

CryoEM sample preparation: A few tales from the bench

Nita R. Shah

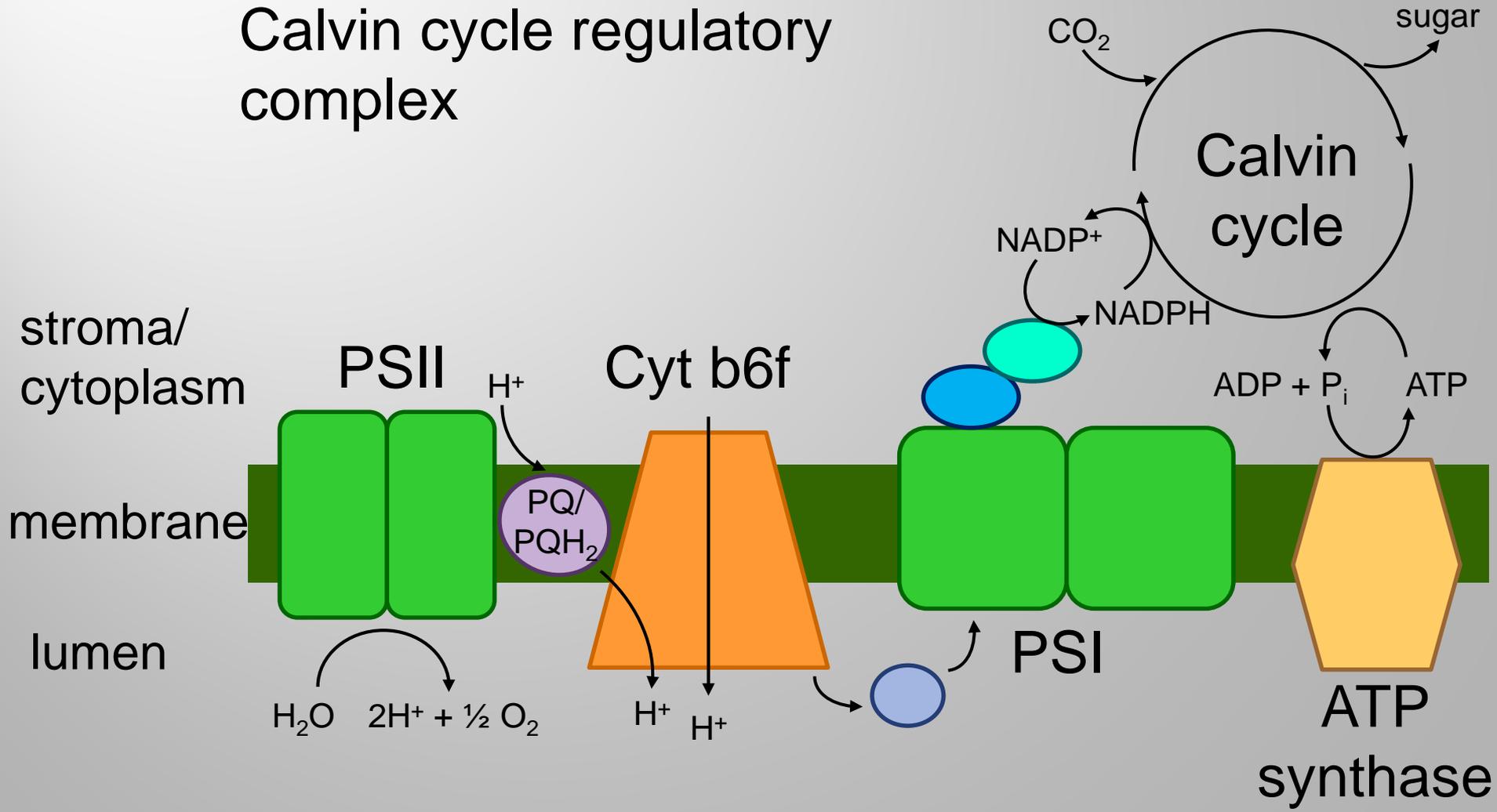
@nitarshah

Bubeck Lab

Imperial College
London

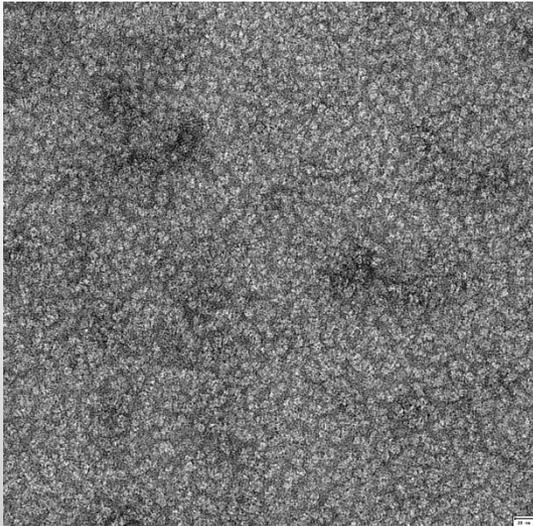
Project 1: Calvin cycle complex

Goal: solve the structure of Calvin cycle regulatory complex

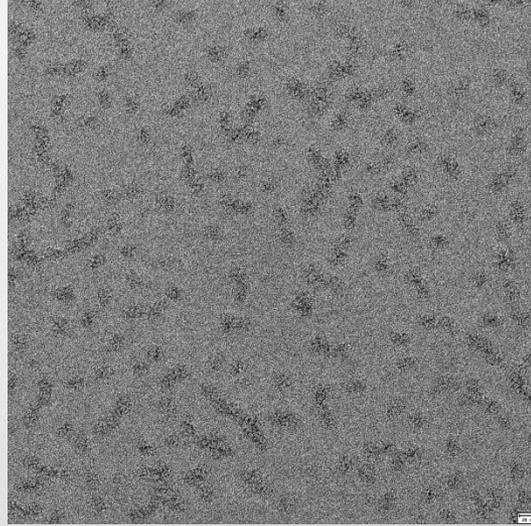


