NCMI

National Center for Macromolecular Imaging

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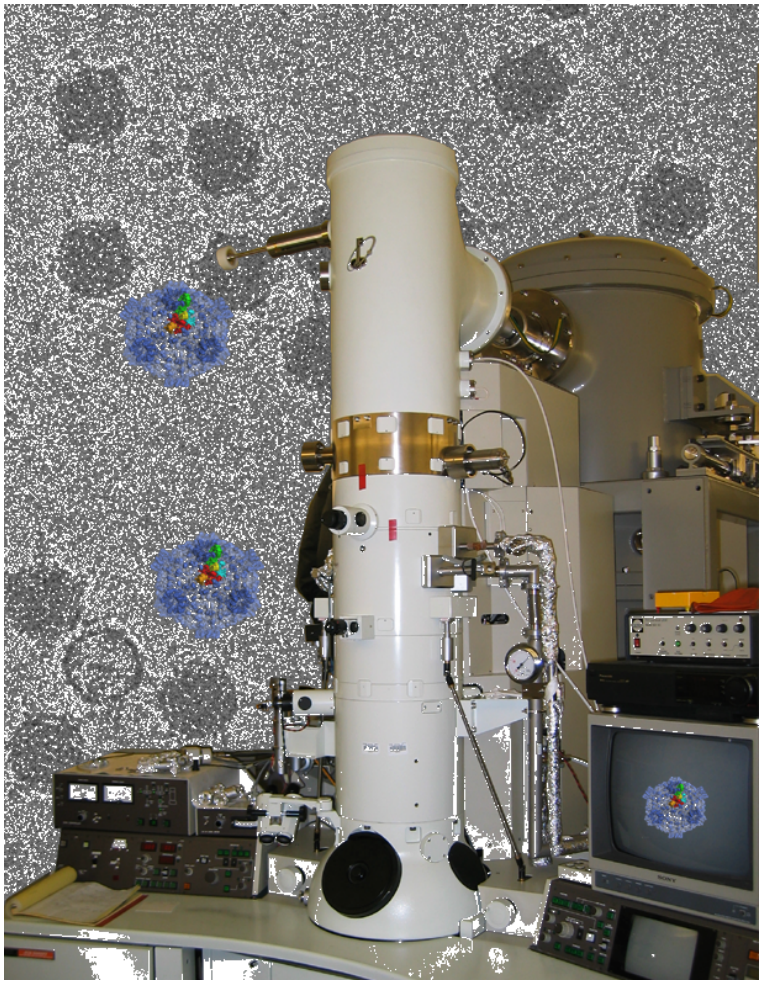
http://ncmi.bcm.tmc.edu



National Center for Research Resources

NSF

NIGMS

Research Mission at NCMI
http://ncmi.bcm.tmc.edu

Development of Experimental and Computational Infrastructure for Near Atomic Resolution Structure Determination of Large Macromolecular Machines without Crystals by Electron Cryomicroscopy

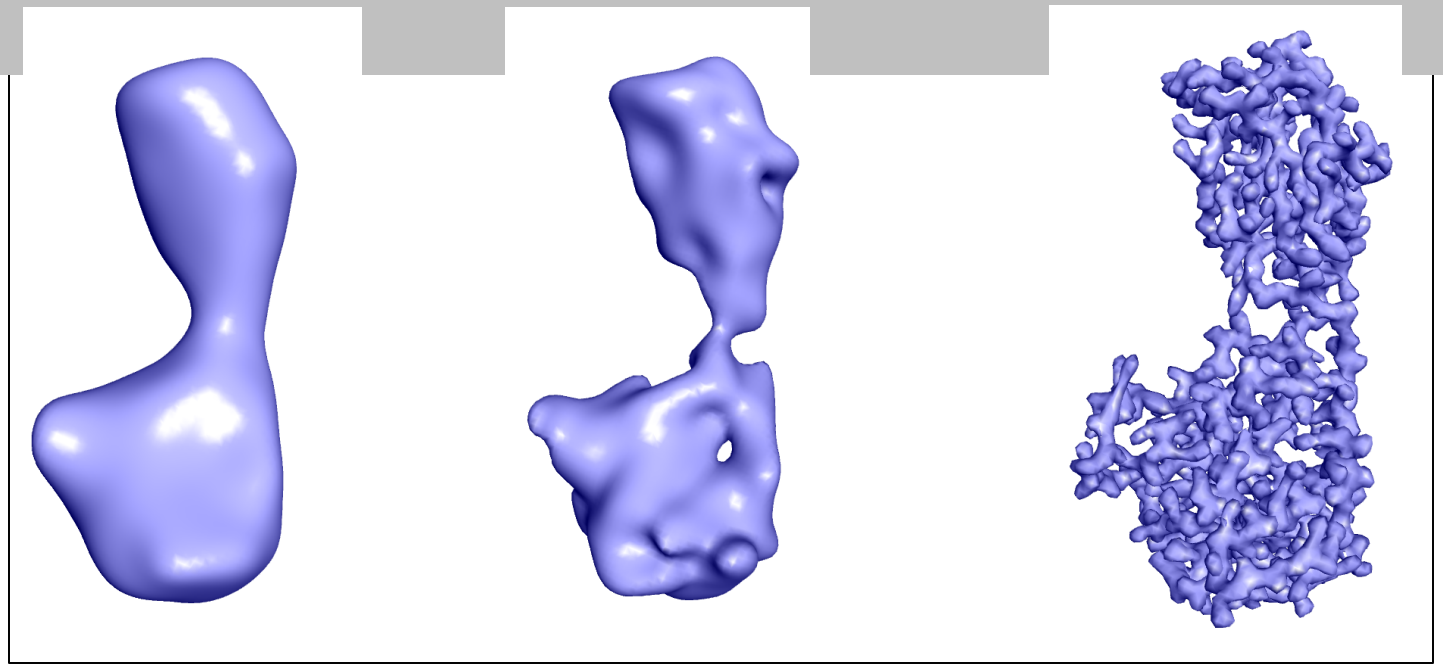
Molecular Cryo-EM

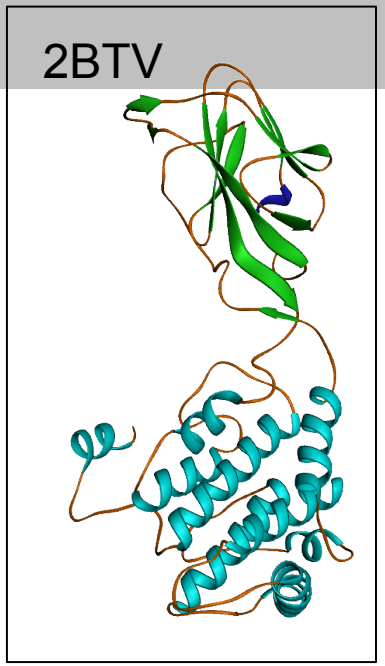
- What has cryo-EM achieved ?
- What are the trends in structural biology ?
- What are the future developments in cryo-EM

Cryo-EM Achievements

- 2-dimensional monolayer protein crystals: 3.5-1.9 Å
  polypeptide traced
- Helical arrays: 9 - 4 Å
  Fold recognized
- Single particles: 9 – 4.5 Å
  α helices and ß sheets visualized
- Subcellular assemblies within a cell: 60 Å
  identify components and domains

