BioCAT

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# Macro-Molecular Fiber Diffraction

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# 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

# Dimensional hierarchy of Biophysical (X-ray) techniques

1D — Low angle solution or powder diffraction

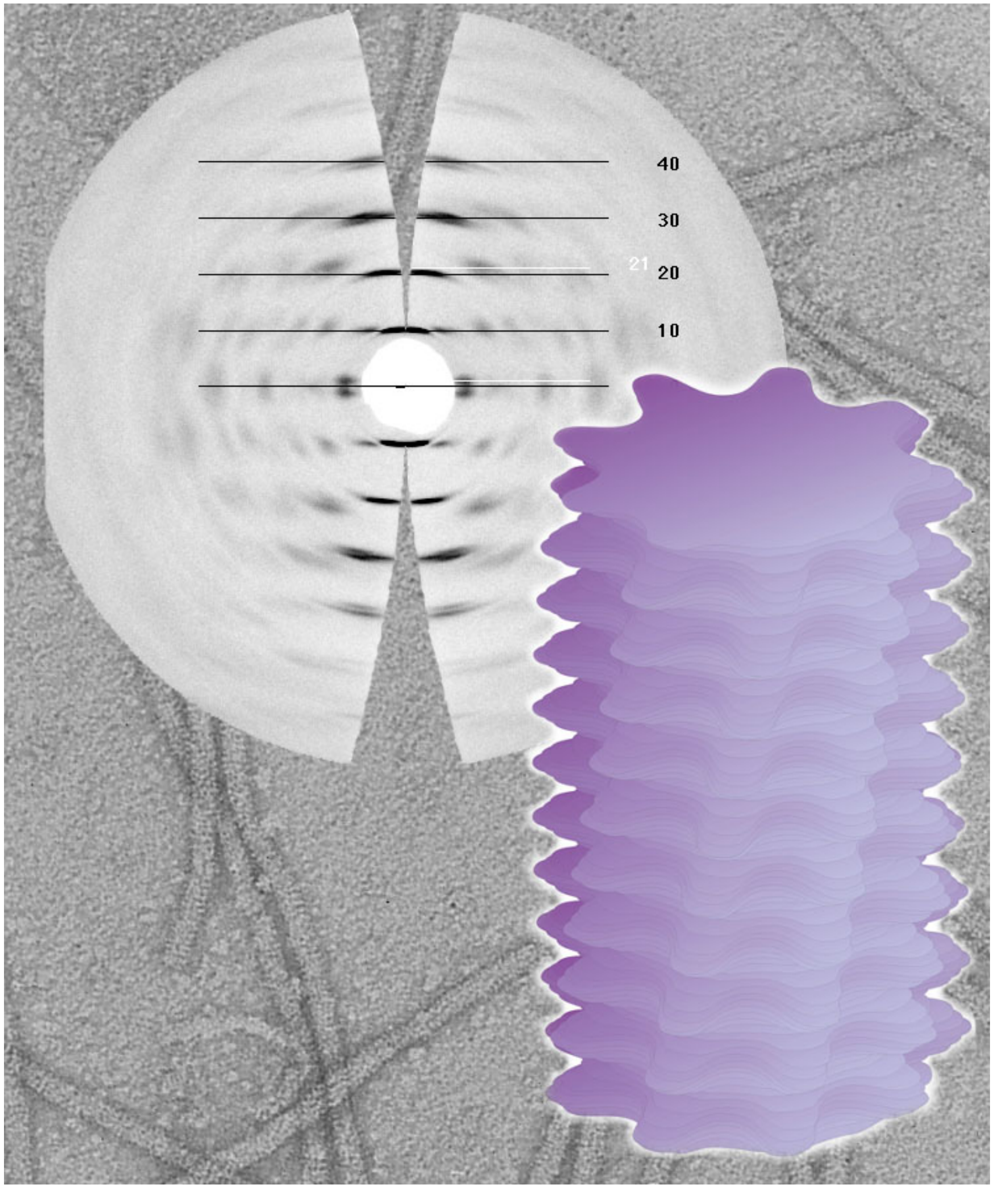
large macromolecular assembles

2-3D — Fiber diffraction

fiber forming arrays – muscle, collagen, DNA, amyloids, various carbohydrates, often *super-macromolecular scale*

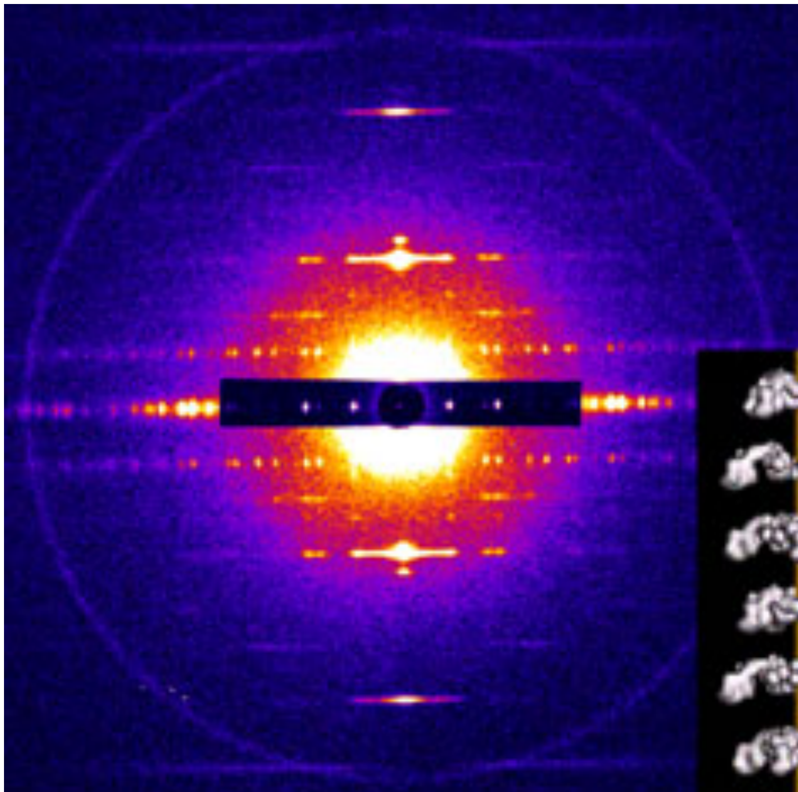
3D — Single crystal X-ray diffraction

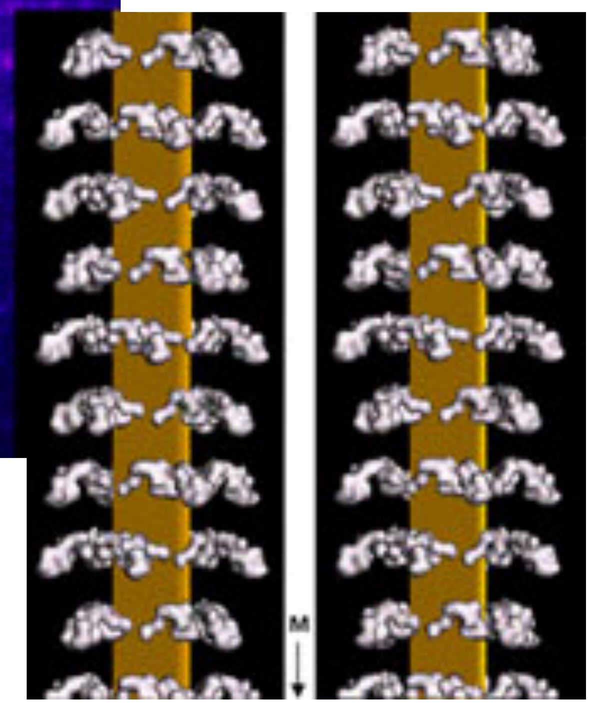
anything that can crystallize, must be (initially) soluble, usually relatively small in comparison to the above, *molecular to macromolecular scale*

