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Tracking Viscosity Changes During the Liquid-liquid Phase Separation and Aggregation of A-synuclein Using Fluorescence Lifetime Imaging Microscopy

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The self-assembly of α-synuclein (α-syn) into oligomers and fibrillar structures has received much attention in recent years, owing to their potential role in Parkinson's disease. One pathway which is known to accumulate such species, and possibly alter their formation kinetics and yields, is a process known as liquid-liquid phase separation (LLPS). LLPS generates protein-rich condensates which, eventually, undergo a liquid-to-solid phase transition, leading to the production of α-syn fibrils. To quantify changes in microviscosity accompanying the formation and maturation of these condensates, new optical probes capable of selectively staining the condensates with high contrast are required. In this work, we present results obtained using fluorescence lifetime imaging microscopy (FLIM) using a series of molecular rotors (MRs), whose fluorescence lifetime is sensitive to both changes in microviscosity and increased molecular crowding during protein aggregation. The spatiotemporal resolution of the resulting FLIM images allows a quantitative assessment of the ability of different MRs to act as lifetime probes for α-syn condensates, oligomers and fibrils. This study demonstrates that MRs are a powerful tool for studying biologically relevant LLPS pathways in vitro, and paves the way for the further development of such approaches for in cellulo applications.