

# **CryoEM sample preparation: A few tales from the bench**

**Nita R. Shah**

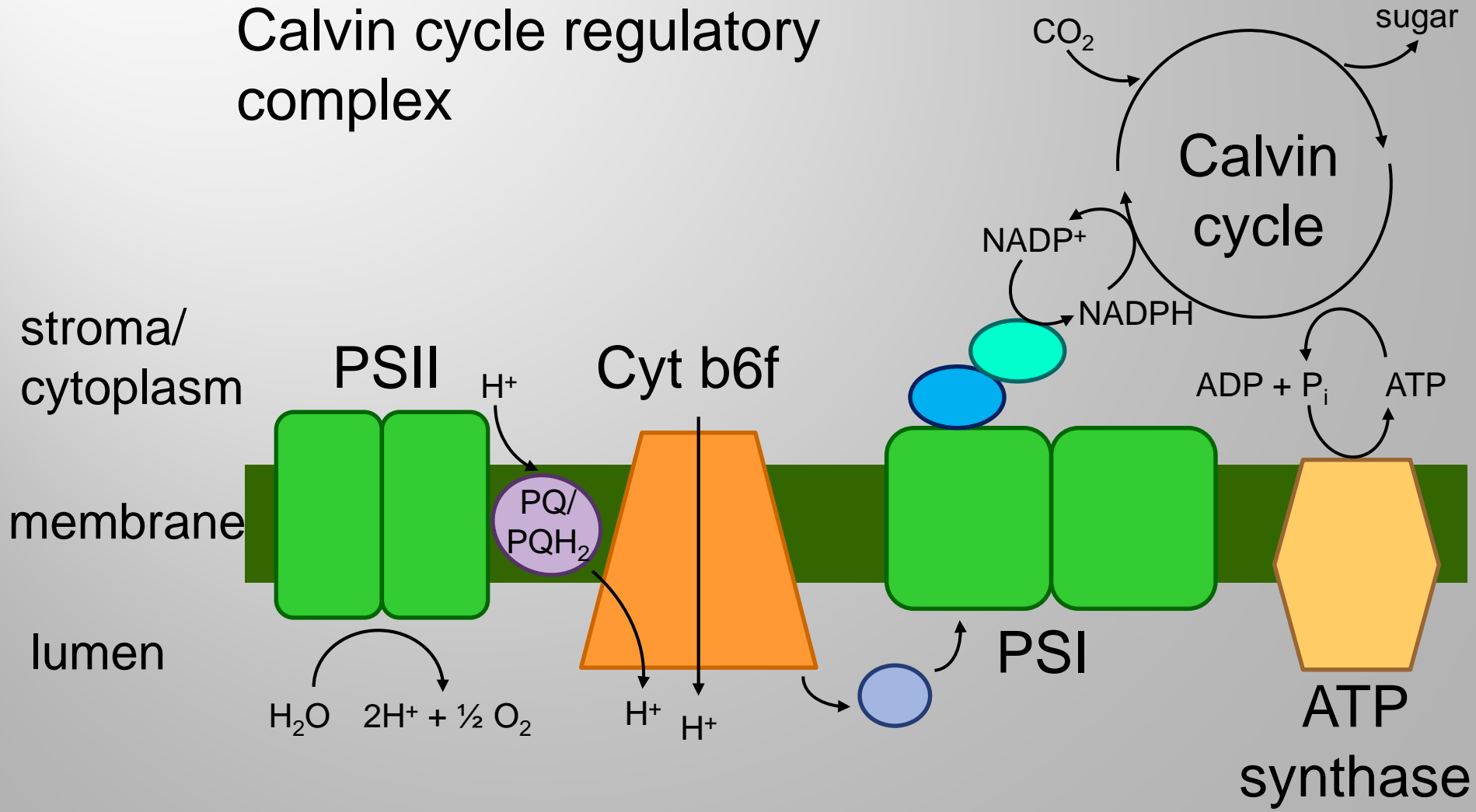
@nitarshah

Bubeck Lab

**Imperial College**  
London

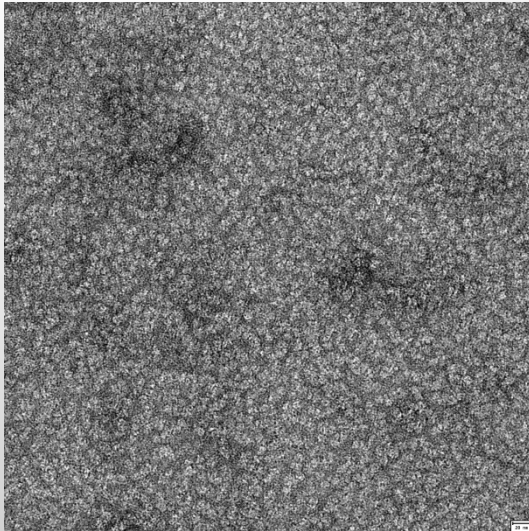
# Project 1: Calvin cycle complex

Goal: solve the structure of Calvin cycle regulatory complex

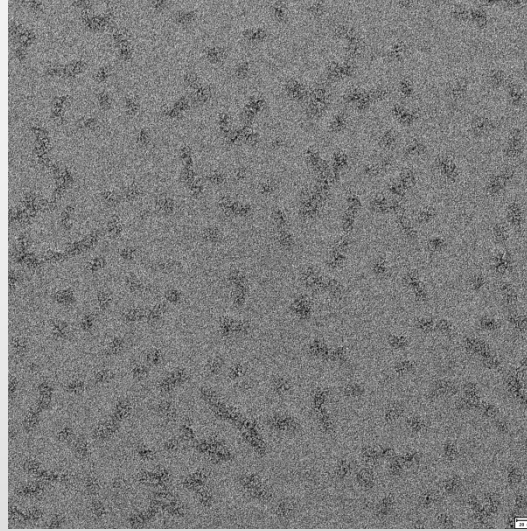


