Turn-key characterization of liquid-liquid phase separation

Stender, Emil G. P.; Norrild, Rasmus Krogh; Larsen, Jacob Aunstrup; Jensen, Henrik; Buell, Alexander (2021): Capillary Flow Experiments (Capflex) for Thermodynamic and Kinetic Characterization of Protein LLPS at High Throughput. https://doi.org/10.26434/chemrxiv.14265194.v1

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Robust, high throughput workflow for all aspects of LLPS

Step 01

Sample is in a thermostatted tray above cloudpoint

Step 02

Sample is loaded into a capillary below the cloud point and LLPS occurs

Step 03

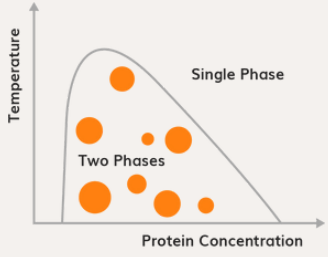
At the detector, each droplet creates a spike in the signal while the light phase sets the baseline

Step 04

Signal intensity determines the dilute phase concentration & thereby, the amount in bio-condensate

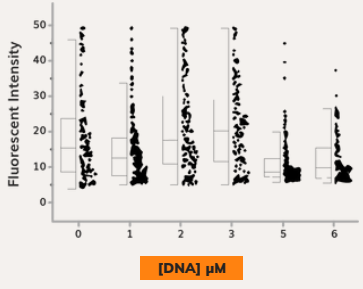
Phase Separation

Fluctuating the factors that drive LLPS, you can determine the transition points from one phase to the other. The phase diagram on the right shows that as the concentration increases we move into the two-phase state. At higher temperatures the two-phase transition occurs at higher concentrations.



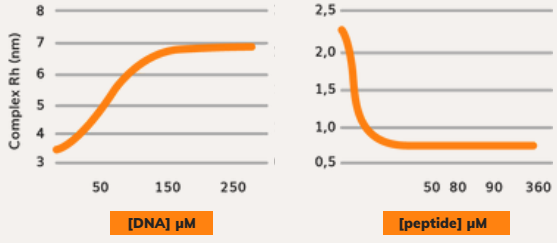
Relative droplet size distribution

The spikes' signal intensity is among others related to the droplets' size. Therefore, it is used to determine the droplet size distribution, which can be measured as a function of the components in the sample (see ssDNA effect on the left).



Measurement of binding affinity

Fida 1 has the built-in function to measure binding affinity. Here, the affinity of ssDNA with Ddx4n1 and RP3 peptide -a bio-condensate model system- is measured.



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