Yield of protein crystallization from metastable liquid-liquid phase separation

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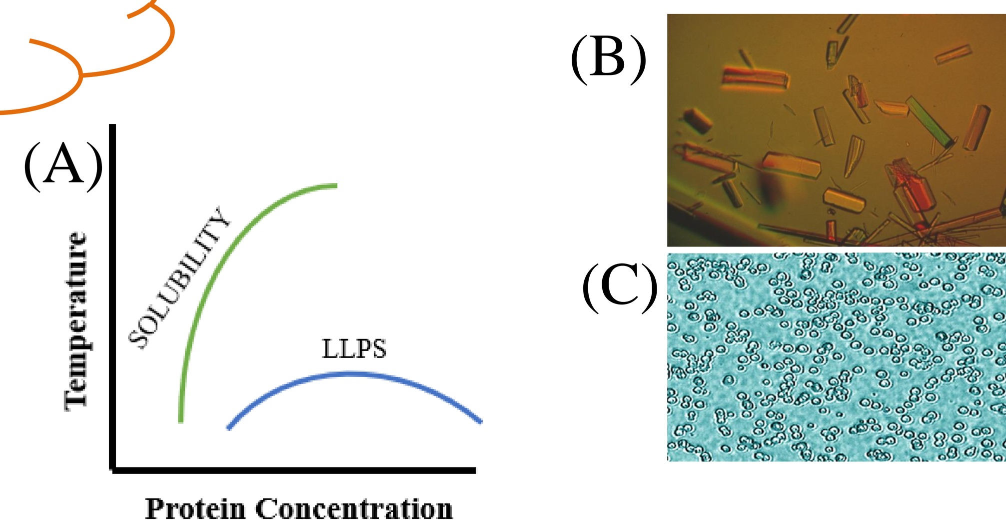
Introduction

A promising alternative for protein purification is preparative protein crystallization. This purification technology can potentially offer significant economic advantages in protein downstream processing.

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.

What is LLPS?

(A)

