# The Ideal Crystal for Structural Biology

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# The ideal crystal for structural biology?

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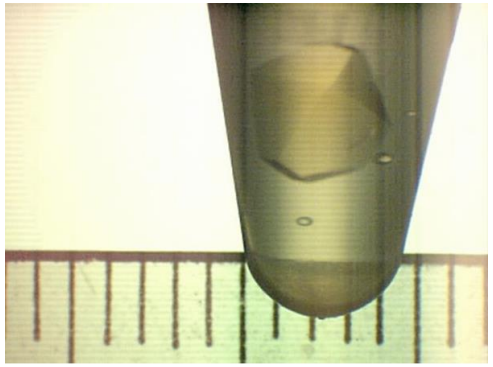
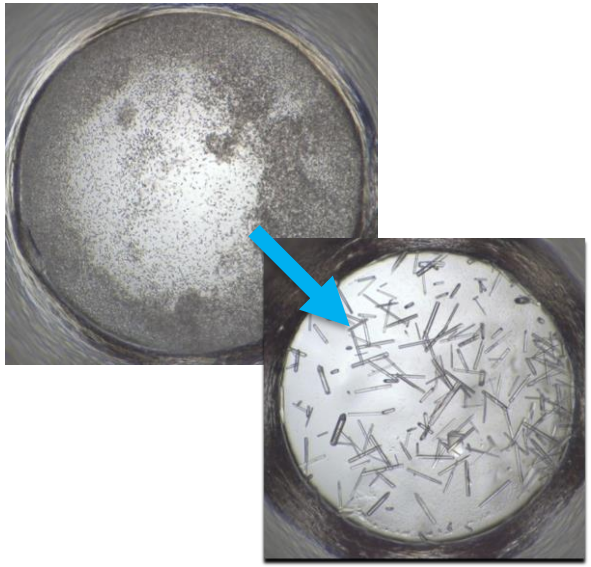
- **The ideal crystal is one or many that enable the biological question being asked to be answered.**



- It has to diffract to sufficient resolution with sufficient data completeness that the structural elements can be resolved at the level of detail required.



- Diffraction properties are largely independent of visual appearance – beautiful looking crystals can crush hopes, and lost causes can redeem themselves.

# Getting the crystal for structural biology

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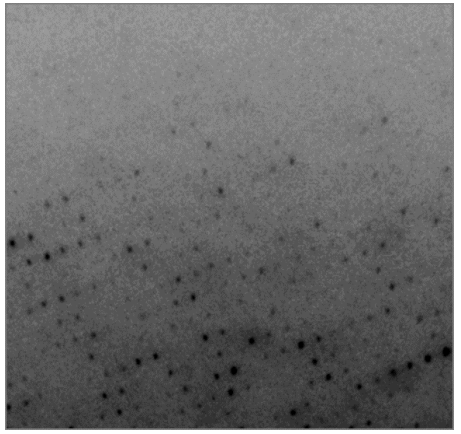
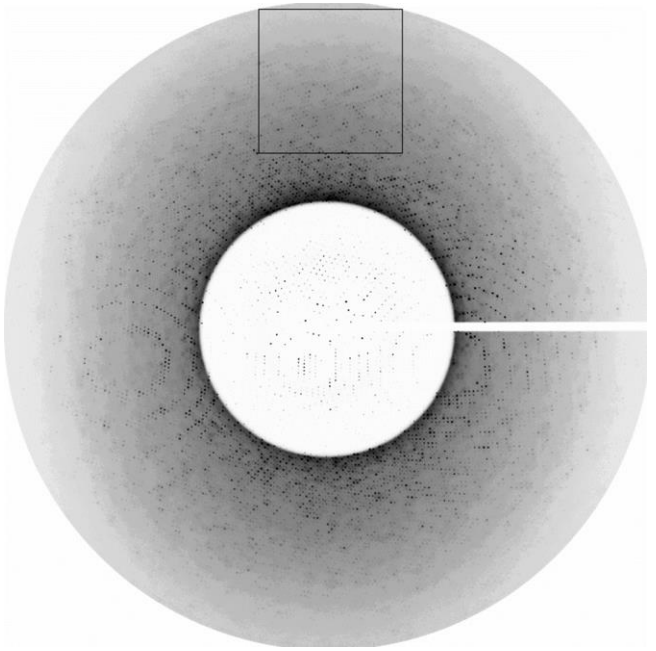
- No crystal, no crystallography, no crystallographer.
- Stating the obvious - Before the diffraction properties of crystals can be tested, the crystal has to be produced.
- Visually if we can identify a crystal, we can then measure properties such as dimension and numbers.
- We also understand the solubility phase diagram and how crystals are formed which can guide our interpretation of the results.





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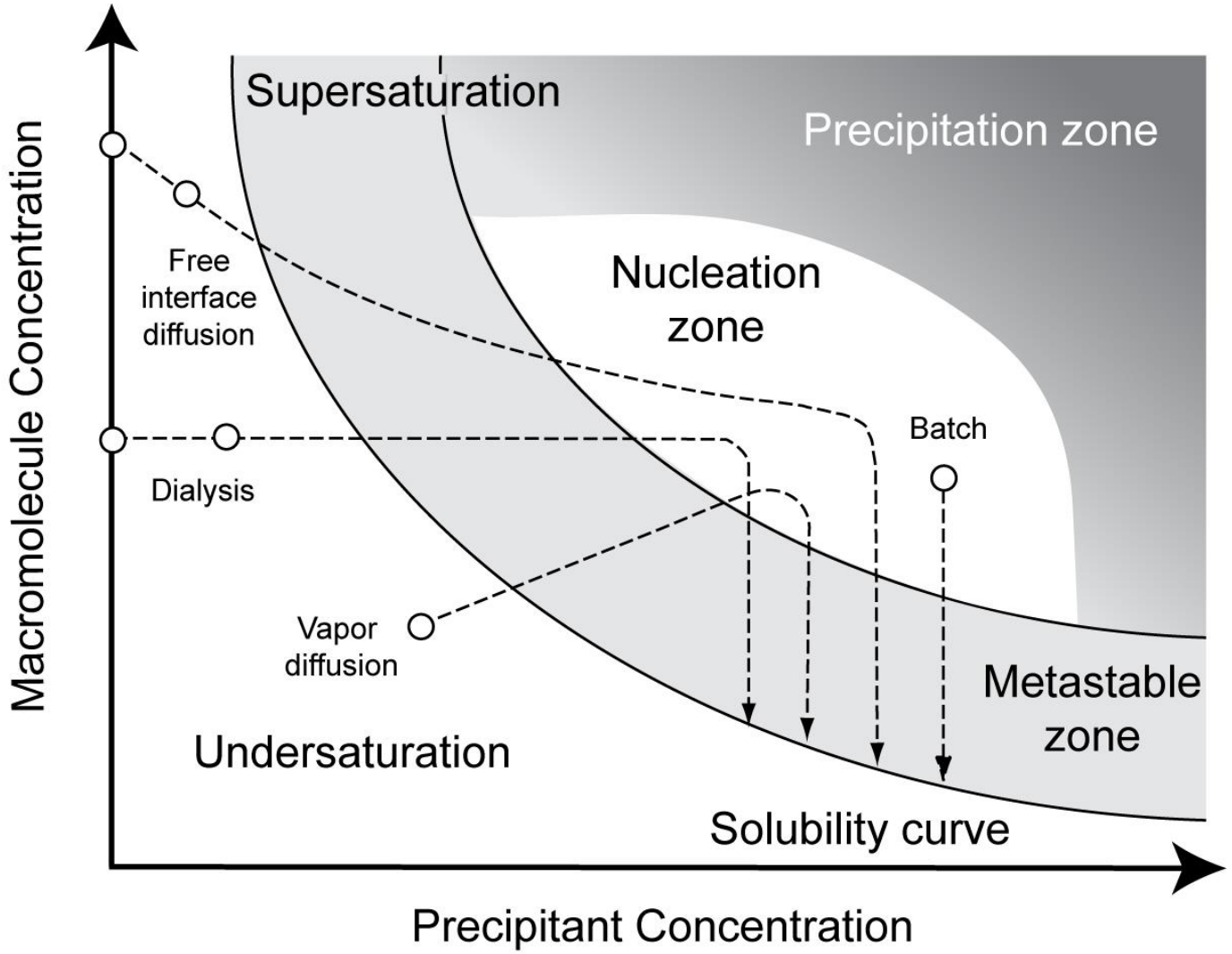
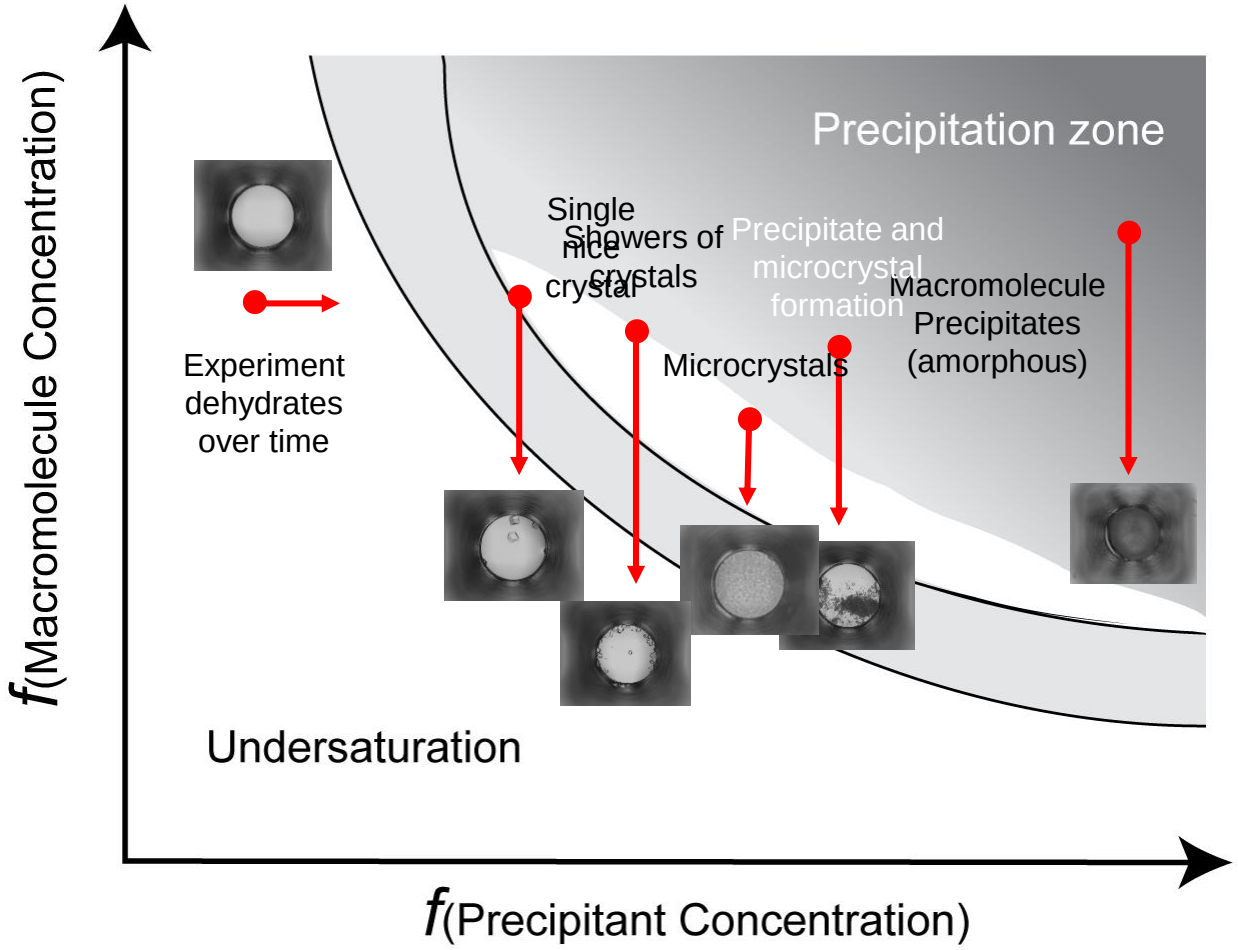
# The solubility diagram and crystallization



