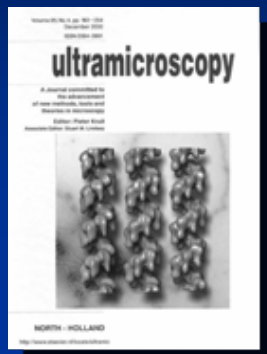
A New Approach to EM of Helical Polymers Yields New Insights

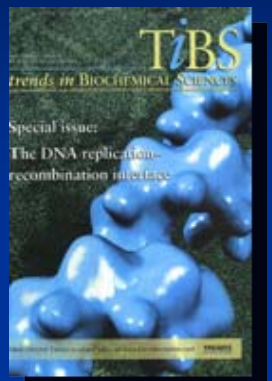
- Helical polymers are ubiquitous in biology –
  - This is because a helical structure results from simplest bonding rule between any two objects!
  - Other interactions (symmetrical dimer, trimer, tetramer, etc.) are more restrictive
- Helical polymers have played an important role in the development of structural biology
  - DeRosier & Klug, 1968
  - Yonekura *et al.*, 2003
  - Miyazawa *et al.*, 2003

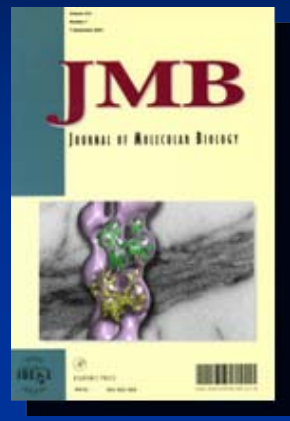
Most Helical Polymers Have Been Refractory to High Resolution EM Studies (and are incompatible with crystallization)!

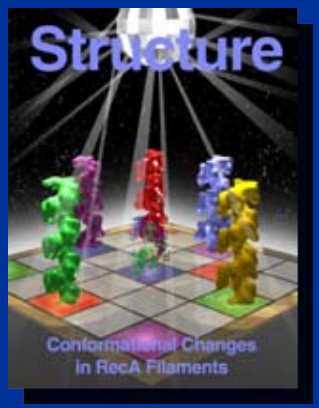
- Disorder or variability
- Heterogeneity
- Weak Scattering

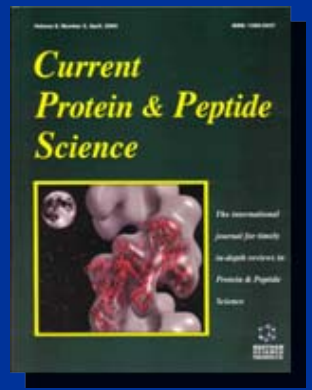
New method: Iterative Helical Real Space Reconstruction

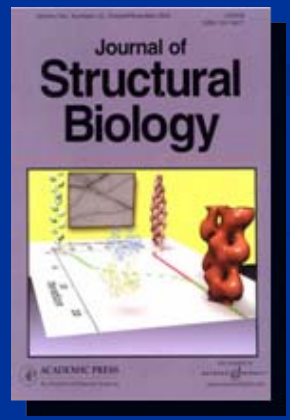


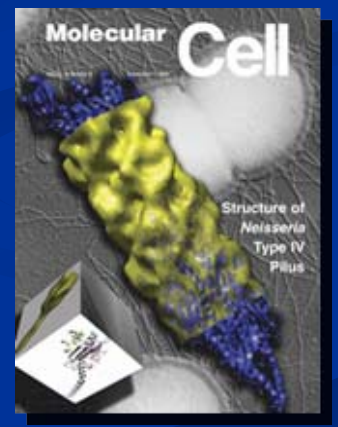








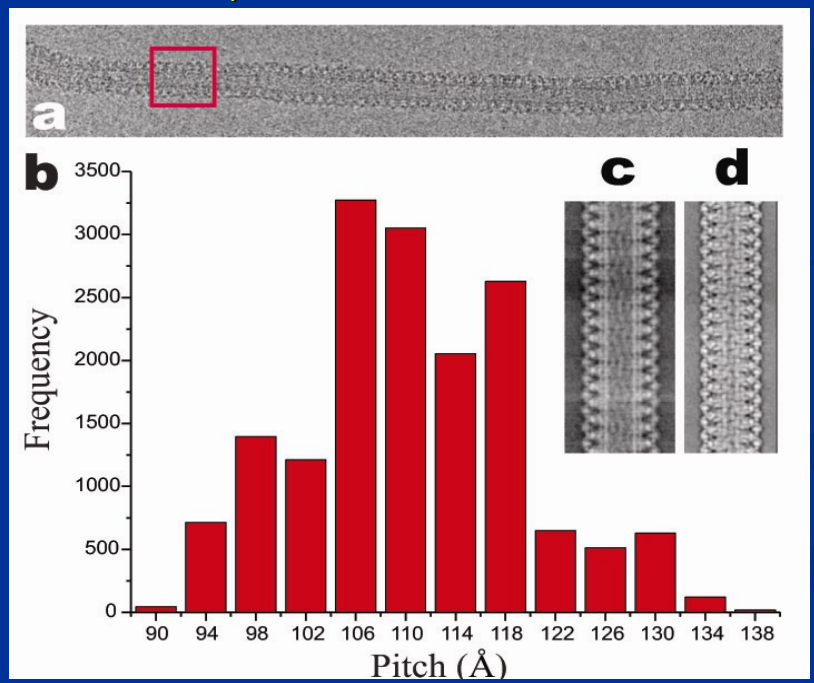




and ~ 35 other papers published or in press

What are the problems with the traditional approach?

- For most real filaments, never straight over long distances
  - can correct for small curvature with "straightening" algorithm (*i.e.*, Egelman, 1986), but this can introduce artifacts
- Indexing can be problematic – both difficult and ambiguous
- Absence of space group means that disorder can accumulate, with liquid-like order
- Many polymers can have great variability, so that Fourier-Bessel methods are useless
- No reason for there to be simple symmetry expressed as ratio of small integers
- Very small changes in symmetry can lead to very large changes in the "selection rule"
  - Example of actin: "u/t = 13/6", c=355 Å, $\Delta\varphi$=166.1538°
  - but $\Delta(\Delta\varphi)$=0.128°, $\Delta\varphi$=166.2818°, u/t=1299/600, c=35,463 Å!
- Bessel overlap can occur for many real structures at low resolution



Iterative Helical Real Space Reconstruction Method

Egelman (2000), Ultramicroscopy 85, 225-234

Helically symmetric 3D volume

rotate azimuthally by 4° increment, project onto 2D image

90 reference projections

0° 4° 8° 12° 16° 20° 24° 28° 32°...

multi-reference alignment

set of raw images (thousands of segments from helical filaments)

In-plane rotation, x-shift, y-shift parameters

Aligned, rotated images; azimuthal angular assignments

back projection

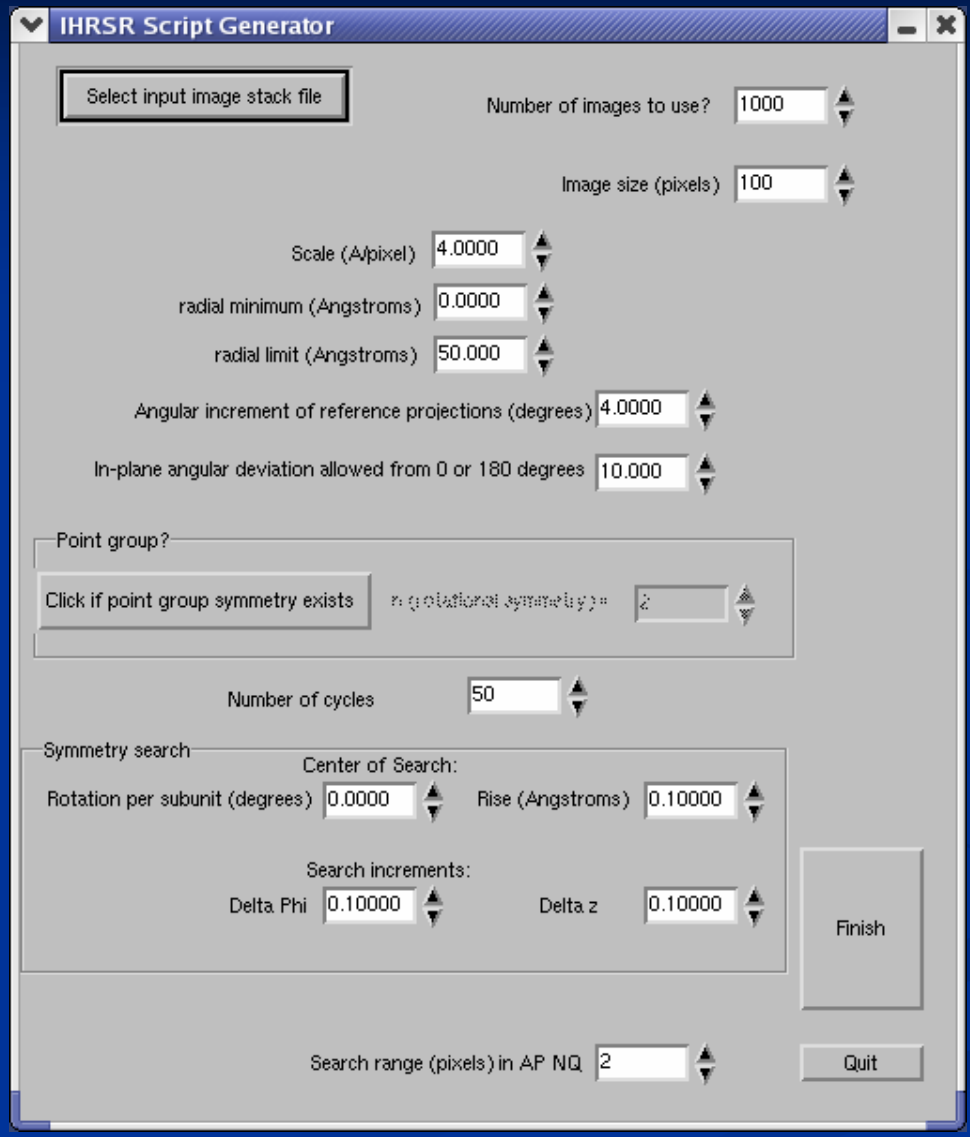
Asymmetric 3D volume

determine helical symmetry by least squares fit

$\Delta\phi$, Δz (helical parameters)

impose helical symmetry

generator creates IHRSR script

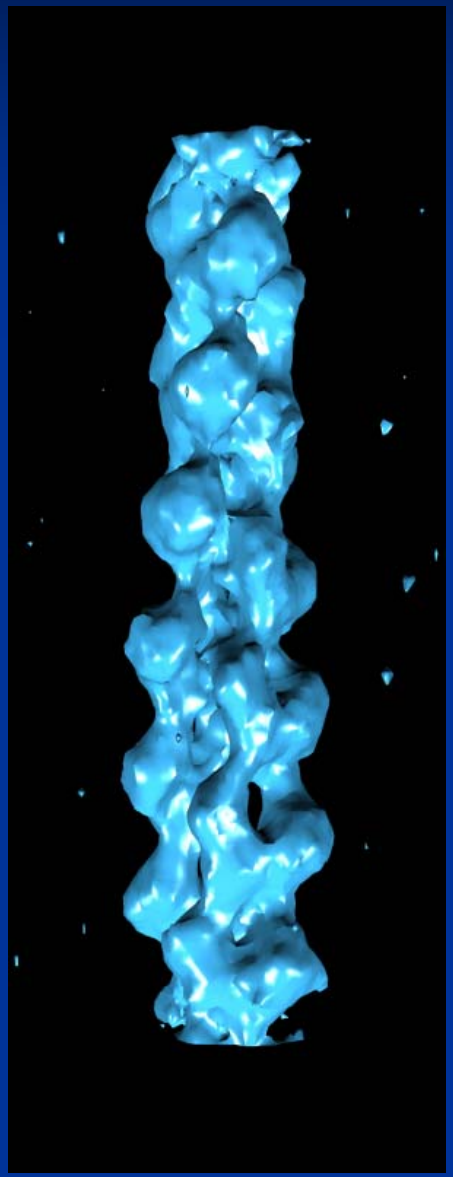


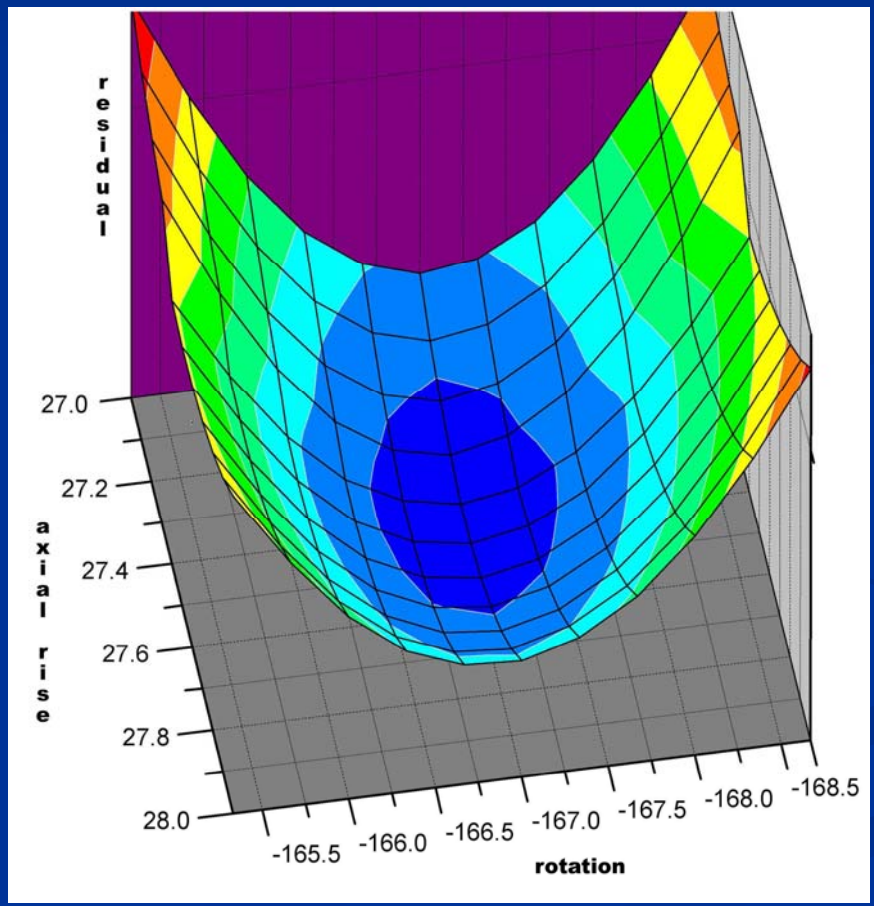
asymmetric reconstruction

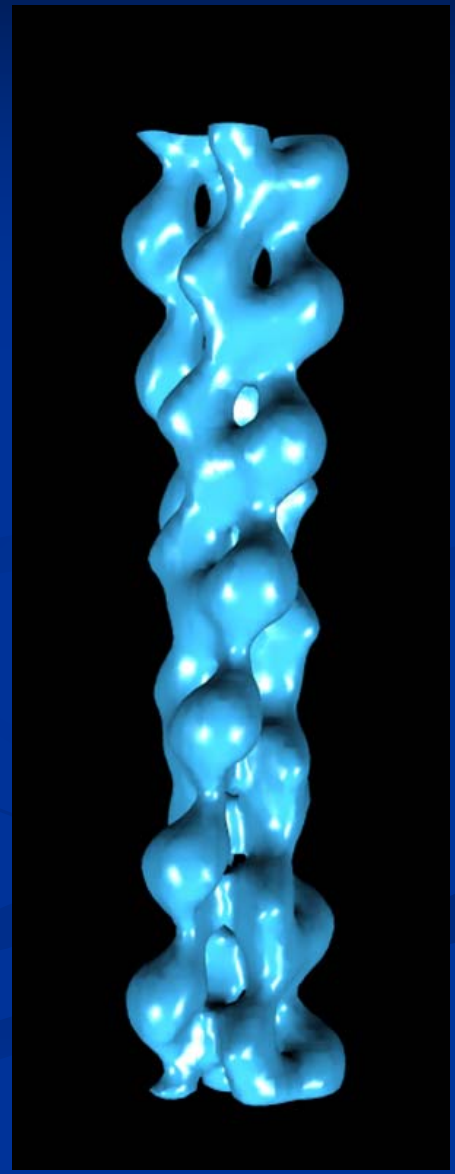
Most important part of cycle…

symmetry search

imposed symmetry







Iterative Helical Real Space Reconstruction Method

Advantages of method over Fourier-Bessel approach:

