**BBSRC MIBTP Studentship Project**

**September 2023**

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| **Project Title** | Dual approach to develop new catalytic systems for energy conversion: exploring natural and de novo protein scaffolds combined with synthetic cofactors |
| **Project Summary**  |
| **Background:** In the future we will depend solely on renewable energy (sun, wind, biomass, etc.), CO2 and H2O to generate and store energy. To aid this transition, efficient catalysts and new catalytic routes for energy conversion reactions based on abundant and cheap materials are needed. Solutions to the energy problem require understanding of how energy conversion processes happen in nature and of what it takes to make catalytic systems as proficient as the natural biocatalytic systems (i.e. energy converting enzymes), while maintaining the relative simplicity of a molecular catalyst or catalytic material.Energy converting enzymes have evolved for billions of years resulting in earth-abundant metal active-sites perfectly optimised by the inner and outer coordination spheres in the protein matrix. They are able to catalyse key chemical reactions with very high rates and minimum energy waste. These reactions still represent enormous challenges for industry (e.g. H2 conversion, reduction of N2 to ammonia, and reduction of CO2 to CO or formate).**Objectives:** This project is in an exciting emerging area of biotechnology where naturally occurring and de novo protein scaffolds are combined with synthetic active-site cofactors to generate semi-synthetic and artificial enzymes with new catalytic reactivities. The project combines the strengths of synthetic chemistry, natural enzymes and de novo protein design to develop a new set of enzymes with biotechnological potential. Artificial enzymes consist of a simple synthetic molecular catalyst hosted in a protein scaffold that confers stability, efficiency and specificity. Importantly, the amino acid residues in the 1st and 2nd coordination-spheres can be engineered to tune the catalytic properties of the catalyst. Recent advances allow high yield (~1 g/L) production of small, stable, and easy to purify and characterise scaffold proteins such as the [FeFe] hydrogenase – one of the fastest and most efficient H2-converting catalyst in nature. These enzymes can be produced in E. coli in a form lacking part of the active site, which can then be reconstituted with synthetic cofactors to produce fully active enzymes. This strategy enables any hydrogenase of interest to be produced and reconstituted with a wide variety of synthetic cofactors. This project aims to:* Generate semi-synthetic [FeFe] hydrogenases by combining hydrogenase scaffolds with synthetic cofactors
* Identify exotic [FeFe] hydrogenase scaffolds from extremophiles (e.g. thermophiles, acidophiles and piezophiles)
* Identify crucial amino acid residues for catalysis in the 1st/2nd coordination-spheres to design de novo protein scaffolds
* Generate artificial enzymes by combining de novo protein scaffolds with synthetic cofactors

**Methodology**: Molecular biology will be used to introduce the hydrogenase genes into E. coli to produce and isolate the desire hydrogenase scaffolds. Successful incorporation of the synthetic cofactors into the protein scaffold will be examined via infrared (IR) spectroscopy of the CN- and CO ligands present in the synthetic cofactors. Native and novel reactivity will be assessed using coupled assays and electrochemistry. Hydrogenases will be engineered by site-directed mutagenesis. De novo design and synthesis of protein scaffolds will be performed in the laboratory of Dr Anna Peacock in the School of Chemistry at the University of Birmingham. X-ray crystallography (using facilities in LISCB) will be used to solve structures of interesting semi-synthetic and artificial enzymes. Synthetic cofactors will be synthesised in collaboration with Dr (School of Chemistry, University Sandy Kilpatrick of Leicester).Techniques that will be undertaken during the project:* Molecular biology techniques (this includes, heterologous protein expression in E. coli, protein purification, genetic modification via site-directed mutagenesis, subcloning, in vitro reconstitution of proteins)
* De novo protein design and synthesis
* Spectroscopy: infrared (IR) spectroscopy and electron paramagnetic resonance (EPR)
* Analytical techniques: Electrochemistry
* Structural biology: crystallisation, X-ray crystallography, ab initio structure prediction

BBSRC Strategic Research Priority: Renewable Resources and Clean Growth – Bio-energyUnderstanding the Rules of Life – Structural Biology |
| **References** |
| 1. M. Lorenzi et al., Chem. Sci., 2022,13, 11058-110642. A. F. A. Peacock et al., Curr. Opin. Chem. Biol. 2013, 6, 934-939 |