# Fibre Diffraction

Dr. Himadri S. Gupta
School of Engineering and Materials Science
Queen Mary University of London

S4SAS 2014

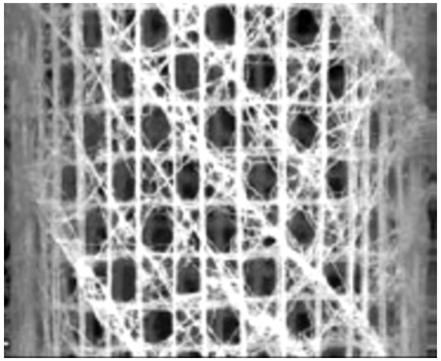
# (Mineralized)
# Fibre (il)
# (Small-Angle) Diffraction
# Combined with Mechanics

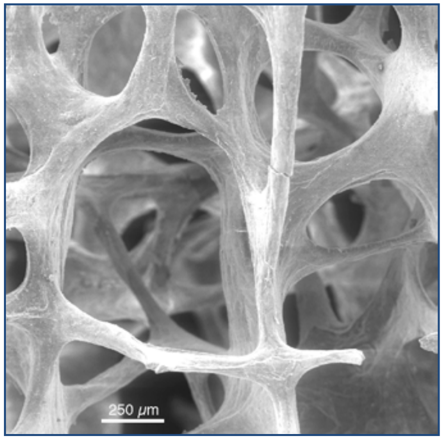
Dr. Himadri S. Gupta
School of Engineering and Materials Science
Queen Mary University of London

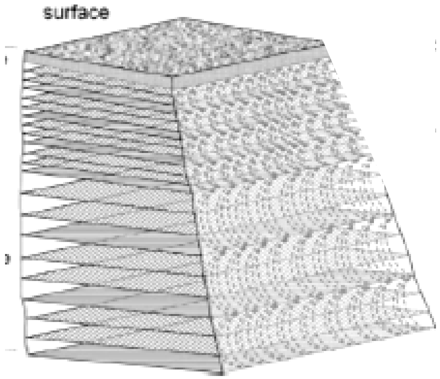
S4SAS 2014

# Outline

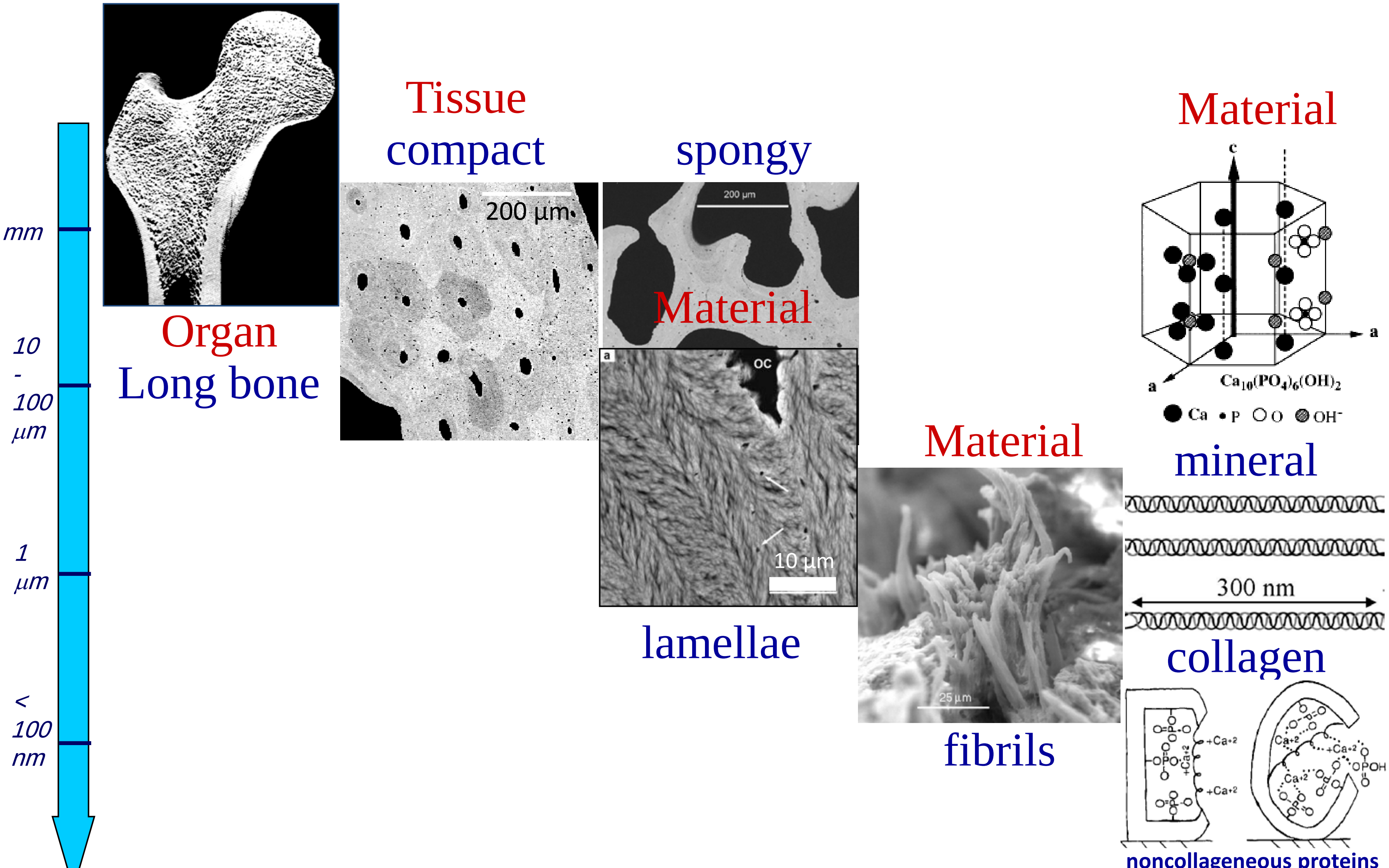
- (why ?) Mineralized Biological Tissues
  - as hierarchical fibre composites
- SAXS on mineralized fibrils: mechanics
  - Meridional scattering features
  - Data reduction for fibril mechanics
  - Example data sets
- WAXD on mineralized fibrils
  - Data reduction for mineral mechanics
  - Orientation and sample geometry
  - Example data sets
- SAXS on fibrils: orientation
  - Identification and background correction
- SAXS on fibrils: radial deformation
  - Approximate methods
- Summary





# Bone as a hierarchical material

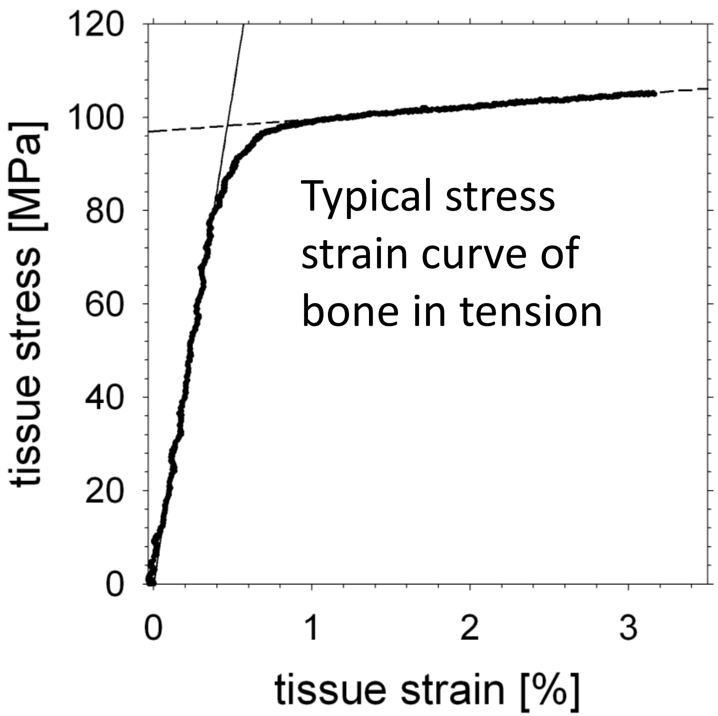



J. P. Gorski, *Calcif. Tissue Int.* 1992

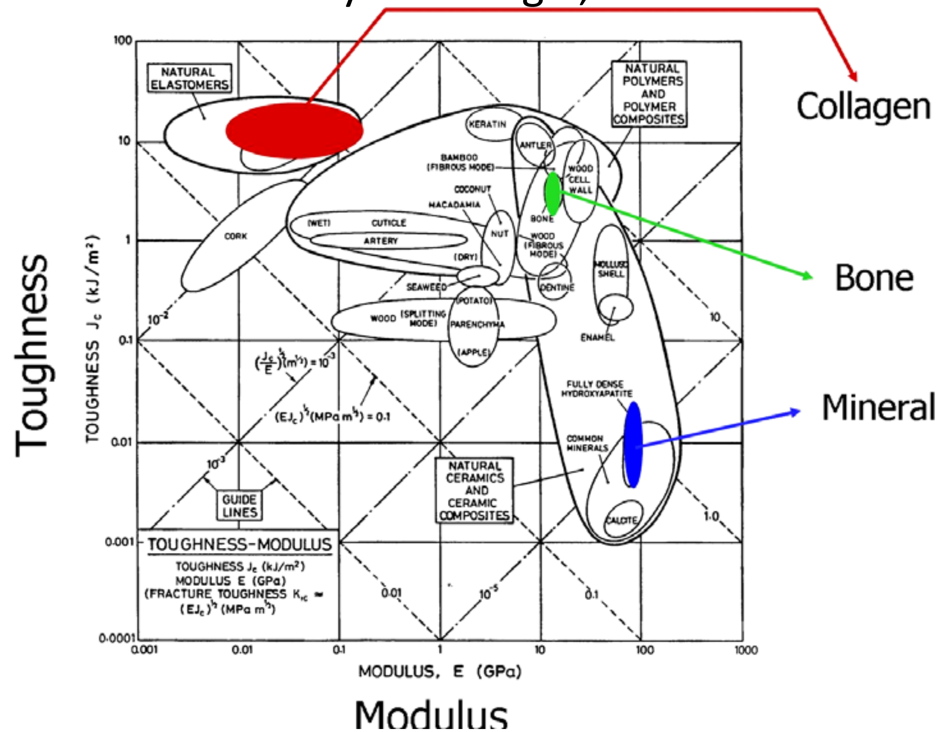
# The Key Mechanical Quality of Bone

## *Combination of Two Complementary Properties*

Elastic modulus




Ashby and Wegst, 2000




Elastic modulus:
Resistance to reversible (small) deformation

Toughness:
Energy needed to tear the material apart

Stress: Force per unit area
Strain: Percent elongation under load

# The nano-composite nature of the bone matrix

## *How does it contribute to toughness?*

## collagen fibrils

100 – 200 nm diameter

axial periodicity in electron density with period D = 64 – 67 nm

triple helical collagen molecules, ~ 300 nm long, 1.2 – 1.5 nm lateral spacing (Ottani et al *Micron* 2004)

## apatite mineral

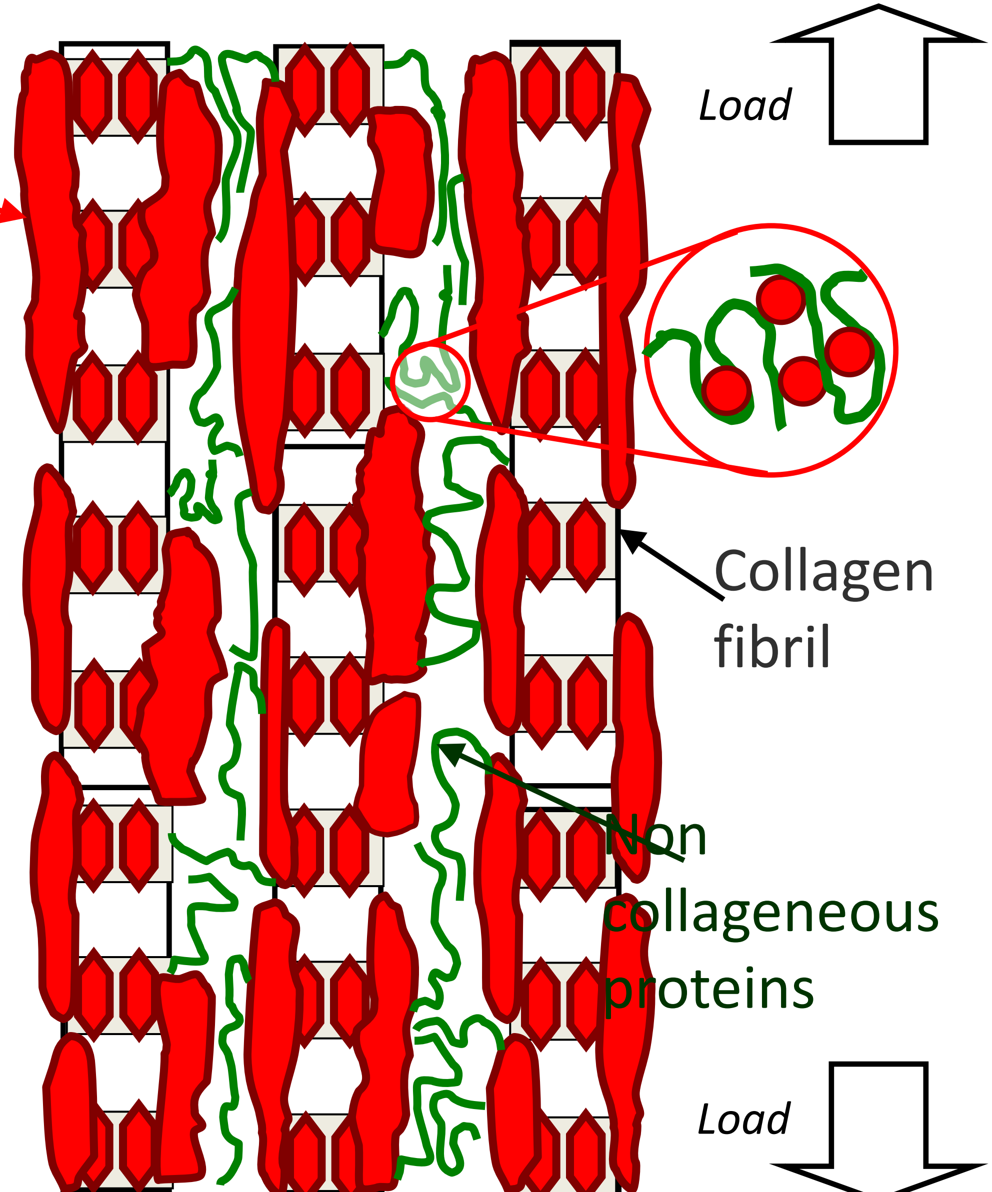
carbonated apatite platelets;

3 – 5 nm thick, 30 – 50 nm wide (W. J. Landis et al *J. Struct. Biol.* 1996, Rubin et al , *Bone*, 2004)

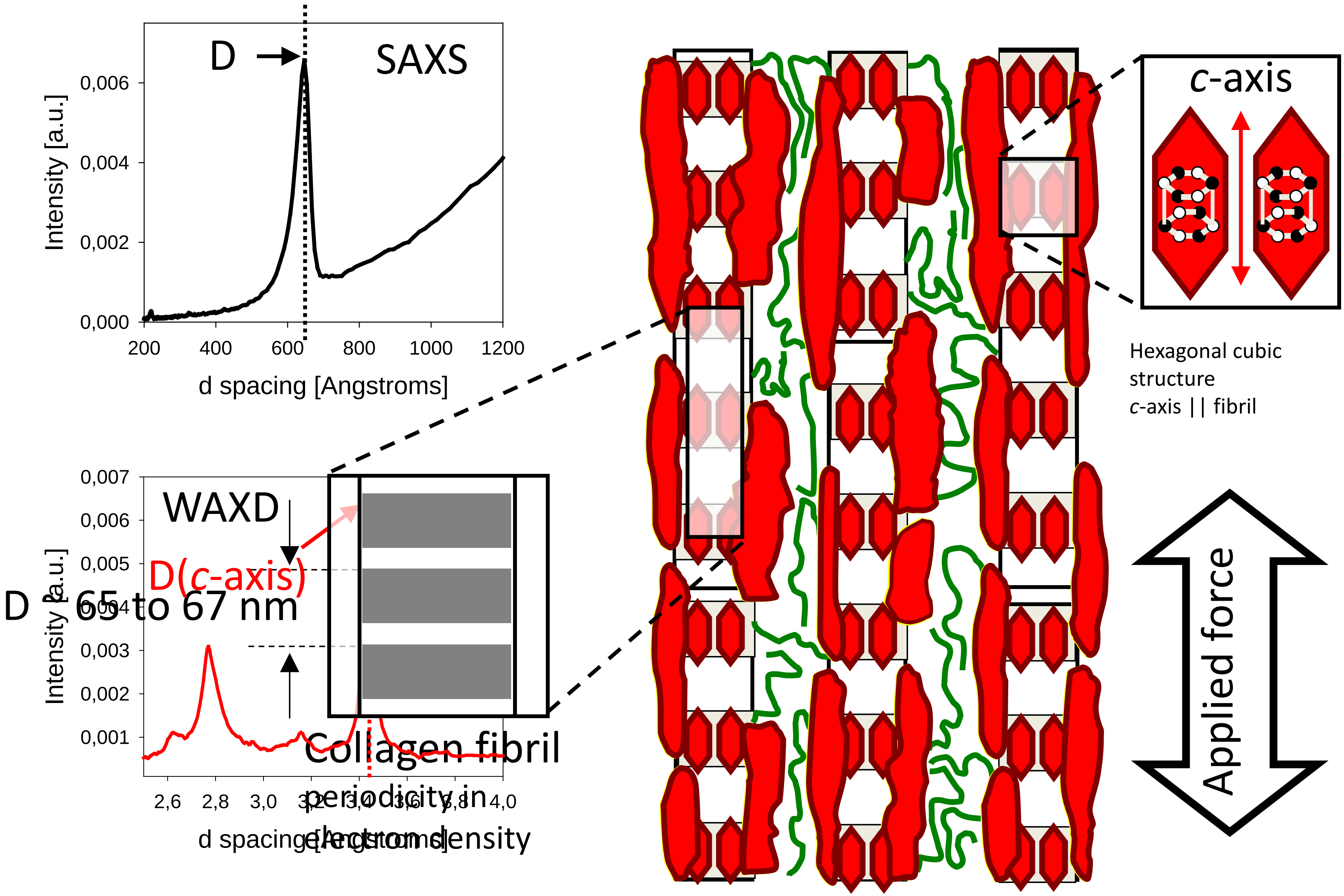
## noncollageneous proteins

phosphorylated, -vely charged, amorphous; ~ 1 - 2 wt % bone

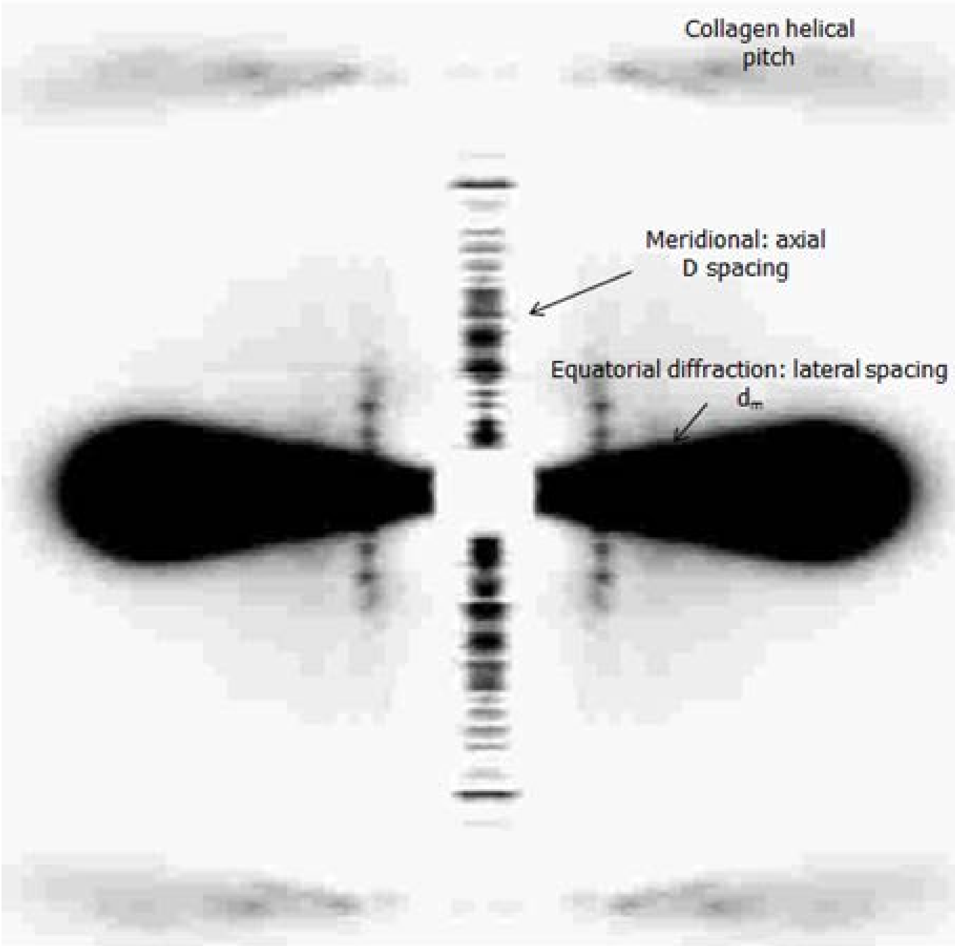
osteopontin, osteocalcin (Nanci et al, *J. Struct. Biol* 1999)



