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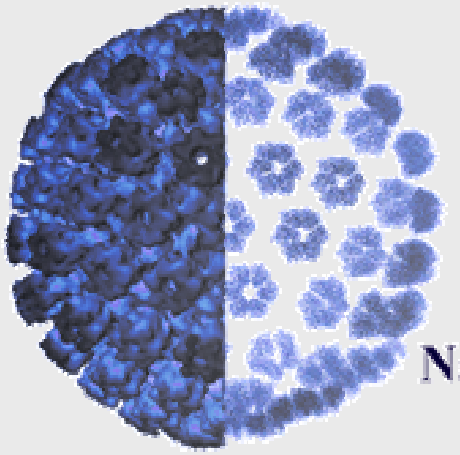
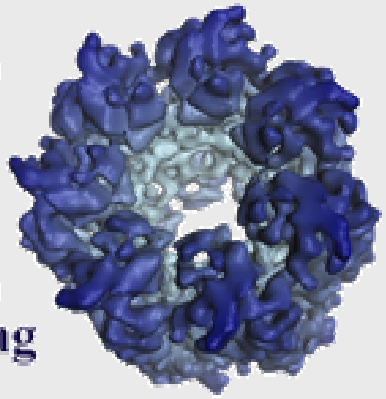


NCMI

National Center for Macromolecular Imaging



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National Center for Research Resources

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

Future of Cryo-EM

- What Has Cryo-EM Achieved ?
- What are the funding opportunities ?
- What are the trends in biomedicine ?
- A technology easily practiced by biologists
- Cryo-EM technology development

Electron Cryomicroscopy Achievements

- 2-dimensional monolayer protein crystals: 3.7-3.0 Å
  polypeptide traced
- Helical arrays: 9 - 4 Å
  Fold recognized
- Single particles: 9 – 5.5 Å
  α helices and ß sheets visualized
- Subcellular assemblies : 50 - 20 Å
  identify components and domains

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NIH Roadmap

ACCELERATING MEDICAL DISCOVERY TO IMPROVE HEALTH

- Overview

  Soon after becoming the Director of the National Institutes of Health (NIH), in May 2002, Elias A. Zerhouni, M.D. convened a series of meetings to chart a "roadmap" for medical research in the 21st century. More...

- Press Release
- Press Briefing Video
- *Science* Magazine Article
- NIH Roadmap Initiatives
- Grants and Funding Opportunities

New Pathways to Discovery

- Building Blocks, Biological Pathways, and Networks
- Molecular Libraries and Imaging
- Structural Biology
- Bioinformatics and Computational Biology
- Nanomedicine

Research Teams of the Future

- High-Risk Research
- Interdisciplinary Research
- Public-Private Partnerships

Re-engineering the Clinical Research Enterprise

- Re-engineering the Clinical Research Enterprise

1999 Protein Structure Initiative (PSI)

9 National Centers with the mission to solve 10,000 structures in the next 10 years

New York Structural Genomics Center

~60 Structures Solved

~2500 Models

~45% of All Structures at X9A/B

Goal: 100-200 structures/yr.

Steve Almo

2003 Protein Structure Initiative

Phase II: Membrane Proteins

NIH Roadmap ACCELERATING MEDICAL DISCOVERY TO IMPROVE HEALTH

Structural Biology
- Overview
- Implementation Group Members
- Grants and Funding Opportunities

Structural Biology

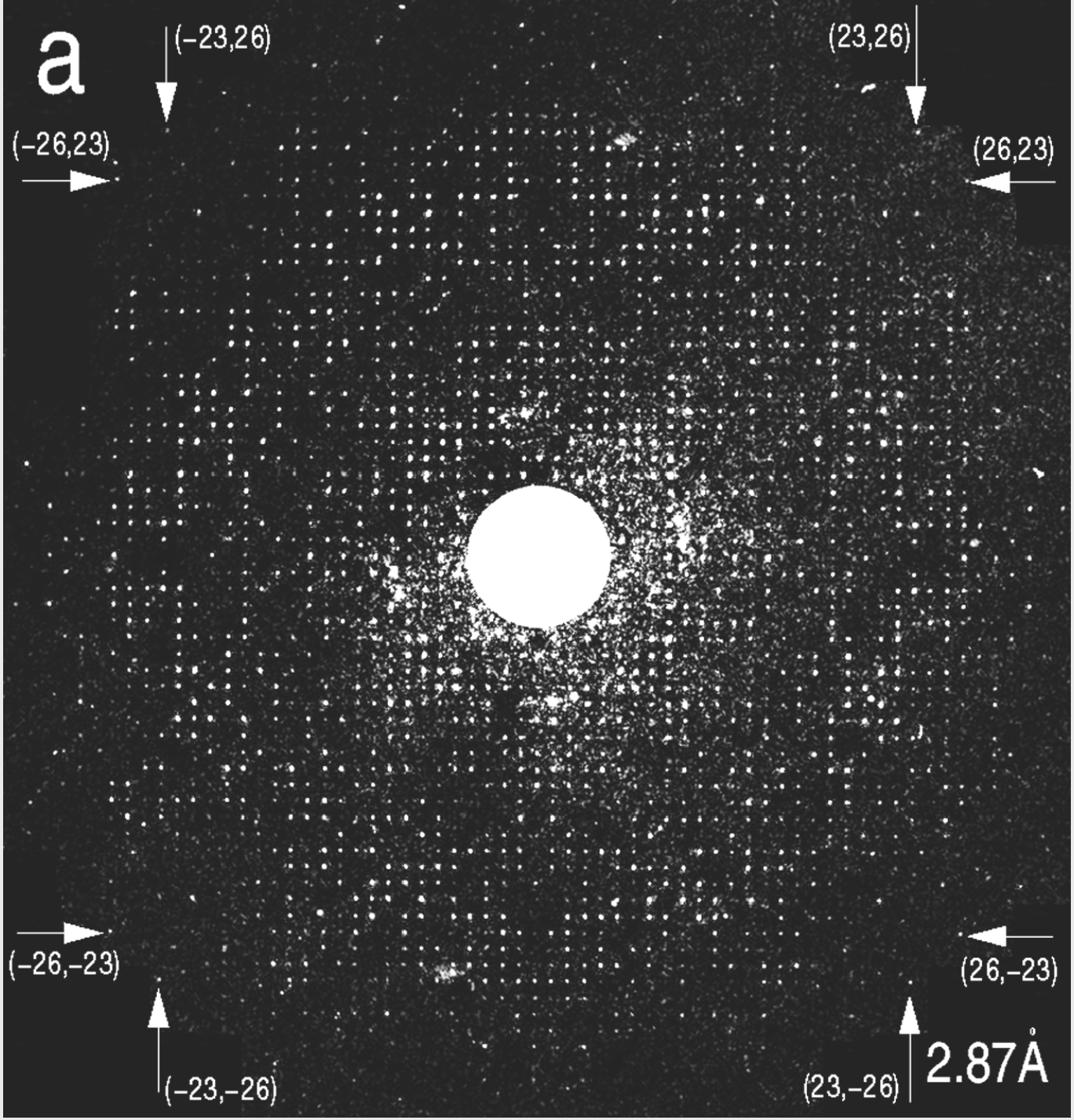
GRANTS AND FUNDING OPPORTUNITIES

Request for Applications (RFA's)

- Centers for Innovation in Membrane Protein Production

How to Study Membrane Protein Structure by Cryo-EM

- Membrane protein purification
- 2-D crystal
- Helical array
- Single particle

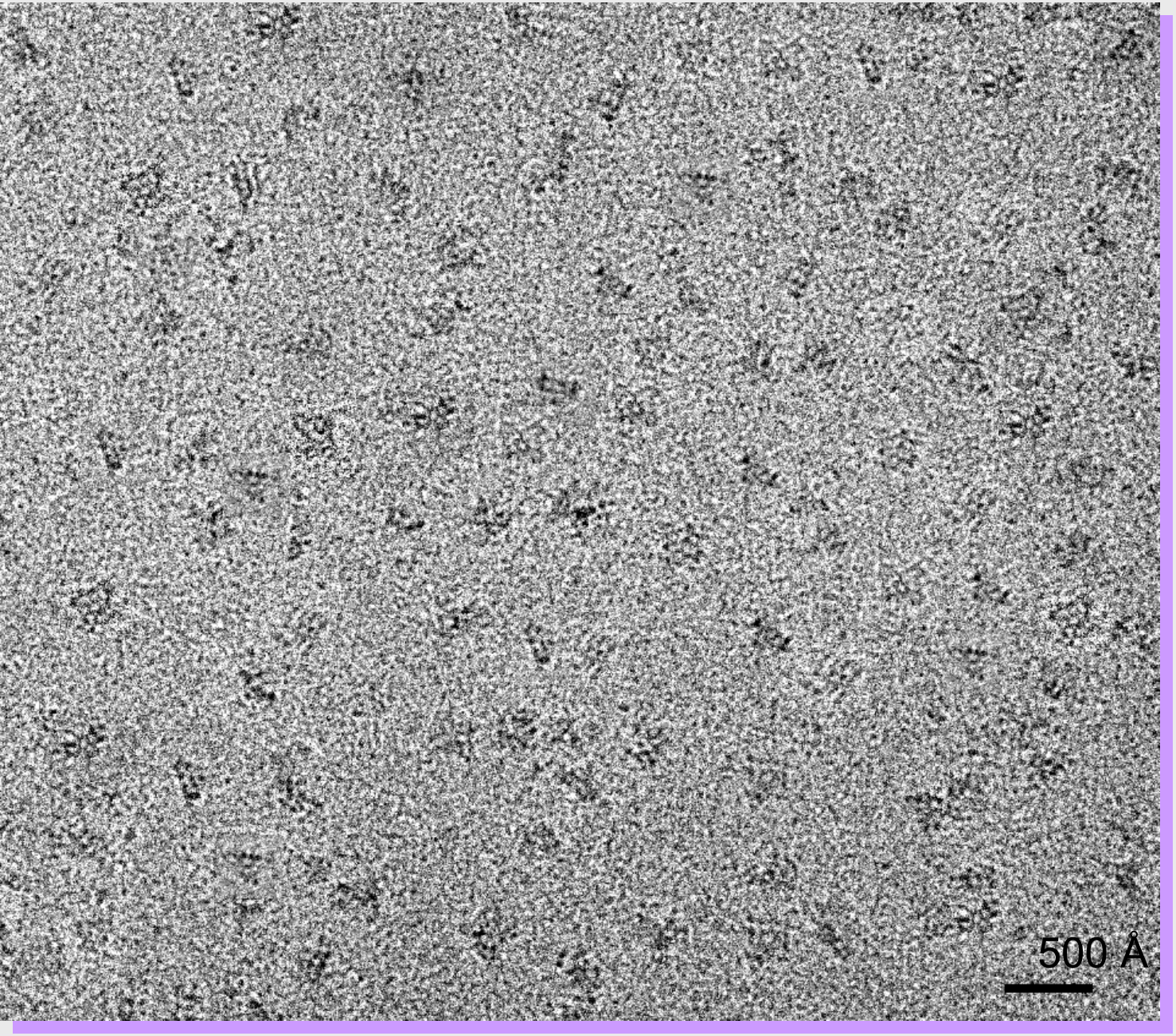


EDP of 2-D crystal

Ren & Mitra

Future Challenges in 2-D crystal and Helical Array

- Making the suitable crystal
- Solve structure beyond 3 Å
- Hybrid of Fourier averaging and single particle approach
- User friendly software



