Grant Impact Report

Name	Shuchi Agrawal Singh
Job title	Lecturer
Organisation	Imperial College London, Centre for Haematology, Department of Immunology & Inflammation,
Co-investigators (if applicable)	
Grant awarded	BSH Visiting Fellow
Year awarded	2024
Date started	16th Sep 2024
Date completed	16th Nov 2024
Total amount expended (£)	**£17,520**

This is the BSH grant impact report form. Please enter the full grant details above, and fill out the form below. The form should be completed electronically and sent to grants@b-s-h.org.uk. Please note that the report can only be accepted if all sections have been completed in full.

In addition: Please include a recent photo of yourself.

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1. **Please summarise what the grant enabled you to achieve; what would not have been possible without the funding? (Up to 500 words)**

The BSH-Visiting Fellow Scholarship allowed me to visit Prof. Salvatella's lab (specialised in Molecular Biophysics) at IRB Barcelona from 15th September to 26th October 2024, on sabbatical. Although I couldn't stay for the full 8 weeks as originally planned because I needed to start my new position at Imperial College London in early November, the 6 weeks I spent at IRB, were extremely valuable for my career development.

During my time at IRB, I worked as a visiting scientist alongside a talented group of researchers, including postdocs and PhD students, who are specialized in molecular biophysics, particularly in the study of intrinsically disordered proteins (IDPs). This experience exposed me to cutting-edge experimental and computational techniques in the field. Specifically, I gained hands-on expertise in large-scale protein purification using a variety of advanced methods, which significantly enhanced my understanding of protein biochemistry. I also learned how to troubleshoot these methods, ensuring high-quality preparations for subsequent experiments.

In addition, I acquired practical experience in studying the phase separation properties of proteins in vitro. One of the most exciting parts of my visit was getting practical experience using NMR spectroscopy, a technique that allows to study molecular interactions. This enabled me to directly observe protein-protein (intramolecular) interactions, providing insights into the phase separation processes due to protein self-assembly. Detailed understanding of phase separation of biomolecules (proteins) could explain many biological phenomena especially in cancer.

Even though I had to return to the UK earlier than planned, the work didn't stop there. I've continued the project I started at IRB, including analysing data and repeating key experiments. We're planning to expand this work into a larger project, and I look forward to continued collaboration with Prof. Salvatella's lab.

In addition to the technical skills, the visit also gave me the chance to learn from world-class scientists who visited IRB during my time there. Attending their seminars and meeting with them helped me grow both scientifically and professionally, and I've made connections that will benefit my future research work.

Without the funding from the BSH-Visiting Fellow Scholarship, this opportunity to work with experts in the field, gain specialized technical skills, and establish an international collaboration would not have been possible. This visit has been a key step in advancing my research, expanding my technical expertise, learning new skills and fostering long-term professional connections. Hence this visit will have a lasting impact on my career.

I am deeply grateful to the BSH for providing me with this valuable opportunity.

2. Briefly describe the aims and intended outcomes of this project. Please clearly indicate if there is any sensitive information in this report that should remain confidential for now. (Up to 300 words)

In this proposal we wanted to characterise intrinsically disordered region (IDR) of transcription factor HOXA9 and establish the sequence determinants of HOXA9 that lead to the formation of biomolecular condensates. For this we aimed to perform solution-state nuclear magnetic resonance (NMR) spectroscopy. NMR is a spectroscopic technique that allows for the characterization of protein sequences at atomic resolution and is particularly well suited for studying intrinsically disordered proteins. NMR has been used to characterize intrinsically disordered regions and determine which amino acids drive the biomolecular condensation process of these proteins. Salvatella's group is pioneer in this field. We aimed to identify key residues in HOXA9 that could drive phase separation of HOXA9 protein. Further, use this knowledge to guide us in understanding the molecular mechanisms of acute myeloid leukaemia that are dependent on high expression of HOXA9. (Confidential)

Goal of the Project:

1. To determine the structural properties of IDR-HOXA9 to delineate the inter and intra molecular interactions driving HOXA9-condensate formation.

2. To use this knowledge to identify potential targetable protein-protein interactions for therapeutic gain in AML.

Typically, the interactions between macromolecules in LLPS are non-covalent, of low affinity between intrinsically disordered regions (IDRs). Our experimental plan will involve complementary multidisciplinary strands and consist of following two specific aims.

Aim-1: Detailed i*n silico* modelling of the HOXA9 protein sequence to identify interactions to support specific design of our structural studies.
Aim-2: Characterization of HOXA9 condensates and structural analyses at molecular level.

This project needs to be confidential as we are now submitting a follow-up funding as described below.

3. **Describe the key outcomes to date, including whether this grant has resulted in further research. Please summarise your conclusions. (Up to 600 words)**

During my visit, we successfully expressed and purified the HOXA9 protein using various constructs, including full-length HOXA9, the unstructured domain, and the DNA-binding domain of HOXA9. These constructs were obtained with high purity, enabling us to perform detailed analyses. We then used these proteins in assays to assess their condensation properties in several in vitro biophysical assays, and we identified that the HOXA9 unstructured domain indeed forms droplets in vitro, demonstrating condensate formation via phase separation in vitro by HOXA9.

