Spectroscopic Imaging of Interactions in the Cell

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FRET Labeling for WW Domain

Computational studies of small peptide kinetics and stabilities *in cellulo* need to be corroborated with experimental results. Therefore, a method of visualizing conformational changes of a protein in a cell without perturbing the stability is required. **Using WW Domain proteins as a model, we will test two different FRET labeling methods, fluorescent proteins and dyes, to determine which provides the smallest perturbation to the protein's stability.** We predict that while both methods will be able to detect unfolding, the dyes will provide the smallest perturbation due to their size.

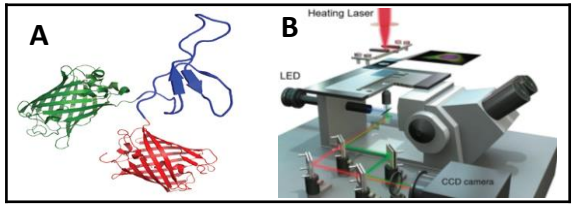

Figure 1. hPin1 WW domain labeled with AcGFP1 and mCherry (**A**) and an example of a T-Jump microscope[1] (**B**)

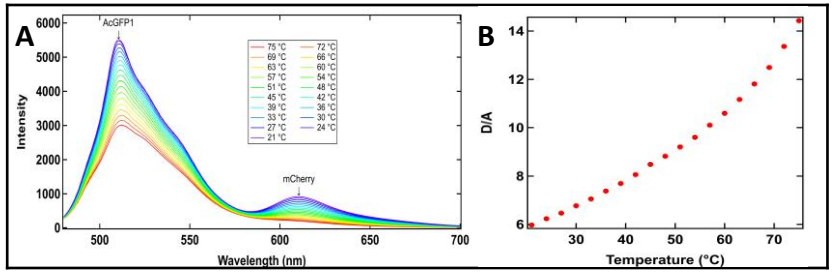

Figure 2. FRET fluorescence spectrum (**A**) is used to determine the temperature dependent D/A (**B**), which can be fitted to determine protein stability

1). Ebbinghaus et al. *Nature Methods* **7**, 319-323 (2010).

RNA Phase Separation

Formation of RNA Foci in the cell nucleus is a hallmark of repeat expansion disorders (e.g. Huntington's Disease and ALS). Above a critical number of repeats, RNAs consisting of disease-associated nucleotide repeats readily form phase separated droplets in vitro in a base-pairing dependent process. **We use UV-Vis spectroscopy to identify structural transitions in CAG RNA foci.**

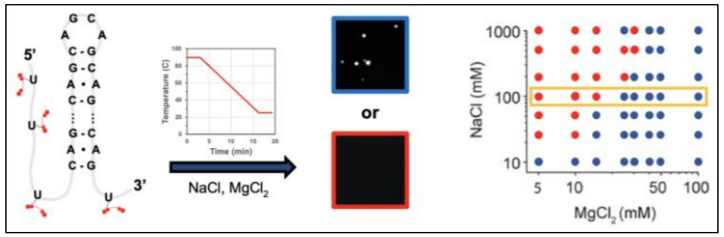

Figure 1. Huntington's CAG RNA Foci form in a temperature and salt dependent process.

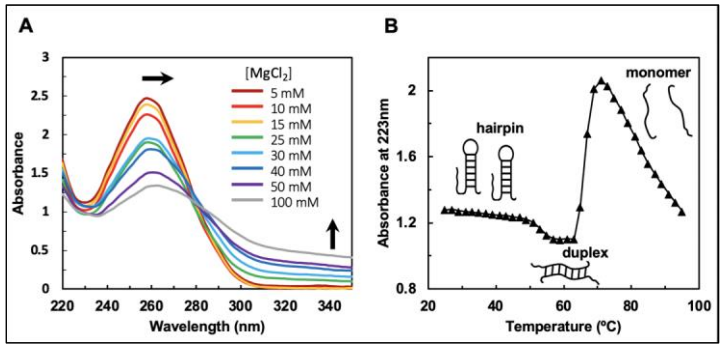

Figure 2. Phase Separation dependent changes in RNA UV-Vis spectra (**A**) can be used to identify structural transitions (**B**).

Kinetic Regulation of Splice Sites

Failure to properly recognize 5' splice sites during pre-mRNA splicing may affect assembly of spliceosome components – resulting in disease-prone outcomes. Due to this, it is important to fully characterize interactions that dictate splice site recognition. SRSF1 and U1-70K, two RNA-binding proteins involved in splicing, are thought to interact with each other via their RNA-recognition motifs (RRMs). **How do the proteins, U1-70K and SRSF1, interact with each other and with RNA to catalyze the splicing mechanism?**

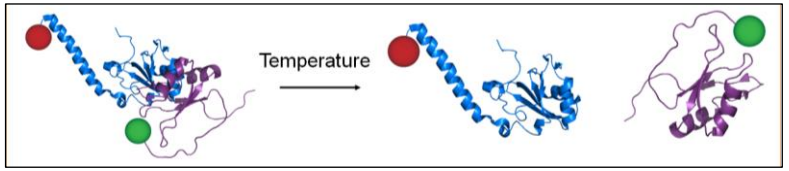

Figure 1. U1-70K and SRSF1 are fluorescently tagged with mCherry and EGFP, respectively. Binding kinetics and thermodynamics will be measured with FRET using FReI.

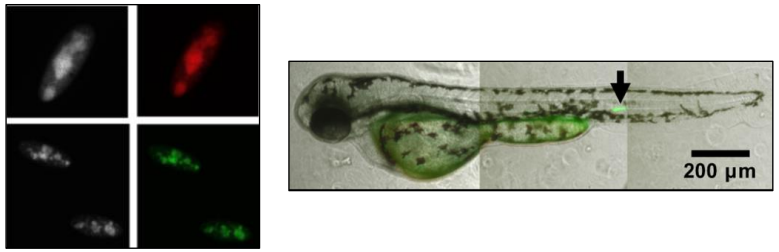

Figure 2. Left: Transfection of plasmid DNA with successful expression of fluorescently-tagged proteins in U2-OS cells Right: Microinjection of plasmid DNA into living zebrafish embryos with transient expression of FRET-labeled protein.