Single Particles Image

I. Serysheva

Challenges in Single Particle

- Structurally homogenous specimen
- More powerful algorithms to sort out heterogeneous structures
- Large data set is required

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Biological Complexes

news feature

The society of proteins

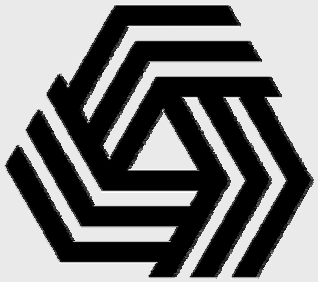
Having realized that proteins usually do their jobs by combining to form transient complexes, biologists are queuing up to study these structures using a powerful electron-microscopy technique. Alison Abbott reports.

Workshop

Structural Proteomics of Biological Complexes

April 7-8, 2003

http://ncmi.bcm.tmc.edu/events



National Center for Research Resources

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NIH Workshop on Structural Proteomics of Biological Complexes

Meeting Review

Andrej Sali*
Departments of Biopharmaceutical Sciences and Pharmaceutical Chemistry and
California Institute for Quantitative Biomedical Research
University of California, San Francisco
San Francisco, California 94143

Summary

Recently, some 50 biologists and officials from government funding agencies met at the NIH campus in Bethesda, MD to explore the interdisciplinary science and organization of the emerging field of structural proteomics. Structural proteomics aims to discover most macromolecular complexes and characterize their three-dimensional structures and functional mechanisms in space and time. The goal seems daunting, but the consensus was that the prize would be commensurate with the effort invested, given the importance of molecular machines and functional networks in biology and medicine. Identification of assemblies and transient complexes combined with their structural and functional characterization will allow us to understand, control, design, and change the functioning of larger biological systems as well as to contribute to drug target discovery, lead discovery, and lead optimization for treatment of human disease.

tional characterization of complexes is likely to play a more important role in structural proteomics than structural genomics.

Identification and Characterization of Macromolecular Complexes

David Drubin (Berkeley) reviewed the motivation of cell biologists for describing the structures and mechanisms of macromolecular complexes. He suggested that proteins and their associated complexes be categorized on the basis of their involvement in core biological processes, such as the maintenance of chromosome structure (nucleosomes), replication (DNA polymerase), transcription (RNA polymerase), nuclear transport (nuclear pore complex), protein synthesis (ribosome), protein degradation (proteosome), metabolism (aspartate transcarbamylase), signal transduction, chromosome movement, and segregation (kinetochore). Merits of the genome-wide versus a more targeted approach were discussed, balancing efficiency, bias, and quality. He suggested that interactions observed by proteomics should be validated by independent means, such as microscopy with green fluorescence protein. He also highlighted the power of emerging chemical genomics approaches, which utilize tailor made pairs of small molecule inhibitors and signaling molecules, to parse the contributions of individual signaling pathways to complex biological processes.

Jack Greenblatt (University of Toronto) described a

Why study large complexes?

- Proteins typically function in association with other proteins.
- Protein complexes are important for virtually every biological process and most diseases.
- Genome sequences identify tens of thousands of genes: linking these to 200-300 core biological processes will make their study manageable.
- Recently developed and/or improved technologies and methodologies make studies of large complexes more feasible and informative.

David Drubin

Challenges in Studying Complexes

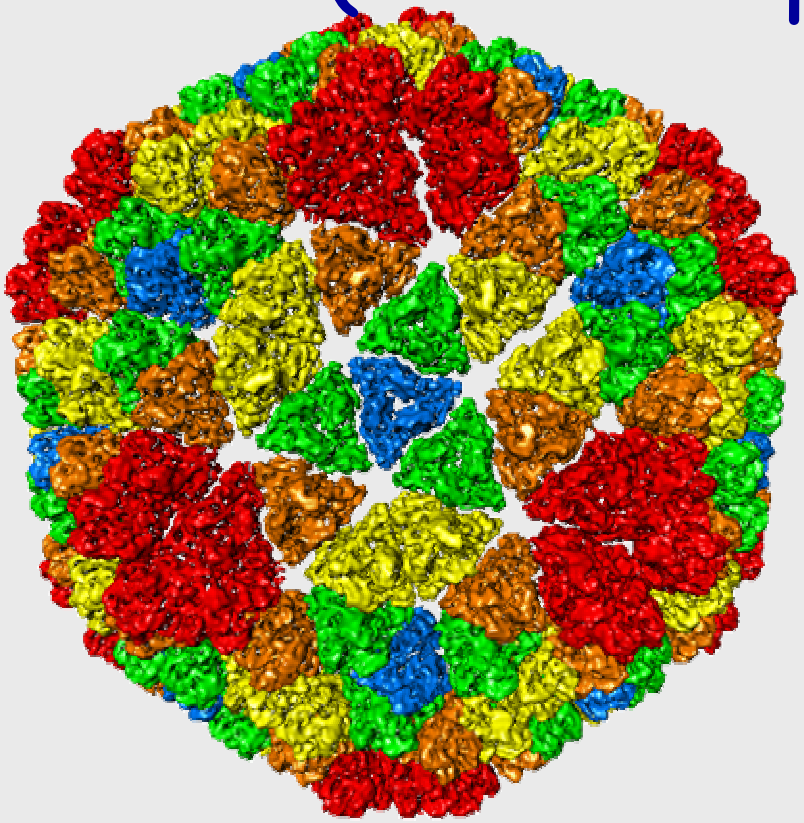
- Complex purification
- Stability of complex
- Flexibility of protein components
- Structure mining

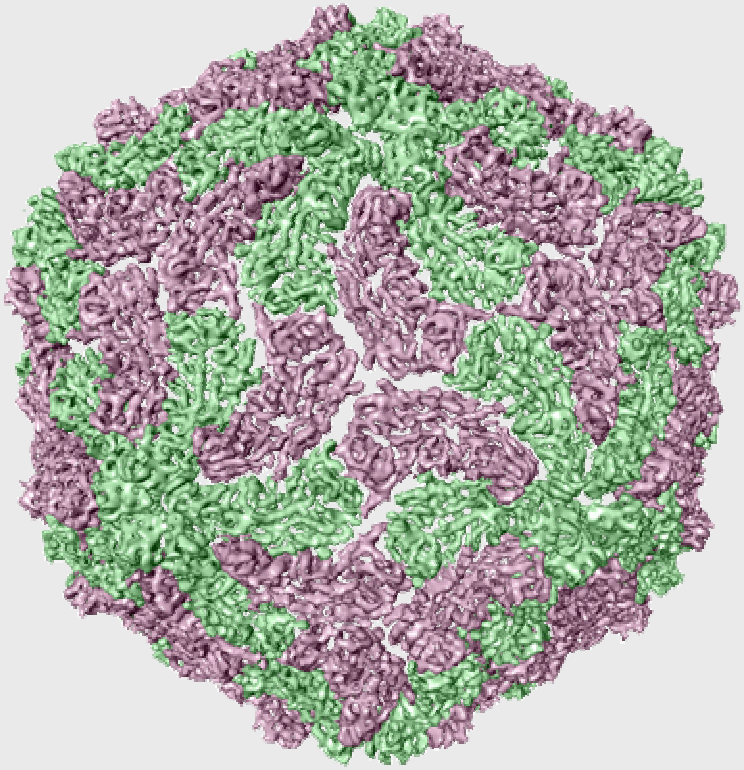
Example 1

Rice Dwarf Virus

Hong Zhou (UTHSC)
Matthew Baker
Wen Jiang
Joanita Jakana
Mat Dougherty

6.8Å Structure of Rice Dwarf Virus (26 MDa protein mass)





Outer capsid

700 Å in diameter

780 copies P8 (46kDa)

