Extreme molecular dynamics in a biomolecular condensate

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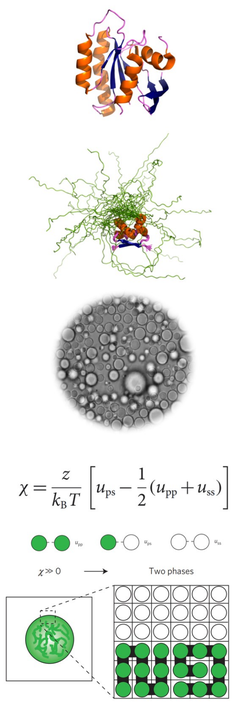
1. Background information



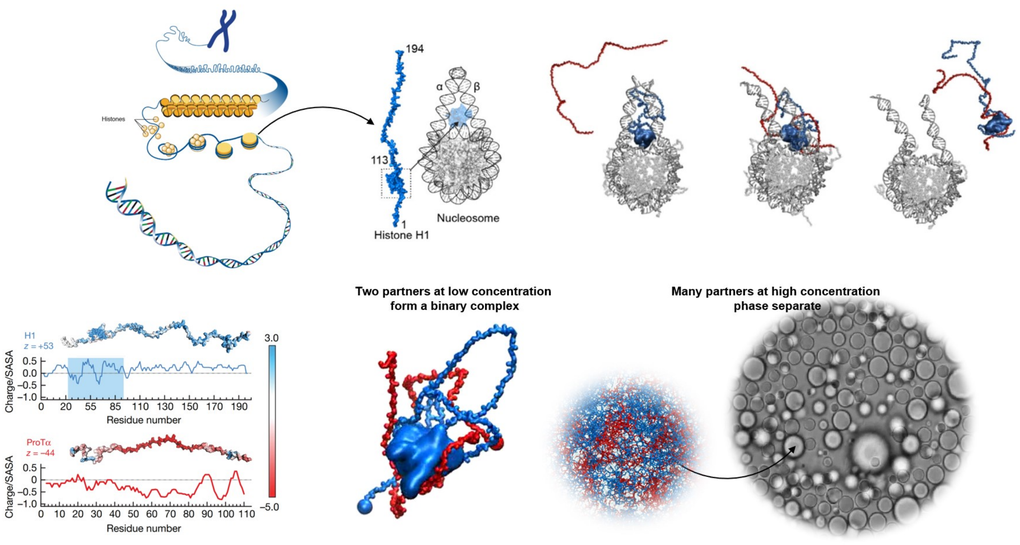
$$\chi = \frac{z}{k_B T}\left[u_{ps} - \frac{1}{2}(u_{pp} + u_{ss})\right]$$

- Biomolecules are the nanomachines of life.
- Proteins are a major class of biomolecules: their amino acid sequence is encoded in the DNA, and a living cell can contain millions of copies of the same protein.
- A single protein is usually involved in a wide range of functions, and it exploits them because of its chemical sequence and its 3D structure.
- The degree of order of a protein 3D structure can vary from fully ordered structures to fully **disordered structures**. The proteins that are mostly disordered are called Intrinsically disordered Proteins (IDPs).
- An IDP can have some attractive interaction sites with another therefore it can form a binary complex: the resulting complex can remain disordered and dynamic.
- Solutions of interacting IDPs above a certain critical concentration can spontaneously phase separate into a dilute phase and into a dense phase (also called *condensates*), **forming droplets**.
- The underlying physical mechanism driving the phase separation can be understood as a minimization of the free energy of the system.

Burger et al., Polymers 2014; Brangwynne et al., Nature Physics 2015.

2. The system under consideration

We are studying the biomolecular condensate of two human intrinsically disordered proteins present in the nucleus of the cell that spontaneously phase separate.



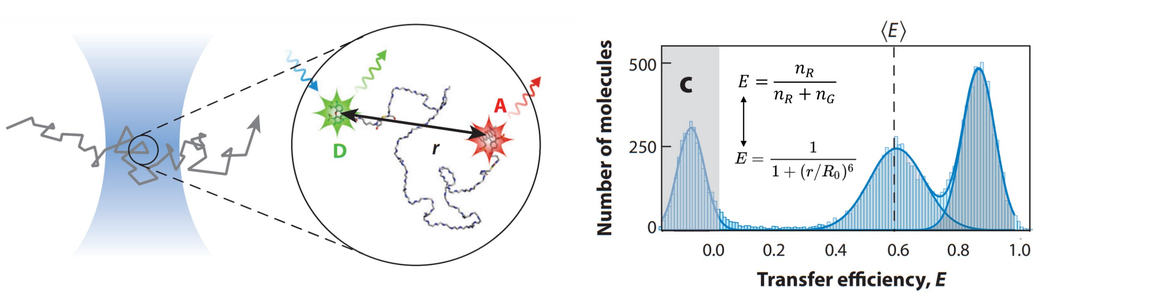
NHGRI (NIH); Heidarsson et al., 2020; Borgia et al., 2018.