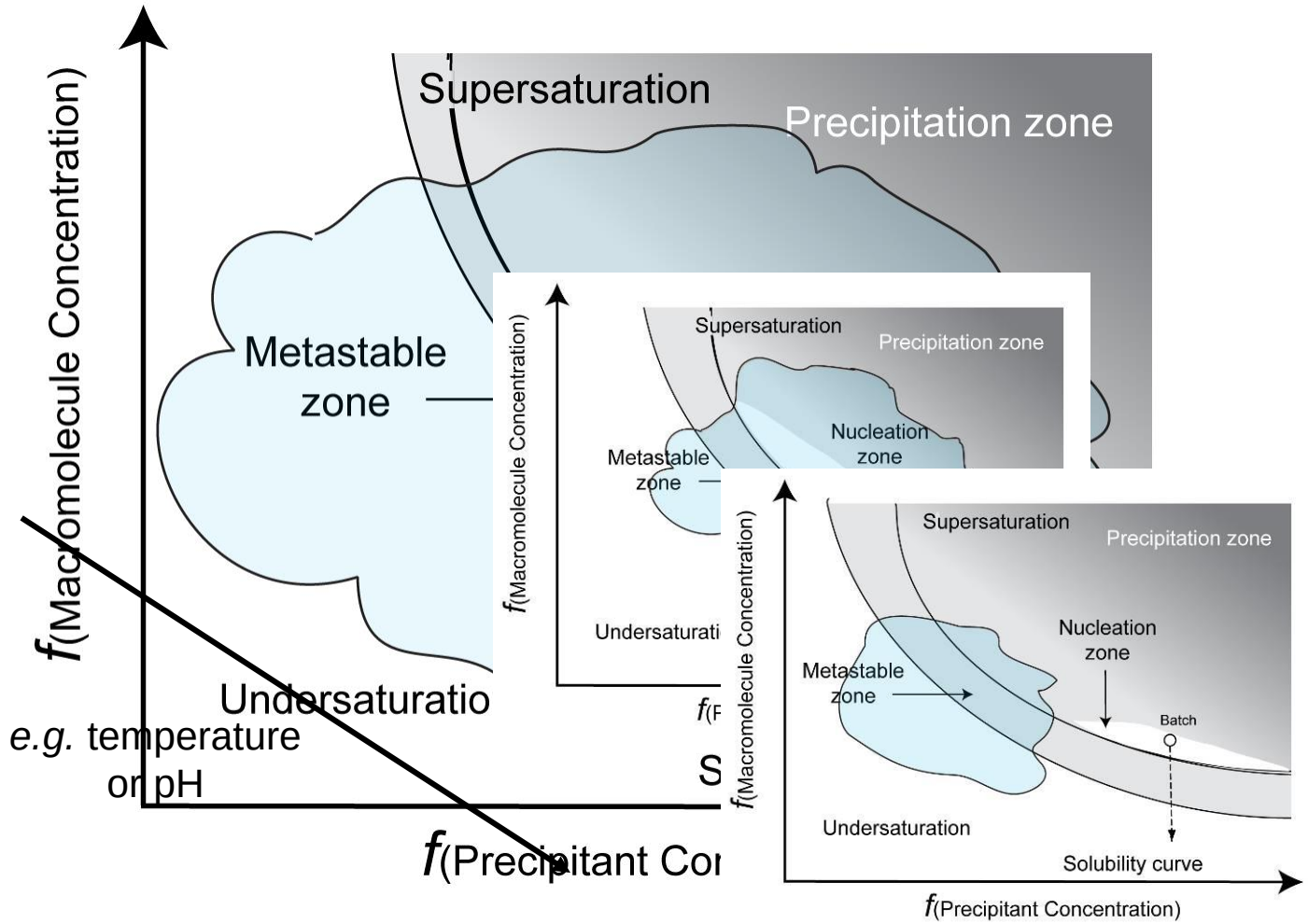

Note, separation between phases are shown as discrete lines but in reality they are probability gradients.

# What to expect from the solubility diagram




*What's in a drop? Correlating observations and outcomes to guide macromolecular crystallization experiments. Luft, Wolfley, & Snell. Cryst Growth Des, 11, 651-663 (2011).*

# When theory meets reality



# What are our expectations?

- Size and number can be measured (mostly).
- Large single crystals grow when a small number of nuclei are formed and conditions rapidly transition to the metastable zone.
- If the system remains in the nucleation zone for a long time, many nuclei result and a larger number of smaller crystals result given a finite protein concentration.
- Microcrystals can easily be misidentified as precipitate.
- Given our knowledge of crystallization theory, we should be able to intelligently control crystallization.
- Why would we want to control crystallization?

