *S. cerevisiae*. This work provides an efficient strategy for EGT production by modular precursor regulation and multi-copy integration.

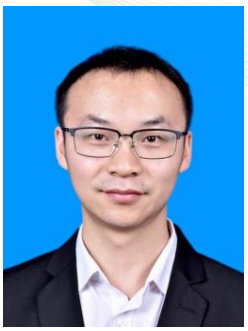
**Key words:** Ergothioneine, *Saccharomyces cerevisiae*, Metabolic engineering, Multi-copy integration

**Representative publication:**

1. Ma M. ,Tang F. ,Deng G.D.*, Application of the modular strategy to the production of ergothioneine by engineered *Saccharomyces cerevisiae*, Food Bioscience, 2026, 83109498-109498.

## Biography

Ming Ma



He is a Ph.D. candidate at Beijing University of Chemical Technology. His research interest is synthetic biology, mainly focusing on metabolic engineering of Saccharomyces cerevisiae. He has authored the journal article "**Application of the modular strategy to the production of ergothioneine by engineered *Saccharomyces cerevisiae***" published in "**Food Bioscience**". His experimental work is conducted at the State Key Laboratory of Green Biomanufacturing, No. 15 North Three-Ring East Road, Chaoyang District, Beijing 100029, China.

# Engineering of a LysG-derived arginine-specific biosensor for high-throughput screening of arginine overproducers in *Corynebacterium glutamicum*

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## Abstract

Arginine is a semi-essential amino acid with important applications in pharmaceutical and nutraceutical industries. *Corynebacterium glutamicum* is a promising host for arginine production, yet its industrial titers remain limited. Biosensor-based high-throughput screening enables rapid isolation of overproducers. However, the native LysG-based biosensor in *C. glutamicum* lacks arginine specificity, limiting its efficiency for screening high arginine-producing strains. Here, we developed an arginine-specific biosensor pLysG$^{F222I}$ in *C. glutamicum*, by combining semi-rational design and directed evolution of LysG, an endogenous regulator responsive to basic amino acids. Structural and interaction analyses revealed that the F222I substitution in LysG retained high affinity for arginine while significantly decreasing its responsiveness to histidine and lysine. Subsequently, this arginine-specific biosensor was successfully applied to screen a library of *N*-acetylglutamate kinase mutants, identifying 18 novel NAGK mutants with enhanced arginine production compared with previously reported variants. The strain integrated with the optimal NAGK mutant was further subjected to mutagenesis and screening, yielding an engineered strain that achieved an arginine titer of 56.07 g/L in a 5-L bioreactor, a 42% increase relative to the original starting strain. Overall, this study provides a powerful tool for accelerating the metabolic engineering of *C. glutamicum* for high-level arginine production.

**Key words:** Arginine-specific biosensor, Arginine, High-throughput screening, LysG, *Corynebacterium glutamicum*

**Representative publication:**

1. Cai N., Wang Y., Ren P., Rao D., Shi T., Wang Z., Shen J., Zhou W., Qi L., Wang L., Chen N., Chen J., Zheng P., Sun J., Engineering of a LysG-derived arginine-specific biosensor for high-throughput screening of arginine overproducers in *Corynebacterium glutamicum*, Biotechnology for Biofuels and Bioproducts, 2026, 19, 60.

2. Pu W., Chen J., Liu P., Shen J., Cai N., Liu B., Lei Y., Wang L., Ni X., Zhang J., Liu J., Zhou Y., Zhou W., Ma H., Wang Y., Zheng P., Sun J., Directed evolution of linker helix as an efficient strategy for engineering LysR-type transcriptional regulators as whole-cell biosensors, Biosensors and Bioelectronics, 2023, 222, 115004.

## Biography



Jiuzhou Chen, Senior Engineer at Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences. His research focuses on the development and industrial application of biosynthetic technologies for amino acids. He has long engaged in the construction and iterative optimization of industrial chassis strains including *Corynebacterium glutamicum* and *Escherichia coli*. His work includes developing genome mutators for accelerated strain evolution and engineering high-performance biosensors for amino acids. He has constructed top-tier strains for production of 5-aminolevulinic acid, glutamate and other amino acids, with several strains successfully transferred to industrial applications.

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Decoupling transcriptional gain and leakage enables programmable high-precision gene regulation in *Yarrowia lipolytica*

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## Abstract

Synthetic transcriptional systems are widely used to engineer biological functions, yet their precision remains limited by an intrinsic trade-off between regulatory gain and basal leakage. This constraint restricts the construction of predictable genetic programs, particularly in non-conventional yeasts that lack sophisticated regulatory architectures. Here, using *Yarrowia lipolytica* as a model chassis, we systematically dissect the relationship between transcriptional amplification and background expression and reveal a fundamental gain-leakage constraint inherent to conventional regulatory designs. By engineering promoter operator architectures and repressor modules, we establish a dual-layer repression strategy that decouples transcriptional activation strength from OFF-state leakage. This architecture enables a Tet-ON system with a 4869.56-fold induction range while maintaining background expression close to empty-vector levels, substantially improving regulatory precision compared with conventional single-layer systems. The underlying design principle is broadly transferable across distinct regulatory platforms, including Tet-OFF and xylose-inducible systems, demonstrating its modularity and general applicability. Furthermore, this high-stringency regulatory framework enables controlled production of a toxic PET hydrolase, achieving 1859.80 mg/L protein production during fed-batch fermentation. Our study reveals a fundamental design principle governing synthetic transcriptional control and provides a general strategy for engineering microbial systems with enhanced precision, predictability and functional capacity.

**Key words:** Synthetic biology; Gene regulation; Transcriptional architecture; Regulatory precision; *Yarrowia lipolytica*

**Representative publication:**

1. Gou Y.W., Li D.F., Zhao M.H., Lian J.Z.*, Intein-mediated temperature control for complete biosynthesis of sanguinarine and its halogenated derivatives in yeast. *Nat Commun*, 2024, 15(1), 5238.

2. Skjoedt M.L., Snoek T., Jensen M.K.*, Keasling J.D.*, Engineering prokaryotic transcriptional activators as metabolite biosensors in yeast. *Nat Chem Biol*, 2026, 12(11): 951-958.

3. Tominaga M., Shima Y., Nozaki K., Ishii J.*, Designing strong inducible synthetic promoters in yeasts. *Nat Commun*, 2024, 15(1), 10653.

4. Dong J.T., Willner I.*, Synthetic dynamic transcription frameworks and their applications. *Nat Chem*, 2026, 18(2): 227-245.

5. Zhai H.T., Liu X.Y., Guo P.Z., Hou J.*, A bacterial-derived quorum-sensing platform enables dynamic metabolic control in yeast. *Trends Biotechnol*, 2026.

## Biography

Jia Yan is a Ph.D. candidate at the State Key Laboratory of Microbial Technology, Shandong University, working under the supervision of Prof. Qingsheng Qi. She earned her Master's degree from Shandong University. Her research primarily centers on synthetic biology and metabolic engineering, specifically aiming to develop efficient gene expression regulation tools to advance microbial cell factory construction.

To date, she has published three peer-reviewed papers, including two as the first author in *Food Bioscience* and *Critical Reviews in Biotechnology*. Recognized for her academic excellence, she received the 2025 National Scholarship for Graduate Students. She will present her latest findings at the Metabolic Engineering Summit 2026 (MES2026). Her long-term goal is to synergize tool development with chassis modification to build highly efficient microbial cell factories.



# UV Mutagenesis and Electrochemical Screening of an Oxidative-Stress-Resistant Cupriavidus necator for $CO_2$-Derived Single-Cell Protein Production

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## Abstract

To address protein shortages and global warming, microbial electrosynthesis offers a sustainable approach for converting carbon dioxide ($CO_2$) into single-cell protein (SCP). The facultative chemolithoautotroph *Cupriavidus necator* is a promising microbial platform. However, oxygen free radicals (OFR) generated at the cathode can impair its growth and metabolism. In this study, ultraviolet (UV) mutagenesis, plate-based screening, and electrochemical cultivation were combined to obtain the oxidative-stress-resistant mutant T3. Under electrochemical cultivation, T3 exhibited a 2.48-fold higher growth rate than the wild-type strain H16. T3 showed significantly enhanced resistance to hydroxyl radicals and superoxide anions. Its resistance to hydrogen peroxide improved only slightly. These results identify hydroxyl radicals and superoxide anions as the primary causes of oxidative damage during microbial electrosynthesis. Comparative analyses also identified candidate genes and metabolic pathways associated with oxidative-stress resistance and electrochemical growth. This study provides an improved microbial chassis for electricity-driven $CO_2$ bioconversion and supports the sustainable production of SCP. Further nutritional, toxicological, and feeding evaluations are required to assess the suitability of T3-derived biomass as a feed ingredient.

**Key words:** oxygen free radicals (OFR); Mutagenesis Screening; Omics; Electrochemical cultivation.

**Representative publication:**

1. Chunling Ma*, Xiaolei Cheng*, Yuhua Wang*, Ke Chen, Yihua Ma, Yuping Lin, Kun Guo, Zhiguang Zhu#. High-efficiency $CO_2$ fixation and single-cell protein production in *Cupriavidus necator* ARTP-driven metabolic rewiring[J]. Green Chemistry, 2026, 28 (10): 4550-4563.
2. Chunling Ma*, Yuhua Wang*, Kun Guo, Ranran Wu#, Zhiguang Zhu#. Enzymatic electrosynthesis system based on multi-enzyme catalysis or coupled with microbial transformation[J]. Catalysis Science & Technology, 2025, 15 (5): 1390-1405.
3. Ke Chen*, Chunling Ma*, Xiaolei Cheng*, Yuhua Wang, Kun Guo, Ranran Wu, Zhiguang Zhu#. Construction of *Cupriavidus necator* displayed with superoxide dismutases for enhanced growth in bioelectrochemical systems[J]. Bioresources and Bioprocessing, 2023, 10: 36.
4. Li Ma*, Ying He*, Youran Wang, Yuhua Wang, Ruotong Li, Zhihong Huang, Yanjun Jiang#, Jing Gao#. Nanocomposites of Pt nanoparticles anchored on UiO66-$NH_2$ as carriers to construct acetylcholinesterase biosensors for organophosphorus pesticide detection[J]. Electrochimica Acta, 2019, 318: 525-533.

## Biography



Yuhua Wang is a researcher specializing in microbial electrosynthesis, carbon dioxide bioconversion, and single-cell protein production. Her research focuses on developing efficient biological systems for carbon fixation and sustainable protein production. She has contributed to studies on ARTP-driven metabolic rewiring in *Cupriavidus necator*, multi-enzyme electrosynthesis systems, and the engineering of *C. necator* with enhanced oxidative-stress resistance. Her research interests also include enzyme catalysis, biosensors, and microbial cell factories. Through interdisciplinary approaches combining microbiology, electrochemistry, and metabolic engineering, she aims to advance sustainable biomanufacturing and develop innovative solutions for carbon utilization, food security, and environmental protection.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# An integrated workflow for proline hydroxylase discovery and engineering through sequence mining, rational design, and low-N learning

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## Abstract

Biocatalysis enables selective synthesis under mild conditions and is central to pharmaceutical and industrial chemical production. However, its extension to non-native reactions is hindered by two consecutive bottlenecks: identifying suitable starting enzymes and optimizing them from sparse activity data. Here, we developed a sequence-structure-mutation workflow that integrates deep-learning-guided enzyme discovery, structure-guided dual-layer confinement engineering, and low-N activity learning using the Mutation Feature-Integrated Protein Language Model (MF-PLM). Applied to the Fe(II)/α-ketoglutarate-dependent C5 hydroxylation of L-pipecolic acid (L-PA), the workflow identified a lead hydroxylase from 500 homologs and yielded the five-substitution variant M5, which showed 12.6-fold higher activity than WT, 6.3- and 20.3-fold higher catalytic efficiencies toward L-PA and α-ketoglutarate. In whole-cell biotransformations, the cis-5-hydroxypipecolic acid yield obtained with M5 was 7.2 times that obtained with the wild type. Structural and dynamic analyses associated these gains with improved substrate accessibility and catalytic-complex preorganization. This workflow provides a generalizable, experimentally economical strategy for discovering and engineering biocatalysts for non-native reactions.

**Key words:** Protein engineering; Enzyme mining; Dual-layer confinement engineering; Protein language models; Whole-cell biocatalysis

**Representative publication:**

1. Li S. F., Zhu X. X., Hu Y. S., Li R. H., Xue Y. P.*, Zheng Y. G., Biosynthesis of L-pipecolic acid and its hydroxylated derivatives: Enzyme, engineering, and synthesis method, Biotechnology and Bioengineering, 2026, 123, 831–854.

## Biography



Yu-Sheng Hu, a doctoral student in Biochemical Engineering at the College of Biotechnology and Bioengineering, Zhejiang University of Technology, China, began his doctoral studies in 2021. His research focuses on synthetic biology and enzyme engineering, with particular emphasis on integrating machine learning with experimental approaches to protein design and engineering. He combines enzyme mining, structure-guided design, and molecular simulations with emerging computational tools, including protein language models, to identify promising biocatalysts and prioritize beneficial mutations. His work explores how these approaches can guide enzyme optimization when experimental data are limited, with applications in selective synthesis and whole-cell biocatalysis for the production of value-added chemicals. He has co-authored one article in Biotechnology and Bioengineering and is a named inventor on two published invention patent applications.

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Yeast Cell Factory for Betulinic Acid Biosynthesis through Pathway Optimization and *BpPDR4*-Mediated Enhancement

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[b] *Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China*

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## Abstract

Betulinic acid (BA) is a bioactive lupane-type pentacyclic triterpenoid mainly derived from birch (*Betula platyphylla* Suk.) bark and has diverse pharmacological activities, including anti-HIV and anticancer effects. However, its low abundance in natural resources and the limitations of conventional production methods hinder the large-scale and sustainable production of BA. Here, an efficient *Saccharomyces cerevisiae* cell factory was developed for BA biosynthesis based on the transcriptome of birch. Using the UniKP computational model and experimental validation, through systematic pathway engineering and optimization of CYP-CPR stoichiometry, NADPH supply, and downstream oxidation, a high-performance chassis strain producing 323.78 mg/L lupeol was constructed, and BA production reached 560.51 mg/L in shake-flask fermentation. Furthermore, *BpPDR4*, a new pleiotropic drug resistance gene from birch, was identified and characterized. Its heterologous expression enhanced the accumulation of BA and related C-28 oxidation products, indicating a potential role in improving BA biosynthesis and cellular product handling. Finally, the engineered strain achieved a BA titer of 3.09 g/L in a 5-L bioreactor. Overall, this study establishes an efficient microbial platform for BA production and provides new insights into the use of plant-derived transporters for improving the biosynthesis of high-value triterpenoids.

**Key words:** Betulinic acid, *Saccharomyces cerevisiae*, Biosynthetic biology, Pleiotropic drug resistance gene

## Biography:

Linlin Xu, doctoral candidate from Northeast Forestry University, specializing in the metabolic regulation and biosynthesis of natural products; Research production have been publish in Plant Biotechnology Journal ea al., and applied for three patents.



# Myristoleic acid produced by *Enterococcus faecalis* inhibits obesity by activating brown adipose tissue

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## Abstract

Activation of brown adipose tissue (BAT) and beige adipocytes is central to thermogenesis in the body and, as a potential therapeutic target for obesity and metabolic disorders, helps promote energy expenditure and maintain metabolic homeostasis. The gut microbiota and its derived metabolites are key mediators in regulating obesity. However, the specific mechanisms by which gut-derived bacteria trigger BAT activation and thermogenesis remain unclear. In this study, we found that *Enterococcus faecalis* (*E. faecalis*) activates BAT and induces beige adipocyte formation by upregulating the expression of the thermogenic marker UCP1, thereby reducing fat accumulation in db/db obese model mice. Through targeted metabolomics, we identified myristoleic acid (MA) as a key metabolite of *E. faecalis*, and the genes encoding acyl-CoA thioesterases (ACOTs) in *E. faecalis* showed biosynthetic potential for MA production. Knockdown of ACOT genes via CRISPR-dCas9 significantly reduced MA production, while treatment with ACOT gene-knockout E. faecalis failed to replicate the beneficial effects of wild-type E. faecalis, demonstrating that wild-type E. faecalis exerts its effects by increasing circulating MA levels. Our findings indicate that the gut microbiota-long-chain fatty acid-BAT axis plays an important role in host metabolism and may provide a strategic advantage for the development of next-generation anti-obesity therapeutics.

**Key words:** Obesity; Gut microbiota; Fatty acids; Brown adipose tissue; Thermogenesis

**Representative publication:**

1. Chang S. Y, Han S. Z, Choe H. M, Gao K, Jin Z. Y, Liu X. Y, Yang L. H, Lv S. T, Yin X. J, Quan L. H*, Kang J. D*. miR-320 regulates myogenesis by targeting growth factor receptor-bound protein-2 and ameliorates myotubes atrophy. Int J Biochem Cell Biol, 2022, 147, 106212.

2. Wang J. X, Chang S. Y, Jin Z. Y, Li D, Zhu J, Luo Z. B, Han S. Z, Kang J. D*, Quan L. H*. *Lactobacillus reuteri*-Enriched Eicosatrienoic Acid Regulates Glucose Homeostasis by Promoting GLP-1 Secretion to Protect Intestinal Barrier Integrity. J Agric Food Chem. 2025, 8, 393-408.

3. Han S. Z, Gao K, Chang S. Y, Choe H. M, Paek H. J, Quan B. H, Liu X. Y, Yang L. H, Lv S. T, Yin X. J, Quan L. H*, Kang J. D*. miR-455-3p Is Negatively Regulated by Myostatin in Skeletal Muscle and Promotes Myoblast Differentiation. J Agric Food Chem. 2022, 24, 10121-10133.

4. An Z. Y, Han S. Z, Li Z. Y, Chang S. Y, Zhang X. L, Lu G. J, Zhang T, Quan B. H, Yin X. J, Quan L. H*, Kang J. D*. Eicosatrienoic acid enhances the quality of in vitro matured porcine oocytes by reducing PRKN-mediated ubiquitination of CISD2. Theriogenology. 2024, 230, 285-298.

5. Yan J, Ma S. Z, Chang S. Y, Jin Z. Y, Liao X. X, Jia M. X, Kang J. D*, Quan L. H*. Eicosatrienoic acid improves PCOS in mice by modulating the intestinal barrier and ovarian inflammation. J Nutr Biochem. 2026, 158, 110477.

## Biography



Shuang-Yan Chang, a doctoral candidate at Yanbian University, was selected for the first batch of the Jilin Province Doctoral Candidate Support Program. Chang's main research focuses on the regulatory effects of gut microbiota on host development, reproductive physiology, and metabolic diseases. During doctoral studies, Chang has participated in projects funded by the National Natural Science Foundation of China (General Program and Regional Program) and the Science and Technology Development Program of Jilin Province. Chang has published 3 SCI papers as first author or co-first author, 11 SCI papers as co-author, and 1 abstract at an international conference.

# Myostatin deletion remodels gut microbiota and improves fast-twitch myofiber growth and muscle atrophy via the valeric acid-GPR43 axis

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## Abstract

The host genome can influence the composition of the gut microbiota, which plays a crucial role in muscle growth and development. This study found that myostatin (*MSTN*) is not only a key molecule regulating skeletal muscle development but also participates in the regulation of gut physiological homeostasis. Using a *MSTN* gene-edited pig model, this study investigated the effects of *MSTN* deficiency on intestinal structure, barrier function, and the gut microbiome. The results showed that *MSTN* knockout can, by inhibiting the MLCK/MLC signaling pathway, improve intestinal tight junctions and alter the composition of the gut microbiota, leading to an enrichment of bacteria that produce short-chain fatty acids (SCFAs). Following fecal microbiota transplantation from $MSTN^{-/-}$ pigs into mice, the recipient mice exhibited hypertrophy of fast-twitch type IIb muscle fibers and elevated levels of short-chain fatty acids, such as valeric acid, in the colon. Further mechanistic studies confirmed that valeric acid activates the Akt/mTOR pathway via GPR43, promoting myocyte differentiation and alleviating dexamethasone-induced muscle atrophy. This study identified the *MSTN* gene-microbiota-short-chain fatty acid metabolism axis that regulates fast-twitch muscle growth, providing a new metabolic perspective on host–microbial interactions and their role in fast-twitch muscle growth.

**Key words:** Akt/mTOR pathway, Fecal microbiota transplantation, Gut-muscle axis, Myostatin

**Representative publication:**

1. Duan X. R., Zhai W., Wang Y., **Sun K**., *et al,* Effects of adding exogenous microorganisms on nitrogen conversion and its mechanism of action in pig carcass composting, Journal of Cleaner Production, 2024, 449, 141734.

2. **Sun K**., Sun Y. Z., Du X. H., Zhang X. Q., *et al,* Lnc-Clic5 as a sponge for miR-212–5p to inhibit cow barn PM2.5-induced apoptosis in rat alveolar macrophages, Toxicology, 2024, 504, 153797.

3. **Sun K.**, Sun Y. Z., Jia Y. N., Duan X. R., *et al,* MicroRNA miR-212-5p Regulates the MEK/ERK Signaling Pathway by Targeting A-Raf proto-oncogene serine/threonine-protein kinase (ARAF) to Regulate Cowshed PM2.5-Induced NR8383 Apoptosis, Toxics, 2023, 11, 981.

4. Sun Y. Z., **Sun K.**, Du X. H., Ma Z. H., *et al,* The long noncoding RNA lnc-H3f3b2 regulates cow barn PM2.5-induced apoptosis of rat alveolar II epithelial cells through a ceRNA mechanism, Ecotoxicology and Environmental Safety, 2025, 302, 118755.

5. Sun Y. Z., **Sun K.**, Ma Z. H., Zhang X. Q., *et al,* miR–122–5p Promotes Cowshed Particulate Matter2.5-Induced Apoptosis in NR8383 by Targeting COL4A1, Toxics, 2024, 12, 386.

## Biography



Ke Sun is a doctoral candidate in Animal Husbandry at Yanbian University. His primary research interest lies in the molecular mechanisms of myostatin in the regulation of metabolism and diseases. In his recent work, he has investigated how myostatin deletion reshapes intestinal barrier integrity and microbial metabolite production, identified the *MSTN*-gut microbiota-short-chain fatty acid metabolic axis, and dissected the regulatory effects of microbiota-host interactions on organismal metabolism. He has participated in multiple research projects supported by the National Natural Science Foundation and Provincial Natural Science Foundation of China. His research work mainly includes animal model establishment, dissection of pathogenic pathways, and exploration of intracellular signal-transduction networks. To date, he has published 8 SCI-indexed papers, 4 of which are first-author or co-first-author publications. He looks forward to exchanging research ideas with faculty and peers at this conference.

# Genome-scale metabolic modeling maps nitrogen–oxygen control of growth-to-storage allocation for PHB biomanufacturing in *Halomonas boliviensis* LC1

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**Abstract**

*Halomonas boliviensis* LC1 is a moderately halophilic poly(3-hydroxybutyrate) (PHB) producer, but the metabolic basis of nutrient- and oxygen-dependent carbon allocation remains unclear. We reconstructed and systematically curated the genome-scale metabolic model iAL1080 using sequence-resolved protein mapping, multi-database reaction evidence, manual gene–protein–reaction review, an LC1-informed biomass objective, penalty-weighted gap filling, and quality control. The final model contains 1,080 SBML geneProduct entries, 2,377 reactions, and 1,662 metabolites, with a MEMOTE score of 93.05%. Simulations revealed a clear growth–PHB trade-off: ammonium limitation strongly reduced growth capacity while largely preserving PHB capacity, whereas oxygen effects depended on the imposed growth-state constraint. A two-dimensional nitrogen–oxygen phase plane and flux-variability analysis further identified robust shifts between growth-dominant and storage-oriented states. iAL1080 therefore provides a reproducible framework for analyzing growth-to-storage allocation and guiding future PHB biomanufacturing and community metabolic engineering.

**Key words:** *Halomonas boliviensis* LC1; genome-scale metabolic model; poly(3-hydroxybutyrate); nitrogen limitation; flux variability analysis

**Representative publication:** Not applicable.

## Biography

Aonan Li is a PhD student at the School of Bioengineering, Dalian University of Technology. His research focuses on genome-scale and microbial community metabolic modeling, model quality control, and computational design for biomanufacturing, with current emphasis on PHB-producing halophilic bacteria.

# *Lactobacillus reuteri* Promotes GLP-1 Secretion to Improve Intestinal Barrier Integrity and Glucose Homeostasis

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## Abstract

Lactobacillus reuteri is a well-known probiotic with beneficial effects on insulin resistance, inflammation, and intestinal barrier function, although the underlying mechanisms remain unclear. Here, we found that L. reuteri supplementation improved intestinal barrier integrity and glucose homeostasis in high-fat diet (HFD)-fed mice. Lipid metabolomic analysis revealed significant alterations in intestinal metabolites following L. reuteri administration. Further studies demonstrated that L. reuteri-associated metabolites promoted GLP-1 secretion through CD36-dependent PLC/IP3R/$Ca^{2+}$ signaling, as confirmed by CD36 inhibition in vivo and CD36 knockdown in STC-1 cells. GLP-1 receptor inhibition in vivo and intestinal organoid experiments further showed that GLP-1 enhanced intestinal tight junction protein expression through the GLP-1R/PI3K/AKT/HIF-1α pathway, thereby improving intestinal barrier integrity. Enhanced barrier function reduced circulating LPS levels, attenuated white adipose tissue (WAT) inflammation, and improved glucose homeostasis in both HFD-fed and db/db mice. Collectively, our findings identify a L. reuteri–GLP-1–intestinal barrier axis linking the gut microbiota to systemic metabolic homeostasis and provide new insights into the metabolic benefits of L. reuteri.

**Key words:** *Lactobacillus reuteri, CD36, GLP-1, intestinal barrier, glucose homeostasis*

**Representative publication:**

1. Wang JX, Chang SY, Jin ZY, Li D, Zhu J, Luo ZB, et al. *Lactobacillus reuteri* enriched eicosatrienoic acid regulates glucose homeostasis by promoting GLP-1 secretion to protect intestinal barrier integrity. J Agric Food Chem 2025;73:393-408.

## Biography



I am a graduate student at Yanbian University, with a research interest in the interactions between the gut microbiota and metabolic health. My current research focuses on understanding how gut microorganisms and their metabolites influence intestinal barrier function and systemic glucose metabolism.

In my recent work, I have been investigating the role of beneficial gut bacteria in regulating GLP-1 secretion and exploring how microbiota–host interactions contribute to the maintenance of intestinal barrier integrity and glucose homeostasis. I am particularly interested in uncovering the molecular mechanisms linking microbial metabolites, intestinal endocrine signaling, and metabolic regulation.

Through this research, I hope to gain a deeper understanding of microbiota–host communication and contribute to the development of microbiome-based strategies for improving metabolic health.

# Biosynthetic production of PSA, BNP, and NT-proBNP as biomarkers for cardiovascular and renal diseases

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## Abstract

Heart failure and kidney disease are major diseases threatening human life and health. Accurate biomarker detection by in vitro diagnostic (IVD) reagents relies on highly stable, high-purity materials. However, heterologous proteins often suffer from insolubility and poor secretion, limiting efficient biomarker production. In this study, prokaryotic and eukaryotic expression systems were constructed for soluble expression and efficient secretion of PSA, BNP, and NT-proBNP. By optimizing expression elements, screening solubilizing tags, optimizing secretion pathways, and overexpressing molecular chaperones, the low expression efficiency of heterologous proteins was overcome, providing a new method for biological synthesis of major disease biomarkers. The constructed strains PSA-XXA-DsbA and BNP-TrxA-TF achieved soluble expression titers of 25.77 and 46.29 mg/L, while NT-proBNP-TrxA reached a purified yield of 101.45 mg/L. These values were 2.74-, 13.9-, and 7.44-fold higher than the original strains, respectively. For NT-proBNP, the yield was 3.95 mg/L higher than the reported 97.5 mg/L, approximately 1.04-fold of the reported value. These results demonstrate that fusion tags combined with molecular chaperone overexpression significantly enhance soluble protein expression. This study enables efficient production of recombinant PSA, BNP, and NT-proBNP with acceptable analytical performance, providing a promising strategy for biomanufacturing heart and kidney disease biomarkers.

**Key words:** Biomarker, Heart failure， Prostate-specific antigen, Brain natriuretic peptide, N-terminal pro-B-type natriuretic peptide

**Representative publication:**

1. Luo Liu, Chenqi Cao, Linbo Wu, Ruikai Wang, Chenyuan Liu, Lintong Jiang. Polyphosphate Kinases (PPKs) for Efficient Regeneration of Guanosine Triphosphate (GTP) and Use Thereof. U.S. Patent Application No. 18/917,329, 2024. (Priority: CN 202410164536.0, filed Feb. 5, 2024)

2. Luo Liu, Chenqi Cao, Linbo Wu, Ruikai Wang, Chenyuan Liu, Lintong Jiang. Polyphosphate Kinases for Efficient Regeneration of GTP and Their Applications. Chinese Invention Patent Application No. 202410164536.0, filed Feb. 5, 2024; published May 24, 2024.

## Biography



Chenyuan Liu， Ph.D. Candidate, Beijing University of Chemical Technology (BUCT).

Research Interests: Machine Learning-Based Protein Solubility Prediction and Soluble Expression Optimization.

# Uncovering Lignin Degradation for Chemical Production through Consolidated Bioprocessing in *Myceliophthora thermophila*

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## Abstract

Consolidated bioprocessing (CBP) integrates enzyme production, biomass saccharification, and product fermentation into a single microbial system. *Myceliophthora thermophila* is a promising CBP chassis owing to its thermotolerance and efficient lignocellulose degradation. Using this platform, we developed cell factories for producing malic acid, ethanol, lactic acid, succinic acid, glutamic acid and protocatechuic acid. Lignin can comprise up to one-third of the plant biomass, providing structural integrity and defense while contributing to the recalcitrance of lignocellulosic biomass. Here, we investigated lignin degradation in *M. thermophila*, which remains poorly understood in ascomycetes. Extracellular lignin-degrading enzymes were studied, and a key dye-decolorizing peroxidase (DyP) was identified. Further, key metabolic pathways for lignin-derived aromatic compounds were uncovered, spanning syringyl (S), guaiacyl (G), and *p*-hydroxyphenyl (H) derivatives. Specifically, we found that *M. thermophila* funnels diverse H and G aromatic monomers into the 3,4-dihydroxybenzoic acid branch of the *β*-ketoadipate pathway. Enhanced lignin depolymerization improved the overall conversion efficiency of corncob biomass, yielding over 150 g/L malic acid with a yield of 0.55 g/g. An engineered Crabtree-positive aerobic system enabled one-step ethanol production from corncob, reaching 50 g/L. These advances establish *M. thermophila* as a versatile CBP chassis for low-carbon production of value-added chemicals from lignocellulosic biomass.

**Key words:** Consolidated bioprocessing; *Myceliophthora thermophila*; Lignin; Malic acid; Ethanol.

**Representative publication:**

1. Li, J., Lin, L., Sun, T., Xu, J., Ji, J., Liu, Q., Tian, C.*. 2020. Direct production of commodity chemicals from lignocellulose using *Myceliophthora thermophila*. Metab Eng, 61, 416-26.

2. Zhang, Y., Sun, T., Wu, T., Li, J., Hu, D., Liu, D., Li, J., Tian, C.*. 2023. Consolidated bioprocessing for bioethanol production by metabolically engineered cellulolytic fungus *Myceliophthora thermophila*. Metab. Eng., 78, 192-199.

3. Zhang, Y., Zhang, Y., Han, L., Liu, J, Liu, D., Sun, T., Wang, D., Li, M., Li, J.*, Tian, C.*. 2027. Unlocking raw-corncob consolidated bioprocessing for malic acid production by DyP-enabled lignin deconstruction in *Myceliophthora thermophila*. Synth Syst Biotechnol, 17, 121-132.

4. Liu, D., Ge Y., Li, J.*, Tian, C.*. 2026. Metabolic engineering of the thermophilic fungus *Myceliophthora thermophila* for L-glutamate production from lignocellulosic biomass. ACS Synth. Biol, (Accepted).

5. Liu, D., Yan, R., Ge Y., Zhang, Y., Li, J.*, Tian, C.*. 2026. Uncovering lignin metabolism in *Myceliophthora thermophila* toward integrated lignocellulose valorization. Under revision.

## Biography



Dr. Defei Liu is a researcher in computational metabolic engineering and fungal biotechnology. His research focuses on *Myceliophthora thermophila* and its application in lignocellulosic biomass conversion. His work combines multi-omics analysis, metabolic network modeling, metabolic engineering, lignin metabolism, and biological database development to understand biomass utilization and improve product formation. His recent research focuses on lignin degradation and the metabolism of lignin-derived aromatic compounds, as well as the use of computational metabolic engineering to guide biomass conversion and fungal cell factory development.

# Metabolic Engineering Optimization of *Escherichia coli* BL21 Star (DE3) for High-efficient Production of Hydroxytyrosol

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## Abstract

Hydroxytyrosol (HT), a vital natural polyphenol abundant in lipid-bound forms in olive tissues, exhibits excellent antioxidant activity and broad application prospects in food, pharmaceutical and cosmetic fields. This study established an efficient de novo HT biosynthesis system via a genome integration strategy in *Escherichia coli* BL21 Star (DE3) combined with multidimensional metabolic engineering. Key functional genes (*HpaBC*, $ARO10^{D331C}$, *ADH6*, *tyrC*, $aroG^{fbr}$) were introduced to construct the basic HT synthetic pathway; carbon flux was optimized by regulating key enzyme gene copy ratios. Competitive branch genes (*tyrR*, *pheA*) were knocked out to reduce precursor competition, and the shikimate pathway was enhanced to boost precursor supply. The *LAAD* gene was integrated to build an efficient tyrosine-to-HT catalytic module, and molecular docking confirmed the catalytic potential of cytochrome P450 enzymes and *HpaBC* for HT synthesis. In a 5 L bioreactor, the engineered strain produced 7460 mg/L HT at 43 h. This work provides a novel technical route for the green biomanufacturing of natural polyphenolic compounds.

**Key words:** de novo synthesis pathway; Synthetic biology; genome integration; The shikimate pathway; Eukaryotic cytochromes P450s

**Representative publication:**

1. **Jin W**, Wu J, Huang Z, Wang J, Qiu X, Yuan L, Chen X, Yao J. Multi-step metabolic engineering optimization of Escherichia coli BL21 Star (DE3) for high-efficient production of hydroxytyrosol. Bioresour Technol. 2026 May;447:134273. doi: 10.1016/j.biortech.2026.134273. Epub 2026 Feb 23. PMID: 41740933.

2. Liao Y, Lao C, Wu J, Yuan L, Xu Y, **Jin W**, Sun J, Zhang Q, Chen X, Yao J. High-Yield Synthesis of Lacto-N-Neotetraose from Glycerol and Glucose in Engineered Escherichia coli. J Agric Food Chem. 2024 Mar 13;72(10):5325-5338. doi: 10.1021/acs.jafc.3c08239. Epub 2024 Jan 26. PMID: 38275134.

## Biography



Weijian Jin is a PhD student in Biology at the University of Science and Technology of China, supervised by Researcher Jianming Yao from the Hefei Institutes of Physical Science, Chinese Academy of Sciences. His research mainly focuses on metabolic engineering, synthetic biology and fermentation engineering. His work involves exploring microbial potential, and regulates microbial metabolic flux through genetic engineering approaches to realize the efficient biosynthesis and yield improvement of target products, thus facilitating the application expansion and technological upgrading of the biosynthetic industry. To date, he has published 3 SCI papers as the first author or co-author in international journals including *Bioresource Technology* and *Journal of Agricultural and Food Chemistry*.

# From Sterol Precursors to Brassinosteroids: Emerging Metabolic Engineering Strategies for Microbial Biomanufacturing

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## Abstract

Brassinosteroids (BRs) are polyhydroxylated steroidal phytohormones that regulate plant growth, development, and stress responses, with considerable potential for agricultural and broader biotechnological applications. Their low natural abundance and the structural and stereochemical challenges associated with chemical synthesis have driven the development of microbial production routes. Recent advances in yeast engineering have enabled the production ofsterol precursors relevant to BR biosynthesis and semisynthesis, including 24-epi-ergosterol and 22R,23R-dihydroxylated sterols. Efficient steroid production, however, depends on more than precursor flux. Sterol stereochemistry, partitioning between free and esterified pools, substrate availability, and the catalytic performance of membrane-associated cytochrome P450 enzymes can critically influence downstream functionalization. Here, we summarize emerging strategies for BR-oriented microbial biomanufacturing, focusing on sterol precursor engineering, stereochemical control, sterol homeostasis, substrate availability, and P450-mediated functionalization. We further discuss spatial metabolic engineering and lipid droplet-endoplasmic reticulum organization as potential approaches to coordinate hydrophobic substrate supply with membrane-associated catalysis. Integrating pathway engineering, sterol homeostasis, enzyme engineering, and subcellular organization may enable a shift from flux-oriented engineering toward coordinated control of substrate identity, availability, localization, and catalytic conversion, providing a framework for microbial production of brassinosteroids and related steroid natural products.

**Key words:**Brassinosteroids; Sterol stereochemistry; Sterol homeostasis; Cytochrome P450; Spatial metabolic engineering

**Representative publication:**

1. Liu H., Wang X., Zhou T., Xiao T., Zhu C., Yang R., Zhang S., Lyu X.*, Metabolomics-Guided Integration of a “Push–Pull–Restrain” Strategy for Enhanced LNnT Production in *Saccharomyces cerevisiae*, Journal of Agricultural and Food Chemistry, 2026, 74, 10220–10231.

2. Liu H., Liu Y., Hu H., Zhu C., Zhang S., Lyu X.*, Yang R.*, De novo production of lacto-*N*-neotetraose by metabolic engineering of *Saccharomyces cerevisiae* as a cell factory, Food Science and Human Wellness, 2026, 15, 9250342.

3. Liu H., Zeng Q., Zhu C., Zhang S., Chen X., Xu C., Yang R., Lyu X.*, High-Throughput Screening and Directed Evolution of β-1,3-N-Acetylglucosaminyltransferase for Enhanced LNnT Production in Engineered *Saccharomyces cerevisiae*, Journal of Agricultural and Food Chemistry, 2025, 73, 7966–7974.

4. Liu Y., Liu H., Hu H., Ng K. R., Yang R.*, Lyu X.*, De Novo Production of Hydroxytyrosol by Metabolic Engineering of *Saccharomyces cerevisiae,* Journal of Agricultural and Food Chemistry, 2022, 70, 7490–7499.

## Biography



Han Liu received a Ph.D. in Food Science and Engineering from Jiangnan University, with doctoral research focusing on synthetic biology and metabolic engineering. Her doctoral research primarily involved the development of *Saccharomyces cerevisiae* cell factories for the *de novo* biosynthesis of value-added compounds, integrating pathway engineering, metabolic regulation and high-throughput screening. She is currently with the College of Life Science and Technology, Beijing University of Chemical Technology. Her current research focuses on metabolic engineering of yeast for sterol biosynthesis and the development of microbial platforms for steroid biomanufacturing. Her broader research interests include the efficient biosynthesis and functionalization of structurally complex natural products.

# Metabolic Engineering for Enhanced Biosynthesis of Vitamin $K_2$ in *Bacillus subtilis*

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## Abstract

Vitamin $K_2$ is an essential fat-soluble vitamin in mammals, among which menaquinone-7 (MK-7) has broad applications in pharmaceuticals, foods, and cosmetics. However, its high market price is largely attributed to low production efficiency. In this study, *Bacillus subtilis* was engineered to enhance MK-7 biosynthesis by addressing limitations in precursor supply, energy and cofactor balance, methyl-group availability, and product transport. The MK-7 biosynthetic network was divided into five modules: gluconeogenesis, glycolysis (EMP), the methylerythritol phosphate (MEP) pathway, the shikimate pathway, and the MK-7 biosynthetic pathway. Guided by metabolic modeling, key genes in the EMP, MEP, and MK-7 pathways were genomically integrated, increasing MK-7 production from 14.42 to 37.27 mg/L. Further engineering of cofactor supply, transport, and methylation-related pathways increased the titer to 56.56 mg/L, 3.92-fold that of the parental strain. Fed-batch fermentation of the engineered strain in a 5-L bioreactor achieved an MK-7 titer of 102.78 mg/L at 144 h. These results provide a stable metabolic engineering strategy for MK-7 production and a foundation for further industrial development.

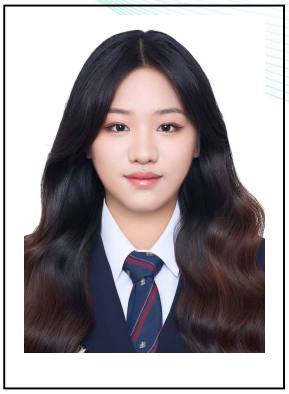
**Key words:** menaquinone-7 (MK-7); MEP pathway; cofactor engineering; methylation platform; transporter engineering

**Representative publication:**

1. Xu, C.; **Zhang, W**.; Wang, K.; Huo, Y.; Zhao, Q.; Liu, Y.; Lin, X.; Xi, L.; Tan, T.*, Sustainable biomanufacturing of fat-soluble vitamins: Rewiring carbon flux and green carbon management. *Green Carbon* **2026**. DOI: https://doi.org/10.1016/j.greenca.2026.08.004

## Biography

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# Biosynthesis and optimization of vanillin

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## Abstract

Vanillin, as one of the most widely used aromatic compounds in the world, is known as the "Queen of Spices", and its demand in the fields of food, daily chemicals and medicine continues to grow. In response to the severe pollution caused by traditional chemical synthesis methods and the scarcity and high cost of raw materials in natural extraction methods, this study takes *Corynebacterium glutamicum* as the chassis cell and conducts systematic research focusing on the key bottlenecks in the biosynthesis process of vanillin. The core achievement is: Fifteen methyltransferases from different sources were screened, and finally RnCOMT with excellent activity (derived from *R. norvegicus*) was obtained. The PEC-PXMJ dual-plasmid co-expression system was constructed. By increasing the copy number of the *comt* gene, the yield was further increased to 886.91 mg/L.

**Key words:** Vanillin, *Corynebacterium glutamicum*, Methyltransferase, Metabolic engineering

**Representative publication:**

1.

## Biography

Jinjin Yang, State key laboratory of green biomanufacturing, Beijing University of Chemical Technology



# Discovery and Engineering a UDP-glycosyltransferase for Efficient Gastrodin Biosynthesis in Yeast

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## Abstract

Gastrodin is a vital bioactive phenolic glucoside, but its supply is severely constrained by the depletion of wild *Gastrodia elata* resources and the environmental pollution associated with chemical synthesis. To establish a sustainable green manufacturing route, we report the first experimental characterization of a UDP-glycosyltransferase (HGT) from *Triticum aestivum* as an efficient biocatalyst for gastrodin biosynthesis in *Saccharomyces cerevisiae*. While wild-type HGT demonstrated robust activity with 86.7% conversion at 5 mM *p*HBA, its efficiency decreased significantly at elevated substrate concentrations, limiting its applicability in high-titer bioprocesses. To address this limitation, we employed directed evolution strategy combined with fluorescence-based screening. This approach identified two mutants, A133T and T256I, with significantly enhanced performance. In fermentations with 40 mM *p*HBA, the A133T and T256I mutants achieved maximum gastrodin titers of 5.37 g/L and 5.45 g/L, corresponding to 42.5% and 44.4% improvements in conversion efficiency compared to the wild-type enzyme. In vitro kinetic assays further validated their enhanced performance, with A133T and T256I exhibiting 1.93-fold and 1.73-fold increases in catalytic efficiency, respectively. Finally, computational modeling provided insights into potential structural determinants of the improved activity. This work provides an efficient, eco-friendly enzymatic route that valorizes phenolic precursors into high-value glycosides, offering a viable alternative to protect plant resources.

**Key words:** Gastrodin, Glycosyltransferase, HGT, Directed evolution, *Saccharomyces cerevisiae*

**Representative publication:**

1. Pan, Y., Zhang, Y., Ji, D., Qin, L., Cao, Y. and Huang, M. Discovery and engineering of a uridine diphosphate glycosyltransferase for efficient gastrodin biosynthesis in yeast, Food Bioscience, 2026,82, 109274.

## Biography



My name is Yuyang Pan. I am a Postdoctoral Fellow and Assistant Researcher at South China University of Technology and a selected fellow of the Guangdong Association for Science and Technology Young Talent Program. My research focuses on the development of natural functional compounds and the construction of microbial cell factories, particularly enzyme mining, heterologous pathway reconstruction, chassis engineering, and fermentation optimization. I have contributed to the development of microbial production systems for natural products such as dihydroquercetin, anthocyanins, and pterostilbene. In this project, I am responsible for overall coordination, pathway construction, engineered strain development, and fermentation validation. I have published 18 papers in journals including Research, PNAS, Advanced Science, Journal of Agricultural and Food Chemistry, and Metabolic Engineering, and filed 11 invention patents, four of which have been granted. I also lead a Guangdong Natural Science Foundation project and have participated in National Key R&D Program and industry-funded projects.

# Shikimate Crosstalk Enables Record-Level Homogentisic Acid Production in *Yarrowia lipolytica*

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## Abstract

Homogentisic acid (HGA; 2,5-dihydroxyphenylacetic acid) is an aromatic compound with diverse applications in pharmaceuticals, food, and functional materials. Industrial HGA production still relies mainly on multistep chemical synthesis routes, which are associated with safety risks and environmental burdens. Here, we report that truncated HMG-CoA reductase (tHMGR), a key enzyme of the mevalonate pathway, unexpectedly serves as a dominant metabolic driving force that enhances shikimate-derived HGA biosynthesis in *Yarrowia lipolytica*. Through a targeted single-gene screen of the mevalonate pathway, tHMGR emerged as the determinant of HGA overproduction, and copy-number optimization increased the shake-flask titer 2.65-fold to 1,766.43 mg/L. Transcriptomic analysis and metabolic network evaluation revealed that the massive NADPH consumption driven by tHMGR perturbs the intracellular redox balance. This cofactor depletion triggers a compensatory upregulation of L-tryptophan biosynthesis and shikimate-associated genes, uncovering a previously unrecognized mevalonate-shikimate metabolic crosstalk. The generality of this tHMGR-driven effect was further validated using *p*-coumaric acid as a shikimate-derived reporter. Finally, fed-batch fermentation achieved 33.71 g/L HGA in a 2 L bioreactor, representing the highest titer reported to date, and establishing tHMGR as a powerful metabolic lever for enhancing shikimate-derived aromatic production in *Y. lipolytica*.

**Key words:** *Yarrowia lipolytica*, Truncated HMG-CoA reductase, Mevalonate pathway, Shikimate pathway, Homogentisic acid

**Representative publication:**

1. Chenchen Xu, Xinyu Jiao, Tianwei Tan*. Membrane transporter mining and enzyme engineering drive high-titer δ-tocotrienol production in *Yarrowia lipolytica*. Biochemical Engineering Journal, 2026, 228, 110079.

2. Haoran Bi, Chenchen Xu, et al. Enhancing precursor supply and modulating metabolism to achieve high-level production of β-farnesene in *Yarrowia lipolytica*. Bioresource Technology, 2023, 382, 129171.

## Biography



Dr. Chengchen Xu is a postdoctoral researcher at Beijing University of Chemical Technology, working under the supervision of Professor Tianwei Tan. Her research centers on synthetic biology and biochemical engineering, with a specific focus on metabolic engineering and microbial biosynthesis. She has published three first-author articles in peer-reviewed journals, including Biochemical Engineering Journal, Synthetic and Systems Biotechnology, and Green Carbon, and holds two granted patents.

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Metabolic Engineering Summit 2026

# Mining and Engineering of Mesophilic Argonaute for Rapid Phage Detection

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## Abstract

Prokaryotic Argonaute (pAgo) proteins are programmable nucleases with great potential for nucleic acid detection. However, the limited catalytic activity of most mesophilic pAgos remains a major constraint on their practical application at moderate temperatures. Here, we systematically mined and characterized mesophilic Ago proteins and identified candidates with efficient DNA-guided cleavage activity under mild conditions. Through enzymatic characterization and protein engineering, key residues associated with catalytic performance were identified, and engineered Ago variants with substantially enhanced cleavage activity at 37°C were obtained. To further evaluate their application potential, the engineered Ago was integrated with nucleic acid amplification and visual signal readout to develop a rapid detection strategy for phage contamination. Taking advantage of PAM-independent recognition, stable DNA guides, high single-nucleotide mismatch discrimination, and multiplexing capability, the platform provides a potential approach for simultaneous detection of multiple phages. This work establishes a workflow from Ago mining and protein engineering to functional application, providing a foundation for engineering programmable nucleases and expanding their applications in molecular diagnostics and industrial biotechnology.

