C9940: 3-Dimensional Transmission Electron Microscopy

Lecture 5: Interpretation and optimization of cryo-EM maps

Content

- symmetries
- map validation
- map interpretation
- model building
- map improvement

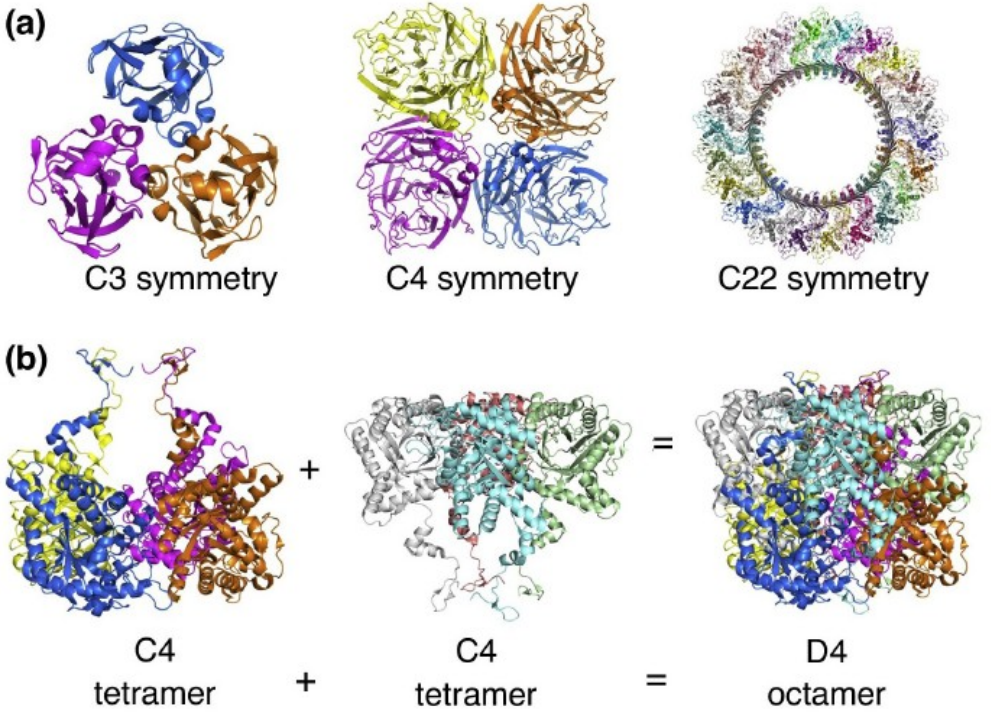
Symmetries

- regular assemblies of protein oligomers are common in nature

- oligomeric protein structures obey certain rules
  → no mirror symmetry

- understanding symmetry rules may prevent incorrect interpretation of the data

- presence of symmetry generally facilitates determination of the density map

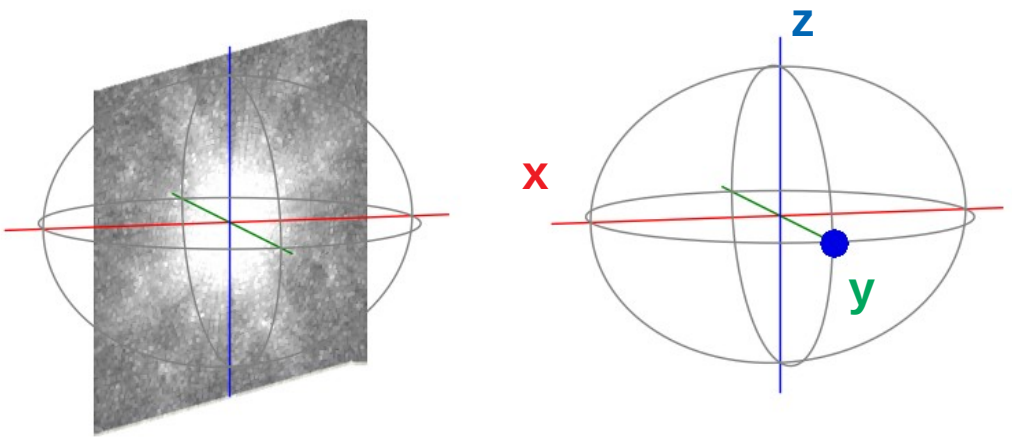


(Xu et al., Curr Opin Struct Biol 2019)

Symmetries

Projection Theorem, Euler angles

- A central section through the 3D Fourier transform is the Fourier transform to the projection in that direction



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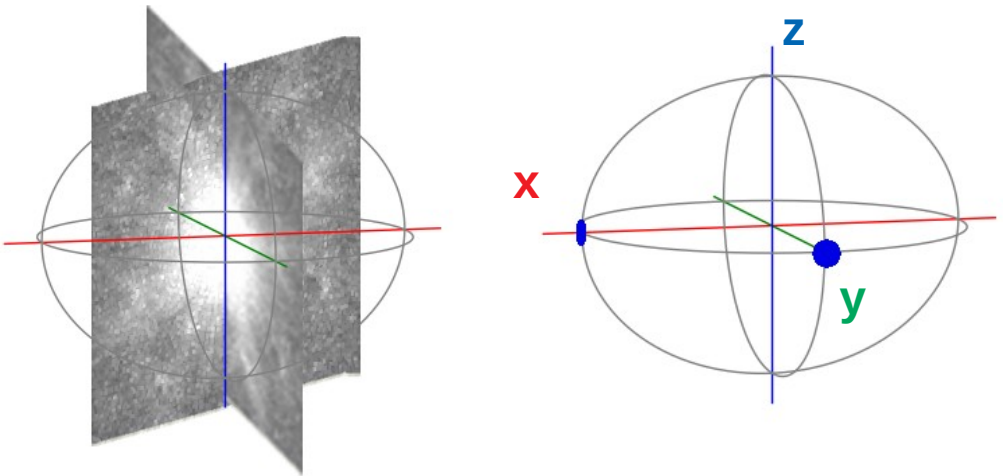
- Images for all possible projection directions are required to obtain structure with homogeneous resolution in all directions



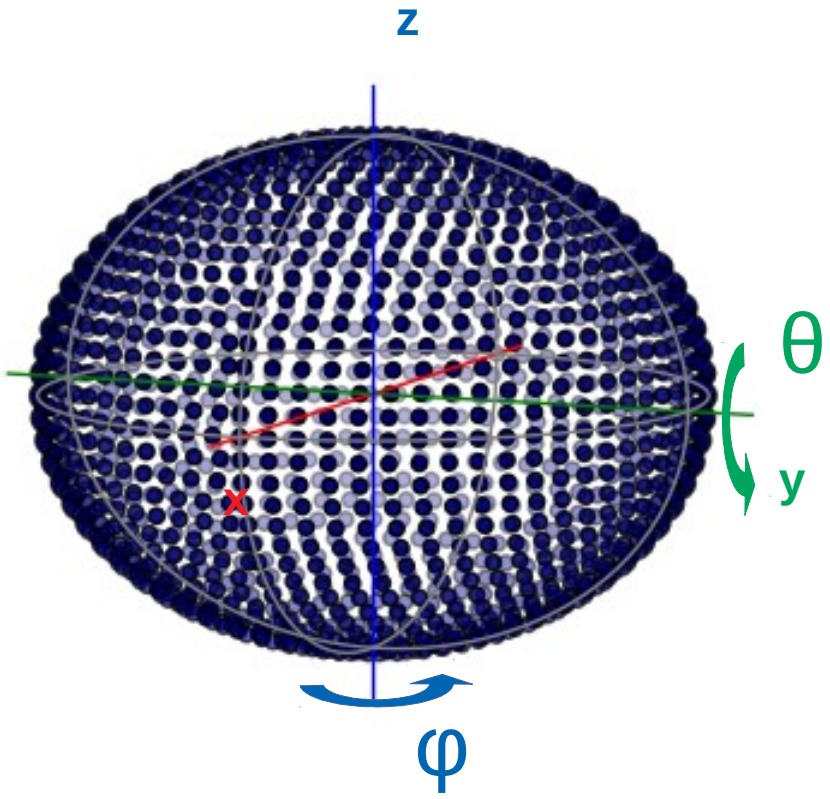
Symmetries

Projection Theorem, Euler angles

- A central section through the 3D Fourier transform is the Fourier transform to the projection in that direction

- Images for all possible projection directions are required to obtain structure with homogeneous resolution in all directions

- Euler angles φ and θ cover ranges of (0° - 360°) and (-90° - +90°)



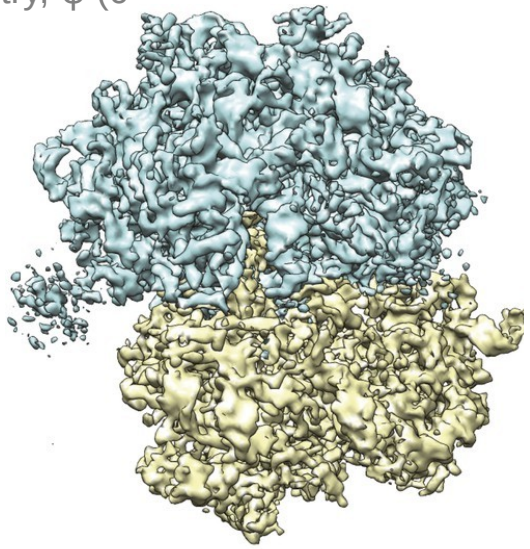
Symmetries

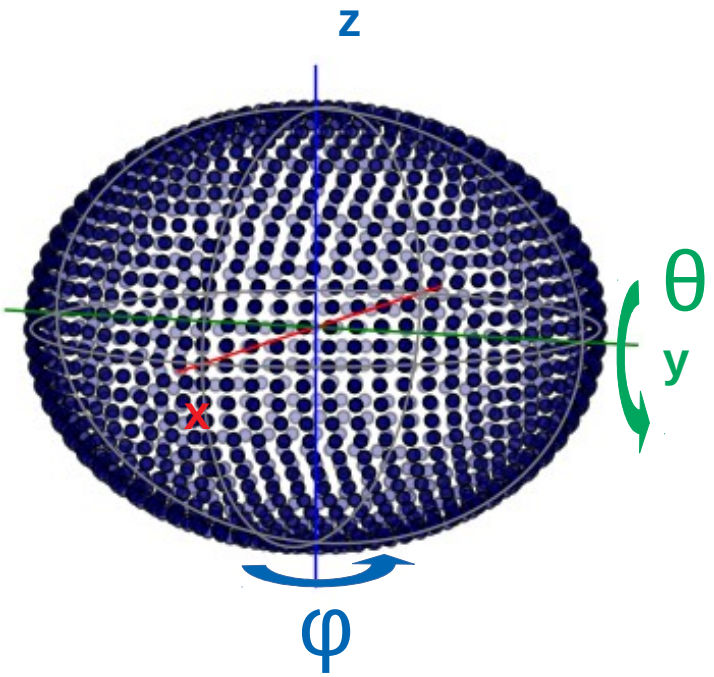
Rotational (cyclic) symmetries

- one symmetry axis (usually molecules oriented with the symmetry axis alongside z)

- Asymmetric unit – the smallest portion of the angular space to which symmetry operation can be applied in order to completely fill the angular space

- C1 – the most trivial case, no symmetry, φ (0° - 360°), θ (-90° - +90°)





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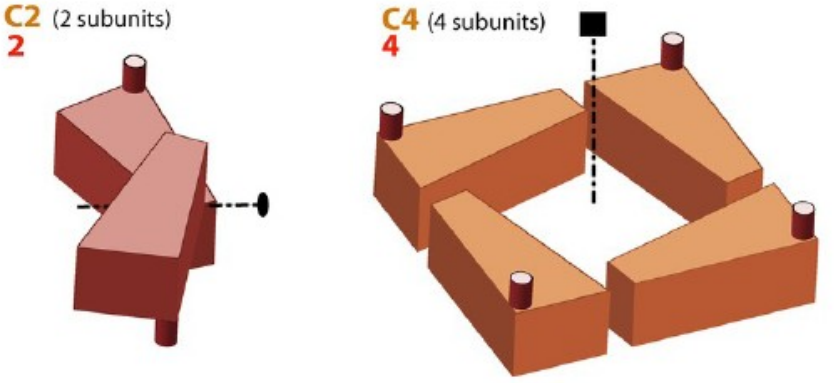
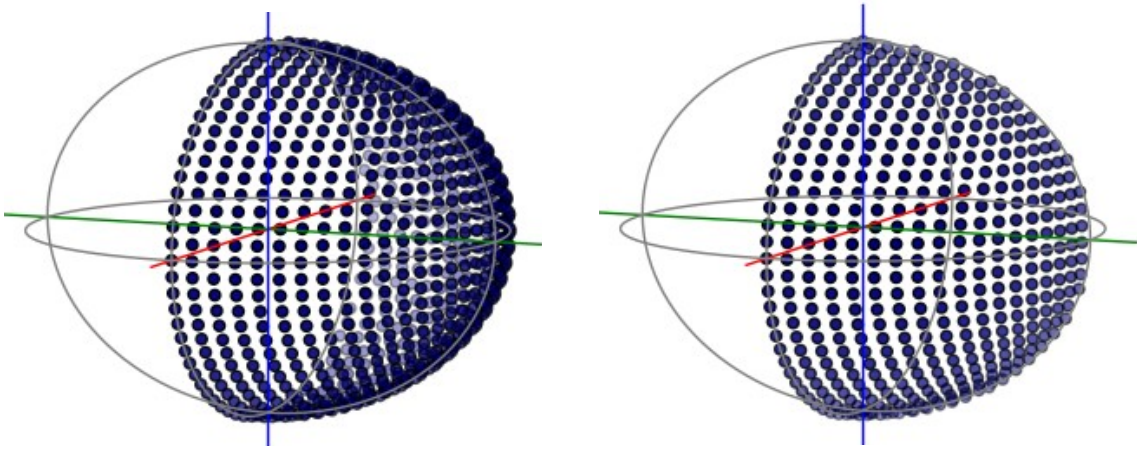
- C2 – φ (0° - 180°), θ (-90° - +90°)
- C3 – φ (0° - 120°), θ (-90° - +90°)
- C4 – φ (0° - 90°), θ (-90° - +90°)
- C6 – φ (0° - 60°), θ (-90° - +90°)



(Levy et al., PLoS computational Biology 2006)