Structure at Different Resolutions

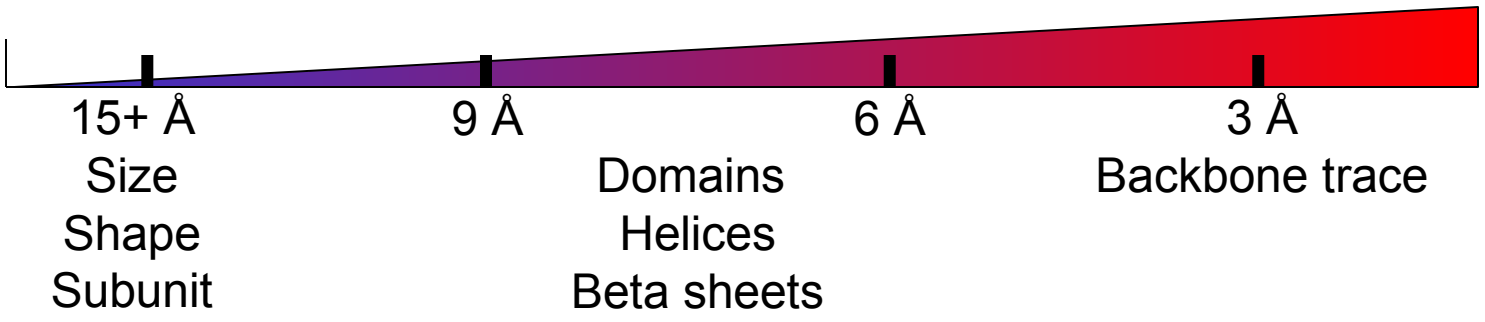




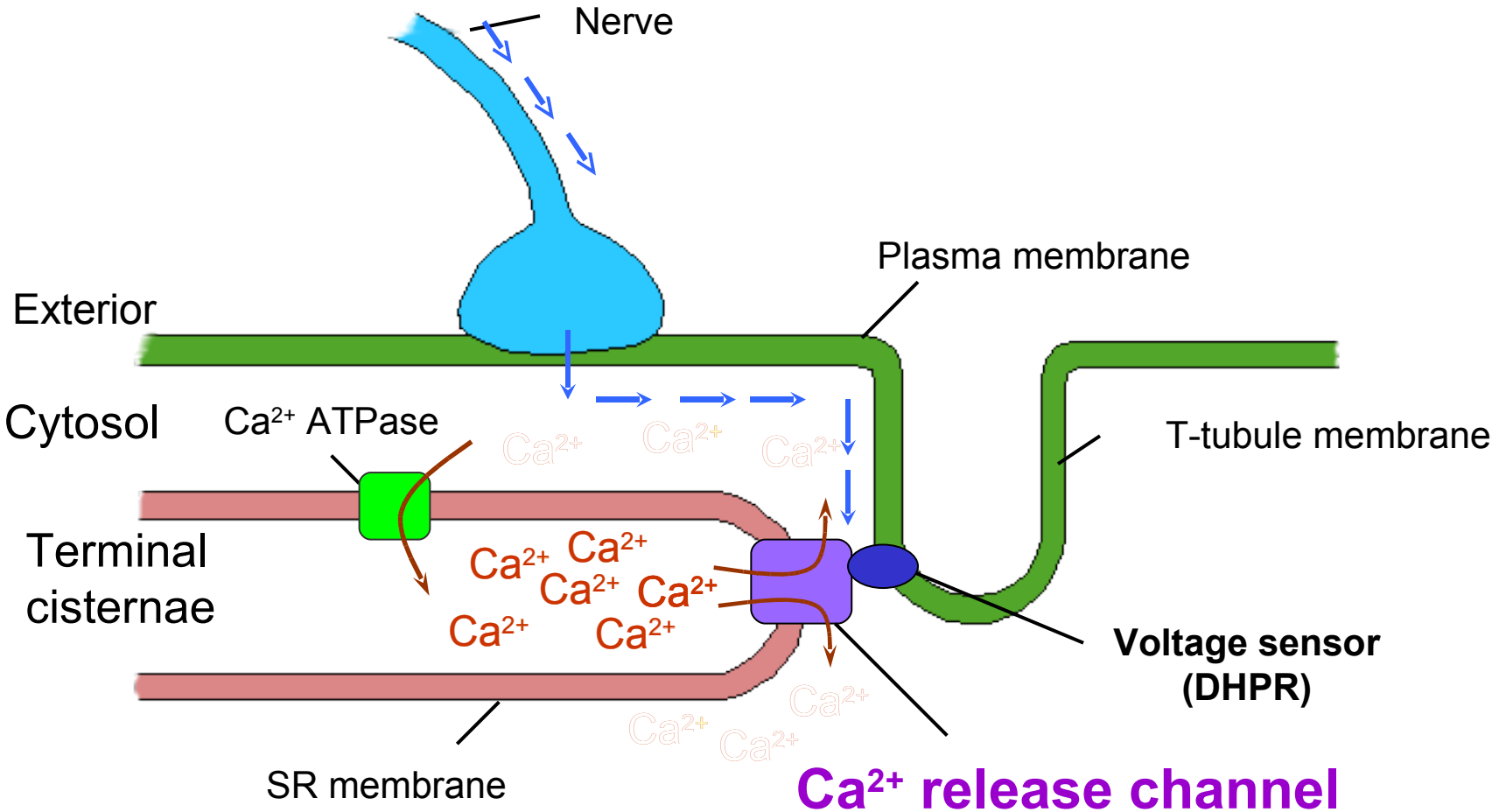
Low Resolution

Intermediate Resolution

High Resolution

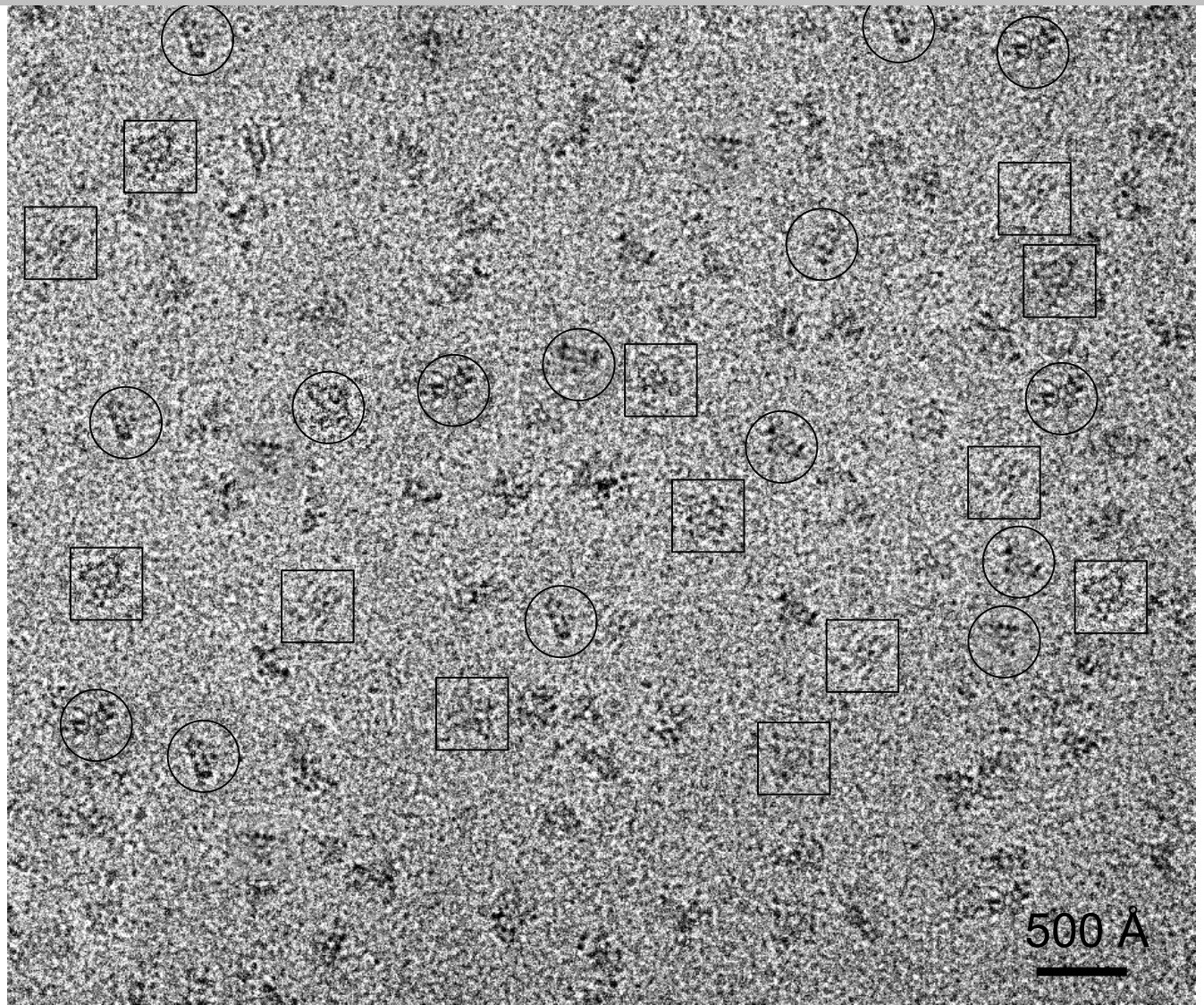


Excitation Contraction Coupling in Skeletal Muscle



From Lodish

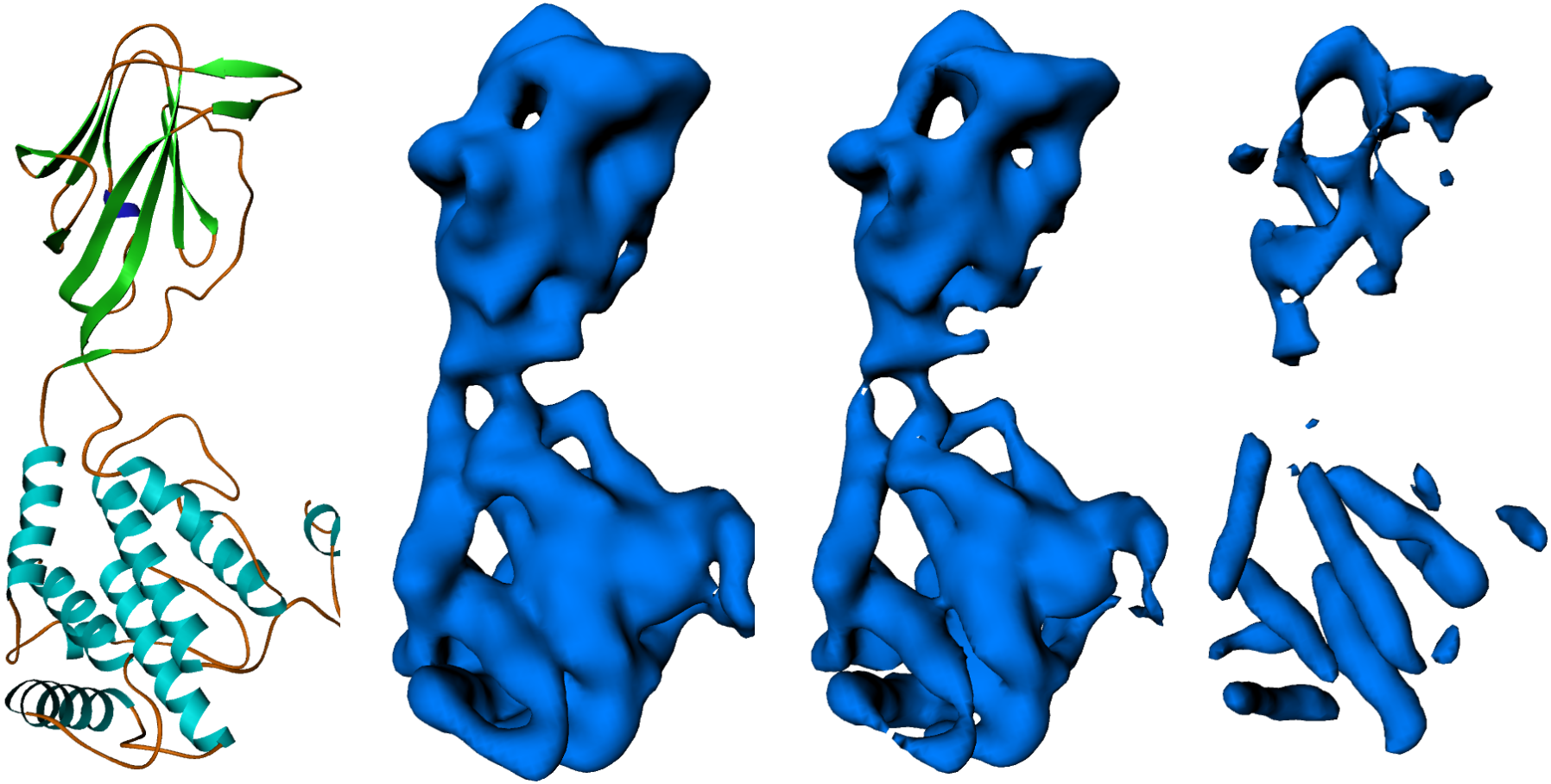
Electron Image of Ca^{+2} Release Channel (2.2 MDa Tetramer)



Data Statistics

- Channel in closed conformation, [Ca^{2+}]<10nM
- JEOL2010F : 200 kV, ~82,800X, -175°C
- Electron dose ~15 e/Å
- Defocus range : 1.6 – 4.1 μm
- ~700 CCD images/ 29,000 particle images
- Density map at 9.6 Å based on 0.5 FSC criterion

Display of Structure at Medium Resolution

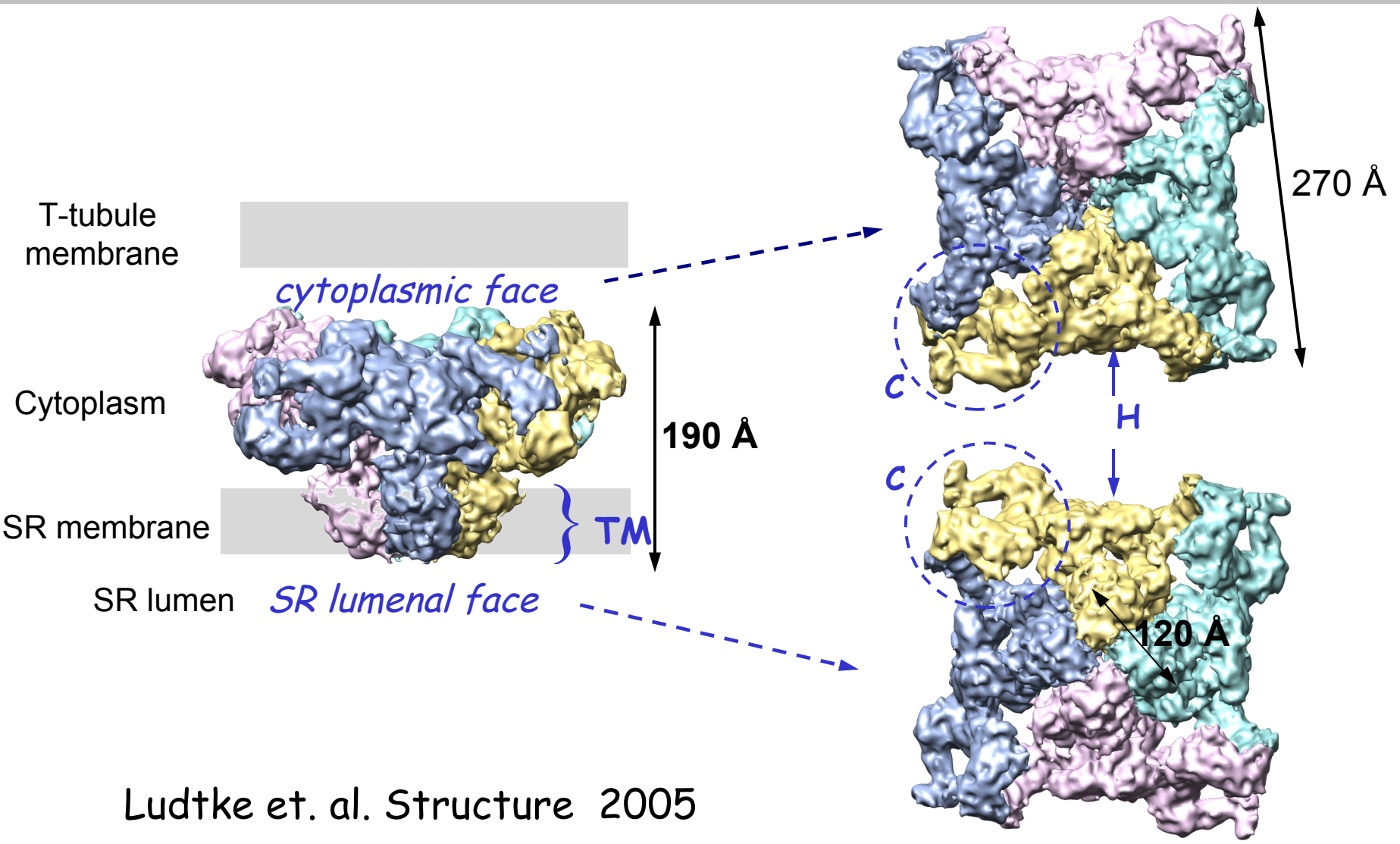


3 Å

7-9 Å

Increasing display threshold

Structure of Ca^{+2} Release Channel in closed conformation



Ludtke et. al. Structure 2005

Steve Ludtke, Irina Serysheva, Susan Hamilton, BCM

Sequence Assignment of Observed Helices

RyR1: 4864 –NKSEDEDEPDMKCDDMMTCYLFHMYVGVRAGGGIGDEIEDPAGDEYELYRVVFDITFFFFVIVILLAIIQGLIIDAFGELRDQQEQVKEDMETK- 4957

Filter — Hinge

M9 — M10

Helix 2 (M4879-E4893) — Helix 1 (I4918-E4948)