**Key words:** Argonaute; Protein engineering; Programmable nuclease; Phage detection; Molecular diagnostics

**Representative publication:**

1. Shen P. J., Gu S. Y., Jin D., Su Y., Wu H. X., Li Q. C., Yang J. H., He W. J., Huang J. Z.*, Qi F.*, Engineering metabolic pathways for cofactor self-sufficiency and serotonin production in *Escherichia coli*, ACS Synthetic Biology, 2022, 11, 2889-2900.

2. Shen P. J., Si Z. J., Huang D., Xu Z. P.*, Wang Z. Y., Fang M. J., Xu Z. N.*, CRISPR Cas12a-enabled biosensors coupled with commercial pregnancy test strips for the visible point-of-care testing of SARS-CoV-2, Analyst, 2023, 148, 2573.

3. Shen P. J., Huang D., Fang M. J., Xu Z. N.*, Fang X. M.*, Temperature-modulation categorization of Argonaute-based bioreactions coupled with amplification methods for nucleic acid detection, Critical Reviews in Analytical Chemistry, 2025, 1-19.

## Biography



Peijie Shen is a postdoctoral researcher at College of Chemical and Biological Engineering, Zhejiang University. His research focuses on programmable nucleases and their applications in nucleic acid detection and industrial biotechnology, particularly on elucidating the structural basis and molecular mechanisms underlying the catalytic properties of Argonaute proteins at moderate temperatures. His work integrates synthetic biology and biosensing technologies to advance the engineering and application of programmable nucleases.

# Unsupervised Learning-Derived Complex Metabolic Signatures Refine Cardiometabolic Risk

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## Abstract

Cardiometabolic diseases arise from interconnected metabolic perturbations that are poorly captured by conventional lipid measurements. We applied unsupervised consensus clustering to 325 plasma metabolic markers in 118,001 UK Biobank participants to construct interpretable metabolic signatures, followed by phenome-wide and genome-wide association analyses, prospective Cox models, and Mendelian randomization. Eleven reproducible metabolic clusters were identified, associated with 101 loci and 445 phenotypes. The triglyceride-rich lipoprotein signature showed strong associations with ischemic heart disease, type 2 diabetes, and hypertension, outperforming several traditional lipid markers; Mendelian randomization further supported a causal association with ischemic heart disease. The non-high-density lipoprotein cholesterol signature increased ischemic heart disease risk but was inversely associated with type 2 diabetes, indicating disease-specific metabolic trade-offs. High-density lipoprotein-related clusters also showed marked heterogeneity: signatures enriched in free cholesterol or triglycerides were associated with higher cardiometabolic risk, whereas a cholesteryl ester-enriched signature showed protective associations. These findings demonstrate that data-driven decomposition of the plasma metabolome can resolve functionally distinct metabolic subpopulations and provide a systems-level framework for cardiometabolic risk stratification and identification of candidate regulatory targets.

**Key words:** Metabolomics; Unsupervised learning; Cardiometabolic disease; Lipoproteins; Mendelian randomization

**Representative publication:**

1. Hua W, Ji X, Chen J, et al. Zhang J*. Transcriptome analysis of orbital tissue in thyroid eye disease uncovers key pathogenic mediators. Scientific Reports. 2025;16(1):1058. doi:10.1038/s41598-025-30716-9.

2. Lu X, Xu Y, Liu Y, et al., Zhang J, Cui F, Chen Q. Neutrophil Depletion Reduced the Relative Abundance of Unsaturated Long-Chain Fatty Acid Synthesis Microbiota and Intestinal Lipid Absorption. Cell Biochemistry and Function. 2025;43(3):e70060. doi:10.1002/cbf.70060.

3. Jin J, Zhang J, Bu S. Tasquinimod efficacy and S100A9 expression in glucose-treated HREC cells. International Ophthalmology. 2022;42(2):661 – 676. doi:10.1007/s10792-021-02038-y.

4. Xiang X, Zhang Z, Xu W, Huang Z, Zhang J*. Role of Myeloid-Derived Suppressor Cells (MDSCs), Specifically Monocyte-Derived MDSCs, in Type 2 Diabetes-Related Diabetic Retinopathy. Diabetes, Metabolic Syndrome and Obesity. 2025;18:2213 – 2220. doi:10.2147/DMSO.S527411.

**5.** Jin J, Ji F, Zhang J. The Relationship between S100A9 Protein Levels and Diabetic Retinopathy among Type 2 Diabetes Patients: A Meta-Analysis. Current Protein & Peptide Science. 2026. doi:10.2174/0113892037399996251201114724.

## Biography

Ji Zhang is Director of the Department of Ophthalmology, and Chief Physician at the Second Affiliated Hospital of Soochow University. He received his medical training at Soochow University and completed advanced ophthalmic training at Shanghai Ninth People's Hospital. He subsequently worked as a visiting scholar at the University of Pittsburgh Medical Center and undertook postdoctoral research in ocular oncology at the Cleveland Clinic. His clinical and research interests focus on orbital diseases, orbital tumors, lacrimal disorders, and ophthalmic plastic surgery. Dr. Zhang serves on several national and provincial ophthalmology committees and is Chair of the Ophthalmology Branch of the Suzhou Medical Association, as well as leader of its orbital disease group. His work integrates clinical ophthalmology with translational research on orbital and ocular tumor biology.



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Metabolic Engineering Summit 2026

# Enhanced Yellow Pigment Production by *Monascus pilosus* and Selective Accumulation of Monascuspiloin under Nitrogen Limitation

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## Abstract

*Monascus* spp. have a long history of use in East Asian fermented foods and produce diverse bioactive secondary metabolites. Yellow *Monascus* pigments are promising food and pharmaceutical ingredients because of their favorable stability and biological activities. Our previous study demonstrated that the crude *Monascus pilosus* extract dominated by monascuspiloin and monascin inhibited *Clostridium butyricum* growth or induced cell elongation (a stress reaction). However, low production of both pigments limits further investigation and application. Here, medium composition was optimized using one-factor-at-a-time experiments, a Box-Behnken design, and response surface methodology. The quadratic model was significant ($p$<0.0001) with $R^2$=0.905, and a nonsignificant lack of fit ($p$=0.728). The optimized medium increased extracellular and intracellular pigment values by 1.86 and 17.91 fold, reaching 0.63 $AU_{380}$/mL and 434.07 $AU_{380}$/g dry biomass, respectively. In addition, monascuspiloin and monascin production increased from 1.01 to 6.70 mg/g and from 0.46 to 9.57 mg/g. Notably, nitrogen limitation at a carbon-to-nitrogen molar ratio of 200 increased the monascuspiloin-to-monascin ratio to 20.4, resulted in 8.519 mg/g monascuspiloin, indicating selective promotion of monascuspiloin biosynthesis. These findings provide a basis for the targeted production and metabolic investigation of purified yellow pigments.

**Key words:** *Monascus pilosus*; yellow pigments; RSM; monascuspiloin.

**Representative publication:**

1. Xu S, Xu J, Wu Q, Zhou H, Lu D, **Jia L**[*], Chen FS.[*] Changes of physicochemical indicators and volatile compounds in grains and liquors during the sauce-aroma *Baijiu* brewing process. Foods, 2026, 15(12), 2211 ([*]These authors jointly supervised this work)

2. Mervic D, Schmidt K, Lambert C, Kaufmann T, **Jia L**, Faix J, Rottner K, Stadler M, Stradal T, Klahn. Comprehensive cell biological investigation of cytochalasin B derivatives with distinct activities on the actin network. Journal of Natural Products, 2024 87: 2421-2431

3. Huang Y[†], **Jia L**[†], Chen F. Effects of *MrwetA* on sexual reproduction and secondary metabolism of *Monascus ruber* M7 based on transcriptome analysis. *Journal of Fungi*, 2024, 10: 338, († These authors contributed equally to this work.)

4. **Jia L,** Huang Y, Yu J, Stadler M, Shao Y, Chen W, Chen F. Characterization of key upstream asexual developmental regulators in *Monascus ruber* M7. Food Bioscience, 2022, 50: 102153

5. Duan Y, **Jia L**, Pei X, Wei X, Li M. An efficient microbial-based method for production of high-purity *Monascus* azaphilones pigments. Lwt-food science and technology, 2022, 170, 114053

6. **Jia L**, Tu X, He K, Wang C, Yin S, Zhou Y, Chen W. Monascorubrin and rubropunctatin: preparation and reaction characteristics with amines. Dyes and Pigments, 2019, 170: 107629

## Biography



Lili Jia is a postdoctoral researcher specializing in the biotechnology of the filamentous fungus *Monascus*. Her research focuses on the submerged fermentation, production optimization, and biological activities of yellow *Monascus* pigments, particularly monascuspiloin and monascin. She has demonstrated the inhibitory effects of these pigments against *Clostridium* species and is currently investigating how medium composition regulates pigment yield and profiles. By integrating fermentation optimization, metabolite analysis, molecular biology, and omics-based approaches, her work aims to elucidate the mechanisms governing pigment biosynthesis and support the targeted production of individual bioactive *Monascus* pigments for potential food and pharmaceutical applications.

# *Lactobacillus reuteri*-enriched eicosatrienoic acid restores intestinal barrier via GLP-1R/PI3K-AKT signaling in a PCOS mouse model

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## Abstract

N-3 PUFAs are sustainable bioactive lipids with emerging metabolic benefits, yet the host-regulatory mechanisms of specific microbial-associated species remain incompletely defined. We previously identified *Lactobacillus reuteri* administration as enriching eicosatrienoic acid (ETA, 20:3) in vivo, which exerts glucose-homeostatic effects. Whether *L. reuteri*-enriched ETA protects intestinal barrier function via GLP-1 signaling was unknown. In DHEA-induced PCOS mice, oral ETA (5 mg/kg for 10 d) elevated intestinal GLP-1R expression and serum active GLP-1 levels, concomitantly upregulating colonic Gcg and Pcsk1 expression. These changes restored crypt architecture and mucin secretion. At the molecular level, ETA rescued the mRNA and protein expression of ZO-1, Occludin, and MUC2, coinciding with reduced serum LPS that negatively correlated with circulating GLP-1. In Caco-2 cells, ETA-stimulated GLP-1 activated PI3K-AKT signaling in a GLP-1R-dependent manner to reinforce barrier integrity; both effects were abolished by the GLP-1R antagonist Exendin 9-39. These findings establish *L. reuteri*-enriched ETA as a GLP-1-inducing bioactive lipid that restores intestinal barrier function via GLP-1R/PI3K-AKT-dependent tight junction and mucus remodeling. This work provides mechanistic evidence supporting gut-barrier endpoints for evaluating microbe-associated lipid therapeutics in PCOS and related metabolic conditions.

**Key words:** Eicosatrienoic acid; *Lactobacillus reuteri*; GLP-1; Intestinal barrier; Ovarian inflammation.

**Representative publication:**

1. Yan J., Ma S.Z., Chang S.Y., Jin Z.Y., Liao X.X., Jia M.X., Kang J.D.*, Quan L.H.*, Eicosatrienoic acid improves PCOS in mice by modulating the intestinal barrier and ovarian inflammation, Journal of Nutritional Biochemistry, 2026, 158, 110477.

2. Wang JX, Chang SY, Jin ZY, Li D, Zhu J, Luo ZB, et al. *Lactobacillus reuteri* enriched eicosatrienoic acid regulates glucose homeostasis by promoting GLP-1 secretion to protect intestinal barrier integrity. J Agric Food Chem 2025;73:393-408.

## Biography



Shaozhuang Ma is currently a Ph.D. candidate in the College of Pharmacy at Yanbian University, under the supervision of Professor Linhu Quan. His doctoral research focuses on the molecular mechanisms by which gut microbiota-derived metabolites regulate disease pathogenesis, with particular emphasis on the regulatory roles of gut microbe-derived long-chain fatty acids-especially polyunsaturated fatty acids produced or enriched by commensal bacteria-in inflammatory bowel disease (IBD) and metabolic disorders. He integrates microbial lipidomics, host-microbe interaction assays, and in vivo disease modeling to elucidate how specific bacterial strains (e.g., *Lactobacillus reuteri*) and their associated lipid metabolites (e.g., eicosatrienoic acid) modulate intestinal barrier integrity through enteroendocrine signaling pathways such as GLP-1R/PI3K-AKT.

# Development of a Rapid Yeast Evolution Method Mediated by a Multiplex Base-Editing System

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## Abstract

Converting abundant, low-cost one-carbon substrates into valuable chemicals and fuels is essential for sustainable carbon cycling. However, heterologous CO2-fixation pathways in *Saccharomyces cerevisiae* often show low efficiency because of metabolic constraints, limited enzyme activity, and complex regulation. Improving substrate utilization and product synthesis requires efficient multi-site mutagenesis and screening.

Base editors avoid DNA double-strand breaks and eliminate the need to synthesize large quantities of DNA fragments for library construction, making them suitable for high-throughput, multiplex genome editing. We developed a multiplex base-editing system for rapid directed evolution of S. cerevisiae and for constructing mutant libraries targeting CO2-utilization genes and key metabolic nodes.

Base editors (CBE, ABE, and CXBE) were characterized in *Saccharomyces cerevisiae*, and several improved systems were obtained through component optimization. We also evaluated position-specific editing efficiencies and performed genome-integration experiments.

This work provides a simple, efficient genome-evolution tool and supports the development of industrial *S. cerevisiae* as autotrophic or mixotrophic cell factories for low-carbon biomanufacturing.

**Key words:** Base editing; one-carbon utilization; multiplex; directed evolution.

## Biography

Qingwen He is a PhD candidate at Dalian University of Technology. She has received several honors and scholarships, including the Outstanding Graduate Student Award for two consecutive years, and has led teams in major competitions such as the Challenge Cup. She is the first inventor on two published patents and has authored one Chinese-language review article.



# Structure-Guided Stepwise Engineering of a Microbial Glycosyltransferase for Highly Regioselective and Efficient Biosynthesis of Salidroside

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## Abstract

Efficient salidroside biosynthesis requires UDP-glycosyltransferases (UGTs) that combine catalytic activity, regioselectivity, and stability. Here, we engineered a microbial UGT from *Paenibacillus durus* (*Pd*UGT) through a spatially ordered strategy. Phylogeny-guided mining identified *Pd*UGT as an active, soluble scaffold with 81.2% regioselectivity. Structure-guided remodeling of the substrate-binding pocket first enhanced activity, while subsequent engineering of flexible loops and the access tunnel progressively restricted tyrosol orientation, yielding M3 with 99.9% regioselectivity. Distal mutations prioritized by SaProt and ΔΔG calculations produced M4, increasing the melting temperature by 11.2 °C and catalytic efficiency by 20-fold, with a specific activity of 58.5 $U \cdot mg^{-1}$. Molecular simulations indicated that pocket compaction and Loop 3 gating suppress unproductive tyrosol reorientation and improve structural stability. Finally, coupling M4 with sucrose synthase-mediated UDP-glucose recycling enabled 193.1 mM salidroside production, 99.0% conversion, and a space-time yield of 5.7 $g \cdot L^{-1} \cdot h^{-1}$. This work provides a generalizable strategy for engineering selective and stable microbial glycosyltransferases for efficient biocatalysis.

**Key words:** salidroside; UDP-glycosyltransferase; protein engineering; regioselective glycosylation; biocatalysis

**Representative publications:**

1. Li, M.; Meng, Y.; Li, J.; Han, L.; Zhou, Z.; Liu, Z.* Structure-Guided Stepwise Engineering of a Microbial Glycosyltransferase for Highly Regioselective and Efficient Biosynthesis of Salidroside. *ACS Catal.* 2026. DOI: 10.1021/acscatal.6c04420.
2. Li, J.; Li, M.; Yong, C.; Zhou, Z.; Han, L.; Liu, Z. Reshaping UDP-Binding Pocket of Bacterial Sucrose Synthase to Improve Efficiency of UDP-Glucose Production. *Bioresour. Technol.* 2025, 427, 132396.

## Biography

Muyang Li is a PhD student at the School of Biotechnology, Jiangnan University, China. The research focuses on enzyme engineering and biocatalysis for sustainable biosynthesis of high-value products. Current work integrates structure-guided mutagenesis, computational protein design, molecular simulation, and catalytic cascade development to improve the activity, selectivity, and stability of microbial glycosyltransferases.



# Se-biofortified *Pediococcus acidilactici* SKR-2 enhances fish thermotolerance over inorganic Se

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## Abstract

Selenium (Se) is a crucial trace element, essential for the maintenance of physiological and metabolic functions. Probiotics have the ability to bioconvert inorganic Se into less toxic and more bioavailable forms, such as seleno-substituted amino acids and nano-selenium (nano-Se), offering dual benefits of Se supplementation and probiotic activity. In this study, *Pediococcus acidilactici* SKR-2, which exhibits high tolerant to sodium selenite, was identified from Qualified Presumption of Safety (QPS) recommended probiotics. Optimal conditions for Se enrichment were established, resulting in a total Se content of 300-350 mg/g, primarily in the form of nano-Se. Characterization confirmed the presence of spherical Se nanoparticles on the surface of Se-enriched *P. acidilactici* SKR-2 (P-Se), while transcriptomic analysis revealed that the strain synthesizes nano-Se through multiple pathways. In Zebrafish trials, P-Se at 0.056 mg/kg did not affect growth performance compared to inorganic Se supplemented at 0.112 mg/kg and significantly improved survival under heat stress.These findings highligh *P. acidilactici* SKR-2 as a highly efficient Se-enriching probiotic with the potential to serve as an alternative to commercially available Se-enriched lactic acid bacteria and Se-enriched yeast, offering promising applications in improving aquatic animal health and stress resistance.

**Key words:** Selenium biofortification; *Pediococcus acidilactici* SKR-2; Characterization; Zebrafish; Transcriptome analysis

**Representative publication:**

1. Shi K.R., Xu S.C., Wang N., Dong R.Y., Peng H.K., Wang T.T., Li Y.Q., Wang M.X., Yang H.W., Zhang Z., Yao Y.Y., Ran C., Ding Q.W., Yang Y.L., Zhou Z.G., Se-biofortified Pediococcus acidilacticiSKR-2 enhances fish thermotolerance over inorganic Se, Water Biology and Security, Article in Press.
2. Shi K.R., Wang N., Sang Y.Y., Li Y.Q., Zhang Z., Yao Y.Y., Shen C., Ding Q.W., Yang Y.L., Zhou Z. G., Preparation, characterization, and application of selenium-enriched yeast in fish stress resistance, Journal of Agricultural Science and Technology, 2025, 27(9), 240-249.

## Biography



Shi Kerou is currently a PhD student at the Feed Research Institute, Chinese Academy of Agricultural Sciences (CAAS), where she is studying biochemistry and molecular biology. Her research focuses on utilising probiotic microorganisms to convert inorganic trace elements into highly bioavailable microbial forms, with a view to addressing issues such as the high toxicity and low utilisation rates of inorganic trace elements in aquaculture feed, as well as environmental pollution. Her research findings have been published in the journal Water Biology and Security, and she has applied for several patents.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# A novel EDTA-independent phage lysin *Av*Lys2 combats *Aeromonas veronii* infection through targeted gut microbiota remodeling

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## Abstract

**Objective** Phage endolysins are emerging antimicrobial biologics, but their potential to modulate host–microbiome interactions for disease protection remains poorly understood. We sought to identify an anti-*Aeromonas veronii* endolysin and define its mechanism of host protection.

**Design** We developed PhageLys-Mine 1.0, a computational discovery pipeline integrating phage genome mining, host/lifestyle annotation and functional enzyme prioritisation, followed by validation in vitro, conventional zebrafish and germ-free models.

**Results** From 873,718 phage genomes, we prioritised *Av*Lys2 as a lead endolysin against *A. veronii*. *Av*Lys2 showed potent bactericidal activity, directly hydrolysed purified cell walls and remained active without EDTA, indicating intrinsic activity against Gram-negative bacteria. In zebrafish, dietary *Av*Lys2 was well tolerated, improved growth and conferred dose-dependent protection against *A. veronii* infection. Mechanistically, *Av*Lys2 remodelled the intestinal microbiota, increasing community richness, depleting Proteobacteria and pathobiont-associated taxa, including *Aeromonas veronii* and *Plesiomonas shigelloides*, while enriching the beneficial commensal *Cetobacterium somerae*. This was coupled to reinforcement of mucosal barrier integrity, with increased expression of tight-junction genes, expansion of goblet cells and improved intestinal histology, together with a controlled mucosal immune programme. In germ-free zebrafish, *Av*Lys2 exerted both a direct anti-infective effect and a microbiota-transferable protective effect.

**Conclusion** *Av*Lys2 protects against enteric infection through a dual mechanism: direct pathogen killing and enhancement of host resistance through microbiota remodelling and mucosal barrier–immune reinforcement. These findings establish phage endolysins as programmable dual-action antimicrobial biologics that combine pathogen elimination with microbiome-guided host protection.

**Key words:** Phage endolysin; Computational discovery; Gut microbiota; Mucosal barrier; Host defence; *Aerom onas veronii*

**Representative publication:**

1. Xiaoyu Zhang, Ruyue Dong, Shichang Xu, Rui Xia, Jinwen Zhou, Yuanyuan Yao, Chao Ran, Zhen Zhang, Qianwen Ding, Nan Wang*, Yalin Yang*, Zhigang Zhou* A novel EDTA-independent phage lysin *Av*Lys2 combats *Aeromonas veronii* infection through targeted gut microbiota remodeling (Submitted to *GUT*)

2. Xiaoyu Zhang, Nan Wang, Shichang Xu, et al., Zhigang Zhou*. Expression and antibacterial activity analysis of Aeromonas veronii phage lysin in Pichia pastoris. Journal of Agricultural Science and Technology, 2025: 2025-0245. (DOI:10.13304/j.nykjdb.2025.0245)

3. Xiaoyu Zhang (Student 1st Author), et al. A phage lysin targeting Aeromonas veronii: Preparation method and application. (CN: 202510209388.4)

4. Xiaoyu Zhang (Student 1st Author), et al. High lipid-producing Rhodotorula toruloides and its application. (CN: 202510851105.6)

## Biography



Zhang Xiaoyu is a joint Ph.D. student at the Chinese Academy of Agricultural Sciences and Wageningen University, and received a master's degree from the Feed Research Institute, Chinese Academy of Agricultural Sciences. Zhang's main research focuses on the mining and molecular engineering of bacteriophage lysins against harmful bacteria from aquatic sources. By leveraging computational biology and synthetic biology approaches, Zhang conducts high-throughput screening of lysins with high antibacterial activity, elucidates their antibacterial regulatory mechanisms, and is committed to the development of novel green antibacterial agents.

# Marine Metagenome-Derived Novel α-Carbonic Anhydrases: Mining, Characterization of Extreme-Condition Adaptability, and Application in Succinic Acid Fermentation

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## Abstract

Carbonic anhydrases (CAs) catalyze reversible $CO_2$ hydration and are key for carbon capture and utilization (CCU), but poor tolerance to high salinity and temperature limits industrial use. Marine metagenomes offer diverse enzymes from uncultured microorganisms. We mined a marine metagenomic database and identified 919 putative α-CA candidates; 75 diverse sequences were selected for heterologous expression. Screening found 26 active CAs. After purification, relative activity ($\Delta A_{405}$/min) was measured by the p-NPA esterase assay. CA-56 showed 138% higher activity than human CA II (hCA II). In 0-5 M NaCl, 13 CAs were halophilic. CA-44 showed 210% higher activity at 5 M NaCl than at 0 M, while CA-57 had the highest activity at 5 M NaCl, retaining 43% of its NaCl-free activity; hCA II retained only 19%. Temperature profiling showed 12 CAs with increased activity at higher temperatures; CA-20 had 641% higher activity at 70 °C than at 25 °C, the highest among all enzymes. Overexpression of CA increased succinate titer and yield in strain EC84 by 21.12% and 24.74%, respectively. These CAs expand extremophilic enzyme diversity and provide components for CCU under extreme conditions and $CO_2$-driven metabolic engineering.

**Key words:** Carbonic anhydrases, Marine Metagenome, Extremophile-adapted Enzyme, Carbon Capture and Utilization, Succinic Acid Fermentation

## Biography



Xi Deng is currently a Ph.D. candidate at Dalian University of Technology. He has received several honors and awards, including the National Scholarship and the title of Outstanding Graduate of Sichuan Province. As the first co-author, He has published two research articles, including a paper in *LWT-Food Science and Technology* (Q1, IF: 7.4) and a paper in *Food Research International* (Q1, IF: 9.6).

# Integrated dynamic control and enzyme co-localization strategies enable high-efficiency stilbenoid biosynthesis

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## Abstract

Stilbenoids are plant-derived polyphenols with potent bioactivities, which makes them promising for pharmaceutical and nutraceutical use. Their production remains challenging due to their low natural abundance in plants and the inherent toxicity associated with chemical synthesis. Here, we developed an integrated strategy to engineer *Escherichia coli* for efficient biosynthesis of multiple stilbenoids. A *p*-coumaric acid-responsive CRISPRi system dynamically regulated malonyl-CoA allocation, balancing host metabolism and stilbenoid production. We systematically explored enzyme co-localization strategies and used structure-informed in silico analysis to support dual-enzyme fusion design. Orthogonal SpyTag/SpyCatcher and SnoopTag/SnoopCatcher systems enabled optimal intermediate channeling and significantly enhanced pathway flux. As a result, we achieved the highest reported titers of piceatannol (494.30 mg/L from glycerol, increased to 583.10 mg/L with L-tyrosine supplementation) and pterostilbene (563.89 mg/L from glycerol, reaching 1110.92 mg/L with L-tyrosine feeding). Techno-economic analysis indicated that combined optimization of metabolic regulation, enzyme colocalization, and process design contributes substantially to the overall feasibility of stilbenoid manufacturing. Altogether, this study demonstrates the potential of stilbenoid biosynthesis and provides a valuable paradigm for the efficient synthesis of other complex natural products.

**Key words:** Stilbenoid, Malonyl-CoA, Dynamic control, Enzyme co-localization, Metabolic engineering

**Representative publication:**

Zhao, G., Li, P., Ding, X., Lv, J., Yang, Y., Liu L., Zi, L., Zhang, Q., Liang, L.,* Liu, R.* Integrated dynamic control and enzyme co-localization strategies enable high-efficiency stilbenoid biosynthesis. Bioresour. Technol. 2026, 445, 134122.

## Biography



Guofu Zhao is currently a Ph.D. candidate at Dalian University of Technology. She has received several honors and awards, including the National Scholarship and the title of Outstanding Graduate of Liaoning Province. As the first author, she has published three research articles, including two papers in *Bioresource Technology* (Q1, IF: 8.2) and one paper in *Industrial Crops and Products* (Q1, IF: 6.4). She has also filed and been granted four invention patents. She has participated in several academic conferences and delivered oral presentations at the International Conference on Engineering Microbiology 2026 and the 9th National Conference on Chemical Engineering and Biochemical Engineering.

# Data-Driven Modeling of Large-Scale Pan-Cancer Phosphoproteomics Reveals Molecular Subtypes and Drivers of Tumor Proliferation

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## Abstract

Protein phosphorylation is a central mechanism governing cellular signaling and is frequently dysregulated in cancer. However, the high dimensionality and heterogeneity of phosphoproteomic data have hindered systematic characterization of phosphorylation landscapes and their functional consequences across cancer types. Here, we developed a large-scale, data-driven computational framework integrating 14 phosphoproteomic and proteomic datasets from CNHPP and CPTAC, encompassing 2,553 samples across 12 cancer types. We identified substantial heterogeneity in phosphosite detection while revealing a conserved core of phosphorylation events across malignancies. Differential and clinical association analyses identified phosphorylation sites associated with tumor progression, disease stage, and survival, including seven phosphosites consistently upregulated across 13 cohorts. Kinase–substrate enrichment modeling revealed recurrent activation of cell-cycle kinases, including CDK1, CDK2, AURKA, and TTK. Unsupervised modeling identified six pan-cancer phosphorylation-based molecular subtypes largely independent of tissue origin. Notably, subtype S2 showed activation of E2F, MYC, DNA replication, and DNA repair programs and was associated with poor clinical outcomes. Integrative modeling of phosphosite phosphorylation and protein abundance further identified BAP18 pS96 as a potential phosphorylation-driven regulator of tumor proliferation, which was functionally supported by phospho-deficient mutant experiments. Collectively, this study establishes a pan-cancer phosphoproteomic atlas and a data-driven modeling framework for deciphering phosphorylation-dependent signaling and tumor proliferation.

**Key words:** Pan-cancer; Phosphoproteomics; Computational modeling; Molecular subtypes; Phosphorylation-driven regulation

**Representative publication:**

1. Dong B.†, Xu J. Y.†, Huang Y.†, Guo J.†, **Dong Q.†**, Wang Y.†, Li N., Liu Q., Zhang M., Pan Q., Wang H., Jiang J., Chen B., Shen D., Ma Y., Zhai L., Zhang J., Li J., Xue W., Tan M., Qin J. Integrative proteogenomic profiling of high-risk prostate cancer samples from chinese patients indicates metabolic vulnerabilities and diagnostic biomarkers. ***Nature Cancer***, 2024, 5(9): 1427-47.

2. **Dong Q.**, Tan M., Zhou Y., Zhang Y., Li J. Causal inference and annotation of phosphoproteomics data in multiomics cancer studies. ***Molecular & Cellular Proteomics***, 2025, 24(3): 100905.
3. **Dong Q.**†, Li F.†, Xu Y.†, Xiao J.†, Xu Y., Shang D., Zhang C., Yang H., Tian Z., Mi K., Li X., Zhang Y. RNAactDrug: A comprehensive database of RNAs associated with drug sensitivity from multi-omics data. ***Briefings in Bioinformatics***, 2020, 21(6): 2167-74.
4. **Dong Q.**, Shen D., Ye J., Chen J., Li J. PhosCancer: A comprehensive database for investigating protein phosphorylation in human cancer. ***iScience***, 2024, 27(11): 111060.
5. Xu Y.†, **Dong Q.**†, Li F.†, Xu Y.†, Hu C., Wang J., Shang D., Zheng X., Yang H., Zhang C., Shao M., Meng M., Xiong Z., Li X., Zhang Y. Identifying subpathway signatures for individualized anticancer drug response by integrating multi-omics data. ***Journal of Translational Medicine***, 2019, 17(1): 255.

## Biography



My research focuses on computational biology and bioinformatics, with an emphasis on multi-omics data integration, methodological development, and molecular mechanism discovery in complex diseases. I develop computational approaches for integrating genomics, transcriptomics, proteomics, phosphoproteomics, and clinical data to uncover disease mechanisms, identify clinically relevant biomarkers, and advance precision medicine through artificial intelligence and statistical modeling.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Biotin-Assisted Pyruvate Carboxylase Engineering Coordinates Inorganic Carbon Anaplerosis and DHA-Rich Lipid Production in Schizochytrium sp. HX-308

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## Abstract

Improving substrate carbon conversion is essential for green microbial manufacturing and sustainable production of bio-based products. *Schizochytrium* produces DHA-rich lipids, but carbon loss through central metabolism limits glucose-to-lipid conversion. Here, pyruvate carboxylase (PYC)-mediated anaplerotic carboxylation was engineered to improve lipid yield by coordinating inorganic-carbon utilization with precursor and redox metabolism. Screening four PYC genes identified Hx5-3, which overexpressed endogenous *HxPYC*, as the best producer. Biotin supplementation (0.2 mg/L) further enhanced this phenotype. During fed-batch cultivation, biotin-treated Hx5-3 produced 80.5 g/L lipid, 26.4% more than HX-308, while maintaining DHA at 47.4% of total fatty acids. Despite similar glucose consumption, its apparent lipid yield reached 0.235 g/g sugar, 39.0% above HX-308. Metabolite and transcriptional analyses indicated stage-dependent coordination of pyruvate–malate metabolism, acetyl-CoA supply, and NADPH turnover. These findings demonstrate that matching PYC activity with biotin availability improves glucose-to-lipid conversion and supports carbon-efficient production of DHA-rich microbial oils.

**Key words:** *Schizochytrium*; pyruvate carboxylase; biotin; docosahexaenoic acid; lipid yield

**Representative publication:**

1. Liu Y. Y., Zhang Z. F., Chen Y. X., Li J., Xu Y. S., Yang W., Xu Q., Ma W.*, Sun X. M., Systematic Metabolic Rewiring of *Schizochytrium* sp. via GmDof4 Overexpression for Enhanced DHA-Rich Lipid Production, Journal of Agricultural and Food Chemistry, 2026, 74, 22988–22999.
2. Ma W., Li J., Yang W. Q., Zhang Z. Y., Yan C. X., Huang P. W., Sun X. M.*, Efficient Biosynthesis of Odd-Chain Fatty Acids via Regulating the Supply and Consumption of Propionyl-CoA in Schizochytrium sp., Journal of Agricultural and Food Chemistry, 2023, 71, 9847–9855.
3. Ma W., Liu M. Z., Zhang Z. X., Xu Y. S., Huang P. W., Guo D. S., Sun X. M.*, Huang H.*, Efficient co-production of EPA and DHA by Schizochytrium sp. via regulation of the polyketide synthase pathway, Communications Biology, 2022, 5, 1356.
4. Ma W., Zhang Z. Y., Yang W. Q., Huang P. W., Gu Y., Sun X. M.*, Huang H., Enhanced docosahexaenoic acid production from cane molasses by engineered and adaptively evolved Schizochytrium sp., Bioresource Technology, 2023, 376, 128833.

## Biography



I am a master's student who enrolled in 2025 at the School of Food Science and Pharmaceutical Engineering, Nanjing Normal University. My research focuses on metabolic engineering of Schizochytrium sp. to improve DHA-rich lipid production through carbon metabolism and transcriptional regulation. I have published one research article in the Journal of Agricultural and Food Chemistry on metabolic rewiring of Schizochytrium sp. through GmDof4 overexpression for enhanced DHA-rich lipid production.

# Telomere-to-Telomere Genome Assembly and Comparative Genomics of *Rhodotorula toruloides*

Ziyan Wang[1], Zongbao Kent. Zhao[1]

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## Abstract

*Rhodotorula toruloides* is an oleaginous yeast of basidiomycota with considerable potential in biorefinery applications. However, its genetic basis and functional diversity remain poorly understood due to insufficient genome assembly and annotation. In this study, we performed a genome reassembly and refinement of the *R. toruloides* NP11 using multi-platform sequencing data, leading to telomere-to-telomere assembly with significantly improved genome continuity, completeness, and structural accuracy. Telomeric regions and repetitive sequences were systematically characterized, revealing key features of genome organization and structure and providing base for future evolutionary and functional studies. Comparative genomic analyses revealed higher genome-wide conservation among strains sharing the same mating type, as indicated by higher nucleotide similarity, stronger synteny, greater genome coverage, and lower SNP density. *R. toruloides* also showed a high proportion of species-specific genes, reflecting substantial lineage-specific divergence. Gene expansion was dominated by dispersed duplication, with a smaller contribution from local tandem duplication, while expansion of transmembrane transporter genes may have contributed to enhanced nutrient uptake during evolution.

**Key words:** *Rhodotorula toruloides*; T2T genome assembly; comparative genomics; mating type; genomic conservation

## Biography

Ziyan Wang is a Ph.D. candidate at the School of Bioengineering, Dalian University of Technology. Her research interests focus on fungal genomics, comparative genomics, systems biology, and metabolic engineering.



Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Alkaline sulfite pretreatment of bamboo culm for enzymatic sugar production

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**At 30 wt% NaOH, 5 wt% sodium sulfite gave 29.68 ± 1.64 g glucose plus xylose per 100 g initial dry bamboo. The 15 wt% sulfite group gave 29.69 ± 1.89 g/100 g; the lower dose used 22.2% less total reagent.**

## 1 Study design

Initial KOH screens across bamboo residues and garden waste motivated an alkaline NaOH + sodium sulfite study of bamboo culm. This approach draws on sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL), but differs from the original acidic protocol (Zhu et al., Bioresour. Technol. 100, 2411–2418, 2009). Pretreated solids were hydrolysed and analysed by HPLC for glucose and xylose and by DNS for total reducing sugars. The NaOH series used 15 FPU/g cellulose and a 1:50 solid-to-liquid ratio. Reagent loadings refer to initial dry biomass.

Yield plots use sugar mass per initial dry bamboo and include recovered solid mass. Pretreatment and wash liquors were not included in the sugar balance.

## 2 NaOH loading

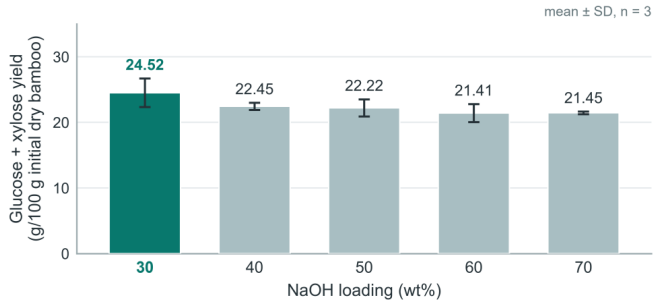



Fig. 1 Glucose + xylose yield after 30–70 wt% NaOH treatment. Bars show mean ± SD from three independent experiments per group. The 30 wt% group had the highest mean, 24.52 ± 2.18 g/100 g initial dry bamboo. The 40–70 wt% groups combine two experimental stages.

The mean at 30 wt% exceeded those at higher loadings, but the groups were not statistically separated in the current comparison. We therefore used 30 wt% as a lower-input working condition.

## 3 Sulfite addition at 30 wt% NaOH

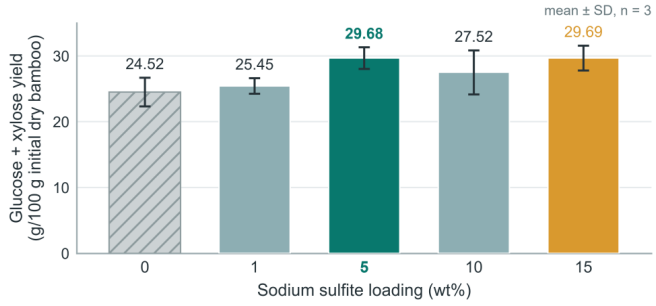



Fig. 2 Glucose + xylose yield at 0, 1, 5, 10 and 15 wt% sodium sulfite (SS). Bars show mean ± SD (n = 3 independent experiments). The hatched 0 wt% NaOH-only reference comes from another batch; same-batch confirmation is needed.

The 5 and 15 wt% SS means were almost identical. Their combined reagent inputs were 35 and 45 g per 100 g dry bamboo, respectively. This favors 5 wt% as the next scale-up candidate, while equivalence and mechanistic synergy remain unproven.

## 4 Corrected composition and sugar basis

| Bamboo culm | Glucan (%) | Xylan (%) | Total lignin (%) |
|---|---|---|---|
| Untreated | 42.94 ± 0.55 | 16.42 ± 0.06 | 28.12 ± 1.12 |
| 30NaOH + 5SS | 61.92 ± 2.03 | 16.70 ± 0.37 | 17.35 ± 0.37 |

The untreated Glucan + Xylan fraction was 59.36%; the treated-solid fraction was 78.62%. The three reported treated-solid fractions sum to 95.97%. These are fractions of different solids, not direct measures of glucan recovery or lignin removal.

Using DNS sugar mass and these composition denominators, apparent yields were 72.44 ± 5.11% per initial biomass and 85.64 ± 6.53% per recovered solid (n = 3). DNS does not provide glucose-specific conversion.

## 5 Enzyme-loading check

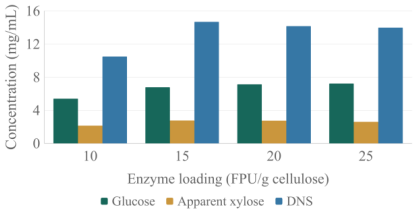



Fig. 3 Separate enzyme titration on 50 wt% KOH + 10 wt% SS treated bamboo. At 15 FPU/g cellulose, DNS peaked at 14.67 mg/mL. HPLC glucose + xylose was 9.54 mg/mL at 15 FPU and 9.87 mg/mL at 20 FPU. The 15 FPU working dose did not maximize every response.

## 6 Structure after 30 wt% NaOH + 10 wt% SS

**The FTIR, XRD and SEM comparisons below use untreated bamboo culm and a separate 30 wt% NaOH + 10 wt% SS sample. They do not directly characterize the 5 wt% SS yield candidate.**

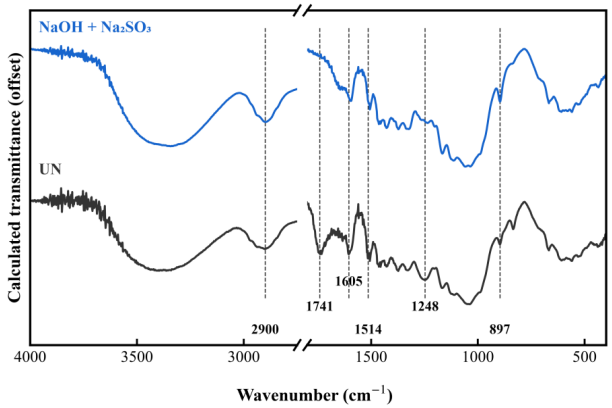



Fig. 4 FTIR spectra. Changes around 1741 and 1514 $cm^{-1}$ are consistent with altered noncellulosic components. The offsets and axis break preclude a quantitative removal estimate.

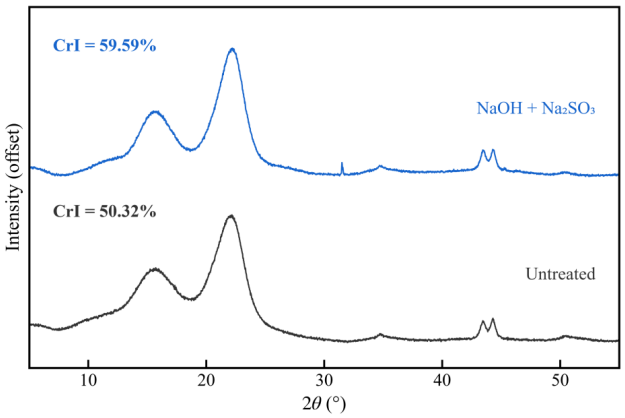



Fig. 5 XRD patterns reproduced from the revised group presentation. Segal relative crystallinity index was 50.32% before and 59.59% after treatment, with Iam taken at 2θ = 18°. The main peak remained near 22.2°. One pattern per condition supports a descriptive comparison only; the index is not absolute crystallinity.

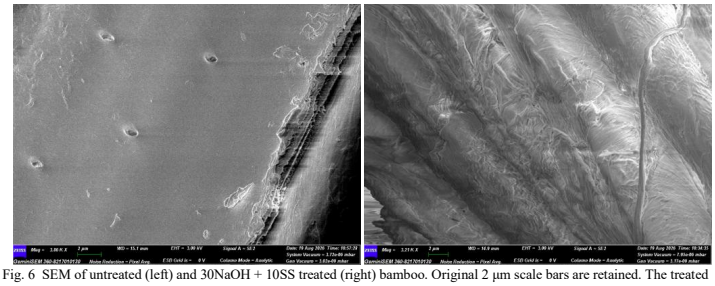


Fig. 6 SEM of untreated (left) and 30NaOH + 10SS treated (right) bamboo. Original 2 µm scale bars are retained. The treated field appears more folded; the slightly different magnifications do not support quantitative surface-area comparison.

## 7 Analysis and next experiment

HPLC sugars: Waters $NH_2$ 3.9 × 300 mm column, 2414 RI detector, 80% acetonitrile, 1.0 mL/min, 3 µL injection, column and cell at 35 °C. Composition followed the NREL two-stage acid-hydrolysis method.

Furfural and 5-HMF: C18–PDA HPLC at 277 and 285 nm. No identifiable sample peaks occurred at the standard retention times under the current method. Validated detection limits are not yet available.

Next, confirm the 5 wt% SS condition within one batch, close the sugar mass balance, and test the resulting hydrolysate for polyhydroxyalkanoate (PHA) fermentation. PHA titer, yield, productivity and polymer identity remain to be measured.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Engineering Ancestral Fatty Acid Photodecarboxylases for Photostable, Catalytically Robust Conversion of Oils into Hydrocarbon Fuels

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## Abstract

Fatty acid photodecarboxylase (FAP) is a blue-light-driven photoenzyme that converts free fatty acids into alkanes or alkenes, offering a promising route for valorizing waste oils into drop-in biofuels. However, the poor photostability and limited long-term catalytic stability of natural FAPs restrict their practical application. In this study, we screened 14 FAP candidates, some of which were generated by ancestral sequence reconstruction (ASR). The ancestral enzyme N185m exhibited both broad substrate activity toward all tested short- and long-chain fatty acids and the best combination of catalytic stability and photostability, and was therefore selected as the engineering template. Introducing the M5 mutation set improved catalytic stability but decreased photostability, whereas the AAK mutation set in the N185m background enhanced both photostability and catalytic activity, yielding N185m-AAK as the best-performing variant. AAK was strongly background-dependent: photostability improved only marginally when AAK was introduced into the M5 background, whereas L116C improved photostability at the cost of catalytic activity, with this penalty persisting in the M5 background. Finally, coupling N185m-AAK with the lipase ROL established a one-pot cascade that converted soybean oil into hydrocarbon fuels; N185m-AAK retained high stability in the complex oil matrix and achieved greater long-term product accumulation than that of native CvFAP. These results demonstrate the potential of ASR-derived FAPs as templates for engineering robust photoenzymes and for waste-oil valorization.

**Key words:** fatty acid photodecarboxylase; ancestral sequence reconstruction; protein engineering; photostability; waste-oil valorization.

**Representative publication:** No representative publication to date.

## Biography



Lijie Long is a graduate student in College of Food Science and Pharmaceutical Engineering at Nanjing Normal University, China, under the supervision of Dr. Guang Yang (Lecturer). Her research focuses on enzyme engineering and biocatalysis, particularly the discovery, rational design, and directed evolution of photoenzymes. Her current work centers on fatty acid photodecarboxylase (FAP), aiming to overcome its limited catalytic stability and photostability through ancestral sequence reconstruction and mutagenesis. By integrating protein engineering with cascade reaction design, she has developed a lipase – FAP coupled system that converts plant oils such as soybean oil into hydrocarbon fuels. These studies contribute to green biocatalytic platforms for sustainable energy and waste-oil valorization. She is currently preparing manuscripts based on this work.

# Directed Evolution of Carbonyl Reductase for Biosynthesis of Chiral Pydiflumetofen Intermediate

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## Abstract

Pydiflumetofen is a widely used succinate dehydrogenase inhibitor fungicide with obvious stereoselective biological activity. Reliable acquisition of high-purity chiral enantiomers is a prerequisite for exploring its structure–activity relationship and precise antifungal performance. Biocatalytic asymmetric synthesis has emerged as a mild, efficient, and environmentally friendly strategy for the preparation of chiral pesticide intermediates, providing a promising alternative to traditional chemical synthesis routes.In this study, carbonyl reductase *Ss*CR was selected as the biocatalytic template for the asymmetric synthesis of pydiflumetofen chiral intermediates. To further improve its catalytic performance, semi-rational design and directed evolution were performed. After iterative mutagenesis and high-throughput screening, the optimal mutant LTYK was obtained with a 3.64-fold increase in specific enzyme activity and excellent enantioselectivity over 99.9% e.e. A whole-cell catalytic system coupled with cofactor regeneration was constructed to realize efficient gram-scale synthesis. Molecular dynamics simulations clarified that structural optimization of the active site significantly promoted substrate binding and catalytic efficiency. Using the obtained chiral products, subsequent antifungal assays confirmed that (*S*)-pydiflumetofen exhibited greatly superior antifungal activity compared with the (*R*)-enantiomer.

**Key words:** Carbonyl reductase, *Ss*CR, Directed evolution, Chiral intermediate, Pydiflumetofen

**Representative publication:**

1. Cai H, Chen Z, Li J, Fan J, et al. New synthesis strategy of chiral pydiflumetofen and investigation on its fungicidal performance, J Hazard Mater. 2026 (in submission)

## Biography



Ziyu Chen obtained his Bachelor of Science degree from the College of Life Science and Technology, Huazhong Agricultural University, in 2026. Subsequently, he began his direct-track PhD program at the same institution. His early-stage work mainly centered on biocatalytic asymmetric synthesis of chiral intermediates using carbonyl reductases. Currently, his doctoral research is devoted to the enzymatic conversion of nitrogen-containing compounds, as well as the construction and optimization of microbial chassis strains for biotechnological applications.

