# From x-ray crystallography to electron microscopy and back -- how best to exploit the continuum of structure-determination methods now available

Scripps EM course, November 14, 2007

What aspects of contemporary x-ray crystallography have made it a particularly powerful tool in structural biology?

- Molecular replacement: the body of pre-existing structural knowledge simplifies a new structure determination

- Density modification: elimination of noise by imposition of “reality criteria” in direct space

- Refinement: constraints enable you to incorporate chemical “reality criteria”

1. Phasing x-ray data from EM (TBSV; reovirus core)
2. Phasing electron diffraction data from coordinates derived from x-ray crystallography (aquaporin)
3. Docking an x-ray structure into an EM map (clathrin coat)

4. Lessons from x-ray crystallography for single-particle EM

# X-ray crystallographic structure determination

1. Experimental phases $\rightarrow$ map $\rightarrow$ (modified map) $\rightarrow$ build model

   Experimental phases are poor; density modification is useful whenever possible.

   Building rarely produces complete or fully correct model:
   model $\rightarrow$ refine $\rightarrow$ rephase $\rightarrow$ rebuild and extend model
   $\rightarrow$ refine $\rightarrow$ (cycle)

2. MR phases $\rightarrow$ map and MR model $\rightarrow$ rebuild or extend model
   $\rightarrow$ refine $\rightarrow$ (cycle)

   Map is strongly biased, so it is *much* better to modify map based on solvent flattening or ncs, then continue with rebuilding and extending

Examples:  phases from EM map
as MR “model”, density modification
from non-crystallographic symmetry
(icosahedral: 5-fold in these two cases)

TBSV: negative stain, 30 Å (1974)
Reovirus: cryo, 30 Å (2000)

*J. Mol. Biol.* (1975) 97, 163–172

# Structure of Tomato Bushy Stunt Virus

## II.† Comparison of Results Obtained by Electron Microscopy and X-ray Diffraction

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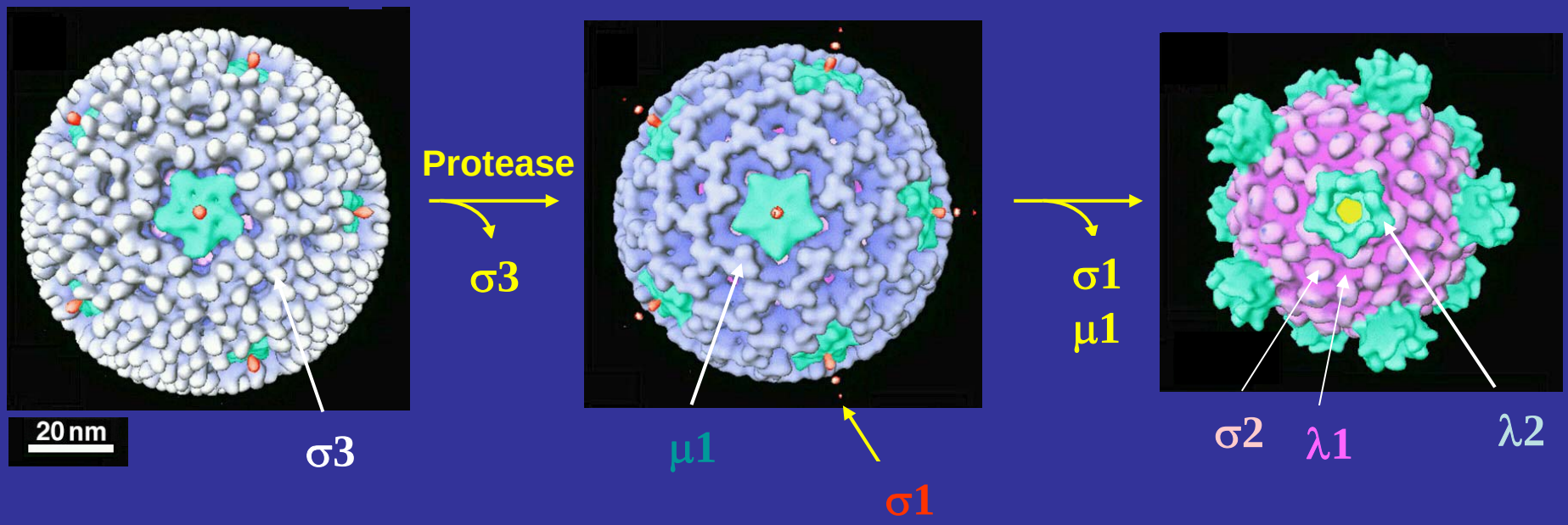
A three-dimensional reconstruction from electron micrographs of tomato bushy stunt virus has been used to determine X-ray phases to 28 Å resolution, by analogy with the single isomorphous replacement method of protein crystallography. An electron density map computed from X-ray amplitudes and these phases differs in two important respects from the electron micrograph reconstruction. The exclusion of stain from the 5-fold vertices, previously attributed to the presence of a minor protein, is shown to be an artifact of staining. The other difference involves positive staining of the RNA at the quasi-3-fold positions.




**Virion**

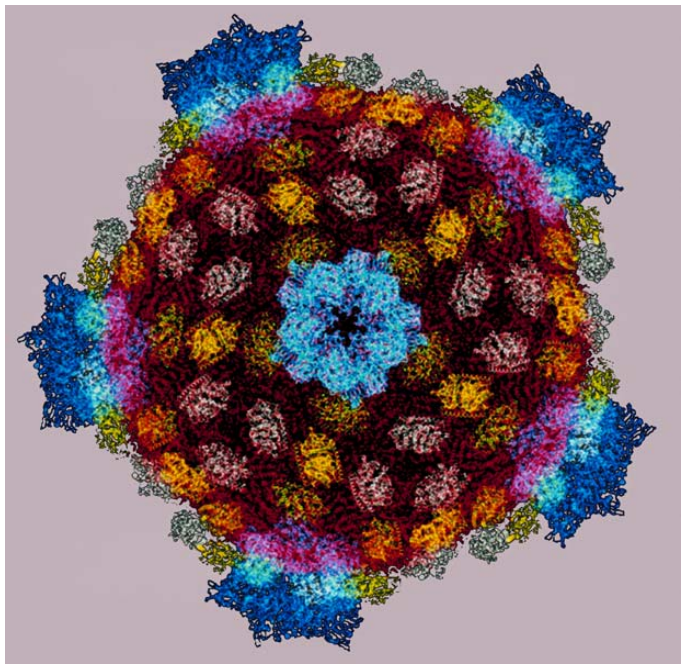
**ISVP**
infectious or intermediate subviral particle

**Core**

Dryden, Baker *et al*. (1993).

