

NCMI

National Center for Macromolecular Imaging

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National Center for Research Resources



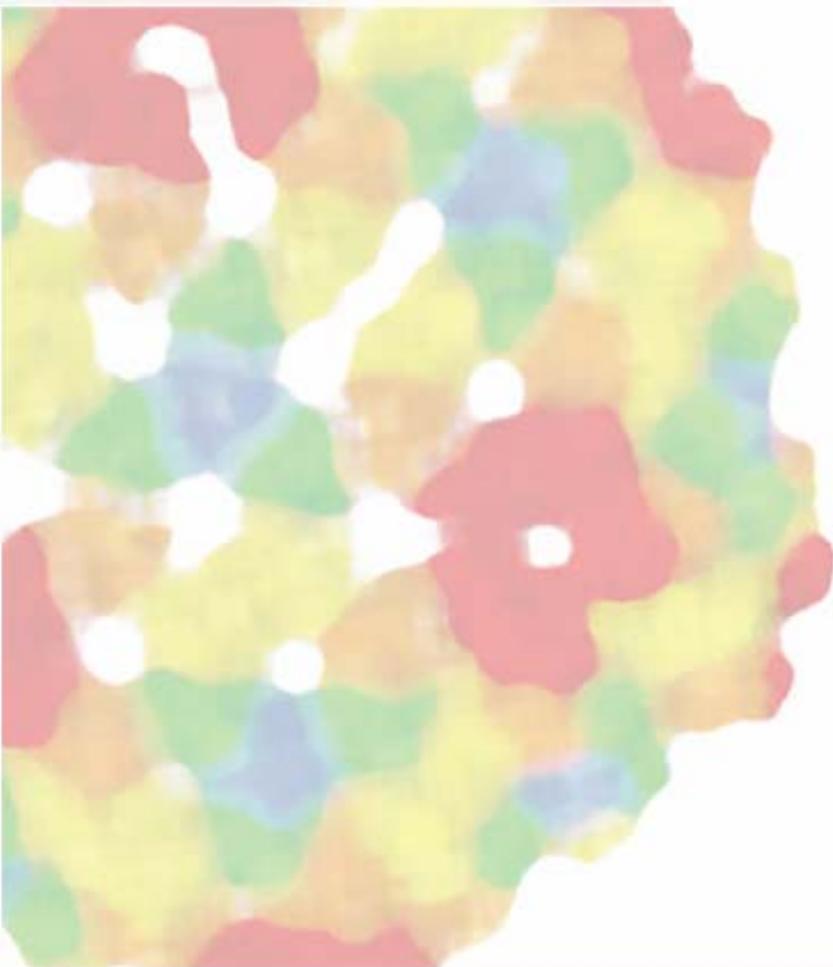
<http://ncmi.bcm.edu>

The logo for the National Center for Macromolecular Imaging (NCMI) features the letters 'NCMI' in a large, orange, sans-serif font. The letters are set against a background of a blue and white molecular structure, possibly a protein or a complex of macromolecules, rendered in a semi-transparent style.

National Center for
Macromolecular Imaging

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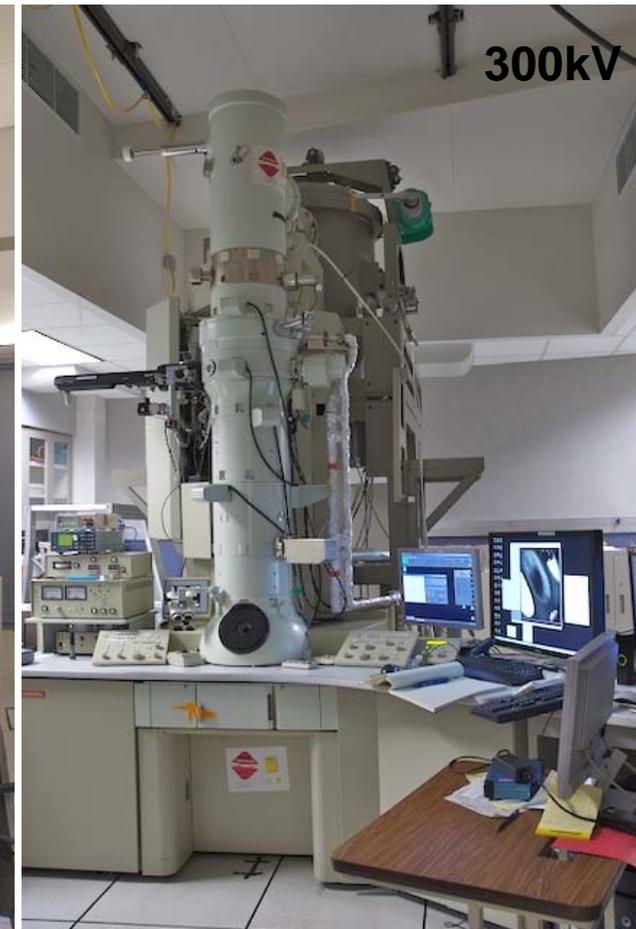
Lenora Trujillo

Research Missions at NCMJ

- Develop and apply **Cryo-EM** for structure determinations of **Molecular Nano-Machines in solution states** towards atomic resolution
- Share our experimental and computational technology freely with the global academic community



Electron Cryo-Microscope at NCMI, Baylor College of Medicine



Pipeline in Biological Cryo-EM

**biochemical
preparation**



**cryo-em sample
preparation**



imaging



data collection

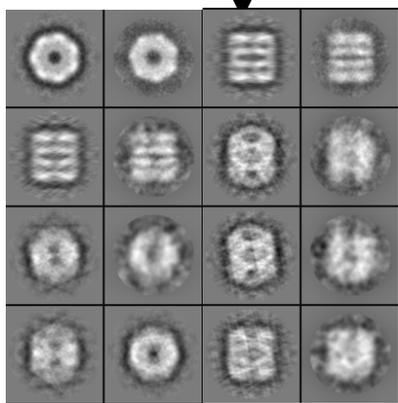
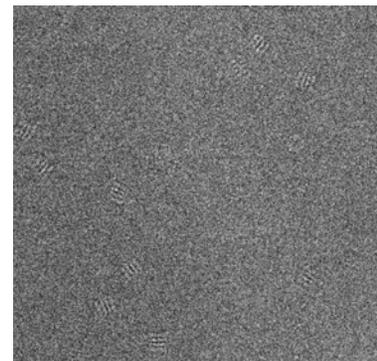
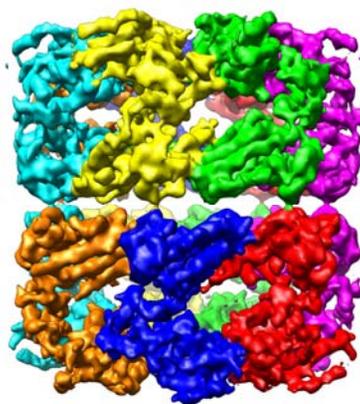
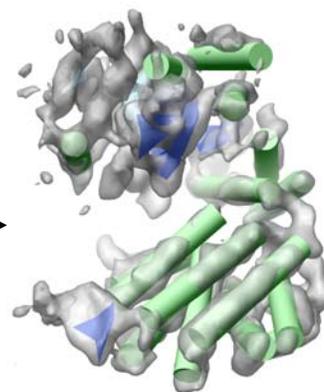


