Decoding Protein Interactions in Biomolecular Condensates Using FRET-FRAP and FCS

Michał K. Białobrzewski[1], Cheng Tan[2], Zuzanna Staszałek[1], Maja K. Cieplak-Rotowska[1,3,7], Marc R. Fabian[4], Nahum Sonenberg[5], Michał Dadlez[6], Yuji Sugita[2] and Anna Niedźwiecka[1]

1 Laboratory of Biological Physics, Institute of Physics, Polish Academy of Sciences, Warsaw, Poland
2 Computational Biophysics Research Team, RIKEN Center for Computational Science, Kobe, Japan
3 Division of Biophysics, Institute of Experimental Physics, Faculty of Physics, University of Warsaw, Warsaw, Poland
4 Lady Davis Institute for Medical Research, Jewish General Hospital, and Department of Oncology, McGill University, Montréal, Canada
5 Department of Biochemistry, and Goodman Cancer Centre, McGill University, Montréal, Canada
6 Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
7 The International Institute of Molecular Mechanisms and Machines (IMol), Polish Academy of Sciences, Warsaw, Poland

GW182 is an intrinsically disordered protein (IDP) that plays a central role in microRNA-mediated post-transcriptional gene silencing. Its N-terminal Argonaute-binding domain (ABD) anchors GW182 to the miRNA-induced silencing complex, while its C-terminal silencing domain (SD) recruits the CCR4–NOT deadenylase complex to targeted mRNAs. The same CCR4–NOT complex is also engaged in ARE-mediated gene silencing by interactions with another IDP, tristetraprolin (TTP), which raises an important question: do these pathways cooperate, converge, or compete?

In this seminar, I will present how we combine fluorescence correlation spectroscopy (FCS) studies with liquid-liquid phase separation (LLPS) to explore how GW182 SD interacts with a fragment of the central subunit of the CCR4–NOT deadenylase complex, CNOT1(800-999). We show that GW182 SD undergoes LLPS with a lower critical solution temperature (LCST)-type behaviour driven by tryptophan-mediated π–π interactions, and that it forms multiprotein condensates with CNOT1 in a scaffold–client manner. Single-point mutations in CNOT1 disrupt this interaction and inhibit the formation of the condensates. By integrating FRET with FRAP, we discriminate between proteins that are engaged in specific complexes and those retained within condensates based on residual interactions, providing a strategy for selectively probing molecular interactions in crowded environments.

Finally, I will discuss how the presence of a TTP peptide fragment responsible for the CNOT1 binding as a third component inhibits the formation of GW182 SD–CNOT1 condensates, demonstrating direct competition for the same binding site. Collectively, these findings show that GW182 SD not only drives LLPS but also exploits the same CNOT1 binding site as TTP, which uncovers molecular cross-talk between distinct post-transcriptional silencing pathways and provides new insight into how biomolecular condensates shape RNA regulation.

ACKNOWLEDGMENTS

This work was supported by the NCN grants no. UMO-2016/22/E/NZ1/00656 to A. N. and no. UMO-2023/49/B/NZ1/04320 to M. K. B. The studies were performed in the NanoFun laboratories co-financed by ERDF within the POIG.02.02.00-00-025/09 project.