# Engineering Yarrowia lipolytica for efficient geranylgeraniol production through the isopentenol utilization (IUP) and mevalonate (MVA) pathways

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## Abstract:

Geranylgeraniol (GGOH) is a plant-derived diterpenoid alcohol with broad applications in pharmaceuticals, nutraceuticals, and cosmetics, yet its supply relies on low-yield plant extraction; microbial cell factories offer a promising alternative. Here, we engineered the oleaginous yeast Yarrowia lipolytica to produce GGOH via the heterologous isopentenol utilization pathway (IUP), which bypasses the tightly regulated native mevalonate (MVA) pathway. Systematic screening of geranylgeranyl diphosphate (GGPP) synthases identified PaGGS1 as the most effective enzyme, while evaluation of 15 candidate kinases for the first phosphorylation step identified the E. coli-derived EcThiM as the best performer, improving the GGOH titer by 150%. Substrate tolerance assays showed that 30 mM prenol was lethal to the host, delimiting the substrate-loading window. Unexpectedly, the heterologous IUP and the native MVA pathway interfered with each other, reducing productivity; dual compartmentalization—targeting the IUP to mitochondria while retaining the MVA pathway in the cytosol—alleviated this interference, enhancing GGOH production by 26.1% and achieving a final titer of 1212.2 mg/L. In contrast, Erg20-IDI integration, phosphatase overexpression (YlDpp1 and YlLpp1), and NOG pathway introduction showed no significant improvement. These findings establish an efficient chassis for GGOH production in Y. lipolytica.

**Key words:** Yarrowia lipolytica; geranylgeraniol; isopentenol utilization pathway; metabolic engineering; terpenoid biosynthesis

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

1. Li D., Li Y., Xu J.Y., Zhang X.L.*, Engineering CrtW and CrtZ for improving biosynthesis of astaxanthin in *Escherichia coli*, Chinese Journal of Natural Medicines, 2020, 18, 666-676.

2. Yu Y., Shao M.Y., Li D., Zhang X.L.*, Construction of a carbon-conserving pathway for glycolate production by synergetic utilization of acetate and glucose in *Escherichia coli*, Metabolic Engineering, 2020, 61, 152-159.

3. Zhu T., Yao D., Li D., Zhang X.L.*, Multiple strategies for metabolic engineering of *Escherichia coli* for efficient production of glycolate, Biotechnology and Bioengineering, 2021, 118, 4699-4707.

## Biography

[Please enter your Biography here, limit to 200 words] (First-line indent of 2 characters, Time new roman, font size 12)



Di Li received his B.S. degree from Tianjin University of Science and Technology in 2018. He then pursued his M.S. degree (2018-2021) through a joint training program between Tianjin University of Science and Technology and the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, focusing on metabolic engineering of Escherichia coli for astaxanthin and glycolate biosynthesis. From 2021 to 2023, he worked as a research engineer at HEBE VDK Bio Co., Ltd., gaining industrial experience in strain development and bioprocess scale-up. Since 2023, he has been a doctoral candidate at the State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University. His current research centers on the metabolic engineering of Yarrowia lipolytica for terpenoid production, including geranylgeraniol and 24-epi-ergosterol, through heterologous pathway design and subcellular compartmentalization. His work has been published in journals such as Chinese Journal of Natural Medicines, Metabolic Engineering, and Biotechnology and Bioengineering.

# Metabolic Engineering of *Saccharomyces cerevisiae* for De Novo Biosynthesis of Rare Ginsenoside Rh1 from Red Algal Biomass

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## Abstract

This study used *Saccharomyces cerevisiae*, a biosafety-friendly and genetically well-defined yeast, as the chassis cell to construct a red algae polysaccharide substrate hydrolysis module containing agarase and neoagarobiose hydrolase genes. This module degrades red algae biomass polysaccharides into fermentable monosaccharides. A product synthesis module integrating dammarene diol synthase, protopanaxatriol synthase/cytochrome P450 reductase, protopanaxadiol synthase and glycosyltransferase was established to create a *de novo* synthesis pathway for the rare ginsenoside Rh1. Combining the red algae polysaccharide hydrolysis module with the rare ginsenoside synthesis module resulted in 141.78 mg/L rare ginsenoside Rh1, overcoming the dependence of fermentation processes on traditional carbon sources such as glucose. Subsequently, using the protopanaxadiol synthase as the target for modification, active site mutation design guided by MM/PBSA and distal site mutation design guided by conformational bias were employed. Experiments verified that the yield of the optimal combination mutant was 3.05 times that of the wild-type. Finally, through comprehensive metabolic engineering, the yield of rare ginsenoside Rh1 was 364.81 mg/L.

**Key words:** rare ginsenoside Rh1; *Saccharomyces cerevisiae*; red algal polysaccharides; metabolic engineering; rational enzyme design

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

1. Wan H., Zhang M., Gao R., Sun W.*, Complete biosynthesis of the clinical agent escin Ia, Nature Communications, 2026.

2. Tang H., Wu L., Guo S., Keasling J. D.*, Yu T.*, Metabolic engineering of yeast for the production of carbohydrate-derived foods and chemicals from C1 – 3 molecules, Nature Catalysis, 2024, 7, 21 – 34.

3. Chang J., Shi X., Kim M., Lee M. E., Oh J. W., Han S. O.*, A sequential and modular platform for red algal polysaccharide saccharification and de novo microbial bilirubin biosynthesis, Process Biochemistry, 2026, 165, 69 – 81.

4. Qiu Z., Wang G., Shao W., Xu J.*, Third-generation D-lactic acid production using red macroalgae *Gelidium amansii* by co-fermentation of galactose, glucose and xylose, Bioresource Technology, 2024, 399, 130631.

## Biography



[Please enter your Biography here, limit to 200 words] (First-line indent of 2 characters, Time new roman, font size 12)

Wei Xie is a Ph.D. candidate in the School of Food Science and Engineering at South China University of Technology. Her research focuses on synthetic biology and enzyme catalysis, particularly the construction of microbial cell factories, engineering of biosynthetic pathways, and optimization of key enzymes for the sustainable production of high-value natural products. She has published research articles in peer-reviewed journals, including *Bioresource Technology*, *Journal of Agricultural and Food Chemistry*, and *Journal of Hazardous Materials*. Her current research explores the intersection of microbial engineering, enzyme design, and sustainable biotechnology.

# Engineering *Methylosinus trichosporium* OB3b for Methane-to-Poly(3HB-co-3HP) Bioconversion

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## Abstract

The accumulation of plastic waste has increased the demand for biodegradable plastics as sustainable alternatives. Among these, polyhydroxyalkanoates (PHAs), including poly(3-hydroxybutyrate) (PHB), are promising biodegradable polymers that can be produced from methane by type II methanotrophs. In this study, we aimed to biosynthesize poly(3-hydroxybutyrate-co-3-hydroxypropionate) [poly(3HB-co-3HP)], one of the PHB copolymers, from methane as the sole carbon source in metabolic engineering of *Methylosinus trichosporium* OB3b. To establish 3-hydroxypropionate (3HP) biosynthesis, the β-alanine and malonyl-CoA pathways were compared, and the malonyl-CoA pathway showed greater potential for 3HP production. In addition, 3HP-CoA synthetases from three different sources were evaluated, and the enzyme from *Metallosphaera sedula* showed the highest efficiency for 3HP incorporation. Among the engineered strains, OB3b-MCRM-3S accumulated poly(3HB-co-3HP) up to 25.62% of dry cell weight with a maximum 3HP fraction of 9.02 mol%. This study represents the first demonstration of poly(3HB-co-3HP) production by a methanotroph using methane as the sole carbon source.

**Key words:** methane, *Methylosinus trichosporium* OB3b, metabolic engineering poly(3HB-co-3HP)

**Representative publication:**

1. da Costa JP. The 2019 global pandemic and plastic pollution prevention measures: Playing catch-up. Sci Total Environ 2021;774:145806.

2. OECD. Plastic Pollution is Growing Relentlessly as Waste Management and Recycling Fall Short, Says. OECD; 2022.

3. OECD. Global Plastics Outlook: Policy Scenarios to 2060. 2022. p. 283.

## Biography



Seo Hui Lee received the B.S. degree in Chemical Engineering from Kyung Hee University, South Korea, and is currently pursuing the M.S. degree in Chemical Engineering. Her research focuses on metabolic engineering and synthetic biology, with a primary emphasis on the genetic manipulation of methanotrophs for the production of high-value biopolymers, including polyhydroxybutyrate (PHB). Her current work involves engineering recombination and plasmid-based selection systems to enhance metabolic pathways in methanotrophs, aiming to advance sustainable biomanufacturing processes.

# Engineering the Glycosylation Regioselectivity of TcCGT1 toward O-Glycosylation of Aloesone

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## Abstract

Glycosyltransferases play an important role in increasing the structural diversity of natural products by determining where and how sugars are attached. Previously, TcCGT1, a highly promiscuous C-glycosyltransferase from the medicinal plant *Trollius chinensis*, was shown to catalyze the direct C-glycosylation of the methyl-chromone aloesone to produce aloesin. TcCGT1 can also catalyze O-glycosylation of phenolic substrates, making it a promising enzyme for studying and engineering glycosylation selectivity.

In this study, we aimed to shift TcCGT1 from C-glycosylation toward O-glycosylation of aloesone. Based on molecular docking and computer-assisted design, we selected twelve active-site residues for site-directed mutagenesis and generated twelve variants. Among them, TcCGT1_N353G showed the strongest preference for the second O-glucoside product (P3), suggesting a change in its regioselectivity. The P3 product was assigned to an O-glycosylation position that, to our knowledge, has not been previously reported for aloesone.

Our results show that targeted changes to the active site can alter the glycosylation selectivity of TcCGT1, providing a promising approach for generating greater structural diversity in glycosylated natural products.

**Key words:** Glycosyltransferase; TcCGT1; enzyme engineering; O-glycosylation; aloesone

**Representative publication:**

1. Putkaradze, N., Dato, L., Kırtel, O., Hansen, J., & Welner, D. H. (2024). Enzymatic glycosylation of aloesone performed by plant UDP-dependent glycosyltransferases. *Glycobiology*, *34*(9), cwae050. https://doi.org/10.1093/glycob/cwae050
2. Putkaradze, N., Teze, D., Fredslund, F., & Welner, D. H. (2021). Natural product *C* -glycosyltransferases – a scarcely characterised enzymatic activity with biotechnological potential. *Natural Product Reports*, *38*(3), 432–443. https://doi.org/10.1039/D0NP00040J

3. Ushasree, M. V., Jia, Q., Do, S. G., & Lee, E. Y. (2024). New opportunities and perspectives on biosynthesis and bioactivities of secondary metabolites from Aloe vera. *Biotechnology Advances*, *72*, 108325. https://doi.org/10.1016/j.biotechadv.2024.108325

## Biography



Genoveva Jessica Valerie Halim is a graduate student in the Department of Chemical Engineering, Integrated Engineering program at Kyung Hee University, Republic of Korea. Her research focuses on enzyme engineering and characterization, with particular interest in understanding and improving the catalytic properties of glycosyltransferases. Her current research investigates the engineered TcCGT1 enzyme and its glycosylation activity, aiming to understand how protein engineering can influence substrate specificity and glycosylation patterns. Through this work, she is developing experience in protein expression, purification, enzymatic assays, and chromatographic analysis for enzyme characterization. Her research interests extend to metabolic and enzyme engineering, with a particular interest in applying protein engineering and biochemical approaches to the development of enzymes with improved or tailored catalytic properties. In the future, she aims to contribute to the development of biotechnology applications through the integration of enzyme engineering and metabolic engineering strategies.

# Engineering Methanotrophs for One-Pot Conversion of Gaseous Alkenes to Vicinal Diols

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## Abstract

Methanotrophs are attractive whole-cell biocatalysts for the transformation of gaseous hydrocarbons because methane monooxygenases (MMOs) can oxidize a broad range of non-native substrates, including alkenes to their corresponding epoxides. In this study, we engineered methanotrophs to establish a one-pot bioconversion platform for the direct production of vicinal diols from gaseous alkenes. Comparative evaluation of type I and type II methanotrophs showed that type II strains exhibited superior alkene epoxidation activity. Based on this result, *Methylosinus trichosporium* OB3b was selected as a host for establishing a one-pot alkene-to-diol bioconversion system. Three epoxide hydrolases (EHs) from rom *Caulobacter crescentus*, *Mugil cephalus* and *Rhodotorula glutinis* were heterologously expressed in OB3b to enable the intracellular hydrolysis of MMO-generated epoxides to the corresponding 1,2-diols. Among the engineered strains, OB3b expressing the EH from *C. crescentus* exhibited the highest catalytic performance, producing 251.5 mg/L of (R)-1,2-propanediol directly from 1-propene. These results demonstrate that coupling native MMO-mediated epoxidation with heterologous epoxide hydrolysis enables one-pot conversion of gaseous alkenes into value-added vicinal diols.

**Key words:** methane monooxygenase; epoxide, epoxide hydrolase; one-pot bioconversion; (R)-1,2-propanediol

**Representative publication:**

1. Park Y.R., Krishna S., Lee O.K., Lee E.Y.*, Biosynthesis of chiral diols from alkenes using metabolically engineered type II methanotroph, *Bioresource Technology*, 2023, 389, 129851.
2. Nguyen T.T., Lee O.K., Naizabekov S., Lee E.Y.*, Bioconversion of methane to cadaverine and lysine using an engineered type II methanotroph, *Methylosinus trichosporium* OB3b, *Green Chemistry*, 2020, 22, 7803–7811.
3. Le H.T.Q., Lee E.Y.*, Insights into C1 and C3 assimilation pathways in type I methanotrophic bacterium from co-production of 1,2-propanediol and lactate, *Bioresource Technology*, 2022, 365, 128172.
4. Hwang S., Choi C.Y., Lee E.Y.*, Enantioconvergent bioconversion of p-chlorostyrene oxide to (R)-p-chlorophenyl-1,2-ethanediol by the bacterial epoxide hydrolase of *Caulobacter crescentus*, *Biotechnology Letters*, 2008, 30, 1219–1225.

## Biography



Jun Seo Shin is a master's student in the Department of Chemical Engineering at Kyung Hee University, Republic of Korea, where he joined the Bioprocess and Metabolic Engineering Laboratory led by Prof. Eun Yeol Lee in 2026. He received his B.S. in chemical engineering from Kyeong Hee university in 2026. His research focuses on the metabolic engineering of methanotrophic bacteria for the conversion of C1 and gaseous hydrocarbon feedstocks into value-added chemicals, with particular emphasis on whole-cell biocatalysis for the synthesis of enantiopure vicinal diols. His current work addresses cofactor regeneration and process intensification in engineered *Methylosinus trichosporium* OB3b, aiming to extend gas-to-liquid bioconversion beyond methanol to a broader portfolio of chiral chemical building blocks.

# Synergistic Lanthanum Recovery and Methane Mitigation by *Methylotuvimicrobium buryatense*: A Biotechnological Strategy Targeting Coal Mining Waste Streams

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## Abstract

Methane emissions from coal mining and the loss of rare earth elements in coal washing wastewater are conventionally treated as separate environmental and resource challenges. This study demonstrates that the methanotroph *Methylotuvimicrobium buryatense* 5GB1C can simultaneously mitigate methane and recover $La^{3+}$. At environmentally relevant $La^{3+}$ concentrations (240 $\mu g \cdot L^{-1}$), both cell growth and methane oxidation activity wereenhanced, revealing a functional synergy between the two processes. $La^{3+}$ removal followed pseudo-second-order kinetics, consistent with chemisorption-dominated adsorption. Meanwhile, integrated mechanistic analyses uncovered a sequentially coordinated pathway in which $La^{3+}$ was first adsorbed onto the cell wall and then actively transported into the cytoplasm, a process mediated by the *lanA* gene and accompanied by transcriptomic reprogramming of metal transport systems, membrane remodeling, and electron transfer pathways. Collectively, these findings establish a self-reinforcing mechanism whereby the methanotroph drives lanthanum uptake, and lanthanum in turn boosts methanotrophic activity. This work provides a proof-of-concept for an integrated microbial platform that turns coal-related waste streams into a resource recovery opportunity, with dual benefits for greenhouse gas mitigation and critical rare earth supply.

**Key words:** methanotroph; methane oxidation; lanthanum; bioaccumulation; transmembrane transport

**Representative publication:**

1. Du R.K., Chai F. G., Zhu R. C., Li S. Y. *, Achieving efficient biodegradation of hydrophobic toluene via the enhancement of β-cyclodextrin and atomization. *Process Safety and Environmental Protection* 2023, 170, 1151-1160.

## Biography



Du Rongkun, Ph.D. candidate at Xi'an Jiaotong University. The research primarily focuses on high-efficiency methane utilization and the customized polymerization of bio-based rubber. To date, he has published over 5 SCI-indexed papers as first author or co-author in leading environmental journals, including Journal of Cleaner Production, Waste Management, and Process Safety and Environmental Protection.

# Engineering a Methanotroph–*Escherichia coli* Consortium for Co-conversion of Methane and Propane to Polyhydroxybutyrate

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## Abstract

Methane and propane are abundant gaseous carbon feedstocks, but their simultaneous biological conversion is constrained by the limited substrate range of individual microorganisms. Here, we developed a synthetic methanotrophic–heterotrophic co-culture for the co-utilization of methane and propane toward polyhydroxybutyrate (PHB) production. To enable assimilation of propane-derived acetone, *E. coli* BL21(DE3) was engineered with a heterologous pathway for converting acetone into PHB. *Methylocystis* sp. OK1 was capable of oxidizing propane to acetone while utilizing methane as a carbon source; however, increasing propane concentrations resulted in reduced PHB accumulation in the methanotroph single-culture. To overcome this limitation, engineered *E. coli* was co-cultured with *Methylocystis* sp. OK1 under methane–propane co-feeding conditions. The co-culture enhanced PHB production by 35%, 65%, and 100% at propane concentrations of 7%, 13%, and 20% (v/v), respectively, compared with the *Methylocystis* sp. OK1 single-culture. These results demonstrate that coupling propane oxidation by a methanotroph with acetone assimilation by an engineered heterotroph can improve PHB production from mixed gaseous substrates.

**Key words:** Methanotroph; *Methylocystis* sp. OK1; *E. coli*; Polyhydroxybutyrate (PHB); Methane–propane co-utilization

**Representative publication:**

1. Lee O. K., Kang S. G., Choi T. R., Yang Y. H., Lee E. Y., Production and characterization of a biodegradable polymer, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), using the type II methanotroph, *Methylocystis* sp. MJC1, Bioresource Technology, 2023, 389, 129853.
2. Le H. T. Q., Lee E. Y., Biological production of 2-propanol from propane using a metabolically engineered type I methanotrophic bacterium, Bioresource Technology, 2022, 362, 127835.
3. Park Y. R., Krishna S., Lee O. K., Lee E. Y., Biosynthesis of chiral diols from alkenes using metabolically engineered type II methanotroph, Bioresource Technology, 2023, 389, 129851.
4. Nguyen T. T., Lee E. Y., Methane-based biosynthesis of 4-hydroxybutyrate and P(3-hydroxybutyrate-co-4-hydroxybutyrate) using engineered *Methylosinus trichosporium* OB3b, Bioresource Technology, 2021, 335, 125263.

## Biography



Seo Yeon Shin is an M.S. student in the Department of Chemical Engineering at Kyung Hee University, Republic of Korea, where she joined the laboratory of Prof. Eun Yeol Lee in 2026. She received her B.S. in Chemical Engineering from Kyung Hee University in the same year. Her research focuses on carbon-neutral C1 biorefinery, particularly the metabolic engineering of methanotrophic bacteria and the design of synthetic microbial co-cultures that convert methane and other gaseous substrates into biodegradable bioplastics such as polyhydroxybutyrate. She is currently interested in expanding the range of gaseous carbon sources that can be assimilated by engineered microbial consortia, and in understanding the metabolic interactions that govern the stability and productivity of methanotrophic–heterotrophic co-cultures. Through this work, she aims to contribute to greenhouse gas mitigation and to the development of sustainable routes for bioplastic and chemical production.

# FBAwOMC: Organelle-Level Molecular Crowding in Yeast Genome-Scale Flux Balance Analysis

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## Abstract

Organelle abundance, size, and morphology dictate metabolic capacity in yeast engineering. However, systematic optimization of these features remains largely empirical. Although flux balance analysis with molecular crowding (FBAwMC) incorporates kinetic parameters and molecular weights alongside reaction stoichiometry, its whole-cell enzyme volume budget fails to capture compartment-specific and membrane-associated physical constraints. We developed an organelle-constrained molecular crowding framework (FBAwOMC) by allocating distinct enzyme volume and membrane surface area budgets to individual subcellular compartments. The framework accounts for volume usage in internal reactions, joint volume–area consumption in membrane/transport reactions, and incorporates shape factors to model membrane folding dynamics. FBAwOMC successfully resolved organelle-level metabolic bottlenecks that remain hidden under whole-cell constraints. Predicted cellular growth and flux distributions varied dynamically with organelle abundance, dimension, and membrane folding, capturing metabolic phenotypes inaccessible to conventional models. The utility of the framework was validated across *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, providing mechanistic insights into compartment-specific resource allocation. FBAwOMC provides an advanced constraint-based modeling infrastructure that bridges organellar morphology with genome-scale enzyme allocation, establishing a rational design framework for coordinating organelle engineering with metabolic flux optimization in synthetic biology.

**Key words:** Genome-scale metabolic model, Flux balance analysis, Molecular crowding, Organelle engineering, Yeast

**Representative publication:**

## Biography



Seung Woo Baek is a Postdoctoral Researcher in the School of Chemical Engineering at Sungkyunkwan University, working under the supervision of Prof. Dong-Yup Lee. He received his B.S. and M.S. in Molecular and Cell Biology from the University of Texas at Dallas, and his Ph.D. in Bioengineering from Korea University, specializing in computational and synthetic biology. Prior to his current role, he gained diverse biotechnology industry experience across several startups, leading research and development initiatives in functional biomaterials, cellular process optimization, and RNA design algorithms. His current research focuses on genome-scale metabolic modeling (GEM), constraint-based flux analysis, and developing machine learning-driven codon optimization platforms for next-generation gene and RNA therapeutics

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Machine-learning guided decomposition of *Streptomyces coelicolor* transcriptome reveals the regulatory architecture encompassing secondary metabolism

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## Abstract

*Streptomyces* species are known for producing a wide array of secondary metabolites with biopharmaceutical importance. However, the biosynthesis of these compounds is often regulated by complex regulations. To unlock the full genetic potential of *Streptomyces*, a deeper understanding of its transcriptional regulation is essential. In this study, we applied independent component analysis (ICA) to 454 transcriptomic profiles of the model organism *Streptomyces coelicolor*, identifying 117 independently modulated gene groups, termed iModulons. Each iModulon represents a set of genes co-regulated in response to specific conditions, often under the control of specific transcriptional regulators. This system-level analysis uncovers the roles of previously uncharacterized genes, putative regulons for 40 transcriptional regulators and transcriptional regulation involved in secondary metabolism. Overall, ICA of large-scale transcriptomic dataset provides novel insights into the transcriptional regulatory network encompassing secondary metabolite production in *Streptomyces*.

This study was supported by the Bio & Medical Technology Development Program (RS-2024-00352229 to B.-K.C.) of the National Research Foundation (NRF), funded by the Ministry of Science and ICT (MSIT). This study was also supported a grant of the Korea-US Collaborative

Research Fund (KUCRF), funded by the Ministry of Science and ICT and Ministry of Health & Welfare, Republic of Korea (RS-2024-00468410 to B.-K.C.)

**Key words:** *Streptomyces*, Machine learning, Secondary metabolism

**Representative publication:**

1. Lee Y., Choe D., Palsson B. O., Cho B.-K.*, Machine-Learning Analysis of *Streptomyces coelicolor* Transcriptomes Reveals a Transcription Regulatory Network Encompassing Biosynthetic Gene Clusters, Adv. Sci., 2024, 11, 2403912.

## Biography



Chanyoung Joung received his B.S. degree in Department of Biological Sciences from Korea Advanced Institute of Science and Technology (KAIST). He is currently working as a graduate student under the supervision of Prof. Byung-Kwan Cho at KAIST. His research focuses on the development of *Streptomyces* chassis platforms for discovering novel secondary metabolites.

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# *In Silico* Retrobiosynthesis and AI-Guided Design of Modular Natural Product Pathways

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## Abstract

Modular polyketide synthases (PKSs) and non-ribosomal peptide synthetases (NRPSs) offer programmable platforms for natural product biosynthesis. Translating target structures into functional enzyme assemblies requires reconstruction of biosynthetic logic and consideration of module–module interactions. In this work, we introduce a multi-task graph attention network that identifies PKS-derived regions and scores candidate reaction centers. Guided by these predictions, sequential reverse transformations unravel post-assembly tailoring cascades to recover candidate biosynthetic precursors. Applied to reference Type I PKS systems, the framework reconstructs precursor scaffolds and deterministically translates them into starter- and extender-unit assignments, reductive-domain states, and candidate PKS gene assemblies. These outputs connect target structures with candidate PKS modules and gene clusters. By linking reverse tailoring analysis with modular pathway reconstruction, the framework supports the rational design of synthetic PKSs and exploration of alternative biosynthetic routes to complex polyketides within iterative design–build–test–learn workflows.

This study was supported by the KAIST Strategic Research Program (N10260074 to B.-K.C.) and the Bio & Medical Technology Development Program (grant no. RS-2024-00352229 to B.-K.C.) of the National Research Foundation (NRF), funded by the Ministry of Science and ICT (MSIT).

**Keywords:** polyketide synthases, retrobiosynthesis, graph neural networks, natural products.

**Representative publication:**

1. Kim G., Lee D., Kim J. H., Kim S. D., Kim H., Kim J. H., Yim S. S., Yeom S.-J., Keasling J. D., Cho B.-K.*, Engineering modular enzyme assembly: synthetic interface strategies for natural products biosynthesis applications, *Nat. Prod. Rep.*, 2025, 42, 1489–1506.

## Biography



Jae Heon Kim received his B.S. degree in Computer Science from New York University in 2020. He then earned an M.S. degree in Information Systems from Yonsei University, where his research focused on AI applications in systems biology. His work included graph neural networks for molecular property prediction, time-series forecasting for public health, and fine-tuning domain-specific large language models using retrieval-augmented generation. He is currently pursuing his Ph.D. in the Department of Biological Sciences at Korea Advanced Institute of Science and Technology (KAIST) under the supervision of Prof. Byung-Kwan Cho. His research focuses on developing *in silico* models to explore previously undiscovered retrosynthetic pathways for antibiotic biosynthesis using Type I polyketide synthases.

# Binary Solvent Extraction Enables Cell Recycling and Semi-Continuous Lipid Production in *Rhodotorula toruloides*

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## Abstract

Conventional microbial lipid extraction relies on cell disruption, which is energy-intensive and prevents cell reuse. Here, we developed a binary solvent system (methyl tert-butyl ether / n-hexane) to extract intracellular lipids directly from fresh fermentation broth of *Rhodotorula toruloides* without breaking cell walls, while aiming to maintain cell viability. Extraction time and solvent ratio were optimized. Using 40% MTBE at equal volume, the total lipid extraction rate reached 60%, and surprisingly, 5% of cells survived. After solvent removal, the surviving cells were supplemented with carbon and a small amount of nitrogen sources; they gradually regrew and produced lipids again. Repeating this gentle extraction on regrown cells enabled a second production cycle. Fatty acid profiles matched those obtained by the standard acid-heat method. This approach avoids energy-intensive pretreatment, simplifies phase separation, and allows recycling of both cells and organic solvents. It provides a promising route for semi-continuous fermentation-extraction integration.

**Key words:** oleaginous microorganisms; lipid extraction; MBTE; cell recycling

**Representative publication:**

1. Song J.Y., Kamal R., Chu Y.D., Liang S.Y., Zhao Z.K., Huang Q.T*, et al. Binary solvent extraction of intracellular lipids from *Rhodotorula toruloides* for cell recycling. Biotechnol. Biofuels Bioprod. 18, 53 (2025).

2. Huang Q.T., Kamal R., Lu H.B., Zhang J.L., Song J.Y., Lyu L.T., Xue H.Z., Song H., Zhao Z.K.*, Successful conversion of corn stover into microbial lipids at high solids loading by *Rhodosporidium toruloides* at pilot scale, Journal of Bioresources and Bioproducts, 2026, 11(1), 100222.

## Biography



Jingyi Song is a Ph.D. candidate at the School of Bioengineering, Dalian University of Technology. Her research interests focus on microbial lipid production, microbial fermentation, bioprocess development, and renewable biomass conversion.

# Engineering yeast platforms for aloesone biosynthesis through metabolic engineering and pathway optimization

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**Abstract**

Polyketides are a structurally diverse class of secondary metabolites with broad pharmaceutical and biotechnological importance. One such type III PKS-derived aromatic polyketide is aloesone, a naturally occurring chromone with diverse bioactive properties and a key biosynthetic precursor of aloesin and related glucosides. However, its production in yeast remains largely unexplored. Because aloesone formation requires substantial supplies of acetyl-CoA and malonyl-CoA, efficient production depends on selecting a suitable host and redirecting carbon flux toward these precursor pools. Here, we established an aloesone-producing platform in a *Yarrowia lipolytica* chassis pre-optimized for xylose assimilation by genomically integrating aloesone synthase (ALS) from *Rheum palmatum*. LC-MS/MS confirmed aloesone formation in the engineered strain. To improve precursor availability, lipid metabolism was rewired through DGA1 disruption and PEX10 overexpression, reducing competing flux toward storage lipids. The resulting strain, YlALSDP, produced 361.2 μg/L aloesone using glucose, while xylose supported a comparable titer of 354.55 μg/L. These results establish *Y. lipolytica* as a promising platform for aloesone biosynthesis and highlight metabolic rewiring and carbon-source flexibility for producing malonyl-CoA-derived plant polyketides.

**Key words:** Aloesone biosynthesis, Metabolic rewiring, Yeast biotechnology, Lipid metabolism, Carbon source utilization.

**Representative publication:**

1. Yang D., Kim W.J., Yoo S.M., Choi J.H., Ha S.H., Lee M.H., et al., Repurposing type III polyketide synthase as a malonyl-CoA biosensor for metabolic engineering in bacteria, Proceedings of the National Academy of Sciences of the United States of America, 2018, 115, 9835–9844.
2. Yang D., Jang W.D., Lee S.Y., Production of carminic acid by metabolically engineered Escherichia coli, Journal of the American Chemical Society, 2021, 143, 5364–5377.

## Biography



Tisa Rani Saha is a graduate researcher in the Department of Chemical Engineering, College of Engineering, Kyung Hee University, Republic of Korea. Her research interests include metabolic engineering, microbial biosynthesis, bioprocess engineering, and the development of sustainable bio-based products. Her current research focuses on engineering yeast cell factories for the biosynthesis of value-added plant-derived compounds, with particular emphasis on metabolic pathway optimization and carbon-source utilization. She has also been involved in research on bio-based polymeric materials and lignin valorization. Her research aims to develop sustainable microbial and biochemical platforms for the production of high-value chemicals and functional biomaterials.

# Long-term serial passage and recurrent strain reinforcement shape the assembly of selenium- and zinc-enriched probiotic communities

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## Abstract

Stable assembly of functional probiotic communities is critical for developing robust microbial systems with enhanced micronutrient bioaccumulation. Here, we investigated how long-term serial passage and recurrent reinforcement of defined functional strains influence the assembly of selenium (Se)- and zinc (Zn)-enriched probiotic communities. Nine initial microbial systems, including controls, single-strain cultures, a defined two-strain consortium, and five complex communities, were subjected to two parallel passage regimes for 40 transfers. In the natural-passage regime, communities were serially transferred without further strain supplementation, whereas in the reinforcement regime, the selected functional strains were periodically reintroduced every five transfers. Growth dynamics were monitored at generations 0, 15, 30, 35, and 40. Long-term passage resulted in pronounced divergence between the two regimes. Most reinforced communities converged toward reproducible high-biomass growth states by late passages, whereas naturally passaged communities frequently shifted toward lower-biomass states or continued to exhibit temporal restructuring. These results indicate that recurrent strain reinforcement can strongly influence long-term community trajectories and phenotypic stability. This study provides an ecological-selection framework for engineering stable probiotic consortia toward simultaneous Se and Zn enrichment.

**Key words:** Long-term serial passage; Microbial community assembly; Selenium and zinc bioaccumulation

**Representative publication:**

Wang Y. D., Yu Y. H., Wang Z. Q.*, et al., Enhancing the Activity of Carboxymethyl Cellulase Enzyme Using Highly Stable Selenium Nanoparticles Biosynthesized by *Bacillus paralicheniformis* Y4, *Molecules*, 2022, 27, 4585.

Duan Y. H., Li M. J., Zhang S. S., Wang Y. D., Wang Z. Q.*, et al., Highly efficient biotransformation and production of selenium nanoparticles and polysaccharides using potential probiotic *Bacillus subtilis* T5, *Metabolites*, 2022, 12, 1204.

## Biography



Yidan Wang is a Ph.D. candidate at Xi'an Jiaotong-Liverpool University. Her research focuses on microbial selenium biotransformation, selenium- and zinc-enriched probiotics, and microbial community engineering. Her current work explores how long-term serial passage and recurrent reinforcement of functional strains drive the adaptive assembly, phenotypic stability, and micronutrient bioaccumulation of probiotic communities. She is particularly interested in applying ecological selection and microbial community engineering to construct stable and high-performing functional communities for selenium and zinc biofortification. Her research also involves microbial selenium nanoparticle biosynthesis and the functional characterization of probiotic strains. She has published related studies in peer-reviewed journals including *Molecules* and *Metabolites*.

Metabolic Engineering Summit 2026

# Genome-wide identification of genetic regulatory elements in *Streptomyces tsukubaensis* NRRL 18488

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## Abstract

*Streptomyces* species are well known for their ability to produce a diverse array of secondary metabolites, many of which are utilized in clinical applications. Among them, *Streptomyces tsukubaensis* NRRL 18488 is an industrially significant strain due to its production of the immunosuppressant FK506. Understanding the complex regulation of secondary metabolism requires a comprehensive analysis of the primary transcriptome architecture, which elucidates transcriptional and post-transcriptional regulatory elements. In this study, we identified a total of 2,389 transcription start sites and 2,110 transcript end positions, which were integrated to define 1,225 transcription units (TUs), including numerous non-coding TUs. Notably, within the FK506 biosynthetic gene cluster, we uncovered previously uncharacterized post-transcriptional regulation governing the production of allylmalonyl-CoA, an essential precursor for FK506 biosynthesis. This genome-wide analysis provides a detailed understanding of the complex regulatory mechanisms orchestrating secondary metabolism in *Streptomyces*.

This research was supported by a grant of the Korea-US Collaborative Research Fund (KUCRF), funded by the Ministry of Science and ICT and Ministry of Health & Welfare, Republic of Korea (RS-2024-00468410 to B.-K.C.), and by the KAIST Strategic Research Program (N10260074 to B.-K.C.).

**Key words:** *Streptomyces*; Secondary Metabolite; FK506; Transcription unit

**Representative publication:**

**1.** Namil Lee, Woori Kim, Ji Hun Kim, Yongjae Lee, Soonkyu Hwang, Gahyeon Kim, Hyeseong Kim, Suhyung Cho, Qingyun Dan, Matthias Schmidt, Yeo Joon Yoon, Suhyung Cho, Bernhard Palsson, Jay D. Keasling, and Byung-Kwan Cho, Regulatory orchestration of FK506 biosynthesis in *Streptomyces tsukubaensis* NRRL 18488 revealed through systematic analysis (2025), *iScience*, 28, 6

## Biography



Hyeseong Kim received her B.S. degree in Microbiology from Pusan National University in 2021. She is currently working as a graduate student under the supervision of Prof. Byung-Kwan Cho at Korea Advanced Institute of Science and Technology (KAIST). Her research focuses on the discovery of novel natural products derived from fungi and *Streptomyces*, with an emphasis on enhancing their production through microbial engineering approaches.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Transcriptome and Translatome Analysis of FK506 Biosynthesis in *Streptomyces tsukubaensis* NRRL 18488

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## Abstract

*Streptomyces* species are industrially important microorganisms that produce diverse bioactive secondary metabolites through tightly regulated secondary metabolite biosynthetic gene clusters (smBGCs). To understand their regulatory dynamics, we investigated transcriptional and translational changes across different growth phases in five *Streptomyces* species: *S. avermitilis*, *S. clavuligerus*, *S. lividans*, *S. venezuelae*, and *S. tsukubaensis*. Cells were collected at early-exponential, transition, late-exponential, and stationary phases in biological duplicates. Transcriptomic and translatomic profiles were generated using RNA sequencing (RNA-Seq) and ribosome profiling, respectively. RNA-Seq generated approximately 12–83.5 million raw reads per sample, whereas ribosome profiling generated 113–763.8 million reads. Quality assessment demonstrated high sequencing quality and reproducibility across biological replicates. Ribosome-protected fragments also showed size distributions consistent with monosome-protected RNA fragments. These comprehensive datasets enable comparative analysis of transcriptional and translational regulation during growth-dependent metabolic transitions in *Streptomyces*. The integrated profiles provide a valuable resource for elucidating regulatory mechanisms associated with secondary metabolism and may facilitate the identification of regulatory elements for rational engineering of *Streptomyces* strains toward enhanced production of valuable secondary metabolites.

This research was supported by a grant of the Korea-US Collaborative Research Fund (KUCRF), funded by the Ministry of Science and ICT and Ministry of Health & Welfare, Republic of Korea (RS-2024-00468410 to B.-K.C.), and by the KAIST Strategic Research Program (N10260074 to B.-K.C.).

**Key words:** *Streptomyces*; Secondary Metabolite; FK506; Systems biology

**Representative publication:**

**1.** Namil Lee, Woori Kim, Ji Hun Kim, Yongjae Lee, Soonkyu Hwang, Gahyeon Kim, Hyeseong Kim, Suhyung Cho, Qingyun Dan, Matthias Schmidt, Yeo Joon Yoon, Suhyung Cho, Bernhard Palsson, Jay D. Keasling, and Byung-Kwan Cho, Regulatory orchestration of FK506 biosynthesis in *Streptomyces tsukubaensis* NRRL 18488 revealed through systematic analysis (2025), *iScience*, 28, 6

## Biography

Sangmin Park received his B.S. degree in Biotechnology from Yonsei University, South Korea, in 2025. He is currently pursuing his Ph.D. in the Department of Biological Sciences at Korea Advanced Institute of Science and Technology (KAIST) under the supervision of Prof. Byung-KwanCho. His research focuses on the discovery and structural optimization of bioactive molecular analogs through integrative computational and AI-driven approaches.



# Development of a promoter-based PLP-responsive biosensor platform for directed evolution of PLP biosynthetic enzymes in *Corynebacterium glutamicum*

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## Abstract

Pyridoxal 5′-phosphate (PLP) is an essential cofactor for numerous metabolic enzymes and plays important roles in amino acid metabolism and the biosynthesis of diverse high-value compounds. Although PLP biosynthetic enzymes have been engineered for microbial production, their engineering remains challenging due to the complex regulation of PLP metabolism and the lack of effective high-throughput screening tools. Previous studies have primarily focused on strengthening PLP biosynthesis or supplementing exogenous PLP to increase PLP availability, whereas few studies have explored PLP-responsive biosensors for the directed evolution of PLP biosynthetic enzymes. Here, we developed a highly sensitive and specific promoter-based PLP-responsive biosensor through transcriptomic data mining and systematic promoter engineering in *Corynebacterium glutamicum*. The platform was applied to the directed evolution of *BspdxST*, a PLP synthase complex from *Bacillus subtilis*, leading to the identification of a novel mutant with 3.26-fold and 1.35-fold increases in *pdxS* and *pdxT* activities, respectively. This study demonstrates the potential of PLP-responsive biosensing as an effective tool for directed evolution of PLP biosynthetic enzymes and provides a generalizable strategy for cofactor engineering in microbial cell factories.

**Key words:** pyridoxal 5′-phosphate, Corynebacterium glutamicum, biosensor, high-throughput screening

**Representative publication:** Zhou, Y. Y.; Guo, J. Q.; Zhang, Z. K.; Lu, K. Y.; Chen, T.; Wang, Z. W. Development of a promoter-based PLP-responsive biosensor platform for directed evolution of PLP biosynthetic enzymes in *Corynebacterium glutamicum*. *In preparation*

## Biography

Corresponding author: Zhiwen Wang, Professor at Tianjin University and Ningxia University, and supervisor of doctoral and master's students. He is a recipient of the National Major Talent



Project (Youth Program). He has led two projects/subprojects under the National Key Research and Development Program, one subproject under the National 863 Program, four projects funded by the National Natural Science Foundation of China, and eight provincial-level and enterprise projects, including the Key Research and Development Program of Ningxia Hui Autonomous Region. As first or corresponding author, he has published more than 60 SCI-indexed papers in leading domestic and international journals, including Advanced Science, Metabolic Engineering, AIChE Journal, Green Chemistry, ACS Sustainable Chemistry & Engineering, ACS Synthetic Biology, and Biotechnology Advances, as well as 19 papers in Chinese core journals. He is the first inventor on 17 granted national invention patents and has served as editor-in-chief of two textbooks published by Higher Education Press. He is a council member of the Ningxia Biological Society, Director of the Ningxia Cattle and Sheep Specialty Feed Technology Innovation Center, a committee member of the Ningxia Feed Industry Association, a Youth Editorial Board Member of Engineering Microbiology, a Review Editor for the Synthetic Biology section of Frontiers in Bioengineering and Biotechnology, and an editorial board member of the Journal of Ningxia University.

# Injectable Hydrogel Microspheres Loaded with Cartilage-Targeting Hybrid Exosomes Mediating FGF18 Gene Delivery for Osteoarthritis Treatment

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## Abstract

Abnormal silencing of fibroblast growth factor (FGF) signaling significantly contributes to joint dysplasia and osteoarthritis (OA); However, the clinical translation of FGF18-based protein drugs is hindered by their short half-life, low delivery efficiency and the need for repeated articular injections. This study proposes a CRISPR/Cas9-based approach to effectively activate the FGF18 gene of OA chondrocytes at the genome level in vivo, using chondrocyte-affinity peptide (CAP) incorporated hybrid exosomes (CAP/FGF18-hyEXO) loaded with an FGF18-targeted gene-editing tool. Furthermore, CAP/FGF18-hyEXO are encapsulated in methacrylic anhydride-modified hyaluronic (HAMA) hydrogel microspheres via microfluidics and photopolymerization to create an injectable microgel system (CAP/FGF18-hyEXO@HMs) with self-renewable hydration layers to provide persistent lubrication in response to frictional wear. Together, the injectable CAP/FGF18-hyEXO@HMs, combined with in vivo FGF18 gene editing and continuous lubrication, have demonstrated their capacity to synergistically promote cartilage regeneration, decrease inflammation, and prevent ECM degradation both in vitro and in vivo, holding great potential for clinical translation.

**Key words:** Osteoarthritis，FGF18，Hyaluronic acid，Gel microspheres

**Representative publication:**

1. Manyu Chen, Yan Lu, Yuhan Liu, Quanying Liu, Siyan Deng, Yuan Liu, Xiaolin Cui, Jie Liang, Xingdong Zhang, Yujiang Fan*, Qiguang Wang*. Injectable Microgels with Hybrid Exosomes of Chondrocyte-Targeted FGF18 Gene-Editing and Self-Renewable Lubrication for Osteoarthritis Therapy. Advanced Materials 2024, 36 (16).

2. Manyu Chen, Yuhan Liu, Yi Cao, Chengkun Zhao, Quanying Liu, Na Li, Yuan Liu, Xiaolin Cui, Pengcheng Liu, Jie Liang, Yujiang Fan*, Qiguang Wang*, Xingdong Zhang. Remodeling the Proinflammatory Microenvironment in Osteoarthritis through Interleukin-1 Beta Tailored Exosome Cargo for Inflammatory Regulation and Cartilage Regeneration. ACS Nano 2025, 19 (4) , 4924-4941.

3. Manyu Chen, Jing Wang, Yuhan Liu, Xiaolin Cui, Jie Liang, Ning Lei, Yumei Xiao, Qiguang Wang*, Yujiang Fan, Xingdong Zhang. Injectable biomimetic microcarriers harness synergistic effects of paracrine factors and cellular membranes to alleviate osteoarthritis. Chemical Engineering Journal 2025, 503 , 158451.

4. Manyu Chen, Yuan Liu, Quanying Liu, Siyan Deng, Yuhan Liu, Jiehao Chen, Yaojia Zhou, Xiaolin Cui, Jie Liang, Xingdong Zhang, Yujiang Fan*, Qiguang Wang*, Bin Shen*. Nanoengineered cargo with targeted in vivo Foxo3 gene editing modulated mitophagy of chondrocytes to alleviate osteoarthritis. Acta Pharmaceutica Sinica B 2025, 15 (1) , 571-591.

5. Manyu Chen, Quanying Liu, Yang Xu,Yuxiang Wang, Xiaowen Han, Zhe Wang, Jie Liang*, Yong Sun, Yujiang Fan,Xingdong Zhang. The effect of LyPRP/collagen composite hydrogel on osteogenic differentiation of rBMSCs, Regenerative Biomaterials 2021, 8(1): rbaa053.

## Biography



The applicant is currently a pre-appointed Associate Research Fellow and postdoctoral researcher at the Sports Medicine Center, First Affiliated Hospital of Army Medical University. He received both his M.Sc. and Ph.D. degrees from the College of Biomedical Engineering, Sichuan University, under the supervision of Prof. Yujiang Fan in Prof. Xingdong Zhang's research team. His research focuses on bioactive materials and their applications in regenerative medicine. He has received the National Scholarship and the Outstanding Graduate Award of Sichuan University and has participated in several national and provincial research programs. As first or co-first author, he has published six papers in leading journals, including Advanced Materials, ACS Nano, Acta Pharmaceutica Sinica B, Chemical Engineering Journal, Regenerative Biomaterials, and Biomedical Materials. He has also contributed to more than 20 collaborative publications in Nature Communications, Advanced Functional Materials, Small, ACS Applied Materials & Interfaces, Acta Biomaterialia, and other journals. In addition, he serves as a peer reviewer for several international journals and has presented his research at multiple national and international conferences through oral and poster presentations. His solid research experience in regenerative biomaterials provides a strong theoretical and technical foundation for conducting innovative and feasible research in the field.

# Matrix Stress Relaxation Regulates Tendon-Bone Interface Repair via OGT/O-GlcNAc-Mediated Mechanometabolic Signaling

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## Abstract

Tendon-bone interface healing often results in scar-like attachment, characterized by fibrotic deposition on the tendon side and insufficient osteogenesis on the bone side. Current approaches mainly emphasize structural biomimicry and static stiffness matching, while the role of the dynamic viscoelastic microenvironment remains unclear. Our preliminary findings indicate that abnormal fibroblast activation and impaired osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) coexist during defective repair, accompanied by dysregulated OGT-mediated O-GlcNAcylation, a metabolism-sensitive post-translational modification linked to glucose metabolism and nutrient sensing.

We hypothesize that abnormal matrix stress relaxation disrupts mechanometabolic homeostasis through OGT/O-GlcNAc signaling, thereby promoting fibroblast fibrosis and suppressing BMSC osteogenesis, ultimately impairing functional tendon–bone regeneration. To test this, we will establish tendon-to-bone gradient stress-relaxation matrix models and investigate how stress relaxation regulates fibroblast activation, extracellular matrix deposition, and BMSC osteogenic differentiation. We will further define the mediating role and molecular mechanisms of OGT/O-GlcNAcylation in this process.

This study may reveal how viscoelastic mechanical cues regulate metabolic signaling and cell fate at the tendonbone interface, providing new mechanometabolic targets for improving regenerative healing.

**Key words:** Tendon-bone interface; Stress relaxation; OGT/O-GlcNAcylation; Mechanometabolism; Osteogenic differentiation

**Representative publication:**

1. Y. Wang, J. Zhang, X. Shu, F. Wu, J. He, Controlled domain gels with a wide stiffness gradient simultaneously promote bone regeneration and suppress tumor recurrence through DAPK activity. Chemical Engineering Journal 482 (2024) 149018

2. Y. Wang [1], N. Zhang [1], J. Zhang, R. Yao, J. He *, F. Wu *, Reinforced enzyme mineralized chitosan hydrogels with superior mechanical and osteogenic properties. Carbohydrate Polymers 349 (2025) 123032.

