# Overview

- Much, if not most, protein in the cell is not monomeric
    - Many proteins exist as part of macromolecular complexes (*e.g.*, ribosome), or as polymers (*e.g.*, actin, microtubules)

•polymeric structures contain many copies of an identical gene product

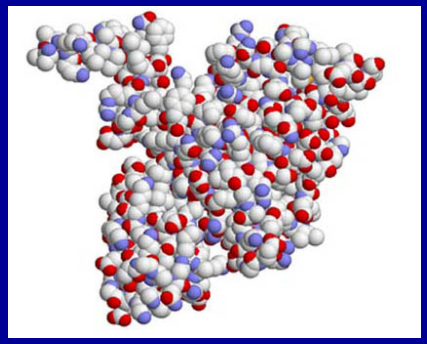
•Helical symmetry actually accounts for a large fraction of protein in a cell

- Helical structures were the first to be reconstructed in 3D in the EM (DeRosier and Klug, Nature, 1968)!

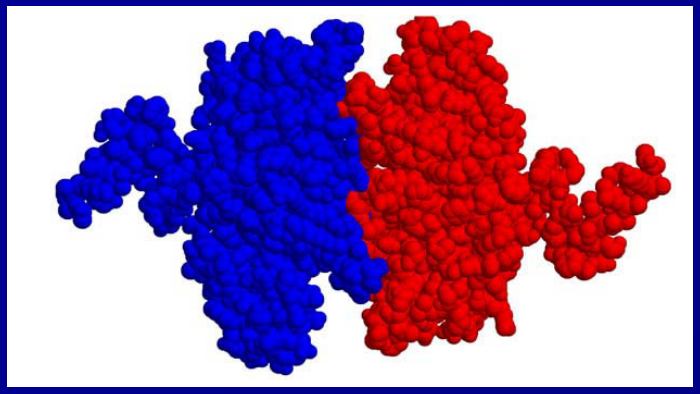
# What is helical symmetry?

- $\rho(r,\varphi,z) = \rho(r,\varphi + \Delta\varphi, z + \Delta z)$
  - $\Delta\varphi$, $\Delta z$ are a screw operator (a coupled rotation and translation)
- There can also be a point group symmetry, $C_n$, if there is *in addition* an n-fold rotational symmetry about the helical axis
- There can also be a dyad symmetry perpendicular to the filament axis (for a bipolar filament or tube)

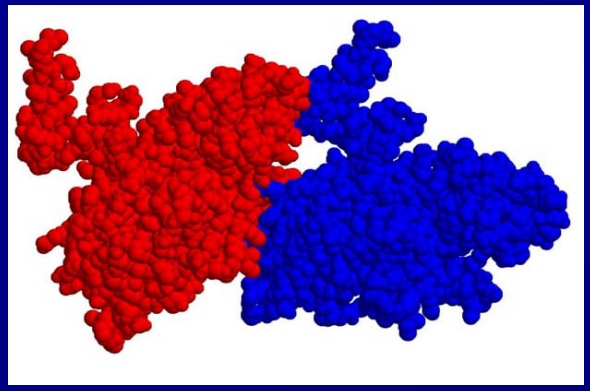
# Helical Symmetry Reflects Simplest Bonding Rule



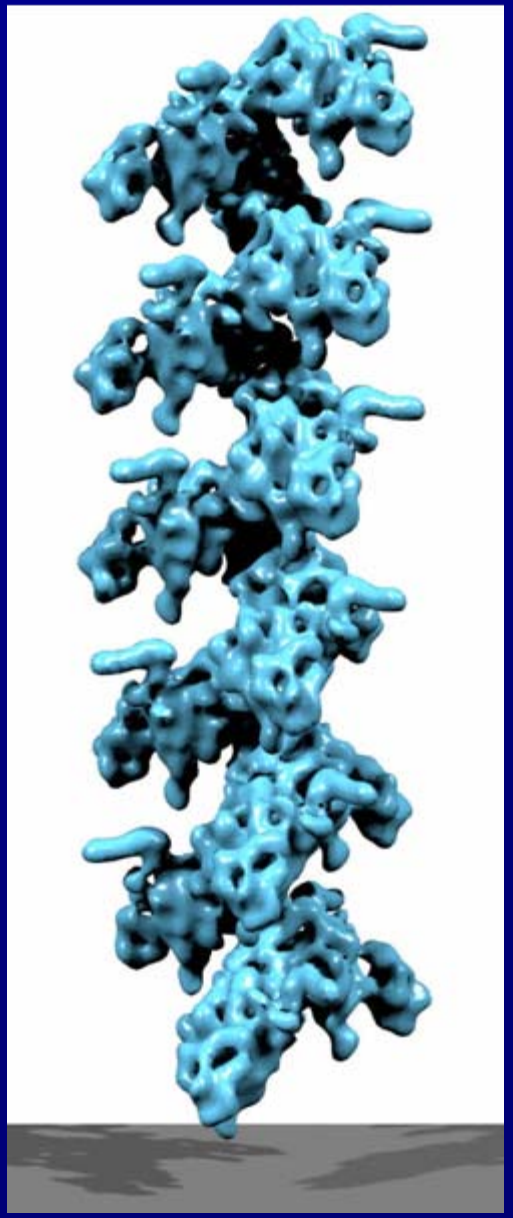
Asymmetric subunit



Symmetric dimer

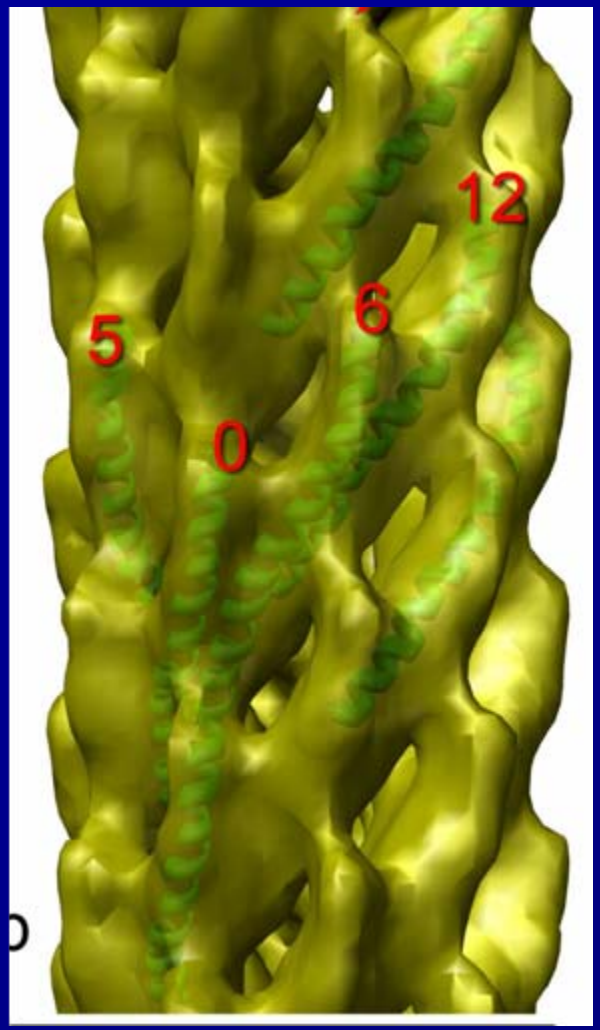


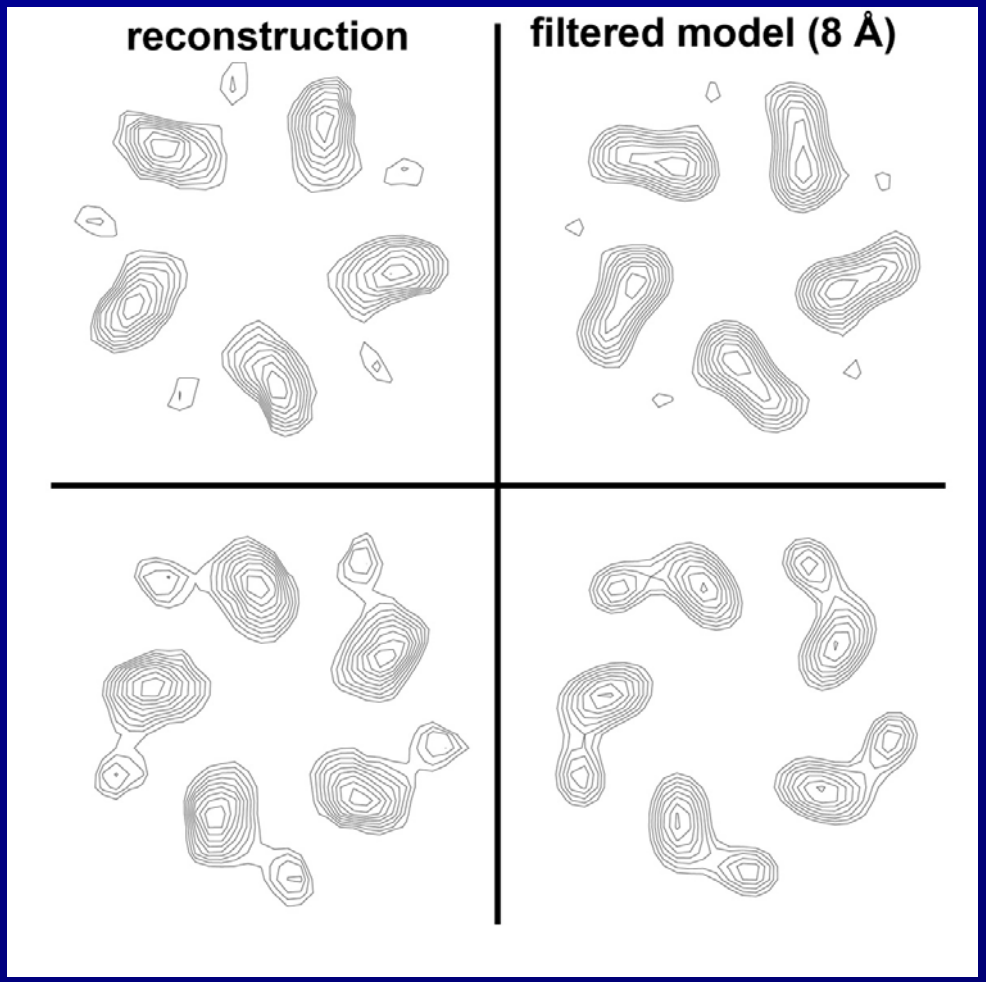
Asymmetric bond



Symmetrical helix

# Structure (filamentous phage fd) with both helical screw symmetry and point group (C5) symmetry




screw operator =37.4°, 17.4 Å

Wang *et al.*, JMB (2006)

# Some definitions

- axial rise is $\Delta z$
- 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

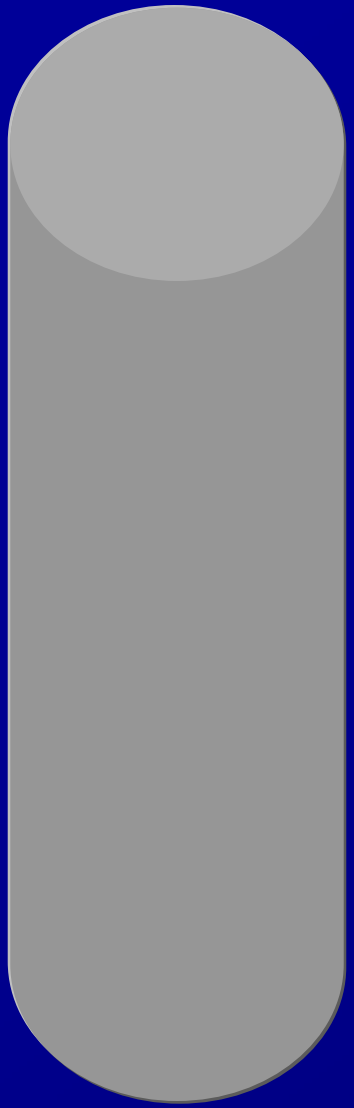
  $$u * \Delta z = c$$

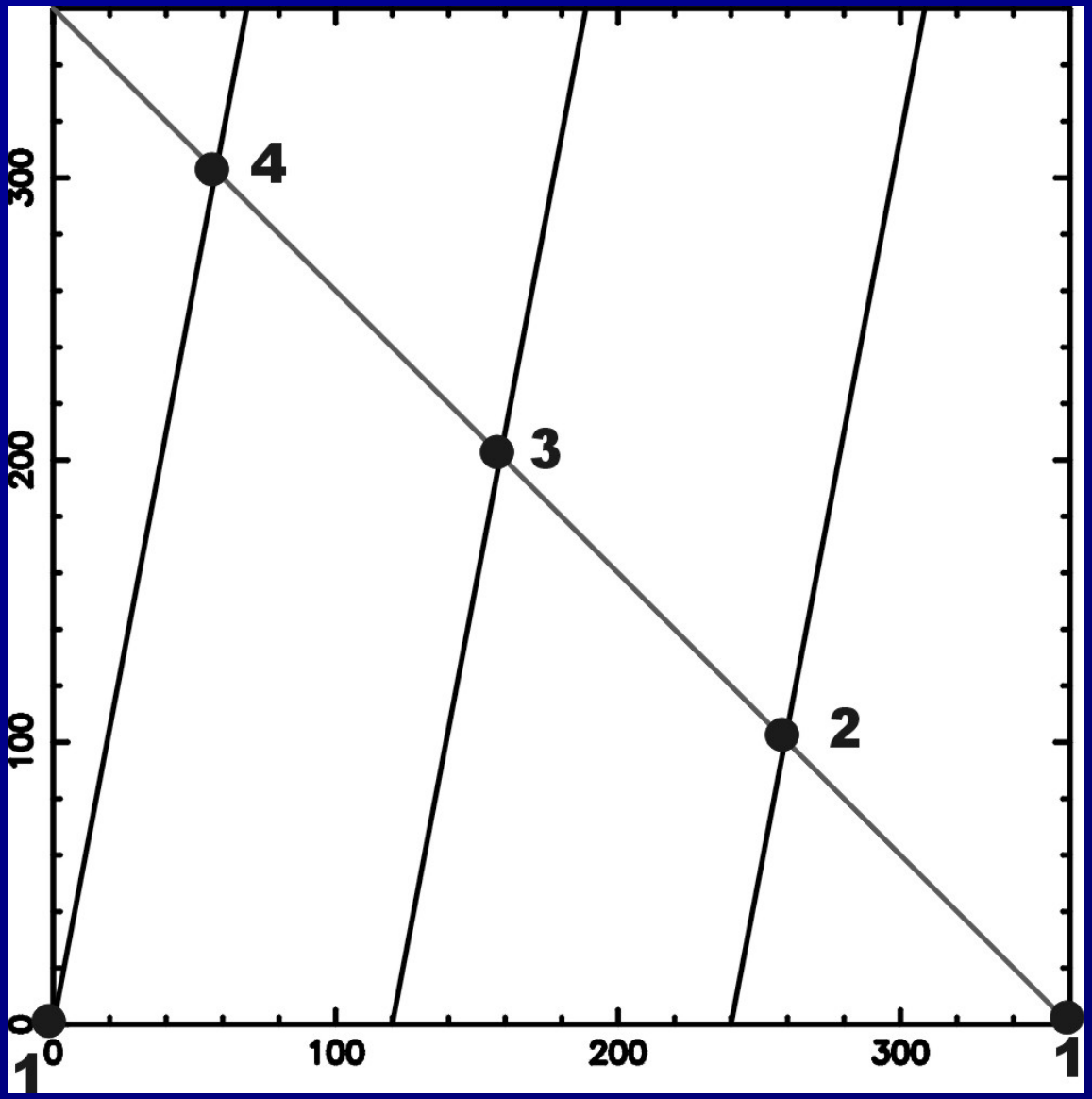
- in a crystal, the only allowed helical symmetries involve 2, 3, 4 or 6 subunits/turn
- outside of a crystal, there is no reason for any helix to have an integer number of u/t!
- outside of a crystal, there is no space group maintaining long-range order. So cannot have true 1D crystal

# Problems with traditional 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, $\Delta\varphi$=166.1538º
  - but $\Delta(\Delta\varphi)$=0.128º, $\Delta\varphi$=166.2818º, u/t=1299/600, c=35,463 Å!

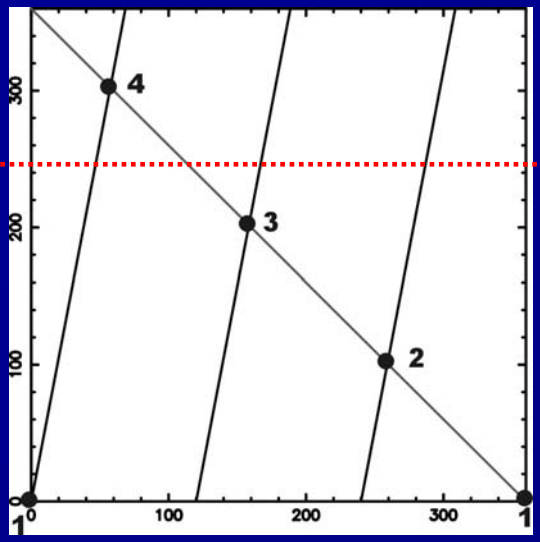
Helical symmetry best understood by "helical net"



