Molecular dynamics determine mesoscopic properties of biomolecular condensates

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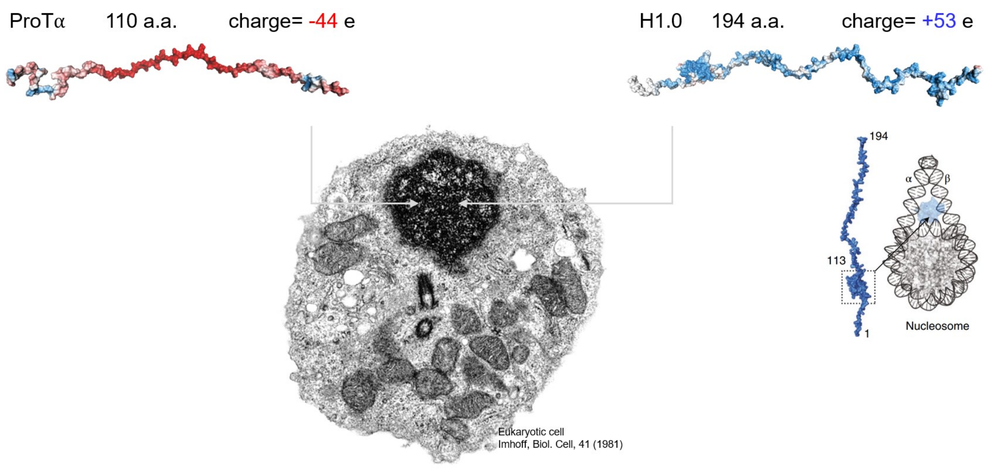
The systems under investigation

We study the dynamical properties of complex coacervates — from the molecular scale to the mesoscale.



Phase separation is driven by charge-charge interactions in these systems. The dynamical properties depend on salt concentration as well as on the different contribution of the positive amino acids Arginine an Lysine.

ProTα: GPMSDAAVDTSSEITTKDLKEKKEVVEEAENGRDAPANGNANEENGEQEADNEVDEEEEEGGEEEEEEEEGDGEEEDGDEDEEAESATGKRAAEDDEDDDVDTKKQKTDEDD

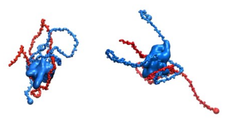
H1: TENSTSAPAAKPKRAKASKKSTDHPKYSDMIVAAIQAEKNRAGSSRQSIQKYIKSHYKVGENADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAKS DEPKKSVAFKKTKKEIKKVATPKKASKPKKAASKAPTKKPKATPVKKAKKKLAATPKKAKKPKTVKAKPVKASKPKKAKPVKPKAKSSAKRAGKKK

Protamine: MPRRRRSSSRPVRRRRRPRVSRRRRRRGGRRRR

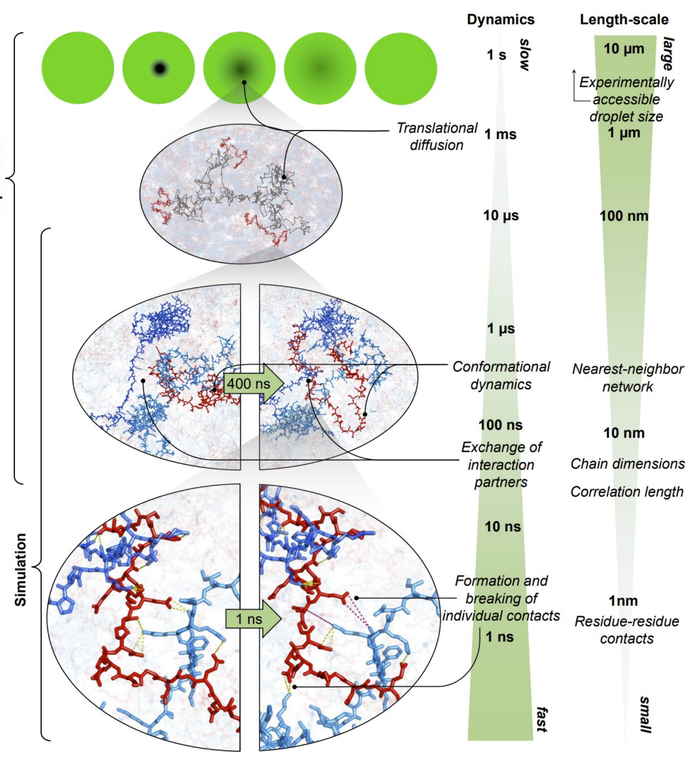
K50: KKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKKK

R50: RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR

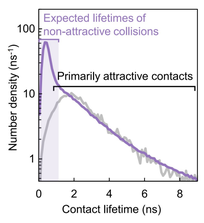
Positive partners of ProTα



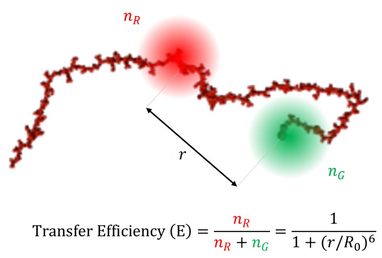
Hierarchy of length and timescales in phase-separated droplets



- Translational diffusion of protein molecules inside droplets is evident in the millisecond timescale in experiments.
- Chain reconfiguration is linked to the rapid exchange between interaction partners on the submicrosecond timescale.
- Intricate networks of competing contacts among residues exchange in nanoseconds.

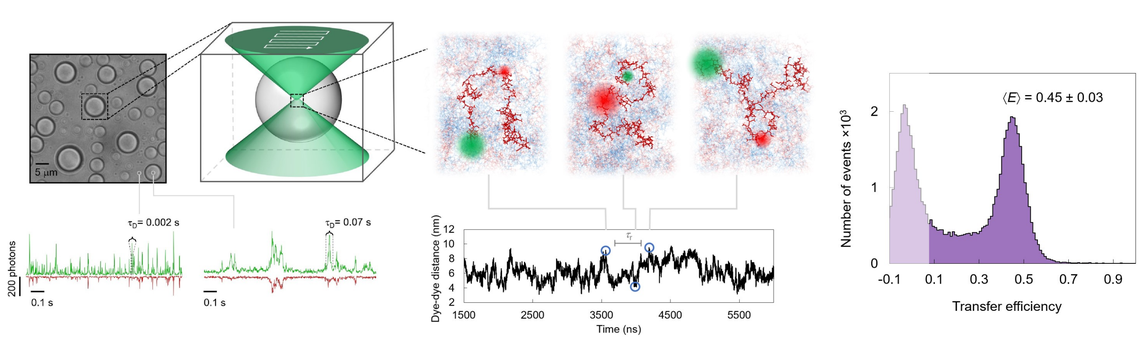


Molecular scale

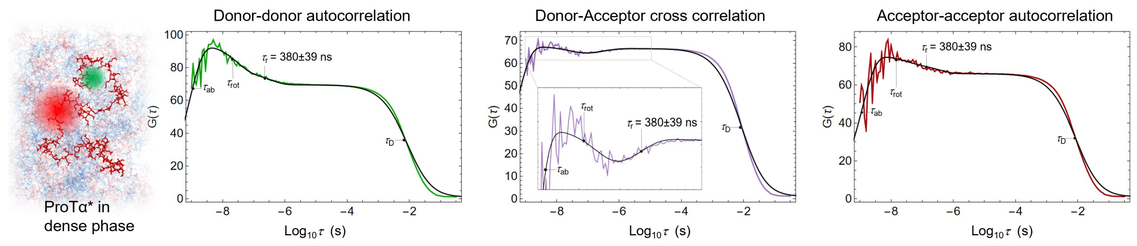


$$\text{Transfer Efficiency (E)} = \frac{n_R}{n_R + n_G} = \frac{1}{1 + (r/R_0)^6}$$

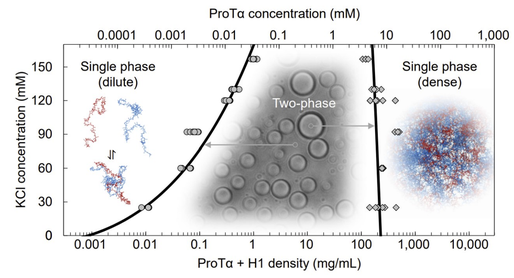
We use single-molecule FRET to measure chain expansion and dynamics of proteins inside droplets.

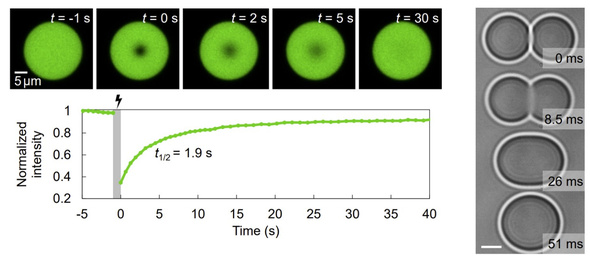


We performed single molecule measurements in the dense phase with pM doping of labeled molecules.