# SAXD diffraction spectrum from mineralized collagen fibril



# SAXD diffraction spectrum from collagen fibril



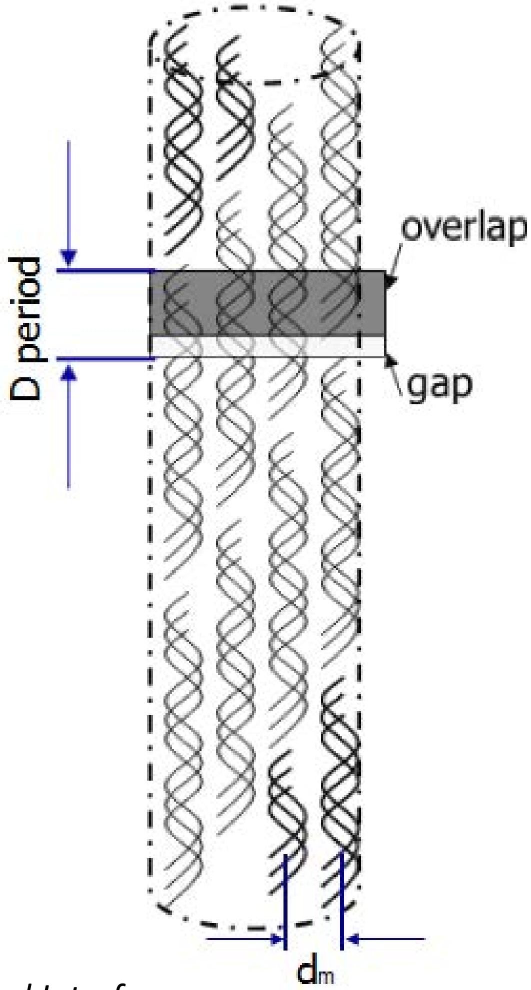
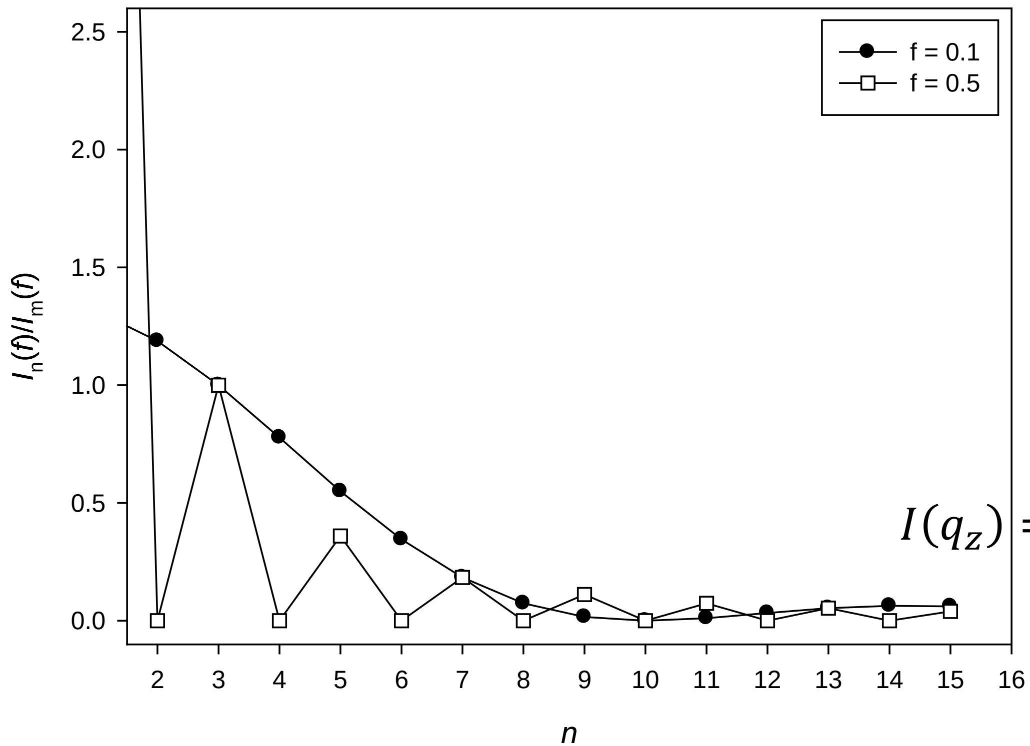




*Image courtesy of Peter Fratzl, Max Planck Institute of Colloids and Interfaces*

# SAXD diffraction spectrum from unmineralized collagen fibril

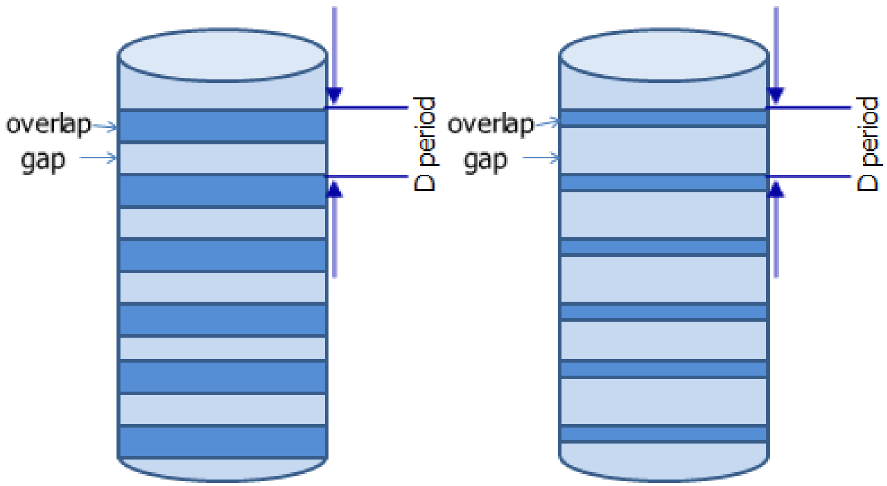



$$I(\boldsymbol{q}) = \left| \iiint \rho(\boldsymbol{x})\, e^{-i\boldsymbol{q}.\boldsymbol{x}} d\boldsymbol{x} \right|^2$$

$$= \left| \iint dx\, dy e^{-i(q_x x + q_y y)} \int dz\, \rho(z) e^{-i q_z . z} \right|^2$$

$$I(q_z) = \left( \frac{2\rho_0}{q} \cdot \frac{\sin\left(\frac{qfD}{2}\right)}{\sin\left(\frac{qD}{2}\right)} \cdot \sin\frac{qD}{2} \cdot (2M+1) \right)^2$$

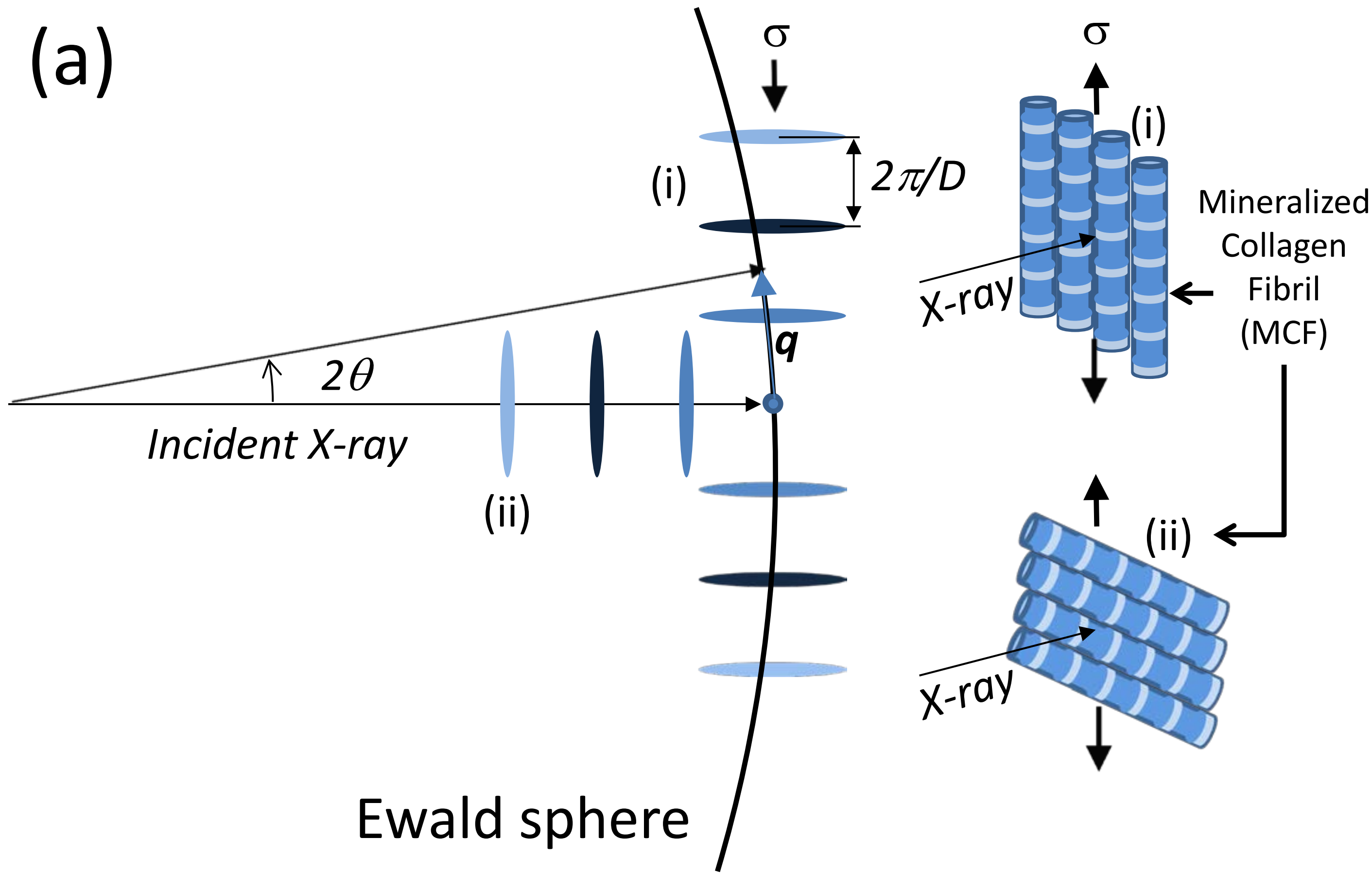
- Depends on the gap/overlap ratio
- In tendon collagen, O/D ~ 0.48
- Slice/plane through 3D object




f= overlap/(overlap+gap)=0.5

f= overlap/(overlap+gap)=0.1

# Importance of Aligning Fibrous Texture with X-ray Beam




A. Karunaratne et al, *Methods in Enzymology* (2014)

# Principle

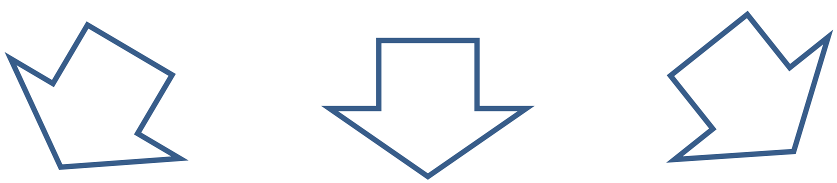
Design micromechanical rig

Fit into sample stage on I22

DEFORM!

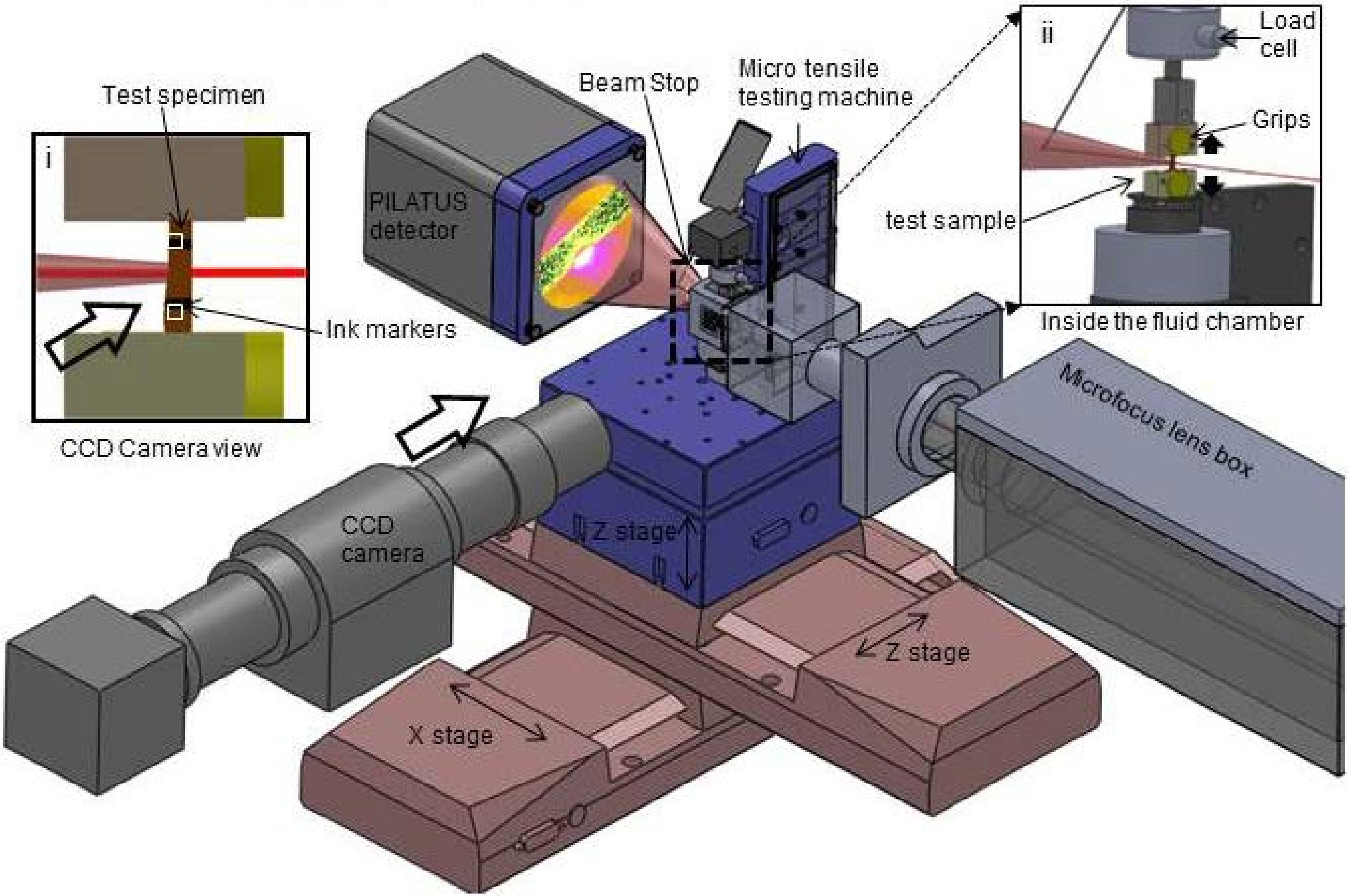
Figure out what the changes in SAXS/WAXD patterns mean …

Build a model or propose a mechanism



Applications (biomaterial / biomedical)

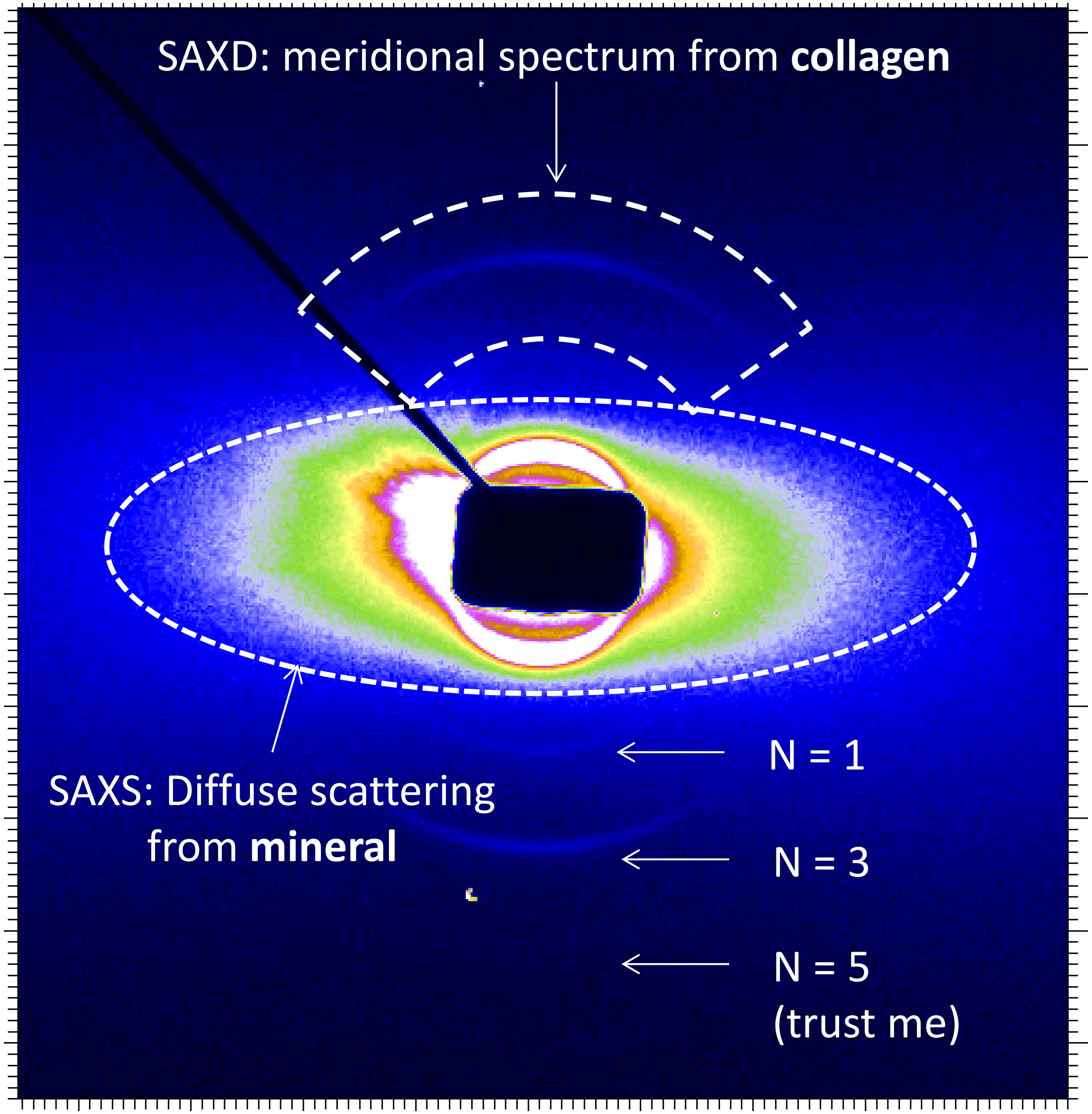
# Schematic of an In situ SAXS/WAXD deformation setup




