Making the most of symmetry

EMBO *Practical Course:*
Image Processing for cryoEM

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IMPERIAL COLLEGE LONDON

DEPARTMENT OF LIFE SCIENCES

What is symmetry?

Perfect rotational symmetry

Nature is not perfect

Learning objectives

1) Describe different types of symmetry common in structural biology

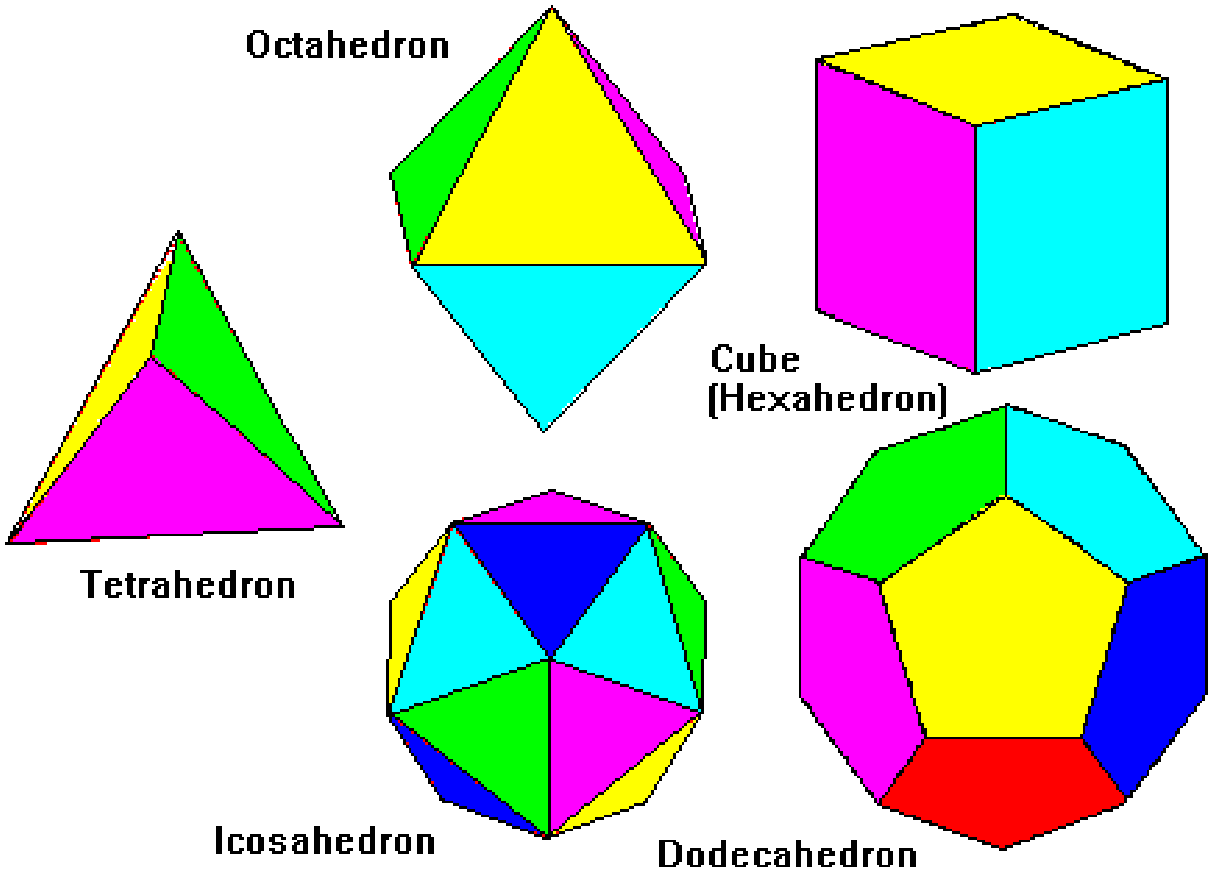
2) Distinguish when it is appropriate to apply symmetry

3) Design computational experiments to solve structures where there is a symmetry break

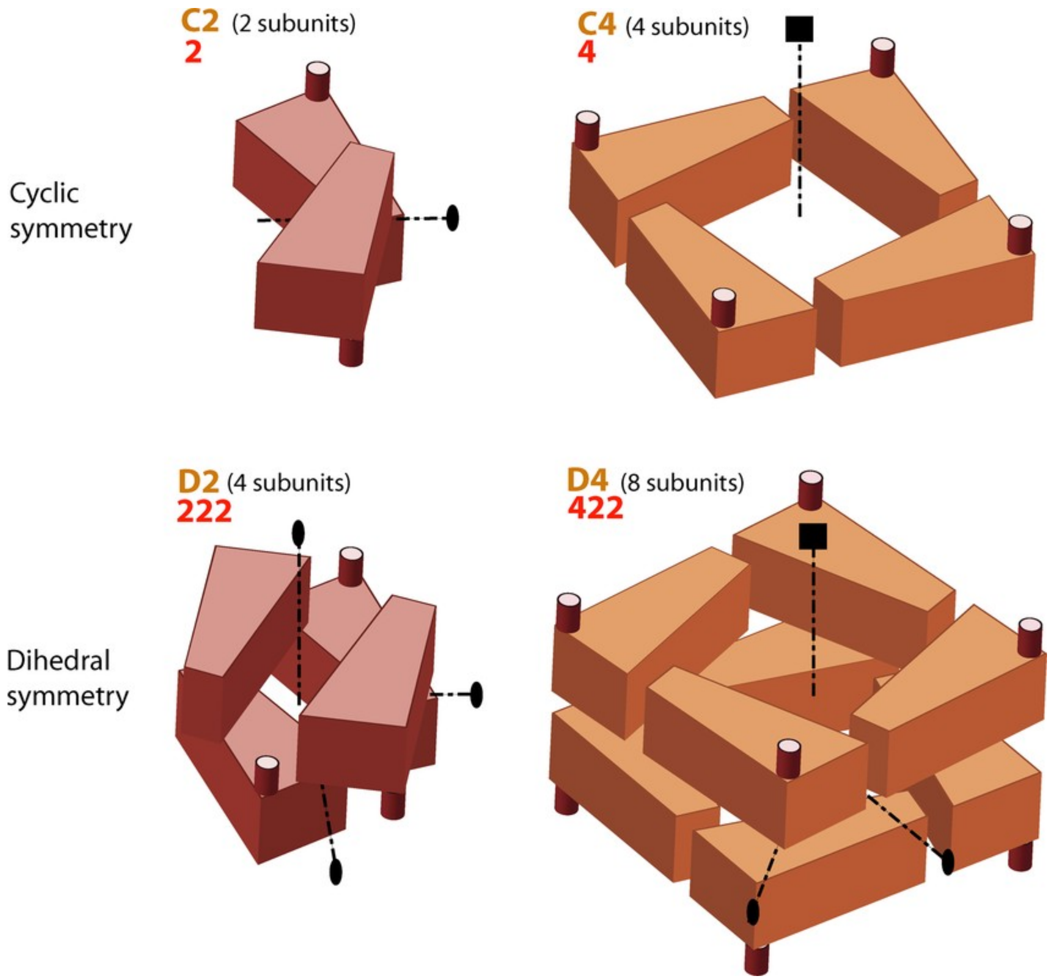
4) Review and report on the use of symmetry (point group, platonic and helical) and mismatches in cryoEM

Symmetry in structural biology

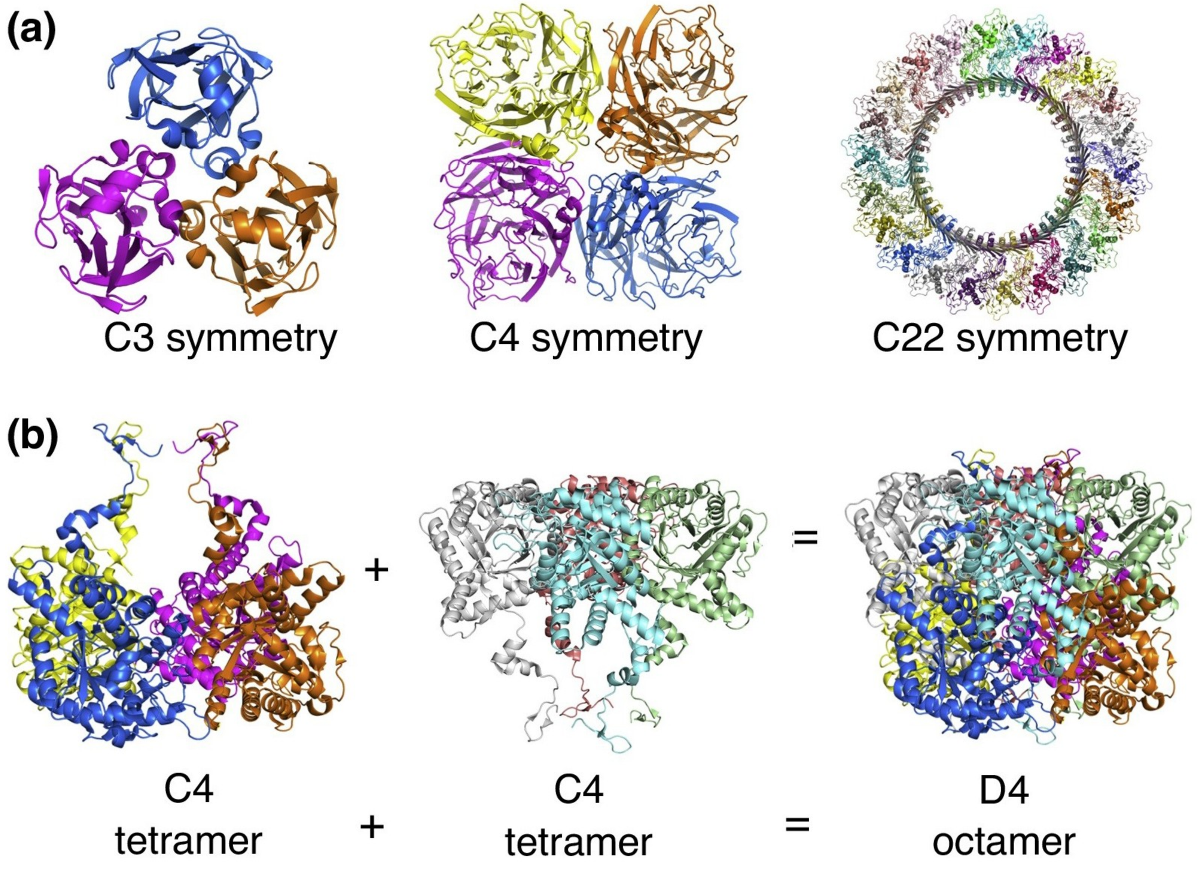
- Rotational (cyclic) symmetry (C_n)
  - Simplest is C_1
  - No perpendicular symmetry axes
- Platonic Solids
  - Faces, edges and corners are related by symmetry operations
  - Dihedral (D_n)
  - Tetrahedral (T)
  - Octahedral (O)
  - Icosahedral (I)



Cyclic and dihedral symmetries



(Levy et al., PLoS computational Biology 2006)



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

How do I know if my protein has symmetry?

J. Mol. Biol. (1971) **60**, 123–130

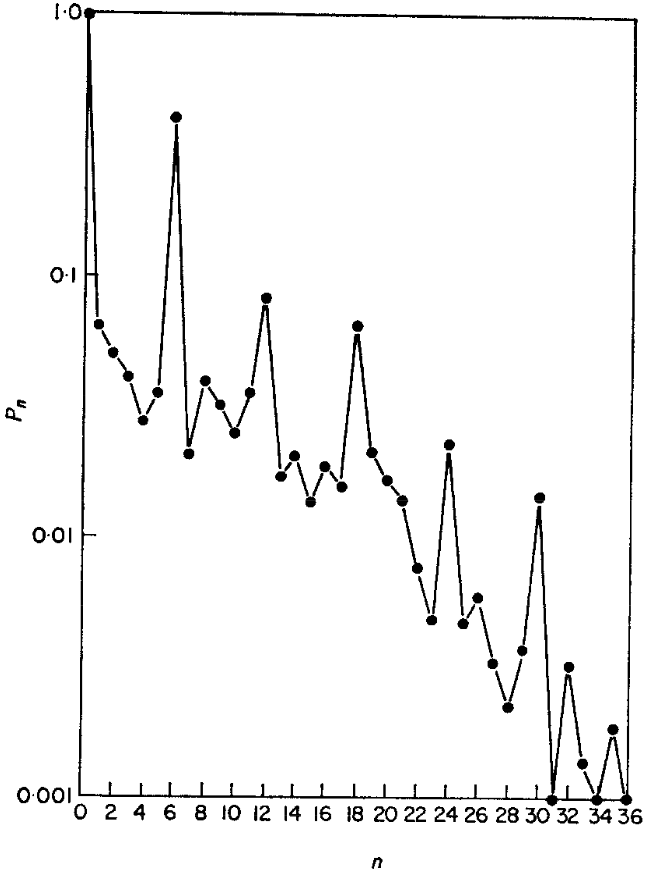
Harmonic Analysis of Electron Microscope Images with Rotational Symmetry

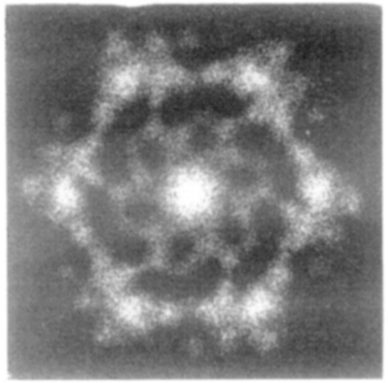
R. A. Crowther and Linda A. Amos

Medical Research Council Laboratory of Molecular Biology
Hills Road, Cambridge, England

(*Received 9 February 1971*)

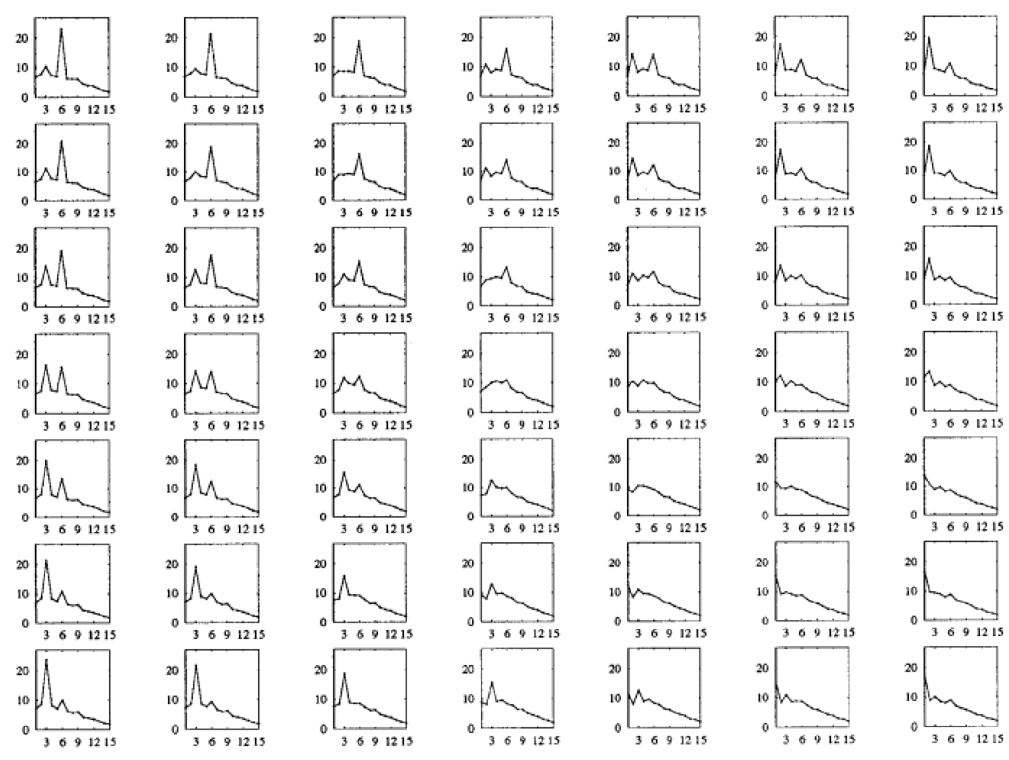
This paper describes a method of analysing images from electron micrographs of biological specimens believed to possess rotational symmetry. An objective analysis of the symmetry is possible because the method, which is computational, produces a rotational power spectrum of the image. We can then combine just those components which are consistent with the previously determined symmetry to produce a filtered image. The method is applied to the base plate of bacteriophage T4 and to discs of tobacco mosaic virus protein. The advantages of this new approach over the well-known Markham rotation technique are discussed.



