KEK SSP’25 REPORT

Hands-on Approaches to Macromolecular Structure Determination via X-ray and Cryo-EM at SBRC, KEK

KEK

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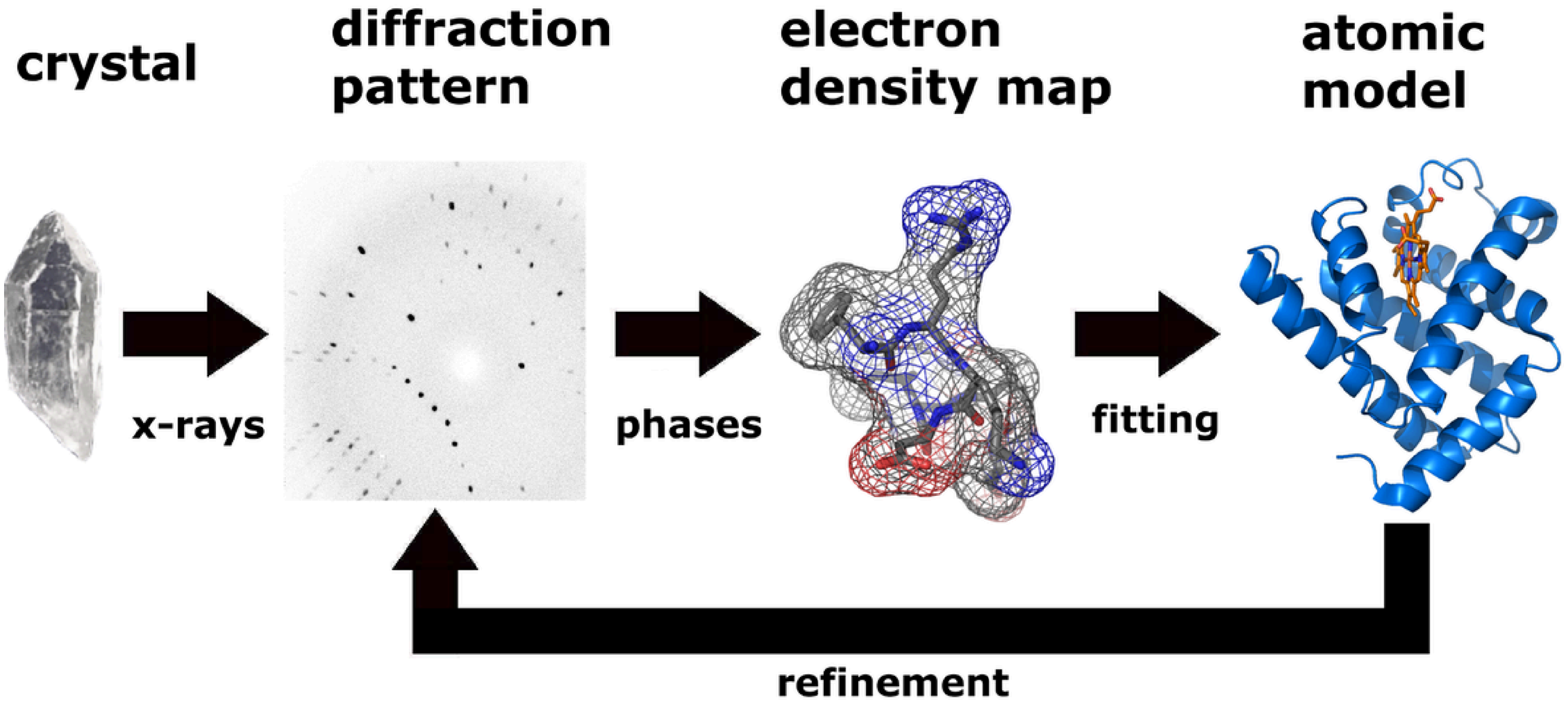
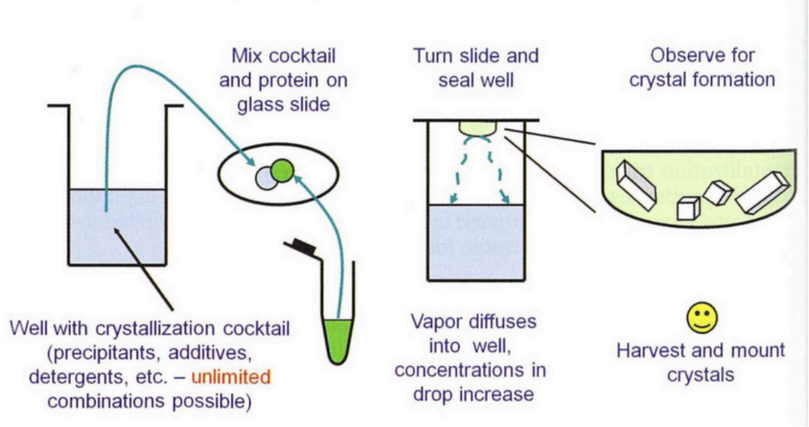
X-Ray Crystallography

Why Crystallisation? Why a Crystal at All?

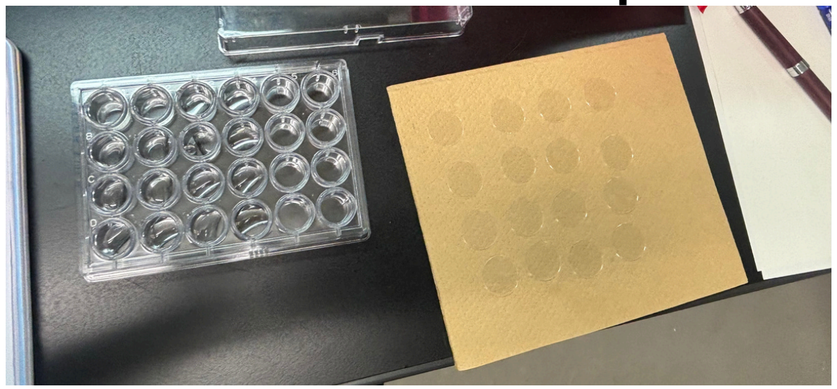
- Proteins are too small to see with visible light, so we need special techniques to study their 3D structure.
- To use X-ray crystallography, we first need the protein in a crystal form, because:
- Crystals align millions of protein molecules in a repeating pattern.
- This uniformity amplifies the X-ray signal, producing measurable diffraction patterns.
- The diffraction pattern is then used to back-calculate the electron density map, which reveals atomic positions.


crystal
diffraction pattern
electron density map
atomic model
x-rays
phases
fitting
refinement

