Single particle cryo-EM

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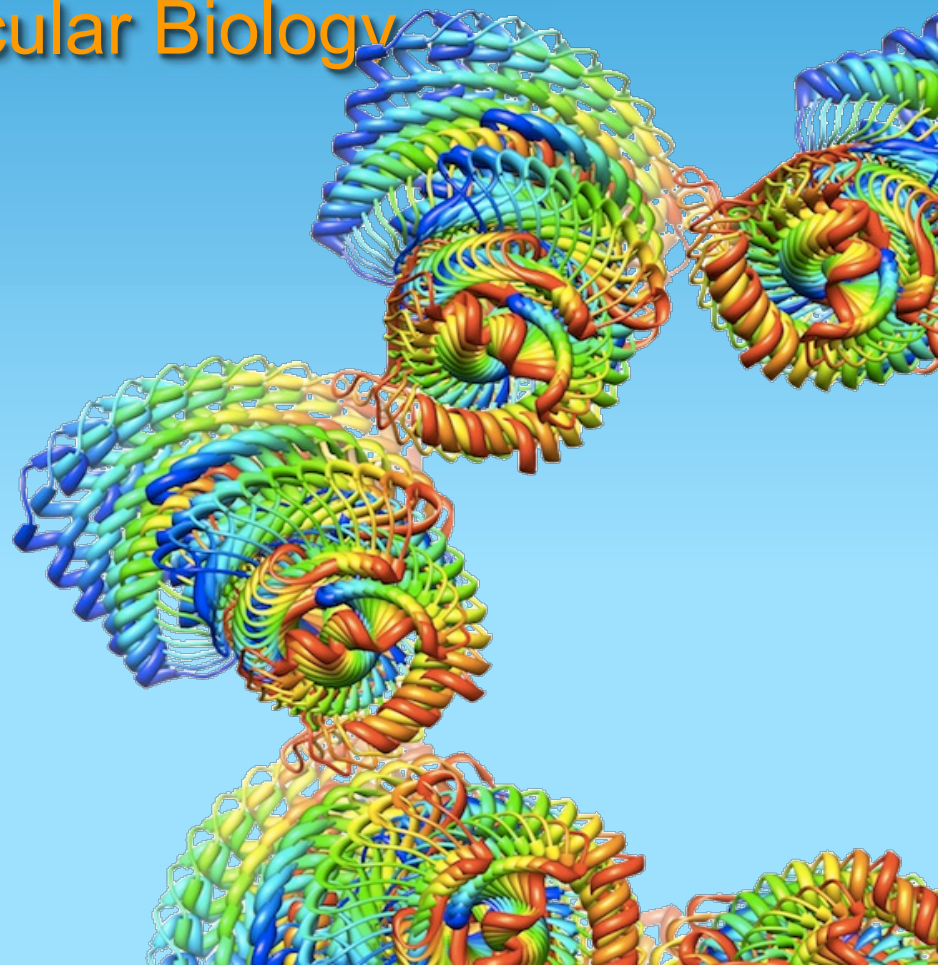
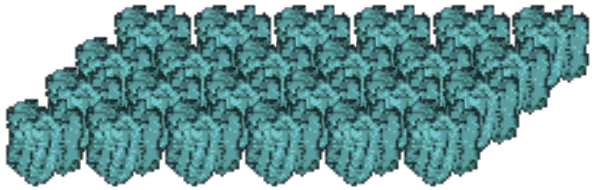


Institute of Structural Molecular Biology
ISMB

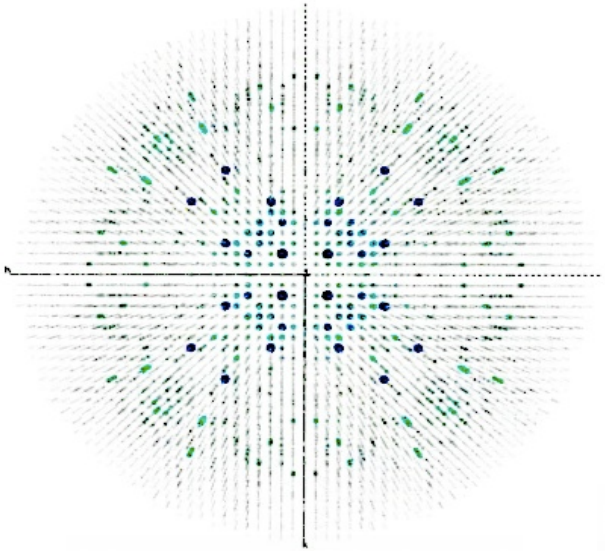
Birkbeck
UNIVERSITY OF LONDON

TEM in structural and cellular biology

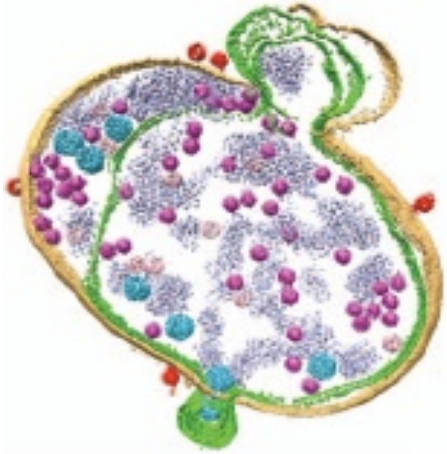


2D crystals

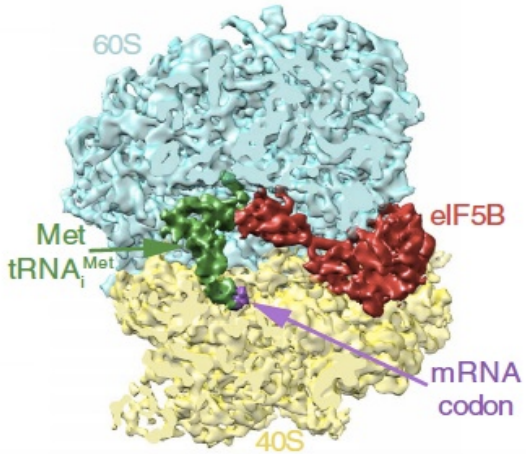
Electron crystallography
(views at different tilts)



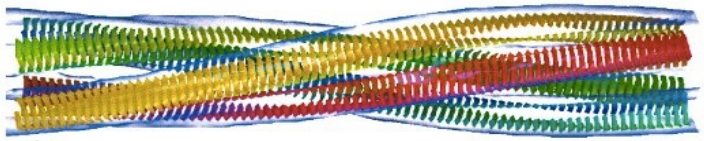
Microcrystal (<1 μm)
electron diffraction



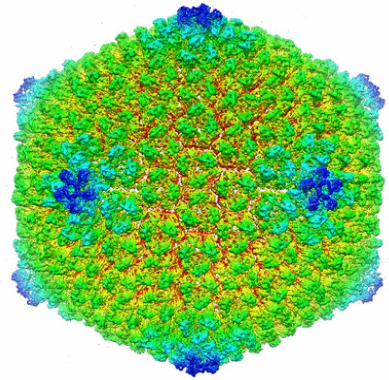
Whole cells or organelles
(**tomography** of unique objects,
cumulative irradiation)



Asymmetric **single particles**



Helical assemblies

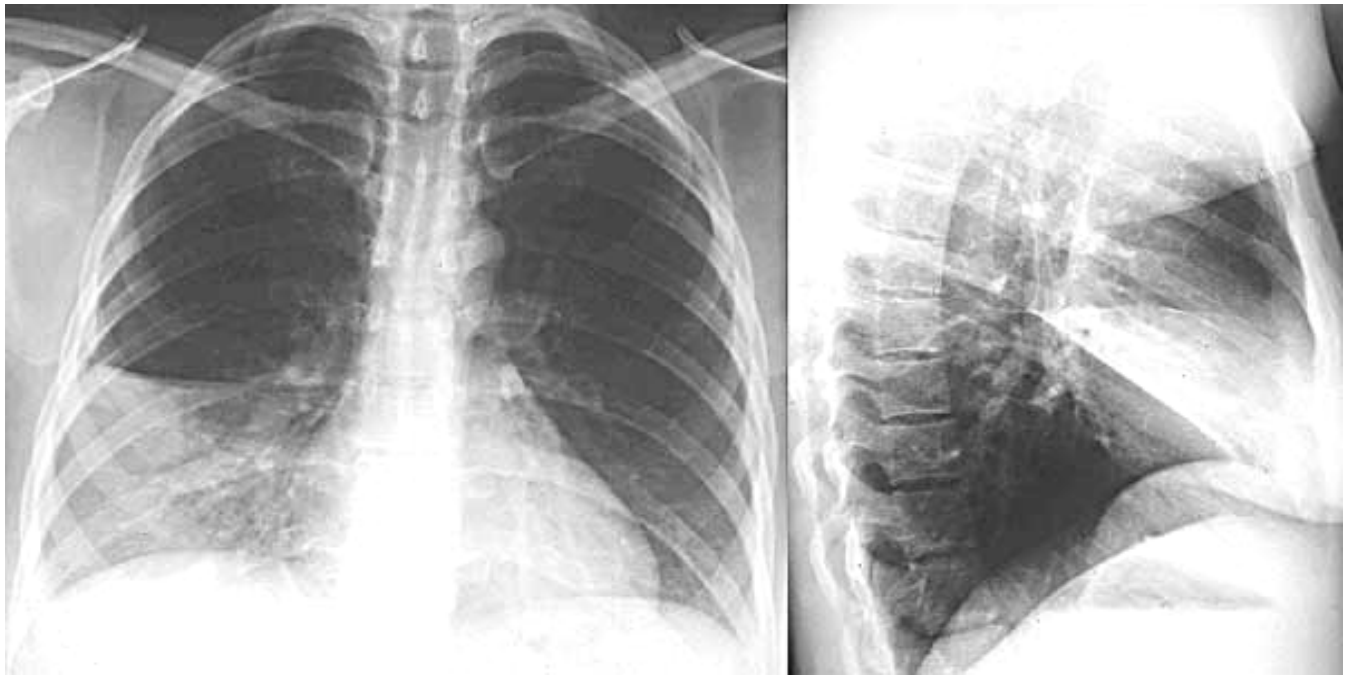


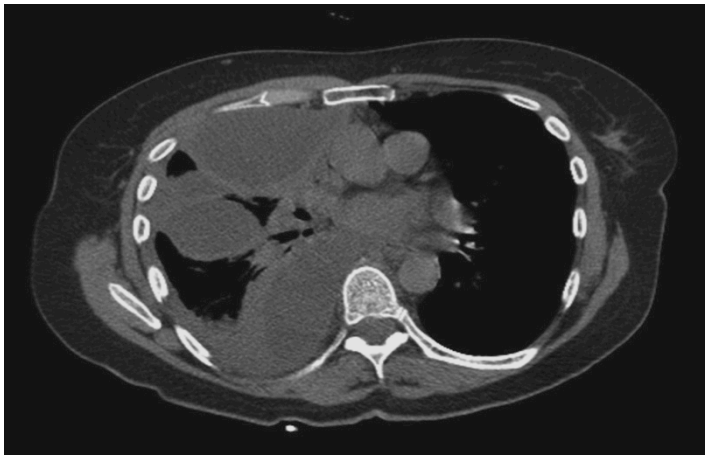
Icosahedral viruses

How is the EM image formed?

