ChinaRxiv 中X

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-202303.00661

Postprint: Engineering-Compliant Biological Component Design

Authors: Cui Yinglu, Wu Bian

Date: 2023-03-19T00:00:00+00:00

Abstract

From the perspective of synthetic biology, the essence of life resides in its datafiability and designability. As enzymes realize the vast majority of catalytic functions in living organisms, catalytic elements constitute one of the most fundamental components in synthetic biology. Sequence determines conformation, and conformation determines function. The digital design of catalytic element sequences based on spatial structure represents a critical frontier and active research area in synthetic biology. It not only furnishes abundant prototype molecules for developing synthetic biology functional devices, particularly novel chemical catalytic devices, but also provides design templates and guiding principles for developing modular, engineered regulatory elements. This article offers a brief introduction to recent advances in biological elements, with particular emphasis on catalytic elements.

Full Text

Biological Components Design for Engineering Requirements

Institute of Microbiology, Chinese Academy of Sciences; State Key Laboratory of Microbial Resources; CAS Key Laboratory of Microbial Physiological and Metabolic Engineering

From the perspective of synthetic biology, life is fundamentally characterized by its digitizability and designability. Since enzymes execute the vast majority of catalytic functions in living organisms, catalytic components represent one of the most essential elements in synthetic biology. Sequence determines conformation, and conformation determines function. The digital design of catalytic component sequences based on spatial structure has emerged as a critical frontier and hotspot in synthetic biology research. This approach not only provides

abundant prototype molecules for developing synthetic biological functional devices, particularly novel chemical catalytic devices, but also offers design templates and guiding principles for modular, engineered regulatory elements. This article briefly reviews recent advances in biological components, with particular emphasis on cutting-edge developments in catalytic component design.

Keywords: computational enzyme design, enzyme engineering, artificial metalloenzymes, substrate selectivity, thermostability
DOI: 10.16418/j.issn.1000-3045.2018.11.002

Introduction

In 2000, genetic circuit design studies marked by the toggle switch and synthetic oscillator brought synthetic biology into the spotlight [1,2]. In recent years, as synthetic biology has flourished, researchers have increasingly recognized that biological systems can be designed and constructed using "modular, standardized" components under quantitative modeling guidance. Biological components constitute one of the three fundamental elements of synthetic biology, and their in-depth exploration and modification have become crucial research directions. To facilitate the assembly of "machines" using modular, standardized "parts," the Registry of Standard Biological Parts was established in 2003 by synthetic biology laboratories at the Massachusetts Institute of Technology (http://partsregistry.org). By 2018, over 20,000 biological components had been registered, including promoters, transcription units, plasmid backbones, protein-coding DNA sequences, as well as RNA elements such as ribosome binding sites and terminators.

Naturally evolved biological components often fail to meet specific engineering requirements in terms of modularity, assembly, and integration. If cells are viewed as synthetic factories, the basic transport, regulatory, and catalytic components encoded by DNA—proteins and RNA—exhibit considerable interactivity and modularity within living systems. Achieving controllable and assemblable interactions between different units, and between units and pathways or networks, represents the primary challenge in engineering biological systems. Currently, numerous functionally characterized biological components remain unstandardized, many exhibit incompatibility, and the complexity of integration processes combined with post-integration variability often prevents coordinated component function, resulting in substantially reduced efficiency or failure to achieve the designed function. Therefore, designing biological systems with well-coordinated behavior and collaborative execution capabilities constitutes a major challenge in synthetic biology and a critical breakthrough toward "synthetic life."This review briefly introduces recent research on non-catalytic components, with particular focus on frontier advances in catalytic component design, including molecular optimization design for *de novo* enzyme structure prediction, artificial metalloenzyme design, substrate selectivity redesign, and enzyme

thermostability design. We also examine the multi-level integration and technological convergence between enzyme design and industrial production, aiming to provide important insights for constructing controllable, function-specific artificial biological systems while promoting interactive development between key foundational technologies in synthetic biology and industrial bioprocesses.