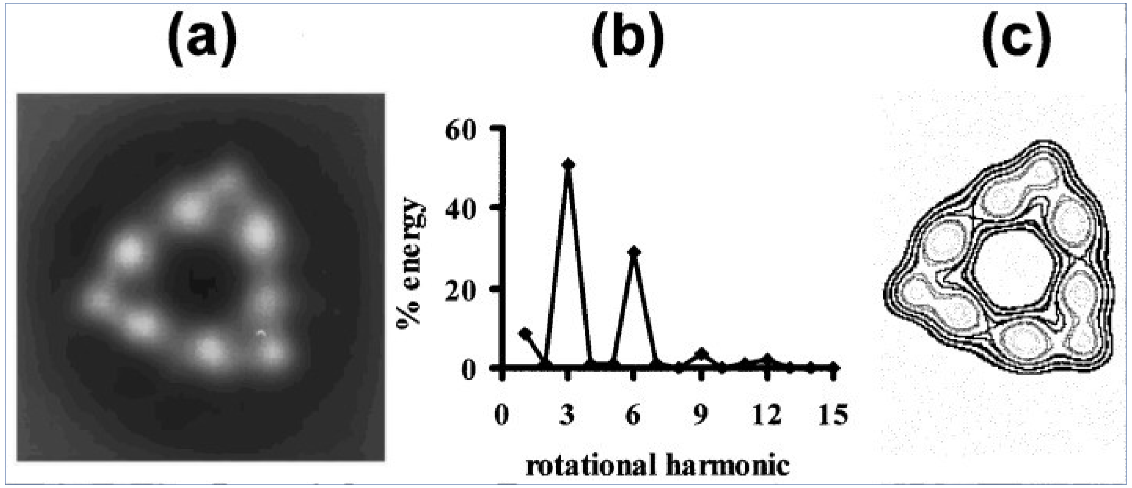


Logarithmic plot of rotational power spectrum of T4 bacteriophage base plate

Implementation in Xmipp



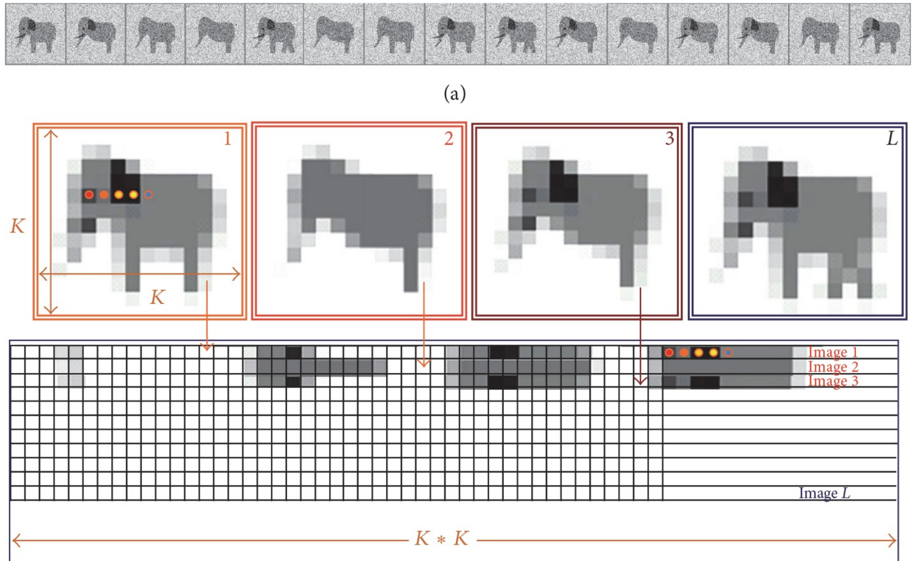
(a)



- Generate rotational power spectra
- K-means classification
- Statistical analysis with eigenvectors
- Limited by noise levels in the images

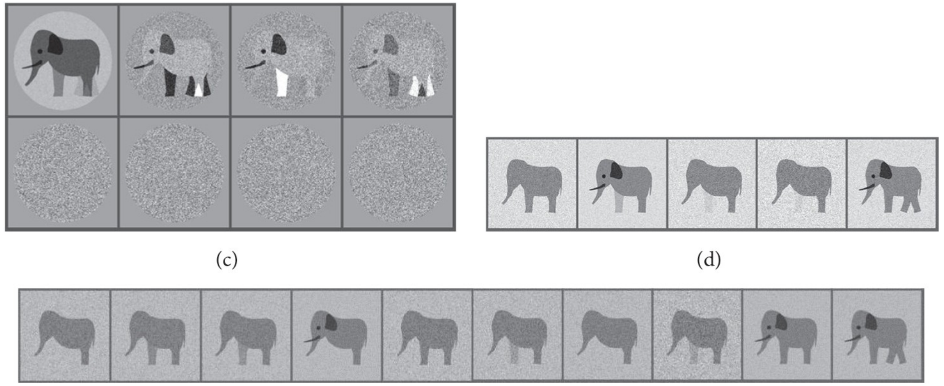
(Barcena et al., JMB 1998; Scheres et al., Nature Protocols 2008)

Multivariate statistical analysis (MSA) approach



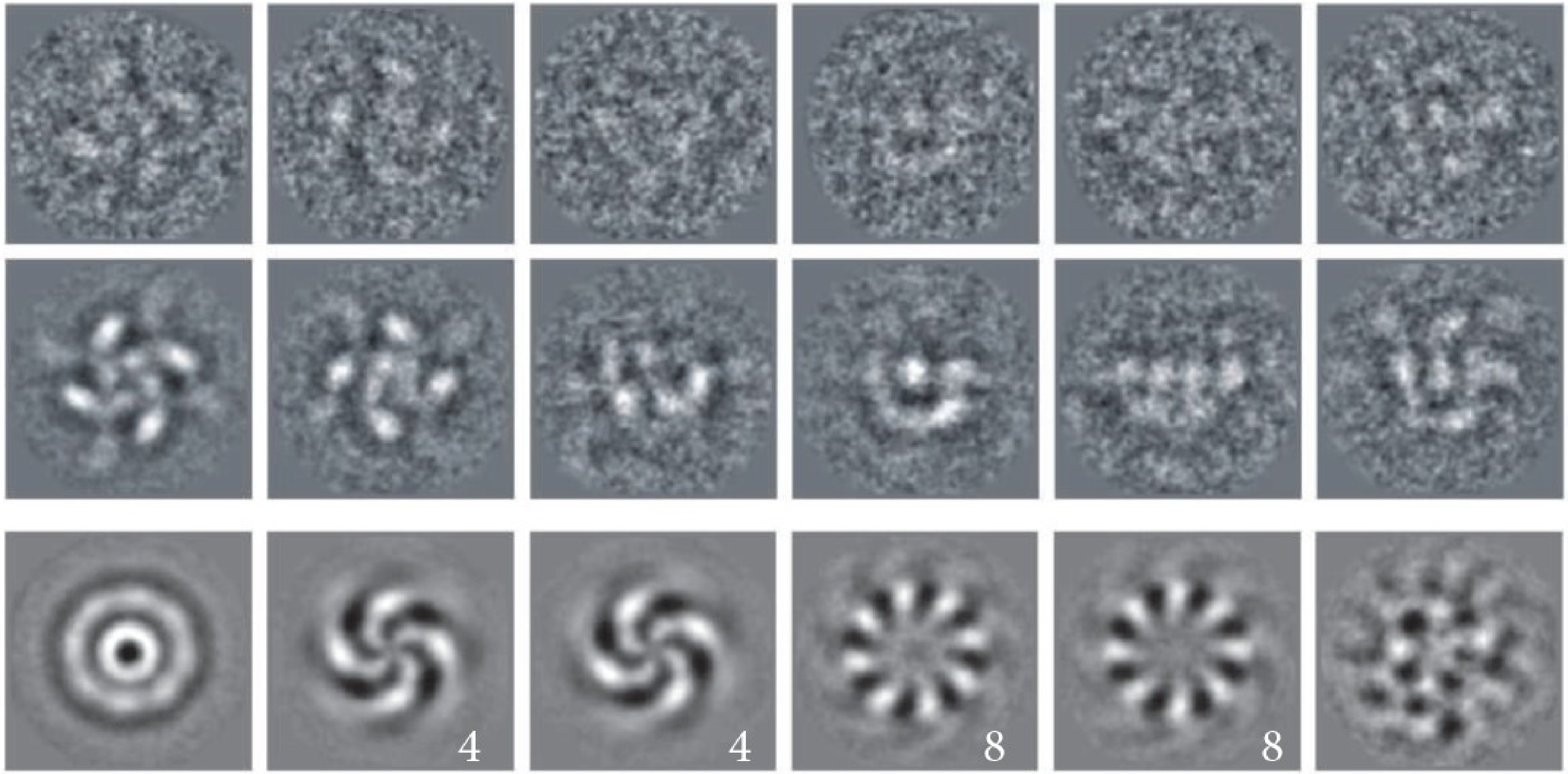
- Finding essential variables
- Representing features as vectors
- Classification based on features

- Eigen images describe particle features
- Can be used as the basis for classification
- Choosing the number of classes



(White et al., Biomedical Research International 2017)

MSA applied to detect symmetry



Main advantage: ability to examine relationships among multiple variables at the same time

(White et al., Biomedical Research International 2017)

Is having symmetry an advantage for cryoEM?

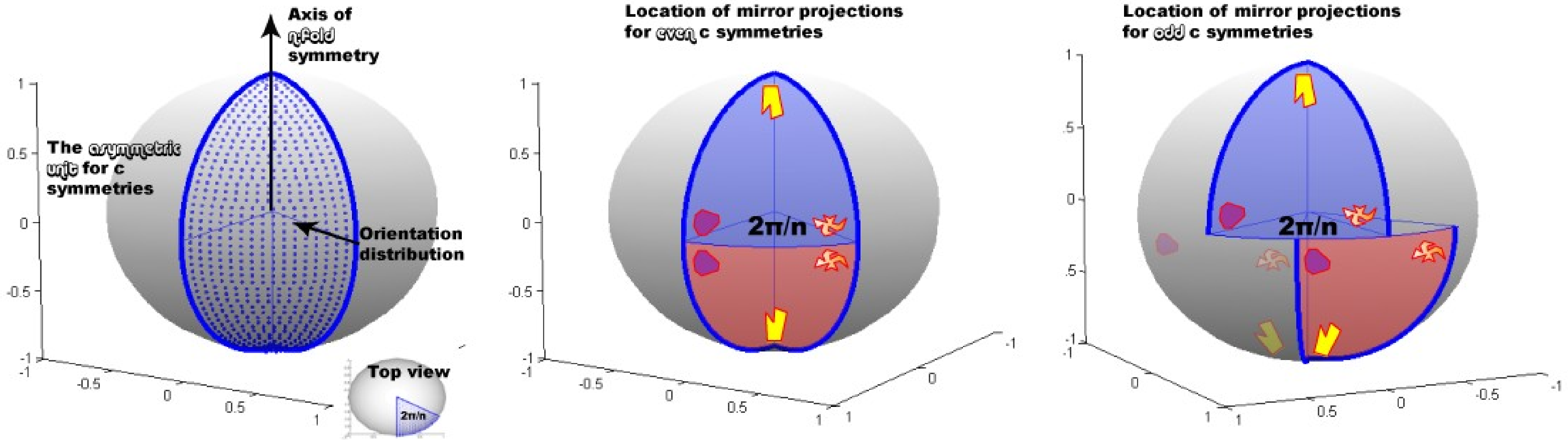
https://b.socrative.com/login/student/

Room FE419C5F

How do I use it to my advantage?

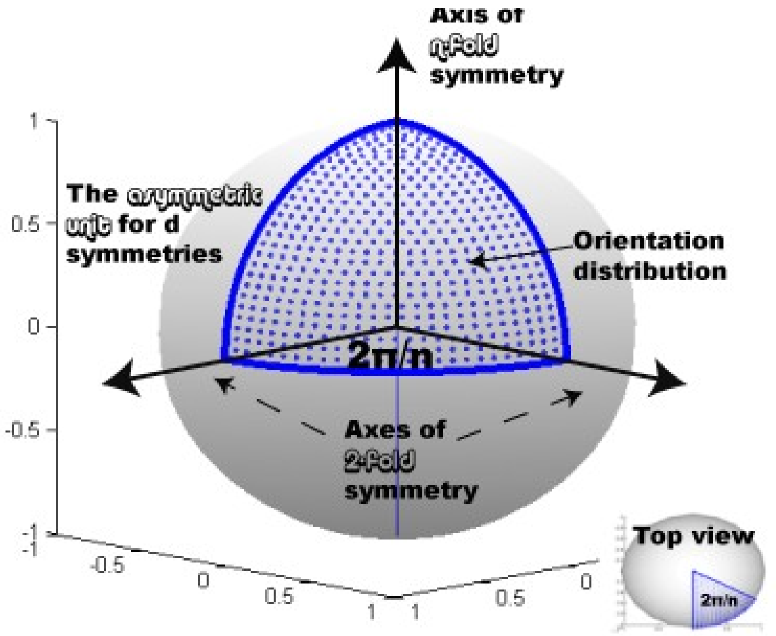
- Smaller asymmetric unit
- Decrease computational resources
- Better averaging improves signal to noise
- Need less particles

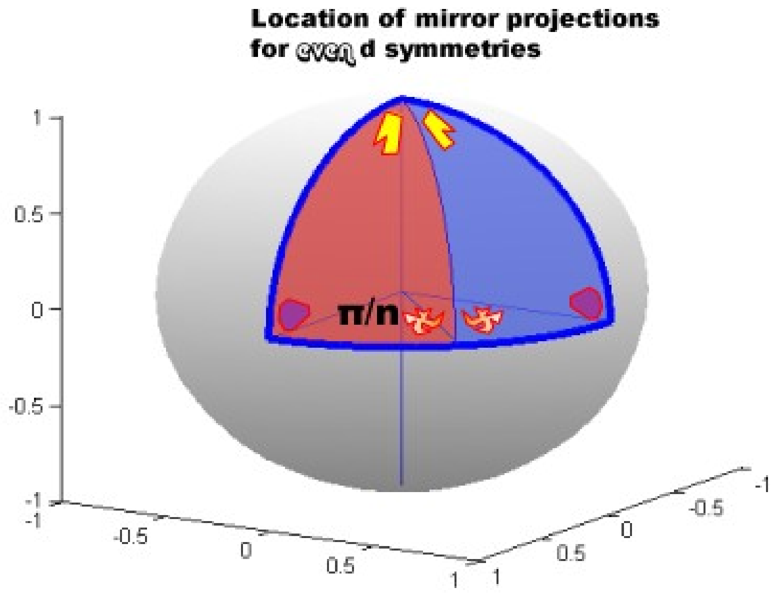
C_n symmetry

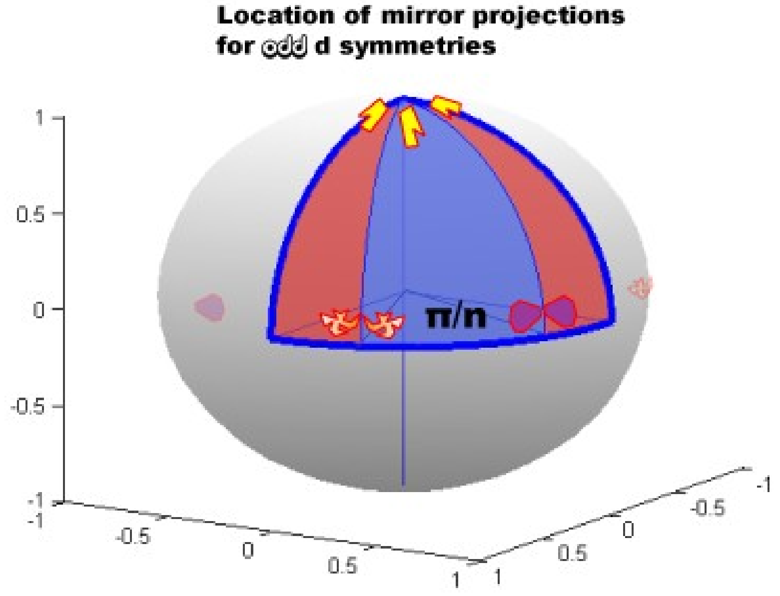


Axis of n-fold symmetry
The asymmetric unit for c symmetries
Orientation distribution
Top view
2π/n
Location of mirror projections for even c symmetries
2π/n
Location of mirror projections for odd c symmetries
2π/n