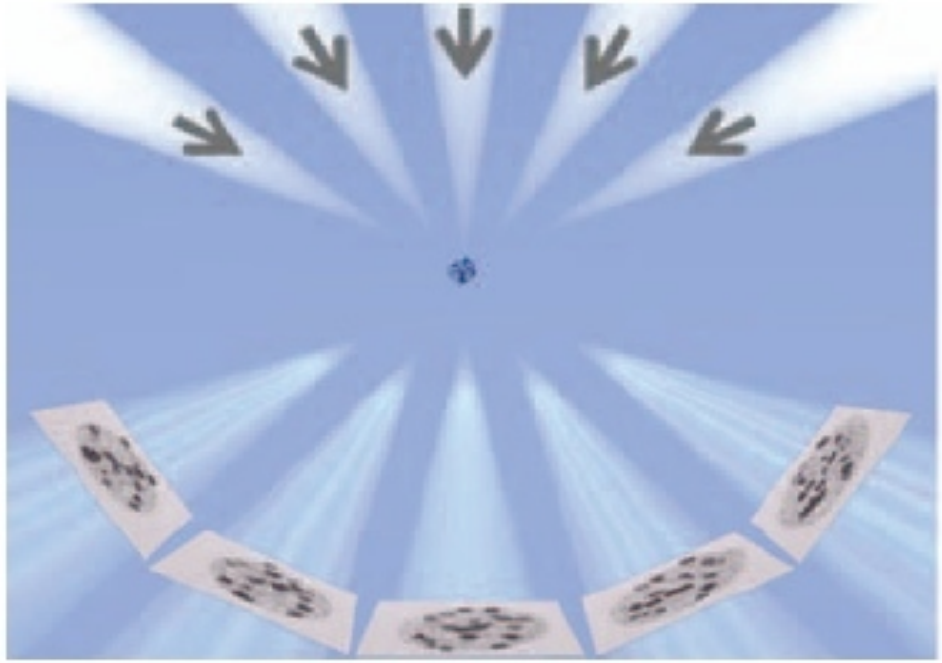
- Thin specimen scatters electrons
- Interference between scattered and unscattered electrons gives phase contrast image
- Image is 2D projection of original 3D object
- 3D structure can be determined from a set of views at different orientations
- Beam damage is the ultimate limit on resolution

Projections and sections

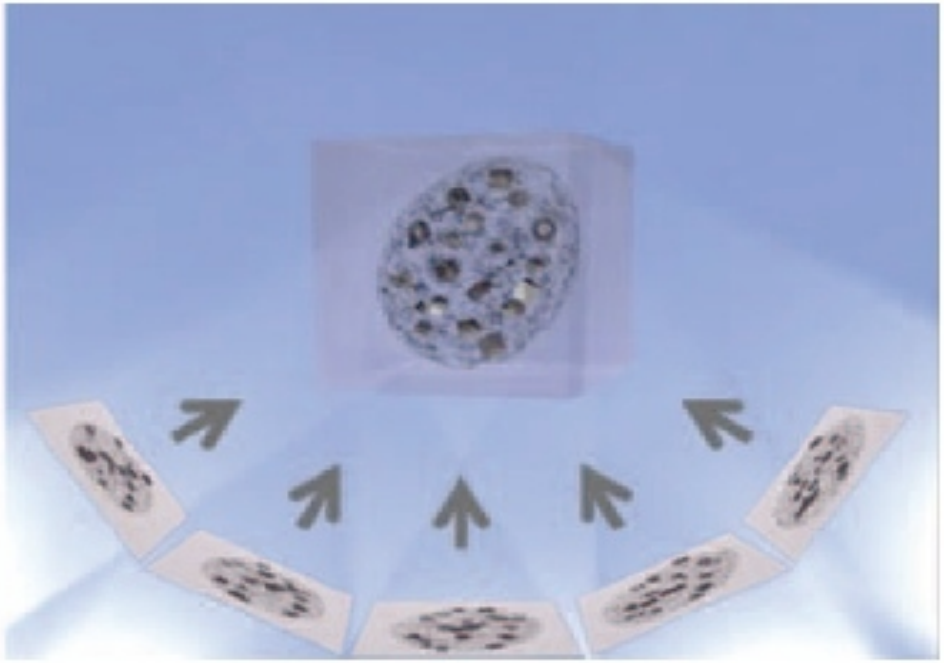




Tomographic reconstruction



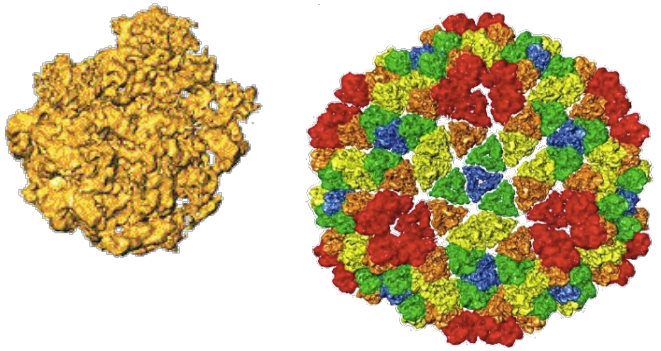
3D-object => set of 2D-projections



2D-projections => 3D-reconstruction

W Baumeister, MPI Martinsried

Single particle EM



- Isolated macromolecular complexes
- Randomly oriented in solution
- Can be trapped in different reaction states by vitrification
- No crystallization or ordered assembly needed
- The position and orientation of each particle must be determined for 3D reconstruction
- The more particles used, the higher the resolution (<2 Å)
- Mixed states can sometimes be separated (“purification in the computer”)
- Ultimate limit to resolution is radiation damage
- Interpretation by atomic structure docking or direct determination of backbone

Negative stain vs cryo EM

Negative staining

- Simple procedure
- Quick to check samples
- High contrast
- Dehydration
- Heavy metal salts
- Possible distortion, flattening

Cryo EM

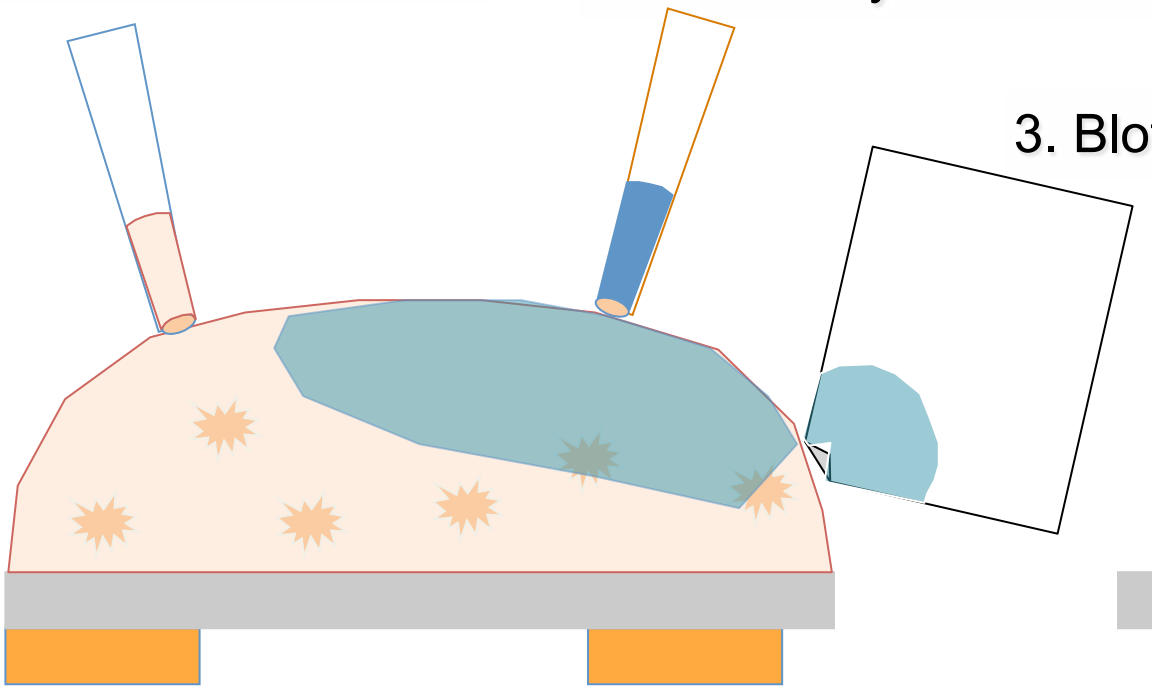
- More complex preparation
- Longer time for checking samples
- Low contrast
- Native, hydrated state
- Near physiological conditions
- 3D structure preserved
- Rapid freezing can trap transient states

