A New Approach to EM of Helical Polymers Yields New Insights

- Helical polymers are ubiquitous in biology
  - Actin, microtubules, intermediate filaments, thick filaments, viruses, bacteriophage, flagella, pili, RecA, Rad51…
- Helical objects were the first to be reconstructed in 3D in the EM
  - DeRosier & Klug (1968), tail of bacteriophage T4
- Many advances since then
  - Structure of one natural helical polymer (bacterial flagellar filament) determined at near atomic resolution (Yonekura *et al.*, 2003)
  - Membrane proteins have been induced to form *in vitro* helical tubes for high resolution structural studies

Some definitions

- What is helical symmetry? A screw operation involves a coupled rotation and axial translation. Some terminology:
  - axial rise is Δz
  - rotation is $\Delta\varphi$
  - helical repeat (c) is the translation along the axis needed to bring one subunit into exact superposition with another subunit
  - for an integer number of subunits/turn, or units/turn (u/t), repeat is given by

    $$u * \Delta z = c$$

  - in a crystal, the only allowed helical symmetries involve 2, 3, 4 or 6 subunits/turn
  - outside of a crystal, there is no reason for any helix to have an integer number of u/t!
  - outside of a crystal, there is no space group maintaining long-range order. So cannot have true 1D crystal

Problems with definition of helical repeat

- Very small changes in symmetry can lead to very large changes in the "repeat"
  - 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!

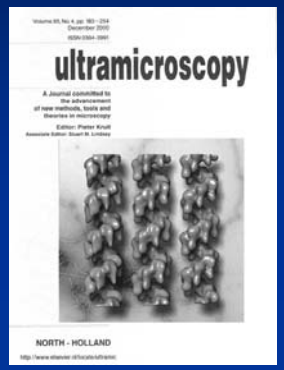
In simplest case:

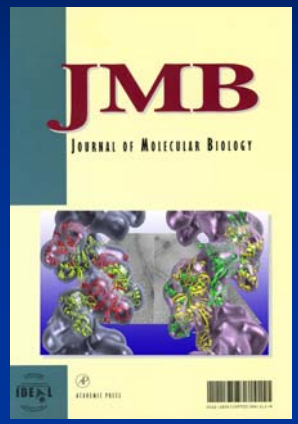
- Each layer line contains a single Bessel function
- "indexing" pattern requires determining Bessel function order for only two layer lines
- 3D reconstruction can then be made by Fourier-Bessel inversion
- If a polymer is highly ordered, homogeneous, does not have Bessel overlap, Fourier-Bessel methods work very well

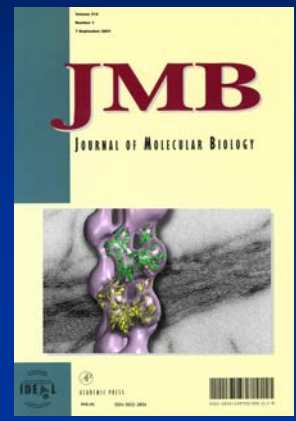
But Most Helical Polymers Have Been Refractory to High Resolution EM Studies!

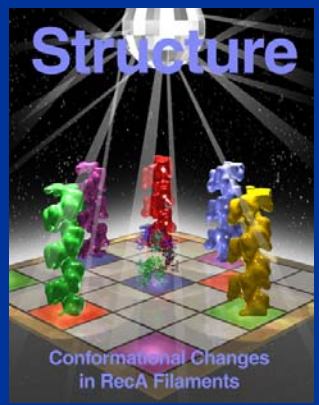
- Disorder or variability
- Heterogeneity
  - (it is much greater than has been assumed!)
- Weak Scattering
- Bessel Overlap

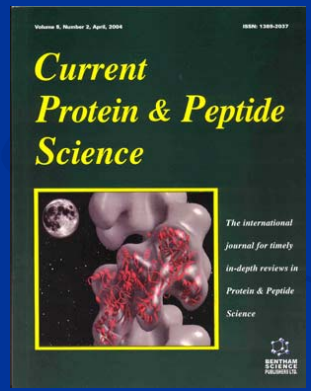
New method: Iterative Helical Real Space Reconstruction

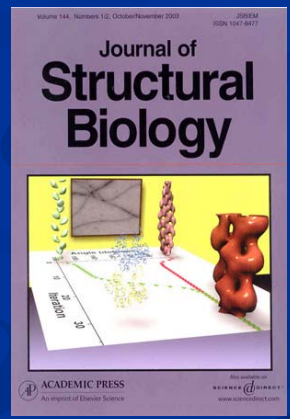












and ~ 20 other papers published or in press

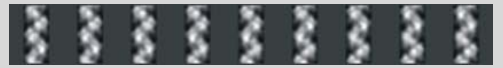
Iterative Helical Real Space Reconstruction cycle

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

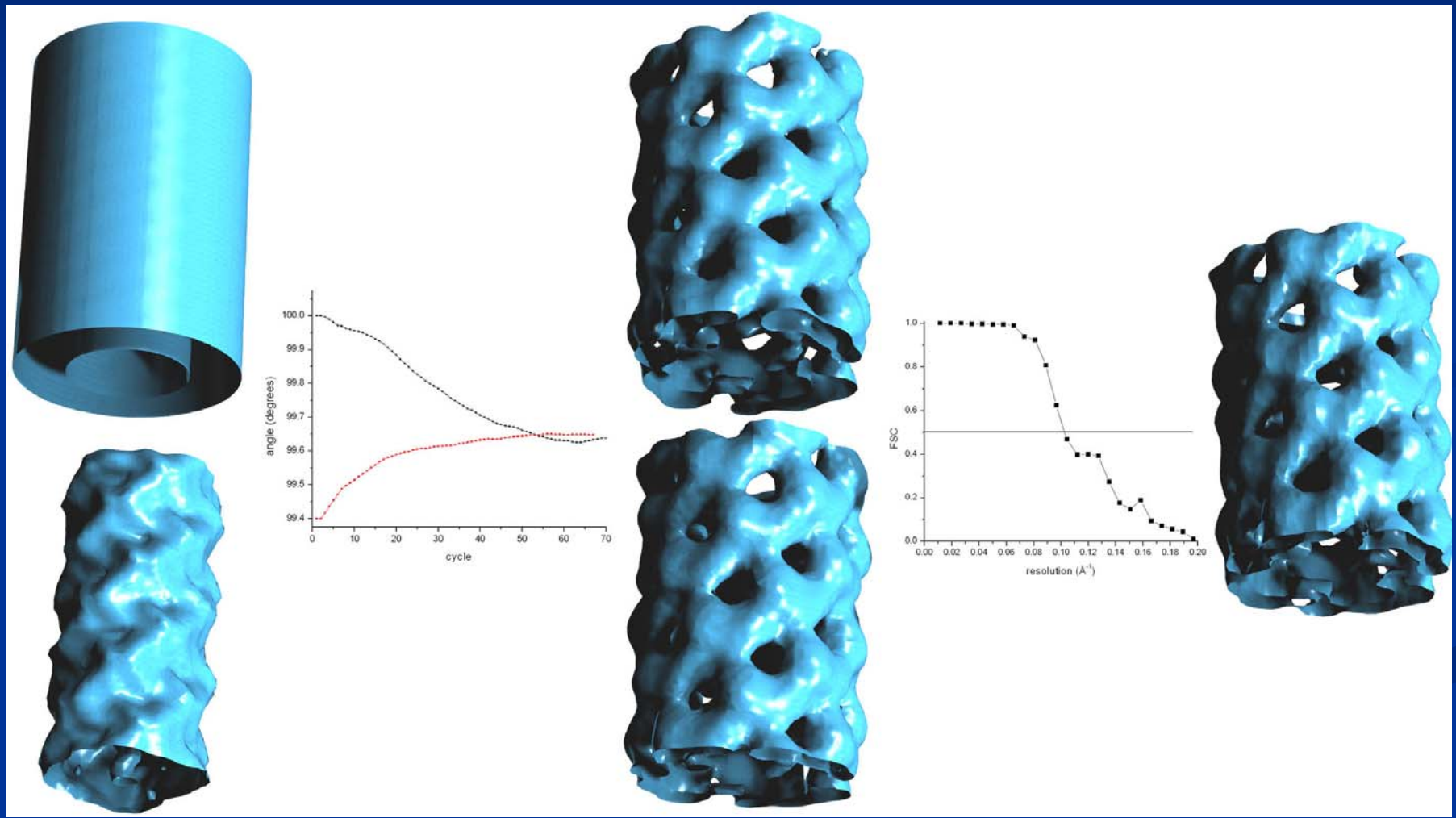
Asymmetric 3D volume

determine helical symmetry by least squares fit

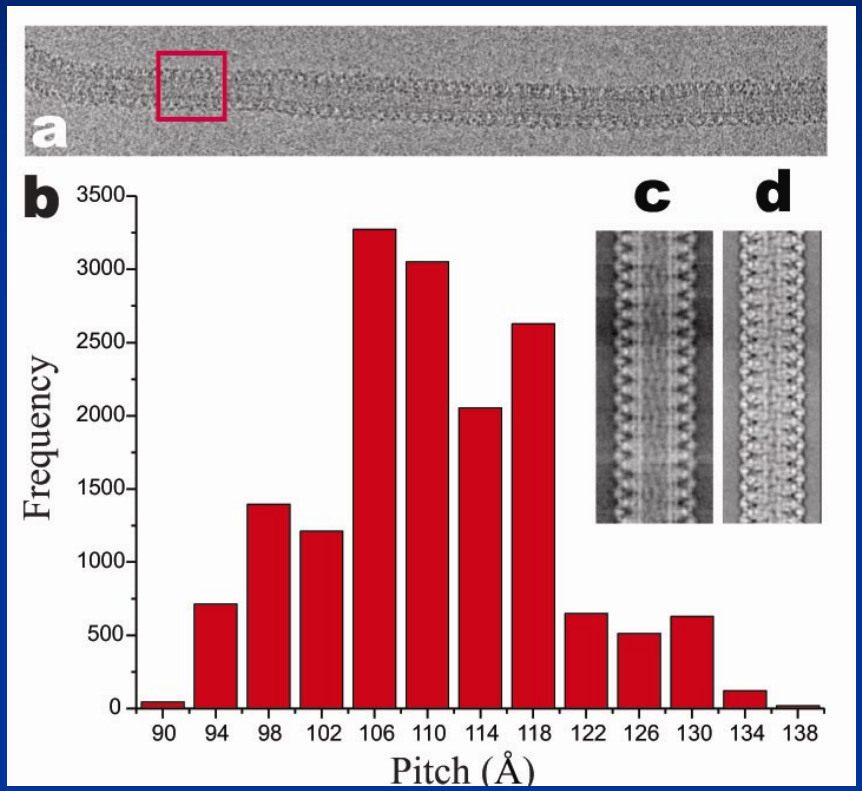
$\Delta\phi$, Δz (helical parameters)