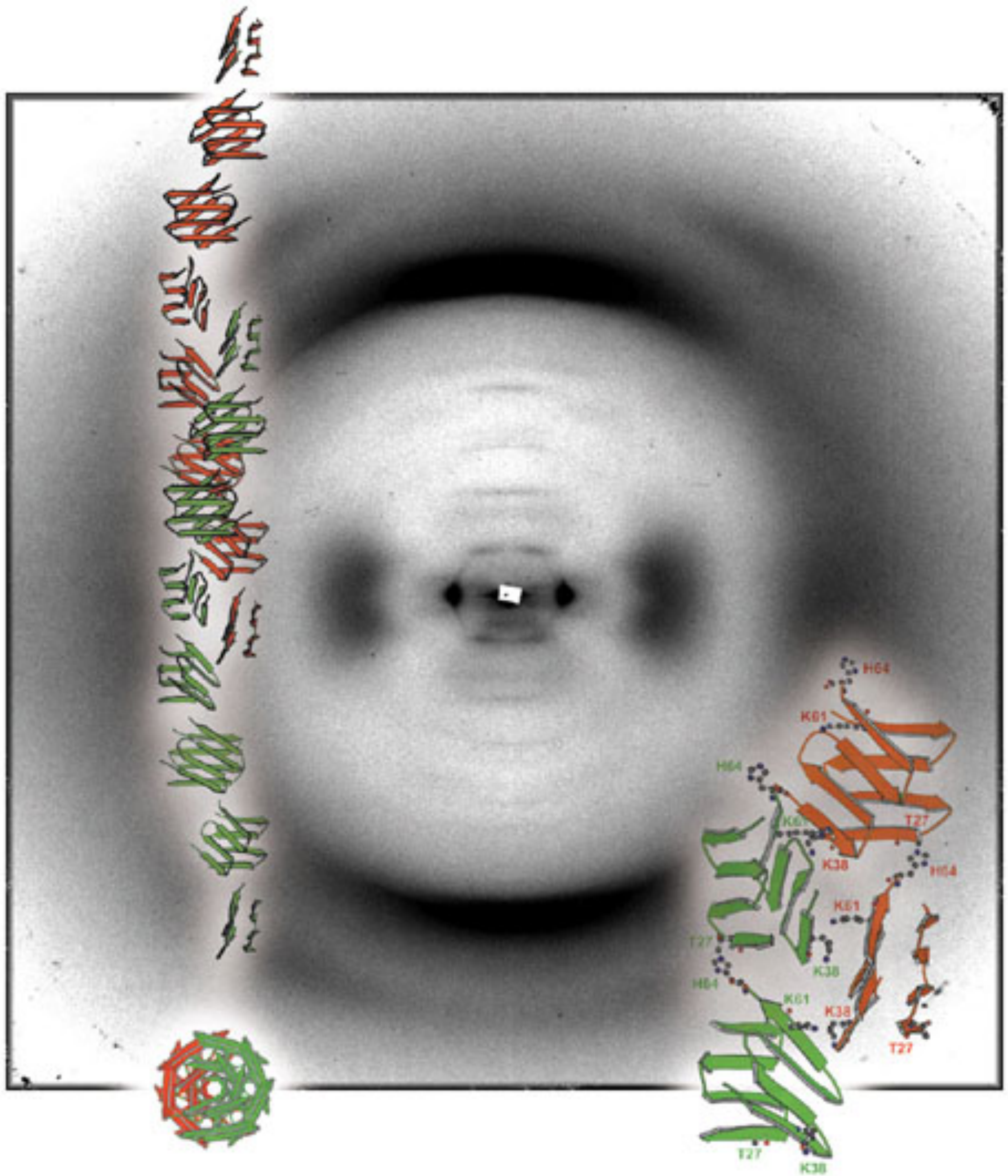



# Potato virus X

# Insect flight-muscle

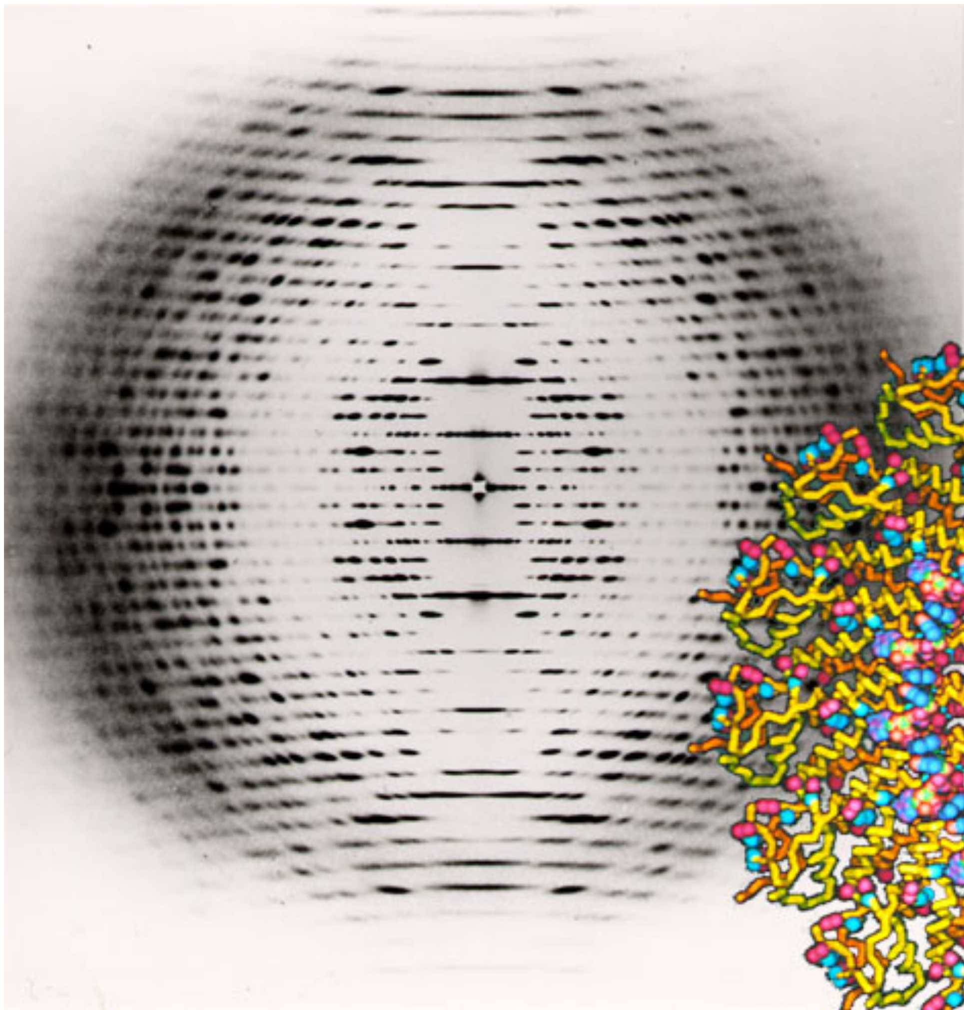


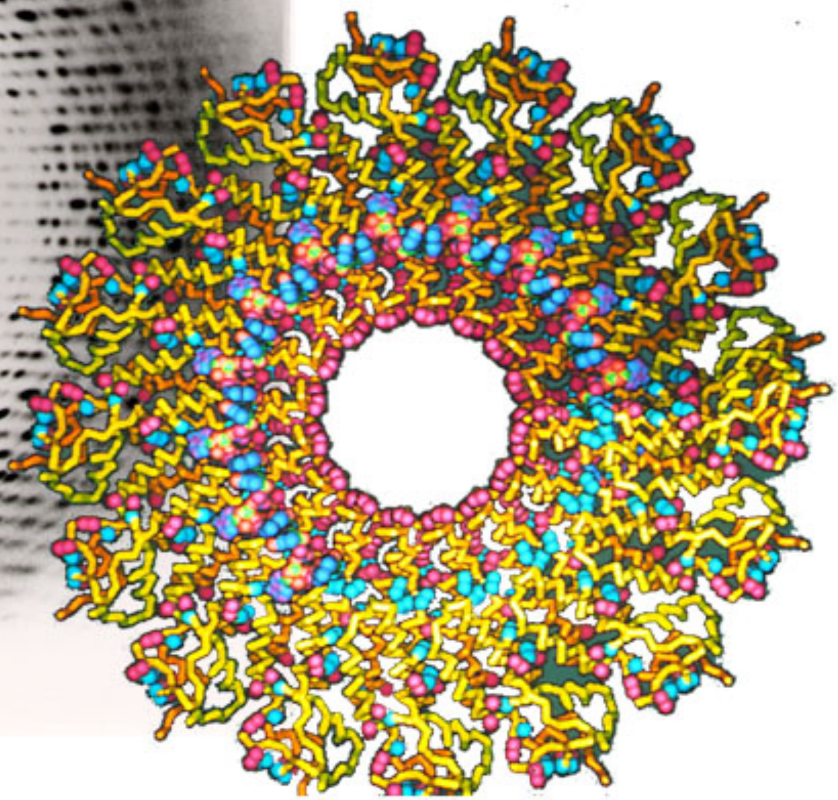





# Engineered amyloid fiber

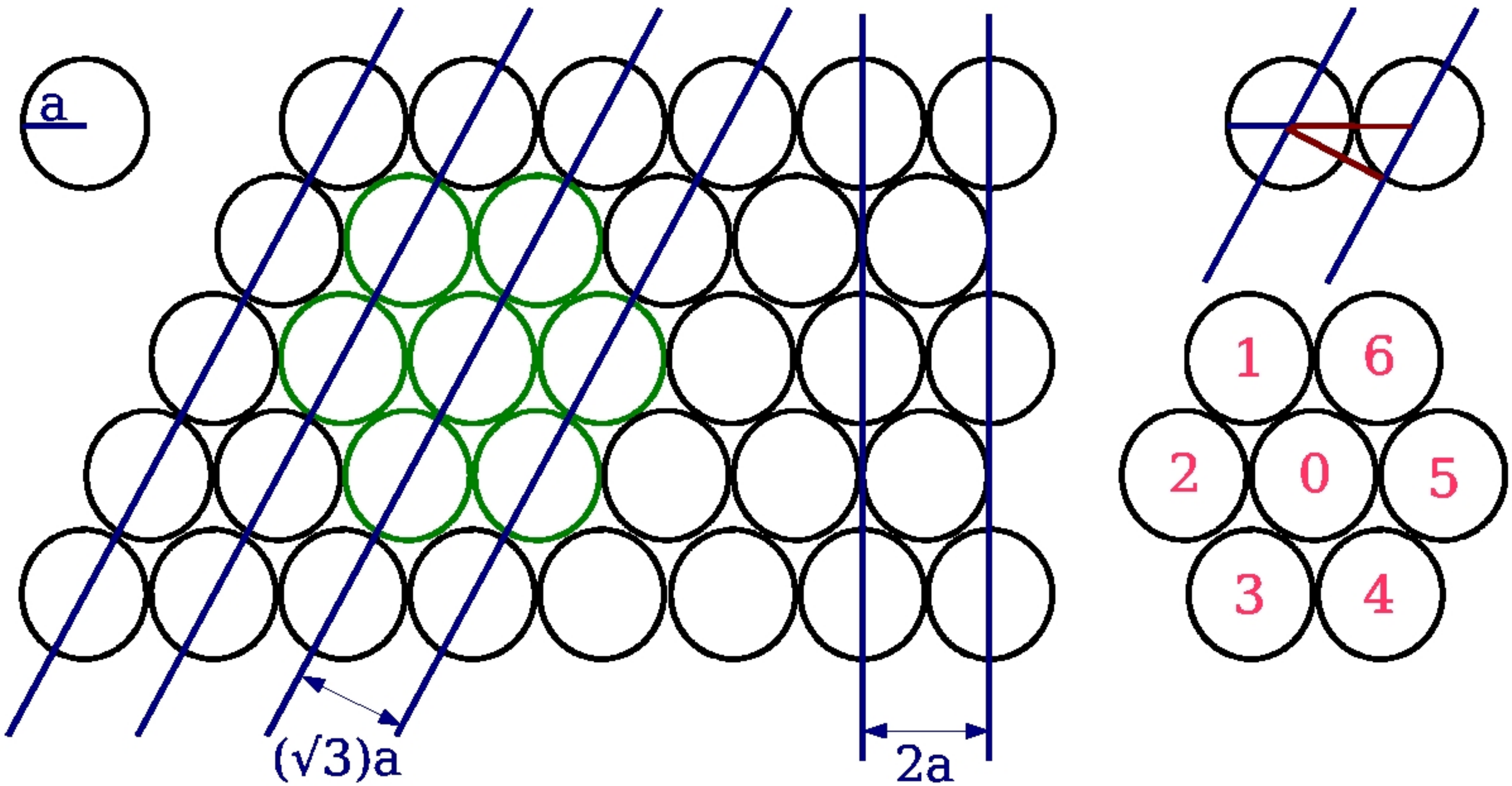
# Tobacco mosaic virus



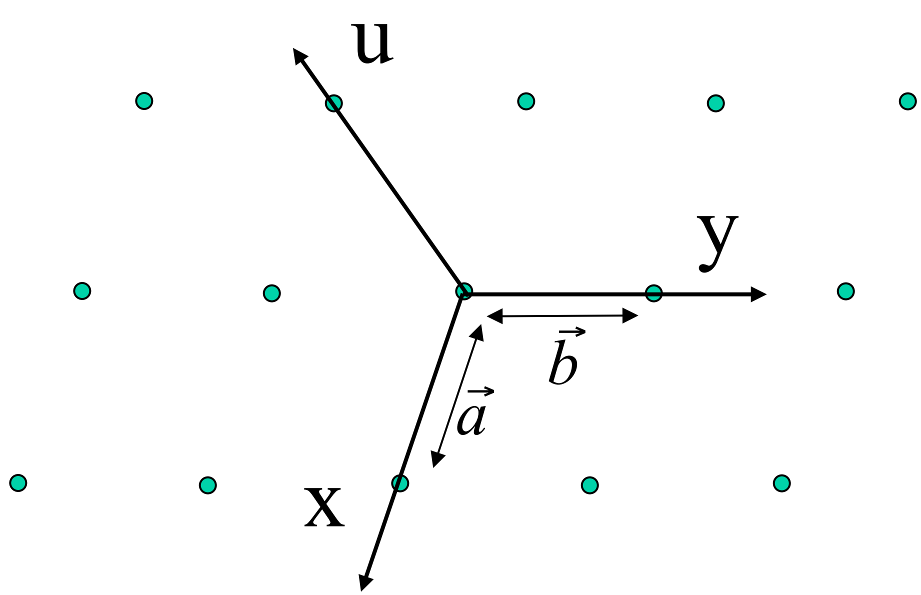


# Fiber Diffraction Theory

Fibers (essentially rods/cylinders) usually hexagonally packed

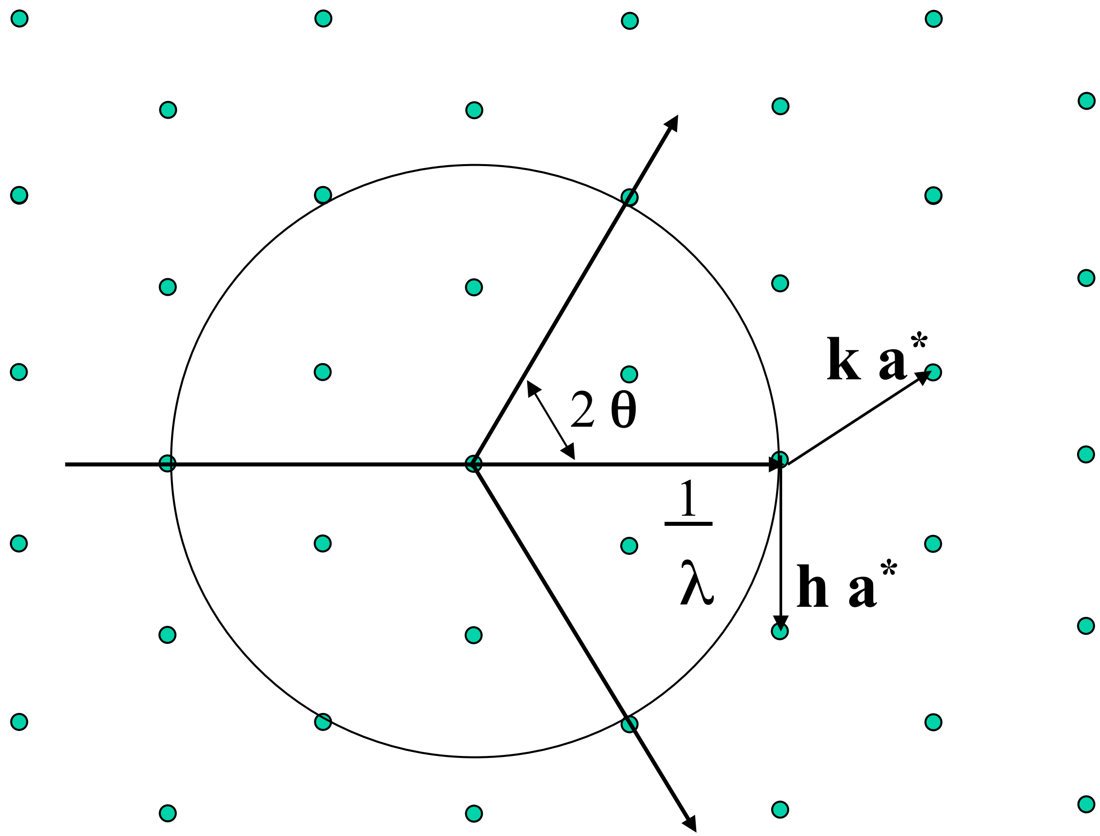