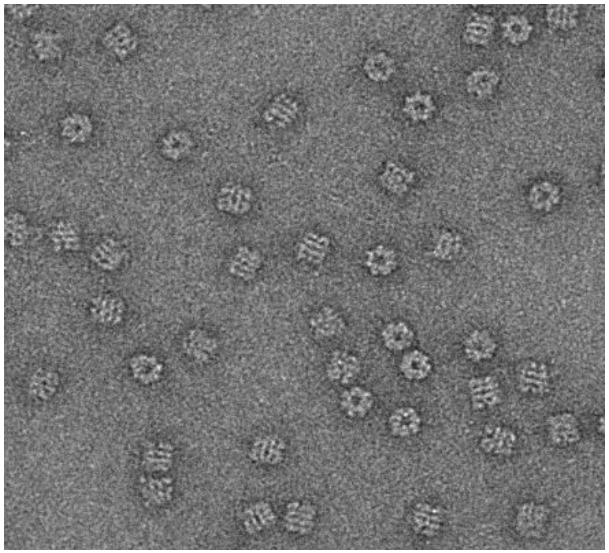
Sample preparation: Negative stain EM

1. Add protein in buffer

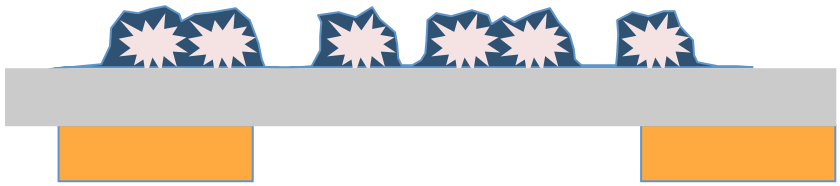
2. Add heavy metal stain

3. Blot

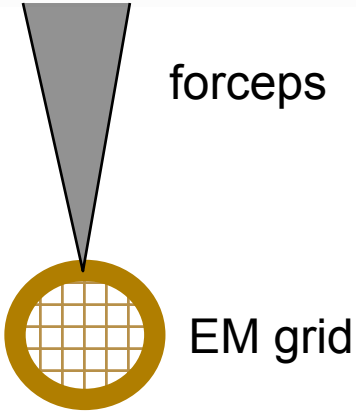


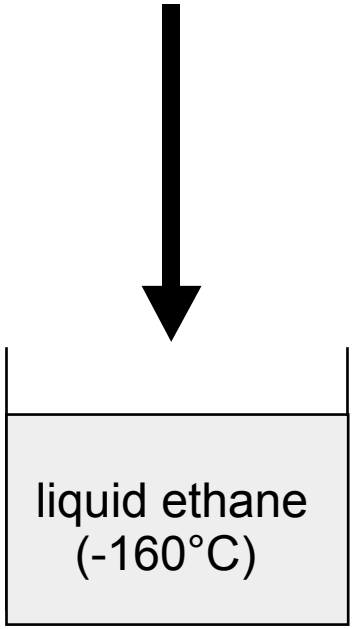


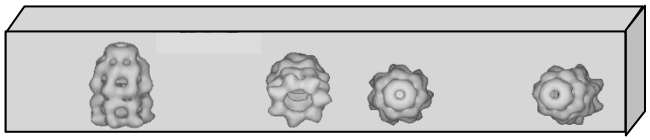
4. Air dry



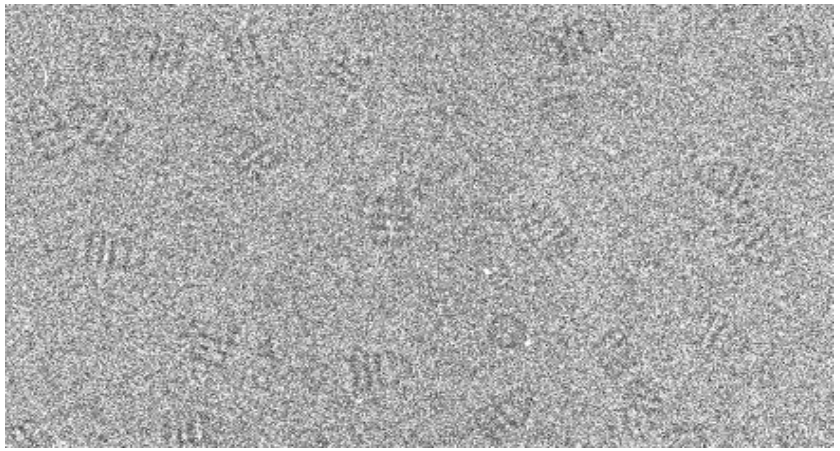
Sample preparation: Cryo EM





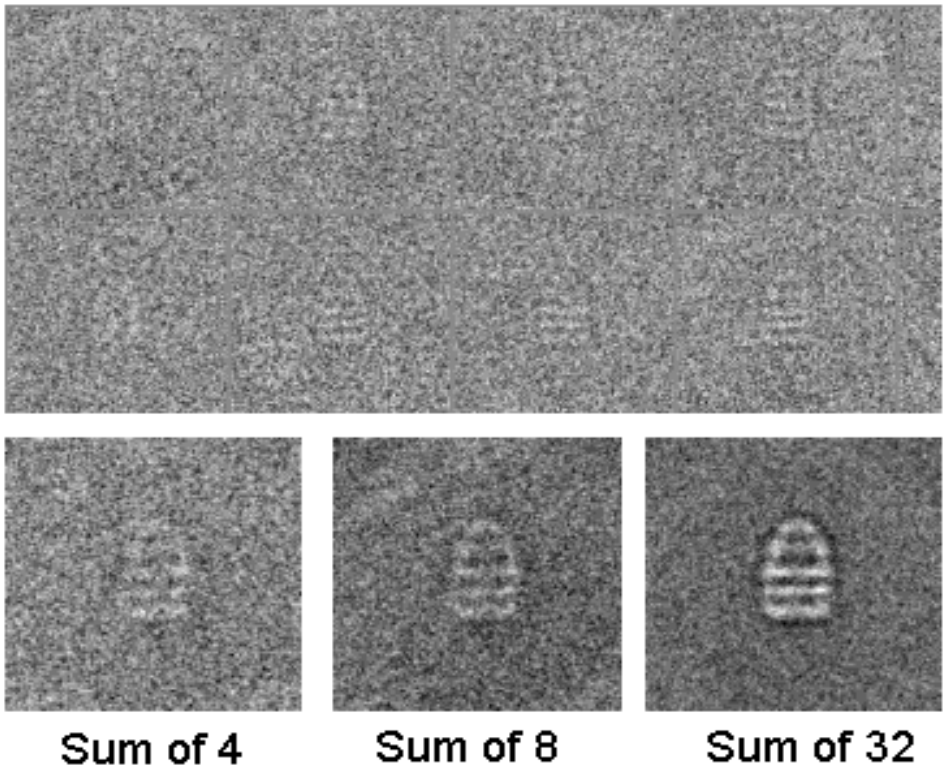


Edge-on view of an unsupported part of the water layer



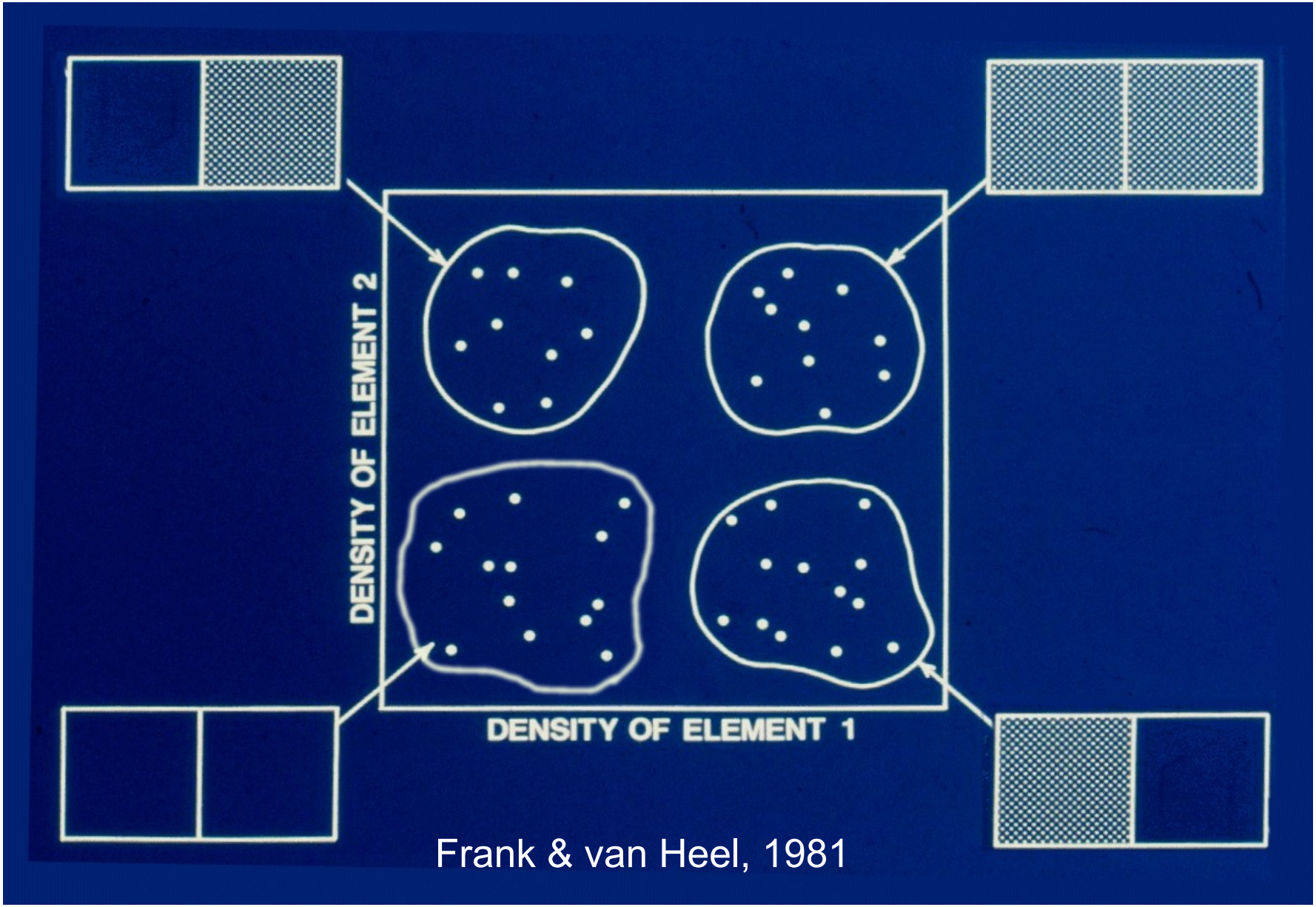
image

Averaging similar views improves the signal:noise ratio



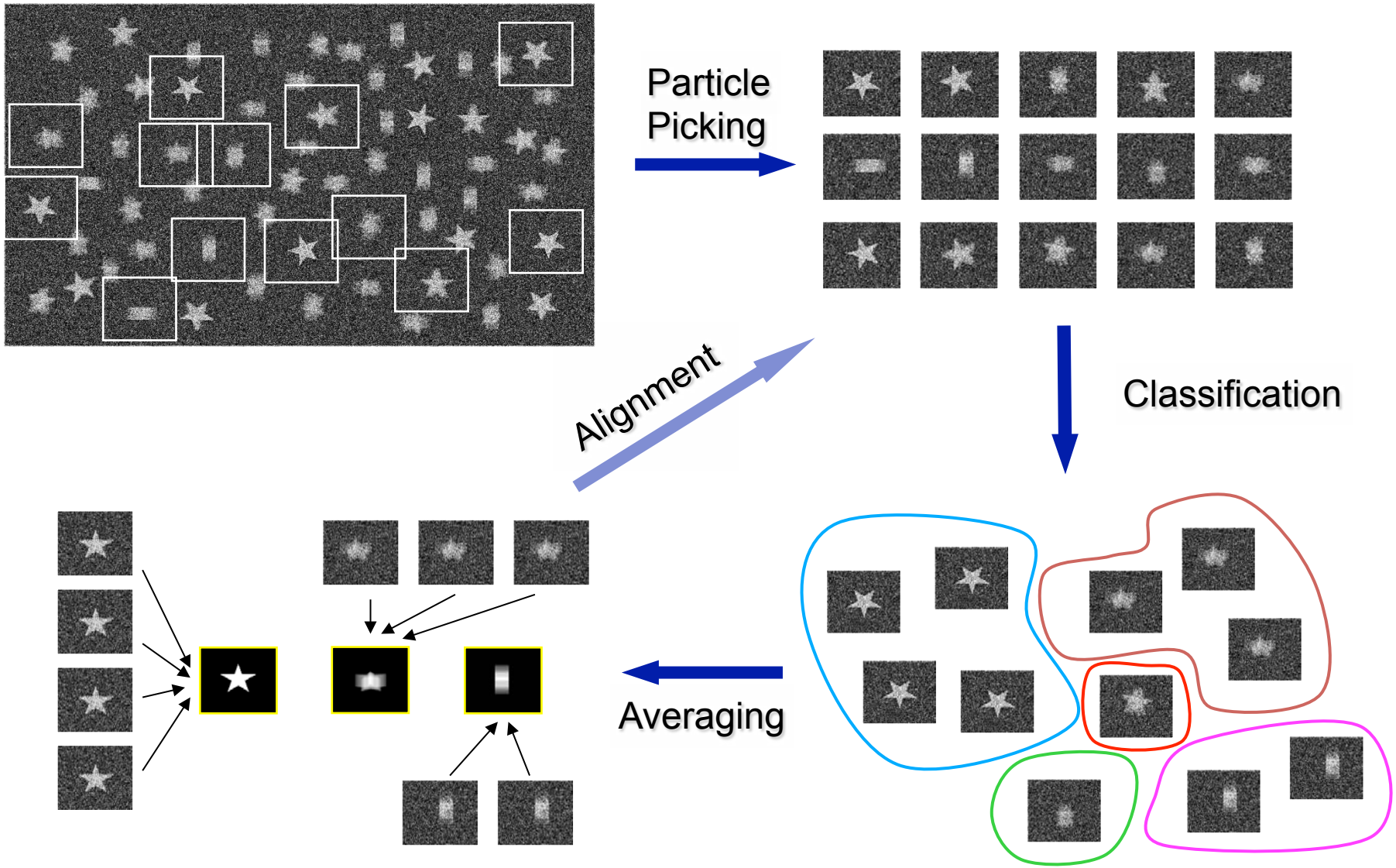
Individual raw images

Classification of images: Multivariate statistical analysis



Frank & van Heel, 1981

Single Particle Image Processing



Getting a starting model for a new structure

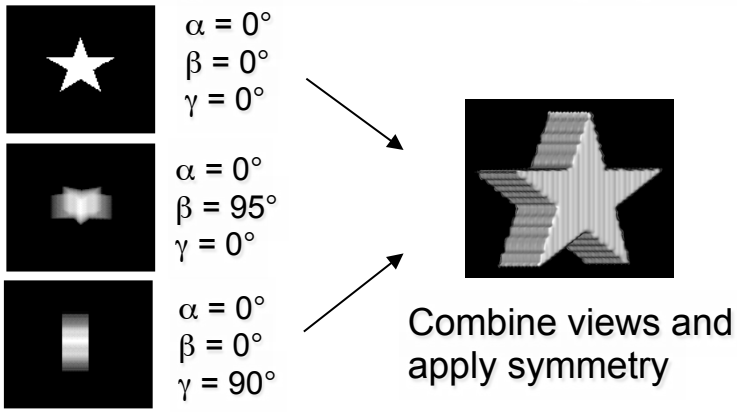
Experimental approaches

In **random conical tilt**, pairs of images are recorded of the same field of particles at high tilt and untilted, and the known angles are used to generate a 3D reconstruction (Frank, 1998).

More recently, **tomography** and **sub-tomogram averaging** have been used to determine an initial structure.

Computational approaches

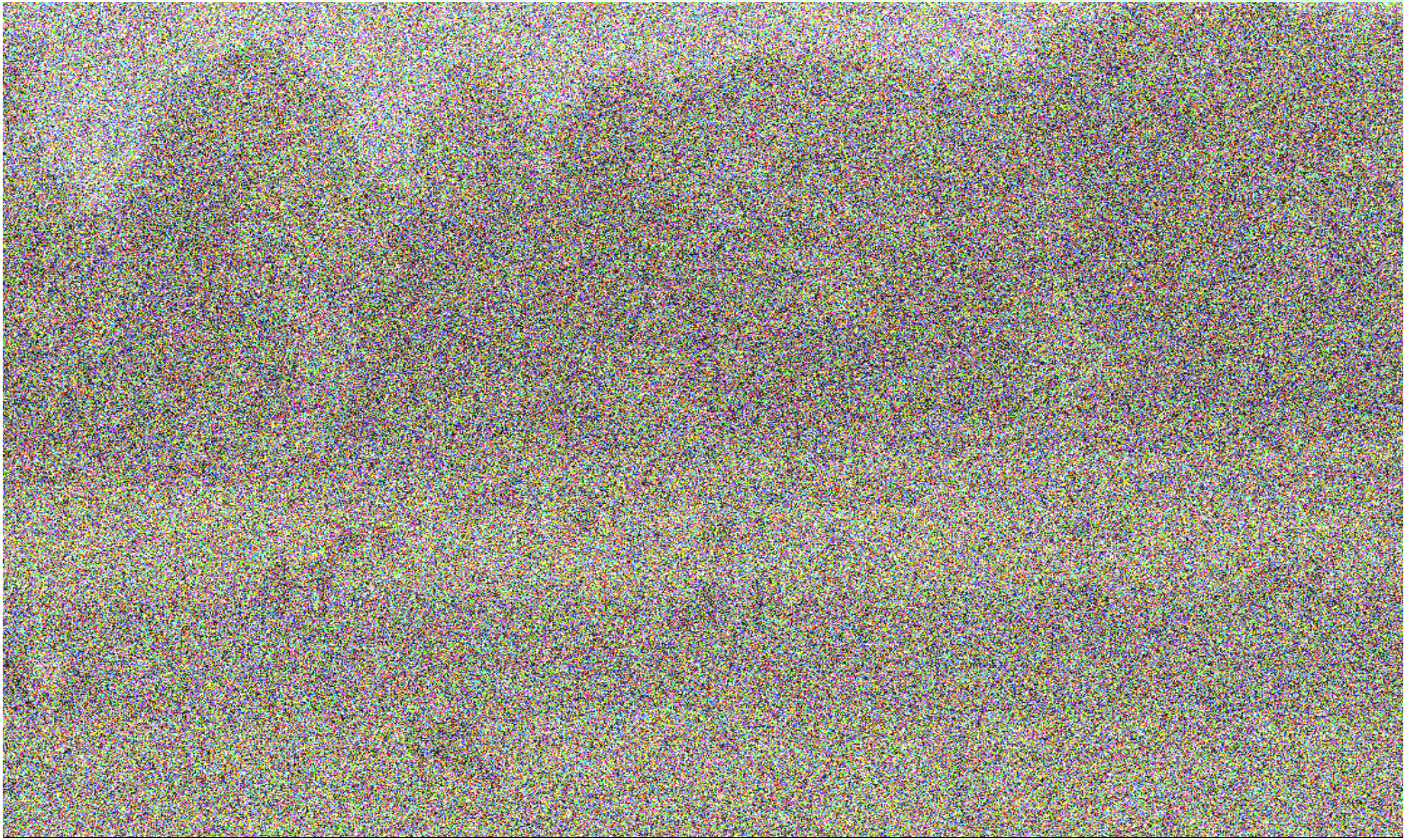
In **angular reconstitution**, angles are found by searching for common line projections.