Crystals of reovirus cores

F432, a= 1255 Å
Initial phases to 30 Å from modified EM density
Phase extension by averaging



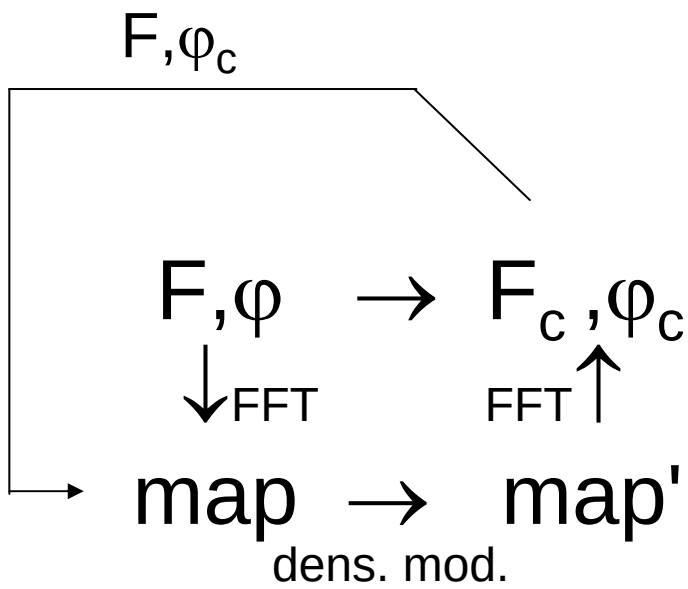
# Averaging as basis for phase extension in x-ray crystallography

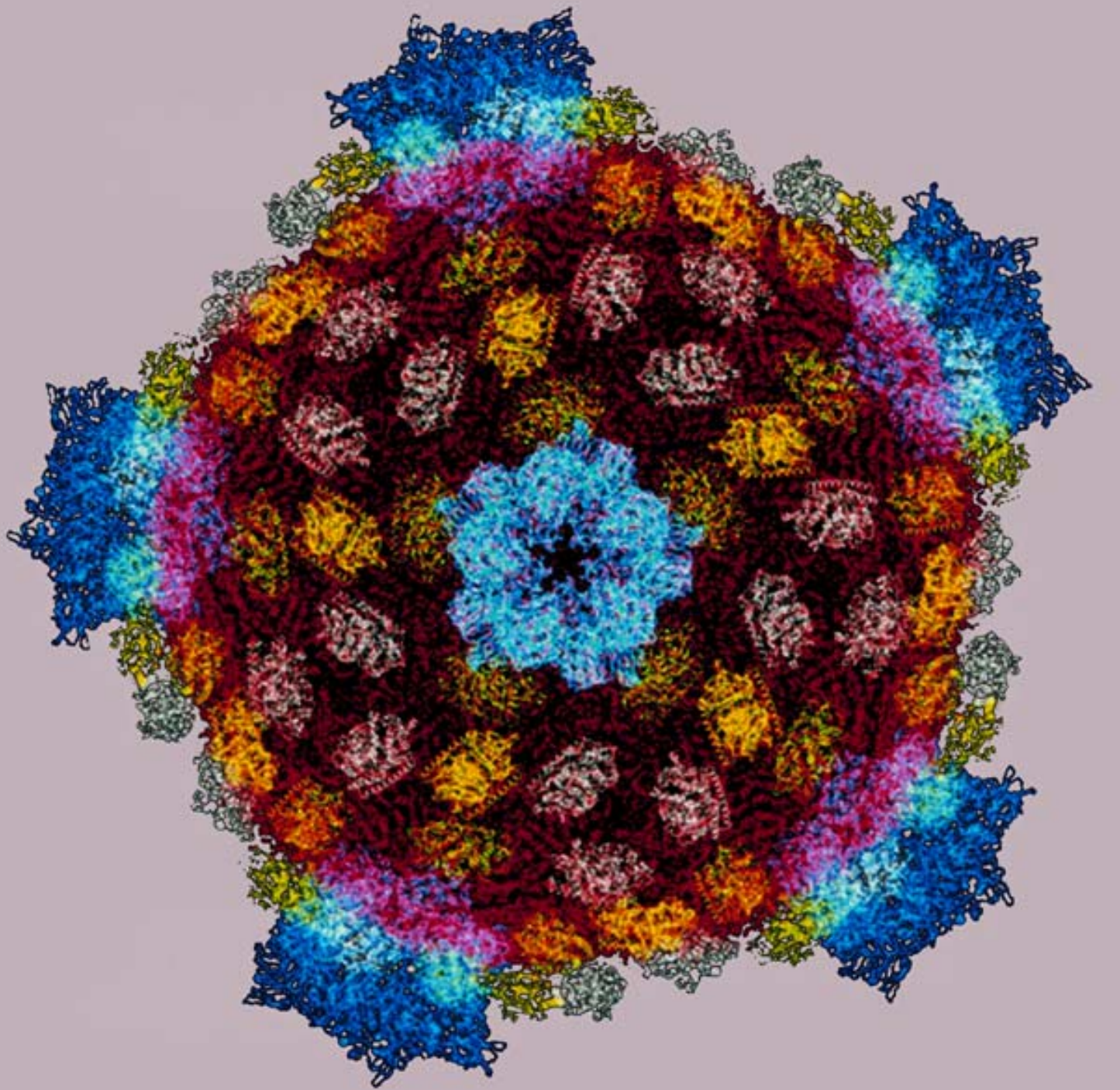
Map $\rightarrow$ Mask, average, and reconstitute $\rightarrow$ SFs

F's and $\varphi$'s

Works because true a.u. is smaller than crystallographic a.u., transform is effectively oversampled

# Non-cryst. symmetry averaging and solvent flattening





# Aquaporin-0 (AQP0):

Molecular replacement with MOLREP, monomer as model
Must refine unit cell (grid search)

Refinement with CNS

1. Rigid body with unit-cell variation
2. Simulated annealing; rebuild from 2Fo-Fc with solvent flipping maps and SA omit maps to correct