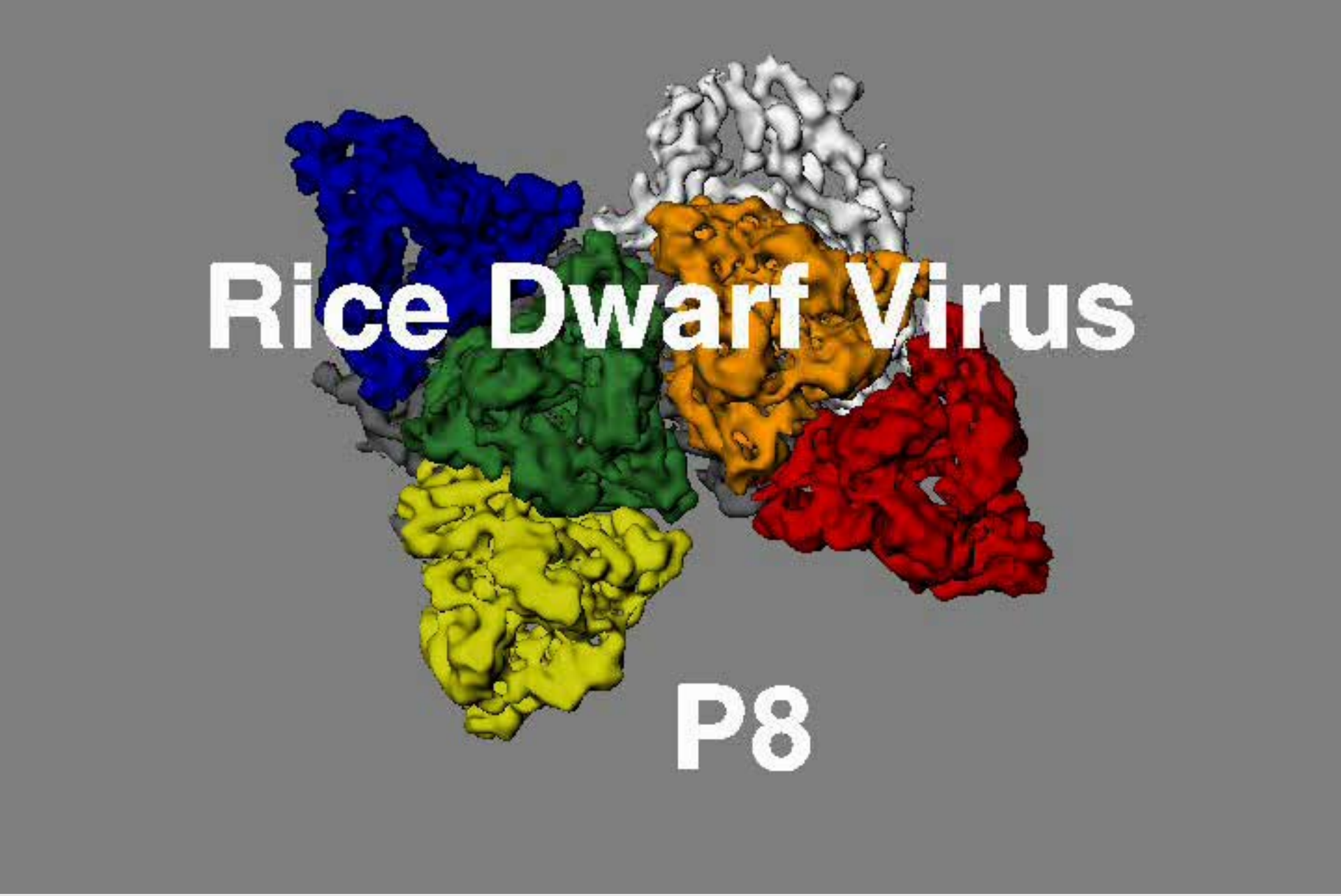
4 1/3 distinct trimers/a.u.

Inner capsid

540 Å in diameter

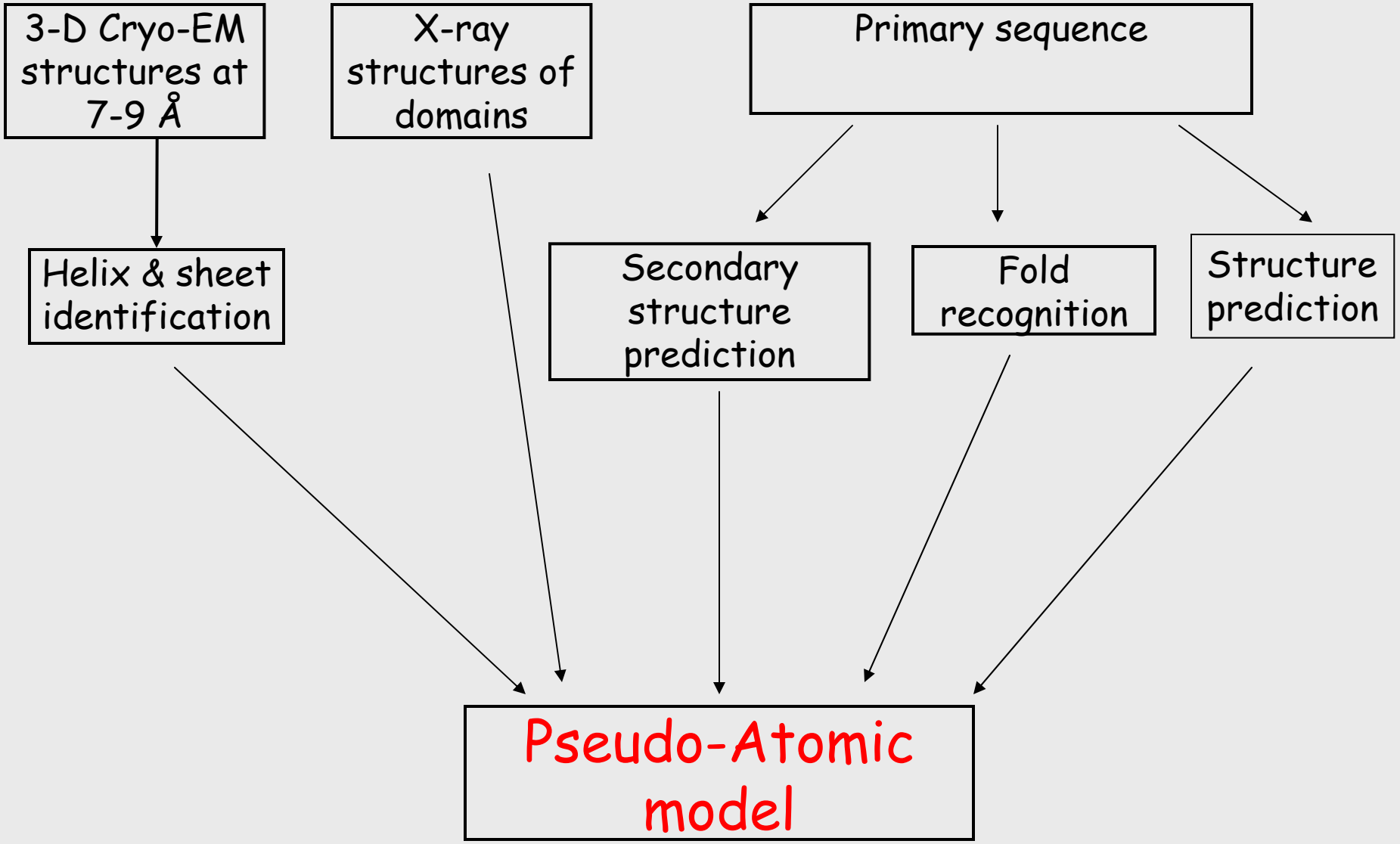
120 copies P3 (114kDa)

2 structural isoforms/a.u.

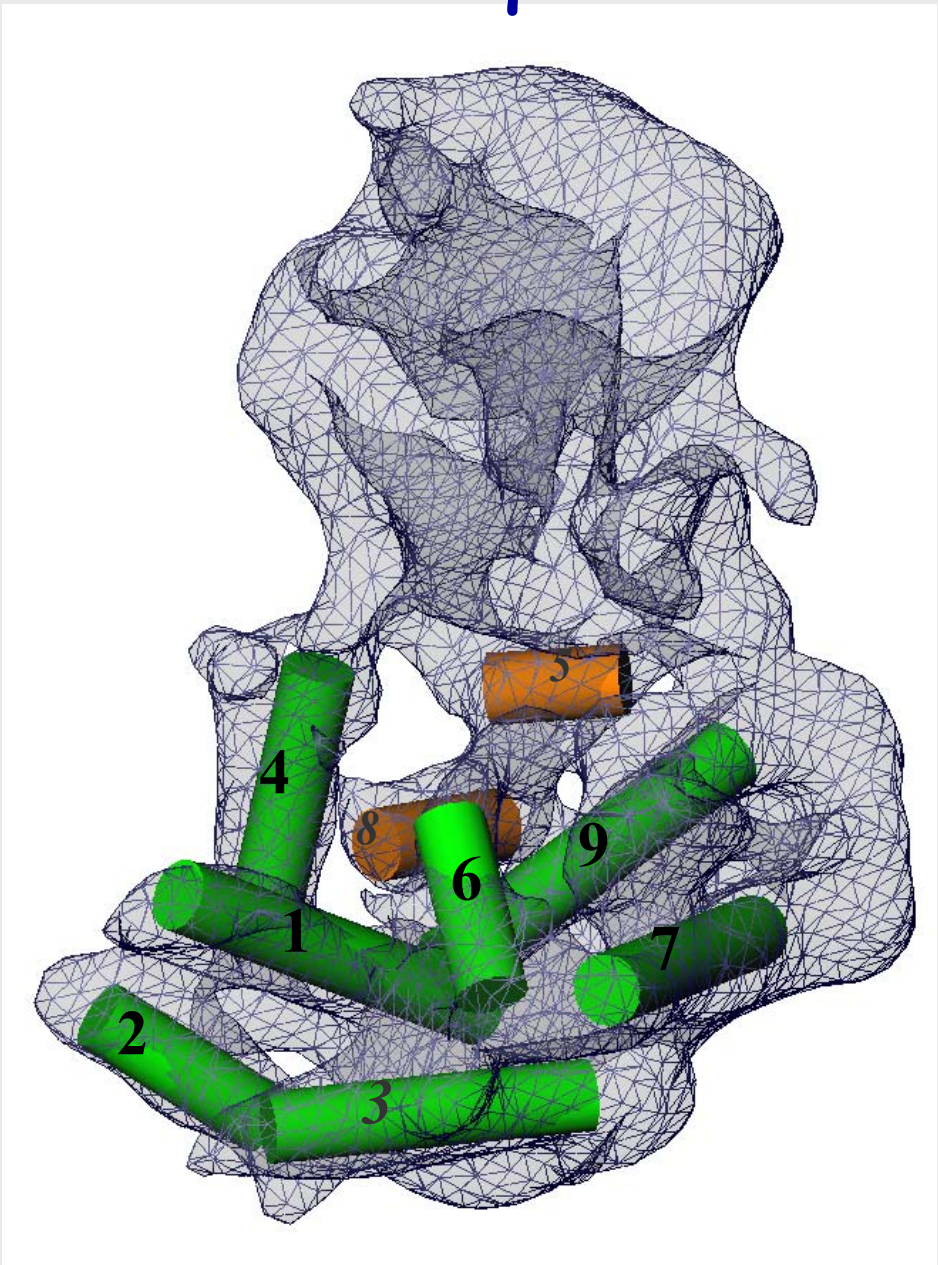


Rice Dwarf Virus Outer Shell Protein P8

AIRS (Analyze Intermediate Resolution Structure)