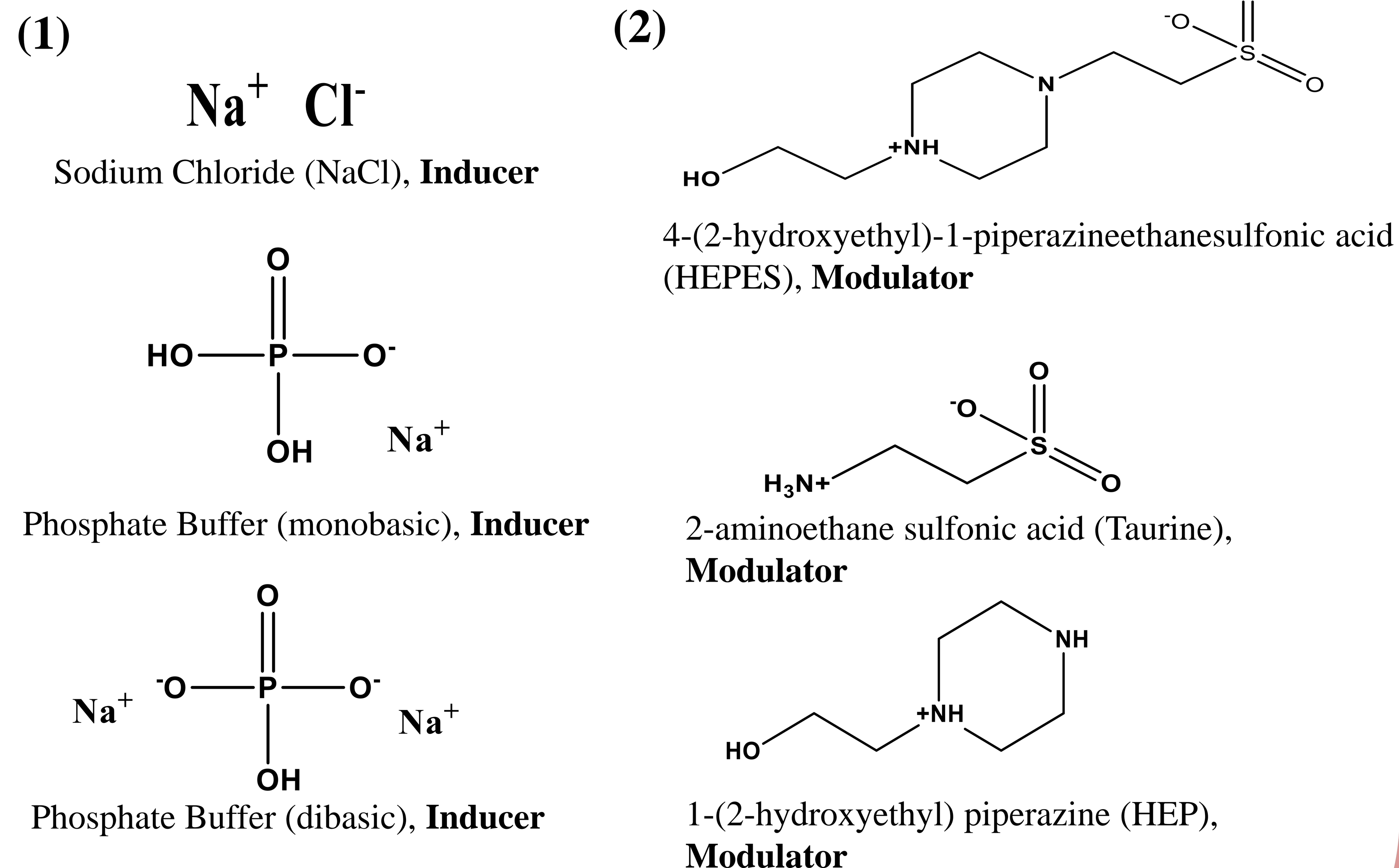


(B)

(C)

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

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**

Phosphate Buffer (monobasic), **Inducer**

Phosphate Buffer (dibasic), **Inducer**

(2)

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

2-aminoethane sulfonic acid (Taurine), **Modulator**

1-(2-hydroxyethyl) piperazine (HEP), **Modulator**

This strategy is employed to the protein Lysozyme.

Experimental Methods

Determination of LLPS temperature

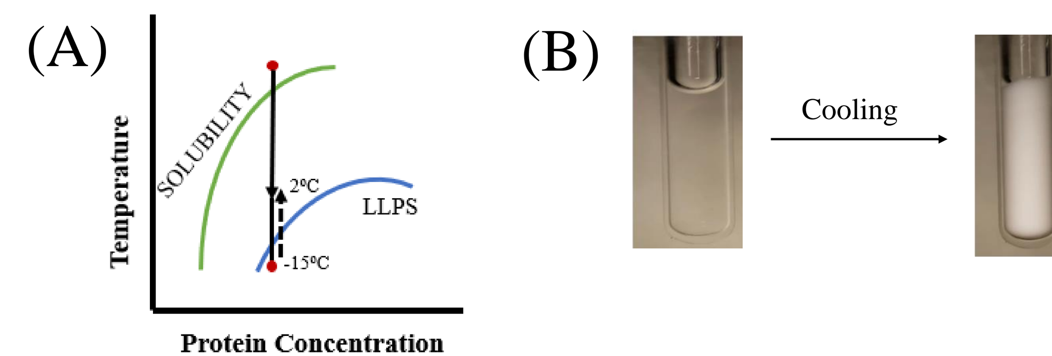


Figure 2: (A)Schematic diagram showing sample thermal history on the phase diagram. (B)LLPS temperature is obtained by monitoring how sample turbidity changes with temperature.