Gonen et al, 2004

# Aquaporin-0 (AQP0):

| | |
|---|---|
| Two-dimensional crystals | |
| Layer group | *p*422 |
| Unit cell (Å) | $a = b = 65.5$ |
| Thickness (assumed) (Å) | 160 |
| Electron diffraction | |
| Number of patterns merged | 286 (0°, 11; 20°, 43; 45°, 107; 60°, 87; 70°, 38) |
| Resolution limit for merging (Å) | 1.7 |
| $R_{Friedel}$ (%) | 14.25 |
| $R_{merge}$ (%) | 16.60 |
| Observed amplitudes to 1.9 Å | 126,980 |
| Unique reflections | 22,293 |
| Maximum tilt angle (°) | 71.3 |
| Fourier space sampled | 80.0% (70.5% at 2.0–1.9 Å) |
| Multiplicity | 5.7 (2.5 at 2.0–1.9 Å) |
| Crystallographic refinement (5.0–1.9 Å) | |
| Resolution limit for refinement (Å) | 1.9 |
| Crystallographic *R* factor (%) | 25.81 |
| Free *R* factor (%) | 29.93 |
| Reflections in working/test set | 14,600/1,580 |
| Non-hydrogen protein atoms | 1,784 |
| Non-hydrogen lipid atoms | 348 |
| Solvent molecules | 76 |
| Average protein *B* factor (Å$^2$) | 48.4 |
| Ramachandran plot (%) | 97.5; 2.5; 0 (allowed; generous; disallowed) |

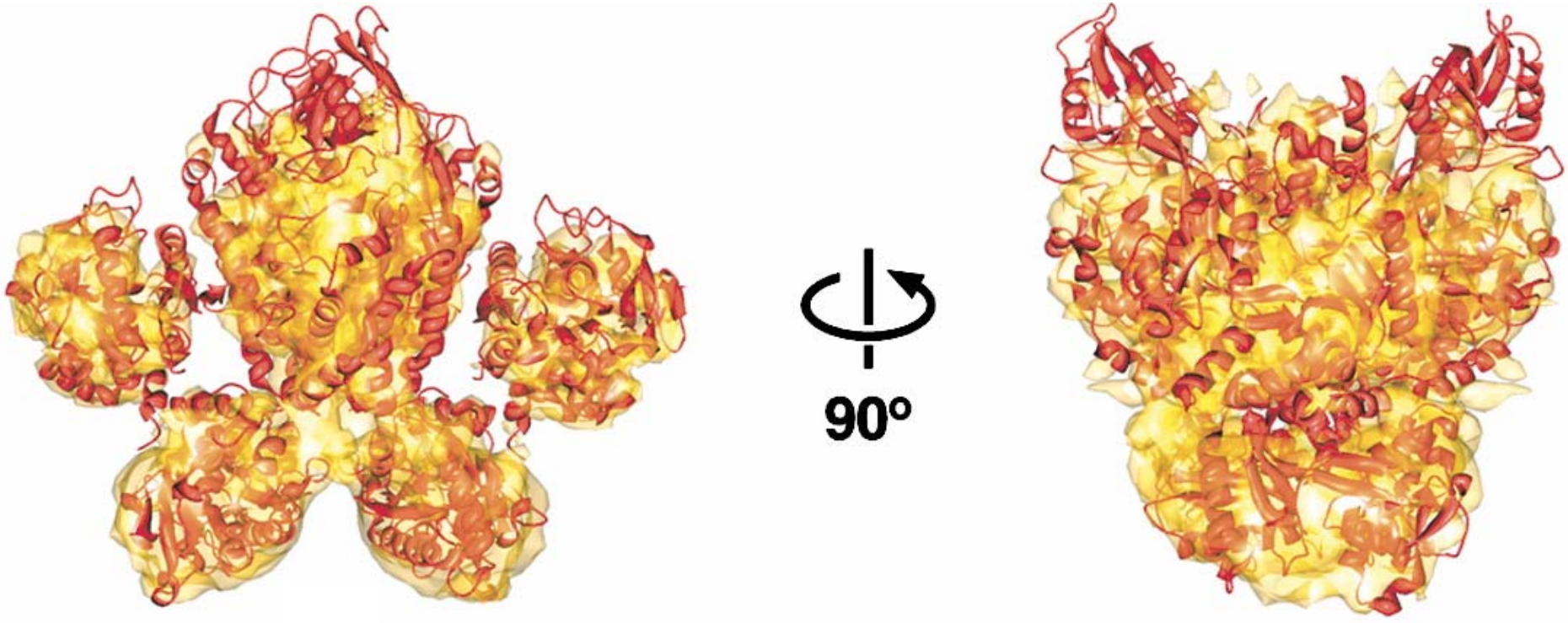
Gonen et al, 2005

Docking a model from x-ray crystallography
(or NMR) into a cryoEM density

Two key resolution barriers: ~ 8-9 Å and ~ 4 Å

Rigid-body refinement vs. more flexible refinement

# Transferrin/TfReceptor




Cheng et al (2004) Cell 116:565-576.

Molecular replacement:

1. Can a molecular model work as an initial reference for single-particle alignment, with appropriate filtering of spatial frequencies?

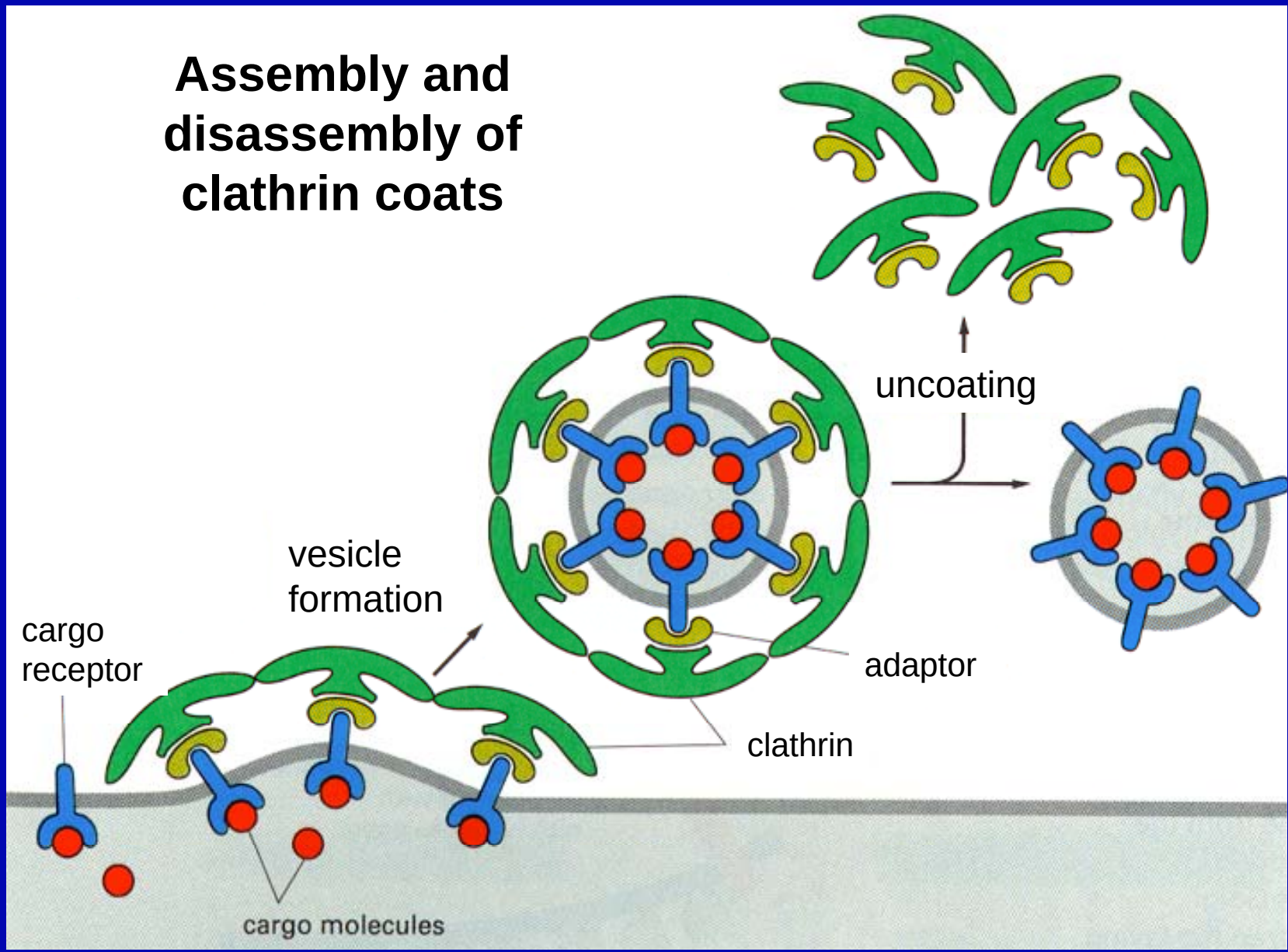
2. How can we best exploit molecular replacement in 2-D crystallography?