A. Karunaratne et al, *in review*

# A representative SAXS spectrum of mineralized collagen

## Multiple structural contributions need to be separated



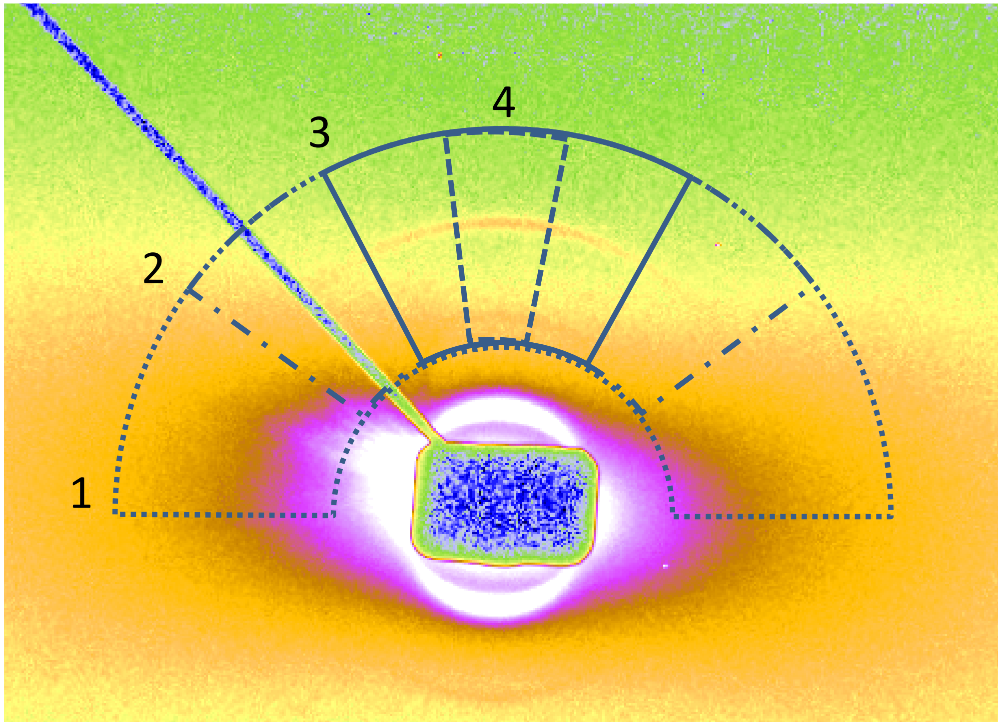

D stagger in fibril

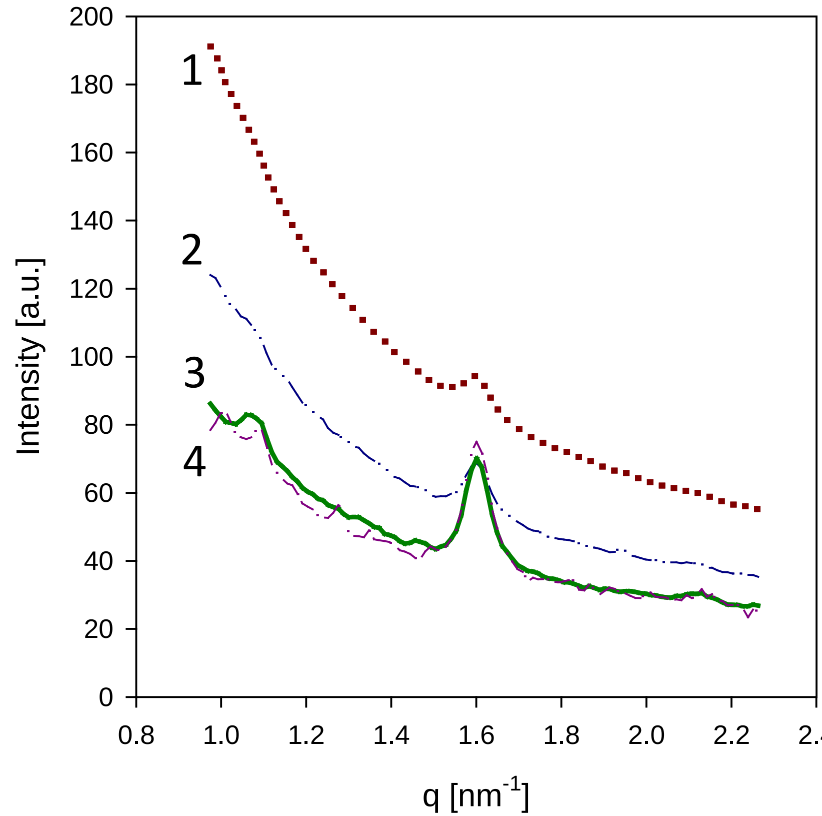
Direction of fibrils

Mineral platelet: Orientation, shape, size

S. Krauss et al, *Bone* (2009)

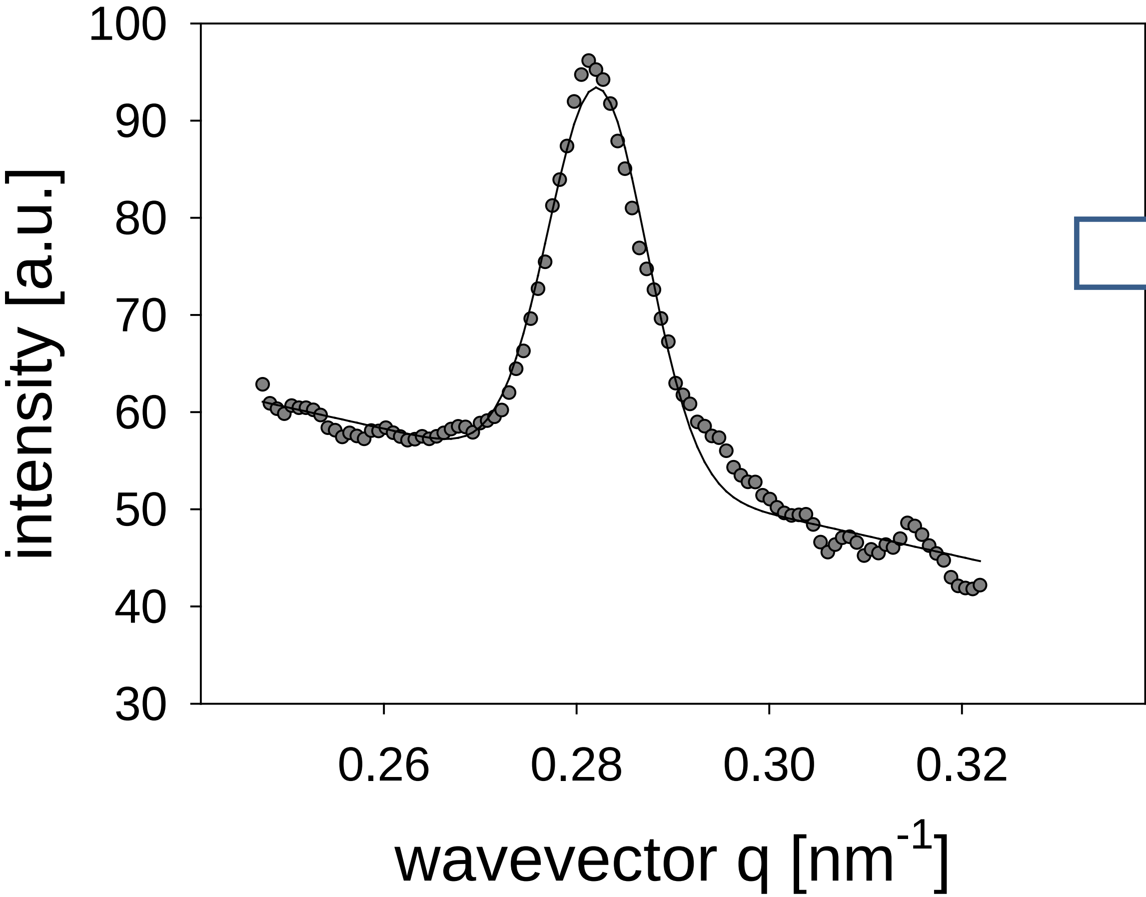
# Setting up the integration zone
## Inherently approximate; count statistics




- Figure out which is the main direction
    - Sample structure
- Make your sector large enough to include "most" of the intensity
- Trade-off between angular size and statistics

# Meridional SAXD: Fit functions
## Gaussian + linear background



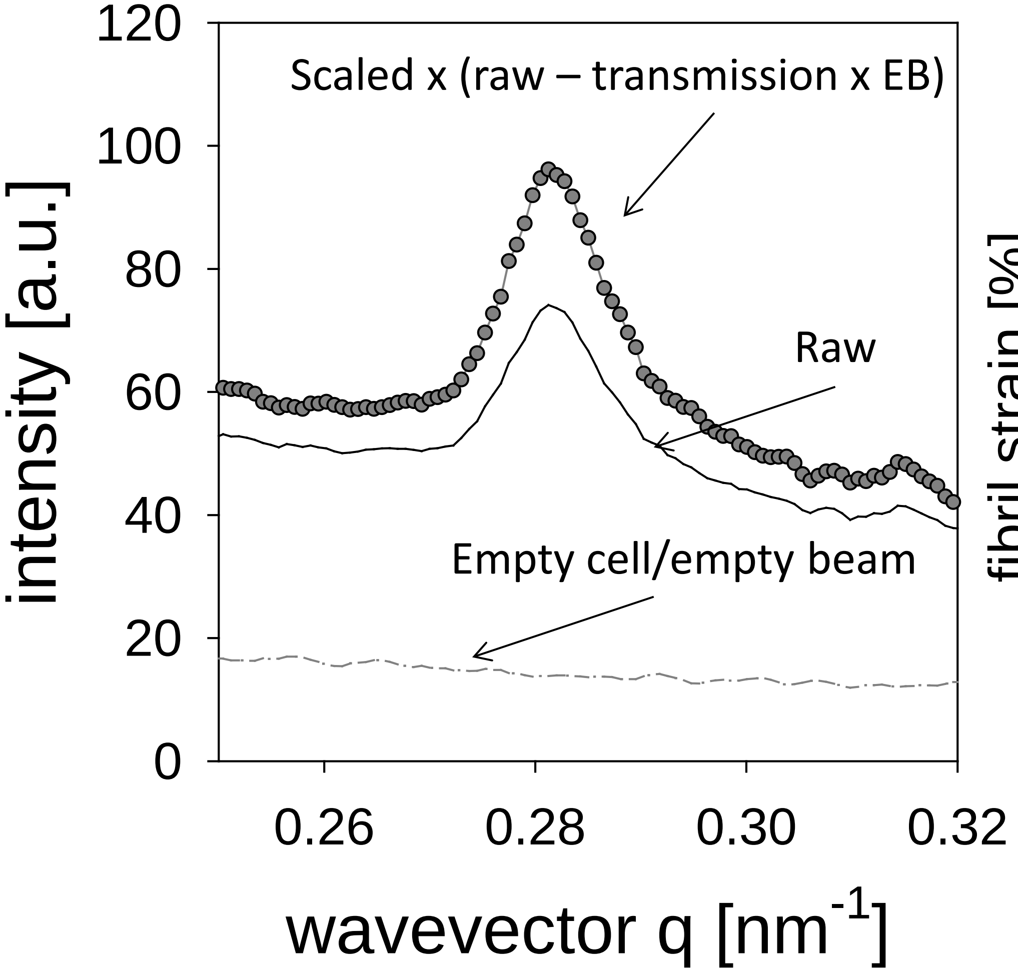

- Peak position
  - $q_3 = 3 \times 2\pi/D$
  - $\delta q_3 \rightarrow \delta D$
  - Fibril strain
- Peak width *w*
  - Heterogeneity in fibril strain

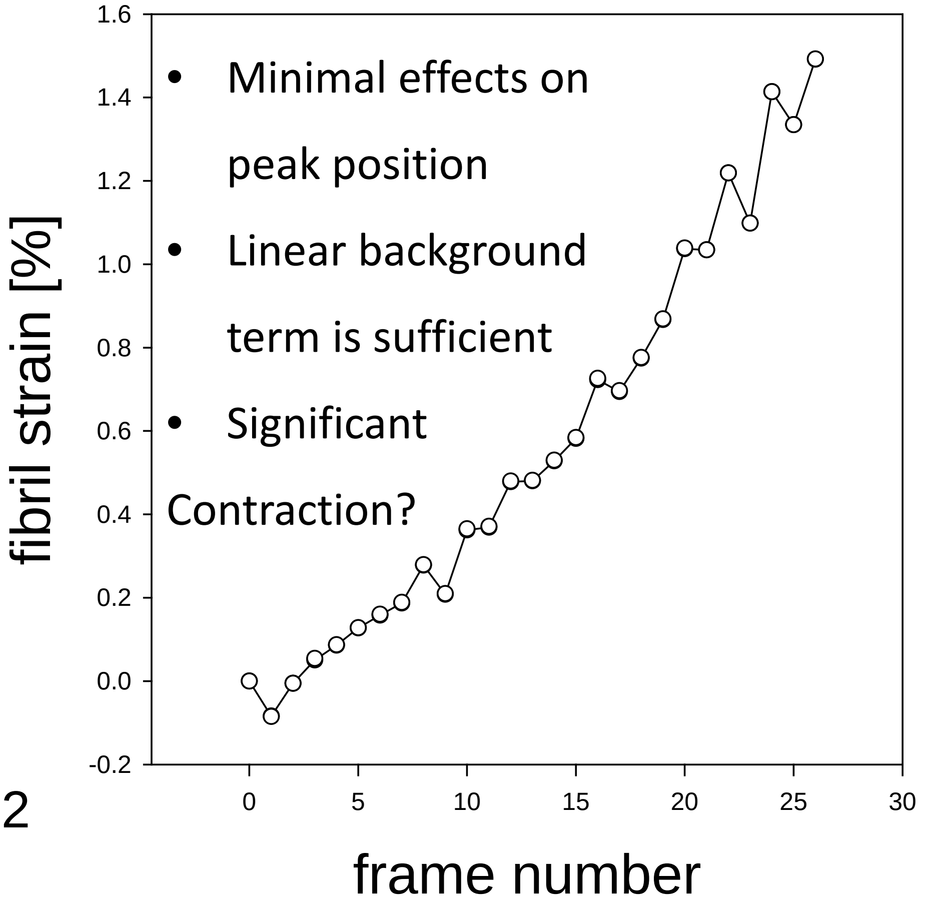
$$I(q) = B_0 + B_1\, q + I_0 \exp(-0.5*((q-q_0)/w)^2)$$

*empirical; getting rid of SAXS scattering*

# Meridional SAXD: Correcting for background

## Empty cell correction or not?




- Minimal effects on peak position
- Linear background term is sufficient
- Significant

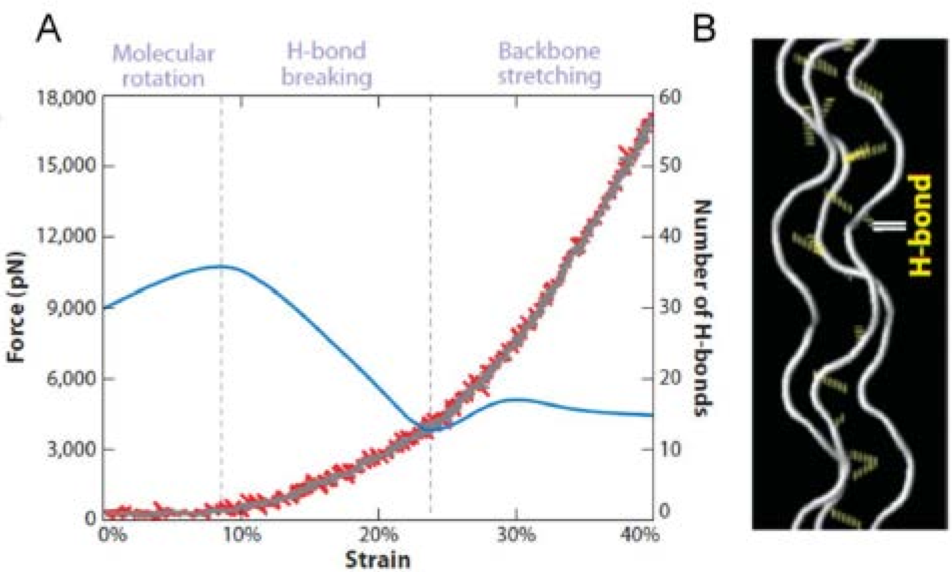
Contraction?

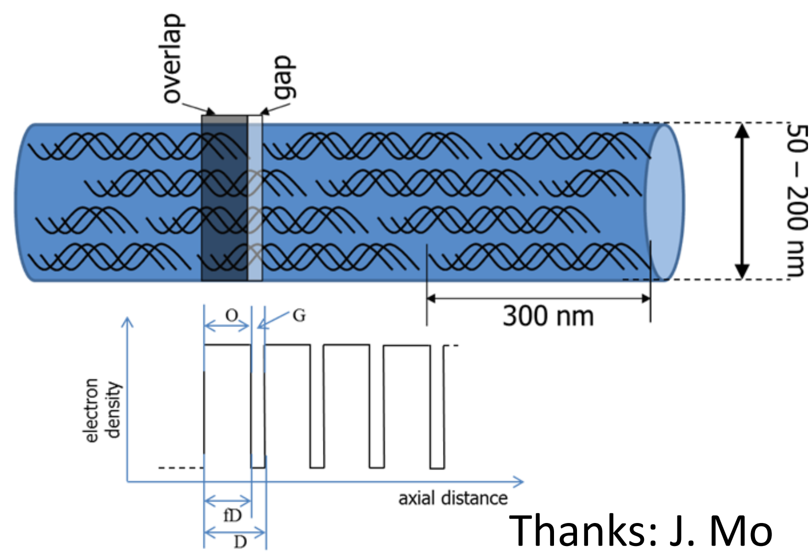
# Defining fibril strain

$$\varepsilon_F = \frac{D(\varepsilon_T) - D(\varepsilon_T = 0)}{D(\varepsilon_T = 0)}$$

$$\varepsilon_F = \frac{q_0(\varepsilon_T = 0)}{q_0(\varepsilon_T)} - 1$$

- Implicitly, we are assuming a homogeneous fibril deformation
- The ab-initio modellers may have something to say about that

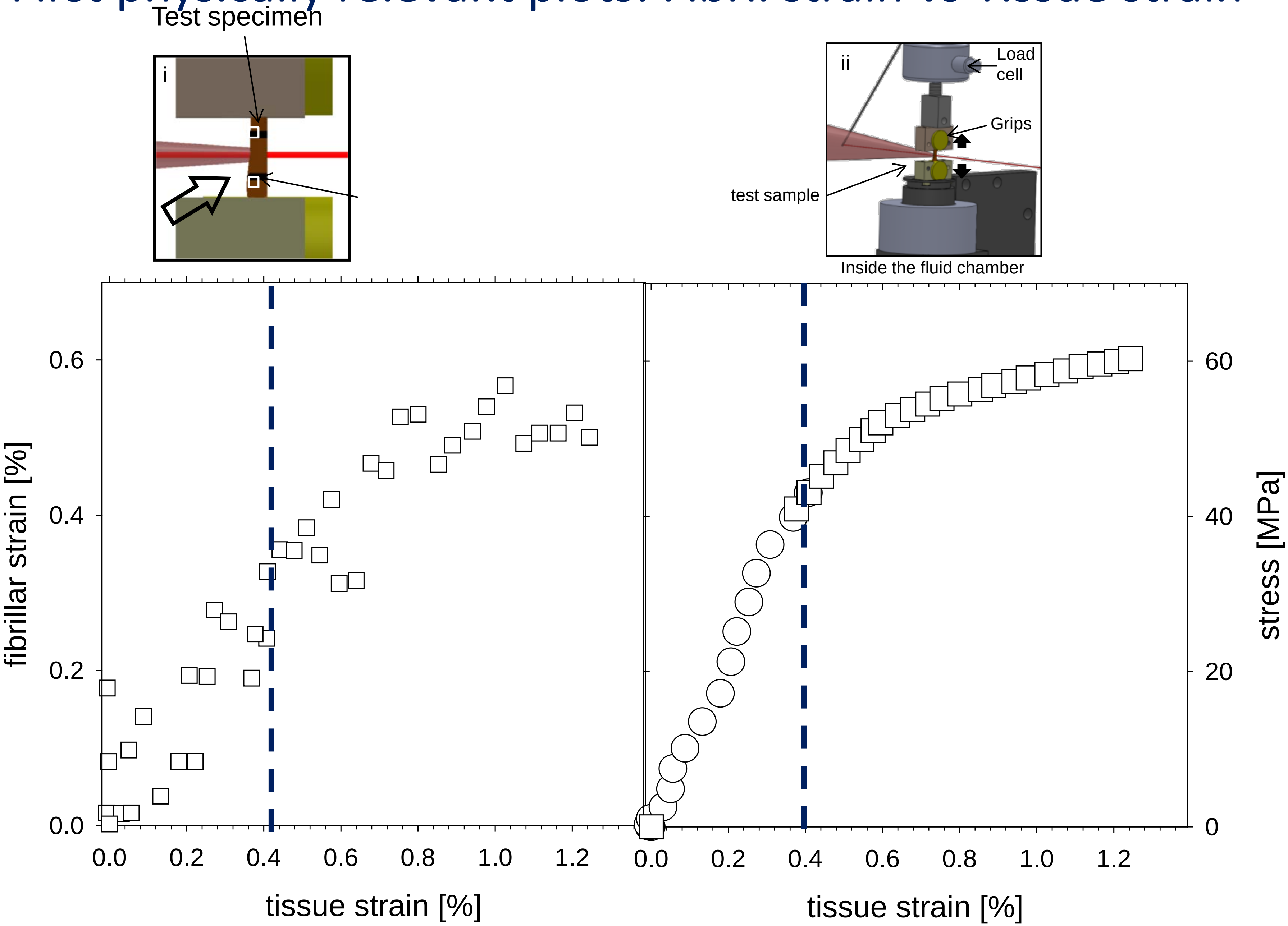



Thanks: J. Mo

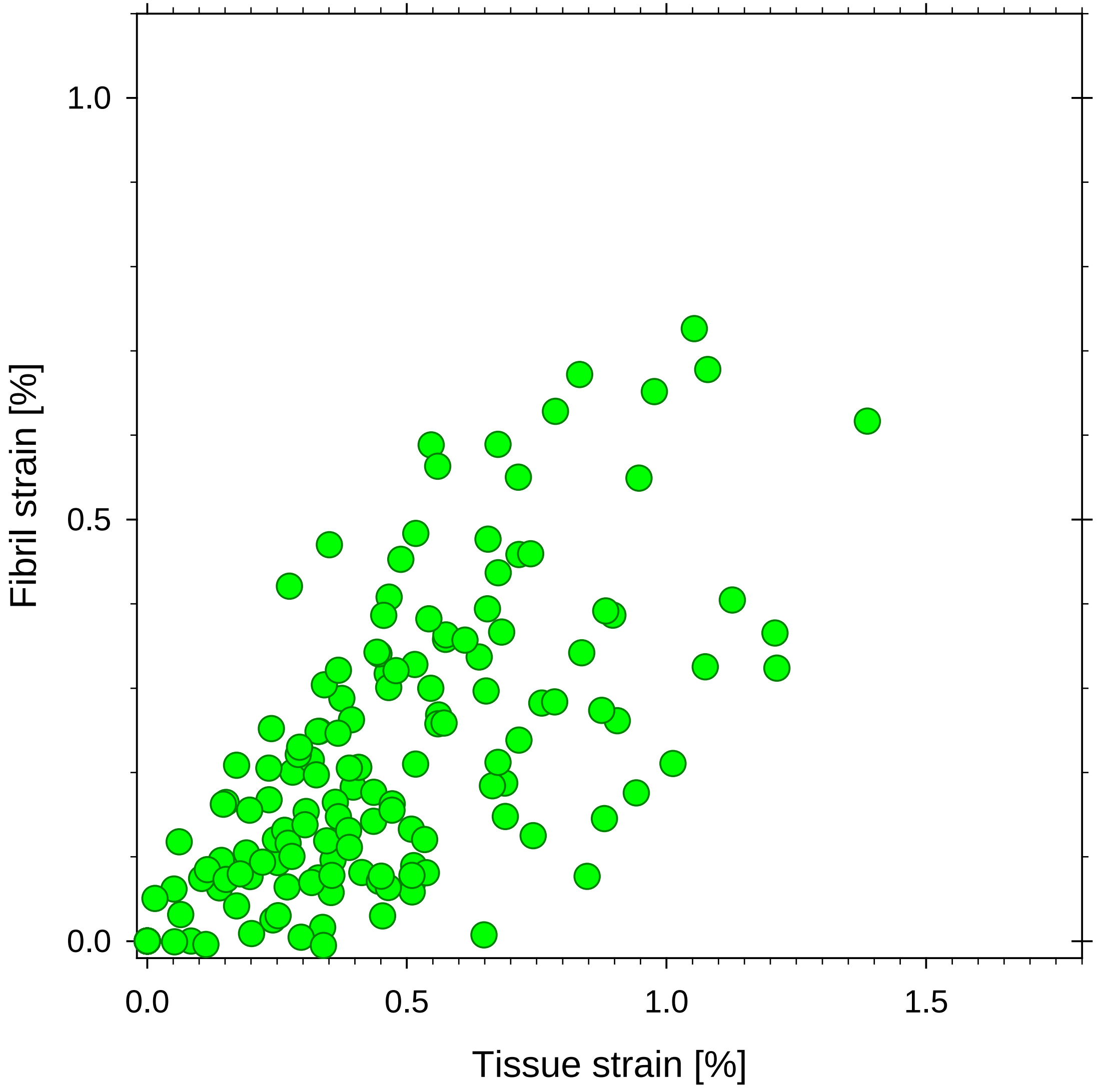
Launey et al, *Annu.Rev. Mater. Res.* (2010)