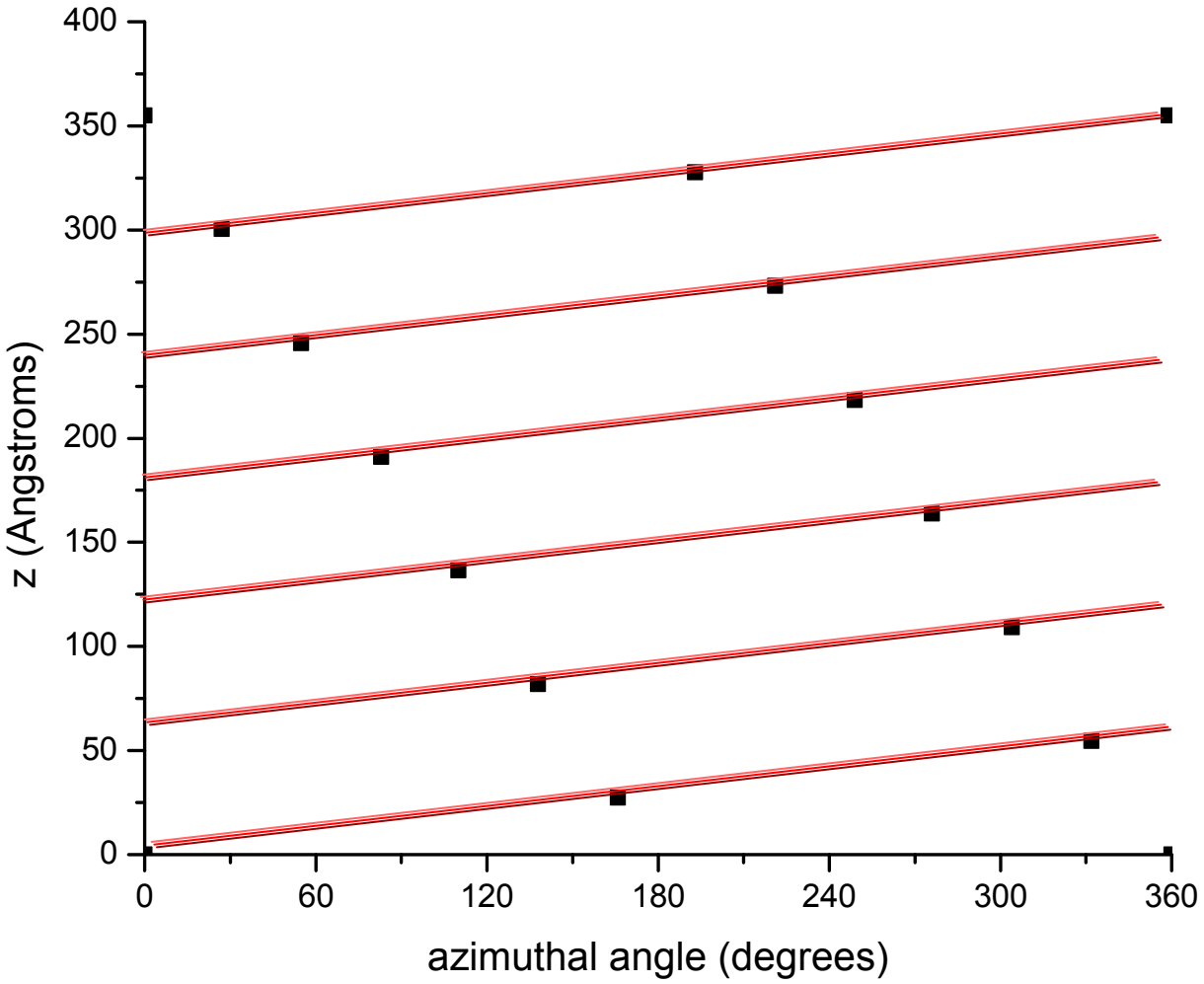



"unroll" the surface of a cylinder, look at inside face

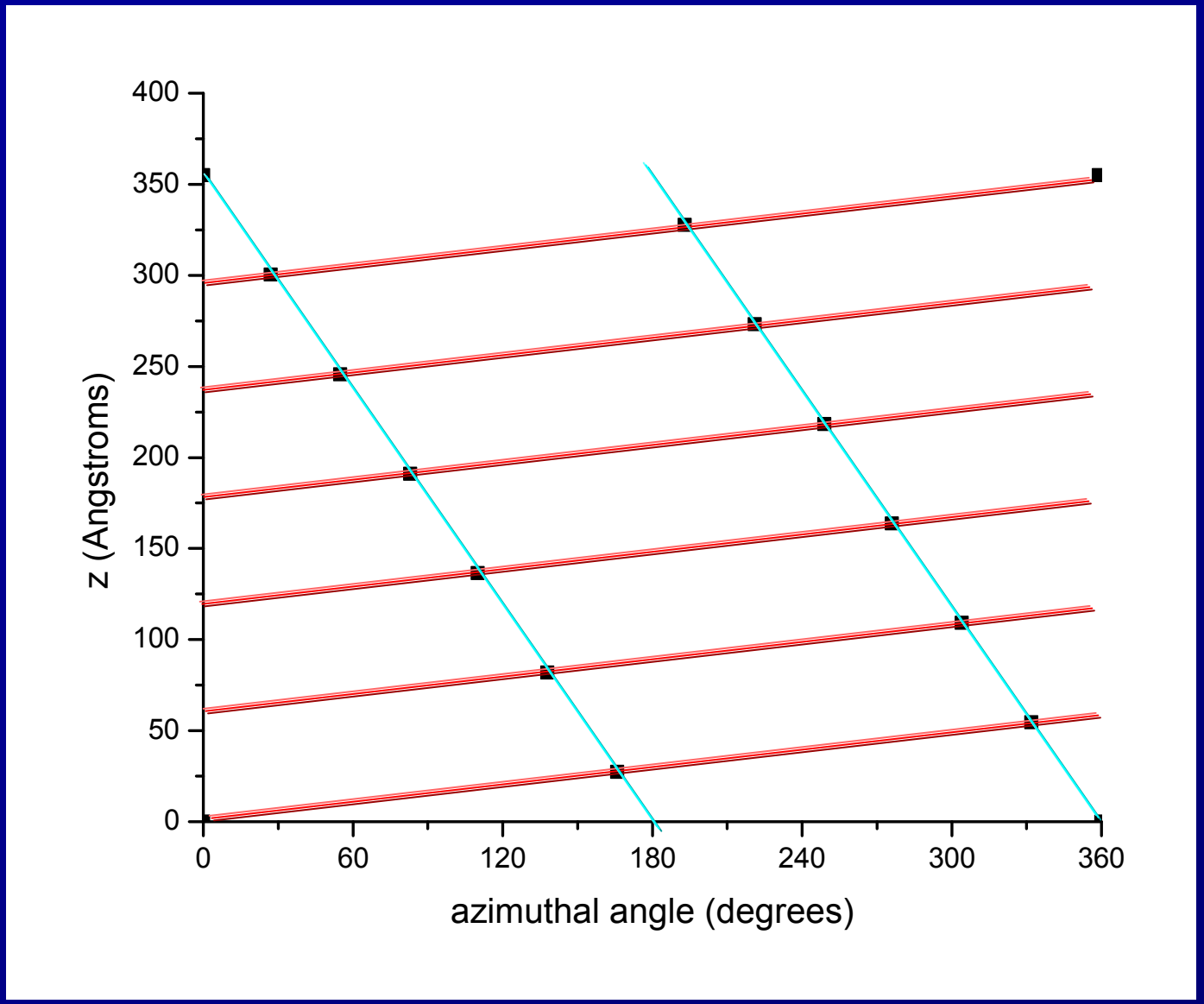
- Understand "hand" convention for helices, helical net




- A "1-start" helix will intersect a horizontal line once, a "2-start" helix will intersect twice,…
- The pitch and the helical repeat are completely different
- Helical repeat is distance needed to translate a subunit axially so that it is in an identical position as another subunit

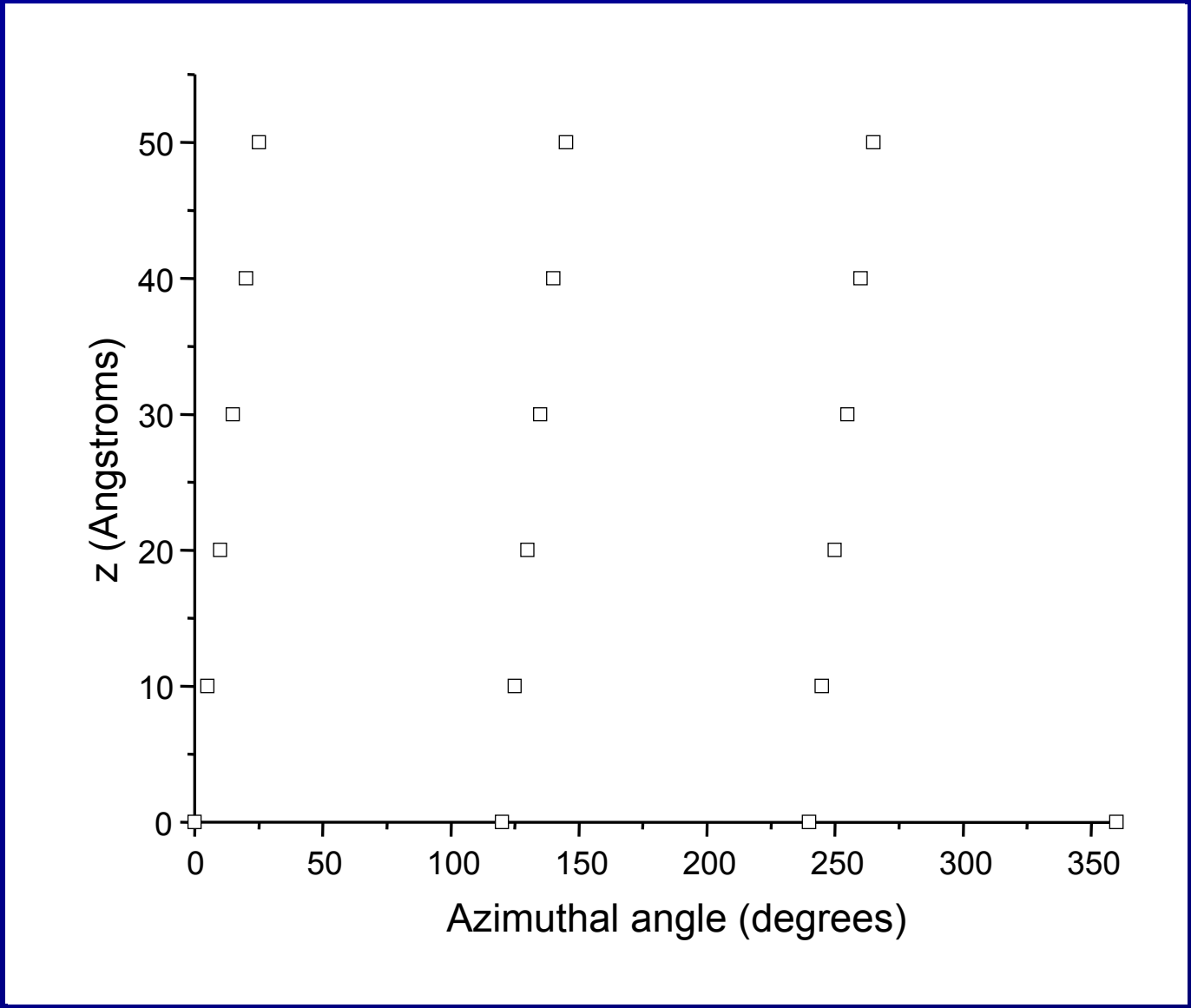



helical net for a 13/6 helix (13 subunits in 6 turns)



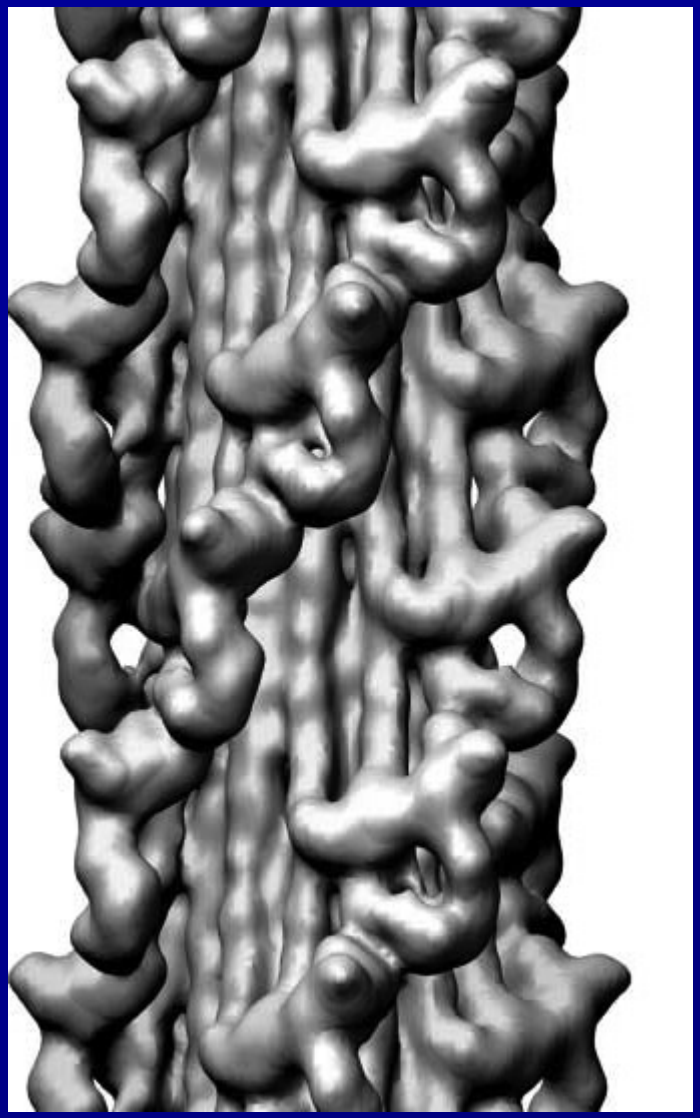

There are an infinite number of different helices that pass through subunits

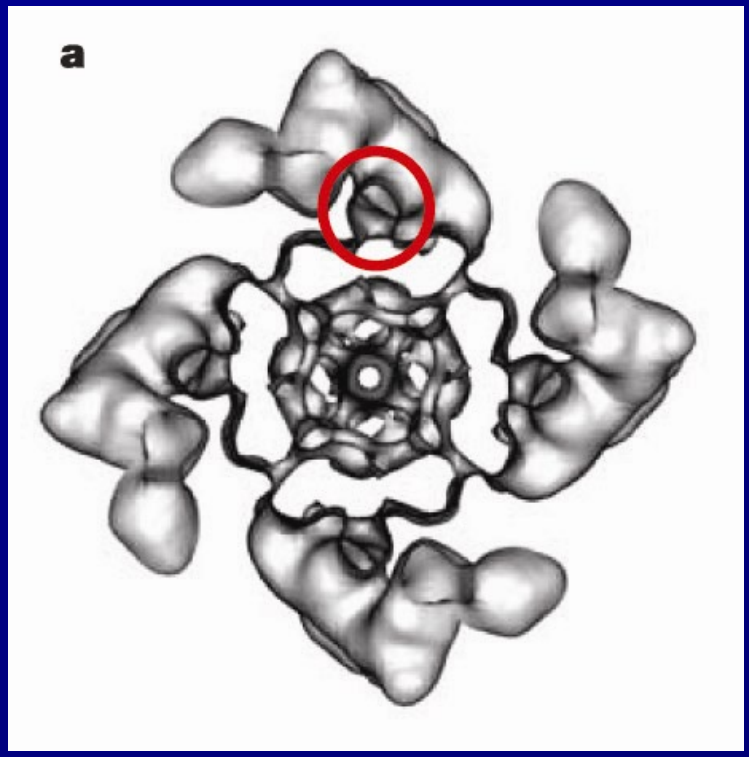
If there is a point group symmetry in addition to the helical symmetry…




point group = C3

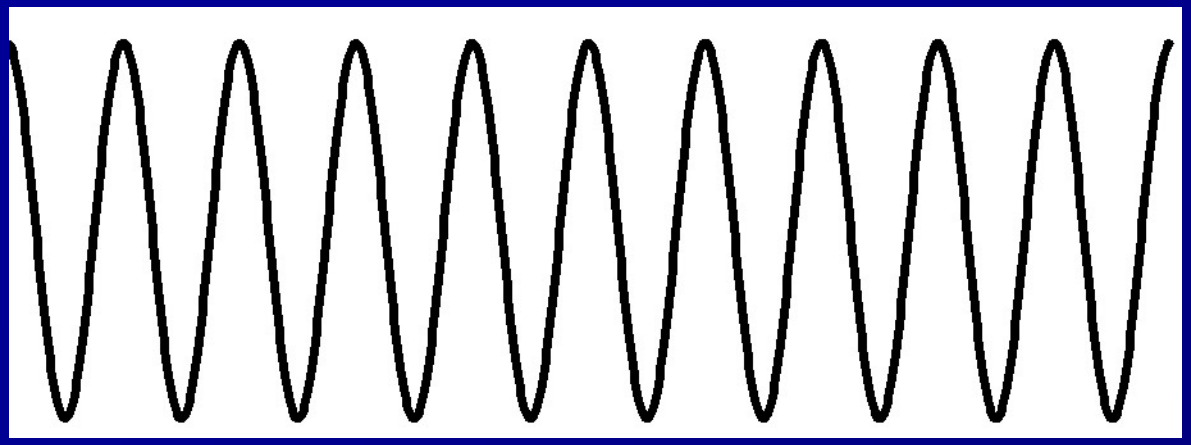
# Myosin thick filament with 4-fold symmetry



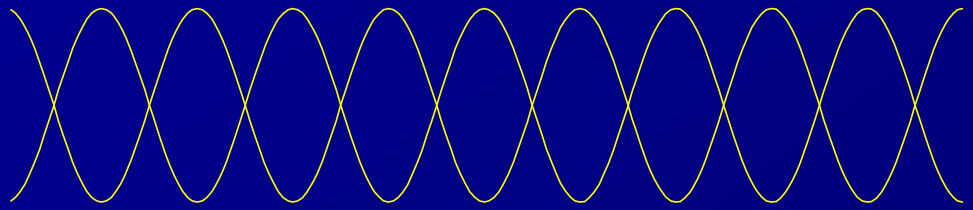



**Woodhead *et al.*, Nature (2005)**

# Projection of a helix



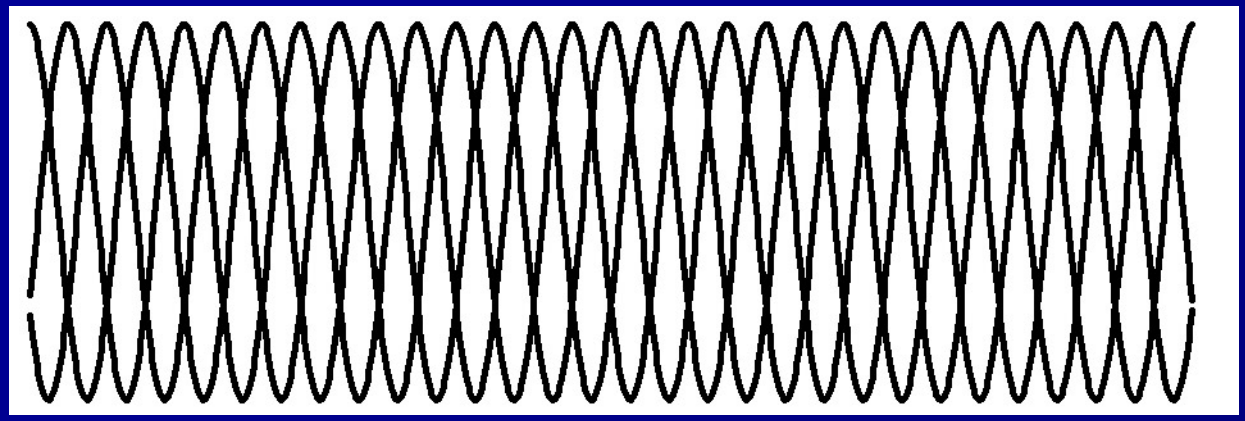
is a sinusoid

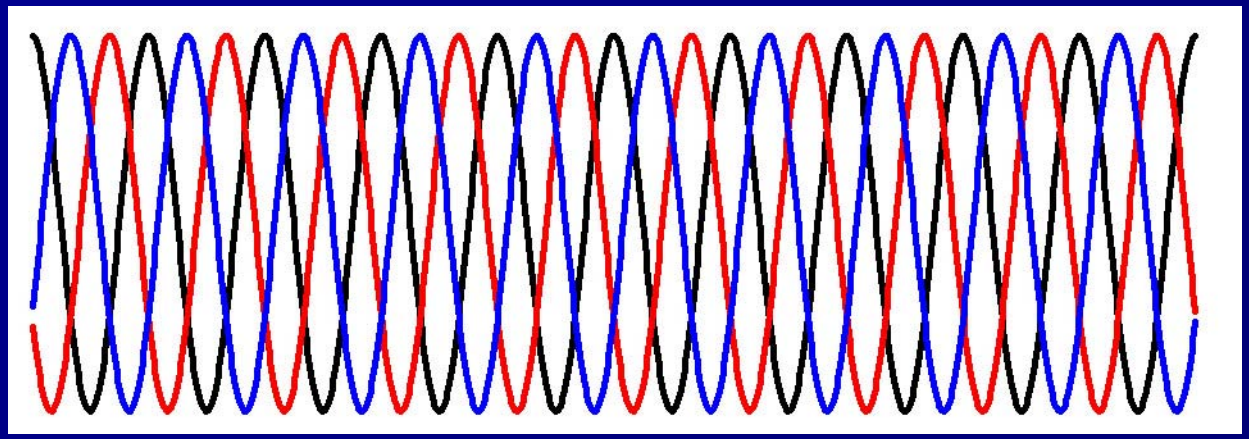


"crossovers" have no physical significance, arise from projection of 3D structure onto 2D

