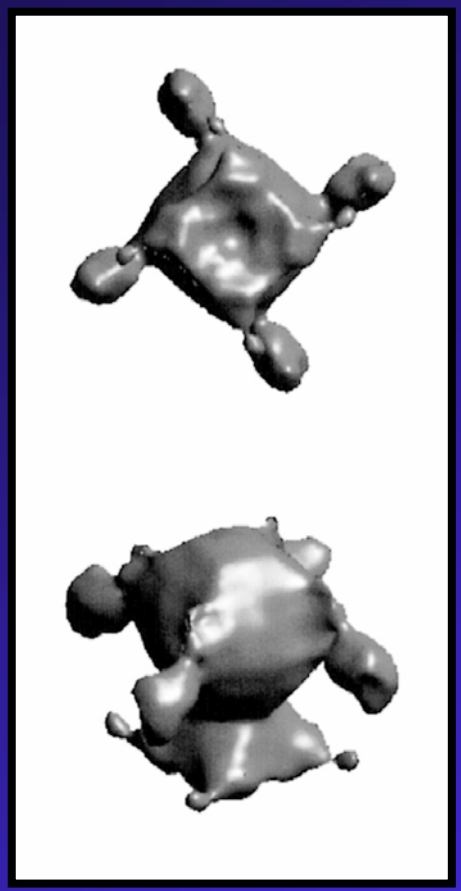
Initial Model Generation

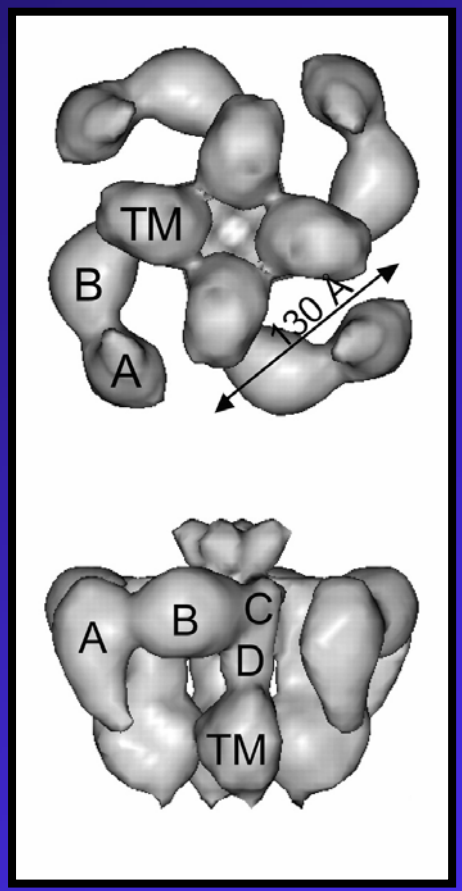
Workshop on Advanced Topics in EM Structure Determination

The Scripps Research Institute
La Jolla, November 2007

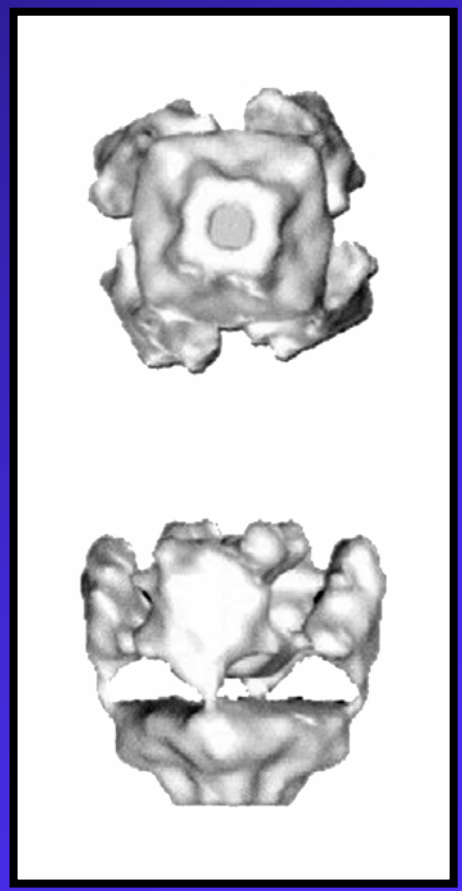
The issue: Structures of the IP3 receptor as determined by single particle EM



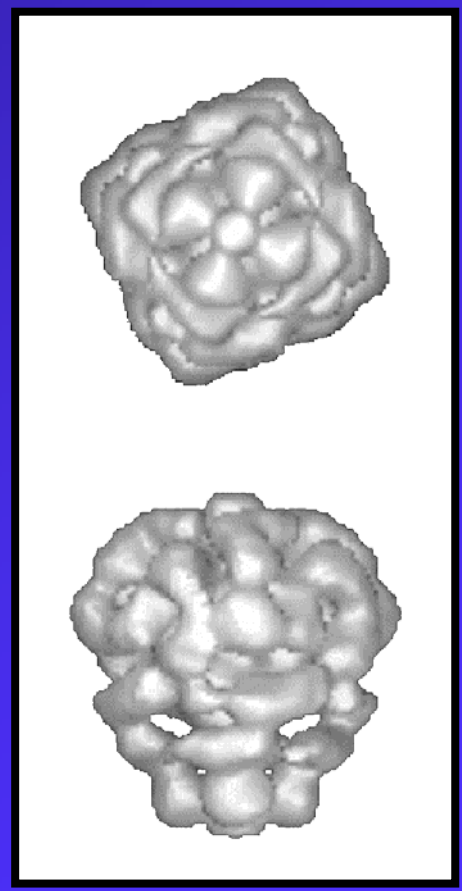
Jiang *et al.*, 2002



Serysheva *et al.*, 2003



Jiang *et al.*, 2003



Sato *et al.*, 2004

Structure determination by single particle EM

Sample

↓ *Specimen preparation*

- *Negative staining*
- *Cryo-negative staining*
- *Vitrification*

EM specimen

↓ *Data collection*

- *Images of untilted samples*
- *Tilt pairs*
- *Tilt series*

Set of 2D images

↓ *3D reconstruction*

- *Random conical tilt*
- *Ab initio angle assignment*
- *Tomographic reconstruction*

Initial 3D model

↓ *Refinement*

Refinement of orientation parameters, magnification, and CTF parameters

Refined 3D model

Verification

Angular distribution
Comparison with 2D data set
Comparison with known structures

Sample

Images that are used for a 3D reconstruction have to be of identical molecules !

Sample can be heterogeneous
even if it is biochemically homogeneous

– conformational heterogeneity
– unstable complexes

Before attempting any 3D reconstruction: Understand your sample !

Sample

We always prepare negatively stained specimens first

good contrast & often preferred orientations
(depends somewhat on preparation method)

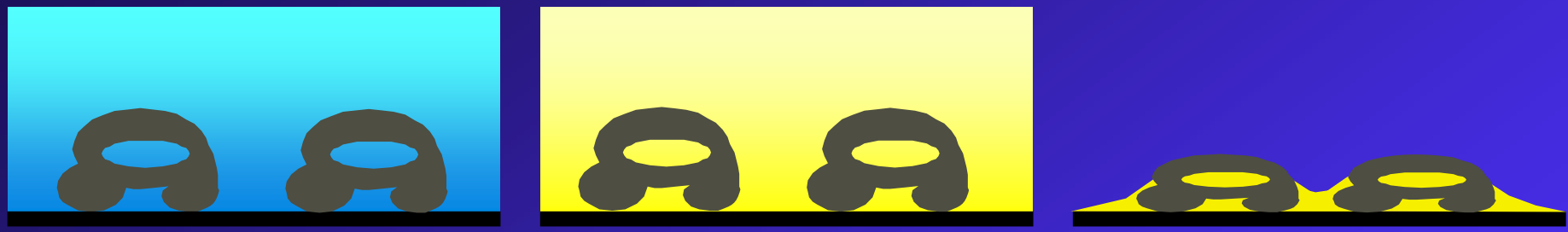
We always calculate class averages (0° images)

different averages = different conformations
(but can also be different orientations)

We usually calculate 3D reconstructions (RCT)

different 3D maps = different conformations
(but can also be deformations)

Sample - Negative staining



Many preparation artifacts (incomplete stain embedding, adsorption deformations, specimen flattening upon drying)

Limitation of the resolution to about 20 Å

Particles adopt preferred orientations on the continuous carbon film !

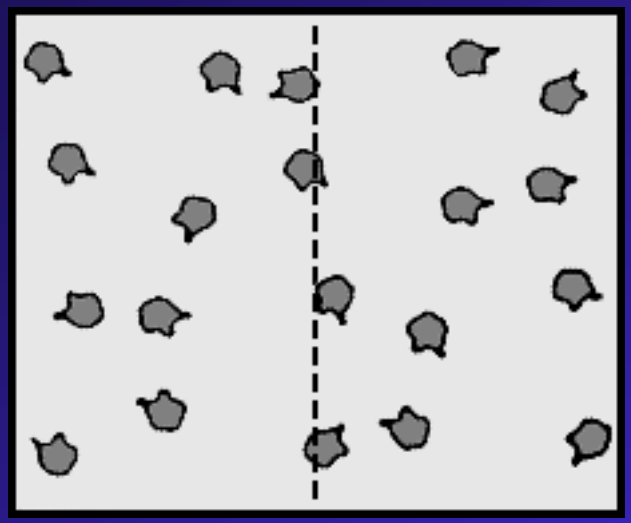
Need to record images of tilted specimens

BUT:

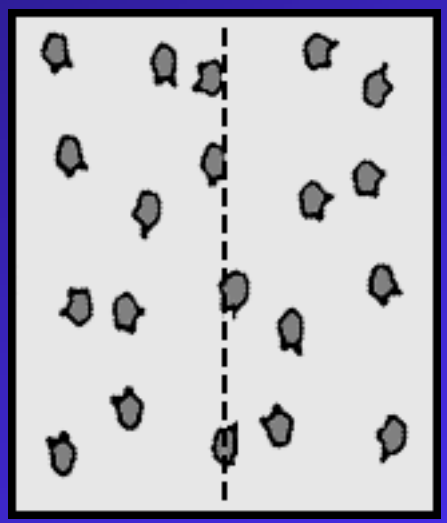
Very useful for heterogeneous samples

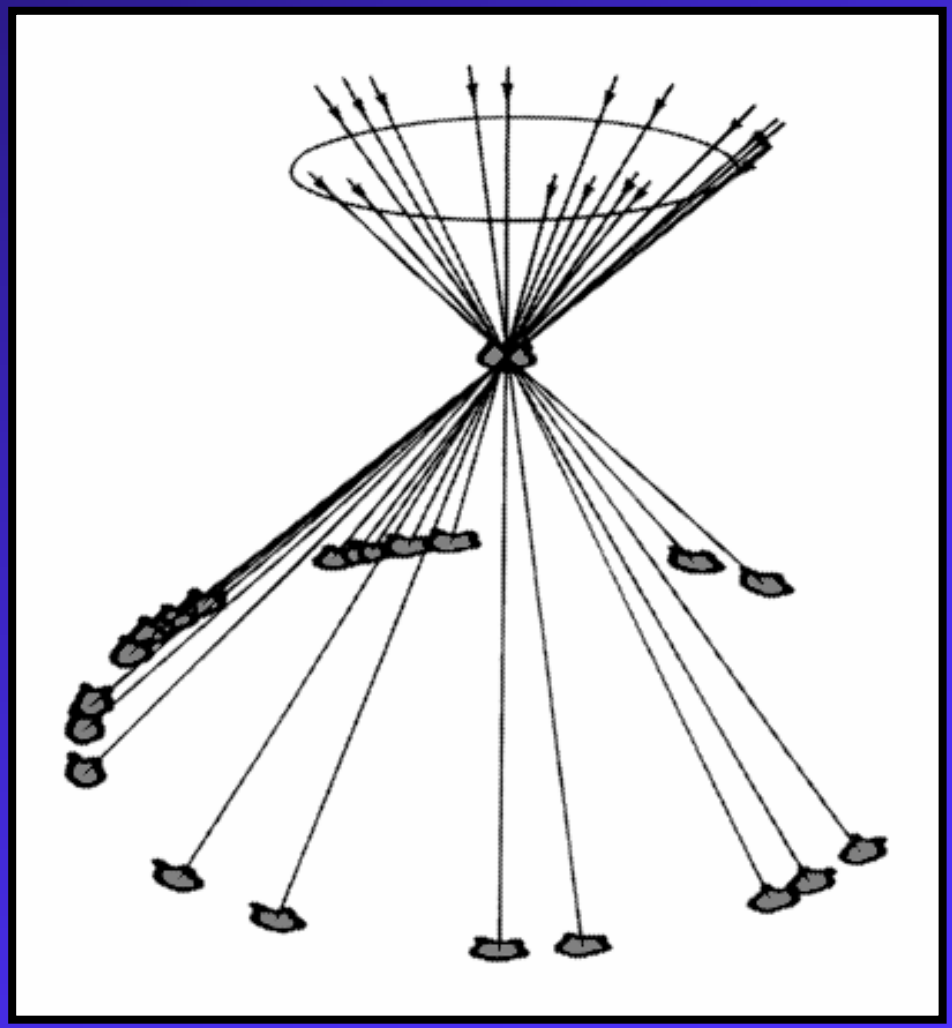
3D reconstruction by RCT is very reliable

Random conical tilt reconstruction



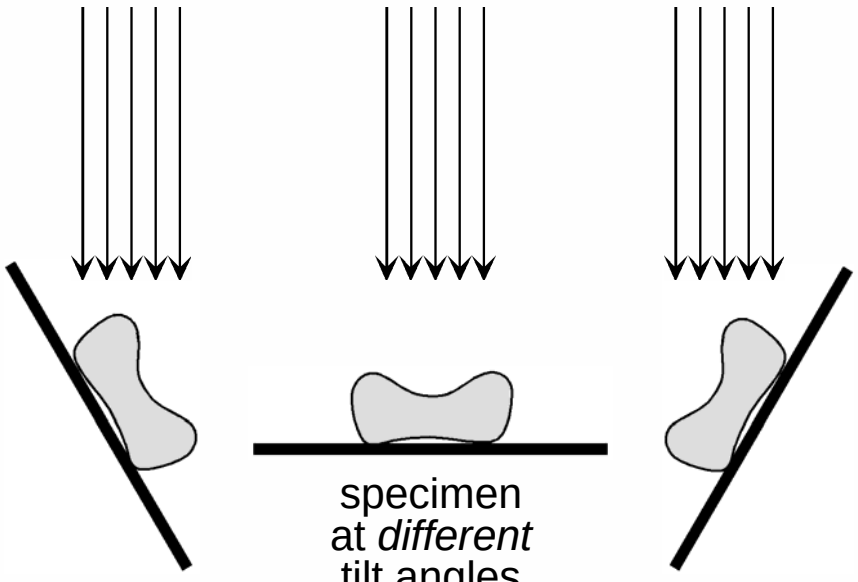
Tiltaxis



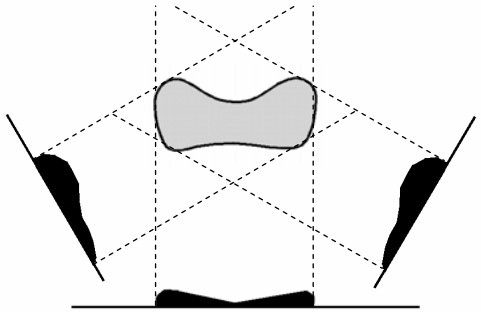


Radermacher *et al*. (1987)

Random conical tilt reconstruction



3D reconstruction
of specimen



IMAGING

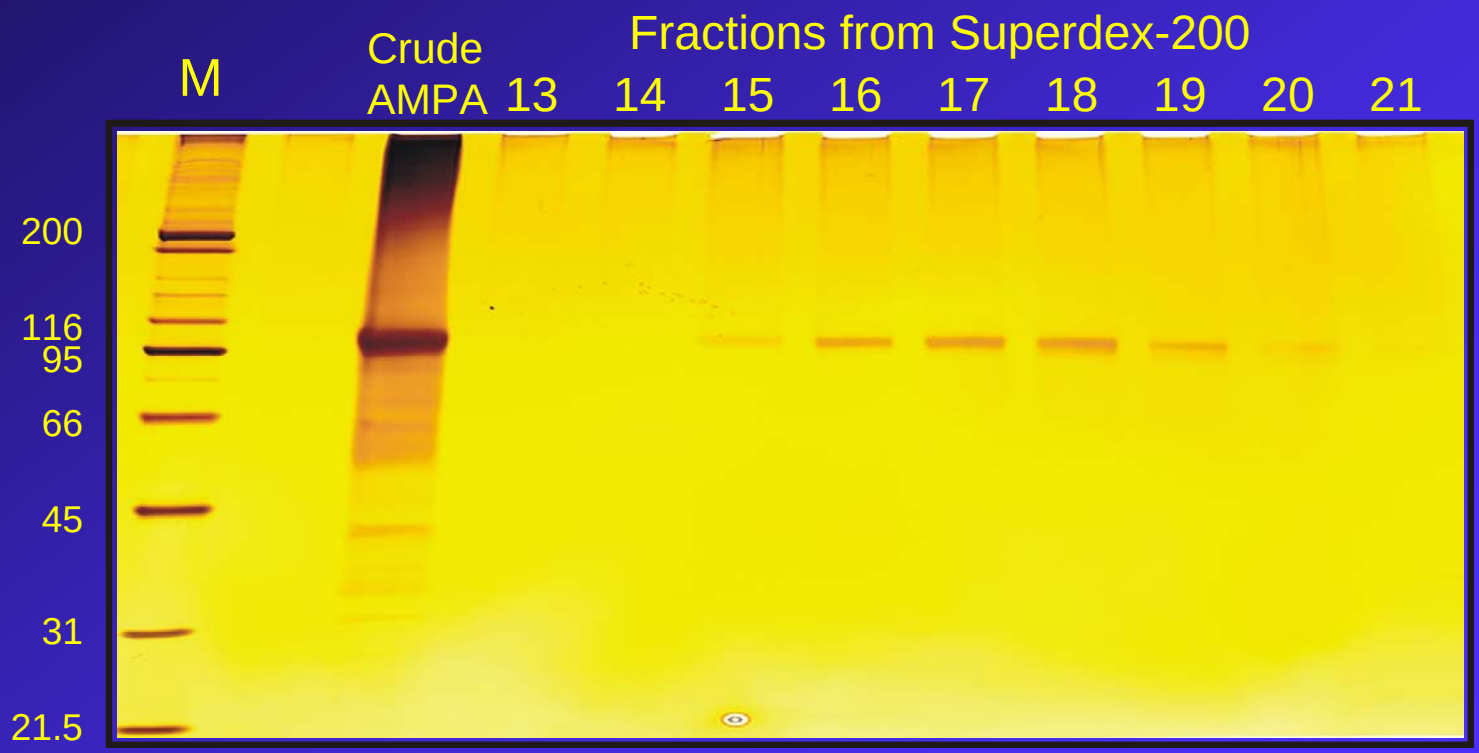
BACKPROJECTION

Different projection views

ASSIGN ORIENTATIONAL PARAMETERS x, y and Φ

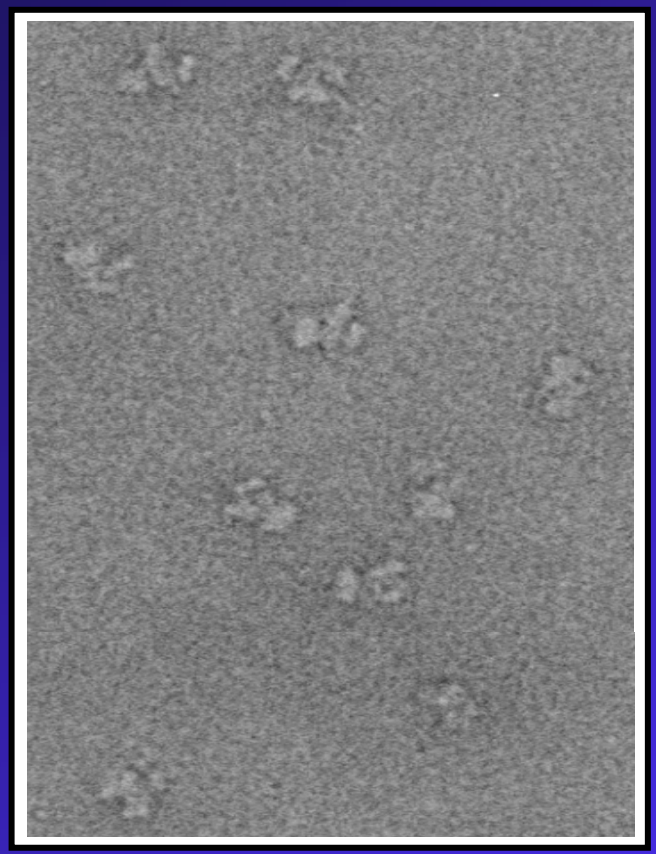
RCT – AMPA receptor

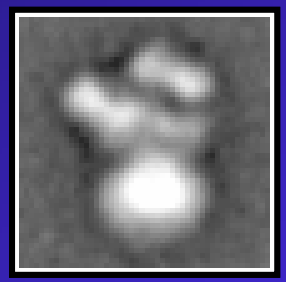
Heterogeneity due to different conformations



