Single-Molecule-Sensitive Fluorescence Measurement of RNA in Attoliter Droplets

Single fluorophores are unique and sensitive reporters of their nanoscopic environment. Techniques such as single molecular-pair fluorescence resonance energy transfer (FRET) have become a cross-disciplinary tool for understanding molecular folding and interactions with unprecedented detail. While providing detailed information about the individual members of a molecular ensemble, these techniques are always limited by fluorophore brightness and stability and the perturbative effects of methods, such as surface attachment, that are commonly used to isolate molecules for observation. Confining molecules in attoliter volume (100 nm radius) aqueous droplets in perfluorinated liquids provides significantly higher signal-to-noise and a much wider dynamic range than FRET from molecules diffusing in solution. It also has the potential to obviate many of the problems associated with surface attachment.

I will report here on recent single-molecule-sensitive measurements from dye-labeled duplex RNA confined to attodroplets, which both improved the fidelity of single-molecule measurements and revealed surprises associated with the nature of the droplet environment.