**BBSRC MIBTP Studentship Project**

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| **Project Title** | Elucidating the mode of dynamic protein-protein interaction between a signalling hub protein Ras and its multiple effectors |
| **Project Summary**  |
| The RAS family of small GTPases act as signalling hubs regulating cell proliferation and differentiation. It is highly conserved from yeast to humans, highlighting its fundamental biological roles. Furthermore, its physiological importance is underlined by the fact that about 25% of all human cancers harbour mutations in RAS genes (COSMIC).   Ras triggers the activation of multiple downstream pathways by directly interacting with “effector” molecules that transduce signals to downstream components. Extensive efforts in the field have revealed individual Ras-effector complex structures for a few prototype effectors, including Raf kinases (leading to ERK kinase activation) and PI3 kinase (leading to Akt kinase activation). However, how Ras behaves in the presence of multiple effectors is largely unknown. Revealing this process will help us understand how a signalling hub protein transduces an input signal into multiple downstream pathways. X-ray crystallography has played an important role in understanding Ras-effector complex formation. Currently, about ten Ras:effector co-crystals have been reported, and in most cases, Ras:effector complexes form a 1:1 heterodimer. Exceptions are Ras:RALGDS (PDB ID 1LFD) and Ras:Rgl2 (PDB ID 8B69) which form a 2:2 heterotetramer (Tariq et al., biorxiv 2022). Interestingly, both RALGDS and Rgl2 are expected to share the function as a guanine nucleotide exchange factor (GEF) for small G proteins, RalA and RalB. We predict that Ras dynamically changes its structural conformation to form complexes with different effectors that activate different downstream pathways. We now wish to address the question; How does RAS manage to activate multiple downstream targets in a coordinated manner? Does RAS simultaneously interact with multiple targets? Or does RAS jump between different effectors?  In the PhD project, we will address these questions by combining structural biology, cell biology and single-molecule analysis. One of the unique features of the single-molecule analysis is that it allows us to obtain *kon* and *koff* rates of interacting molecules through live observation of these molecules. This allows us to study how multiple RAS effectors compete with each other for the active RAS at a molecular level.  Competition of multiple Ras effectors for the active Ras has been proposed in the past. However, the kinetics under the physiological setting is still elusive. In the PhD project, we will further develop this observation. For *in vitro* studies, single-molecule analyses, as well as competition NMR analysis, will be conducted. For *in vivo* studies, co-localisation of Ras, Raf kinase, and Rgl2 will be examined through live cell imaging through super-resolution microscopy, split-GFP assays and fluorescence resonance energy transfer (FRET). Collectively, the successful delivery of the project will bring a novel concept of RAS signalling. Techniques that will be undertaken during the project:Recombinant protein production using size-exclusion chromatography, biochemical analyses of protein-protein interactions using biolayer interferometry and mass photometry, gene editing (human culture cells), cell imaging analyses including super-resolution microscopy, X-ray crystallography, NMR, SAXS, and single-molecule analyses using a TIRF microscope. BBSRC Strategic Research Priority: Understanding the Rules of Life - Structural Biology, Systems Biology |
| **References** |
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