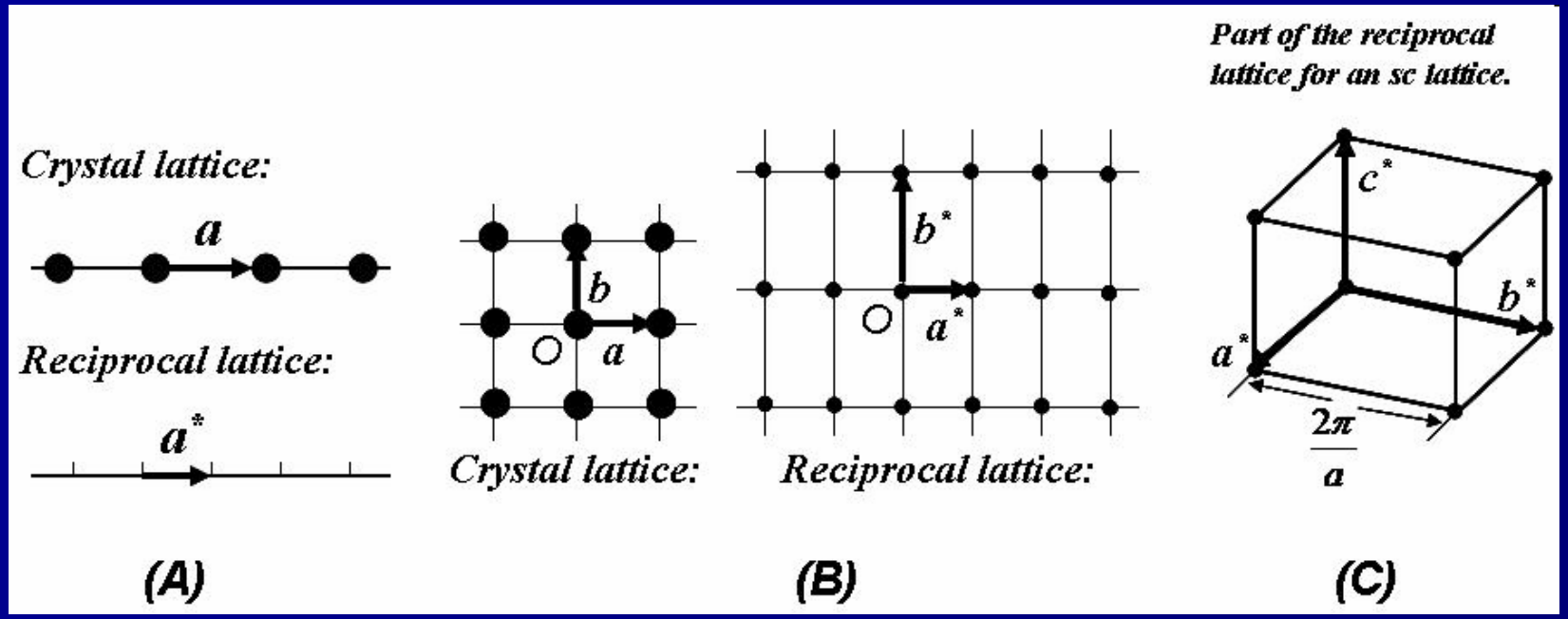
for n=2, crossover distance = P/2; for n=3, crossover distance = P/3,…

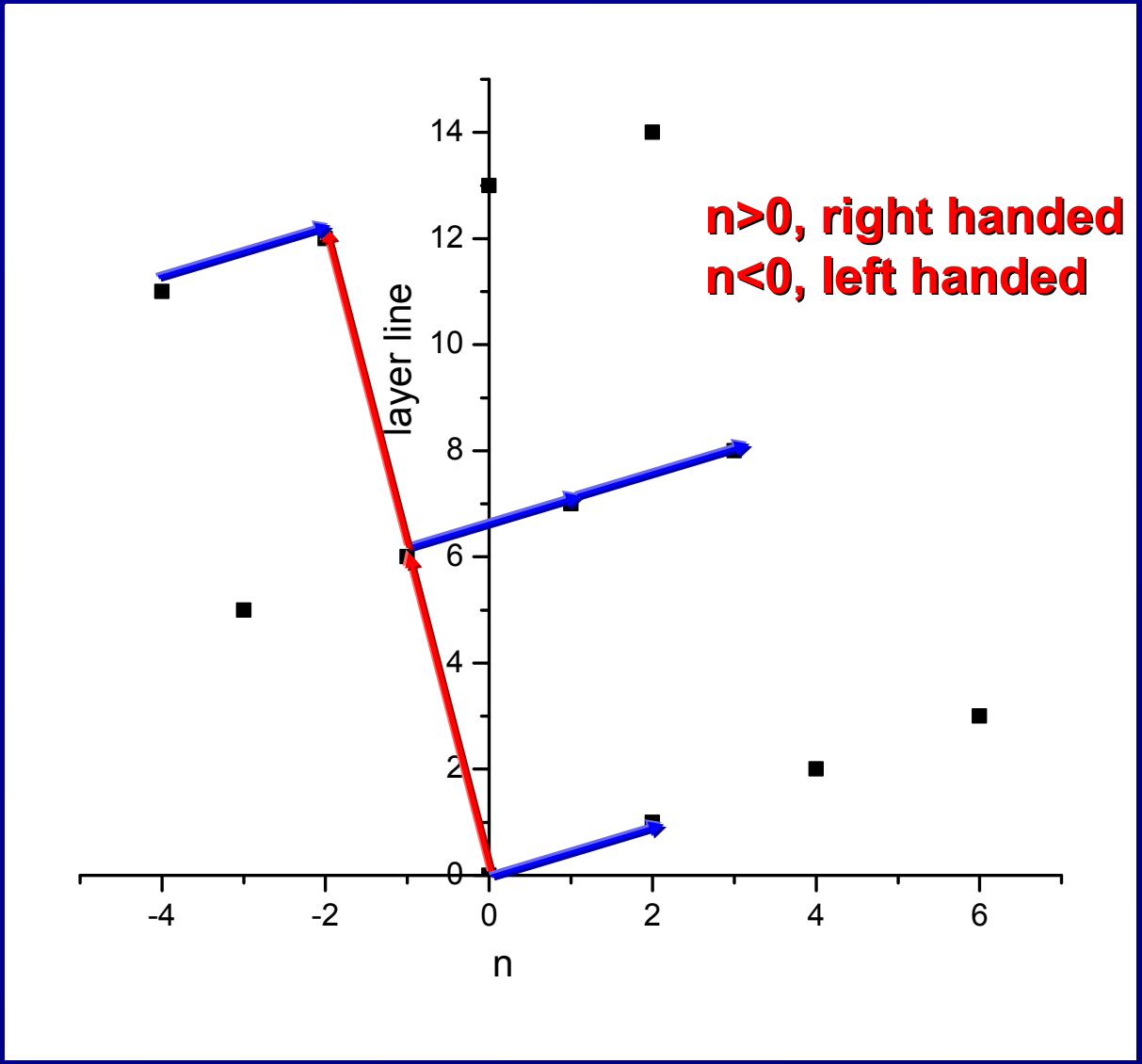
# Consider 3-start





# lattices and reciprocal lattices






n,l plot is reciprocal lattice of helical net

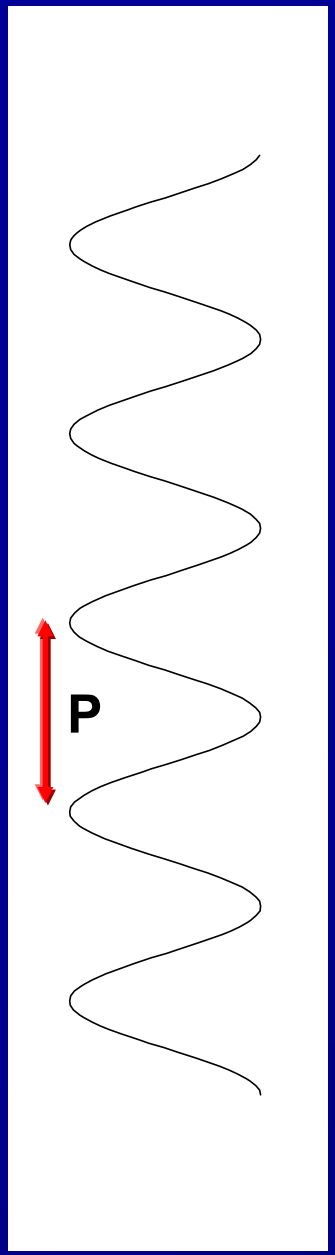
each point corresponds to n-start helix, pitch=|n|*c/l

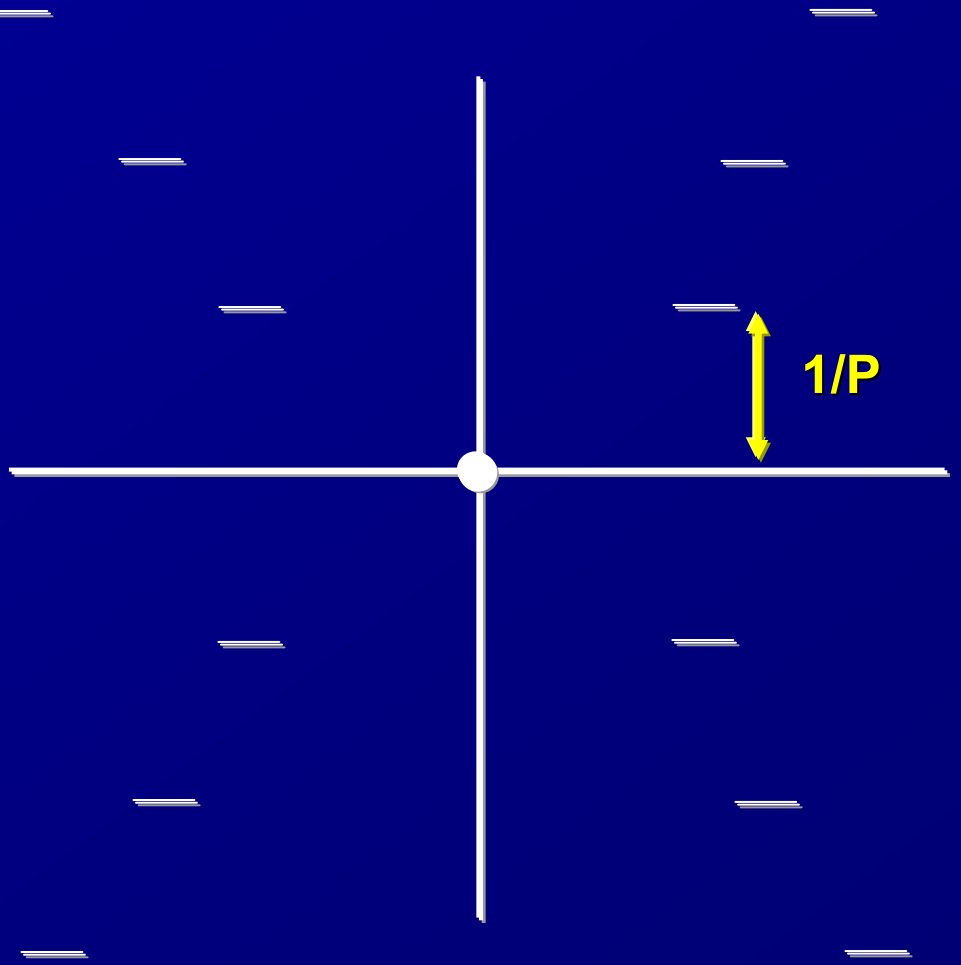
infinite number of points, infinite number of helices!

we can understand n,l plot in terms of diffraction from helical objects

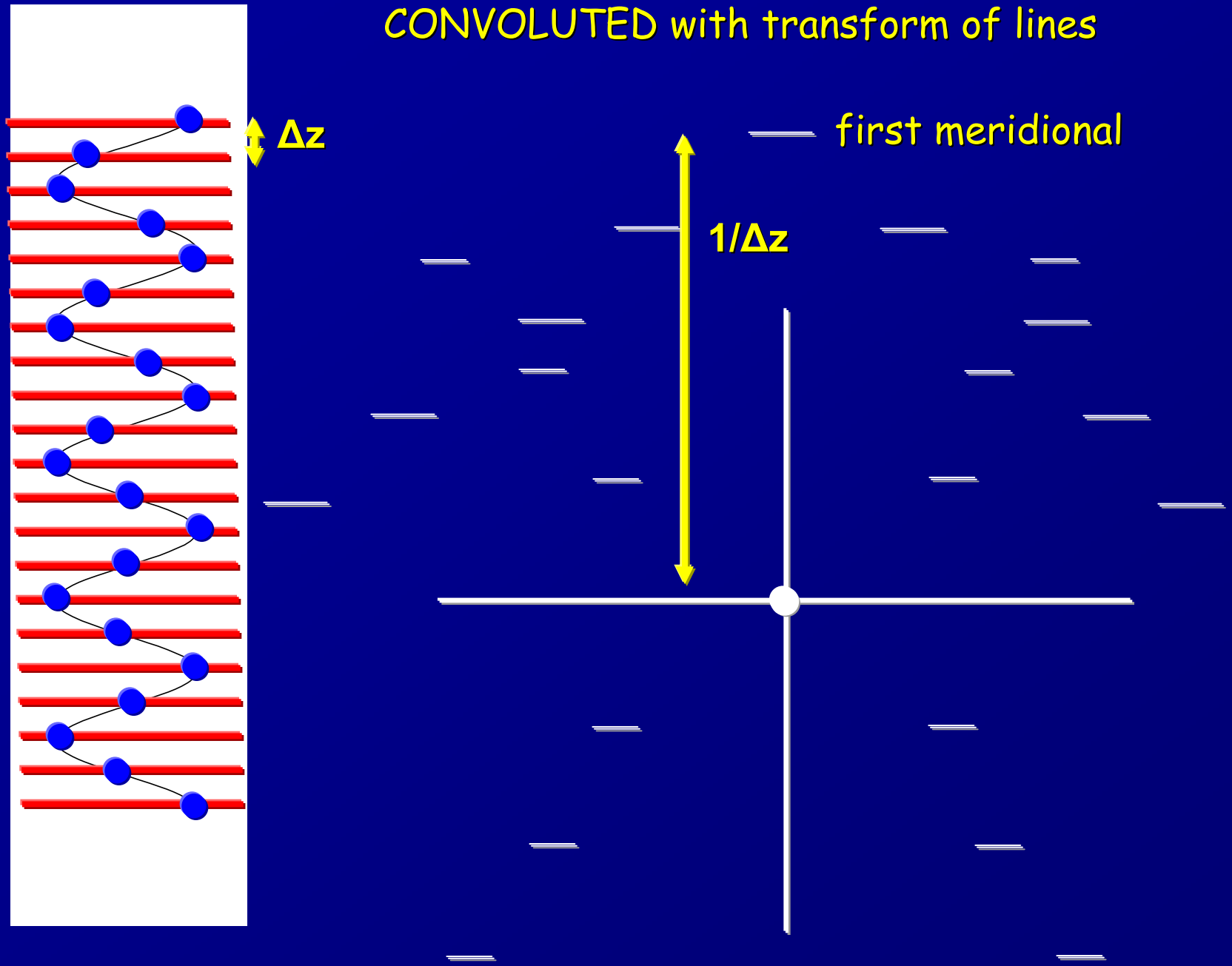
projection of a single helix is a sinusoid

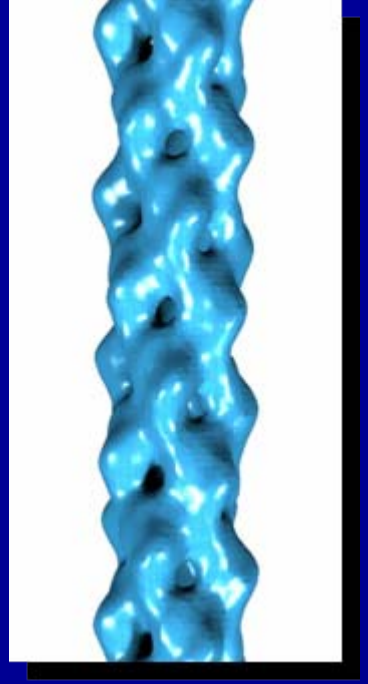
Fourier transform of helix generates "layer lines"

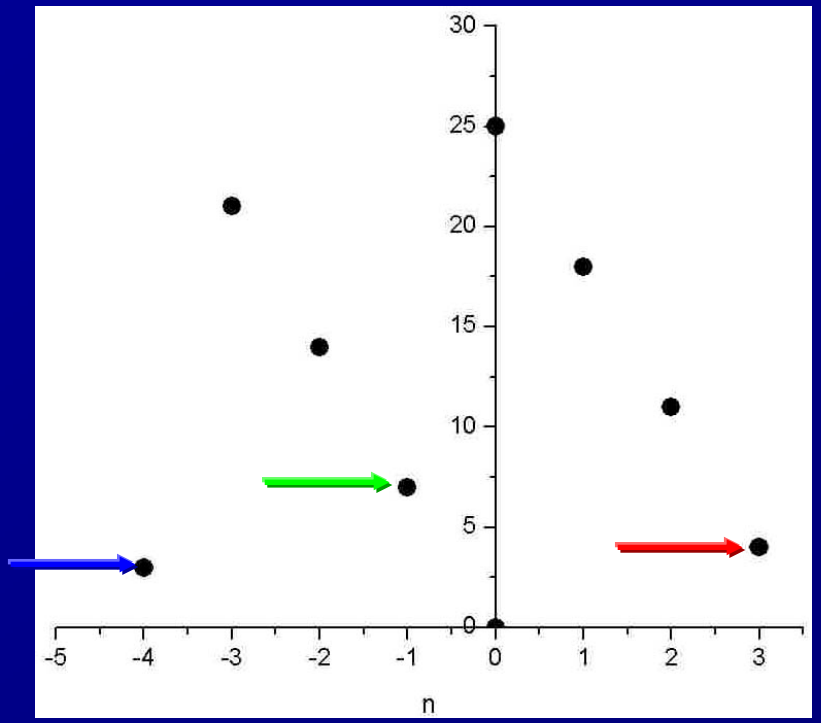


transform will be transform of continuous helix
CONVOLUTED with transform of lines






