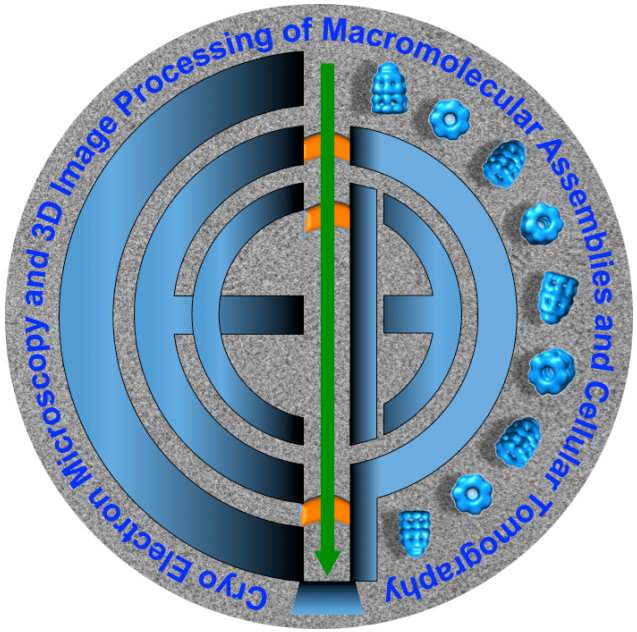
Lecture 7

(5 Jul 2016 11:15 am)

Refinement of Classifications, Dealing with orientation and Heterogeneity of particles

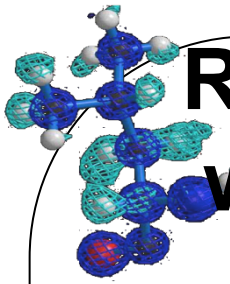
Ramanathan Natesh



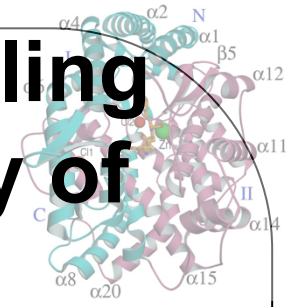
CEM3DIP 2016

2 July - 13 July 2016

IISER Thiruvananthapuram
(IISER-TVM), Trivandrum

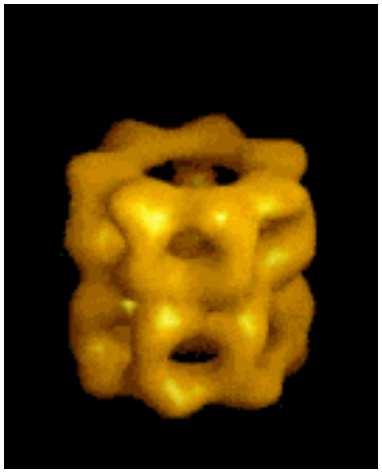


Refinement of Classifications, Dealing with orientation and Heterogeneity of particles



@**CEM3DIP 2016** - at UDS Kovalam organised by IISER-TVM, India

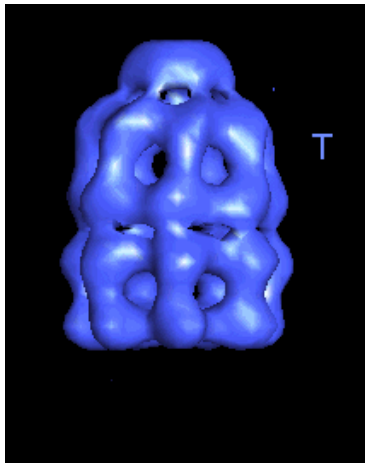
- 5 Jul 2016



Ramanathan Natesh

Ramalingaswami Fellow and Assistant Professor

School of Biological Sciences



Indian Institute of Science Education and Research Thiruvananthapuram (IISER-TVM)

Organization of the talk

- Some Basics
- What is classification?
- When do we start with classification?
- Refinement of Classification
- Methods in dealing with orientation and heterogeneity of particles (with challenges in visualizing the non-native substrate protein bound to Chaperonin).

SINGLE PARTICLE CryoEM and –ve stain EM and 3D-RECONSTRUCTION

- Sample preparation
- **Specimen preparation**
- Data collection
- Image processing and 3D reconstruction

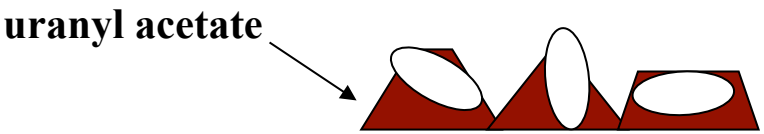
x VITRIFICATION (High Pressure Freezing & Plunge Freezing)
x Cryo-sectioning of vitreous samples
x CRYO-ELECTRON TOMOGRAPHY
x Cryo 2D Crystallography

- **Combining different structural methods (MX**, NMR, SAS, **EM**, MS on 9th July 2016)

Classification :Equally good for both type of Specimen preparations

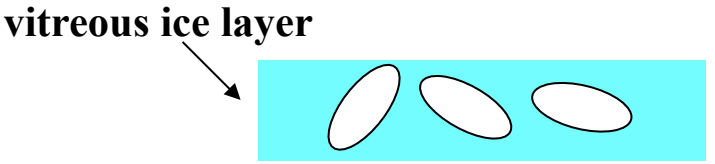
Negative Stain vs. Vitreous Ice

Specimen in Stain



- High contrast image
- No special temperature control
- Essentially no radiation damage
- Particle distorted
- Image = stain “shell” around the particle
- Low resolution method: 20-15 Å
- Great choice for initial sample screening

Cryogenic Specimen



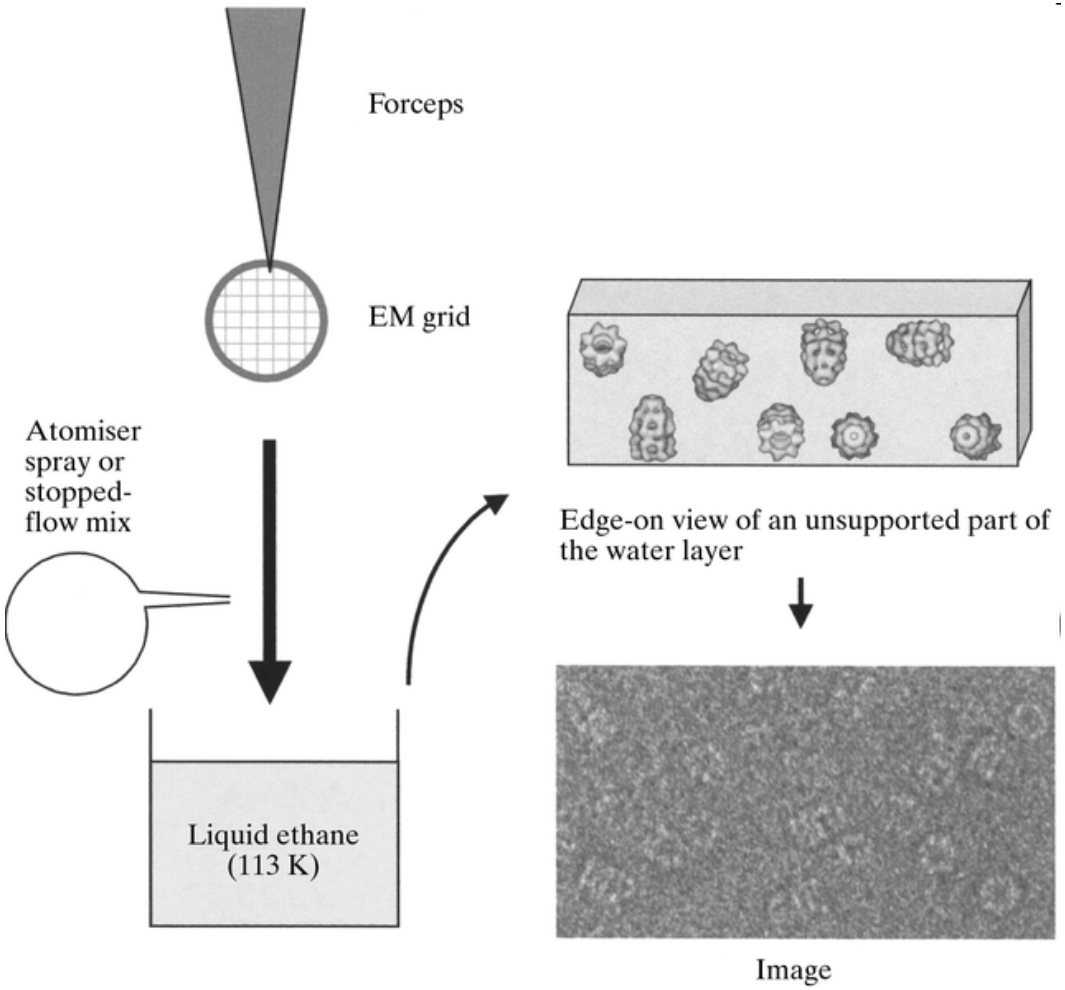
- Low contrast image
- Sample maintained at cryogenic temperature (85 ˚K)
- High radiation damage
- Particle undistorted
- Image is of the actual particle
- Higher resolution obtained: 15-4 Å
- Best choice for reconstruction

Why Classifications?

- Radiation damage limits the total electron dose that can be used to image biological sample.

- Thus, images of frozen hydrated macromolecules are very noisy, with extremely low signal-to-noise ratio (SNR).

Sample preparation
Vitrified

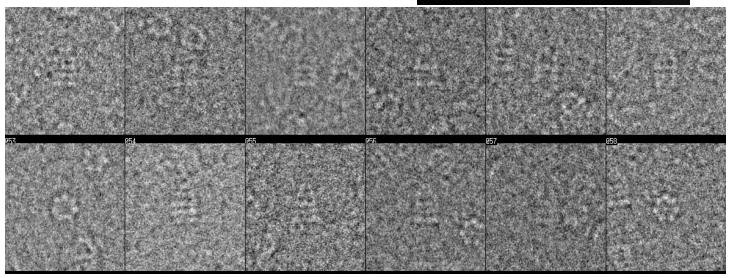


Film/CCD

Data collection

strict selection < 10% Ast, >15ÅDef

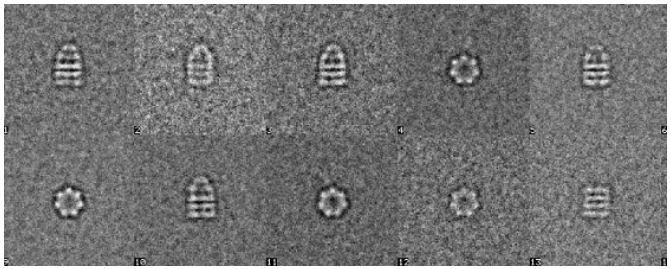
22,084 (194/609 Dig. μgraphs)



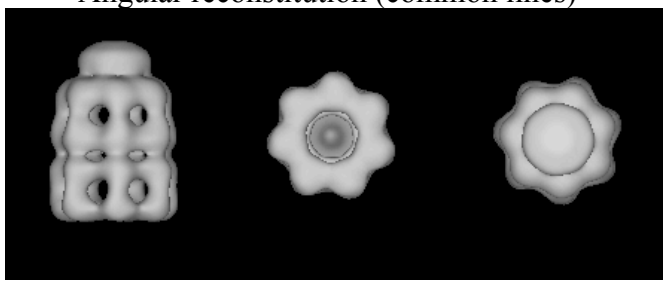
- MSA analysis – IMAGIC

467/30 = ~15.6 images per classums.

This approach is based on the Central-Section Theorem, which implies that in real space any two projections of a 3D object will share a common 1D projection.