# Different techniques require different crystals

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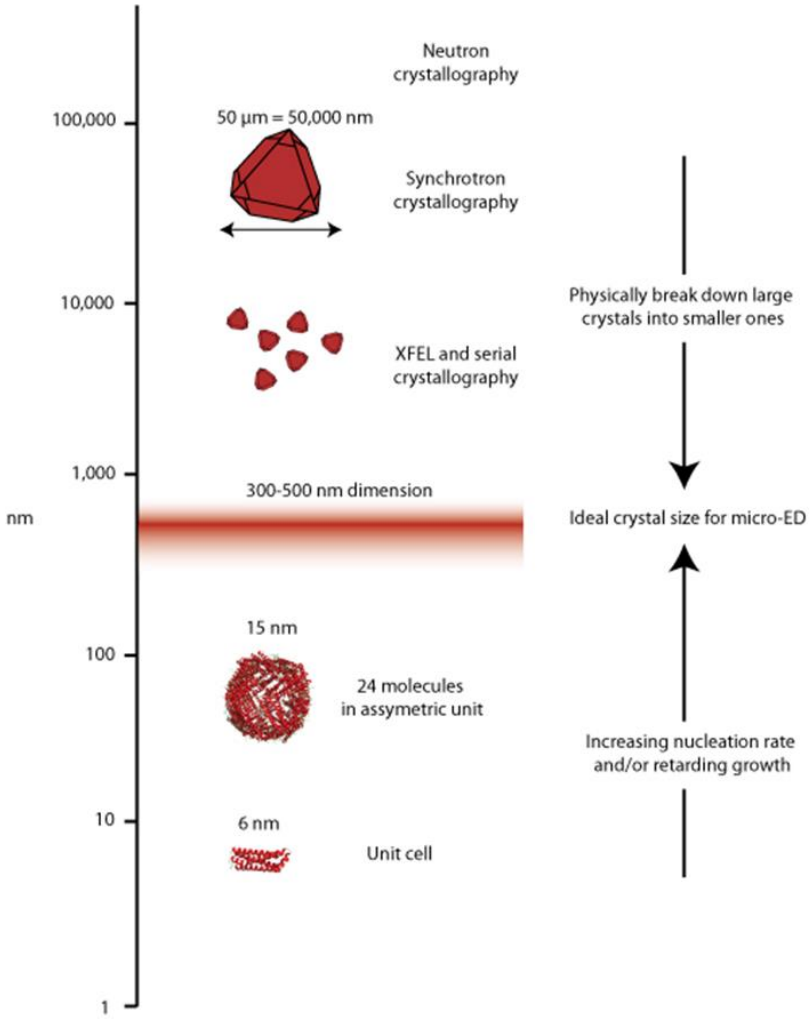
| Crystal based technique | Crystal requirements |
|---|---|
| **Neutron crystallography** – studies of hydrogen position, protonation state, or elimination of detrimental radiation chemistry effects. | **~ mm** in dimension, can be smaller if perduterated |
| **Single crystal laboratory studies** – characterization of samples, structural studies at single wavelengths. | **~ 200 µm** in dimension |
| **Synchrotron single crystal studies** –phasing of new structures, structural data collection to high resolution and/or high speed. | **~ 10-20 µm** in dimension or smaller on modern microfocus beamlines |
| **Electron diffraction** – structural data collection, structural determination with high-resolution data. | **300-500 nm** in dimension |
| **X-ray free electron lasers** – structural information on dynamics, where crystals can't be grown large, or where radiation chemistry impacts the information obtained. | Data has been recorded from 10's of unit cells but typically a **few µm** in dimension |

# There is a spectrum of crystals available




- Once a crystallization condition has been identified it is theoretically possible to produce one of the volume/dimensions desired.
- There are practical considerations including surface poisoning and depletion of the sample.
- One can start by growing the crystal and stopping it at the required volume, or disrupting a larger crystal and breaking it up.

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# Starting large

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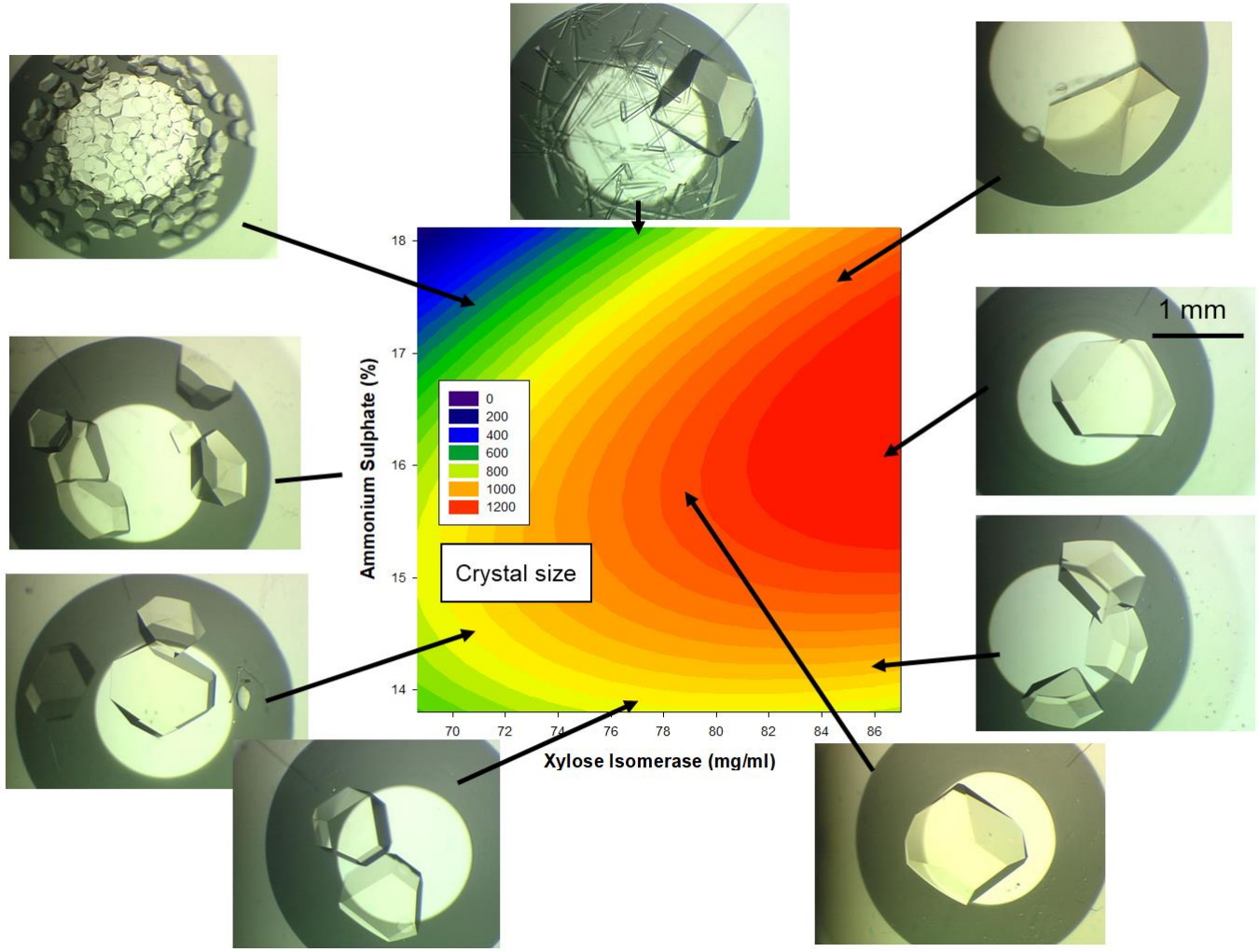
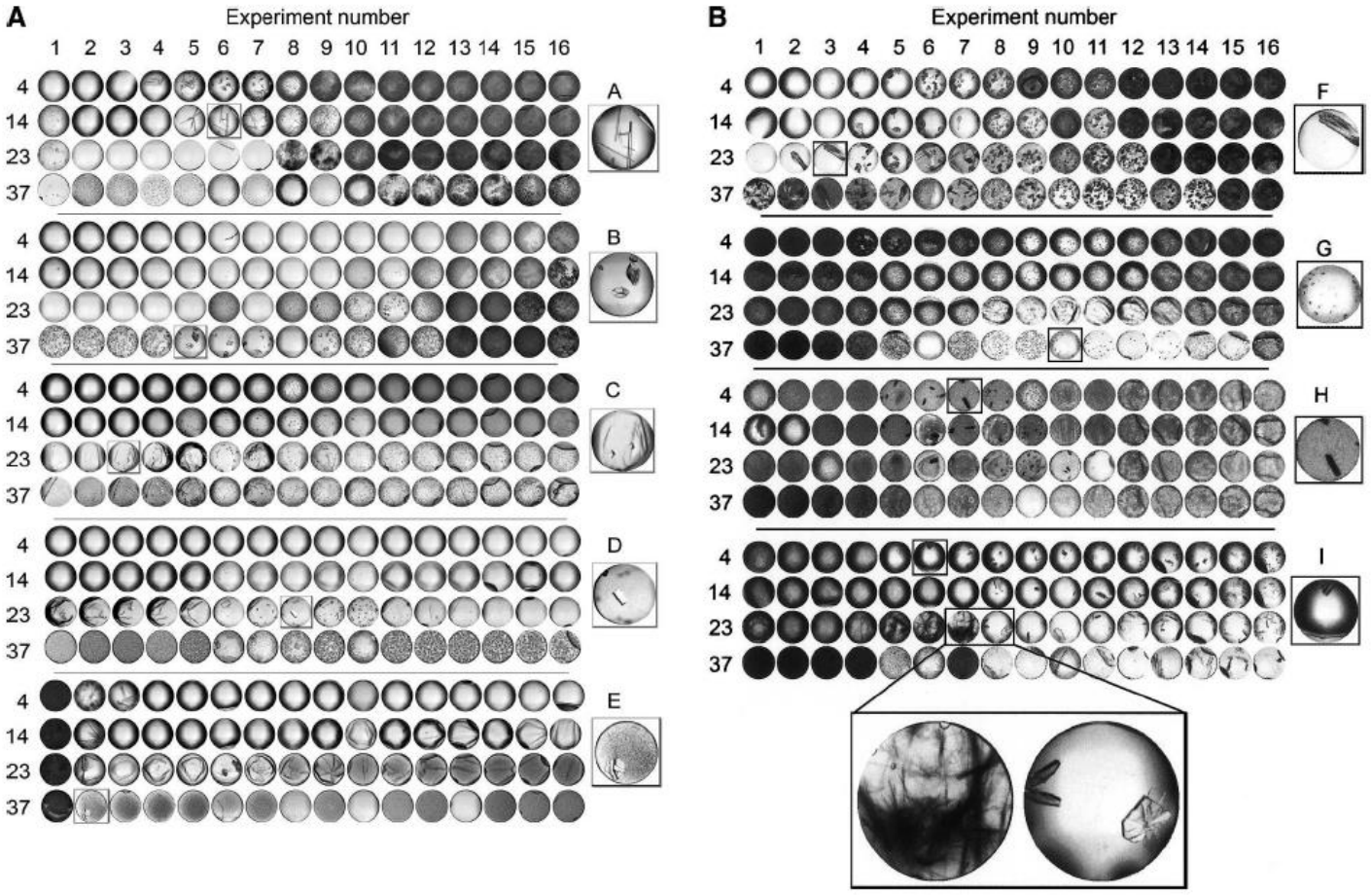
# Volume is measurable and can be optimized



