**University of Leicester**

**BBSRC MIBTP Studentship Project 2025-6 entry.**

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**Section 2 – *Project Information***

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| **Project Title** | New Approaches to Investigate Substrate Binding and Reaction Mechanism of CO2-reducing Enzymes  |
| **Project Summary**  |
| **Background:** Global warming caused by rising levels of greenhouse gases, especially CO2 is a major global concern. Sequestration and reduction of atmospheric CO2 is central for limiting global warming and providing a non-petrochemical route to chemical building blocks such as CO, a key feedstock for use in downstream industrial applications. Therefore, it is essential to develop eco-friendly and cost-effective catalysts for CO2 conversion into chemical feedstocks, i.e. catalysts based on abundant and cheap metals. Nevertheless, catalysts for CO2 reduction, particularly in aqueous media, remain a major challenge to design mainly due to selectivity problems. To help design and develop efficient and selective catalysts for CO2 conversion, a fundamental understanding of how CO2 fixation happens in nature is required in order to understand how we can make synthetic catalytic systems with activities, selectivity and efficiencies as high as the biocatalysts. As such, studying biocatalytic CO2 reduction offers enormous potential for guiding the design of novel catalysts needed for a sustainable circular energy economy to help close the CO2 cycle. Only two enzymes are known to catalyse the direct reduction of CO2 in nature: CO dehydrogenases (CODH), which catalyse the reduction of CO2 to CO and formate dehydrogenases (FDH), which catalyse the reduction of CO2 to formate (HCOO-).Both of these enzymes participate in the Wood-Ljungdahl pathway, a major circular CO2 fixation pathway in anaerobic organisms, and work under physiological conditions with very high activities, selectivity and reversibility/efficiency. Ni-containing CODHs contain a unique [Ni–4Fe–4S] active site, while metal-dependent (Mo- or W-containing) FDHs active site contains a hexacoordinated metal ion (Mo or W) in a distorted triangular prismatic geometry with two di­thiol­ene ligands, a cysteine (Cys) or seleno­cysteine (SeCys) residue and an inorganic sulfide that is necessary for activity. Apart from the active site, both enzymes contain gas channels for the substrates (i.e. CO2 and CO) to diffuse in and out the active site, proton pathways and iron-sulfur clusters as electron relays.  **Objectives:** This exciting project will focus on the structural and biophysical characterisation of natural CO2-reducing enzymes, CODH and FDH enzymes, by developing new approaches to study substrate binding at the active site and its mechanism of conversion. Structural characterisation under different conditions will be achieved via cryo-EM. New approaches for cryo-EM grid preparation under anaerobic and substrate-controlled conditions will be developed with the long-term goal of coupling cryo-EM with electrochemical control to poise the enzymes into well-defined catalytic states in the presence/absence of the substrate. Cryo-EM will be complemented with electrochemistry and spectroscopic and computational methods to gain a holistic understanding of how CO2 binds at the active site of these enzymes and is converted to C1-products. This is an interdisciplinary project encompassing molecular and structural biology, chemistry and biophysics. Novel methodologies and approaches will be developed to provide crucial insights into these fascinating CO2-reducing enzymes, and to reveal design principles toward the development of (bio)hybrid catalysts for CO2 reduction.   **Methodology:** Molecular biology will be used to introduce the CODH and FDH genes into *E. coli* to recombinantly produce and isolate the desire enzymes. Biochemical characterisation will be performed in parallel to structural characterisation via cryo-EM. The enzymes will be prepared in different catalytic states in the presence and absence of the substrate and their cryo-EM structures will be solved. The catalytic activity of the produced enzymes will be assessed using coupled assays and electrochemistry. To stabilise the substrate-bound state, the enzymes will be engineered by site-directed mutagenesis.  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)
* Spectroscopy: infrared (IR) spectroscopy and electron paramagnetic resonance (EPR)
* Analytical techniques: Electrochemistry
* Structural biology: crystallisation, Cryo-EM, *ab initio* structure prediction
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| **References** |
| Y. Basak *et al., Angew Chem Int Ed Engl* **2023**, *62*, 32, e202305341  Y. Basak *et al., ACS Catal.* **2022**, *12*, 20, 12711–12719  J. Park *et al.,* *Scientific Reports* **2024**, 14, 3819 |