30
25
20
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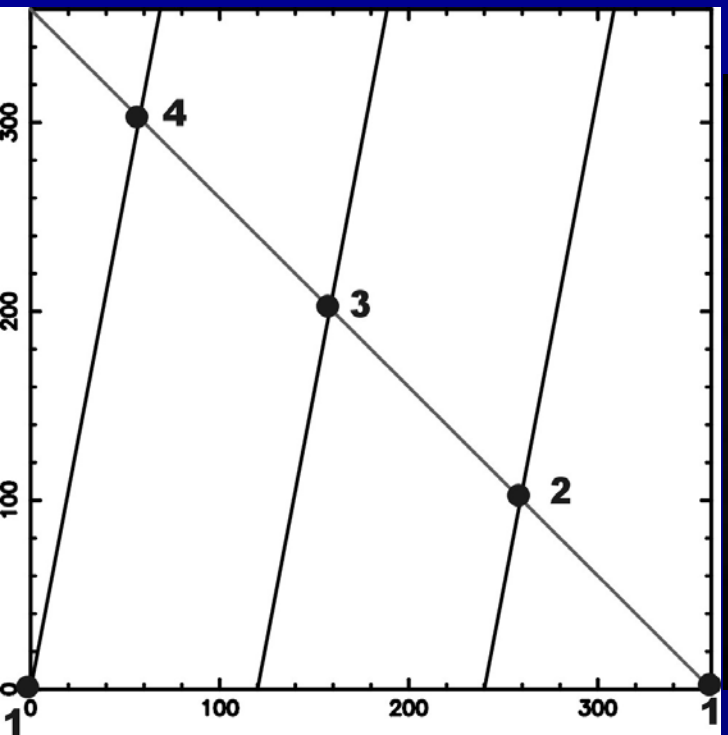
4
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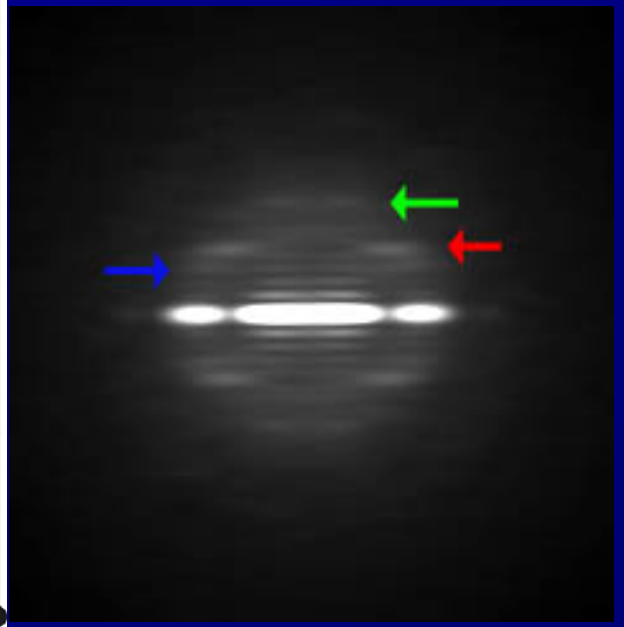
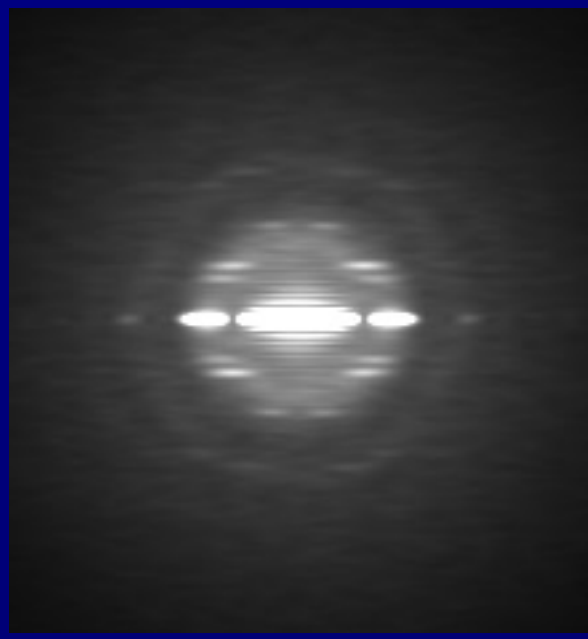
300





# 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

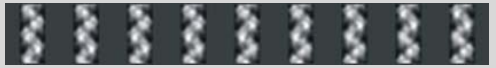
# Iterative Helical Real Space Reconstruction cycle

**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

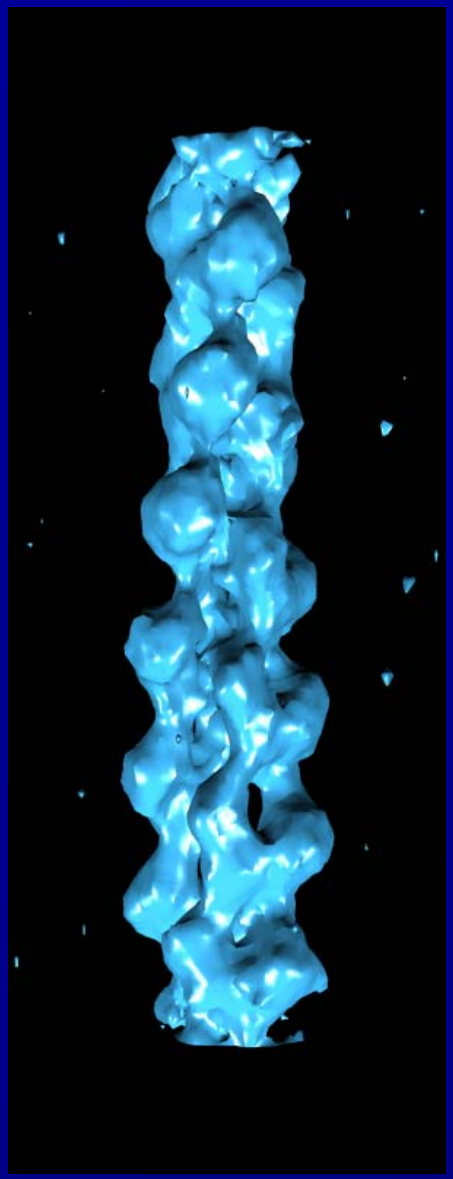
Asymmetric 3D volume

determine helical symmetry by least squares fit

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

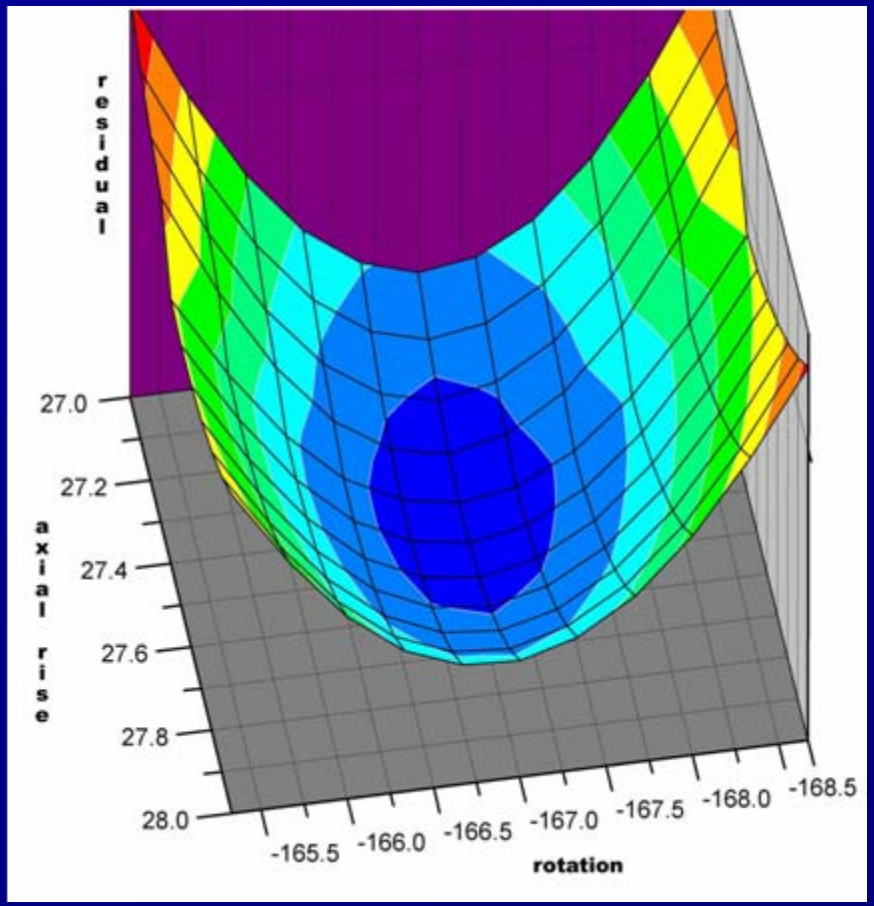
impose helical symmetry

asymmetric reconstruction

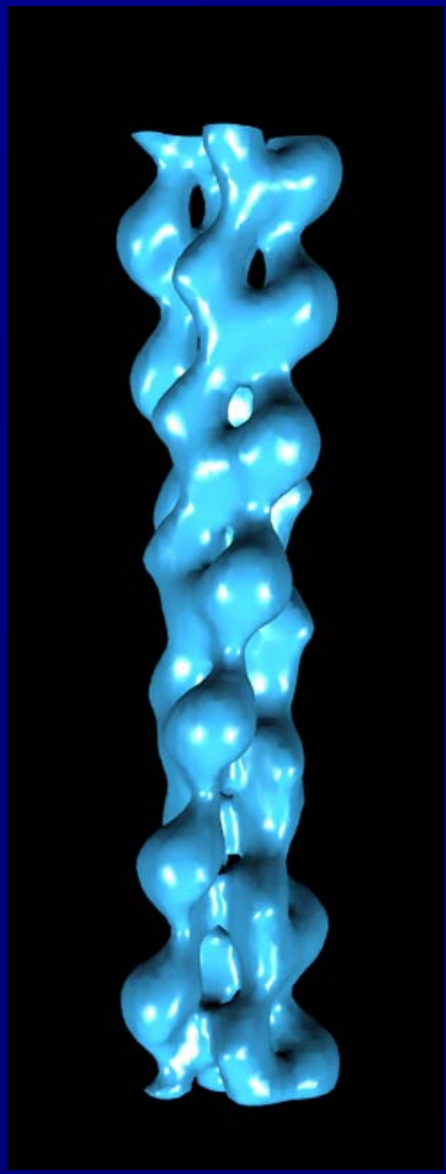


Most important part of cycle…

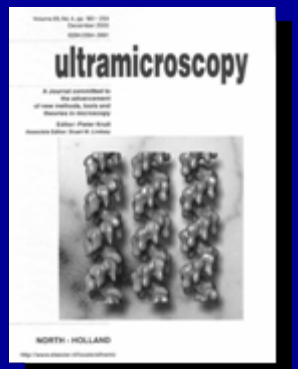
symmetry search

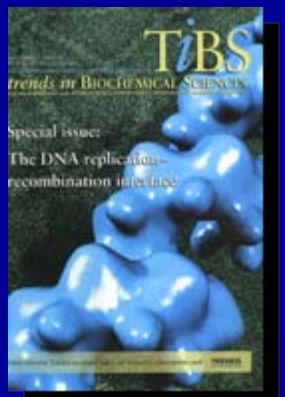



imposed symmetry



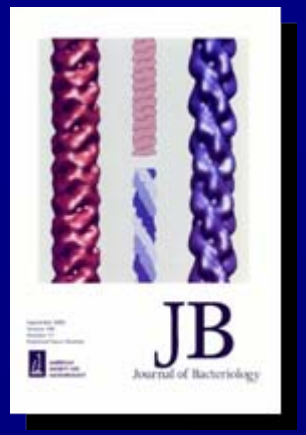
# Iterative Helical Real Space Reconstruction

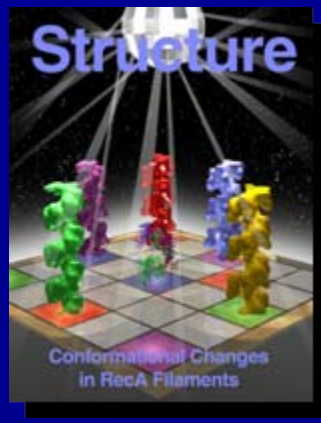


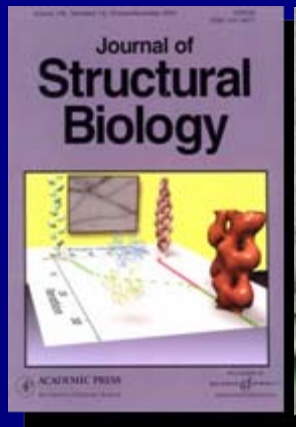


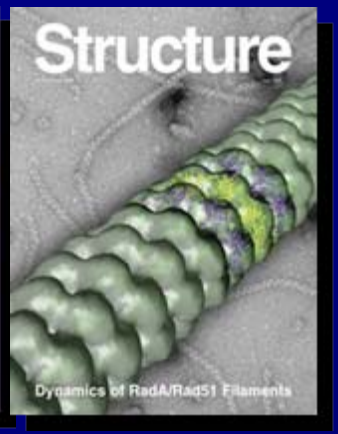


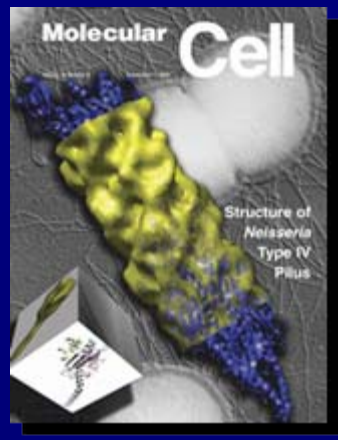





and > 100 other papers published or in press

# Iterative Helical Real Space Reconstruction

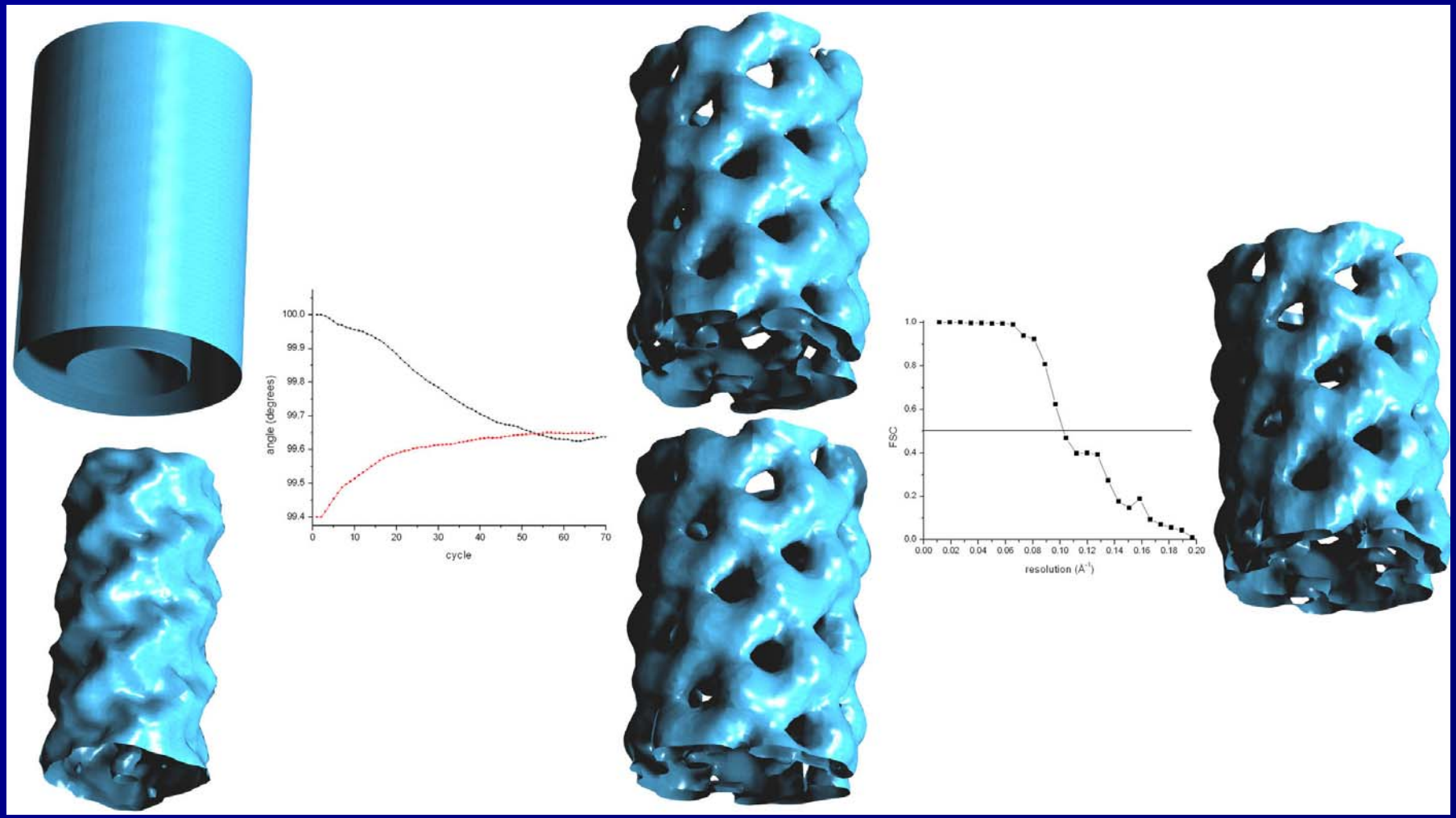
Advantages of method over Fourier-Bessel approach:

- 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, EspA
- Is transparent to the almost intractable problem of Bessel overlap
  - myosin thick filament
- Is easier, both conceptually and in practice

Disadvantages:

- None (for most real specimens)

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



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

But it is not robust with respect to starting symmetry, and application of the method typically requires some estimate of the helical symmetry!