# Step 1: Optimizing the sample

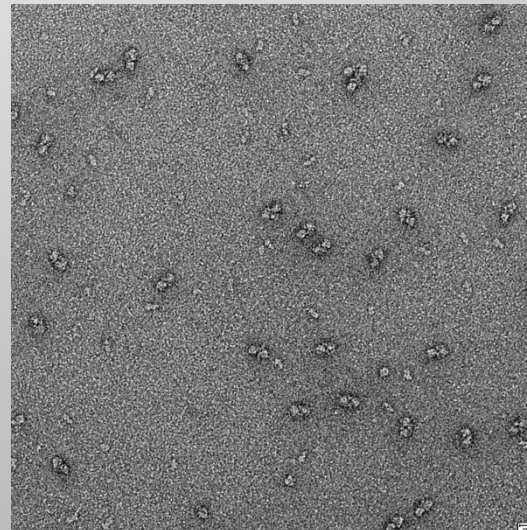
Original sample



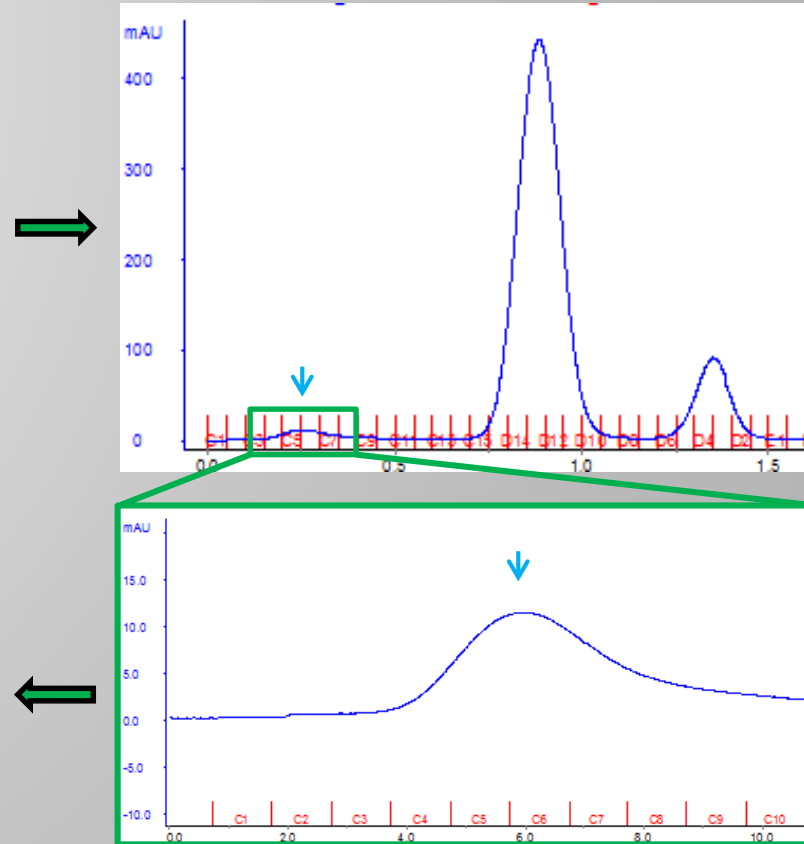
Original sample 1/10 dilution



SEC fraction, undiluted

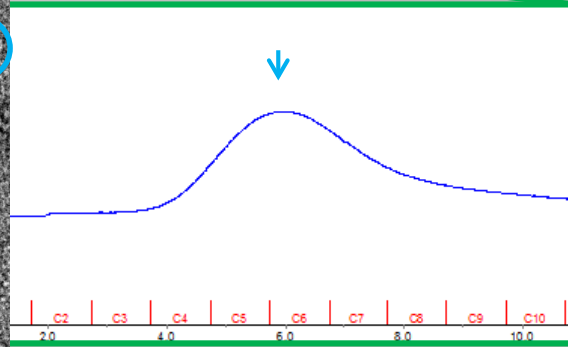
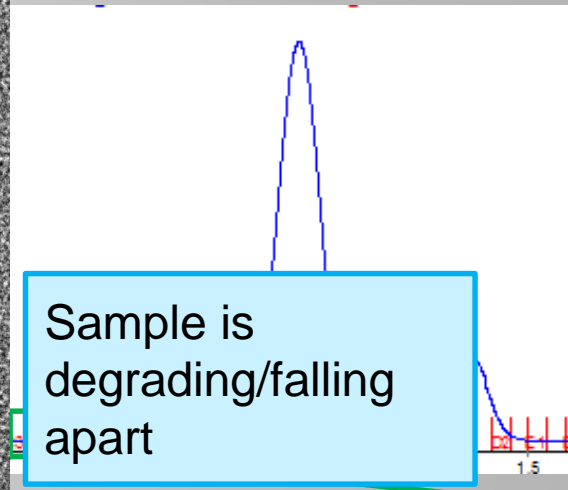
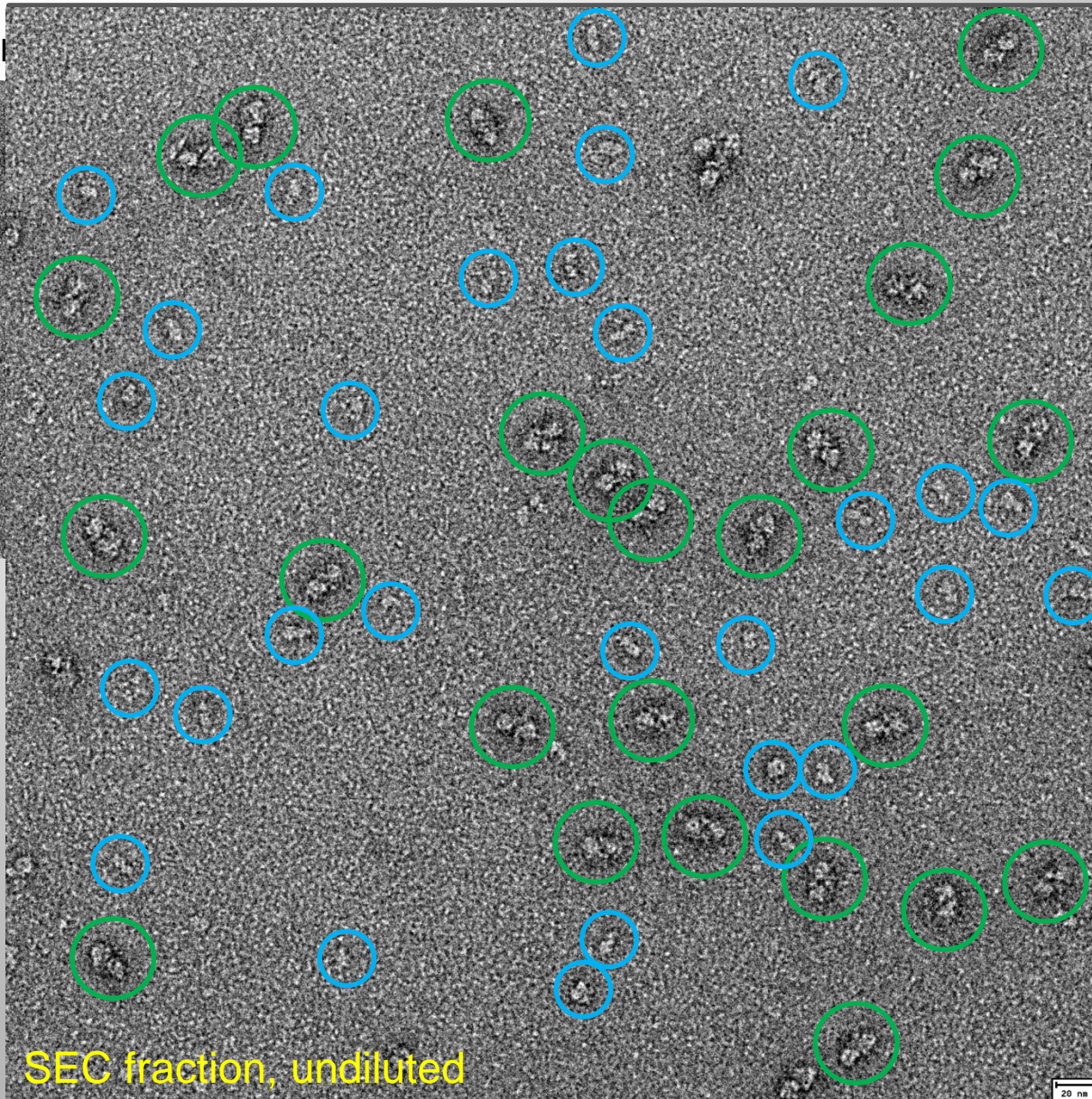