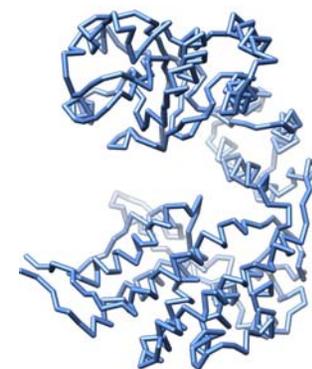
image processing



reconstruction



structural analysis



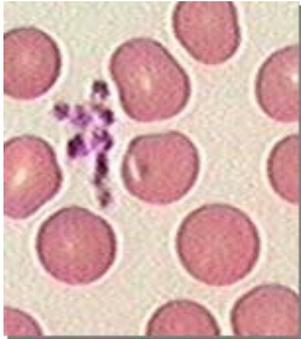
**Model;
Deposition**

Cryo-EM: A Critical Tool in Biomedicine

- Can visualize bio-structures at a broad range of resolutions and complexities

Structural Biology from Cells to Atoms

*Optical
microscopy*



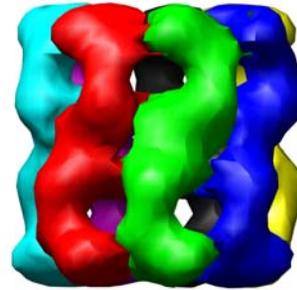
200 nm

*Electron
cryotomography*



8nm

*Electron
cryomicroscopy*

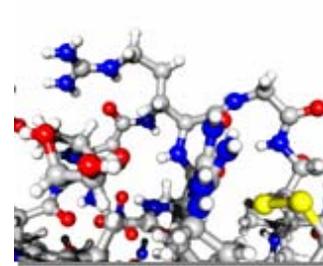


0.9nm

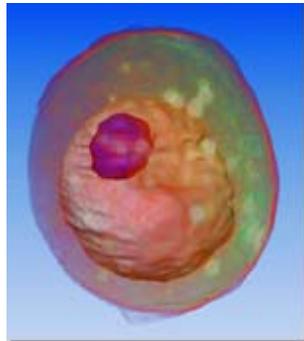


0.4nm

Crystallography

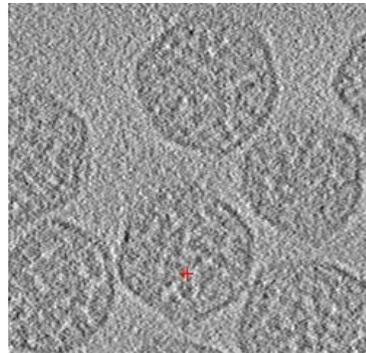


0.1nm



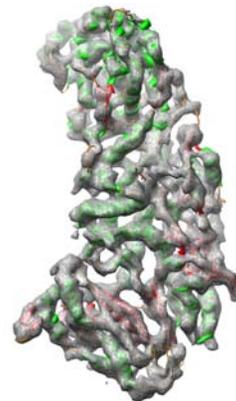
X-ray microscopy

50nm



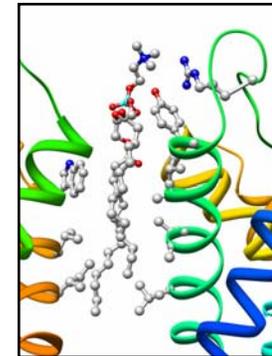
*Electron
cryotomography*

5nm



*Electron
cryomicroscopy*

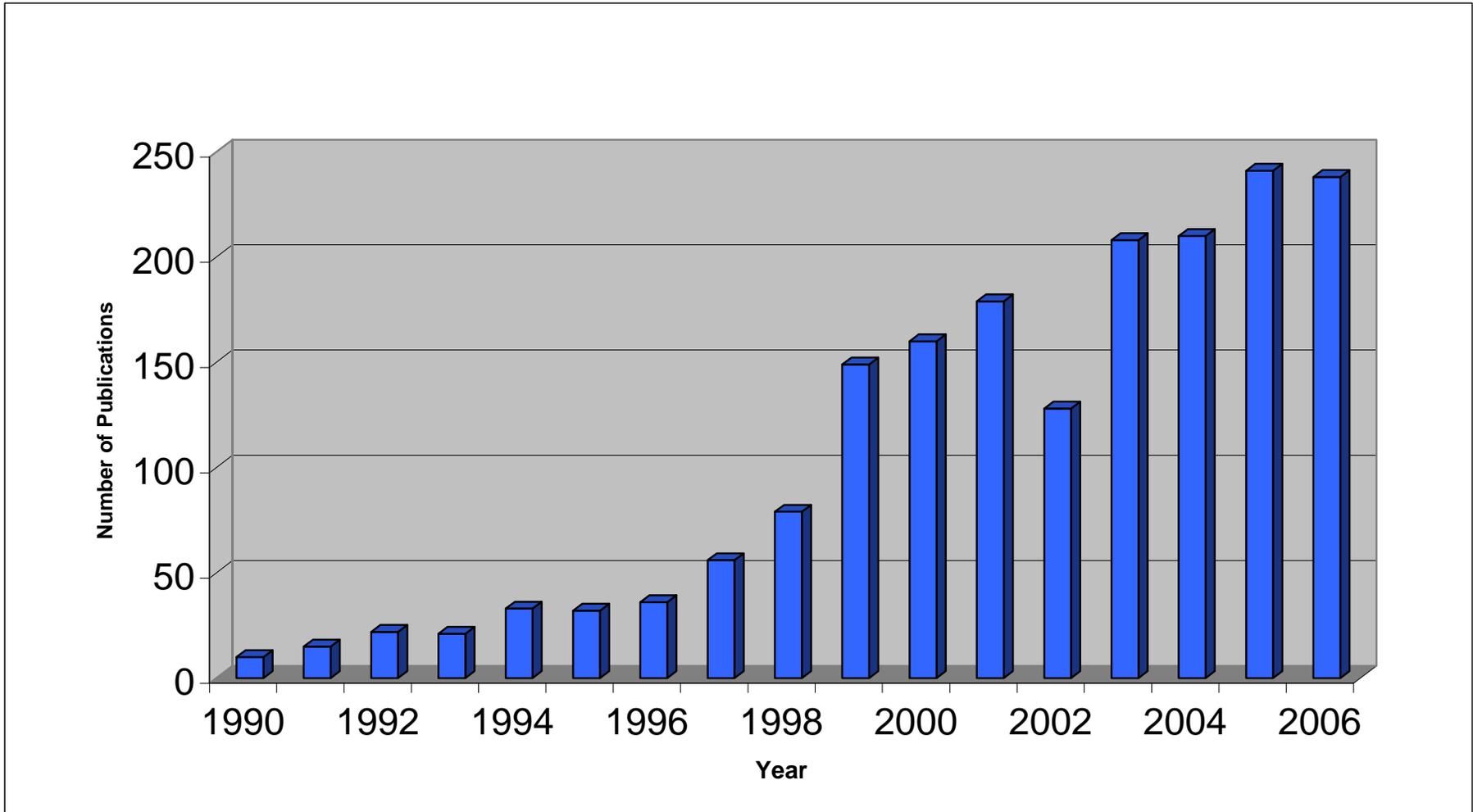
0.6nm



*Electron & X-ray
Crystallography
NMR*

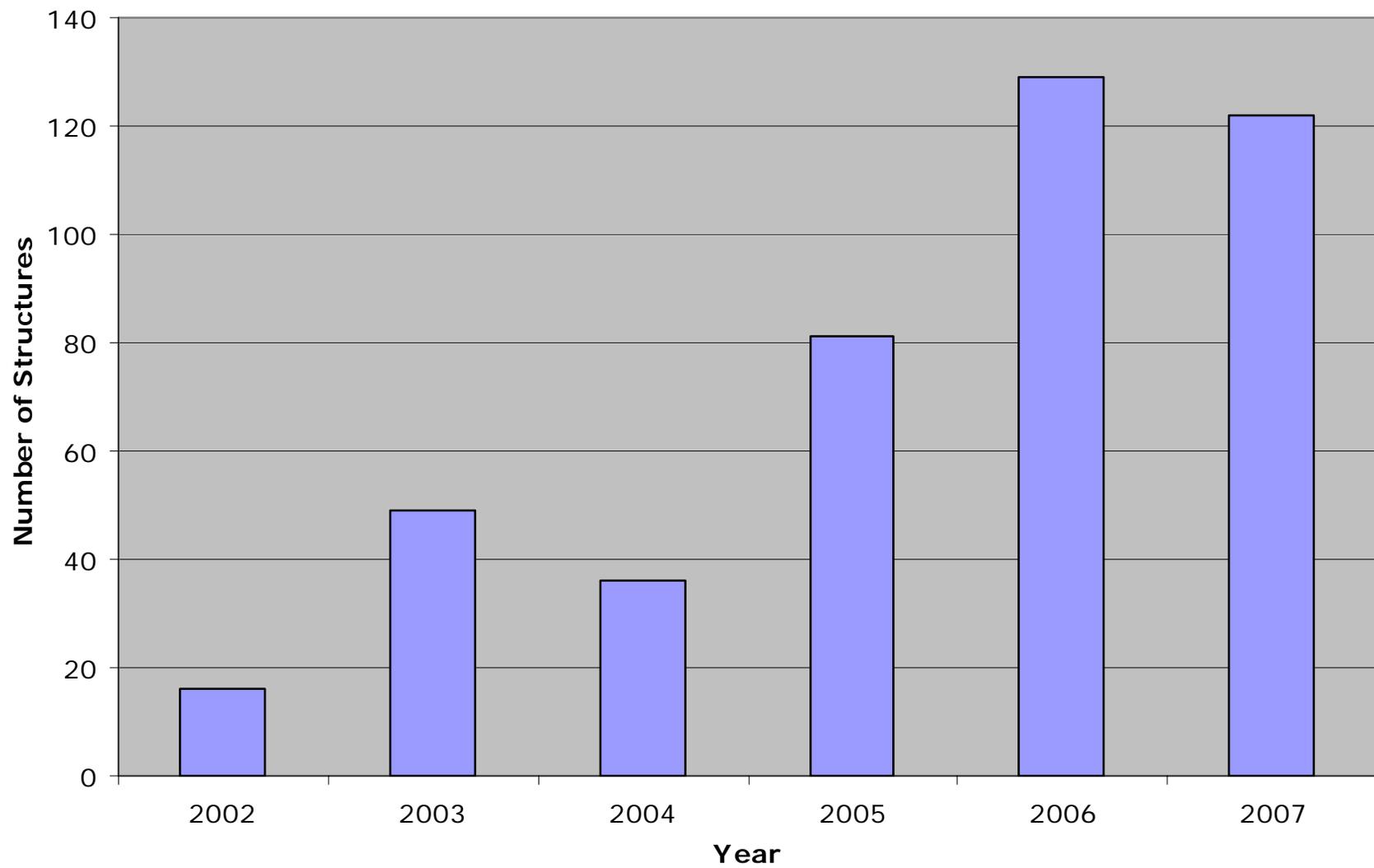
0.3nm

Trends in Macromolecular Cryo-EM



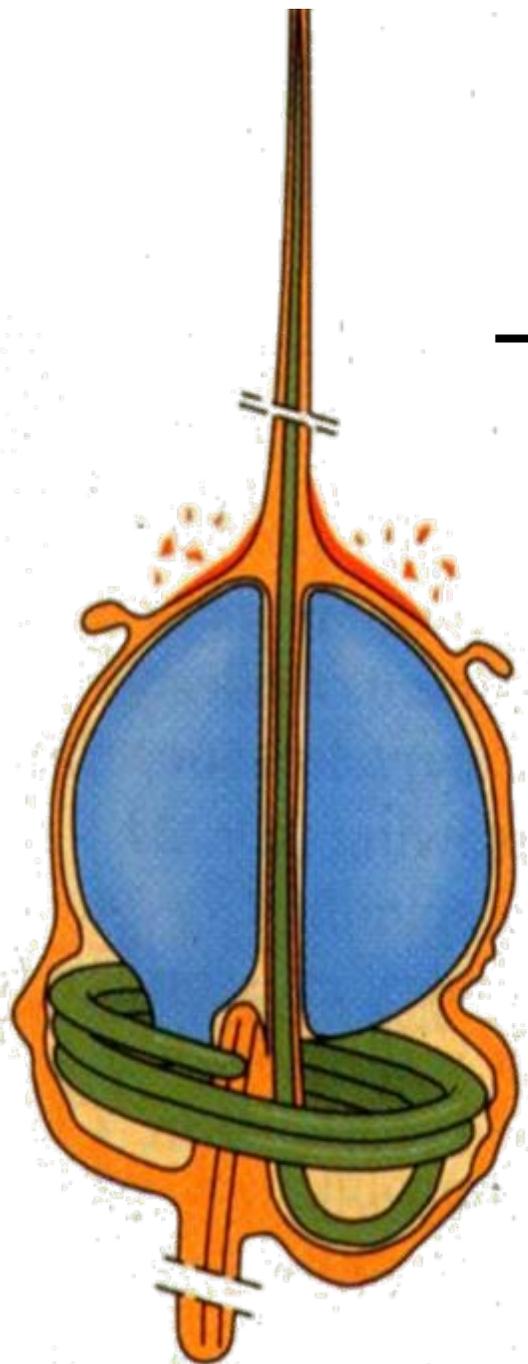
Matthew Baker (2007)

