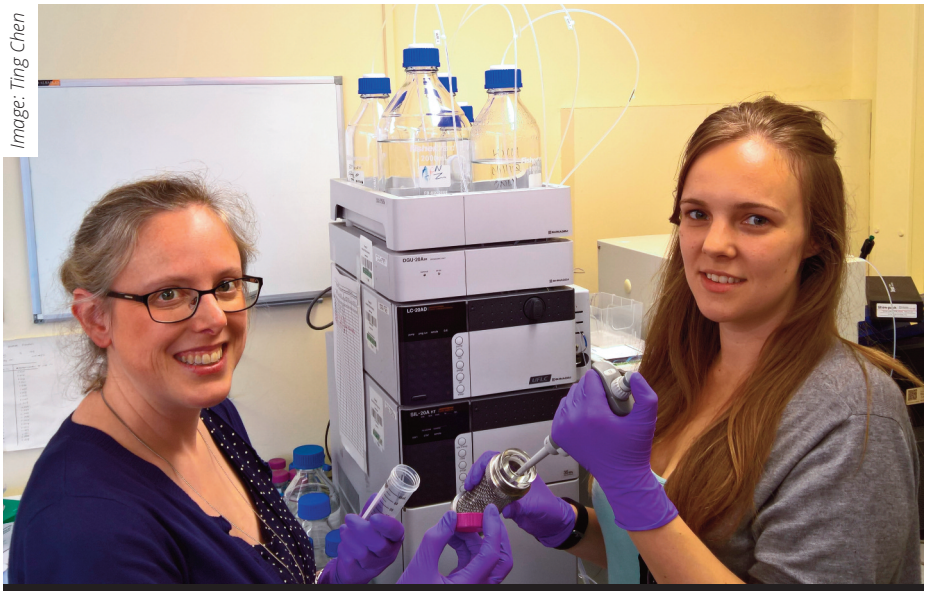
A collaborative journey to greener chemicals

Kylie Vincent, Holly Reeve and colleagues at Oxford University are working on ways to make greener chemicals. With government funding, the technology could soon be used in industry...

We are working on greener ways to make chemicals that are used in medicines, food and cosmetics by using enzymes that are found in nature – an area of research known as industrial biotechnology. Our group came to work in industrial biocatalysis in a roundabout way. We were studying types of bacteria that use the very small amounts of hydrogen gas found in the environment as 'food' or energy. To do so, these bacteria contain enzymes – hydrogenases – in their cells which use metal ions to split apart the hydrogen molecule. We were interested in how nature uses metal ions to enable, or catalyse, the split of the hydrogen molecule to better understand how we could make use of this reaction in the lab.

In the course of this work, we carried out collaborative experiments with Dr Oliver Lenz's group at the Technical University of Berlin into a special hydrogenase that uses hydrogen as a fuel to drive the production of a biological molecule called nicotinamide adenine dinucleotide hydride (NADH) [1]. NADH is an important energy currency inside cells, and is required for the function of many types of enzymes. In particular, NADH is essential to the types of enzymes that are gaining attention for their ability to make complicated chemicals such as pharmaceuticals, or flavour or fragrance chemicals in greener ways than in the past.

Image: Ting Chen



Kylie Vincent and Holly Reeve

Although the use of enzymes as biocatalysts in chemical synthesis is really taking off, a key challenge is developing an efficient supply of NADH. We quickly realised that we could make use of components from bacterial cells to assemble a system for recycling NADH that could be used outside of the bacterial cell by incorporating some of the cell components onto cheap carbon beads. The use of hydrogen gas as the energy source to drive the chemistry gives a much cleaner way of supplying the NADH for enzyme-catalysed chemical synthesis [2].

We developed this into a technology that we call HydRegen (short for hydrogen-driven regeneration). We were able to show quite quickly that our HydRegen beads work with a wide range of NADH-dependent enzyme reactions. This was a key finding, as it increases the potential applications of the technology. We filed a patent application with support from Oxford University's Technology Transfer company, Oxford University Innovation, and began talking to some of the companies that are interested in using NADH-dependent enzymes to make complex chemicals.