Clathrin coat

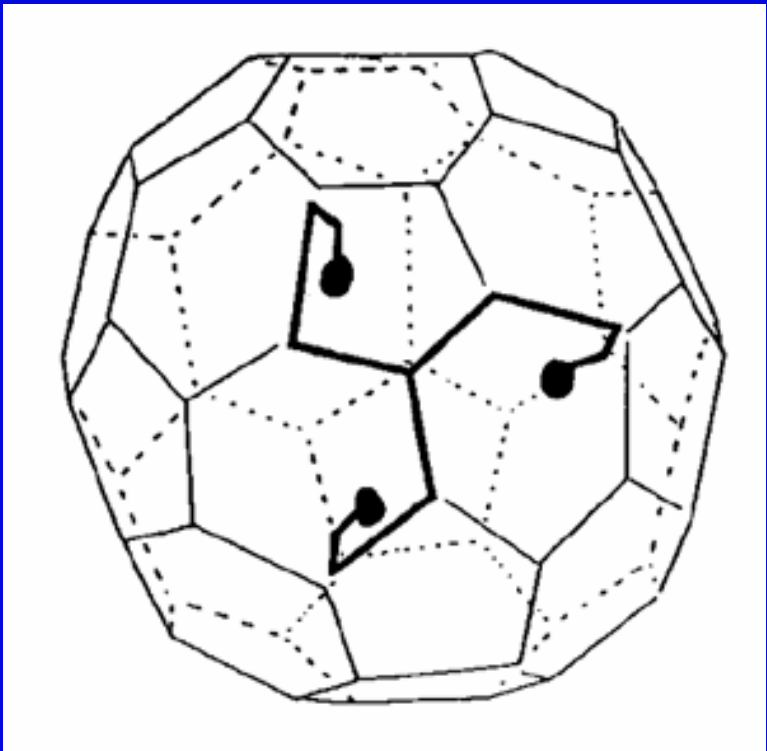
1. Density modification
2. ncs symmetry averaging

Fotin et al, 2004

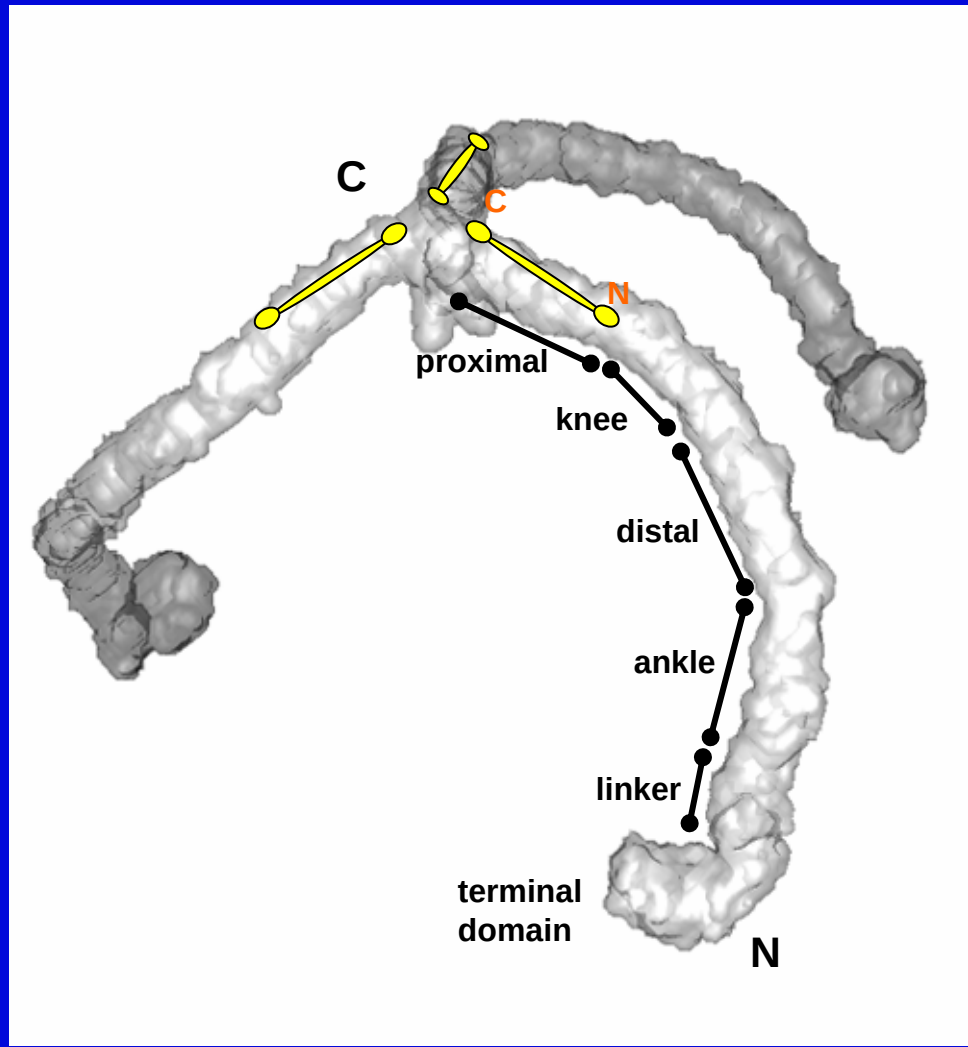
# Assembly and disassembly of clathrin coats



