ARTIFICIAL ENZYMES

Synergistic catalysis in an artificial enzyme

The introduction of single abiological catalytic groups enables enzymes to catalyse new-to-nature chemical transformations. Now, this concept is extended to two abiological groups in a single protein scaffold to allow synergistic catalysis in a stereoselective Michael addition reaction.

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Biocatalysis constitutes an attractive and competitive technology for the synthesis of chiral molecules, pharmaceuticals, and other high-value compounds, as enzymes often display high degrees of stereoselectivity, function under ambient conditions, and their activity and selectivity toward a particular transformation can be tuned by protein engineering[1,2]. However, natural enzymes catalyse a much narrower spectrum of chemical reactions compared to chemocatalytic methods, which limits their scope for many synthetic applications. Toward overcoming this limitation, a major approach has entailed the design and development of artificial enzymes, in which an abiological catalytic group, often in the form of a synthetic organometallic complex, is covalently or non-covalently embedded into a protein scaffold, thereby equipping the protein with a new catalytic function[3]. More recently, an alternative strategy has relied on the redesign and/or engineering of

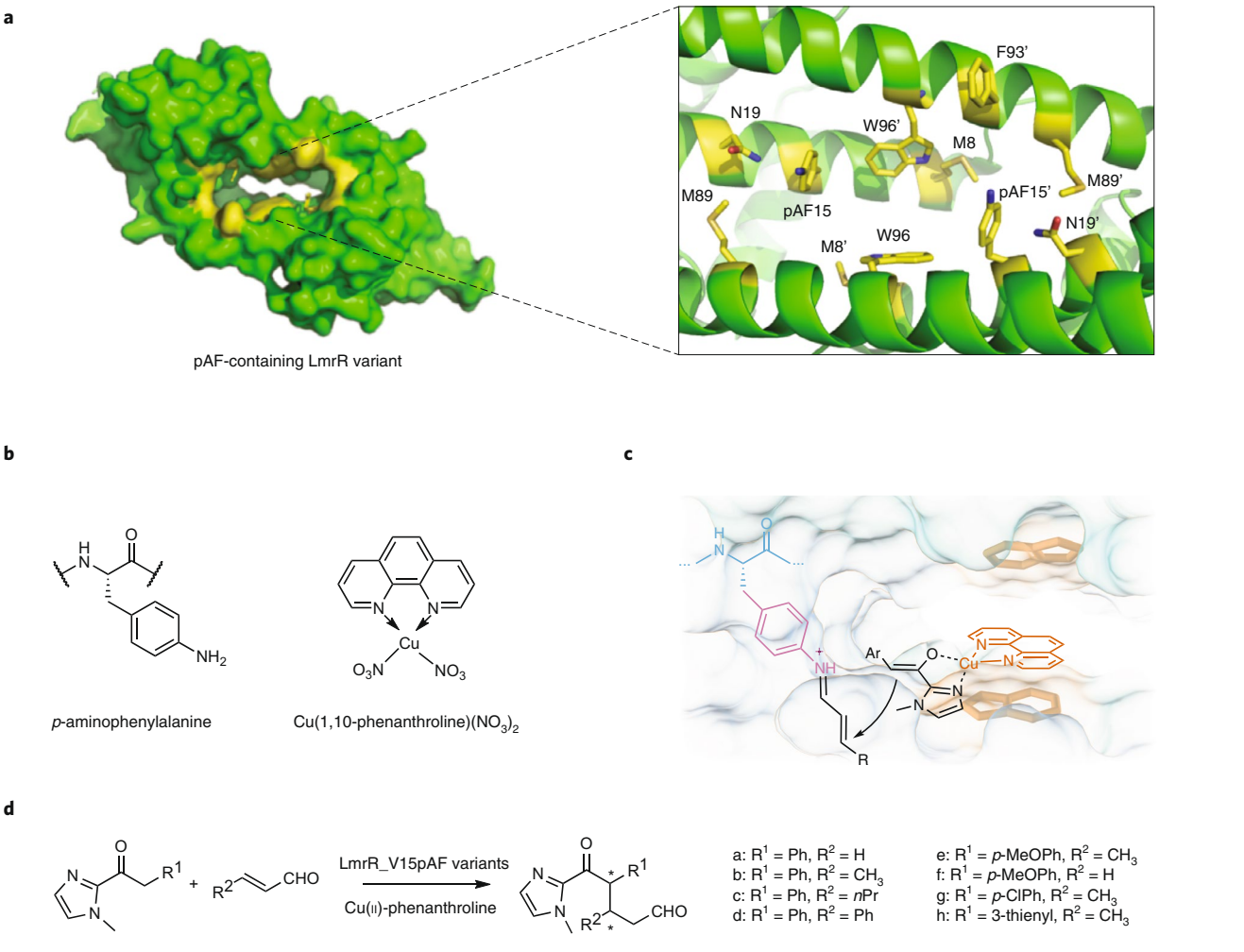

Fig. 1 | LmrR-based artificial enzyme for asymmetric Michael addition. a, Active site of *p*-aminophenylalanine (pAF)-containing LmrR protein scaffold. **b**,**c**, Abiological components (**b**) and putative mechanism (**c**) of the artificial enzyme. **d**, Substrate scope of LmrR_V15pAF/Cu-phen catalysed Michael addition reaction. Credit: panel **c** adapted with permission from ref. [5], Springer Nature Ltd.

natural metalloproteins to realize abiological transformations[4]. Both approaches have produced attractive avenues for creating biocatalysts capable of mediating chemical reactions beyond those carried out by natural enzymes.

