BIO-315
Structural Biology

Introduction to Electron Microscopy
- Lecture 3 -

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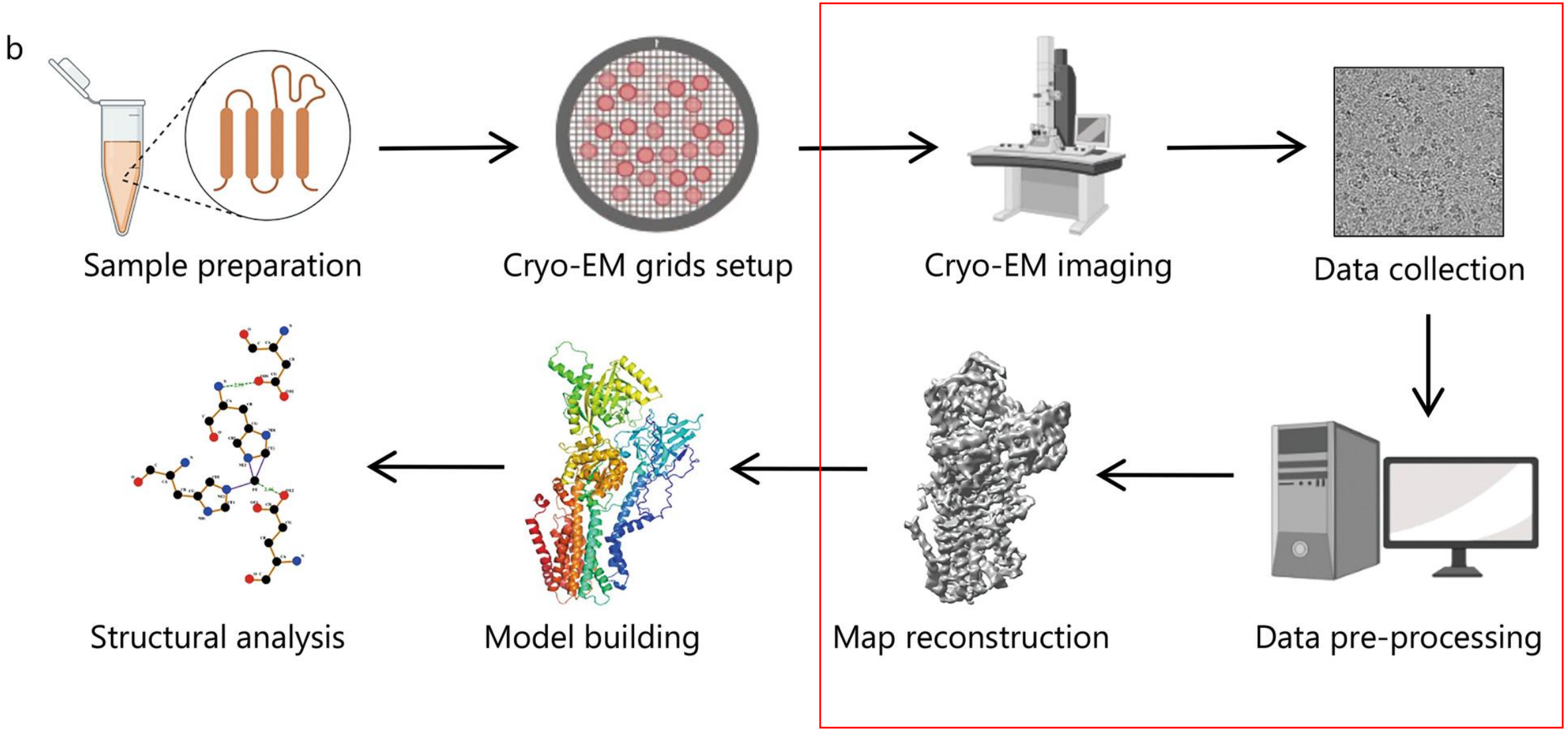
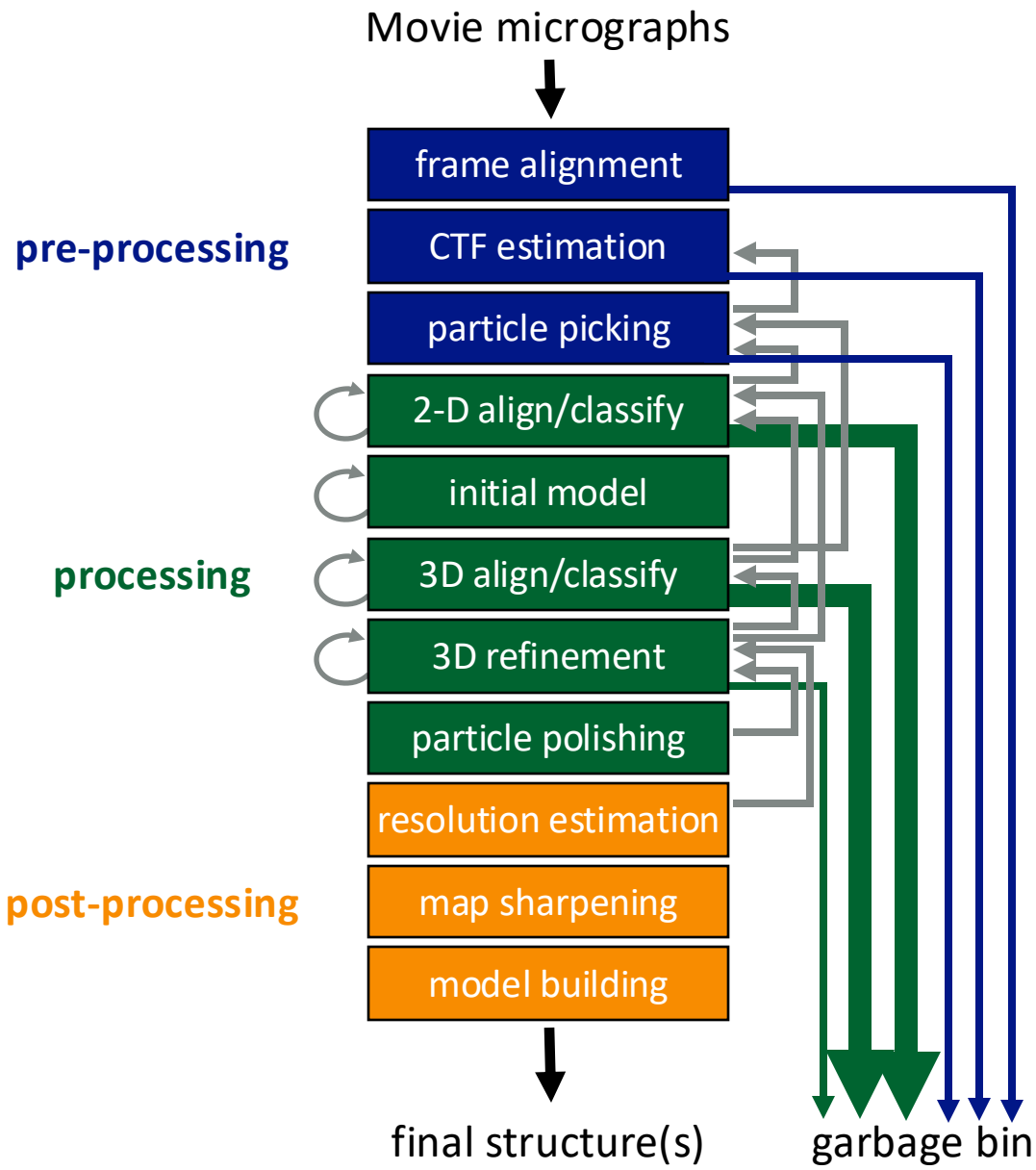
26-03-25

Quick Recap

Electron Microscopy for Structure Determination



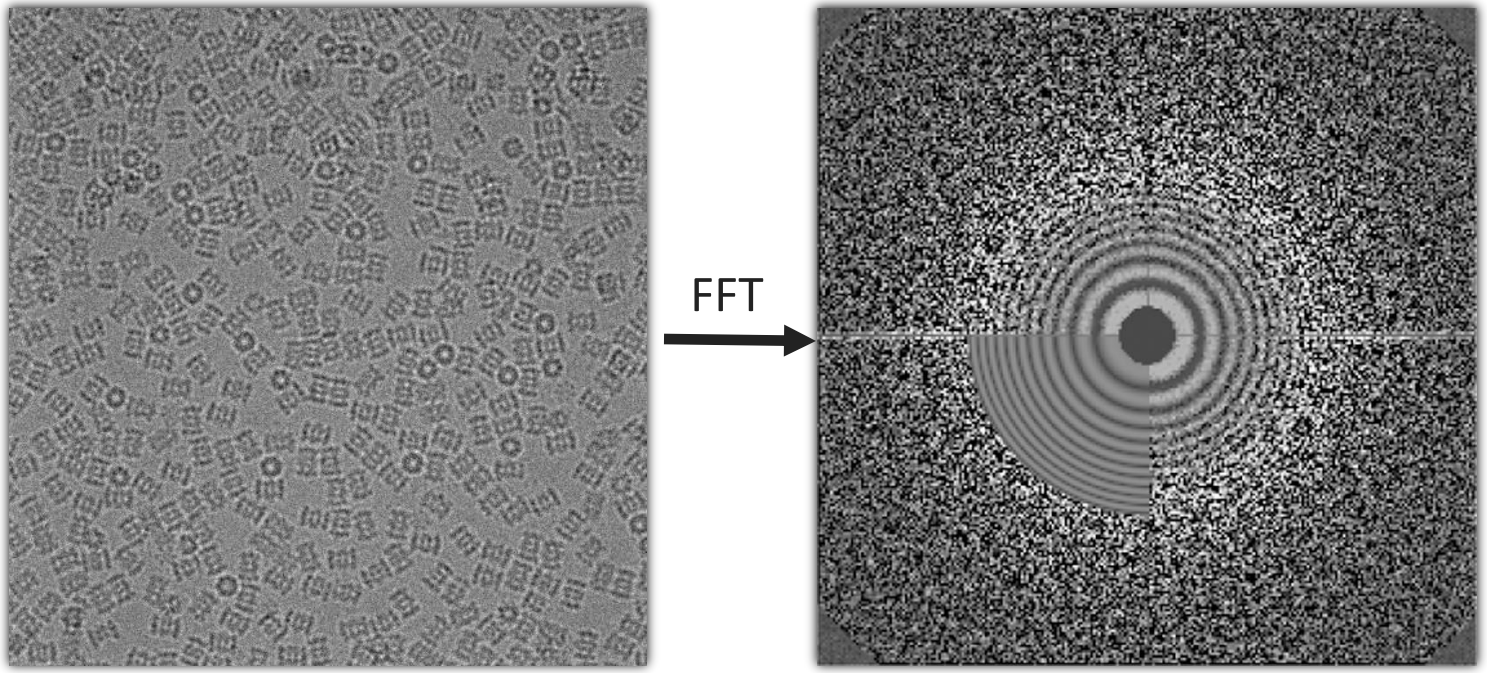


many of the downstream steps feed back to earlier steps (not a linear path)

in silico pruning: images can be rejected at multiple points

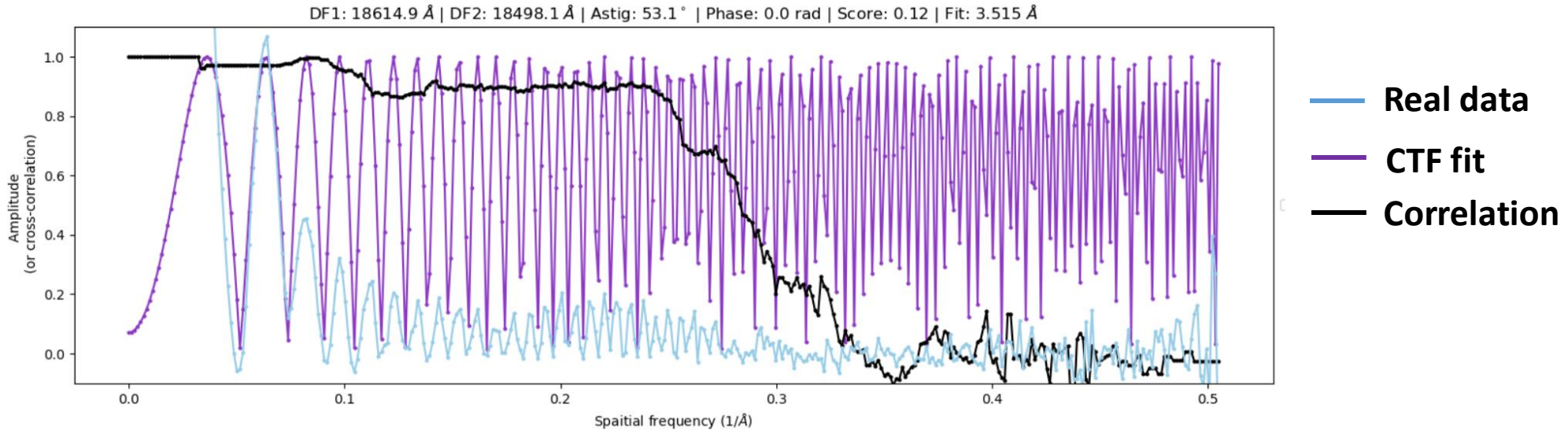
Power spectrum and Contrast Transfer Function

Raw micrograph (real space)



Fourier transform (power spectrum)

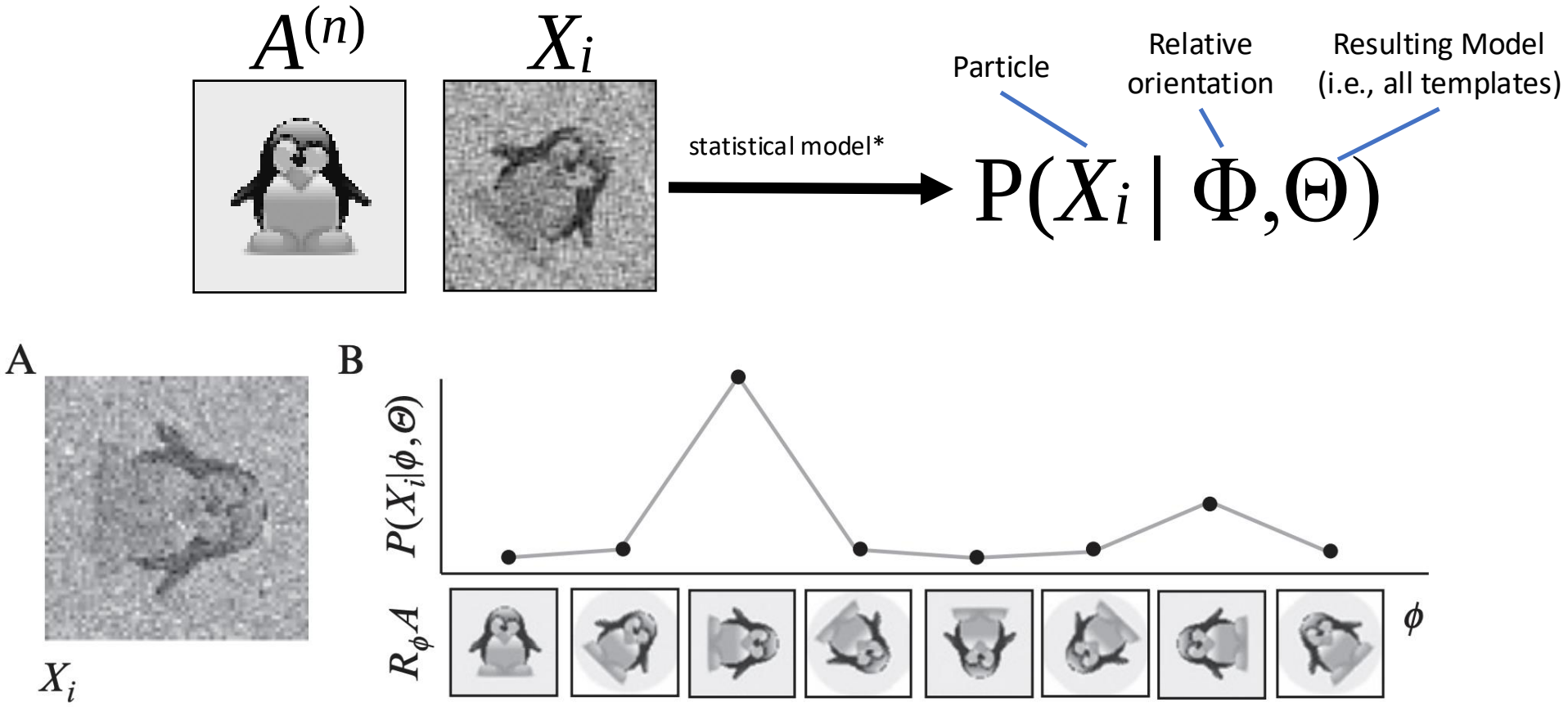
CTF fitting



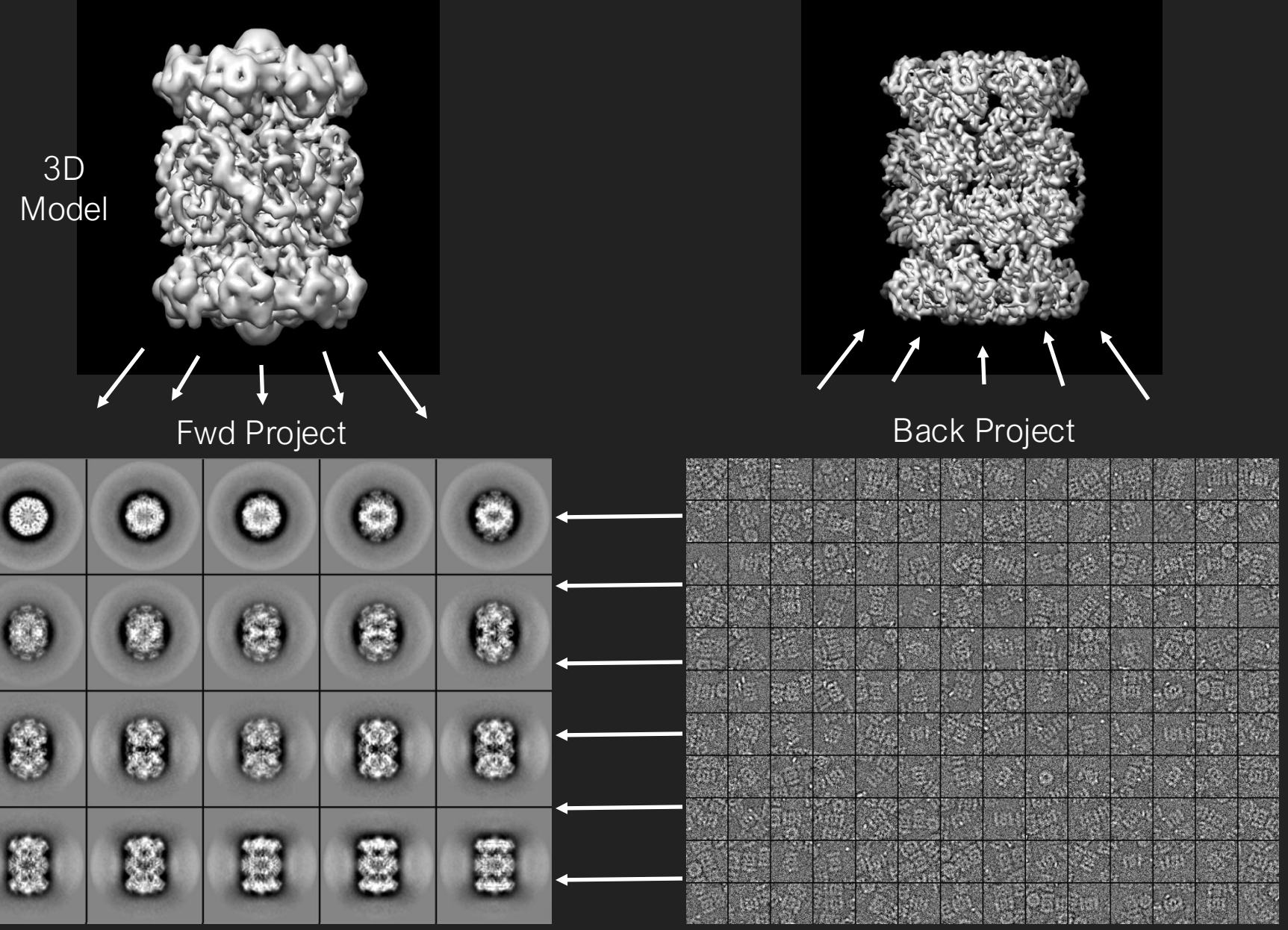
Rohou and Grigorieff, JSB 2015

Maximum likelihood approaches to find particle orientations

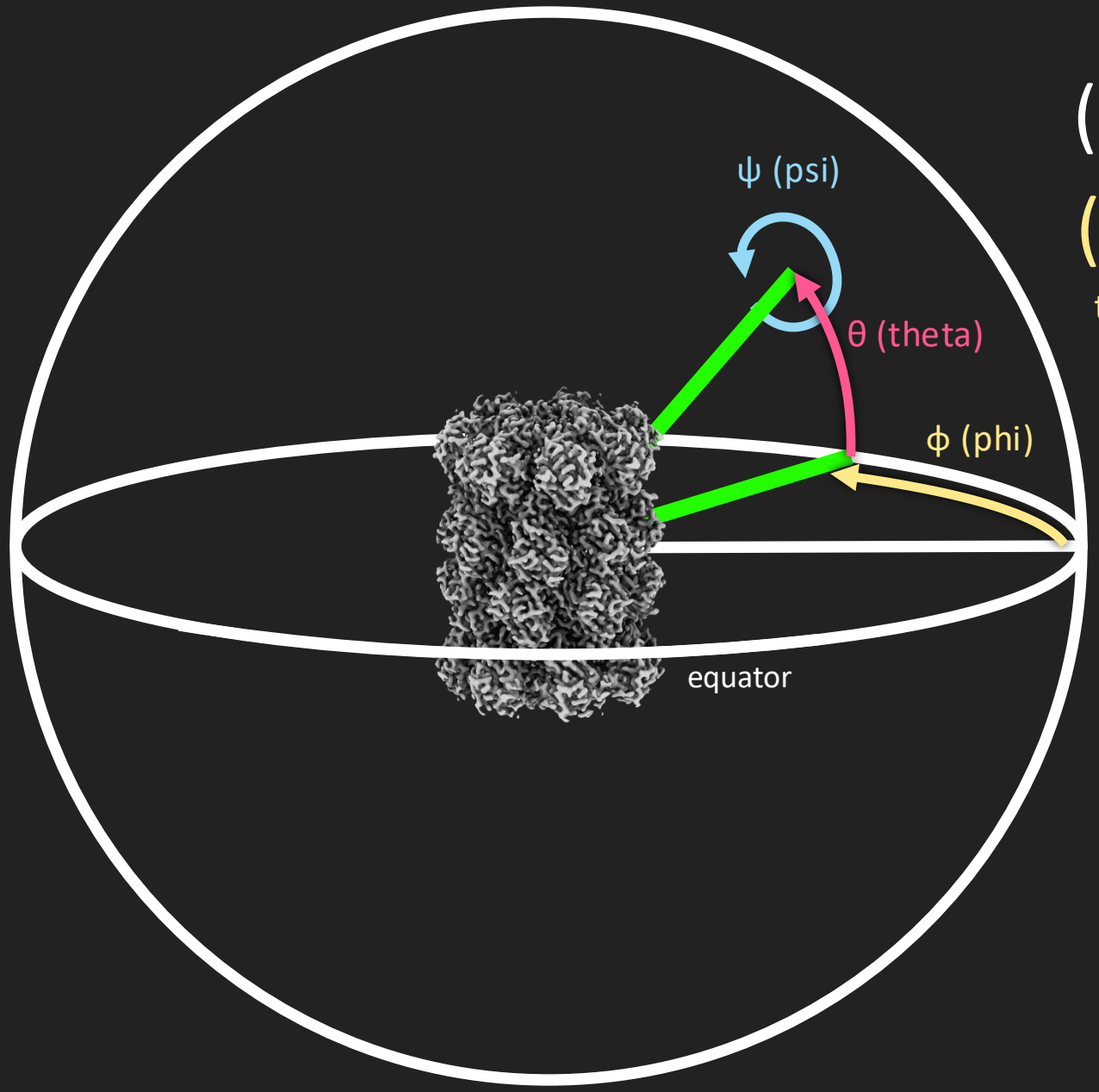
- Estimating the likelihood that the current model is correct given the data
- Therefore, must be able to assign a probability that a 2D projection describes an image.
- The program searches for a set of 2D classes and particle positions with **combined highest likelihood**



Sigworth, Doerschuk, Carazo, Scheres. "An introduction to maximum-likelihood methods in cryo-EM" Methods in Enzymology 2010


3D
Model
Fwd Project
Back Project
Projection
Matching

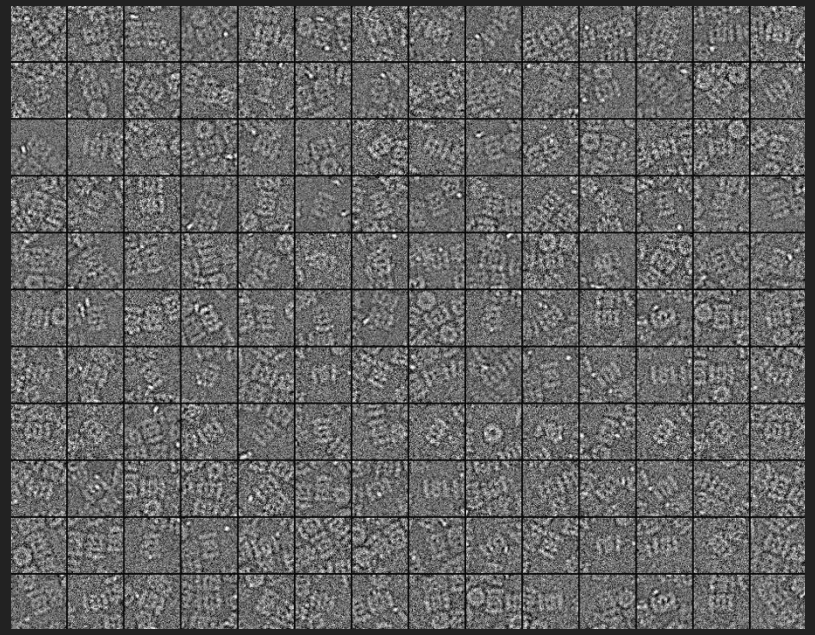
Defining particle orientation with respect to the 3D object



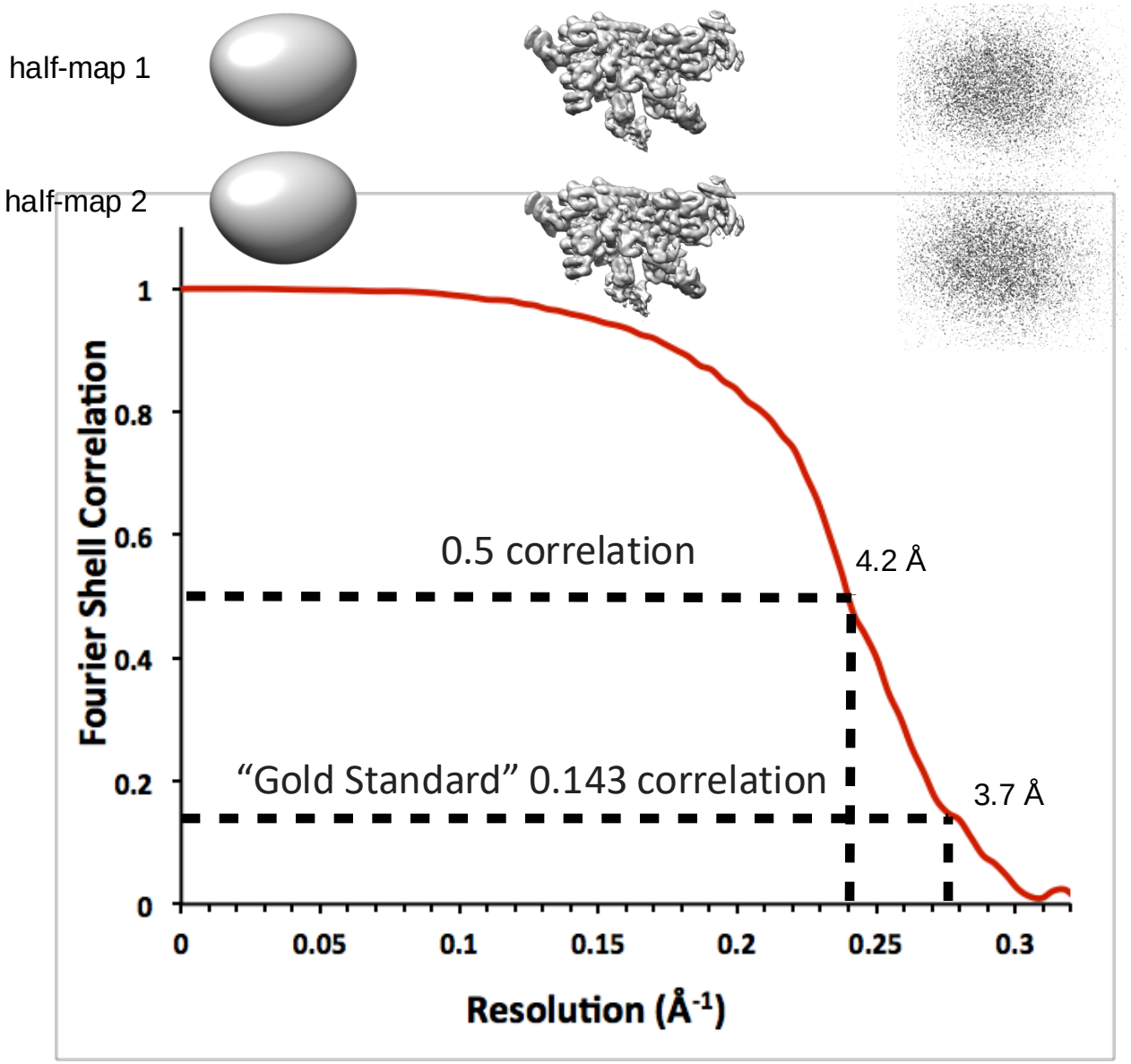
(ϕ, θ, ψ)

