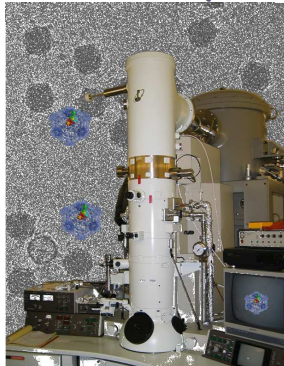




Wah Chiu
 wah@bcm.edu
<http://ncmi.bcm.tmc.edu>



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**

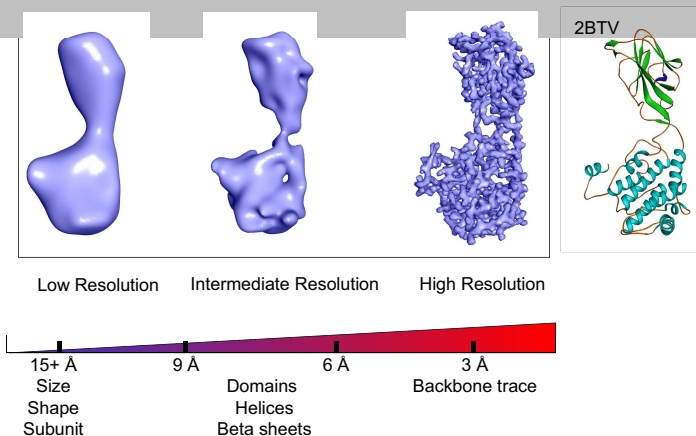
Molecular Cryo-EM

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

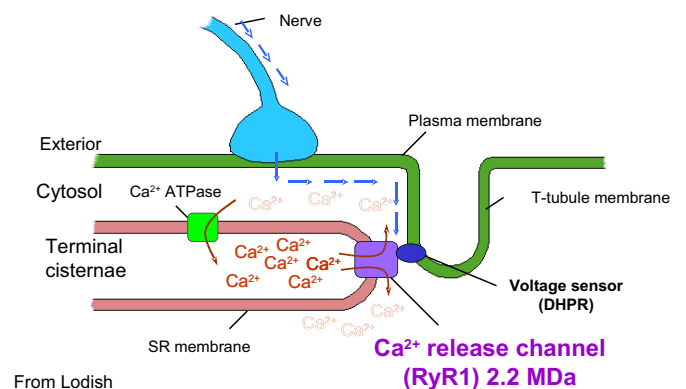
Cryo-EM Achievements

- 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 β sheets visualized
- Subcellular assemblies within a cell: 60 Å identify components and domains

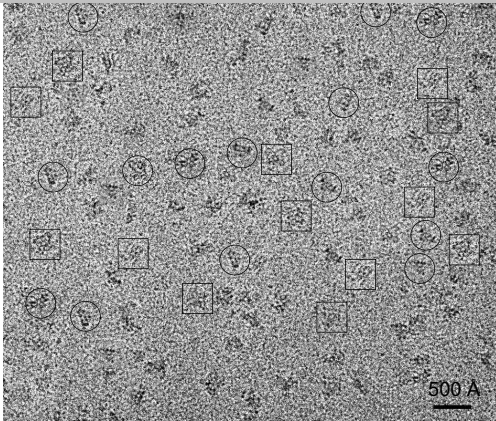
Structure at Different Resolutions



Excitation Contraction Coupling in Skeletal Muscle



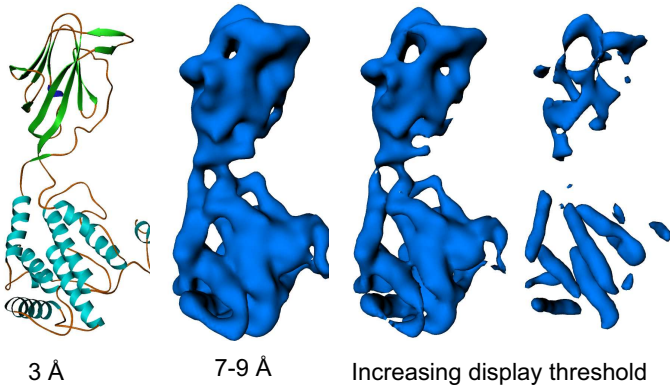
Electron Image of Ca²⁺ Release Channel (2.2 MDa Tetramer)