A key feature underlying the catalytic proficiency of natural enzymes is their ability to exploit multiple catalytic mechanisms in concert to accelerate the rate of a chemical reaction by several orders of magnitude (for example, by 10^5- to 10^{20}-fold). Previous efforts toward creating artificial enzymes have focused on the introduction of a single, abiological catalytic group, such as an organometallic complex or an organocatalytic group, into a protein scaffold[3]. In these systems, concerted catalysis has been achieved in some cases by combining the unique reactivity of the abiological catalytic group with manipulation of the amino acid residues surrounding the active centre, resulting in improved catalytic activity and/or refined chemo- and stereoselectivity.[3] Now, writing in *Nature Catalysis*, Zhi Zhou and Gerard Roelfes report an important development in the field of artificial enzyme design, in which two abiological catalytic groups are designed to act in concert toward catalysing a stereoselective Michael addition reaction[5] (Fig. 1c).

The artificial enzyme reported by the authors is based on a bacterial multidrug resistance regulator protein, called LmrR, which forms a homodimeric complex featuring a hydrophobic central cavity lined up by two tryptophan residues (Fig. 1a). Building upon previous work, the authors exploited this cavity to non-covalently bind a Cu-complex to the protein, which was meant to act as a Lewis acid for the activation of the nucleophilic substrate in the desired Michael addition reaction (Fig. 1b). The authors further envisioned that synergistic catalysis in this reaction could be achieved by incorporating a non-canonical amino acid, *p*-aminophenylalanine (pAF), in close proximity to the reaction centre. This pAF-based abiological catalytic group was meant to enhance the reactivity of the Michael acceptor substrate (aldehyde) toward the Michael addition reaction via iminium catalysis (Fig. 1b), a mechanism commonly exploited in organocatalysis.

To create the artificial enzyme, the authors started from a previously developed LmrR variant in which *p*-aminophenylalanine is introduced in close proximity to the predicted cofactor binding pocket defined by the central tryptophan residues (Fig. 1a). Next, the authors tested the relative efficiency of various organometallic copper complexes non-covalently bound to the protein, resulting in the identification of Cu(1,10-phenanthroline) as the most effective choice for productive generation of the enolate nucleophile. The resulting protein complex was found to be able to catalyse a Michael addition reaction between acrolein and an imidazolyl-ketone in moderate yield (36%) but with promising enantioselectivity (86%) (Fig. 1c). Importantly, additional experiments demonstrated the synergistic action of the two abiological catalytic groups, that is, the pAF residue and the protein-bound Cu-complex, in promoting this reaction, along with the superior activity and enantioselectivity of the artificial enzyme compared to commonly used (chiral) amine-based synthetic catalysts.

The substrate scope of this biocatalyst could then be extended to catalyse this transformation in the presence of various α,β-unsaturated aldehydes and 2-acyl imidazole derivatives as the Michael acceptor and donor substrates, respectively (Fig. 1c). This reaction generates two new stereocentres and thus four possible products, posing the problem of controlling both the diastereo- and enantioselectivity of the reaction. In most cases, the LmrR-based enzyme was found to yield the desired Michael addition products with good diastereocontrol (up to 9:1 d.r.) and good-to-excellent levels of enantioselectivity (up to >99% e.e.), thus demonstrating its functionality across a range of different substrates (Fig. 1c). Through mutagenesis studies, the authors further established that a Met8Val mutation, which is located near to the Cu-complex binding pocket (Fig. 1a), has a beneficial effect toward increasing the activity and stereoselectivity of the enzyme for some of the target reactions.

Overall, this study describes an elegant approach to artificial enzyme design, which could be extended to other systems. A current limitation of the present system is a modest catalytic activity, as it was determined to support about 10–20 turnovers with a catalytic efficiency (k_{cat}/K_M) of 0.1 M^{-1} s^{-1}. The latter remains orders of magnitude lower than that of natural or engineered enzymes used in biocatalysis. Further optimization via protein engineering along with elucidation of the stereochemical preference of the biocatalyst, which remains currently undefined, are expected to provide potential avenues for further development and refinement of this artificial enzyme in the future. While the LmrR-based enzyme is currently assembled in vitro, acquiring the capability to assemble this system directly in a cell, as has recently become possible for other types of artificial enzymes[6,7], would facilitate these protein engineering efforts. Prior to this work, genetically encoded non-canonical amino acids have been incorporated into engineered and artificial enzymes to alter their substrate scope, tune their reactivity, or create and append new catalytic centres[8]. The work by Zhi Zhou and Gerard Roelfes expands upon this concept by demonstrating the value of achieving synergistic catalysis in an artificial enzyme through the combination of a non-canonical amino acid and an abiological organometallic complex. This strategy is expected to prove valuable toward creating and expanding the catalytic repertoire of artificial enzymes in the context of other valuable chemical transformations. ❐

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Published online: 18 March 2020
https://doi.org/10.1038/s41929-020-0435-z

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Competing interests

The authors declare no competing interests.