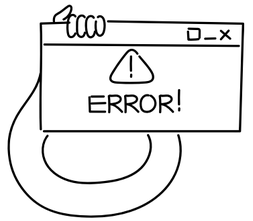
Hanging drop method

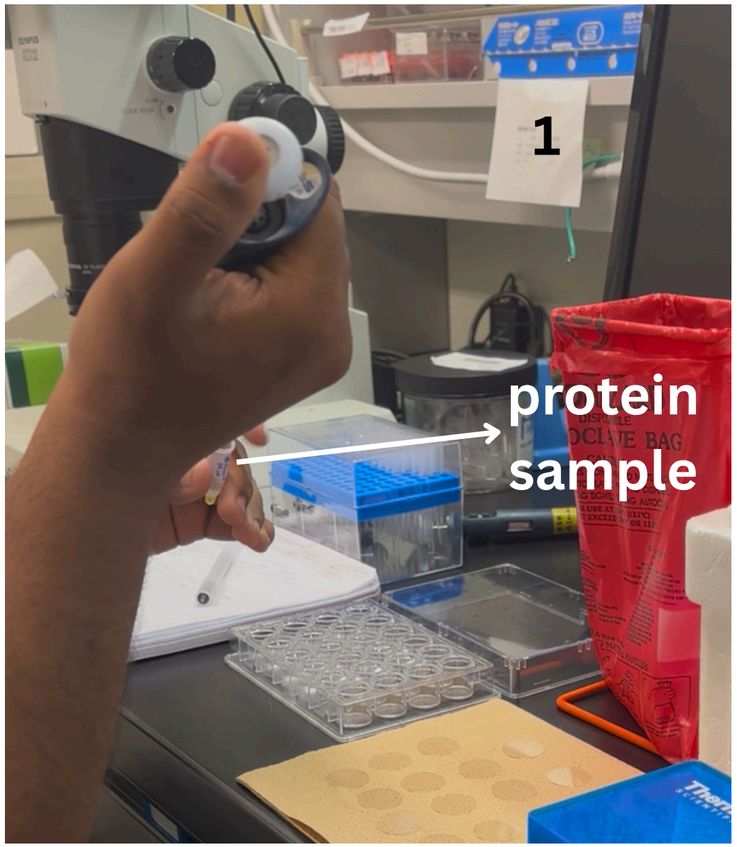


Workbench Setup



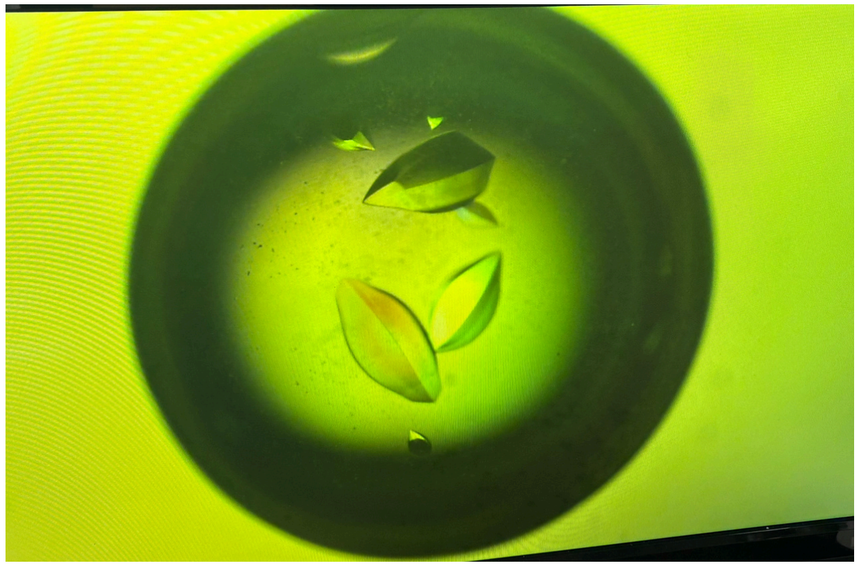
Precipitant used: Sodium formate

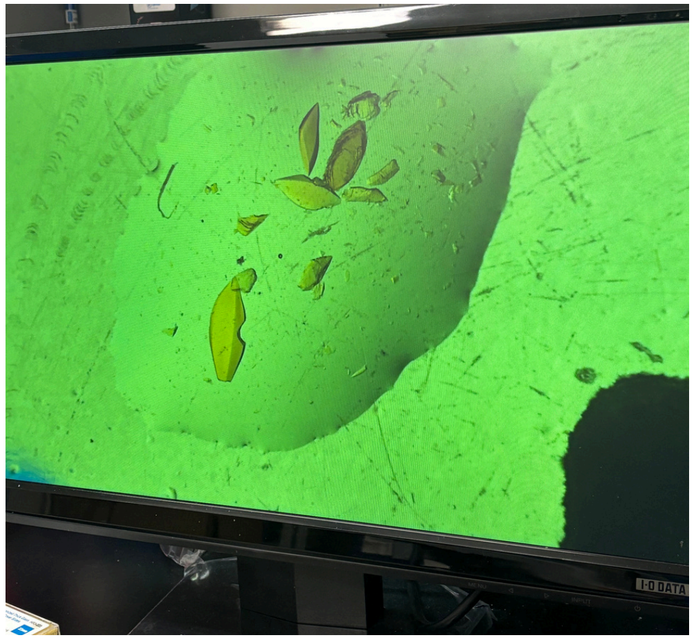




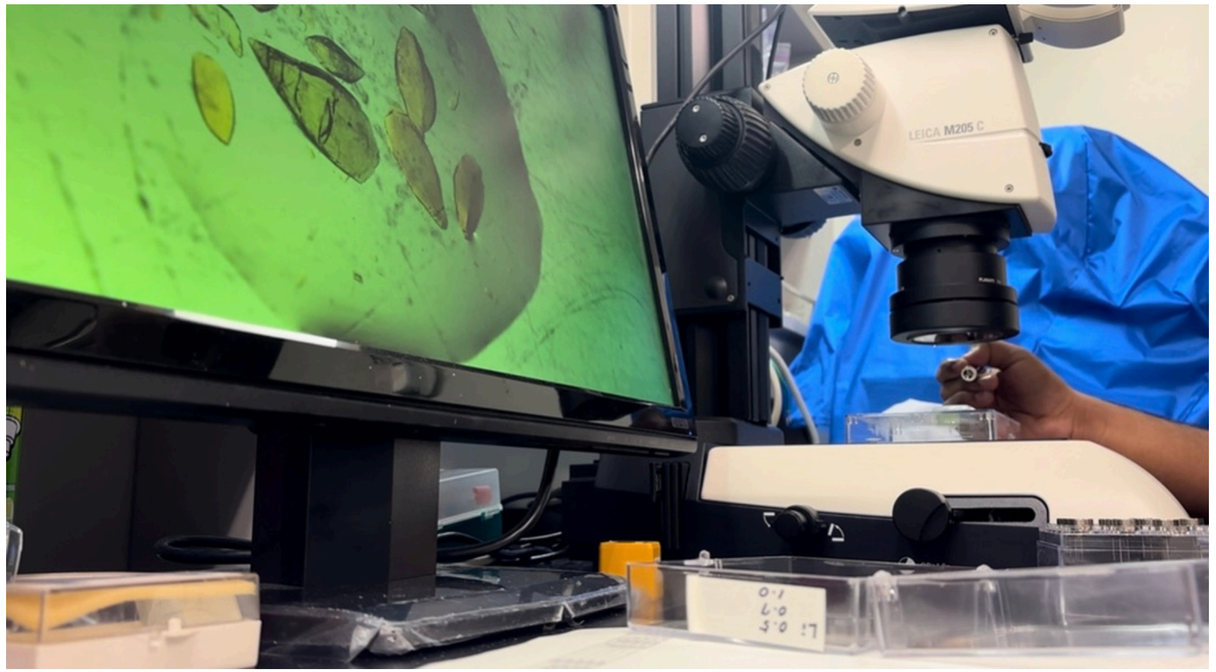
After 24 hours....