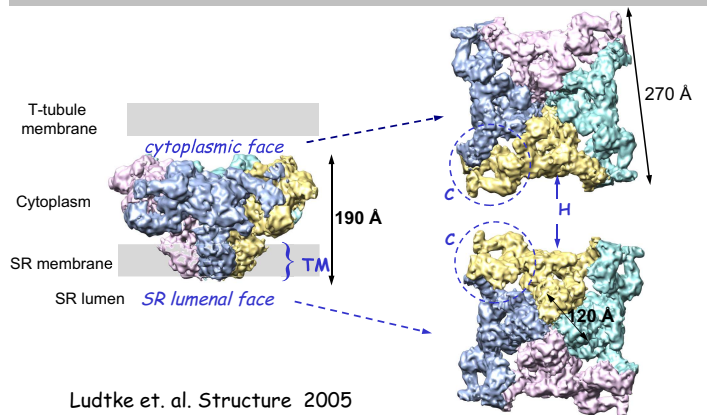
Data Statistics

- Channel in closed conformation, [Ca²⁺] < 10 nM
- 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



Structure of Ca²⁺ Release Channel in closed conformation



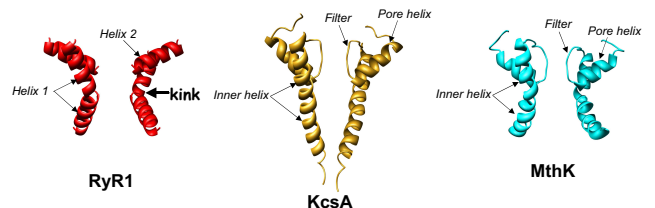
Steve Ludtke, Irina Serysheva, Susan Hamilton, BCM

Sequence Assignment of Observed Helices

RyR1: 4864 - NKSEDEDEPDKCDDMMTCVLFHMVYGVVRAAGGIGDEIEDPAGDEYELVYRVFDITFFVIVILLAAOGLIDAFGELRDQEQVKEDMETK- 4957
 ***** *
 M9 M10
 Helix 2 (M4879-E4893) Helix 1 (M4918-E4948)

KcsA: 36 -QLITYPRALWWSVETATTVYGGDLYPVTLWGRCAVVMVAGITSFGLVTAALATWVFGREQ- 119
 Pore helix Inner helix

MthK: 45 -SWTSLYWFVVTIATVGYGDSPTPLGMVYFTVTLVIGIGTFAVAVERLLEFLINREQ- 103
 Pore helix Inner helix



Sequence Assignment of Observed Helices

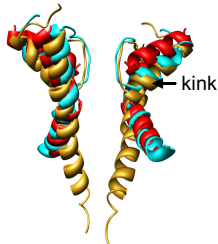
RyR1: 4864 - NKSEDEDEPDKCDDMMTCYLFHMYVGVVRAAGGIGGIDIEDPAGDEYELRVVFDITFFFVILLAIQGLIDAFGELRDQGEQVKEDMETK - 4957

Filter Hinge

M9 M10

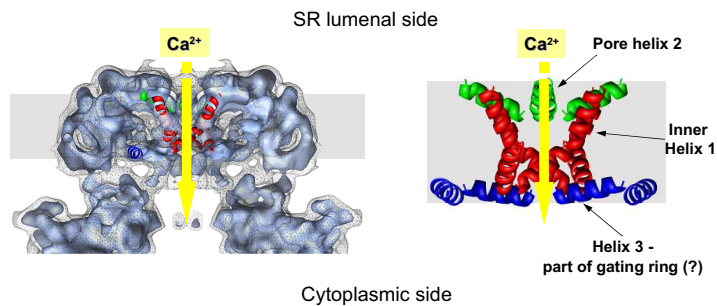
Helix 2 (M4879-E4893) Helix 1 (M4918-E4948)

luminal side ('out')

