Deciphering the molecular logic driving mesoscale structure of RNA-dependent nuclear condensates

Nuclear biomolecular condensates are multicomponent membraneless organelles that often exhibit complex internal organization and contribute to functions such as transcription and splicing. Paraspeckles are a paradigmatic example of these condensates, exhibiting compositionally distinct core and shell layers on a common scaffolding lncRNA (NEAT1). However, the molecular logic dictating this emergent structure remains poorly understood.

We address this question in this work by combining experiments, informatics, and coarse-grained simulations to dissect molecular features underlying paraspeckle assembly. From binding assays and motif density profiles we surprisingly find the essential core proteins, FUS and NONO, preferentially bind to the layer-localizing ends of NEAT1, contradicting current models of paraspeckle assembly. Through analyzing proteomics data we find that NONO is 2-3 fold higher in concentration compared to FUS across human tissues. Using *in vitro* droplet assembly and a coarse-grained model of the key paraspeckle species, we demonstrate that the competition between the core proteins and the shell binding protein, TDP-43, promote a core-and-shell. By explicitly studying the interplay between relative rates of active NEAT1 synthesis and NEAT1 condensation, we find that co-transcriptional bursting and assembly promotes paraspeckle-like clusters while slow transcription leads to small oligomers. Overall, this project proposes a fundamentally multicomponent logic demonstrating protein and RNA sequence/structure dependent interactions, relative stoichiometry and active transcription as key features driving emergent morphology of paraspeckles.