3. The scientific questions

- How do molecules behave in the dense environment compared to the dilute one?
- How does the **microscopic** behavior influence the **macroscopic** material properties of the dense phase?

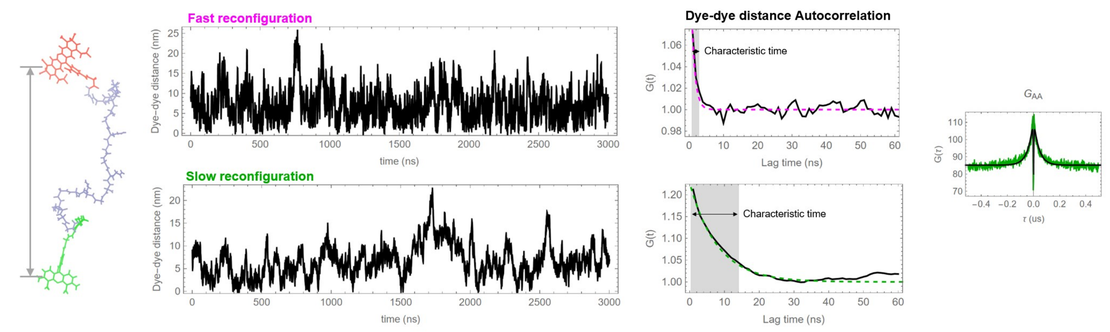
4. How to measure molecules in a droplet (nano-scale)

The key experimental tool used in the Schuler lab is single-molecule Förster Resonance Energy Transfer (smFRET). Two excitable fluorophores can interact and exchange quanta of energy through the EM field, non-radiatively.
The process is highly distance dependent ($\propto R^{-6}$) with a characteristic length scale of a few nm, therefore FRET can be exploited as a time resolved *spectroscopic ruler* at the nanoscale.



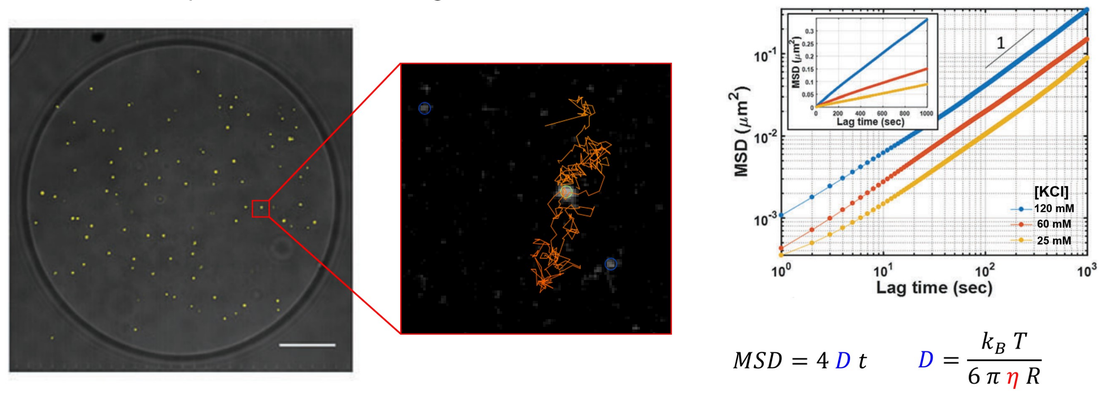
Schuler et al., 2016.

Not only distances: smFRET can be used to measure the chain dynamics of single molecules.



5. How to measure the material properties of a droplet

Rheology provides the tools for measuring relevant properties for characterizing liquids (e.g. viscosity, surface tension). However, when the systems under investigation are human proteins, the amount of sample available for the experiments might be a limiting factor, therefore the standard rheological instruments cannot be applied. In order to measure the viscosity of a very limited amount of liquid, a suitable technique consist of tracking the diffusion of micron sized beads inside of it.