Angular reconstitution (common lines)

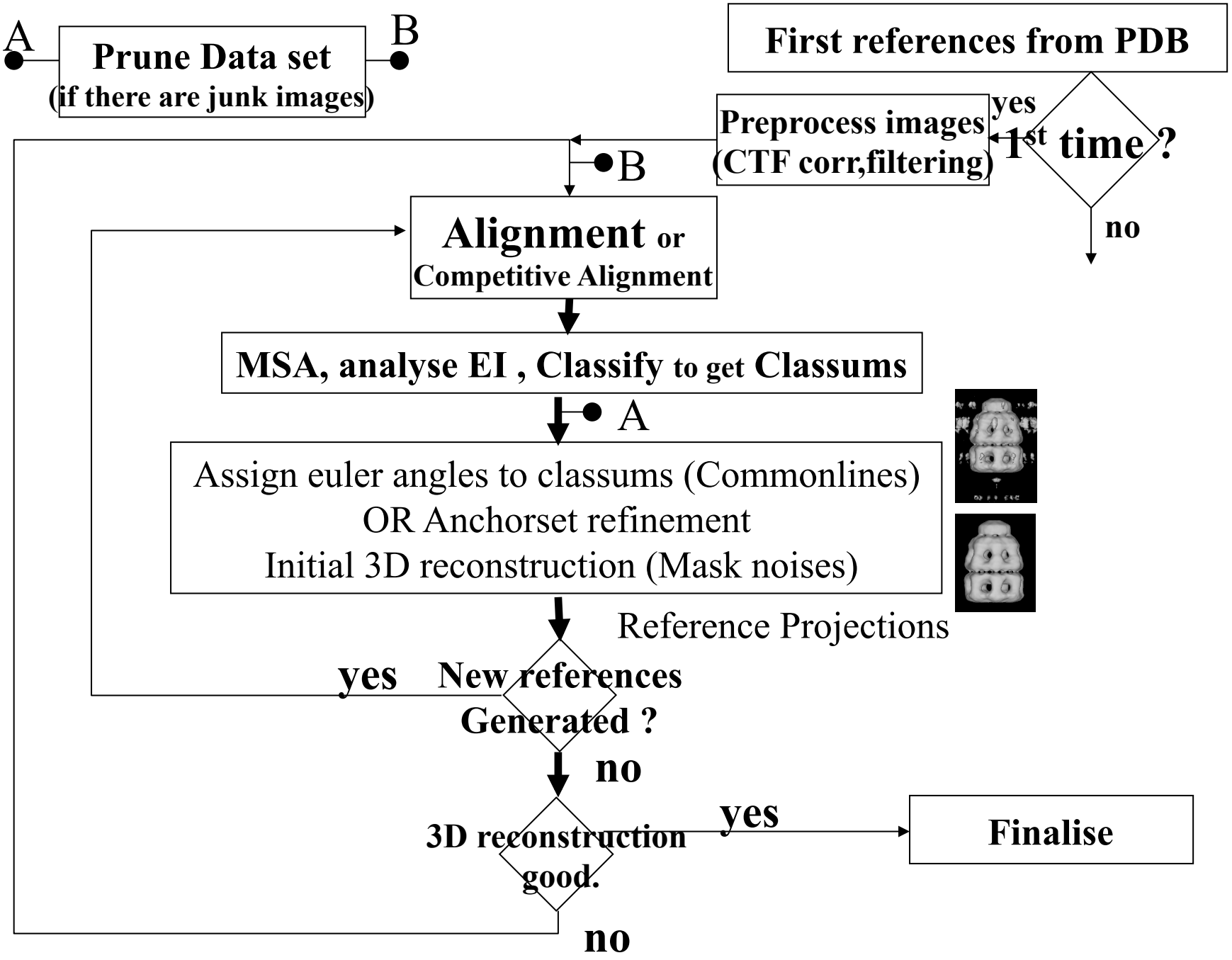


By searching for the common line projections, one can determine the **spatial relationships** between the set of projection Images.

Other ways of Assigning Euler Angles. : eg. Using Anchor Sets

Data Collection and Initial Image Processing

- Collect image set (20-500 images, vary focus)
- Pick Particles (10,000-100,000)
- Perform contrast-transfer-function (CTF) correction for each image
- Center, align, **classify**, make “class averages”
- Assign orientational relationships between all projection images
- 3D reconstruction


A
Prune Data set
(if there are junk images)
B
First references from PDB
yes
1st time ?
no
Preprocess images
(CTF corr,filtering)
B
Alignment or
Competitive Alignment
MSA, analyse EI , Classify to get Classums
A
Assign euler angles to classums (Commonlines)
OR Anchorset refinement
Initial 3D reconstruction (Mask noises)
Reference Projections
yes
New references
Generated ?
no
3D reconstruction
good.
yes
Finalise
no

Classification

Classification : a process of dividing a set of images in to subsets with similar features.

OR

Classification is a computational procedure that sorts images into groups ("classes") according to their similarities. (Wadsworth)

- A single particle image data set is a collection of images, each contains projection images of one molecules.
- The orientations and position of particles in all images are different.

When do you do Classification ?....

- Before averaging, one needs to:

- judge how similar is the two particles: *cross-correlation coefficient (ccc)*;
- shifts/rotates one particle to match another by maximizing ccc: *alignment*;
- separate different particles for averaging: *classification*;

Refinement of Classification

- Competitive projection matching or Competitive alignment

After MSA

- **Cluster analysis** is the identification of groups of similar objects. This type of analysis is used for the classification of images.

  The most common implementations of cluster analysis in EM are:

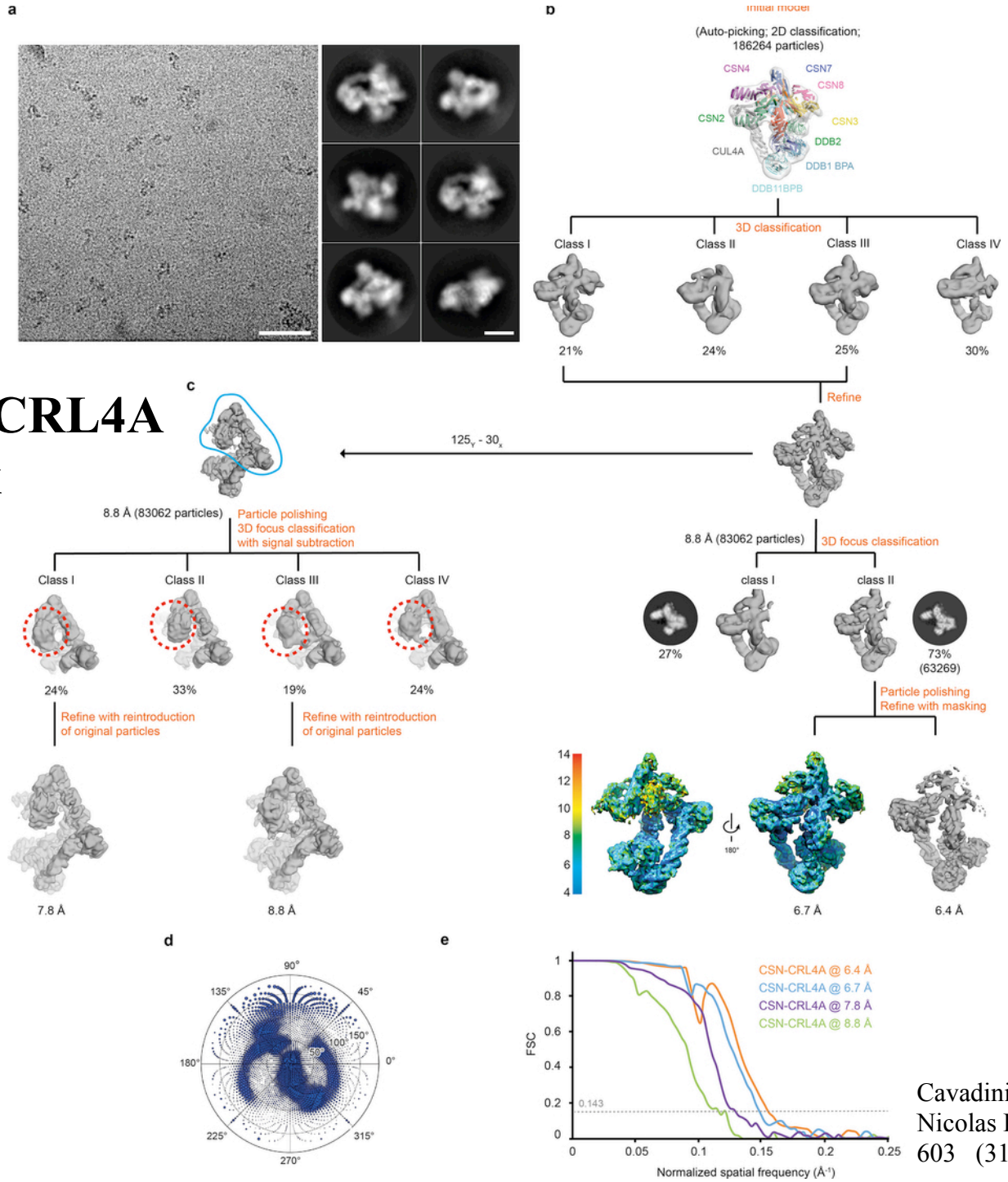
  K-means (Sparx, Spider, EMAN, Xmipp)

  Hierarchical ascendant classification - HAC (Imagic, Spider)