Step 1: Optimizing the sample

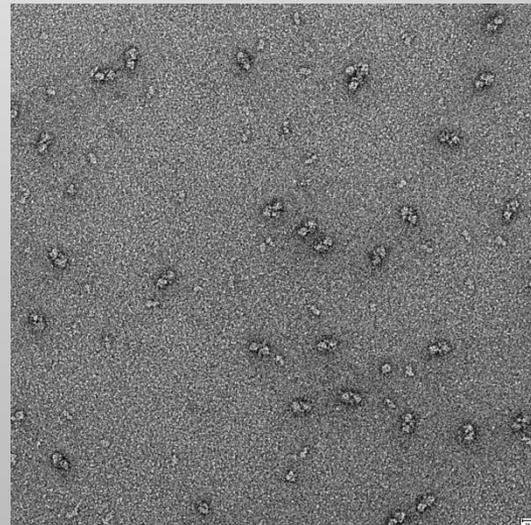
Original sample



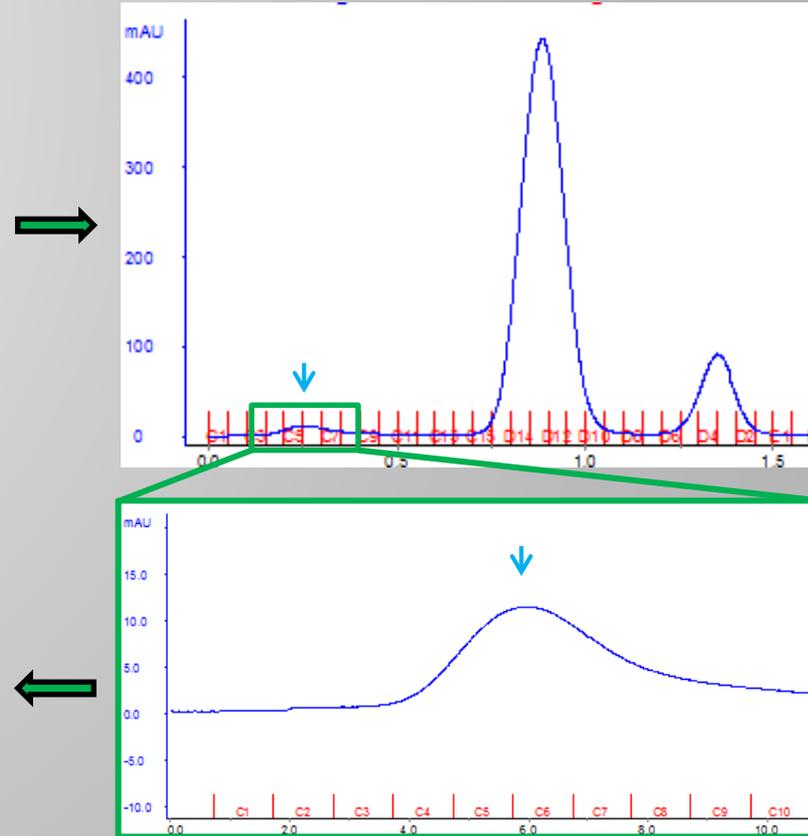
Original sample 1/10 dilution



SEC fraction, undiluted

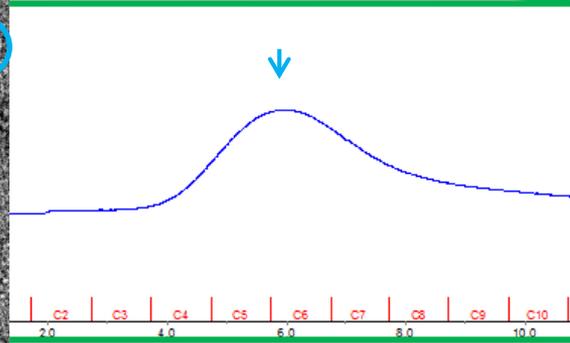
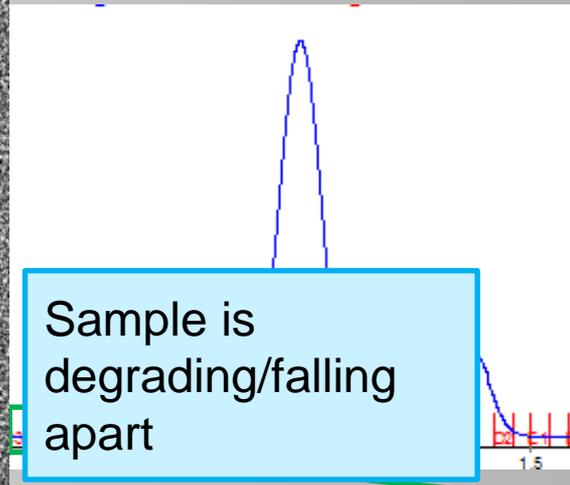
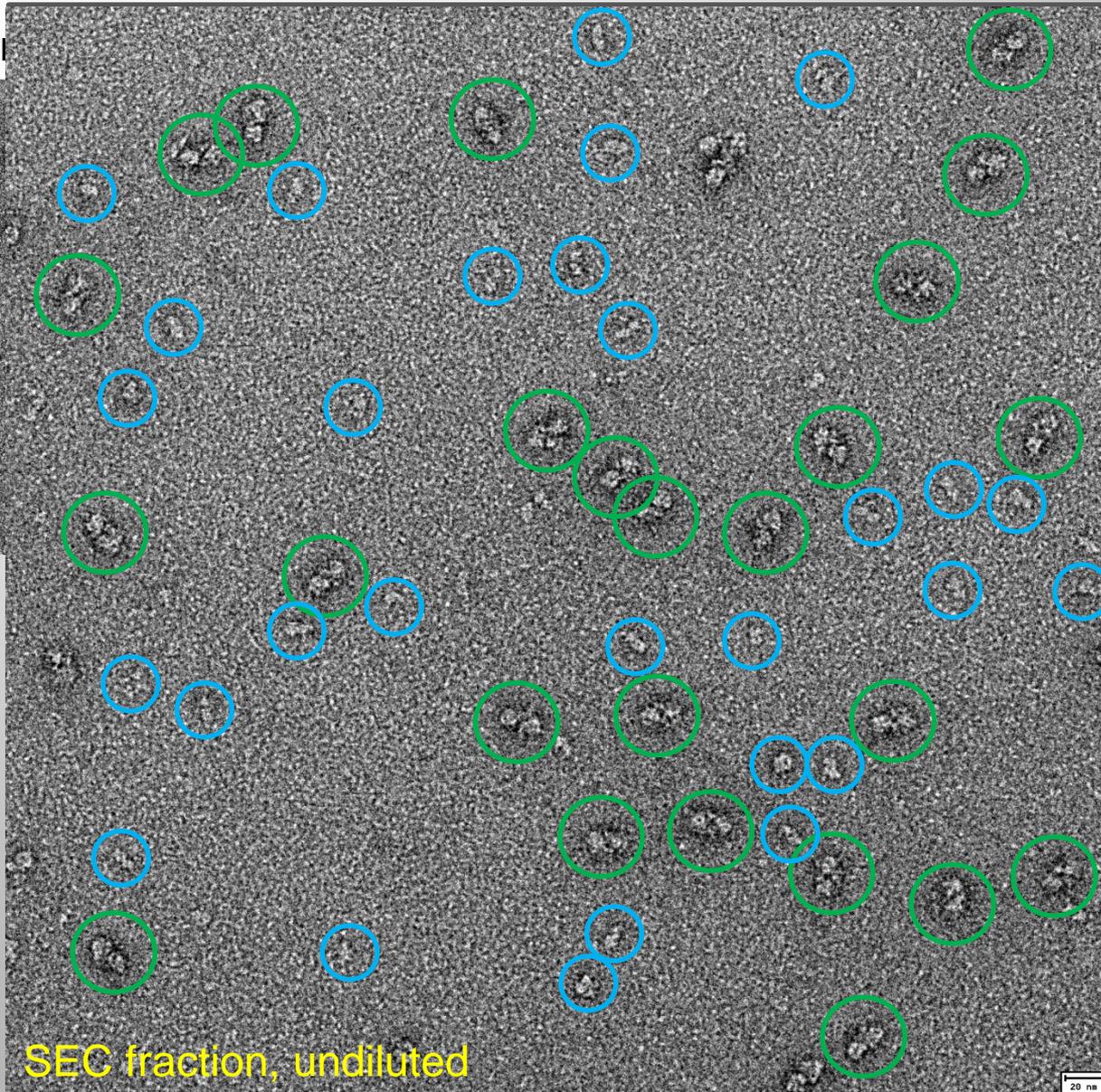