# First physically relevant plots: Fibril strain vs Tissue strain

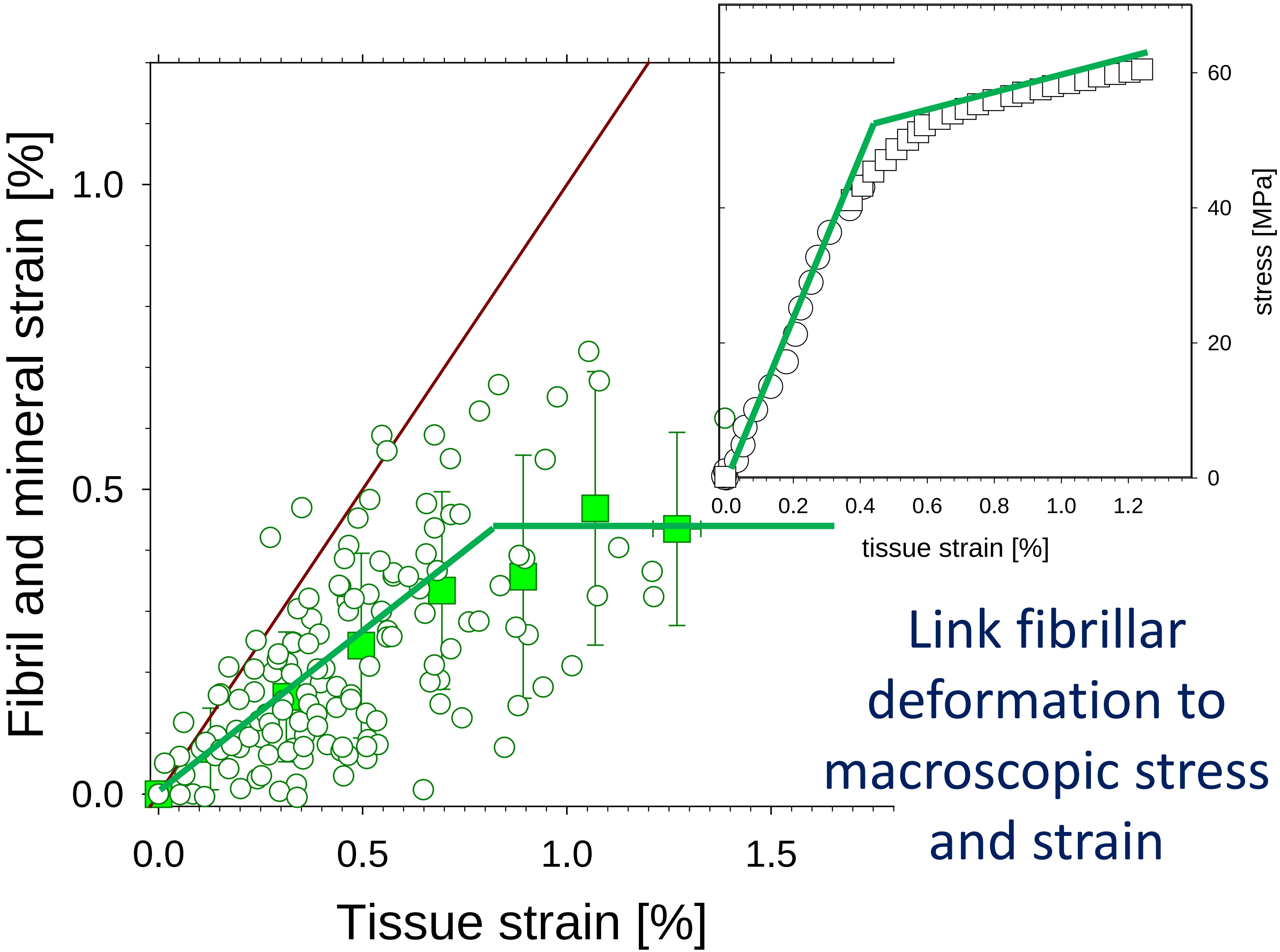



H. S. Gupta et al, *Nano Lett* (2005)

# Merging data from multiple samples:
## Don’t be surprised by significant scatter in biological samples



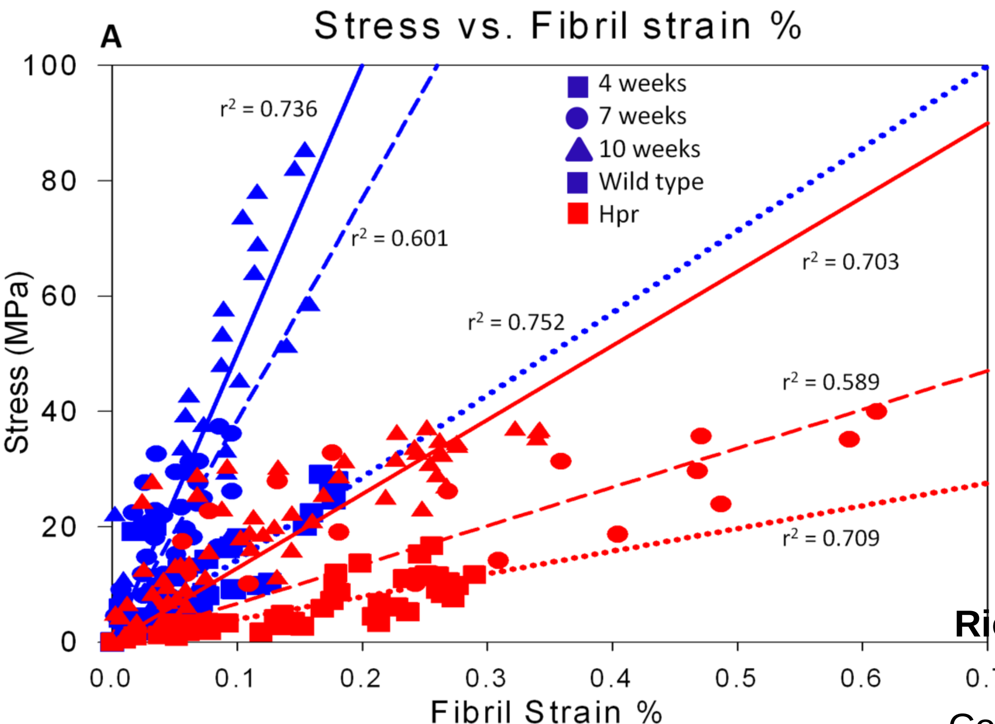
# Binning the data to extracting mean trends



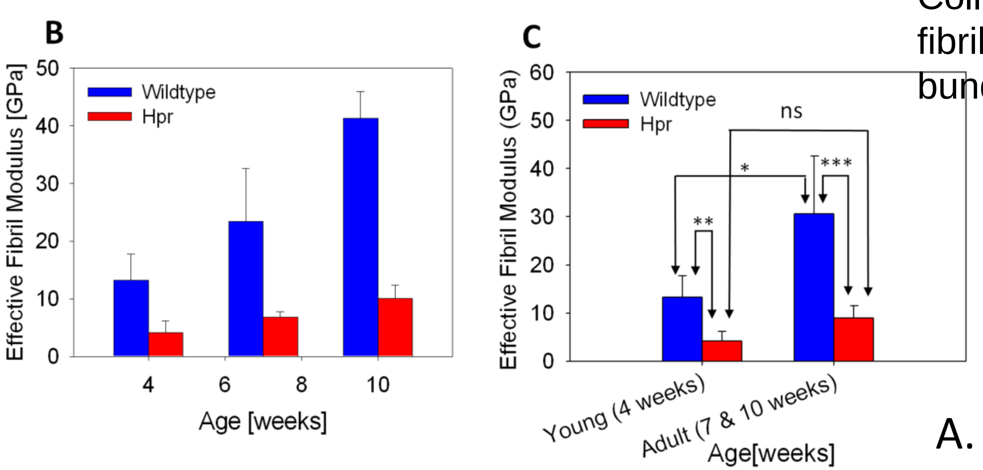

Link fibrillar deformation to macroscopic stress and strain

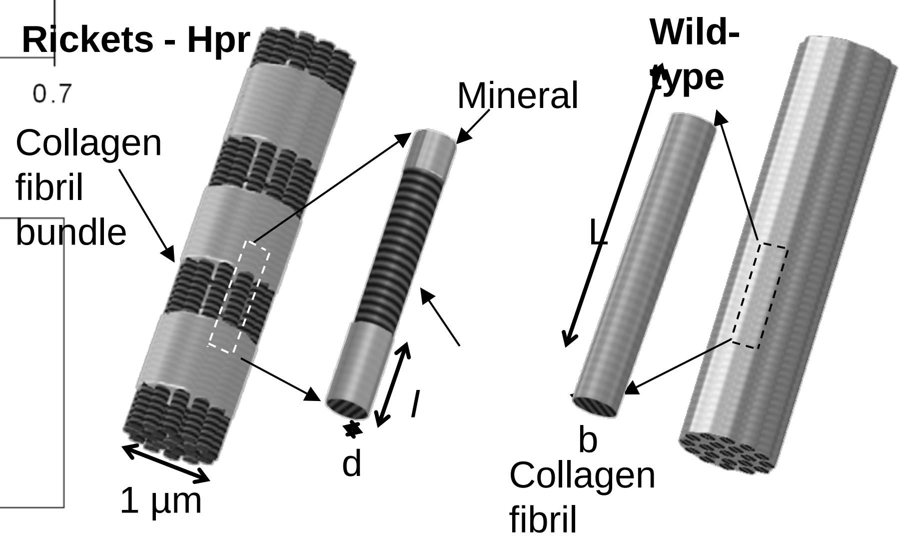
Data from experiments reported in H. S. Gupta et al, PNAS (2006)

# Example: Altered Fibrillar Mechanics in Metabolic Bone Diseases
# Hypophosphataemic rickets



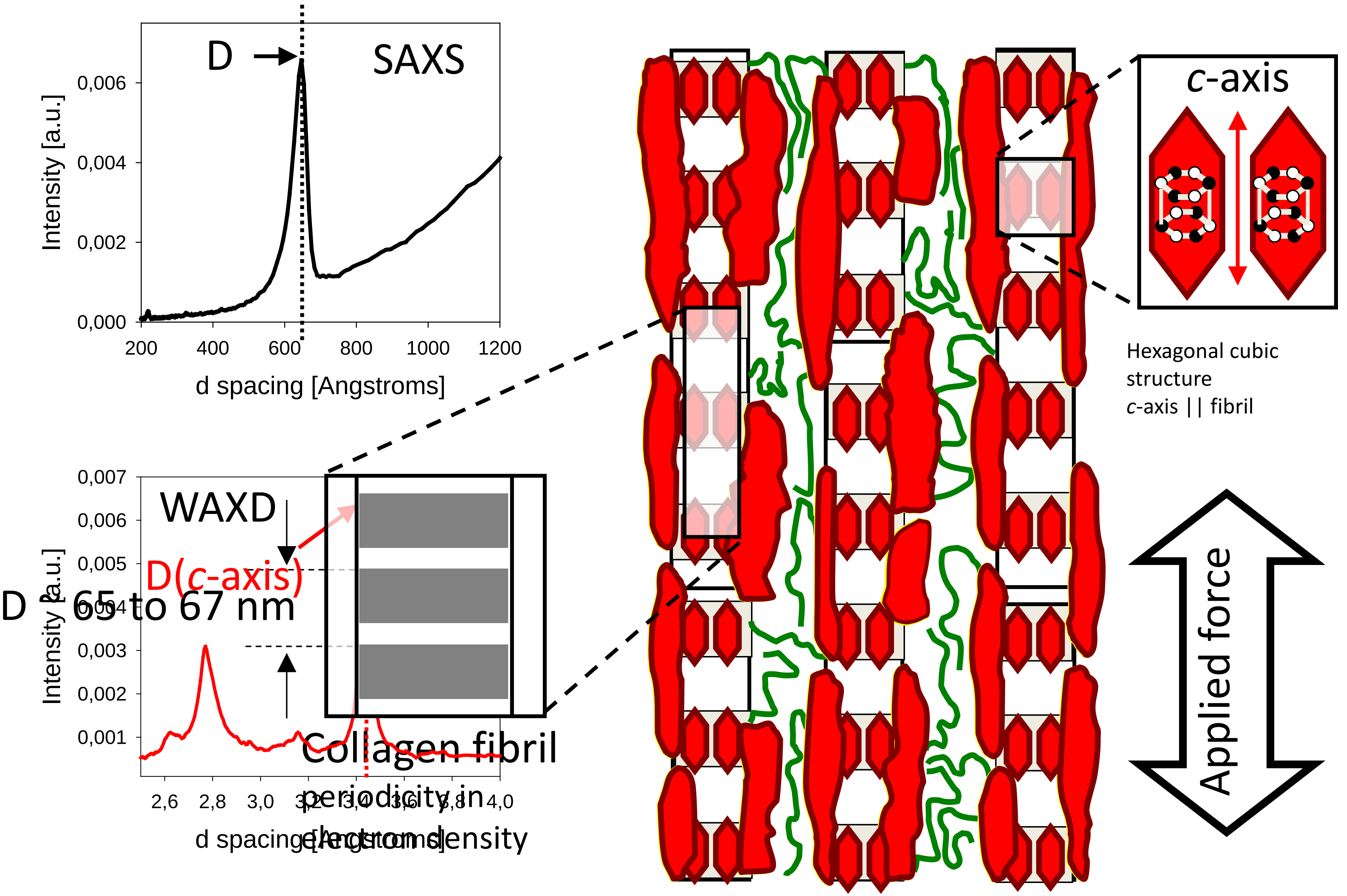

- Effective fibril modulus increased by 15 GPa in wild type mice and only 2 GPa in rickety mice from 4 weeks to 10 weeks → increased amount of mineral nano particle reinforcement.
- Such a decrease would indicate reduced extrafibrillar stiffness in rickety bone, possibly due to lower secondary mineralization in the extrafibrillar space



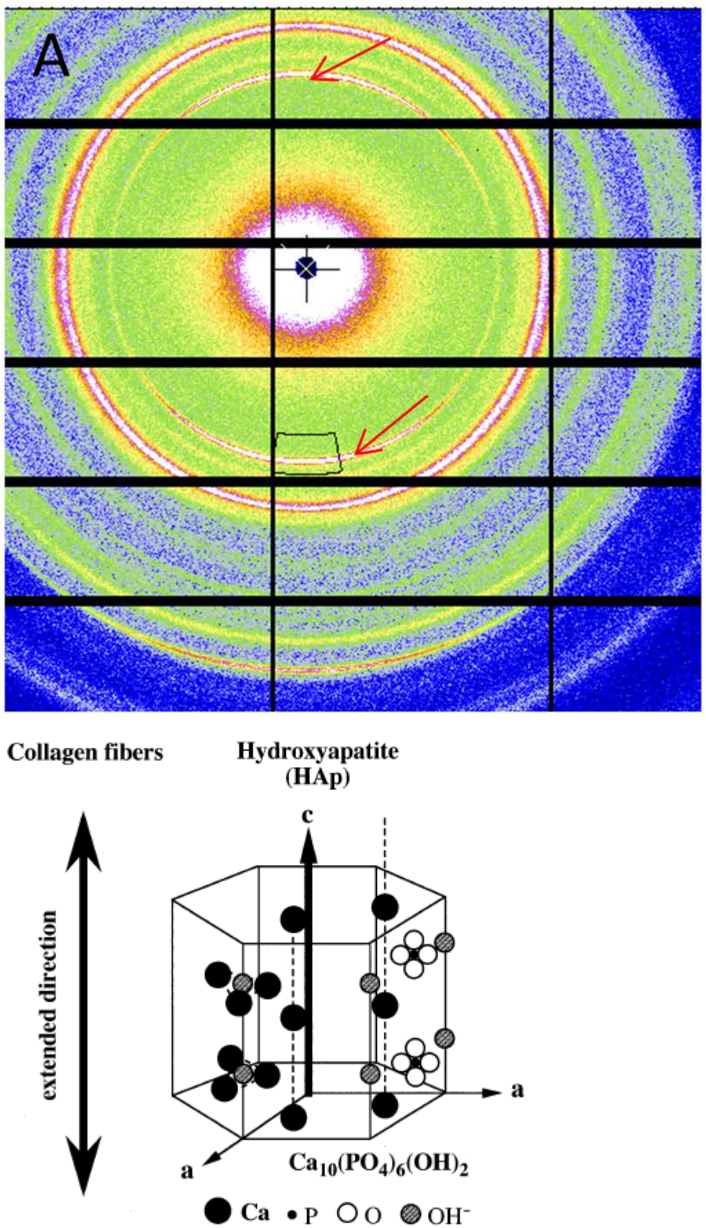

A. Karunaratne et al, *J. Bone Miner. Res.* (2012)

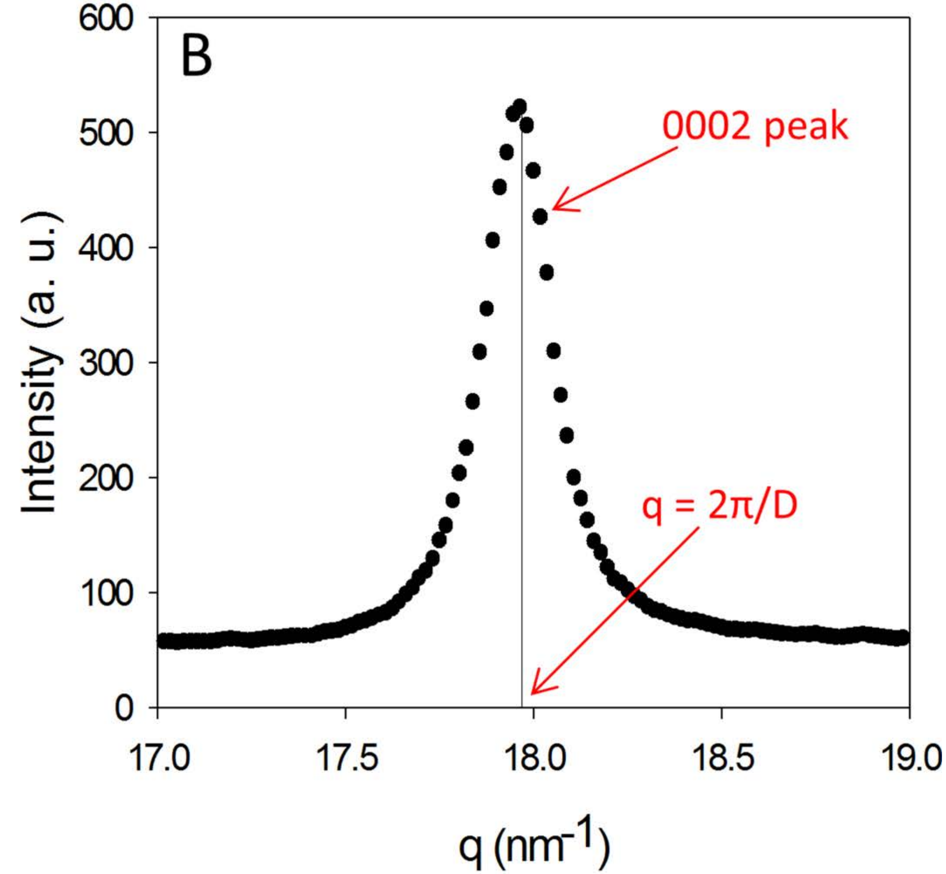
# SAXD diffraction spectrum from mineralized collagen fibril