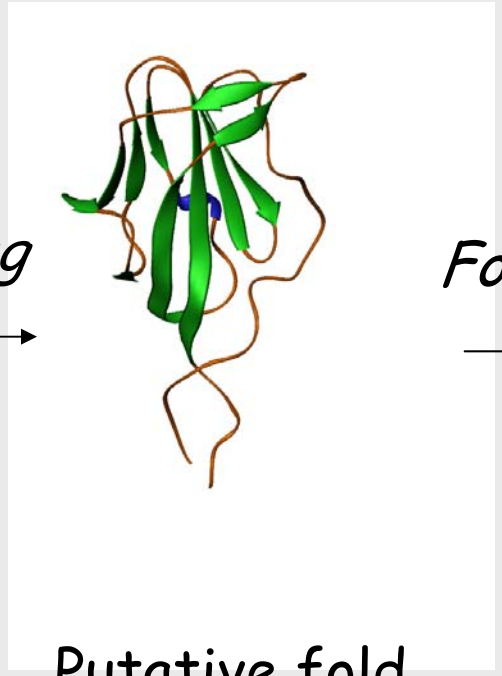


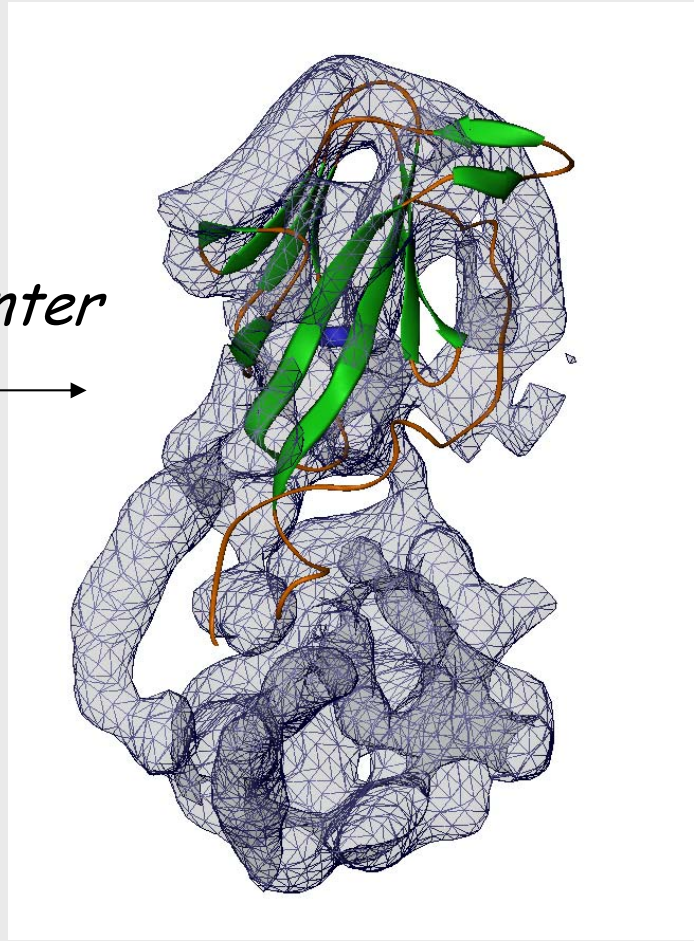
Assignment of Sequences to Helices



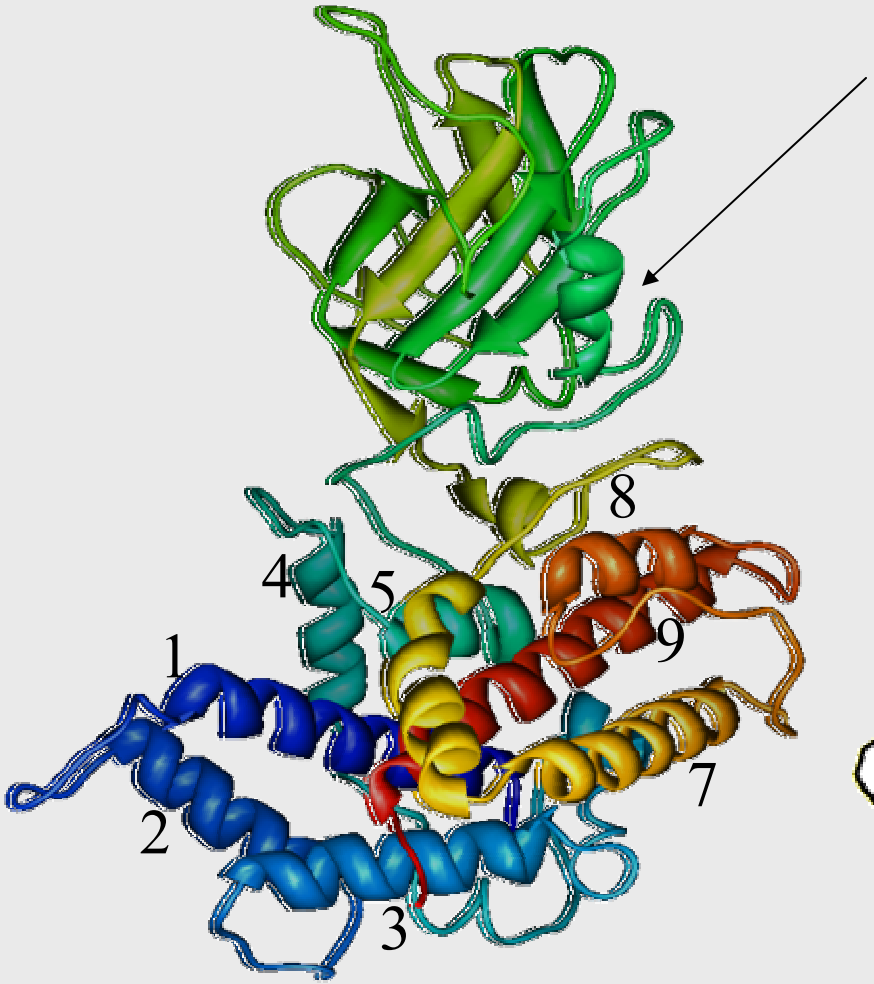
Predicted Fold Matches with P8 Upper Domain

