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SAXS Applications 2: Biological Non-Crystalline Diffraction

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SAXS: What is it?

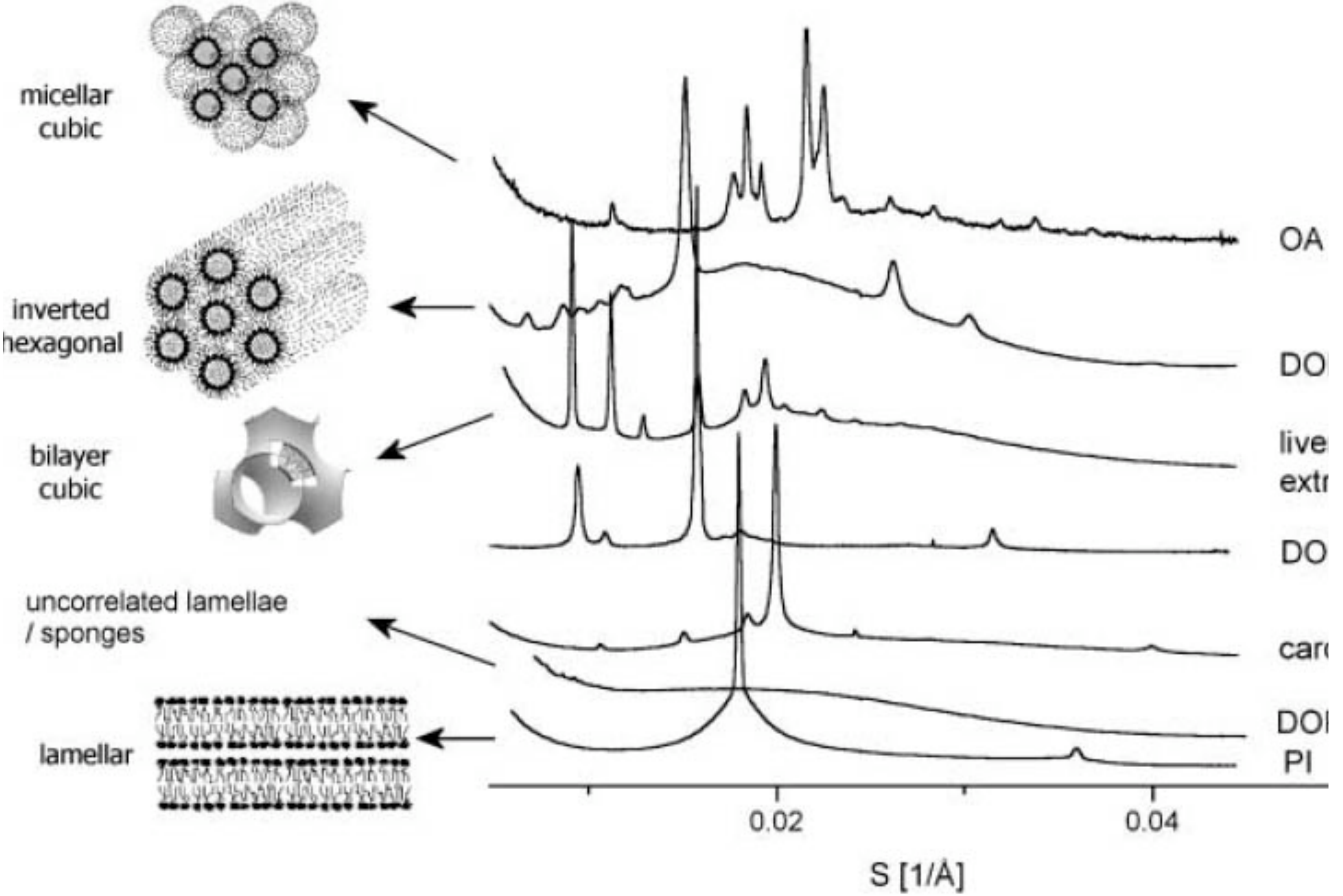
- "Small-angle X-ray Scattering"
- Often used to refer to a continuum of techniques not all of which particularly small angle!
- A better term might be "Non-Crystalline Diffraction" (NCD)
- Seems to be some moves to reserve SAS for" Small Angle Solution Scattering"

Dimensional Hierarchy of Biophysical (X-ray) techniques

1D	Low angle solution or powder diffraction
	large macromolecular assembles, model membrane systems
2-3D	Fiber diffraction
	fiber forming arrays – muscle, collagen, DNA, amyloids, various carbohydrates, often *super-macromolecular scale*
3D	Single crystal X-ray diffraction
	anything that can crystallize, must be (initially) soluble, usually relatively small in comparison to the above, *molecular to macromolecular scale*

Model Membrane Systems

- Usually (but not always!) a 1D problem
- Biophysics of membrane fluidity
- Effects of cholesterol, temperature, pressure
- Studies membrane fusion
- Understanding phase behavior for novel drug, DNA delivery systems



vsky et al. 2004 Biophys. J. 87:1054

Why Fiber Diffraction ?

- Atomic level structures from crystallography or NMR = "gold standard" for structural inferences

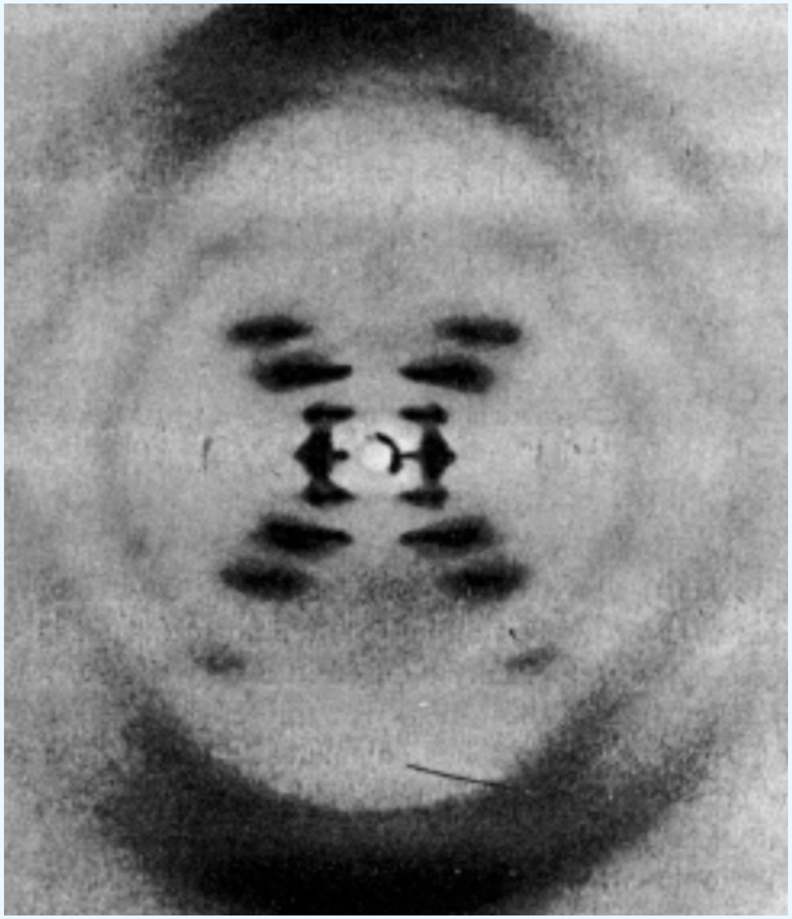
- But there is a large class of "fibrous proteins"

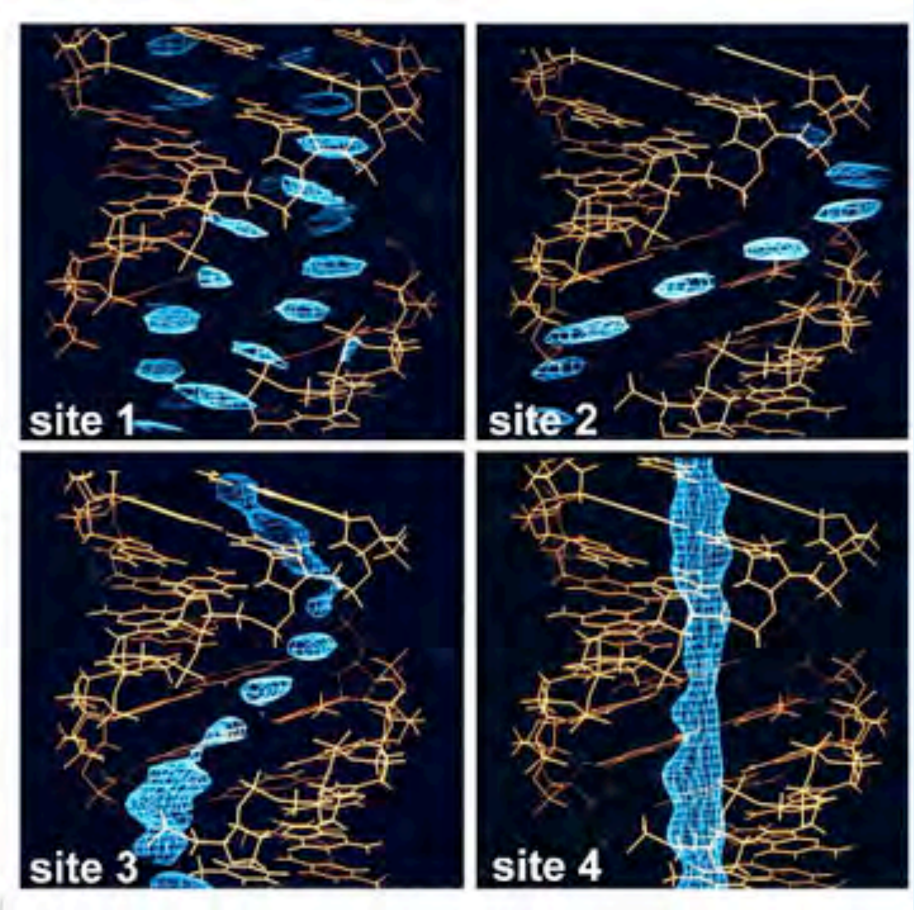
  e.g: Actin, myosin, intermediate filaments, microtubules, bacterial flagella, filamentous viruses, amyloid, collagenous connective tissue