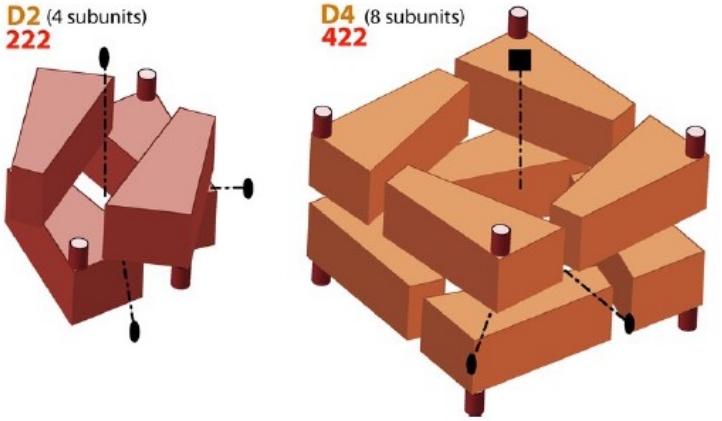


Symmetries

Dihedral symmetries

- one n-fold rotational axis and two-fold axis perpendicular to it

- Asymmetric unit
  - D2 – φ (0° - 180°), θ (0° - +90°)
  - D5 – φ (0° - 72°), θ (0° - +90°)
  - D7 – φ (0° - ~51°), θ (0° - +90°)



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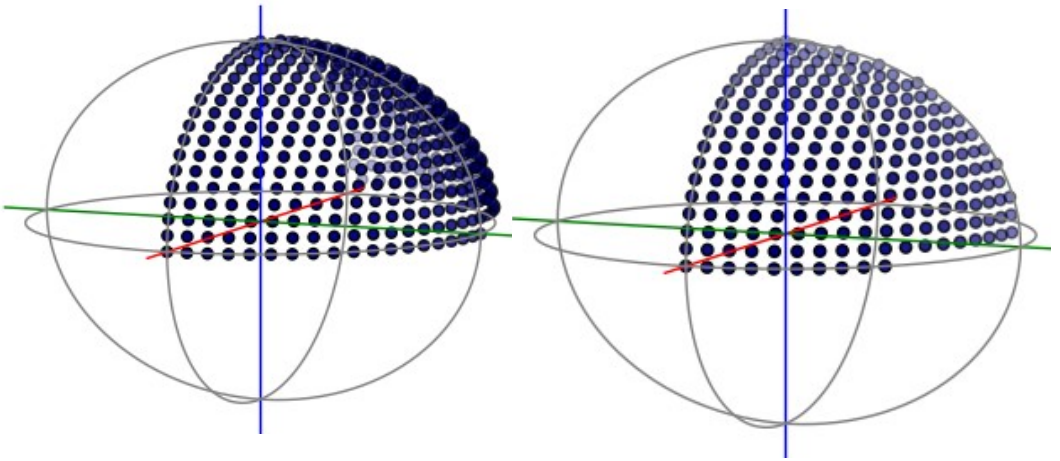
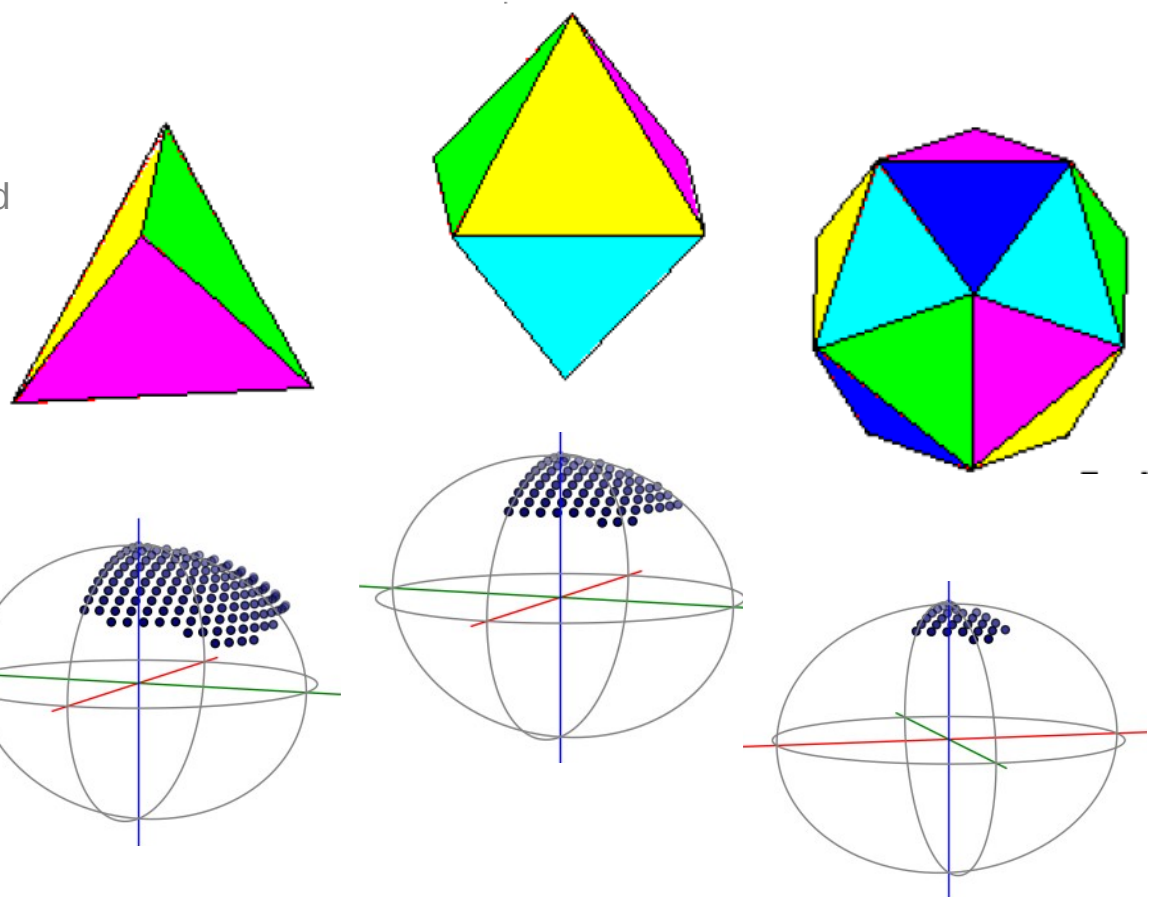


Symmetries

Platonic symmetries

- faces, edges, and corners are related by symmetry operations

- tetrahedral – 4 3-fold axes and 3 2-fold axes
- octahedral – 3 4-fold axes of symmetry, 4 3-fold axes of symmetry, and 6 2-fold axes
- icosahedral – 6 5-fold, 10 3-fold and 15 2-fold axes



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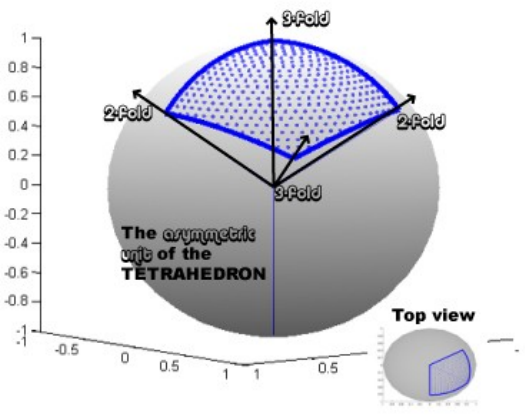
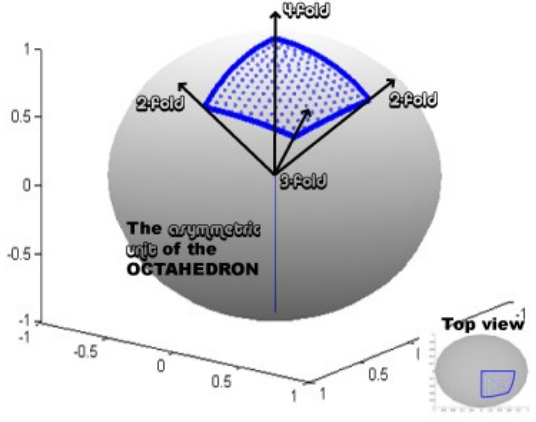
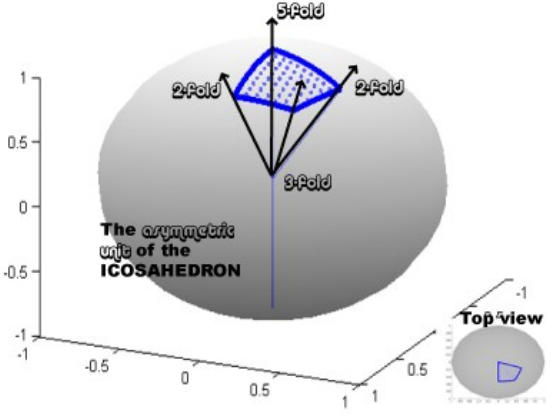






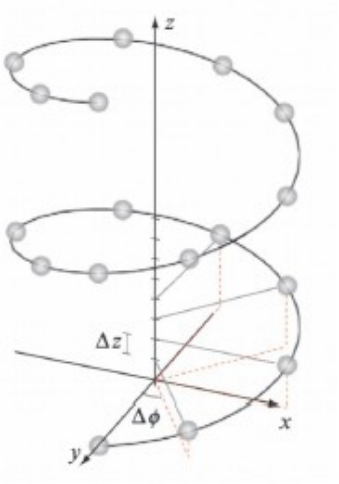
EMAN2

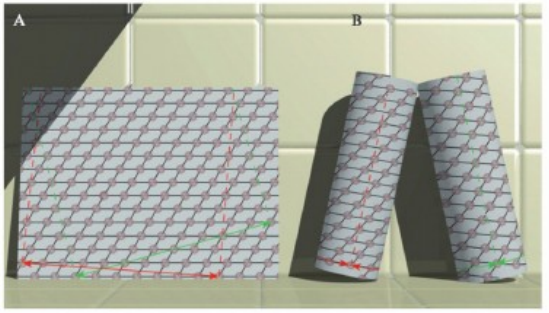
Symmetries

Helical symmetry

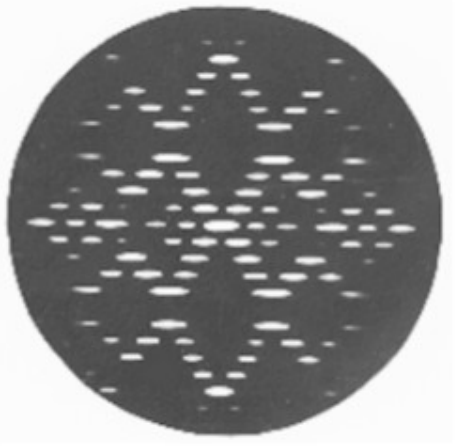
- A single view contains all the necessary info for 3D reconstruction

- 2D surface lattice rolled into 3D
- 3D reconstruction approaches:
    - Fourier-Bessel analysis
    - Iterative Real-Space Refinement (IHRSR)





(Diaz et al. Methods in Enzymology 2010)



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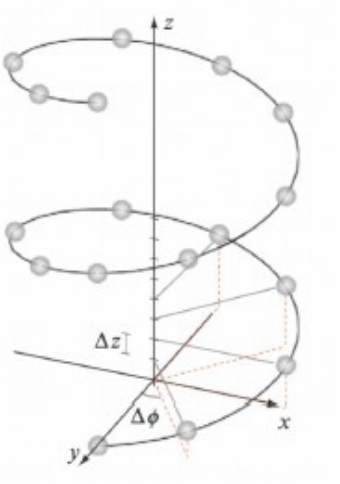

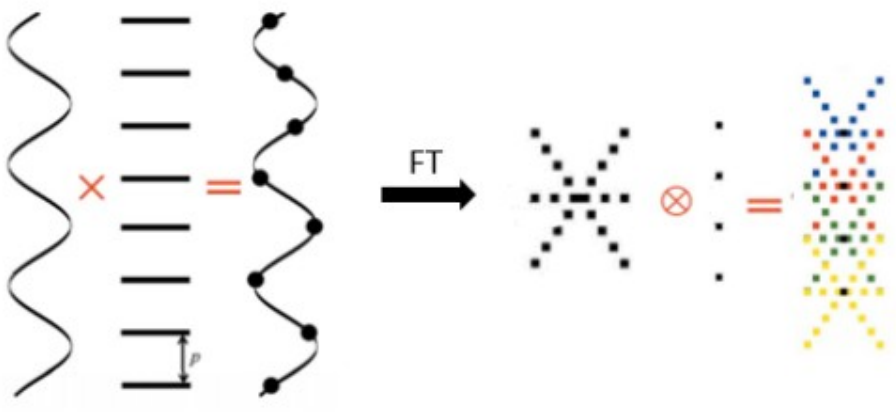

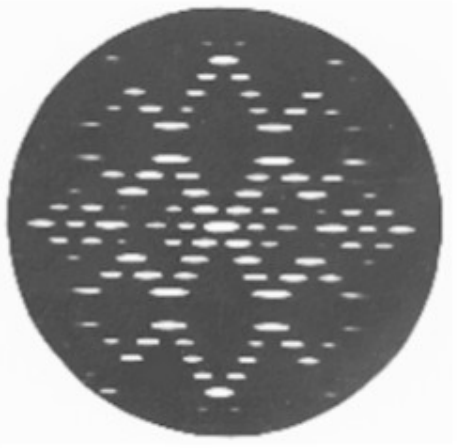

- small inaccuracies in indexing lead to incorrect structure
- requires strict helical symmetry
- requires flat straight helices
- laborious