(x,y)

translation/shifts



Fourier Shell Correlation (FSC) Plot



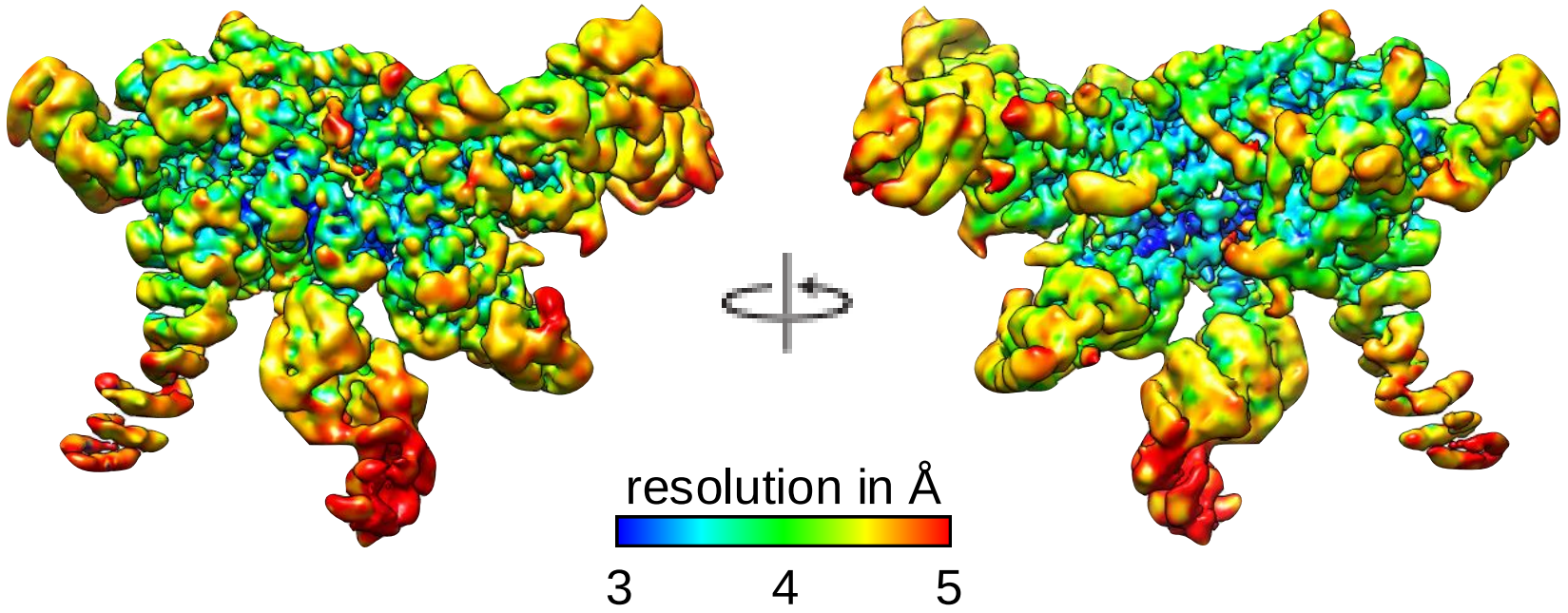
- Correlation between resolution shells in 2 independently refined half-sets of data

$$FSC(r) = \frac{\sum_{r_i \in r} F_1(r_i) \cdot F_2(r_i)^*}{\sqrt{\sum_{r_i \in r} |F_1(r_i)|^2 \cdot \sum_{r_i \in r} |F_2(r_i)|^2}}$$

F1 – Structure factors for volume 1
F2* – Complex conjugate of the structure factors for volume 2
$\mathbf{r_i}$ – Voxel element at the radius r

- "Gold Standard" is the most commonly used criterion to define global resolution

Local resolution plot is more informative



Resmap - compares power of Fourier components
Bsoft - calculates windowed FSCs
Relion - calculates windowed FSCs
Sparx - calculates local variance from 2D images

- Useful for visualization of local resolution across the entire map
- Can be used for map flitering to improve interpetability
- Can be used to inform data processing (e.g., positioning of sorting masks)
- Can be used to inform model building (e.g., which areas to build and where to stop)

B-factor to balance attenuation of amplitudes at high resolution

- frame alignment
- CTF estimation
- particle picking
- 2-D align/classify
- initial model
- 3D align/classify
- refinement
- particle polishing
- resolution estimation
- **map sharpening**
- model building

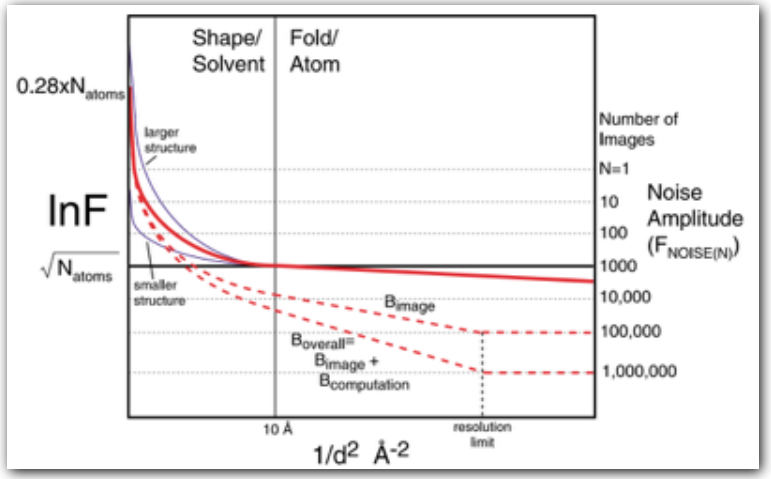
The problem:
combined effects of imaging and processing reduces observed high-frequency amplitudes

The solution:
apply negative B-factor to "sharpen" the map

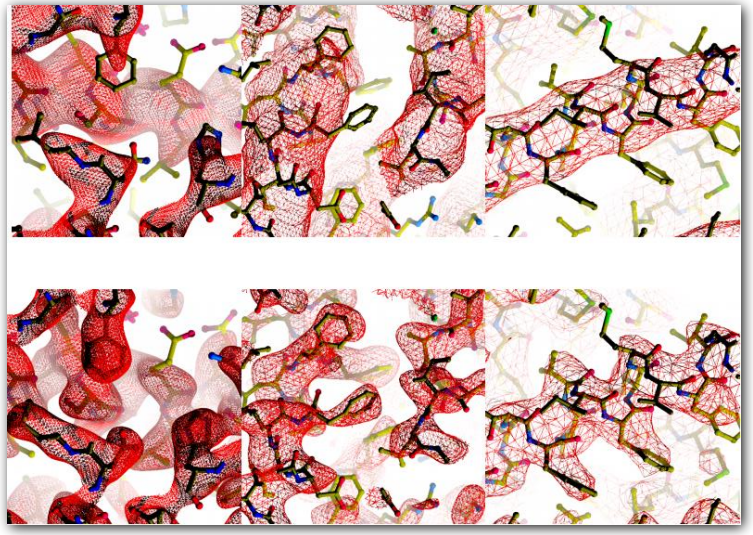
What B-factor to use?

- Calculate from Guinier plot
- ad hoc - increase until noise becomes problematic

rotationally averaged spectrum



Rosenthal and Henderson JMB 2003



Terwilliger et al. bioRxiv doi.org/10.1101/247049

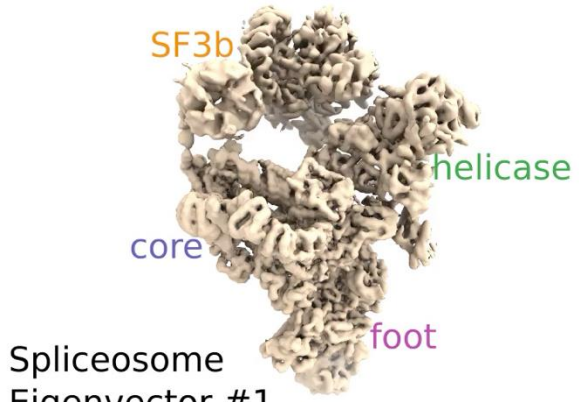
output: map with enhanced high resolution features

3D classification as a tool to study molecule heterogeneity

- Compositional and conformational heterogeneity can be approximated by analysis of variability across particles

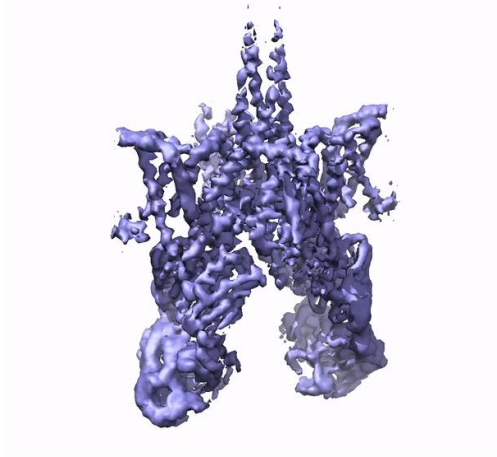
frame alignment
CTF estimation
particle picking
2D align/classify
initial model
3D align/classify
3D refinement
3D landscape characterization
particle polishing
resolution estimation
map sharpening
model building

Multi-body refinement (RELION)



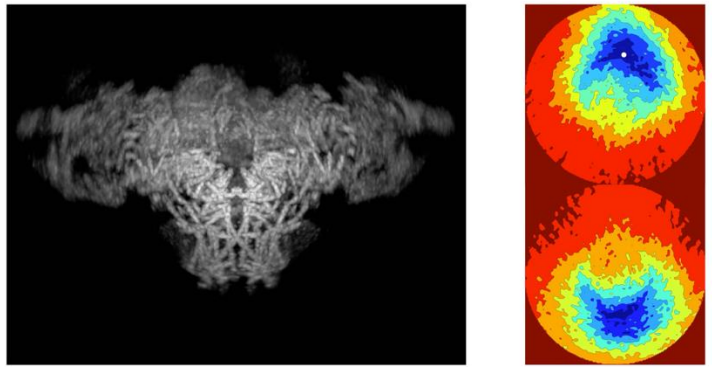
Nakane et al. eLife 2018

Variability Analysis (CryoSPARC)



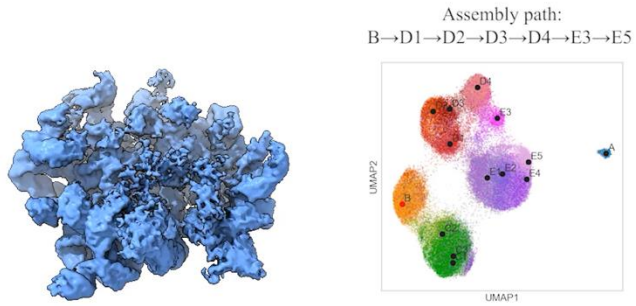
Punjani & Fleet JSB 2021

ManifoldEM



Dashti et al. Nat Comm 2020

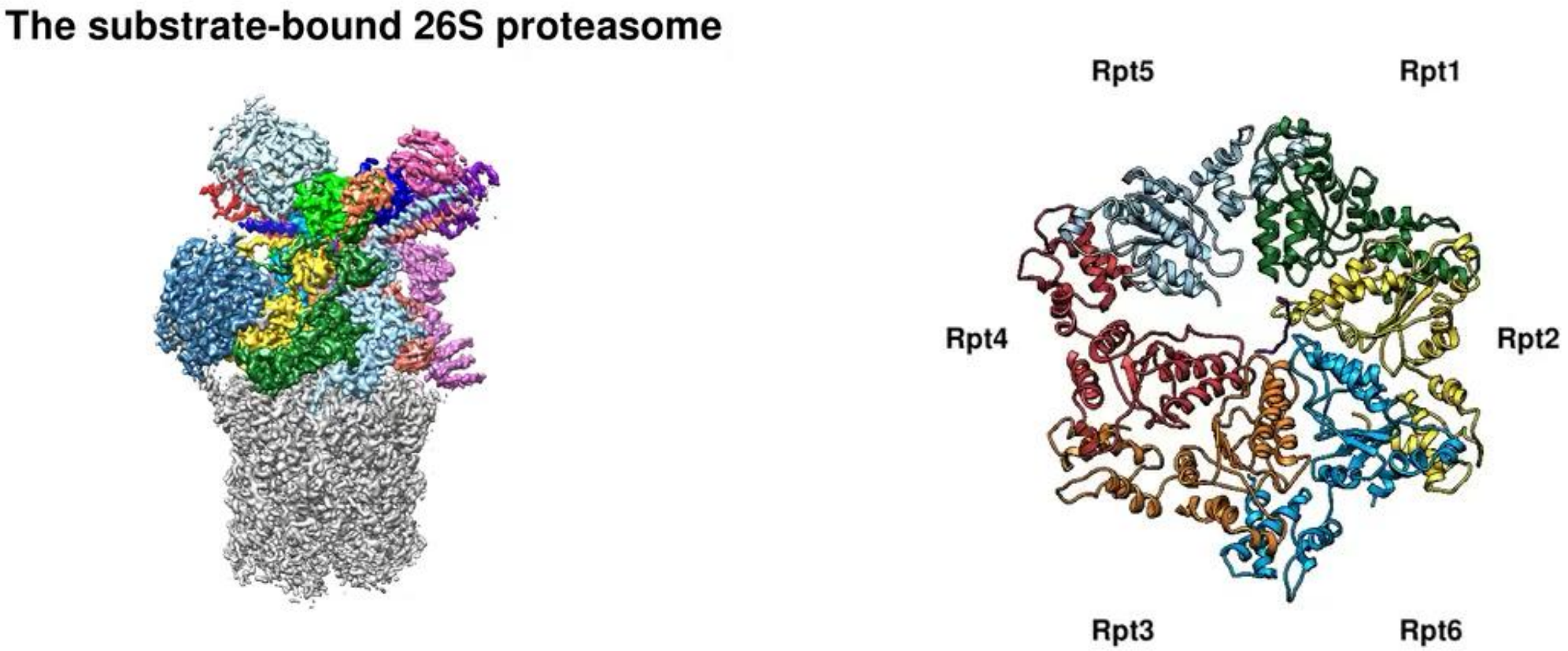
CryoDRGN



Zhong et al. Nat Methods 2021

EM allows to study biomolecules in action

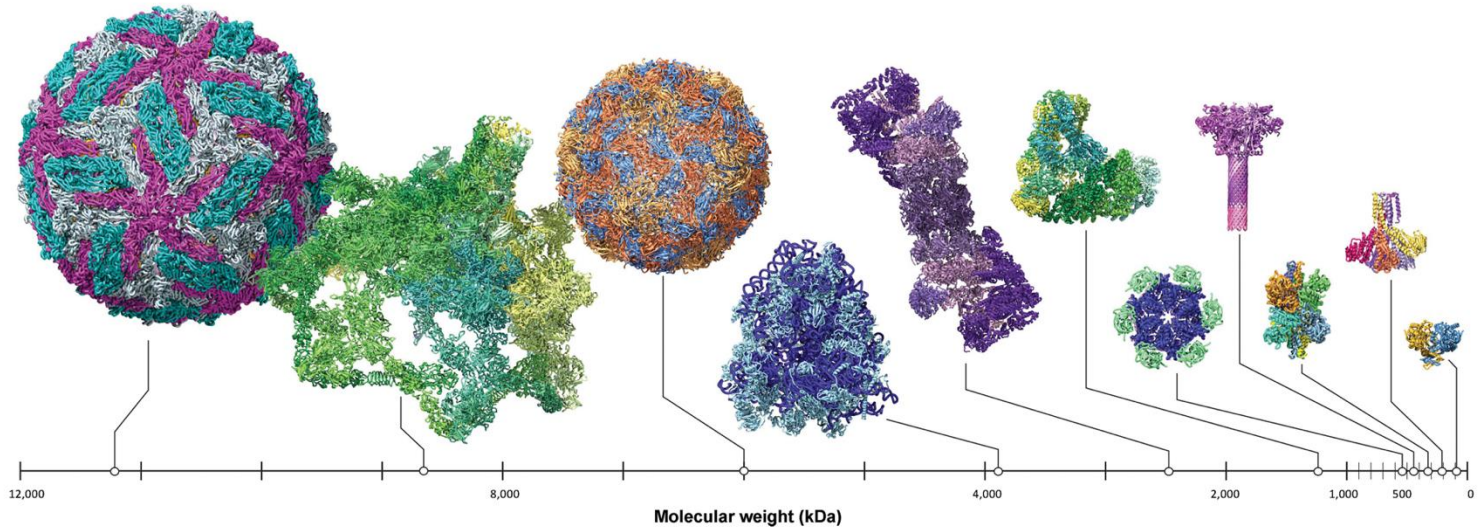
- Proteasome engaging a ubiquitinated protein substrate



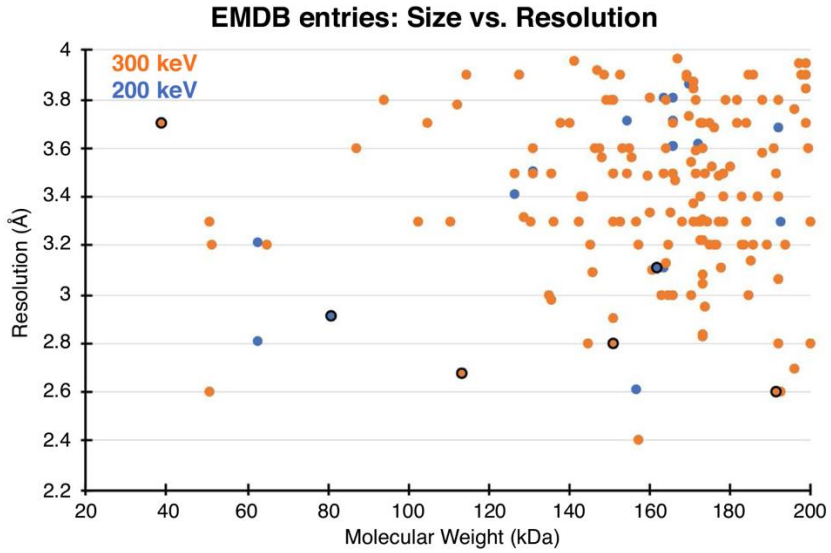
What molecules can be studied by EM and what resolutions can be achieved?

EM allows to study a wide range of protein targets

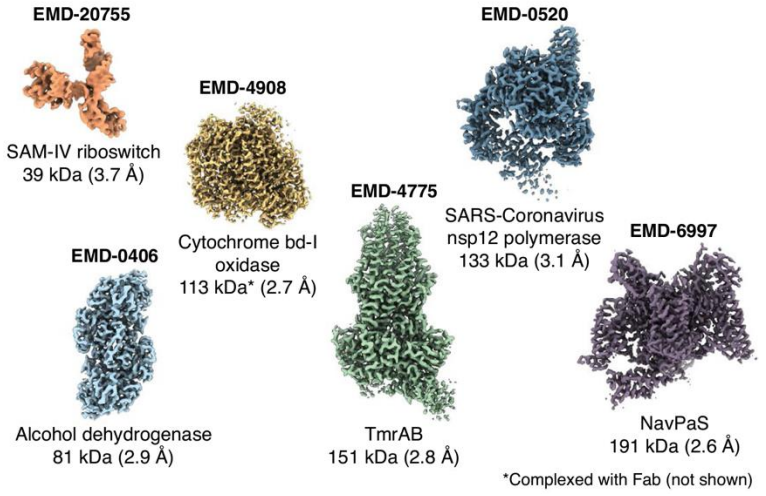
- Larger protein complexes (e.g., viruses) have stronger signal in EM images and are easier to tackle
- Smaller biomolecules <100kDa can be quite challenging for imaging and subsequent alignment via existing algorithms
- The lowest MW complex resolved by EM was a 39kDa riboswitch



Subramaniam, 2021



Examples of small proteins resolved by EM



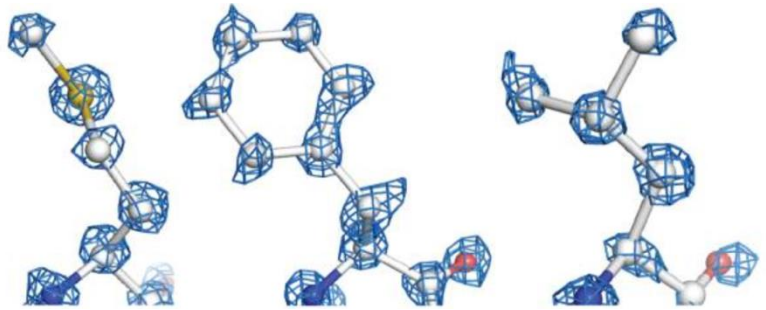
Wu, Lander, 2020

Attainable Resolutions dramatically improved (again) around 2020

Very noisy 2D projection images that are radiation damaged

How is this possible?

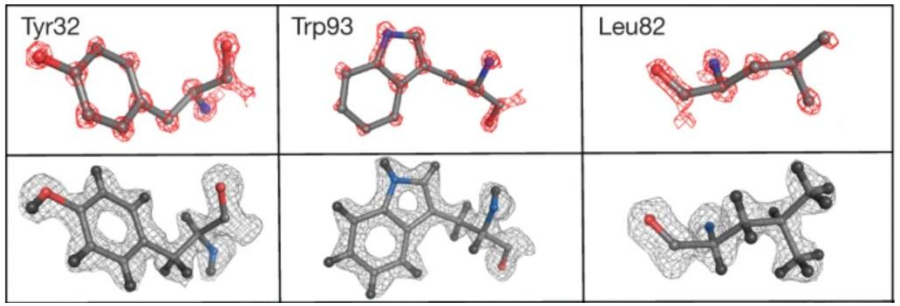
Atomic resolution structures



Nakane et al., Nature 2020

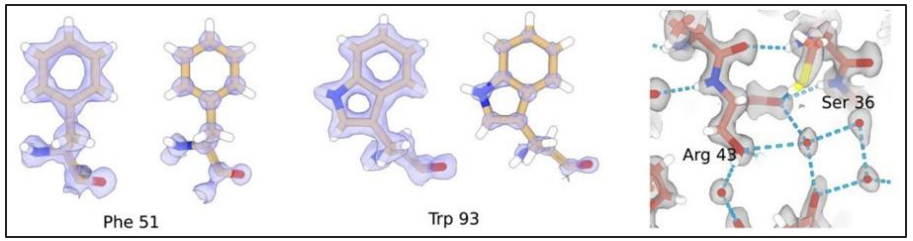
β3 $GABA_A$
1.7Å

Apoferritin
1.22Å



Yip et al., Nature 2020

Apoferritin
1.25Å

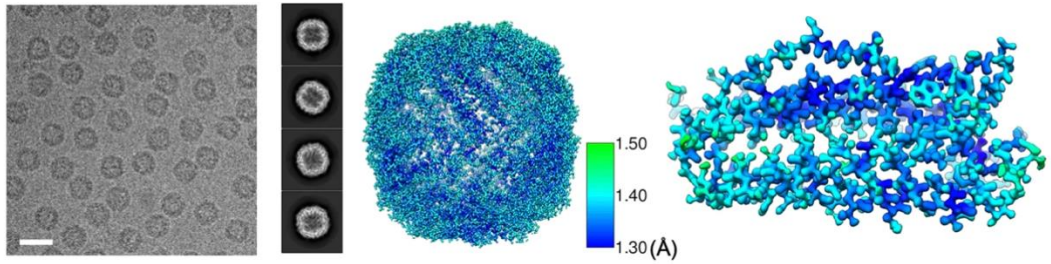


Kucukoglu et al., Nature Comm 2024

Apoferritin
1.09Å

Attainable Resolutions dramatically improved (again) around 2020

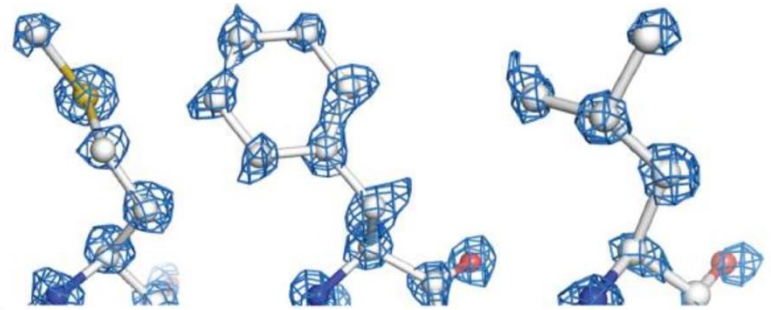
- Highly stable protein systems amenable to EM



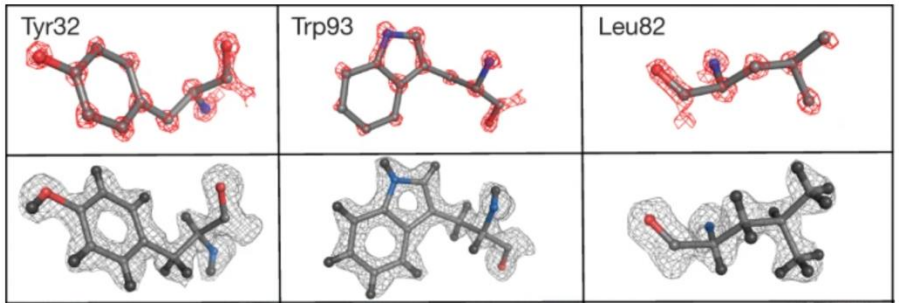
- Optimized imaging strategies:
  - 300kV microscopes (Titan Krios G3, cryoARM)
  - Cold Field Emission Gun (CFEG)
  - High DQE detectors (Falcon IV and Gatan K3)
  - Energy Filter
  - Spherical aberration corrector
  - Large particle datasets

- Advanced data processing tools:
  - Measuring detector MTF
  - Magnification anisotropy and aberration correction
  - CTF refinement (per-particle defocus)
  - Bayesian polishing

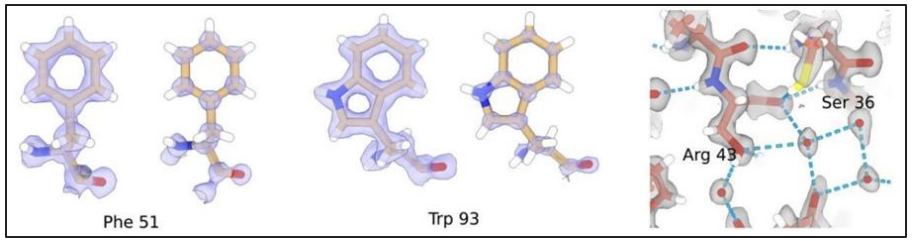
Atomic resolution structures



Nakane et al., Nature 2020



Yip et al., Nature 2020



Kucukoglu et al., Nature Comm 2024

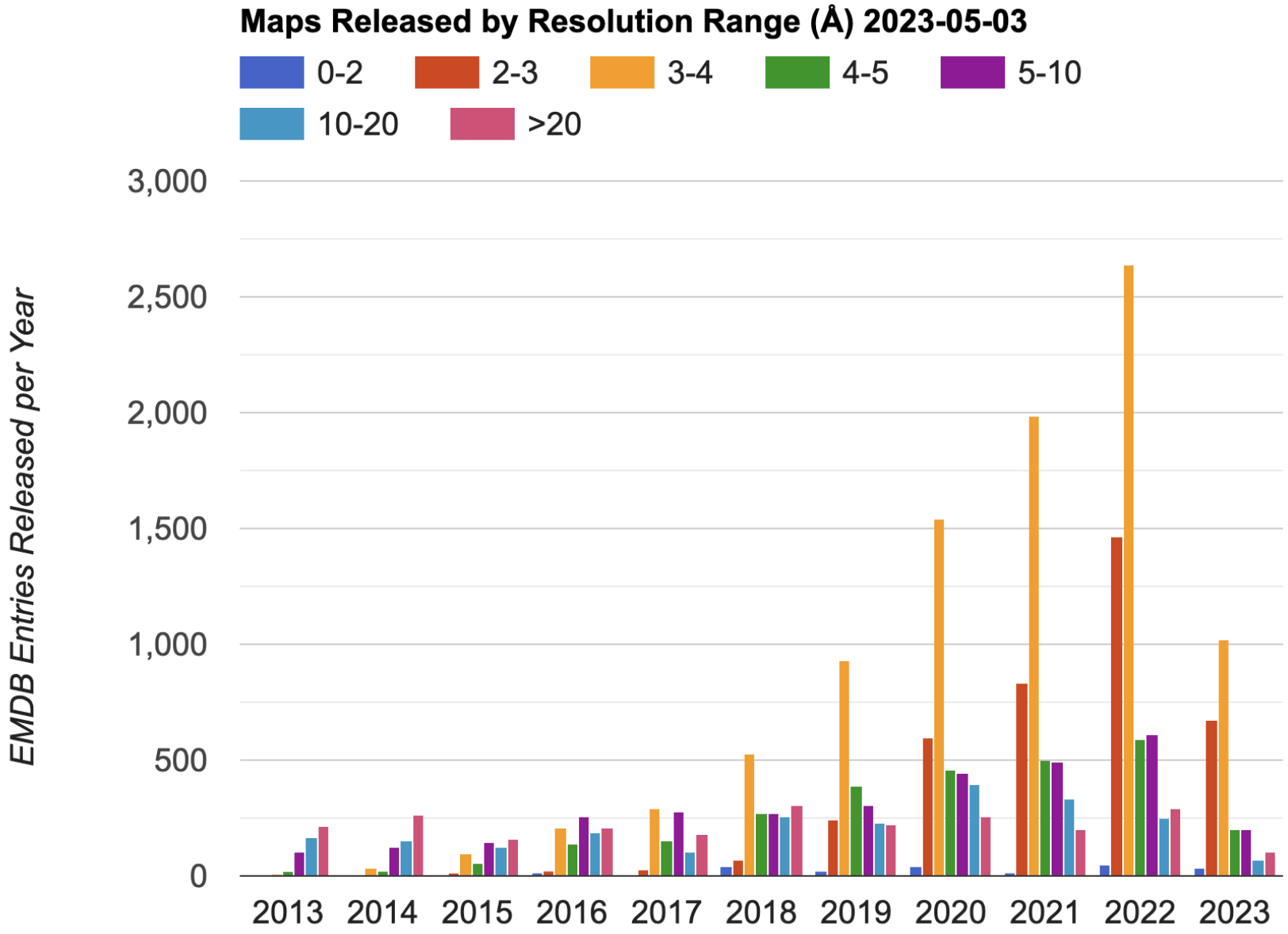
β3 $GABA_A$
1.7Å

Apoferritin
1.22Å

Apoferritin
1.25Å

Apoferritin
1.09Å

More realistic resolution values are somewhere in the 2-4Å range



Nyquist frequency defines the physical resolution limit of a micrograph

Harry Nyquist
1889 - 1976

Nyquist-Shannon sampling theorem:
“ If a function x(t) contains no frequencies higher than B hertz, then it can be completely determined from its ordinates at a sequence of points spaced less than 1/(2B) seconds apart.”