- Will not crystallize but can be induced to form oriented assemblies

- Some systems *naturally* form ordered systems

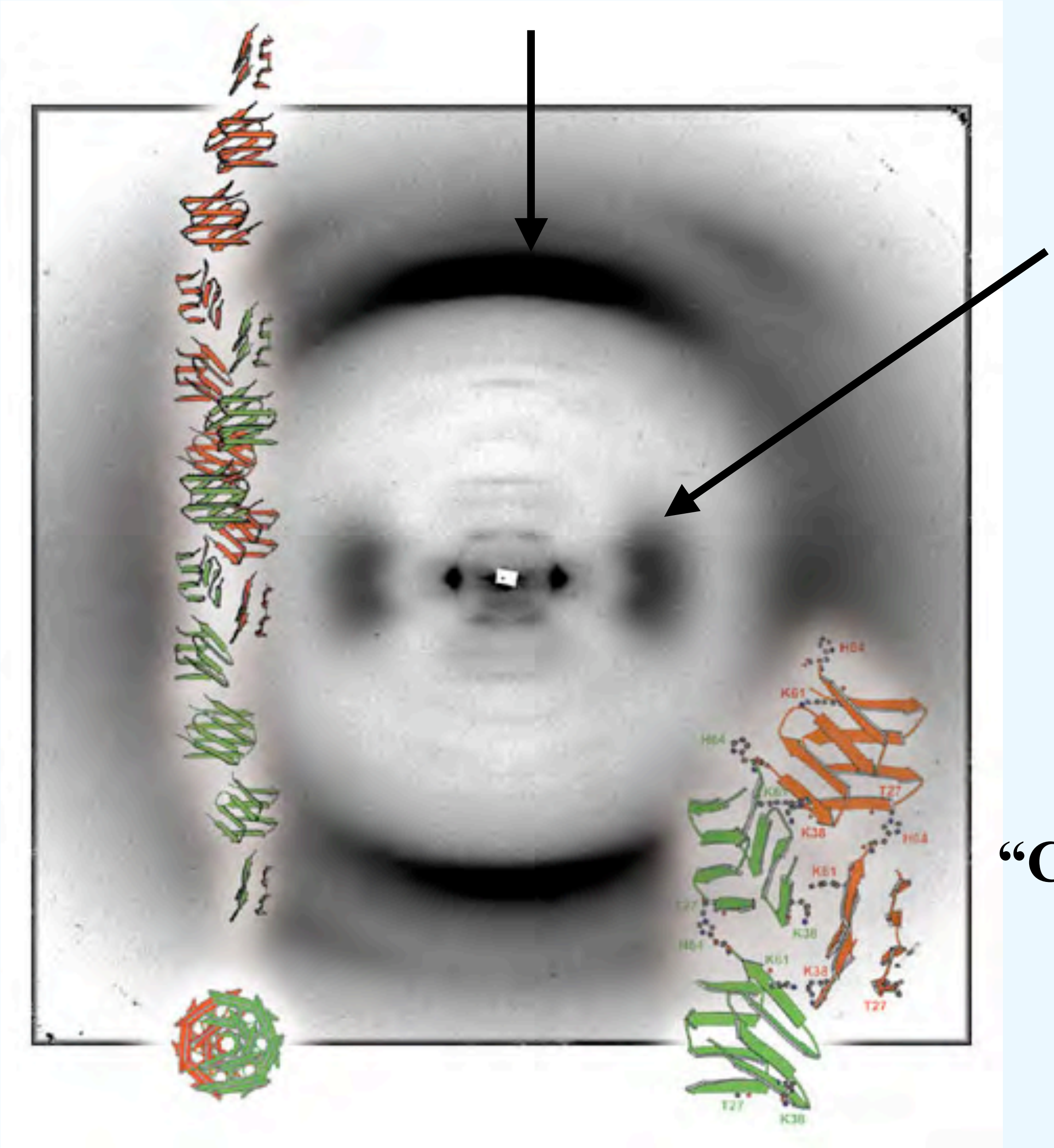
Rosalind Franklin’s Pattern from B-DNA





Franklin & Gosling, 1953 Nature 171:740

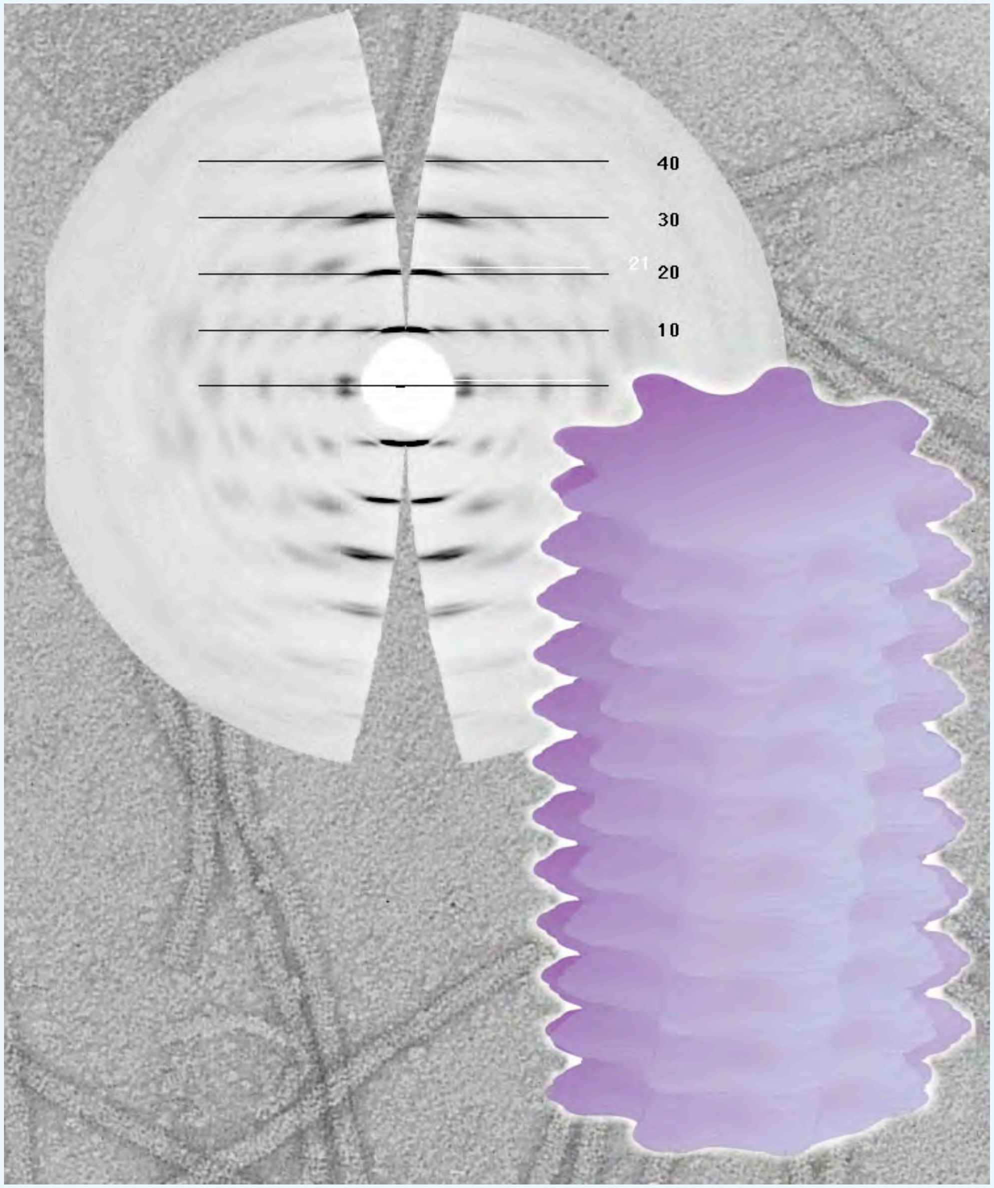
Engineered amyloid fiber



~ 4.7Å

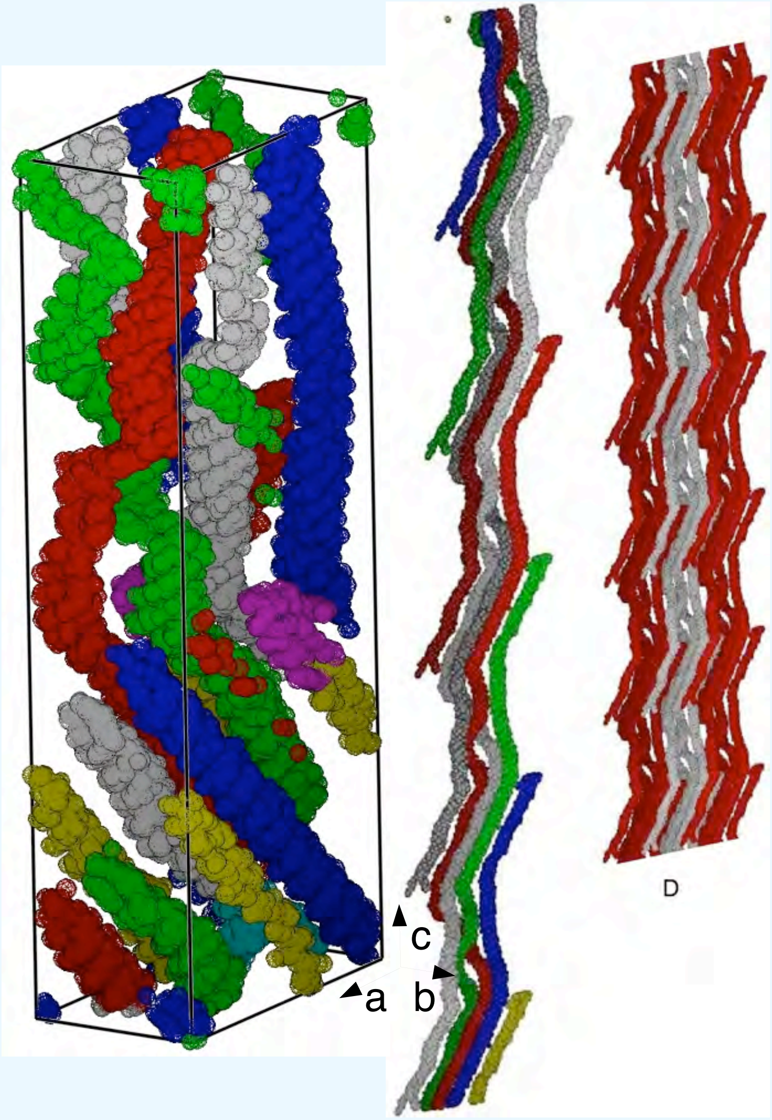
~10Å

“Cross-beta” Structure

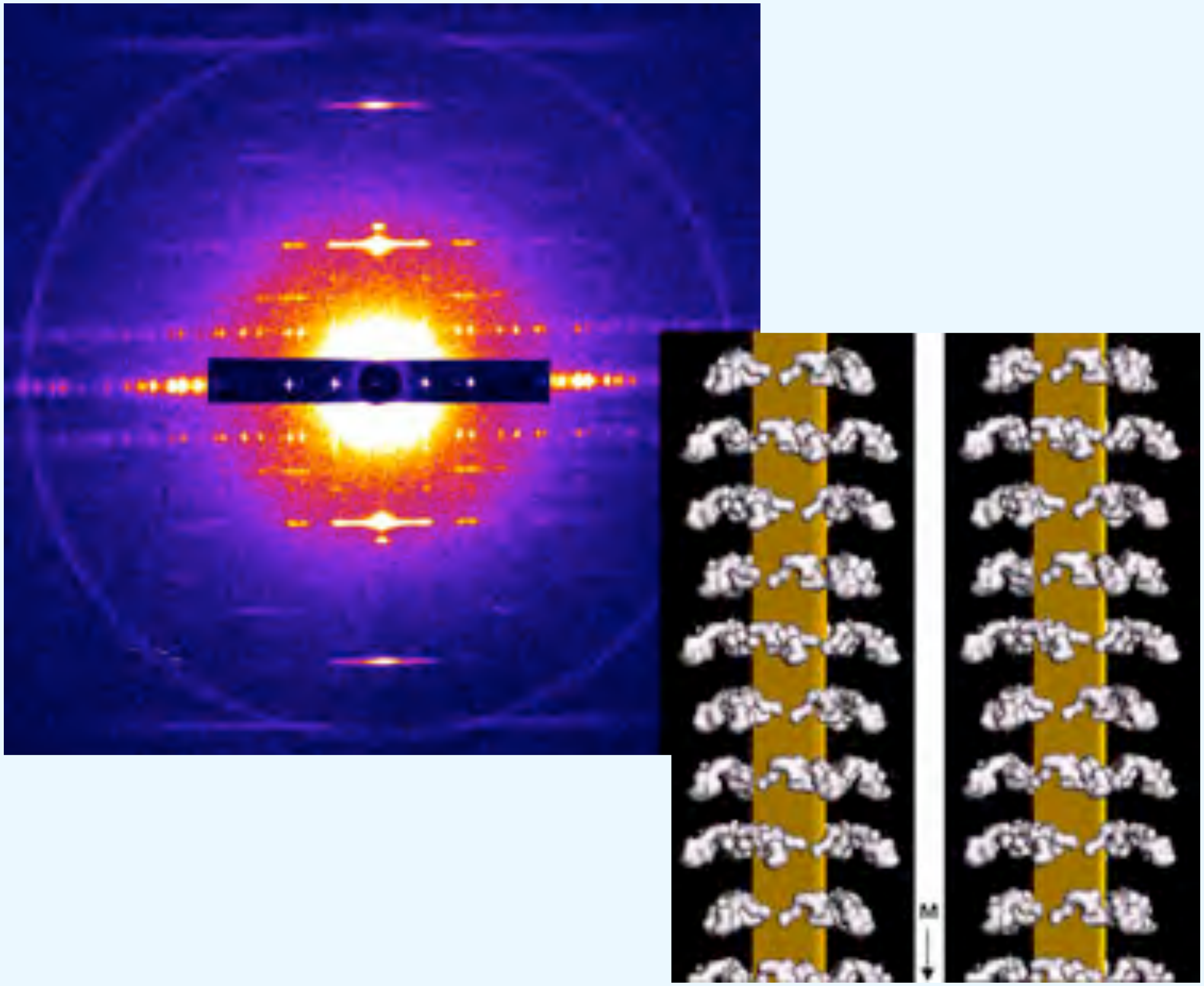


Potato virus X

Type I collagen



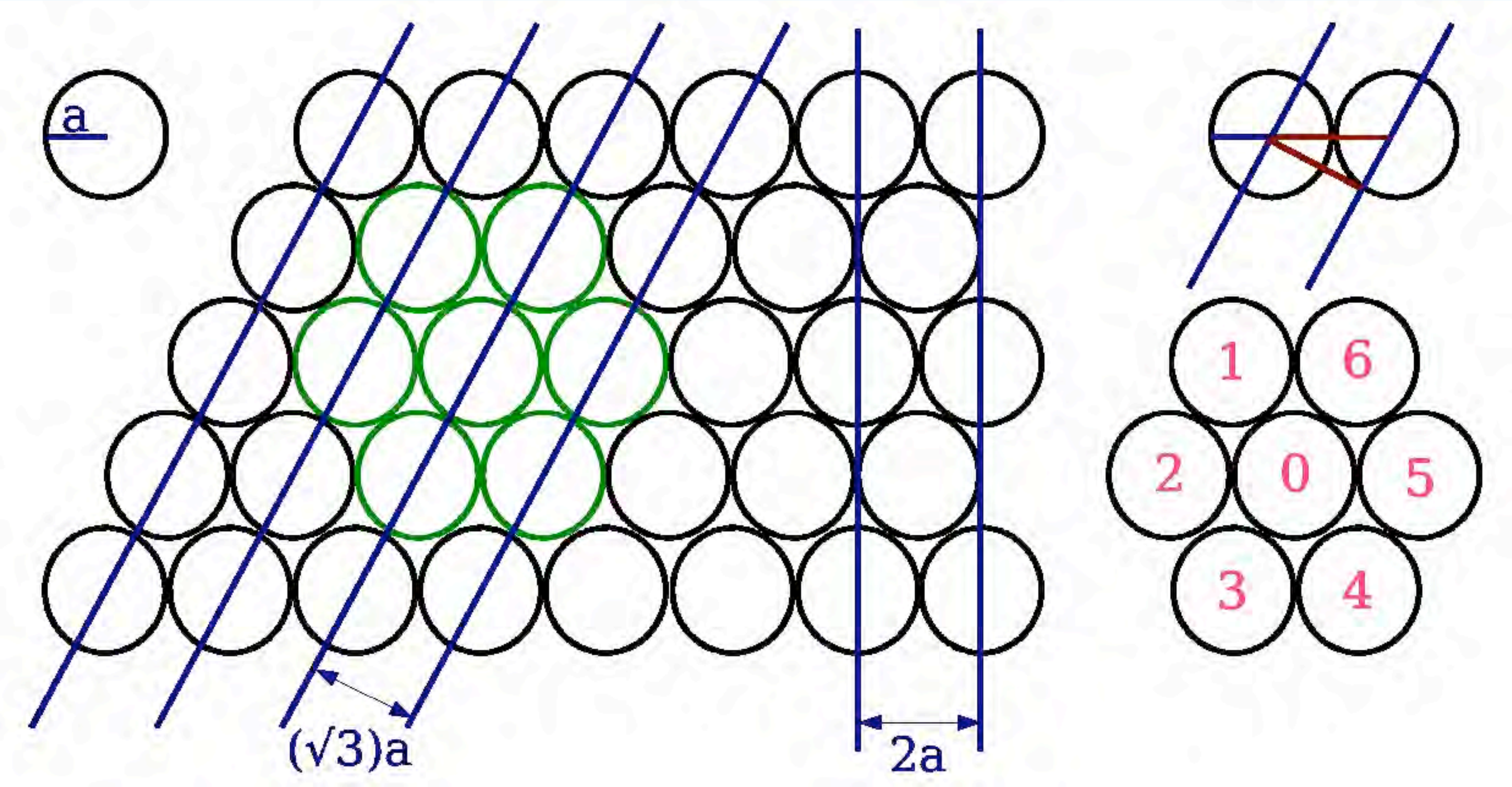
Insect flight-muscle



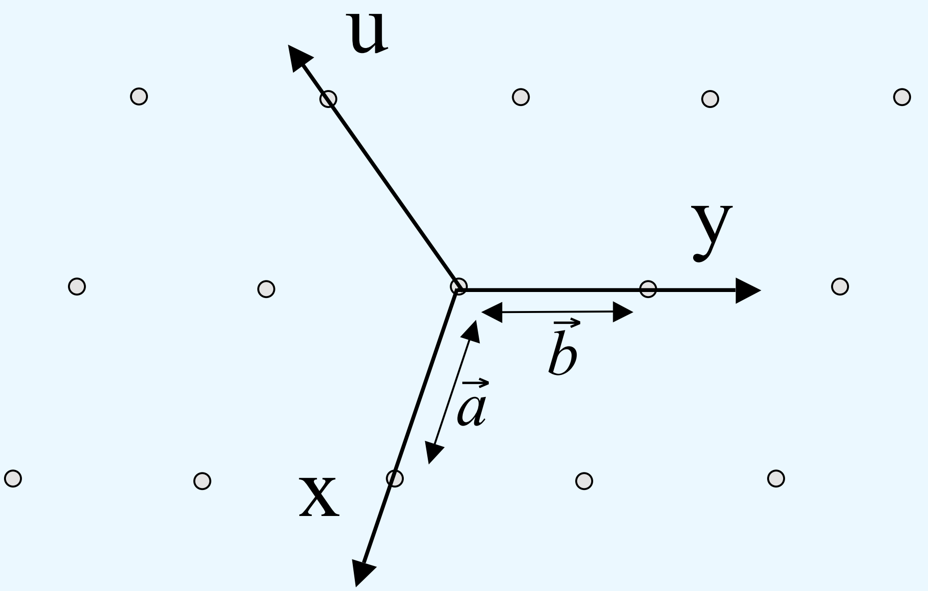
Principles I

Packing of Fibers and Diffraction

Fibers (essentially rods/cylinders) Usually Hexagonally Packed

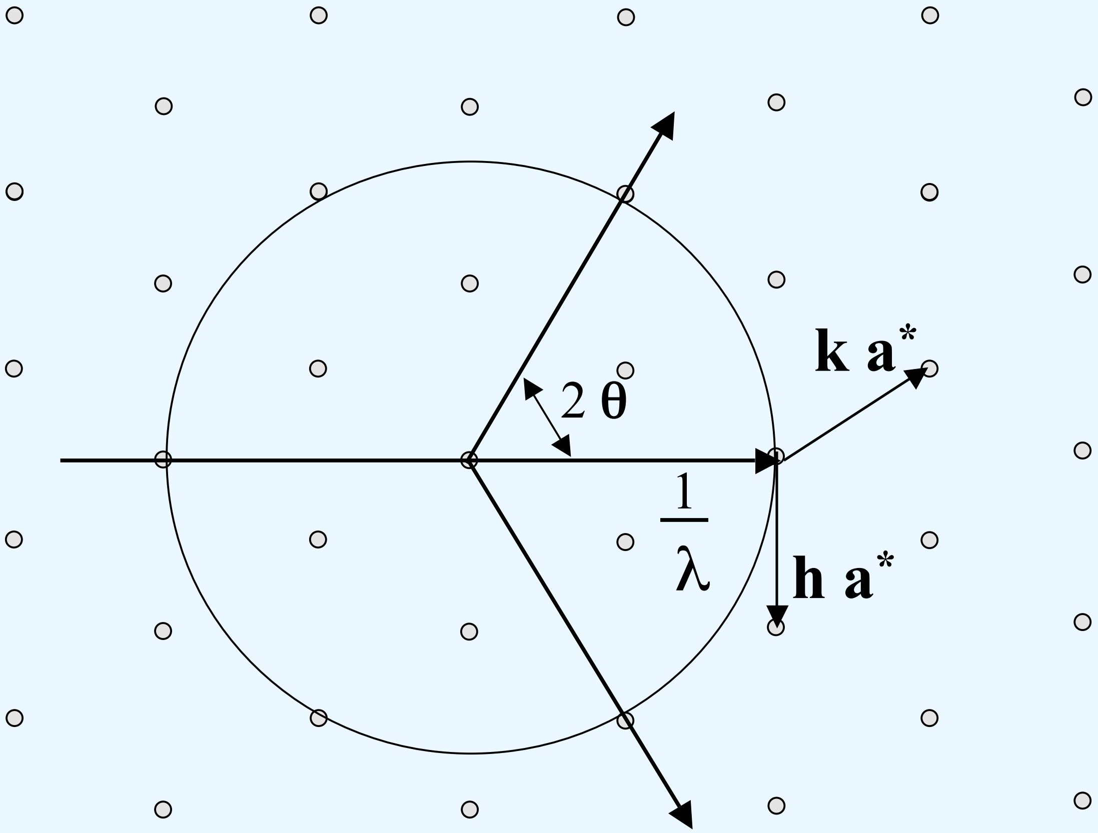


Hexagonal Lattice variables

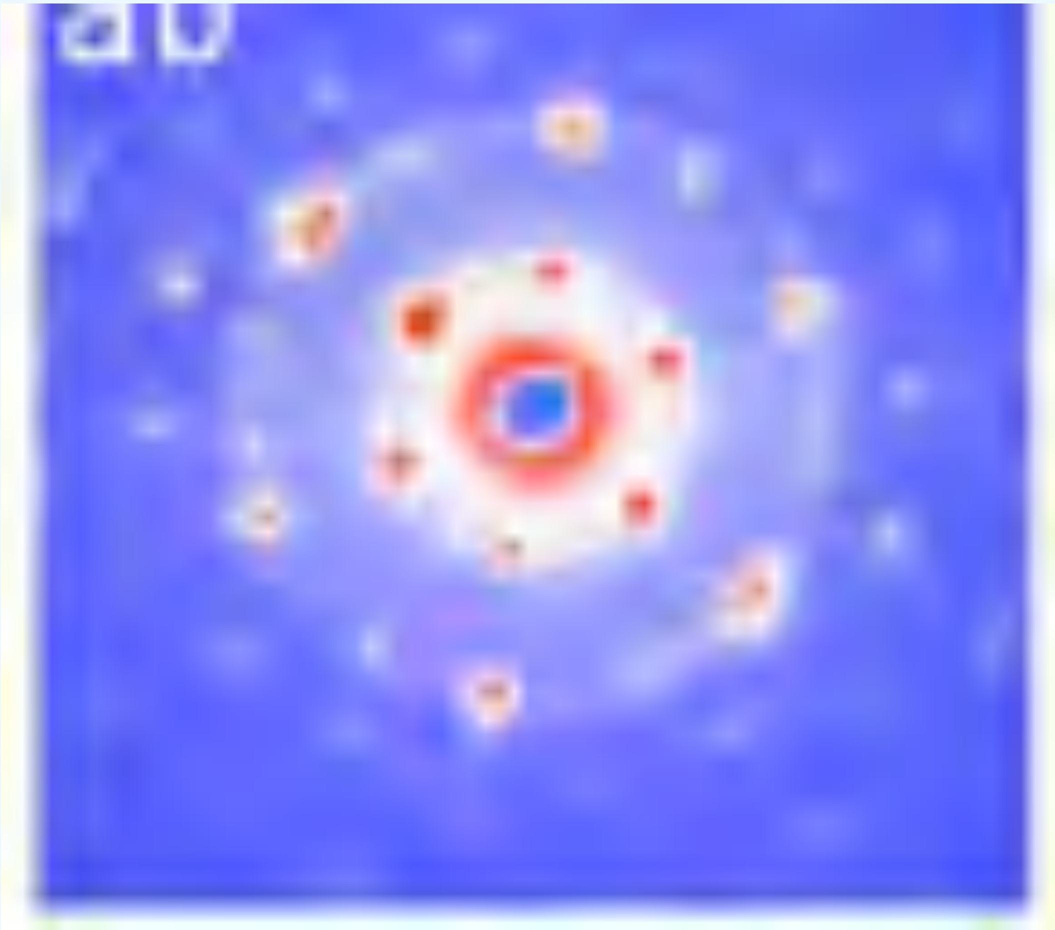


$$\vec{a} = \vec{b}$$

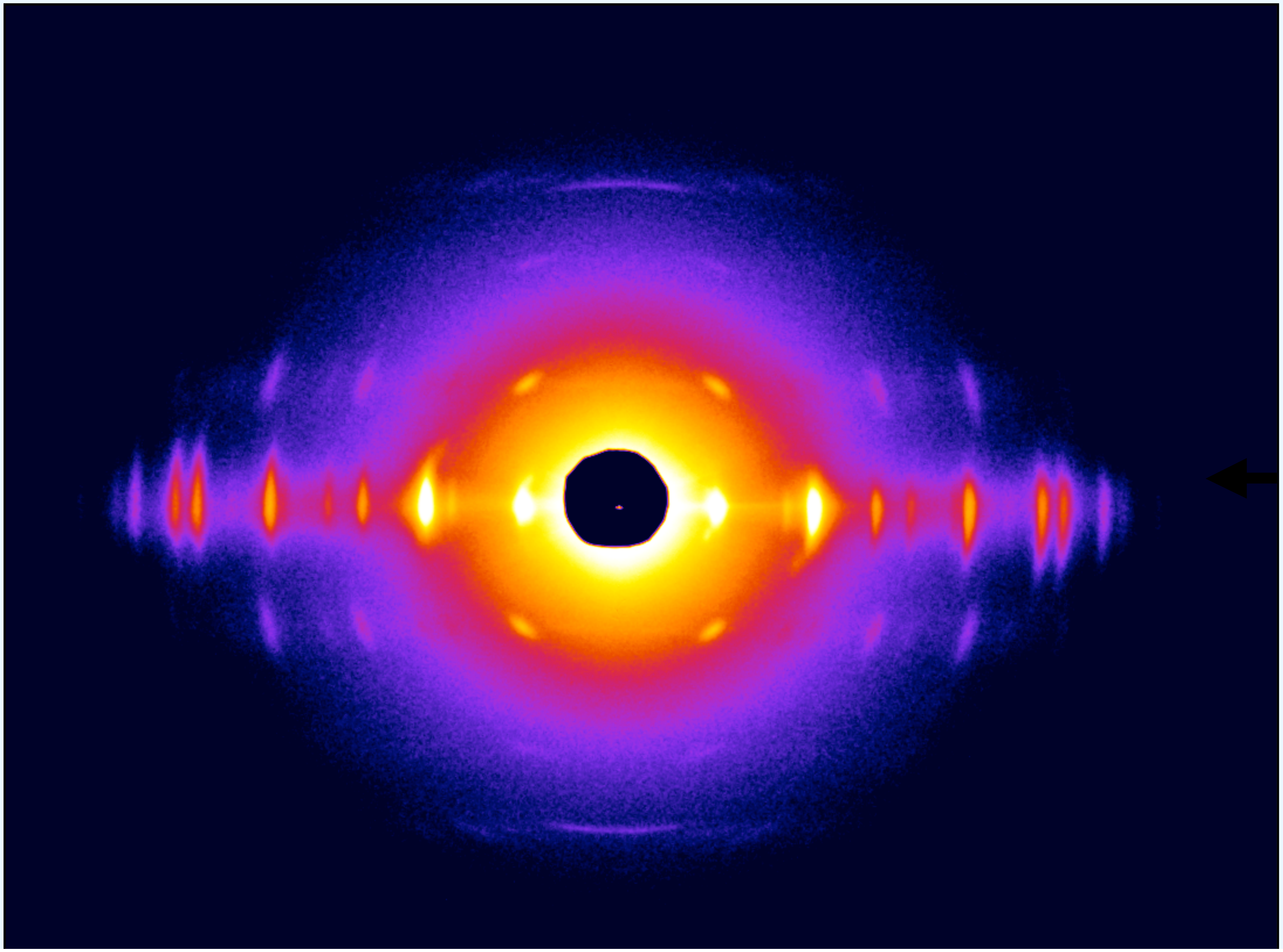
Ewald Sphere



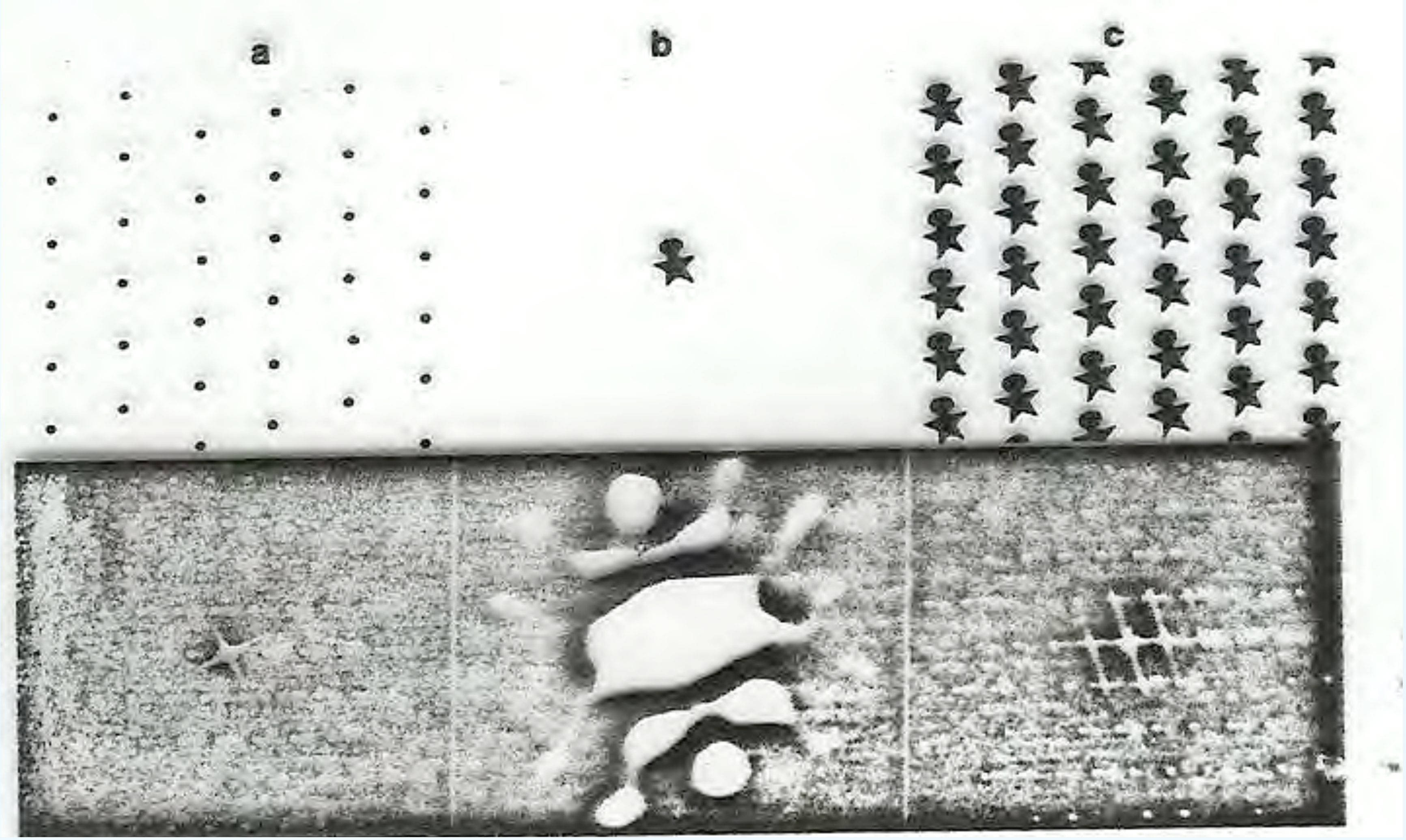
End on view of hexagonal reciprocal lattice