Symmetries

Helical symmetry

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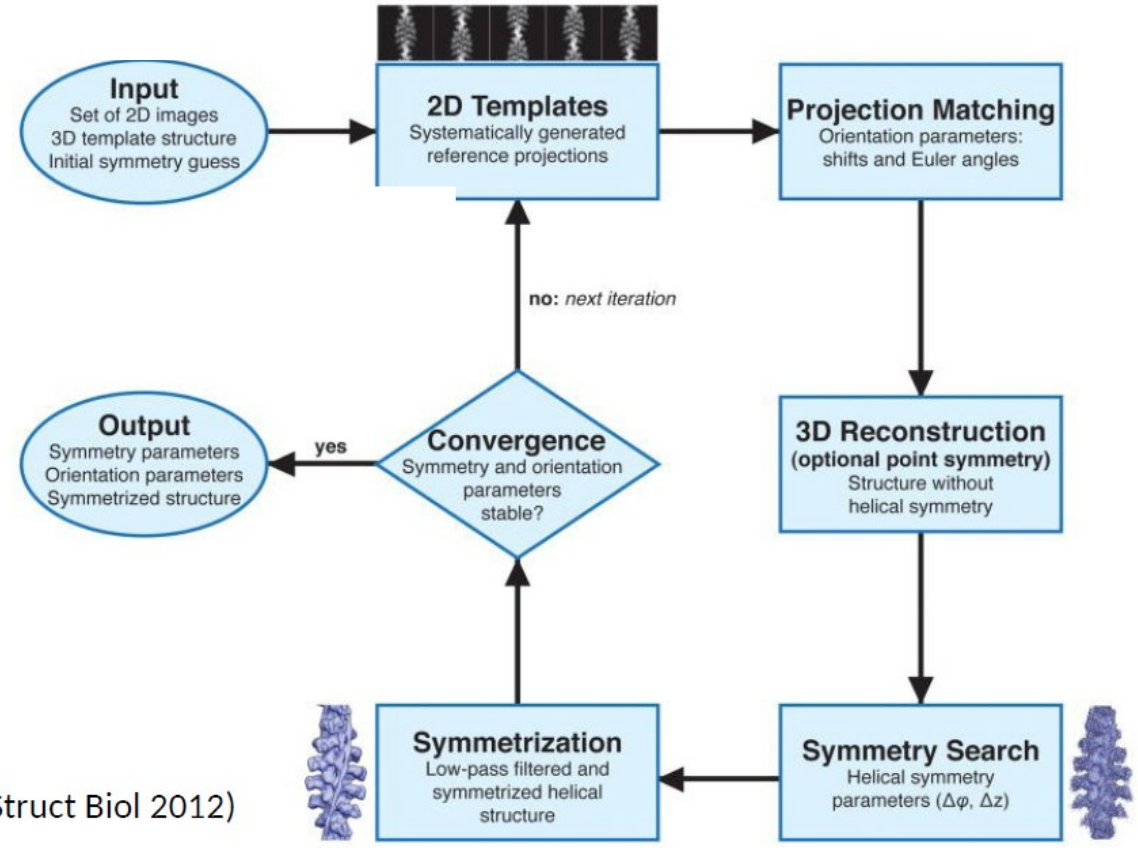
    - Fourier-Bessel analysis
    - **Iterative Real-Space Refinement (IHRSR)**



(Behrmann et al., J Struct Biol 2012)

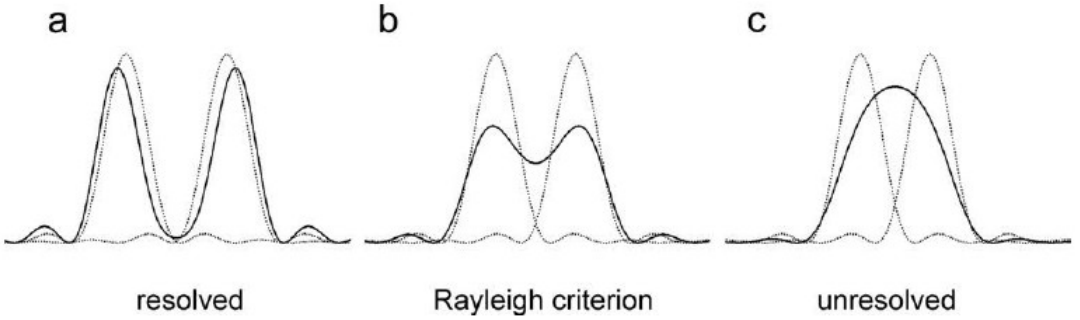
- requires fairly good estimate of the cylinder diameter, rise, and twist
- can cope with heterogeneous data
- manages to reconstruct weakly diffracting filaments (where layer lines are not visible)

Symmetries

- Smaller asymmetric unit
- Decreased computational demands
- Improved signal to noise due to better averaging
- Lower number of particles required

Map validation

Resolution



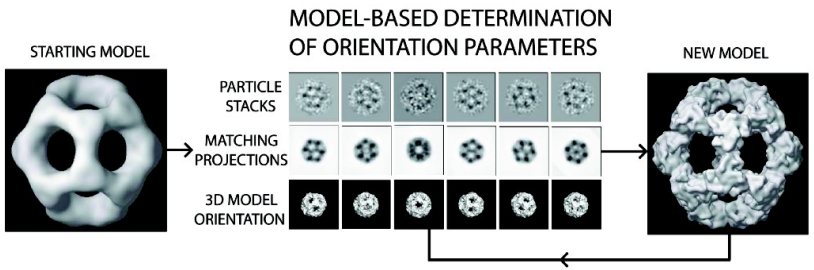
Resolution definition by separation of features. (a) When two points are far apart, there is a deep trough of density between them. (b) Two points are regarded as just resolved when the peak of one point spread function overlaps the first minimum of the other (Rayleigh criterion), see ref 60. (c) The point spread functions of two dots close together overlap to form one maximum, so that the points are not resolved.

Published in: E. V. Orlova; H. R. Saibil; *Chem. Rev.* Article ASAP

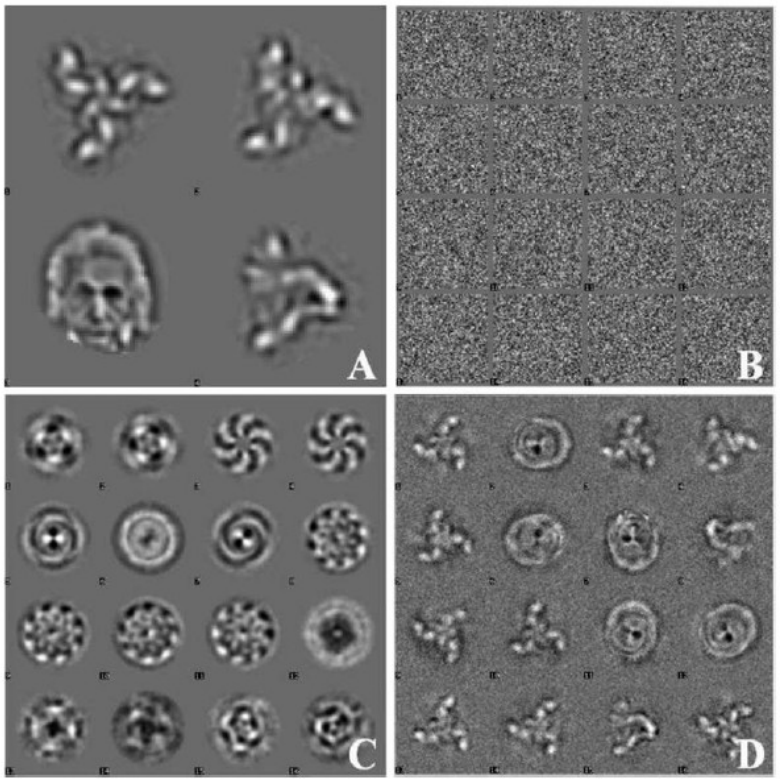
Map validation

- cryo-EM data – low signal to noise (VERY)

- model bias – persistence of an incorrect map or map features during refinement



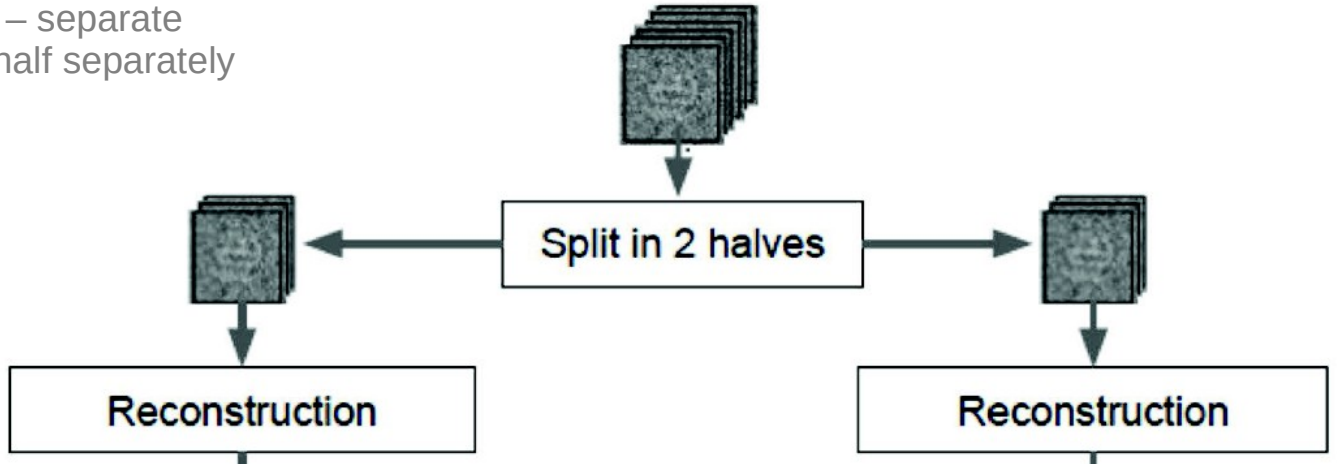
(A) Four reference images (each 64 × 64 pixels) used for picking from 1,024 random noise images (of 1,024 × 1,024 pixels).



van Heel M PNAS 2013;110:E4175-E4177

Map validation

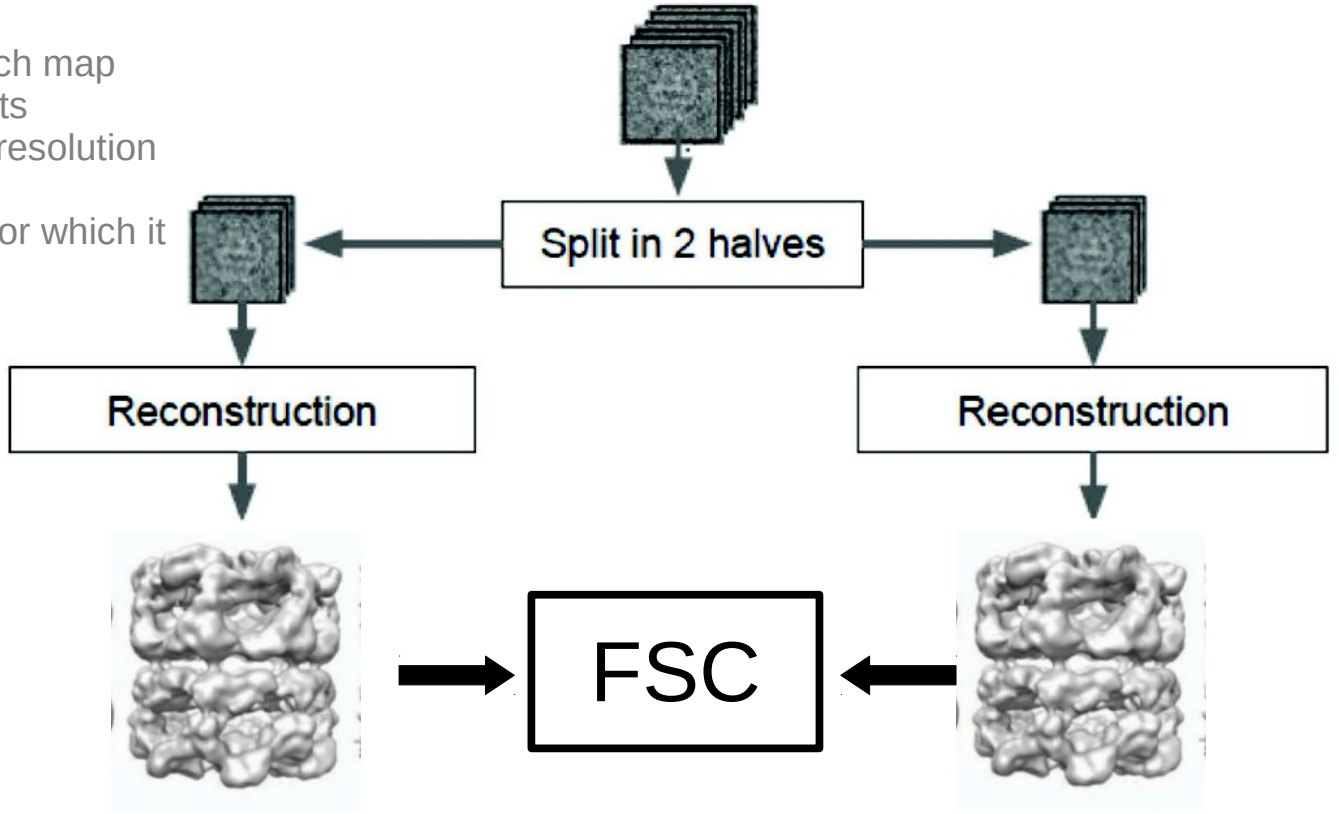
- in order to minimize the model bias – separate the data into two halves, refine each half separately using the standard SPA protocol



Map validation

Fourier shell correlation

- nowadays used as a metrics for map resolution estimation
- calculate 3D Fourier transform of each map
- calculate cross-correlation coefficients between the two 3D FTs for individual resolution shells
- plot the CCC against the resolution for which it was calculated
- determined CCC threshold for which resolution is reported



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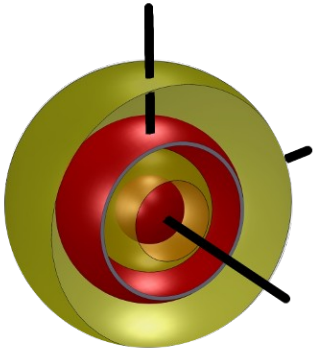

