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Bioprocess engineering for the application of P450s

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The highly specific hydroxylation performed by P450 monooxygenases is a very powerful tool for synthetic chemists, not only at laboratory scale, but potentially also at industrial scale. However, despite this potential, only in a few cases has this class of enzymes been implemented at an industrial scale. In the case of P450s, the requirements for cofactor and electron transporting redox partner, coupled with conversion of hydrophobic substrates to hydrophobic products already set some constraints. In order to enable focused and directed improvement of the biocatalyst (and process), such limitations need to be quantified via carefully designed experiments. In this presentation, we will report the results of a hypothesis driven experimental approach to characterization, with the aim of quantifying the limitations associated with cofactor regeneration, inhibition, toxicity and trans-membrane transport. Selected test reactions and biocatalysts made available within the EC FP7 research program P4FIFTY have been used for the study, including CYP153A[1], CYP102A1[2] expressed in *Escherichia coli* and CYP106A2[3] expressed in *Bacillus megaterium*. Common limitations have been found to be the stability of the biocatalyst, as well as substrate inhibition and toxicity. These limitations will influence what we have reported as typical targets necessary to implement a commercially feasible process (reaction yield, biocatalyst yield, final product concentration and space-time yield). The analysis reveals that while further improvements are required to reach the targets, the remaining limitations should ultimately be possible to overcome. Such a process analysis tool can in principle be applied to many biocatalytic systems and it is hoped that in the future it will help to enable accelerated biocatalytic process development.

[1] Scheps, D., Honda Malca, S., Richter, S. M., Marisch, K. *et al.*, Synthesis of ω -hydroxy dodecanoic acid based on an engineered CYP153A fusion construct. *Microbial biotechnology* 2013, 6, 694-707.

[2] Lewis, J. C., Arnold, F. H., Catalysts on demand: selective oxidations by laboratory-evolved cytochrome P450 BM3. *CHIMIA International Journal for Chemistry* 2009, 63, 309-312.

[3] Bleif, S., Hannemann, F., Zapp, J., Hartmann, D. *et al.*, A new *Bacillus megaterium* whole-cell catalyst for the hydroxylation of the pentacyclic triterpene 11-keto- β -boswellic acid (KBA) based on a recombinant cytochrome P450 system. *Appl. Microbiol. Biotechnol.* 2012, 93, 1135-1146.