Fiber diagram - Insect Muscle (hexagonal lattice)



Equator

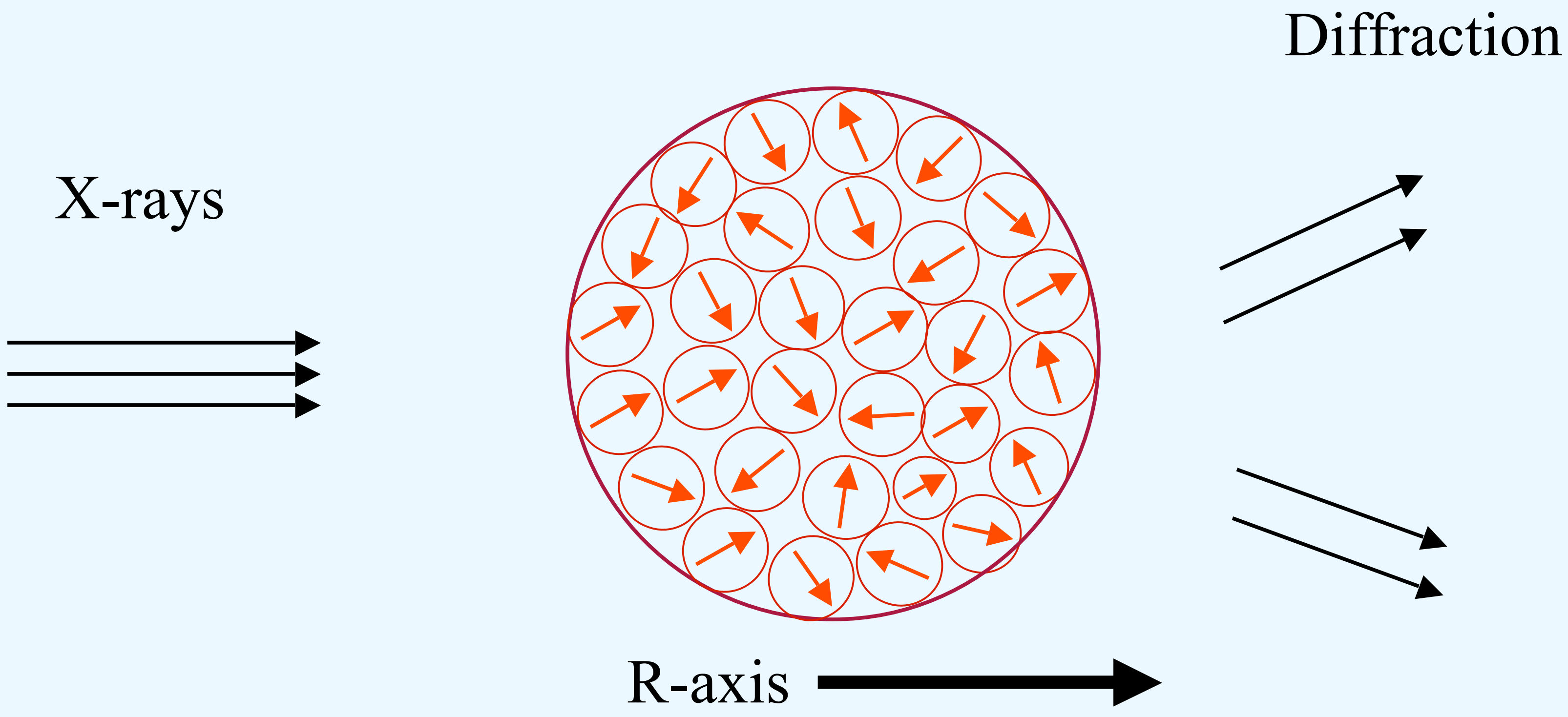


$$I = |F_M F_L|^2$$

Principles II

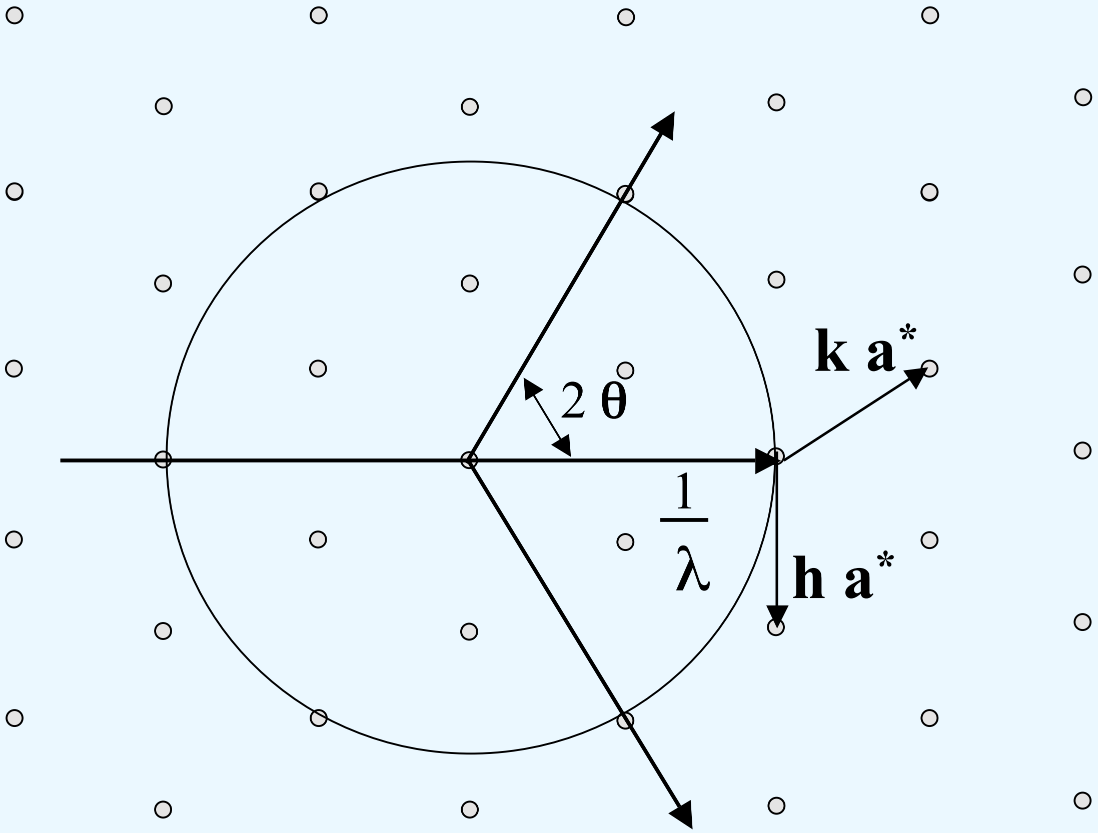
Cylindrical Convolution effects

Fiber Cross-section



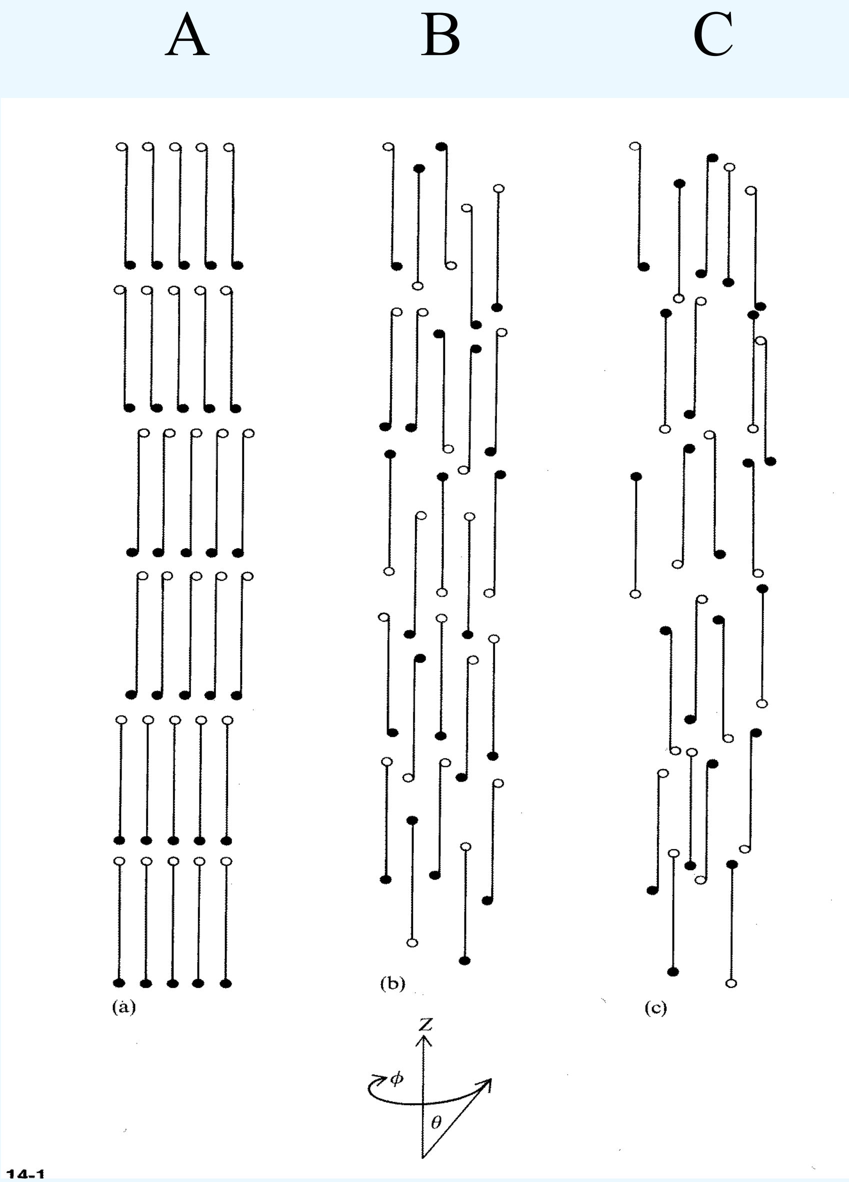
Crystallites randomly orientated around the axis perpendicular to the fiber axis (the ‘**R**’ – axis: Sum of Rotation of crystallites assumed = 360 degrees)

Ewald Sphere



Principles III

Order and disorder in fibrous specimens



Ordering in Fibers:

A - Crystalline fiber

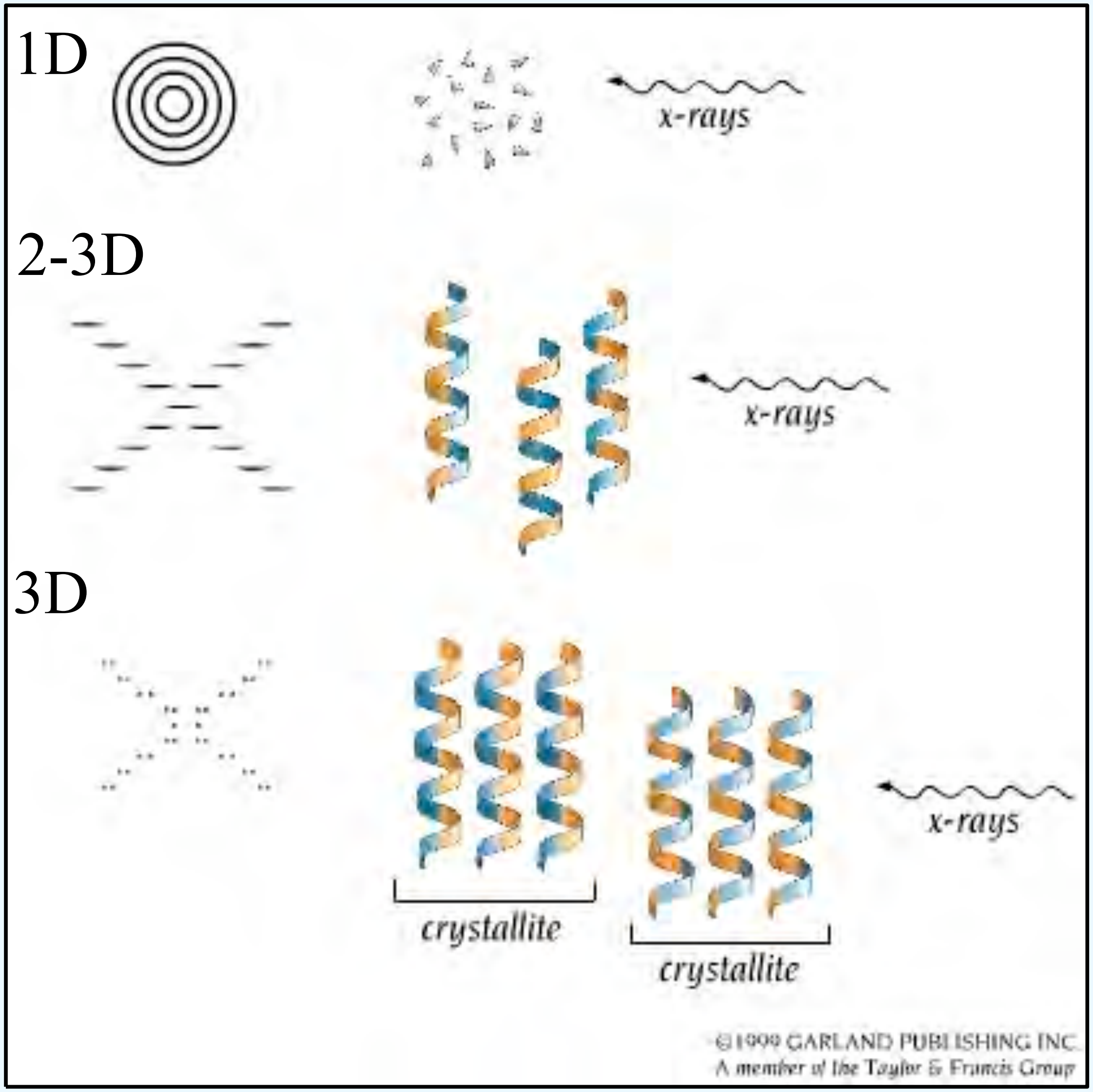
B Semicrystalline Fiber

C Non-crystalline fiber

$$\langle I(s)\rangle = \langle |F_m(S)F_L(S)|^2\rangle$$

Average over all molecular and lattice orientations

Dimensional hierarchy of NCD patterns



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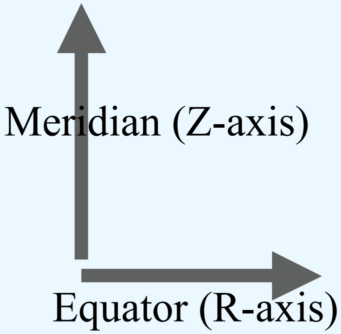
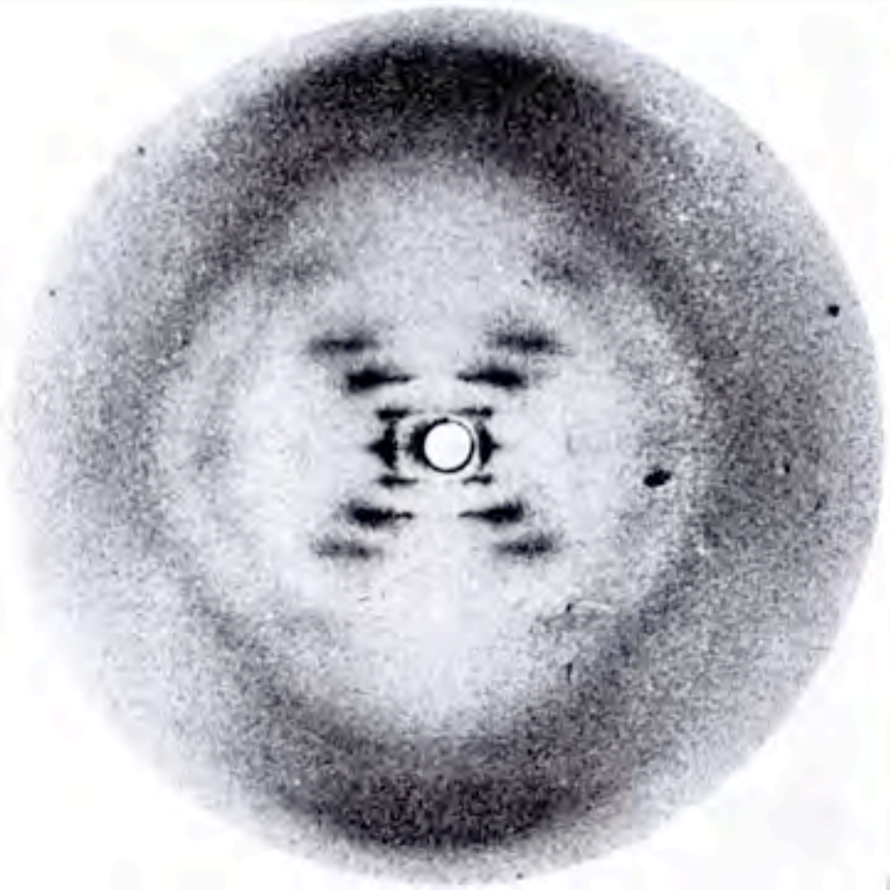
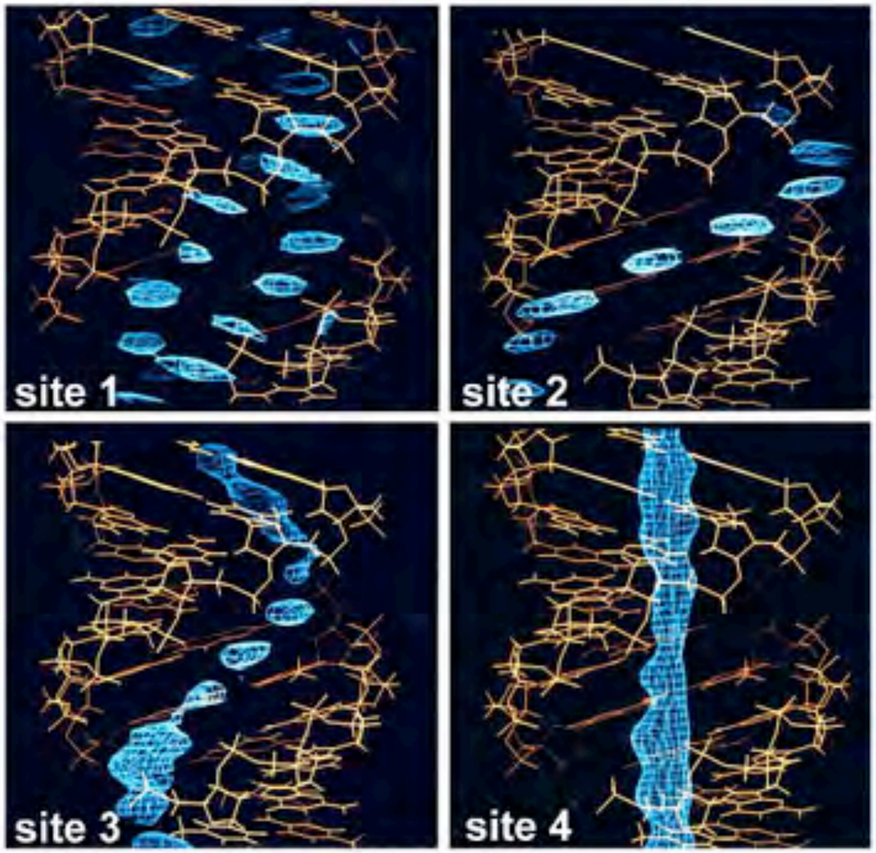