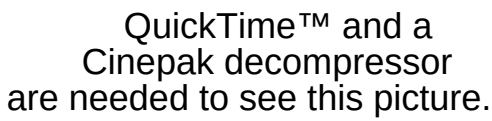
# Anatomy of a clathrin coat



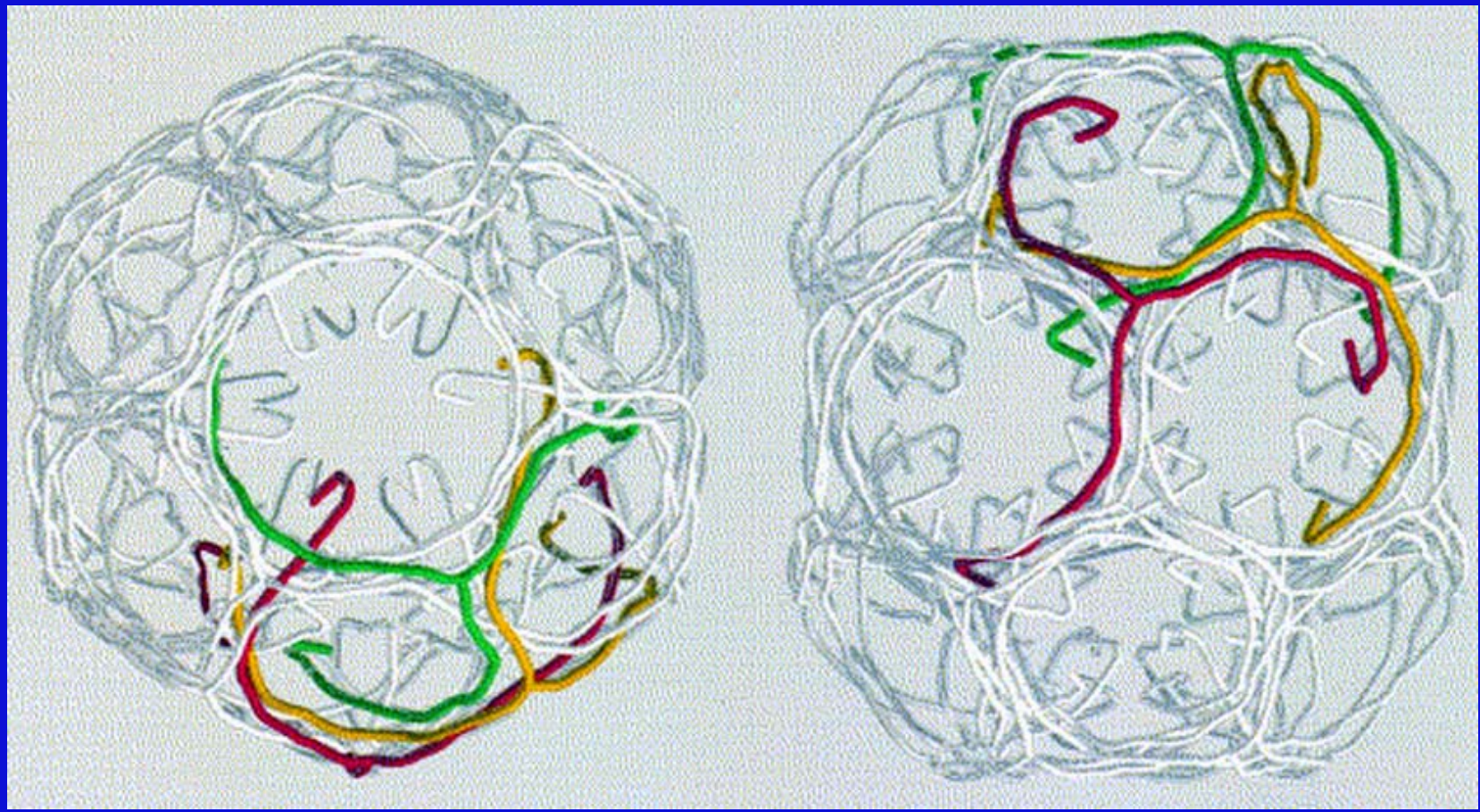
Clathrin lattice




Triskelion = 3 x (Heavy Chain + Light Chain)