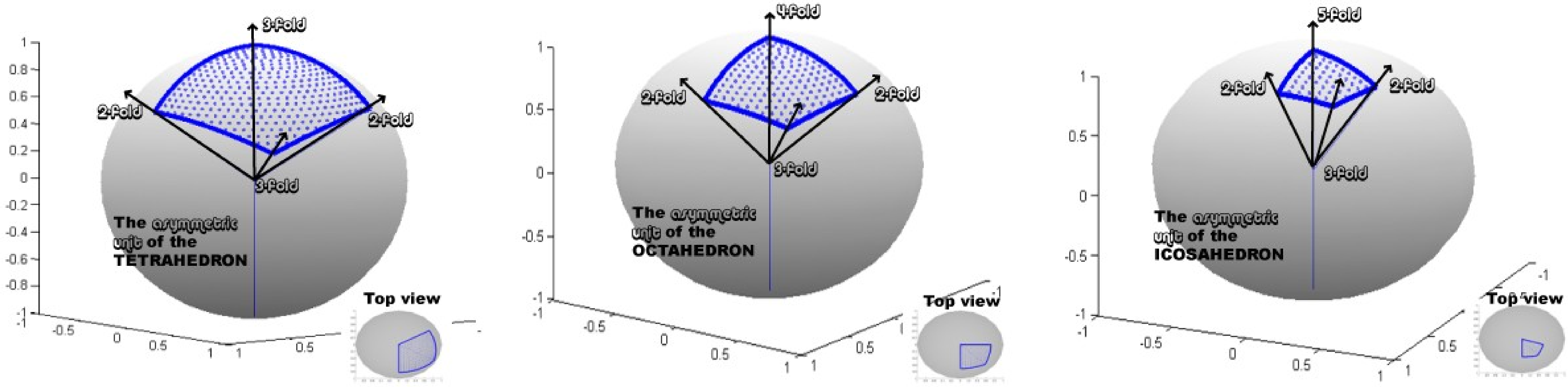
D_n symmetries







Platonic symmetries



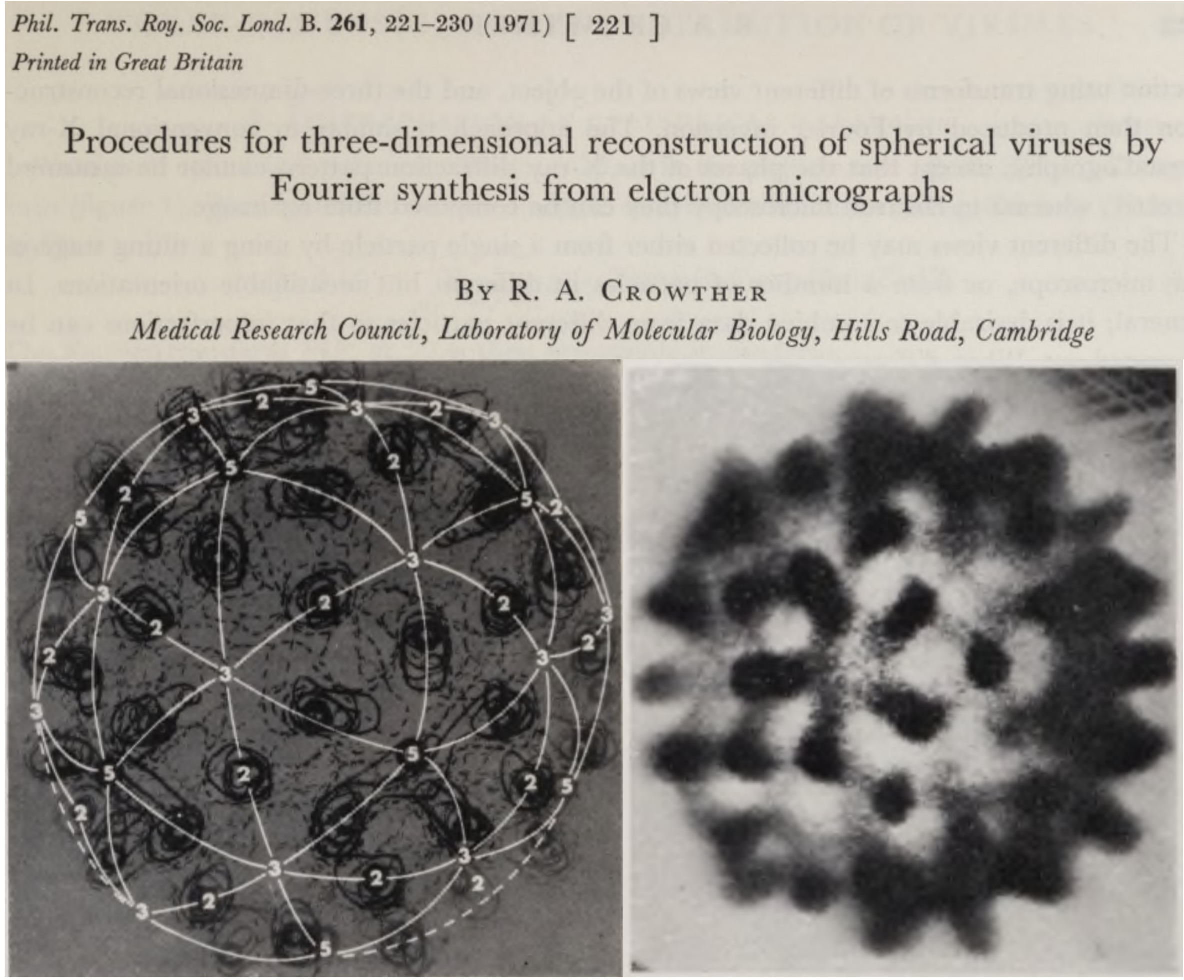
Interactive asymmetric unit viewing using emimag3dsym.py in EMAN2

Potential pitfalls

Symmetry Group	Notation	Origin	Orientation
Asymmetric	C1	User-defined	User-defined
Cyclic	C<n>	On symm axis, Z user-defined	Symm axis on Z
Dihedral	D<n>	Intersection of symm axes	principle symm axis on Z, 2-fold on X
Tetrahedral	T	Intersection of symm axes	3-fold axis on Z (deviating from Heymann et al!)
Octahedral	O	Intersection of symm axes	4-fold axes on X, Y, Z
Icosahedral	I<n>	Intersection of symm axes	++

RELION symmetry conventions and axis definitions, but not all programs are the same!

Icosahedral symmetry and viruses

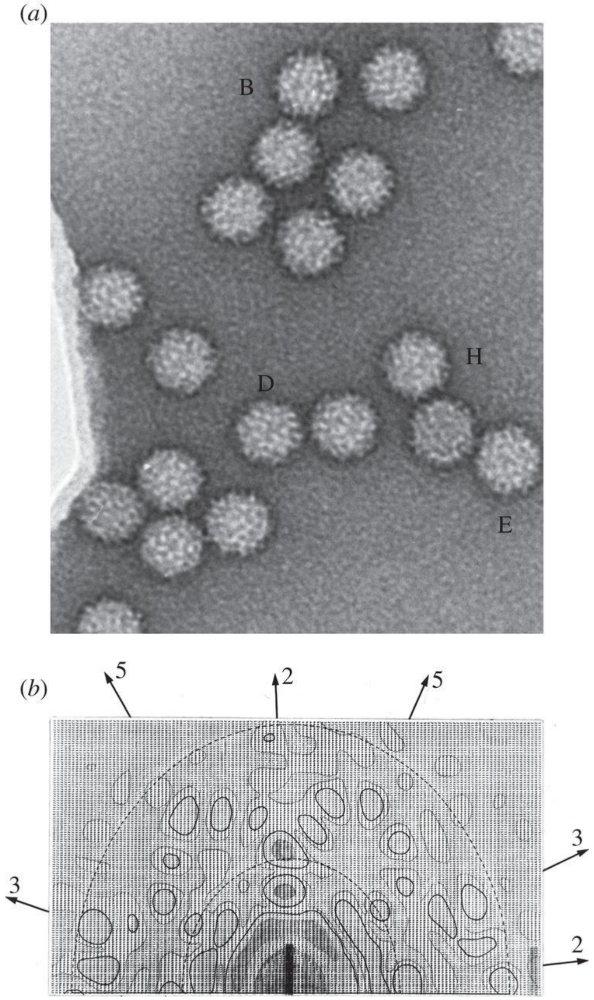


Phil. Trans. Roy. Soc. Lond. B. **261**, 221–230 (1971) [221]
Printed in Great Britain

Procedures for three-dimensional reconstruction of spherical viruses by Fourier synthesis from electron micrographs

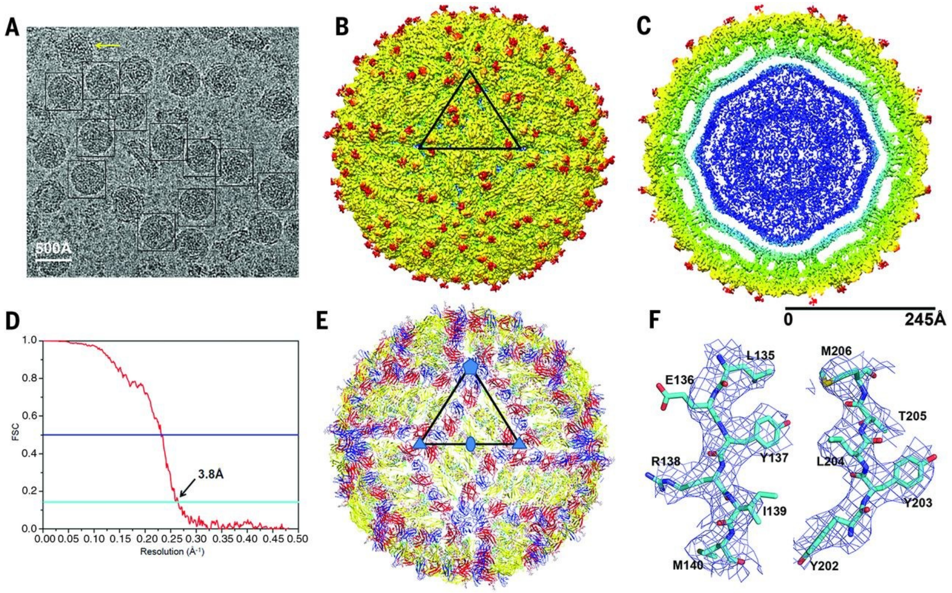
BY R. A. CROWTHER

Medical Research Council, Laboratory of Molecular Biology, Hills Road, Cambridge



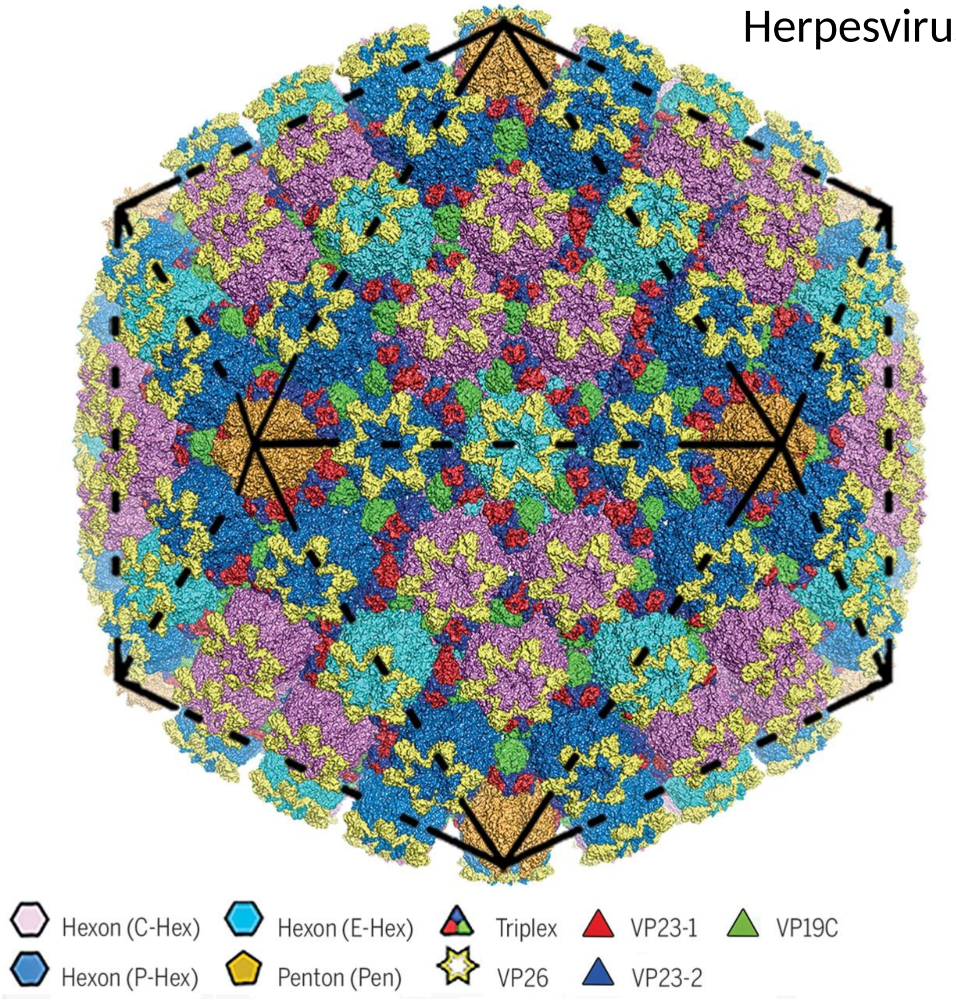
Icosahedral symmetry and viruses

Herpesvirus



Zika virus (Sirohi et al., Science 2016)