impose helical symmetry

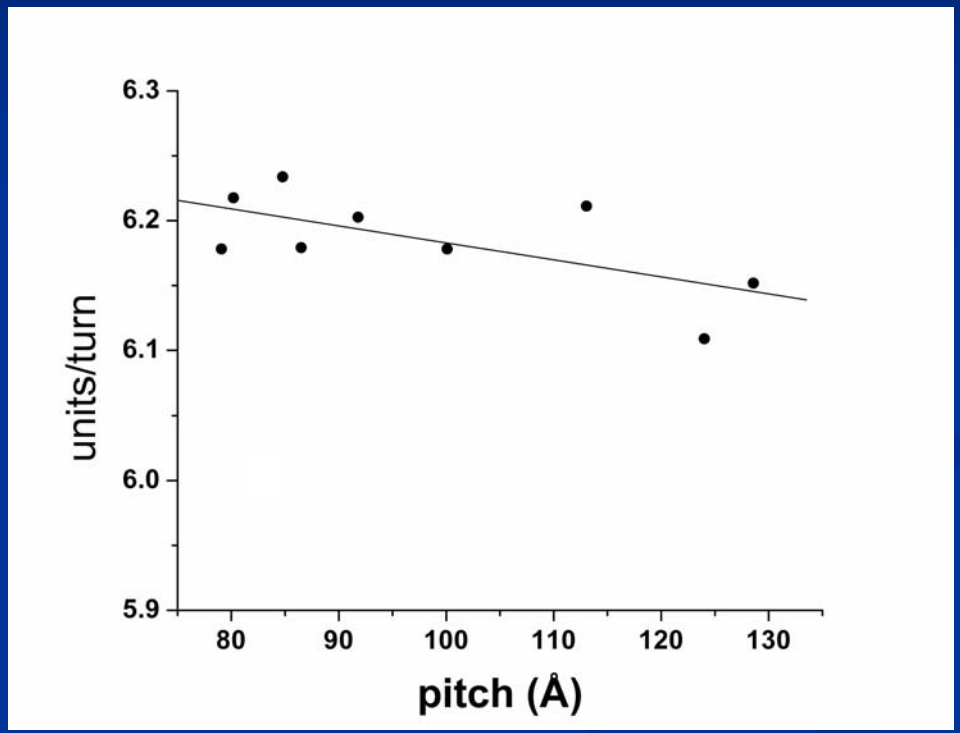
Algorithm is "robust" in that it is independent of starting model



Disorder can be enormous



Dynamin-lipid tubes
Chen *et al.*, Nature SMB (2004)



RecA-DNA filaments
VanLoock *et al.*, JMB (2003)

Myosin thick filament has been almost intractable

"The helical symmetry is such that, on a given layer-line, Bessel function contributions of different orders start to overlap at fairly low resolution and must therefore be separated computationally by combining data from different views"

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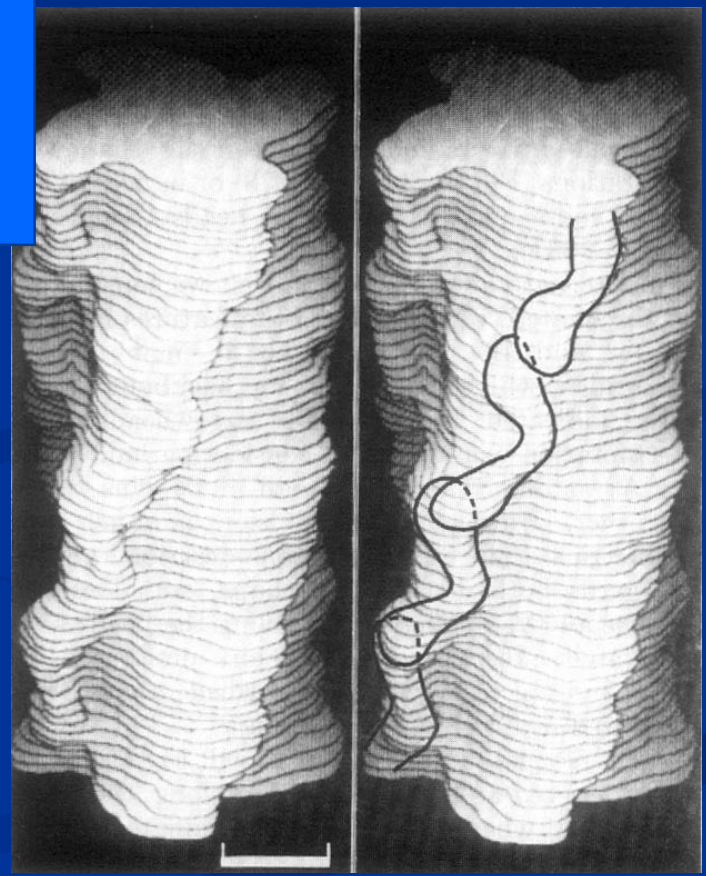
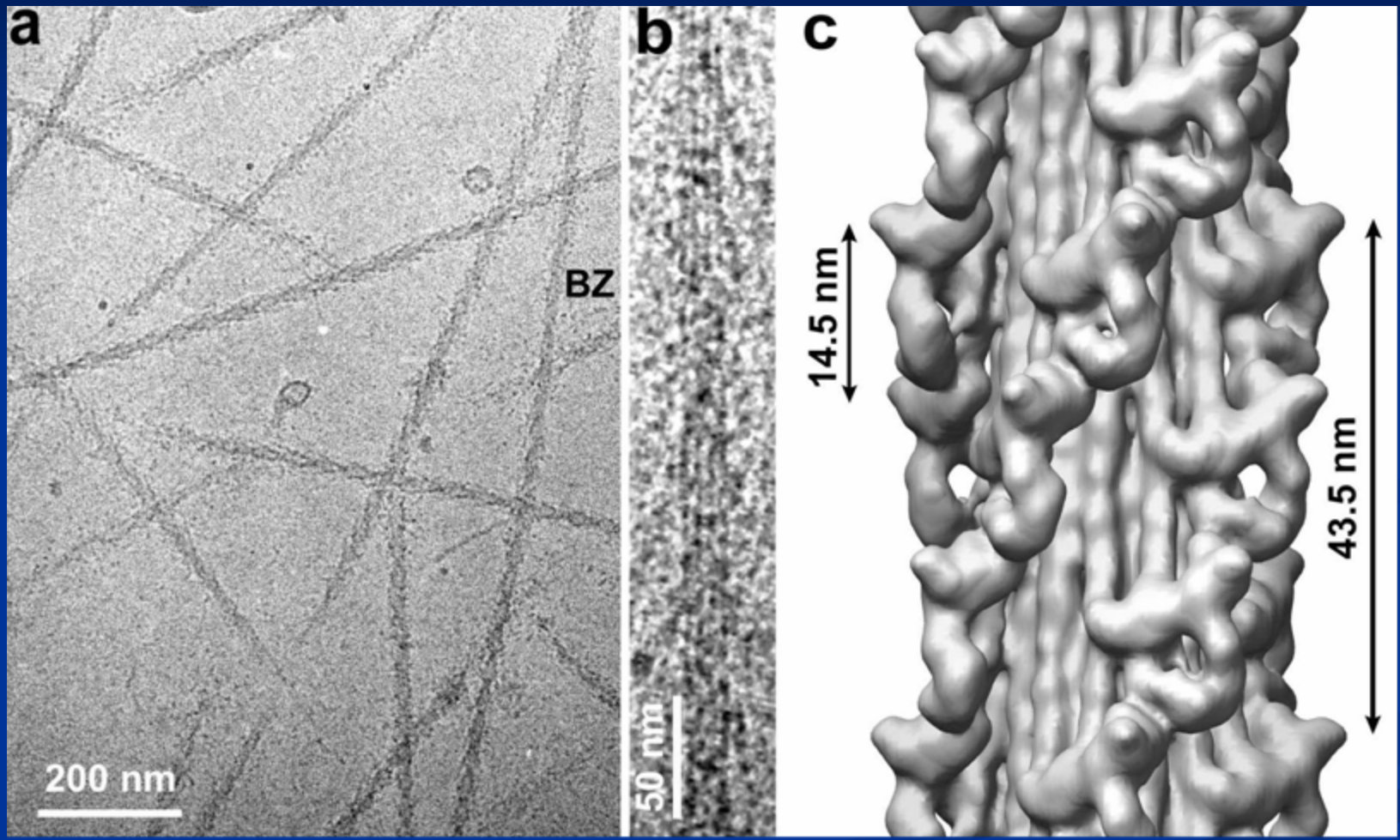
Hills Road, Cambridge CB2 2

(Received 6 November 1984, and in revise *1985)*

Thick filaments from leg muscle of tarantula, mainta relaxing conditions (Mg-ATP and EGTA), were negatively stained and photo with minimal electron dose. Particles were selected for three-dimensional imag construction by general visual appearance and by the strength and symmetry of thei optical diffraction patterns, the best of which extend to spacings of $1/5\,nm^{-1}$. The helical symmetry is such that, on a given layer-line, Bessel function contributions of different orders start to overlap at fairly low resolution and must therefore be separated computationally by combining data from different views. Independent reconstructions agree well and show more detail than previous reconstructions of thick filaments from *Limulus* and scallop. The strongest feature is a set of four long-pitch right-handed helical ridges (pitch $4 \times 43{\cdot}5$ nm) formed by the elongated myosin heads. The long-pitch helices are modulated to give ridges with an axial spacing of 14·5 nm, lying in planes roughly normal to the filament axis and running circumferentially. We suggest that the latter may be formed by the stacking of a subfragment 1 (S1) head from one myosin molecule on an S1 from an axially neighbouring molecule. Internal features in the map indicate an approximate local twofold axis relating the putative heads within a molecule. The heads appear to point in opposite directions along the filament axis and are located very close to the filament backbone. Thus, for the first time, the two heads of the myosin molecule appear to have been visualized in a native thick filament under relaxing conditions.