Sequence → *Threading* → Putative fold → *Foldhunter* →

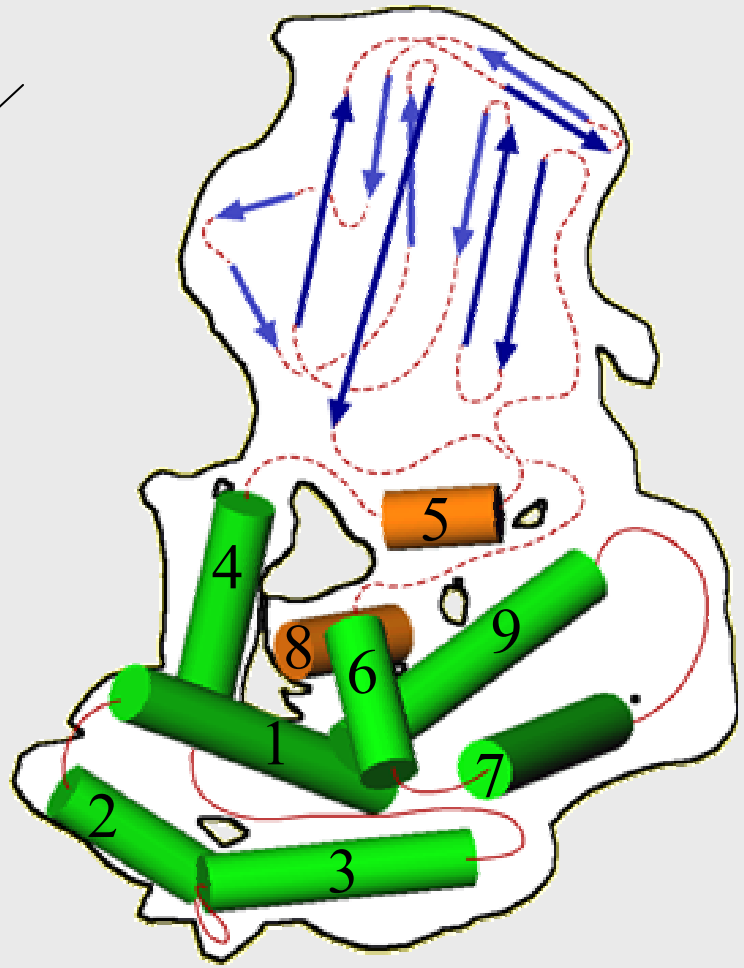




Model Building



Nakagawa et al Structure 2003

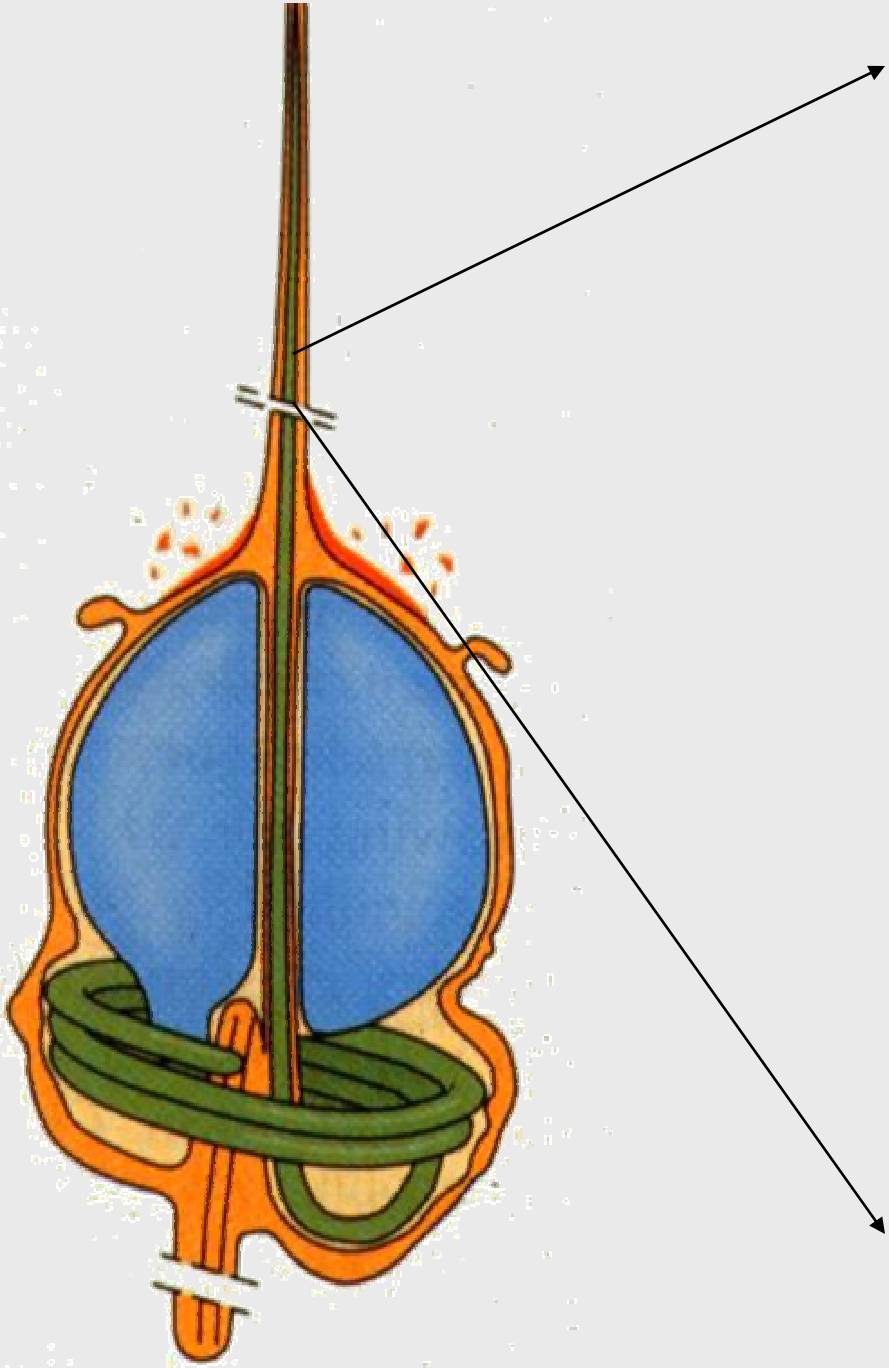


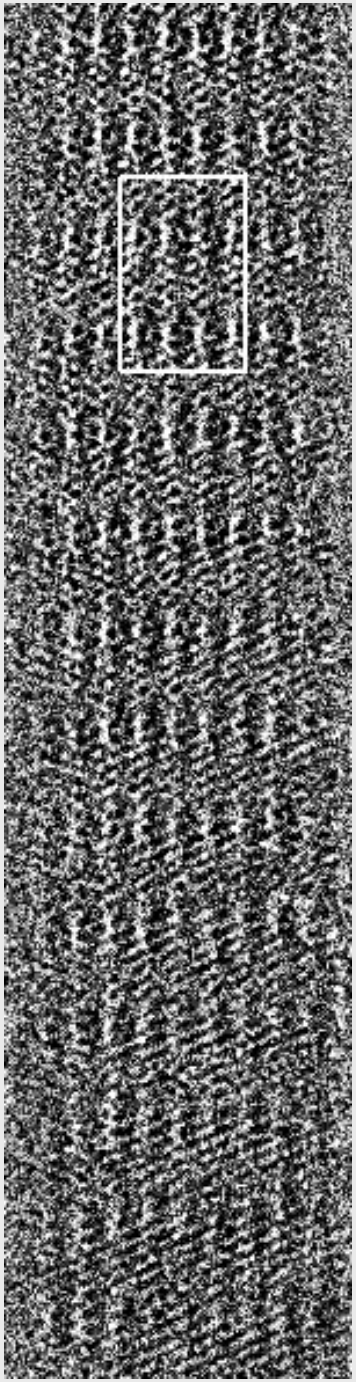
Zhou et al NSB, 2001

Example 2

Acrosomal Bundle

Michael Schmid
Misha Sherman
Joanita Jakana
Matthew Dougherty
Paul Matsudaira (MIT)