Non-Catalytic Component Design Advances

Since the introduction of promoter libraries for fine-tuning metabolic pathways in 2005, promoter libraries for various host cells (including *E. coli*, yeast, and actinomycetes) have been successively developed and designed. With advances in big data and artificial intelligence technologies, artificial neural network systems and computer-aided design have enabled quantitative design of promoter sequences for specific requirements, achieving training and test set regression correlation coefficients up to 98% [3]. Rackham and Chin from the University of Cambridge employed screening methods to obtain orthogonal ribosome RNA-mRNA pairs [4]. In cells, functional proteins are produced only when both orthogonal components are expressed, allowing the ribosome to specifically recognize the target mRNA. They further constructed multiple translation-level logic gates using different orthogonal pair topologies, laying the foundation for artificially designed genetic circuits orthogonal to host genetic backgrounds.

Currently, various libraries of promoters, ribosome binding sites, and terminators from different sources have been constructed and predictively designed, achieving component libraries with broad dynamic ranges and dense gradients. Additionally, increasingly novel components continue to be developed, including *cis*-acting elements such as riboswitches and riboregulators, as well as *trans*-acting elements like transcription activator-like effectors (TALE) and CRISPR/Cas9 systems, providing solid support for metabolic balance optimization and complex genetic circuit design. If non-catalytic components serve as "basic tools," catalytic components can be considered "functional tools" that are crucial for efficient metabolic pathway operation.

Catalytic Component Design: International Frontiers

Catalytic components represent fundamental biological elements in synthetic biology research. Although natural enzyme resources can be applied across species to reconstruct specific metabolic pathways in artificial assembly, natural catalytic systems often fail to meet special synthetic biology requirements due to their inherent characteristics, such as low expression in heterologous hosts, incompatibility with physiological conditions, and mismatched upstream/downstream enzyme activities. Therefore, artificial design and assembly of catalytic components to provide novel functions has become the foundation for constructing synthetic pathways. While directed evolution strategies have achieved some success in improving catalytic component activity and stability, the enormous mutant library construction requirements and need

for multiple evolution rounds make comprehensive sequence space exploration impractical. Additionally, inherent codon degeneracy and base mutation bias in mutagenesis techniques severely limit library diversity.

With high-performance computing development, computational protein design technology has achieved unprecedented progress, particularly demonstrating tremendous potential in core catalytic component design. Computational protein design enables directed modification of catalytic components based on specific reaction characteristics and substrate structures while establishing comprehensively searchable, high-precision intelligent libraries. This substantially reduces material, time, and labor costs associated with the massive mutant libraries required for directed evolution strategies [5]. In 2016, *Nature* published a seminal review titled "The Coming of Age of *De Novo* Protein Design" [6]. The same year, *Science* selected computational protein design as one of the top ten scientific breakthroughs. In 2017, the American Chemical Society ranked AI-designed novel protein structures first among eight major chemical research advances [7]. Through high-precision, high-efficiency computational simulation technologies, protein molecular structure and function design have dramatically expanded application scenarios for engineered life, creating new development opportunities for modern biomanufacturing. Regarding enzyme catalytic function characteristics, current computational design research on catalytic components primarily encompasses structure-based *de novo* enzyme prediction and subsequent optimization design, catalytic substrate stereoselectivity and regioselectivity design, metal-catalysis-based metalloenzyme design, and function-based thermostability design.

De Novo Enzyme Prediction and Optimization Design

In 2008, the Baker team at the University of Washington proposed the "Inside-out strategy" and applied it to design and remodel a series of enzymes including retro-aldolases [8], Diels-Alder reaction catalysts [9], and Kemp eliminases [10]. However, despite initial successes in *de novo* computational enzyme design, the Inside-out strategy suffers from several limitations: (1) the theozyme model cannot fully reflect the true transition state of enzyme catalysis; (2) new enzyme design requires embedding catalytic active centers into protein scaffolds, which inevitably affects protein structure and stability; (3) the computational process neglects long-range electrostatic interactions and protein scaffold "induced-fit" allosteric effects, causing designed enzyme conformations to easily become trapped in energy minima with other local minima in adjacent positions. Consequently, the few newly designed enzymes with desired functions exhibit relatively low initial activity and require further modification to improve enzymatic properties.