Crystal formation after 24h



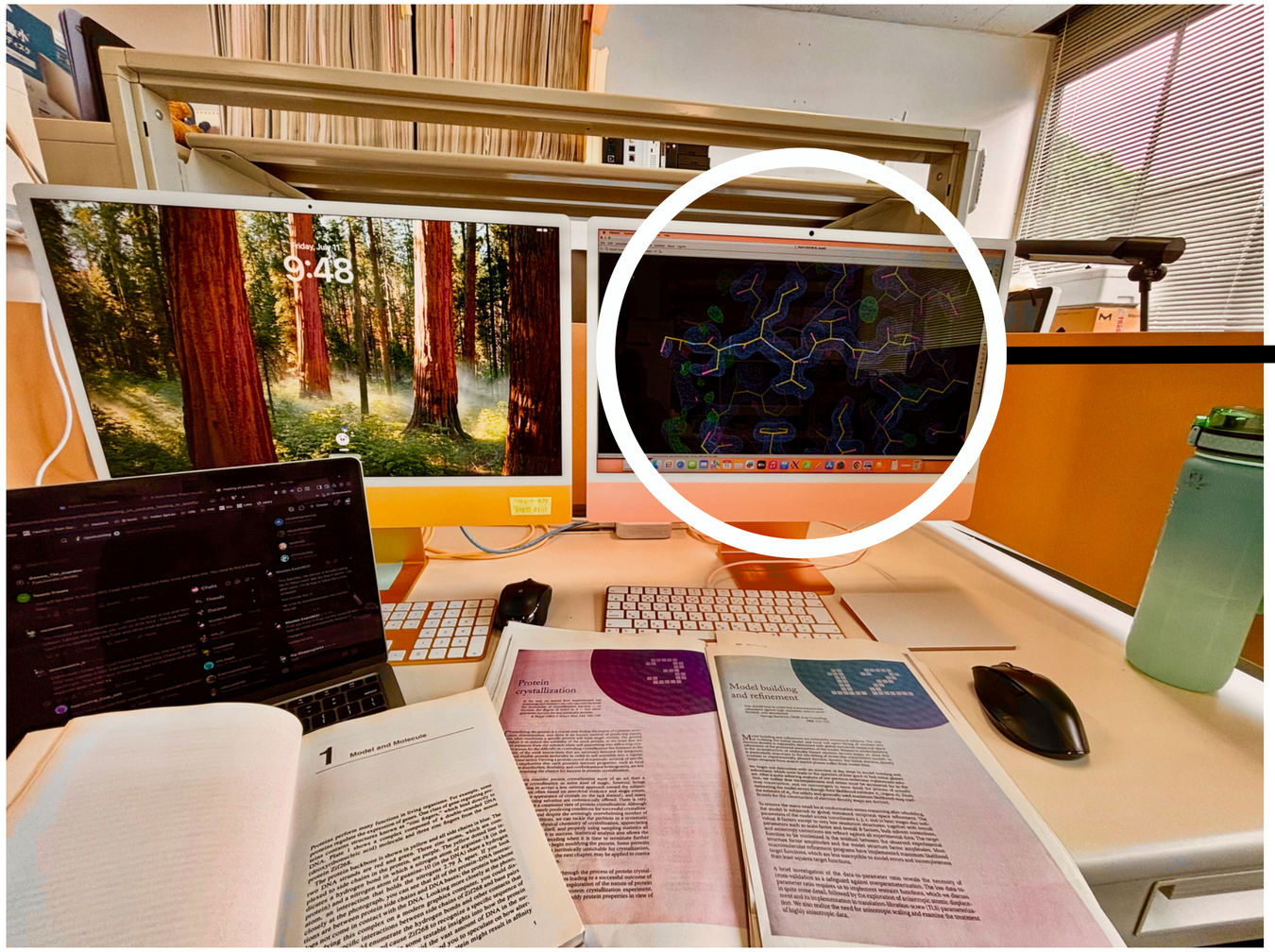


Transfer of crystals into a cryoprotectant solution



OOooopsies..broke some crystals!!..

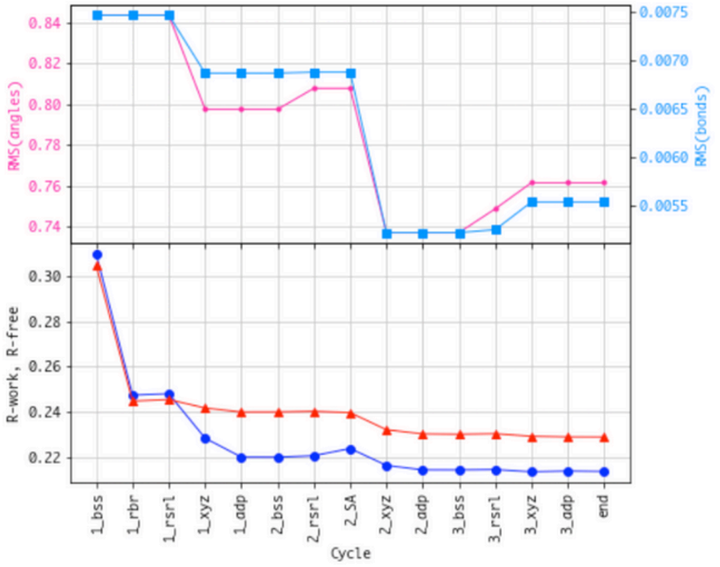
Anyways, these are stored in liquid nitrogen and sent to beamline (i.e, @ Photon Factory, KEK)



Collected diffraction data resulting in .hkl files (indexing of reflections: h, k, l and intensity values).

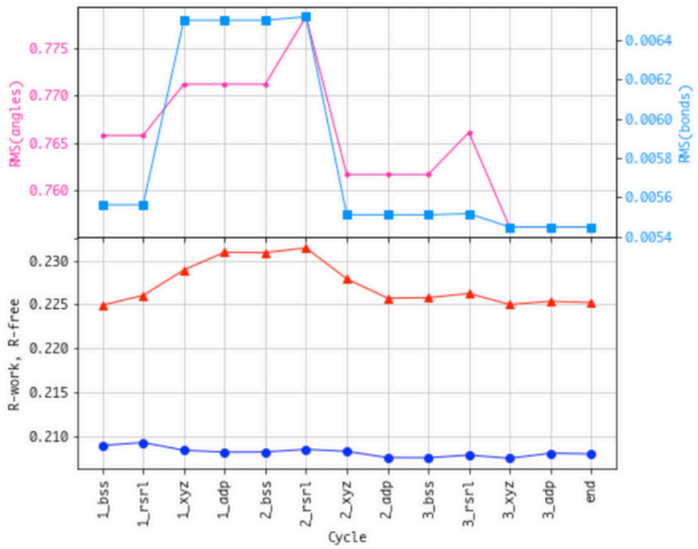
- Processed electron density maps using COOT for model building and manual corrections.
- Performed structure refinement using Phenix.refine to minimize R-factors and improve model geometry.
- Validated model for quality using Ramachandran plot.

