# Sample preparation for frontier structural biology: cryoEM, neutron diffraction, serial data collection and microED

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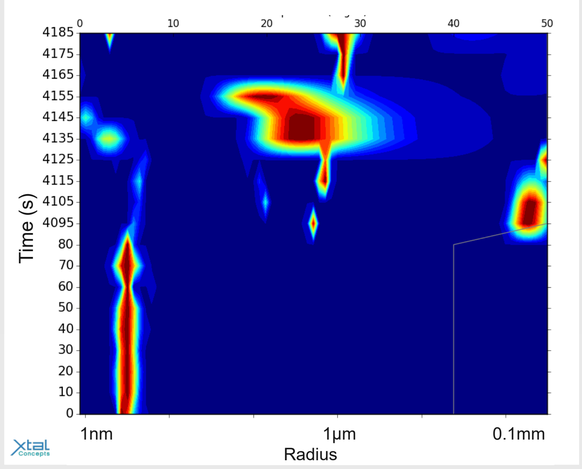
## Drop setter (microbatch)

## Dynamic light scattering

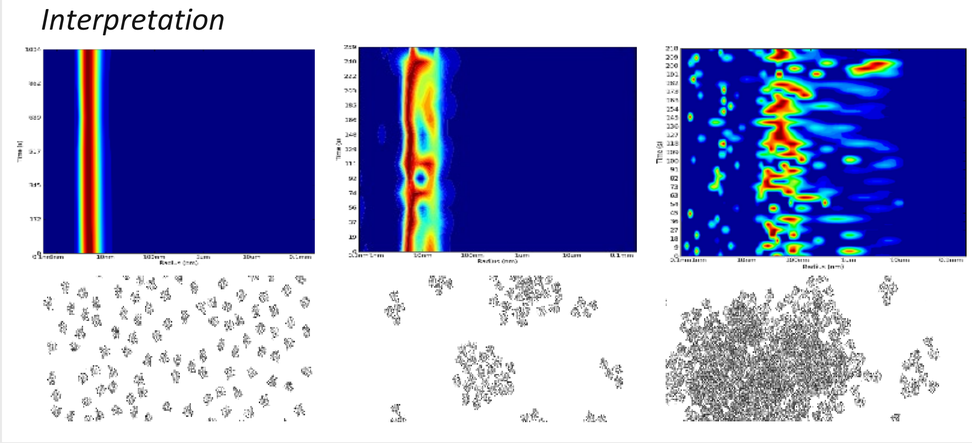

## cryoEM / crystallization / NMR

*DLS in microbatch-under-oil*

- Refractive index of oil is similar to water
- Surface is particle-free
- Alignment of laser and detector is maintained




*Interpretation*



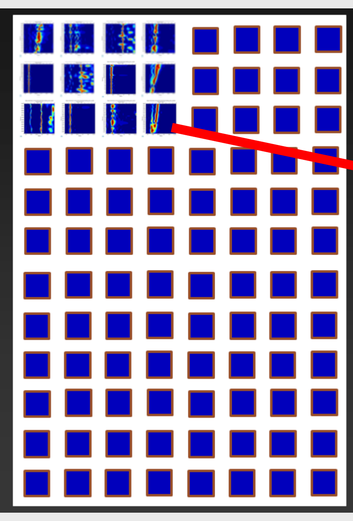
Membrane protein (LacY)