KcsA: 36 -QLITYPRALWWSVETATTVGYGDLYPVTLWGRCVAVVVMVAGITSFGLVTAALATWFVGREQ -119

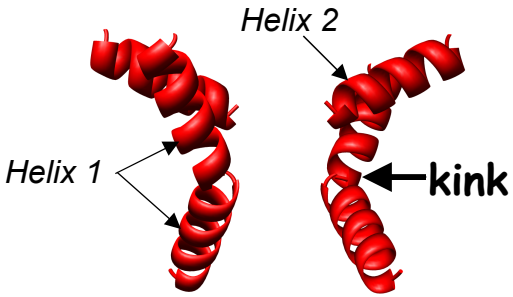
Filter

Pore helix — Inner helix

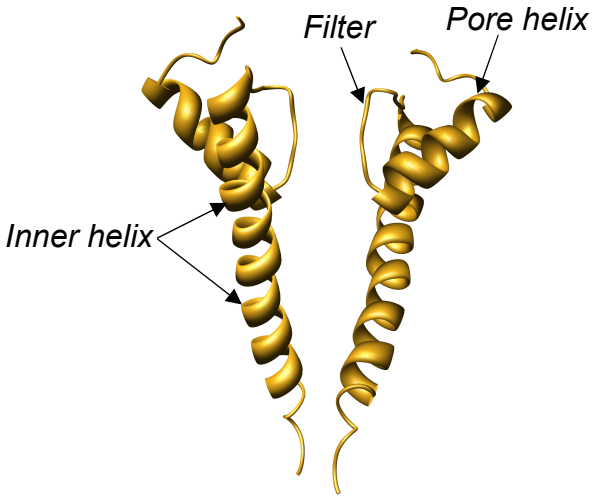
MthK: 45 -SWTVSLYWTFVTIATVGYGDYSPSTPLGMYFTVTLIVLGIGTFAVAVERLLEFLINREQ- 103

Filter — Hinge

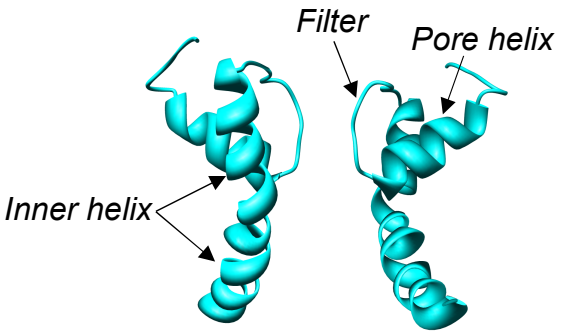
Pore helix — Inner helix



RyR1



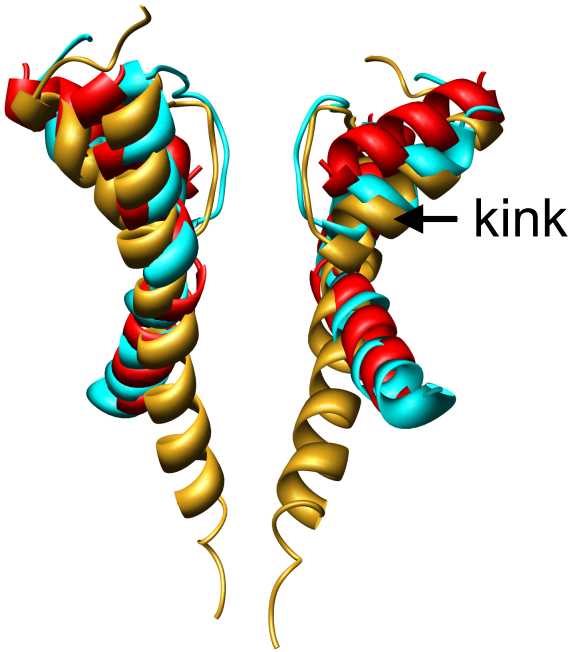
KcsA



MthK

Sequence Assignment of Observed Helices

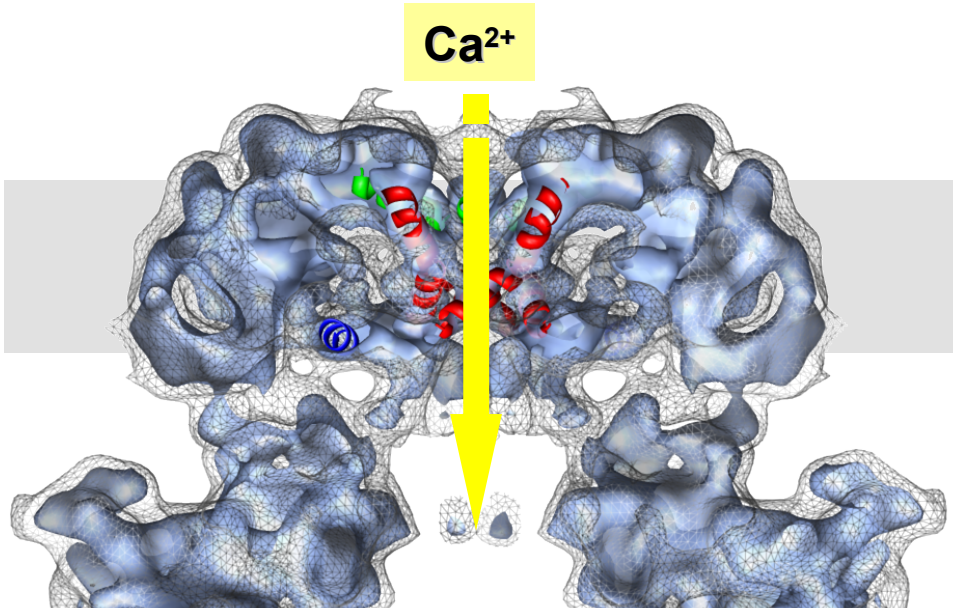
lumenal side (‘out’)

