Small X-ray scattering: problems and solutions

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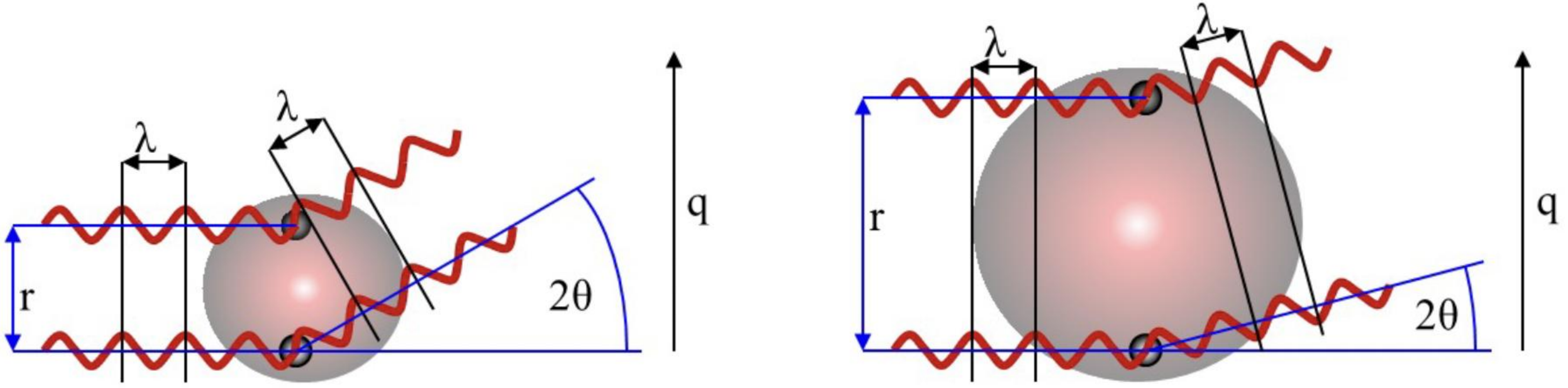
McGill University

Canadian Powder Diffraction Workshop 18 (CPDW18)

McGILL CHEMISTRY
CHARACTERIZATION

X-RAY
DIFFRACTION

Why SAXS?



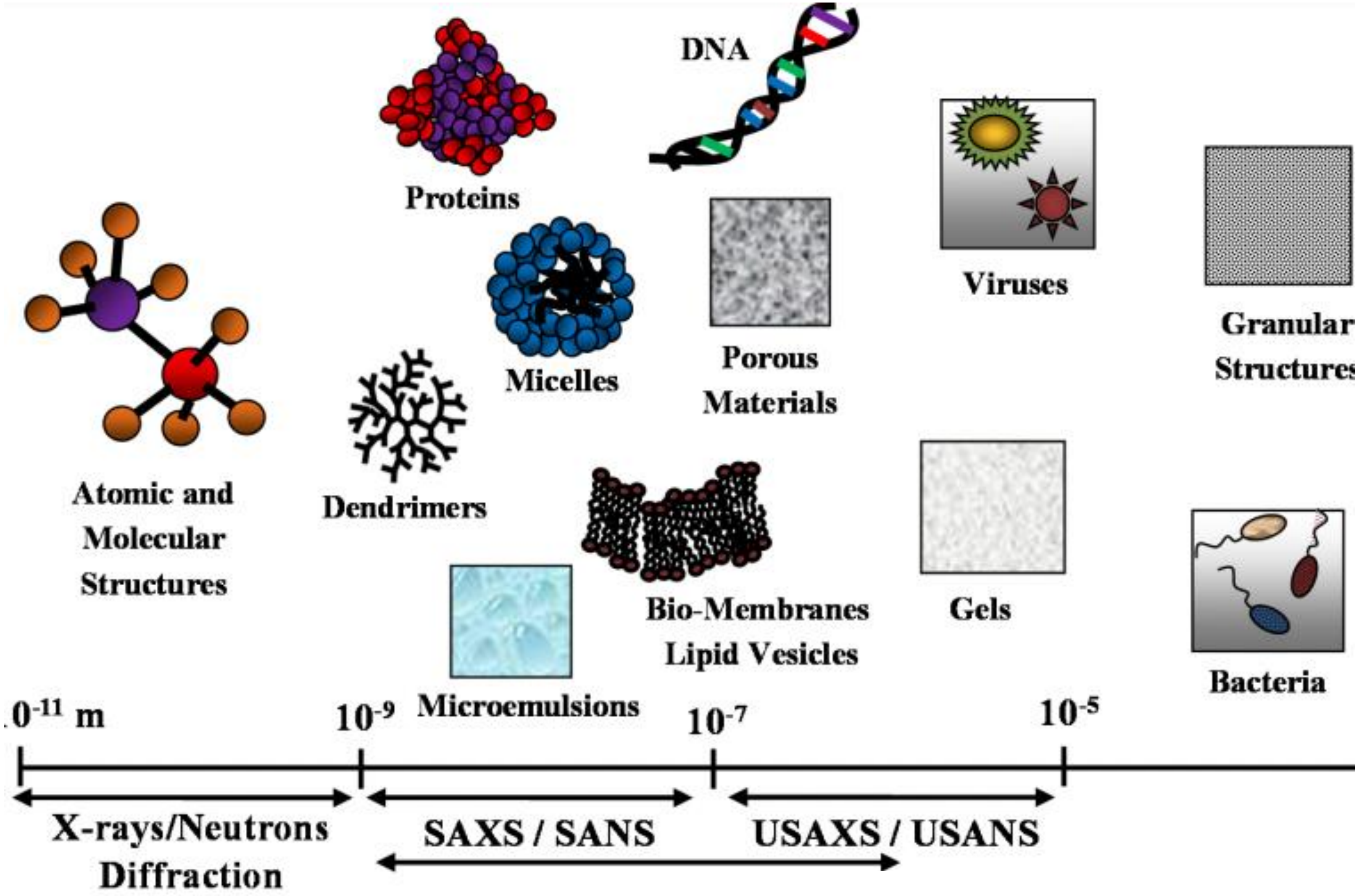
Small size ⇔ Large angle

Large size ⇔ Small angle

Scattering for two points of a wave front on small and large particles.

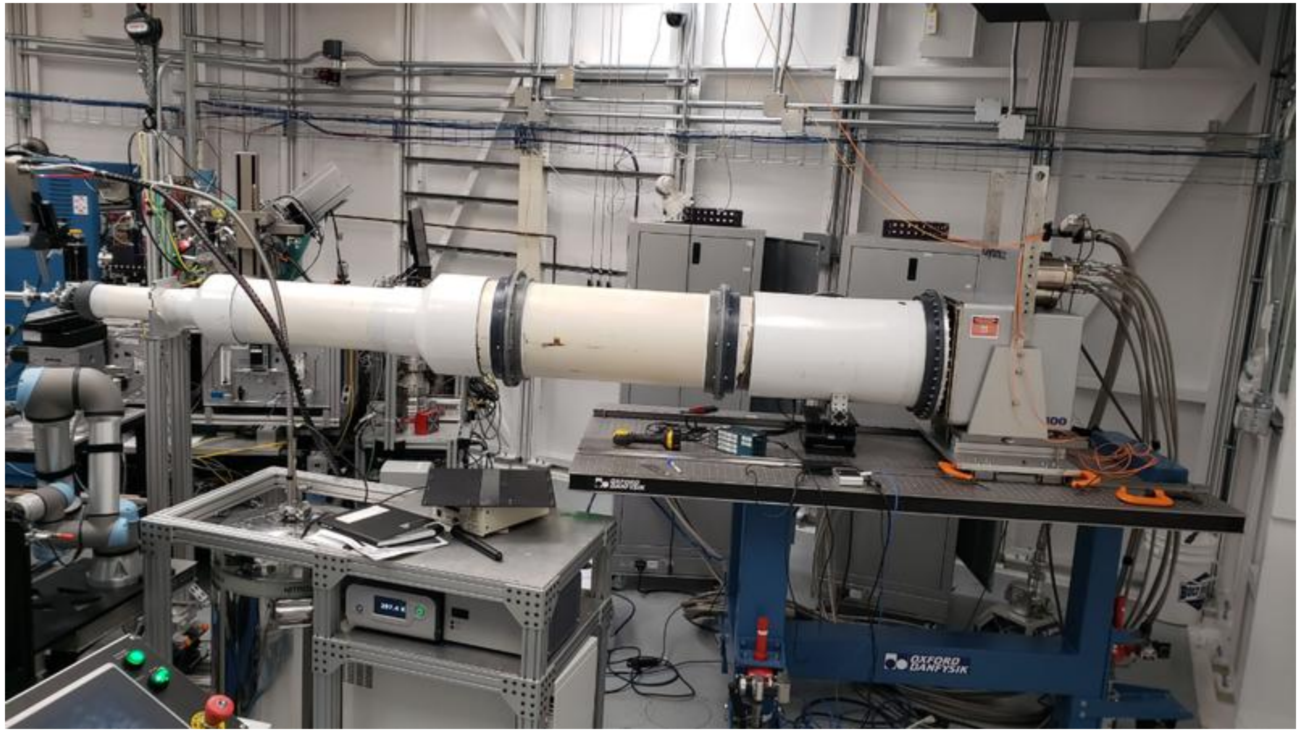
Anton Paar

X-ray diffraction and scattering



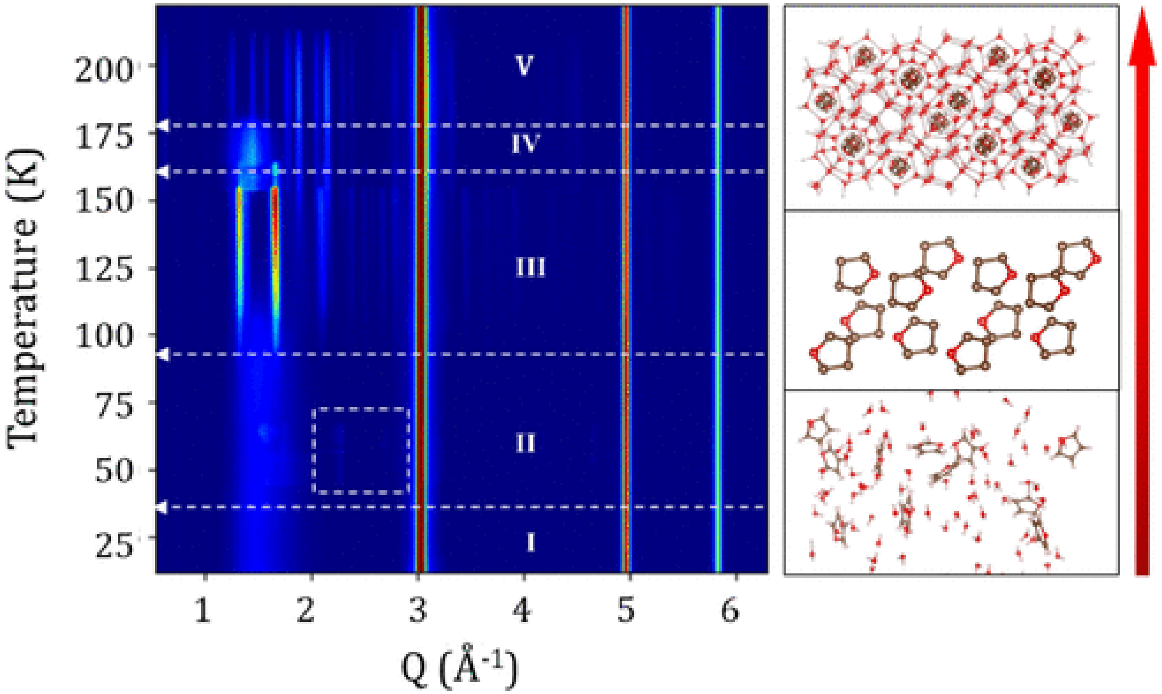
Lombardo *et al*. *Molecules* **2020**, *25*, 5624

X-ray scattering and diffraction in CLS



https://brockhouse.lightsource.ca/about/low-energy-wiggler-beamline/

Tse *et al*. reported the Clathrate hydrate behaviour



J. Phys. Chem. Lett. 2025, 16, 20, 5160-5167

Small-angle X-ray scattering – Anton Paar SAXSPoint 2.0

The typical setup can resolve particle size range from 1 to 300 nm
q-resolution as low as 0.02 nm^{-1}

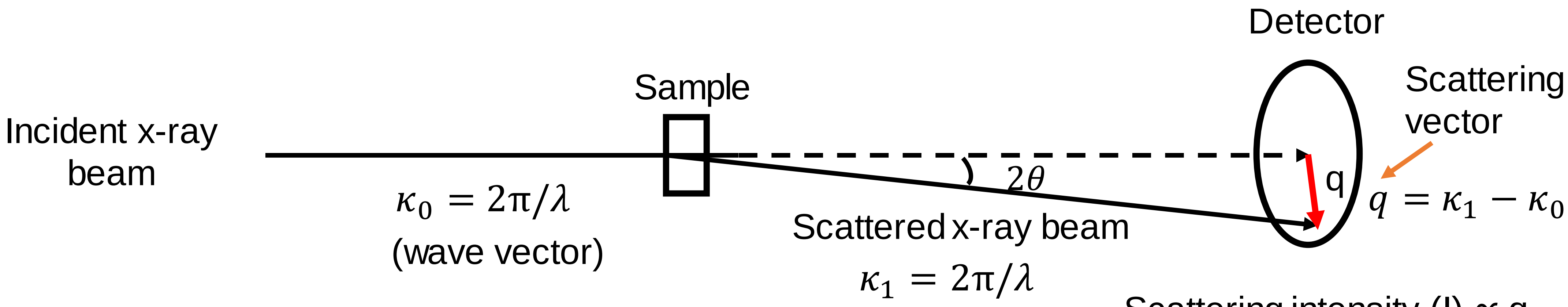
Applications

- Protein analysis
- Polymers
- Fibers
- Structural biology
- Colloidal dispersions
- Emulsions
- Pharmaceuticals
- Nanoparticles
- Liquid crystals
- Porous materials
- Thin films

Results

- Size distribution
- Shape
- Surface
- Internal structure
- Interaction
- Porosity
- Crystallinity
- Orientation
- Molecular weight

X-ray scattering: basics



$\kappa_0 = 2\pi/\lambda$

(wave vector)

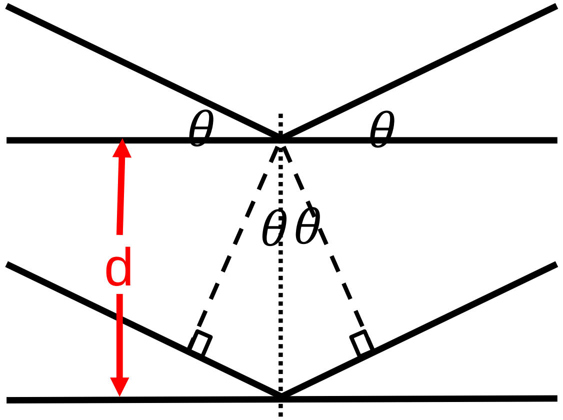
Scattered x-ray beam

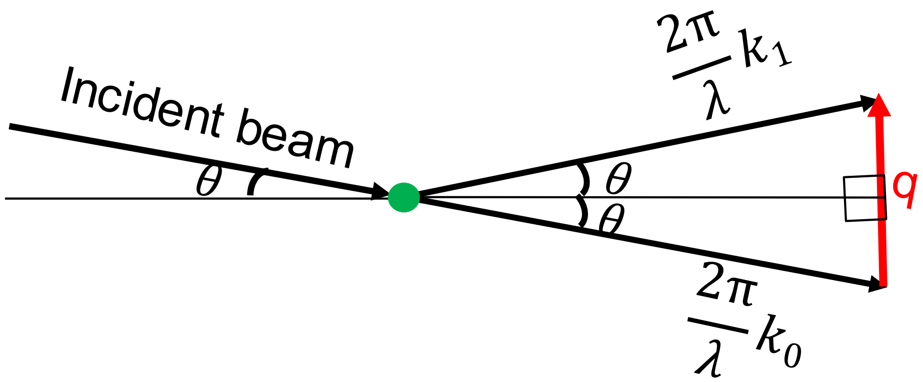
$\kappa_1 = 2\pi/\lambda$

$q = \kappa_1 - \kappa_0$

Scattering intensity (I) ∝ q

λ of incident x-ray





Bragg's law

$$\lambda = 2d\sin(\theta)$$

$$d = \frac{2\pi}{q}$$

⇩

$$q = \frac{4\pi}{d}\sin(\theta)$$

X-ray scattering: basics

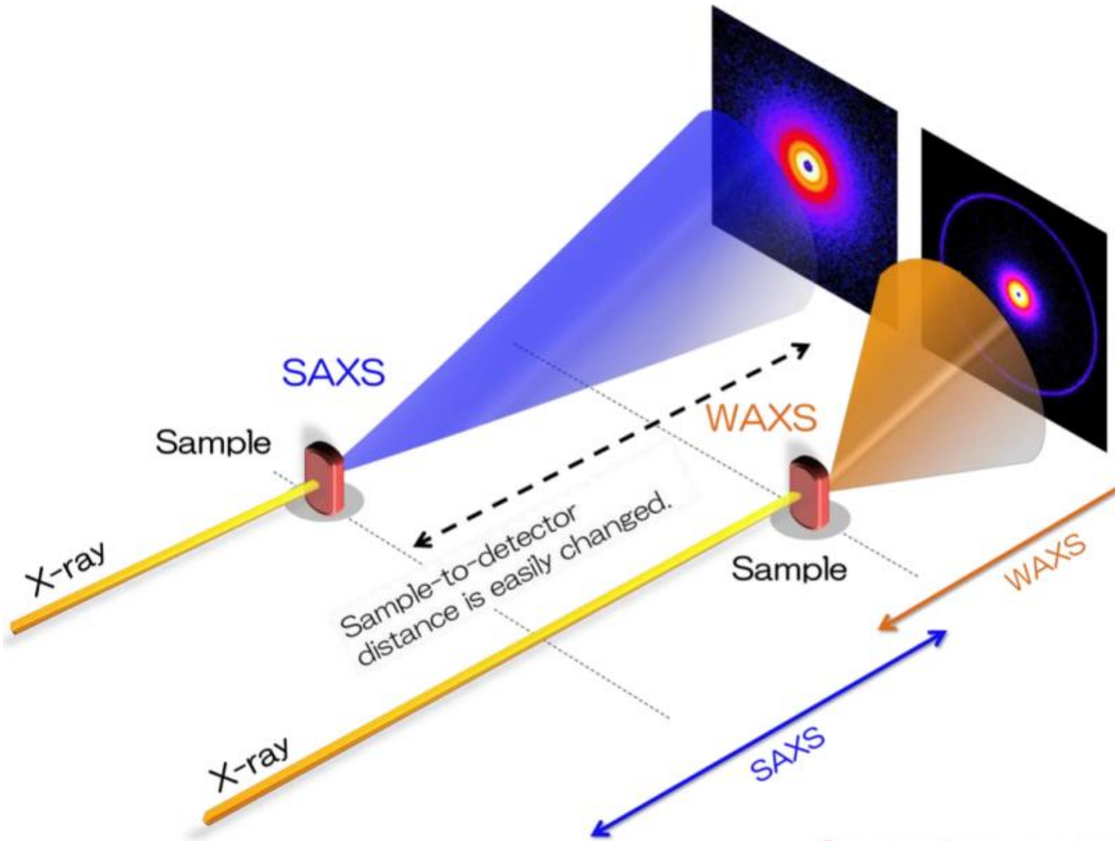


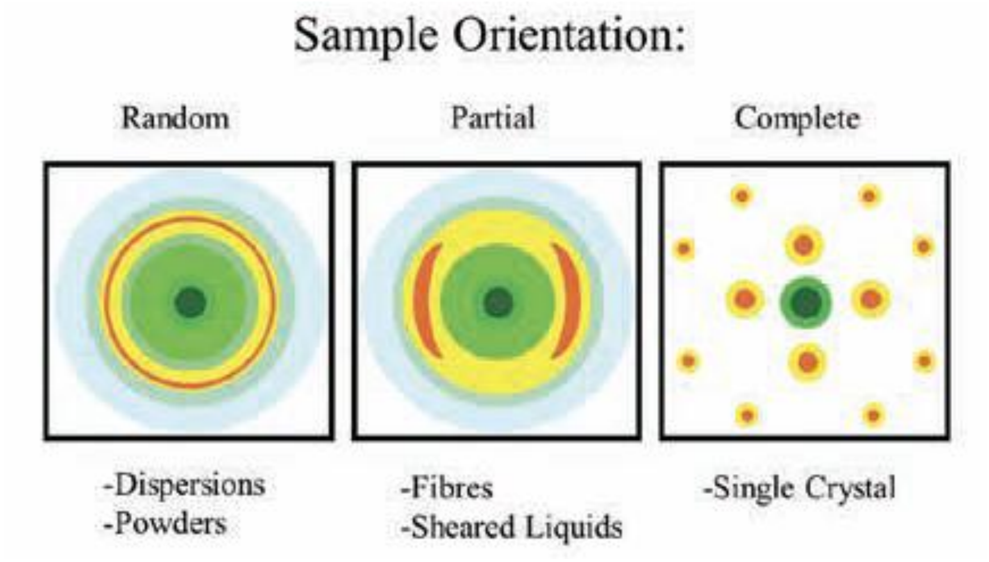
Figure 1: Experimental Differences between SAXS and WAXS

