DE NOVO ENZYME DESIGN

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In his 1902 Nobel lecture, the great German chemist Emil Fischer foresaw an era in which "chemistry will not only make extensive use of the natural enzymes as catalytic agents, but will also prepare synthetic ferments for its own purposes" [1]. Today, more than a century later, enzymes have become readily available for the production of everything from pharmaceuticals and agrochemicals to biofuels. Indeed, biocatalysis is increasingly viewed as an enabling technology for a greener and more efficient chemical industry [2,3].

The advent of powerful engineering tools to tailor the properties of enzymes from nature for new reactions of chemical interest has enabled their broad application [4]. Thanks to directed evolution, for example, altering the substrate and stereochemical preferences of natural enzymes is almost routine [5-7]. Nevertheless, the success of such endeavors generally requires some starting activity. If none is detectable, engineering can often supply it. In favorable cases, natural proteins can be modified rationally to access interesting abiological reactivity. Sometimes a few mutations suffice to alter function dramatically [8]. Alternatively, noncanonical amino acids, metal ions, or other cofactors can be exploited as sources of novel chemistry [9-11].

Creating enzymes from scratch is far more challenging than tailoring the properties of an existing catalyst. In one approach, the mammalian immune system has been harnessed to create antibodies possessing catalytic activity [12]. The properties of these catalysts are programmed by the structure of a stable transition state analog that is used to elicit an immune response. Although more than 100 different chemical transformations have been successfully catalyzed in this way, including normally disfavored processes and reactions lacking biological counterparts, even the best antibody catalysts are orders of magnitude less efficient than their natural counterparts [13].

A more generally productive pathway to de novo protein catalysts that exhibit true enzyme-like rates and selectivities combines state-of-the-art computational methods and high-throughput evolutionary optimization [14-16]. Conceptually, computational enzyme design is like catalytic antibody technology, but rather than utilize an imperfect transition-state analog to provide chemical instruction, the rate-limiting transition state of the target reaction, including potentially stabilizing functional groups, is modeled computationally, and docked in silico into structurally characterized protein scaffolds. After optimization of active site packing, the designs are ranked according to their calculated energies, and the top scorers are tested experimentally.

In collaboration with several computational groups, we have used the latter approach to repurpose natural protein scaffolds for the catalysis of mechanistically distinct chemical

transformations. These include simple proton transfers [17-19], a stereoselective Diels-Alder cycloaddition [20,21], and a multistep aldol reaction [22-24] (Figure 1). Although the starting designs typically exhibit only modest efficiencies, these have proven ideal starting points for laboratory evolution, and iterative rounds of mutagenesis and screening have yielded artificial enzymes that match the speed and stereoselectivity of their natural counterparts. Optimization frequently entails dramatic active site remodeling, to create more complex arrays of functional groups and/or minimize unproductive states by modulating protein conformation landscapes. The best resulting catalysts not only achieve billionfold rate accelerations but, on a preparative scale, produce their target products as single stereoisomers.

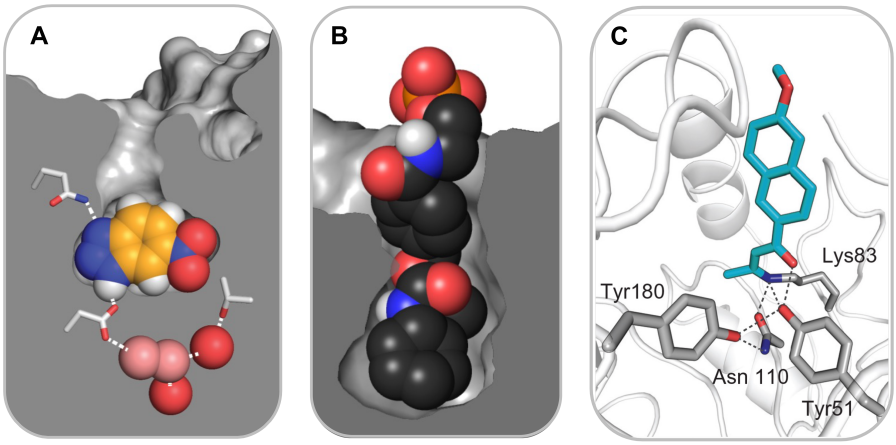

Figure 1. *De novo enzymes generated by directed evolution of modestly active computational designs. (A) A Kemp eliminase effectively utilizes acid-base chemistry in a shape complementary pocket to accelerate an elementary proton transfer 6 x 10^8-fold [18]. (B) A Diels-Alderase produces a single product diastereomer by employing hydrogen bond donors and acceptors to preorganize the diene and dienophile substrates and stabilize the cycloaddition transition state electronically [21]. (C) The >10^9 rate enhancement achieved by an aldolase is ascribed to a catalytic tetrad that arose residue by residue during evolutionary optimization [24].*

