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ABSTRACTS

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Biomanufacturing Promoted by Synthetic Biology with High-Quality Productivity Forces

He Huang (黄和)
Nanjing Normal University

Abstract

Synthetic biology is a transformative field that unites biology, engineering, chemistry, and information technology to design and construct novel biological systems optimized for industrial applications. By integrating advancements in gene sequencing, biological engineering, and computer modeling, synthetic biology surpasses the limitations of natural biological systems, generating new frameworks that enhance efficiency, scalability, and sustainability. This multidisciplinary approach is being applied across sectors, including medicine, energy, chemical production, agriculture, and environmental management with offering solutions that are low-carbon, sustainable, and cost-effective. Currently, synthetic biology-based biomanufacturing is emerging as a primary driver of innovation and economic growth, positioning itself as a cornerstone of the future bioeconomy. Here, we highlight advancements in the production of next-generation functional sugars and acids et al., showcasing synthetic biology's role in transforming traditional manufacturing and setting new standards for efficiency and environmental stewardship.

Brief Biography

He Huang, an academician of the Chinese Academy of Engineering, Vice President of Nanjing Normal University, Professor, doctoral supervisor, has been awarded the National Distinguished Young Scholars Fund Project and other multiple national talent projects. Engage in research on metabolic engineering of industrial microorganisms and synthetic biology for a long time. The research achievements include two second prizes of the National Technology Invention Award and two first prizes of the Ministry of Education Technology Invention Award as the first completer. He has also received the Ho Leung Ho Lee Foundation Science and Technology Award-Young Innovation Award, the Min Enze Energy and Chemical Award- Outstanding Contribution Award, the Highest Scientific and Technological Award in Business in China and the Bill & Melinda Gates Foundation Young Scientist Award. He has been continuously selected as one of the most highly cited Chinese researchers in the field of chemical engineering by Elsevier from 2014 to 2022.

Next-Generation Precise Genome Editing Technologies and Their Applications in Crop Improvement

Caixia Gao (高彩霞)
Institute of Genetics and Developmental Biology, Chinese Academy of Sciences

Abstract

The development of targeted genome modification in plants has gone through an evolution from generating random mutations, to creating precise base substitutions, followed by producing insertions, substitutions, and deletions of small DNA segments, and finally achieving precision manipulation of large DNA segments. These four developments have laid a solid technological foundation for carrying out plant basic research and precise molecular breeding. In this presentation, I will describe these four stages of genome editing and systematically outline the technological principles underlying each developmental stage. I will also provide some examples demonstrating their application in the creation of new agricultural crops for the future.

Brief Biography

Caixia Gao is a principal investigator at the Institute of Genetics and Developmental Biology of the Chinese Academy of Sciences. Prior to joining the IGDB, she served as a research scientist at DLF's biotechnology group in Denmark. Her research group develops precise and specific genome editing technologies and applies modern biotechnologies to study plant genetic traits and develop high-quality traits including disease resistance and stress tolerance in a variety of crop plants. Her team has published more than 100 papers on plant genome editing, which have cumulatively been cited nearly 30,000 times.

Enzyme promiscuity, underground metabolism or hidden pathways in microbial metabolism: the good and the bad in Synthetic Biology applied to White Biotechnology

Jean Marie FRANCOIS
Toulouse Biotechnology Institute

Abstract

White biotechnology also termed industrial biotechnology aims at producing valuable goods by microbial fermentation from renewable -preferentially- non-edible biomass. Those valuable goods can be naturally produced compounds such as ethanol, lactic acid, succinic acid by the microorganism, but more often nowadays it is a non-natural substance that is wishing to be produced such as isobutyric acid, vanillin, 2, 4-dihydroxybutyric acid or even more complex molecules like amorphodiene and flavonoids. For these latter, synthetic biology tools are well appealing to generate metabolic pathways that do not exist in Nature. Beside all technical difficulties to design, implement, optimise the pathway reactions through the so-called DBLT cycle, the myriad of potential interactions between the components of the new metabolic pathway and the existing natural metabolic network unveils an underground metabolism or hidden metabolic pathway caused by the promiscuity of numerous metabolic enzymes, or triggers inhibitory crosstalk leading to a detour of metabolites from the new pathway by the promiscuity of enzyme activities in the existing metabolic network, making metabolic prediction models largely inaccurate. Through examples, I will expose that harnessing the power of underground metabolism can confer fitness advantage and adaptation under specific environments and thus can be exploited to enhance the physiological performance of a microbial factory1. On the other hand, the activation of an underground metabolism can be highly detrimental, thwarting the microorganisms' production capacity for its added-value compound[2].

Brief Biography

Jean Marie FRANCOIS is Exceptional class professor of at Federal University of Toulouse, National Institute of Applied Sciences, department of Bioengineering. His research activity concerns integrated physiology and functional genomics in microbial systems, with a specific focus on genetic and metabolic regulation and refactoring carbon and energy metabolism towards production of bio-based chemicals for renewable carbon sources. He is author of more than 230 papers in international journals, with a H-index of 70 (Scopus) and holds 27 patents. He is Editor-in-Chief of BMC Biotechnology for Biofuels and Bioproducts and Editor in Chief of Frontiers in Bioengineering and Biotechnology, section Synthetic Biology. He is member of the European Federation of Biotechnology.

Artificial Intelligence Systems for Enhancing Biomanufacturing

Baishan Fang (方柏山)
Xiamen University

Abstract

The biomanufacturing concept holds the promise of green industrial production of biofuel or chemicals, in which fossil resources are substituted by renewable biomass, securing sustainable socioeconomic development. In addition to an optimal microbial cell factory, the fermentation mode is also a key factor of biomanufacturing. Although fed-batch fermentation is generally an advantageous mode of submerged fermentation, it requires more sophisticated equipment for online measurement, control techniques for process management, and intelligent decisions during the entire operational process, which are great challenges for robust and green industrial production of Green Biosynthesis. Here, we developed an extraordinary artificial intelligence system for entirely automatic fed-batch fermentation of 1,3-propanediol, including a Sensor, Predictor, Controller, and automation system. Compared with the constant-speed fed-batch fermentation strategy, the artificial intelligence system could not only automatically regulate the feeding rate and maintain a low concentration of glycerol, but also increase the 1,3-PDO concentration and yield. Combined with dynamic metabolic flux analysis, we demonstrate that a low concentration of glycerol controlled by an artificial intelligence system contributes to the balance of the redox pool. The artificial intelligence system for automatic, robust, and enhanced 1,3-propanediol concentration and yield has been successfully developed which not only increases glycerol utilization efficiency but also decreases the medium cost. It also eliminates the dependency on expensive online instruments and staffing, which not only beneficial for the sustainable biosynthesis of 1,3-propanediol but also adapted to similar production processes.

Brief Biography

Prof. Baishan Fang is a Distinguished Professor at Xiamen University, China. He received B.S. degree in Chemical Engineering from Zhejiang University in 1982, and Ph.D. degree in Chemical Engineering from Tianjin University in 2000. He joined Institute of Biochemical Engineering, University of Stuttgart, Germany as a Visiting Scholar from 1991 to 1993, German Biotechnology Research Center as Visiting Scholar from 2000 to 2001, and then visited the University of Washington as a Senior Researcher in Dec. 2018. His research interest focuses on Synthetic biology and bioinformatics, directed enzyme evolution and biocatalysis, biorefining and bioprocess optimization, microecology and culturomics. He has published more than 100 papers in Nat Catal, AIChE *et al*.

Intelligent Drug Discovery in Practice on Challenging Targets

Shuangjia Zheng (郑双佳)
Shanghai Jiao Tong University

Abstract

The typical drug discovery paradigm is a tedious process, requiring extensive manual effort and relying heavily on expert intuition. Artificial intelligence (AI) has already started to transform this process and promises to transition drug discovery from intuition-driven to information-driven.

In this talk, I will discuss our efforts to broaden the application of deep representation learning in real-world drug discovery. I will begin by outlining our approaches to molecular representation learning, where we have developed several algorithmic tools to effectively capture protein-ligand interactions and perform data-driven virtual screening. Following this, I will delve into our recent advancements in dynamic complex structure modeling using physics-aware diffusion networks, demonstrating how these models facilitate real-world drug development and lead to the discovery of numerous potent molecular candidates targeting challenging proteins. Finally, I will share our latest work in rational PROTAC and antibody design using deep generative models, highlighting the identification of lead candidates with high potency and favorable developability

Brief Biography

Shuangjia Zheng is an Assistant Professor at the Global Institute of Future Technology (GIFT), Shanghai Jiao Tong University. His research interests focused on applying machine learning for the discovery and design of novel therapeutic biomolecules. He earned his Ph.D. in Computer Science at Sun Yat-sen University and served as a visiting scientist at MIT and Harvard. His work has been published in prestigious journals such as Nat. Mach. Intell, Nat. Biomed. Eng., Nat. Commu. and presented at leading conferences like NeurIPS and KDD. His work has been recognized by numerous awards, including the Asian Young Scientist Fellowship, Young Elite Scientists Sponsorship by CAST, Forbes 30 Under 30 Asia, and the WAIC Rising Star Award.

Reaction Enzyme Mining and Evaluation Based on Pre-Trained Protein and Reaction Language Models

Xiaoping Liao(廖小平)
Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences

Abstract

The pursuit of designing novel metabolic pathways for synthesizing industrially important chemicals stands as a pivotal research frontier. Central to this endeavor is the formidable challenge of identifying suitable enzymes capable of catalyzing reactions within these engineered pathways, which dictates the rate and selectivity of chemical transformations, particularly when faced with non-natural reactions. Synthetic biologists navigate this challenge by integrating principles of enzyme mining, experimental validation and enzyme engineering, leveraging computational tools and experimental techniques to unlock the potential of enzymes in novel contexts. Existing enzyme mining approaches mainly rely on reaction similarity computations, but current tools struggle to locate the most similar reactions and face limitations in the subsequent enzyme screening and evaluation, making further experimental validation difficult.

To address this need, in 2024, we developed the REME platform (https://reme.biodesign.ac.cn), which combines atom-to-atom mapping and reaction similarity computation based on multiple reaction representations. REME enables rapid ranking of similar reactions and convenient visualization. Additionally, REME allows users to filter similar reactions and associated proteins based on atom type changes and specific functional groups, and assess them using tools such as ESP, DLKcat/TurNup/UniKP, DeepET, and EpHod, helping experimental scientists quickly identify potential candidate enzymes.

Brief Biography

Liao Xiaoping, Ph.D., is a researcher at the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences. He graduated with a bachelor's degree from the University of Science and Technology of China in 2006 and earned his Ph.D. from the Academy of Mathematics and Systems Science, Chinese Academy of Sciences, in 2011. From 2011 to 2014, he conducted postdoctoral research at the University of Alberta in Canada. In 2014, he joined the Tianjin Institute of Industrial Biotechnology, where he focuses on intelligent analysis of industrial biological big data and develops core databases, algorithms, and tools.

He has constructed several industrial biology-specific databases, including the glycosyltransferase database pUGTdb and the Escherichia coli regulatory landscape ERMer. Additionally, he has developed a series of bio-design software, such as the automated editing sequence design platform AutoESD and the pathway design platform CAVE. He has also advanced AI tools, including the protein function prediction algorithm HDMLF, the enzyme mining and evaluation tool REME, and the protein homomeric state prediction algorithm DeepSub.

In recent years, he has published over 50 papers in high-impact journals such as *Nucleic Acids Research*, *Science Advances*, *Molecular Plant*, and *Research*, with more than 2,000 citations. He has led several national and provincial-

level projects, including key interdisciplinary projects funded by the National Natural Science Foundation of China, the Strategic Priority Research Program of the Chinese Academy of Sciences, the Tianjin Synthetic Biotechnology Innovation Capacity Improvement Project, and the Innovation Fund of Haihe Laboratory of Synthetic Biology.

Artificial General Intelligence for Protein Engineering Based on Pre-Training

Liang Hong (洪亮)
Shanghai Jiao Tong University

Abstract

AlphaFold has solved the challenge of predicting the three-dimensional structure of proteins and their complexes, but having the correct three-dimensional structure does not necessarily imply that a protein has a specific function. Our team has spent the past 3 years developing a general-purpose artificial intelligence platform for protein engineering – the Pro series – based on pre-trained models. Unlike AlphaFold, the Pro series groundbreaking achieves precision protein design directly from sequence to function. Through pre-training, the large model can learn the known protein sequence and structural characteristics in nature, and explore and understand the mapping law of protein sequence and function in nature. As a result, we have developed a set of general large model that can efficiently design various protein products with enhanced stability, activity and function. Using this method in just one year, we have successfully designed and modified more than 20 proteins , with experimental validation completed in wet labs (including nucleic acid polymerases, gene editing enzymes, IVD enzymes, antibodies, etc.). Among these, two proteins have been scaled up for production and applied in industrial production.

Brief Biography

Professor Hong received his bachelor's degree in physics from the University of Science and Technology of China in 2004, his master's degree in physics from the Chinese University of Hong Kong in 2006, and his doctorate degree in polymer science from the University of Akron in 2010. He did his postdoctoral research in computational biology at Oak Ridge National Laboratory in 2010 and joined Shanghai Jiao Tong University in December 2014. He is currently a distinguished professor of Academy of Natural Sciences/School of Physics and Astronomy/School of Pharmacy, Shanghai Jiao Tong University, and director of Artificial Intelligence Biomedical Center, Zhang Jianggao Institute of Research, Shanghai Jiao Tong University. Engages in computational, artificial intelligence, and experimental approaches to molecular biophysics and protein design research. In 2016, he was selected as a national high-level talent Young expert, and in 2021, he was selected as a Changjiang Scholar of the Ministry of Education. He has published more than 70 SCI papers in nature, science, PNAS and other journals. Participated in and led the development of several innovative algorithms to improve the research and development efficiency of functional proteins.

Π-primenovo: An Accurate and Efficient Non-Autoregressive Deep Learning Model for De Novo Peptide Sequencing

Siqi Sun (孙思琦)
Fudan University

Abstract

Peptide sequencing via tandem mass spectrometry (MS/MS) is fundamental in proteomics data analysis, playing a pivotal role in unraveling the complex world of proteins within biological systems. In contrast to conventional database searching methods, deep learning models excel in de novo sequencing peptides absent from existing databases, thereby facilitating the identification and analysis of novel peptide sequences. Current deep learning models for peptide sequencing predominantly use an autoregressive generation approach, where early errors can cascade, largely affecting overall sequence accuracy. And the usage of sequential decoding algorithms such as beam search suffers from the low inference speed. To address this, we introduce π-PrimeNovo, a non-autoregressive Transformer-based deep learning model designed to perform accurate and efficient de novo peptide sequencing. With the proposed novel architecture, π-PrimeNovo achieves significantly higher accuracy and up to 69x faster sequencing compared to the state-of-the-art methods.

Brief Biography

Siqi Sun is a Young PI at Fudan University and the AI For Science Group at Shanghai AI Lab. He completed his Ph.D. at the TTIC under Professor Jinbo Xu and earned his Bachelor's from Fudan University. Between 2018 and 2022, he contributed to research on language model and its applications at Microsoft Research. Siqi's work in deep learning spans life sciences and natural language processing, and has published numerous papers on top conferences and journals.

Combining Multi-Omics and Metabolic Modelling to Decipher Cellular Stress Mechanisms for Antimicrobial Pharmacology and Biomanufacturing Applications

Yan Zhu (朱岩)
Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences

Abstract

Multidrug resistance presents a critical challenge to global health, with the emergence of widespread superbugs urgently demanding novel antimicrobial treatments. Concurrently, the escalating climate crisis and the pressing need for sustainable development underscore the importance of biomanufacturing through synthetic microbial cell factories to produce biochemicals, biofuels, and biomaterials. While microbial pathogens are targeted by antimicrobials that specifically disrupt their cellular machinery or structure, microbial cell factories are exposed to industrial stresses that impose a broader spectrum of detrimental impacts. However, the underlying cellular mechanisms governing these responses are intricate and remain only partially understood. Recent advances in mass spectrometry, sequencing technologies, and data science have enabled the integration of multi-omics approaches with computational modelling to effectively unravel the cellular dynamics underlying responses to these diverse stresses. In this context, I utilise polymyxin treatment against Gram-negative superbugs as a model, combining multi-omics analyses and computational approaches to decipher the mechanisms of antimicrobial action, drug synergy, resistance development, and nephrotoxicity. For industrial applications, I integrate correlative multi-omics approaches with metabolic modelling to elucidate the mechanisms by which *Corynebacterium glutamicum* cell factory responds to various industrial stresses, including methanol exposure, low pH, hydrogen peroxide, furfural, high osmolarity, and heat. Our integrated omics and metabolic modelling analyses consistently revealed a reduction in central metabolism, alongside perturbations in redox homeostasis and energy biogenesis under multiple stress conditions. Ultimately, the combination of multi-omics and metabolic modelling provides a powerful framework for deciphering the complex interplay of biological pathways in response to both antimicrobial and industrial stresses, offering valuable insights for the development of more effective antimicrobial treatments targeting superbugs, as well as for optimising microbial cell factories for biomanufacturing.

Brief Biography

Dr Zhu received his PhD in Microbiology in 2013 from the University of the Chinese Academy of Sciences (UCAS). He subsequently joined the University of Queensland and Monash University, where he worked on food microbiology and systems pharmacology. In 2022, he accepted a full-time professorship at the Tianjin Institute of Industrial Biotechnology (TIB), CAS, where he focuses on systems biotechnology. His research is well-supported through funding from TIB, CAS, and the Ministry of Science and Technology (MOST). Dr Zhu has published two book chapters and 96 peer-reviewed papers to date. His recent first-authored research on polymyxin dependence was featured on the front cover of Advanced Science in 2020. Additionally, his work on a novel synthetic lipopeptide antibiotic, published in Nature Communications in 2022, was selected as an Editorial Highlight. Dr Zhu serves as a member of the Technical Committee of Computational Synthetic Biology at the Chinese Bioinformatics Society and as an Associate Editor for the International Journal of Antimicrobial Agents.

Understanding and Engineering Proteins with Geometric Deep Learning

Bingxin Zhou (周冰心)
Shanghai Jiao Tong University

Abstract

Protein engineering plays a pivotal role in addressing global challenges, from healthcare to sustainability. Recent research leverages deep learning methods, such as language models and graph neural networks, to analyze protein sequences, structures, and functions. This emerging biotechnology significantly reduces the cost and complexity of studying and modifying proteins. This talk introduces our recent deep learning solutions for protein engineering, aimed at enhancing protein functionality and properties to meet practical needs. We address a range of challenges faced by biologists, including zero-shot mutations, deep mutations, few-shot dry-wet experimental iterations, and patent blockades. The reliability and generalizability of our solutions have been validated through wet lab experiments on a variety of proteins and protein assays.

Brief Biography

Bingxin Zhou is currently a Research Scientist at Shanghai Jiao Tong University. She obtained her Ph.D. from the University of Sydney, Australia, in 2022, and was also a visiting scholar at the University of Cambridge. Her research primarily focuses on the development of deep learning techniques, especially geometric deep learning, to address challenges in biology such as enzyme engineering, metabolic gene networks, and proteome-wide evolutionary analysis. For model development, she has published useful Graph Neural Network models for static, dynamic, heterophilic, and noisy graphs in IEEE TPAMI, JMLR, ICML, NeurIPS, etc. In the field of protein analysis and application, she has established general deep learning frameworks for protein engineering and sequence design, with promising experimental evaluations. Some results have been published in eLife, Chem. Sci., JCIM, etc.

Exploration of the Biological Diversity of RNA-guided Miniature Cas12 Genome Editors

Quanjiang Ji (季泉江)
ShanghaiTech University

Abstract

CRISPR-Cas9/Cas12a genome editing systems have been widely harnessed for genetic engineering and gene therapeutics. However, the large sizes of these CRISPR effector nucleases restrict their flexibility in therapeutic applications that use the cargo-size-limited adeno-associated virus delivery vehicle. We recently developed miniature CRISPR-Cas12f and -Cas12n systems for efficient genome editing. We studied the detailed DNA recognition and cleavage mechanisms of the two systems. Moreover, we engineered a CRISPR-Cas12f variant with enhanced editing activity using structure-guided protein engineering. The small sizes of the nucleases offer advantages for cellular delivery, and characterizations of the nucleases will facilitate engineering more compact genome-manipulation technologies.

Brief Biography

Dr. Quanjiang Ji is a Professor at ShanghaiTech University and his research focuses on the mining and engineering of new CRISPR-Cas systems and the development of CRISPR-based gene therapeutics. He received his bachelor's degree from Nanjing University in 2009, and PhD from University of Chicago in 2014. He pursued his postdoctoral training from University of California, Berkeley from 2014 to 2016. He has developed two miniature genome editing systems, CRISPR-Cas12f and -Cas12n. Moreover, he has developed facile CRISPR-based genetic manipulation methods in pathogenic bacteria.

High-Precision Base Editing Technology

Erwei Zuo (左二伟)
Institute of Agricultural Genomics, Chinese Academy of Agricultural Sciences

Abstract

Base editing technology is a cutting-edge genome editing tool that has emerged in recent years. By combining the CRISPR/Cas editing system with deaminases, it merges the catalytic efficiency of these enzymes with the precise targeting capabilities of CRISPR/Cas. This approach enables deamination reactions at specific sites on DNA or RNA strands, facilitating accurate base substitutions. Because base editors do not induce double-strand breaks (DSBs) and do not rely on the host's non-homologous end joining or homologous recombination pathways, they significantly reduce DSB-related byproducts, such as small insertions or deletions. Nevertheless, base editing still faces challenges, including low editing efficiency, a limited number of editable sites, and off-target effects. Researchers are actively exploring effective strategies, such as optimizing Cas proteins or deaminases through rational design or continuous evolution, and developing various enhanced versions of base editors, thereby providing powerful tools for disease treatment and molecular breeding.

Brief Biography

Zuo Erwei is a researcher at the Institute of Agricultural Genomics, Chinese Academy of Agricultural Sciences. In recent years, he has concentrated on enhancing the precision and efficiency of gene editing technologies, engaging in the research and application of high-performance techniques while actively advocating for the establishment of a safety evaluation system for these applications. He has developed various technologies to detect off-target effects and has demonstrated that base editors can lead to completely random and unpredictable off-target effects across the genome. Furthermore, he proposed a scientific hypothesis regarding the molecular mechanisms underlying these off-target effects and created several base editing tools designed to minimize such issues. His contributions have been acknowledged in prestigious listings, including the "Top Ten Advances in Life Sciences in China 2019," "Major Medical Advances in China 2019," the "Major Scientific Discoveries of the Chinese Academy of Agricultural Sciences 2020," and the "2022 Youth Science and Technology Innovation Award." He has published numerous research papers in top scientific journals, including Science, Nature, Nature Methods, Cell Research, and Nature Communications.

fficient Genome-Editing tools to Engineer the Recalcitrant Non-model Industrial Microorganism

Binan Geng (耿碧男)
Hubei University

Abstract

Current biotechnology relies on a few well-studied chasses such as *Escherichia coli* and *Saccharomyces cerevisiae*, which have abundant information and efficient toolkits for genetic manipulation, but lack industrial characteristics. Instead, non-model industrial microorganisms usually possess excellent features but do not have effective and efficient genome-engineering toolkits, which hampers the development of microbial cell factories to meet the fast-growing bioeconomy. In this study, using the non-model ethanologenic bacterium *Zymomonas mobilis* as an example, we developed a workflow to mine and temper the elements of Restriction-Modification, CRISPR-Cas, Toxin-Antitoxin systems, and native plasmids that are hidden within industrial microorganisms themselves as efficient genome-editing toolkits, and established a Genome-Wide Iterative and Continuous Editing (GW-ICE) system for continuous genome-editing with high-efficiency**.** This research not only provides tools and pipelines for engineering the non-model polyploid industrial microorganism *Z. mobilis* efficiently, but also sets a paradigm to overcome biotechnological limitations in other genetically recalcitrant non-model industrial microorganisms.

Brief Biography

Binan Geng works as a postdoctoral researcher in school of life sciences from Hubei university. She got her bachelor's degree and followed by her Ph.D. training from Hubei university. Her research focuses on synthetic biology, genome-editing, and biotechnology, aimed at developing efficient genome-editing technology for non-model industrial microorganisms. Up to now, Dr. Geng has published more than 11 academic papers and holds 4 patents in China.

Engineering Prime Editors for Versatile and High Efficient Editing in Rice

Pengcheng Wei (魏鹏程)
Anhui Agricultural University

Abstract

The efficiencies of plant prime editing systems are frequently lower than those of mammalians. To improve plant PE activity, we engineered two types of prime editors, PE5a/b/c and PE6s, for plants, and their editing ability was examined with different types of mutations in rice. Compared to previously established plants PEs, ePE5c-Cre and ePE6d exhibited superior capability on short mutation editing as well as fragment insertions. Our engineering on plant prime editors provided reliable and easy-to-use toolbox for plant functional genomic research and for genome structural variants manipulations of practical breeding.

Brief Biography

Wei Pengcheng, Professor of Anhui agricultural University, college of agronomy. He seeks the engineering precise breeding tools and manipulation methods for plants. He especially is interested in the germplasm innovation of rice industrial production traits, e.g. herbicide resistance, mechanized seed production, through genome editing strategies. The recent publications included Nature plants, genome biology, Molecular plant, Plant Biotechnology Journal, etc.

Development of Novel Gene Editing Tools Using A Data-driven Approach

Zhenghe Li (李正和)
Zhejiang University

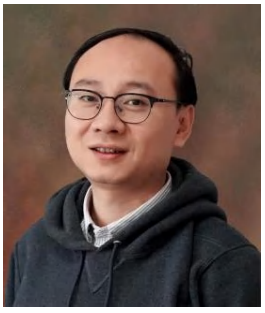
Abstract

Emerging genome editing tools such as CRISPR/Cas nucleases, base editors, and prime editors enable targeted precise changes to a cell's genome and facilitate fundamental biological research. Adaption and refinement of these technologies in plant science have also opened up new opportunities for rapid and precise trait improvement of a myriad of crops. Despite the potential, challenges remain for most plant species/genotypes in delivering the CRISPR/Cas reagents into plant cells and subsequent recovery of genetically modified plants from edited cells. Plant viral vectors have emerged as promising tools for gene delivery, but the generally small packaging capacities constrain their utilities in delivering the large CRISPR/Cas enzymes. Here, we compare the biological properties of various plant viral vectors and highlight the unparalleled cargo capacities of a group of segmented or nonsegmented, negative-stranded RNA viruses. Our work addressed issues related to strategies for viral vector designing, recovery of edited plants, heritability of virus-induced mutations, viral vector clearance etc. Using these RNA viral vectors, we demonstrate efficient delivery of the commonly used genome-editing enzymes (CRISPR/Cas9, CRISPR/Cas12a, adenosine and cytosine base editors) and DNA-free genome editing of several crop species and varieties. The viral delivery systems promise to overcome gene delivery bottlenecks for genome-editing some recalcitrant plant species and elite crop varieties.

Brief Biography

Zhenghe Li is currently a professor at Institute of Biotechnology, Zhejiang University, China. Zhenghe graduated at Zhejiang University with a Ph.D. degree in plant pathology in 2005 and then moved to University of Kentucky, USA for a postdoctoral training during 2005-2011, where he studies plant positive-strand RNA virus replication. Since 2011, Zhenghe has been employed as a faculty member in Zhejiang University. Current work in Zhenghe's laboratory focuses on molecular plant virology studies, viral vector development and applications in genome editing. His research group has developed the first reverse genetics systems for any plant negative-strand RNA viruses and engineered several viral vectors for transformation-free genome editing in plants.