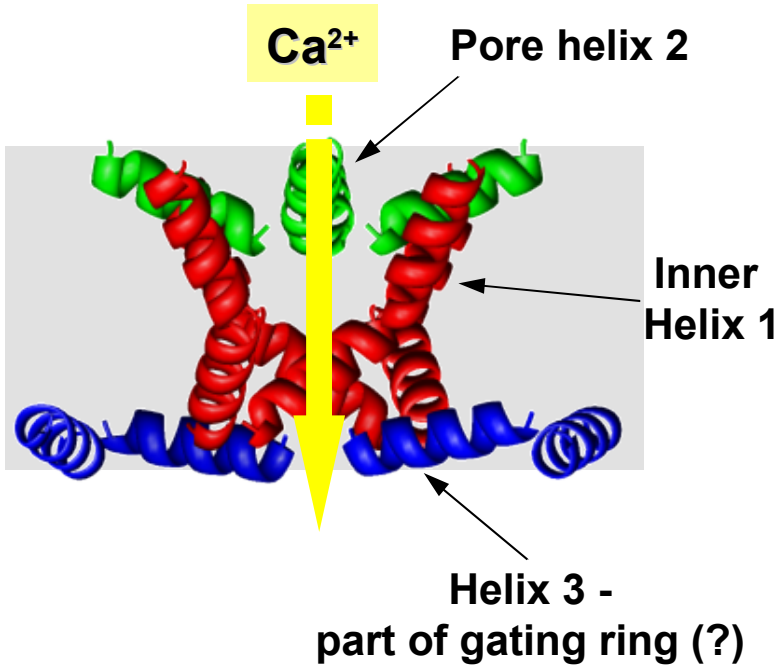


RyR1/KcsA/MthK

Functions of Observed Membrane Helices

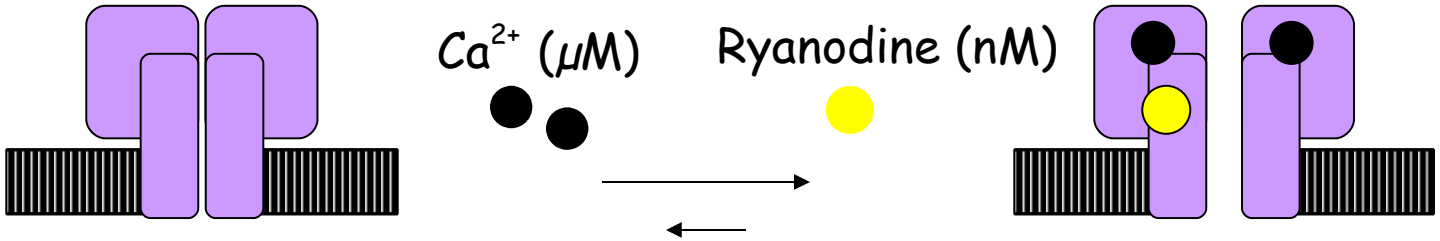
SR lumenal side





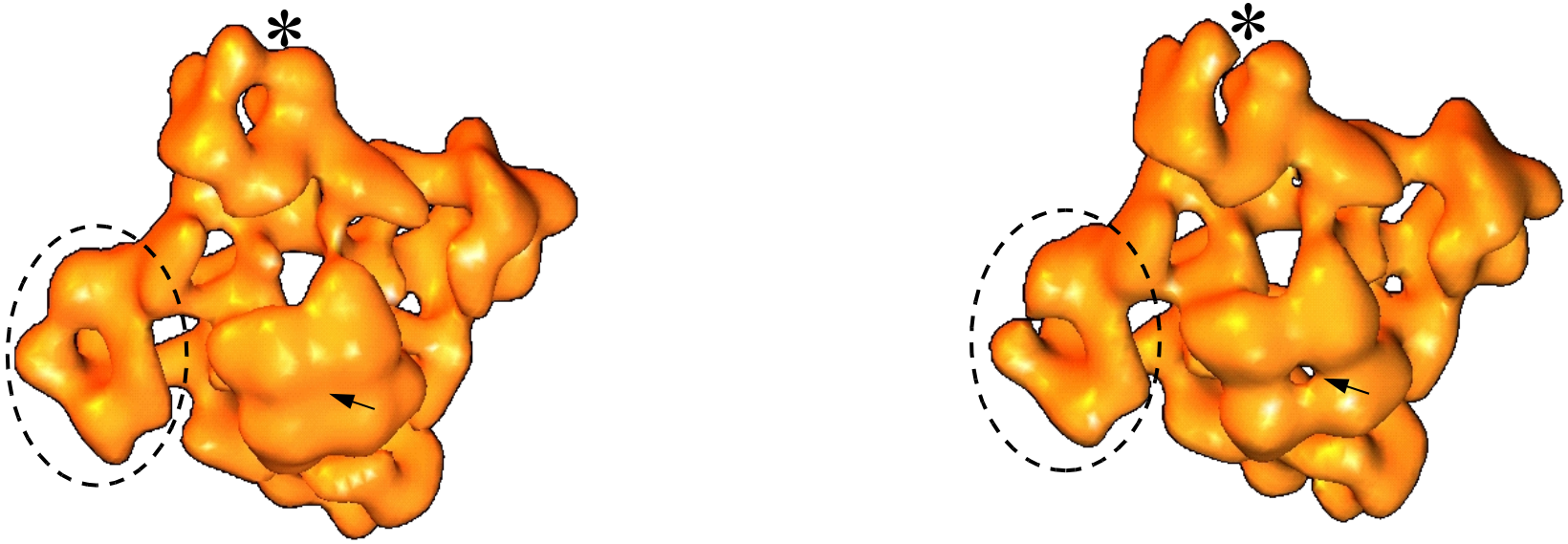
Cytoplasmic side

Conformational Variations of RyR1



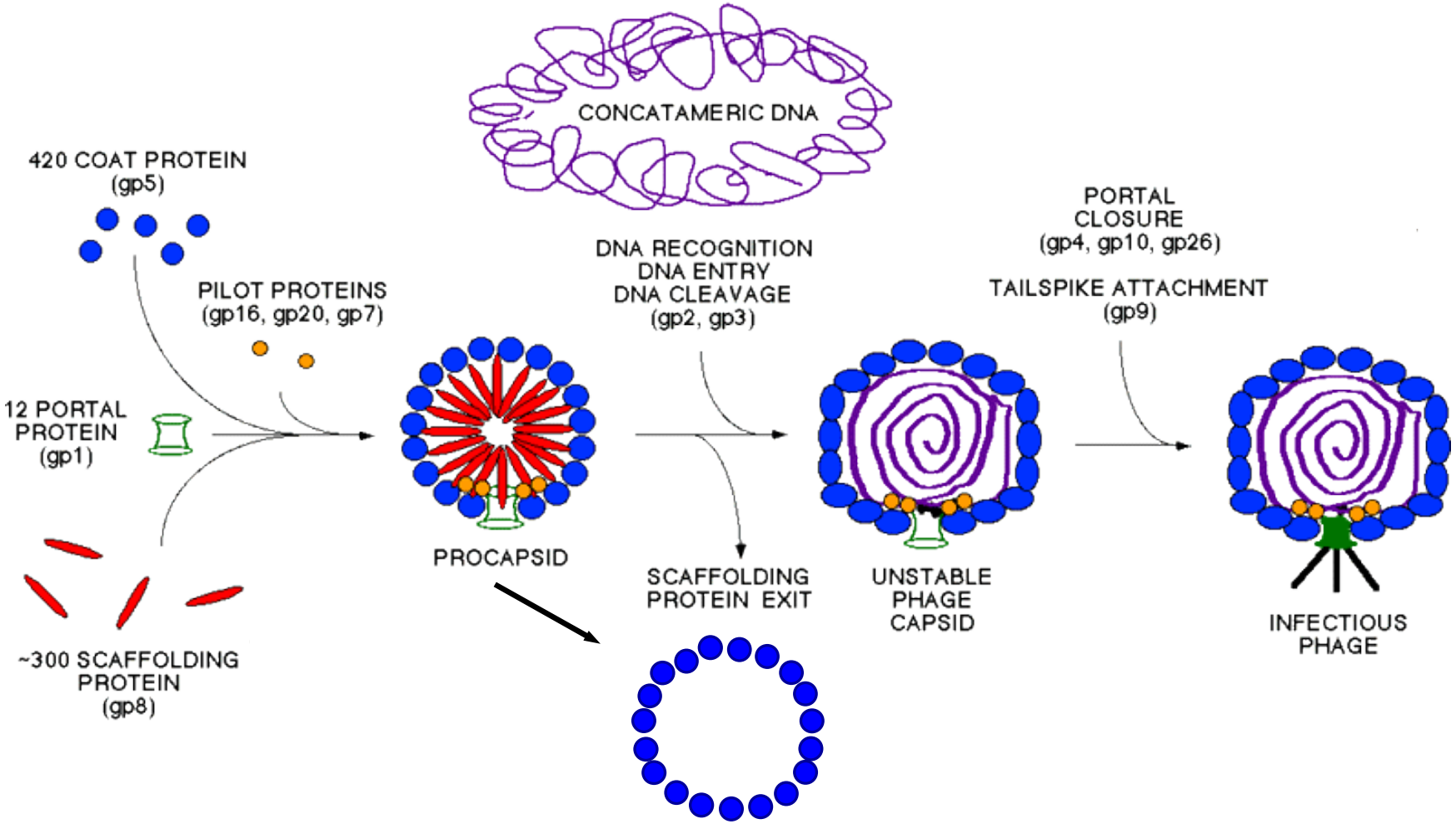
closed

open *



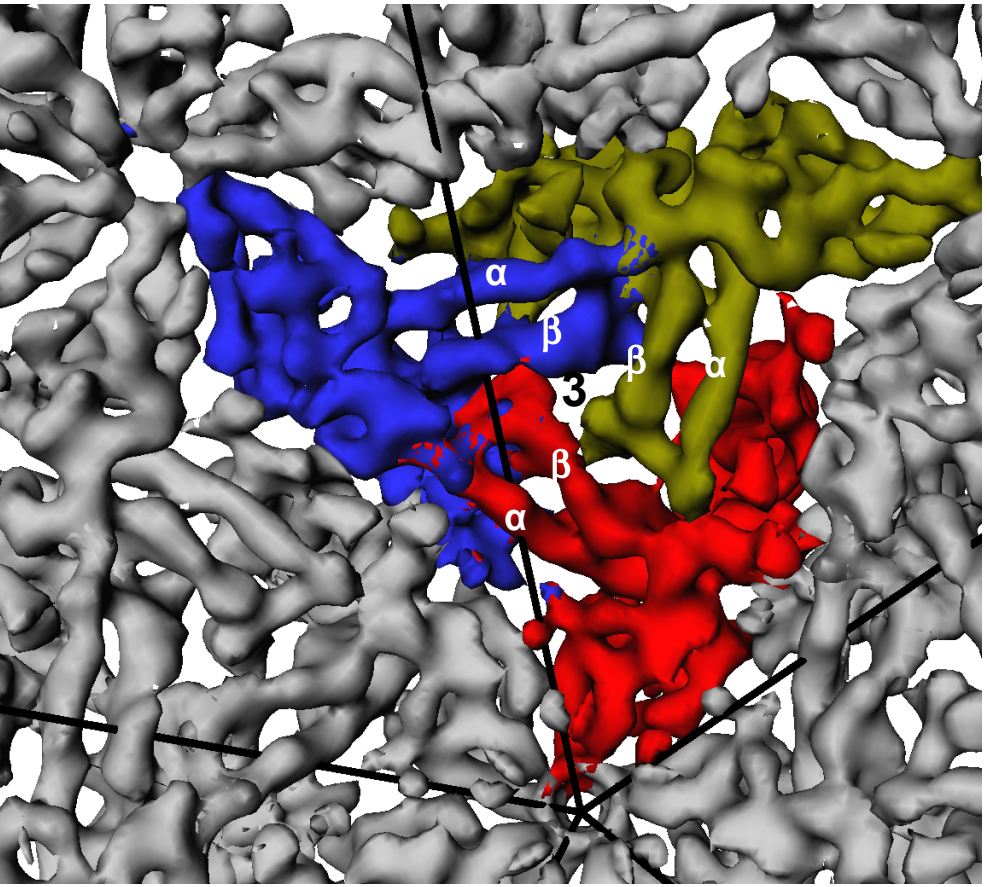
Orlova et.al. NSB 1996

Morphogenesis of Bacteriophage

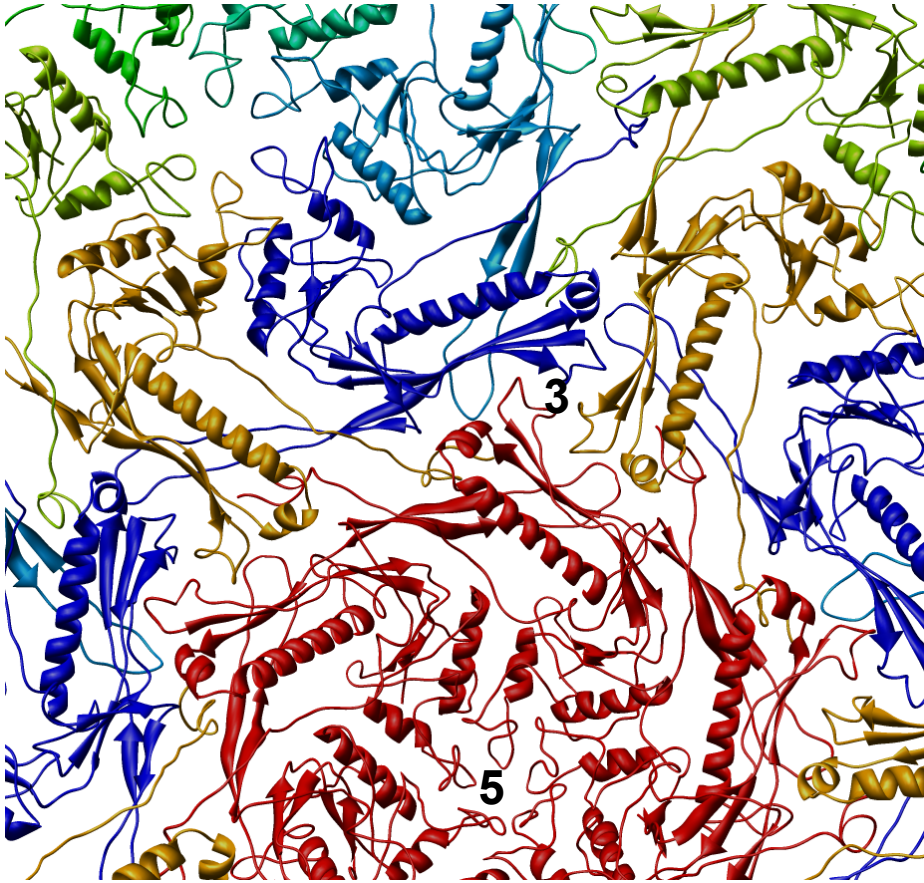


Wen Jiang, Joanita Jakana, Juan Chang (BCM)
Peter Weigele, Jonathan King (MIT)

