University of Essex

Biological Sciences at the University of Essex

Next generation SMALPs for purification of fully functional GPCRs – to start October 2019

Christine Desty Scholarship, fully-funded (Home/EU fees £4630 plus stipend of £15,009) for an MSc by Dissertation (MSD) in the School of Biological Sciences, University of Essex

The aim of this project will be to identify next generation (ng) SMALPs for functional extraction of G Protein-Coupled Receptors (GPCRs) from cell membranes. GPCRs are the largest family of plasma membrane proteins in humans and are targets of ~34% of approved drugs (annual global market $180 billion). Current drug discovery platforms for GPCRs require structure/function approaches, often exploiting crystal and cryo EM structure models. However, extraction of GPCRS from cell membranes requires solubilization using detergents, agents that often destabilize the receptor causing it to unfold.

Recently, a method for detergent-free capture of integral membrane proteins from cell membranes was described. Poly (styrene-co-maleic acid) (SMA) integrates into membranes to form poly (styrene-co-maleic acid) lipid particles (SMALPs) that can also capture membrane proteins. SMALPS are water soluble and capture boundary lipid thus preserving the structural integrity of encapsulated membrane proteins. While this method has been used for extraction of diverse membrane proteins including GPCRs[1], the current limitation is that SMALP encapsulated membrane proteins, while extremely stable, often do not retain full function. For example, bovine rhodopsin can be extracted from rod photoreceptor cell membranes, but it does not undergo full activation when exposed to light[2]. We hypothesise that current SMALPs introduce a steric constraint preventing complete conformational changes required for full receptor activation. Our goals are to identify ngSMAs that retain efficient and stable receptor extraction whilst conferring full receptor function. We will use the dim-light photoreceptor rhodopsin as a model GPCR for this research. The native rhodopsin chomophore acts as a sensitive sensor of receptor capture and stability. Furthermore, receptor activation by light can be measured using UV-vis spectroscopy.

The aims of this project are:

1. To test ngSMA polymers designed to change the properties of SMALPs, including the numbers of lipids encapsulated.
2. To examine the extent of extraction and the thermal stability of rhodopsin encapsulated in ngSMALPs.
3. To examine the spectral and biochemical properties, including activation extent, of rhodopsin encapsulated in ngSMALPs

Preliminary work demonstrating the feasibility of SMALP-mediated rhodopsin extraction from bovine retina membranes has been done[2]. Here we will perform similar experiments using a panel of ngSMALPs. Rhodopsin will be incubated with ngSMA and the extent of extraction will be determined using UV-vis absorbance spectroscopy. Rhodopsin-containing ngSMALPs will be purified by immunoaffinity chromatography[2]. The stability of ground-state rhodopsin as well as its capacity for light activation will be determined using UV-vis spectroscopy. The stability of light-activated rhodopsin (MII) as well as activation of cognate G protein transducin will be both be measured by fluorescence spectroscopy.

The MSD student will become a member of an international team investigating ngSMALPS with a focus on one of the most important classes of membrane receptors: GPCRs.

1. Jamshad M. et al. (2015) G protein coupled receptor solubilization and purification for biophysical analysis and functional studies, in the total absence of detergent. Biosci Rep. 35(2).

2. Poyner, D. et al. (2018) The utility of SMALPs for investigating the structure/function of bovine rhodopsin. Proceedings British Pharmacological Society: http://www.pa2online.org/abstract/abstract.jsp?abid=33551&period=67

Entry requirements and application procedures
Highly motivated applicants with, or expecting, a good degree in the broad area of Life Sciences are encouraged to apply.
Applications should be submitted electronically by **24th April 2019** see here for details https://www.essex.ac.uk/pgapply/enter.aspx

You are encouraged to contact the supervisor before application: preeves@essex.ac.uk If you have any queries with the online application process, please contact ecrix@essex.ac.uk

For general information about the School of Biological Sciences at the University please visit our webpages http://www.essex.ac.uk/bs/.

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