Less clumpy/  
aggregated after  
fresh SEC



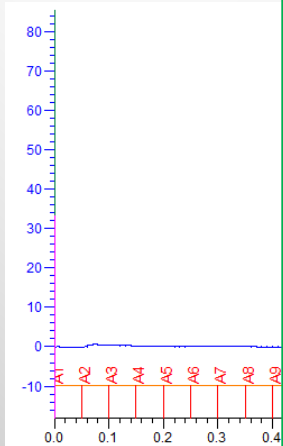
# Step 1: Optimizing the sample

Original

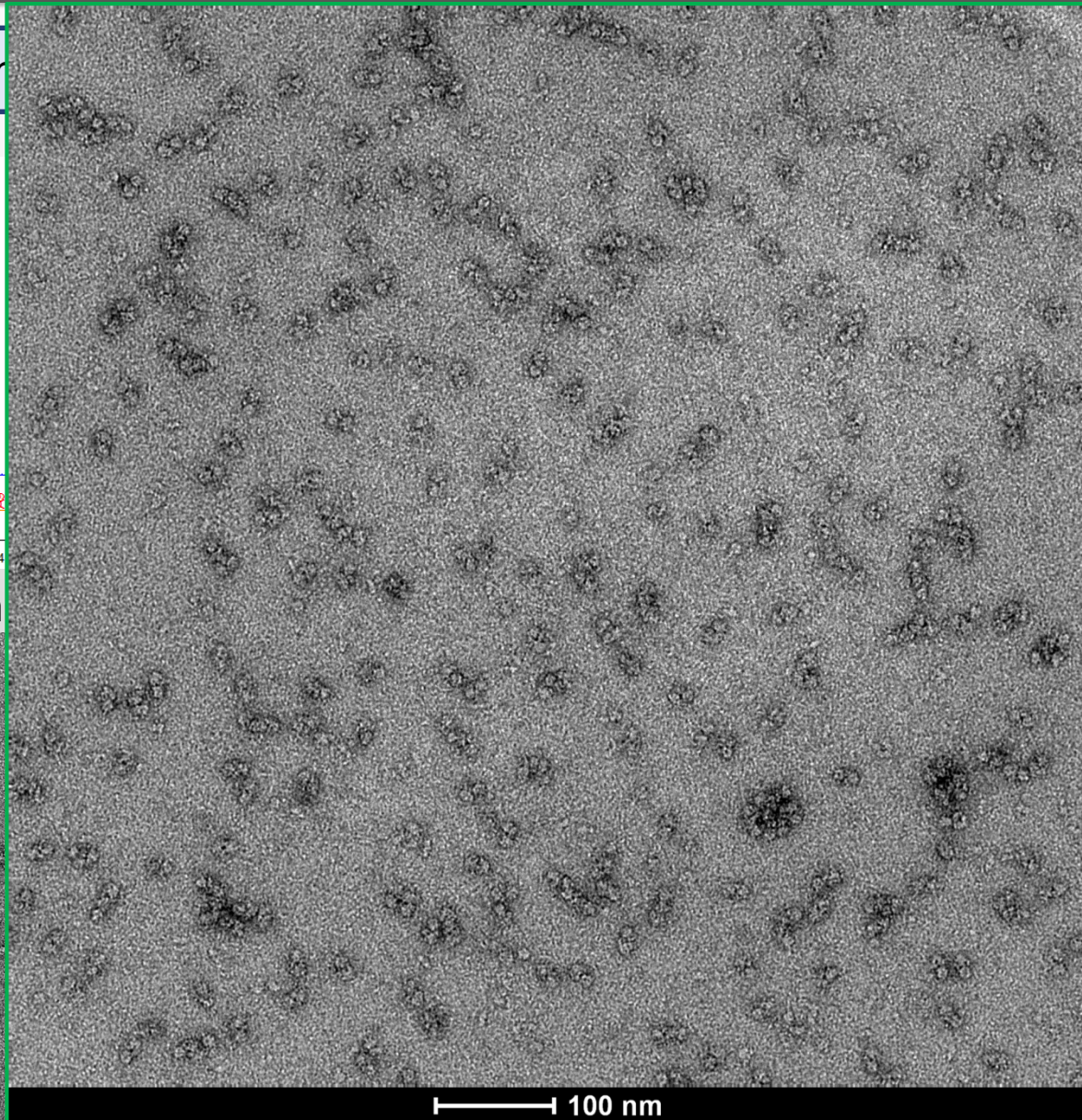


# Step 1: Optimizing the sample

Next step: Im



Previous sam



concentration on  
half of the peak  
aggregated particles

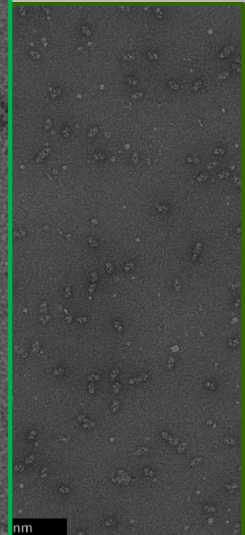
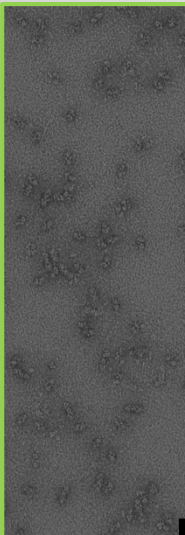
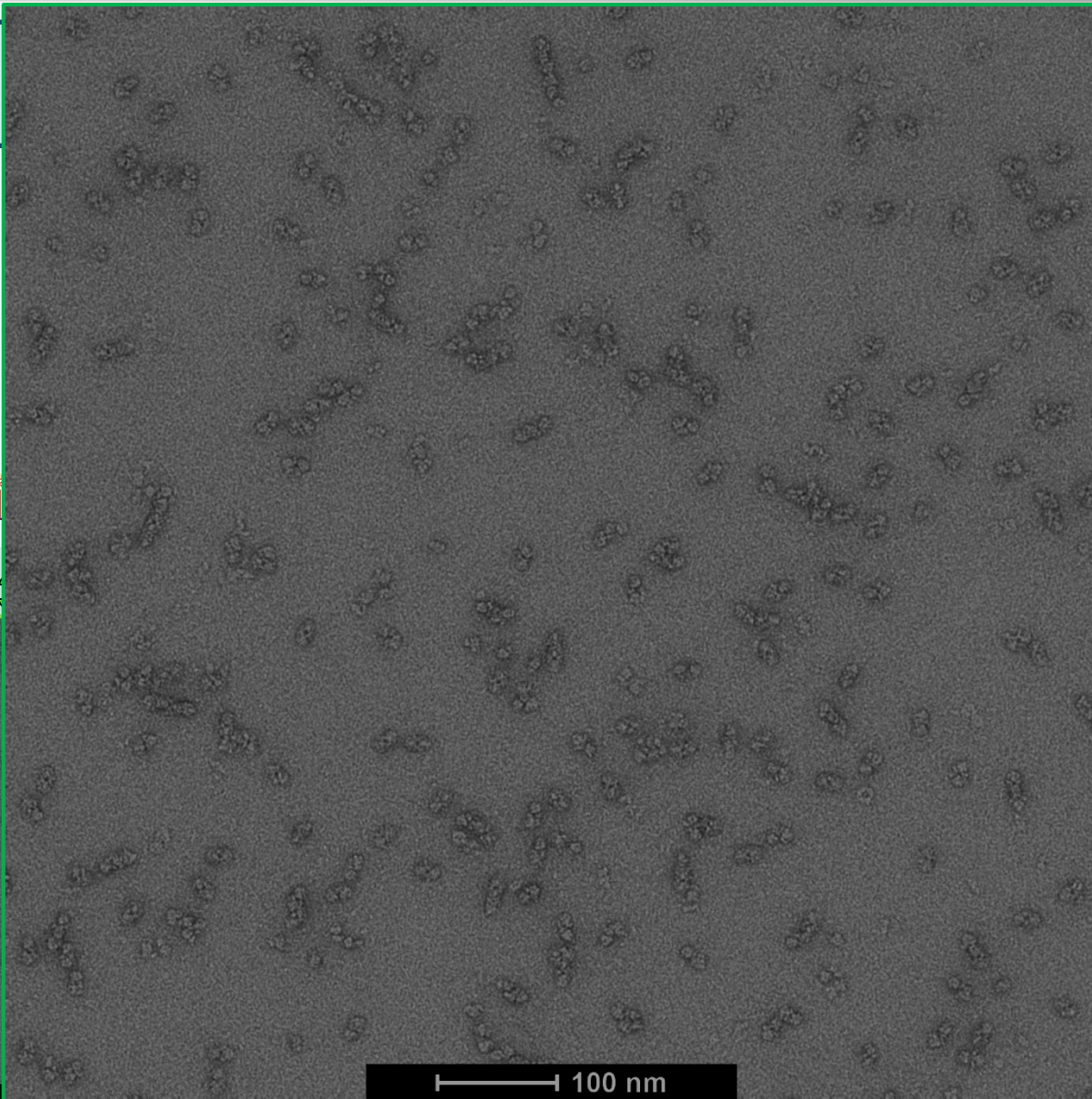
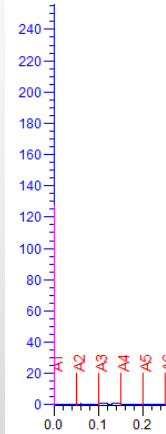
100 nm

