Properties of γD-crystallin undergoing Liquid-Liquid Phase Separation studied by EPR and *In-situ* Raman spectroscopy

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Liquid-Liquid Phase Separation (LLPS) describes the demixing of a homogeneous protein solution into a dense protein-rich and a diluted protein-depleted two-phase system. This sub-compartmentation in the complex environment of the cell creates a confined domain crucial for a manifold of biological processes like the formation membraneless organelles or the recruitement of proteins involved in the assembly of the cytoskeleton.[1]

In contrast to the intrinsic disorder associated to most proteins undergoing LLPS, γD-crystallin is a globular protein with a mass of 20,6 kDa, ubiquitously expressed in the eye lens of vertebrates, which forms molecular condensates in aqueous solutions at relatively high concentration and low temperatures. The presence of co-solutes such as polyethylene glycol and TMAO were shown to stabilize the phase separated state thereby increasing the onset temperature of LLPS.[2]

Here, we used γD-crystallin as a model protein undergoing LLPS, to eluicidate the effects of molecular crowding on its dynamics and to address the changes in solvation and protein density upon droplet formation in the presence of co-solutes. In an integrative approach we investigate the effects of co-solutes on the phase diagram by UV-vis spectroscopy, the dynamics and the local protein density of γD-crystallin in the condensed phase by continuous wave and pulsed EPR spectroscopy and we used *in-situ* Raman spectroscopy to address local protein concentration in the two phases, partitionig of co-solutes and changes in protein hydration.

[1] Zhang W. et al., Front. Microbiol. 12 (2021)
[2] Cinar, H. & Winter, R., Sci Rep 10, 17245 (2020)