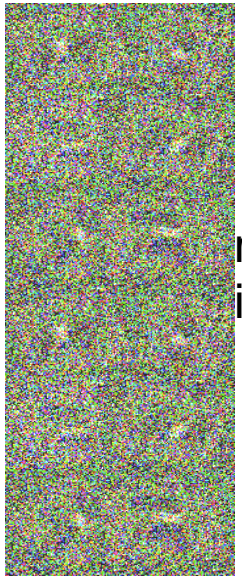
Sometimes, **projection matching to multiple initial models**, created by aligning class averages to random noise. Many models are created but only a small number lead to successful refinement.

Single particles

Low signal:noise



Projection matching



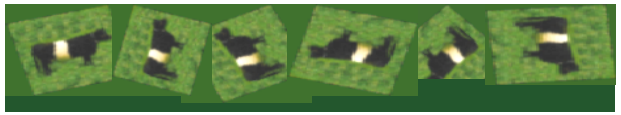
raw images

3D starting model

project

2D templates

Align and group into classes

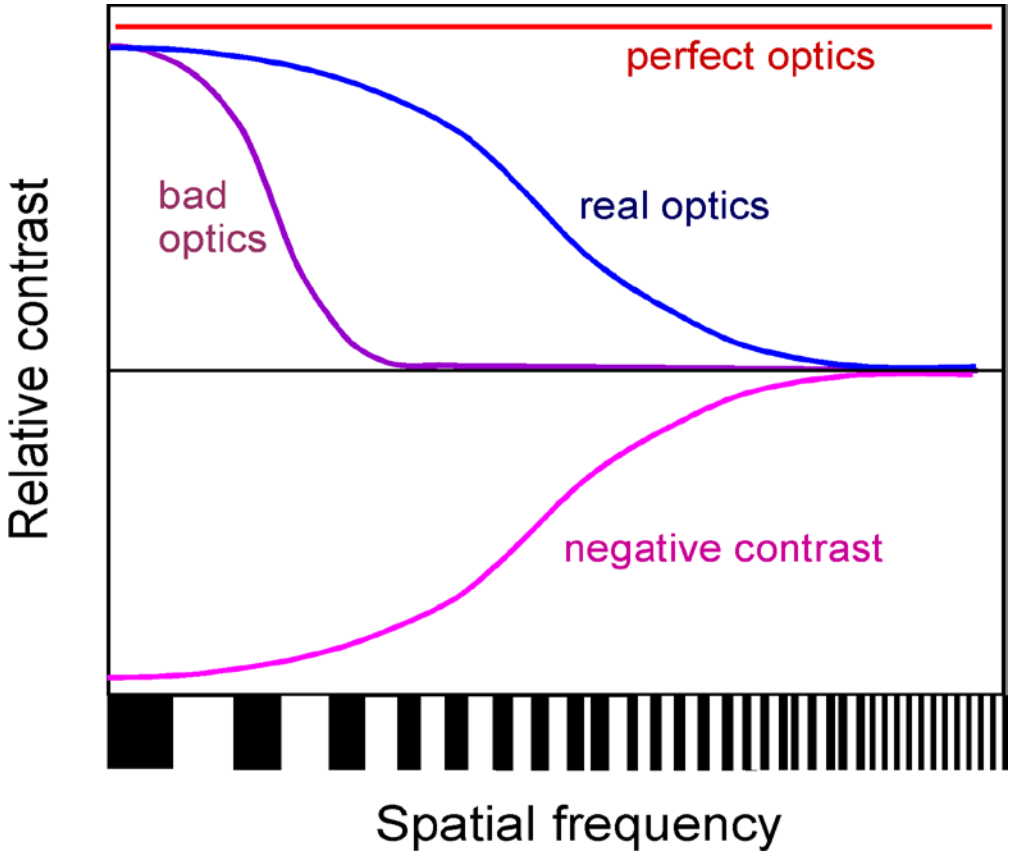


2D class averages

reconstruct

new 3D model

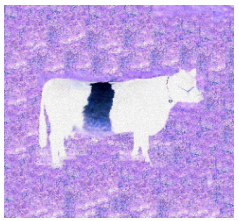
Optical corrections: Contrast transfer



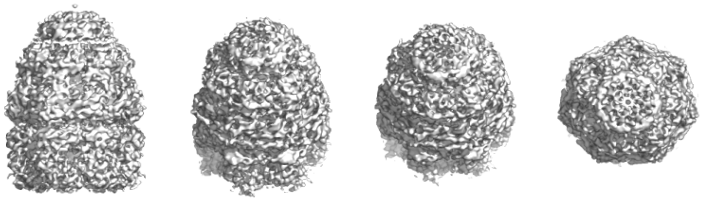

perfect optics


normal optics

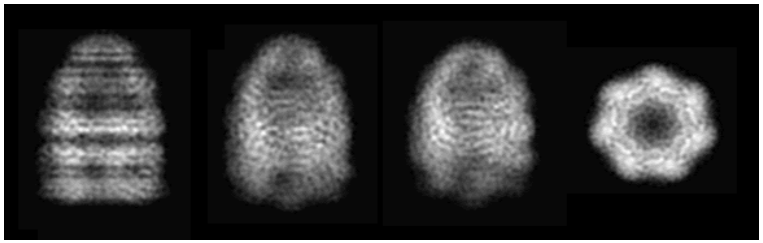

bad optics


negative contrast

3D reconstruction from 2D projections

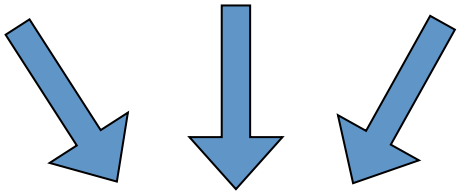


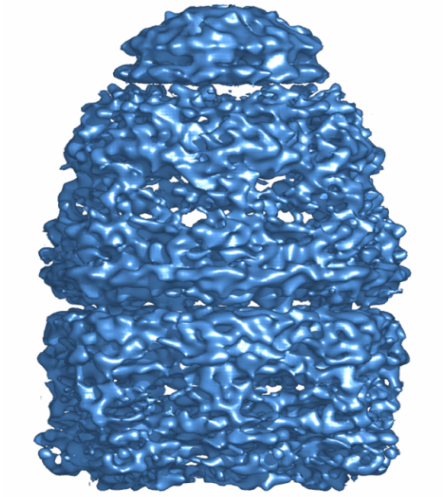
Molecular orientations



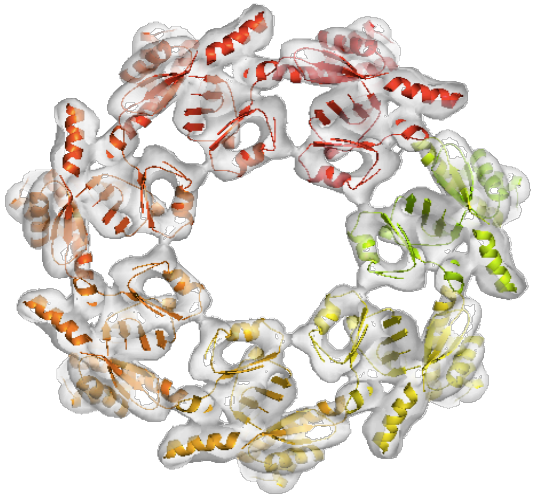
2D projections (observed images, without noise)

Back projection





Section through map with fitted atomic structure



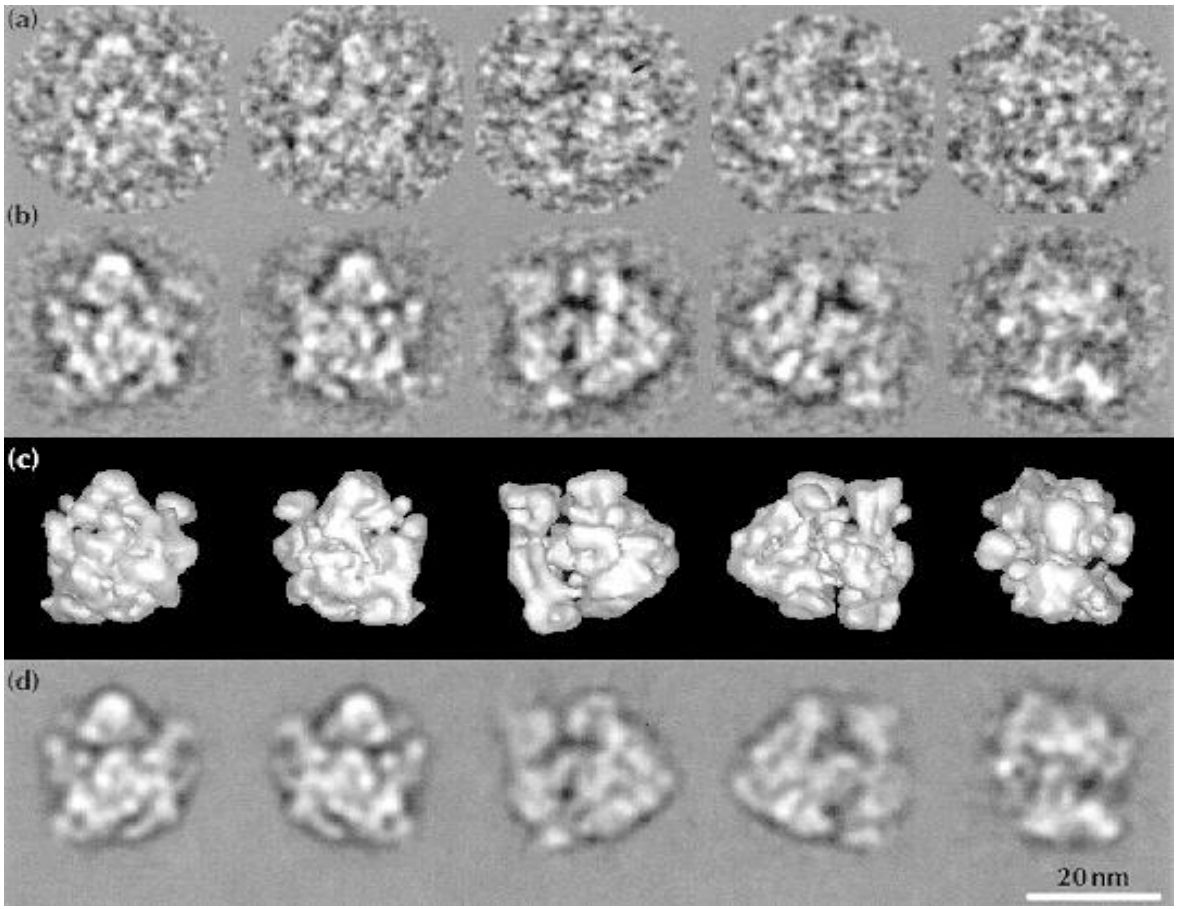
Ribosome: Angular reconstitution

Original images

Class averages

3D reconstruction

Reprojections



Stark *et al.* (1995)

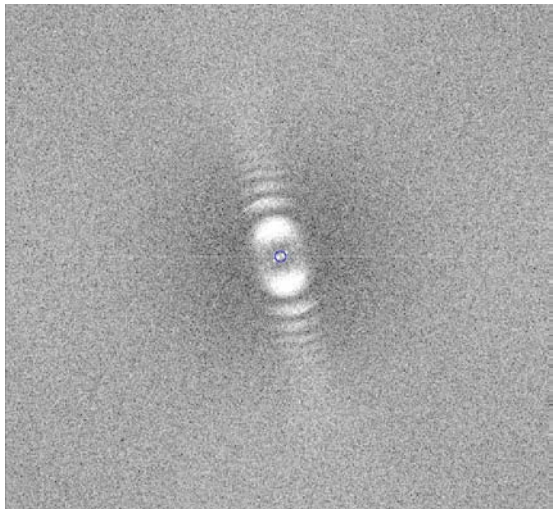
Technological advances improve both high resolution and cellular information

- Coherent FEG sources and stable cryo stages have provided big advances in resolution and throughput
- Larger data sets and improved data processing lead to improved structures, including sorting of heterogeneous structures; merging of single particle and tomographic approaches
- Direct electron detectors offer major gains in resolution and sensitivity – movie mode enables drift correction, restoring high resolution signal; electron counting enhances DQE at all spatial frequencies
- Phase plates substantially improve contrast and extend the limits of cryo tomography and for small particles