Common Types of Molecular Interactions in Capsid Shell

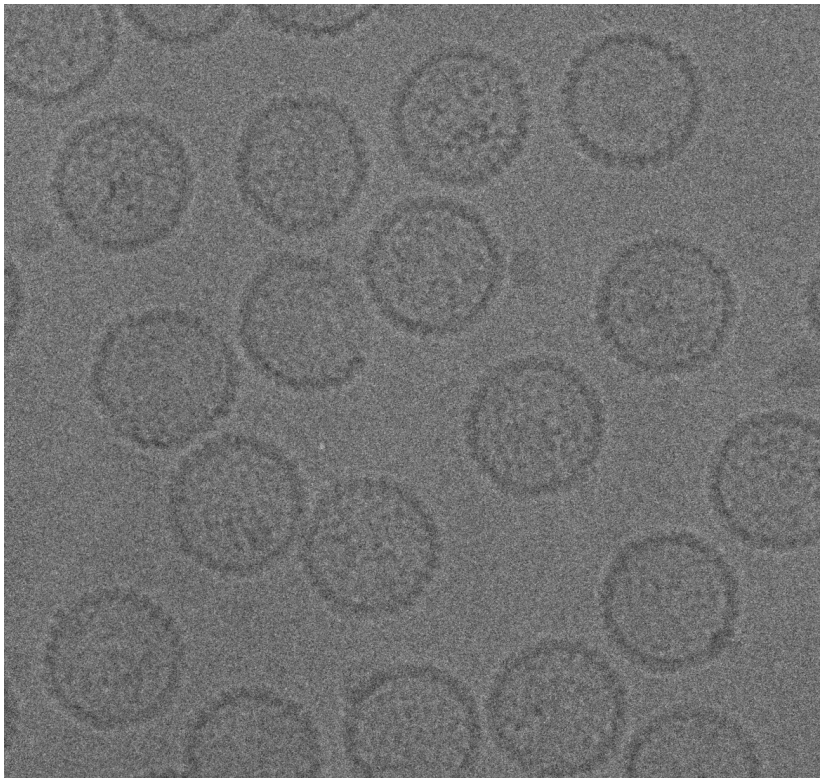


Epsilon15

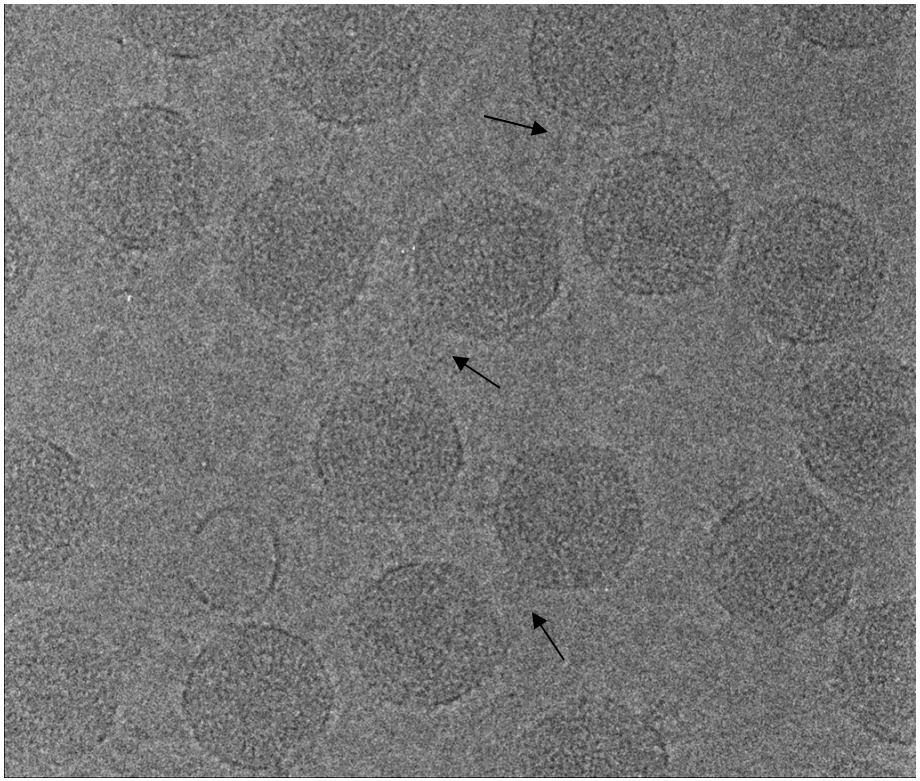


HK97

Images of Bacteriophage P22

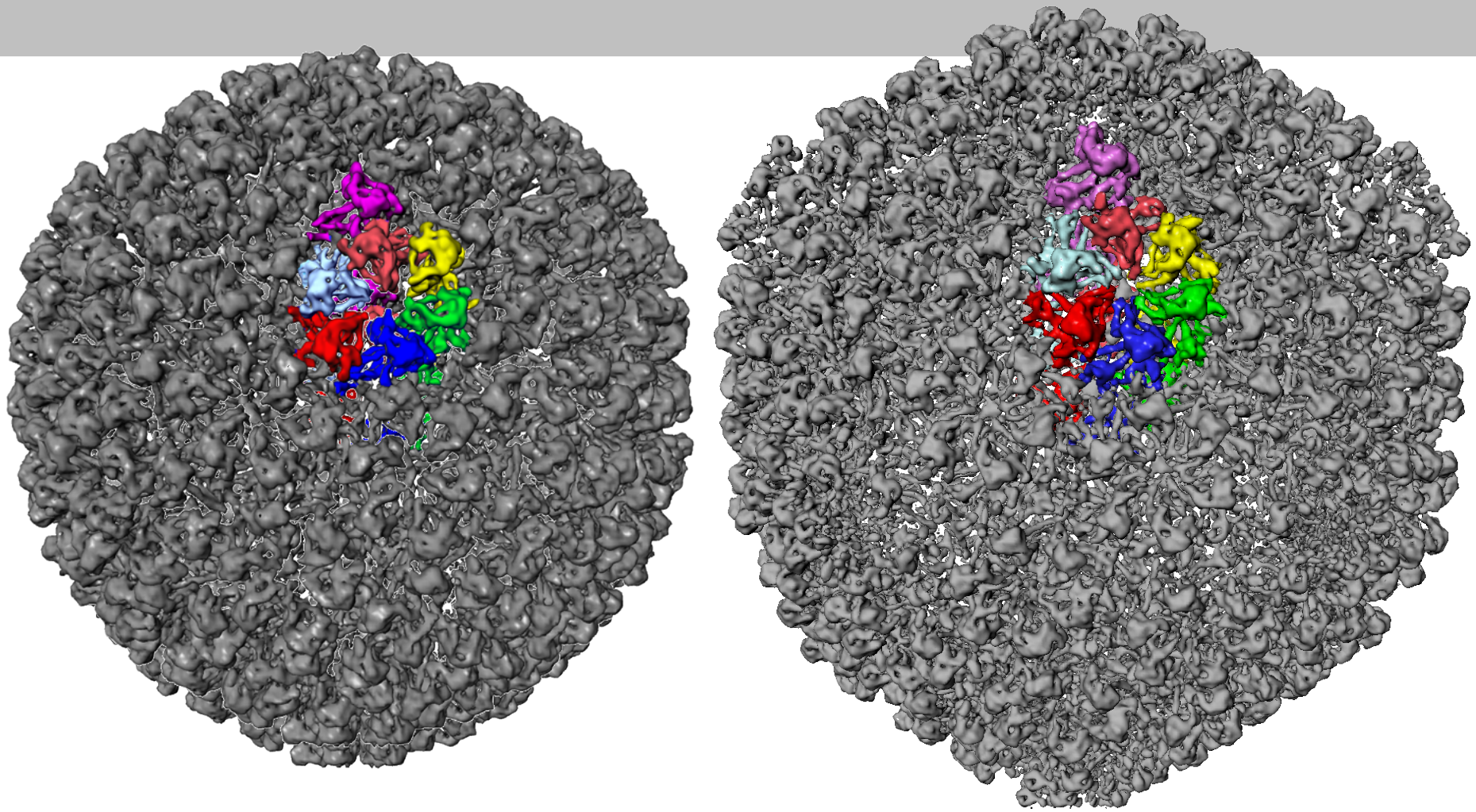


Procapsid shell
Diameter = 585 Å



Mature phage
Diameter = 700 Å

P22 Phage Structures



Procapsid Shell

Mature Phage

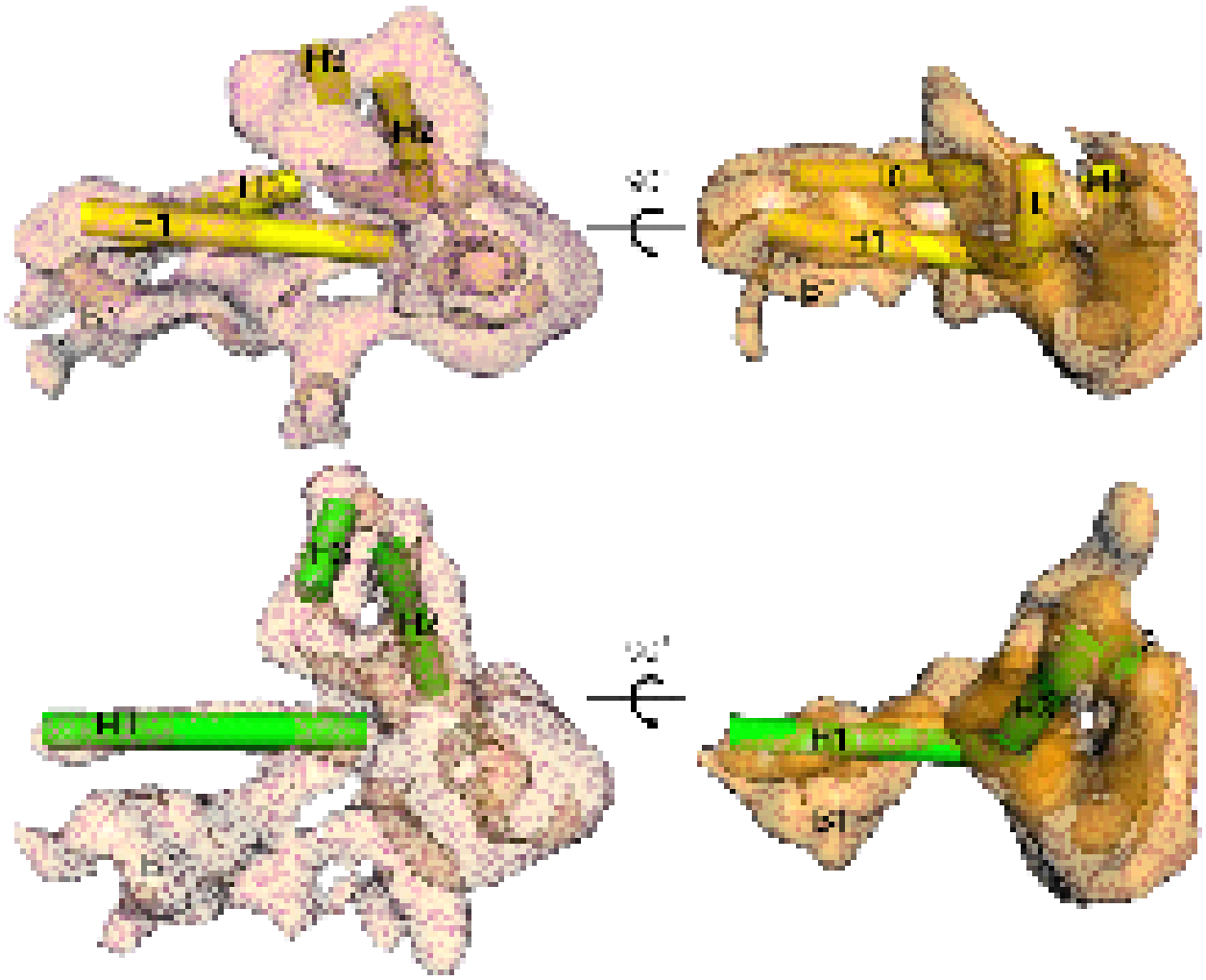
Jiang et al NSB 2003

Wen Jiang, Zongli Li, Zhi Zhang, Mat Baker (BCM)
Peter Prevelige (U Alabama)