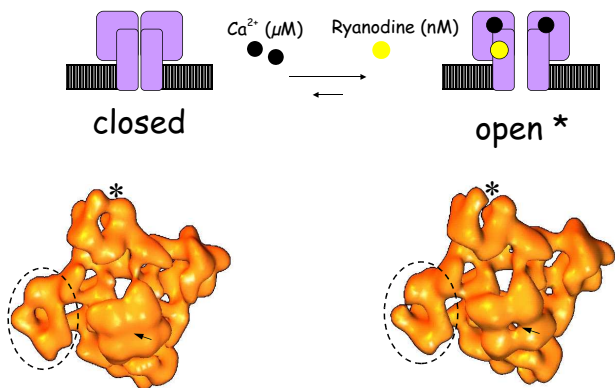


RyR1/KcsA/MthK

Functions of Observed Membrane Helices



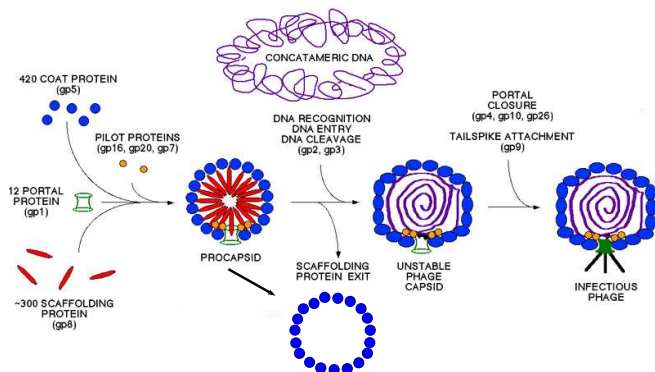
Conformational Variations of RyR1



Orlova et.al. NSB 1996

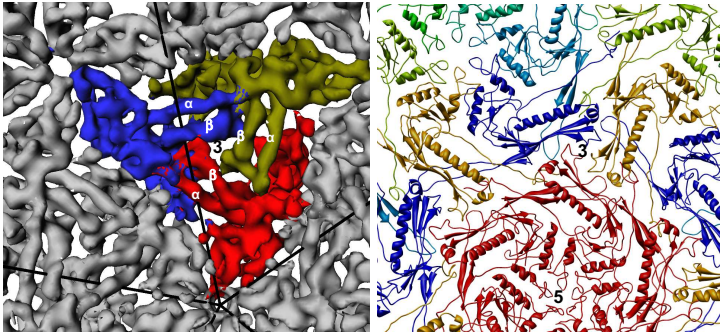


Morphogenesis of Bacteriophage



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

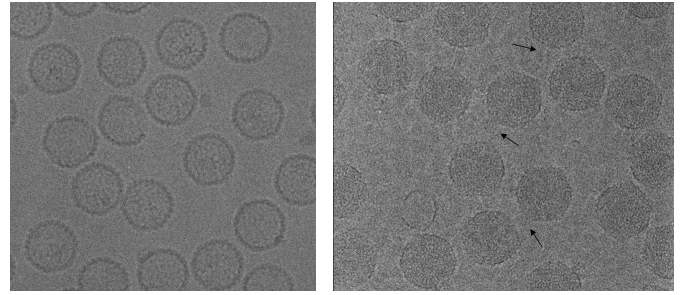
Common Types of Molecular Interactions in Capsid Shell