LLPS mediated protein crystallization

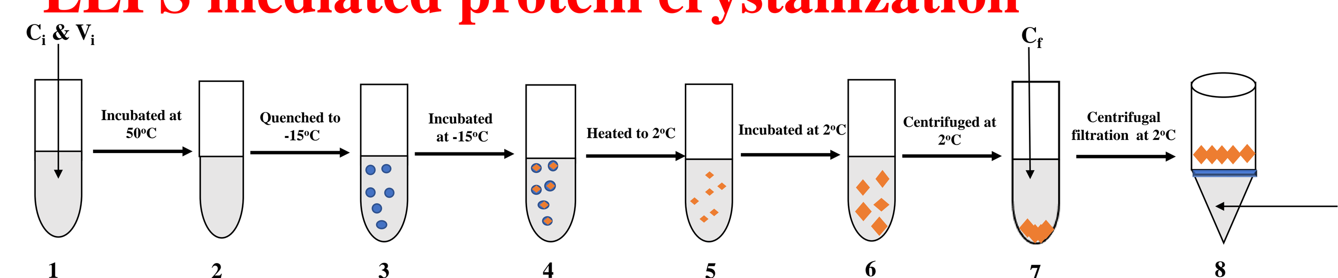


Figure 3: Schematic diagram showing LLPS mediated protein crystallization of Lysozyme aqueous samples. All initial protein concentrations were about **60 g/L.** Samples incubated for 30 minutes.

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

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

- LLPS is observed in the presence of phosphate buffer and NaCl as inducers.
- Among LLPS modulators, HEPES is the most effective in reducing LLPS temperature.

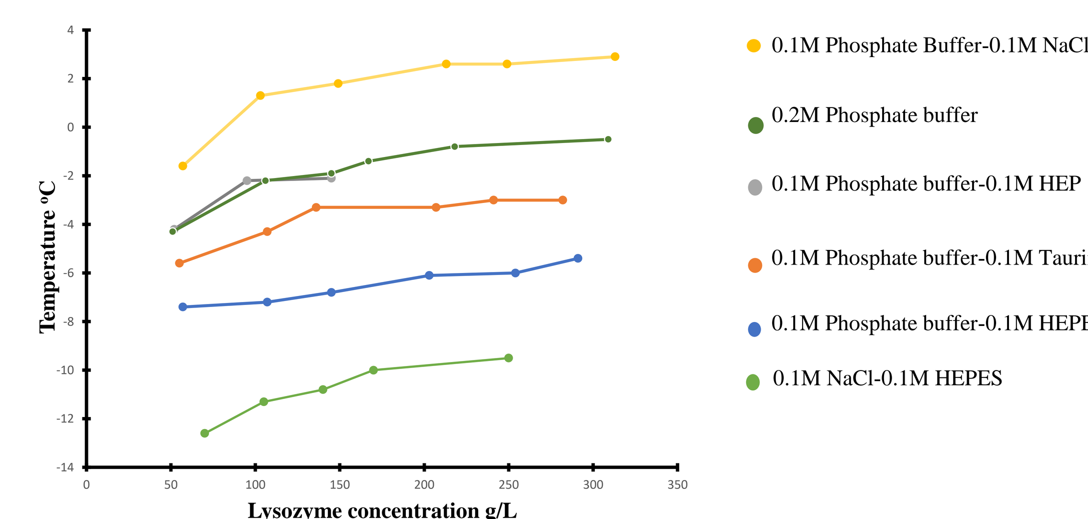


Figure 3: Temperature-Concentration LLPS boundaries of aqueous Lysozyme with various inducer-modulator-pairs at pH 7.4.

HEPES also produced the highest yield of protein crystallization.

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

Conclusion

- HEPES decreases LLPS boundary and provides a large yield of protein crystallization.
- Taurine and HEP have marginal effect on LLPS temperature and provides a negligible yield of protein crystallization.
- Sodium Chloride, which is the most employed crystallizing agent for lysozyme, increases LLPS temperature but provides a negligible yield of crystallization.
- This LLPS-mediated protein-crystallization strategy needs to applied to other proteins

References

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