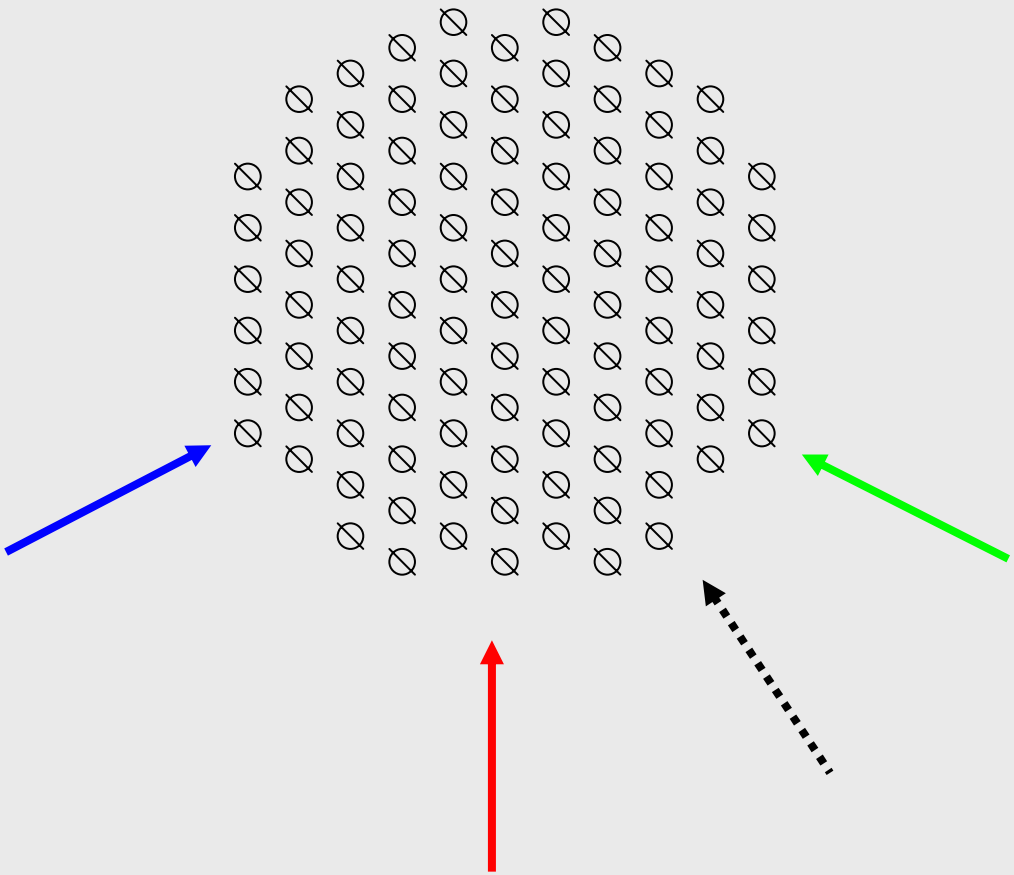


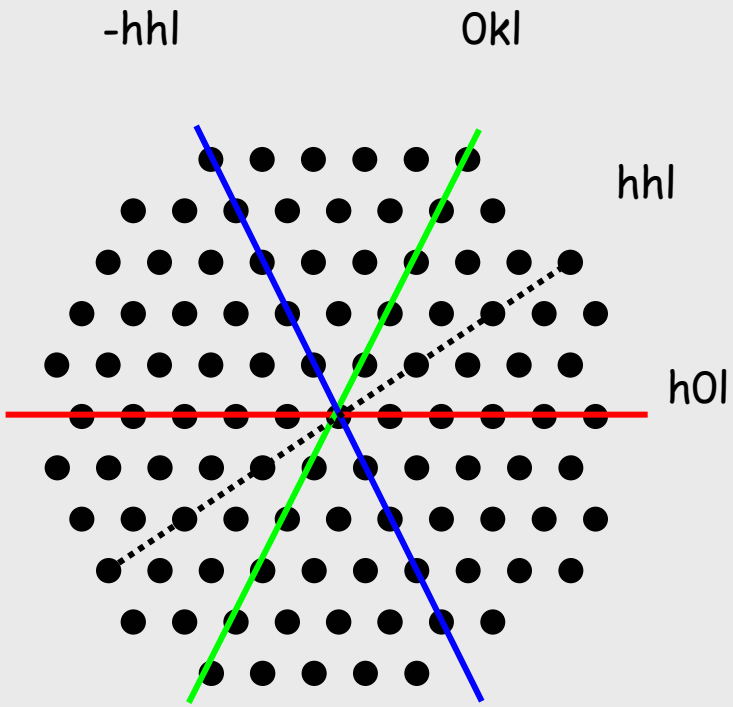
Cryo-image

1 x 4 unit cells

Bundle twist

Bundle Schematic View

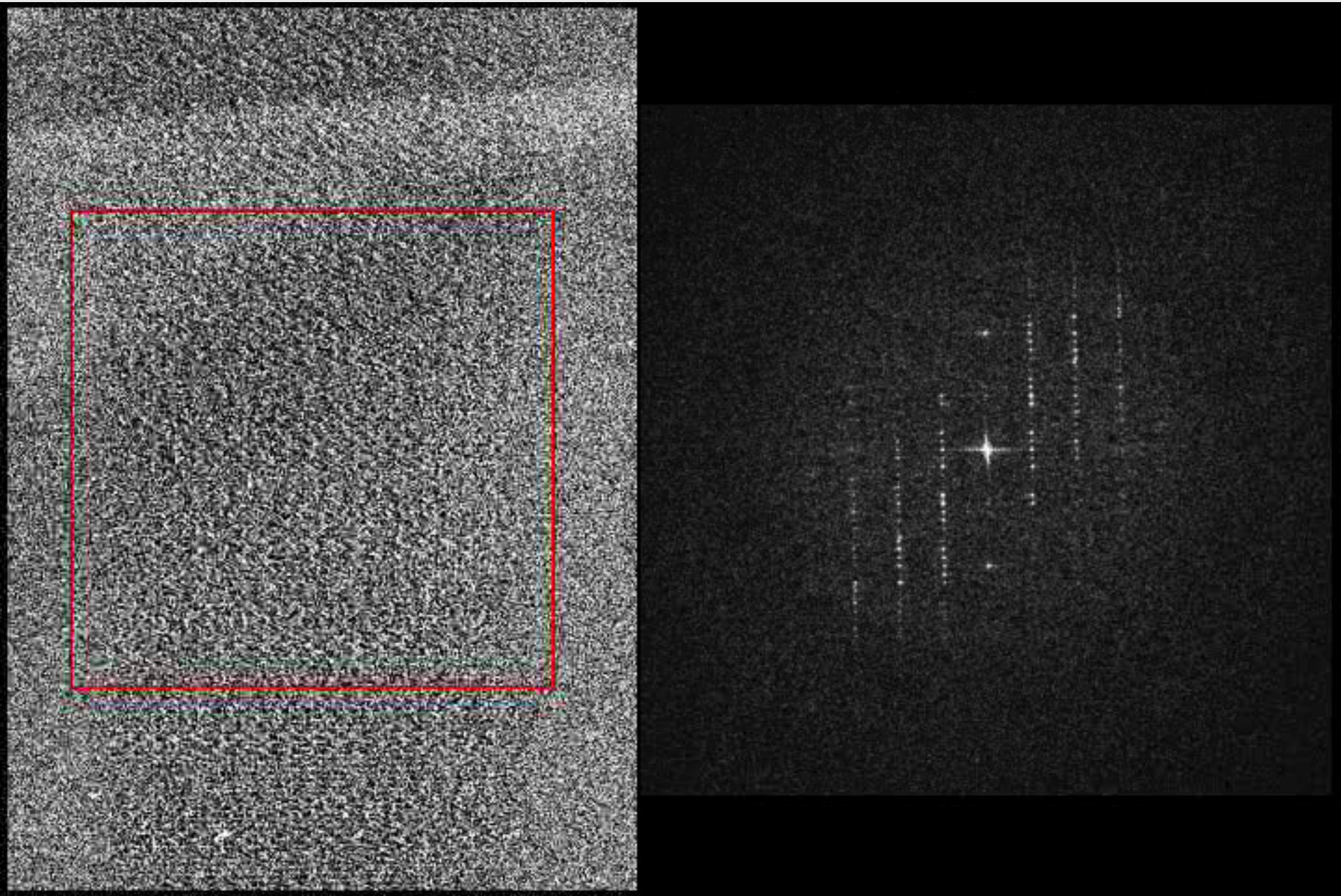




Real space filament packing

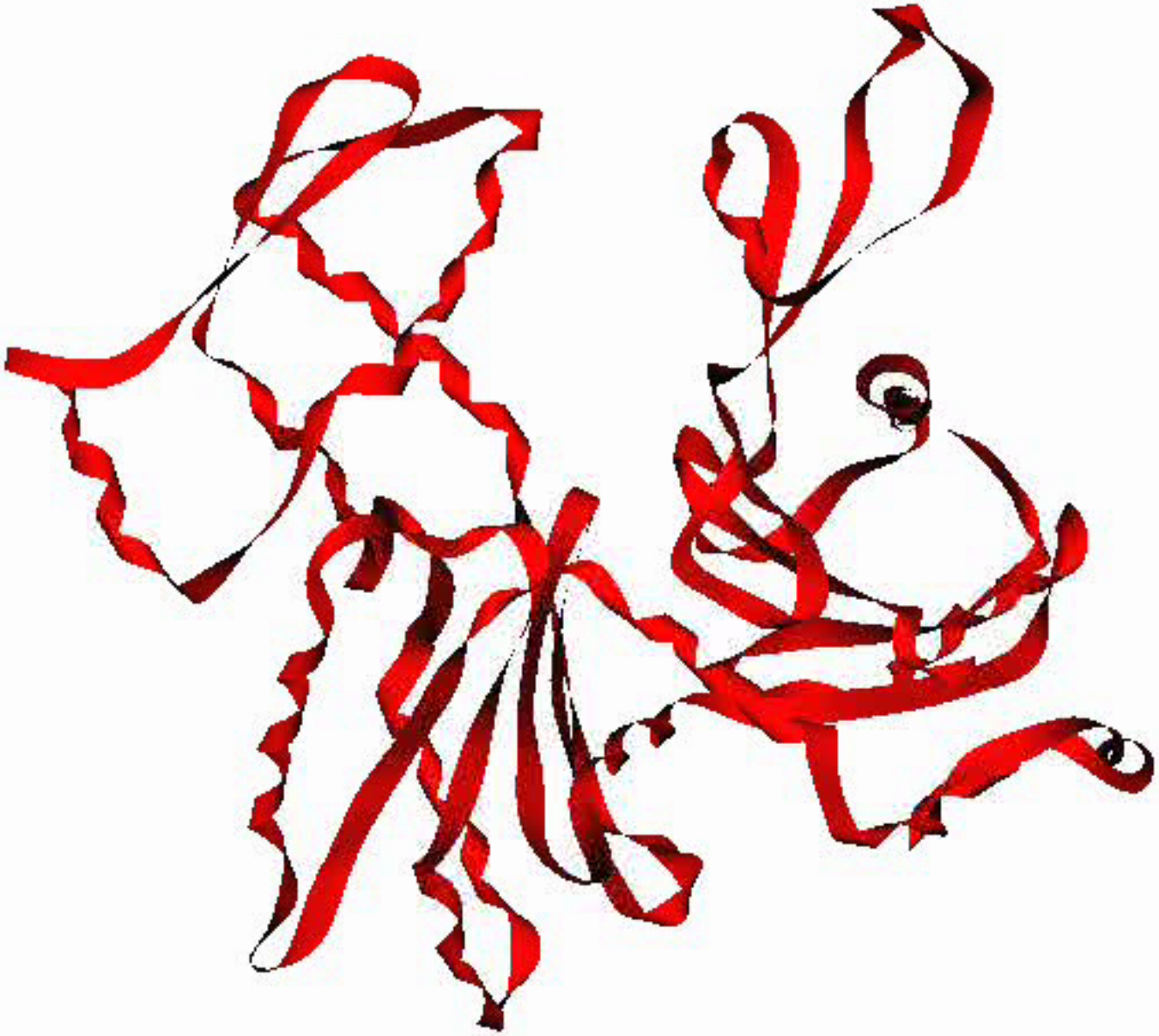
Diffraction space

Image and Computed Diffraction along a Bundle



Validation of the Initial 9.5 Å Map

acrosomal bundle



Actin molecular adaptation to helix distortion

tilt and rotation

found by foldhunter

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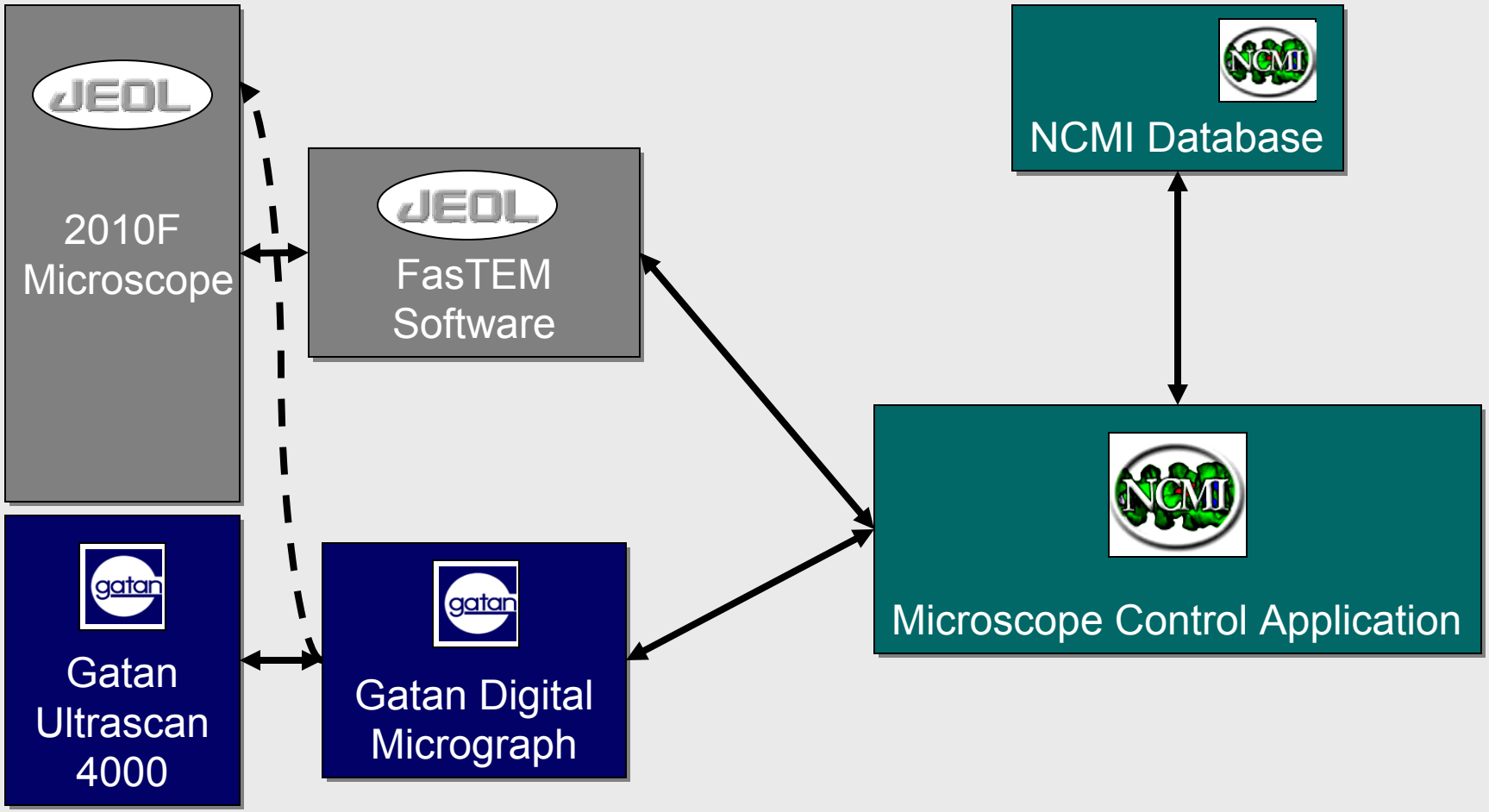
At CIMBio (main picture), Ron Milligan hopes that cryo-electron microscopy can be automated.

