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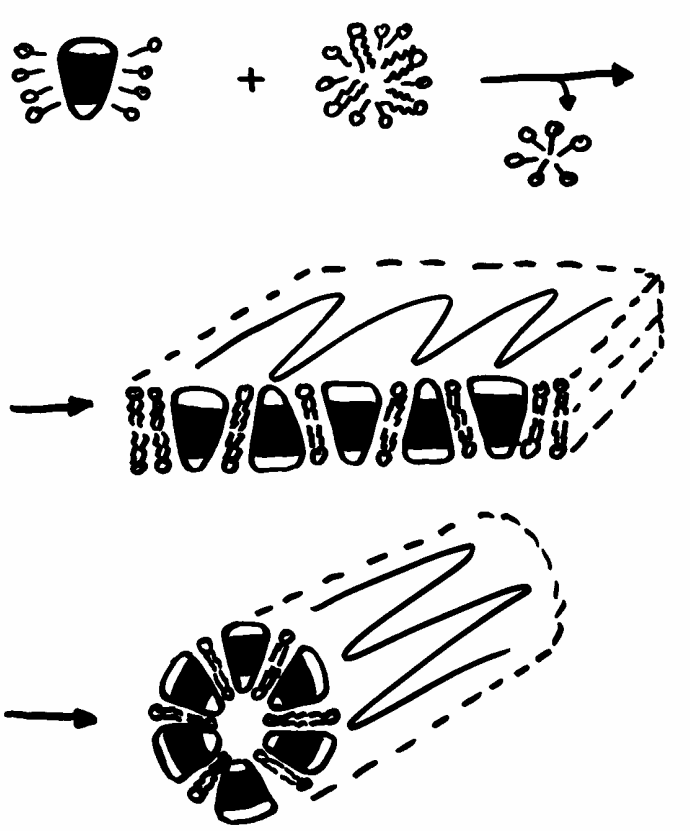
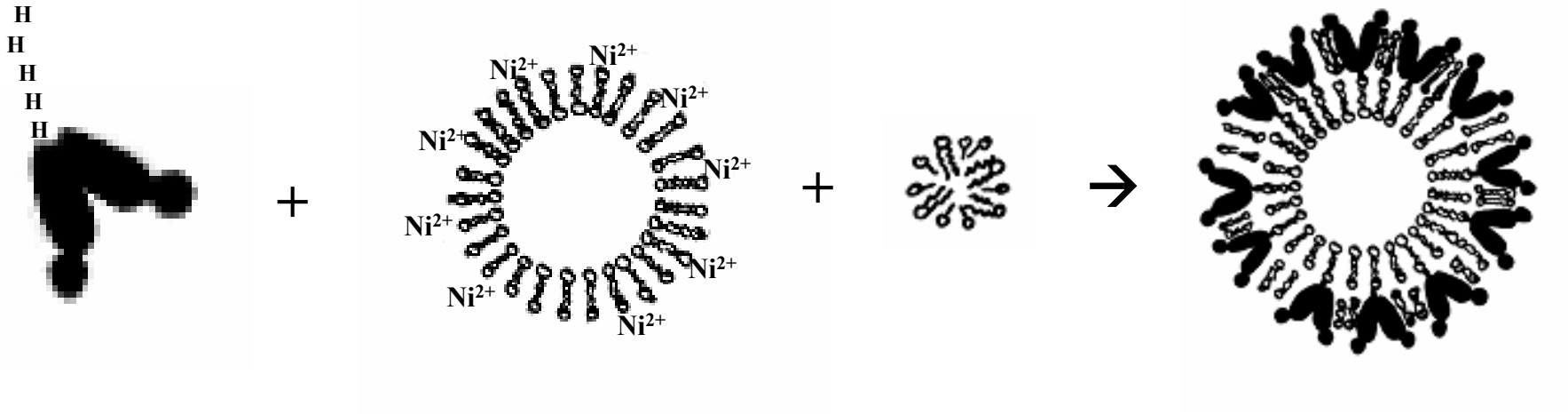
Crystallization of Membrane Proteins

