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Photoenzymes for Radical C–C Coupling

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Standfirst:

General catalytic methods for free radical-mediated asymmetric transformations have long eluded synthetic organic chemists. Now, NAD(P)H-dependent ketoreductases (KREDs) are repurposed and engineered as highly efficient photoenzymes to catalyse asymmetric radical C−C couplings.

Radical reactions have found important applications spanning all disciplines of chemical synthesis. In small-molecule chemistry, radical-mediated carbon-carbon bond forming reactions have been widely implemented in the total synthesis of complex natural products. In the realm of macromolecule synthesis, radical polymerization methods have now become an indispensable tool to access polymeric materials. Despite the already significant utility of radical chemistry, general methods to enable catalytic asymmetric free radical-mediated reactions have long eluded synthetic chemists. In part due to their highly reactive nature, taming fleeting radical intermediates for stereoselective catalysis is a notoriously difficult problem. Moreover, maintaining tight association between the chiral catalyst and the free radical intermediate and/or the radicalphile is oftentimes a nontrivial task, further limiting the substrate and reaction classes amenable to asymmetric radical catalysis.[1,2]

As nature's privileged catalysts, enzymes are capable of facilitating an array of biochemical transformations with unparalleled stereoselectivities, thereby providing a promising solution to challenging problems in asymmetric catalysis. However, until recently, the catalytic repertoire of enzymes has been largely limited to reactions found in nature. Thus, bringing new catalytic functions to naturally occurring enzymes can dramatically expand the reach of enzymology, ultimately leading to powerful biocatalysts to dramatically expand synthetic chemist's toolbox. In this regard, over the past five years, drawing inspirations from synthetic organic chemistry, researchers have been able to design and develop stereoselective biocatalytic reactions which are never previously encountered in nature, including those following a radical mechanism. Using visible light to unveil the one-electron reaction manifold of nicotinamide and flavin cofactors, the groups of Hyster and Zhao pioneered a photoenzymatic strategy to catalyse diverse new-to-nature enantioselective radical

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Competing interests

The authors declare no competing interests.

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reactions.[3,4] By leveraging the innate redox properties of first-row transition-metal cofactors found in natural metalloenzymes, the Yang group recently introduced a metalloredox strategy for stereoselective unnatural radical biocatalysis.[5] At present, the identification and evolution of promiscuous enzymes to engage other challenging asymmetric free radical reactions continue to represent the forefront of this burgeoning area of research.

Now, writing in *Nature Catalysis*, Huimin Zhao, Binju Wang and colleagues unlock the potential of NAD(P)H-dependent ketoreductases (KREDs) as photoredox enzymes to catalyse asymmetric radical C−C bond formation in an intermolecular fashion (Fig 1a).[6]

In their native biochemistry, KREDs catalyse hydride transfer reactions from their nicotinamide cofactor to the carbonyl substrate often with excellent enantioselectivities. Inspired by nicotinamide-mediated, photoinduced decarboxylative conjugate addition reactions previously developed by synthetic chemists, the authors envisioned that the enantioselective radical C−C bond formation could be accomplished with nicotinamide-dependent enzymes upon visible light irradiation using *N*-(acyloxy)phthalimides as the radical precursor. The authors commenced their investigation by evaluating commercially available enzymes from Codexis's KRED kit. Initial hits with high levels of enantioselectivity were quickly identified without any protein engineering, among which the optimal result was found using a triple mutant of the KRED from *Lactobacillus kefir* (20% yield and 91:9 enantiomeric ratio (e.r.)).

With these initial results in hand, the researchers obtained a crystal structure of the best performing enzyme variant and proceeded to perform structure-guided protein engineering. First, docking the C−C bond formation product into the enzyme's active site revealed 10 potentially important residues for this new-to-nature radical biocatalytic process. Next, in lieu of using iterative site-saturation mutagenesis (SSM), the authors opted to generate small focused libraries for their enzyme engineering. The steric property of key active site residues was used as the sole parameter for enzyme library generation, and only 3−5 amino acid residues with varying steric profiles, including alanine (A)/glycine (G), leucine (L)/isoleucine (I), and phenylalanine (F) were evaluated. This site-directed mutagenesis led to a small, yet high-quality library of 30 enzyme mutants. Screening this focused library against the model reaction afforded an improved enzyme variant with higher enantioselectivity. In this way, three rounds of protein engineering were performed, eventually resulting in the final variant with three beneficial mutations, including L199A, M205F and M206F (Fig 1b). Under the optimized reaction conditions, this final variant afforded the desired radical conjugate addition product in 79% yield and 95.5:4.5 e.r.. This engineered enzyme readily accommodated a wide range of substrate combinations. A variety of redox-active esters, which are precursors to diverse benzylic and aliphatic radicals, including primary, secondary, and tertiary ones, underwent this biocatalytic transformation with good to excellent enantioselectivities.

