PUBLISHABLE SUMMARY

Summary of the context and overall objectives of the project (For the final period, include the conclusions of the action)

MICALs (Molecules Interacting with CasL) are a family of large cytosolic signaling proteins that control cytoskeletal dynamics. They are known to play essential roles in many actin-dependent events, including axon growth and guidance, synapse development, synaptic homeostasis, dendrite morphology, and neuronal cell migration. In this project, my focus was on understanding how MICALs precisely turn their activity on and off. I used single-particle cryoelectron microscopy to determine the overall architecture of human MICAL1. The determined structure was key to understanding the molecular mechanisms of MICAL autoinhibition, which was further confirmed with biochemical and biophysical experiments.

These findings provide a launchpad for addressing more complex questions, such as what controls axon length and directionality, how axons change shape, and how we can promote regrowth in damaged or diseased axons.

I believe that the project has achieved its main objectives and significantly improved my experience and competence. Furthermore, this project exceeded my expectations in terms of its impact on my career, as I successfully established a new Laboratory of Structural Neurobiology and secured my position as a Junior Group Leader.

Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far (For the final period please include an overview of the results and their exploitation and dissemination)

To study the molecular mechanisms of human MICAL1, we first produced this protein in insect cells using a baculoviral expression system. With single-particle cryoelectron microscopy, we determined the overall architecture of MICAL1 and solved the structure in very fine detail. We were the first ones to see the full-length MICAL structure, which helped us understand how it works. The overall architecture of MICAL1 revealed that a C-terminal domain folds over the catalytic site, restricting substrate access. This intramolecular interaction between the C-terminal and N-terminal catalytic domains appears to impose MICAL1 autoinhibition, which we confirmed through biochemical and biophysical studies.

We also tried to understand how MICAL works with other proteins called plexin receptors. Unfortunately, we were not able to detect any binding between them. So, we tried using another protein called Rab8, which we found in previous studies. Testing Rab8 and MICAL together, we observed direct binding. However, we have not yet determined the structure of the complex due to difficulties with plexin. Nevertheless, this project is ongoing, and we expect to determine the structure of the complex soon.

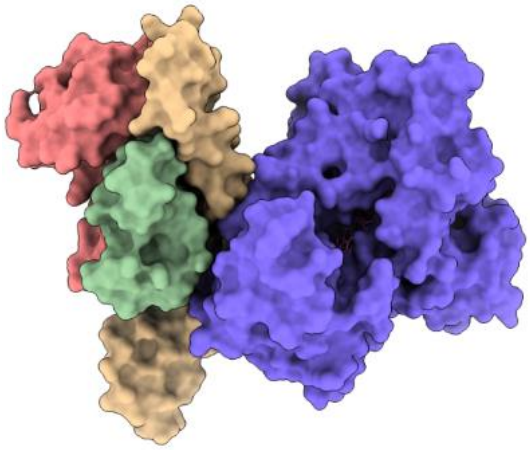
Another goal of the project was personal growth, and I believe this goal was fully addressed. In particular, I significantly improved my leadership and management skills by attending four workshops focused on project management. My leadership skills were further developed by supervising two undergraduate students.

The findings of this research will be published soon, and we expect to submit two manuscript by the end of this year. I presented the results through conferences and a number of invited talks. Our research was also popularized through newspaper interviews.

Progress beyond the state of the art, expected results until the end of the project and potential impacts (including the socio-economic impact and the wider societal implications of the project so far)

In this project, we determined the structure of human MICAL1, which is the first full-length structure solved in the entire MICAL family. Based on this structure, we provided the first structural and mechanistic insight into MICAL function, particularly how MICAL activity is precisely turned on and off. These findings can be applied to more complex studies and provide a tool for the discovery of new molecular and biochemical mechanisms controlling the actin cytoskeleton.

Furthermore, the MICAL family poses a series of interconnected questions that are of fundamental importance for our understanding of a broad range of biomedically relevant activities. Our findings provide insight into how to modulate MICAL interactions to switch between active and inhibited states, which could pave the way to treating MICAL-related diseases. Recent studies on MICALs have revealed a number of disease-causing mutations, and our determination of the overall hMICAL1 structure can provide informative delineation of the effects of these mutations on the mechanisms of particular diseases, particularly autosomal-dominant lateral temporal epilepsy. I am excited to map these mutations onto the structure of hMICAL1 in the future, perform in-depth structure-based analysis, and investigate the possible impact of these mutations on the protein structure and hence its function.



Surface representation of the full-length MICAL1 structure.