Reconstitution

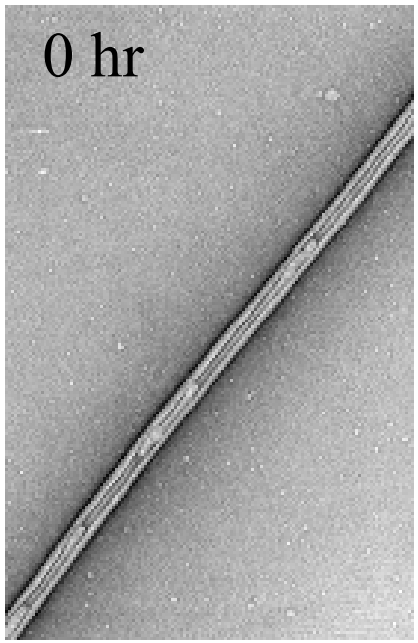


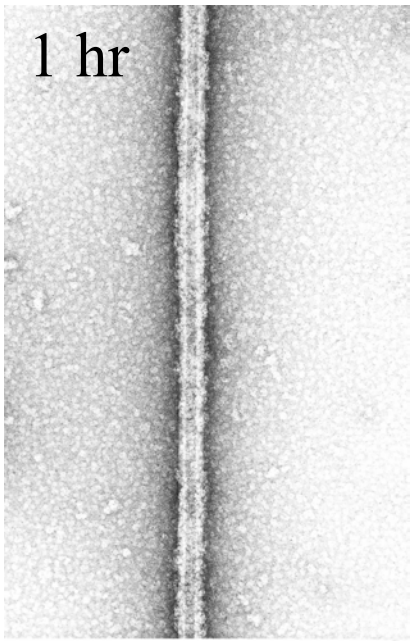
GalCer Scaffolding

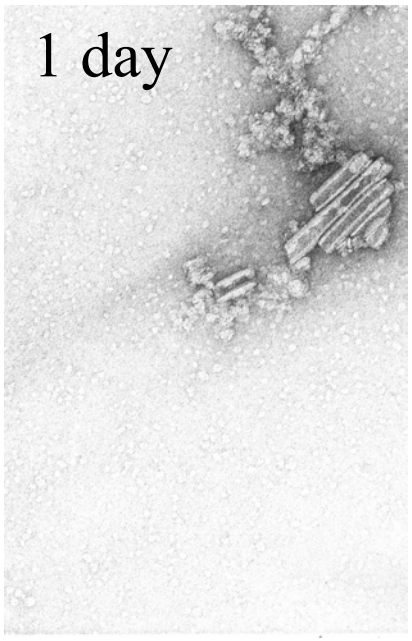
- Create tubes made of bilayers doped with a tag (Ni^{2+}, charge, biotin, etc.)
- Add tagged protein and lipid in the presence of detergent to pre-formed tubes
- Dialyze detergent out to form a second bilayer made of a reconstituted helical array of protein

