Polyethylene Glycol as an LLPS Temperature Promoter for Protein Crystallization

Joel Dougay, Shamberia Thomas, and Onofrio Annunziata, Ph.D.
Texas Christian University
Department of Chemistry and Biochemistry

Introduction

Protein crystallization is a promising alternative to chromatography as a protein purification strategy, as it does not suffer some of the limitations of chromatography, such as limited loading capacity, scalability and high operation costs. Protein crystallization, though it is promising, is a complex and not well-understood process. In our lab, a new strategy for enhancing protein crystallization from metastable protein-rich droplets was examined using lysozyme, a model protein, requiring the use of two additives. The first additive (inducer, HEPES) promotes metastable liquid-liquid phase separation (LLPS) in a protein aqueous sample. The second additive (modulator, NaCl) alters the composition of droplets and their thermodynamic stability. The phase diagram in Fig. 1 shows the crystal solubility and LLPS boundaries. While HEPES is shown to enhance yield of crystallization, LLPS temperature is reduced significantly. In this poster, our goal is to develop strategies to increase LLPS temperature by using polyethylene glycol (PEG). PEG promotes protein-protein attractive interactions through the macromolecular crowding effect, shown in Fig. 2. This leads to an increase in LLPS temperature. Thus, we examine the effect of PEG on LLPS temperature and overall crystallization yield.

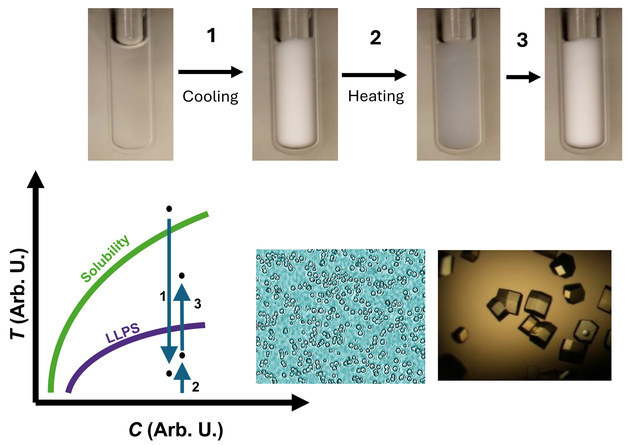

FIGURE 1. Schematic Temperature(*T*)-Concentration(*C*) phase diagram showing crystal solubility and LLPS phase boundaries. Images illustrates the formation of protein-rich globular micro-droplets and crystals, and sample turbidity upon cooling and heating.

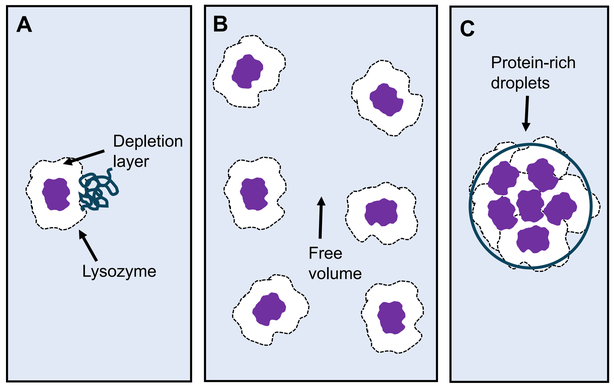

FIGURE 2. Macromolecular crowding mechanism. **(A)** The center of mass of polymer coil is unable to access the layer surrounding the protein (depletion layer). **(B)** Free volume inside protein solution in the presence of PEG coils. **(C)** Protein condensation to droplets is favored because of an increase in free volume.

The Temperature-Turbidity profile used to identify the LLPS temperature is shown in Fig. 3.