ϕ6



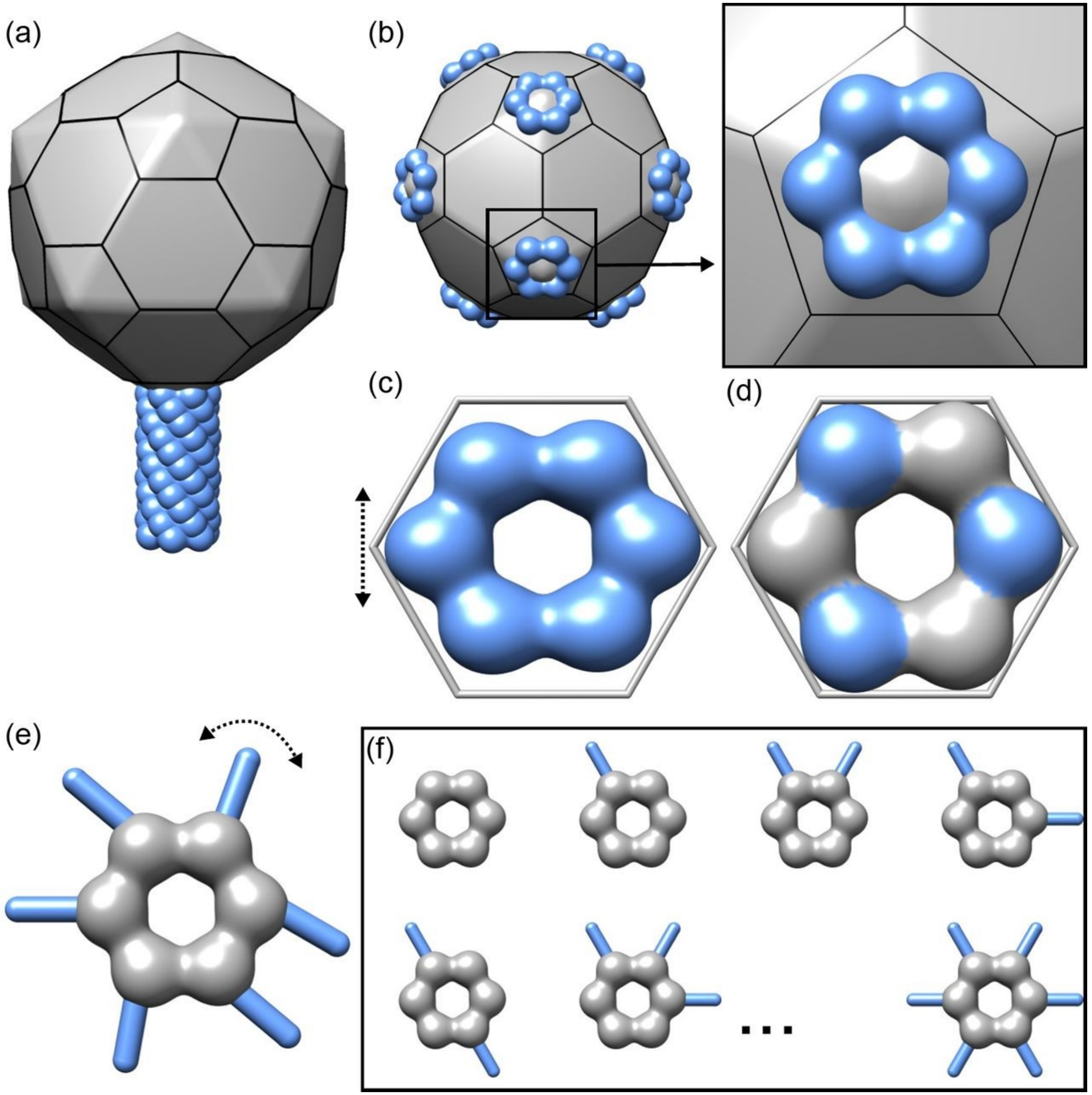
(Yuan et al., Science 2018)

- A powerful averaging tool
- Improve your signal to noise
- Need less data
- Smaller asymmetric unit speeds up processing

But remember, nature is not perfect!

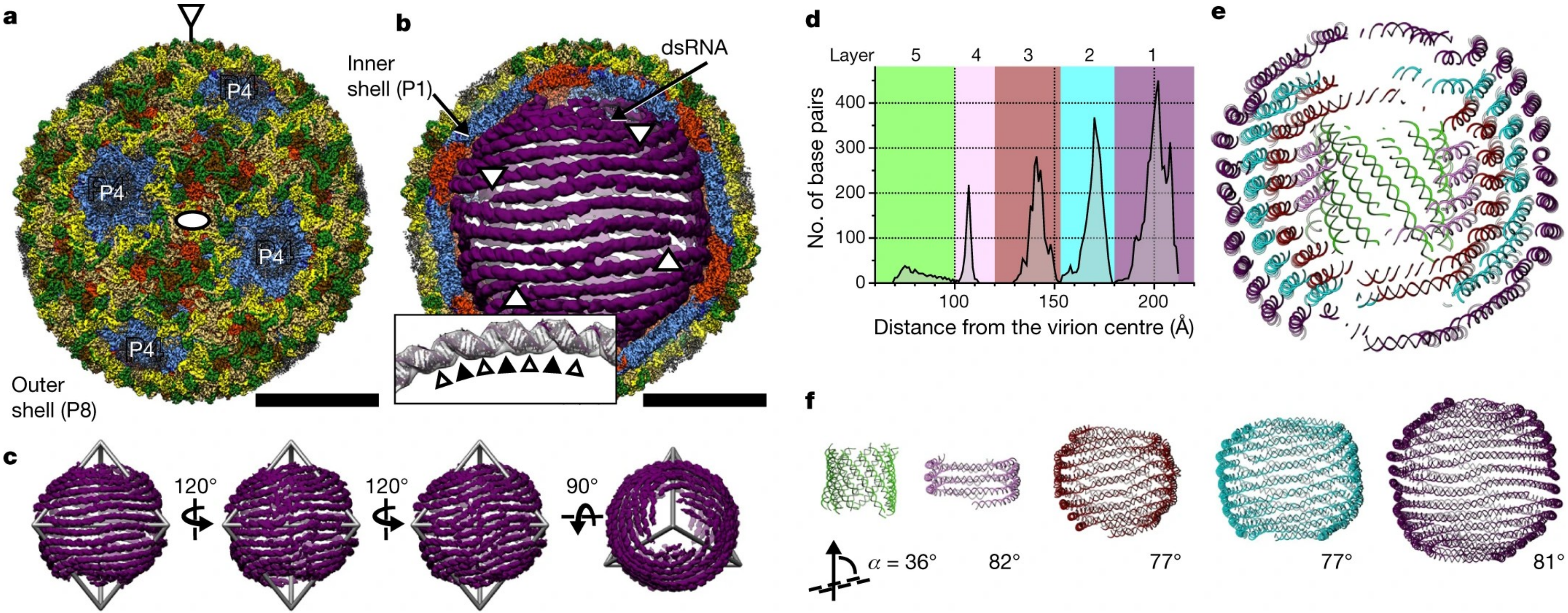
Symmetry breaks and mismatches

- ❖ Symmetry mismatch
- ❖ At low resolution seems symmetric
- ❖ Flexibility
- ❖ Sub-stoichiometric binding



(Huiskonen, Biosci. Rep. 2018)

Symmetry breaks and viruses

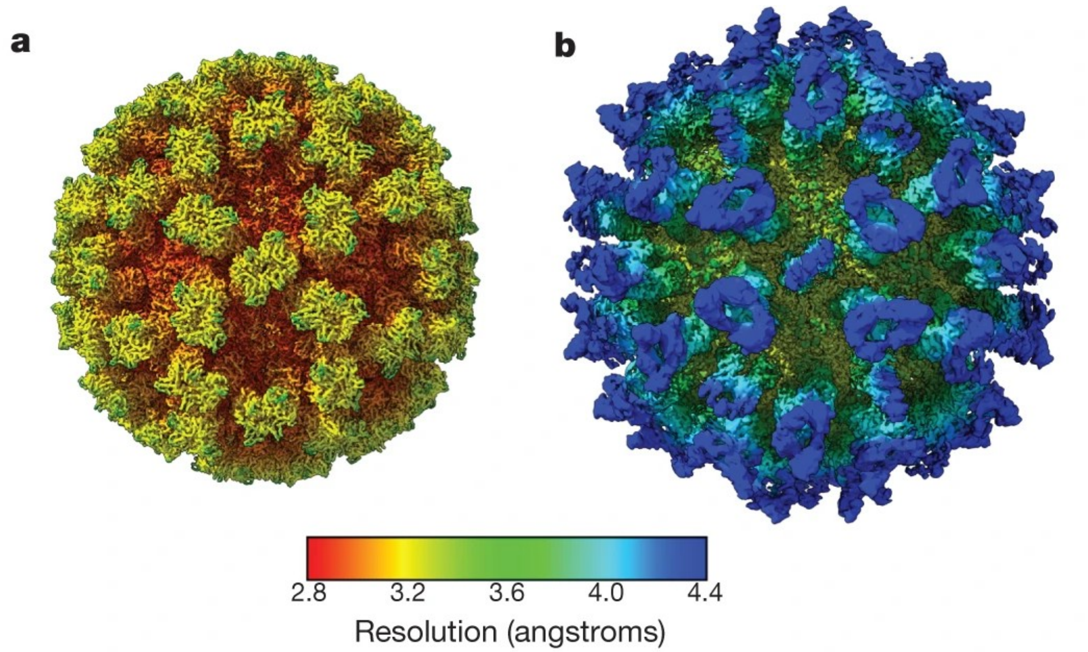


φ6 phage and its dsRNA genome (Ilca et al., Nature 2019)

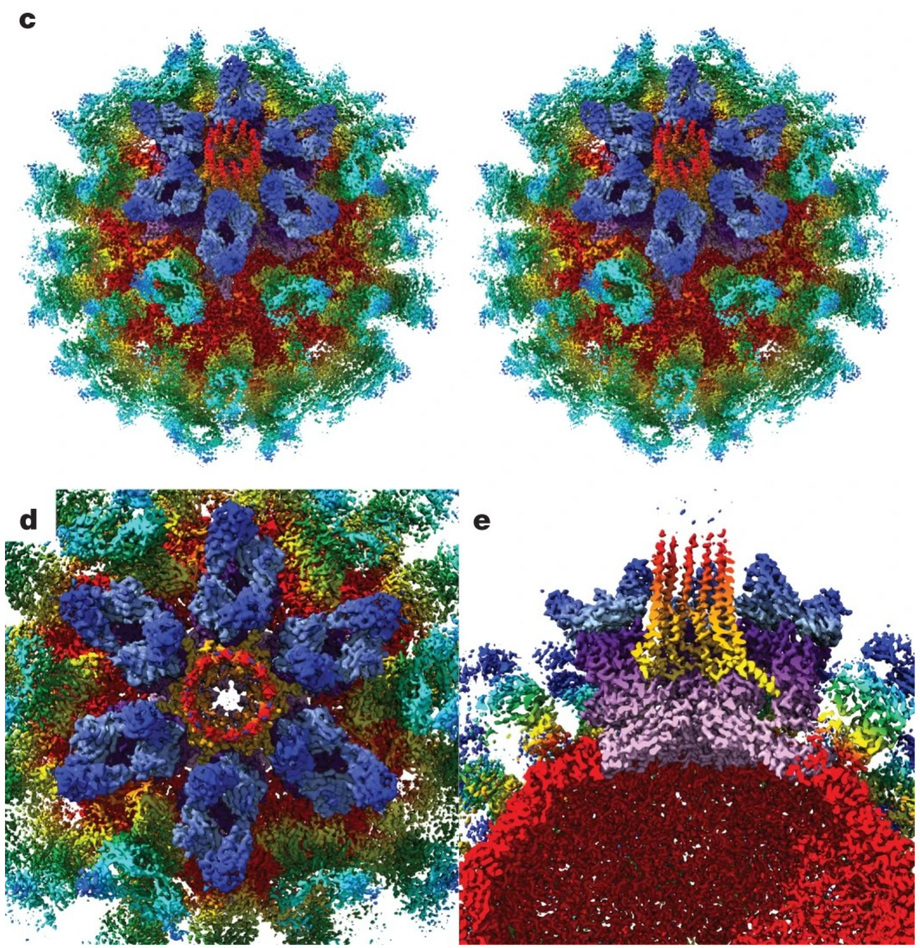
Symmetry breaks and viruses

But infection is an asymmetric process…

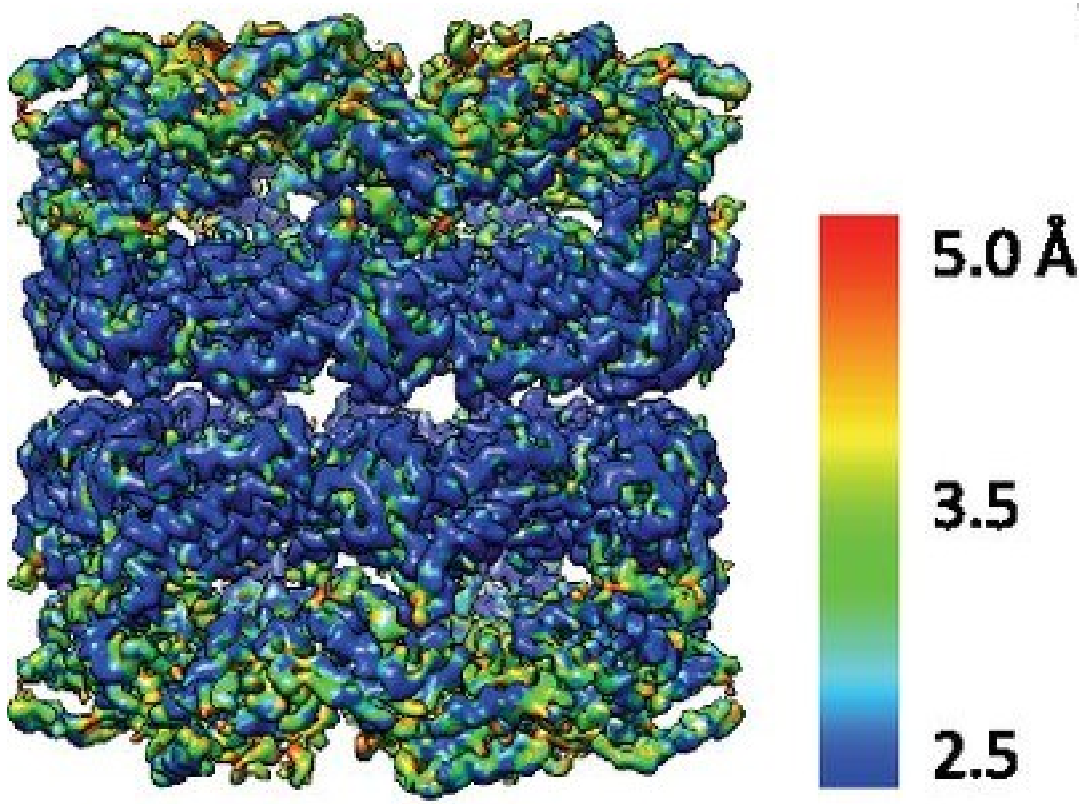
Calicivirus (norovirus)
(Conley et al., Nature 2019)

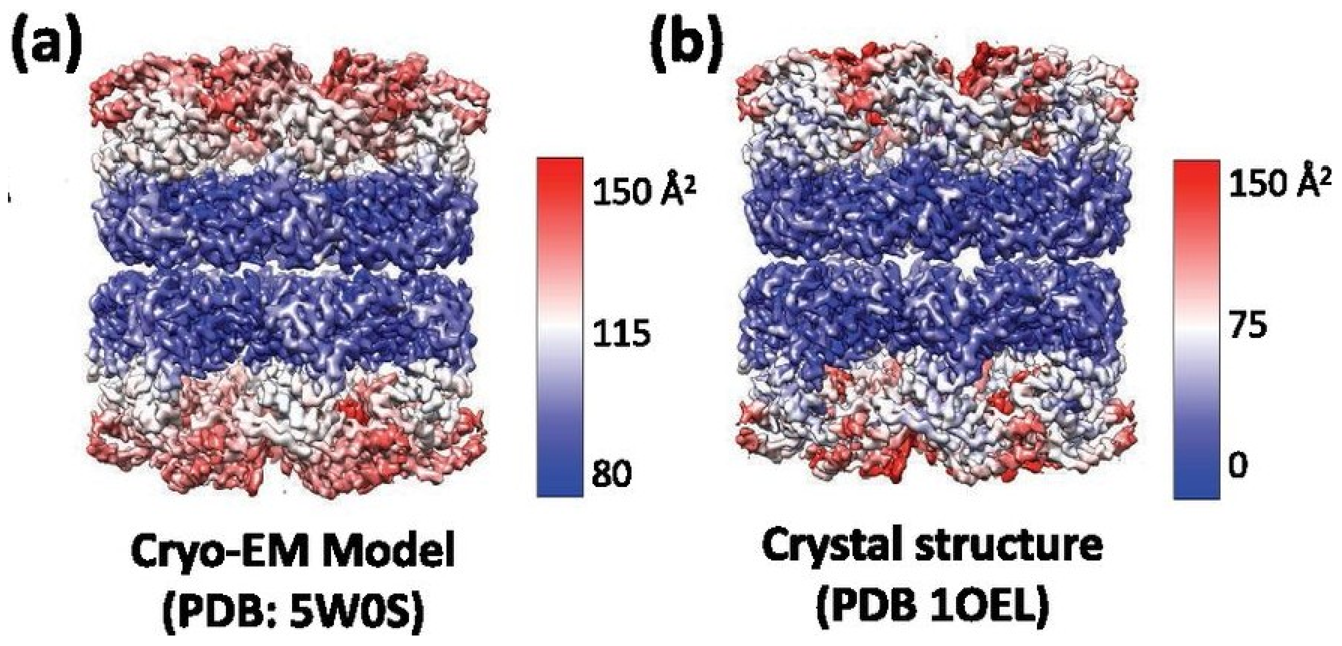


Use symmetry to get high resolution….