Meridian (Z-axis)

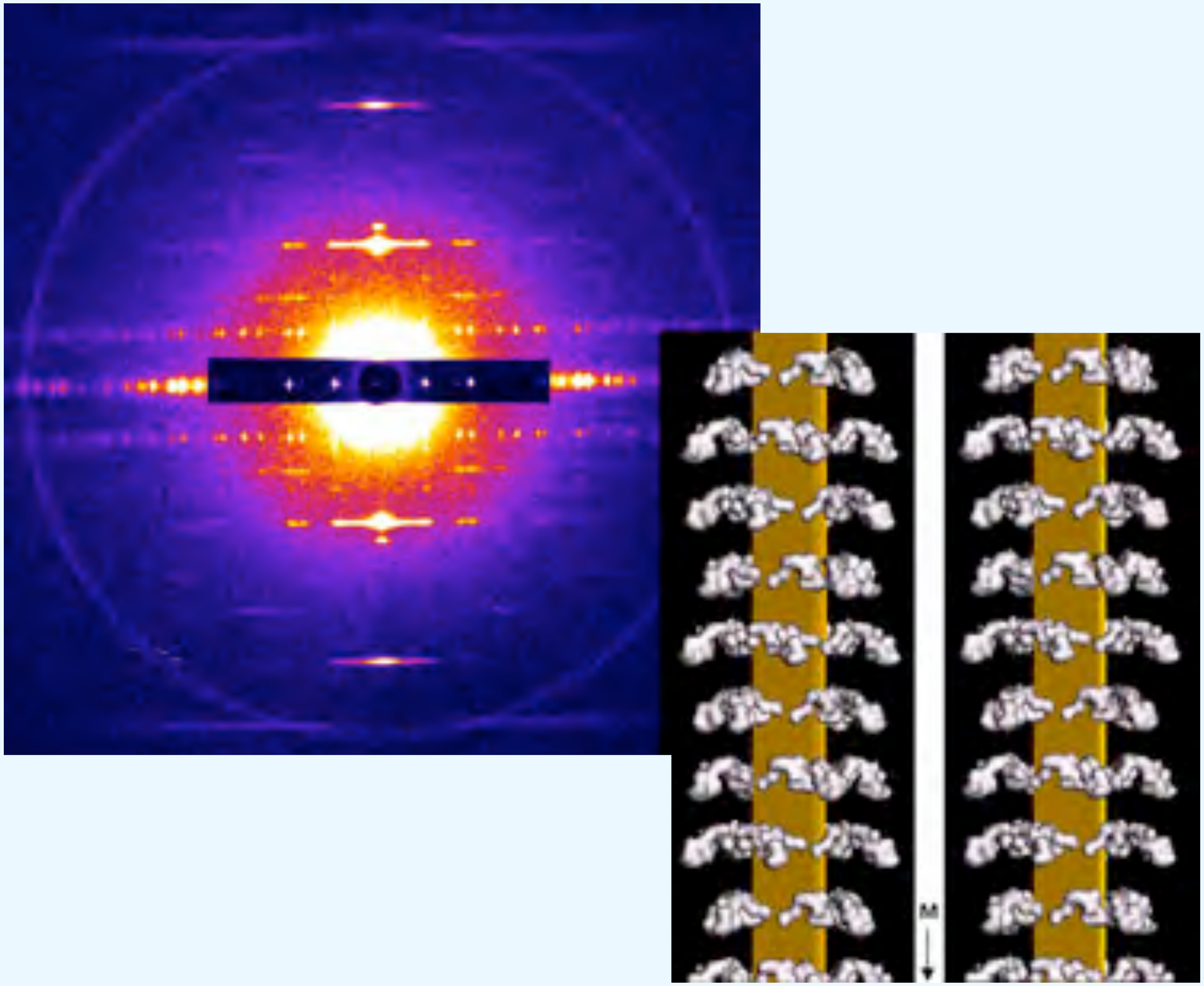
Equator (R-axis)

