Fluorescent Molecular Rotors as Probes for Biomolecular Condensates

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Background

Biomolecular condensates have a highly dynamic nature and are crucial to a variety of cellular processes, such as enhancing and controlling biomolecular reactions. It has also become apparent that physical parameters of biomolecular condensates are not uniform across condensates and that condensates show variance in these physical parameters such as viscosity. Viscosity is thought to be of great significance to the underlying function of condensates where transitions from liquid-like to gel-like and sometimes glass-like properties informs their function. A potential probe for monitoring and mapping condensate viscosity are fluorescence molecular rotors (FMRS) which are environmentally sensitive dyes. The photophysical properties (fluorescence intensity, fluorescence lifetime, emission wavelength) of FMRs are highly sensitive to changes in viscosity and, therefore, accurately report on the physical state of the surrounding environment.

Aim

Determine if fluorescence molecular rotors are useful for assessing condensate properties

Method

Several FMRs were examined for their quantitative sensitivity to changing viscosity by mixing them with different concentrations of glycerol and assessing their diffusion speed using fluorescence correlation spectroscopy and their fluorescent lifetimes using FLIM.

FMRs were then used to probe the material states of several proteins capable of undergoing condensation including DDX4, hnRNPA2 (WT and D290V), UBQLN2 (WT and T487I).

Results

Photophysical responses of FMRs to increasing concentrations of glycerol showed a viscosity dependent increase in fluorescence intensity and fluorescence lifetime that correlated with decreased diffusion speeds. Interestingly, our control dye fluorescein (not an FMR) showed interesting photophysical properties in glycerol, indicating that fluorescein potentially interacts with glycerol.

We found that the FMR sulforhodamine-B (SRB) had excellent properties of being highly soluble, bright, and extremely responsive to changes in viscosity. Usage of this dye in the presence of protein condensates, showed changes in fluorescence lifetime that correspond to an increased viscosity in protein droplets. However, we also observed a phenomenon where by high-powered excitiation of a sample containing dilute protein and SRB resulted in the formation of droplets potentially through a charge-based mechanism.

Conclusion

Our work shows that FMRs can be used to probe the material states of purified droplets, but care must be taken for interpretation. Importantly, the ability of excited dyes to increase the condensation of samples is a confounding component to FMR usage as probes but does raise the usage of such dyes as methods to seed or alter the properties of condensates in the future.