Results and Discussion

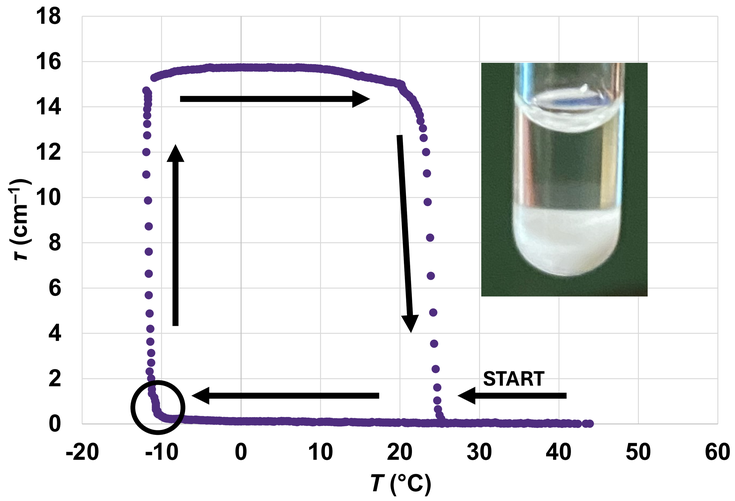

FIGURE 3. Temperature(*T*)-Turbidity(*τ*) profile for the lysozyme-NaCl-HEPES system with lysozyme concentration of 50 g·L^{-1}. Arrows show direction of progression with time. Circled area shows LLPS transition. Image showing sample crystallization heating is included. The LLPS temperature is –11.2 °C.

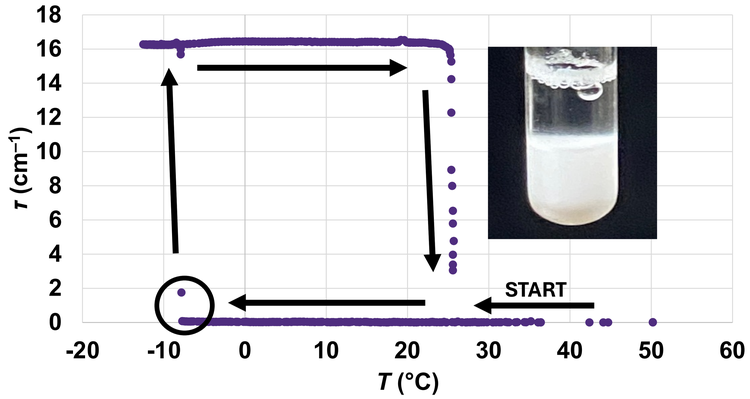

FIGURE 4. Temperature(*T*)-Turbidity(*τ*) profile for the lysozyme-NaCl-HEPES-PEG system with lysozyme concentration of 50 g·L^{-1} and 3,350 MW PEG concentration of 5% (w/w). Arrows show direction of progression with time. Circled area shows LLPS transition. Image showing sample crystallization heating is included. The LLPS temperature is –7.8 °C.

Conclusion

- LLPS temperature increases with PEG concentration.
- Crystallization output is not qualitatively altered after PEG addition.

Future Work

Future work will focus on examining the effect of PEG molecular weight on LLPS temperature and quantitatively determine crystallization yields in our systems.

References

- Annunziata, O.; Fahim, A. *Int. J. Biol. Macromol.* **2021,** 186, 519-527.
- Bloustine, J.; Virmani, T.; Thurston, G. M.; Fraden, S. *Phys. Rev. Lett.* **2006**, 96 (8), 087803.
- Fahim, A.; Pham, J.; Thomas, S.; Annunziata, O. *Journal of Molecular Liquids* **2024**, 398, 124164.
- Gao, Y.; Gervais, D. *J. Chem. Technol. Biotechnol.* **2021,** 96 (5), 1141-1150.

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Liquid-liquid phase separation (LLPS) is a promising technique for purifying specific proteins, having distinct advantages over chromatography, the typically used purification method. By adding two additives to a protein sample and running LLPS experiments, the Annunziata group was able to produce protein purification yields upwards of 85%. Preliminary studies investigate the effect of a third additive on LLPS temperature and yield.