Plant Synthetic Biology Promotes the Development of Future Functional Food Crops



Qinlong Zhu (祝钦泷)
South China Agricultural University

Abstract

Functional and nutritious food crops are beneficial to human health. Refined grains are mainly starch of endosperm and lack nutrients. Therefore, using plant biodesign and synthetic biology methods to synthesize nutrients and bioactive components in crops has important research significance. The development of plant synthetic biology has opened up opportunities to germplasm innovation of future functional food crops, which have multiple functions and high nutritional density. In previous studies, we have developed TAC-based TransGene Stacking (TGSII) systems, TGSII and TGSII-UNiE, through Cre/*loxP* irreversible recombination and unique nucleotide sequence-guided nicking endonuclease (UNiE)-mediated DNA assembly. Furthermore, a series of high-efficiency plant multiplex genome editing systems and base editors with a wide range have been developed for synthetic metabolic engineering. Utilizing these tools, we have successfully developed "Purple endosperm rice (called Zijingmi in Chinese) ", "Astaxanthin rice 1.0 (AR, called Chijingmi 1.0 in Chinese)" and new high-quality rice germplasm with suitable amylose content. On this basis, we have further developed new DNA assembly tools and strategies that enable effective multigene stacking and multiplex gene editing at the same time. As a result, we have developed the AR2.0 germplasm with higher astaxanthin content and enhanced stability. And successful heterologous biosynthesis of crocins was obtained in rice callus and plants. Additionally, effective synthesis of melatonin was also achieved in rice callus. These studies provide valuable tools and examples for the genetic engineering operations of complex metabolic pathways, the biosynthesis of essential bioactive substances, and enhancement of important agronomic traits involving multiple genes.

Brief Biography

Dr. Zhu is a professor of plant synthetic biology and molecular breeding at College of Agriculture, South China Agricultural University, and previously served as a visiting associate professor at Cornell University. His research mainly focuses on using plant synthetic biology strategies to develop new functional food crops for addressing the challenges in human health by synthesizing bioactive compounds and improving nutritional quality. His research program is centered on developing DNA assembly methods, TransGene Stacking system, and genome engineering tools for plant biosynthetic biology and crop breeding application. He has developed the first endosperm anthocyanin-rich Purple Endosperm Rice (Zijingmi in Chinese) and the first endosperm astaxanthin-rich Astaxanthin Rice (AR, Chijingmi in Chinese). He supervised the students to win the first Best Plant Synthetic Biology Award of iGEM in 2016. He won the Best Youth Outstanding Paper Award of CSBT in 2019.

High-Throughput, Label-Free Sorting of High DHA-Content Single-Cells from Genome-Wide Random Mutagenesis Libraries by Flowracs

Jian Xu (徐健)
Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences

Abstract

A full spontaneous single-cell Raman spectrum captures the metabolic phenome in a label-free and non-invasive manner, however Raman-activated cell sorting (RACS) of rare target cells from highly heterogeneous systems has remained a concept. Here, we present a positive dielectrophoresis-induced deterministic lateral displacement (pDEP-DLD)-based flow-mode RACS (FlowRACS), where a modulated pDEP-DLD force is exerted to focus, trap, and sort the fast-moving single cells in a wide channel. In pigment- and oil-producing yeasts, FlowRACS shows high sorting accuracy (>90%), high throughput (>600 events per min), high yield (>85%), long running time (>10 hours), and therefore is capable of sorting rare cells while preserving full cellular vitality. As an example, direct label-free sorting of a genome-wide random mutagenesis library containing 10^5 *Aurantiochytrium* sp. mutants via intracellular docosahexaenoic acid (DHA) content produced mutant cells with 58% higher DHA productivity in just two FlowRACS runs completed within two days, representing two orders of magnitude improvement in time- and cost-efficiency over conventional approaches. The superior trait is attributed to global remolding of transcriptome, including enhanced carbon metabolism, reduced intracellular NADPH synthesis rate, and increased triacylglycerol (TAG) synthesis. FlowRACS emerges as a powerful platform for synthetic biology by enabling the direct screening of metabolic traits from genome-wide mutagenesis libraries.

Brief Biography

XU Jian (xujian@qibebt.ac.cn) currently serves as Director of Single-Cell Center and Director of BioEnergy Division at CAS-QIBEBT. Jian has published over 170 papers on leading journals such as *Science, Cell Host Microbe* etc with ~17,000 citations (H-index 62). He is a founding senior editor of *mSystems*. His contribution was recognized by career awards from NSFC, MOST and CAS, including National Distinguished Young Scholars Award (2014), National Young-Scientist Award for Science and Technology (2016) and VCANBIO Award for Biosciences and Medicine (2016). Single-Cell Center (http://www.single-cell.cn/) has invented the Ramanomics Instrument Series (RACS-Seq, FlowRACS, EasySort, etc; http://www.singlecellbiotech.com/) to support industrial biotechnology, precision medicine, environmental remediation and synthetic biology.

Pioneering Bioingredient Production

Congqiang Zhang (张聪强)
Singapore Institute of Food and Biotechnology Innovation

Abstract

The worldwide shift away from fossil fuels toward more environmentally friendly resources and technologies has opened up significant opportunities for biomanufacturing, particularly through microbial fermentation. Isoprenoids represent the largest classes of natural products, numbering over 110,000 compounds. Their structural diversity contributes to a wide range of applications, including pharmaceuticals (e.g., Taxol), nutraceuticals (e.g., astaxanthin), flavours and fragrances (e.g., linalool), polymer molecules (e.g., isoprene), and biofuels (e.g., farnesene). To enhance isoprenoid production, our approach involves mining novel terpene synthases from fungi using bioinformatics. We also refine synthetic biology tools and explore novel microaerobic fermentation bioprocess to maximize the product yields and titres. Leveraging pathway design and our recently identified novel enzymes, we have successfully engineered *Escherichia coli* to produce high-value flavour, fragrance, and nutraceutical molecules at near-theoretical yields (up to 90%) and high titers (17-30 g/L) for several sesquiterpenes and monoterpenes, a challenging task for microorganisms. We also engineer microbes to produce carotenoids and vitamin A at “g/L” levels. We also engineer a filamentous fungus for diterpenoid production reaching “g/L” level in flasks.

Brief Biography

Dr. Congqiang Zhang earned his PhD in 2014 from a joint program at the National University of Singapore and the Massachusetts Institute of Technology. He joined the Biotransformation Innovation Platform at A*STAR in 2015 as a founding member and is now a Principal Investigator and a group leader at SIFBI, A*STAR. His research focuses on metabolic engineering, synthetic biology, and enzyme engineering, with a particular emphasis on industrial production of isoprenoids, carotenoids, and lipids. He has authored >40 publications and holds >10 international patents (4 granted) in this area. He collaborates closely with both local and multinational corporations on translational research. He serves as the Secretary of the BioEnergy Society of Singapore and is an editor for Frontiers in Bioengineering and Biotechnology and Advanced Biotechnology. Dr. Zhang was awarded the prestigious Singapore Young Investigator Research Grant in 2019 and has secured multiple national-level competitive grants as Principal Investigator or Co-Principal Investigator, like Singapore-France and Singapore-Australia Bilateral grants.

XianLiang Zheng (郑贤良)
Angel Yeast Company

Brief Biography

Dr. Xian-Liang Zheng graduated from the University of Chinese Academy of Science, completed 3-year postdoctoral research at Denmark Technical University and University of Tennessee. he is currently the vice general manager of Center for Biocatalysis and Enzyme Technology at Angel Yeast Company, China. Mainly responsible for the development of special enzyme strains, protein engineering, bio-manufacturing and other related work. He also serves as a corporate supervisor for graduate student at Shandong University, Hubei University and Three Gorges University. In recent years, as the project leader and participant, he undertakes multiple key research and development projects. He and his team developed various new enzyme preparation products, annual sales exceeding 20 million yuan. He is currently a council member of the Hubei Society of Synthetic Biology, he has been awarded multiple honorary titles, such as Innovative Talent, Mount Taishan Industrial Leading Talents, Model of Innovation and Entrepreneurship and so on.

High-throughput and Low-cost DNA Synthesis on Semiconductor Biochip

Dan Wu (吴丹)
XinSu Technology (Suzhou) Co., Ltd.

Abstract

DNA synthesis and sequencing are complementary processes that form a critical feedback loop in synthetic biology and genomics. While the past decade has seen an extraordinary reduction in the cost of DNA sequencing—approximately six orders of magnitude—this rapid progress has not been mirrored in DNA synthesis. The growing demand for large-scale DNA production in fields like synthetic biology, personalized medicine, and biomanufacturing calls for more efficient and cost-effective synthesis techniques, yet existing methods fall short of meeting these needs.

In this talk, I will present the cutting-edge development of next-generation DNA synthesis technology, leveraging semiconductor bio-chips to transform the way DNA is synthesized. This innovative approach promises to overcome current limitations, offering scalable, precise, and economical DNA synthesis. Additionally, I will explore the exciting potential of this technology in DNA data storage, an emerging field poised to revolutionize information storage for the digital age.

Brief Biography

Dan Wu is a seasoned biomedical engineer and entrepreneur with extensive experience in end-to-end development of biomedical systems, including biomedical imaging, single-cell detection, and protein testing technologies. He holds a Bachelor's degree in Electrical Engineering and a Master's degree in Acoustics from Nanjing University, as well as a Ph.D. in Mechanical Engineering and Computation from MIT. Currently, Dan is the co-founder and CTO of Atantares, a leading company pioneering next-generation DNA synthesis technology.

Application and Prospects of DNA Biosynthesis Technology

Jun Sun (孙隽)
Tianjin Zhonghe Gene Technology Co., Ltd.

Abstract

DNA synthesis technology is the fundamental key technology of synthetic biology, and its update and iteration will promote giant development of synthetic biology research and industry, guide the revolution of life science research methods, and have great scientific research and industrial application value. Traditional DNA synthesis technology is based on solid phase phosphoramidite chemical synthesis, and has undergone two generations of technological changes, but there are still some technical defects such as short synthetic fragments, serious environmental pollution, and high cost, which can not meet the growing needs of gene and even genome synthesis. DNA biosynthesis technology is a new generation of DNA synthesis technology, which exploits terminal deoxynucleotidyl transferase (TdT) as a DNA synthesis tool. The current development of DNA synthesis equipment based on biosynthesis by Zhonghe Gene will be introduced. Based on the current progress of Zhonghe Gene, the application and prospects of DNA biosynthesis will be discussed in this presentation.

Brief Biography

Dr. Xiaoyun Lu is the Deputy General Manager of Zhonghe Gene and previously worked at the Tianjin Institute of Industrial Biotechnology under the Chinese Academy of Sciences. Her research focuses on developing DNA biosynthesis technology and equipment, which holds the potential to overcome the current limitations of DNA synthesis based on phosphoramidite chemistry. Dr. Lu has published numerous papers in high-impact journals, including Nature Communications, ACS Catalysis, PNAS, and Plant Communications. She holds 23 international and domestic patents, several of which are pioneering patents in DNA biosynthesis. With extensive experience in this field, she is a recognized leader in advancing DNA biosynthesis technology.

High Throughput, Fully Integrated, and Low Cost DNA Storage System

Hong Liu (刘宏)
Southeast University

Abstract

DNA has been considered as a compelling candidate for digital data storage due to advantages such as high coding density, long retention time, and low energy consumption. Despite many works reported, the development of a DNA-based database of full integration, high efficiency, and practical applicability is still challenging. In this talk, firstly I summarize the general procedures of the state-of-the-art DNA-based digital data storage methods, highlighting the uncertainties involved (Nucleic Acids Res. 2021). Then, synthesis and sequencing of DNA on a single electrode with scalability for an integrated DNA-based data storage system is presented (Sci. Adv. 2021). Next, a high throughput, and fully integrated CMOS chip specifically designed for DNA storage is introduced. Lastly, I will present a flexible paradigm by recombining already synthesized DNA to build cost-effective and intelligent DNA data storage systems (ACS Appl. Mater. Inter. 2023), and the quasi-solid-state electrochemical DNA synthesis based on gels (Chem. Eng. J. 2024).

Brief Biography

Hong Liu, Professor and doctoral supervisor in Southeast University, Associate Dean of School of Biological Science and Medical Engineering. He got his Bachelor and Master degree in Nanjing University, and PhD degree in University of Texas, Austin. He developed origami paper analytical device (oPAD) during his PhD, and the device was permanently kept in NIH history museum. His current research interest is large scale microelectrode array for DNA synthesis and sequencing with applications on data storage, and point-of-care testing. He is the recipient of ‘Wanren’ project and ‘Qianren’ project, and PI of two National Key R&D Program of China, and two Key R&D Program of Jiangsu. He published more than 80 papers on Nat. Rev. Bioeng., Nat. Commun., Sci. Adv., J. Am. Chem. Soc., Angew. Chem. Int. Ed. and others.

Development and Application of Integrated DNA Data Storage Device

Chunyang Geng (耿春阳)
Southern University of Science and Technology

Abstract

As the demand for data generation and storage skyrockets, traditional storage technologies encounter significant challenges related to capacity, durability, and reliability. DNA data storage, an emerging and advanced technology, offers a potential solution by harnessing the high-density storage capabilities of DNA. Our group employs microfluidic technology to achieve DNA synthesis, mineralization preservation, random accessing, sequencing and reading within a single device, integrating the processes of DNA data storage and advancing the automation and device-based implementation of DNA data storage.

Brief Biography

Visiting research student at Southern University of Science and Technology, under the supervision of Prof. Xingyu Jiang. The research focuses on DNA data storage and biomolecular detection using microfluidic technology.

Research progress on information model theory and application tools for DNA storage

Xin Chen (陈鑫)
Tianjin University

Abstract

DNA information storage is a cutting-edge interdisciplinary research topic. Our team rise the characteristics of mathematical research to assist the experimental team in advancing the development of DNA information storage technology. Relying on the interdisciplinary platform within Tianjin University and collaborative efforts with leading domestic institutions, our team has completed a series of work on the foundational mathematical principles of DNA information storage, the development of application tools tailored to the biochemical properties of applicable DNA information storage, and coding methods designed to address specific scientific challenges. Here, we provide a brief introduction to our team's work and attempt to raise some general considerations regarding mathematical modeling issues and biochemical technology issues in DNA information storage.

Brief Biography

Chen Xin is an Associate Professor at Tianjin University - Tianjin National Center for Applied Mathematics. He holds a degree in Bioinformatics from Jilin University. His main research direction is the application of mathematical methods in biological data and software development. He has published more than 20 papers in the fields of bioinformatics and DNA storage. Chen Xin has led several sub-projects under the Fundamental Strengthening Project of the Science and Technology Committee of the Central Military Commission and the Ministry of Science and Technology's Key Research and Development Plan. He currently serves as the Project Secretary for the Ministry of Science and Technology's Key Research and Development Plan porject "Combinatorial Methods in DNA Storage"

DNA Storage Empowered by Soft-Decision Decoding

Lulu Ding (丁璐璐)
Shenzhen University

Abstract

DNA storage (DS) is an emerging technology with significant potential due to its exceptional density, long-term stability, and low maintenance costs. However, high error rates during DNA synthesis, amplification, sequencing, and preservation pose severe challenges, complicating data recovery and reducing storage density. To address these challenges, our study focuses on enhancing the error-correcting capabilities of error-correcting codes for DS systems. We analyzed the error characteristics and developed prediction models to quantify the DS channel. By introducing the soft-decision strategy from the communications field, we devised the *Derrick* algorithm for four nucleotide-based DS and *Derrick_cp* for composite letter-based DS. *In vitro* experiments demonstrated that *Derrick* doubled the error-correcting capability of the Reed-Solomon code compared to previous algorithms that lacked soft-decision, and *Derrick_cp* achieved the highest information density in DS systems.

Brief Biography

Lulu Ding received the Ph.D. degree in bioinformatics from the Chinese Academy of Agricultural Sciences in 2023, then she joined the National Engineering Laboratory for Big Data System Computing Technology at Shenzhen University. Her research focuses on DNA storage, multiple sequence alignments in third-generation sequencing, and pangenome construction. Dr. Ding's work in the field of DNA storage has been published as the first author (including co-first author) in journals such as *National Science Review* and *Advanced Science*.

DNA-based On-chip Classifiers

Xiaolei Zuo (左小磊)
Shanghai Jiao Tong University

Abstract

So far, as chip circuits continue to become more complex and Moore's Law approaches its limits, the development of silicon-based computers has hit a bottleneck, leading to the emergence of biocomputation and quantum computation. However, biocomputation strictly follows traditional design principles (e.g. binary system) of digital electronics, which could reach their limits when assembling gene circuits of higher complexity. Here, we introduce a DNA-encoded molecular classifier that can physically implement the computational classification of molecular data with module-n system. To produce unified sensing signals across heterogeneous molecular recognition events, we exploit DNA-framework-based programmable atom-like nanoparticles with n valence to develop valence-encoded signal reporters that enable linearity in translating virtually any biomolecular recognition events to signal gains. Multidimensional molecular information in computational classification is thus precisely assigned weights for bioanalysis. We demonstrate the implementation of a molecular classifier based on programmable atom-like nanoparticles to perform biomarker panel screening and analyze a panel of six biomarkers across three-dimensional data types for a near-deterministic molecular taxonomy of prostate cancer patients.

Brief Biography

Xiaolei Zuo received his Ph.D. degree from Shanghai Institute of Applied Physics, Chinese Academy of Science (2008). He was a postdoctoral fellow at University of California, Santa Barbara, USA (2008-2010), and at Los Alamos National Laboratory, USA (2010-2012). Now he is a professor of the Institute of Molecular Medicine, Renji Hospital, School of Medicine, Shanghai Jiao Tong University. His research interests include DNA electrochemical biosensors, 3D DNA probes, and DNA memory.

DNA Data Storage: A Revolutionary Paradigm for Future Data Storage

Wen Wang (王雯)
BGI Research

Abstract

The global explosion of digital information presents significant challenges for data storage. DNA, with its remarkable durability and space-efficient storage, emerges as a highly promising medium for this purpose. DNA data storage technology involves encoding digital information into artificially designed sequences of DNA molecules. Its high storage density and low energy consumption make it an extremely promising solution to the growing demands of data storage. As an interdisciplinary frontier, DNA data storage bridges biotechnology and information technology. This presentation will provide a systematic overview of DNA data storage, discussing recent progress and future application prospects.

Brief Biography

Dr. Wen Wang got her bachelor's degree from Zhejiang University and PhD degree from the Hong Kong University of Science and Technology. After postdoctoral training at Tsinghua University, she joined BGI Research in 2022 as a research scientist of DNA storage. Her research interests include DNA nanotechnology and its related applications in DNA storage, multiplexed imaging and detection, and self-assembly of functional materials. She has published 10 related papers in high level journals such as *Nucleic Acids Research, Nature Communications* and *Angewandte Chemie*.

DNA Nanodevices for Nongenetically Controlled Cellular Behaviors and Their Cell Therapeutic Applications

Zhou Nie (聂舟)
Hunan University

Abstract

Nucleic acids, as crucial biological functional molecules, are not only the primary carriers of genetic information but also have increasingly gained widespread attention for their non-genetic functions in recent years. The development of emerging technologies such as functional nucleic acids, self-assembly of DNA nanostructures, and dynamic DNA nanotechnology has made it possible to construct intelligent DNA nanomaterials with complex functions. Benefiting from the precise programmability of DNA sequence complementarity, high structural controllability, and the convenience of synthesis, modification, and functionalization, intelligent DNA nanomaterials are gradually becoming an important tool for biological function regulation, with broad application prospects in biomedical research. Here, we propose a new concept of non-genetic reprogramming of cell receptor functions for regulating cellular behaviors based on intelligent DNA nanomaterials. By using various de novo designed DNA intelligent nanodevices, we can precisely regulate and reprogram the molecular recognition and activation patterns of important receptor families on the cell membrane surface, thereby rewiring cellular signaling pathways to achieve precise control over downstream cellular behaviors. Utilizing dynamic DNA nano-assembly technology, we have successfully reprogrammed the molecular recognition targets of the receptor tyrosine kinases (RTKs) from native protein ligands to customized small molecules, extracellular miRNA, near-infrared light, and specific cell types. We have also achieved automated control and precise nanoscale clustering regulation of RTK receptor activation through DNA nanorobots and DNA origami techniques. In addition, we have developed a novel CAN-TE technology based on DNA-antibody chimeras, enabling multi-target intelligent logical recognition of antigens on the surface of tumor cells and regulating the activation efficiency of immune cells through multivalent effects, greatly enhancing the specificity of tumor cell recognition in T cell engagement techniques. We have also targeted integrins to construct a molecular tension sensing unit, creating an artificial mechanoreceptor that can specifically respond to the cellular mechanical action mediated by individual adhesion proteins at the piconewton (pN) scale, and successfully used it for the maintenance of stemness in neural stem cells mediated by cellular adhesion force.

Brief Biography

Zhou Nie is a professor at College of Chemistry and Chemical Engineering, Hunan University. He obtained bachelor degree from Nankai University in 2002, and obtained Ph.D. degree from Institute of Chemistry, Chinese Academy of Science in 2007. Since 2007, He started his career at State Key Laboratory of Chemo/Biosensing and Chemometrics at Hunan University. From 2011 to 2012, he received his postdoctoral training at Purdue University. His current research is focused on the development of new chemical-biological tools for detection and regulation of key factors in crucial biological events, such as cellular signal transduction and transcription regulation. In recent 5 years, he has published 90+ papers as correspondence author in high impact journals, including Nat. Chem. Biol., J. Am. Chem. Soc., Angew. Chem. Int. Ed., Science Advances. He was awarded by the National Science Fund for Distinguished Young Scholars in 2017, the "Cheung Kong Scholar" for Young Scholars in 2015, the Ten Thousand Talent Program for Young Top-notch Talent in 2014, the National Science Fund for Excellent Young Scholars in 2012, and Chinese Chemical Society Award for

Outstanding Young Chemist in 2015.

Engineering Long-Lived T Cells to Cure Chronic Diseases

Min Peng (彭敏)
Tsinghua University

Abstract

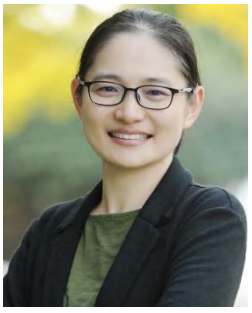
Engineered T cells represent a cornerstone of immunotherapy. The long-term therapeutic efficacy of engineered T cells depends on their functional persistence in vivo. Recently, we have demonstrated the induction of CAR T cells into an immortal-like and functional state, termed T_{IF}. These reprogrammed T_{IF} cells possess near-infinite stemness, akin to induced pluripotent stem cells, while retaining the functionality of mature T cells, representing a novel synthetic state of T cells. I will discuss the induction, mechanism, and application of T_{IF} cells in cancer and noncancerous diseases.

Brief Biography

Dr. Peng received his bachelor's degree in clinical medicine from West China School of Medicine, Sichuan University in 2005, and a Ph.D. in immunology from Peking Union Medical College in 2010. He conducted postdoctoral research at Memorial Sloan Kettering Cancer Center from 2010 to 2017, focusing on mTORC1 regulation and immunometabolism, with first-author publications in Cell, Science, and Nature. In 2017, Dr. Peng joined Tsinghua University, where he leads research on T cell biology. His work has been published in Nature Immunology, Journal of Experimental Medicine, among others.

Precise Engineering of Immune Cells for Therapeutics

Jie Sun (孙洁)
Zhejiang University



Abstract

Genetic engineering of immune cells, such as cytotoxic T and NK cells, to express chimeric antigen receptors (CARs) enables them to recognize and eliminate tumor cells. Currently, the most common way to efficiently delivery exogenous CAR gene into primary T and NK cells is through integrating viral vectors, such as retrovirus or lentivirus. However, these viral vectors integrate CAR gene into the immune cell genome in a random way, resulting in risk of carcinogenesis. Here we utilize CRISPR/Cas9 gene editing tools to precisely integrate CAR gene at designated genetic loci in T and NK cells. This precise engineering strategy has allowed us to reduce the fratricide of CAR-T cells as well as to develop allogeneic CAR-T and CAR-NK cells to treat cancer.

Brief Biography

Dr. Jie Sun is a principal investigator at School of Medicine, Zhejiang University. She obtained her Bachelor's degree from Hong Kong University and PhD degree from University of Illinois at Urbana Champaign. She was a Beckman Postdoctoral Fellow at Beckman Institute for Advanced Science, developing biosensors for live cell imaging. Then she was trained to develop novel CAR-T therapies in Michel Sadelain's lab at Memorial Sloan Kettering Cancer Center. Her current research focuses on developing tools and strategies to enhance the anti-tumor functions of CAR-T and CAR-NK cells for both hematological and solid tumors as well as revealing the molecular mechanisms underlying the cytotoxicity, proliferation and exhaustion of CAR-T cells.

Immune Cell Engineering and Cellular Immunotherapy

Lupeng Ye (叶露鹏)
Nanjing University

Abstract

Immunotherapy has achieved remarkable success in treating certain cancers, but its effectiveness remains limited for most solid tumors, with an overall response rate of approximately 15-20% in clinical settings. In addition, current methods for producing therapeutic immune cells (e.g. CAR-T cells) still face various obstacles, including low efficiency, uncertain cell quality, and safety concerns. These limitations hinder the development of high-quality, cell-based cancer therapies like CAR-T. Therefore, advancing new immunotherapies and engineering improved methods or platforms for producing therapeutic immune cells are critical to enhancing cancer immunotherapy and cell therapy.

In the past few years, we have developed high-throughput genetic screening platforms in primary CD8+ T cells to overcome technological limitations in identifying gene targets that may improve cancer immunotherapy. For instance, we created a novel gene editing system for efficiently manipulating the genome of mouse T cells: the adeno-associated virus (AAV)-SB transposon-CRISPR (AAV-SB-CRISPR) system (*Nature Biotechnology*, 2019). The advantage of this system over most other current systems is more efficacious editing of primary immune cells and other hard-to-gene-edit cell types. Using this system, we have identified promising membrane gene targets, such as Pdia3 and Mgat5, that could improve therapies for glioblastoma multiforme and other solid tumors. We also applied this technology to primary NK cell editing and identified a genetic checkpoint, CALHM2, which enhances CAR-NK therapy (*Nature Biotechnology*, 2024).

Recently, we established the first gain-of-function screening platform in primary T cells, leading to the discovery of immune boosters such as Prodh2 (proline dehydrogenase). Over-expressing *Prodh2/PRODH2* substantially enhanced TCR-T and CAR-T cells' anti-tumor activity by re-programming T cell proline metabolism (*Cell Metabolism*, 2022). To further enhance the safety and efficiency of immune cell engineering, we developed a novel technology called MAJESTIC (mRNA AAV-Sleeping-Beauty Joint Engineering of Stable Therapeutic Immune Cells) (*Nature Biomedical Engineering*, 2023). This innovative technology allows us to engineer cancer-fighting immune cells - such as CAR-T, CAR-NK, TCR-T, CAR-Macrophages, and CAR-iPSCs - more efficiently, with reduced cellular toxicity and genotoxicity, compared to conventional systems like lentivirus, retrovirus, CRISPR-based gene editing, plasmid transposon electroporation, and minicircle transposon electroporation.

Brief Biography

Dr. Ye received his Ph.D. in Biochemistry and Molecular Biology from Zhejiang University in 2015, after which he began his postdoctoral training at Zhejiang University College of Pharmaceutical Sciences. In 2017, he joined Dr. Sidi Chen's lab at Yale University School of Medicine for further postdoctoral training. His research mainly focuses on new tech development, cancer immunotherapy, and cell therapy. Since 2018, Dr. Ye has published over ten first-author and corresponding author papers in leading journals, including Nature Biotechnology (2019, 2024), Nature Biomedical Engineering (2023, cover story), Cell Metabolism (2022), Cell (2019), Nature Methods (2019), Journal of Hematology & Oncology (2022), Cancer Immunology Research (2023), Cell Discovery (2018). In 2022, Dr. Ye joined Nanjing University Institute of Modern Biology as a Tenure-track Assistant Professor and Principal Investigator.

