Principal Supervisor: Dr. Cyril Dominguez

Co-supervisor: Prof. Ian Eperon

PhD project title: **Structural basis of Sam68 alternative splicing inhibition by Endostatin**

University of Registration: Leicester

**Project outline**

1. Project outline describing the scientific rationale of the project

Sam68 (Src associated protein during mitosis of 68kDa) belongs to the STAR (Signal Transduction and Activation of RNA) family of proteins and plays important roles in cell cycle regulation, signal transduction, RNA metabolism and viral replication. Sam68 regulates pre-mRNA alternative splicing, is highly controlled by signaling pathways, and overexpression of Sam68 correlates with poor prognosis in various cancers.

Sam68 has been shown to have oncogenic properties and high expression of Sam68 correlates with poor prognosis in various cancers. This is associated with the fact that Sam68 affects crucial cellular processes such as cell differentiation, cell cycle progression and apoptosis, through its direct involvement in alternative splicing regulation of CD44, Bcl-x, SRSF1, Cyclin D1, and Human Papillomavirus 16 (HPV-16) E6 pre-mRNAs, often favoring the production of the most oncogenic protein isoform. Additionally, Sam68 is critical for controlling adipogenesis through splicing of mTOR, and controls nervous system functions through splicing of the Neurexin pre-mRNAs. Sam68 also plays a crucial role in HIV replication by assisting the nuclear export of unspliced and singly spliced HIV RNA.

A recent study demonstrated that the regulation of adipogenesis and angiogenesis by Sam68 is inhibited by endostatin, a well-known inhibitor of angiogenesis. Endostatin interacts with the dimerization domain of Sam68 and prevent Sam68 to regulate the alternative splicing of the mTOR pre-mRNA. Using endostatin as an inhibitor of this pathway could therefore potentially be a useful therapeutic option for diet-induced obesity.

We have recently revealed the structural basis of dimerization and RNA recognition by Sam68. We have shown that the dimerization of Sam68, which is unique and novel, play an important role in Sam68 affinity for its RNA targets and splicing regulation. We anticipate that the binding of endostatin will alter the dimerization of Sam68 and therefore its function.

This project will investigate the structure of Sam68 in complex with endostatin, using X-ray crystallography and Nuclear Magnetic Resonance (NMR). The effect of the endostatin-Sam68 interaction on Sam68 RNA binding ability and splicing function will be investigated by biochemical experiments (site-directed mutagenesis, RNA binding and in vitro splicing assays). This structure will be used to design small molecules that mimic the binding of endostatin to Sam68 and alter its RNA binding ability.
References:


2- Wang H., Chen Y., Lu X.A., Liu G., Fu Y., Luo Y. Endostatin Prevents Dietary-Induced Obesity by Inhibiting Adipogenesis and Angiogenesis. *Diabetes*, 64, 24420-2456


**Relevant BBSRC Strategic Research Priority:**
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Techniques that will be undertaken during the project.

Expression and purification of proteins from bacterial and mammalian cultures; structure determination using NMR, X-ray crystallography, protein-RNA and protein-protein interactions using Isothermal Titration Calorimetry and Fluorescence Polarization; functional assays using *in vitro* and *in vivo* splicing assays.

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