**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** | Molecular characterization of Sam68-driven cytoskeletal reorganization |
| **Project Summary** | |
| The cytoskeleton is a complex network of various fibres (microtubules, actin, …) that is essential for cells to maintain their shape and internal organization and for their migration. It is a very dynamic network that reorganize constantly especially during the cell cycle. Like most essential processes, the cytoskeleton organization is highly regulated by regulatory proteins and its misregulation is a hallmark of cancer cell invasion and metastasis. The molecular processes underlying such changes in both normal and disease states are still poorly understood and require further investigation.  Sam68 is an oncogenic RNA-binding protein whose increased expression is correlated with poor prognosis in multiple cancers such as prostate and colon cancers. Sam68 display multiple functions in the cell. Its best characterized function occurs in the cell nucleus and is the regulation of alternative splicing, a process that allow cells to produce multiple proteins from a single gene. However, Sam68 is also localized in the cell cytoplasm but its cytoplasmic functions remain largely unknown. It has been suggested that Sam68 plays a role in cytoskeleton reorganization since depletion of Sam68 leads to defects in cytoskeleton organization in cancer cells.  We have investigated the consequences of Sam68 phosphorylation on its functions and found that Sam68 phosphorylation by the enzyme Cdk1 reduces its RNA-binding ability and alternative splicing regulatory activity. During this investigation, we have incubated a region of Sam68 (its N-terminal domain) with cytoplasmic extract and to our surprise, observed that on one hand, the cytoplasmic extract induces a striking structural rearrangement of this domain and on the other, that this domain induces the formation of a macroscopic fibre composed essentially of cytoskeleton and RNA-binding proteins. This is very surprising and very exciting. This provides us with a unique in vitro system to study various fundamental processes such as the dynamics and kinetics of fibre formation, cell-extract induced protein folding and the role of Sam68 in cytoskeleton reorganization.  In this proposal we will address three complementary questions:   1. What is the composition of the fibre and what are the kinetics of its formation? 2. What is the structure of Sam68 N-terminal domain in cytoplasmic extracts? 3. What is the role of Sam68 in cytoskeleton remodeling?   Techniques that will be undertaken during the project  Nuclear Magnetic Resonance (NMR)  Cryo-Electron microscopy  Live-cell imaging / confocal microscopy | |
| **References** | |
| M. Feracci, J. Foot, S.N. Grellscheid, M. Danilenko, R. Stehle, O. Gonchar, H.S. Kang, C. Dalgliesh, N.H. Meyer, Y. Liu, A. Lahat, M. Sattler, I.C. Eperon, D.J. Elliott, and **C. Dominguez**. Structural basis of RNA recognition and dimerization by the STAR proteins T-STAR and Sam68. **Nature Communications** 7, 10355 (2016).  Malki, M., Liepina I., Kogelnik, N., Watmuff, H., Robinson, S. Lightfoot, A., Gonchar, O., Bottrill, A., **Fry, A.M.,** **Dominguez, C**. Cdk1-mediated threonine phosphorylation of Sam68 modulates its RNA binding, alternative splicing activity, and cellular functions. ***Nucleic Acids Research***, 50, 13045-62 (2022). | |

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