Furthermore, we carried out NMR spectroscopy on the HOXA9 unstructured domain and observed self-association between specific residues within the intrinsically disordered region (IDR) of HOXA9. Notably, this self-interaction was found to be salt-dependent, suggesting that the self-association of these amino acid residues is driven by electrostatic interactions. To refine these findings, we performed carbon-labelled NMR to assign specific residues involved in the self-interaction process, and we are currently analysing the data to identify the precise residues contributing to the phase separation behaviour of HOXA9.

These results strongly support our hypothesis that HOXA9 possess the tendency to phase separate via intramolecular interactions of HOXA9 protein, which may be relevant to its function in leukaemia. Our next step will be to carry out structure-function mutation studies to determine whether disrupting these specific residues can inhibit the phase separation process and, if so, to assess whether this disruption affects leukaemia development or maintenance.

This research has successfully generated crucial preliminary data, which now forms the foundation for a deeper investigation into the role of phase separation in blood cancer. The outcomes from this work have not only supported our hypothesis but also opened new avenues for future research. We plan to extend these studies to explore whether the phase separation process observed in HOXA9 is also a feature of other mutated proteins associated with acute myeloid leukaemia (AML).

The next phase of the research will focus on using this understanding to test the functional significance of these interactions and investigate whether disrupting phase separation can impact leukemogenesis.

In conclusion, this project has resulted in significant progress towards understanding the role of phase separation in leukaemia and has laid the groundwork for future studies to explore this mechanism in more detail.

We are now in a position to apply for further funding to support the next steps, including the generation of mutants and in-depth biological studies to determine the relevance of phase separation in HOXA9 dependent leukaemia progression.

4. List published papers, oral and/or poster presentations as a result of this grant. Include manuscripts in preparation or in submission / under review, prefaced by an asterisk.

The results from this visit have provided a proof of concept for future research and are still in the preliminary stages. We have developed the project concept and idea, but have not yet presented the findings at any meetings. We are currently working on securing additional funding to carry out this work in greater detail.

5. Did any patent applications arise from this work? (If yes, please detail. Up to 200 words)

Not applicable

6. Were you successful in any further grant applications as a result of this work? (If yes, please detail. Up to 200 words)

I am planning to submit a joint grant application to the Mark Foundation for Cancer Research, in collaboration with Prof. Salvatella. Additionally, I am preparing an application for a Career Establishment Fellowship from CRUK, and Future Leader Fellowship from UKRI, to seek funding to support my independent research and establishing myself in the field. For this fellowship, I will include preliminary data generated during my visit to IRB, show my visit as part of my career development. I will also highlight the multidisciplinary collaboration with Prof. Salvatella, focusing on the biophysical approaches to understand disease mechanisms which we will functionally validate using genomics and biology skills in my lab.

7. Did new collaborations arise from this work? (If yes, please detail. Up to 400 words)

Yes, this visit has led to the establishment of a new collaboration between the Salvatella lab and my own, focused on investigating the role of HOXA9 ‘s disordered domain in blood cancer. We are planning to submit joint grant proposals to support this research and will share our expertise in biophysical approaches and functional genomics. In addition, we aim to facilitate the exchange of students between our labs, fostering a multidisciplinary team that can approach this complex issue from multiple angles. This collaboration not only strengthens our individual research but also opens up exciting opportunities for joint publications and long-term research initiatives.

8. What was the funding amount you received and how was it actually spent? (detail item/activity and amount spent in pounds)

I received total amount of £17520. The detailed breakdown of spent is described below:

Cost Category	**Amount Spent (£)**
Protein expression in E coli and purification for NMR experiments using normal and C13 label media and sample preparation. (Consumables)	£2,450
Construct generation/ cloning/ sequencing using Genscript and other reagents	£2,342
Consumables purchased	£3,652
Total amount spent (£)	**£8,444**

Cost Category	**Amount (£) Costs incurred at IRB**
Microscopy experiments at IRB (charges at facility)	£400 *(Expected amount, awaiting final invoice)
NMR assignment experiments at IRB	£2,480 *(Expected amount, awaiting final invoice)
NMR HSQC experiments at IRB	£2,200 *(Expected amount, awaiting final invoice)
Total amount (£)	**£5,080**

Cost Category	**Amount (£) Costs incurred in travel (awaiting final invoice)**
Apartment hire at Barcelona	£2699.64
Living Expenses for 6 weeks	£1296.44
Total amount (£)	**£3,996.08**

9. What are the future research priorities in this area?

The future research priorities will focus on deepening our understanding of the role of intrinsically disordered proteins (IDPs) in blood cancer, specifically investigating HOXA9 and expanding to other proteins mutated in acute myeloid leukaemia (AML). We aim to explore how the structural flexibility and functional diversity of these proteins contribute to disease mechanisms. A key focus will be on understanding the molecular basis of protein self-interactions that lead to the formation of abnormal clusters or condensates.

To achieve this, we plan to identify key residues involved in this self-assembly process through NMR-based studies, in collaboration with the Salvatella lab. Additionally, we will assess the relevance of these interactions in leukemogenesis using structure-function mutants in the Agrawal Singh lab. The ultimate goal is to disrupt the abnormal self-assembly of mutated proteins, initially focussing on HOXA9, to prevent leukaemia cell growth and explore new therapeutic avenues for AML.