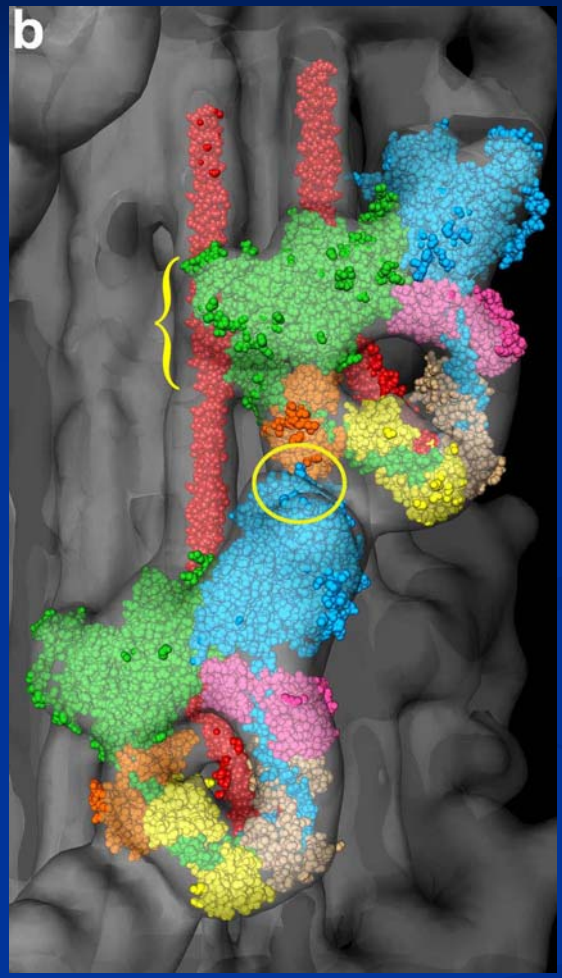


Problem non-existent using IHRSR



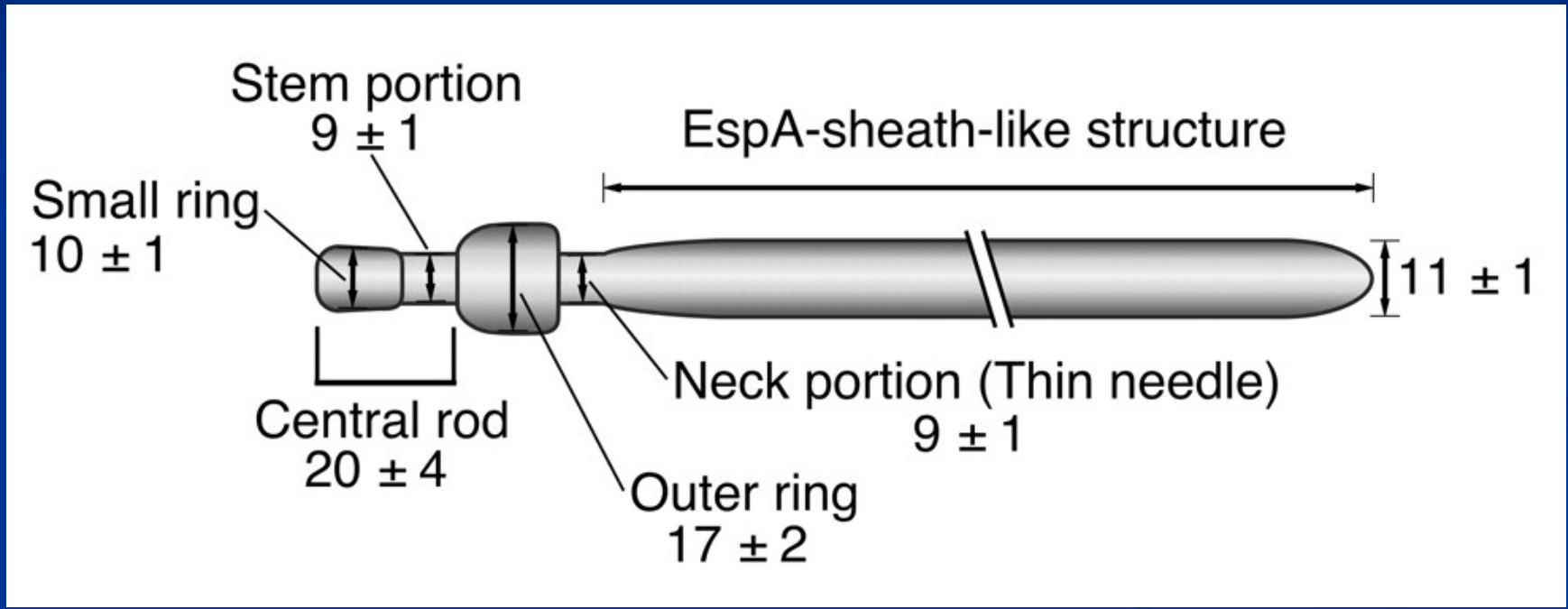
Woodhead *et al.*, Nature 436, 1195-1199 (2005)

Resolution sufficient to fit atomic model

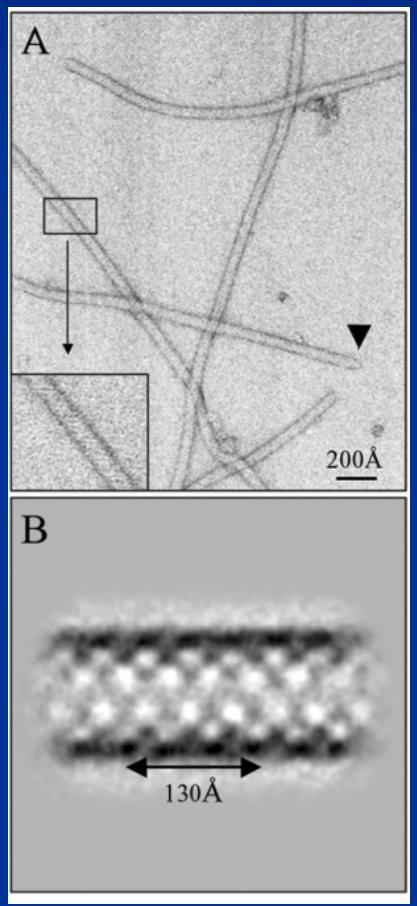


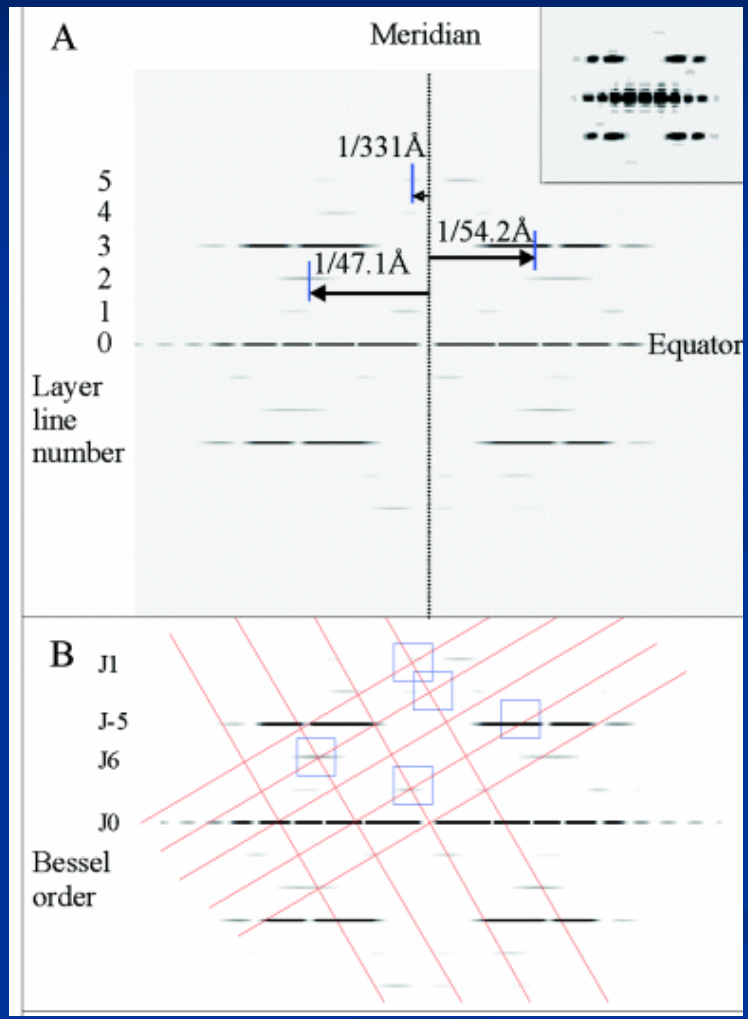
Woodhead *et al.*, Nature 436, 1195-1199 (2005)