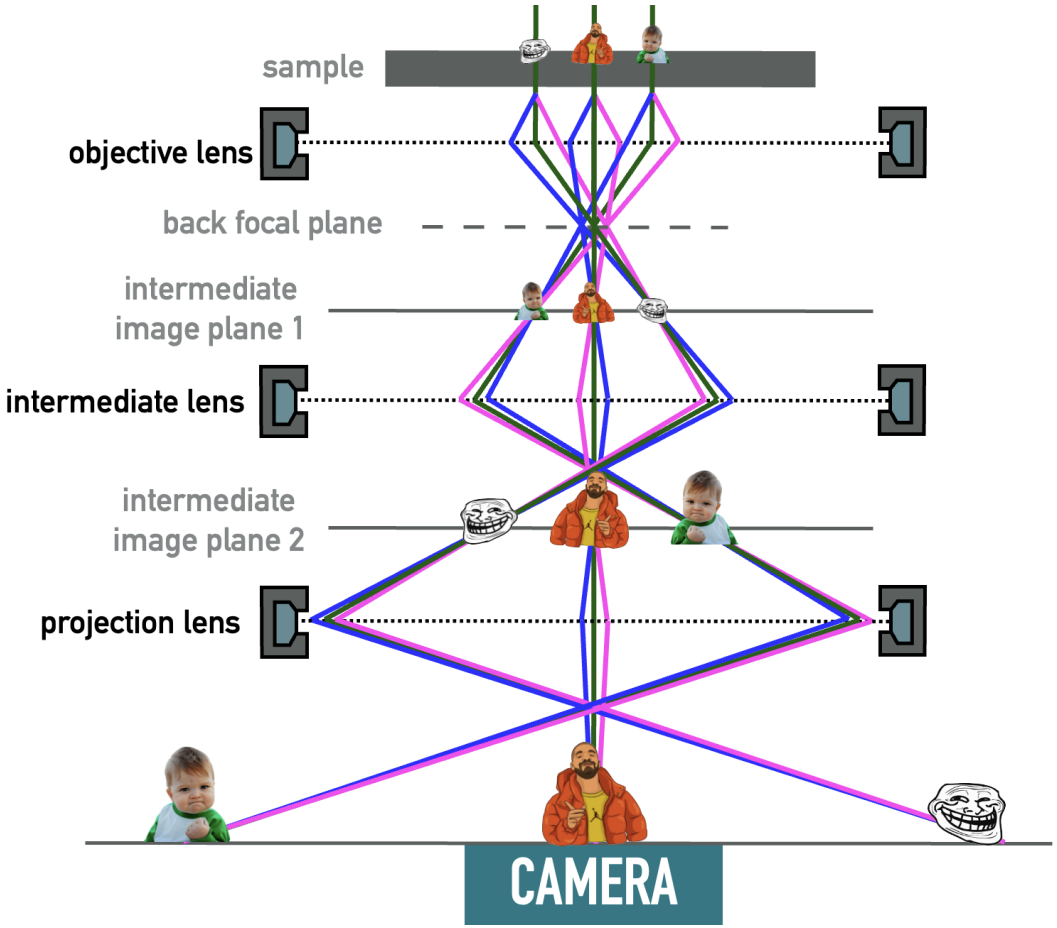
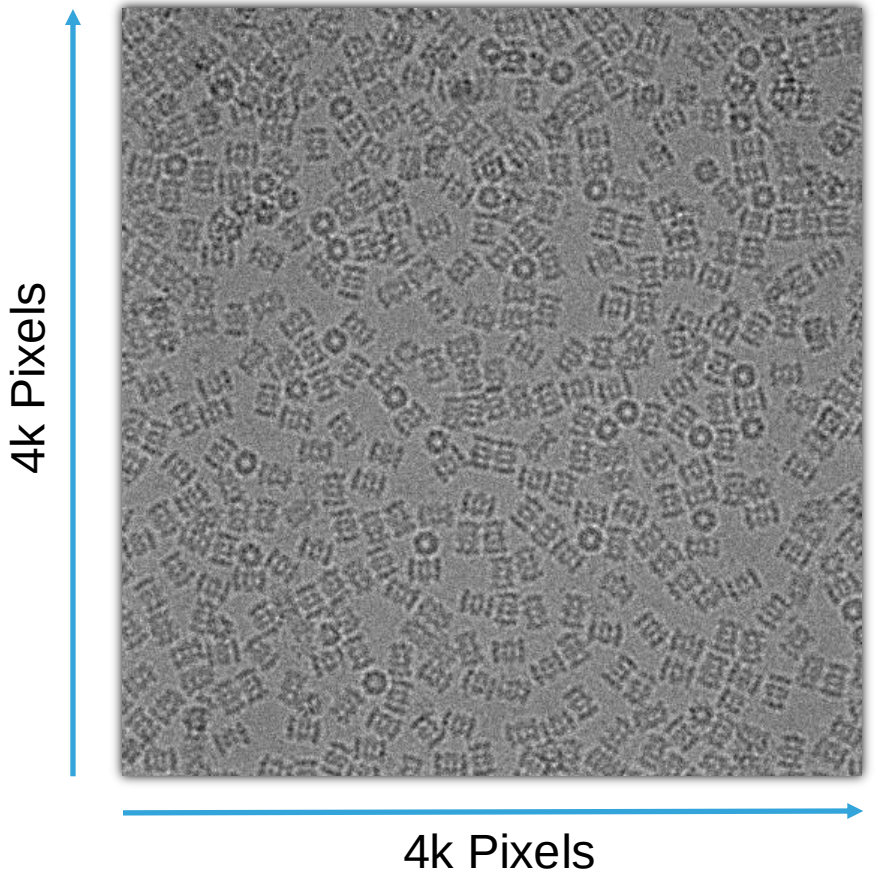


Image in real space



Each pixel corresponds to certain size in Å, depending on the magnification.
Max resolution = 2 x Pixel Size

Nyquist frequency defines the physical resolution limit of a micrograph

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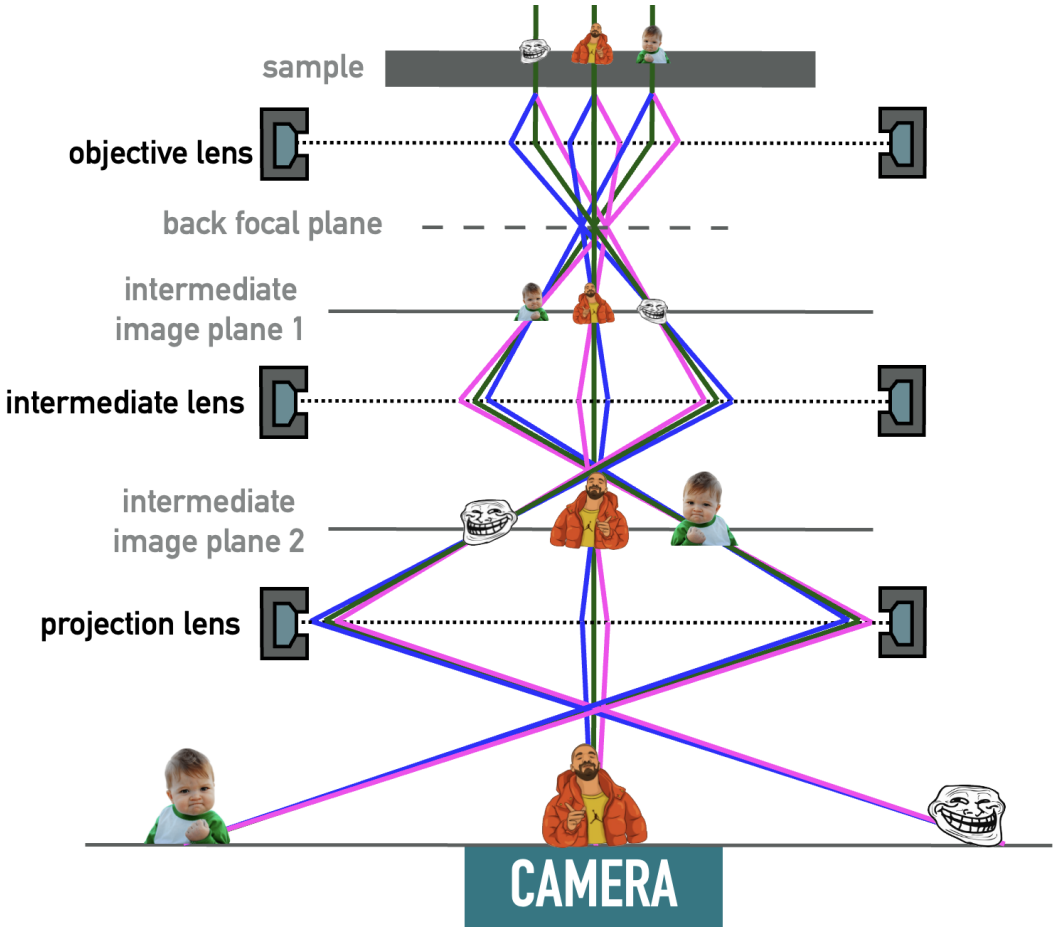
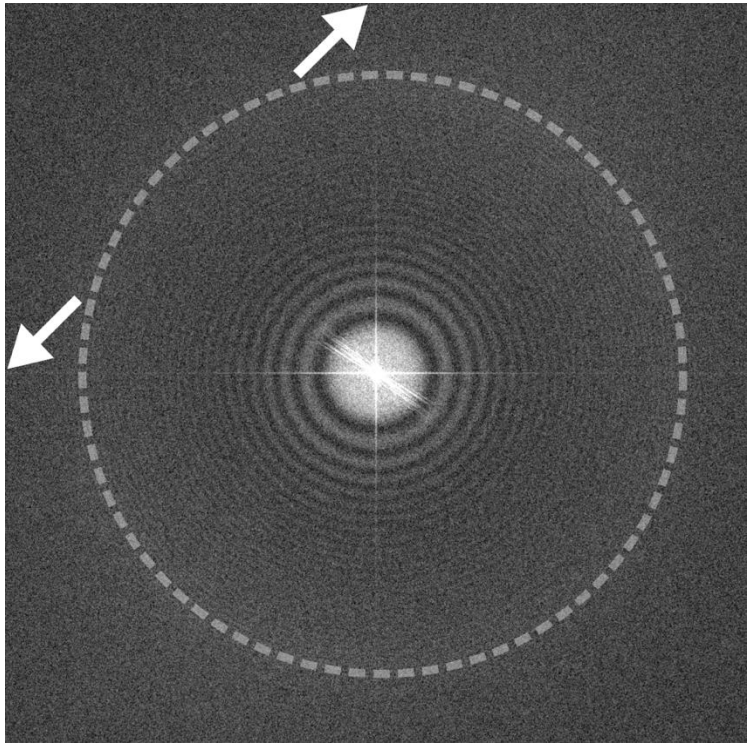


Power Spectrum

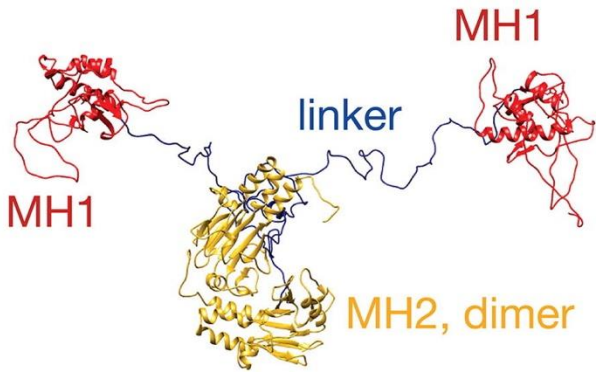


Each pixel corresponds to certain size in Å, depending on the magnification.
Max resolution = 2 x Pixel Size

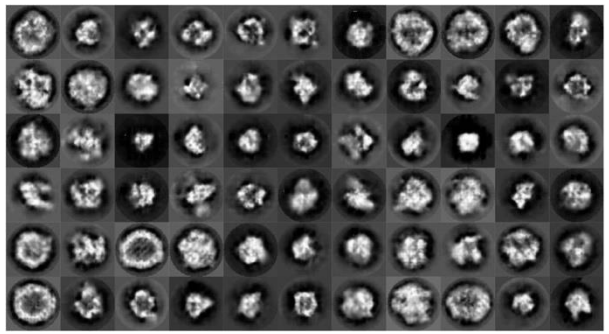
What factors affect attainable map resolution?

Sample properties

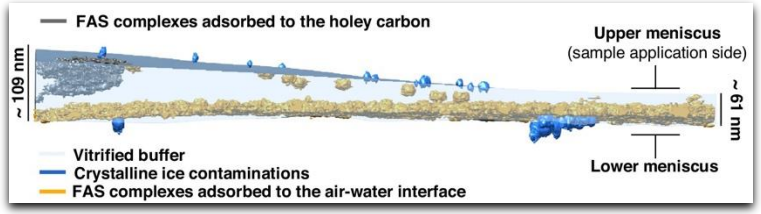
Flexibility

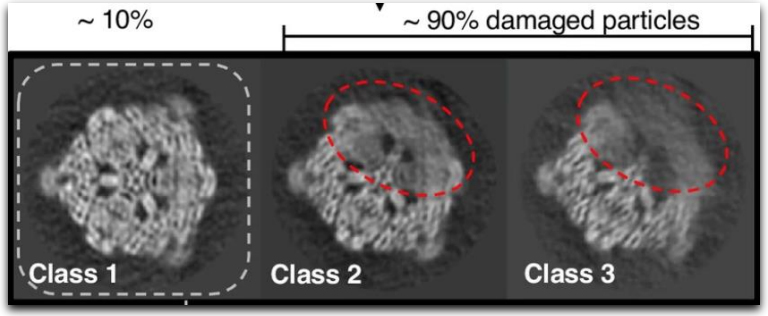


Purity

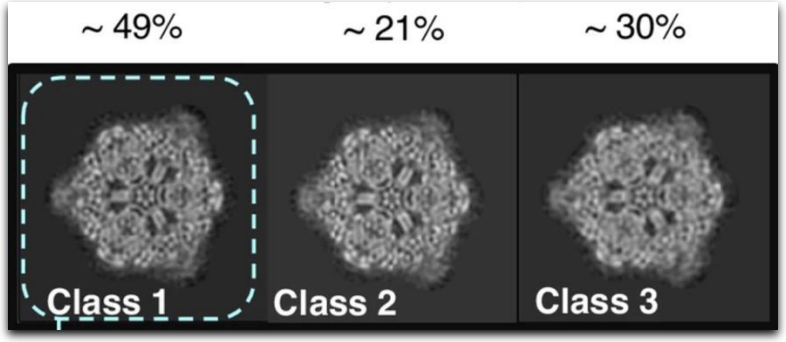


Particle denaturation at the air-water interface



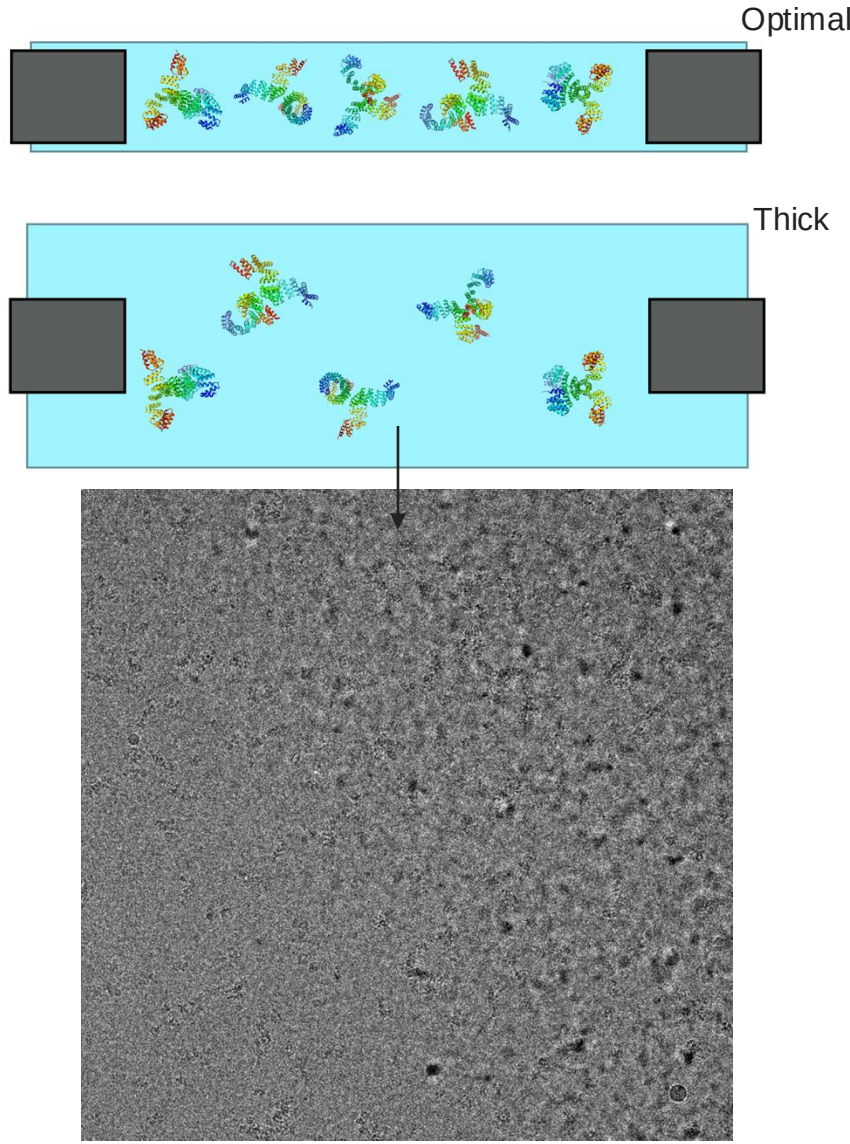


After coating the grid with graphene

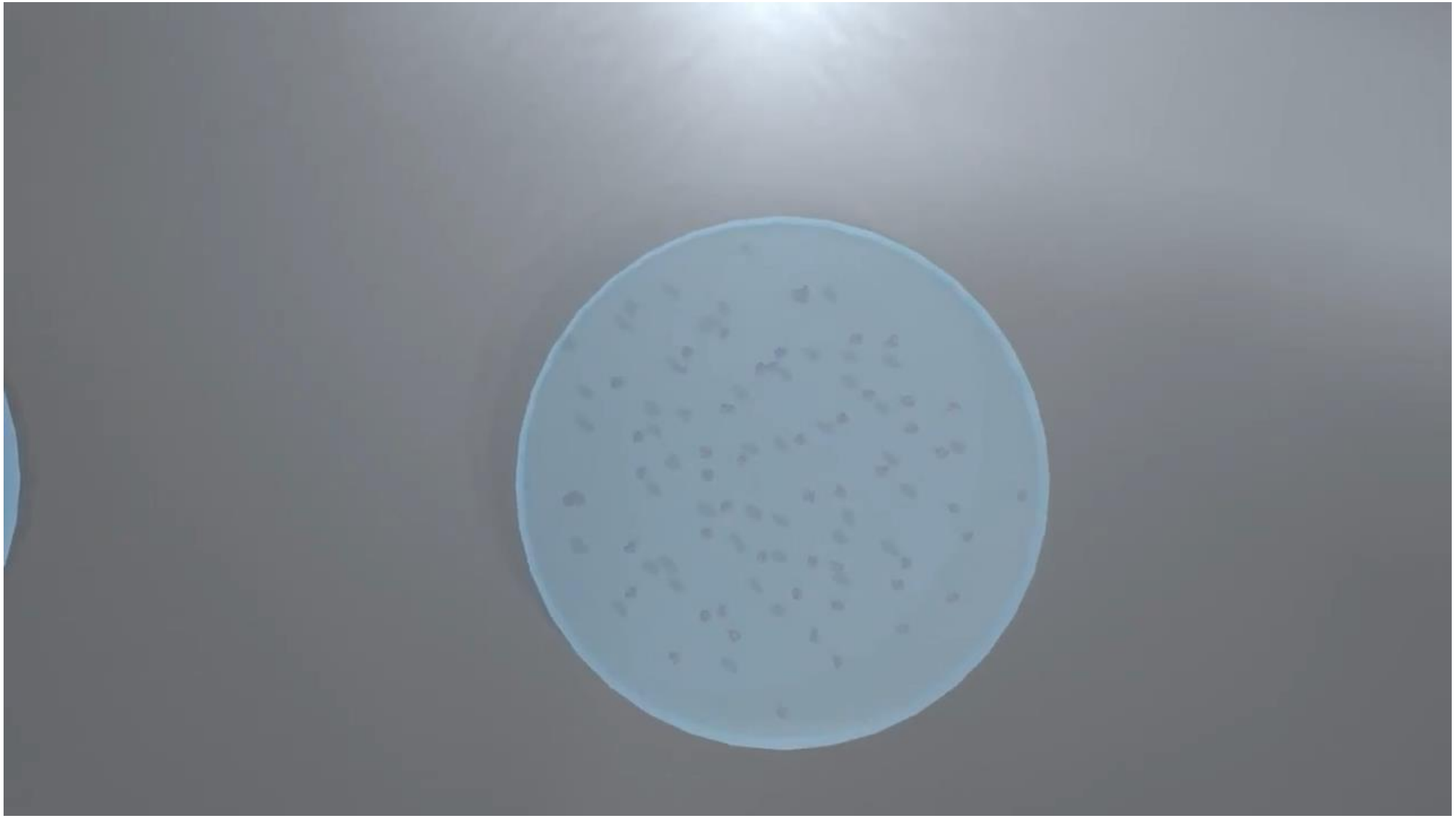


D'Iprima et al. eLife 2019

Ice thickness variation

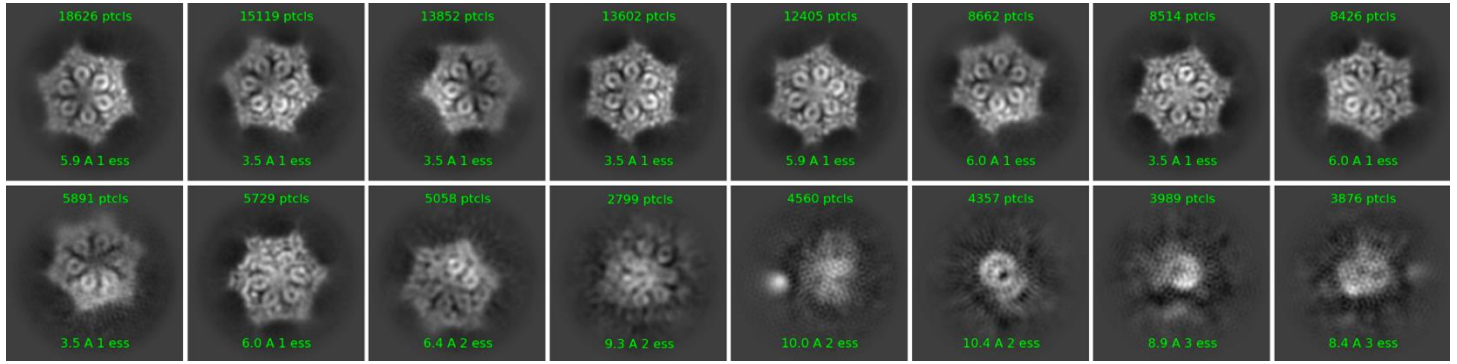


Preferred sample orientation on cryoEM grids

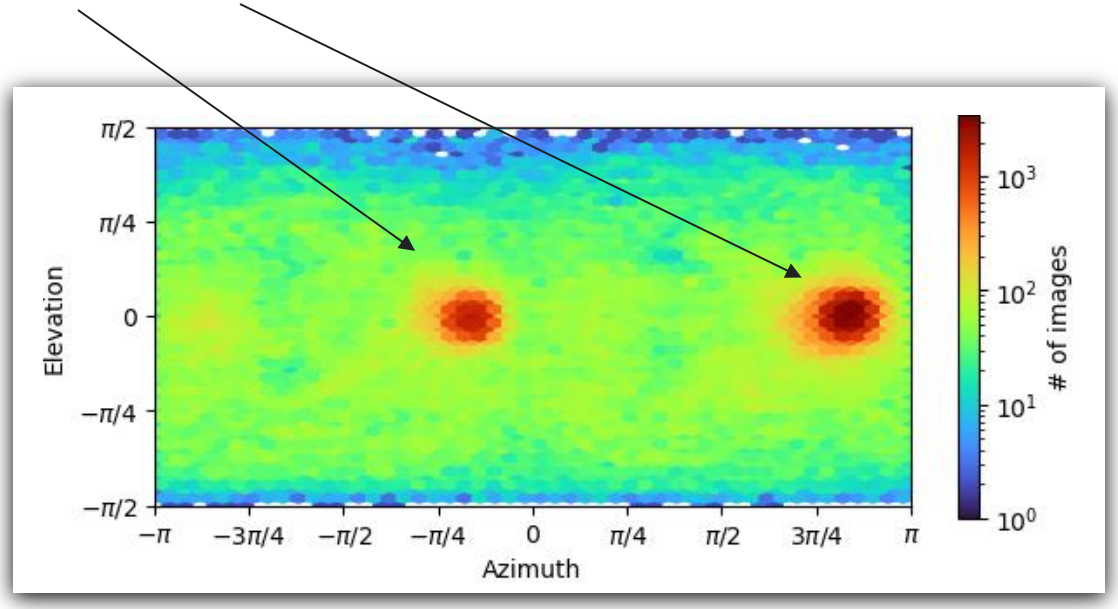


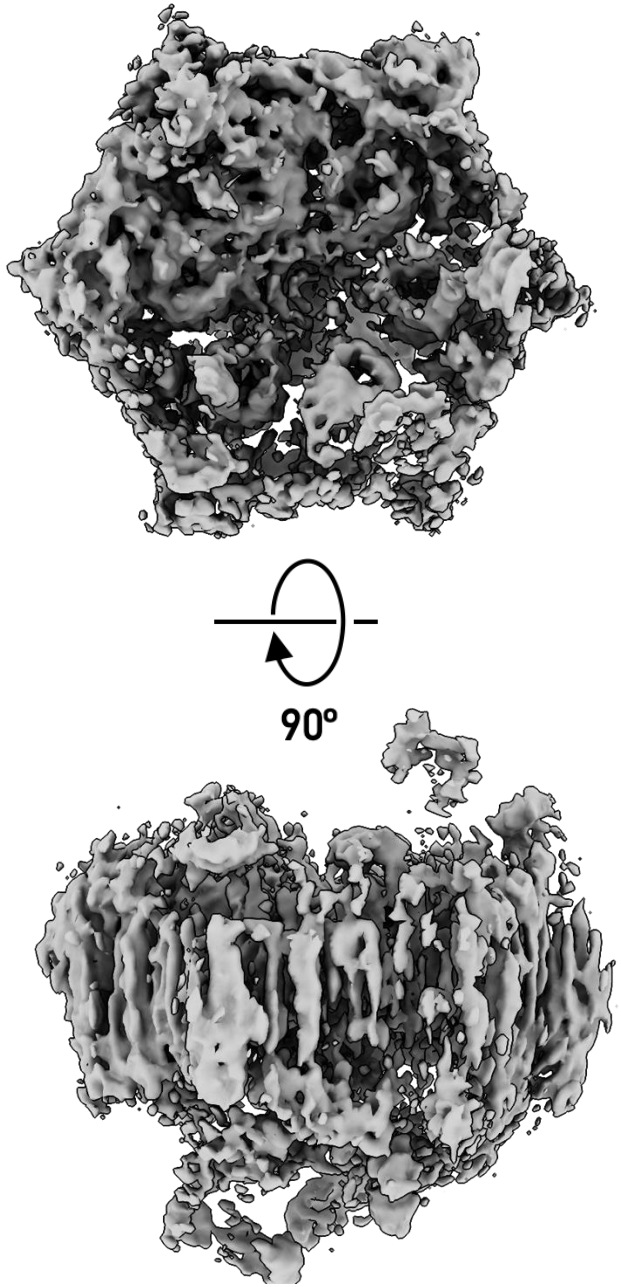
Preferred sample orientation on cryoEM grids

Example: **2D classes of a LONP1 protease**



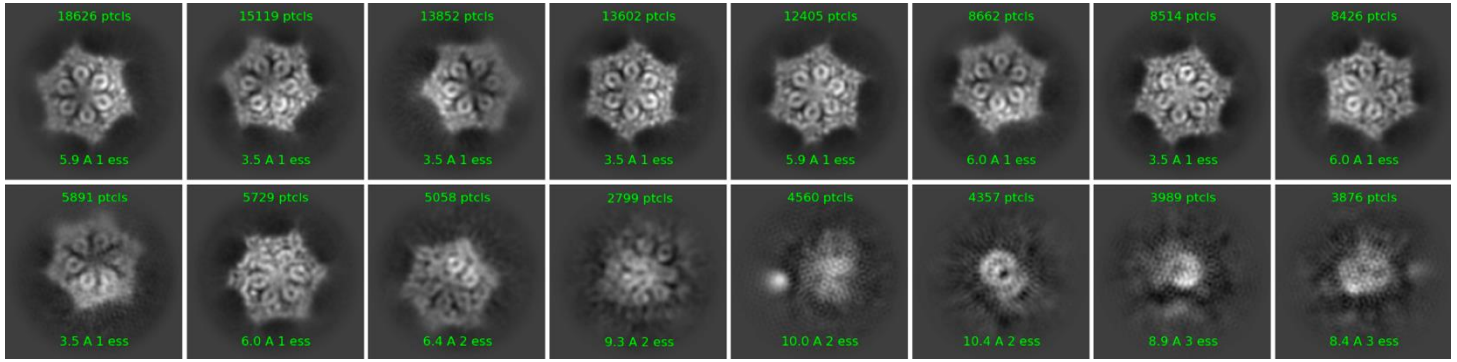
Only top and bottom views of the particles in 2D classes