- Overcomes problems of straightening
- Can work with very weakly scattering specimens
  - bacterial pili, filamentous phage
- Can deal with disordered or heterogeneous filaments
  - RecA/RAD51/Dmc1, actin, ParM
- Is transparent to the almost intractable problem of Bessel overlap
  - myosin thick filament
- Is easier, both conceptually and in practice

Disadvantages:

- None

Archaeal flagellum shows homology with bacterial Type IV pilus

doi:10.1016/j.jmb.2004.12.010 J. Mol. Biol. (2005) 346, 665–676

JMB

Available online at www.sciencedirect.com

SCIENCE DIRECT®

ELSEVIER

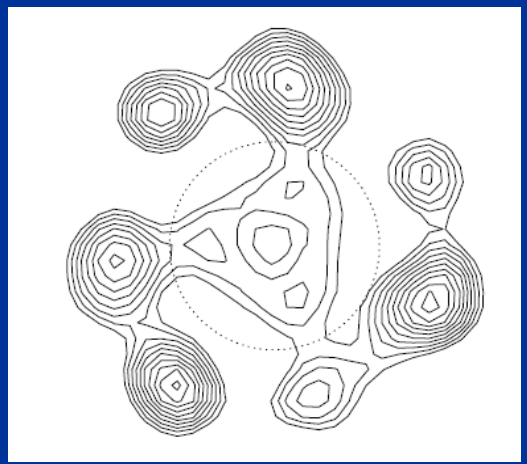
Refining the Structure of the *Halobacterium salinarum* Flagellar Filament Using the Iterative Helical Real Space Reconstruction Method: Insights into Polymorphism

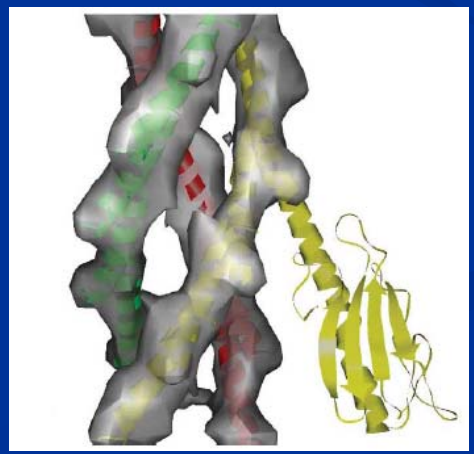
Shlomo Trachtenberg[1]*, Vitold E. Galkin[2] and Edward H. Egelman[2]*

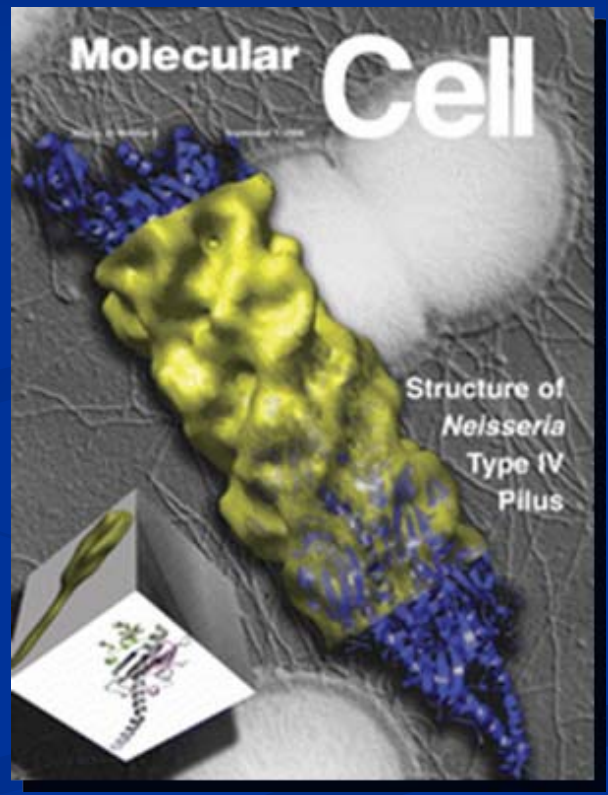
[1]*Department of Membrane and Ultrastructural Research The Hebrew University of Jerusalem—Hadassah Medical School, P.O. Box 12272 Jerusalem 91120, Israel*

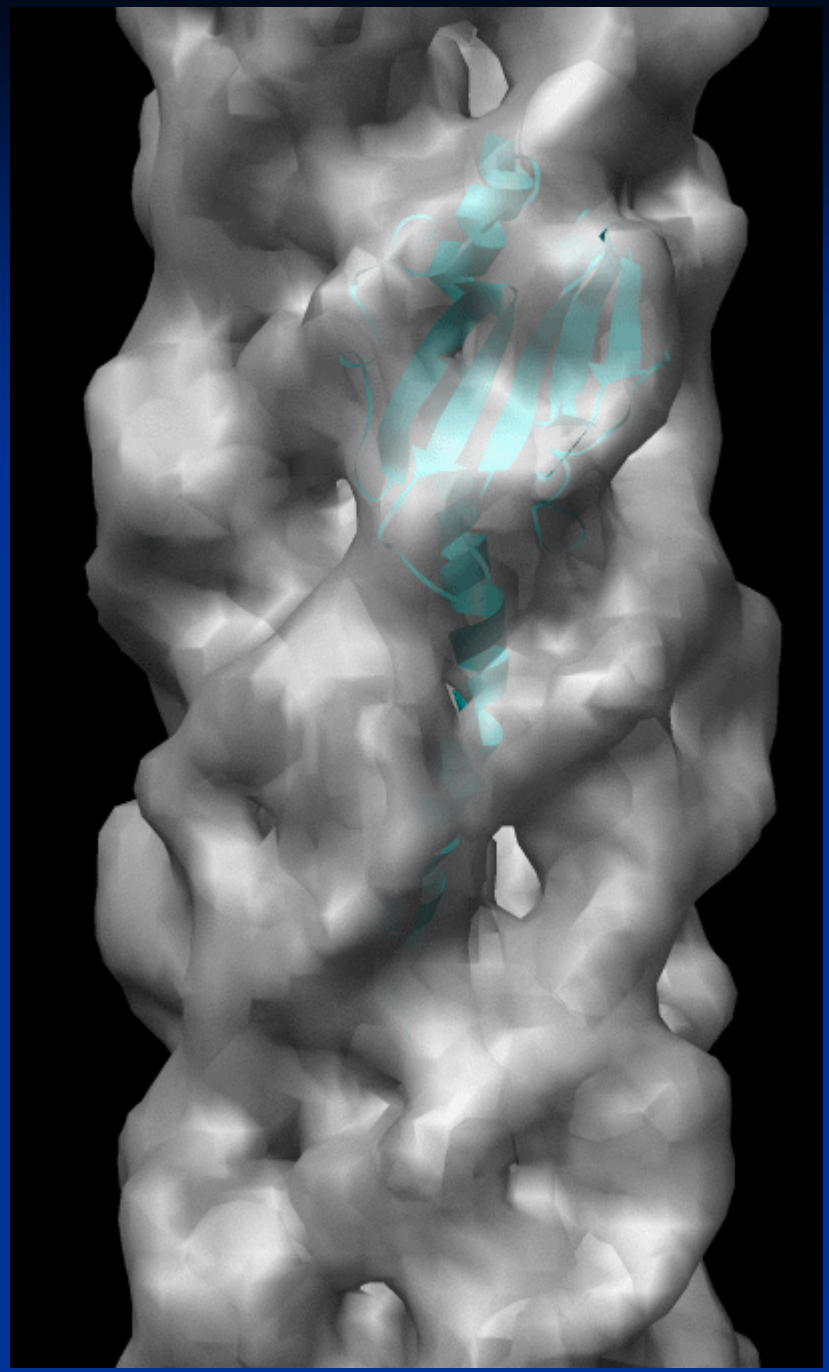
[2]*Department of Biochemistry and Molecular Genetics University of Virginia Health Sciences Center, Charlottesville VA 22908-0733, USA*

The eubacterial flagellar filament is an external, self-assembling, helical polymer ~220 Å in diameter constructed from a highly conserved monomer, flagellin, which polymerizes externally at the distal end. The archaeal filament is only ~100 Å in diameter, assembles at the proximal end and is constructed from different, glycosylated flagellins. Although the phenomenology of swimming is similar to that of eubacteria, the symmetry of the archebacterial filament is entirely different. Here, we extend our previous study on the flagellar coiled filament structure of strain R1M1 of *Halobacterium salinarum*. We use strain M175 of *H. salinarum*, which forms poly-flagellar bundles at high yield which, under conditions of relatively low ionic-strength (0.8 M *versus* 5 M) and low pH (~2.5 *versus* ~6.8), form straight filaments. We demonstrated previously that a single-particle approach to helical reconstruction has many advantages over conventional Fourier–Bessel methods when dealing with variable helical symmetry and heterogeneity. We show here that when this method is applied to the ordered helical structure of the archebacterial uncoiled flagellar filament, significant extensions in resolution can be obtained readily when compared to applying traditional helical techniques. The filament population can be separated into classes of different morphologies, which may represent polymorphic states. Using cryo-negatively stained images, a resolution of ~10–15 Å has been achieved. Single α-helices can be fit into the reconstruction, supporting the proposed similarity of the structure to that of type IV bacterial pili.