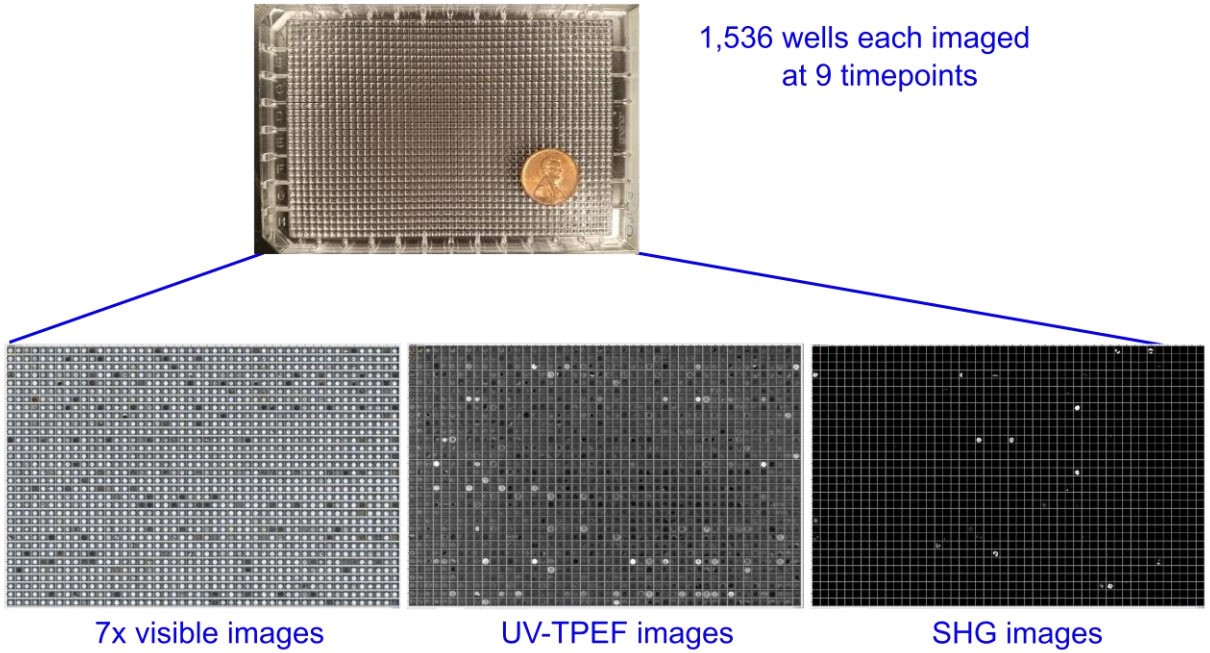

- Size is a metric that is amenable to mathematical optimization using response surface methods pioneered by Charlie Carter.
- This has been used effectively to optimize samples for neutron diffraction (*Snell et al., Eur Biophys J. 2006 Sep;35(7):621-32*).

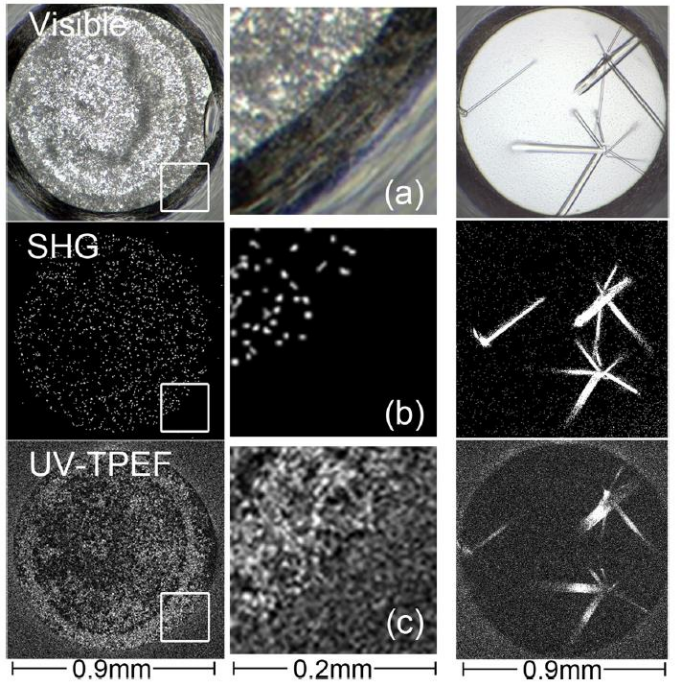
# Volume is measurable and can optimized

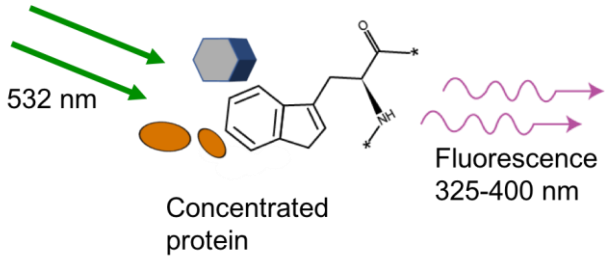



- For the batch technique, the ratio of protein to precipitant can be varied with temperature and volume optimized effectively (*Luft et al. Protein Sci. 2007, 16(4):715-22)*.
- This can be extended to vapor diffusion.

# Where volume is too small, other imaging methods can be used




*Identifying, studying and making good use of macromolecular crystals. Calero, Cohen, Luft, Newman, & Snell. (2014) Acta Cryst. F70, 993-1008.*

