iCASE Project / AY 2025 -2026

Synthetic biology with nanodiscs for enhanced targeting and understanding of receptor signals

Project Reference: ICS-PHA-MH

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Department/Institute: Pharmacology

Website: http://www.howarthgroup.org/

Industrial Partner: Bicycle Therapeutics

BBSRC DTP main strategic theme: Transformative technologies

BBSRC DTP secondary strategic theme: Understanding the rules of life, Bioscience for an integrated understanding of health

Project outline:

The overall goal of this studentship is to create tailored and ultra-stable membrane nanodiscs, to accelerate structural characterisation and generation of binders to integral membrane proteins. Bicycle Therapeutics have a unique technology: Bicycle peptides constrain short linear peptides into a stabilized bi-cyclic structure using a central chemical scaffold. This structure confers powerful drug-like properties, including high affinity binding and rapid tissue penetration, to generate therapeutics against targets intractable for small molecule or antibody therapies. Bicycles are initially selected by screening billions of variants through phage display against immobilized targets. This selection is routine for soluble proteins or membrane proteins with large structured ectodomains, but is still a substantial challenge for multi-transmembrane (multiTM) membrane proteins (notably ion channels and GPCRs). MultiTM proteins are harder to express and purify, and often lose native conformation in detergent. MultiTM proteins represent some of the most important targets for Bicycle, so the expertise of the Howarth in protein technologies and protein engineering can contribute to this challenge. The Howarth group created SpyTag, a peptide that forms a spontaneous isopeptide bond upon mixing with the SpyCatcher protein. Each component is composed of the regular 20 amino acids and reaction is rapid and specific under diverse conditions (Keeble/Howarth PNAS 2019, Keeble and Howarth, Chem Sci 2020). Nanodiscs are small proteins that can encapsulate integral membrane proteins, forming a ring containing natural membrane lipids. Nanodiscs have been transformative for studying solubilised membrane proteins in an environment much closer to the cellular context than detergent solubilisation. However, nanodiscs face challenges with instability and lack of controlled assembly, that inhibits their use for many applications, including screening binders by phage display, affinity determination of binders, and cryoEM to understand and optimize Bicycle binding. Combining SpyTag/SpyCatcher technology with nanodiscs enabled intramolecular cyclization of the nanodisc, enhancing stability of multiTM proteins and generating SpyRing-nanodiscs with tunable size range, adaptable to different membrane proteins and complexes. Here we will first validate E. coli-expressed SpyRing-nanodiscs for capture from HEK 293S cells of a multiTM target of interest to Bicycle with literature precedent for isolation and known ligands e.g. somatostatin receptors. Bicycles and known ligands will be characterised for affinity and specificity through biophysical or biochemical assays. Apo and liganded protein structures will also be studied by cryoEM. We will then employ protein engineering using isopeptide cross-linking and structure-based design, combined

with computational tools employed in the Howarth group (Rosetta, ProteinMPNN). We will test these engineered nanodiscs to achieve major improvements in nanodisc expression yield, % recovery of purified multiTM from expressing cells, and stability of ligand-binding conformation of multiTM. We will optimise stability of these nanodiscs to Bicycle phage display processes, and their ability to return target binders specific to the real cell-surface target. We will subsequently generalise these advances with a multiTM target from a distinct receptor class. Beyond application for Bicycles, this technology would have broad value for generation of vaccines to multiTM proteins and fundamental understanding of protein interactions important for ion channel and GPCR regulation.