# Entropy of Water in Protein Condensates

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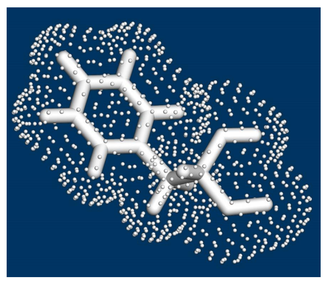
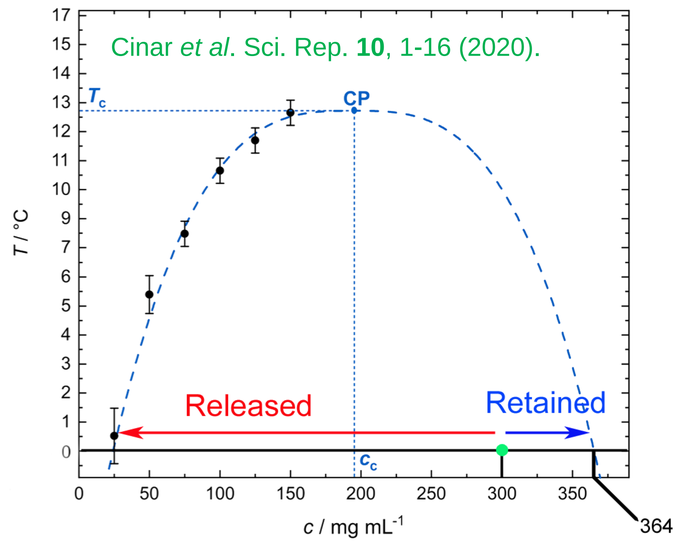
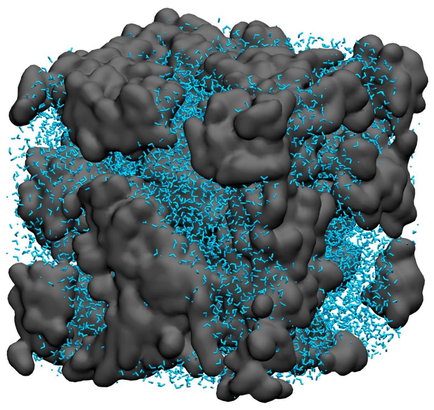


## Does entropy of water contribute to the Liquid Liquid Phase Separation of Proteins?

## Introduction: Protein Condensates

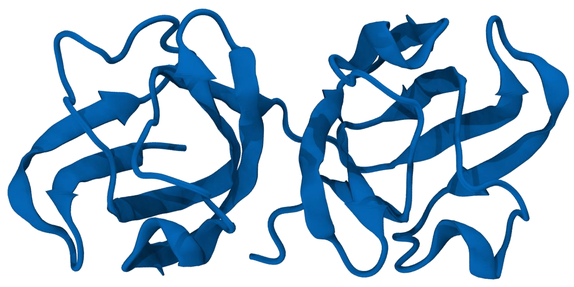


Water in protein condensates

## System

Globular Protein

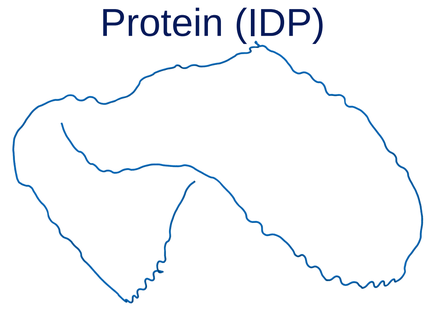


Human γD-Crystallin (PDB: 1HK0)

**Concentrations** (mg mL$^{-1}$)**:**
310, 420, 525, 645

**Temperature:** 273 K

Intrinsically Disordered Protein (IDP)



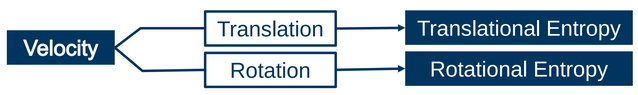
Fused in Sarcoma
Low Complexity Domain (FUS-LCD)

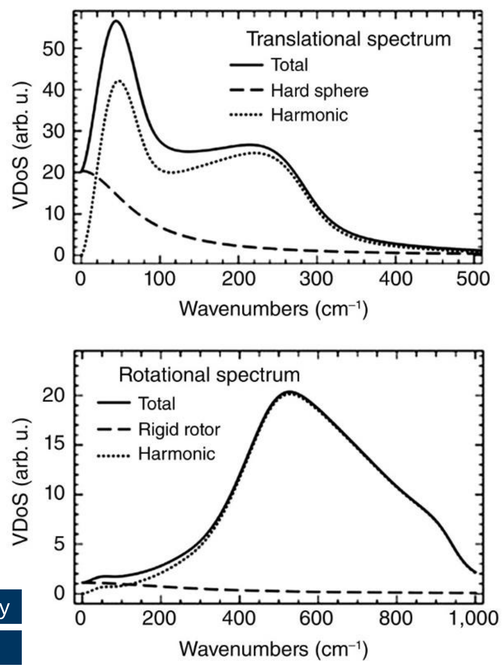
**Concentrations** (mg mL$^{-1}$)**:**
100, 110, 125, 145, 170, 205, 260, 350