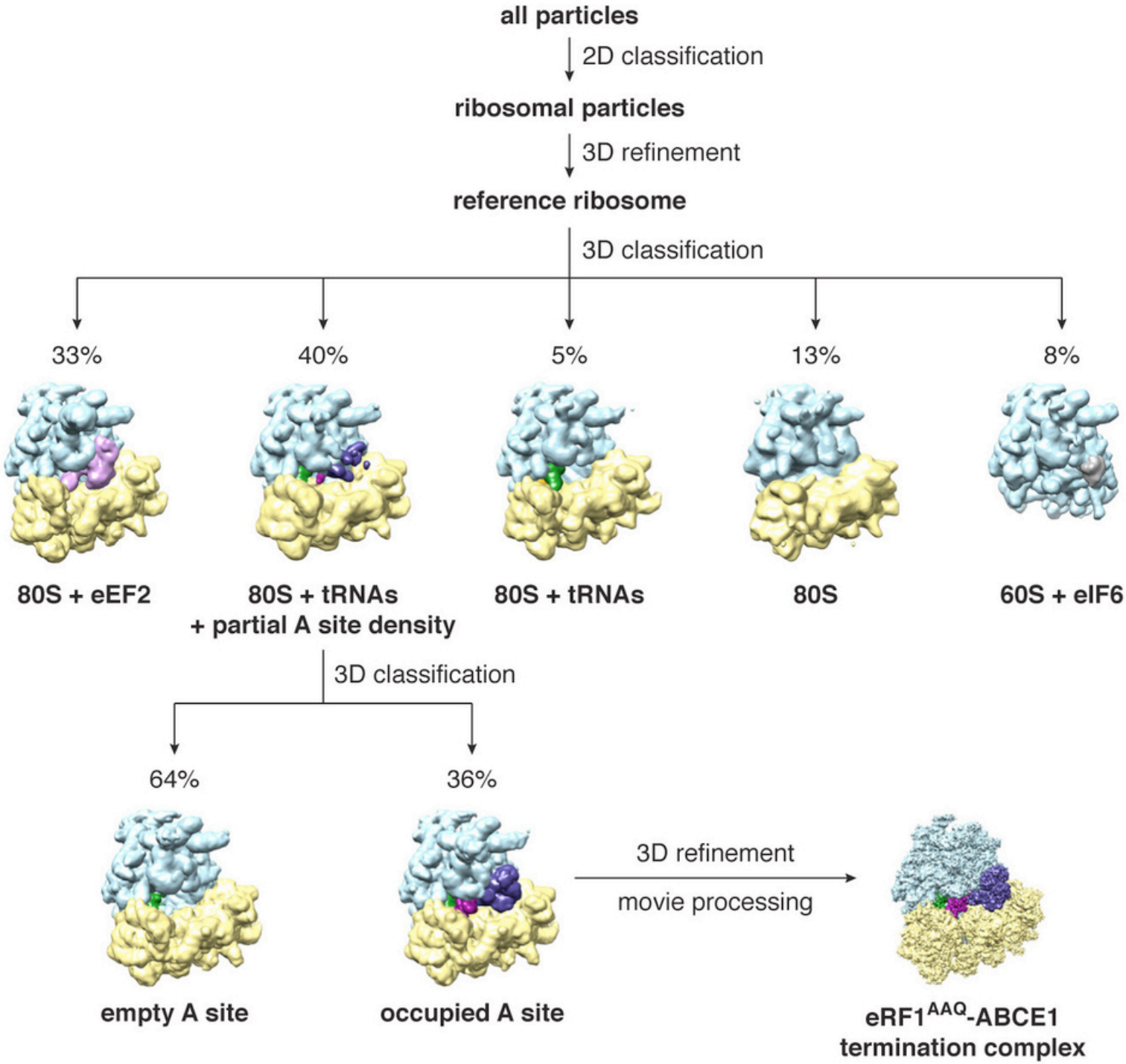
Alignment  Classification

CSN–$_{N8}$CRL4A complex

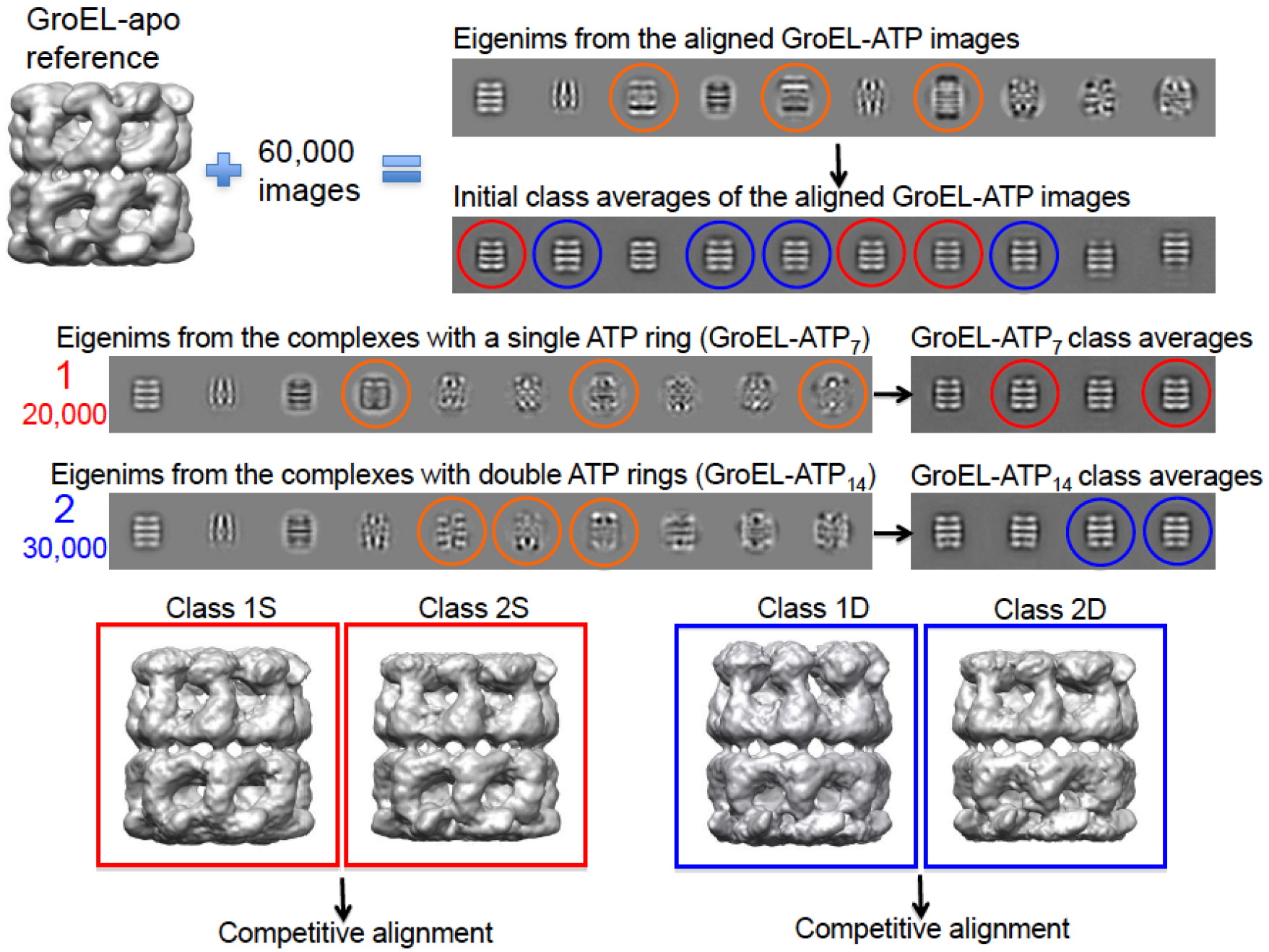
Cullin–RING ubiquitin E3 ligase (CRL) regulation by the COP9 signalosome complex (CSN)



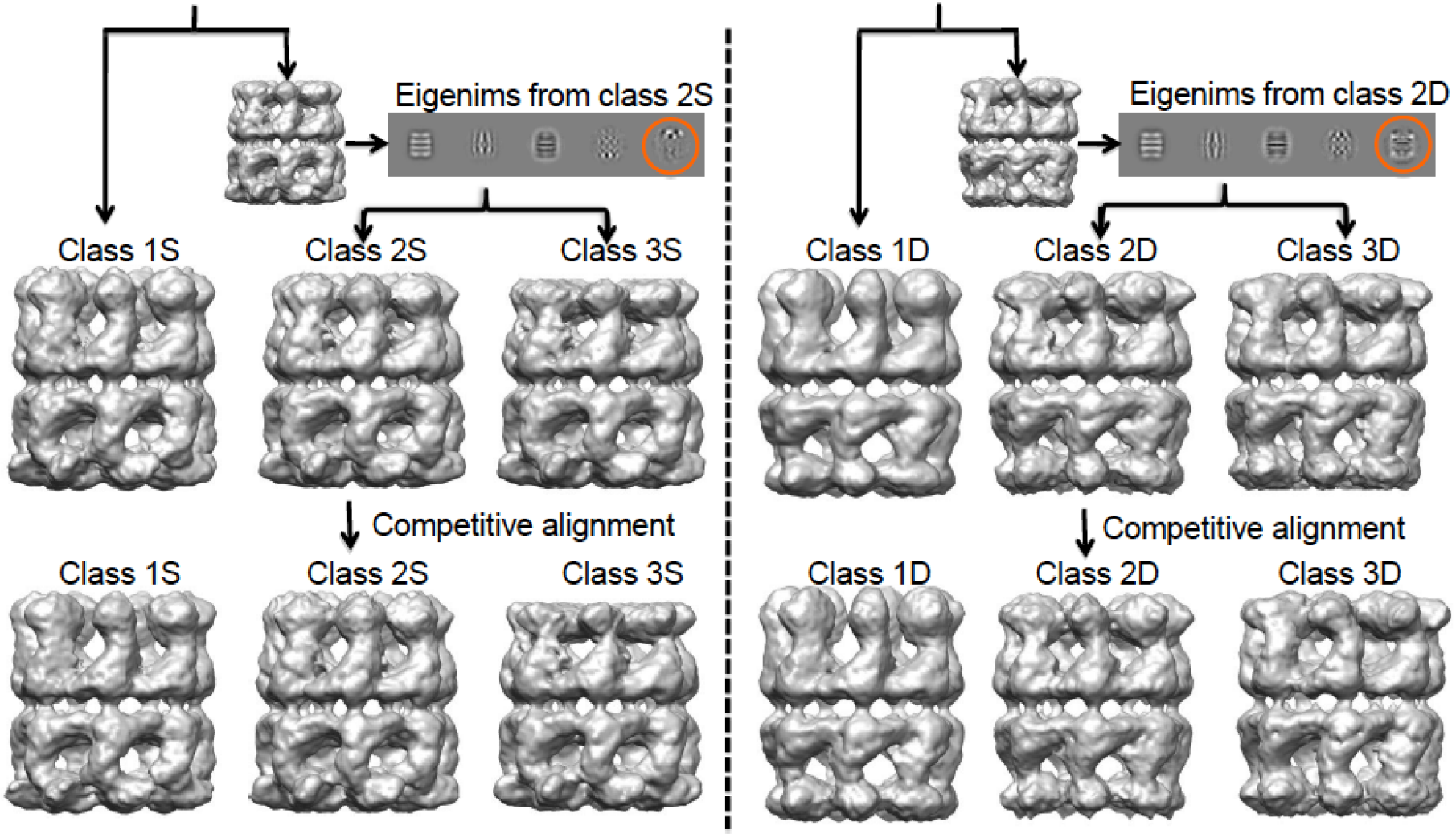
Cavadini et al., Hanning Stahlberg & Nicolas H. Thoma. Nature, 531, 598–603 (31 March 2016)


all particles
2D classification
ribosomal particles
3D refinement
reference ribosome
3D classification
33%
40%
5%
13%
8%
80S + eEF2
80S + tRNAs
+ partial A site density
80S + tRNAs
80S
60S + eIF6
3D classification
64%
36%
empty A site
occupied A site
3D refinement
movie processing
eRF1AAQ-ABCE1
termination complex

MSA and competitive alignment GroEL-ATP



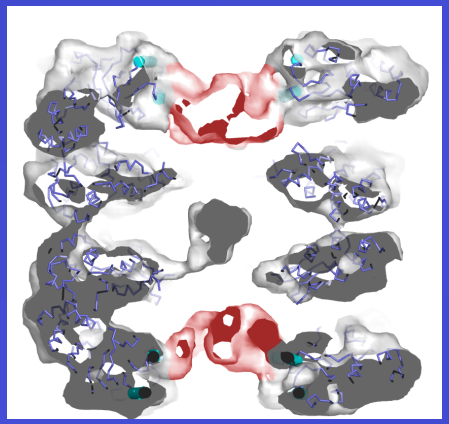
MSA and competitive alignment GroEL-ATP



After multiple rounds of competitive alignment and MSA analysis there were 3 stable structures for each of the ATP_7 and ATP_{14} data sets.

(Clare et al., 2012)

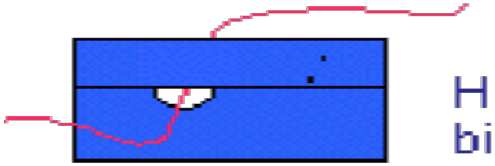
Visualising the non-native substrate protein bound to chaperonin system – Handling Heterogeniety



Clare *et al.*, Nature (2009). 457,107-110

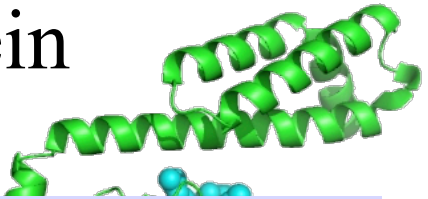
Only structural biology visualisation method that can provide 3-dimensional structures from heterogeneous populations.

What are Molecular Chaperones



Properties

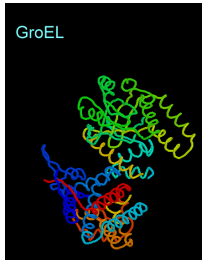
A large group of unrelated protein families whose role :

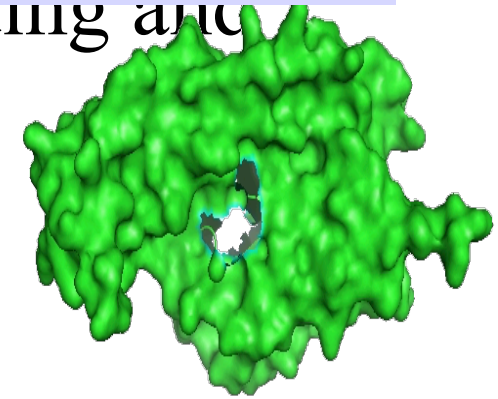


Historically they were identified as Heat Shock Proteins (Hsp's) expressed under stress conditions and Classified as HspMW Hsp25, Hsp60, Hsp70, Hsp90, Hsp100 etc.

- and/ or to assist in their correct folding and assembly

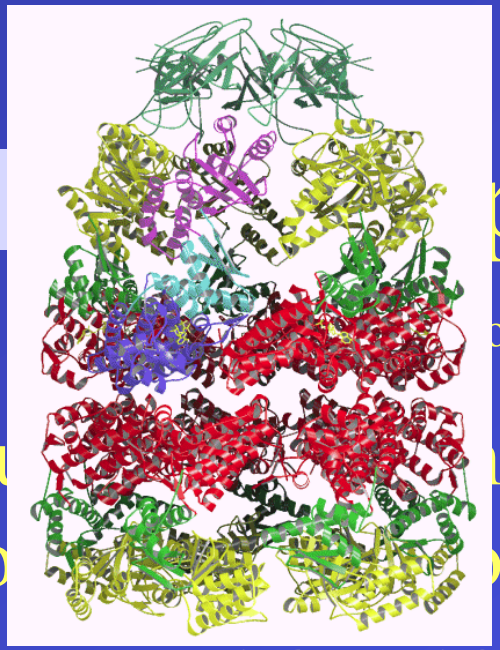
ATP

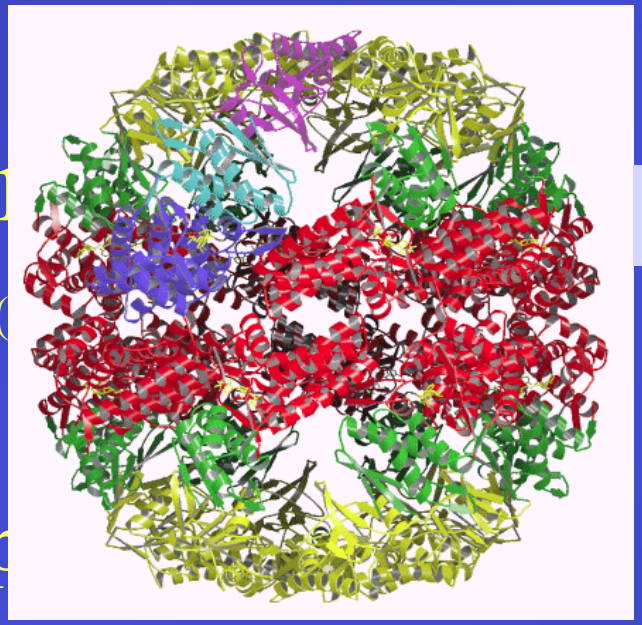




The Chaperonins

Group I





Group II

- Grou its
  mito rop
- Group II 16 or 18 subunits (higher organisms as compared to Group I, eucaryotic CCT, thermosomes, TF55)

Capping by GroES (Hsp10)	No separate Capping
Two Heptameric ring -Identical subunits promiscuous	8(CCT)/9(thermosome) ring -Non Identical subunits Some are more specialised – CCT-actin/tubulin

Why do we need Chaperones in Cell

Anfinsen's Dogma

While most denatured proteins refold spontaniously in vitro, Situation inside the cell is Different.