3. Y. Wang [a,1], J. He [a,1], J. Zhang [a], N. Zhang [a], Y. Zhou [b,*], F. Wu [a,*], Cell migration induces apoptosis in osteosarcoma cell via inhibition of Wnt-β-catenin signaling pathway. Colloids and Surfaces B: Biointerfaces 223 (2023) 113142.

## Biography



The applicant is currently an Assistant Research Fellow and postdoctoral fellow at the Sports Medicine Center, First Affiliated Hospital of the Army Medical University, People's Liberation Army of China. The applicant received both master's and doctoral degrees from the College of Biomedical Engineering, Sichuan University, and was awarded honors including Outstanding Graduate Student and Outstanding Graduate of Sichuan University.

During master's and doctoral training, the applicant conducted systematic research on key scientific questions related to the cellular mechanical microenvironment and cell fate regulation. This work focused on the development of functional gradient hydrogels, the mechanisms of matrix mechanotransduction, and their regulatory roles in tissue regeneration and disease treatment. Through these studies, the applicant has established a solid research foundation in biomaterial design, mechanical microenvironment modulation, and cell behavior intervention.

In recent years, the applicant has published six SCI-indexed papers as first author or co-first author in leading journals in related fields, including Chemical Engineering Journal (IF = 13.2, Q1), Carbohydrate Polymers (IF = 12.5, Q1), Colloids and Surfaces B: Biointerfaces (IF = 5.6, Q1),

Journal of Materials Chemistry B (IF = 5.8, Q1), and Journal of Bionic Engineering (IF = 5.8, Q1), with a cumulative impact factor of 47.9. The applicant has also been granted two national invention patents as a major inventor.

Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Genome-Scale Multi-Omics Analysis of Gene Regulation across Industrially Relevant *Streptomyces* Species

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## Abstract

*Streptomyces* species are major producers of industrially important secondary metabolites. However, engineering their biosynthetic potential requires a genome-scale understanding of gene regulation. We integrated differential RNA-seq, Term-seq, RNA-seq, and ribosome profiling to characterize transcriptional and translational regulation in *Streptomyces clavuligerus*, *S. lividans*, *S. avermitilis*, and *S. griseus*. Genome-wide mapping identified conserved -10 promoter motifs, diverse -35 elements, and abundant leaderless transcripts, demonstrating distinct regulation of transcription and translation initiation. Integration of transcript start and end positions reconstructed complex transcription unit architectures and showed that alternative boundaries, premature termination, and RNA processing can fine-tune gene expression within polycistronic regions. Growth-phase-resolved transcriptome and translatome analyses further revealed coordinated regulatory transitions from primary to secondary mechanisms. Collectively, these studies reveal multilayered regulatory mechanisms extending from transcription initiation to termination and translation, and provide a foundation for rational activation and engineering *of Streptomyces* biosynthetic gene clusters.

This research was supported by a grant of the Korea-US Collaborative Research Fund(KUCRF), funded by the Ministry of Science and ICT and Ministry of Health & Welfare, Republic of Korea (grant number: RS-2024-00468410 to B.-K.C.), and also supported by the Bio & Medical Technology Development Program (grant no. RS-2024-00352229 to B.-K.C.) of the National Research Foundation (NRF), funded by the Ministry of Science and ICT (MSIT).

**Key words:** *Streptomyces*, Multi-omics, Gene regulatory architecture

**Representative publication:**

1. Hwang S., Lee N., Jeong Y., Lee Y., Kim W., Cho S., Palsson B.O., Cho B.K.*, Primary transcriptome and translatome analysis determines transcriptional and translational regulatory elements encoded in the Streptomyces clavuligerus genome, Nucleic Acids Research, 2019, 47, 6114–6129.

2. Lee Y., Lee N., Jeong Y., Hwang S., Kim W., Cho S., Palsson B.O., Cho B.K.*, The transcription unit architecture of Streptomyces lividans TK24, Frontiers in Microbiology, 2019, 10, 2074.

3. Hwang S., Lee N., Choe D., Lee Y., Kim W., Jeong Y., Cho S., Palsson B.O., Cho B.K.*, Elucidating the regulatory elements for transcription termination and posttranscriptional processing in the Streptomyces clavuligerus genome, mSystems, 2021, 6, e01013-20.

4. Lee Y., Lee N., Hwang S., Kim W., Cho S., Palsson B.O., Cho B.K.*, Genome-scale analysis of genetic regulatory elements in Streptomyces avermitilis MA-4680 using transcript boundary information, BMC Genomics, 2022, 23, 68.

5. Hwang S., Lee N., Choe D., Lee Y., Kim W., Kim J.H., Kim G., Kim H., Ahn N.H., Lee B.H., Palsson B.O., Cho B.K.*, System-level analysis of transcriptional and translational regulatory elements in Streptomyces griseus, Frontiers in Bioengineering and Biotechnology, 2022, 10, 844200.

## Biography



Gahyeon Kim received her B.S. degree in Systems Biotechnology from Chung-Ang University, South Korea, in 2021. She is currently pursuing her Ph.D. in the Department of Biological Sciences at Korea Advanced Institute of Science and Technology (KAIST) under the supervision of Prof. Byung-Kwan Cho. Her research focuses on genome mining of Streptomyces and modular engineering of polyketide synthases to enable the discovery and development of novel natural products.

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# Elucidating essential genes for autotrophic growth in *Eubacterium limosum* through genome-wide CRISPRi screening

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## Abstract

One of model acetogenic species, *Eubacterium limosum*, has a potential for sustainable bioconversion of carbon oxides (e.g., CO and $CO_2$) into multi-carbon biochemicals. Elucidating their genotype-phenotype relationships is a key for improving their biocatalytic performances; however, systemic investigation on the fitness contribution of individual genes has been limited. Here, we report a genome-scale CRISPR interference (CRISPRi) screen using 41,939 guide RNAs designed from the genome of *E. limosum* for transcriptional repression of all genes. Our data revealed that the genes related to Wood-Ljungdahl pathway and membrane regeneration are essential for its autotrophic growth. Additionally, we identified genes that enhanced autotrophic growth rates by 4-fold and increased acetoin production by 1.5-fold compared to the wild-type under $CO_2$-$H_2$ conditions. These findings offer valuable insights for both understanding and engineering acetogenic bacteria.

This work was supported by Korea Environmental Industry & Technology Institute (KEITI) through the Technology Development Program for $CO_2$ Mitigation and Conversion to Value-Added Products Using Indigenous Organism, funded by Korea Ministry of Climate, Energy and Environment (MCEE) (RS-2026-25505528). This work was also supported by the Bio & Medical Technology Development Program (RS-2024-00352229 to B-KC) funded by the Ministry of Science and ICT (MSIT).

**Key words:** Acetogenic bacteria, CRISPRi, Genome-wide screening

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

1. Jongoh Shin, Jiyun Bae, Hyeonsik Lee, Seulgi Kang, Sangrak Jin, Yoseb Song, Suhyung Cho, and Byung-Kwan Cho*, Genome-wide CRISPRi screen identifies enhanced autolithotrophic phenotypes in acetogenic bacterium Eubacterium limosum, PNAS, 2023, 101073

## Biography



Chanho Park received B.S. degree in Department of Engineering Science in Konkuk University, South Korea, in 2023. He is currently in his Ph.D. degree Department of Biological Sciences at Korea Advanced Institute of Science and Technology (KAIST) under the supervision of Prof. Byung-Kwan Cho. His research aim is elucidating the regulatory mechanisms of acetogens using systems-level approaches with the ultimate goal of improving their carbon fixation efficiency.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Engineering *Saccharomyces cerevisiae* for Cannabigerolic Acid Production

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## Abstract

Cannabinoids are plant-specialized metabolites with diverse pharmaceutical applications, and their microbial production in *Saccharomyces cerevisiae* offers a sustainable alternative to plant-based production. In this study, we engineered *S. cerevisiae* to produce cannabigerolic acid (CBGA), a central biosynthetic precursor of major cannabinoids, including THCA, CBDA, and CBCA. To increase the availability of geranyl pyrophosphate (GPP), a key precursor for CBGA biosynthesis, a monoterpenoid chassis (MC) strain was constructed by targeting the mevalonate pathway to peroxisomes and expressing Erg20$^{WWG}$, a variant with reduced activity for converting GPP to farnesyl pyrophosphate (FPP). The resulting MC1 strain produced 277 mg/L geraniol, which was used as a proxy for GPP availability. For olivetolic acid biosynthesis, hexanoic acid was supplied as a precursor and converted to hexanoyl-CoA by acyl-activating enzymes. Tetraketide synthase (TKS) catalyzes three successive condensations of hexanoyl-CoA with malonyl-CoA to generate a linear tetraketide intermediate, which is subsequently cyclized to olivetolic acid by olivetolic acid cyclase (OAC). Screening of acyl-activating enzymes identified *Pseudomonas putida* LvaE (*Pp*LvaE) as the most effective enzyme for enhancing olivetolic acid production. CBGA biosynthesis was subsequently established by expressing TKS, OAC, and prenyltransferase 4 (PT4) from *Cannabis sativa*. We also evaluated NphB, a soluble prenyltransferase from *Streptomyces* sp. capable of catalyzing CBGA formation from olivetolic acid, together with several NphB variants previously engineered for enhanced CBGA production. However, a truncated form of *C. sativa* PT4 fused to Sod1 supported higher CBGA production than the tested NphB variants. Collectively, these engineering strategies established a yeast platform for CBGA biosynthesis that can serve as a foundation for producing cannabinoid derivatives with improved therapeutic potential.

**Key words:** *Saccharomyces cerevisiae*; Cannabinoid; Metabolic engineering; Monoterpenoid; CBGA

**Representative publication:**

1. Bernard A., Cha S., Shin H., Lee D., Hahn J.S.*, Efficient production of (*S*)-limonene and geraniol in *Saccharomyces cerevisiae* through the utilization of an Erg20 mutant with enhanced GPP accumulation capability, *Metabolic Engineering*, 2024, 83, 183–192.

2. Cha S., Jang B., Lee D., Cho I., Park W., Lee Y., Shin H., Kim M., Sung C., Hahn J.S.*, Efficient (*S*)-acetoin production in *Saccharomyces cerevisiae* by modulating α-acetolactate decarboxylase stereospecificity, *Bioresource Technology*, 434, 2025, 132767, 0960-8524

## Biography



Hyesoo Shin is a graduate student at Seoul National University working in metabolic engineering and synthetic biology, with a focus on engineering yeast cell factories for the biosynthesis of plant-derived natural products.

Her research centers on the production of monoterpenoids, particularly cannabinoids in *S. cerevisiae*. Her research covers biosynthetic enzyme screening, heterologous enzyme expression and optimization of metabolic flux in yeast. Current efforts focus on substrate channeling of cannabinoid biosynthesis enzymes, and on producing high-performance cannabinoid derivatives through enzyme engineering.

# High-Throughput Growth-Coupled Evolution of D-Amino Acid Biocatalysts for Non-Canonical Amino Acid Synthesis

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## Abstract

Engineering amino acid biocatalysts for non-canonical substrates is constrained by the low throughput of conventional activity-based screening. Here, we developed a growth-coupled selection platform linking intracellular D-amino acid biocatalytic activity to ammonia generation and bacterial growth under nitrogen-limited conditions. Poor intracellular transport of non-canonical substrates initially prevented effective growth selection. Systematic optimization of eight amino acid transporters, substrates, and IPTG concentrations identified AroP as effective for aromatic α- and β-amino acids, while BrnQ, Mtr, and TnaB provided substrate-specific transport. Structure-guided engineering of bovine D-aspartate oxidase (BtDDO) and D-amino acid aminotransferase from Bacillus sp. 168 (BsDATA) was performed. For BtDDO, screening 16 active-site residues identified R278A with 0.4–0.8 U/mg activity toward β-phenylalanine and derivatives. Subsequent saturation mutagenesis generated R278A/Y223F with 1.2–1.8 U/mg activity toward β-phenylalanine and derivatives, and R216L/R237F with 0.9–1.2 U/mg activity toward several α-aromatic and hydrophobic substrates. For BsDATA, 12 substrate-pocket residues were individually screened, identifying V33G, T241G, N151A, and T204A with improved activity toward most tested α-D-amino acids; T241G additionally showed 12.5-fold improved activity toward glufosinate. Subsequent saturation mutagenesis generated T241G/N151A, improving activity toward most tested α-D-amino acids, with the highest enhancement reaching 34-fold toward D-2-naphthylalanine. This platform enables scalable genotype-to-phenotype selection and rapid evolution of stereoselective biocatalysts.

**Key words:** Growth Selection; Enzyme Engineering; D-Amino Acids; BtDDO; BsDATA

**Representative publication:**

1. Israr M., Basheer F., Zhou Y., Zeeshan M., Ali F., Wu S.*, Recent advances in protein engineering and synthetic applications of amino acid oxidases, Bioresource Technology, 2026, 135229.

2. Israr M.*, Basheer F., Wu S., Wen S., Ju J.*, Engineering the entryway corridor of alanine racemase enhances biocatalytic performance, Molecular Catalysis, 2026, 601, 116127.

3. Tariq M., Israr M.*, Raza M., Ahmad B., Azizullah A., Ur Rehman S., Yuan Q.*, Engineering of phenylalanine dehydrogenase from *Thermoactinomyces intermedius* for the production of a novel homoglutamate, PLOS ONE, 2022, 17, e0263784.

4. Xu S., Chu M., Zhang F., Zhao J., Zhang J., Cao Y., He G., Israr M.*, Ju J.*, Enhancement in the catalytic efficiency of D-amino acid oxidase from *Glutamicibacter protophormiae* by multiple amino acid substitutions, Enzyme and Microbial Technology, 2023, 166, 110224.

5. Israr M., Lv G., Xu S., Li Y., Ding S., Zhao B., Ju J.*, Biochemical characterization and mutational analysis of alanine racemase from *Clostridium perfringens*, Journal of Bioscience and Bioengineering, 2019, 128, 149–155.

## Biography



Dr. Muhammad Israr is a protein engineer and molecular enzymologist with expertise in structure-guided protein engineering, directed evolution, high-throughput enzyme screening, synthetic biocatalysis and computational approaches. He obtained his PhD in Biochemistry and Molecular Biology from Hebei Normal University, China (2019). His PhD research focused on exploring the catalytic mechanism and properties of D-amino acid metabolic enzymes. He served as an Assistant Professor at the Department of Biology, the University of Haripur, Pakistan (2020-2024). Currently, Dr. Israr is a Postdoctoral Fellow at the College of Life Science and Technology, Huazhong Agricultural University, China, under the mentorship of Professor Shuke Wu. He is the PI of the NSFC Project for International Young Scientists (RMB 400,000), focused on developing growth-selection systems for the directed evolution of amino acid-catalyzing enzymes. He has authored 25+ scientific publications in reputed international journals, such as *Bioresource Technology, Molecular Catalysis, ChemCatChem, International Journal of Biological Macromolecules* and *Enzyme and Microbial Technology*. His current research focuses on the development of high-performance biocatalysts for non-canonical amino acid synthesis.

# Development of an EvolvR-Based Continuous Evolution System in *Yarrowia lipolytica*

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## Abstract

Directed evolution is a powerful strategy for generating functional genetic variants without requiring detailed knowledge of structure–function relationships. CRISPR-based in vivo mutagenesis systems, such as EvolvR, enable continuous and targeted diversification of specific genomic regions. However, their application in non-conventional yeasts remains limited due to host-specific constraints. *Yarrowia lipolytica* is an attractive host for industrial biotechnology, yet efficient targeted *in vivo* evolution systems have not been well established in this organism. In this study, we aimed to establish an EvolvR-based continuous evolution platform in *Y. lipolytica* by optimizing key system components, including the fusion of nCas9 with a mutagenic DNA polymerase, to improve targeted mutagenesis efficiency. System performance was evaluated using a reporter system in which *URA3* function was restored through mutation of a premature stop codon. Our results demonstrate the feasibility of EvolvR-mediated targeted *in vivo* evolution in *Y. lipolytica*, providing a foundation for future applications in enzyme engineering and metabolic pathway optimization.

**Key words:** Continuous evolution, EvolvR, *Yarrowia lipolytica*

**Representative publication:**

## Biography

Dain Jang is a graduate student in the Department of Chemical and Biological Engineering at Seoul National University. Her research focuses on synthetic biology and metabolic engineering of *Yarrowia lipolytica*, with particular interests in developing *in vivo* directed evolution systems for efficient strain and enzyme engineering. Her current work involves establishing an EvolvR-based continuous evolution platform in *Y. lipolytica* and improving its mutagenesis efficiency and applicability for metabolic pathway engineering.



# De novo synthesis of sakuranetin by metabolic engineering of *E. coli*

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## Abstract

As a high-value methoxylated flavanone and potent phytoalexin, sakuranetin exhibits remarkable antioxidant, anti-inflammatory and antimicrobial effects. However, its limited natural abundance and the environmental challenges associated with chemical synthesis necessitate a sustainable production alternative. In this study, we established a highly efficient *Escherichia coli* cell factory for the de novo production of sakuranetin from simple carbon sources. Initially, we built a five-step heterologous pathway that includes *RgTAL*, *At4CL*, *PhCHS*, *MsCHI*, and *PfOMT3*. To overcome precursor limitations, systemic genomic remodeling was performed to reinforce L-tyrosine flux by deleting *tyrR*, *pheA*, *ptsG*, and *yddG*, *adhE*, *pykF*, *ascF*, alongside the overexpression of $tyrA^{fbr}$, $aroG^{fbr}$, *ppsA*. To reduce the metabolic burden and the toxicity of the intermediate p-coumaric acid, a microbial co-culture system was implemented, which successfully redistributed metabolic flux and increased the titer to 87.47 mg/L. Further enhancement of malonyl-CoA availability through *accABCD* knock-in and *fumC* deletion, coupled with the optimization of trace elements ($Mg^{2+}$, $Fe^{2+}$, $Ca^{2+}$), ultimately boosted the sakuranetin titer to 131.46 mg/L. To our knowledge, this 23.5-fold increase over the initial strain represents the highest reported titer for de novo sakuranetin production in *E. coli* to date. This study establishes a robust and green biomanufacturing platform for the sustainable supply of high-value dietary flavonoids.

**Key words:** *Escherichia coli*, sakuranetin, metabolic engineering, microbial co-culture

**Representative publication:** no relevant publications yet.

## Biography

Currently pursuing a master of science degree in Food Science and Engineering at South China University of Technology under the guidance of Professor Wu Xiaoling. My primary research interests are in synthetic biology and metabolic engineering, and my research project focuses on the biosynthesis of sakuranetin.



# An In Vivo-Guided Optimization Framework for Efficient Cell-Free Protein Synthesis and Biomanufacturing

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## Abstract

Cell-free protein synthesis (CFPS) enables rapid, flexible biomanufacturing but remains limited by cost and productivity. We developed an in vivo-guided framework that jointly optimizes reaction composition, the extract-preparation medium, and template codon usage. Principal-component analysis and response-surface methodology identified ATP, UTP, and folinic acid as key reaction variables; optimization increased protein expression 1.5-fold and protein production per unit cost 4.6-fold. Screening commercial nitrogen sources in 2 × YTPG identified a yeast extract that increased CFPS activity 1.4-fold. Proteomic and metabolomic analyses linked this improvement to remodeling of thiamine, energy, amino-acid, nucleotide, and osmolyte metabolism. Template engineering further showed that codon adaptation index, rather than codon harmonization or sensitivity, was the dominant determinant of CFPS output, providing an additional 1.1-fold increase. The optimized system enhanced expression of six fluorescent or chromoproteins by more than 1.6-fold and improved enzyme-enabled natural-product synthesis. Vanillin production increased by 54.1% using an Fcs-Ech cascade, and combining lysate optimization with host engineering raised production to 2.9-fold of the original system. These results establish a systematic strategy for improving CFPS efficiency and expanding its application in metabolic engineering and biomanufacturing.

**Key words:** cell-free protein synthesis, metabolic engineering, medium optimization, codon adaptation, in vitro biomanufacturing

**Representative publication:**

1. Liao C., Li H., Zheng H., Li C., Liu L., Wang J., Ni J. Optimization and Application of the Purified Cell-Free System. ChemBioChem, 2025, 26(11), e202500176.

2. Wang J., Ouyang X., Meng S., Zhao B., Liu L., Li C., Li H., Zheng H., Liu Y., Shi T. Rational multienzyme architecture design with iMARS. Cell, 2025, 188(5), 1349-1362.e17.

3. Liu L., Wang X., Wu Y., Li H., Wang J., Li C., Zheng H., Ni J. Chemoenzymatic platform with coordinated cofactor self-circulation for lignin valorization. Nature Synthesis, 2025, 4(9), 1151-1160.

4. Wang J., Ouyang X., Meng S., Li J., Liu L., Li C., Li H., Zheng H., Liao C., Zhao Y.-L., Ni J. Semi-rational design of an aromatic dioxygenase by substrate tunnel redirection. iScience, 2025, 28(1), 111570. https://doi.org/10.1016/j.isci.2024.111570.

5. Li C., Zheng H., Li Y., Liu L., Wang J., Li H., Zhang Y., Liao C., Ni J. Facilitated channeling of fixed carbon and energy into chemicals in artificial phototrophic communities. Journal of the American Chemical Society, 2025, 147(6), 4707-4713.

## Biography



Hengrun Li is a doctoral researcher in the School of Life Sciences and Biotechnology at Shanghai Jiao Tong University. His research focuses on cell-free protein synthesis, synthetic biology, enzyme engineering, and in vitro biomanufacturing. He develops cost-effective CFPS systems by integrating reaction engineering, culture-medium optimization, codon design, multi-omics, and quantitative screening, and applies these platforms to rapid protein expression and cell-free synthesis of value-added natural products. His collaborative research spans purified cell-free systems, semi-rational redesign of aromatic dioxygenases through substrate-tunnel engineering, coordinated cofactor self-circulation for chemoenzymatic lignin valorization, rational construction of multienzyme architectures, and synthetic phototrophic communities that channel fixed carbon and energy into chemicals. These efforts aim to connect mechanistic enzyme optimization with modular biosynthetic systems for more efficient and sustainable production of proteins, aromatic compounds, and other value-added chemicals.

# Transcriptome and lipidome profiling reveal phospholipid remodeling in diterpenoid-producing yeast

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## Abstract

Plant cytochrome P450 monooxygenases (CYPs) catalyze key oxidations in specialized metabolism but often underperform in heterologous hosts, where their activity is strongly influenced by the surrounding membrane microenvironment. Among membrane components, phospholipids represent major structural determinants that modulate membrane fluidity, curvature, and interactions of membrane-bound proteins. Here, we demonstrate that targeted remodeling of phospholipid composition enhances the activity of plant CYPs reconstituted in *Saccharomyces cerevisiae*, achieved through either precursor supplementation or overexpression of phospholipid biosynthetic genes. We show that choline supplementation improves CYP-dependent oxidation, whereas ethanolamine and inositol feeding produce mild inhibitory effects. Consistent with precursor supplementation, overexpression of phosphatidylcholine biosynthetic genes also enhances CYP oxidation efficiency. These effects are corroborated by integrated omics analyses revealing that subtle shifts in the PC:PI balance improve CYP catalytic activity. Moreover, transcriptomic profiling indicates that phospholipid gene overexpression induces global transcriptional repression alongside mild, consistent upregulation of sterol biosynthetic genes. Overall, this study demonstrates that membrane lipid dynamics modulates CYP efficiency in heterologous hosts, providing a broadly applicable strategy to optimize CYP-dependent plant pathways reconstructed in microbial systems.

**Key words:** cytochrome P450 (CYP); membrane homeostasis; phospholipid remodeling; lipid trafficking; metabolic engineering

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

1. Jin Z., Dong Y., Rafi A.M., Patwary M., Xu C., Raadam M.H., G. de Boer C., Ignea C., Unraveling the regulatory dynamics of bidirectional promoters for modulating gene co-expression and metabolic flux in *Saccharomyces cerevisiae, Nucleic Acid Research,* 53, DOI: 10.1093/nar/gkaf511.

2. Sofianovich O., Willis-Urena K., Dong Y., Ignea C., Bioengineered yeast for preventing age-related diseases, *Trends in Biotechnology (Cell Press)*, 43(3):586-600.

3. Iram A., Dong Y., Ignea C., Synthetic biology advances towards a bio-based society in the era of artificial intelligence, *Current Opinion in Biotechnology,* 87:103143.

4. Bureau JA., Escobar M., Dong Y., Ignea C., Engineering yeast for production of plant terpenoids using synthetic biology approaches, *Natural Product Reports (RSC),* 40(12):1822-1848.

5. Jin Z., Vighi A., Dong Y., Bureau J-A., Ignea C., Engineering membrane architecture for biotechnological applications, *Biotechnology Advances*, 64:108118.

## Biography

Yueming Dong completed her undergraduate studies in Bioengineering at McGill University and is currently a PhD student in Bioengineering at McGill University. Her research focuses on synthetic biology, metabolic engineering, and computational approaches for engineering yeast cell factories, with particular interests in enzyme engineering, membrane-associated biosynthetic pathways, and the production of plant-derived terpenoids.



# Design of the biosensor-dependent coupling system stabilizes the high-synthesis phenotype of cell factory

Lei Qin[1,2,3,4,5#], Bo Yang[6#], Rui Huang[7#], Haocheng Zhang[1,2,3], Qiuyang Li[7], Xinyi Li[7], Yingqi Tan[1,2,3], Wentao Sun[1,2,3], Haiyang Jia[7], Bing Hu[7], Bo Lv[7*], Chun Li[1,2,3,4,5,6,7*]

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## Abstract

Microbial cell factories offer a sustainable route to plant-derived natural products, but yield drift and strain degeneration persist. Growth-coupled biosynthesis can continuously enrich high producers, yet specific product-responsive biosensors remain scarce and their population-level effects are unclear. Here, we describe a rapid transcriptome-mining workflow that, as proof-of-concept, delivers yeast biosensors for glycyrrhetinic acid and medicarpin. By fine-tuning *PDR5* promoter, we expand the dynamic range of the glycyrrhetinic acid sensor and wire it to an essential gene, establishing a growth-addiction circuit that increases titer by 46.8 % after subculture. Single-cell transcriptome reveals that the evolved strain population exhibits a completely different division of labor compared to the initial strain. Coupling does not eliminate phenotypic heterogeneity; instead, it amplifies a dedicated sub-population marked by discrete transcriptional

signatures. Deletion of genes highly expressed in non-producing cells or enrichment of high-producing cell clusters can further boost population-level production. This study provides both a generalizable biosensor-discovery platform and single-cell-guided strategies for stabilizing and optimizing natural-product cell factories.

**Key words:** biosensor; growth-synthesis coupling; glycyrrhetinic acid; single-cell transcriptomics

**Representative publication:**

Qin, L., Yang, B., Huang, R. et al. Design of the biosensor-dependent coupling system stabilizes the high-synthesis phenotype of cell factory. Nat Commun 17, 5081 (2026).

## Biography

I am a Ph.D. student at the School of Chemistry and Chemical Engineering, Beijing Institute of Technology, focusing on metabolic engineering and synthetic biology.



# Establishing cell suitability for high-level production of licorice triterpenoids in yeast

Meiling Zhang ( 张美玲 )[a], Qin Lei ( 秦磊 )[b], Wentao Sun ( 孙文涛 )[b] , Chun Li ( 李春 )[a, b*]

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## Abstract

Yeast has been an indispensable host for synthesizing complex plant-derived natural compounds, yet the yields remained largely constrained. This limitation mainly arises from overlooking the importance of cell and pathway suitability during the optimization of enzymes and pathways. Herein, beyond conventional enzyme engineering, we dissected metabolic suitability with a framework for simultaneously augmenting cofactors and carbon flux to enhance the biosynthesis of heterogenous triterpenoids. We further developed phospholipid microenvironment engineering strategies, dramatically improving yeast's suitability for the high performance of endoplasmic reticulum (ER)-localized, rate-limiting plant P450s. Combining metabolic and microenvironment suitability by manipulating only three genes, NHMGR (NADH-dependent HMG-CoA reductase), SIP4 (a DNA-binding transcription factor) and GPP1 (Glycerol-1-phosphate phosphohydrolase 1), we enabled the high-level production of 4.92 g/L rare licorice triterpenoids derived from consecutive oxidation of β-amyrin by two P450 enzymes after fermentation optimization. This production holds substantial commercial value, highlighting the critical role of establishing cell suitability in enhancing triterpenoid biosynthesis and offering a versatile framework applicable to various plant natural product biosynthetic pathways.

**Key words:** Cell suitability, Phospholipid microenvironment, Cytochrome P450, Triterpenoids, *Saccharomyces cerevisiae*

**Representative publication:**

Sun W, Wan S, Liu C, Wang R, Zhang H, Qin L, Wang R, Lv B, Li C*.Establishing cell suitability for high-level production of licorice triterpenoids in yeast, Acta Pharmaceutica Sinica B, 2024, 14(9): 4134-4148.

## Biography

Meiling Zhang is a Master's student at Xinjiang University since 2024, focusing on the development and application of yeast cell factories for plant-derived natural products.



Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# Precursor Engineering and $^{13}C$ Flux Analysis Reveal Fatty Acid Recycling as a Control Point for Cyanobacterial Alkane Biosynthesis

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## Abstract

Photosynthetic conversion of $CO_2$ into drop-in hydrocarbons offers a sustainable alternative to fossil fuels, but alkane titers in engineered cyanobacteria remain constrained by limited acyl-ACP precursor supply. We engineered *Synechococcus elongatus* PCC 11802 by expressing acyl-ACP reductase (AAR) and aldehyde-deformylating oxygenase (ADO) to establish the hydrocarbon pathway, coupled with acyl-ACP synthetase (AAS) overexpression to reactivate free fatty acids back into the acyl-ACP pool. Under identical culture conditions, the best-performing AAS-dosage strain achieved a 1.58-fold increase in alkane titer (4.43 mg/L) relative to the AAR/ADO only control, incurring only a ~7% reduction in biomass while yielding a ~70% higher specific alkane productivity (titer per unit biomass), suggesting that AAS-mediated precursor recycling enhances alkane output with limited impact on growth. These results identify fatty acid recycling efficiency as a quantitatively tunable control point for alkane productivity. Future work will apply $^{13}C$ metabolic flux analysis and intracellular metabolite profiling to directly resolve carbon partitioning among fatty acid synthesis, membrane lipid biogenesis, and alkane formation, and to determine whether this efficiency gain reflects reduced competition for acyl-ACP, alongside dynamic AAS regulation and outdoor cultivation trials to assess scalability for sustainable, light-driven hydrocarbon fuel production from $CO_2$.

**Key words:** Metabolic engineering, *Synechococcus elongatus* PCC 11802, Alkane biosynthesis, Fatty acid recycling, Carbon flux

**Representative publication:** Manuscript under preparation and will be submitted soon

## Biography



I am a Ph.D. candidate in the Department of Chemical Engineering at the Indian Institute of Technology (IIT) Bombay, working under the guidance of Prof. Pramod Wangikar. My research sits at the interface of metabolic engineering, synthetic biology, and systems biology, with a focus on engineering fast-growing cyanobacteria as photosynthetic cell factories for converting $CO_2$ into value-added chemicals and fuels. Specifically, my work involves enhancing succinate and alkane production through pathway and promoter engineering, CRISPR-based genome editing, metabolomics, and $^{13}C$ metabolic flux analysis. By combining experimental and computational approaches, I aim to uncover metabolic bottlenecks and design rational strategies to improve carbon partitioning and product titers.

I hold a Master's degree in Biotechnology from Gulbarga University and a Bachelor's degree in Life Sciences from the University of Delhi. My broader research interests span metabolic and synthetic biology, systems-level flux analysis, and industrial biotechnology. I have presented my work at national scientific forums and am currently preparing manuscripts for submission to peer-reviewed international journals. My long-term vision is to advance sustainable biomanufacturing by engineering photosynthetic microorganisms for the carbon-neutral production of fuels and platform chemicals.

# Hierarchical modular rewiring of yeast sterol metabolism for high-titer de novo cholesterol biosynthesis by resolving multi-layer metabolic bottlenecks

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## Abstract

Cholesterol serves as an essential membrane constituent and steroid precursor for pharmaceutical and fine-chemical manufacturing. Conventional animal-tissue extraction and chemical semi-synthesis are intrinsically limited, positioning microbial biosynthesis as a sustainable alternative. *De novo* microbial cholesterol production is constrained by complex sterol cascades, tight sterol homeostasis, and sterol-mediated cytotoxicity. Here, using *Saccharomyces cerevisiae* as chassis, we developed a hierarchical modular metabolic engineering strategy to overcome multilayer limitations. Heterologous pathway reconstruction plus promoter remodeling redirected native sterol flux from ergosterol to cholesterol. Enlarged lipid-droplet compartments enhanced cholesterol storage, while deletion of sterol-sensitive transcriptional repressors relieved post-squalene flux constraints. Central-carbon rewiring combined with rate-limiting enzyme overexpression yielded a high-performance strain. Fed-batch culture in a 5-L bioreactor attained a peak cholesterol titer of 6.23 g/L at 126 h, with a glucose yield of 0.015 g/g and specific cellular productivity of 0.025 g/L/OD—the highest yeast cholesterol titer reported to date. Triple bottom-line evaluation confirmed superior economic, environmental, and social sustainability relative to conventional routes. This work establishes a systematic modular engineering pipeline and robust microbial chassis enabling eco-friendly, large-scale production of cholesterol and valuable sterol intermediates.

**Key words:** Cholesterol; Saccharomyces cerevisiae; Sterol biosynthesis; Metabolic engineering

**Representative publication:**

1. Han Y.C., Huang Y. W., Israr M., Li H. H.*, Zhang W. Y.*, Advances in biosynthesis of 7-dehydrocholesterol through *de novo* cell factory strategies, Bioresour. Technol, 2025, 418, 131888.

2. Jiang Y. Q., Sun Z. J., Lu K. X., Wu Z. Y., Xue H. L., Zhu L., Li G. S., Feng Y., Wu M. B., Lin J. P.*, Lian J. Z.*, Yang L. R., Manipulation of sterol homeostasis for the production of 24-epi-ergosterol in industrial yeast, Nature Communications, 2023, 14, 437.

3. Liu W., Dong X. F., Li H. B., Gong J. S., Zhang X. M., Shi J. S., Xu Z. H., Koffas M.A.G.*, Li H.*, Rewiring metabolism and restoring sterol homeostasis for overproduction of campesterol in *Saccharomyces cerevisiae*, Chem. Eng. J, 2025, 515, 163686.

4. Chen, Q. H., Wei, W. Q., Chao, Z. K., Qi, R., He, J. H., Chen, H. T., Wang, K., Wang, X. L., Rao, Y. J., Zhou, J. W.*, Electron transfer engineering of artificially designed cell factory for complete biosynthesis of steroids, Nature Communications, 2025, 16(1), 3740.

## Biography



Yuhui Cheng is a fourth-year Ph.D. candidate at the State Key Laboratory of Bioreactor Engineering, School of Biotechnology, East China University of Science and Technology (ECUST). She is enrolled in the combined Master-PhD program and pursues her doctorate under the supervision of Prof. Fengqing Wang, with expected graduation in 2027. She obtained her B.S. degree from Shanghai Normal University. Her research focuses on de novo biosynthesis of high-value steroid compounds via metabolic and enzyme engineering to enhance product titers and lay foundations for industrial application. She has participated in multiple national synthetic-biology research programs. Her cholesterol-biosynthesis work has generated filed patents and one manuscript currently under review. She has established systematic modular engineering strategies that achieve the highest reported *de novo* cholesterol titer in yeast, providing a sustainable microbial chassis for steroid biomanufacturing.

# Engineering High-Temperature Biosynthetic Pathway for Polylactic Acid Production

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b. Carbon-Negative Synthetic Biology for Biomaterial Production from CO2 (CNSB), Asia Pacific Graduate Institute of Shanghai Jiao Tong University, 1 CREATE Way, Singapore 138602. (上海交通大学新加坡研究生院).

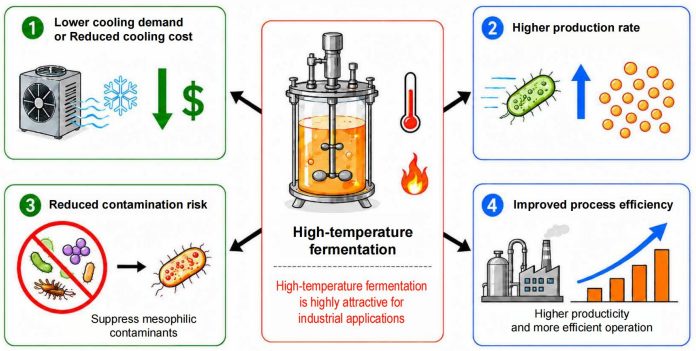
c. Shanghai Artificial Intelligence Laboratory, Shanghai 200030, China. (中国上海人工智能实验室)

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## Introduction

High-temperature biomanufacturing offers a promising strategy for cost-effective and efficient chemical production. Compared with conventional fermentation, elevated-temperature processes can reduce cooling requirements, suppress mesophilic contamination, and improve overall productivity, thereby lowering production costs. Polylactic acid (PLA) is an important biodegradable polymer widely used in sustainable materials; however, its production mainly relies on chemical synthesis, while microbial biosynthesis under high-temperature conditions remains unexplored. Here, we aim to develop a high-temperature intracellular PLA biosynthesis strategy to advance sustainable biomanufacturing.

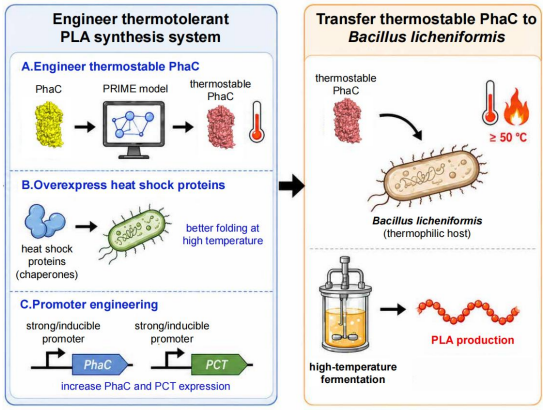
**Industrial Advantages of High-Temperature Fermentation**




## Workflow

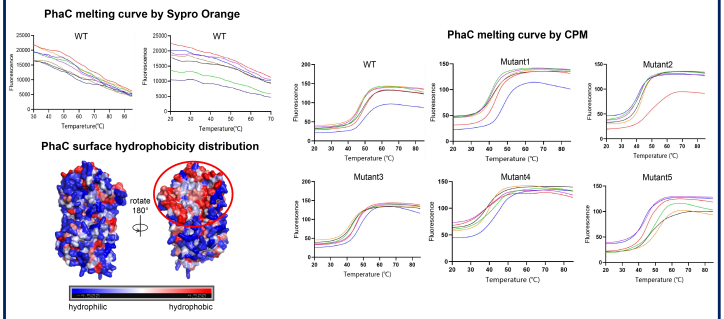
Firstly, PRIME-guided protein engineering is being applied to enhance the thermostability of PhaC in *Escherichia coli*, aiming to maintain catalytic activity under elevated-temperature conditions. In parallel, complementary strategies, including heat shock protein overexpression and promoter optimization of key PLA biosynthetic enzymes (PhaC and PCT), are being explored to further improve high-temperature PLA biosynthesis. Ultimately, the optimized thermostable PhaC will be transferred into the thermophilic host *Bacillus licheniformis* to establish a robust platform for high-temperature PLA production.

**Workflow of PLA high-temperature biosynthesis**

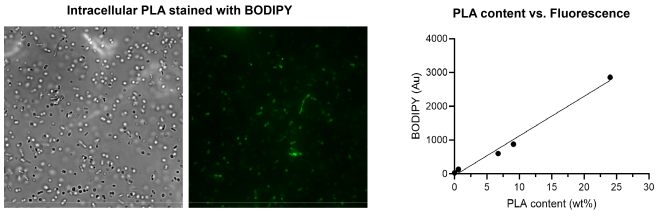



## Results

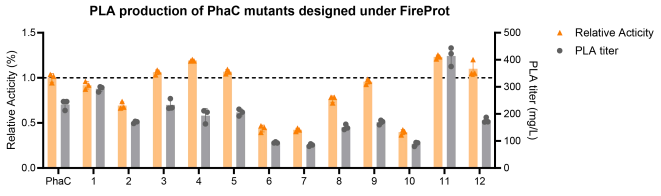
**Key result 1:** Using the universal hydrophobic dye SYPRO Orange, differential scanning fluorimetry (DSF) was performed to determine the melting temperature (Tm) of PhaC. The melting curve exhibited a decreasing trend with increasing temperature, which is attributed to the presence of a hydrophobic patch at the N-terminus of PhaC that directly interacts with the dye. In contrast, when the thiol-reactive dye CPM was used in DSF, a sigmoidal melting curve was obtained, enabling high-throughput Tm determination for PhaC mutants.




**Key result 2:** We have successfully established a PLA biosynthesis system in *E. coli*, achieving a PLA content of approximately 4 wt%. Staining with BODIPY dye revealed distinct green fluorescent PLA granules within the cells. Flow cytometry analysis demonstrated a good linear correlation between BODIPY fluorescence intensity and PLA content.




**Key result 3:** From the FireProt web server, we selected 12 single-point mutants on the basis of structural and evolutionary information. These mutants were evaluated for PLA production via shake-flask fermentation, and their relative activities were calculated by comparing the PLA content to that of the wild-type. Among them, 5 positive hits were identified. These results will serve as preliminary input for the protein language model PRIME to drive AI-guided protein engineering.




## Conclusion

1. We stablished a high-throughput Tm screening method for PhaC mutants and validated a dye-based PLA detection method.
2. A PLA biosynthesis system in *E. coli* was constructed for mutant-production evaluation.
3. 12 single-point mutants designed under FireProt will serve as pre-input for PRIME to facilitate AI-guided engineering.

This research is supported by the National Research Foundation, Prime Minister's Office, Singapore under its Campus for Research Excellence and Technological Enterprise (CREATE) programme (CNSB).

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Development of a DNA-GPT–Machine Learning Framework for Promoter Generation and Gene Expression Prediction in Bacteria

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## Abstract

The transformer architecture uses self-attention to capture long-range dependencies in sequential data, while Generative Pre-trained Transformer (GPT) models extend this framework through large-scale self-supervised pretraining to learn contextual representations that support effective sequence modeling, prediction, and adaptation to diverse downstream tasks. A promoter database was assembled from curated promoter sequences in RegulonDB and the Anderson promoter library for Escherichia coli, curated transcription starting site (TSS) data from Methylosinus sporium 5, and predicted promoter sequences from *Methylotuvimicrobium alcaliphilum* 20Z and *Methylocystis* sp. MJC1. These data were used to train a GPT model to generate novel promoter sequences. To estimate the transcriptional strength of the generated promoters, a machine learning (ML) model was developed using dTomato and sfGFP fluorescence data obtained by fluorescence-activated cell sorting (FACS). The GPT model generated 10,000 promoter sequences, of which 439 were identified as potentially novel promoters. An ML predictive model was then built based on 62 fluorescence measurements obtained using the constructed pAWP89 vector. The ML model screened 7,902 promoter–RBS–reporter combinations generated from 439 promoters, three RBSs, two reporter genes, and one terminator in the pAWP89 vector, and suggested candidate promoters for experimental validation. This study expands methanotroph promoter resources for precise heterologous expression.

**Keywords:** Transformer GPT model, machine learning, promoter sequence, FACS, methanotrophs.

**Representative publication:**

1. Pham, K.N., Lee, E.Y. Development of a semi-endogenous PRT-based expression system for *Methylotuvimicrobium alcaliphilum* and its application to enhance 3-hydroxybutyric acid production from methanol. *J Biol Eng* 20, 5 (2026). https://doi.org/10.1186/s13036-025-00592-y

2. Pham, K.N., Lee, E.Y. Hybrid metabolic engineering enables xylose-driven co-production of polyhydroxybutyrate and violacein in Escherichia coli. J Biol Eng 20, 66 (2026). https://doi.org/10.1186/s13036-026-00653-w

3. Lee, G.M., Pham, K.N., Bang, I. et al. Integrated genomic and transcriptomic Insights into methanol tolerance mechanisms in *Methylobacterium extorquens* AM1, identifying key targets for strain engineering. J Biol Eng 20, 2 (2026). https://doi.org/10.1186/s13036-025-00557-1

## Biography



Khoi Nhat Pham is a PhD candidate student in the Department of Chemical Engineering at Kyung Hee University, Republic of Korea. His research focuses on metabolic engineering, synthetic biology, and genetic tool development for microorganisms, particularly methanotrophs. His work includes the development of promoter systems, metabolic engineering strategies, and computational approaches for optimizing heterologous gene expression. He has also applied machine learning and generative artificial intelligence to biological sequence design, including the generation and prediction of functional promoter sequences. His current research interests include developing computational and experimental frameworks for designing genetic elements and improving the production of value-added compounds in microbial hosts.

# Dynamic Metabolic Engineering of *Komagataella phaffii* for cis, cis-Muconic Acid Production from Methanol

Chuanling Zhan(詹传玲) [ab], Fei Tao(陶飞) [abc], Ping Xu(许平) [ab]

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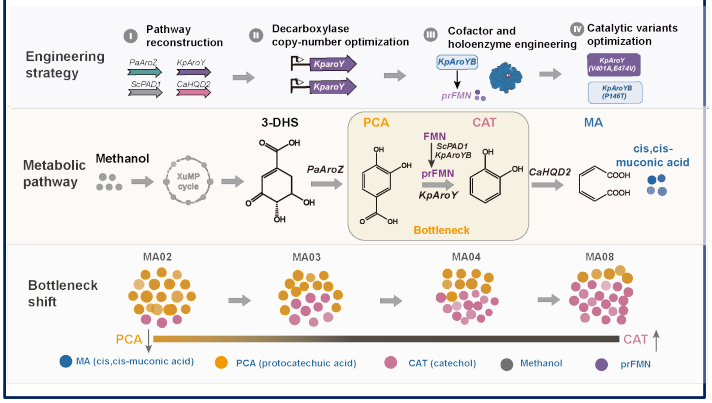
b. Carbon-Negative Synthetic Biology for Biomaterial Production from $CO_2$ (CNSB), Campus for Research Excellence and Technological Enterprise (CREATE), 1 CREATE Way, Singapore, 138602, Singapore.

c. State Key Laboratory of Synergistic Chem-Bio Synthesis, Shanghai Jiao Tong University, Shanghai 200030, China. *E-mail: cl_zhan@sjtu.edu.cn

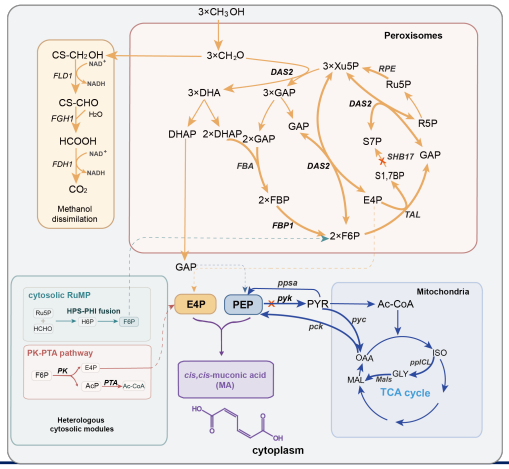
## Introduction

cis, cis-Muconic acid (MA) is a versatile platform dicarboxylic acid for bio-based adipic acid, terephthalate substitutes and degradable polymers. Current microbial MA production mainly relies on sugar feedstocks, which increases cost and competes with food resources. Methanol, which can be produced from $CO_2$ using renewable energy, provides an attractive C1 substrate for sustainable biomanufacturing. Here, we explored *Komagataella phaffii* as a methanol-based chassis for MA production. A heterologous MA pathway was established and subsequently optimized by addressing intermediate accumulation and introducing controllable pathway regulation. This poster highlights representative engineering steps that improve PCA conversion, enable blue-light-responsive control and strengthen terminal catechol conversion, revealing key bottlenecks in methanol-to-MA biosynthesis.