RCT – AMPA receptor

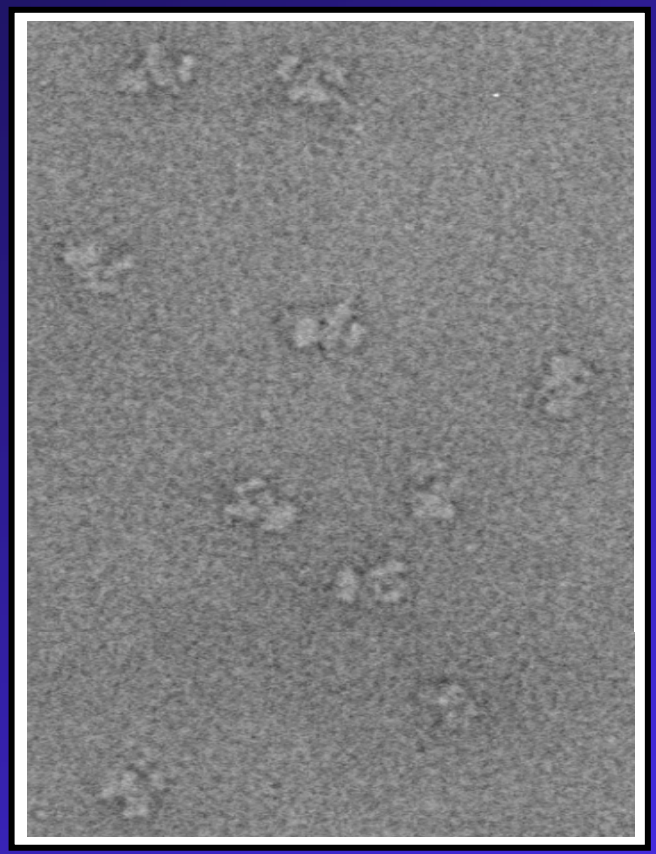
Heterogeneity due to different conformations

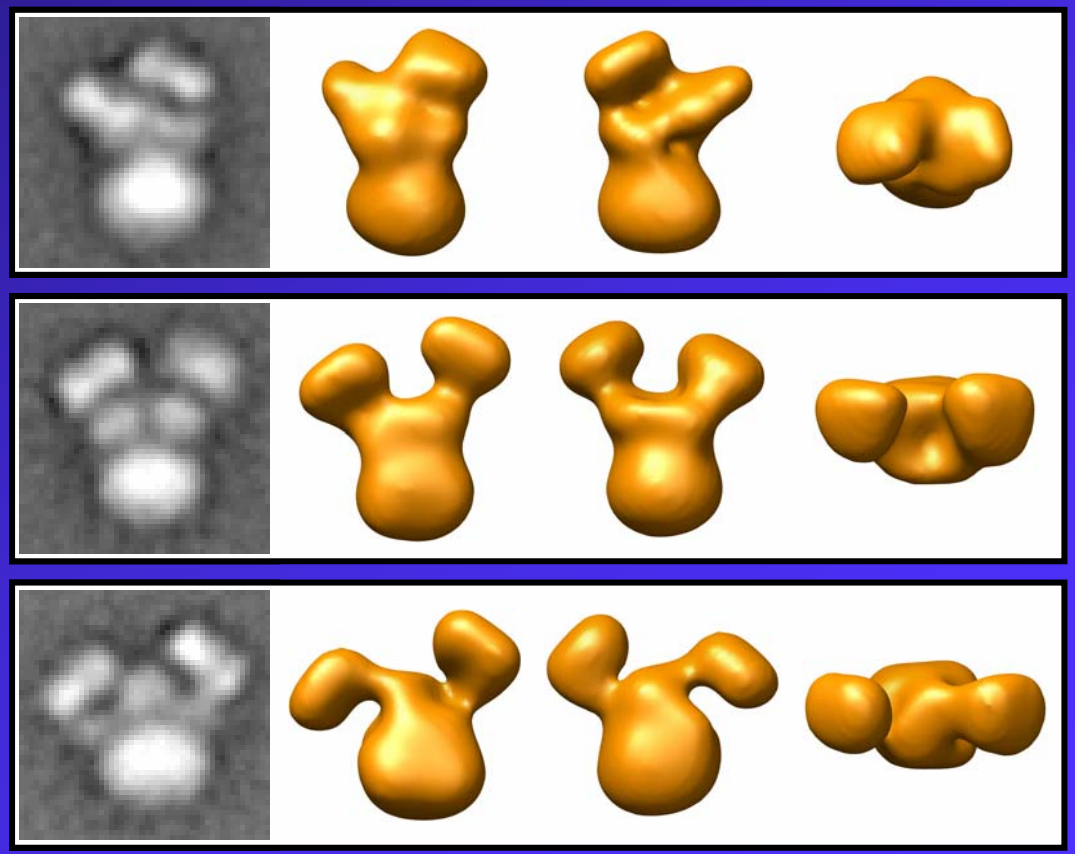




RCT – AMPA receptor

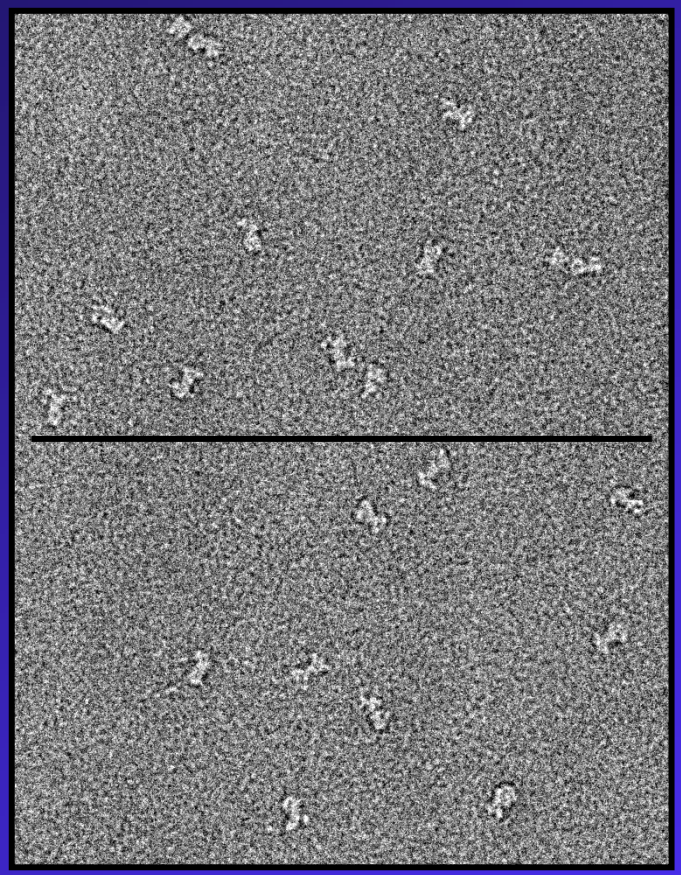
Heterogeneity due to different conformations



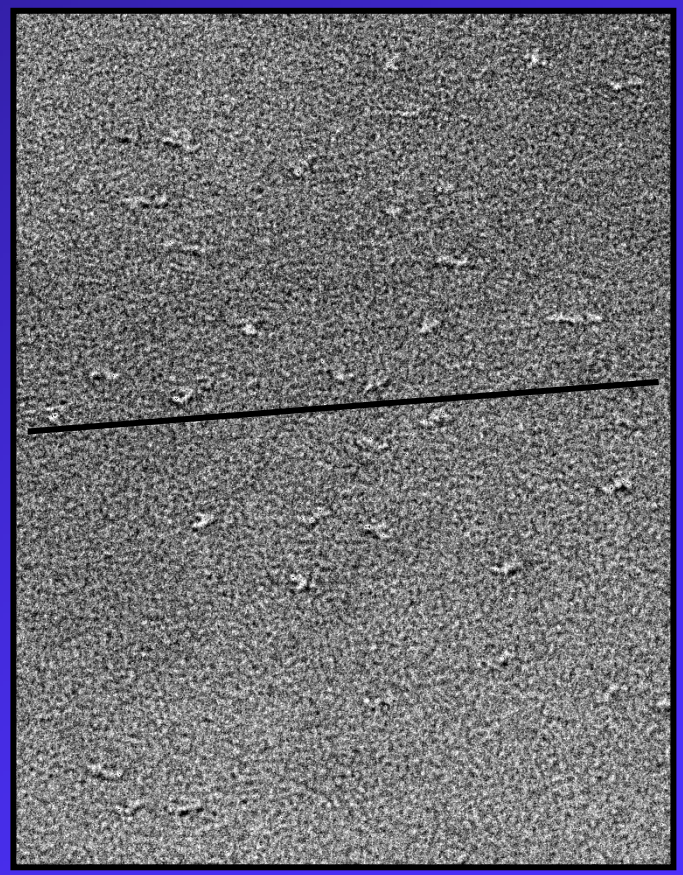


RCT - Sec23/Sec24 complex

Heterogeneity due to different orientations
Negligible deformations



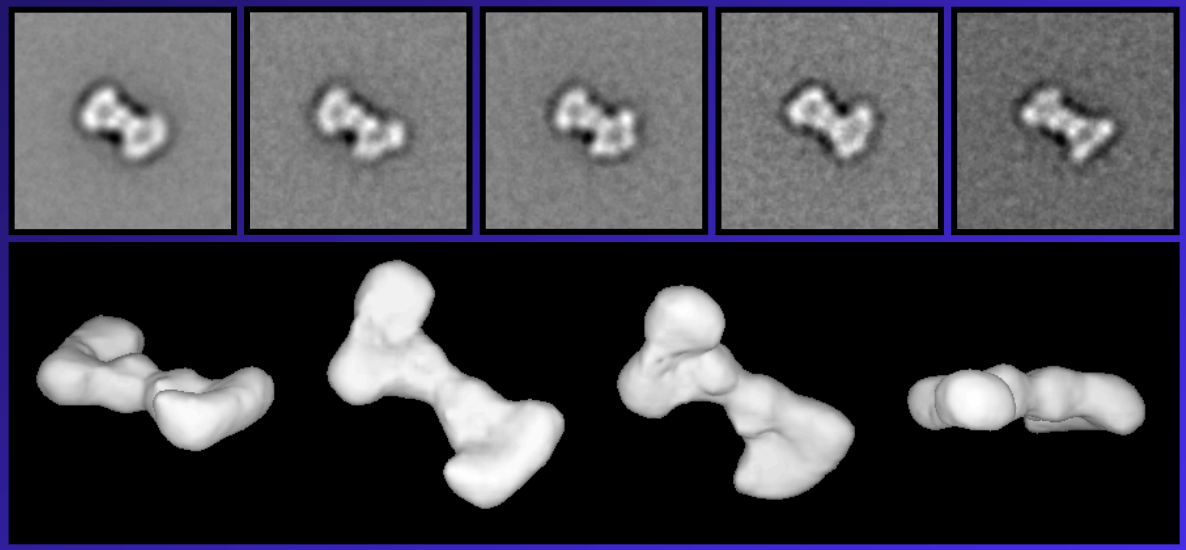
0° tilt



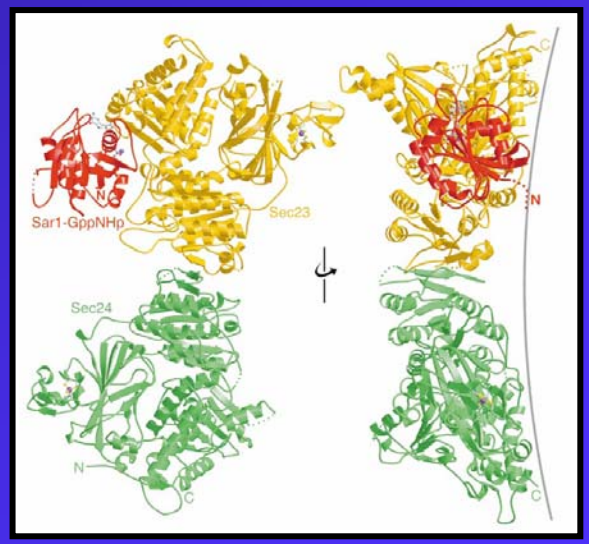
60° tilt

RCT - Sec23/Sec24 complex

Heterogeneity due to different orientations



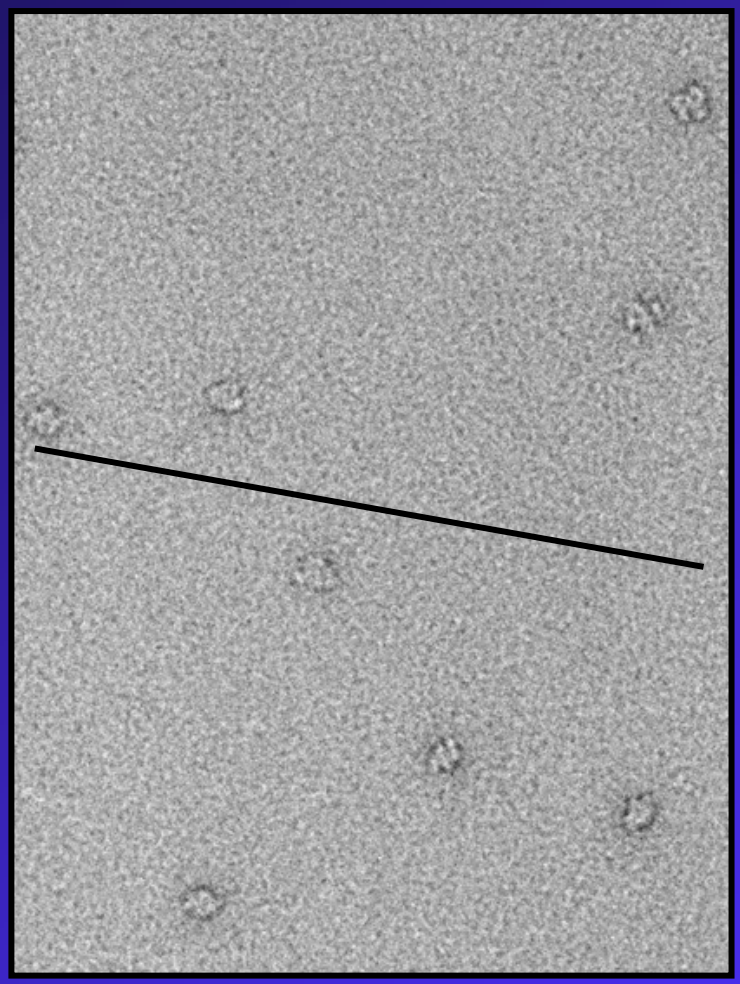
Lederkremer *et al.*, 2001

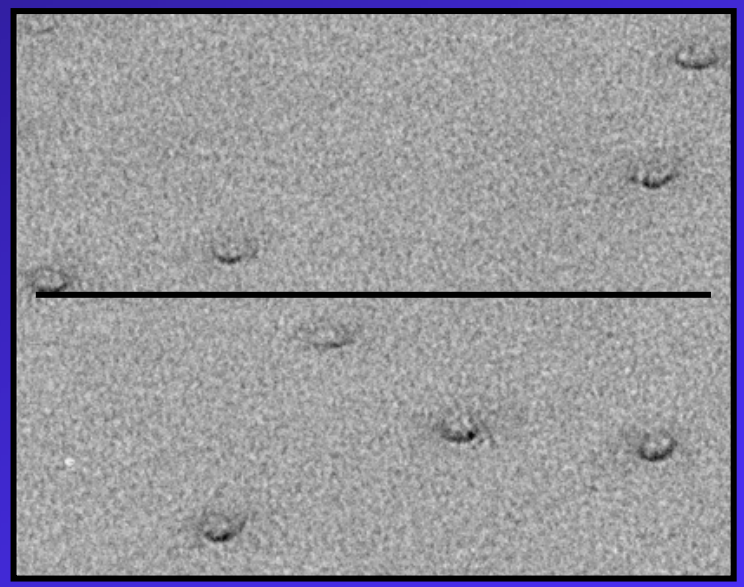


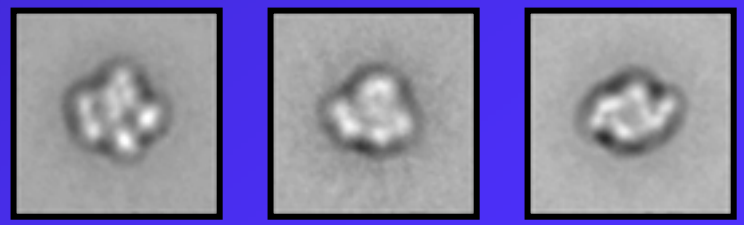
Bi *et al.*, 2002

RCT - Tf-TfR complex

Heterogeneity due to different orientations
Severe deformations







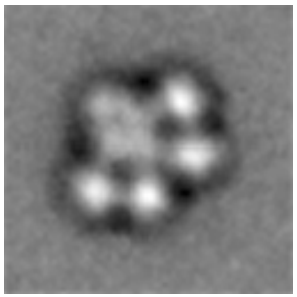
Face view

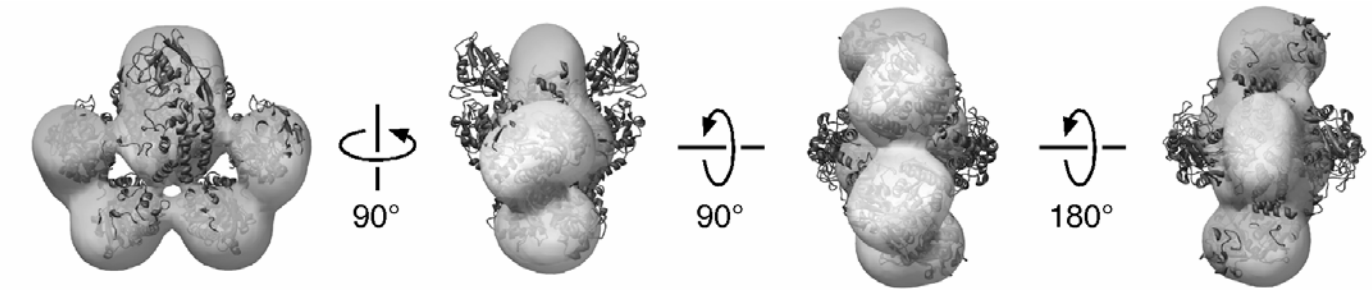
Top view

RCT - Tf-TfR complex

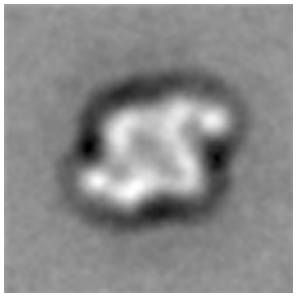
Heterogeneity due to different orientations
Severe deformations

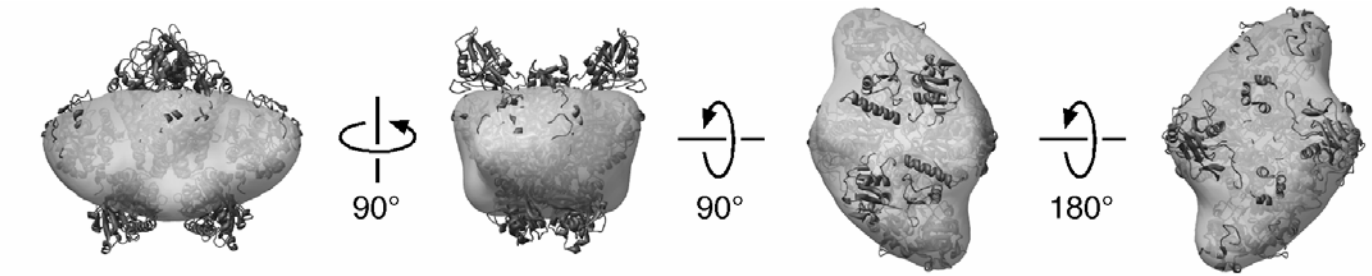
Face view





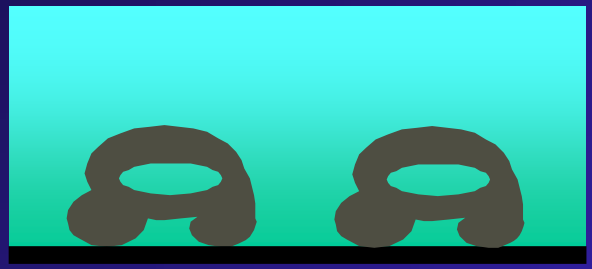
Top view

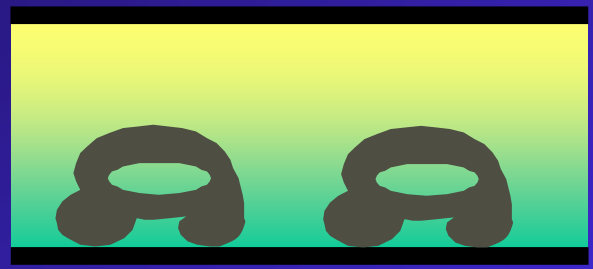


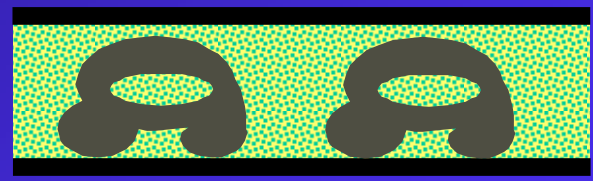


Conventional negative staining

Cryo-negative staining







Addition of glycerol
minimizes adsorption artifacts
prevents specimen flattening
serves as cryo-protectant