Production of Steroids by Synthetic Biology

Jingwen Zhou (周景文)
Jiangnan University

Abstract

Steroids are the second largest class of drugs after antibiotics and are currently widely used in the treatment of cancer, inflammation, and heart disease. However, existing synthetic biology technologies for steroid production face several challenges, including the promiscuity and efficiency of heterologous enzymes required for post-modification of active steroid functions, the metabolic pathway balance in synthetic cell factories, and the tolerance to exogenous steroids. Thus, advanced novel synthetic biology strategies need to be developed for overcoming multiple electron-requiring rate-limiting steps in the biosynthesis of steroids. Our study focuses on engineering efficient heterologous enzymes for steroid post-modification, constructing novel synthetic pathways for steroid production, and ultimately developing artificial cell factories capable of high-yield steroid production. The specific contents include: 1) Based on the post-modification characteristics of target steroids, evolutionary analysis techniques were employed to deeply explore related heterologous enzymes. Computational simulations were used to elucidate their catalytic mechanisms and guide their rational modification. 2) Efficient integration targets and heterologous expression systems were developed. By combining subcellular regionalization engineering, the expression and functional adaptation of heterologous enzymes were achieved. Cofactor and electron transfer engineering were utilized to enhance and balance the energy-driven pathways. 3) the metabolic transport network of cell factories was rationally regulated, and biosensors and microfluidic technologies were employed to achieve rapid, high-throughput screening of strains with high steroid production and tolerance. This approach has successfully achieved the efficient *de novo* biosynthesis of various important steroid compounds, including ergosterol, campesterol, 7-dehydrocholesterol, cholesterol, pregnenolone, and progesterone. Additionally, new synthetic pathways for some important steroid hormones, such as cortisone and hydrocortisone have been developed.

Brief 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 300 peer reviewed publications and invited reviews with a H-index 43 (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 from 2023.

Yeast Cell Factories Facilitate the Biomanufacturing of Lipophilic Compounds

Shuobo Shi (史硕博)
Beijing University of Chemical Technology

Abstract

Lipophilicity describes a chemical compound's ability to dissolve in fats, oils, lipids, and non-polar solvents like hexane. Lipophilic compounds have a wide range of applications, with fatty acid derivatives and terpenoids being two of the most important groups. For example, many natural products belonging to terpenoids are vital for human nutrition and health, while fatty acids can be converted into biofuels such as biodiesel, which are considered more environmentally friendly and sustainable alternatives to traditional fossil fuels. However, current production methods for certain lipophilic compounds face challenges and complexities.

Recognizing the significance of constructing artificial microbial cell factories for efficient biological manufacturing, both the scientific and industrial communities have embraced this approach. Nonetheless, there are still many challenges in developing microbial cell factories that are highly efficient and robust. In this report, the speaker presents recent laboratory work focused on the analysis and optimization of biosynthesis pathways using synthetic biology techniques. Additionally, the development of efficient CRISPR technology is explored to achieve the biological manufacturing of lipophilic compounds such as fatty acids and carotenoids. This research provides valuable insights and serves as a reference for related studies.

Brief Biography

Dr. Shuobo Shi is now a professor at Beijing University of Chemical Technology. He obtained his Ph.D. degree in Biochemical Engineering from Tianjin University in 2009. Dr. Shi's primary scientific interests lie in metabolic engineering and synthetic biology. His research encompasses metabolic engineering of microbial cell factories, specifically for the production of lipids and terpenoids. Additionally, he focuses on the development of tools for genome engineering and synthetic biology, as well as the advancement of automation systems for synthetic biology applications. To date, he has authored over 70 peer-reviewed papers in well-known journals such as Nature Communications and Metabolic Engineering, including four highly cited papers in the ESI (Essential Science Indicators) category.

Green Biomanufacturing of Functional Chemicals Via Biocatalyst Engineering and Biological Circuit Design

Jinsong Gong (龚劲松)
Jiangnan University

Abstract

The green biomanufacturing of functional chemicals is a disruptive technology that has rapidly developed in recent years as an alternative to chemical processes. Employing non-food biomass and other biological carbon resources as raw materials, and building biocatalysts with independent intellectual property rights, as well as establishing a modern biomanufacturing industry technology system, are at the core of solving the supply issues in the biomanufacturing industry. Our research group starts with the performance requirements of biological circuit design and key biocatalysts (cells or enzymes) for bioprocessing, focusing on solving the core issue of poor performance in industrial applications. Through the construction of metabolic network models, modularization of synthetic pathways, virtual-real screening and bidirectional evolution of key enzymes, semi-automatic design and efficient expression of difficult-to-express proteins, and industrial technology integration, several typical biosynthetic processes have been established. We have achieved the green biomanufacturing of functional chemical products such as niacin, membrane proteins, functional sugars, and polyamino acids, and have gained comparative advantages in cost and environmental friendliness over traditional chemical processes.

Brief Biography

Dr. Jin-Song Gong is currently a Professor of biopharmaceutical engineering at Jiangnan University, China. He received Ph.D. degree in Fermentation Engineering from Jiangnan University in 2014. And joined School of Life Sciences and Health Engineering of Jiangnan University in 2014. His research interests mainly focused on the biomanufacturing of functional chemicals and pharmaceutical intermediates via protein engineering, synthetic biology, material chemistry, fermentation engineering strategies. He published more than 80 papers in the international academic journal, including ACS catalysis, Biotechnology Advances, Metabolic Engineering, ACS Synthetic Biology, Applied and Environmental Microbiology, and so on. Also, he obtained 75 authorized national invention patents and 3 US patents. He also serves the scientific community as the editor of international journals such as Frontiers in Bioengineering and Biotechnology, BioDesign Research.

AI-driven Enzyme Discovery for Synthetic Biology Innovative R&D Models

Qiannan Hu (胡黔楠)
Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences

Abstract

Identifying functional enzymes for the catalysis of specific biochemical reactions is a major bottleneck in the de novo design of biosynthesis and biodegradation pathways. Conventional methods based on microbial screening and functional metagenomics require long verification periods and incur high experimental costs; recent data-driven methods apply only to a few common substrates. To enable rapid and high-throughput identification of enzymes for complex and less-studied substrates, we propose a robust enzyme's substrate promiscuity prediction model based on positive unlabeled learning. We anticipate that this model will serve as a useful tool for identifying new functional enzymes and understanding the nature of biocatalysis, thereby advancing the fields of synthetic biology, metabolic engineering, and pollutant biodegradation.

Furthermore, Dr. Qian-Nan Hu will report a novel data-driven one-stop biosynthetic/biodegradation design technology system: (1)Cell2Chem: Mining Explored and Unexplored Biosynthetic Chemical Spaces. (2) BCSExplorer: A Customized Biosynthetic Chemical Space Explorer with Multifunctional Objective Function Analysis. (3)Bio2Rxn: Sequence-Based Enzymatic Reaction Predictions by a Consensus Strategy. (4) RxnBLAST: Molecular Scaffold and Reactive Chemical Environment Feature Extractor for Biochemical Reactions. (5) PrecursorFinder: a customized biosynthetic precursor explorer. (6) CF-Targeter: a rational biological cell factory targeting platform for biosynthetic target chemicals. (7)novoPathFinder: a webserver of designing novel-pathway with integrating GEM-model. (8) Data-Driven Rational Biosynthesis Design: From Molecules to Cell Factories.

Brief Biography

Prof. Hu received Ph.D. degree in applied chemistry from Central South University in 2004. He joined Computer Chemistry Center at University of Erlangen Nurnberg as a Postdoctoral Research Associate in 2004. Dr. Hu joined School of Informatics and Computer Sciences University of California Irvine in 2007 as a Postdoctoral Research Associate, and then Bioinformatics Center at Kyoto University as a staff Scientist in 2008. He joined Wuhan University as Associate Professor of School of Pharmacy in 2010. Then, Dr. Hu joined in Chinese Sciences of Academy as Full Professor since 2014, and his research interest focuses on data-driven synthetic biology for solving global health challenges.

Protein Engineering Facilitates in Vitro Synthetic Enzymatic Biosystem

Chun You (游淳)
Zhejiang University

Abstract

The in vitro synthetic enzymatic biosystem (ivSEB) operates on the basis of a meticulously designed multi-enzyme catalytic pathway. This pathway is intricately composed of a diverse array of biological enzyme components, each playing a crucial role in the overall functionality of the system. The ivSEB is capable of transforming specific substrates into desired target compounds, all within an environment that is external to living organisms. One of the most notable characteristics of this biosystem is its modularity, which provides researchers with a high degree of freedom when it comes to the design, assembly, and regulation of the system. Our research team has adopted a strategic approach that focuses on the ATP-independent energy activation of glycosidic bonds. This innovative strategy leverages the use of inexpensive and renewable biomass materials, such as starch, cellulose, glucose, and carbon dioxide, as substrates for the purpose of biomanufacturing. These substrates serve as the foundational building blocks from which a variety of valuable compounds can be synthesized. However, within these biosystems, certain enzymes that are rate-limiting in nature often fall short of meeting the demands of the ivSEB, resulting suboptimal product yields and titers. To address this challenge, We have employed protein engineering to enhance the activity, thermostability, and substrate specificity of these rate-limiting enzymes within the ivSEBs. These advanced ivSEBs have been instrumental in the efficient synthesis of a range of valuable compounds, such as myo-inositol, glucosamine, rare sugars, disaccharides, bio-materials, and even biomimetic coenzyme regeneration. Through continuous research and development, we are committed to further refining these biosystems to unlock even greater possibilities in the realm of in vitro synthetic biology.

Brief Biography

Chun You got his PhD degree in Fudan University, and then worked as postdoctoral in Virginia Tech from 2010-2015. He join Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences as Principal Investigator since 2016, and join Zhejiang University as Principal Investigator since 2024. His research focused on the construction, optimization and application of in vitro synthetic enzymatic biosystems from renewable biomass, including improving enzyme engineering, switching coenzyme preference, constructing enzyme complex, elucidating substrate channeling mechanism, and figuring out the suitability of enzymes/modules. He has published more than 50 scientific papers in Nat. Commun, ACS Catal, Angew Chem Int Ed, Proc Natl Acad Sci USA, ChemSusChem et. al.

Artificial Enantioselective Photoenzymes with Unnatural Amino Acids

Yuzhou Wu (吴钰周)
Huazhong University of Science and Technology

Abstract

Designing artificial enzyme that could catalyze unnatural reaction is of great interest for expanding enzymatic reactions and could be highly useful for biosynthesis of unnatural products. Particularly, taking the advantages of enzyme's extraordinary enantioselectivity, developing unnatural enzymes for asymmetric synthesis is highly valuable. For instance, stereochemical control of photochemical reactions remains a formidable challenge with the existing small molecule catalysts. Therefore, we developed a method to create artificial photoenzymes with desired reactivity for unnatural reactions. The genetically encoded, chemically evolved triplet photoenzymes were developed which embedded with a benzophenone synthetic photosensitizer via genetic code expension. Structural optimization through four founds of rational mutagenesis afforded proficient variants. They promoted enantioselective intramolecular [2+2] photocycloaddition of indole derivatives with good substrate generality and excellent enantioselectivites (up to 99% enantiomeric excess). X-ray crystal structure of photoenzyme-substrate complex elucidated the important multiple noncovalent interactions that work synergistically to induce high enantioselectivity. This study shows that by merging the empowering mechanism of triplet energy transfer catalysis with the delicate supramolecular cavity of proteins, the triplet photoenzymes artificially expand the fundamental reactivity with respect to enzyme catalysis and unlock an integrated approach to valuable enantioselective photochemical synthesis that are not accessible with either the synthetic or the biological world alone.

Brief Biography

Prof. Yuzhou Wu is a professor at Huazhong University of Science and Technology (HUST). She graduated from Zhejiang University, obtained a master's degree and a doctor's degree from the National University of Singapore and Ulm University in Germany respectively, supervised by Professor Tanja Weil. She has been worked as the project leader in Ulm University and Max Planck Institute of Polymers in Germany. Since 2017, she was appointed as Professor in HUST, and was also selected as the leader of the Max Planck Society Overseas Partner Group. Her research interests laid in the development of unnatural enzymes and biomacromolecules for biosynthesis and biomedical applications, and has published more than 100 papers in Nature, J Am. Chem. Soc., Angew. Chem. Int. Ed., Adv. Func. Mater., ACS Nano, Nano Lett. , Nano Res., Chem. Rev.. She has received four national and provincial research projects, including the National Key R&D Program of China, the Key R&D Program of Hubei province, and projects from National Natural Science Foundation of China.

Machine Learning to Engineer Trna Synthetase Activity for Improved Incorporation of Noncanonical Amino Acids

Haoran Yu (于浩然)
Zhejiang University

Abstract

The pyrrolysyl-tRNA synthetase (PylRS) and *Methanocaldococcus jannaschii* tyrosyl-tRNA synthetase (MjTyrRS) are most widely used enzymes for the incorporation of noncanonical amino acids (ncAAs) into proteins at specific positions. Although directed evolution of these enzymes have enabled over 400 ncAAs to be incorporated into proteins, most of the ncAA containing proteins are expressed in a limited yield due to low activities of the variants. To further improve the activities of these enzymes, we first applied machine learning (ML) to engineer the tRNA-binding domain of PylRS with a fast fourier transformation-partial least square regression (FFT-PLSR) model and three zero-shot prediction ML models. A variant Com2-IFRS was obtained from a sequence space containing 11520 mutations, which showed a 30-fold increase in activity. Transplantation of the evolved mutations into other 7 PylRS-derived synthetases improved yields of proteins containing six types of ncAAs including Phe derivatives, Tyr derivatives, Trp derivatives, Cys derivatives, His derivatives and Lys derivatives, by up to 1149.7-fold. We also devised a protein language model-enabled automatic evolution (PLMeAE) platform, a closed-loop system for automated protein engineering within the Design-Build-Test-Learn (DBTL) cycle. The protein language model (PLM) ESM-2 makes zero-shot prediction of 96 variants to initiate the cycle. Then the biofoundry constructs and evaluates these variants, and feeds the results back to a multi-layer perceptron to train a fitness predictor, which then makes prediction of second round of 96 variants with improved fitness. With the application of PLMeAE platform, four-rounds of *in vitro* continuous directed evolution was carried out to engineer MjTyrRS within half a month. The mutants obtained increased the enzyme activity by up to 12.0-fold.

Brief Biography

Dr. Haoran Yu is now a principal investigator in Zhejiang University. He received his PhD in 2019 from the University College London for work on protein engineering of transketolase. After that, he worked as a research associate in Department of Chemistry, UCL, to incorporate unnatural amino acids into proteins using the expanding genetic codes method. He joined Zhejiang University as a PI since 2020, and the research interest of his group focuses on developing advanced protein engineering methods to improve enzyme properties for industrial applications. He has published more than 30 papers in the journals such as PNAS, Angew. Chem. Int. Ed.

Toward Better Utilization of Nicotinamide Biomimetics: Coupled Photo-Enzymatic Approach

Ye Ni (倪晔)
Jiangnan University

Abstract

As potential substitutes for natural cofactors, nicotinamide cofactor biomimetics (NCBs) have been extensively explored due to their cost-efficiency and easy synthesis. *Ss*GDH is the first enzyme identified to utilize oxidized NCBs, enabling NCBs-based in situ cofactor regeneration. However, *Ss*GDH remains limited in cofactor scope, and the catalytic efficiency is inadequate. Here, a series of totally synthetic NCBs were designed. *Ss*GDH was engineered by modification of interface residues. M2-IG showed a 47.2-fold increase in catalytic efficiency when utilizing newly synthesized *p*-BNNA$^+$. Beneficial variants were coupled with XenA-catalyzed reaction to demonstrate their universality as NCBs regenerative enzymes. Based on all-atom MD simulation, more flexible conformation of F279 and widened cofactor entry tunnel are conducive to the entry and binding of NCBs. In addition, photocatalytic regeneration of NADH and NCBs using g-C3N4 was developed. The highest regeneration yield of 48.32% was achieved with BANA+, outperforming the natural cofactor NAD+. A coupled photo- XenA system was explored. Among all the NCBs and NAD+, the highest conversion ratio of over 99% was obtained with BANA$^+$. After recycled for 8 times, g-C3N4 maintained over 93.6% catalytic efficiency.

Brief Biography

Prof. Ye NI is a Chang Jiang Distinguished Professor, Ministry of Education. Her expertise is biocatalysis and protein engineering. Prof. Ni is a Chief Scientist of National key research and development program. She has published over 100 original paper in top journals including JACS, ACS Catal. She has over 30 authorized Chinese patents and 6 US patents, and has received Science & Technology Progress Award from Ministry of Education of China. She also serves as an Associate Editor of Applied Biochemistry and Biotechnology.

FRISM-A Powerful Method for Protein Engineering

Qi Wu (吴起)
Zhejiang University

Abstract

Directed evolution has emerged as the most productive enzyme engineering method, with stereoselectivity playing a crucial role when evolving mutants for application in synthetic organic chemistry and biotechnology. In order to reduce the screening effort (bottleneck of directed evolution), an improved method dubbed focused rational iterative site-specific mutagenesis (FRISM), has been developed. It involves the identification of hotspots, which is usually rationalized through computer-aided technologies, followed by the generation of a focused mutant library. By reducing the size of the mutation library and the screening efforts, the FRISM strategy has been successfully employed in engineering a wide range of enzymes with enhanced catalytic performance, improved enantio-, regio-, and chemoselectivities.

Brief Biography

Wu Qi is a full professor of chemistry and principal investigator at Zhejiang University. His research mainly focuses on biocatalysis and protein engineering. In the past five years, he has published more than 40 papers as the first and corresponding author in journals such as *J. Am. Chem. Soc*., *Angew*. *Chem*. *Int*. *Ed*., and *Nature Commun*.

AI-BT-Chem Assisted 1C Assimilation

Yajie Wang (王雅婕)
Westlake University

Abstract

Biocatalysts offer advantages like self-regeneration, renewability, and environmental friendliness compared to traditional chemical catalysts. They are increasingly used in agriculture, chemical engineering, pharmaceuticals, and energy. McKinsey estimates that biomass manufacturing could account for 60-70% of chemical production, becoming a major economic market. Despite their potential, biocatalysts face challenges such as low activity and stability. Directed evolution has improved these aspects but is not a complete solution. Our lab has developed high-efficiency enzyme discovery tools and platforms, including "ESM-Ezy," a deep learning tool for identifying superior enzymes. This tool has discovered multi-copper oxidases with enhanced performance in dye decolorization and biotoxin degradation. We also develop a cooperative bioelectrochemical system featuring a bifunctional rhodium-based catalyst for simultaneous CO_2 and NAD^+ electroreduction for the first time, and enzymatic cascades for the direct synthesis of C3 and C4 from CO_2 in a “one pot” manner.

Brief Biography

Dr. Yajie Wang joined the Westlake University in the fall of 2021 and established WangSynbio lab. Wang lab focuses on integrating protein engineering, synthetic biology, chemistry, material sciences, and machine learning to establish a “Design-Build-Test-Learn” platform to design and construct artificial chemo-bio hybrid systems to harness the synthetic power from both chemistry and biology, and synthesize value-added compounds from the renewable sources, waste, and even air. Her research has been published in Nature, Nature Chemical Biology, ACS Catalysis, Chemical Review, Natural Product Report, etc. Dr. Wang was the recipient of Singapore National Scholar and the recipient of “35 Innovators Under 35 (TR35)” in China 2022.

Chemo-biocatalytic Synthesis of Valuable Chiral Pharmaceutical Intermediates

Bo Yuan (袁波)
Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences

Abstract

Biocatalysis is an important technology for green synthesis and aligns with the long-term goals set by China during the 14th Five-Year Plan. To achieve the biosynthesis of key pharmaceuticals and fine chemicals using biocatalysis, it is necessary to integrate chemoenzymatic, photoenzymatic, and metal-enzyme cascade reactions. Meanwhile, in recent years, enzyme engineering techniques combining directed evolution and rational design have developed rapidly, promoting the significant application of enzyme catalysis methods in green biomanufacturing industries. This talk will introduce the team's research achievements in the synthesis of chiral compounds, including the synthesis of axially chiral compounds via biocatalysis, chemoenzymatic cascade reactions, the directed evolution of enzymes for the synthesis of intermediates for cephalosporin antibiotics, and their industrial applications. With the advancement of directed evolution technology, the design of new enzyme-catalyzed reactions, and the emergence of new enzymatic processes, it is expected that full-chain R&D routes for important pharmaceutical intermediates will be established in the future, contributing to the development of China's green bioeconomy.

Brief Biography

Bo Yuan graduated from the University of Manchester with a PhD in biochemistry. She is currently an associate professor at Tianjin Institute of Industrial Biotechnology at Chinese Academy of Sciences. Previously, she obtained her BSc from Sun Yat-Sen University and MSc from the University of Manchester. She also worked in Xi'an Jiaotong University during 2016-2021. Her main research interests lie in the applications of protein engineering and chemoenzymatic cascade methodologies in biocatalytic synthesis of pharmaceutical intermediates. In the recent 5 years she has been hosting more than 5 national and provincial programs including the National Natural Science Foundation of China General and Youth programs and Tianjin Synthetic Biotechnology Innovation Capacity Improvement Project. She has published more than 20 papers, and 2 book chapters.

Constructing Microbial Cell Factories to Produce Pharmaceuticals

Qipeng Yuan (袁其朋)
Beijing University of Chemical Technology

Abstract

The production of pharmaceutical chemicals plays an important role in ensuring people's health, but traditional production processes have problems such as large pollution emissions and low efficiency. Green biomanufacturing of pharmaceutical chemicals is an important pathway for achieving sustainable development of human society. High-efficiency cell factories are the core of green biomanufacturing and will greatly promote the development of the bioeconomy. This report introduces the scientific and technical problems and challenges in the current construction of cell factories, and presents new technologies and strategies for constructing high-efficiency cell factories from the perspectives of the discovery and application of new enzyme functions, the design and construction of non-natural pathways, the enhancement of cell factory synthesis capacity through drive force, the construction of stable self-regulating co-culture systems, and the principles and applications of quorum sensing regulation. It also demonstrates the infinite potential of synthetic biology through the construction of cell factories for three-seven-sulfate and paracetamol, and the industrial application of arbutin, to provide strategies for the green biomanufacturing of pharmaceutical chemicals.

Brief Biography

Qipeng, Yuan, Distinguished Professor of Changjiang Scholars of the Ministry of Education, winner of the 11th China Youth Science and Technology Award, one of the top 100 Leading Talents in Beijing. The main research areas are synthetic biology and metabolic engineering, large-scale preparation of high purity natural products and study of their bioactivities. In recent years, it has undertaken key research and development tasks of the Ministry of Science and Technology, key and general programs of the National Natural Science Foundation, provincial and ministerial level and enterprise cooperation projects. He has published more than 300 SCI papers as the corresponding author, and authorized more than 60 PCT and Chinese invention patents. A number of results have been achieved in industrial production, creating good economic benefits. As the first contributor, he won 1 second prize of National Science and Technology Progress, 1 special prize, 2 first prizes and 2 second prizes of provincial and ministerial science and technology progress.

Reprogramming Methylotrophic Yeast for Chemical Overproduction from Methanol

Yongjin Zhou (周雍进)
Dalian Institute of Chemical Physics, Chinese Academy of Sciences

Abstract

Methanol is an ideal feedstock for bio-manufacturing that can be beneficial for global carbon neutrality. However, the toxicity of methanol limits the efficiency of methanol metabolism toward biochemical production, and it is still challenging in engineering this non-conventional yeast due to serious lack of genetic editing tools and unclear methanol metabolism. In this talk, we will show our recent progress in establishing CRISPR-Cas9 based genome editing tools and enhancing the homologous recombination in methylotrophic yeast *Ogataea polymorpha* and *Pichia pastoris*. With this genetic platform, we tried to engineer cellular metabolism for fatty acid production from methanol. We found that engineering overproduction of free fatty acids (FFA) from sole methanol resulted cell death with a decreased cellular phospholipid in O. polymorpha, and the cell growth was restored by adaptive laboratory evolution (ALE). Multi-moic analysis showed that phospholipid metabolism homeostasis is very essential for methanol tolerance. Enhancing the methanol tolerance and engineering cellular metabolism enabled methanol biotransformation for high level production of a variety of chemicals such as FFA (20 g/L) and lactic acids (35 g/L).

Brief Biography

Yongjin Zhou is a Chair Professor at Dalian Institute of Chemical Physics, Chinese Academy of Sciences. His research areas include synthetic microbiology for cell factory construction, yeast genetics and metabolic engineering. He co-authored more than 100 peer reviewed papers on prestigious journal such as Cell, Nature Energy, Nature Metabolism, Nature Chemical Biology, JACS, PNAS, Nature Communications with >6800 citations and hold 15 patents. He serves as Editor-in-Chief of Biotechnology Journal, Associate Editor of Synthetic and Systems Biotechnology and editor board member for 5 other journals. He was honored several awards includes Outstanding Young Scholar Grant from NSFC (2024), Agilent “ACT-UR” Award (2023), and Excellent Youth Scholars from NSFC (2019) etc.

Developing The Reading and Writing Toolbox for Nucleic Acid Modifications

Guanzheng Luo (骆观正)
Sun Yat-sen University

Abstract

Nucleic acids harbor over 150 distinct chemical modifications, expanding their functional roles beyond the genetic code. These "epigenetic codes" are implicated in diverse biological processes, ranging from bacterial immunity to mammalian development, highlighting the importance of deciphering their intricate functions. Recent advances in third-generation nanopore direct sequencing (DRS) have revolutionized the field by enabling simultaneous detection of canonical and modified bases across long DNA/RNA molecules. This disruptive technology provides unprecedented opportunities for studying epigenetic modifications at single-molecule resolution. We have developed a high-precision algorithm for identifying nucleic acid modifications based on DRS data, generating detailed single-molecule modification maps. This approach has been successfully applied to investigate various biological processes across multiple species, providing valuable insights into the dynamic nature of epigenetic regulation. Furthermore, we are actively developing novel tools for "writing" modifications, enabling the targeted installation or editing of nucleic acid modifications in vivo. These tools offer the ability to modulate biological functions and track RNA and DNA dynamics with spatiotemporal precision. The reading and writing technologies provide a powerful toolbox for understanding the fundamental roles of nucleic acid modifications and unlocking their application potential.

Brief Biography

Dr. Luo Guanzheng is currently a professor at the School of Life Sciences, Sun Yat-sen University. He received his Bachelor's degree in Biomedical Engineering from Southeast University and his bioinformatics PhD from the Institute of Genetics and Development, Chinese Academy of Sciences. Following his doctoral studies, he pursued postdoctoral research at the University of Chicago before joining Sun Yat-sen University in 2017. His research focuses on understanding the fundamental principles of genome coding and complex life phenomena through the lens of nucleic acid modifications. His work is characterized by a multidisciplinary and methodology-driven approach, integrating theoretical innovation with scientific discovery. He has contributed to the field by developing novel reading and writing technologies to systematically investigate the biological significance of diverse DNA and RNA modifications. He has published numerous articles as a corresponding author in prestigious journals, including *Nature Methods*, *Science Advances*, and *Cell Research*.

