PHASE SEPARATION

Students will participate in the following 2 modules:

MODULE 1: PROBING RNA-PROTEIN INTERACTION BY SINGLE-MOLECULE FRET AND COMPUTATIONAL ANALYSIS

Part 1: smFRET measurement of FUS-RNA interaction of the wildtype and mutant FUS protein

Laboratory: Sua Myong (Johns Hopkins University)

Students will engage in single molecule FRET (smFRET) experiments to measure FUS-RNA interaction in varying protein concentrations. They are expected to learn how to prepare and apply RNA and protein samples for single molecule detection, how to generate FRET histograms and extract individual single molecule time traces and analyze data. In FUS binding assay, the students will test one or more single point mutants of FUS that will be compared to the wildtype FUS in terms of the binding mode and conformational dynamics and glean more refined molecular insight from simulations (Part 2)

Part 2:

Laboratory: Alek Aksimentiev (UIUC Physics)

Students will learn how to build computational models of nucleic acid - protein systems and to carry out single molecule biophysics experiments, in silico. The students will learn practicalities of Brownian dynamics simulations, visualization and analysis of the computational experiments. The students will build computational model of the systems investigated by smFRET and correlated the outcomes of Brownian dynamics simulations with the smFRET measurements.

MODULE 2: MEASURING PHASE SEPARATION OF RNA-PROTEIN COMPLEXES

Part 1: Liquid-liquid phase separation of FUS-RNA

Laboratory: Sua Myong (Johns Hopkins University)

Students will form FUS-RNA condensates of the wildtype and mutants tested above. The condensed droplets will be visualized by differential contrast microscope as well as wild field fluorescence microscope over 4 hour time window (in vitro aging). They will track the size, number and circularity of the condensed droplets over 4 hours. Every hour, they will also measure fluorescence recovery after photobleaching (FRAP) to test the fluidity of the condensates. The fluidity/size data will be interpreted in the Aksimentiev lab (Part 2).

Part 2:

Laboratory: Alek Aksimentiev (UIUC Physics)

The students will investigate the process of liquid-liquid phase separation using a biologically inspired model system. The students will build a computational model of a multi-component liquid mixture and determine its phase behavior by performing a set of Brownian dynamics simulations at several conditions. The students will analyze the simulation outcomes to determine the scaling laws governing the domain coarsening behavior.