With advances in quantum mechanics and molecular dynamics theory and methodology, computational accuracy continues to improve while computational scales gradually expand. Related algorithms have achieved substantial improvements in throughput, sensitivity, and selectivity. Consequently, quantum mechanics/molecular dynamics and big data analysis methods can

rapidly identify specific functional regions and co-evolutionary sites, providing numerous precise templates and interaction models for synthetic biology functional devices. Further optimization based on Inside-out strategy-derived novel enzymes can substantially enhance catalytic component activity, ushering in a new development stage for enzyme engineering while providing guiding principles for designing biological components that meet engineering requirements.

For example, in Kemp eliminase design, carboxylate groups serve as general bases interacting with nonpolar substrates. However, due to the high degrees of freedom of carboxylate groups, failure to accurately calculate the energetic cost of desolvation and entropy loss may cause the carboxylate to lose its general base function, preventing catalysis. The Warshel group used QM/MM empirical valence bond (EVB) methods to predict highly active Kemp eliminase mutants, with results consistent with experimental values [11]. The Mayo group employed computational iterative methods starting from an inactive protein scaffold HG-1, and after iterative refinement, all eight designed enzymes exhibited significant catalytic activity, substantially improving the success rate of computationally designed enzymes [12].

The Diels-Alder reaction, a bimolecular cycloaddition, lacks natural enzyme catalysts. In 2010, the Baker team designed 84 candidate proteins with potential Diels-Alder activity using the Inside-out strategy, but experimental validation revealed only two proteins (DA_{20} and DA_{42}) with catalytic activity [9]. Subsequently, the Baker team introduced backbone activity to the *de novo* designed Diels-Alderase through the Foldit online protein folding game. Multiple online contributors remodeled the DA_{20} scaffold, enabling substrate-protein scaffold adaptation that improved Diels-Alderase activity by at least 18-fold [13]. In 2012, the Roelfes group introduced cysteine at the terminus of the transcription factor LmrR channel, covalently linking small molecules containing phenanthroline or bipyridine groups to coordinate with Cu(II). The resulting artificial metalloenzyme achieved 97% enantioselectivity [14].

Artificial Metalloenzyme Design

Metalloenzymes play crucial roles in atom transfer reactions. Since the 21st century, artificial metalloenzyme design has rapidly developed, expanding from natural catalytic reactions such as hydrolysis to non-natural enzyme-catalyzed atom transfer reactions including carbon-carbon bond formation and oxygen transfer. For instance, in the aforementioned Diels-Alder reaction, artificial metalloenzyme catalysis has achieved stereoselectivity and activity comparable to chemical catalysts. Moreover, metalloenzymes exhibit significantly expanded substrate scope compared to natural enzymes [15]. In 2011, the Kuhlman group serendipitously discovered that computationally designed Zn(II) binding sites at homodimer interfaces could effectively catalyze carboxylate and phosphate ester hydrolysis [16]. Subsequently, they introduced histidine to coordinate with Zn(II) in the Rab4 binding domain of rabenosyn protein, with the resulting

complex enhancing the hydrolysis rate of 4-nitrophenyl acetate by five orders of magnitude [17].

Beyond active site structure optimization, chemical conjugation represents a viable strategy for artificial metalloenzyme design. The Rovis group constructed an artificial metalloenzyme for C-H bond activation by conjugating biotinylated Rh(III) complexes with streptavidin, achieving a 100-fold increase in catalytic rate and 93% enantioselectivity [18]. As Alexandrova and colleagues reviewed, metalloenzyme evolution is influenced not only by catalytic activity but also by metal availability and toxicity [15]. Under these constraints, metal catalysts may not necessarily achieve optimal performance. Therefore, using novel metals to replace native metal catalysts for improving enzymatic properties provides new avenues for metalloenzyme design. Based on molecular dynamics simulations, Itoh and Fujieda replaced the dizinc binding site of β-lactamase with copper ions to create an artificial dicopper oxidase. Compared to wild-type β-lactamase, the triple mutant of this copper-substituted oxidase exhibited an 87-fold increase in kcat/KM for 4-tert-butyl carboxylate ester hydrolysis [19].