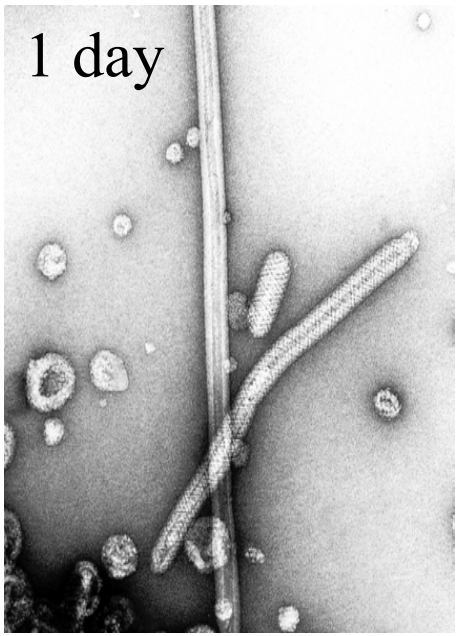


GalCer Experiments









no dialysis

with dialysis

Detergent Removal Strategies

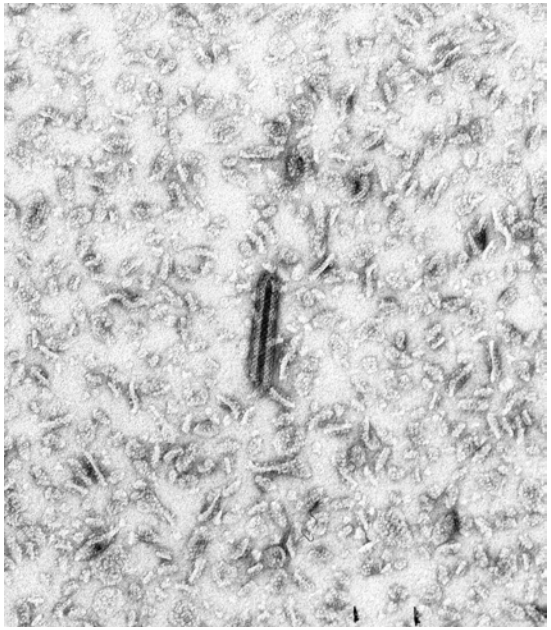
- Dialysis membranes
  - slow, difficult to set up with small volumes
- Dilution
  - very fast, can change [x] unfavorably
- BioBeads
  - fast, easy, can vary rate many ways

BioBead preparation

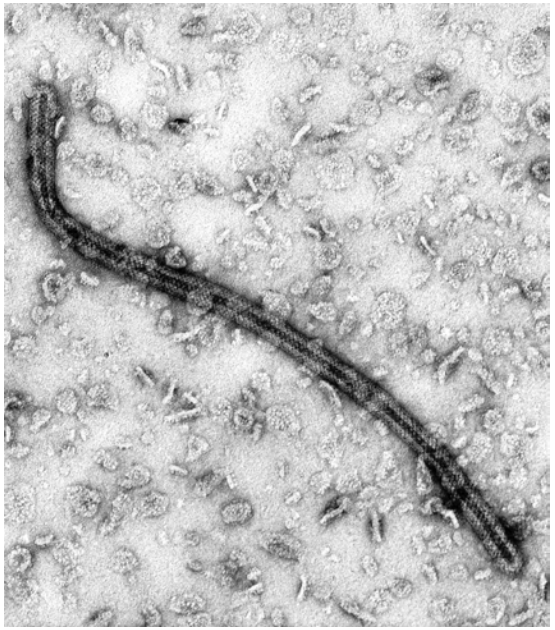
- Clean BioBeads with methanol
- Wash BioBeads in buffer (+/- detergent)
- Store BioBeads wet on moist filter paper for up to one month