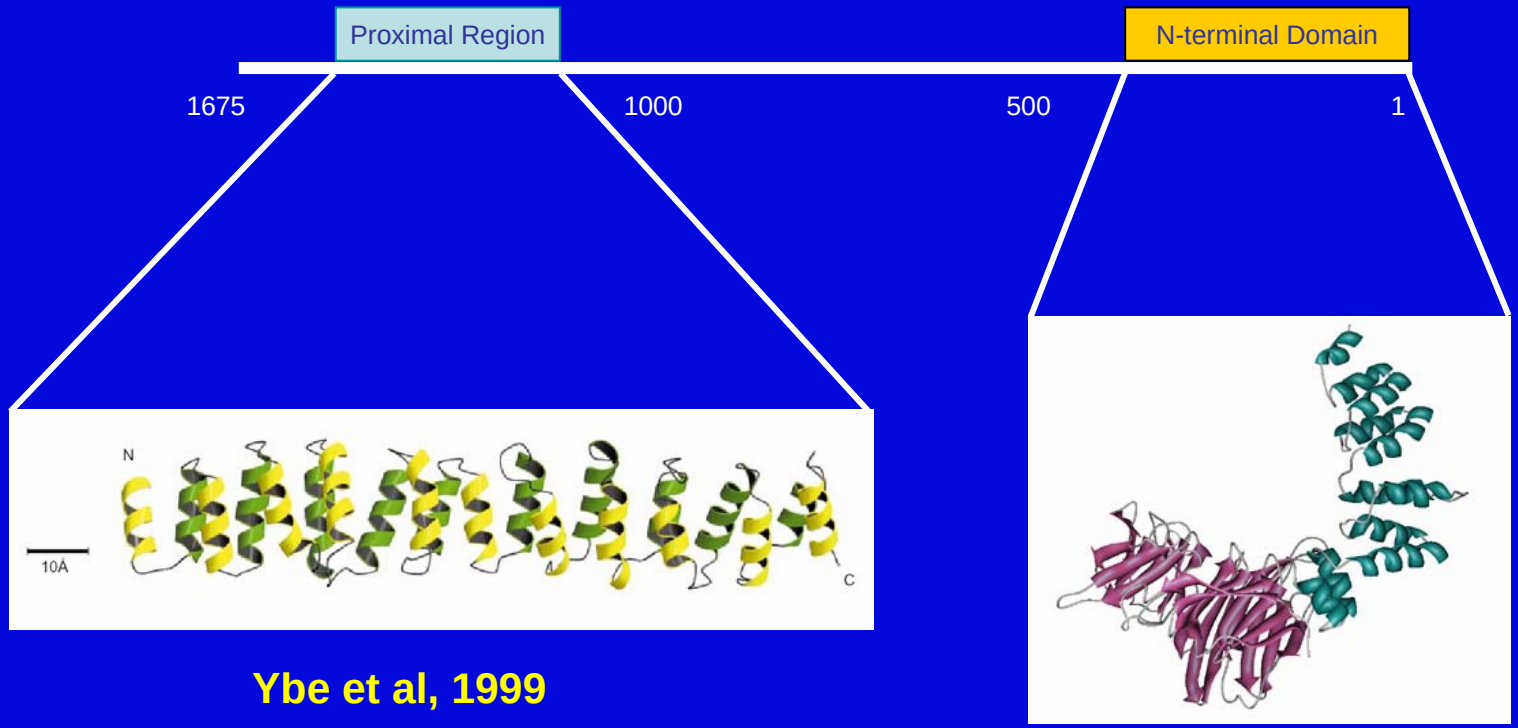

QuickTime™ and a
Cinepak decompressor
are needed to see this picture.

# D6 barrel



Musacchio, Smith, Grigorieff,
Pearse, Kirchhausen

# X-ray structure of clathrin fragments




Ybe et al, 1999

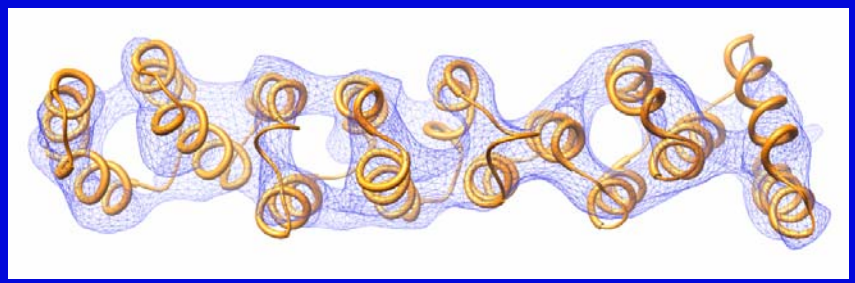
terHaar et al, 1998

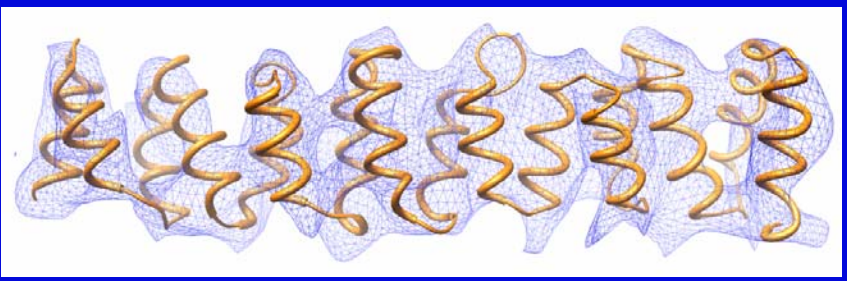
# Comparison of EM and X-ray densities at 7.9 Å

