Project Details	
Project Code	MRCNMH25Ex Gold
Title	The consequence of mitochondrial import failure in neurogenerative disease
Research Theme	Neuroscience & Mental Health
Summary	Mitochondria provide energy essential for life and are dependent on the delivery of proteins from the cytosol for their biogenesis, maintenance and regeneration. Defects in protein import affect cellular bioenergetics and thereby cause muscular, neurological and age-related degenerative diseases. In this project, the effects of defective protein import will be studied at both the cellular and protein structure level using state-of-the-art methodologies including CRISPR-based genome editing of human cells for cryo-electron microscopy. The results will boost understanding of how mitochondrial protein import is essential for mitochondrial homeostasis and health, and how import failure relates to disease.
Description	**Background:** According to The Endosymbiotic Theory, mitochondria arose due to the intracellular enslavement of bacteria. During eukaryotic evolution, the mitochondrial genome was significantly reduced, leading to a dependence on cytosolic protein synthesis, protein targeting and import for >99% of mitochondrial proteins. Mitochondrial protein import machinery in the outer and inner membranes evolved to facilitate mitochondrial biogenesis. The Translocase of the Outer Membrane (TOM) complex serves as the entry gate for most mitochondrial proteins, with pathways diverging depending on the protein's destination. Mitochondrial dysfunction, a hallmark of neurodegeneration, is increasingly linked to protein import machinery malfunctions, with catastrophic effects on health (1). The Gold lab has developed methods to visualise protein import on mitochondria by cryo-electron tomography (cryoET) (2–4) and has investigated neurodegenerative-related mitochondrial changes using the same methodology (5). In Alzheimer's disease, amyloid precursor protein (APP) accumulates within mitochondria, interacting with both outer and inner membrane protein import machinery (6). Similarly, mitochondria from Huntington's disease brains exhibit aggregation-prone Huntingtin protein (Htt) variants that inhibit import at the mitochondrial inner membrane (7). The Collinson lab has shown that a Tau protein associated with Alzheimer's Disease interacts with the TOM complex (8), disrupting import and reducing neuronal complexity (e.g. less branching and fewer synapses). Protein import is also crucial for mitochondrial quality control. In healthy cells, respiratory chain complexes of the mitochondrial inner membrane generate a membrane potential and ultimately production of ATP. A collaboration between the Gold and Collinson labs has revealed large-scale structural changes in respiratory chain proteins in a model representing ageing and age-related neurodegenerative disease (9,10). In cells with a compromised membrane potential, the mitochondrial-targeted kinase PINK1 accumulates at the TOM complex, where it is recognised by Parkin (9), which ultimately flags mitochondria for

	removal by mitophagy. Several PINK1 mutations are linked to early onset Parkinson's Disease (11). Whilst numerous studies have linked import dysfunction and impaired ATP production to neurodegenerative disease, the impact on mitochondrial ultrastructure and respiratory chain proteins remains unclear. Building on our existing collaboration, understanding the links between protein import and the structure and arrangement of respiratory chain proteins will provide further insight into the etiology of neurodegenerative diseases. **The key questions for this project are:** 1. What is the consequence of mitochondrial import failure on the mitochondrial network, mitochondrial ultrastructure and respiratory chain protein structures? 2. How do these changes compare to mitochondrial abnormalities seen in various neurodegenerative diseases? **Objectives:** We will work with established human cell culture that has been used as a model to measure the effects of import failure in the Collinson lab (12). Cells will be transfected and cultured using established conditions designed to perturb the protein import machinery (8,12). This will include the expression of protein trapping constructs that arrest during protein import, as well as the production of aggregation prone proteins linked to neurodegeneration. Analysis of compromised HeLa cells by fluorescence microscopy revealed that both the protein trap and the aggregation-prone Tau caused similar effects, namely mitochondrial fragmentation (8,12). This studentship aims to advance the analysis of import-compromised HeLa cells by transitioning to structural cell biology, enabling a higher resolution examination of the impact on mitochondrial morphology. We will leverage use of brand-new technology that will shortly become available through a successful GW4 application to UKRI-BBSRC for a cryoFIB-SEM (1 of only a handful in the UK). Cryo-FIB milling of specimens that would otherwise be too thick to image by cryoET (e.g. cells and tissues) generates thin lamella suitable for subsequent imaging at high-resolution. The results will provide an extraordinary in situ view facilitating an investigation of the effects of import machinery perturbation on mitochondrial network formation, ultrastructure, and proteins of the respiratory chain. This project offers outstanding opportunities for training within the MRC cross-cutting themes of data science, interdisciplinary skills and translation and innovation (strategic skills section). **Where the student can steer the project:** The student can design translocation-arrested substrates to focus on an area of neurodegenerative disease according to interest. The team will support the student with ideas development regarding specific mutations or substrates to study. They can revisit this and design additional substrates as the project and their research evolves. References: 1. Lin, M. et al. Nature (2006). 2. Gold, V. et al. Methods Mol Biol (2017). 3. Gold, V., et al. A. EMBO Rep (2017).

	4. Gold, V. et al. Nat Commun (2014). 5. Stockburger, C. et al. J Alz Dis (2014). 6. Devi, L. et al. J Neurosci (2006) 7. Yano, H. et al. Nat Neurosci (2014). 8. Needs, H. I. et al. J Cell Sci (2023). 9. Knapp-Wilson, A. et al. J Cell Sci (2021). 10. Buzzard, E. et al. In preparation. 11. Valente, E. M. et al. Science (2004). 12. Needs, H. I. et al. Nat Commun (202
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