**University of Leicester**

**BBSRC MIBTP Studentship Project 2025-6 entry.**

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**Section 2 – *Project Information***

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| **Project Title** | Structure and dynamics of the intrinsically disordered regions of the RNA binding protein Sam68: implication for RNA binding and phosphorylation.  |
| **Project Summary**  |
| A large proportion of the human proteome is composed of unstructured regions, termed intrinsically disordered regions (IDRs). Over recent years, it has been demonstrated that these regions are crucial for almost all cellular functions but structural studies of these IDRs to unravel the molecular mechanisms of their function remain largely unknown.  A typical example is the oncogenic RNA-binding protein Sam68, a multifunctional protein contributing to regulation of RNA metabolism, and signal transduction that is often overexpressed in many types of cancers (1-3). While the role of Sam68 central RNA binding domain in RNA recognition is well characterized (4), it is currently unknown how Sam68 N-terminal (Nter) and C-terminal (Cter) IDRs contributes to RNA binding and function of the protein, although these regions are targeted by multiple post-translational modifications (PTMs) that modulate Sam68 functions. By investigating the role of PTMs in regulating the cellular functions of Sam68, we have demonstrated that Sam68 Nter and Cter IDRs bind RNA specifically and that phosphorylation of these regions by Cdk1 modulates their RNA binding and the cellular functions of the protein (5). How do these regions bind specifically RNA and how phosphorylation of a single amino acid affect the interaction are still unknown.   Our hypothesis is that Sam68’s IDRs adopt transient structural features that are crucial for specific RNA recognition and that phosphorylation of these regions modulate these features, preventing RNA binding. Our hypothesis is supported by our preliminary NMR data. **Experimental methods:** We will combine biochemical techniques, NMR, Fluorescence correlation spectroscopy (FCS), single-molecule fluorescence resonance energy transfer (sm-FRET), small angle X-ray scattering (SAXS) and molecular dynamics (MD) to decipher the structural properties of Sam68 IDRs either free, in complex with RNA or following phosphorylation by CDK1.  **Research Proposal:** 1. We will define the minimum regions of Sam68 Nter and Cter IDRs capable of RNA binding and the minimum RNA region sufficient for specific binding to Sam68 IDRs: We will test the interaction (NMR and biochemical assays) of truncated version of Sam68 IDRs with truncated version of the RNAs. This will allow us to define the minimum regions of the protein and the RNA sufficient for specific binding.

 1. We will determine the structural properties of Sam68 Nter and Cter in complex with RNA and upon phosphorylation: We will combine NMR, FCS, sm-FRET, SAXS and MD to decipher the structural properties of Sam68 IDRs either free, in complex with RNA or following phosphorylation by CDK1. This will provide crucial structural and mechanistic insight into the specific RNA-binding by these IDRs and the role of phosphorylation.

 **Expected outcome and Impact:** Over recent years, it has become evident that IDRs play a key role in cellular processes. However, the structural and the molecular mechanisms regulating such processes are clearly lacking. This multi-disciplinary project will provide unique structural and mechanistic insights into RNA binding properties of IDPs/IDRs. Furthermore, Sam68 is overexpressed in many types of cancer. The structural study on this project will provide structural ensembles of Sam68 IDRs that are crucial for its function. It might therefore be possible, in the future, to design molecules that trap these IDRs in a non-functional conformation, therefore inhibiting their function.  Techniques that will be undertaken during the projectProtein expression (E. coli) and purification Nuclear Magnetic Resonance (NMR) Fluorescence Correlation Spectroscopy (FCS) Single-molecule Foerster Resonance Energy Transfer (sm-FRET)  Small-Angle X-ray scattering (SAXS) Molecular dynamics simulations  |
| **References** |
| 1. Bielli, P., et al. (2011) *Endocr Relat Cancer*, **18**, R91-R102. 2. Busa, et al. (2007) *Oncogene*, **26**, 4372-4382. 3. Fu, et al. (2016) *Elife*, **5**.e21957 4. Feracci, *et al.* (2016) *Nat Commun*, **7**, 10355. 5. Malki, et al. (2022) *Nucleic Acids Res*, **50**, 13045-13062.  |