Quantitative spatio-temporal dynamics of membrane proteins and lipid domains measured by label-free optical microscopy

Supervisory team:
Main supervisor: Prof Paola Borri (Cardiff University)
Second supervisor: Dr Mark Young (Cardiff University)
Prof Wolfgang Langbein (Cardiff University)

Collaborators: Prof Christiane Berger-Schaffitzel (University of Bristol)

Host institution: Cardiff University
Project description:
Membrane proteins represent the target of about two thirds of currently marketed pharmaceuticals[1] and play a critical role in both infection and immunity. Despite this importance, the direct study of membrane proteins in their native lipid environment remains a significant challenge. How do membrane proteins dynamically interact with the lipid bilayer, and how does bilayer composition affect membrane protein function? Can the membrane protein-lipid interaction be quantified with high spatio-temporal precision?

The aim of this project is to apply novel label-free optical imaging techniques to quantify membrane protein diffusion at the single protein level, in physiologically relevant heterogeneous 'raft-like' lipid ordered/disordered domains.

The project will specifically address the diffusion and lipid partitioning of P2X7, an ATP-gated cation channel which is known to partition into lipid microdomains, and which plays an important role in inflammatory signaling[2]. Label-free imaging will involve a combination of linear and nonlinear optical methods including Coherent Antistokes Raman Scattering (CARS) microscopy[3], quantitative Differential Interference Contrast (qDIC)[4] and the development of a novel interferometric Gated Off-axis Reflectance (iGOR) microscopy. Both synthetic lipid membrane bilayers and cellular membranes will be investigated.

This is a highly cross-disciplinary project at the physics-life science interface and two assessed rotation mini-projects in year 1 will provide focused training. **The first rotation** will be held in the Biophotonics lab of Professors Borri and Langbein who are physicists with extensive experience in the development of advanced laser micro-spectroscopy techniques. This mini-project will focus on synthetic membranes with lipid ordered/disordered microdomains imaged by CARS and qDIC. By the end of the project the student will be able to fabricate artificial lipid bilayers and quantify the chemical composition and thickness of the domains. **The second rotation** project will be held in the lab of Dr Young who is an expert in the structure, function, expression and purification of eukaryotic membrane proteins, with particular focus on the P2X receptor family of ATP-gated cation channels. This mini-project will focus on the optimization of expression and purification of P2X7 for reconstitution into synthetic membranes, and for low- to medium-resolution structural studies using transmission electron microscopy (TEM) and single particle analysis (SPA) of purified protein. By the end of the project the student will be able to purify sufficient P2X7 for reconstitution into synthetic membranes for subsequent diffusion and lipid partitioning experiments.

[1] J. P. Overington *et al.*, Nat Rev Drug Discov 5, 993 (2006).

[2] A. Surprenant and R. A. North, Annu. Rev. Physiol. 71, 333-59 (2009).

[3] A. Zumbusch *et al*. Progress in Lipid Research 52, 615 (2013).

[4] C. McPhee *et al.*, Biophysical Journal 105, 1414 (2013).