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**PhD Project Commencing October 2024**

**(Closing date: 14 March 2024)**

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**Combining cryoEM and mutagenesis to determine how respiratory complex I works**

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 interrogate the mechanism of catalysis. The enzyme will be reconstituted into nanodiscs [3] to stabilise it and mimic its native membrane environment, allowing us to combine mutagenesis, structural (cryoEM) and functional studies. CryoEM will be essential to understand the molecular effects of mutations that disrupt or interrupt the catalytic cycle.

The student working on this project will learn to design, create and characterise new genetic variants and to isolate and characterise membrane-bound proteins. They will also use single-particle cryoEM to determine high-resolution structures of complex I, and to model and interpret the data. The project therefore represents an exciting opportunity to contribute to answering a challenging research question, and to develop research skills and expertise in both biochemical methods and structural 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. doi:10.1038/s41594-020-0473-x
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. doi: 10.1038/s41598-021-89575-9
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. doi:10.1038/s41467-022-30506-1

**Subject areas**

Biochemistry, Molecular Biology, Structural Biology

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