

P035 Exploitation of flavocytochrome P450 BM3 for selective hydroxylation and epoxidation of alkenes and other molecules
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Flavocytochrome P450 BM3 from *Bacillus megaterium* is a high activity fatty acid oxygenase that has assumed a pivotal position in the P450 enzyme superfamily from the perspectives of i) acting as a "model" system for rationalizing structure/mechanism and electron transfer in the P450s, and ii) exploitation for biotechnological purposes. P450 BM3 is a natural fusion of a heme-containing P450 and its redox partner, a NADPH-dependent diflavin reductase. It has the highest reported mono-oxygenase activity of any P450 enzyme. P450 BM3 has been engineered extensively using both rational and forced evolution methods, in efforts to understand mechanism and to generate variants with novel activities. Mutants with activity towards short chain alkanes and polycyclic molecules have been produced using both methods. In this work we describe the characterization of the reactivity of wild-type and mutant forms of P450 BM3 with short chain alkenes and styrene as substrates. P450 BM3 and a F87G variant show interesting reactivity patterns with alkene substrates, with product type varying according to the position of the double bond. Epoxidation and hydroxylation reactions are catalysed with good efficiency. Styrene epoxidation is catalysed by both wild-type and F87G variants – with a substantial increase in formation of the R enantiomer with the F87G mutant.