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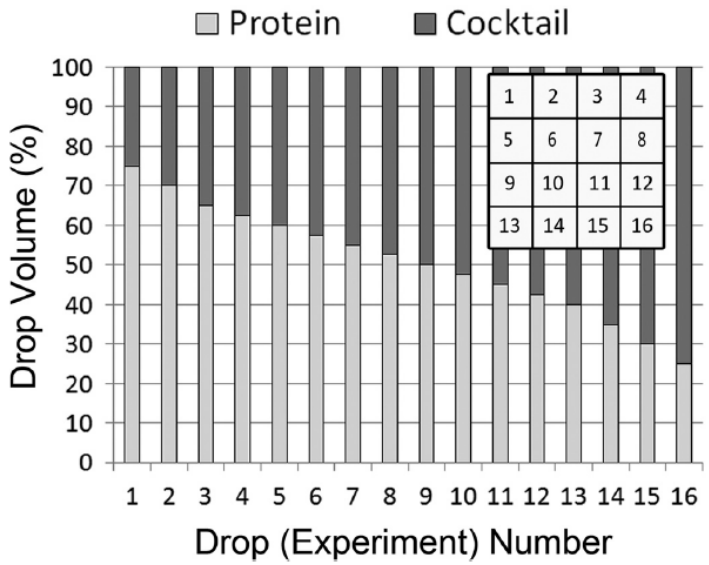
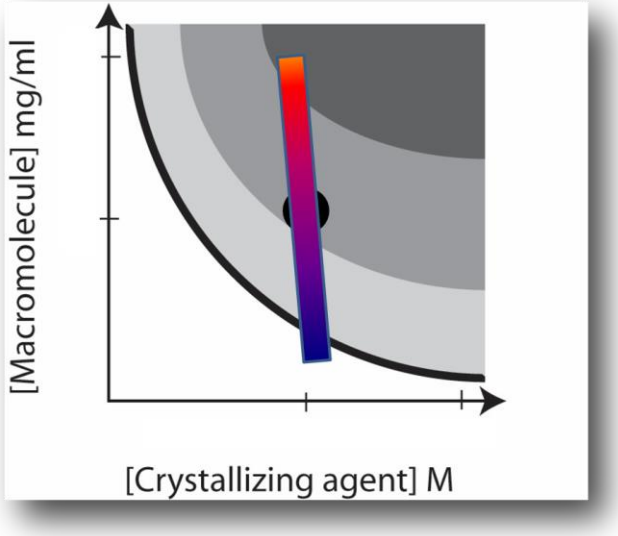
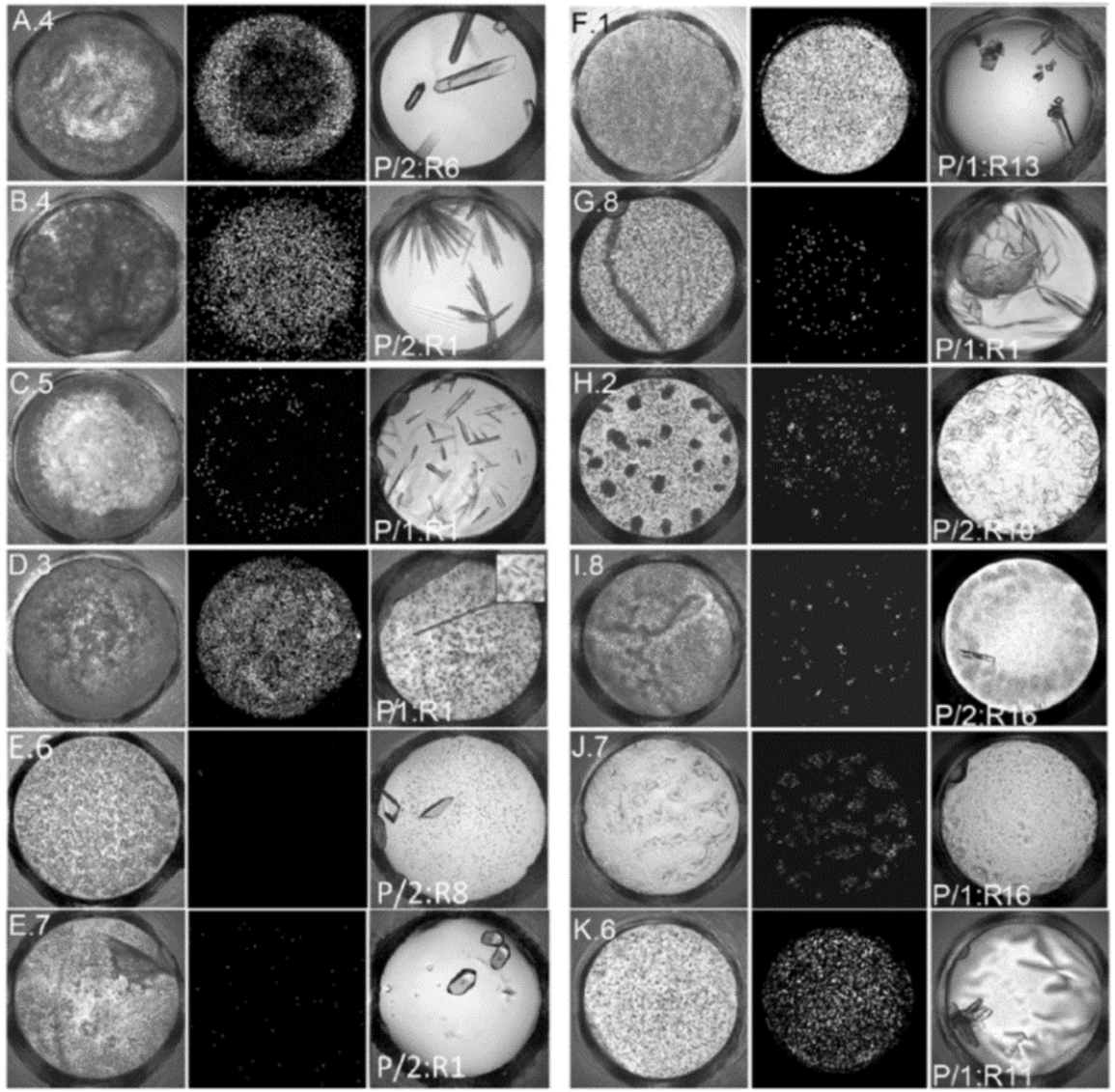
# From an initial hit, optimization can be systematic



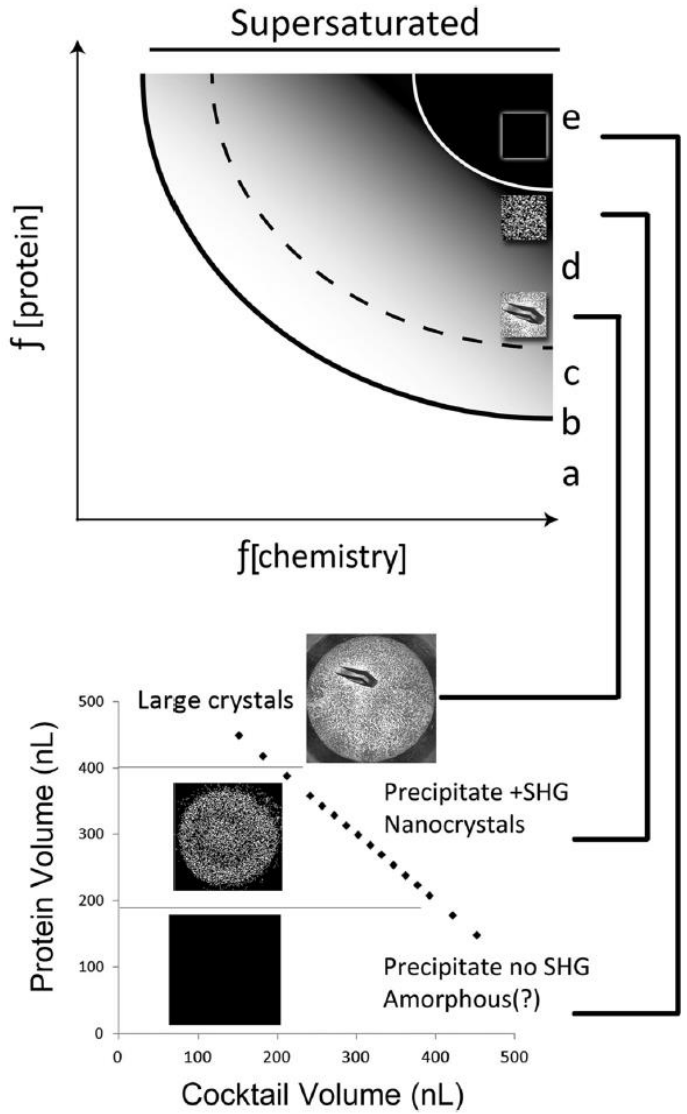

- For the batch technique, the ratio of protein to precipitant can be varied with temperature and volume optimized effectively (*Luft et al. Protein Sci. 2007 Apr;16(4):715-22)*.
- This can be extended to vapor diffusion.

# If small crystals result, they can usually be grown larger




- For a study on 60 protein samples submitted to the Crystallization Center 39 produced microcrystals (65%).
- Of these 14 were used to study different imaging techniques.
- Eleven (78%) produced microcrystals, all of which were able to be optimized.
- In all cases where microcrystals were observed, larger crystals could be grown by simple ratio techniques.
- *Luft, Wolfley, Franks, Lauricella, Gualtieri, Snell, Xiao, Everett, Montelione. (2015) Struct Dyn, 2, 041710*

# You can make a small crystal large




- Once a crystallization condition has been identified it is theoretically possible to produce one of ideal volume.
- There are practical considerations including surface poisoning and depletion of the sample.
- One can start by growing the crystal and stopping it at the required volume, or disrupting a larger crystal and reducing the volume.