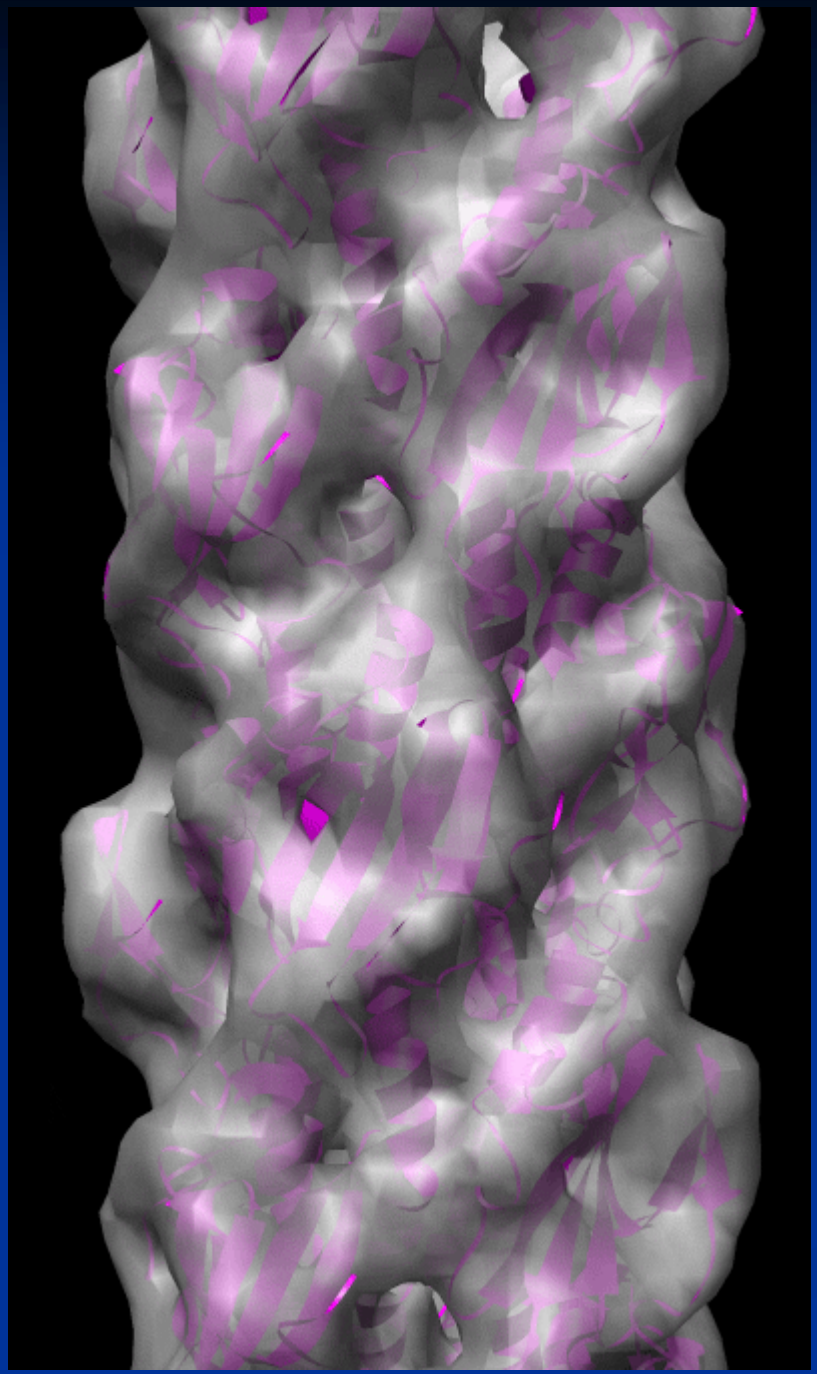




Unambiguous fit of monomeric crystal subunit from *Neisseria gonorrhoeae*

~ 3.6 subunits per turn of a 37 Å pitch helix

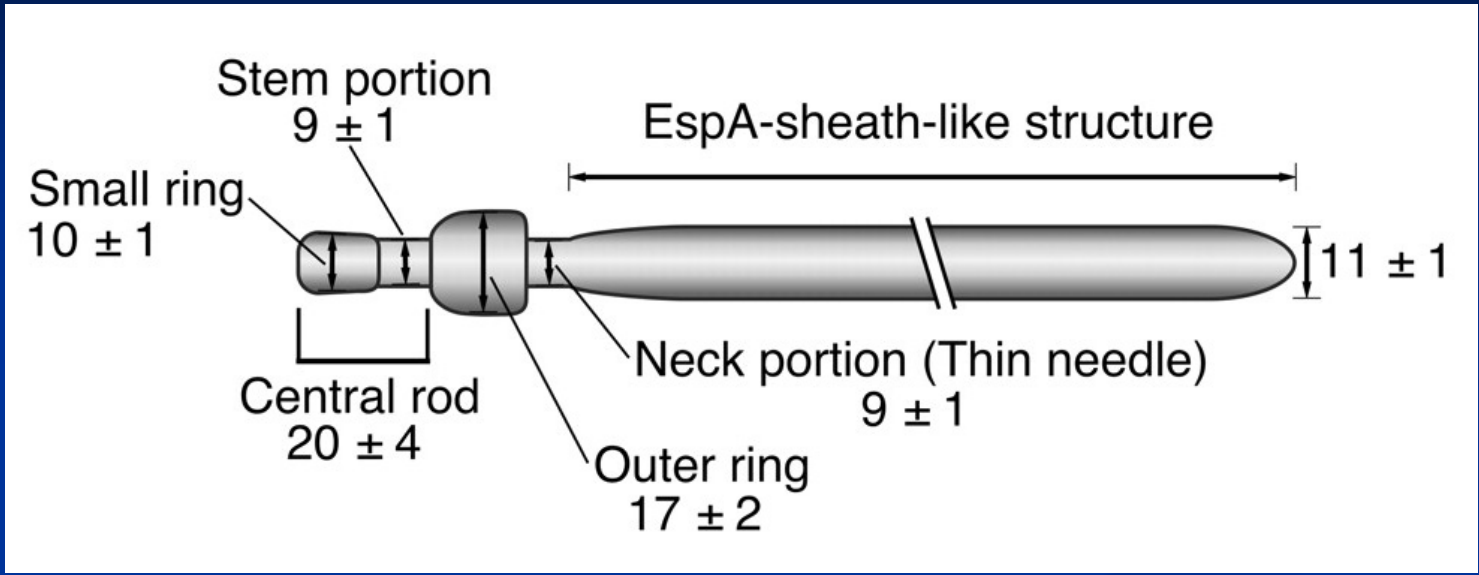
Craig *et al.*, Mol. Cell 23, 651-662 (2006)

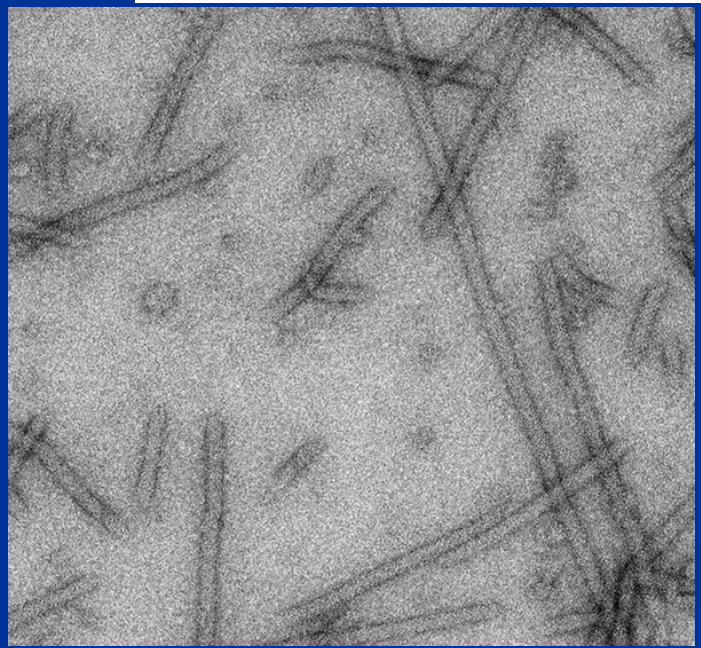


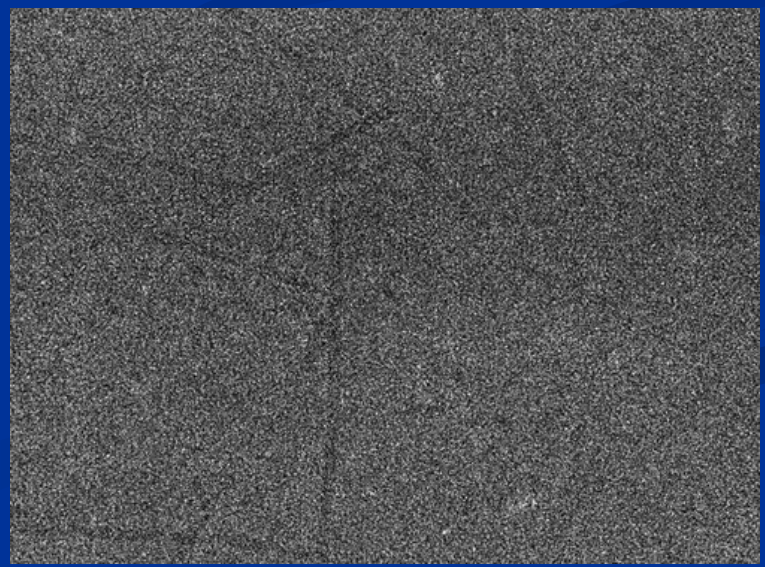
Packing of crystal subunit from *Neisseria gonorrhoeae*

Craig *et al.*, Mol. Cell 23, 651-662 (2006)

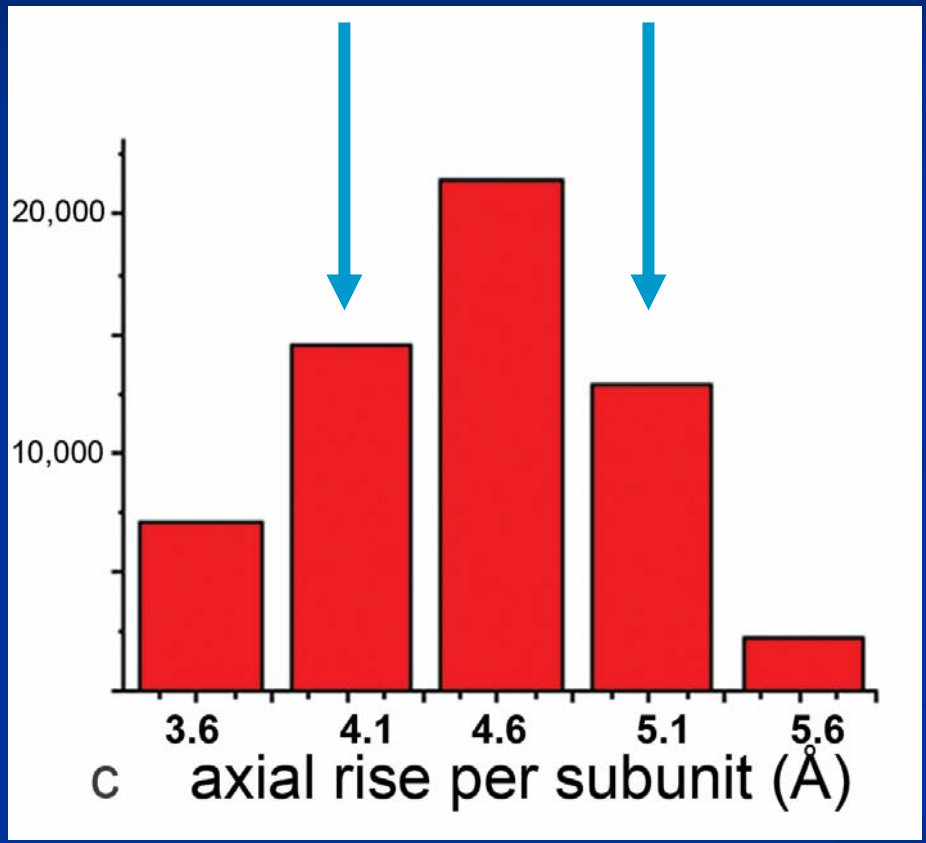
EspA of Enteropathogenic *E. coli*





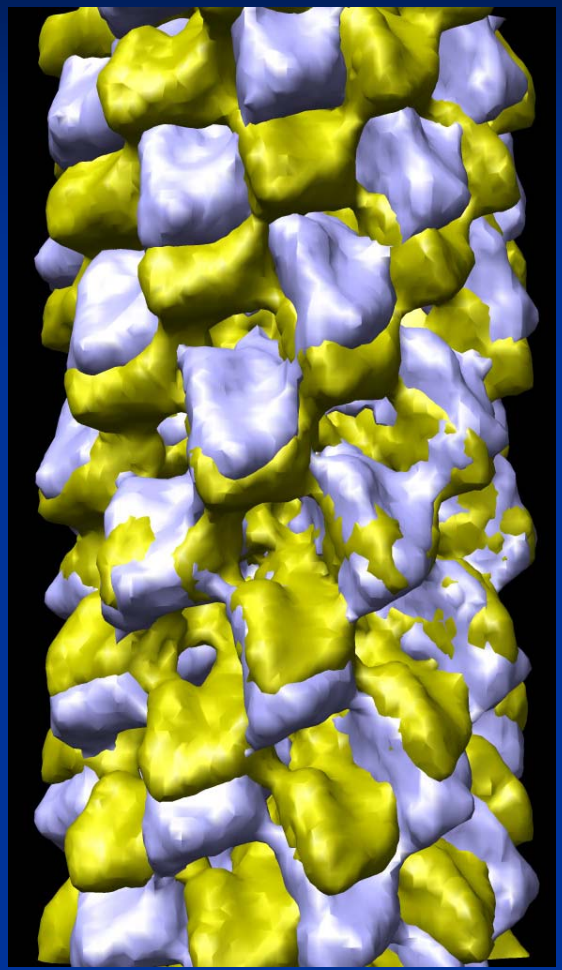


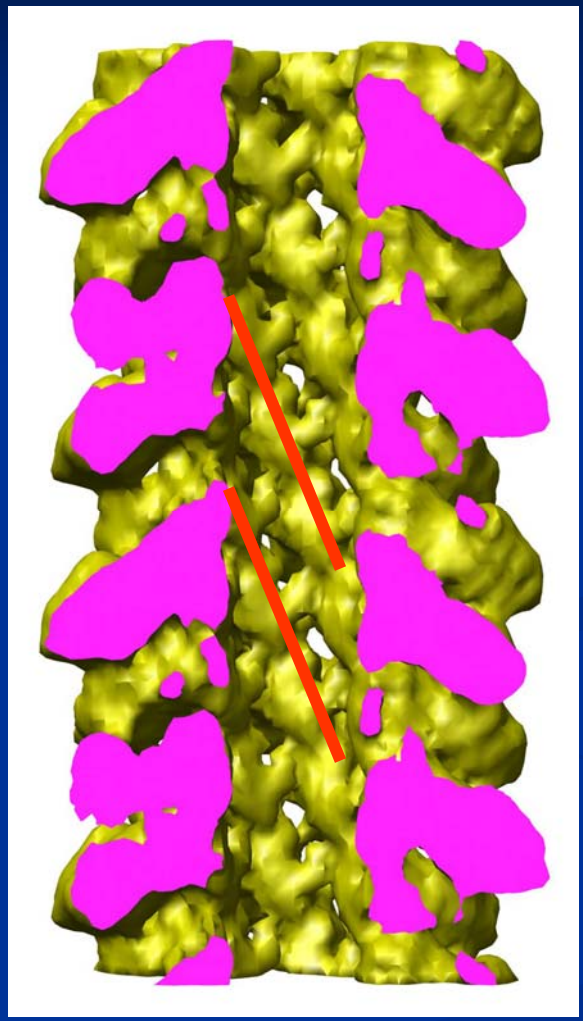
EspA of Enteropathogenic *E. coli*



~60,000 segments (each ~ 240 Å in length) analyzed
Most variation determined to be due to variable axial rise
Wang *et al.*, Structure **14**, 1189-96 (2006)

EspA: heterogeneity in axial rise





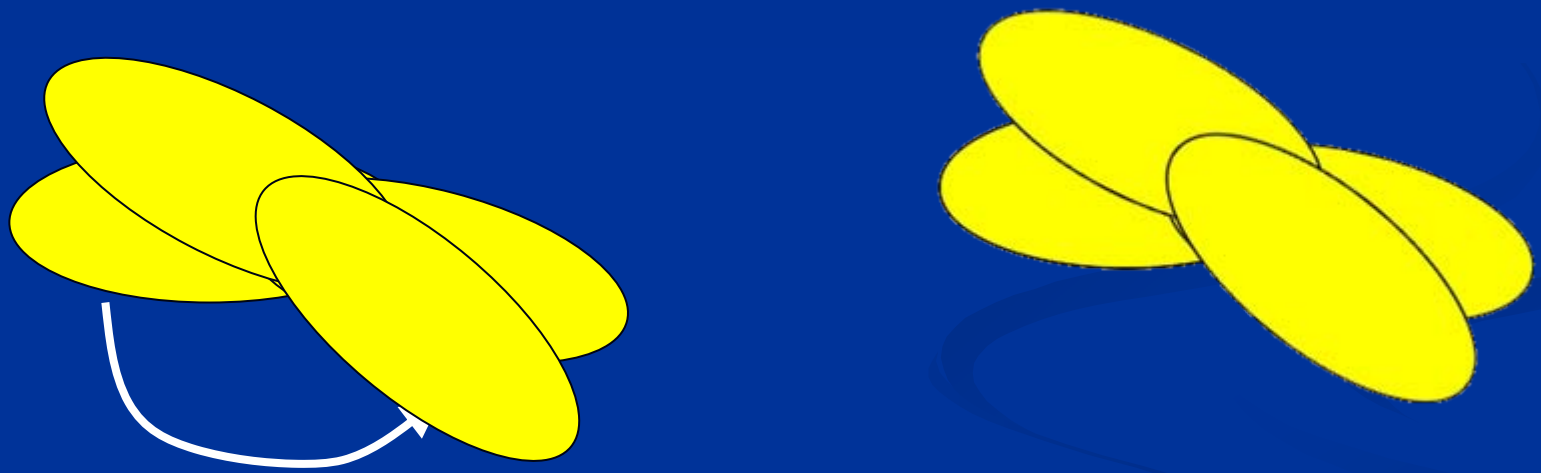
Grey: 5.3 Å Yellow: 4.2 Å Right-handed 6-start Left-handed 5-start

Wang *et al.*, Structure **14**, 1189-96 (2006)

What role does such plasticity play?