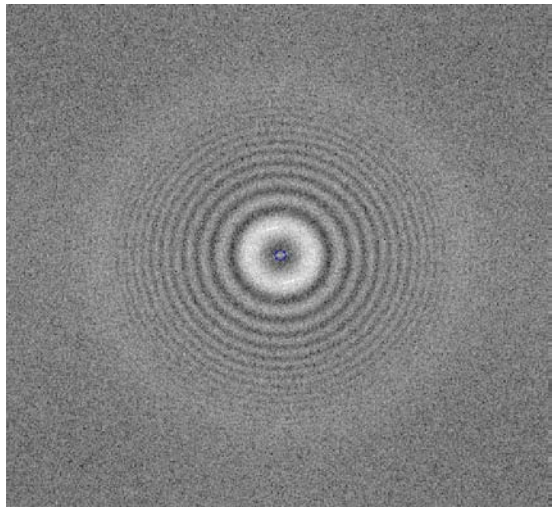
Direct electron detectors

Power spectra

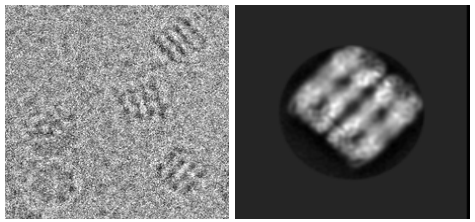
With beam-induced movement

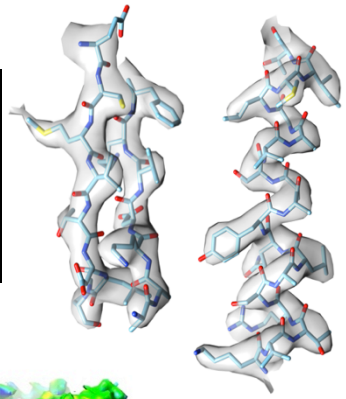


After sub-frame alignment

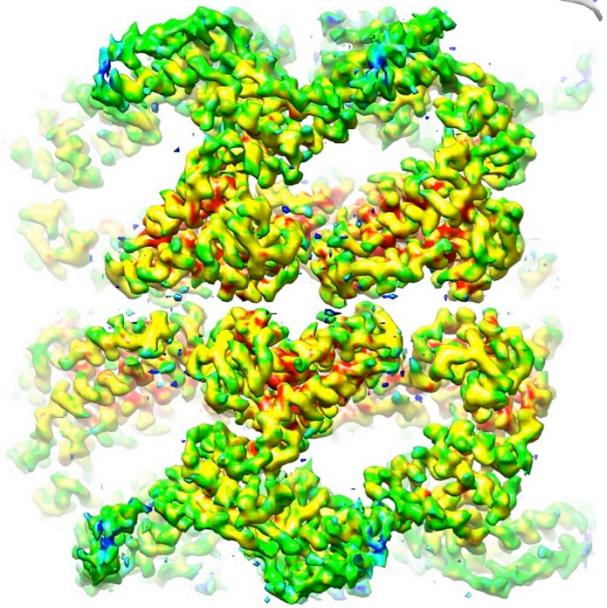


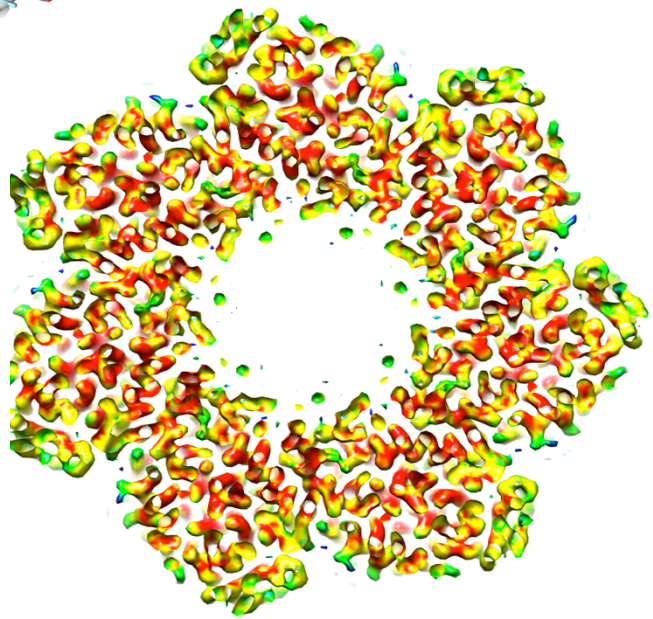
eBIC test: GroEL EPU-K2





17,400 particles
10 hours data collection
Average resolution 3.3 Å; Relion



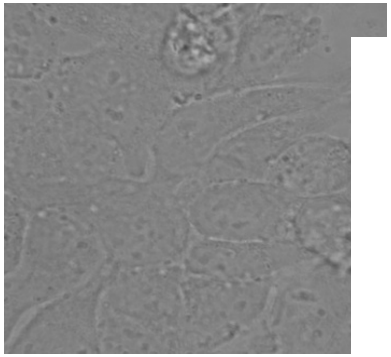


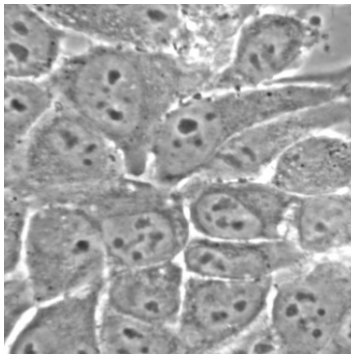
Joseph et al, Methods 2016

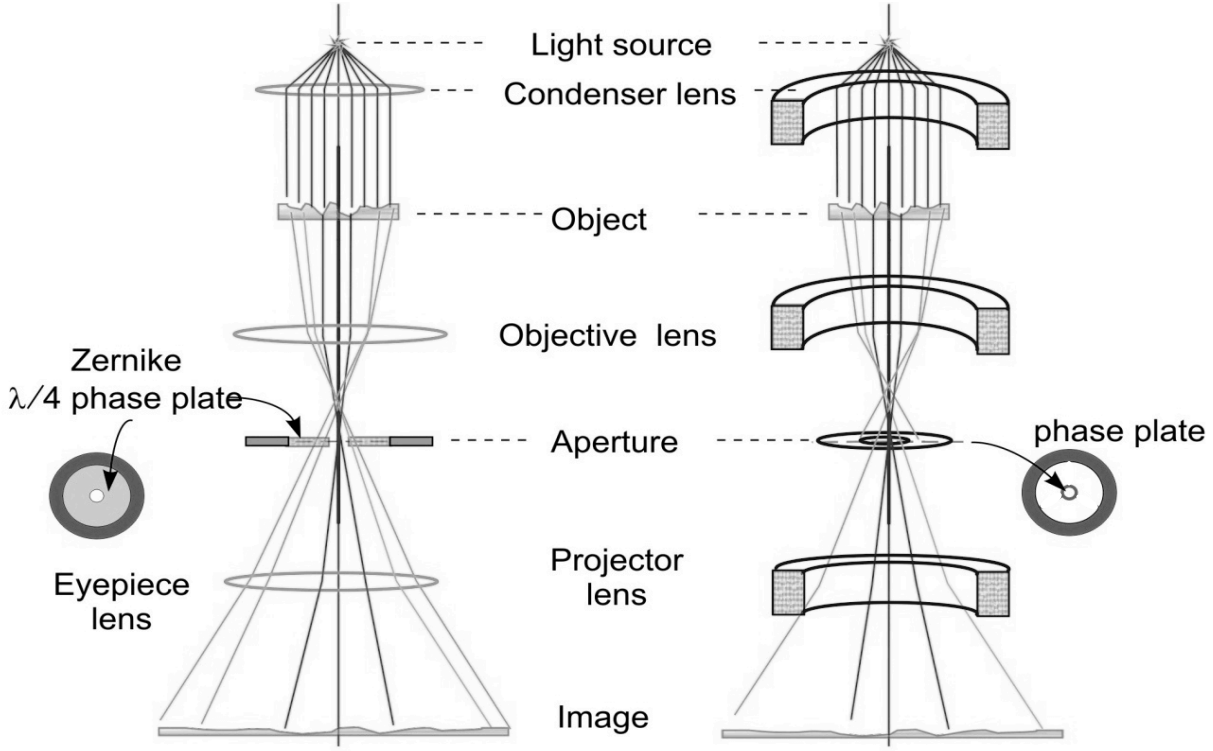
Phase contrast microscopy

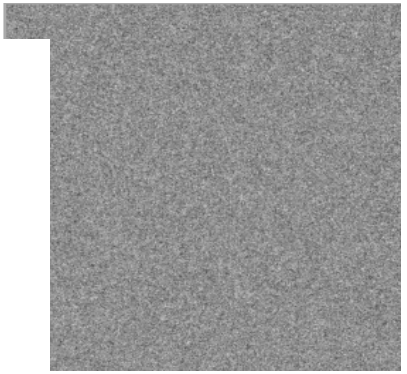
Optical phase contrast microscope

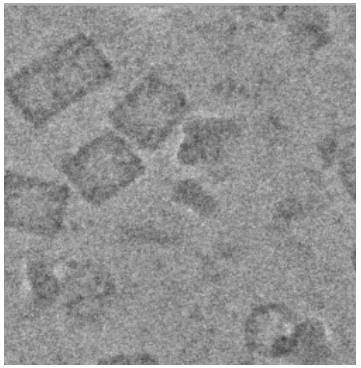
Transmission electron microscope











In phase contrast microscopy, small differences in scattering from transparent specimens are converted into intensity variations, to give better contrast