# Hexagonal Lattice variables




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

# Ewald Sphere



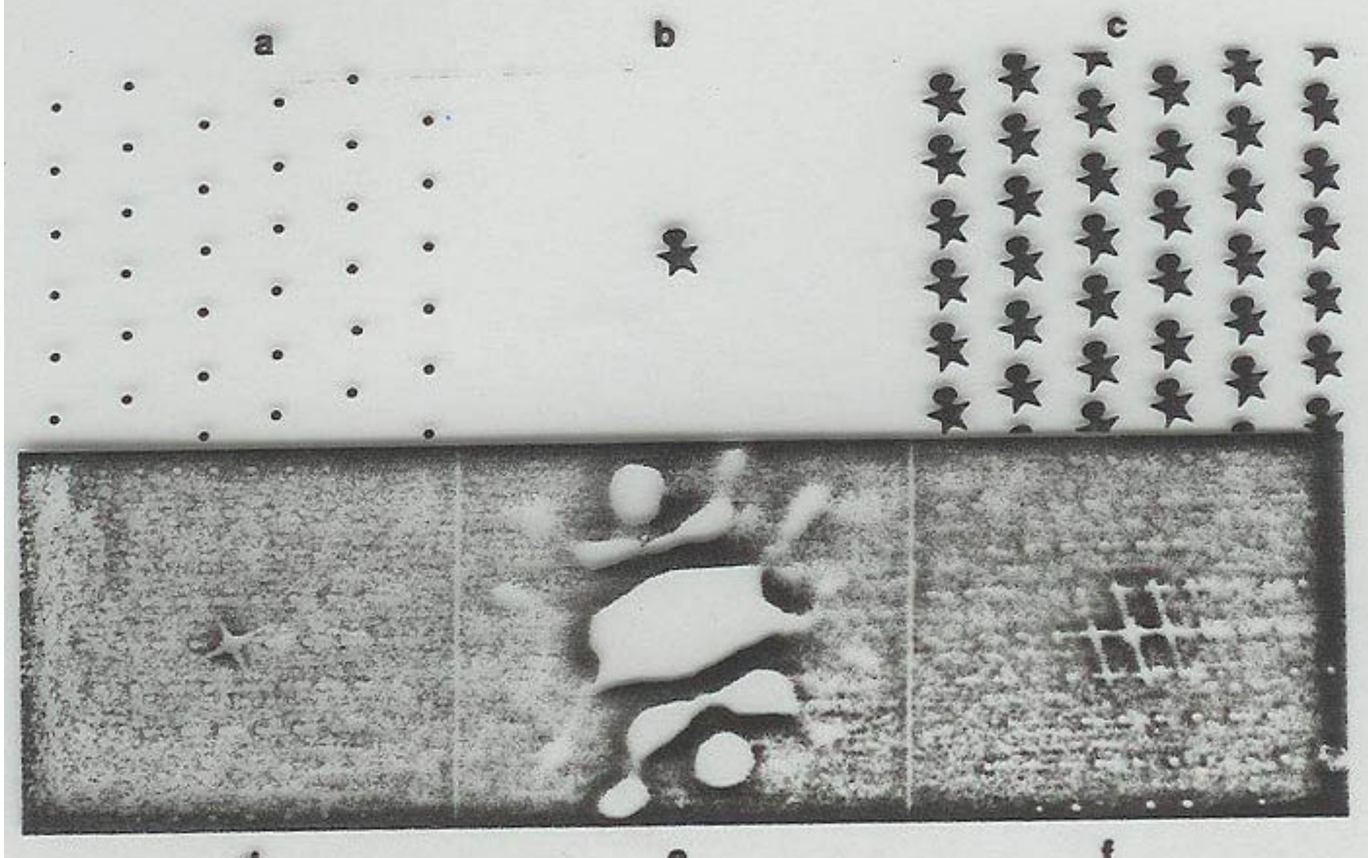
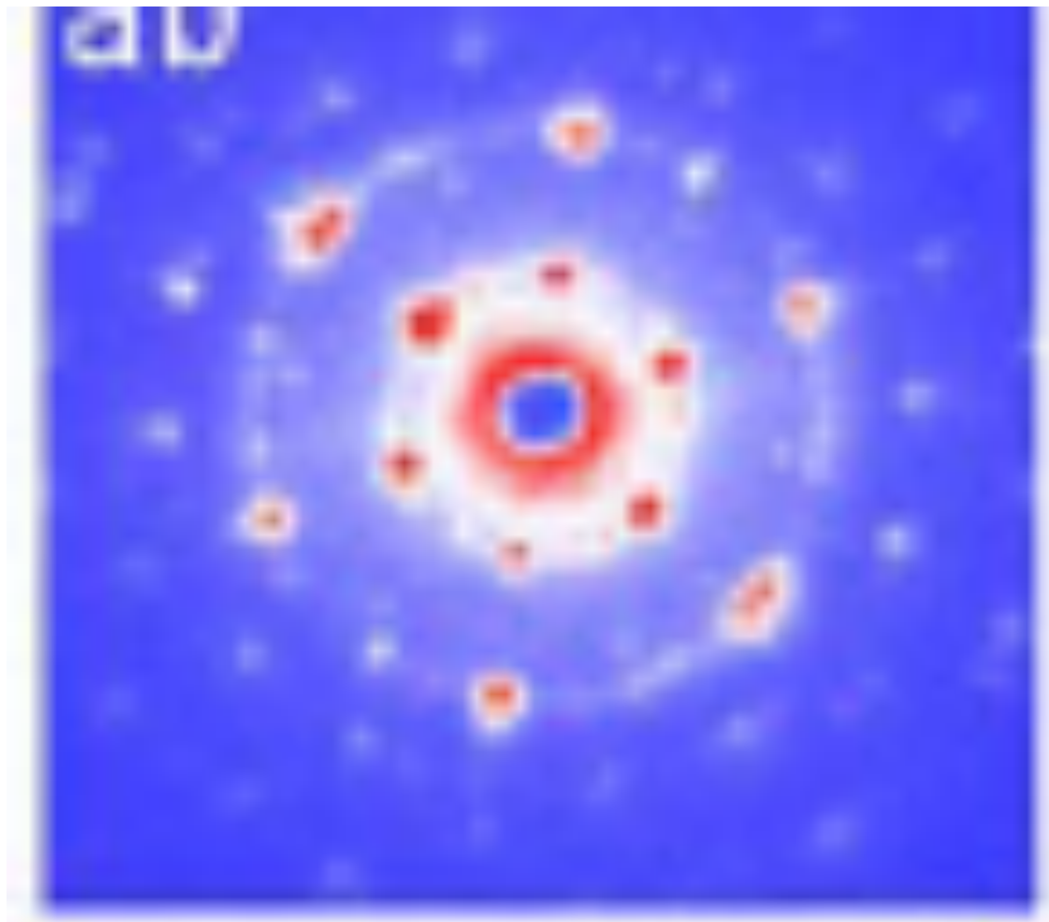


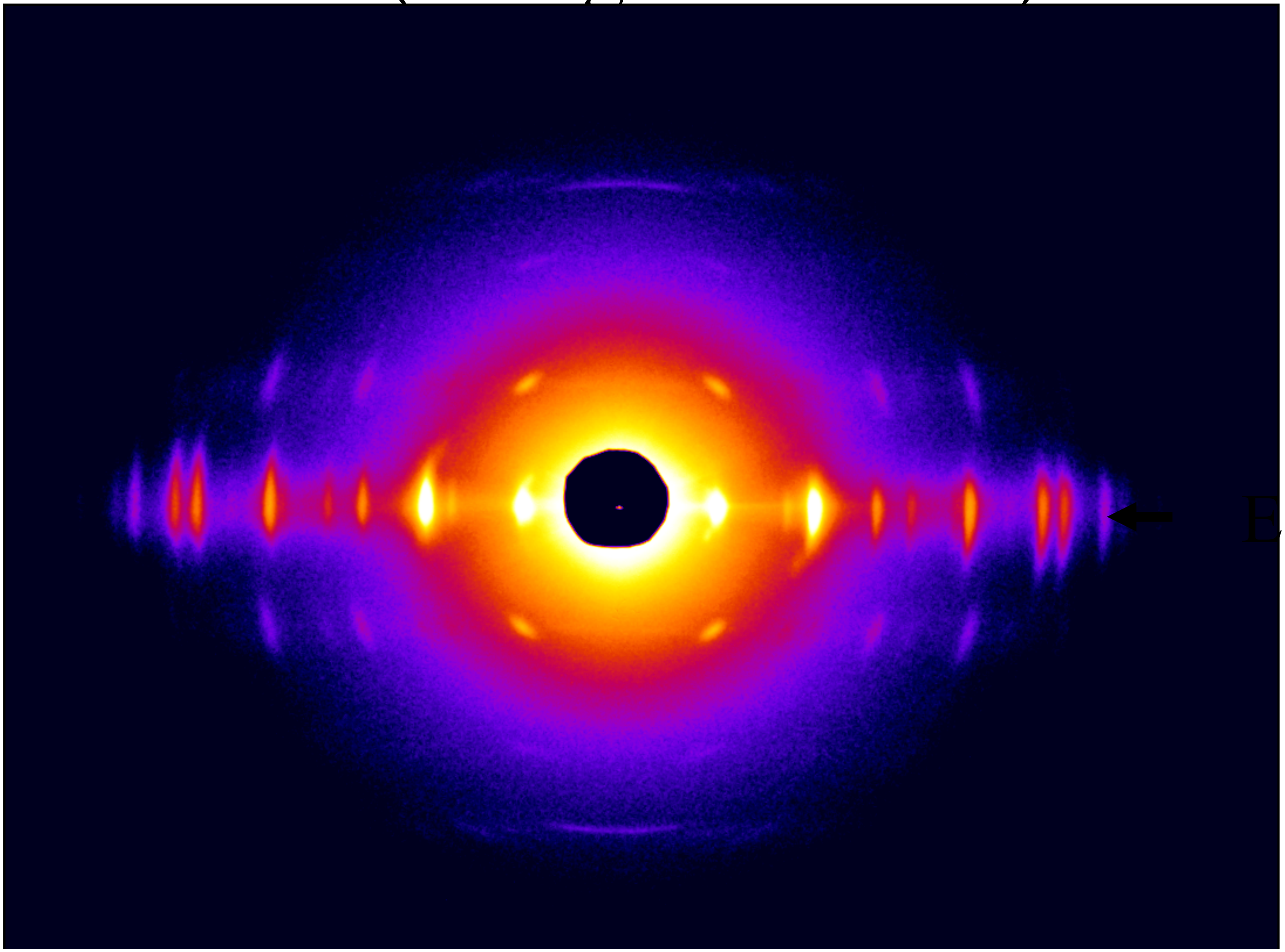

**FIGURE 2.10.** An array of complex objects (c) is produced by convoluting one object (b) with a 2-D array (a). The diffraction pattern (f) from (c) is therefore the product of the diffraction patterns (d) and (e) of (a) and (b), respectively.