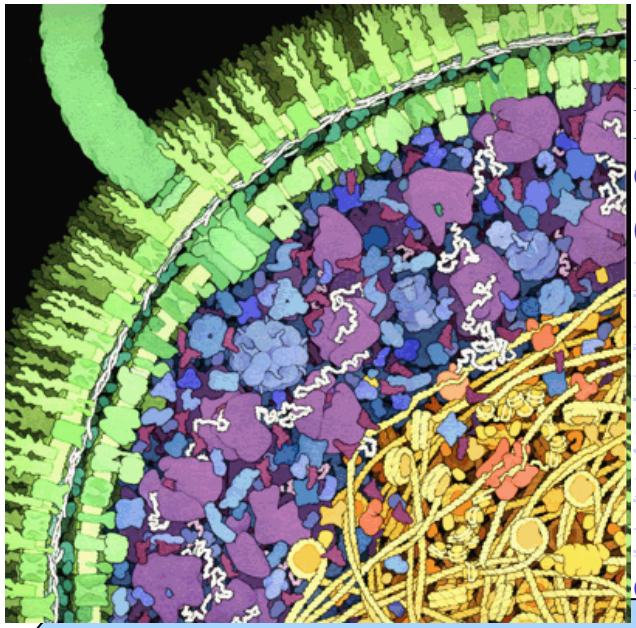


Image by David Goodsell (http://mgl.scripps.edu/people/goodsell/illustration/public; copyright

under such conditions - result in a larger folded species

Chaperones act to prevent or reverse these competing side reactions

Why do we need to study Chaperones

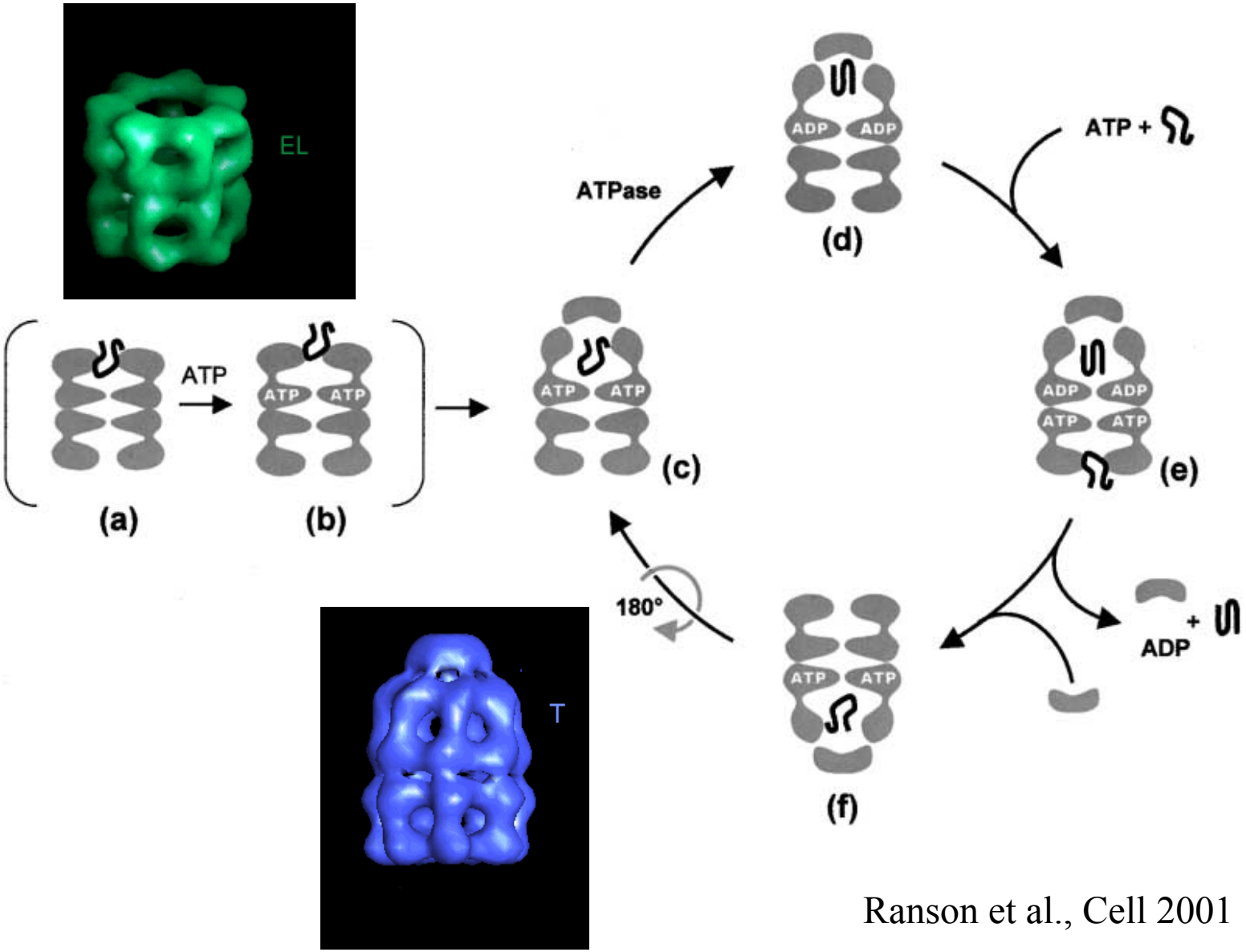
Cellular accumulation of incorrectly folded proteins is the molecular basis of many diseases, including

Alzheimer's Disease,

Prion Diseases and Huntington Disease

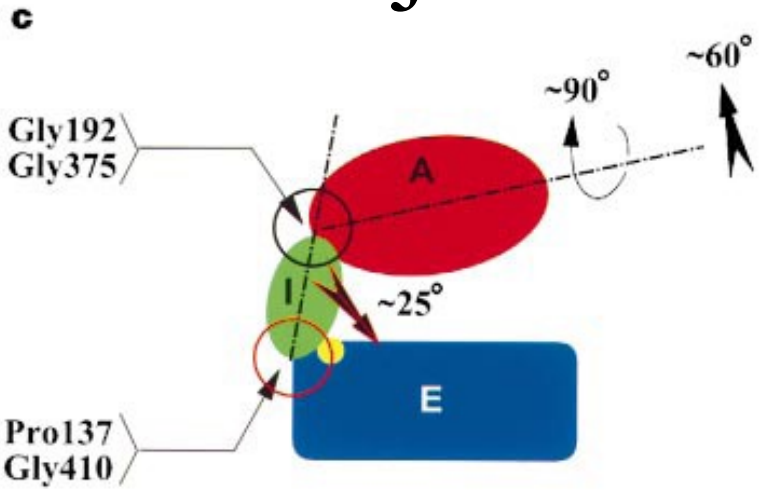
- This underscore the importance of understanding the mechanisms of folding in vivo

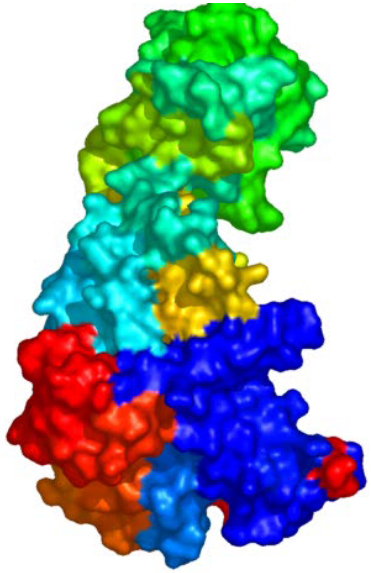
GroEL functional states



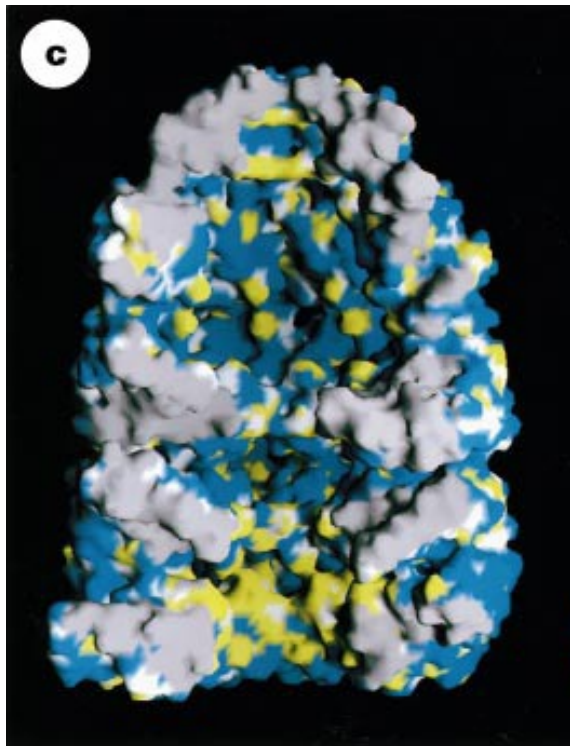
Ranson et al., Cell 2001

Major structural changes





The Yale Morph Server http://molmovdb.org

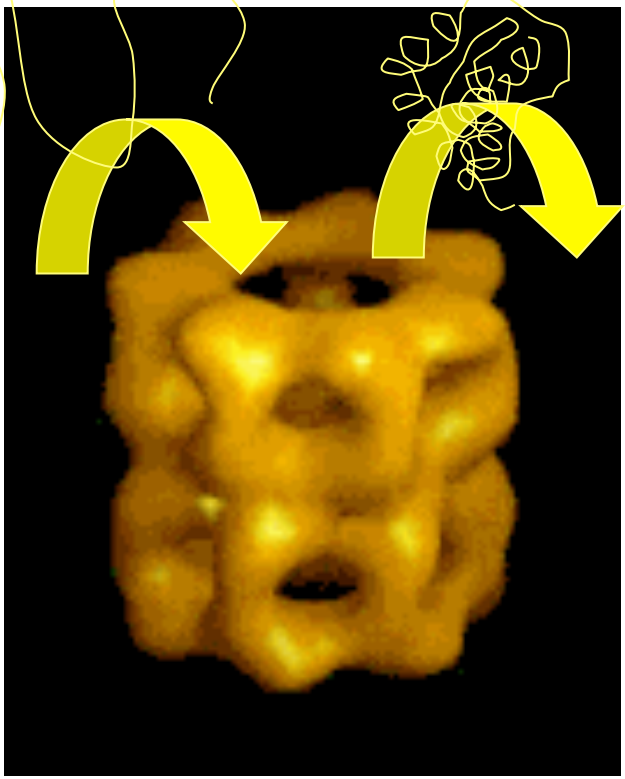


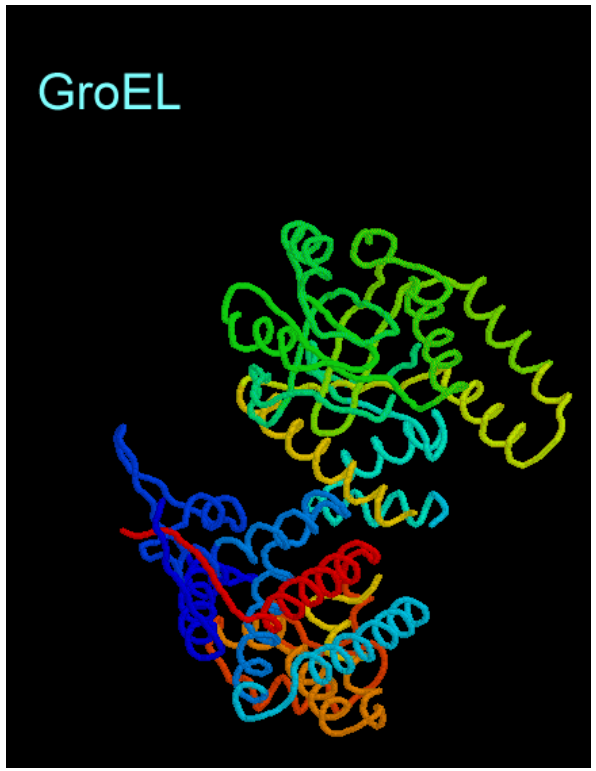
Xu et. al. Nature 1997
Clare et al., Cell 2012

In my mind - 1988 Discovery of Chaperonin – When RJ Ellis, Gatenby, Hemmingston and others identified Helper protein.

•Protein Folding utilising ATP

Classic GroEL/ES from *E.Coli*





Protein folding in chamber

Chen S, Roseman AM, Hunter AS, Wood SP, Burston SG, Ranson NA, Clarke AR, Saibil HR.
Location of a folding protein and shape changes in GroEL-GroES complexes imaged by cryo-electron microscopy.
Nature. 1994, 371:261-264.