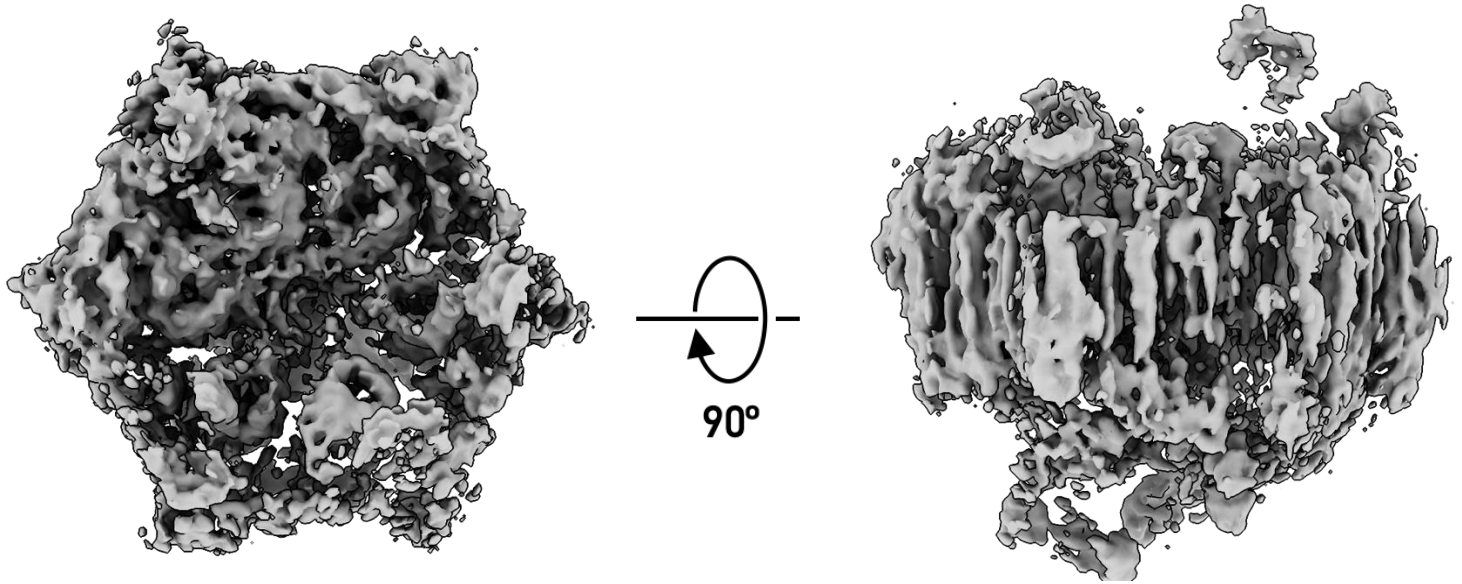




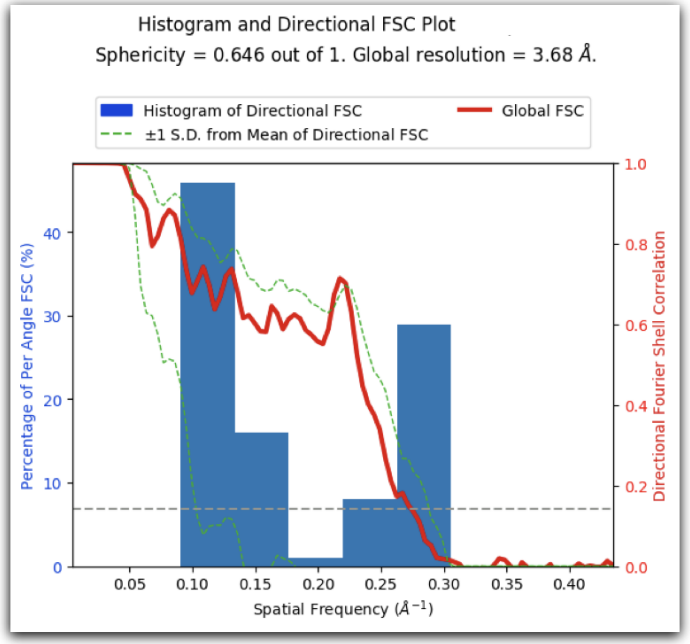
Preferred sample orientation on cryoEM grids

Only top and bottom views of the particles in 2D classes





Resolution anisotropy:
Estimated resolution differs depending on the angle

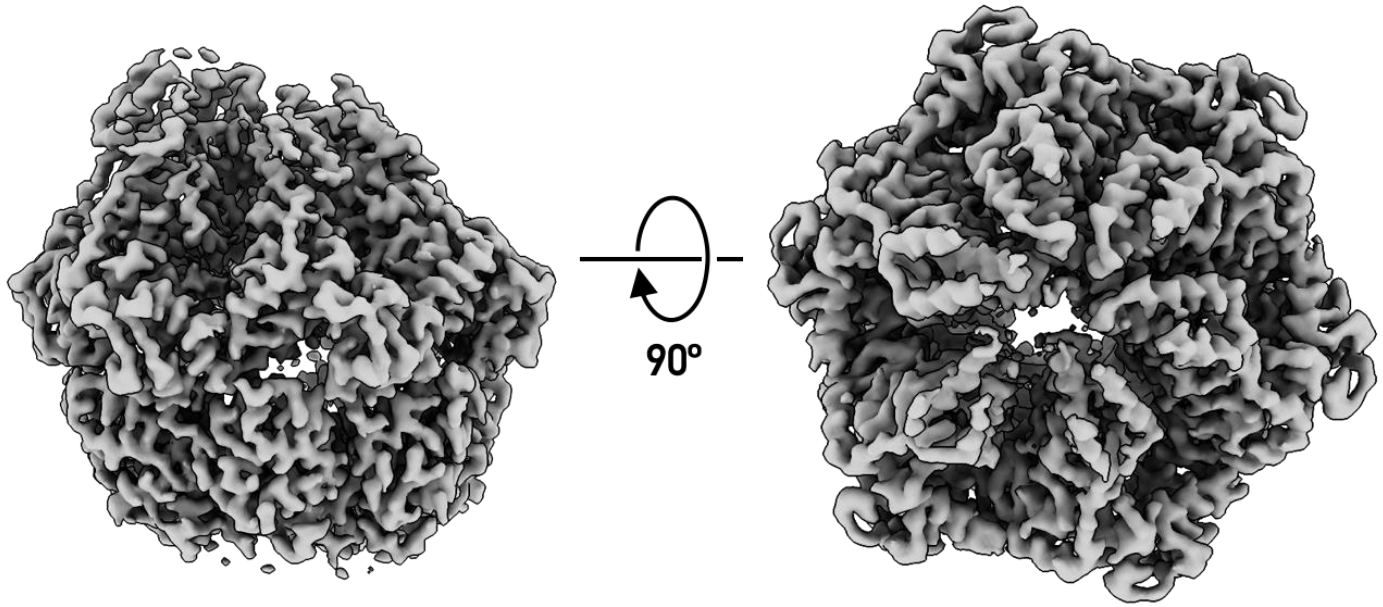


3dfsc.salk.edu

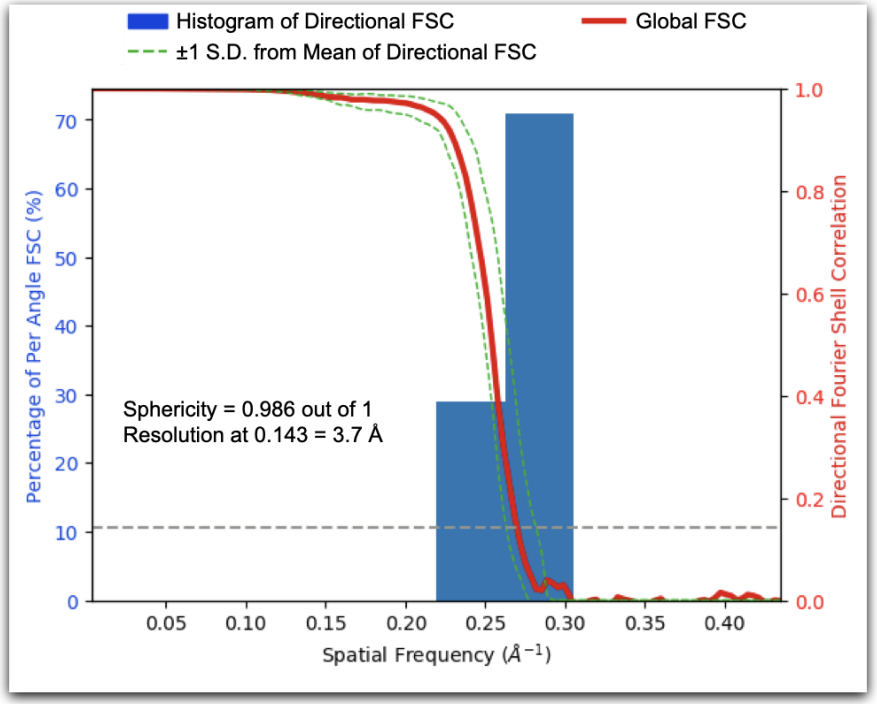
Shin et al., Nature Comm, 2021

Preferred sample orientation on cryoEM grids

- The same map reconstructed with sufficiently diverse particle views



Well-balanced resolution values



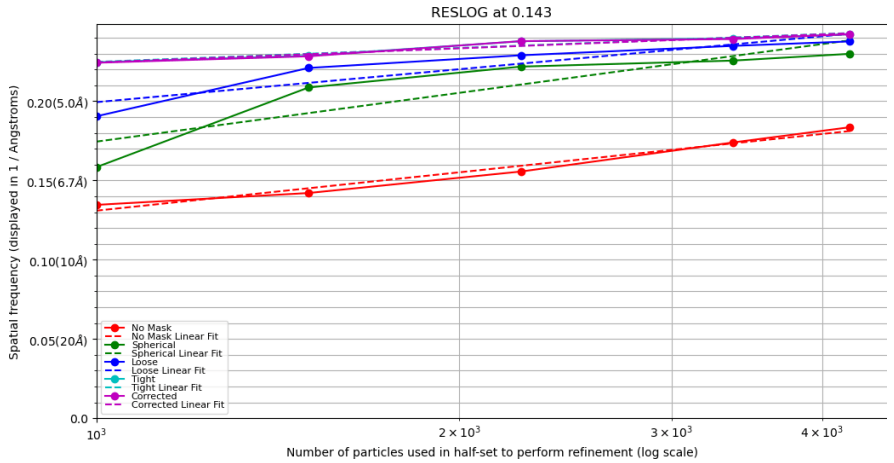
3dfsc.salk.edu

Preferred orientation issues can be addressed by:
- Surfactants (detergents)
- Using grids with carbon/graphene oxide support
- Chemical grid functionalization
- Plasma cleaning
- Imaging samples with stage tilt
- Complexing with antibodies

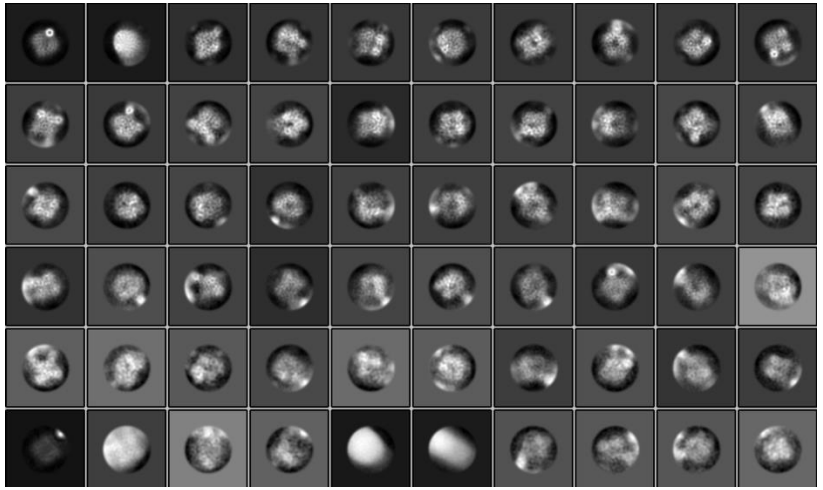
Shin et al., Nature Comm, 2021

Particle count and symmetry impact on resolution

- Greater number of particles generally improves the chances of achieving high res

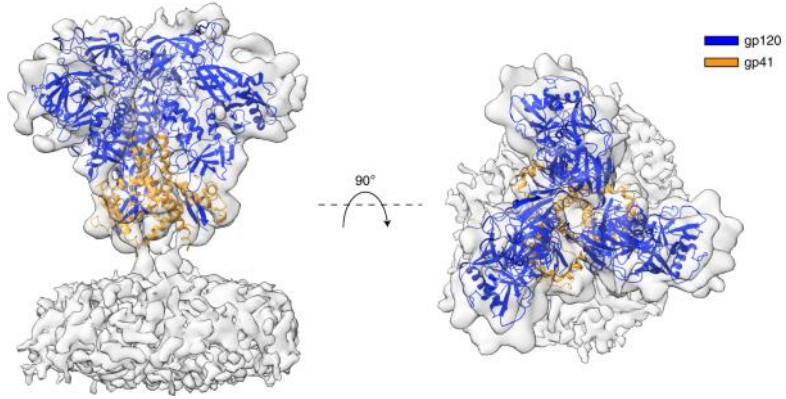


- However, this does not work for heterogeneous samples

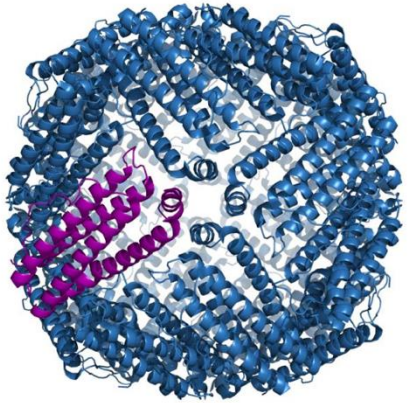


- Particle symmetry (C_x, D_x, T, O, I) artificially builds up the number of sub-particles and their view distribution

HIV glycoprotein (C3 sym)

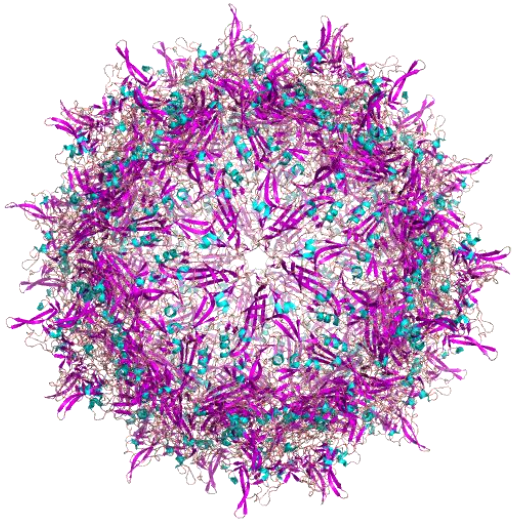


Apoferritin (Octahedral sym)



6 x 4-fold axes
8 x 3-fold axes
12 x 2-fold axes

AAV (Icosahedral sym)



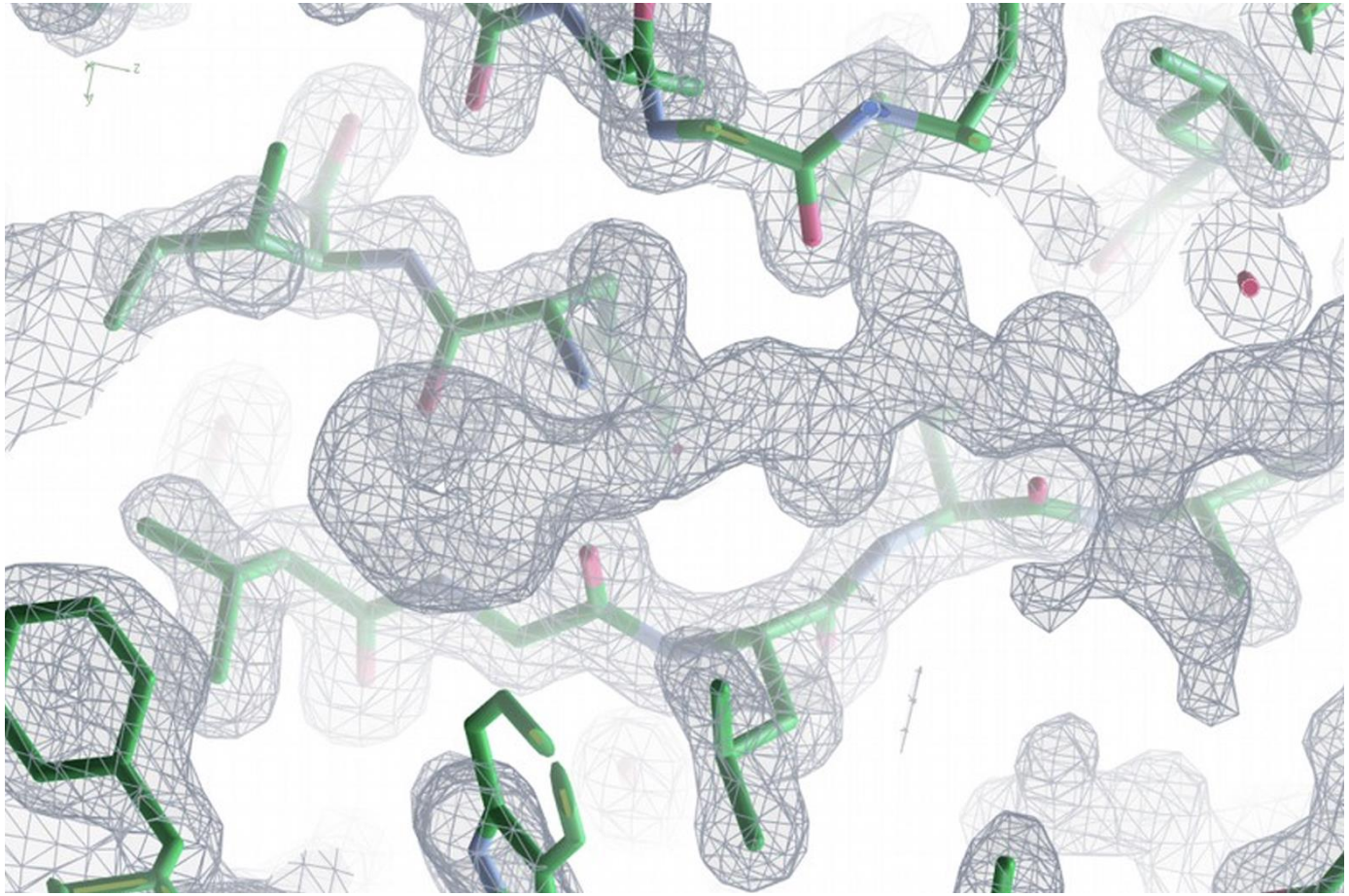
12 x 5-fold axes
20 x 3-fold axes
30 x 2-fold axes

Building and Evaluating Atomic Models

Molecular Interpretation of an EM density map

- frame alignment
- CTF estimation
- particle picking
- 2-D align/classify
- initial model
- 3D align/classify
- refinement
- particle polishing
- resolution estimation
- map sharpening
- **model building**

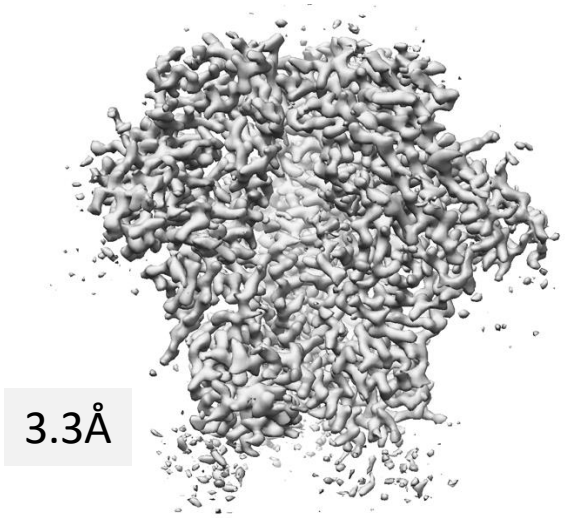
- The essence of model building and refinement is to approximate an atomic model that best recapitulates the reconstructed map

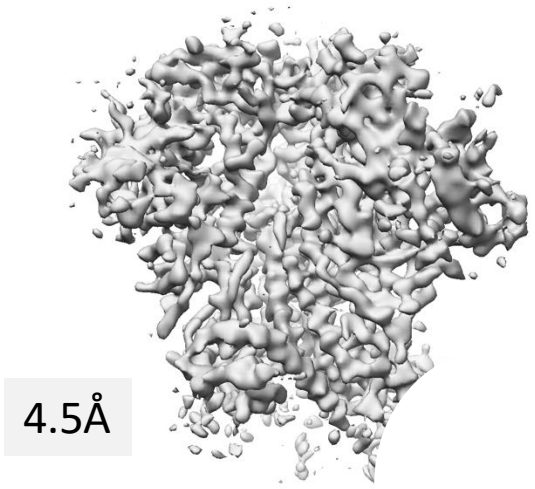


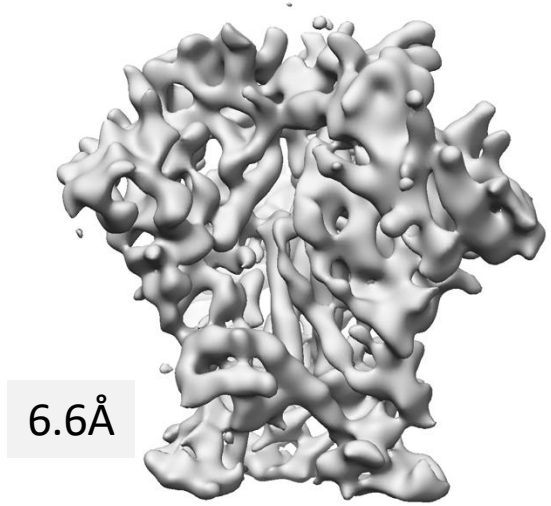
output: atomic model

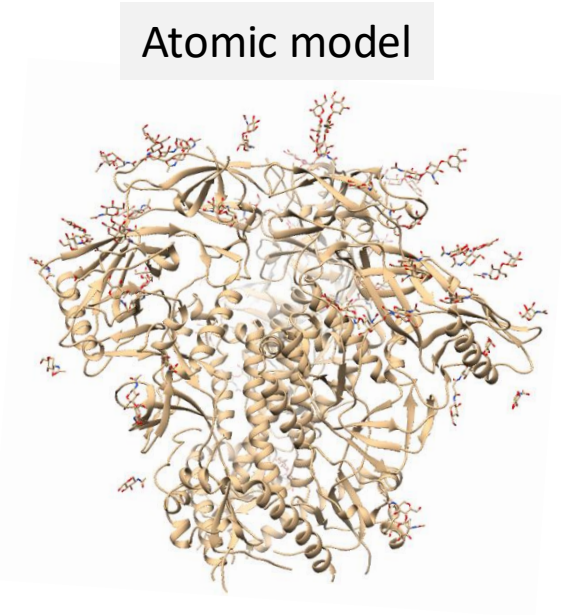
Should you proceed to model building?

- Decide if the map is of sufficient quality to relax a model
- The usual cutoff is ~4Å for maps that have well balanced local resolution
- Rigid body docking of structures/models is appropriate in lower res maps
- Hydrogens are typically not built unless you reach <1.2Å resolution









Should you proceed to model building?

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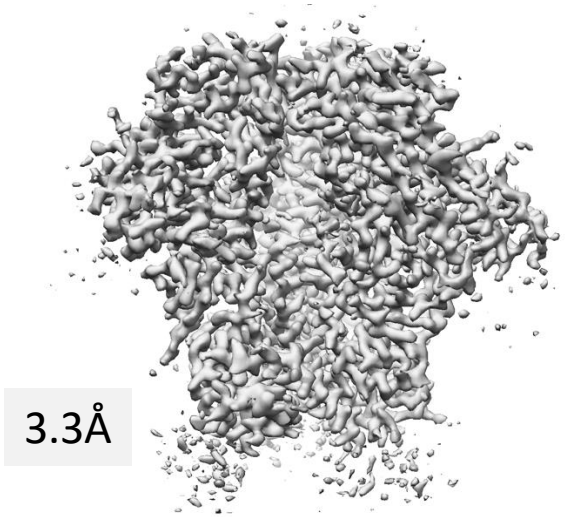
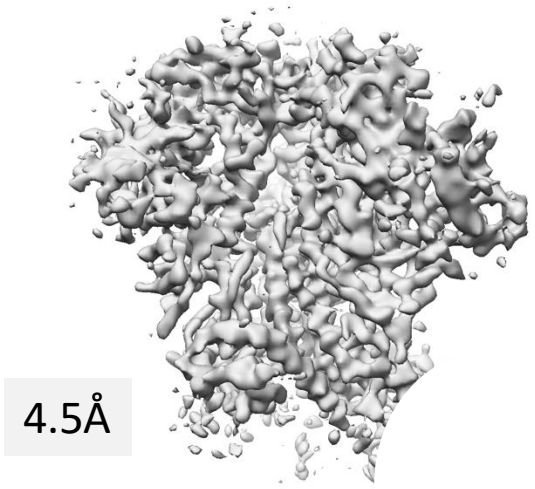
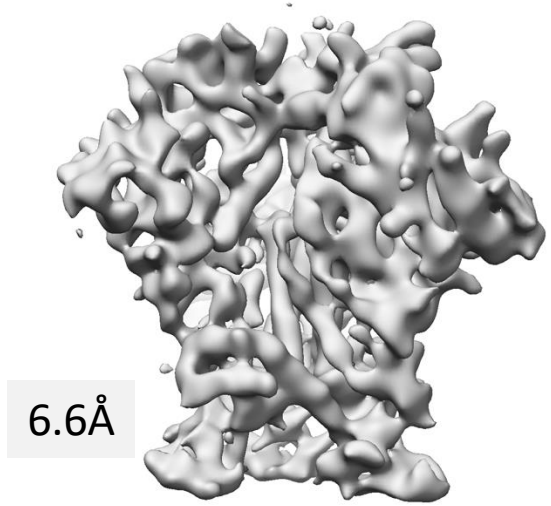
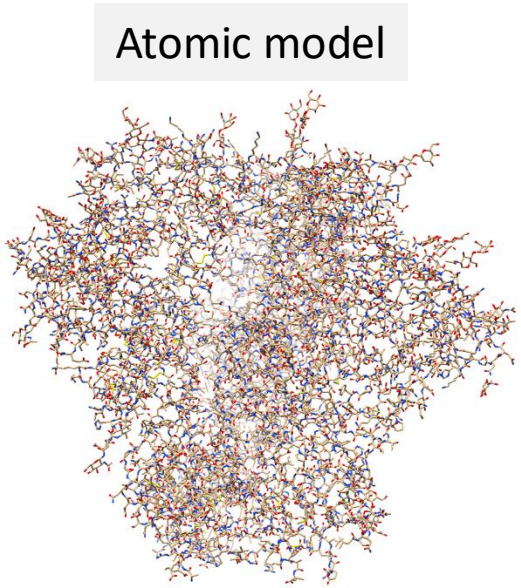


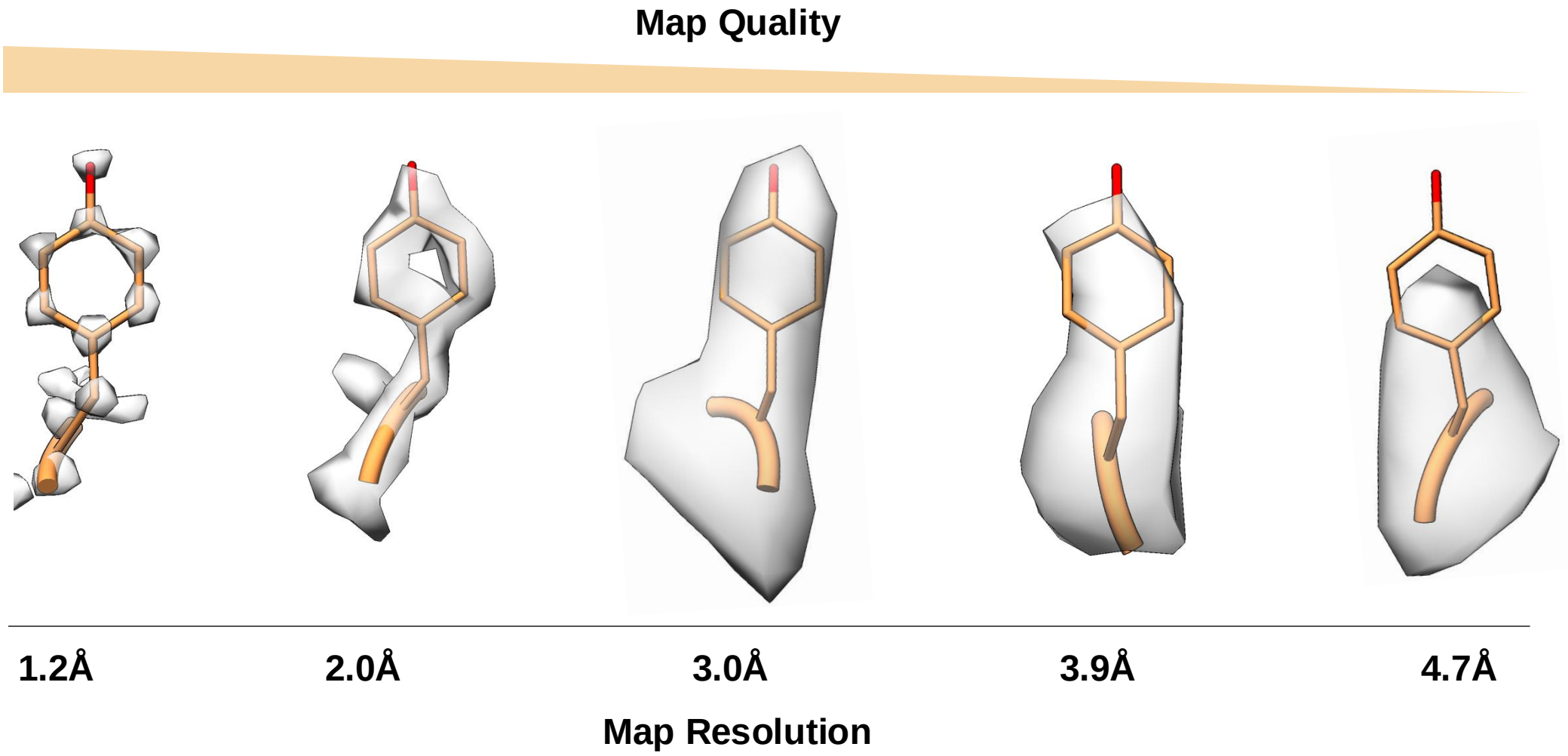






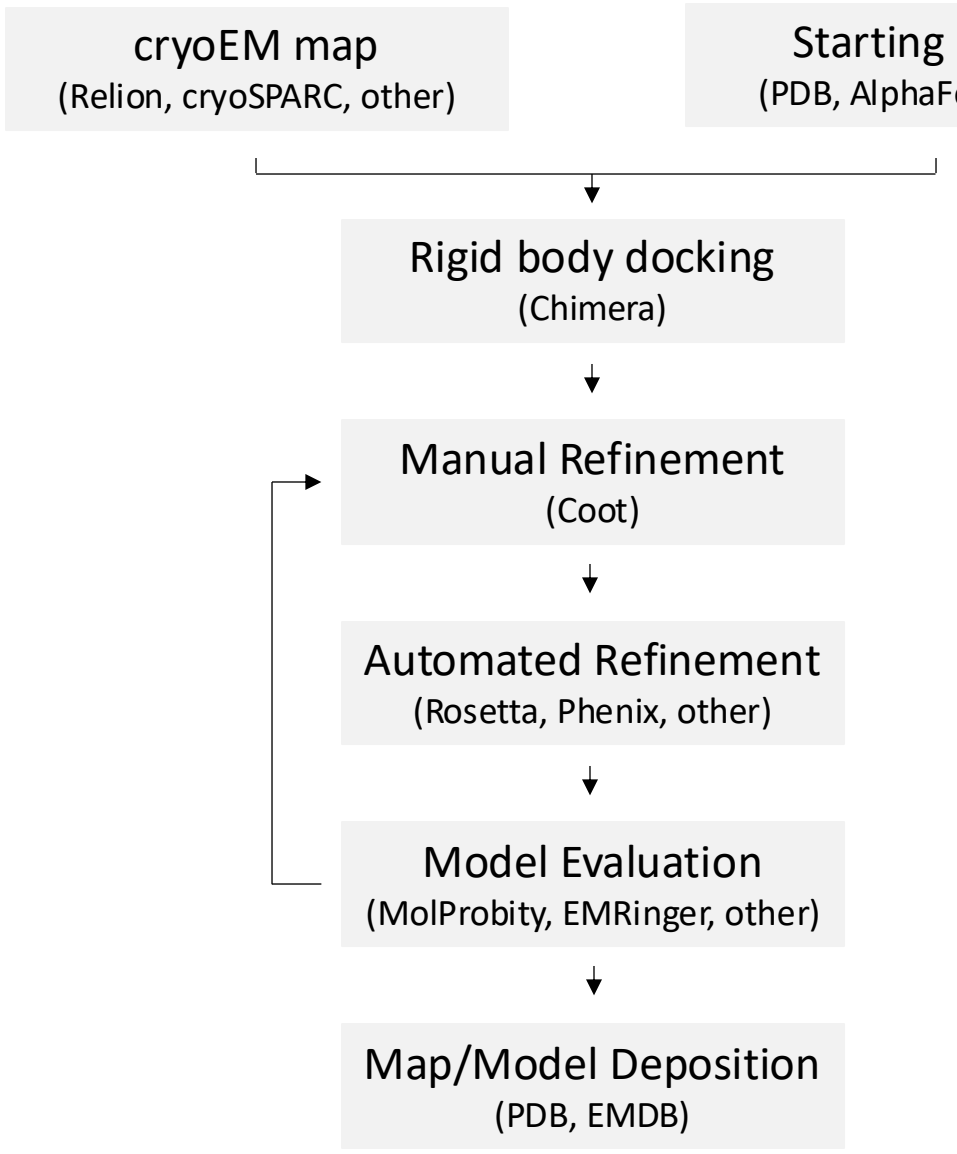
EM Map quality and observable model details