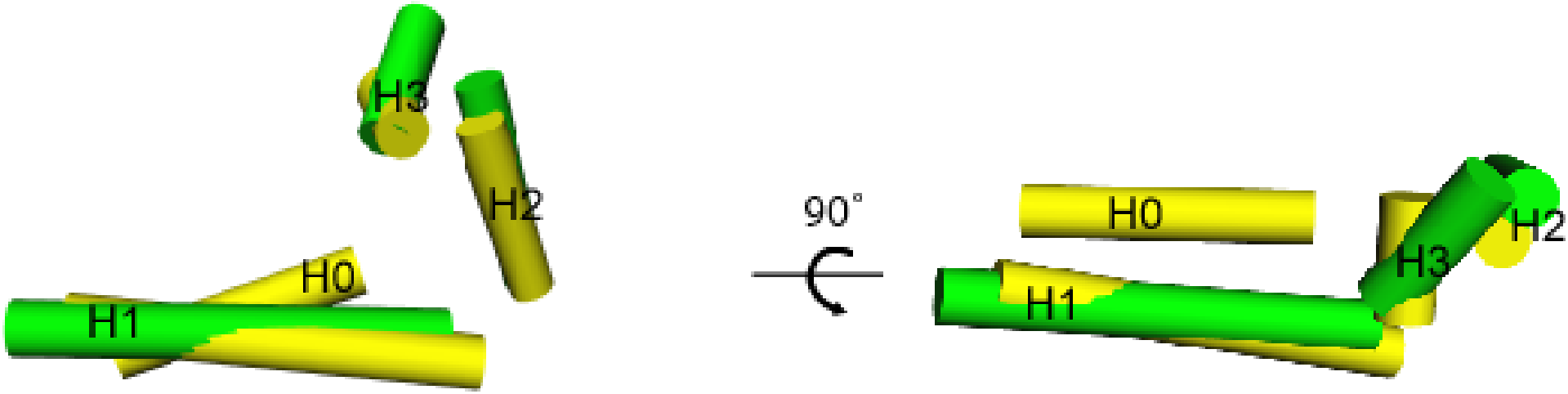
Domain Movements

Procapsid

Mature phage



Helix Movements and Refolding



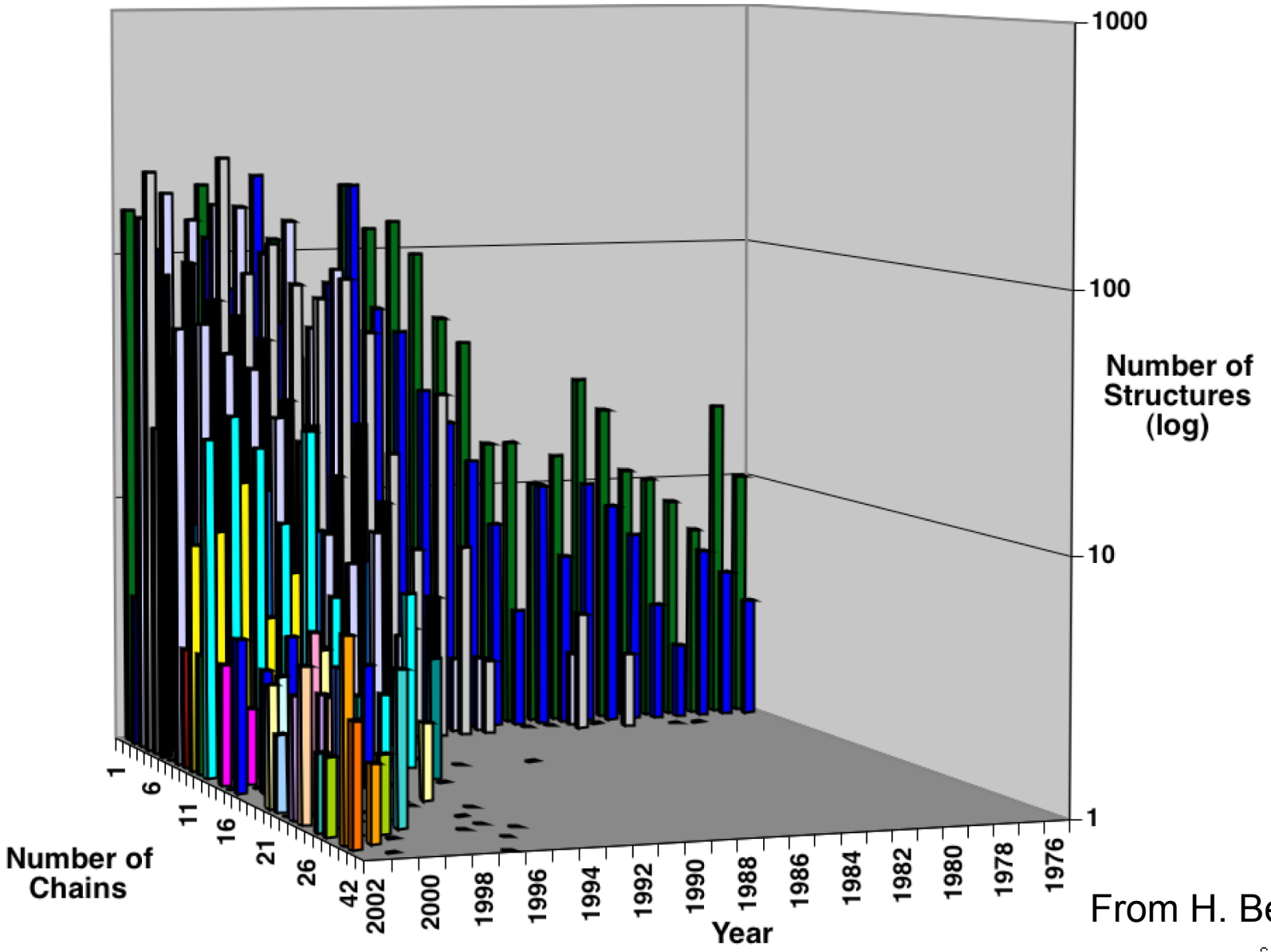
- Helices 1 and 2 < 6° change
- Helices 3 47° rotation
- Helix h0 becomes coil

Mature
Procapsid

Summary: Cryo-EM of Single Particles

- Locations of secondary structure elements of molecular components in a biological machine
- Pseudo atomic models of molecular components
- Structural conformation switches at different functional/chemical states
- Structural mechanism of biological function

Trend in PDB Structures



From H. Berman

Frontiers in Cryo-EM

- Atomic model of machine without using crystals
  - New instrumentation
  - Novel data processing algorithms
  - Biochemical sample purification

Electron Cryomicroscopes at NCMI

JEM2010F

JEM3000SFF

Wen Jiang, Joanita Jakana, Juan Chang (BCM)
Peter Weigele, Jonathan King (MIT)

Frontiers in Cryo-EM

- Atomic model of machine without using crystals
- Dynamics and instability of machines

  Conformational heterogeneity

  Short life time

Frontiers in Cryo-EM

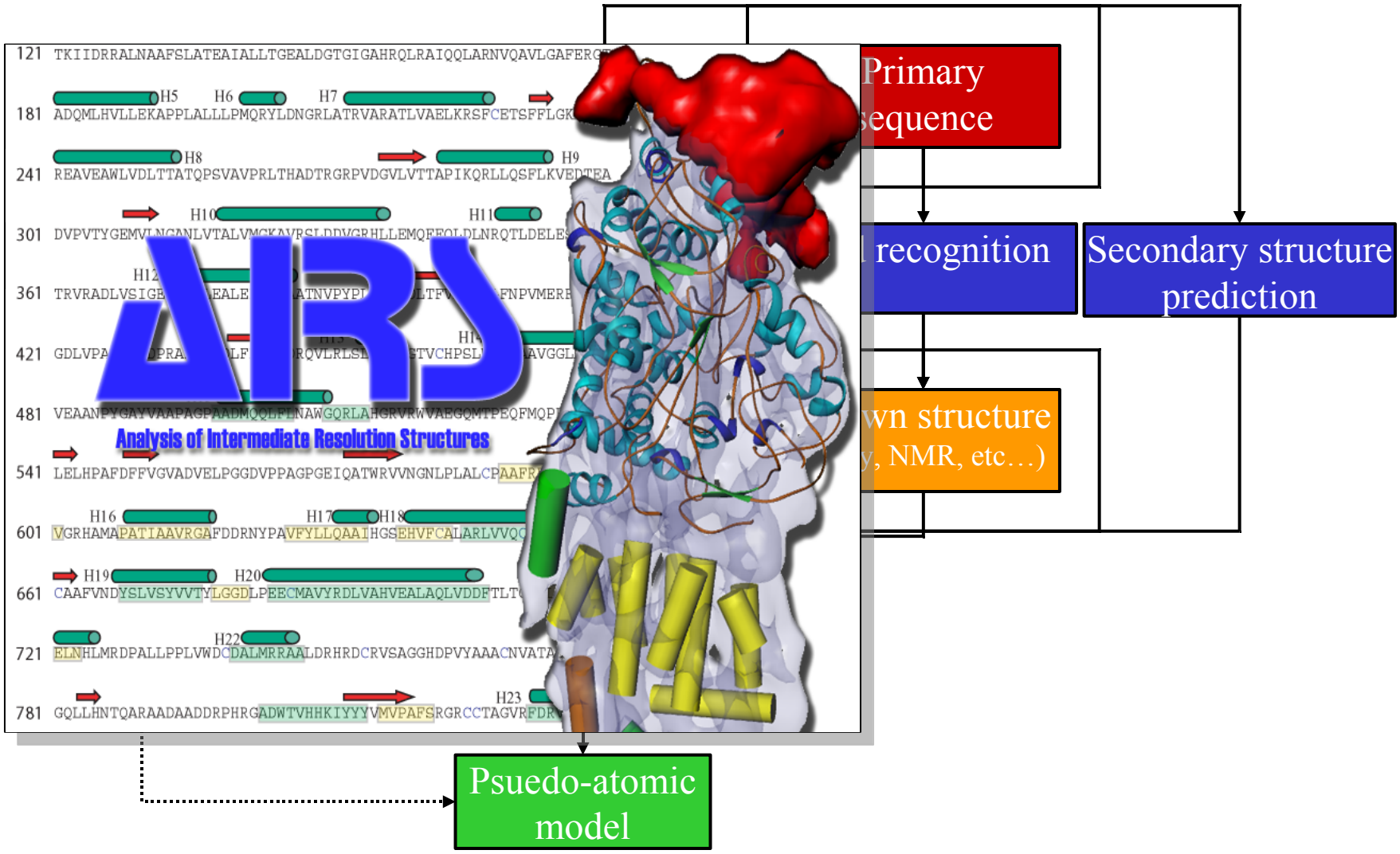
- Atomic model of machine without using crystals
- Dynamics and instability of machines
- Combine cryo-EM data with other experimental and computational data