B-form DNA





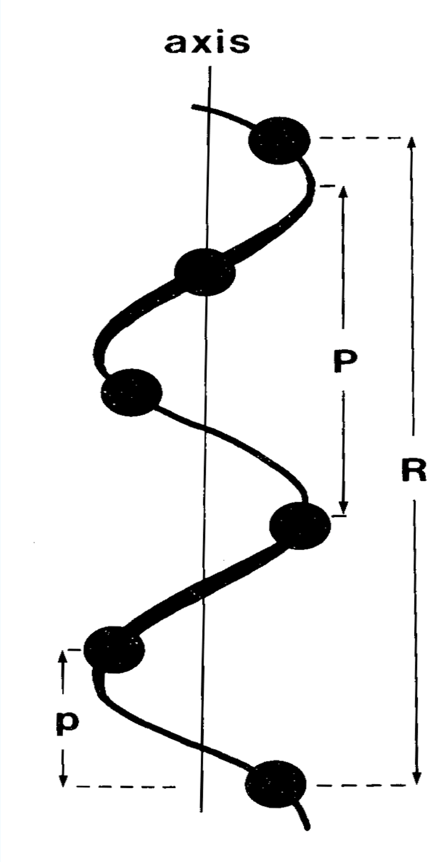
Insect flight-muscle



Principles IV

Helical diffraction theory

Fibrous Proteins Usually Show Helical Symmetry

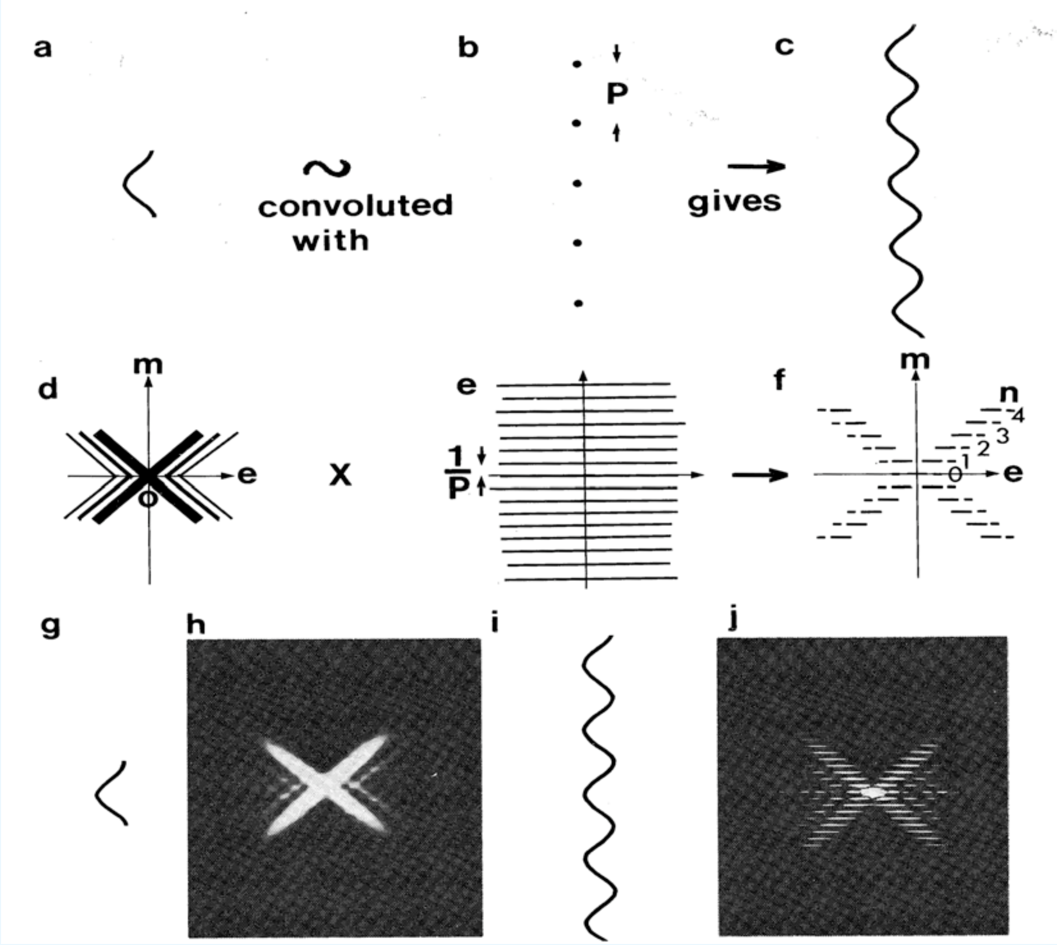


P = pitch

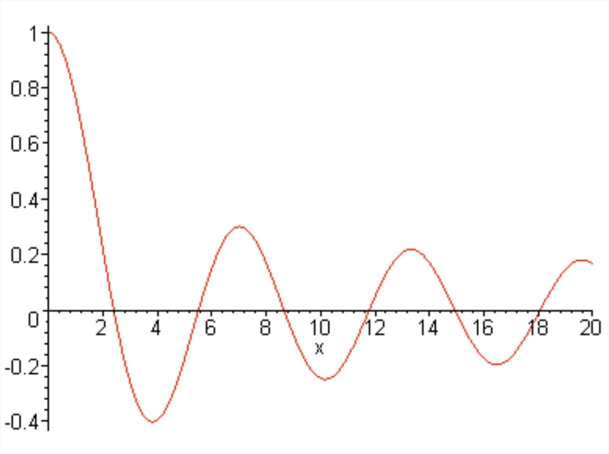
p = subunit axial translation distance

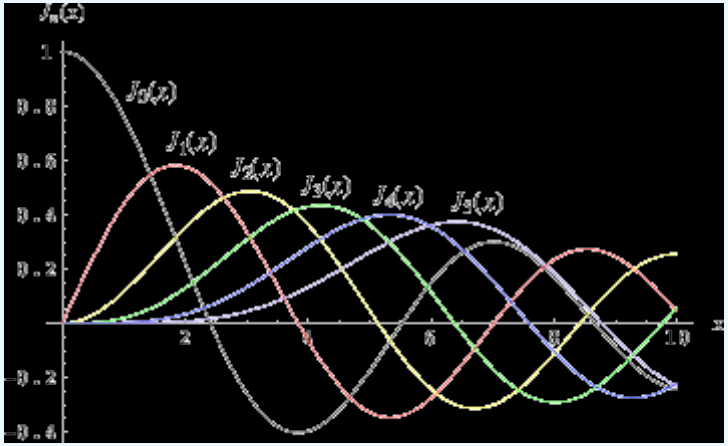
R = true repeat distance

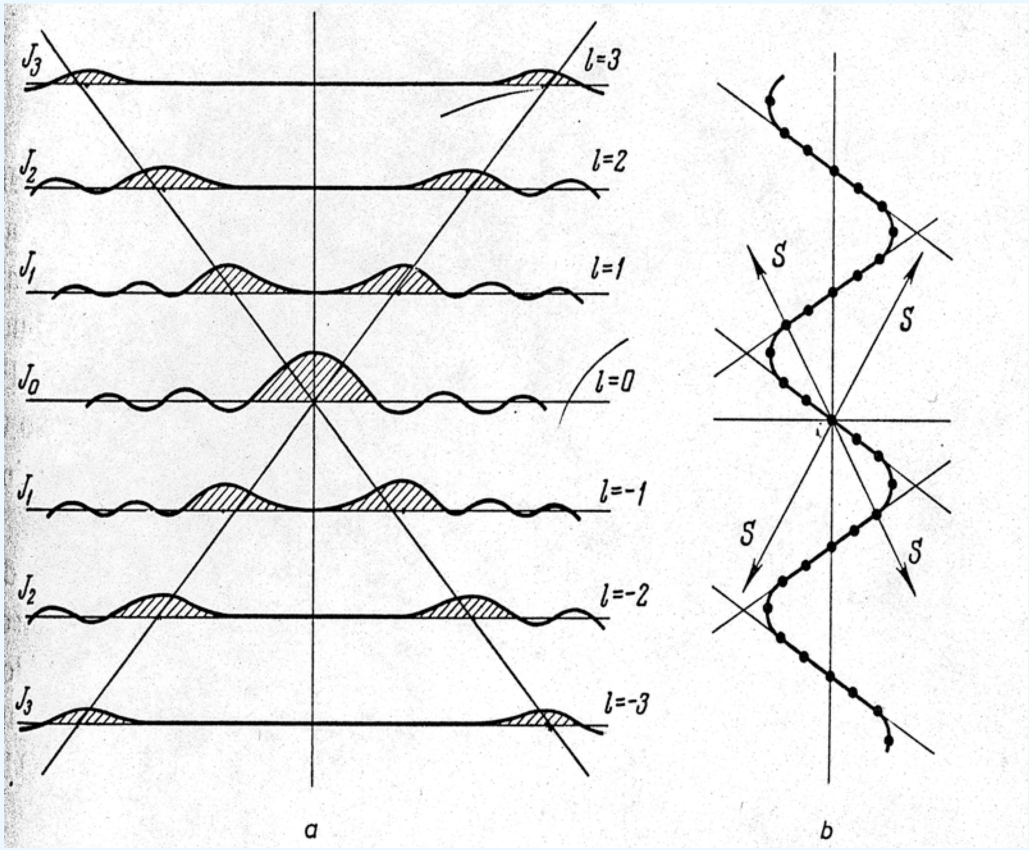
Diffraction from a continuous Helix



Bessel Functions and Layer lines

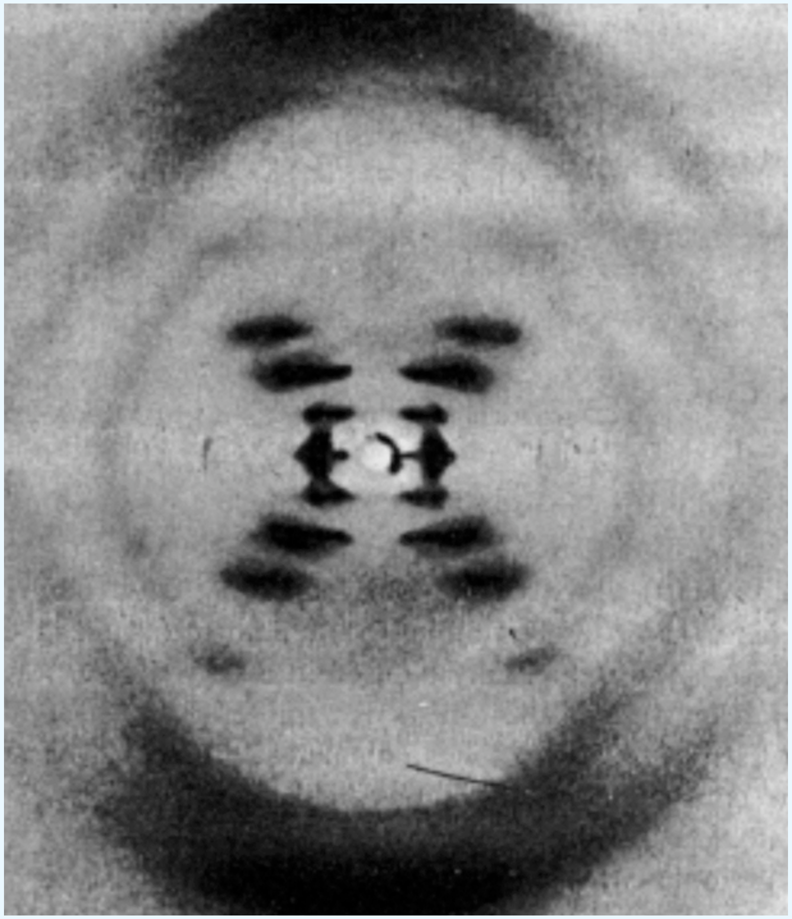






Transform of a cylinder

Rosalind Franklin’s Pattern from B-DNA

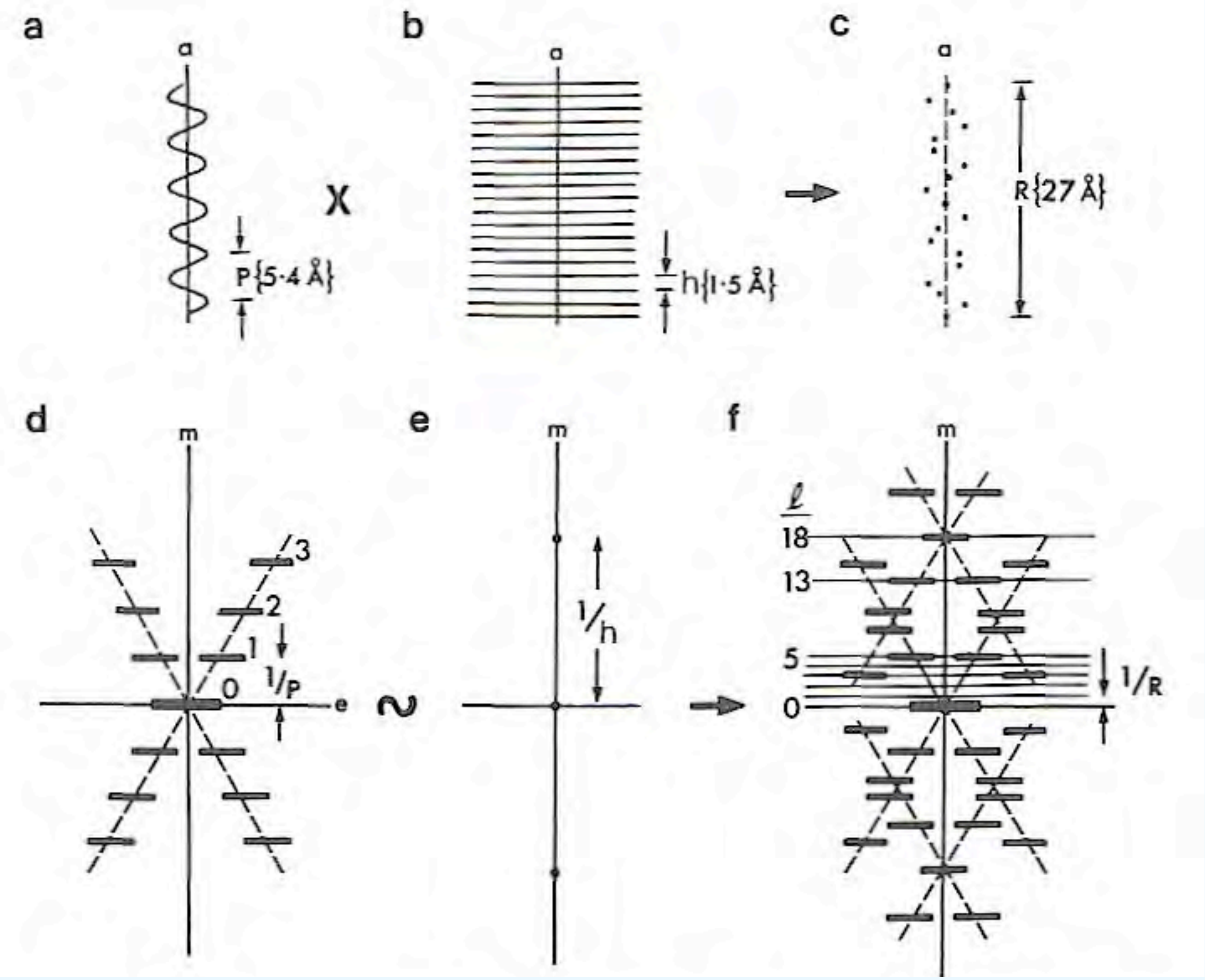


Franklin & Gosling, 1953 Nature 171:740

Discontinuous Helices

A set of points that are regularly spaced along a helical path

Diffraction From a Discontinuous Helix



α- helix

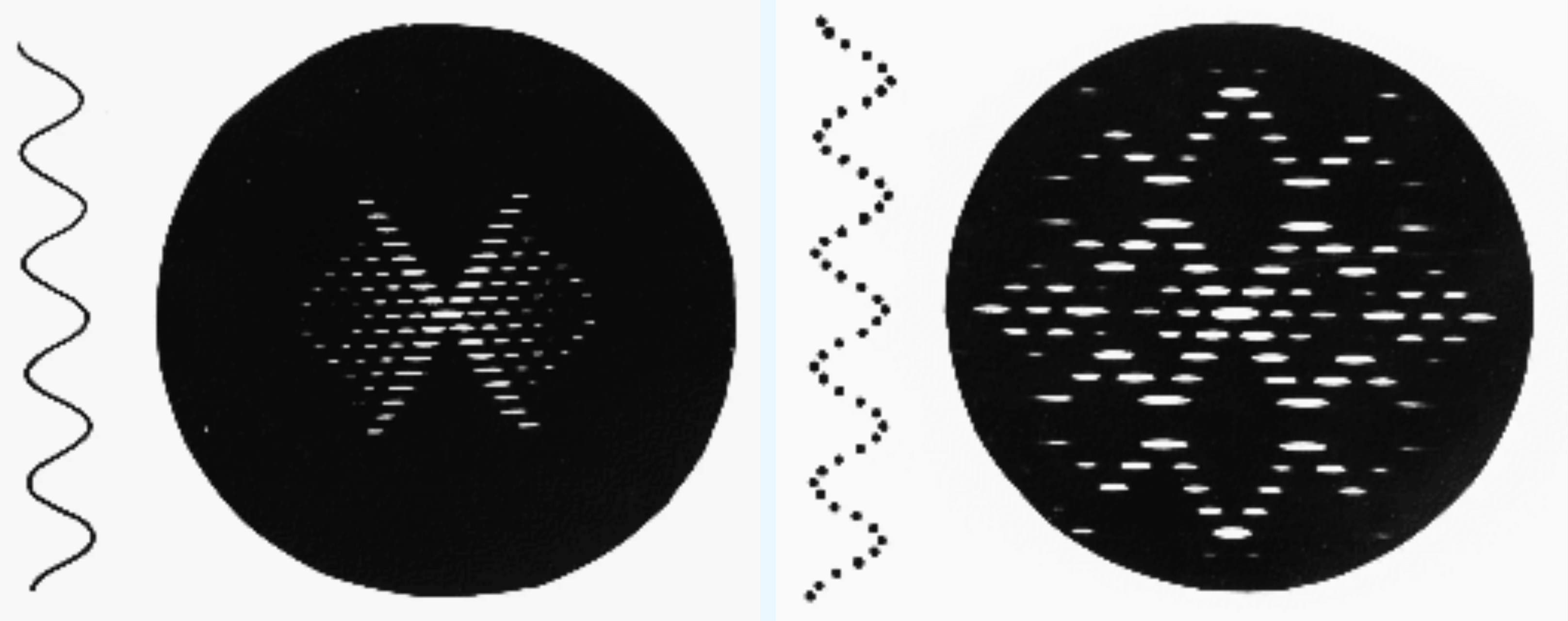
18 subunits/5 turns

h=1.5 Å

Pitch =5.4 Å

R= 27 Å

Diffraction from a helix: comparison



The main effect of shifting from a continuous to a discontinuous helix is to introduce new helix crosses with their origins displaced up and down the meridian by a distance 1/*p*

Helical Selection Rule

Which Bessel function order will turn up on what layer line for a more complicated helix?

For a non-integral helix (repeats after two or more turns), with **u** subunits in **t** turns, allowed Bessel functions (n) on layer line ***l*** are:

$$l = m.u + n.t$$

m is an integer indicating translational periodicity index of helix lattice

Integral / Non-integral helices

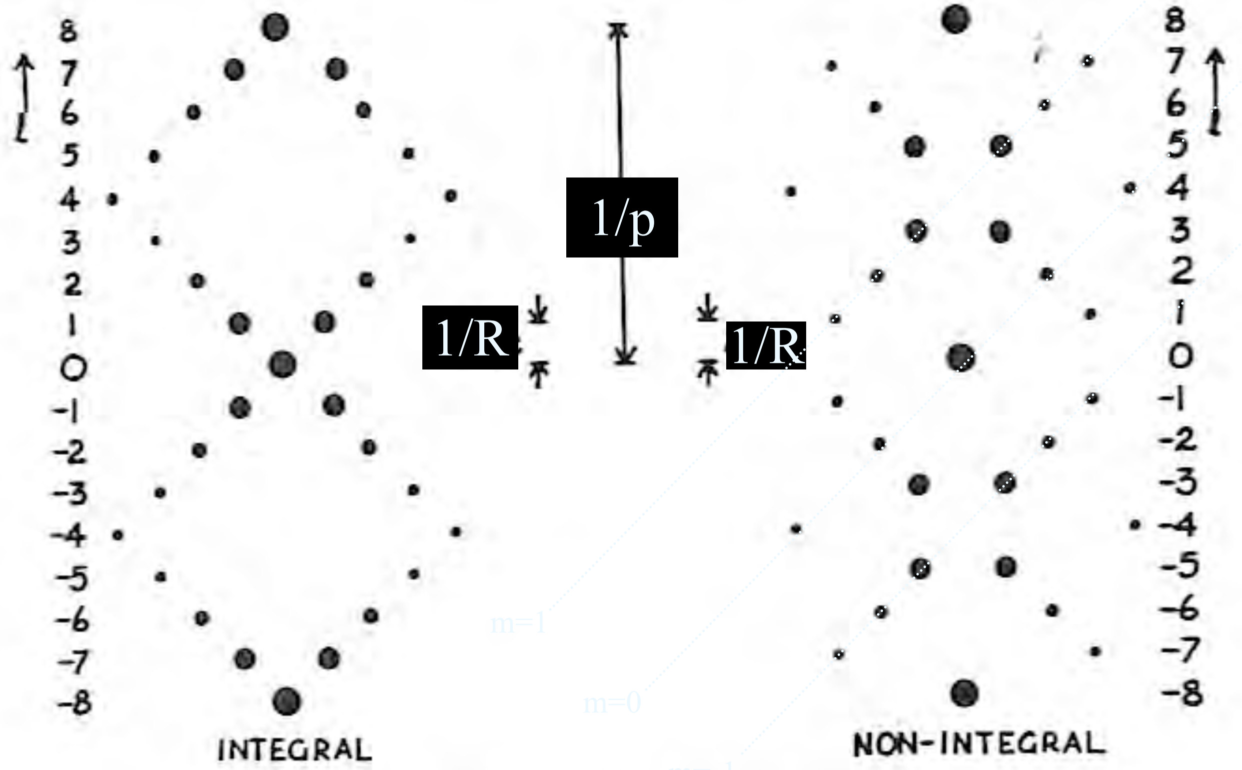
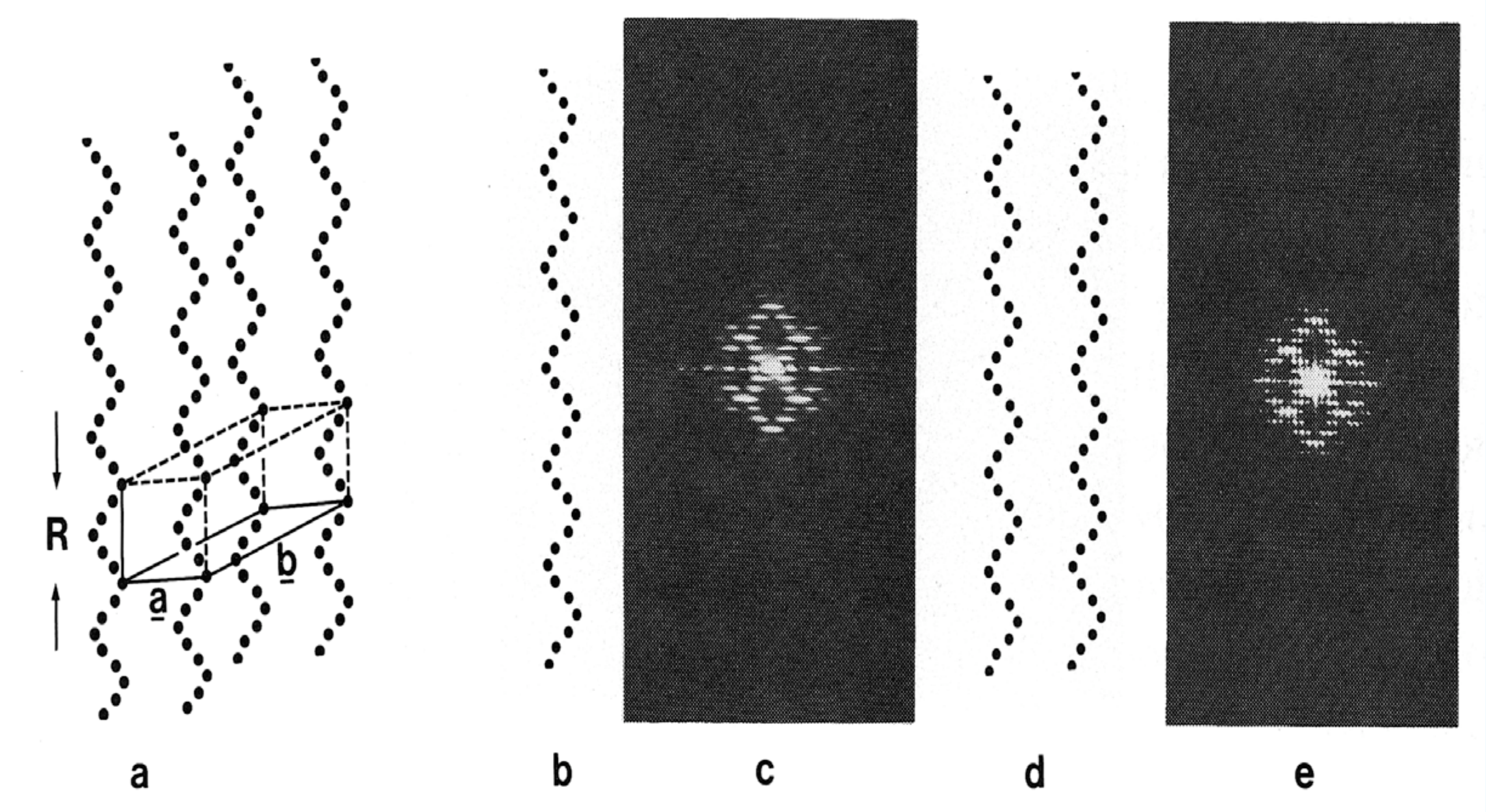


Fig. IX.8. $I(\xi,\ell)$ for integral and non-integral helices.

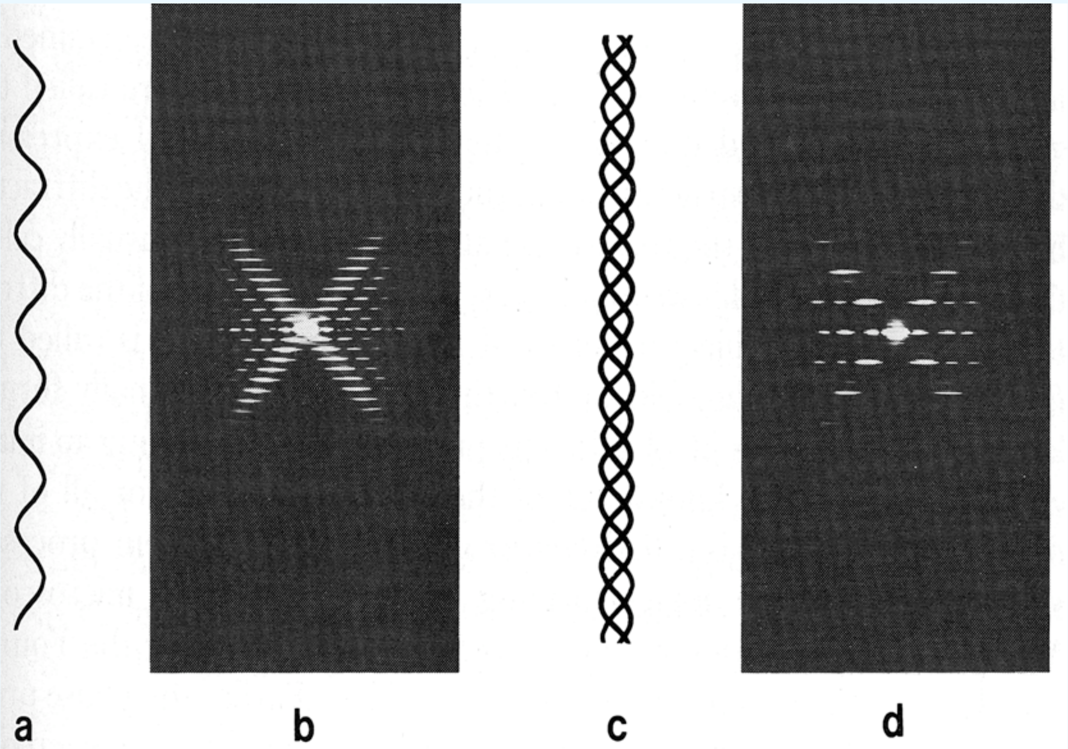
m=0, *l*=n

l = 8m + 3n

Crystals of Helical Molecules

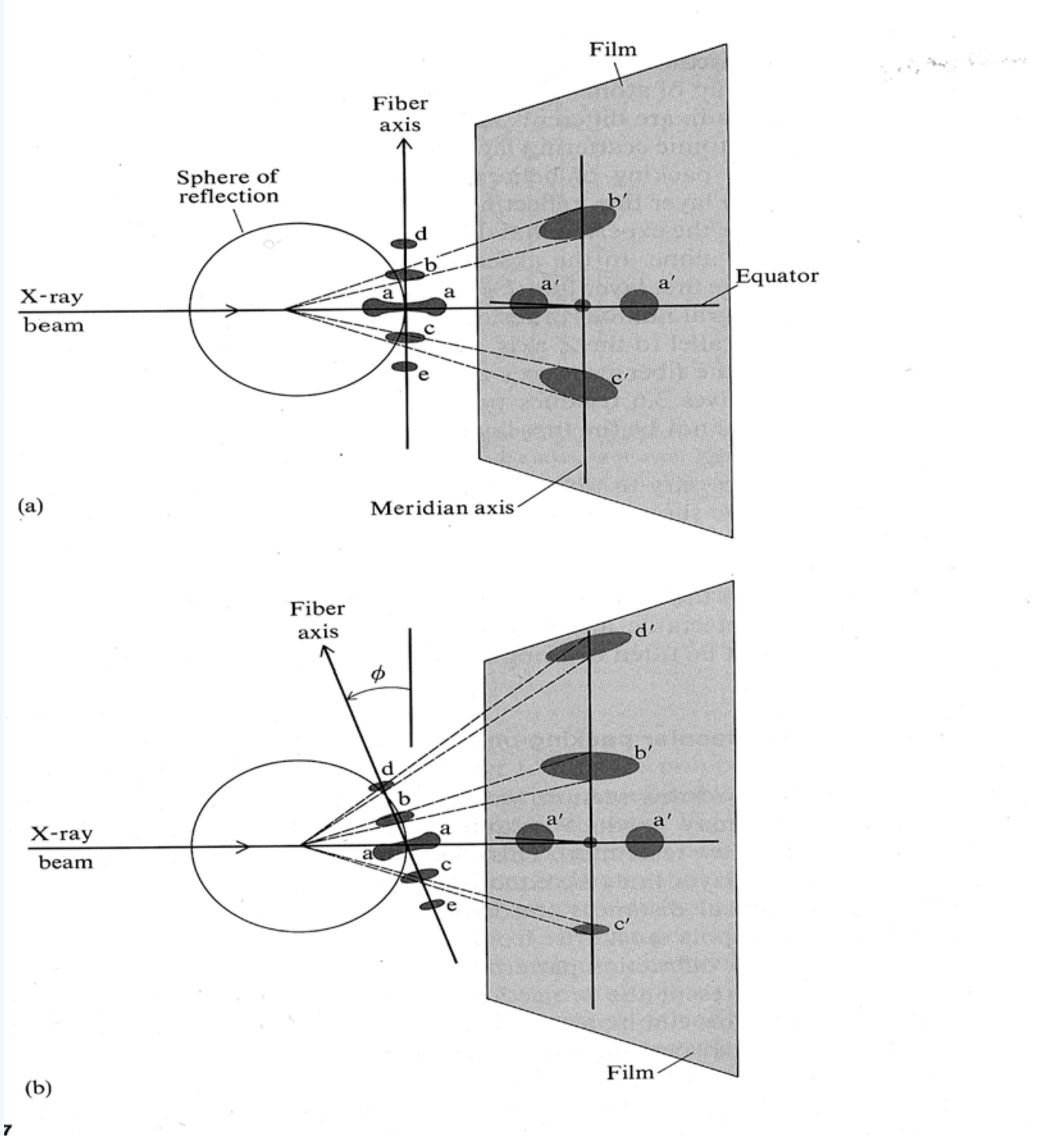


Multi-Stranded (coiled coil) Helices



If N strands
Only every
Nth Layer -
line allowed

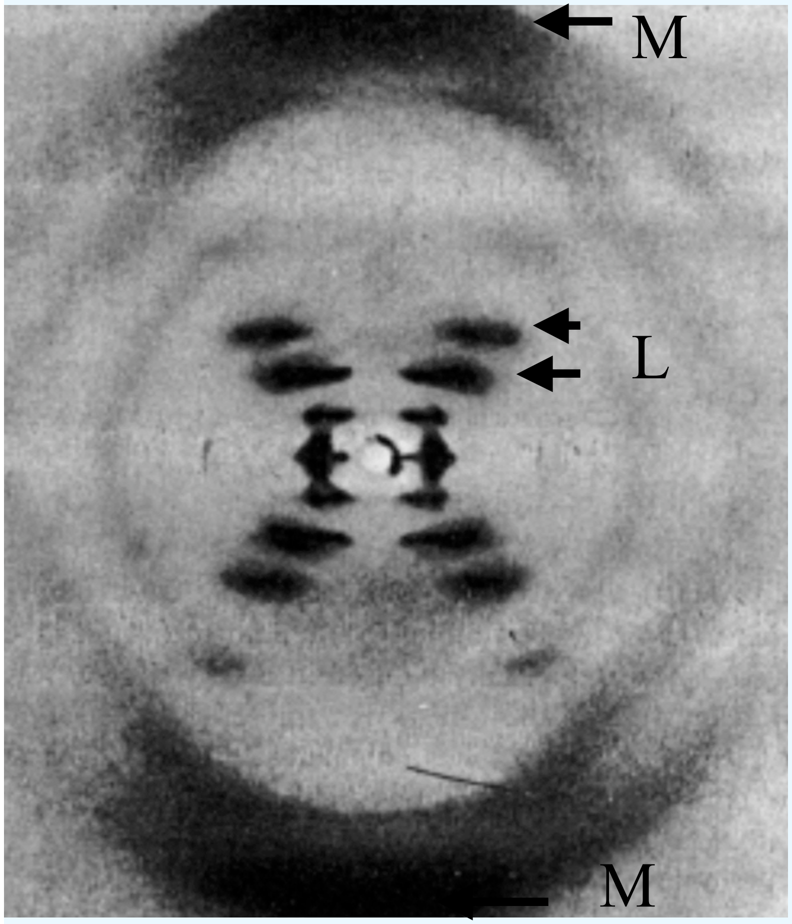
Geometry of Fiber Patterns



Fiber Diffraction Often Just Used to Find Gross Molecular Parameters

- In many cases one can make structural inferences without a full-blown structure solution
- Helical parameters in Polyamino-acids and nucleic acids
- Topology of viruses and other large molecular complexes
- Test hypotheses concerning influence of inter-filament lattice spacing