Symmetry breaks and GroEL

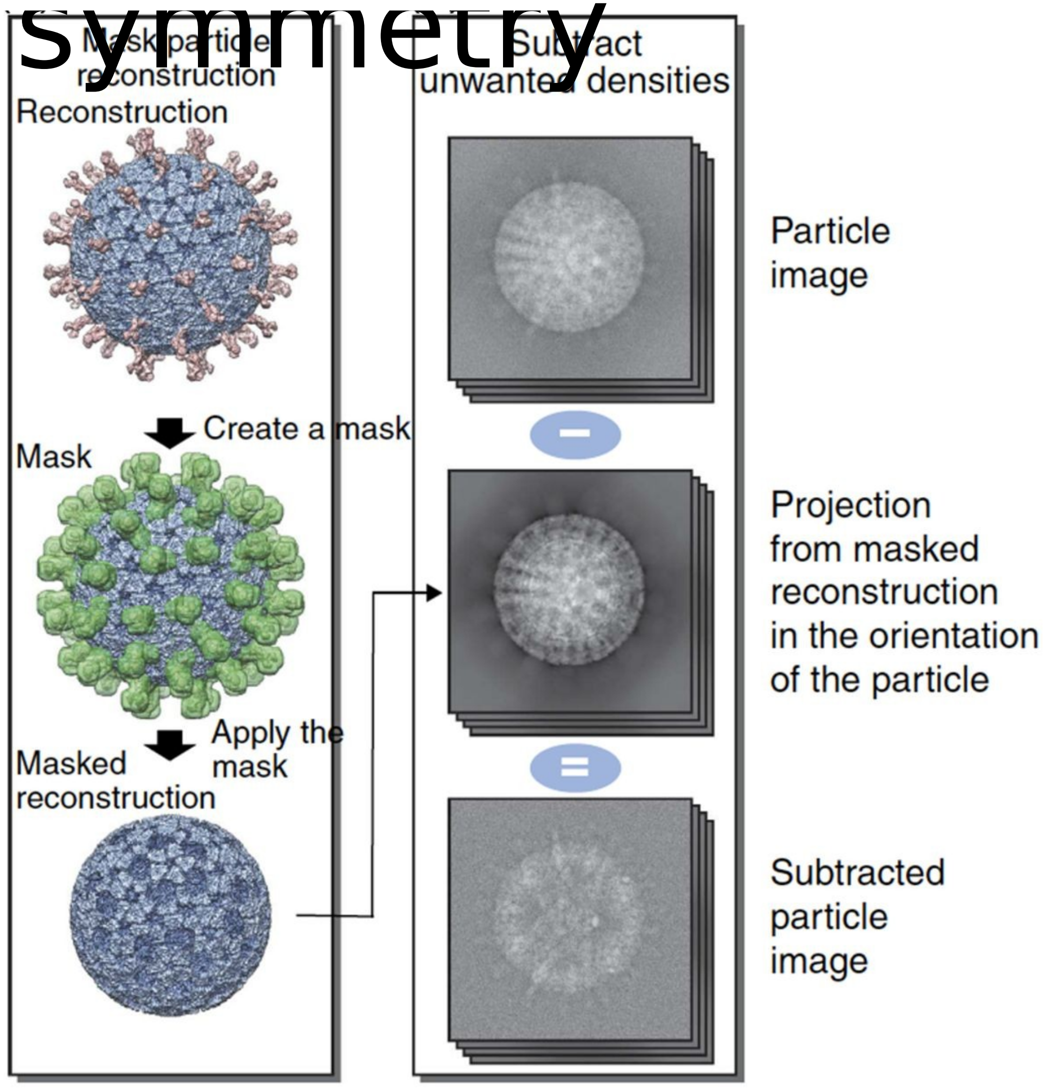




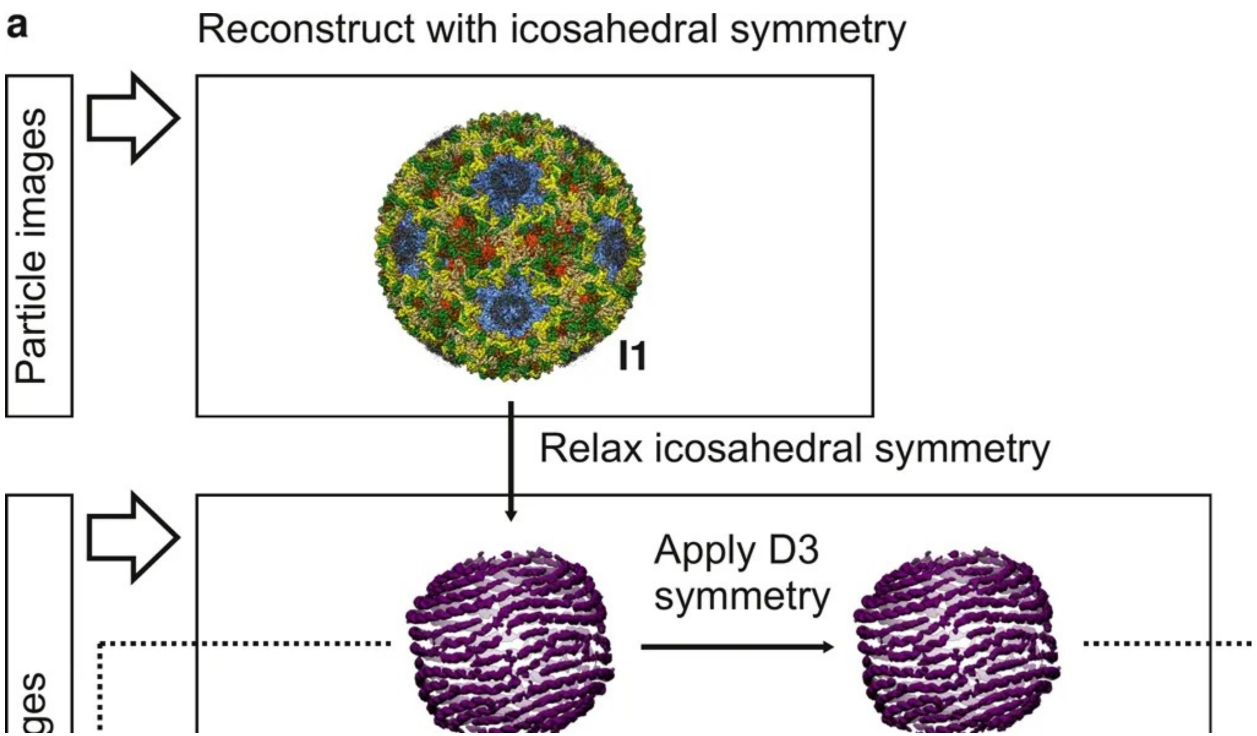
Exploring flexibility of GroEL monomers

How would you do it??

Remove signal from dominant symmetry

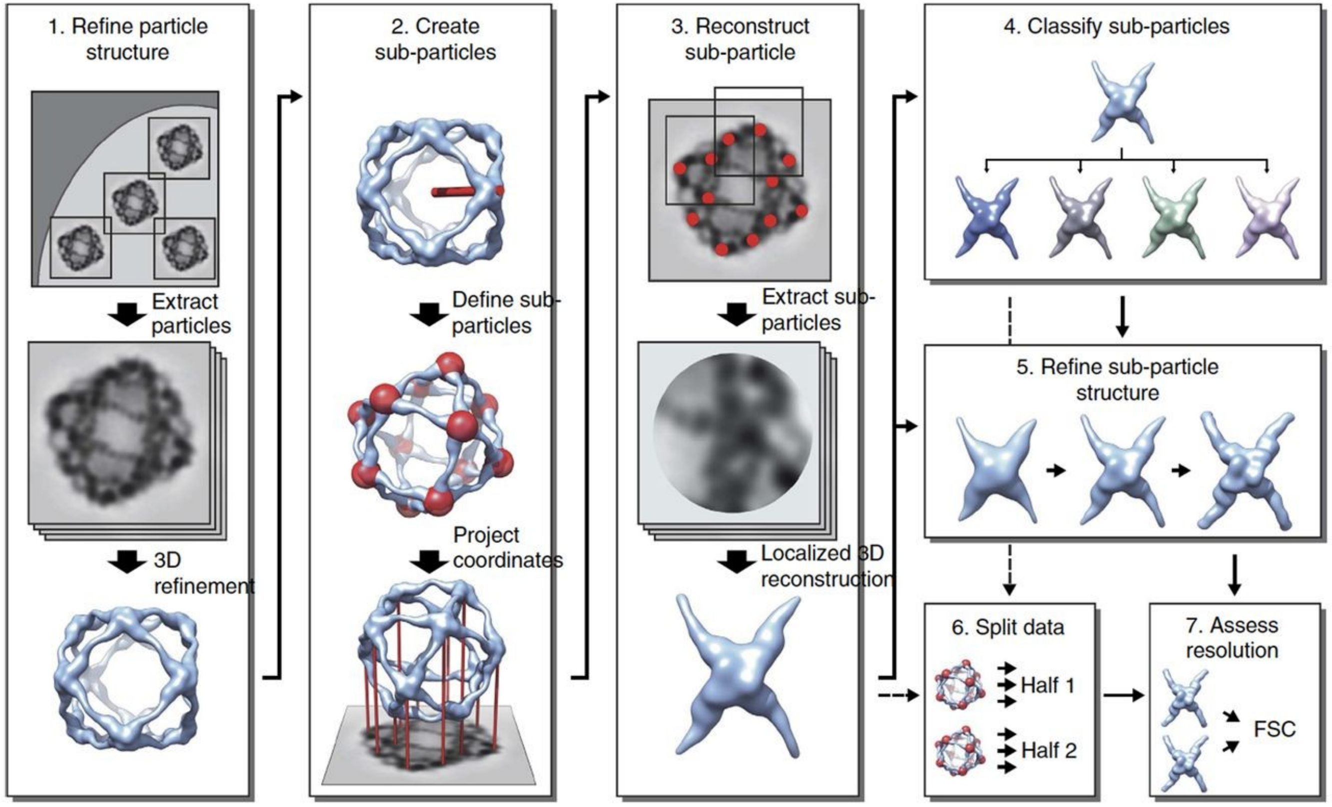


(Huiskonen, Biosci. Rep. 2018)

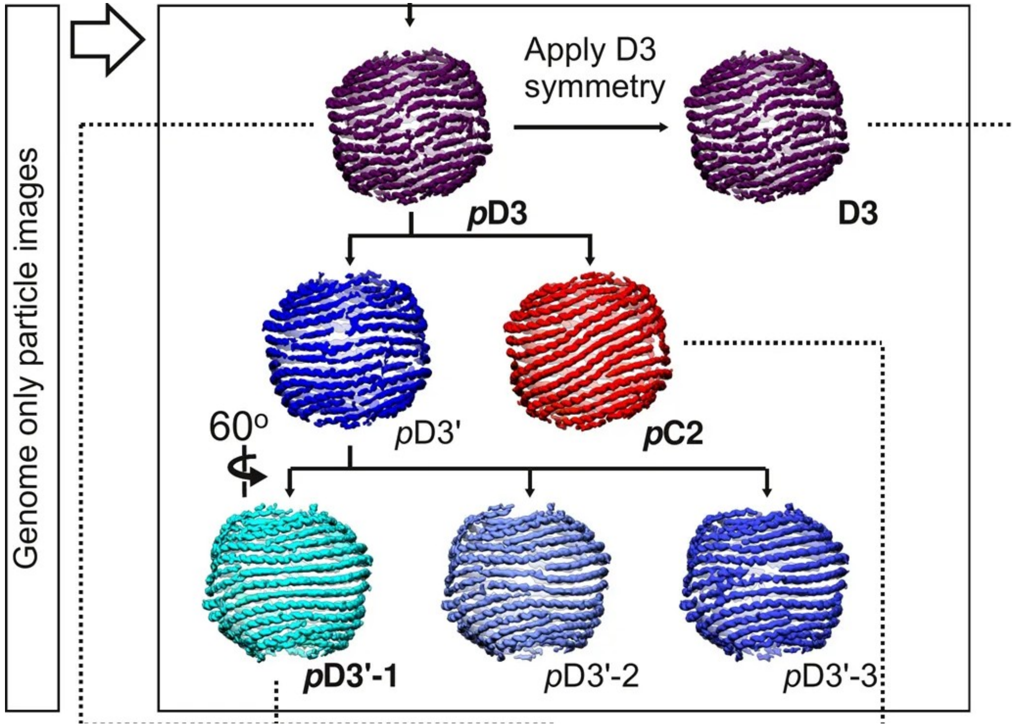


(Ilca et al., Nature 2019)

Local reconstruction (Scipion)



(Huiskonen, Biosci. Rep. 2018)



(Ilca et al., Nature 2019)

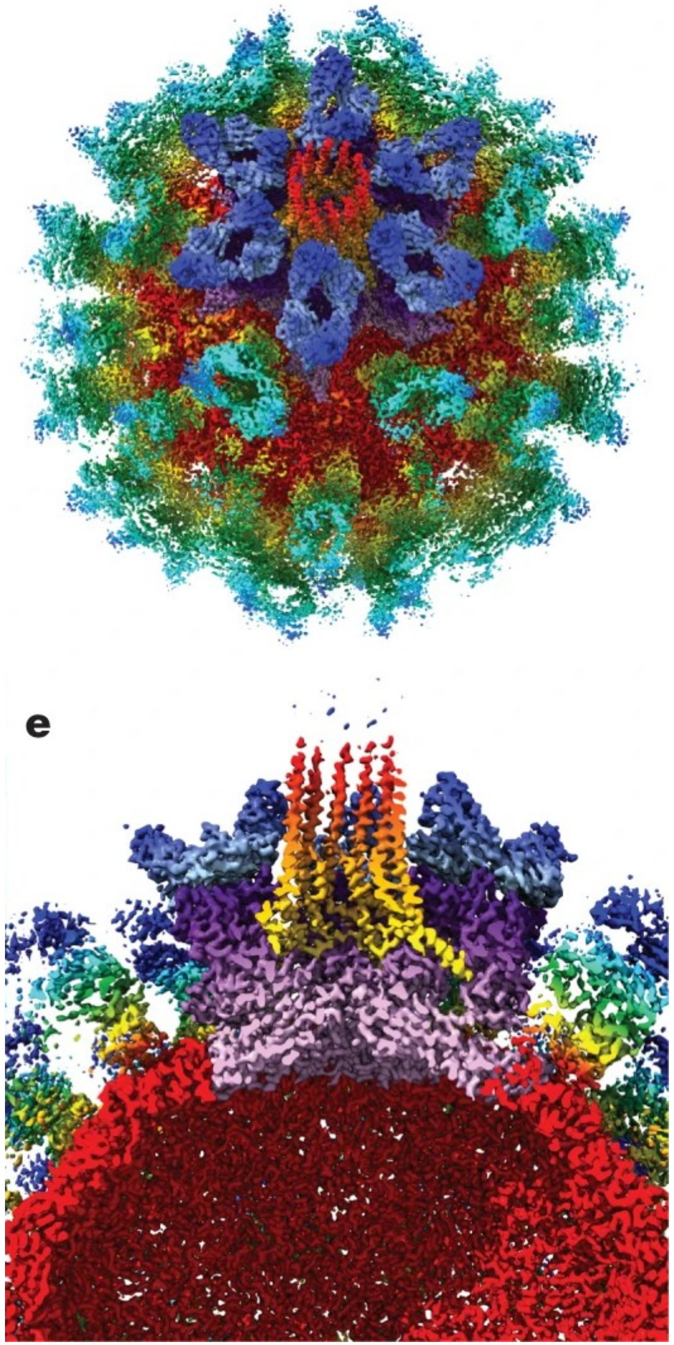
Using symmetry to reduce your search range