- Side-chain of residue Y28 of apoferritin at different EM map resolutions



PDB IDs: **7A6A, 6WX6, 5YI5, 6HPI, 4V1W**

Typical model building workflow



- **Iterative model improvement process until it meets the (contemporary) quality standards**

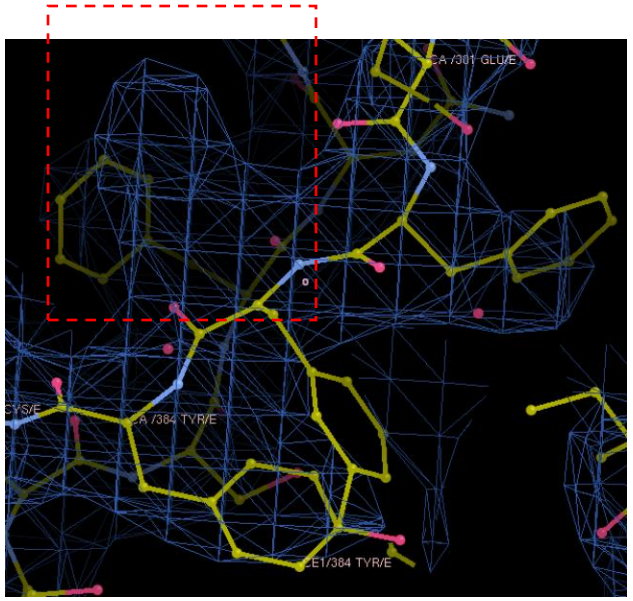
Example of reported model statistics

	My model
PDB ID	XXXX
Residues	1815
Amino-acids	1710
Carbohydrates	105
RMSD Bonds (4σ)	0.020
RMSD Angles (4σ)	1.677
Ramachandran	
Outliers (%)	0.00
Allowed (%)	1.43
Favored (%)	98.57
Rotamer outliers (%)	0.20
Clash score	1.09
Molprobity score	0.81
EMRinger score	2.59

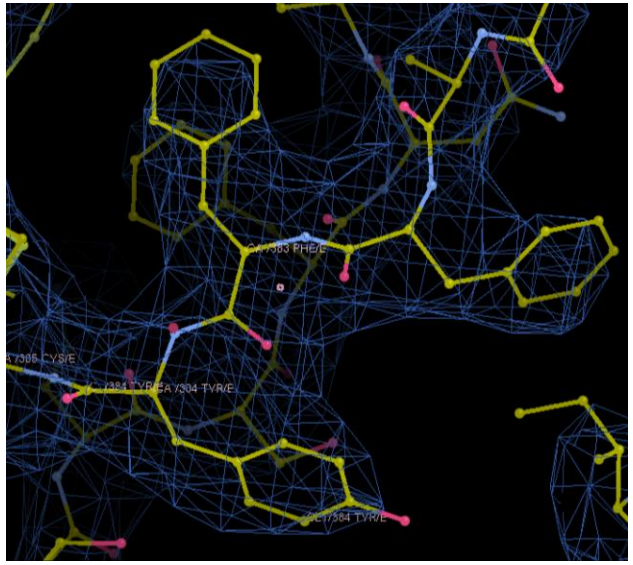
Manual model refinement in Coot

- Crystallographic Object-Oriented Toolkit (Coot) is a molecular-graphics application for model building and validation of biological macromolecules

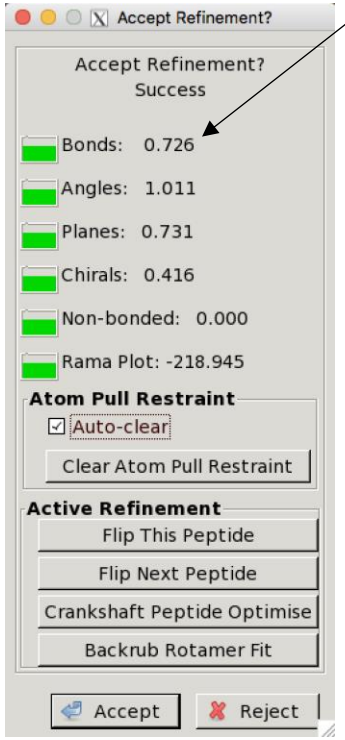
Poor fit
(Side chain clearly in the wrong place)



Good fit
(Side chain in the pocket)



$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$



Bond lengths

Bond angles

Bond planarity

Chiral centers

Other non-bonded molecules

Ramachandran

- Improving the agreement between the model and the map
- Optimizing the geometry on the level of main chain (e.g., Ramachandran angles) and side chains (e.g., rotamers)

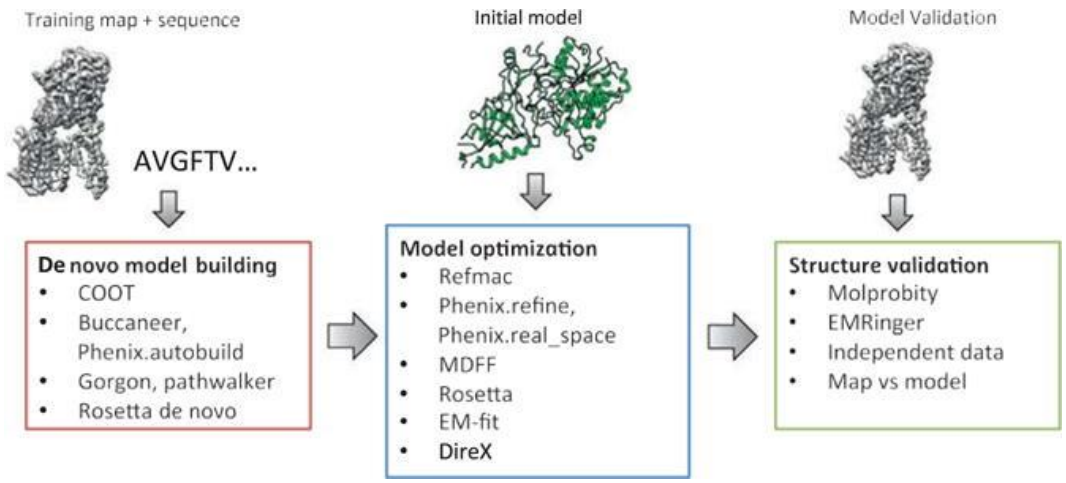
Emsley et al., Akta Crystallogr D, 2010

Automated model refinement

- Automated optimization of the atomic coordinates in the input model to (1) improve the agreement with the experimental data and (2) refine model geometry

- Weight factor (w) scales the relative contribution of the experimental data (i.e. model-to-map agreement)

- Common examples Rosetta, Phenix Real Space Refinement, Refmac



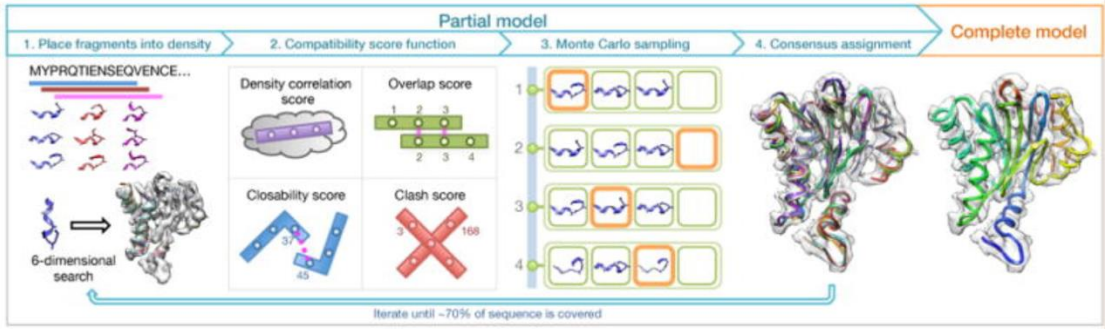
$$E_{total} = E_{geom} + w*E_{data}$$

The lower the resolution the more weight is added to correct geometry.

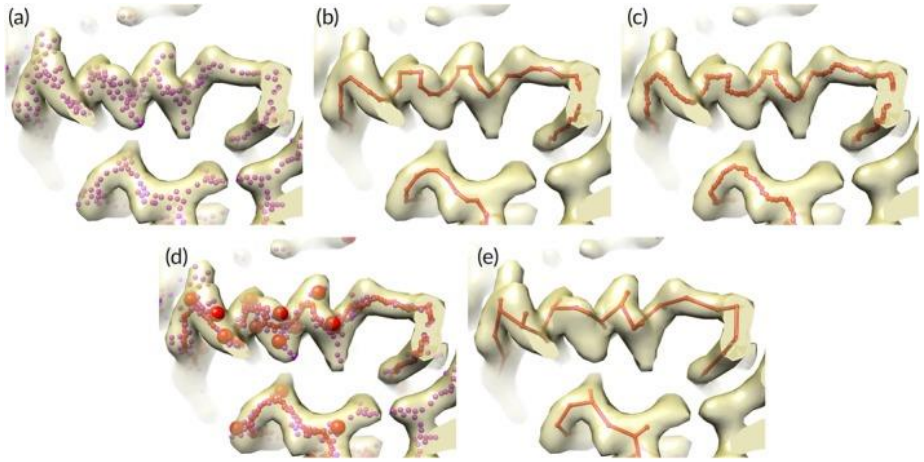
F. DiMaio and W. Chiu, 2018

Automated model refinement – New Tools

- Automated refinement from known sequence

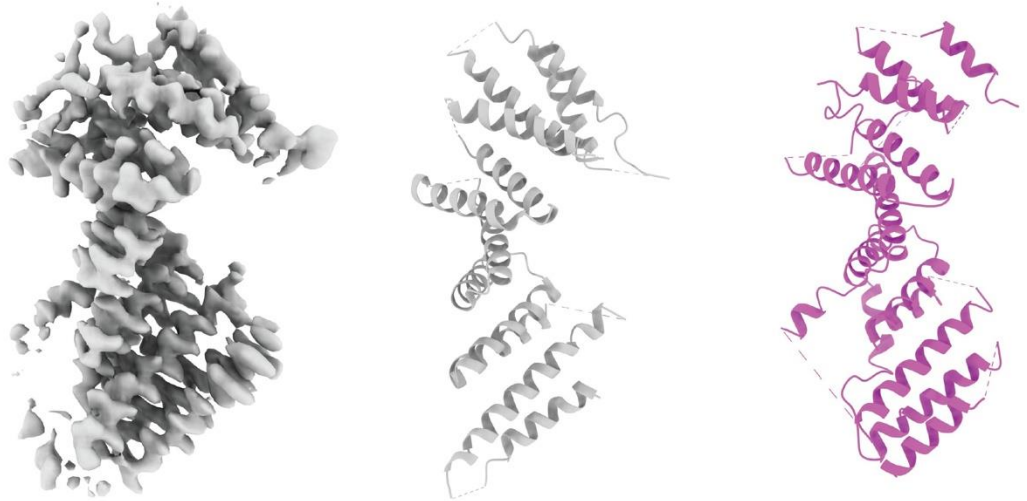


Wang et al., Nat Methods, 2015



Terwilliger et al., Protein Sci, 2020

- No sequence – no problem



Cryo-EM density (3.8Å) [EMD-24677]

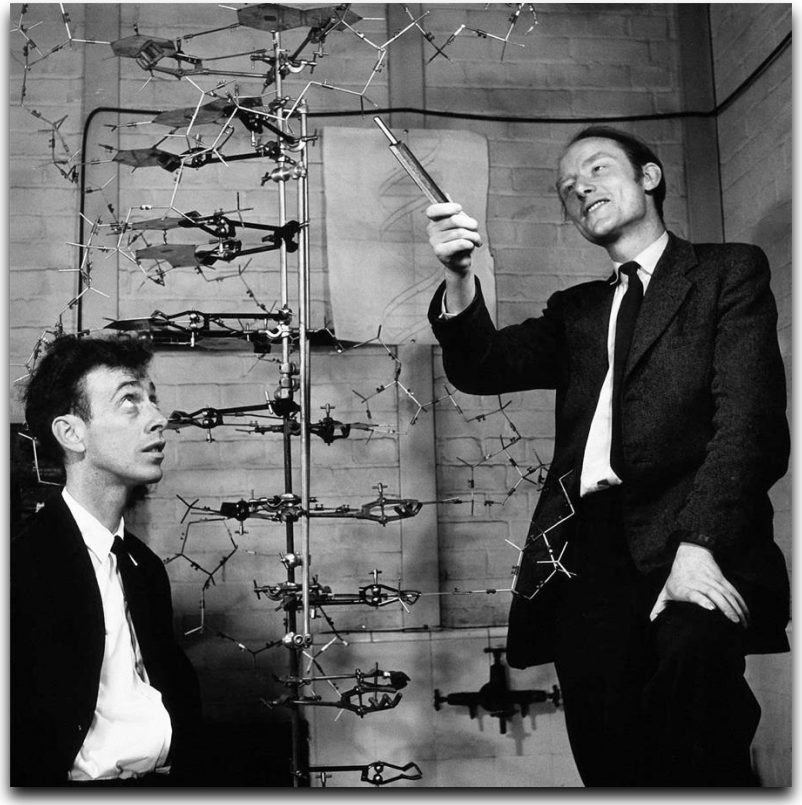
***De novo* manual build (2+ weeks) [PDB 7RSQ]**

Model Angelo (10 minutes)

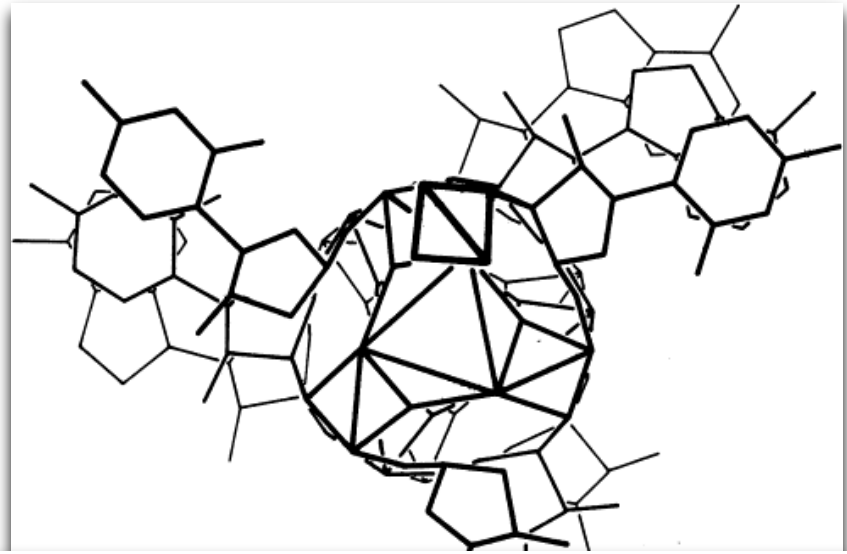
Jamali et al., Nature, 2024

- Automated model building and sequence prediction with the help of deep learning

Validation of cryoEM maps and models



Watson & Crick
Feb 1953



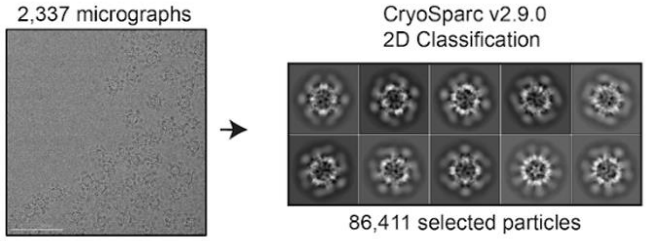
Pauling noted that the model accounted only "moderately well" for the x-ray data, and that the atomic positions were "probably capable of further refinement."

"A proposed structure for the nucleic acids."
Linus Pauling, PNAS, Feb. 1953

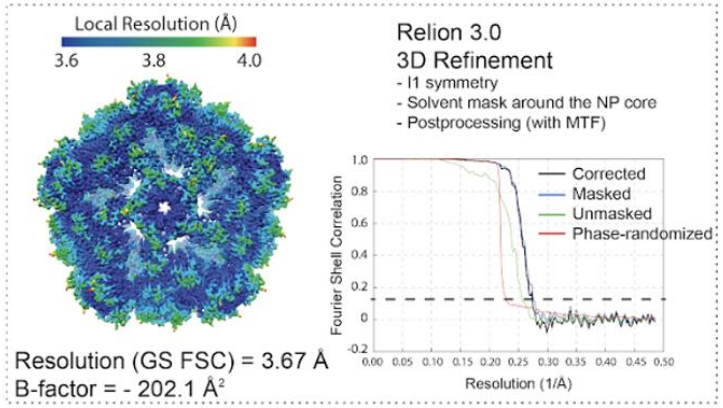
Validation of cryoEM maps and models

- Cryo-EM data is evaluated on 3 levels:

Data collection and map refinement
(refinement procedure, resolution, B-factor, angular distribution…)

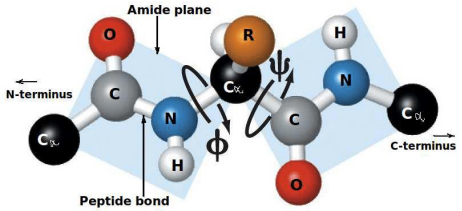


Data processing protocol

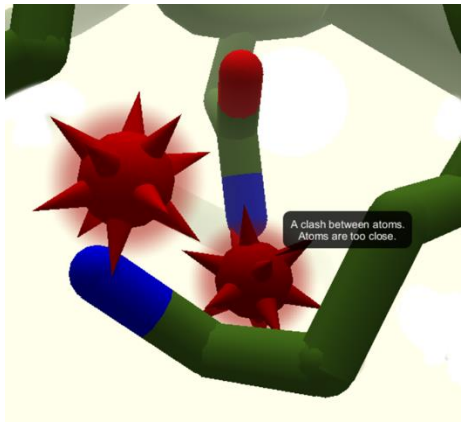


Relevant plots

The quality of refined model
(RMSD bonds, RMSD angles, Rama and Rotamer statistics, clash-score...)

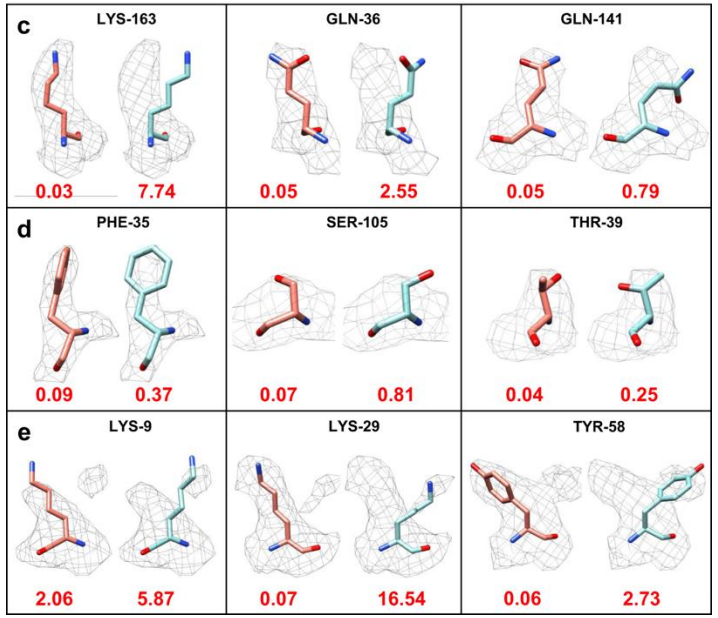


Bond lengths and angles



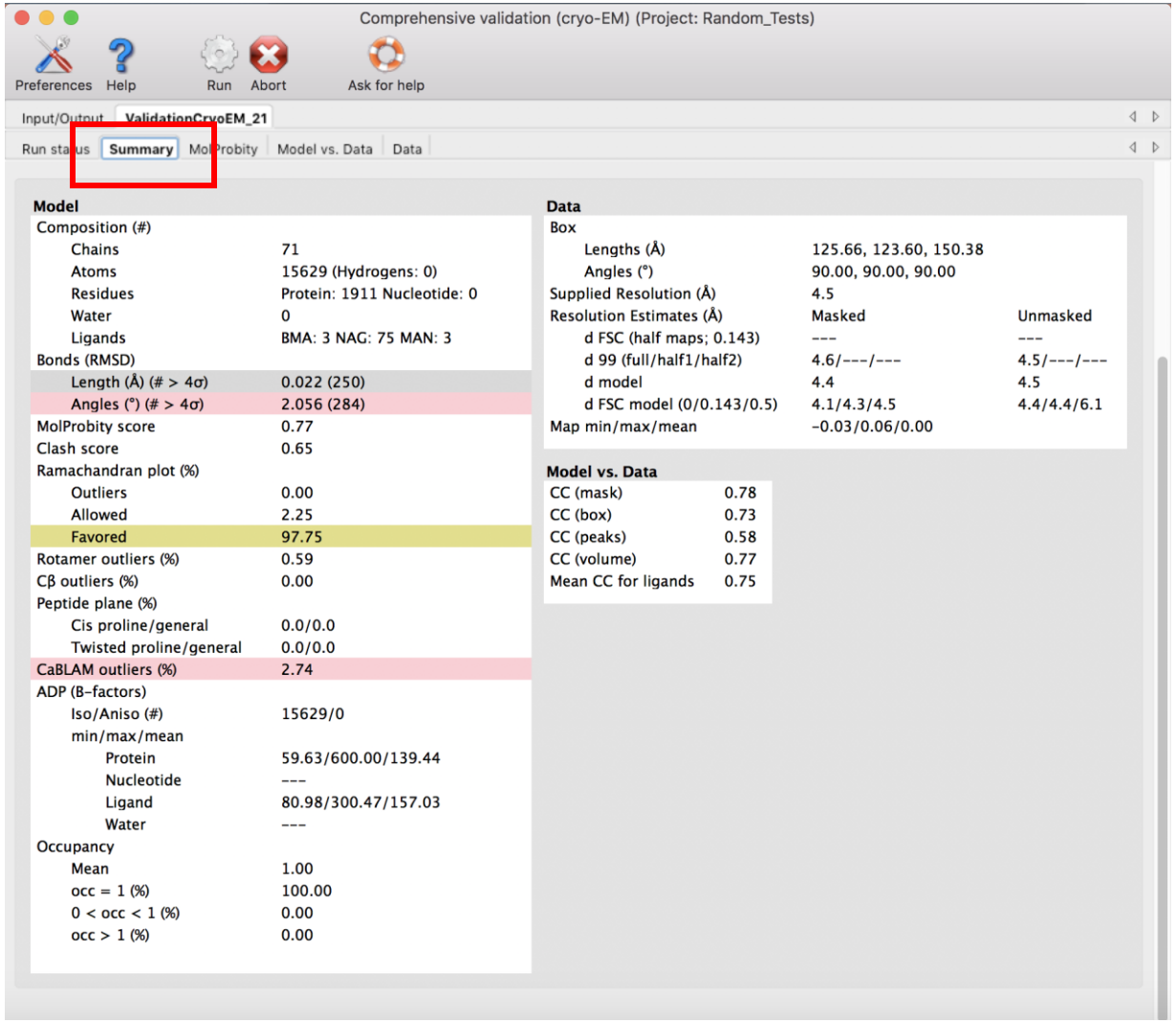
Absence of atom clashes

Model-to-map agreement
(EMRinger, Correlation Coefficients (CC) and Q-scores)



Global and local metrics

Comprehensive validation results in Phenix

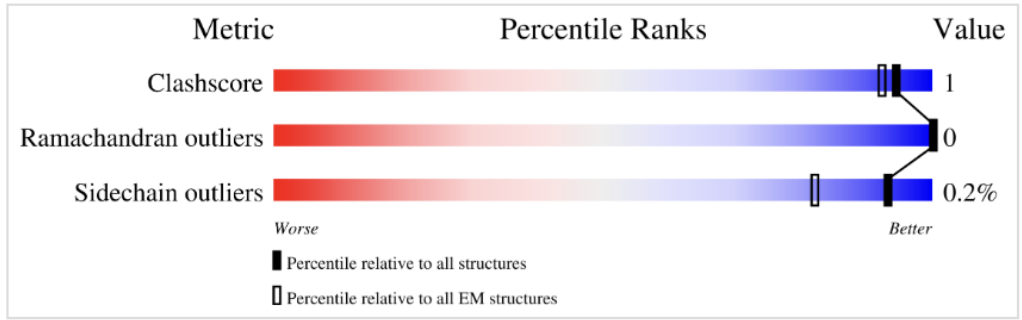


Example output of Comprehensive Validation

MolProbity analysis is used for validation of models and maps during the deposition to the Protein Data Bank (PDB) and Electron Microscopy Data Bank (EMDB)

MolProbity uses REDUCE and PROBE for all-atom contact analysis, RAMALYZE, ROTALYZE, DANGLE, SILK and SUITENAME for other criteria and KiNG for three-dimensional visualization of the structure and its validation markers

Example PDB-reported model quality metrics

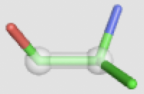


Evaluation of geometry – Individual outliers

Bond length and angle outliers that are >4 **σ** away from theoretical values.

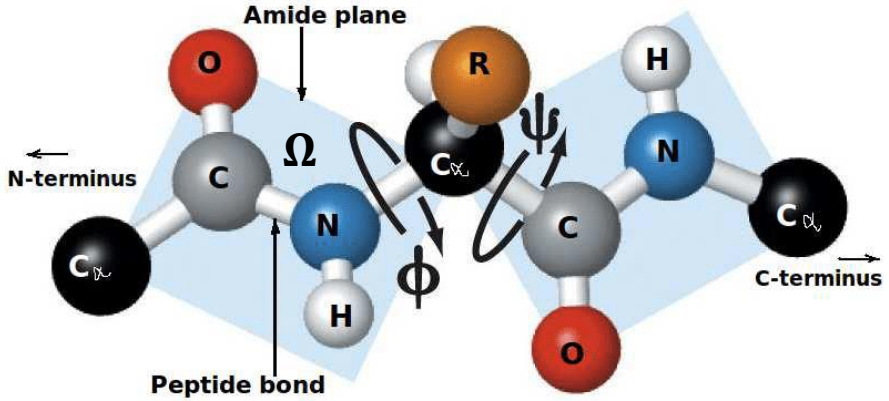
Bond length restraints

Number of restraints:	15381
RMS(deviation):	0.020
Max. deviation:	0.113
Min. deviation:	0.000
Number of outliers > 4σ:	18

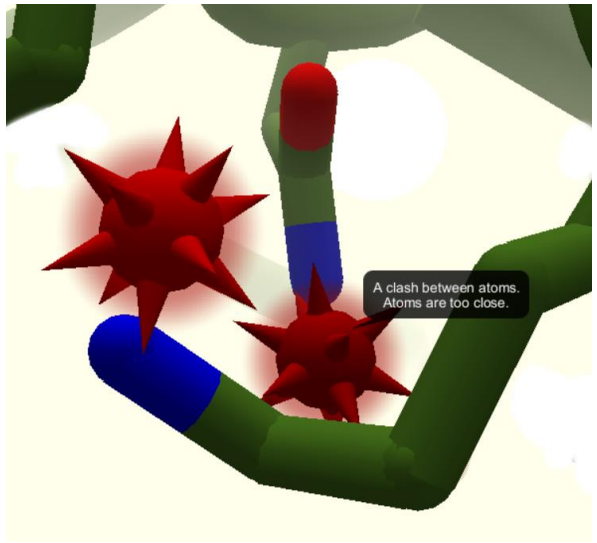


Atom 1	Atom 2	Ideal value	Model value	Deviation (sigmas)
A 374 HIS CB	A 374 HIS CG	1.497	1.416	5.8
C 374 HIS CB	C 374 HIS CG	1.497	1.417	5.7
E 374 HIS CB	E 374 HIS CG	1.497	1.417	5.7
C 479 TRP NE1	C 479 TRP CE2	1.370	1.320	4.5
A 479 TRP NE1	A 479 TRP CE2	1.370	1.321	4.5

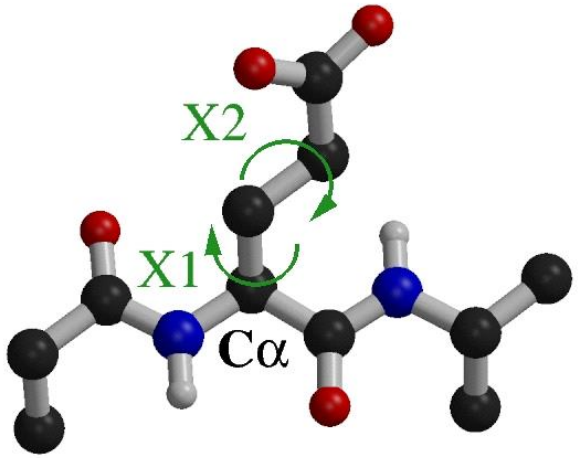
Peptide bond angle (**Φ**, **Ψ**, **Ω**) outliers from values predicted by Ramachandran plot for a given amino-acid.