Less clumpy/
aggregated after
fresh SEC



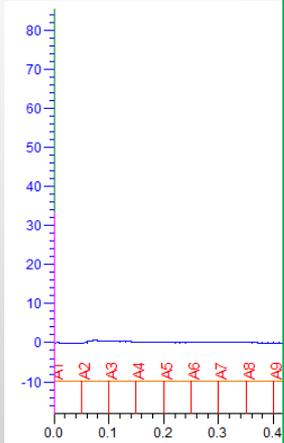
Step 1: Optimizing the sample

Original

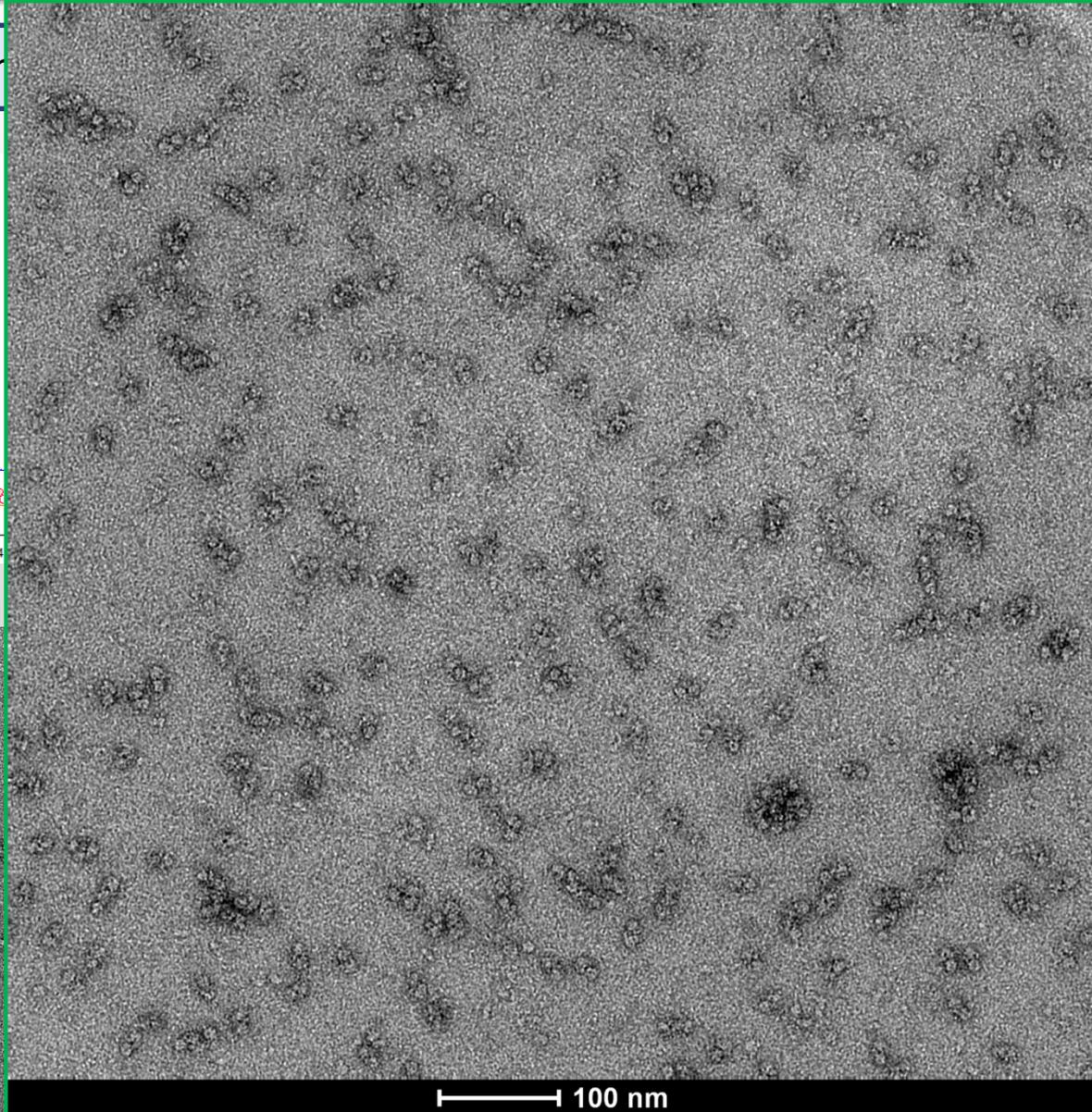


Step 1: Optimizing the sample

Next step: Im



Previous sam



concentration on
half of the peak
aggregated particles

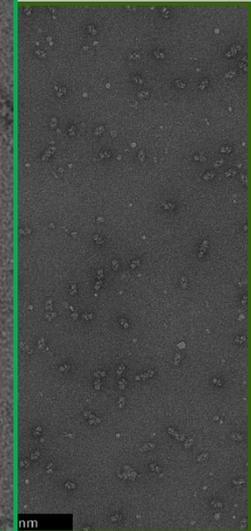
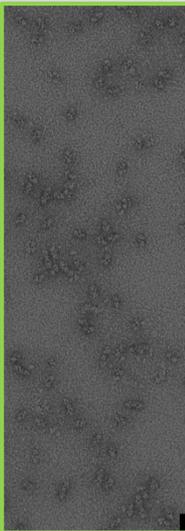
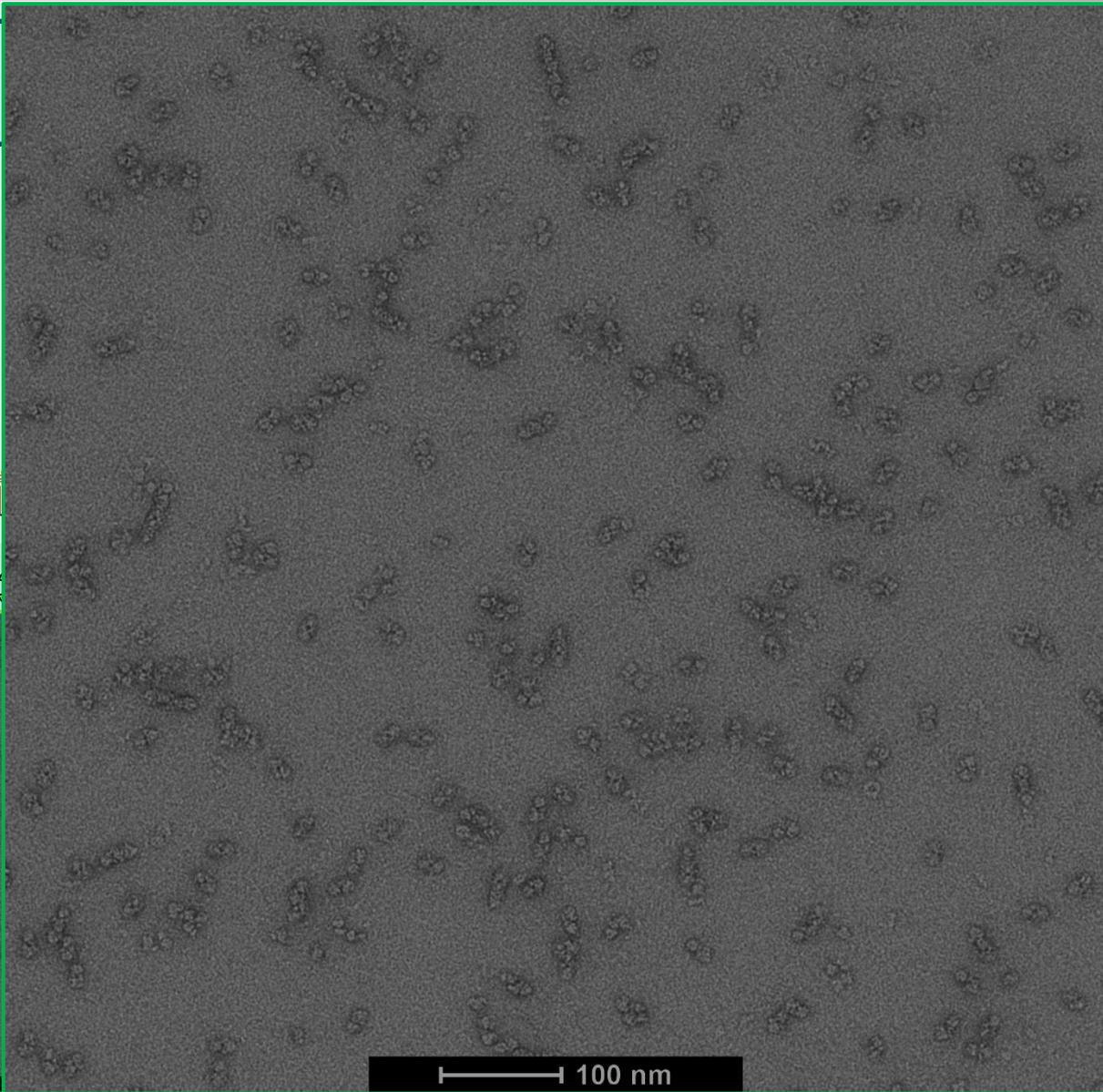