Substrate Selectivity Redesign

The precise spatial structure of enzyme active centers endows them with high substrate specificity. Given the vast and complex substrate spectrum, the pace of new enzyme discovery from nature cannot meet current synthetic biology demands. Consequently, altering enzyme substrate specificity represents one of the most widely applied directions in computational protein design [20].

In 2015, the Baker group computationally designed a formolase that fixes one-carbon formaldehyde molecules into dihydroxyacetone, a three-carbon unit, thereby achieving a core metabolic step and demonstrating for the first time that catalytic component design can guide novel metabolic pathway synthesis [21]. Starting from benzaldehyde lyase (BAL), they used RosettaDesign and Foldit to redesign the benzaldehyde binding pocket to increase specificity for formaldehyde, obtaining a formolase (FLS) composed of seven mutated amino acids. This enzyme exhibited a 100-fold improvement in catalytic efficiency for the formolase reaction compared to the original BAL. Direct reduction of formate to formaldehyde is thermodynamically challenging. To achieve formate conversion, the Baker group first activated formate to formyl-CoA to lower the thermodynamic barrier. They then mined the BRENDA database to identify an acylating aldehyde dehydrogenase capable of reducing formyl-CoA to formaldehyde, and tandemly catalyzed formate conversion to formaldehyde using acetyl-CoA synthetase and acylating aldehyde dehydrogenase. Through the designed formolase, formate was ultimately converted to dihydroxyacetone phosphate. This work convincingly demonstrates that enzyme substrate redesign can effectively guide novel metabolic pathway design.

Enzyme Thermostability Design

Improving enzyme thermostability can enhance heterologous host expression levels, increase catalytic component activity under mild conditions, and strengthen enzyme tolerance to harsh industrial conditions (organic solvents, high temperature, extreme pH). Traditional protein evolution methods typically achieve protein melting temperature increases of less than 15°C. In contrast, computer-aided design methods guided by overall protein structure energy calculations can surpass this limitation, even enabling protein melting temperature improvements exceeding 35°C to obtain hyperthermostable proteins [22].

Multiple strategies and algorithms have been developed for enzyme thermostability modification, with the highest correlation coefficient (R value) between predicted energy and database values reaching 0.73 (FoldX program) [23]. The Weiss team employed the SCADS strategy (Statistical, Computationally Assisted Design Strategy) to evaluate environmental energy for aristolochene synthase, examining amino acid side chain interactions with surrounding scaffolds, side chains, and environments [24]. By screening 12 highest-scoring amino acids for mutation, the melting temperature increased from 38°C to 83°C. The Janssen group used the FRESCO strategy (Framework for Rapid Enzyme Stabilization by Computational libraries) to construct thermostability mutant libraries for limonene epoxide hydrolase. Combining 10-12 single-point mutations, the combinatorial mutant exhibited a melting temperature increase from 50°C to 85°C, with enhanced catalytic activity and a half-life extension exceeding 250-fold [25]. They also applied FRESCO to compute thermostability for haloalkane dehalogenase LinB, where 12 stabilizing mutations increased the melting temperature by 23°C and extended the half-life over 200-fold at 60°C [26]. Additionally, to improve halohydrin dehalogenase tolerance to organic solvents, the Janssen group used FRESCO to identify 218 mutation sites and 35 disulfide bonds with predicted stabilizing effects. The resulting combinatorial mutant (HheC-H12) showed a 28°C melting temperature increase and significantly enhanced cosolvent resistance [27].

In 2016, the Fleishman team developed a combined computational strategy (protein repair one-stop shop, PROSS) to design an acetylcholinesterase (hAChE) mutant containing 51 mutations. Structural analysis revealed that this mutant significantly improved protein core packing, surface polarity, and backbone rigidity [28]. Compared to wild-type, this mutant exhibited 2,000-fold higher expression levels in *E. coli* and a 20°C thermostability improvement.