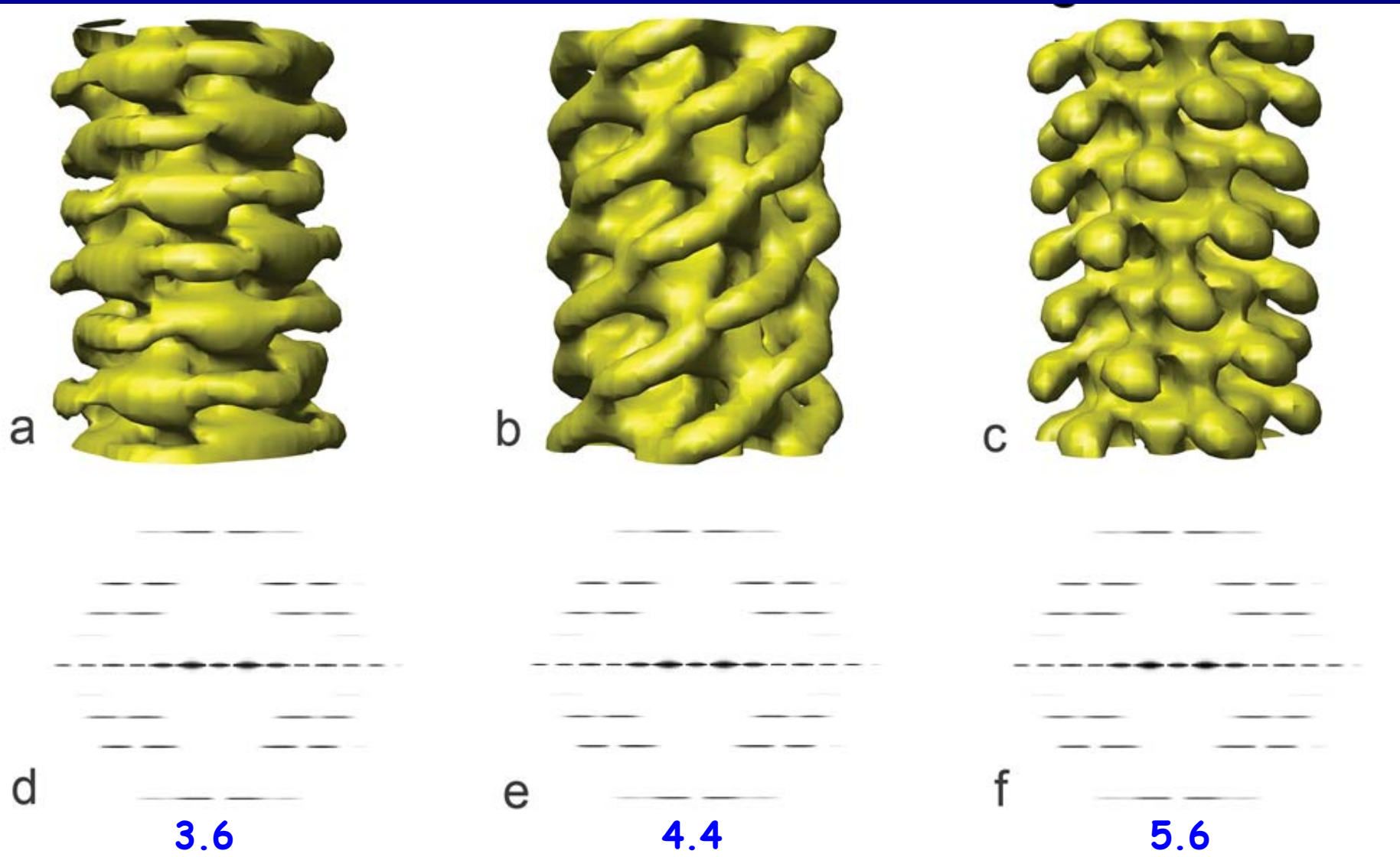
How can this estimate be made?

- STEM (mass/length)
- power spectra
- x-ray fiber diffraction

# At limited resolution, helical polymers may have ambiguous symmetry

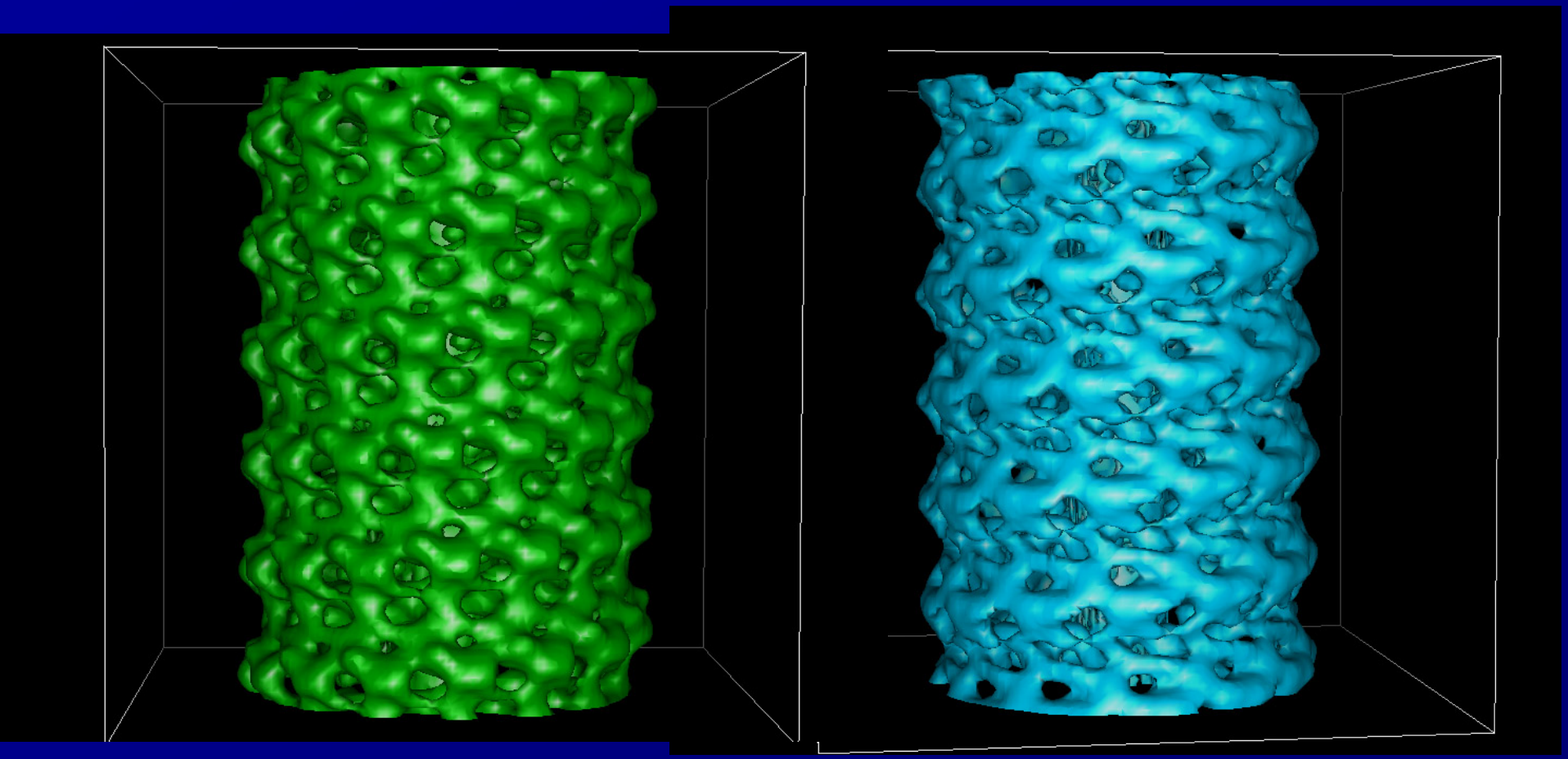
- Images alone do not contain enough information for disambiguation
- Other data are needed:
  - prior knowledge of subunit (e.g., from crystal)
  - mass per unit length (from STEM)
  - metal shadowing or AFM
  - tilts in TEM
  - **higher resolution**

# EspA of Enteropathogenic *E. coli* degeneracy of solutions (Wang *et al.*, 2006)



# with limited resolution, there are intrinsic ambiguities…

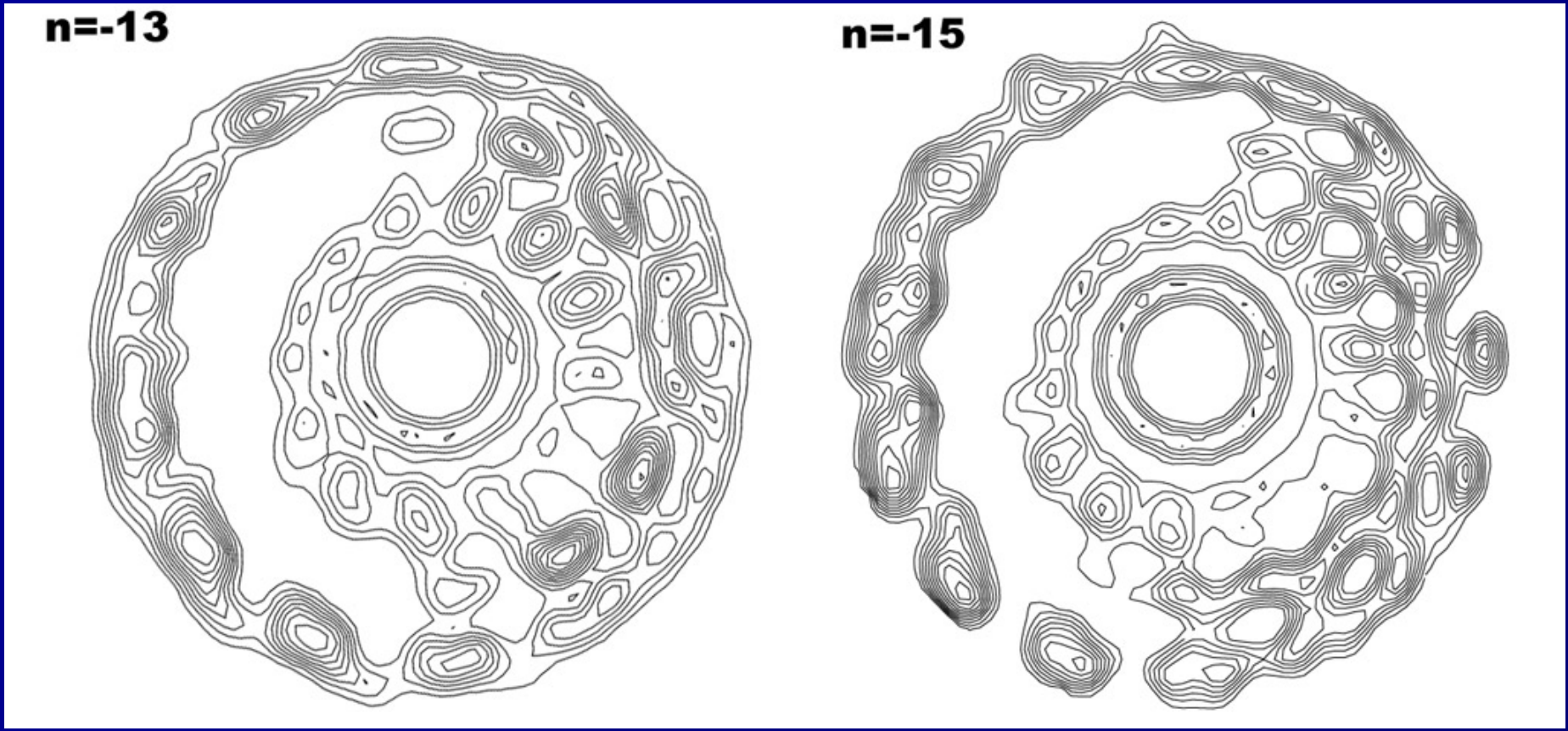
dynamin tube (Egelman, JSB 2007)



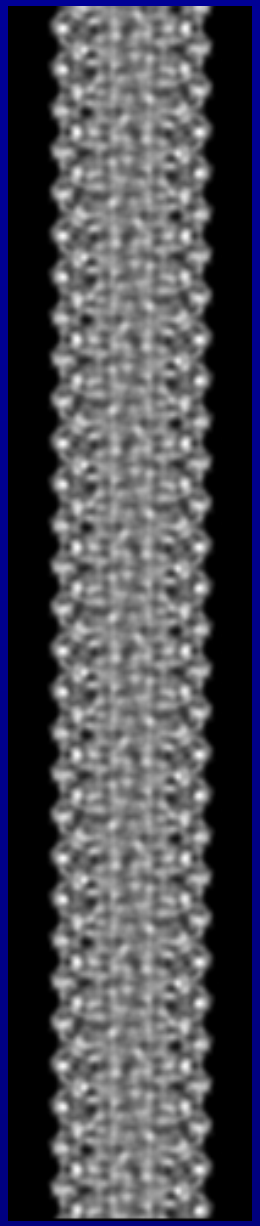
~15 u/t

~13 u/t

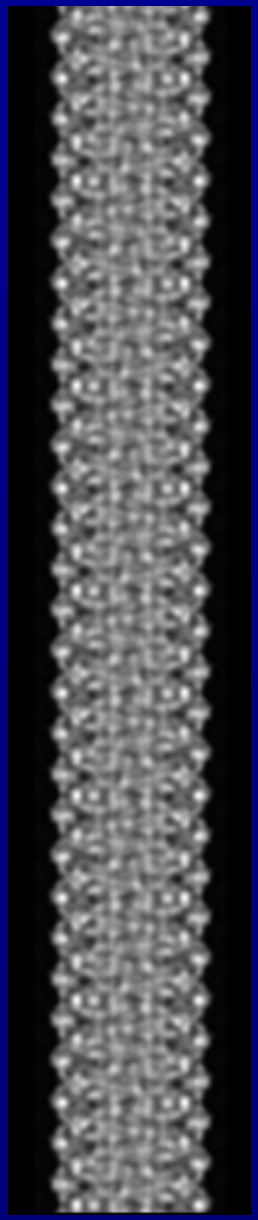
# with limited resolution, there are intrinsic ambiguities…