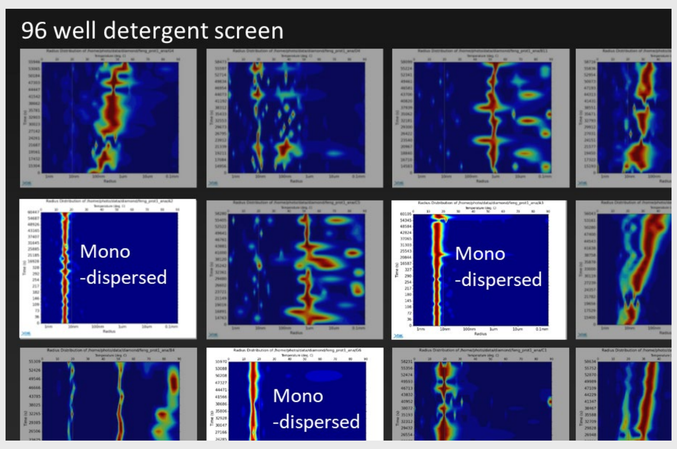
Crystallization project

Screen with 96 detergents

10 µl of protein

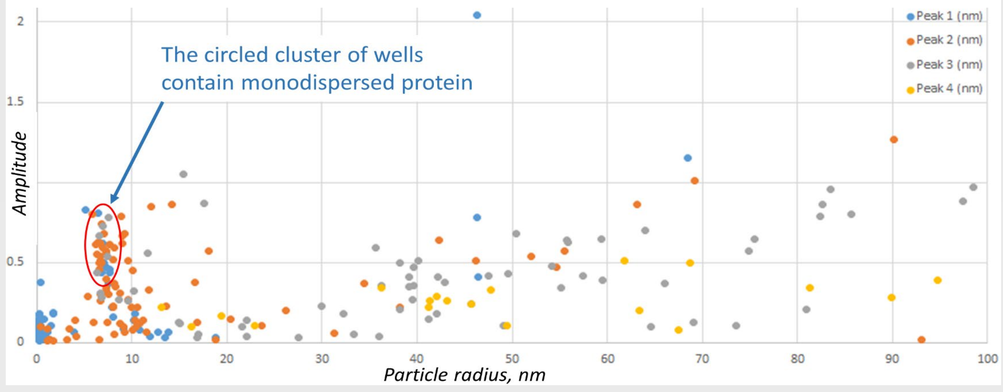
100 + 100 nL drops






*Screening – Solubility and Stability screen by Hampton Research*

DLS results for 100 nL LacY samples mixed with 100 nL screen for cryoEM and/or crystallization