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# Large to small

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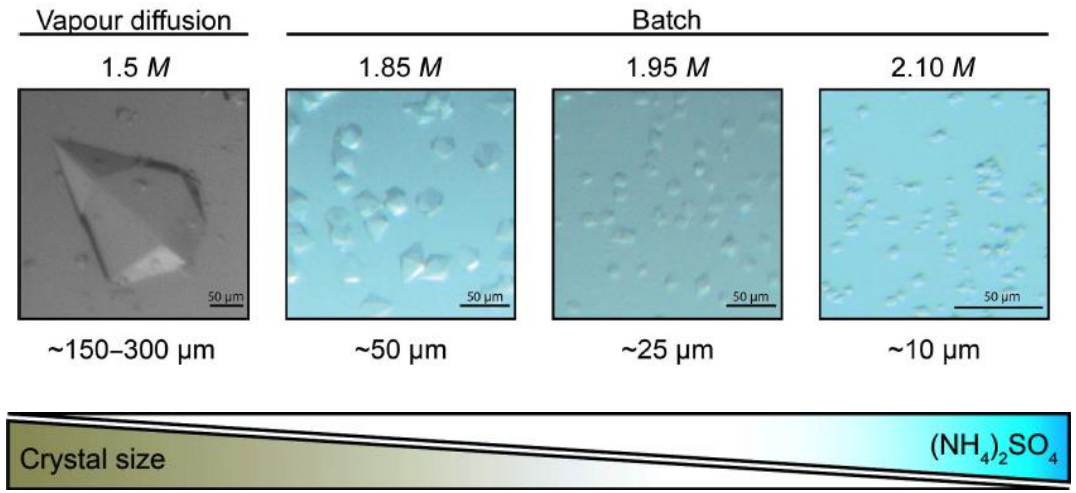
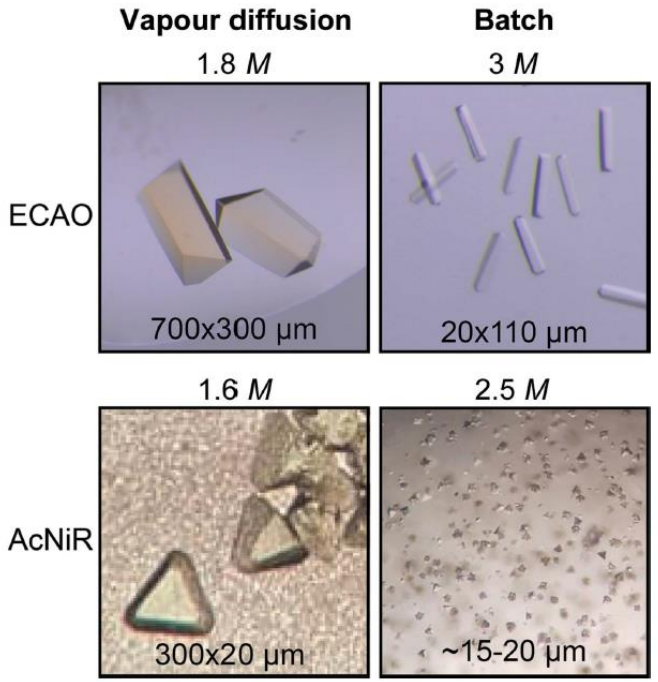
# You can make a large crystal small - changing conditions

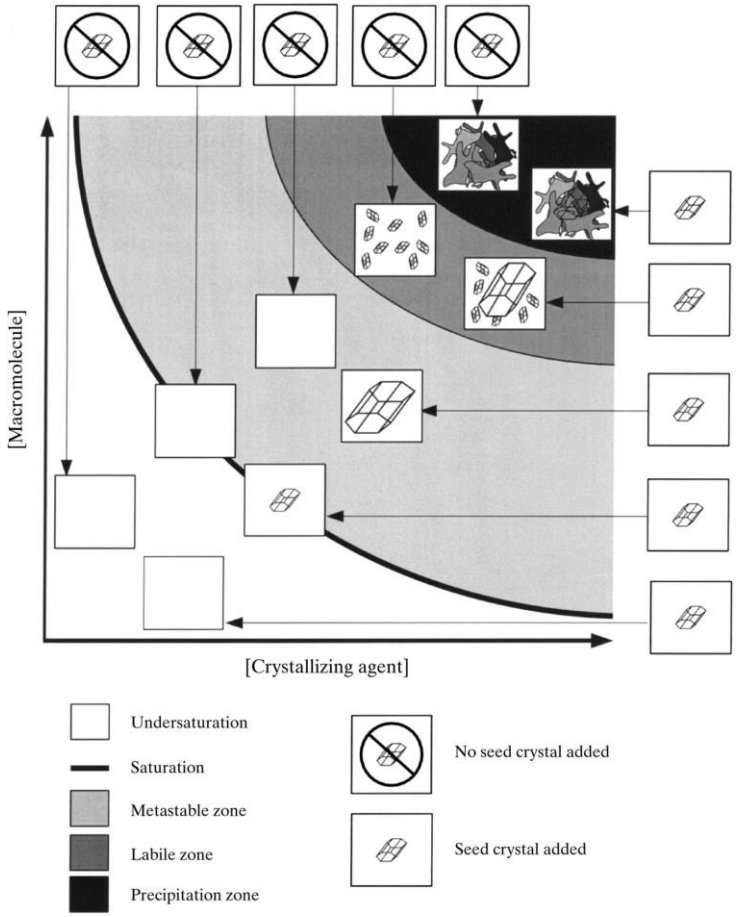


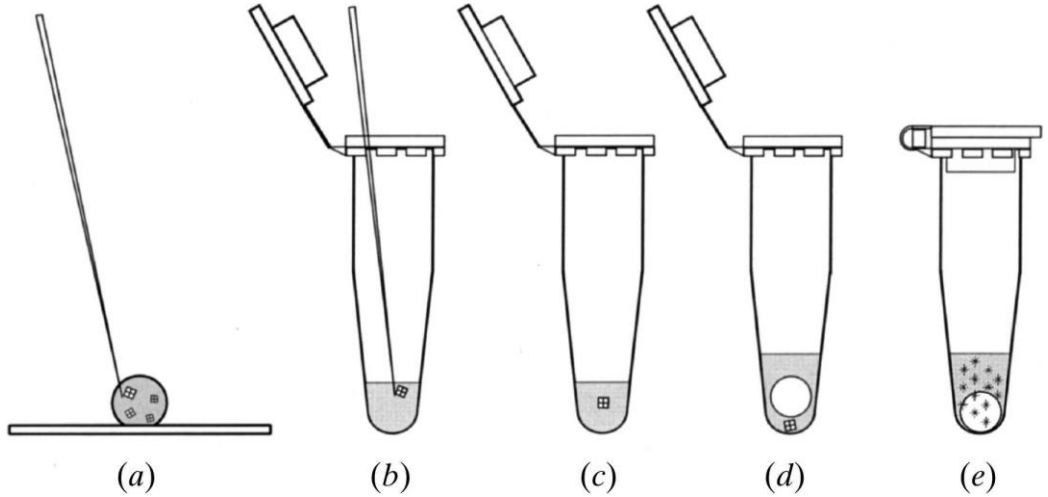




When conditions for a large crystal are known, those conditions can be manipulated to produce small crystals

*Homogeneous batch micro-crystallization of proteins from ammonium sulfate. Stohrer, Horrell, Meier, Sans, von Stetten, Hough, Goldman, Monteiro, & Pearson. Acta Cryst D77, 194-204 (2021).*

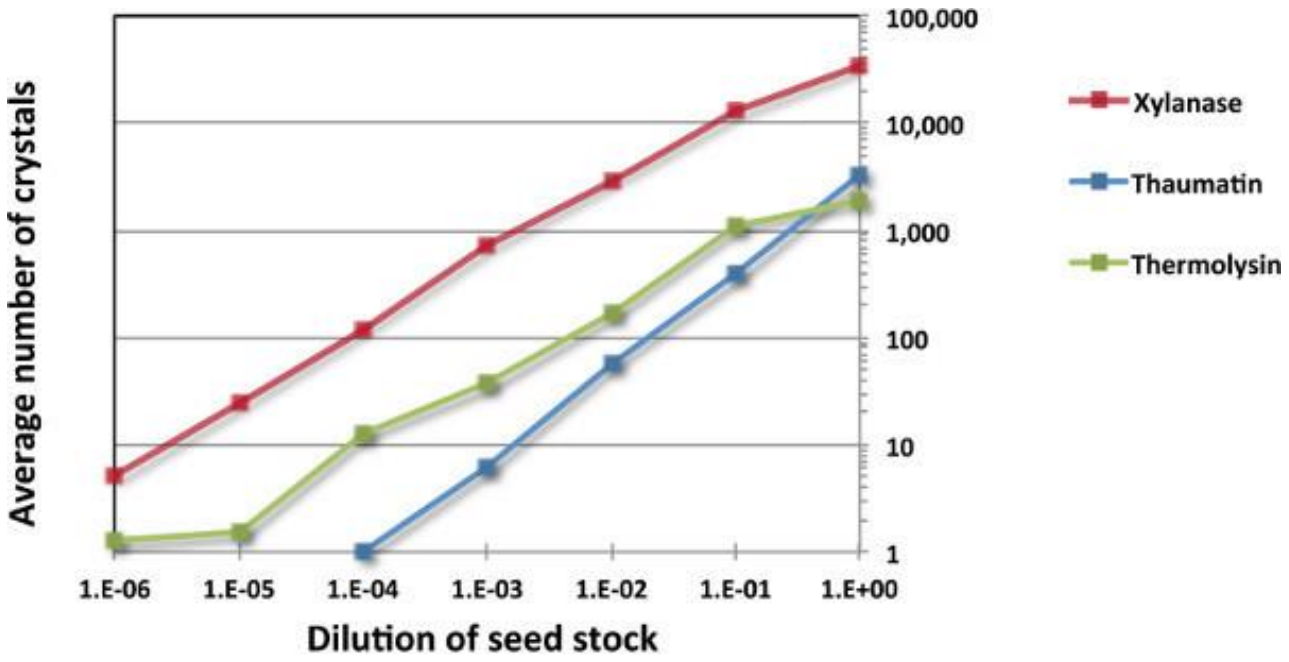
# Making a large crystal small (seed bead and seeding)




*A method to produce microseed stock for use in the crystallization of biological macromolecules. Luft & DeTitta, Acta Cryst. D55, 988-993, (1999).*

# Making a large crystal small (seed bead and seeding)




While seeding has been used to grow large crystals, the same process can be used to increase the initial nuclei and produce many small crystals.