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

# End on view of hexagonal reciprocal lattice

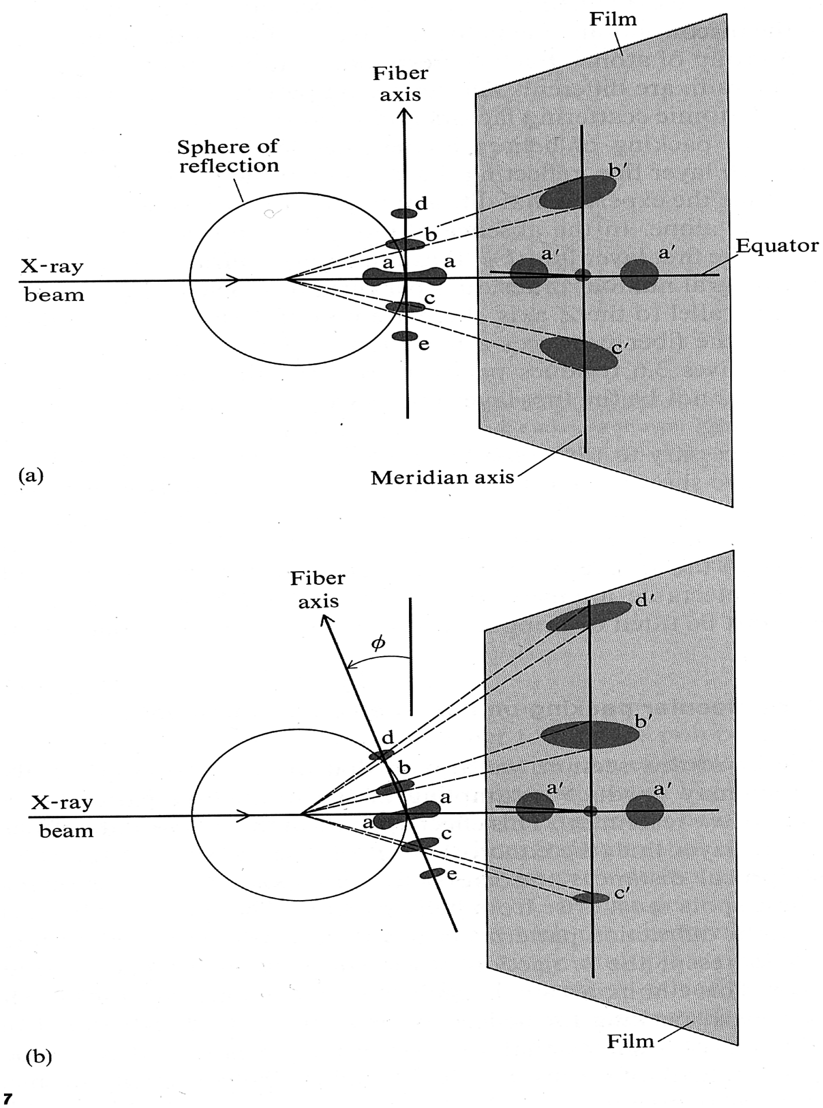


# Fiber diagram - Insect Muscle (hexagonal lattice)

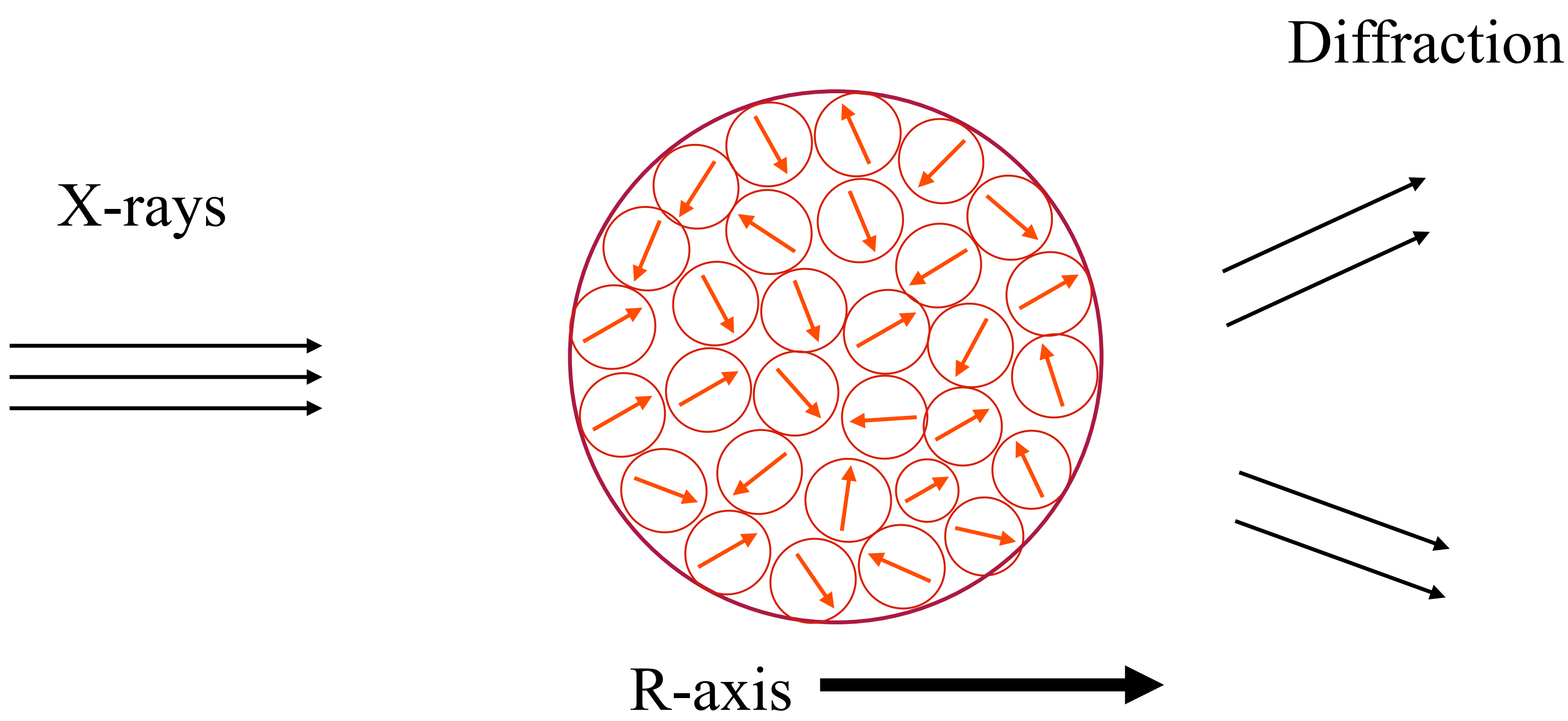


Equator

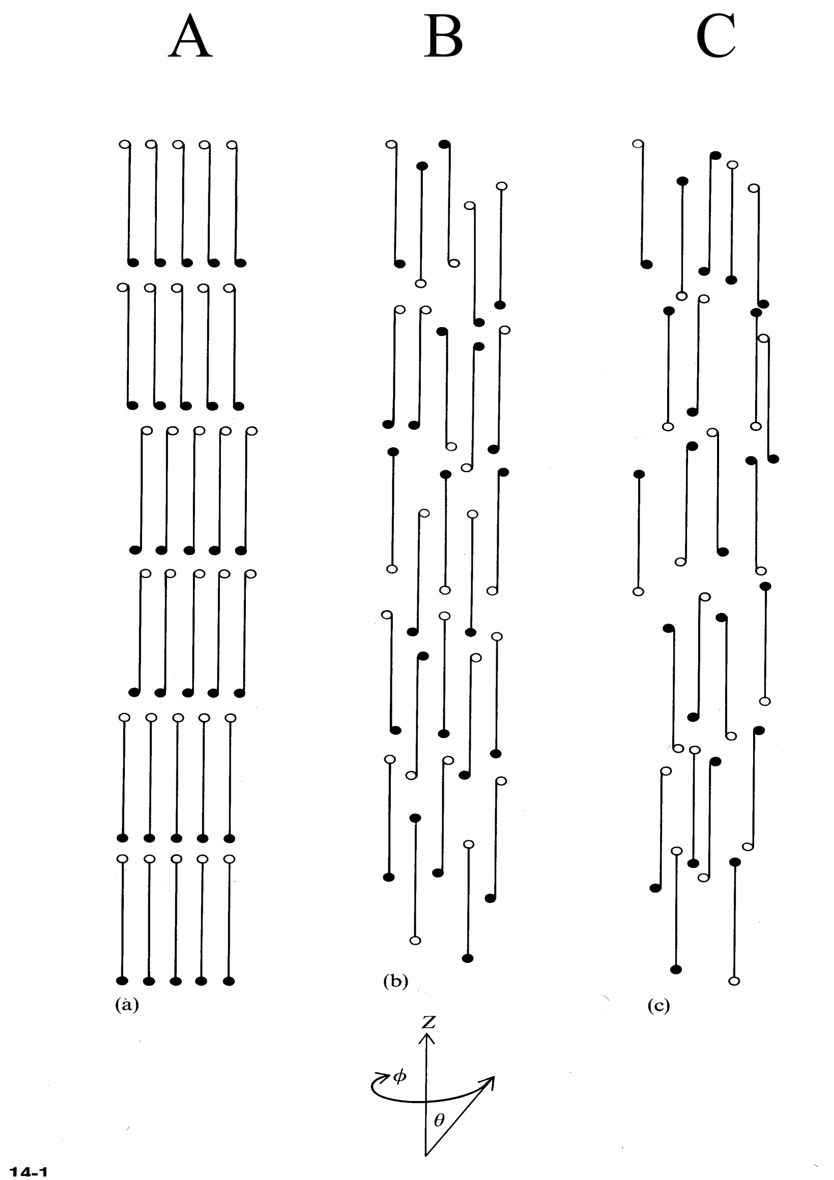
# Geometry of Fiber Patterns



# 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)




Ordering in Fibers:

A - Crystalline fiber

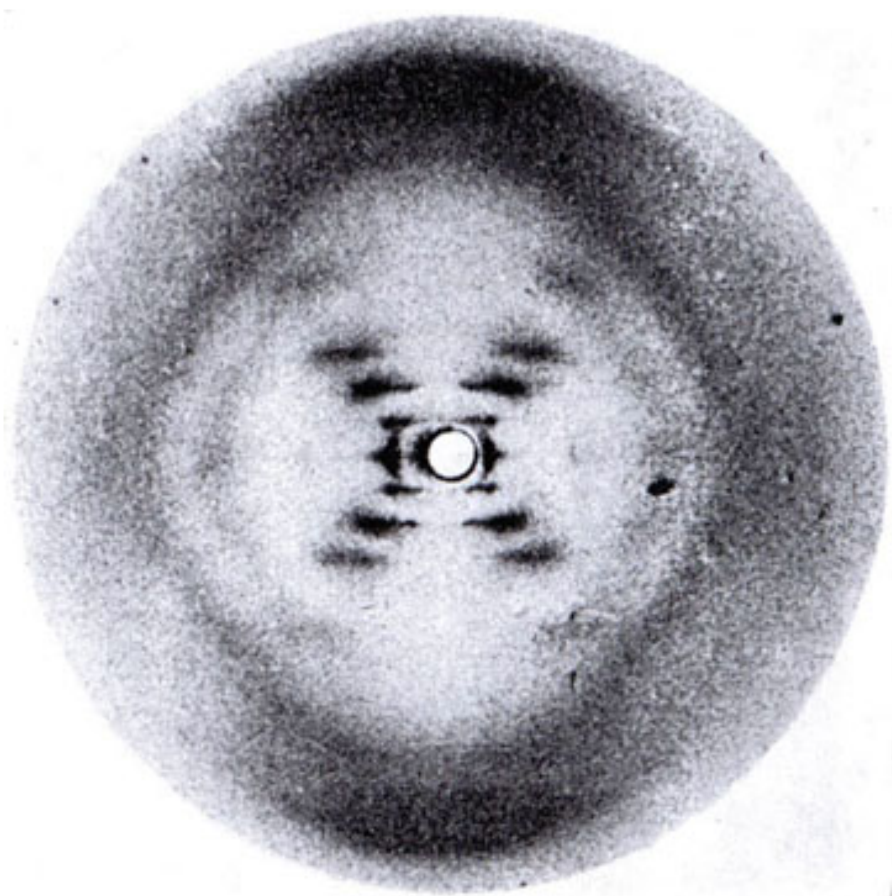
B Semicrystalline Fiber

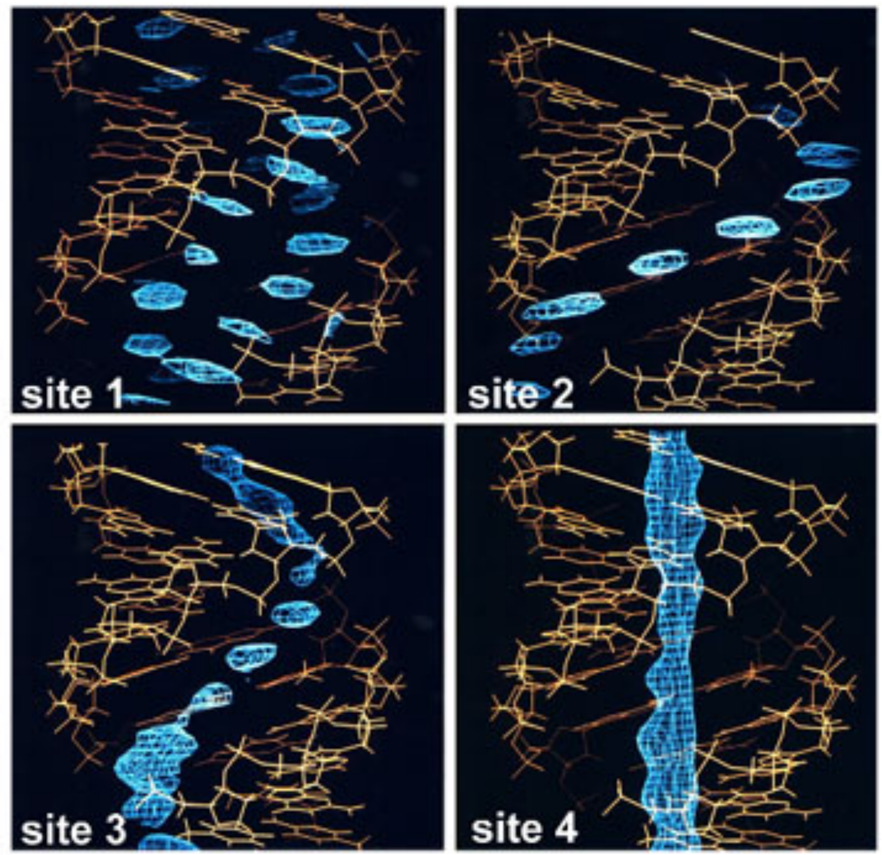
C Non-crystalline fiber

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

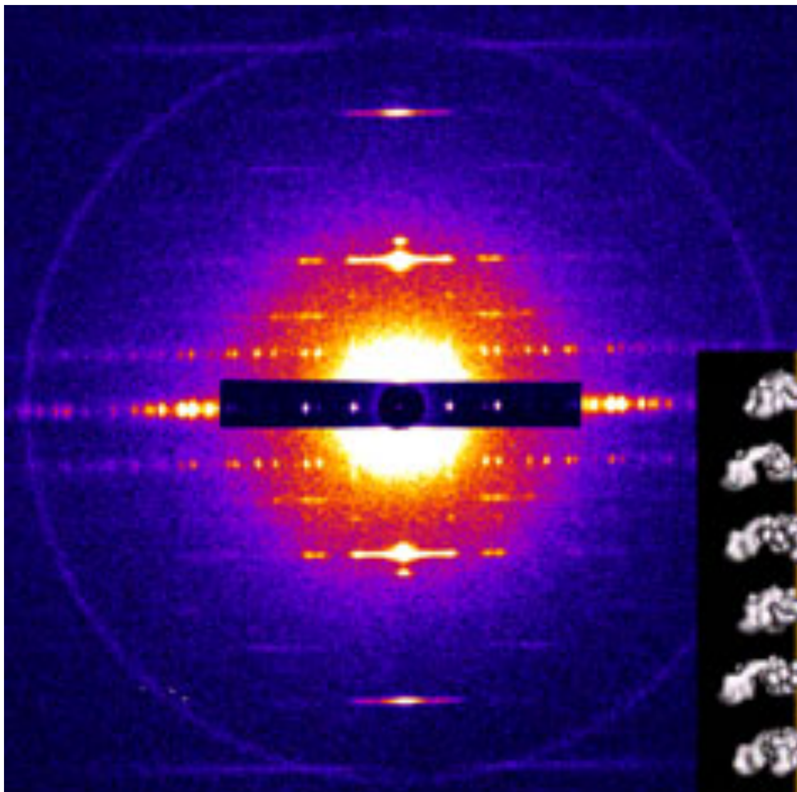
Average over all molecular and lattice orientations