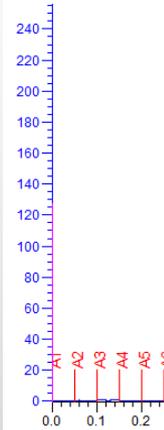
100 nm

Step 1: Optimizing the sample

Next step:

complex together

concentration on
be the best



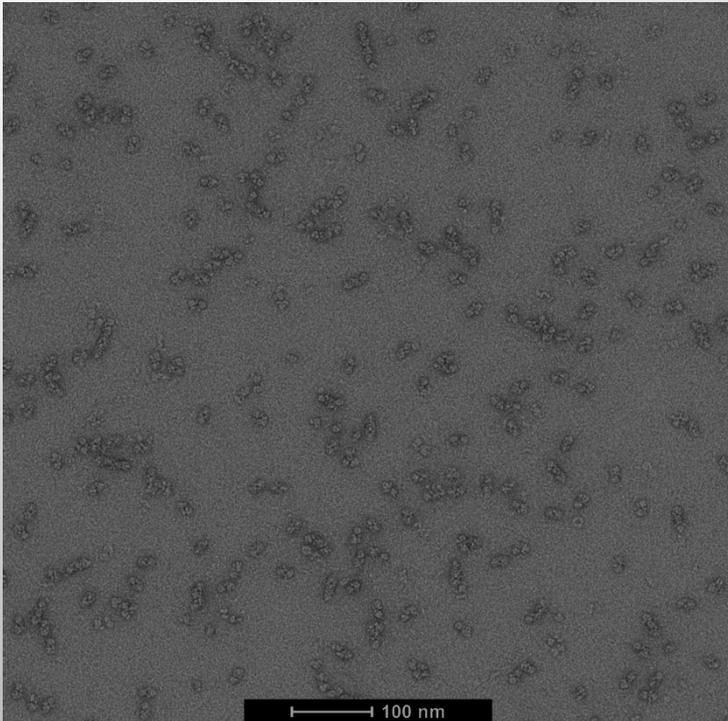
100 nm

nm

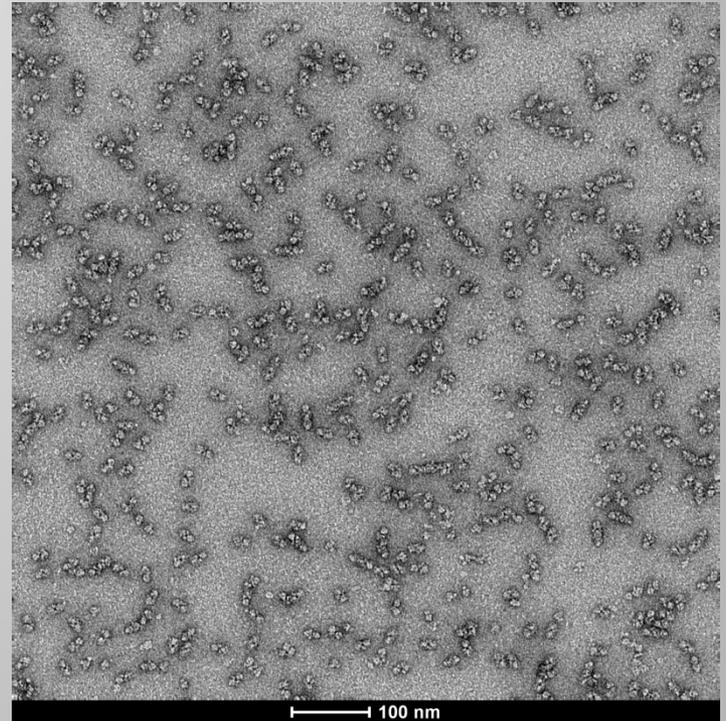
A few side notes

Note 1: Negative stain EM is a great way to check your sample stability, e.g. after storing at 4°C or freeze/thawing

