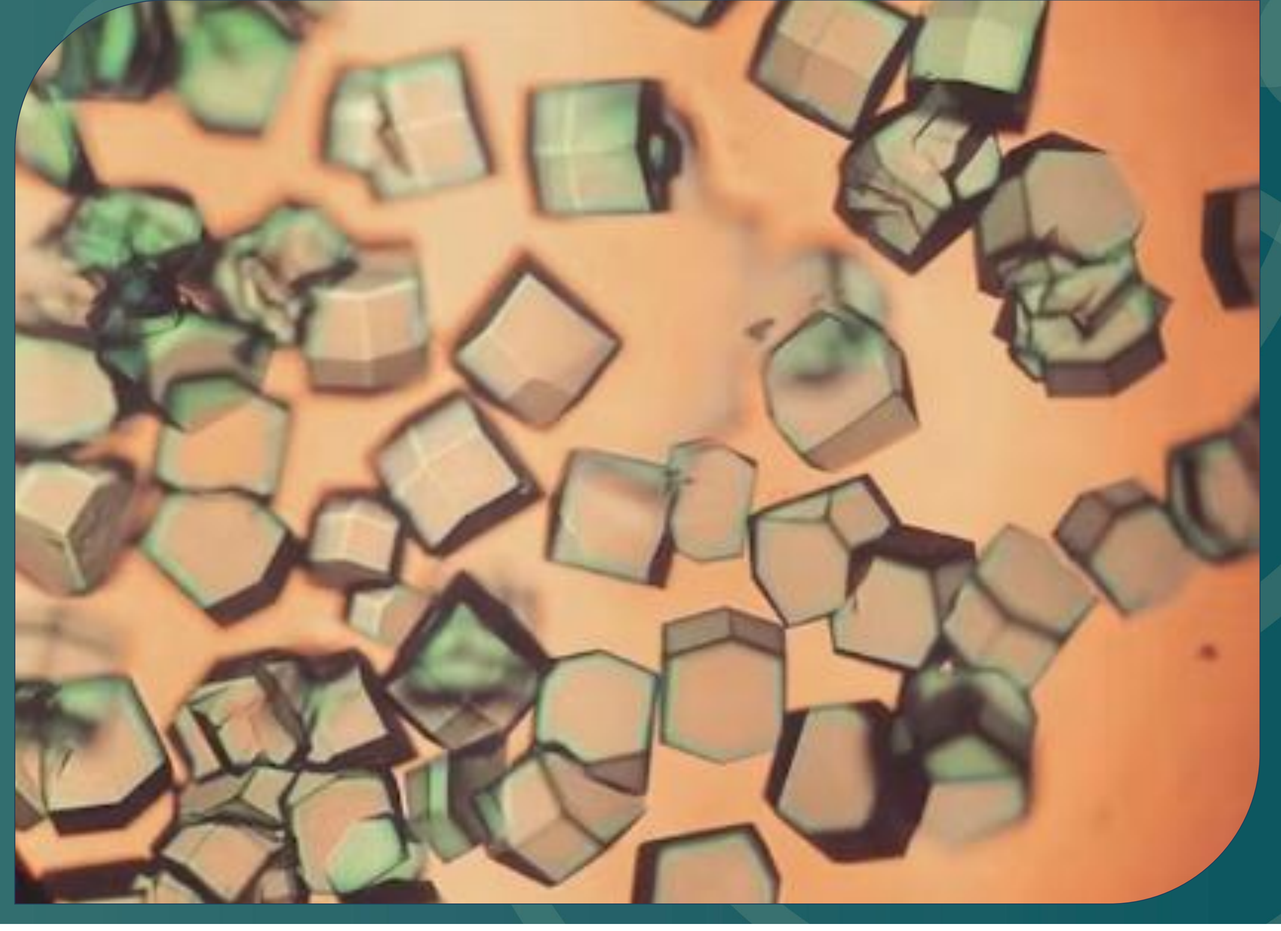
Optimizing Nanocrystals for Serial Crystallography

Claudia Karako[1,2], Kathryn Olsen[1], Sandra Mous[1]

Contact: rubyc13@slac.stanford.edu

1. SLAC National Accelerator Laboratory, USA. 2. University of California Riverside, Riverside, CA, USA



BACKGROUND

Benefit: Serial crystallography lowers radiation damage, allowing for room-temperature and time-resolved protein studies.