# Hierarchical fibre composites: Example WAXD spectrum

## Texture/orientation along fibre but again angular distribution



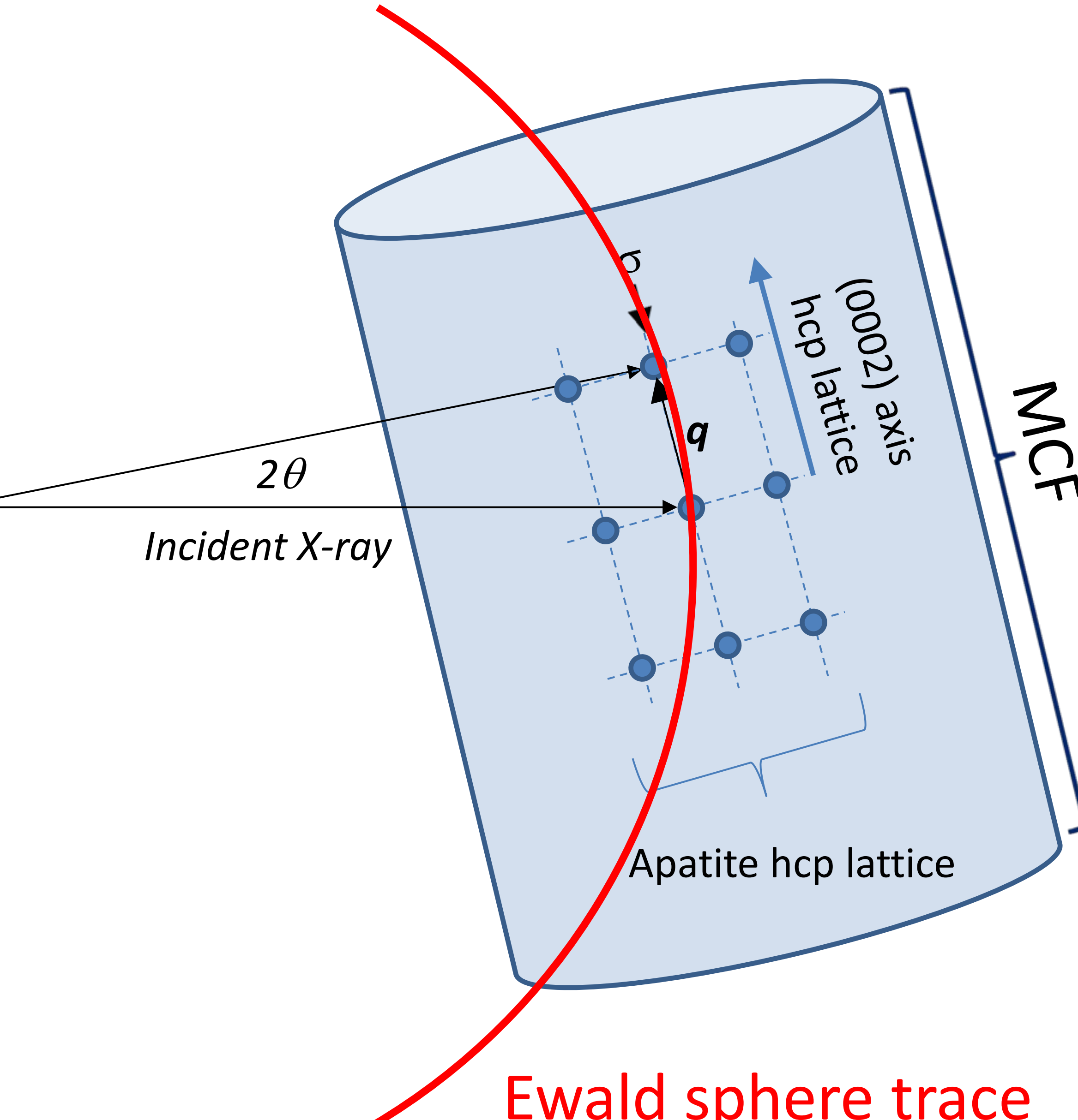




- You will not get a narrower alignment of mineral than fibrils
- Intrinsic poor crystallinity of apatite

T. Nakano et al, *Bone* (2002)

# Tilting to measure on-fibre deformation

## Assuming changes in SAXS spectrum minimal

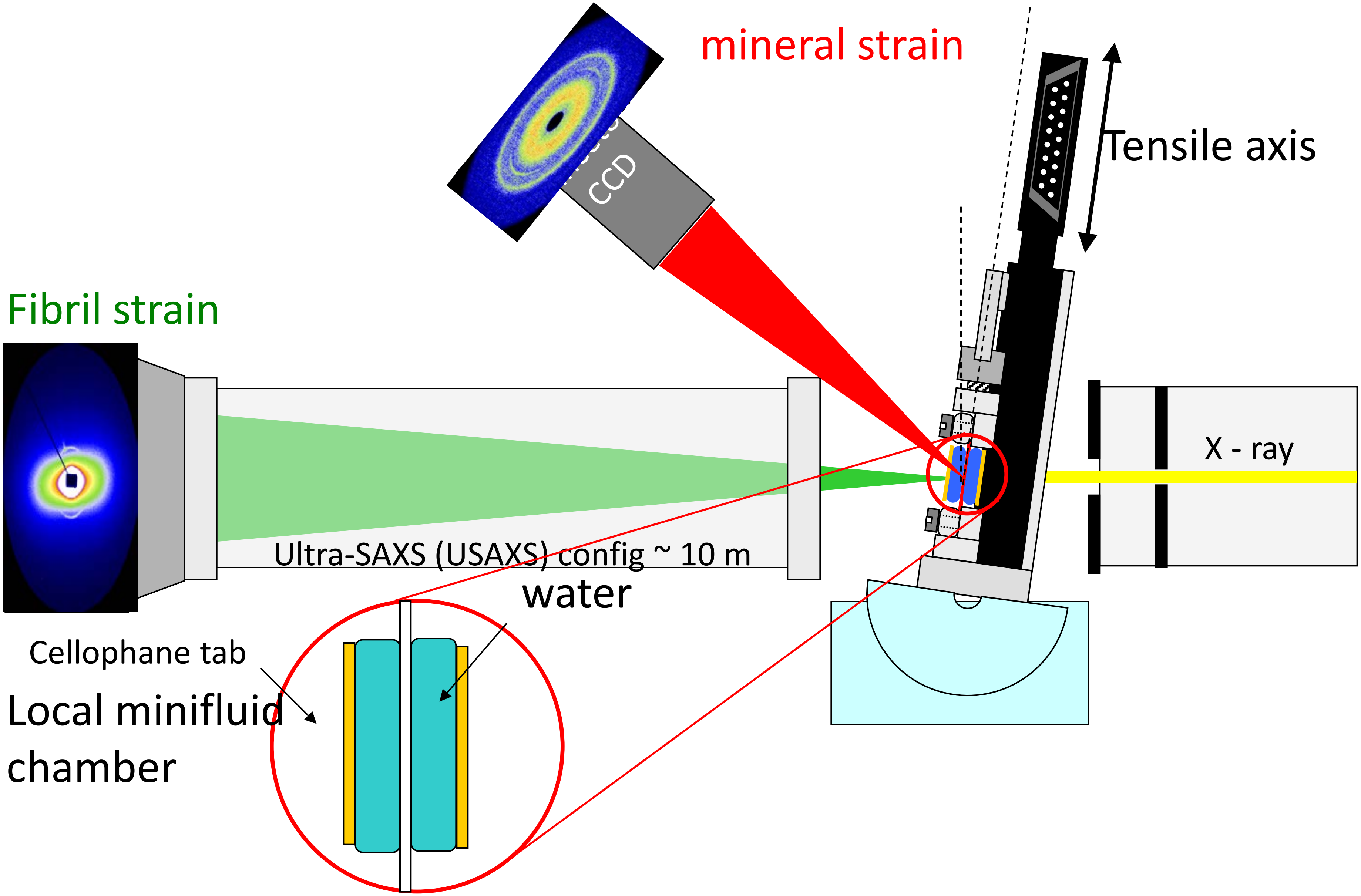



Need two conditions:

- Alignment of mineral platelets with fibrils with sample
- Tilting the tensile test stage by ½ the Bragg angle for the c-axis reflection (maps the fibril)

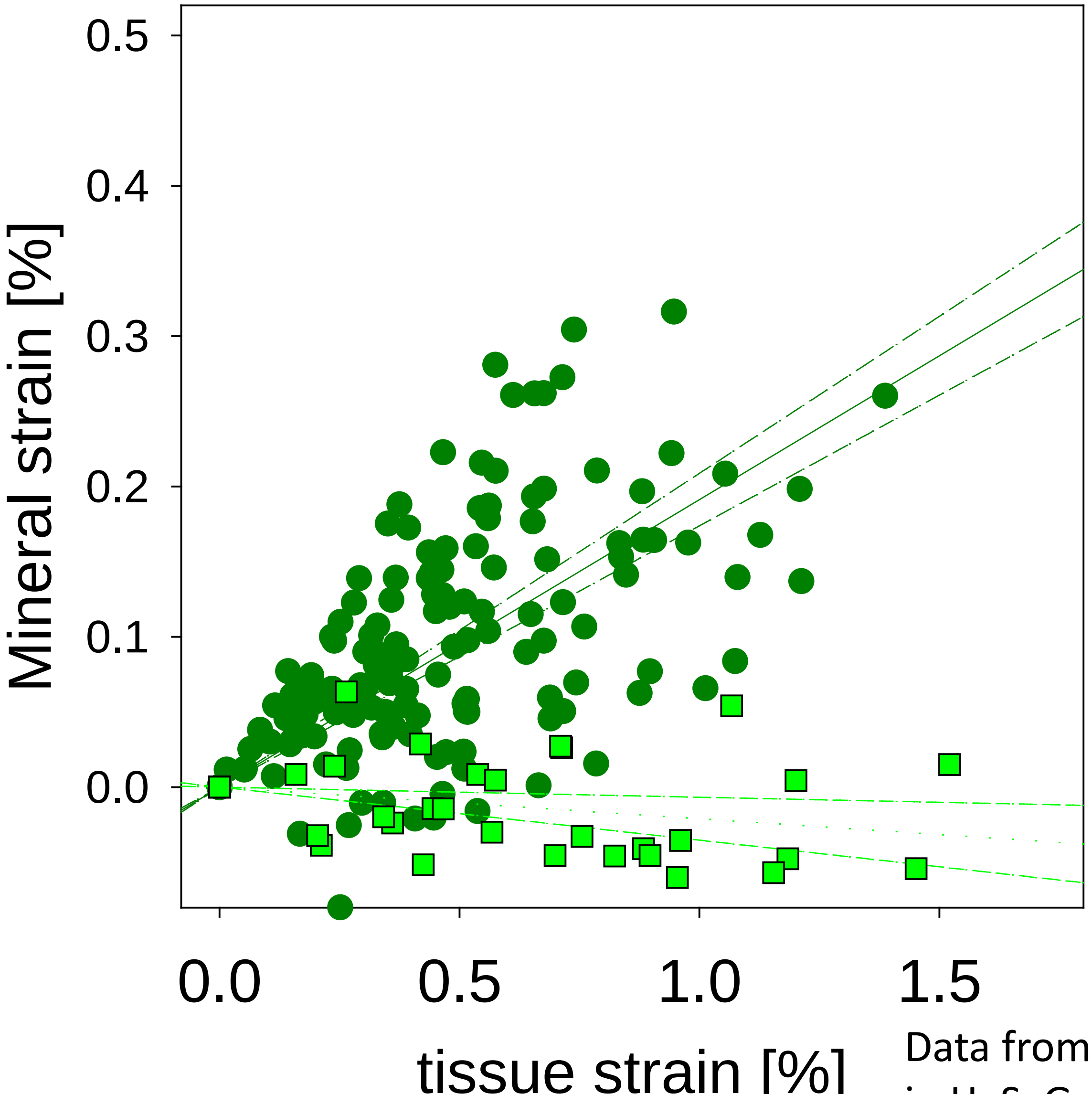
A. Karunaratne et al, *Methods in Enzymology* (2014)

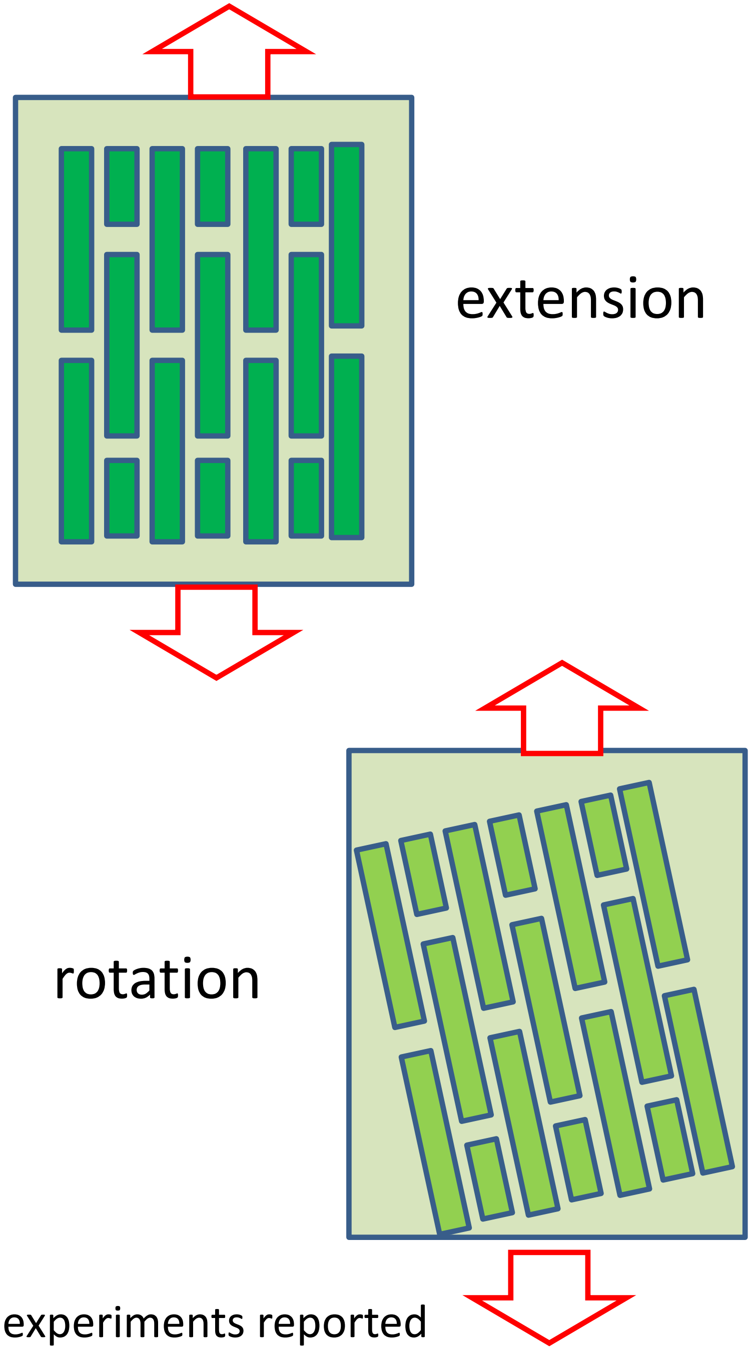
# Setup of a combined SAXS/WAXD experiment



# Yes, it does make a difference …

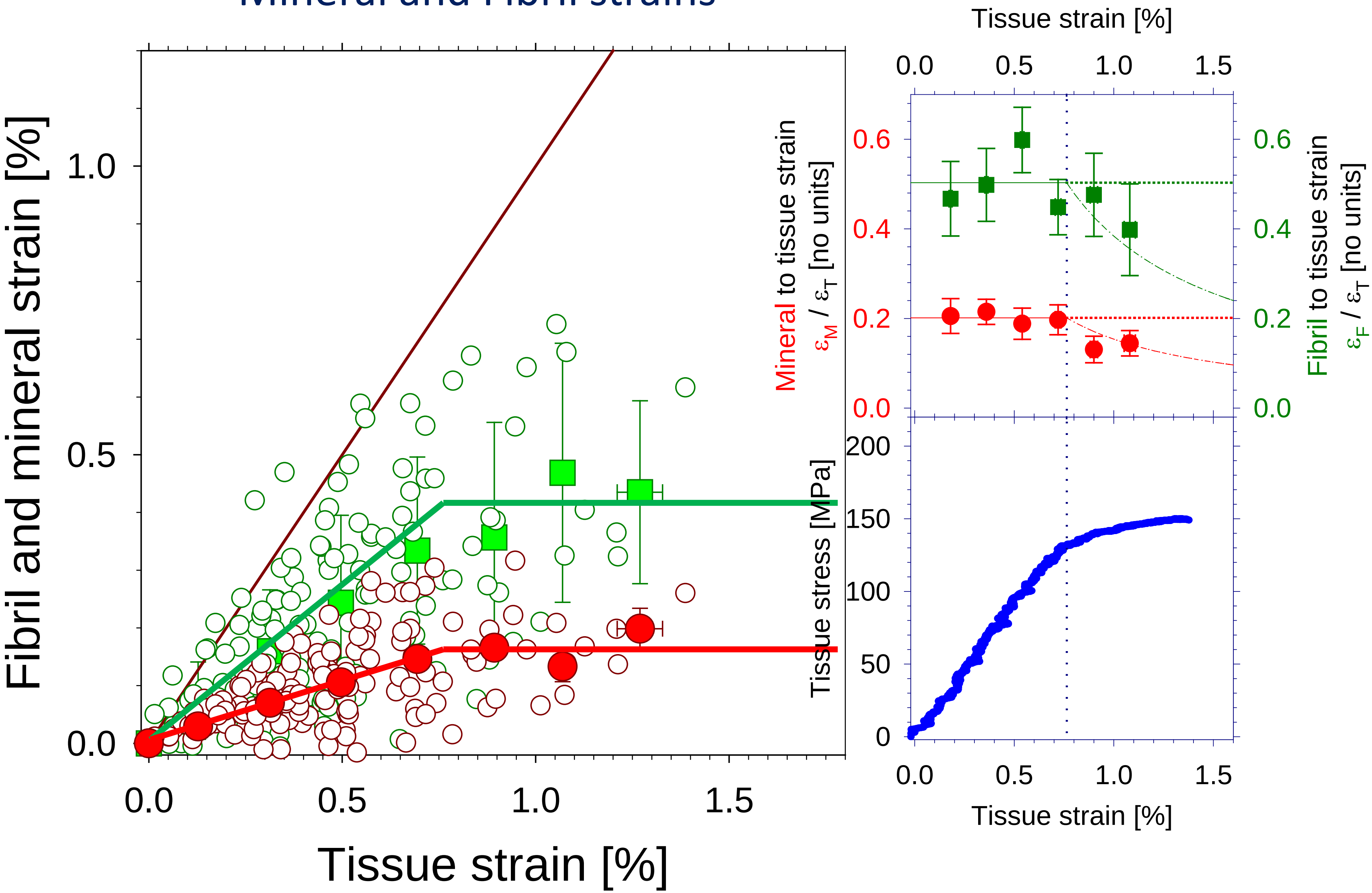
## Stretching versus rotation



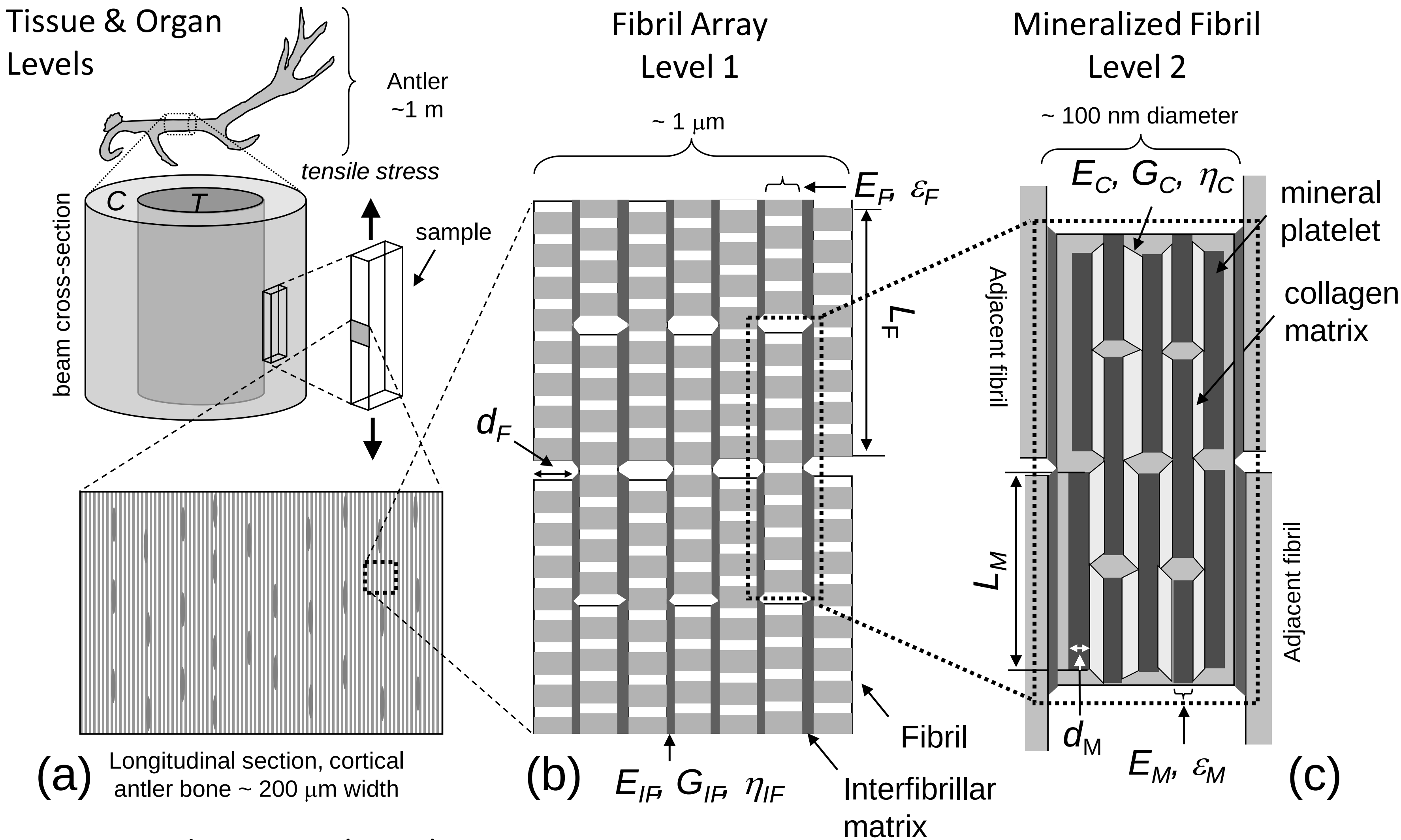

Data from experiments reported in H. S. Gupta et al, PNAS (2006)