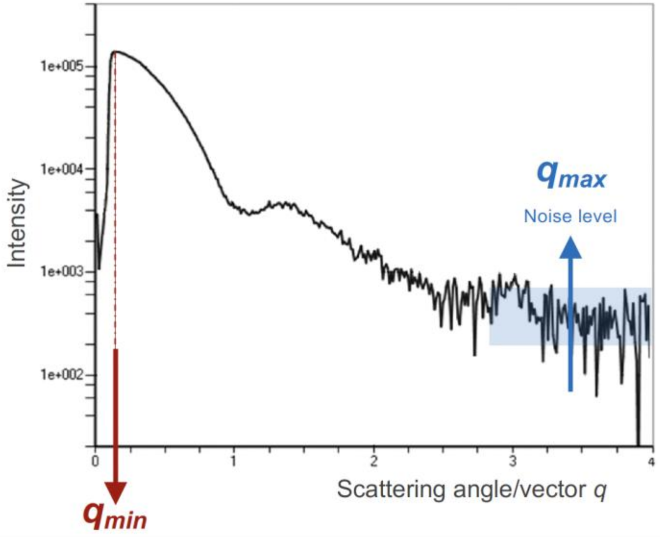
https://www.rigaku.com/techniques/industrial-saxs-waxs

Scattering vector *q*

aka 'Momentum transfer'

$$q = \frac{4\pi}{\lambda} \sin\left(\frac{2\theta}{2}\right)$$





Largest resolvable dimension (particle)

$$D_{max} = \frac{\pi}{q_{min}}$$

Smallest resolvable dimension (particle)

$$D_{min} = \frac{\pi}{q_{max}}$$

Scattering from particles

$$I(q) = n\left|F(q)\right|^2 S(q)$$

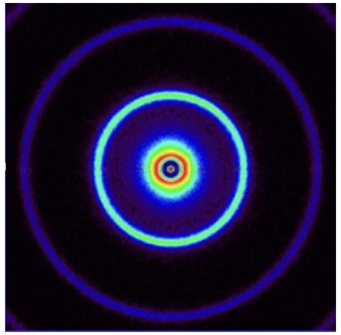
Number density
of particles

Form factor
(particle shape)

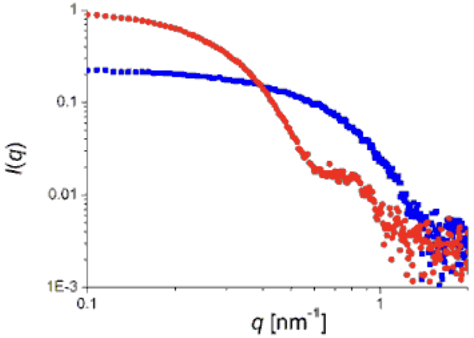
Structure factor
(particle interactions)

Discrete particles are often the easiest to understand and analyze

General flow chart for data analysis

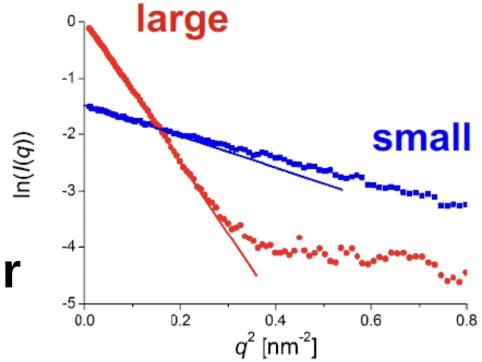


Reduction
2D to 1D



Basic calculations

Guinier



PDDF

Peak analysis

Slope analysis

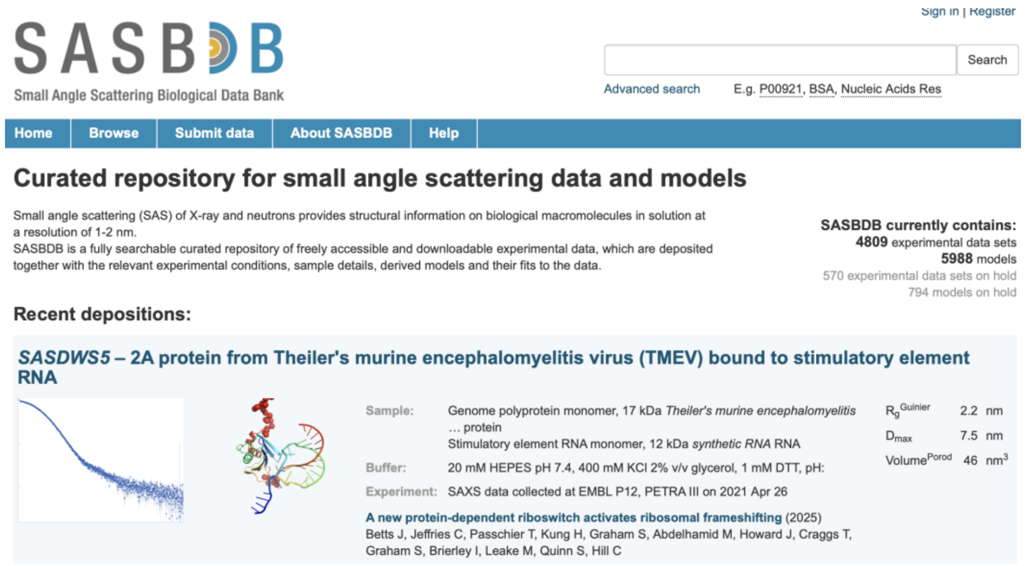
Orientation/crystallinity

Porod
$I(q) \sim q^{-n}$

Molecular weight

Model fitting

And more



Curated repository for small angle scattering data and models

Small angle scattering (SAS) of X-ray and neutrons provides structural information on biological macromolecules in solution at a resolution of 1-2 nm.
SASBDB is a fully searchable curated repository of freely accessible and downloadable experimental data, which are deposited together with the relevant experimental conditions, sample details, derived models and their fits to the data.

SASBDB currently contains:
4809 experimental data sets
5988 models
570 experimental data sets on hold
794 models on hold

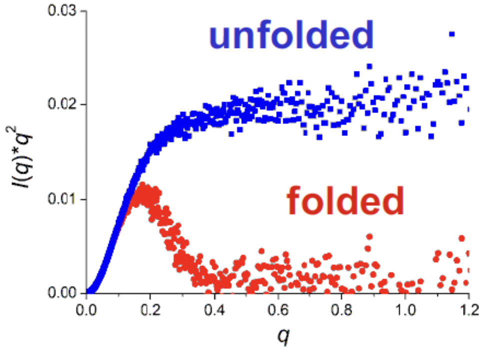
Recent depositions:

SASDWS5 – 2A protein from Theiler's murine encephalomyelitis virus (TMEV) bound to stimulatory element RNA

Sample: Genome polyprotein monomer, 17 kDa *Theiler's murine encephalomyelitis* … protein
Stimulatory element RNA monomer, 12 kDa *synthetic RNA* RNA
Buffer: 20 mM HEPES pH 7.4, 400 mM KCl 2% v/v glycerol, 1 mM DTT, pH:
Experiment: SAXS data collected at EMBL P12, PETRA III on 2021 Apr 26

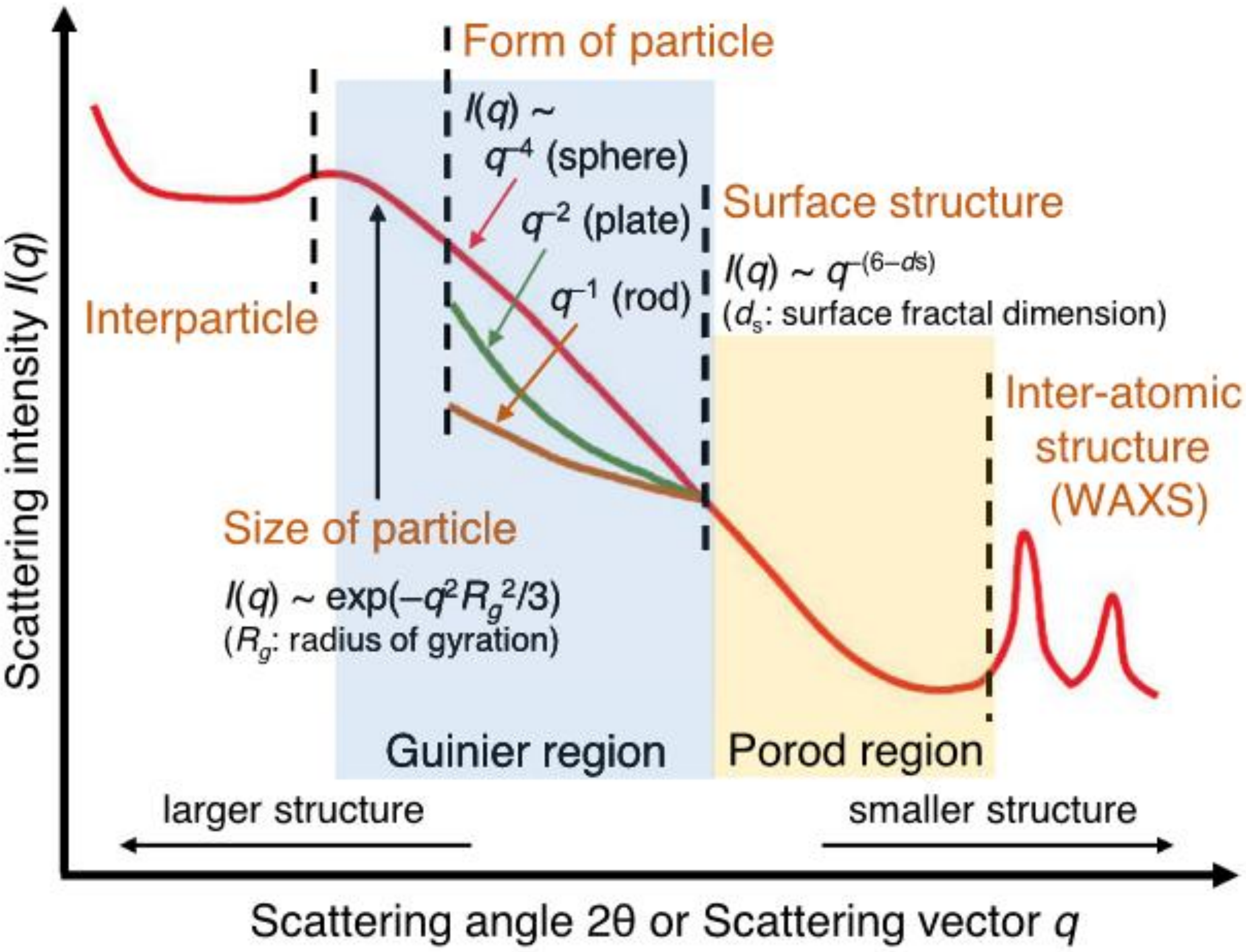
$R_g^{Guinier}$	2.2	nm
D_{max}	7.5	nm
$Volume^{Porod}$	46	nm^3

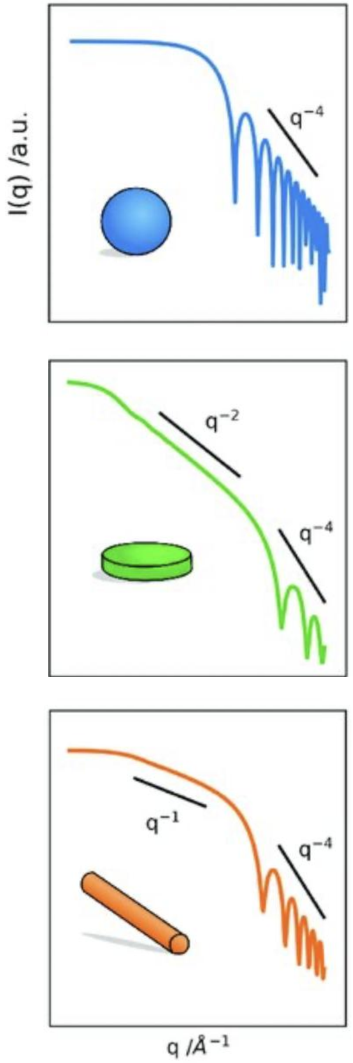
A new protein-dependent riboswitch activates ribosomal frameshifting (2025)
Betts J, Jeffries C, Passchier T, Kung H, Graham S, Abdelhamid M, Howard J, Craggs T, Graham S, Brierley I, Leake M, Quinn S, Hill C



Kratky plot

q-plot (or s) and what type of information can we obtain?



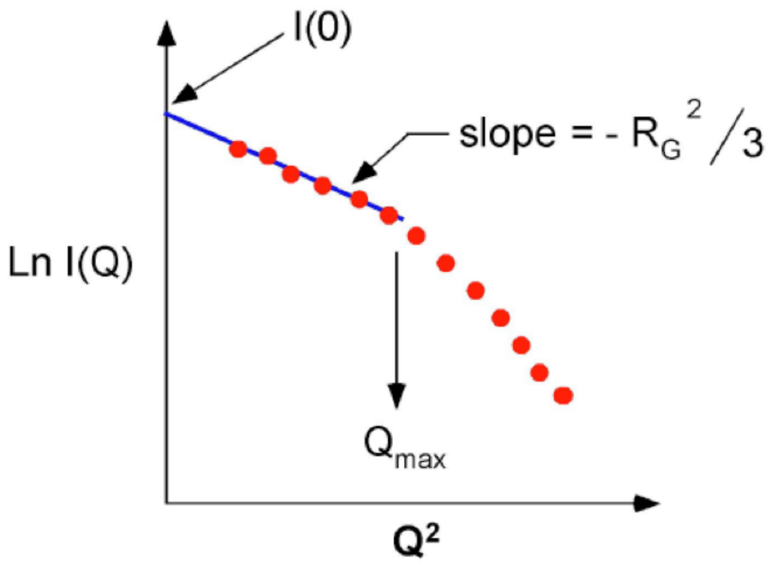


Axel Rüter, Lund University, Ph.D. Thesis 2020

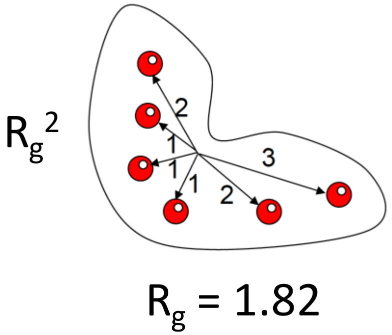
Guinier region: defines the radius of gyration R_g, *the overall* dimension of the particle

Guinier Plot:

$$\ln[I(Q)] = \ln[I(0)] - Q^2 R_G^2/3$$



Quarterly Reviews of Biophysics 2007, 40, 191–285.