Top View

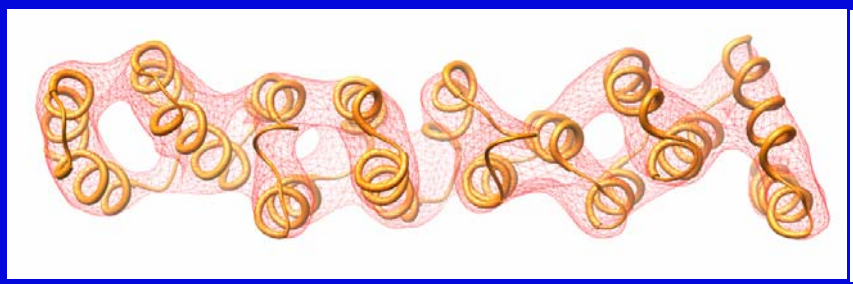
Side View

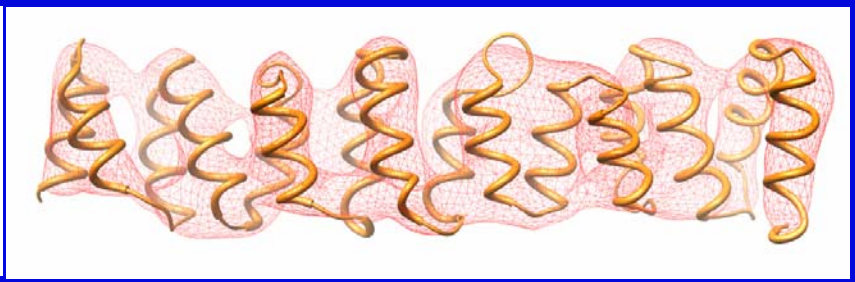
EM



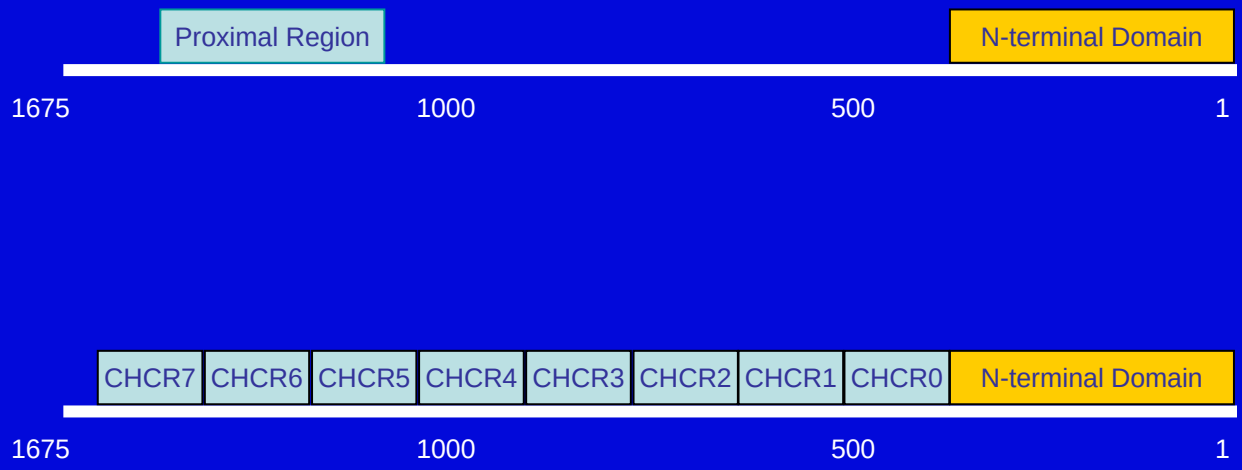


X-ray

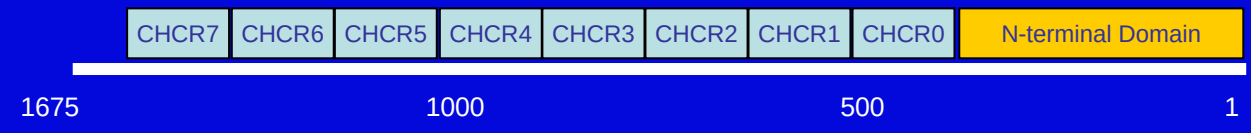


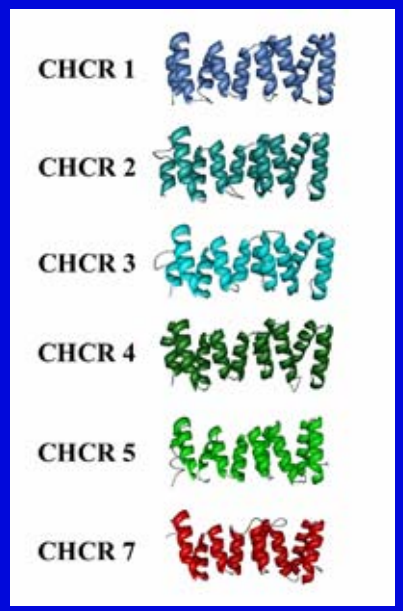


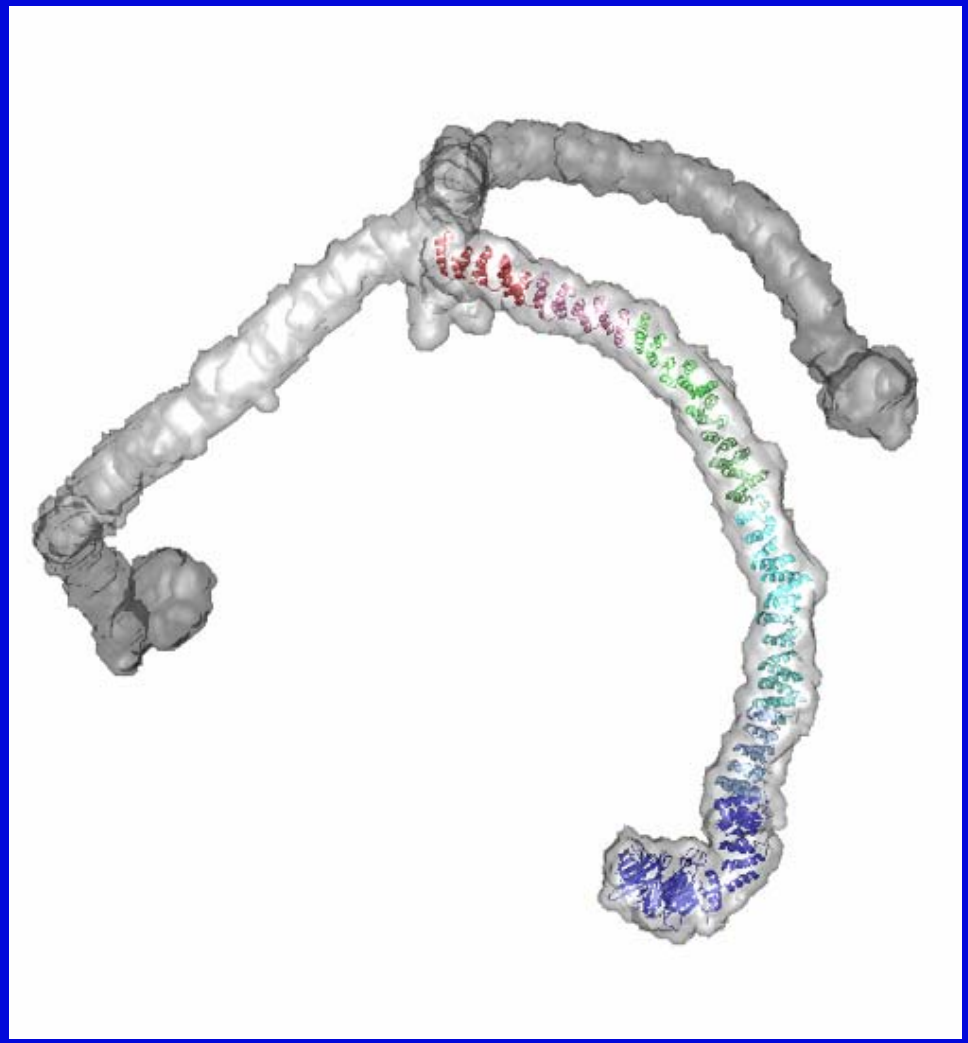
# Clathrin CHCR domain organization



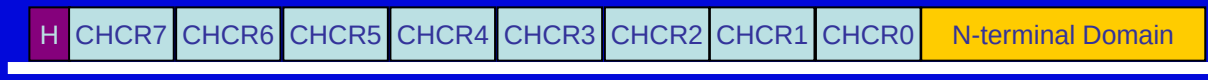
# Modeling structure of the whole leg

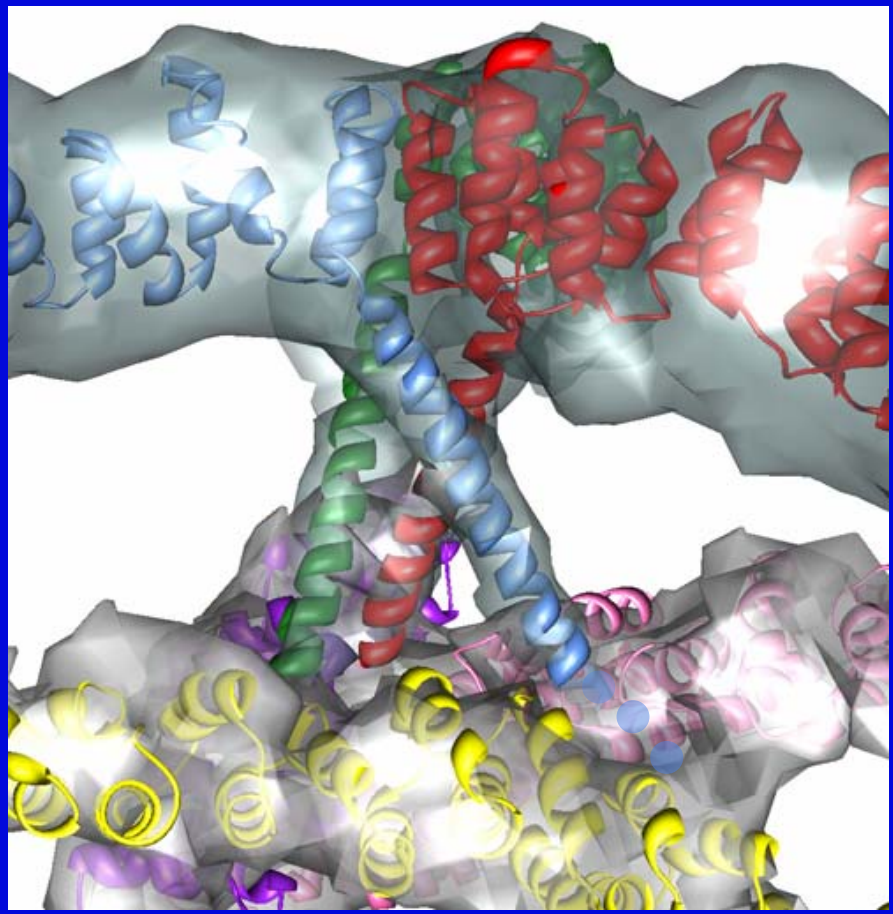


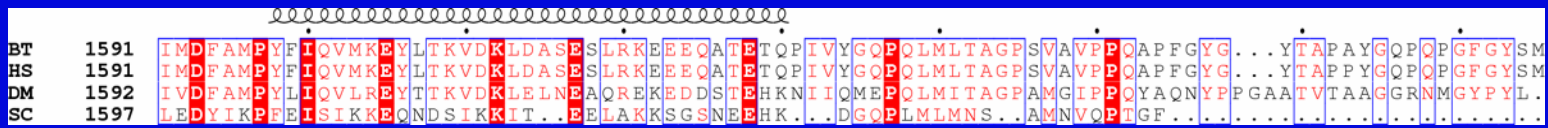



# The helical tripod





Two questions:

1. Can we improve a reconstruction by use of a model built into the density as reference?

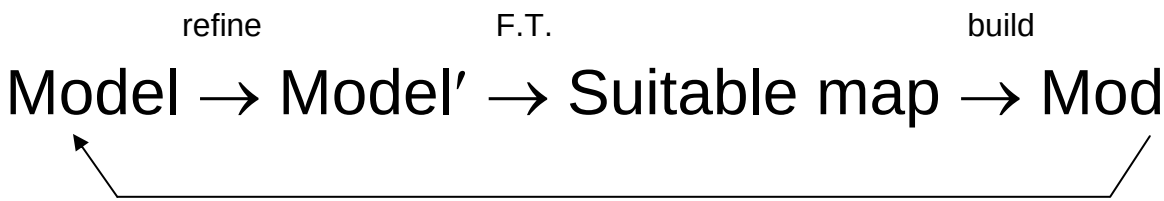
2. Can we refine a model against the observed data (projected images)?

In crystallography, measured amplitudes are, by experimental arrangement, coming from an averaged structure.

In single-particle EM, measured projections contain unique “noise” that will disturb estimate of projection parameters

X-ray: observations are amplitudes; refine model parameters against these observations, using chemistry as a constraint.