# Step 1: Optimizing the sample

Next step:

complex together

concentration on  
be the best



100 nm

nm

C

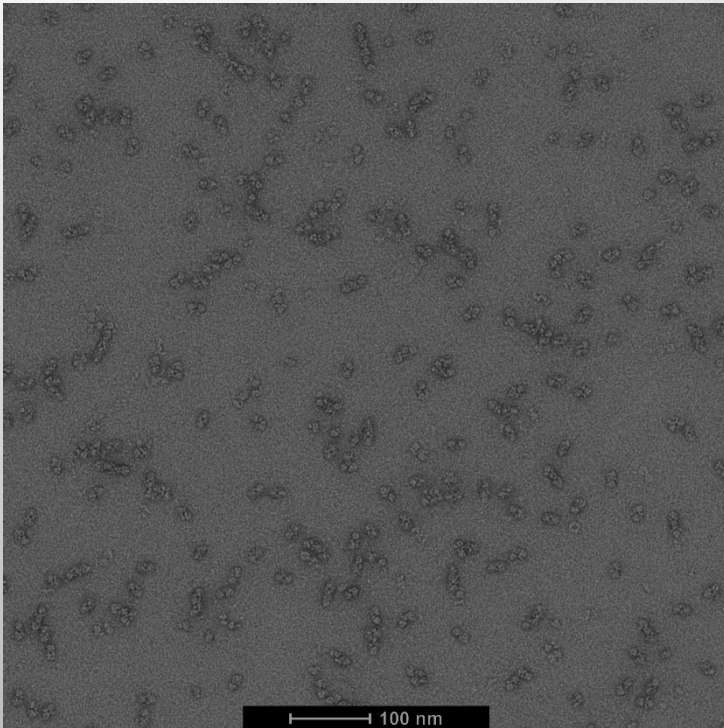
) dil.

# A few side notes

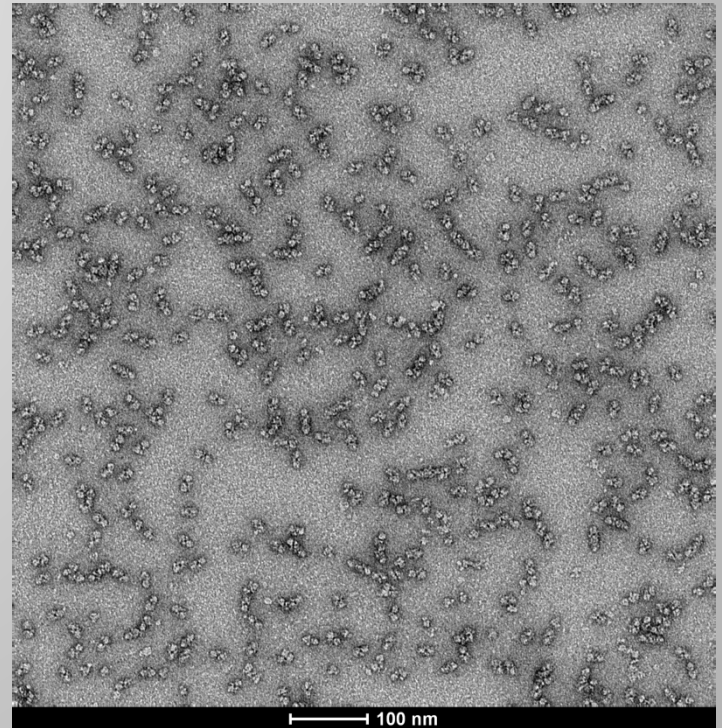
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Note 1: Negative stain EM is a great way to check your sample stability, e.g. after storing at 4°C or freeze/thawing