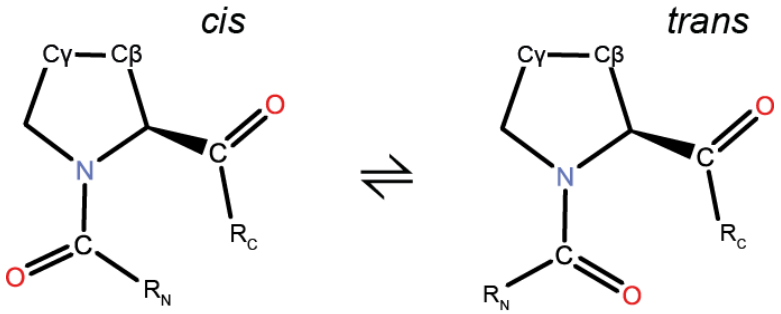
Clashes between 2 atoms that are >0.4Å



Side-chain outliers



Unusual features in the structure



Cis-Prolines are very rare (3.3% of all Pro residues)

Evaluation of geometry – Global parameters

- These global parameters reflect the overall quality of the model

	Sample model	
Residues	1815	Number of residues or atoms
Amino-acids	1710	
Carbohydrates	105	
RMSD Bonds (4σ)	0.020	RMSD of bond lengths that are > 4σ away from expected theoretical values
RMSD Angles (4σ)	1.677	RMSD of bond angles that are > 4σ away from expected theoretical values
Ramachandran		
Outliers (%)	0.00	Ramachandran statistics for the entire model
Allowed (%)	1.43	
Favored (%)	98.57	
Rotamer outliers (%)	0.20	Side-chain geometry outliers
Clash score	1.09	Total number of clashes (overlap >0.4Å) per 1000 atoms
Molprobity score	0.81	MolProbity Score is an overall indicator of model quality

```
MPscore = 0.426 *ln(1+clashscore) +
          0.33 *ln(1+max(0, rota_out|-1)) +
          0.25 *ln(1+max(0, rama_iffy|-2)) +
          0.5
```

MolProbity score is a log-weighted combination of the clashscore, percentage Ramachandran not favored and percentage bad side-chain rotamers, giving one number that reflects the crystallographic resolution at which those values would be expected.

Evaluation of geometry – Global parameters

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Ramachandran	
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Rotamer outliers (%)	0.20
Clash score	1.09
Molprobity score	0.81

Number of residues or atoms

RMSD of bond lengths that are > 4σ away from expected theoretical values **(should be <0.01)**

RMSD of bond angles that are > 4σ away from expected theoretical values **(should be <1)**

Ramachandran statistics for the entire model **(0 outliers, >95% favored)**

Side-chain geometry outliers **(0 outliers)**

Total number of clashes (overlap >0.4Å) per 1000 atoms **(should be below 10)**

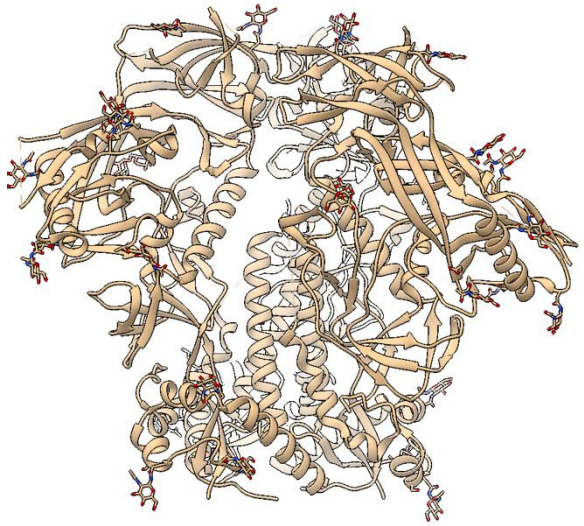
MolProbity Score is an overall indicator of model quality **(should be lower than map resolution)**

```
MPscore = 0.426 *ln(1+clashscore) +
          0.33 *ln(1+max(0, rota_out|-1)) +
          0.25 *ln(1+max(0, rama_iffy|-2)) +
          0.5
```

MolProbity score is a log-weighted combination of the clashscore, percentage Ramachandran not favored and percentage bad side-chain rotamers, giving one number that reflects the crystallographic resolution at which those values would be expected.

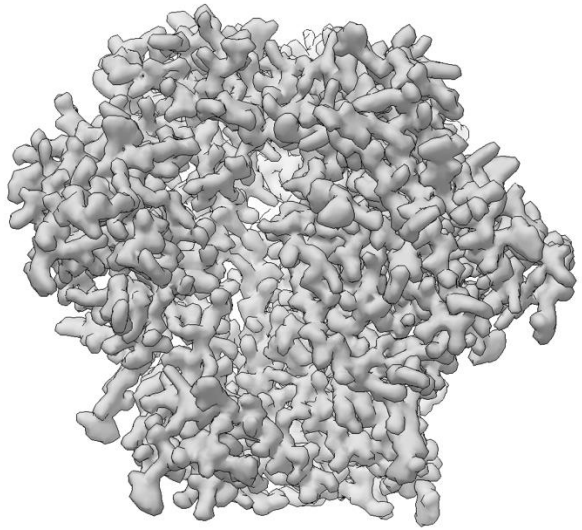
Model-to-map fit analysis using cross-correlation (CC)

Model



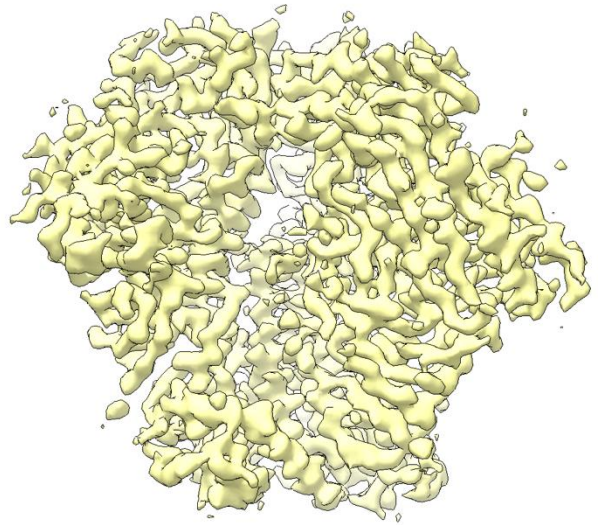
Transform

Calculated map



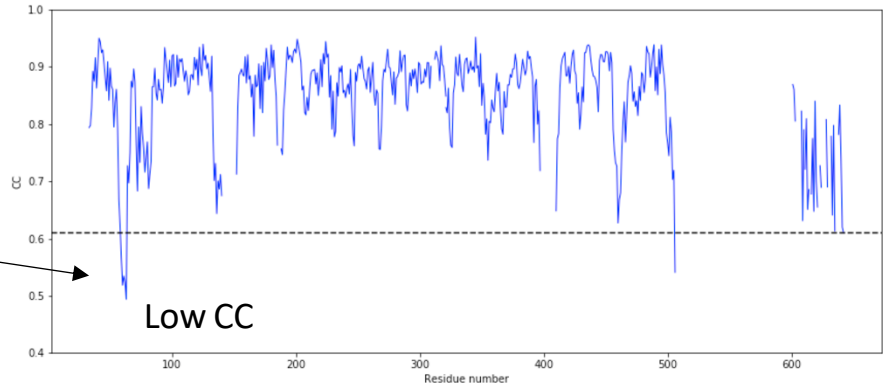
CC

Experimental map



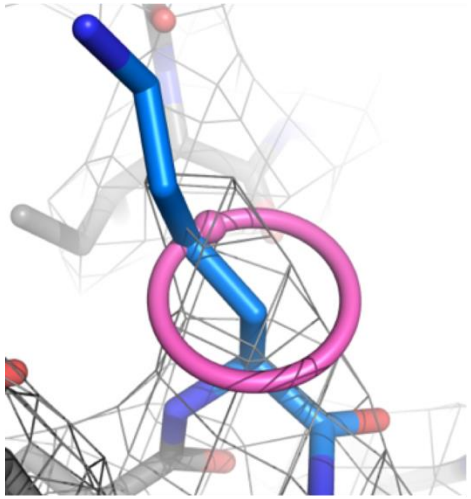
- Global and local map-to-model correlation
- Not very sensitive to side-chain positions
- Identify problematic regions in the map

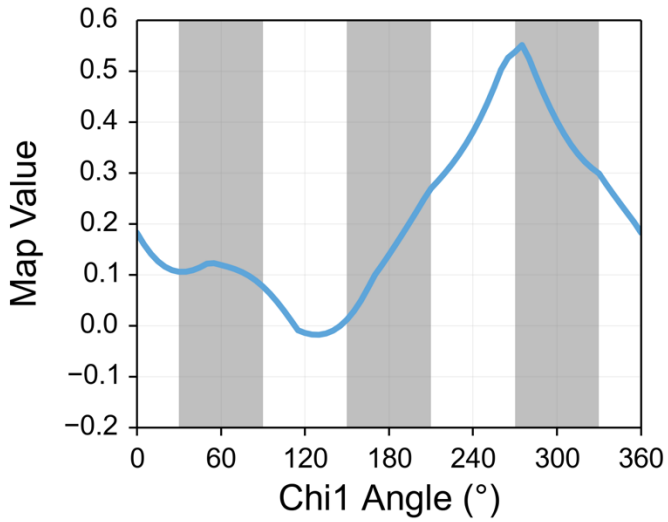
CC for each amino-acid in the model



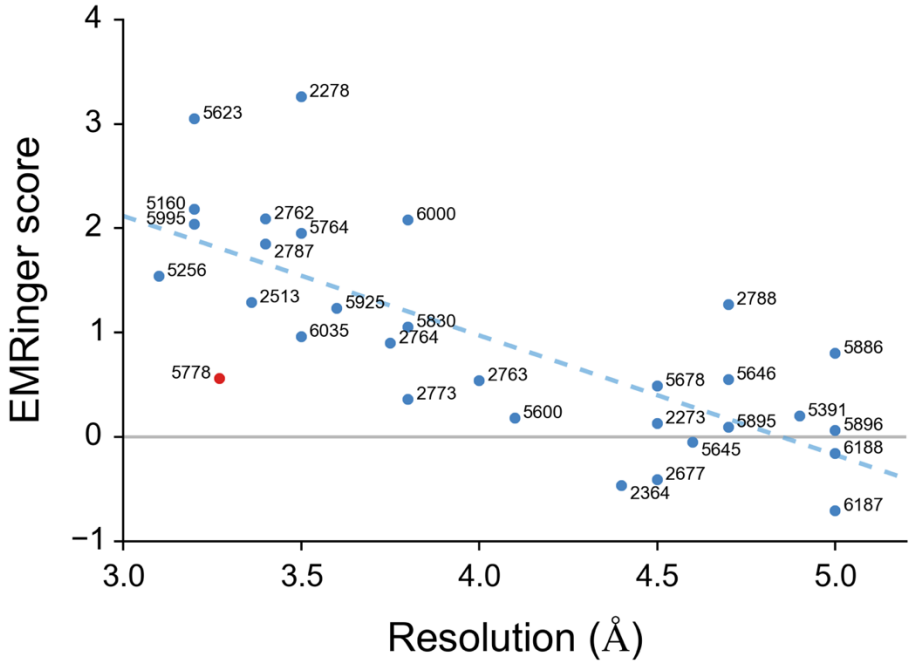
Model-to-map fit analysis using EMRinger scores

- EMRinger measures the density as Cγ is rotated around Chi-1
- EMRinger reports on whether the density peak is positioned at a rotameric angle





- The score decreases with resolution due to loss of side chain densities



Barad *et al*. **Nature Methods** 2015

Finalizing model refinement

cryoEM map
(Relion, cryoSPARC, other)

Starting model
(PDB, AlphaFold, other)

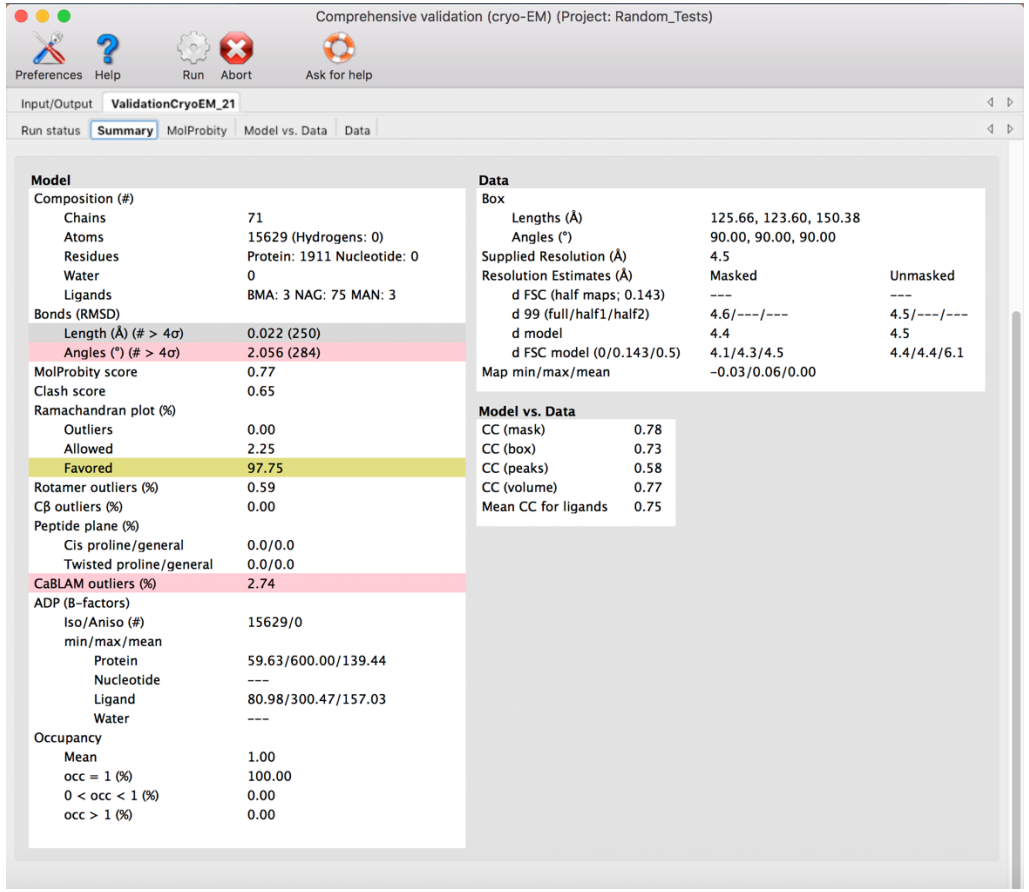
Rigid body docking
(Chimera)

Manual Refinement
(Coot)

Automated Refinement
(Rosetta, Phenix, other)

Model Evaluation
(MolProbity, EMRinger, other)

Map/Model Deposition
(PDB, EMDB)

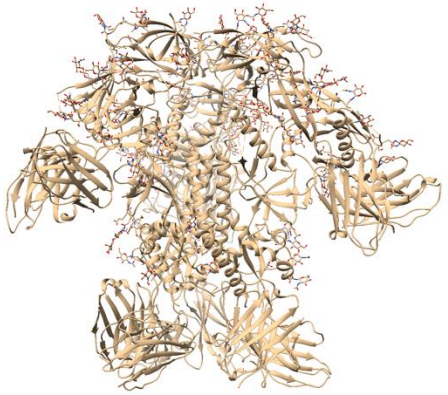


- Make sure all issues are resolved
- Make sure the metrics are within acceptable range
- Double-check the model-to-map fit manually

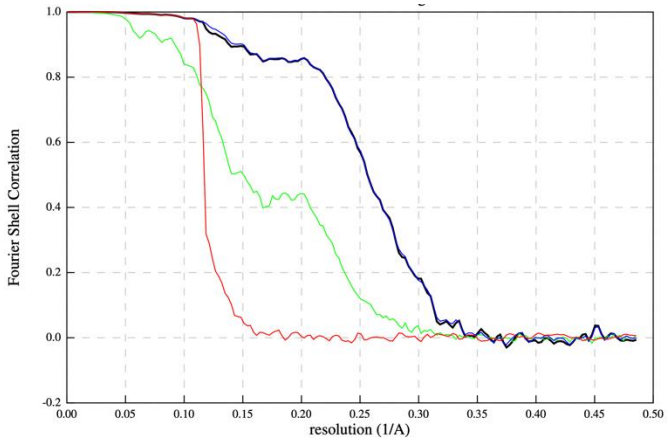
What data is required for deposition?

Cryo-EM	Negative Stain EM
Final postprocessed map	Final map
Nice image of the map	Nice image of the map
Half_map 1	Half_map 1
Half_map 2	Half_map 2
Mask used for postprocessing	
FSC.xml file	FSC.xml file*
PDB model *	

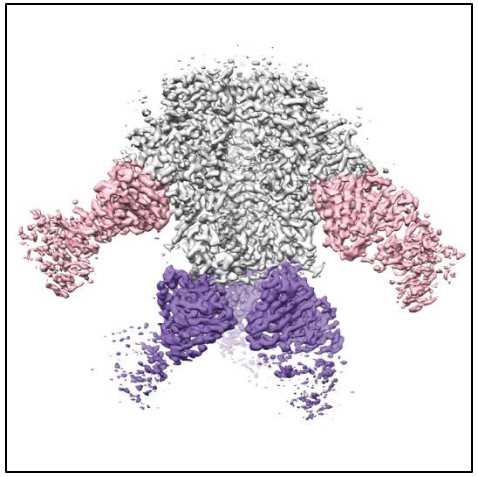
Model



FSC curve

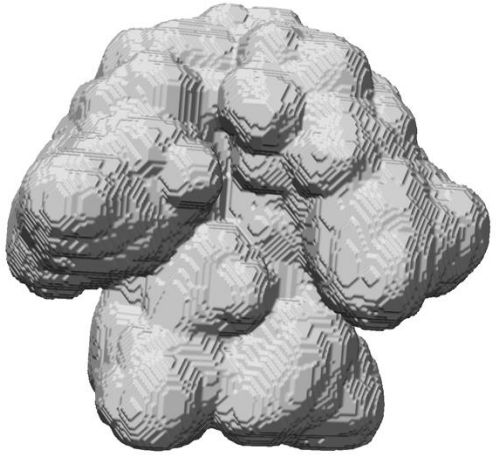


Image

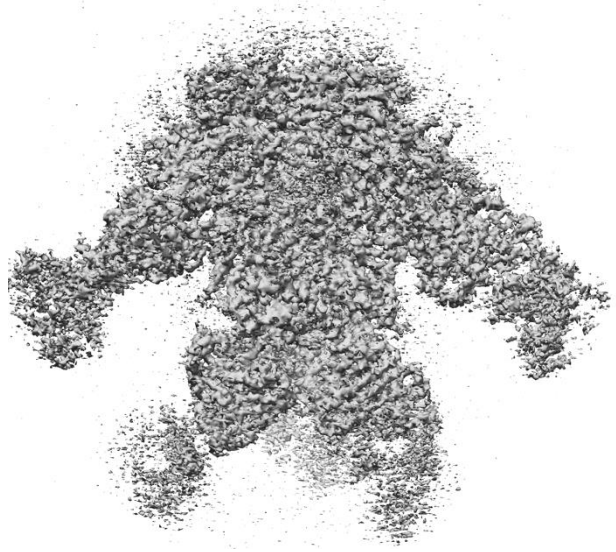


Map

Mask



Half-Map-1



- Fix any problems that the PDB validation server detects
- Save a copy of the validation report as you will need it for paper deposition

What do you need to report in the manuscript

Cryo-EM data collection statistics

	My Data
Microscope	Talos Arctica
Voltage (kV)	200
Detector	Gatan K2 Summit
Recording mode	Counting
Magnification	36,000 X
Movie micrograph pixel size	1.15
Dose rate ($e^-/Å^2/s$)	4.39
No. of frames per movie micrograph	45
Frame exposure time (ms)	250
Movie micrograph exposure time (s)	11.25
Total dose ($e^-/Å^2$)	49.39
Nominal under focus range (µm)	0.6 – 2.0
Number of movie micrographs	798

Cryo-EM data processing

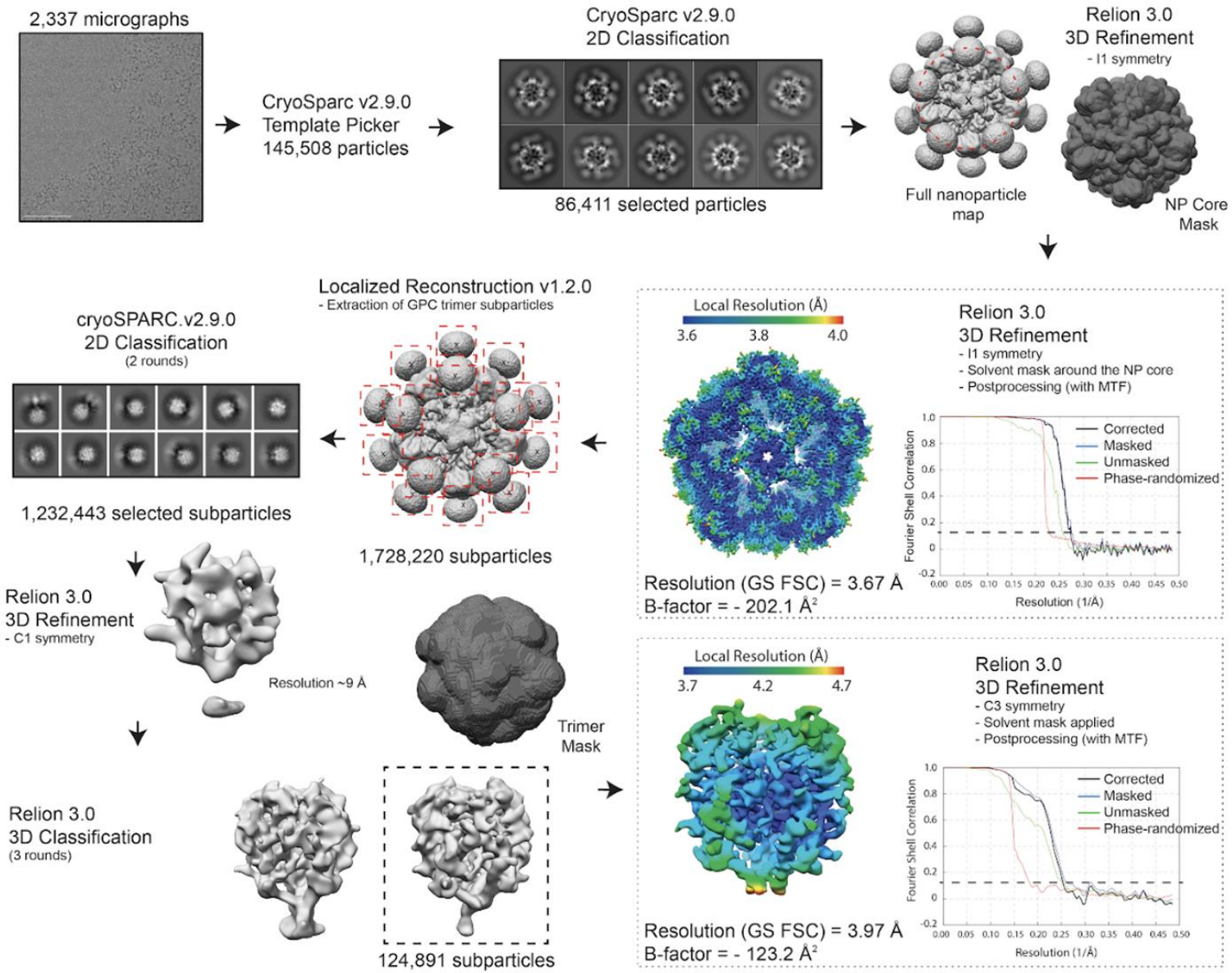
	My map
EMDB ID	YYYYY
Number of molecular projection images in map	84,435
Symmetry	C3
Map resolution (Å)	4.14
Map sharpening B-factor	-105.4

Model refinement statistics

	My model
PDB ID	XXXX
Residues	1815
Amino-acids	1710
Carbohydrates	105
RMSD Bonds (4σ)	0.020
RMSD Angles (4σ)	1.677
Ramachandran	
Outliers (%)	0.00
Allowed (%)	1.43
Favored (%)	98.57
Rotamer outliers (%)	0.20
Clash score	1.09
Molprobity score	0.81
EMRinger score	2.59

Journal-specific requirements may also apply.

Supplement Figure with examples of data and the processing workflow



<u>The most relevant info:</u>

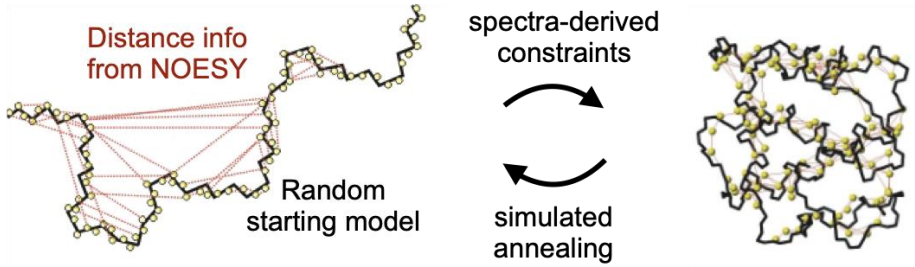
- Illustration of processing steps
- Programs used (if multiple)
- Sample micrographs
- Micrograph count
- Particle count through processing
- Information on imposed symmetry
- Sample 2D classes
- Intermediate reconstructions
- Resulting maps
- Masks used for 3D refinement/FSC
- FSC plots
- Local resolution plots
- Angular distribution plots

Brouwer, Antanasijevic *et al.* **Cell Host & Microbe,** 2022

How does EM compare to other methods for structure determination?