If the model is incomplete, use refinement to improve phases, get better map, extend model.




Refinement minimizes:

$$R = \frac{\sum \left| \left| F_i^{calc}(h;x) \right| - \left| F_i^{obs}(h) \right| \right|^2}{\sum \left| F_i^{obs}(h) \right|^2}$$

EM: observations are projections; what parameters should be refined?

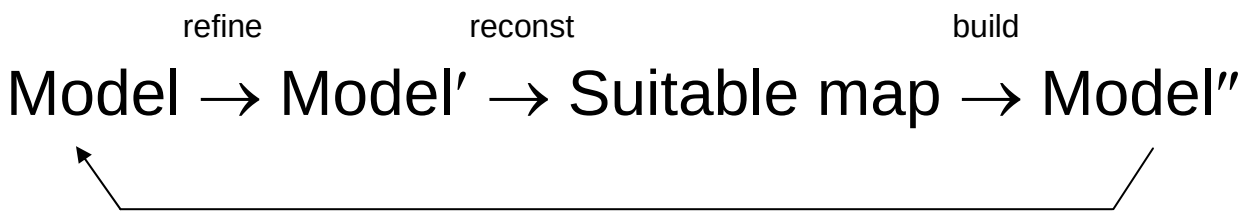
Do we have enough power to refine against the following agreement factor?

$$R = \frac{\sum \left| \sigma_i^{calc}(u,v;\mathbf{x},\theta_i) - \sigma_i^{obs}(u,v) \right|^2}{\sum \left| \sigma_i^{obs}(u,v) \right|^2}$$

where $\sigma_i^{calc}$ is the calculated projection, as a function of x, the model coordinates (and B's), and of $\theta_i$, the orientation and origin of the $i^{th}$ projection

If not, what is a suitable compromise?

Would hope to have the following cycle:



Karin Reinisch
Tamir Gonen
Yifan Cheng
Piotr Sliz
Alex Fotin

Tom Walz
Niko Grigorieff

Tom Kirchhausen

David DeRosier

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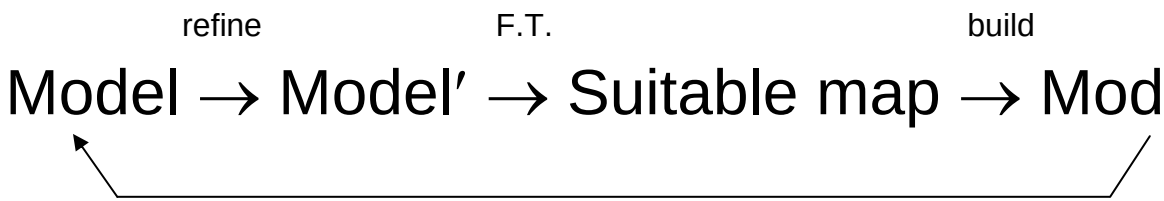


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