Yield of protein crystallization from metastable liquid-liquid phase separation

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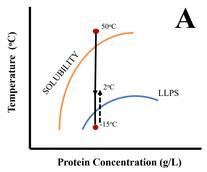
Introduction

Hi! We are types of chromatography and are used for purifying compounds in mixtures. We do a great job but many people who use us think we are expensive and don't purify compounds in large quantities. 😕

Preparative protein crystallization is an alternative for chromatography



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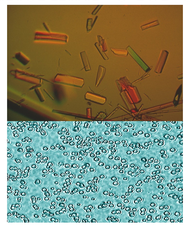

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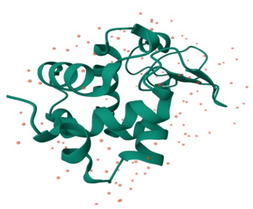
Figure 1: (**A**) Temperature-concentration phase diagram showing crystal solubility and LLPS phase boundaries. LLPS is metastable with respect to protein crystallization. (**B**) Protein-rich microdroplets generated by LLPS. (**C**) Protein Crystals.

Goal: Developing a new strategy for enhancing protein crystallization from metastable protein-rich droplets generated by **liquid-liquid phase separation** (LLPS) of protein aqueous solutions.

Lysozyme, our model protein.

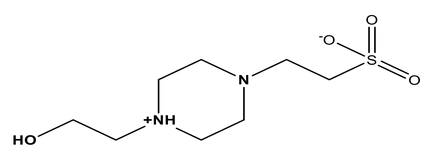


Proposed strategy to enhance protein crystallization from droplets: Introduce two additives: LLPS inducer **(1)** and LLPS modulator**(2)**.

(1) Na^+ Cl^-

Sodium Chloride (NaCl), **Inducer**

(2)



4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), **Modulator**

Experimental Methods

LLPS mediated protein crystallization

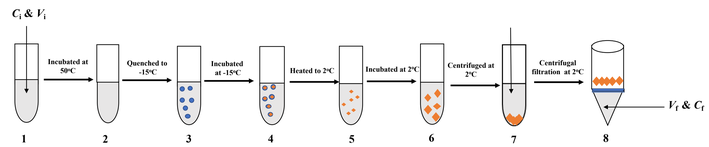


Figure 2: Schematic diagram showing LLPS mediated protein crystallization of Lysozyme aqueous samples. All initial protein concentrations were about **50 g/L.**

$$yield = 1 - \frac{C_f}{C_i}\frac{V_f}{V_i}$$

Where:
C_f is final supernatant concentration
C_i is initial sample concentration
V_f is final supernatant volume
V_i is initial sample volume

Results and Discussion

Effect of HEPES on LLPS promoted crystallization of lysozyme

- HEPES significantly reduces LLPS boundary; acting as a salting-in agent.
- HEPES produces the highest yield of protein crystallization; acting a salting-out agent.

Table 1: LLPS temperature and yield of crystallization for Lysozyme aqueous samples of various inducer-modulator pairs.

Inducer-modulator pair system	LLPS Temperature (°C)	Yield of Crystallization (%)
0.1M NaCl- 0.1M HEPES	-12.6	92.3
0.1M Phosphate buffer- 0.1M HEPES	-7.4	42.6
0.1M Phosphate buffer- 0.1M NaCl	-1.6	5.6
0.1M phosphate buffer- 0.1M Taurine	-5.6	7.3
0.1M phosphate buffer- 0.1M HEP	-4.2	2.2
0.2M Phosphate buffer	-4.3	6.5

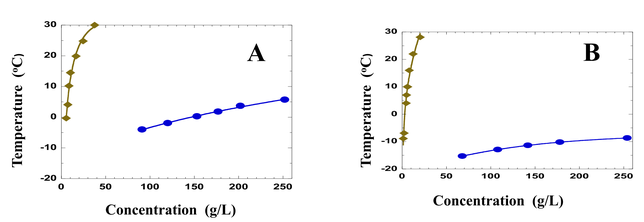


Figure 3:**(A)** Temperature-concentration phase diagram of inducer NaCl but no modulator **(B)** Temperature-concentration phase diagram of inducer-modulator pair NaCl-HEPES.

Effect of incubation time and temperature on yield of crystallization

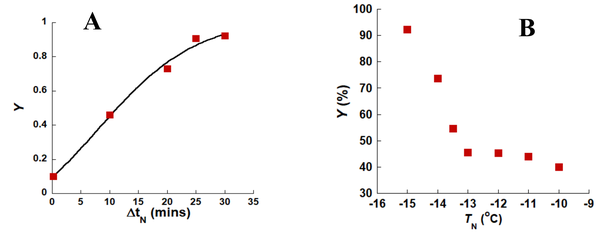


Figure 4: **(A)** Effect of incubation on crystallization yield of lysozyme-0.15M NaCl-0.10M HEPES system at pH 7.4. Samples incubated at (50°C) =30 min, quenched at T_N (-15°C) for Δt_N=0-30min, and incubated at T_{CG} (2°C) for Δt_{CG}=30 min. **(B)** Effect of incubation temperature on yield of crystallization of lysozyme-0.15M NaCl-0.1M HEPES system at pH 7.4. Samples incubated at (50°C) =30 mins; quenched at T_N for Δt_N = 30mins and incubated at T_{CG} (2°C) for Δt_{CG}=30 mins.

Conclusion

- Yield of crystallization significantly increases with incubation time up to 30 mins.
- LLPS enhances protein crystallization.
- This LLPS-mediated protein-crystallization strategy will be applied to other proteins like ribonuclease A and human serum albumin.
- I plan to explore how inorganic nanoparticles that can weakly adsorb proteins may enhance protein crystallization from protein-rich liquid droplets.

References

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