- EspA needle extensions are quite long (~ 0.6 μ) and must remain intact in a high shear environment
- Which is more resistant to breakage – a rigid glass tube or a flexible rubber tube?
- What happens when a rubber tube is stretched?
- Homology suggests that flexibility of flagellar hook may arise by similar means

Variable "twist" in F-actin



Variable tilt and twist seen in actin-scruin bundle

Structure of the acrosomal bundle

Michael F. Schmid[1], Michael B. Sherman[1]*, Paul Matsudaira[2] & Wah Chiu[1]

[1]*National Center for Macromolecular Imaging, Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030, USA*
[2]*Whitehead Institute, Department of Biology and Division of Biological Engineering MIT, Cambridge, Massachusetts 02142, USA*

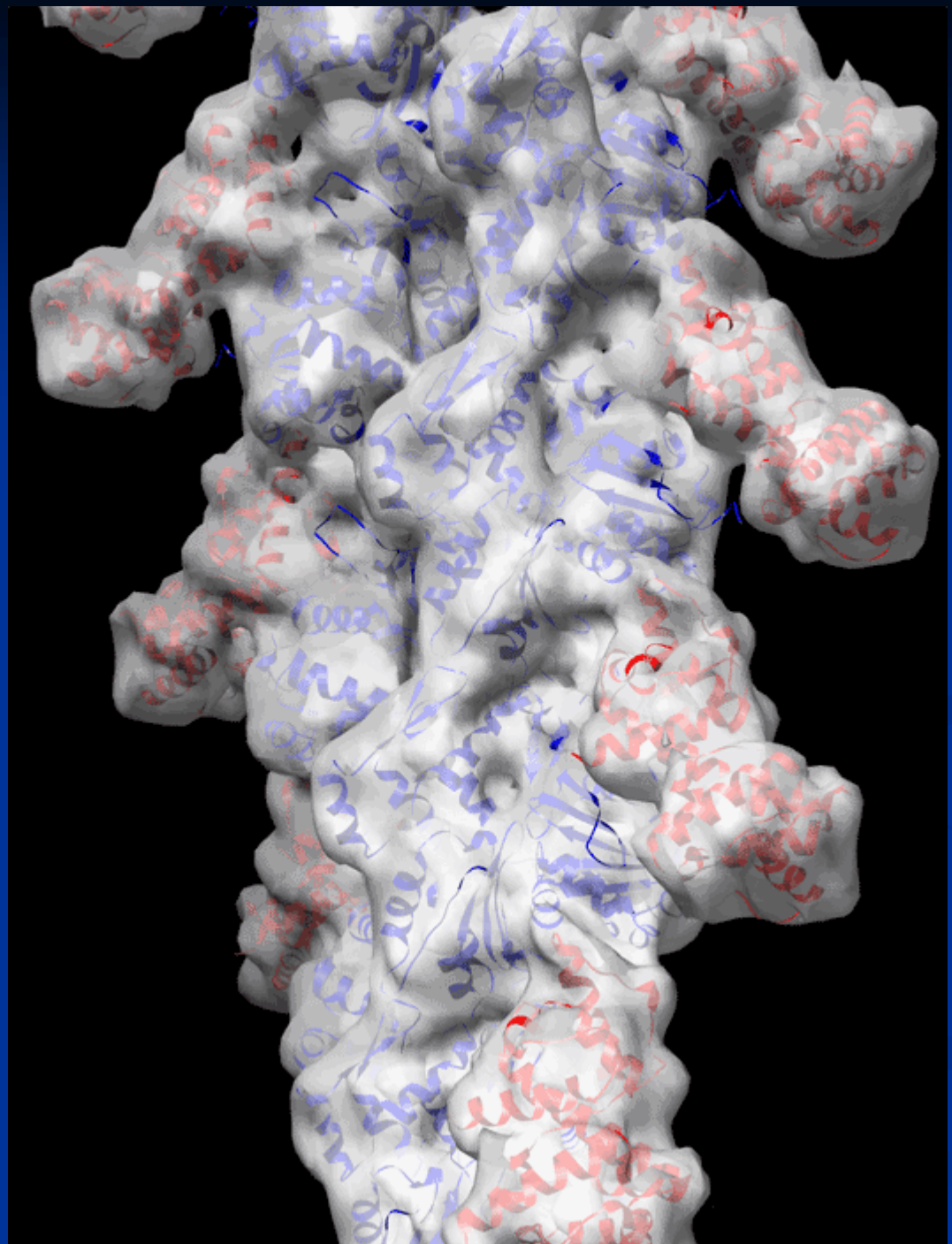
* Present address: Purdue University, Department of Biological Sciences, West Lafayette, Indiana 47907-139, USA

In the unactivated *Limulus* sperm, a 60-µm-long bundle of actin filaments crosslinked by the protein scruin is bent and twisted into a coil around the base of the nucleus. At fertilization, the bundle uncoils and fully extends in five seconds to support a finger of membrane known as the acrosomal process. This biological spring is powered by stored elastic energy and does not require the action of motor proteins or actin polymerization[1]. In a 9.5-Å electron cryomicroscopic structure of the extended bundle, we show that twist, tilt and rotation of actin–scruin subunits deviate widely from a 'standard' F-actin filament. This variability in structural organization allows filaments to pack into a highly ordered and rigid bundle in the extended state and suggests a mechanism for storing and releasing energy between coiled and extended states without disassembly.

Nature **431**, 2 Sept. 2004

"Movie" of tilt shows:

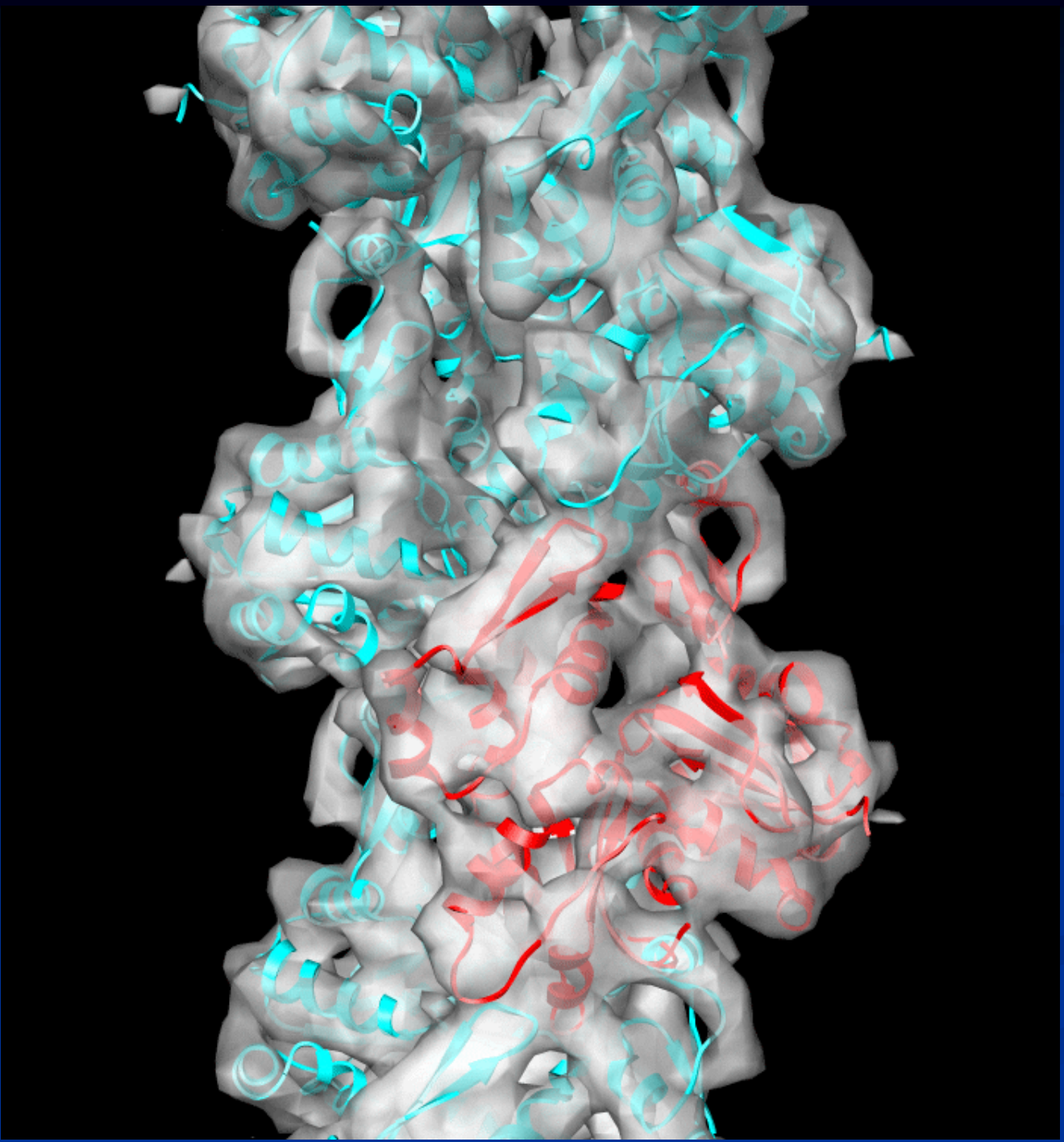
1) rearrangement of contacts
2) change in twist (from 167° to 154°)
3) propeller rotation of actin domains



Unprecedented resolution in looking at isolated actin filaments and complexes

F-actin decorated with ABD2 of fimbrin, ~ 12 Å

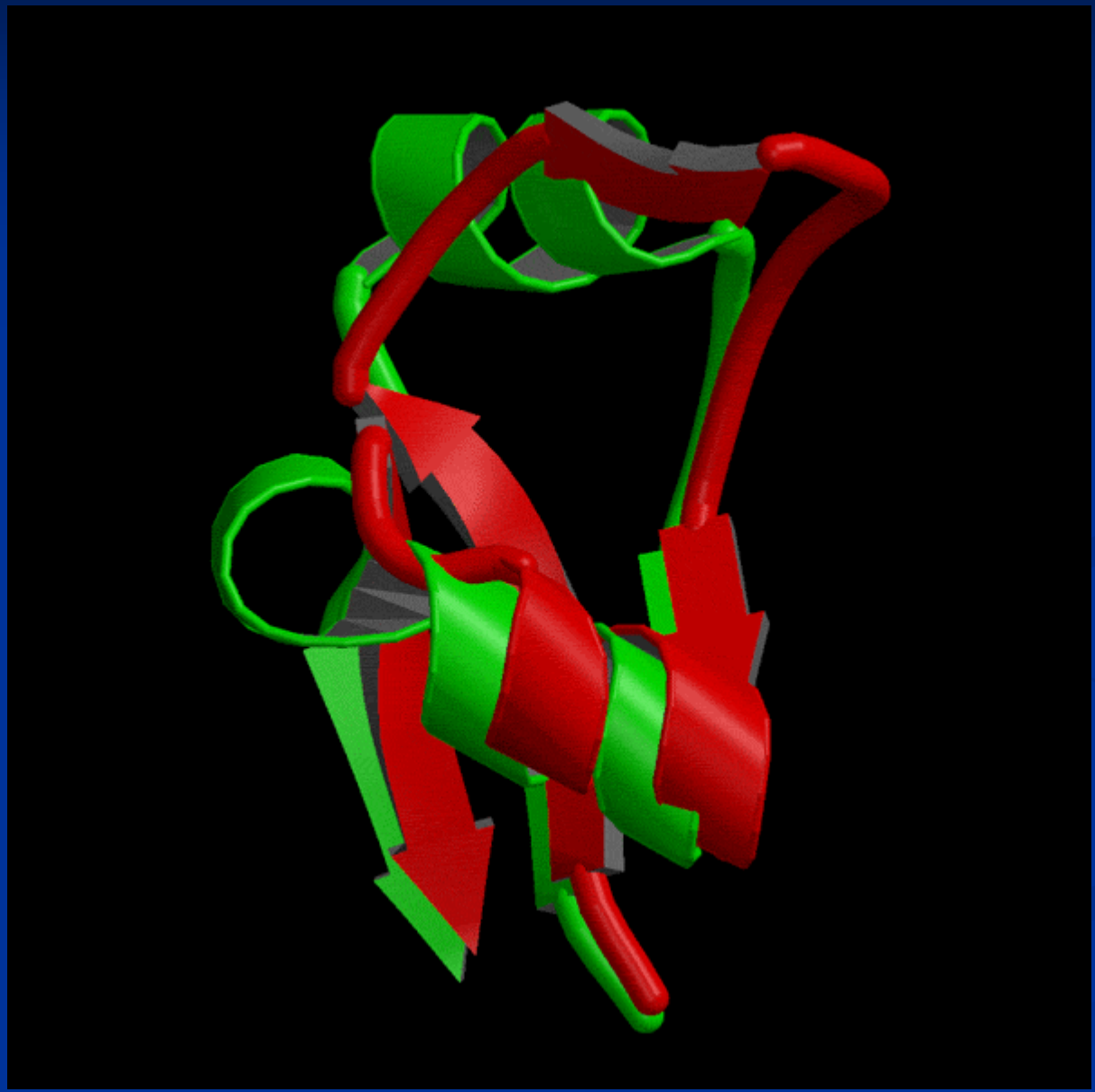
shows unambiguously that there is not a conserved mode of interaction of Calponin Homology (CH) domains with actin



