

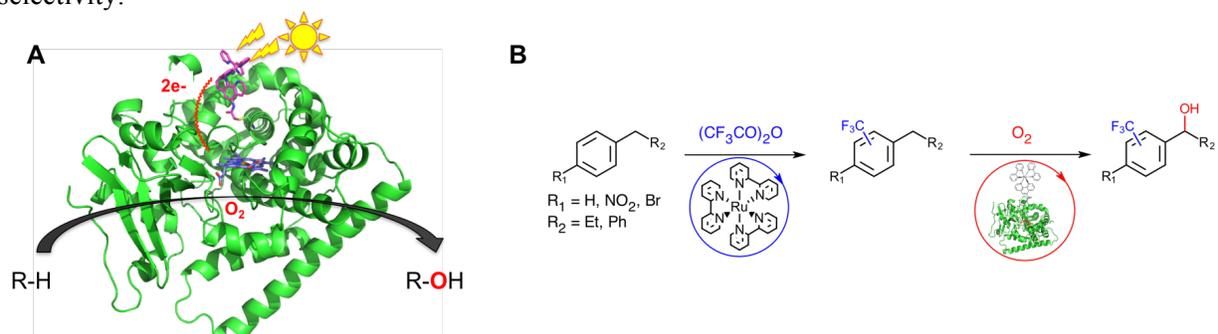
# Hybrid P450 Enzymes Featuring Ru(II)-diimine Complexes

Lionel Cheruzel

Department of Chemistry, San Jose State University, San Jose, CA, USA

lionel.cheruzel@sjsu.edu

Our laboratory has developed hybrid P450 enzymes containing a Ru(II)-diimine photosensitizer covalently attached to non-native cysteine residues of P450 heme domains. This approach has enabled to harness their synthetic potential upon visible light excitation.<sup>1</sup> High total turnover numbers and initial reaction rates were obtained in the light-driven hydroxylation of natural long-chain fatty acid substrates.<sup>2</sup> The crystal structure of the most efficient hybrid enzyme revealed that the photosensitizer is ideally positioned to deliver electrons to the heme active site utilizing the natural electron transfer pathway.<sup>3</sup> Our current efforts in optimizing the biocatalyst photocatalytic activity has included a combination of rational and directed evolution approaches while taking advantages of the unique properties of the Ru(II)-diimine complexes.<sup>4-6</sup> Selected mutants from a directed evolution screen display several folds enhancement in photocatalytic activity towards various substituted arenes. We also probed the effect of systematically varying the para-substituents on the Ru(II)-diimine photosensitizer on the photocatalytic of the hybrid enzymes and gained insights into the rate limiting step of the photocatalytic process.<sup>5</sup> Recently, the merging of photoredox catalysis with the hybrid enzyme approach has enabled the selective light-driven chemoenzymatic trifluoromethylation hydroxylation of a wide range of substituted arenes with exquisite selectivity.<sup>6</sup>



**Figure 1.** A) Crystal structure of the hybrid P450 enzyme (green) and the light-harvesting Ru(II)-diimine complex in magenta. Upon light-activation, electrons are injected into the enzyme active site for activation of molecular dioxygen and substrate oxyfunctionalization; B) Recent light-driven chemoenzymatic approach for the selective trifluoromethylation and oxyfunctionalization of substituted arenes.

**Ref.** 1. *Biochim. Biophys. Acta*, **2016**, 1857 (5), 589-97; 2. *J. Am. Chem. Soc.* **2013**, 135 (39), 14484-14487; 3. *Biochim. Biophys. Acta, Prot.* **2016**, 1864 (12), 1732-1738; 4. *J. Inorg. Biochem.*, **2018**, 186, 130-134; 5. *Inorg. Chem.* **2017**, 56 (11), 6558-6564; 6. *ACS Catal.*, **2018**, 8, 2225-2229.