# Representative experimental results
## Mineral and Fibril strains



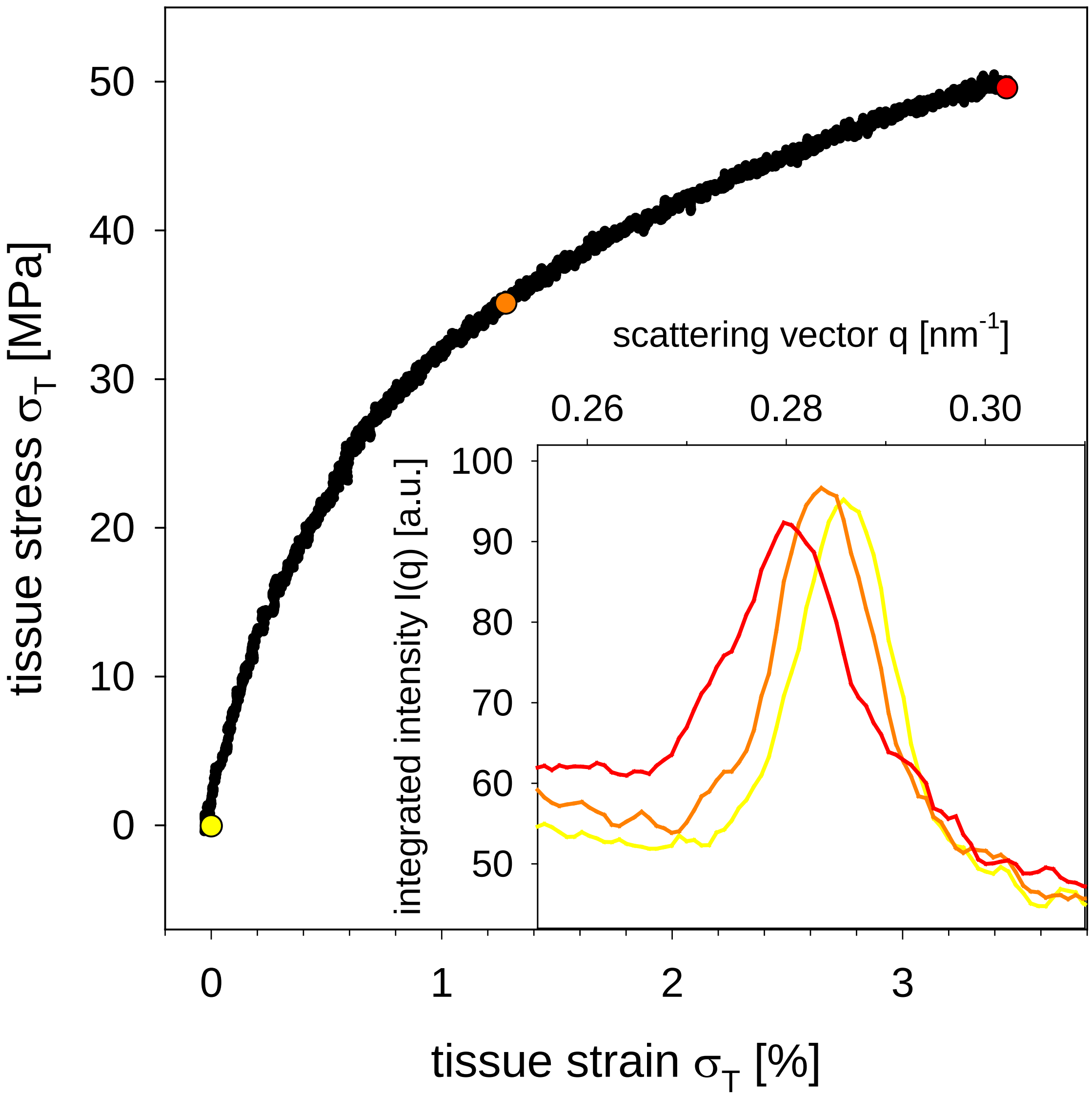

Data from experiments reported in H. S. Gupta et al, PNAS (2006)

# An explicit structural model is essential
## to integrate multiscale strain measurements



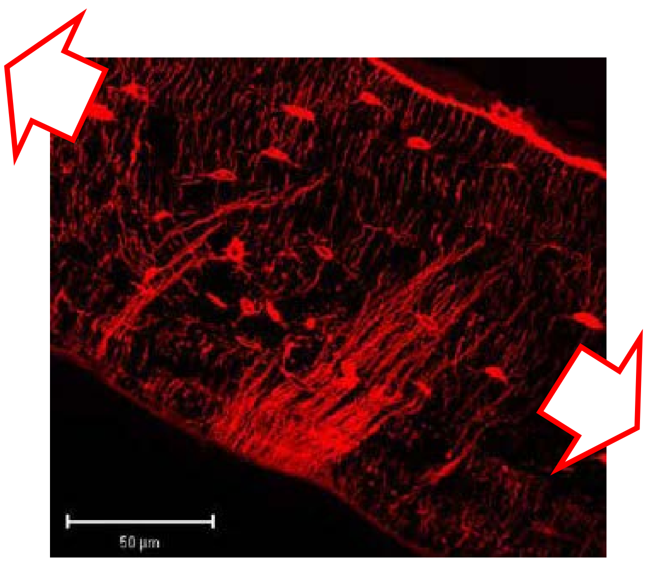

H. S. Gupta et al, JMBBM (2013)

# Back to the original fit function:
## variations in width term and their physical interpretation




*Broadening of I(q)*

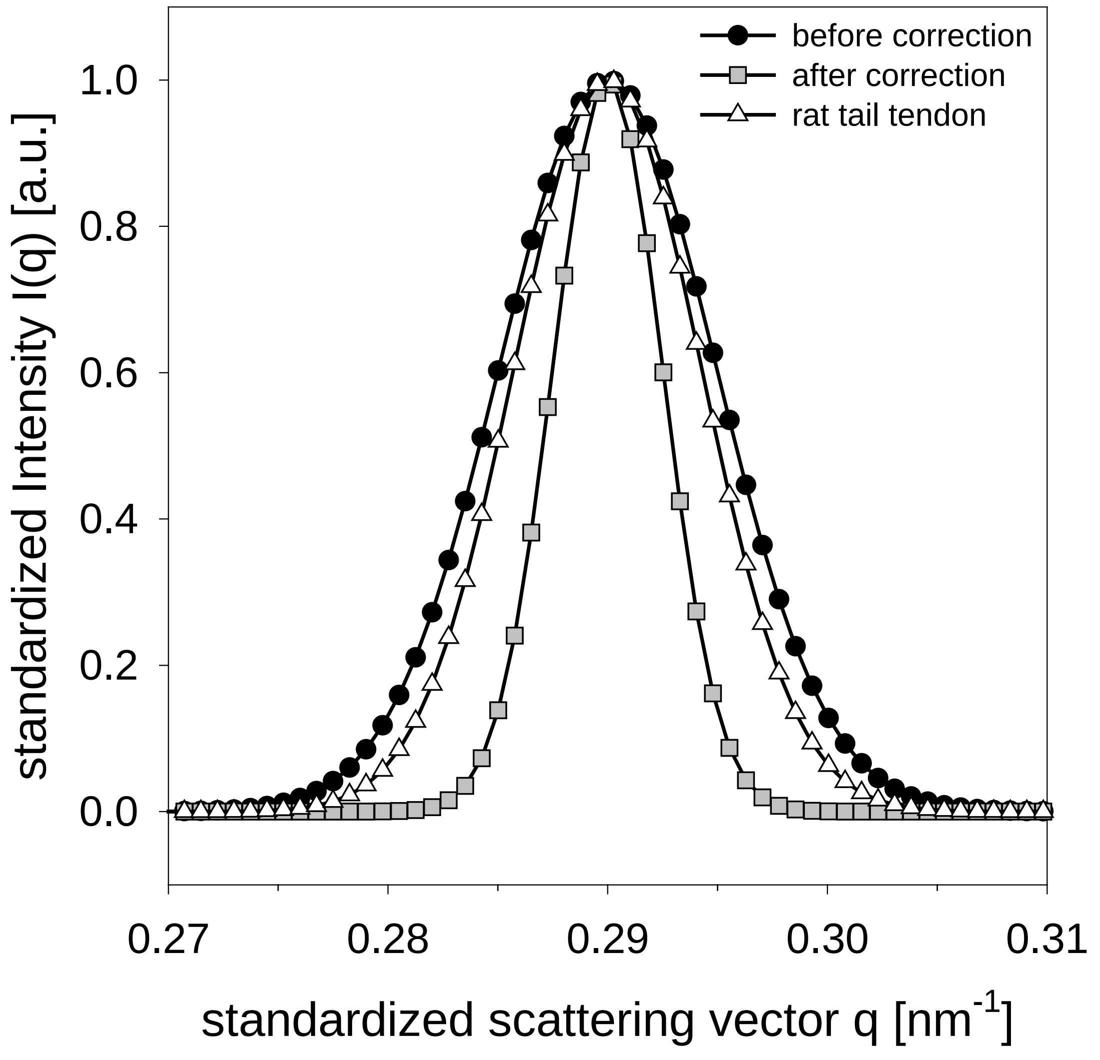
- Increasing distribution f fibril strain
- Need to check that integrated area ~ const




S. Krauss et al, *Bone* (2009)

# Widths: correct for instrumental broadening

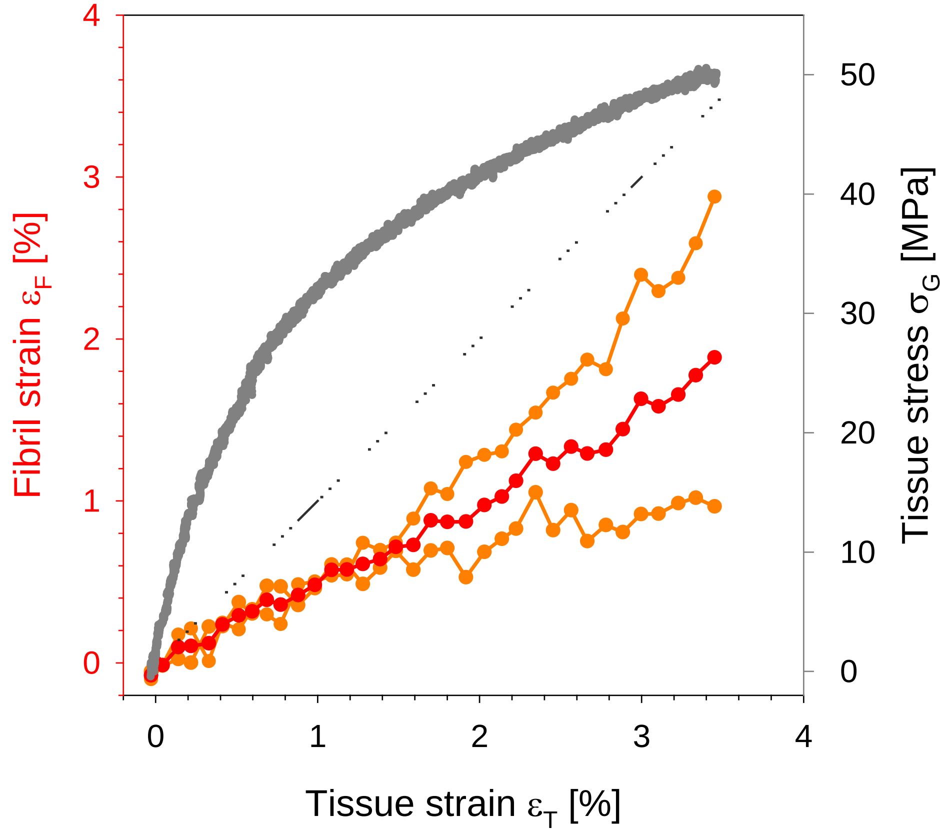
## No golden rule; use a highly oriented collagen