Original sample, 1/10 dilution



After 4 days at 4°C, 1/4 dilution



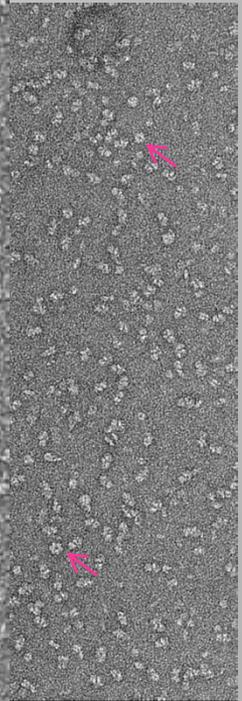
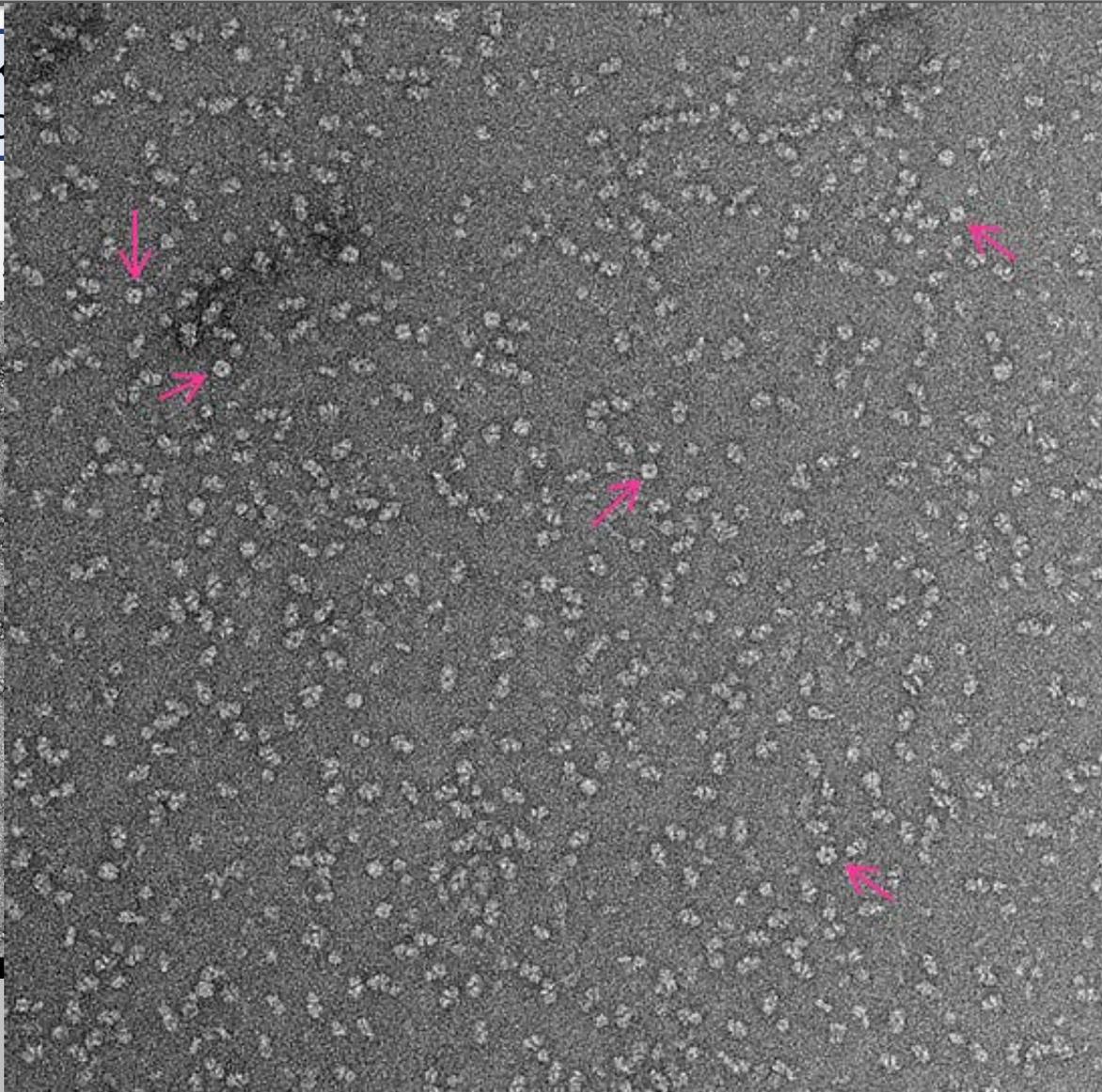
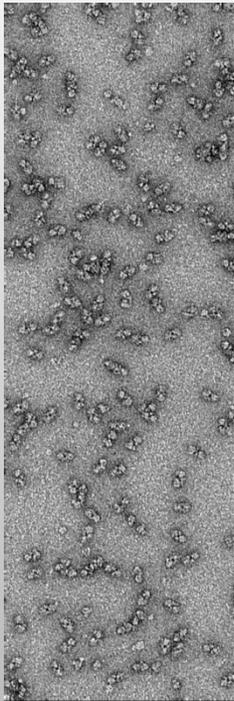
A few side notes

Note 2: Check
avoid

maintain quality –

cond SEC
remove contaminant

Previous

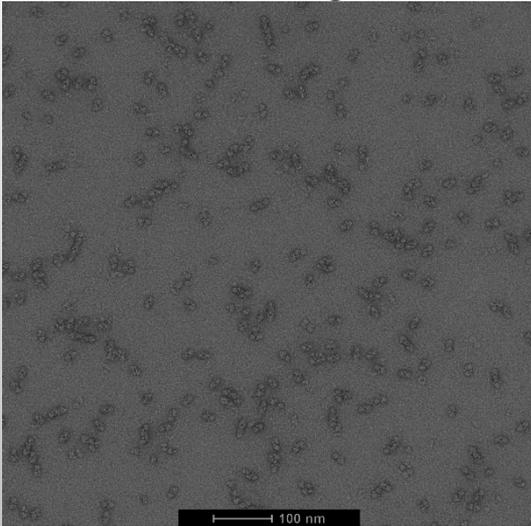


100 nm

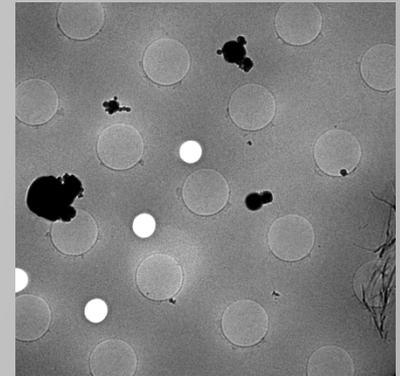
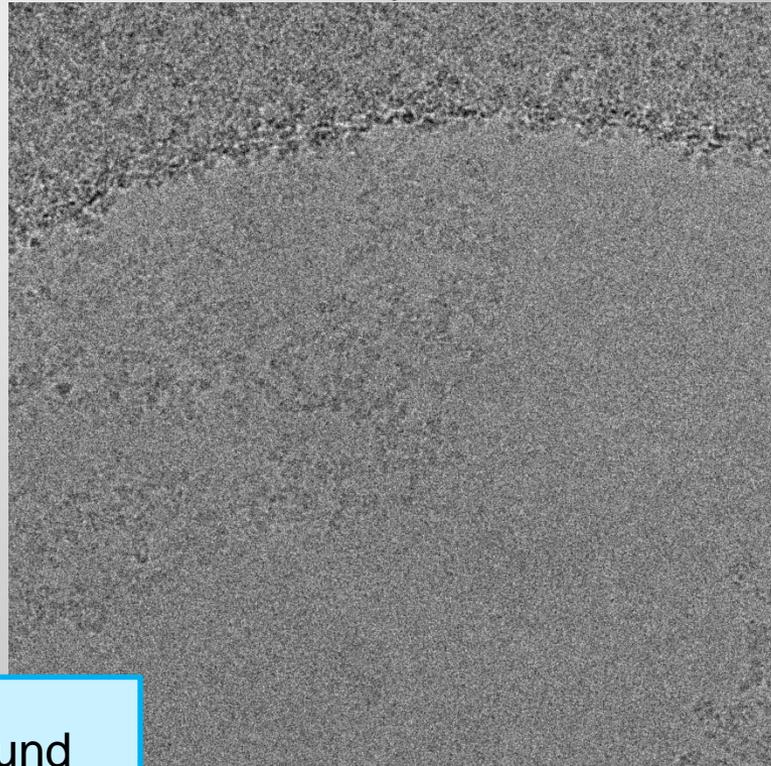
Step 2: Optimizing the grid

