Project Details	
Project Code	MRCNMH24Ca Lloyd-Evans
Title	AI and structure based drug discovery of chaperones for misfolded lysosomal enzymes involved in neurodegenerative disease
Research Theme	Neuroscience & Mental Health
Summary	A major risk gene for Parkinson disease encodes a mis-folded form of the lysosomal enzyme GBA1, whilst other lysosomal enzymes are associated with Alzheimer disease risk and show defective transport to the lysosome. The aim of this project is to use our skills in lysosomal biology and drug discovery (including AI), alongside structural biology, to identify new small molecule chemical chaperones for these enzymes and new therapies for these devastating diseases.
Description	There is increasing overlap between lysosomal storage disorders (the most common cause of childhood brain disease) and neurodegenerative diseases of ageing. Lysosomal enzymes including the glycosphingolipid and lipid hydrolases glucocerebrosidase (GBA1), phospholipase D3 (PLD3) and beta-hexosaminidase (HEXB) or the aspartyl protease cathepsin H (CTSH) are risk genes associated with Parkinson disease (PD) and Alzheimer disease (AD) respectively. In PD, heterozygosity for the mis-folding and endopasmic reticulum (ER) retention N370S or L444P mutations in the GBA1 gene are sufficient to cause the disease. In AD the lysosomal enzyme activities are known to be reduced in the lysosome, suggestive of transport defects,with loss of function of CTSH proposed to be protective against AD. Molecular chaperones are small molecule inhibitor drugs that bind to the enzyme, either temporarily at the active site or at an allosteric modulatory site, and assist in stabilising the enzyme structure, aid ER exit and transport to the lysosome. This both reduces ER stress caused by the accumulation of mis-folded protein and normalises lysosomal function. As a proof of principle, ambroxol, a chaperone of GBA1, has been shown to rescue the function of GBA1 and cellular phenotypes of PD cells and mice. However, the drug fails to cross the human blood brain barrier. As a proof of concept it illustrates how chaperone therapy could be used to revolutionise the treatment for varous forms of PD and AD. We have combined expertise in lysosomal cell biology and enzymology, cellular phenotyping of PD and AD cell lines and iPS neurons, small molecule drug discovery (including AI based pipelines) and glycoside hydrolase structural biology. We aim to consolidate these skills to identify new small molecule chaperones of GBA1 and HEXB, by fragment based screening, classical small molecule screening and virtual AI assisted screening. We will also utilise these methodologies to identify first in-class inhibitors of CTSH, to mimic the protective effects of the loss-of-function mutation. Candidate small molecules will be screened by enzyme assay and hits will be assessed in cell culture models before mechanisms of action are analysed by co-crystallisation and resolution of the structures by X-ray crystallogaphy. Key research question(s): 1. Utilising a combination of AI, structural biology and classical pharmacology approaches can we identify improved blood brain barrier penetrant chaperones for the lysosomal enzymes whose mis-trafficking is associated with PD and AD? 2. Can small molecule inhibitors of CTSH rescue function in AD cell models? Objectives: 1. Identify small molecules for screening using 1) an open

	access AI developed for drug screening, 2) a fragment based screen at Diamond and 3) a classical enzyme screening approach using the FDA approved library available to us at Cardiff. 2. Confirm that the hit compounds from objective 1 are acting directly on the enzymes, including binding (Creoptix Wave/CD) to (CTSH) and stabilisation (thermal denaturation assays) of mutant N370S and L444P GBA1 and HEXB proteins. 3. Confirm that the final hits from objective 2 are able to restore lysosomal transport (GBA1, HEXB) or inhibit (CTSH) the enzymes (by magnetic separation of the organelle) and restore cellular function in iPS-neuron or microglial models of PD (GBA1) or AD (HEXB/CTSH). 4. Determine the site and mechanism of action of the potential chaperones through co-crystallisation and structure solution. We would expect the student to take ownership of the project at various stages. First there will be three different initial screening cascades, all of which will produce hit compounds. The student will make decisions over what to prioritise (with our expert guidance). These hits will be tested against three enzymes involved in PD and AD, it will be for the student to ultimately decide which indication to prioritise and focus on.
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