C-terminal functionalization of peptides profoundly affects protein biochemical properties. Specific C-terminal modifications can extend peptide metabolic half-life, reduce immunogenicity, or decrease toxic side effects. Due to peptide structural complexity, chemical modification involves multiple steps with low yield and high difficulty.

In 2016, a research team from the Institute of Microbiology, Chinese Academy of Sciences, in collaboration with the University of Groningen and Enzypep, em-

ployed a "FRESCO and consensus analysis combined computational" strategy to engineer peptide amidase, successfully designing a highly modified peptide amidase mutant PAM12A (containing 12 mutation sites) [29]. PAM12A exhibits extremely high thermostability (melting temperature of 76°C) and maintains stable activity for days in various anhydrous solvents including acetonitrile and acetone. Experimental results demonstrated that PAM12A can catalyze diverse peptide modification reactions including methyl esterification, hydroxyamination, methylamination, and amination, unrestricted by peptide sequence or original C-terminal functional groups.

Integration of Enzyme Design and Industrial Production

In recent years, foundational synthetic biology theories and related technologies such as genome sequencing and computational design have developed exponentially. Notably, developed countries have been extremely active in synthetic biology intellectual property, continuously filing broad patents that prevent others from entering the field [30]. In contrast, synthetic biology research in China remains in its infancy, with core challenges manifesting as relatively lagging capabilities in connecting cutting-edge laboratory technologies with industrial production techniques and slow technology integration processes.

Novozymes (https://www.novozymes.com), as an industrial enzyme giant, held 44% market share in 2014, representing the absolute leader in the global industrial enzyme and microbial preparation market; DuPont and DSM held 20% and 6% market shares, respectively. Global regional demand for industrial enzymes shows enormous variation, with Europe and North America representing the largest demand, together accounting for 80% of market share, while China represents only 9.4% [31]. As national innovation-driven development strategies deepen implementation, the bioindustry faces important development opportunities and investment prospects. Establishing low-cost biological manufacturing processes centered on synthetic biology and bio-chemical hybrid technologies while building key technology systems that promote industrial biotechnology development has become an urgent core issue requiring simultaneous consideration of research institution outputs and industrial bioprocesses.

With the expanding scope and scale of advanced manufacturing industries, disruptive innovative applications continue to emerge in biological and cross-disciplinary fields, gradually forming an industrial technology innovation system characterized by product diversification, strong production capacity, and active market transformation. For example, β-amino acids possess diverse special biological activities and are applied in multiple industries including pharmaceuticals, food, and agriculture. Star molecules with huge market sales such as β-lactam antibiotics, the blockbuster drug paclitaxel (a major anticancer drug), sitagliptin (a diabetes drug), and vitamin B5 all require β-amino acids as synthetic building blocks. For decades, β-amino acid synthesis has relied on transition metal-catalyzed chemical routes requiring expensive catalysts, cumbersome protection/deprotection steps, and harsh reaction conditions. Asymmetric hy-

droamination offers extremely high atom economy without additional additives, representing one of the ten most "green chemistry and green engineering" reactions identified by the American Chemical Society [32]. However, neither artificially designed chemical catalysts nor naturally occurring biocatalysts can directly catalyze this reaction.

A research team from the Institute of Microbiology, Chinese Academy of Sciences, successfully redesigned aspartase using RosettaDesign and high-throughput MD simulation methods, obtaining a series of artificial β-amino acid synthetases with absolute regioselectivity and stereoselectivity [33]. This artificially designed reaction system demonstrates tremendous advantages in efficiency and atom economy, achieving 99% conversion, 99% regioselectivity, and 99% stereoselectivity at substrate concentrations reaching 300 g/L—meeting industrial production standards. This research represents a successful case of AI technology applied to industrial strain design. Beyond its scientific significance, the team actively promoted technology translation; through industrial collaboration, the technology has passed pilot-scale and full-scale production process validation, with a thousand-ton production line recently completed. The related products are expected to substantially reduce production costs in the manufacturing of anticancer and HIV therapeutic drugs such as paclitaxel, dolutegravir, and maraviroc.