# with limited resolution, there are intrinsic ambiguities…

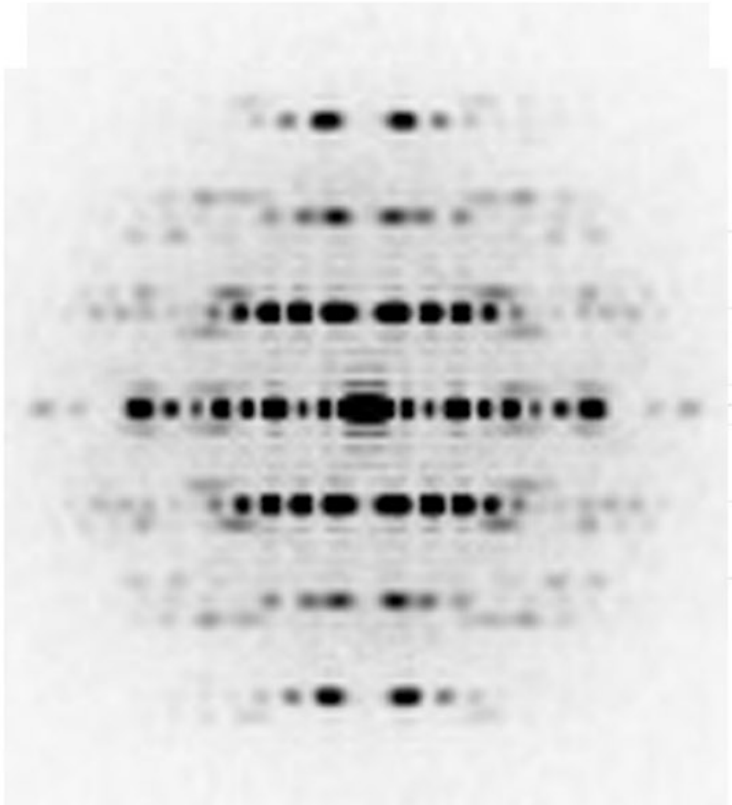


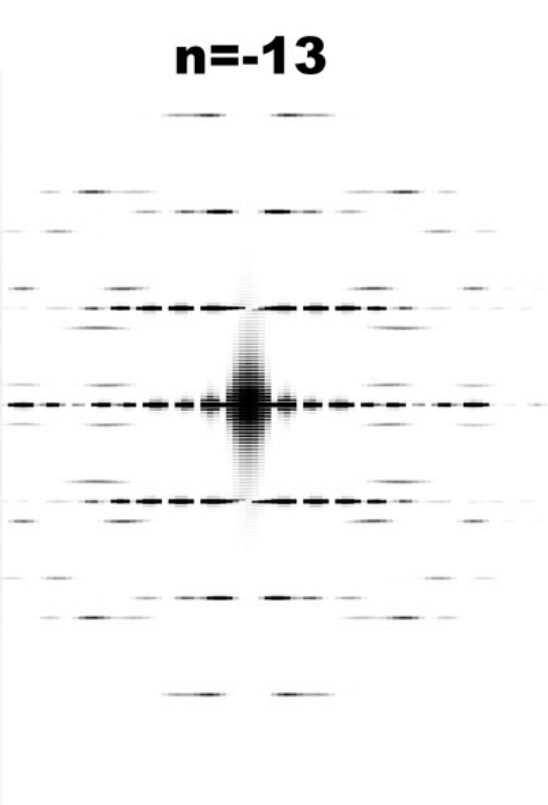
~15 u/t

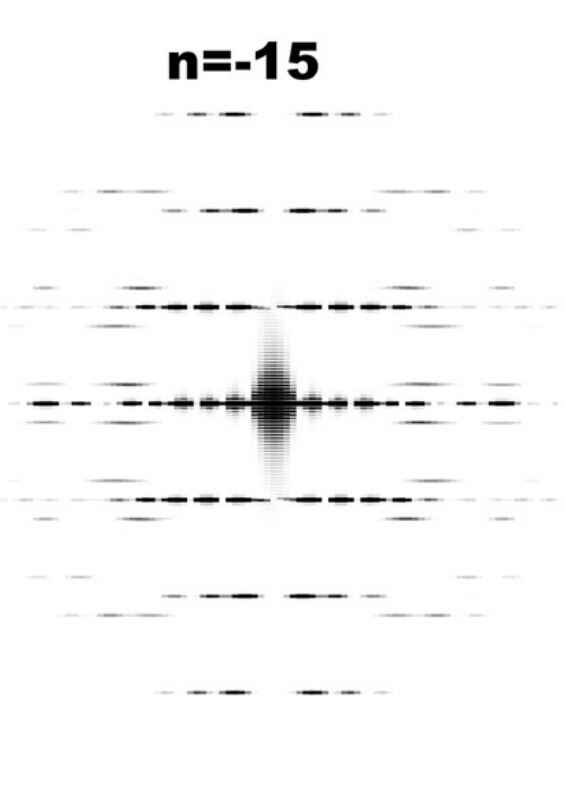


~13 u/t

# with limited resolution, there are intrinsic ambiguities…

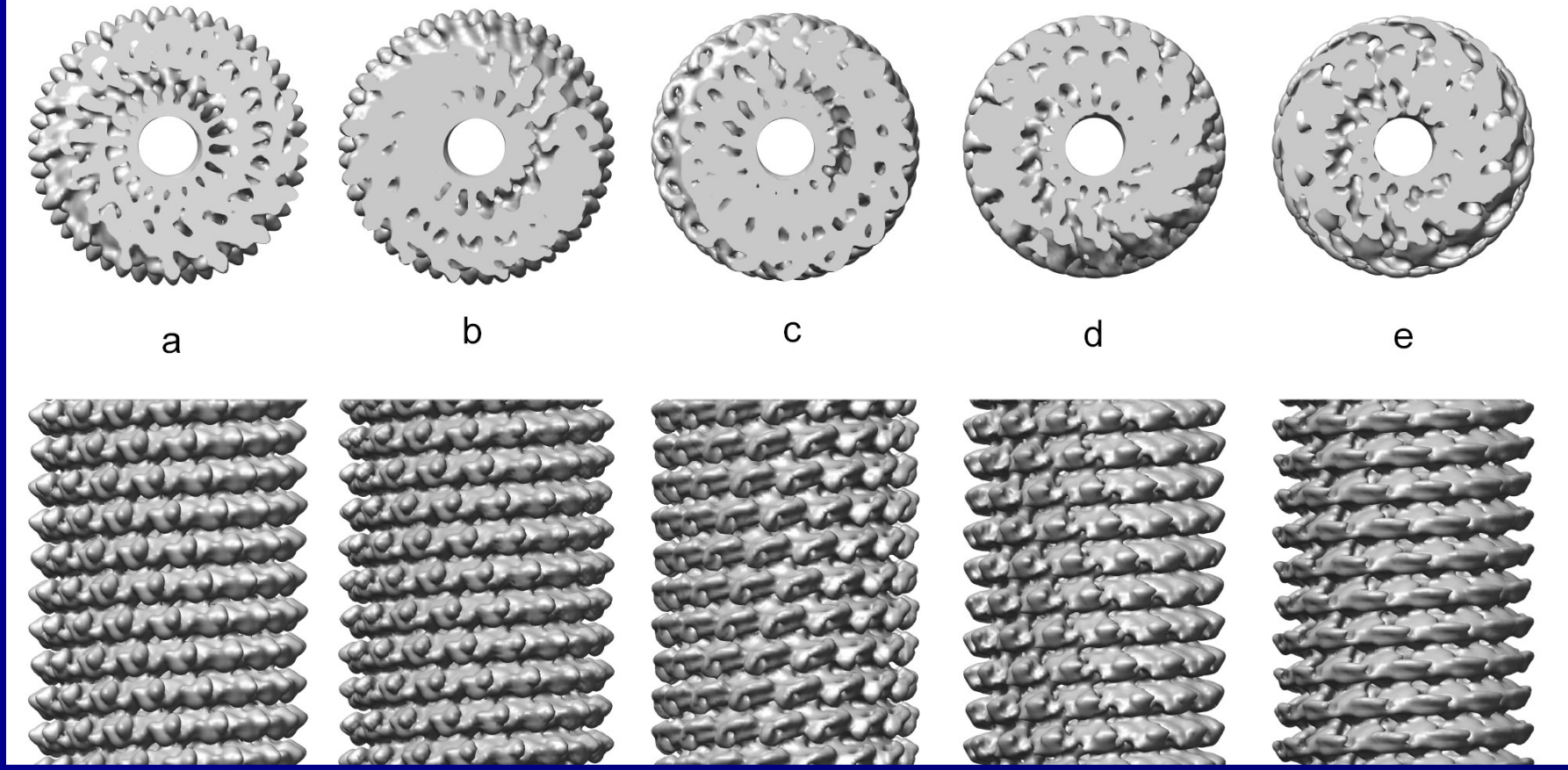




# with limited resolution, there are intrinsic ambiguities…

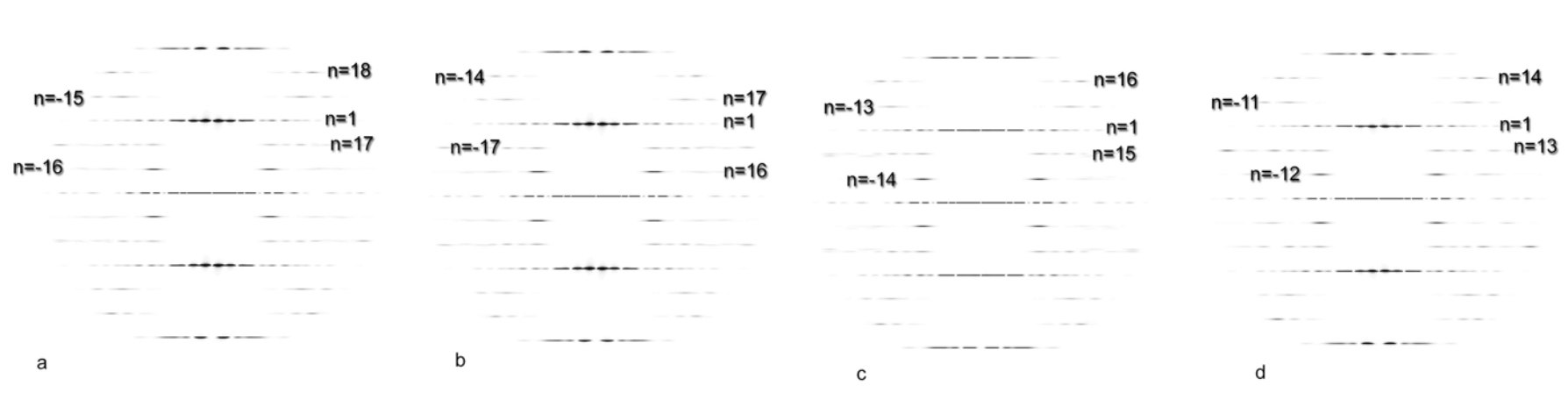
**Tobacco Mosaic Virus**




Egelman, Methods in Enzymology 482, 167-183 (2010)

# with limited resolution, there are intrinsic ambiguities…

## Tobacco Mosaic Virus




**16.33** **15.67** **14.33** **12.33**

Egelman, Methods in Enzymology 482, 167-183 (2010)

# human Dmc1 filaments

doi:10.1016/j.jmb.2010.06.049 J. Mol. Biol. (2010) 401, 544–551

JMB

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## Helical Filaments of Human Dmc1 Protein on Single-Stranded DNA: A Cautionary Tale

**Xiong Yu and Edward H. Egelman***

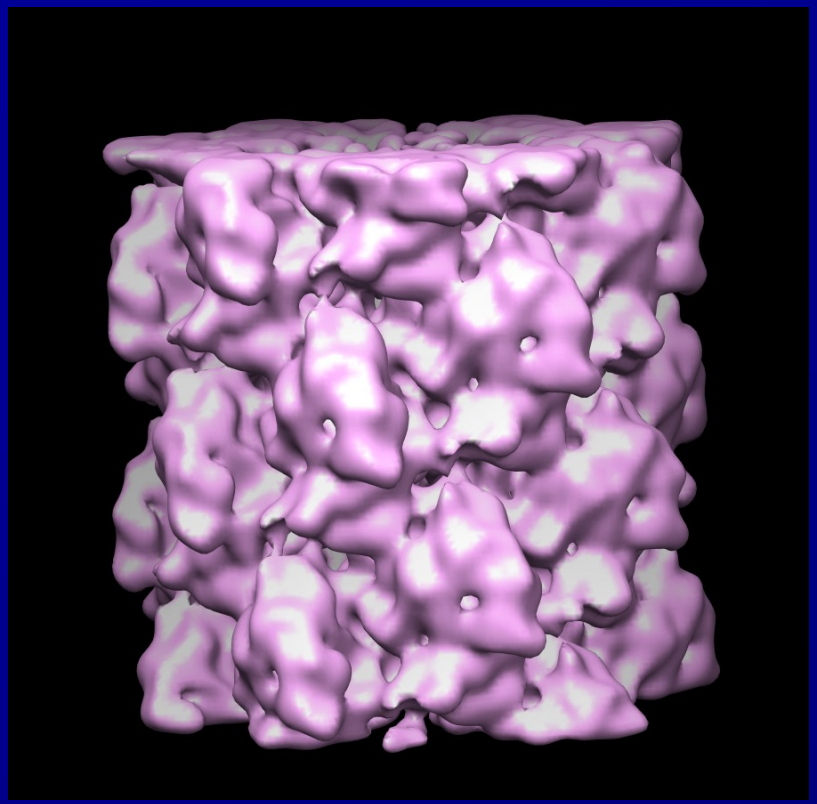
*Department of Biochemistry and Molecular Genetics, Box 800733, University of Virginia, Charlottesville, VA 22908-0733, USA*

*Received 28 May 2010; received in revised form 16 June 2010; accepted 23 June 2010*
Available online 30 June 2010

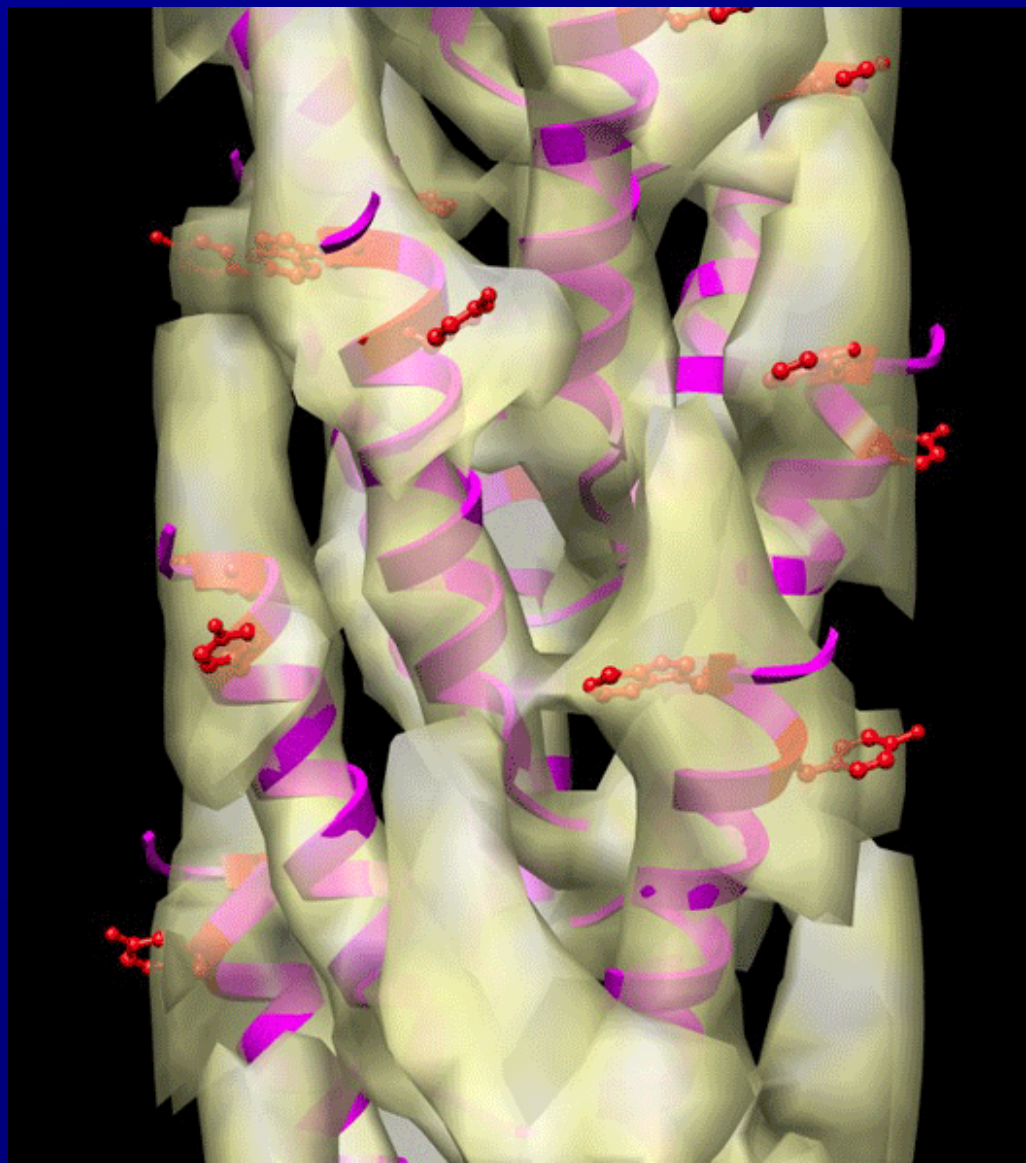
Proteins in the RecA/Rad51/RadA family form nucleoprotein filaments on DNA that catalyze a strand exchange reaction as part of homologous genetic recombination. Because of the centrality of this system to many aspects of DNA repair, the generation of genetic diversity, and cancer when this system fails or is not properly regulated, these filaments have been the object of many biochemical and biophysical studies. A recent paper has argued that the human Dmc1 protein, a meiotic homolog of bacterial RecA and human Rad51, forms filaments on single-stranded DNA with ~9 subunits per turn in contrast to the filaments formed on double-stranded DNA with ~6.4 subunits per turn and that the stoichiometry of DNA binding is different between these two filaments. We show using scanning transmission electron microscopy that the Dmc1 filament formed on single-stranded DNA has a mass per unit length expected from ~6.5 subunits per turn. More generally, we show how ambiguities in helical symmetry determination can generate incorrect solutions and why one sometimes must use other techniques, such as biochemistry, metal shadowing, or scanning transmission electron microscopy, to resolve these ambiguities. While three-dimensional reconstruction of helical filaments from EM images is a powerful tool, the intrinsic ambiguities that may be present with limited resolution are not sufficiently appreciated.

# Ambiguities disappear at higher resolution

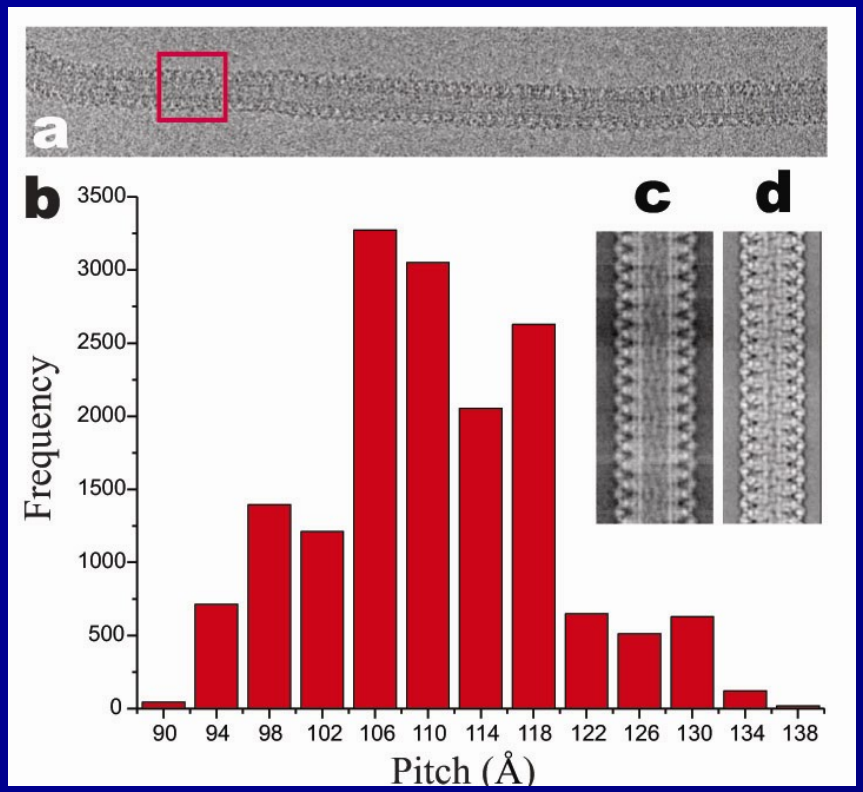
archaeal surface filaments – adhesion filaments resemble flagella



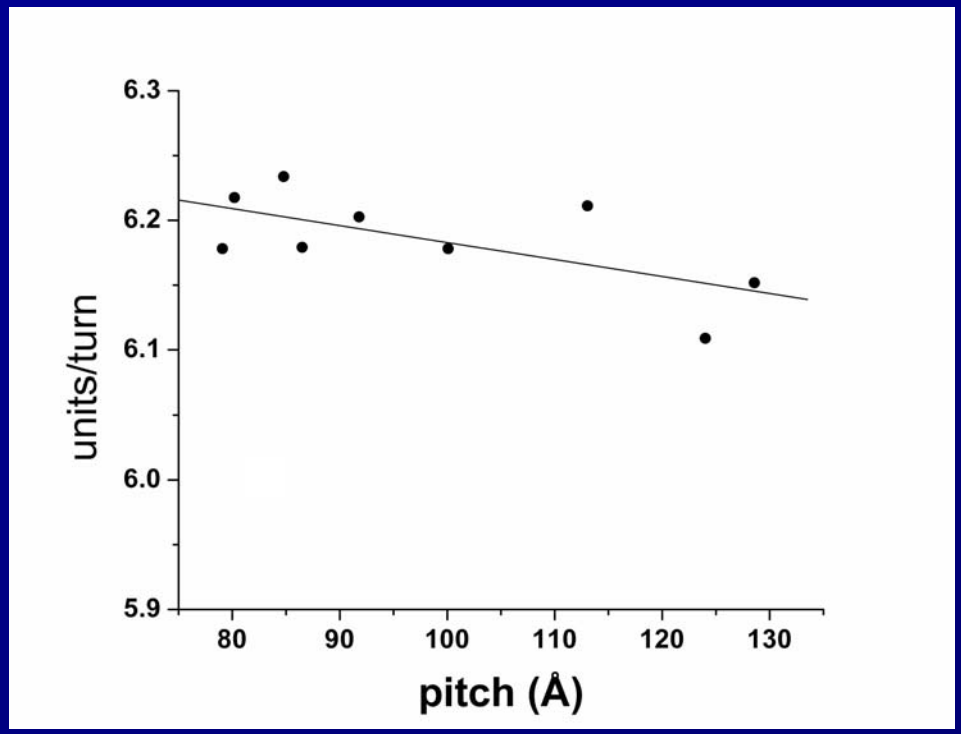
*I. hospitalis* adhesion filaments



# Variability can be enormous (and obvious)

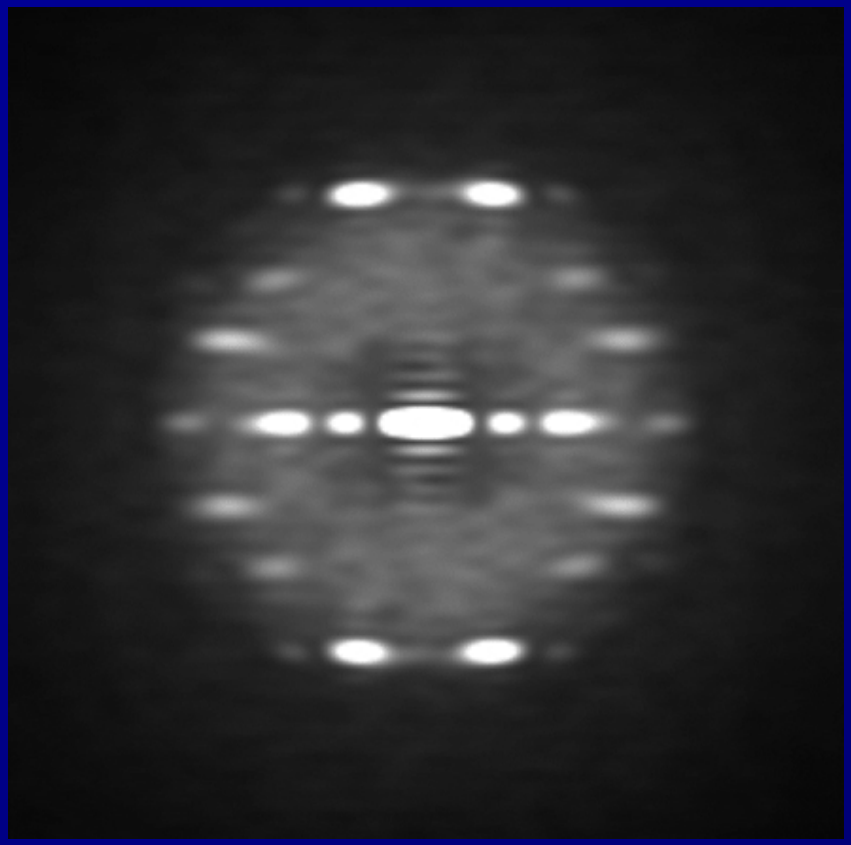



Dynamin-lipid tubes
Chen *et al.*, Nature SMB (2004)



