What is the structure of a biomolecular condensate?

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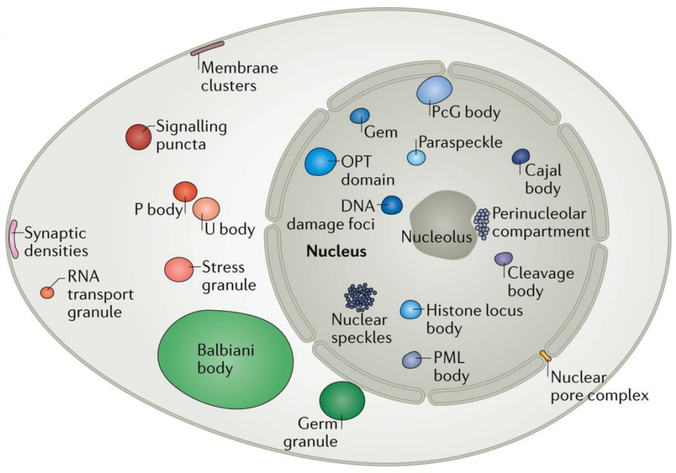
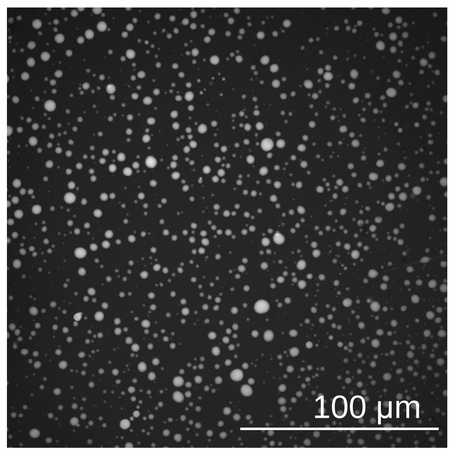
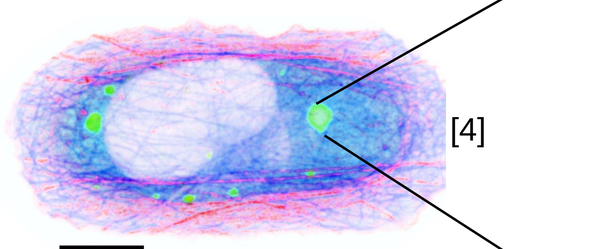


Biomolecular condensates are important for a variety of cellular functions, such as biochemical regulation, structural organization, and RNA metabolism. While the properties and physiology of these condensates depend on their structure, this important aspect has received little experimental consideration. On the other hand, recent simulations of disordered proteins with interactions based on the sticker-and-spacer suggest fascinating structures in the bulk and surface of condensates [1]. We aim to reveal the structure of biomolecular condensates using X-ray scattering. Here, we will present results for a simple model system [2] and apply our approach to the structure of condensates made of disordered proteins.

(Left panel: [3], right panel: FUS droplets imaged by Dr Kathryn Rosowski)



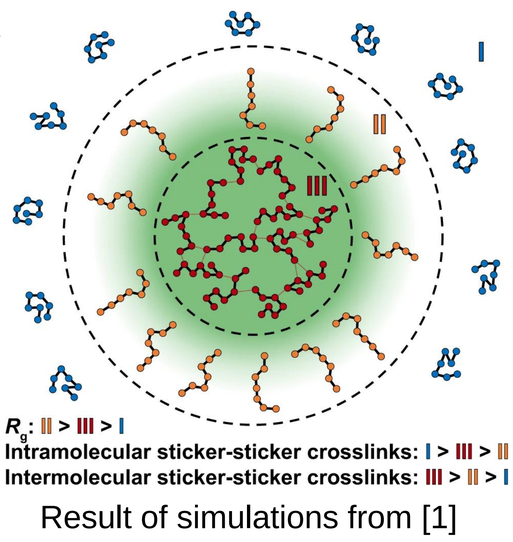
Structure-function relationship for condensates?



