PROMISCUOUS REACTIONS OF ENGINEERED P450s

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Oxygenase Catalysis:

Enzymatic catalysis for its use in chemical synthesis has gained notice in recent years for the advantages it has over traditional small molecule methods such as ambient conditions as well as remarkable regio- and stereochemical selectivity. Of the enzymes gaining the most attention in recent years are cytochrome P450s. These enzymes have evolved in all domains of life and as such are the product of many millennia of evolution. Even with their diversity in nature cytochrome P450s all have a conserved iron prosthetic group ligated by porphyrin with an axial cysteine residue. The cysteine residue is absolutely critical for reactivity and longevity of the catalyst by decreasing the rate constant of unproductive pathways while providing a thermodynamic driving force for productive pathways. In nature, P450s have been used to perform several oxygenation reactions by the use of molecular oxygen and stoichiometric NAD(P)H and/or FAD as electron sources to effect transformations such as hydroxylations, epoxidations, Baeyer-Villiger insertions, and sulfoxidations via an iron-oxo intermediate commonly referred to as Compound I (Figure 1).[1] In order to increase the practicality of using P450s in a synthetically useful way, groups have engineered variants of these enzymes which change the topology and reactivity of the binding pocket to enhance yields, selectivity, and turnover of the catalyst. With the success of engineered P450s to perform native reactivity, the Watanabe group and others have looked to hydroxylate benzene to phenol with the use of a 'dummy' substrate that mimics the natural substrate but cannot

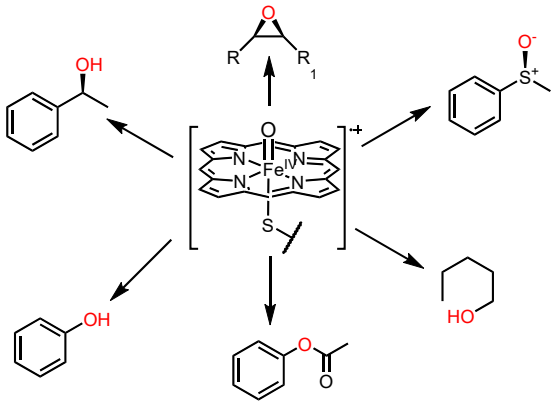


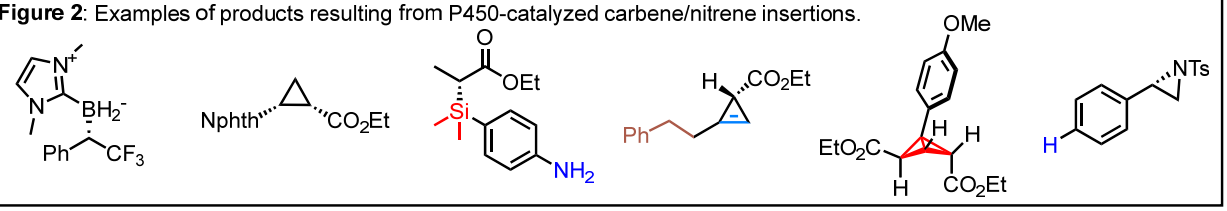
Figure 1. Examples of oxygenation reactions

itself undergo oxidation.[2] Driven by the success of the field, the Arnold group took the forefront of looking at non-native reactivity.

Reactions of Carbenoids and Nitrenoids:

In order to effect transformations outside the typical scope for an enzyme, site-saturation mutagenesis was used to introduce singular residue changes in an iterative fashion to alter reactivity. This method requires knowledge about the binding site, usually by a crystal structure in order to rationally guide mutagenesis. This semi-random approach to protein engineering has allowed for the expedient access to non-natural P450s and P411s to perform carbene and nitrene insertions. The Arnold group has proven this method effective in the preparation of chiral aziridines, spiro-cyclopropanes, sulfimines, thioethers, cyclopropenes, bicyclobutanes, organoboranes, and organosilicates (Figure 2).[3-7] Of particular interest was the

Figure 2: Examples of products resulting from P450-catalyzed carbene/nitrene insertions.



discovery that a P450 could perform nitrene insertions. Given the current trajectory of the field, it seems likely that the reaction scope and utilization by chemists both in laboratory and in industry will continue to grow.

References:

(1) Liang, Y.; Wei, J.; Qiu, X.; Jiao, N., *Chem. Rev*. **2018**, *118*, 4912−4945.
(2) Watanabe et al. *Angew. Chem., Int. Ed*. **2017**, *56*, 10324−10329.
(3) Farwell, C. C.; Zhang, R. K.; McIntosh, J. A.; Hyster, T. K.; Arnold, F. H., *ACS Cent Sci*. **2015**, *1*, 89-93
(4) Huang, A.; Arnold, F. H. *Angew. Chem. Int. Ed.* **2016**, *55*, 4711 –4715.
(5) Chen, K.; Huang, X.; Jennifer Kan, S. B.; Zhang, R. K.; Arnold, F. H., *Science* **2018**, *360*, 71–75.
(6) Kan, S. B. J.; Huang, X.; Gumulya, Y.; Chen, K.; Arnold, F. H. *Nature* **2017**, *552*, 132.
(7) Kan, S. B. J.; Lewis R. D.; Chen, K.; Arnold, F. H., *Science* **2016**, *354* (6315), 1048-1051.