Rigaud, et al., JSB 1997

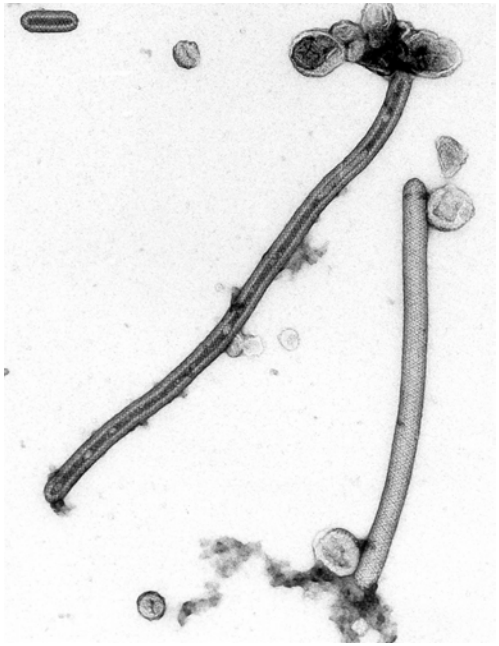
Detergent Removal via BB



2 hours



6 hours



16 hours

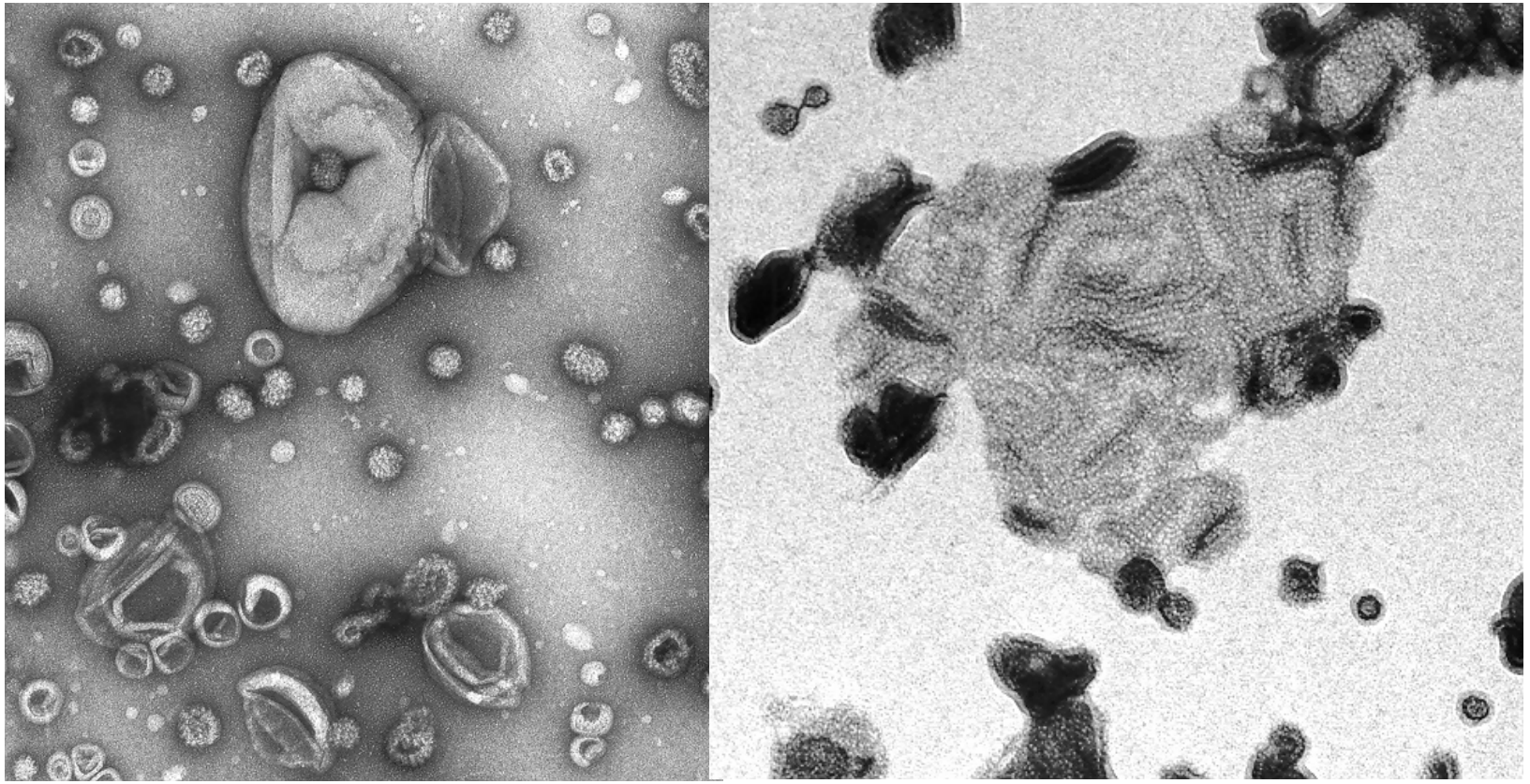
Screens

Set up basic matrices to begin

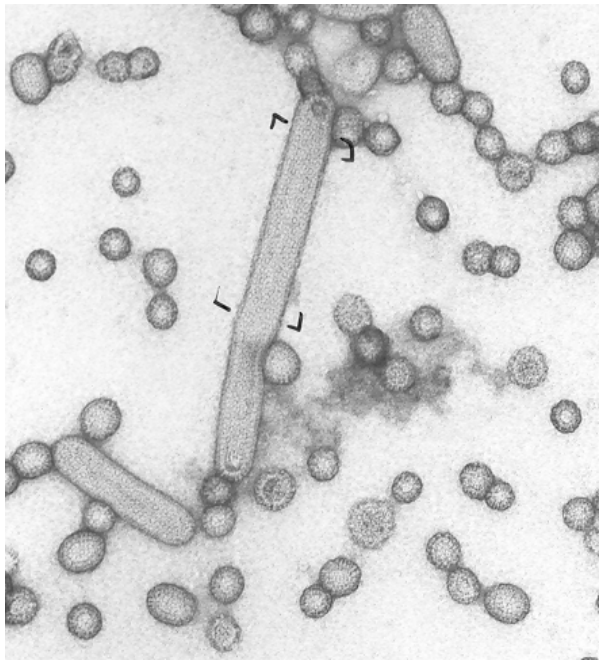
- pH, [lip]:[prot], lipid choice