## Flux Rewiring and Pathway Optimization in *K. phaffii*

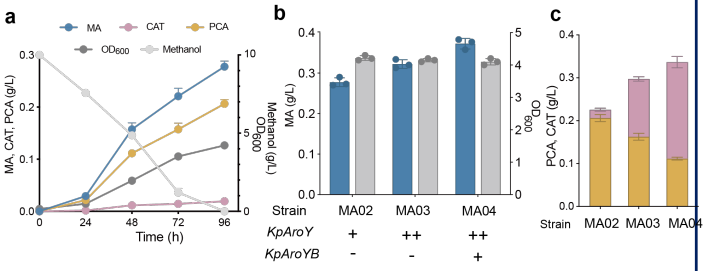



## Carbon flux design for methanol-to-MA biorefinery

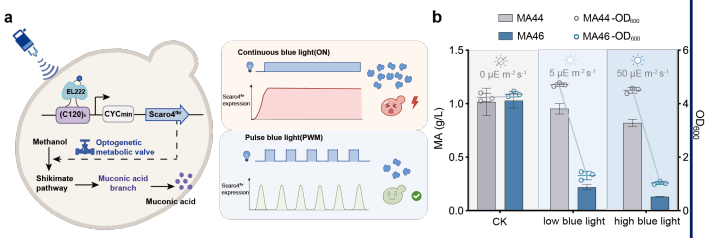



## Results and Discussion

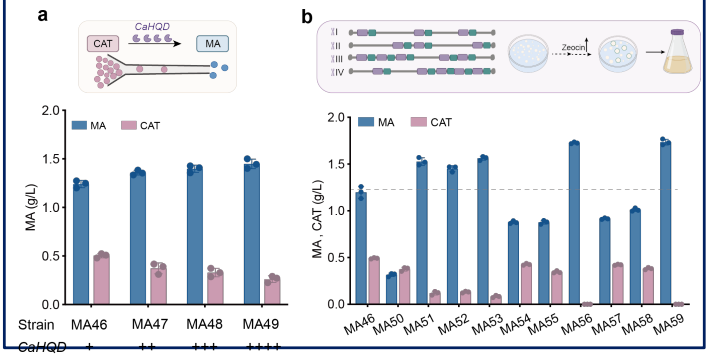
**Key result 1**: Establishing the methanol-to-MA pathway and identifying a downstream bottleneck. Increasing the dosage of the PCA decarboxylation module and supplying its accessory factor further improved MA formation. At the same time, PCA accumulation decreased whereas catechol became more prominent, indicating that enhanced PCA conversion shifted the principal bottleneck toward the terminal catechol-to-MA step.




**Key result 2:** Introducing optogenetic control to balance pathway activity and cell growth. An EL222-based blue-light switch was coupled to *ScAro4*$^{fbr}$, enabling light-dependent regulation of pathway entry. Different illumination intensities markedly affected MA production and biomass, demonstrating that optogenetic regulation provides a tunable strategy for dynamic flux control.




**Key result 3:** Relieving the terminal catechol-conversion bottleneck. Increasing the copy number of *CaHQD2* enhanced MA production while reducing catechol accumulation. Subsequent screening of multi-copy strains identified variants with further improved MA production, highlighting enhanced catechol conversion as an effective strategy for strengthening the terminal pathway.




## Conclusion

1.The biosynthetic pathway for cis, cis-muconic acid (MA) was successfully established in *K. phaffii* using methanol as the sole carbon source.
2.Optogenetic regulation enabled dynamic control of pathway activity and improved the balance between cell growth and MA production. accumulation.
3. Strengthening catechol-to-MA conversion relieved the terminal pathway bottleneck, achieving an MA titer of 1.73 g/L in shake-flask fermentation.

This research is supported by the National Research Foundation, Prime Minister's Office, Singapore under its Campus for Research Excellence and Technological Enterprise (CREATE) programme (CNSB).

# Codon Design Online (CoDOn): a Comprehensive Synthetic Gene Design Tool for Codon Optimization of Microbial and Mammalian Cell Factories

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[1] *School of Chemical Engineering, Sungkyunkwan University, 2066 Seobu-ro, Jangan-gu, Suwon 16419, Republic of Korea*
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## Abstract

Codon optimization for heterologous protein expression requires balancing multiple sequence-level criteria that may favor different synonymous solutions. To support this process, we developed Codon Design Online (CoDOn), a web-based platform for multi-objective and multi-parameter coding sequence design. CoDOn provides an interactive interface for defining host-specific codon references, selecting and parameterizing design criteria, and applying sequence constraints, while generating non-dominated sequence candidates for comparison. The platform also integrates graphical analyses to visualize trade-offs among objectives and support rational sequence selection. To demonstrate its applicability across diverse expression systems, we applied CoDOn to representative targets in Escherichia coli, Pichia pastoris, human embryonic kidney (HEK) cells, and Chinese hamster ovary (CHO) cells using curated and custom references. Case-specific objective sets were configured from available criteria, including individual codon usage (ICU), codon context (CC), codon adaptation index (CAI), overall and third-position GC content, and hidden stop codons. The resulting solutions illustrate how design priorities can be tailored to different chassis and products. This work was supported by the National Research Foundation of Korea (NRF) grant (RS-2024-00341312) funded by the Korea government (MSIT).

**Key words:** Codon optimization, Multi-objective sequence design, Recombinant protein production, Heterologous gene expression, Industrial biotechnology

**Representative publication:**

1. Moon J. H., Ang K. S., Lee D.-Y.*, Codon Design Online (CoDOn): a comprehensive web application of synthetic gene design with multi-criteria and multi-parameters for heterologous expression, BMC Bioinformatics, 2026, in press.

## Biography



Jehun Moon is a Ph.D. student in the School of Chemical Engineering at Sungkyunkwan University, Republic of Korea. His research focuses on computational codon engineering and synthetic gene design for heterologous protein expression across microbial and mammalian hosts. He has developed Codon Design Online (CoDOn), a web-based platform that integrates host-specific codon usage with multiple sequence design criteria through multi-objective optimization. His research explores the systematic design and evaluation of coding sequences by considering codon usage, codon context, sequence composition, and other biologically relevant constraints. His current interests include applying computational sequence design to recombinant protein production, industrial biotechnology, genetic vaccines, and gene therapy.

||SHREE||

# Brown macroalgae Rags to Riches: Engineering Stress-Tolerant Yeast Issatchenkia orientalis for Sargassum Valorization

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## Abstract

Over the past decade, Sargassum blooms have proliferated at unprecedented scale, stranding along coastlines from the Caribbean to West Africa. Driven by rising ocean temperatures and nutrient pollution, these accumulations smother coral reefs, disrupt marine breeding grounds, and burden coastal economies. Yet this crisis is a bioeconomy opportunity: Sargassum is an abundant, land-independent, third-generation feedstock needing no agricultural inputs or freshwater.

Realizing this opportunity demands a microbial chassis able to handle Sargassum's compositional complexity. Its carbohydrates are dominated by alginate, alongside mannitol and minor sugars. Conventional microorganisms struggle with macroalgal hydrolysates: low pH, high salinity, and inhibitory byproducts. We engineer Issatchenkia orientalis, an acid- and stress-tolerant yeast, as a production host.

Our approach spans three fronts. First, I. orientalis is engineered to secrete alginate lyases, enabling saccharification directly in the fermentation vessel and eliminating costly upstream depolymerization. Second, we expand its metabolism to assimilate mannitol and DEHU, the primary alginate monomer. Third, we integrate these into a bioprocess run in saline water, not freshwater, cutting footprint and matching the feedstock's origin.

Together, these strategies establish a fermentation platform converting raw Sargassum biomass into biofuels, organic acids, and high-value products, turning a coastal nuisance into a renewable feedstock for sustainable chemical manufacturing.

**Key words:** Sargassum; Brown Macroalgae Valorization; Issatchenkia orientalis; Multi-stress tolerant yeast engineering.

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

Mantri K., Gallimore-Repole E., Alimpjevic M., Avalos J.*, Engineering Stress-Tolerant Yeast Issatchenkia orientalis for Sargassum Valorization, (In prep.)

1.

## Biography



Krishi is a fourth-year PhD student in the Department of Chemical and Biological Engineering at Princeton University. His research applies synthetic biology to develop new industrial processes for producing value-added chemicals and energy sources, with a current focus on engineering non-conventional yeast strains to convert Sargassum biomass into biofuels and high-value products. Krishi is broadly interested in metabolic engineering and hopes to ultimately design novel microbial strains for industrial-scale chemical production. Outside the lab, he enjoys following emerging biotech startups, watching movies, and swimming.

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Research Article

# Metabolic Engineering of *Saccharomyces cerevisiae* for High-Titer Ginsenoside Rh2 Production from Glycerol

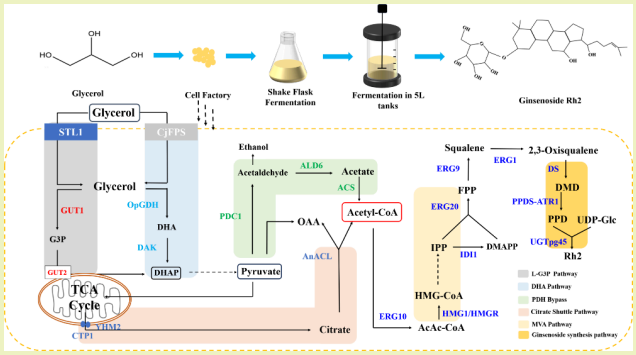
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**ABSTRACT:** As a byproduct of biodiesel production, glycerol is considered as a promising alternative feedstock for microbial fermentation processes. In this study, *Saccharomyces cerevisiae* was metabolically engineered to utilize glycerol as the carbon source for the efficient production of ginsenoside Rh2 (Rh2), isolated from *Panax ginseng* that exhibits significant antitumor and antioxidant activities. First, the biosynthesis of 124.67 mg/L protopanaxadiol (PPD), which serves as the aglycone of Rh2, from glycerol was achieved by introducing the heterologous glycerol utilization pathway ("DHA" pathway) and PPD biosynthesis pathway into *S. cerevisiae* BY4741. Subsequently, to enhance acetyl-CoA supply from glycerol, we engineered the "pyruvate dehydrogenase bypass" and the heterologous citrate cleavage pathway. While both resulting strains showed similar PPD production, metabolomic analysis indicated higher uridine diphosphate glucose accumulation, which serves as the glycosyl donor for Rh2 in the strain incorporating the citrate cleavage pathway. Therefore, the heterologous citrate cleavage pathway-engineered strain was selected, and the mevalonate pathway and PPD biosynthesis pathway in this strain were synergistically enhanced, resulting in a PPD production of 598.28 mg/L. Finally, by overexpressing glycosyltransferase and knocking out *EGH1*, a high-yielding Rh2 strain was constructed, achieving 700.53 mg/L in flasks and 2.88 g/L via fed-batch fermentation in a 5 L bioreactor, the highest reported titer to date. This study successfully demonstrates the metabolic engineering of yeast cell factories for high-efficiency biosynthesis of plant-derived saponin using glycerol as a nonfermentable carbon substrate.




**KEYWORDS:** *ginsenoside Rh2, Saccharomyces cerevisiae, glycerol, metabolic engineering, cell factory*

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## 1. INTRODUCTION

Ginsenosides, the core active constituents in *Panax ginseng*, exhibit significant biological activities including antitumor, anticancer, and antioxidant effects, demonstrating substantial application potential in pharmaceutical and cosmetic industries.[1] Ginsenoside Rh2 (Rh2) is a natural saponin extracted from red ginseng, with a yield of only 0.001%.[2] Rh2 belongs to the PPD-type terpenoid saponins and is generated through the glycosylation reaction of protopanaxadiol (PPD) catalyzed by glycosyltransferase. It is the principal metabolic product of other diol-type ginsenosides such as Ginsenoside Rb1, Rd, and Rg3 in the human intestine.[3] As PPD-type ginsenosides are most closely related to PPD, nearly all PPD-type ginsenosides are broken down into Rh2 in the intestinal tract. Therefore, compared to other types of ginsenosides, Rh2 is more easily absorbed and utilized by the human body.[4] However, their industrial-scale development faces critical challenges: (1) the complex molecular architectures and low natural abundance in ginseng pose significant challenges to the direct isolation process;[5] (2) conventional chemical hydrolysis methods struggle to efficiently obtain monomeric saponins;[3] (3) the increasing cultivation costs of *Panax ginseng* further limit the potential for its sustainable production.

In recent years, the rapid development of synthetic biology and metabolic engineering technologies has established microbial cell factories as a predominant strategy for ginsenoside production.[6,7] Compared with traditional methods for ginsenoside production, microbial biosynthesis in engineered cell factories offers distinct and comprehensive advantages: (1) it reduces reliance on medicinal plants with long growth cycles and can utilize inexpensive carbon sources such as glucose as raw materials;[8] (2) standardized fermentation conditions ensure stable and reproducible yield and quality, overcoming the challenge of compositional variability inherent in plant extraction;[9] (3) microbial systems enable the targeted synthesis of specific saponins, greatly

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simplifying downstream separation and purification steps, and possess unique potential for producing novel derivatives through engineering.[10]

*Saccharomyces cerevisiae*, a safe model microorganism with a well-characterized genetic background, mature gene-editing tools, and an intrinsic mevalonate (MVA) pathway,[11,12] has emerged as an ideal microbial host for terpenoid biosynthesis. Researchers have engineered *S. cerevisiae* strains by introducing genes that encode target terpenoid biosynthetic pathways in order to confer the ability to produce these compounds.[13,14] This is coupled with genome editing and metabolic engineering to optimize pathway fluxes, thereby achieving cost-effective and high-yield synthesis of natural products.[15,16] Zhou et al.[17] engineered the glycosyltransferase UGTpg45, screening a mutant variant (UGTpg45-HV) that showed a 40% increase in catalytic efficiency. Utilizing this mutant, they developed an *S. cerevisiae* strain, producing 2.25 g/L ginsenoside Rh2. However, the PPD in the engineered strain was still high. *S. cerevisiae* exhibits the Crabtree effect when glucose serves as the carbon source, thereby imposing significant metabolic challenges on the engineered Rh2-producing strain, including excessive accumulation of PPD and insufficient intracellular availability of uridine diphosphate glucose (UDPG).

In previous studies, we found that adding glycerol to the fermentation medium could effectively enhance the glycosylation efficiency of PPD and promote the synthesis of ginsenoside CK.[18] Concurrently, glycerol, a major byproduct of biodiesel production, has accumulated substantially due to the expansion of the industry.[19,20] Compared to conventional carbon sources like glucose, glycerol possesses a higher degree of reduction (C-molar reduction state: + 0.667 vs glucose +0.0),[21] theoretically enabling greater metabolic yields and thus presenting promising industrial applications.[22] Importantly, the use of glycerol as a substrate in *S. cerevisiae* for the biosynthesis of high-value-added products does not induce the Crabtree effect.[23] However, there is a lack of *S. cerevisiae* cell factories capable of efficiently synthesizing Rh2 directly from glycerol.

Wild-type *S. cerevisiae* strains typically exhibit limited glycerol utilization capabilities despite possessing endogenous glycerol catabolic pathways (FAD-dependent pathway), including glycerol kinase (GUT1) and mitochondrial glycerol-3-phosphate dehydrogenase (GUT2).[24,25] To fully exploit glycerol's higher reducing power (compared to sugars) for the production of reductive platform chemicals such as 1,2-propanediol and succinic acid, a synthetic NAD-dependent dihydroxyacetone (DHA) pathway was introduced into *S. cerevisiae*.[21,24] This pathway includes the heterologous aquaglyceroporin CjFPS1 from *Cyberlindnera jadinii*, glycerol dehydrogenase OpGDH from *Ogataea parapolymorpha*, and the endogenous DHA kinase DAK1.[21] The introduction of a heterologous DHA pathway combined with knockout of the glucose repression transcription factor gene *MIG1* significantly enhanced glycerol utilization in *S. cerevisiae*, resulting in an 82.2% increase in the production of the sesquiterpene bisabolene.[26] These findings demonstrate that strengthening the glycerol assimilation pathway in *S. cerevisiae* is an effective strategy for improving the synthesis efficiency of terpenoids.

In our previous study,[27] by introducing the DHA pathway and the glycosyltransferase UGT1 into the chassis with high efficiency in PPD synthesis, the engineered strain could utilize glycerol as the carbon source to efficiently synthesize ginsenoside CK.[15,27] However, since the starting chassis strain MVA5 was designed as an efficient PPD synthesis strain based on glucose and ethanol as substrates,[15] after modification, it hardly accumulates ethanol when glycerol is used as the substrate. Meanwhile, through transcriptome analysis, we found that the genes of the PDH bypass pathway in this strain were significantly down-regulated when glycerol was used as the substrate compared to when glucose was used.[27] In *S. cerevisiae*, the native cytoplasmic acetyl-CoA synthesis route termed the PDH bypass converts pyruvate to acetyl-CoA through sequential catalysis by pyruvate decarboxylase (PDC1), aldehyde dehydrogenase (ALD6), and acetyl-CoA synthetase (ACS).[28,29] Acetyl-CoA, serving as a common precursor in multiple intracellular metabolic pathways, plays a pivotal role in terpenoid biosynthesis when its flux into the mevalonate (MVA) pathway is optimized.[30] A landmark study by Zhao et al.[15] demonstrated that overexpression of the Salmonella-derived cytosolic $ACSse^{L641P}$ mutant boosted cytoplasmic acetyl-CoA availability, enabling engineered yeast to achieve a PPD titer of 614.3 mg/L, validating the effectiveness of PDH bypass modulation. This transcriptional repression directly limits cytosolic acetyl-CoA availability, creating a bottleneck that restricts the efficient biosynthesis of Rh2.

In conclusion, switching the carbon source of the existing high-yield strains to glycerol may encounter the following key bottlenecks: (1) The native capacity of *S. cerevisiae* to absorb and convert glycerol remains limited;[23] (2) Glycerol, being a nonfermentable carbon source, often exhibits constrained metabolic flux toward cytosolic acetyl-CoA, the essential precursor for terpenoid biosynthesis.[27] Therefore, in this study, a *S. cerevisiae* cell factory for the synthesis of PPD using glycerol as the carbon source was constructed through the introduction of the DHA pathway and the PPD biosynthetic pathway. The effects of the PDH bypass pathway and the citrate shuttle pathway on the synthesis of ginsenoside PPD were then systematically investigated, and metabolomic analysis revealed that yeast modified via the citrate shuttle pathway exhibited an enhanced suitability for the subsequent synthesis of Rh2. Through further glycosyltransferase module expression and fermentation process optimization, a Rh2 yield of 2.88 g/L was achieved, representing the highest yield reported to date. The findings of this study offer a valuable blueprint for *S. cerevisiae* to synthesize high-value natural terpenoids from glycerol.

## 2. MATERIALS AND METHODS

### 2.1. Strains, Reagents, and Medium

*S. cerevisiae* strain *BY4741* (*MATa; his3Δ1; leu2Δ0; met17Δ0; ura3Δ0*) was used as the chassis for the biosynthetic pathway assembly. *E. coli* DH5α (TransGen Biotech, Beijing) was used for gene cloning. Luria−Bertani (LB) broth with antibiotics (100 μg/mL ampicillin or 50 μg/mL kanamycin) was used for the cultivation of recombinant *E. coli*. YPD medium (1% yeast extract, 2% peptone, and 2.5% glucose) and YPG medium (1% yeast extract, 2% peptone, and 2.5% glycerol) were used for yeast cultivation. SC medium (0.67% yeast nitrogen base, 2% glucose, and proper amino acid dropout mix) was used for the selection of recombinant yeast strains. All restriction enzymes and T4 DNA ligase were purchased from Thermo Fisher (Thermo Fisher Scientific, USA). DNA polymerase was purchased from Vazyme (Nanjing, China), and primers were purchased from GENEWIZ (Suzhou, China). The In-fusion Cloning Kit was purchased from UE (US Everbright Inc., Suzhou, Jiangsu, China).


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### 2.2. Molecular Cloning and Strain Construction

Strains used in this study are listed in Table S1, and primers used in this study are listed in Table S2. Genes *CjFPS* (GenBank: OQ890734), *OpGDH* (GenBank: OQ890735), *UGTpg45* (GenBank: KM401918.1), *AnACLa* (NCBI Reference Sequence: XP_660039.1), *AnACLb* (NCBI Reference Sequence: XP_660040.1), and *HMGR* (GenBank: OQ890736) were synthesized by GENEWIZ (Suzhou, China) with codon optimization for *S. cerevisiae*. All the codon-optimized sequences are listed in the Supporting Information.

The promoters, coding sequences, and terminators were amplified from the genomic DNA of *S. cerevisiae BY4741*. Gene expression cassettes (Figure S1) were constructed as described in our previous work.[13] Briefly, primers with 30 bp overlap were designed between adjacent fragments, and the fragments were connected by fusion polymerase chain reaction (PCR) after amplification.[31] The CRISPR-Cas9 plasmid was pWS158, derived from Addgene (RRID: 90517). The URA3 marker was removed on SD + 5-fluorooroticacid (5-FOA) plates containing 6.7 g/L YNB, 20 g/L glucose, and 1 g/L 5-FOA.[32] The strains we used and constructed are listed in Table S1. The primers used for the construction of the plasmids and strains are listed in Table S2. The DNA products purification and recovery kit, agarose gel DNA purification and recovery kit, and plasmid extraction kit used in this study were purchased from TIANGEN BIOTECH Co., Ltd. (Beijing, China). Protopanaxadiol (PPD), ginsenoside Rh2, and other chemicals and reagents were purchased from Sigma-Aldrich unless otherwise specified. Antifoaming agent for fermentation was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). The gene expression cassettes, homologous arms of the genomic integration locus, and the selection marker were integrated into the *S. cerevisiae* genome using the lithium acetate method as previously described.[33] A single colony was selected from the selection plate and resuspended in 20 mM NaOH. The suspension was subsequently incubated at 100 °C for 20 min in a water bath. The cell suspension was utilized as the PCR template for verification, and the correct strain was subsequently sequenced for further validation.

### 2.3. Flask Fermentation

A single colony was isolated from the YPD plate, inoculated into YPD medium, and cultured at 30 °C for 18 h. The harvested cells were inoculated into 250 mL shake flasks containing 30 mL of YPG medium, with a unified initial $OD_{600}$ value of 0.1. Flask fermentations were carried out at a stirring speed of 220 rpm and a constant temperature of 30 °C for 96 h. All fermentations were performed in triplicate.

### 2.4. Analytical Methods

The UV−visible spectrophotometer (MAPADA UV-1200 spectrophotometer, Shanghai, China) was used to detect the optical density of yeast cultures at a wavelength of 600 nm.

For the detection of glycerol and ethanol, the fermentation broth was centrifuged at 12,000 rpm for 2 min. The supernatant was subsequently collected and filtered through a 0.22 μm membrane for HPLC analysis. The HPLC analysis was performed using an Elite P230II high-pressure pump system coupled with a refractive index detector (Elite, Dalian, China). Chromatographic separation of glycerol and ethanol was carried out at 65 °C using an Aminex HPX-87H ion-exchange column (Bio-Rad, United States). The elution system consisted of a 5 mM $H_2SO_4$ aqueous solution, and the flow rate was maintained at 1 mL/min.

For the extraction and detection of PPD and Rh2, the fermentation broth was subjected to vortex agitation with N-butanol and 40−100 mesh $SiO_2$. The mixture was subsequently centrifuged at 12,000 rpm for 10 min, and the *N*-butanol phase was collected for HPLC analysis. The HPLC analysis was performed on an Elite P230II system (Elite, Dalian, China) equipped with an Elite P230II pump and a UV detector Elite UV230II. Chromatographic separation of ginsenoside Rh2, PPD, and DM was carried out at 25 °C on a Hypersil ODS2 C18 column (300 × 4.6 mm, 5 μm, Elite, Dalian, China). The elution system consisted of 20% water (A) and 80% acetonitrile (B), and the flow rate was kept at 1 mL/min. UDPG was extracted and quantified from yeast cells following the methodology described by Keasling et al.[34]

### 2.5. Metabolomic Analysis

After cultivating the target strains at 30 °C and 220 rpm for 36 h (Figure S4), $10^7$ cells were transferred into 2 mL microcentrifuge tubes. Tubes were centrifuged at 8000 rpm for 20 min at 4 °C, and then the supernatant was carefully removed. Finally, the samples were quenched in liquid nitrogen for 10 min and then stored at −80 °C. The yeast cell samples (25 mg) were taken and mixed with beads and 500 μL of extraction solution (MeOH: ACN: $H_2O$, 2:2:1 (v/v)). The extraction solution contains deuterated internal standards. The mixed solution was vortexed for 30 s. Two homogenization beads were added, homogenized for 4 min (35 Hz), and then transferred to an ice−water bath to sonicate for 5 min (repeated 3 times). The samples were then allowed to thaw at room temperature and vortexed for 30 s. This freeze−thaw cycle was repeated three times. Then, the samples were sonicated for 10 min in a 4 °C water bath and incubated for 1 h at −40 °C to precipitate proteins. The samples were centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was transferred to a fresh glass vial for analysis. The quality control (QC) sample was prepared by mixing an equal aliquot of the supernatant of the samples.

For metabolites, LC-MS/MS analyses were performed using a UHPLC system (Vanquish, Thermo Fisher Scientific) with a Waters ACQUITY UPLC BEH Amide (2.1 × 50 mm, 1.7 μm) coupled to an Orbitrap Exploris 120 mass spectrometer (Orbitrap MS, Thermo). The mobile phase consisted of 25 mmol/L ammonium acetate and 25 mmol/L ammonia hydroxide in water (pH = 9.75; (A) and acetonitrile (B). The autosampler temperature was 4 °C, and the injection volume was 2 μL. The Orbitrap Exploris 120 mass spectrometer was used for its ability to acquire MS/MS spectra on information-dependent acquisition (IDA) mode in the control of the acquisition software (Xcalibur, Thermo). In this mode, the acquisition software continuously evaluates the full scan of the MS spectrum. The ESI source conditions were set as follows: sheath gas flow rate of 50 Arb, Aux gas flow rate of 15 Arb, capillary temperature of 320 °C, full MS resolution of 60000, MS/MS resolution of 15,000, collision energy: SNCE 20/30/40, spray voltage of 3.8 (positive) or −3.4 kV (negative), respectively. The raw data were converted to mzXML format using ProteoWizard and processed with an in-house program. which was developed using R and based on XCMS, for feature detection, extraction, alignment, and integration. The R package was applied to metabolite identification. For comprehensive methodological details regarding metabolomic analysis assays, refer to the study by Zhu et al.[35]

### 2.6. Fed-Batch Fermentation in a 5 L Bioreactor

Strain ZHE01 was used for fed-batch fermentation. A single colony on a YPD agar plate was selected and cultured overnight in 3 mL of YPD liquid medium. The obtained primary seed solution was inoculated into 200 mL of YPG medium to prepare a secondary seed culture for a 5 L bioreactor (Shanghai Bailun Bio). The seed medium was cultivated at a temperature of 30 °C and 220 rpm for a duration of 24 h, followed by inoculation into a 5 L bioreactor with an initial volume ratio of 10%. The initial fermentation medium consisted of 2 L of YPG and was used for fed-batch fermentation. The pH was maintained at 5.5 using 2.5 M $H_2SO_4$ and 5 M $NH_3{\cdot}H_2O$. The temperature was automatically controlled at 30 °C, and the aeration rate was set at 2 vvm. Dissolved oxygen (DO) levels were maintained above 35%. Starting from 24 h, 20 g of glycerol and 8 g of yeast extract powder were added to the bioreactor every 12 h until the end of the fermentation.

## 3. RESULTS AND DISCUSSION

### 3.1. Construction of *S. cerevisiae* Using Glycerol as the Carbon Source to Synthesize PPD

PPD serves as a precursor for Rh2 production. Therefore, the construction of *S. cerevisiae* strains for the synthesis of PPD


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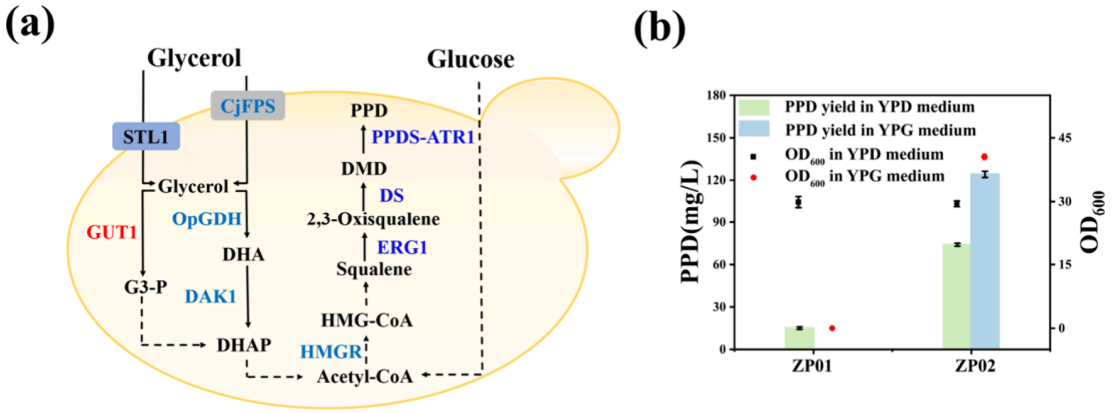



**Figure 1.** Construction of *S. cerevisiae* using glycerol as the carbon source to synthesize PPD. (a) Schematic diagram of the metabolic pathways for PPD biosynthesis from glycerol in *S. cerevisiae*. Endogenous pathway enzymes were in red, "DHA pathway" enzymes were in cyan, and PPD biosynthesis pathway were in blue. CjFPS, aquaglyceroporin; OpGDH, glycerol dehydrogenase; DAK1, DHA kinase; STL1, glycerol/$H^+$ symporter; GUT1, glycerol kinase; HMGR, NADH-dependent 3-hydroxy-3-methylglutaryl-CoA reductase; ERG1, squalene monooxygenase; DS, *Panax ginseng* dammarenediol synthase; PPDS-ATR1, *Panax ginseng* protopanaxadiol synthase CYP716A47 fusion with *Arabidopsis thaliana* P450 reductase 1; DMD, dammarenediol; PPD, protopanaxadiol; G3-P, glycerol 3-phosphate; DHA, dihydroxyacetone; DHAP, dihydroxyacetone phosphate. The solid arrows represent a single step and a direct reaction. The dotted arrows represent multistep and indirect reactions. (b) The PPD titers and $OD_{600}$ values of ZP01 and ZP02 were determined by fermentation in shake flasks using glucose and glycerol as carbon sources, respectively. Mean values ± standard deviations are shown ($n$ = 3 independent biological replicates).

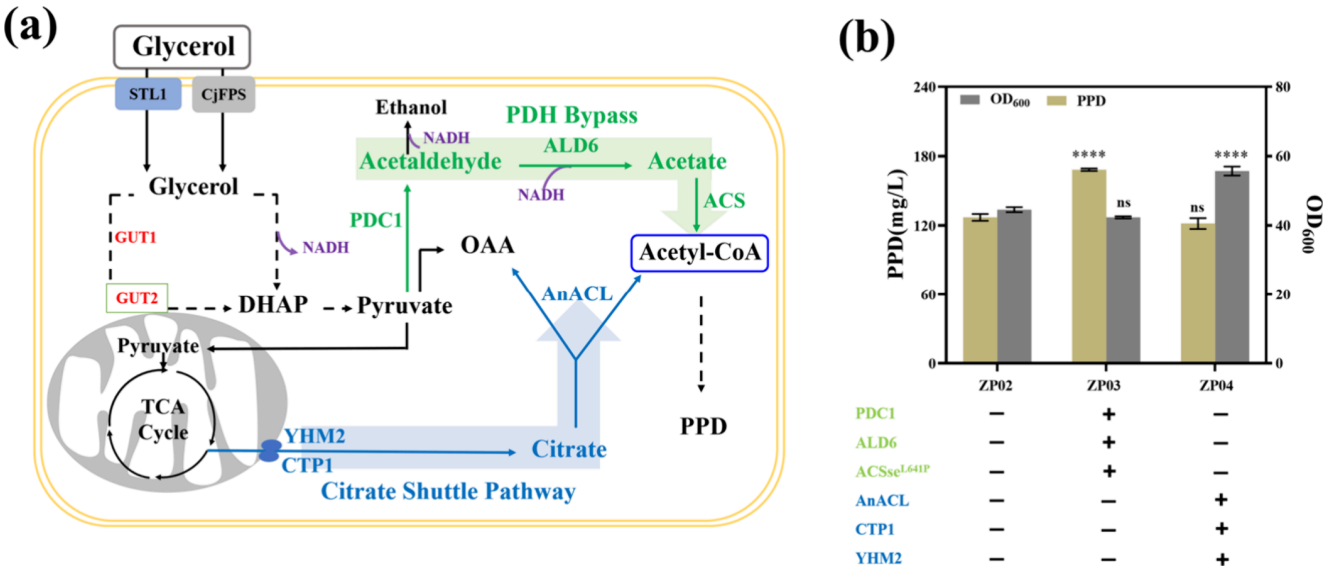



**Figure 2.** Strategies to optimize acetyl-CoA supply. (a) Schematic diagram of the PDH bypass and citrate shuttle pathway. GUT2, mitochondrial (membrane bound) FAD-dependent G3-P dehydrogenase; PDC1, indolepyruvate decarboxylase 1; ALD6, aldehyde dehydrogenase ($NADP^+$); ACS, acetyl-CoA synthetase; YHM2, CTP1, citrate transporter; AnACL, citrate lyase; and OAA, Oxaloacetic acid. The solid arrows represent a single step and a direct reaction. The dotted arrows represent multistep and indirect reactions. (b) The 96h shake flask fermentation results of ZP02, ZP03, ZP04. Mean values ± standard deviations are shown ($n$ = 3 independent biological replicates). Statistical analysis of PPD production was performed using one-way ANOVA (**** $p < 0.0001$, ns stands for nonsignificant).

using glycerol as the substrate was first studied. The synthesis pathway of PPD, including the PPD synthase (PPDS) and NADPH-cytochrome P450 reductase (ATR1) fusion protein (PPDS-ATR1), dammarenediol synthase (DS), and squalene oxidase (ERG1), was integrated into the *S. cerevisiae* BY4741 genome to obtain strain ZP01, as shown in Figure 1a.[36] The strain ZP01 synthesized 15.62 ± 1.48 mg/L of PPD after 96 h of fermentation in YPD medium (Figure 1b). However, the native glycerol catabolism pathway in wild-type strains of *S. cerevisiae* is insufficient to effectively support growth and fermentation from glycerol.[25] To overcome this limitation, the heterologous "DHA pathway", comprising the aqueous glycerol channel protein (CjFPS1), glycerol dehydrogenase (OpGDH), and dihydroxyacetone kinase (DAK1), was successfully constructed and implemented in *S. cerevisiae* to enable efficient glycerol metabolism for chemical production. Moreover, an additional NADH-consuming step was necessary to balance the NADH generated via the "DHA pathway".[37] Therefore, the heterologous "DHA pathway" along with an NADH-dependent 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) from *Silicibacter pomeroyi* was integrated into strain ZP01 to generate strain ZP02. Strain ZP02 was capable of synthesizing 124.67 ± 3.54 mg/L PPD when cultured in YPG medium supplemented with 25 g/L glycerol. However, when it


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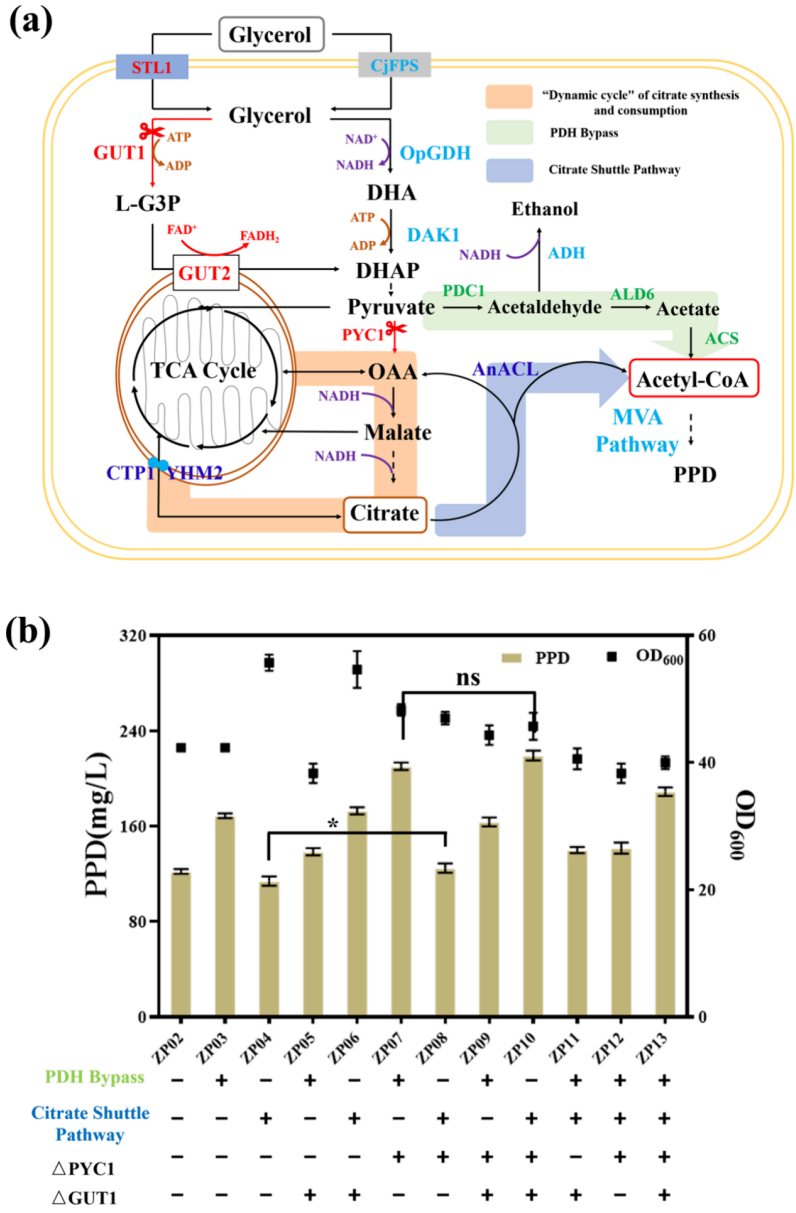



**Figure 3.** Adaptability study of glycerol metabolism and PPD production. (a) Schematic diagram of the intracellular engineered metabolic pathways for PPD production from glycerol. The solid arrows represent a single step and a direct reaction. The dotted arrows represent multistep and indirect reactions. (b) Fermentation results of all strains in shaker bottles in this chapter. Mean values ± standard deviations are shown ($n = 3$ independent biological replicates). Statistical analysis of PPD production was performed using one-way ANOVA (*$p < 0.05$, ns stands for nonsignificant).

was fermented with YPD medium containing 25 g/L glucose, ZP02 was only able to synthesize 73.96 ± 1.06 mg/L of PPD. These results demonstrated that glycerol was the preferred carbon source for *S. cerevisiae* ZP02 to produce PPD when compared with glucose.

### 3.2. Optimizing Acetyl-CoA Supply for Improved PPD Production

Acetyl-CoA, as a central metabolite, serves as a crucial hub connecting key metabolic pathways such as the tricarboxylic acid (TCA) cycle, the MVA pathway, and the glyoxylate cycle.[38,39] Previous studies have demonstrated that increasing the intracellular acetyl-CoA concentration in the cytoplasm significantly enhances the metabolic flux of the MVA pathway, consequently promoting the biosynthesis of terpenoids, such as ginsenosides.[40,41] In the native metabolic network of *S. cerevisiae*, cytoplasmic acetyl-CoA is produced through the pyruvate dehydrogenase bypass, comprising three key enzymes: pyruvate decarboxylase (PDC1), acetaldehyde dehydrogenase (ALD6), and acetyl-CoA synthetase (ACS).[42,43] Notably, some oleaginous yeast strains have evolved a unique citrate shuttle system, which transports citrate from the mitochondria to the cytoplasm via transporters on the mitochondrial membrane (such as YHM2/CTP1), and then decomposes it into oxaloacetate (OAA) and acetyl-CoA through the catalysis of ATP citrate lyase (ACL).[44,45] Researchers have introduced heterologous citrate shuttle systems into *S. cerevisiae* to augment cytoplasmic acetyl-CoA pools.[46] Therefore, these two pathways, as shown in Figure 2a, were selected as the targets to enhance the cytoplasmic acetyl-


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CoA supply, with the ultimate aim of achieving a targeted increase in the metabolic flux for PPD and Rh2 biosynthesis from glycerol.

A PDH bypass-enhanced strain, ZP03, was constructed by overexpressing pyruvate decarboxylase (PDC1), acetaldehyde dehydrogenase (ALD6), and acetyl-CoA synthetase (ACS) in parental strain ZP02. The titer of PPD in ZP03 was significantly increased to 168.26 ± 1.10 mg/L, which was 33.33% higher than that in strain ZP02. Peng et al. overexpressed the PDH bypass pathway in *S. cerevisiae*, which increased the amount of acetyl-CoA in the cytosol and improved the ability of *S. cerevisiae* to synthesize β-carotene from glucose.[39] These results also showed that enhancing the endogenous PDH bypass was beneficial to promote the conversion of pyruvate to acetyl-CoA in the cytosol, thereby increasing the production of PPD from glycerol. However, upon coexpression of citrate transporter (YHM2 and CTP1) and citrate lyase AnACL in ZP02 (resulting in strain ZP04), the PPD titer was only 114.21 ± 4.73 mg/L, which showed no statistically significant difference compared to ZP02 (Figure 2b), although the $OD_{600}$ of ZP04 was significantly increased. It has been reported that the introduction of *Aspergillus nidulans* ATP citrate lyase (AnACL) into *S. cerevisiae* significantly enhanced the precursor supply for fatty acid biosynthesis, resulting in a 2.8-fold increase in lipid titers compared to the parental strain.[44] Furthermore, this strategy has also been successfully applied to the synthesis of ursolic acid from glucose.[46] The increase in $OD_{600}$ observed in ZP04 may indicate that more carbon was directed toward cell growth, following the expression of the heterologous citrate shuttle. As reported by Roberts and Hudson,[47] when *S. cerevisiae* is cultured in a medium with glycerol as the carbon source, the expression of isocitrate lyase and malate synthase is significantly upregulated. This leads to increased consumption of cytosolic acetyl-CoA, synthesis of malate and other intermediates, and replenishment of the TCA cycle, thereby redirecting greater carbon flux toward respiration.[48] This reallocation of carbon flux likely affected the strain's fermentation performance. Therefore, strains ZP03 and ZP04 were further used to explore which pathway combined with downstream metabolic engineering is more suitable to optimize the supply of acetyl-CoA in the cytosol and promote the synthesis of PPD from glycerol.

### 3.3. Influence of Glycerol Kinase and Pyruvate Carboxylase Knockout on PPD Production

To enhance NADH supply, the native glycerol metabolic pathway was disrupted through deletion of the glycerol kinase gene (*GUT1*) in strains ZP03 (PDH bypass strengthened) and ZP04 (Citrate shuttle strengthened), resulting in strains ZP05 and ZP06, respectively (Figure 3a). The PPD yields were 138.33 ± 4.51 and 172.33 ± 8.32 mg/L (Figure 3b). The results showed that the simultaneous expression of the citrate shuttle pathway combined with *GUT1* knockout was conducive to PPD production from glycerol (Figure 3b). When the native glycerol metabolic pathway was disrupted, *S. cerevisiae* exclusively utilized the "DHA pathway" for the uptake and metabolism of glycerol, resulting in substantial NADH production. At the same time, after *GUT1* knockout, the supply of $FADH_2$ in the mitochondria was insufficient.[19] Therefore, a substantial amount of pyruvate may enter the mitochondria to maintain normal respiration, while oxaloacetate (OAA) generated in the cytosol through citrate cleavage promotes the efficiency of the malate shuttle. We propose that this metabolic cycle contributes to an increased supply of cytoplasmic acetyl-CoA, thereby further enhancing the production of PPD (Figure 3a). However, it is noteworthy that following the knockout of the *GUT1* gene, the PPD production of strain ZP05, which expresses the PDH bypass module, exhibited a decrease, whereas ethanol accumulation increased (Figure S2). The underlying reason is that the substantial amount of NADH generated via the "DHA" pathway[37] is preferentially utilized through the PDH bypass for ethanol production rather than for PPD synthesis (Figure 3a). Although the ethanol produced can be reused to generate acetyl-CoA, during this process, on the one hand, ethanol will evaporate, resulting in substrate loss; on the other hand, the acetyl-CoA produced by ethanol metabolism will be used for cell growth and PPD synthesis, thus causing a decrease in yield.[49]

Our previous study showed that the expression level of pyruvate carboxylase (PYC1) in engineered *S. cerevisiae* strains was significantly elevated during glycerol fermentation compared to that observed during glucose fermentation (Figure S3),[27] thereby providing evidence that the malate shuttle plays a crucial role in mitochondrial NADH transport during glycerol metabolism. Therefore, to investigate the effect of *PYC1* gene deletion on PPD production, PYC1 was knocked out in strains ZP03 and ZP04, resulting in the generation of ZP07 and ZP08. The PPD yields of these strains were 209.34 ± 10.21 and 124.74 ± 4.38 mg/L (Figure 3b). These results indicate that knockout of the *PYC1* gene exerts a positive effect on both strains harboring the PDH bypass and the citrate shuttle cleavage pathway. When *S. cerevisiae* ferments with glycerol as a carbon source, knocking out *PYC1* while expressing the PDH bypass can effectively block the metabolic branch of pyruvate through pyruvate carboxylase to produce OAA, thereby directing more carbon flux to the cytosolic pathway for acetyl-CoA production (Figure 3a). However, the accumulation of pyruvate in the cytoplasm may exert a minor influence on the citrate shuttle and cleavage pathway in the absence of redox-driven phosphorylation (NADH), thereby resulting in a slight enhancement of PPD production in the ZP08 strain. Therefore, both genes, *GUT1* and *PYC1*, were deleted in ZP03 and ZP04 strains, obtaining strains ZP09 and ZP10, respectively. As expected, strain ZP10 produced 220.12 ± 4.36 mg/L PPD, representing a 92.98% increase compared to strain ZP04. (Figure 3b). However, the PPD yield decreased to 164 ± 3.22 mg/L in strain ZP09. These results indicate that redox balance plays a critical role in carbon flux distribution within the "DHA pathway"-engineered *S. cerevisiae* strain during glycerol fermentation. The citrate shuttle and cleavage pathway, integrated with the "DHA pathway", establish an effective carbon and redox cycling system for acetyl-CoA-derived PPD production. However, when the native glycerol pathway was retained, the PDH bypass was also an effective combination for cytoplasmic acetyl-CoA production.