CryoEM Maps in EBI EMDB



Cryo-EM: A Critical Tool in Biomedicine

- Can visualize bio-structures at a broad range of resolutions and complexities
- Is the only method to look at structures of certain molecular machines



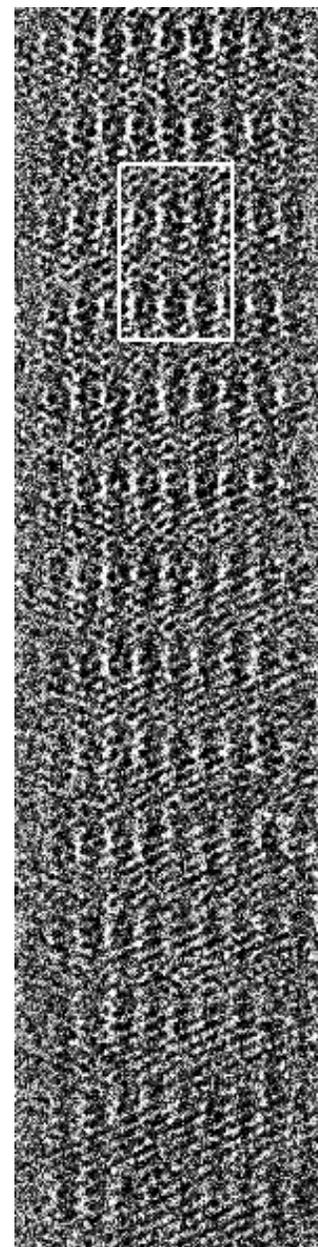
sruin
102kDa

actin
42kDa

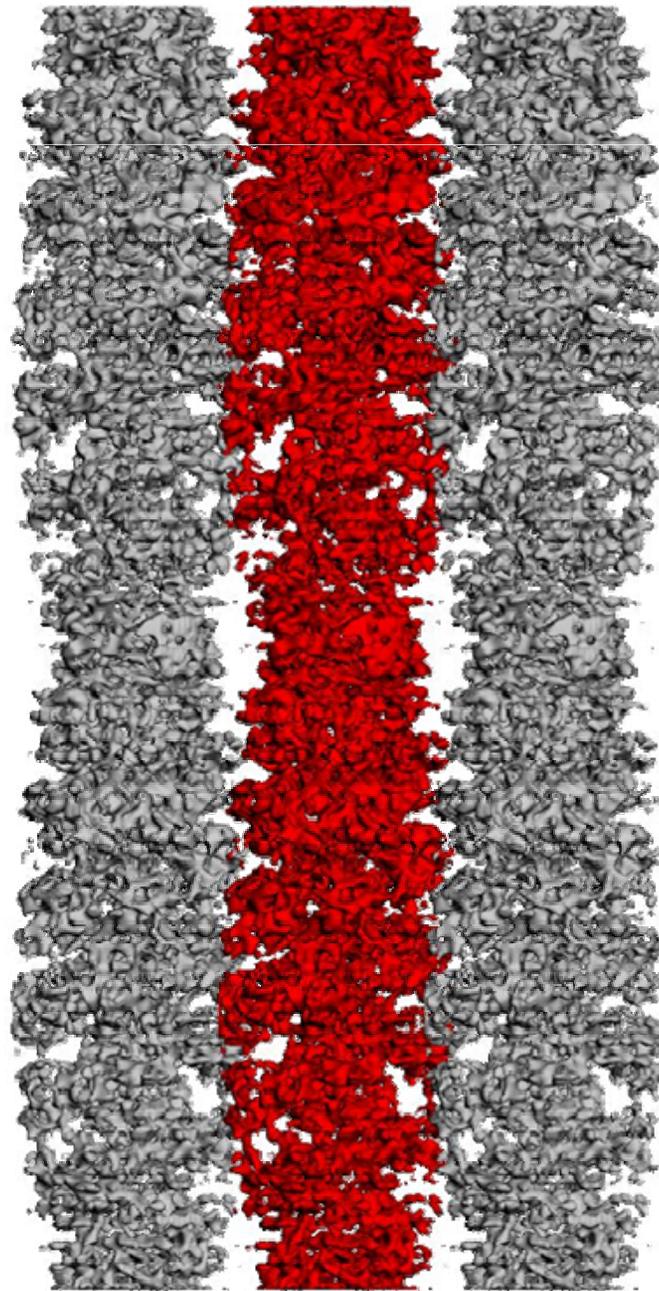
calmodulin
24kDa

1:1:1

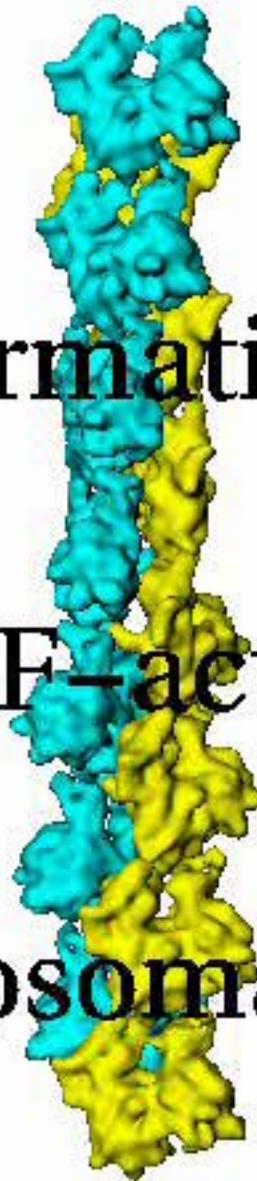
Cryo-EM image

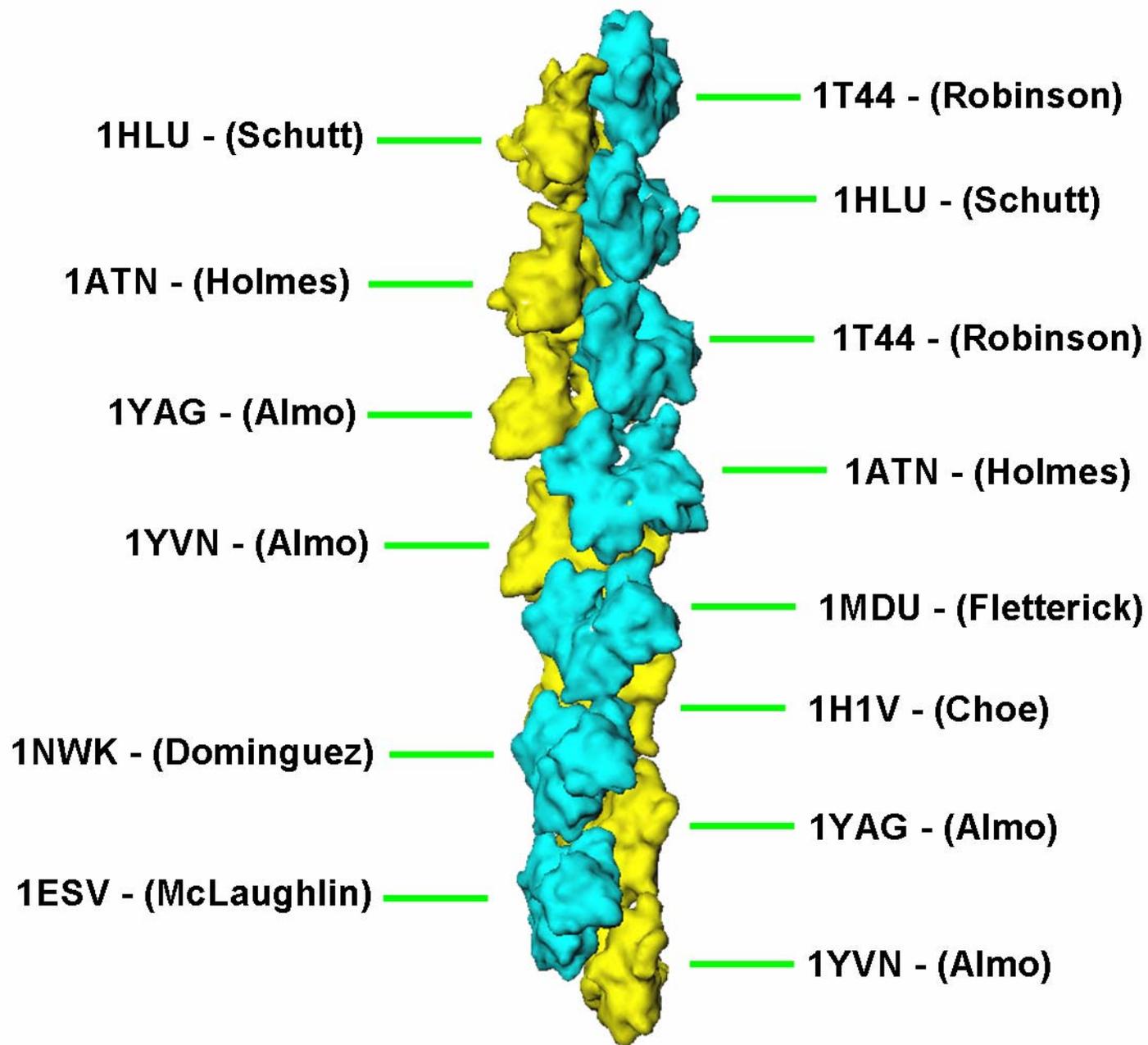


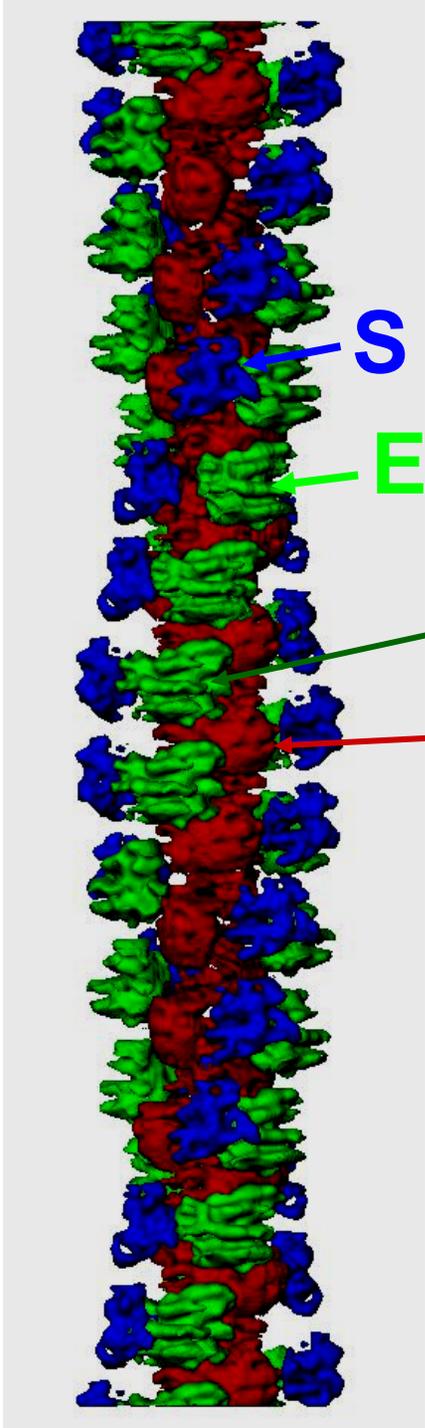
3 filaments



transformation from
perfect F-actin helix
to Acrosomal Actin







S

E

Scruin

Actin

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JEM3200
(Yoshi type)