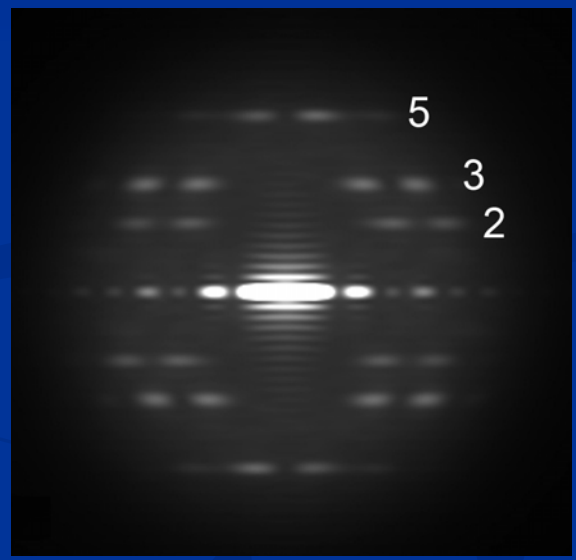
EspA of Enteropathogenic *E. coli*



EspA of Enteropathogenic *E. coli*

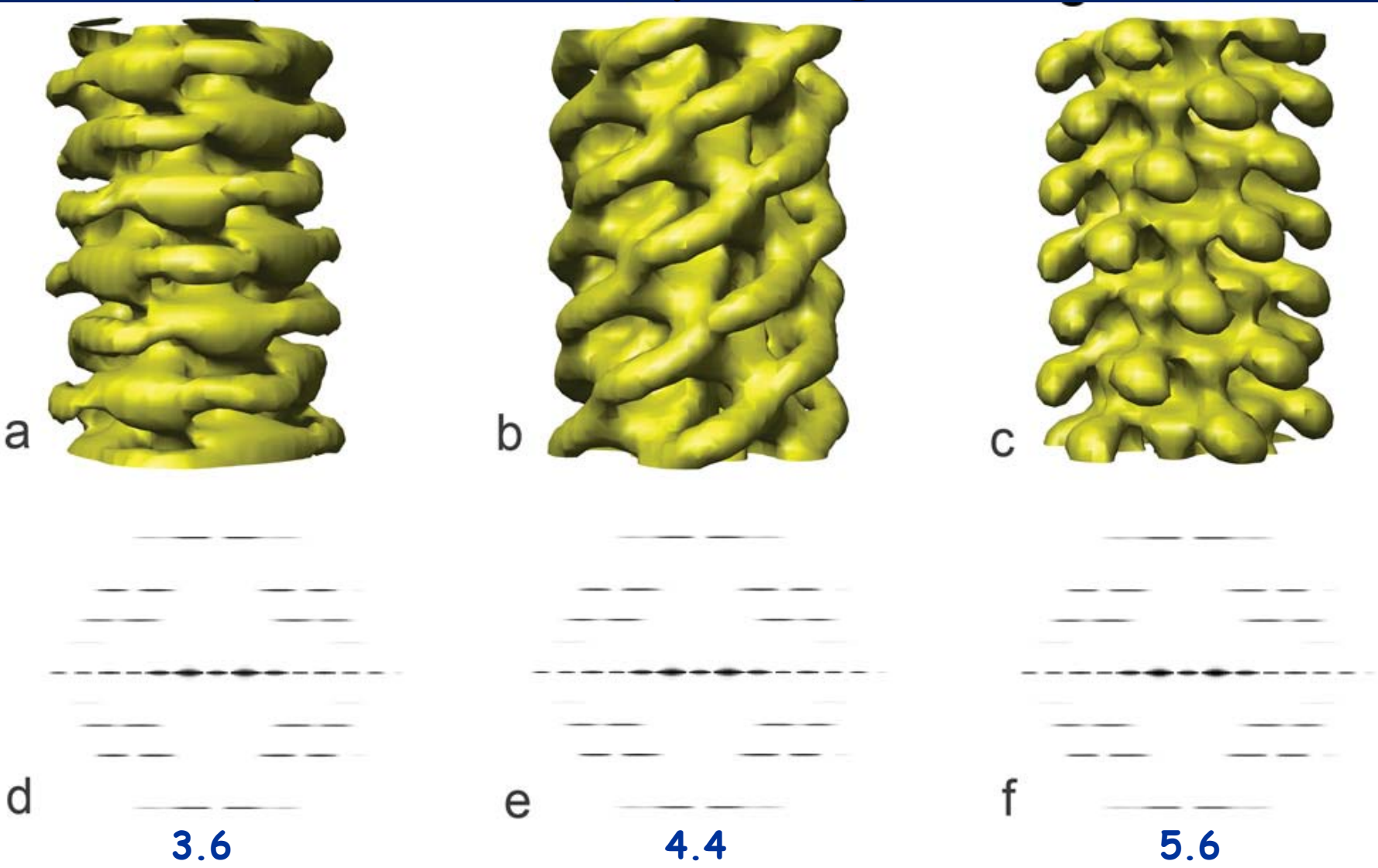




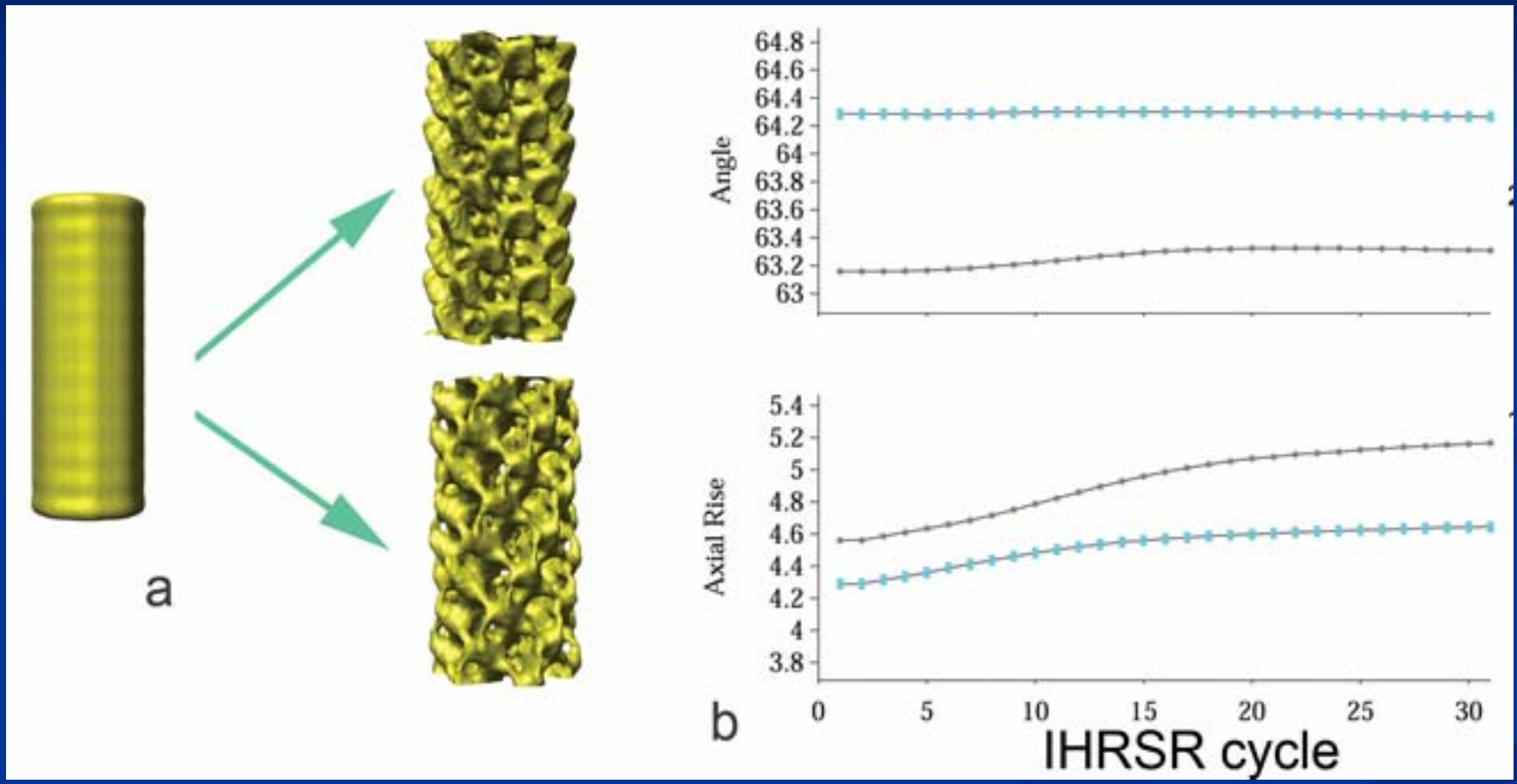


Daniell *et al.*, Mol. Micro. (2003)

EspA of Enteropathogenic *E. coli*

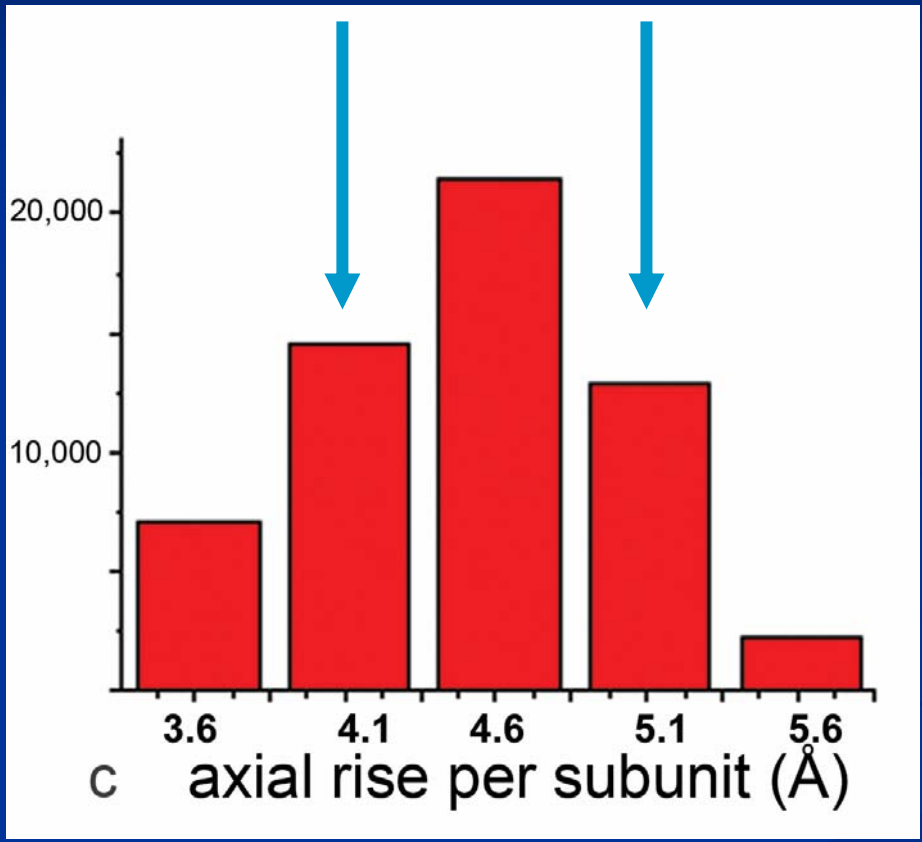


EspA of Enteropathogenic *E. coli*



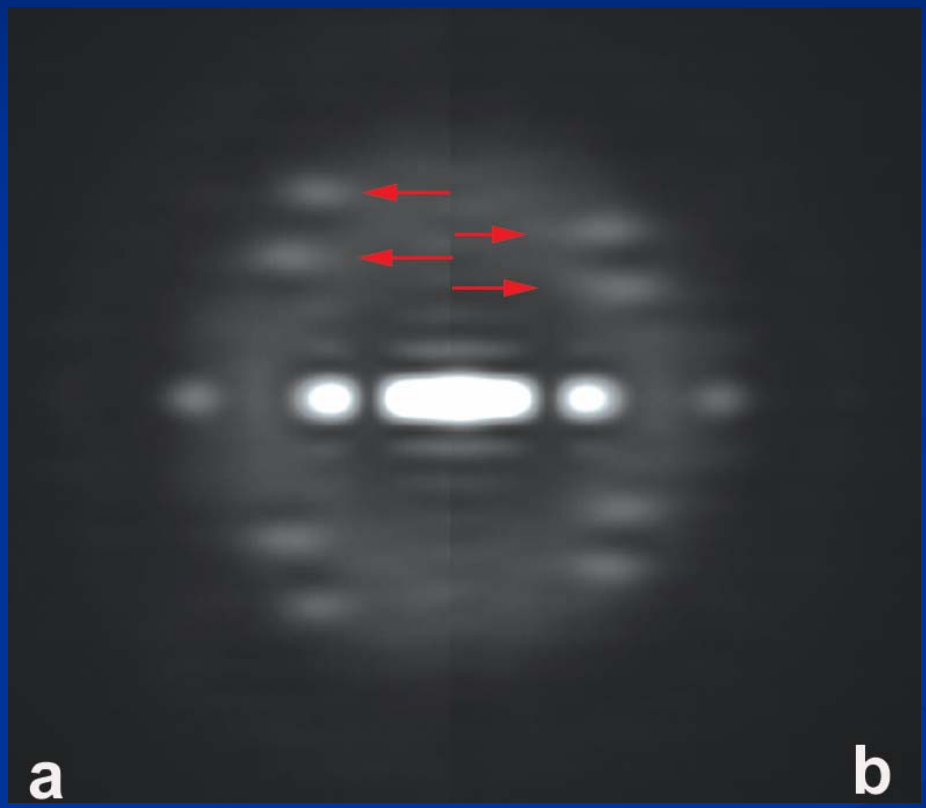
Such behavior is a *prima facie* argument for heterogeneity!

