

P003 Engineering lignin peroxidase activity into
Coprinus cinereus peroxidase

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In recent studies, a second unique substrate oxidation site has been found in *P. chrysosporium* LiPH8, specifically for veratryl alcohol (VA). This site is situated on the surface of the enzyme, requires Trp171 as an absolute requirement and has an acidic local environment generated by four negatively charged amino acids. This negative charge is thought to stabilise the VA cation radical product and removal of two or more acidic residues in site-directed mutants of LiPH8 leads to loss of activity.

Coprinus cinereus peroxidase (CIP) has a very similar fold to LiPH8, but does not turnover VA and is not a lignin peroxidase. Lignin peroxidase activity has been introduced into a triple mutant of CIP, [D179W,R258E,R272D] (or CIP-LiP) at low level. Work is now underway to test whether the two introduced negative charges are essential for the new activity or whether it only requires the removal of the two bulky arginines. For this both single and double charge neutralisation mutants are being generated, which will be analysed by both steady and pre-steady state techniques, along with the original CIP-LiP enzyme. In addition, substitution of the introduced tryptophan with either tyrosine or histidine is being carried out to see if they can function in a similar redox active manner in the oxidation of substrates.