

RECENT ADVANCES IN CHEMOENZYMATIC SYNTHESIS

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INTRODUCTION

Biocatalysis has recently been extensively used in synthesis to bypass multiple chemical reactions and result in better synthesis routes. Its history can be traced back to the early 1800s, when researchers began to investigate the fermentation ability of yeasts. Soon after, several fundamental discoveries, including cell-free fermentation, DNA structure elucidation and X-ray crystallography for enzyme 3D structure viewing and catalysis mechanism explaining, had drastically accelerated the development of biocatalysis. Another milestone will be the design of *in situ* cofactor regeneration system in 2007, and with that established, enzyme catalysis based on electrochemistry, photochemistry and other systems were all developed rapidly.¹ In 2018, the Nobel Prize in Chemistry was awarded to Frances H. Arnold for the directed evolution of enzymes, highlighting this new method of taming enzymes for new-to-nature catalysis. Up till now, biocatalysis have brought various new transformations into traditional reaction space, especially in site-selective C-H bond functionalization and challenging C-C bond formation. At the same time, the high regioselectivity and enantioselectivity of enzymatic catalysis have been fully exploited in late-stage modification of natural products and drug candidates. When investigating into chemoenzymatic synthesis, the biological and chemical steps serve as perfect supplementary to each other and generating shorter routes with high selectivity compared to traditional organic synthesis.

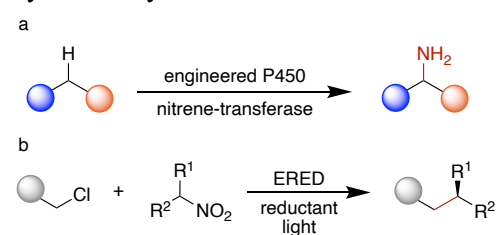
BROADEN REACTION SPACE: BIOCATALYSIS BRINGS NEW TRANSFORMATIONS

Previously, the enzymatic construction of C-N bond relied on a preoxygenated carbon center through reductive amination, and the direct nitrogen insertion had been limited to electronically activated positions like benzylic etc. In 2022, Athavale, Hirschi, Arnold and co-workers reported the first enzymatic nitrogen insertion into unactivated C-H bond, in which the P450 enzyme was evolved to bind with Fe-nitrenoid as the nitrogen source and through 9 cycles of directed evolution, the total turnover number (TTN) was drastically elevated (**Scheme 1a**).² Another revolutionary application is enzyme catalyzed Csp^3 - Csp^3 coupling. The best solution in organic catalysis was metal-catalyzed cross electrophile coupling, which suffers from large amount of dimerization and poor enantioselectivity. In 2022, Hyster and co-workers reported the ene-reductase catalyzed asymmetric sp^3 - sp^3 cross electrophile coupling (**Scheme 1b**).³ The reaction started with reaction between selectively reduced alkyl halide and in situ-generated nitronate to form the new C-C bond and then followed by C-N bond cleavage and hydrogen atom transfer. All the reactions were catalyzed by the ene reductase and a series of amide were formed with up to 98% yields and 97:3 enantiomeric ratio.

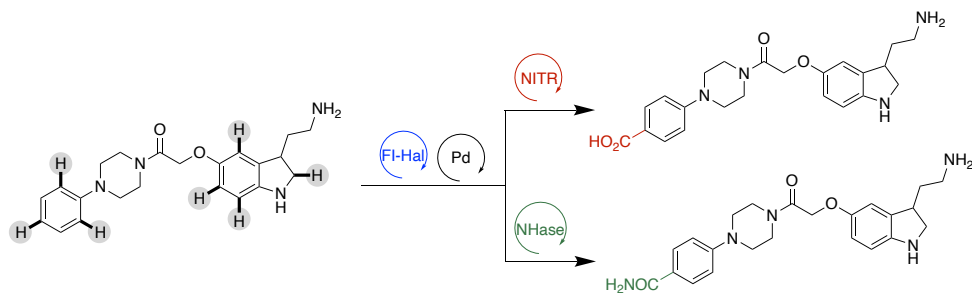
REACHING TARGET MOLECULES: LATE-STAGE CHEMOENZYMATIC MODIFICATION

Late-stage C-H functionalization has long been regarded as a challenging task due to multiple reactive sites and various existing functional groups in the complex molecules and the achieved examples were mainly limited to functionalization with few atoms like oxygen and halogens. In 2021, Micklefield and co-workers reported a one-pot chemoenzymatic synthesis to obtain amides or carboxylic acids from C-H bonds (**Scheme 2**).⁴ The near-identical C-H bonds were first selectively halogenated by enzyme, and then transformed to cyan through palladium catalysis

Scheme 1. New Transformations Brought by Biocatalysis.