Core Requirement: Experiments require large amounts of uniform, well-ordered [sub]micron crystals.

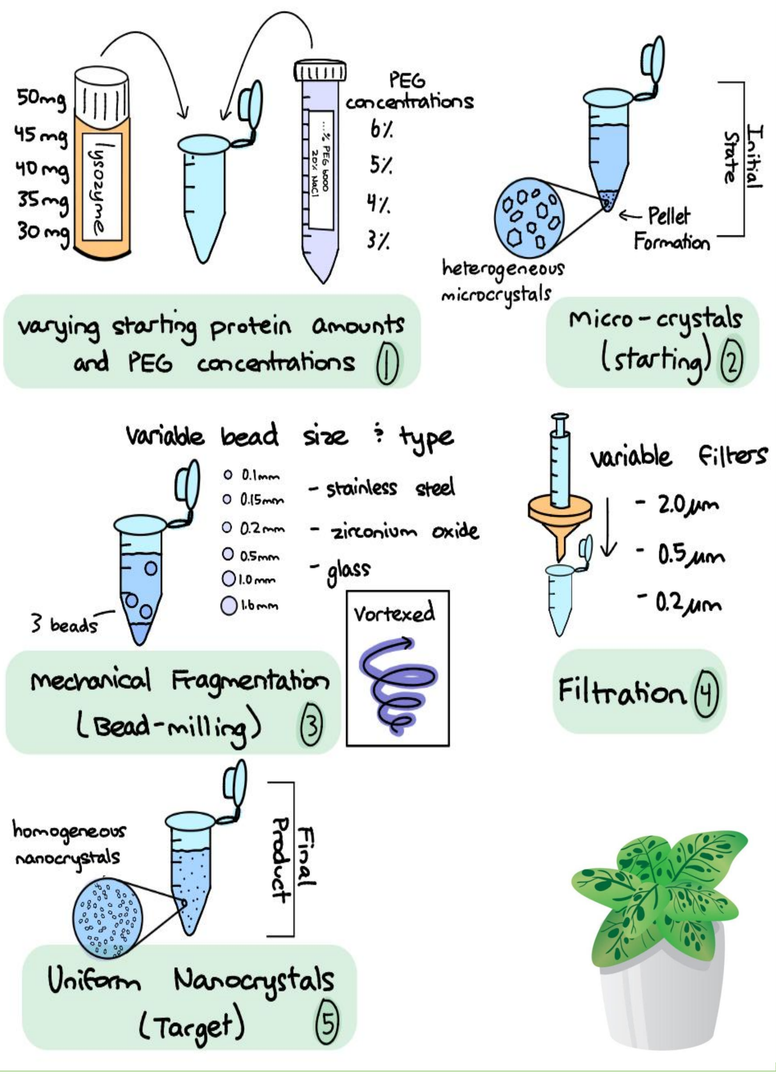
The Challenge: Direct batch nano-crystallization often causes amorphous precipitation, leading to inconsistencies in crystal morphology and polydispersity.

Solution: Larger microcrystals of Hen Egg White Lysozyme will be crushed into [sub]micron nanocrystals through methods of bead milling.