300kV

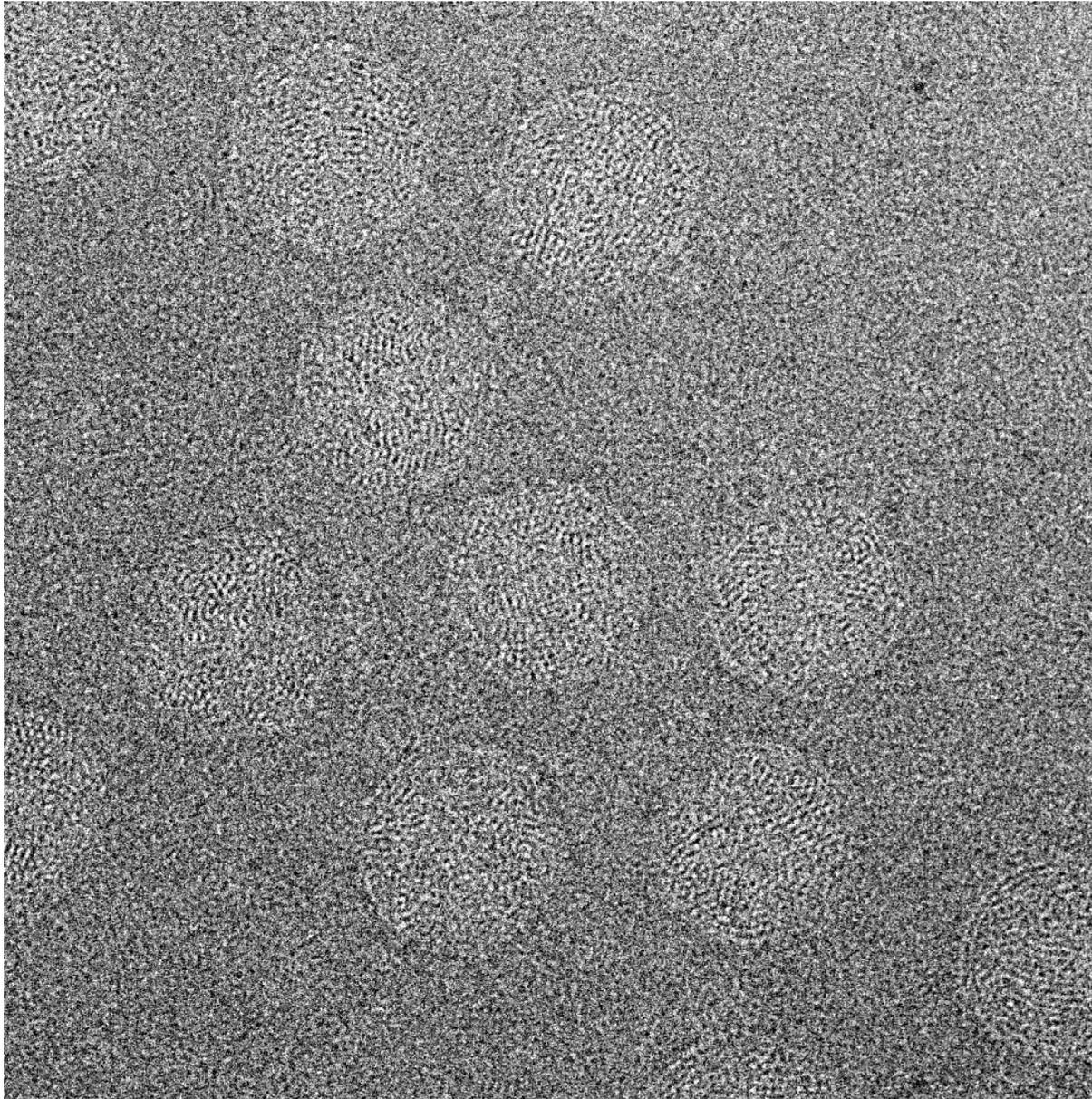
FEG

Liquid helium

4k Gatan CCD



Imaging Epsilon15 Phage at Liquid He



JEM3000

300kV

4°K

60Kx mag

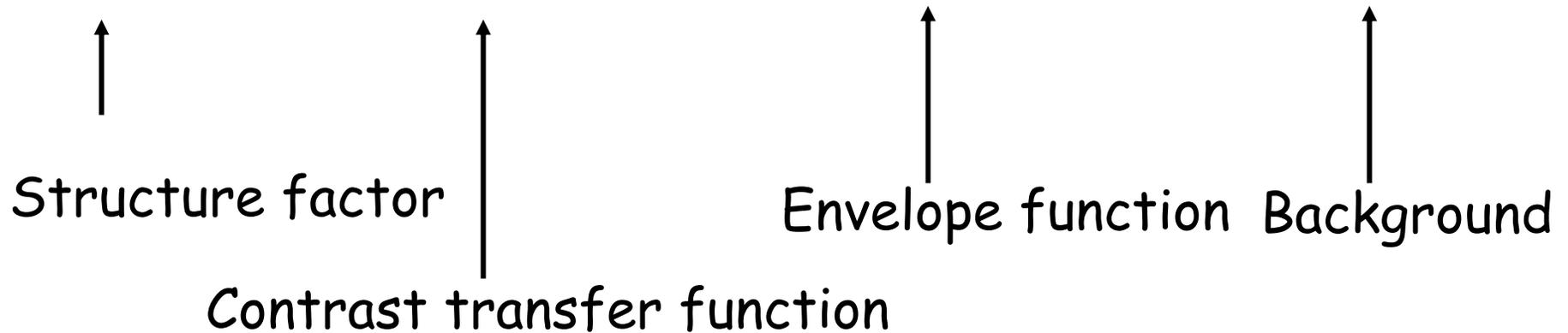
$\sim 28 \text{ e}/\text{\AA}^2$

Film data

J Jakana

Computed FFT of Images

$$F^2(s) \quad CTF^2(s) \quad Env^2(s) \quad + \quad N^2(s)$$



$$SNR \text{ (Contrast)} = (F^2 \quad CTF^2 \quad Env^2) / N^2$$

$$Env^2(s) \sim \exp(-2BS^2)$$

Epsilon15 Image Data Recorded with Liquid Helium for 4.5 Å Map

- 3,000 micrographs were digitized
- 40% has SNR beyond 6 Å
- Images with non-isotropic CTF were discarded
- 36,000 particles were picked from 1,228 micrographs
- 20,000 particles were finally used for 3-D reconstruction