Carbon sandwich
reduces incomplete stain embedding

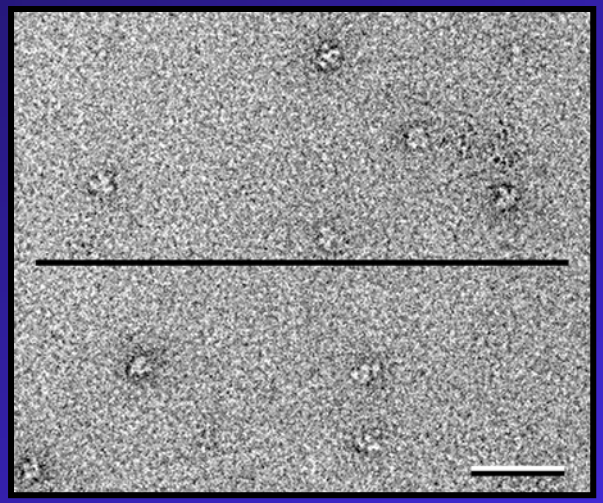
Freezing
prevents specimen flattening

RCT - Tf-TfR complex

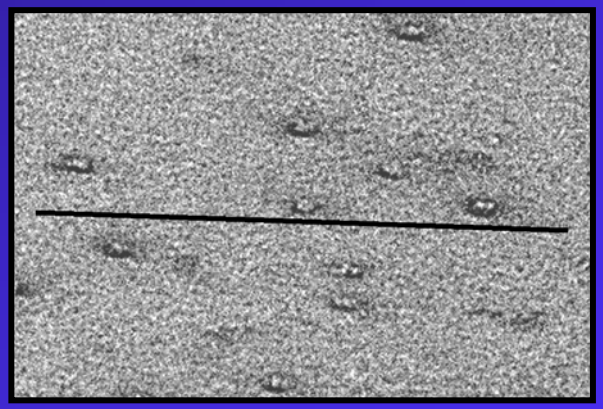
Heterogeneity due to different orientations
Severe deformations

Conventional negative staining

0° tilt

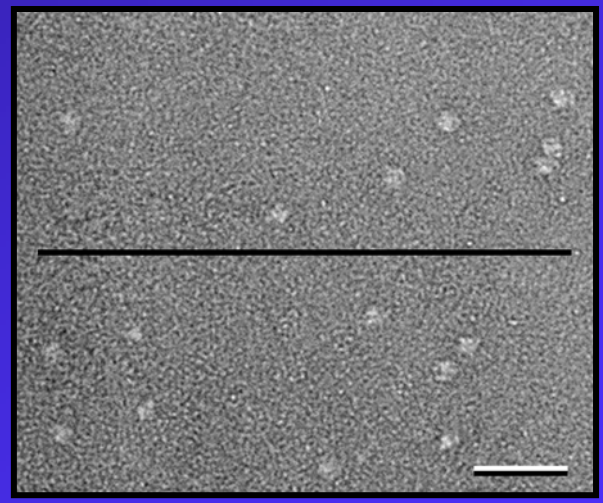


60° tilt

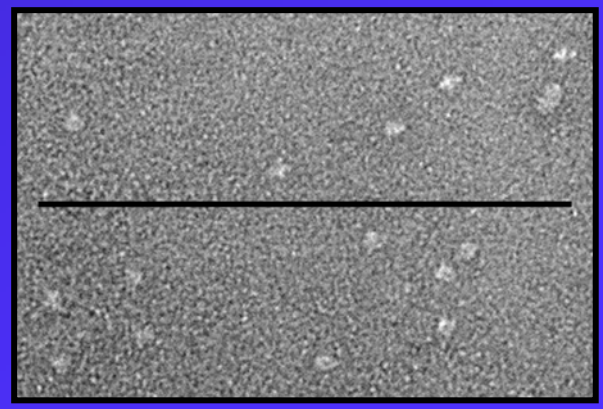


Cryo-negative staining

0° tilt



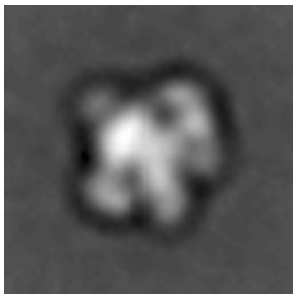
60° tilt

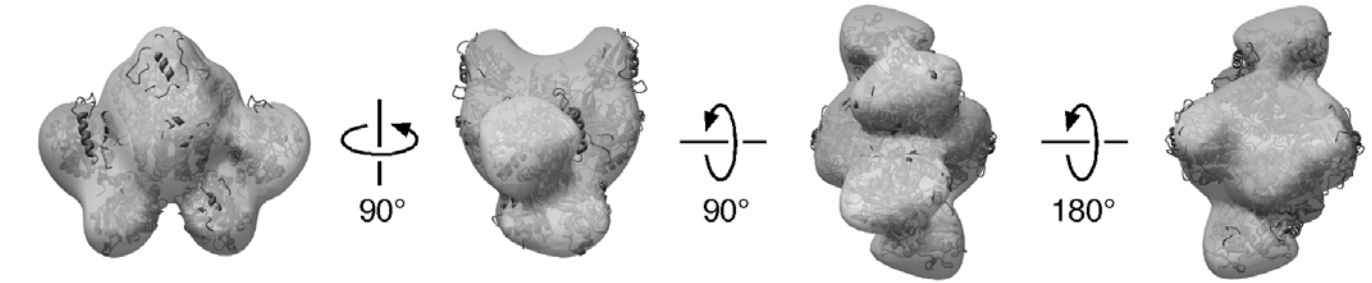


RCT - Tf-TfR complex

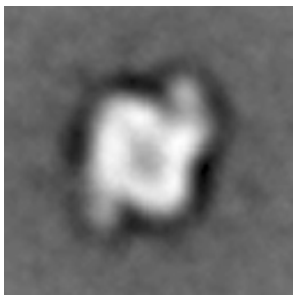
Heterogeneity due to different orientations
Severe deformations

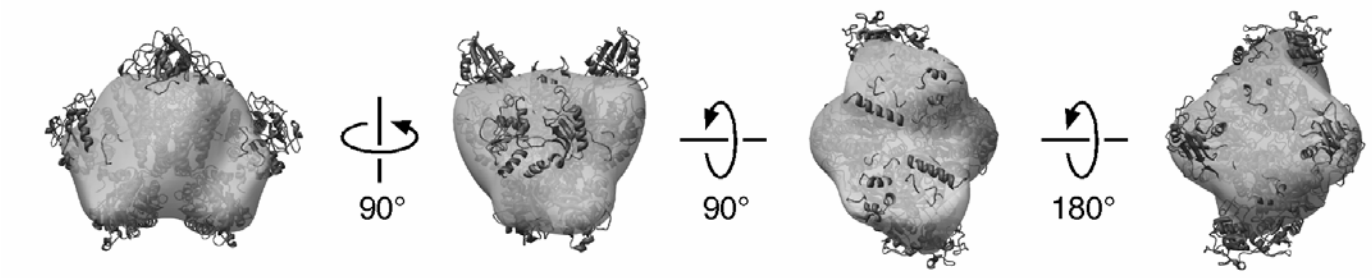
Face view





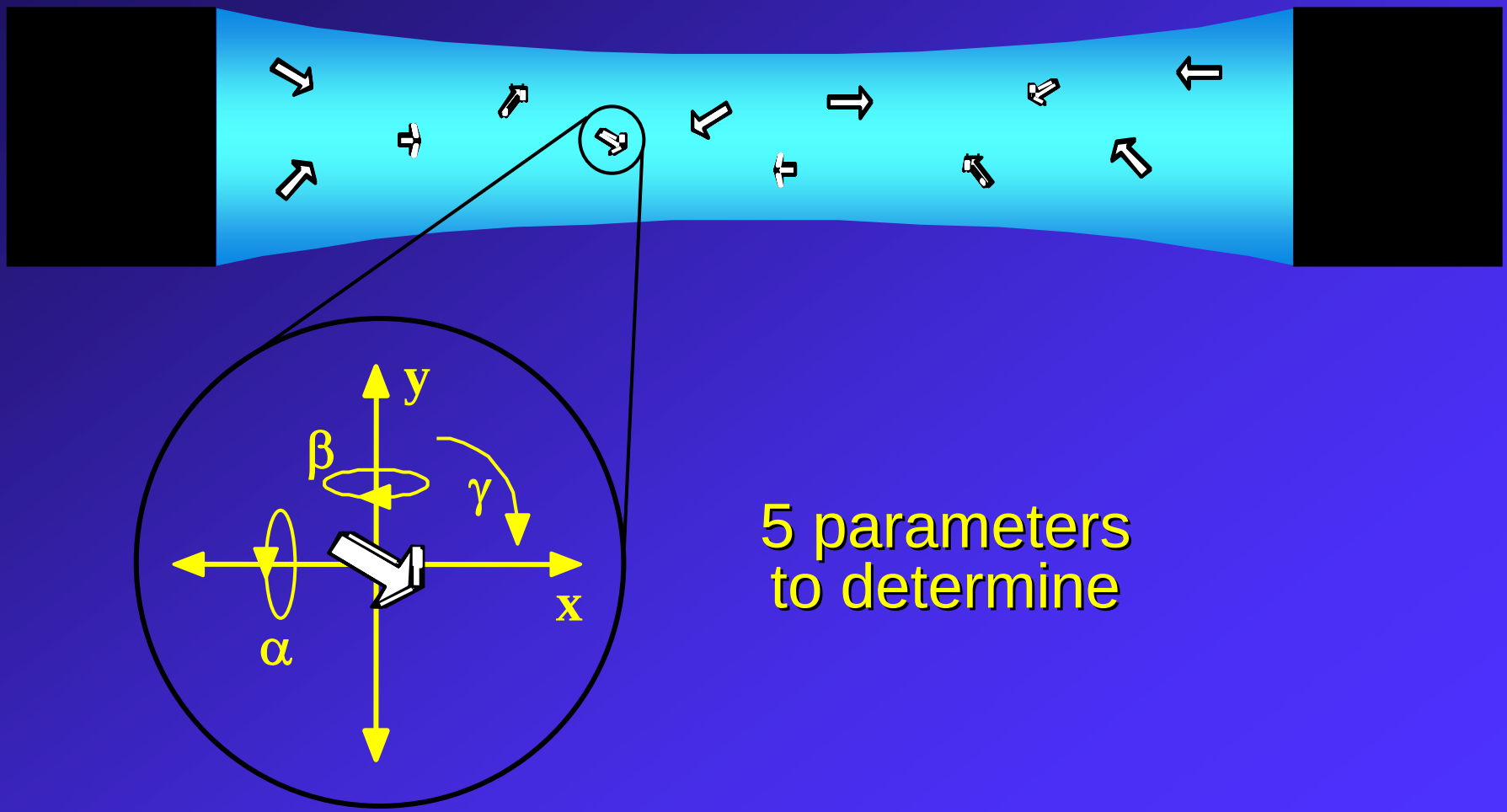
Top view





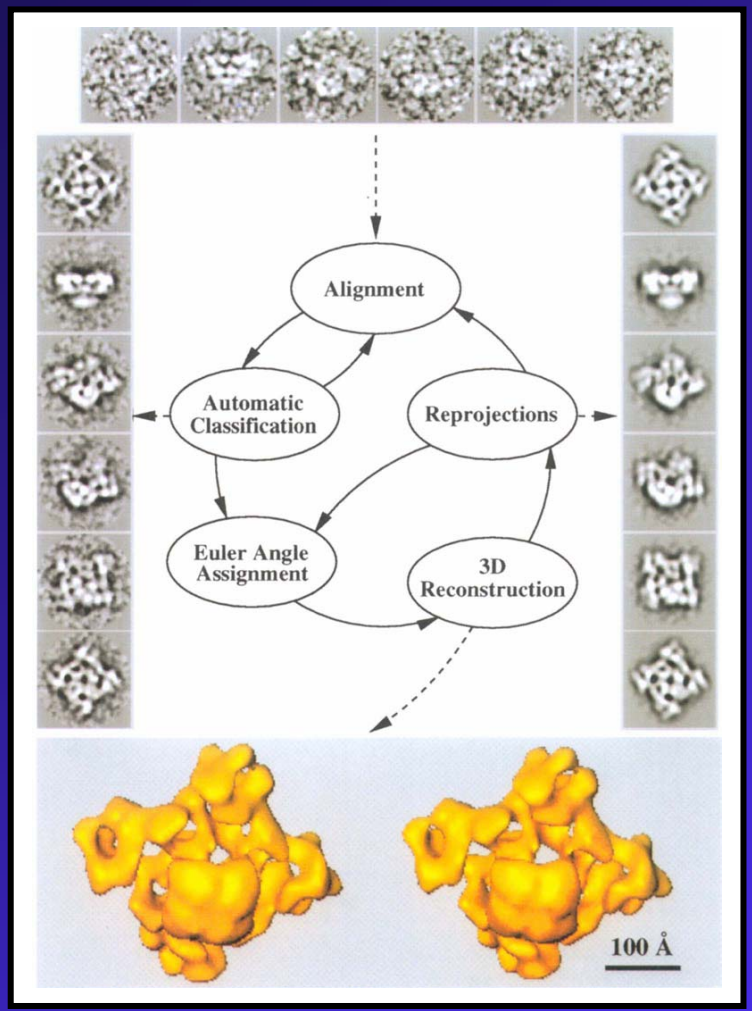
Cryo-negative staining

Single particles in ice



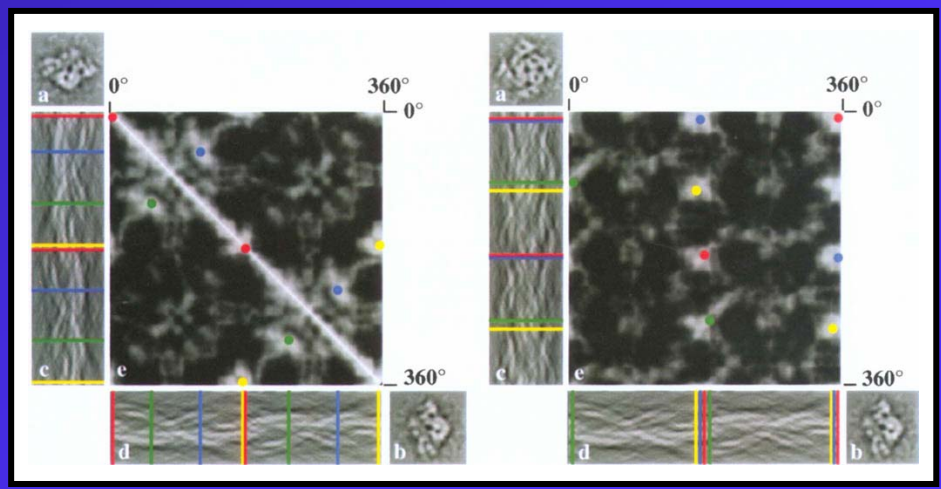
5 parameters
to determine

Angular reconstitution (Imagic)



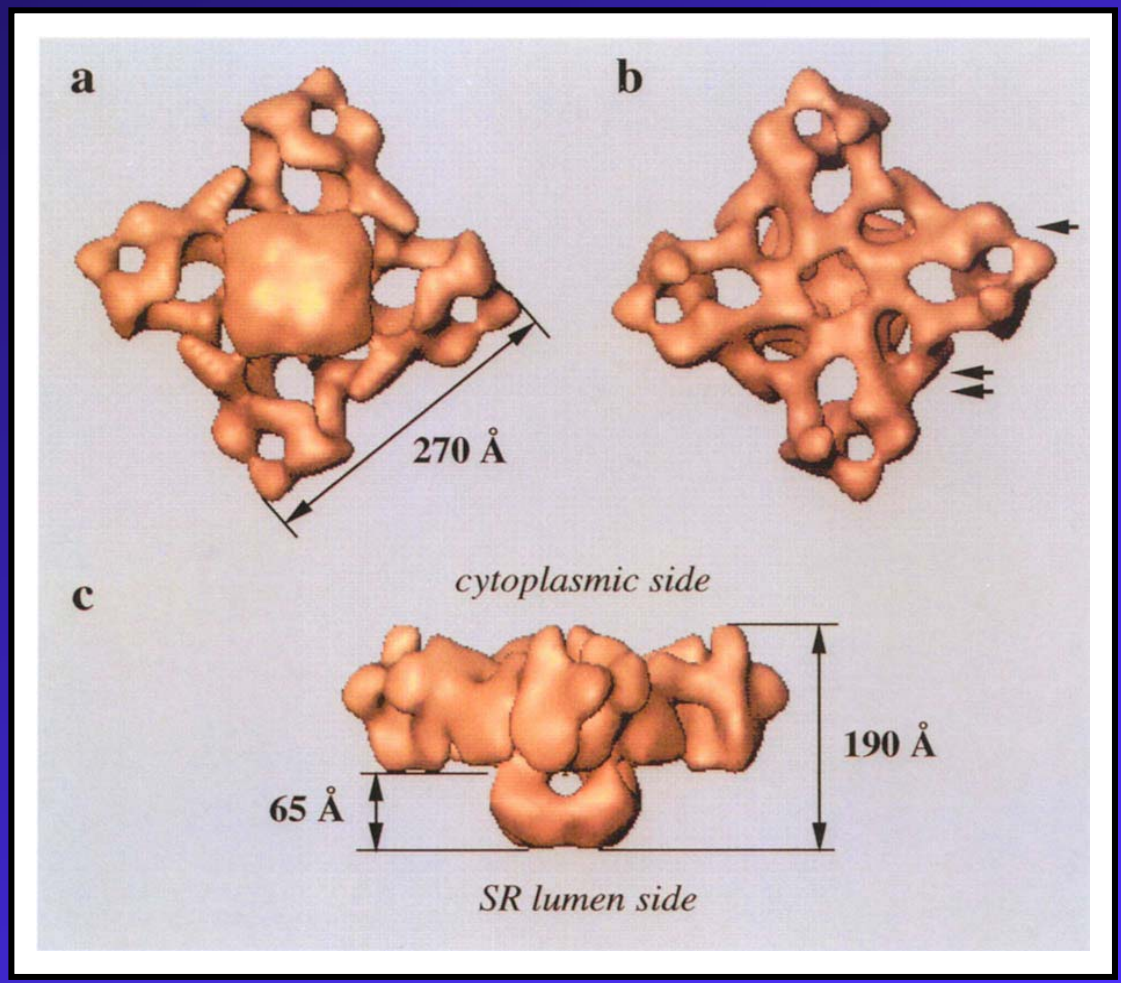
van Heel, 1987

1. chose 3 projection images that are perpendicular views of the particle (anchor set)
2. add in further projections and keep refining