1st refinement cycle

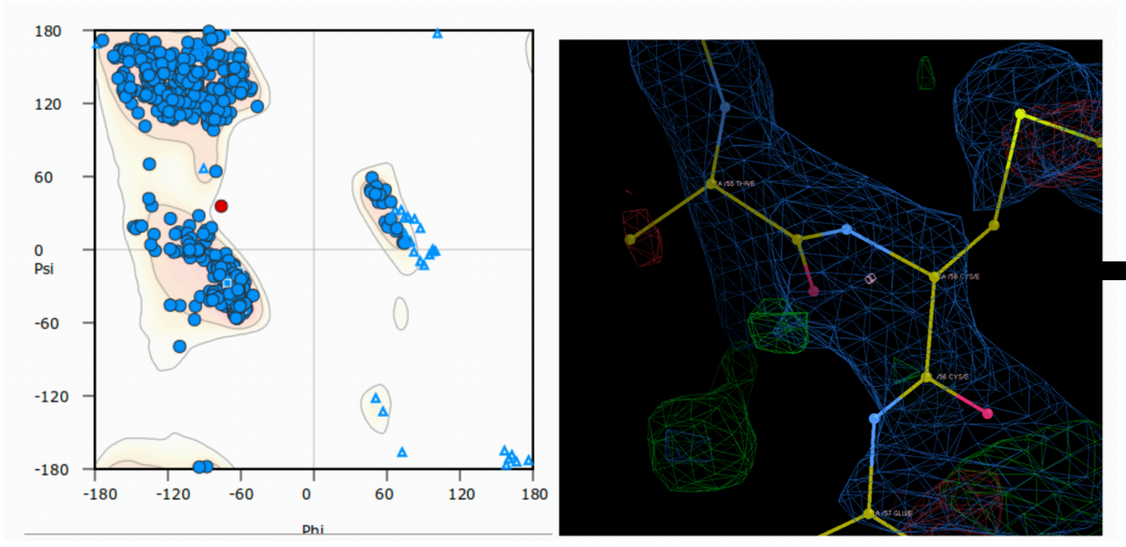


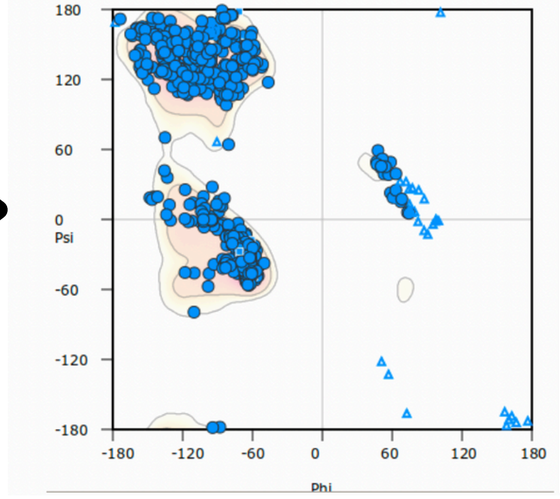
R-work: 0.2137
R-free: 0.2290

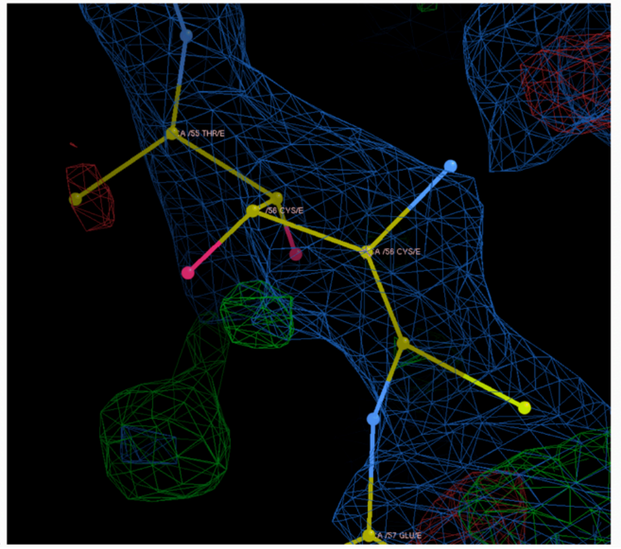
2nd refinement cycle



R-work: 0.2079
R-free: 0.2252







- **In the first refinement cycle, R-work and R-free significantly dropped, stabilizing at 0.2137 and 0.2290, respectively. The small gap between them indicates a good fit without overfitting. Geometry also improved as reflected in the declining RMS deviations.**
- **In the second refinement, R-work dropped further to 0.2079 while R-free stabilized at 0.2252 — an expected and healthy separation. This suggests that the model was improved without overfitting, and overall quality got better compared to the first cycle.**

CRYO-EM

Why Cryo-Electron Microscopy (Cryo-EM)?

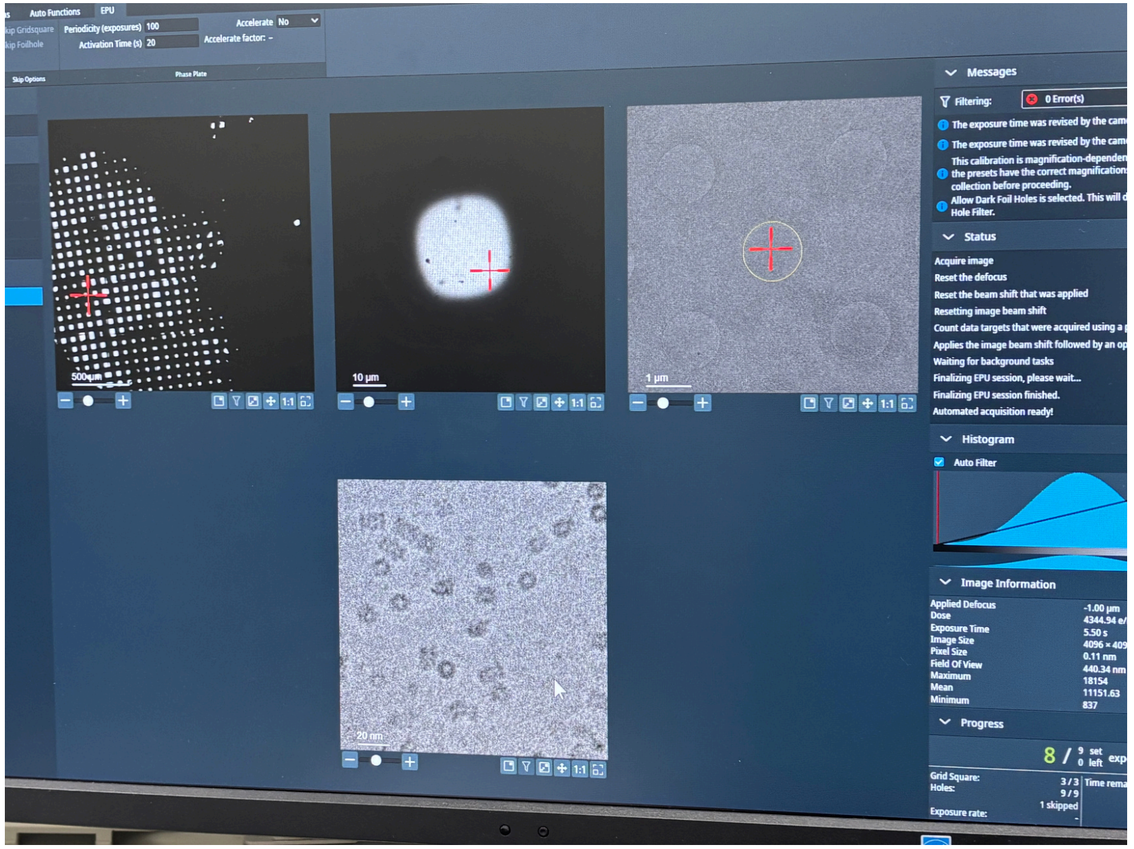
- Not all proteins crystallize well. Some are too flexible, dynamic, or large (like membrane proteins or complexes).
- That’s where Cryo-EM saves the day. No need to grow crystals!
- In Cryo-EM, we: Flash-freeze protein samples in a thin layer of vitreous ice.
- Directly image them under an electron microscope at cryogenic temperatures.
- Collect thousands to millions of 2D particle images, then computationally reconstruct a high-resolution 3D structure.

Grid preparation

Freeezing the sample

Sample exposed to cryo em machine

Image collection

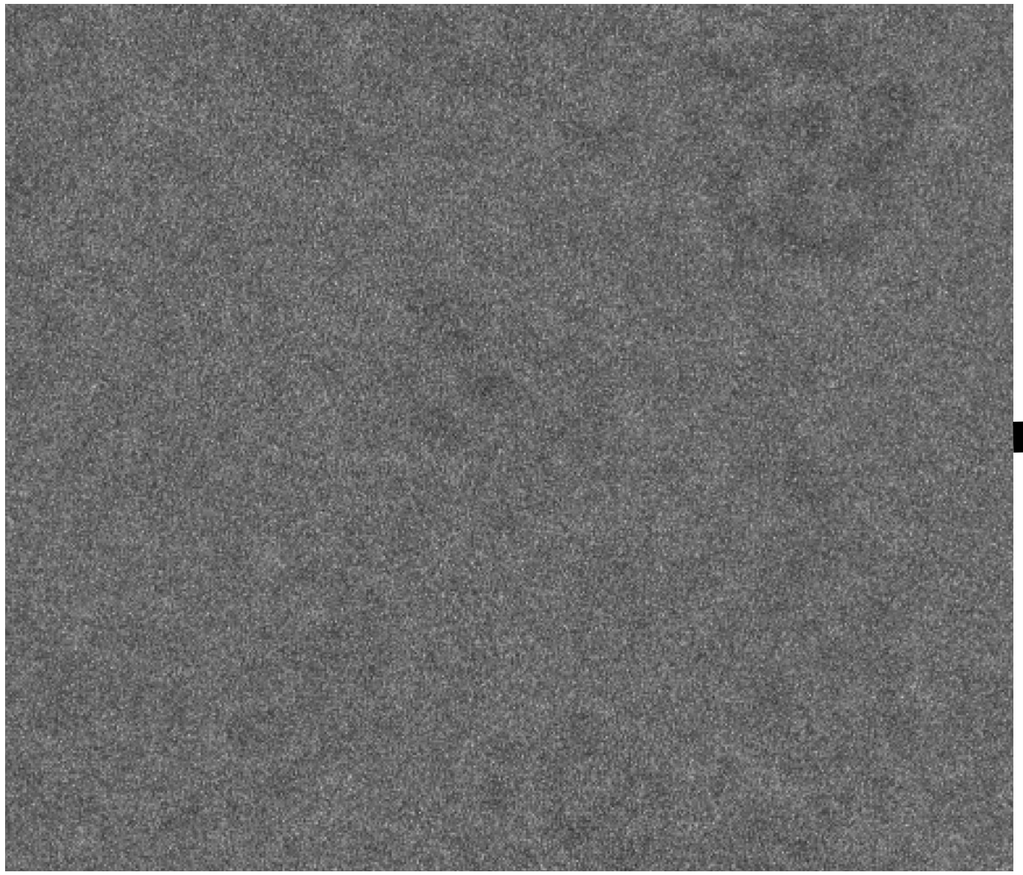


After collection of 10,000+ images, here comes the difficult part!!

