Investigating the structure, function and dynamics of the P2X7 receptor ballast domain

Supervisory team:
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Project description:
P2X7 receptors are ATP-activated cation channels predominantly expressed in immune cells. They respond to high concentrations of extracellular ATP found in infection and inflammation, and their activation leads to calcium influx followed by a series of cell signalling responses including cytokine release, kinase activation (e.g. ERK1/2) and cell death. P2X7 has been implicated in several diseases with a significant inflammatory component including arthritis and age-related macular degeneration, and is also implicated in several types of cancer, where alterations in the receptor structure and expression drive cell proliferation. Precisely how P2X7 receptor activation couples to cell signalling is poorly understood, but it is known to require the intracellular domains of the protein.

Recent cryoEM structures of rat P2X7 have revealed a novel 200 amino-acid globular intracellular C-terminal domain, termed the ballast, which contains 2 zinc ions, a GDP/GTP binding pocket, and an unstructured region previously implicated in binding to kinases. The zinc ions are thought to stabilise the folded structure of the ballast domain, but the role of GDP/GTP binding is unknown. Recent biophysical studies on the isolated ballast domain demonstrated a stabilising effect of GDP addition, but also demonstrated a destabilising interaction with calcium-calmodulin, suggesting that calcium influx may indirectly lead to conformational change via calmodulin binding.

The aim of this project is to investigate the structure and function of the P2X7 ballast domain using a combination of molecular dynamics and functional experiments. We will investigate the impact of GDP or GTP binding by performing molecular dynamics simulations on our established model of human P2X7, assessing the impact of in silico mutations on residues implicated in GDP/GTP binding (Rotation Project 1). Informed by the results of these simulations, we will make mutations in P2X7 and test their effect on the function of the protein, analysing ion channel and kinase activation (Rotation Project 2). Following this, we will investigate how P2X7 activation leads to activation of kinases including ERK1/2, by quantifying activation of upstream mediators including Ras. Furthermore, building on our preliminary data demonstrating successful expression and purification of the P2X7 ballast domain, we will look to confirm a physical interaction with calcium-calmodulin, and attempt to either model and quantify the interaction using molecular dynamics, or determine the 3D-structure of the protein complex using X-ray crystallography.

Our aim as the SWBio DTP is to support students from a range of backgrounds and circumstances. Where needed, we will work with you to take into consideration reasonable project adaptations (for example to support caring responsibilities, disabilities, other significant personal circumstances) as well as flexible working and part-time study requests, to enable greater access to a PhD. All our supervisors support us with this aim, so please feel comfortable in discussing further with the listed PhD project supervisor to see what is feasible.