Scheme 2. Chemoenzymatic Late-stage C-H Functionalization



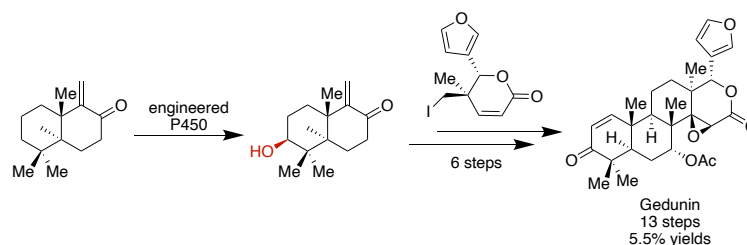
with non-toxic cyanide source and finally get diversified. The selective halogenation can hardly be done with chemical catalysis and cyanation is challenging with biocatalysis, emphasizing the necessity of their combination. Another

example is the late-stage diversification achieved by Sherman and co-workers.⁵ They synthesized M-4365 G₁ as antimicrobials and diversified it with chemoenzymatic method in search of better drug candidates. The engineered P450 enzyme performed well in both hydroxylation and epoxidation of this substrate, while copper was exploited in the hydroxyl oxidation due to low yield of enzymatic catalysis. After the late-stage diversification, its antimicrobial activity was tremendously improved by 100 folds according to minimum inhibitory concentration test.

IMPROVE SYNTHESIS EFFECTIVENESS: SHORTER ROUTES, HIGHER YIELDS AND SELECTIVITY

The fact that chemoenzymatic synthesis strategies exploded recently might be explained as shorter routes and higher selectivity it can afford, especially when comparing with traditional chemical method head-to-head. In 2017, Arnold, Stoltz and co-workers reported the enantiomeric total synthesis of Nigelladine A.⁶ After several chemical steps, they met the problem to hydroxylate the secondary carbon alone in the presence of tertiary carbon. Various types of catalyst including palladium, rhodium and chromium all led to poor selectivity and trace desired products. That explained why they turned to enzyme catalyzed hydroxylation and several P450 variants successfully generated the desired products with high yields. Similarly, Renata and co-workers first reported the chemoenzymatic synthesis of Gedunin in only 13 steps (Scheme 3).⁷ Gedunin had never been synthesized before and all its analogs were synthesized by chemical routes with at least 20 steps. The P450 mutants was used to do selective hydroxylation and avoid the epoxidation of existing enone group. In this case, they were able to generate the hydroxyl at the last step of that moiety and avoid further protection steps.

Scheme 3. Chemoenzymatic Synthesis of Gedunin



SUMMARY AND OUTLOOK

The advantages of chemoenzymatic synthesis have been shown in many aspects, yet there are still space for improvements and shinning new opportunities, including new application: utilizing current biocatalysis in more substrate synthesis by directed evolution to overcome the specificity issue; new function: engineering enzyme for new-to-nature functions and discovering enzyme promiscuity as great supplementary for organic catalysis; and new design: employing the revolutionary computational and AI tools for enzyme screening and mechanism investigation.

REFERENCE

1. *ACS Cent. Sci.* **2021**, 7, 1105–1116
2. *J. Am. Chem. Soc.* **2022**, 144, 19097–19105
3. *Nature* **2022**, 610, 302–307
4. *Nature Catalysis* **2021**, 4, 385–394
5. *J. Am. Chem. Soc.* **2017**, 139, 7913–7920
6. *J. Am. Chem. Soc.* **2017**, 139, 10196–10199
7. *J. Am. Chem. Soc.* **2022**, 144, 19238–19242