Unprecedented resolution in looking at isolated actin filaments and complexes

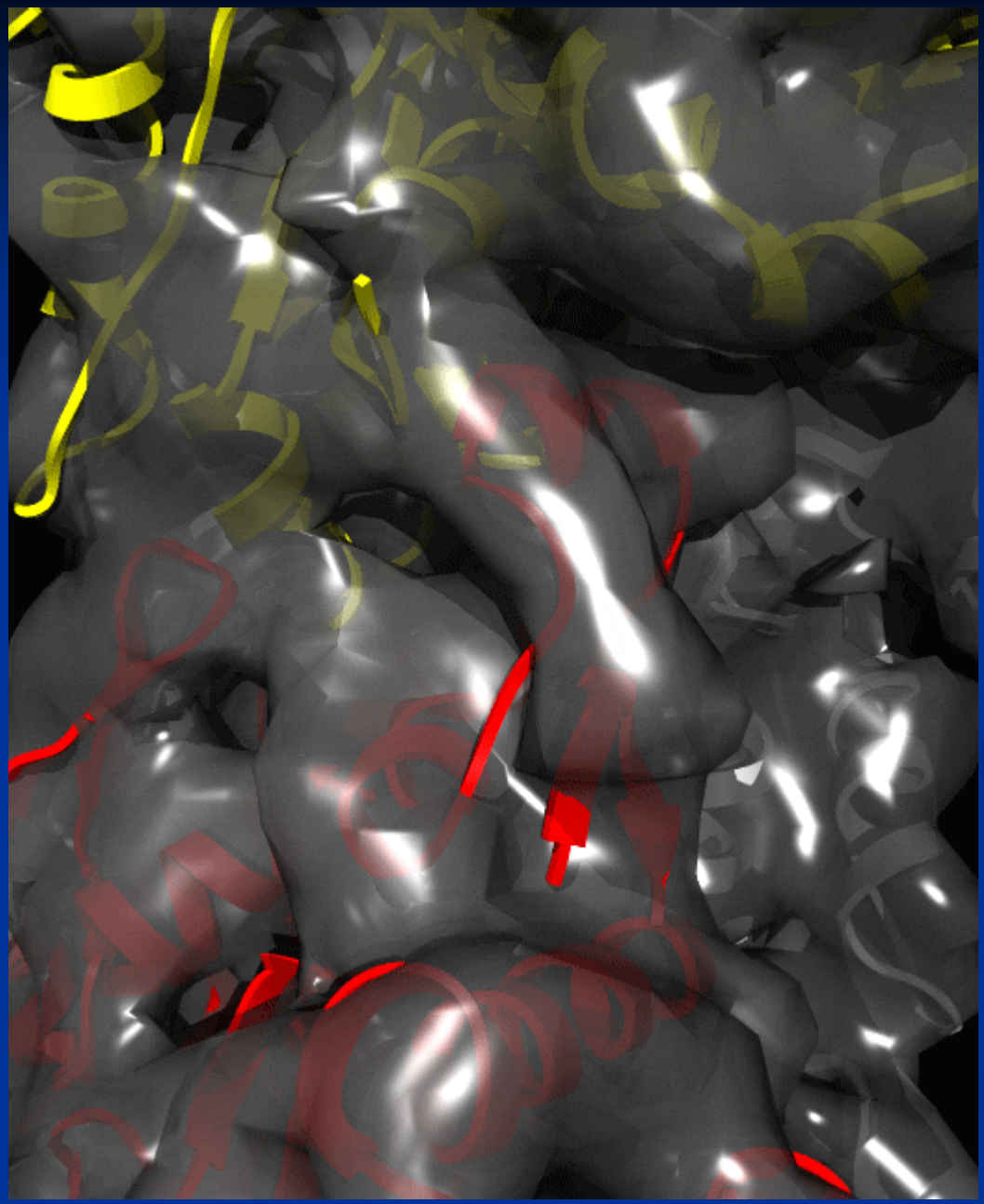
pure F-actin, ~ 9 Å

Different G-actin structures reinforce concept of subdomain 2 being a switch



Kabsch *et al.* (1990), actin-DNase I complex

Otterbein *et al.* (2001), modified G-actin



Unprecedented resolution in looking at isolated actin filaments and complexes

pure F-actin, ~ 9 Å

Conserved family of proteins

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 7290–7294, August 1992
Biochemistry

An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin, and hsp70 heat shock proteins

(structural comparison/property pattern/remote homology)

PEER BORK, CHRIS SANDER, AND ALFONSO VALENCIA

European Molecular Biology Laboratory, D-6900 Heidelberg, Federal Republic of Germany

Communicated by Russell F. Doolittle, March 6, 1992

ABSTRACT **The functionally diverse actin, hexokinase, and hsp70 protein families have in common an ATPase domain of known three-dimensional structure. Optimal superposition of the three structures and alignment of many sequences in each of the three families has revealed a set of common conserved residues, distributed in five sequence motifs, which are involved in ATP binding and in a putative interdomain hinge. From the multiple sequence alignment in these motifs a pattern of amino acid properties required at each position is defined. The discriminatory power of the pattern is in part due to the use of several known three-dimensional structures and many sequences and in part to the "property" method of generalizing from observed amino acid frequencies to amino acid fitness at each sequence position. A sequence data base search with the pattern significantly matches sugar kinases, such as fuco-, glucono-, xylulo-, ribulo-, and glycerokinase, as well as the prokaryotic cell cycle proteins MreB, FtsA, and StbA. These are predicted to have subdomains with the same tertiary structure as the ATPase subdomains Ia and IIa of hexokinase, actin, and Hsc70, a very similar ATP binding pocket, and the capacity for interdomain hinge motion accompanying functional state changes. A common evolutionary origin for all of the proteins in this class is proposed.**

In spite of their different biological functions, actin, Hsc70, and hexokinase contain similar three-dimensional structures (Fig. 1) (1–4). No overall sequence similarity between these three protein families can be detected with standard pairwise sequence alignment algorithms, so the structural similarity

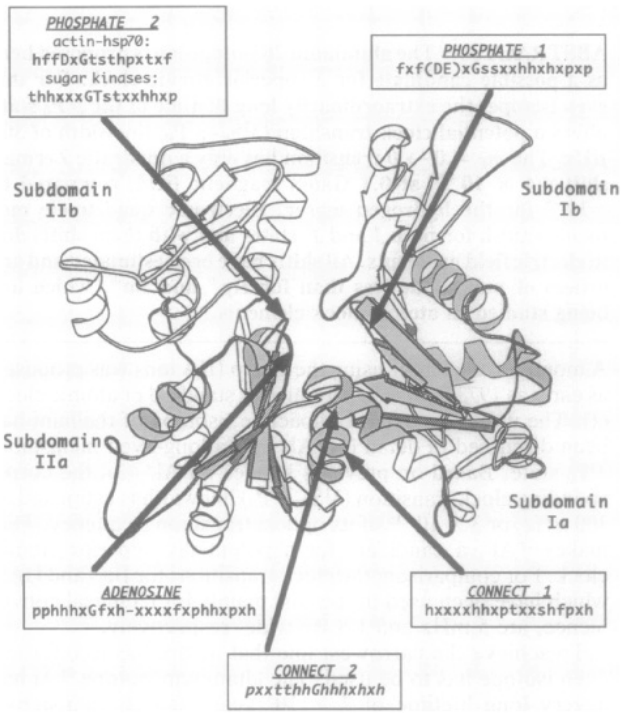
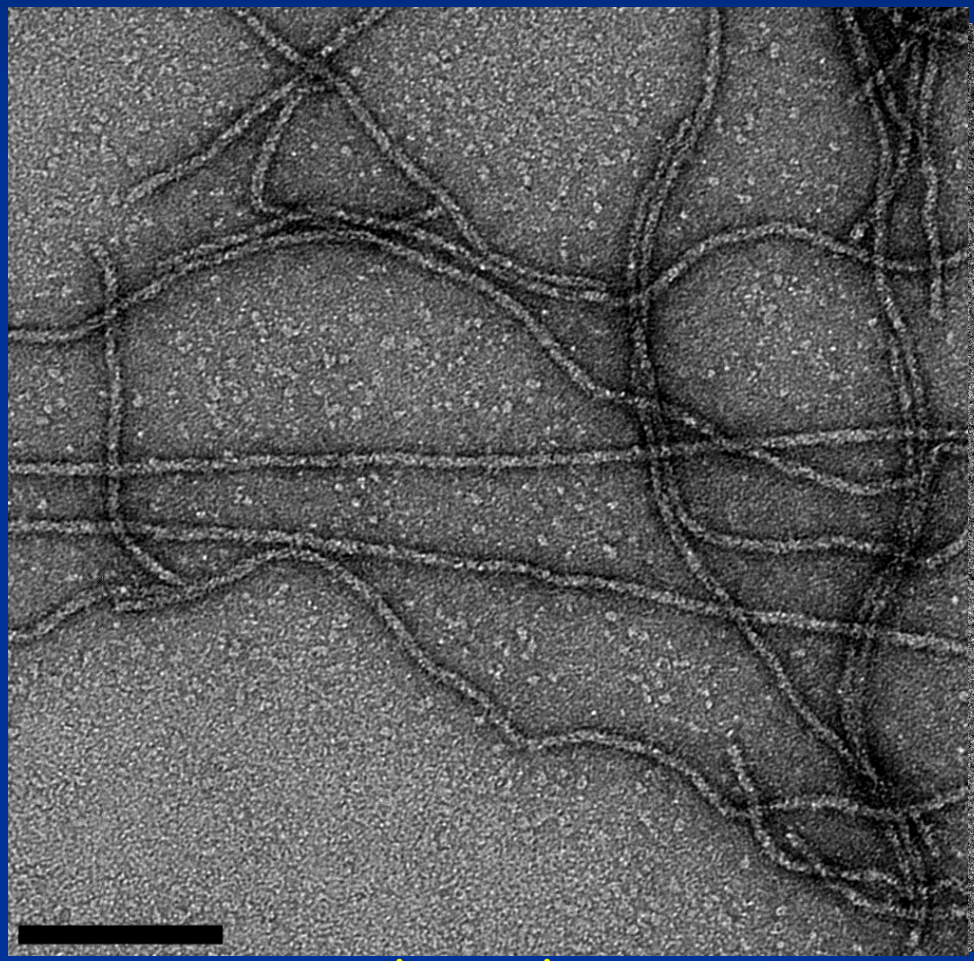


FIG. 1. Diagramatic representation of actin. The five parts of the sequence pattern characteristic for the actin/hexokinase/hsc70

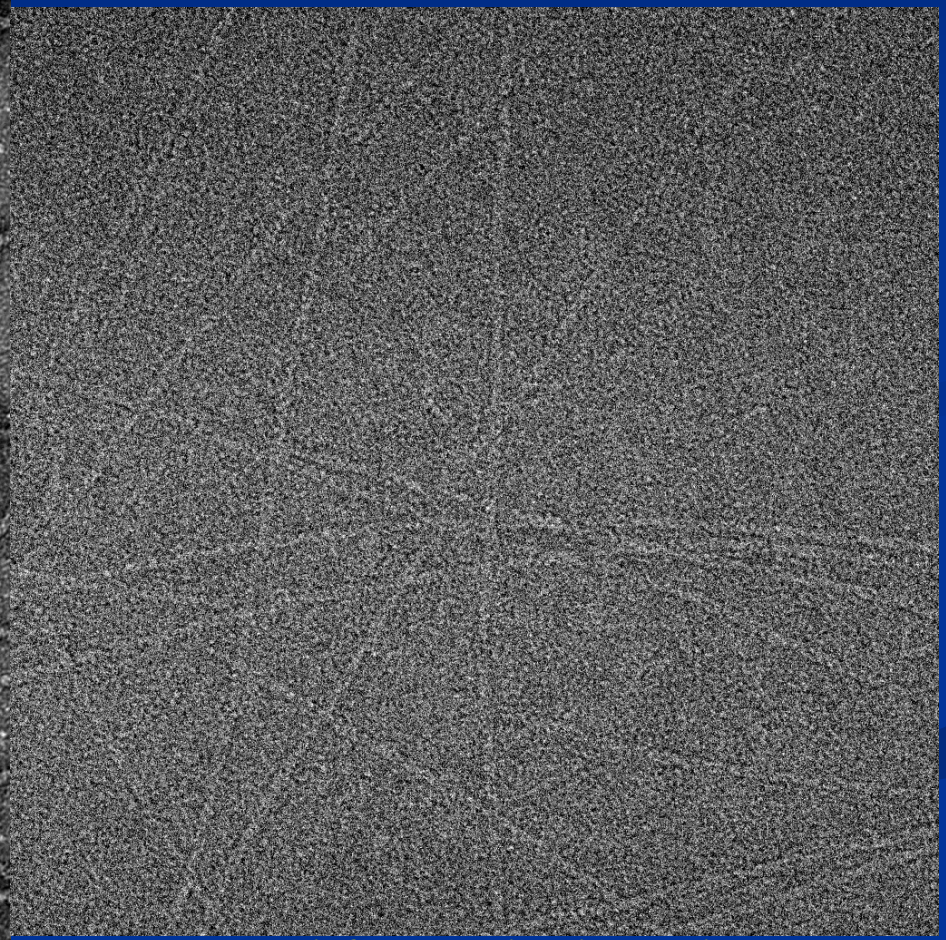
Dramatic support for conformational changes in actin subunit comes from ParM structures!

van den Ent *et al.*, "F-actin-like filaments formed by plasmid segregation protein ParM", EMBO J. (2002)