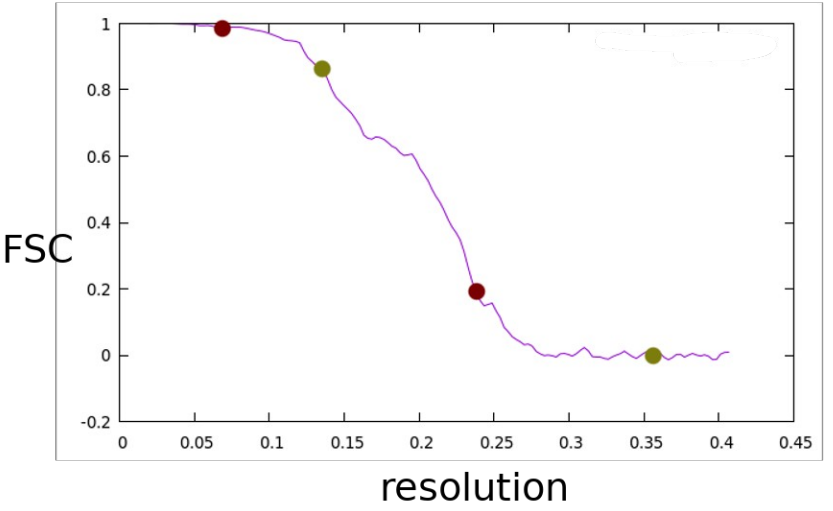

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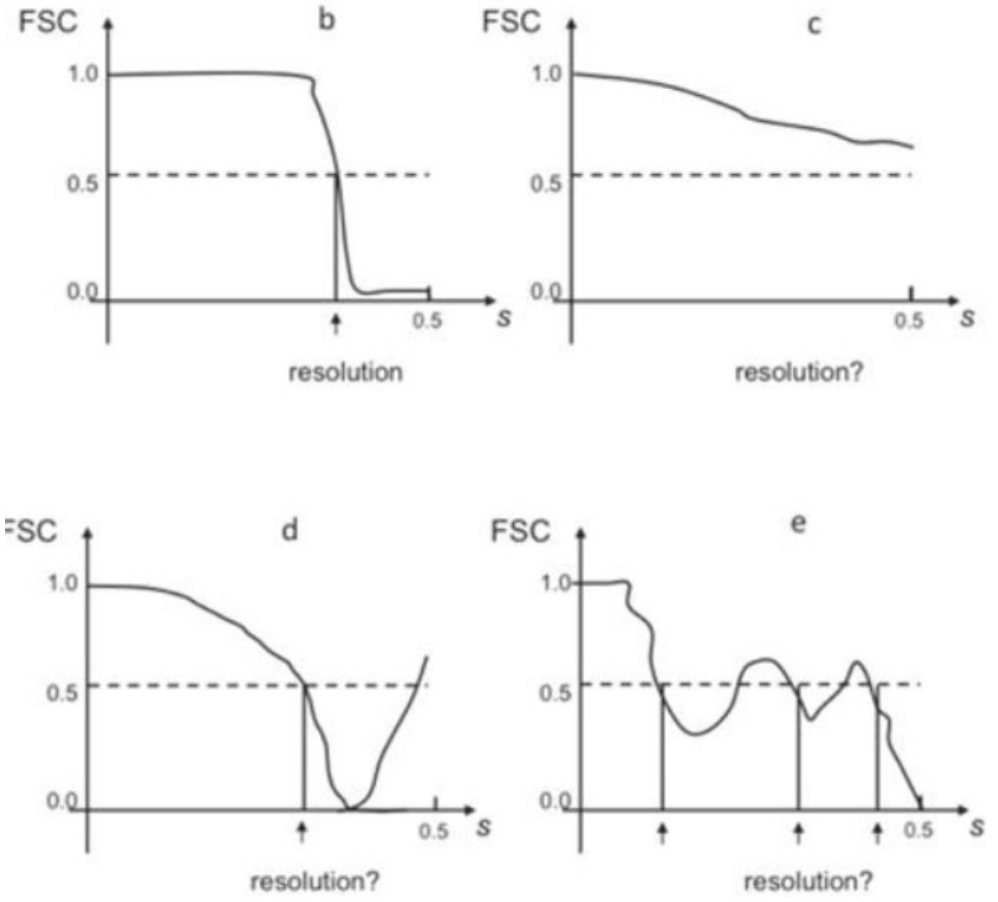
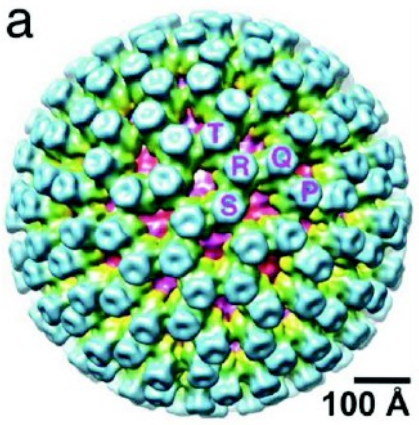


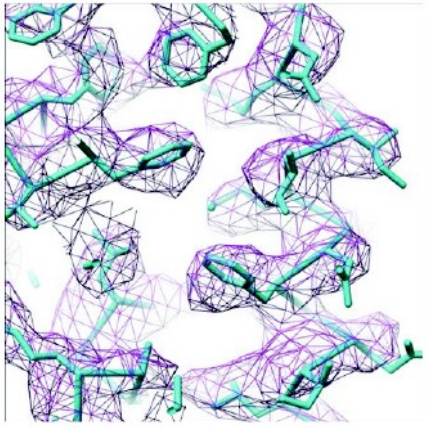
Penczek (2010), Meth. Enzym.

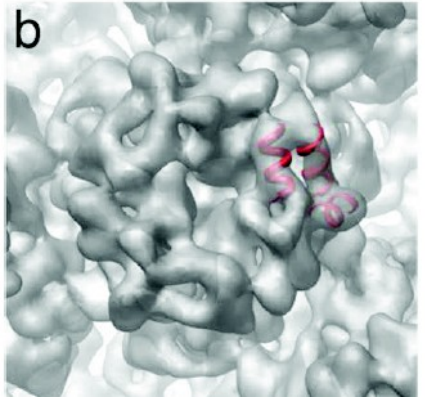
Map validation

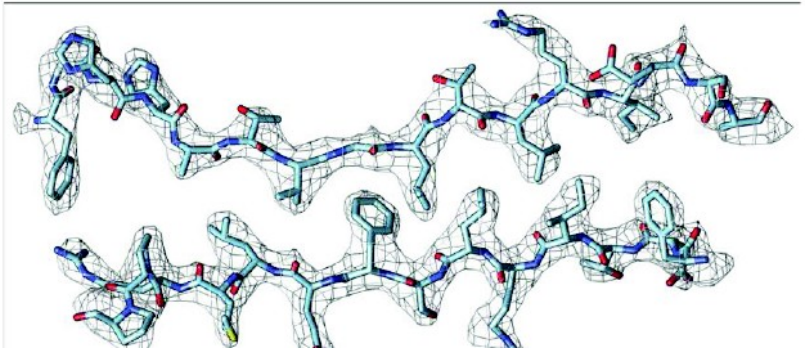
- observed features of the map should be consistent with the resolution assessment

- visibility of expected structural features
  - helices visible at 8Å
  - strands separated at 4.8Å
  - side-chains visible beyond 4Å



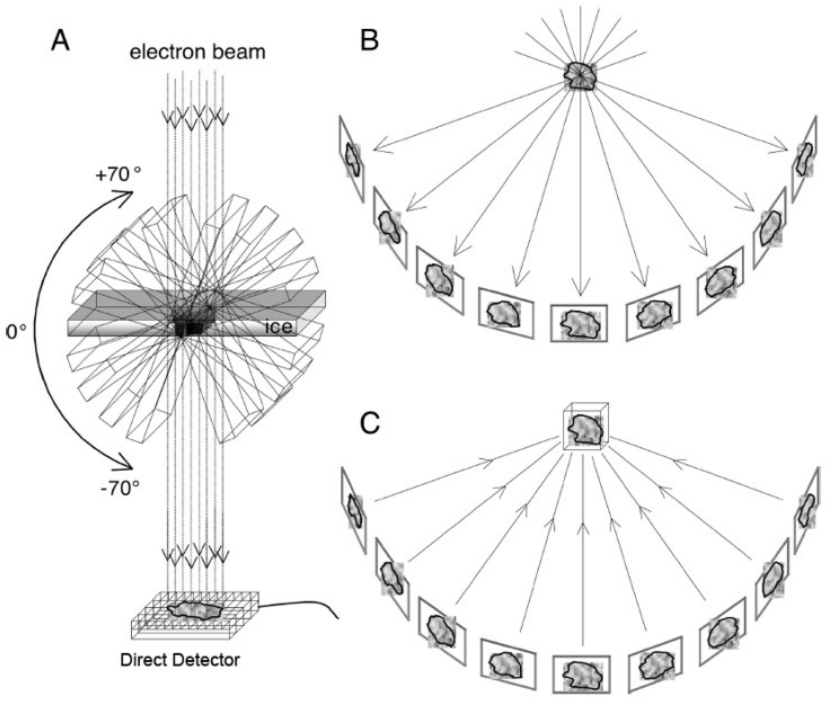


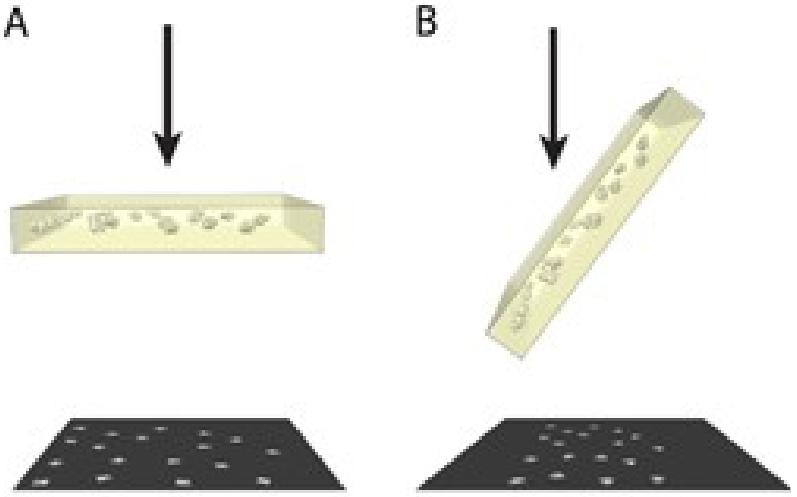




Map validation

- experimental
    - cryo-ET
    - tilt pairs (common-lines)

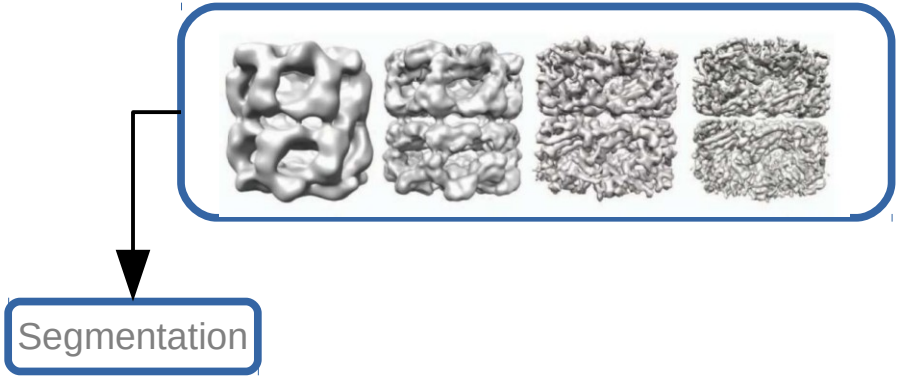




Map validation

- Steps:
  - map is correct at low resolution
  - spurious noise features are not present (noise overfitting, over-refinement)
  - FSC curve has a proper shape
  - resolution estimate corresponds to the observed structural features
  - acquisition of complementary data to confirm the model (e.g. in low resolution)

Map interpretation



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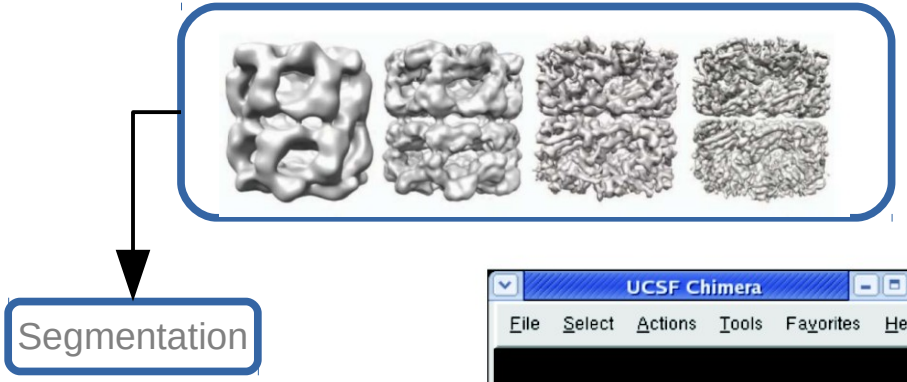
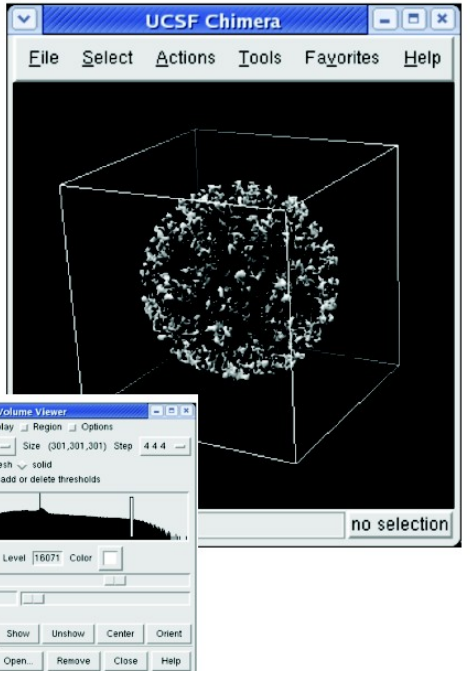