Rapid Genome Evolution of the *Corynebacterium Glutamicum* Via the Dual Genetic Level Editing

Meijuan Xu (徐美娟)
Jiangnan University

Abstract

As an industrial microorganism, *Corynebacterium glutamicum* plays a pivotal role in the amino acid industry. This study proposes a practical, efficient, and controllable evolutionary tool (oMut-Cg^{ts}) for *Corynebacterium glutamicum* based on dual genetic level modification engineering, which facilitates the creation and optimization of *C. glutamicum* cell factories. Initially, endogenous RNA polymerase α-subunit and DNA helicase Cgl0854 were utilized as "docks" for cytosine deaminase (pmCDA1) in transcription and replication level modification engineering, respectively. This significantly enhanced the genomic mutation rate, demonstrating that the localization of pmCDA1 around transient ssDNA is a necessary condition for achieving efficient genomic mutations. Subsequently, the combined modification and optimization of both genetic levels elevated the spontaneous mutation rate of *C. glutamicum* by 1.02×10^4-fold, while maintaining a relatively low background mutation rate (approximately 2.62-fold that of the wild-type strain). This represents the highest mutation rate reported so far for *C. glutamicum* evolutionary tools. Whole-genome sequencing of the mutation library revealed the "breadth" and "depth" of genome mutations mediated by oMut-Cg^{ts}, which achieved uniform and efficient C:G → T:A transitions across the entire genome without evident strand preference or base background bias. Furthermore, the rapid evolution of stresses (low pH, oxidative stress, and high ethanol concentration) tolerance phenotypes mediated by oMut-Cg^{ts} demonstrates the tool's powerful capabilities in multi-dimensional bioengineering, including rapid phenotype evolution, gene function mining, and protein evolution

Brief Biography

Professor Xu Meijuan, from the School of Biological Engineering at Jiangnan University, has presided over more than ten national and provincial projects and been selected for the National Special Support Program for high-level Talents Youth Top Program. Professor Xu's research primarily focuses on synthetic biology and industrial enzyme engineering, with a specialization in constructing microbial cell factories that efficiently produce target amino acids and their high-value derivatives.

Cisencoder: Integrating Massively Parallel Reporter Assays and Artificial Intelligence for *De Novo* Design of Cis-Regulatory Elements

Yuwen Liu (刘毓文)
Institute of Agricultural Genomics, Chinese Academy of Agricultural Sciences

Abstract

In synthetic biology, artificially designed cis-regulatory elements (CREs), such as enhancers, can be used to precisely control the yield of target products, playing a critical role in cost reduction and efficiency enhancement. However, our understanding of CRE nucleic acid syntax remains limited, and *de novo* design of these elements is still in its infancy. To address this challenge, we are developing CisEncoder, a platform that integrates Massively Parallel Reporter Assays (MPRAs), which provide high-quality, large-scale quantification of CREs, with DREAM (DNA cis-Regulatory Elements with controllable Activity design platforM), an innovative deep learning framework designed to unravel the nucleic acid syntax of CREs. To demonstrate the capabilities of CisEncoder, we achieved state-of-the-art sequence-based enhancer activity prediction in Drosophila S2 cells and identified key sequence features that are crucial for strong enhancer activity. Leveraging this predictive power, we designed DreaMer001, a synthetic enhancer with 3.6 times the activity of the strongest natural enhancer in the Drosophila genome. Remarkably, DreaMer001 not only showed high activity in Drosophila S2 cells but also demonstrated significant activity across multiple species' cell lines. In mammals like humans, mice, and pigs, DreaMer001 averaged over twice the activity of the CMV enhancer. In SF9 cells, its activity was 15.7 times higher than the Hr5 enhancer, and it exhibited 7.6 times and 26.6 times higher activity than the CMV enhancer in chicken DF1 cells and fish spermatogonial cells, respectively. Additionally, using MPRA-derived data, we developed the ultra-strong silencer DreaMer002, which reduced gene expression by 44.7-fold. Our study not only introduces an efficient platform for enhancer design but also establishes a general framework applicable to other CRE types, offering significant potential for designing gene expression circuits in synthetic biology.

Brief Biography

Yuwen Liu is a Principal Investigator, PhD supervisor, and Deputy Director of the Animal Genome Research Center at the Agricultural Genomics Institute at Shenzhen (AGIS), Chinese Academy of Agricultural Sciences. Dr. Liu earned his bachelor's degree from Tsinghua University in 2006 and completed his PhD at the University of Chicago in 2014. Since joining AGIS in 2019, Dr. Liu has been recognized with several prestigious awards, including the National Major Talent Project (Youth), Guangdong High-Level Talent (Youth), “Agricultural Leading Talent” by the Chinese Academy of Agricultural Sciences, Shenzhen High-Level Talent, and the Shenzhen "Youth May Fourth Medal." With over 20 years of research experience in genetics and functional genomics, Dr. Liu specializes in developing experimental and computational methods to decipher the regulatory code of natural non-coding cis-regulatory elements (CREs) and in the de novo design of synthetic CREs. His research has been published in leading journals such as Genome Biology, American Journal of Human Genetics, JACS, Bioinformatics, and Genetics Selection Evolution, and his work has been cited over 1,700 times. Dr. Liu is also actively advancing the industrial application of the recently developed CisEncoder platform, which provides a comprehensive suite of synthetic CREs tailored to fine-tune gene expression in synthetic biology.

Design Strategy for "Genetic Software" Based on Engineered Tristate Logics

Jiawei Shao (邵佳伟)
Zhejiang University

Abstract

Bio-computation strictly follows traditional design principles of digital electronics, which could reach their limits when assembling gene circuits of higher complexity. By creating genetic variants of tristate buffers instead of using conventional logic gates as basic signal processing units, we introduce a tristate based logic synthesis (TriLoS) framework for resource-efficient design of multi-layered gene networks capable of performing complex Boolean calculus within single-cell populations. This sets the stage for simple, modular, and low-interference mapping of various arithmetic logics of interest and an effectively enlarged engineering space within single cells. We not only construct computational gene networks running full adder and full subtractor operations at a cellular level but also describe a treatment paradigm building on programmable cell-based therapeutics, allowing for adjustable and disease-specific drug secretion logics in vivo. This work could foster the evolution of modern biocomputers to progress toward unexplored applications in precision medicine.

Brief Biography

Jiawei Shao is a young Principal Investigator (PI) at the International School of Medicine, Zhejiang University, and the Fourth Affiliated Hospital of Zhejiang University School of Medicine. He employs synthetic biology techniques to construct gene switches by leveraging the transcription initiation, translation processes, and post-translational modifications of gene expression. Shao designs artificial gene circuits and develops smart cell and gene therapies to enhance the precision and effectiveness of disease treatment. His primary research outcomes have been published in international academic journals such as Cell, Science Translational Medicine, PNAS, Cell Research, Science, Science Advances, and Nature Communications, and he has been granted over 10 PCT and Chinese patents.

Synthetic Designer Bacteria-Based Anti-Tumor Therapies with Customizable Outputs and Precise Dosage Control

Ningzi Guan (管宁子)
East China Normal University

Abstract

Bacteria-based therapies offer significant potential for cancer treatment due to their ability to selectively colonize tumors and deliver therapeutic proteins. However, clinical application has been limited by the lack of safe, tunable systems to regulate therapeutic protein expression. Recent advances in remote-control technologies have enabled precise spatial and temporal control of these therapies. We developed two distinct platforms to address this need. The first is a sono-activatable gene circuit (SINGER), based on the thermosensitive repressor TlpA39. In various tumor models, engineered bacteria expressing apoptotic Azurin and the immune checkpoint inhibitor PD-L1 nanobody, when activated by US, significantly suppressed tumor growth. The second platform employs a NIR-mediated PadC-based photoswitch (NETMAP) system, which controls protein expression in engineered bacteria via NIR light. In murine tumor models with varying immunogenicity, bacteria colonized tumors and selectively produced immunomodulators and cytotoxic proteins. In highly immunogenic A20 lymphoma models, NIR-induced CTLA-4 and PD-L1 nanobodies enhanced adaptive immune responses, while in low-immunogenic colon and breast cancer models, NIR-induced Azurin and ClyA triggered apoptosis. These platforms demonstrate precise, remote-controlled bacterial therapies for cancer, offering customizable therapeutic outputs with enhanced safety and efficacy.

Brief Biography

Dr. Ningzi Guan is an Associate Researcher at the School of Life Sciences, East China Normal University. She earned her Ph.D. in Fermentation Engineering from Jiangnan University in 2016 and conducted postdoctoral research at the Georgia Institute of Technology from 2016 to 2018. Since 2018, she has been working at East China Normal University. Her research focuses on the application of metabolic engineering and microbial synthetic biology in disease treatment, with particular emphasis on the development and application of probiotic sensors, optogenetics, and cancer immunotherapy. Her work has been published in Nature Communications, Cell Reports Medicine, Science Advances, Nature Biotechnology, ACS Synthetic Biology, and other journals.

Efficient Biosynthesis of Poly(3-Hydroxybutyrate-*Co*-Lactate) in Metabolically Engineered *Escherichia Coli*

Hui Wu (吴辉)
Dalian University of Technology

Abstract

With the wide application of traditional plastic products, the harm of "white pollution" caused by these products has become increasingly serious. Application of bio-based degradable plastics are the main solution for plastic pollution. Compared to petroleum-based plastics, bio-based degradable plastics have a lower carbon footprint and contribute to a more sustainable life cycle. Polyhydroxyalkanoates (PHAs) synthesized by microorganisms have excellent degradation properties and are important alternatives to petroleum-based plastics. Poly(3-hydroxybutyrate-*co*-lactate) [P(3HB-*co*-LA)] is a high-molecular-weight biomaterial with excellent biocompatibility and biodegradability. The material properties of P(3HB-*co*-LA) are mainly determined by its lactate fraction. Here different strategies of metabolic engineering were applied in this study, including electron transfer chain based metabolic transistor regulation, engineered LA-CoA biosynthesis, and cofactor engineering. The lactate fraction in P(3HB-*co*-LA) synthesized by the engineered strain ranged from 6.2 to 52.84 mol%. Furthermore, when a 5 L bioreactor was used for fermentation utilizing xylose as a carbon source, the titer of P(3HB-*co*-41.3 mol% LA) reached 8.57 g/L.

Brief Biography

Dr. Hui Wu is a professor in Dalian University of Technology (DUT), China, and a member of State Key Lab of Bioreactor Engineering of China. He received Ph.D in East China University of Science and Technology (ECUST) in 2009. Then he joined Shanghai Institutes for Biological Sciences, CAS (2009-2011) and Department of Bioengineering, Rice University (2011-2014) as Postdoc. He joined ECUST in 2014 and moved to DUT in 2024. His recent research focuses on microbial metabolic engineering, metabolic regulation, and synthetic biology. He published more than 60 papers. He also serves the scientific community as an editor of international journals such as *Frontiers in Bioengineering and Biotechnology, Journal of Industrial Microbiology & Biotechnology, Journal of Chemical Technology and Biotechnology, Bioresources and Bioprocessing*, and is the associate editor of *Frontiers in Microbiology* and *BioDesign Research*.

Construction of methanol-tolerant yeast cell factories and synthesis of fine chemicals

Shuangyan Han (韩双艳)
South China University of Technology

Abstract

Yeast can be empowered to produce a variety of chemicals through metabolic engineering and synthetic biology, thereby aiding in the sustainable creation of bioproducts via a clean and renewable approach. Methanol stands out as both a copious and eco-friendly substance, and as a viable candidate for clean energy. The development of microorganisms that can thrive solely on methanol make it is possible to circumvent the food issue of competition with people. *Pichia pastoris* is one of the natural methylotrophic yeast. However, poor tolerance at higher methanol concentrations as well as low carbon atom economy caused by methanol dissimilating into CO_2 have become bottlenecks restricting its efficient utilization and conversion of methanol. Addressing these challenges, our group used kinds of strategies such as knockout of the dissimilation pathway, strengthening of methanol assimilation pathway, along with the construction of the substrate channels and rebalancing of cofactors. These modifications prompted *P. pastoris* into more efficient chassis cells, elevating its methanol utilization by 10%-30%. Furthermore, through iterative evolution and multi omics combination, the high tolerance mechanism of evolutionary strains was analyzed while multiple membrane related tolerance elements were excavated, which helps the construction of multiple high tolerant engineering yeast strains growing well in 10% liquid methanol medium. Based on the above research, our group successfully constructed and obtained several *P. pastoris* cell factories using methanol as the sole carbon source, which were used to synthesize of multiple high-value chemicals high-efficiency, such as linolenic acid (72.2 g/L) and β-arbutin (128.6 g/L), etc.

Brief Biography

Han Shuangyan, a female scholar born in 1976, holds the distinguished title of level-3 professor as well as serving as a doctoral supervisor at South China University of Technology. Her research endeavors focus on microbial utilization and efficient conversion of methanol, microbial cell factory construction and fine chemicals biosynthesis and so on. In the past five years, she has published upwards of 30 scholarly articles in esteemed journals such as *Green chemistry, Food Hydrocolloids, International Journal of Biological Macromolecules*, etc., and authorized more than 20 patents. She has presided over more than 10 scientific research projects, including the National Key R&D Program of China, the National Natural Science Foundation of China, and the Key Technologies R&D Program of Guangdong Province. The book "Enzyme Engineering" edited by her has been awarded the "14th Five Year Plan" undergraduate planning textbook for general higher education by Science Press. She was also ever honored with the China Outstanding Patent Award and the Second Prize of Guangdong Provincial Science and Technology Progress Award.

Plant-based Meat Processing: Fundamentals, Progress, and Industrialization

Xiaonan Sui (隋晓楠)
Northeast Agricultural University

Abstract

With the concepts of sustainable development and "big food" gaining popularity, plant-based meat, as a typical representative of plant-based food, have been widely favored by consumers and is gradually becoming a major trend in the future development of the food industry. Accordingly, following the current research progress in the field of plant-based meat at home and abroad, this report will summarize and discuss the key scientific issues that need to be broken through in the field of plant-based meat, and provide an outlook on the future development of plant-based meat.

Brief Biography

Dr. Xiaonan Sui, a full professor at the College of Food Science, Northeast Agricultural University, is also the vice dean of the Heilongjiang Green Food Science Research Institute. He earned his Ph.D. from the National University of Singapore (NUS) and is a recipient of the Distinguished Young Scientists Fund and Excellent Young Scientists Fund from the National Natural Science Foundation of China (NSFC). Dr. Sui's primary research focuses on soy protein, specifically molecular structure analysis, conformational relationships, and regulatory mechanisms. His goal is to uncover the fundamental scientific principles governing protein molecule alteration, assembly, and rearrangement in food processing to promote green processing and high-value utilization of soy proteins. He has received research grants from various organizations, including NSFC, the Ministry of Human Resources and Social Security's High-level Talents Fund, the Fok Ying Tung Education Foundation's Young Teachers Fund, and the China Association for Science and Technology's Young Talent Promotion Project. Dr. Sui serves as an associate editor for *Sustainable Food Proteins* and is a member of the academic editorial board for *Journal of Food Biochemistry*, *Food Science* and *Future Food Science*. He has authored nearly 200 SCI papers published in respected journals including *Biomaterials*, *Annual Review of Food Science and Technology*, and *ACS Sustainable Chemistry & Engineering*, with nearly 6000 citations to his name. He has also published three English books. His exceptional research contributions have earned him numerous awards, including the Distinguished Young Scientists from Chinese Institute of Food Science and Technology (CIFST), Young Scientist Award from the Division of Agricultural and Food Chemistry of the American Chemical Society (ACS), the Young Scientist Award from the International Union of Food Science and Technology (IUFoST), the Young Scientist Research Award from the American Oil Chemists' Society (AOCS), the Springer Thesis Award.

Synthetic Biology-driven Alternative Protein/Fatty Acids Manufacturing

Demao Li (李德茂)
Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences

Abstract

With the improvement of living standards and the growth of the global population, the consumption of protein continues to increase, which puts enormous pressure on the global food supply. In order to meet the dietary needs of global consumers, it is necessary to produce protein and other food resources in a sustainable manner to address the severe challenges brought by population growth, climate change, and other environmental factors. Microbial protein has the advantages of high production efficiency, no occupation of farmland, and the ability to use one carbon gas as raw material. It is a sustainable and low-carbon protein manufacturing method with unique advantages in replacing proteins. It is an important path to achieve "protein demand from microorganisms" through biotechnology, which can effectively fill the rigid demand for edible and feed protein in China. Oils and fats are important food ingredients that play a crucial role in food flavor, texture, and other aspects. The report introduces in detail the research progress in cell factory construction, process optimization, safety evaluation and food technology of microbial protein/oil production in Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, and puts forward its key bottleneck problems and future solutions.

Brief Biography

Demao Li, Ph.D., a professor of the Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, doctoral supervisor, PI of the Industrial Biosystem Engineering Research Group, a candidate for a distinguished research post of the Chinese Academy of Sciences, a member of the Youth Innovation Promotion Association of the Chinese Academy of Sciences, coordinator of the bioenergy joint research group of COMSATS Industrial Biotechnology Joint Center, a member of the Industrial Fermentation Sub technical Committee of the National Technical Committee for Food Industry Standardization, a member of the Beijing Tianjin Hebei Food Nutrition, Health and Safety Innovation Platform, and an editorial board member of the magazine Fermentation. Dedicated to low-carbon biomanufacturing research of microbial proteins/oils. Hosted more than 20 provincial and ministerial level projects, obtained more than 20 authorized national invention patents, and published over 100 SCI papers in internationally renowned journals such as Applied Catalysis B: Environment and Energy, Nature Communication, Biotechnology Advances, Journal of Agricultural and Food Chemistry, Sustainable Energy&Fuels, Reviews in Aquaculture, etc. As the main contributor, won the second prize of the National Oceanic Administration Ocean Engineering Award and the second prize of the National Oceanic Administration Ocean Science and Technology Award.

Biological Upcycling of Waste for Sustainable Development

Jinjin Diao (刁进进)
Washington University in St. Louis

Abstract

More than 70 million tons of poly(ethylene terephthalate) (PET) are manufactured worldwide every year. The accumulation of PET waste has become a global pollution concern, motivating the urgent development of technologies to valorize post-consumer PET. The development of chemocatalytic and enzymatic approaches for depolymerizing PET to its corresponding monomers opens up new opportunities for PET upcycling through biological transformation. However, there are only a handful of reports demonstrating non-model microbes capable of growing on both TPA and EG generated from PET as sole carbon sources. Moreover, the scarcity of synthetic biology tools specifically designed for these non-model species, limiting the development of the corresponding microbial cell factories for upcycling of the post-consumer PET. To overcome the aforementioned limitations, we performed strain screening to discover a Rhodococcus strain RPET (RPET) that can grow well on the alkaline hydrolysis products of PET as the sole carbon source. Notably, this strain can grow on a mixture of TPA and EG at extremely high concentrations (up to 0.6M) and high osmolarity resulting from alkaline hydrolysis and pH neutralization. The resultant media supported RPET's growth without any purification and sterilization step except for their dilution. To expand the repertoire of bioproducts from post-consumer PET, we described the development of potent genetic tools in RPET, including: (1) two inducible and titratable expression systems for tunable gene expression, (2) Serine Integrase based Recombinational Tools (SIRT) for genome editing. Using these tools, we systematically engineer the RPET strain to ultimately establish microbial supply chains of multiple chemicals, producing the highest titers of chemicals ever reported thus far, including lycopene, lipids, and succinate, from post-consumer PET waste bottles (e.g., 22.6 mg/L of lycopene, about 10,000-fold higher than that of the wild-type strain). This work highlights the great potential of plastic upcycling as a generalizable means of sustainable production of diverse chemicals.

Brief Biography

Dr. Jinjin Diao is a Senior Scientist in the Department of Energy, Environment, and Chemical Engineering at Washington University in St. Louis. Dr. Diao received his PhD from Tianjin University in Biochemical Engineering in 2019. In his PhD, Dr. Diao demonstrated how microbes can be reprogrammed to produce value-added chemicals through synthetic biology. Dr. Diao finished his postdoc training under Prof. Tae Seok Moon at Washington University in St. Louis, where he focused on elucidating the metabolic and regulatory mechanisms of aromatic catabolism in Rhodococcus opacus PD630. Now, Served as CO-PIs of three US federal funded grants, Dr. Diao mainly focused on the development of robust microbial chassis for the goal of "Green Biomanufacturing" by using alternative feedstocks. So far, Dr. Diao has published 25 peer-reviewed papers, and of them, 8 first-author papers have been published in the prestigious journals including Cell Reports, Nature Communications Biology, Metabolic Engineering, et al. In recognition of his outstanding research, he has received Johns Hopkins University Conference Travel Fellowship and IMES Travel Award, been invited to join the Early Career Reviewer Board of the BMC journals in Biotechnology, and also been invited to give oral presentations in multiple international conferences.

Biosynthesis Strategies of Sweetener Rebaudioside D

Yuanyuan Ma (马媛媛)
Tianjin University

Abstract

Rebaudioside D (Reb D) is a promising sweetener due to its zero calorie and high sweetness, but its content in stevia leaves is extremely low. Here, I will briefly discuss two biological conversion strategies established to convert the low-value Rebaudioside A to Reb D. The first strategy involves constructing an expression system for glycosyltransferase EUGT11 using *Pichia pastoris* and *E. coli*. The effects of different hosts on the activity of the recombinant enzyme were examined. Reb D was synthesized in a one-pot reaction using the engineered *Pichia pastoris*. A two-step temperature control method was subsequently developed, achieving a conversion rate of 95.31% at 28/35 °C. The second strategy involves identifying a new enzyme StUGT, which can convert Reb A to generate Reb D. The characteristics of this enzyme were characterized, and a cell catalytic system was developed using the StUGT *E. coli* strain. The highest yield of 98.08% was obtained by enhancing cell permeability and optimizing conditions statistically. A cascade process was further established using this StUGT strain and *E. coli* expressing sucrose synthase to reduce costs by replacing expensive UDPG with sucrose. These studies pave the way for cost-effective and sustainable synthesis of scarce, high-value sweeteners.

Brief Biography

Dr. Ma received Ph.D. degree from Nankai University in Dec 2006. She joined "Biomass conversion Lab, R&D Center for Petrochemical Technology, Tianjin University" as an assistant professor in Mar 2007, and then she became an associate professor and the head of biomass conversion Lab in 2012. In 2012, she also worked as a visiting scholar in the Industrial Biotechnology Lab at the Institute of Chemical & Engineering Sciences (ICES) in Singapore for one month. Subsequently, she worked at the School of Marine Science and Technology at Tianjin University. Her research interest focuses on synthetic biology, biocatalysis and exploration of functional genes from marine. She has published papers in journals including Metab Eng, Biotechnol Biofuels, Int J Biol Macromol, Applied Microbiol Biotechnol, and Front Microbiol. And she has undertaken numerous National level research projects as well as the projects from central State-owned enterprises.

Establishing cell factories for value-added food protein bioproduction

Yanfeng Liu (刘延峰)
Jiangnan University

Abstract

Cell factories-based efficient biosynthesis of value-added food protein is a sustainable approach for protein supply. However, efficient protein expression tools and chassis cell hamper economic protein bioproduction in large-scale. In this talk, recent advances in developing protein expression regulatory elements and strategies for engineering production host are discussed. Specifically, continues evolution and antibiotic free-recombinant protein expression tools are developed for improving protein expression regulatory tools. Next, single stranded DNA annealing protein-guided CRISPR genome editing method and protein secretory pathway engineering strategies are developed for modifying chassis cell for protein production. Finally, the developed protein expression regulatory elements and strategies for engineering production host are used for establishing cell factories for bioproduction of value-added food protein, such as -lactalbumin and ovalbumin. The proposed protein expression tools and engineering approach may be useful for enhancing bioproduction of other food protein.

Brief Biography

Yanfeng Liu is a Professor of School of Biotechnology in Jiangnan University. He focuses on using synthetic biology tools to construct cell factories for biosynthesis of important nutraceuticals and food ingredients. As the first or corresponding author, his research has been published in journals such as Nature Chemical Biology, Nature Communications and Metabolic Engineering. As the first inventor, 15 invention patents were authorized. He is the project leader of National Excellent Youth Fund, National Natural Science Foundation. 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.

Construction of Bioinspired Multi-enzyme Molecular Machines and the Application for Functional Sugars Biosynthesis

Wei Liu (刘伟)
Nanjing Tech University

Abstract

To address the challenges of multi-enzyme adaptation and coupling in the current enzymatic synthesis of functional sugars, this work developed various substrate-channeling and compartmentalized multi-enzyme molecular machines based on protein-peptide pairs, self-assembling proteins, nucleic acids, and phase-separated proteins. These molecular machines provided mild and efficient assembly tools for multi-enzyme cascade catalysis, which were applied to the enzymatic preparation of typical functional sugars, including trehalose, tagatose, and psicose, achieving enhanced multi-enzyme catalytic efficiency.

Brief Biography

Wei Liu, Entrepreneurship and Innovation Doctoral Talent of Jiangsu Province, associate professor at college of Food Science and Light Industry, Nanjing Tech University. His primary research focuses on enzyme engineering and synthetic biology, with a particular emphasis on the construction of in vivo/in vitro multi-enzyme cascade systems and the creation of cell factories for food nutrients. He has successively led several national and provincial/ministerial projects, including the Youth Fund Project of the National Natural Science Foundation of China and subprojects of the National Key Research and Development Program. He has published over 20 papers in academic journals, including Metabolic Engineering, Food Research International, Journal of Agricultural and Food Chemistry, and applied for 14 invention patents and 3 PCT patents. His research achievements have garnered one First Prize for Scientific and Technological Progress awarded by the China National Light Industry Council and one Special Prize for Scientific and Technological Progress awarded by the China General Chamber of Commerce.

Current Situation and Future Prospect of the Bio-manufacturing on Human Milk Oligosaccharides

Zhengqiang Jiang (江正强)
China Agricultural University

Abstract

The strategy for “Healthy China” not only promotes the development of healthy industry, but also brings the golden opportunity for the development of functional oligosaccharides. Human milk oligosaccahrides (HMOs) as a kind of oligosaccharides naturally exist in human milk. Their monosaccharide compositions are simple, while the structures are complex. HMOs play an important role in the growth and development of infants. USA and European Union have approved seven HMOs as the novel foods to be applied in infants formula. So far, the global market of HMOs has reached up to 380 million dollars. Chemical synthesis and biosynthesis can be used to produce HMOs. The biosynthesis including enzymatic and whole cell synthesis is the principal method. As the representative HMOs, the productivity of 2'-fucosyllactose (2'-FL) and lacto-N-neotetraose (LNnT) has been more than 100 g/L via the whole cell synthesis based on precision fermentation. With the aim to facilitate the industrial development of HMOs, our research team has focused on the production of HMOs using enzymatic method and whole cell synthesis. The novel α-L-fucosidases, β-N-acetylhexosaminidases, and β-galactosidases were exploited to produce 2'-FL, 3-fucosyllactose (3-FL), LNnT, and lacto-N-tetraose (LNT) via enzymatic method. Many of them were modified to increase the yield. Also, the novel α-1,2-fucosyltransferases and β-1,4-galactotransferases were introduced to the engineered Escherichia coli for the efficient synthesis of 2'-FL, 3-FL, difucosyllactose (DFL), LNT, and LNnT. In the future, we will enhance the study on the biomanufacturing and downstream purification of HMOs, which will provide technological and theoretical foundation for the industrial development of HMOs.

Brief Biography

Jiang Zhengqiang, member of the Communist Party of China, born in June, 1971. He was graduated from China Agricultural University with doctorate in Food Science and gained “One Hundred Outstanding Doctoral Dissertation” in 2001. Since 1995, he worked in China Agricultural University and was promoted as Professor in 2004. He has been the Winner of “The National Science Fund for Distinguished Young Scholars” and the distinguished professor of “The Yangtze River Scholar”. Moreover he has been selected as young and middle-aged expert contribution to “The National People’s Ten Thousand Talents Program”, the leading talent of “The National Ten Thousand People Plan”, and received the state council special allowance. Also, he has won “The Young and Middle-Aged Leading Talents in Technological Innovation” of the ministry of science and technology, and obtained the agricultural bioprocessing technology innovation team” of “Outstanding Agricultural Research Talents and Innovation Teams” approved by the ministry of agriculture. In addition, he was served as the president of “Agricultural Processing Branch of Chinese Society of Agricultural Engineering”, the vice president of “Enzyme Branch of Chinese Society of Food Science and Technology” and “Enzyme Preparations and Prebiotics Branch of Chinese Fermentation Industrial Association”. The editorial board member of more than ten journals, such as “Food Chemistry”, “Food Science”. He focus on food biotechnology, food enzyme, and fermentation engineering. More than 300 academic papers (including more than 200 SCI papers) and 6 monographs were published during the past 20 years. More than 70 invention patents were authorized. He has won two awards for “Second Class Prizes of National Science and Technology Progress (named first and second)”, one award for “Second Prize of National Science and Technology”, and one award for “China Youth Science and Technology”, two awards for “Guanghua

Engineering Science and Technology Prize", and two international academic awards.