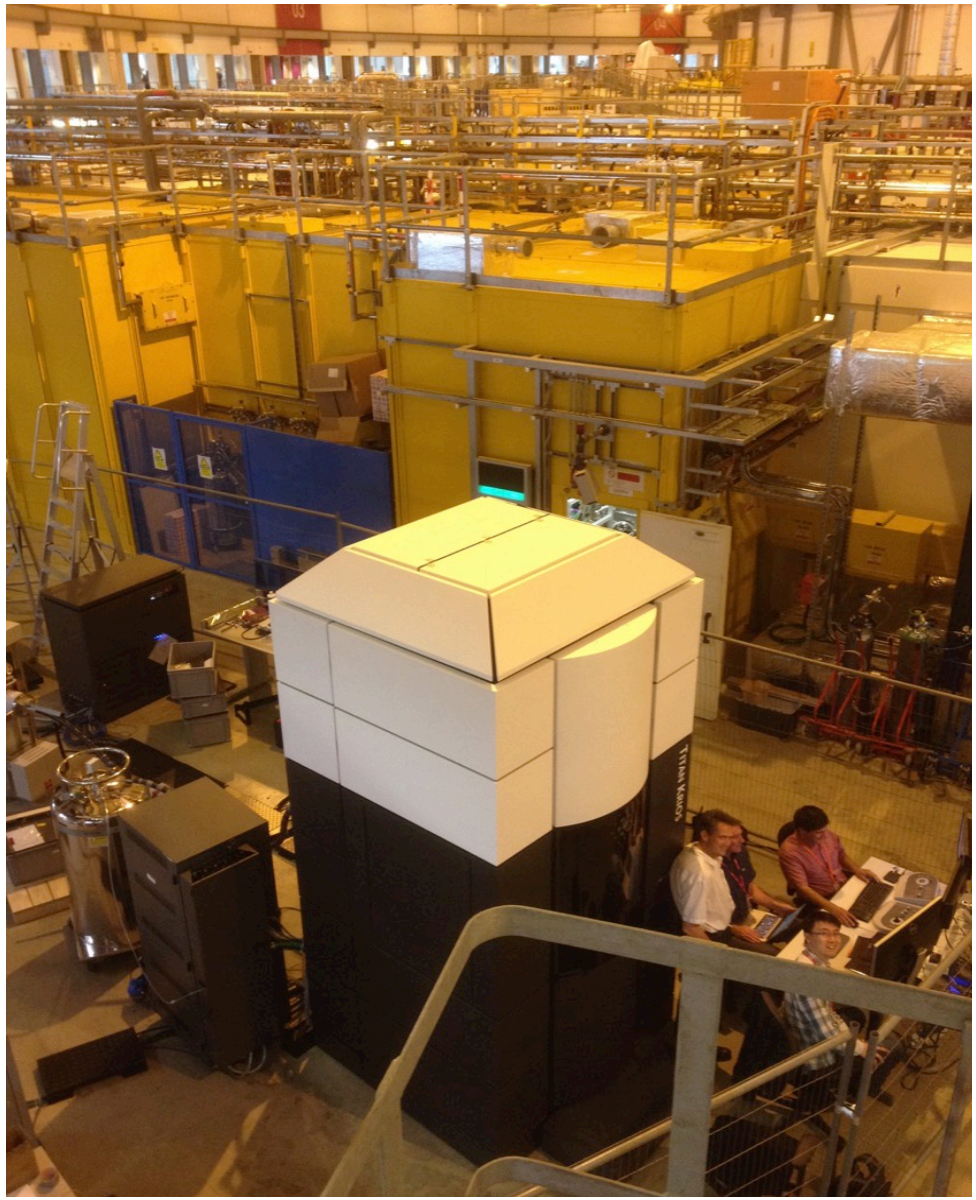
eBIC: electron BioImaging Centre

Wellcome Trust/MRC/BBSRC Strategic Award

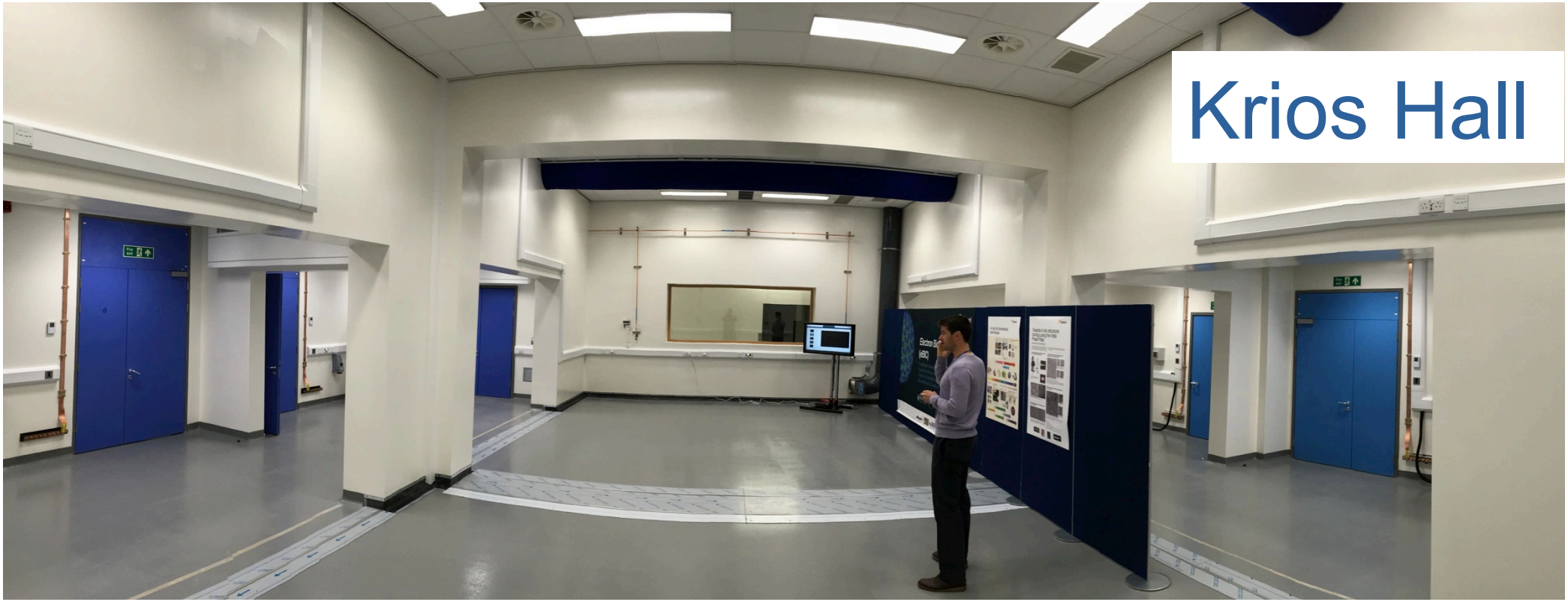
Helen Saibil, Kay Grünewald, David Stuart, Gerhard Materlik

A National User Facility for Biological Electron Cryo-microscopy

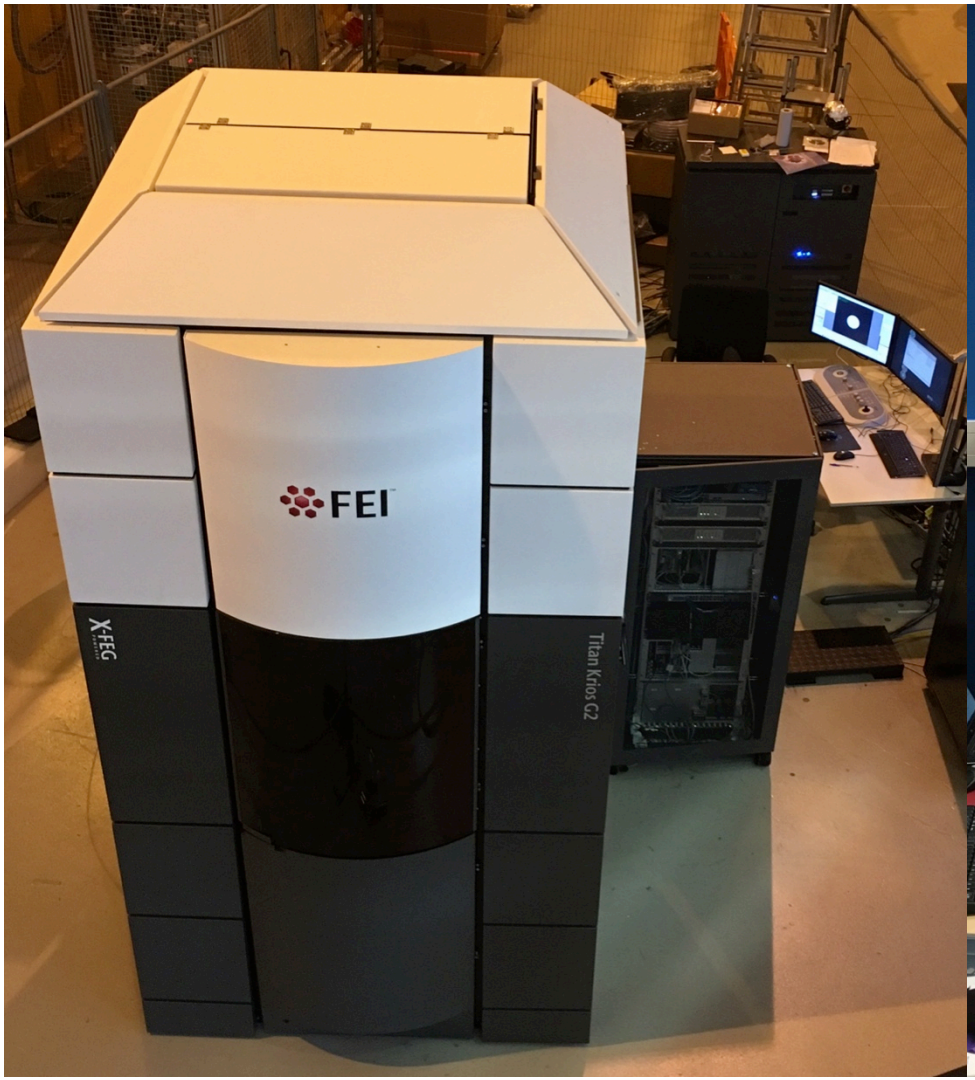
- State-of-the art facilities for single particle analysis and cryo-tomography
- Funded access, based on scientific excellence, to very expensive facilities, including biological containment facilities in Oxford (OPIC)
- Beamline-like 24/7 access supported by expert staff
- Focus for future hardware and software developments and advanced training



eBIC New Building



Krios II



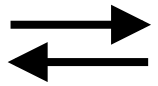
The first external user session on Krios II took place on 27/06/16 and now has a full user program running

Talos

The Talos installation is almost complete and will be available for in-house testing soon

Example:

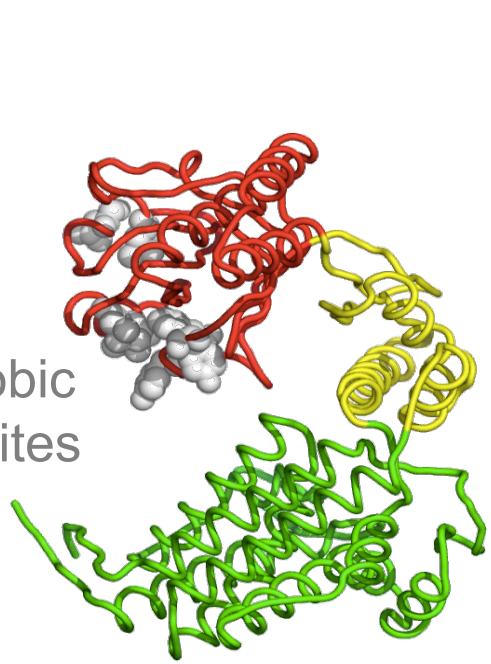
Molecular chaperones: Machines for folding and unfolding

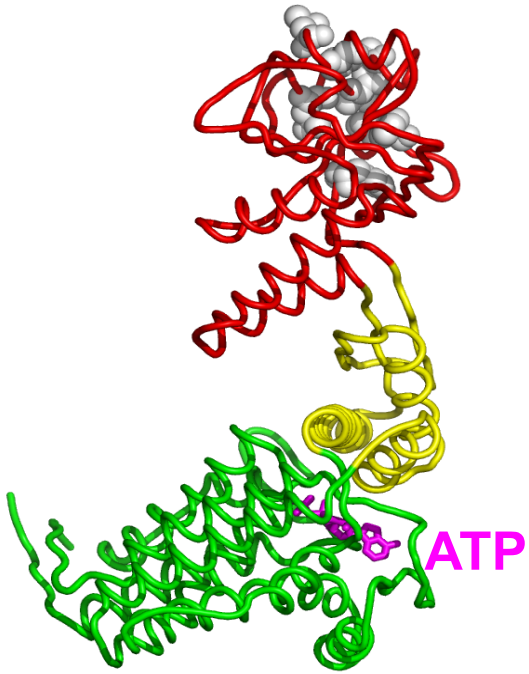


Dan Clare (*now eBIC*), Daven Vasishtan (*now Oxford*), Maya Topf, Elena Orlova
Art Horwich, *Yale HHMI*
Carragher lab, *Scripps Institute (now NY)*

GroEL and GroEL-GroES crystal structures

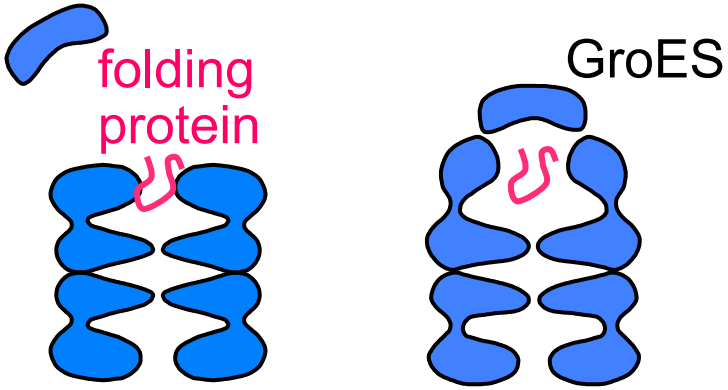
hydrophobic binding sites

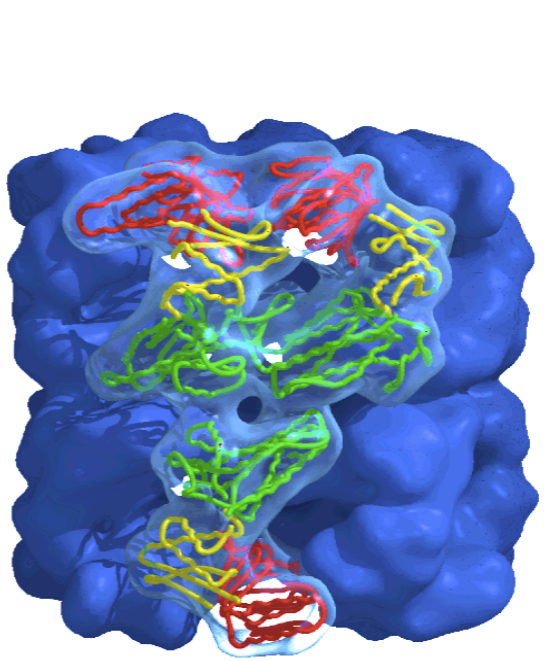


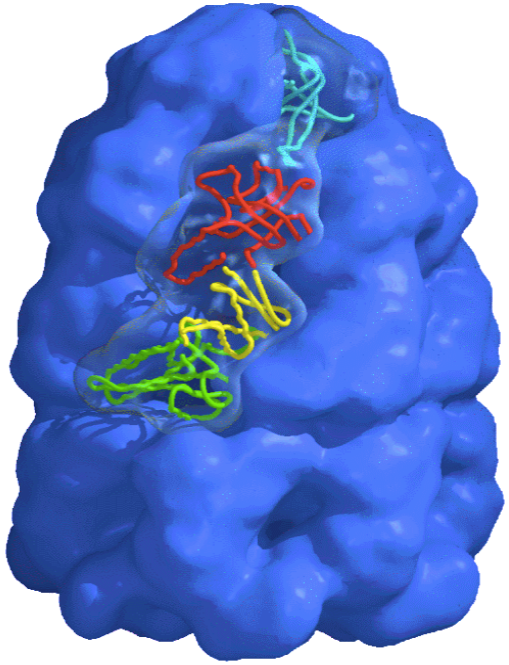


Subunit structures:

Oligomeric structures:

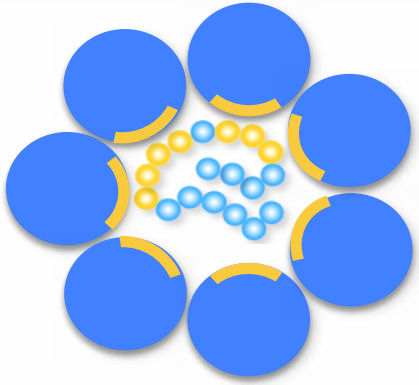


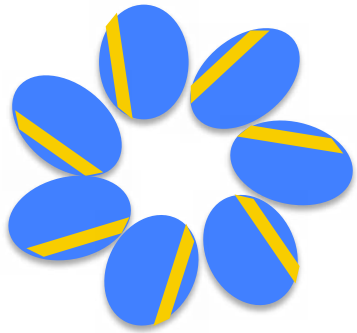




Binding and encapsulation of a folding substrate protein

Top view

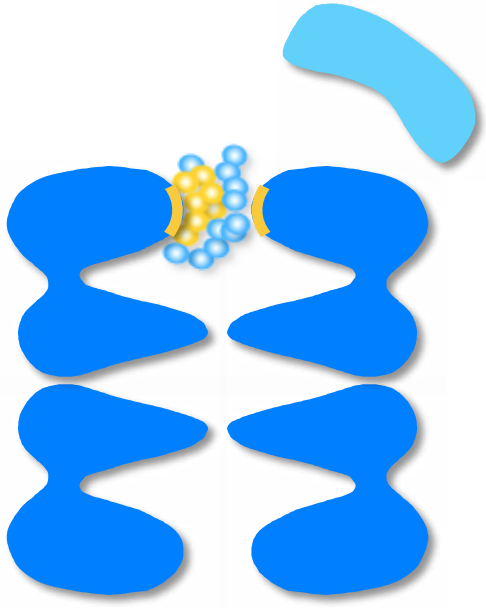


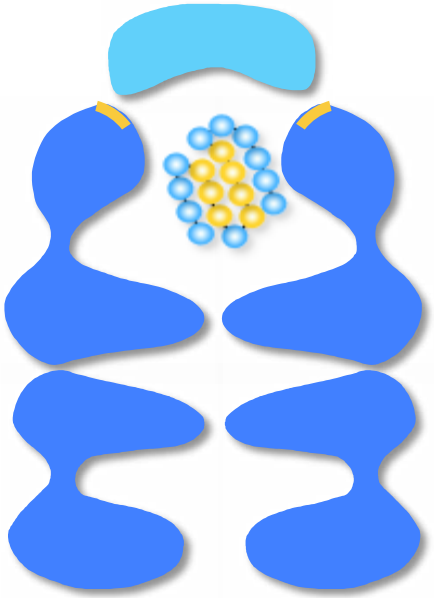


Polar residue

Non-polar residue

Side view

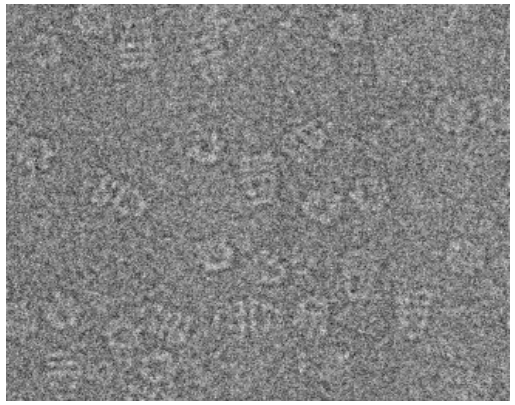




Unfolded or misfolded protein captured on the chaperonin open ring

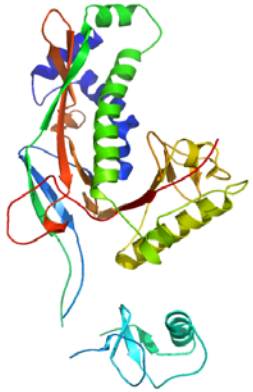
Newly folded protein trapped inside the chaperonin folding chamber

Bacteriophage T4 capsid protein gp23 folding inside the chaperonin complex



Cryo EM image of chaperonins with non-native/folding gp23

Gp24



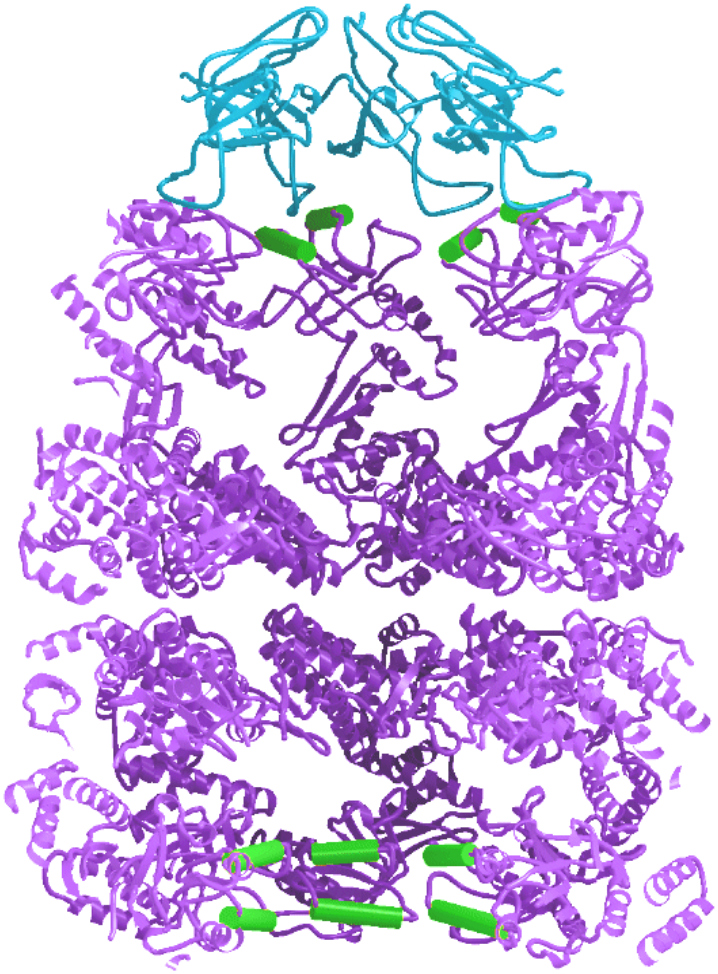
Major domain

Insertion domain

gp23

56 kDa subunit

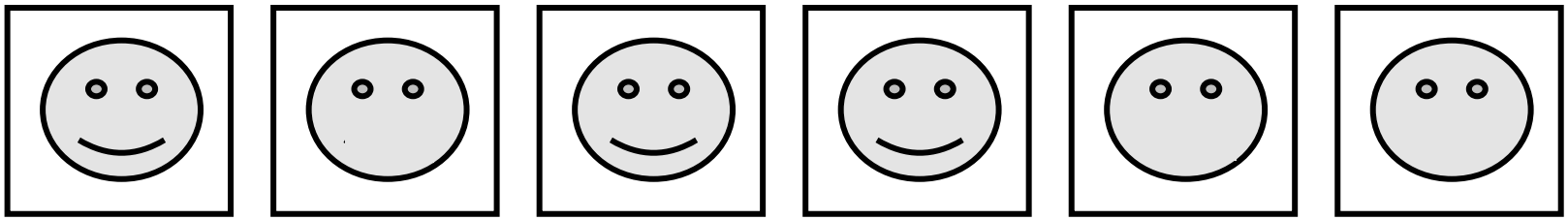
Denatured in 6 M urea and diluted into a GroEL-containing buffer



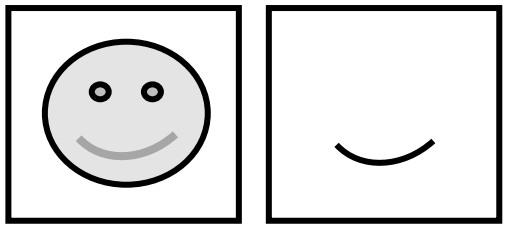
Multiparticles

Eigenimage (principal component) analysis to detect and sort heterogeneous complexes