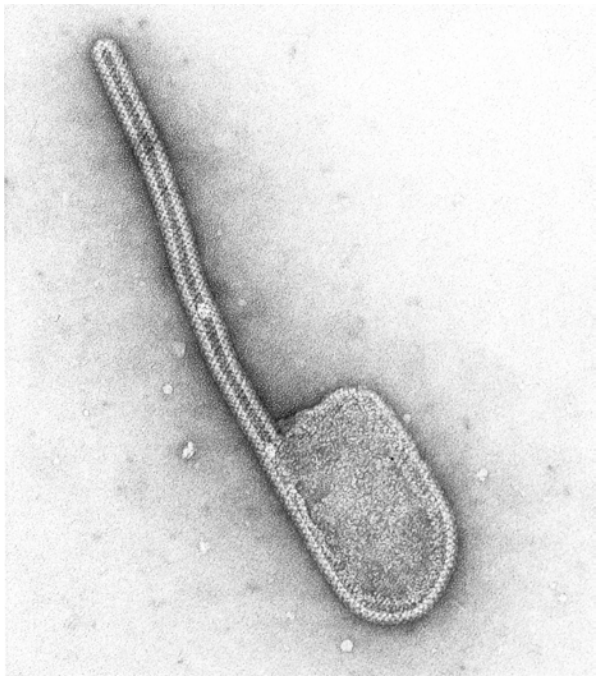
- Refine with additives, variations in [x], detergent removal rate, temp, etc.

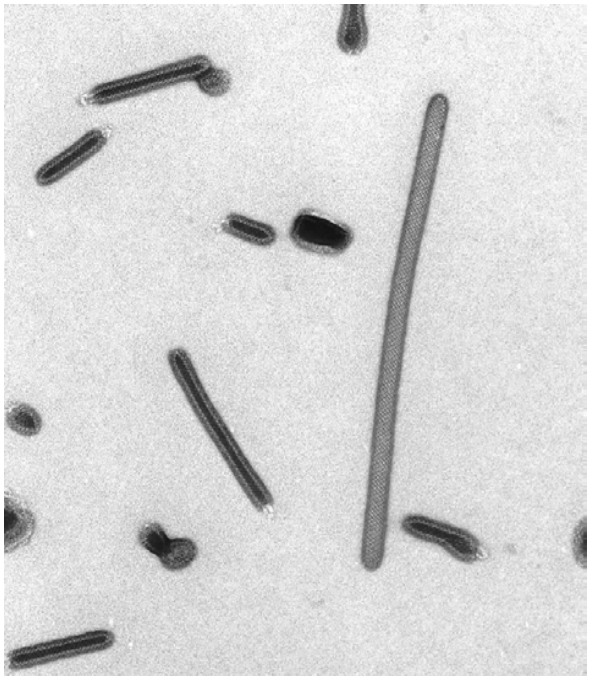
Crystallogenesis



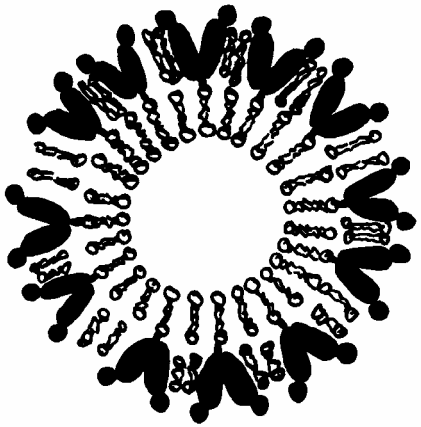
Crystallogenesis





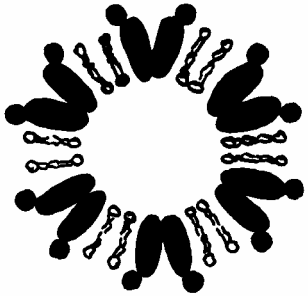


Helical Arrays



GalCer tubes:

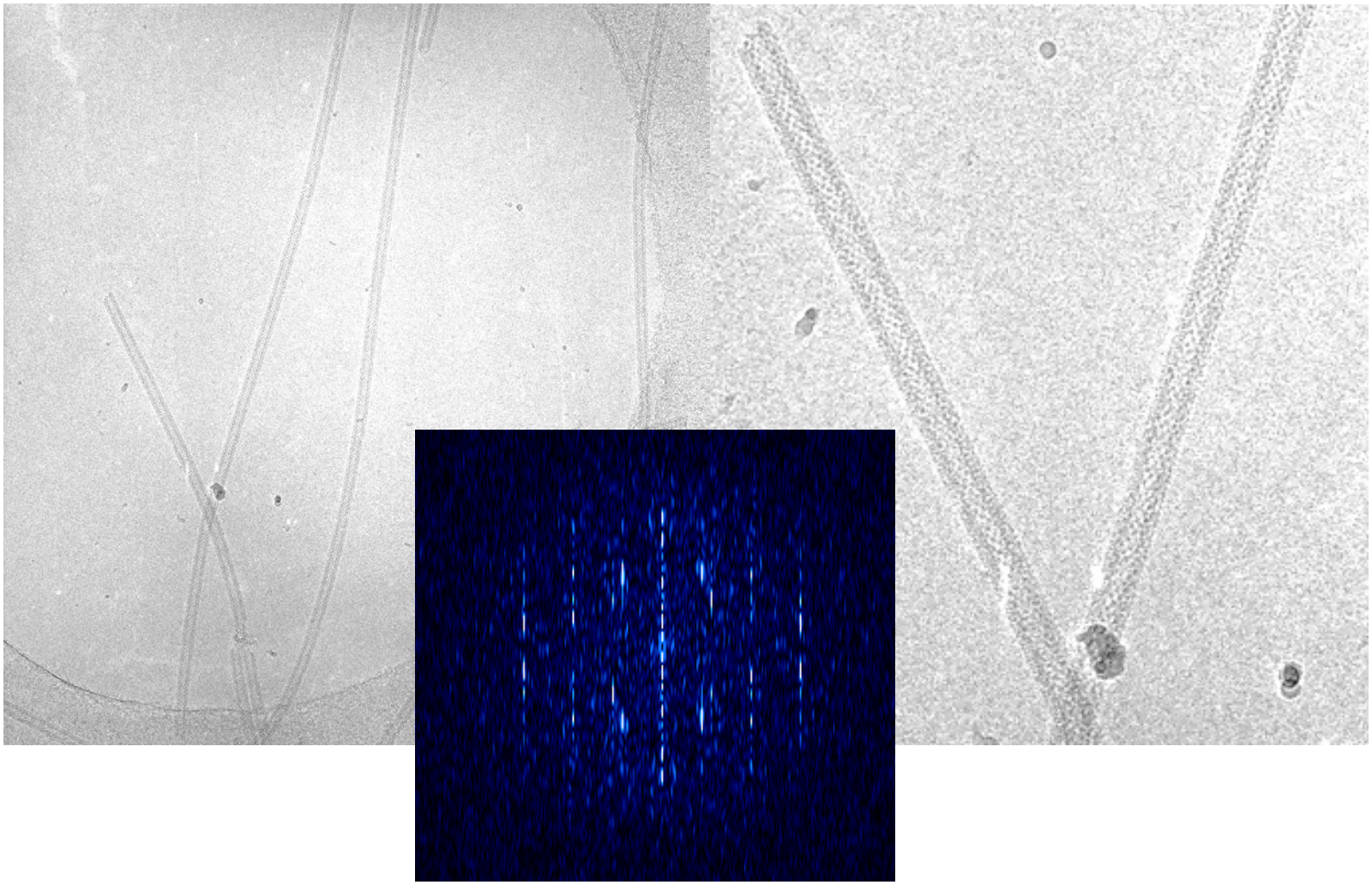
- confined geometry
- susceptible to detergent
- template for any type helix