Original sample, 1/10 dilution



After 4 days at 4°C, 1/4 dilution



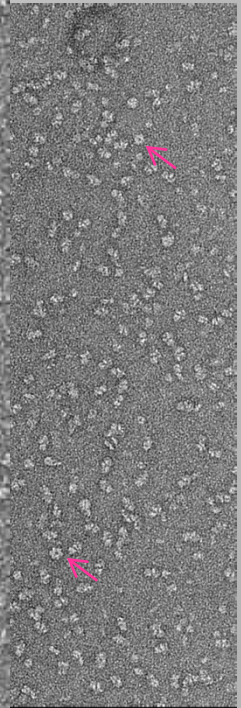
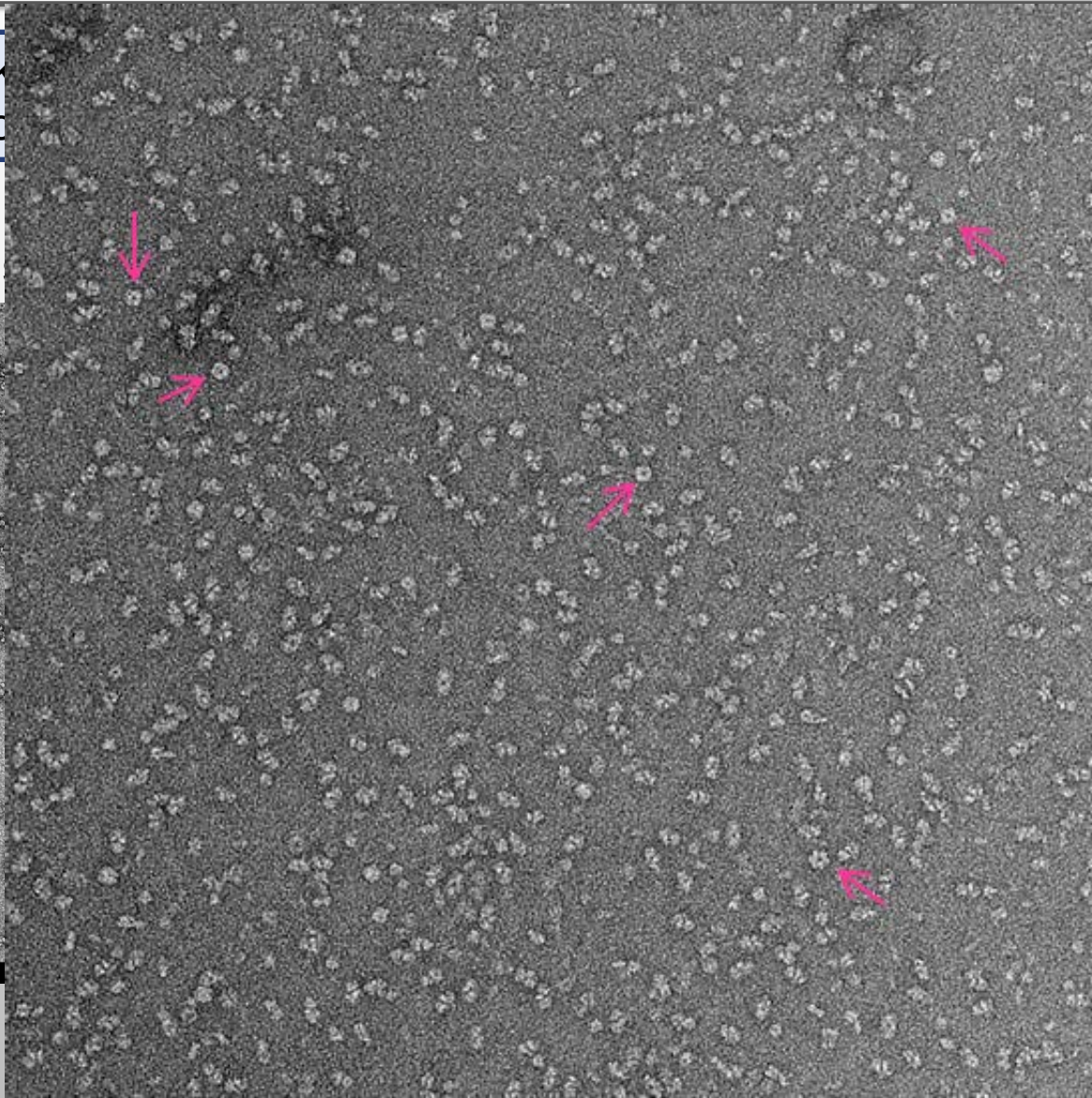
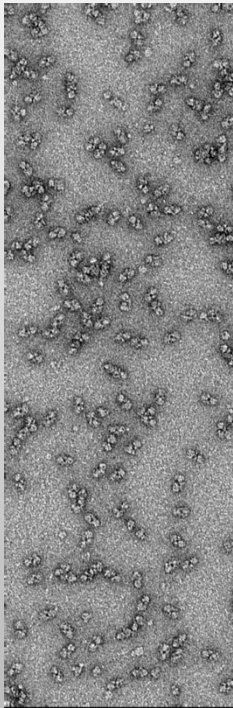
# A few side notes

Note 2: Check  
avoid

maintain quality –

cond SEC  
remove contaminant

Previous



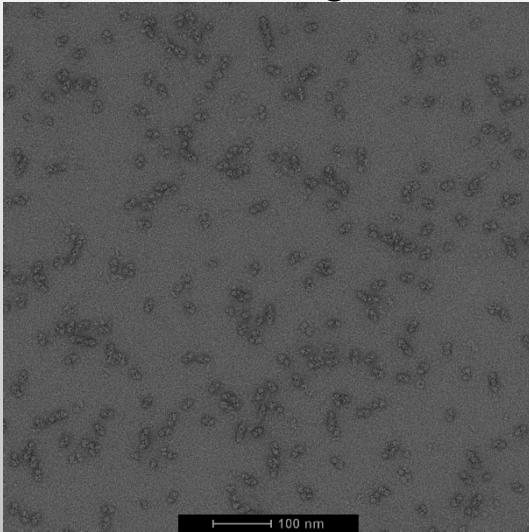
100 nm



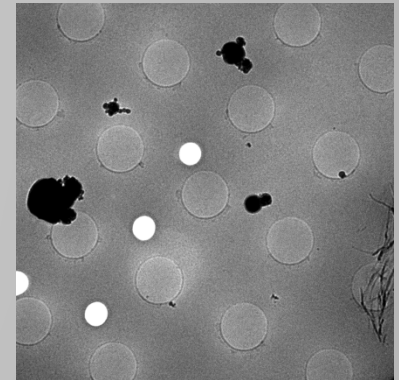
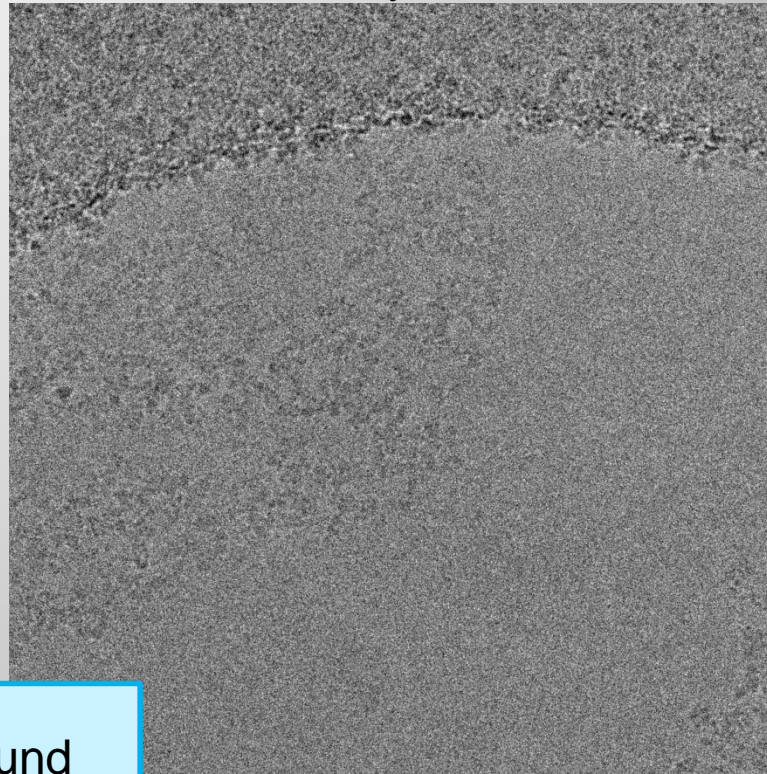
# Step 2: Optimizing the grid