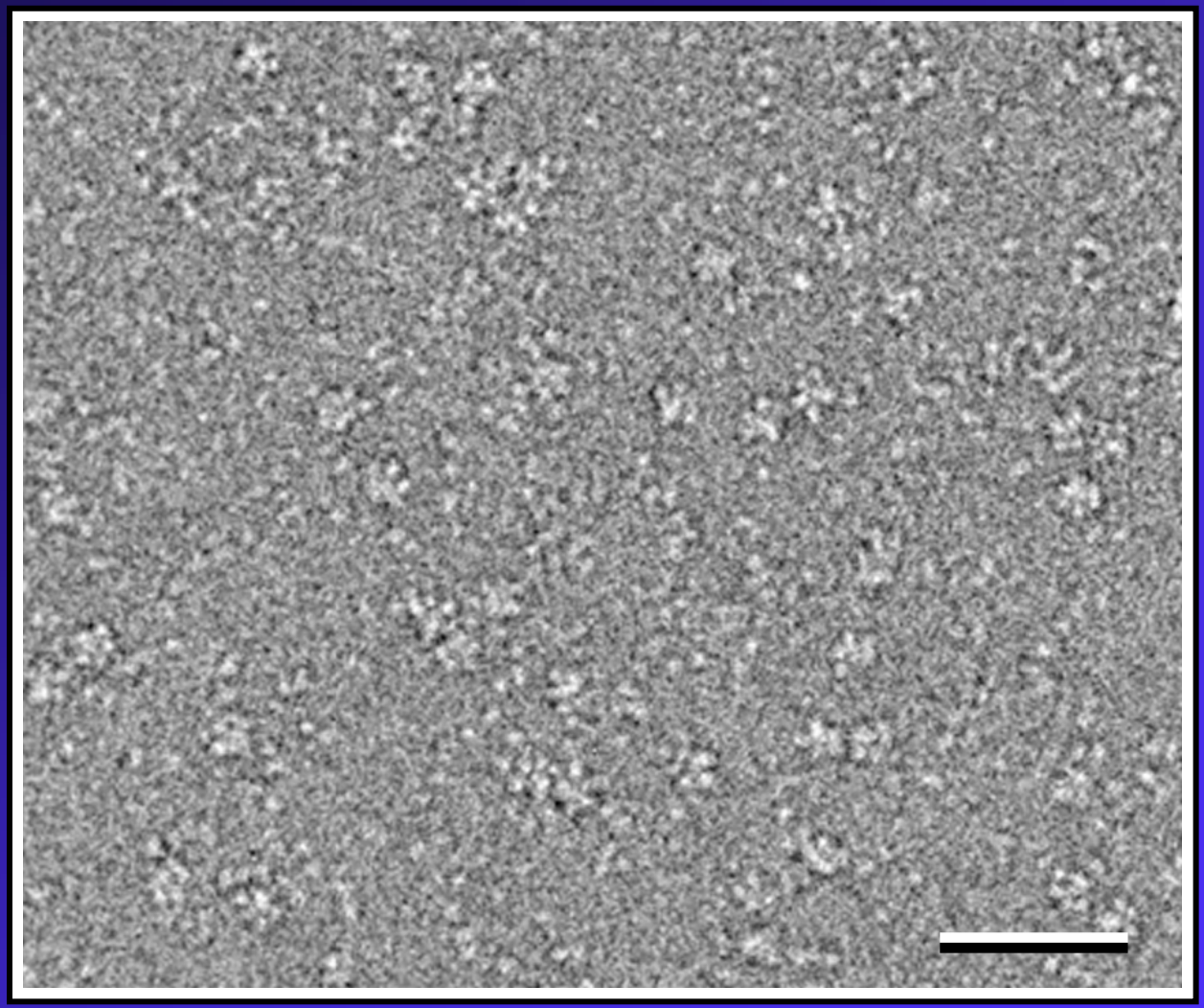
Serysheva *et al.*, 1995

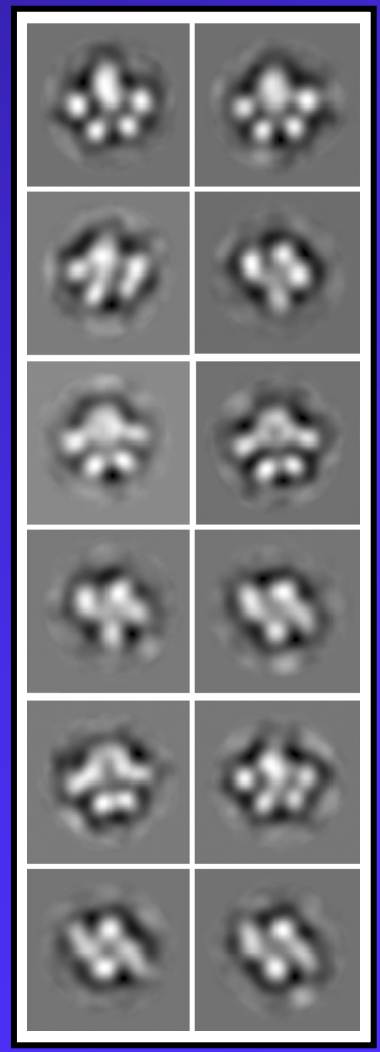
Angular reconstitution - Ryanodine receptor



Serysheva *et al.*, 1995

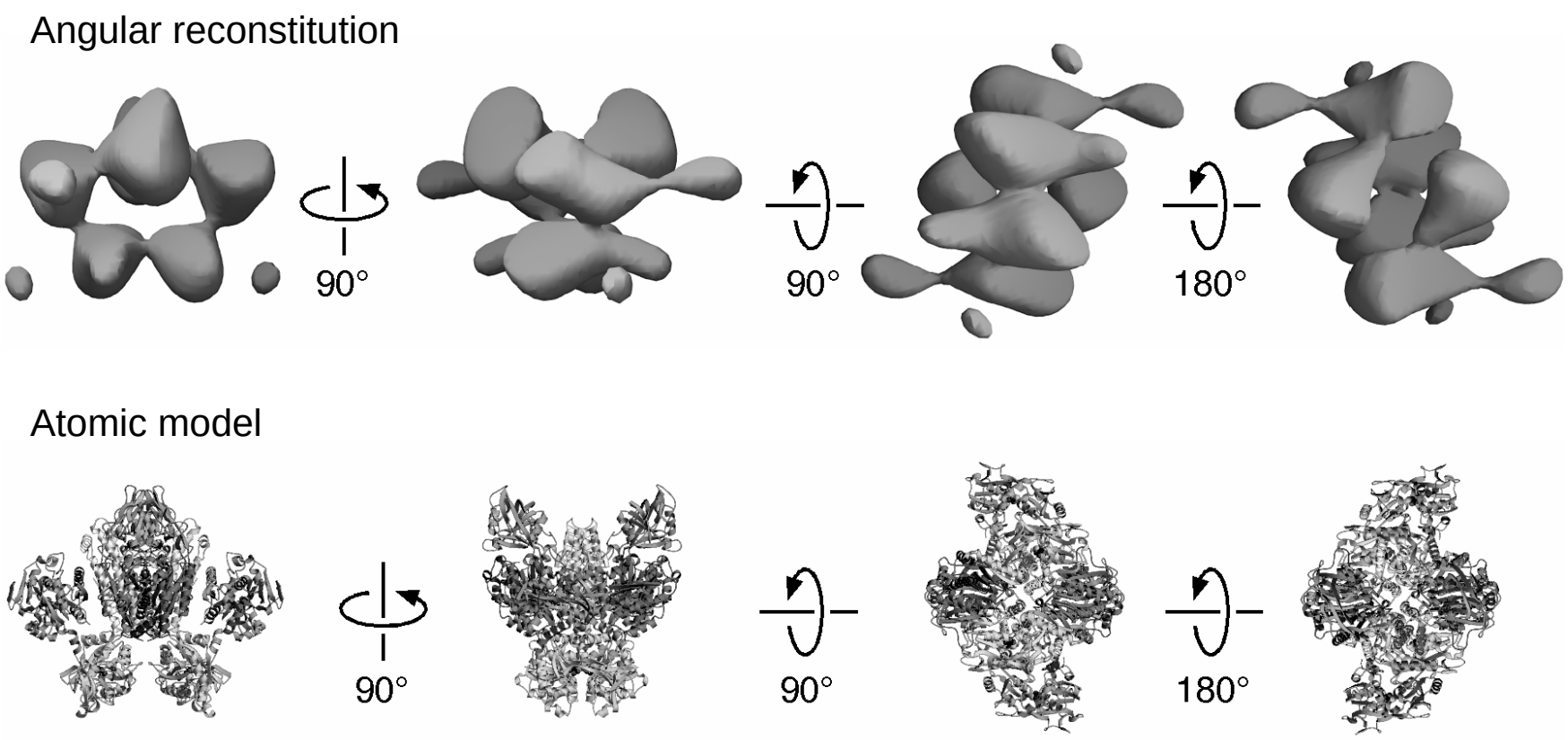
Angular reconstitution - Tf-TfR complex





Vitrified ice

Angular reconstitution - Tf-TfR complex



Angular reconstitution (IMAGIC)

Structure depends critically on the anchor set
(these should ideally be 3 perpendicular views,
which is hard to know with an unknown molecule)

Structure also depends on the order in which
additional projections are included

Angular reconstitution is best for:
large specimens with symmetry,
and available structural information
(can be obtained from
random conical tilt)

OP command (SPIDER)

Penczek *et al.*, 1996

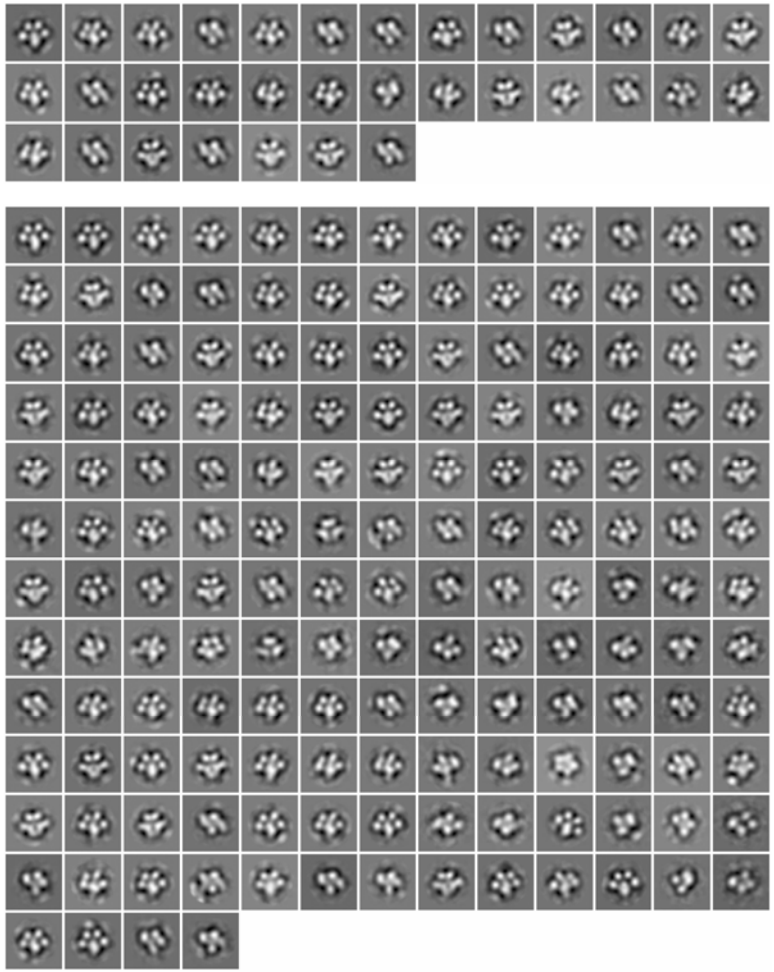
A common-lines based method for determining orientations for $N > 3$ particle projections simultaneously

Essentially the opposite of standard common lines approach

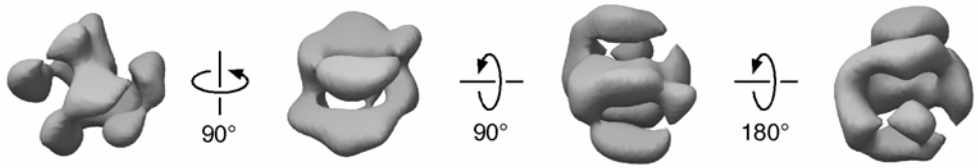
(instead of trying to determine the Euler angles based on pair-wise angles of common lines in the projections' planes, one assumes that rotation matrices are known, finds set of angles of common lines and computes the overall discrepancy along these lines)

Applied to 70S ribosome from *E. coli*

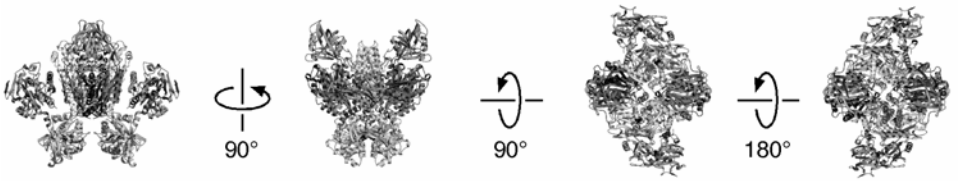
OP command - Tf-TfR complex



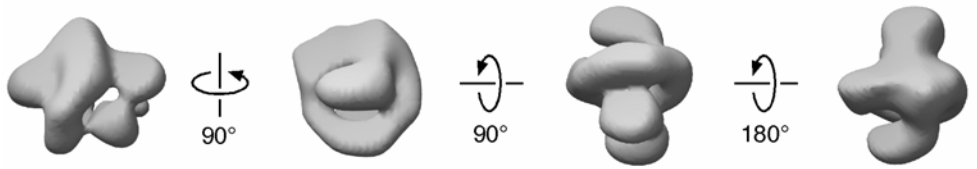
OP reconstruction (33 classes)



Atomic model



OP reconstruction (160 classes)

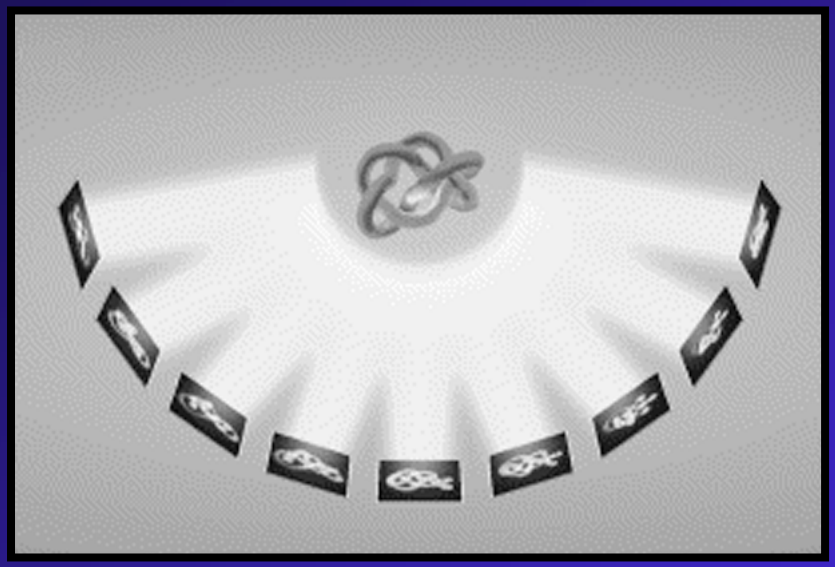


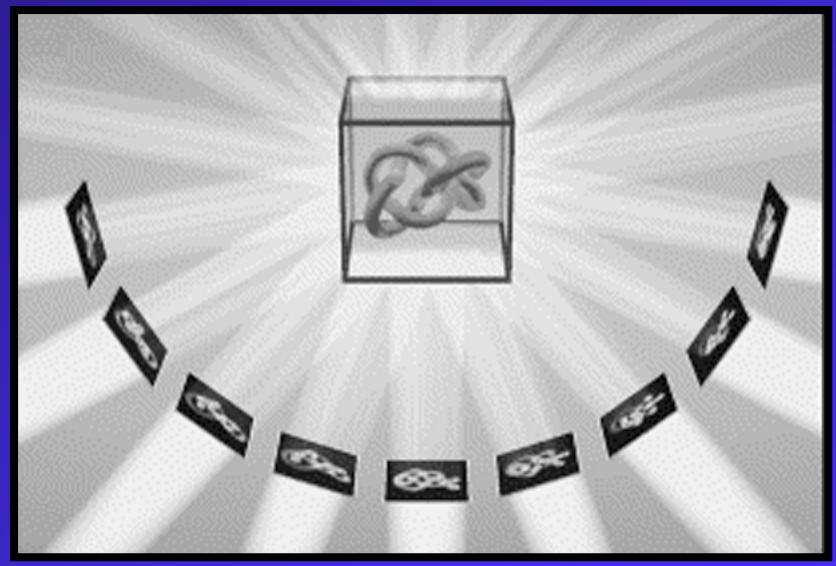
OP reconstructions

3D reconstruction in EMAN

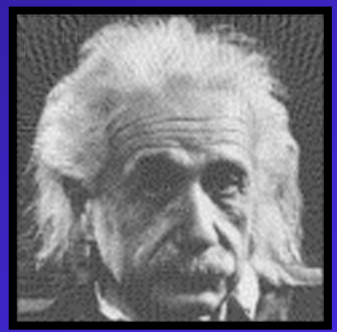
Also uses a set of projections
to generate an initial model

Electron tomographic reconstruction

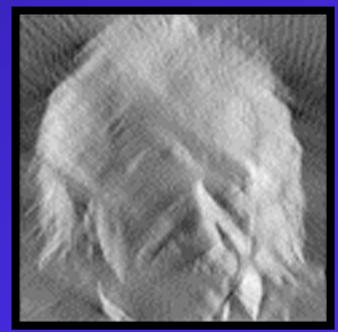




Baumeister
et al. (1999)