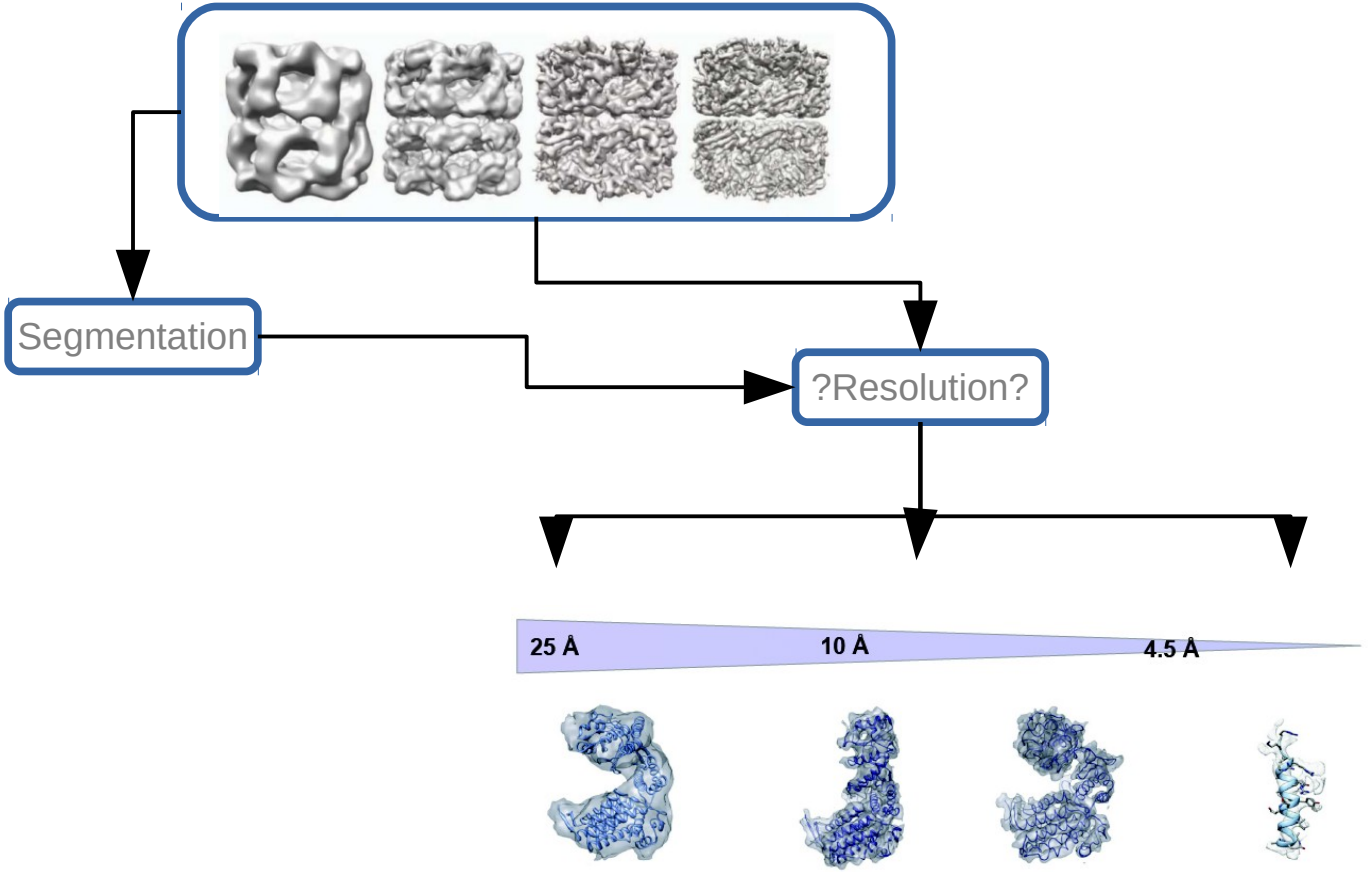
Visualization tools

- Chimera/ChimeraX
- Coot
- PyMol
- VMD
- Amira (Commercial)
- ...

Segmentation

- identify boundaries map regions which represent different structural components
- component structures can be positions into the identified segments
- the size of the segmented components is related to the map resolution
- manual segmentation | automated segmentation | knowledge-based segmentation

Map interpretation



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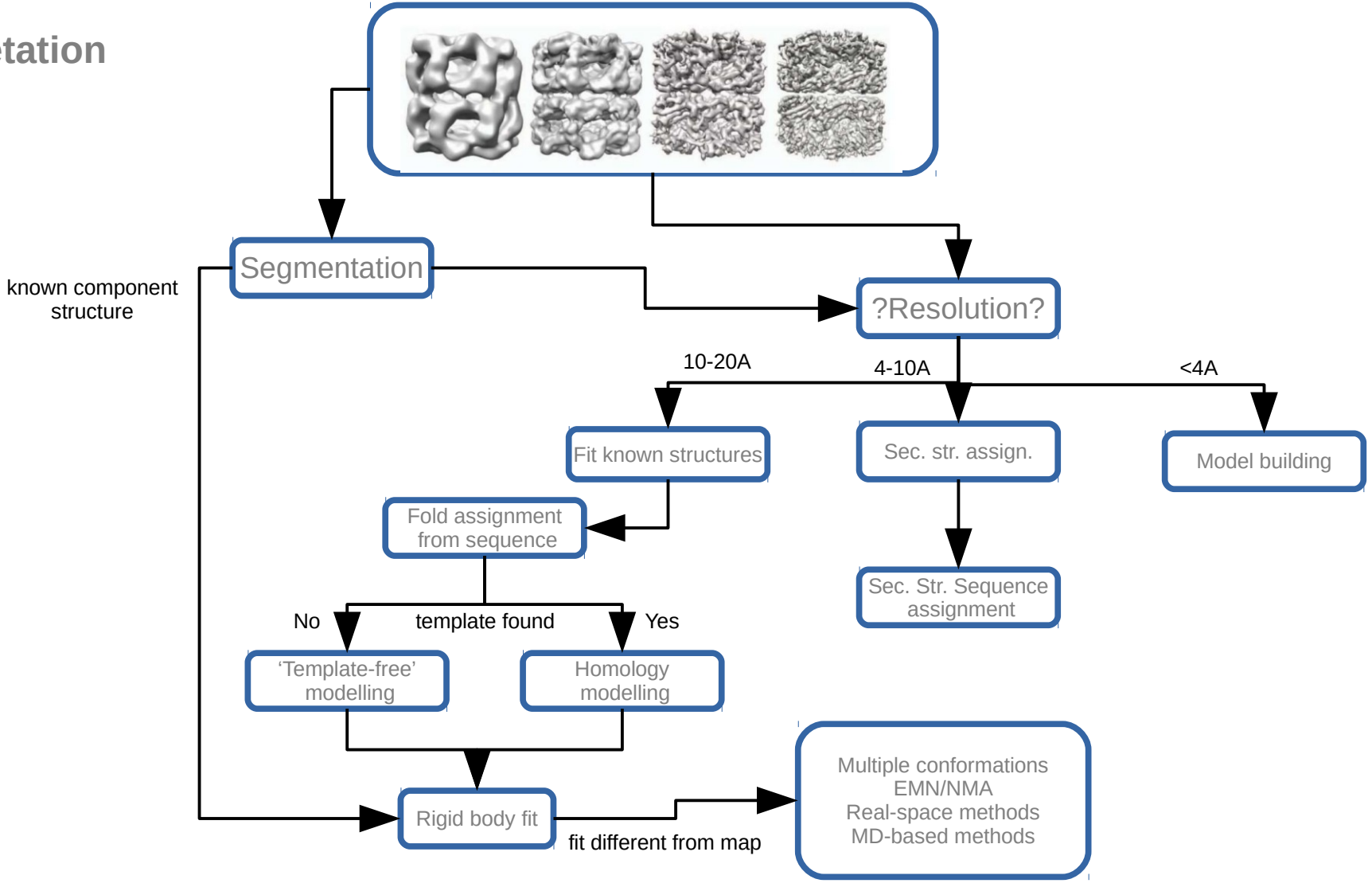
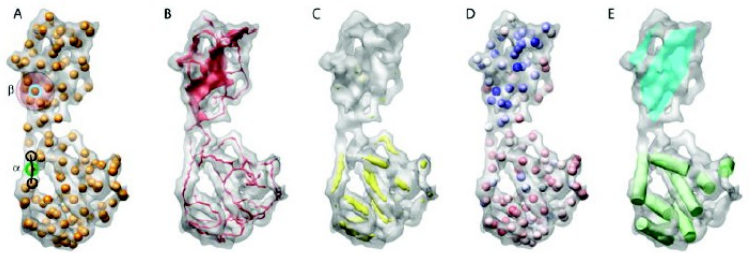


Map interpretation

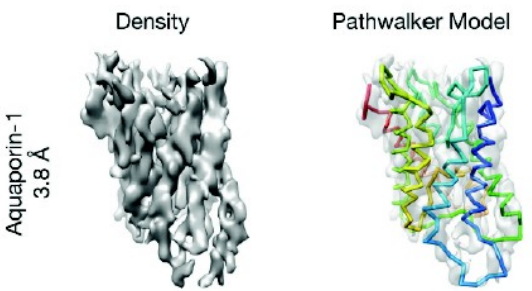
Fold recognition from density

- 4.5-10A: secondary structure detection



Baker et al. *Structure* 2007

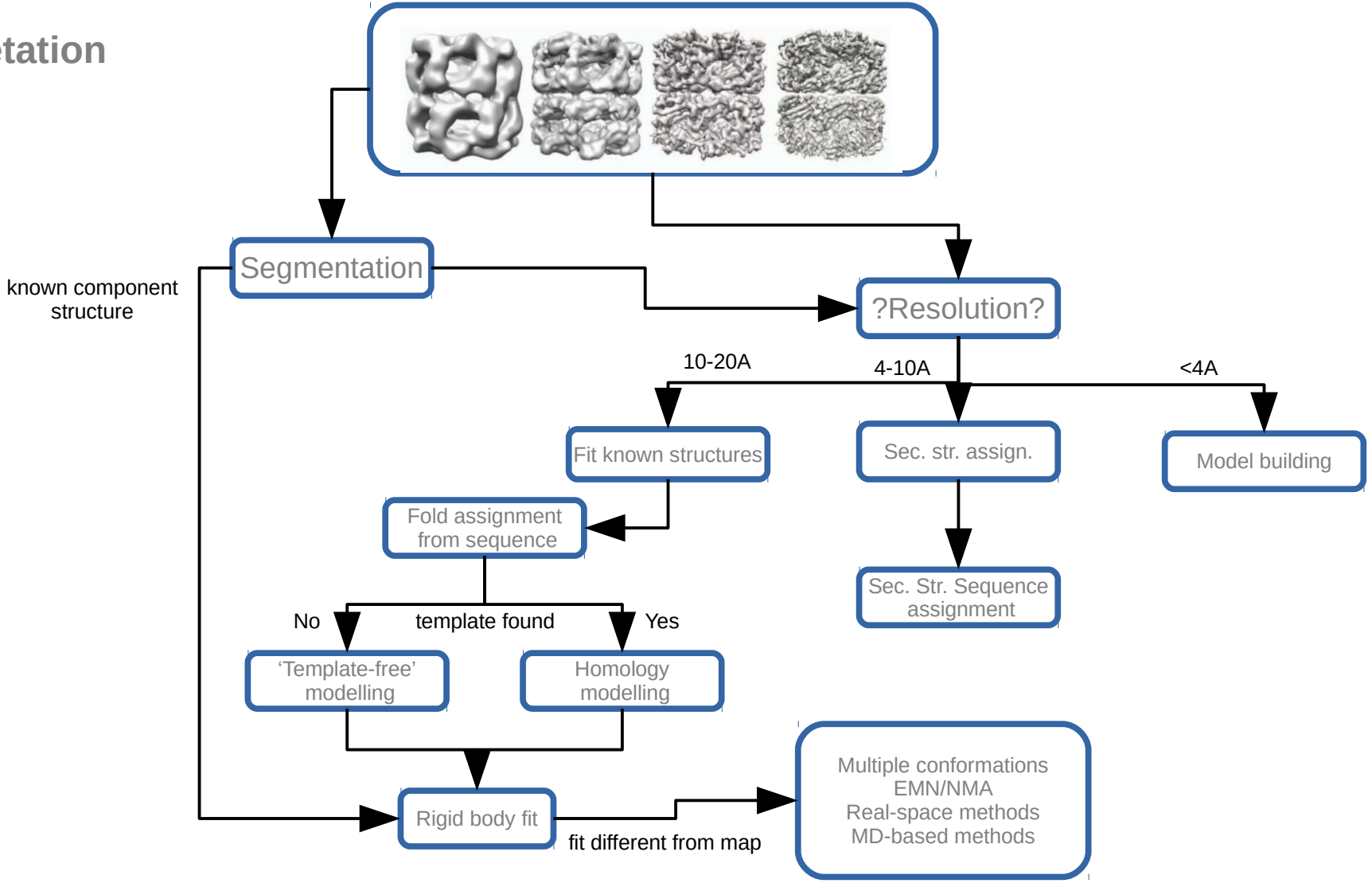
- 4.5A and better: de novo CA tracing and model building



Baker et al. *Structure* 2012

- programs: SSEhunter, SSEtracer, Ematch, Pathwalker, Coot, Buccaneer, EM-fold, Rosseta, Phenix, ARP/wARP, MAINMAST