To investigate the synergistic effects of integrating two metabolic pathways in combination with *GUT1* and *PYC1* deletion, we constructed strains incorporating both the PDH bypass and the citrate shuttle pathway, along with the knockout of *GUT1* and *PYC1*, to assess the impact of this integrated metabolic engineering strategy on PPD production. Finally, strains ZP11, ZP12, and ZP13 were obtained, and their PPD yields were 138.12 ± 2.16, 142.33 ± 2.67, and 192.46 ± 3.55 mg/L, respectively (Figure 3b). Based on the above


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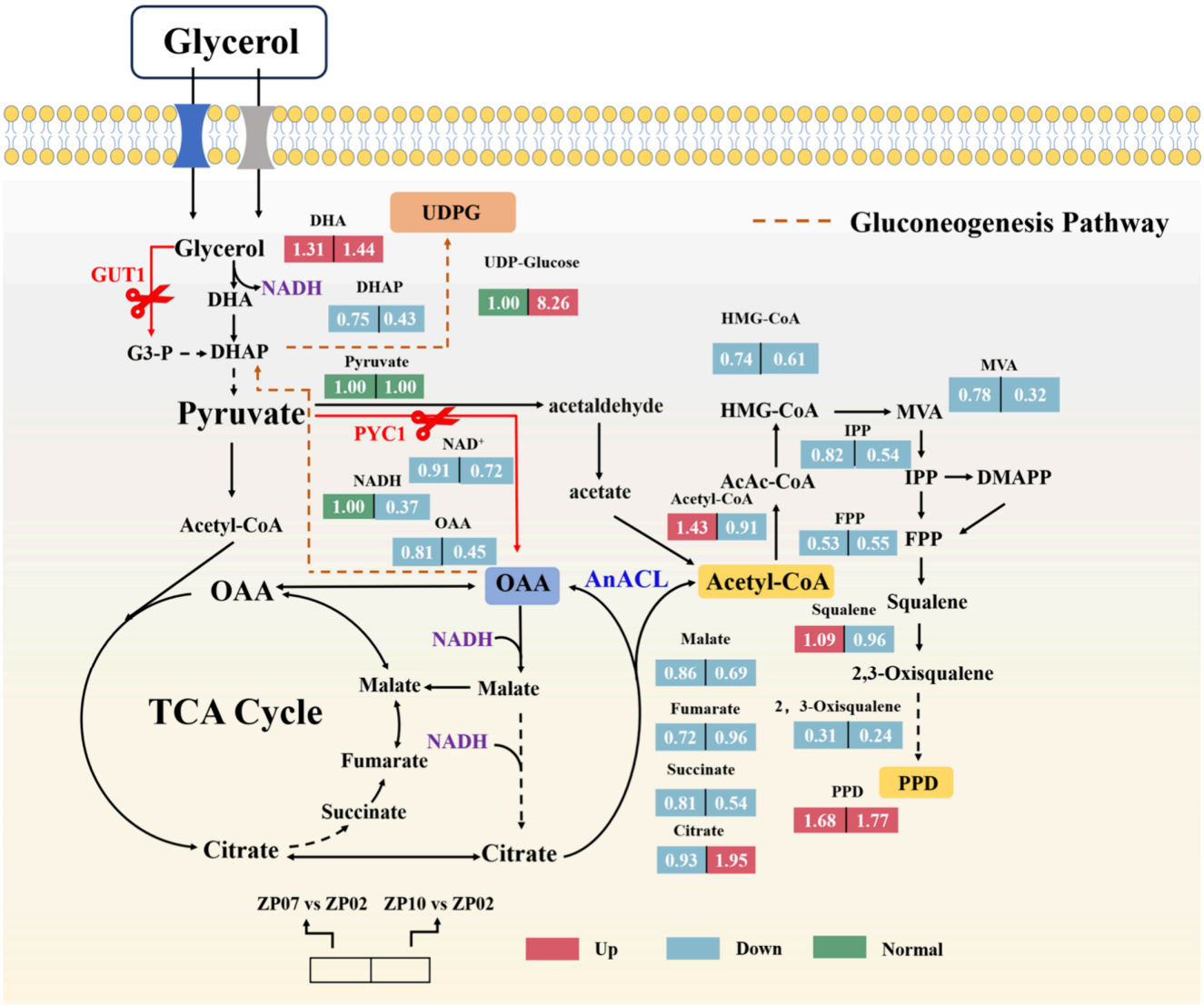



**Figure 4.** Differential metabolite expression results of major intracellular metabolic pathways of strains ZP07 and ZP10 (the left side of the square represents strains ZP07 VS ZP02, the right side of the square represents strains ZP10 VS ZP02, red indicates an increase, blue indicates a decrease, and green indicates no change). The solid arrows represent a single step and a direct reaction. The dotted arrows represent multistep and indirect reactions.

results, we found that the simultaneous expression of endogenous PDH bypass and heterologous citrate shuttle cleavage pathway had an antagonistic effect on PPD production.

### 3.4. Metabolomic Analysis

Given that strains ZP07 (PDH bypass combined with *PYC1* deletion) and ZP10 (citrate shuttle cleavage pathway combined with *GUT1* and *PYC1* knockout) exhibit identical PPD production capacities, metabolomics analysis was conducted to further investigate the underlying mechanisms by which these two pathways contribute to PPD biosynthesis. Therefore, nontargeted metabolomics analysis of ZP02, ZP07, and ZP10 was carried out, focusing on the expression differences of key intracellular metabolites in the PDH bypass pathway and citrate shuttle cleavage pathway. Compared with the starting strain ZP02, ZP07 based on endogenous PDH bypass modification and strain ZP10 modified based on the heterologous citrate shuttle cleavage pathway both showed an increase in the accumulation of DHA and PPD, while the accumulation of most other intermediate metabolites decreased. This situation shows that both strategies are conducive to improving the utilization efficiency of the substrate glycerol and increasing the synthesis of PPD.

In strain ZP10, the knockout of the *GUT1* gene combined with the overexpression of the citrate cleavage pathway establishes a cofactor-driven dynamic cycle of citrate synthesis and cleavage, and the acetyl-CoA generated through citrate cleavage accumulates in the cytosol, thereby continuously supplying the MVA pathway for metabolic utilization, which significantly enhances the capacity for PPD synthesis. As shown in Figure 4, the ratio of NADH/$NAD^+$ of ZP10 is significantly lower than that in ZP07, the lower NADH/$NAD^+$ is more conducive to the role of the DHA pathway, thereby improving the efficiency of glycerol metabolism (Figure S4a). Furthermore, the accumulation of citrate in the downstream metabolic pathway is increased, while the accumulation of OAA is substantially reduced. These observations suggest that the citrate shuttle lysis cycle exerts a significant influence on


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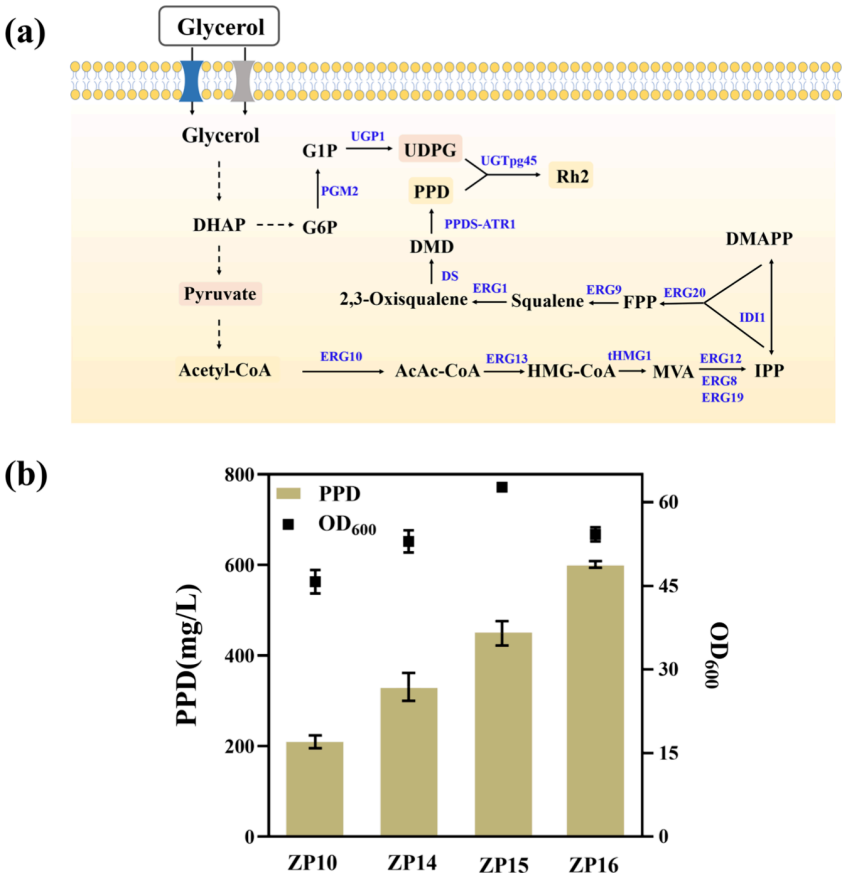



**Figure 5.** Strategies to alleviate the rate-limiting steps of the MVA pathway and the PPD synthesis pathway. (a) Schematic diagram of the PPD synthesis pathway. G6P, glucose 6-phosphate; G1P, glucose 1-phosphate; AcAc-CoA, acetoacetyl-CoA; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MVA, mevalonic acid; IPP, isopentenyl pyrophosphate; DMAPP, isopentenyl pyrophosphate; FPP, farnesyl pyrophosphate; UDPG, uridine diphosphate glucose; ERG10, acetyl-CoA C-acetyltransferase; ERG13, hydroxymethylglutaryl-CoA synthase; tHMG1, truncated HMG-CoA reductase; ERG12, mevalonate kinase; ERG19, diphosphomevalonate decarboxylase; ERG8, phosphomevalonate kinase; IDI1, isopentenyl-diphosphate $\delta$-isomerase; ERG20, farnesyl diphosphate synthetase; ERG9, bifunctional farnesyl diphosphate farnesyltransferase/squalene synthase; ERG1, squalene monooxygenase; UGTpg45, *Panax ginseng* UDP-glycosyltransferase. PGM2, phosphoglucomutase 2; UGP1, UTP glucose 1-phosphate uridylyltransferase. The solid arrows represent a single step and a direct reaction. The dotted arrows represent multistep and indirect reactions. (b) Shake flask fermentation results. Mean values ± standard deviations are shown ($n$ = 3 independent biological replicates).

promoting glycerol metabolism, leading to a significant decrease in the accumulation of DHAP metabolites. Compared with ZP10, ZP07 exhibited an increased accumulation of intermediate metabolites such as acetyl-CoA and squalene. Additionally, ethanol accumulation was observed in ZP07 (Figure S4d), indicating the occurrence of overflow metabolism as a mechanism for $NAD^+$ regeneration. These results suggest that the enhanced PDH metabolic pathway and the heterologous glycerol utilization pathway may be less effective and still require further optimization through metabolic engineering. Surprisingly, we observed a significant increase in UDPG levels in ZP10, which were 8.26 times higher than those in ZP07. The supply of UDPG is typically the rate-limiting step in the synthesis of glycosylated natural products; therefore, the coexpression of the citrate shuttle lysis pathway with the DHA pathway may be more advantageous for the synthesis of Rh2. As shown in Figure 4, when glycerol serves as the sole substrate, cytoplasmic UDPG is derived from the gluconeogenesis pathway. In ZP10, cytoplasmic citrate is cleaved into oxaloacetate (OAA) by the catalysis of AnACL. OAA can directly enter the gluconeogenesis pathway, providing sufficient precursors for UDPG synthesis, thereby promoting UDPG accumulation and supplying ample glycosyl donors for the subsequent synthesis of Rh2. It is noteworthy that, compared to ZP10, the *URA3* gene is deleted in ZP07, which may also affect the synthesis of UDPG. Therefore, the *URA3* gene was reintroduced into ZP07 to generate strain ZP07U. The accumulation levels of UDPG in ZP02, ZP07, ZP07U, and ZP10 were detected, and the results are shown in Figure S5. The accumulation of UDPG in ZP07U was approximately twice that of ZP07, indicating that the restoration of the URA3 function had a positive effect on UDPG accumulation. In comparison, UDPG accumulation in ZP10 was 5-fold higher than that in ZP07U, demonstrating that ZP10 maintained a robust capacity for UDPG accumulation. Considering that the ultimate objective of this study is the construction of an engineered strain capable of efficiently synthesizing Rh2 in *S. cerevisiae* and that the biosynthetic pathway of Rh2 relies on PPD and UDPG as key precursors, a sufficient supply of UDPG is crucial for the efficient accumulation of Rh2. Therefore, strain ZP10 was selected as the starting strain for the next stage of metabolic engineering.

Thus, by producing ginsenosides and other glycosides in *S. cerevisiae* using glycerol as the carbon source, a metabolic engineering strategy can be employed that integrates the


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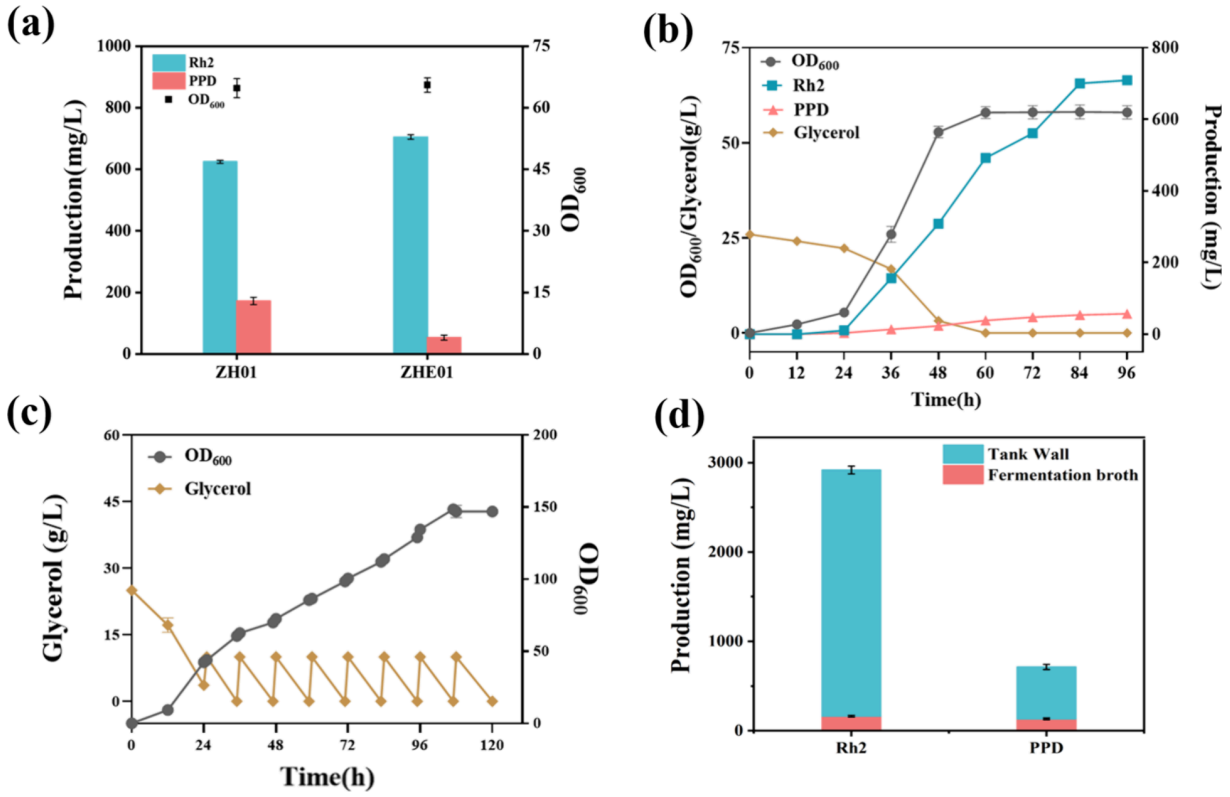



**Figure 6.** Production of Rh2 from glycerol with engineered yeast cells. (a) Fermentation results of Rh2 production strains. (b) Fermentation curves of strain ZHE01 in shake flasks. (c) Fed-batch fermentation of strain ZHE01 in a 5 L bioreactor. (d) Final yield of ginsenoside Rh2 and protopanaxadiol in the 5 L reactor. The red part refers to the products in fermentation broth, and the blue part represents the product precipitated onto the wall of the bioreactor. Mean values ± standard deviations are shown ($n$ = 3 independent biological replicates).

"DHA" pathway with the citrate shuttle cleavage pathway, accompanied by the knockout of *GUT1* and *PYC1*. This combined approach not only augments the cytosolic supply of acetyl-CoA but also enhances the availability of glycosyl donors.

### 3.5. Overexpression of the MVA Pathway Genes to Increase the Production of PPD

HMG-CoA reductase (HMG1) is recognized as the key rate-limiting enzyme of the MVA pathway, and increasing its copy number is beneficial for the large-scale synthesis of squalene.[50] Researchers have found that using truncated HMG1 (*tHMG1*) can effectively relieve feedback inhibition of the MVA pathway and increase the synthesis of terpenoids. Meanwhile, Yuan discovered that overexpressing *ERG10* could enhance the flux of the MVA pathway and increase the synthesis of terpenoids.[51] Squalene monooxygenase (ERG1) and farnesyl diphosphate synthase (FPS, encoded by the *ERG20* gene) have both been previously reported to enhance the production of triterpenoid saponins in yeast.[52] To further enhance the flux from acetyl-CoA to the mevalonate (MVA) pathway, several key enzymes, including truncated HMG-CoA reductase (tHMG1), squalene monooxygenase (ERG1), farnesyl diphosphate synthase (ERG20), and acetyl-CoA acetyltransferase (ERG10), were overexpressed in strain ZP14 (Figure 5a). This resulted in a 57.89% increase in the PPD titer to 330.67 ± 8.33 mg/L compared with its parental strain ZP10 (Figure 5b). Our results also suggest that overexpression of these rate-limiting pathway genes can effectively contribute to increasing the yield of PPD. Zhao[36] discovered that when the expression level of *PPDS-ATR1* reached three copies, over 96% of dama-2,3-diene-1,4-diol could be converted into PPD. Therefore, increasing the copy number of *PPDS-ATR1* is beneficial for the large-scale synthesis of PPD (Figure 5a). Thus, we inserted the *PPDS-ATR1* and *DS* expression module into the *δ* locus of ZP14 (Figure 5a) to increase the copy number of *PPDS-ATR1* within ZP14 and screened out a strain of ZP15 capable of synthesizing 450.38 ± 3.88 mg/L PPD (Figure 5b). The PPD yield increased by 36.36%, demonstrating that increasing the copy number of *PPDS-ATR1* helps to reduce the accumulation of precursors and achieve efficient PPD synthesis. We subsequently integrated the *ERG12*, *ERG13*, *ERG19*, *ERG8*, *IDI1*, *ERG10*, *ERG20*, and *tHMG1* genes into the *rDNA* locus of strain ZP15 to comprehensively reinforce the mevalonate (MVA) pathway and promote PPD synthesis (Figure 5a). We did not overexpress *ERG9* and *ERG1* at the *rDNA* locus because no accumulation of farnesol (FOH, a byproduct of FPP) or squalene was detected in the fermentation broth of strain ZP15. This result indicates that the endogenous expression levels of *ERG9* and *ERG1* are sufficient to support the downstream metabolic flux, and thus, further increasing their copy number is unnecessary. As a result, a high-yield PPD production strain, ZP16, was obtained, with the PPD titer reaching 598.28 ± 9.68 mg/L, representing a 68.57% increase compared to that of strain ZP15 (Figure 5b). This result demonstrates that reinforcing the downstream MVA pathway and the upstream acetyl-CoA supply pathway constitutes an effective "pull and push" strategy for enhancing PPD production from glycerol.


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### 3.6. Ginsenoside Rh2 Cell Factory Construction and 5 L Bioreactor Fermentation

The glycosyltransferase UGTpg45 originating from *Panax ginseng* is capable of catalyzing the biosynthesis of Rh2 from PPD. Zhou[17] reported that the mutant UGTpg45-HV exhibited a more than 40% increase in catalytic efficiency (*kcat/Km*) compared to the wild-type UGTpg45, and its activity was significantly higher than that of other previously reported candidate enzymes(UGTPn50 and UGT51). Therefore, UGTpg45-HV was selected as the key glycosyltransferase for catalyzing the conversion of PPD to Rh2 in this study. Meanwhile, in *S. cerevisiae*, PGM2 catalyzes the conversion of glucose 6-phosphate to glucose 1-phosphate, while UGP1 catalyzes the conversion of glucose 1-phosphate to UDPG, which serves as a glycosyl donor for the biosynthesis of PPD. Therefore, the glycosyltransferase mutant UGTpg45-HV, phosphoglucose mutase 2 (PGM2), and UTP glucose 1-phosphate uridylyltransferase (UGP1) were coexpressed in ZP16 to obtain strain ZH01. The overexpression of these two genes was demonstrated to be an efficient strategy for enhancing ginsenoside production.[18] The ginsenoside Rh2 titer of strain ZH01 reached 600.49 ± 8.88 mg/L after 96 h of fermentation in YPG medium (Figure 6a). However, the PPD titer in strain ZH01 remained relatively high, reaching 180.66 ± 9.43 mg/L (Figure 6a). It has been reported that the presence of a glycoside hydrolase (EGH1) in *S. cerevisiae* could lead to the degradation of ginsenoside Rh2, whereas its knockout can block the hydrolysis pathway of Rh2 and leads to an increase the accumulation of Rh2.[53] Therefore, the *EGH1* gene was knockout and obtained the strain ZHE01 that is capable of synthesizing 700.53 ± 6.88 mg/L Rh2 and the PPD titer was also decreased to 50.34 ± 9.56 mg/L (Figure 6a). This yield is higher than that of the *S. cerevisiae* strain constructed by Feng et al.,[53] which produced 300 mg/L Rh2 from glucose through enzyme engineering and blockage of the Rh2 hydrolysis pathway. These results demonstrate that enhancing glycerol uptake and assimilation efficiency while concurrently increasing the intracellular availability of key metabolic precursors, including acetyl-CoA and UDPG, enables the successful construction of a robust microbial cell factory for the efficient biosynthesis of ginsenoside Rh2.

In order to evaluate the fermentation performance of ZHE01, fed-batch fermentation was carried out in a 5 L bioreactor, and from 24 h, 20 g of glycerol and 8 g of yeast extract were added to the fermenter every 12 h. At 120 h, the $OD_{600}$ reached 150, and the yield of Rh2 reached 2.88 g/L (Figure 6d), which was the highest Rh2 yield reported so far. Meanwhile, the accumulation of PPD in the ZHE01 strain was only 580.22 ± 10.88 mg/L, which was significantly lower than the 11 g/L PPD accumulation level in the study by Zhou et al.[17] This indicates that using glycerol as a substrate favors an enhanced UDPG supply, thereby promoting the synthesis of Rh2. As shown in Figure 6d, we found that more than 90% of Rh2 was deposited on the wall of the fermentation tank. However, the accumulation of Rh2 was both in the fermentation broth (62%) and the tank wall (38%) when glucose and xylose were used as carbon sources in our previous study.[54] This phenomenon suggests that glycerol, as a carbon source, may promote the extracellular secretion of Rh2. Due to the low solubility of Rh2 in water, it is easy to combine or aggregate with other components in the fermentation broth after being secreted to the outside of the cell, thus attaching to the tank wall. These results suggest that the metabolic status and secretory behavior of *S. cerevisiae* may have changed under glycerol culture conditions.

In summary, glycerol as a carbon source can not only promote the synthesis of Rh2, but also enhance its secretion ability. This observation offers a potential source for future research aimed at optimizing fermentation strategies and enhancing product recovery.

## 4. CONCLUSIONS

In this study, *S. cerevisiae* was engineered to de novo synthesize ginsenoside Rh2 from glycerol via synthetic biology and metabolic engineering strategies. The coupling of the heterologous ″DHA″ pathway and citrate shuttle pathway proved to be an efficient combination to produce PPD from glycerol, which increased the PPD titer by 77.42% compared to the original strain. Moreover, the metabolome analysis indicated that the engineered yeast cells expressing the citrate shuttle pathway were more conducive to the accumulation of UDPG than PDH bypass-enhancing strains and thus more favorable for the synthesis of ginsenoside Rh2. Finally, a highly efficient ginsenoside Rh2-producing strain, ZHE01, was constructed through the enhancement of the MVA pathway and glycosyltransferase module engineering. A glycerol fed-batch fermentation process in a 5 L bioreactor with ZHE01 enabled the achievement of the highest Rh2 titer at 2.88 g/L. This work demonstrates that glycerol is an excellent carbon source for terpenoid ginsenoside production in *S. cerevisiae*, highlighting its significant potential as a “nonfermentation carbon source” for the production of other glycosylated natural products. Additionally, it provides valuable synthetic biology and metabolic engineering strategies for engineering *S. cerevisiae* to efficiently utilize glycerol as a carbon source for the production of other acetyl-CoA-derived chemicals or products.

## ■ ASSOCIATED CONTENT

### Data Availability Statement

Data will be made available on request.

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.5c12013.

*S. cerevisiae* strains and plasmids used in this work (Table S1); primers used in this study (Table S2); gRNA used in this study (Table S3); expression cassettes construction and insertion (Figure S1); changes in ethanol accumulation during the 96 h shake flask fermentation of strains ZP03 and ZP05 (Figure S2); The expression levels of PYC1 gene in glycerol and glucose at 24 and 60 h, respectively (Figure S3); fermentation curves of strains ZP02, ZP07, and ZP10 in shake flasks (Figure S4); impact of Ura Tag supplementation on the accumulation capacity of UDPG during 36 h shake flask fermentation of strains ZP07 and ZP10 (Figure S5); codon-optimized CDS for *S. cerevisiae* expression (PDF)

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## Author Contributions

[#]J.Z. and J.F. contributed equally to this work.

## Author Contributions

C.Z. designed the project. J.Z. performed all the experiments. J.F. contributed to the 5 L fermentation experiments. C.Z. and J.L. analyzed the data. J.Z. wrote the initial manuscript. M.W., M.Y., C.Z., and W.L. edited the manuscript. C.Z. and W.L. supervised the project. All authors checked and verified the final manuscript.

## Notes

The authors declare no competing financial interest.

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# A Machine Learning-Based Tool for Enzyme-Substrate Activity Prediction and Enzyme Discovery in Plant Transcriptomes

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## Abstract

Machine Learning (ML) models constitute an efficient strategy for narrowing the search space in complex biological problems, reducing computational time compared to molecular dynamics approaches and providing higher reliability than homology-based methods. In this work, we used ML models to predict enzyme-substrate activity in order to identify candidate enzymes, within the transcriptome of selected plant species, active toward a target compound of interest. A sequence-based learning approach was used to characterize the enzyme-substrate complex through 47 molecular descriptors, including kernel-based descriptors, physicochemical encoding of protein sequences, N-gram representations and position-specific scoring matrices. Three algorithms — Support Vector Machine, Gradient Boosting Trees and Neural Network — were trained and combined through a majority-vote pseudo-learner, reaching accuracies between 0.75 and 0.85. The search space was restricted to the transcriptomes of a set of taxonomically related plant species known to produce the target compound, retrieved from UniProtKB. Several candidate enzymes with predicted activity toward the substrate were identified. The computational prediction required 20 processing cores and 80 GB of RAM, with training taking 46 hours and activity prediction approximately two weeks for four transcript files of about 400 MB each.

**Key words:** Machine Learning; enzyme-substrate prediction; molecular descriptors; enzyme discovery; plant transcriptome

**Representative publication:**

1. Salas-Nuñez, L.F.; Barrera-Ocampo, A.; Caicedo, P.A.; Cortes, N.; Osorio, E.H.; Villegas-Torres, M.F.; González Barrios, A.F. Machine Learning to Predict Enzyme–Substrate Interactions in Elucidation of Synthesis Pathways: A Review. Metabolites, 2024, 14(3), 154.
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5. Venanzi, N.A.E.; Basciu, A.; Vargiu, A.V.; Kiparissides, A.; Dalby, P.A.; Dikicioglu, D. Machine Learning Integrating Protein Structure, Sequence, and Dynamics to Predict the Enzyme Activity of Bovine Enterokinase Variants. Journal of Chemical Information and Modeling, 2024, 64(7), 2681-2694.

## Biography



Andrés Fernando González Barrios is an Associate Professor in the Department of Chemical and Food Engineering at the Universidad de los Andes, Colombia, a position he has held since 2011. He earned his Ph.D. and M.Sc. in Chemical Engineering from the University of Connecticut (2007 and 2006), an M.Sc. in Civil Engineering from Universidad de los Andes, and a B.Sc. in Chemical Engineering from Universidad Pontificia Bolivariana. He leads research within the Product and Process Design Group and the Biomedical Engineering Group, focusing on systems biology, metabolic engineering, biocatalysis, and machine learning applications in biological problems. He has authored more than 25 publications in indexed journals covering topics such as virtual drug screening, cocoa fermentation, biosensors, nanomedicine, and machine learning tools for enzyme-substrate prediction. He has led projects funded by MinCiencias, Colombia's General Royalties System, and other institutions, with budgets exceeding COP 1.3 billion, focused on biodiversity, licit alternative economies to coca cultivation, and cosmeceutical applications of microalgae. He served as Director of the Department of Chemical and Food Engineering from 2018 to 2021. He teaches courses on bioproduct systemic design, advanced thermodynamics, molecular transport phenomena, and artificial intelligence in biocatalysis. He holds a patent on obtaining sugar syrups through acidic hydrolysis of food industry residues. He has also been active in scientific outreach, including promoting the licit use of coca leaf in Putumayo.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Carbon-Ratio Tuning of a Division-of-Labor *Corynebacterium glutamicum* Co-culture for 3'-Prenylnaringenin Biosynthesis

Yong Jun Jeong, Ki Jun Jeong (Time new roman, Align center, font size 12)

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## Abstract

3'-Prenylnaringenin (3'-PN) is a prenylated flavonoid of pharmaceutical interest as a protein tyrosine phosphatase-1B (PTP1B) inhibitor, a validated type 2 diabetes target. However, its microbial production is challenging because the pathway requires three converging precursors, malonyl-CoA, p-coumaric acid, and dimethylallyl diphosphate (DMAPP) which occurs heavy metabolic burden on a single host. To address this, we established a carbon-partitioned co-culture in *Corynebacterium glutamicum* that distributes the rough across two substrate-specialized strains (division of labor). Chromosomal mOrange and sfGFP labeling enabled real-time monitoring of co-culture cell composition by flow cytometry. By assigning glycerol and galactose as strain-exclusive carbon sources, we tuned the population balance widely through medium composition alone, without genetic circuits or inoculum control. With reinforced malonyl-CoA flux and p-coumaric acid feeding, the upstream strain produced naringenin. The downstream strain, engineered for enhanced DMAPP flux, took up this naringenin and prenylated it to 3'-PN via the prenyltransferase AnaPT. Because the two strain modules could be balanced simply by titrating the carbon-source ratio, production was readily optimized without further engineering. These results demonstrate that carbon partitioning practical handle for tuning modular pathways in engineered microbial consortia, offering a generalizable approach to produce complex plant natural products.

**Key words:** *Corynebacterium glutamicum*, division-of-labor, metabolic engineering, 3'-prenylnaringenin, microbial co-culture

**Representative publication:** [Please list no more than 5 of your publication related to the topic] (Time new roman, font size 11)

## Biography



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Yong Jun Jeong received his Ph.D. in August 2026 from the Protein Engineering Laboratory, Department of Chemical and Biomolecular Engineering, KAIST, under the supervision of Professor Ki Jun Jeong. His doctoral research centered on the construction of synthetic microbial consortia and the metabolic engineering of *Corynebacterium glutamicum* for high-level production of plant-derived natural products. In particular, he developed carbon-partitioned, division-of-labor co-culture platforms that distribute long biosynthetic pathways across substrate-specialized strains, enabling modular pathway optimization through simple carbon-source control. His thesis established *C. glutamicum* systems for the biosynthesis of prenylated flavonoids, including naringenin and the antidiabetic compound 3'-prenylnaringenin. His broader research interests lie in synthetic biology and metabolic engineering strategies for building robust microbial consortia that convert simple carbon sources into complex, high-value natural products. He aims to advance scalable and sustainable microbial platforms for the production of pharmaceutically relevant plant metabolites.

# Biocatalytic synthesis of chiral fluorinated non-natural amino acids via aldolase-aminase cascades

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## Abstract

Fluorinated amino acids are key building blocks for drug synthesis due to their ability to improve drug pharmacokinetic properties, and enzymatic synthesis offers an alternative route to produce such fluorinated compounds, complementing conventional chemical methods. This study designed two cascading biocatalytic routes using aldolases and aminases for the asymmetric synthesis of chiral fluorinated aromatic amino acids. The methodology utilized cost-effective aldehydes as starting materials, enabling economical aldol reactions catalyzed by aldolases. Subsequent stereoselective aminations were catalyzed by phenylalanine ammonia lyase and transaminase, resulting in a library of fluorinated aromatic L-α-amino acids with significant stereoselectivity (up to 99%) and γ-OH-α-amino acids with improved diastereoselectivity (13:87). This biocatalytic cascade has been shown to be preparatively scalable for synthesizing pharmaceutically important fluorinated non-natural amino acids, thus demonstrating the versatility of the approach. Furthermore, we encapsulated aldolase within a thin polymer shell via a two-step in-situ encapsulation process; the resulting aldolase nanocapsules retained stable catalytic activity in 0%–40% DMSO and acetonitrile, with 1.45- to 21.25-fold enhanced catalytic efficiency toward nine fluorobenzaldehyde derivatives. This study proposed a simplified, efficient, cost-effective method for synthesizing fluorinated aromatic amino acids under mild conditions, thus providing insights for producing valuable fluorine-containing pharmaceuticals and bioactive compounds.

**Key words:** fluorinated non-natural amino acids, aldolase, aminase, cascade, nanocapsules

**Representative publication:**

1. Jin, X. R.; Huang, C. Q.; Cui, C.; Liu, H. Y.; Liu, Z. Y.; Niu, D. W.; Luo, Y. Z., Chemoenzymatic synthesis of fluorinated L-α-amino acids. ACS Sustainable Chemistry & Engineering 2024, 12 (36), 13645-13653.

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4. Huang, C. Q; Jin, X. R.; Liu, Z. Y.; Cui, C.; Zhang, Y.; Wang, B. J.; Zhang, C.; Feng, J. Q.; Niu, D. W.; Luo, Y. Z., Photoenzymatic enantioselective synthesis of fluorinated amides with remote stereocenter. Nature Communications 2025, 16 (1).

## Biography



Xuerui Jin is a PhD student in the School of Synthetic Biology and Biomanufacturing at Tianjin University under the supervision of Prof. Yunzi Luo. She obtained her master's degree in 2022 from Tianjin University, with a specialization in non-natural biocatalysis research. Her research interests are centred on the fields of natural product biosynthesis and enzyme engineering.

# Engineering of Olivetol Synthase for Effective Biosynthesis of the Cannabinoid Precursor, Olivetolic Acid

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## Abstract

Phytocannabinoids possess significant therapeutic value, yet their microbial production remains challenging due to the limited efficiency and selectivity of key biosynthetic enzymes. As a key type III polyketide synthase (PKS), olivetol synthase (OLS) catalyzes the synthesis of olivetolic acid (OA)—the core precursor for cannabinoids. However, heterologous expression of OLS in *Escherichia coli* often suffers from premature intermediate release, leading to the accumulation of the derailment byproduct pentyl diacetic acid lactone (PDAL). Here, we implemented a structure-guided pocket engineering strategy to engineer the active-site pocket and product selectivity of OLS. By combining comparative structural analysis with the efficient homolog OLS4, *in silico* cavity assessment, hotspot prediction, and molecular docking, we identified key active-site residues governing intermediate stabilization. Through subsequent *in vivo* screening of variants in *E. coli*, the optimized double variant, M187V/S332A, achieved a 3.9-fold increase in OA production (1.6 mg/L) relative to wild-type OLS (0.4 mg/L), while simultaneously reducing PDAL byproduct formation by 37.0%. These findings suggest that precise active-site pocket remodeling can effectively redirect metabolic flux away from byproduct pathways toward the intended product, establishing a scalable engineering framework for type III PKSs in microbial cannabinoid precursor synthesis.

**Key words:** Olivetol synthase, olivetolic acid, type III polyketide synthase, enzyme engineering

**Representative publication:**

1. Won J., Lee B., Park B., Kwon K. K., Lee D.-H., Lee S.-G., Hwang B. Y., Jang J.-H., Hong Y.-S., Engineering the Substrate-Binding Pocket of Olivetol Synthase to Suppress Derailment Byproducts in Olivetolic Acid Biosynthesis in *Escherichia coli*, Journal of Biological Engineering, 2026, in press.

2. Lee B.#, Hwang G. J.#, Jang J.-P.#, Park B., Won J., Kim S. Y., Woo M., Wood C., Hwang B. Y., Jang J.-H., *Hong Y.-S.*, Discovery of pyridomycin derivatives as InhA inhibitors from actinomycetes through molecular networking and an In-House tandem mass library, Nat Prod Bioprospect., 2026, 16 (1), 25.

3. Won J., Lee B., Park B., Ahn J., Hwang B. Y., Jang J.-H., *Hong Y.-S.*, De Novo Artificial Biosynthesis of 3-Hydroxyphloretin in *Escherichia coli*, Journal of Agricultural and Food Chemistry, 2025, 73 (18).

4. Heo K. T., Park K. W., Won J., Lee B., Jang J.-H., Ahn J.-O., Hwang B. Y., Hong Y.-S., Construction of an Artificial Biosynthetic Pathway for Zingerone Production in *Escherichia coli* Using Benzalacetone Synthase from *Piper methysticum*, Journal of Agricultural and Food Chemistry, 2021, 69 (48), 14620–14629.

## Biography



Juhee Won received her B.S. degree in Biological Sciences from Kyungpook National University in 2020 and her M.S. degree in Pharmacy from Chungbuk National University in 2023. She is a Ph.D. candidate in Pharmacy at Chungbuk National University and is currently a researcher at the Chemical Biology Research Center in Korea Research Institute of Bioscience and Biotechnology (KRIBB). Her research interests focus on microbial synthetic biology, enzyme engineering, and metabolic engineering for the sustainable biosynthesis of high-value natural products.

# Development of a Microbial Platform for Serotonin-Derived Metabolite Biosynthesis

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## Abstract

Serotonin is an important aromatic metabolite with diverse biological functions and significant potential as a precursor for value-added compounds. In this study, we developed an engineered *Escherichia coli* platform for the biosynthesis of serotonin-derived metabolites. A heterologous serotonin biosynthetic pathway was constructed and optimized to improve serotonin production. Based on the serotonin-producing strain, additional biosynthetic modules were introduced to enable downstream metabolite formation. LC-MS analysis confirmed the production of several serotonin-derived compounds. This work demonstrates the feasibility of microbial production of serotonin-derived metabolites and provides a foundation for the development of sustainable biosynthetic routes to aromatic compounds.

**Key words:** serotonin; Escherichia coli; metabolic engineering; microbial biosynthesis; aromatic metabolites

**Representative publication:**

1. 1. Han L., Li Y., Wang X., Yao Z., Chen Z., Li J.-p., Tan T., Lee S. Y., Lv Y. Spatially Decoupled Electro-Biosystem for Efficient $CO_2$-to-Chemicals Conversion via Tandem Catalysis.

## Biography

Xuyuan Wang is currently pursuing a Ph.D. in Chemical & Biomolecular Engineering at KAIST, Daejeon, Korea, under the supervision of Prof. Sang Yup Lee (September 2024-present). She received her M.S. degree in Biological Engineering from East China University of Science and Technology, Shanghai, China, under the supervision of Prof. Lixin Zhang (September 2019-June 2022).



# Spatially Decoupled Electro-Biosystem for Efficient $CO_2$-to-Chemicals Conversion via Tandem Catalysis

Lin Han[a,b], Yan Li[a,c,d], Xuyuan Wang[d], Zhibo Yao[e], Ziman Chen[a,c], Jin-ping Li[b,f], Tianwei Tan[a,c], Sang Yup Lee[d,g,*], Yongqin Lv[c,*]

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## Abstract

The conversion of $CO_2$ into value-added chemicals offers a sustainable approach for carbon utilization, but the efficient production of energy-rich products remains challenging. In this study, we developed a spatially decoupled electro-biosystem that combines electrochemical $CO_2$ reduction with microbial fermentation. A tannic acid-$Cu^{2+}$ metal-phenolic network was used to spatially confine Ag and $Cu_2O$ nanoparticles within a covalent organic polymer, generating tandem catalytic sites for ethanol production. The catalyst achieved a Faradaic efficiency of 44.5% for ethanol and operated at current densities up to 400 mA $cm^{-2}$. Ethanol produced from $CO_2$ in a membrane electrode assembly reactor was subsequently utilized by engineered Escherichia coli for the biosynthesis of itaconic acid, isopropanol, and polyhydroxybutyrate. By separating electrochemical conversion and microbial production into two independent modules, the system reduces the limitations associated with direct electro-fermentation while maintaining flexibility for product synthesis. These results demonstrate the potential of integrating electrochemical and biological processes for $CO_2$-based chemical production.

**Key words:** $CO_2$ valorization; electro-biosystem; tandem catalysis; membrane electrode assembly; microbial fermentation

**Representative publication:**

1. 1. Han L., Li Y., Wang X., Yao Z., Chen Z., Li J.-p., Tan T., Lee S. Y., Lv Y. Spatially Decoupled Electro-Biosystem for Efficient $CO_2$-to-Chemicals Conversion via Tandem Catalysis.

## Biography

Xuyuan Wang is currently pursuing a Ph.D. in Chemical & Biomolecular Engineering at KAIST, Daejeon, Korea, under the supervision of Prof. Sang Yup Lee (September 2024-present). She received her M.S. degree in Biological Engineering from East China University of Science and Technology, Shanghai, China, under the supervision of Prof. Lixin Zhang (September 2019-June 2022).



Dalian, China | 18-22,October 2026

Metabolic Engineering Summit 2026

# DeepDDI-X Enables High-Throughput Mapping of Drug–Drug Interactions and Clinical Severity

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## Abstract

Polypharmacy is increasingly common among older adults and patients with chronic diseases, increasing the risk of clinically important drug–drug interactions (DDIs). However, only a small fraction of possible drug combinations has been systematically characterized. Here, we present DeepDDI-X, a deep-learning framework for high-throughput prediction of DDI types and clinical severity from drug-pair structures. Module 1 comprises two deep neural networks that jointly predict 201 human-readable DDI types, while module 2 predicts four levels of clinical severity. The module 1 models were trained using DrugBank and an in-house curated dataset, expanding DDI-type coverage beyond previous models. We applied DeepDDI-X to 349 curated COVID-related drugs and 3,090 prescription and comedication drugs, evaluating more than one million unique drug pairs. DDIs were predicted for 77.6% of pairs, with 92.4% classified as moderate or major. Analysis of 10 representative COVID-19 therapeutics further identified drugs and comedication classes contributing most to high-severity interactions. Finally, a prototype application was developed to query drug combinations and visualize predicted DDI types and severities, demonstrating its potential for multidrug risk assessment.

**Key words:** drug–drug interaction; DeepDDI-X; deep learning; clinical severity; polypharmacy

**Representative publication:**

1. Yu, T., Chae, M., Wang, Z., Ryu, G., Kim, G.B., and Lee, S.Y., "Microbial technologies enhanced by artificial intelligence for healthcare applications", Microb. Biotechnol., 18(3): e70131 (2025.3) https://doi.org/10.1111/1751-7915.70131

## Biography



Ziling Wang is a Ph.D. student in the Department of Chemical and Biomolecular Engineering at KAIST, working in Prof. Sang Yup Lee's laboratory. Her research focuses on computational biology and artificial intelligence for systems healthcare, including data-driven prediction and interpretation of drug–drug interactions.

# Engineering *Escherichia coli* for highly efficient production of recombinant native-sized spider dragline silk protein

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## Abstract

Spider dragline silk is an attractive biomaterial owing to its exceptional mechanical performance, biocompatibility, and biodegradability. However, microbial production of native-sized spider silk proteins remains challenging because of their large molecular size, highly repetitive sequences, and unusual amino acid composition. Here, we developed an integrated engineering framework in Escherichia coli to improve the stable expression and production of large recombinant spidroins. By combining complementary strategies spanning genetic construct design, cellular production, and fermentation, we achieved 10 g/L level production of a native-sized recombinant spidroin, together with gram-per-liter level production of a 377.0 kDa ultra-high-molecular-weight variant in fed-batch culture. These results demonstrate the feasibility of producing exceptionally large and repetitive structural proteins in microbial hosts and provide a general framework for the scalable production of recombinant silk proteins.

**Key words:** Spider dragline silk; metabolic engineering; *Escherichia coli*; recombinant protein production; tRNA

**Representative publication:**

## Biography



I am currently a M.S.- Ph.D. integrated student in Chemical and Biomolecular Engineering at KAIST under the supervision of Prof. Sang Yup Lee. I received my B.S. degree in Chemical and Biological Engineering from Zhejiang University in 2024. My current research interests focus on metabolic engineering and protein engineering, with an emphasis on engineering biological systems for the production of valuable compounds and proteins.

# Blocking β-oxidation and amplifying ω-oxidation enables *de novo* sebacic acid production from glucose in *Saccharomyces cerevisiae*

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## Abstract

Sebacic acid (SA), a C10 dicarboxylic acid (DCA) used in bio-based polyamides, plasticizers, lubricants, and cosmetic ingredients, is produced industrially from castor oil. We engineered Saccharomyces cerevisiae for *de novo* SA synthesis from glucose. Using CRISPR-Cas9, six fatty acid activation and β-oxidation genes (*FAA1*, *FAA4*, *FAA2*, *POX1*, *PXA1*, *PEX11*) were deleted to block precursor catabolism; the resulting 6KO strain accumulated 28.9 mg/L decanoic acid, the direct SA precursor, from 20 g/L glucose. Genomic integration of a four-gene ω-oxidation module from *Candida tropicalis* MYA-3404 (*CtCYP52B1*, *CtNCP1*, *CtADH1*, *CtALD1*) established SA production at 0.4 mg/L. Because ω-carbon oxidation proved rate-limiting, Ty1 retrotransposon-mediated multicopy integration of the P450–reductase pair *CtCYP52B1* and *CtNCP1* raised the titer 37-fold to 14.9 mg/L. Independently, placing the pathway under the inducible *GAL1* promoter with *GAL80* deletion gave 16.2 mg/L. Combining multicopy integration with *GAL1* induction yielded 38.8 mg/L SA — the highest titer reported directly from glucose in engineered yeast. Coordinated pathway rewiring, gene multicopy amplification, and inducible control together establish yeast as a viable platform for sustainable medium chain DCA biosynthesis, with P450 electron transfer the clearest target for further gains.

**Key words:** Sebacic acid; *Saccharomyces cerevisiae*; ω-Oxidation; β-Oxidation; Cytochrome P450

**Representative publication:**

1. Wang, S., Oh, Y., Ji, S., Yun, E. J. *, & Kim, K. H. *, *De novo* production of sebacic acid from glucose by *Saccharomyces cerevisiae* via engineered ω-oxidation and β-oxidation pathways. Bioresource Technology, 2026, 134685.

## Biography



Shuaiwen Wang is a PhD candidate in Systems Food Biotechnology at the Graduate School of Biotechnology, Korea University, Seoul, where he works with Prof. Kyoung Heon Kim. He completed a combined bachelor's–master's program in the Life Science and Technology Talent Training Program at the School of Biotechnology, Jiangnan University, Wuxi, China, with Prof. Xiaoyuan Wang.

His research applies metabolic engineering to industrial microbes for the production of bulk and high-value chemicals. During his master's work he improved L-threonine production in *E. coli* by eliminating the ProP and ProVWX transporters and contributed to studies of lipid A glycosylation in *Bordetella pertussis*. His doctoral work established *de novo* sebacic acid production from glucose in *S. cerevisiae* through engineered ω- and β-oxidation pathways and extends to converting low-cost feedstocks such as waste cooking oil into nylon-monomer diacids.

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# *De novo* production of sebacic acid from glucose by *Saccharomyces cerevisiae* via engineered ω-oxidation and β-oxidation pathways

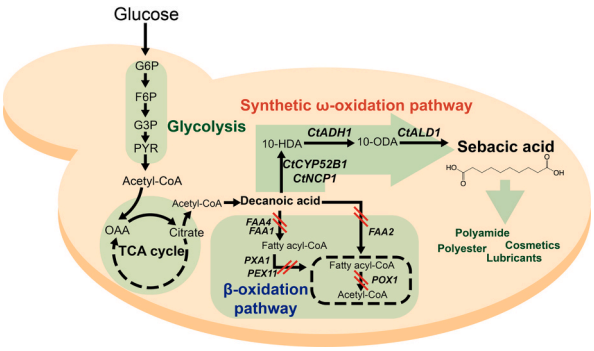
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## HIGHLIGHTS

- *De novo* sebacic acid was produced from glucose in *Saccharomyces cerevisiae*.
- β-Oxidation disruption redirected flux toward decanoic acid accumulation.
- Heterologous ω-oxidation enabled conversion of decanoic acid to sebacic acid.
- Ty1-mediated multicopy integration enhanced P450 expression.
- Growth-decoupled *GAL1* regulation increased sebacic acid titer to 38.8 mg $L^{-1}$.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

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Multicopy genome integration
GAL gene regulator

## ABSTRACT

Sebacic acid (SA) is an important industrial compound used in the production of bio-based polyamides, plasticizers, lubricants, and cosmetic ingredients, and is traditionally derived from castor oil. In this study, we developed a metabolic engineering strategy in *Saccharomyces cerevisiae* using CRISPR-Cas9 to enable *de novo* SA production from glucose. Deletion of six fatty acid degradation genes (*FAA1*, *FAA4*, *POX1*, *PEX11*, *PXA1*, and *FAA2*) generated strain 6KO, which accumulated 28.9 mg $L^{-1}$ decanoic acid (DA) from 20 g $L^{-1}$ glucose, the key precursor of SA. Genomic integration of four ω-oxidation genes from *Candida tropicalis* MYA-3404 (*CtCYP52B1*, *CtNCP1*, *CtADH1*, and *CtALD1*) enabled initial SA production of 0.4 mg $L^{-1}$. Because ω-carbon oxidation was identified as the rate-limiting step, multicopy integration of *CtCYP52B1* and *CtNCP1* via Ty1 retrotransposon significantly increased SA titer to 14.9 mg $L^{-1}$ from 20 g $L^{-1}$ glucose. Further enhancement was achieved by applying the inducible *GAL1* promoter and deleting the transcriptional repressor *GAL80*, which increased SA titer to 16.2 mg $L^{-1}$. Ultimately, combining multicopy integration with *GAL1* induction produced a strain capable of generating 38.8 mg $L^{-1}$ SA—the highest reported SA titer directly from glucose by engineered *S. cerevisiae*. These results demonstrate the effectiveness of integrated pathway rewiring, gene amplification, and inducible control for establishing yeast as a viable platform for sustainable SA biosynthesis.