Symmetry expansion

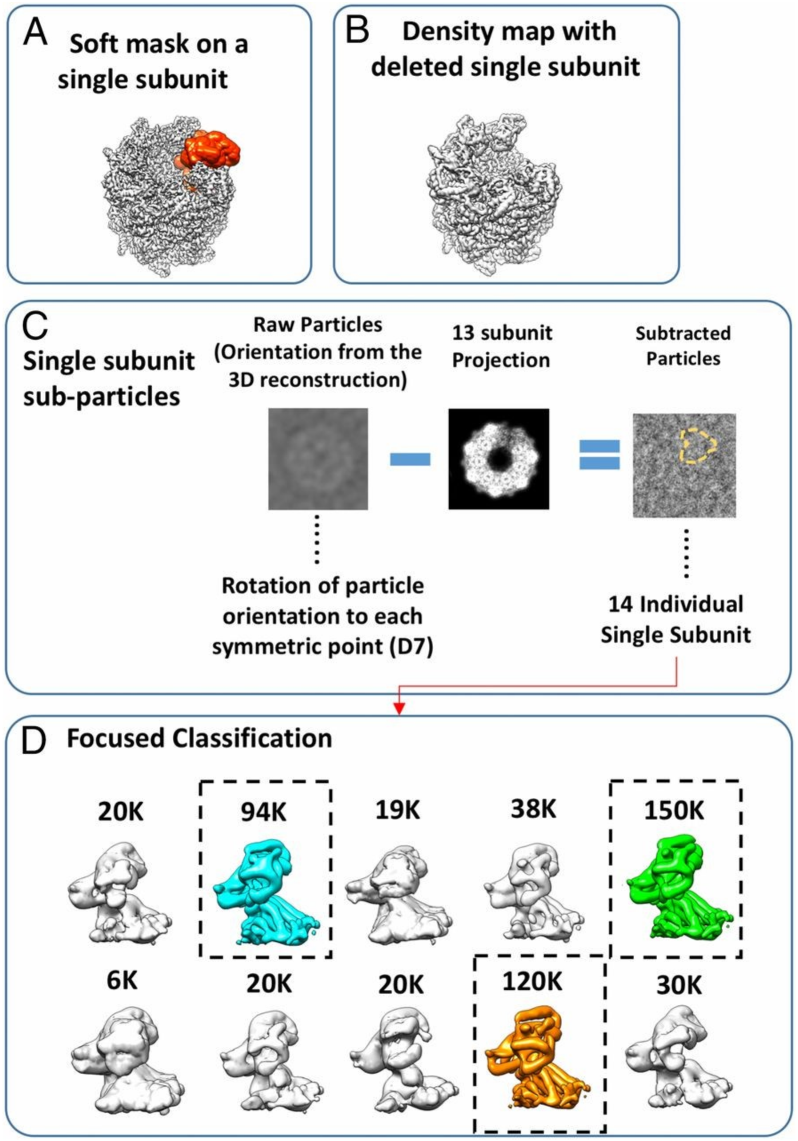
3D classification no refinement

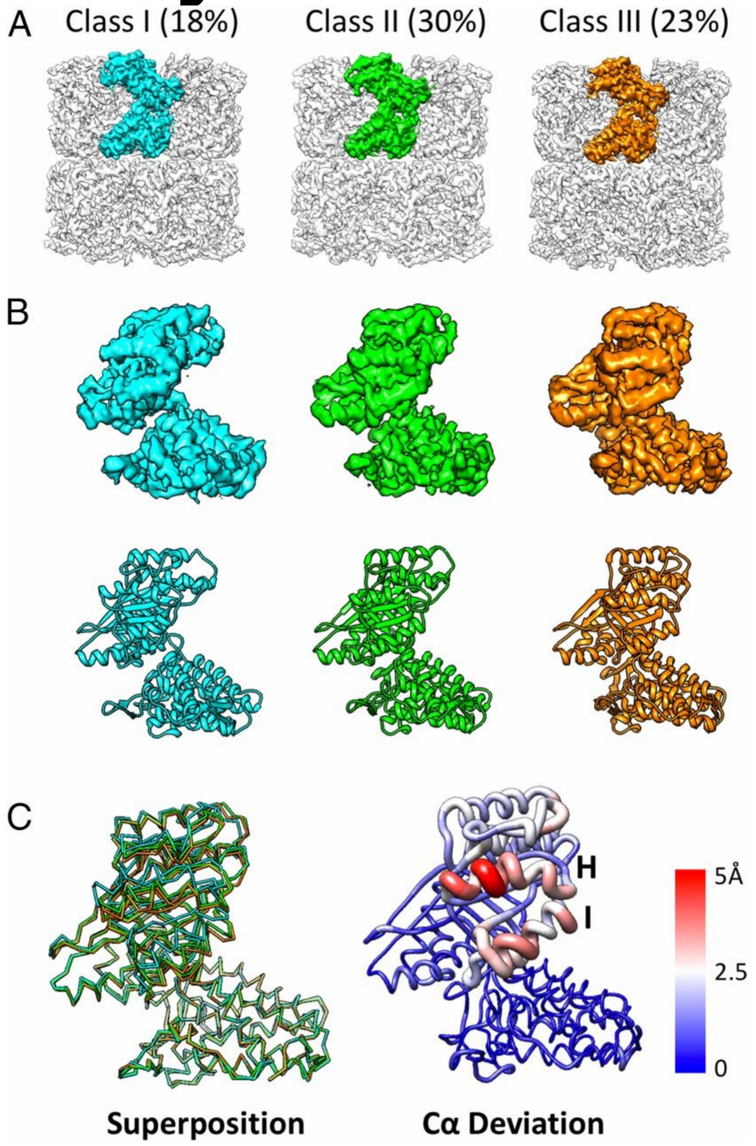
- ❖Increase the T value (Relion)
- ❖Increase the number of classes
- ❖Don’t make your mask too small

(Conley et al., Nature 2019)

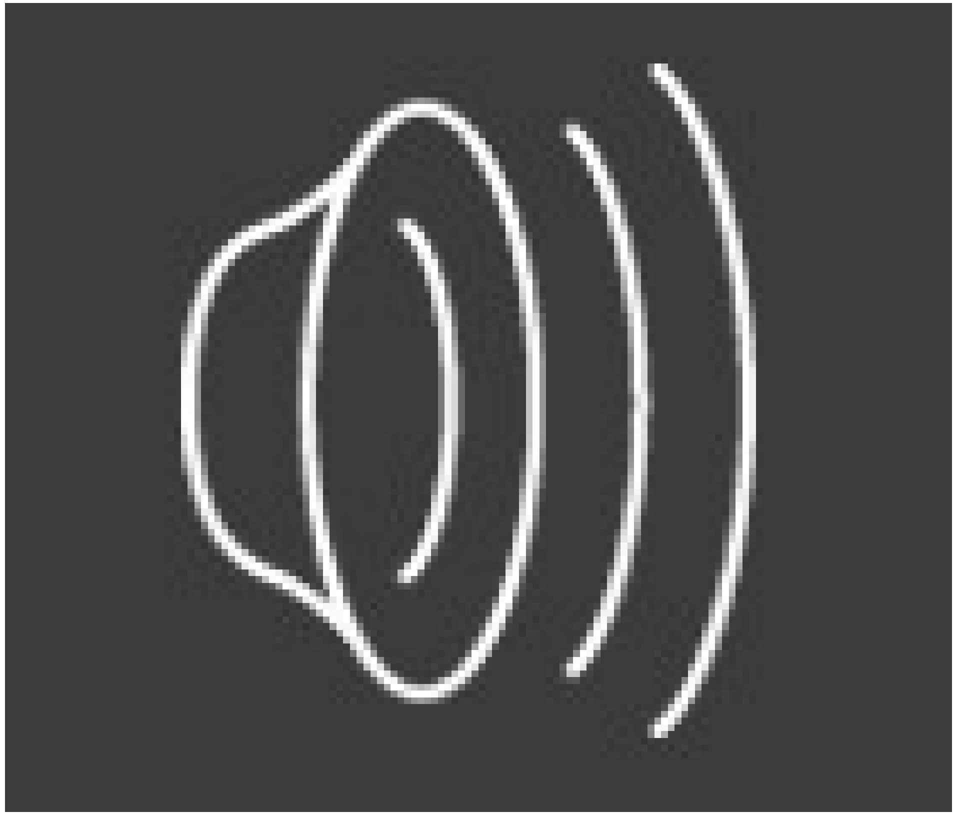


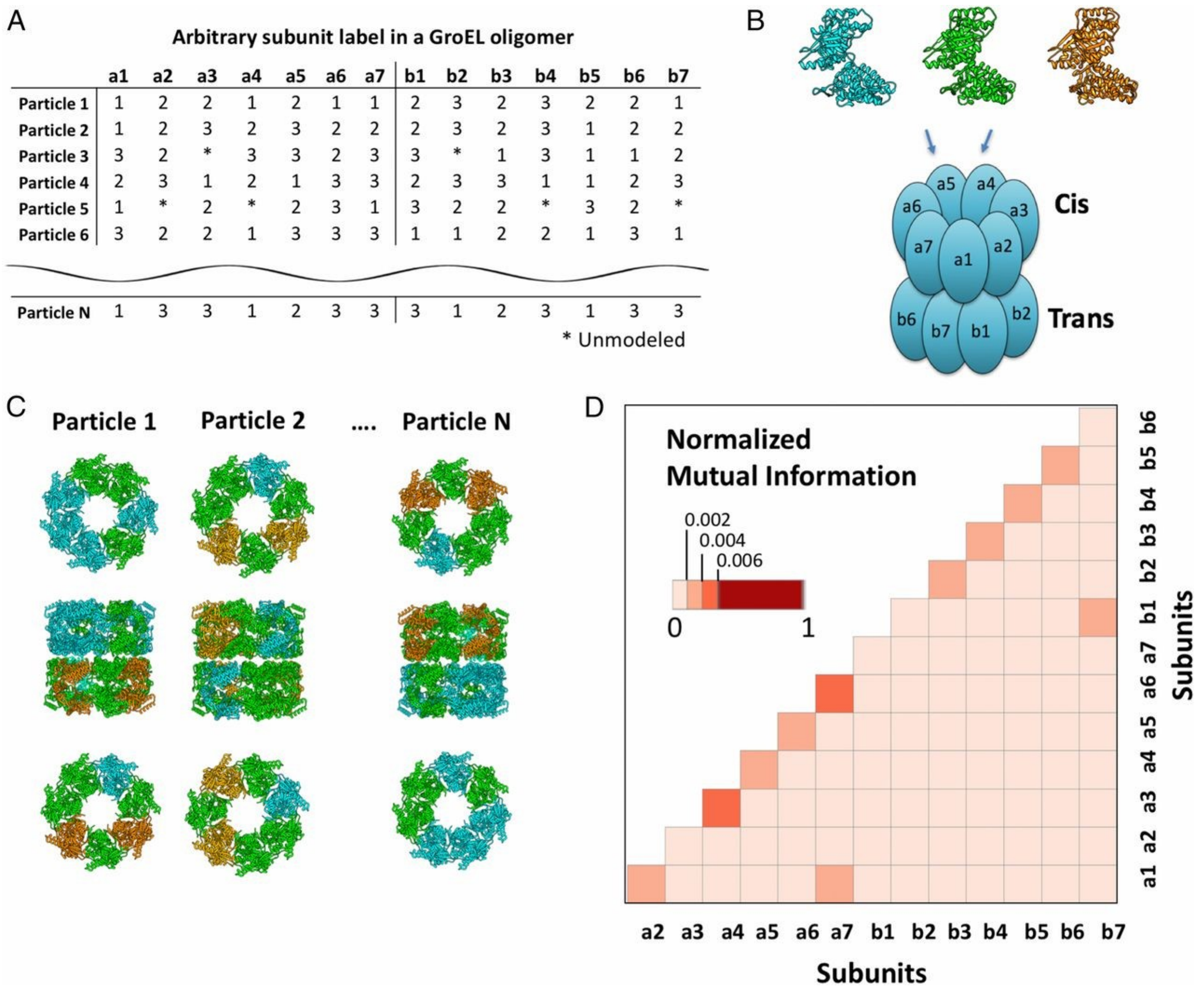
Conformational variability of GroEL





Conformational variability of GroEL





A

Arbitrary subunit label in a GroEL oligomer

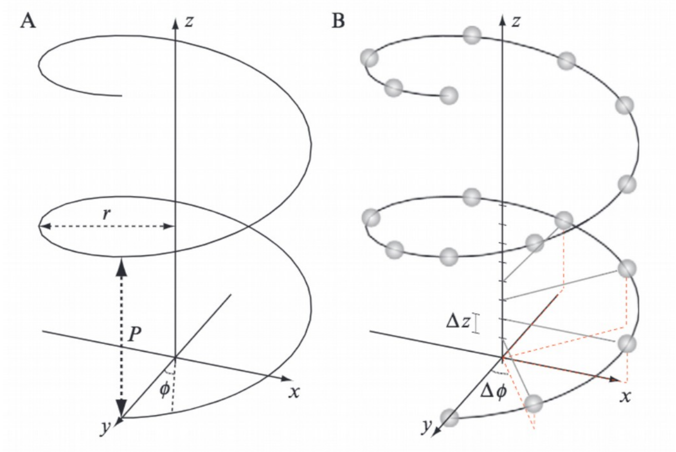
	a1	a2	a3	a4	a5	a6	a7	b1	b2	b3	b4	b5	b6	b7
Particle 1	1	2	2	1	2	1	1	2	3	2	3	2	2	1
Particle 2	1	2	3	2	3	2	2	2	3	2	3	1	2	2
Particle 3	3	2	*	3	3	2	3	3	*	1	3	1	1	2
Particle 4	2	3	1	2	1	3	3	2	3	3	1	1	2	3
Particle 5	1	*	2	*	2	3	1	3	2	2	*	3	2	*
Particle 6	3	2	2	1	3	3	3	1	1	2	2	1	3	1
Particle N	1	3	3	1	2	3	3	3	1	2	3	1	3	3