Epsilon15 Phage

4.5 Angstroms Cryo-EM

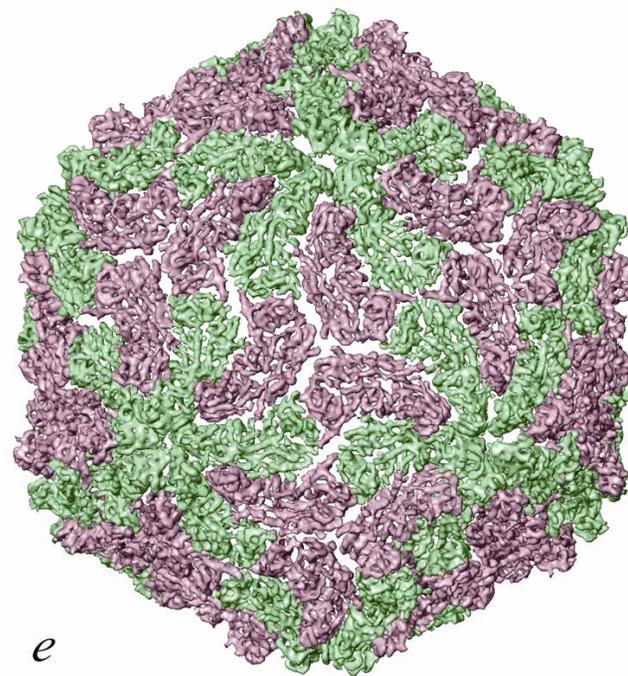
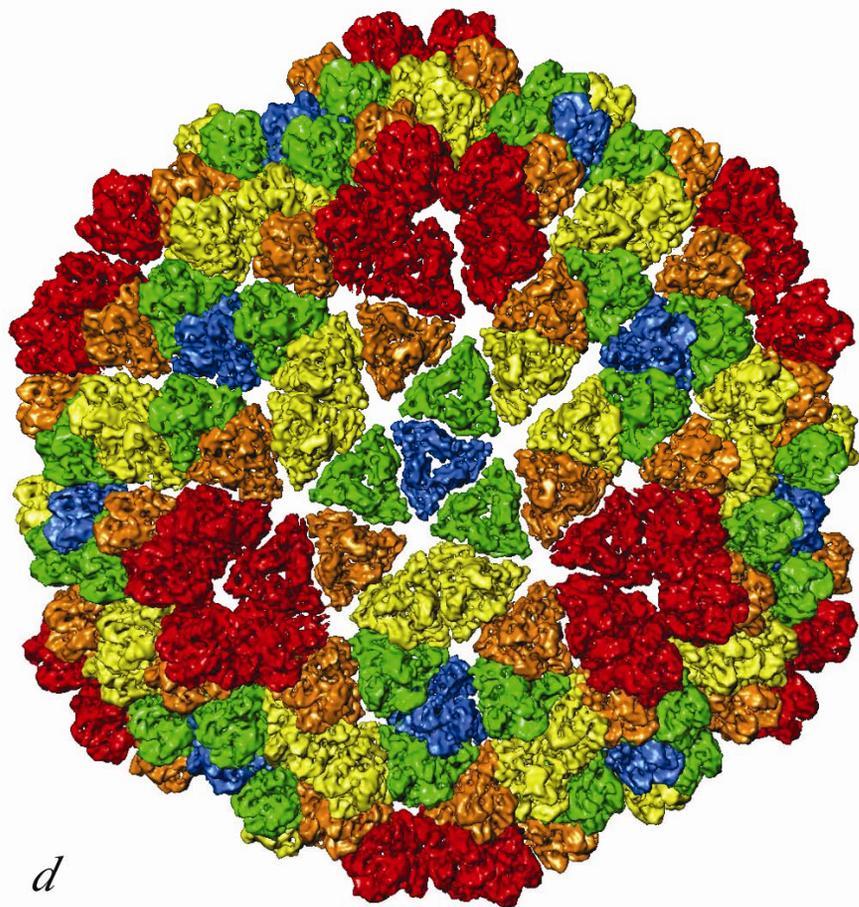
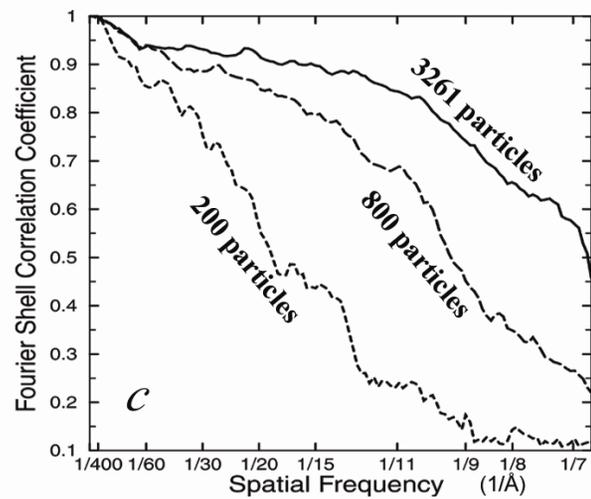
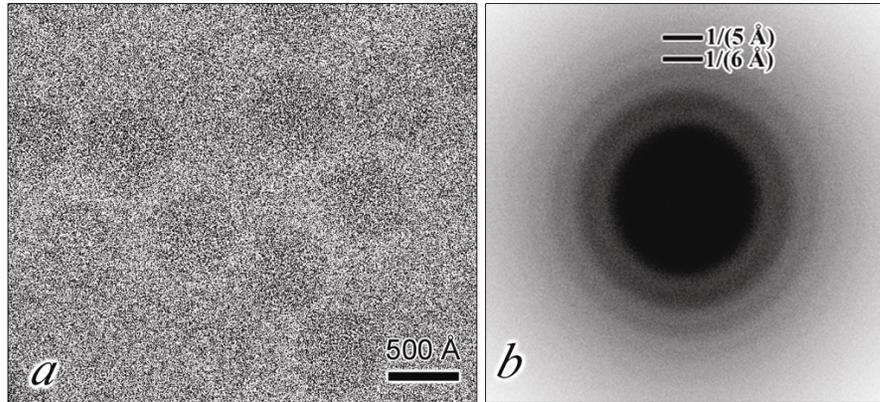
Single Particle Reconstruction

A001.D12

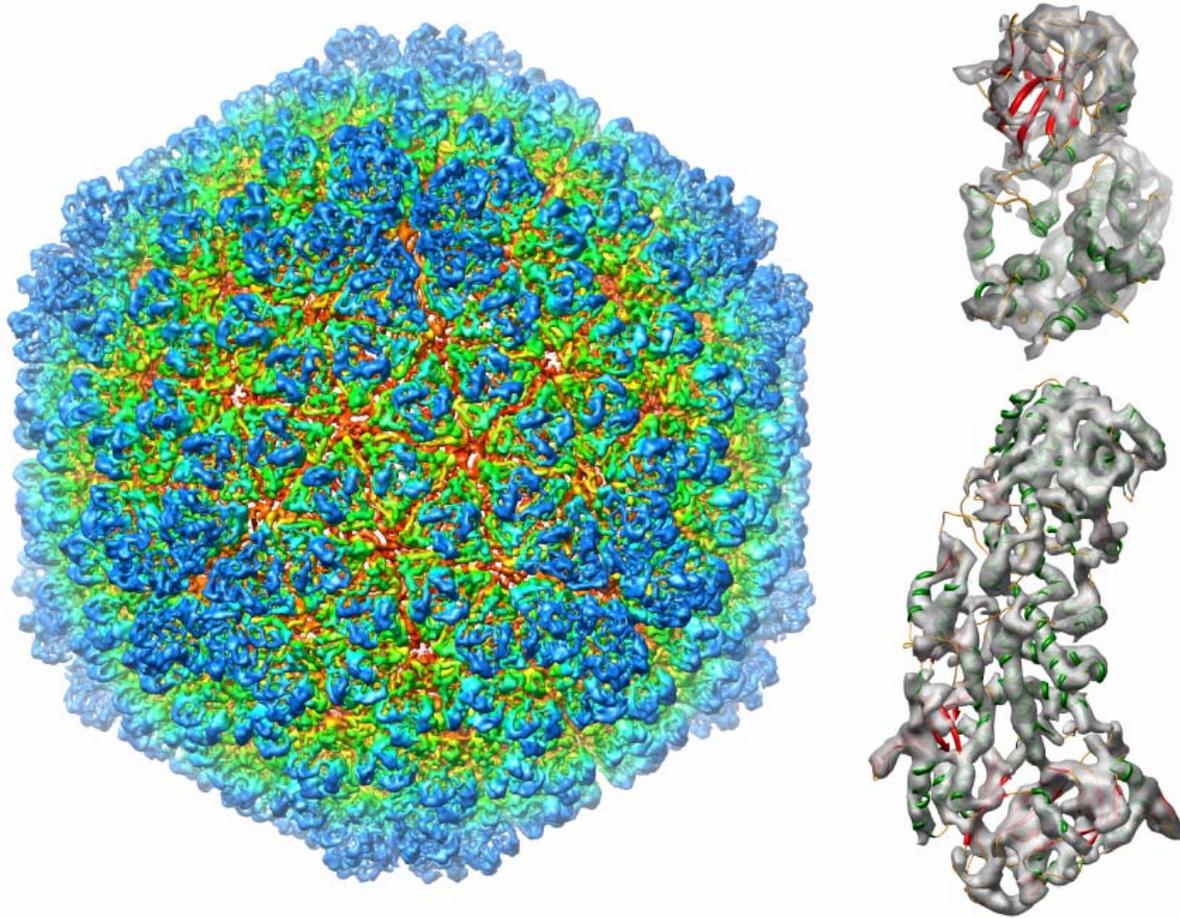
Jiang, Baker, Jakana, Weigele, King, Chiu (unpublished)

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- Can do *de novo* $C\alpha$ backbone without crystallography
- Can determine subnanometer resolution structure with few tens to thousands of particle images



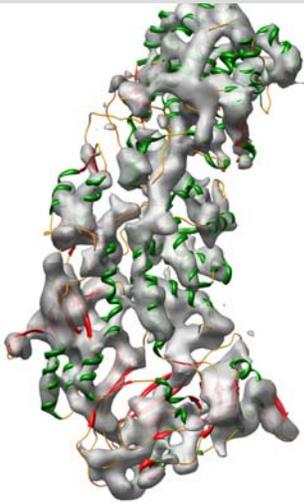
7.9 Å cryoEM map of Rice Dwarf Virus Reconstructed from 284 Particles



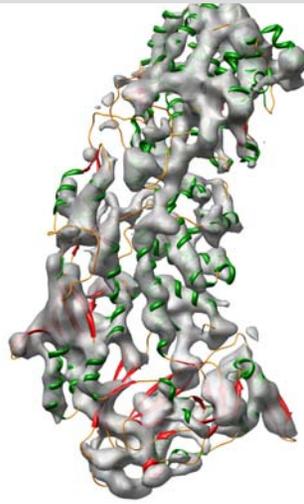
Multi-Path Monte Carlo Simulated Annealing Optimization Algorithm

Liu, Jiang, Jakana and Chiu (2007) JSB 160:11-27

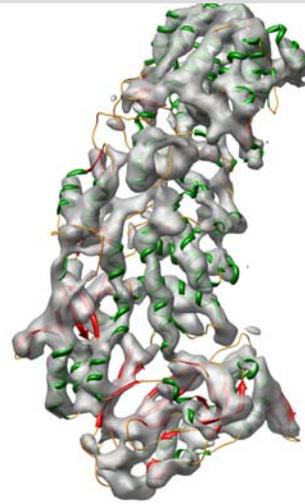
Cryo-EM Maps (numbers of particles)