EspA of Enteropathogenic *E. coli*



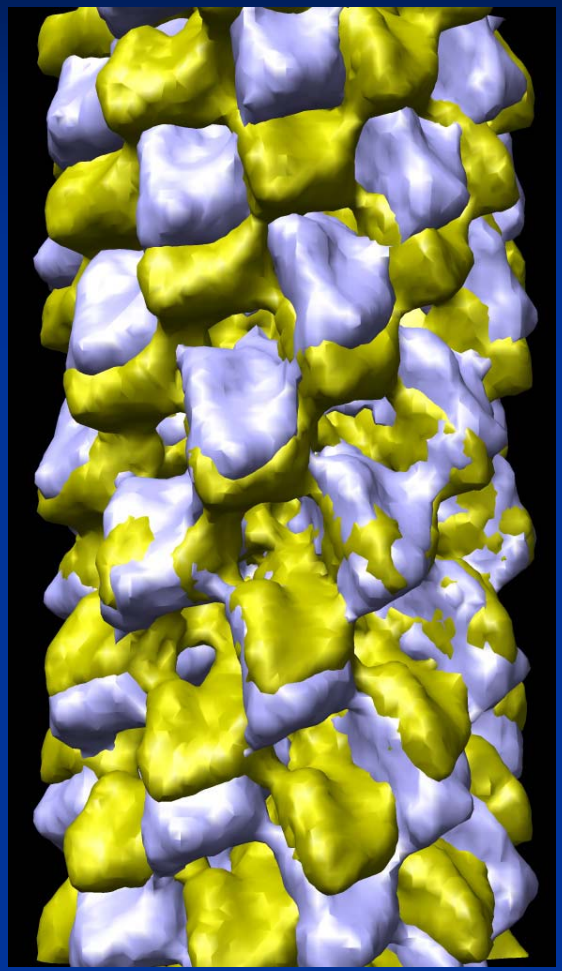
Most variation determined to be due to variable axial rise
… but is this sorting valid? Is there a reality check?