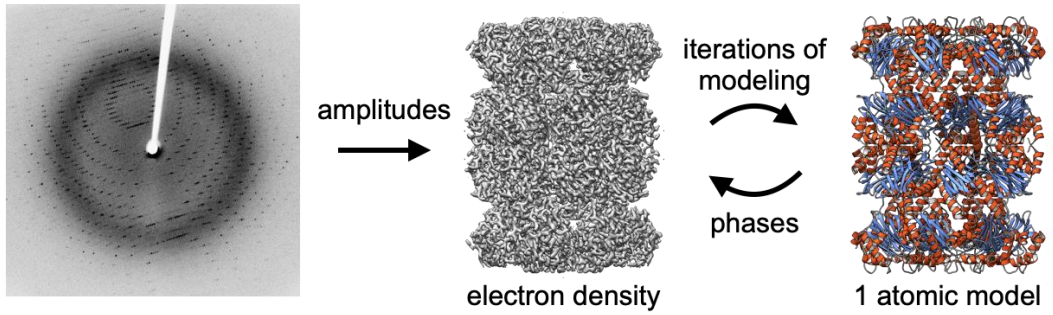
Methods for determining biomolecule structures

NMR



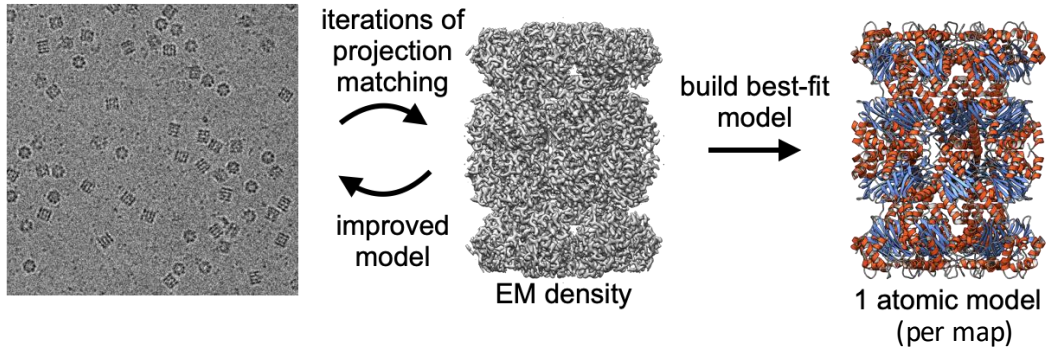
- **Versatile tool for studying protein structure and dynamics**
- Computationally light
- Full structural analysis limited to smaller proteins (<50kDa)
- Requires isotopic labeling
- Results in model ensemble

X-ray



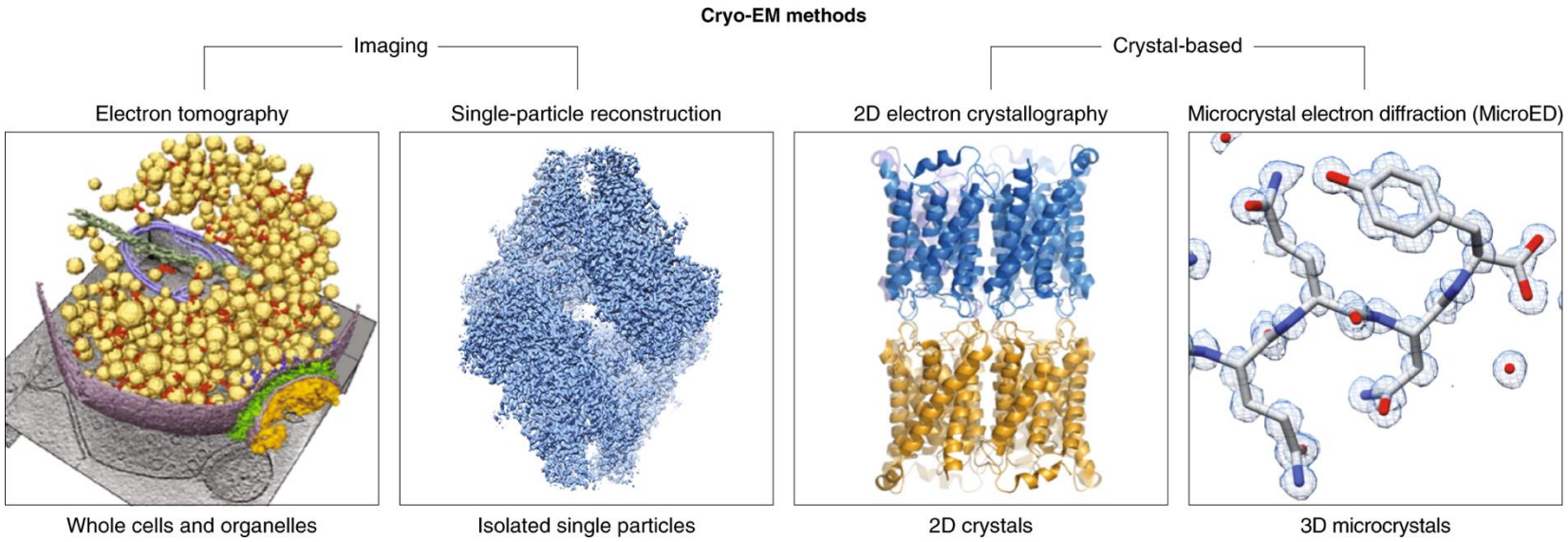
- Gold-standard method for solving protein structures
- **Not limited in size or achievable resolution**
- Computationally light
- Requires highly homogenous, crystallizable sample
- Requires screening of crystallization conditions
- Phase problem
- Results in a single model

CryoEM



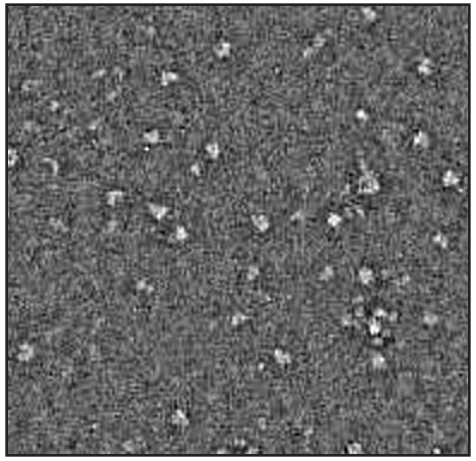
- Versatile tool for studying protein assembly, structure, dynamics
- Limited to proteins >40kDa
- No requirement for protein labeling
- **Does not require homogenous samples**
- Grid preparation procedure requires screening
- Real space imaging – no phase problem
- Can be used to study protein dynamics
- Can be expanded to larger assemblies (e.g., viruses and cells)
- Results in 1 model per map
- Computationally heavy (TBs of data + requirement for GPU processing)

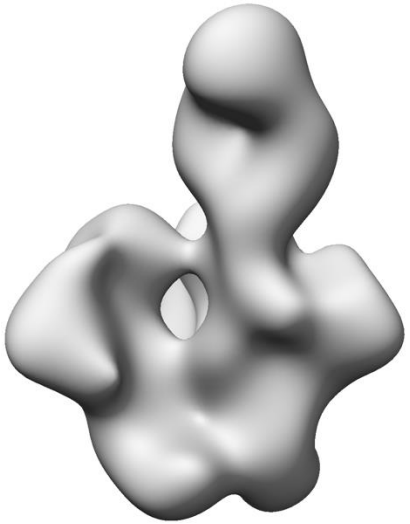
Electron microscopy applications



Single particle analysis in transmission electron microscopy

Negative stain EM

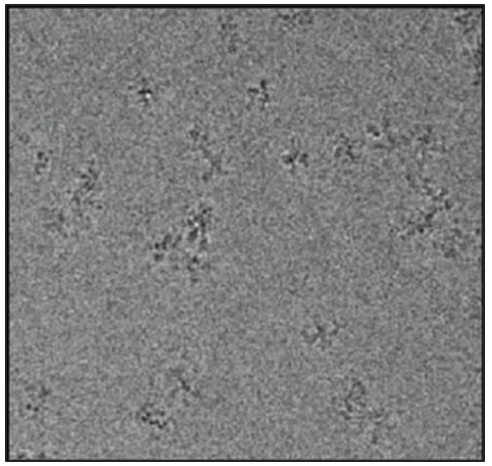


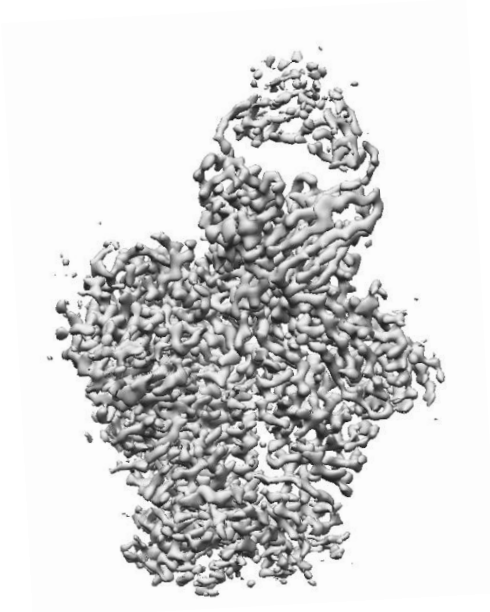


Pros:
- Straightforward & quick to prepare and image (~10 minutes from sample in tube to an image on microscope)
- Data collection & image analysis is fast (on the fly)
- Domain-level information

Cons:
- Limited resolution - only domain architecture if discernible
- Dilution (10 - 50 nM) may cause multi-component samples to dissociate

cryoEM





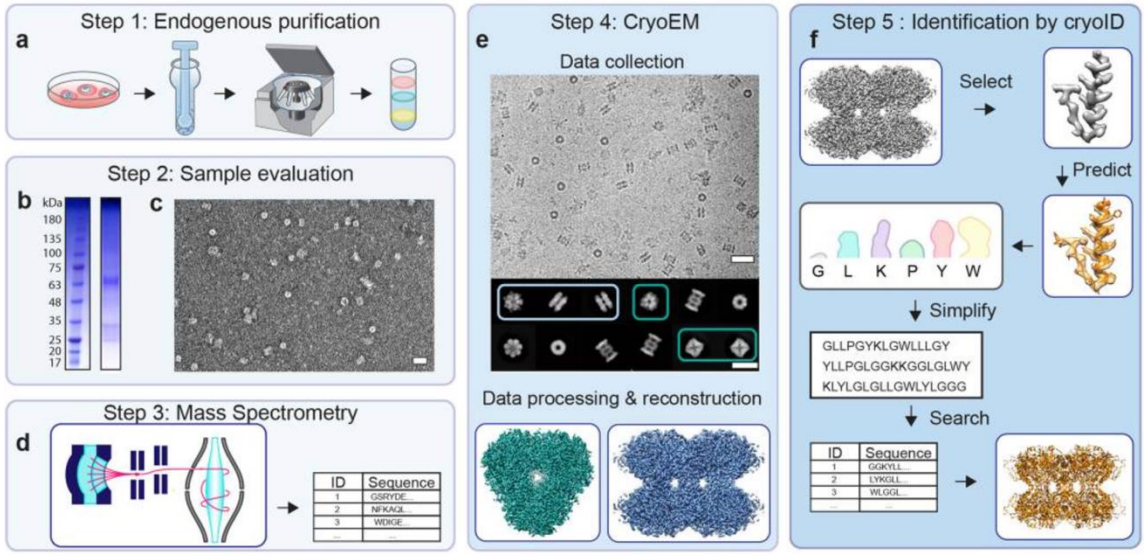
Pros:
- Enables (but doesn't guarantee!) high resolution structure determination without crystallization
- Multiple states can be resolved from a single sample/dataset

Cons:
- Low contrast technique, requires lots of images ($$$), low throughput
- Sample preparation is more complicated than negative stain
- Size and flexibility limitations (limited to >50kDa proteins)
- Requires screening to optimize conditions

Application to heterogeneous (unpurified) samples

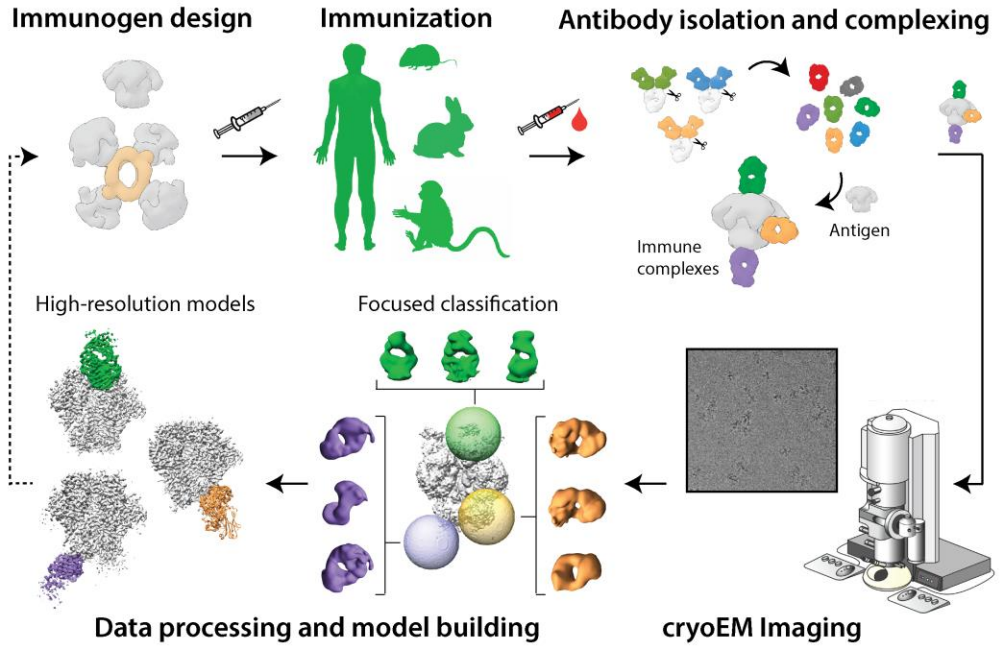
- High-resolution maps of biomolecules can be recovered from heterogeneous samples (such as cell lysates) through computational classification of diverse particles observed in EM images

Studying proteins from cell lysates



Ho *et al*. **Nat Methods,** 2020

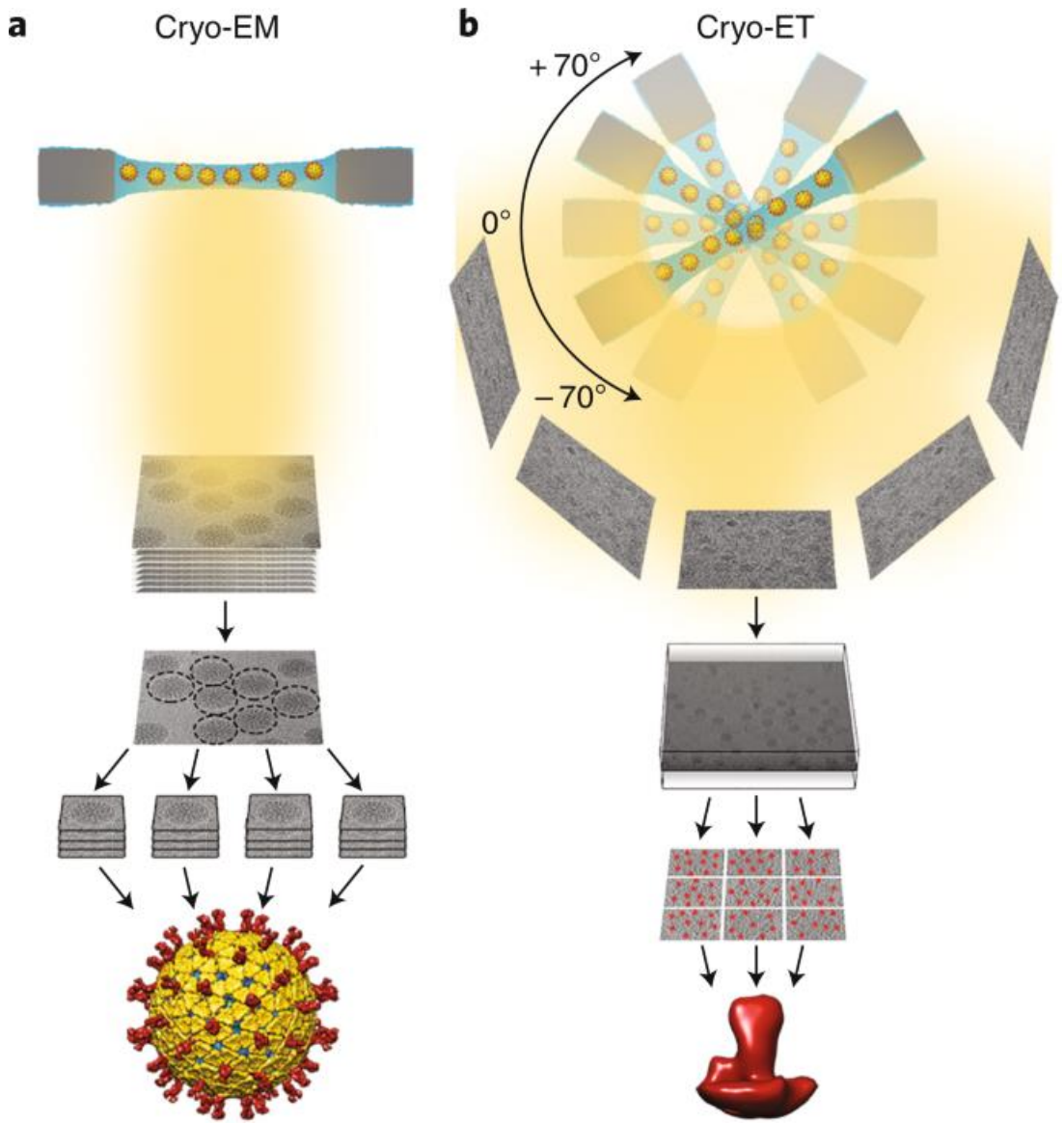
cryoEM-based polyclonal epitope mapping



Antanasijevic *et al*. **Nat Comm,** 2021

- High resolution maps allow to identify underlying biomolecules (sequence-from-structure)

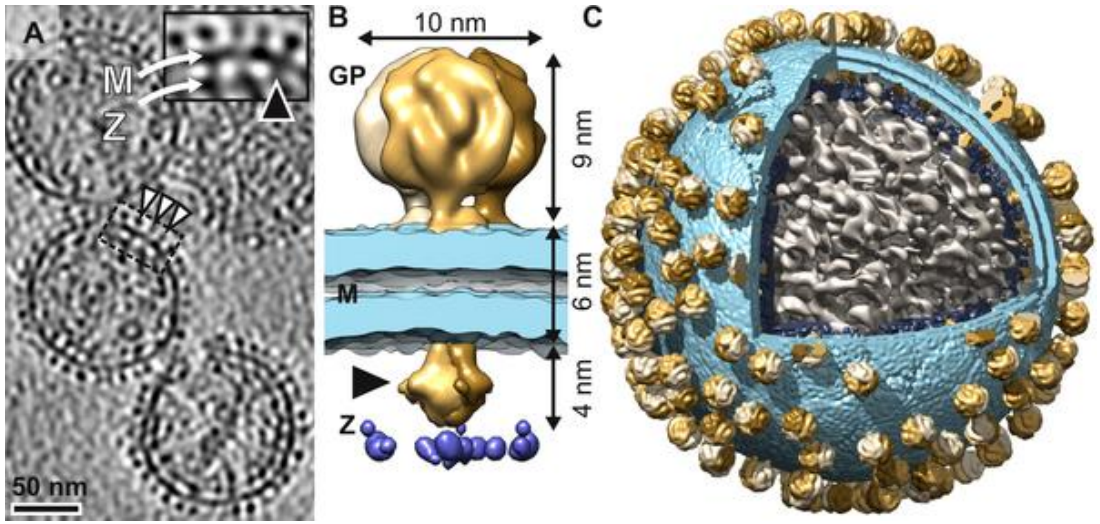
Cryo-electron tomography (cryo-ET)



- CryoET allows to reconstruct ~nm resolution three-dimensional views of complex assemblies such as protein complexes, pleomorphic viruses, bacterial pathogens, cells etc.
- Tilt-series of images (typically every 1-2˚ in the range of -70˚ to +70˚) is used for tomographic reconstruction
- Subtomogram averaging can be applied to extract the signal corresponding to different components in the complex
- Sample thickness limits the contrast in images and the ultimate resolution

LASV GP reconstructed by subtomogram averaging

Whole LASV particle

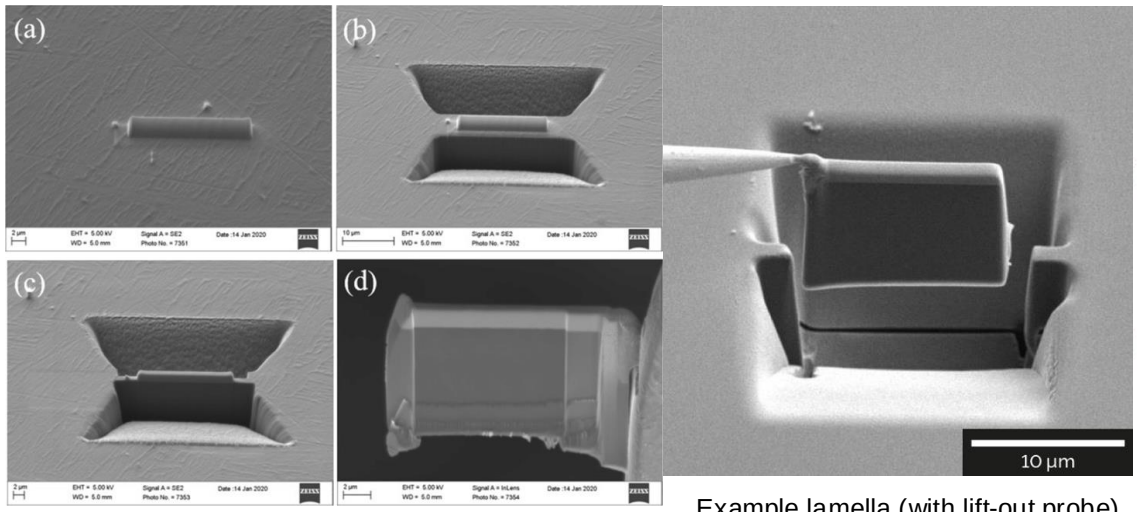


Luque et al., **Nature Chem Bio**, 2020

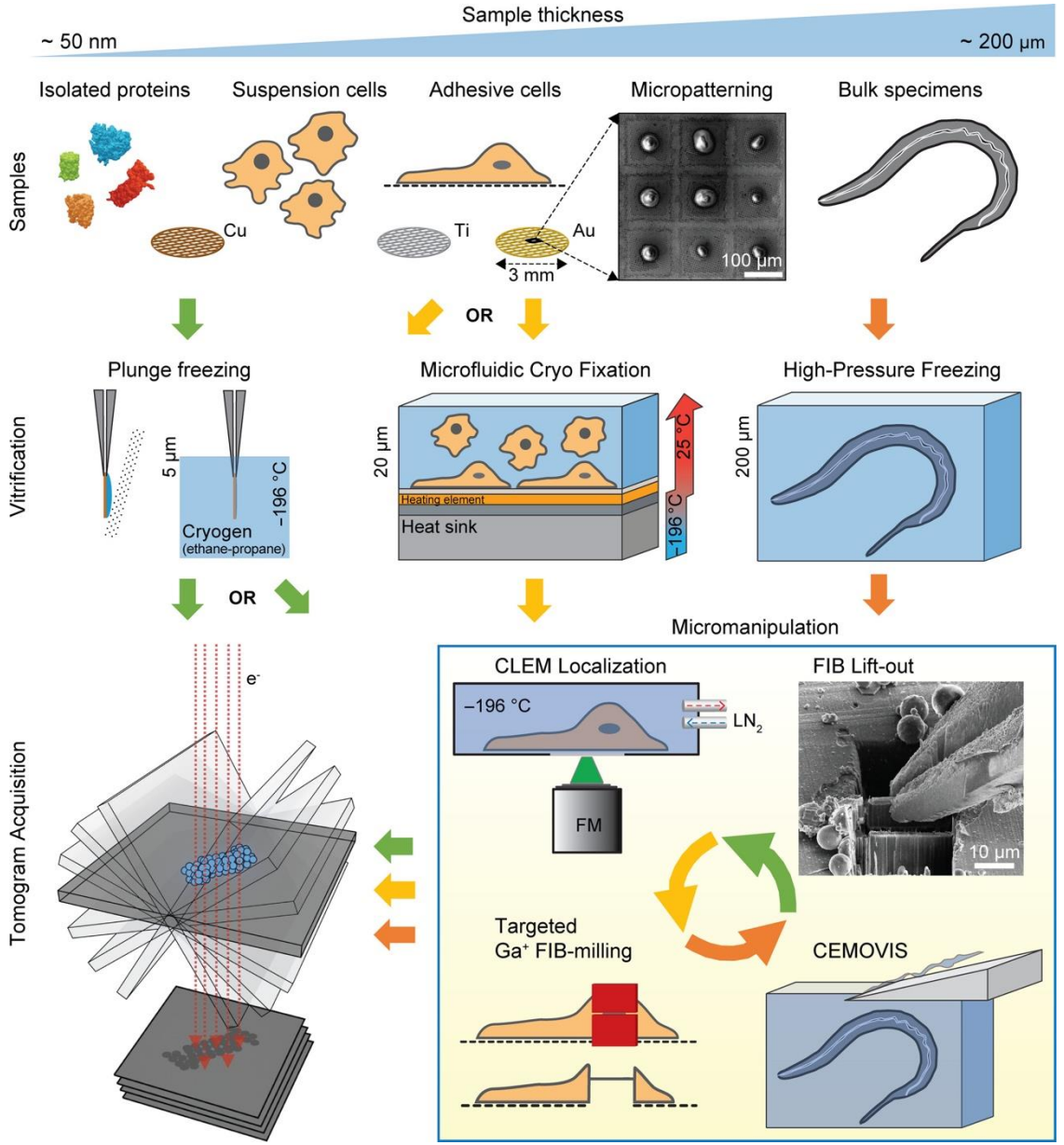
Li et al., **PLOS Path**, 2016

Dealing with sample thickness for cryoET

- Depending on the size and thickness of the sample, additional processing needs to be performed
- Optimally, the samples should be below <200-300nm thick to be susceptible for transmission EM
- Cryo-electron microscopy of vitreous sections (CEMOVIS) is based on mechanical sectioning with very fine blade
- Focused Ion Beam (FIB) approach allows to further thin out the sample using a beam of ions (typically Ga^{2+}) directed at an angle.
- Due to working under cryogenic conditions the sample typically does not need to be stained or chemically fixed



Example lamella (with lift-out probe)

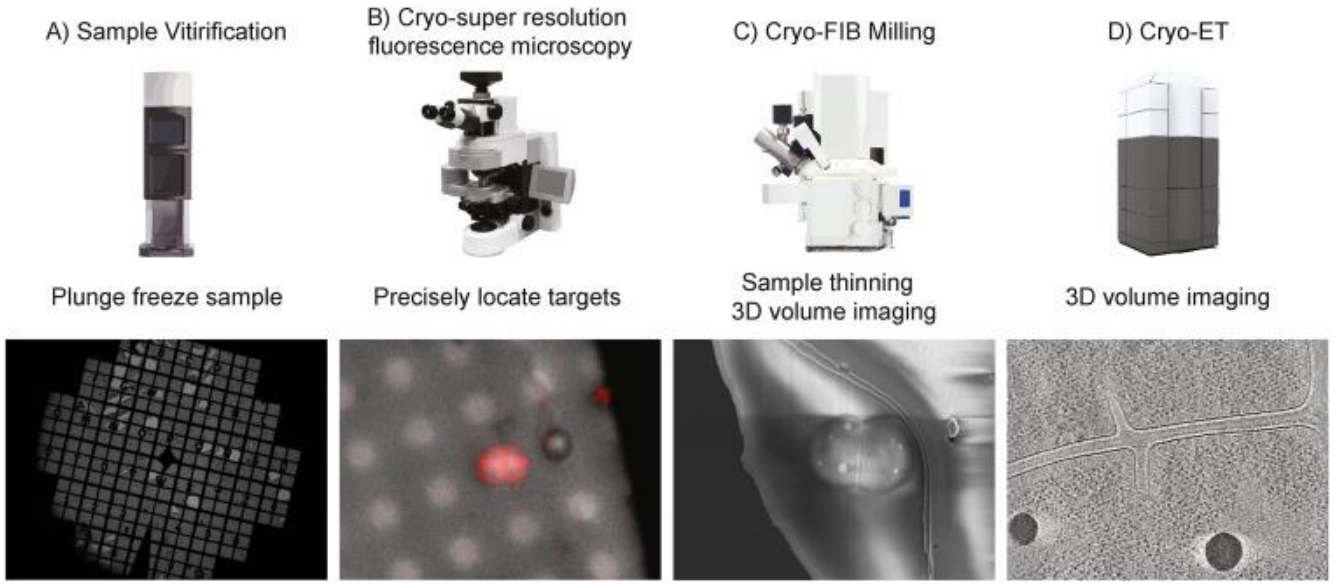


Turk et al., **FEBS Letters**, 2020

Locating the area of interest for cryoET analysis

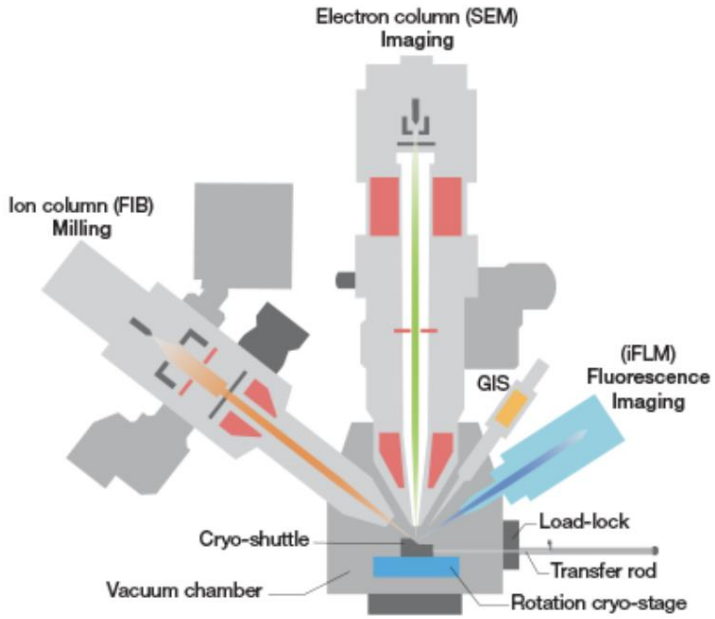
- Correlated light and electron microscopy (CLEM) – Coming to DCI soon, equipment available at UniL

Example workflow



Sexton et al., **CRSB**, 2022

Aquilos 2 Cryo-FIB
(integrated system)

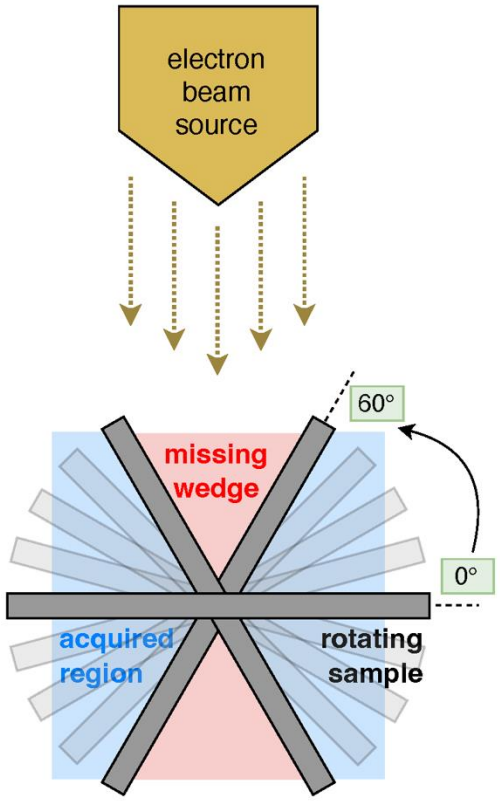


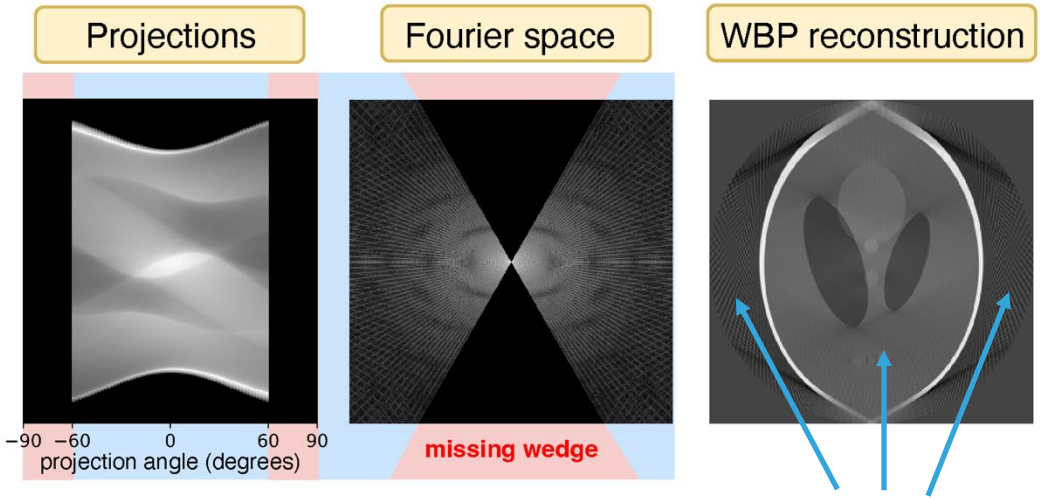
- Focused ion beam (FIB) is used to carve out the thin region of the sample to be imaged.