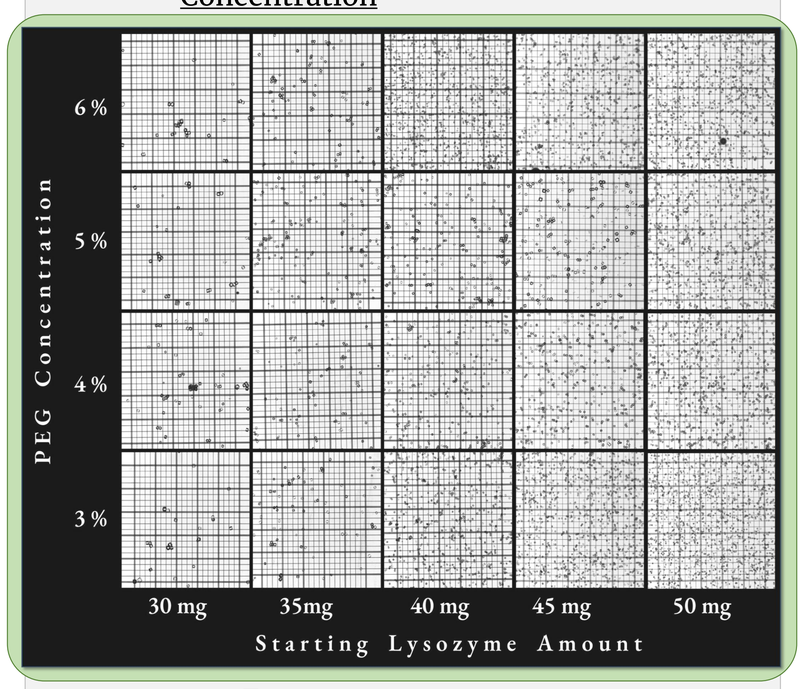
OBJECTIVE

Optimize nanocrystal formation and morphology by testing various bead sizes, milling conditions, and initial microcrystal sizes.