Native tubes:

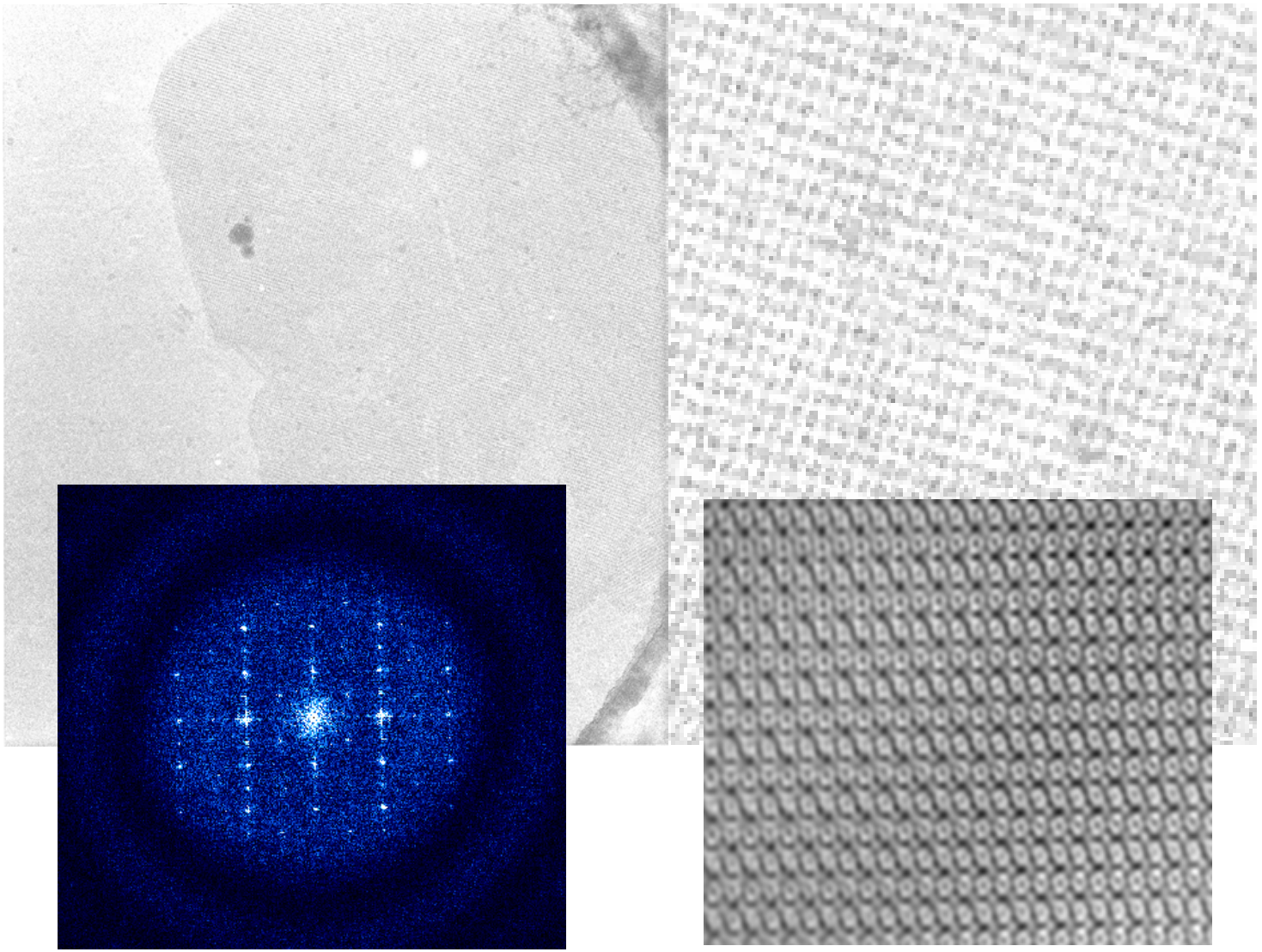
- protein must drive helices
- slower?