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

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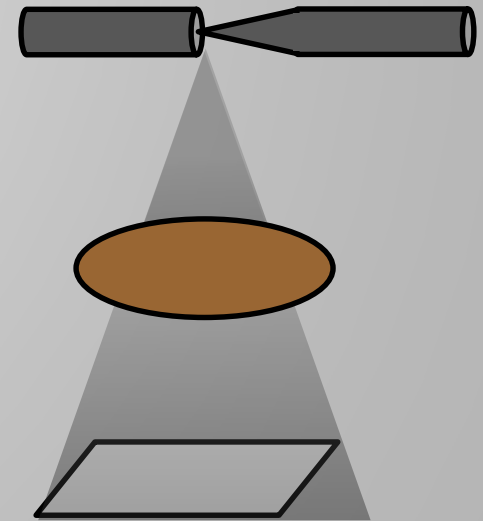
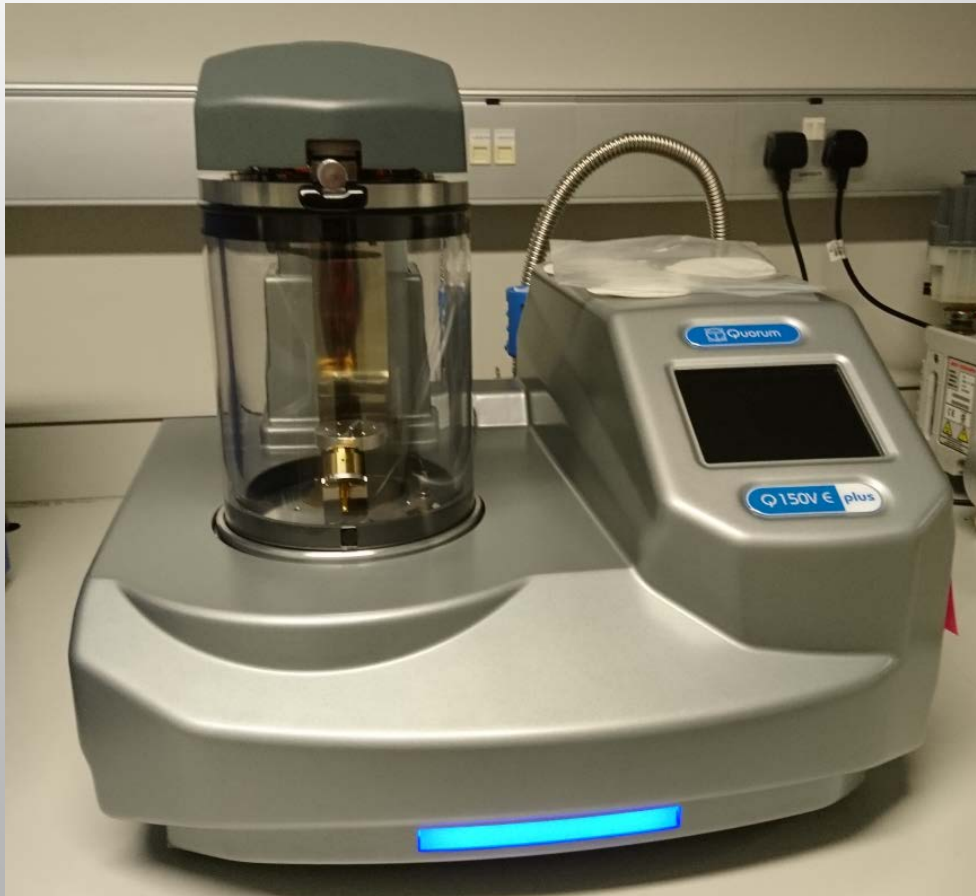
Next step: Make carbon-coated grids

## 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**

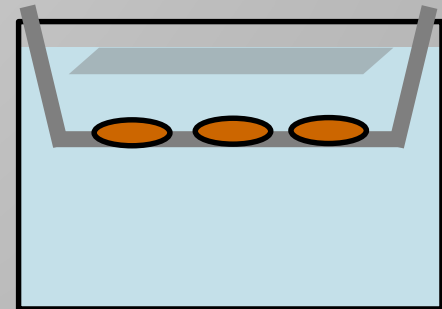
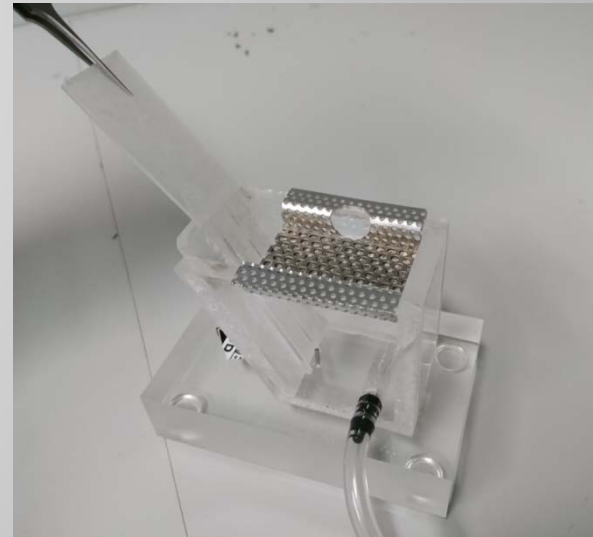
# Step 2: Optimizing the grid

First: make the thin coat of carbon on mica



# Step 2: Optimizing the grid

Second: float the carbon layer onto the grids

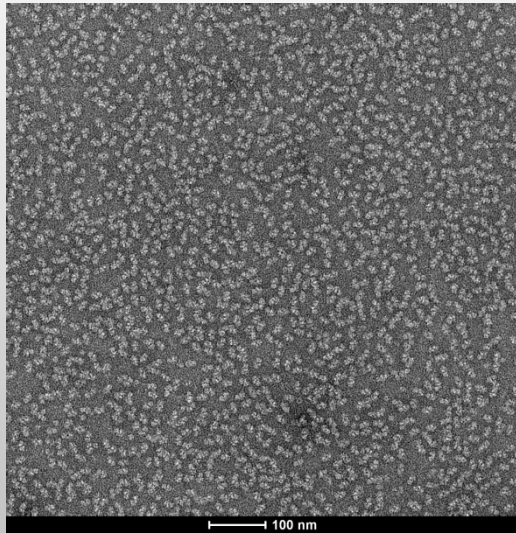


# Step 2: Optimizing the grid

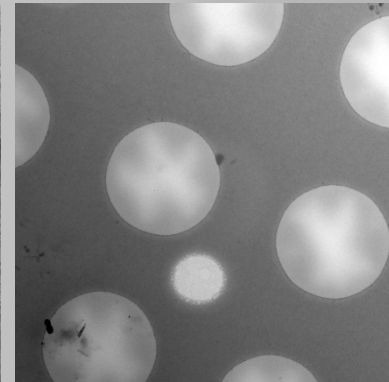
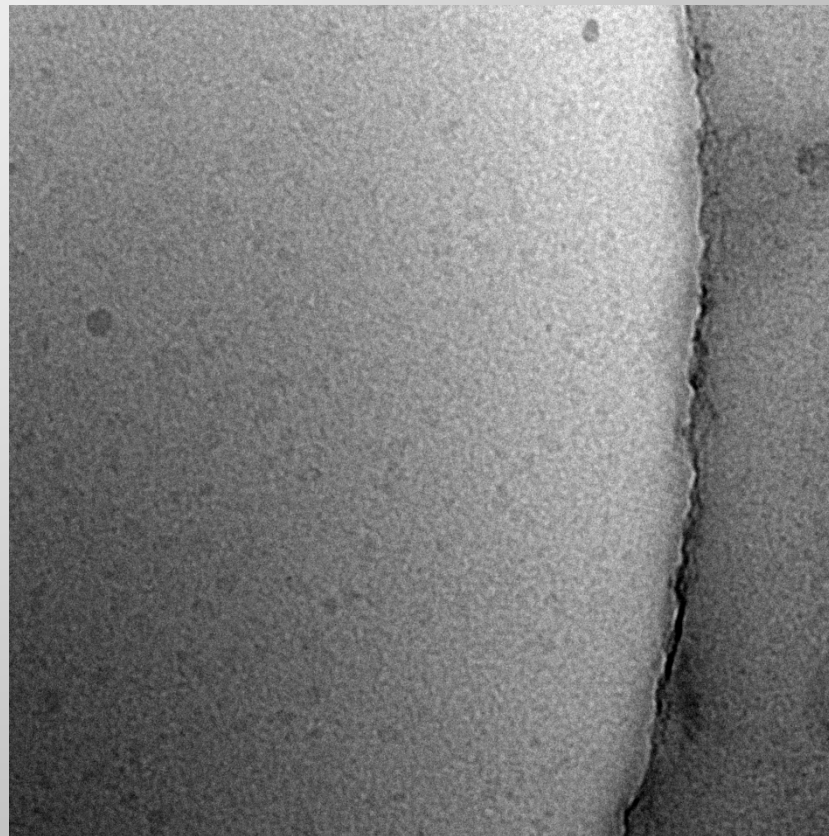
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Next step: Freeze sample on carbon-coated Quantifoil 2/2