Next step: Freeze the C4 sample on grids (Cflats, 1.2/1.3)

C4 1/10 dil. negative stain



C4 undiluted, cryoEM



- Particles are clumping around the edge of the hole AND sticking to the carbon hole edge

Step 2: Optimizing the grid

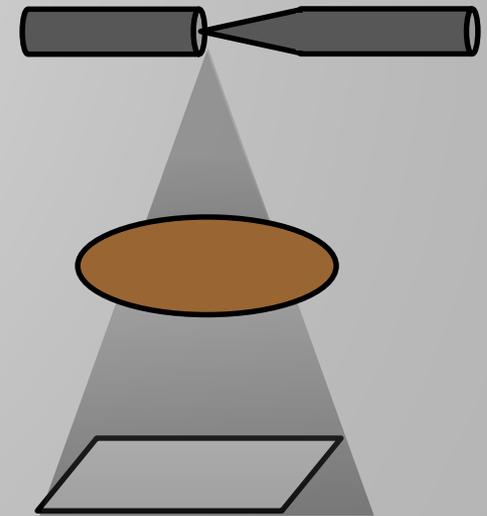
Next step: Make carbon-coated grids

Carbon coating, things to consider:

- **Carbon thickness**
 - Thinner carbon = less background
 - Too thin carbon = breaks apart during blotting
- With the support, you can get a **higher concentration of particles** in holes
- Carbon support can cause **orientation bias**

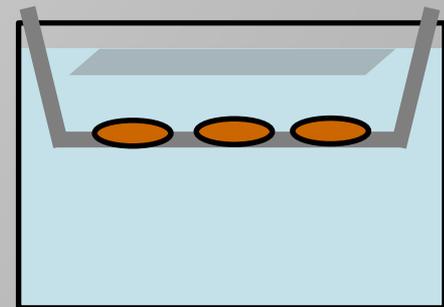
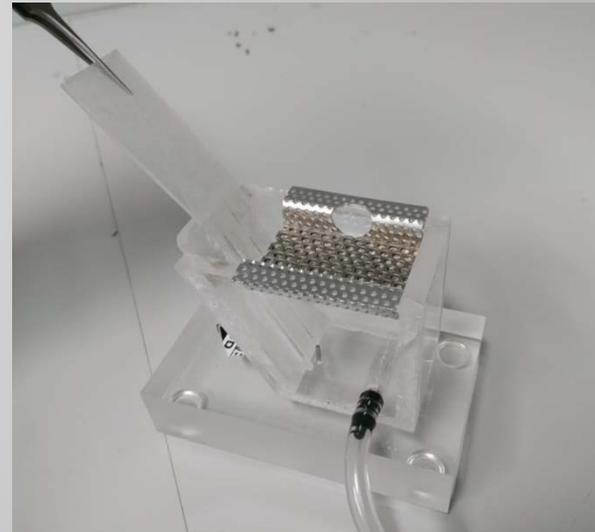
Step 2: Optimizing the grid

First: make the thin coat of carbon on mica



Step 2: Optimizing the grid

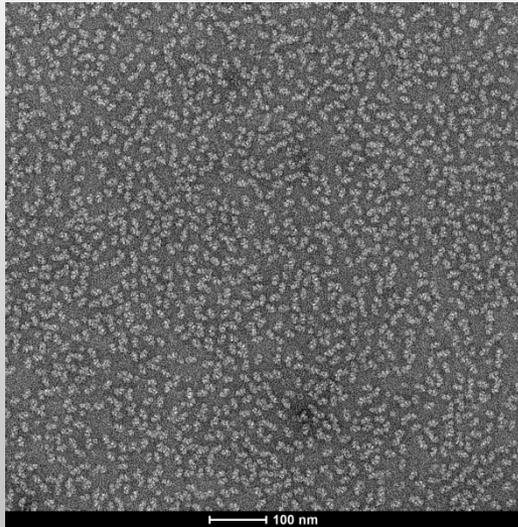
Second: float the carbon layer onto the grids



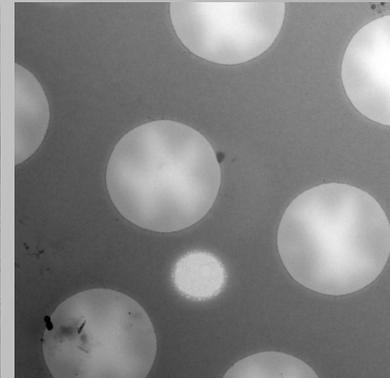
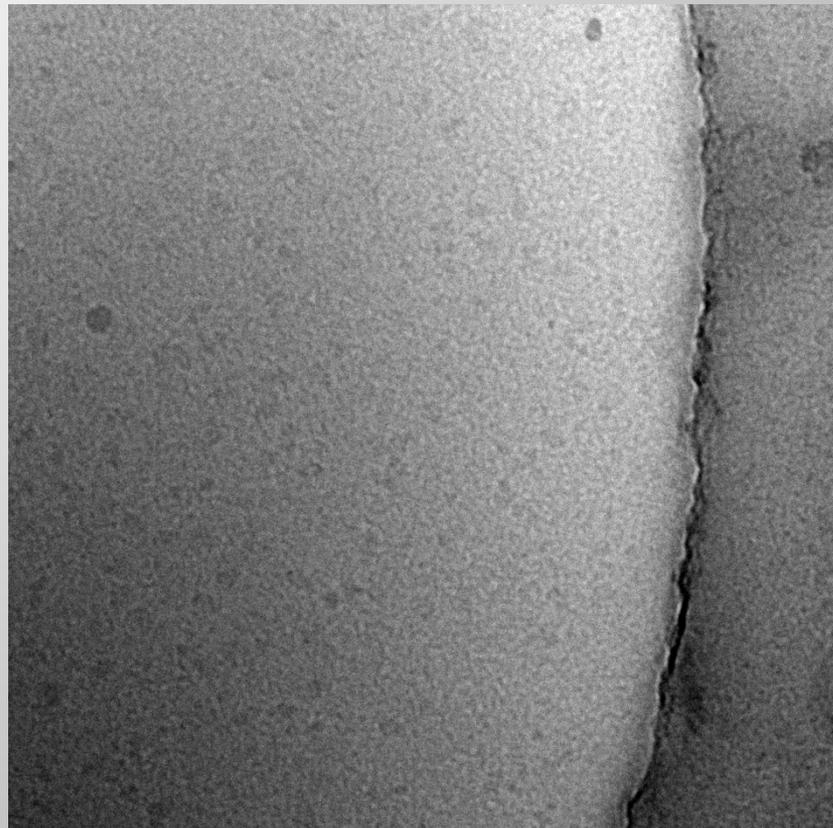
Step 2: Optimizing the grid