Helical Crystals

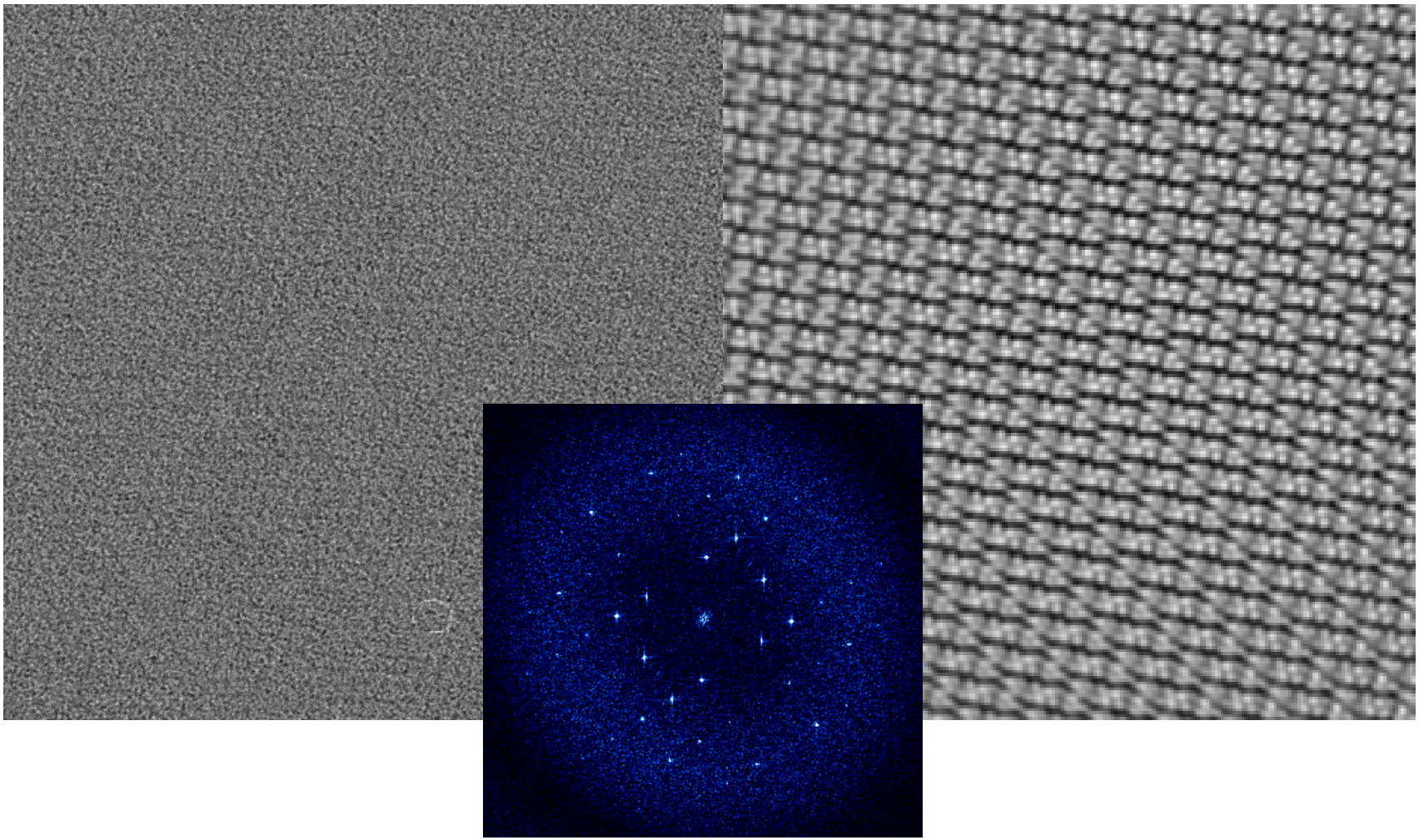


2D Arrays

2D Crystals



2D Crystals



What makes a good crystal?

- Low mosaicity/no variation in helical pitch
- Larger (2D)/longer, straighter (helical)
- Low background
- System amenable to cryo…especially for helical arrays