Targeted Project / AY 2025 -2026

Alpha-synuclein phase separation – from computational modelling to in vivo exploration

Project Reference: TRG-IMR-JL

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BBSRC DTP main strategic theme: Understanding the rules of life

BBSRC DTP secondary strategic theme: Bioscience for an integrated understanding of health

Project outline:

Background: Alpha-synuclein, a crucial synaptic protein with as-yet-undefined function, has been shown to undergo protein phase separation. However, the regulatory mechanisms governing alpha-synuclein phase separation in a biological context remain unexplored. Our laboratory has recently demonstrated through in vitro biochemical assays, cellular studies, and NMR that another synaptic protein, vesicle-associated membrane protein 2 (VAMP2), plays a regulatory role in alpha-synuclein phase separation (Agarwal et al. 2024, accepted in Nat Cell Biol).

Objective: This Targeted Project aims to comprehensively investigate the interaction between VAMP2 and alpha-synuclein, employing a multidisciplinary approach that spans computational modelling, rigorous experimental validation, and in vivo studies using Caenorhabditis elegans as a model organism.

Methodology:

[1] Computational Modelling: In our previous work we have identified that the interaction between VAMP2 and alpha-synuclein nucleates alpha-synuclein phase separation. Understanding the molecular determinants will gain further mechanistic insights. We will work with Dr. Bhattacharya, a lecturer in the Department of Physics at the University of Limerick and an expert in modelling intrinsically disordered proteins. His expertise in theoretical physics-based and computer-assisted molecular design models will enable us to model low-affinity intra- and intermolecular interactions whose impact we will then test experimentally.

[2] Experimental Pipeline: We will conduct biochemical assays using purified recombinant proteins to test the role of targeted mutations in the identified protein regions. We will assess alpha-synuclein propensity to undergo droplet formation using imaging and quantitative experiments as well as testing the interaction between the respective VAMP2 and alpha-synuclein variants. The engineered mutants will further be taken into cellular systems and neurons to validate biochemical findings and to understand the biological context of the VAMP2 interaction on alpha-synuclein phase separation. These studies will be further complemented by isothermal titration calorimetry (ITC) and NMR measurements.

[3] In Vivo Studies: Partnering with Dr. David from the Babraham Institute, we will generate in vivo models in Caenorhabditis elegans, a transparent nematode well-suited for aging studies. We will

generate alpha-synuclein mutant strains which we will monitor for alpha-synuclein phase separation and neuronal phenotypes over their lifespan. We will use the mutants identified above, but also alpha-synuclein variants which we have already identified to have increased or decreased phase separation due to modulation of their lipid binding propensity.

Impact and Significance: This project aims to uncover the specific protein regions and conformational states involved in the interaction between VAMP2 and alpha-synuclein. The project will investigate the physiological relevance of alpha-synuclein phase separation, providing the exciting opportunity to test our hypothesis on alpha-synuclein phase separation in a living organism. Understanding the regulatory mechanisms will pave the way for strategies to maintain protein homeostasis and promote healthy aging.

Conclusion: By integrating computational modelling with experimental and in vivo approaches, this project will generate a pipeline to study protein interactions involved in phase separation, ultimately contributing to our understanding of protein dynamics in synaptic function and aging. This multidisciplinary effort promises to advance the field of neurobiology elucidating fundamental mechanisms in protein chemistry.