Check out example videos on this link: https://www.thermofisher.com/ch/en/home/electron-microscopy/products/dualbeam-fib-sem-microscopes/aquilos-2-cryo-fib.html

The “missing wedge” problem in cryoET

- The advantage of cryoET is that the 3D volumes can be reconstructed using a single tilt series
- However, the “missing wedge” during data collection represents a major challenge for reconstruction





Artefacts

Actual 3D volume

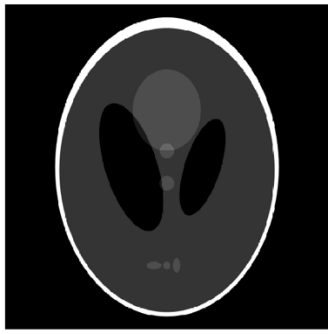
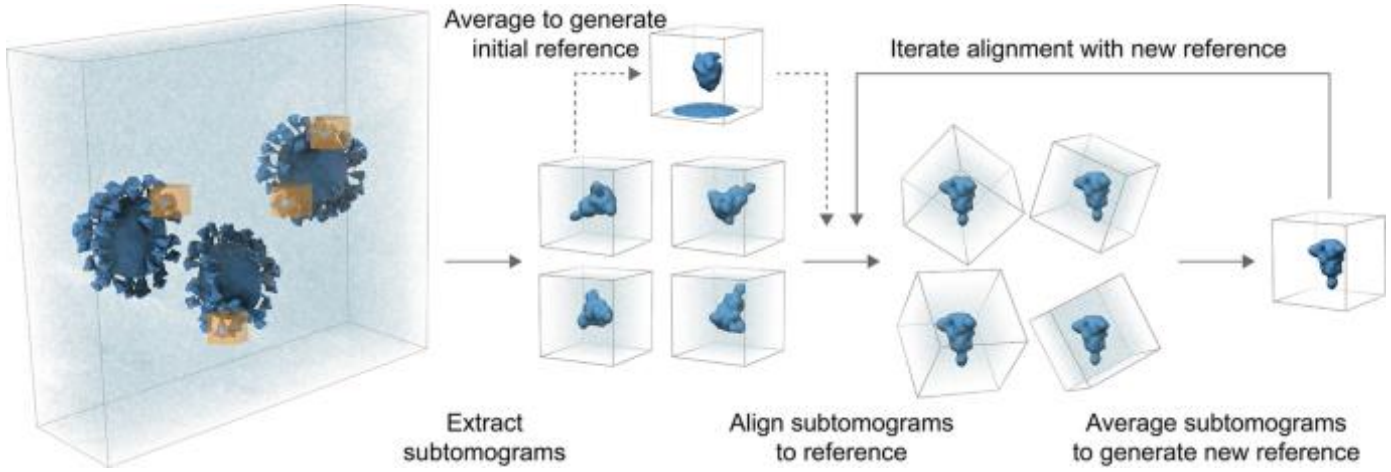
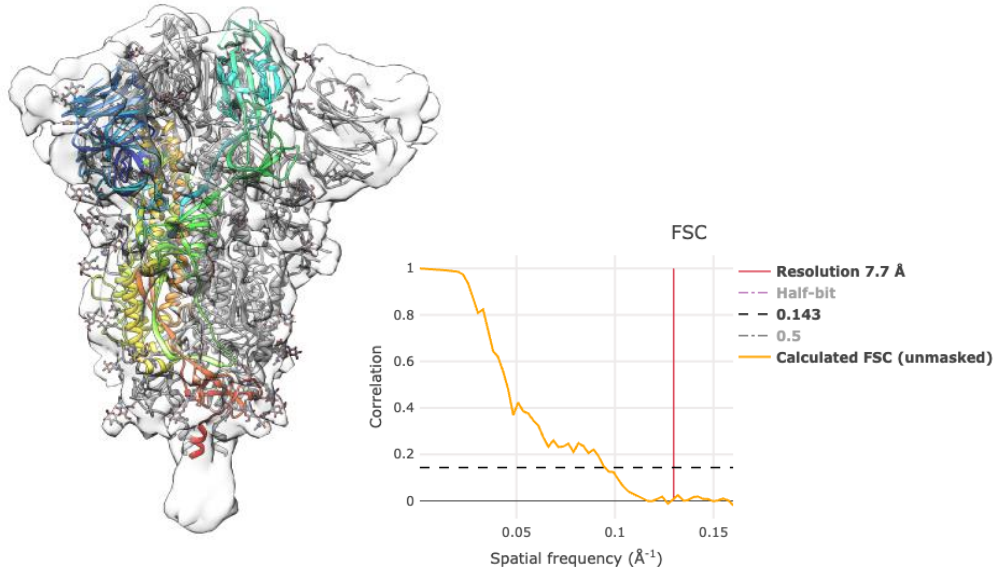


Image reconstruction and achievable resolutions

- In most cases the reconstructions are achieved through averaging of local tomogram sections corresponding to biomolecules of interest (subtomograms)



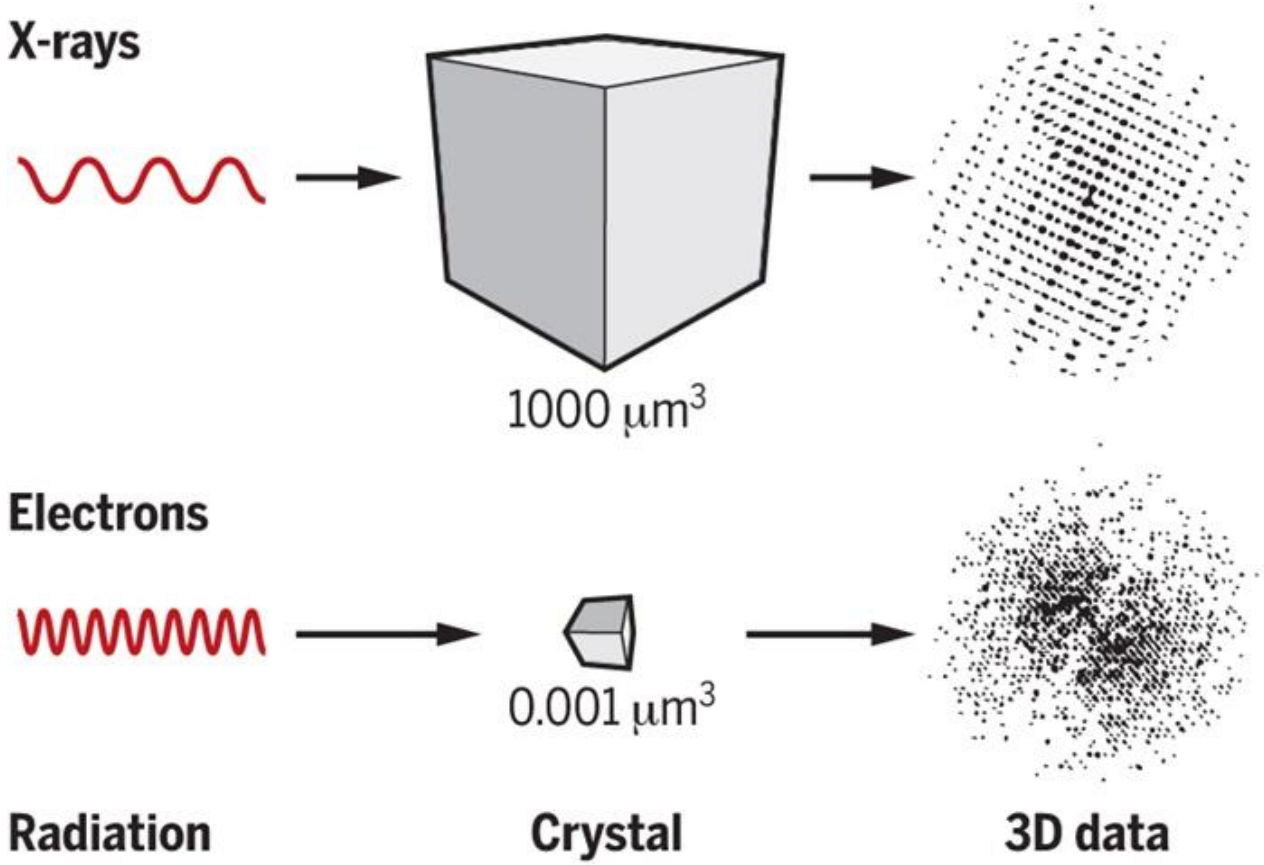


SARS-2 Spike

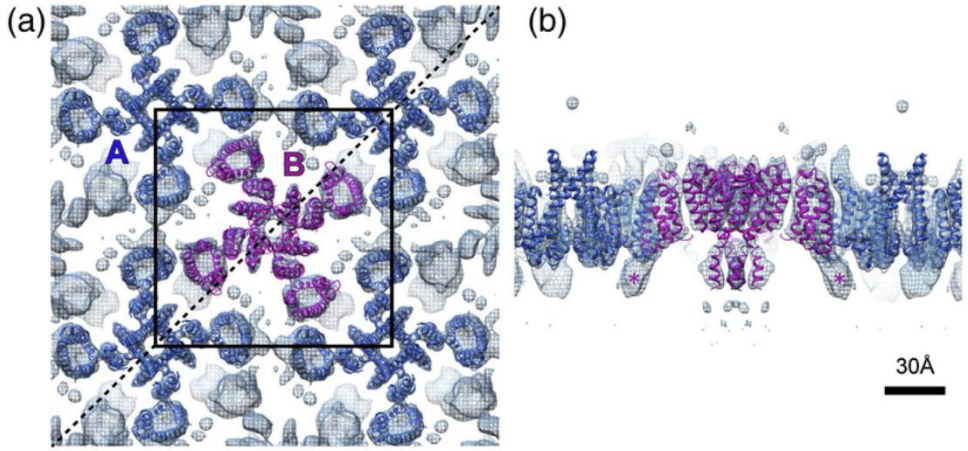
- Achievable resolutions are ~4Å for large biomolecules under (very) optimal conditions
- For complex heterogeneous samples (e.g., cell sections) and biomolecules present in few copies per cell the resolutions are typically >20Å.

Electron diffraction experiments

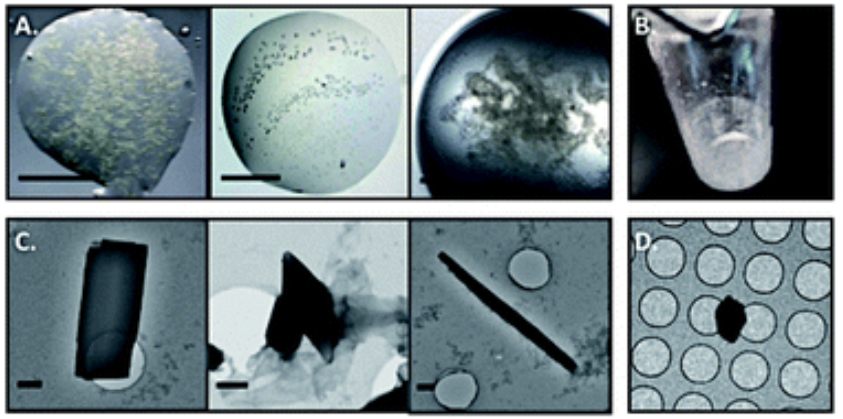
- Electron diffraction experiments are similar to X-ray crystallography but limited by inelastic and dynamical scattering
- Thinner (i.e., 2D) or smaller (i.e., µm to nm size) crystals are necessary



2D crystal of NavCt

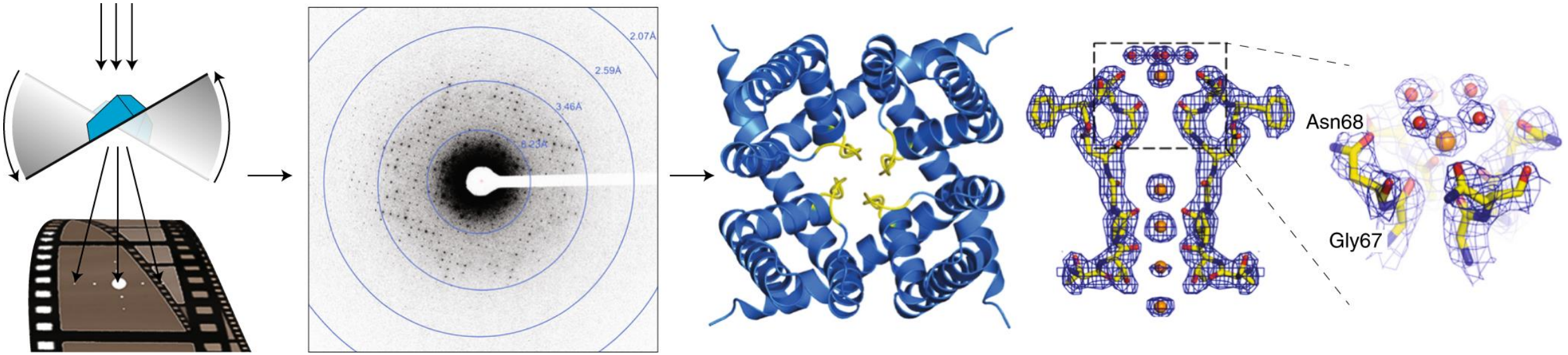


Crystals for microED



Microcrystal electron diffraction (MicroED)

- MicroED is a form of electron crystallography where thin 3D crystals are used for structure determination by electron diffraction.

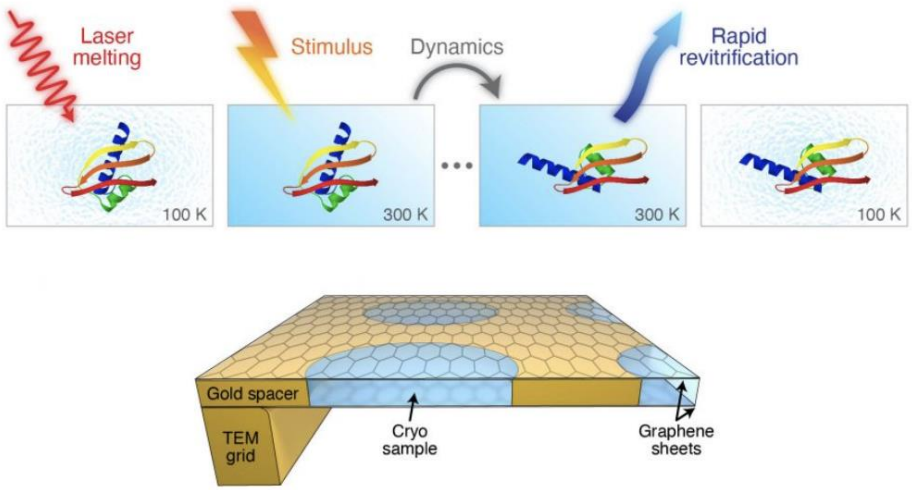


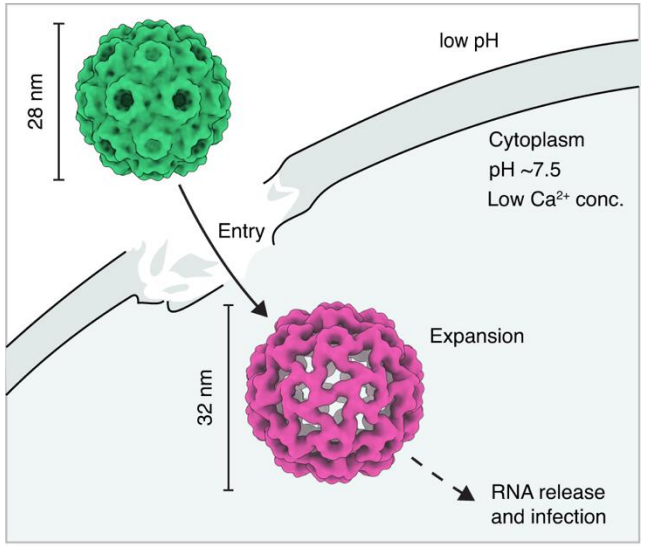
- The samples are frozen hydrated as for all other CryoEM modalities but instead of using the transmission electron microscope (TEM) in imaging mode one uses it in diffraction mode with an extremely low electron exposure (typically < 0.01 e−/Å^2/s).
- The nano crystal is exposed to the diffracting beam and continuously rotated while diffraction is collected as a movie
- Achieved 1Å resolution on carbamazepine (small molecule)
- Experimental phasing is problematic (currently done by molecular replacement or de novo)

Nannenga *et al*. **Nature Methods** 2019

Time-resolved cryoEM

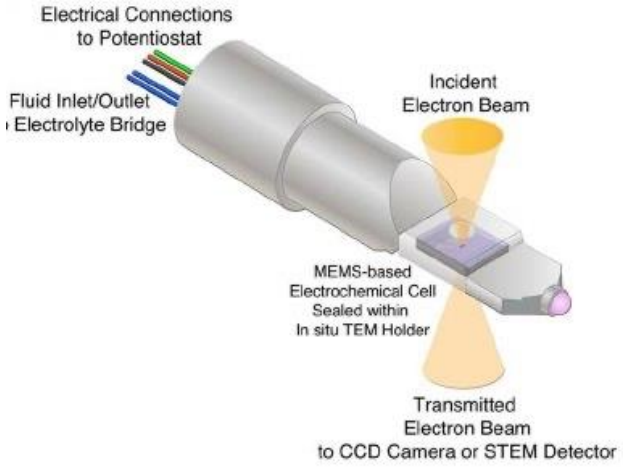
- Microsecond time-resolved cryoEM (Lorentz Ulrich, EPFL)

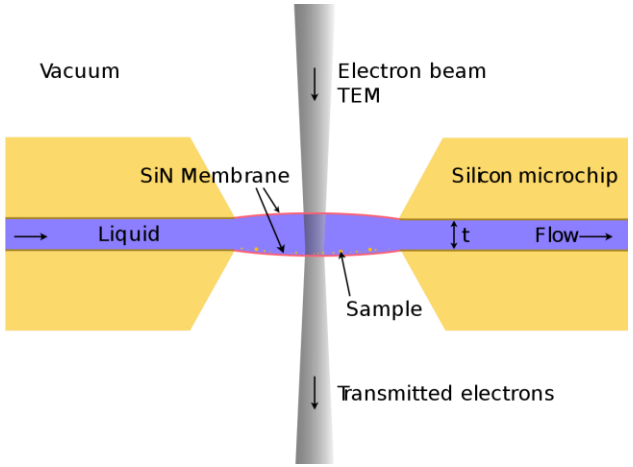




Harder *et al.* **Nature Comm** 2023

- Liquid-Phase EM – Sample holder with a nanofluidics device (CIME, EPFL)



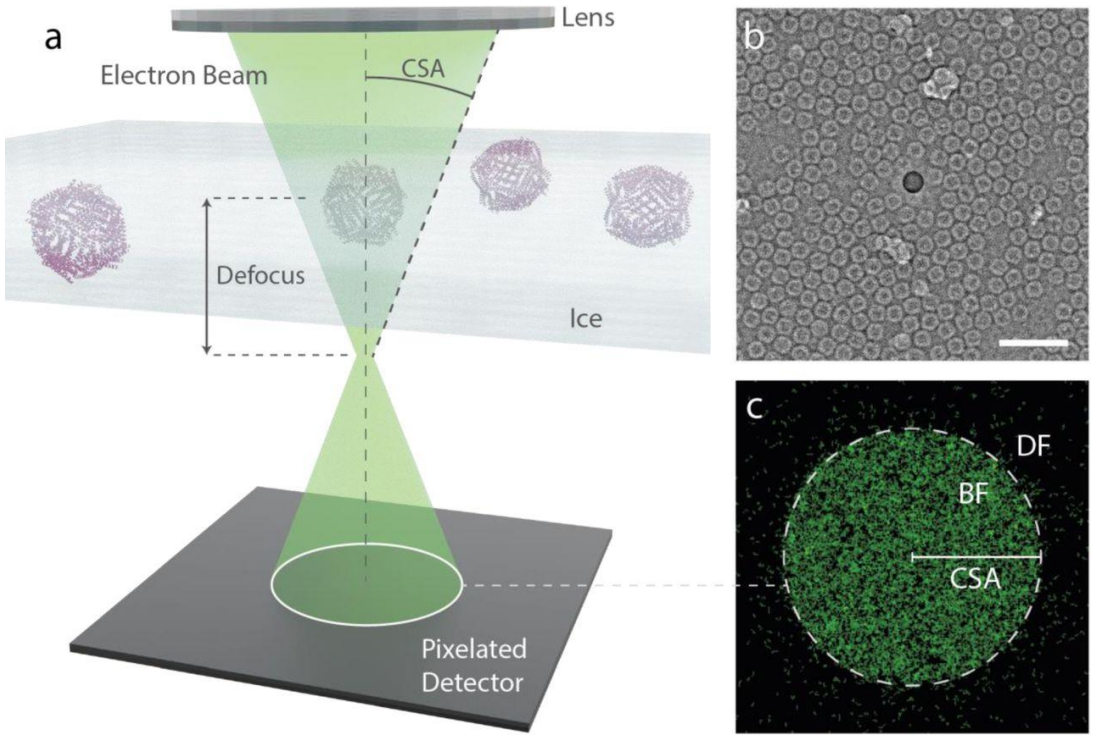


Studying biomolecules in solution and at physiological temperatures

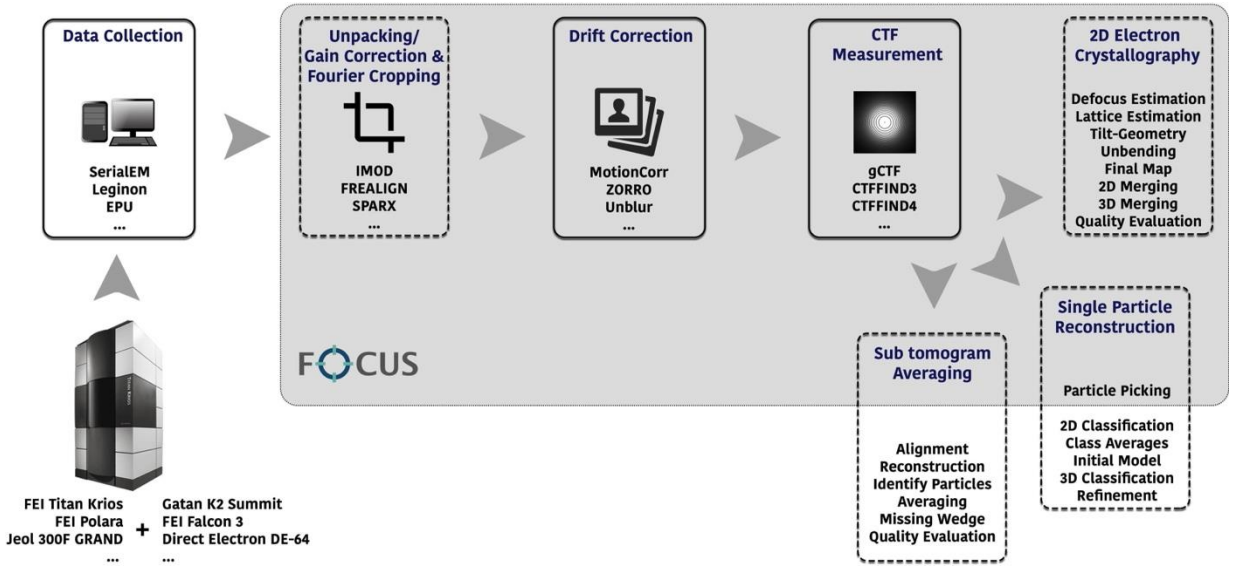
Available at CIME!

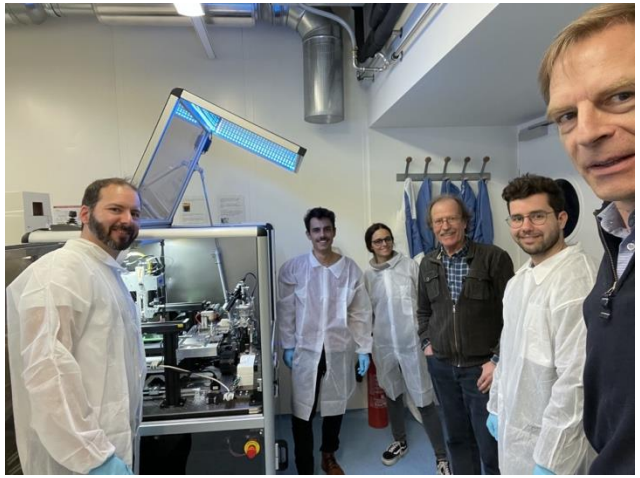
EM method development

Low-dose cryo-electron ptychography



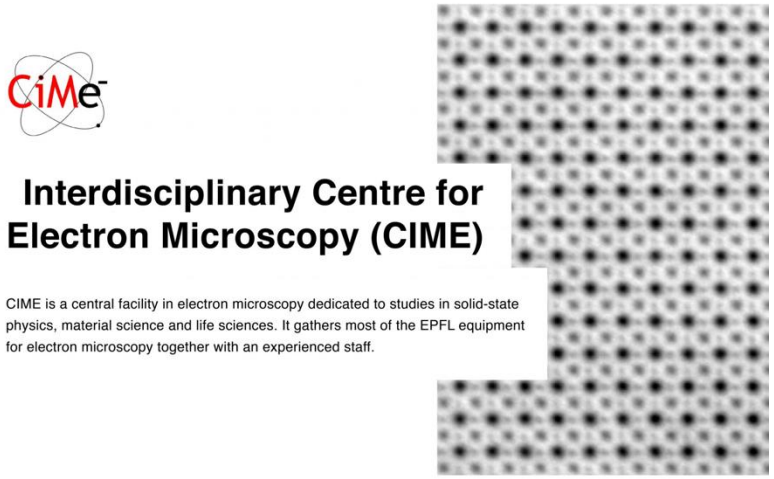
Software and workflow development

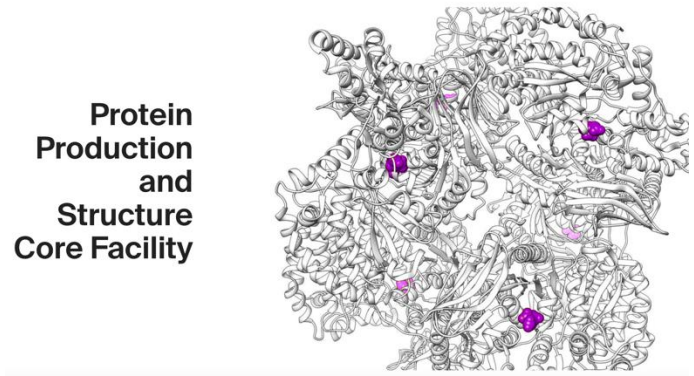


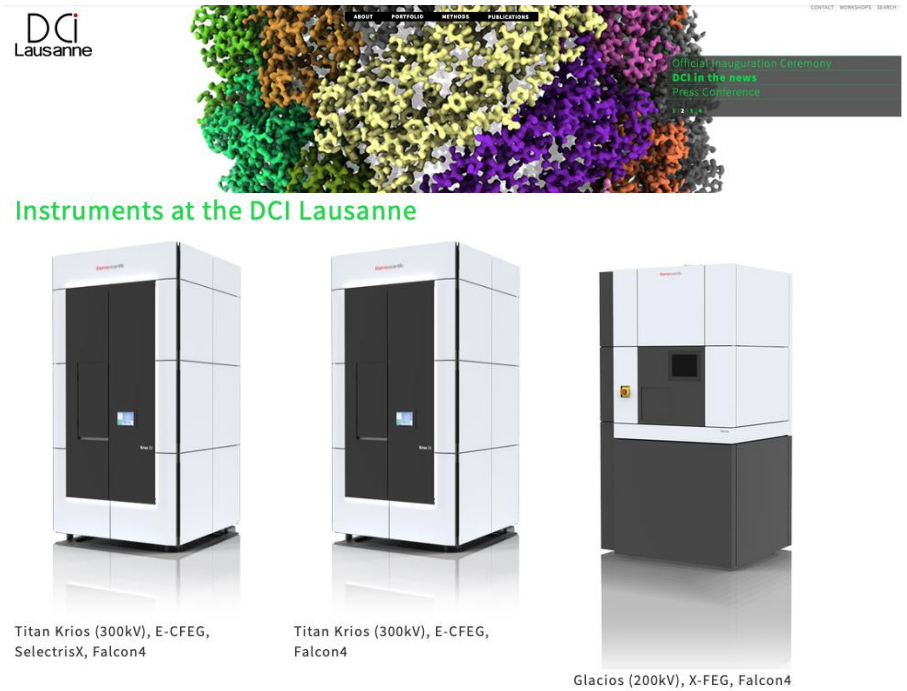


- Some recent papers to familiarize yourself with their work:
  - https://www.biorxiv.org/content/10.1101/2024.02.12.579607v1
  - https://pubmed.ncbi.nlm.nih.gov/28344036/
  - https://pubmed.ncbi.nlm.nih.gov/31815671/

CryoEM resources on our campus







INFORMATIQUE SCIENTIFIQUE & SUPPORT APPLICATIF
SCITAS
SCIENTIFIC IT AND APPLICATION SUPPORT

- Research labs: Henning Stahlberg, Nicolas Thoma, Lorenz Ulrich, Matteo Dal Peraro, Andrea Ablasser, Francesco Stellacci, Aleksandar Antanasijevic, Bruno Correia, Pierre Gonczy and others…

The main topics/questions from today's lecture

- What is a Nyquist frequency?
- How does air-water interface interfere with grid preparation?
- What are the basic steps for building atomic models into cryoEM maps?
- How are cryoEM data, maps and models evaluated?
- What is MolProbity score and what other scores does it incorporate?
- How to assess model-to-map fit?
- What do you need for deposition of cryoEM data to PDB/EMDB?
- Explain the basics of single particle, cryoET and MicroED experiments?

Plan for tomorrow (27/03/25)

- Meeting in DIA004 at 8AM sharp.
- Bring your computer
- Download and install UCSF Chimera software from:
  - https://www.cgl.ucsf.edu/chimera/download.html