Yeast Metabolism Adaptation for Efficient Terpenoids Synthesis Via Isopentenol Utilization

Wenyong Lou (娄文勇)
South China University of Technology

Abstract

Terpenoids, also known as isoprenoids, are abundant natural compounds found in animals, plants, and microorganisms, playing a crucial role in maintaining normal biological activities. These compounds have extensive applications, including use in fragrances, biofuel, colorants, micronutrients, cosmetics, and medicine. However, conventional extraction methods are insufficient to meet the growing market demand for terpenoids. Synthetic biology technology has enabled the design and construction of microbial cell factories, offering a sustainable and scalable solution for terpenoid production. Several valuable terpenoids have been successfully synthesized by engineered microorganisms such as retinol, carotenoid, ursolic acid, and rebaudiosides. Recently, a two-step isopentenol utilization (IU) pathway relying solely on ATP as the cofactor has been proposed as an alternative to the mevalonate (MVA) pathway, streamlining the synthesis of the common terpenoid precursors. Herein, we find that isopentenol inhibits energy metabolism, leading to reduced efficiency of the IU pathway in *Saccharomyces cerevisiae*. To overcome this, we engineer an IU pathway-dependent (IUPD) strain, designed for growth-coupled production. The IUPD strain is compelled to enhance the ATP supply, essential for the IU pathway, and incorporates a high-throughput screening method for enzyme evolution. The refined IU pathway surpasses the MVA pathway in synthesizing complex terpenoids. Our work offers valuable insights into developing growth-coupled strains adapted to efficient natural product synthesis.

Brief Biography

He is currently Vice president and Deputy Party Secretary of the School of Food Science and Engineering, South China University of Technology. He received numerous honors including the National Excellent Doctoral Dissertation Award, the National Outstanding Youth Science Foundation (the first batch of Outstanding Youth), the Ministry of Education's New Century Excellent Talents Award, and the Leading Talent of Guangdong Province's "Hundred, Thousand, and Ten Thousand Talents Project". In 2023, ranked among the top 100,000 scholars globally for academic impact (Lifetime Academic Influence Rankings).Research focuses on food biotechnology, regulation and application of biocatalytic processes, exploration and biomanufacturing of food functional factors, and encapsulation and targeted delivery of nutrients. Led and participated in over 30 research projects, including the National Key R&D Program, the National Natural Science Foundation of China, the Guangdong Key R&D Program, and industry-university collaborative projects. Published over 150 SCI papers in prestigious journals such as Nature Communications, Microbiome, Coordination Chemistry Reviews, Biotechnology Advances, and Small, with more than 6,000 citations and an h-index of 46. Serves as associate editor for Frontiers in Bioengineering and Biotechnology and Frontiers in Microbiology, and on the editorial boards of Bioresources and Bioprocessing and the Chinese Journal of Food Science. Holds 56 Chinese invention patents, 3 international PCT patents (USA), and has transferred 10 patents to industry, with many research outcomes successfully commercialized.

Crystaline Immobilization Platform for Biocatalysis

Yao Chen (陈瑶)
Nankai University

Abstract

Biomacromolecules, such as enzymes, are ubiquitous in nature and essential for maintaining basic life activities. Apart from the fundamental biological functions, biomacromolecules are also of great values in industrial applications, especially in food and pharmaceutical production. However, their industrial applications are often handicapped by low operational stability, poor robustness, difficult recovery and reuse. Incorporation of biomolecules within protective exteriors has been proved to be an effective method to promote their stabilities and applications. As new classes of crystalline solid-state materials, covalent-organic frameworks (COFs), feature high surface area, tunable pore size, high stability, and easily tailored functionality, which entitle them as ideal supports for encapsulation of biomolecules to form novel composite materials for various applications. Moreover, the formed composites can combine the properties of both constitutes, where crystalline frameworks materials and biomolecules are indeed mutually beneficial. Our researches mainly focus on the development of novel functional carriers, and their efficient assemble/cascade with biomacromolecules. This novel crystalline platform composed of biomolecules-incorporation and framework materials exhibited various functionality and superior poteintials in catalysis and separation.

Brief Biography

Yao Chen is a professor and doctoral supervisor of Nankai University and Institute of Process Engineering, China. She received the master's degree from Nanjing University of technology in 2009, and then the doctor's degree from the University of South Florida in 2014, and engaged in postdoctoral research at the UCSD from 2014 to 2016. She joined Nankai University in July 2016 and established independent research group that focuses on the precise immobilization and functional formulation of biomacromolecules. In 2024, she also joined Institute of Process Engineering, CAS. She has published 130 SCI papers. As the corresponding/first author she has published 60 papers including Nat Commun、Nat Protoc、Nat Rev Chem, JACS, Angew Chem, Adv Mater, Chem, Chem Soc Rev. 14 papers have been selected as ESI highly-cited paper. She has 41 authorized/applied Chinese patents and 3 U.S. patents. She became the member of the council of The Chemical Industry and Engineering Society of China (2022) and received the National Excellent Youth Science Fund (2020)

Designing Enzyme-producing Cell Factories for Green Production of Chemicals

Yaping Xue (薛亚平)
Zhejiang University of Technology

Abstract

The evolution of industrial enzyme-producing cell factories marks a notable progress in biotechnology, providing novel solutions for diverse industrial applications. These factories leverage genetically engineered microorganisms to synthesize enzymes at scale, thereby enhancing the efficiency and sustainability of industrial processes through genetic and metabolic optimizations. The progress in industrial enzyme production signifies an exciting frontier in biotechnological innovation, promising to enhance efficiency across multiple sectors. We have engineered a suite of tools and synthetic biology elements, including genome-editing technologies, promoters, signal peptides, and molecular chaperones. These tools have been instrumental in constructing industrial enzymes or whole-cell biofactories for the efficient production of functional chemicals such as pesticides, pharmaceuticals, and nutrition additives

Brief Biography

Dr. Ya-Ping Xue is a distinguished Professor from Zhejiang University of Technology. His research interests include biopharmaceutical, biocatalysis and transformation, enzyme engineering, synthetic biology, and green bio-manufacturing. He has successfully developed several green bio-manufacturing industrialization technologies for the production of pharmaceuticals or their intermediates, pesticides or their intermediates, and nutritional additives, resulting in enormous profits in both economy and society. He has published more than 100 papers in academic journals and authorized more than 100 patents for invention.

Biosynthesis of Bioactive Cyclic Peptides

Huan Wang (王欢)
Nanjing University

Abstract

Cyclic peptides represent a unique family of bioactive compounds. Our group aims to develop chemical and biochemical methods to synthesize cyclic peptides with structural and functional diversity. This presentation will introduce our efforts in elucidation of the biosynthesis of a family of natural peptide natural products named lanthipeptides, specifically on the enzymology involved in the biosynthetic process and attempts to engineering biosynthetic enzymes for enhanced catalytic activities.

Brief Biography

Huan Wang is a Professor in the Department of Chemistry and Chemical Engineering at Nanjing University, China. Wang received a BS in Chemistry from Peking University, PhD in Chemistry from Univ. of Maryland, and completed postdoctoral work at UIUC. Wang joined Nanjing University in 2014. Wang's research focuses on the chemical synthesis and biosynthesis of bioactive peptides and related chemical biology.

Molecular Evolution of Baeyer-villiger Monooxygenases for Synthesis of Chiral Sulfoxide Pharmaceuticals

Huilei Yu (郁惠蕾)
East China University of Science and Technology

Abstract

A unique and typical Baeyer Villiger monooxygenase library was constructed by gene mining and cluster analysis. The crystal structure of cyclohexanone monooxygenase from *Acinetobacter* was successfully resolved for the first time. We successfully switched the substrate preference from small molecule cyclohexanone to bulky lazole sulfide substrate through semi rational design. The molecular mechanism affecting the substrate selectivity of thioether monooxygenase for sulfide and sulfoxide was clarified and the precise regulation of substrate selectivity was realized. Finally, the chiral sulfoxides, such as (*S*)-omeprazole, (*R*)-lansoprazole can be synthesized efficiently and controllably in large-scale, achieving a reform in the production mode of chiral sulfoxide drugs.

Brief Biography

Professor. Huilei Yu obtained her Ph.D. on Biochemical Engineering from East China University of Science and Technology in 2008. She was appointed as Assoc. Professor in 2010, and subsequently Professor in 2017 at State Key laboratory of Bioreactor Engineering of ECUST. During 2013-2014, she worked as a Visiting Professor in Laboratory of Bioinformatics and Metabolic Engineering at MIT. Her research focuses on elucidating the structure-activity relationship of enzyme, designing new biochemical reactions, and expanding the space for enzyme catalyzed synthesis of functional molecules. The enzymatic synthesis technologies of chiral hydroxyl acid, chiral sulfoxide and chiral alcohol have been applied in industry, which greatly deceased the production waste generation and promoted the technology renovation of pharmaceutical industry. She was also the executive editor of *Bioresources and Bioprocessing*. She was awarded Outstanding academic leader of Shanghai, Outstanding young enzymologist, First Level Technical Innovation Award of Shanghai City and so on.

Boosted Enzyme Activity by Engineering Microenvironment

Yongqin Lyn (吕永琴)
Beijing University of Chemical Technology

Abstract

For successful industrial applications, high activity and stability are the necessary characteristics for enzymes. However, industrial operational conditions often differ significantly from the natural environment in which enzymes function, such as variations in pH, temperature, and the presence of organic co-solvents. These disparities can lead to a significant reduction in enzyme activity. One effective approach to address this challenge is the immobilization of enzymes onto heterogeneous supports or carriers. This immobilization strategy not only stabilizes enzymes but also facilitates their easy recovery and continuous use. However, traditional immobilization methods often result in a reduced apparent enzyme activity compared to their native counterparts. This is primarily due to the distortion of the enzyme's tertiary structure and the obstruction of substrate access during the immobilization process.

In this study, we have developed novel enzyme immobilization carriers that serve a dual purpose. These carriers not only enhance enzyme activity but also function as "artificial" chaperones to assist in the refolding of denatured enzymes. We achieve this by carefully engineering the local chemical microenvironment of enzymes, which involves modifying pore sizes, pore shapes, and functionalities of solid supports. Furthermore, by replicating the active sites found in native enzymes, we have also successfully created structural mimics of carbonic anhydrase and laccase. These enzyme mimics have demonstrated exceptional catalytic capabilities in the processes of CO_2 hydration and lignin degradation.

Brief Biography

Prof. Yongqin Lv received her Bachelor degree from Department of Materials Science and Engineering in 2005, and Ph.D. degree from the College of Life Science and Technology both at Beijing University of Chemical Technology in December of 2010. From 2008 to 2010 she studied at the Materials Science Division of Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia. For 2 years from 2011, she was a postdoctoral fellow at the Department of Chemistry, University of California, Berkeley. She is currently a professor at College of Life Science and Technology at Beijing University of Chemical Technology, and deputy directors of International Joint Laboratory for Bioenergy of Ministry of Education and Beijing Key Laboratory of Bioprocess. Her research interests include microenvironment regulation to enhance biocatalysis, photo- and electrically-driven biocatalytic carbon fixation, and synthetic antibody engineering. She has published 76 papers in SCI-index journals including P. Natl. Acad. Sci. USA., J. Am. Chem. Soc., Matter, Nano Lett., Adv. Sci., Prog. Energ. Combust., Biotechnol. Adv., and etc. She also applied more than twenty patents. Her leadership is evident in overseeing more than 10 research projects, including 9 national initiatives. She has presented academic presentations at over 50 domestic and international conferences, with over 30 being invited talks. Beyond her academic contributions, she actively participates in professional committees, serving as a member of the One Carbon Biotechnology Committee of the Chinese Society of Biotechnology and a member of the Youth Work Committee of the Chemical Industry and Engineering Society of China. Moreover, she serves in various editorial roles, including guest editor for Biotechnology Advances, editorial board member and guest editor for Synthetic and Systems Biotechnology, young editorial board member and guest editor for Frontiers of Chemical Science & Engineering, editorial board member for Microchimica Acta, and young editorial board member for Bioresources and Bioprocessing, among others. She also contributes her expertise as an expert reviewer for the National Natural Science Foundation of China, a

reviewer for the Beijing Natural Science Foundation, and an expert reviewer for European Commission projects.

Biotransformation of Lignocellulosic and Waste Plastic Upgraded by Computer-assisted Enzyme Engineering

Xiujuan Li (李秀娟)
Nanjing Normal University

Abstract

Considering the demand in the Chinese market for enzyme preparations, it is essential to strengthen the research and development of new technologies, address issues such as low industrial enzyme performance, establish key technologies including independently owned proprietary technologies and strains, and promote the application of enzyme preparations in the industrial sector. The past few years have witnessed the development of protein engineering, achieved a series of significant advancements and breakthroughs, and became a powerful tool for improving enzyme catalytic performance. In recent years, we have explored and designed several cutting-edge enzyme strategies that combine computational techniques with synthetic biology. These efforts aim to provide methods for the preparation of higher-performance enzyme components. 1) utilized intelligent computing to assist in obtaining highly robust cellulase enzymes to enhance lignocellulosic conversion efficiency, 2) developed BHETase mining strategy and the enzyme engineering strategy guided by kinetic calculation to improve the anchoring rate and catalytic efficiency, and to find new breakthroughs for the biological degradation of plastics to achieve plastic recycling economy.

Brief Biography

Xiujuan Li professor at Nanjing Normal University has long been engaged in deeply integrating cutting-edge technologies such as enzyme engineering, computational biology, and AI into the waste resource recycling research, helping to achieve the goal of carbon neutrality and carbon peak. In the past five years, as the first/corresponding author, she has published more than 30 SCI academic papers in *Nat Commun*, *Angew Chem* and other journals, and applied for more than 20 invention patents. The relevant achievements won the grand prize and the second prize of the China General Chamber of Commerce in 2023, and the first prize of the Jiangsu Agricultural Society technology innovation Award.

Application of Metabolic Pathway Compartmentalization Engineering Strategy in the Construction of Yeast Cell Factories

Aiqun Yu (于爱群)
Tianjin University of Science and Technology

Abstract

Yeast offers a complete and organized production line for compound synthesis and storage, which relies on a variety of organelles and membranous structures, including the endoplasmic reticulum (ER), Golgi, lipid droplets (LDs), peroxisomes, mitochondria, and plasma membrane. Because excessive accumulation of various compounds can cause cytotoxicity, compartmentalization protects the intracellular environment by concentrating detoxifying enzymes and metabolites within a limited and closed space. At the same time, such arrangement improves the catalytic efficiency of enzymes. In recent years, researchers have begun to use this subcellular compartmentalization engineering strategy to construct cell factories that can more effectively produce value-added chemicals, and have achieved good production efficiency. We have repositioned the synthetic pathway of a terpenoid natural product, bisabolene, to the cytoplasm, mitochondria, and peroxisomes of of the yeast *Yarrowia lipolytica*. The findings revealed a significant increase in production for organelle engineering strains compared to cytoplasmic engineering strains, marking the highest bisabolene titer in microbial chassis to date. This highlights the effectiveness of compartmentalizing metabolic pathways as an optimal strategy for bisabolene synthesis in yeast cell factories. However, the molecular mechanisms underlying the strategy's enhancement of product yield remain unexplored, and we herein conduct preliminary analysis and discussion on this.

Brief Biography

Aiqun Yu, Bachelor's degree from Shandong Normal University (2002-2006), Master's degree from Fujian Normal University (2006-2009, supervised by Professor Jianzhong Huang), and PhD from Nankai University (2009-2012, supervised by Professor Mingchun Li); I conducted postdoctoral research at Nanyang Technological University and National University of Singapore from 2012 to 2016, with Professor Matthew Chang as my co-supervisor; Selected for the Overseas Young Scientist Program of Tianjin in 2017. He is now a professor of the State Key Laboratory of Food Nutrition and Safety at Tianjin University of Science and Technology, and the deputy director of Tianjin Microbial Metabolism and Fermentation Process Control Technology Engineering Center. At present, my main research interest lies in yeast cell factories, green biomanufacturing, modern brewing technology, etc. I have published 36 first/corresponding author papers in well-known domestic and foreign journals in the field of biotechnology; applied for/authorized 10 national invention patents as the first inventor; been invited to serve as a young editorial board member for SCI journal Microbial Biotechnology and guest editor for Journal of Fungi, Frontiers in Bioengineering and Biotechnology, and Food Science.

Drug Delivery by Synthetic Probiotic Bacteria

Yun Yang (杨昀)
Beihang University

Abstract

An overwhelming number of studies have reported the correlation of decreased abundance of butyrate-producing commensals with a wide range of diseases. However, the molecular-level mechanisms whereby gut butyrate causally affects the host physiology were poorly understood. We engineered a commensal bacterium to delivery butyrate at the intestinal mucosal surface, and implemented it to dissect the causal role of gut butyrate in microbe-host interaction.

Brief Biography

Dr. Yang is an associate professor at the School of Engineering Medicine, Beihang University. She got her Bachelor and Master degree from Tsinghua University, and received a Ph.D. degree from Nanyang Technological University in Singapore. Dr. Yang has conducted a series of studies on rational design and engineering of bacteria for biocatalysis and drug delivery.

Mechanistic Study and Modification of Depolymerases for Synthetic Polyesters

Yu Yang (杨钰)
Hubei University

Abstract

Bio-based degradation using renewable biological entities, i.e., enzymes or microorganisms, is an ideal and environmentally benign approach to reduce and recycle plastics. Thus, significant effort has been made to develop a PBAT biodegradation strategy. However, the lack of in-depth investigation into their potency and mechanism of action hampers their applications. In this work, we investigate the PBAT hydrolysis potency of cutinases and PET-degrading enzymes, and find that cutinases outcompete other reported enzymes. Significantly, engineering cutinases with the DM strategy results in higher activity such that PBAT polymers can be decomposed to their monomeric constituents. We apply biochemical and structural analyses to investigate the hydrolytic intermediates/products and substrate-binding features to propose its mechanism of action. Taken together, this study illustrates the potential application of engineered cutinase in PBAT biodegradation. These results provide an important basis to guide the development of biodegradation/bio-recycling of PBAT as well as other aliphatic-aromatic co-polyesters.

Brief Biography

Dr. Yu Yang obtained his Ph.D. of biotechnology at Jiangnan University in 2014. After that, he worked as a postdoc at Georgia State University and the University of Texas at San Antonio. Currently, he joined in at Hubei University as an associate professor. He has a broad interest in studying biocatalysis's structural-functional relationship related to the degradation of polyesters and neurological diseases, including hydrolase, decarboxylase, dehydrogenase, and dioxygenase. He has solved over 100 protein structures and published around 20 papers like *Nat. Commun.*, *J. Hazard. Mater.*、*J. Med. Chem.*, *J. Biol. Chem. et al*.

Synthetic Biology with Single-cell Precision: From Enzyme Development to Technology Development and Application

Jia Zhang (张佳)
Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences

Abstract

Synthetic biology, an interdisciplinary frontier of science, amalgamates the essence of numerous research areas and directions, emerging as a pivotal force in exploring the mysteries of life and innovating biotechnologies. In recent years, my research team has focused on the innovative development of single-cell Raman technology, skillfully integrating it into the grand blueprint of synthetic biology, aiming to establish a new paradigm of synthetic biology research centered on single-cell precision.

Our journey of exploration began with the meticulous construction of a comprehensive Single-cell Raman technology pipeline: from precise Single-cell sorting and efficient gene amplification to seamless coupling with Single-cell sequencing or Single-cell culture. During this process, we astutely identified that the high efficiency and low cost of enzyme reagents were critical bottlenecks in the entire technology chain. To address this, we successfully developed a high-efficiency, low-cost HotJa single-cell whole-genome enzyme kit based on advanced enzyme engineering technology. This breakthrough not only overcame technical barriers but also endowed our single-cell Raman technology pipeline with distinctive technical features and strong industry competitiveness.

As the technology matured, we applied this innovative achievement across multiple fields: in the targeted discovery of functional gut strains, our technology enables precise identification and selection of strains with specific functions; in the fermentation process of strains, it offers the possibility of real-time monitoring, ensuring the stability and efficiency of the production process; and in the rapid detection technology of integrated probiotic products, it demonstrates rapid and accurate detection capabilities. These applications not only prove the immense potential of single-cell Raman technology but also facilitate our horizontal collaborations with Moutai Group, COFCO Group, and Yili Group, paving new paths for the practical application of synthetic biology and heralding a new era of more precise and efficient biotechnology.

Brief Biography

Jia Zhang is currently the group leader/associate researcher of the Enzyme Research Group at the Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, and a founding young editorial board member of BioDesign Research. Her main research areas include gene synthesis, metabolic engineering, enzyme engineering, and the development and application of single-cell Raman technology, all related to synthetic biology. To date, she has published a total of 15 SCI papers, with 9 as the first author or corresponding author. She has led 9 research projects, including the National Key R&D Program Young Scientist Project, National Defense Science and Technology Projects, National Natural Science Foundation Youth Projects, Shandong Province General Projects, and various horizontal projects (including COFCO Group, Moutai Group, Yili Group, etc.), with a total funding of 10.26 million RMB. She has applied for 33 patents, with 32 granted, and has been awarded the title of "Qingyuan Scholar" Young Talent.

Phosphoantigens Glue Butyrophilin 3A1 and 2A1 to Activate VΓ9VΔ2 T Cells

Yunyun Yang (杨云云)
Hubei University

Abstract

In both cancer and infections, diseased cells are presented to human Vγ9Vδ2 T cells through an “inside-out” signaling process wherein structurally diverse phosphoantigen (pAg) molecules are sensed by the intracellular domain of butyrophilin BTN3A1. Here, we show how—in both human and alpaca—multiple pAgs function as “molecular glues” to promote heteromeric association between the intracellular domains of BTN3A1 and the structurally similar butyrophilin BTN2A1. X-ray crystallographic studies visualized that BTN3A1 engagement with pAgs forms a composite interface for direct binding to BTN2A1, with various pAg molecules each positioned at the center of the interface and gluing the butyrophilins with distinct affinities. Our structural insights guided mutagenesis experiments that led to disruption of the intracellular 3A1-2A1 association, abrogating pAgs-mediated Vγ9Vδ2 T cell activation. Structure-based MD simulations, ^{19}F-NMR investigations, chimeric receptor engineering, and direct measurement of intercellular binding force revealed how pAgs-mediated 2A1 association drives 3A1 intracellular fluctuations outwards in a thermodynamically favorable manner, thereby allowing 3A1 to “push off” from the 2A1 ectodomain to initiate TCR-mediated γδ T cell activation. Practically, we harnessed the molecular glue model for immune-therapeutics design, demonstrating chemical principles for developing both small molecule activators and inhibitors of human γδ T cell function.

Brief Biography

Yang Yunyun, Ph.D., graduated from Tsinghua University and is currently an associate professor at Hubei University. Her research primarily focuses on the structural biology and biochemical mechanisms of (1) disease-related proteins and (2) the enzymatic reaction mechanisms involved in the biosynthesis of antitumor natural products. She has published 17 articles in international journals, including Nature, Cell, Immunity, Angewandte Chemie International Edition, and ACS Catalysis. Dr. Yang has led eight national and provincial-level projects, including a National Key R&D Program of China, and National Natural Science Foundation of China. Additionally, she has been recognized as a candidate for the Hubei Province Future Female Scientist Program and has received exceptional support as a “Chutian Scholar” in Hubei Province.

Reprogramming Unconventional Yeast Cell Factories for The Low-ph Biomanufacturing of Succinic Acid

Zhiyong Cui (崔志勇)
Shandong University

Abstract

Succinic acid (SA) is an important metabolic intermediate and platform compound for organic synthesis, with broad market prospects. It is expected that by 2025, the domestic SA market potential will reach 200,000 tons per year, and the output value is expected to exceed 500 million US dollars/year. The development of green and sustainable SA fermentation technology is not only helpful to solve the problem of plastic pollution, but also in line with low-carbon development strategy. Due to its excellent acid tolerance, *Yarrowia lipolytica* has attracted much attention as a novel chassis for synthetic biology. In our previous study, we obtained a series of recombinant engineered strains of *Y. lipolytica* by inactivating succinate dehydrogenase and enhancing oxidative TCA cycle, and achieved high SA production at low pH for the first time. However, the yields of these strains are too low to meet the needs of industrial production. To address this bottleneck, a reductive SA synthetic pathway was created in strictly aerobic yeast to improve carbon source utilization efficiency. Adaptive evolution and cofactor engineering were used to enhance the adaptability of heterologous metabolic pathways to chassis. Eventually SA titer, yield, and productivity could reach to 111.9 g/L, 0.79 g/g glucose, and 1.8 g/L/h, respectively. Furthermore, the engineered *Y. lipolytica* strain was proved to produce 45.34 g/L SA using undetoxified lignocellulose hydrolysates. This study will help to reveal the metabolic regulation mechanism of SA biosynthesis in aerobic yeast, and lay the foundation for the development of bio-based materials industry in China.

Brief Biography

Zhiyong Cui is an associate researcher at the State Key Laboratory of Microbial Technology, Shandong University. He has long been engaged in the research of unconventional yeast synthetic biotechnology and bulk chemicals bio-manufacturing. Based on the development of large-scale DNA genome integration and high-throughput mutant library construction technology on the platform of important industrial microorganisms, efficient biosynthesis of high-value chemicals such as succinic acid, 3-hydroxypropionic acid, and itaconic acid has been realized. The low pH succinic acid fermentation technology based on unconventional yeast cell factory developed by him is the first in China, which broke the technological monopoly of multinational companies such as DSM, Reverdia, and Cargill. These achievements have been published in Nat Commun, Metab Eng, Biotechnol Biofuels, ACS Synth Biol and other journals, and have been cited more than 800 times.

Unveiling Cryptic Microbial Biosynthesis Towards Unprecedented Classes of Ribosomal Peptides

Hengqian Ren (任恒千)
Dalian University of Technology

Abstract

Secondary metabolism, widely present in numerous microorganisms, produces natural products that serve as invaluable resources for drug development. Ribosomally synthesized and post-translationally modified peptides (RiPPs) constitute an emerging family of natural products since the 21st century. With vast structural diversity and myriad biological functions, such as antimicrobial, anticancer, and antiviral activities, RiPPs are notable for their therapeutic potentials. The rapid growth of genome sequencing data, along with advances in bioinformatics, has led to a significant expansion of predicted RiPP biosynthetic pathways. However, most of these pathways are transcriptionally silent or sparingly expressed under standard laboratory growth conditions, rendering the corresponding products cryptic.

Here I present using synthetic biology to rapidly uncover the biosynthetic potential of cryptic RiPP pathways. To bypass the cryptic transcriptional regulation, we developed a plug-and-play genetic assembly workflow that reconstructs pathway expression systems using well-characterized regulatory elements. For pathways with complex gene arrangements, we employed the direct cloning strategy to achieve heterologous expression in genetically tractable model microbes. Integration of these technologies to an automation platform has significantly expanded the discovery scale. In total, we identified 38 cryptic RiPPs across 8 RiPP classes, including two unprecedented classes: daptides and lipoavitides. Daptides represent the first example of ribosomal peptides bearing two amino termini, with one formed via a novel biosynthetic route involving decarboxylation, transamination, and demethylation. While lipoavitides are a class RiPP/fatty-acid hybrid lipopeptides that display an amino terminus modified by a distinct short-chain fatty acid. Further investigation of lipoavitide biosynthesis revealed an acyltransferase that connects the fatty acid and RiPP substructures with promiscuous activity, affording potential application in lipopeptide bioengineering. Overall, our work showcases the scalable pathway activation, biosynthetic machinery elucidation, and enzymatic tool generation for RiPPs via synthetic biology approaches.

