Fachbereich Biologie (10)

JOHANNES GUTENBERG UNIVERSITÄT MAINZ

AG Functional Neurobiology (AG Heine)
Institute for Developmental and Neurobiology (IDN)

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Bachelor project

Cloning and testing of novel IDR-specific Bassoon fragments to study the biophysical properties of the Bassoon protein

Liquid-liquid phase separation, namely the formation of a condensed molecular assembly within another diluted aqueous solution, is a means for cells to organize highly condensed biological assemblies (also known as biological condensates or membraneless compartments) with very broad functions and regulatory properties in different subcellular regions. Emerging evidence suggests that proteins in the presynaptic active zone might be organized in such membraneless compartments. Particularly, in vitro studies indicate that synapsin1, RIM, RBPs and Liprin-alpha can assembly in phase-separated compartments that could possibly include other key proteins of the release machinery, such as scaffolding proteins and calcium channels. However, how other big scaffolding proteins might be involved in the formation of phase separated compartments remains poorly understood. Very recent computational modelling studies suggest that the scaffolding protein Bassoon presents in its structure peculiar features known as intrinsically disordered regions (IDRs) that act as predictive signs of condensates formation. In this project, we aim to characterize how the protein Bassoon might be organized in phase separated condensates. To do so, we will create IDR specific fragments of the Bassoon protein and evaluate their different assembly properties through overexpression in HEK293T cells.

In this project, we aim to Identify and clone specific IDRs of the Bassoon protein sequence, as well as to investigate the aggregation properties of selected IDR-containing fragments in a heterologous cell system.

While carrying out this project, you will learn basic molecular cloning techniques such as primers design, PCR reaction, restriction digestion and ligation. You will also be introduced to SnapGene software for molecular biology. Moreover, you will learn how to culture and transfect HEK293T cells for the testing of newly generated DNA constructs and how to visualize fluorescently labelled proteins using live fluorescence microcopy. You will then be taught how to perform fluorescence after photobleaching (FRAP) imaging and carry out droplet fusion assays to evaluate the biophysical properties of newly generated DNA constructs.

If you are interested, please contact Michela Borghi (mborghi@uni-mainz.de).

AG Functional Neurobiology

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