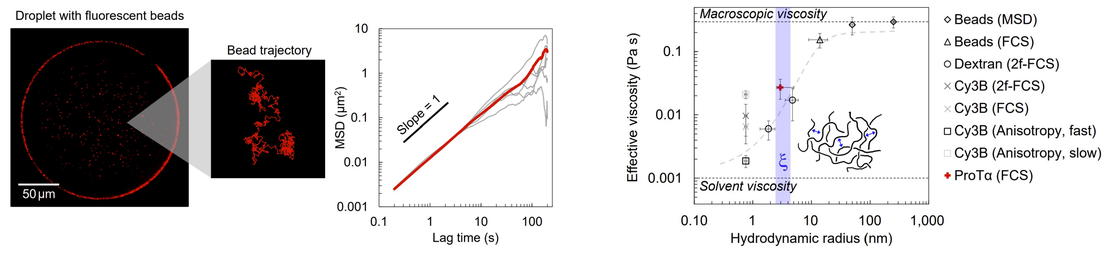


Mesoscale



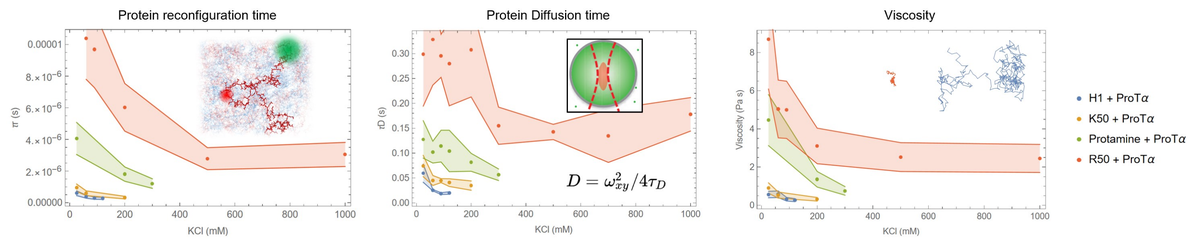


With FRAP we monitored the recovery of single labeled ProTα in the dense phase and, with optical tweezers, we measured the droplet-fusion time.

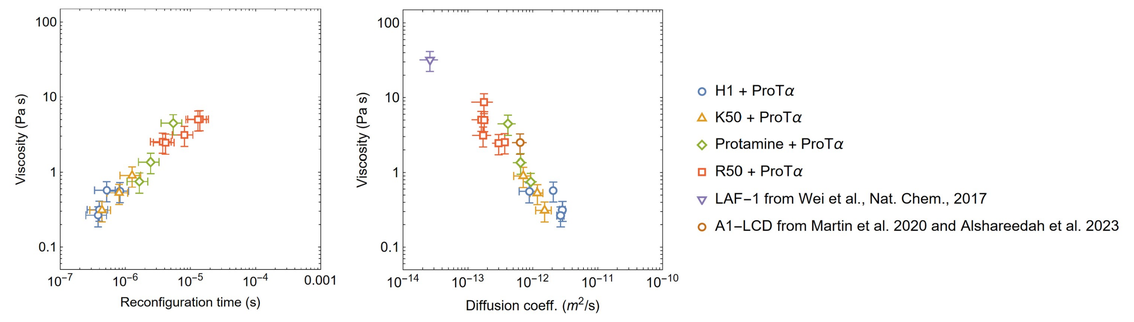


We also systematically probed the diffusion time of fluorescent tracers (beads of different sizes) with FCS, two-focus FCS, and particle tracking. There is a abrupt jump of the size-dependent viscosity ($6\pi r \eta_{app} D_{3D} = k_b T$) over a size range that reflects the size of the mesh. The summary of these rheological investigations is that these droplets are liquid, they have a macroscopic viscosity ∼300 times larger than water (0.30 Pa·s), and ProTα molecules diffuse in the dense phase ∼40 times slower than in the dilute phase.

Correlating microscopic and mesoscopic observables



We measured the chain reconfiguration time of ProTα (with nsFCS) and its diffusion coefficient (with FCS) inside the droplet, as well as the viscosity (with single particle tracking) of the droplets. We performed these measurements at different salt concentrations and in coacervates formed with different partners. The strong correlation among these dynamical quantities suggests the presence of an underlying physical model that link them.



Making sense of these correlations

From Rouse 1953; Doi and Edwards 1986; Rubinstein and Colby 2003; Manias lab (notes).

The Rouse model (developed in 1953) provides a quantitative framework that links the following: *(i)* the friction of single diffusing elements connected by springs to *(ii)* the conformational dynamics of the whole chain, and to *(iii)* the macroscopic viscosity of the polymeric solution. In other words — the equations have predictive power for a range of dynamical properties of biomolecular condensates.