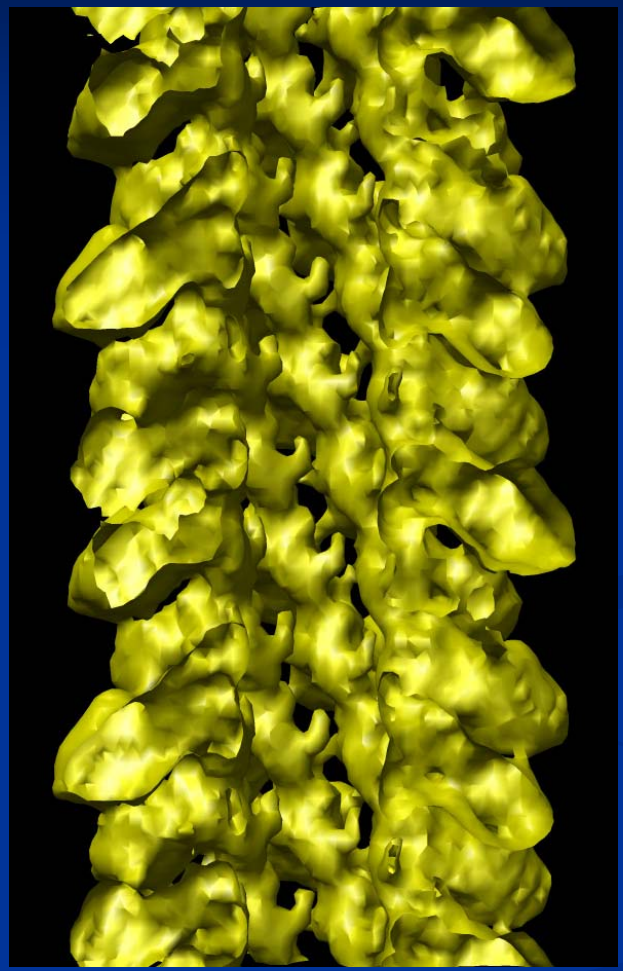
EspA of Enteropathogenic *E. coli*

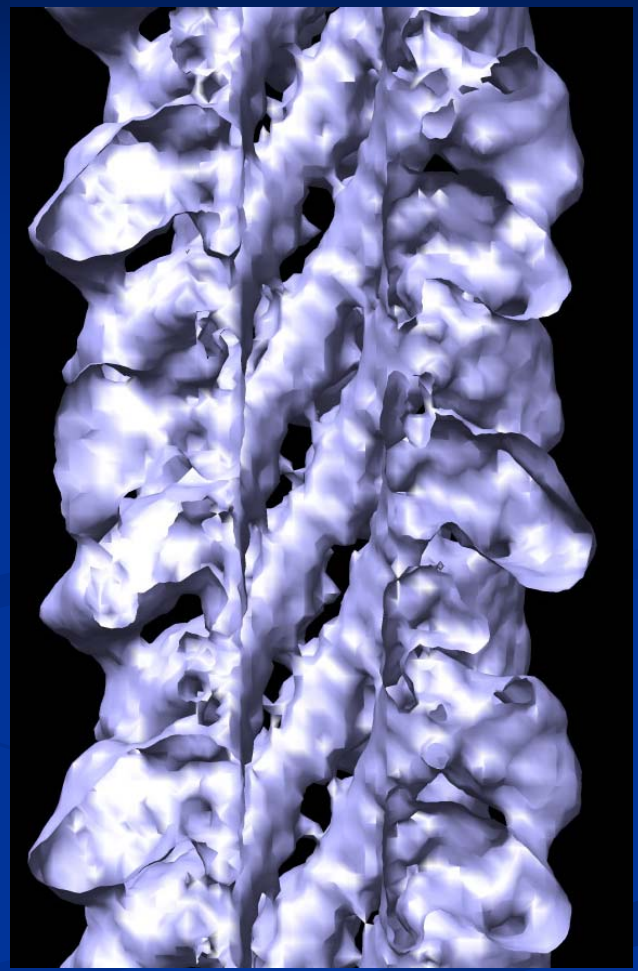


Power spectra show change in axial rise, no change in twist

EspA: heterogeneity in axial rise

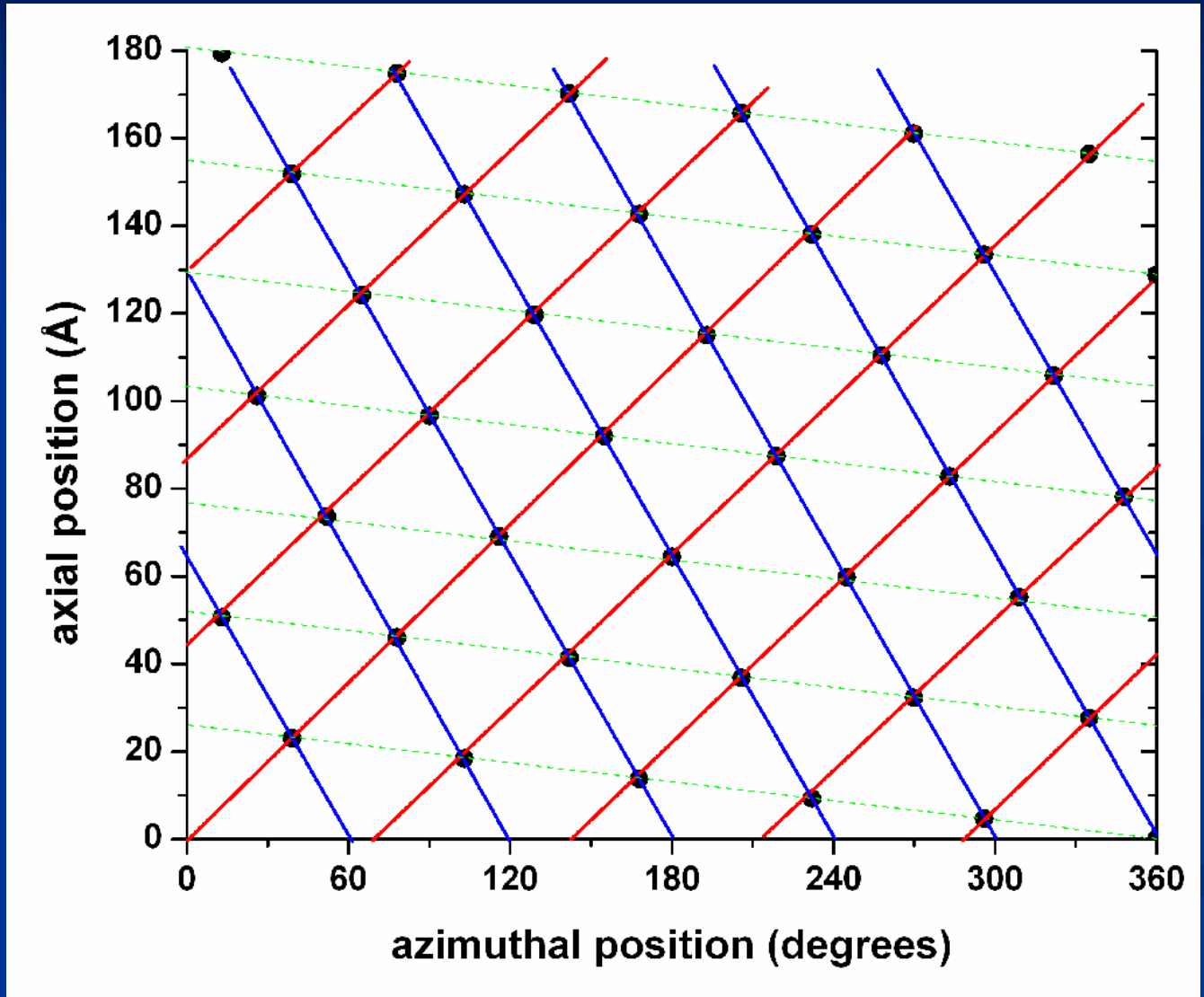




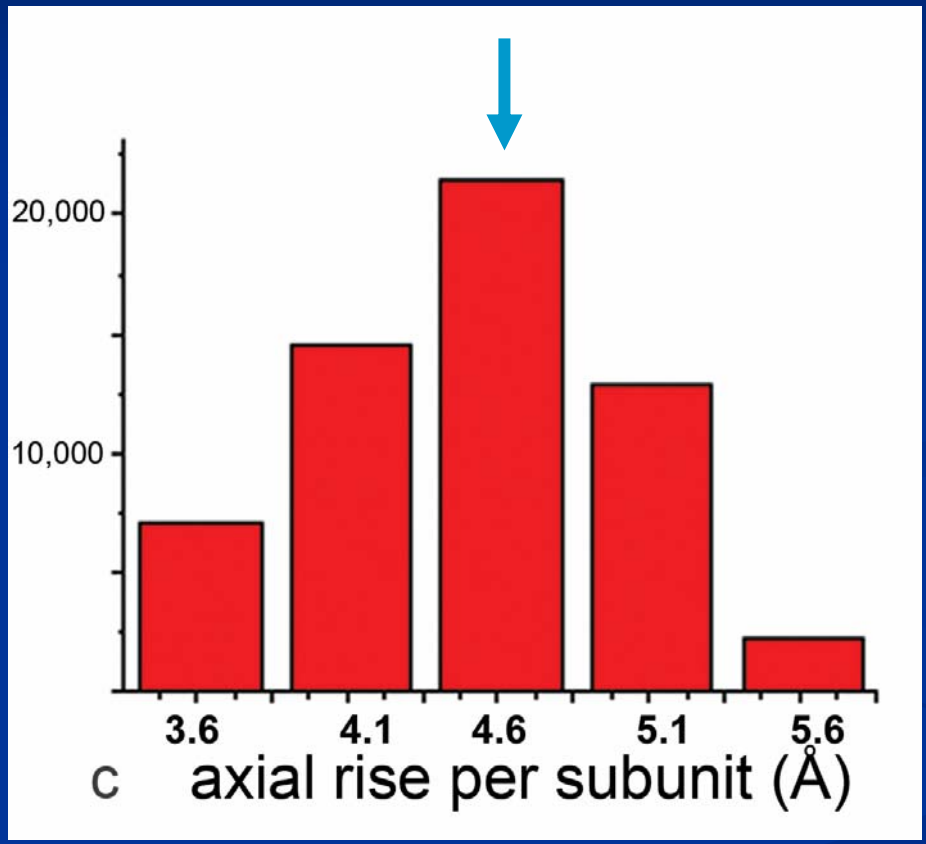


Grey: 5.3 Å Yellow: 4.2 Å Right-handed 6-start Left-handed 5-start

EspA of Enteropathogenic *E. coli*

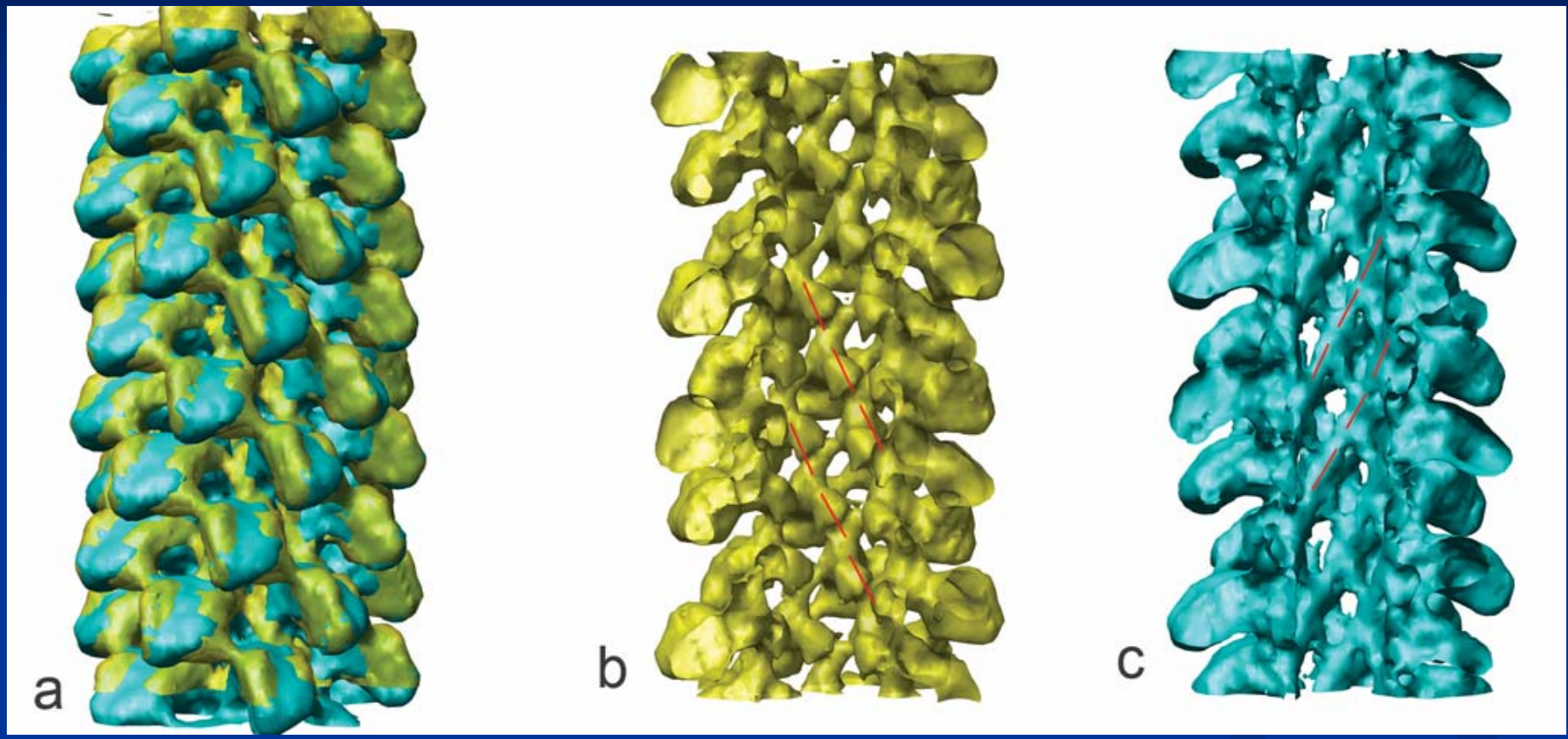


EspA of Enteropathogenic *E. coli*



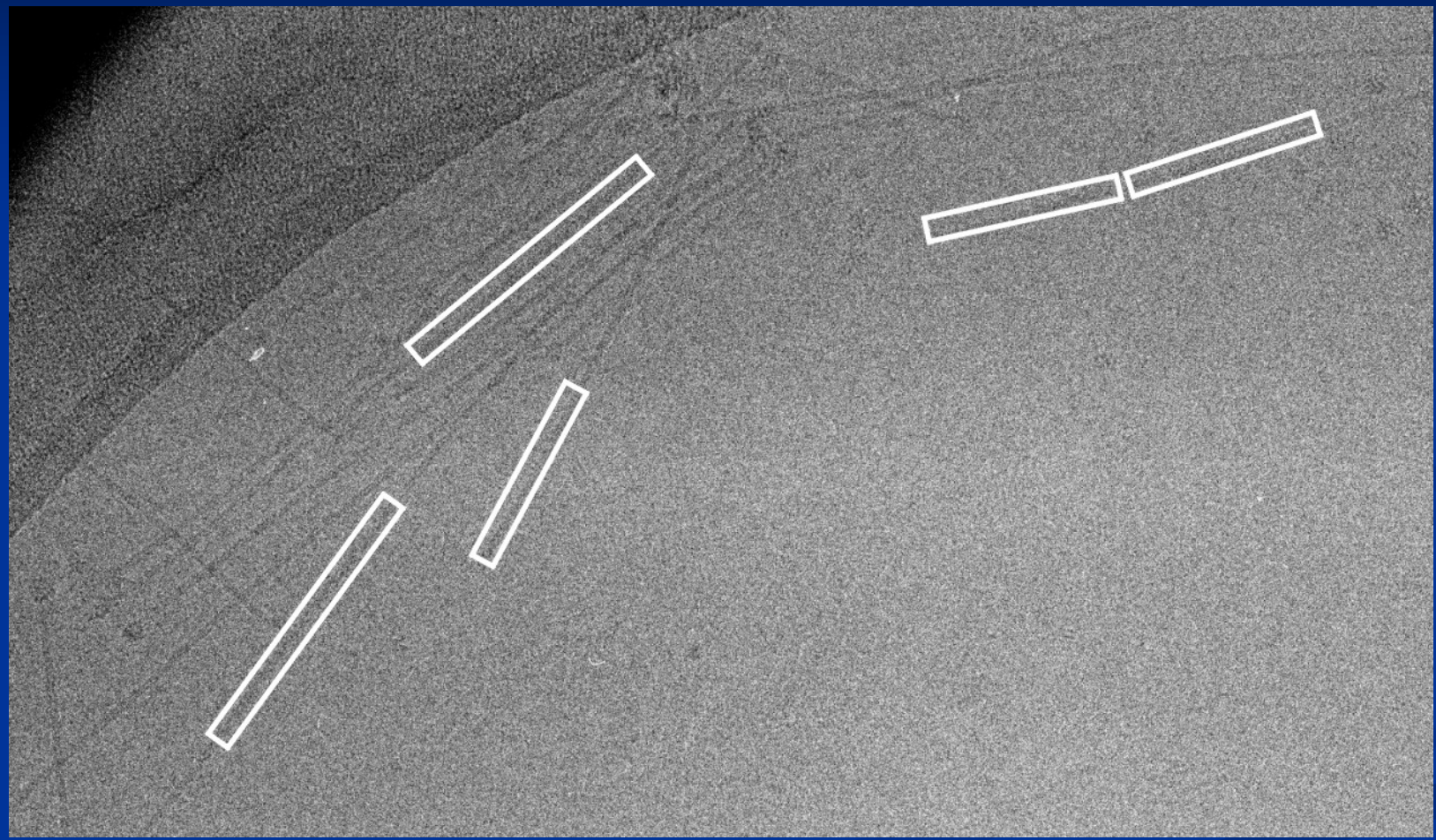
What happens in the central bin?