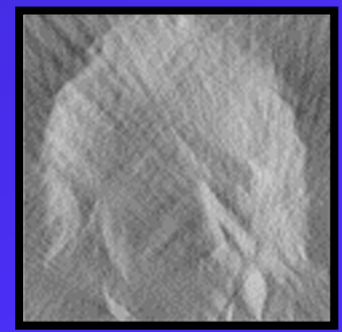


± 90°
2° steps



± 60°
2° steps

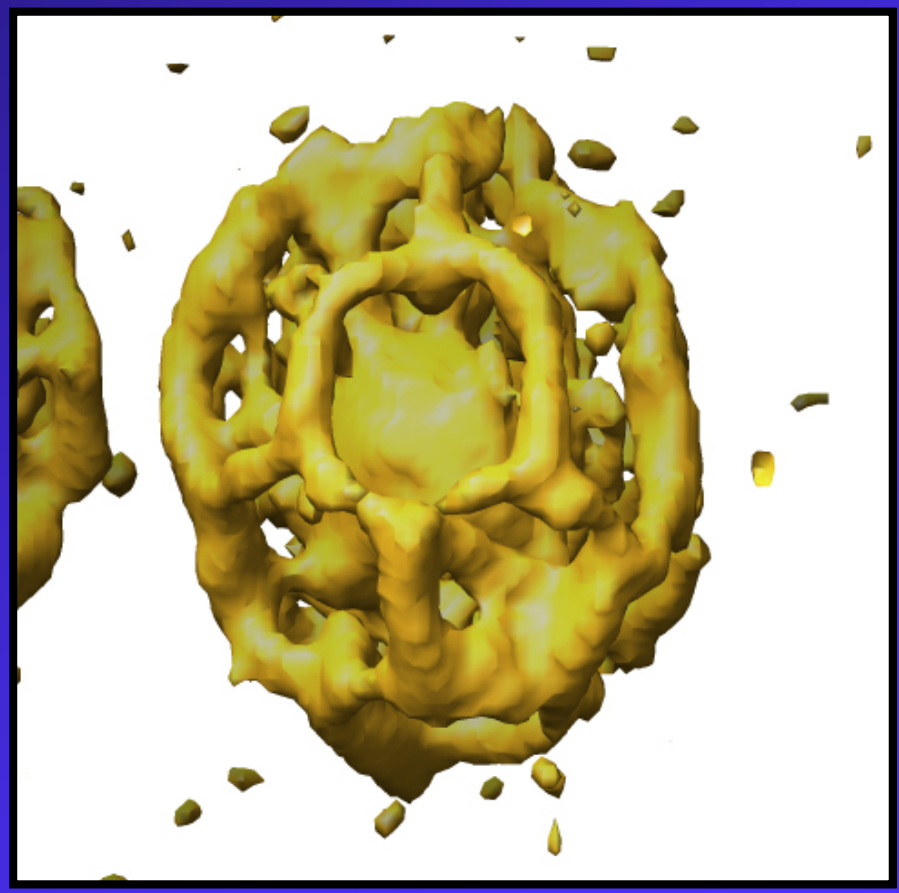
± 90°
5° steps



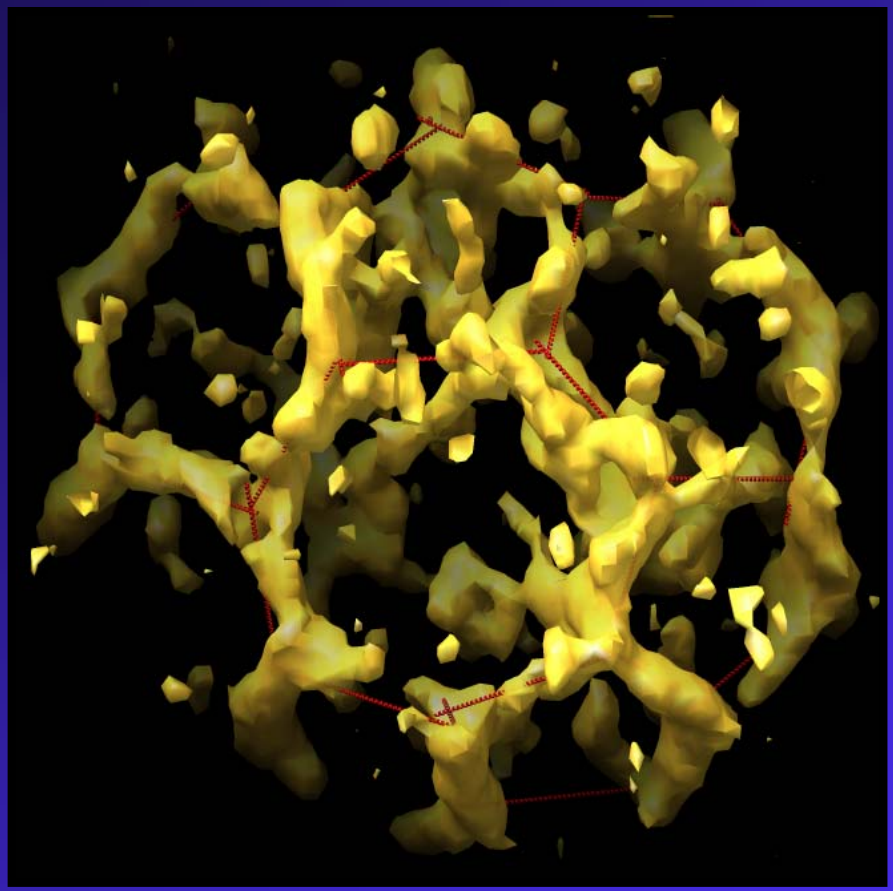
± 60°
5° steps

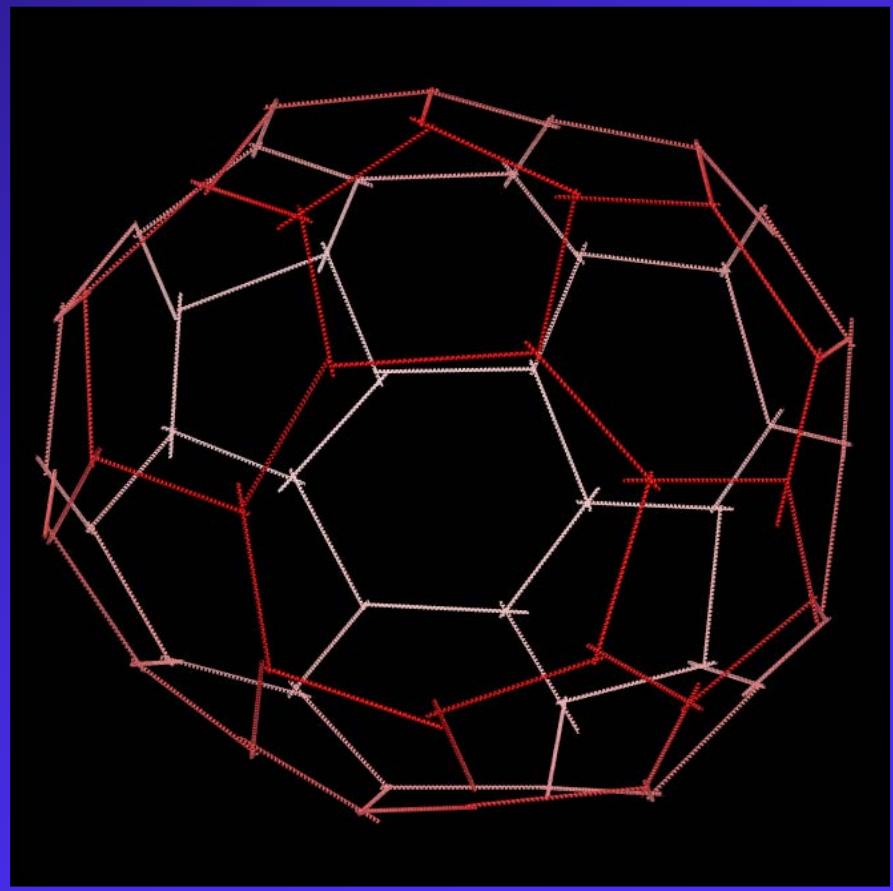
Electron tomography - Clathrin-coated vesicles

QuickTime™ and a
Cinepak decompressor
are needed to see this picture.

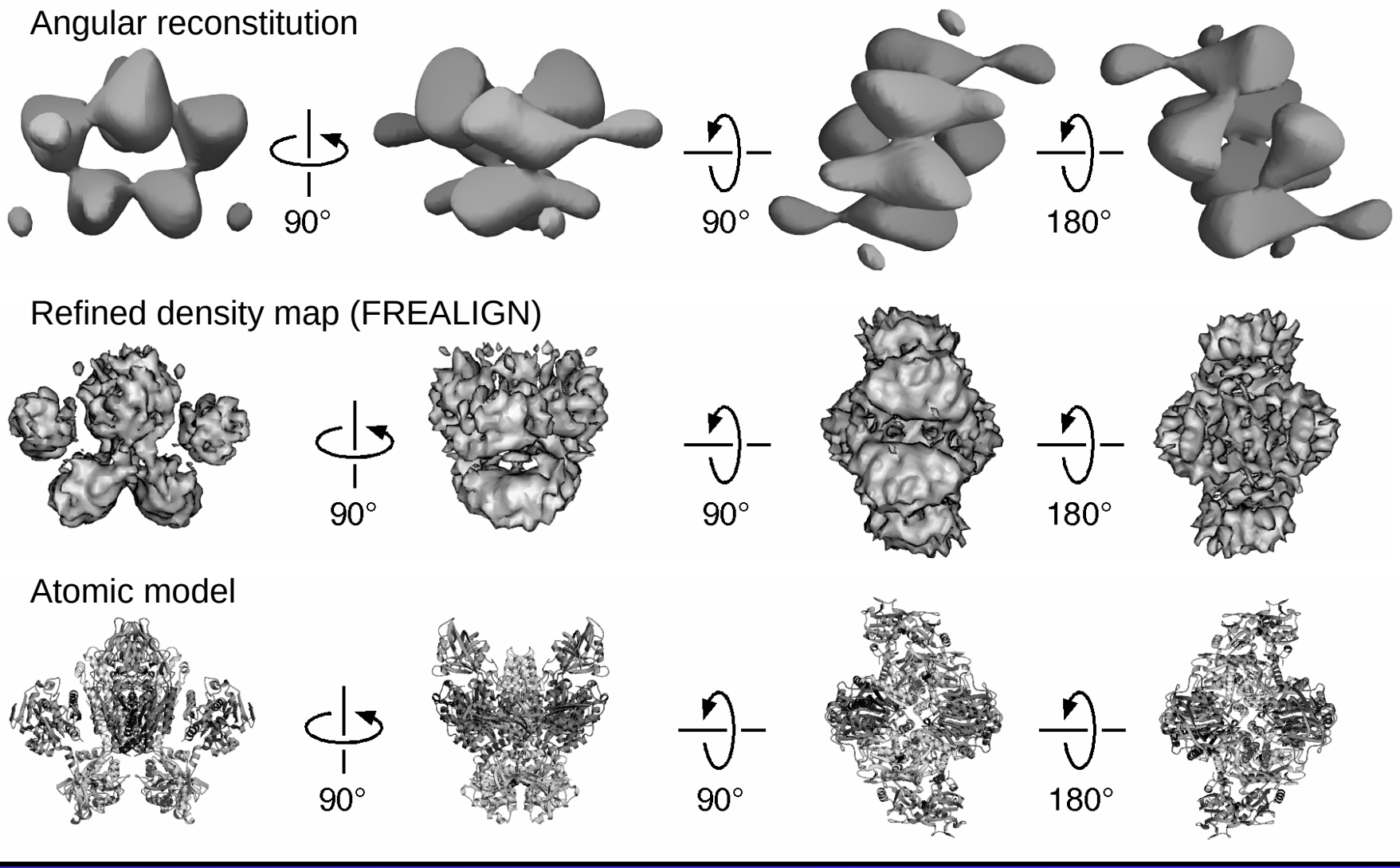


Electron tomography - Clathrin-coated vesicles



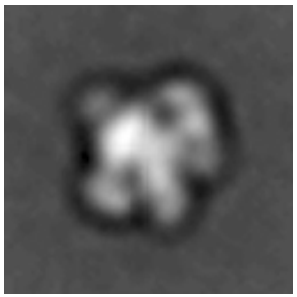


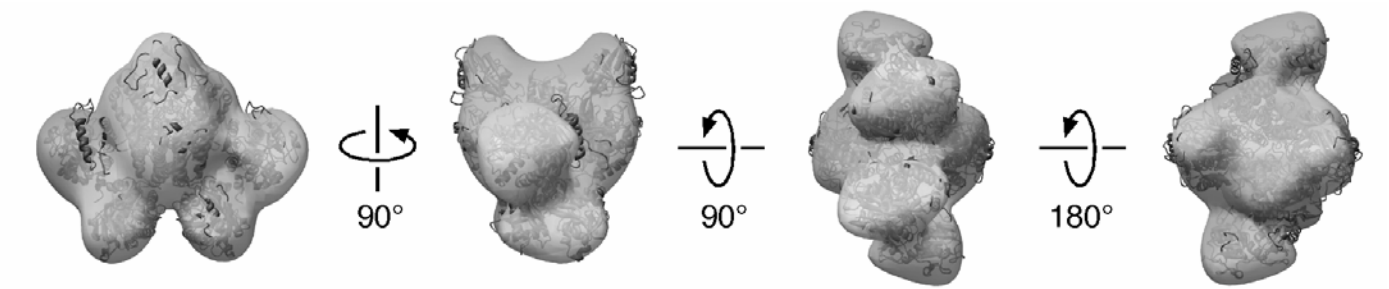
Model refinement - Tf-TfR complex



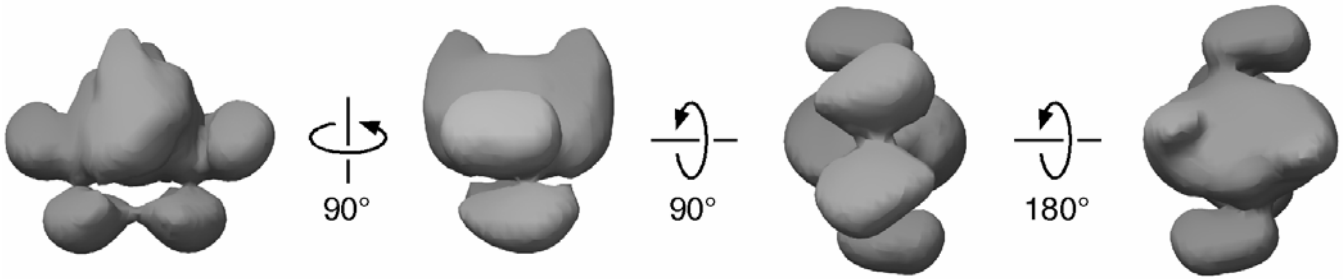
Model refinement - Tf-TfR complex

Reference model (face view in cryo-negative stain)

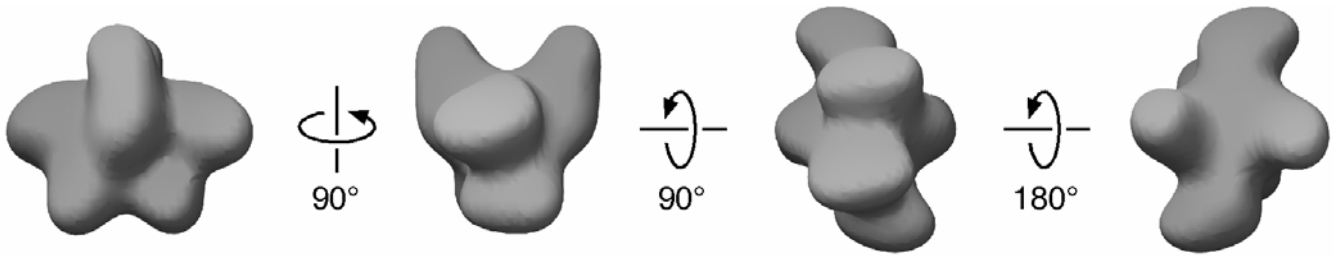




Alignment of 500 class averages

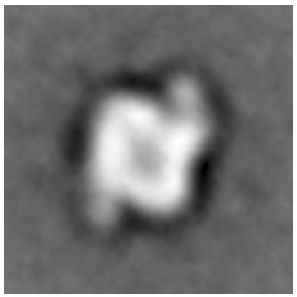


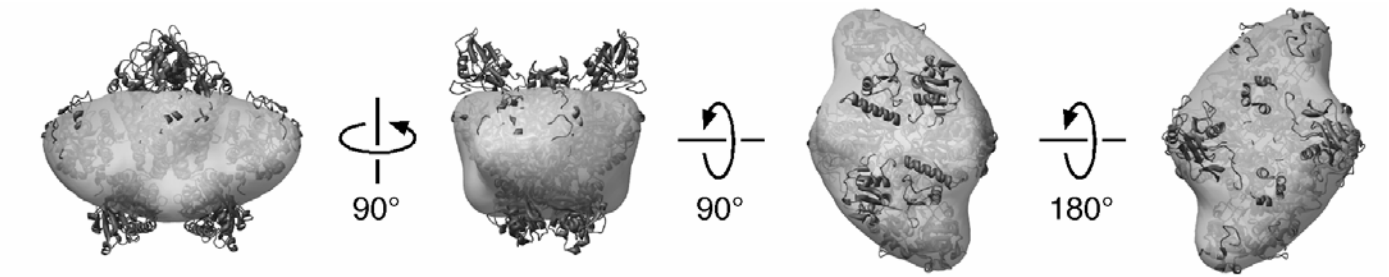
Atomic model filtered to 40 Å



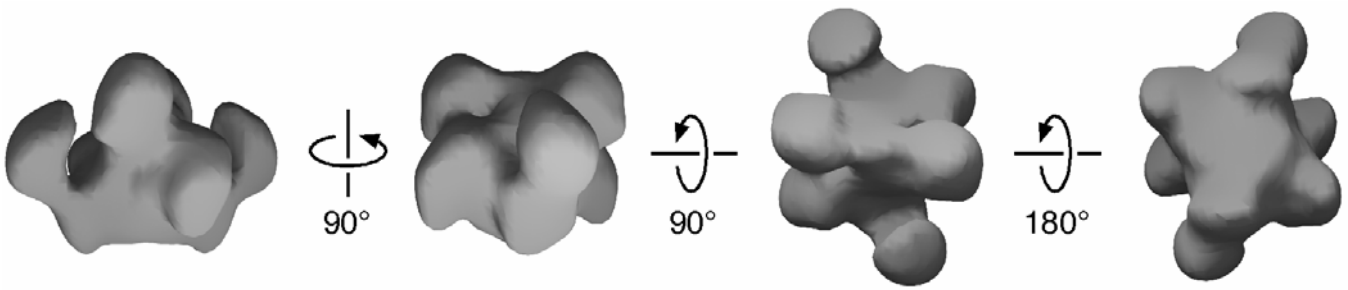
Model refinement - Tf-TfR complex

Reference model (top view in cryo-negative stain)

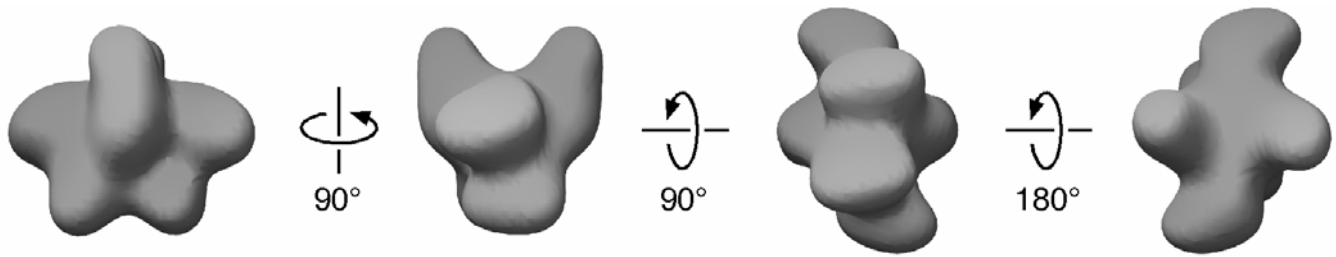




Alignment of 500 class averages

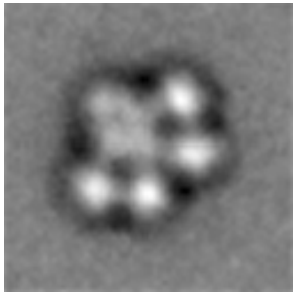


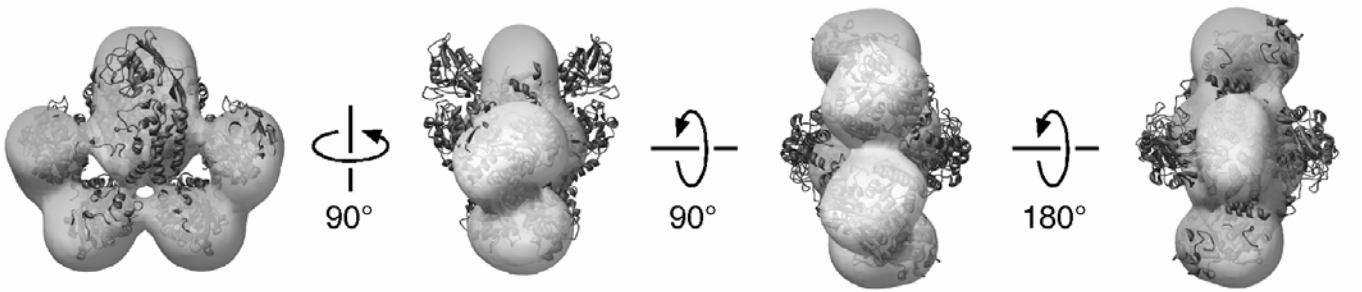
Atomic model filtered to 40 Å



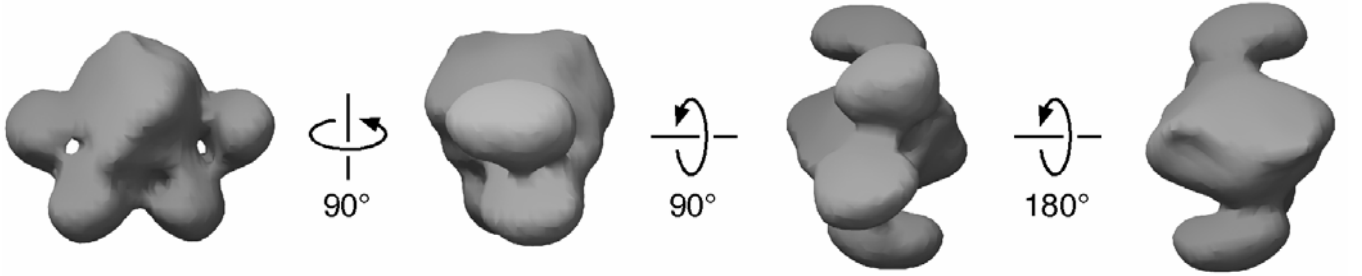
Model refinement - Tf-TfR complex

Reference model (face view in conventional negative stain)