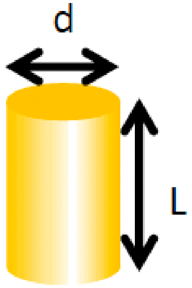


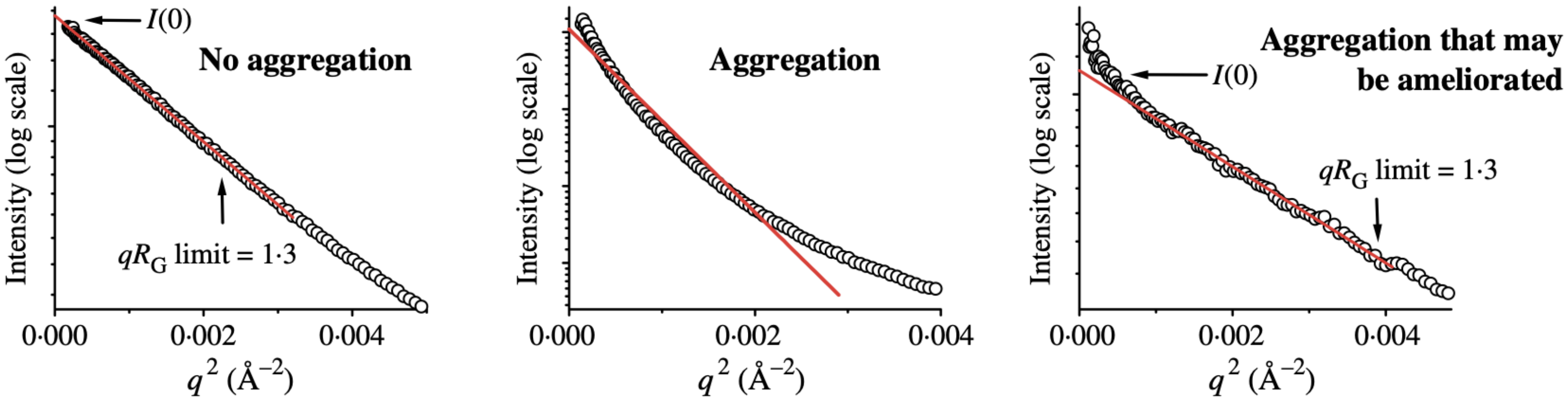
Sphere:

$$R_g^2 = \frac{3}{5}R^2$$

Cylinder:

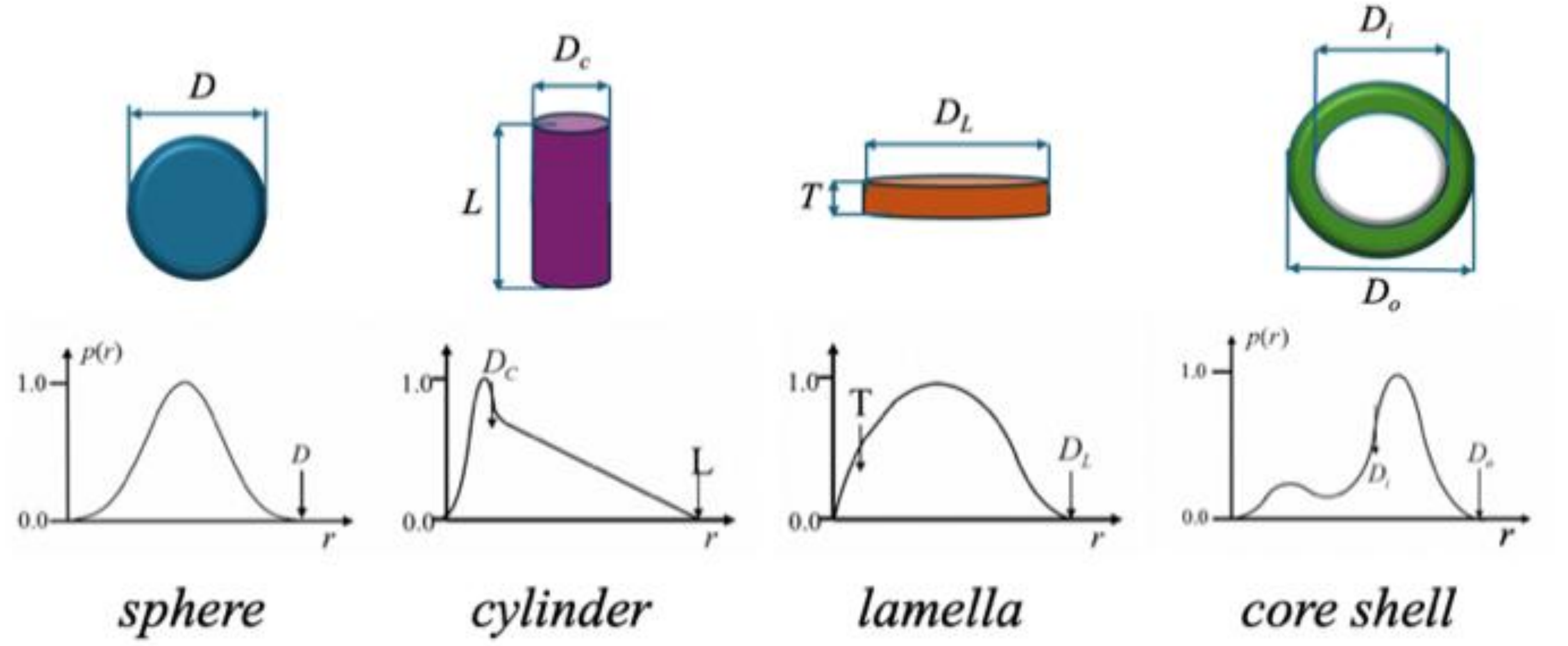
$$R_g^2 = \frac{L^2}{12} + \frac{d^2}{8}$$





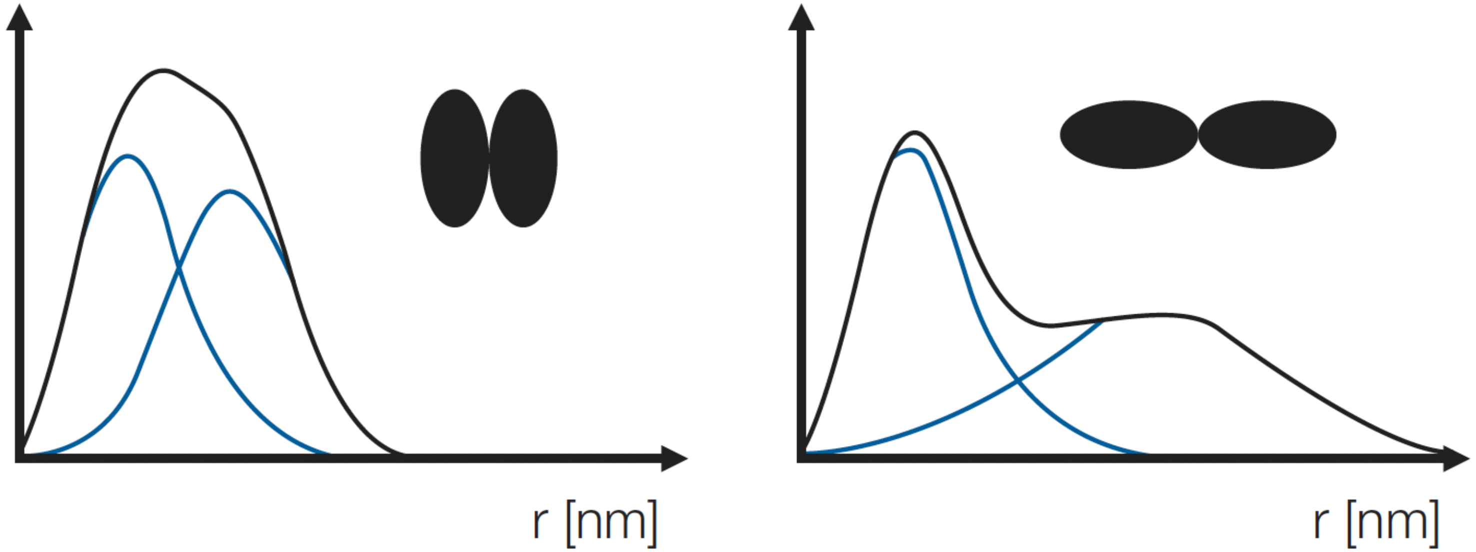
The radius of gyration is a distribution of components of an object around an axis. Note that R_g does not contain information about particle shape

Pair distance distribution function P(r); PDDF



- Calculated from form factor
- Appearance of the resulting plot enables prediction of molecular size and shape
- P(r) is the inverse Fourier Transform of I(q) and represents the histogram of electron-pair distances in compounds.

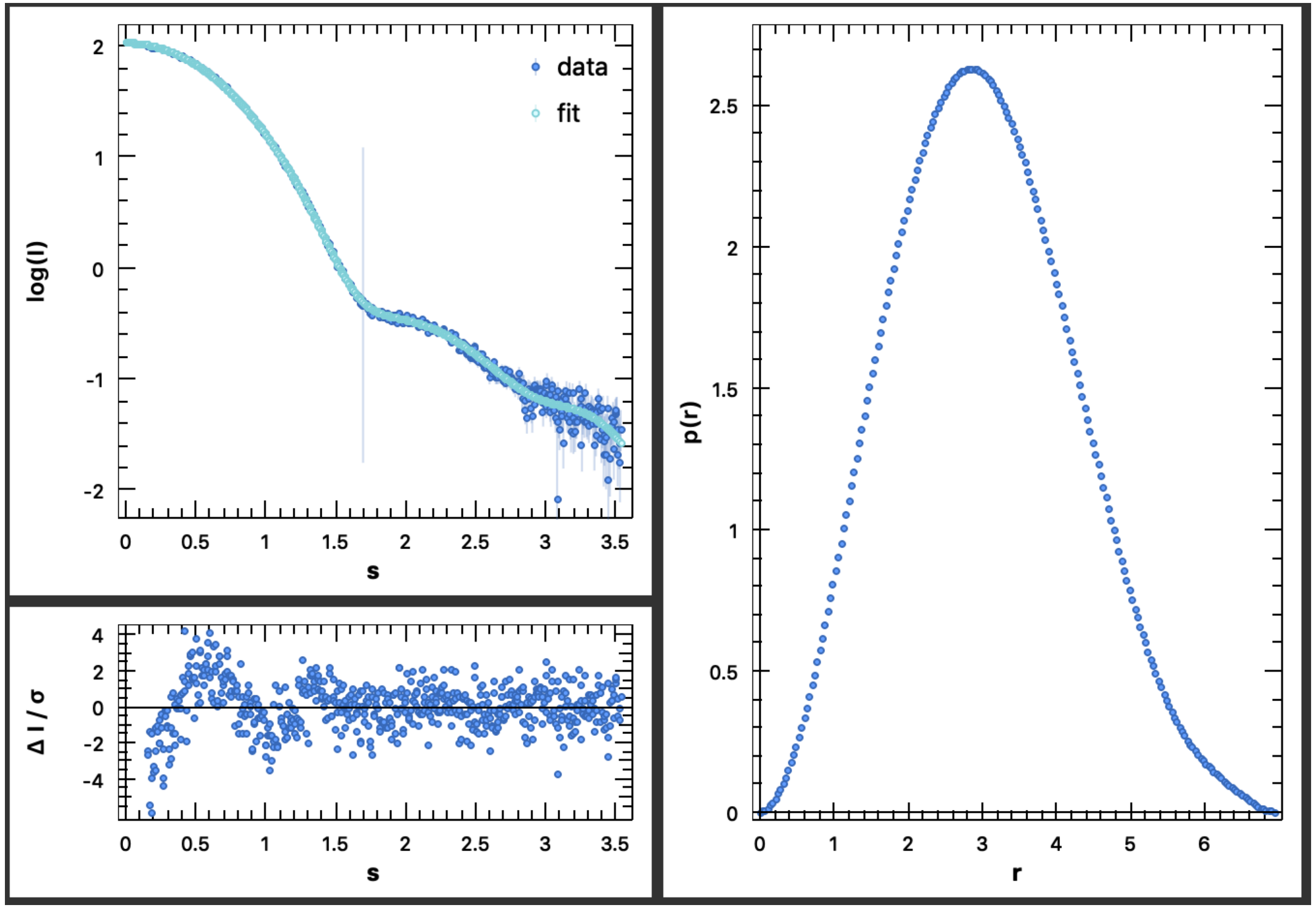
Aggregates and PDDF



The aggregate of two subunits makes a PDDF, which a second peak can recognize.

Anton Paar

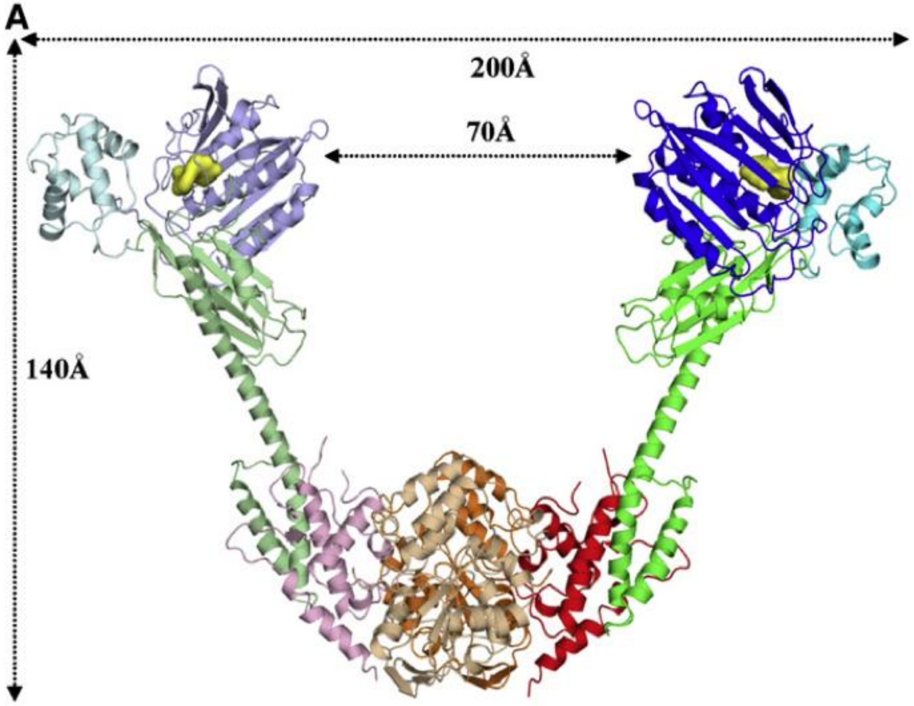
The R_g and P(r) of quantum dots

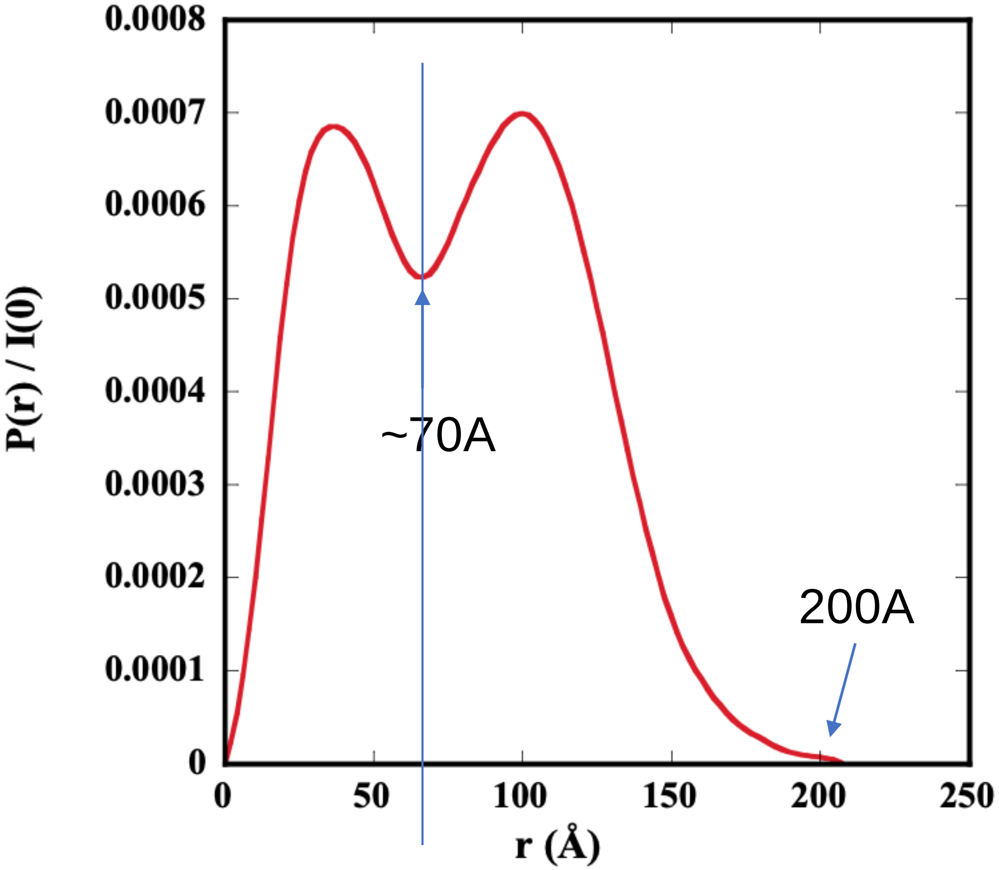


Monodisperse spheres of CdS quantum dots
$R_g = 2.26$ nm
(solved using ATSAS).