Data set

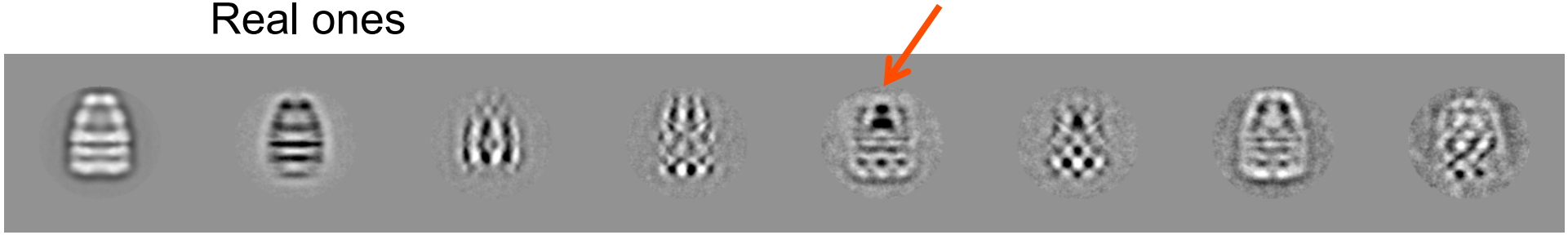


Eigenimages

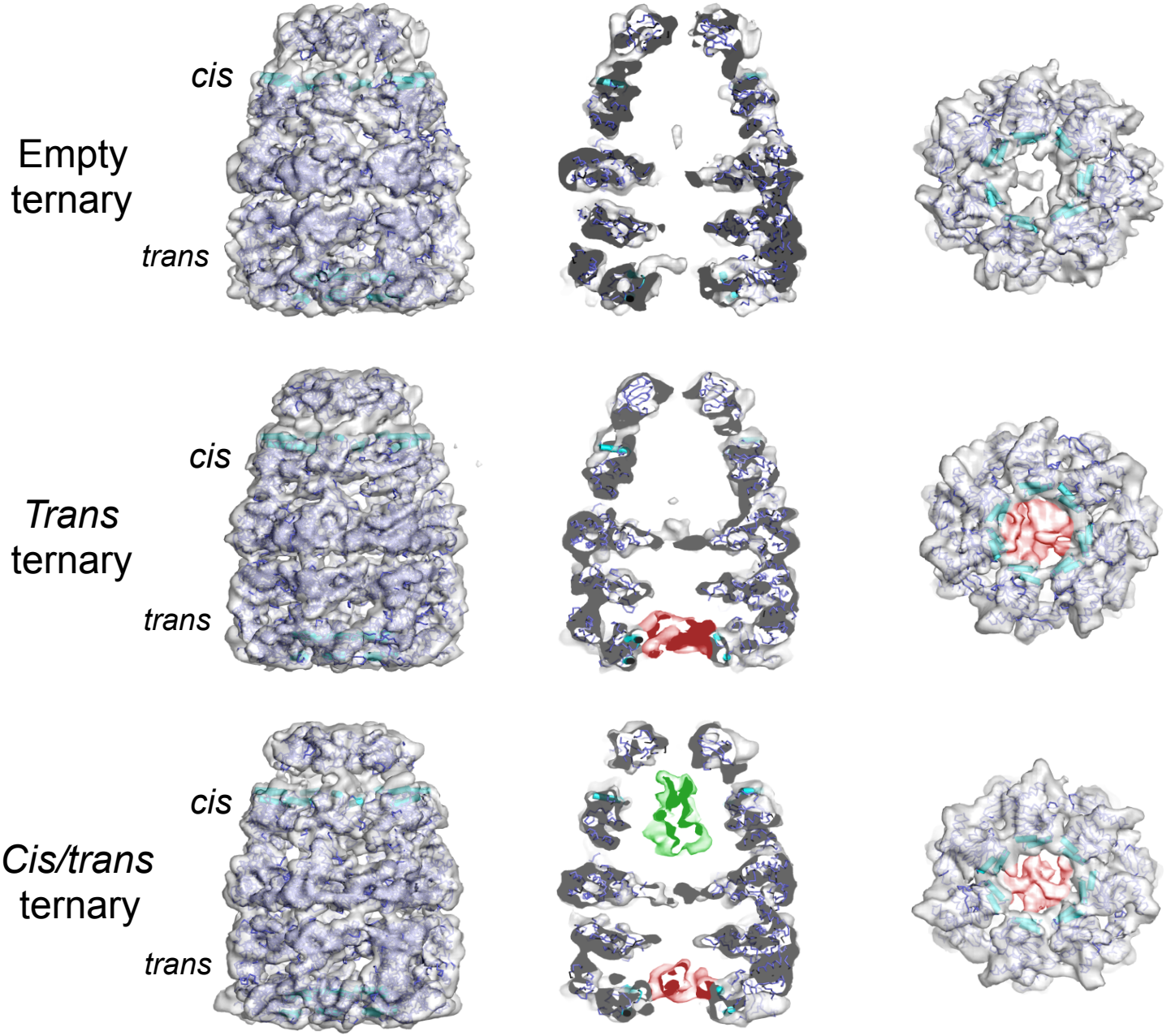


Elad et al, J Struct Biol 2008
Orlova & Saibil, Chem Rev 2011

Real ones



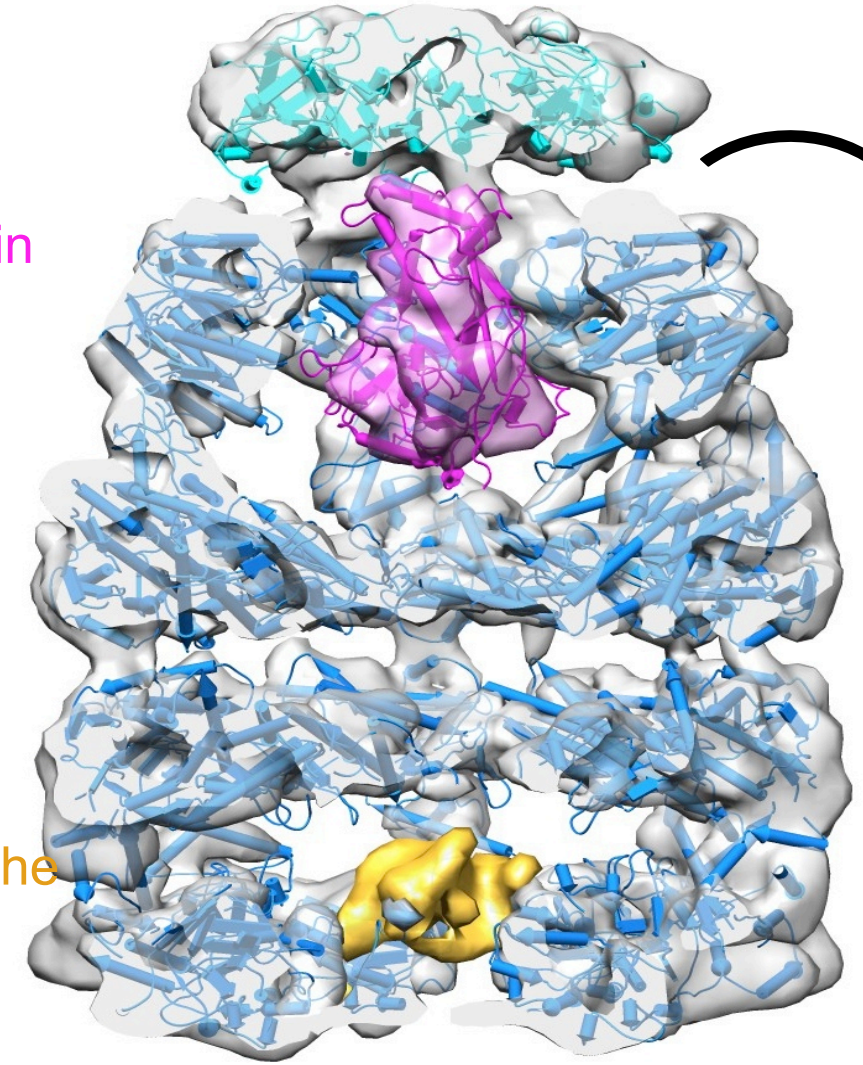
Asymmetric reconstructions of GroEL-gp23-gp31 complexes



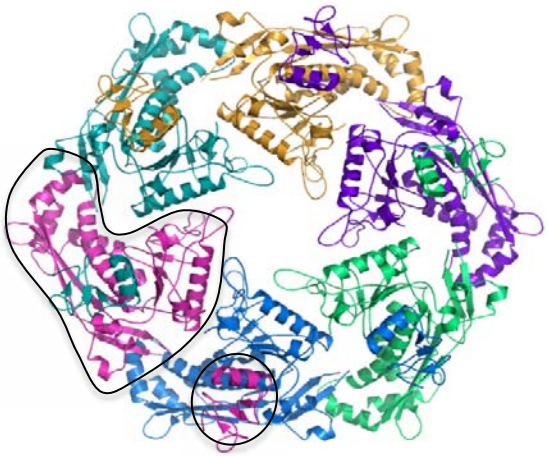
Chaperonin complexes containing both unfolded and partly folded substrate protein

Newly folded gp23 in the chaperonin chamber

Non-native gp23 in the open ring

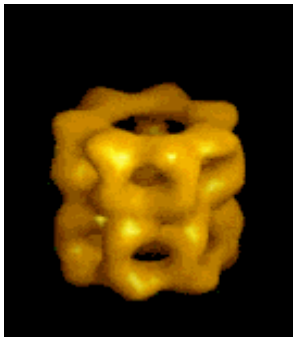


Large domain folds inside the cage; small domain only folds when hexamer assembles

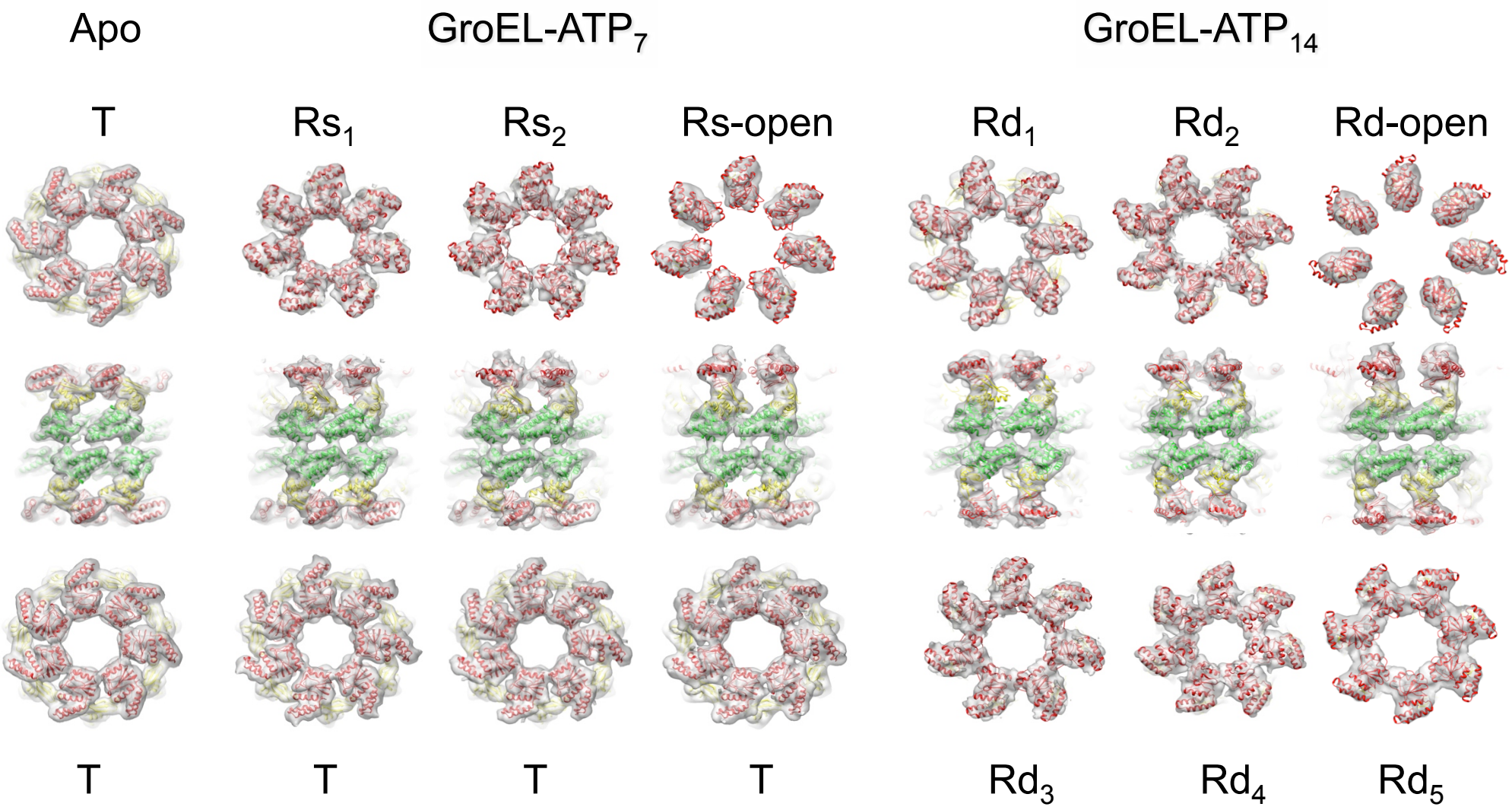


Clare et al, Nature 2009

The chaperonin functional cycle

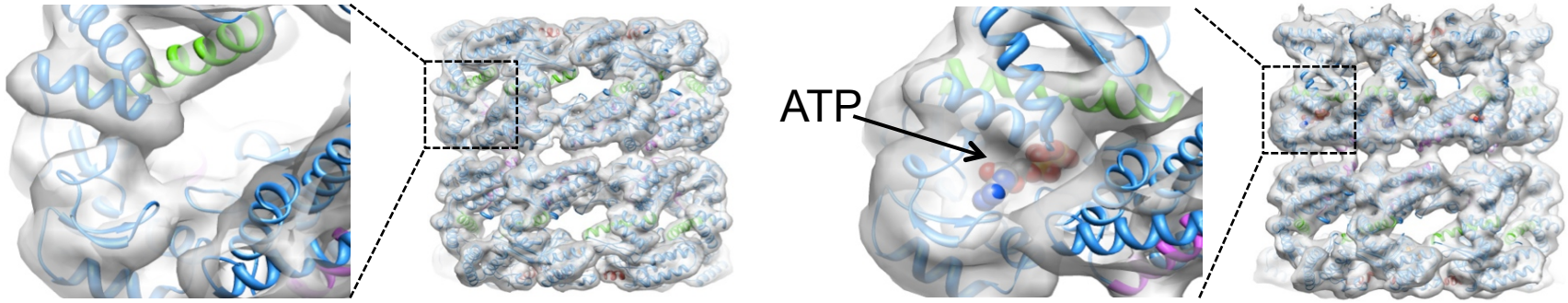


Capturing the machine motions : GroEL-ATP

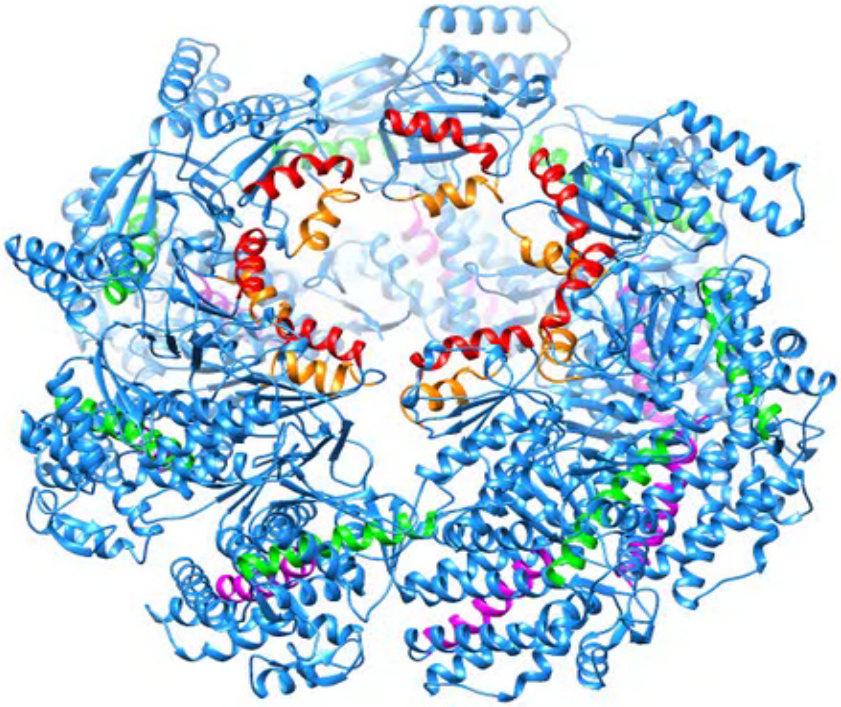


Clare et al, Cell 2012

Chaperonin machine for ATP-driven protein folding



Power stroke for forced unfolding of misfolded proteins: a trajectory of domain movements based on multiple structures resolved by single particle analysis



Clare et al, Cell 2012

Single particle electron microscopy references

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483, Cryo-EM, Part C: Analyses, Interpretation, and Case studies

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