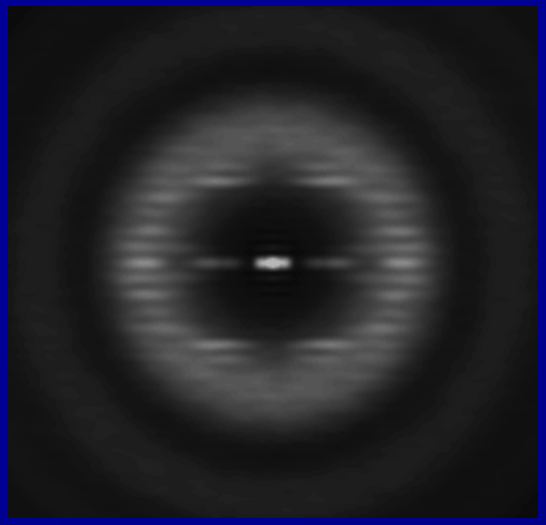

RecA-DNA filaments
VanLoock *et al.*, JMB (2003)

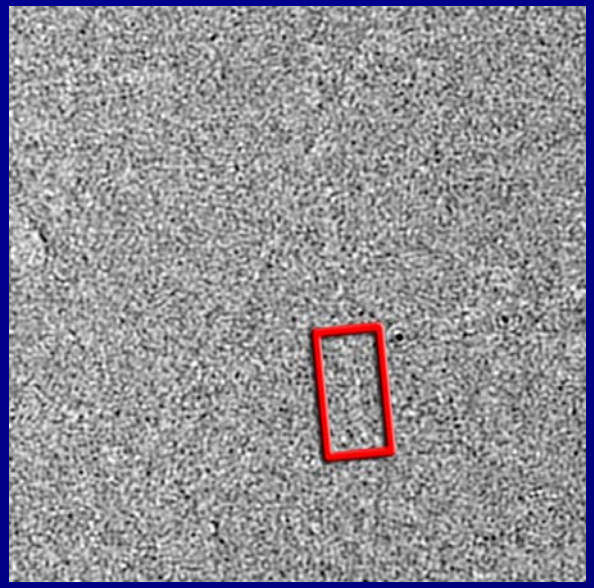
# Variability in twist

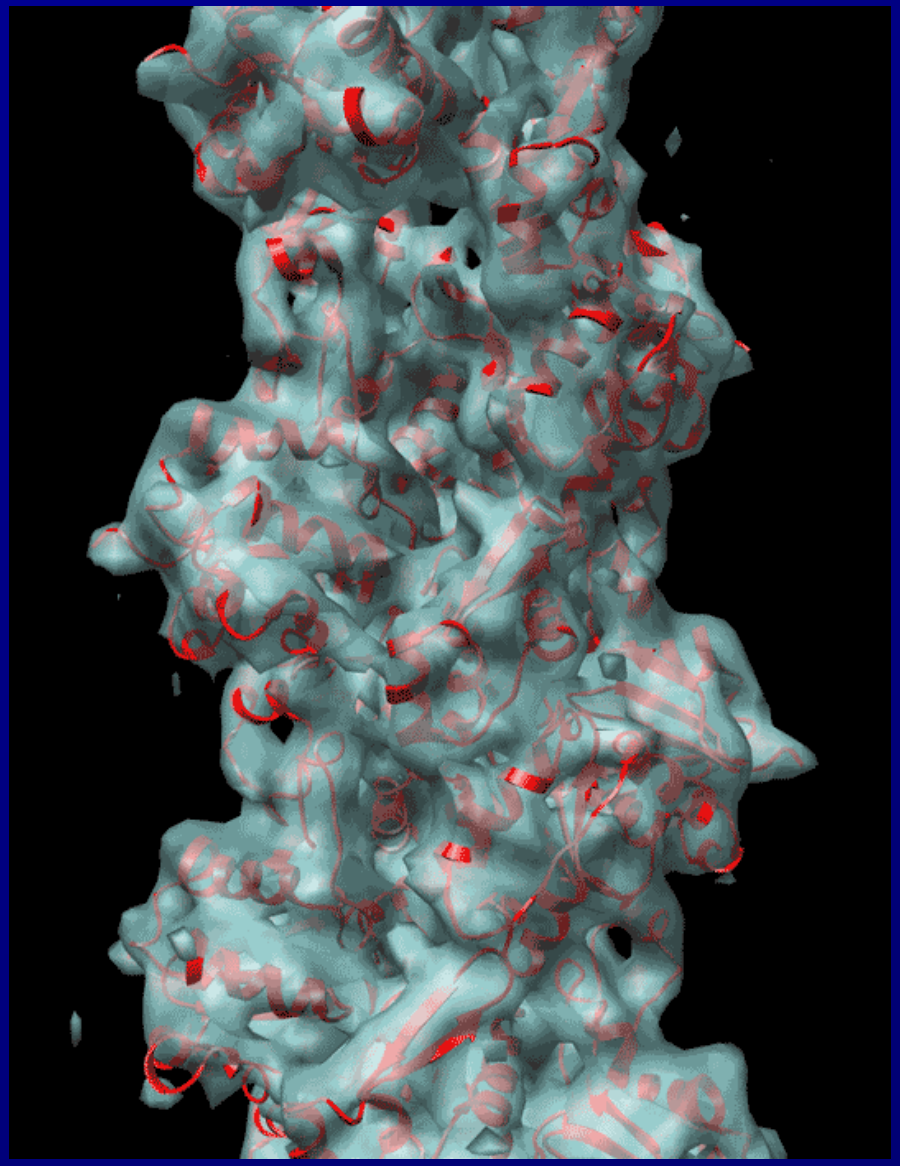


tubes formed by mutant HIV
Gag CA protein (~ 210 Å in diameter)

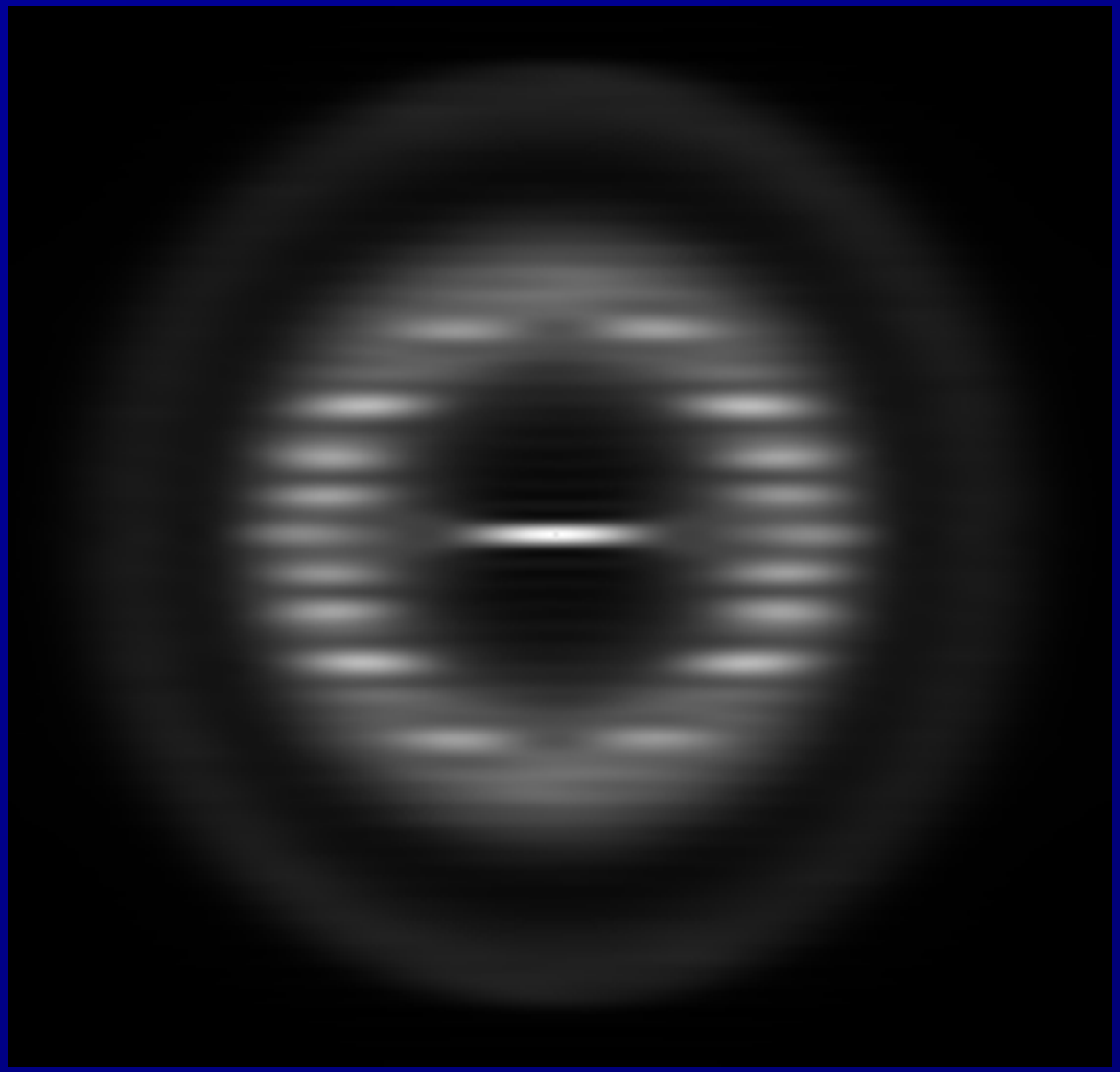
# Variability in helices: F-Actin is typical, not unusual



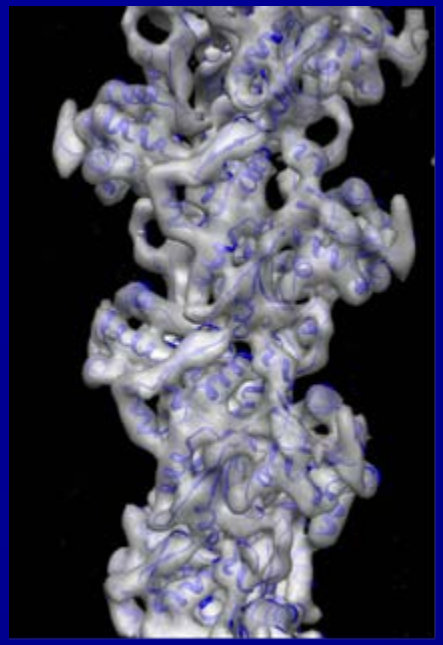




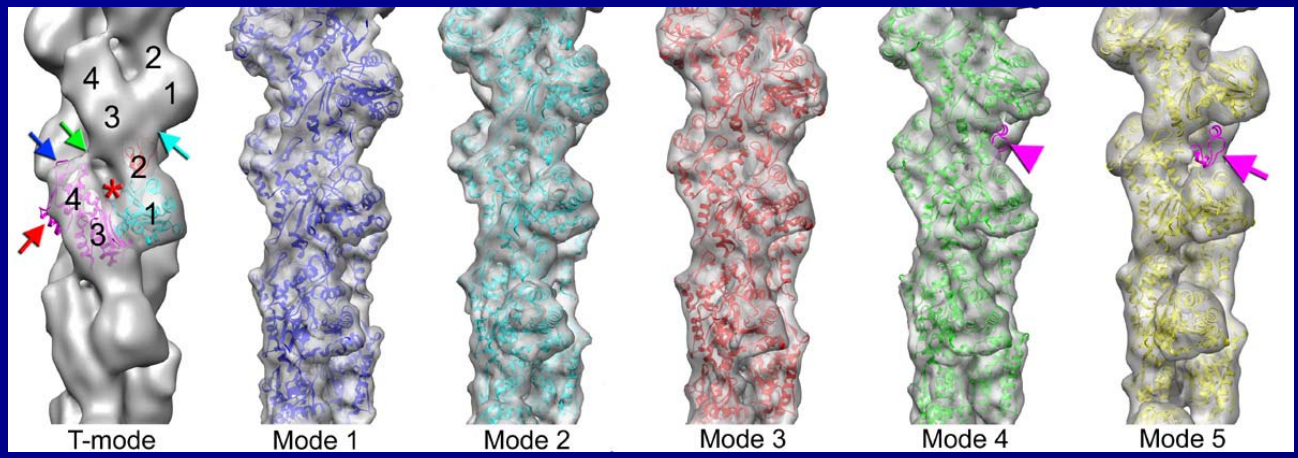
Variability in twist in ParM much greater than in F-actin



# Does F-actin have one structure?



Fujii *et al.*,
Nature (2010)



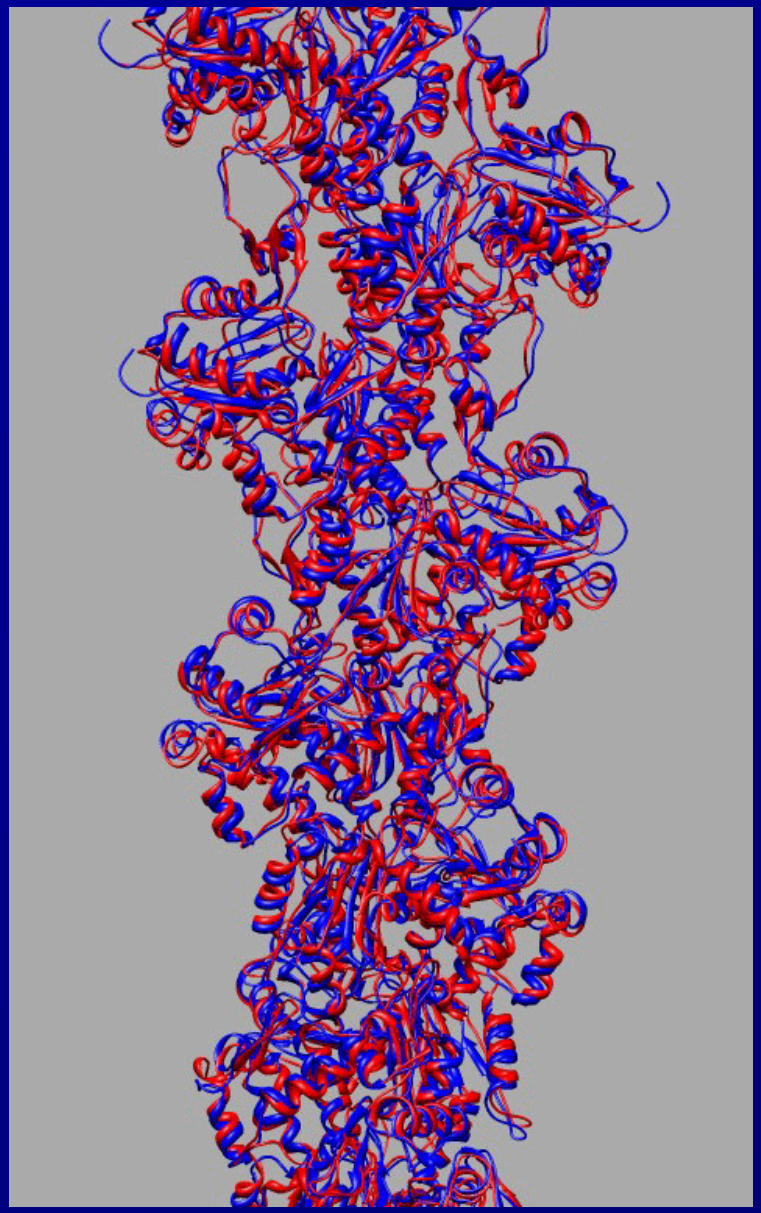

Galkin *et al.*,
NSMB (2010)

# Excellent match of our “canonical” F-actin with Fujii *et al.* model

Galkin *et al.*

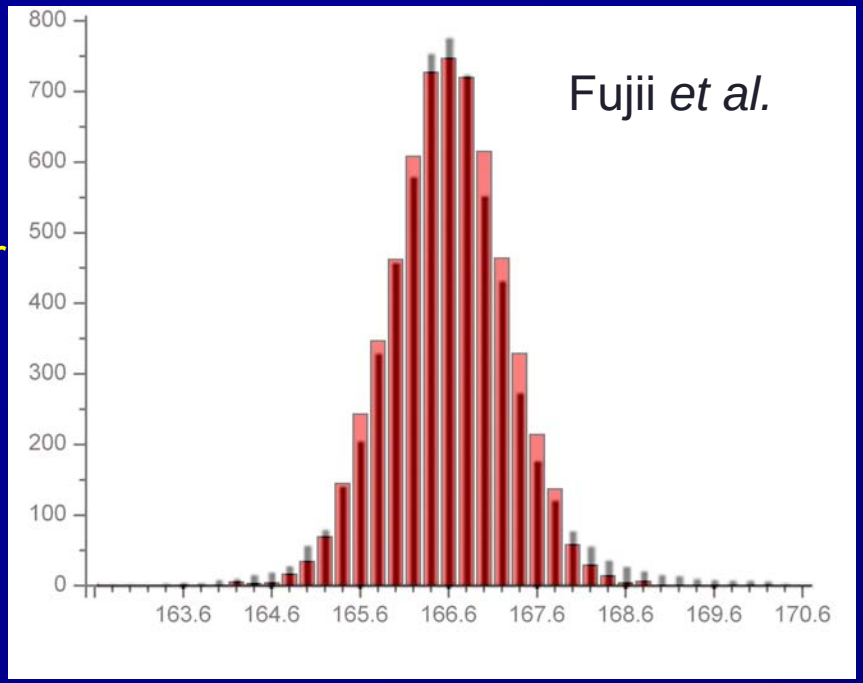
Fujii *et al.*

2.0 Å rms deviation

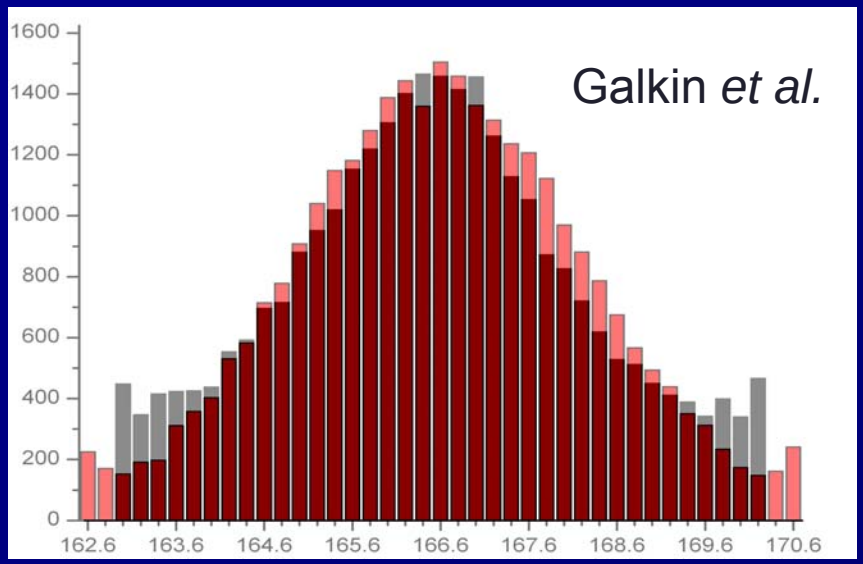


# How can structural homogeneityof Fujii *et al.* be explained? It is coupled with a reduction in variable twist

simulation with 2.5° random angular disorder per subunit (in red)