The results are in good agreement with the TEM

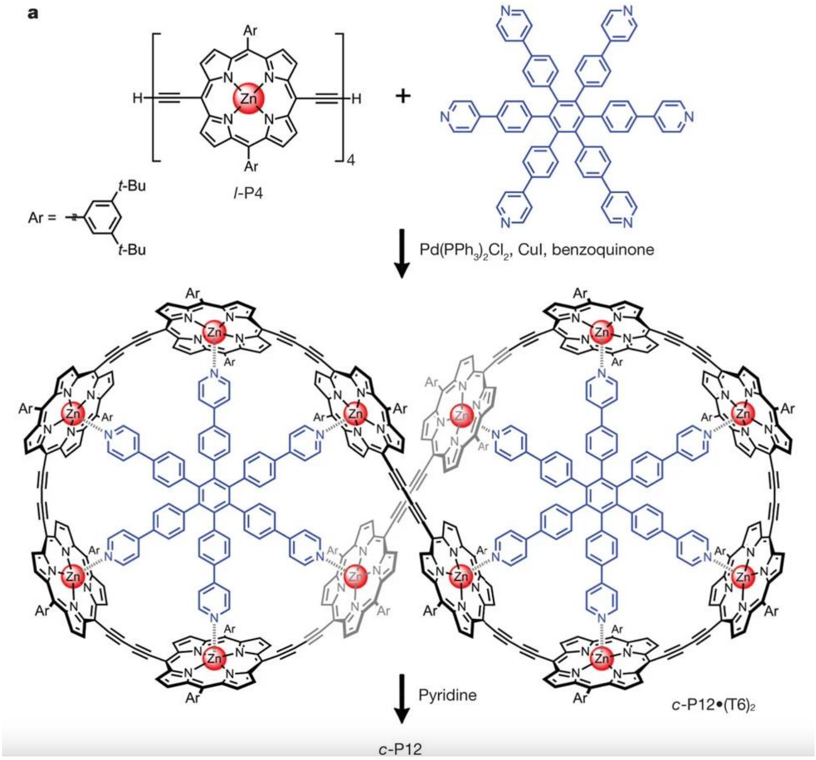
PDDF example of biomolecular structure

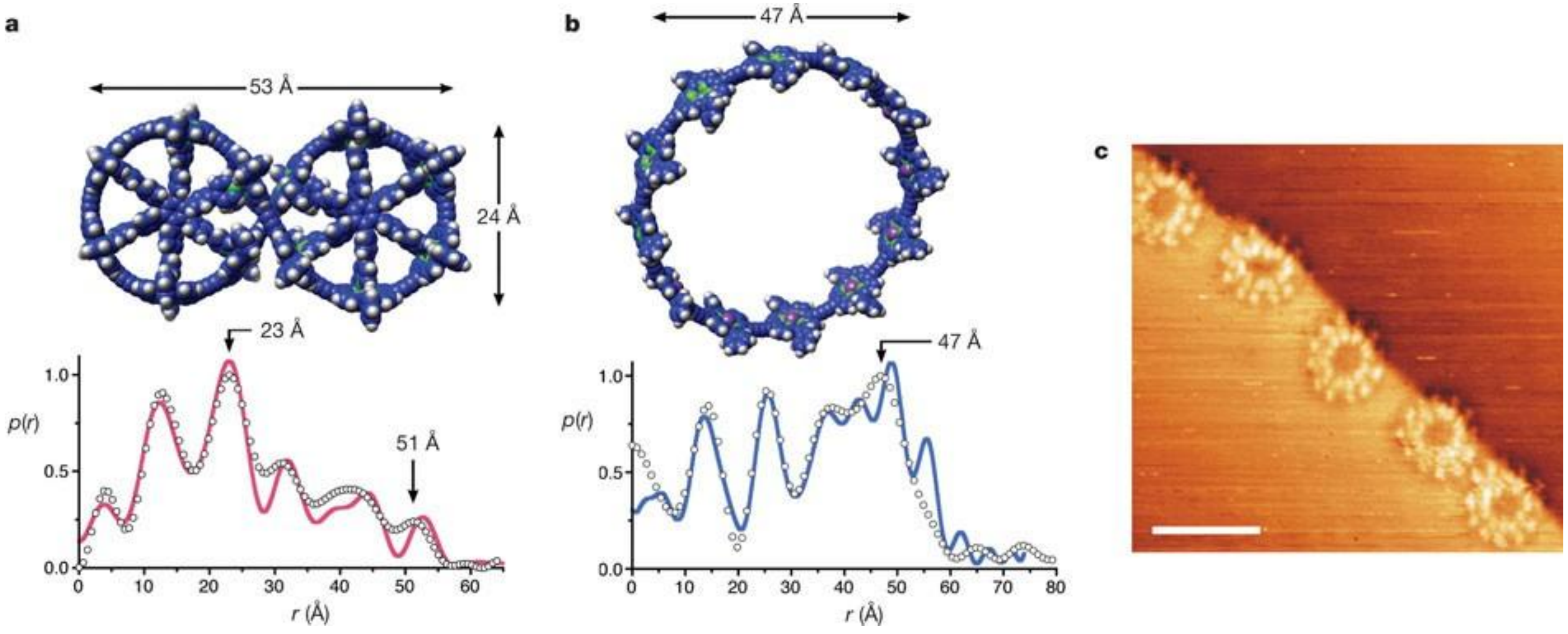




M. Graille et al., Structure 2008, 16, 360-370

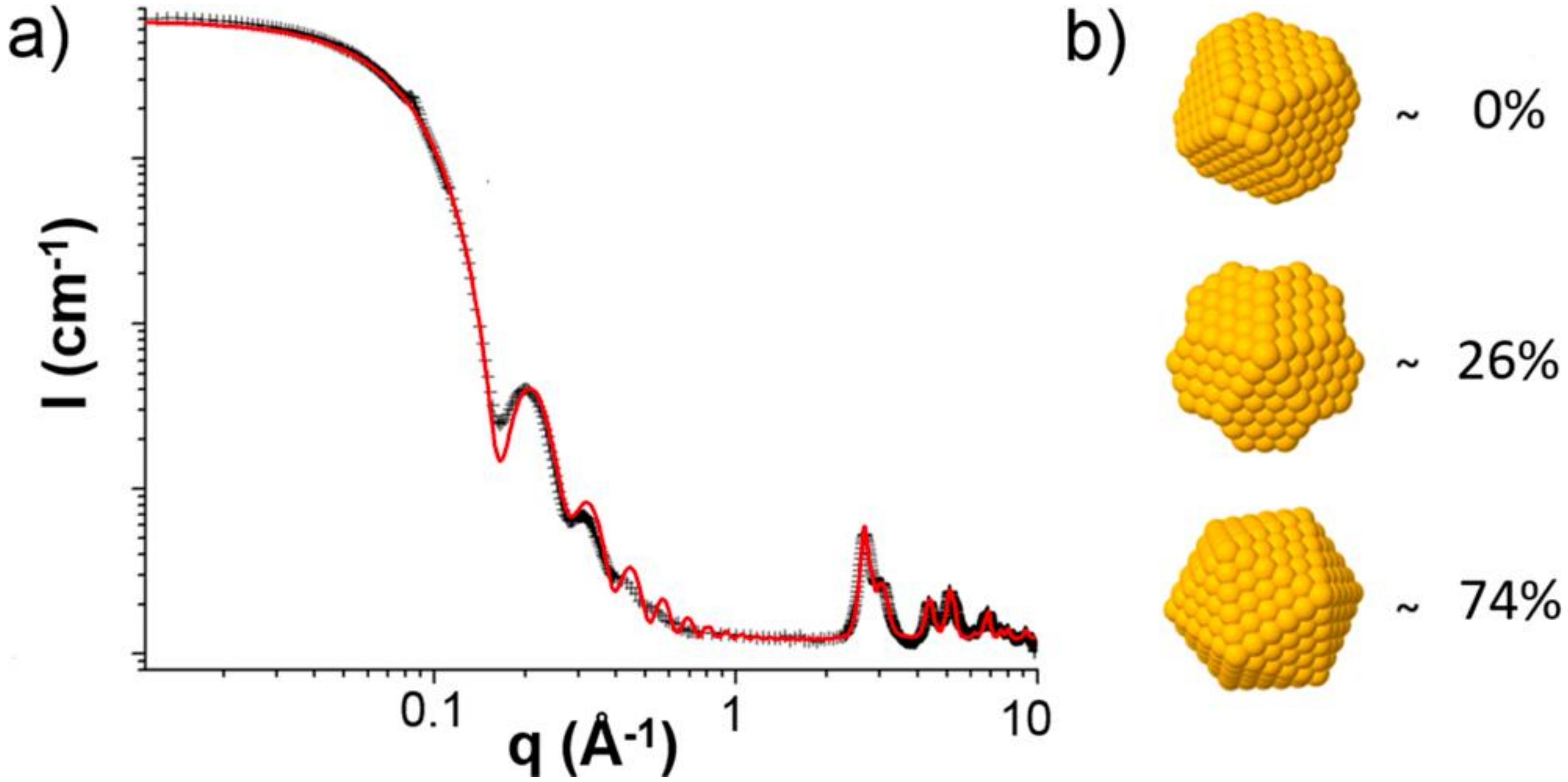
Pair distance distribution function P(r): examples





Nature 2011, **469**, 72–75

Modeling different external shapes using SAXS and WAXS

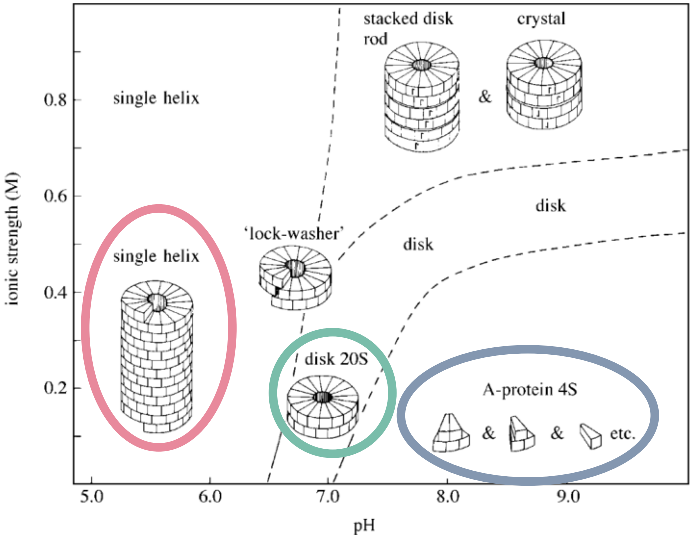


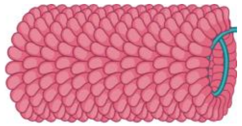
The best fit is obtained from a linear combination of the calculated scattering intensities of three different external shapes, including icosahedra, decahedra, and truncated octahedral structures

Nano Lett. 2015, 15,6088−6094

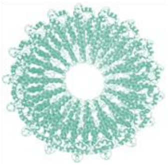
SAXS – Lab X-ray source vs Synchrotron

Guido Merino (guido.merino@mail.mcgill.ca)
Dr. Harrington Lab, McGill University.
Matt.harrington@mcgill.ca

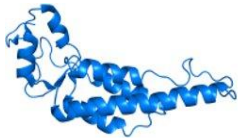




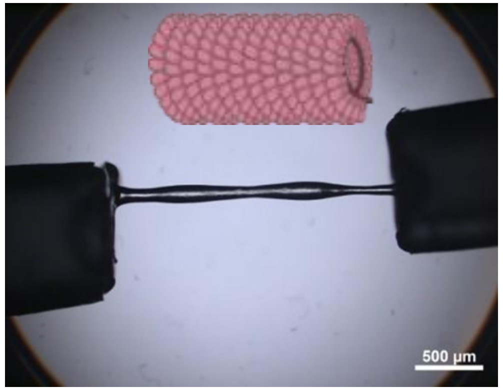
Rod, pH 5.0
10mM Acetate

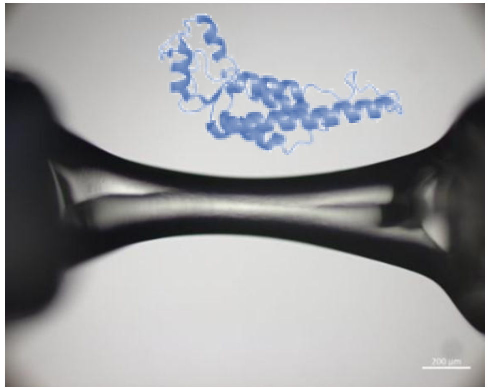


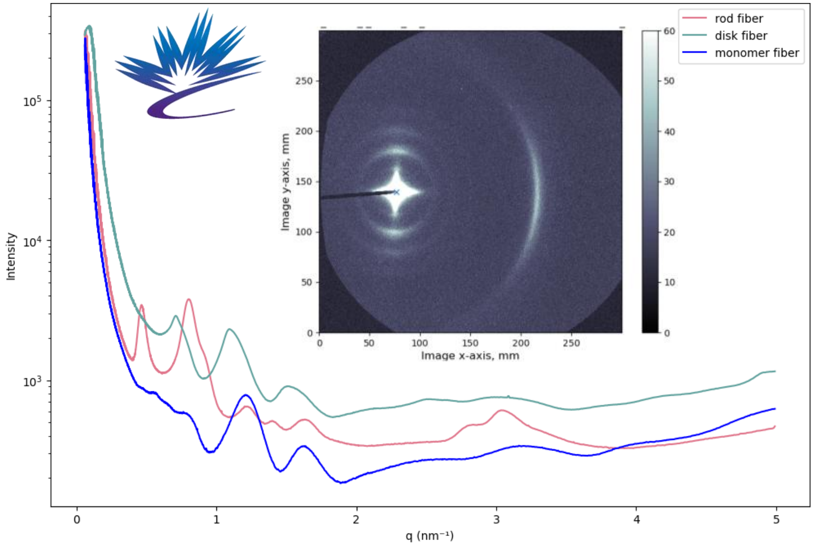
Disk, pH 6.8
10mM Potassium Phosphate

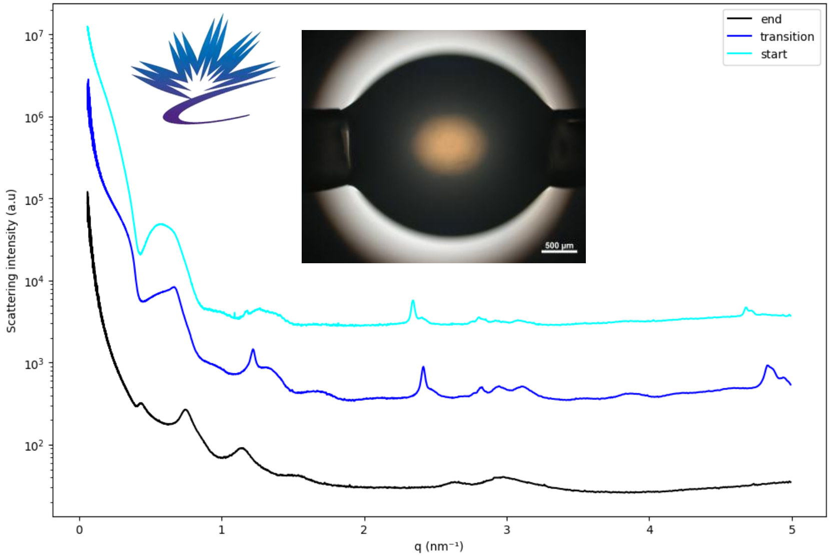


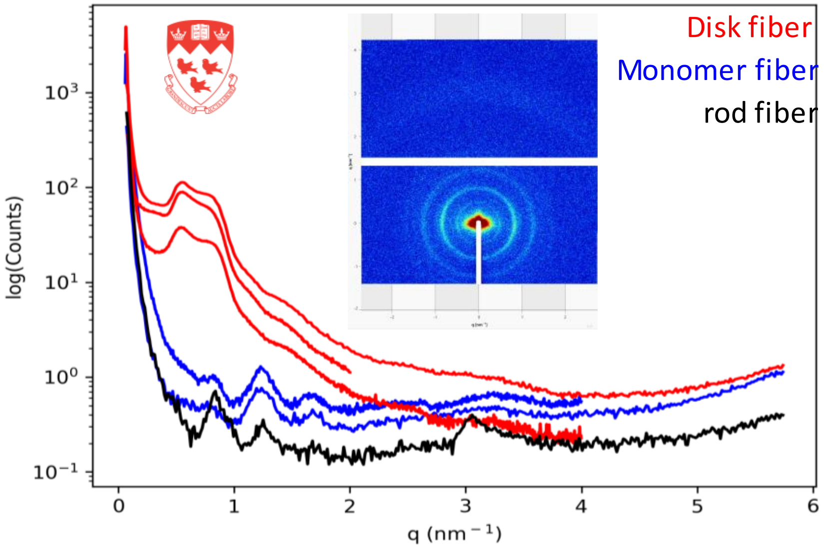
Monomer, pH 8.0
10mM Triethanolamine



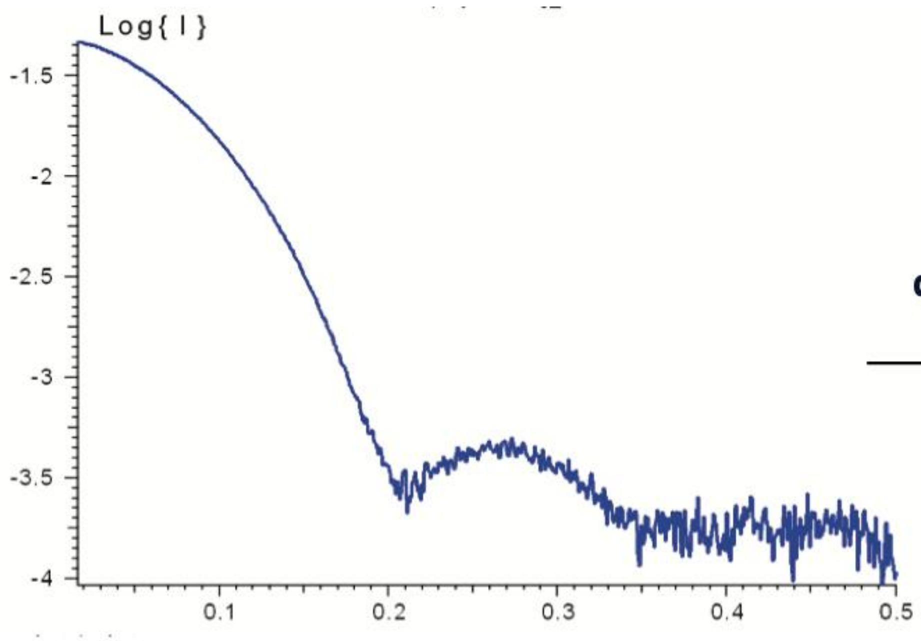






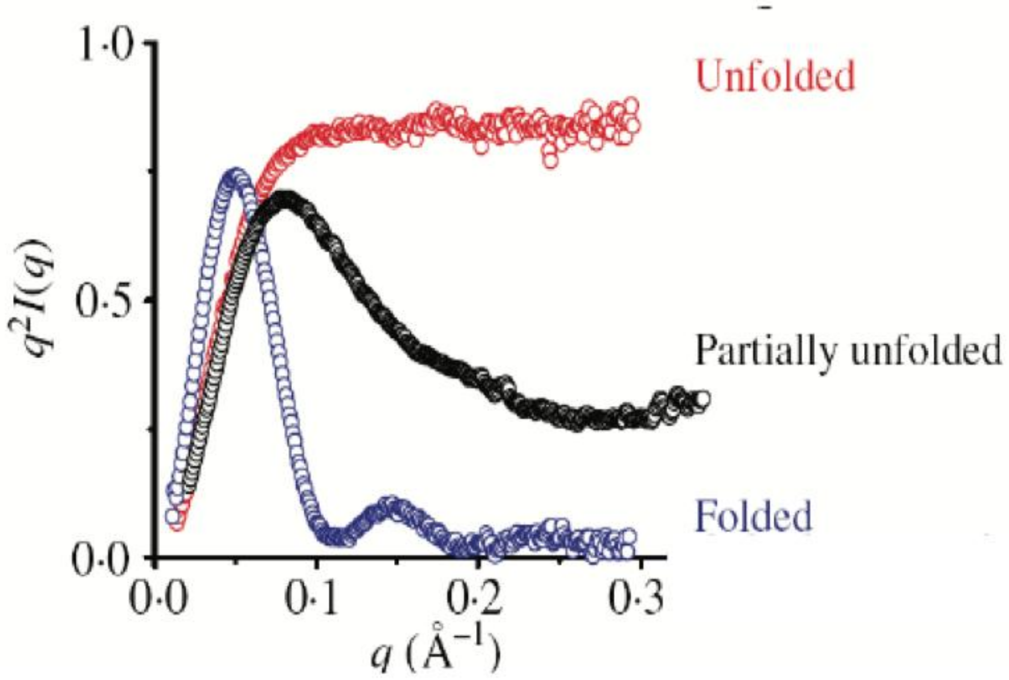


Kratky plot: protein analysis



A protein sample

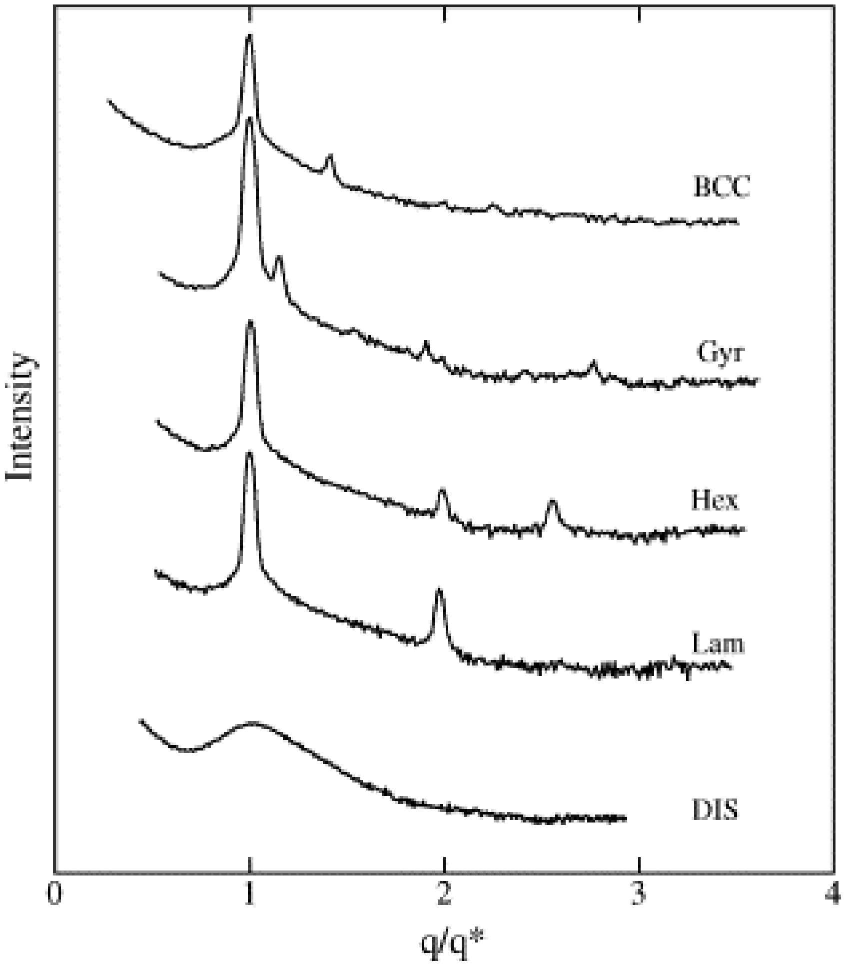
q^2 I(q) *versus* q



Putnam, D., et al. (2007) Quart. Rev. Biophys. 40, 191-285.