Next step: Freeze sample on carbon-coated Quantifoil 2/2

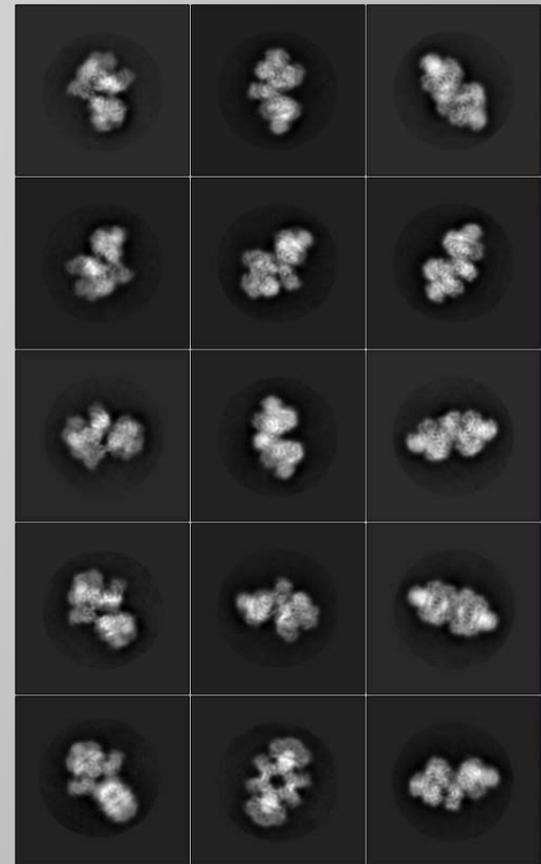
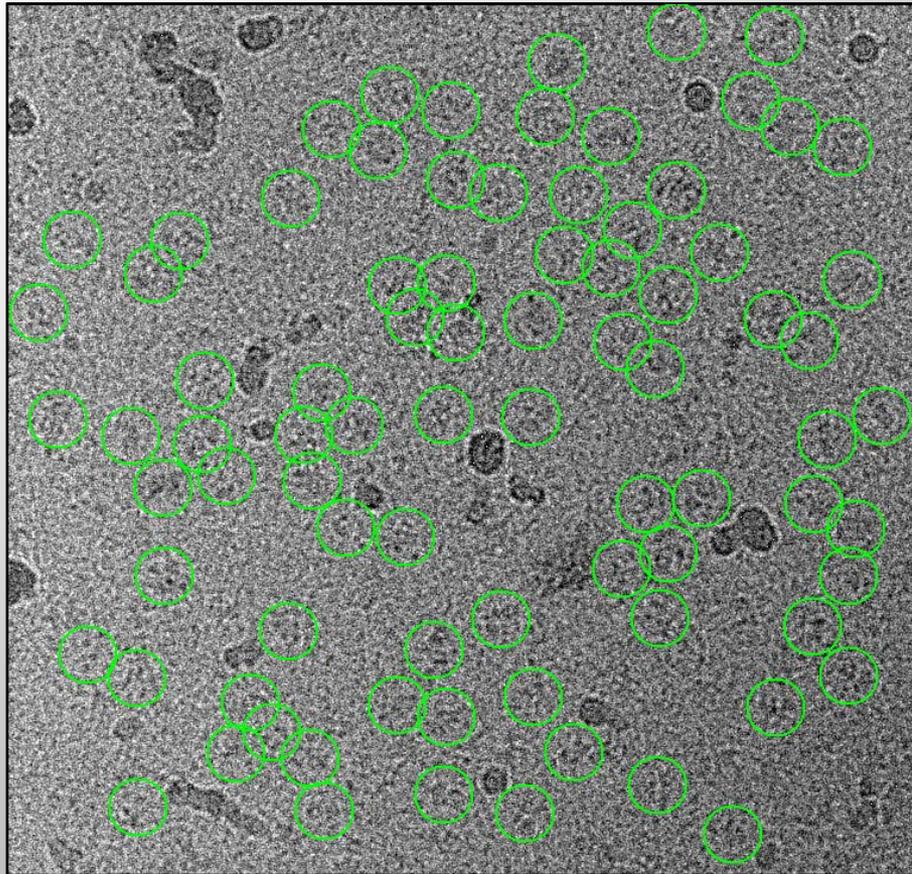
Negative stain 1/4 dilution