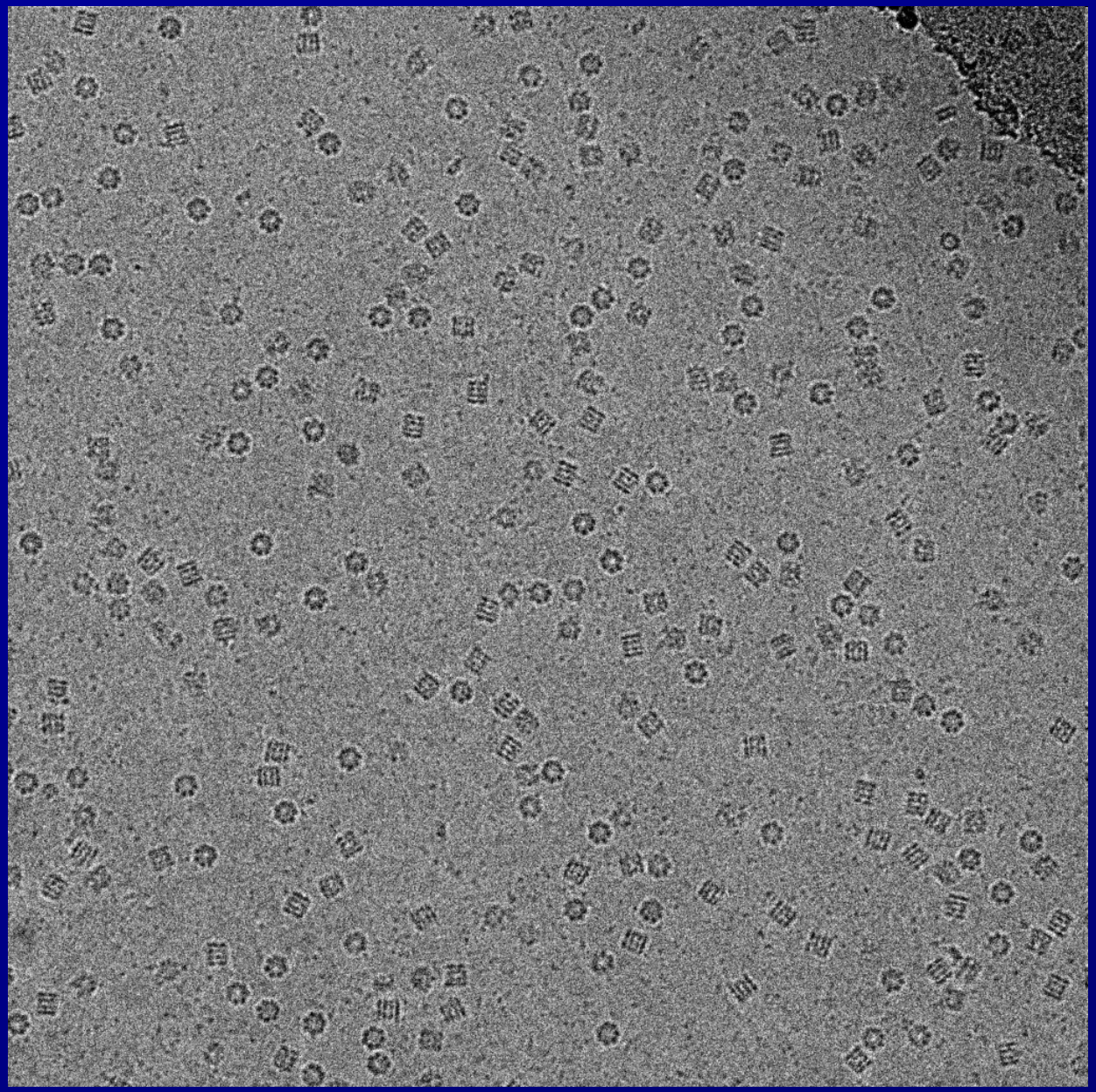



simulation with 6° random angular disorder per subunit (in red)

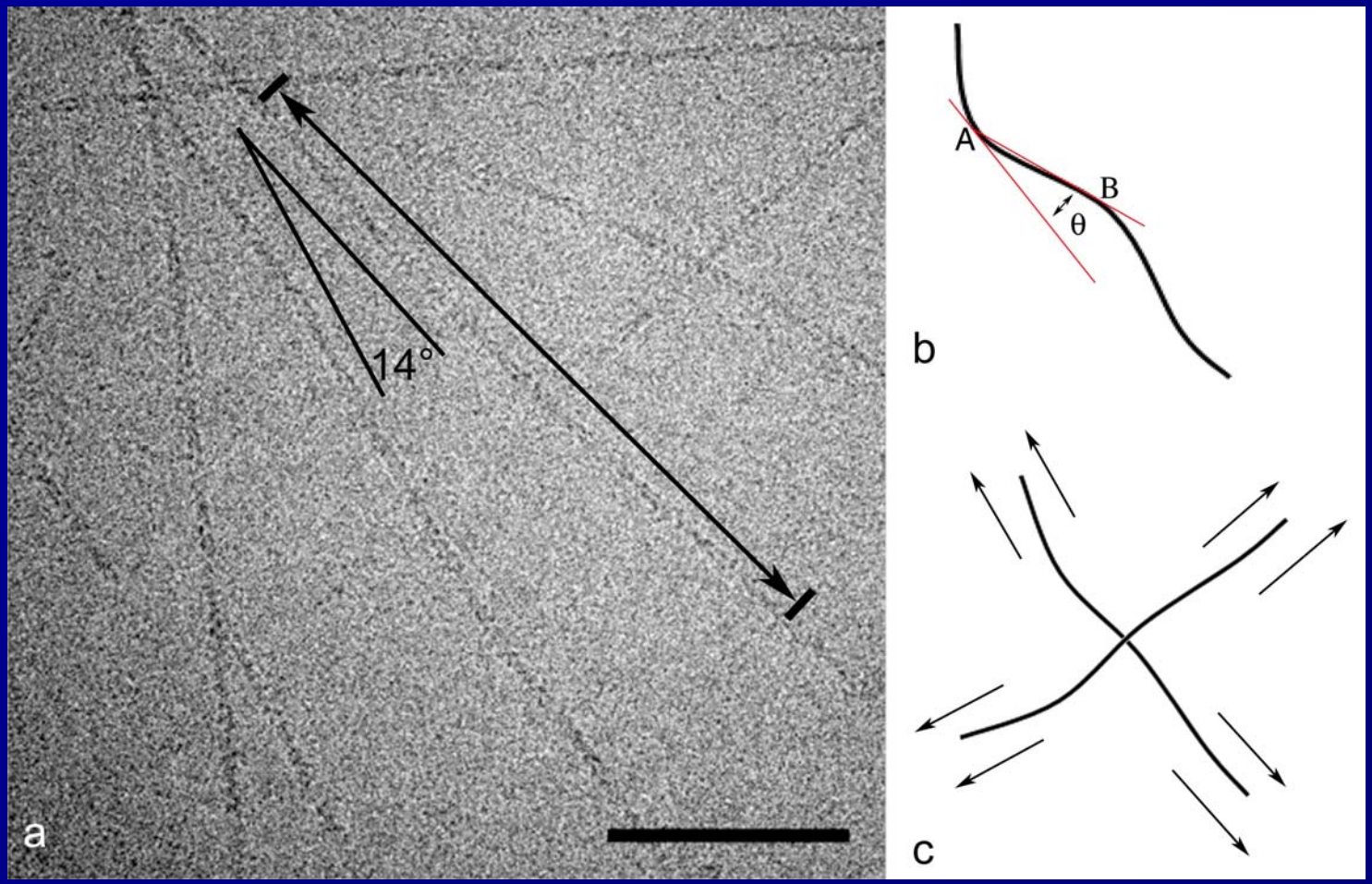


# Cryo-EM images are not snapshots of a solution!

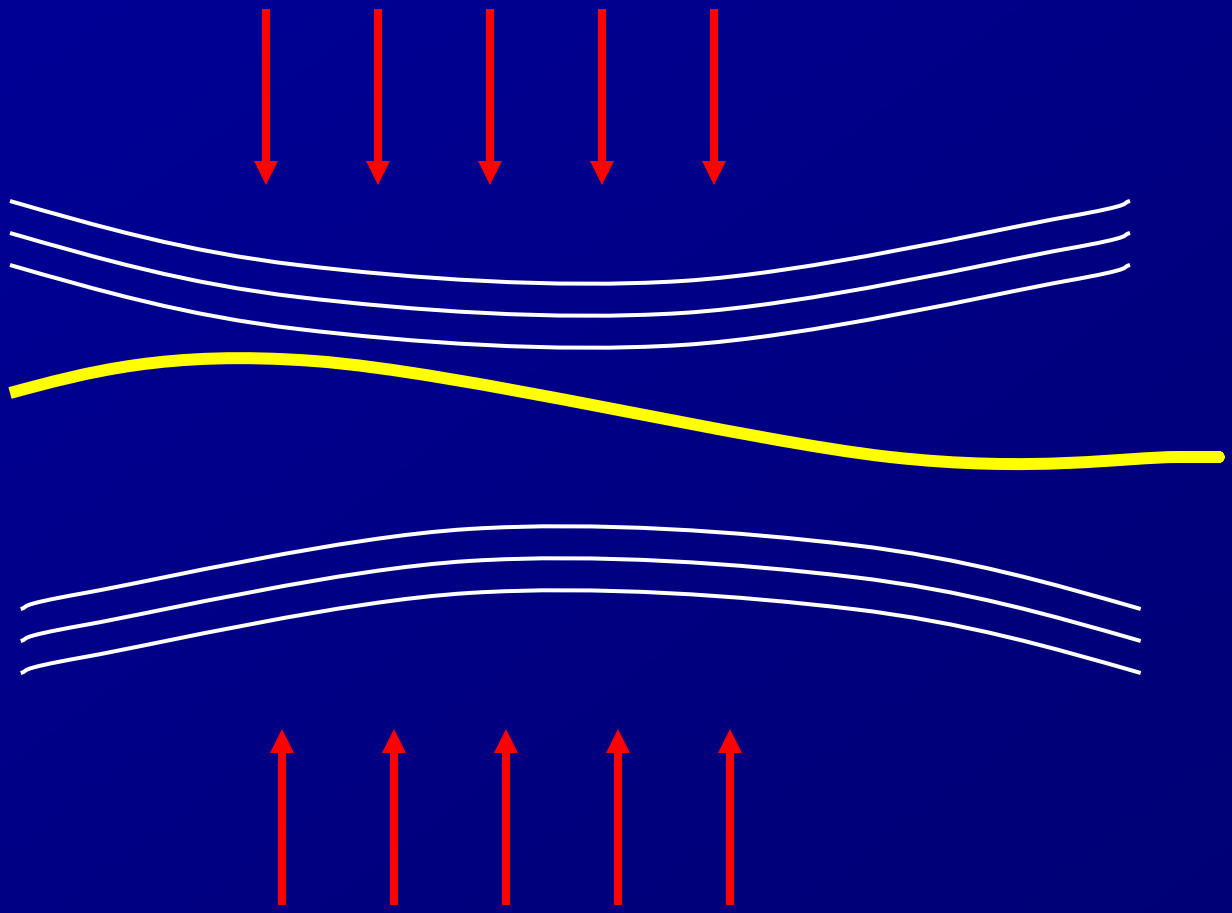
GroEL, N. Voss, Scripps



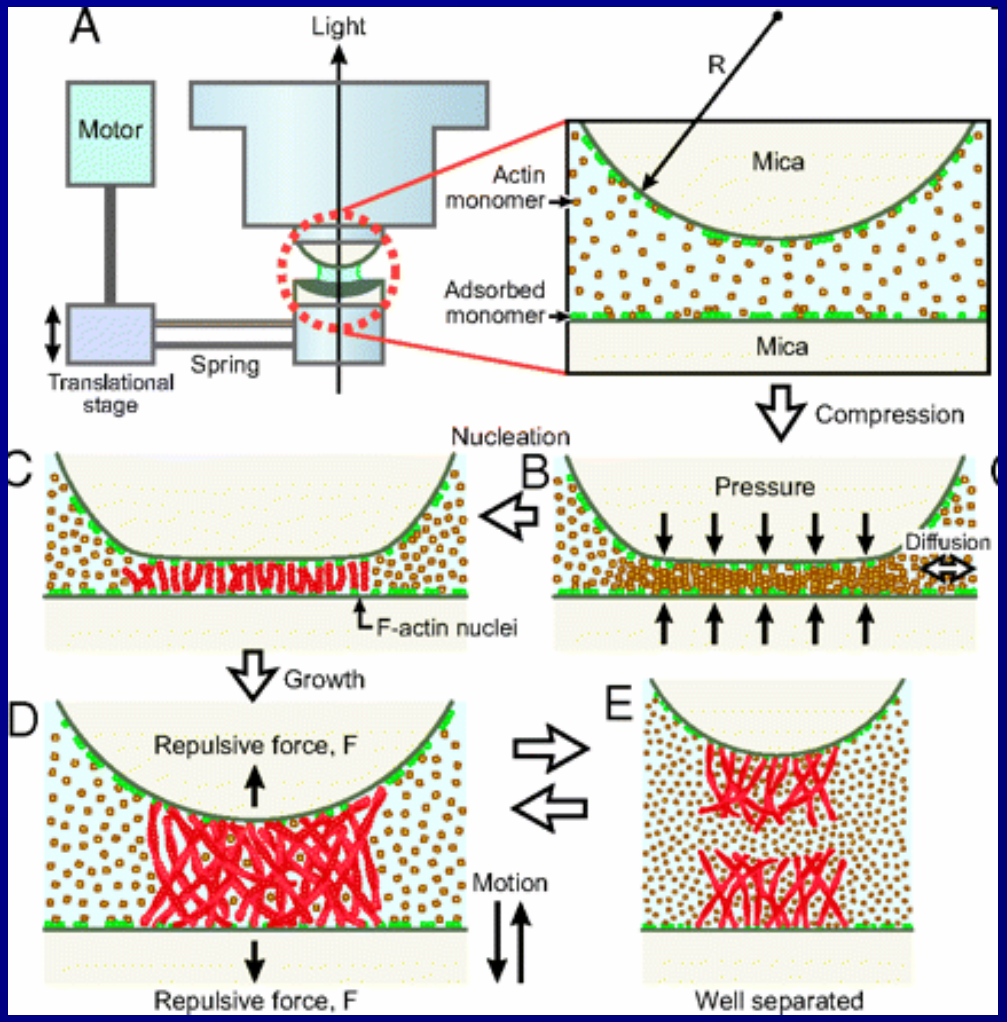
# Cryo-EM can introduce large forces due to fluid flow and surface tension



# Cryo-EM preparation can introduce large forces due to fluid flow and surface tension



# Apparatus to compress actin filaments




PNAS 106, 445-449 (2009)

# Actin filaments stiffen under compression

PNAS PNAS PNAS

## Force amplification response of actin filaments under confined compression

George W. Greene[a], Travers H. Anderson[b], Hongbo Zeng[b], Bruno Zappone[a,c], and Jacob N. Israelachvili[a,b,1]

[a]Materials Department and [b]Department of Chemical Engineering, University of California, Santa Barbara, CA 93106; and [c]Liquid Crystal Laboratory, CNR-INFM and Centro di Eccellenza CEMIF.CAL Università della Calabria, cubo 33B, Rende (CS) 87036

Contributed by Jacob N. Israelachvili, November 26, 2008 (sent for review July 8, 2008)

**Actin protein is a major component of the cell cytoskeleton, and its ability to respond to external forces and generate propulsive forces through the polymerization of filaments is central to many cellular processes. The mechanisms governing actin's abilities are still not fully understood because of the difficulty in observing these processes at a molecular level. Here, we describe a technique for studying actin–surface interactions by using a surface forces apparatus that is able to directly visualize and quantify the collective forces generated when layers of noninterconnected, end-tethered actin filaments are confined between 2 (mica) surfaces. We also identify a force-response mechanism in which filaments not only stiffen under compression, which increases the bending modulus, but more importantly generates opposing forces that are larger than the compressive force. This elastic stiffening mechanism appears to require the presence of confining surfaces, enabling actin filaments to both sense and respond to compressive forces without additional mediating proteins, providing insight into the potential role compressive forces play in many actin and other motor protein-based phenomena.**

on the filaments that prevents their total depolymerization, keeping the number and surface coverage density of filaments in the contact region essentially constant over the duration of a typical experiment (≈2–4 h). We were thus able to investigate the mechanical properties of and forces produced by the filament layers while also visualizing, in real time, their growth and fluctuation dynamics with angstrom-level resolution.

After filaments have been nucleated during the initial confinement, subsequent "approaches" and "separations" of the surfaces are invariably met with a repulsive force that is found to be significantly larger on separation (decompression) than on the prior approach (compression)—the opposite effect of typical hysteretic and viscous forces. A typical force–distance curve is shown in Fig. 2*A* for 2 surfaces approaching at a rate of 15 nm/s and then separated at the same rate. The increased intensity in the mean "background" repulsive (elastic) force on decompression than on approach is clearly seen.

The polymerization-driven fluctuations in the forces, $\Delta F_f$, lead to fluctuations in the distance, $\Delta D$, between the surfaces as they deflect the force-measuring spring (Fig. 1*A*) by $\Delta D = (\Delta F_f +$

PNAS 106, 445-449 (2009)

# Conclusions

- real space methods (IHRSR) offer many advantages over the traditional Fourier-Bessel approach for helical reconstruction
- but helical symmetry can be ambiguous, and this is as true for Fourier-Bessel methods as IHRSR
- most helical protein polymers suffer from variability
- this can be addressed with real space methods