Brief Biography

Dr. Hengqian Ren received his B.S. in Chemical Engineering from Tianjin University in 2013, and Ph.D in Chemical Engineering from University of Illinois at Urbana-Champaign (UIUC) in 2019 under the guidance of Dr. Huimin Zhao. He joined the School of Bioengineering, Dalian University of Technology in the summer of 2024. Dr. Ren focuses on developing synthetic biology strategies to uncover cryptic biosynthetic pathways of ribosomally synthesized and post-translationally modified peptides (RiPPs), an emerging family of natural products with vast pharmaceutical potential. His research has been published in *Nature Chemistry*, *Nature Communications*, *Biotechnology and Bioengineering*, *ACS Chemical Biology*, etc.

The Innovation of Study on Anti-tumor Active Compounds

Mengyao Li (李梦尧)
Shanghai Cancer Institute

Abstract

The disease of tumors poses a grave threat to human wellness, thus the pursuit of novel molecules exhibiting anti-tumor activity remains an enduring concern for medicinal chemists and biologists. It is of paramount importance to devise innovative molecular skeletons, achieve efficient synthesis of active compounds, and select appropriate pharmacodynamic validation models to enhance the clinical value of research. Dr. Li will present the recent researches in the above three fields, encompassing: 1) Employing solvent-free and catalyst-free reactions for synthesizing active molecules against gallbladder and pancreatic cancer; 2) Investigating the efficacy of multi-substituted alkenes against biliary pancreatic tumors; 3) Utilizing patient-derived organoids (PDOs), patient-derived explants (PDEs), and patient-derived xenografts (PDXs) models to assess the *in vitro* and *in vivo* anti-tumor effects of these active molecules.

Brief Biography

Dr. Meng-Yao Li is currently serving as an assistant professor at Renji Hospital Affiliated to Shanghai Jiao Tong University School of Medicine/Shanghai Cancer Institute. He obtained his graduate degree from the prestigious Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, under the esteemed guidance of Prof. Guo-Qiang Lin. Li has made significant contributions to various scientific publications in renowned journals such as Aggregate, Sci. China Chem., Green Chem., iScience, Org. Lett., and Chem. Commun. His research output comprises 19 SCI papers that have garnered a total citation count of 300, resulting in a personal H-index value of 10. His research interests involve investigating the anti-tumor activity and mechanisms of traditional Chinese medicine; exploring drug design, synthesis, and their antitumor properties; developing and utilizing patient-derived organoid models as well as patient-derived tumor xenografts; studying drug resistance mechanisms in biliary and pancreatic tumors.

Engineering Carbon Source Division of Labor for Efficient A-carotene Production in *Corynebacterium Glutamicum*

Kai Li (李凯)
Shanghai Jiao Tong University

Abstract

Effective utilization of glucose, xylose, and acetate, common carbon sources in lignocellulose hydrolysate, can boost biomanufacturing economics. However, carbon leaks into biomass biosynthesis pathways instead of the intended target product remain to be optimized. This study aimed to enhance α-carotene production by optimizing glucose, xylose, and acetate utilization in a high-efficiency *Corynebacterium glutamicum* cell factory. Heterologous xylose pathway expression in *C. glutamicum* resulted in strain m4, exhibiting a threefold increase in α-carotene production from xylose compared to glucose. Xylose utilization was found to boost the biosynthesis of pyruvate and acetyl-CoA, essential precursors for carotenoid biosynthesis. Additionally, metabolic engineering including *pck, pyc, ppc*, and *aceE* deletion, completely disrupted the metabolic connection between glycolysis and the TCA cycle, further enhancing α-carotene production. This strategic intervention directed glucose and xylose primarily towards target chemical production, while acetate supplied essential metabolites for cell growth recovery. The engineered strain *C. glutamicum* m8 achieved 30 mg/g α-carotene, 67% higher than strain m4. In fed-batch fermentation, strain m8 produced 1,802 mg/L of α-carotene, marking the highest titer reported to date in microbial fermentation. Moreover, it exhibited excellent performance in authentic lignocellulosic hydrolysate, producing 216 mg/L α-carotene, 1.75 times higher than the initial strain (m4). These labor-division strategies significantly contribute to the development of clean processes for producing various valuable chemicals from lignocellulosic resources.

Brief Biography

Dr. Kai Li received his Ph.D. degree from the Shanghai Jiao Tong University (SJTU) in 2021. As a visiting scholar, Dr. Li studied at the Massachusetts Institute of Technology (MIT) during 2020-2021. He subsequently worked as a postdoctoral fellow and Assistant Professor at SJTU from 2021 to 2023. He also worked as a Visiting Professor at department of biology of MIT from June 2024.

Bioprocess Design to Enhance Lignin Bioaccessibility and Biotransformation

Zhimin Zhao (赵志敏)
Tianjin University

Abstract

Biological lignin valorization represents an emerging green approach to upgrading lignin for sustainable and economic biorefineries. However, due to the organic macromolecular structure, lignin generally exhibits poor water solubility and inhomogeneous distribution in an aqueous medium, significantly limiting its bioconversion efficiency. Herein, we developed a novel alkali sterilization (AS) strategy to enhance the dispersion and fermentation performance of lignin substrates effectively. AS enhanced the ionization process of acidic groups in lignin colloids, reducing the volume of colloidal lignin particles dramatically compared with conventional thermal sterilization. By providing more uniformly distributed and readily degraded lignin substrates, the AS strategy facilitated both *Rhodococcus opacus* PD630 growth and lipids production during fermentation. Furthermore, Cosolvent enhanced lignocellulosic fractionation (CELF) pretreatment was employed to tailor lignin chemistry, which enhanced lignin bioaccessibility at a molecular level and further upgraded lignin bioconversion. Therefore, this work presents a facile and effective strategy to overcome inhomogeneous lignin distribution in aqueous media, showing great potential as a platform technique to promote biological lignin valorization.

Brief Biography

Zhi-Min Zhao is an associate professor from Tianjin University. He received his Ph.D. degree in biochemical engineering from Institute of Process Engineering, Chinese Academy of Sciences. He was trained as a postdoctoral researcher at Oak Ridge National Laboratory, United States. His research focuses on designing advanced processes to tailor lignin chemistry and depolymerize lignin, aiming to facilitate lignin bioconversion and biorefinery. He has published over 30 peer-reviewed papers in biorefinery and bioprocess design fields.

Design And Construction of Microbial Cell Factory for the Efficient Synthesis of High-value Amino Acid Derivatives

Xuewei Pan (潘学玮)
Jiangnan University

Abstract

The amino acid industry, including amino acids and their derivatives, is one of the pillar industries of China's fermentation industry, with significant application value in the fields of food, pharmaceutical, feed, and chemical industry. China is a large production nation of amino acids, but not a strong producer of amino acids. The main factors restricting the development of China's amino acid industry are: (1) severe overcapacity of bulk amino acid production capacity; (2) a significant gap existed in some production strains compared to the advanced level abroad; (3) lack of independent intellectual property in some production strains, leading to the risk of “bottleneck”. Therefore, there is an urgent need to develop high-value amino acid derivative cell factories with independent intellectual property rights and competitiveness to enhance the international competitiveness of China's amino acid industry. This report introduces the advancement of our research group in the construction of high-value amino acid derivative cell factories in recent years, using high-value chemicals such as prodigiosin, deoxyviolacein, L-carnosine, and α-arbutin as examples.

Brief Biography

Xuewei Pan, Associate Researcher and Master Supervisor at the School of Biotechnology, Jiangnan University, is mainly engaged in research on the construction of efficient synthetic cell factories for high-value amino acid derivatives. As the first/corresponding author, he has published 15 SCI papers in authoritative journals in the fields of synthetic biology and metabolic engineering, such as Nucleic Acids Res, Appl Environ Microbiol, and Bioresour Technol. He has presided over and undertaken 8 national and provincial-level scientific research projects, including the General Program of National Natural Science Foundation of China (No. 32470067), the Youth Fund of National Natural Science Foundation of China (No. 32100055), the National Key Research and Development Program of China (No. 2023YFA0914500), and the Natural Science Foundation of Jiangsu Province (No. BK20210464). He was awarded the title of 2021 Jiangsu Youth Science and Technology Innovation "U35 Exploration" candidate.

Acetate as A Potential Platform Feedstock for Future Biomanufacturing

Guiping Gong (龚贵平)
Biogas Institute of Ministry of Agricultural and Rural Affairs

Abstract

The production of biofuels and biochemicals derived from microbial fermentation has received a lot of attention and interest considering concerns about the depletion of fossil fuel resources and climatic degeneration. However, the economic viability of feedstocks for biological conversion remains a barrier, urging researchers to develop renewable and sustainable low-cost carbon sources for future bioindustries. In concerns of food security, what will be the potential feedstock for future next-generation biomanufacturing? Owing to the numerous advantages, acetate has been regarded as a promising two-carbon building block feedstock targeting the production of acetyl-CoA-derived chemicals in industrial biotechnology. Here, we introduced our latest progress in bioconversion of acetate to produce biomacromolecules, such as single cell protein and microbial lipids in oleaginous microorganisms including yeast and algae. Different alternative approaches and routes for renewable acetate generation based both on biogas and ethanol will be described. Challenges and future development for acetate generation and assimilation as well as chemicals production from acetate will be also discussed.

Brief Biography

Dr. Guiping Gong received his PhD degree from Beijing University of Chemical Technology (BUCT) in 2021. During his PhD career, he got a scholarship from China Scholarship Council (CSC) and went to the lab of **Jens Nielsen** (Foreign academicians of Chinese Academy of Sciences) at Chalmers University of Technology. After graduated from PhD in 2021, he now works in the team of Microbial Synthetic Biology and Bioconversion (mSynBio) at Biogas Institute of Ministry of Agriculture and Rural Affairs (BIOMA). His current research focused on yeast metabolic engineering and acetate bioconversion. Based on the theme of “acetate bioconversion”, he has published more than 10 peer-reviewed papers in leading journals, including *Biotechnology Advances*, *Bioresource Technology* (x5), *Science of the Total Environment*, *ACS Synthetic Biology*, *Engineering Microbiology*, etc.

Bioconversion of Methane to C50 Carotenoid Bacterioruberin Using Soil-enriched Microbial Consortia

Shuqi Guo (郭树奇)
Xi'an Jiaotong University

Abstract

Bacterioruberin is widely used in medicine, food, and cosmetics, owing to its prominent antioxidant and bioactivity characteristics. In this study, we aimed to upcycle methane to bacterioruberin using microbial consortia. Microbial consortia consisting of *Methylomonas* and *Methylophilus*, which are capable of synthesizing carotenoids from methane, were first enriched in paddy soil. Through this microbial community, methane was successfully converted to C50 bacterioruberin for the first time. The bioconversion process was then optimized using response surface methodology. Finally, the methane-derived bacterioruberin reached a yield of 280.88 ± 2.94 μg/g dry cell weight. This study presents a cost-effective and eco-friendly approach for producing long-carbon chain carotenoids from methane, offering significant advancement in the direct conversion of greenhouse gases into value-added products.

Brief Biography

Dr. Shuqi Guo is now an associate professor at the School of Chemical Engineering and Technology of Xi'an Jiaotong University, China. Guo graduated from Shanghai Jiao Tong University in 2020. He then joined the School of Chemical Engineering and Technology of Xi'an Jiaotong University and has been engaged in research on the bioconversion of methane and metabolic engineering of methanotrophic bacteria.

Computation-driven Virtual Screening and Design of Carboxylic Acid Reductase for Nylon Monomers Biosynthesis

Kun Shi (石焜)
East China University of Science and Technology

Abstract

Nonribosomal peptide synthetases (NRPSs) are large multienzyme machineries that produce many valuable pharmaceutical compounds. However, engineering these megaenzymes is challenging due to the complex interactions between modules and domains, which require a deep understanding of protein-protein interactions and substrate specificities. In this work, we reported a computational redesign of the “gate-keeper” adenylation domain of the model NRPS-like enzyme carboxylic acid reductases (CARs). In particular, we proposed a strategy to screen the *in silico* mutant library through approximate mechanism-based geometric criteria and the Rosetta energy score. This approach effectively predicts the catalytic efficiency (k_{cat}/K_M) bypassing the need for specialized molecular dynamics and quantum mechanics simulations. With relatively little and high-efficiency experimental effort, only 72 mutants of *Mab*CAR3 were screened, from which 50 positive mutants were identified with a 70% positive rate. *Mab*CAR3 was efficiently engineered to generate a series of tailored enzymes. The refined biocatalytic system yielded a wide spectrum of nylon monomers (C6-C12) with outstanding yields (up to 88%), loading capacities (up to 100 mM), and productivities (up to 46 g L^{-1} d^{-1}), demonstrating the broad applicability of the system.

Brief Biography

I graduated with a master's degree in microbiology from Nanjing Tech University (Prof. He Huang/ Prof. Xiao-Jun Ji team), and obtained a Ph.D. in biochemical engineering from East China University of Science and Technology (Prof. Jian-He Xu/ Prof. Hui-Lei Yu team). I am currently working as a postdoctoral researcher at East China University of Science and Technology, with Prof. Jian-He Xu as my co-advisor. My research focuses on the computationally driven rational design of enzymes and artificial intelligence-based *de novo* design of multi-enzyme molecular machines. I am presently the principal investigator of a project funded by the National Natural Science Foundation of China (32401276) and am also involved in a project under the Shanghai Commission of Science and Technology (23HC1400200). As the first author, I have published a total of five papers in SCI journals, including *Science Advances*, *ChemSusChem*, *Biotechnology and Bioengineering*, and *Chemical Engineering Science*.

Computation-driven Virtual Screening and Design of Carboxylic Acid Reductase for Nylon Monomers Biosynthesis

Lingling Zhang (张玲玲)
Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences

Abstract

The increasing emission of CO_2 has resulted in severe climate problems, calling for carbon-fixation actions. CO_2 biotransformation through biochemical reactions catalyzed by enzymes and microbes provides a promising and sustainable way for not only reducing CO_2 emission but also producing various chemicals. Key steps in CO_2 biotransformation includes CO_2 activation and energy conversion. By utilizing electricity as activator and energy input, we have successfully established several systems, including CO_2-formate enzymatic conversion sytem, CO_2-single cell protein system, and ATP regeneration system, validating the effective and efficient CO_2 activation and transformation, paving the way for CO_2-based green biomanufacturing.

Brief Biography

Lingling Zhang is now a team leader in Tianjin Institute of Industrial Biotechnology (TIB), Chinese Academy of Sciences (CAS). She got her PhD degree in Changchun Institute of Applied Chemistry, Chinese Academy of Sciences (CAS) in 2016. After that, she did her postdoc in Aarhus University, Denmark and RWTH-Aachen University, Germany. In 2020, she joined in TIB. Her research interests focus on bioelectrocatalysis and electrochemical energy conversion, mainly on bioelectrocatalytic CO2 reduction, electro-assisted ATP regeneration, and intramolecular/heterogeneous electron transfer mechanism understanding.

Efficient stereoselective hydroxylation of deoxycholic acid by the robust whole-cell Cytochrome P450 CYP107D1 biocatalyst

Chixiang Sun, Guocheng Du, Xinyue Zhao

Jiangnan University

Keywords: OleP, deoxycholic acid, hydroxylation, redox partners, whole-cell catalysis

Deoxycholic acid (DCA) has been authorized by the Federal Drug Agency for cosmetic reduction of redundant submental fat. The hydroxylated product (6β-OH DCA) was developed to improve the solubility and pharmaceutic properties of DCA for further applications. Herein, a combinatorial catalytic strategy was applied to construct a powerful Cytochrome P450 biocatalyst (CYP107D1, OleP) to convert DCA to 6β-OH DCA. Firstly, the weak expression of OleP was significantly improved using pRSFDuet-1 plasmid in the E. coli C41(DE3) strain. Next, the supply of heme was enhanced by the moderate overexpression of crucial genes in the heme biosynthetic pathway. In addition, a new biosensor was developed to select the appropriate redox partner. Furthermore, a cost-effective whole-cell catalytic system was constructed, resulting in the highest reported conversion rate of 6β-OH DCA (from 4.8% to 99.1%). The combinatorial catalytic strategies applied in this study provide an efficient method to synthesize high-value-added hydroxylated compounds by P450s.

Enhancing Long-term Stability of DNA Data Storage through Chemical Modifications of DNA Synthesis

Mancang Zhang[1], Tingfei PENG[2], Mancang Zhang

1. Shanghai Jiao Tong University
2. SJTU-Dynegene Technologies Next Generation DNA Synthesis Joint Research Center

Keywords: DNA data storage; Chemical modification; Long-term stability; Ultra-high-throughput DNA synthesis

DNA data storage technology is a revolutionary approach that leverages the high-density, long-term stability, and low-energy consumption characteristics of DNA as an information carrier, making it an ideal choice for the long-term storage of cold data, particularly for the ultra-long preservation of high-value information. The stability of DNA is a central design consideration for DNA data storage systems application.

While several studies have focused on enhancing DNA stability through physical encapsulation methods such as metal capsules, thermo-responsive microcapsules, and cellulose acetate colloids, research aimed at enhancing DNA stability through chemical modifications remains relatively scarce. This study addresses this gap by exploring nucleotide chemical modifications level to enhance the stability of DNA as an information carrier, aiming to achieve stable and reliable long-term DNA data storage at room temperature without the need for physical protective measures.

Utilizing multi-nozzle inkjet printing for ultra-high-throughput DNA synthesis, this study compared the effects of various base chemical modifications and molecular weights on DNA stability. The results revealed that chemical protections, such as 5mC modification, significantly enhance the resilience of DNA sequences to environmental conditions and reduce the rate of DNA degradation, substantially extending the storage duration of DNA. Building on these insights, we employed microarray chips with multi-nozzle inkjet printing to synthesize short sequences with chemical modifications as information carriers, demonstrating a practical application for the long-term and reliable storage of personal whole genomic information. This study not only provides new chemical strategies for enhancing the stability of DNA storage technology but also lays the foundation for the widespread application of DNA in the field of information storage.

Identification of functional sgRNA mutants lacking canonical secondary structure using high-throughput FACS screening

Zeyu Liang, Yi-xin Huo*

Beijing institute of technology

Keywords: CRISPR/Cas9, Nonrepetitive sgRNA, metabolic engineering

Coexpressing multiple identical single guide RNAs (sgRNAs) in CRISPR-dependent engineering triggers genetic instability and phenotype loss. To provide sgRNA derivatives for efficient DNA-digestion, we design a high-throughput digestion-activity-dependent positive screening strategy and astonishingly obtain functional nonrepetitive sgRNA mutants with up to 48 out of the 61 nucleotides mutated, and these nonrepetitive mutants completely lose canonical secondary sgRNA structure in simulation. Cas9-sgRNA complexes containing these noncanonical sgRNAs maintain wild-type level of digestion activities *in vivo*, indicating that the Cas9 protein is compatible with or is able to adjust the secondary structure of sgRNAs. Using these noncanonical sgRNAs, we achieve multiplex genetic engineering for gene knockout and base editing in microbial cell factories. Libraries of strains with rewired metabolism are constructed, and overproducers of isobutanol or 1,3-propanediol are identified by biosensor-based fluorescence-activated cell sorting (FACS). This work sheds new light on the remarkable flexibility of the secondary structure of functional sgRNA.

Language model generates regulatory sequences across diverse prokaryotic species

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Beijing Institute of Technology

Keywords: Deep learning, regulatory elements, promoter, synthetic biology

Native prokaryotic promoters share common sequence patterns, but are species dependent. For understudied species with limited data, it is challenging to predict the strength of existing promoters and generate novel promoters. Here, we developed PromoGen1, a collection of nucleotide language models to generate species-specific functional promoters, across dozens of species in a data and parameter efficient way. Twenty-seven species-specific models in this collection were finetuned from the pretrained model which was trained on multi-species promoters. When systematically compared with native promoters, the *Escherichia coli*- and *Bacillus subtilis*-specific artificial PromoGen-generated promoters (PGPs) were demonstrated to hold all distribution patterns of native promoters. Encouraged by *in silico* analysis, we further experimentally characterized twenty-two *B. subtilis* PGPs, results showed that four of tested PGPs reached the strong promoter level while all were active. To further extend the applicability of our models, we developed PromoGen2, a universal promoter model designed to generate functional promoter sequences across a wide range of prokaryotes. Remarkably, when applied to *Jejubacter sp*. L23, a species lacking characterized promoters, and PromoGen2 utilizing the genome sequence of the species, achieved a 95% success rate. The generated sequences exhibited a dynamic regulation range of up to 37.4-fold. Additionally, we created a user-friendly web interface that allows the generation of species-specific promoters using either PromoGen1 or PromoGen2. This work demonstrates an efficient deep learning-based approach for *de novo* species-specific promoter generation, even with limited datasets, providing a versatile model for prokaryotic promoter design.

Modular remodeling of the hyaluronic acid synthesis pathway based on metabolic network modeling prediction

Zhi-Yuan Yao, Jin-Song Gong, Heng Li, Jin-Song Shi

Jiangnan University

Keywords: Hyaluronic acid, Comparative transcriptome analysis, Bacillus subtilis, genome-scale metabolic network model, Metabolic engineering

Hyaluronic acid (HA) is a viscous polysaccharide composed of alternating N-acetylglucosamine (GlcNAc) and D-glucuronic acid (GlcA) units linked by β-1,3 and β-1,4 glycosidic bonds. Due to its unique bioactivity, HA has found wide applications in the fields of pharmaceuticals, cosmetics, and materials. This study employed atmospheric and room temperature plasma (ARTP) mutagenesis and high-throughput screening to enhance the production capability of HA in wild-type Streptococcus zooepidemicus. Transcriptomic analysis was used to compare differential genes affecting product expression in mutant strains and the enzymes and genetic variations influencing HA biosynthesis. To reconstruct the HA biosynthetic pathway in the generally recognized as safe (GRAS) organism Bacillus subtilis, a genome-scale metabolic network model, iBsu1147, was utilized. Using flux balance analysis (FBA) as a foundation, gene editing targets were validated using FSEOF and iBridge algorithms. Various genetic manipulation strategies were employed, including combinatorial overexpression of enzymes in the HA synthase, GlcNAc, and GlcA synthesis modules. To reduce precursor consumption, key genes in downstream competing pathways were knocked out. Additionally, a series of synthetic genes related to extracellular polysaccharide synthesis, energy metabolism genes, and byproduct synthesis pathway genes were further knocked out to achieve efficient heterologous production of HA. Shake flask production in the B. subtilis expression system reached 5.10 g/L. To explore the industrial production potential of the recombinant strain further, different fermentation strategies were optimized on a 5-L scale, resulting in a further increase in production to 14.84 g/L using a dissolved oxygen-based constant feeding fermentation control scheme. This study established a cell factory with high-yield HA through systematic metabolic network design and pathway modularization, laying the foundation for industrial-scale production.

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Designing ASSMD strategy for exploring and engineering extreme thermophilic ancestral nitrilase for nitriles biocatalysis

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Keywords: Ancestral sequence reconstruction; Extremozyme; Protein engineering; Nitrilase; Thermal stability

Enzyme thermostability is vital for prolonged reactions and reusability. Extremozymes, known for high thermal stability, have gained biocatalysis prominence. Extremozymes evolve under extreme conditions, possibly becoming extinct during the evolutionary process of adapting to the current environment. Fortunately, the ancestral enzyme sequence reconstruction could deduce the ancestral enzyme sequence of existing enzymes through computer algorithms. Here, we designed an Ancestral Sequence-Structure-Molecular Dynamics (ASSMD) strategy to unveil molecular insights into extinct ancestral enzymes in the evolutionary landscape. Furthermore, this approach was applied to explore extremophilic ancestral nitrilase. In a dynamic flexibility trough, we obtained ASR135, an ancestral nitrilase capable of tolerating 90°C. Combining evolution analysis and laboratory evolution, we achieved laboratory further evolution of the thermostability of ASR135 in this evolutionary event and obtained the mutant ASR135-M4 (S97E/S101A/N124H/H155Y), which exhibited hydrolytic activity at 100°C. Mechanistic analysis revealed that ASR135-M4 exhibited the addition of salt bridge, hydrogen bond, π-alkyl interaction tetrahedral cage, and strengthening the hydrophobic core inside the protein. These modifications resulted in a more robust interaction network between 4 secondary structures. In general, ASSMD strategy holds potential for discovering high-performance nitrilases, particularly extremozymes. Additionally, laboratory thermostability evolution of ASR135-M4 sheds light on enzyme-directed evolution and thermostability mechanisms.

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Green biomanufacturing of functional chemicals via biocatalyst engineering and biological circuit design

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Keywords: Green biomanufacturing; biocatalyst engineering; biological circuit design; industrial application

The green biomanufacturing of functional chemicals is a disruptive technology that has rapidly developed in recent years as an alternative to chemical processes. Employing non-food biomass and other biological carbon resources as raw materials, and building biocatalysts with independent intellectual property rights, as well as establishing a modern biomanufacturing industry technology system, are at the core of solving the supply issues in the biomanufacturing industry. Our research group starts with the performance requirements of biological circuit design and key biocatalysts (cells or enzymes) for bioprocessing, focusing on solving the core issue of poor performance in industrial applications. Through the construction of metabolic network models, modularization of synthetic pathways, virtual-real screening and bidirectional evolution of key enzymes, semi-automatic design and efficient expression of difficult-to-express proteins, and industrial technology integration, several typical biosynthetic processes have been established. We have achieved the green biomanufacturing of functional chemical products such as niacin, membrane proteins, functional sugars, and polyamino acids, and have gained comparative advantages in cost and environmental friendliness over traditional chemical processes.

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Catalytic cycle of formate dehydrogenase captured by single-molecule conductance

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Keywords: single-molecule, formate dehydrogenase, catalytic cycle

Understanding the mechanisms and kinetics of enzymatic reactions are essential for studies of life science and also for bioengineering. However, the mechanism of oxidoreductase remains speculative due to the missing experimental evidence of reaction dynamics from the molecular level. Herein, the different reaction states in the catalytic cycle of formate dehydrogenase have been distinguished by their characteristic conductances, using the scanning tunneling microscope break junction technique, whereas the characterized conductances have been further exploited as markers to monitor the catalytic mechanism of formate dehydrogenase from Candida boidinii. Combined with multi-scale simulations, we demonstrate that the bound NADH converts to NAD+ directly via a hydride transfer reaction in-situ during the catalytic cycle of formate dehydrogenase. This conversion does not proceed via the apoenzyme state invoked in the conventional Theorell-Chance mechanism, a widely accepted mechanism in textbooks. This work provides intriguing insight into the mechanism of formate dehydrogenase and highlights the potential of the single-molecule technique in revealing the catalytic mechanism of NADH/NAD+-dependent oxidoreductases, offering promising avenues for enzyme design and modification.