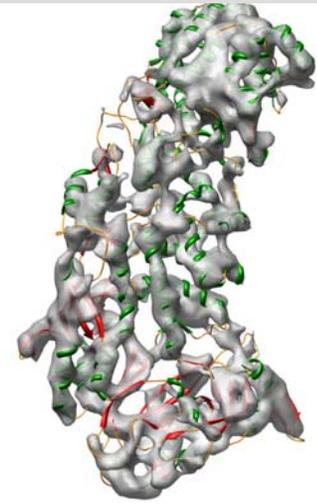
41, 9.8Å, 1 α , 0 β



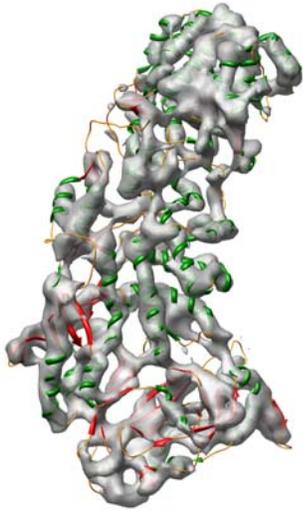
62, 9.6Å, 4 α , 0 β



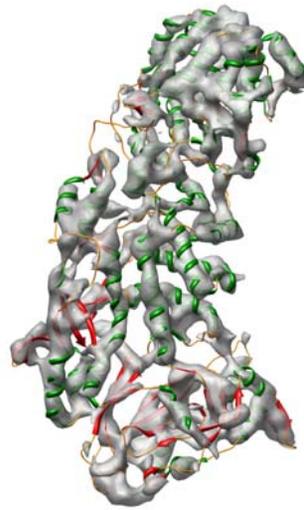
81, 9.2Å, 10 α , 0 β



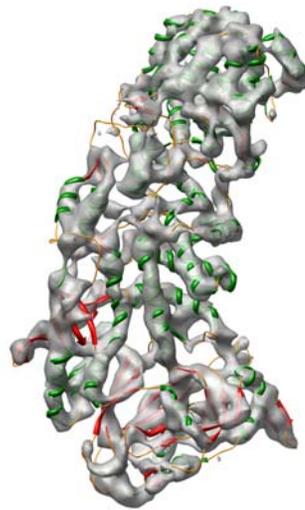
101, 8.9Å, 14 α , 2 β



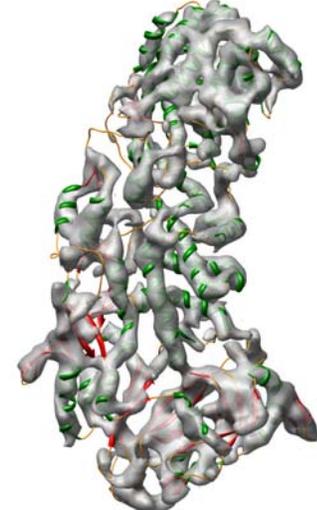
128, 8.6Å, 12 α , 3 β



147, 8.3Å, 15 α , 3 β



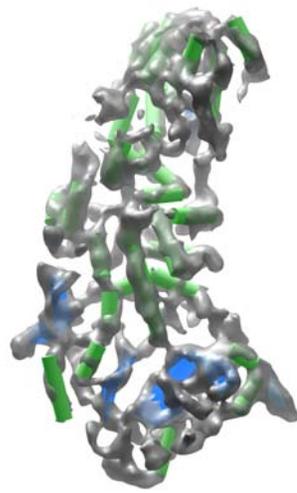
181, 8.3Å, 16 α , 4 β



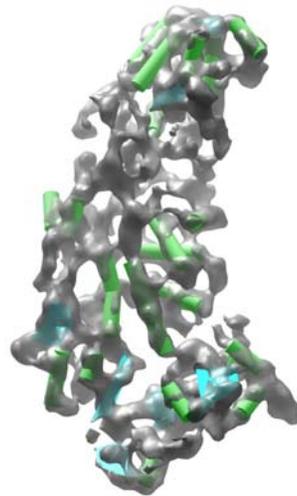
284, 7.9Å, 20 α , 7 β

Liu, Jiang, Jakana and Chiu (2007) JSB **160**:11-27.

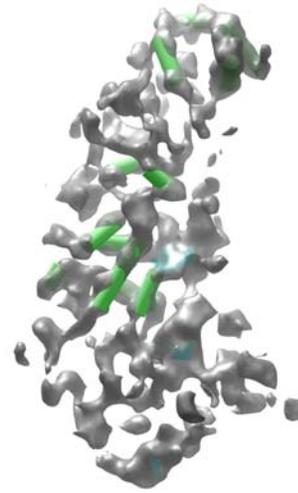
Reconstructions from Various Subsets of 284 Particle Images



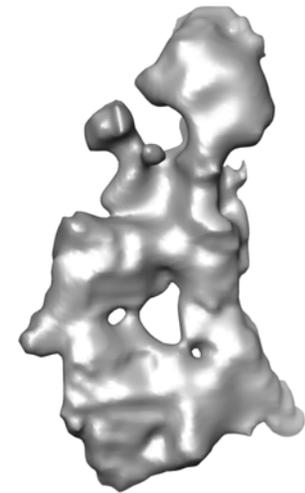
(a)



(b)



(c)

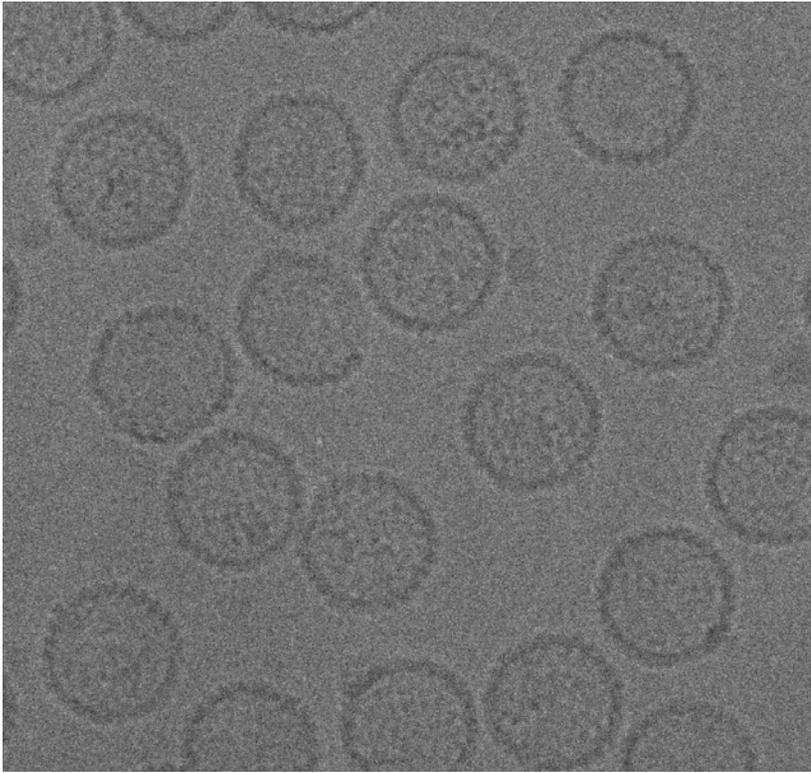


(d)

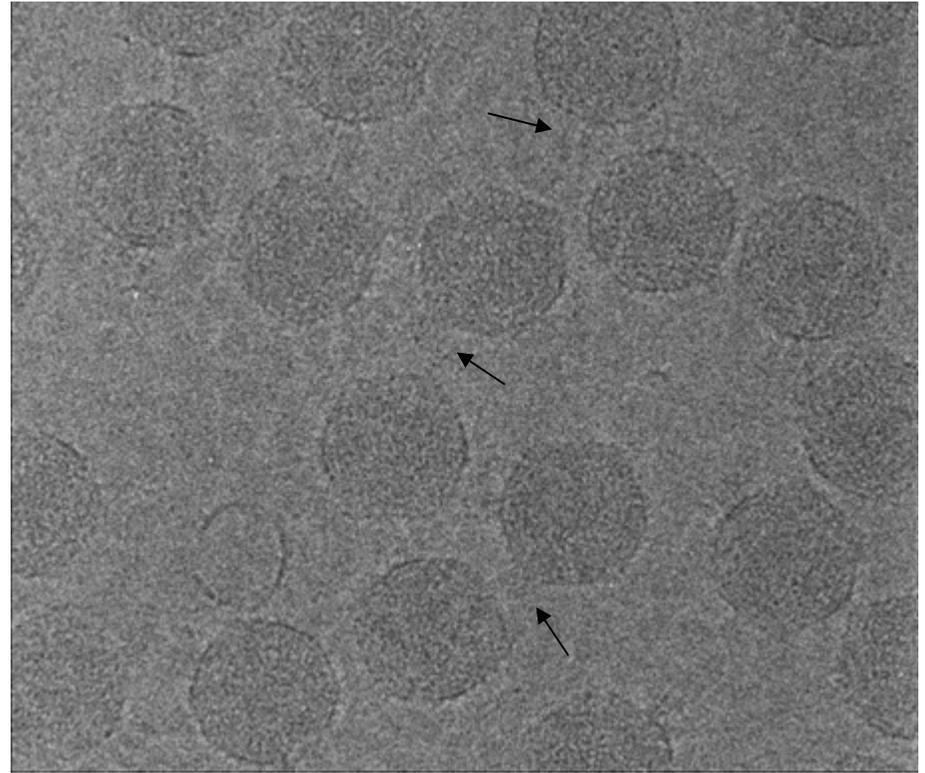
Cryo-EM: A Critical Tool in Biomedicine

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- Can determine subnanometer resolution structure with few tens to thousands of particle images
- **Can detect protein conformational changes in a physiological process**