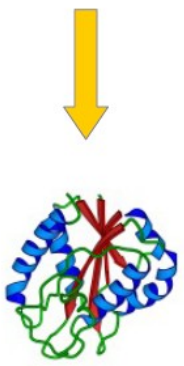
Map interpretation



Map interpretation

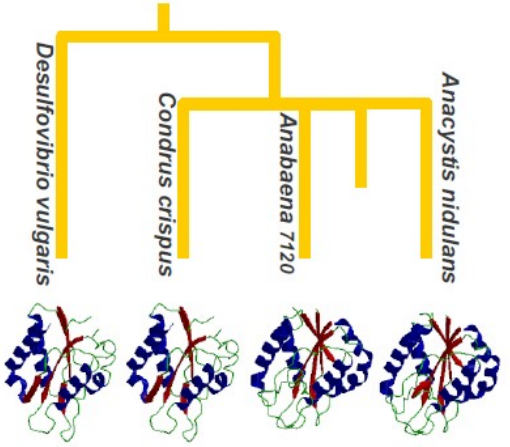
Fold recognition from sequence

GFCHIKAYTRLIMVG…



Template-free

Ab initio (*de novo*) prediction
Fragment Assembly
Evolutionary Couplings



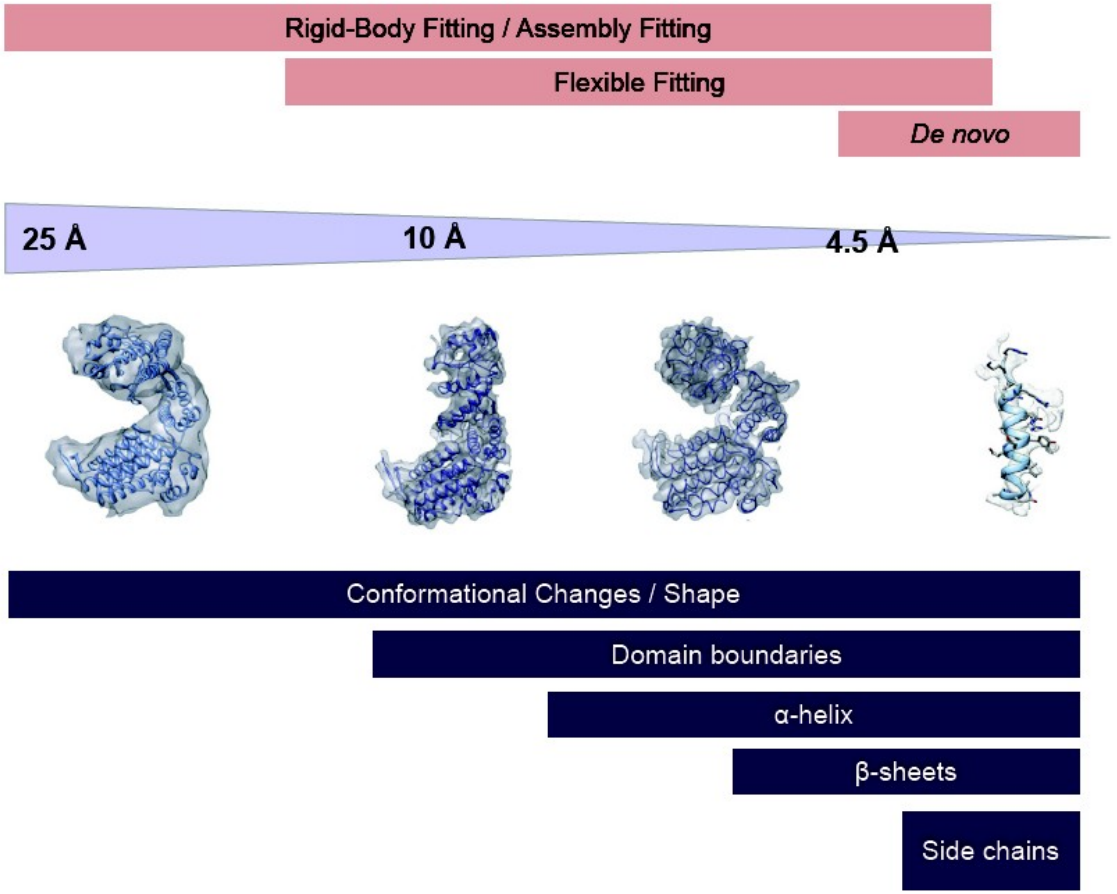
Template-based

Threading
Comparative (Homology) Modelling

- programs: MODELLER, SWISS-MODEL, Phyre2, RaptorX, I-TASSER, Rosetta, EVfold

Map interpretation

Density fitting



Villa & Lasker, *Curr Opin Struct Biol*, 2014,
Cassidy et al, *Curr Opin Microbiology* 2018

Map interpretation

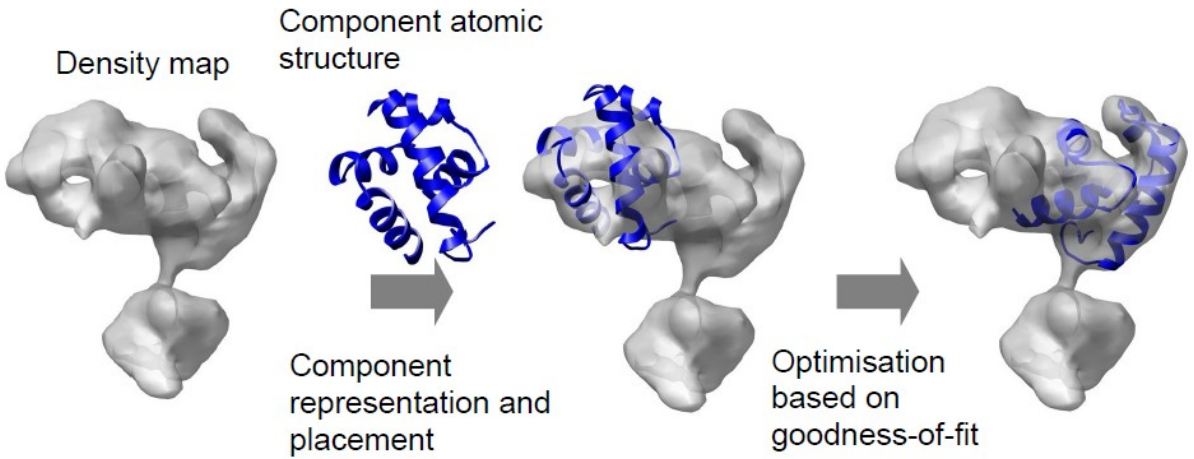
Density fitting

- manual fitting
    - positioning of the atomic structure into the cryo-EM density using visualization programs
    - usually efficient (human brain efficient in pattern recognition)
    - direct feedback
    - good for initial placement of the component in to the map
    - high level of subjectivity may lead to errors
    - depends on contour level at which the map is visualized
    - conformational rearangements cannot be modelled

Map interpretation

Density fitting

- automated fitting
    - requires common representation of both the structure and the density map
    - measure of the quality of the fit
    - optimization protocol for fit improvement



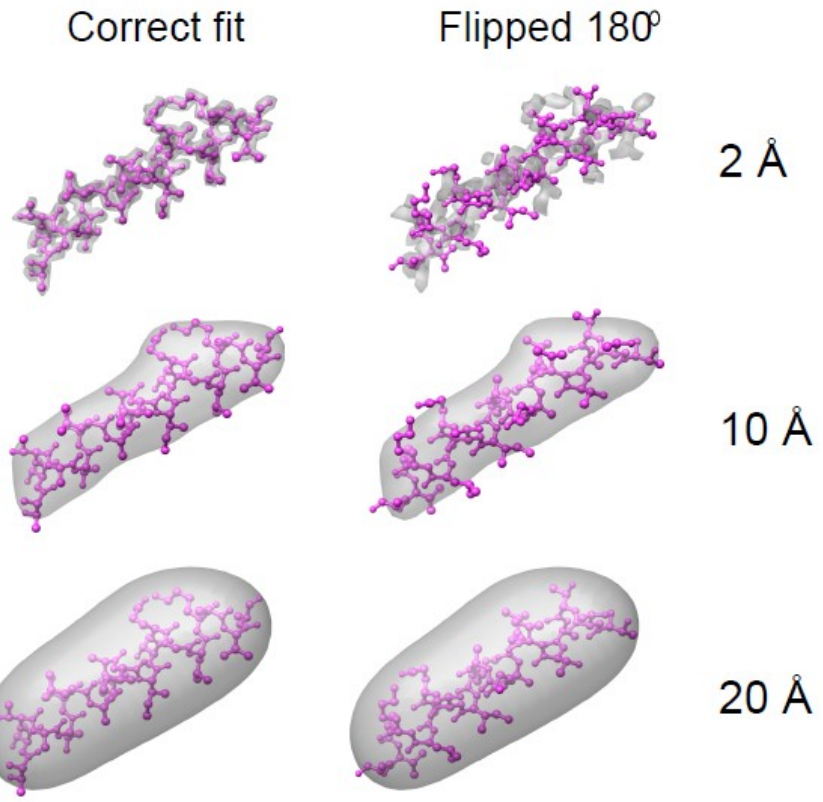
$$CCC_{\rho,lin} = \int_x (\rho_{obs}(\mathbf{x})\rho_{calc}(\mathbf{x},\mathbf{m}))^2 d^3\mathbf{x}$$

Map interpretation

Problems of density fitting

- limited resolution
  - many local optima with similar numerical values at low resolution
  - local resolution, noise, scaling, filtering, masking
  - blurring of the atomic structure

→ better resolution
→ improve scoring for goodness-of-fit
→ coarse-graining (change represenation)
→ fit/model validation

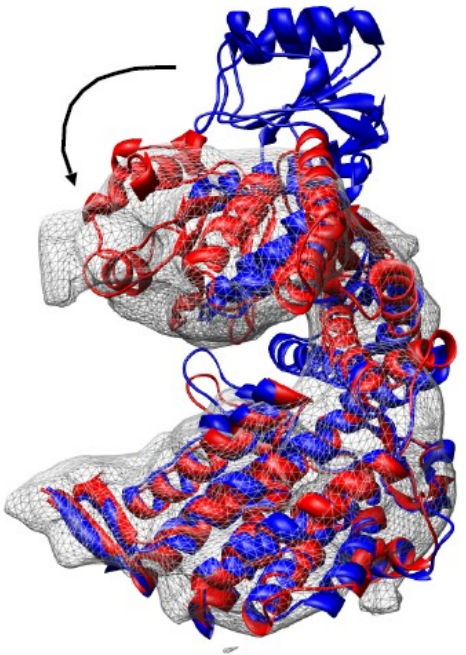


Map interpretation

Problems of density fitting

- conformational variability
    - many lconformations which are observed in density maps deviate from the conformations of the atomic models which are fitted

    - dynamics
    - crystal packing effects
    - errors in structure prediction



→ allow for the conformational changes during model fitting process = flexible fitting

Map interpretation

Model refinement

- without any restraints a model may fit well with a high score in near-atomic to low resolution density

- such a model will, however, not have standard protein geometry: backbone torsions (Ramachandran diagram), peptide planarity, chirality (trans/cis), bond lengths and angles, side chain torsions / rotamers

- refinement methods try to maintain standard geometry while fitting the model into the density map. The geometry restraints reduce the levels of freedom.

- map density contributes as an additional penalty in the scoring function

Programs: MDFF, Refmac, Rosetta, Coot, Phenix, Isolde, iMODFIT

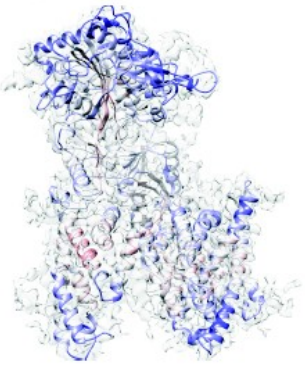
Map interpretation

Model validation

Model fit

Model geometry

peptide planarity
backbone torsions (Ramachandran)
bond lengths
bond angles
side chain rotamers



Molprobity: http://molprobity.biochem.duke.edu/
What check: http://swift.cmbi.ru.nl/gv/whatcheck/
PROCHECK: http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/

Map improvement

- in order to facilitate map interpretation, the data processing should correct for the imperfections of the imaging system to the highest possible level

- these imperfections comprise:
    - aberrations of microscope optical system (higher-order)
    - sample drift and distortions caused by interaction of the electrons with a matter

- the effect is primarily pronounced at high frequencies (resolution) → parameter optimization and additional data processing primarily concerns improving the quality of high resolution maps (<4.5A resolution)

- the effect on medium and low resolution (>8A) is limited and additional data processing usually does not result in any map improvement

Map improvement

Electron lens aberrations

- objective lens of the transmission electron microscope is really bad

$$B(\mathbf{k}) = \exp\left[i\frac{2\pi}{\lambda}W(\mathbf{k})\right]$$

2.2: Description of aberration constants to 6th order

A_0	Lateral image shift
A_1	Two-fold astigmatism
C_1	Defocus
A_2	Three-fold astigmatism
B_2	Axial coma
A_3	Four-fold astigmatism
S_3	Axial star aberration
$C_3 = C_s$	Spherical aberration
A_4	Five-fold astigmatism
D_4	Three-lobe aberration
B_4	Fourth-order axial coma
A_5	Six-fold astigmatism
S_5	Fifth-order star aberration
C_5	Fifth-order spherical aberrat
R_5	Fifth-order rosette aberratior

$$\begin{aligned}W(\mathbf{k}) =& \Re\{A_0\lambda\mathbf{k}^* \\ &+ \frac{1}{2}A_1\lambda^2\mathbf{k}^{*2} + \frac{1}{2}C_1\lambda^2\mathbf{k}^*\mathbf{k} \\ &+ \frac{1}{3}A_2\lambda^3\mathbf{k}^{*3} + \frac{1}{3}B_2\lambda^3\mathbf{k}^{*2}\mathbf{k} \\ &+ \frac{1}{4}A_3\lambda^4\mathbf{k}^{*4} + \frac{1}{4}S_3\lambda^4\mathbf{k}^{*3}\mathbf{k} + \frac{1}{4}C_3\lambda^4\mathbf{k}^{*2}\mathbf{k}^2 \\ &+ \frac{1}{5}A_4\lambda^5\mathbf{k}^{*5} + \frac{1}{5}D_4\lambda^5\mathbf{k}^{*4}\mathbf{k} + \frac{1}{5}B_4\lambda^5\mathbf{k}^{*3}\mathbf{k}^2 \\ &+ \frac{1}{6}A_5\lambda^6\mathbf{k}^{*6} + \frac{1}{6}S_5\lambda^6\mathbf{k}^{*4}\mathbf{k}^2 + \frac{1}{6}C_5\lambda^6\mathbf{k}^{*3}\mathbf{k}^3 + \frac{1}{6}R_5\lambda^6\mathbf{k}^{*5}\mathbf{k}\end{aligned}$$