EspA of Enteropathogenic *E. coli*

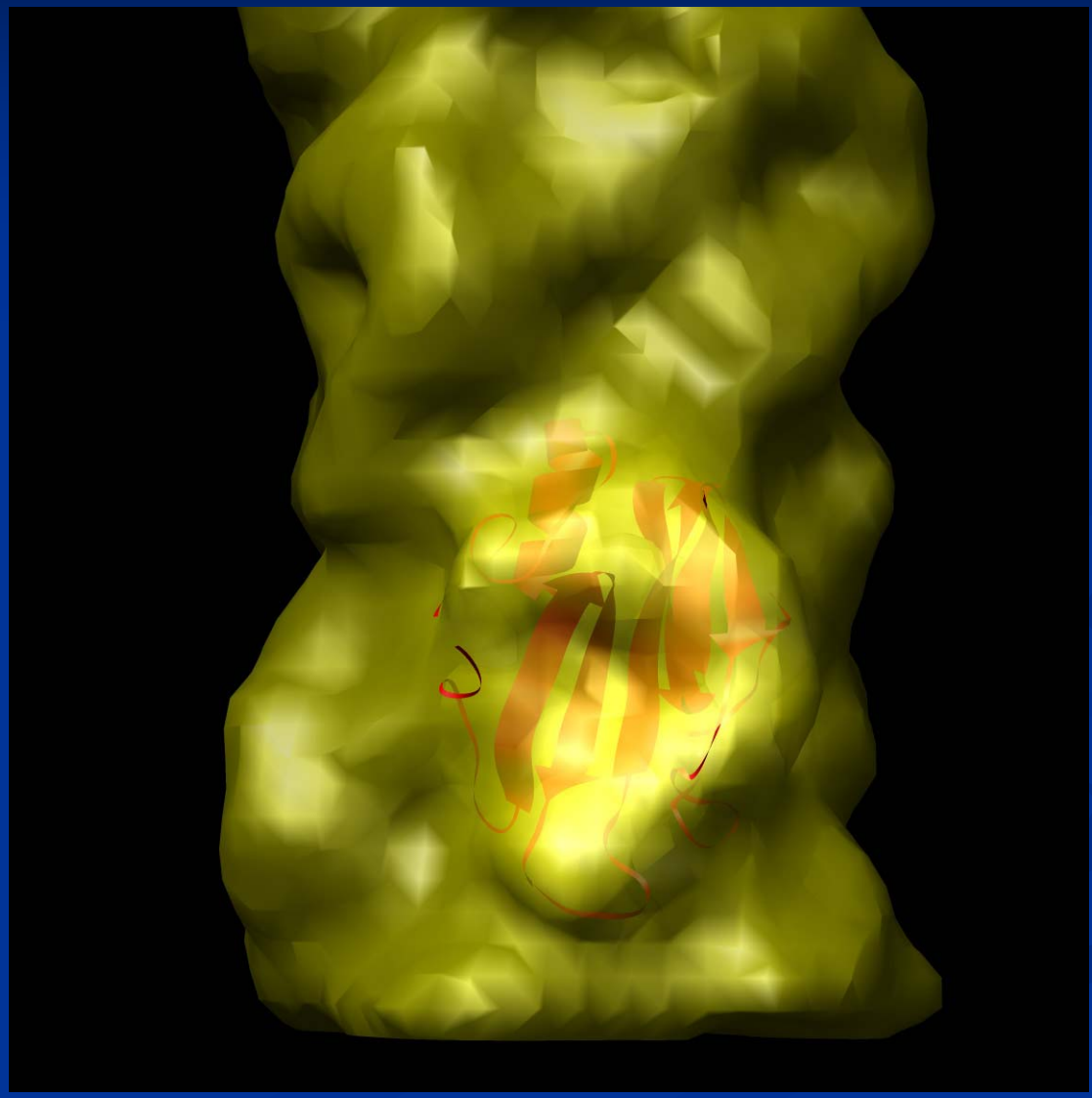


central bin (~ 4.6 Å) shows no major variability in rise, twist, but can separate out two states of connectivity around lumen!

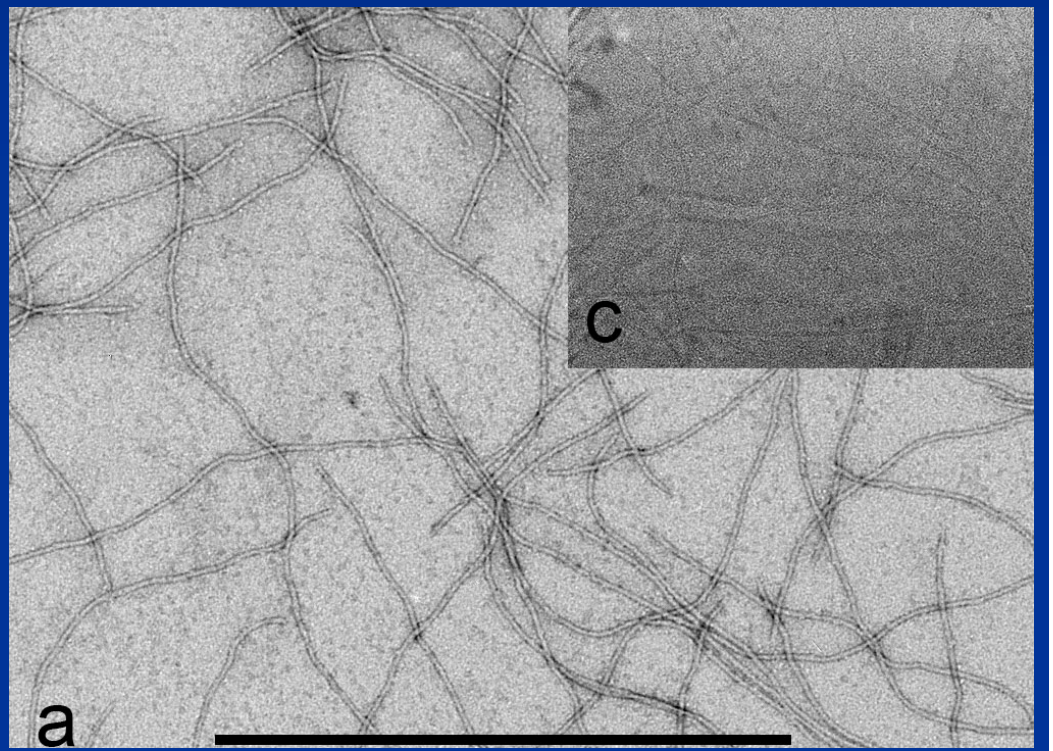
Bacterial pili: example of even more weakly scattering objects

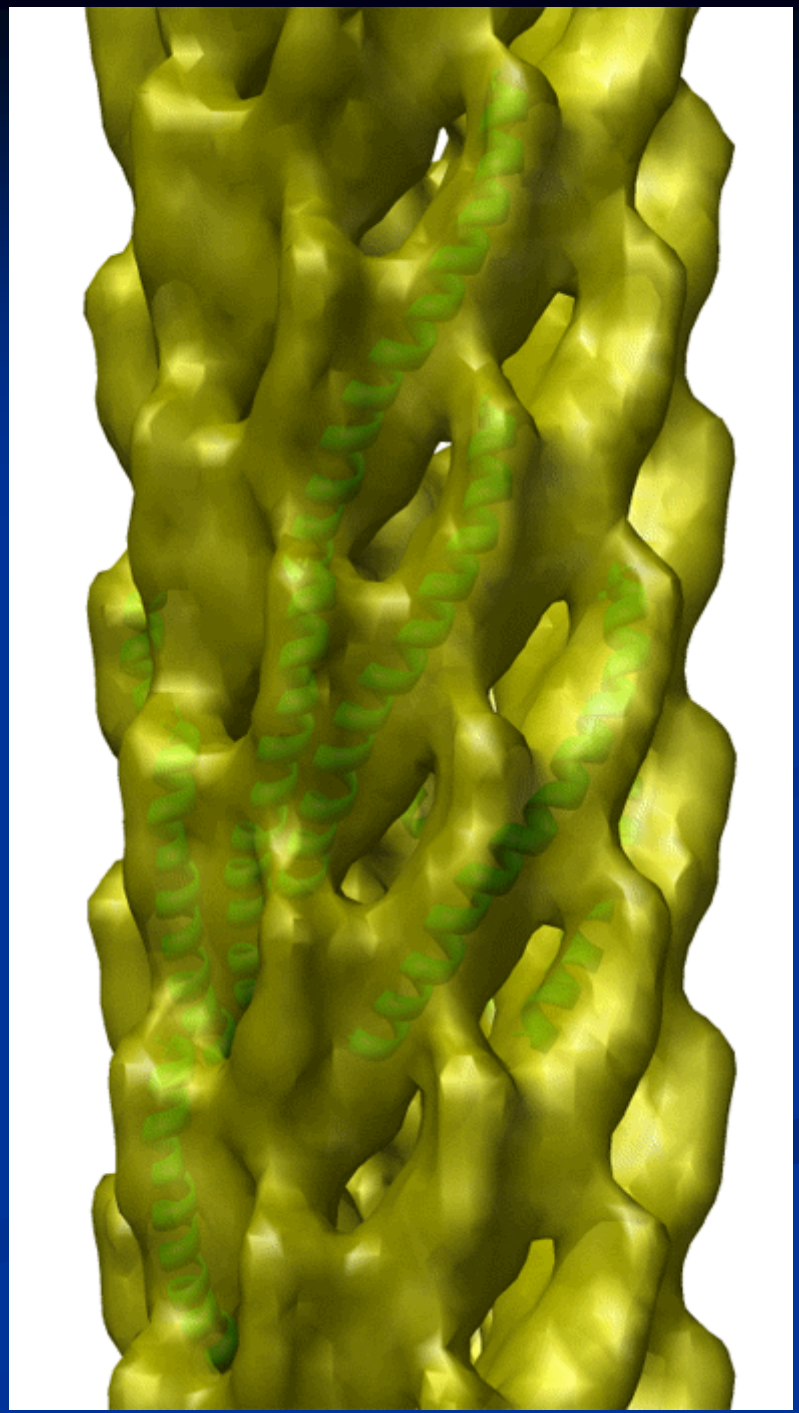


Unambiguous fit of monomeric crystal subunit from *Neisseria gonorrhoeae*



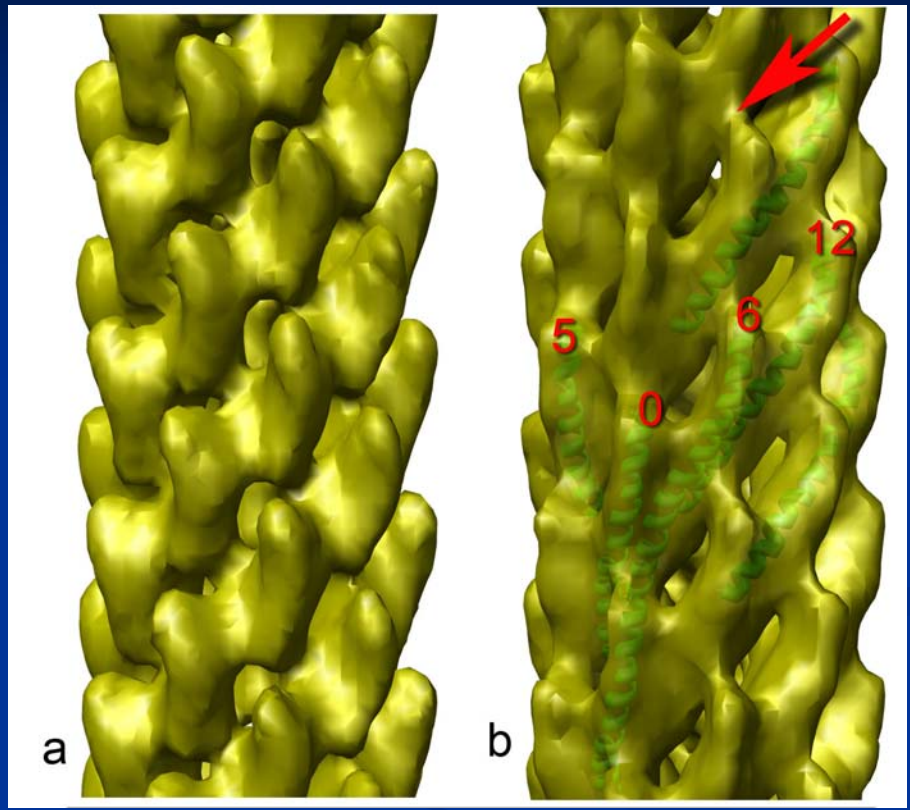
Very weak scattering is no longer a problem!