**Temperature:** 298 K

## Entropy Calculation: The 2PT Method

- All atom **Molecular dynamics Simulations** in GROMACS 2020.1
- **Entropy calculation:** Two Phase Thermodynamics (2PT) method (Lin *et al*. JCP 119, 11792 (2003).)
- Velocity ACF → Spectral density → Liquid = Solid (Harmonic Oscillator) + Gas (Hard Sphere) → Analytical treatment → Entropy

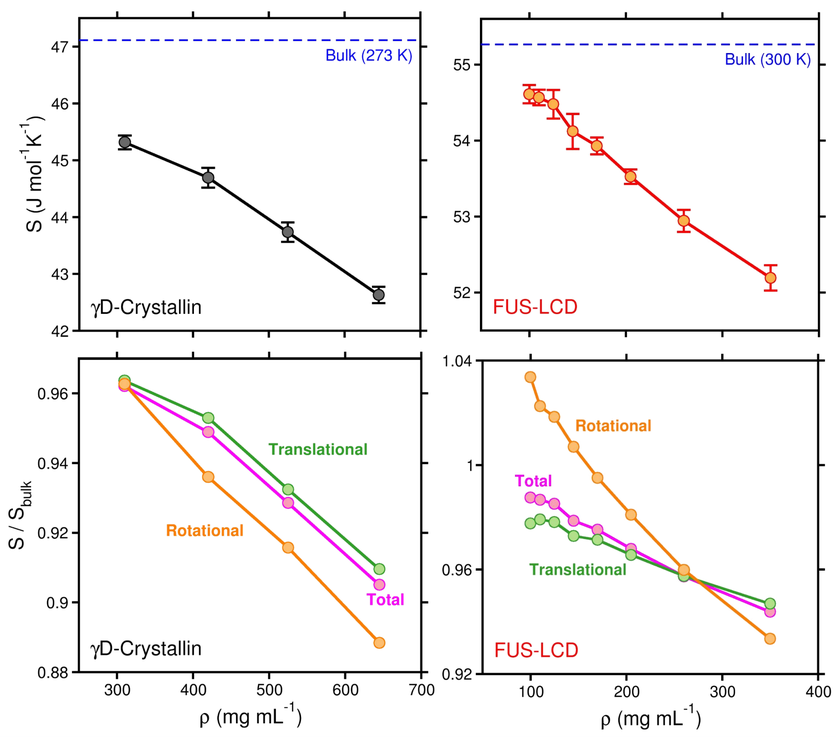






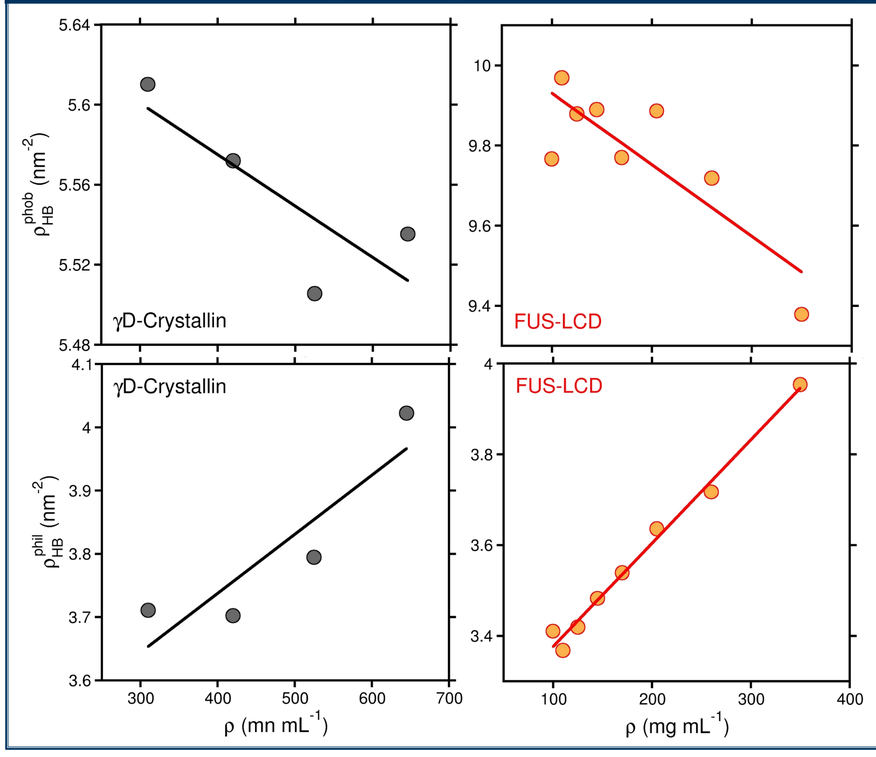
## Entropy against Concentration




Entropy of water in the condensate decreases with increase in concentration

Translational and rotational entropy show a cross over

## Hydrogen Bond Density



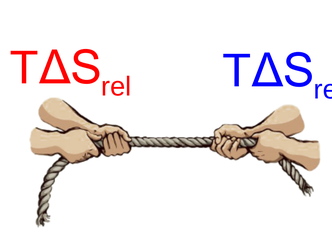

**Hydrophobic** surface density of protein-water HB

**(water release)**

**Hydrophilic** surface density of protein-water HB

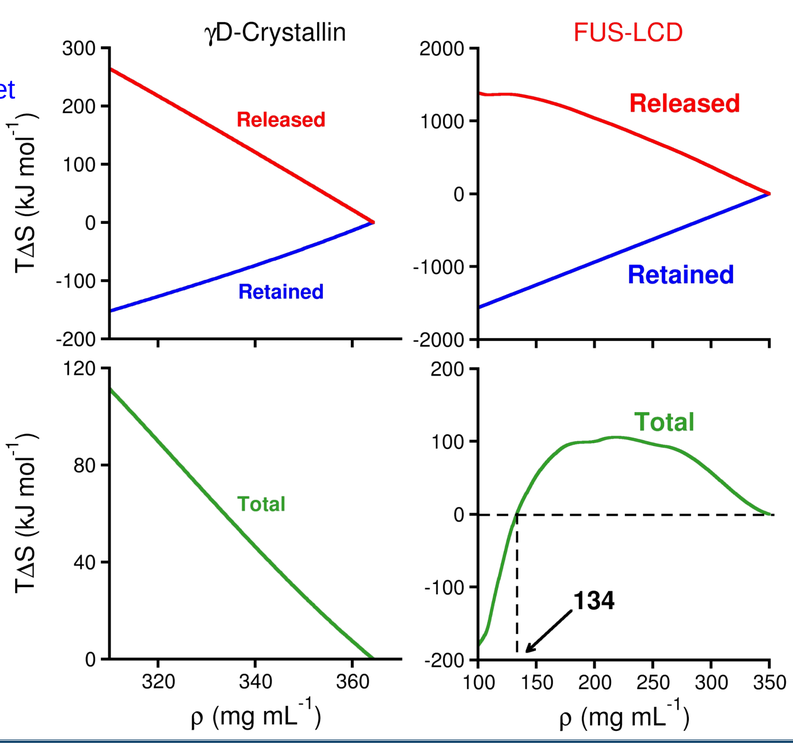