* Unmodeled

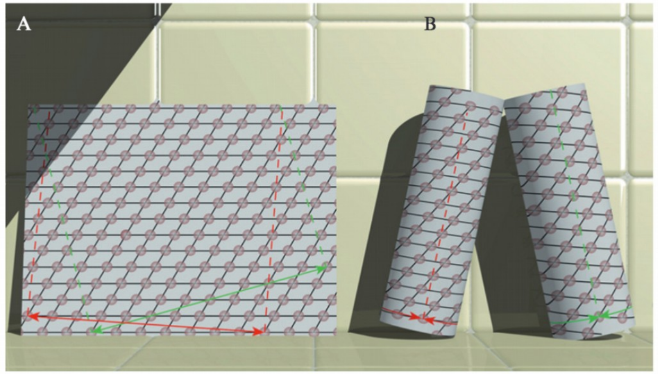
Helical reconstruction

Helical Symmetry

Geometry of a helix: pitch and radius

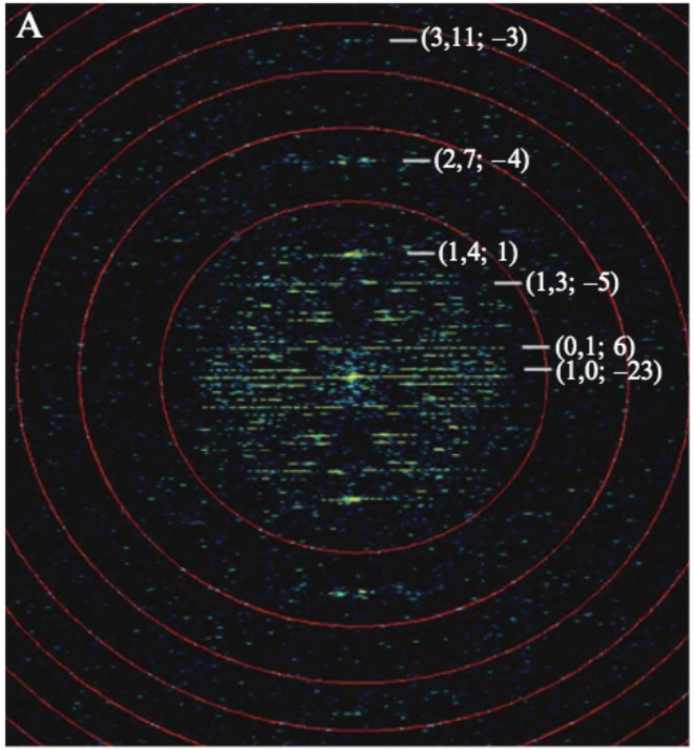


How a helical assembly relates to a 2D lattice



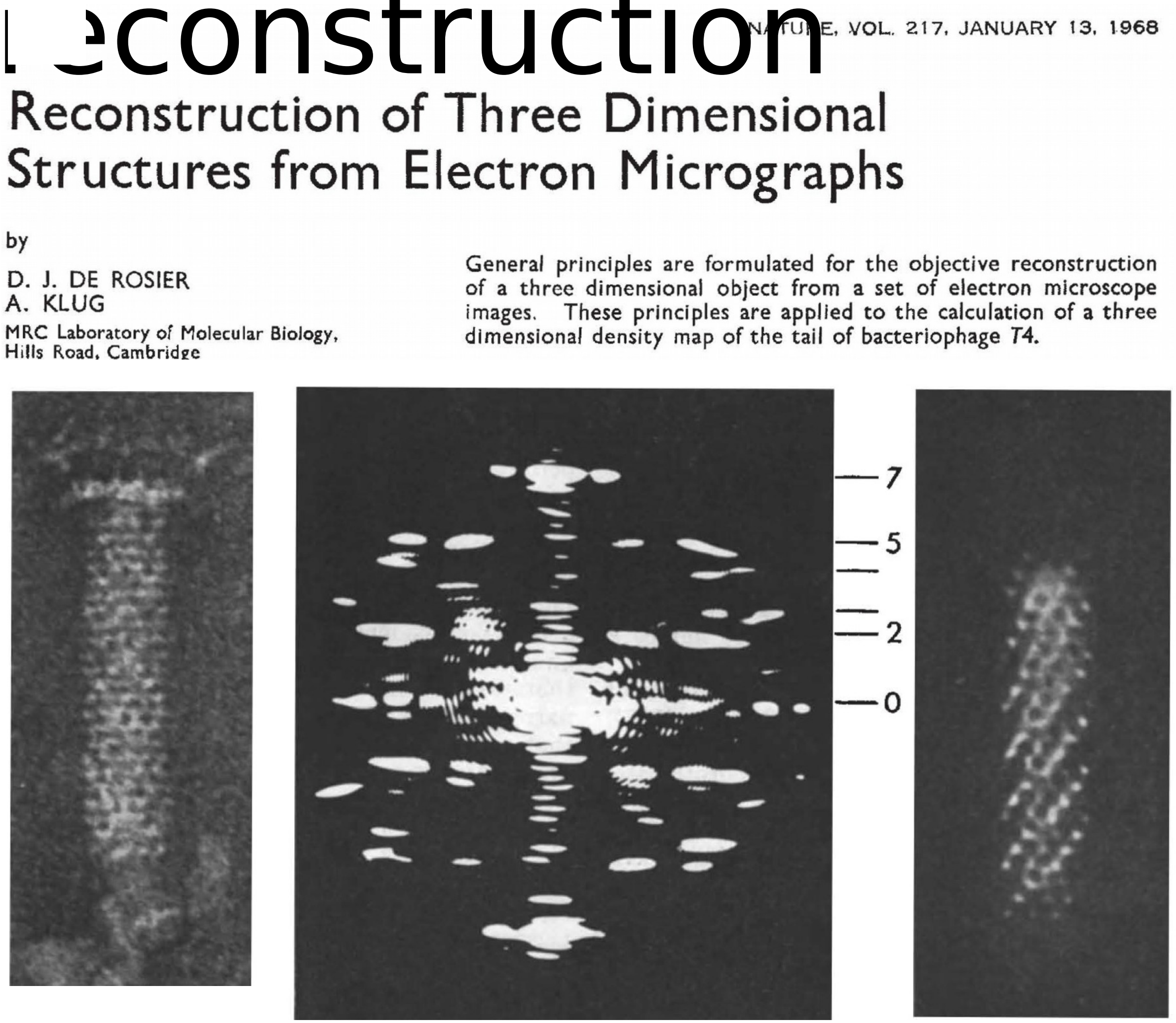
(Diaz et al. Methods in Enzymology 2010)

2D Fourier Transform



- A single view contains all the necessary info for 3D
- 2D surface lattice rolled into 3D
- Bessel function: cylindrical harmonic
- Fourier Bessel analysis

Fourier Bessel method for helical reconstruction


NATURE, VOL. 217, JANUARY 13, 1968

Reconstruction of Three Dimensional Structures from Electron Micrographs

by

D. J. DE ROSIER
A. KLUG
MRC Laboratory of Molecular Biology, Hills Road, Cambridge

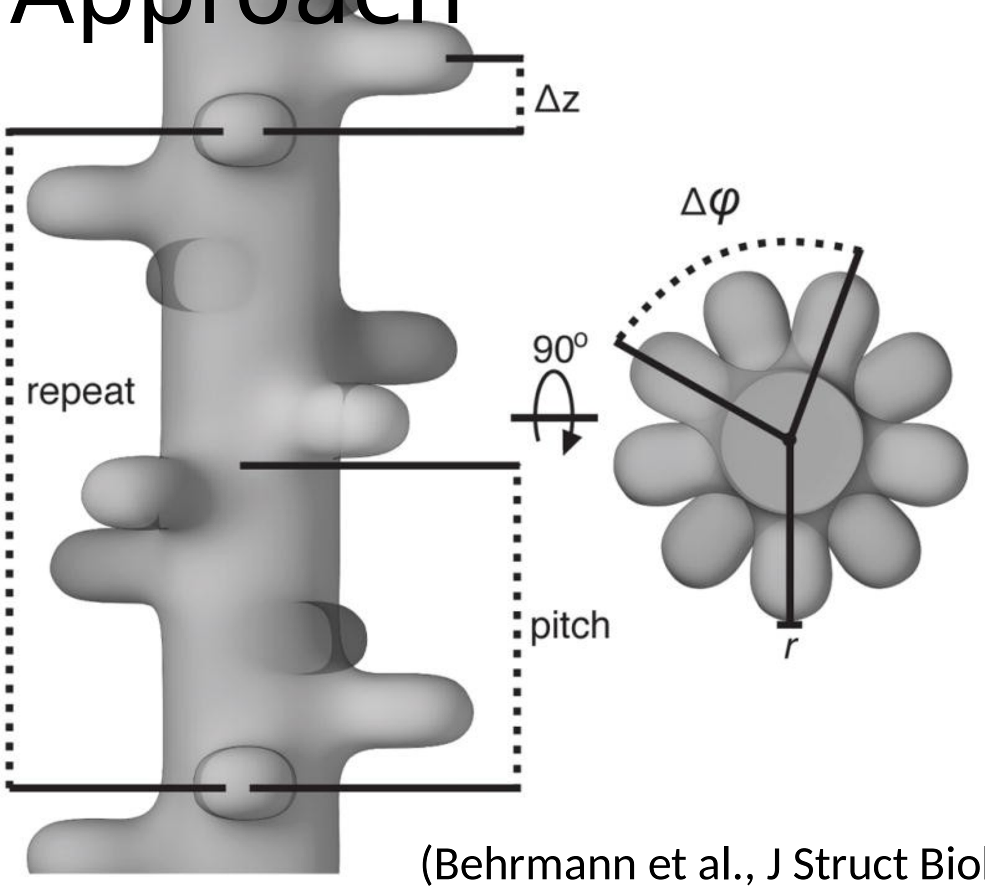
General principles are formulated for the objective reconstruction of a three dimensional object from a set of electron microscope images. These principles are applied to the calculation of a three dimensional density map of the tail of bacteriophage *T4*.

Limitations

- Laborious
- Small inaccuracies in indexing lead to incorrect structures!
- Requires strict helical symmetry
- Requires flat straight helices

Iterative Real-Space Refinement (IHRSR)

Single Particle Approach



(Behrmann et al., J Struct Biol 2012)

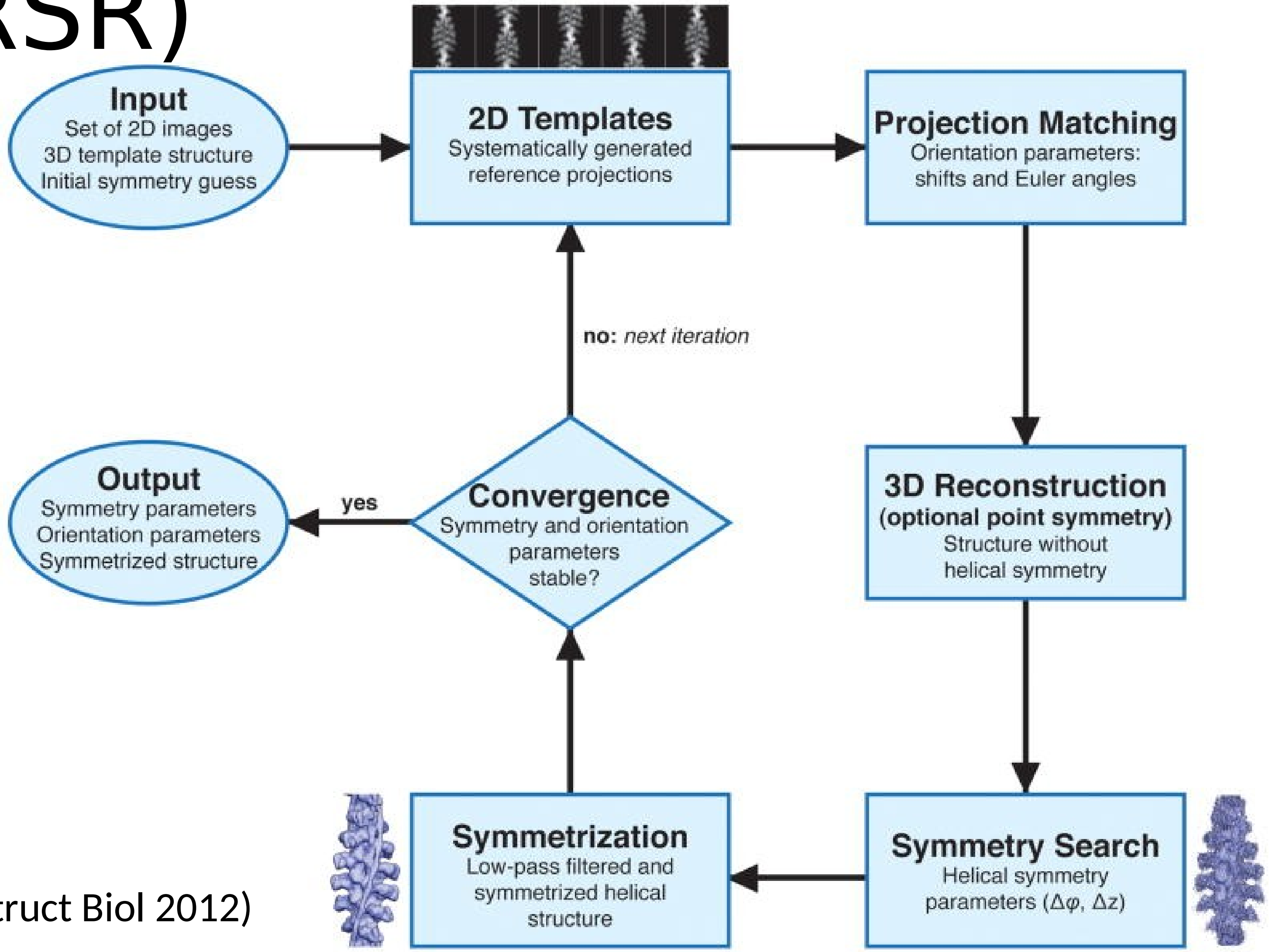
Parameters

- Outer and inner radii of cylinder
- Pitch
- Repeat (2x pitch)

Advantages

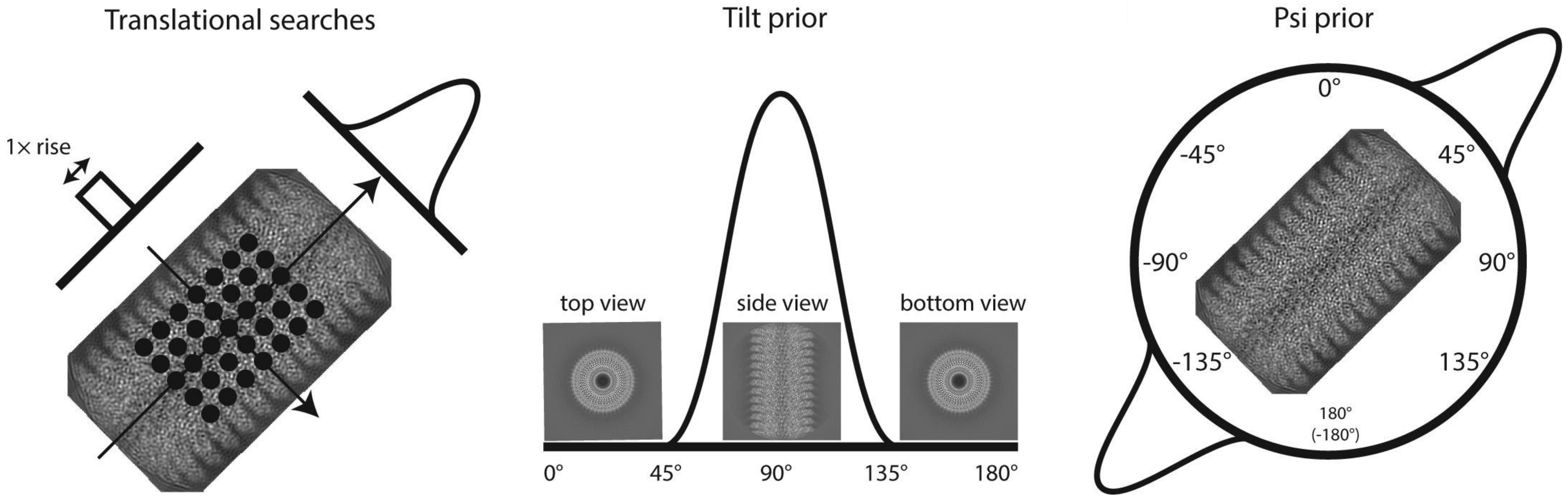
- Cope with heterogeneous specimens
- Reconstruct filaments that diffract weakly, i.e. when layer lines not visualized.