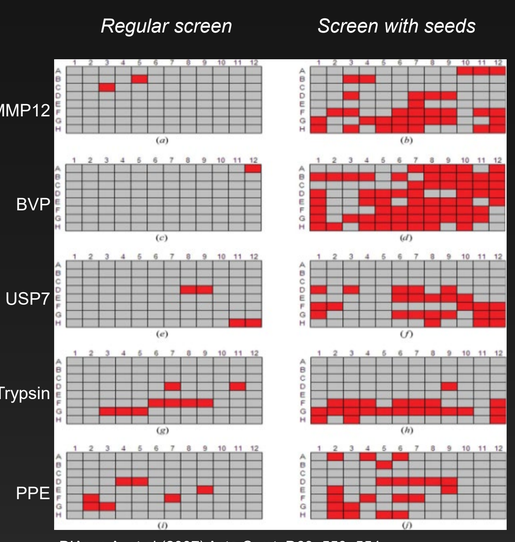

*Neutron diffraction* — *Serial data collection* — *MicroED*

*Less seed* ⋯⋯▶ *More seed*

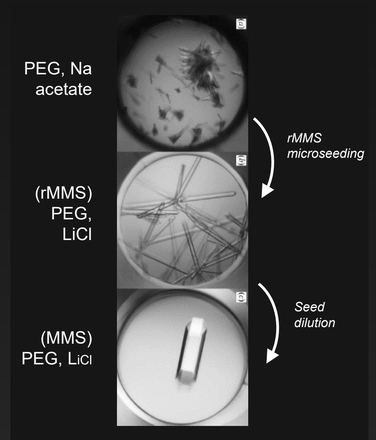
## *Same work flow*

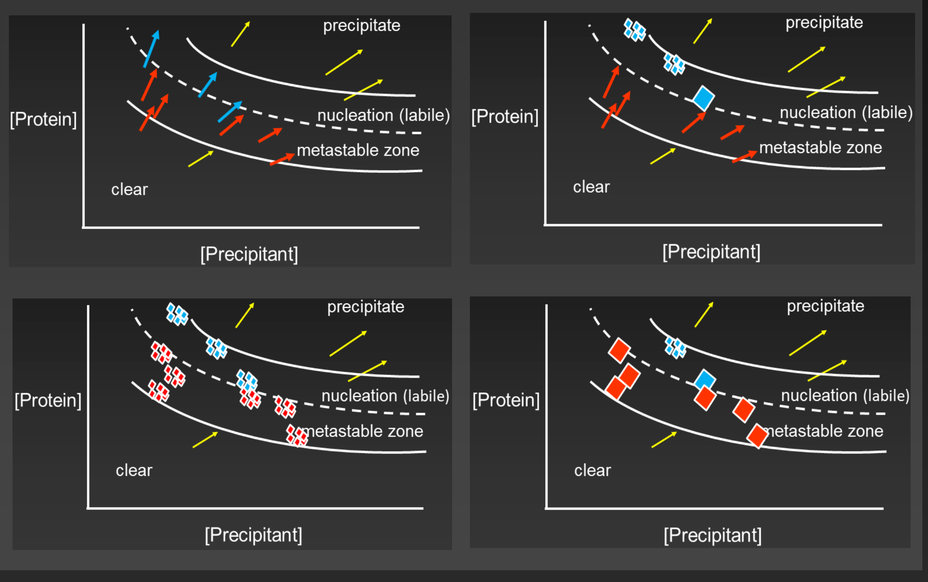
Starting with published vapor diffusion conditions:

- Make a seedstock – increase control
- Convert to MB: make a grid
- Or add seeds to a *random screen* in MB
- Make *phase diagrams* in MB
- *Scale up* sample volume
- Optimize
  - crystal crushing
  - seed dilution
  - crystal growth time

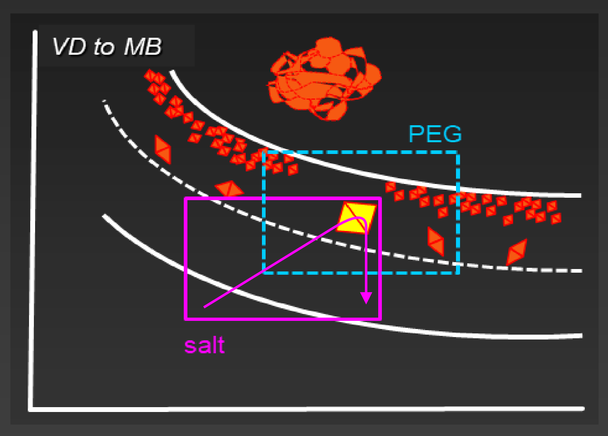



D'Arcy, A et al.(2007) Acta Cryst. D63, 550–554.

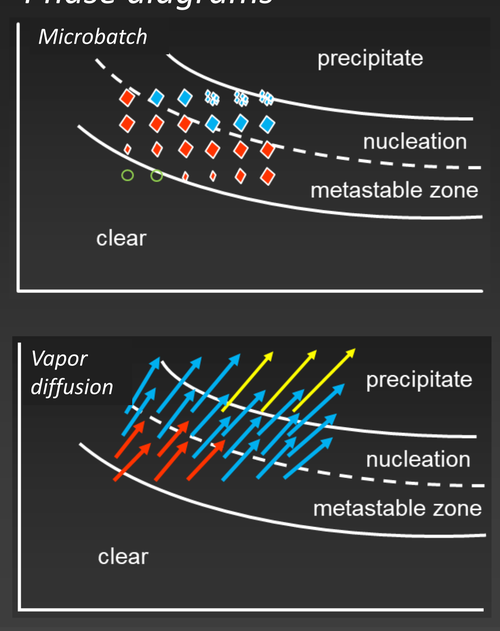


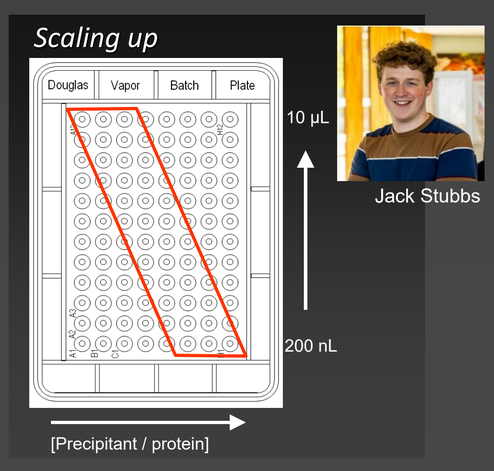

*Microbatch-under-oil*


*Phase diagrams*




*Scaling up*






Jack Stubbs