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Protein engineering of toluene monooxygenase to enable mild synthesis of oxirane

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D. A. Carlin,[a] S. J. Bertolani and J. B. Siegel[c]

University of California, Davis, Department of Chemistry

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Abstract Here. The abstract should be a single paragraph which summarises the content of the article. It should be no longer than 50 words (approximately 5-6 lines). The abstract should be a single paragraph which summarises the content of the article. It should be no longer than 50 words (approximately 5-6 lines).

Ethylene oxide (IUPAC name: oxirane) is an important feedstock chemical and industrial disinfectant. The 20 million tons produced annually are used to make a variety of consumer productions and industrial chemicals. State of the art industrial production methods oxidize ethene in a high-pressure, high-temperature reactor to produce oxirane. Current reactor designs demand temperatures of 200–300 C and pressures of 10–30 atm to provide yields of up to 75% oxirane. Sustainable production would take place under ambient temperature and pressure in a mild, aqueous environment.

Enzymes are renowned for their ability to reduce the activation energy of chemical reactions, allowing mild reaction conditions. In nature, a class of enzymes called alkene monooxygenases (AOs) oxidize alkenes such as ethene. However, the production of recombinant AOs in E. coli has proven elusive.

Toluene o-xylene monooxygenase (TOM), an enzyme closely related to AOs, has been shown to oxidize aromatic compounds such as toluene via an epoxide intermediate [Whited 1991]. In addition, TOM is easily expressed in E. coli. We hypothesized that minor sequence changes would be sufficient to alter TOM's substrate specificity to include alkenes such as ethene. After further engineering, an enzyme capable of oxidizing ethene to oxirane would become an important industrial catalyst for oxirane synthesis under mild conditions.

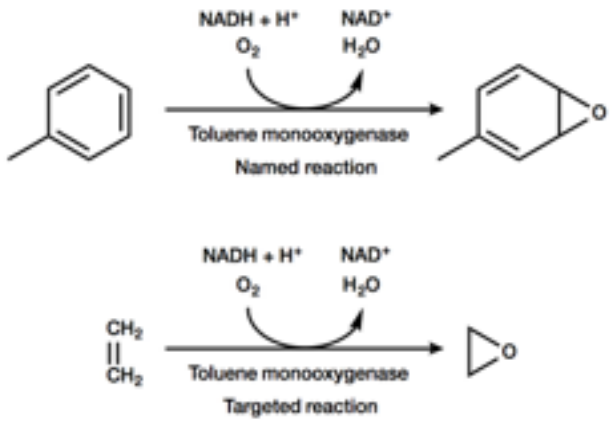

Figure 1. Reaction scheme showing the native reaction of toluene to a p-cresol intermediate and the novel reaction of ethene to oxirane.

In our study, a directed evolution library of TOM in E. coli was assayed to determine if any of the mutants oxidized ethene to oxirane. Using GC-FID, we were able to simultaneously detect both ethene and oxirane in the headspace of sealed culture vials.

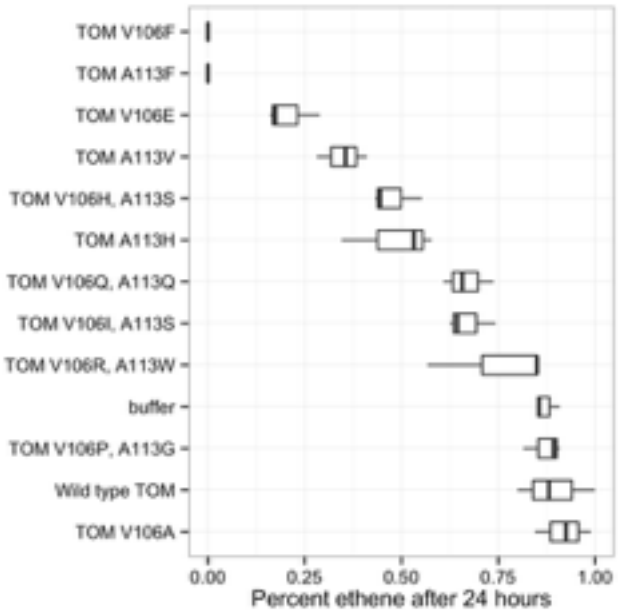

Figure 2. Ethene remaining after 24 hours of contact with whole cells expressing variants mutants of TOM. Cells suspended in phosphate-buffered saline were sealed in glass vials under 1.5% ethene atmosphere. After 24 hours, headspace was assayed for ethene and oxirane by GC-FID.

