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Catalytic C–H Amination: A Reaction Now Accessible to Engineered Natural Enzymes**

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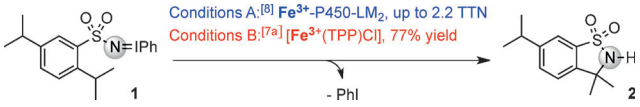
biocatalysis · C–H amination · directed evolution · nitrenes · P450 enzymes

Nature has always been a source of inspiration for scientists. Its ability to construct complex natural products has challenged the creativity of synthetic chemists. The toolbox of organic reactions is thus still evolving, and catalytic C–H amination has recently emerged as a powerful method for total synthesis and late-stage functionalization of biomolecules.[1,2] Most of these achievements can be attributed to new reagents that enable the selective insertion of a metallanitrene into a specific C–H bond. Paradoxically, this reaction cannot be found in nature, and typical biosynthetic methods for the formation of C–N bonds rely on the amination of functional groups. Biomimetic models that have been tailored to emulate natural enzymes have also been extensively studied. A typical example is provided by the monooxygenases that catalyze the regio- and stereoselective C–H hydroxylation of alkanes through the formation of iron–oxo species following O_2 activation in the presence of NADPH.[3] These metalloenzymes have received considerable attention through the design of bioinspired catalysts,[4] which highlights our capacity to mimic nature, as have recent applications in late-stage C–H bond oxidations.[5]

While most of these research developments are centered on simulating nature, it would be worthwhile to challenge nature's ingenuity to mimic the synthetic reactions conceived by chemists. To this end, catalytic C–H amination is an excellent platform for the development of a non-natural enzymatic reaction, especially in the context of the design and implementation of sustainable procedures. Recent progress made in the directed evolution of enzymes has successfully addressed this issue,[6] and this highlight showcases two studies that demonstrate the capacity of engineered natural oxidases to catalyze the formation of C–N bonds and paved the way for the application of biocatalysis in catalytic C–H functionalization.

Pioneering studies from the groups of Breslow and Mansuy have demonstrated the capacity of porphyrins with Fe^{3+} centers to catalyze nitrene transfer reactions.[7] Soon after these studies were published, the first and only example, prior to the two reports highlighted here, of an intramolecular amination reaction catalyzed by a native cytochrome (Cyt) P450 enzyme was described. Iminoiodane **1** was found to cyclize into the aminated product **2** in the presence of Fe^{3+} containing P450-LM_2 (Scheme 1).[8] Importantly, both the



Scheme 1. Pioneering studies in porphyrin-catalyzed C–H amination.

porphyrin-catalyzed process and the P450-mediated transformation relied on an Fe^{3+} species. This P450-catalyzed reaction was quite unique, but major drawbacks were the low turnover number (2.2 under the optimized reaction conditions) and the poor atom economy. The insolubility of iminoiodinanes and the hydrolysis of the iron–imido complex, which resulted in oxene transfer rather than nitrene addition, particularly for the intermolecular reaction, were additional problems that prevented advancements in this area for more than 30 years.

Recently, two groups have reported solutions to circumvent some of these limitations with a focus on modifying the reaction conditions. Therefore, combining azides with engineered P450 enzymes that include a reduced Fe^{2+} center has resulted in an efficient intramolecular benzylic C–H amination reaction under anaerobic conditions.[9,10] In the study carried out by Arnold and co-workers,[9] the mutation of two amino acids, threonine 268 and the axially coordinated cysteine 400, which exerts a "thiolate push", was found to be critical for the switch in reactivity. Both of these amino acids are thought to play a crucial role for the formation of the iron(V)–oxo species through O–O bond cleavage, possibly assisted by a molecule of water (Figure 1). These modifica-

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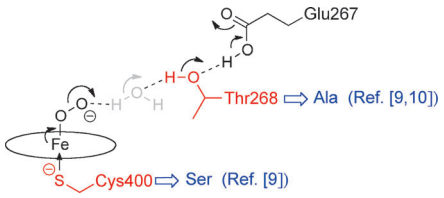
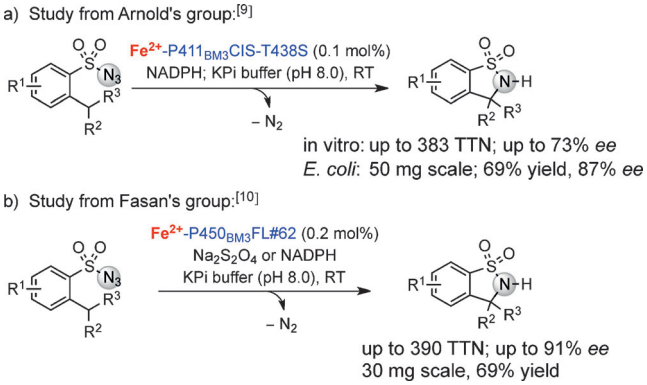


