PROJECT PARTNERS: Simone Morra, University of Nottingham; Holly Reeve and Sarah Cleary, HydRegen

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# Expanding the biocatalytic hydrogenation toolbox with [FeFe]-hydrogenases

**"To understand the industrial potential of new specialist enzymes, we used the expertise of our academic partner to carry out more detailed studies. We hope to continue this relationship and build on these exciting results in the future," HydRegen**

PROJECT AIMS: The industrial partner HydRegen develops biocatalytic alternatives to traditional precious-metal catalysts for hydrogenation reactions. Their current technology uses [NiFe]-hydrogenases to catalyse $H_2$ oxidation reactions. A different class of hydrogenases, [FeFe]-hydrogenases are easier to scale-up than [NiFe]-hydrogenases, but the majority of [FeFe]-hydrogenases are quickly and irreversibly inactivated by oxygen. This feature makes them impractical for use in industrial applications. CbA5H is a recently isolated [FeFe]-hydrogenase that is $O_2$-tolerant and has been characterised by the academic partner. This project will evaluate the potential of CbA5H in biocatalysis.

**OUTCOMES & NEXT STEPS:**

- A proof-of-concept application has been awarded (POCE3B034) to extend the present results, focusing on the scale-up and industrial aspects.
- Innovate UK funding of £365k (Investment Accelerator) moves this project from majority academic to joint development.
- UKRI feasibility funding of £93k for project 'Intensification of metal-enzyme production to unlock sustainable biocatalytic hydrogenation'
- A BBSRC New Investigator application is in preparation.
- Data will be presented at the International Hydrogenase conference in 2023.
- A publication will be prepared when additional data are available.

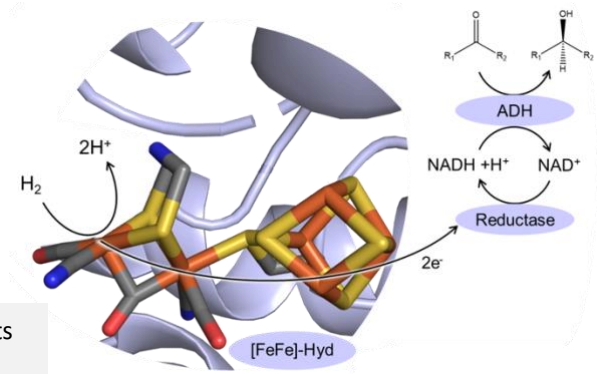
RESULTS: During this project, the recombinant overproduction of CbA5H was improved 10-fold over previously published yields. This was achieved by subcloning the CbA5H gene in a new expression vector and co-expressing it with HydEFG maturases while further optimising the expression conditions. As anticipated, the enzyme retained high specific activity when purified aerobically.

A simple adsorption protocol was developed to immobilise CbA5H on carbon particles (a key step in HydRegen's technology). Given CbA5H's tolerance to oxygen, this could be carried out on the laboratory bench without the need to work under inert gas.

The immobilised [FeFe]-hydrogenase was combined with HydRegen's proprietary cascade enzymes (a $NAD^+$ reductase and an alcohol dehydrogenase) and tested in a standard reaction assay. The results showed that CbA5H allowed full conversion of a ketone to a chiral secondary alcohol.

These results demonstrate that CbA5H has the potential to be exploited in biocatalysis.

Change in technology readiness level: 1 to 3




The novel CbA5H [FeFe]-hydrogenase supports biocatalytic cascades.