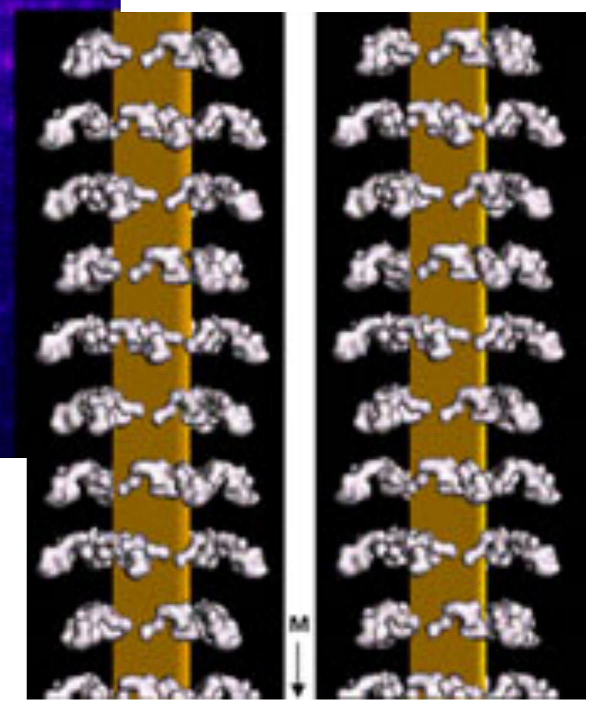
# B-form DNA - Non-crystalline



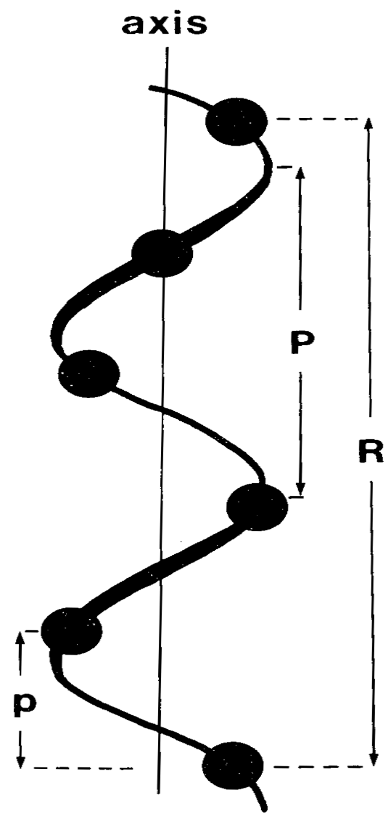


# Insect flight-muscle - crystalline





# Fibrous Proteins Usually Show Helical Symmetry

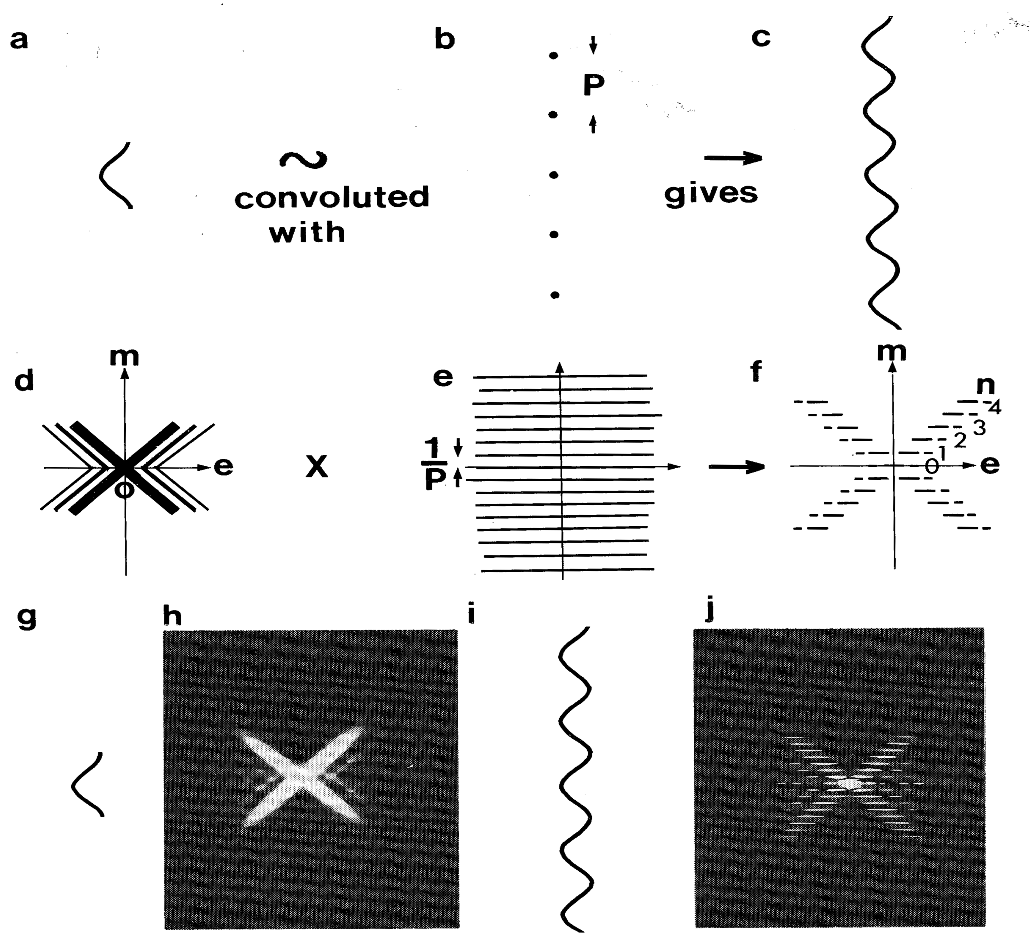



P = pitch

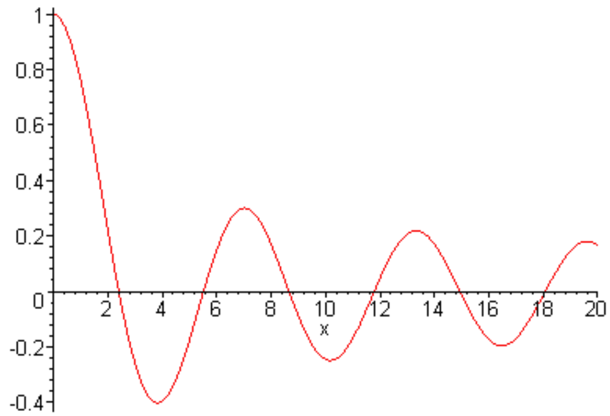
p = subunit axial translation distance

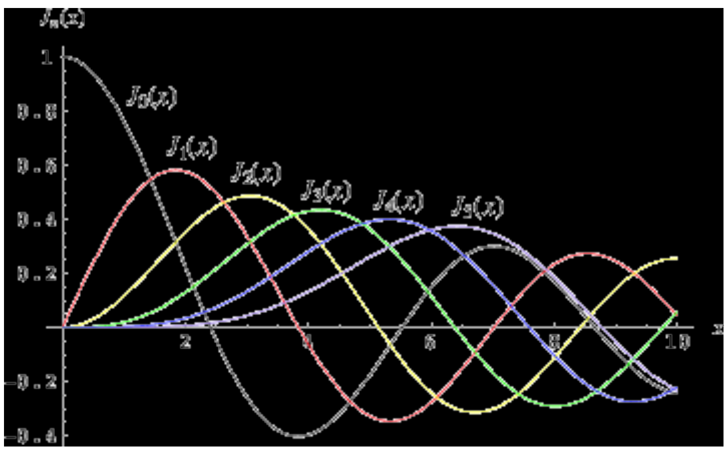
R = true repeat distance

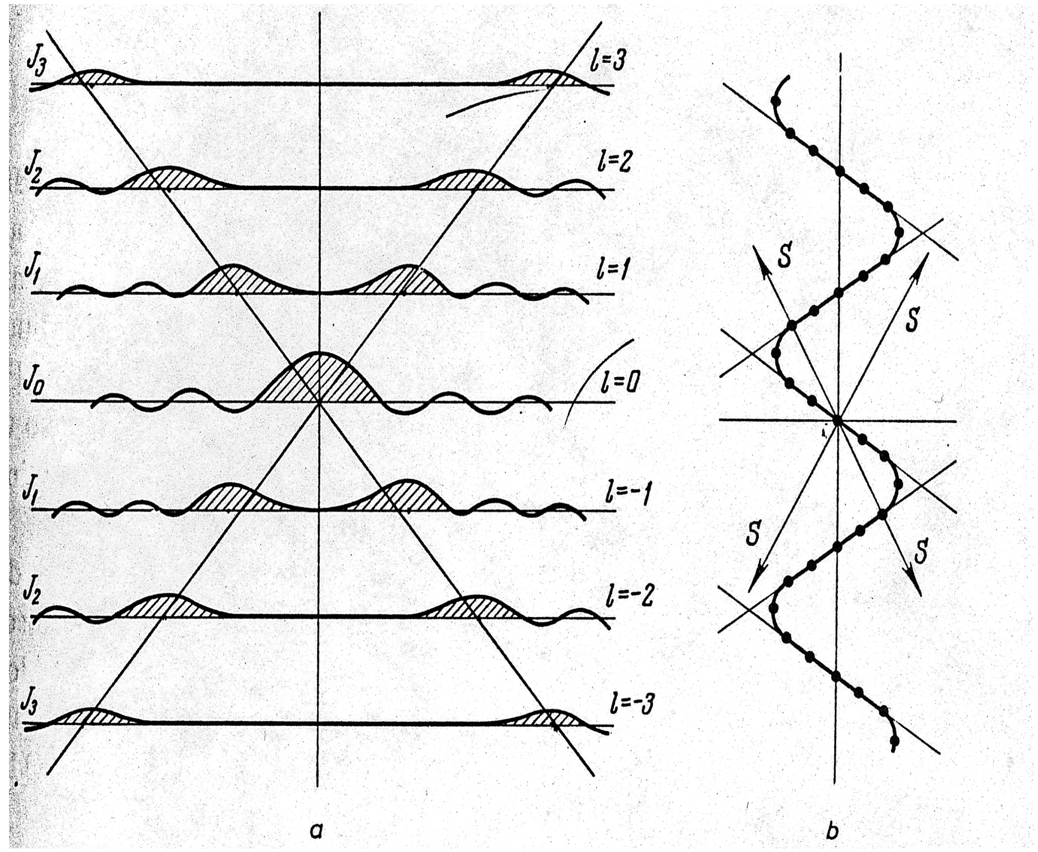
# Diffraction from a continuous Helix



# Bessel Functions and Layer lines

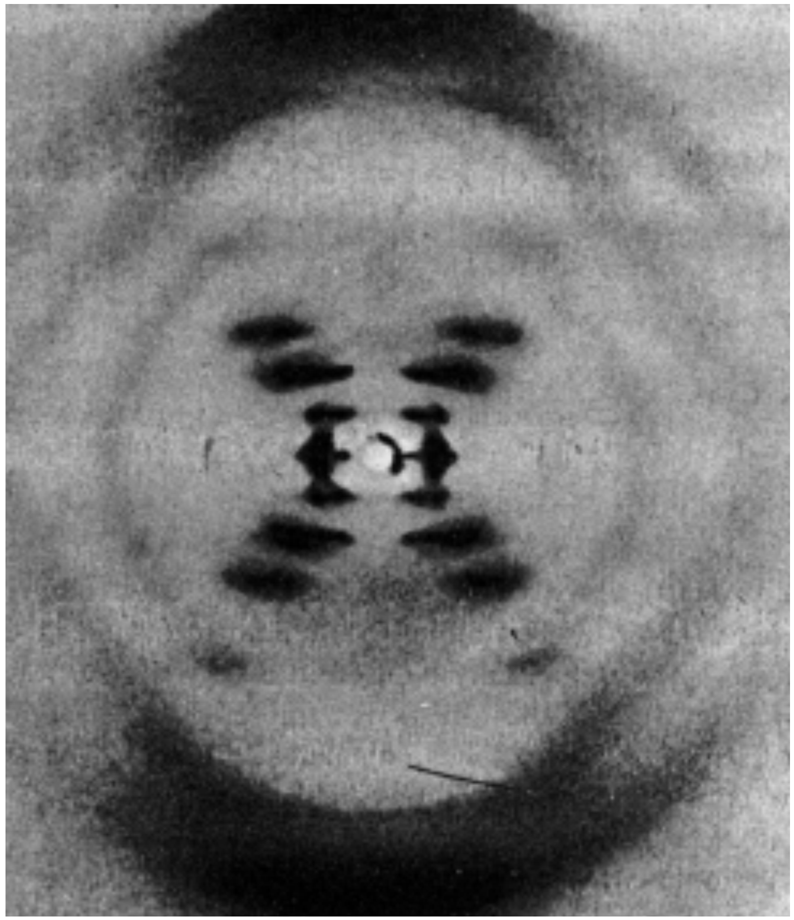



Transform of a cylinder

# Rosalind Franklin's Pattern from B-DNA



Franklin & Gosling, 1953 Nature 171:740

# Diffraction From a Discontinuous Helix

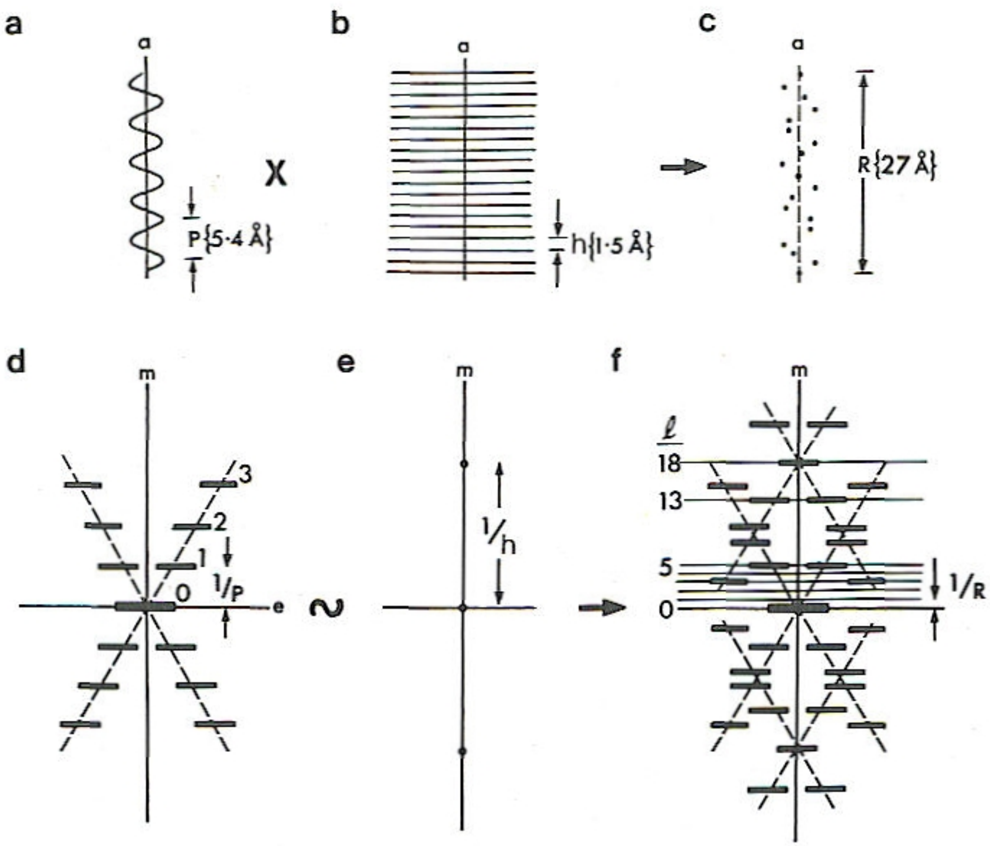
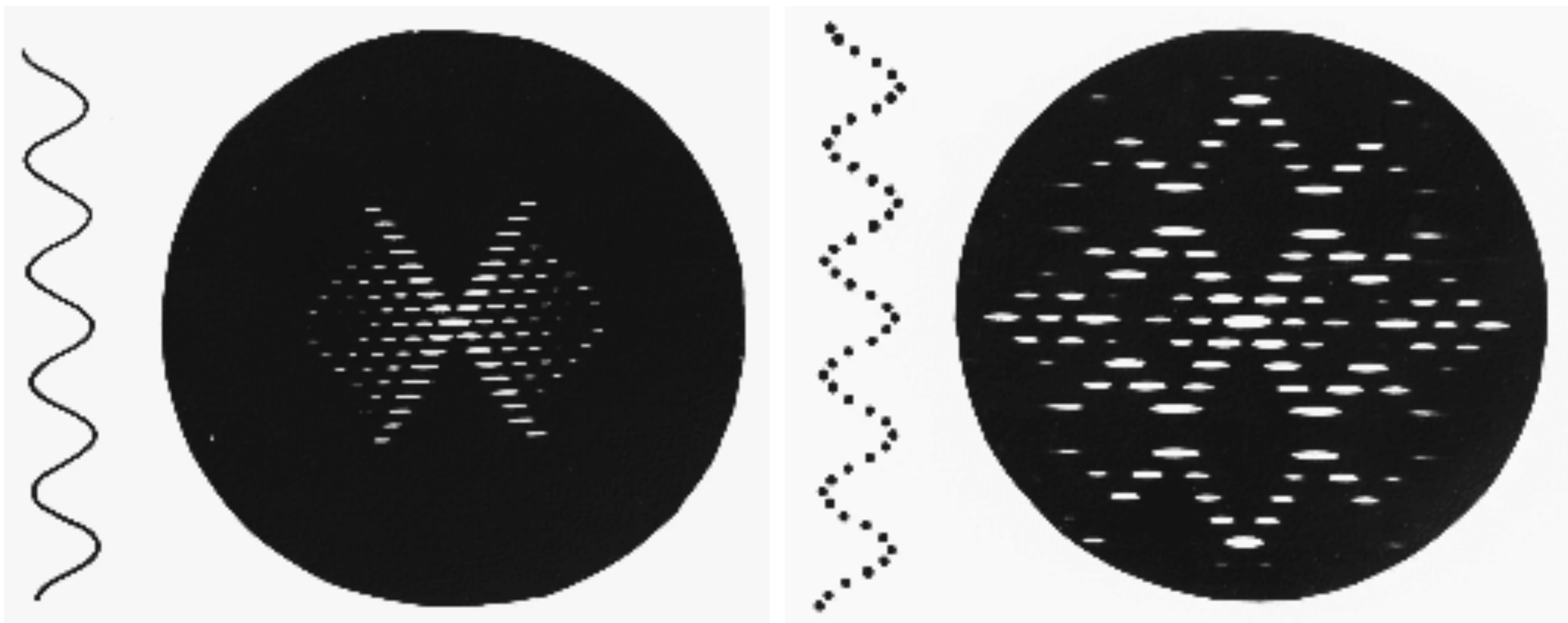



**Figure 3.17** Generation of the form of the diffraction pattern from an 18/5 α-helix. Such a helix (*c*) can be considered as the product of a continuous helix of pitch 5.4 Å (*a*) and a set of density planes spaced 1.5 Å apart (*b*). The helix cross diffraction pattern (*d*) from (*a*) needs to be convoluted with the diffraction pattern from (*b*), a set of meridional points (*e*), to give the diffraction pattern (*f*) from the α-helix. Strong reflections occur on layer lines for which *l* is 5, 13, and 18.