[4]

Cell physiology

Structure ?? ⟷ Function



Result of simulations from [1]

Structure of the model system

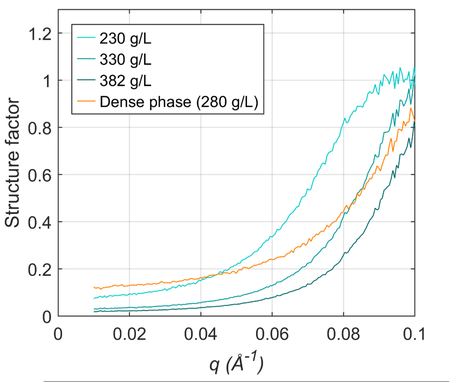
BSA form factor: R_G = 2.9 nm

Dilute phase: R_G = 3.3 nm

High PEG in dilute phase increases BSA size.

Condensate:

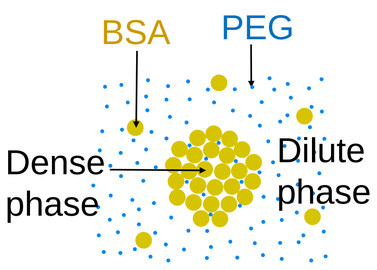
- Heterogeneous
- Higher compressibility than pure BSA
- Peak at ca. 0.1 Å^{-1} ≙ 6.3 nm ≙ 2r_{BSA}
- Pure dense phase: 1500 mg of protein
- Subtraction dilute phase: 3 mg of protein

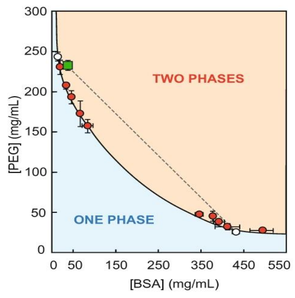


👀 Interested in structure factors? Let's chat!

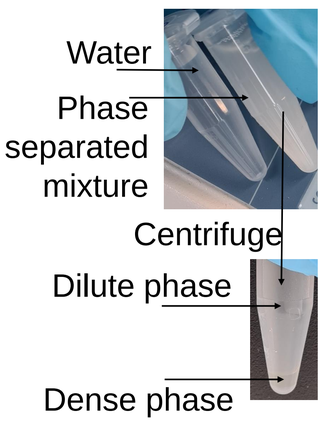
Model system

Bovine serum albumin (BSA, 66.43 kDa) and polyethylene glycol (PEG, 4 kDa)





Phase diagram from [2]



Modeling scattering of disordered proteins

Dilute phase

Intensity ← Concentration & contrast

$I(q) = c' P(q) S(q).$

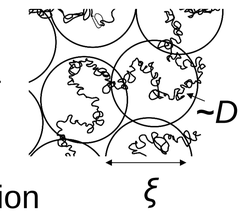
Form ← $P(q)$ $S(q)$ → Structure

Condensate:

Extended Ornstein-Zernike relation

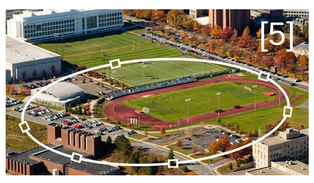
$$I(q) = Aq^{-4} + \frac{B}{\left(1 + \frac{(D+1)}{3}\xi^2 q^2\right)^{D/2}}$$

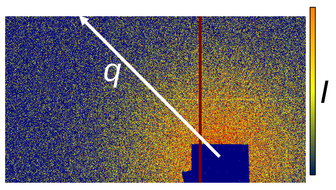
Droplets ← Aq^{-4}; Correlation length ← ξ; Fractal dimension ← D



X-rays to measure structure

SAXS measurements at the Cornell Synchrotron CHESS (NY, USA) and at the ESRF (Grenoble, FR)

