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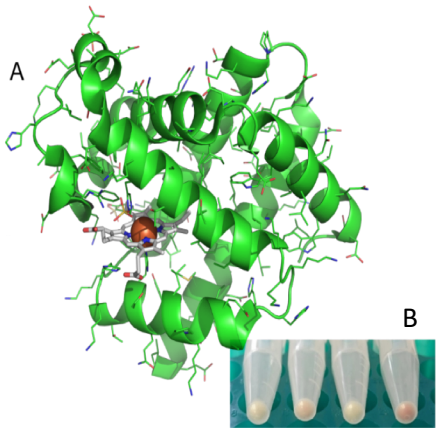
Enhancing *E. coli* for optimal cofactor insertion into heme-containing proteins

"This work showed that the P450 enzyme was sub-optimally loaded with heme. Further enhancement of heme incorporation could increase the commercial yields of this enzyme, so we support further investigations into this and are pleased to continue our collaboration with Professor Le Brun's laboratory." **Biocatalysts Ltd**

Nick Le Brun, Myles Cheesman, Jonathon Todd & Jason Crack, University of East Anglia; Mark Blight & Jon Wood, Biocatalysts Ltd

OUTCOMES: Our initial set of studies showed that the amount of cytochrome $P450_{BM3}$ recovered from *E. coli* cell lysates was low and that only 56% of the P450 contained heme. Moreover, $P450_{BM3}$ existed as truncated protein; most likely due to proteolysis in the cell lysates. We then explored several different strategies to improve heme incorporation into several heme-containing proteins, including over-expression of parts of the heme biosynthetic pathway and the expression of membrane-bound importers capable of taking up intact heme from the growth medium. Using *E. coli* (a workhorse for protein production), we generated a system that gives ~3-fold enhancement of heme incorporation into two very different 'test' heme-containing proteins (*E. coli* bacterioferritin and human myoglobin). Preliminary results with a third 'test' protein indicated that the effect is reproducible for other heme-containing proteins. To date, we have not seen any negative effects on yield of protein. These are all important properties for a process that could be commercially useful.

A) Cartoon structure of human myoglobin showing the heme group with an iron atom at its centre. **B**) *E. coli* cell pellets. The two tubes on the left show pellets of cultures without the heme enhancement system; the two on the right with it. Each pair shows before and after induction of heme protein production.



INITIAL AIMS: Many metalloproteins have the potential to be used as biocatalysts in the synthesis of useful materials or medicines. However, to exploit this the metalloprotein must be purified with its metallo-cofactor fully incorporated to avoid major inefficiencies in the production process. A strategy to improve cofactor insertion is to more carefully match protein synthesis with cofactor synthesis/insertion. This should be achievable by engineering cell factories to increase their capacity to produce and incorporate the cofactor. In these projects, we will first analyse the level of heme insertion in a commercially relevant metalloprotein, cytochrome P450. We will then generate *E. coli* strains of that have increased capacity to incorporate heme into cytochrome P450, which, together with optimised growth protocols, will be tested for the production by the commercial partner.

- Awarded funding (£12k) from UEA to continue the project
- Aim to submit application to BBSRC Follow on Funding Pathfinder scheme
- Aim to test increased heme incorporation into wider range of proteins