- Kratky plot: I*q^2 vs. q
- Sensitive to morphology of the particle
- Sensitive to the compactness of a protein
- Unfolded and folded states of proteins are easy to distinguish

Supramolecular structures and their corresponding peak ratios observed in SAXS



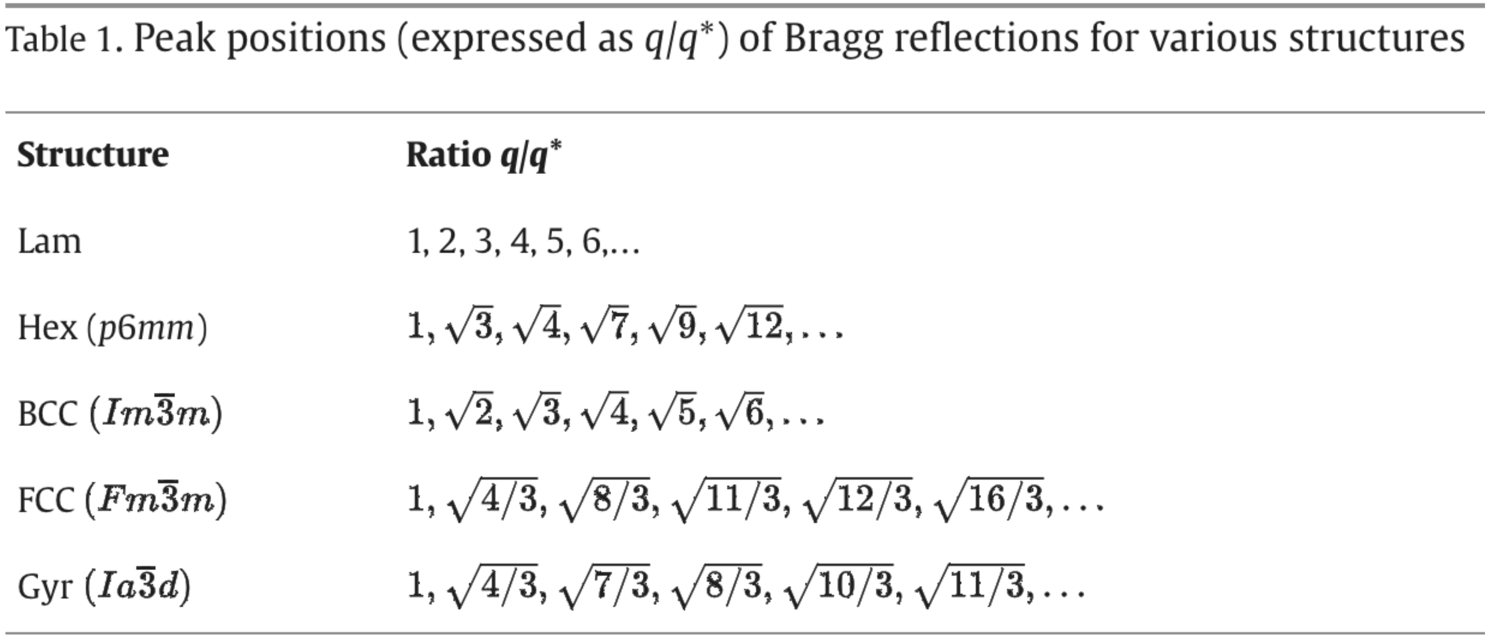
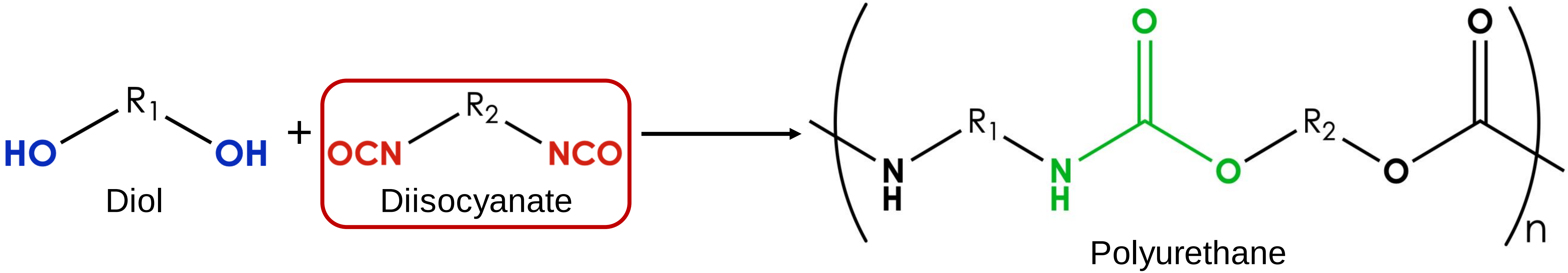


Table 1. Peak positions (expressed as q/q^*) of Bragg reflections for various structures

Structure	**Ratio q/q^***
Lam	1, 2, 3, 4, 5, 6,…
Hex ($p6mm$)	$1, \sqrt{3}, \sqrt{4}, \sqrt{7}, \sqrt{9}, \sqrt{12}, \ldots$
BCC ($Im\overline{3}m$)	$1, \sqrt{2}, \sqrt{3}, \sqrt{4}, \sqrt{5}, \sqrt{6}, \ldots$
FCC ($Fm\overline{3}m$)	$1, \sqrt{4/3}, \sqrt{8/3}, \sqrt{11/3}, \sqrt{12/3}, \sqrt{16/3}, \ldots$
Gyr ($Ia\overline{3}d$)	$1, \sqrt{4/3}, \sqrt{7/3}, \sqrt{8/3}, \sqrt{10/3}, \sqrt{11/3}, \ldots$

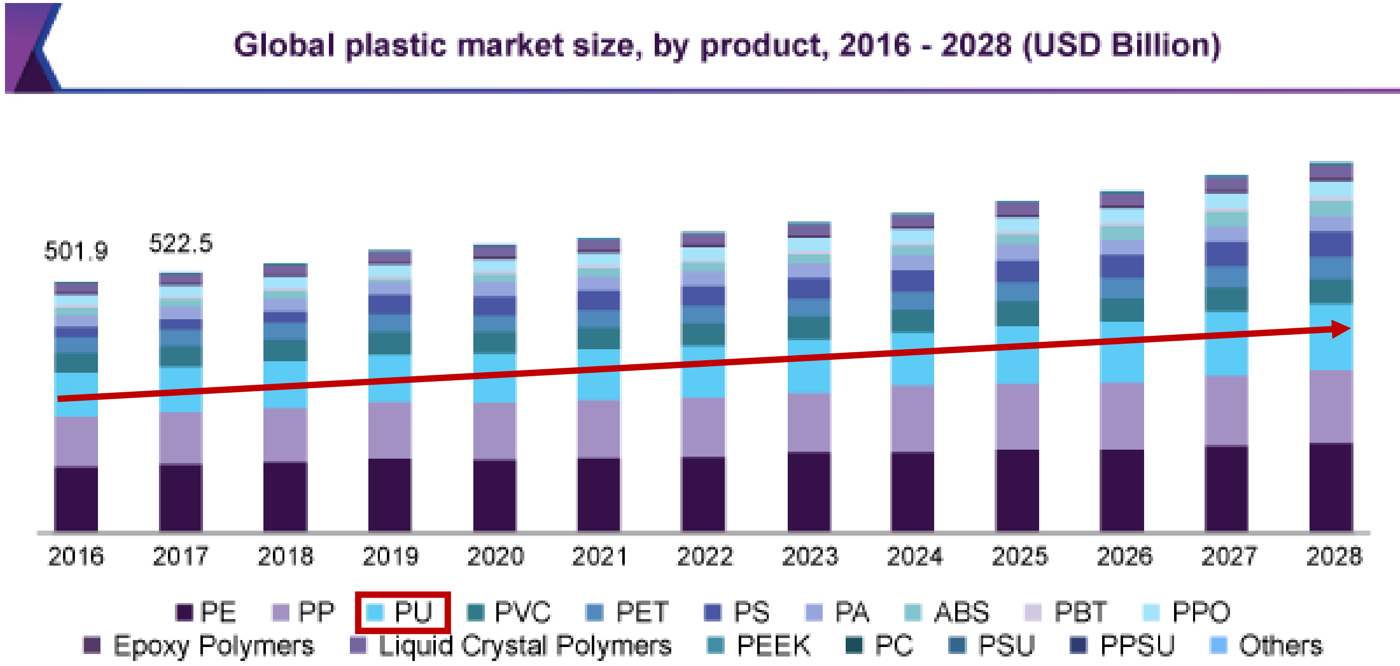
Prog. Polym. Sci. 2004, 29, 909–948

Conventional polyurethanes (PUs)



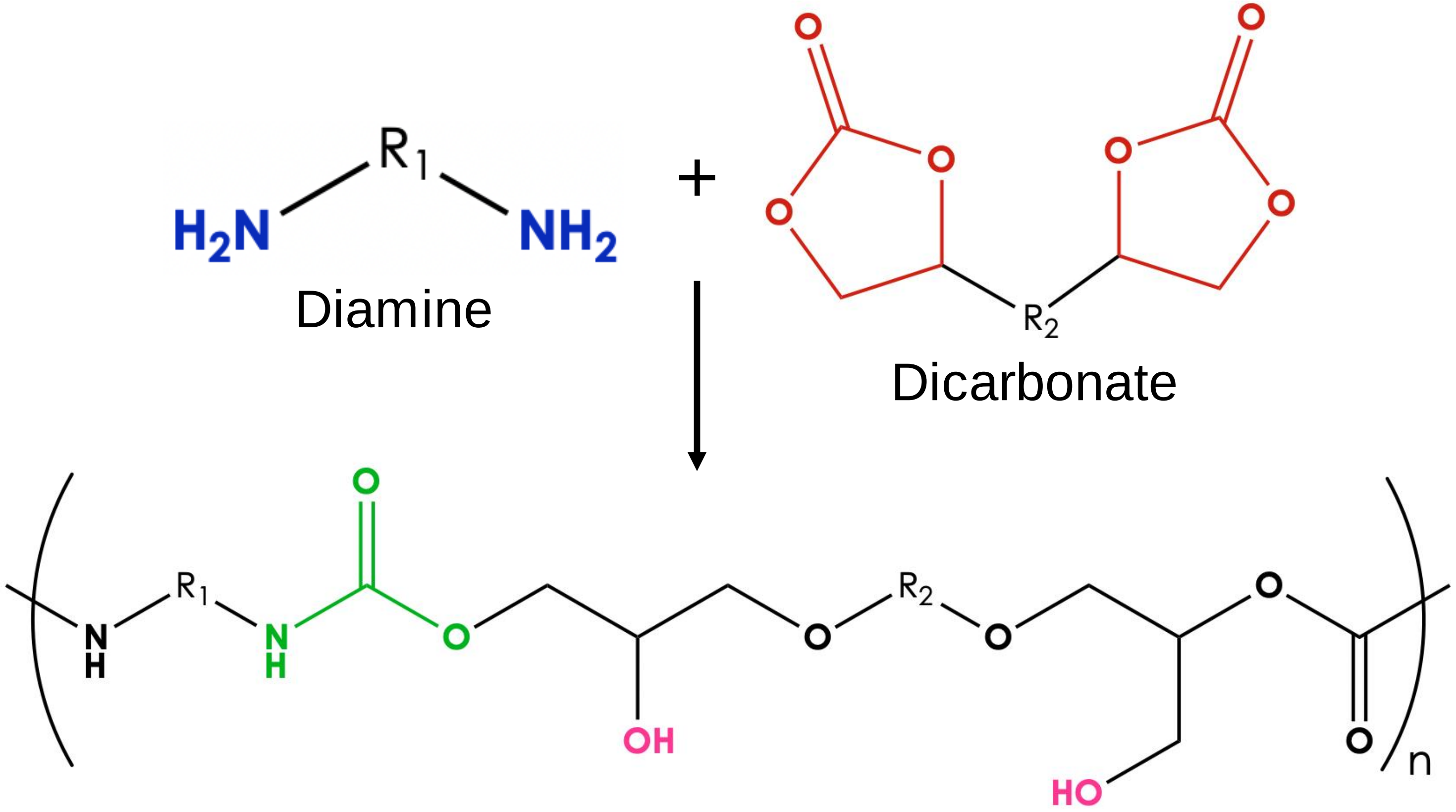
Formulations of polyurethanes <u>without isocyanates</u>:

Non-Isocyanate Polyurethanes (NIPUs)