Epsilon15

HK97

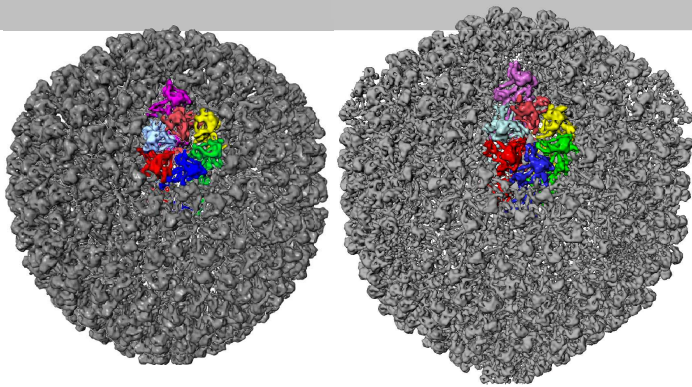
Images of Bacteriophage P22



Procapsid shell
Diameter = 585 Å

Mature phage
Diameter = 700 Å

P22 Phage Structures



Procapsid Shell

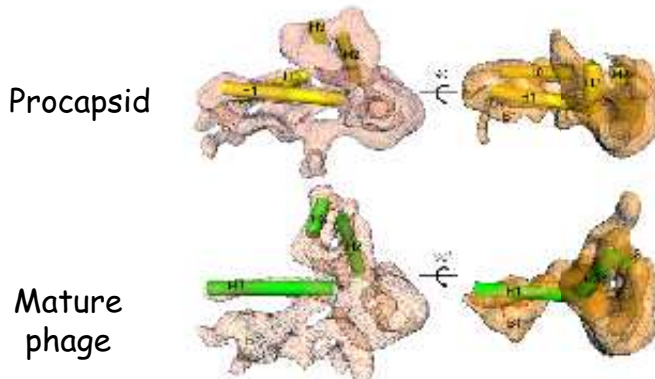
Mature Phage

Jiang et al NSB 2003



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

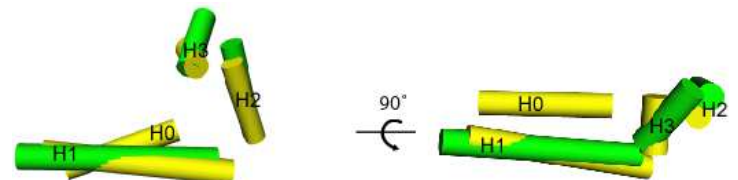
Domain Movements



Procapsid

Mature phage

Helix Movements and Refolding



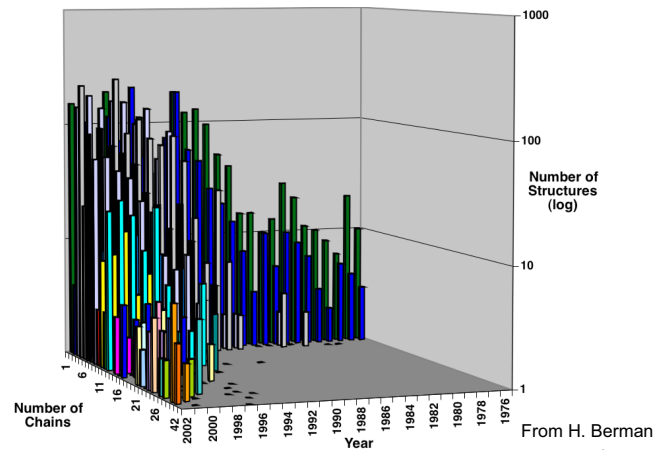
- Helices 1 and 2 < 6° change
- Helices 3 47° rotation
- Helix h0 becomes coil

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

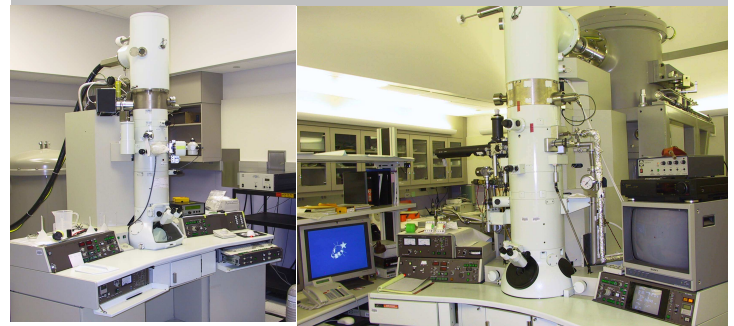
Trend in PDB Structures



Frontiers in Cryo-EM

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

Electron Cryomicroscopes at NCMi

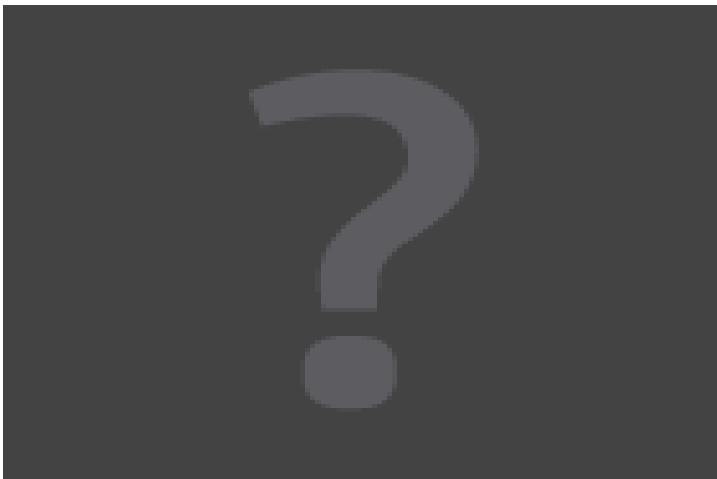


JEM2010F

JEM3000SFF

Frontiers in Cryo-EM

- Atomic model of machine without using crystals
- Dynamics and instability of machines
 - Conformational heterogeneity
 - Short life time