Through concerted efforts from multiple international research teams, catalytic component design has gradually evolved from simple model chemical reactions to catalytic pathways with industrial application prospects. Beyond single catalytic reactions, integrating computationally designed enzymes into complex pathways can create entirely novel metabolic routes and produce new chemical molecules. In particular, computation-driven pioneering research in catalytic design can provide synthetic biology with entirely new components that do not exist in nature, thereby vastly expanding the designability of life while offering insights into fundamental scientific questions regarding the essential mechanisms of enzyme catalysis and basic protein folding principles.

References

1. Elowitz M B, Leibler S. A synthetic oscillatory network of transcriptional regulators. Nature, 2000, 403(6767): 335-338.

2. Gardner T S, Cantor C R, Collins J J. Construction of a genetic toggle switch in Escherichia coli. Nature, 2000, 403: 339-342.

3. Meng H L, Wang J F, Xiong Z Q, et al. Quantitative design of regulatory elements based on high-precision strength prediction using artificial neural network. PLoS One, 2013, 8: e60288.

4. Rackham O, Chin J W. A network of orthogonal ribosome mRNA pairs. Nature Chemical Biology, 2005, 1, 3: 159-166.

5. Packer M S, Liu D R. Methods for the directed evolution of proteins. Nature Reviews Genetics, 2015, 16: 379-394.
6. Huang P S, Boyken S E, Baker D. The coming of age of de novo protein design. Nature, 2016, 537: 320-327.
7. Chemical & Engineering News, 2017, 95(49): 20.
8. Giger L, Caner S, Obexer R, et al. Evolution of a designed retro-aldolase leads to complete active site remodeling. Nature Chemical Biology, 2013, 9: 494-498.
9. Siegel J B, Zanghellini A, Lovick H M, et al. Computational design of an enzyme catalyst for a stereoselective bimolecular Diels-Alder reaction. Science, 2010, 329: 309-313.
10. Röthlisberger D, Khersonsky O, Wollacott A M, et al. Kemp elimination catalysts by computational enzyme design. Nature, 2008, 453: 190-195.
11. Frushicheva M P, Cao J, Warshel A. Challenges and advances in validating enzyme design proposals: The case of Kemp eliminase catalysis. Biochemistry, 2011, 50: 3849-3858.
12. Privett H K, Kiss G, Lee T M, et al. Iterative approach to computational enzyme design. PNAS, 2012, 109: 3790-3795.
13. Eiben C B, Siegel J B, Bale J B, et al. Increased Diels-Alderase activity through backbone remodeling guided by Foldit players. Nature Biotechnology, 2012, 30: 190-192.
14. Bos J, Roelfes G. Artificial metalloenzymes for enantioselective catalysis: Creation of a novel active site at the protein dimer interface. Angewandte Chemie International Edition, 2012, 51: 7472-7475.
15. Valdez C E, Smith Q A, Nechay M R, et al. Mysteries of metals in metalloenzymes. Accounts of Chemical Research, 2014, 47: 3110-3123.
16. Der B S, Machius M, Miley M J, et al. Metal-mediated affinity and orientation specificity in a computationally designed protein homodimer. Journal of the American Chemical Society, 2011, 134: 294-303.
17. Der B S, Edwards D R, Kuhlman B. Catalysis by a de novo zinc-mediated protein interface: Implications for natural enzyme evolution and rational enzyme engineering. Biochemistry, 2012, 51: 3933-3940.
18. Hyster T K, Knörr L, Ward T R, et al. Biotinylated Rh(III) complexes in engineered streptavidin for accelerated asymmetric C–H activation. Science, 2012, 338: 500-503.
19. Fujieda N, Hasegawa A, Ishihama K I, et al. Artificial Dicopper Oxidase: Rational Reprogramming of Bacterial Metallo-β-lactamase into a Catechol Oxidase. Chemistry-An Asian Journal, 2012, 7: 1203-1207.

20. Khersonsky O, Tawfik D S. Enzyme promiscuity: a mechanistic and evolutionary perspective. Annual Review of Biochemistry, 2010, 79: 471-505.

