Project Details	
Project Code	MRCNMH25Ba Licchesi
Title	Targeted protein degradation in CNS disorders
Research Theme	Neuroscience & Mental Health
Summary	Targeted protein degradation (TPD) has emerged has a new therapeutic modality for the on-demand removal of disease-causing proteins. TPD employs hetero-bifunctional molecules which can bind a protein of interest and trigger its ubiquitin-mediated degradation, and these molecules are already being used to treat cancer. Through this project, we aim to develop similar TPD strategies for neurogenerative diseases. The student will work at the interface of cell and chemical biology, molecular neuroscience and cellular biochemistry to develop new biologics targeting mutant Tau and to evaluate their efficacy and impact on neuronal health using cellular models of Alzheimer's' Disease
Description	Most neurodegenerative diseases are characterised by loss of proteostasis which is an imbalance between protein synthesis and protein degradation. In Alzheimer's Disease (AD) and other dementia-related pathologies this translates into the accumulation of protein aggregates such as fibrils and tangles which can be toxic to cells in particular neurons. The small protein modifier ubiquitin functions as a housekeeping signal which sends no longer needed or potentially toxic proteins for degradation by the cellular recycling machineries, the proteasome or the lysosome. Pharma/Biotech are currently developing new therapeutic programmes aimed at harnessing the function of ubiquitin for the on-demand removal of disease-causing proteins. Until now, most applications using Targeted Protein Degradation (TPD) have focused on cancer therapies, but recent data indicate that these approaches could also be effective for CNS disorders including neurogenerative diseases. Mutant Tau has been found to cause familial dementia and animal models have added support to the idea that aberrant Tau could alone cause neurodegeneration. Although most of our understanding of mutant Tau has been in neuronal cells, there is also mounting evidence that aberrant Tau could also impact on the normal function of other brain cells and for example drive astrocyte reactivity and dysfunction. TPD is mediated by hetero-bifunctional molecules which can bind to a protein of interest and trigger its ubiquitin-mediated degradation via the activity of an E3 ubiquitin ligase enzyme, also known as degrader. Although most degraders employed to date have been from the RING E3 ubiquitin ligase family, we have recently established proof-of-principle that HECT E3 ubiquitin ligase enzymes can also be used. BioPROTAC are biologics-based hetero-bifunctional molecules which consists of a guidance mechanism (i.e., Vhh4, a single domain antibody which binds to the Green Fluorescent Protein), and a "warhead" which contains the enzymatic activity of an E3 ubiquitin ligase, in our case the HECT ligase domain. When expressed in cells, these fusion proteins can bind exogenous GFP through Vhh4 and trigger the ubiquitination of the GFP-tagged protein via the HECT ligase domain. By incorporating different HECT domains within our BioPROTAC designs, we should be able to selectively target protein degradation through the proteasome or

	lysosome systems, on demand. The overall aim of this PhD project is to develop/refine/validate BioPROTAC using HECT domains as degraders to specifically remove mutant Tau in cells. These new BioPROTAC will be tested using cellular models of Alzheimer's Disease, and the impact of mutant Tau degradation on neuronal cell health will be determined by measuring neuronal network activity. **Objective 1** (Month 1-12). Validate BioPROTAC in easy-to-manipulate cells Test/validate/refine HECT-based BioPROTAC designs in mammalian cells (e.g., HEK293T, SHSY5Y) stably expressing either nuclear (GFP-H2B), cytoplasmic (GFP-Beta-catenin), plasma membrane (alpha-actinin) proteins, or cytoskeletal associated protein (GFP-Tau). This will inform us of the limit and requirement of BioPROTAC in terms of the proteins it can target. Protein degradation will be quantified by flow cytometry, western blotting and high content microscopy. Proof-of-principle has already been established and so we are confident this work package can be delivered in the first year. **Objective 2** (Month 12-24). BioPROTAC degradation of GFP-Tau mutant in brains cells Optimised HECT-based BioPROTAC degraders will be subcloned into Inducible viral vectors and tested for the degradation of GFP-TauP301L and other potential Tau mutants in neurons and astrocytes. We will also test whether BioPROTAC can revert the deleterious effect of GFP-TauP301L by assessing neuronal network activity using microelectrode array (MEA) technology. The supervisory team has expertise in handling and manipulating mouse and Human iPSC-derived neuronal cells. We will also explore the ability for BioPROTAC to revert GFP-TauP301L-induced astrocyte reactivity and dysfunction using established phenotypic assays. **Objective 3** (Months 24-36). Screening for ligands of endogenous HECT ligases The next logical step of this project is to be able to recruit an endogenous HECT E3 ubiquitin ligase to degrade a protein of interest. In the absence of known ligands for our candidate E3 ligases, we will screen for binders using an aptamer library. Aptamers are nucleic acid-based ligands which have better pharmacological properties than small molecules and peptides, including superior blood brain barrier permeability, which is important for the long term of this project. We have already identified a unique domain within one of our HECT E3 which will be used as bait to fish out a specific aptamer from a library containing 10^{15} different DNA nucleic acid sequences. This protein domain has already been produced which will facilitate the delivery of this work package. Once this aptamer screen has been completed and unique sequence(s) identified, aptamers will be incorporated into new BioPROTAC designs which will tested for their ability to degrade GFP-TauP301L.

	Student taking ownership of the project The student will have opportunity to refine the objectives and prioritise the work packages. All the key techniques which are relevant for the project are up and running and routinely used in the laboratories of the supervisory team. The students will have major input in the type of cells tested, the parameters of neuronal activity measured, the refinement and improvement of BioPROTAC, as well as the undertaking and analysis of the aptamer screen.
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