Biophysical characterization of a globular protein undergoing Liquid-Liquid Phase Separation

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Biomolecular condensates are involved in a manifold of processes like gene transcription, cellular organization or signal amplification. They form confined but permeable spaces which are in dynamic exchange with their environment. The underlying process of the formation of biomolecular condensates is liquid-liquid phase separation (LLPS). LLPS is a thermodynamically-driven process, in which a homogenous solution separates into a protein-enriched (dense) and a protein-depleted (light) phase. A practical aid to capture the different effects of temperature, pressure or cosolute addition to samples at different concentrations are phase diagrams.

To elucidate the biophysical properties of the proteins undergoing LLPS, complex biological systems are not suitable. Here, we used the globular protein γD-crystallin as an *in vitr*o model to investigate the phase separation behavior in the presence of different cosolutes and the concomitant changes in the biophysical properties of the proteins in the two phases. We present an integrative approach to extract the onset temperature of LLPS by turbidimetry and continuous wave electron paramagnetic resonance (cw EPR) spectroscopy and to characterize the rotational dynamics of crystallin in the two phases using EPR and fluorescence spectroscopy, corroborated by molecular dynamics simulations.[1,2] We further demonstrate the potential of an *in situ* microspectroscopy approach to construct the complete temperature vs concentration phase diagram of γD-crystallin alone and in the presence of the cosolutes trimethylamine-N-oxide (TMAO) and polyethylene glycol (PEG6000). The Raman data are compared with the results obtained with conventional turbidity and centrifugation methods and we showcase the limitations of pulsed EPR spectrosocpy to determine local concentrations in heterogeneous sample.[3]

References:

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