**BBSRC MIBTP Studentship Project**

**September 2023**

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| **Project Title** | Unravelling the roles of redox chemistry and electronic structure in determining heme reactivity |
| **Project Summary**  |
| Redox properties of metal-containing active sites are critically important to many biocatalytic processes: one third of all proteins contain a redox-active metal, and ca 22% of submissions to the Protein Data Bank contain a transition metal. Metalloproteins capable of sequestering CO2 from the atmosphere, extracting energy from H2 gas, or performing complex monooxygenation reactions, rely upon the ability to access and control a range of often exotic metal oxidation states in an aqueous environment. Heme proteins are an essential component of all life, with a wide range of roles from O2 and electron transport to drug metabolism. Heme peroxidases allow biological systems to use O2 by removing the damaging products of its metabolism. Key to understanding the mechanisms of these enzymes is knowledge of the detailed electronic and coordination states of the iron atom during catalysis, which are notoriously difficult to study. As a consequence, debate even remains over the nature of O2 binding, and iron oxidation state, in apparently well-understood systems such as oxy-myoglobin and -hemoglobin. X-ray and neutron crystallography have been used to study intermediate oxygen-bound Fe(IV) states in Cytochrome c peroxidase and Ascorbate peroxidase, but it has become increasingly clear that Fe−O bond distances derived from these studies provide only a very limited indicator of the structure of these critical intermediates. In this project, you will use a combination of structural and spectroscopic methods to unravel details of transient intermediates in a range of native and mutant heme proteins. By using stopped flow and freeze quench methods you will trap specific intermediates for interrogation by advanced X-ray and optical spectroscopies. In conjunction with computational modelling this will reveal the electronic structural information that underpins the biological role of each protein. The results of this project will provide insight into why heme proteins with otherwise near-identical structures exhibit huge variety in their reactivity with O2.Techniques that will be undertaken during the project:* Molecular biology (cloning and mutagenesis)
* Protein expression and purification
* Protein crystallisation
* Computational chemistry (DFT, QM-MM)
* X-ray spectroscopy
* Electrochemistry
* Infrared spectroscopy
* Raman spectroscopy
* Uv-visible spectroscopy
* Single crystal spectroscopy
* Stopped-flow and freeze-quench kinetics
* Structure determination (X-ray crystallography and related techniques)

BBSRC Strategic Research Priority: Understanding the Rules of Life - Structural Biology |
| **References** |
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