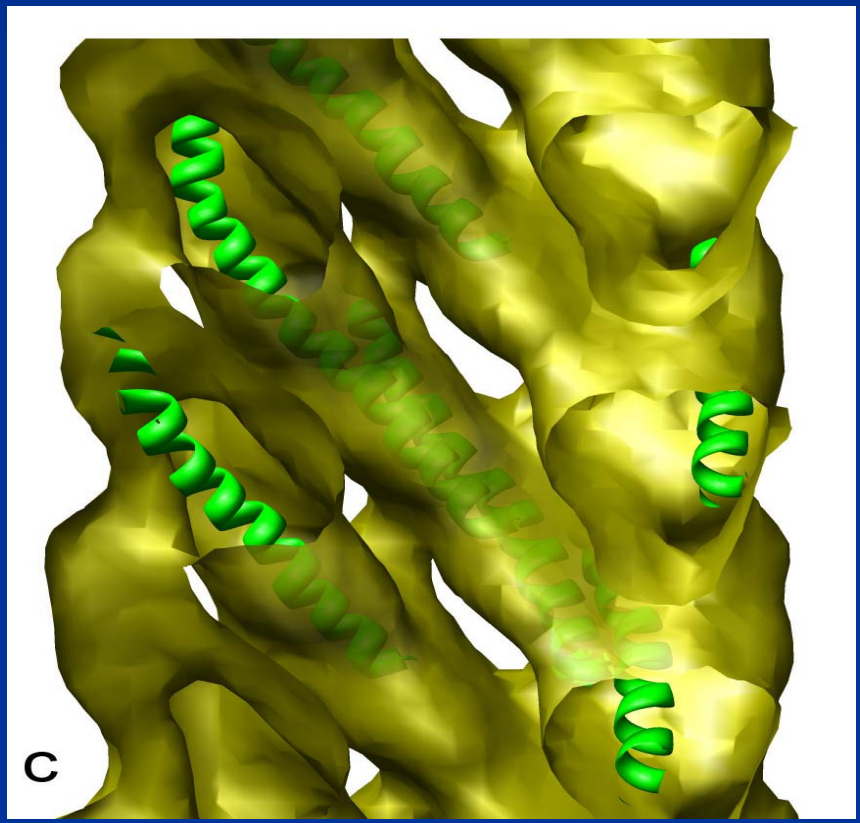
Filamentous bacteriophage (M13/fd)

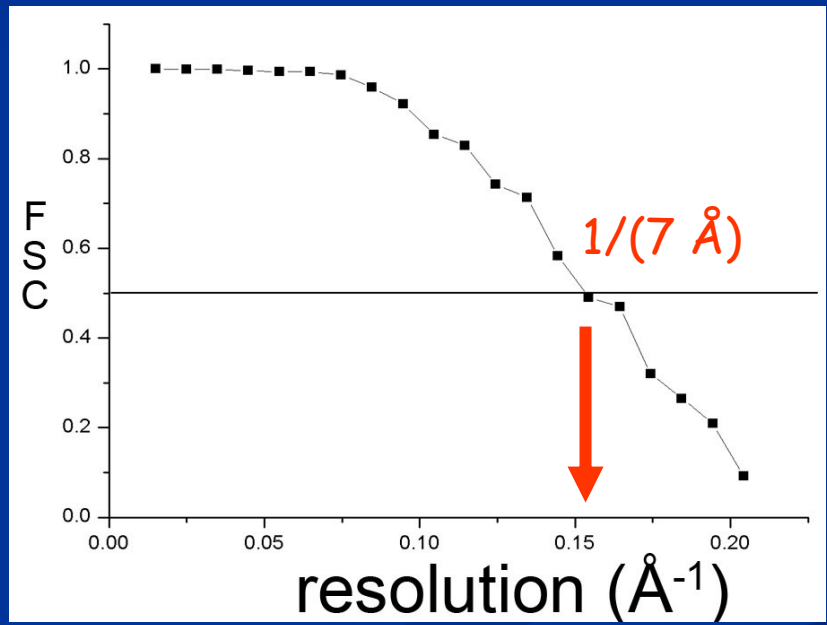
- Model systems in understanding:
  - DNA packaging
  - Assembly of a protein polymer from a small integral membrane protein
- Important in cloning, phage display, etc.



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)

Filamentous bacteriophage fd

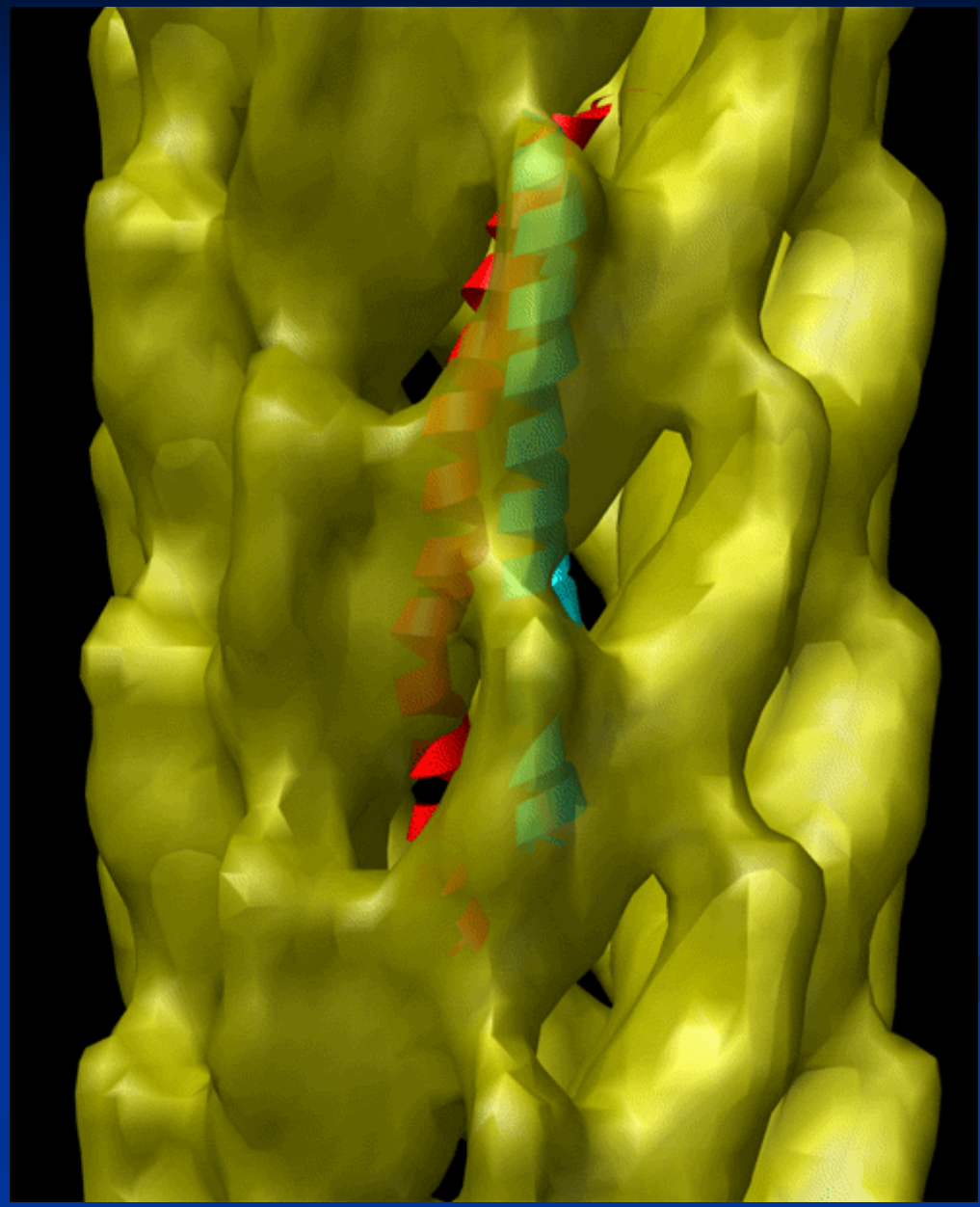




EM reconstruction cannot be reconciled with existing models

Cyan: Solid State NMR Model, Zeri *et al.*, PNAS (2003) - "…calculation of the three-dimensional structure of the protein from orientational restraints with an accuracy equivalent to an rms deviation of approximately 1 Å"

Red: X-ray fiber diffraction (refined against NMR data), Marvin *et al.*, JMB (2006) – "Here we show that reinterpreted NMR data are also consistent with the model derived from X-ray fibre diffraction studies…"



Conclusions I

- Outside of a crystal, there is nothing to maintain long-range order in a macromolecular assembly
- Many helical protein polymers are more polymorphic than assumed
  - Originally showed this for F-actin and RecA
  - Can now demonstrate similar polymorphisms in bacteriophage fd, T3SS EspA, dynamin tubes, …
- Plasticity of protein-protein interface in these structures must reflect evolutionary selection

Conclusions II

- Polymorphism and heterogeneity not necessarily revealed by casual observation or global averaging
- Bessel overlap occurs in many structures where it cannot be ignored
- In every instance, we can do better with the IHRSR approach than Fourier-Bessel methods

Acknowledgments

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

- Myosin: Raul Padron (Caracas), Roger Craig (U. Mass.)
- EspA: Natalie Strynadka (UBC)
- GC Pili: John Tainer (Scripps), Lisa Craig (Simon Fraser)
- Bacteriophage fd: George Thomas (UMKC)