Colony pattern development of a synthetic bistable switch

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Keywords: pattern formation, toggle switch, bifurcation, bacterial physiology

Microbial colony development hinges upon a myriad of factors, including mechanical, biochemical, and environmental niches, which collectively shape spatial patterns governed by intricate gene regulatory networks. The inherent complexity of this phenomenon necessitates innovative approaches to comprehend and compare the mechanisms driving pattern formation. Here, we unveil the multistability of bacterial colony patterns orchestrated by a simple synthetic bistable switch. Utilizing quantitative imaging and spatially resolved transcriptome approaches, we explore the deterministic process of a ring-like colony pattern formation from a single cell. This process is primarily driven by bifurcation events programmed by the gene regulatory network and microenvironmental cues. Additionally, we observe a noise-induced process amplified by the founder effect, leading to patterns of symmetry-break during range expansion. The degrees of asymmetry are profoundly influenced by the initial conditions of single progenitor cells during the nascent stages of colony development. These findings underscore how the process of range expansion enables individual cells, exposed to a uniform growth-promoting environment, to exhibit inherent capabilities in generating emergent, self-organized behaviour.

Microbial gene editing and gene expression regulation technology

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Keywords: microbial gene editing, microbial gene expression regulation, RedEx method, ExoCET method, gene transcription activating strategy

Microbial gene editing and gene expression regulation is one of the core technologies for directed engineering and systematic reconstruction of recombinant bacteria strains. Biosynthesis reprograming is an important way to diversify chemical structures. The large repetitive DNA sequences existing in polyketide synthase genes make seamless DNA manipulation of the polyketide biosynthetic gene clusters extremely challenging. I developed the RedEx method by combining Redαβ mediated linear-circular homologous recombination, *ccdB* counterselection, and exonuclease-mediated in vitro annealing to achieve seamless site-directed mutagenesis in spinosad polyketide synthase genes for structural diversification and optimization. Refactoring biosynthetic pathways for enhanced secondary metabolite production relies on efficient DNA assembly method. The ExoCET method was developed to assemble at least 13 pieces DNA fragments with high efficiency in one step for construction of the 79-kb multioperon artificial gene cluster, in which, 23 biosynthetic genes were grouped into 7 operons with strong constitutive promoters, optimized ATG and ribosome binding sites. Compared with the original gene cluster, the artificial gene cluster resulted in a 328-fold enhanced spinosad production in *Streptomyces albus* J1074. Positive transcription regulatory proteins bind specific DNA regions and interact with RNA polymerase to activate transcription of downstream genes in bacteria. A universal highly efficient gene transcription activating strategy in bacteria was developed by placing the positive transcription regulator gene directly downstream of its regulating sequences. The positive transcription regulator binds upstream sequence and stimulates transcription of itself and downstream genes. When this strategy was applied to overexpress pathway-specific positive regulators and their regulating genes of nitrogen fixation and salinomycin biosynthesis, its performance is much better than that obtained by placing regulator genes downstream of strong constitutive promoters. When this strategy was used to overexpress exogenous biosynthetic genes for the antitumor alkaloid prodigiosin in *Pseudomonas stutzeri*, the highest yields in heterologous hosts reported so far was obtained.

Production of steroids by synthetic biology

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Keywords: Synthetic biology, P450, steroids, squalene, hydrocortisone

Steroids are the second largest class of drugs after antibiotics and are currently widely used in the treatment of cancer, inflammation, and heart disease. However, existing synthetic biology technologies for steroid production face several challenges, including the promiscuity and efficiency of heterologous enzymes required for post-modification of active steroid functions, the metabolic pathway balance in synthetic cell factories, and the tolerance to exogenous steroids. Thus, advanced novel synthetic biology strategies need to be developed for overcoming multiple electron-requiring rate-limiting steps in the biosynthesis of steroids. Our study focuses on engineering efficient heterologous enzymes for steroid post-modification, constructing novel synthetic pathways for steroid production, and ultimately developing artificial cell factories capable of high-yield steroid production. The specific contents include: 1) Based on the post-modification characteristics of target steroids, evolutionary analysis techniques were employed to deeply explore related heterologous enzymes. Computational simulations were used to elucidate their catalytic mechanisms and guide their rational modification. 2) Efficient integration targets and heterologous expression systems were developed. By combining subcellular regionalization engineering, the expression and functional adaptation of heterologous enzymes were achieved. Cofactor and electron transfer engineering were utilized to enhance and balance the energy-driven pathways. 3) the metabolic transport network of cell factories was rationally regulated, and biosensors and microfluidic technologies were employed to achieve rapid, high-throughput screening of strains with high steroid production and tolerance. This approach has successfully achieved the efficient de novo biosynthesis of various important steroid compounds, including ergosterol, campesterol, 7-dehydrocholesterol, cholesterol, pregnenolone, and progesterone. Additionally, new synthetic pathways for some important steroid hormones, such as cortisone and hydrocortisone have been developed.

Structure-guided design of a non-natural transcription factor responding to androst-4-ene-3,17-dione

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Anhui Polytechnic University

Keywords: Protein design, Artificial transcription factor, Molecular dynamics, Steriods, Site-mutation

Synthetic biology is based on the concept of engineering design, which aims to design, transform, and even re-synthesize organisms. The bottom-up closed-loop “design–synthesis–test–learning” forward engineering strategy usually requires a large number of elements, such as transcription factors (TFs), to participate in application building, especially the construction of transcription-dependent biosensors. However, identifying TFs that specifically respond to a given molecule is a complex and onerous task. In this study, we devised an allosteric TF based strategy to develop an artificial TF-type biosensor by engineering an allosteric TF of Androstenedione AdT based on structure-guided molecular dynamics (MD) simulation. As a proof of principle, a androstenedione biosensor was designed and constructed on the basis of the ligand-binding domain (LBD) of progesterone receptor. According to MD analysis of the conformational changes of AdT after binding to androstenedione, an LBD in which the N- and C-termini exhibited convergence tendencies was used as a microswitch to guide the assembly of a DNA-binding domain and a transcription activation domain into an artificial TF. In addition, the transcription factor activity of AdT was increased by 1.44-fold for its variant F320Y. Overall, we identified a shortcut to creating non-natural TF elements for AD microbial cell factory, and expected that the design TF strategy will be applied to running in parallel to the signaling machinery of the host cell.

DNA Nanodevices for Nongenetically Controlled Cellular Behaviors and Their Cell Therapeutic Applications

Zhou Nie

Hunan University

Keywords: Chemical Synthetic Biology; DNA Nanotechnology; Non-genetic Receptor Regulation; Cellular Function Reprogramming

Nucleic acids are the primary carriers of genetic information and have gained attention for their non-genetic functions. Developing functional nucleic acids, self-assembly of DNA nanostructures, and dynamic DNA nanotechnology have made it possible to construct intelligent DNA nanomaterials with complex functions. Benefiting from the precise programmability of DNA sequence complementarity, high structural controllability, and the convenience of synthesis, modification, and functionalization, intelligent DNA nanomaterials are becoming important tools for biological function regulation, with broad potential in biomedical research. Here, we propose a new concept of non-genetic reprogramming of cell receptor functions for regulating cellular behaviors using intelligent DNA nanomaterials. By using various de novo-designed DNA intelligent nanodevices, we precisely regulate and reprogram the molecular recognition and activation patterns of important receptor families on the cell membrane surface, thereby rewiring cellular signaling pathways to achieve precise control over downstream cellular behaviors. Utilizing dynamic DNA nano-assembly technology, we have successfully reprogrammed the molecular recognition targets of the receptor tyrosine kinases (RTKs) from native protein ligands to customized small molecules, extracellular miRNA, near-infrared light, and specific cell types. We have also achieved automated control and precise nanoscale clustering regulation of RTK receptor activation through DNA nanorobots and DNA origami techniques. In addition, we have developed a novel CAN-TE technology based on DNA-antibody chimeras, enabling multi-target intelligent logical recognition of antigens on the surface of tumor cells and regulating the activation efficiency of immune cells through multivalent effects, greatly enhancing the specificity of tumor cell recognition in T cell engagement techniques. We have also targeted integrins to construct a molecular tension sensing unit, creating an artificial mechanoreceptor that can specifically respond to the cellular mechanical action mediated by individual adhesion proteins at the piconewton (pN) scale, and successfully used it for the maintenance of stemness in neural stem cells mediated by cellular adhesion force.

Advanced Computational Methods for Enzyme Annotation, Mining, and Evaluation

Zhenkun Shi

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Keywords: Enzyme mining, reaction mining, biological tool, machine learning

Designing novel metabolic pathways for synthesizing industrially important chemicals is an important research topic in synthetic Biology. A key challenge in pathway design is finding proper enzymes that can be engineered to catalyze reactions with unknown enzymes (e.g. non-natural reactions). Here, we present REME(https://reme.biodesign.ac.cn/), the first integrated web platform for reaction enzyme mining and evaluation. Combining atom-to-atom mapping, changed atoms identification, and reaction similarity calculations enables quick ranking and visualization of similar reactions. Additional functionality enables users to filter similar reactions by their specified functional group and candidate proteins can be further filtered (e.g. by organisms) and expanded by EC number or sequence homology. Afterward, protein attributes (such as kcat, KM, substrate specifity, optimal temperature, and optimal pH) can be assessed with deep learning-based methods, facilitating the swift identification of potential catalysts.

Development of Algorithms and Tools for Rational Design of Biosynthetic Pathways

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Keywords: pathway design, non-natural pathways, metabolic network

To address issues such as the complexity of natural product biosynthetic pathways and low conversion rates, we have developed the non-natural pathway prediction algorithm comb-FBA. This algorithm combines a combinatorial approach with flux balance analysis to achieve controllable non-natural reaction numbers and multiple optimizable pathway options for pathway design goals. Using this algorithm, we have designed and successfully validated several new one-carbon conversion pathways, such as GAA, GAPA, and ASAP. To enable experimental biologists to better utilize existing models and algorithms for metabolic pathway design, we have developed the cloud-based online tool CAVE (https://cave.biodesign.ac.cn), which has received nearly 40,000 visits within two months. Furthermore, to predict new pathways that can improve the conversion rate of target products, we have developed the quantitative heterologous pathway design tool QHEpath (https://qhepath.biodesign.ac.cn). Users can specify the chassis strain, substrate, and target product, and QHEpath will calculate the optimized synthesis strategy for the product. To explore the diversity of biological metabolic pathways, we have developed the model simplification algorithm CNGEM, achieving comprehensive and systematic mining of product synthesis pathways. The development of the aforementioned algorithms and tools will empower experimental biologists.

Building Multi-Constraint Models and Their Applications in Metabolic Engineering

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Keywords: multi-constraint models, metabolic engineering, strain design

Biomanufacturing is considered to have significant potential in achieving "carbon neutrality" strategies and promoting the development of the bio-economy due to its green, low-carbon, and sustainable nature. However, the development of industrial strains faces challenges due to a lack of reliable cellular model guidance, often relying on experimental approaches and iterative processes. To address this issue, we propose research on the construction and analysis of multi-constraint models. First, we introduce the Enzyme-Constrained Model Construction framework (ECMpy) and build enzyme-constrained models for multiple model organisms. We also developed the Enzyme-Thermodynamics Constrained Model framework (ETGEMs) by incorporating reaction thermodynamics data, creating the first multi-constraint model of E. coli that integrates enzyme and thermodynamic constraints, along with new algorithms for predicting metabolic engineering modification targets. Additionally, we developed the E. coli Regulatory Miner Cloud Platform (ERMer) based on a graph database, enabling rapid retrieval and visualization of complex regulatory patterns. Finally, we introduced the CAVE tool, leveraging cloud computing technology to allow biologists to quickly obtain optimal synthesis pathways for specific strains and identify modification targets or correct pathway errors. The multi-constraint model construction and analysis tools proposed in this research provide precise design guidelines for strain engineering, promote the development of chassis organisms, and establish a general, transferable methodology and framework to support experimental biologists in creating innovative industrial strains.

Trigger engineering enables the production of high-value intermediate for PET upcycling

Yibo Song

Nanjing Normal University

Keywords: PET upcycling, Protein engineering, Mono-2-hydroxyethyl terephthalate (MHET), Enzyme selectivity, Molecular mechanisms

Various microorganisms and PET hydrolases have been identified for their ability to depolymerize PET into useful intermediates, though the heterogeneity of these products limits their application in PET recondensation and high-value derivative synthesis. To address this challenge, we developed a protein engineering strategy called trigger engineering to tailor the enzyme selectivity of the model PET hydrolase, Bacillus subtilis PET-86 (BsEst), aiming to produce the valuable intermediate MHET rather than the terminal product TPA, which is over 105 times more valuable. After screening only 73 variants, we identified ten robust substitutions (V185G/Q/Y, T306D/H/Q, and T359D/K/N/Q) that successfully diminished the production of heterogeneous products, achieving 100% purity of MHET. And the kinetic characterization revealed a remarkable increase in turnover number (kcat) and catalytic efficiency (kcat/KM) by up to 5.2-fold and 2.5-fold compared to wild type, respectively. Furthermore, the molecular understanding empowered by molecular dynamics simulation and quantum mechanics cluster (QM-cluster) revealed that the main driving forces for governing the high selectivity and thermostability of the evolved trigger variants. Collectively, our results demonstrate an efficient and environmentally friendly biocatalysis approach for the production of high-value monomers from PET plastic waste, thereby contributing to the advancement of PET upcycling.

The innovation of anti-tumor active compounds research

Mengyao Li

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Keywords: Anti-tumor research; Drug molecular design; Synthesis strategy design; In vitro and in vivo models design

The disease of tumors poses a grave threat to human wellness, thus the pursuit of novel molecules exhibiting anti-tumor activity remains an enduring concern for medicinal chemists and biologists. It is of paramount importance to devise innovative molecular skeletons, achieve efficient synthesis of active compounds, and select appropriate pharmacodynamic validation models to enhance the clinical value of research.

Drug molecular design. The integration of several scaffolds exhibiting anti-tumor activity constitutes a pivotal methodology for the development of new active compounds. Multi-substituted alkenes and heterocyclic compounds are two classes of anti-tumor molecular scaffolds. Their combination offers a promising avenue for the design of molecules with enhanced anti-tumor activity. Utilizing alkenyl bromides and hetero-arylboronic acids as substrates, trisubstituted alkenes incorporating hetero-aromatic rings (including *N*, *O*, *S* atoms) can be efficiently synthesized via the Suzuki reaction. *In vitro* assays indicate that the synthesized compounds exhibit remarkable anti-gallbladder cancer and anti-pancreatic cancer activities.

Synthesis method design. Green organic synthesis offers novel opportunities for drug research and development. Notably, solvent-free and catalyst-free reactions represent a promising approach for the green synthesis of anti-tumor drugs. It has been demonstrated that 1,1-diphenylethylene can spontaneously react with benzyl chloride to yield a series of trisubstituted alkenes in the absence of both solvent and catalyst. The abundance of evidence highlights the crucial role played by the aggregate effect in triggering the reaction. Both *in vitro* and *in vivo* assays have indicated that these compounds exhibit significant anti-pancreatic cancer activity.

In vitro and *in vivo* model design. Selecting an appropriate experimental model is essential for validating the efficacy of compounds. Patient-derived models (e.g. PDOs, PDEs, and PDXs) have proven to be highly effective preclinical tools, as they accurately replicate key characteristics of patient tumor tissue. Consequently, these models provide a more robust foundation for the screening of anti-tumor molecules.

Unveiling Metabolic Engineering Strategies by Quantitative Heterologous Pathway Design

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Keywords: metabolic network, pathway design, engineering strategies

Constructing efficient cell factories requires the rational design of metabolic pathways, yet quantitatively predicting the potential pathway for breaking stoichiometric yield limit in hosts remains challenging. This leaves it uncertain whether the pathway yield of various products can be enhanced to surpass the stoichiometric yield limit and whether common strategies exist. Here, we develop a high-quality cross-species metabolic network model (CSMN) and a quantitative heterologous pathway design algorithm (QHEPath) to address this challenge. Through systematic calculations using CSMN and QHEPath, we evaluate 12,000 biosynthetic scenarios across 300 products and four substrates in five industrial organisms, revealing that over 70% of product pathway yields can be improved by introducing appropriate heterologous reactions. Thirteen engineering strategies, categorized as carbon-conserving and energy-conserving, are identified, with five strategies effective for over 100 products. A user-friendly web server is developed to quantitatively calculate and visualize the product yields and pathways, which successfully predicts biologically plausible strategies validated in literature for multiple products.

An Automated Platform for High-Quality Genome-Scale Metabolic Network and Enzyme-Constrained Model Construction

Aonan Li

Tianjin Institute of Industrial Biotechnology

Keywords: Genome-scale metabolic models, enzyme-constrained models, metabolic pathway design, automated construction, bioinformatics

Genome-scale metabolic models (GEMs) are vital for understanding cellular metabolism and guiding metabolic pathway design. However, constructing high-quality models is often time-consuming and complex. Existing tools face challenges in user-friendliness, limited databases, and lack of support for eukaryotes and archaea. To address these issues, we developed an automated platform for rapid and convenient GEM and enzyme-constrained model (ECM) construction from amino acid sequences.

This platform is the first to generate enzyme-constrained models directly from sequence data, supporting prokaryotes, eukaryotes, and archaea. It utilizes databases like BIGG, KEGG, and MetaCyc, and performs DIAMOND BLASTp alignments to create initial models in SBML format. A novel gap-filling algorithm ensures completeness by incorporating reactions with genetic evidence, while subcellular localization is handled via the Uniprot API and Deeploc 2.0 tools. All data is integrated into the ECMpy 2.0 pipeline for ECM construction.

We provide a user-friendly web interface to enhance accessibility. Initial tests on gene essentiality and metabolic flux demonstrate the platform's capability, offering a practical tool for researchers in metabolic modeling.

REME: an integrated platform for reaction enzyme mining and evaluation

Dexing Wang

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Keywords: Reaction enzyme mining; Enzyme evaluation

A key challenge in pathway design is finding proper enzymes that can be engineered to catalyze a non-natural reaction. Although existing tools can identify potential enzymes based on similar reactions, these tools encounter several issues. Firstly, the calculated similar reactions may not even have the same reaction type. Secondly, the associated enzymes are often numerous and identifying the most promising candidate enzymes is difficult due to the lack of data for evaluation. Thirdly, existing web tools do not provide interactive functions that enable users to fine-tune results based on their expertise. Here, we present REME (https://reme.biodesign.ac.cn/), the first integrated web platform for reaction enzyme mining and evaluation. Combining atom-to-atom mapping, atom type change identification, and reaction similarity calculation enables quick ranking and visualization of reactions similar to an objective non-natural reaction. Additional functionality enables users to filter similar reactions by their specified functional groups and candidate enzymes can be further filtered (e.g. by organisms) or expanded by Enzyme Commission number (EC) or sequence homology. Afterward, enzyme attributes (such as kcat, Km, optimal temperature and pH) can be assessed with deep learning-based methods, facilitating the swift identification of potential enzymes that can catalyze the non-natural reaction.

MTLKP: Enzymatic kinetics constant prediction based on multi-task learning

Yang Li

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Keywords: Michaelis constant, Turnover number, multi-task learning

Enzymatic kinetics play a pivotal role in analyzing mechanisms of enzymatic reactions and in optimizing target enzymes for biomanufacturing and related sectors. The enzyme turnover number (kcat) and the Michaelis constant (Km) are essential kinetic parameters that gauge the catalytic proficiency of enzymes. They are vital for dissecting the intricacies of enzymatic reactions and for guiding the directed evolution of enzymes to meet specific needs. However, the experimental measurement of kcat and Km can be quite expensive, both in terms of time and resources. Recognizing the inherent relationship between kcat and Km, we introduce a novel approach to enhance prediction accuracy. We propose a multi-task deep learning model, named MTLKP, designed to forecast both kcat and Km concurrently. The MTLKP model demonstrates a commendable coefficient of determination, achieving a score of 0.5764 for the kcat test set and 0.5781 for the Km test set, indicating a high level of predictive accuracy. The multitask learning approach significantly enhances the model's predictive capabilities, particularly for enzymes and ligands characterized by strong binding affinities or rapid catalytic rates. This advancement allows for more precise predictions across a broader spectrum of enzymatic interactions.

Improving metabolic engineering design with enzyme-thermo constrained optimization

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Keywords: Metabolic target prediction; Enzyme-thermo constraints; Protein-Centric methodology

Metabolic target and strategy design is pivotal in metabolic engineering, constituting a fundamental phase of the DBTL (Design-Build-Test-Learn) cycle. Target prediction algorithms expedite this process by narrowing the experimental scope, conserving time, and reducing the dependence on costly combinatorial experiments. However classic stoichiometric models based methods such as OptForceMust and FSEOF lack the consideration of thermodynamic limitations and enzyme costs, and they are not able to predict enzyme regulation strategies directly. To address these limitations, we developed a novel metabolic engineering prediction method termed “ET-OptME”, to incorperate enzyme constraint and thermodynamic constraints into genome-scale metabolic models to enhance predictive accuracy. The ET-OptME algorithm synthesizes the advantages of OptForceMust and FSEOF with enzyme-thermo constraints, pinpointing enzyme regulation to boost metabolite yields. In contrast to traditional reaction-centric methods, ET-OptME’s Protein-Centric methodology eliminates conflicts in target strategies and accurately reflects enzyme modifications, thereby improving the precision and feasibility of metabolic engineering predictions.

Incorporating enzyme-thermo constraints within the ET-OptME framework resulted in a 250% average improvement in accuracy metrics for predicting 5 products in the Corynebacterium glutamicum model, with a 86% increase in the minimum precision rate. proving Protein-Centric enzymatic-thermaldynamic approach an accountable advance in rational metabolic design.

Development and Prospect for Biorefinery of Waste Carbon Resources

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Keywords: Biorefinery, Waste Carbon Resources

The process of "carbon peaking and carbon neutrality" has become one of the key indicators to measure the economic development level and modernization of a country or region. Bio-conversion of waste carbon resources can realize the greening of raw materials, processes and products. Furthermore, the whole process is zero carbon and negative carbon emissions. However, due to the complex components, poor composition stability, and stress factors of waste carbon resources including straw and waste plastics, the bio-conversion through single cells has defects such as the excessive integration of modules and functions and heavy metabolic load, making it difficult to achieve high efficient utilization of waste carbon resources.

Designing and constructing artificial multicellular systems to achieve effective degradation of waste carbon resources, elimination of stress factors and efficient synthesis of useful chemicals through labor division is essential for the bio-transformation of waste carbon resources. Hence, an artificial mixed bacterial system with adaptive evolution of tolerance inhibitory factors was designed and first established, and multidimensional omics analysis was carried out to elaborate the mechanism of bacterial flora cooperation. The fermentation process was finally optimized by combining intelligent control methods such as computational simulation to finally realize the bio-transformation of straw, waste plastics, etc. to microbial oils, organic acids, organic alcohols and their derivatives.

Enhanced β-carotene production in Yarrowia lipolytica through the metabolic and fermentation engineering

Jingnan Wang

Nanjing Tech University

Keywords: β-Carotene, Yarrowia lipolytica, MVA pathway, Fatty acid pathway, Metabolic engineering, Fermentation engineering

β-Carotene is a kind of high-value tetraterpene compound, which shows various applications in medical, agricultural, and industrial areas owing to its antioxidant, antitumor, and anti-inflammatory activities. In this study, Yarrowia lipolytica was successfully metabolically modified through the construction and optimization of β-carotene biosynthetic pathway for β-carotene production. The β-carotene titer in the engineered strain Yli-C with the introduction of the carotenogenesis genes crtI, crtE, and crtYB can reach 34.5 mg/L. With the overexpression of key gene in the mevalonate pathway and the enhanced expression of the fatty acid synthesis pathway, the β-carotene titer of the engineered strain Yli-CAH reached 87 mg/L, which was 152% higher than that of the strain Yli-C. Through the further expression of the rate-limiting enzyme tHMGR and the copy number of β-carotene synthesis related genes, the β-carotene production of Yli-C2AH2 strain reached 117.5 mg/L. The final strain Yli-C2AH2 produced 2.7 g/L β-carotene titer by fed_x0002_batch fermentation in a 5.0-L fermenter. This research will greatly speed up the process of developing microbial cell factories for the commercial production of β-carotene.

High astaxanthin production by Xanthophyllomyces dendrorhous strain DW6 from cane molasses using two-stage pH strategies

Dawei Zhou

Nanjing Tech University

Keywords: Xanthophyllomyces dendrorhous; Astaxanthin; pH; Microbial fermentation; Sugarcane molasses; Transcriptome

Astaxanthin is a kind of carotenoids with high antioxidant capacity, which shows promising applications in feed and human health. With the consumers pursuit for natural products, the biological production of astaxanthin has gained great attentions. In this study, an astaxanthin-producing Xanthophyllomyces dendrorhous strain DW6 was first isolated, whose astaxanthin synthetic pathway was analyzed by the whole genome sequencing. Furthermore, fermentation parameters including temperature, pH and carbon and nitrogen sources on the yeast growth and astaxanthin synthesis were also comprehensively investigated. Accordingly, a two-stage pH fermentation strategy was developed to relieve the metabolic stress and promote the astaxanthin accumulation. Transcriptome analysis found that key genes of fadA (encoding acetyl-CoA acyltransferase) for fatty acid degradation and GGPS1 (geranylgeranyl diphosphate synthase) for astaxanthin synthesis, as well as genes involved in the glutathione and peroxisomes metabolism played major roles for the improved astaxanthin synthesis through the two-stage pH process. Finally, an inferior substrate of sugarcane molasses was used as sole carbon and nitrogen sources, and 374.3 mg/L of astaxanthin was produced with the astaxanthin content of 9.0 mg/g, representing the highest astaxanthin production from organic wastes by using X. dendrorhous. Overall, this study will pave the way for the large scaling production of astaxanthin.

Enzymatic Synthesis of Chitosan with a Single Degree of Polymerization

Yuwei Wang

Nanjing Tech University

Keywords: mutation; immobilization; chitosanase

Chitosan shows great potential for application in agriculture, food, medicine and cosmetics due to its rich physiological activity. Enzymatic degradation of chitosan is the main technical route for the preparation of chitosan, and the selection of chitosan enzymes with high enzyme activity, high stability and low cost is the key factor affecting the development of chitosan industry. In addition, chitosans with different degrees of polymerization have significantly different functional activities, and the synthesis of chitosan with a specific single degree of polymerization is crucial for further investigation of its constitutive relationship. In this study, focusing on the enzymatic preparation of chitooligosaccharide products with specific degrees of polymerization by chitosanase, we performed 18 point mutations of CsnB from Bacillus sp. BY01 source to investigate their effects on the enzyme activity and the distribution of the degree of product polymerization. Mutants D78W, D78Y, K260W and K260Y altered the product distribution but decreased the enzyme activity; mutant P115A increased the enzyme activity to 106.94% and an increase to 60% in the proportion of chitotriose because of the action of the newly formed π-bond. The purification and immobilization of CRT was then achieved by the temperature phase transition of ELP, silica mineralization properties, and ester-bonding interactions of molecular peptides to ReverseCatcher and ReverseTag to obtain the RCE-CRT@SiO2 NPs immobilized enzyme, which exhibited higher catalytic activity and stability at pH 6.0 and 60 °C, and retained 90.69% of the initial enzyme activity after 15 cycles of recycling.

Substrate tunnel redesign of SDR enabled efficient biocatlytic production of the TRPV1 inhibitor trans-4-tert-butylcyclohexanol

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Zhejiang University

Keywords: short-chain dehydrogenases/reductases; trans-4-tert-butylcyclohexanol; diastereoselectivity; activity; rational design

The TRPV1 antagonist *trans*-4-tert-butylcyclohexanol is an effective active ingredient for treating sensitive skin and has been successfully used in cosmetic products with restorative functions. Carbonyl reductase (CRED) has proven be a valuable tool for the rapid and efficient preparation of diastereopure cyclic alcohols. However, the bio-synthesis of *trans*-4-tert-butylcyclohexanol remains challenging due to the lack of biocatalyst with both satisfying activity and diastereoselectivity. In this study, an NADH-dependent short-chain dehydrogenase, UCPA, from *Escherichia coli* K12, was identified to exhibit excellent diastereoselectivity and moderate catalytic activity for the production of *trans*-4-tert-butylcyclohexanol. To enhance the enzyme activity, structure-guided rational engineering was performed. Coupled with glucose dehydrogenase to regenerate NADH, the best mutant allowed the complete conversion of up to 1 M 4-tert-butylcyclohexanone to *trans*-4-tert-butylcyclohexanol with 99.9% *trans*-selectivity and a high yield of 100% within 10 h. This mutant also demonstrated excellent activity and diastereoselectivity toward five other *para*-substituted cyclohexanones. Molecular dynamics simulation analysis suggested that the more hydrophobic and enlarged entrance of substrate tunnel served as the molecular basis for the improved activity. To conclude, this study developed a promising biocatalyst for preparation of *trans*-4-tert-butylcyclohexanol and broadened the source of *trans*-selective carbonyl reductase toward *para*-substituted cyclohexanones.