High Throughput Activities at NCMI: "James-SAVR Project"

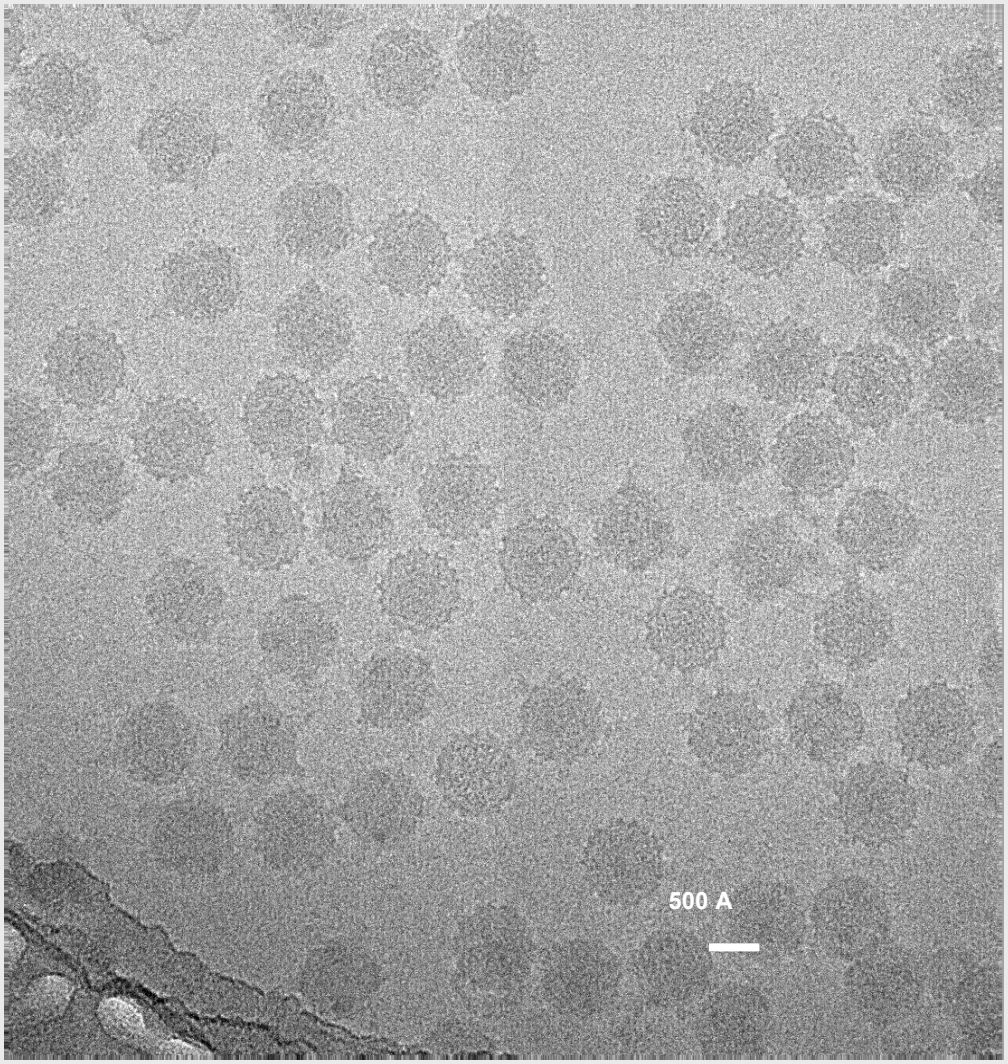
Christopher Booth
Wen Jiang
Matthew Baker
Mike Marsh
Steve Ludtke

JEOL 2010F FasTEM + Gatan 4kX4k CCD

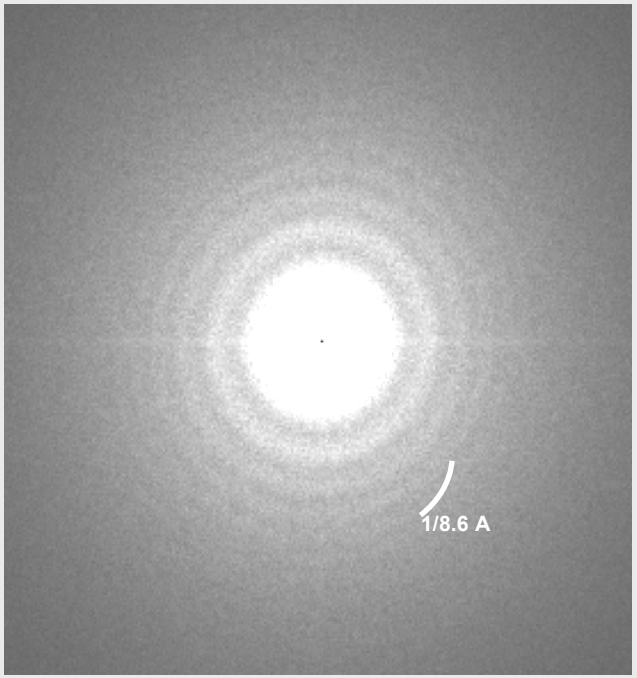
JEOL Automated Microscope Expert System (JAMES)