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## 1. Introduction

Sebacic acid (SA) is a high-value saturated dicarboxylic fatty acid ($C_{10}H_{18}O_4$) containing two terminal carboxyl groups. The global SA market was valued at USD 286.3 million in 2024 and is projected to reach USD 373.7 million by 2034, growing at a compound annual growth rate (CAGR) of 3% (Sebacic Acid Market Size, Share, Growth & Forecast, 2034). Due to its versatile structure, SA is widely applied as a monomer in nylon production and as a plasticizer, lubricant, and cosmetic additive (Jeon et al., 2019).

Castor (*Ricinus communis*) oil remains the primary renewable feedstock for industrial SA production (Veličković et al., 2025). However, its cultivation and processing raise significant sustainability and safety concerns. Global supply is highly concentrated, with India accounting for over 50% of production, leading to market instability (Logan and Udeshi, 2002). In China, shrinking cultivation areas and labor shortages further limit output despite growing demand. Additionally, castor seeds contain toxic proteins such as ricin and ricinine, posing handling risks. The conventional alkaline fusion process, involving concentrated sodium hydroxide and phenol at 230–290 °C, suffers from low yield (~50 wt%), severe corrosion, and substantial phenol-containing wastewater generation (Avramovic et al., 2025). Complex downstream purification is also required to recover byproducts such as 2-octanol and decanoic acid (DA). These drawbacks highlight the need for safer and more sustainable alternatives.

Amid the global drive for carbon neutrality, biological conversion pathways have emerged as promising alternatives. Diploid yeasts such as *Candida tropicalis* possess native ω-oxidation pathways capable of converting alkanes or fatty acid methyl esters into SA with near-complete molecular efficiency and minimal byproducts. However, *C. tropicalis* is not a generally recognized as safe (GRAS) organism and its large-scale use carries biosafety concerns. Moreover, its diploid genome and limited genetic tools restrict metabolic engineering (Pham et al., 2023; Zhang et al., 2021). These constraints underscore the need for safer and more genetically tractable hosts.

*Saccharomyces cerevisiae*, a long-domesticated yeast used in brewing and baking, is an ideal industrial host owing to its well-characterized genetics, robust genome-editing systems, and established large-scale safety record. It has been successfully engineered for numerous high-value chemicals and natural products (Zhu et al., 2020). Abundant sugar streams from first- and second-generation biomass make it particularly suited for sustainable biomanufacturing. Among these, glucose is the most accessible substrate-readily available, quality-consistent, and compatible with standard fed-batch systems.

Establishing a *de novo* pathway requires efficient endogenous generation of DA from glucose. In *S. cerevisiae*, DA originates from glucose-derived acetyl-CoA through iterative elongation by the fatty acid synthase (FAS) complex. Although medium-chain intermediates are transiently formed during chain elongation, they are typically further extended to long-chain fatty acids (C16–C18) or degraded via peroxisomal β-oxidation. Consequently, intracellular DA levels remain intrinsically low, posing a major bottleneck for downstream ω-oxidation toward SA. In the context of sebacic acid bioproduction, ω-oxidation of DA proceeds through a defined three-step terminal oxidation cascade. First, the terminal methyl group (ω-carbon) of DA is hydroxylated by a cytochrome P450 monooxygenase to form 10-hydroxydecanoic acid (10-HDA). Subsequently, 10-HDA is oxidized to 10-oxodecanoic acid (10-ODA) by an alcohol dehydrogenase. Finally, the ω-aldehyde intermediate is further oxidized by an aldehyde dehydrogenase to yield SA. Reconstruction of this DA-to-SA oxidation sequence in *S. cerevisiae* therefore requires coordinated expression of a functional P450 monooxygenase system together with downstream dehydrogenases to complete the terminal oxidation cascade.

Although oleaginous yeasts such as *C. tropicalis* and *Yarrowia lipolytica* have been used for ω-oxidation–based production of dicarboxylic acids (DCAs), these efforts have relied exclusively on salvage pathways using exogenously supplied fatty acids or their derivatives. For example, *C. tropicalis* produced SA from supplemented fatty acids or fatty acid derivatives, overexpression of cytochrome P450 monooxygenases and associated redox partners has enabled the conversion of fatty acid methyl esters into adipic acid (C6 DCA) and other DCAs with improved ω-oxidation efficiency (Min Lee et al., 2024), combinatorial pathway engineering and genome-scale modeling approaches have been applied to enhance DCA production in *Y. lipolytica*, achieving gram-*per*-liter titers of long-chain DCAs from glycerol by redirecting intracellular fatty acid metabolism (Abghari et al., 2017). In addition, *Y. lipolytica* also produced other aliphatic DCAs from externally added fatty acids or related derivatives (Park et al., 2023). However, these systems do not support *de novo* SA synthesis from glucose, and SA production in *S. cerevisiae* has not been reported to date.

In this study, we established a *de novo* biosynthetic route converting glucose to SA in *S. cerevisiae* by integrating a heterologous ω-oxidation pathway with β-oxidation disruption (Fig. 1). Specifically, six genes involved in fatty-acid degradation were deleted to prevent fatty-acid catabolism and redirect carbon flux. To establish the ω-oxidation module, we expressed four heterologous genes from *C. tropicalis*. To further enhance catalytic efficiency and decouple product formation from growth, codon-optimized genes were introduced in multiple copies under an inducible promoter system. To our knowledge, this work represents the first demonstration of *de novo* SA production directly from glucose in *S. cerevisiae*.

## 2. Materials and methods

### 2.1. Strains and culture conditions

*S. cerevisiae* D452-2 (*MATα, leu2, his3, ura3, can1*) was used as the host strain for SA production. Genes encoding the ω-oxidation pathway-cytochrome P450 monooxygenase (*CtCYP52B1*, CTRG_03115), cytochrome P450 reductase (*CtNCP1*, CTRG_00485), alcohol dehydrogenase (*CtADH1*, CTRG_06113), and aldehyde dehydrogenase (*CtALD1*, CTRG_04471)-were derived from *C. tropicalis* MYA-3404 (ATCC). *Escherichia coli* DH5α was used for plasmid construction and maintenance. All strains used in this study are listed in Supplementary Table S1.

*E. coli* strains were cultured in Luria–Bertani (LB) medium (10 g $L^{-1}$ tryptone, 5 g $L^{-1}$ yeast extract, and 10 g $L^{-1}$ NaCl) at 37 °C and 250 rpm. *S. cerevisiae* strains were cultivated in YPD medium (10 g $L^{-}$1 yeast extract, 20 g $L^{-1}$ peptone, and 20 g $L^{-1}$ glucose) supplemented with antibiotics when required. Modified Delft medium (6.7 g $L^{-1}$ yeast nitrogen base without amino acids, 13.4 g $L^{-1}$ $KH_2SO_4$, 20 g $L^{-1}$ glucose, and appropriate amino acid supplements) was used for fatty acid production. Transformants were selected on YPD plates containing clonNAT (100 μg $mL^{-1}$), hygromycin B (300 μg $mL^{-1}$), or G418 sulfate (200 μg $mL^{-1}$), depending on the selection marker.

All strains were cultivated in 50-mL Erlenmeyer flasks containing 10 mL medium. Cultures were incubated at 30 °C with shaking at 200 rpm. Baffled flasks were used throughout the experiments. Unless otherwise stated, inoculation was performed to an initial $OD_{600}$ of 0.1, and samples were collected at every 24 h.

### 2.2. Construction of β-oxidation knockout strains and ω-oxidation pathway integration strains

Gene deletions and integration were performed using a CRISPR-Cas9-based system. Plasmid Cas-NAT was used to express Cas9 protein, while pRS42K and pRS42H (Supplementary Table S3) were used for single-guide RNA (sgRNA) expression. The lithium acetate/single-stranded DNA/polyethylene glycol transformation protocol was followed as described previously (Lee et al., 2020). Integration sites were selected based on previous reports (Reider Apel et al., 2017). sgRNA sequences were inserted by reverse PCR.

Gene deletion sgRNA target sites were selected using the CHOPCHOP

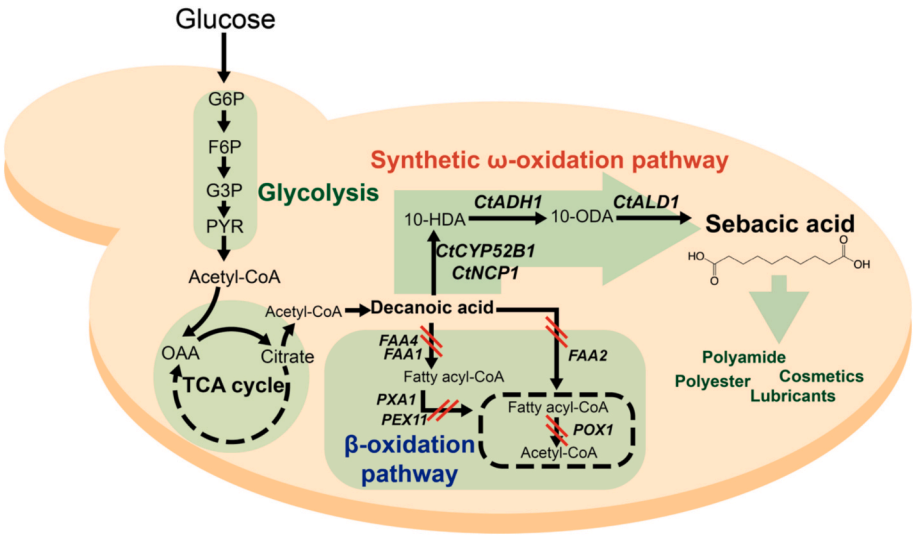



**Fig. 1.** Overall scheme of *de novo* SA production in engineered *Saccharomyces cerevisiae*. Red slashes indicate gene deletions. G6P, glucose-6-phosphate; G3P, glycerol-3-phosphate; PYR, pyruvate; OAA, oxaloacetate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; 10-HDA, 10-hydroxydecanoic acid; 10-ODA, 10-oxodecanoic acid. Gene abbreviations: *FAA1*, long-chain fatty acid CoA ligase I; *FAA4*, long-chain fatty acid CoA ligase IV; *POX1*, acyl-CoA oxidase; *PEX11*, peroxisomal membrane protein PMP27; *PXA1*, peroxisomal long-chain fatty acid import protein II; *FAA2*, long-chain fatty acid CoA ligase II; *CtCYP52B1*, cytochrome P450 monooxygenase; *CtNCP1*, NADPH–cytochrome P450 reductase; *CtADH1*, alcohol dehydrogenase; *CtALD1*, aldehyde dehydrogenase.

web tool (https://chopchop.cbu.uib.no), and the most efficient 20-base pair sequences were cloned into pRS42K or pRS42H via reverse PCR (Supplementary Table S2). Donor DNA fragments containing 5′ and 3′ homology arms of each open reading frame (ORF) were generated by overlap-extension PCR to achieve complete gene deletion. Transformants were screened by colony PCR, and verified strains were stored at −80 °C in 50% (w/v) glycerol.

Integration donor DNA fragments were assembled using pET28a as a cloning backbone, containing upstream and downstream homology arms flanking the heterologous gene fragments. Correctly integrated clones were confirmed by colony PCR and stored at −80 °C in 50% (w/v) glycerol.

### 2.3. Expression of codon-optimized CtCYP52B1 and CtNCP1

To express *CtCYP52B1* and *CtNCP1*, codon-optimized genes (GenBank accession numbers: PZ050893 and PZ050894) (Supplementary Table S4) were inserted into the yeast integrative plasmid pRS403 under either the constitutive *ADH1* promoter or the inducible *GAL1* promoter with *ADH1* terminator, generating plasmid pRSCN. Genes encoding cytochrome P450 monooxygenase (*CtCYP52B1*, CTRG_03115) and cytochrome P450 reductase (*CtNCP1*, CTRG_00485) were codon-optimized for *S. cerevisiae* using codon optimization tool and chemically synthesized by Macrogen (Seoul, South Korea). Plasmids were linearized with *Nde*I before transformation. Transformants were selected on yeast nitrogen base (YNB) plates lacking histidine, verified by colony PCR, and preserved at −80 °C in 50% (w/v) glycerol.

### 2.4. Multicopy integration of CtCYP52B1 and CtNCP1

Homology sequences from Ty1 retrotransposons and δ-elements were introduced into plasmids pRSCN and pRSGAL1pCGAL1pN for multicopy genome integration. sgRNA sequences targeting Ty1 and δ regions were designed using the GGGenome database (https://gggenome.dbcls.jp/sacCer3/). The selected Ty1 sgRNA (5′-TTACCAAAAACGTCTTAGAA-3′) introduced 14 double-strand breaks, whereas the δ sgRNA (5′-TATACTAGAAGTTCTCCTCG-3′) generated 109 cuts across the *S. cerevisiae* S288C genome. After transformation, single colonies were isolated on histidine-deficient YNB agar plates.

### 2.5. Quantitative PCR analysis

Genomic DNA was extracted using the MasterPure Yeast DNA Purification Kit (LGC Biosearch Technologies, UK). Quantitative PCR (qPCR) was performed using Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) on a CFX Opus 96 Real-Time PCR system (Bio-Rad, Hercules, CA). Plasmid pRSCN was used to establish standard curves, and all reactions were run in triplicate.

The *ALG9* gene, which exists as a single copy in the *S. cerevisiae* genome, served as the internal control. The relative copy numbers of *CtCYP52B1* and *CtNCP1* were determined using the ΔΔCq method (Eq. (1):

$$\text{Copy number of gene} = \frac{2^{-[Cq(\text{Gene})-Cq(ALG9)]}(\text{Sample})}{2^{-[Cq(\text{Gene})-Cq(ALG9)]}(\text{Control})} \quad (1)$$

where *Cq(Gene)* and *Cq(ALG9)* represent quantification cycles for the target and reference genes, respectively.

The initial copy number of the *CtCYP52B1* and *CtNCP1* genes in the *S. cerevisiae* genome were determined by the ratio of the gene copy number to the *ALG9* gene copy number. The specific primers used in fluorescence quantitative PCR are shown in Supplementary Table S2. DNA copy concentration (copies $\mu L^{-1}$) was calculated as below (Eq. (2):

$$\text{DNA copies} = \frac{(6.02 \times 10^{23}) \times (\text{ng } \mu L^{-1} \times 10^{-9})}{\text{DNA length} \times 660} \quad (2)$$

### 2.6. Measurement of cell growth and high-performance liquid chromatography (HPLC) analysis of extracellular metabolites

Cell growth was monitored by measuring the $OD_{600}$ using a spectrophotometer (SmartSpec 3000; Bio-Rad). Dry cell weight (DCW) was determined based on the proportional relationship with $OD_{600}$, using the ratio $DCW/OD_{600} = 0.33$.

The concentrations of glucose and ethanol were measured using an HPLC system (Agilent 1100, Agilent Technologies, Santa Clara, CA) equipped with an RID and an H+ (8%) column (Rezex ROA-Organic Acid; Phenomenex, Torrance, CA, USA). The mobile phase was 0.005 N $H_2SO_4$, the flow rate was set at 0.5 mL $min^{-1}$, the column temperature was 65 °C, and the RID temperature was 55 °C.

### 2.7. Gas chromatography/mass spectrometry (GC/MS) analysis of fatty acids

To analyze DCAs culture broths were acidified with 2 M HCl, vortexed for 1 min, and extracted with an equal volume of ethyl acetate. After 3 min of vortexing and centrifugation (13,000 × *g*, 3 min), the upper organic layer was collected and evaporated under vacuum.

Dried extracts were derivatized with 50 μL *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (Sigma-Aldrich, St. Louis, MO) to improve volatility. One μL of the derivatized sample was analyzed using an Agilent 7890A GC/5975C MSD system equipped with a DB-5 MS column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies). The oven program was: 100 °C (3.5 min), ramp to 160 °C at 15 °C $min^{-1}$ (hold 20 min), to 200 °C at 20 °C $min^{-1}$ (hold 20 min), and to 280 °C at 20 °C $min^{-1}$ (hold 5 min). Ionization was performed by electron impact (70 eV) at 230 °C, and mass spectra were recorded in the range of 50–500 *m/z*.

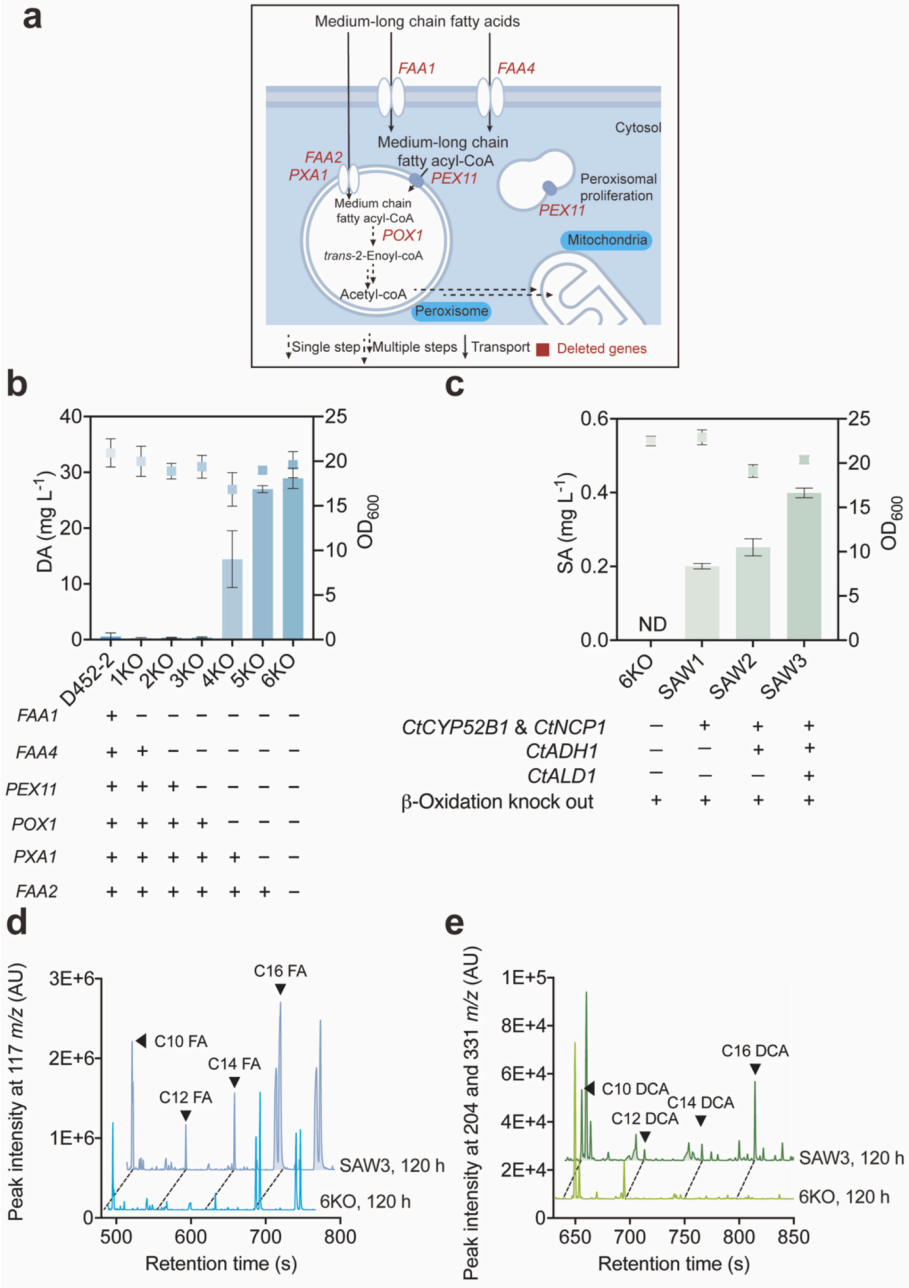



**Fig. 2.** DA accumulation and initial SA production in engineered *S. cerevisiae*. (a) Simplified schematic of the β-oxidation pathway in *S. cerevisiae* highlighting the roles of the six deleted genes (*FAA1*, *FAA4*, *POX1*, *PEX11*, *PXA1*, and *FAA2*). *FAA1*/*FAA4* and *FAA2* encode acyl-CoA synthetases that activate long- and medium-chain fatty acids; *PXA1* mediates fatty-acid import into the peroxisome; *PEX11* regulates peroxisomal proliferation; and *POX1* catalyzes the first β-oxidation step. (b) DA titers in strains with stepwise deletion of six β-oxidation genes after 120 h cultivation. (c) SA production in strains expressing different combinations of the heterologous ω-oxidation genes. GC/MS analysis of fatty acids and dicarboxylic acids produced by engineered strains. (d) Fatty acids produced by 6KO and SAW3. C10 FA, decanoic acid; C12 FA, dodecanoic acid; C14 FA, tetradecanoic acid; C16 FA, hexadecanoic acid. (e) Distribution of C10-C16 DCAs in SAW3. C10 DCA, decanedioic acid; C12 DCA, dodecanedioic acid; C14 DCA, tetradecanedioic acid; C16 DCA, hexadecanedioic acid. Peak identities were confirmed using authentic standards. DA, decanoic acid; SA, sebacic acid; ND, not detected. Data represent mean ± SD of three biological replicates.

## 3. Results and discussion

### 3.1. Redirecting fatty acid flux toward decanoic acid by multi-gene deletion of the β-oxidation pathway

SA can be synthesized through ω-oxidation using DA as a precursor in *C. tropicalis*. Accordingly, intracellular DA concentration is a key determinant for SA production efficiency in *S. cerevisiae*. However, DA is inherently more cytotoxic than most other fatty acids because its hydrophobic tail can intercalate into the plasma membrane, altering lipid packing and reducing membrane fluidity, which subsequently leads to the generation of oxidative stress (Borrull et al., 2015). These effects ultimately impair cell viability and restrict accumulation of DA beyond certain thresholds. Therefore, identifying metabolic nodes that limit DA degradation and redirecting carbon flux toward its accumulation represent critical first steps toward establishing an effective SA production platform.

To achieve this, six genes involved in fatty acid degradation were selected for systematic deletion: *FAA1*, *FAA2*, *FAA4*, *POX1*, *PEX11*, and *PXA1* (Fig. 2a). Among them, *FAA1*, *FAA2*, *FAA4*, and *PXA1* encode acyl-CoA synthetases localized to the cell membrane or peroxisomal membrane, mediating the activation and transport of medium-long chain fatty acids into the peroxisome; *PEX11* encodes a membrane protein that regulates peroxisome proliferation; and *POX1* encodes acyl-CoA oxidase, which catalyzes the first step of β-oxidation (Han et al., 2017; Leber et al., 2015). Deletion of *FAA1*, *FAA4*, and *PEX11* did not noticeably increase DA accumulation (Fig. 2b). This is likely because *FAA1* and *FAA4* are responsible for both long-chain fatty acid transport and activation, and reduced peroxisome proliferation caused by *PEX11* deletion alone may not significantly influence medium-chain fatty acid turnover (Fig. 2a). In contrast, *POX1* deletion resulted in a marked elevation of DA to 14.4 mg $L^{-1}$ (Fig. 2b), confirming that the first β-oxidation step is crucial for DA degradation and that blocking this step can redirect flux toward accumulation.

Sequential deletion of *PXA1* and *FAA2* further enhanced DA levels and slightly improved cell growth, leading to strain 6KO, which accumulated 28.9 mg $L^{-1}$ DA from 20 g $L^{-1}$ glucose after 120 h, approximately 53-fold higher than the wild type (0.5 mg $L^{-1}$). This demonstrates that inhibiting fatty acid degradation effectively increases intracellular DA without additional engineering of upstream fatty acid synthesis. The observed increase in biomass may reflect partial alleviation of redox imbalance, as fewer acyl-CoA intermediates are oxidized in the peroxisome.

Overall, this β-oxidation blocking strategy provided a simple yet powerful means to enrich the key precursor pool for SA biosynthesis. Nonetheless, further enhancement of medium-chain fatty acid biosynthesis could be achieved by tuning chain-length specificity in the fatty acid synthase complex or overexpressing thioesterases with preference for decanoyl-CoA to facilitate DA release (Zhu et al., 2020). Considering DA's intrinsic toxicity, evolving or engineering host tolerance to medium-chain fatty acids will also be critical for sustaining higher production levels (Pereira et al., 2019). Thus, the construction of 6KO established a stable and high-yield precursor chassis, providing the metabolic foundation for subsequent ω-oxidation engineering.

### 3.2. Reconstitution of heterologous ω-oxidation pathway for de novo sebacic acid production

The ω-oxidation pathway is a native oxidative route present in higher eukaryotes and certain yeasts, functioning mainly in fatty acid detoxification and terminal oxidation. In industrial biocatalysts such as *C. tropicalis*, this pathway enables efficient oxidation of alkanes or fatty acids into DCAs, including SA, through a three-step oxidation cascade: hydroxylation of the terminal methyl group by cytochrome P450 monooxygenase, oxidation of the resulting ω-alcohol to an aldehyde by alcohol dehydrogenase, and further oxidation of the aldehyde to a dicarboxylic acid by aldehyde dehydrogenase. To reconstruct this capability in *S. cerevisiae*, four genes from *C. tropicalis* MYA-3404, *CtCYP52B1* (cytochrome P450 monooxygenase), *CtNCP1* (NADPH–cytochrome P450 reductase), *CtADH1* (alcohol dehydrogenase), and *CtALD1* (aldehyde dehydrogenase) were sequentially integrated into the β-oxidation–deficient strain 6KO (Fig. 2c).

CRISPR-Cas9-mediated genomic integration was employed instead of episomal plasmids to ensure expression stability and avoid the high cost and variability associated with antibiotic supplementation. The integration sites were chosen based on previously reported transcriptionally active loci. After sequential insertion of the four genes, the resulting strain SAW3 produced 0.4 mg $L^{-1}$ SA from glucose within 120 h (Fig. 2c). Interestingly, partial expression of only *CtCYP52B1* and *CtNCP1* already led to low but detectable SA formation (0.2 mg $L^{-1}$), suggesting that endogenous dehydrogenases in *S. cerevisiae* can partially complement the ω-oxidation pathway.

GC/MS analysis revealed that strain SAW3 generated not only SA but also several medium- and long-chain fatty acids and DCAs (Fig. 2d, e). The presence of significant C10 DCA (SA) confirmed that the introduced P450 monooxygenase efficiently hydroxylated DA, establishing DA as a competent substrate for ω-oxidation. However, additional DCAs with chain lengths of C12, C14 and C16 were also detected (Fig. 2e), indicating that Cyp52b1p retains broad substrate specificity. The co-production of these structurally similar DCAs may reduce product selectivity and complicate downstream purification, as compounds with comparable chain lengths typically display similar physicochemical properties.

The identification of byproducts underscores both the success and the limitation of the current system. The results demonstrate that the core ω-oxidation module from *C. tropicalis* can function in *S. cerevisiae*, confirming proper folding, cofactor interaction, and electron transfer between Cyp52b1p and Ncp1p. At the same time, the relatively low SA titer (0.4 mg $L^{-1}$) suggests that ω-carbon hydroxylation remains the major bottleneck, likely constrained by P450 expression level, redox capacity, or inefficient coupling between the monooxygenase and reductase components. In preliminary efforts to improve this step, *CtCYP52B1* was engineered using error-prone PCR and structure-based mutagenesis; however, no variants significantly enhanced SA production (Fig. S8). This suggests that catalytic performance in *S. cerevisiae* is constrained more by system-level factors than by sequence variation alone. Inefficient electron transfer between *CtCYP52B1* and its reductase *CtNCP1*, as well as limited heme availability, may restrict catalytic turnover. Future optimization of the ω-oxidation module should therefore focus on structure-guided engineering, including modification of the substrate-binding pocket, optimization of substrate access channels, and improvement of the P450–reductase electron transfer interface. Enhancing redox coupling efficiency and substrate affinity will be critical to increasing pathway flux (Su et al., 2025).

Additionally, the formation of longer-chain DCAs suggests that other native fatty acids in *S. cerevisiae*, such as C12–C16 acyl chains, also serve as unintended substrates for Cyp52b1p. Directed evolution or rational mutagenesis focusing on the substrate-binding pocket could improve chain-length discrimination and reduce these side reactions (Hu et al., 2020). Alternatively, modulating endogenous lipid pools to favor medium-chain fatty acids could further enhance process selectivity (Muller et al., 2025).

Collectively, these results confirm that the heterologous ω-oxidation pathway was successfully reconstructed and functional in *S. cerevisiae* for the first time, enabling *de novo* SA synthesis directly from glucose. The establishment of strain SAW3 thus represents a pivotal step toward developing a safe, genetically tractable, and GRAS-compatible yeast platform for sustainable DCA production.

### 3.3. Enhancement of sebacic acid production via CRISPR-mediated multicopy integration

Stable and high-level expression of the ω-oxidation module is essential for efficient DA conversion to SA. Although multicopy plasmids are commonly used to amplify expression, they often suffer from segregational instability, metabolic burden from antibiotic selection, and copy-number heterogeneity, all which compromise reproducibility and scalability (Hanasaki and Masumoto, 2019). Chromosomal amplification offers a more robust and heritable solution, especially when implemented through the CRISPR-Cas9 system, which enables precise integration at multiple genomic loci with high efficiency (Curcio et al., 2015).

To strengthen expression of the rate-limiting enzymes in the ω-oxidation pathway, *CtCYP52B1* and *CtNCP1* were codon-optimized and integrated into the *S. cerevisiae* genome using two multicopy strategies: δ-sequence integration and Ty1-retrotransposon-based integration (Fig. 3a) (Curcio et al., 2015). Both methods exploit repetitive

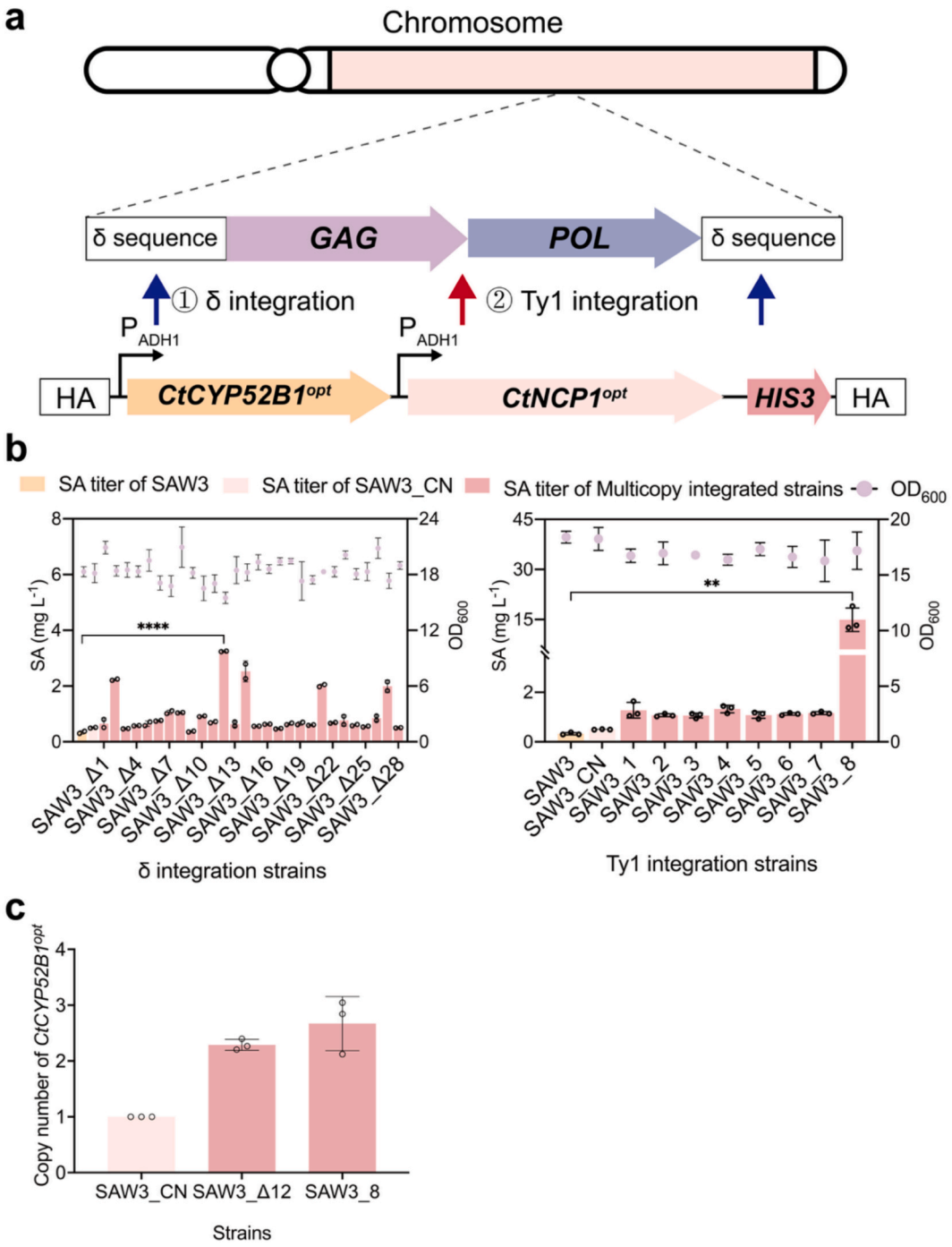



**Fig. 3.** CRISPR-mediated multicopy genome integration using δ-sequences and Ty1 retrotransposons. (a) Schematic illustration of CRISPR-mediated δ-sequence and Ty1-based multicopy integration strategies. (b) SA titers of multicopy transformants derived from SAW3. (c) Quantification of *CtCYP52B1*$^{opt}$ copy numbers in representative δ- and Ty1-integrated strains. Gene copy numbers were determined by qPCR using *ALG9* as the single-copy reference gene. GAG and POL, Ty1 polyprotein genes; *HIS3*, imidazole glycerol-phosphate dehydratase; *CtCYP52B1*$^{opt}$, *CtNCP1*$^{opt}$, codon-optimized genes; HA, homologous arms. Data are means of three biological replicates. Statistical analysis: two-way ANOVA (****$P < 0.0001$, **$P < 0.005$).

elements distributed across the yeast genome, allowing simultaneous insertion of several expression cassettes through CRISPR-mediated double-strand breaks.

For δ-sequence integration, a *HIS3* auxotrophic marker facilitated rapid screening of transformants. Because δ-sequences are terminal repeats of long terminal repeats (LTRs) flanking Ty elements, their abundance-approximately 300 copies per haploid genome—provides numerous potential integration sites. Among 28 isolates obtained, strain SAW3_Δ12 exhibited the best performance, producing 3.2 mg $L^{-1}$ SA after 120 h, with slightly reduced biomass relative to the parental strain (Fig. 3b). The reduced growth rate likely reflects partial resource competition for transcriptional and translational capacity resulting from multiple insertions. To compare efficiency, the same cassette was integrated into Ty1 retrotransposons, which exist as 30–40 full-length copies scattered throughout the genome and are highly transcriptionally active regions. Ty1-targeted integration yielded 19 transformants, several of which displayed enhanced product formation. The optimal strain, SAW3_8, accumulated 14.9 mg $L^{-1}$ SA after 120 h under batch conditions (Fig. 3c), a 4.6-fold improvement over the best δ-integration strain and nearly 40-fold higher than the single-copy control (SAW3_CN).

qPCR quantification confirmed that the *CtCYP52B1* copy number increased 2.3-fold in SAW3_Δ12 and 2.7-fold in SAW3_8 compared with the baseline single-copy strain (Fig. 3d). These moderate increases were sufficient to significantly elevate SA titer, indicating that even small increments in gene copy number of the rate-limiting monooxygenase complex can substantially enhance catalytic throughput. Interestingly, further copy-number amplification beyond 3 to 4 copies (observed in a few variants) did not correlate with higher titers; rather, it led to slower growth and reduced yield (Fig. S2). The comparison between δ- and Ty1-based multicopy variants indicates that the improvement in SA production is determined more by the chromatin environment of the integration locus than by copy number alone. Ty1 sites, located in transcriptionally active regions, support higher expression of the P450–reductase module, whereas δ-integrations exhibit greater variability due to heterogeneous chromatin states. These results highlight locus activity as a key factor influencing pathway performance. This non-linear relationship suggests that excessive expression of P450 systems can impose metabolic stress through cofactor (NADPH) depletion, heme competition, and misfolded protein accumulation within the endoplasmic reticulum. Beyond Ty and δ elements, other multicopy genomic regions such as rDNA clusters, subtelomeric repeats, or centromere-proximal sites could also be explored for future optimization.

Together, these results demonstrate that controlled multicopy chromosomal integration provides a powerful strategy to enhance SA biosynthesis. By amplifying key ω-oxidation genes while maintaining genomic stability, *S. cerevisiae* can achieve high expression levels without the instability associated with plasmid systems. Nonetheless, the observed plateau in productivity emphasizes that copy-number amplification must be balanced with cellular capacity to maintain metabolic homeostasis. Identifying the optimal integration architecture, both in locus selection and expression strength, will be essential for translating this strategy to industrially relevant scales.

### 3.4. Controlled expression of ω-oxidation pathway (CtCYP52B1 and CtNCP1) using engineered GAL1 regulation by glucose

Although multicopy integration increased gene copy number, constitutive overexpression of the ω-oxidation enzymes still imposed a heavy metabolic load, leading to slower growth and diminished SA titers. To relieve this burden, we explored inducible control of pathway expression using the inducible *GAL1* promoter, which is one of the best-characterized transcriptional switches in *S. cerevisiae* (Su et al., 2025). In glucose-rich media, the *GAL* genes are repressed because Gal80p binds to the transcriptional activator Gal4p and blocks its activation function. When galactose is present, Gal3p interacts with Gal80p, freeing Gal4p to activate transcription (Fig. 4a). Therefore, deletion of *GAL80* removes the inhibitory component, allowing constitutive activation of GAL promoters once glucose levels drop, even without galactose addition (Chen et al., 2022).

We first compared the use of the strong constitutive *ADH1* promoter and the inducible *GAL1* promoter for driving *CtCYP52B1* and *CtNCP1*. Under *ADH1* promoter, strains exhibited slower growth and decreased SA titers (Fig. 4b), likely due to continuous electron transfer through the P450 system, which imposes substantial NADPH demand and contributes to oxidative stress, as widely reported for heterologous P450 pathways. In contrast, switching to *GAL1* promoter temporally restricted expression, reducing early-phase burden and enabling better cell propagation before induction. After *GAL80* deletion, *GAL1* promoter-driven expression occurred spontaneously during late-exponential to stationary phase, corresponding to the period when glucose was nearly exhausted. This design effectively separated the growth and production phases. As a result, strains SAW5 and SAW6 displayed improved cell densities and SA titers compared with the parental strain SAW3_CN (Fig. 4b). The best strain, SAW5, produced 16.2 mg $L^{-1}$ SA after 120 h, while DA levels decreased to 1.5 mg $L^{-1}$, reflecting enhanced substrate conversion efficiency (Fig. 4c). The inducible control of P450 expression therefore increased the DA-to-SA conversion rate by synchronizing enzyme activity with metabolic readiness, minimizing precursor accumulation and redox imbalance.

Meanwhile, *GAL80* deletion alone cannot fully remove glucose

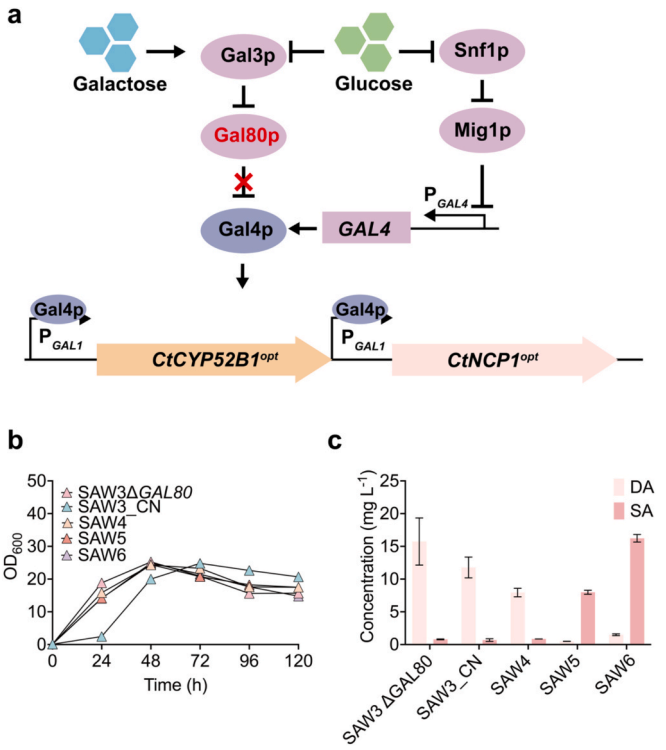



**Fig. 4.** *GAL1* promoter regulation and *GAL80* deletion improve strain performance. (a) Schematic of *GAL1* promoter activation after *GAL80* deletion under glucose-depletion conditions, *GAL80* deletion is shown in red. (b) Growth curves of intermediate strains expressing *CtCYP52B1*$^{opt}$ and *CtNCP1*$^{opt}$ under *ADH1* or *GAL1* promoters. (c) DA and SA titers of the corresponding strains at 120 h. SAW3_CN, SAW3 his::HIS-pRSCN; SAW3ΔGAL80, SAW3 *gal*80Δ; SAW4, SAW3 his::HIS-pRSGAL1pCN; SAW5, SAW3ΔGAL80 his::HIS-pRSGAL1pCN; SAW6, SAW3ΔGAL80 his::HIS-pRSGAL1pCGAL1pN. DA, decanoic acid; SA, sebacic acid; *CtCYP52B1*$^{opt}$, *CtNCP1*$^{opt}$, codon-optimized genes; $P_{ADH1}$, *ADH1* promoter; $P_{GAL1}$, *GAL1* promoter; Δ*GAL80*, *GAL80* deletion; Gal3p, Gal80p, Mig1p, transcriptional regulators; Gal4p, galactose-responsive transcription factor; Snf1p, AMP-activated serine/threonine kinase. Data represent mean ± SD of three biological replicates.

repression because Mig1p and its associated complex continue to inhibit *GAL1* promoter activity in the presence of residual glucose (Lee et al., 2025). This partial repression can delay induction during fed-batch fermentation or high-sugar conditions. For further enhancement, simultaneous inactivation of *MIG1*, *HXK2*, or other regulators in the Snf1p-dependent signaling pathway may achieve tighter control and earlier activation of *GAL1* promoter (Alipourfard et al., 2019). Alternatively, hybrid promoters responsive to intracellular redox state or NADPH levels could be designed to couple expression directly to metabolic cues.

Together, these results demonstrate that introducing inducible control through *GAL1* promoter and *GAL80* deletion efficiently alleviates the growth–production trade-off observed under constitutive expression. By activating ω-oxidation only after biomass accumulation, the engineered strain maintained higher redox balance, reduced DA toxicity, and achieved a two-fold improvement in SA titer. This strategy provides a versatile regulatory module for controlling other NADPH-intensive pathways in yeast cell factories.

### 3.5. Combined multicopy integration and GAL1 regulation further improve sebacic acid production

Because multicopy amplification and *GAL1* regulation each enhanced SA biosynthesis individually, the two strategies were next combined to test for synergistic effects. The rationale was that multicopy insertion of *CtCYP52B1* and *CtNCP1* would increase total enzyme abundance, while growth-decoupled induction via *GAL1* promoter would prevent early-phase redox stress and DA toxicity.

To implement this dual strategy, the Ty1-mediated CRISPR system was again employed because it had produced the most stable and transcriptionally active integrations in earlier experiments. The multicopy cassettes carrying codon-optimized *CtCYP52B1* and *CtNCP1*, each driven by *GAL1* promoter and terminated by *ADH1* terminator, were integrated into the *GAL80*-deleted background of strain SAW3. Forty-two independent transformants were obtained and screened in shake-flask cultures under identical fermentation conditions.

Among all isolates, strain SAW6_5 achieved the highest SA production, reaching 38.8 mg $L^{-1}$ after 120 h (Fig. 5a). This represented a 2.4-fold increase compared with the best strain using a single strategy (SAW5, 16.2 mg $L^{-1}$) and more than a 90-fold improvement over the initial four-gene integrant (SAW3, 0.4 mg $L^{-1}$). qPCR quantification revealed that the *CtCYP52B1* copy number in SAW6_5 was approximately 3.6 times higher than that of the single-copy control (Fig. 5b). The strong correlation between increased copy number and enhanced product titer further confirms that the P450-reductase complex constitutes the primary rate-limiting step of the ω-oxidation pathway in yeast.

During batch fermentation (Fig. 5c, d), distinct changes in metabolite concentrations were observed over time. In the first 24 h, while glucose remained available, DA accumulated and 10-HDA was not detected in the culture supernatant. Under these conditions, the *GAL1* promoter was repressed. Upon glucose depletion after 24 h, *GAL1* was expressed, leading to increased expression of *CtCYP52B1* and *CtNCP1*. This

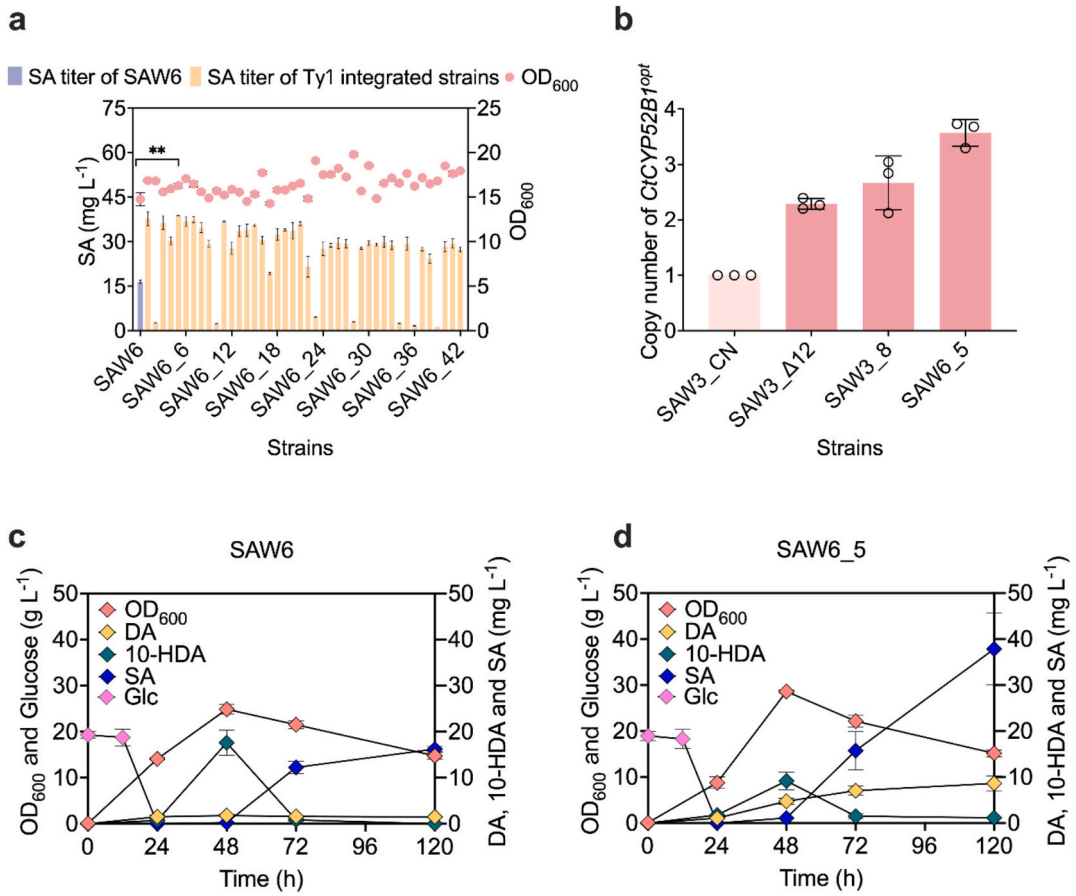



**Fig. 5.** Combined multicopy integration and *GAL1* promoter regulation enhance SA production. (a) SA titers of Ty1-based multicopy transformants constructed in SAW3Δ*GAL80*. (b) qPCR quantification of *CtCYP52B1*$^{opt}$ copy numbers in representative strains. Batch fermentation profiles of engineered strains. (c) Time-course concentrations of glucose, DA, 10-HDA, and SA in strain SAW6. (d) Corresponding metabolite profiles for strain SAW6_5. DA, decanoic acid; 10-HDA, 10-hydroxydecanoic acid; SA, sebacic acid; Glc, glucose. SAW3_CN, SAW3 his::HIS-pRSCN; SAW3_Δ12, SAW3 with pRSCN optimal multicopy delta integration; SAW3_8, SAW3 with pRSCN optimal multicopy Ty1 integration; SAW6_5, SAW3ΔGAL80 with pRSGAL1pCGAL1pN optimal multicopy Ty1 integration. Data represent means of three biological replicates. Statistical analysis: two-way ANOVA (**$P < 0.005$).

activation enhanced ω-oxidation flux, resulting in rapid conversion of 10-HDA to SA during the exponential phase. By 120 h, 10-HDA was fully consumed and SA reached 38.8 mg $L^{-1}$. This temporal separation between precursor accumulation and product formation demonstrates the benefit of inducible control in coordinating enzyme expression with cellular metabolic state.