Rosalind Franklin’s Pattern from B-DNA



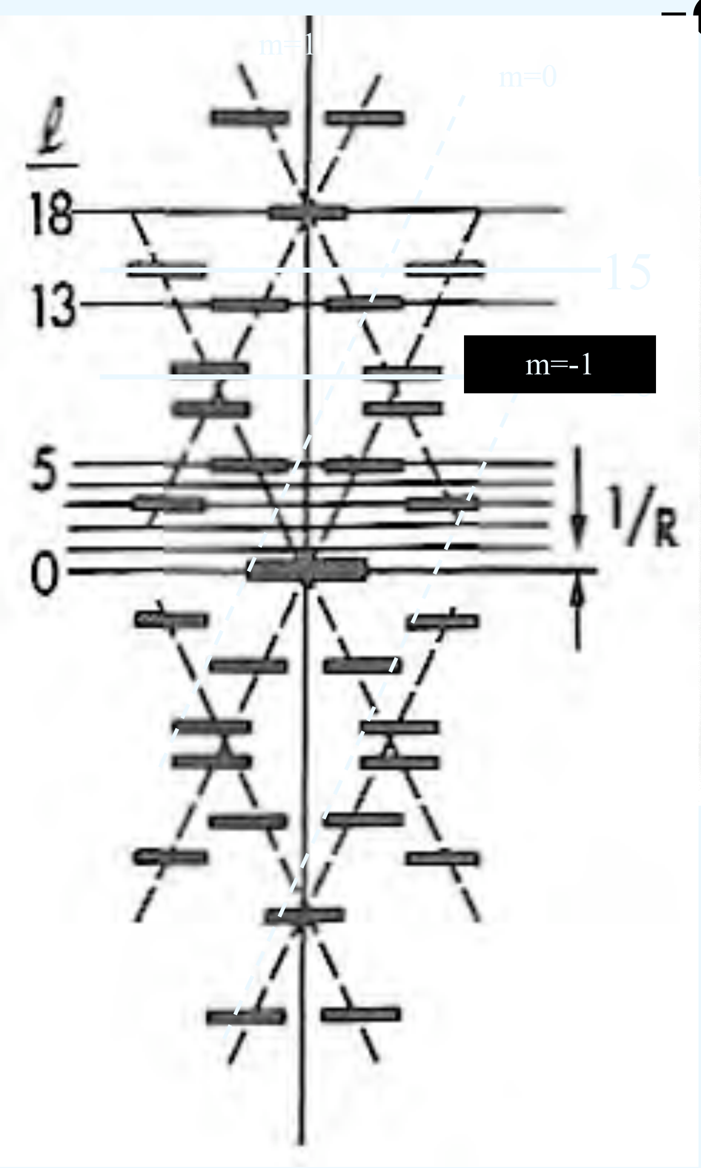
Layer lines (L) separated by 34 Å nm

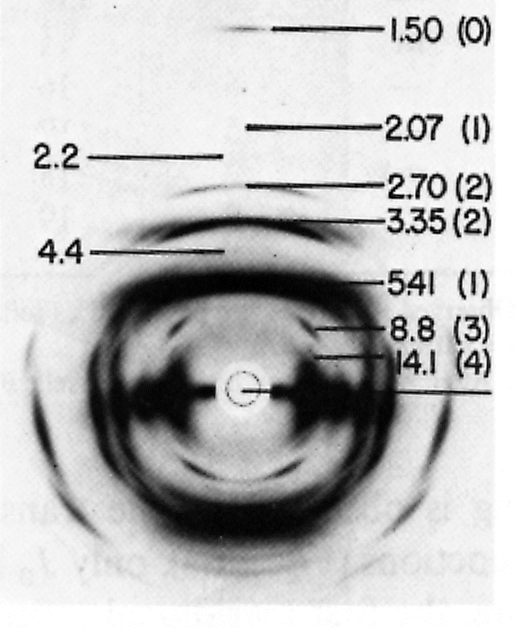
Meridional (M) reflection at 3.4 Å

=> 10 residues/turn

Franklin & Gosling, 1953 Nature 171:740

Diffraction from Poly L-Alanine -α-helix





1.5 Å residue trans.

Pitch 5.4Å, R= 27Å

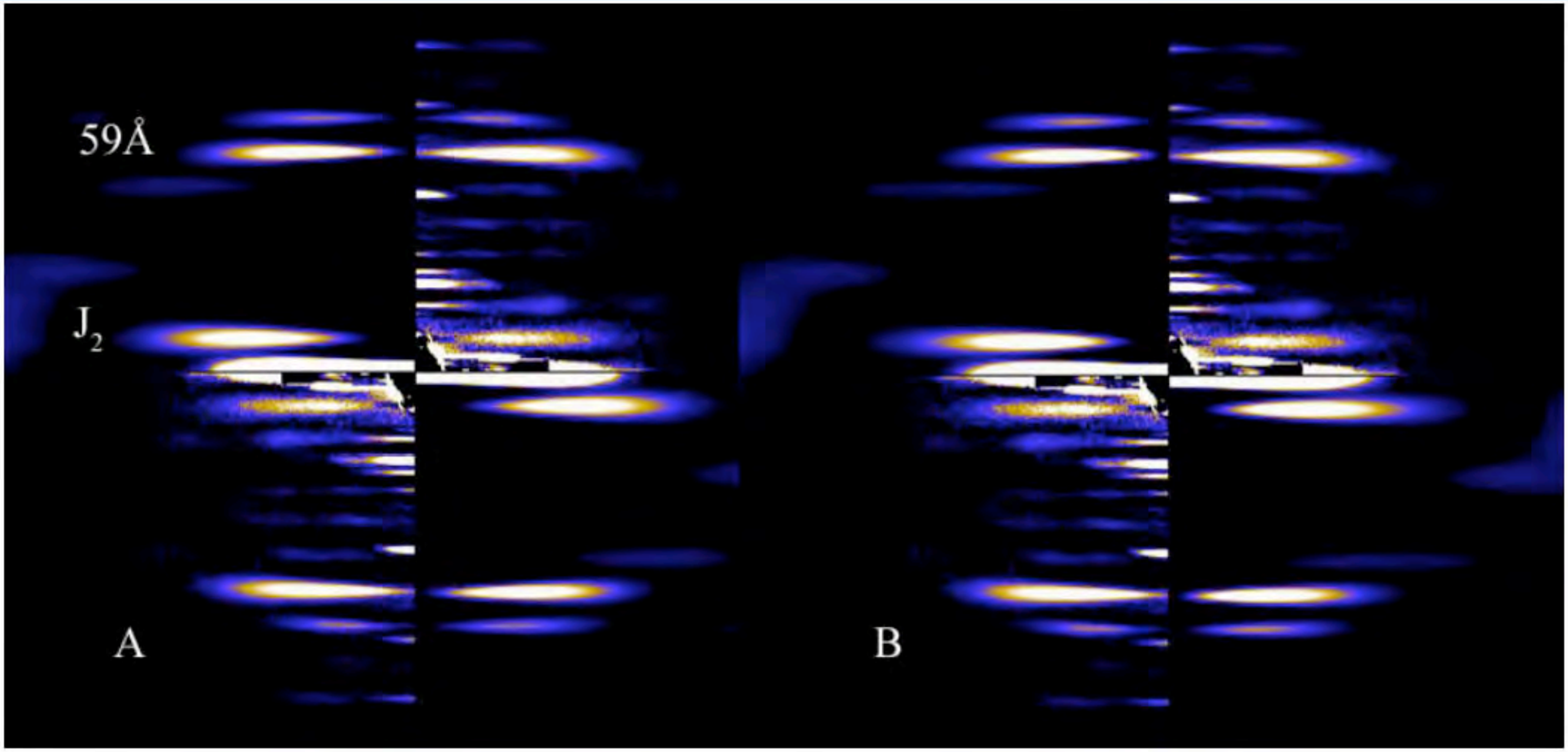
18 residues/5 turns

$l=18m+5n$

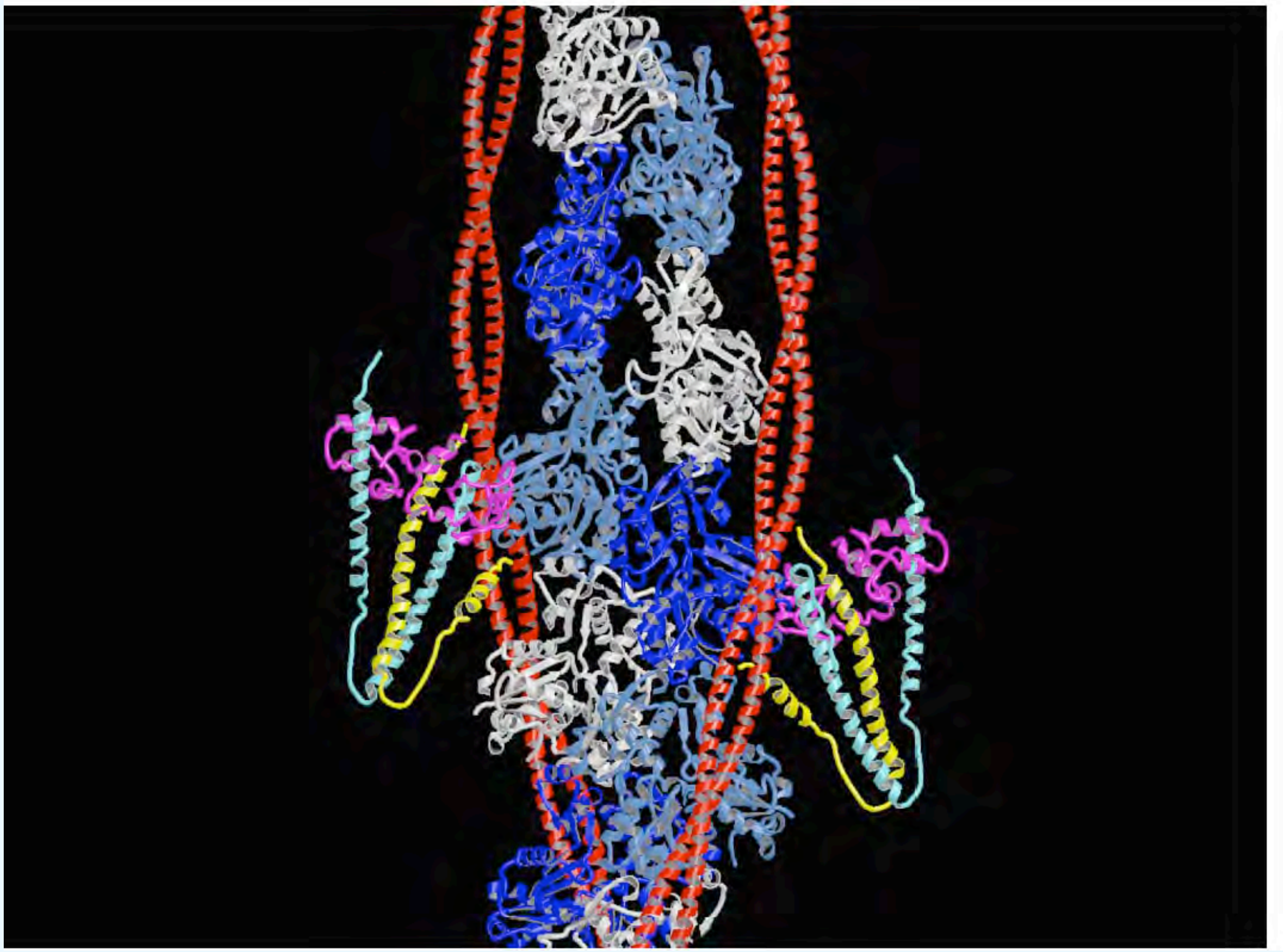
Often a Modeling Approach is Used

- Use known information & any high resolution structural information
- Simulate the observed diffraction pattern with a calculated one
- Use simulated annealing or similar algorithms to minimize differences

Diffraction Pattern from Overstretched Rabbit Muscle



Model of Regulated Thin Filament from Muscle from Fiber Data

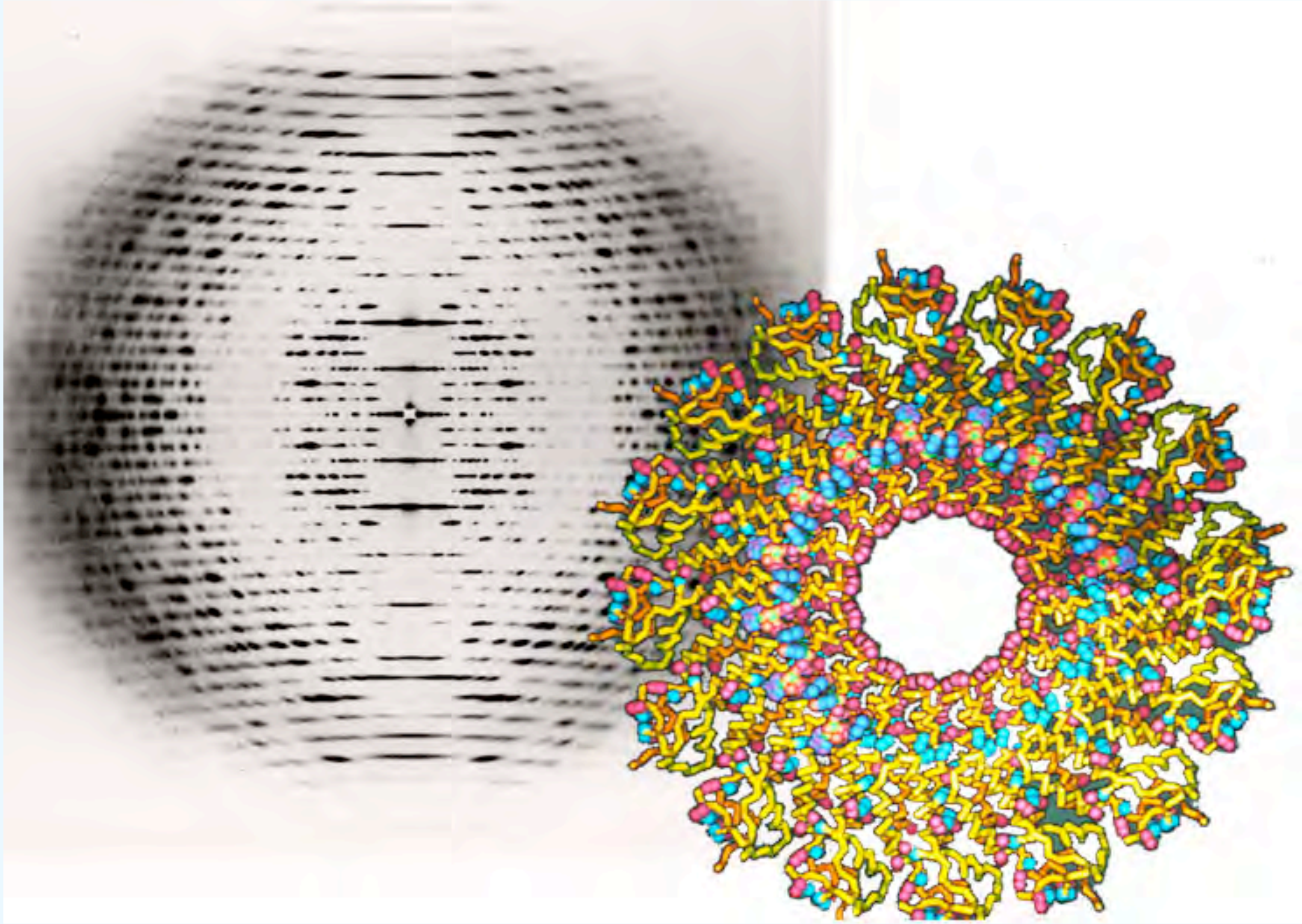


Poole et al. 2006 *J Struct Biol*. 155:273

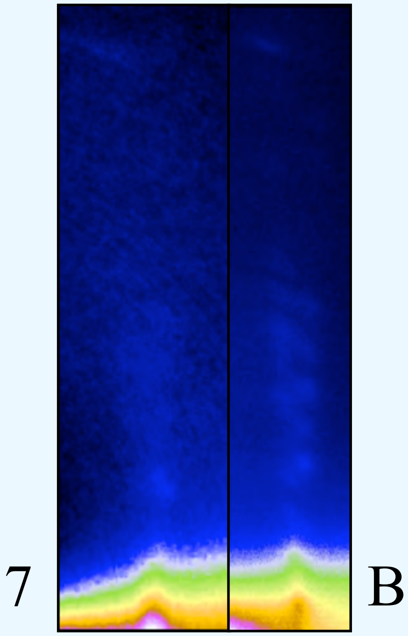
Fiber Crystallography

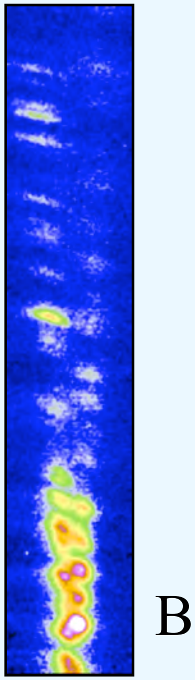
- Most fiber “structures” result of model building studies
- There have been a small number of Fiber “structure solutions”.
- TMV by Stubbs, Caspar, Holmes et al. (1970’s 1980’s)
- High resolution structures by Keichi Namba on bacterial flagella (Yamashita et al., 1998 Nature SB) aligned by high magnetic fields
- Orgel et al. (2001, 2006) MIR structures of Type I collagen from rat tail tendon

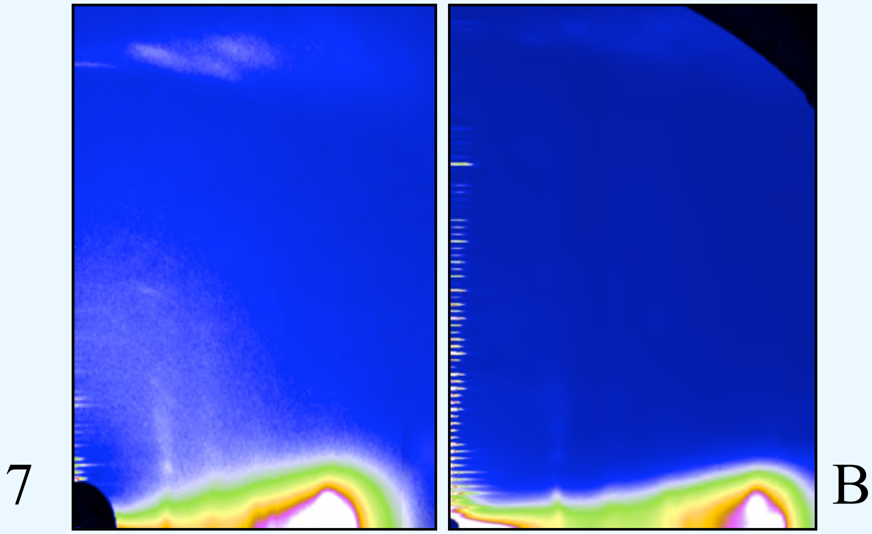
Tobacco mosaic virus

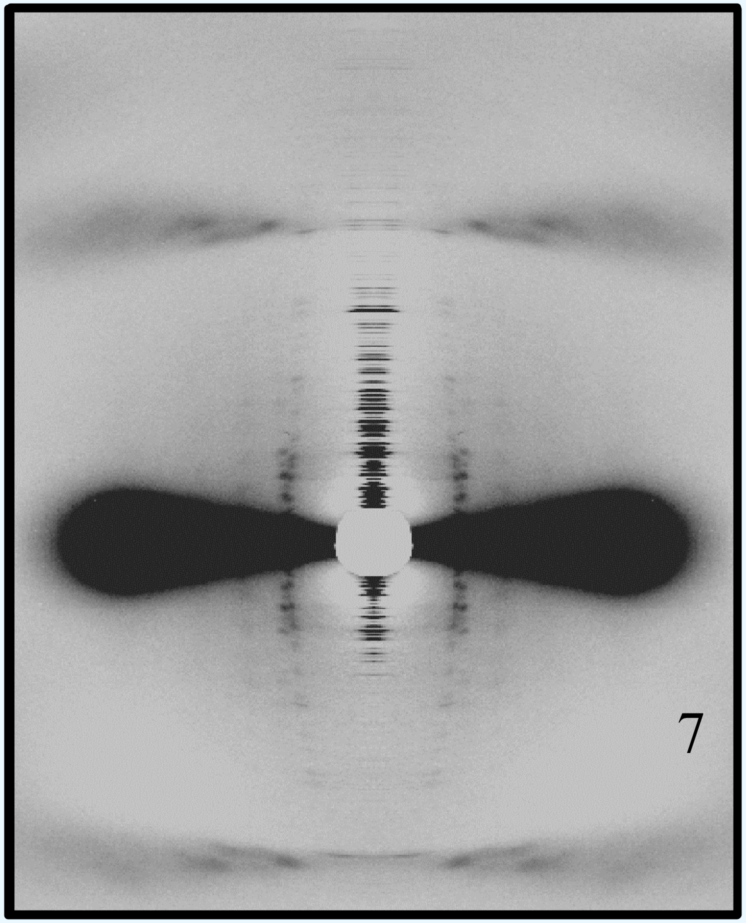


Data collection:

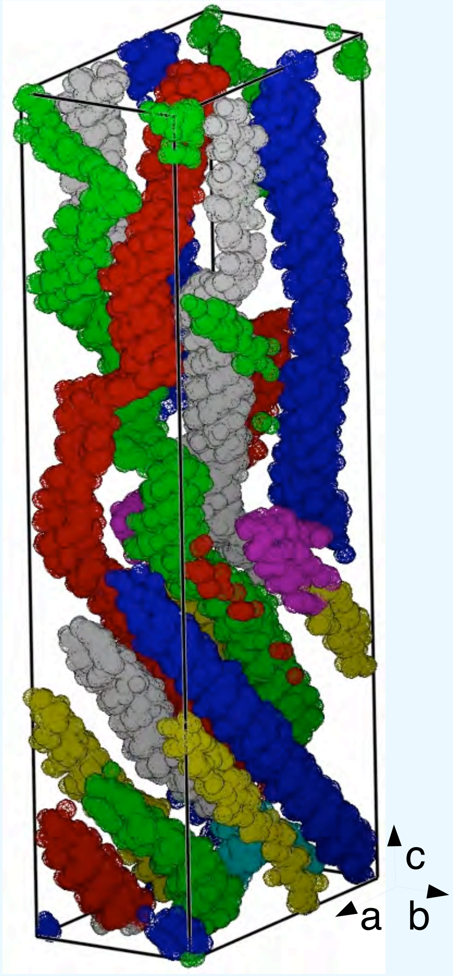


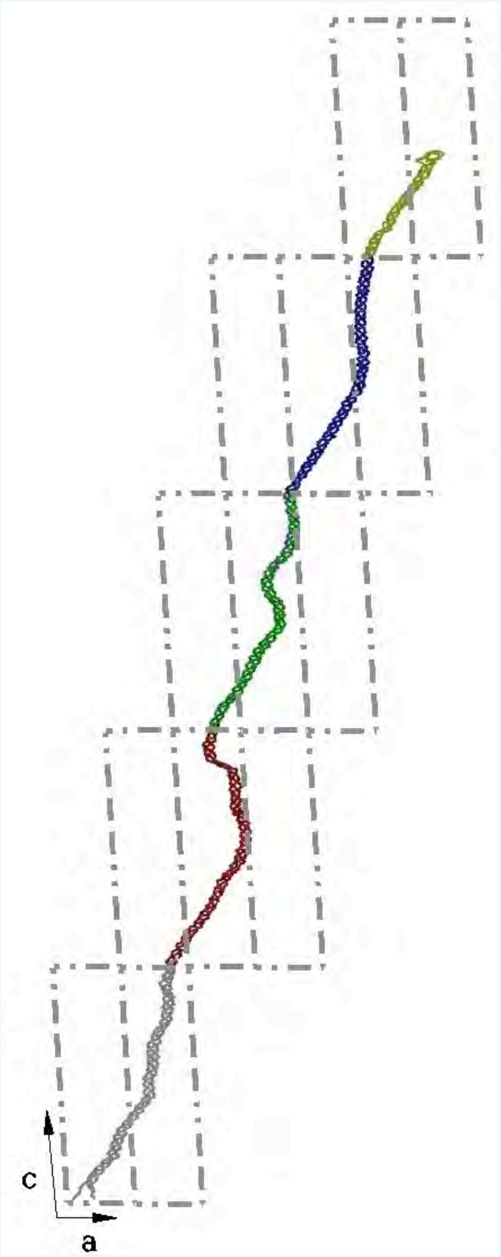


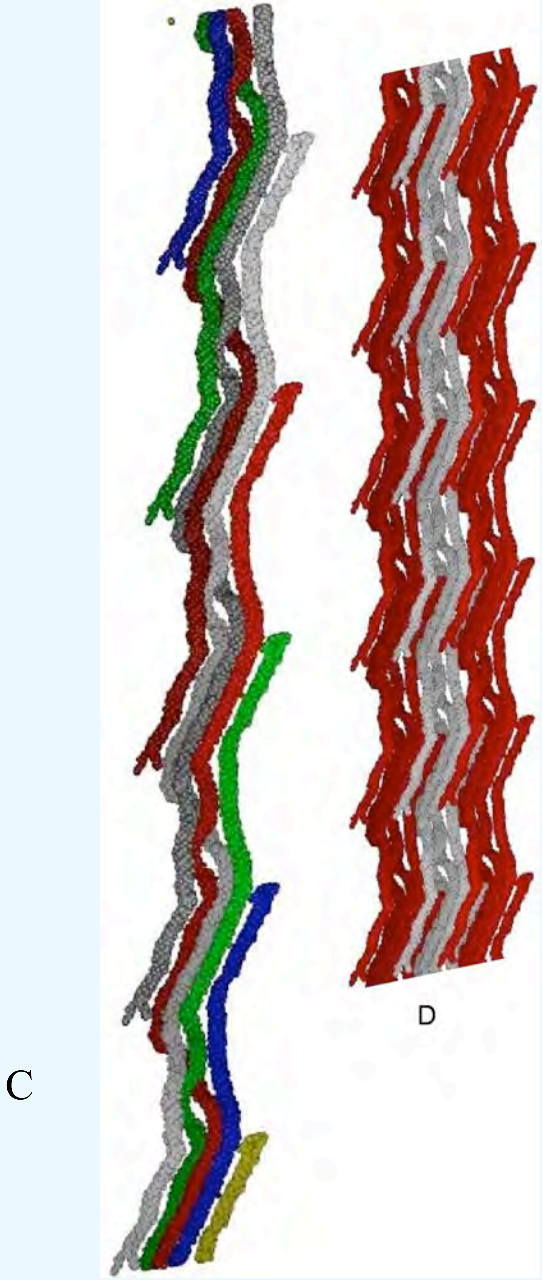




Collagen type I sub-fibrillar structure







Resolution ~ 11 /5.16 Å

Orgel et al., 2006 *PNAS* 103:9001

Synchrotrons and Fiber Diffraction

- Early work all done with conventional sources - why need synchrotron?
- Patterns weak, have high backgrounds, frequently have multiple closely spaced lattices
- Studies benefit from greatly increased beam quality
- Greatly increased flux permits time-resolved experiments

Why X-ray Diffraction of Muscle?

- Force producing events occur on the time scale of ≤ 1 ms
- Relevant size scale is 5 - 50 nm for molecular machinery
- X-ray diffraction only technique that allows simultaneous collection of structural and physiological information on this time scale
- Can be used on *living* systems to do *real physiological experiments*

First Diffraction Pattern Using Synchrotron Radiation

Equatorial pattern from insect flight muscle

August, 1970, DESY

Rosenbaum, Holmes,. & Witz (1971).

Nature **230**, 434-437.

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X-ray Interference and Crossbridge Motion in Active Muscle

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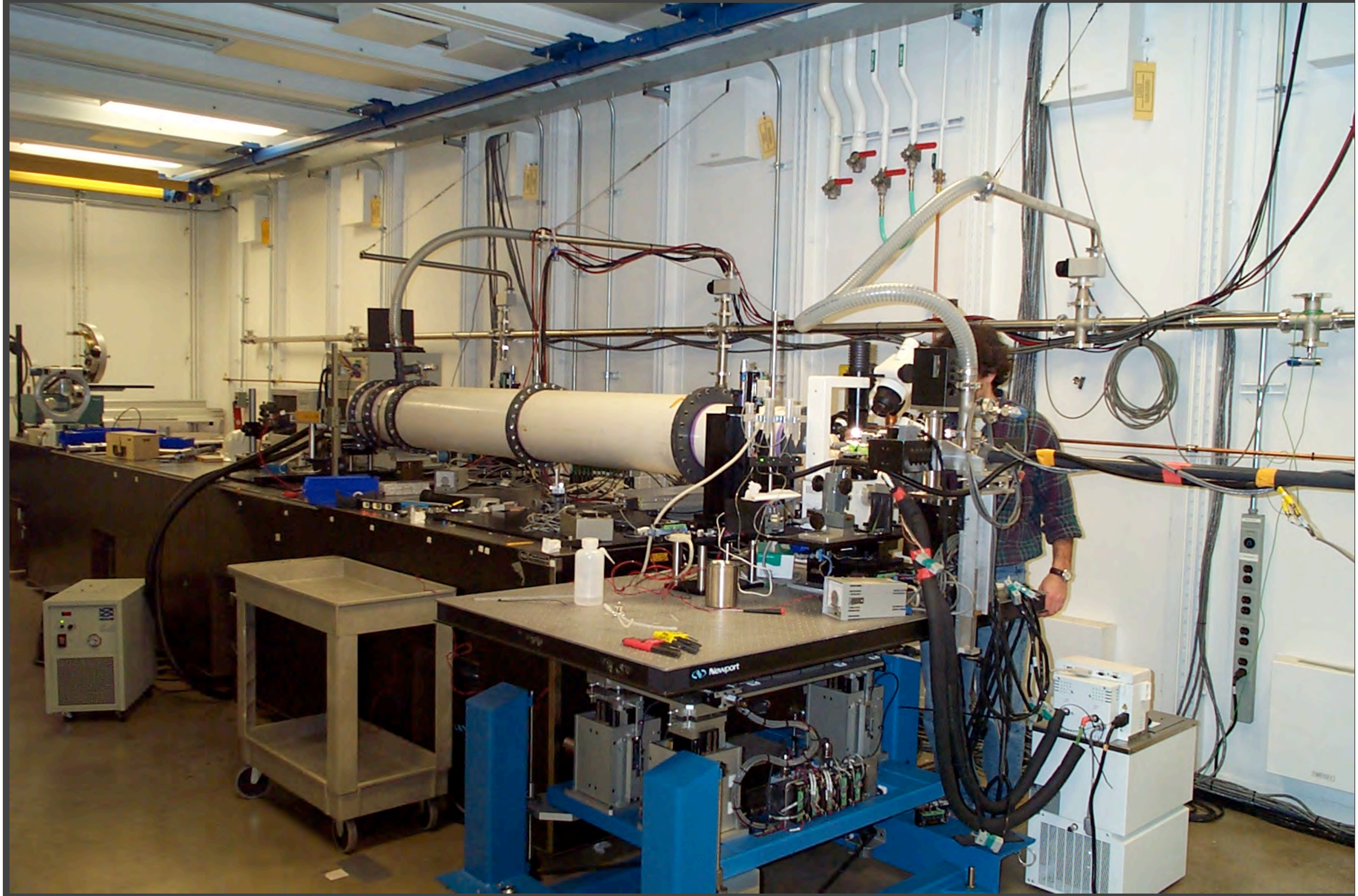
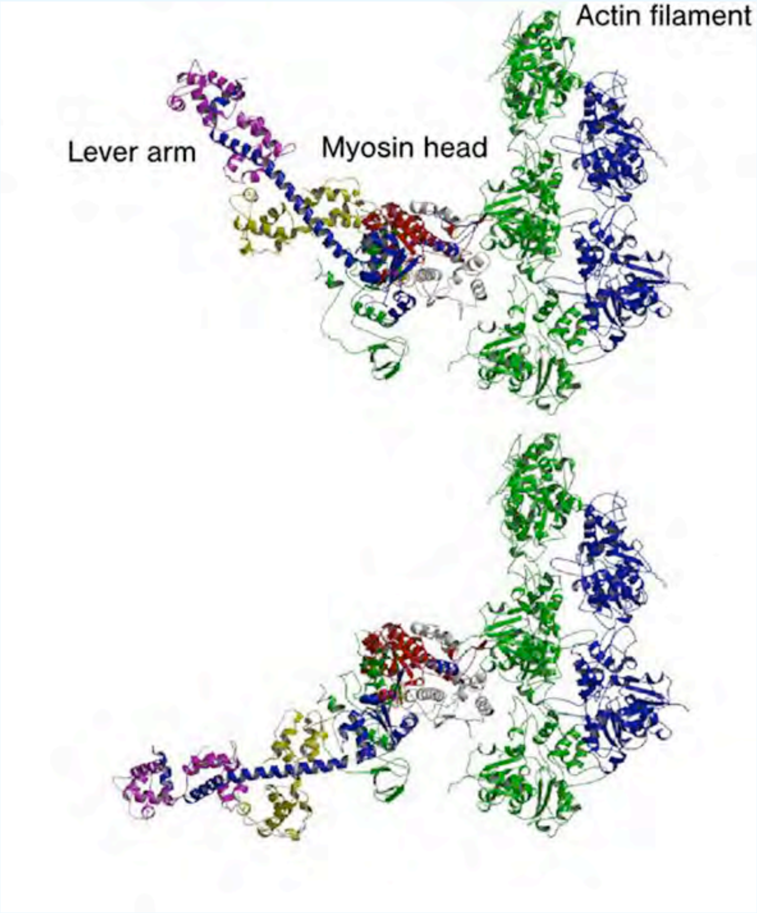
M. Reconditi (Florence), M. Irving
KCL

H. Huxley, A. Stewart (Brandeis)

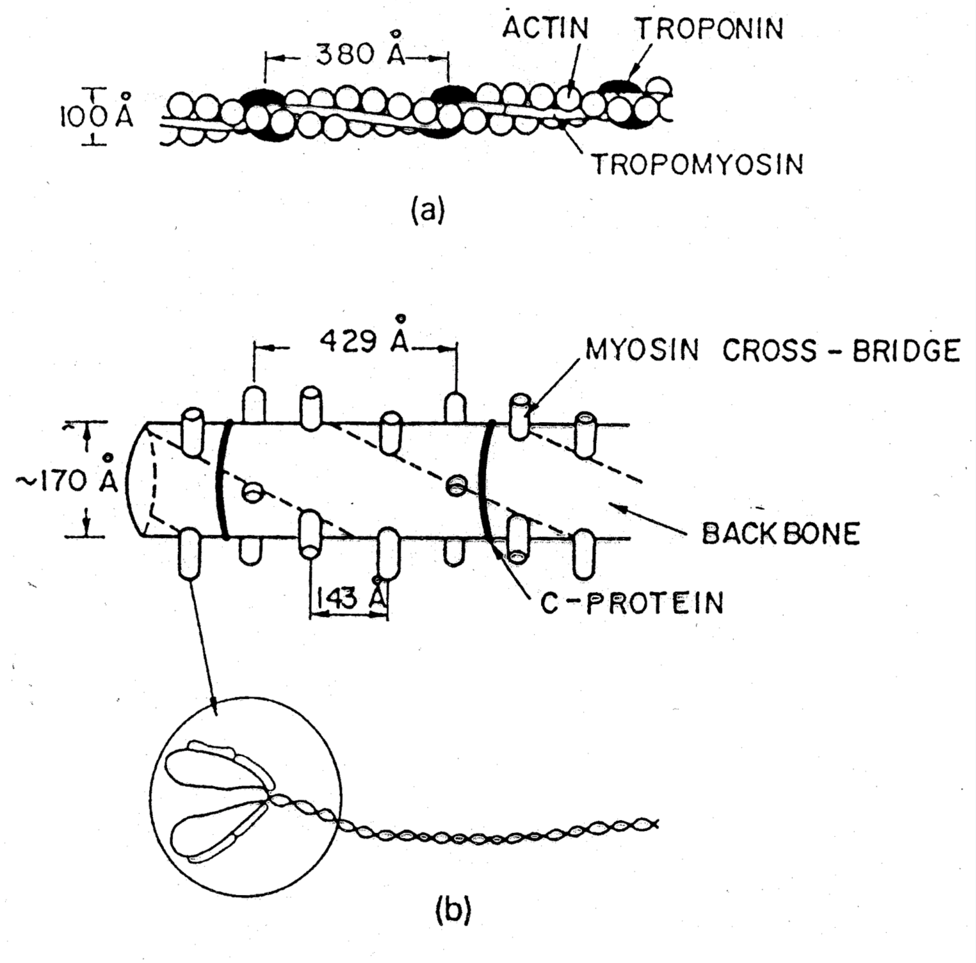
T. Irving (IIT)


Newport

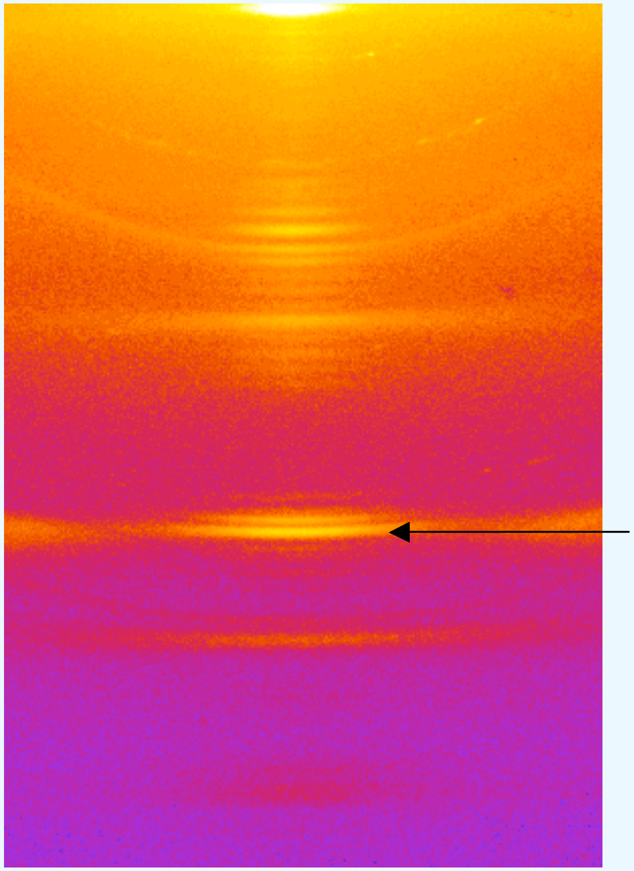
"Swinging Lever Arm Hypothesis"