21. Siegel J B, Smith A L, Poust S, et al. Computational protein design enables a novel one-carbon assimilation pathway. PNAS, 2015, 112: 3704-3709.

22. Wijma H J, Floor R J, Janssen D B. Structure- and sequence-based design of proteins for high bacterial expression and stability. Molecular Cell, 2016, 63: 337-346.

23. Buß O, Rudat J, Ochsenreither K. FoldX as Protein Engineering Tool: Better Than Random Based Approaches? Computational and Structural Biotechnology Journal, 2018, 16: 25-33.

24. Diaz J E, Lin C S, Kunishiro K, et al. Computational design and selections for an engineered, thermostable terpene synthase. Protein Science, 2011, 20: 1597-1606.

25. Wijma H J, Floor R J, Jekel P A, et al. Computationally designed libraries for rapid enzyme stabilization. Protein Engineering, Design and Selection, 2014, 27: 49-58.

26. Floor R J, Wijma H J, Colpa D I, et al. Computational library design for increasing haloalkane dehalogenase stability. ChemBioChem, 2014, 15: 1660-1672.

27. Arabnejad H, Dal L M, Jekel P A, et al. A robust cosolvent-compatible halohydrin dehalogenase by computational library design. Protein Engineering, Design and Selection, 2016, 30: 175-189.

28. Goldschmidt L, Teng R, Roder H, et al. Protein repair one-stop shop (PROSS): a web server for the design of thermostabilizing mutations. Protein Engineering, Design and Selection, 2016, 29: 405-412.

29. Wu B, Wijma H J, Song L, et al. Versatile peptide C-terminal functionalization via a computationally engineered peptide amidase. ACS Catalysis, 2016, 6: 5405-5414.

30. Van D D, Koenigstein S, Reiss T. The development of synthetic biology: a patent analysis. Systems and Synthetic Biology, 2013, 7: 153-164.

31. Global and China Industrial Enzyme Industry Report, 2014-2017. [2015-07-02]. https://www.reportlinker.com/p01037001/Global-and-China-Industrial-Enzyme-Industry-Report.html.

32. Constable D J C, Dunn P J, Hayler J D, et al. Key green chemistry research areas—a perspective from pharmaceutical manufacturers. Green Chemistry, 2007, 9(5): 411-420.

33. Li R F, Wijma H J, Song L, et al. Computational redesign of enzymes for regio- and enantioselective hydroamination. Nature Chemical Biology, 2018, 14: 664-670.

CUI Yinglu is an Assistant Professor at the Institute of Microbiology, Chinese Academy of Sciences. She received her Ph.D. from Jilin University in 2015 and has been honored with the National Scholarship for Ph.D. Students and Tang Aoqing Chemistry Scholarship. Her research focuses on computational redesign of novel industrial enzymes. She has authored over 20 SCI-indexed journal articles and leads projects supported by the National Natural Science Foundation of China (Youth Program) and the China Postdoctoral Science Foundation. E-mail: cuiyinglu@im.ac.cn

WU Bian is a Principal Investigator at the Institute of Microbiology, Chinese Academy of Sciences, under the "Hundred Talents Program." He serves as a doctoral supervisor and Deputy Director of the State Key Laboratory of Microbial Resources, and received funding from the NSFC Excellent Young Scientists Fund. He is a council member of the China Biotech Fermentation Industry Association and a committee member of the Enzyme Engineering Professional Committee of the Chinese Society for Microbiology. His research focuses on discovery, engineering design, and catalytic applications of novel industrial enzymes, with recent emphasis on computational enzyme design. He has published dozens of papers in journals including *Nature Chemical Biology*, *Angewandte Chemie International Edition*, *Trends in Biotechnology*, and *ACS Catalysis*. He maintains long-term close collaboration with industry, having participated in industrial enzyme development projects for multinational chemical companies including BASF (Germany) and DSM (Netherlands), and has conducted joint R&D with numerous domestic enterprises, with multiple technologies already commercialized and industrially applied. E-mail: wub@im.ac.cn

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv —Machine translation. Verify with original.