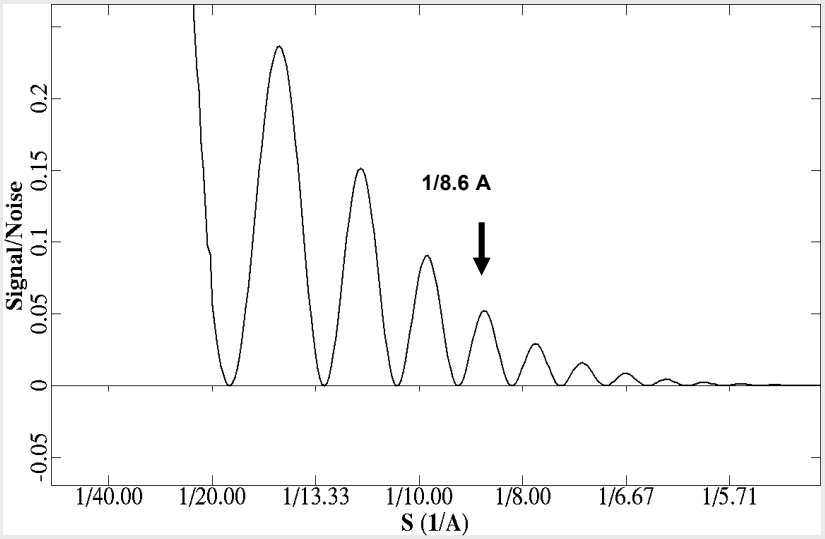


CCD Image of CPV



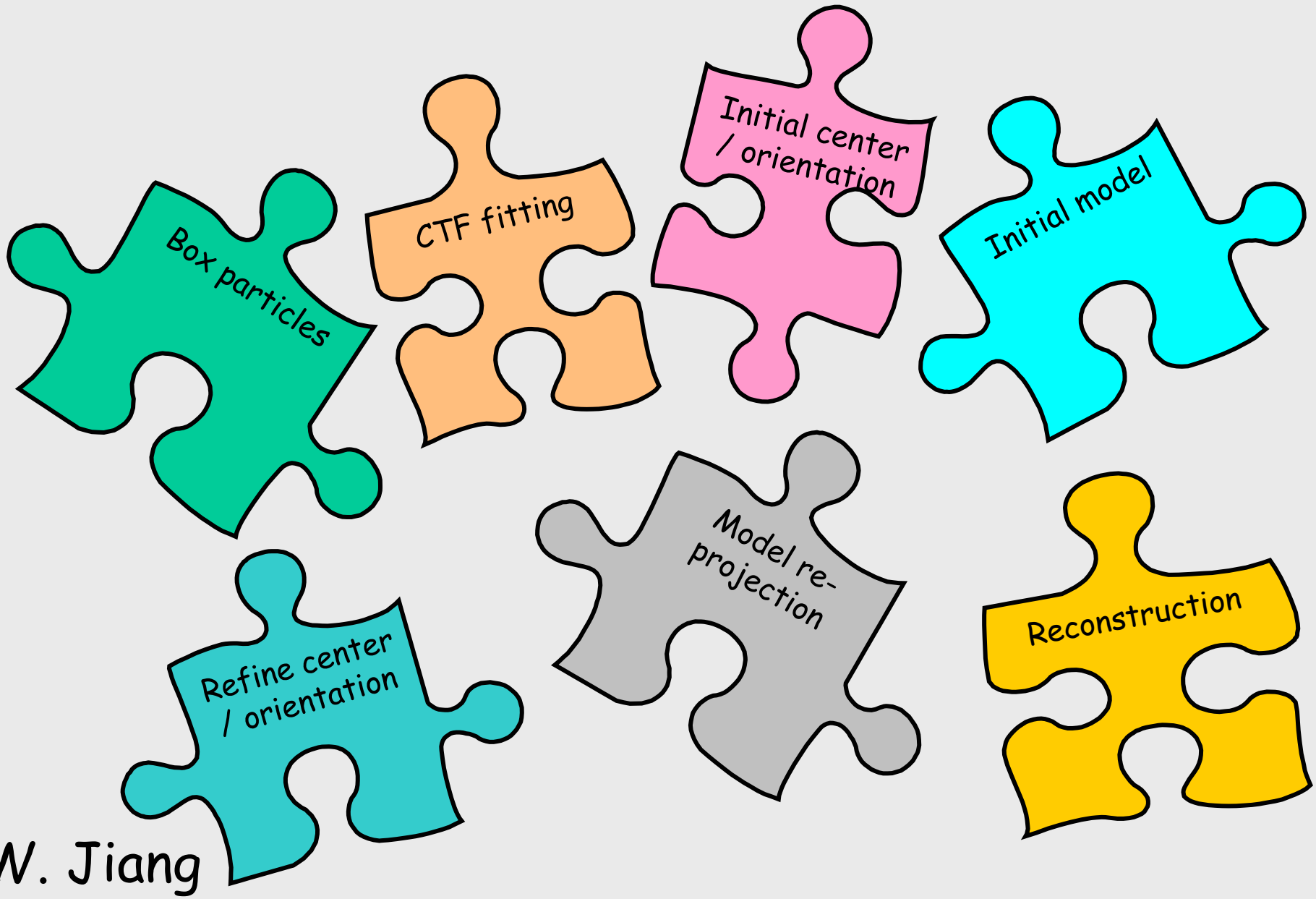
Power Spectrum





C. Booth

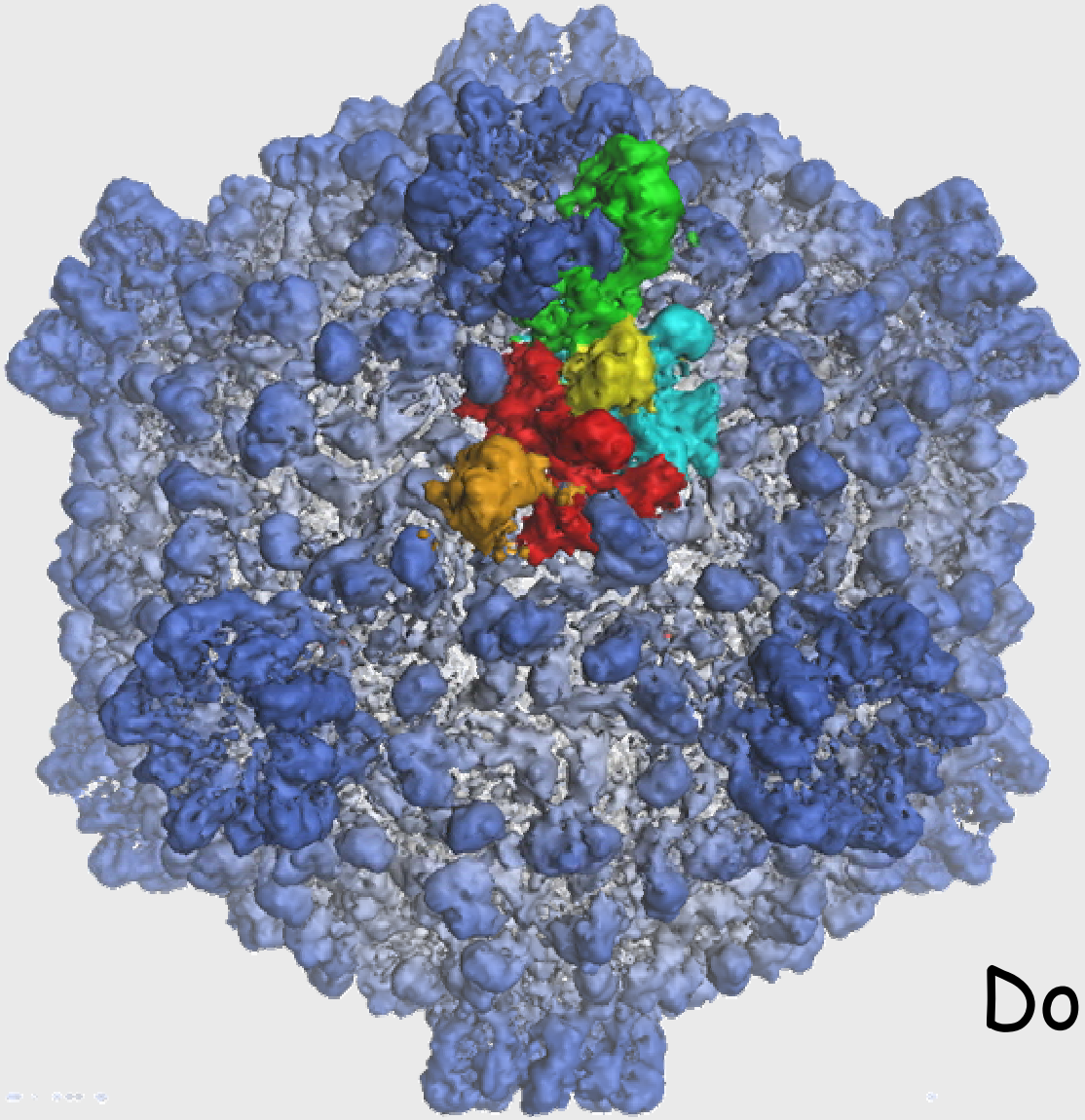
Virus Reconstruction



W. Jiang

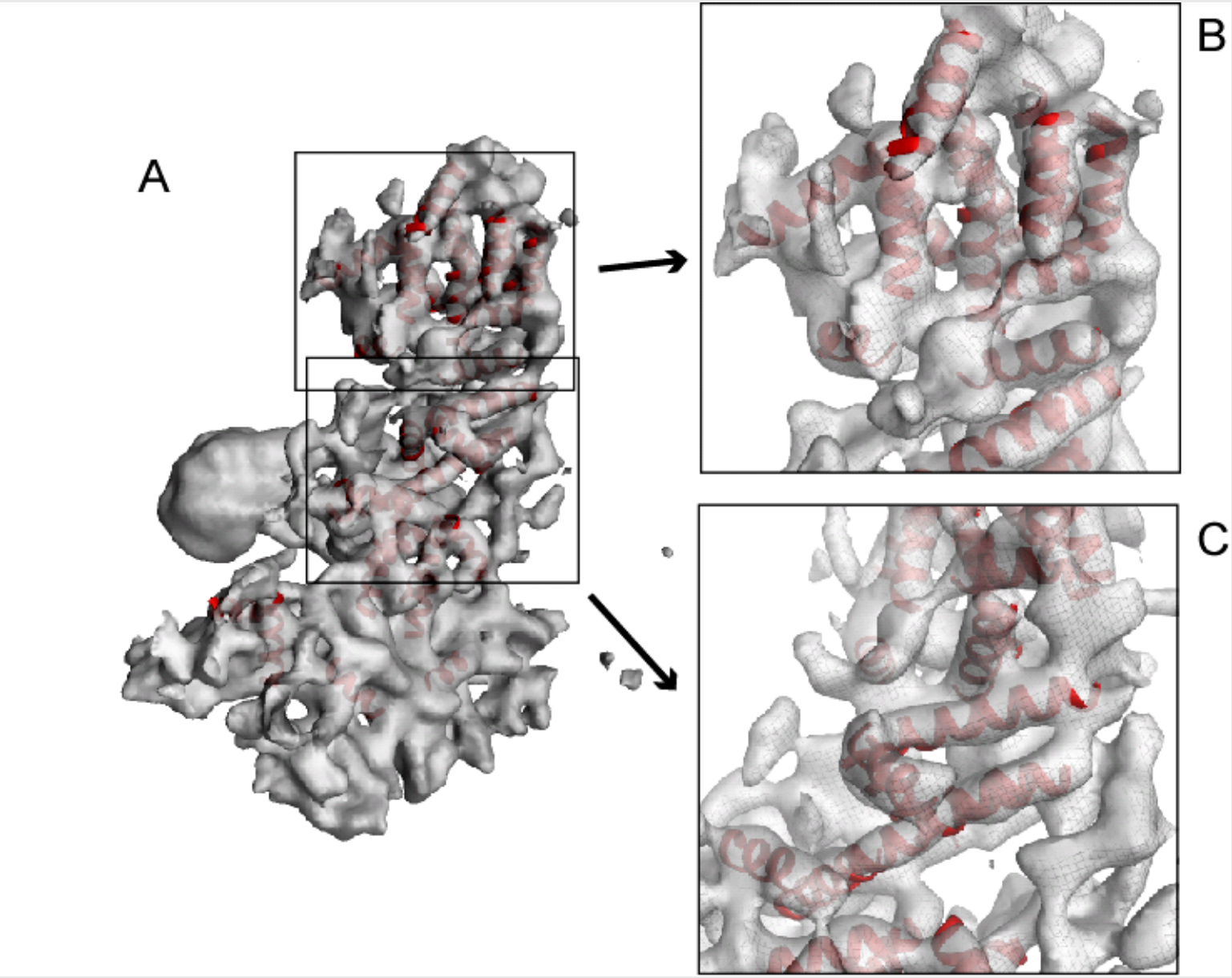
SAVR: Semi-Automated Virus Reconstruction

9 Å Structure of CPV



Done < 2weeks

Alpha Helices in CSP-A



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Future Instrumentation

- Higher spatial coherence
- Choose an optimal cryo-specimen temperature
- Energy filter
- Large CCD
- Complete automation for data collection

Future Cyber-infrastructure

- Better algorithms
- Easy-to-use database
- Visualization/animation software
- A unified type of image processing environment
- Direct deposit to PDB
- Easily connect to other structure analysis programs

Future Specimen Prep Developments

- Automated freezing apparatus
- Grid type
- Grid treatment
- Filter paper

When Does The Future End ?

- No more this type of wonderful workshop is needed

- Every biologist is a microscopist