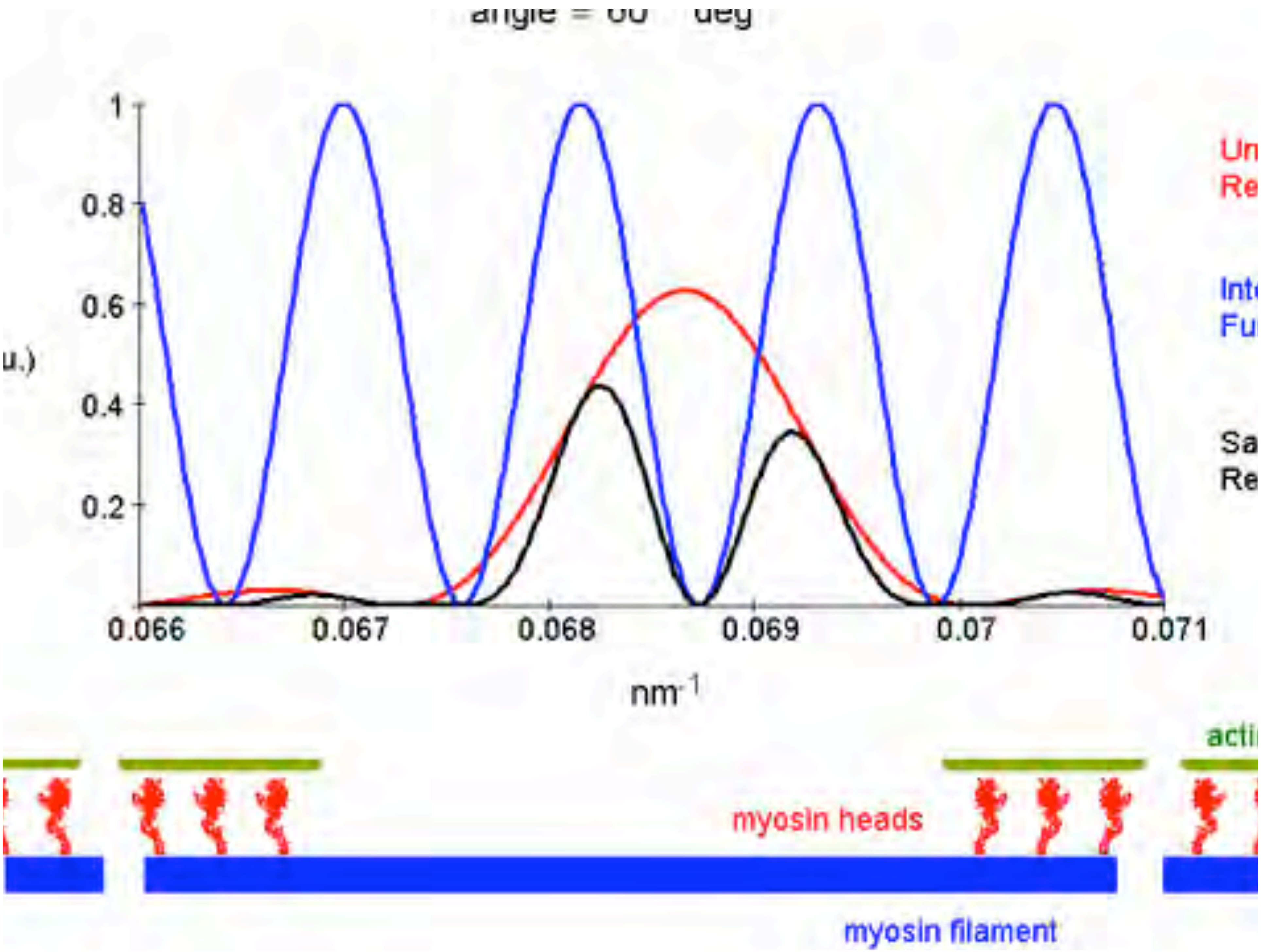
Muscle Filament Substructure



Interference from myosin heads on opposite sides of A-band



Fine structure in the 145 Å reflection from frog muscle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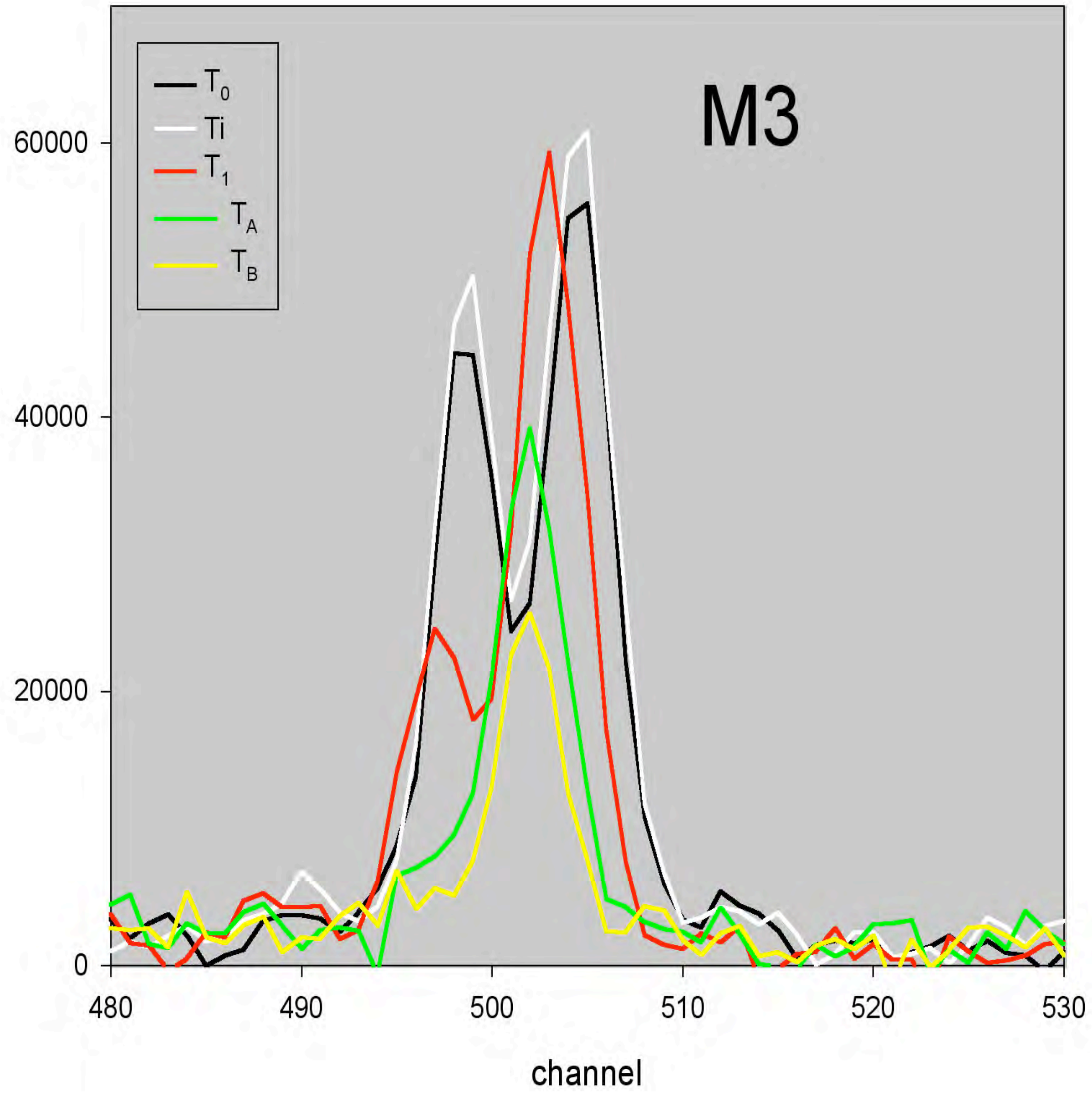
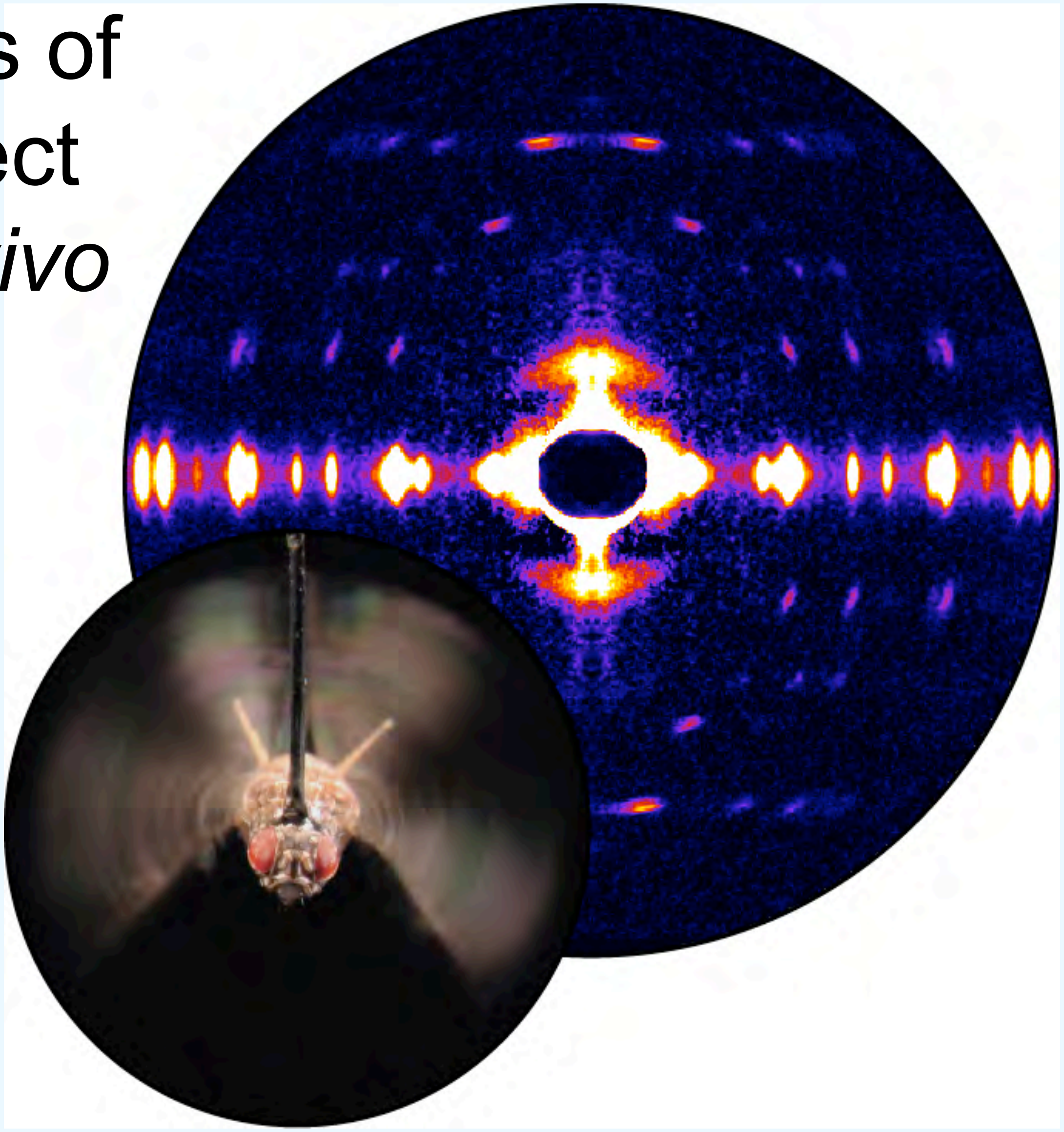
Fibre 08, load clamp at $T=T_0/2$



Conclusions

- The interference pattern on the meridian can study motion of crossbridges in living muscle to 100 microsecond time resolution and 1 Å accuracy
- Crossbridges do, in fact, move axially when muscles allowed to shorten
- Move more at low loads, less at high loads
- Unexpected finding that under most physiological shortening conditions ~5-7 nm stroke size, 6 pN force per bridge
- The 10-12 nm step size expected from crystallography seen only at very low load
- "The Muscle Problem" essentially solved

Time-resolved X-ray Diffraction Studies of *Drosophila* Indirect Flight Muscle *in vivo*



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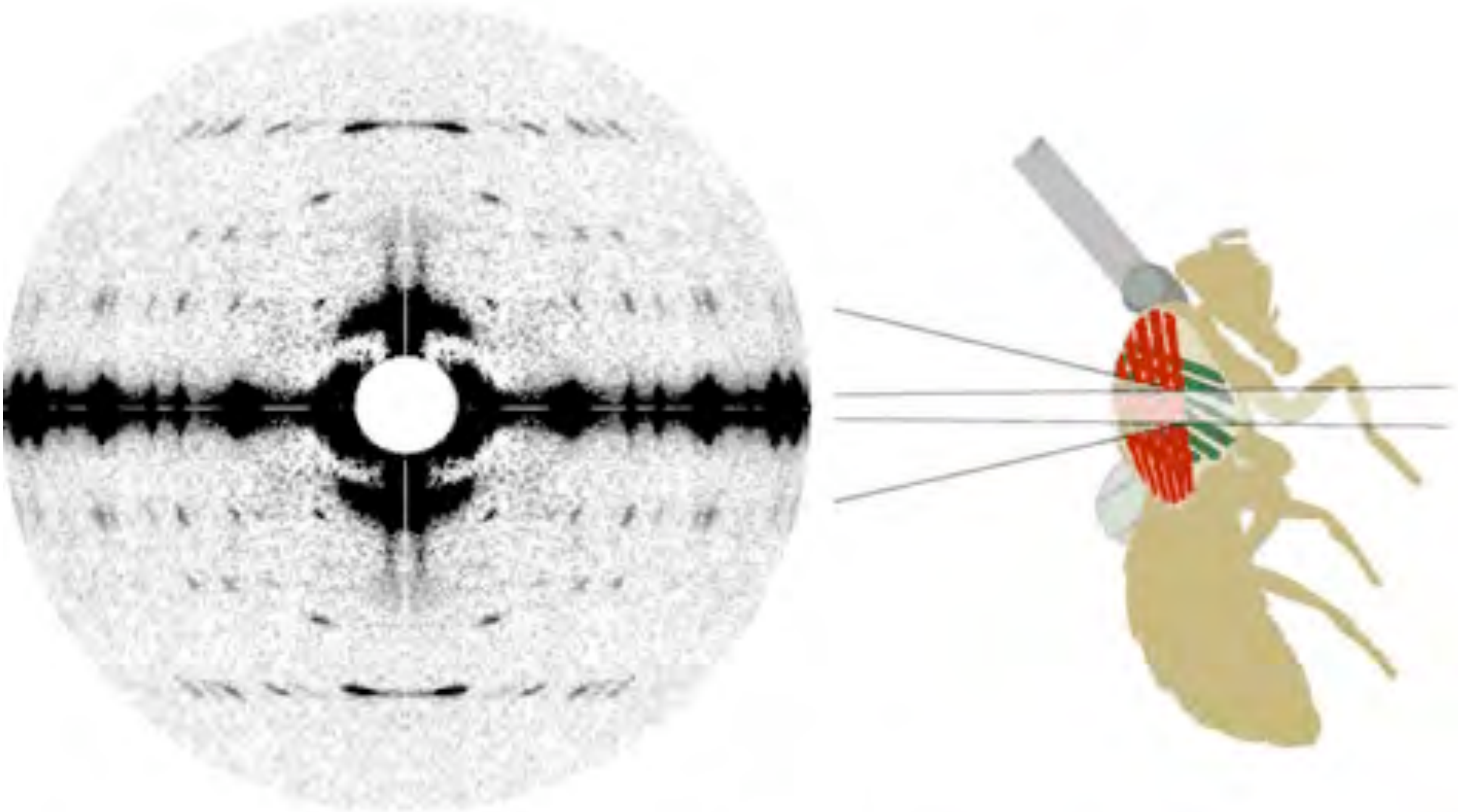
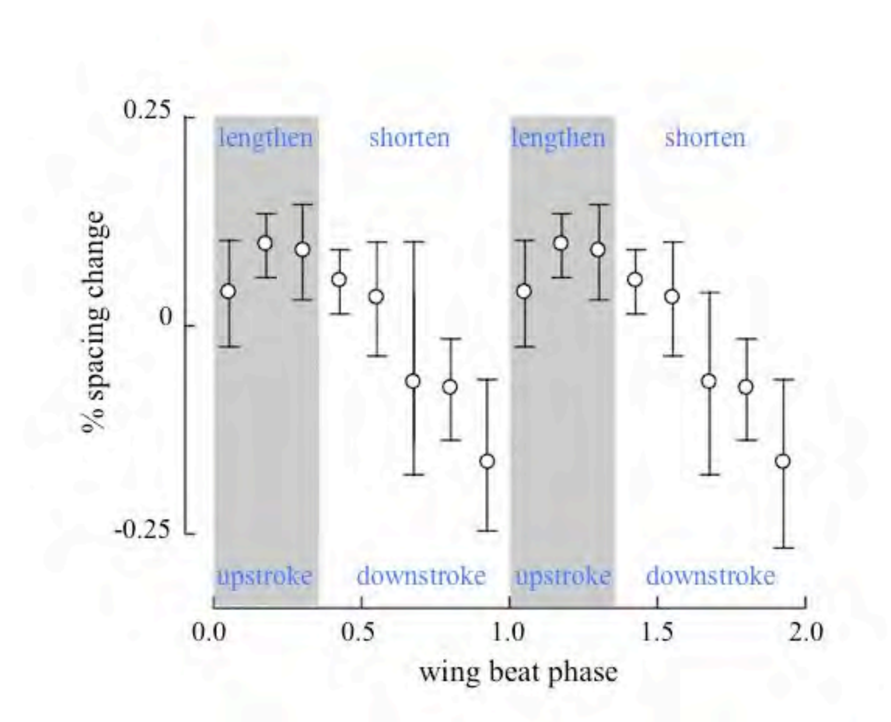
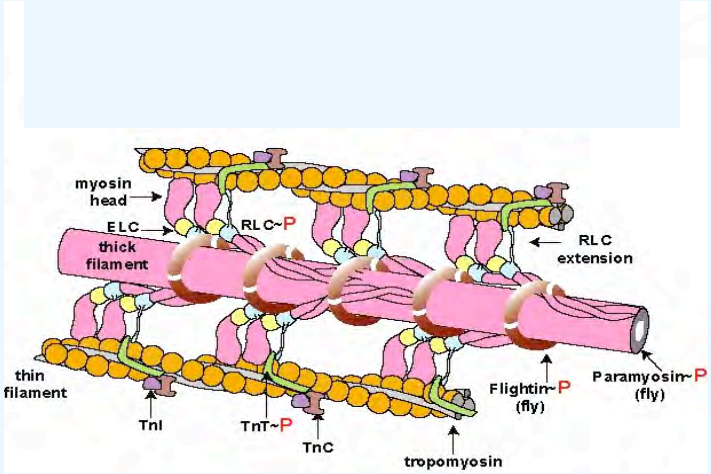


0.06 phase

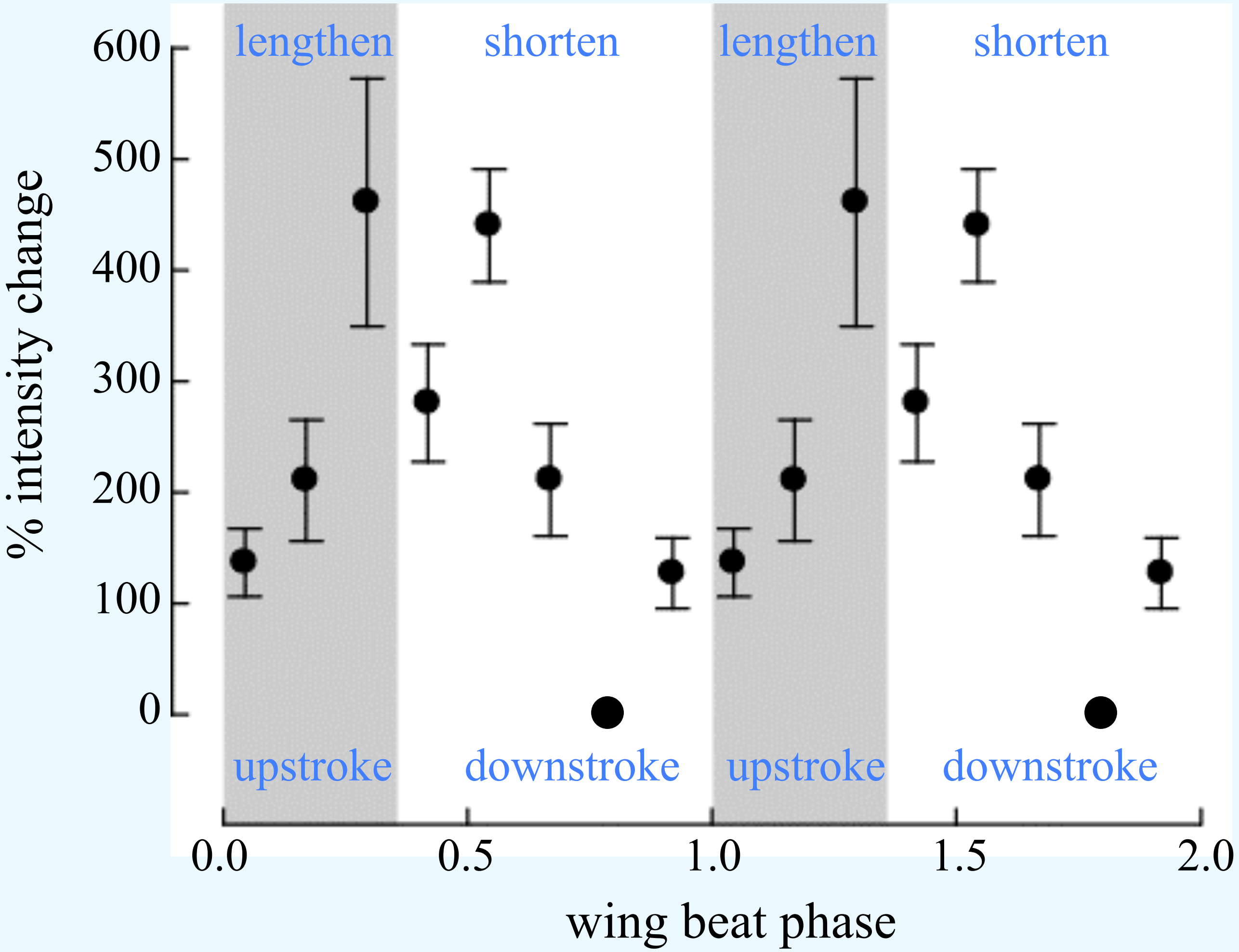
Time-resolved: 14.5 nm Reflection Spacing





- Small changes in filament length index thick filament stiffness *in vivo*
- Stores elastic strain energy during the wingbeat reducing energy consumption

19.3 nm first row line spot intensity (crossbridge attachment)



Analysis Software

- Rate limiting step is data analysis
- Long tradition of “rolling on your own”
- CCP 13 project http://www.ccp13.ac.uk
- Comprehensive data extraction suite
- Complementary NSF RCN Stubbs (Vanderbilt) PI will add angular deconvolution, other features to suite

References

- **Basics**:

  C. Cantor and P. Schimmel “Biophysical Chemistry part II: Techniques for the study of Biological Structure and Function” Chapter 14. Freeman, 1980
- **A terrific introduction to fiber diffraction** :

  John Squire “The Structural Basis of Muscular Contraction” Plenum, 1981
- **Definitive Reference on all things non-crystalline**:

  B.K. Vainshtein “Diffraction of X-rays by Chain Molecules” Elsevier, 1966.

More references:

Good introduction to “Fiber crystallography”:

Chandrasekaran, R. and Stubbs, G. (2001). Fiber diffraction. in *International Tables for Crystallography, Vol. F: Crystallography of Biological Macromolecules* (Rossman, M.G. and Arnold, E., eds.), Kluwer Academic Publishers, The Netherlands, 444-450.