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

Frontiers in Cryo-EM

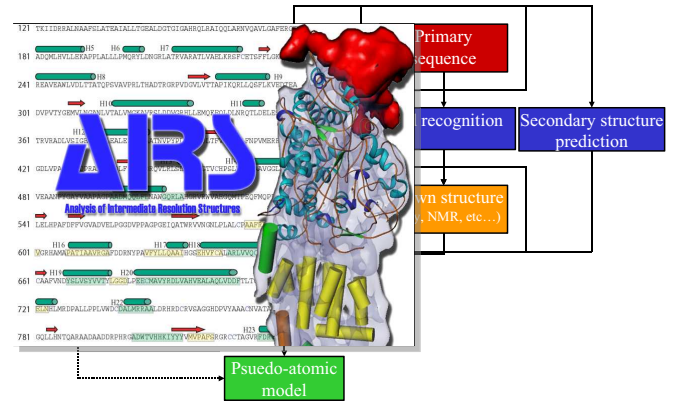
- Atomic model of machine without using crystals
- Dynamics and instability of machines
- Combine cryo-EM data with other experimental and computational data

Integration with Bioinformatics

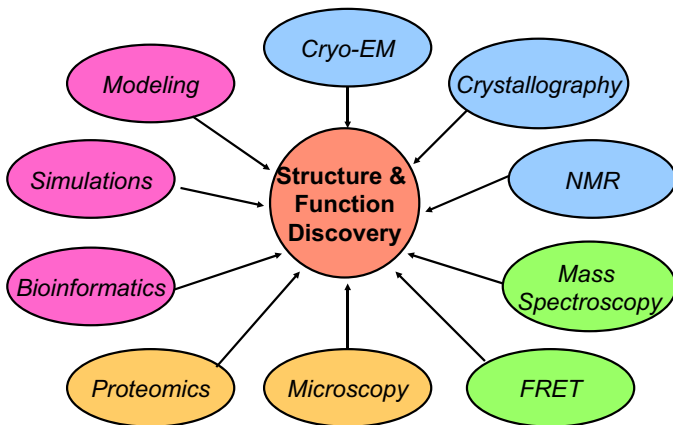
Modeling with cryo-EM restraints

Integration of heterogeneous data type

Hybrid cryo-EM with Other Data



Data Integration

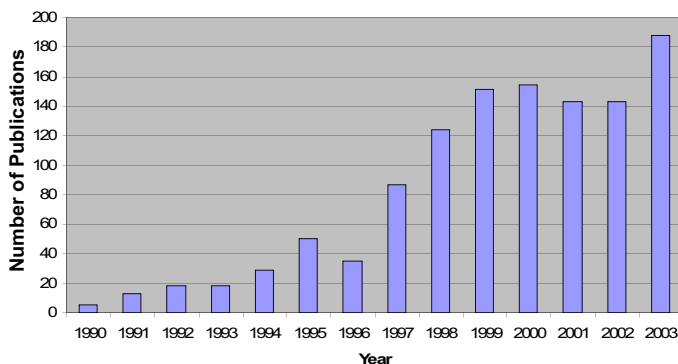


Frontiers in Cryo-EM

- 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

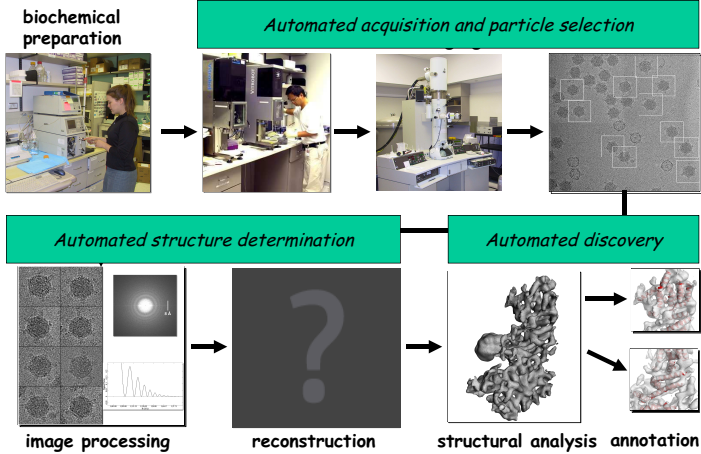


Frontiers in Cryo-EM

- 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

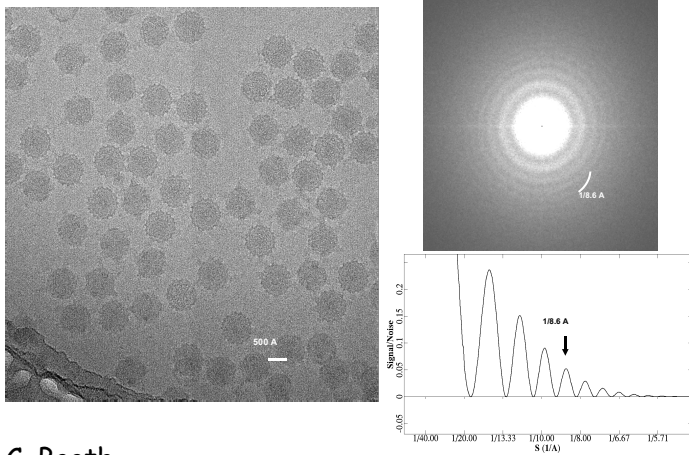
Automation in Molecular Cryo-EM



JEOL 2010F FasTEM + Gatan 4kX4k CCD

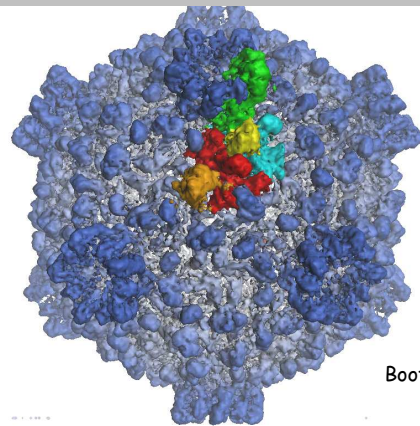


CCD Image of CPV Power Spectrum



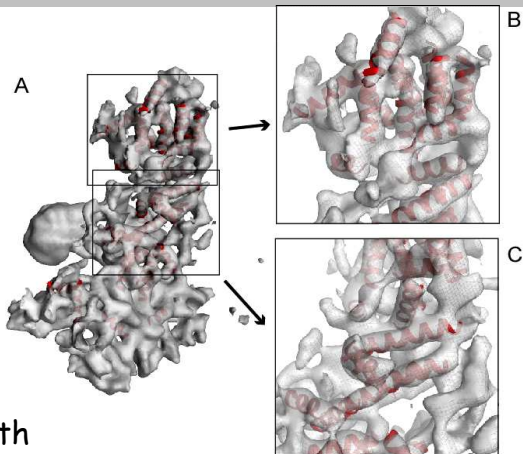
C. Booth

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

- Chiu, W., M.L. Baker, W. Jiang, M. Dougherty, and M.F. Schmid. 2005. Electron cryomicroscopy of biological machines at subnanometer resolution. *Structure (Camb)*. 13:363-72.
- Jiang, W., and S.J. Ludtke. 2005. Electron cryomicroscopy of single particles at subnanometer resolution. *Curr Opin Struct Biol*. 15:571-7.
- Ludtke, S.J., Serysheva, II, S.L. Hamilton, and W. Chiu. 2005. The pore structure of the closed RyR1 channel. *Structure (Camb)*. 13:1203-11.