Map improvement

Zernike polynomials

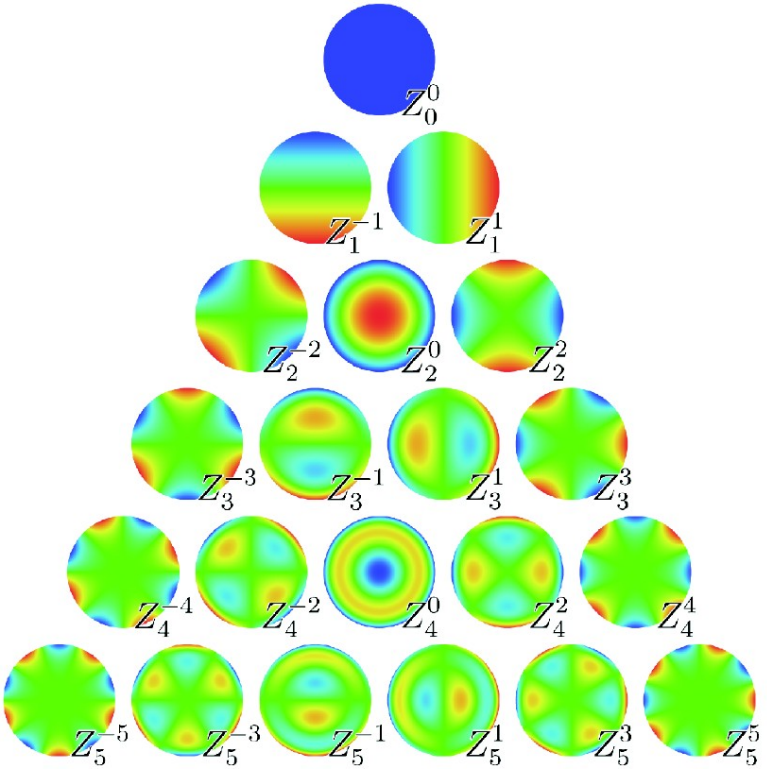
- complete set of orthogonal functions

- Zernike transform analogous to Fourier transform

- can be used to visualize lens aberrations

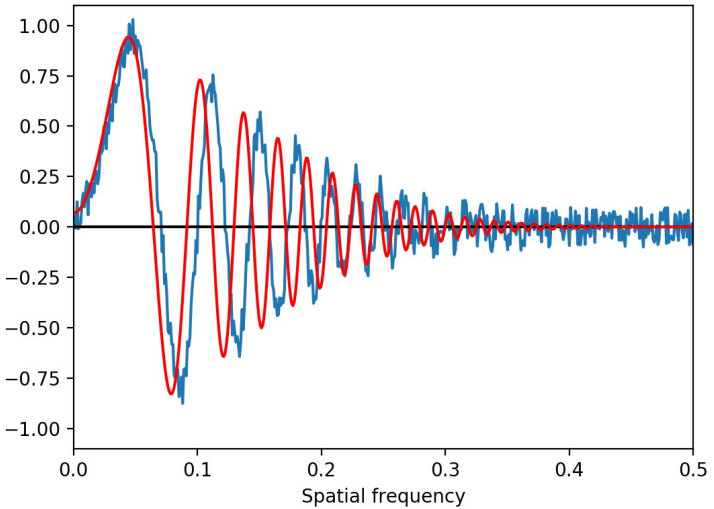
- the aberrations can be corrected for by introducing additional lens to the microscope or by software during the image processing

Frits Zernike,
1953 Nobel Prize in Physics
inventor of phase contrast microscopy

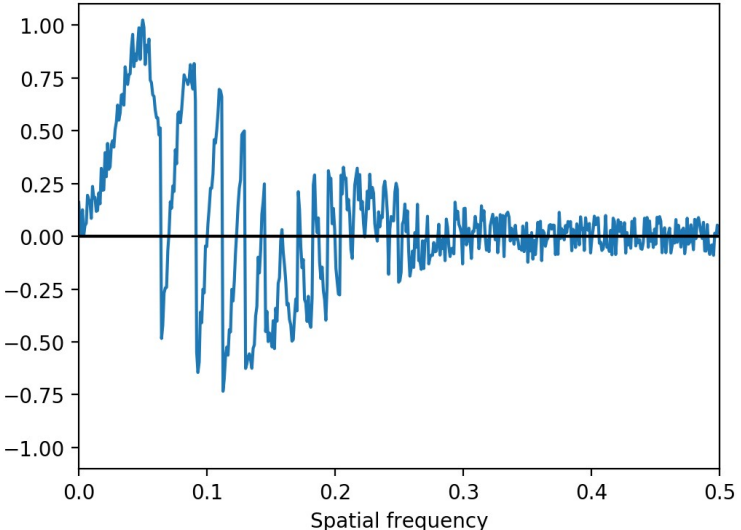


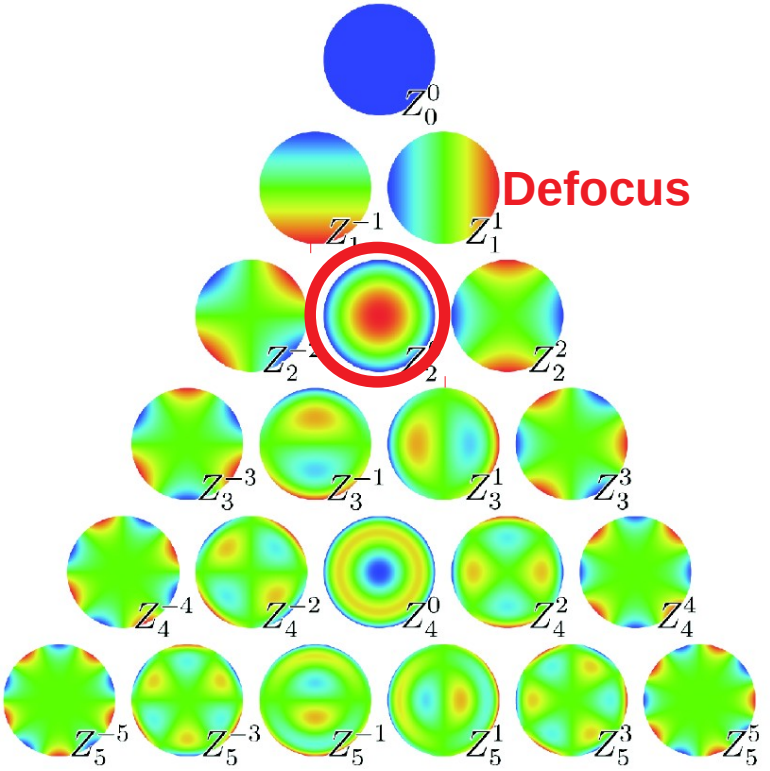
Map improvement

Lens aberrations



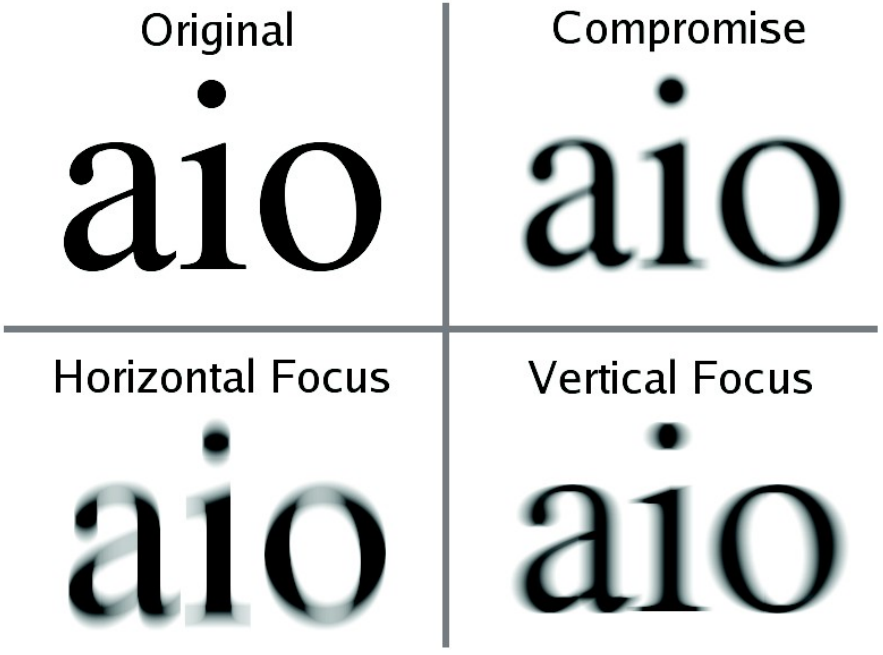
- 200nm error in defocus estimation (1.2um instead of 1.0um)

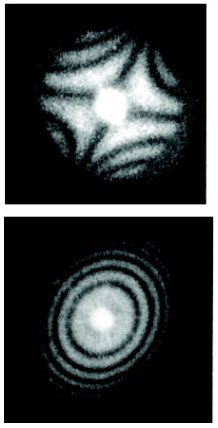


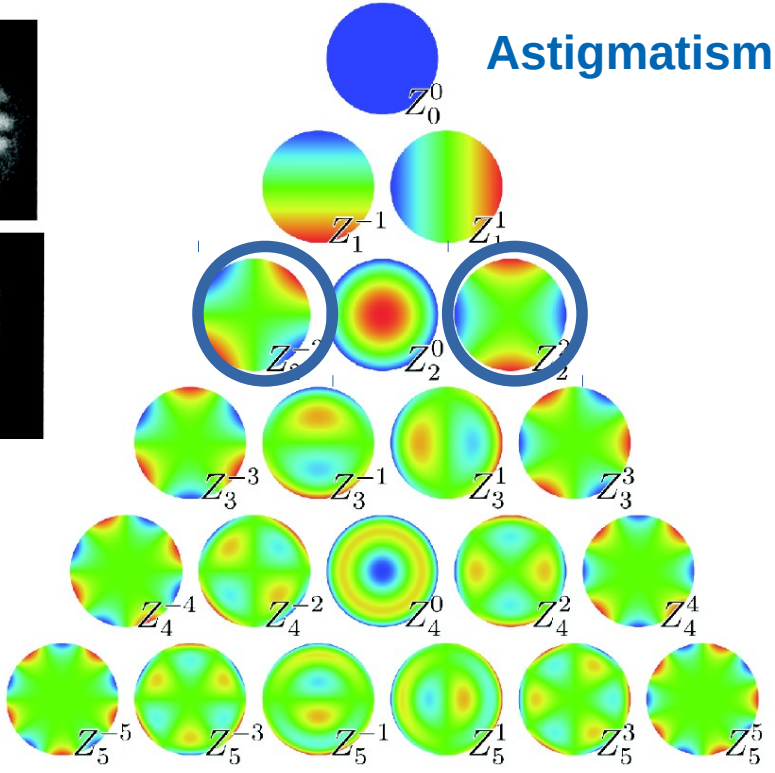


Map improvement

Lens aberrations







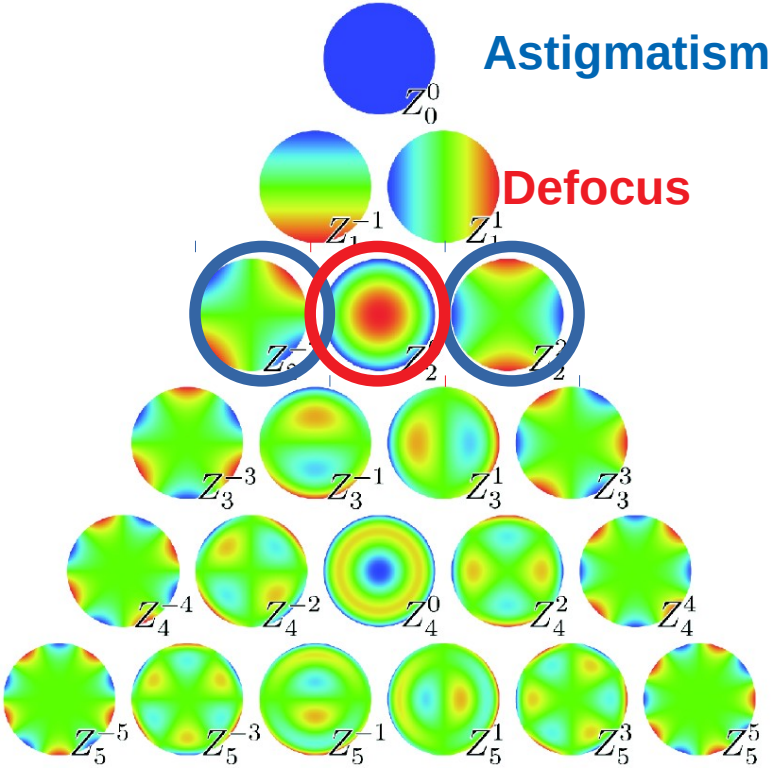
Map improvement

Lens aberrations

- certain level of underfocus is necessary during cryo-EM data collection
  → corrected during CTF correction
- astigmatism can be eliminated to high extent by proper microscope alignment

- only aberrations which are relevant for the quality of medium and low resolution maps

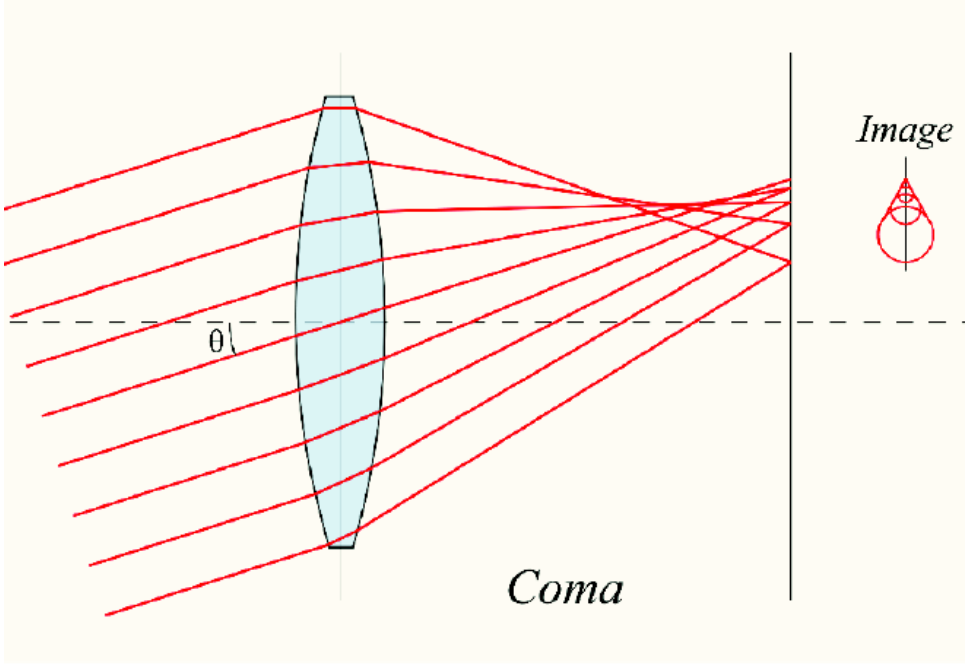
- correct estimation of CTF parameters (defocus,astigmatism)
  → quality control – goodness of fit