S. Krauss et al, *Bone* (2009)

# Increase in width of I(q) occurs as the tissue becomes inelastic

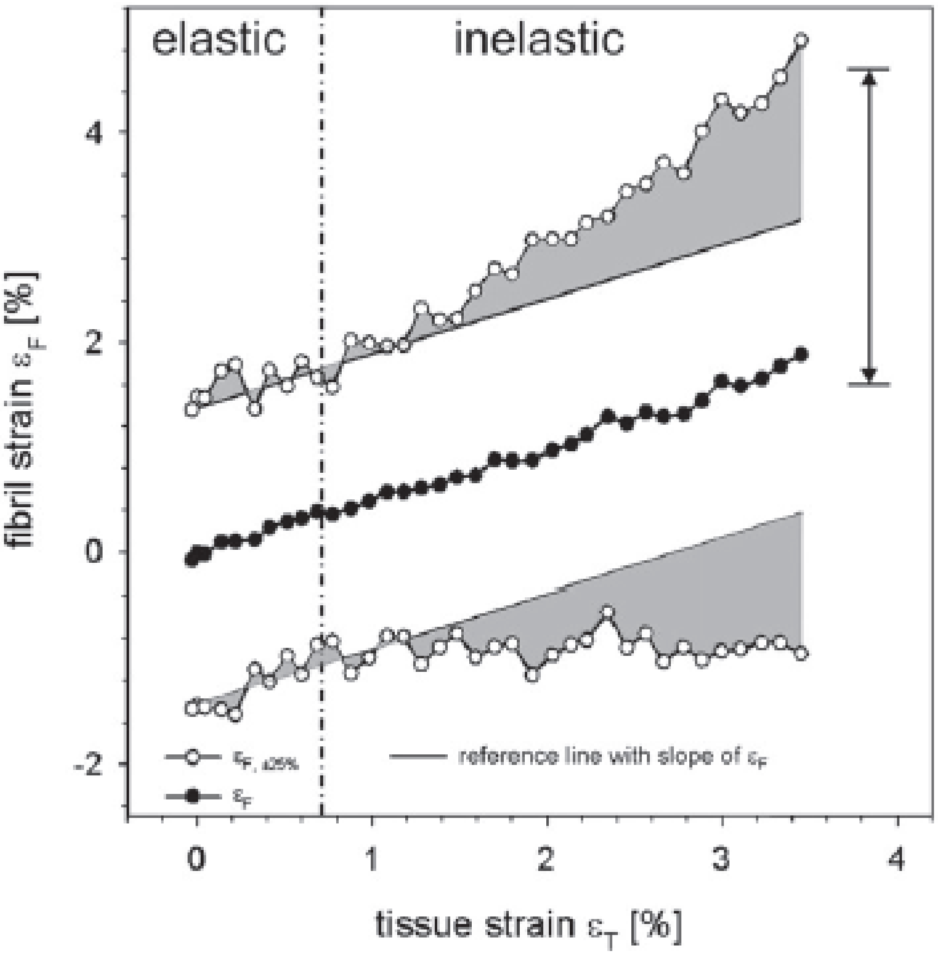
## Correlate to the tissue mechanics

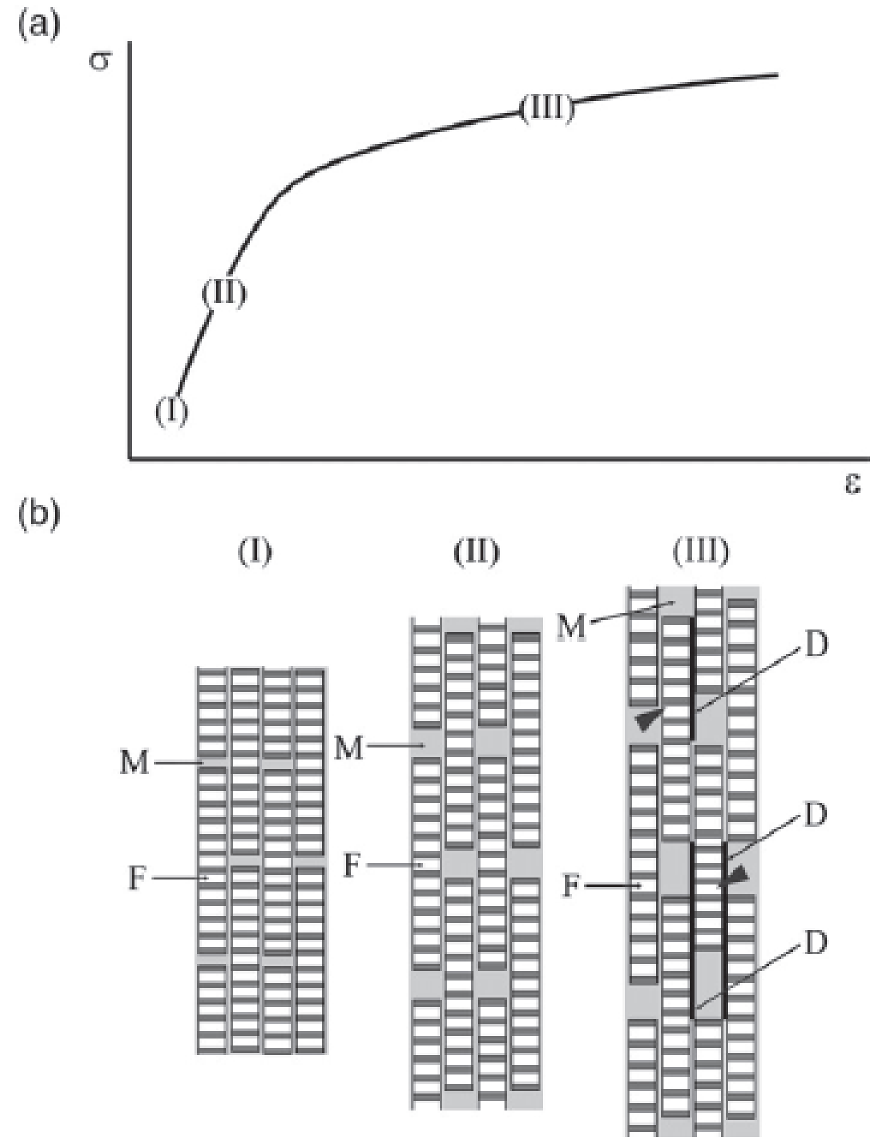



S. Krauss et al, *Bone* (2009)

# Inhomogeneous deformation in the post yield region

## Example of the nanoscale toughening in antler bone

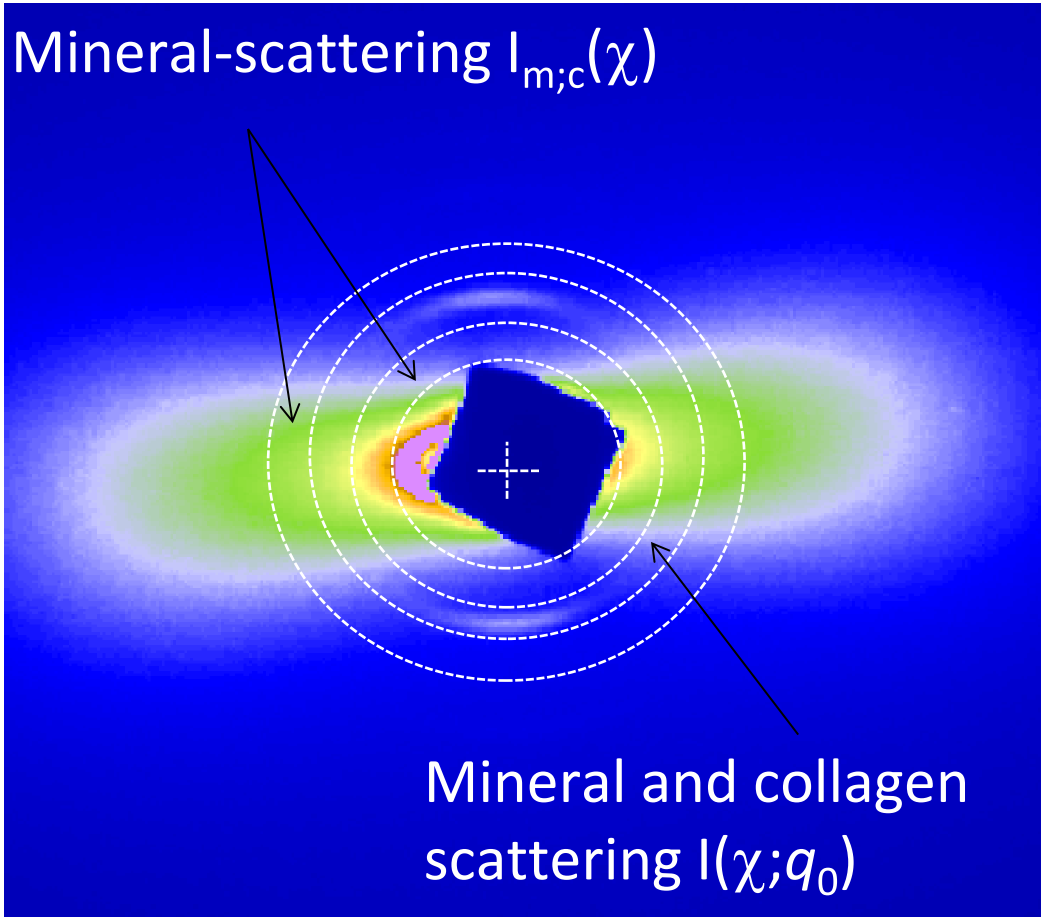


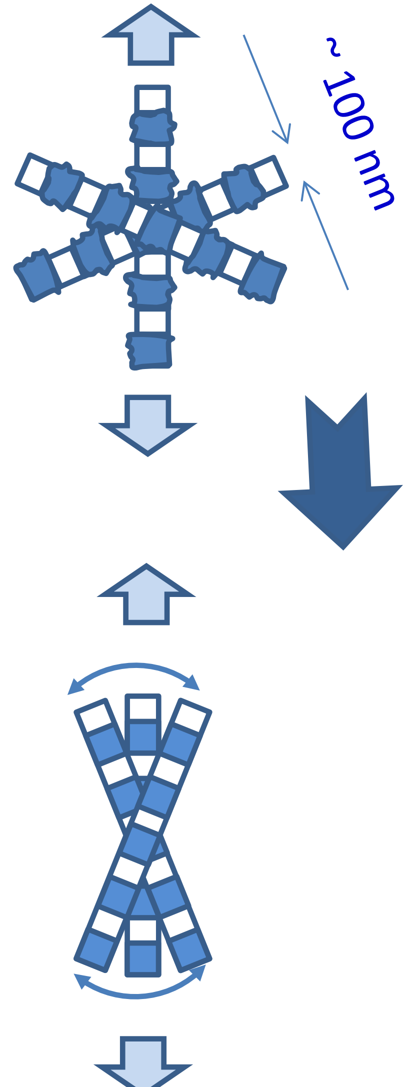




S. Krauss et al *Bone* (2009)

# Background subtraction

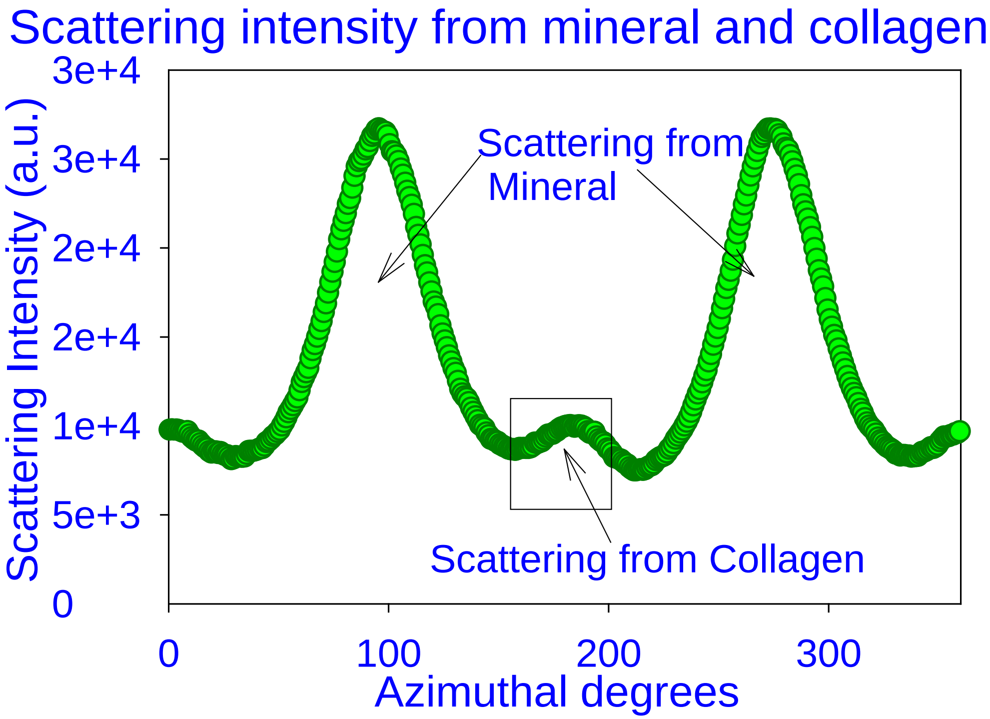
## Especially important in mineralized tissues due to strong SAXS bgr



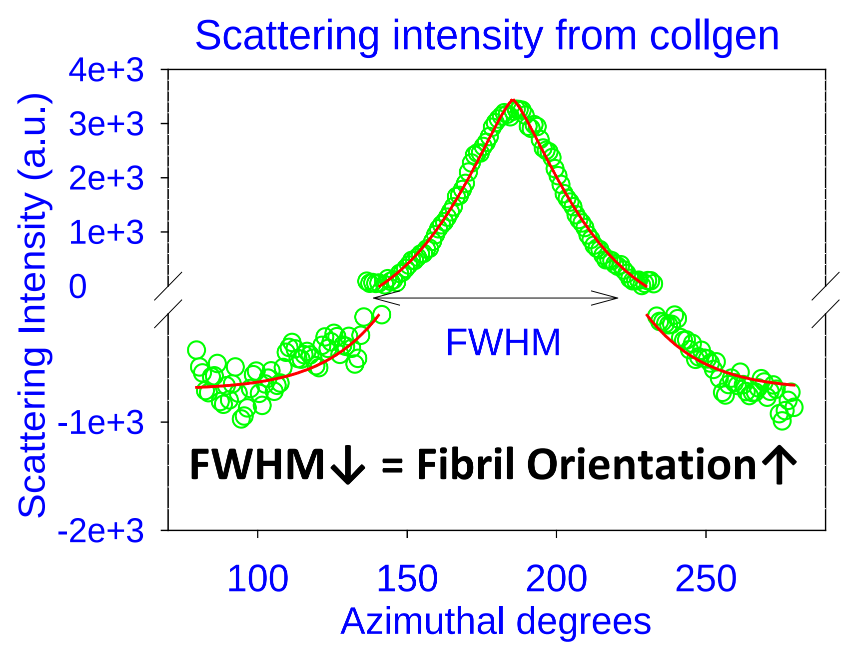




A. Karunaratne et al, *in review*

# Fitting the data




- Subtracting diffuse background from the oriented signal is important
- Especially for inorganic/organic composites like bone

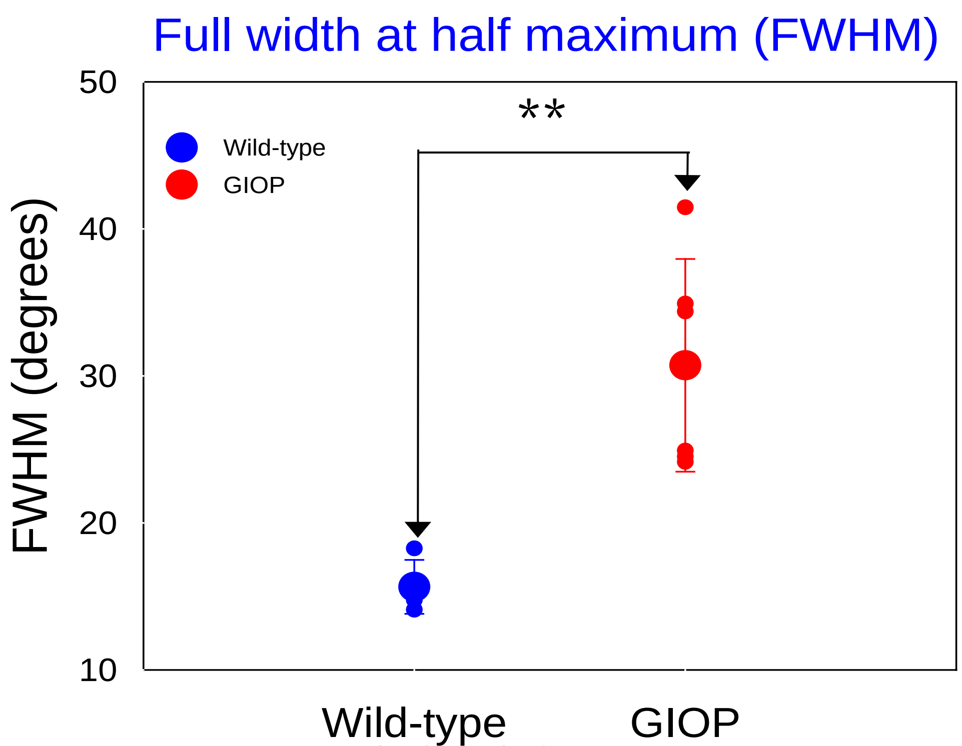


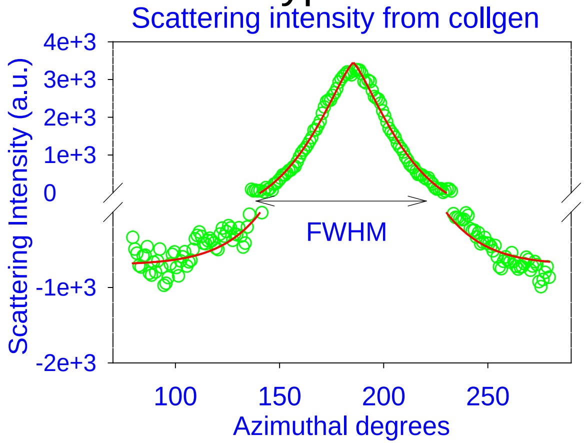

- Correct method will be to take
  - $W = (W_m^2 - W_f^2)^{1/2}$
  - Wf is the width of a single fibril in the lateral direction
    - ~ 1/R (1st zero $(2J_1(qR)/qR))^2$

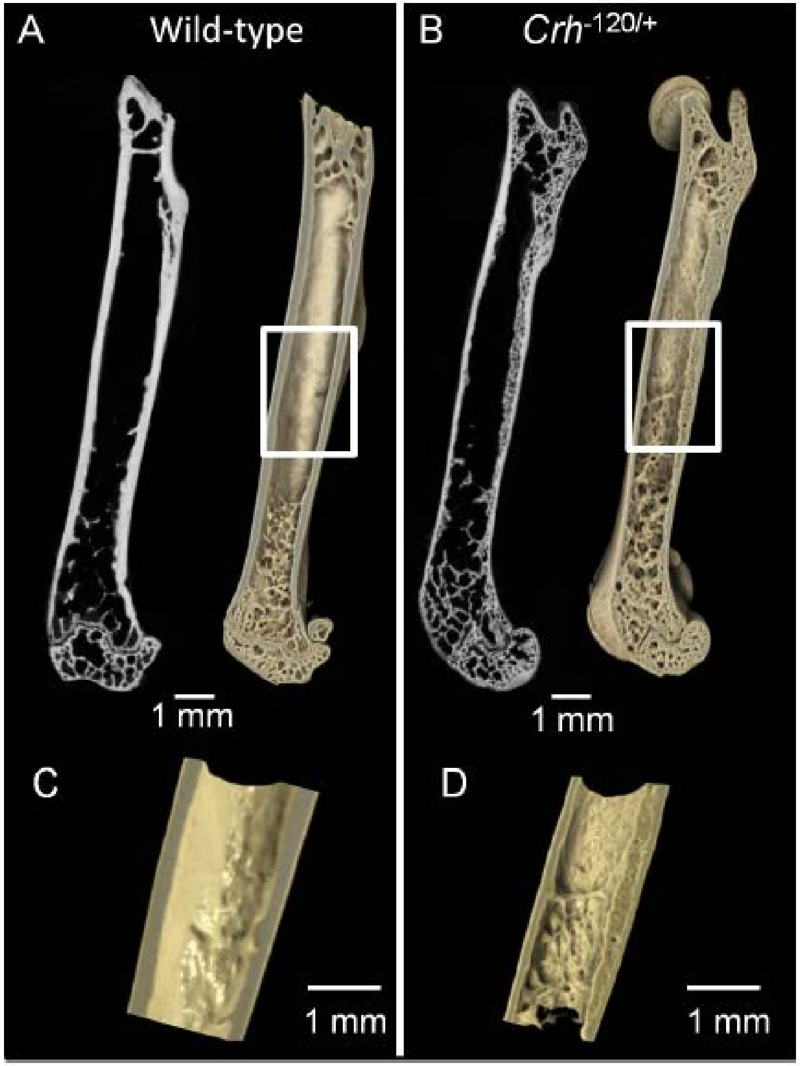
A. Karunaratne et al, *in review*

# Direction of orientation and Degree of orientation

## A first approximation: example of osteoporotic bone



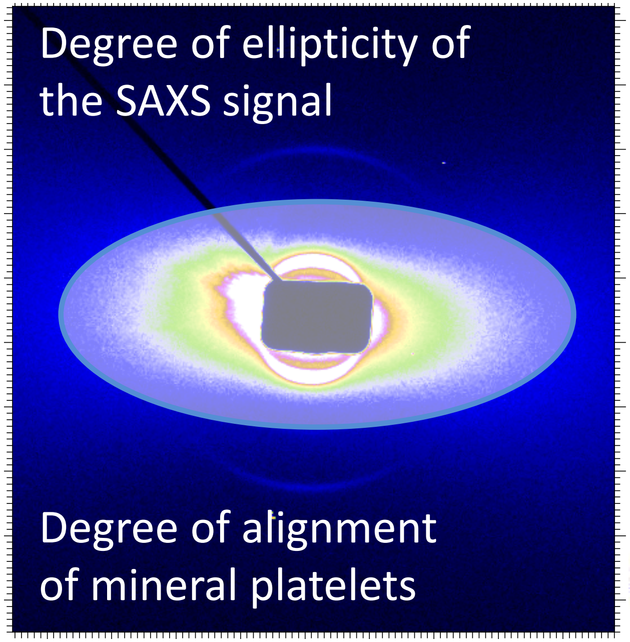



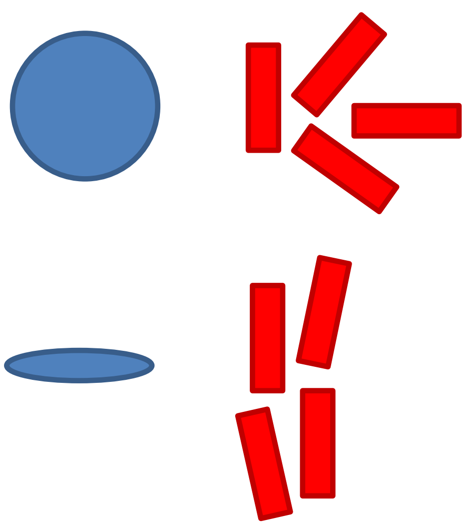



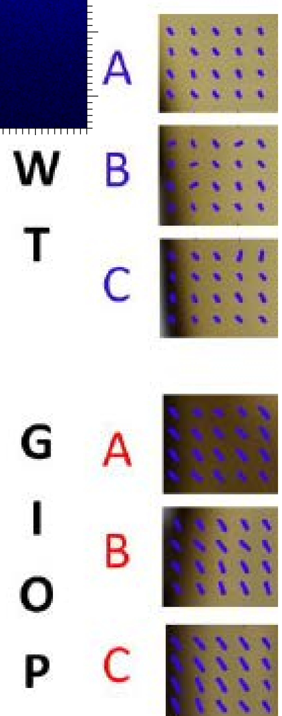

A. Karunaratne et al, *in review*

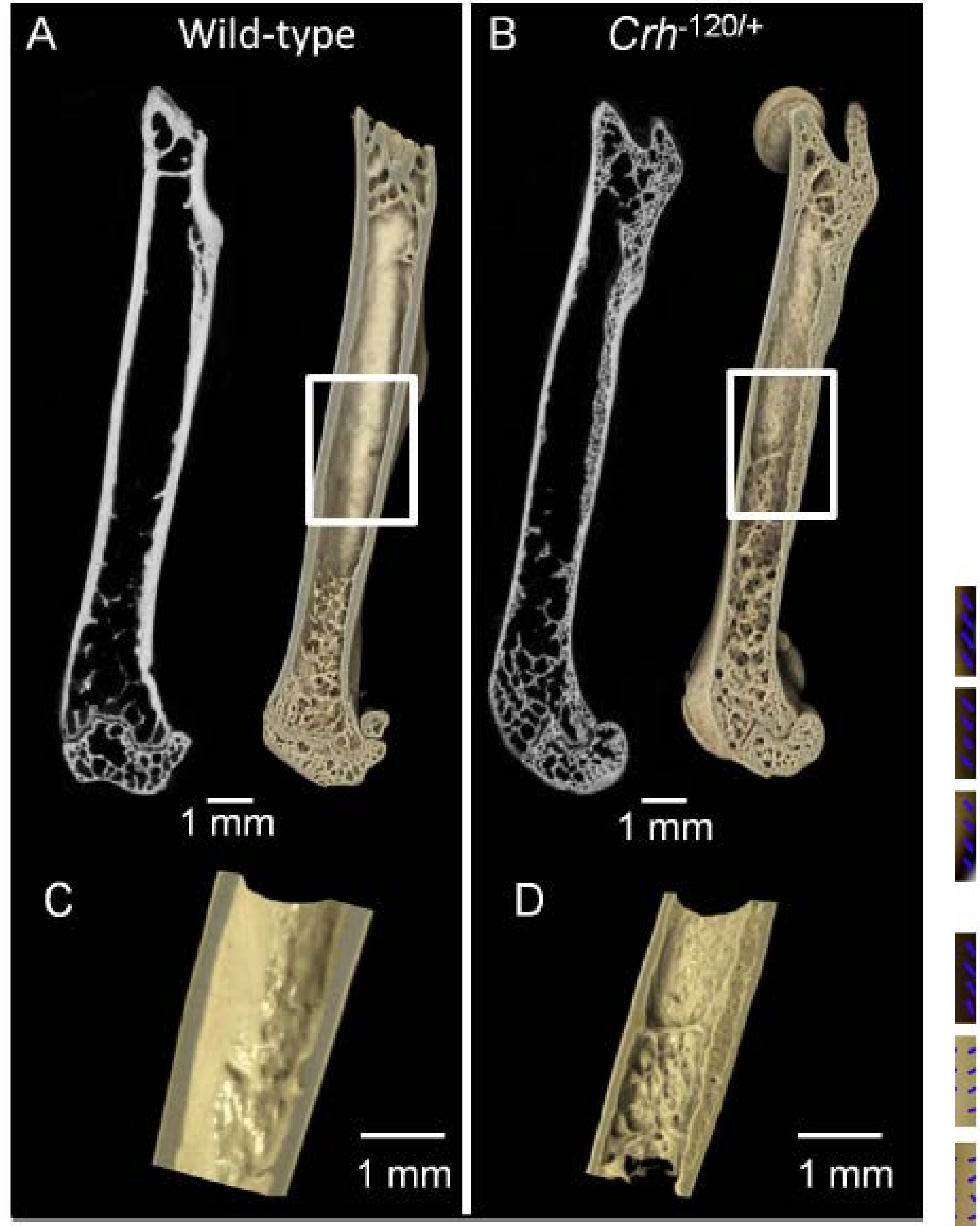
# If by Fibres you mean anisotropic inclusions …