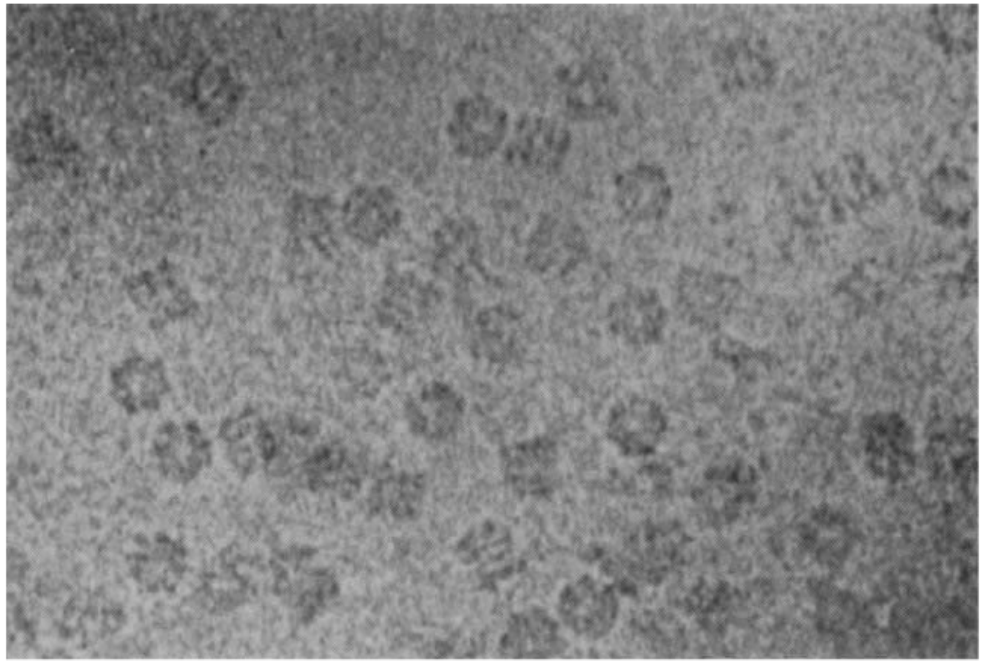
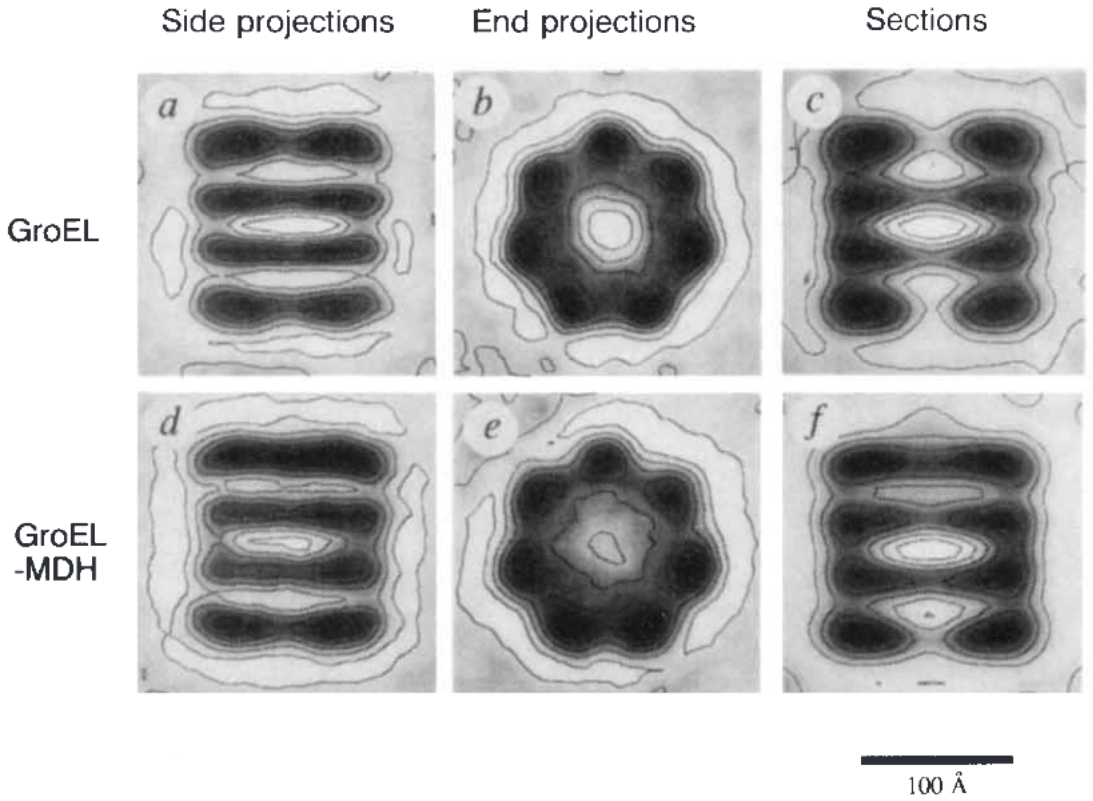


FIG. 1 Unstained, frozen-hydrated GroEL oligomers were imaged in vitreous ice over holes in the carbon support film. The dark regions

418 individual images of GroEL-ES-MDH

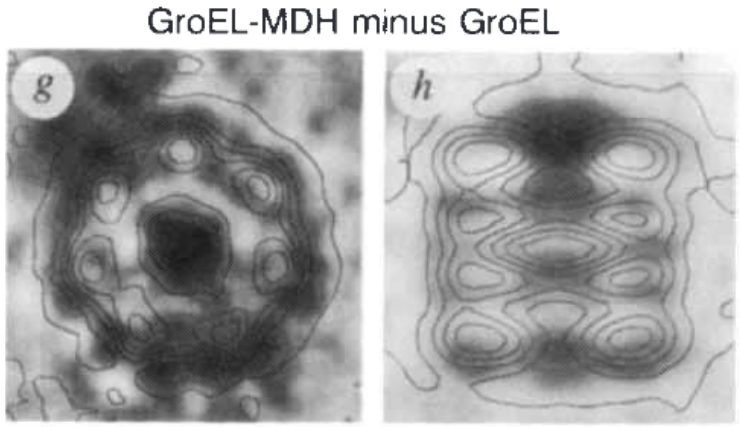
Chen et al.,
Nature
15 Sep 1994.