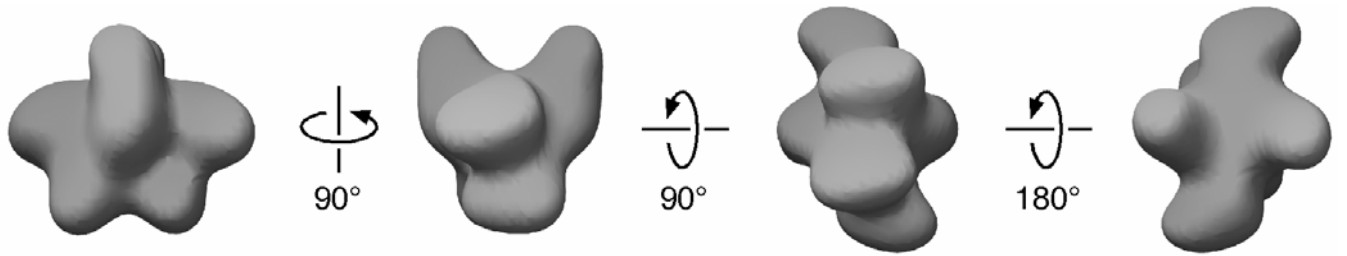




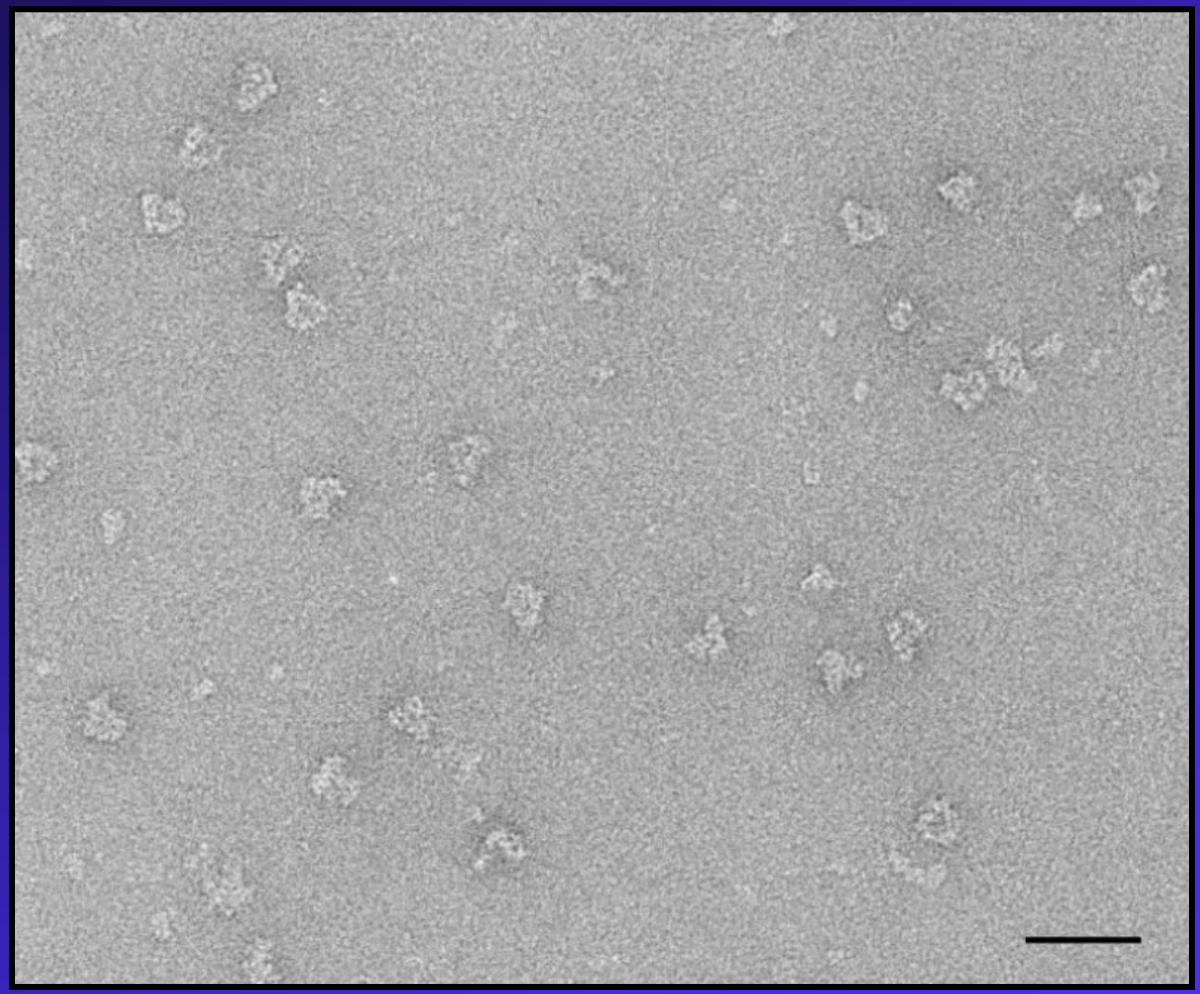
Alignment of 500 class averages



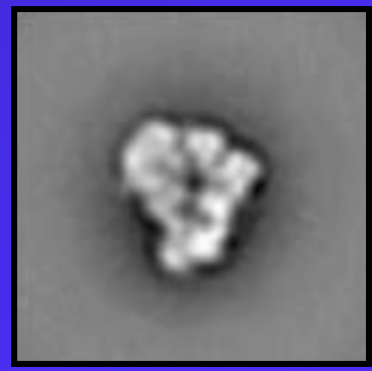
Atomic model filtered to 40 Å



Model refinement - APC

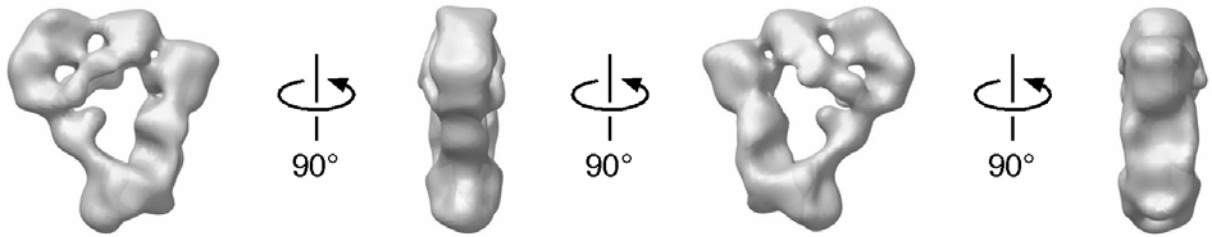


Anaphase promoting complex (*S. pombe*) in negative stain

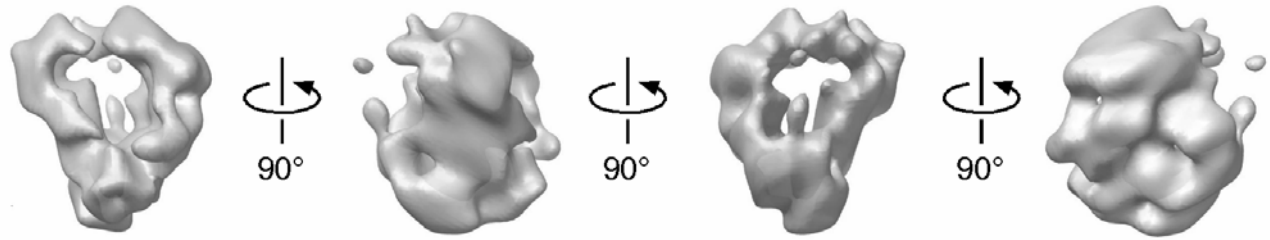


Model refinement - APC

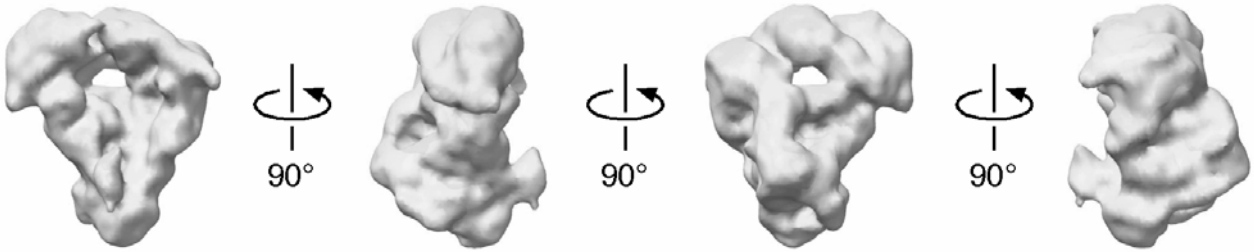
RCT reconstruction
in negative stain



RCT reconstruction
in cryo-negative stain



Reconstruction
with vitrified ice data
using CNS RCT
as reference model



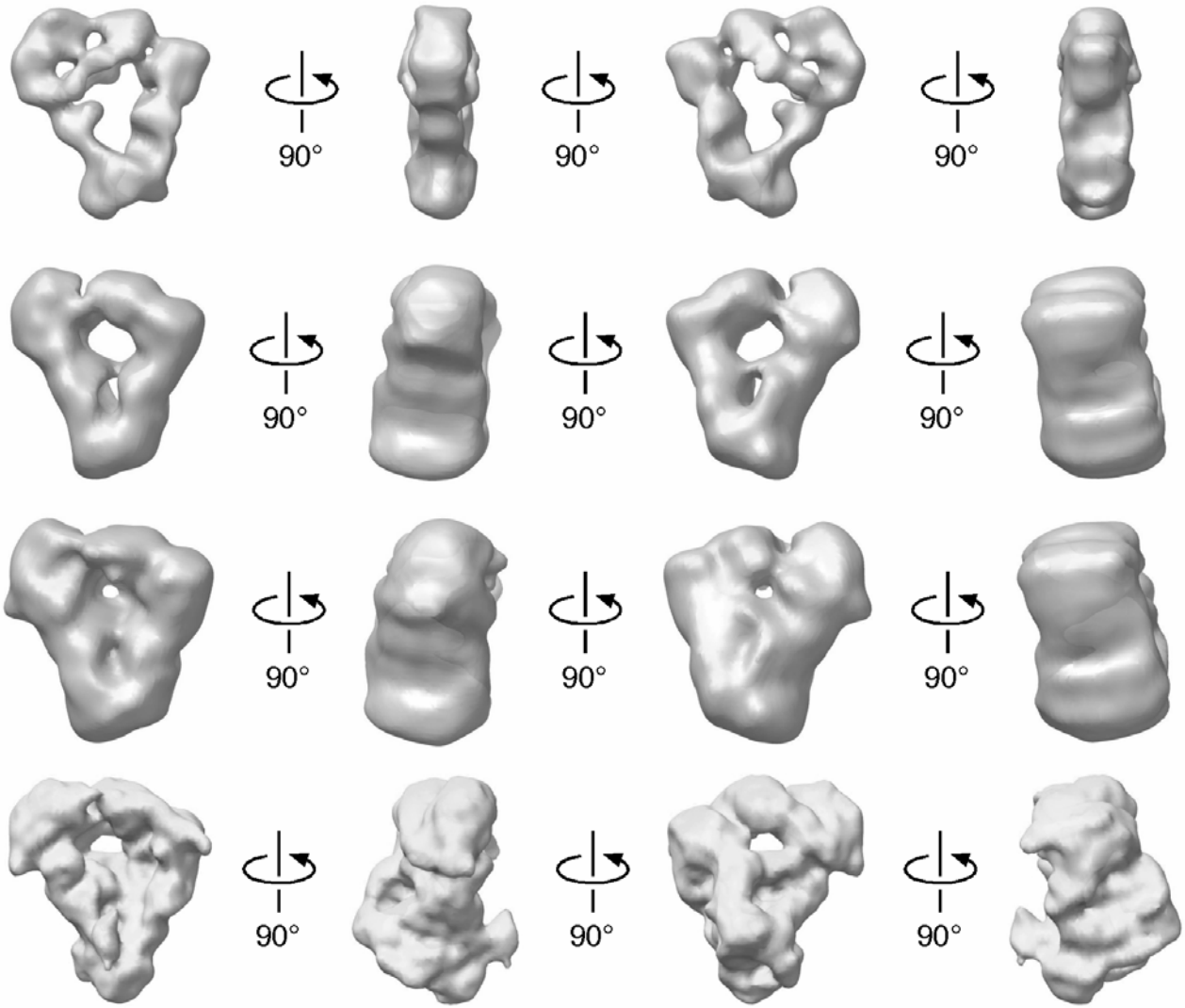
Model refinement - APC

RCT reconstruction in negative stain

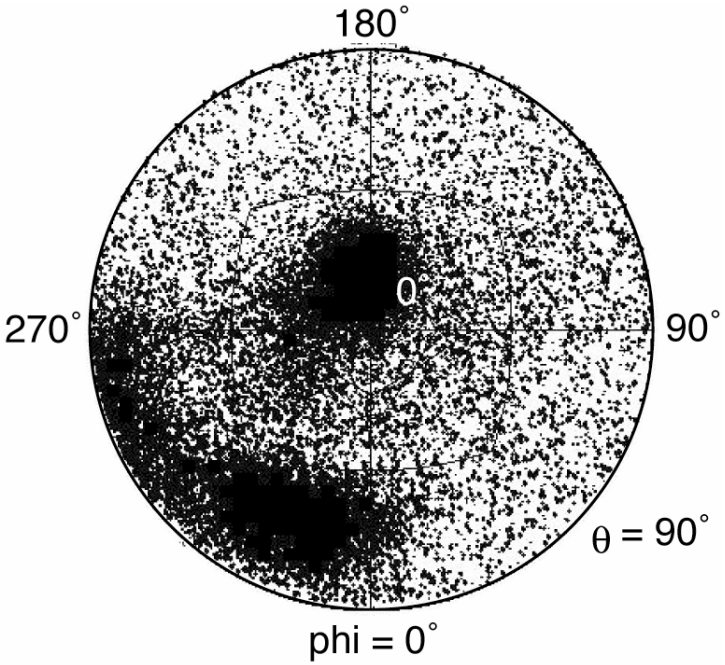
Reconstruction by aligning raw images to RCT map in negative stain

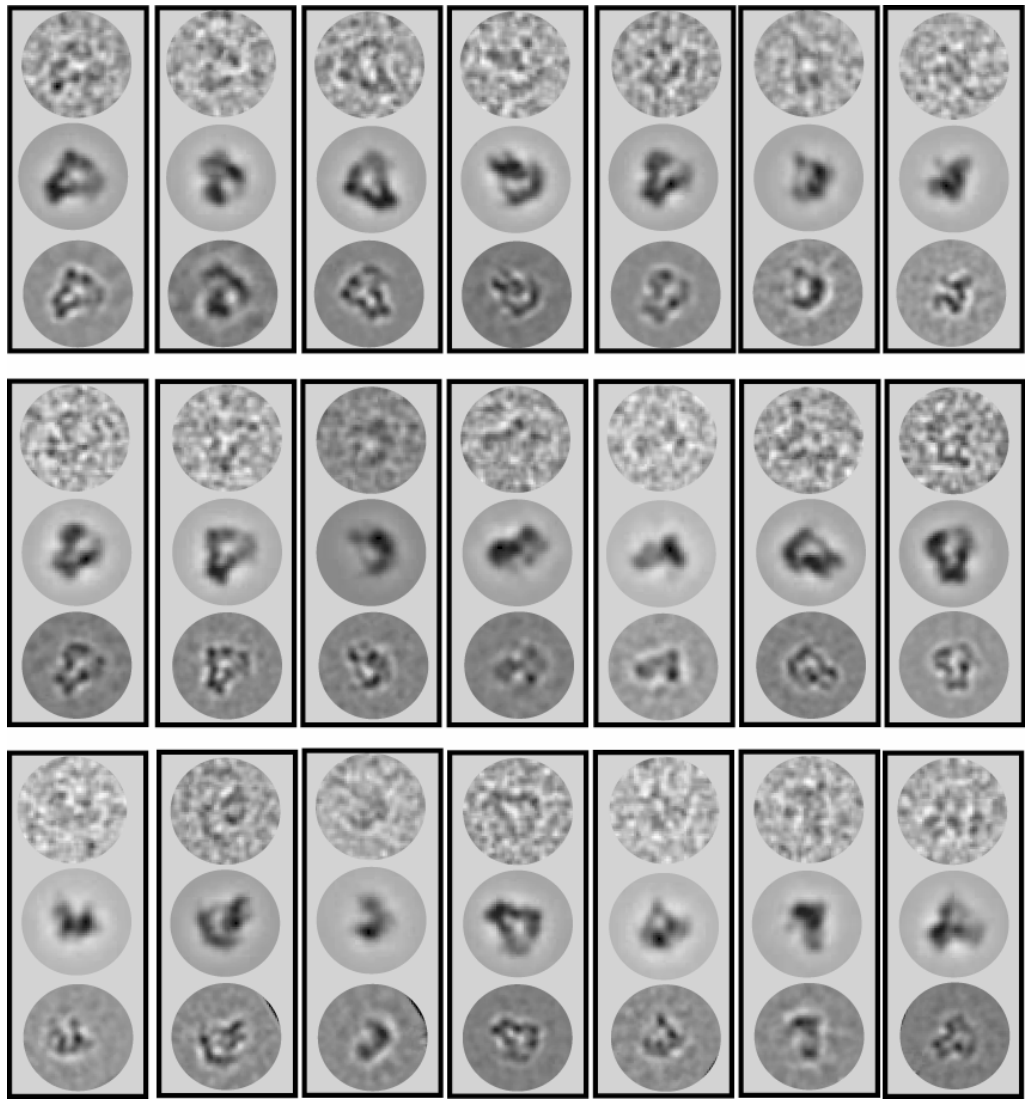
Reconstruction by aligning class averages to RCT map in negative stain

Reconstruction by aligning ice data to RCT map in cryo-negative stain



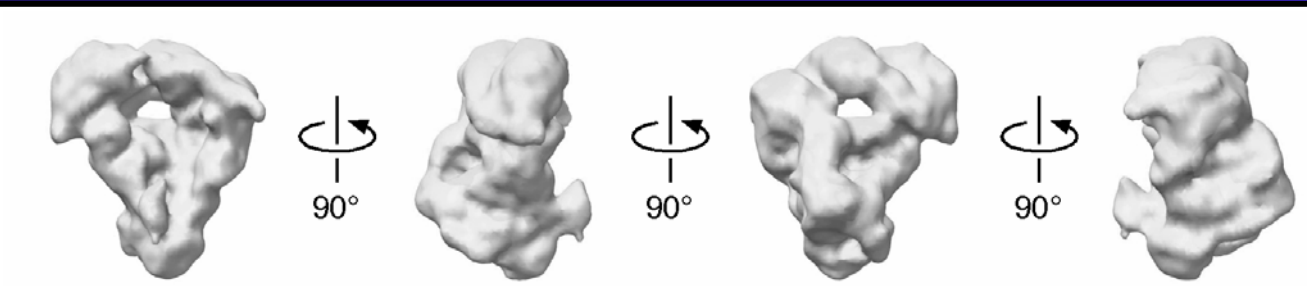
Model verification - APC



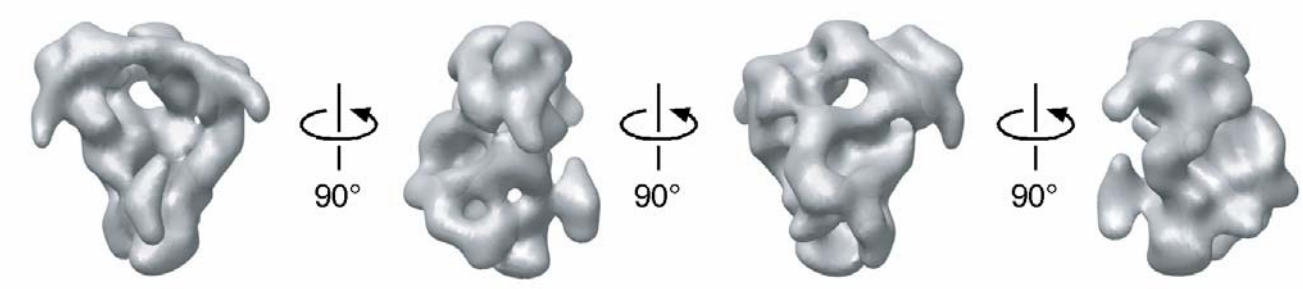


Model verification - APC

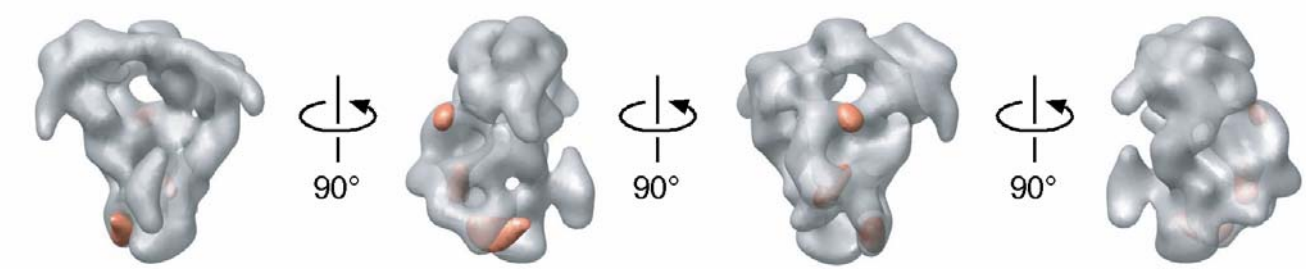
Reconstruction
of vitrified ice data
using CNS RCT
as reference model



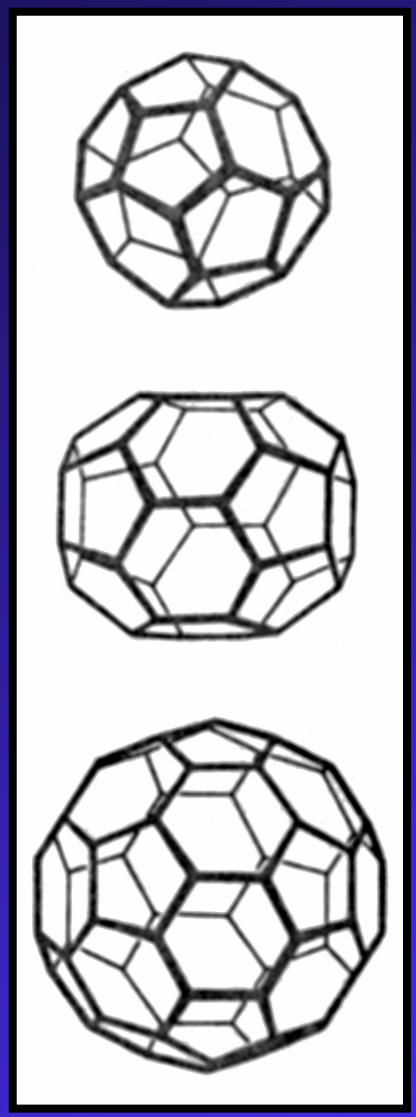
Reconstruction
of vitrified ice data
using OP command