## The method of Fratzl et al



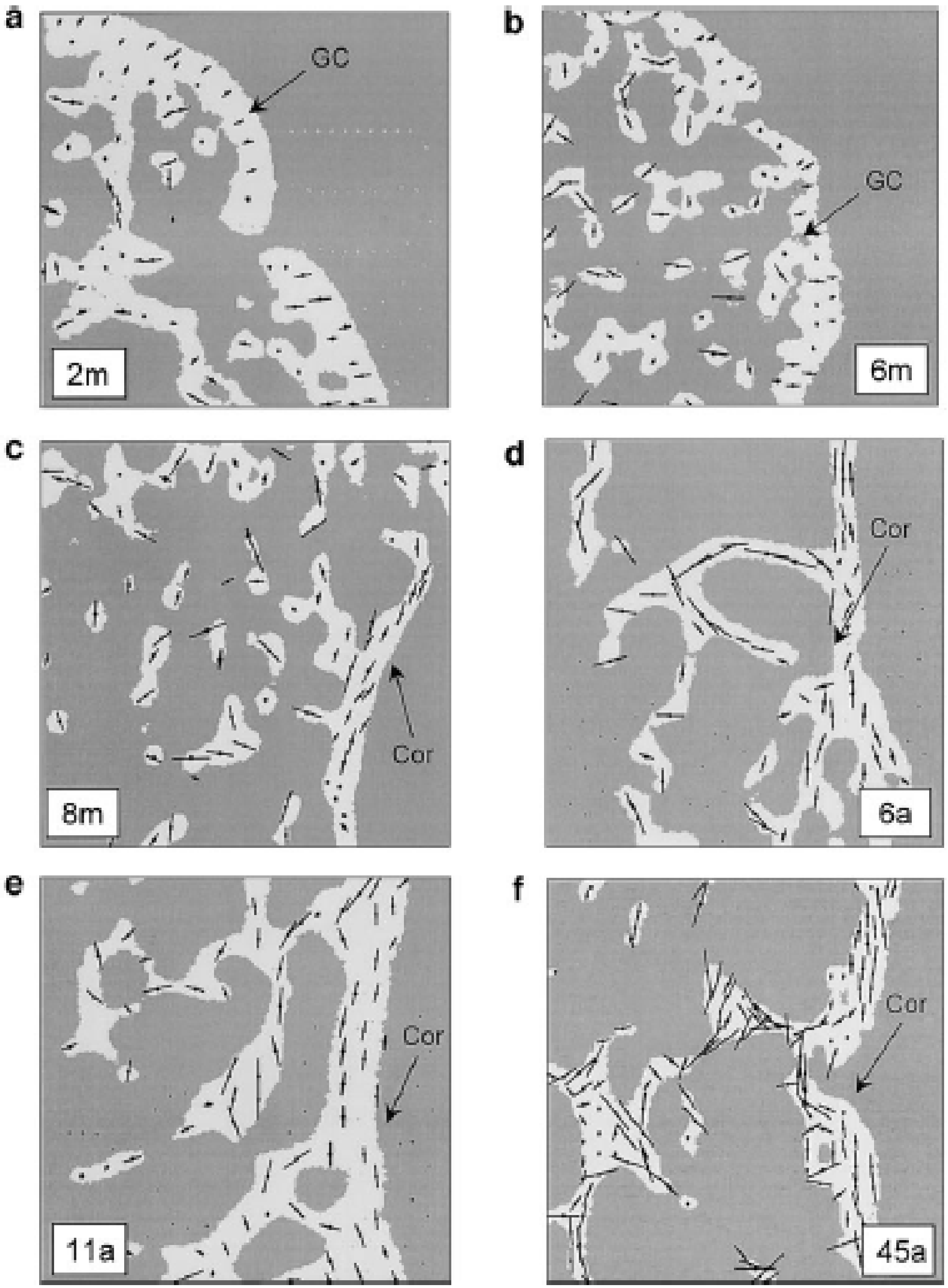


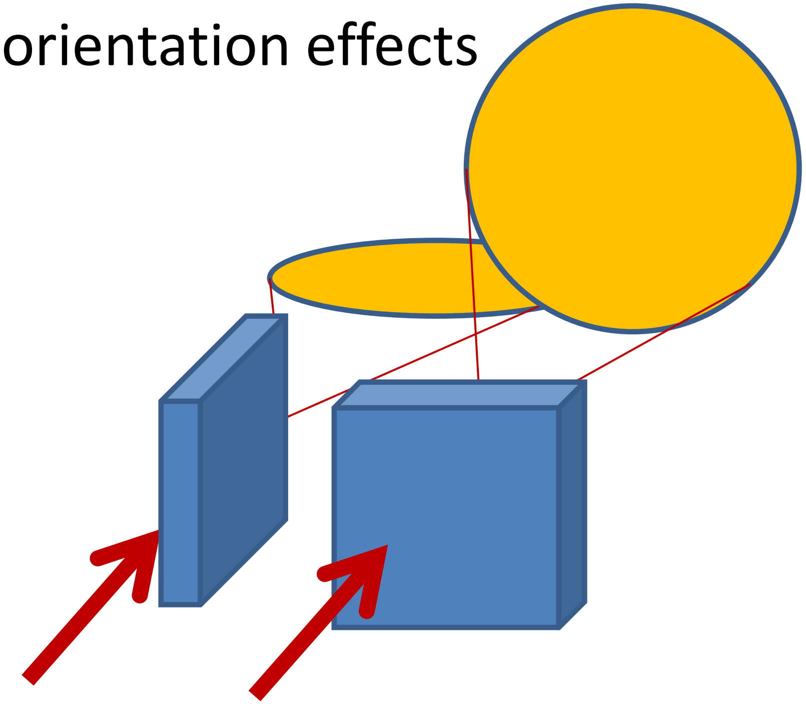
# An example of mineral alignment with development

L4-vertebral data (P. Fratzl et al)

- Shows clearly that mineral platelets become more aligned with development
- However, be aware of 3D orientation effects



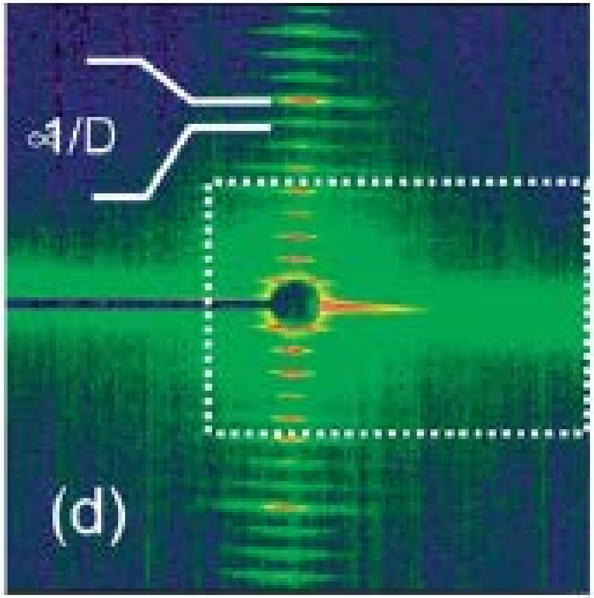

P. Roschger et al, J. Struct. Biol. (2001)

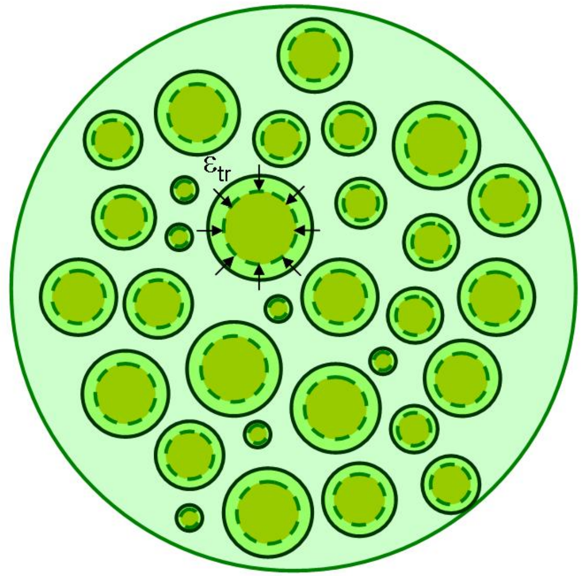


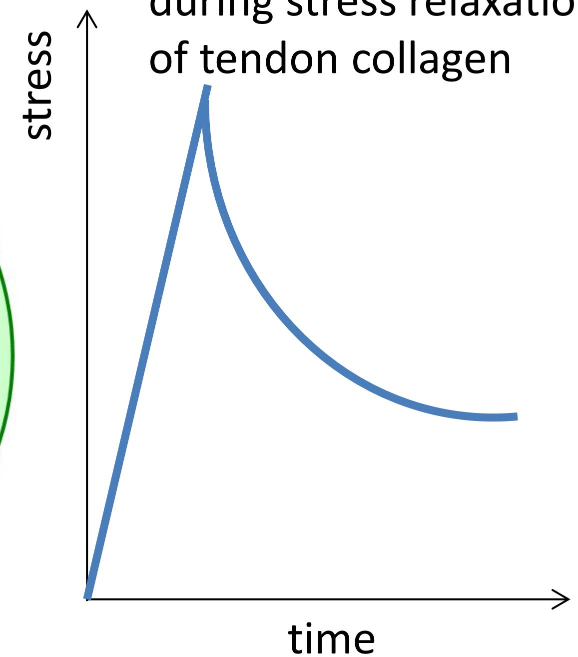
# A third aspect of SAXD spectrum for tendon collagen

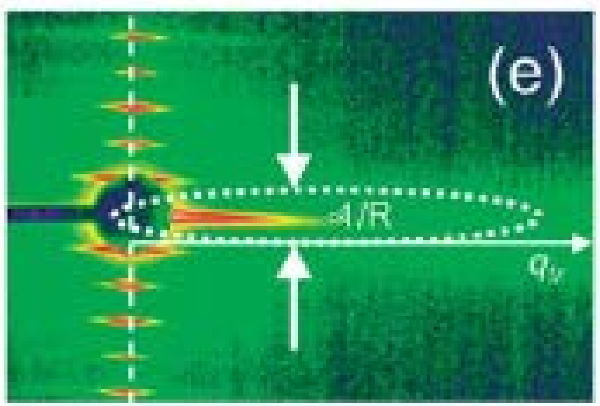
## Lateral deformation

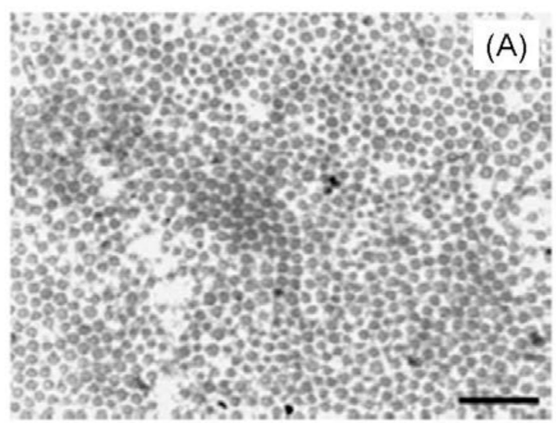
- Time resolved SAXD during stress relaxation of tendon collagen

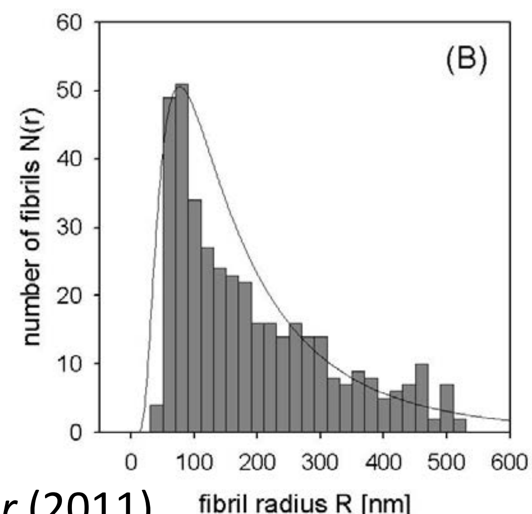


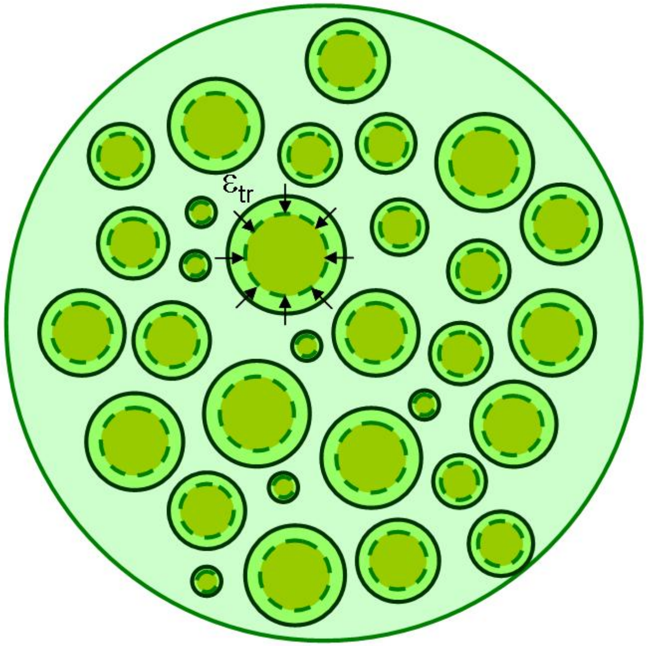



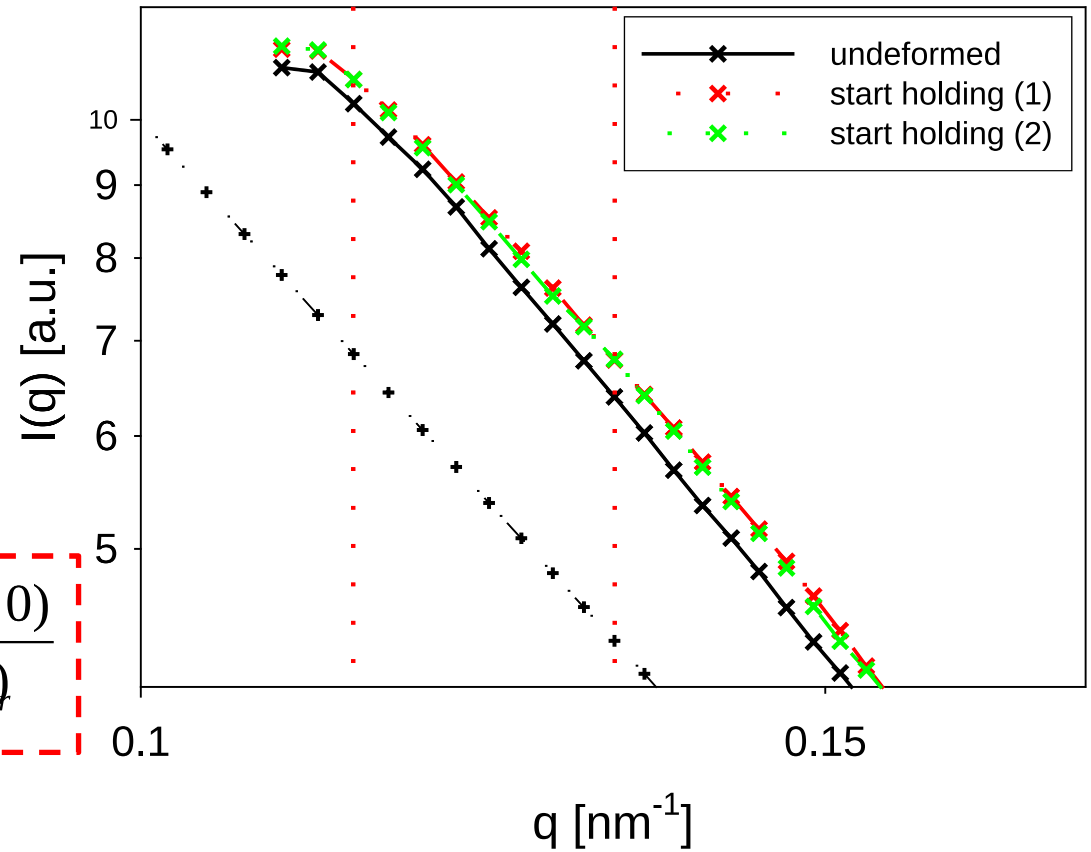

H. S. Gupta et al, *J.Struct. Biol.* (2010); H. R. C. Screen et al *Soft Matter* (2011)

# Approximate method

## Assume all fibrils have the same percentage reduction in radius

$$I(q) = c\int_0^\infty p(R)\left|F(q;R)\right|^2 dR = c\int_0^\infty p(R)\left[\rho_e \pi R^2\right]^2 \left(\frac{2J_1(qR)}{qR}\right)^2 dR$$

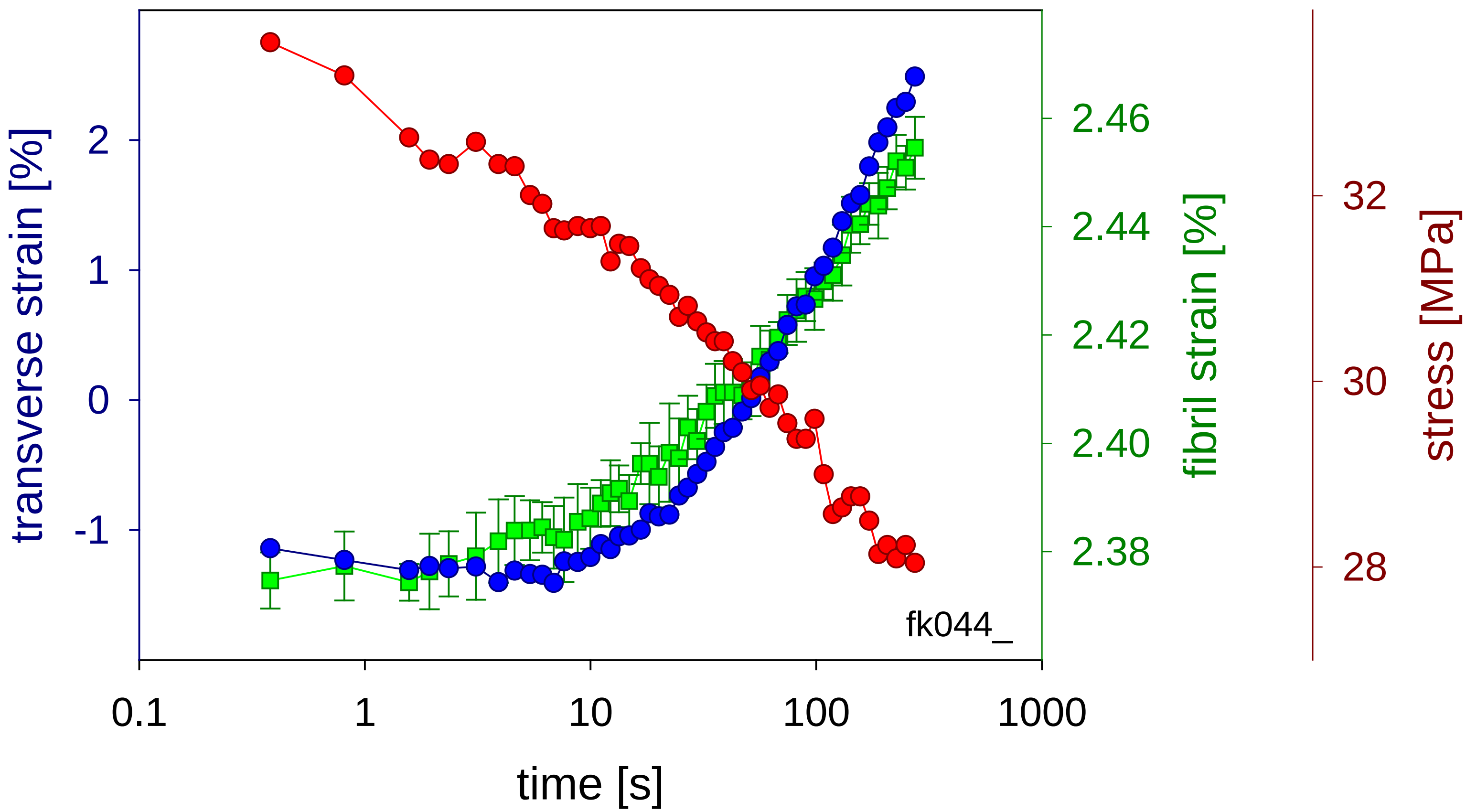



$$\ln\left(1+\varepsilon_{tr}\right) \approx \varepsilon_{tr} = \frac{1}{3}\ln\frac{I(q;\varepsilon_{tr}=0)}{I(q;\varepsilon_{tr})}$$

$$I(q;\varepsilon_{tr}) \to \frac{c}{q^3\left(1+\varepsilon_{tr}\right)^3}\int_0^\infty p(R)\left[\rho_e \pi R^2\right]^2 \left\{\frac{8}{\pi R^3}\right\}\cos^2\left(qR-\frac{\alpha}{2}-\frac{\pi}{4}\right)dR = \frac{I(q;\varepsilon_{tr}=0)}{\left(1+\varepsilon_{tr}\right)^3}$$

# The result: Fibrillar swelling during axial relaxation




- In excess of volume conservation implying extrafibrillar diffusion
- No specific assumptions on size distributions

H. R.C. Screen et al, Soft Matter (2011)

# Summary

- Case study : mineralized fibrils in bone-like tissues
- Extracting mechanics at multiple scales:
  - fibril strain,
  - heterogeneous fibril deformation
  - Mineral platelet strain

- Interaction of sample geometry and spectra obtained
- Method general: applied to
  - Tendon collagen, muscle, mineralized collagen in bone, enamels and other high-ceramic "fiber" composites
  - Geometry of the 3D SAXD pattern in each case has to be considered separately
  - Mechanics is different from the "invariants" and other integral measures that have been discussed before