****

**PhD Project Commencing October 2023**

**(Closing date: 5 January 2023)**

**Principal Supervisor:** [**Judy Hirst**](https://www.mrc-mbu.cam.ac.uk/research-groups/hirst-group)(enquiries to: jh@mrc-mbu.cam.ac.uk)

**Using cryoEM to capture intermediate states in complex I catalysis**

Complex I (NADH:ubiquinone oxidoreductase) is an intricate ~1 MDa multimeric membrane-bound complex that is essential for mitochondrial metabolism: its dysfunctions lead to neuromuscular and metabolic diseases and it is implicated in ischaemia-reperfusion injury.

Single-particle electron cryomicroscopy (cryoEM) has enabled high resolution structures of respiratory complex I to be determined from both mammalian mitochondria and model species [for example see [1]) – but structures of the resting, wild-type enzyme are not sufficient to reveal the mechanism of catalysis or the conformational states that the enzyme cycles through.

This project will focus on exploiting a powerful genetically-tractable bacterial model system for complex I (*Paracoccus denitrificans*) [2] to define the structures of catalytic intermediates in complex I catalysis. The enzyme will be reconstituted into nanodiscs [3] to stabilise it and mimic its native membrane environment, and trapped in intermediate states either by freezing samples during turnover, or by using mutations to stop the catalytic cycle.

The student working on this project will learn to use single-particle cryoEM to determine high-resolution structures of complex I, and to model and interpret the data, as well as to isolate and characterise membrane-bound proteins. As the project develops there will be opportunities to contribute to developing biochemical and cryoEM sample preparation methods and/or to design, create and characterise new genetic variants. The project therefore represents an exciting opportunity to develop research skills and expertise in both biochemical methods and computational analyses.

**Keywords**

General:

cryoEM, enzyme, mechanism, structure, genetics

More specific:

complex I, electron transport, respiratory chain, proton pumping, redox enzyme

**References**

1. Grba, D. N. & Hirst, J. (2020) Mitochondrial complex I structure reveals ordered water molecules for catalysis and proton translocation. *Nature Struct. Mol. Biol.* **27**, 892-900

2. Jarman, O. D., Biner, O., Wright, J. J. & Hirst, J. (2021) Paracoccus denitrificans: a genetically tractable model system for studying respiratory complex I. *Sci. Rep.* **11**, 10143

3. Chung, I., Wright, J. J., Bridges, H. R., Ivanov, B. S., Biner, O., Pereira, C. S., Arantes, G. M. & Hirst, J. (2022) Cryo-EM structures define ubiquinone-10 binding to mitochondrial complex I and conformational transitions accompanying Q-site occupancy. *Nature Commun.* **13**, 2758.

**Subject areas**

Biochemistry, Molecular Biology, Structural Biology

**How to apply:** please visit the[MBU's Postgraduate Studies website](https://www.mrc-mbu.cam.ac.uk/postgraduate-studies)