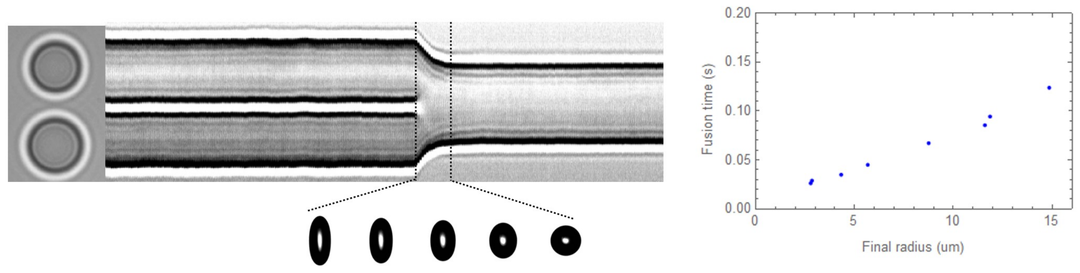


$$MSD = 4\,D\,t \qquad D = \frac{k_B\,T}{6\,\pi\,\eta\,R}$$

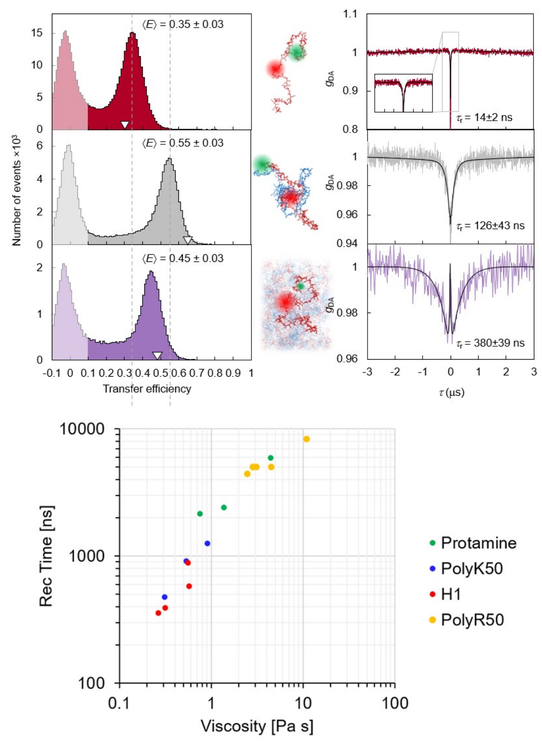
Stokes-Einstein equation

Burger et al., Soft Matter 2018.

The ratio between the surface tension and the viscosity of these droplets can be assessed by measuring the fusion time between two droplets (more precisely, the relaxation time of the single deformed droplet resulting after fusion). These experiments can be performed with Optical Tweezers, i.e. an instrument based on a microscope that can trap microscopic objects with laser light, and measure forces with extremely high precision.



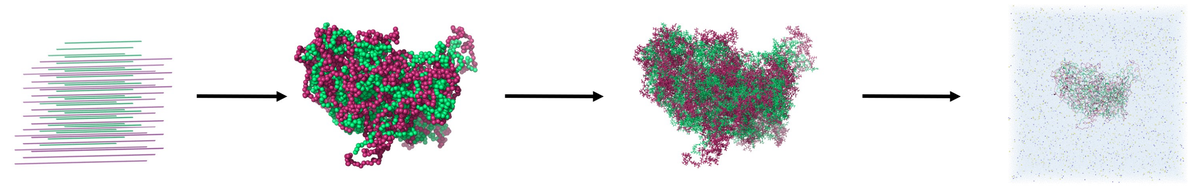
6. What are we learning?



smFRET allows us to measure the average chain expansion in the three different conditions, showing that the proteins in the dense phase are more expanded than in the binary complex, but more compacted than when the protein is in isolation. The biomolecular condensate is an environment in which $\sim 20\%$ of the volume is occupied by atoms of the proteins, it is 2/3 orders of magnitude more viscous than the dilute phase (0.3 Pa s *vs* 0.001 Pa s), but the constituent molecules remain higly dynamic because the reconfiguation time is only two to three times slower than in the dilute phase. We could also investigate how the macroscopic viscosity depends on molecular reconfiguration times.

7. Simulations come to help

Weak multivalent interactions between proteins are the major driving forces of phase separation. Understanding different modes of interaction, such as electrostatic and hydrophobic interactions, H-bonds, π-π and cation-π interactions and their contributions to the self-assembly of the proteins is experimentally difficult to achieve.



All-atom explicit-solvent simulations provide an opportunity to investigate these contributions, given that the simulations are computationally feasible, and that the underlying physico-chemical model is of a reasonable accuracy. In this way we studied the dynamics at atomic scale of the contact formation and breaking among different charged amino acids.