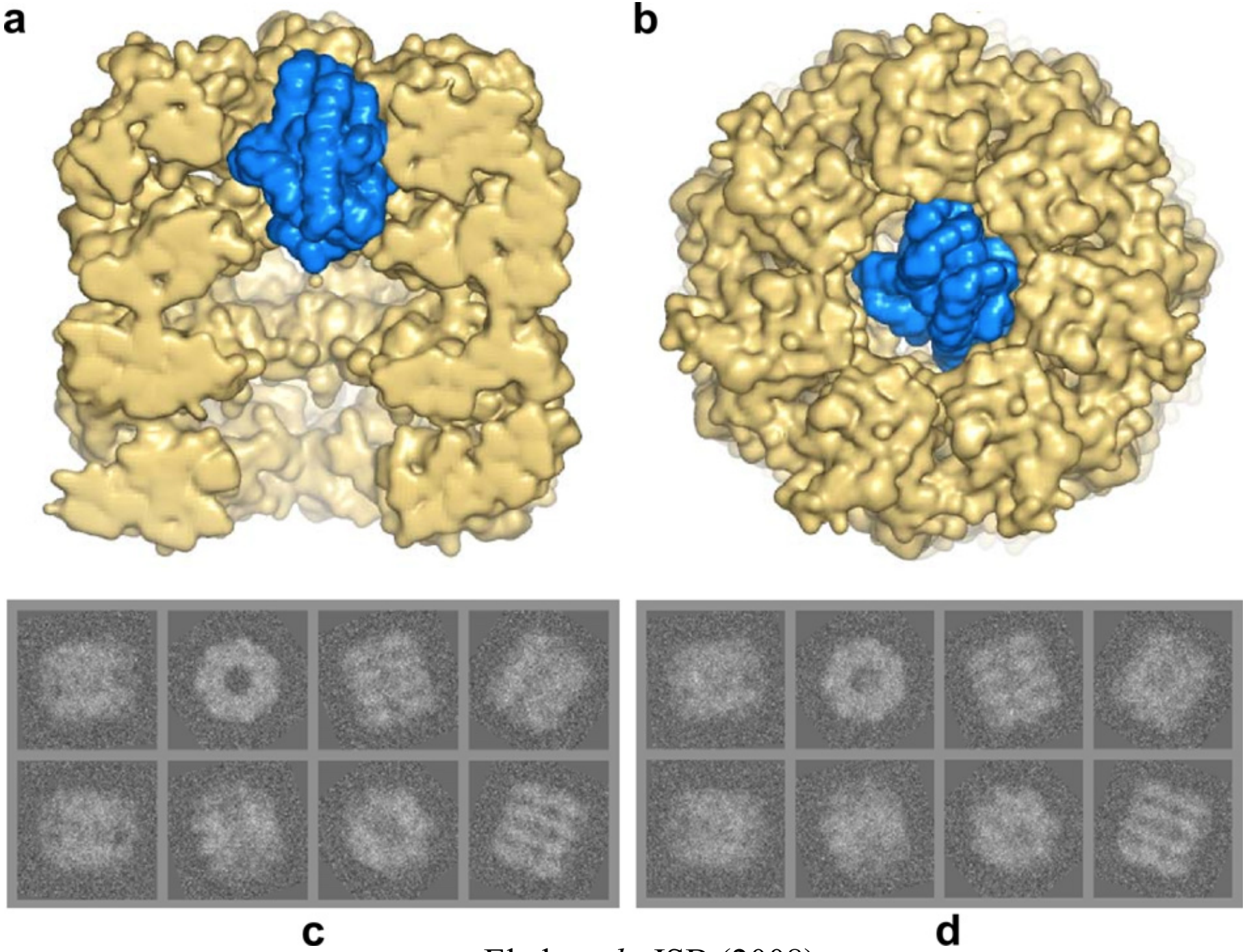


Averaged side views of 418 individual images

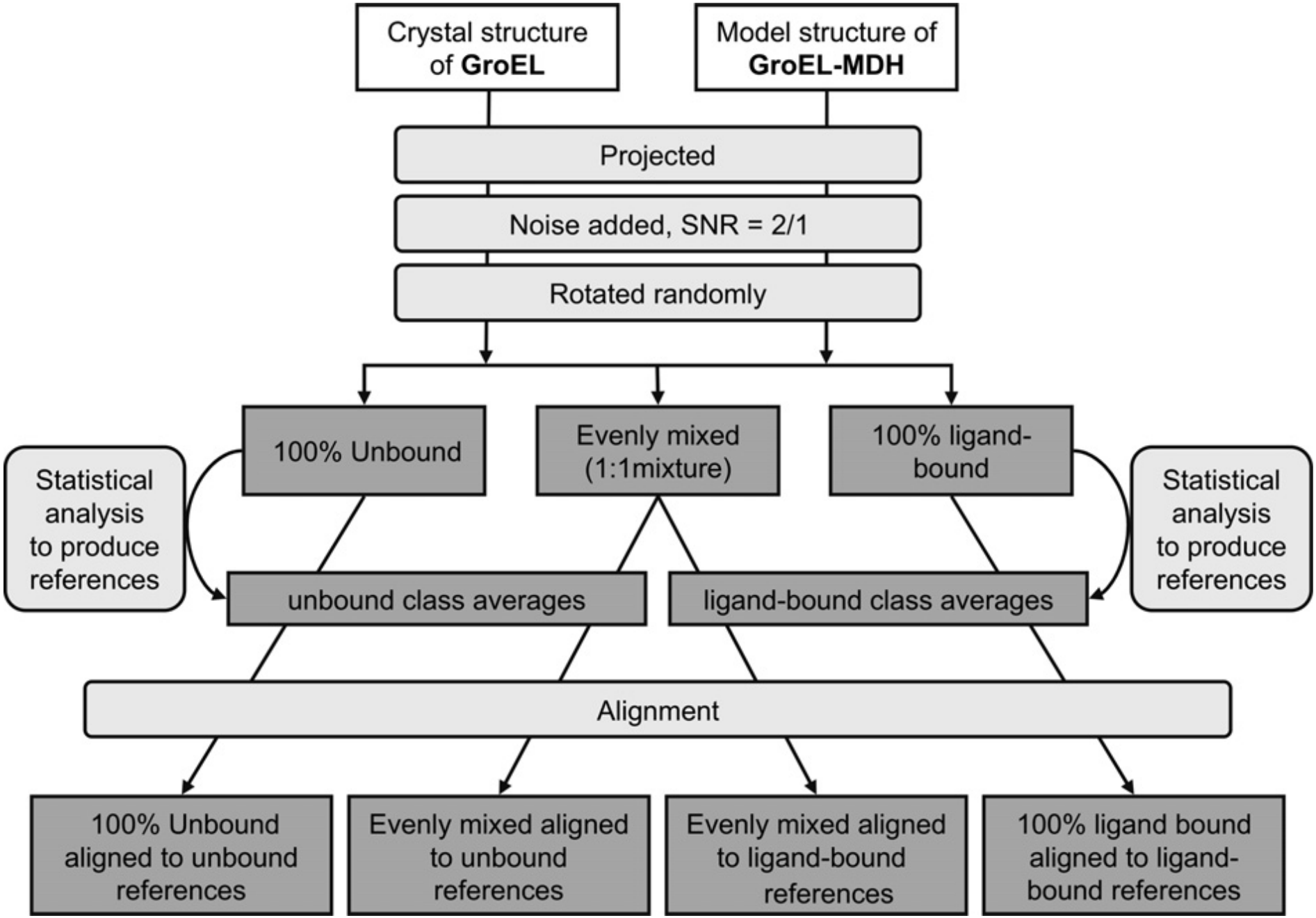
Porcine Mitochondrial MDH

Average of 368 views

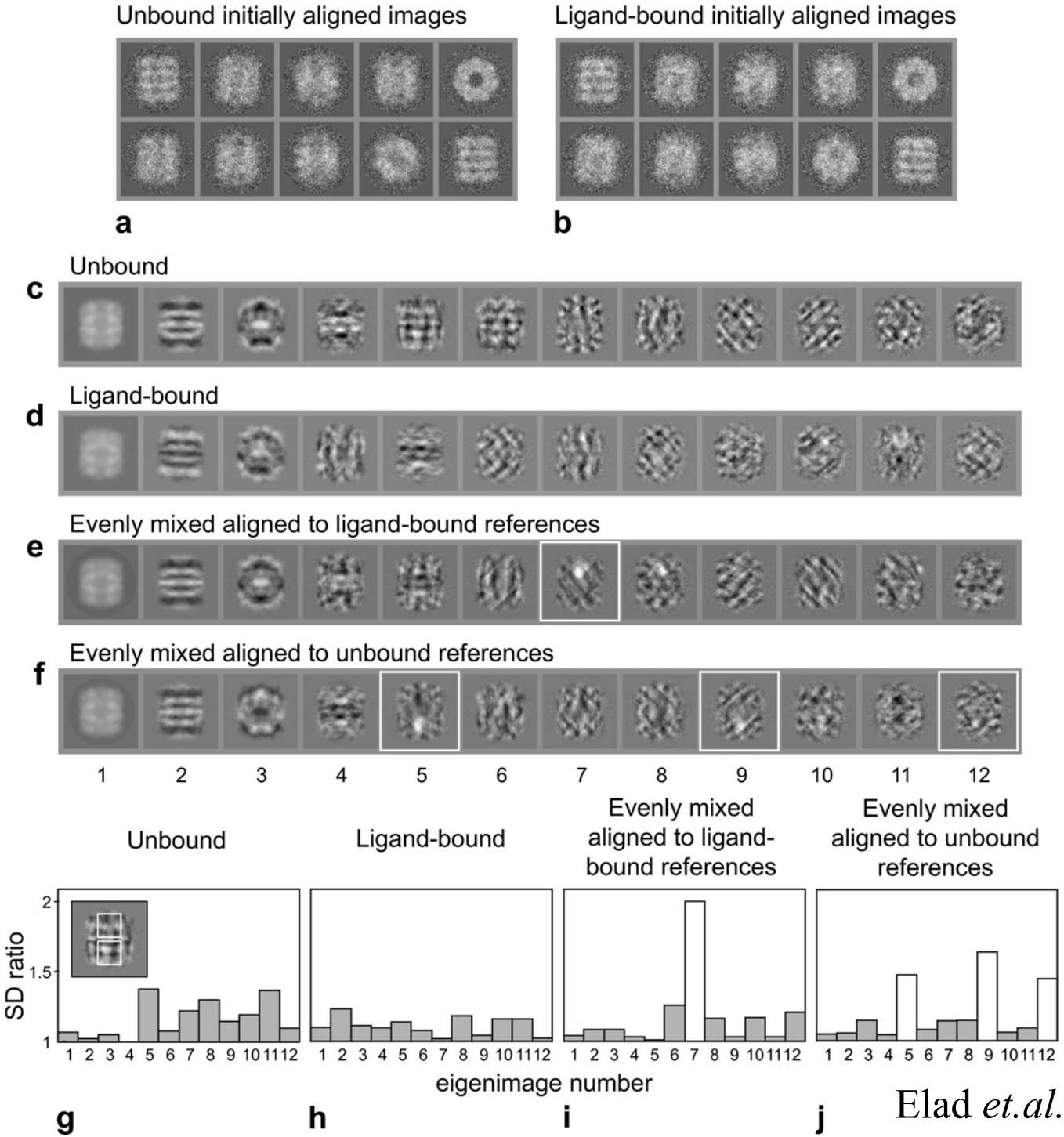




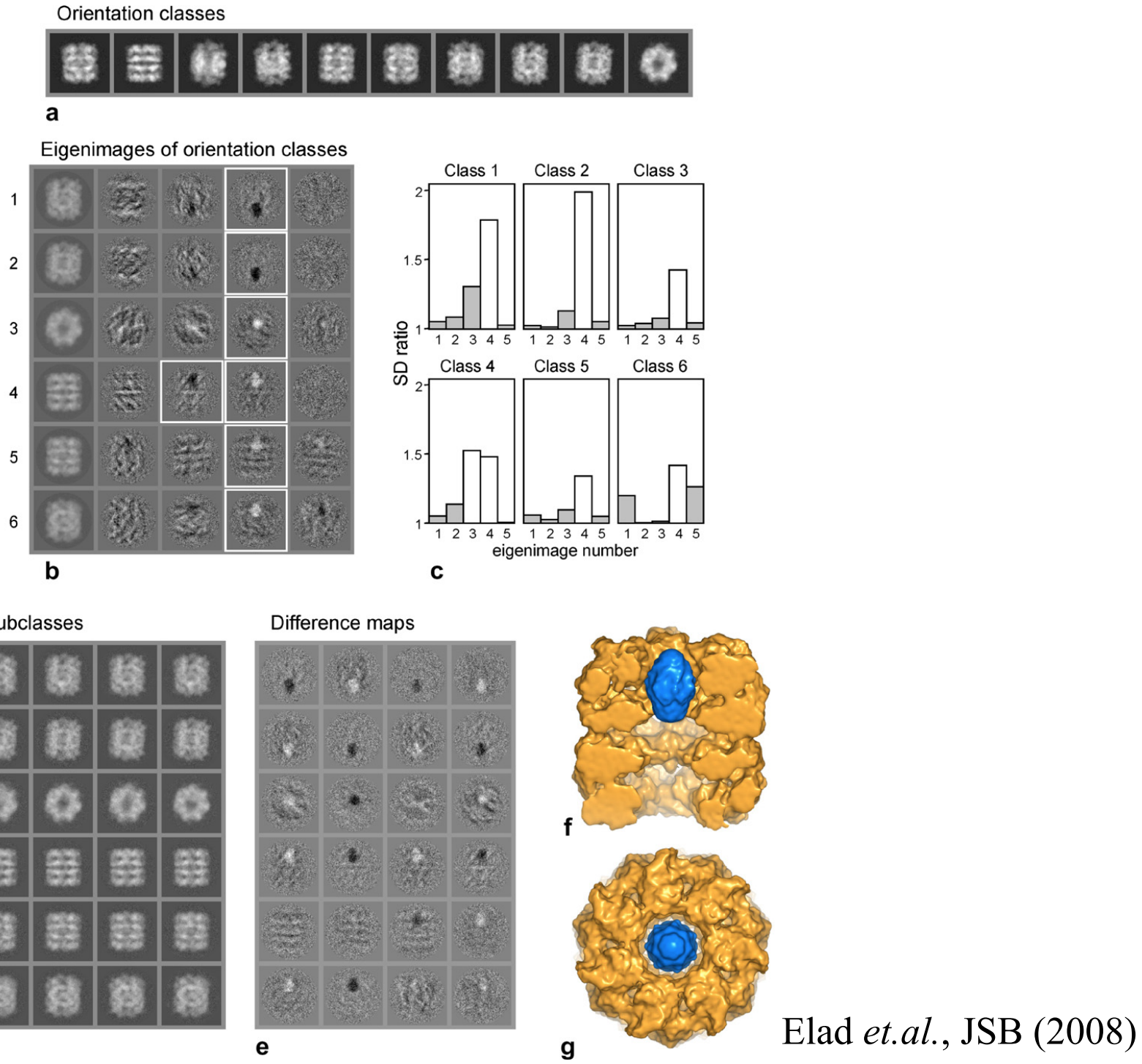
Elad *et.al.*, JSB (2008)



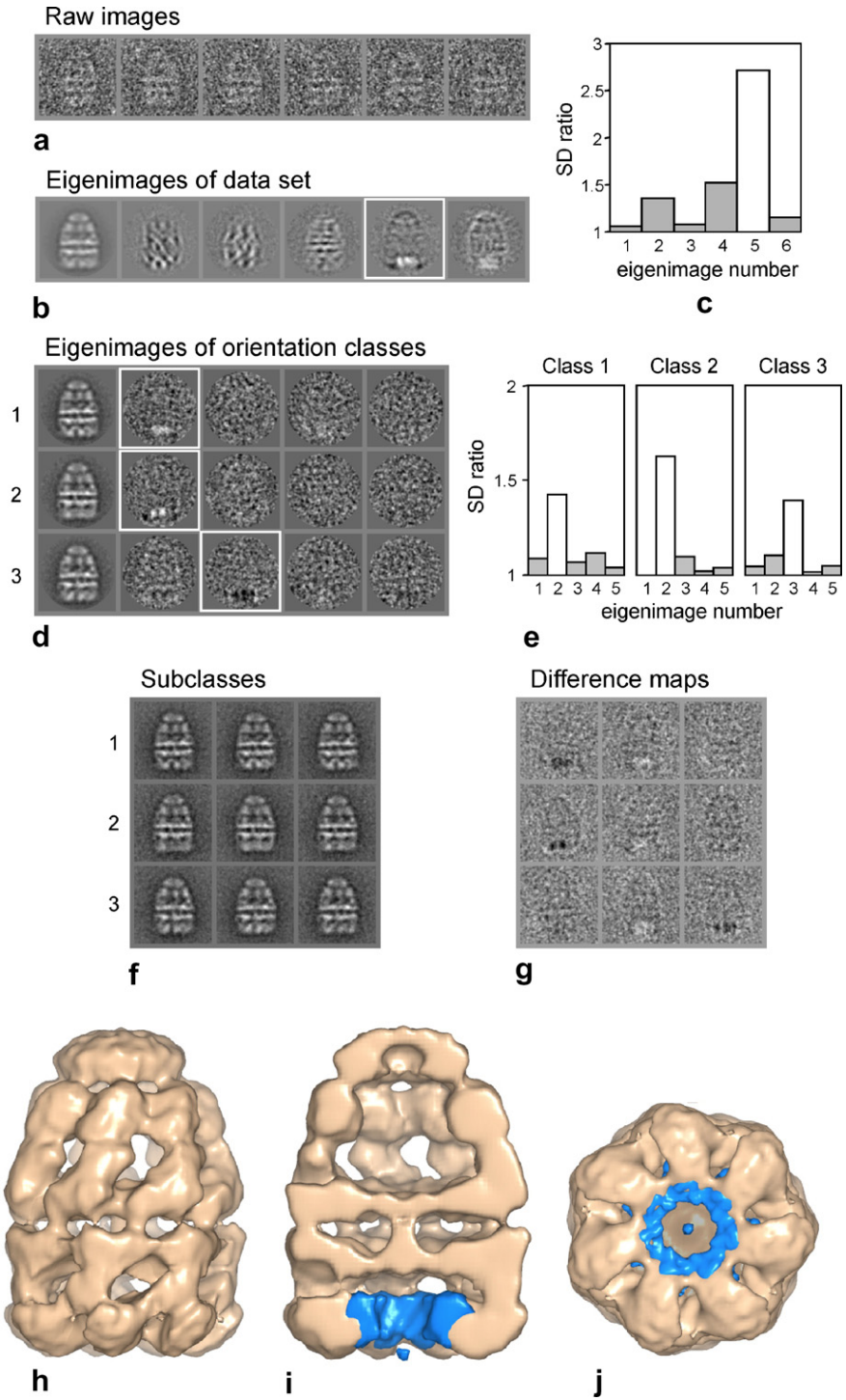
Elad *et.al.*, JSB (2008)



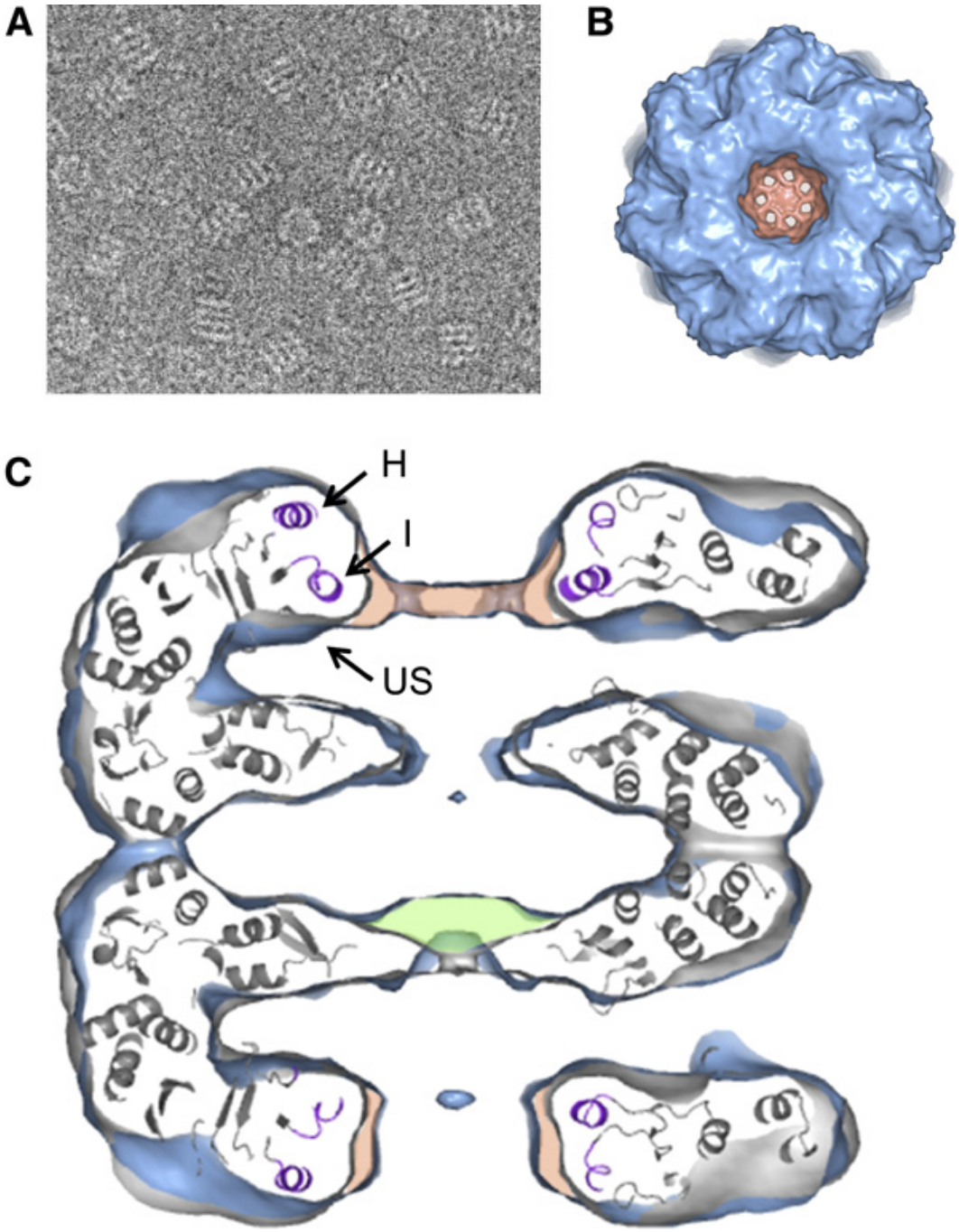
Elad *et.al.*, JSB (2008)