- Topf, M., M.L. Baker, B. John, W. Chiu, and A. Sali. 2005. Structural characterization of components of protein assemblies by comparative modeling and electron cryo-microscopy. *J Struct Biol*. 149:191-203.
- Dutta, S., and H.M. Berman. 2005. Large macromolecular complexes in the Protein Data Bank: a status report. *Structure (Camb)*. 13:381-8.

References

- Carragher, B., D. Fellmann, F. Guerra, R.A. Milligan, F. Mouche, J. Pulokas, B. Sheehan, J. Quispe, C. Suloway, Y. Zhu, and C.S. Potter. 2004. Rapid routine structure determination of macromolecular assemblies using electron microscopy: current progress and further challenges. *J Synchrotron Radiat*. 11:83-5.
- Booth, C.R., W. Jiang, M.L. Baker, Z. Hong Zhou, S.J. Ludtke, and W. Chiu. 2004. A 9Å single particle reconstruction from CCD captured images on a 200kV electron cryomicroscope. *J Struct Biol*. 147:116-27.
- Baumeister, W. 2004. Mapping molecular landscapes inside cells. *Biol Chem*. 385:865-72.
- Jiang, W., Z. Li, Z. Zhang, M.L. Baker, P.E. Prevelige, and W. Chiu. 2003. Coat protein fold and maturation transition of bacteriophage P22 seen at sub-nanometer resolutions. *Nat Struct Biol* 10:131-135.
- Orlova, E.V., Serysheva, II, M. van Heel, S.L. Hamilton, and W. Chiu. 1996. Two structural configurations of the skeletal muscle calcium release channel. *Nat Struct Biol* 3:547-52.