  Integration with Bioinformatics

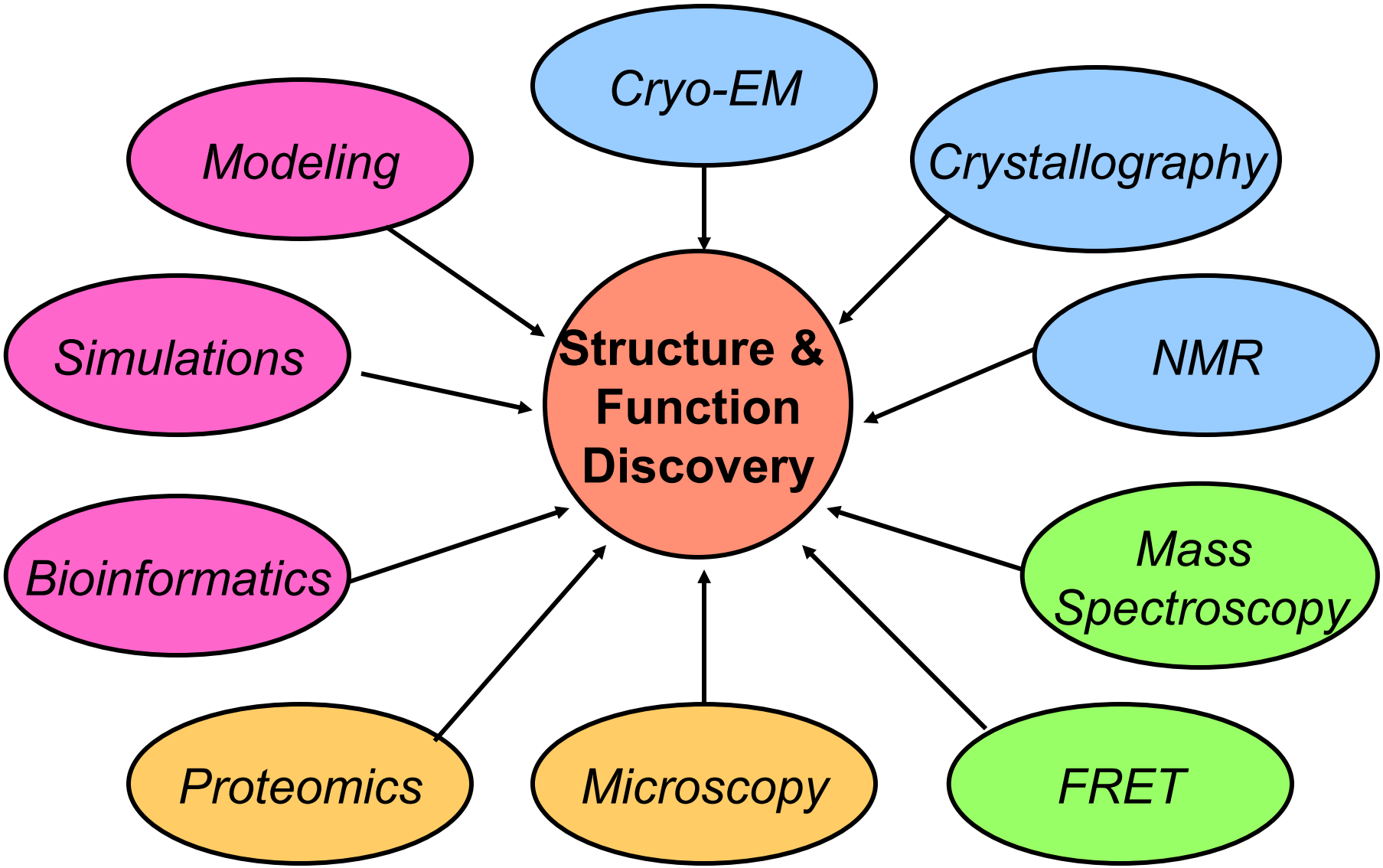
  Modeling with cryo-EM restraints

  Integration of heterogeneous data type

Hybrid cryo-EM with Other Data



Data Integration

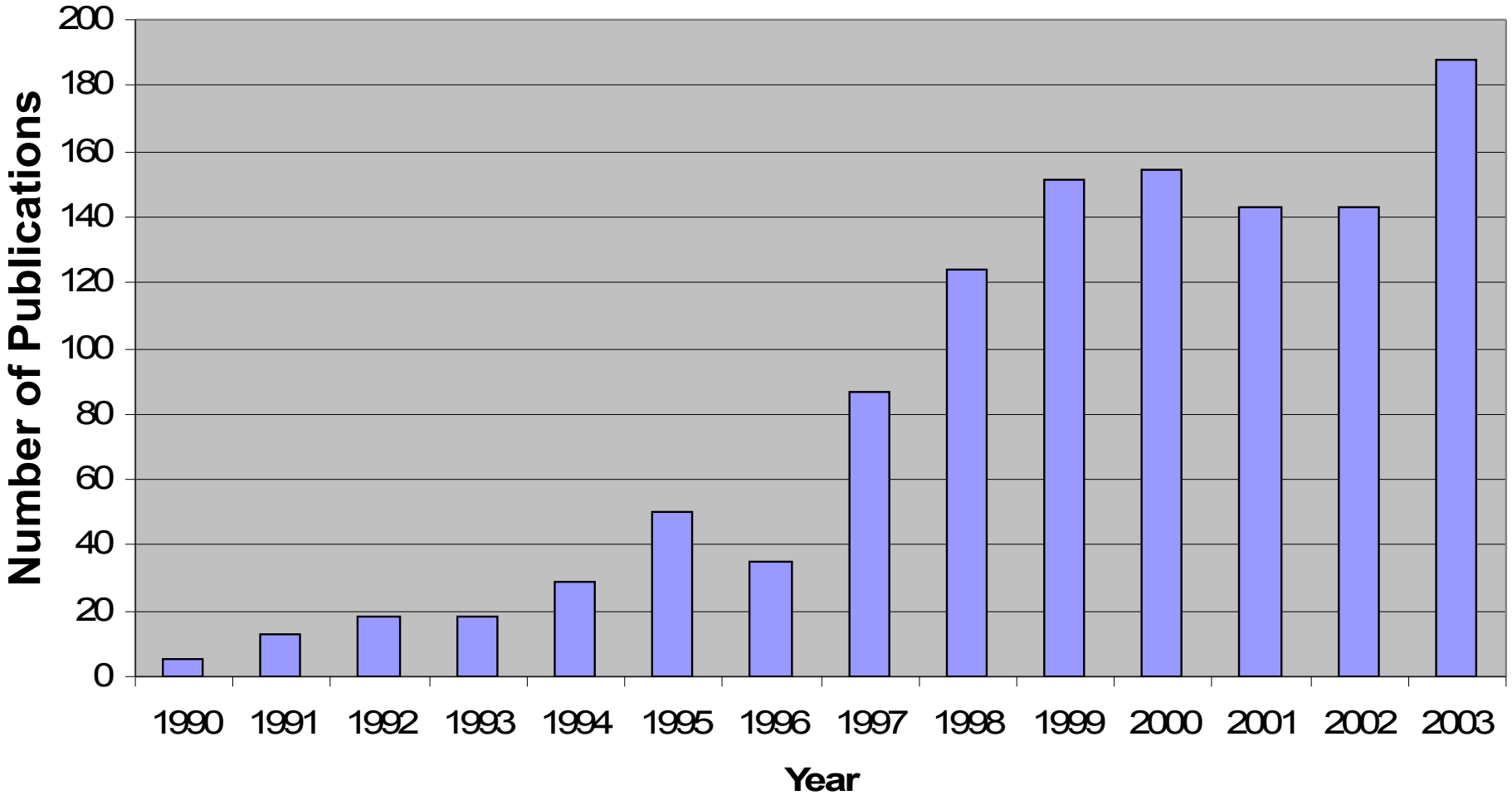


Frontiers in Cryo-EM

- Atomic model of machine without using crystals
- Dynamics and stability of machines
- Combine cryo-EM data with other experimental and computational data
- Archiving Cryo-EM structures

Visualization and annotation

Trends in Cryo-EM Publications



Frontiers in Cryo-EM

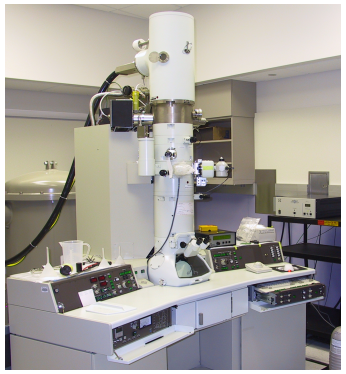
- Atomic details of machine without using crystals
- Dynamics and stability of machines
- Combine cryoEM data with other experimental and computational data
- Archiving CryoEM structures
- CryoEM for the novice

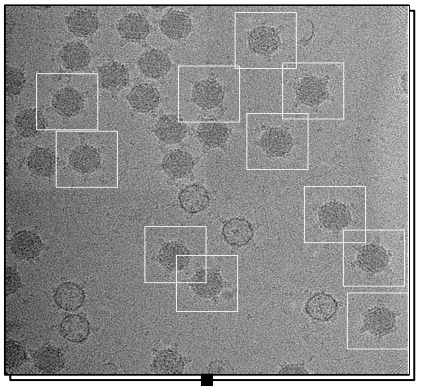
  Automated cryo-specimen preparation, data collection, image processing and structural interpretation

Automation in Molecular Cryo-EM

biochemical preparation

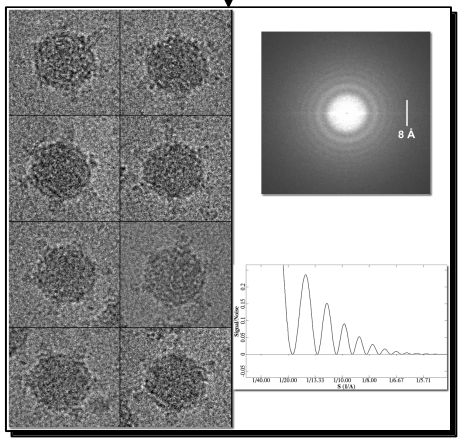
Automated acquisition and particle selection