https://www.grandviewresearch.com/industry-analysis/global-plastics-market

Non-isocyanate polyurethanes (NIPUs): Green chemistry



Polyhydroxyurethane

Prof. Milan Marić

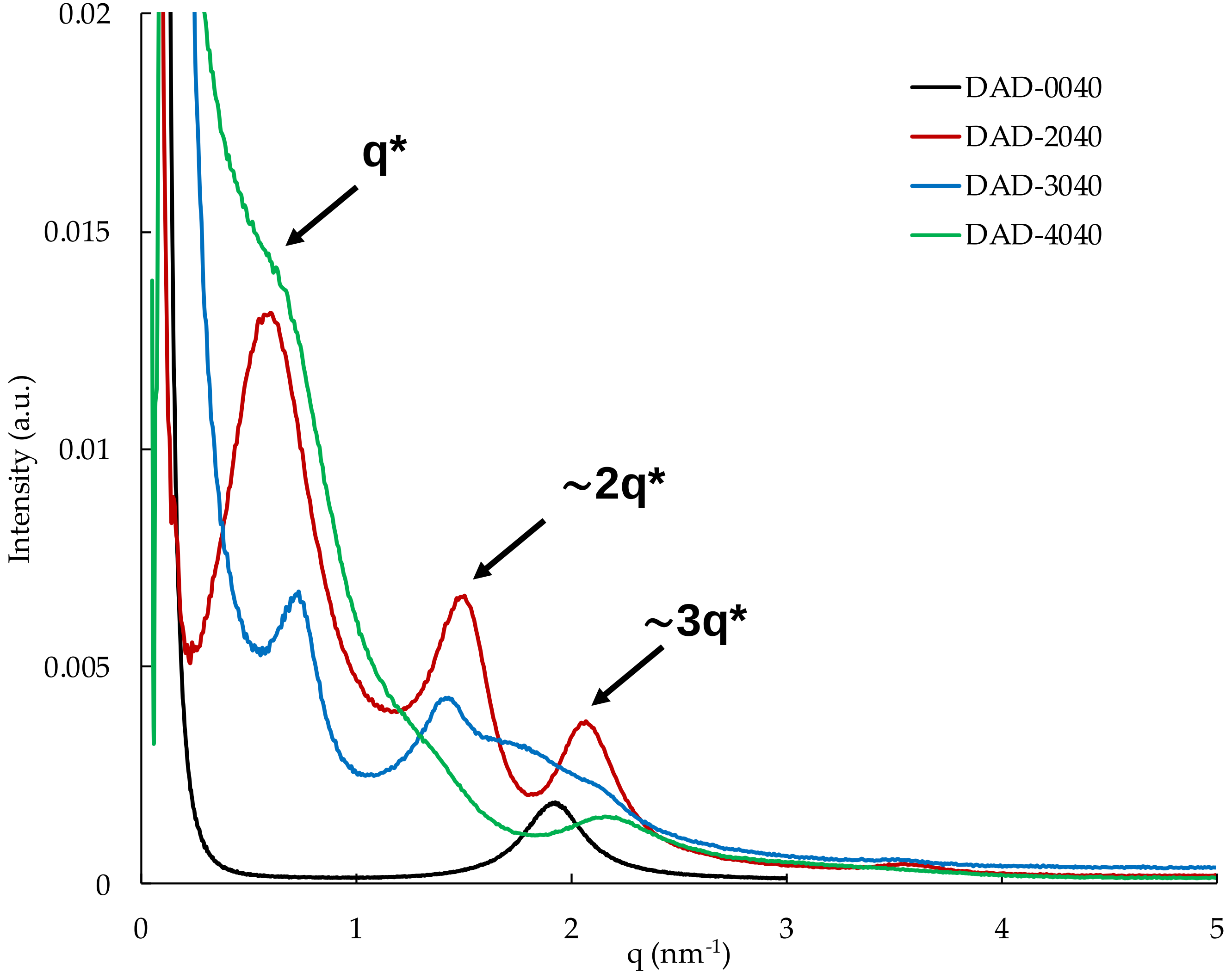
Dr. Georges Younes

McGill University
Department of Chemical Engineering

Safe and green reagents, bulk polymerization, moderate conditions

Hydroxyl groups: hydrogen bonding and post-functionalization

Thermoplastic PHUs microstructure: SAXS

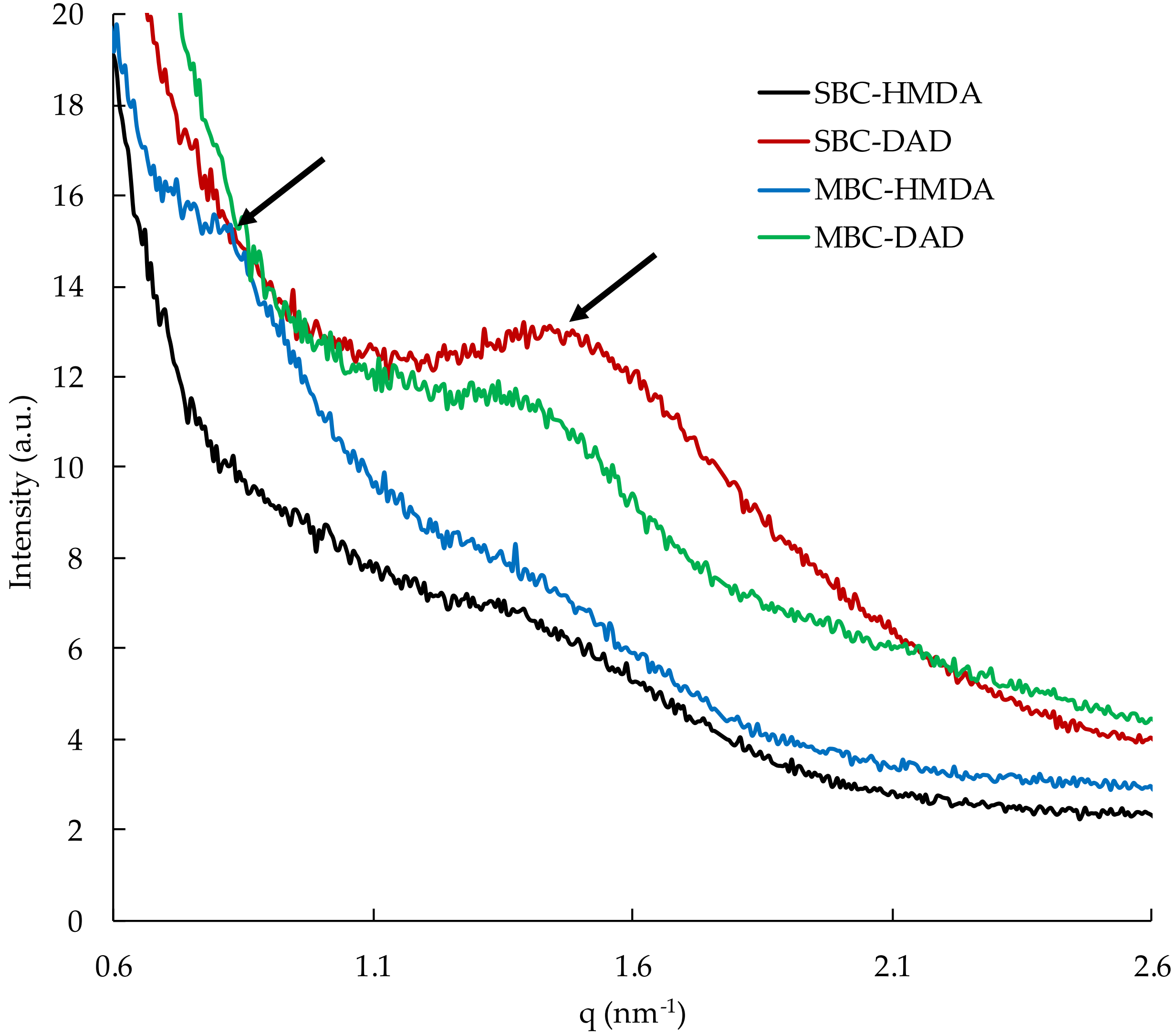


TPHUs from DGC and Priamine 1074 show **disordered nanophase separation** between hard and soft segments

Addition of DAD introduced **crystallinity** into the microstructure of TPHU - **ordered segmented lamellae** pattern (polymer chains are parallel to one another at room temperature)

Younes, G.R. and Marić, M., *Macromolecules*, **2021**, *54*, 10189-10202.

SBC/MBC-based TPHUs microstructure: SAXS



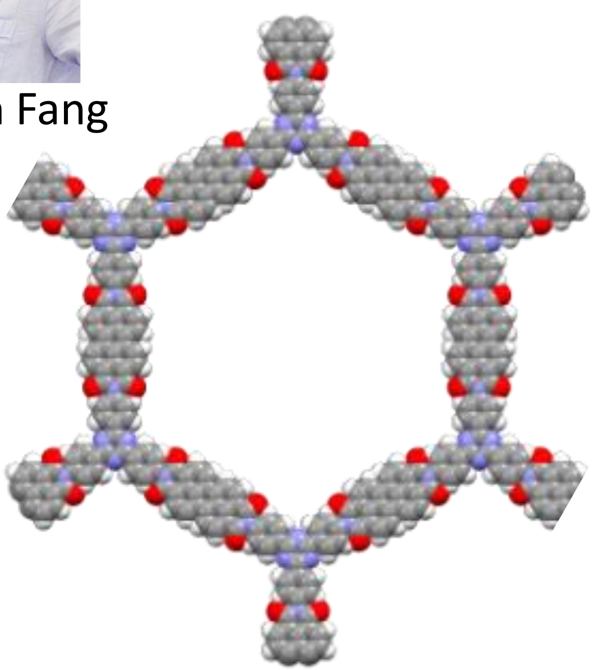
PHU	q_{max} (nm^{-1})	d (nm)
SBC-HMDA	1.40	4.49
SBC-DAD	1.48	4.27
MBC-HMDA	0.83/1.42	7.57/4.42
MBC-DAD	1.42	4.42

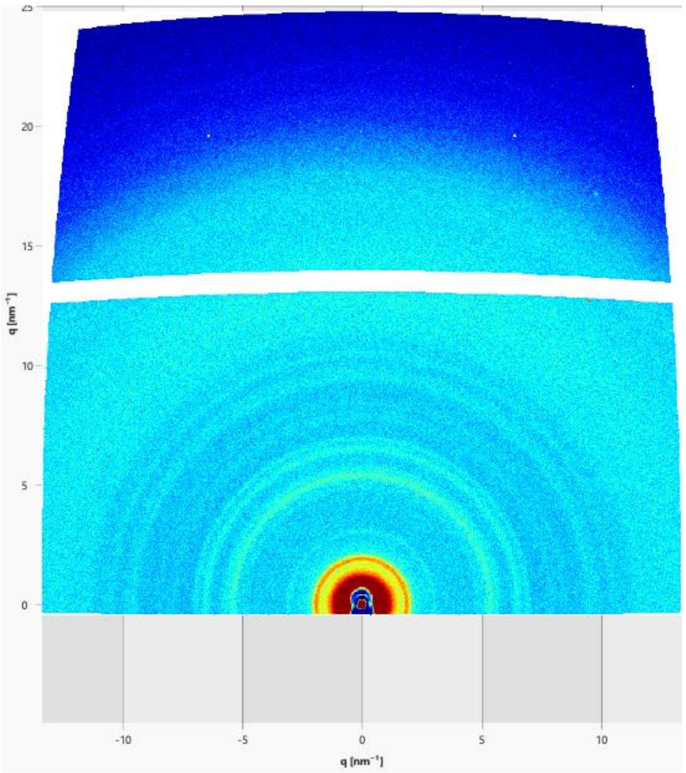
- **Nanophase separation** exists in PHUs (hard segments versus soft segments)
- **Random ordering** of the PHU chains except for MBC-HMDA chains (ordered in a hexagonal *P*6mm (1, $\sqrt{3}$…) spacial geometry)

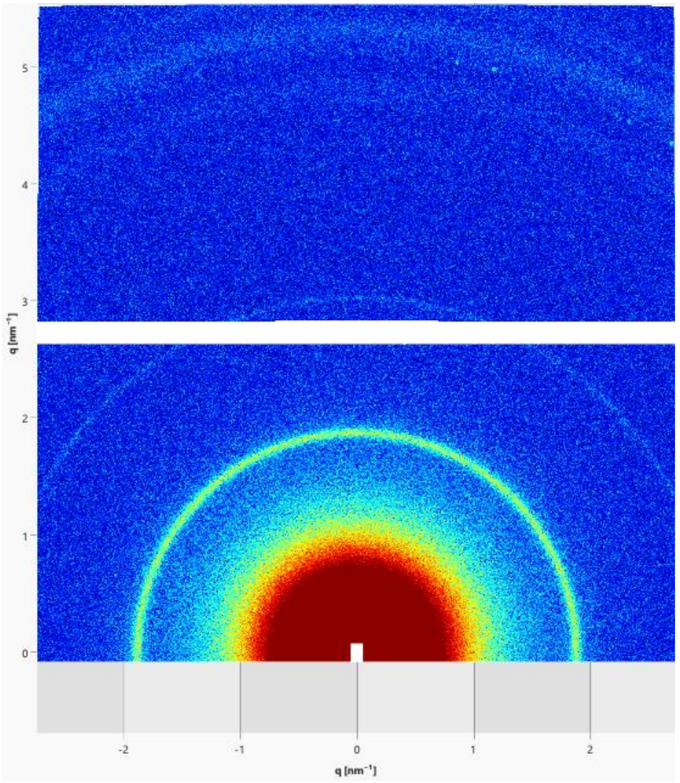
Younes, G.R., Kamel, M., Titi, H.M. and Marić, M., *ACS Appl. Polym. Mater.*, **2022**, *4*, 5161-5172.

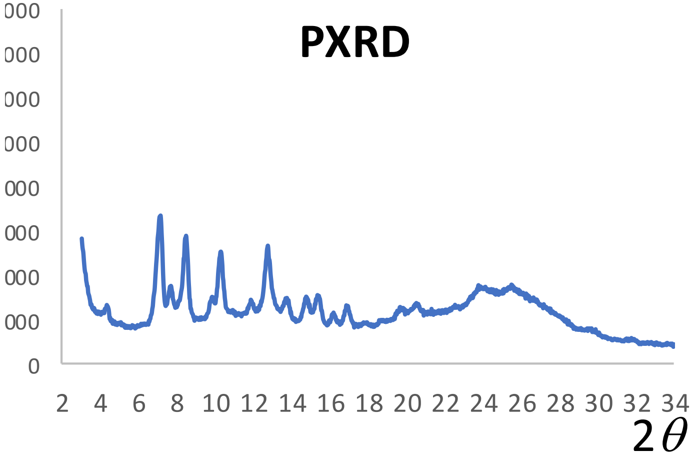

Prof. Yuan Fang

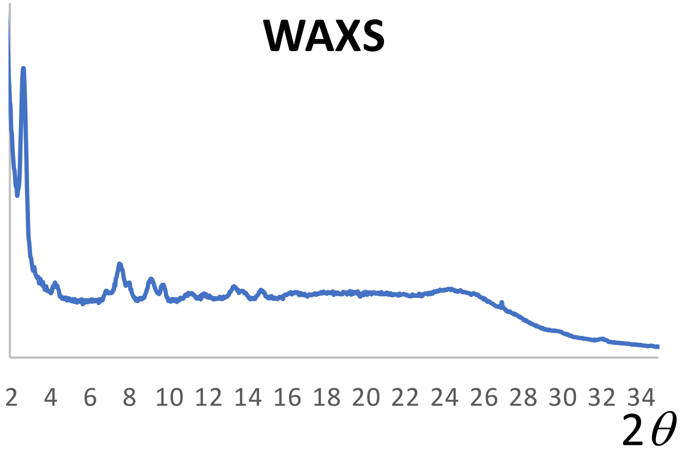
Covalent Organic Framework

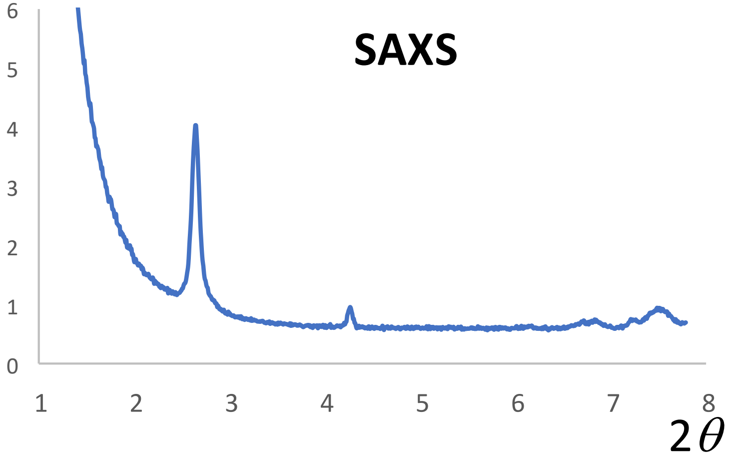




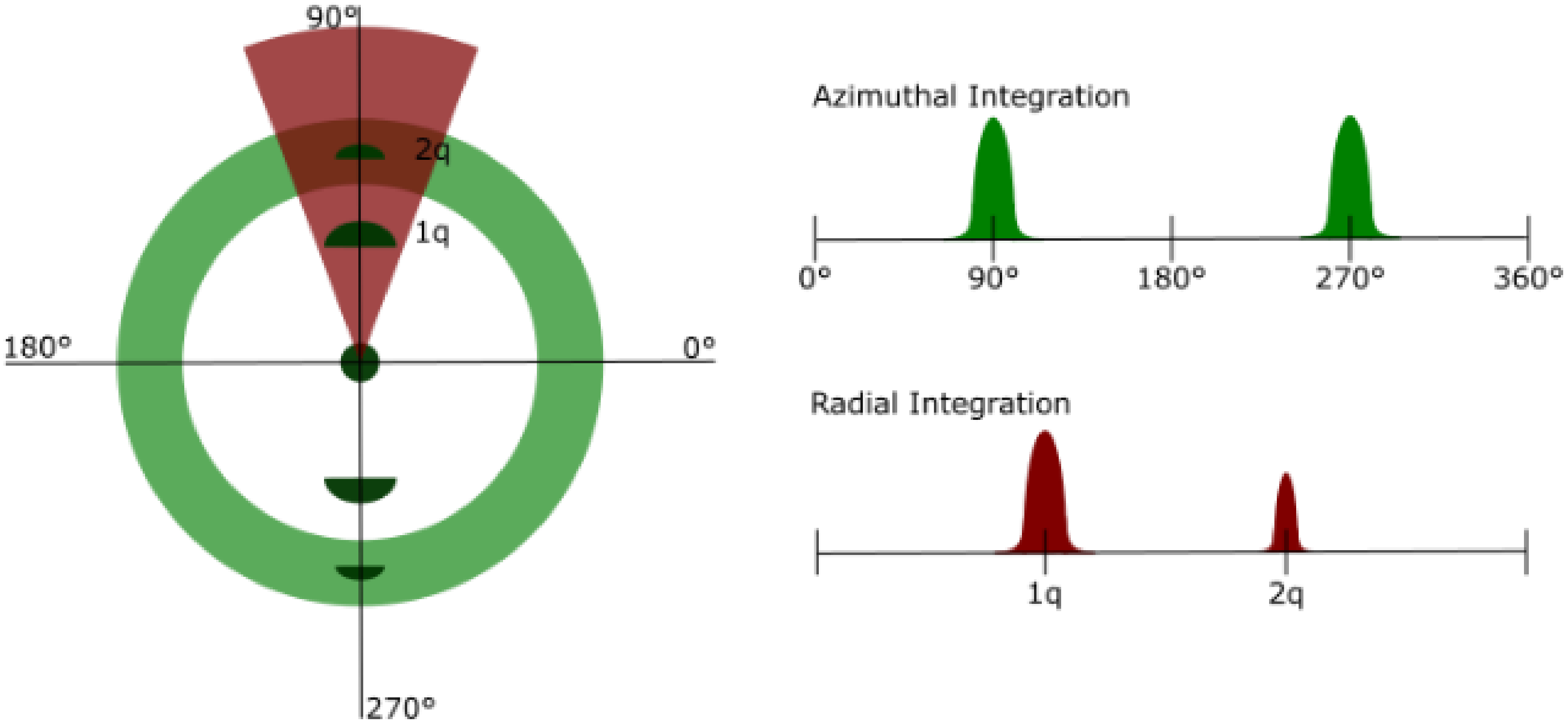



PXRD


WAXS


SAXS

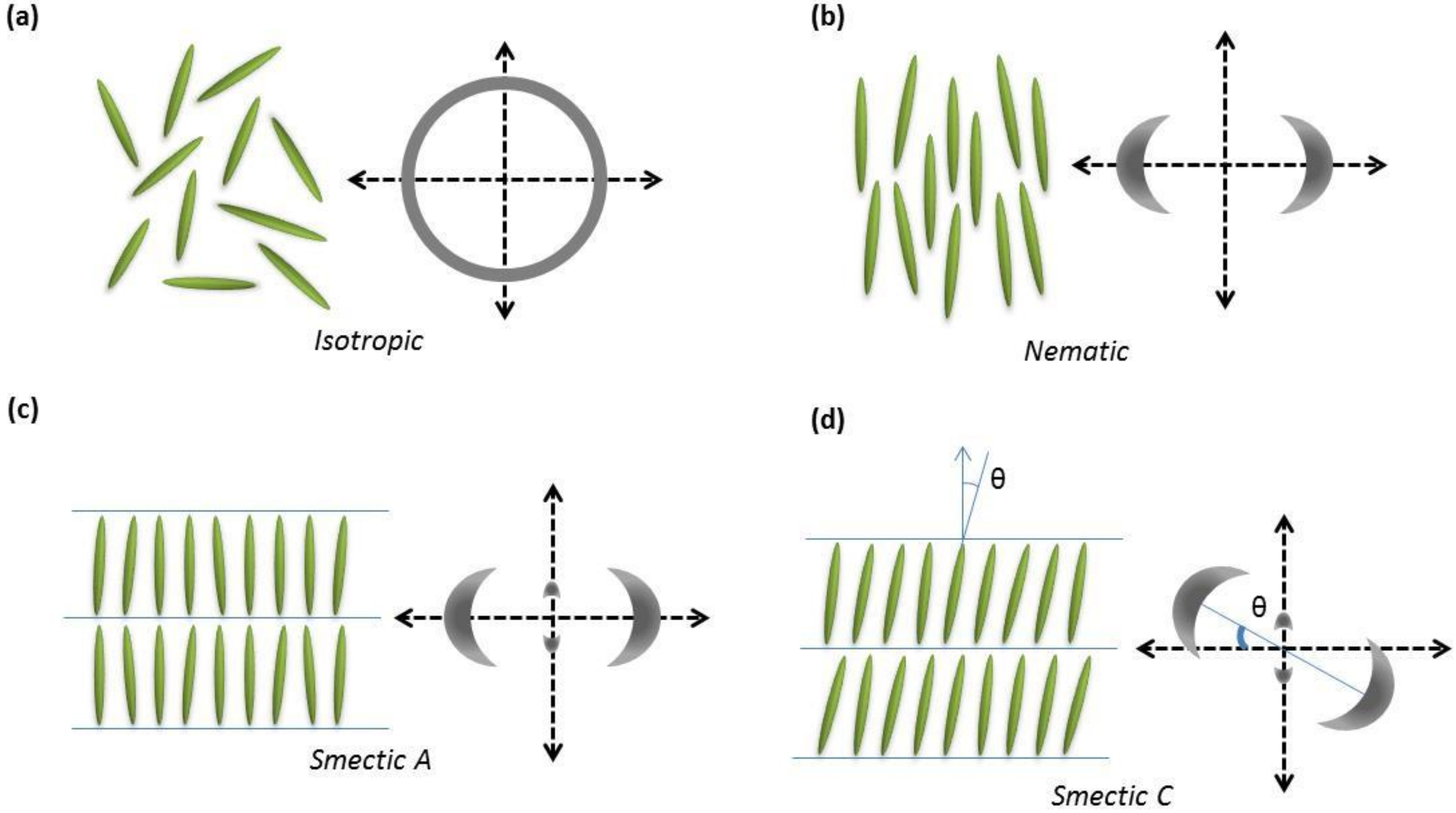
Azimuthal *vs* radial integration of the 2D and their resulting 1D scattering profile



