DIRECTED EVOLUTION AS A TOOL TO EXPAND BIOCATALTYIC MECHANISMS

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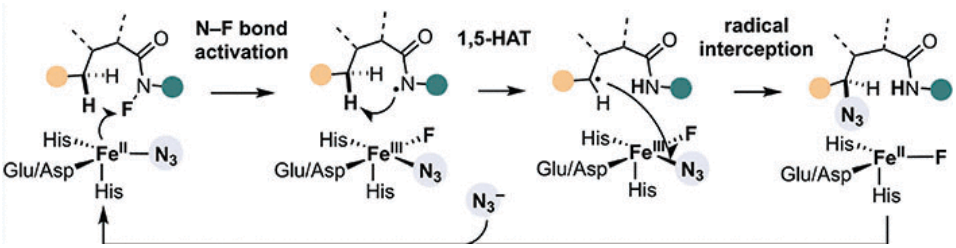
INTRODUCTION

Biocatalysis is a powerful tool to selectively synthesize high value chemical substrates. The hallmark specificity and selectivity characteristics of biocatalysts can be leveraged to perform difficult laboratory transformations in stereo-, regio-, and chemoselective manners where traditional chemical catalysts have failed.[1] While this controlled reactivity is valuable, biocatalysts are generally limited in their native mechanisms, performing the same reaction on conserved targets or targets showing similarity to its natural substrate.[2] Thus, the selectivity and specificity of enzymes can compromise the versatility and variability in the transformations performed, unlike their small molecule chemical catalyst counterparts. Broadening the mechanistic and synthetic repertoire of biocatalysts in which one enzyme can perform a plethora of chemical reactions without compromising their inherent selectivity would revolutionize catalysis. Directed evolution is one of the tools that have been used to broaden the promiscuity of enzymes to allow for non-natural reactivity.[1] Directed evolution is a protein engineering tool in which mutant enzymes with altered amino acid sequences can be generated for the purpose of optimizing parameters such as enantioselectivity, total turnover numbers (TTN), binding affinity, and active site rigidity.[2] The enzymes undergo iterative rounds of evolution as their performance is assessed and enhanced via mutagenesis.

APPLICATIONS OF DIRECTED EVOLUTION

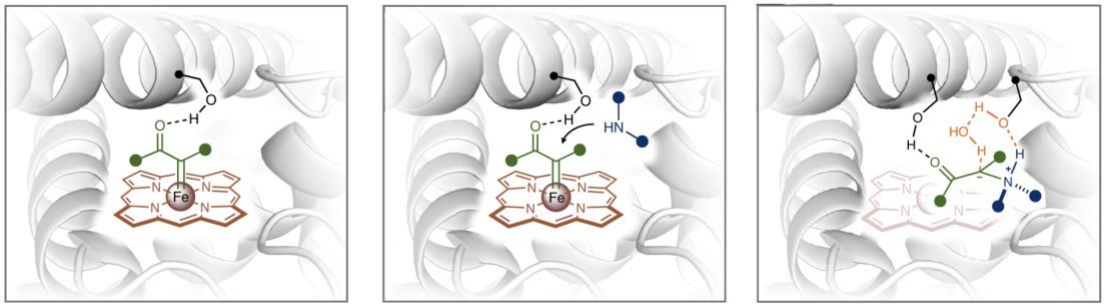
Huang and coworkers used directed evolution to program (4-hydroxyphenyl)pyruvate dioxygenase (HppD), a nonheme iron enzyme from *Streptomyces avermitilis*, to perform a non-natural radical relay C(sp^3)-H azidation reaction.[3] The native mechanism of HppD converts hydroxyphenylpyruvic acid (HPPA) into homogentisic acid (HGA) via an $Fe^{II/IV}$ catalytic cycle featuring dioxygen addition to a keto group, decarboxylation, acyl chain migration and hydroxylation.[4] However, after six to seven mutations, HppD was repurposed to be able to perform a radical relay reaction via an $Fe^{II/III}$ catalytic cycle distinct from its native mechanism featuring the generation of an amidyl radical, 1,5 hydrogen atom transfer, and radical interception to access a variety of azidated substrates in appreciable yields, enantioselectivities, and TTN.

Scheme 1. Mechanism of C(sp^3)-H Azidation Via HppD[3]



Similarly, Arnold and coworkers demonstrated the ability to use a cytochrome P450 variant, P411, to facilitate carbene insertion into an N-H bond.[5] While cytochrome P450s are generally responsible for the oxygen transfer to hydrocarbons and heteroatomsm, After 29 mutations of the wildtype P450BM3, they were able to engineer an enzyme capable of facilitating carbene formation in the active site, carbene transfer to the amines, and a selective proton transfer to yield a variety enantiopure primary and secondary anilines and aliphatic amines (**Scheme 2**).[5,6]

Scheme 2. Mechanism of Carbene Insertion Into N-H Bond Via P450 Variant[5]



CONCLUSION AND FUTURE DIRECTIONS

Biocatalysis has the potential to expand the number of novel abiotic reactions that can be performed in laboratories. Harnessing nature's selectivity while drawing inspiration from the versatility of chemical catalysts will have expansive impacts on molecular synthesis. However, while current progress has been appreciable, biocatalysis still suffers from challenges in reproducibility, scalability, and compatibility with industrial conditions.[1] These limitations challenge the generalizability of this form of catalysis and more advances will be needed to move from isolated bench-top examples of enzymatic synthesis to widespread industrial applications.

REFERENCES

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