Project Details	
Project Code	MRCNMH26Br Cross
Title	Understanding and reprogramming axonal transport with protein design
Research Theme	NMH
Project Type	Wet lab
Summary	Intracellular transport is essential for organising and maintaining the function of eukaryotic cells. This is particularly important in neurons, where cargo must be moved efficiently over long distances. Transport is driven by kinesin and dynein motor proteins which travel along microtubules to deliver organelles, proteins, and RNAs to precise locations. Disruption of this system is linked to numerous neurological disorders. This PhD will design synthetic protein modulators to achieve acute, isoform specific control of kinesin 1 activity in cells. Precise modulation of kinesin-1 will offer mechanistic insights into neurodegenerative transport defects and may offer routes to new classes of therapeutics.
Description	Intracellular transport is essential for the functioning of all eukaryotic cells, enabling delivery of cargo including organelles, proteins, and RNAs to specific subcellular destinations for critical processes such as cell division, signalling, and growth. This system is particularly important in neurons, where efficient and precise transport along axons is required. Faults in this system are associated with neurodegenerative diseases, including Alzheimer's, Parkinson's, and motor neuron disease. Cargo transport is powered by motor proteins, primarily kinesins and cytoplasmic dynein, which move along intracellular microtubule tracks. These motors convert chemical energy from ATP hydrolysis into mechanical force, driving directional movement of cargo toward the cell centre or periphery. In neurons, kinesin-1 is a key motor that transports cargo from the cell body toward the synapse. There are multiple isoforms of kinesin-1 which are gene variants encoding similar but distinct proteins with specific functions. Despite its central role, tools to precisely control kinesin-1 activity in space and time are lacking, limiting our ability to dissect its function in health and disease. This interdisciplinary PhD project aims to address this gap by developing a new class of synthetic protein tools for isoform-specific control of kinesin-1 in living cells. Drawing on advances in protein design, synthetic biology, and cell biology, the student will engineer molecular switches to study and manipulate neuronal transport with high specificity. **Hypothesis:** Synthetic protein modulators can be rationally designed to achieve isoform-specific control of kinesin-1 activity in living cells, enabling precise dissection of axonal transport mechanisms relevant to neurodegenerative disease. **Objectives:** The project will be organised around three main objectives, designed to develop the student's skills in protein design, molecular and cell biology, while generating valuable new tools to study intracellular transport: 1. De novo design of peptides and proteins to control kinesin-1 activity: Building on our established approach for one isoform of kinesin-1 (KIF5C) (Cross et al. Nature Chemical Biology 2024), the student will insert a designed cell-penetrating-peptide-inducible conformational switch into a regulatory hinge of the other isoforms (KIF5A, KIF5B). This will enable

	isoform-specific, peptide-inducible motor activation across the family. In a parallel approach, the student will design de novo protein binders that stabilise either the active or inhibited states of the endogenous kinesins. These synthetic regulators will be developed using rational and computational strategies, including hotspot grafting, RFdiffusion, Protein MPNN, and Bindcraft. Structural predictions will be evaluated using AlphaFold2/3 and in-house metrics. 2. Validate designed reagents in vitro and in cells: Candidate designs will be expressed and characterised using established biochemical and biophysical methods, including single-molecule FRET, negative-stain EM, and hydrogen-deuterium exchange (with Jonathan Phillips, Exeter). The student will also assess motor motility in reconstituted microtubule-based assays. In parallel, mammalian cells will be used to evaluate subcellular activity of the synthetic regulators. Quantitative phenotypes will include perinuclear accumulation or peripheral dispersion of organelles such as endosomes, lysosomes, and mitochondria, assessed by live-cell imaging. These are well-established proxies for kinesin activity. For KIF5C, validation steps are already optimised and can be initiated from the start. As the project progresses, the student could introduce engineered modifications into endogenous kinesin loci using CRISPR genome editing. This will enable specific activation or inhibition of native proteins using synthetic peptides. 3. Apply synthetic regulators in neurons to investigate kinesin-1 function: The student will use these tools in iPSC-derived neurons to dissect how kinesin-1 isoforms contribute to axonal transport. By selectively activating or inhibiting individual family members, the student will identify which motors are required for the transport of specific cargo. Live-cell imaging will be used to track fluorescent markers of organelles. The student will assess cargo distribution within axons, dendrites, and the soma, and analyse speed, run length, pause frequency, and directionality. It remains unclear which kinesin isoforms move which cargoes so these tools will enable unprecedented insight into their distinct roles in neurons. This project offers a strong foundation for scientific training and independence. The early stages will focus on developing modulators for KIF5A and KIF5B using established protocols for KIF5C, enabling rapid progress. As the work evolves, the student will be encouraged to shape the project's direction, advancing new peptide design strategies, choosing cargos to study, and exploring connections to disease-relevant mutations. The student will receive training across a broad range of techniques, including: • Rational and computational protein design and engineering • Recombinant protein expression, purification, and biophysical characterisation • Mammalian cell culture, CRISPR editing, neuronal biology and live-cell imaging The student will be embedded in an interdisciplinary team with expertise spanning synthetic biology, structural biology, and cell biology. This environment will support creative exploration, critical thinking, and the development of a versatile research skillset. This PhD will appeal to

	students with interests in synthetic biology, protein engineering, intracellular transport, and the cellular basis of neurodegeneration. By combining cutting-edge protein design with quantitative cell biology, the project offers the opportunity to create powerful new tools for discovery and therapeutic innovation in neuroscience.
Supervisory Team	
Lead Supervisor	
Name	Dr Jessica Cross
Affiliation	Bristol
College/Faculty	Science and Engineering
Department/School	Engineering Mathematics
Email Address	jessica.cross@bristol.ac.uk
Co-Supervisor 1	
Name	Professor Mark Dodding
Affiliation	Bristol
College/Faculty	Health and Life Sciences
Department/School	Biochemistry
Co-Supervisor 2	
Name	Professor Dek Woolfson
Affiliation	Bristol
College/Faculty	Science and Engineering
Department/School	Chemistry
Co-Supervisor 3	
Name	
Affiliation	
College/Faculty	
Department/School	