- Azimuthal integration of the scattered intensities provides a distribution of LC preferential orientations

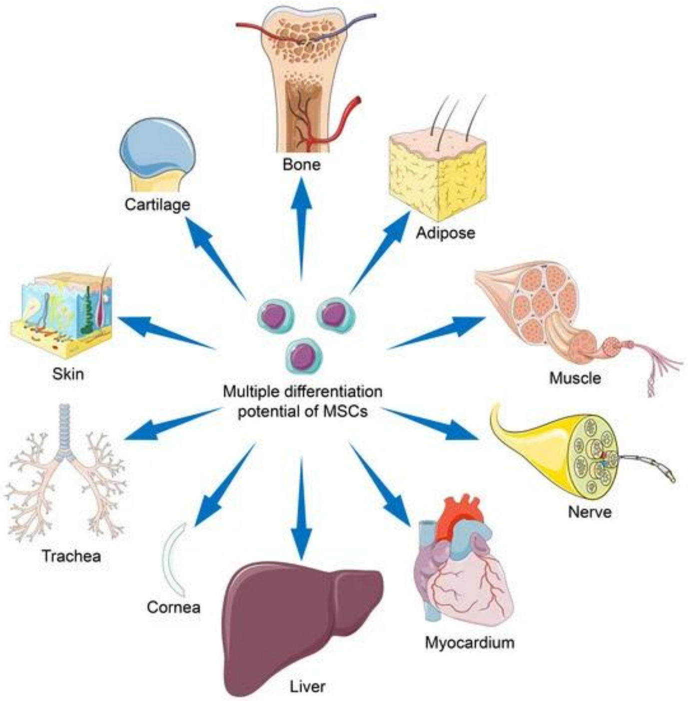
- Full width at half maximum, FWHM, is used to calculate the crystallite orientation

X-ray scattering of liquid crystals



In search of a tunable scaffold

Stem cell differentiation is a highly regulated process, but the ECM plays a key role



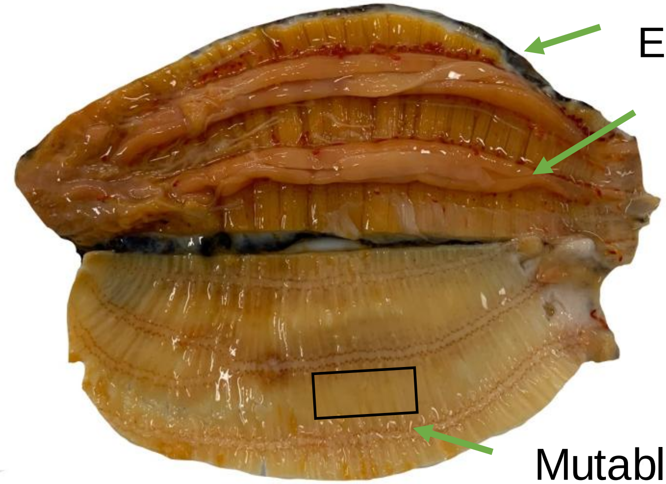
Han et al., *Cells.* **(2019)**

Nathalie Singh

Prof. Matt Harrington

Low stiffness

High stiffness

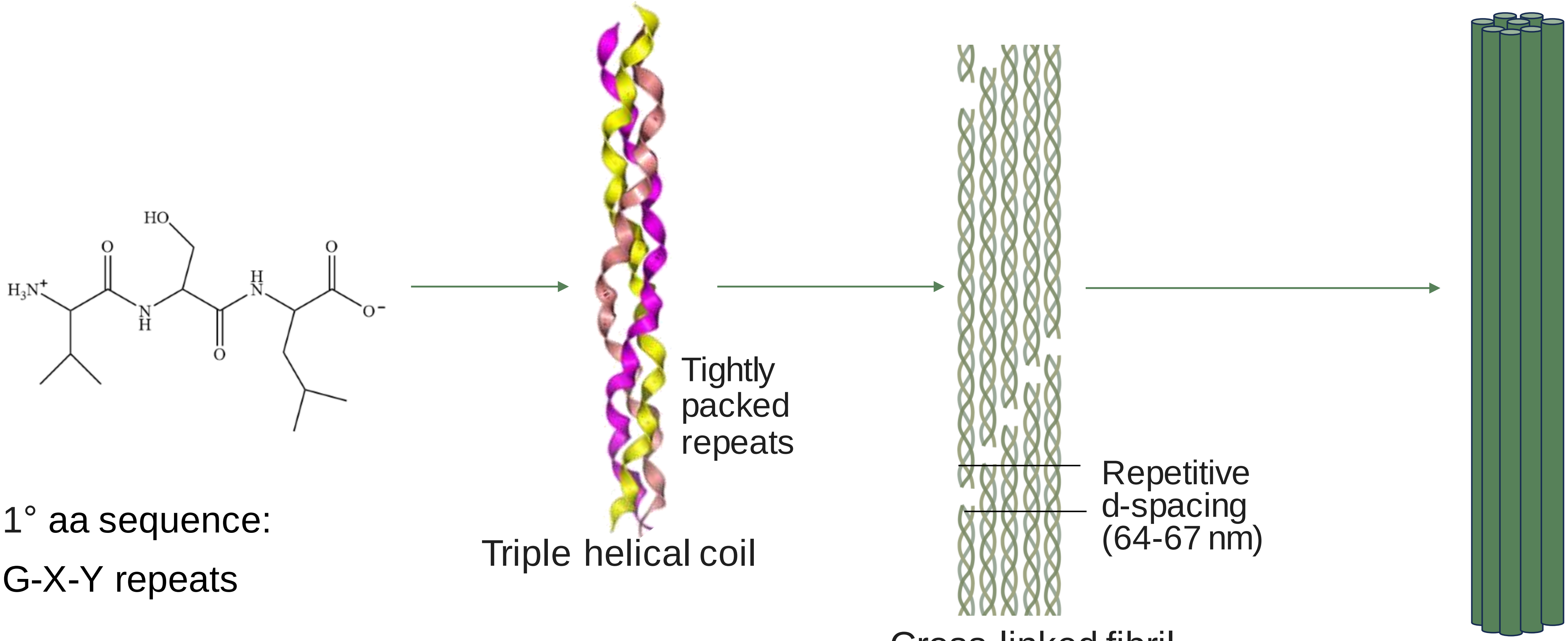


Epidermis

Longitudinal muscle

Mutable collagenous tissue (MCT, largely collagen I)

Collagen I structure



1° aa sequence:

G-X-Y repeats

Tightly packed repeats

Triple helical coil

Repetitive d-spacing (64-67 nm)

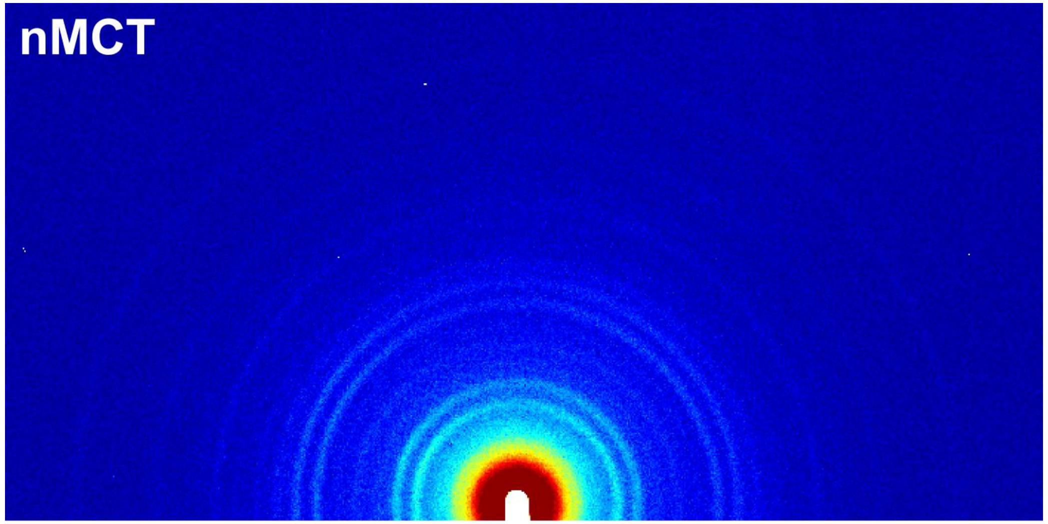
Cross-linked fibril

Self-assembling fiber

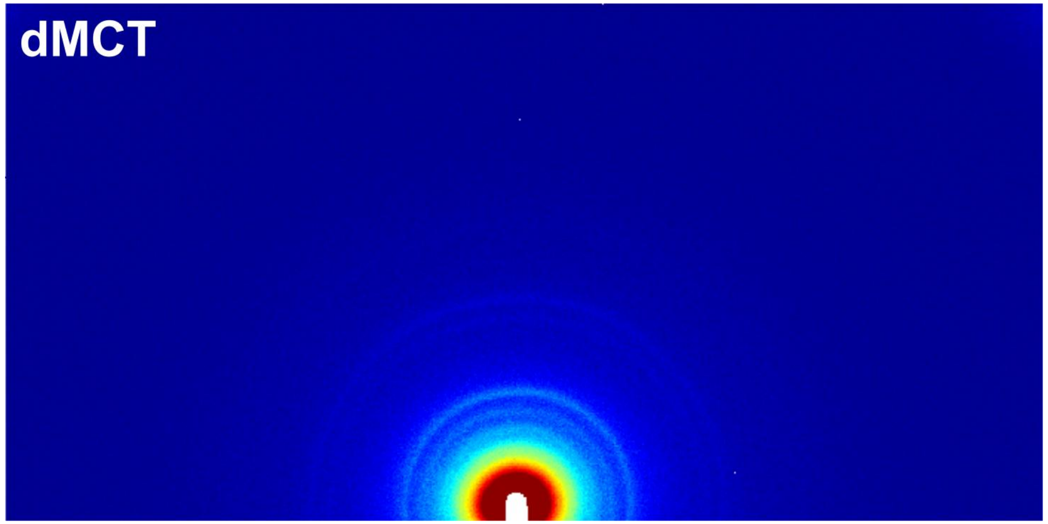
SAXS of MCT (SDD: 576 mm)

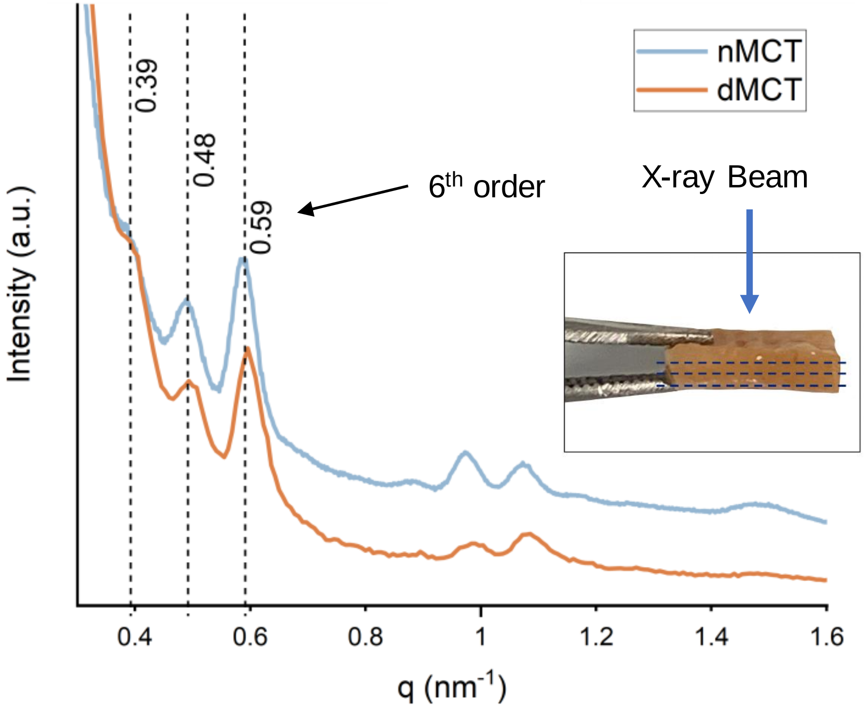
SAXS probes the d-spacing (gap & overlap periodicity) between collagen I triple helices as they assemble into triple helices

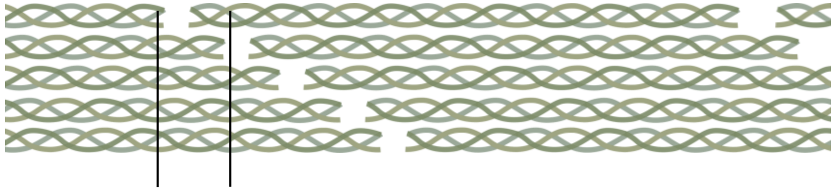
native tissue



decellularized tissue





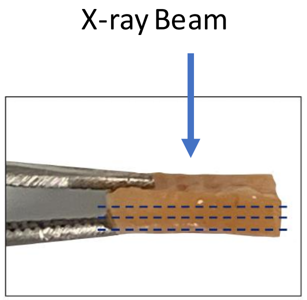


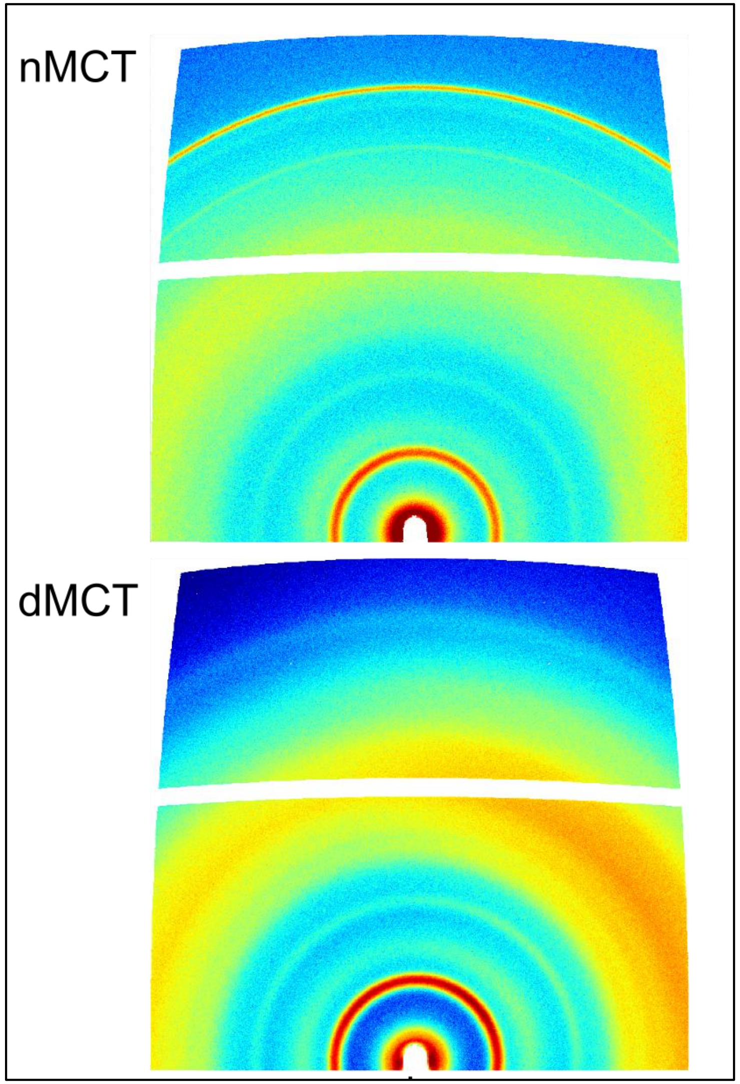
d-spacing: nMCT: 65.38 ± 0.28 nm

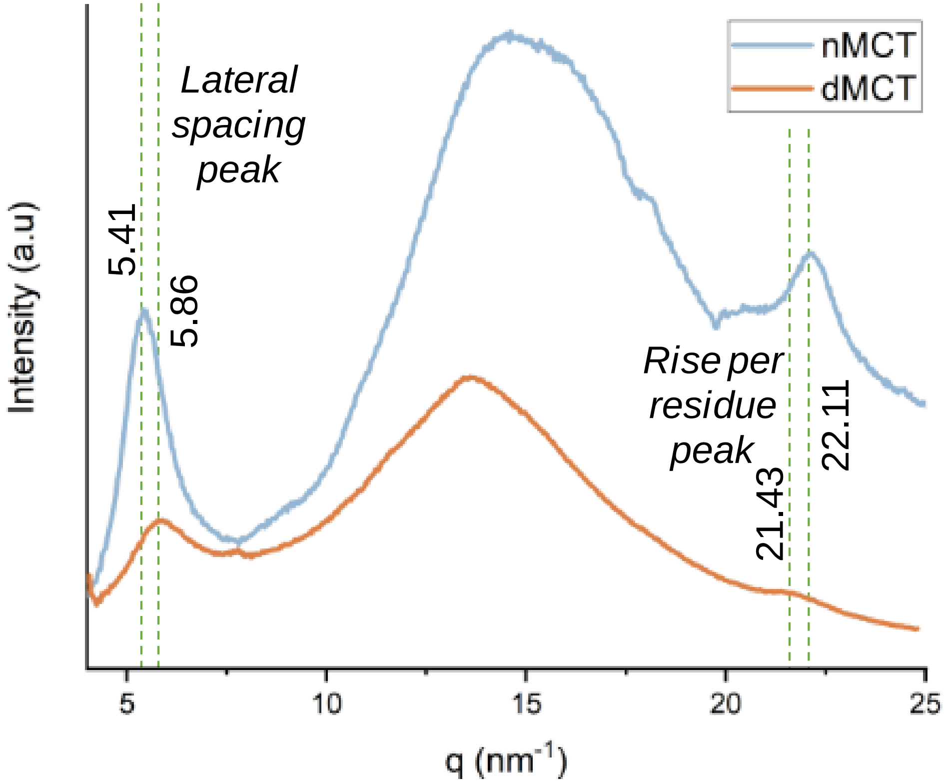
dMCT: 63.48 ± 0.26 nm

WAXS of MCT (SDD 113 mm)

WAXS probes the rise per residue spacing **within** the triple helix and the lateral spacings **between** triple helices







