Effect of HEPES on Lysozyme Crystallization from Metastable Protein Rich Droplets

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Introduction

The high demand for protein production in the pharmaceutical and biotechnological fields has prompted a need for economically-sustainable protein purification technique in protein downstream processing.

Preparative protein crystallization is considered a more economically sustainable strategy for achieving protein purification than chromatography, but it is not a well understood process and still relies on empirical protocols.

Liquid-liquid phase separation (LLPS) of protein solution is a promising strategy to efficiently obtain protein crystals as illustrated in Figure 2. This process is favored by a reduction of crystal surface tension. The phase diagram of a protein aqueous mixture can be used to describe crystallization thermodynamics (Fig.1). Both LLPS and crystallization are driven by protein-protein attractive interactions (Galkin et al., 2000)

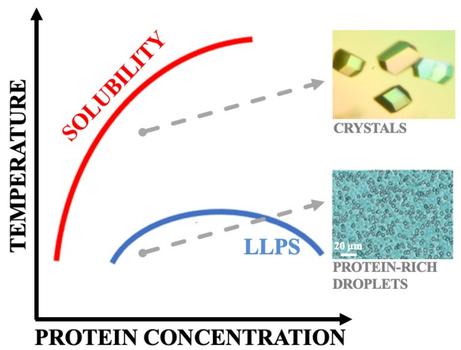


Figure 1. Schematic temperature-concentration phase diagram showing crystal solubility and LLPS phase boundaries.

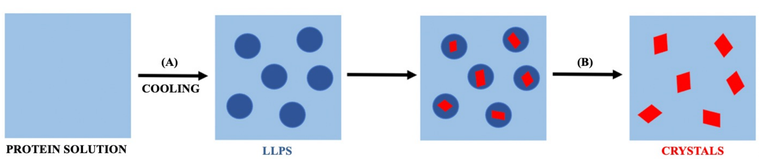
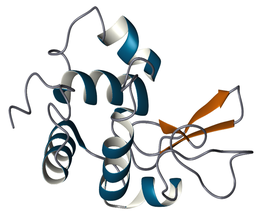


Figure 2. Simplified diagram showing the main operations in protein crystallization through LLPS. Step **(A)** cooling of protein solution inducing the formation of metastable protein-rich droplets (LLPS). Each droplets may produce a protein crystal **(B)**.

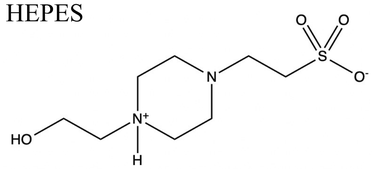
Results and Discussion

The Choice of System

Protein: Lysozyme
Inducer: NaCl
Modulator: HEPES



Lysozyme



4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)

The presence of HEPES enables LLPS based lysozyme crystallization (Fig.3).

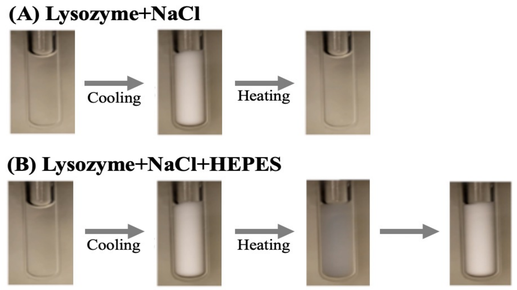


Figure 3. Visual images showing sample turbidity upon cooling and heating for the lysozyme+NaCl **(A)** and lysozyme+NaCl+HEPES **(B)** systems.

Addition of HEPES lowers the LLPS temperature. This indicates that HEPES is a salting-in agent.

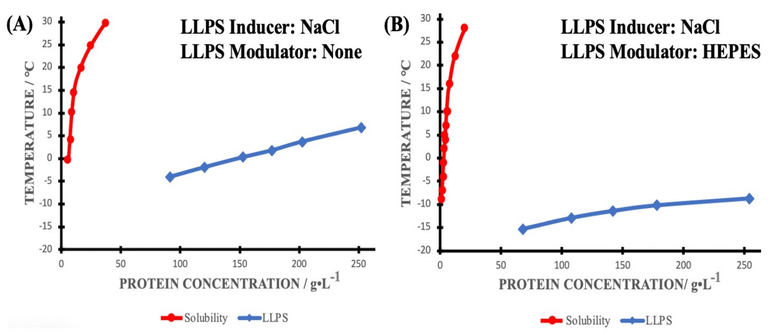


Figure 4. Crystal solubility (circles) and LLPS boundary (diamonds) for the lysozyme+NaCl (0.15M)+water system at pH 7.4 **(A)** and the lysozyme+NaCl(0.15M)+water system at pH 7.4 **(B)**.

Lowering LLPS temperature produces larger crystals supersaturation.

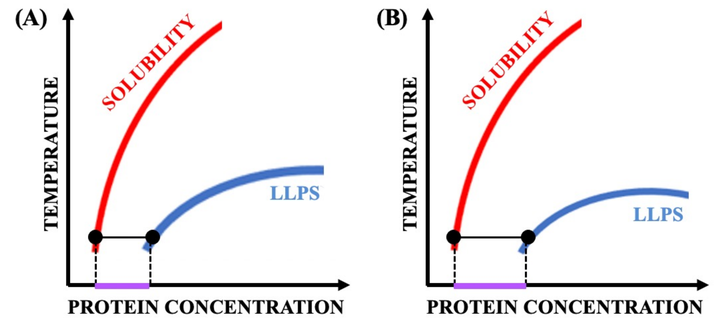


Figure 5. **(A)** LLPS with inducer but no modulator. Sample saturation to crystallization, which is shown by the horizontal gap between two dashed vertical lines, depends on the relative position of solubility and LLPS boundaries. **(B)** LLPS modulator lowers the relative position of the LLPS boundary with respect to crystal solubility curve thereby increasing supersaturation

Conclusion

HEPES lowers LLPS temperature and promotes protein crystallization. This is related to an increase in crystal supersaturation in LLPS conditions. This LLPS mediated protein crystallization strategy can be used in protein purification protocols. This strategy needs to be explored for other proteins to demonstrate generality.

References

Fahim, A.; Annunziata, O., Effect of a Good buffer on the fate of metastable protein-rich droplets near physiological composition. *International Journal of Biological Macromolecules* **2021**, 186, 519-527.

Galkin, O.; Vekilov, P. G., Control of protein crystal nucleation around the metastable liquid-liquid phase boundary. *Proc. Natl. Acad. Sci. U. S. A.* **2000,** *97* (12), 6277-6281.

Hubbuch, J.; Kind, M.; Nirschl, H., Preparative Protein Crystallization. *Chemical Engineering & Technology* **2019,** *42* (11), 2275-2281.

Park, S., Barnes, R., Lin, Y., Jeon, B. J., Najafi, S., Delaney, K. T., ... & Han, S. Dehydration entropy drives liquid-liquid phase separation by molecular crowding. *Communications Chemistry* **2020**, *3*(1), 83.