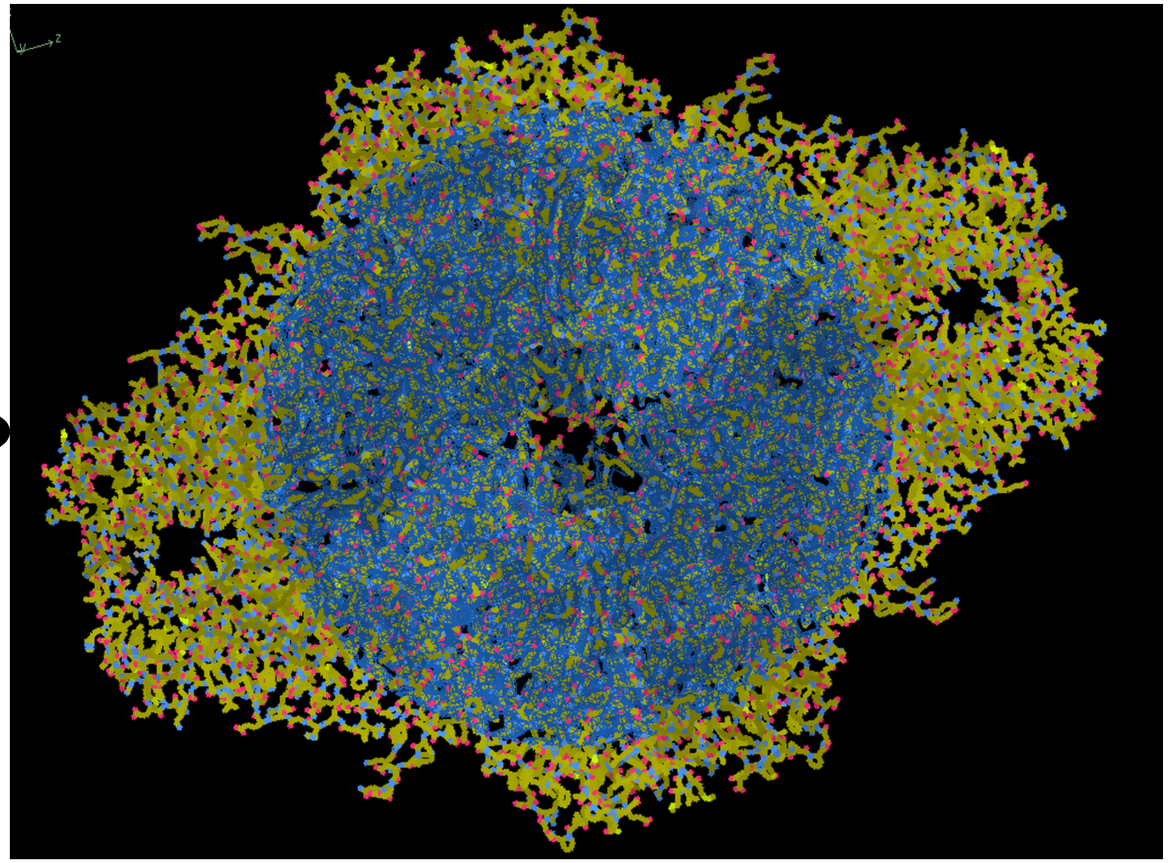
Reconstruction of image to form a 3d model