*Improving the Success Rate of Protein Crystallization by Random Microseed Matrix Screening. Till, Robson, Byrne, Nair, Kolek, Shaw Stewart, & Race. J Vis Exp. 2013; (78): 50548.*

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# Growing small

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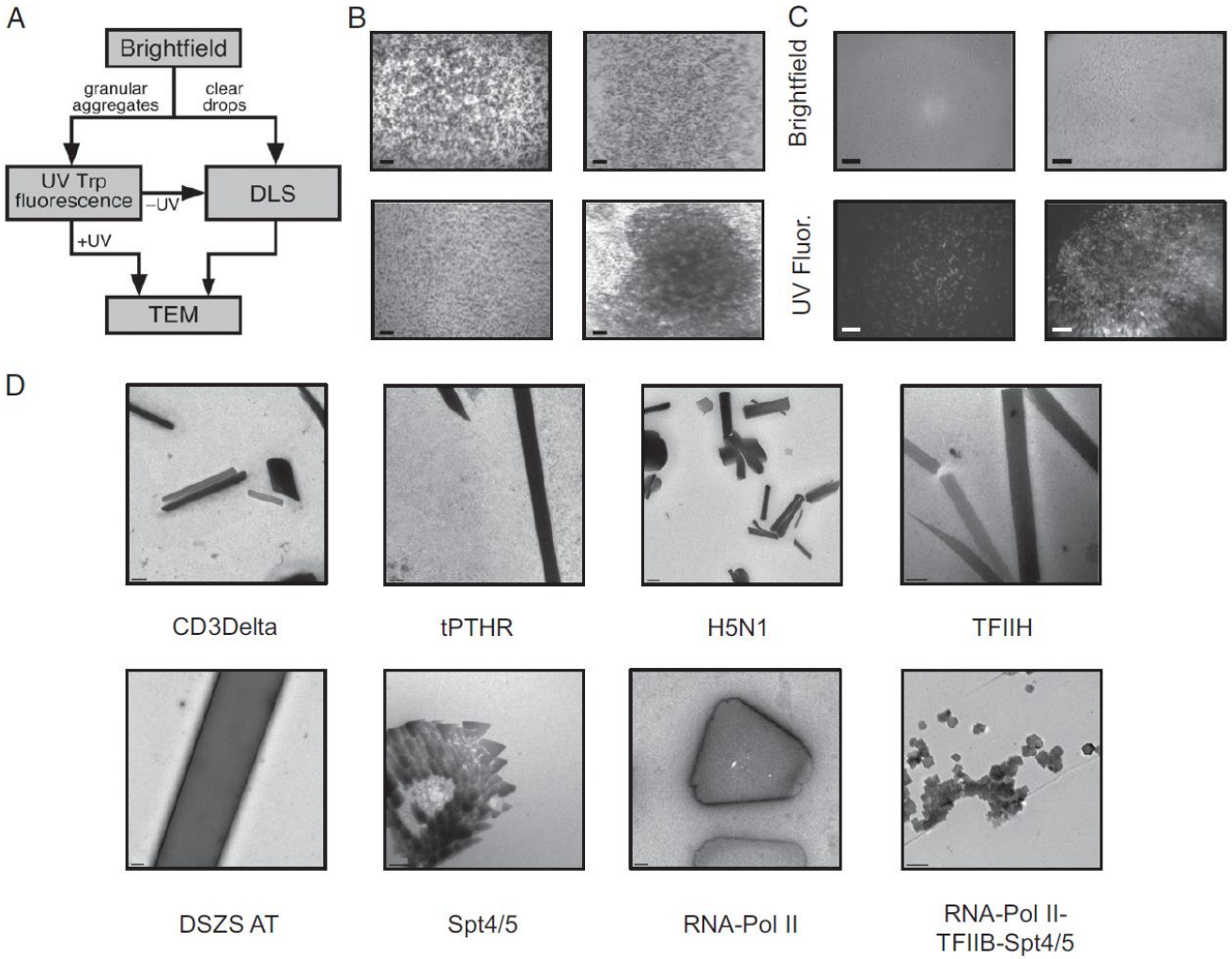
# Identifying the crystal




"*Transmission electron microscopy (TEM) was used to visualize nanocrystals (NCs) found in crystallization drops that would classically not be considered as "hits." We found that protein NCs were* ***readily detected in all samples tested****, including multiprotein complexes and membrane proteins*."

*Use of transmission electron microscopy to identify nanocrystals of challenging protein targets. Stevenson, et al.. PNAS 111, 8470-8475, (2014).*

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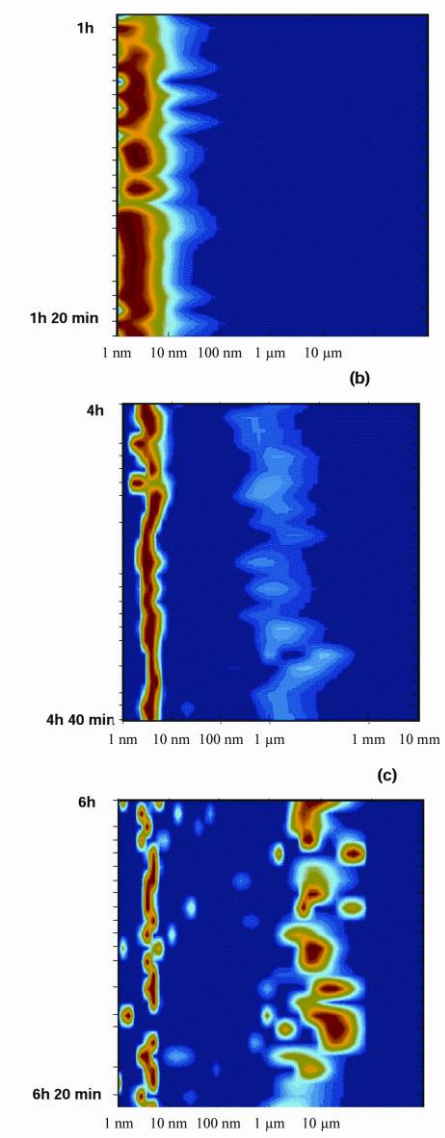
# Following crystallization




- Where the smallest crystals are required, e.g. for micro-ED where optimum size is close to that of the wavelength of light, non-visual techniques have to be used.
- A powerful one is time-resolved dynamic light scattering where the formation of nuclei and initial crystals can be followed.
- Another is powder diffraction.

*Separating nucleation and growth in protein crystallization using dynamic light scattering, Saridakis, Dierks, Moreno, Dieckmann, & Chayen, Acta Cryst D58, 1597-1600 (2002).*

# One Center for All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# The National Crystallization Center

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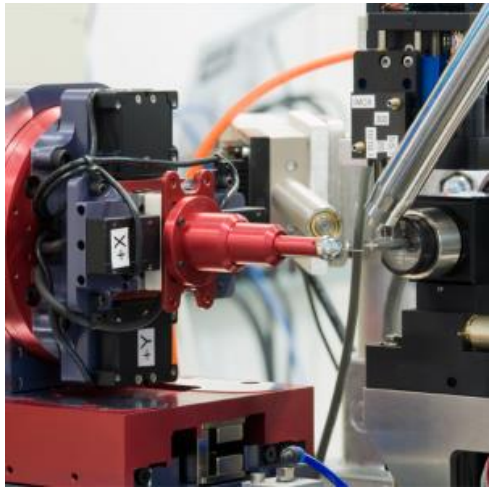
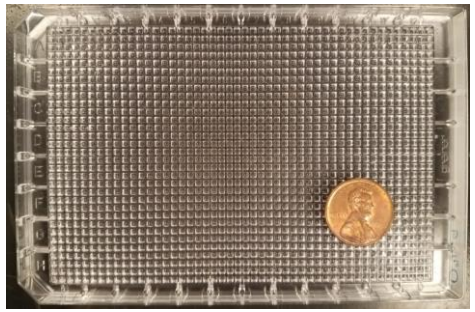
MARCO

