**University of Leicester**

**BBSRC MIBTP Studentship Project 2024-5 entry.**

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| **Project Reference** |  |

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**Section 2 – *Project Information***

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| **Project Title** | Obtaining insights into how a signalling hub protein Ras activates multiple effectors  |
| **Project Summary**  |
| The RAS family of small GTPases act as signalling hubs regulating cell proliferation and differentiation. They are highly conserved from yeast to humans, highlighting their fundamental biological roles. The physiological importance of the RAS family is underlined by various observations. These include that: (i) in mouse models, *KRAS*, one of the RAS isoforms, is essential, (ii) germline mutations in humans cause disorders termed RASopathies, (iii) human *RAS* genes are frequently mutated in cancers, and (iv) overexpression of oncogenic RAS causes premature senescence.  As a signalling hub, RAS activates multiple downstream pathways by directly interacting with “effector” molecules, which harbour domains termed “RAS binding domains (RBD)” or “RAS associating (RA)” domains, responsible for the RAS-effector binding. Extensive efforts have revealed RAS-RBD and RAS-RA complex crystal structures for reoresentative effectors, including RBDs of Raf kinases (that lead to ERK activation), RBDs of PI3 kinases (that lead to Akt activation) and RAs of RalGEFs (guanine nucleotide exchange factors for another small G proteins RalA and RalB). Interestingly, affinities between RAS and RBDs/RAs vary; the dissociation constant (KD) values range from 50 nM to a few µM, meaning that there is a 100-fold difference between the highest and lowest affinity RBDs/RAs. Whilst it has been widely accepted that RAS activates multiple effectors, how RAS interacts with low-affinity effectors inside cells is under-explored. This PhD project aims to address this question and understand the mechanism of a signalling hub protein that activates multiple effectors of various affinities. We have three working hypotheses which we will address during the project. **Working hypothesis 1**: The low-affinity effectors are more abundant in a cell than the high-affinity effectors. To examine this possibility, we will generate a set of isogenic human cell lines where a prototype RAS, KRAS, and prototype high- and low-affinity effectors (BRAF and Rgl2, respectively) are endogenously tagged with a variant of green fluorescent protein, mStayGold, using CRISPR-Cas9 technology. We will use hTERT RPE-1 ER-Cre-ER as the host human cell line, which we recently generated as a human cell culture model where the karyotype is stable and an inducible Cre recombinase can facilitate the gene editing (Hindul et al., 2022, *Biol Open*, **11**: bio059056). The generated cells will be analysed by quantitative live cell imaging to determine the relative molar ratios of KRAS, BRAF and Rgl2. This will be conducted in collaboration with Dr. Rob Mahen, an expert in the quantitative live-cell imaging. **Working hypothesis 2**: The full-length effectors show a higher affinity towards RAS than RBDs/RAs only, which can be a low-affinity RAS binder. To examine this possibility, we will prepare bacteria recombinant human KRAS, the full-length BRAF, and the full-length Rgl2 and test the affinities using various biochemical assays including biolayer interferometry (BLI), isothermal titration calorimetry (ITC) and mass photometry. We will also conduct cryo-electron microscopy (cryo-EM) of KRAS complexed with full-length Rgl2. We recently determined the crystal structure of KRAS and the RA of Rgl2 (Tariq et al., 2023, bioRxiv, https://doi.org/10.1101/2022.10.10.511529), and this data will help solve the cryo-EM structure of KRAS and full-length Rgl2. This part of the work will potentially unveil previously unidentified RAS binding elements in the Rgl2 protein.  **Working hypothesis 3:** It is well-established that RAS-effector interactions occur at the plasma membrane and low-affinity effectors may interact strongly with RAS at the plasma membrane. To examine this possibility, we will prepare membrane nano-discs and include them in the above-mentioned binding assays.  Techniques that will be undertaken during the projectRecombinant protein production using size-exclusion chromatography, biochemical analyses of protein-protein interactions using biolayer interferometry and mass photometry, gene editing (human culture cells), quantitative cell imaging analyses including super-resolution microscopy, and cryo-electron microscopy.   |
| **References** |
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**To apply please refer to**

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