Bacterial ParM filaments



negative stain



unstained frozen-hydrated

The ParM Paradox

The EMBO Journal Vol. 21 No. 24 pp. 6935–6943, 2002

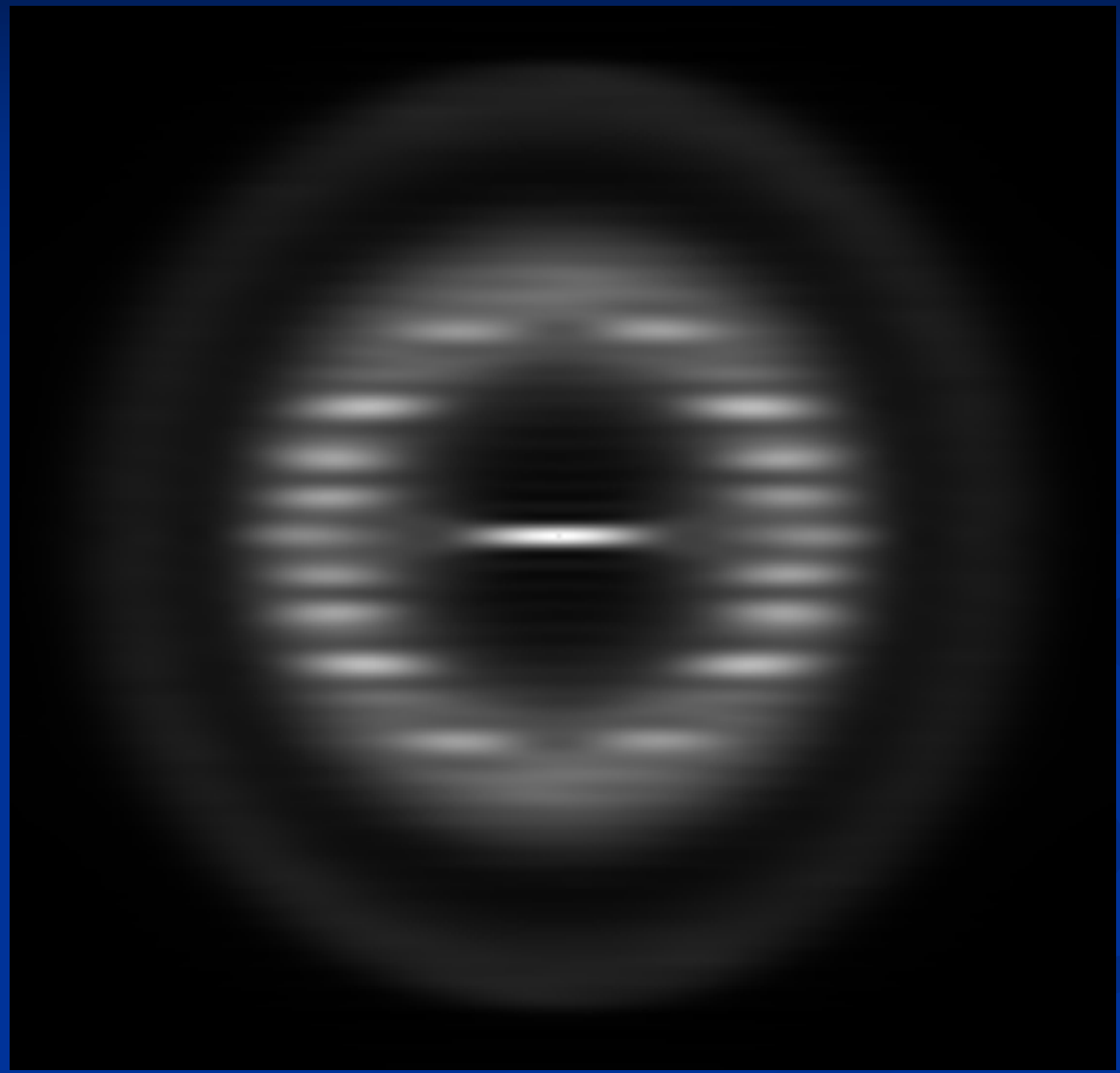
F-actin-like filaments formed by plasmid segregation protein ParM

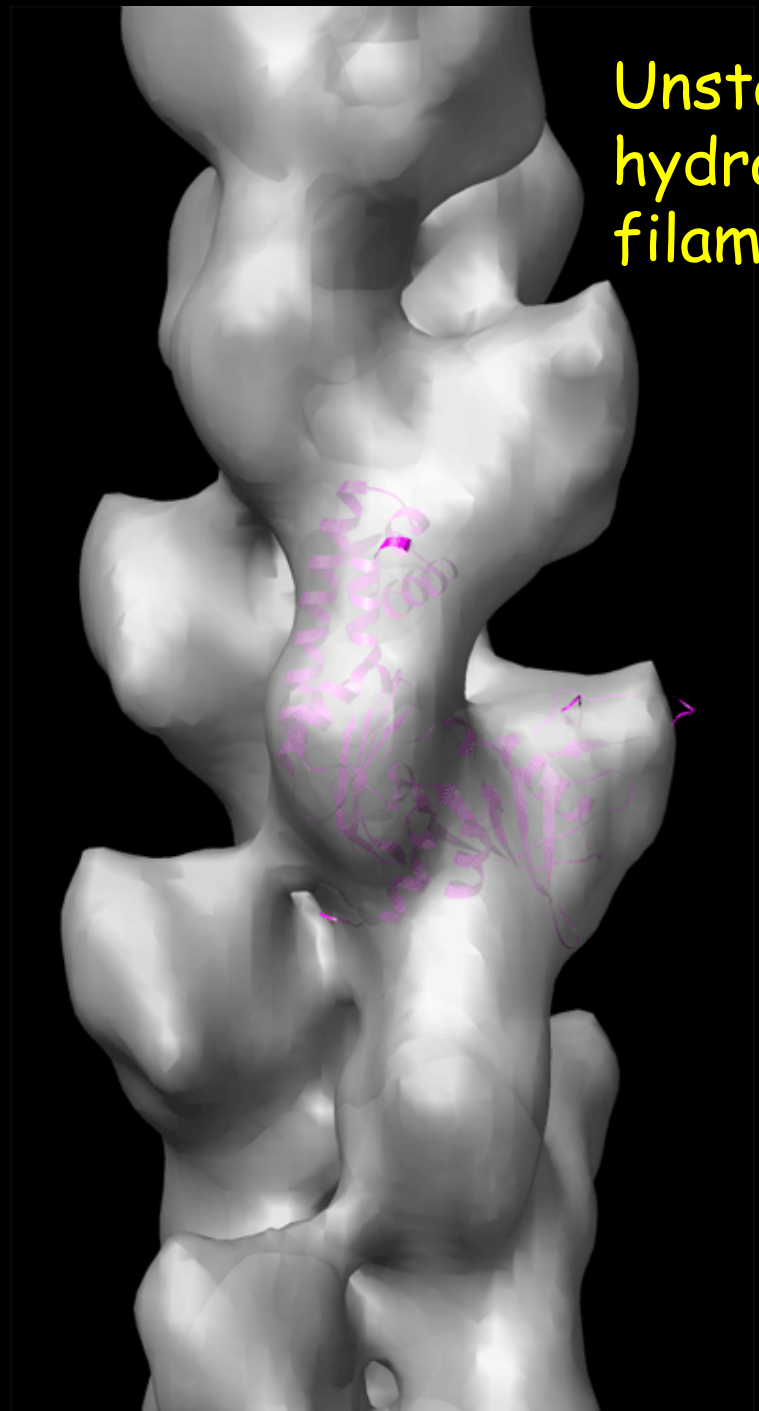
Fusinita van den Ent, Jakob Møller-Jensen[1], Linda A.Amos, Kenn Gerdes[1] and Jan Löwe[2]

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK and [1]Department of Biochemistry and Molecular Biology, University of Southern Denmark, DK-5230 Odense M, Denmark

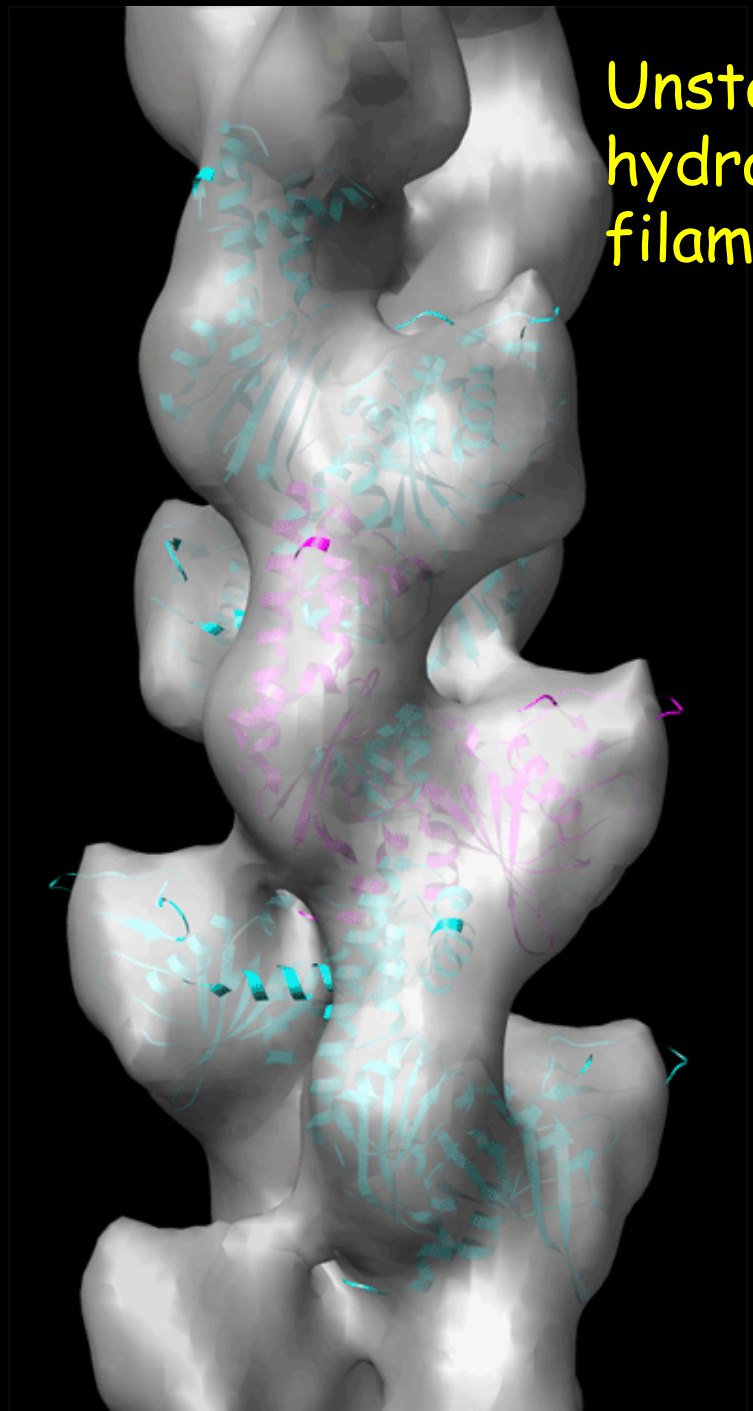
Although the overall fold of ParM resembles actin (Figure 4), ParM has some unique features, which were unexpected for a protein whose filaments are almost indistinguishable from F-actin. Surprisingly, the differences are in regions that are involved in protofilament contacts. The first major difference is in subdomain IB. A