Natural proteins offer a wide range of architectures for enzyme engineering. Because their complex sequence-structure relationships reflect unique evolutionary histories, they may respond to sequence modification in unexpected ways. De novo-designed proteins, which are often hyperstable and possess well-understood sequence-structure relationships, represent potentially more robust starting points for enzyme design. Artificial retro-aldolases have been produced by computationally customizing the backbone and sequence of a de novo eight-stranded β-barrel protein, illustrating the potential of this approach [25]. Alternatively, the intrinsic reactivity of inorganic or organic cofactors can supply sufficient starting activity for subsequent evolutionary optimization [11]. For example, metal ions and metalloporphyrin cofactors have been introduced into designed α-helical bundles to produce protein catalysts for hydrolytic reactions, redox processes, carbene transfers, and other activities [26-28]. This approach was used to transform a computationally designed

zinc-binding peptide into an enantiospecific metalloesterase having catalytic efficiency only two orders of magnitude below the diffusion limit [29] (Figure 2). Functional diversification of this scaffold by divergent evolution has also yielded efficient, stereoselective catalysts for a bimolecular hetero-Diels-Alder reaction [30], a retro-aldol cleavage and an ene reduction of an unsaturated ketone (unpublished), attesting to the utility of metal ion catalysis for accessing diverse non-natural functions (Figure 2).

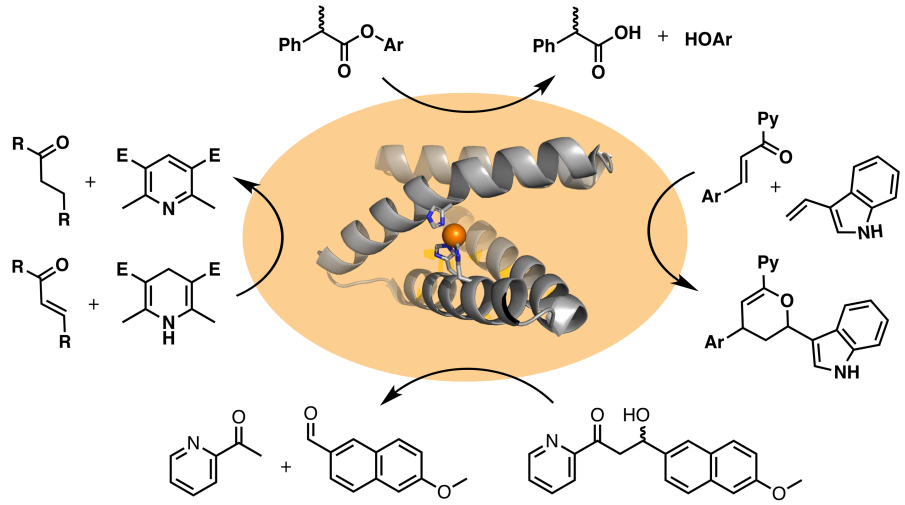

Figure 2. *Divergent evolution of a de novo zinc-binding helical bundle. Promiscuous esterase, hetero-Diels-Alderase, retro-aldolase, and ene reductase activities were optimized by iterative rounds of mutagenesis and screening to yield highly efficient and stereoselective metalloenzymes.*

As these few examples attest, enzyme design has come of age. It is now possible to create de novo enzymes fully rivaling their natural counterparts. The task today is to progress from simple model systems to more demanding transformations and complex, real-world challenges. Emerging experimental and computational innovations will be key to the success of such endeavors. Faster, more robust methods such as high-throughput screening and continuous evolution, improved forced fields, multistate design, and machine learning have much to offer in this context. Their successful implementation promises to bring Emil Fischer's dream of being able to prepare enzymes on demand, for our own purposes, to full realization.

Acknowledgments

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