One of the questions we were frequently asked was whether we could extend to the related biological cofactor NADPH (nicotinamide adenine dinucleotide phosphate), as this is essential to many other industrially relevant enzymes. However, NADPH presents even more of a headache in industrial applications of enzymes as it is more expensive and less stable than NADH. After winning the prestigious Royal Society of Chemistry's Emerging Technologies Competition

in 2013, we were mentored by Dr Ian Churcher from the pharmaceutical company GlaxoSmithKline, who helped to advise us on development of the technology.

GlaxoSmithKline were natural partners as we started to explore the scope for extending our HydRegen beads to NADPH in an industrial setting. Together, we secured short-term funding in the form of a Business Interaction Voucher from the Metals in Biology BBSRC NIBB, to test whether we could produce enough NADPH to drive some NADPH-dependent enzymes that GlaxoSmithKline are interested in. The project was successful, and provided real proof of the versatility of the HydRegen technology.

However, major research challenges remain. The two enzymes we use are excellent biocatalysts – the hydrogenase enzyme splits the hydrogen molecule, causing electron transfer through the electronically conductive carbon beads to support the recycling of NADH (or NADPH) at the other enzyme. But they are complex metalloenzymes that can only be produced in bacterial cells.

Although making these enzymes for use in the lab is now relatively straightforward, making them on an industrial scale remains unchartered territory. We still need to find the best ways to make the bacteria produce these enzymes in high quantities, the minimal steps needed to isolate the enzymes, and the best ways to handle them once they are separated from the cells. To address these research challenges, we applied to a government funding scheme called the Industrial Biotechnology Catalyst scheme (run through the BBSRC/EPSRC/Innovate UK), and in January 2016 we were awarded £2.9 million of funding for a major research programme to explore and de-risk the scalability of the HydRegen technology.

We have assembled an industrial advisory board, taking advantage of industry contacts we have made along the way at Oxford University-led meetings, at Metals in Biology BBSRC NIBB meetings, and with help from Oxford's technology transfer team. The advisory board is helping to keep the project focussed on addressing real industry challenges in biotechnology.

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The Industrial Biotechnology Catalyst-funded project is now up and running successfully, and one of the most exciting parts of the first six months has been seeing the project generate new fundamental insight into the enzyme systems and new patentable discoveries, as well as advances in the core HydRegen technology itself. We have also continued to make industrial contacts and develop our commercialisation strategy in conjunction with Oxford University Innovation.

We have always enjoyed the interplay between detailed studies of enzyme function and development of applications of enzyme biocatalysis. In parallel to the Industrial Biotechnology Catalyst project, we continue to work on fundamental studies into hydrogenase enzymes, and to develop new tools for studying metalloenzymes, supported by BBSRC responsive mode grants on which we are co-investigators [3]. Our strengthened understanding of how hydrogenase enzymes work, and the accumulation of related know-how in the group continues to feed very productively into our development of the HydRegen technology.

Indeed, the Industrial Biotechnology Catalyst funding should help this technology to cross the so-called 'valley of death' that often impedes early-stage technologies. With this funding, and our additional grants, we hope to bring the HydRegen system to market in the next 3-4 years.

1 Lauterbach, L., Idris, Z., Vincent, K.A., Lenz, O. 'Catalytic properties of the isolated diaphorase fragment of the NAD^+-reducing [NiFe]-hydrogenase from *Ralstonia eutropha*' PLoS ONE, 2011, 6, (10): e25939.

2 Reeve, H.A., Lauterbach, L., Lenz, O., Vincent, K.A. 'Enzyme-modified particles for selective bio-catalytic hydrogenation via H_2-driven NADH recycling' ChemCatChem, 2015, 7, 3480-3487.

3 Hidalgo, R., Ash, P.A., Healy, A.J., Vincent, K.A. 'Infrared spectroscopy during electrocatalytic turnover reveals the Ni-L active site state during H_2 oxidation by a NiFe hydrogenase' Angew. Chemie. Int. Ed. 2015, 54, 7110-7113.

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