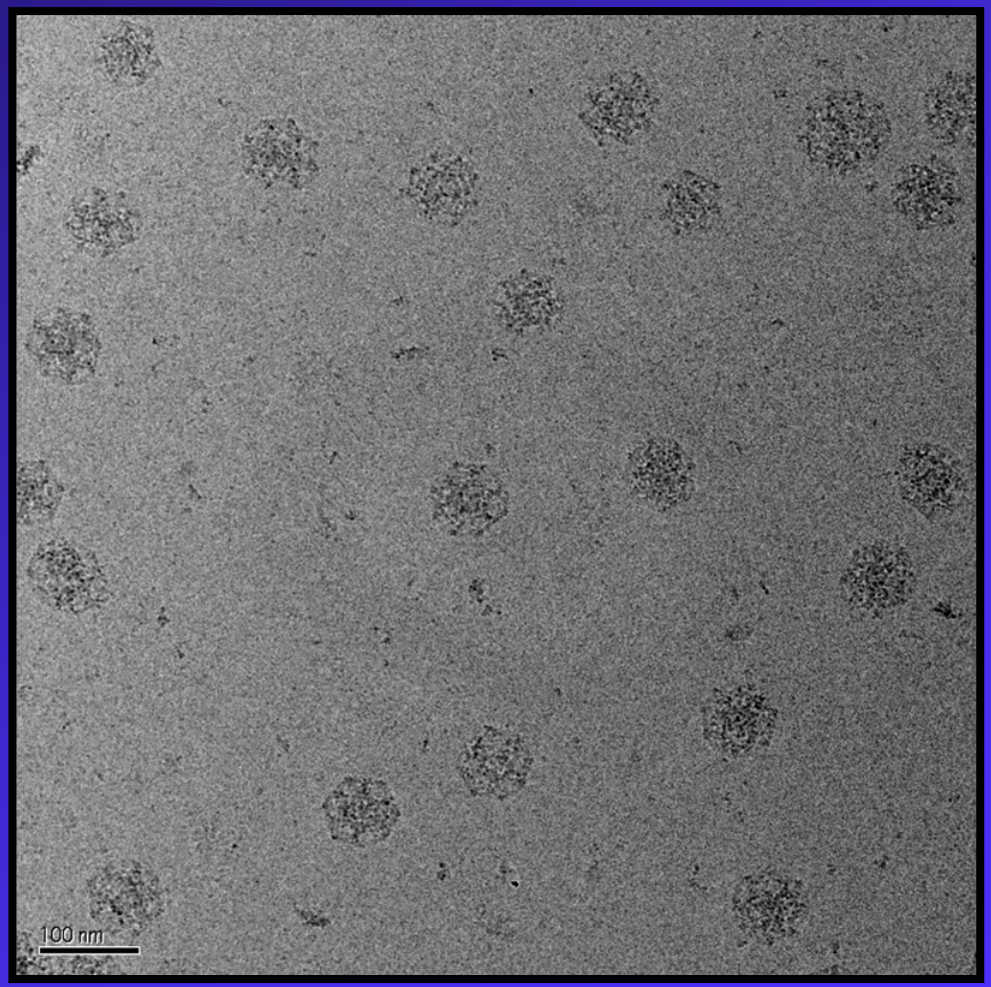


3D variance map
Penczek *et al.*, 2006



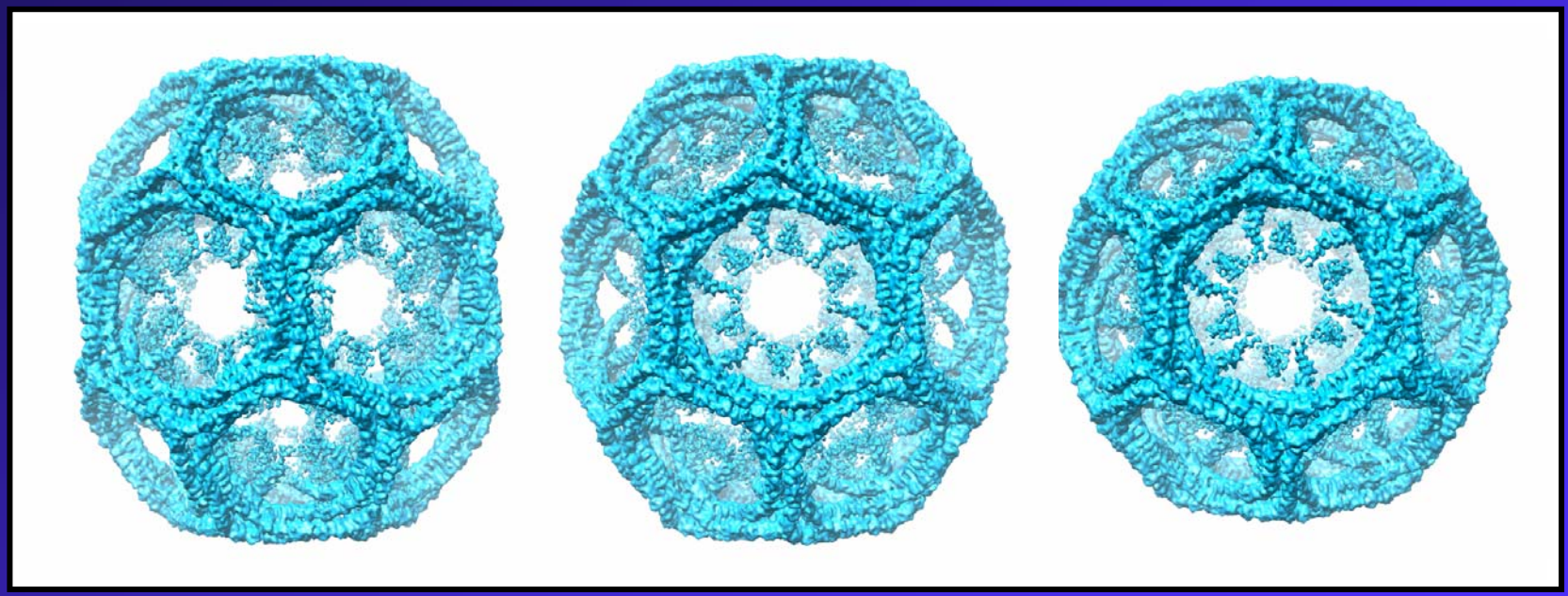
Model verification - Clathrin cages



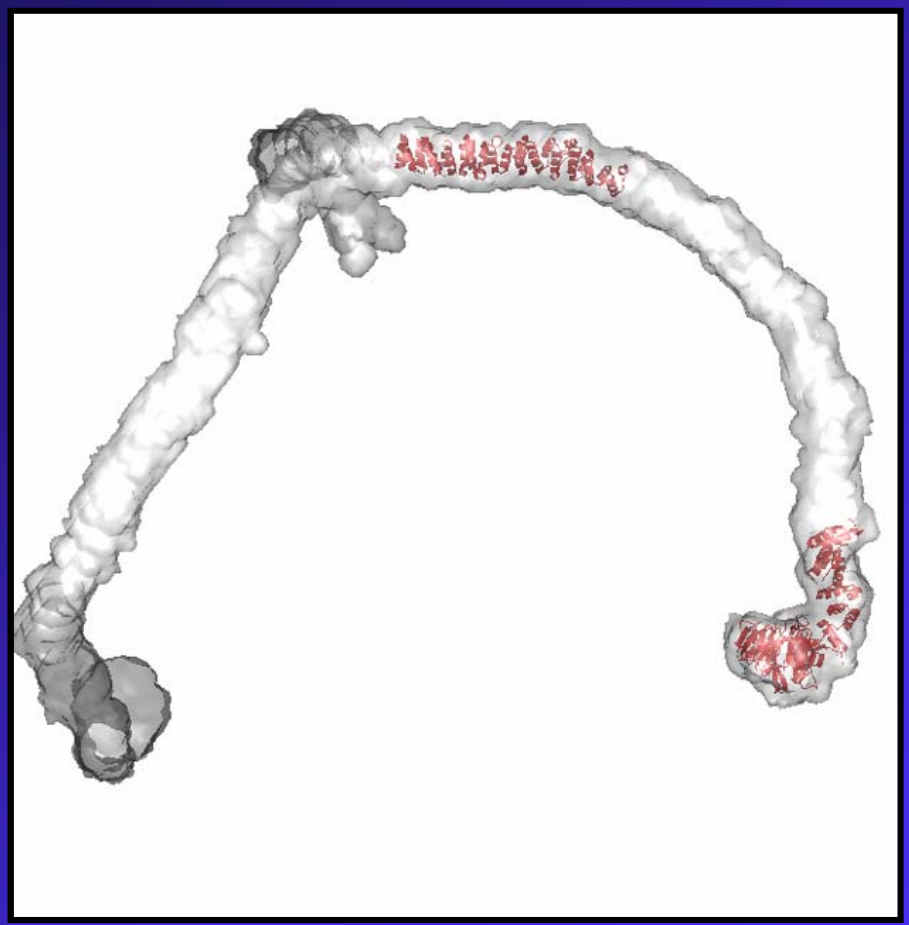


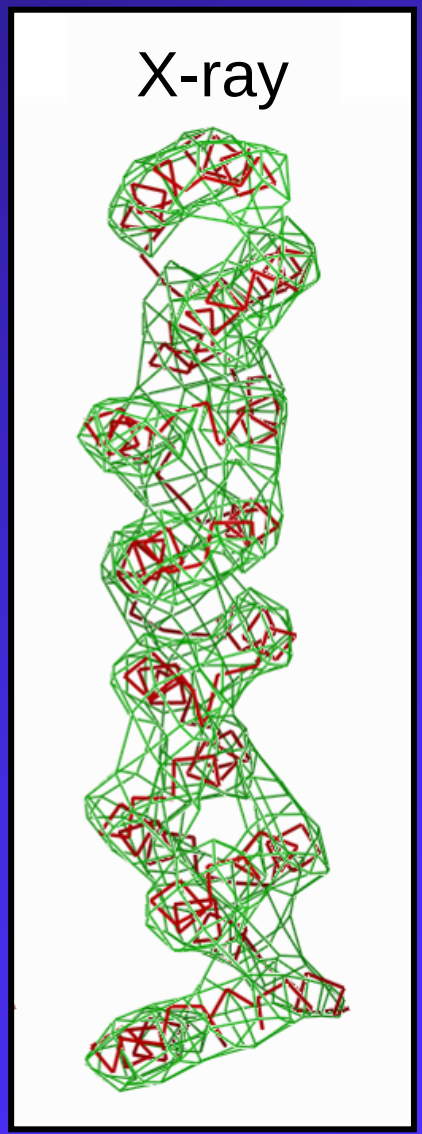
Clathrin cages in vitrified ice

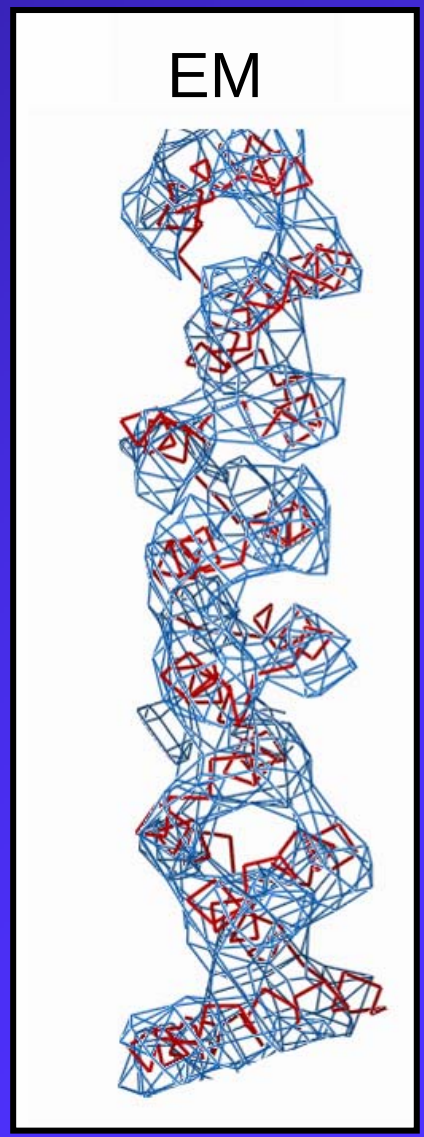
Model verification - Clathrin cages



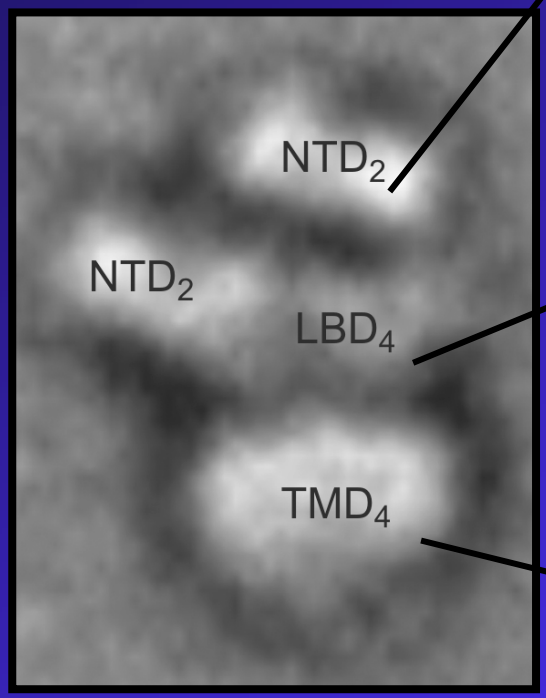
Model verification - Clathrin cages



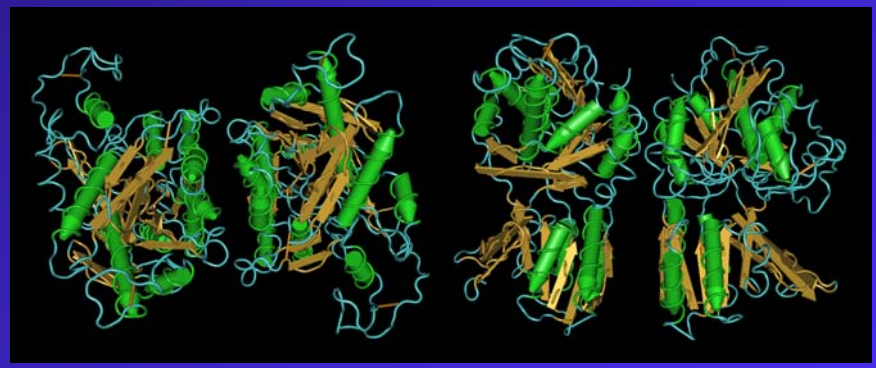




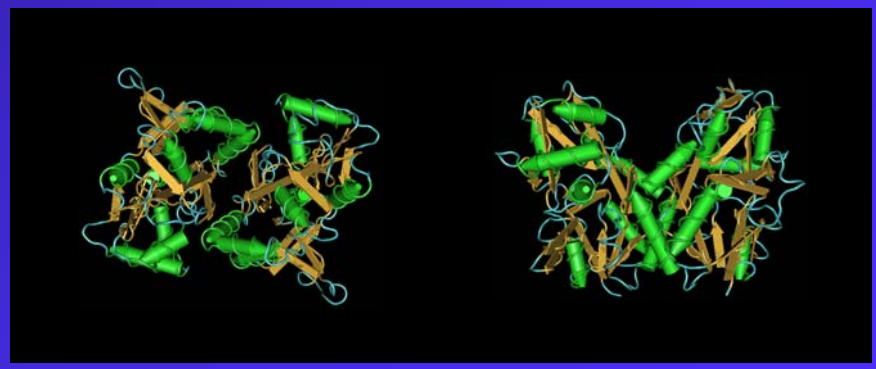
Model verification - AMPA receptor



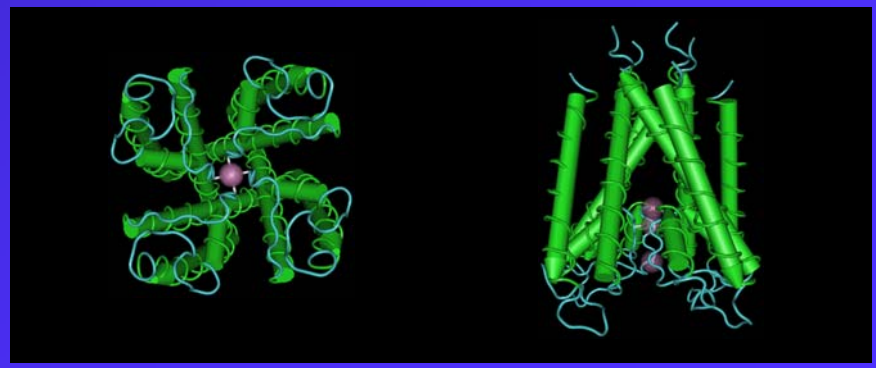
mGluR1
Kunishima et al. 2000
(K. Morikawa)



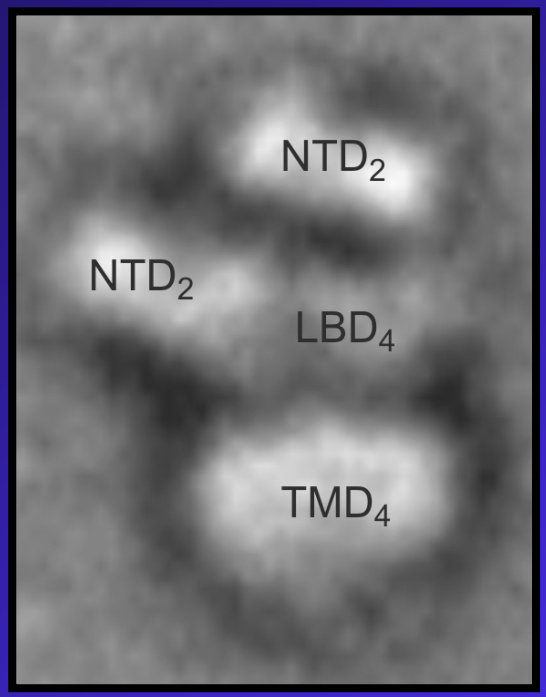
GluR2
Armstrong et al. 2000
(E. Gouaux)

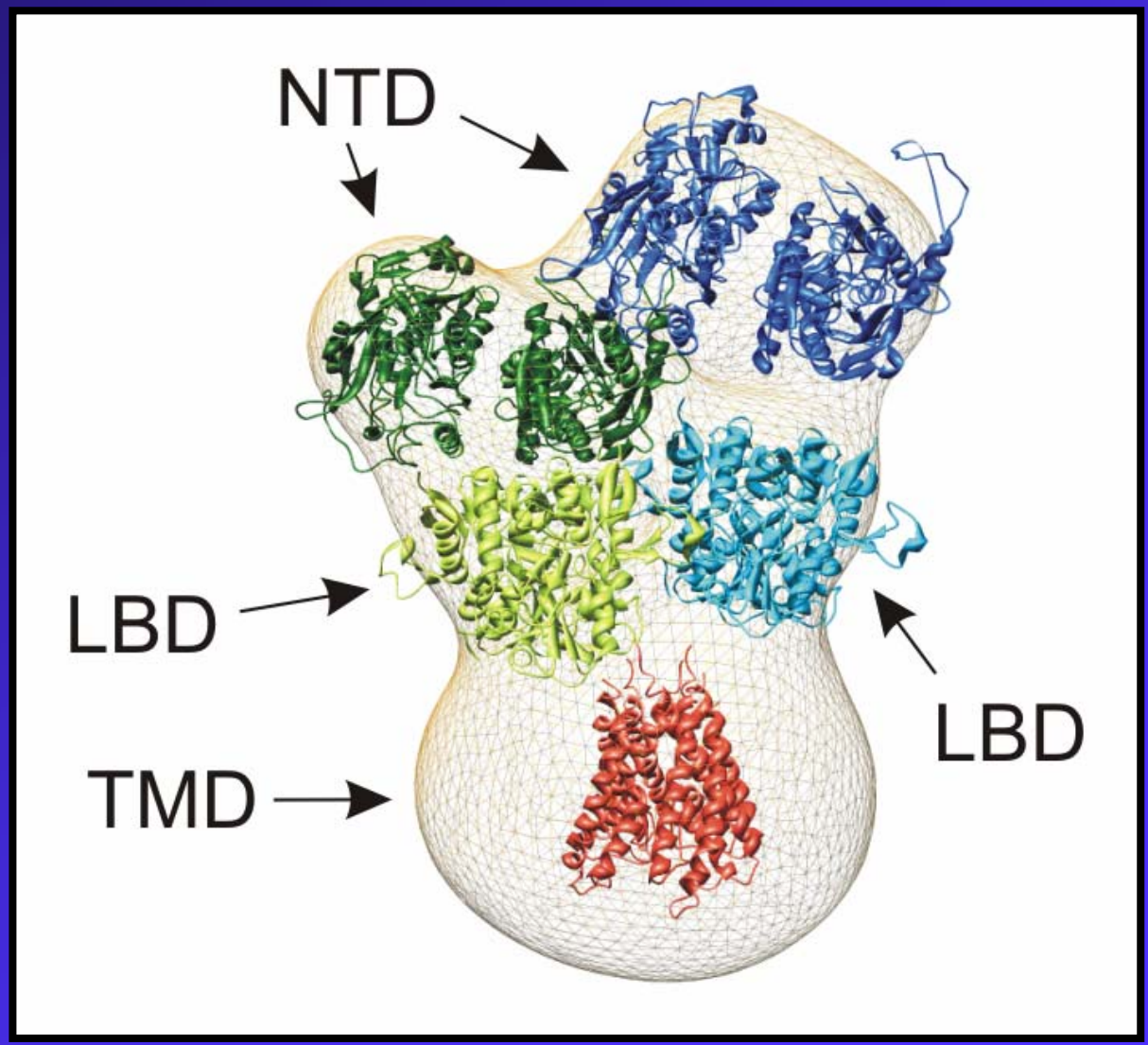


KcsA
Doyle et al. 1998
(R. MacKinnon)