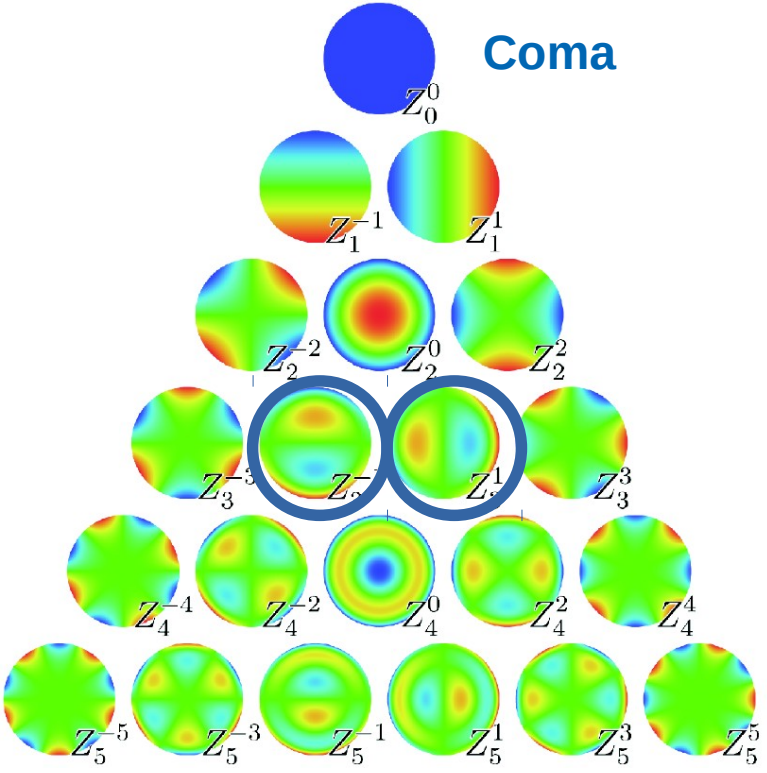


Map improvement

Lens aberrations

- dependence with third power of frequency
- can be primarily removed by proper microscope alignment and further during data analysis in software

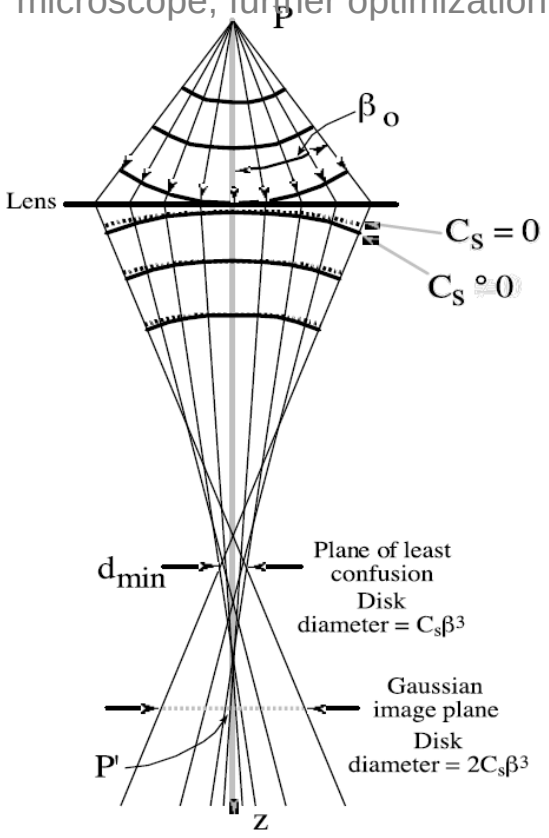


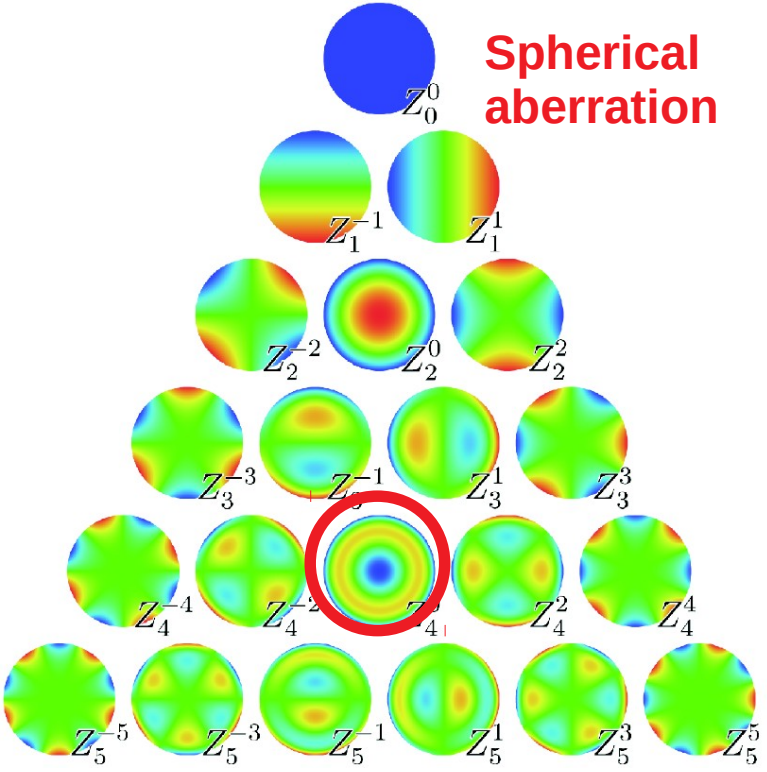


Map improvement

Lens aberrations

- dependence on fourth power of the frequency
- lens is stronger off axis, plane of least confusion
- considered constant for microscope, further optimization in software possible

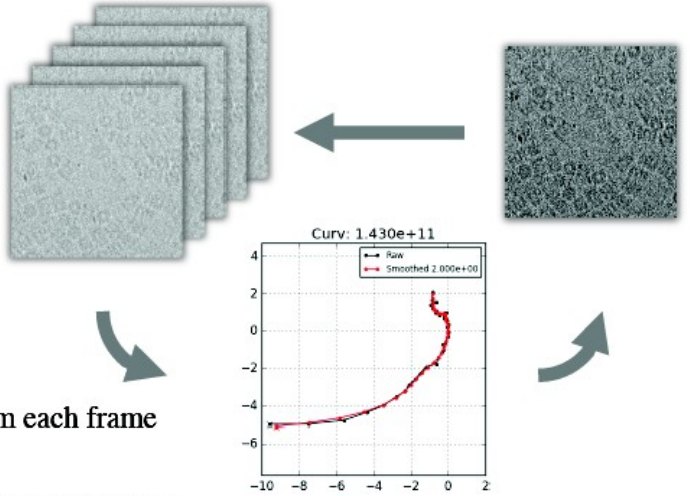




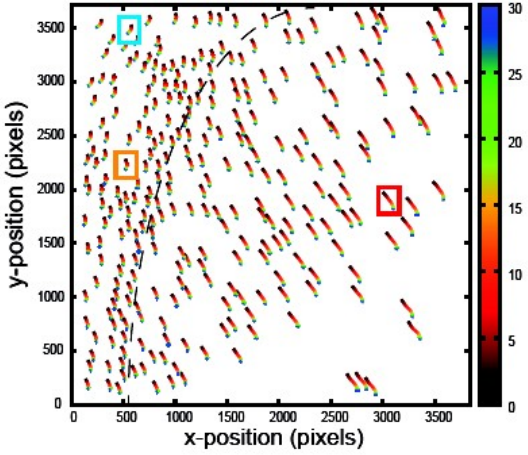
Map improvement

Sample distorsions during imaging

- local motion different in distinct parts of the image

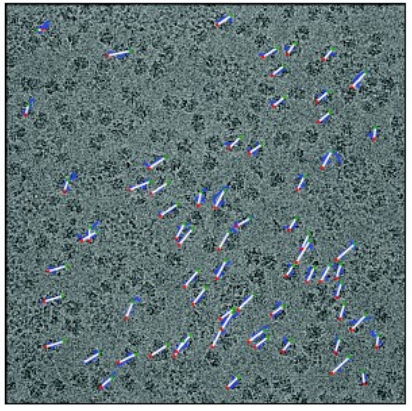


Compare particle in each frame to sum of frames



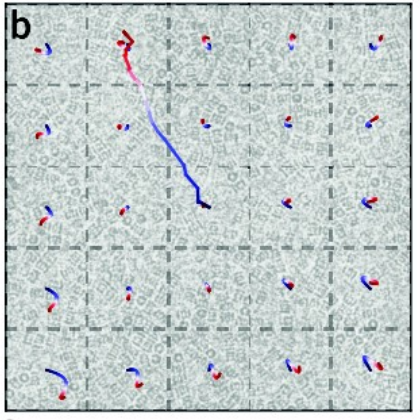
Alignparts_lmbfgs (Rubinstein & Brubaker, 2015, *JSB* 192, 188-95)
[improved version in cryoSPARC ver 2]

Compare particle in each frame to map



Relion Polishing (Scheres, 2014, *eLife 3:e03665*)
[improved version with Alignparts-like smoothing in Bayesian polishing]

Compare patch from each frame to sum of frames



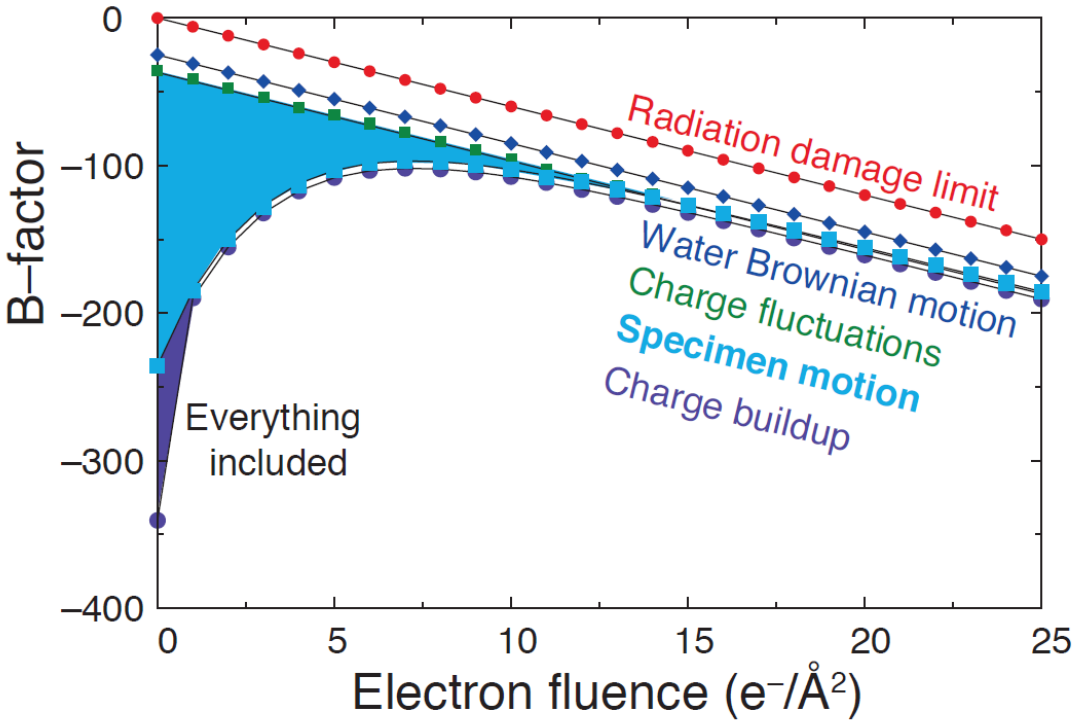
MotionCor2 (Zheng…Agard, 2017, *Nat Meth* 14, 331-2)

Map improvement

Sample distorsions during imaging

- the information in each frame is damped by different B-factor due to distinct effects during data collection

- compensation for local motion (per particle) + per frame amplitude weighting with corresponding B-factor => particle polishing



Map improvement

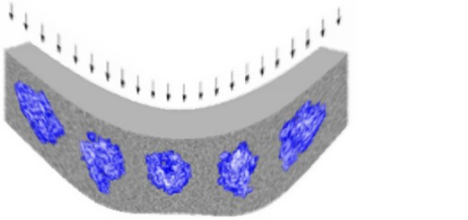
Sample distorsions during imaging

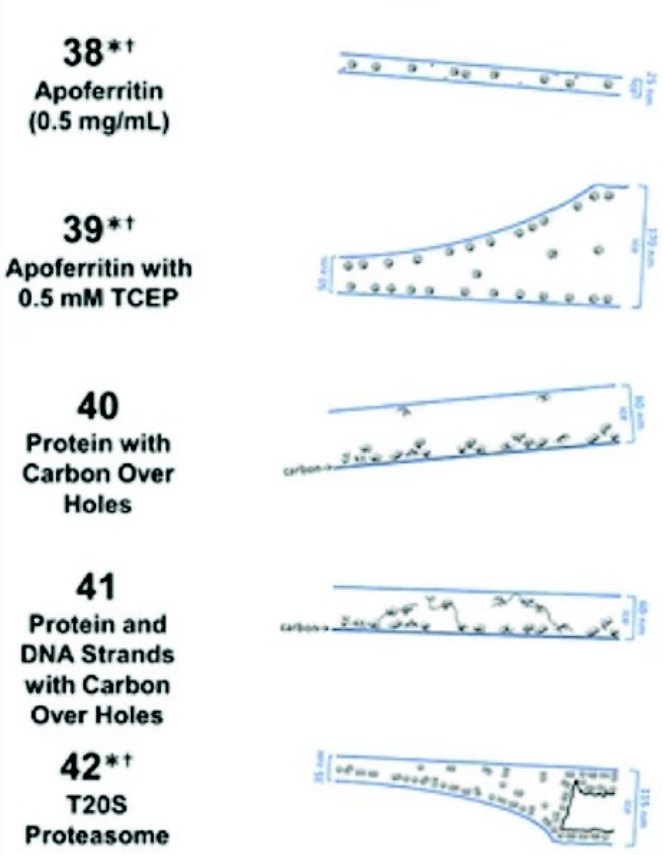
- distortion of sample surface due to illumination with electron beam

- particles located in different depth of the specimen layer

  → defocus variance for particles within single micrograph

- per particle defocus (astigmatism) estimation = ctf refinement





Noble et al eLife 2018