**(water retention)**

## Entropy Bill




$T\Delta S_{rel} + T\Delta S_{ret} > 0$

Concentration after which entropy of released water dominates the total entropy contribution




## Interaction Energy

$$\Delta G_{solv} = \Delta H_{solv} - T\Delta S_{solv}$$

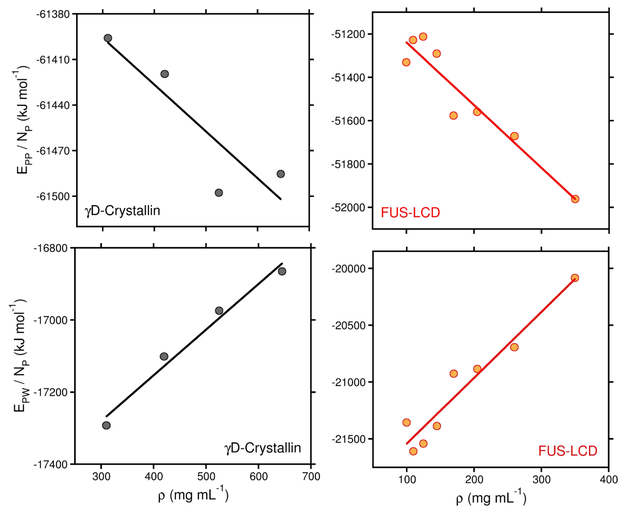
$$\Delta H_{solv} = \langle \Delta U_{pw} \rangle + \langle \Delta U_{ww} \rangle$$

$$\Delta S_{solv} = \Delta S_{pw} + \Delta S_{ww}$$

$$\langle \Delta U_{ww} \rangle - T\Delta S_{ww} = 0$$

Ben-Naim, A. Biopolymers, **14**, 1337-1355 (1975).

$$\Delta G_{solv} = \langle \Delta U_{pw} \rangle - T\Delta S_{pw}$$




## Conclusion and Acknowledgement

- Increase in entropy due to water released into the bulk on condensate formations favors LLPS after a certain protein concentration
- Protein-Protein interactions are enthalpically favorable

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