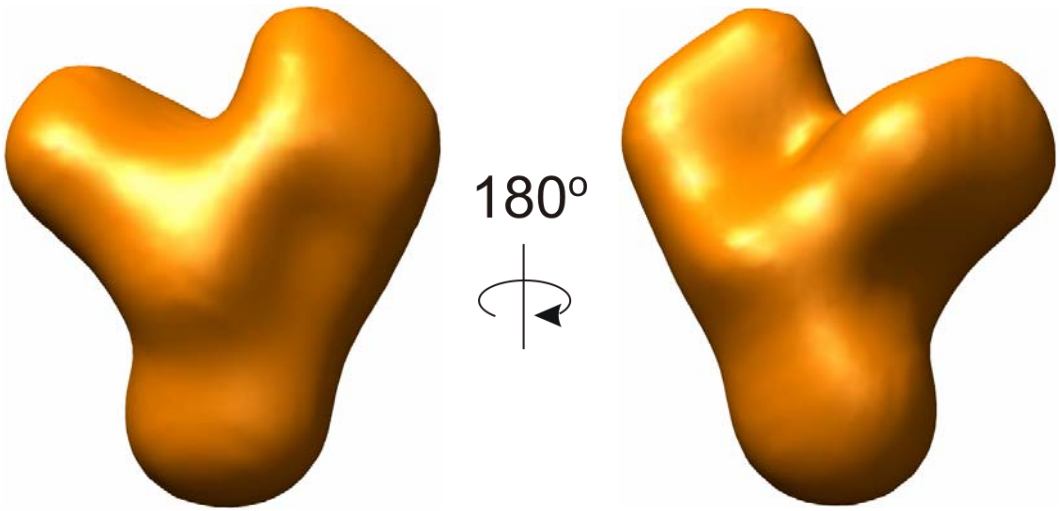


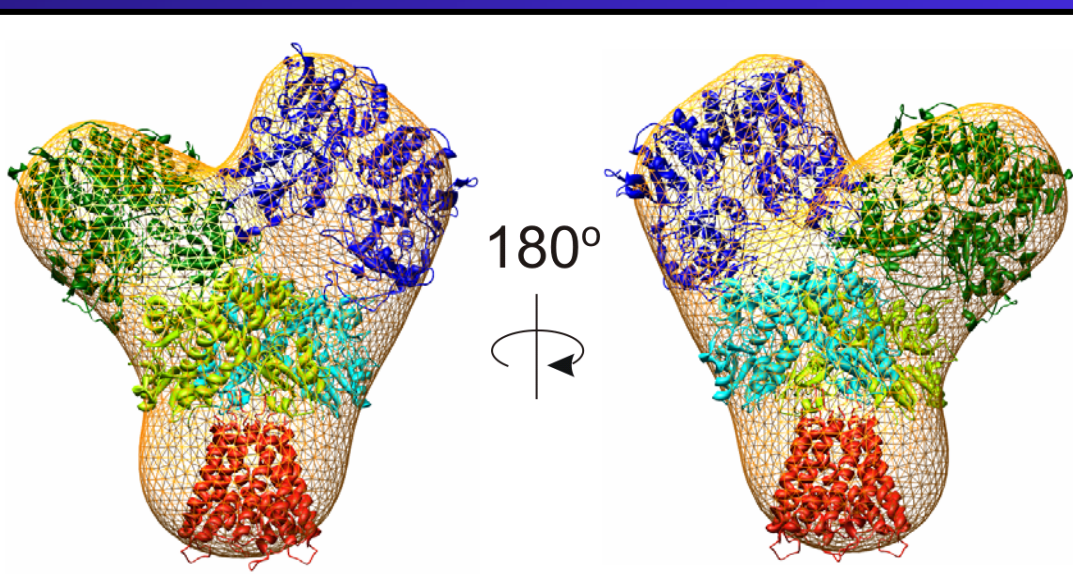
Model verification - AMPA receptor

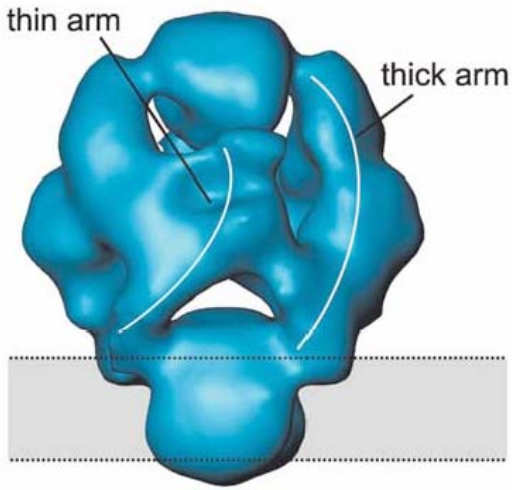


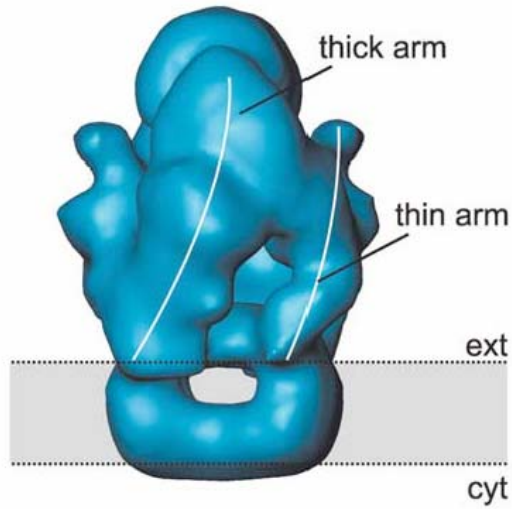


Model verification - AMPA receptor



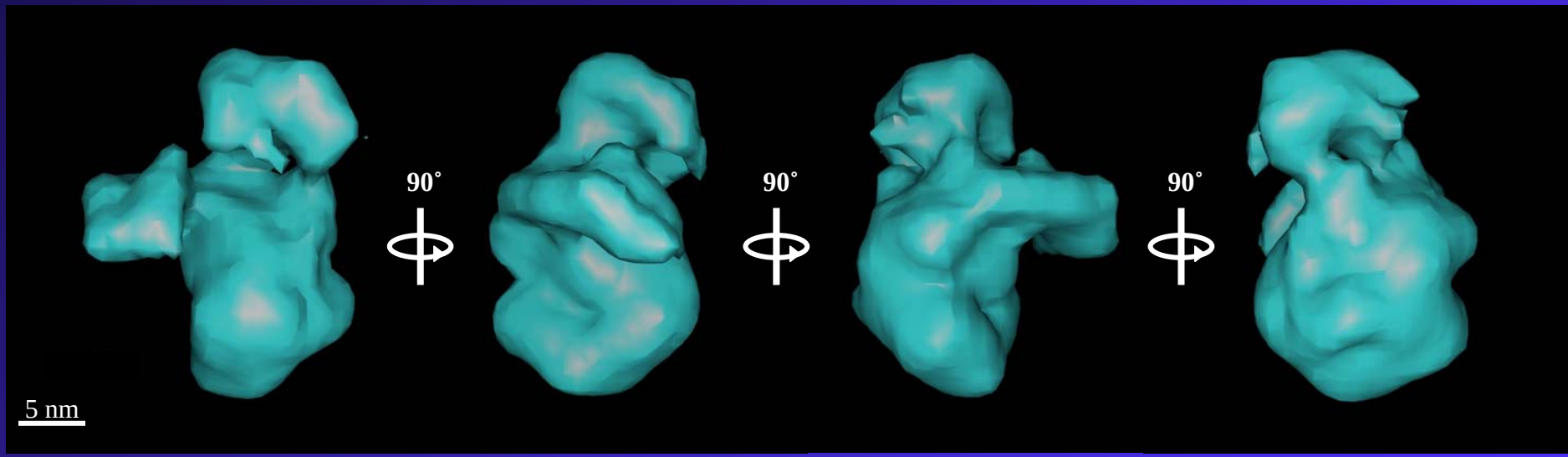




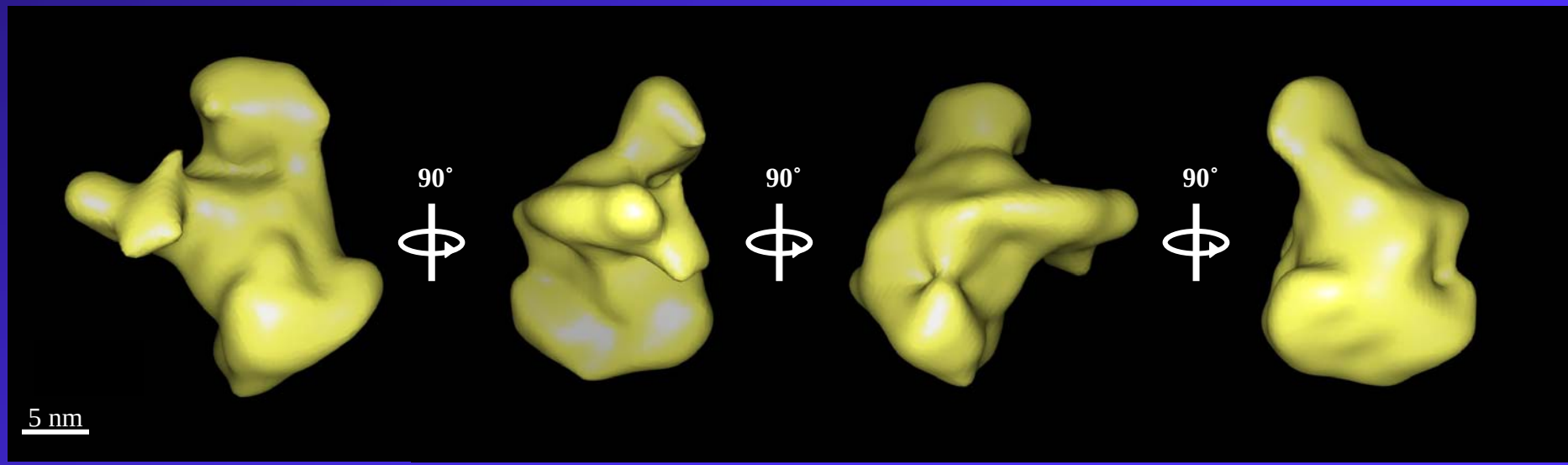


Model verification - Spliceosome

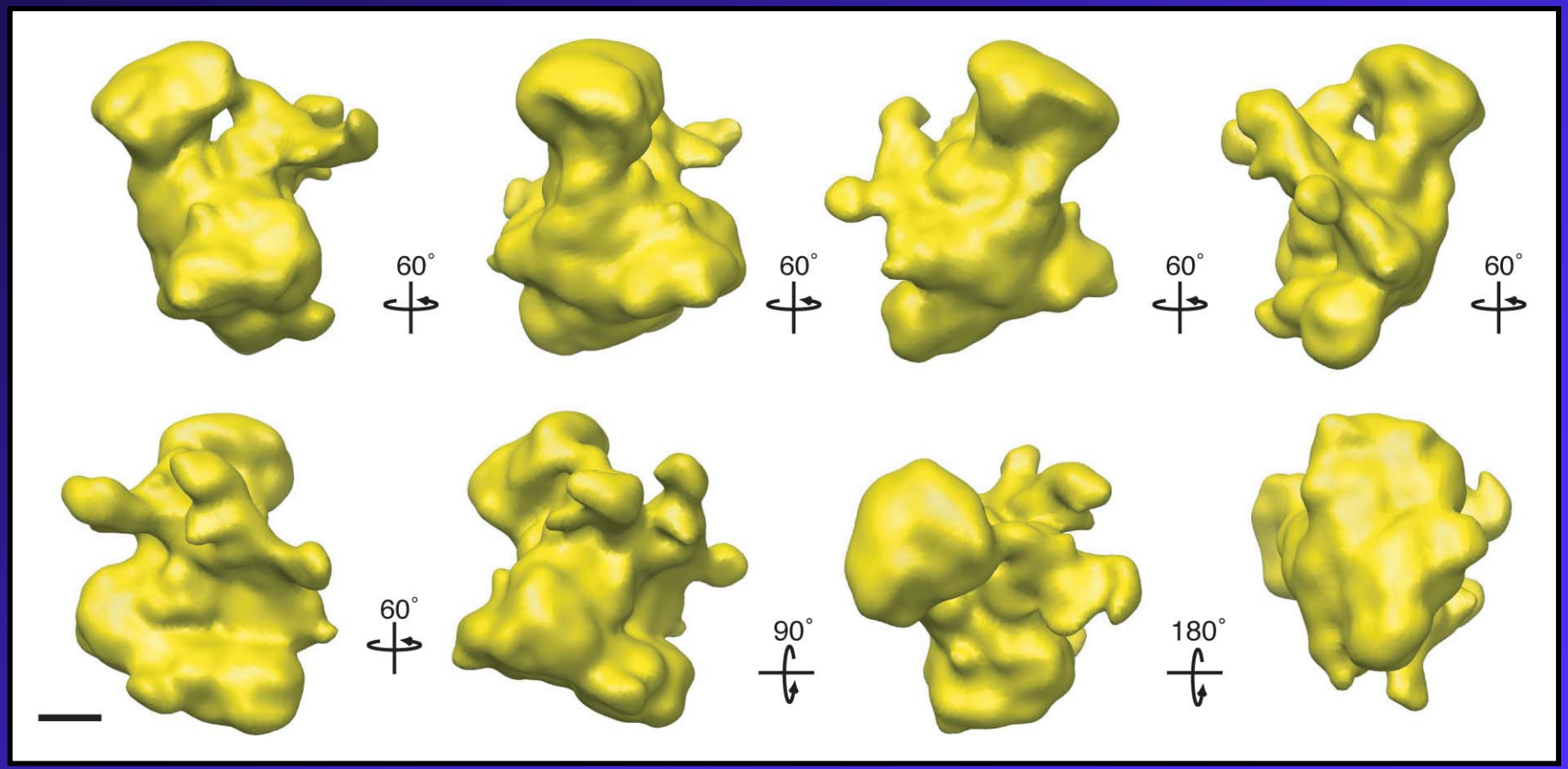
Mammalian C complex (Jurica *et al.*, 2004)



S. pombe Cdc5p complex

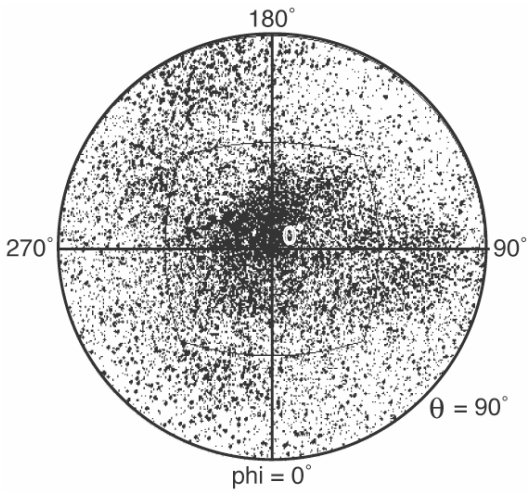


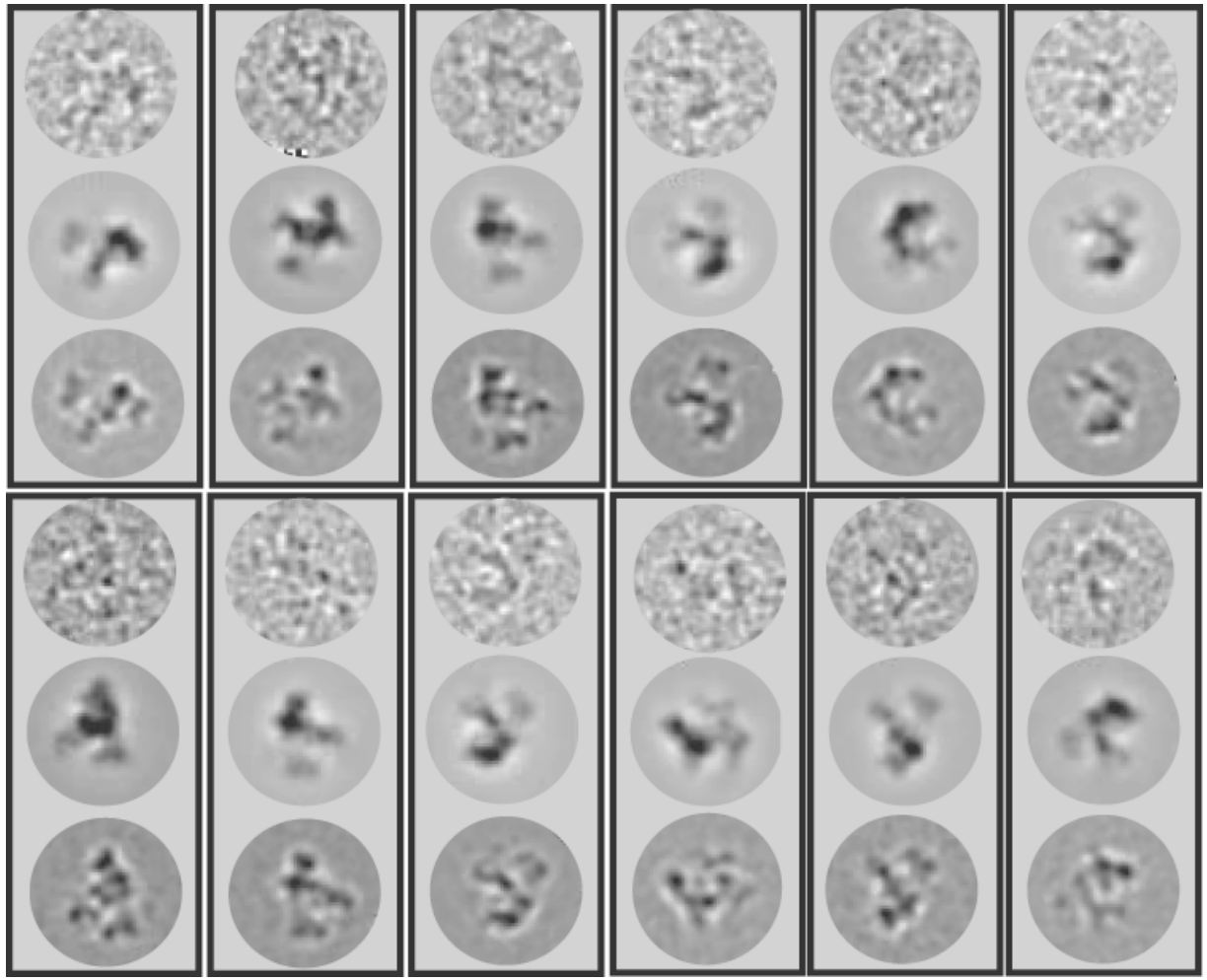
Model verification - Spliceosome



S. pombe Cdc5p complex in vitrified ice
using cryo-negative stain reconstruction as initial model

Model verification - Spliceosome





Conclusions

Never just believe your initial model !

There is currently no general way to generate a reliable initial model

Calculate random conical tilt reconstructions of (cryo-)negatively stained specimens !

The density map is probably distorted, but it is a good basis to interpret subsequent reconstructions

Use your “biological intelligence” !

Some Literature

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Radermacher, M., Wagenknecht, T., Verschoor, A. and Frank, J. (1987) Three-dimensional reconstruction from a single-exposure, random conical tilt series applied to the 50S ribosomal subunit of Escherichia coli. *J. Microsc.* 146: 113-136

Van Heel, M. (1987) Angular reconstitution: a posteriori assignment of projection directions for 3D reconstruction. *Ultramicroscopy* 21: 111-123

Serysheva, I. I., Orlova, E. V., Chiu, W., Sherman, M. B., Hamilton, S. L. and van Heel, M. (1995) Electron cryomicroscopy and angular reconstitution used to visualize the skeletal muscle calcium release channel. *Nat. Struct. Biol.* 2: 18-24

Penczek, P. A., Zhu, J. and Frank, J. (1996) A common-lines based method for determining orientations for N > 3 particle projections simultaneously. *Ultramicroscopy* 63: 205-218