RAPID CHARACTERIZATION OF LLPS WITH FIDA

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Introduction

This poster describes how FIDA is used as the new method to rapidly characterize multiple crucial parameters of = Liquid-Liquid Phase Separation:

- Dilute phase concentrations
- Droplet count
- Relative droplet size
- Distribution
- Knetics of droplet formation
- Maturation into amyloid fibrils
- The affinity between proteins undergoing LLPS and LLPS modulating compounds.

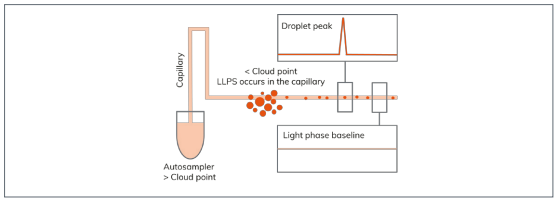
The Fida 1 was the single platform deployed to determine all the above parameters using just a few µL of sample. The poster is based on the paper of Stender, Ray & Norrild et al. published in Nature Communication.[1]

Methods

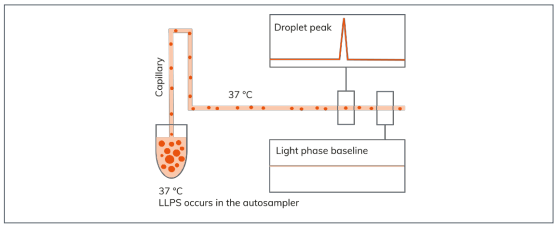
In FIDA, the sample flows from the autosampler, through the capillary and towards the detector, which measures the fluorescence of a labelled molecule, called "indicator". The fluorescence intensity is used to determine the different LLPS parameters.

In this study, the samples are stored either:

1. Above the cloud point temperature in the autosampler and then flowed through the capillary which is kept at a temperature below the cloud point. As a result, LLPS occurs inside the capillary.



2. Or, below the cloud point in the autosampler and injected into the capillary in condensed state.

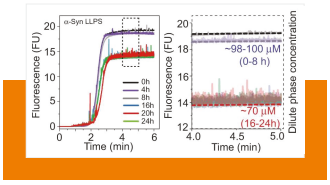


When a droplet passes the detector a signal spike is observed while the baseline corresponds to the dilute phase concentration.

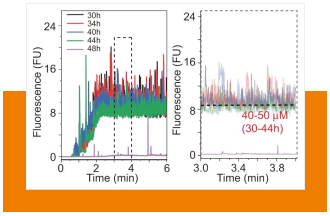
Results

ABERRANT LIQUID TO SOLID TRANSITION OF α-SYNUCLEIN

FIDA enables the study of the thermodynamics of the transition from reversible liquid droplets intoirreversible solid particles. In this experiment, the LLPS behavior of α-Syn was examined by measuring the FIDA signal of α- Syn with Alexa488 labeled α-Syn tracer ("indicator") at an interval of 4 h for a total of 48 h. At each time-point, the sample was diluted with buffer and the reversibility of the droplet formation was monitored by FIDA.



During the first 4 hours, no LLPS was observed as all protein remained in the dilute phase. At 8h, spikes were observed, and the dilute phase concentration slightly decreased indicating the initiation of droplet formation. After 16h, the concentration decreased further, and significant spikes were observed. The spikes disappeared when the sample was diluted, confirming that spike signal originates from liquid droplets.

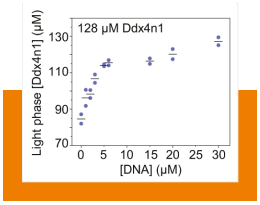


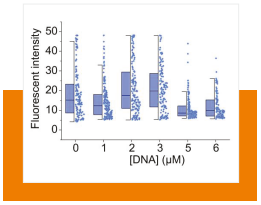
After 30 hours, the dilute phase concentration further dropped, and the spikes could no longer be dissolved by dilution indicating the irreversible liquid to solid transition of α-Syn. After 48h, only a low concentration of α-Syn remained in the dilute phase.

INFLUENCE OF ssDNA ON LLPS OF Ddx4n1

To evaluate the influence of single-stranded DNA (ssDNA) on the LLPS of Ddx4n1, the protein was titrated by increasing concentration of ssDNA. YFP-labelled Ddx4n1 was used as tracer ("indicator").

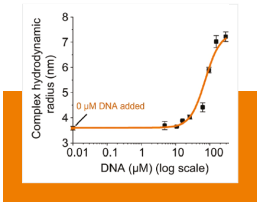
1. When no ssDNA is present, the Ddx4n1 dilute phase concentration is ~88 µM and several spikes are observed indicating that LLPS has occurred. As the concentration of ssDNA increases, so does the dilute phase concentration of Ddx4n1.





2. This is supported by the relative droplet size distribution decrease with increasing ssDNA concentration. The dense phase is completely dissolved at 30 µM ssDNA. This shows that the presence of ssDNA is detrimental to the LLPS of Ddx4n1, indicating a potentially strong and disruptive interaction with Ddx4n1.

3. The interaction between Ddx4n1 and ssDNA was characterized by standard FIDA assay. The dissociation constant (KD) was found to be 50.9 ±11.1 µM, revealing that the affinity is an order of magnitude above the concentration required to dissolve the droplets. This indicates that the effect of ssDNA on Ddx4n1 LLPS is due to multivalent interaction happening at high concentrations inside the droplets.



Conclusions

This scientific work demonstrates the versatility and strength of FIDA in LLPS analysis. Using only µL of sample and without any need for expert users, we described the detrimental effects of ssDNA on the condensation of human Ddx4n1. We also described the LLPS of α-synuclein and the aberrant liquid to solid transition into amyloid fibrils. The results presented in this App Note are described and discussed in detail in Nature Communications[1].

[1]Stender, Ray, Norrild et al. Capillary flow experiments for thermodynamic and kinetic characterization of protein liquid-liquid phase separation. Nature Communications 12, 7289 (2021).