BIVMiB036 BB/L013711/1
1 July – 31 December 2017
Funded by Metals in Biology BBSRC NIBB Business Interaction Voucher
metals.bbsrcnibb@durham.ac.uk @METALSBBSRCNIBB https://mib-nibb.webspace.durham.ac.uk

Improving biocatalytic processes by enzyme stability enhancement

"Increasing the stability of the enzyme catalyst in biocatalytic oxidation technology will improve the catalyst lifetime, leading to improved process productivity and broadening the range of products accessible to this technology platform." Luet Wong, University of Oxford

Stuart Ferguson and Luet Wong, University of Oxford; Jason King, Oxford Biotrans Ltd

RESULTS: To try to produce a P450BM3 enzyme containing a covalently bound heme, we first introduced a nucleophilic side chain close to the heme vinyl groups. Cysteine and histidine substitutions were introduced by site-directed mutagenesis to give a total of 20 single and double mutants. Expression trials showed that several mutants either did not produce holoprotein (the peptide and non-peptide parts of the enzyme) or that protein was produced at a low level. The nine mutants that formed holoprotein and the wild-type enzyme were tested for their ability to oxidise lauric acid (a natural substrate of the enzyme) and propylbenzene (an unnatural substrate). The wild type was the most active of the enzymes at oxidising lauric acid and each of the mutants had a similar profile to the wild type (63% to 90% of the activity). The activity profiles were also virtually identical for propylbenzene oxidation. Next, the wild type and mutant P450BM3 enzymes were treated with dithinoite, in expectation of form a covalently bound heme group. Encouragingly, the P450BM3 enzymes treated with dithinoite had a similar pattern of activity as the untreated enzymes in lauric acid and propylbenzene oxidation assays. Unexpectedly however, further tests showed that there was no covalent attachment of the heme vinyl groups to the introduced cysteine and histidine residues under the reaction conditions we used.

INITIAL AIMS: The industry partner has licensed biocatalytic oxidation technology from the University of Oxford for the production of fine chemicals such as flavours, fragrances, agrochemicals and active pharmaceutical ingredients. This approach not only replaces classical, more energy-demanding and polluting chemical processes, it also enables the use of sustainable feedstocks that are not fossil fuels. Enhancing of the stability of the enzyme catalyst that lies at the heart of the technology will be a key improvement to the process, making it applicable to a wider range of products. In this project we aim to increase the stability of the cytochrome P450BM3 by introducing a covalently bound heme into the enzyme.

- **We were surprised not to be able to covalently attach heme onto the P450BM3 protein**
- **Systematically altering the conditions (time, concentration) for dithionite treatment should be explored as there is precedent for the attachment procedure being successful**