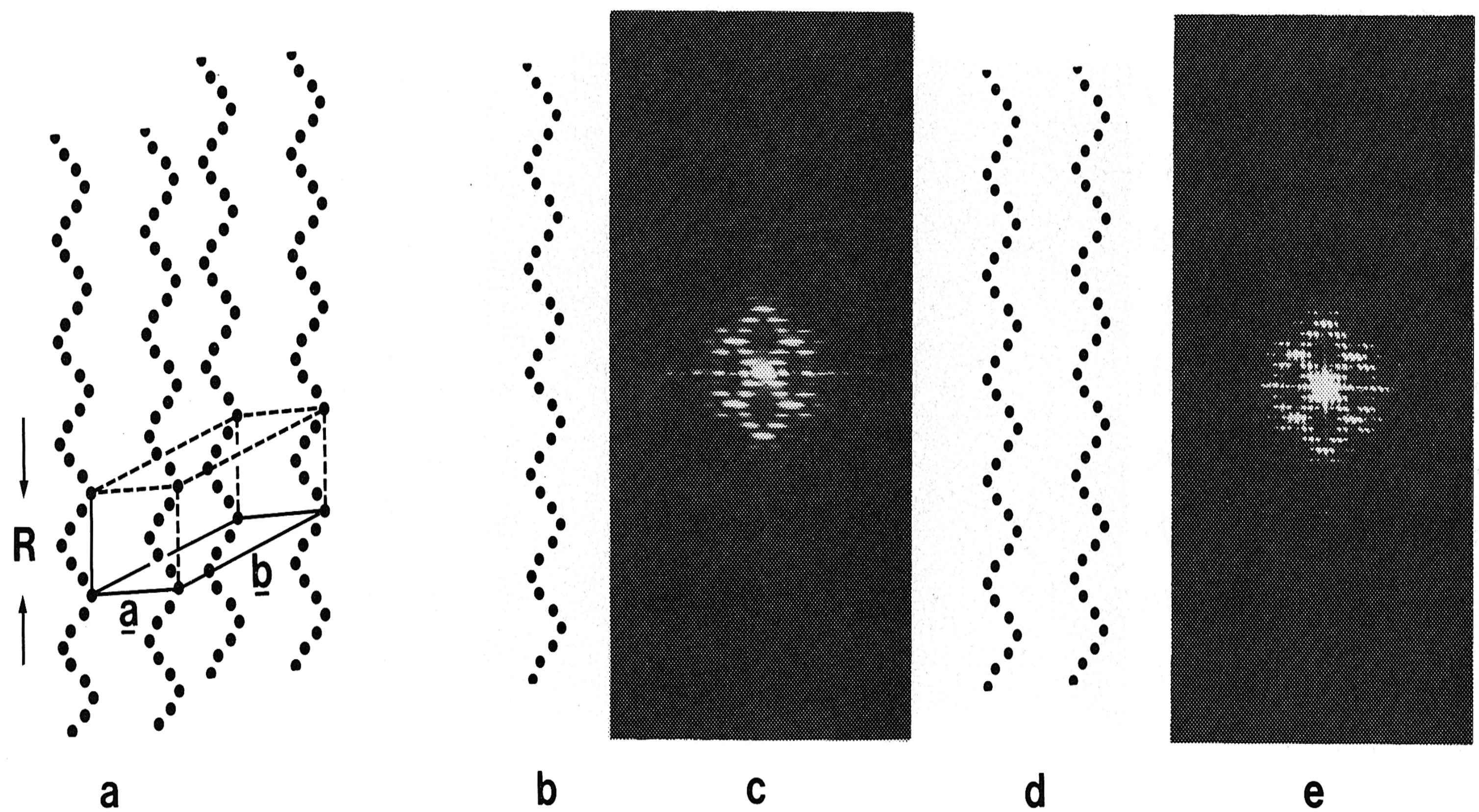
A set of points that are regularly spaced along a helical path

# Diffraction from a helix: comparison

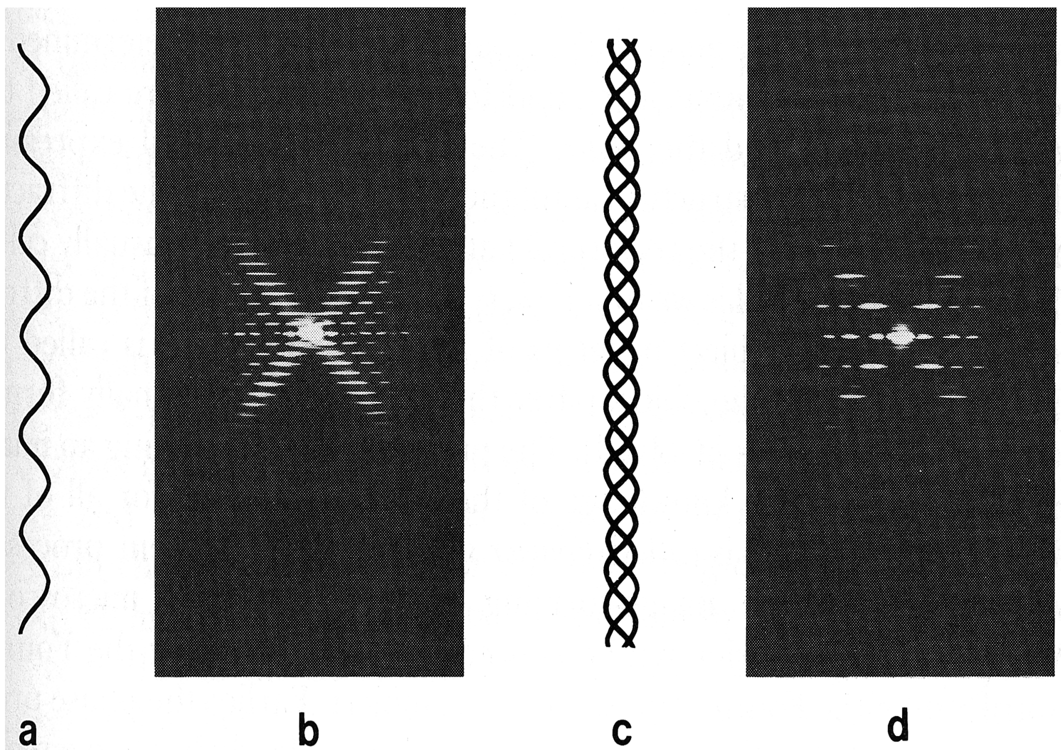


The main effect of shifting from a continuous to a discontinuous helix is to introduce new helix crosses with their origins displaced up and down the meridian by a distance 1/*p*

# Crystals of Helical Molecules



# Multi-Stranded (coiled coil) Helices

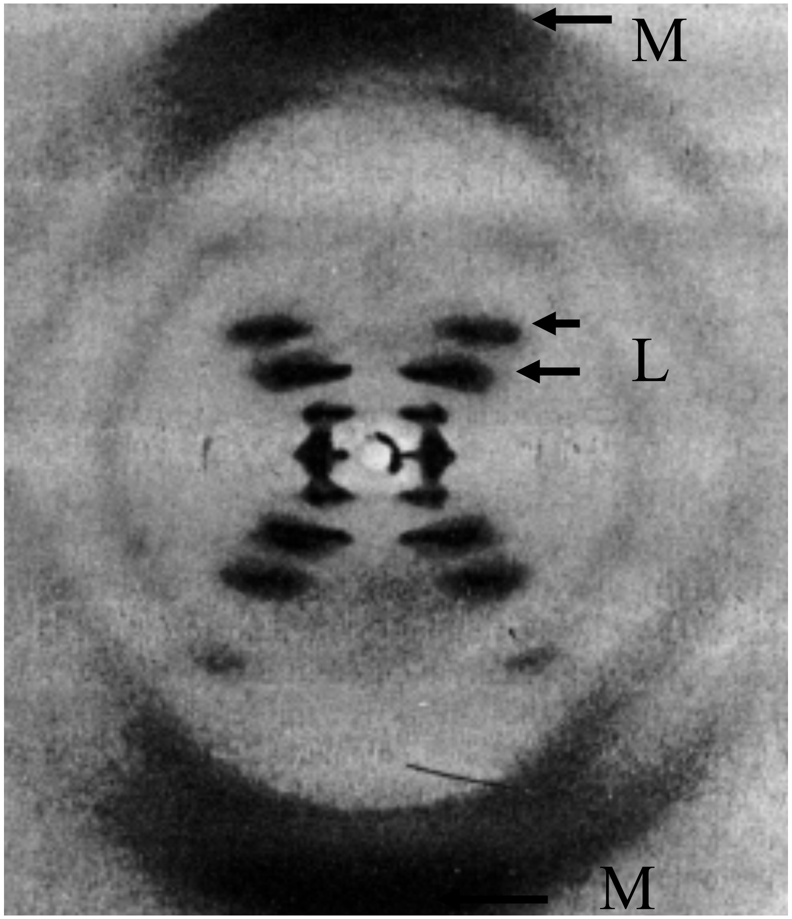



If N strands
Only every
Nth Layer -
line allowed

# 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

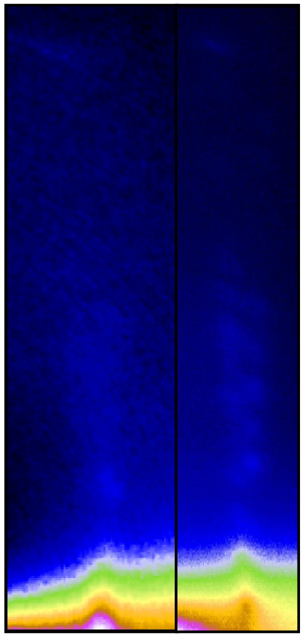
=> 10 residues/turn

Franklin & Gosling, 1953 Nature 171:740

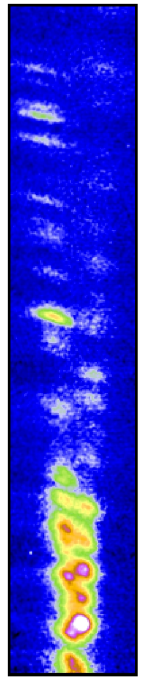
# Fiber Crystallography

- Most fiber “structures” result of model building studies
- There have been a small number of  Fiber “structure solutions”.
- High resolution structure by Keichi Namba on bacterial flagella (Yamashita et al., 1998 Nature SB) aligned by high magnetic fields
- Orgel et al. (2001 Structure) published first MIR structure of a natural fiber - Type I Collagen from rat tail collagen

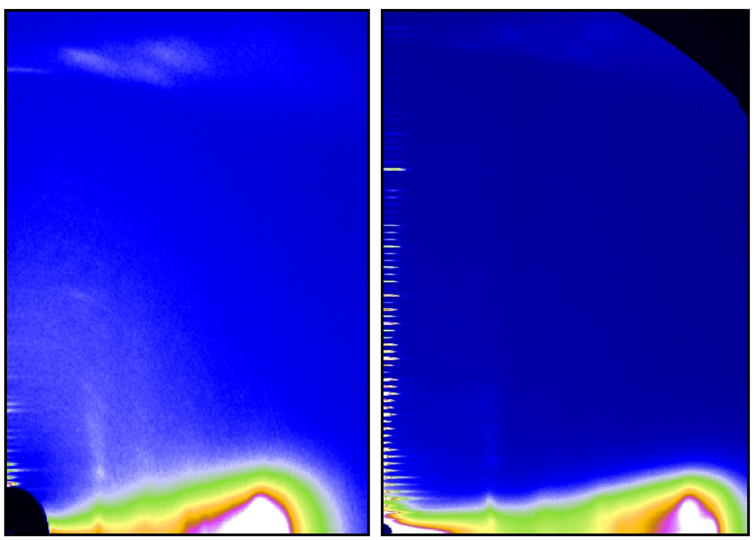
Data collection:



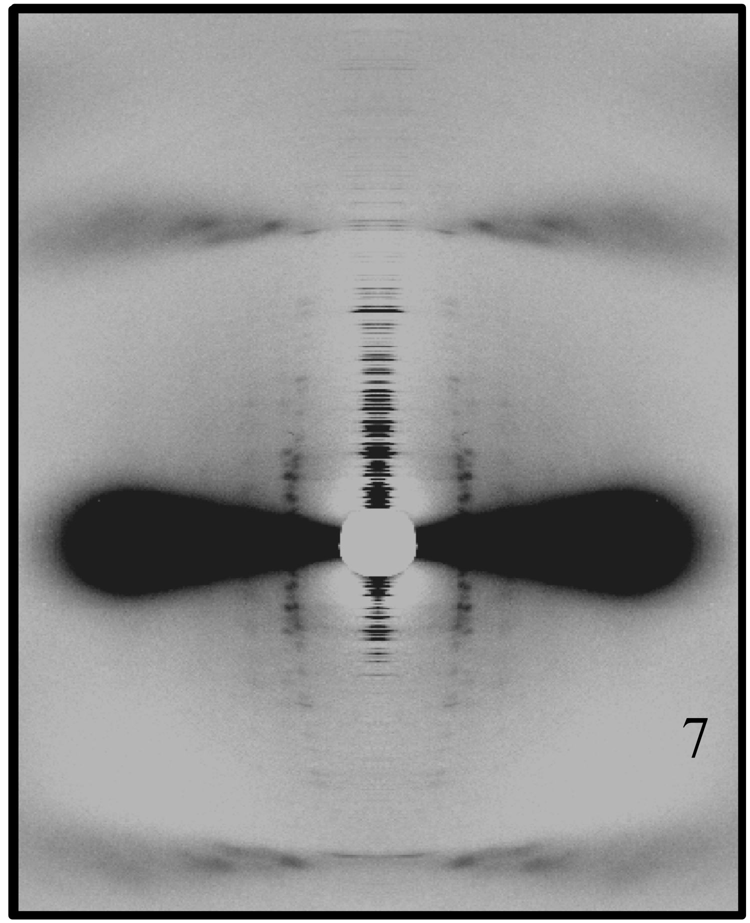
7 B



B



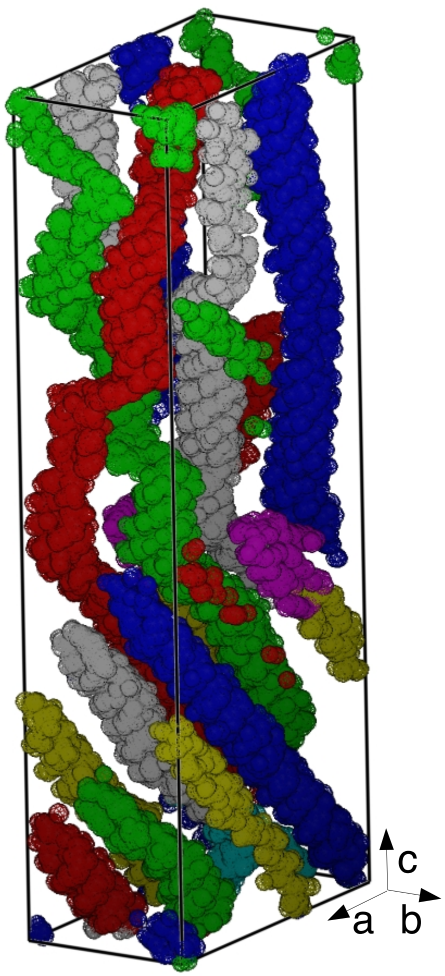
7 B



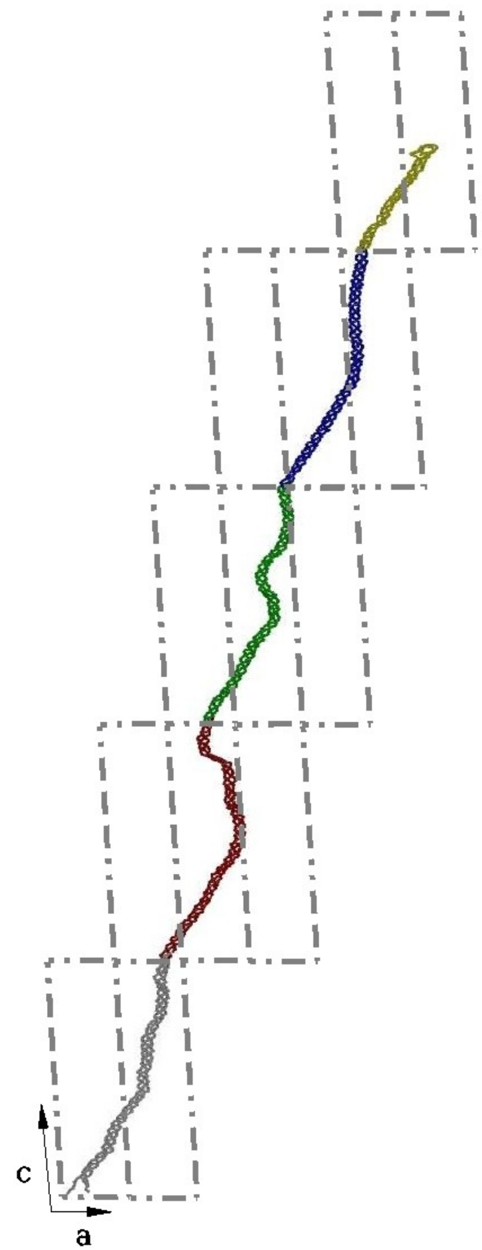
7

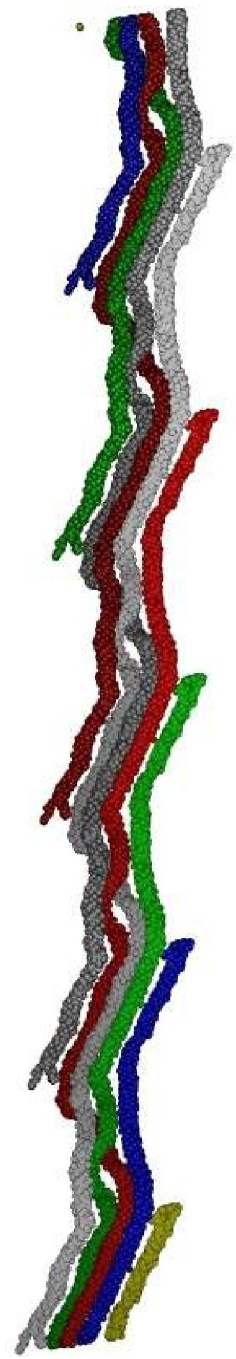
B=Bio-CATid
7=7.2bm

# Collagen type I sub-fibrillar structure

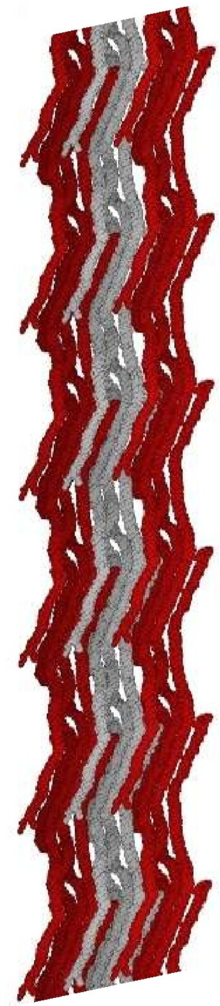



Resolution ~ 11 /5.16 Å



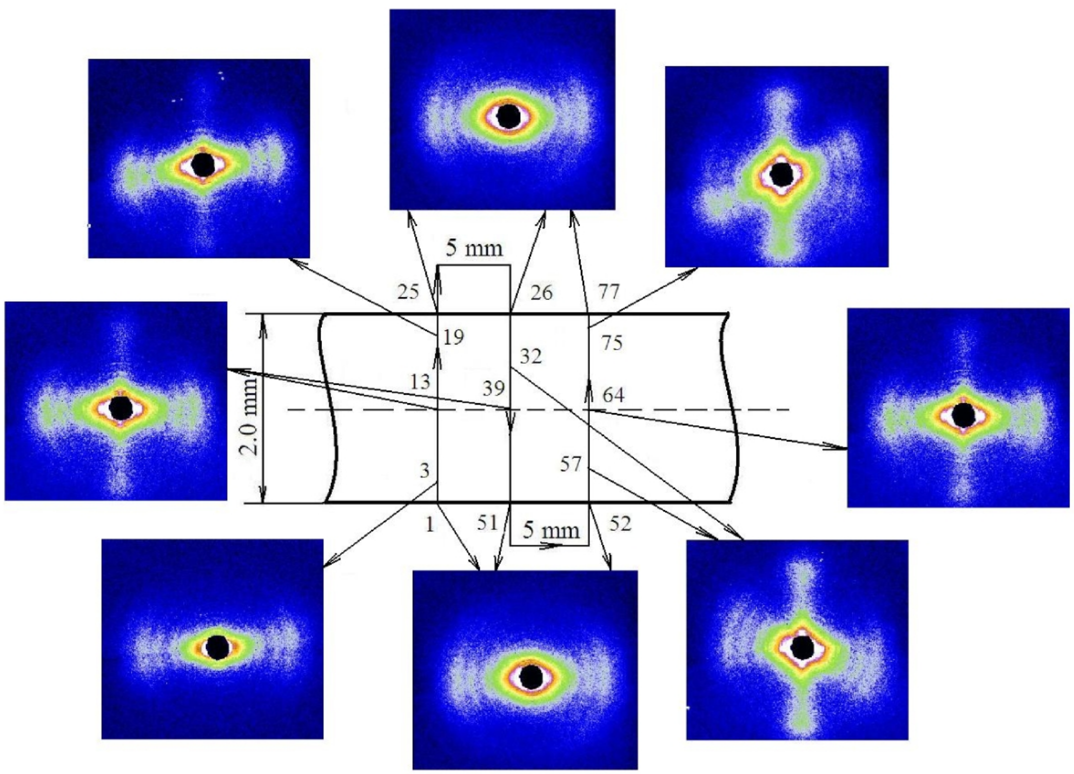
C

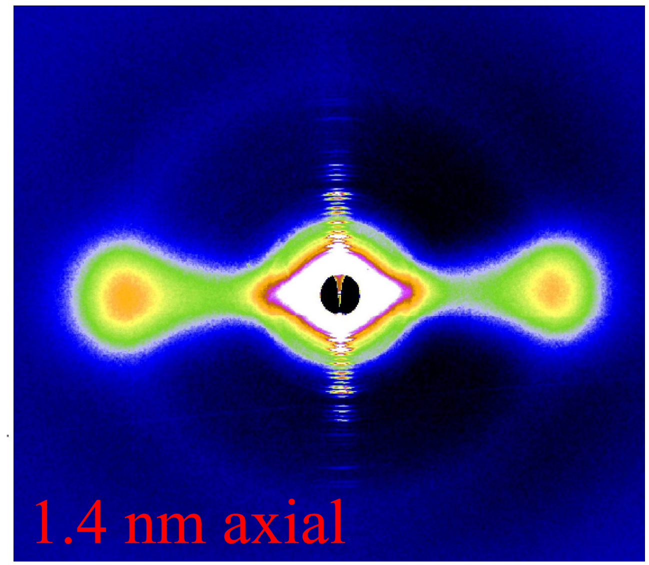


D

# Structural Studies of Collagen Type 2 from Lamprey Notochord(Orgel)

Histology mapping shows where the most crystalline /oriented regions are located




1.1 nm equatorial

# 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**:
  John Squire "The Structural Basis of Muscular Contraction" Plenum, 1981
- **Definitive Reference**:
  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.