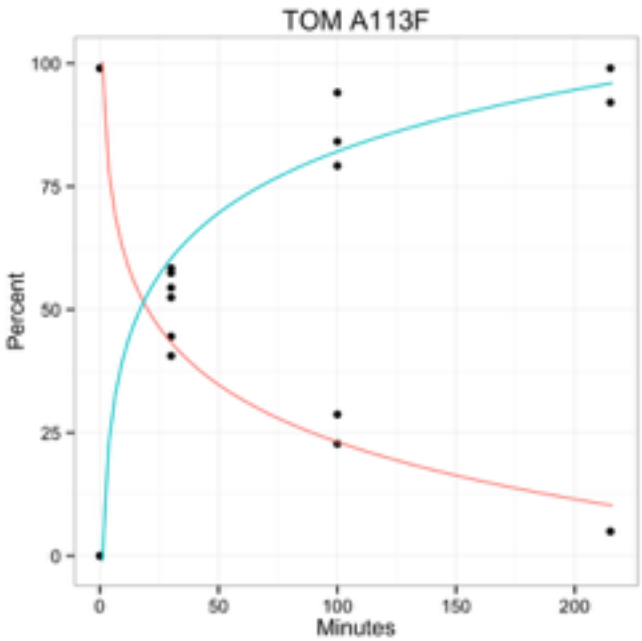

Figure 3. Further characterization of TOM A113F showing the complete conversion of 0.7 mM ethene to oxirane in under 4 hours.

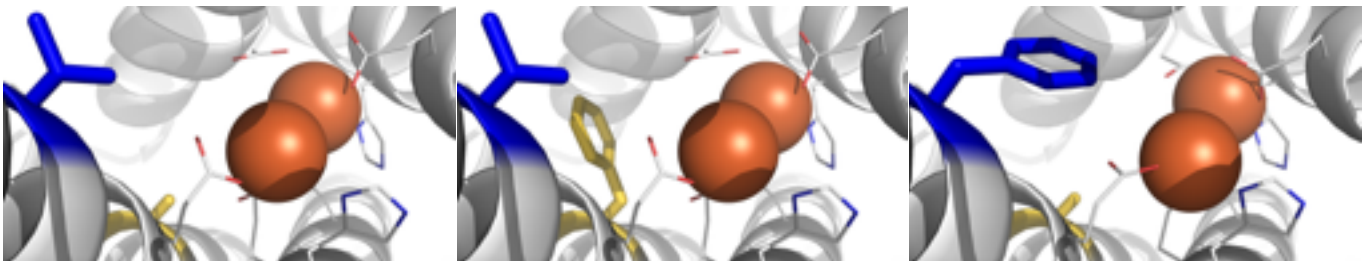

Figure 4. The active sites of TOM (left), TOM V106F (center), and TOM A113F (right). Irons (spheres) are shown with coordinating residues. Mutated residues are colored blue (106) and gold (113). Phenylalanine at either position partly occludes the active site.

With a limit of detection of 5 uM (lowest observed conc.: 5 uM, over 200 minutes: 50 nM/min, over 1440 min: 7 nM/min), we were unable to detect any oxirane production in wild type TOM or mutants TOM V106P+A113G and TOM V106A. We detected ethene degradation rates of about 300 nM/min in mutants TOM V106R +A113W, TOM V106Q+A113Q, TOM 103S+A107T, TOM A113H, and TOM V106H+A113S. TOM mutants A113V, V106F, and A113F, where V106 and A113 are replaced by residues with large sidechains, showed complete degradation of 0.7 mM ethene over 3 hours and rates of oxirane production 4 nM/min under these conditions.

Atomic-resolution molecular models of the mutant protein structures were constructed using Rosetta. A model of TOM was constructed using PDB entries 3U52 (66% sequence identity), 2INN (64% sequence identity), and 2INP (67% sequence identity). The geometry of iron-coordinating residues in the model was as observed in crystal structures.

Our models revealed a pocket around the open coordination sites of the diiron center in the wild type enzyme. In the mutants A113F and V113F, the pocket is partially occluded by the sidechain of phenylalanine. Interestingly, this admits a simple explanation for the change in substrate specificity observed. The large aromatic side chain in the pocket partially fills it such that it will not admit an indole but will easily admit a small nonpolar species such as ethene. This is consistent with our experimental results and is the best explanation for the specific activity of the mutants on ethene.

Notes and references

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[b] Address here.

[c] Address here.

† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

1 Citations here in the format A. Name, B. Name and C. Name, *Journal Title*, 2000, **35**, 3523; A. Name, B. Name and C. Name, *Journal Title*, 2000, **35**, 3523.Notes and references

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