Electron Images of P22 Phage



Procapsid shell
Diameter = 585 Å

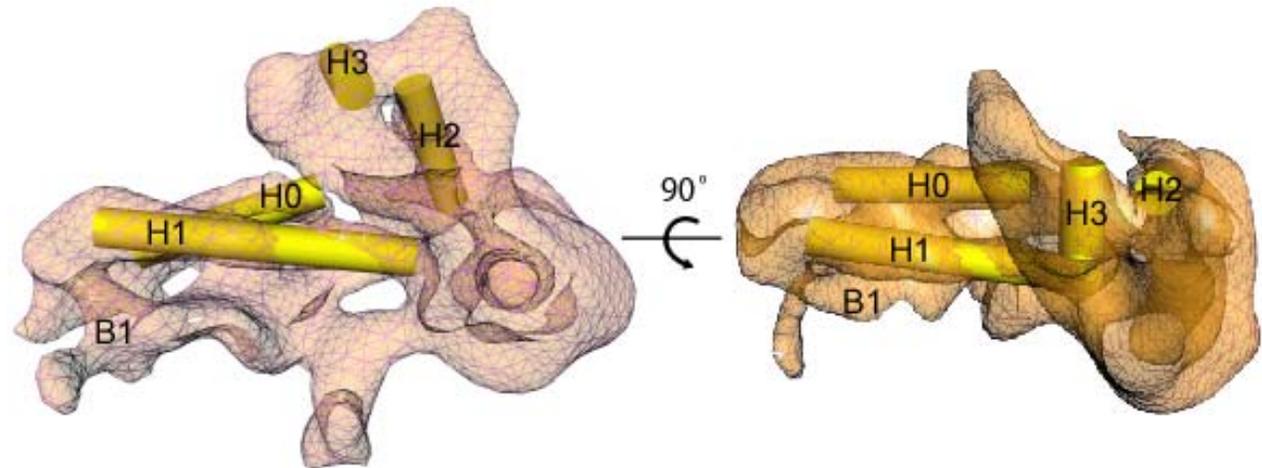


Mature phage
Diameter = 700 Å

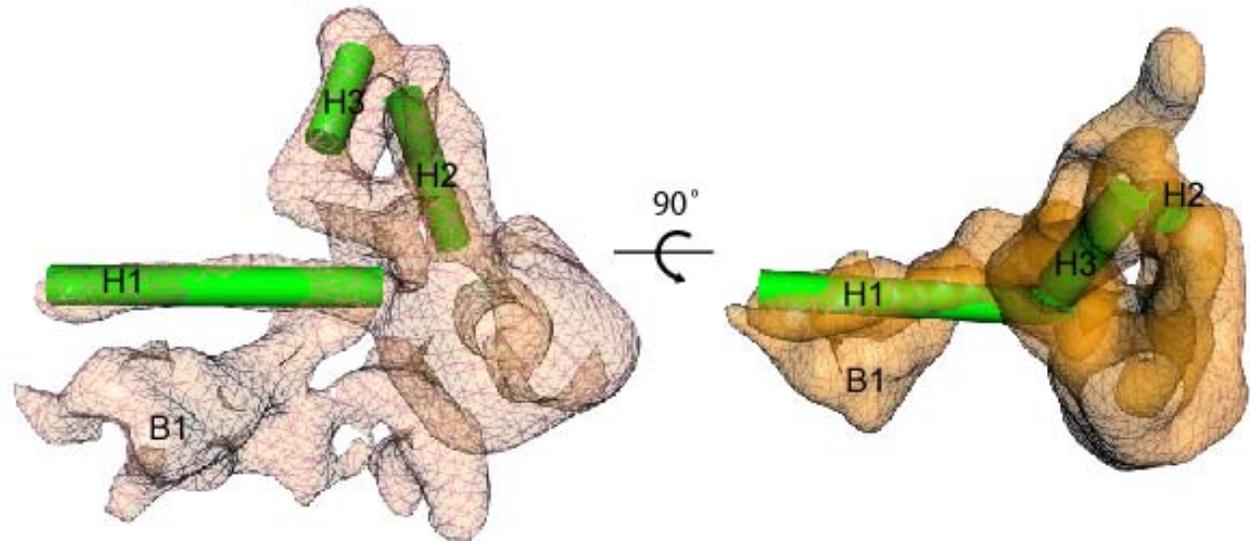
P22 procapsid–phage Capsid maturation

Large Structural Changes in P22 Maturation

Procapsid



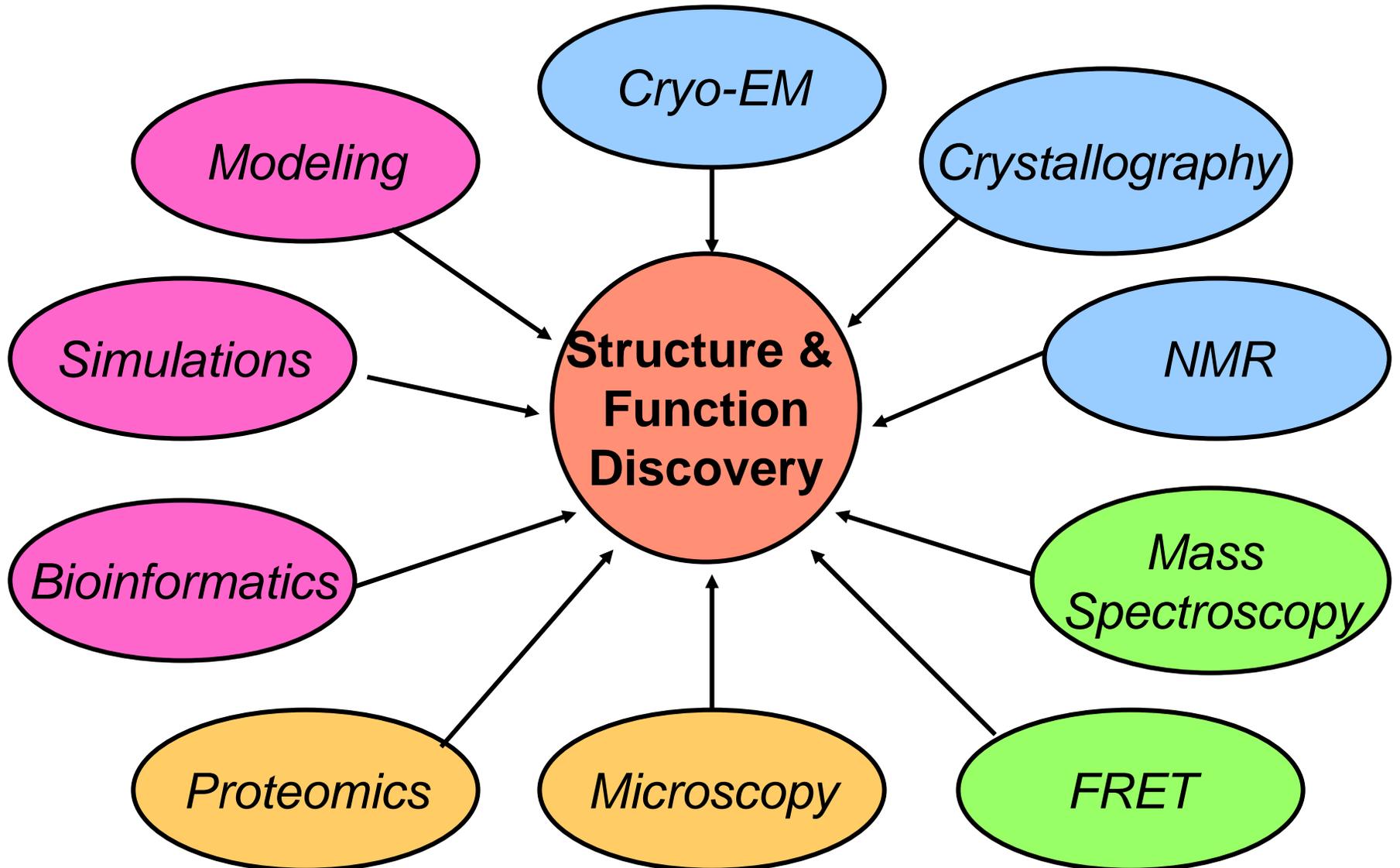
Mature phage



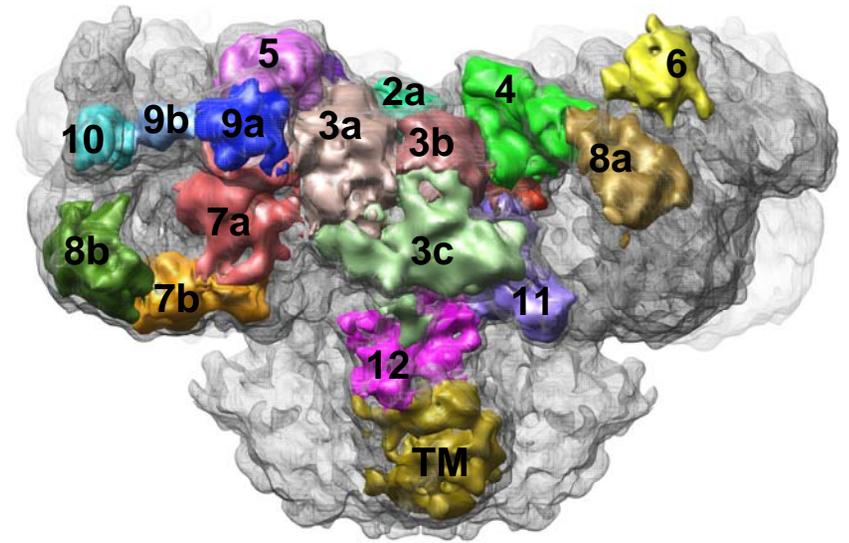
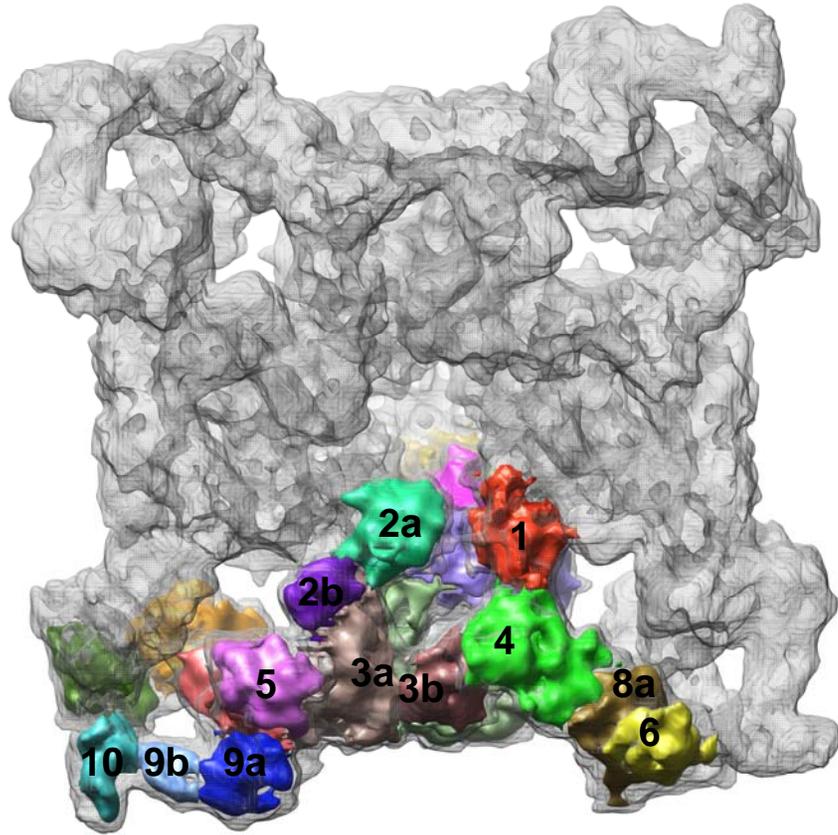
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- Can detect protein conformational changes in a physiological process
- Can provide a key data set for computational biology research to extract additional structural information