Engineered prime editing systems to efficiently insert tags in rice

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Anhui Agricultural University

Keywords: CRISRP; Prime editing; insertion; tagging

Several elements related to genetic engineering, such as recombination sites and epitope tag sequences, can be precisely inserted into plant genomes through a prime editing (PE) system. However, the PE efficiencies of fragment insertions are much lower than those of small edits. In this study, two novel plant PE systems, ePE6c and ePE6d, were engineered, and their editing ability was examined with different types of mutations in rice. Compared to previously established ePE2, ePE6d exhibited a similar ability to induce small edits but significantly increased editing efficiency of a 27-bp HA tag insertion by 2.65- to 8.36-fold at various sites in rice calli. The ratios of tagged plants were also increased by ePE6d in the T0 lines, while byproduct rates did not raise as the activity increased. Furthermore, the insertion of a relatively longer 78-bp calmodulin-binding peptide (CBP) tag and 90-bp 3×c-MYC tag via GRAND editing was 4.47- and 5.16-fold greater, respectively, in ePE6d than in ePE2. In addition, ePE6d could insert the CBP tag and 135-bp 3×AVI tag in up to 81.25% and 18.75% of the transgenic lines, respectively, demonstrating the superior capability of ePE6d to perform protein tagging. Together, our results indicated that ePE6d systems largely reinforce the capability of fragment precise insertion, thus providing reliable and easy-to-use tools for in suit protein tagging of functional genomic research and for genome structural variants manipulations of practical breeding.

De novo biosynthesis of antiarrhythmic alkaloid ajmaline

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Keywords: ajmaline, *de novo* biosynthesis, *S. cerevisiae*

The antiarrhythmic drug ajmaline is a monoterpenoid indole alkaloid (MIA) isolated from the Ayurvedic plant *Rauvolfia serpentina* (Indian Snakeroot). Research into the biosynthesis of ajmaline and another renowned MIA chemotherapeutic drug vinblastine has yielded pivotal advancements in the realm of plant specialized metabolism and engineering over recent decades. While the majority of vinblastine biosynthesis has been recently elucidated, the quest for comprehending ajmaline biosynthesis remained incomplete, marked by the absence of two critical enzymes. In this study, we successfully discovered and characterized these two elusive reductases, alongside the identification of two new and physiologically relevant esterases that completed the biosynthesis of ajmaline. We showed that ajmaline biosynthesis proceeds with vomilenine 1,2(*R*)-reduction followed by its 19,20(*S*)-reduction. This process is further modulated by two previously unreported root-expressing esterases responsible for the intermediate's deacetylation. Expanding upon the successful completion of the ajmaline biosynthetic pathway, we have harnessed the power of synthetic biology to enable the *de novo* biosynthesis of ajmaline in Baker's yeast for the first time and promised its large-scale production using synthetic biology approaches.

Engineering synthetic multi-enzyme complexes

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Dalian University of Technology

Keywords: Multienzyme complexes, self-assembled protein cage, biocatalysis, bioconjugation

Living cells have evolved to use self-assembled protein structures to spatially organize sequential enzymes to entail facilitated intermediate transfer, enhanced reaction rate and controlled metabolites flux at branched metabolic nodes. Examples include multi-enzyme complexes, metabolons, and microcompartment. These self-assembled protein reactors inspire devise artificial ones in engineered biosynthetic systems to gear metabolic flux, thereby improving product yield. In this work, we developed an interactive protein cage that can be used as a scaffold for multi-enzyme spatial organization. In vitro, we show that consecutive enzymes in the menaquinone biosynthesis with different sizes and shapes can be targeted to the surface of the engineered protein cage in high density, yielding spherical, monodispersed and homogenous nano-reactors with superior catalytic properties. Modulated reaction rate was achieved by altering the distance between attached enzymes. Using a pair of fluorescent proteins as models, proteins assembled with the protein nanoparticles in complex intracellular environment spontaneously. In engineered Escherichia coli, three key enzymes invovled in the MVA pathway have been co-localized on the exterior of the protein cage, leading to an 8.5-fold increase of lycopene production by streamlining metabolic flux towards its biosynthesis. This work presents a versatile route to multienzyme spatial organization for applications in biosynthetic industry and studying the mechanisms of naturally occurring nano-reactors.

Development and characterization of a bacterial enzyme cascade reaction system for efficient and stable PET degradation

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Nanjing Tech University

Keywords: Polyethylene terephthalate; Microplastics; Degradation; Whole-cell catalyst; CsgA

The widespread use of polyethylene terephthalate (PET) in various industries has led to a surge in microplastics (MPs) pollution, posing a significant threat to ecosystems and human health. To address this, we have developed a bacterial enzyme cascade reaction system (BECRS) that focuses on the efficient degradation of PET. This system harnesses the Escherichia coli Nissle 1917 (ECN) surface to display CsgA protein, which forms curli fibers, along with the carbohydrate-binding module 3 (CBM3) and PETases, to enhance the adsorption and degradation of PET. The study demonstrated that the BECRS achieved a notable PET film degradation rate of 3437 ± 148 μg/(d*cm²), with a degradation efficiency of 21.40% for crystalline PET MPs, and the degradation products were all converted to TPA. The stability of the system was evidenced by retaining over 80% of its original activity after multiple uses and during one month of storage. Molecular dynamics simulations confirmed that the presence of CsgA did not interfere with the enzymatic activity of PETases. This BECRS represents a significant step forward in the biodegradation of PET, particularly microplastics, offering a practical and sustainable solution for environmental pollution control.

Development of 3-Hydroxypropionic biosensors in Saccharomyces cerevisiae

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Keywords: 3-HP, Biosensor, Saccharomyces cerevisiae, Transcriptome, Yeast one-hybrid

3-Hydroxypropionic acid (3-HP) is a significant platform compound with considerable commercial potentials. In recent years, the production of 3-HP through biological synthesis has been significantly improved by strategies such as modifying microbial metabolic pathways. Despite this, issues such as poor strain tolerance to 3-HP, toxicity caused by the accumulation of intermediates, and inadequate cofactor provision continue to limit the enhancement of 3-HP yields. To address these issues, a promising strategy is to directly use biosensors to screen for targets from the genomic libraries that can increase 3-HP production.

In this study, by adding 3-HP to the culture medium and using transcriptome analysis, we successfully identified two promoters that are sensitive to fluctuations in 3-HP concentration, derived from genes significantly upregulated at the transcriptional level. Following sequence truncation and optimization, we developed two 3-HP sensors: X-2, which exhibits a linear response to 3-HP concentrations ranging from 2.5 to 15 g/L, and X-15, which responds linearly within range of 0-15 g/L. At the same time, through the yeast one-hybrid experiment, we preliminarily screened transcription factors that may be involved in 3-HP sensing, laying the foundation for further exploration of the 3-HP response mechanisms of X-2 and X-15, as well as the optimization of the 3-HP sensor.

The aim of this study is to elucidate the regulatory mechanism of 3-HP-induced promoters in *Saccharomyces cerevisiae* and to develop a 3-HP sensor based on this mechanism, which provide strong support for further improving the efficiency of 3-HP biosynthesis.

Utilization of patient-derived models for investigating the antitumor effects of traditional Chinese medicine compounds

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Keywords: patient-derived models; patient-derive xenograft; patient-derived organoids; traditional Chinese compounds;

Cancer presents a significant global health challenge, exerting a substantial impact on the overall disease burden worldwide. Traditional Chinese medicine (TCM) compounds offer a promising avenue for cancer prevention and treatment. Patient-derived models serve as a robust platform to validate the antitumor effects of these compounds, characterized by their ability to capture patients' tumor structure and gene variants. Our research group's has pioneeringly used patient-derived model to verify the anti-tumor effect of TCM, which showed strongly clinical significance.

Establishment of Patient-derived models. Patient-derived xenograft (PDX) and patient-derived organoids (PDO) can faithfully obtain the patients' tumor structure and gene characteristics and reflect highly clinical relevance drug effect. PDX and PDO models can effectively capture the heterogeneity of tumor cells and gene variants, surpassing the limitations of conventional cell line models.

Traditional Chinese medicine compounds antitumor effect investigation. The potential drug targets and promising herb compounds were identified through integrated network pharmacology and molecular docking, followed by validation. Subsequently, the selected compounds' antitumor effects were assessed using patient-derived models in vitro (PDO) and in vivo (PDX), following computer analysis.

Design of anaerobic fermentation pathway based on novel multi-constraint algorithm

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Keywords: multiple constraint algorithm, anaerobic fermentation, path design

As industrialization progresses, traditional chemical processes are being phased out due to pollution and reliance on non-renewable resources. Bio-fermentation, with its eco-friendly and sustainable benefits, is emerging as a future chemical production trend. Metabolic engineering is crucial for enhancing production efficiency and reducing costs, with anaerobic fermentation showing particular promise for industrial application despite challenges such as energy supply and strain growth issues. Existing metabolic optimization algorithms lack specific optimization for anaerobic fermentation, limiting their effectiveness.

This project aims to develop a novel phenotype prediction algorithm for anaerobic fermentation pathways, integrating enzyme kinetics, thermodynamics, and regulatory information to enhance prediction accuracy. The algorithm will address substrate selection, product synthesis, and metabolic pathway design, offering a global optimization solution. It will expand microbial production capacity by incorporating heterologous metabolic pathways, providing computational support for industrial chemical production via anaerobic fermentation. The research is expected to offer new theoretical insights and practical tools for bio-fermentation technology, fostering green transformation and sustainable development in the chemical industry.

Paradigm of engineering recalcitrant non-model microorganism with dominant metabolic pathway as a biorefinery chassis for circular economy

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Keywords: non-model; Zymomonas mobilis; biorefinery chassis; lactate

The development and implement of microbial chassis cells for biorefinery have profound impact on circular economy. However, either model or non-model microorganisms cannot fully meet the need of diverse industrial applications due to their innate limitations on industrial merits or genetic engineering. Non-model ethanologenic bacterium *Zymomonas mobilis* could be an excellent biorefinery chassis due to its extraordinary industrial characteristics. In this study, genome-scale metabolic model *i*ZM516 was improved and updated by integrating enzyme constraints to simulate the dynamics of flux distribution, which was then used to guide pathway design for biochemical production in *Z. mobilis*. Although cell factories have been constructed for diverse C2-C5 biochemical production using glucose, xylose, or glycerol as substrates, the innate recalcitrant ethanol pathway of *Z. mobilis* restricts the titer and rate of these biochemicals. A dominant-metabolism compromised intermediate-chassis (DMCI) strategy was developed to address this challenge. Instead of engineering *Z. mobilis* directly for the target biochemicals, a recombinant 2,3-butanediol producer with low toxicity but cofactor imbalance was constructed and served as the intermediate-chassis for subsequent construction of D-lactate producer with ethanol pathway completely blocked, which can produce D-lactate more than 140.92 g/L and 104.6 g/L at a yield > 0.97 g/g from glucose and corncob residue hydrolysate, respectively. Finally, techno-economic analysis and life-cycle analysis were performed to evaluate the commercialization of lignocellulosic D-lactate. According to the TEA results, the minimum selling price of D-lactate varied between USD 0.28 and 0.45 per kilogram, with an annual production capacity of 31,000 tons. This study thus not only constructed a recombinant strain for D-lactate production using the lignocellulosic waste, but also established a paradigm for engineering recalcitrant microorganisms as biorefinery chasses for sustainable bioeconomy.

Construction and application of tight expression circuit for Zymomonas mobilis

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Keywords: Zymomonas mobilis, Tight expression circuit

Despite of the development of different prokaryotic or eukaryotic cell expression regulatory systems, current system for the expression regulation still has some limitations. For example, for prokaryotes, their system for the expression regulation has a slow and inefficient induction process and is lack of induction specificity and precise regulation of expression, which limits the application of the expression system. Therefore, a moretight gene regulation system is needed, so that the spatial and temporal expressions of genes can be strictly controlled efficiently and cell resources can be used effectively. The establishment of the T7 system will improve the gene tight regulation system and the genetic operation tool system of *Zymomonas mobilis*, and solve the lack of gene tight regulation system for *Z. mobilis*, and provide more possibilities for gene function analysis, circuitry construction, and temporal and spatial regulation of metabolic pathway of *Z. mobilis*.

Reshaping substrate preference of phenylpyruvate decarboxylase for controlled biosynthesis of aromatic amino acid derivatives

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Keywords: phenylpyruvate decarboxylase, substrate specificity, aromatic amino acid derivatives, molecular dynamics simulations, enzyme engineering, metabolic engineering

The biosynthetic pathway of aromatic amino acids (AAAs) and the subsequent branches are highly valuable due to their role in the production of bioactive compounds. Yeast cell factories are widely applied in the synthesis of AAA-derivatives as they are safe, cost-effective, and scalable. ARO10, a phenylpyruvate decarboxylase catalyzing the nonoxidative decarboxylation of 2-keto acids to respective aldehydes, plays a crucial role in the biosynthesis of AAA-derivatives in yeast. However, the broad substrate specificity of ARO10 presents a significant challenge for the efficient synthesis of the target 2-keto acids. In this study, we engineered ARO10 to develop mutants with enhanced catalytic activity for three aromatic 2-keto acids substrates. The underlying mechanisms were elucidated through enzyme kinetics experiments and molecular dynamics simulations. Furthermore, these mutants were applied in three strains to produce AAA-derivatives, achieving titers of 1.50 g/L for tyrosol, 1.75 g/L for 2-phenylethanol and 337.2 mg/L for tryptophol in shake flask cultures, which represent the highest reported titers in yeast to date. These findings underscore the pivotal role of ARO10 in directing the biosynthesis of AAA-derivatives and the mutants could be applied to modulate the metabolism of other aromatic alcohol derivatives and alkaloids.

Clostridium tyrobutyricum in Combination with Chitooligosaccharides Modulate Inflammation and Gut Microbiota for Inflammatory Bowel Disease Treatment

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Nanjing Tech University

Keywords: probiotics, synbiotic, Clostridium tyrobutyricum, chitooligosaccharides, short-chain fatty acids

Synbiotics, the combination of probiotics and prebiotics, are thought to be a pragmatic approach for the treatment of various diseases, including inflammatory bowel disease (IBD). The synergistic therapeutic effects of probiotics and prebiotics remain underexplored. Clostridium tyrobutyricum, a short-chain fatty acid (SCFA) producer, has been recognized as a promising probiotic candidate that can offer health benefits. In this study, the treatment effects of synbiotics containing C. tyrobutyricum and chitooligosaccharides (COSs) on IBD were evaluated. The results indicated that the synbiotic supplement effectively relieved inflammation and restored intestinal barrier function. Additionally, the synbiotic supplement could contribute to the elimination of reactive oxygen species (ROS) and improve the production of SCFAs through the SCFAs-producer of C. tyrobutyricum. Furthermore, such the synbiotic could also regulate the composition of gut microbiota. These findings underscore the potential of C. tyrobutyricum and COSs as valuable living biotherapeutics for the treatment of intestinal-related diseases.

The simplified metabolic network model characterized by the number of carbon and nitrogen predicts the potential evolutionary phenomena of metabolic pathways.

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Tianjin Institute of Industrial Biotechnology

Keywords: Horizontal gene transfer; Adaptive evolution; Genome-scale metabolic network model; metabolic potential; Self-replicating systems

The emergence of constrained metabolic network models greatly helps researchers to understand all aspects of metabolism on the genome scale and more directly links genotypes and phenotypes, which facilitates researchers' research on biological transformation methods, especially in transforming metabolic pathways. Nowadays, the metabolic network model has been widely used to study the phenotype behavior of wild and mutant strains that associate genotypes with phenotypes under different conditions. This phenotype simulation can predict the growth phenotype after gene manipulation, the growth phenotype after adaptation evolution, and essential genes. In view of the advent of the era of artificial intelligence, the accumulation of genomic data continues to accelerate. A large amount of genomic data proves that the adaptive evolution of metabolic pathways is common among bacteria. However, not all evolutionary events are of biological significance, but may represent the result of a continuous evolutionary process, and this evolutionary process It just has an occasional beneficial purpose. In order to use the genome-scale metabolic network model to predict the evolutionary phenomenon of metabolic pathways of practical biological significance, We have developed a new set of model algorithms for simplifying the metabolic network using the quantitative characterization of carbon-nitrogen atoms - CNGEM, focusing on the reaction of changes in the number of carbon and nitrogen in the metabolic network, that is, the formation and decomposition of carbon-carbon and carbon-nitrogen bonds.

Water-mediated active conformational transitions of lipase on organic solvent interfaces

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1. Nanjing Tech University
2. Soochow University

Keywords: solvent-lipase interfaces; interfacial activation; conformational transitions

When it comes to enzyme stability and their application in organic solvents, enzyme biocatalysis has emerged as a popular substitute for conventional chemical processes. However, the demand for enzymes exhibiting improved stability remains a persistent challenge. Organic solvents can significantly impacts enzyme properties, thereby limiting their practical application. This study focuses on Lipase Thermomyces lanuginose, through molecular dynamics simulations and experiments, we quantified the effect of different solvent-lipase interfaces on the interfacial activation of lipase. Revealed molecular views of the complex solvation processes through the minimum distance distribution function. Solvent-protein interactions were used to interpret the factors influencing changes in lipase conformation and enzyme activity. We found that water content is crucial for enzyme stability, and the optimum water content for lipase activity was 35% in the presence of benzene-water interface, which is closely related to the increase of its interfacial activation angle from 78° to 102°. Methanol induces interfacial activation in addition to significant competitive inhibition and denaturation at low water content. Our findings shed light on the importance of understanding solvent effects on enzyme function and provide practical insights for enzyme engineering and optimization in various solvent-lipase interfaces.

Amphiphilic Nano-interface: Inducing the Interfacial Activation for Lipase

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2. Soochow University

Keywords: immobilized lipase, reduced graphene oxide, molecular dynamics simulation, orientation, interfacial activation

Graphene-based materials are widely used in the field of immobilized enzymes due to their easily tunable interfacial properties. We designed amphiphilic nano-biological interfaces between graphene oxide (GO) and lipase TL (Thermomyces lanuginosus) with tunable reduction degrees through molecular dynamics simulations and a facile chemical modulation, thus revealing the optimal interface for the interfacial activation of lipase TL and addressing the weakness of lipase TL which exhibits weak catalytic activity due to an inconspicuous active site lid. It was demonstrated that the reduced graphene oxide (rGO) after 4 h of ascorbic acid reduction could boost the relative enzyme activity of lipase TL to reach 208%, which was 48% higher than the pristine GO and 120% higher than the rGO after 48 h of reduction. Moreover, TL-GO-4h's tolerance against heat, organic solvent and long-term storage environment was higher than that of free TL. The drawbacks of strong hydrophobic nanomaterials on lipase production were explored in depth with the help of molecular dynamics simulations, which explained the mechanism of enzyme activity enhancement. We demonstrated that nanomaterials with certain hydrophilicity could facilitate the lipase to undergo interfacial activation and improve its stability and protein loading rate, displaying the potential of the extensive application.

Hybrid Cas12a variants with relaxed PAM requirements

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Keywords: Cas12a

Cas12a is a widely used programmable nuclease for genome editing, but its application is limited due to its recognition of 5′-TTTV-3′ PAMs. We utilized domain shuffling to generate chimeras, and identified an efficient hybrid Cas12a (ehCas12a), further engineered ehCas12a RVR variant showed significant improvements in recognizing 5′-TNYN, TWRV-3′ pams. Finally, we demonstrated that the base editor based on ehCas12a RVR was capable of targeting non-canonical PAMs *in vivo*.

Probing Biomass Precursor Synthesis as a Key Factor in Microbial Adaptation to Unadapted Carbon Sources

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Tianjin Institute of Industrial Biotechnology

Keywords: metabolic network, renewable carbon source, growth coupling, engineering strategies

SourceURL:file:///home/leon/data/Project/onecarbon_coupling/Paper/Cell Systems/manuscript4cellsystems.docx
Industrial microorganisms often face challenges in utilizing renewable substrates such as methanol, formate, and xylose. We present findings that the proportion of biomass precursors that must be synthesized from unadapted carbon sources is a critical determinant of the evolutionary driving force and minimal substrate requirements, using a new computational framework, AdaptUC. We predict metabolic engineering strategies for Adaptive Laboratory Evolution (ALE). These strategies enable microorganisms to co-utilize an adapted co-substrate and an unadapted carbon source or, in some cases, rely exclusively on the unadapted source. AdaptUC was validated through experimental records and literature, confirming its effectiveness in identifying gene knockout strategies. Case studies in Escherichia coli and Corynebacterium glutamicum highlight superior strategies with higher driving forces and reduced substrate requirements. This method has the potential to transform industrial biosynthesis by enabling more efficient use of renewable carbon sources.

Bioprocess Design to Enhance Lignin Bioaccessibility and Biotransformation

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Tianjin University

Keywords: Bioprocess design; Lignin valorization; Bioaccessibility; Biotransformation; Biorefinery

Biological lignin valorization represents an emerging green approach to upgrading lignin for sustainable and economic biorefineries. However, due to the organic macromolecular structure, lignin generally exhibits poor water solubility and inhomogeneous distribution in an aqueous medium, significantly limiting its bioconversion efficiency. Herein, we developed a novel alkali sterilization (AS) strategy to enhance the dispersion and fermentation performance of lignin substrates effectively. AS enhanced the ionization process of acidic groups in lignin colloids, reducing the volume of colloidal lignin particles dramatically compared with conventional thermal sterilization. By providing more uniformly distributed and readily degraded lignin substrates, the AS strategy facilitated both *Rhodococcus opacus* PD630 growth and lipids production during fermentation. Furthermore, Cosolvent enhanced lignocellulosic fractionation (CELF) pretreatment was employed to tailor lignin chemistry, which enhanced lignin bioaccessibility at a molecular level and further upgraded lignin bioconversion. Therefore, this work presents a facile and effective strategy to overcome inhomogeneous lignin distribution in aqueous media, showing great potential as a platform technique to promote biological lignin valorization.

Synthesis of succinic acid by carbon sequestration using amino-modified cellulose sponge loaded with biofilm

Yue Pan, Hao Wu

Nanjing Tech University

Keywords: Amino-modified cellulosic materials; CO2 slow-release; Micro-nano bubbles; Biofilm; Succinic acid

The production of bio-based succinic acid through microbial CO_2 fixation and conversion has gained significant attention as a promising approach to mitigate greenhouse gas emissions. However, the low CO_2 utilization efficiency limits the efficient biosynthesis of succinic acid. This study aimed to enhance the utilization of CO_2 during anaerobic carbon sequestration for succinic acid synthesis. The amine-based modification of cellulose sponge result in the reduction of CO_2 emission in the water by 88. 57% compared with that of the control, while the total amount of CO_3^{2-} and HCO^{3-} in the water was increased by 4.17% to enhance the efficiency of extracellular supply of CO_2. Sponge-loaded *E.coli* Suc260-CsgA was then used to form a biofilm and synthesize succinic acid. The modified cellulose sponge had a cell loading ratio of over 53% and produced 11.7% more succinic acid than free cells. Biofilms in the sponges reduced CO_2 overflow and increased HCO_3^- and CO_3^{2-} in the water. Finally, the effects of free cell fermentation and fixed bed repeated batch fermentation on the synthesis of succinic acid from carbon sequestration were investigated. Compared with the free cell fermentation, the concentration of free cells in the fixed-bed fermentation system was significantly reduced, and the average productivity of succinic acid increased by 87.56%, and the average yields increased to 89.21%, and the biofilm and the production performance could be maintained stable. The carbonic anhydrase activity of the biofilm was increased by 93.31% compared with that of the free cell, and the activity of the key enzyme for the production of succinic acid was also increased significantly compared with that of the free cell. All these results indicated that the amine-modified cellulose sponge-loaded biofilm reduced the CO_2 overflow and increase the effective supply of CO_2 to the microbial carbon sequestration for the synthesis of succinic acid.

Design and Application of Enhanced UDP-Glucose Regeneration System

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East China University of Science and Technology

Keywords: In vitro multi-enzyme cascades, Thermal stability engineering, UDP-glc regeneration

Uridine diphosphate glucose (UDP-Glc) is a crucial metabolite in carbohydrate metabolism and plays a key role in glycosyl transfer reactions, which is important for the food and agricultural industries. In this study, an enhanced UDP-glucose regeneration system was designed to utilise polyphosphate and maltodextrin as the basic substrates for UDP-Glc synthesis. The system consists of two modules involving a total of four enzymes. The first module consists of α-glucan phosphorylase (αGP) and UDP-glucose pyrophosphorylase (UGP), which are used to convert polyphosphate and maltodextrin to glucose-1-phosphate and react with uridine triphosphate to generate UDP-Glc. The second module consists of the newly discovered bifunctional type 3 polyphosphate kinase (PPK3) and pyrophosphatase (PPase), which are used to generate the UDP -Glc regeneration from the phosphorylated donors uridine triphosphate and inorganic phosphate. The breakthrough of this study is the use of a data science strategy to mine and modify a PPK3 variant with an ultra-high enzyme activity of 1000 U/mg and a 2566-fold increase in thermal stability compared to the wild enzyme. Meanwhile, the UGP engineering modification was guided by ProteinMPNN deep learning-based protein sequence design method and ancestral sequence reconstruction method to obtain a variant with a 498-fold increase in thermal stability, and the cause was deeply analysed using molecular dynamics simulations and umbrella sampling calculations. Finally, the performance of this enhanced UDP-Glc regeneration system in nucleoside disaccharide synthesis was verified by cascade reaction with nucleoside-specific glycosyltransferases. In conclusion, this study provides an efficient method for UDP-Glc regeneration, which is expected to be widely used in a variety of glycosyltransferase-catalysed systems.

Yarrowia lipolytica as a Cell Factory for Green Synthesis of Squalene

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Squalene is a functionally lipid derivative with diverse physiological activities and has broad application potential in medicine, health, and other fields. The current production method for squalene mainly involves extraction from shark liver, which has the disadvantages of damaging the ecosystem and potential safety hazards. Utilizing safe microorganisms to synthesize squalene will become one of the important green production pathways. This study has chosen *Y. lipolytica* as the chassis strain for its safety and FDA approval, along with a well-defined genetic background that enables it to synthesize squalene via the MVA pathway. Firstly, the engineered strain SQ-1 was able to produce 439 mg/L squalene by overexpressing of HMG1 and DGA1 based on pathway engineering and lipid engineering strategies. Further, strain YLSQ1 was developed, achieving a squalene yield of 262.0 mg/L through the combination of overexpressing of HMGR from different species and the SQS of *Y. lipolytica*. To enhance the pentose phosphate pathway and enhance the supply of NADPH, the G6PD and PGD was then overexpressed, resulting in strain YLSQ5 with a squalene yield of 535.5 mg/L. Leveraging precursor engineering strategy, the IUP path was constructed, which increased squalene yield in strain YLSQ6 to 795.5 mg/L. By further overexpressing DGA1 based on the lipid engineering strategy, strain YLSQ9 reached a squalene yield of 868.1 mg/L. Finally, optimizing fermentation conditions for YLSQ9 yieled 1628.2 mg/L of squalene.

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