cryoEM, undiluted

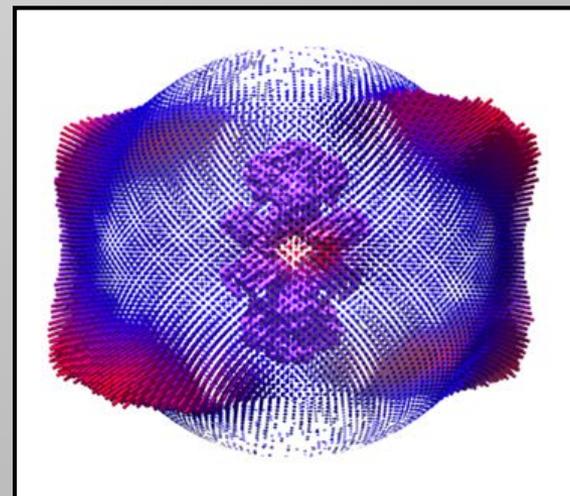
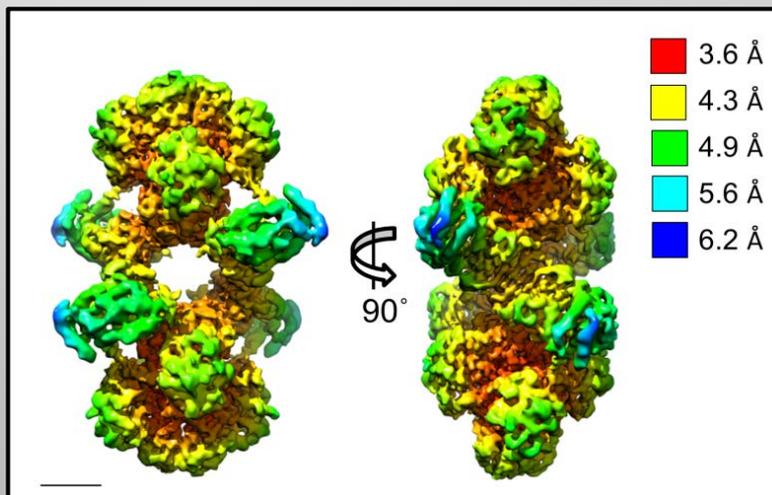
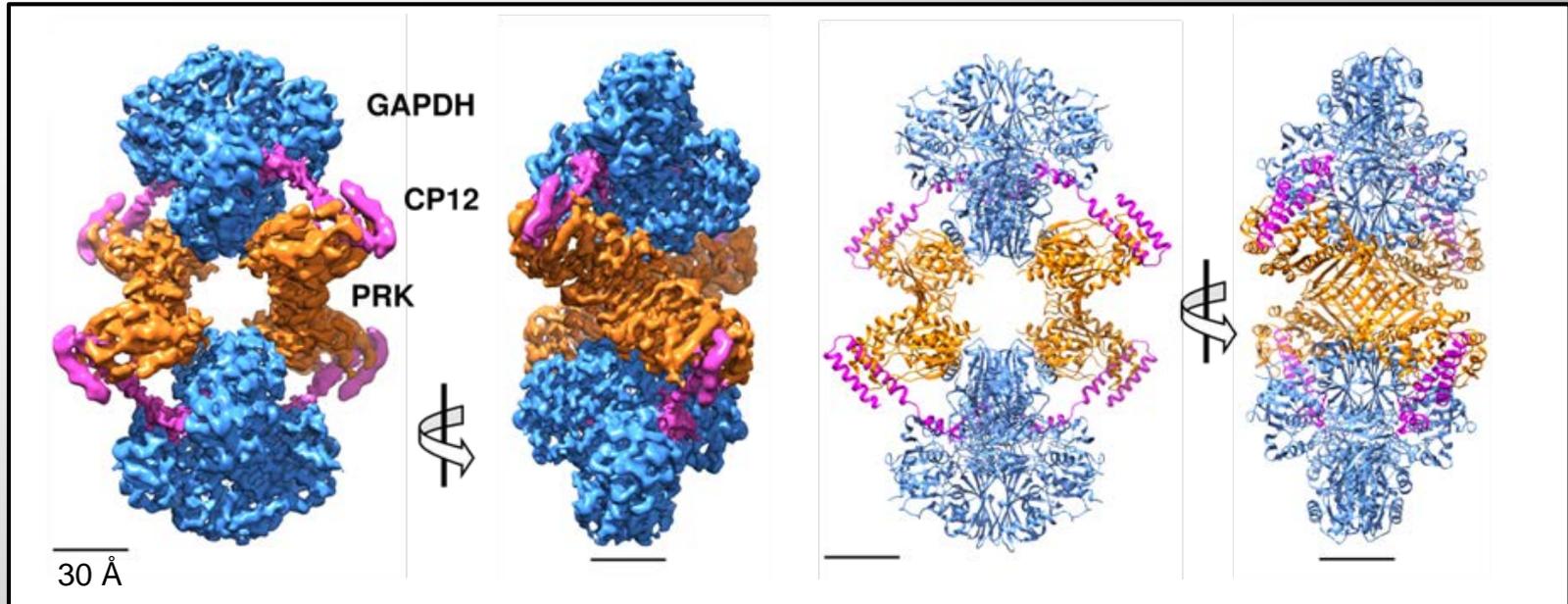


Krios collection at eBIC

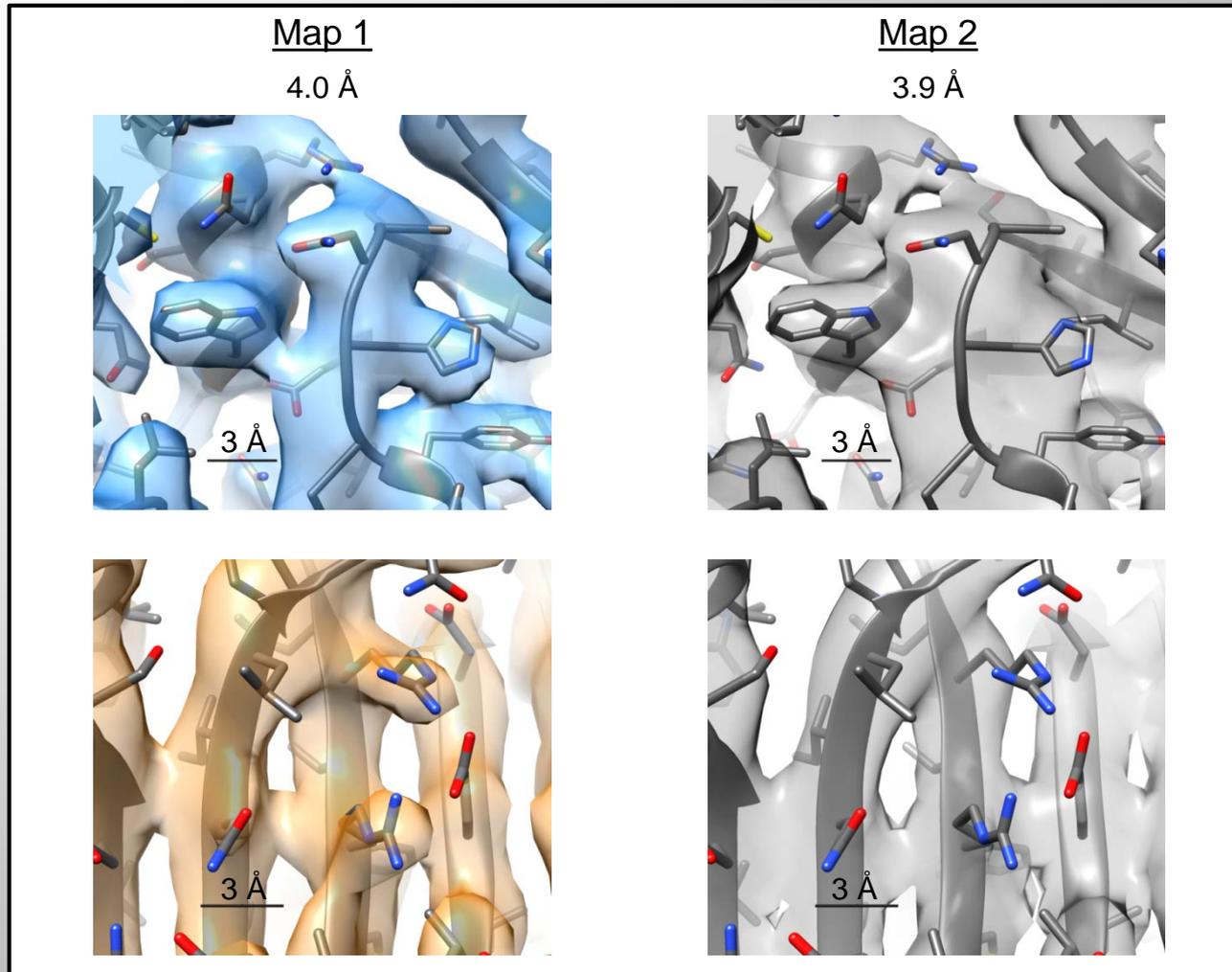


197 212 particles

Reconstructed density to 4.0 Å



Side note on resolution estimate



Always check the map quality, and don't just rely on the resolution estimate given to you by various programs

The End