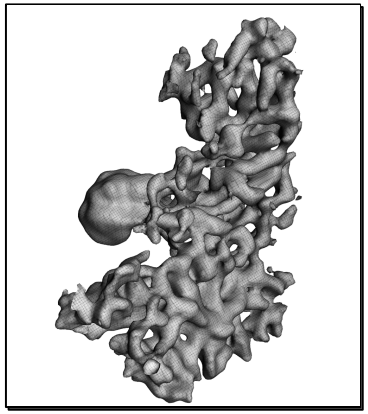


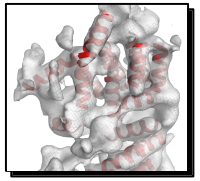


Automated structure determination

Automated discovery







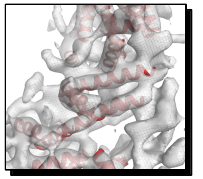


image processing

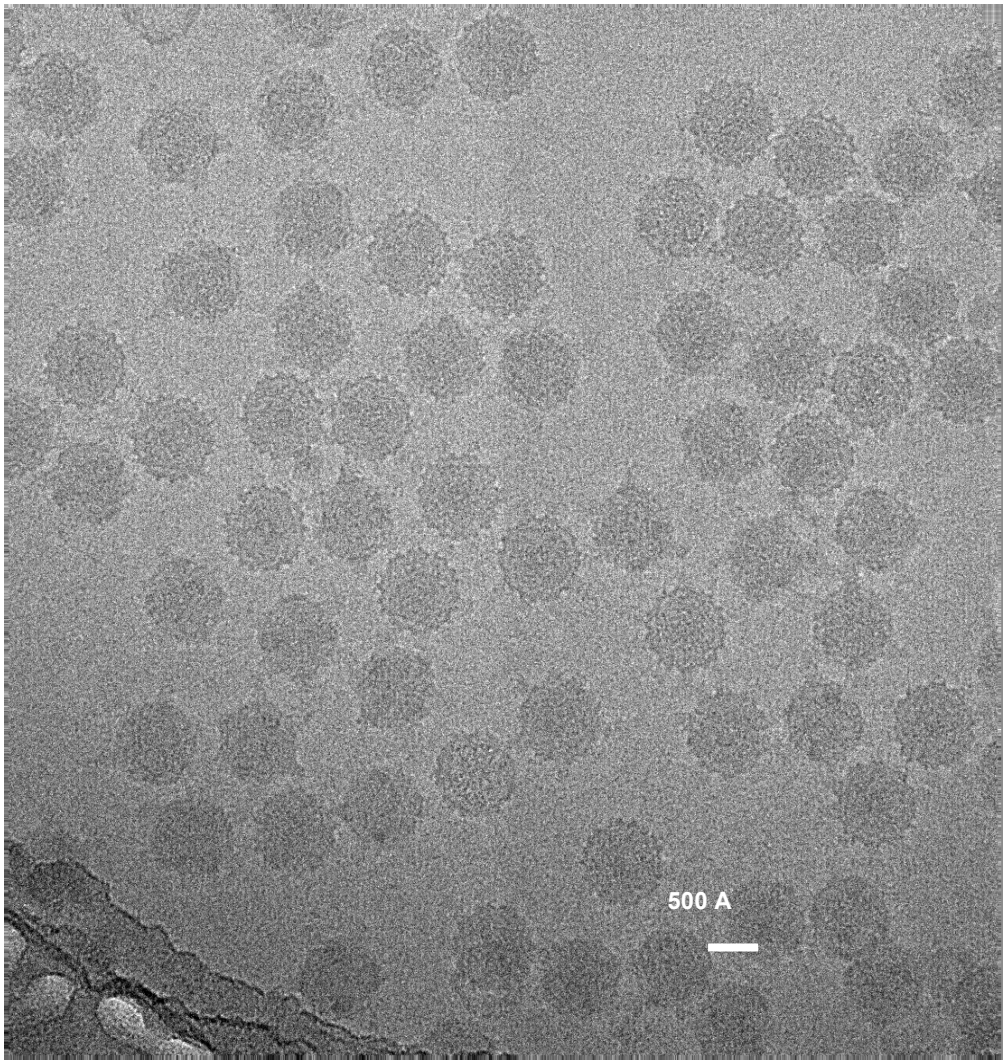
reconstruction

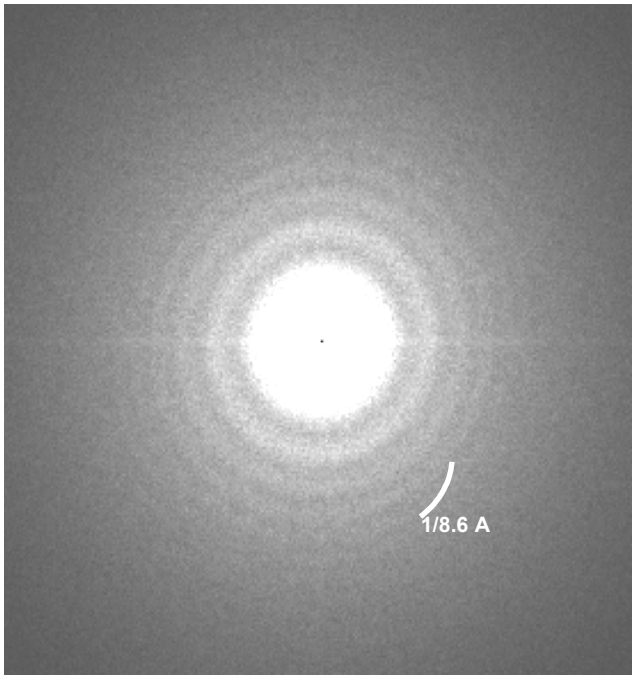
structural analysis

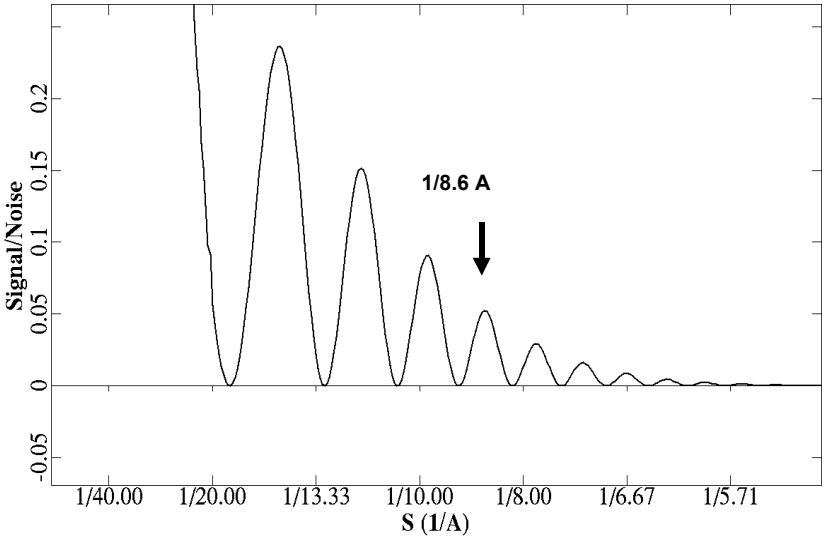
annotation

JEOL 2010F FasTEM + Gatan 4kX4k CCD

CCD Image of CPV Power Spectrum

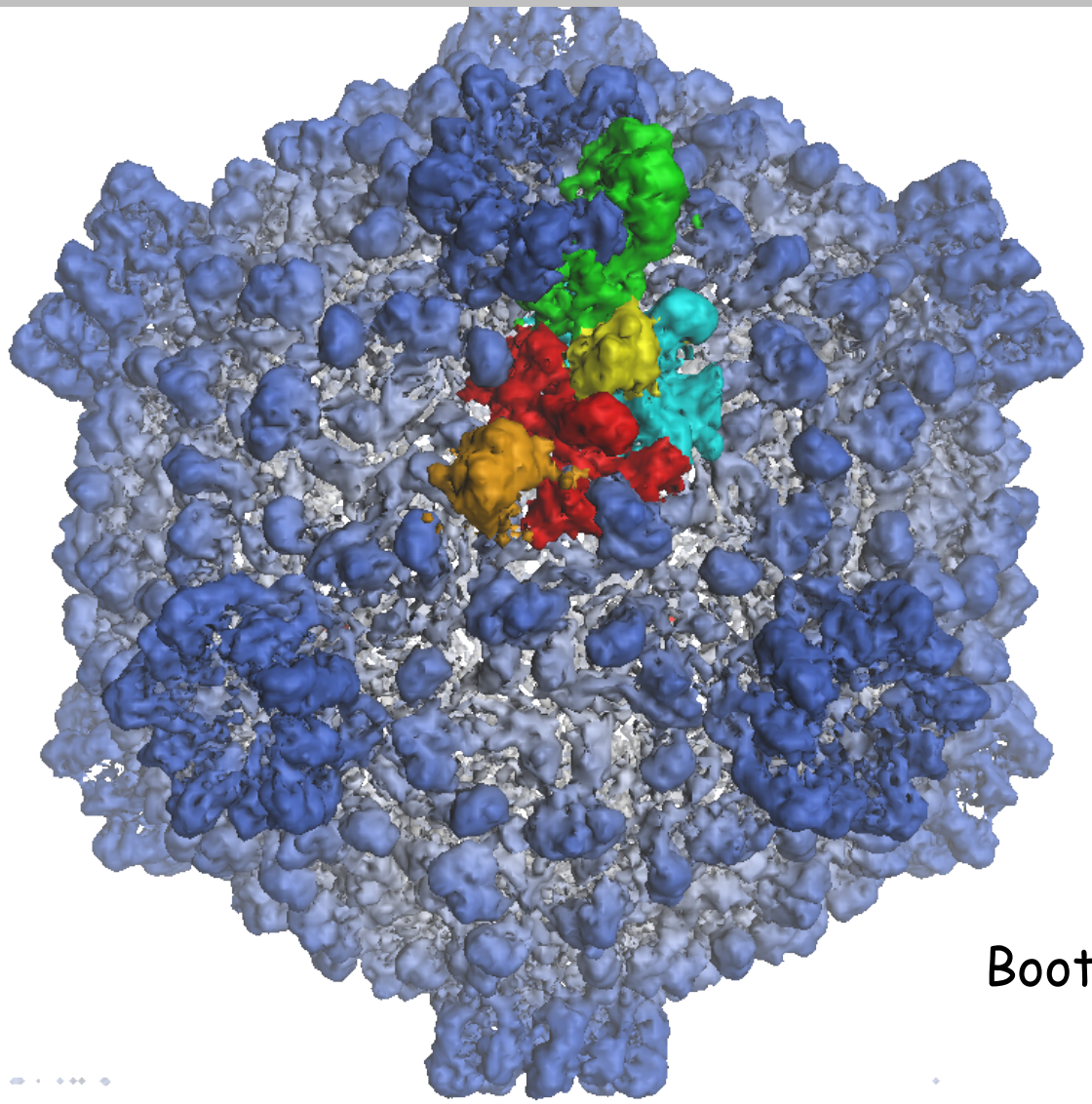






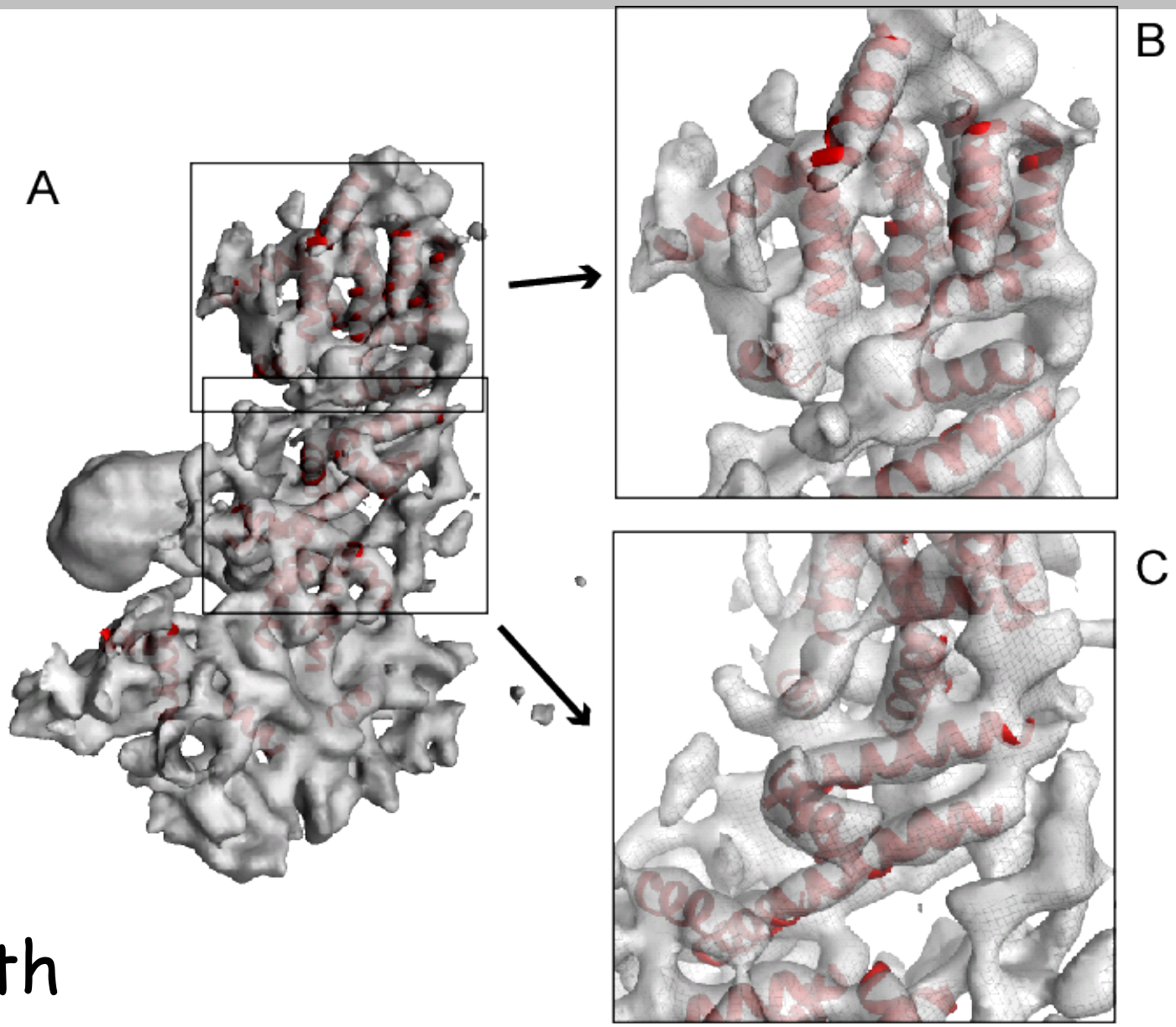
C. Booth

9 Å Structure of CPV



Booth JSB 2004

Alpha Helices in CSP-A



C Booth

Research Frontiers in Cryo-EM

- Atomic details of machine without using crystals
- Dynamics and stability of machines
- Combine cryo-EM data with other experimental and computational data
- Archiving and disseminating cryo-EM structures
- Cryo-EM for the novice
- Electron cryo-tomography of a whole cell

References

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