Software used: RELION

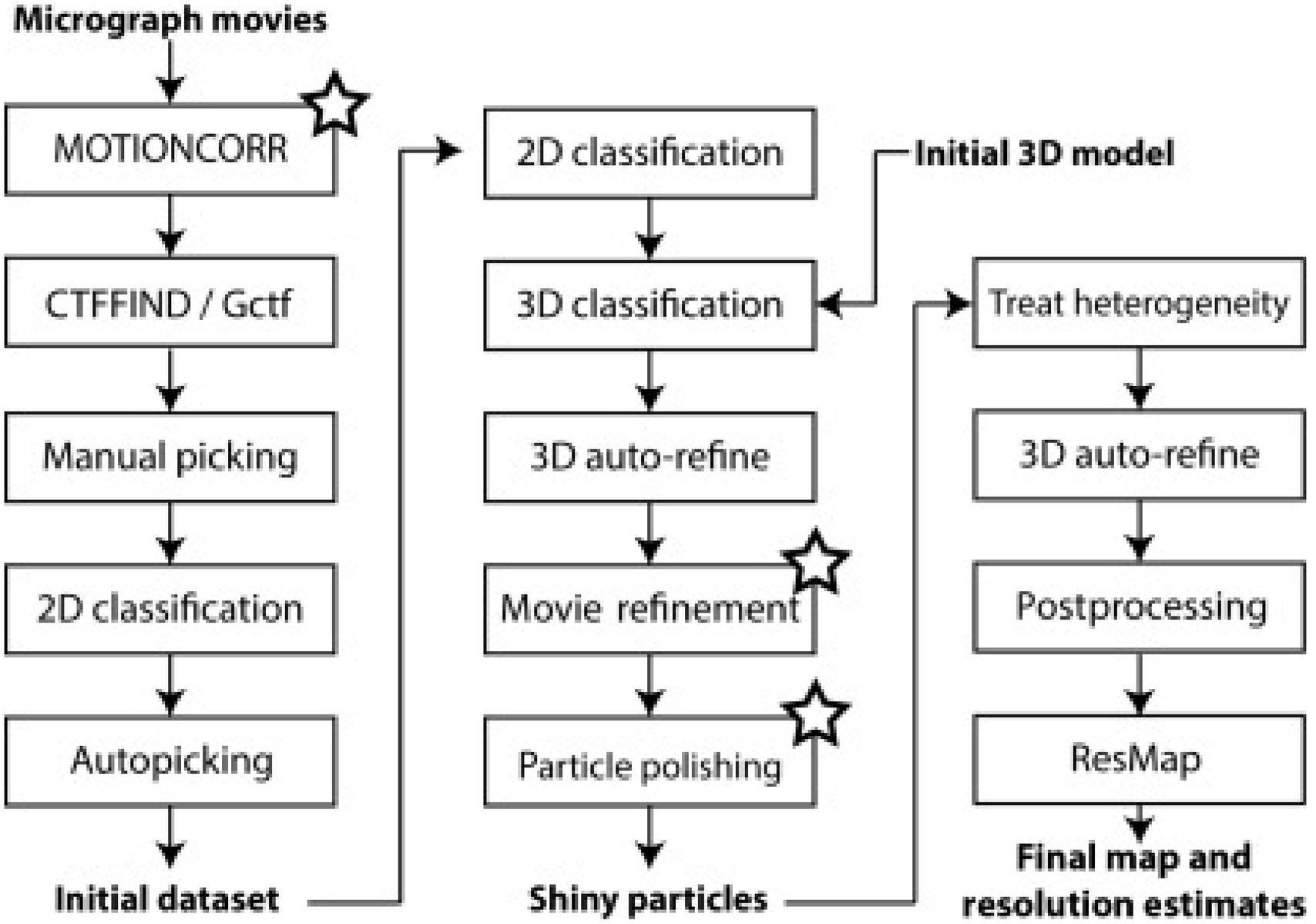
2D micrographs



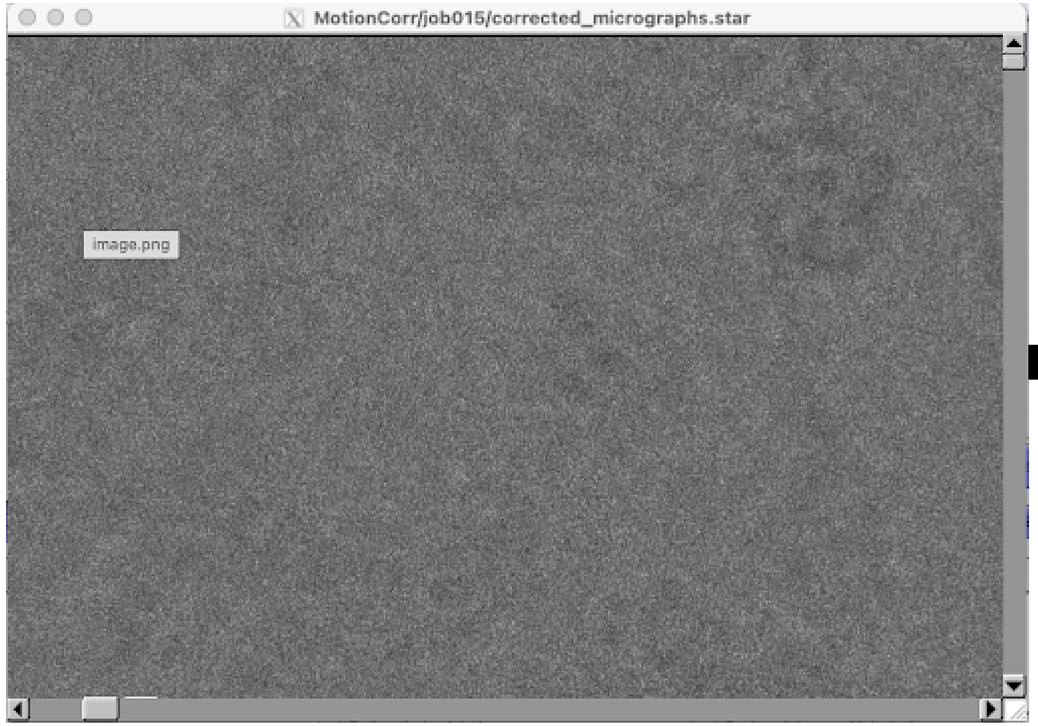
3D protein structure



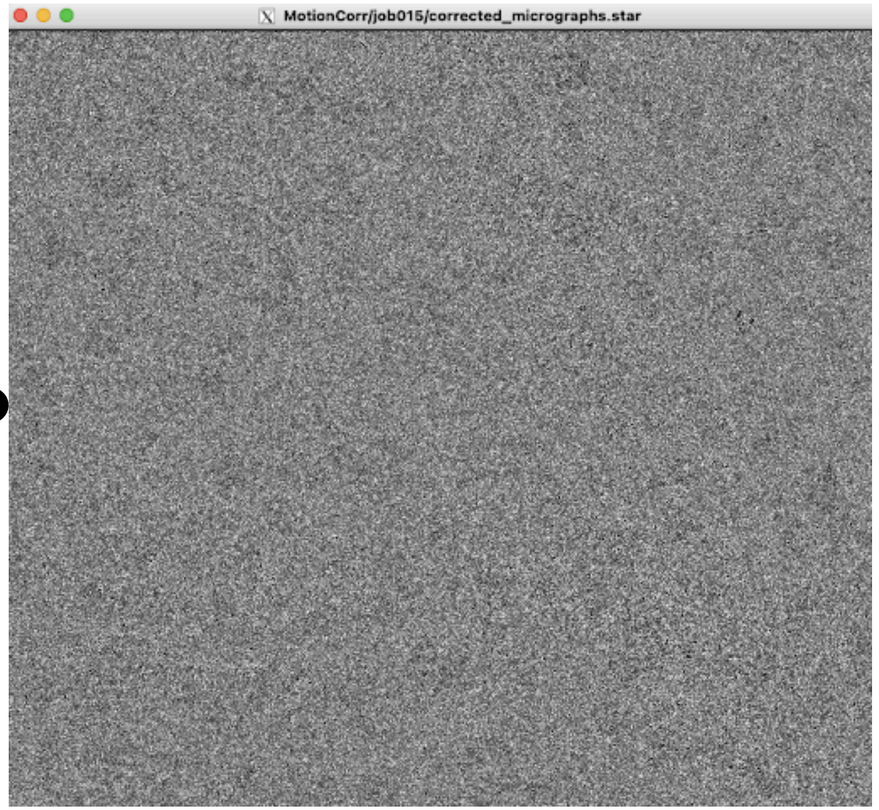
RELION image processing workflow



Motion correction

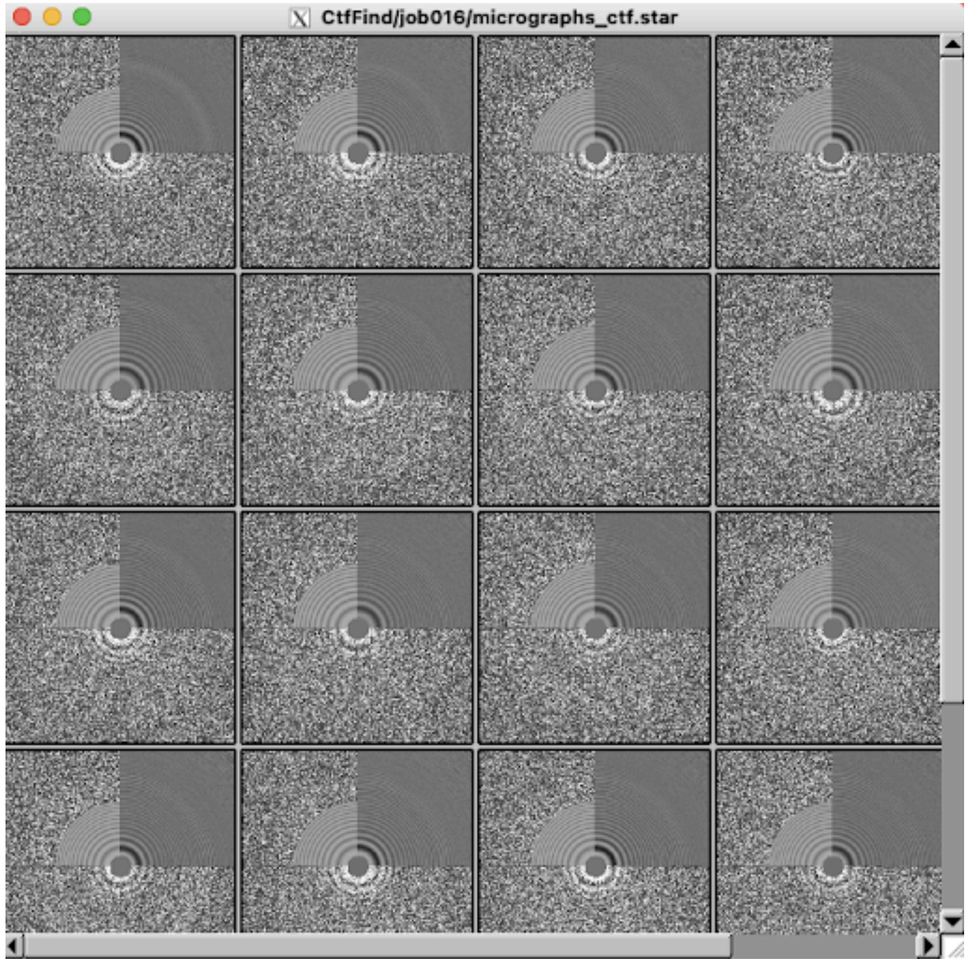


after adjusting
sigma and contrast

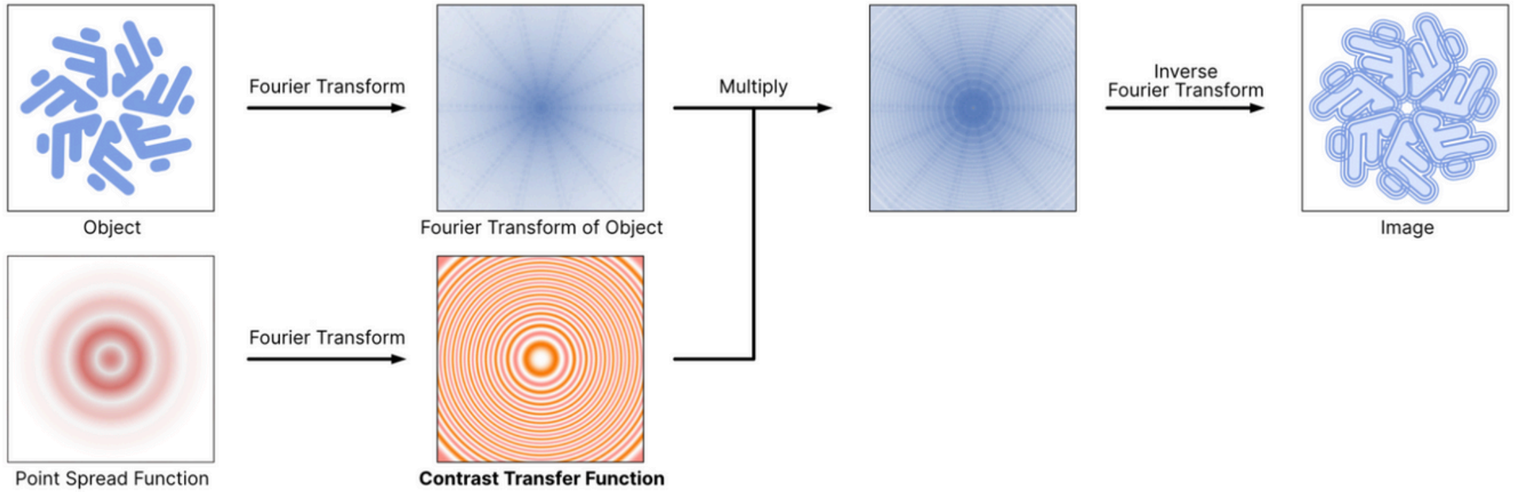


- Motion correction algorithms identify and compensate for these movements by aligning each frame of a movie with respect to a reference frame, and then averaging the aligned frames
- By removing the blur, motion correction significantly improves the signal-to-noise ratio and allows for the determination of higher-resolution structures.
- RELION utilizes a Bayesian approach, works by estimating particle trajectories and aligning movie frames based on these trajectories, ultimately producing an average of the aligned frames