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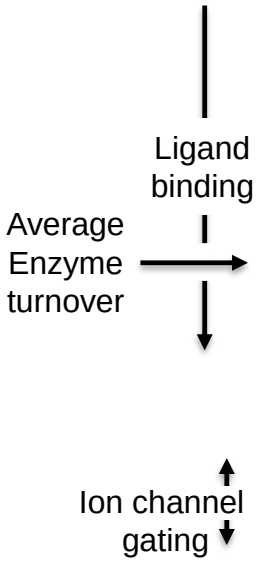
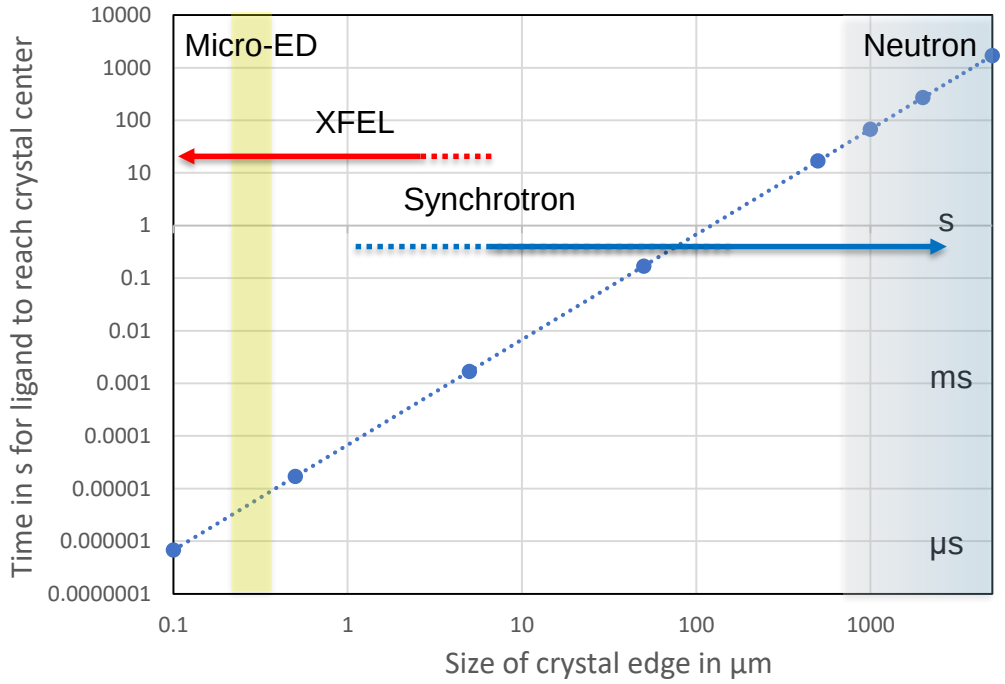
# Given an optimized crystal – why go small?

If you can grow a large crystal why go small?

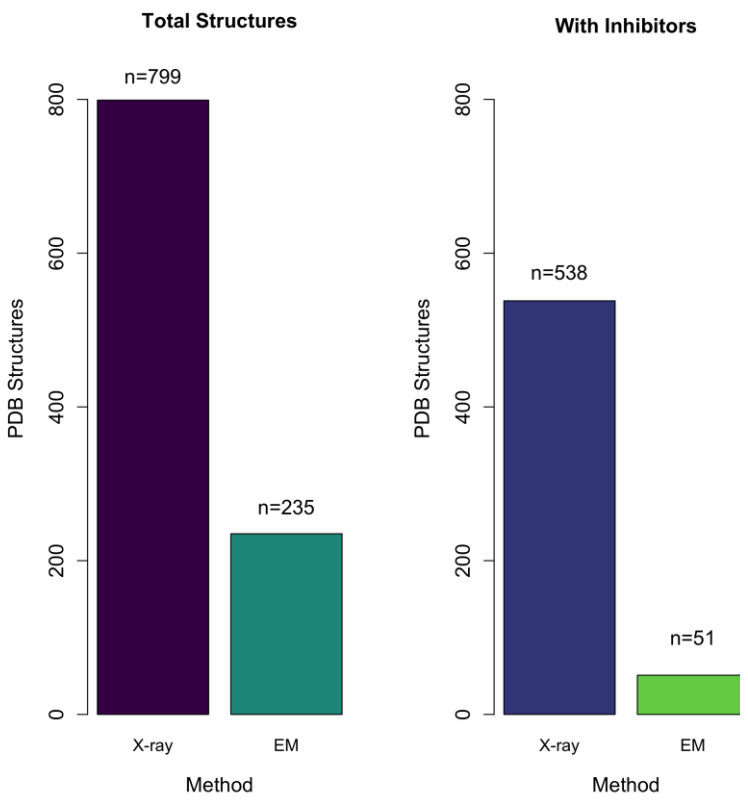
- Accessibility to material.
- Ease of cryopreservation for cryocooling.
- Efficient ligand diffusion to initiate and trap dynamics.
- Serial crystal approaches to minimize radiation chemistry.



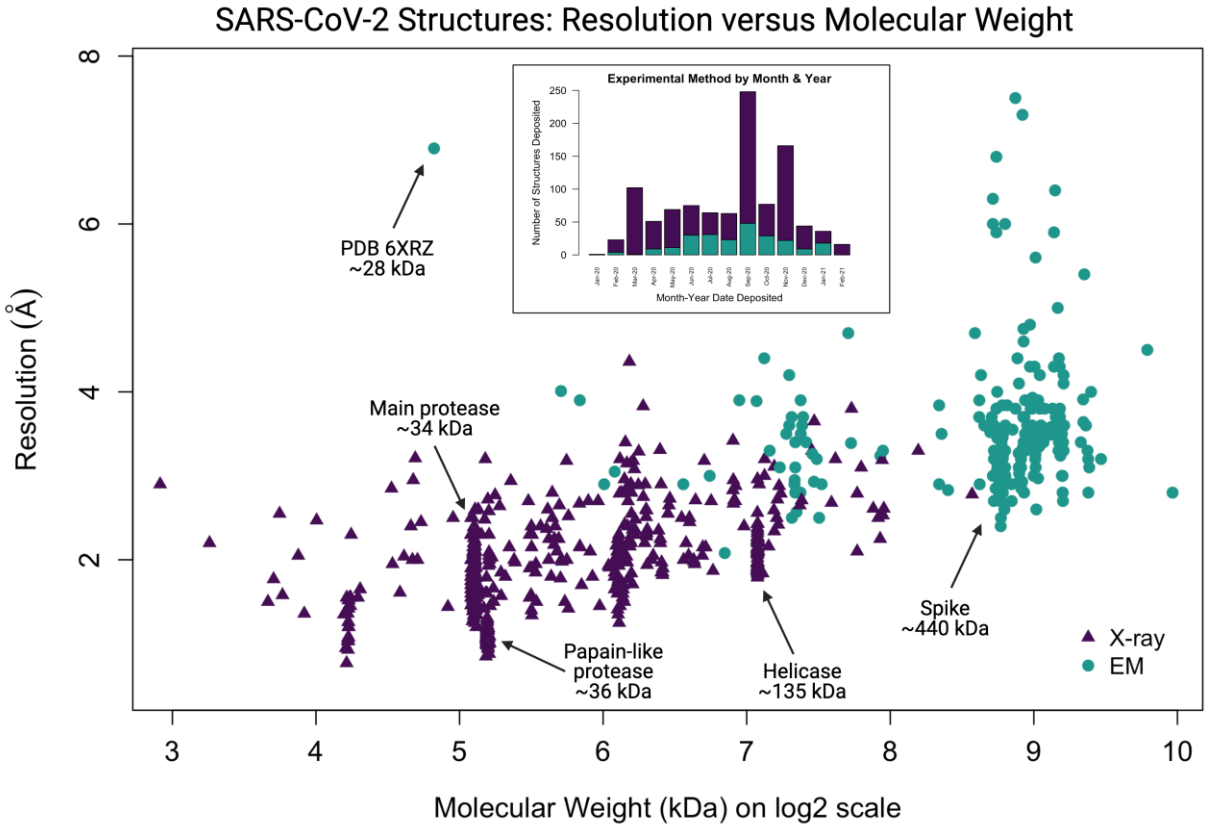

*Plot of maximum time take for glucose to diffuse into a center of a cube shaped crystal of various sizes (calculated from Carsaew and Jaeger, Conduction Heat in Solids, 1959).*

# Expand beyond X-ray crystallography










*Lynch, ML, Snell, EH, Bowman, SEJ 2021. IUCrJ. 8(3):335.*

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# Summary

- There is no idea crystal – the crystal need is dependent on the technique.
- Studies on the solubility diagram of proteins inform rational ways to optimize them for the technique of choice.
- Dimension and numbers can be used for this optimization but diffraction properties have to be optimized later.
- Once a crystallization condition is identified, in the majority of cases those can be optimized to produce the ideal crystal for the technique.
- It is easier to induce nucleation and control size by material quality than to stop growth for crystals of very small dimensions.
- Small crystals enable more effective ligand binding studies.

# Thank-you and questions?

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- Collaborators at the Hauptman-Woodward Medical Research Institute - Drs Sarah Bowman and Diana Monterio
- **We are hiring** at the National Crystallization Center
- Much of the literature mentioned is available at https://getacrystal.org
- Crystallization, cryo-EM, and synchrotron services for academia and industry are available at https://hwi.buffalo.edu/research-services/


National Institute of General Medical Sciences

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