Negative stain 1/4 dilution

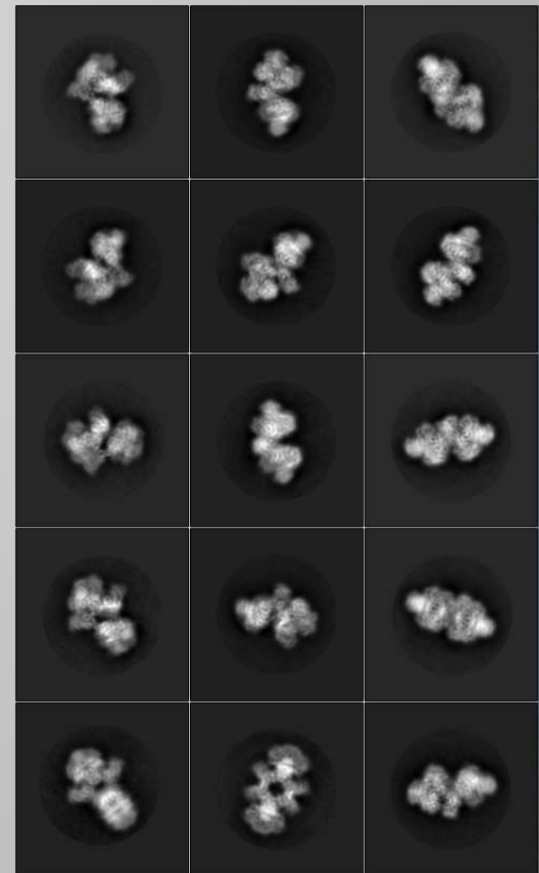
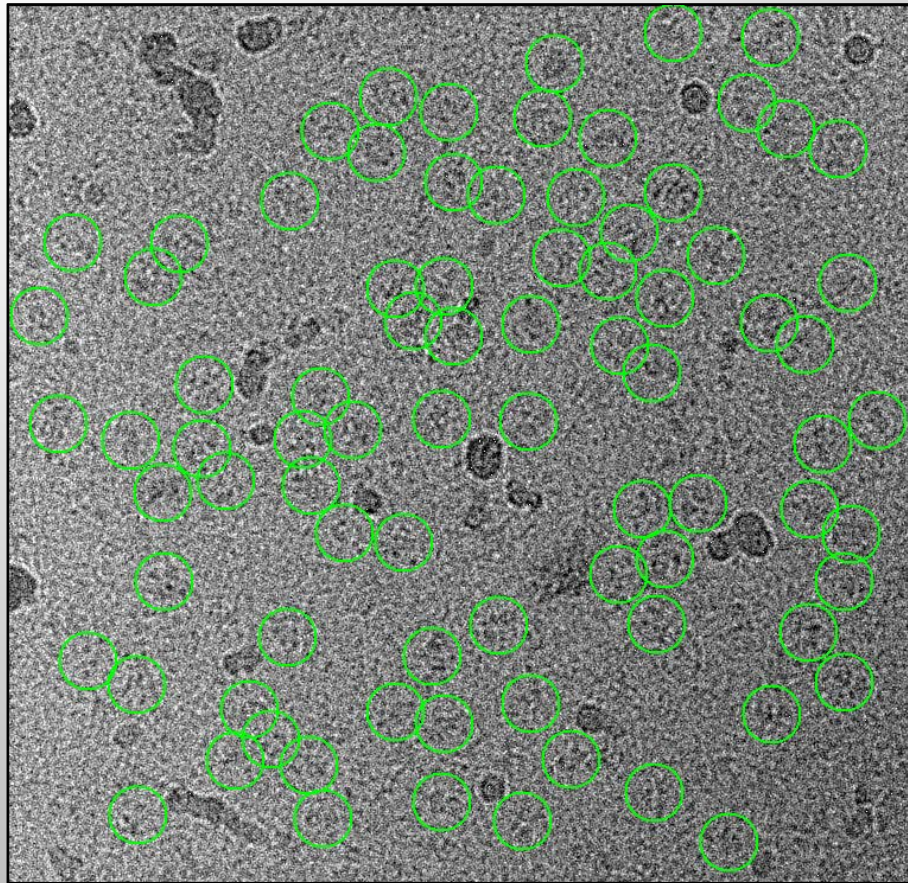