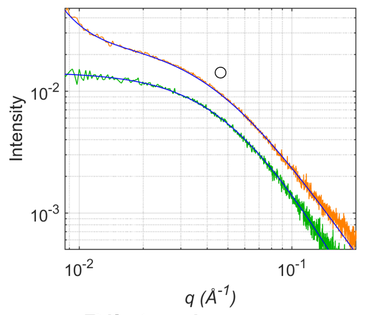




Structure of disordered proteins in and around biomolecular condensates

Dilute phase, condensates: $I_{mix} - I_{dil}$, extended Ornstein-Zernike fit, o: ξ

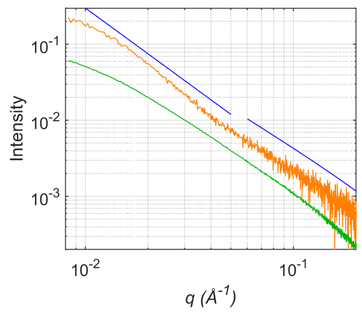
FUS



Condensates:
ξ = 13.7±1 nm
D = 2.5±0.1

Dilute phase:
D = 3.0±0.1

Bik1



Condensates:
ξ = 14.9±1 nm
low q: -2.0
→ Fractal [6]

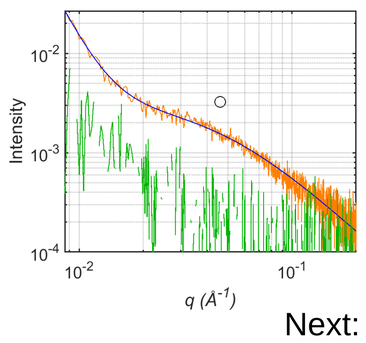
Dilute phase:
D = 1.7±0.1

→ Dilute phase more compact

Next: add solutes

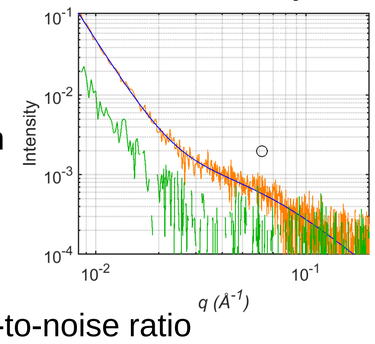
Complex coacervates

H1 + Prothymosin-α [7]



Condensates:
ξ = 15.0±1 nm
D = 2.0±0.2

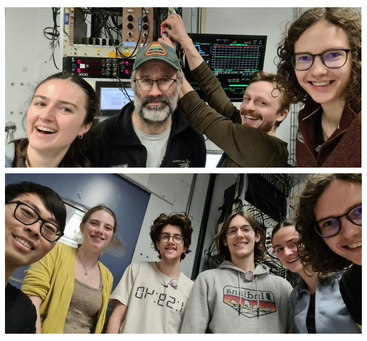
Protamine + Prothymosin-α [7,8]



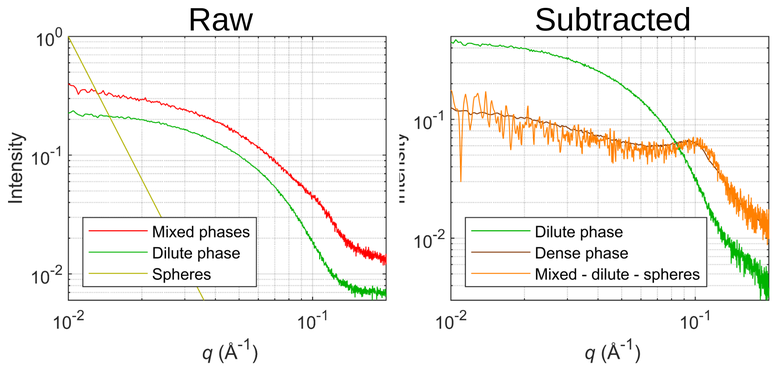
Condensates:
ξ = 10.2±1 nm
D = 2.3±0.2

Next: improve signal-to-noise ratio

Impressions from the beamline



Structure of model system studied with X rays



References:

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[7] N. Galvanetto et al., Nature, 2023.
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Acknowledgements

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