Variability in twist in ParM greater than F-actin



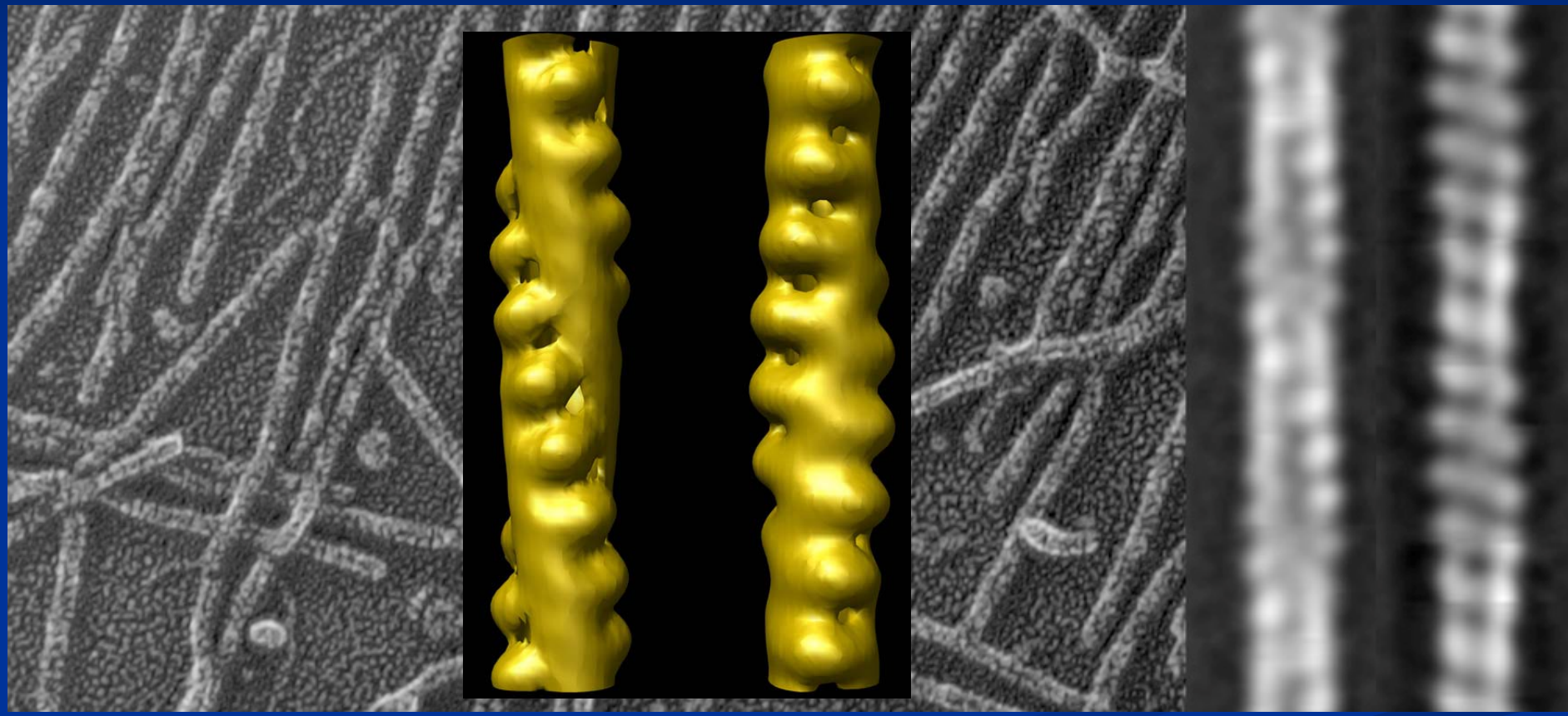


Unstained frozen-hydrated ParM filaments by cryo-EM



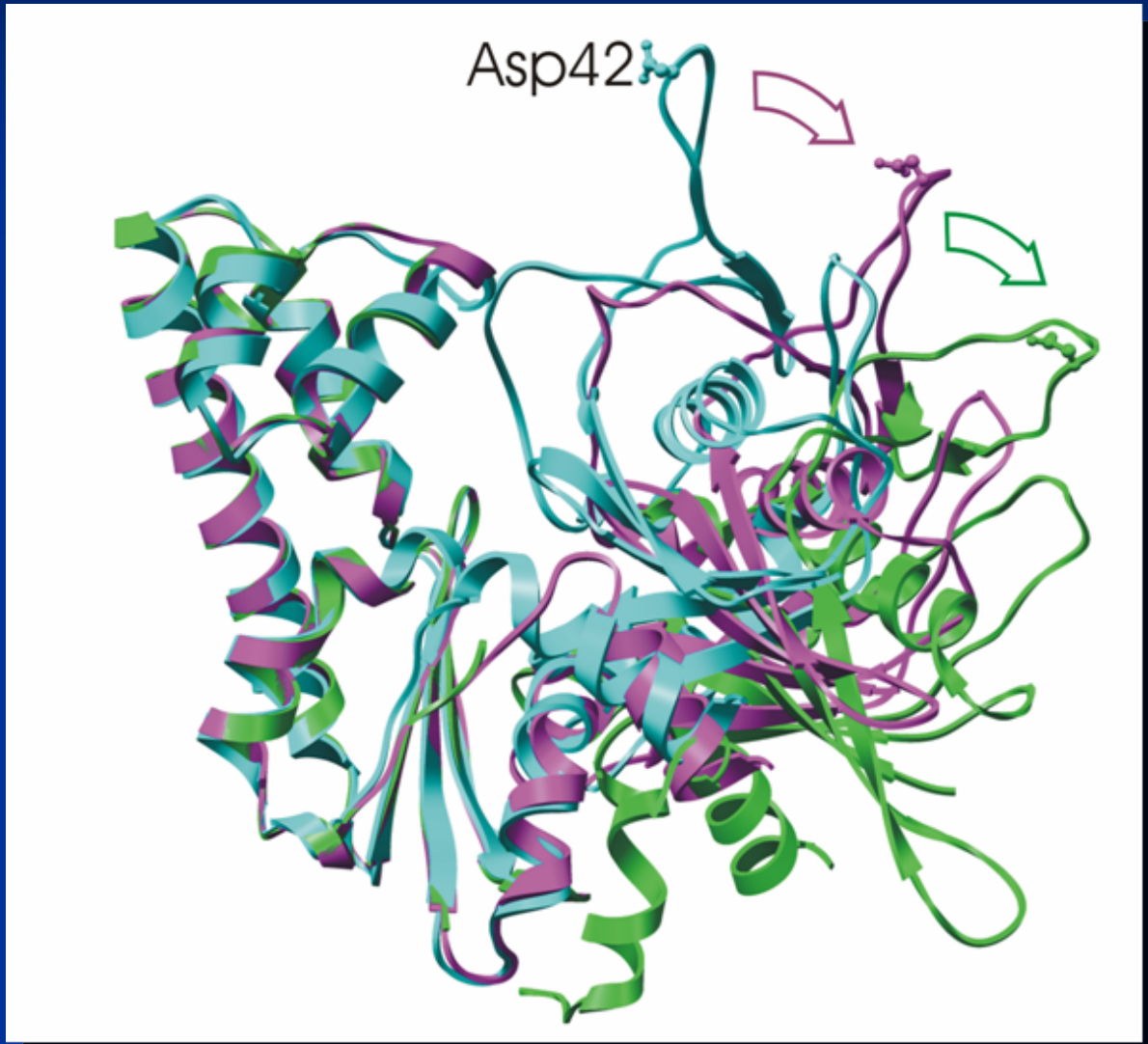
Unstained frozen-hydrated ParM filaments by cryo-EM

Helical hand confirmed by quick freeze/deep etch EM

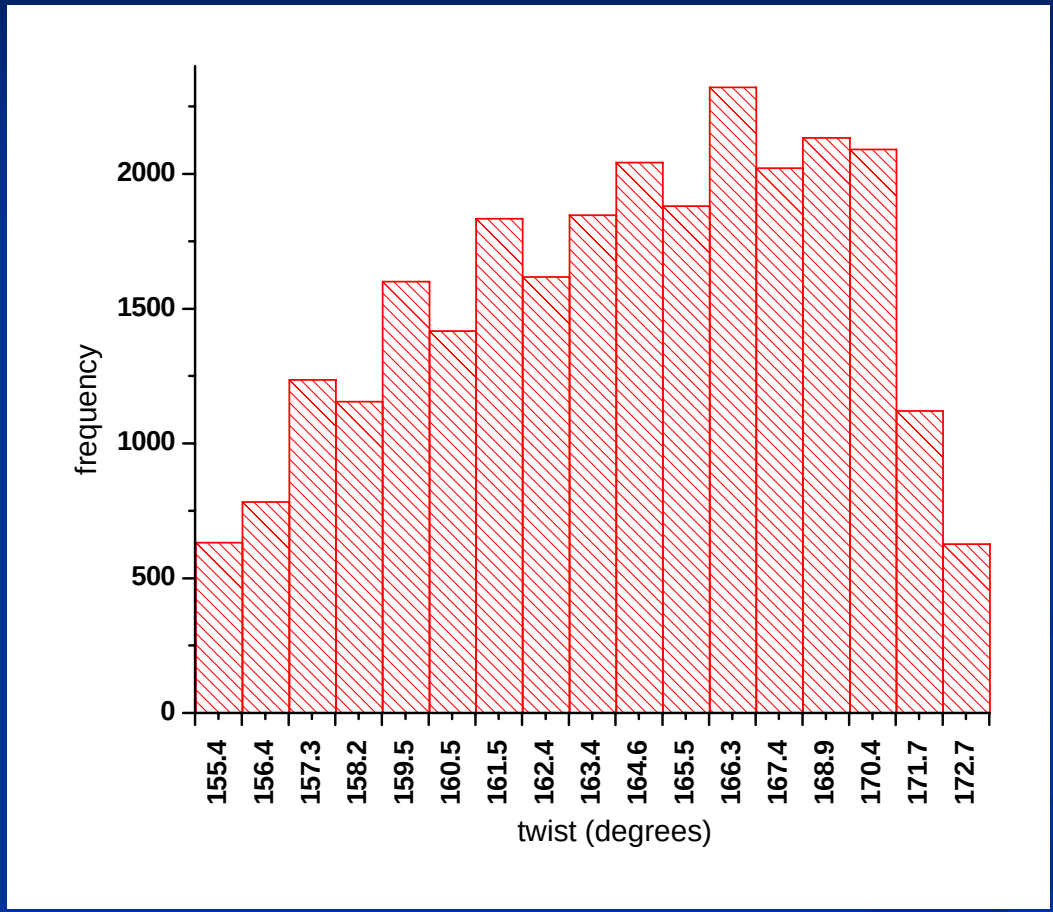


ParM F-actin

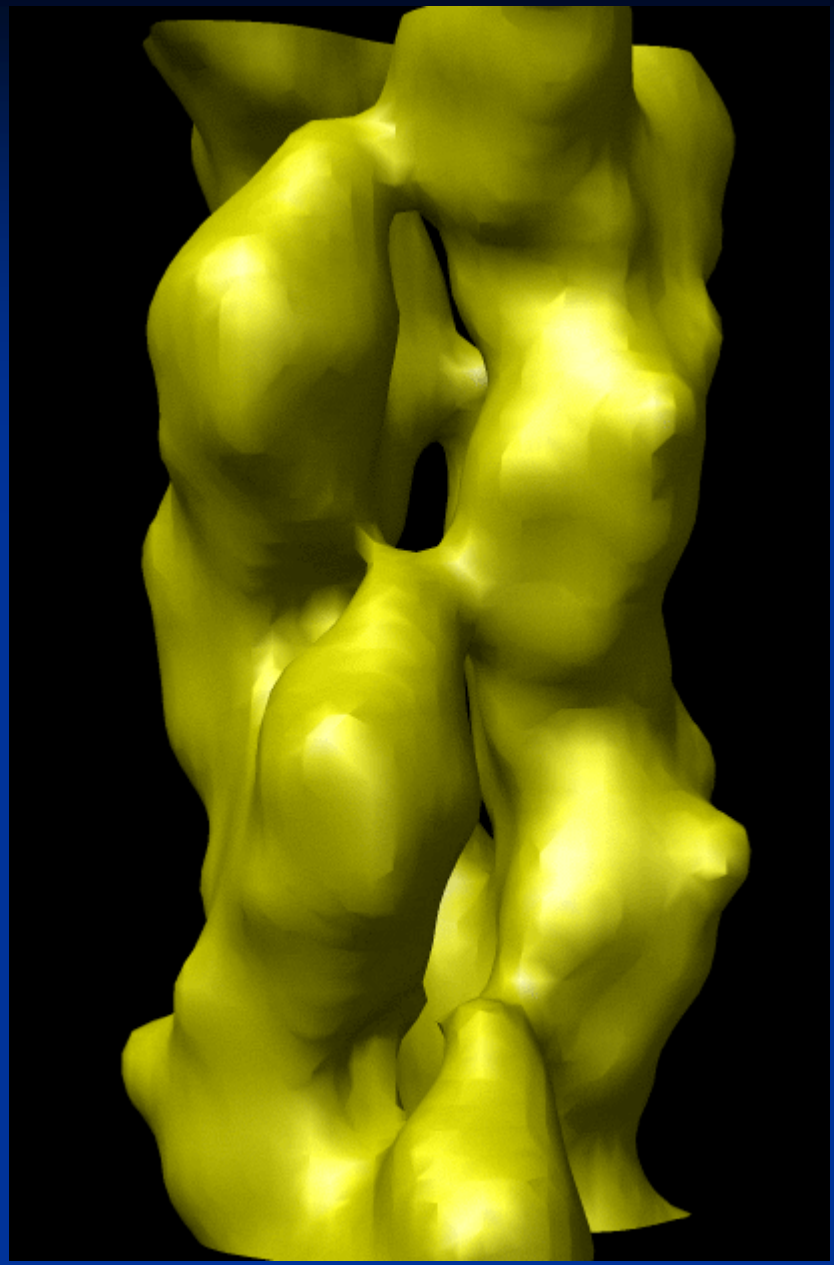
Filament protomer more open than crystal subunit



Variability in twist in ParM greater than in F-actin

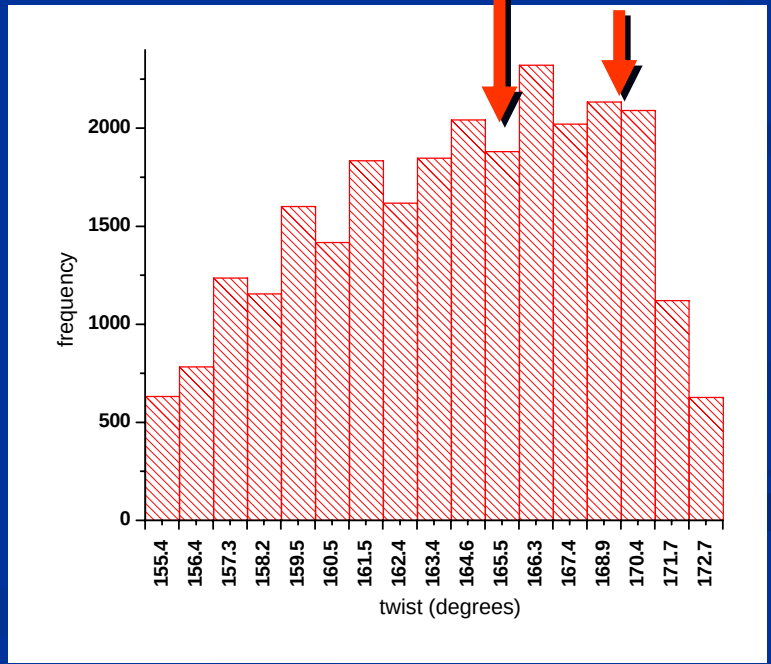


After sorting, can now reconstruct more homogeneous subsets at a reasonable resolution