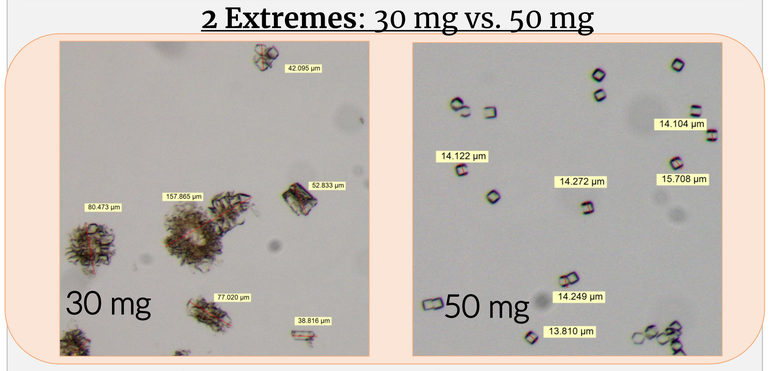
METHODS

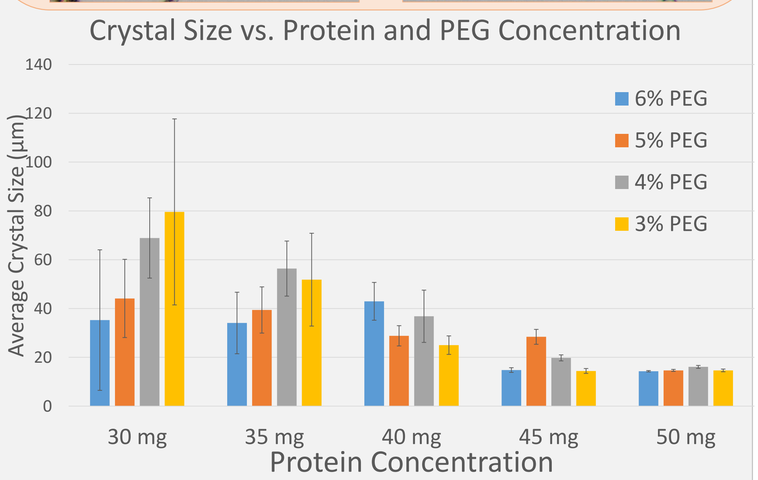


RESULTS Adjusting Starting Protein Amount and PEG Concentration



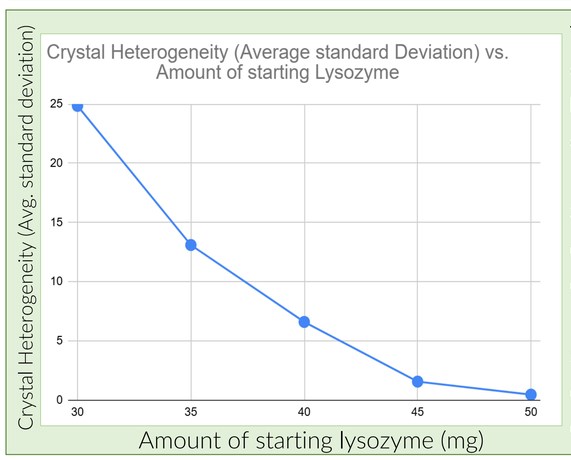
2 Extremes: 30 mg vs. 50 mg





Bar Plot depicting Crystal Size relative to changes in Protein and PEG Concentrations.

Larger error bars = less homogeneous



The starting amount of protein affects the homogeneity of the crystals.

30mg = highest deviations between crystal sizes.

50 mg = lowest deviations between crystal sizes.

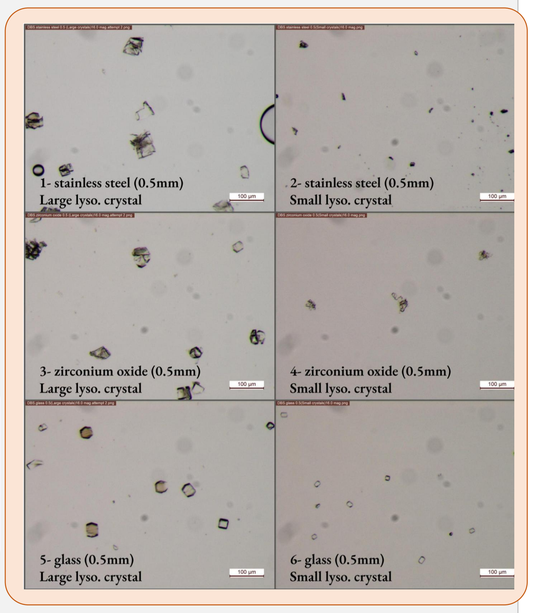
Bead Milling with Various Materials on Large vs. Small Lysozyme Crystals

3 Materials

- Stainless steel
- Zirconium oxide
- Glass

Stainless steel = most promising

Glass = least promising



Smaller, uniform crystals are an ideal starting material for bead milling and nanocrystal formation.

❖ Dynamic Light Scattering Data
Average Crystal Size: 2.595 μm

❖ Viscometer Data
Average Viscosity: 2.922 cP

The Stokes-Einstein equation utilizes the hydrodynamic radius of the particle (Rh) as well as the viscosity of the solute (η) to determine the particle's diffusion coefficient (D).

$$D \propto \frac{1}{R_h}$$

Low D > High D

Determines the speed at which a molecule moves through a solution.

$$D = \frac{k_B T}{6\pi\eta R_h}$$

$2.832 \times 10^{-17}\ m^2/s$

FUTURE WORK

- ❖ **Optimize Milling:** Test different vortexing durations to improve nanocrystal homogeneity and morphology.
- ❖ **Map Lysozyme Limits:** Adjust protein and PEG concentrations to determine the boundaries of lysozyme crystallization.
- ❖ **Test Other Proteins:** Explore crystallization boundaries and size homogeneity for additional proteins, such as PrxC.
- ❖ **Characterize Structure:** Use Negative Stain Electron Microscopy to confirm and analyze (sub)micron nanocrystal morphology.

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[1] Rupp, B. (2009). *Biomolecular Crystallography: Principles, Practice, and Application to Structural Biology*. Garland Science.

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[3] SED Department, SLAC National Laboratory, USA.