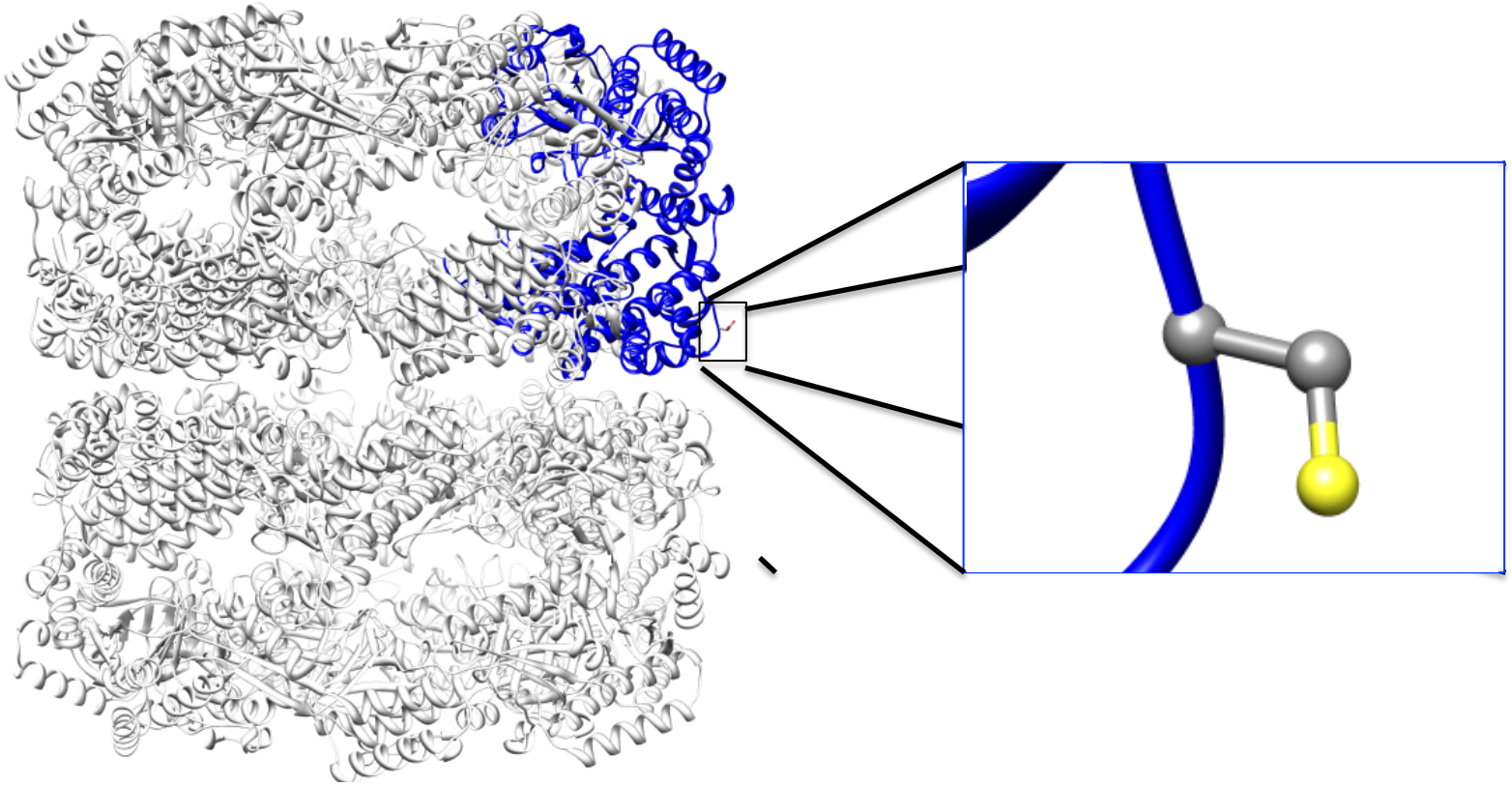
Elad *et.al.*, JSB (2008)



Elad *et.al.*, JSB (2008)

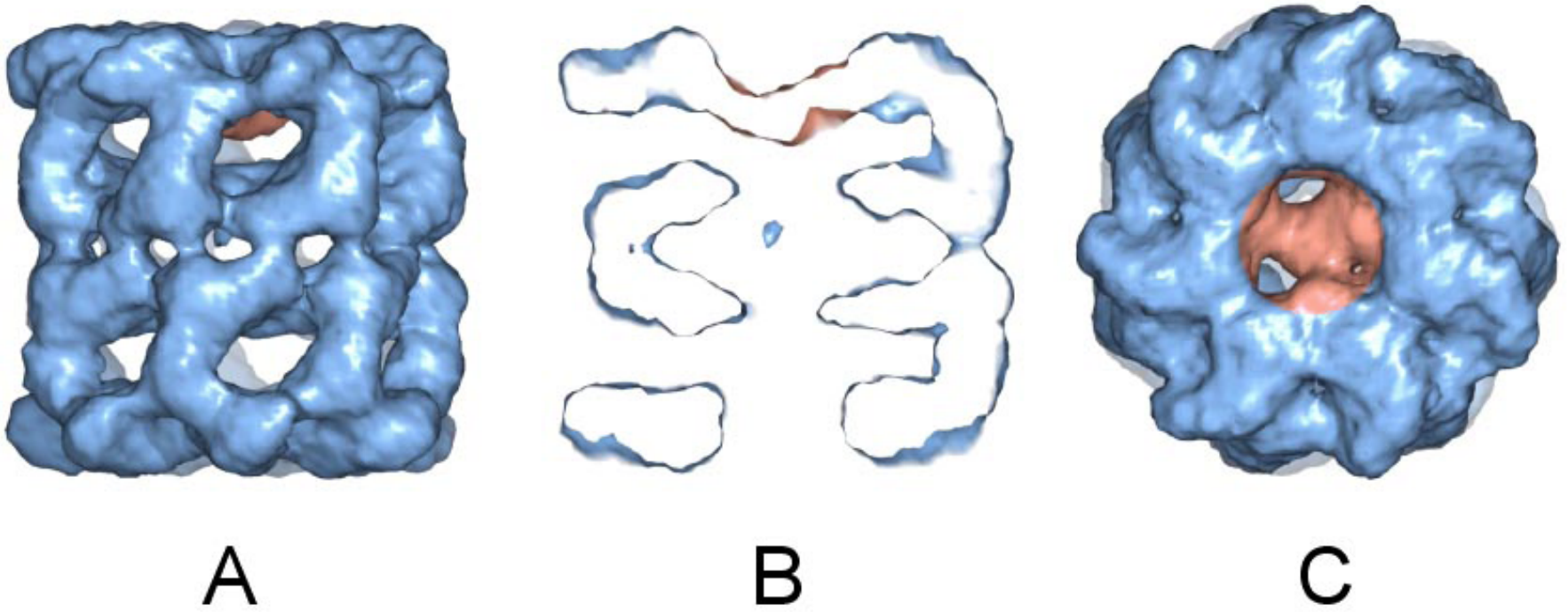

A
B
C
H
I
US

Dealing with particle orientation bias

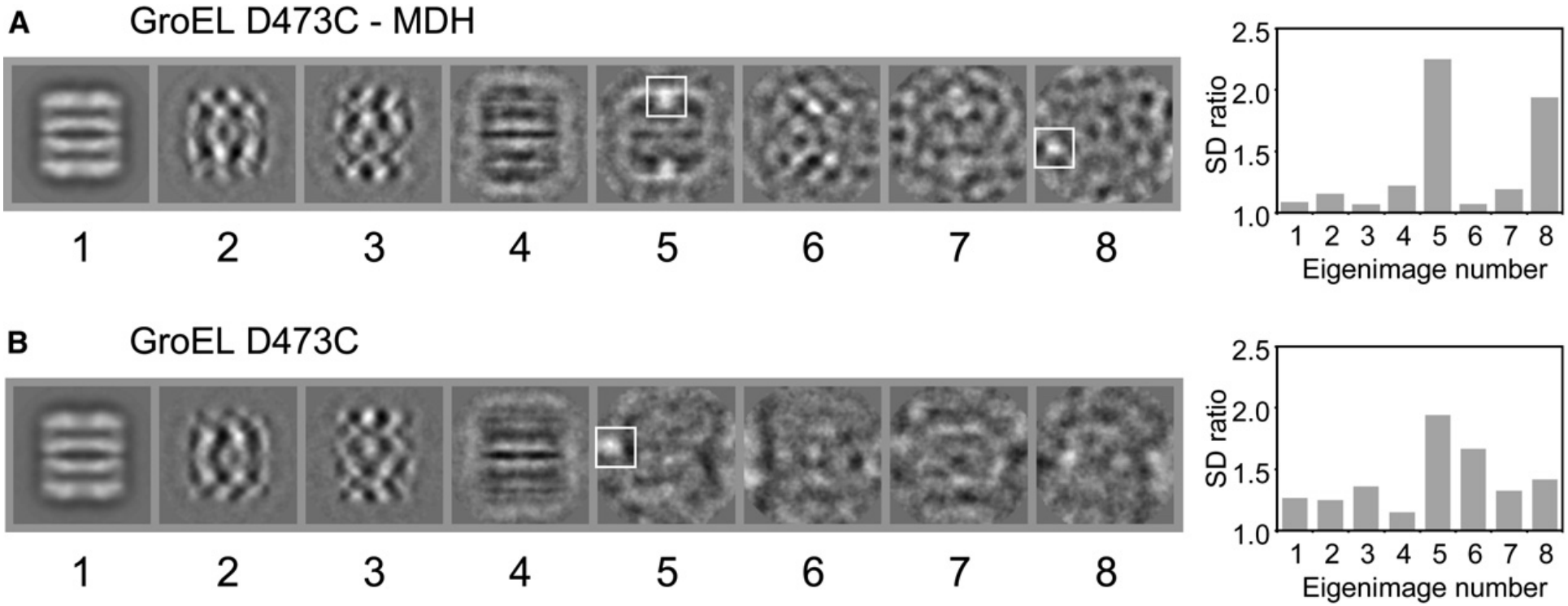


One of the ways : Mutant of GroEL D473C
D473C cross linked to His6 using HBC Sulfo SMCC[Pierce]

Elad *et.al.*, Molecular Cell (2007), 26, 415–426.