Figure 1. Influence of the T268 and C400 residues on the formation of the iron(V)–oxo species.

tions, combined with twelve other mutations, induced the intramolecular amination of benzenesulfonyl azide in 73% yield with a total turnover number (TTN) of 140. This result was then improved by introducing the T438S mutation: The mutant $P411_{BM3}$CIS-T438S gave the desired product in vitro with 73% *ee* and a TTN of 383 (Scheme 2a). Importantly,



Scheme 2. C–H amination reactions catalyzed by engineered P450 enzymes. KPi = potassium phosphate.

expression of the $P411_{BM3}$CIS-T438S catalyst in *E. coli* allowed for the intramolecular C–H amination to be performed in cellulo on a 50 mg scale. The benzosultam was isolated in 69% yield and 87% *ee*.

The study by Fasan et al. provides an alternative enzymatic catalytic system for C–H amination.[10] They demonstrated that, contrary to Arnold's studies, cysteine-ligated P450 enzymes can also be efficient catalysts for this reaction. The optimal enzyme for the C–H amination was found to be $P450_{BM3}$–FL#62; however, the turnover numbers varied with respect to the substitution pattern on the substrate (Scheme 2b). Thus, substrates with tertiary and secondary benzylic positions provided the desired aminated products with TTNs of 47 and 388, respectively, whereas the C–H functionalization of a benzylic CH_3 group occurs with a significantly lower TTN (TTN = 5). It was also found that the introduction of a bulky substituent on the aromatic ring enhanced the efficiency of the reaction. During these studies, the corresponding sulfonamide was often formed as a side product. Mechanistic investigations revealed that threonine 268, which is involved in the heterolytic cleavage of O_2, could play an analogous role in the protonation of the iron–imido species to give the sulfonamide and possibly an iron–oxo species. Accordingly, the hydrophobic nature of the active site was increased, which improved its catalytic activity in the nitrene transfer. The higher turnover numbers and yields were observed with the mutant $P450_{BM3}$–FL#62 (T268A), and *ee* values of up to 91% were achieved. Finally, this process proved to be efficient on a preparative scale (30 mg, 42% yield).

In both studies, the C–H amination reaction requires a reducing agent, and the presence of CO inhibits the process through formation of a stable iron(II)–CO complex. These experiments support the involvement of an Fe^{II} species that would react with the azide to produce an iron(IV)–imido intermediate. The C–H amination would then proceed either through a radical rebound mechanism or a concerted pathway, as is the case in transition-metal-catalyzed reactions. Interestingly, these enzymatic processes compete favorably with classical C–H amination. In the case of the analogous rhodium-,[11a] cobalt-,[11b] and iridium-catalyzed[11c] reactions, the yields are comparable, but TTNs did not exceed 50. However, in terms of the enantioselectivities, the enzymatic process falls in between the iridium-catalyzed reaction, which is the most efficient process, and the rhodium-catalyzed reaction, which is the least effective.

In conclusion, these two studies highlight that engineered C–H oxidases can be turned into C–H aminases. The concept of tuning the native reactivity of enzymes has recently received considerable attention.[12,13] The results reported here also provide insight into the exact role of the key residues of monooxygenases, that is, T268 and C400, even though they are somewhat contradictory with respect to the axial C400 ligand. The use of Fe^{II} enzymes could also explain the poor reactivity of native Fe^{III} P450 enzymes in catalytic nitrene transfer reactions, which could be related to either the oxidation state or the geometry of the active site. This raises the questions as to whether it would be possible to control the chemoselectivity of C–H functionalization processes through specific mutations, an objective that is still difficult to achieve in metal-catalyzed reactions. Finally, these enzymatic methods validate the extension of biocatalytic processes to artificial procedures, which opens the door for new synthetic reagents, protein engineering, and environmentally friendly reactions. Provided that their practicability is demonstrated and their scope improved, these reactions could complement classical metal-catalyzed processes in the future.

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