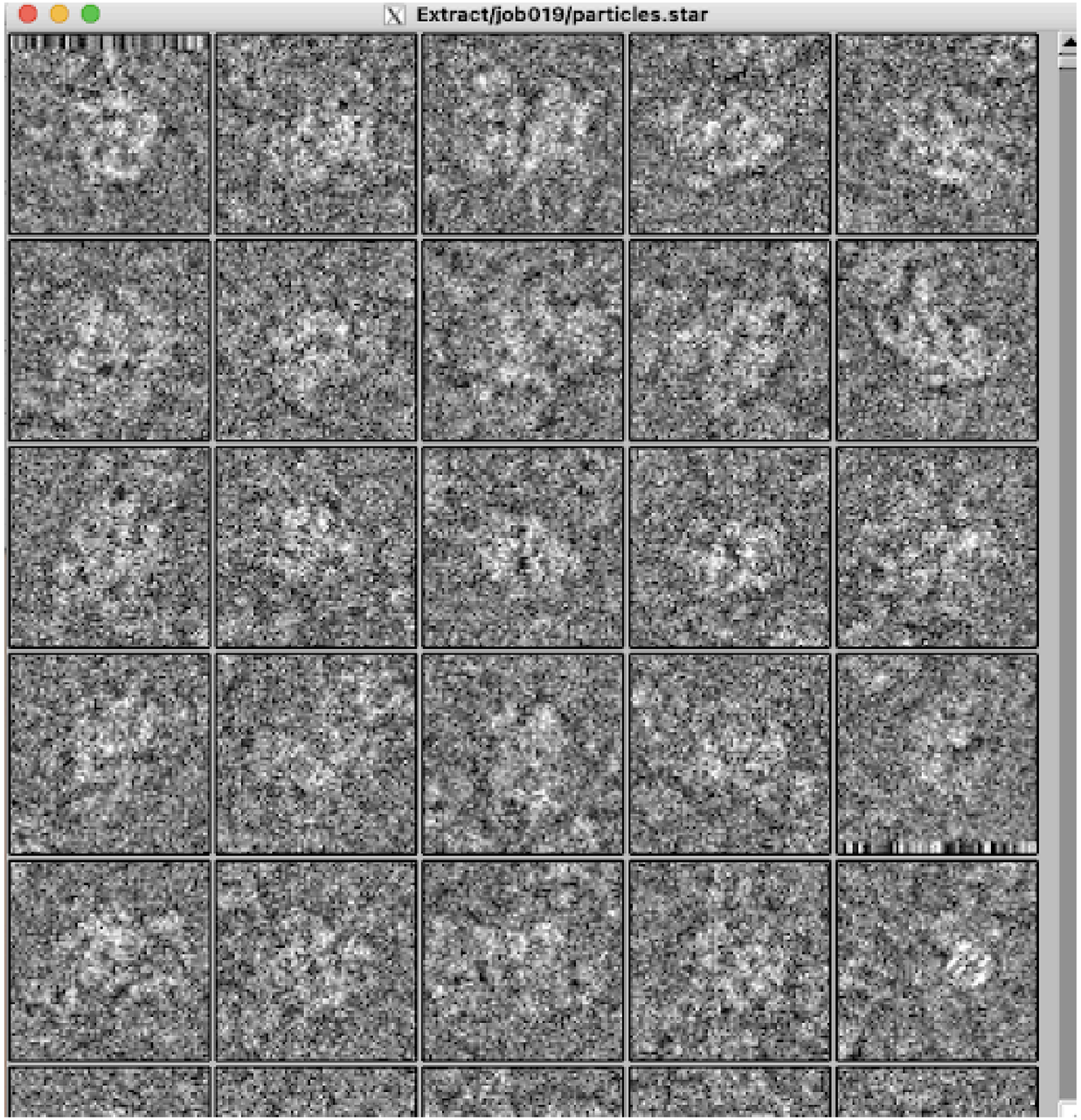
CTF estimation: Contrast Transfer Function



- The Contrast Transfer Function (CTF) models the effect of defocus and microscope aberrations on single particle images.
- These effects must be corrected before the images can be used to reconstruct a 3D Volume.



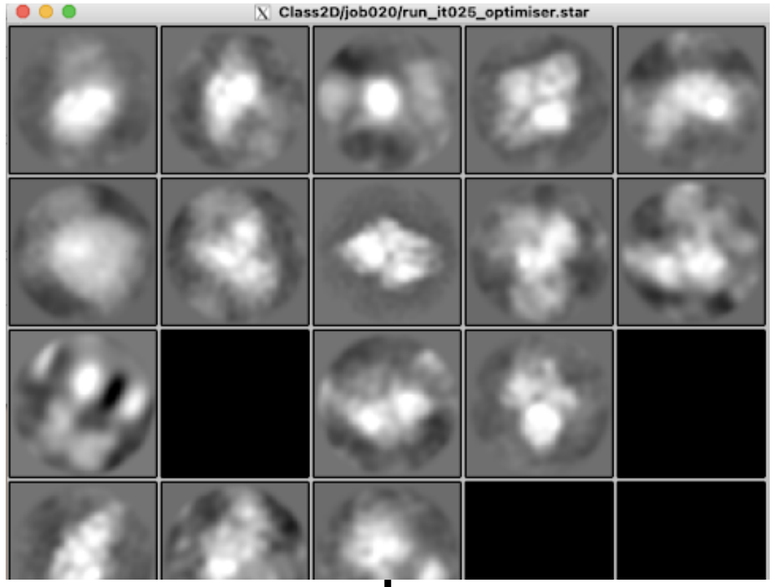
Particle extraction: Manual or Autopicking



- Identify individual particles (protein projections) in each micrograph.
- Can be done manually, semi-automatically, or using Auto-picking in RELION (reference-based or LoG).
- Output: coordinates of each particle for extraction.
- Extract boxed-out images (particles) from the micrographs based on picked coordinates.
- Sets up the data for alignment and classification