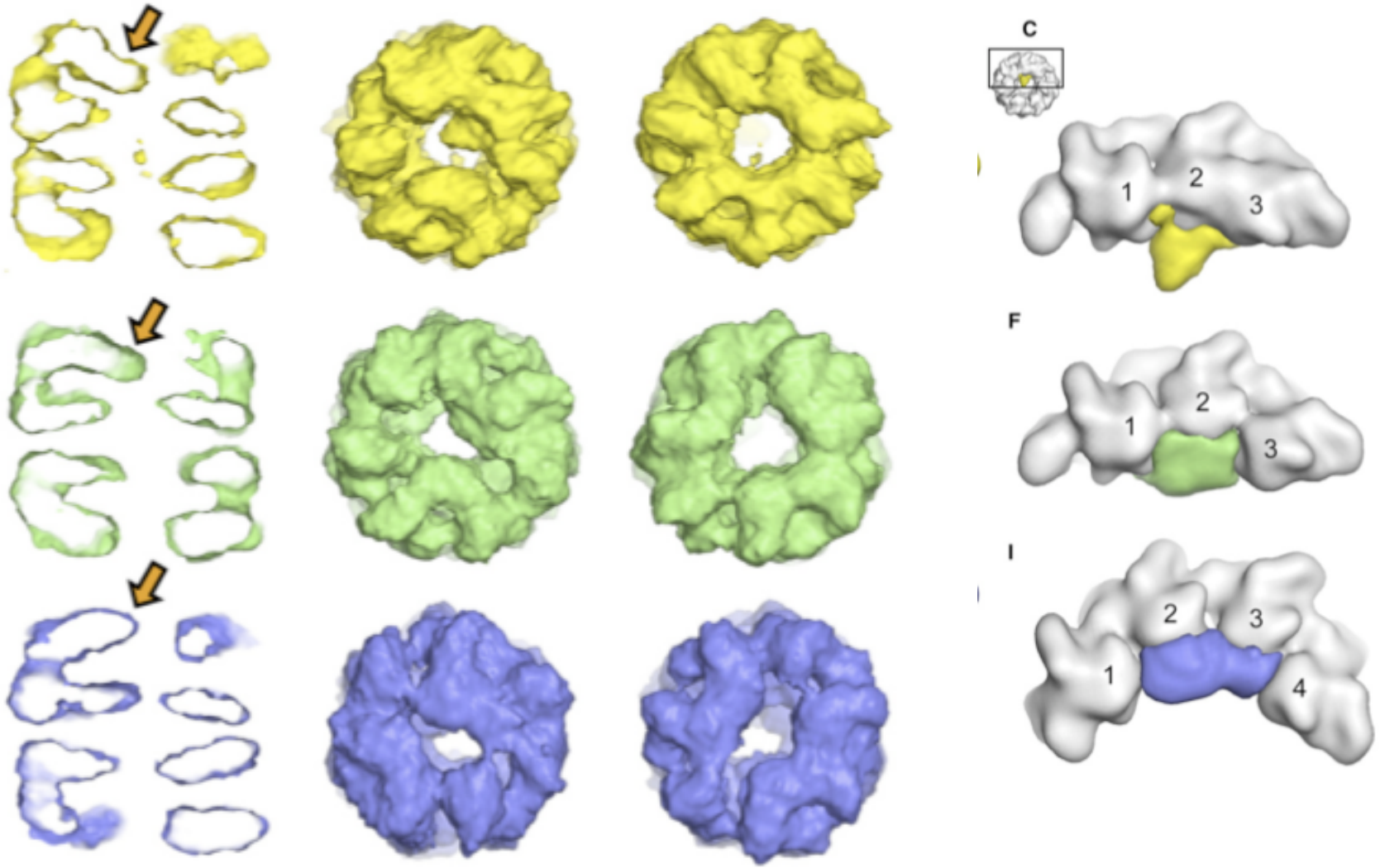


Elad *et.al.*, Molecular Cell (2007), 26, 415–426.



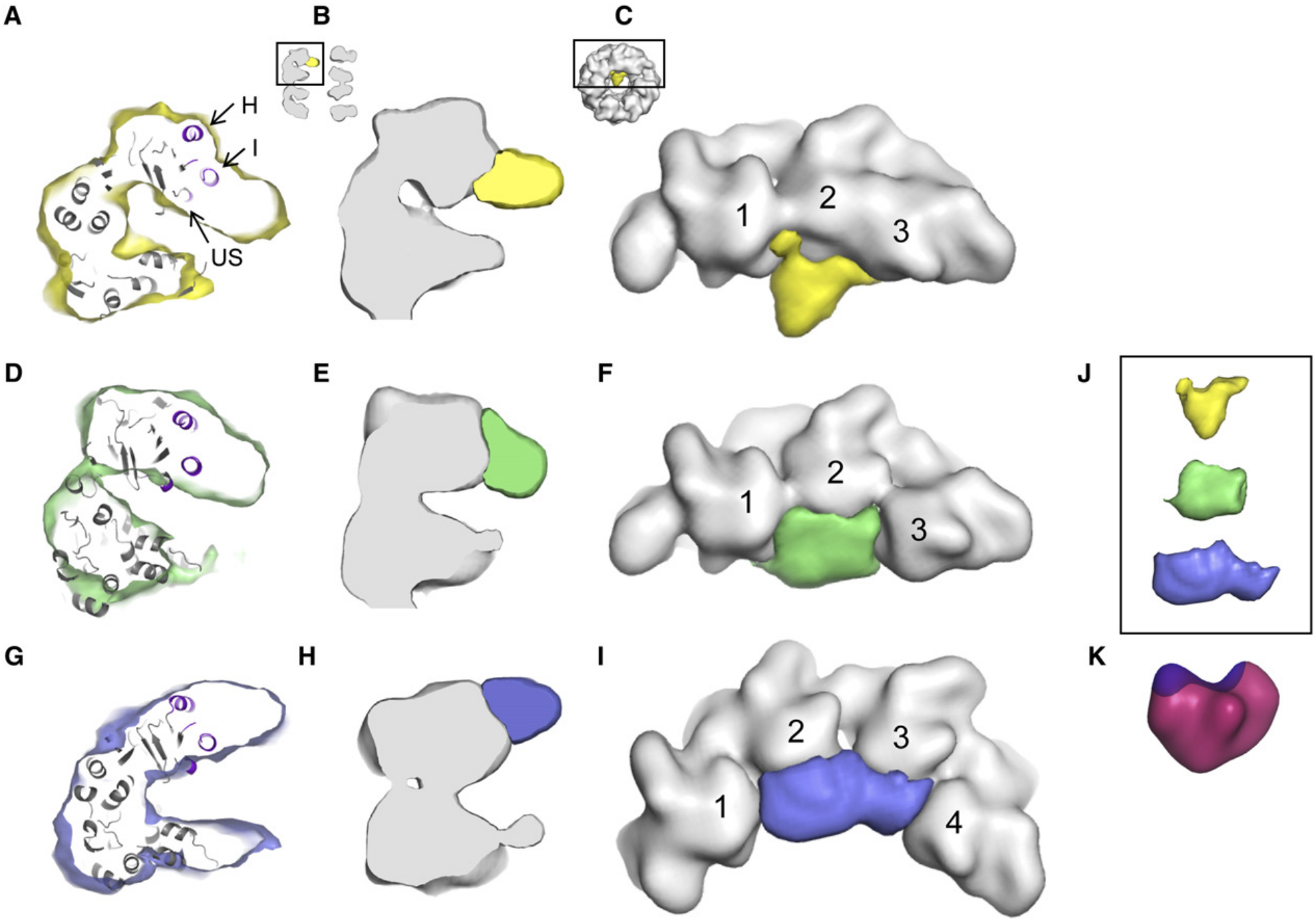
Elad *et.al.*, Molecular Cell (2007), 26, 415–426.

Nadav Elad's 3D map. MDH seen at one end due to low MDH conc.



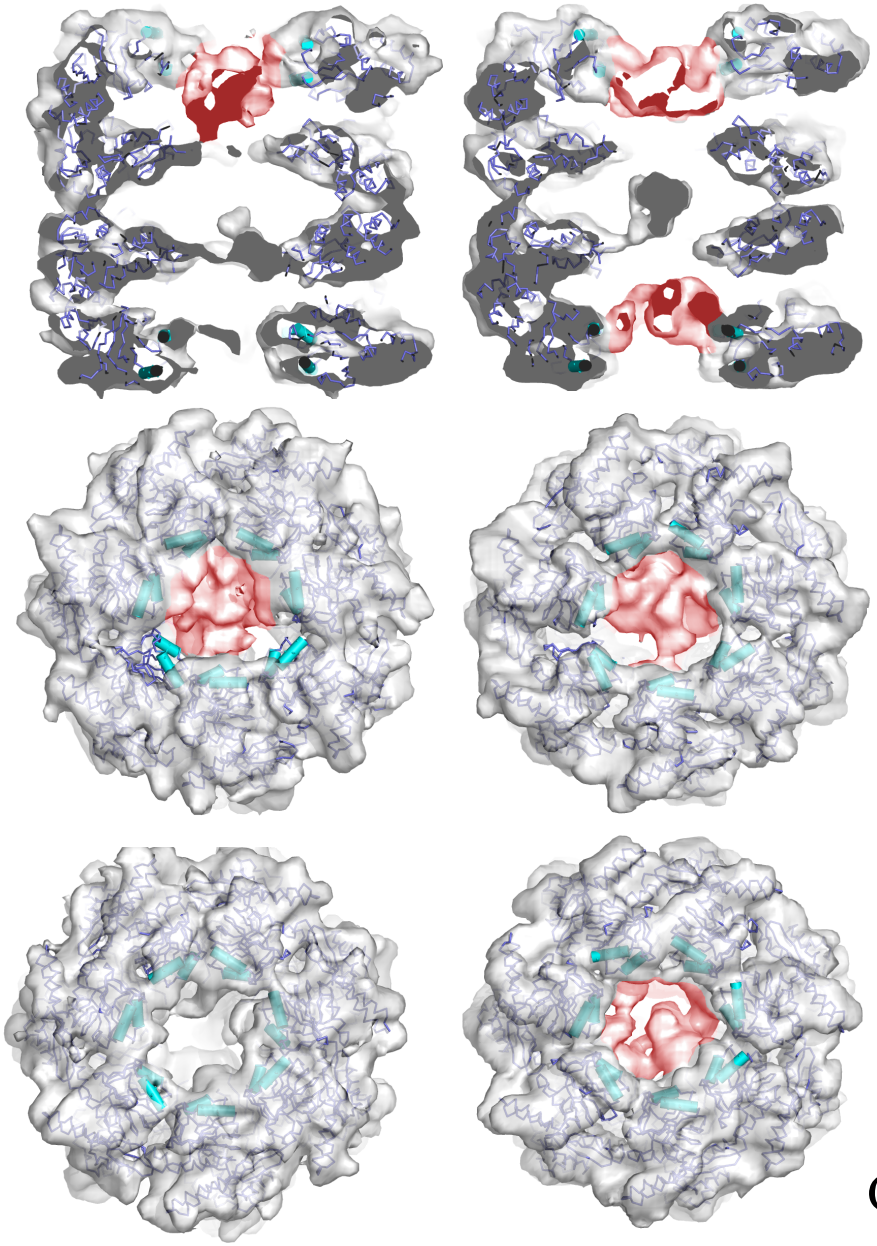
Elad *et.al.*, Molecular Cell (2007), 26, 415–426.

Cryo EM work on MDH, GroEL work from our lab shows various bound substrate states.



Elad *et.al.*, Molecular Cell (2007), 26, 415–426.

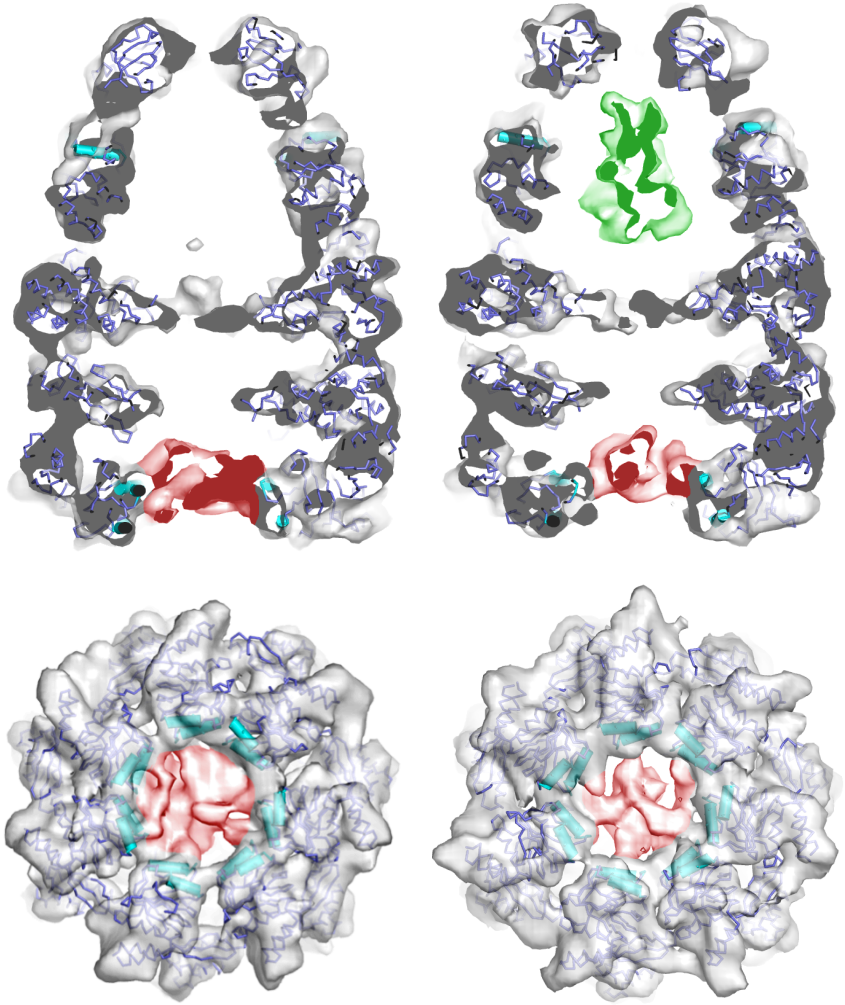
GroEL-gp23 complexes



Single binary

Double binary

GroEL-gp23-gp31 complexes



Clare *et al.*, Nature (2009). 457,107-110

Cryo EM work on gp23, gp31, GroES work from our lab shows various states.

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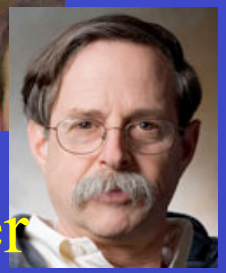

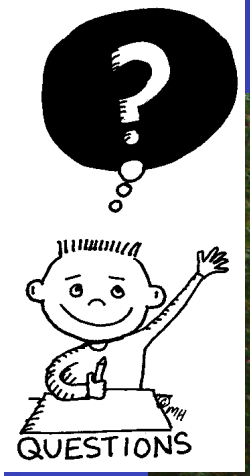

Thank you