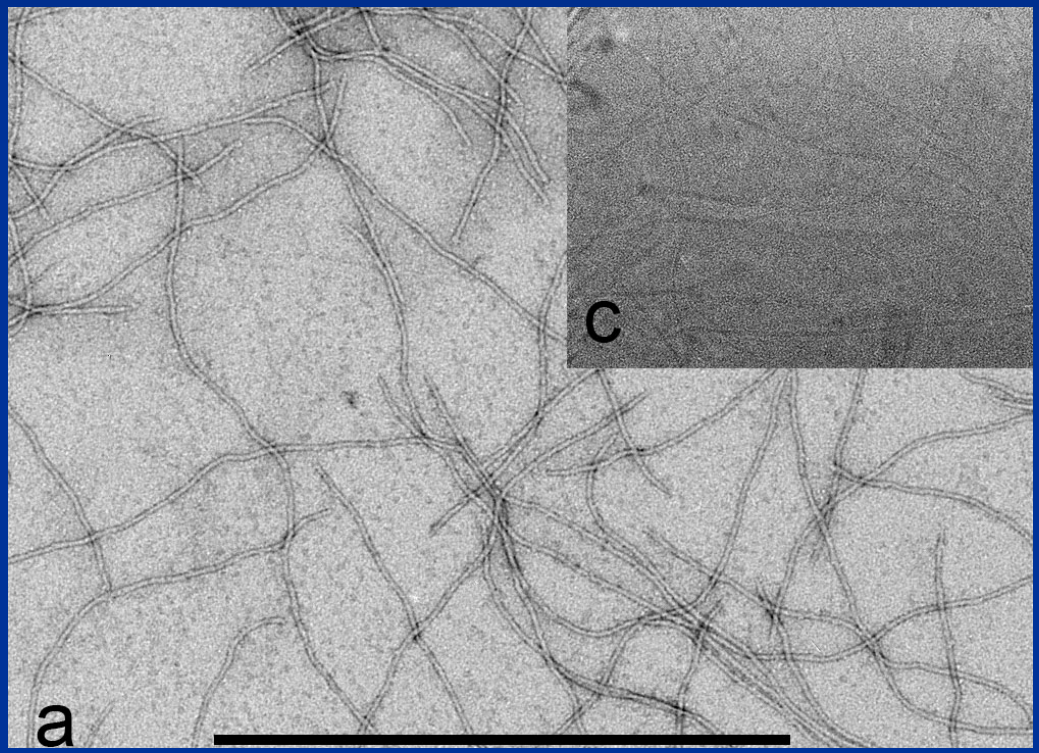


Domain-domain motions part of variable twist

animation between two states of twist, 165.2° and 169.6°



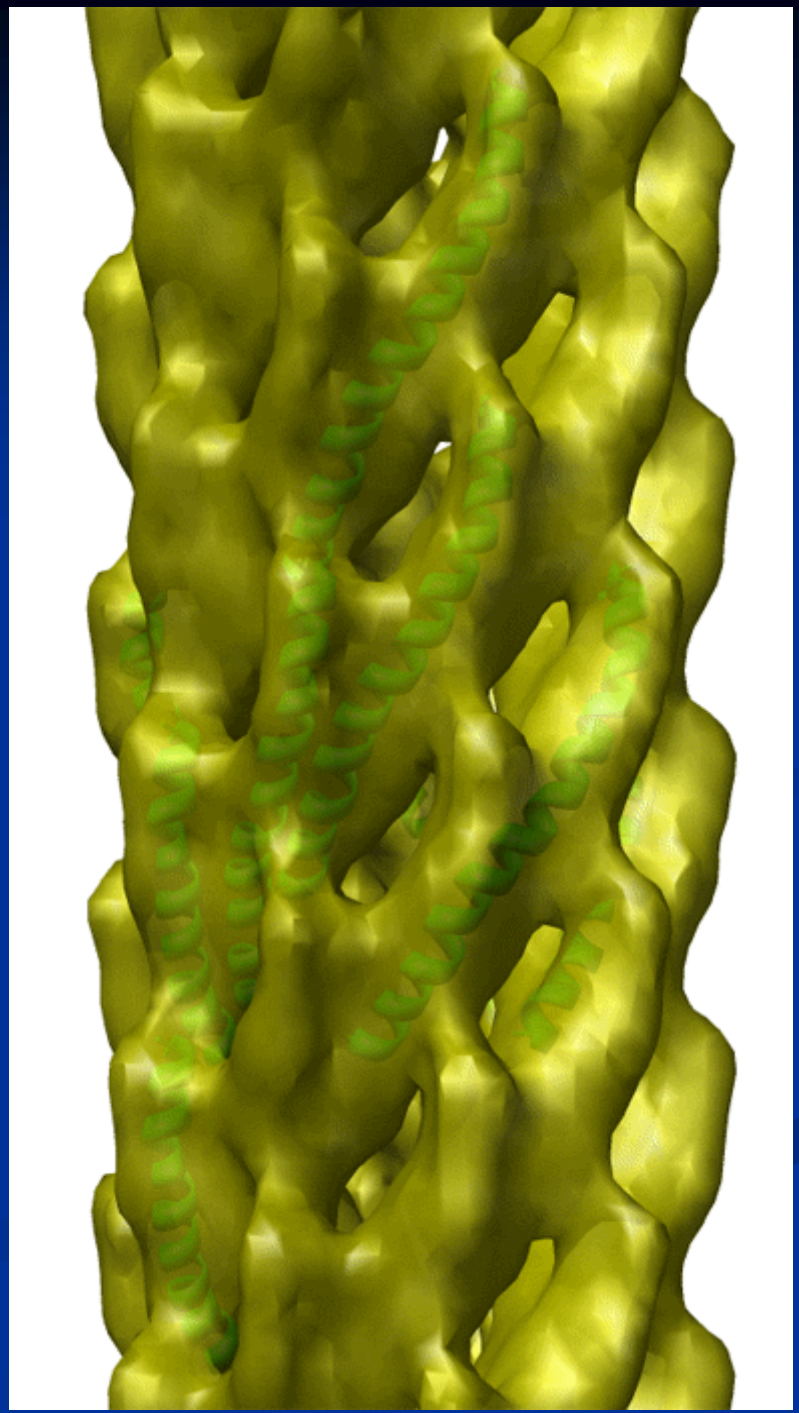
Method allows for studying filamentous bacteriophage



- Model systems in understanding:
  - DNA packaging
  - Assembly of a protein polymer from a small integral membrane protein
- Important in cloning, phage display, etc.

Wang *et al.*, J. Mol. Biol. **361**, 209-215 (2006)

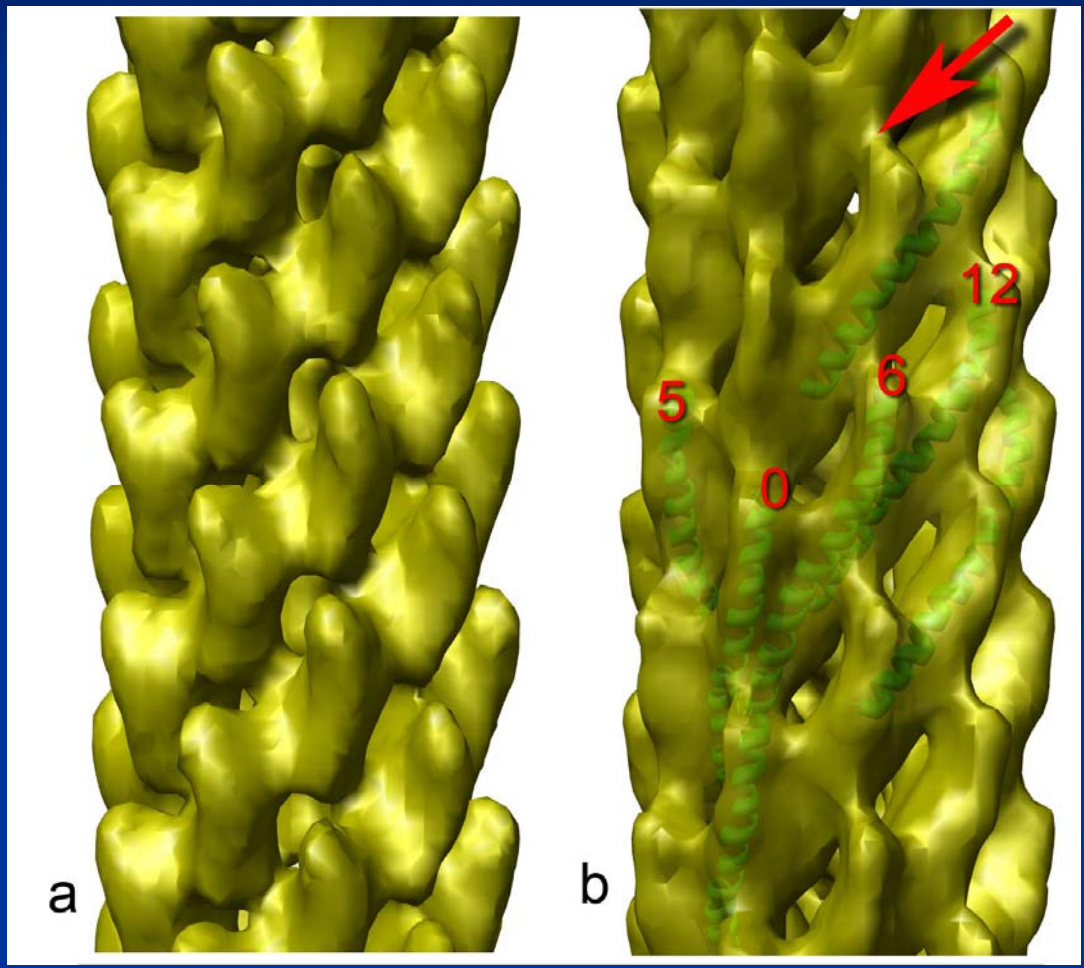
phage fd – small subunit containing 50 residues, exists before polymerization as an integral membrane protein



"The Structure of a Filamentous Bacteriophage",

Wang *et al.*, J. Mol. Biol. **361**, 209-215 (2006)

Two states of filamentous bacteriophage fd



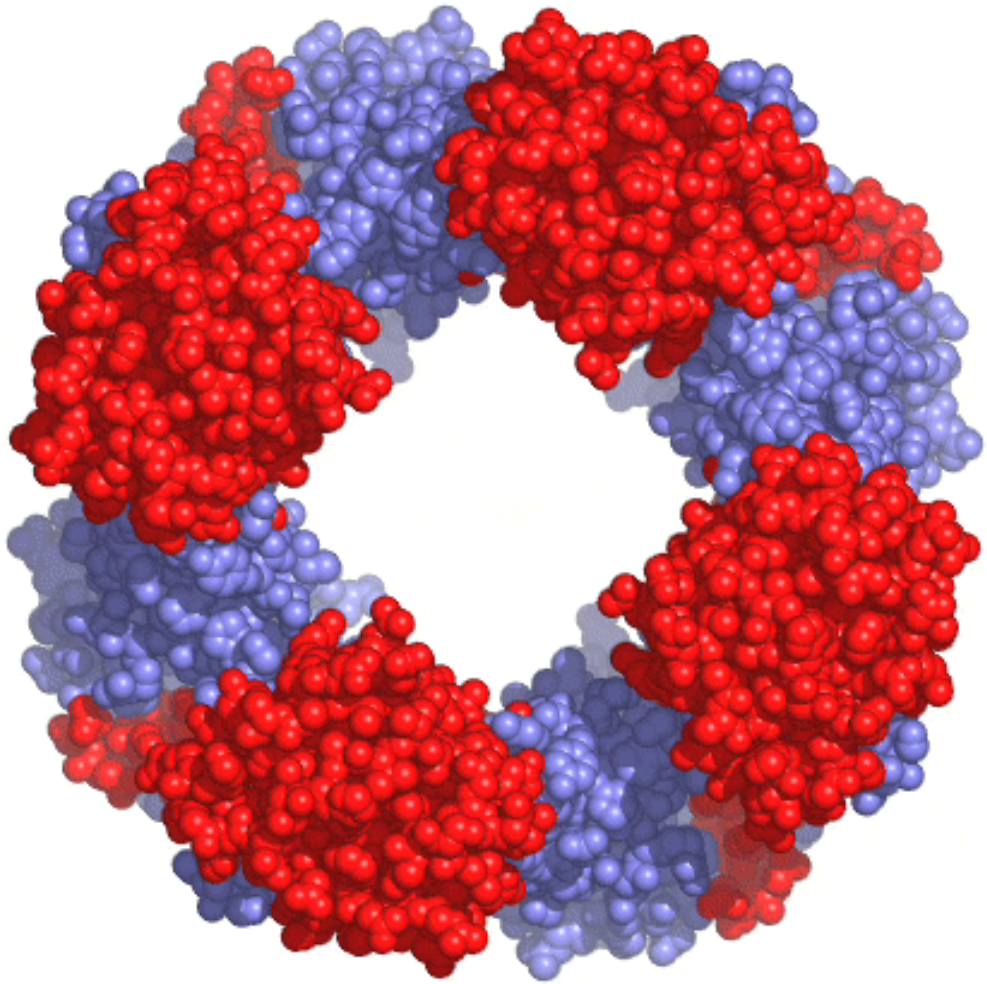
Such polymorphism should not be surprising, given that 41/50 residues can be mutated to Ala and the subunit still co-assembles almost as efficiently as wt! (Roth *et al.*, JMB 322,357-67, 2002)

Octameric membrane transporter shows similar degree of polymorphism

The RCK Domain of the KtrAB K^+ Transporter: Multiple Conformations of an Octameric Ring

Ronald A. Albright,[1] José-Luís Vazquez Ibar,[1] Chae Un Kim,[2] Sol M. Gruner,[2,3] and João Henrique Morais-Cabral[1,*]
[1] Department of Molecular Biophysics and Biochemistry, Yale University, 266 Whitney Avenue, New Haven, CT 06520, USA
[2] Cornell High Energy Synchrotron Source
[3] Physics Department
Cornell University, Ithaca, NY 14853, USA
*Contact: joao.cabral@yale.edu
DOI 10.1016/j.cell.2006.08.028

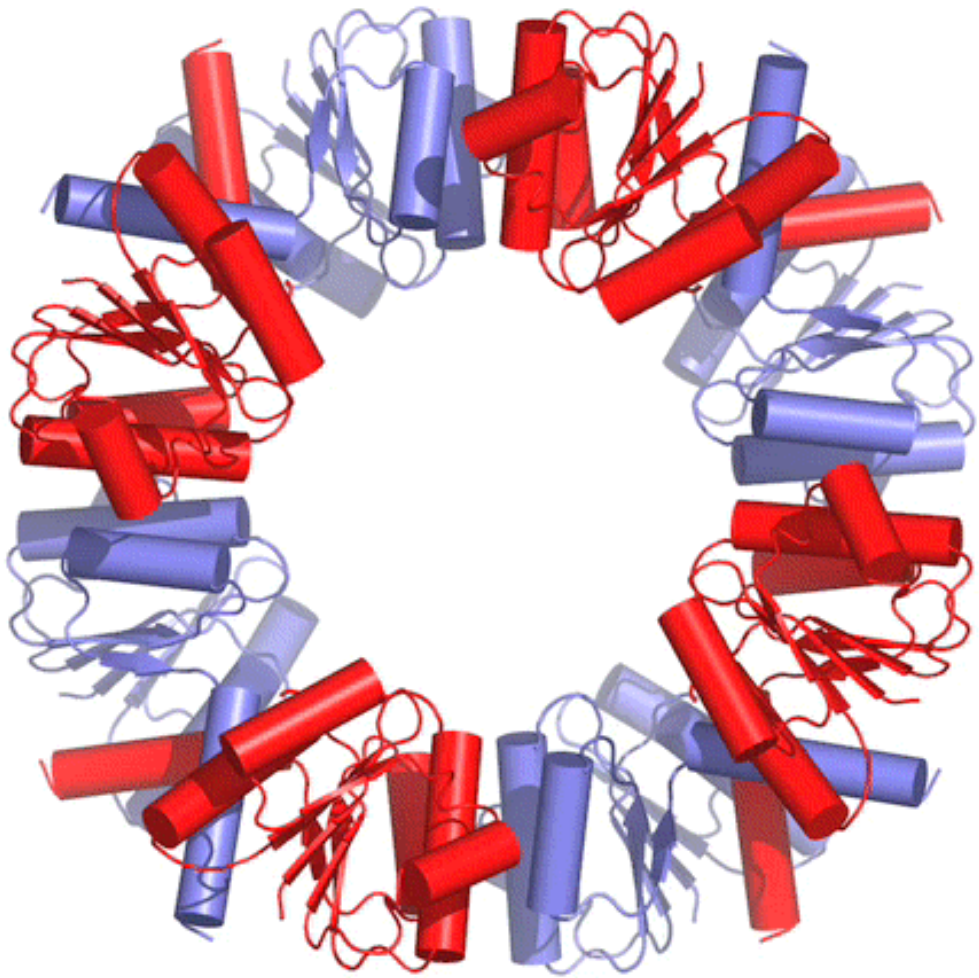
Three states observed in crystals, with relative domain angles of 35°, 46° and 80°



Octameric membrane transporter shows similar degree of polymorphism

The RCK Domain of the KtrAB K^+ Transporter: Multiple Conformations of an Octameric Ring

Ronald A. Albright,[1] José-Luís Vazquez Ibar,[1] Chae Un Kim,[2] Sol M. Gruner,[2,3] and João Henrique Morais-Cabral[1,*]
[1] Department of Molecular Biophysics and Biochemistry, Yale University, 266 Whitney Avenue, New Haven, CT 06520, USA
[2] Cornell High Energy Synchrotron Source
[3] Physics Department
Cornell University, Ithaca, NY 14853, USA
*Contact: joao.cabral@yale.edu
DOI 10.1016/j.cell.2006.08.028



Acknowledgments

Vitold Galkin, Xiong Yu, Albina Orlova, Ying Wang, Jakub Bielnicki
Olga Cherepanova, Margaret VanLoock, Yen-Ju Chen, Natasha Lukoyanova

- EspA: Calvin Yip, Natalie Strynadka (UBC)
- ParM: Ethan Garner, Dyche Mullins (UCSF), John Heuser (WUSTL)
- Phage fd: George Thomas (UMKC)
- Archaeal flagellum: Shlomo Trachtenberg (HU)
- GC Pili: John Tainer (Scripps), Lisa Craig (Simon Fraser), Nils Volkmann (Burnham)