E3B The Elements of Bioremediation, Biomanufacturing & Bioenergy Metals in Biology

Durham University · University of Kent · Manchester 1824 The University of Manchester · UKRI Biotechnology and Biological Sciences Research Council

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FLAVH2: Establishing the feasibility of a metalloenzyme system for dihydrogen-driven flavin recycling for chemical synthesis

"The project demonstrated the feasibility of running biocatalytic reactions much more cleanly and sustainably by using H_2 gas to drive the reduction of flavin cofactors. This development is very timely, as sustainability and lowering energy demands become key points of focus for the chemical industry." Johnson Matthey

PROJECT AIMS: Hydrogenases are enzymes that uses a nickel–iron cluster to split hydrogen gas, and clusters of iron atoms to conduct electrons. A technology previously developed by the PI and colleagues exploits hydrogenases to recharge flavin, a co-factor that is needed by enzymes used as industrial biocatalysts. Their technology, termed FLAVH2, provides a simple and clean way to recharge flavin with hydrogen. The aims of this project were to:

Aim 1: Test the FLAVH2 recycling system on Johnson Matthey's ene-reductases, and make comparisons with their conventional glucose/glucose dehydrogenase (GDH) /NADPH-driven reactions.

Aim 2: Test the FLAVH2 recycling system on a halogenase in collaboration with the University of Manchester to prove that the recycling system works under safe H_2/air and can be used with halogenases.

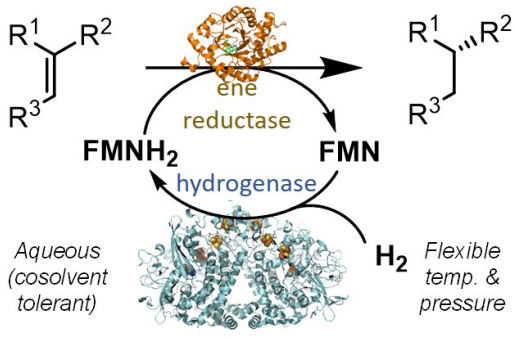
RESULTS: Aim 1: The FLAVH2 technology was able to drive Johnson Matthey's commercial ene-reductases and nitro reductases, and the activity was benchmarked against the GDH/NAD(P)H system which the company currently uses for supplying reductant to these enzymes. Johnson Matthey were particularly interested in the use of FLAVH2 technology with nitro reductases, since removing the need for glucose will greatly impact the economics and sustainability of the reaction. The reactions were easily implemented in batch/fed-batch reactors, and there was full conversion at 60 mM substrate for an ene-reduction, indicating good progress towards industrial application.

Aim 2: Academic collaborator Jason Micklefield from the University of Manchester supplied plasmids to express halogenase and the requisite reductase. These enzymes were successfully expressed in the Oxford lab, and a student is working to take this part of the project forward.

Change in technology readiness level: 2 to 3

OUTCOMES & NEXT STEPS:

- Secured further funding for a 'Technology Translator' postdoc through the Bath–Oxford iCAST sustainable chemistry network to take the nitro reduction work forward to licence-ready stage.
- A DPhil student with funding from their home country will continue the halogenase work.
- An MChem student will expand the scope of the ene-reductase work, and to scale this into flow reactors with a new academic collaborator.
- Publication: Srinivasan, SJ et al., *E. coli* nickel-iron hydrogenase 1 catalyses non-native reduction of flavins: demonstration for alkene hydrogenation by old yellow enzyme ene-reductases. *Angew. Chemie Int. Ed.*, 2021, 60, 13824-13828; https://doi.org/10.1002/anie.202101186
- Patent application: PCT/GB2021/051000 includes examples of enzyme reactions and substrates from this project.



New enzyme activity, multi-day stability

The scope for sustainable industrial biotechnology is expanded by a H_2-driven system for recycling reduced-flavin cofactors, which can then be supplied as reductants for C=C and nitro reductions in the synthesis of active pharmaceutical ingredients.