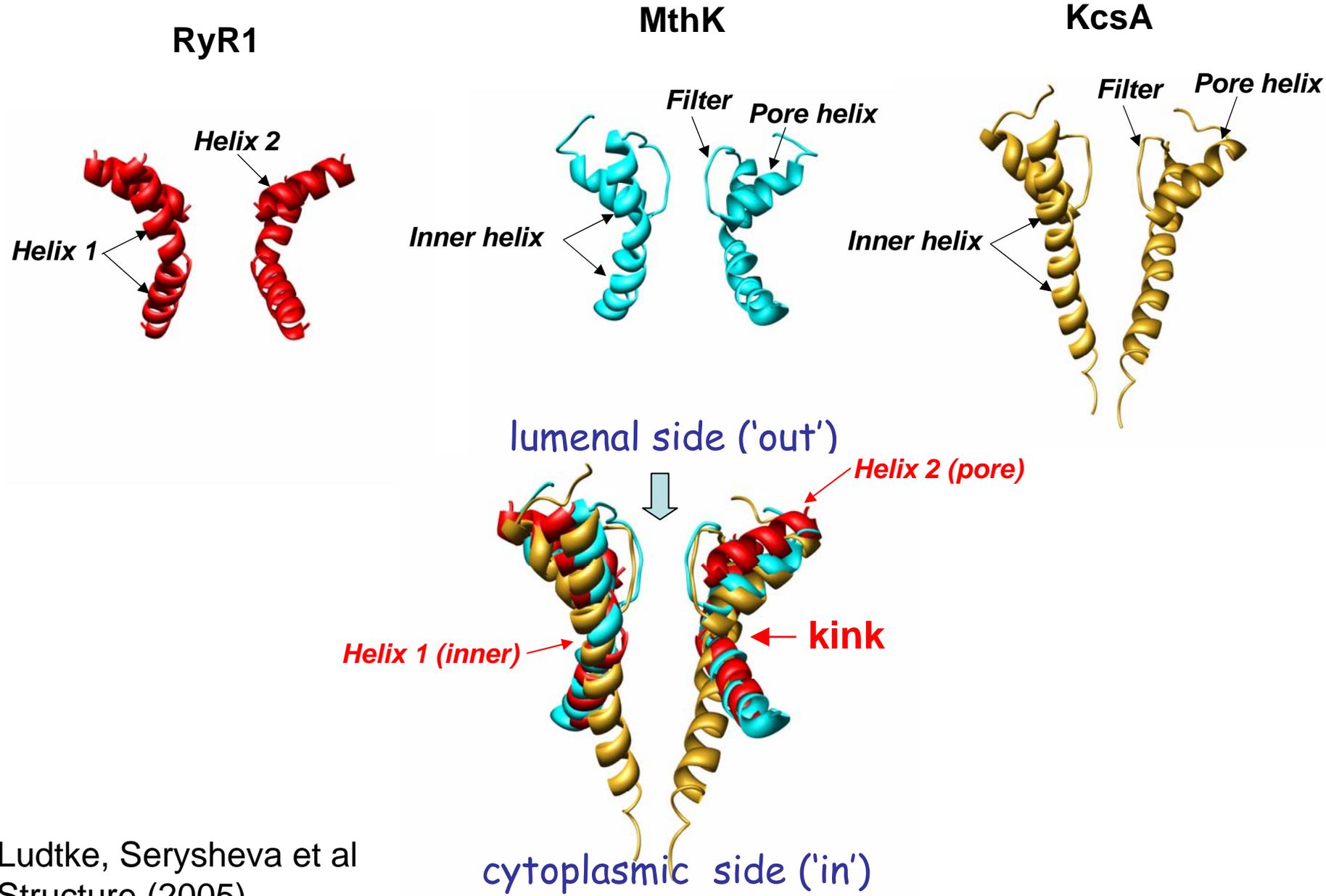
Data Integration



9.6 Å Cryo-EM Map of RyR1 (2.2 MDa)

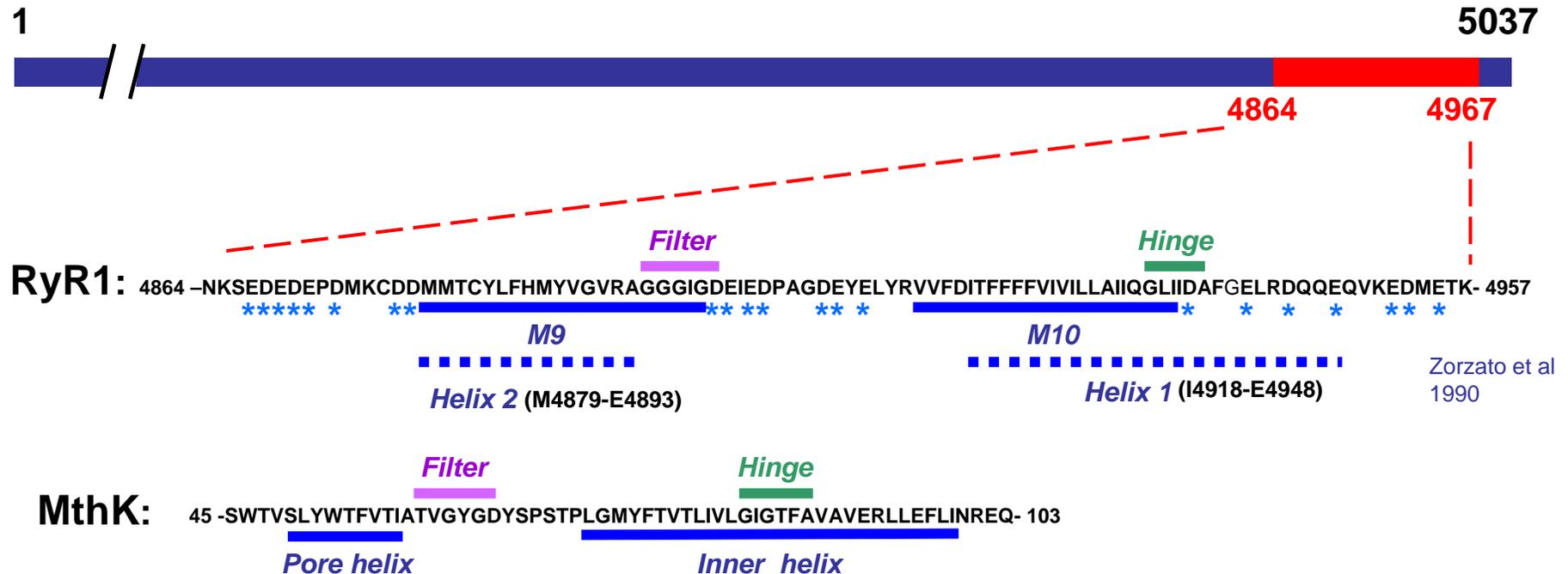


Compare the pore in RyR1 and K⁺ channels



Ludtke, Serysheva et al
Structure (2005)

Sequence Assignment of Observed α -Helices in the TM Region



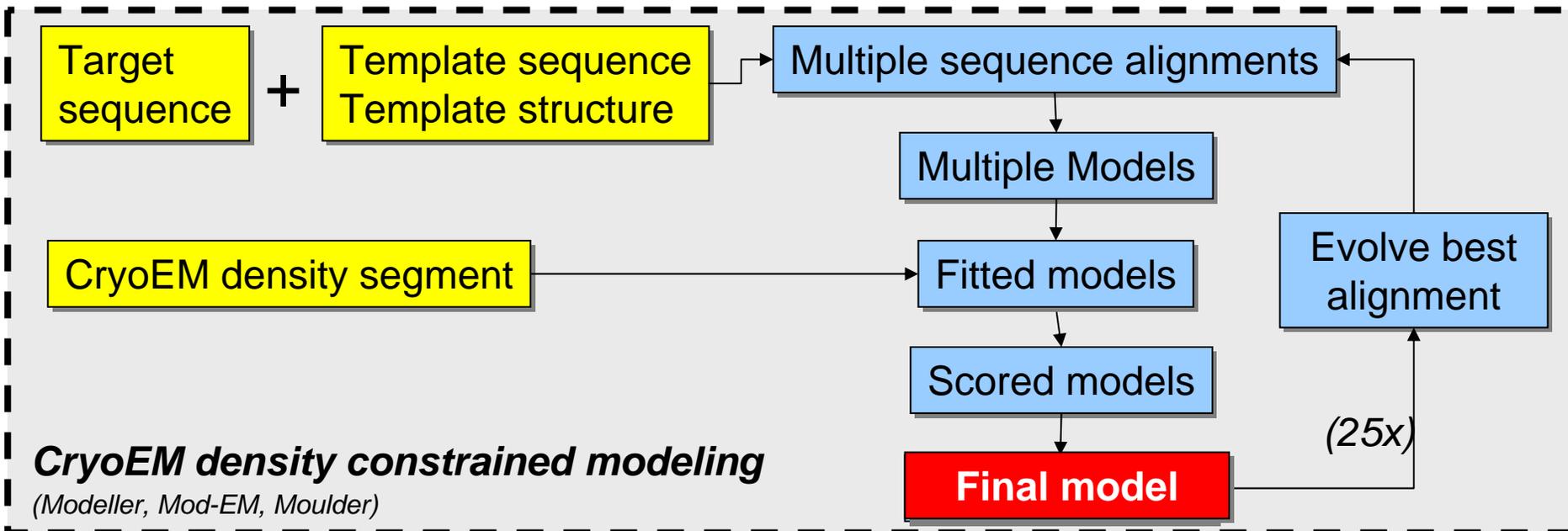
- ✓ Highly conserved region (>90% identity) among RyRs
- ✓ Mutations within these regions of RyRs (G4895A, I4898A, D4900N) alter rates of ion translocation

CryoEM Restrained Comparative Modeling

Target identification



Model localization



Challenges and Opportunities

- Specimens with conformational variability
- 2-3 Å map of single particles
- Integrate with other information for knowledge discovery
- Extend post-averaging of cryoET subtomograms to molecular resolution
- Engage cryoEM study to translational medicine
- Relatively high cost of very high-end instruments (development, acquisition and maintenance)