The synergistic improvement observed here likely results from three complementary effects. First, multicopy integration ensured a higher intracellular pool of functional P450 complexes, increasing catalytic turnover capacity. Second, *GAL1* promoter induction minimized redox stress during early growth, preventing NADPH depletion and maintaining robust biomass formation before induction. Third, controlled timing of ω-oxidation reduced DA accumulation, mitigating cytotoxicity and avoiding feedback inhibition on lipid metabolism. Collectively, these effects allowed carbon flux to be efficiently redirected toward SA formation once cells entered the stationary phase.

The DCA profiles shown in Fig. S3 further illustrate that the engineered strains generate a spectrum of C12–C16 DCAs rather than exclusively producing sebacic acid. This distribution is consistent with the known broad substrate range of *CtCYP52B1* and suggests that improved ω-oxidation capacity in SAW6_5 also increases off-target oxidation of long-chain fatty acids. While SA remained the predominant product, the presence of C12–C16 DCAs underscores the need for subsequent optimization. Targeted engineering of the P450 active site or reshaping of intracellular fatty-acid pools will be critical for enhancing chain-length selectivity and minimizing byproduct formation.

To further investigate the metabolic potential and bypass the glucose-mediated repression of the *GAL* promoters, we conducted feeding experiments using ethanol as a co-substrate using final strain SAW6_5 (Fig. S4). To address this, we performed feeding experiments at the bioreactor and shake-flask level to explore the strain's maximum precursor-supply capacity and identify potential regulatory hurdles. Under 1-L bioreactor conditions with ethanol feeding, DA and 10-HDA accumulated to 6.2 mg $L^{-1}$ and 4.0 mg $L^{-1}$, respectively, whereas SA remained low at 1.0 mg $L^{-1}$, indicating that the downstream P450-mediated ω-oxidation step remained the primary bottleneck despite enhanced precursor supply. Interestingly, DA accumulation surged to 145.0 mg $L^{-1}$, a 15.0-fold improvement over the glucose-only condition in shake-flask condition. Despite the high availability of the precursor, SA production did not increase proportionally, SA only remained low at 4.4 mg $L^{-1}$. This finding confirms that while the engineered upstream pathway is highly robust, the catalytic capacity of the ω-oxidation module remains the ultimate rate-limiting factor. These results suggest that future scale-up efforts should focus on a two-stage feeding strategy in pH-controlled bioreactors to fully realize the biosynthetic capacity of engineered strain.

Overall, the combination of Ty1-mediated multicopy integration and growth-decoupled *GAL1* promoter regulation produced the highest reported SA titer from glucose in engineered *S. cerevisiae* (Fig. 6). This dual-layered strategy demonstrates a generalizable design principle for balancing expression strength and metabolic homeostasis in yeast. Based on 20 g $L^{-1}$ of initial glucose and a final SA titer of 38.8 mg $L^{-1}$, the specific SA production was 7.2 ± 0.1 mg $g^{-1}$ DCW (Supplementary Table S5). Although the conversion efficiency remains below that of chemical routes, this study demonstrates the first *de novo* SA production from sugars in *S. cerevisiae*, providing a foundation for further optimization. Key limitations include fatty acid toxicity, NADPH constraints for P450 activity, and byproduct formation. Future work should focus on fed-batch strategies, cofactor engineering, adaptive evolution, and protein engineering to enhance pathway performance. This platform may also be extended to other ω-oxidation-derived products, expanding yeast-based biomanufacturing from renewable sugars.

## 4. Conclusions

In this study, we engineered *Saccharomyces cerevisiae* for *de novo* sebacic acid (SA) production from glucose through systematic pathway rewiring and CRISPR-Cas9 modification. Deletion of six fatty acid degradation genes generated strain 6KO, accumulating 28.9 mg $L^{-1}$ decanoic acid (DA) from 20 g $L^{-1}$ glucose. Introduction of a heterologous ω-oxidation module from *Candida tropicalis* enabled initial SA production (0.4 mg $L^{-1}$). As ω-carbon oxidation was rate-limiting, multicopy integration of *CtCYP52B1* and *CtNCP1* via Ty1 elements increased SA to 14.9 mg $L^{-1}$. Further improvement was achieved by *GAL80* deletion and *GAL1* promoter regulation, decoupling growth from production and raising SA to 16.2 mg $L^{-1}$. Combining multicopy amplification with inducible control yielded strain SAW6_5, producing 38.8 mg $L^{-1}$ SA-the highest reported titer from glucose in engineered yeast. Our results reveal a non-linear copy number–productivity relationship and establish a modular framework integrating precursor engineering, gene amplification, and dynamic regulation for sustainable SA bioproduction.

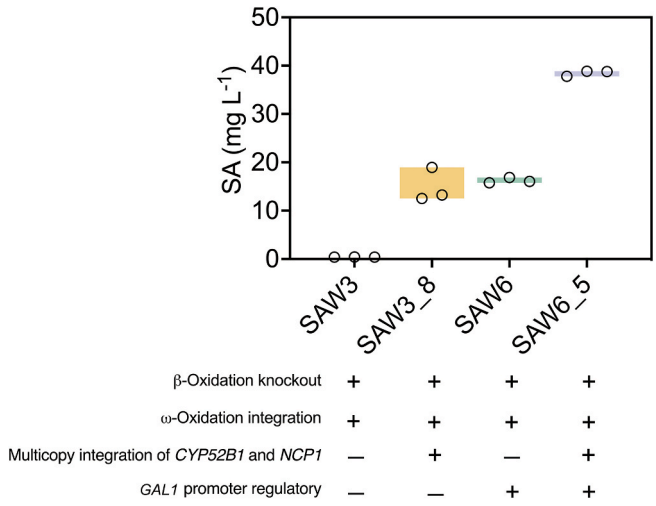



**Fig. 6.** Summary of metabolic engineering strategies and SA production achieved in this study. Major genetic interventions are shown alongside the corresponding SA titers, highlighting progressive improvements across engineered strains.

E-supplementary data for this work can be found in e-version of this paper online.

## CRediT authorship contribution statement

**Shuaiwen Wang:** Writing – original draft, Visualization, Validation, Investigation. **Yukyoung Oh:** Writing – original draft, Investigation. **Sangyun Ji:** Investigation. **Eun Ju Yun:** Writing – review & editing, Supervision, Conceptualization. **Kyoung Heon Kim:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2026.134685.

## Data availability

Data will be made available on request.

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# Integrated Rewiring of Aromatic Metabolism Enhances p-Coumaric Acid Production in *Yarrowia lipolytica*

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## Abstract

*p*-Coumaric acid is a central precursor for flavonoids, stilbenes, lignans, and numerous other high-value phenylpropanoid-derived products. Developing an efficient microbial platform for its production is therefore important for sustainable natural-product manufacturing. *Yarrowia lipolytica* is an attractive non-conventional yeast chassis because of its robust metabolism, high acetyl-CoA availability, and suitability for the expression of eukaryotic enzymes; however, its aromatic amino acid metabolism remains insufficiently exploited for phenylpropanoid biosynthesis. Here, parallel phenylalanine- and tyrosine-derived routes were introduced to improve p-coumaric acid formation in *Y. lipolytica*. The combined expression increased production to 397.59 mg/L, demonstrating that simultaneous utilization of both aromatic amino acid precursors was more effective than engineering either route alone. Competing metabolic branches were subsequently reduced, and feedback-resistant variants of key shikimate-pathway enzymes were introduced to reinforce aromatic precursor supply. The final engineered strain produced 503.35 mg/L p-coumaric acid, representing a 26.6% improvement over the dual-route strain.By integrating complementary precursor-conversion routes with upstream flux redistribution, this study establishes a distinct strategy for expanding aromatic biosynthetic capacity in *Y. lipolytica*. The resulting platform provides a valuable foundation for the sustainable production of flavonoids and other structurally diverse phenylpropanoid-derived compounds.

**Key words:** *Yarrowia lipolytica*; *p*-coumaric acid; aromatic amino acid pathway;pathway rewiring; phenylpropanoid biosynthesis

**Representative publication:** None.

## Biography

The first author, Jing Chen, is a Ph.D. candidate in Synthetic Biology at the School of Synthetic Biology and Biomanufacturing, Tianjin University. Research interests include synthetic biology, metabolic engineering, and the development of microbial cell factories for sustainable production of high-value natural products, with a particular focus on engineering Yarrowia lipolytica for the biosynthesis of p-coumaric acid, flavonoids, and other phenylpropanoid-derived compounds through heterologous pathway construction and metabolic engineering strategies.



# A Bacterial Metabolism-Driven Dual-Enzyme Cascade Sensor for Rapid Metabolic Phenotyping and Antibiotic Susceptibility Profiling

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## Abstract

Rapid and cost-effective assessment of microbial metabolic activity is critical for screening engineered chassis, monitoring bioprocesses, and ensuring biomanufacturing security. Here, we developed a bacterial metabolism-driven dual-enzyme cascade colorimetric sensor (DECCS) that converts glucose consumption into an instrument-free visual readout. Under antibiotic stress, susceptible strains halt metabolism and preserve glucose, yielding intense color; resistant strains continue glucose catabolism, producing faint color. This metabolic phenotype is directly observable by the naked eye and can be quantified using smartphone RGB analysis or a microplate reader. The platform was rigorously validated with 155 clinical isolates and 5 reference strains encompassing clinically relevant Gram-positive and Gram-negative species (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*), tested against 9 essential antibiotics including ceftriaxone, levofloxacin, and gentamicin. The ~5 h DECCS workflow achieved > 96% essential agreement and > 98% categorical agreement with the broth microdilution gold standard. Requiring only common enzymes and glucose, this minimal-equipment method offers a practical approach for rapid metabolic phenotyping. Beyond clinical antimicrobial susceptibility testing, DECCS holds promise as an accessible tool for evaluating chassis fitness, screening tolerance phenotypes, and detecting microbial contaminants in metabolic engineering and biomanufacturing workflows.

**Key words:** Metabolic phenotyping; Colorimetric biosensor; Glucose metabolism; Antimicrobial susceptibility Testing; Cascade reaction

**Representative publication:**

1. Zhang X, Weng B, Li Y*, Wang Q.*, Bacterial Metabolism-Driven Dual-Enzyme Cascade Colorimetric Sensor for Antimicrobial Susceptibility Testing with Readily Interpretable Signals, Analytical Chemistry, 2026, 98(13): 9883-9899.

2. Weng B, Li Y, Feng W, Yao P, Wang Y, Wang Q, Wang X, Li Y, Li L, Wang Q.*, Azithromycin inhibits the intracellular persistence of Acinetobacter baumannii by inducing host cell autophagy in human bronchial epithelial cells, Microbial Pathogenesis, 2025, 198:107152.

## Biography



Yuliang Li is an Associate Researcher and Pharmacist-in-charge at The First Affiliated Hospital of Army Medical University. He received his Master's degree from Chongqing Medical University in 2016 and is currently pursuing a PhD at Southwest University. His research focuses on medical diagnostic technologies and antimicrobial resistance prevention, with particular emphasis on rapid bacterial susceptibility testing and biosensor development. He has published eight peer-reviewed journal articles as first or co-first author as well as co-corresponding author, and presided over four research projects including the Young Scientists Fund of the National Natural Science Foundation of China. His ongoing work aims to translate microbial metabolic phenotyping into low-cost, instrument-free diagnostic platforms, offering accessible tools for metabolic engineering applications such as chassis screening and bioprocess monitoring.

# Metabolic Reprogramming of Bronchial Epithelial Autophagic Flux by Azithromycin Eradicates Intracellular Persistent *Acinetobacter baumannii*

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## Abstract

Intracellular persistent infection induced by bacterial metabolic hijacking of host cells represents a key metabolic barrier to effective antimicrobial clearance and leads to refractory and recurrent infections. Multidrug-resistant *A. baumannii* is a critical clinical pathogen that remodels host cellular metabolic homeostasis to establish intracellular persistence, severely limiting therapeutic efficacy and creating unmet demands for metabolic-targeted intervention strategies. Beyond conventional bactericidal effects, antibiotic-mediated modulation of host metabolic networks has emerged as a promising metabolic engineering and regulatory strategy to eliminate intracellular pathogens. In this work, we systematically investigated the metabolic rewiring mechanism by which azithromycin reshapes host autophagic metabolic flux to eradicate intracellular *A. baumannii*, using combined in vitro and in vivo validation. Our results demonstrated that intracellular *A. baumannii* persistence significantly altered host metabolic tolerance, resulting in elevated bacterial survival thresholds against conventional bactericidal treatments. In contrast, azithromycin effectively eliminated intracellular multidrug-resistant *A. baumannii* both in vitro and in vivo via metabolic reprogramming of bronchial epithelial cells. Specifically, 60 μg/mL azithromycin markedly promoted autophagosome formation and optimized autophagic degradation flux in BEAS-2B cells, restoring host metabolic homeostasis disrupted by pathogen infection. These findings reveal a novel metabolic regulatory mechanism by which small-molecule compounds remodel host cellular catabolic pathways to override pathogen-induced metabolic adaptation. This metabolic modulation strategy provides a new theoretical framework and actionable target for engineering host metabolic robustness against intracellular persistent bacterial infections, offering great potential for the development of metabolic intervention-based anti-infection biotechnologies.

**Key words:**

metabolic reprogramming; metabolic flux; autophagy homeostasis; host-pathogen metabolic interaction; macrolide metabolic regulator

**Representative publication:**

1.**Weng B**, Li Y, Feng W, Yao P, Wang Y, Wang Q, Wang X, Li Y, Li L, Wang Q. Azithromycin inhibits the intracellular persistence of Acinetobacter baumannii by inducing host cell autophagy in human bronchial epithelial cells. Microbial pathogenesis .198 (2025): 107152. .

2.Cheng L[#], **Weng B**[#], Jia C, Zhang L, Hu B, Deng L, Mou N, Sun F, Hu J. The expression and significance of efferocytosis and immune checkpoint related molecules in pancancer samples and the correlation of their expression with anticancer drug sensitivity. Front Pharmacol. 2022 Aug 19;13:977025..

3.Zhang X, **Weng B**, Li Y, Wang Q. Bacterial Metabolism-Driven Dual-Enzyme Cascade Colorimetric Sensor for Antimicrobial Susceptibility Testing with Readily Interpretable Signals. Anal Chem. 2026, 98(13):9883-9899..

## Biography



Bangbi Weng is a PhD candidate supervised by Prof. Qian Wang at the Department of Pharmacy, Southwest Hospital of Army Medical University. Her research centers on host-pathogen metabolic crosstalk, with a specific focus on metabolic reprogramming of bronchial epithelial cells to eliminate intracellular persistent multidrug-resistant *A. baumannii*. She has presided over a research project funded by the Chongqing Municipal Education Commission. She mainly explores small-molecule compounds targeting autophagic metabolic flux to reverse pathogen-induced metabolic adaptation.She has published 1 peer-reviewed SCI paper concerning macrolide-mediated host autophagy against intracellular bacteria and participated in two provincial research projects on bacterial drug resistance. She attends MES 2026 to exchange novel metabolic intervention strategies for infectious diseases and learn advanced metabolic modeling and host metabolic engineering technologies.

Dalian, China 18-22,October 2026

Metabolic Engineering Summit 2026

# Biosensor-guided strain engineering of *Klebsiella michiganensis* for high-level 2,3-butanediol production

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## Abstract

2,3-Butanediol (2,3-BDO) is a valuable platform chemical with broad industrial applications, but efficient strain development remains limited by the difficulty of screening high-producing variants from large mutant libraries. Here, we established a biosensor-guided design–build–test–learn platform for improving 2,3-BDO production in *Klebsiella michiganensis*. A transcriptional regulator responsive to 2,3-BDO was identified and engineered into a fluorescent biosensor. The sensor was further optimized and coupled with a growth-based negative selection module to reduce false-positive populations prior to fluorescence-activated cell sorting (FACS). Multiplex automated genome engineering (MAGE) was then applied to diversify key metabolic nodes involved in 2,3-BDO biosynthesis and competing pathways. Biosensor-guided enrichment and microplate-based rescreening enabled the identification of improved producers, with biosensor output showing a positive correlation with 2,3-BDO production. The selected strain was further subjected to medium optimization using a Plackett–Burman design to enhance 2,3-BDO yield, followed by validation in 5-L fed-batch fermentation. Overall, this study demonstrates an integrated strategy combining metabolite sensing, multiplex genome engineering, high-throughput screening, and fermentation optimization for the development of high-performance microbial cell factories.

**Key words:** 2,3-Butanediol, Biosensor, *Klebsiella michiganensis*, MAGE, High-throughput screening

**Representative publication:**

1. **Kim K. H.**, Cha J. W., Song E. S., Son J., Jo M.-H., Oh B.-R., Kim S. C., Jeong K. J.*, Metabolic engineering and scale-up of *Klebsiella michiganensis* for high-level 2,3-butanediol production and agricultural application, Biocatalysis and Agricultural Biotechnology, 2026, 72, 103948.

2. Song E. S., **Kim K. H.**, Lee J. Y., Jeong K. J.*, Engineering of *Corynebacterium glutamicum* for the enhanced production of optically pure (*meso*)-2,3-butanediol, Bioresources and Bioprocessing, 2026, 13, 37.

## Biography



Kyeong Ho Kim received his B.S. degree from the Department of Bioscience and Biotechnology at Hankuk University of Foreign Studies (HUFS), South Korea, in 2021. He is currently an integrated M.S.–Ph.D. student in the Department of Chemical and Biomolecular Engineering at KAIST, South Korea. His research focuses on synthetic biology and microbial metabolic engineering, with interests in transcription factor-based biosensors, high-throughput screening, genome engineering, dynamic gene regulation and microbial cell factory development. His current research aims to integrate metabolite-responsive biosensors with multiplex genome engineering and high-throughput screening for the efficient production of value-added chemicals, particularly 2,3-butanediol. He has published research on metabolic engineering and bioprocess development of microbial platforms for 2,3-butanediol production and is a co-inventor of a patent on a transcription factor-based 2,3-butanediol biosensor. He received the Hoimyung Graduate Research Award from the Korean Institute of Chemical Engineers (KIChE) in 2026 and Poster Presentation Awards from the Korean Society for Biotechnology and Bioengineering (KSBB) in 2023 and KIChE in 2022.

# Biosensor-Driven Engineering of Taxadiene Synthase for Rapid Paclitaxel Precursor Biosynthesis

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## Abstract

Paclitaxel (Taxol), a diterpenoid natural product of major clinical importance as an anticancer agent, is derived from Pacific yew and its biosynthetic pathway begins with the cyclization of GGPP into taxa-4(5),11(12)-diene (taxadiene) by taxadiene synthase (TS). Although TS is the dedicated cyclase for this reaction, it also generates a minor isomeric byproduct, iso-taxadiene. Downstream, the cytochrome P450 taxadiene-5α-hydroxylase (T5αH) acts on these two isomers very differently: acting on taxadiene, it mostly yields non-productive shunt products (OCT and iso-OCT), whereas acting on iso-taxadiene, it specifically generates taxadien-5α-ol, the intermediate required for further paclitaxel biosynthesis. Based on this substrate preference, our strategy is to redirect TS activity so that iso-taxadiene becomes the dominant cyclization product rather than a minor side product. To enable this, we are building a biosensor capable of distinguishing between the two structurally similar isomers, which will serve as the basis of a high-throughput screening system for evolving TS variants. By coupling this biosensor-based selection platform with directed enzyme engineering, we aim to establish an efficient framework for improving microbial production of paclitaxel precursors.

**Key words:** Paclitaxel, Taxadiene synthase, Biosensor, Enzyme engineering

**Representative publication:**

1. “A rationally designed miniature of soluble methane monooxygenase enables rapid and high-yield methanol production in *Escherichia coli*”, ***Nat. Commun.*** (2024), Yeonhwa Yu, Yongfan Shi, Young Wan Kwon, Yoobin Choi, Yusik Kim, Jeong-Geol Na, June Huh* & Jeewon Lee*

2. “Terminal Tryptophan-Directed Anisotropic Self-Assembly for Precise Protein Nanostructure Regulation”, ***Small*** (2024), Young Eun Jang, June Huh, Yoobin Choi, Yusik Kim, and Jeewon Lee*

## Biography



Yusik Kim is a Ph.D. candidate in the Department of Chemical and Biological Engineering at Korea University, Seoul, Republic of Korea, currently advised by Prof. Dongsoo Yang (2025–present), having previously conducted his doctoral research under Prof. Jeewon Lee (2019–2024). He holds a B.S. in Chemical and Biological Engineering and a B.A. in English Language and Literature, both from Korea University. During his early doctoral studies, he participated in the national C1 Gas Refinery Project (2020–2024), contributing to the development of practical bioconversion technologies based on functional methane monooxygenase for C1 gas utilization, work that led to co-authored publications in *Nature Communications*. His current research shifts focus to natural product biosynthesis and enzyme engineering, specifically the engineering of taxadiene synthase (TS) to preferentially produce iso-taxadiene, a minor cyclization product and the preferred substrate for downstream paclitaxel biosynthesis. To support this effort, he is developing biosensors capable of distinguishing structurally similar isomeric compounds, enabling high-throughput screening for directed enzyme evolution toward improved microbial production of paclitaxel precursors.

# A multi-input probiotic biosensor for inflammatory bowel disease

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## Abstract

Monitoring inflammatory bowel disease (IBD) relies on endoscopy or fecal biomarker assays, which are invasive or require sample collection and laboratory processing, making frequent longitudinal assessment impractical. Whole-cell biosensors offer a non-invasive alternative, and multi-input designs have improved specificity, but have largely relied on microbial metabolites rather than the host-derived proteins used clinically. Here we engineer the probiotic *Escherichia coli* Nissle 1917 (EcN) into a multifactorial biosensor that integrates both classes of marker. Two orthogonal synthetic gene circuits detect calprotectin, the established clinical biomarker of IBD, and thiosulfate, an inflammation-associated luminal metabolite; each was characterized in EcN for dose-dependence and dynamic range, then combined in a single strain. By requiring concordance between independent inputs, this design mitigates the false positives inherent to single-biomarker sensing. These validated circuits further provide a plug-and-play input layer for programmable live biotherapeutics acting within the gut.

**Key words:** Whole-cell biosensor, *Escherichia coli* Nissle 1917, Synthetic gene circuit, Inflammatory bowel disease

**Representative publication:**

1. J.Y. Xia et al., Engineered calprotectin-sensing probiotics for IBD surveillance in humans, Proc. Natl. Acad. Sci. U. S. A., 2023, 120 (32) e2221121120

2. D. Zhu et al., Microbial Biosensor for Sensing and Treatment of Intestinal Inflammation, Adv. Sci. (Weinh), 2025, 2504364

3. M.T. Buss et al., Probiotic acoustic biosensors for noninvasive imaging of gut inflammation, Nat. Commun. 2025, 16, 7931

## Biography



Minjun Chang is an integrated M.S.–Ph.D. student in the Department of Chemical and Biological Engineering at Korea University, where he works in the Synthetic Biology and Enzyme Engineering Laboratory under Prof. Dongsoo Yang. He received his B.S. in Department of Chemical and Biological Engineering from Korea University in 2024, and first joined the laboratory as an undergraduate researcher in 2022. His research focuses on engineering probiotic bacteria as programmable living devices, combining synthetic gene circuit design, whole-cell biosensor development, and CRISPR-based genome engineering in *Escherichia coli* Nissle 1917. He is currently developing a multifactorial biosensor for non-invasive monitoring of inflammatory bowel disease, and is also engaged in metabolic engineering of meroterpenoid natural products. He was previously a research intern at the Korea Institute of Science and Technology (KIST).

# Directed Evolution of Dextransucrase for Thermostability Engineering toward Rebaudioside Derivative Production

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## Abstract

Steviol glycosides are zero-calorie sweeteners obtained from *Stevia rebaudiana*, and numerous studies have used glucosylation to improve the bitter aftertaste of stevioside. Enzymatic biotransformation further enables production of non-natural rebaudioside derivatives with enhanced physicochemical properties—for example, *Leuconostoc*-derived dextransucrase converts Rebaudioside A into *O*-α-D-glucosyl-(1→6) Rebaudioside A, improving solubility and stability against pH and temperature variations. However, industrial application requires a thermostable catalyst, as elevated reaction temperatures improve compatibility with multi-enzyme cascades, accelerate reaction rates, and reduce contamination risk. To address this, structure-guided rational mutagenesis was employed to develop a variant with enhanced activity at high temperatures. A random mutant library was also constructed, and a colorimetric assay quantifying fructose—a byproduct of the enzymatic reaction—was developed to enable high-throughput screening. This study is expected to provide a practical enzyme-engineering framework for biotransforming diverse natural product derivatives and serve as a guideline for developing robust industrial biocatalysts.
This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government (MSIT) (RS-2024-00398252), and by the Bio-Industrial Strategic Technology Development Program (RS-2023-KT226226, Development of rebaudioside bio-manufacturing technology and multifunctional application products using plant-derived biomass) funded by the Ministry of Trade, Industry & Energy(MOTIE, Korea).

**Key words:** Dextransucrase, Directed Evolution, Thermostability, Rebaudioside, High Throughput Screening

**Representative publication:**

1. Chang M., Shim K., Yang D.*, Growth-coupled evolution accelerates efficient biosynthesis of natural products, Trends in Biotechnology, 2026, 44(3), 596–598.

2. Kim K., Shim K., Wang Y. W., Yang D.*, Synthetic biology strategies for the production of natural colorants and their non-natural derivatives, ACS Synthetic Biology, 2025, 14(3), 662–676.

## Biography



Kyubin Shim is a Ph.D. student in the Department of Chemical and Biological Engineering at Korea University under the supervision of Prof. Dongsoo Yang. His research aims to advance sustainable biomanufacturing through synthetic biology, metabolic engineering, and enzyme engineering. He currently develops biosensor-based screening platforms to identify and engineer microbial cell factories with enhanced capacity for natural product biosynthesis. His work also involves improving catalytic activity, stability, and overall performance of enzymes for efficient biotransformation. He is a co-first author of publications in *Trends in Biotechnology* and *ACS Synthetic Biology*. He received the Excellent Paper Presentation Award (Poster) at the 2026 Spring Meeting of the Korean Institute of Chemical Engineers (KIChE).

# Microbial Production of Urolithins through AI-Assisted Biosynthetic Pathway Reconstruction in *Escherichia coli*

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## Abstract

Gut microbiota-derived metabolites have gained attention as natural compounds with diverse health-promoting effects. Among these metabolites, urolithins have attracted particular interest because of their antioxidant and anti-aging properties and potential for pharmaceutical and functional food applications. In this study, an engineered *Escherichia coli* platform was developed for microbial production of urolithins through heterologous reconstruction of the biosynthetic pathway. *Gordonibacter urolithinfaciens*, a native producer, was selected as the source organism for identifying relevant biosynthetic enzymes.

The proposed pathway consists of sequential transformations involving more than four enzymatic reactions. The precursor was initially converted by Enzyme A, followed by downstream reactions catalyzed by Enzymes B and C. The catalytic activities of these enzymes were investigated using cell-free assays and whole-cell bioconversion systems. Reaction parameters and cofactor requirements were optimized to enhance catalytic efficiency and substrate conversion.

Because enzymes catalyzing the terminal step had not been identified, potential candidates were predicted using CLEAN, an AI-based enzyme function annotation tool. The selected enzymes were combined into an integrated biosynthetic system. Reconstruction of the complete pathway enabled production of urolithins in cell-free and fermentation platforms, demonstrating the feasibility of an AI-assisted microbial production strategy for a gut microbiota-derived bioactive compound.

**Key words:** Urolithins; Metabolic engineering; Biosynthetic pathway reconstruction; Whole-cell bioconversion; AI-assisted enzyme discovery

## Biography



Ingyeong Jeong is an integrated M.S.–Ph.D. student in the Department of Chemical and Biological Engineering at Korea University, Republic of Korea. Her research focuses on natural product biosynthesis, metabolic engineering, and enzyme engineering for the microbial production of bioactive compounds. She is particularly interested in reconstructing heterologous biosynthetic pathways in *Escherichia coli* and applying computational and AI-based tools to identify and characterize biosynthetic enzymes. Her current research involves developing an engineered *E. coli* platform for urolithin production using enzyme characterization, cell-free assays, whole-cell bioconversion, and fermentation. Through this work, she aims to establish efficient microbial production systems for gut microbiota-derived metabolites and other high-value natural products.

# Reprogramming the *Escherichia coli* Tryptophan Repressor into Serotonin- and Melatonin-Responsive Microbial Biosensors

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## Abstract

Serotonin and melatonin are tryptophan-derived metabolites involved in intestinal homeostasis and immune regulation. Serotonin is produced predominantly in the gut, while melatonin is also synthesized locally in the gastrointestinal tract. However, microbial tools for monitoring these metabolites remain limited. Here, we reprogrammed the *Escherichia coli* tryptophan repressor TrpR to develop whole-cell biosensors responsive to serotonin and melatonin. Candidate residues were selected through structural analysis and molecular docking, and the resulting variants were evaluated using ligand-dependent fluorescence assays. Serotonin-responsive TrpR variants were first identified and assessed through combinatorial mutation, and the same design framework was subsequently applied to generate melatonin-responsive variants. The resulting biosensors were characterized by using related metabolites and graded ligand concentrations to evaluate their response profiles, cross-reactivity, and dose dependence. These results demonstrate that a bacterial transcriptional regulator can be redirected toward distinct tryptophan-derived metabolites, providing a versatile platform for developing microbial biosensors for serotonin, melatonin, and related compounds.

This work was supported by the Engineering Research Center (RS-2025-02263336) funded by the National Research Foundation (NRF) of the Republic of Korea.

**Key words:** Serotonin, Melatonin, TrpR, Biosensor, PyRosetta

**Representative publication:**

1. Gong, X., Zhang, R., Wang, J., & Yan, Y. (2022). Engineering of a TrpR-Based Biosensor for Altered Dynamic Range and Ligand Preference. *ACS Synthetic Biology*, *11*(6), 2175-2183

## Biography



Sion Lee is a graduate student in the integrated MS–PhD program in the Department of Chemical and Biological Engineering at Korea University, and a member of the Synthetic Biology and Enzyme Engineering Laboratory led by Prof. Dongsoo Yang. Sion's research combines protein engineering with computational design to develop microbial biosensors for physiologically relevant metabolites. Current projects include reprogramming transcription factor scaffolds toward tryptophan-derived metabolites and building computational pipelines for de novo small-molecule binder design.

# Proteome allocation distinguishes glycolytic rewiring strategies in *Escherichia coli*

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## Abstract

Alternative glycolytic pathways can support growth despite differing in energy yield and redox chemistry, but the proteome-level demands of operating these routes remain poorly understood. Here we combined growth phenotyping and quantitative proteomics to compare six *Escherichia coli* strains implementing native-enzyme or heterologous alternatives to canonical glycolysis. All engineered strains shared increased abundance of thiamine-associated proteins and reduced abundance of motility-related proteins, defining a conserved response to glycolytic disruption. Superimposed on this response were design-specific proteomic states: even three non-isogenic strategies carrying the same glycolytic deletion showed distinct changes in cellular functions. In the selected allocation analyses, the three heterologous designs showed larger core-pathway shifts than the three native-enzyme strategies, alongside different changes in other sectors. Thus, the glycolytic designs examined here differed not only in pathway chemistry, but also in their associated proteome reallocation patterns. These findings identify proteome economy as an important consideration when engineering central metabolism.

**Key words:** *Escherichia coli*; glycolytic rewiring; proteome allocation; central carbon metabolism; metabolic engineering

## Biography



Yating Zhao is a PhD candidate at Charité - Universitätsmedizin Berlin, pursuing her doctoral degree through Freie Universität Berlin. She received her master's degree from China Agricultural University. Her research focuses on synthetic biology, metabolic engineering, and the remodeling of microbial metabolic networks. She applies molecular biotechnology, metabolic engineering, adaptive laboratory evolution, growth phenotyping, and quantitative proteomics to investigate how *Escherichia coli* responds to redesigned central carbon metabolism. Her current projects examine proteome allocation associated with alternative glycolytic pathways and the engineering and evolution of non-canonical pentose utilization pathways. Her broader research interest is to understand how metabolic pathway design shapes cellular resource allocation, physiological adaptation, and strain performance.

# Engineering L-Lactic Acid Production in *Cupriavidus necator* under Different Trophic Conditions

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## Abstract

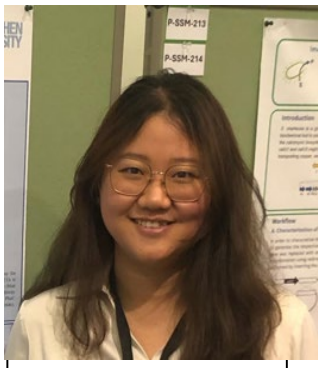
Lactate is a valuable platform chemical widely used in multiple industries, yet conventional lactic acid production relies on sugar-based feedstocks, creating economic and sustainability challenges. Carbon dioxide offers a renewable, non-food alternative feedstock and the chemolithoautotrophic *Cupriavidus necator* is a promising host for lactic acid biosynthesis through gas fermentation using $CO_2$ as the sole carbon source. Under mixotrophic condition, glycerol can enhance soluble hydrogenase expression in *C. necator* while serving as an auxiliary carbon source. To evaluate the production potential under autotrophic and mixotrophic conditions, nine *L*-lactate dehydrogenases (*L*-LDHs) from different lactic acid bacteria were selected and heterologously produced in engineered *C. necator* strains Re2133 (Δ*phaB1* Δ*phaB2* Δ*phaB3* Δ*phaC*) and RL3 (Re2133 Δ*dld* Δ*lldA* Δ*lldD*). Among the enzymes tested, one of the *L*-LDHs produced the highest lactate titer in nitrogen starvation condition, reaching 599 mg/L in *C. necator* RL3 during autotrophic gas fermentation and 960 mg/L in *C. necator* Re2133 under mixotrophic cultivation. Nitrogen limitation markedly increased extracellular pyruvate accumulation under mixotrophic condition, indicating restricted downstream pyruvate conversion. These findings identify an effective *L*-LDH for autotrophic and mixotrophic *L*-lactate production in *C. necator* and provide targets for further metabolic engineering.

**Key words:** chemolithoautotrophy, mixotrophy, lactate production, metabolic engineering

**Representative publication:**

## Biography

Hongyi Fan is a PhD student in Prof. Lars Lauterbach lab in RWTH, Germany and working on PLA copolymer production in Valor$CO_2$ project funded by Bioeconomy Science Center (BioSC), Germany. She finished her Bachelor of Science in biology science in Yunnan University, China and her Master of Science in biotechnology and bioresource in Justus-Liebig University Giessen, Germany.



# Engineering *Escherichia coli* Nissle 1917 for Enhanced Serotonin Biosynthesis toward Gut–Brain Axis Applications

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## Abstract

The gut microbiota contributes to host physiology by producing bioactive metabolites that influence both gastrointestinal and neurological functions. Serotonin, a tryptophan-derived neurotransmitter, is closely involved in mood, cognition, and intestinal homeostasis, and most peripheral serotonin is generated in the gastrointestinal tract. This makes the gut microbiome an attractive target for modulating gut–brain axis signaling. Here, we engineered the probiotic *Escherichia coli* Nissle 1917 (EcN) for efficient and sustained serotonin biosynthesis. A heterologous serotonin pathway was integrated with tetrahydrobiopterin (BH4) biosynthesis and regeneration modules to support catalytic activity. The optimized strain, SER09, showed a near complete conversion of L-tryptophan into serotonin.

Its in vivo performance was further evaluated in a murine model, where stable gastrointestinal colonization was observed after oral administration. Serotonin was detected in intestinal tissues, feces, serum, and brain, indicating the systemic distribution of microbiota-derived metabolites. Ongoing studies are assessing the resulting effects on gut–brain axis signaling. Overall, this study establishes a genetically stable live biotherapeutic platform for localized neurotransmitter production in the gut and supports the potential of engineered probiotics to modulate host metabolic communication.

**Key words:** Serotonin, Gut-brain axis, Live biotherapeutics, Engineered probiotic, *Escherichia coli* Nissle

**Representative publication:**

1. Rosero, N. A., Cao, J., & Wang, X., Bacteria Engineered to Produce Serotonin Modulate Host Intestinal Physiology, *ACS Synthetic Biology*, 2023, 12(9), 2603–2612.

2. Dinan, T. G., & Cryan, J. F., Brain-Gut-Microbiota Axis and Mental Health, *Psychosomatic Medicine*, 2017, 79(8), 920–926.

3. Shen, P., Gu, S., Jin, D., et al., Engineering Metabolic Pathways for Cofactor Self-Sufficiency and Serotonin Production in Escherichia coli, ACS Synthetic Biology, 2022, 11(8), 2889–2900.

4. Kan, A., Gelfat, I., et al., Plasmid Vectors for In Vivo Selection-Free Use with the Probiotic E. coli Nissle 1917, ACS Synthetic Biology, 2021, 10(1), 94–106.

## Biography



Hyunji Yoon is a graduate student in the integrated M.S.–Ph.D. program at the Department of Chemical and Biological Engineering, Korea University, where she conducts research in the Laboratory of Synthetic Biology and Enzyme Engineering under the supervision of Prof. Dongsoo Yang. She joined the program in March 2026 after completing her undergraduate studies in Biotechnology at Korea University. Her research focuses on microbial metabolic engineering for natural product biosynthesis, with particular interests in polyketide synthase (PKS) engineering, gut microbiome–host interactions, and CRISPR-based genetic tools. Her current project involves engineering the probiotic *Escherichia coli* Nissle 1917 as a live biotherapeutic platform for localized serotonin biosynthesis, aiming to develop new strategies for modulating gut–brain axis signaling through synthetic biology.

# Methane-Based Production of D-Pantothenic Acid by the Newly Isolated Methanotroph *Methylosinus sporium* UOS2

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## Abstract

Methane bioconversion offers a sustainable route for mitigating greenhouse gas emissions while producing value-added compounds. In this study, we investigated D-pantothenic acid (vitamin B5) production from methane using *Methylosinus sporium* UOS2, a newly isolated type II methanotroph obtained from wastewater. Genome analysis revealed that UOS2 possesses a complete biosynthetic pathway for D-pantothenic acid, and cultivation with methane as the sole carbon source confirmed D-pantothenic acid production. Optimization of the nitrogen source increased biomass by 55.3% and enhanced D-pantothenic acid production by 37.8% to 0.37 mg/L compared with ammonium-based cultivation. To further enhance production, *panE*, encoding ketopantoate reductase, was overexpressed, resulting in a 3.5-fold increase in D-pantothenic acid titer to 1.29 mg/L at 96 h. These results demonstrate the potential of *M. sporium* UOS2 as a platform for direct methane-to-vitamin conversion and show that medium optimization combined with targeted metabolic engineering can effectively improve product formation.

**Key words:** Methane, Methanotrophic bacteria, Bioconversion, D-pantothenic acid, Metabolic engineering

**Representative publication:**

1. Kim M. S., Choi Y. J.*, Sustainable D-pantothenic acid production from methane using a newly isolated methanotrophic bacterium, *Methylosinus sporium* UOS2, Journal of Material Cycles and Waste Management, 2026, 28, 2607–2617.

## Biography



Min Sun Kim is a graduate student at the University of Seoul, Republic of Korea. His research focuses on the metabolic engineering of methanotrophic bacteria for the sustainable production of value-added compounds from methane. His current work includes the development of *Methylosinus sporium* UOS2 as a microbial platform for D-pantothenic acid production through metabolic engineering. He is particularly interested in discovering and characterizing novel microorganisms, exploring new bioproducts that can be produced from methane, and developing metabolic engineering strategies to enhance their production. His broader research interests include methane bioconversion, microbial metabolic engineering, and the biosynthesis of high-value chemicals.

# Structure-based design of *P. ananatis* glutamate dehydrogenase mutants with altered coenzyme specificity

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## Abstract

The Gram-negative bacterium *Pantoea ananatis* represents a promising biotechnological platform. We previously showed that *P. ananatis* glutamate dehydrogenase ($GDH_{Pa}$) is a bidirectional enzyme with dual cofactor specificity *in vitro,* but *in vivo* it prioritizes $NAD^+$-dependent glutamate deamination. Published data indicate that altering the coenzyme specificity of GDH often requires substitutions at the P2 and P7 positions within the cofactor-binding pocket. Our analysis revealed that NADP(H)-dependent GDHs typically contain serine/tyrosine at the P2 position and serine at the P8 position, which form hydrogen bonds that stabilize NADP(H). In contrast, $GDH_{Pa}$ contains a histidine (H250) at position P8, which efficiently binds NADPH only under acidic conditions. Guided by structural data and AI-predicted models, we mutated H250S to shift the specificity of $GDH_{Pa}$ toward NADP(H). H250S substitution increased $GDH_{Pa}$ amination activity at non-saturating NADPH concentrations. Furthermore, overproduction of $GDH_{Pa}^{H250S}$ restored the growth of the glutamate-auxotrophic SC17(0)Δ*gltB* strain without glutamate and increased tolerance to toxic glutamate levels. Thus, we demonstrate for the first time that substitution of P8 histidine with serine reverses the direction of the $GDH_{Pa}$ reaction *in vivo*, offering a new strategy for engineering microbial biotechnological platforms.

**Key words:** cofactor specificity, *Pantoea ananatis*, glutamate dehydrogenase

**Representative publication:**

1. Kharchenko M. S., Skripnikova V. S., Rostova J. G., Zakataeva N. P., Glutamate dehydrogenase from *Pantoea ananatis*: A new bacterial enzyme with dual coenzyme specificity, PLoS One, 2025, 20(8), e0328289.

## Biography

**Mariia Sergeevna Kharchenko**

Researcher, JSC Ajinomoto-Genetika



Mariia Kharchenko is a researcher and Ph.D. candidate at JSC Ajinomoto-Genetika Research Institute, specializing in metabolic engineering of industrial microbial hosts. She earned her degree in molecular genetics from Moscow State University in 2015.

Her doctoral research focuses on optimizing nitrogen metabolism for enhanced metabolite production, with a specific emphasis on characterizing a novel, bidirectional glutamate dehydrogenase from *Pantoea ananatis*. Utilizing an integrated approach of classical genetics, genetic engineering and computational biology, she investigates gene expression regulation and the structural basis of dual cofactor specificity. Her broader expertise includes strain development and genetic tool design, with successful applications in industrially valuable *Bacillus* hosts, *Bacillus subtilis* and *B. amyloliquefaciens.*

Her findings have been published in high-impact, peer-reviewed scientific journals and presented through poster presentations at international conferences. She holds several patents and her industrial innovations have been recognized with an internal award from the institute.

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Metabolic Engineering Summit 2026

# Utilization of toxic, hard-to-degrade plastic monomers by engineered *Pseudomonas putida* KT2440

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## Abstract

The current chemical industry, a linear economy, is deeply fossil-based and based on environmentally unsustainable production processes. Regarding the plastics industry, to date, there are no established sustainable recycling processes for the rising amounts of end-of-life plastic waste, which is therefore commonly landfilled or thermally recycled. Within the interdisciplinary WSS Research Centre catalaix the transformation of this linear economy into a multidimensional circular economy is aimed by converting diverse plastic waste into high-value chemical building blocks. This study focuses leveraging metabolic engineering to expand the metabolism of the microorganism *Pseudomonas putida* KT2440 to biocatalytically convert toxic monomers derived from hydrogenolyzed real-world plastic polymers. A strain was engineered, capable of degrading PET, PU and PA monomers. In a continuous cultivation, the strain was subjected to fluctuating plastic monomer mixtures to mimic changing inputs of plastic waste streams, in which it showed high flexibility and robustness. Currently, the range of substrates derived from PA is further expanded. Besides enabling growth, the monomers will serve as substrates for *P. putida* to produce the biopolymer cyanophycin, which will be valorized into novel products within catalaix.

**Key words:** Metabolic engineering, *Pseudomonas putida* KT2440, Bioeconomy, Biopolymers, Plastic degradation

**Representative publication:** Not published yet

## Biography



Anka Siebericchs (26 years old) was born in Aachen, Germany. She studied biology at the Heinrich-Heine University Düsseldorf (Germany), whereby she conducted her bachelor thesis in the group of Prof. Dr. Nick Wierckx at the Forschungszentrum Jülich (Germany). During this work, she prevented the accumulation of the byproduct *trans*-cinnamic acid in 4-hydroxybenzoate-producing *Pseudomonas taiwanensis* VLB120. She completed her master thesis under the supervision of Prof. Dr. Karl-Erich Jaeger at the Institute of Molecular Enzyme Technology at the Forschungszentrum Jülich (Germany). In her research project she investigated the *in vivo* transformation of tripyrrolic prodiginins by cyclase enzymes in *Pseudomonas putida* KT2440. To broaden her expertise in industrial biotechnology, she subsequently joined the biotechnology unit at the Bayer AG in Leverkusen (Germany). In March 2025 she started her PhD project the field of biotechnology at the Institute of Applied Microbiology at the RWTH Aachen (Germany) under the supervision of Prof. Dr. Lars Blank. Within the project catalaix, which aims the transformation of plastic recycling into a multi-dimensional open-loop circular economy, her research currently focuses on the conversion of toxic, hard-degradable plastic monomers into high-value products by the development of *P. putida* KT2440 strains by metabolic engineering.

# Multidimensional engineering and peptidoglycan modulation for surfactin production in *Bacillus subtilis*

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## Abstract

Surfactin is a multifunctional lipopeptide with potential in antimicrobial treatments and sustainable bioremediation. However, its production needs to be enhanced for industrial scalability. This study presents a multidimensional engineering strategy to enhance surfactin synthesis in *Bacillus subtilis*. Overexpression of SacA (phosphosucrase) and inactivation of Ldh (lactate dehydrogenase) improved carbon utilization for surfactin synthesis. Further enhancement of surfactin production was achieved by overexpressing FadA (*β*-oxidation rate-limiting enzyme) to optimize fatty acid metabolism. Additionally, modifications in quorum sensing regulators ComQX and YmcA further improved surfactin titer. Enhancements in peptidoglycan integrity, through modifications of LytH (endopeptidase) and DapB (dihydrodipicolinate reductase), resulted in a 4.1-fold increase in cell viability. Engineered strain SUR-a39 achieved a surfactin titer of 32.47 g/L in a 5 L bioreactor, with a productivity of 1.01 g/L/h, showcasing exceptional process compatibility and production potential for industrial application. These strategies provide valuable insights for the development and production of surfactin and other high-value biochemicals.

**Key words:** Multidimensional engineering; Cell lysis; Lipopeptide; Surfactin; Peptidoglycan regulation

**Representative publication:**

1. Xia, L.*, Hou, Z., Zhu, F., Wen, J., Multidimensional engineering and peptidoglycan modulation for surfactin production in Bacillus subtilis. Chem. Eng. J. 2025, 516, 163954.

2. Zhu, F., Xia, L., Wen, J., Zhang, L.*, Recent Advances in the Biosynthesis of Mid- and Long-Chain Dicarboxylic Acids Using Terminally Oxidizing Unconventional Yeasts. J. Agric. Food Chem. 2024, 72(36), 19566-19580.

## Biography



Fuzhou Zhu, a doctoral student majored in biochemical engineering at Tianjin University. His research involves the metabolic engineering of chassis strains such as *Escherichia coli*, *Corynebacterium glutamicum*, and nonconventional yeasts, along with the process development for protein-based pharmaceuticals and secondary metabolites.