cryoEM, undiluted



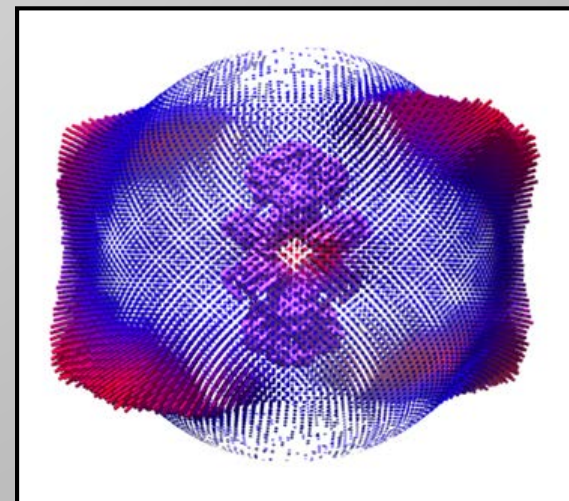
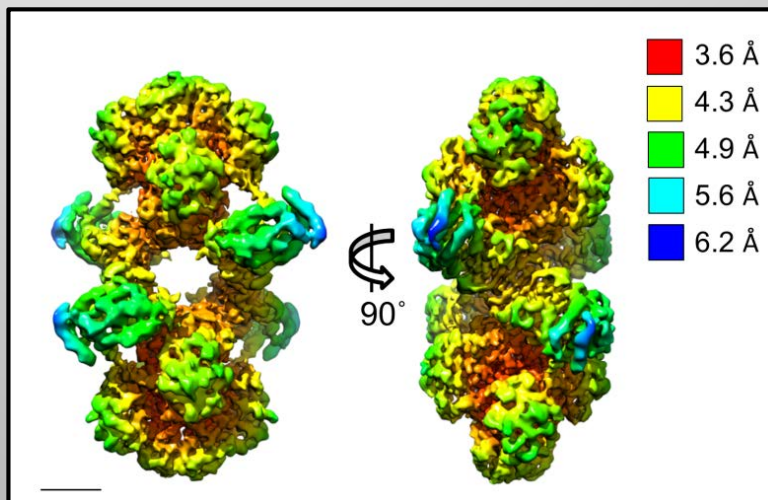
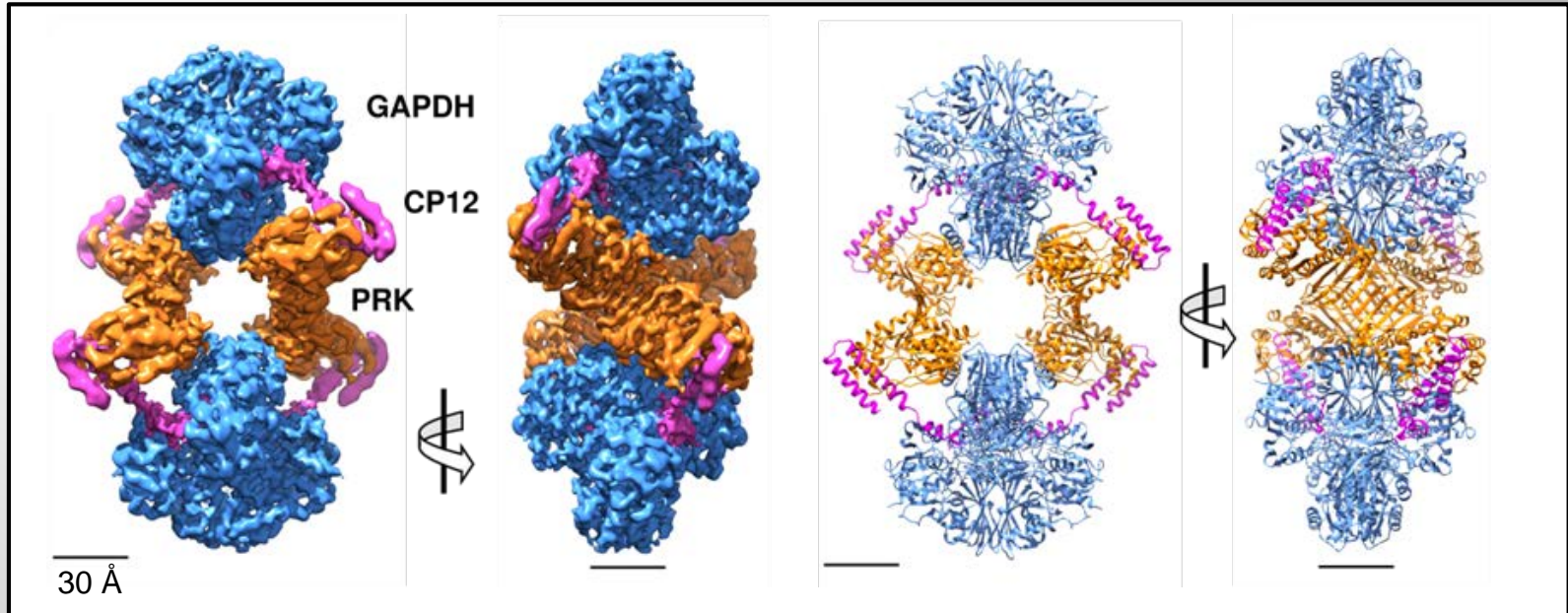
# Krios collection at eBIC

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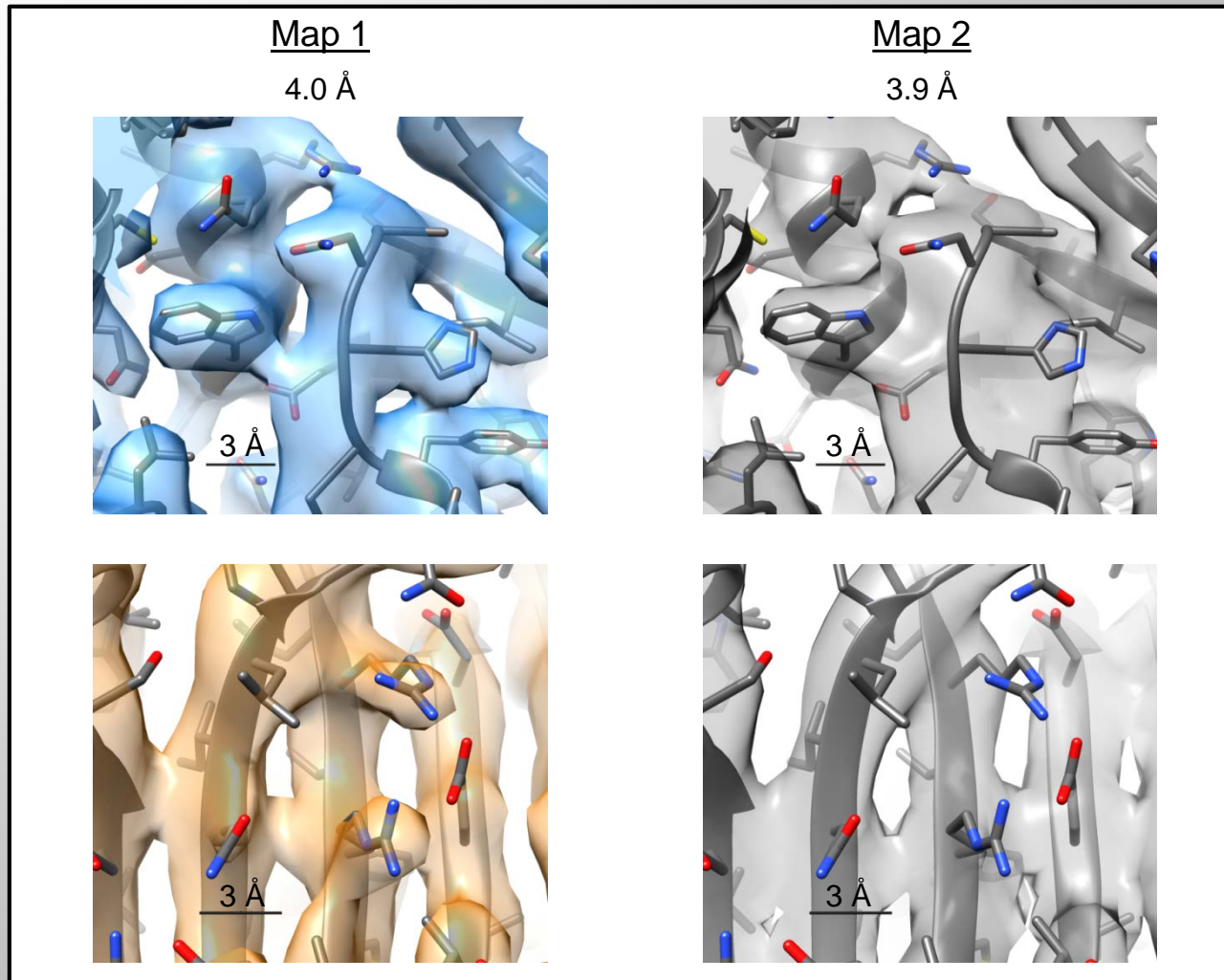
197 212 particles

# Reconstructed density to 4.0 Å



# Side note on resolution estimate

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



# The End

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