Next, the researchers performed computational studies to gain insights into the enzymatic reaction mechanism and the origin of enantioselectivity. Based on their study, the authors propose that the nicotinamide cofactor of the enzyme first engages the redox-active ester to form a charge transfer (CT) complex (Fig 1c). Photoinduced electron transfer of this

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CT complex leads to the formation of a carboxyl radical, which rapidly decarboxylates to provide a nucleophilic alkyl radical. This nascent alkyl radical intermediate then quickly adds to the methacrylate substrate to furnish a new electrophilic radical intermediate. Final hydrogen atom transfer (HAT) from the nicotinamide radical cation to this electrophilic radical provides the conjugate addition product and completes the catalytic cycle. The computational model allows to infer that the binding of the methacrylate substrate dictates the enantioselectivity. This finding is in contrast to some of the previously developed photoenzymatic reactions using ene reductases (EREDs), where the HAT step is enantiodetermining.

Overall, the elegant work from Zhao, Wang and colleagues further expands the catalytic repertoire of unnatural biocatalytic reactions to encompass a synthetically valuable intermolecular asymmetric radical coupling. Although NAD(P)H-dependent enzymes have been previously repurposed as photoenzymes for single electron transfer processes,[7] radical C−C bond formation was considered a challenging objective with these systems, in part due to the rapid radical termination via HAT. The present study dispels this notion and demonstrates the potential of KREDs and related NAD(P)H-dependent enzymes to facilitate productive C−C bond formation via the intermediacy of transient radicals. Most notably, this enzyme activity is demonstrated in the context of intermolecular radical coupling, a process that is not easily achieved in unnatural radical biocatalysis. In principle, once the nascent radical species is formed, it will undergo various side reactions if not positioned in proximity to the radicalphile. To circumvent the unproductive decay of highly reactive radical intermediates, previously developed ERED-based intermolecular radical processes hinge on the formation of a ternary complex to ensure rapid radical addition to the 2π component. While this design is ingenious, it may pose constraints on the types of radical coupling partners that can be employed in photoenzymatic catalysis. The work from Zhao, Wang and colleagues indicate the availability of alternative mechanisms for intermolecular radical biocatalysis, highlighting exciting new avenues to design enantioselective radical reactions that are not previously encountered in the biological world.

References

1. Sibi MP, Manyem S & Zimmerman J Chem. Rev. 103, 3263–3296 (2003). [PubMed: 12914498]
2. Mondal S, Dumur F; Gigmes D; Sibi MP, Bertrand MP & Nechab M Chem. Rev. 122, 5842–5936 (2022). [PubMed: 35073048]
3. Sandoval BA & Hyster TK Curr. Opin. Chem. Biol. 55, 45–51 (2020). [PubMed: 31935627]
4. Harrison W, Huang X & Zhao H Acc. Chem. Res. 55, 1087–1096, (2022). [PubMed: 35353478]
5. Zhou Q, Chin M, Fu Y, Liu P & Yang Y Science 374, 1612–1616 (2021). [PubMed: 34941416]
6. Huang X et al. Nat. Catal. 10.1038/s41929-022-00777-4 (2022).
7. Emmanuel MA, Greenberg NR, Oblinsky DG & Hyster TK Nature 540, 414–417 (2016). [PubMed: 27974767]

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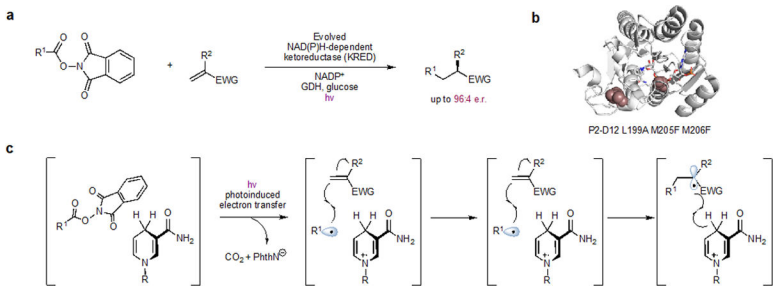

Fig 1. KRED-catalysed enantioselective intermolecular radical addition reactions.

a, overall photoenzymatic transformation. **b,** evolved KRED variant. Illustration was made based on a closely related KRED (PDB ID: 4RF2). **c,** proposed reaction mechanism. EWG = electron-withdrawing group.