Project Details	
Project Code	MRCNMH25Ca Mehellou
Title	Unravelling the Pharmacological Activation of PINK1, a Protein Kinase Mutated in Parkinson's Disease
Research Theme	Neuroscience & Mental Health
Summary	Parkinson's disease (PD) is the second most common neurological disease in the world. Current PD therapies and medical interventions are limited to addressing the symptoms of this disease. Thus, there is a need for new and effective treatments for PD. Recently, we discovered molecules that activate a protein kinase called PINK1, which is mutated in early-onset PD. In this project, we now aim to use a multidisciplinary approach, which spans biochemistry, biophysics and cell biology, to understand how these molecules activate PINK1. The advances achieved from this project will facilitate the future discovery of effective treatments for PD.
Description	Mutations in the protein kinase PINK1 cause early-onset Parkinson's disease (PD) in humans.1 These mutations abrogate PINK1's kinase activity2 and prevent the autophagy-mediated degradation of damaged mitochondria (termed mitophagy) leading to neuronal loss. This led us and others to develop PINK1 activators as potential treatments for PD. Such efforts have shown that the pharmacological activation of PINK1 rescues mitochondrial turnover and bioenergetics, and prevent the elevated accumulation of phosphoubiquitin in cells and neurons, a hallmark of idiopathic PD.3, 4 In PD animal models, PINK1 activators caused a decrease in inflammatory markers and α-synuclein pathology, which was accompanied with a rescue of free movement and motor activity.5 Despite the promise of PINK1 activators in treating PD, their exact molecular mechanism of PINK1 activation remains not fully understood. Recent work indicated that these compounds stabilize PINK1 homodimerisation,5 though their binding site on PINK1 is still unknown. In this project, we aim to use a multidisciplinary approach to address this gap in knowledge. **HYPOTHESIS** PINK1 activators are molecular glues that stabilize PINK1 homodimers. **AIMS AND PROJECT PLAN** Aim 1. Identify and verify the binding site of PINK1 activators. Objective 1.1. Synthesise PINK1 covalent binder. Our validated PINK1 activators will be turned into covalent PINK1 activators by attaching covalent warheads that bind to nucleophilic amino acids at the PINK1 binding site. This approach of turning non-covalent inhibitors into covalent ones is well established in the literature and many types of warheads are available. The student will be provided with our PINK1 activators, and through a single chemical reaction, they will be able to turn these into covalent PINK1 binders. Objective 1.2. Label PINK1 with covalent binders and use proteomics to identify their binding site. Recombinant human PINK1 (for which we already have the cDNA plasmid) will be expressed in E.coli and purified as reported. Subsequently, it will be labelled with the generated covalent PINK1 activators, see 1.1 above, as established for the covalent labelling of proteins with small molecules. The labelling efficiency will be

	confirmed by mass spectrometry, and the samples will be digested by trypsin and analysed by proteomics to identify the site(s) on PINK1 that bind the covalent PINK1 activators. Objective 1.3. Verify the binding site of the PINK1 activators. The sites where PINK1 activators bind covalently PINK1 will be mutated. Subsequently, PINK1 wildtype (WT) and the new mutants will be overexpressed in PINK1 knockout (KO) HeLa cells, which we already have in the lab. These will then be treated with our PINK1 activators (non-covalent binders) for 1 h, lysed, and undergo Western blotting for FLAG (PINK1 expression), parkin, parkin S65 (as a readout of PINK1 activation), and GAPDH as a loading control. The mutant(s) which will not be activated by PINK1 activators will be determined as the true site for their binding. Aim 2. Study the influence of PINK1 activators on PINK1 homodimerization and PINK1-dependent mitophagy. Objective 2.1. Establish the impact of PINK1 activators on PINK1 homodimerization in vitro. The PINK1 mutant that abolishes binding to PINK1 activators will be expressed in E.coli and purified by the student. Then, PINK1 WT and the mutant will be incubated with non-covalent PINK1 activators in buffer for 1 h. The samples will be run on a size exclusion column to identify the size of the PINK1 complexes. This will give an insight on whether PINK1 activators induce and stabilize PINK1 WT homodimerization, while not the mutant PINK1 as the activators will not be able to bind to it. Objective 2.2. Study the impact of PINK1 activators on PINK1 homodimerization in cells. We will introduce tags at the C-terminal domain of human PINK1 WT and mutant, so they can be labelled with inorganic fluorophores in cells. We will transfect HeLa PINK1 KO cells with these plasmids, and then label them with the fluorophores. Subsequently, we will use our single molecule imaging techniques to measure the amount of PINK1 monomers and homodimers as well as the kinetics of the dimerization in the presence and absence of PINK1 activators. Objective 2.3. Determine the impact of PINK1 PD-causing mutations on PINK1 dimerization. PINK1 WT and its PD-causing mutants, for which the cDNA clones are commercially available, will be overexpressed in PINK1 KO HeLa cells, and then treated with PINK1 activators. Subsequently, we will employ our single molecule biophysics technique discussed above to determine whether these PINK1 PD-causing mutations prevent PINK1 activation via the inhibition of its homodimerization. We will also run the exact same experiment in mitoQC MEF PINK1 KO to establish the impact of PINK1 PD-causing mutations on PINK1 homodimerization and mitophagy. (1) Valente et al. Science 2004, 304, 1158-1160. (2) Woodroof et al. Open Biol 2011, 1, 110012. (3) Hou et al. Autophagy 2018, 14, 1404-1418. (4) Fiesel et al. EMBO Rep 2015, 16, 1114-1130. (5) Chin et al. BioRxiv 2023. DOI: 10.1101/2023.02.14.528378.

Supervisory Team	
Lead Supervisor	
Name	Dr Youcef Mehellou
Affiliation	Cardiff
College/Faculty	Biomedical and Life Sciences
Department/School	Pharmacy and Pharmaceutical Sciences
Email Address	mehellouy1@cardiff.ac.uk
Co-Supervisor 1	
Name	Professor Jon Lane
Affiliation	Bristol
College/Faculty	Life Sciences
Department/School	Biochemistry
Co-Supervisor 2	
Name	Dr Oliver Castell
Affiliation	Cardiff
College/Faculty	Biomedical and Life Sciences
Department/School	Pharmacy and Pharmaceutical Sciences
Co-Supervisor 3	
Name	
Affiliation	
College/Faculty	
Department/School	