Iterative Real-Space Refinement (IHRSR)



(Behrmann et al., J Struct Biol 2012)

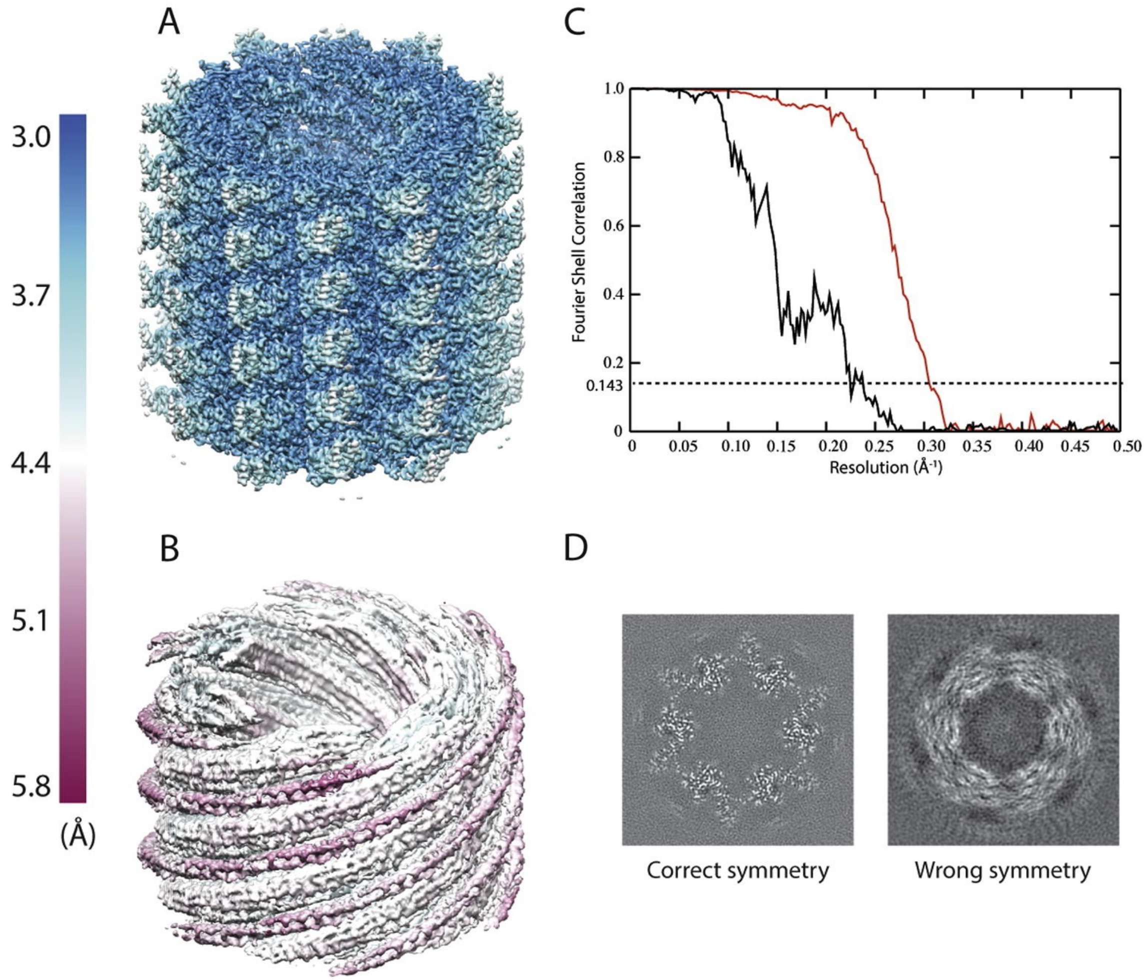
RELION and helical symmetry



Incorporating prior information about the orientations

(He & Scheres., J Struct Biol 2017)

Potential pitfalls

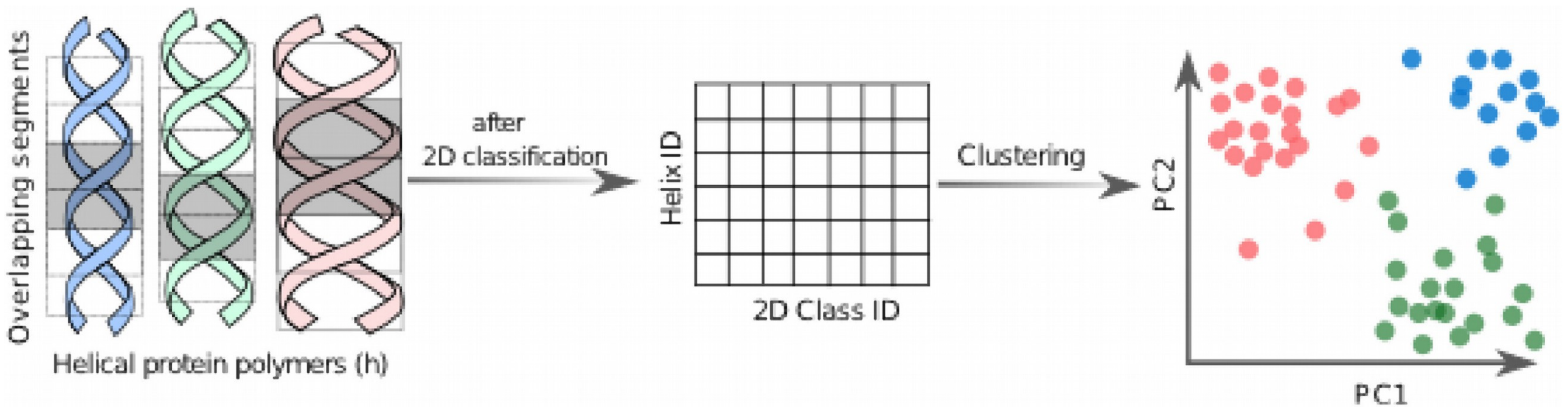


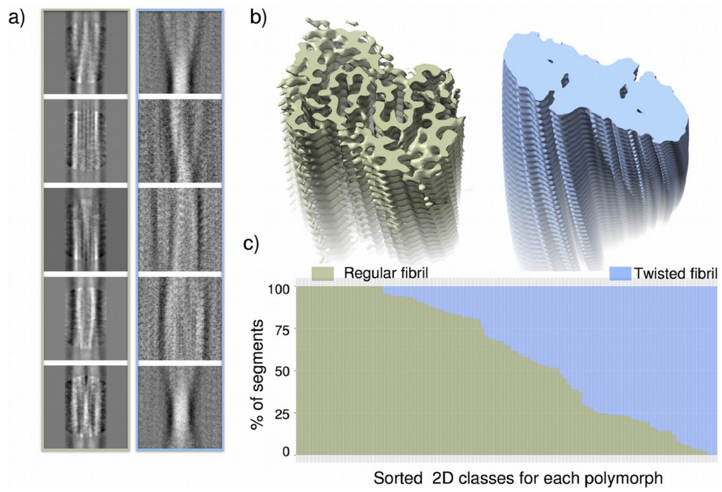
What to look out for

- ❖ Does your map look like a protein?
- ❖ Is your FSC smooth?
- ❖ What's happening to your noise?

(He & Scheres., J Struct Biol 2017)

Helical mixed symmetry populations



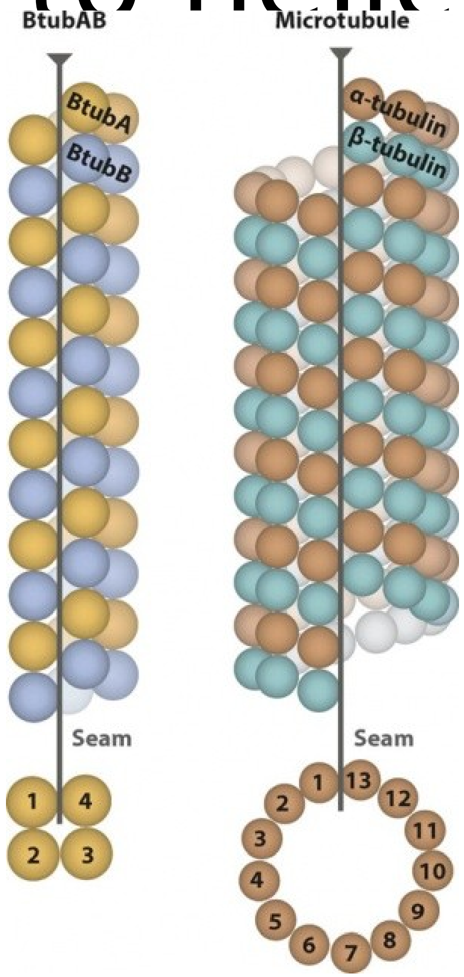


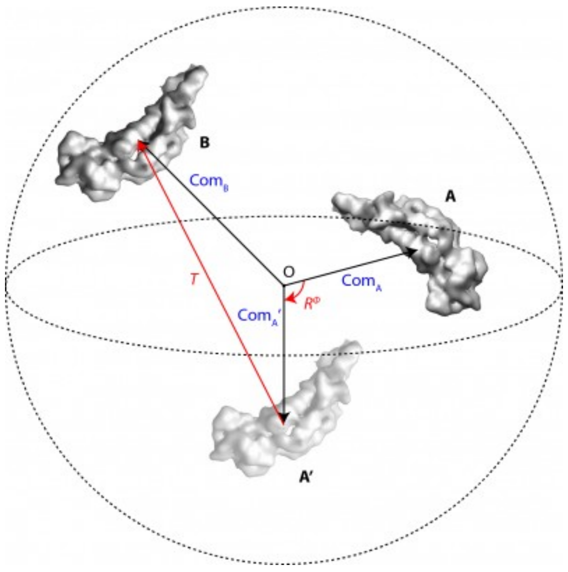
Approach

- Principal component analysis
- K means classification

(Pothula et al., Ultramicroscopy 2019)

RELION local symmetry applied to helices



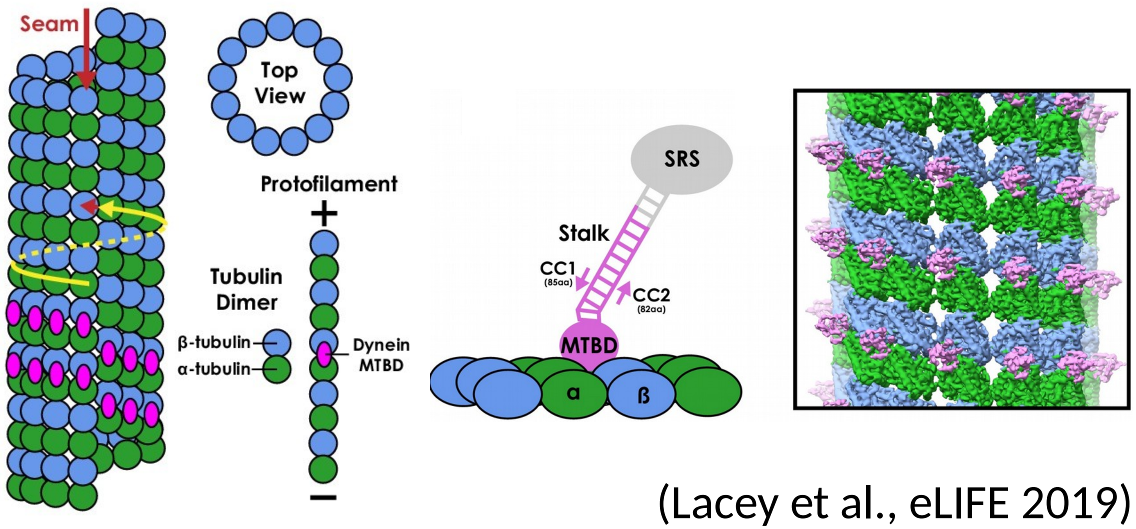


(Deng et al., PNAS 2017)

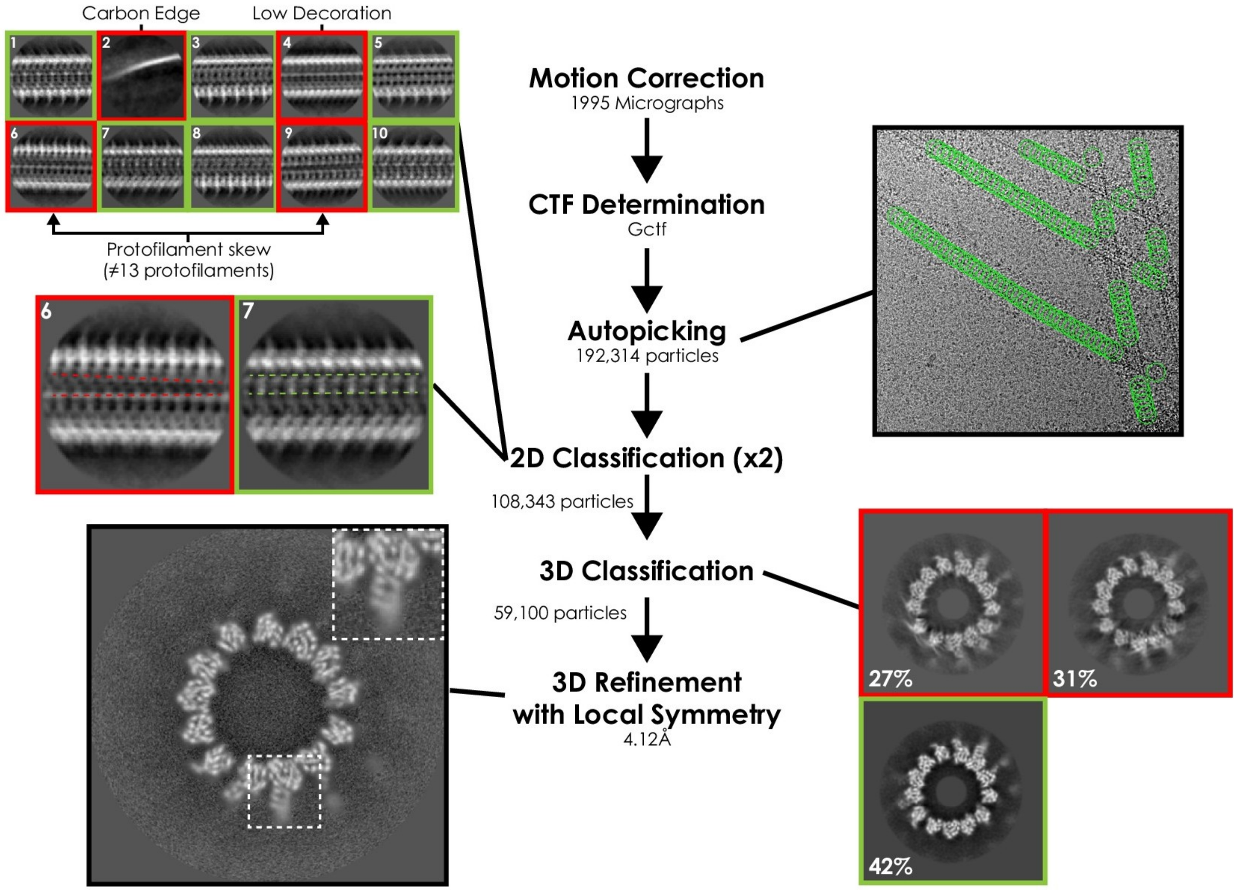
Approach

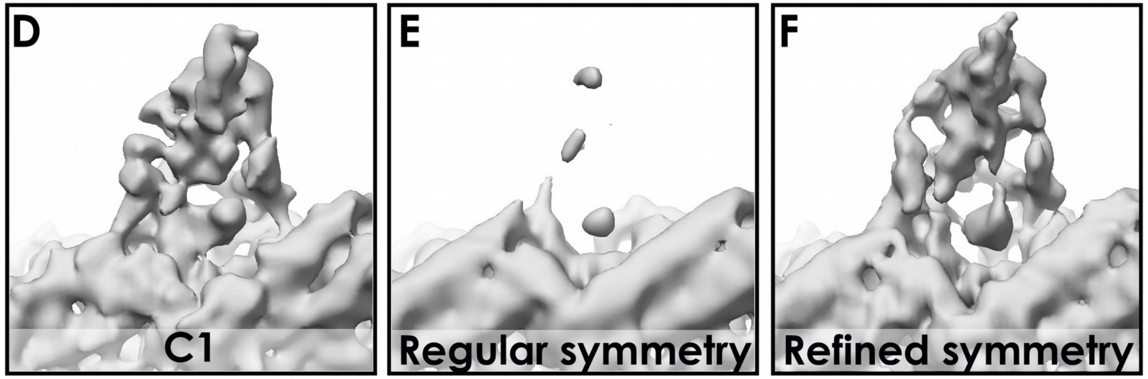
- Define local symmetry operators
  - Place PDB into map
  - Create masks from PDB
- Global & local search for symmetry operators
- Verification of symmetry operators
- Impose local symmetry in 3D classification/refinement

MicroTuble Binding Domain of dynein (MTBD) bound to microtubles



(Lacey et al., eLIFE 2019)





- 2D classification: decoration/stoichiometry
- 3D classification: decoration/stoichiometry
- 3D refinement (C1) with 13-fold local sym

Think, digest, DRAW!