2D classification

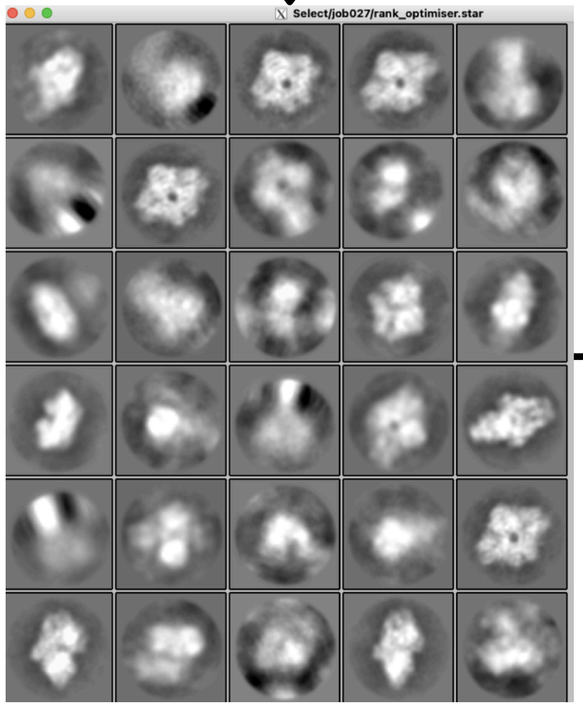
1



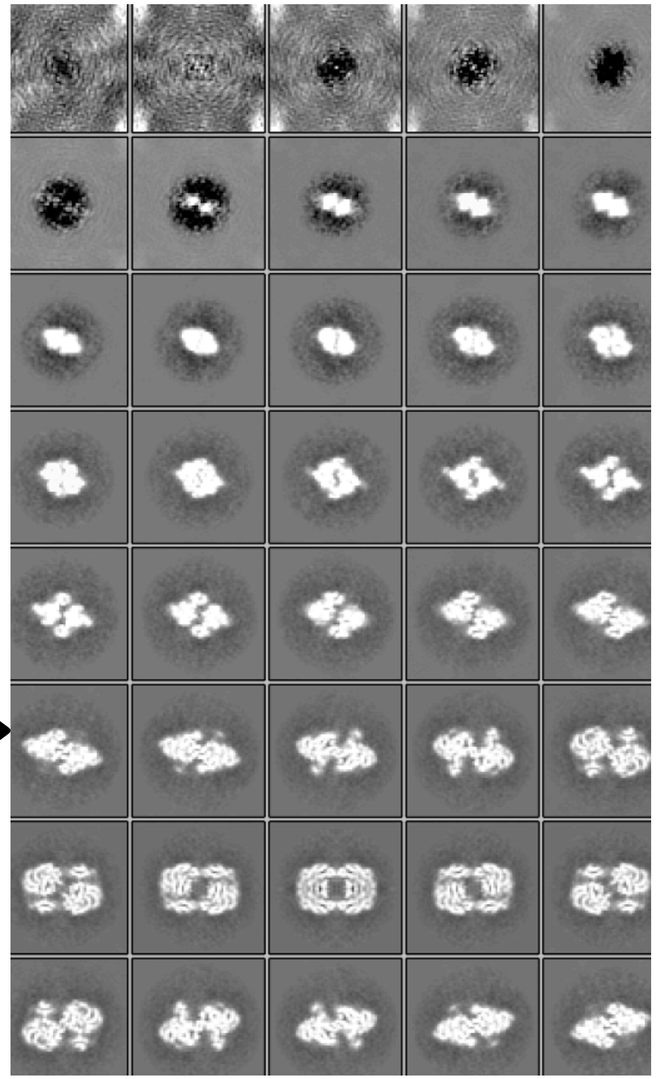
- Group similar particle projections into 2D classes.
- Helps to filter out bad picks, noise, and junk particles.
- Choose best-looking classes for further steps.

After 3-4 iterations
and rank optimisation

2



3



De novo/ab-initio 3D model generation

4

3D classifcation

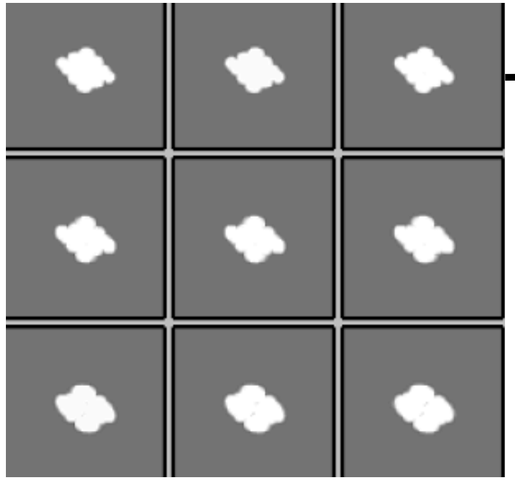
- Sort particles into different 3D classes.
- Helps identify heterogeneity (multiple conformations or assemblies).
- Select best class for refinement.

5

3D auto refinement

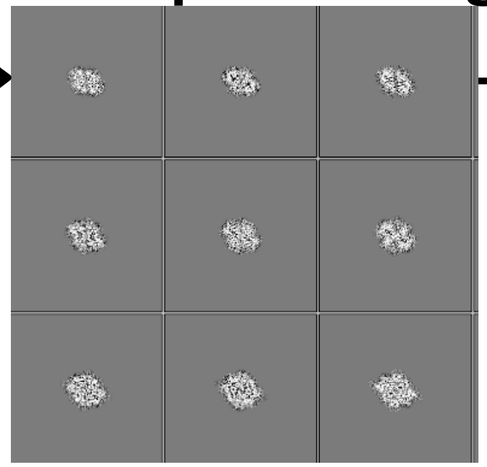
- High-resolution refinement of the selected class.
- Produces a polished 3D map with improved signal and resolution.

Mask creation



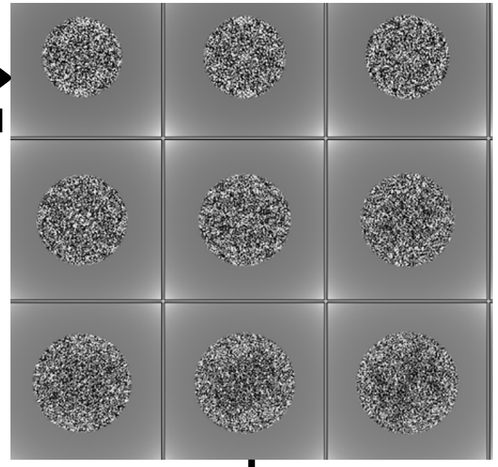
- After refinement, I generated a soft-edged solvent mask to isolate the protein volume.
- This mask was used during the post-processing step to improve resolution estimation and sharpen the final density map.

Post-processing

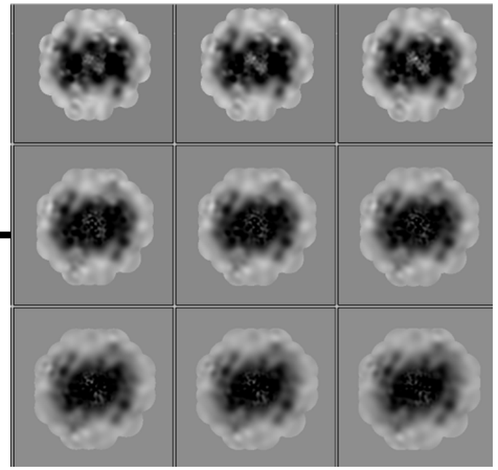


- B-factor sharpening, and FSC (Fourier Shell Correlation) calculation.
- Final resolution is estimated using the 0.143 FSC cutoff.

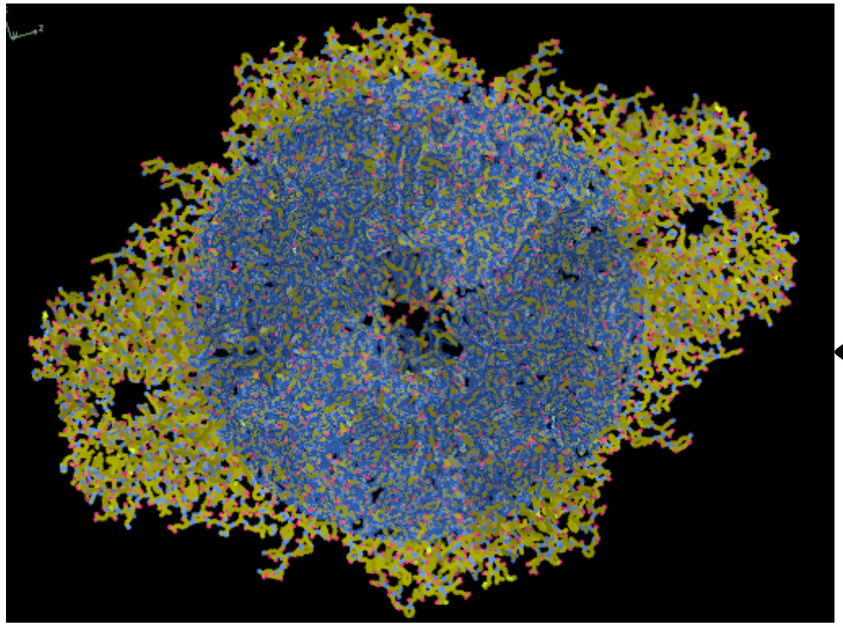
Fix ctf and abberation



Local resolution



Visual inspection of maps via COOT, local resolution estimation



Thank you!!

SBRC group, KEK