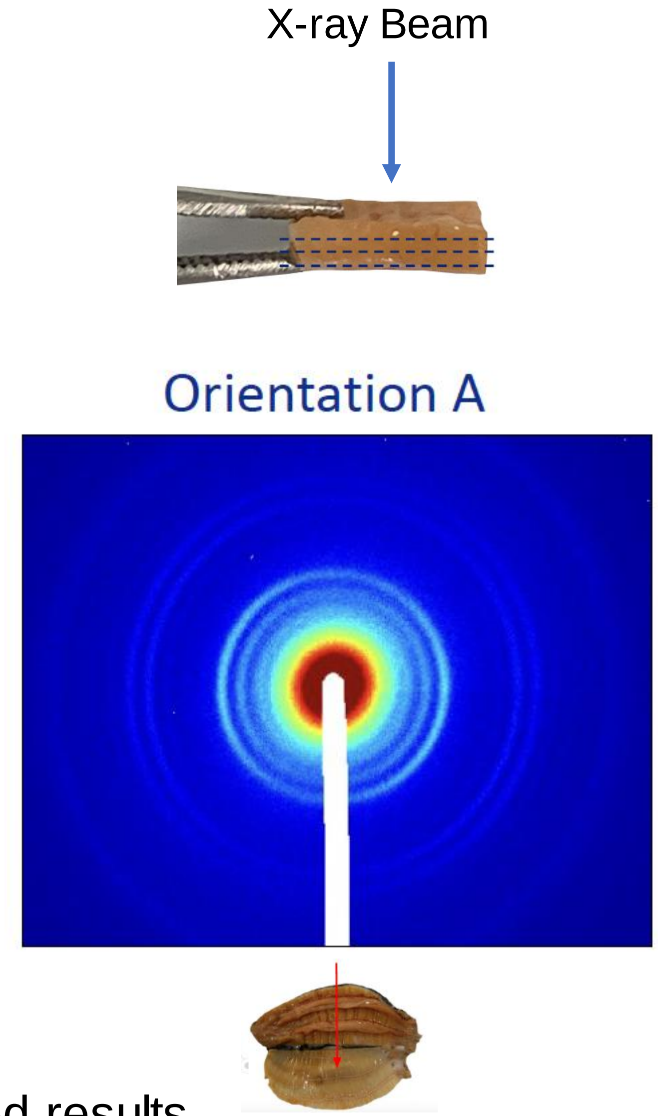
nMCT: 1.20 ± 0.04 nm

dMCT: 1.09 ± 0.02 nm

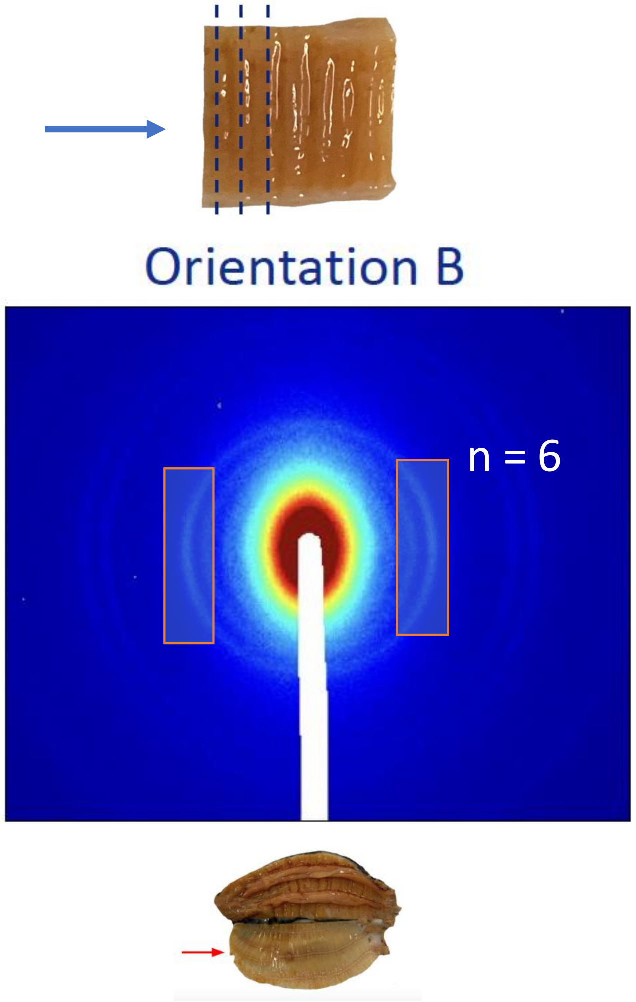
nMCT: 0.29 ± 0.00 nm

dMCT: 0.29 ± 0.07 nm

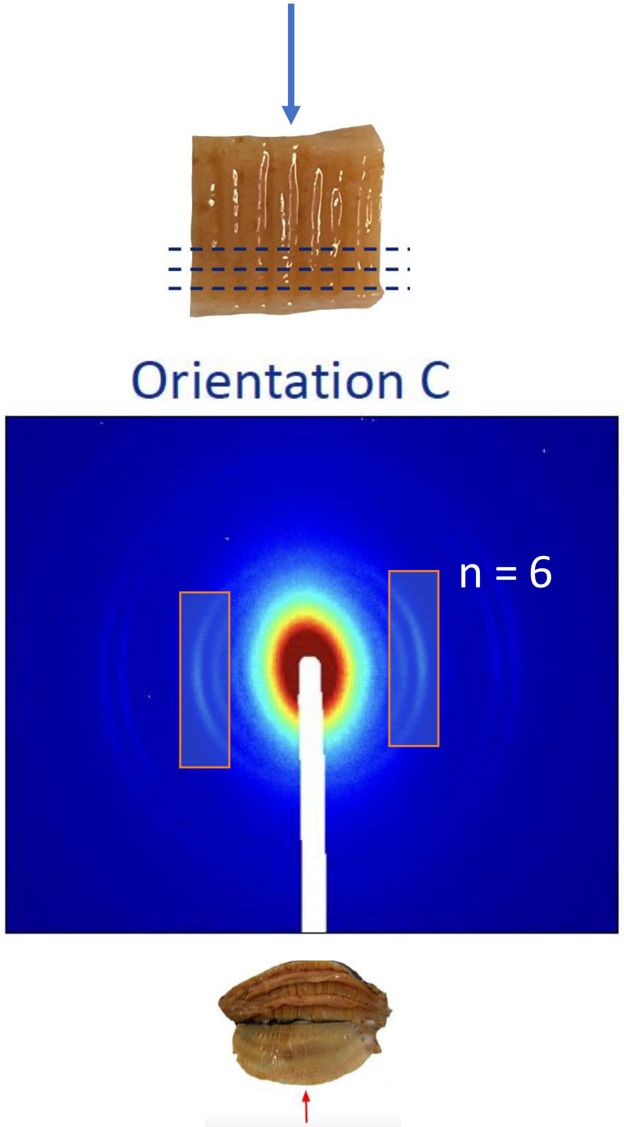
Preferential orientation of Fibrils



Orientation A



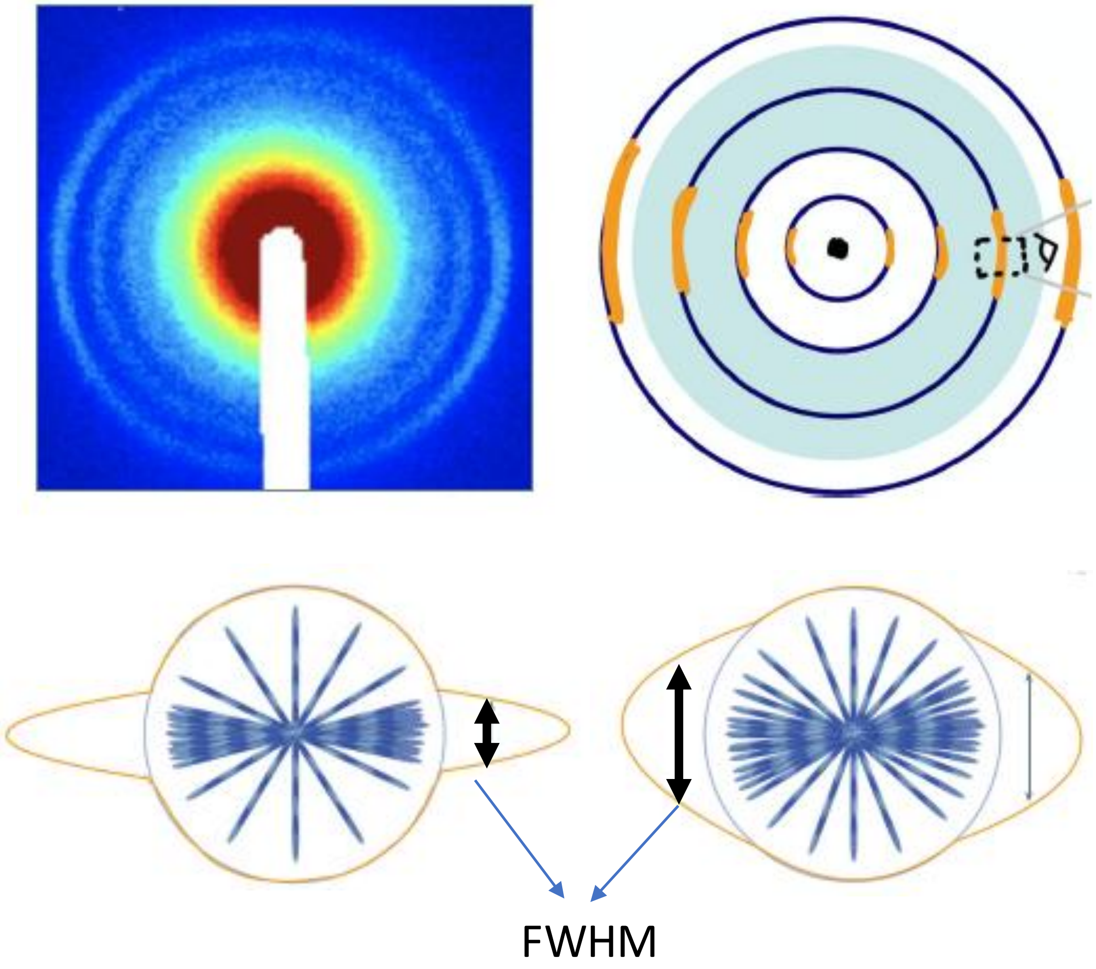
Orientation B

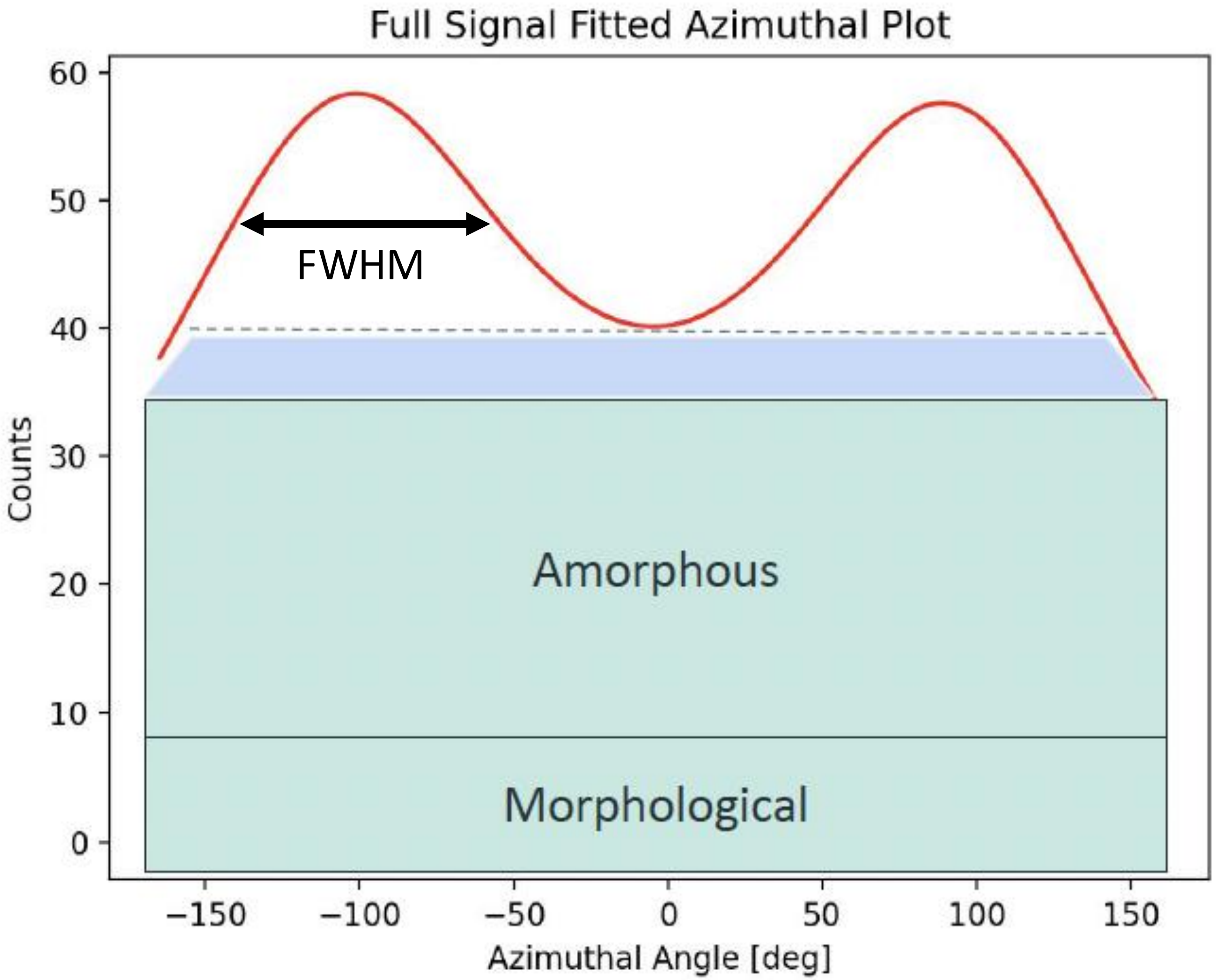


Orientation C

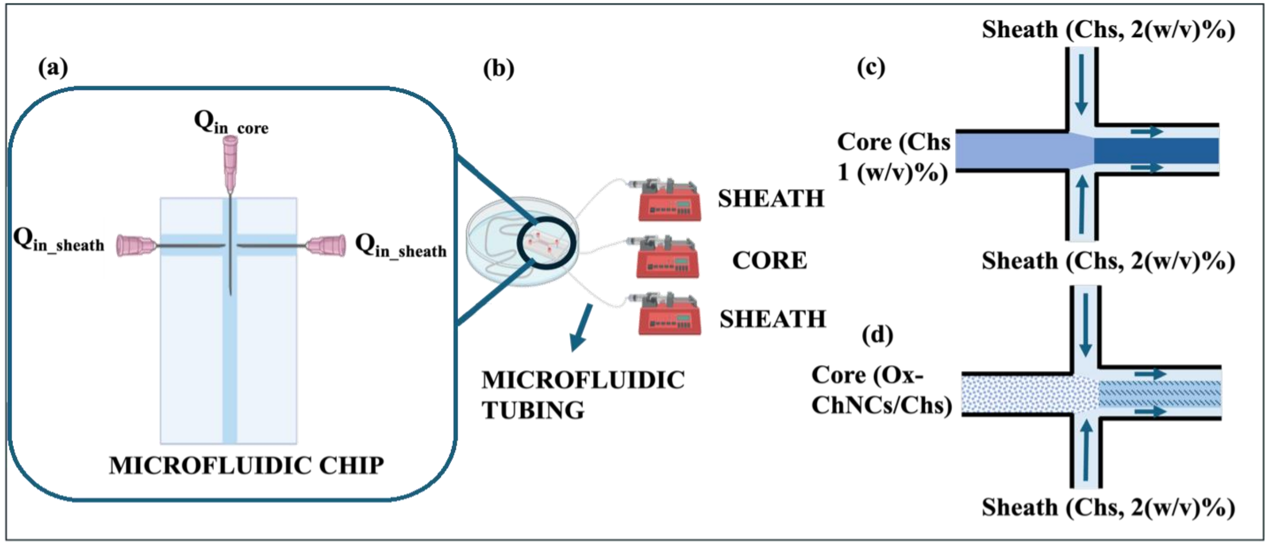
Unpublished results

Radial and Azimuthal SAXS data



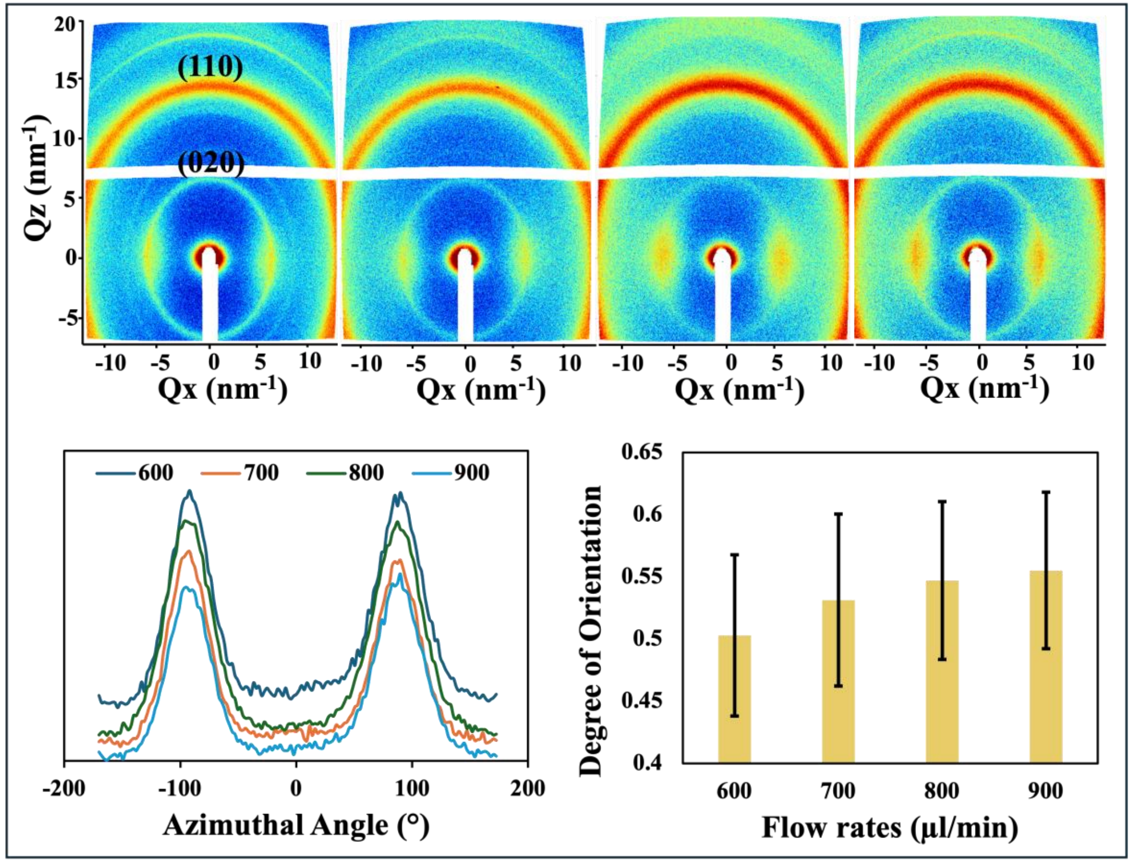


Chitosan Fibers



Ishneet Kaur

Prof. Audrey Moores



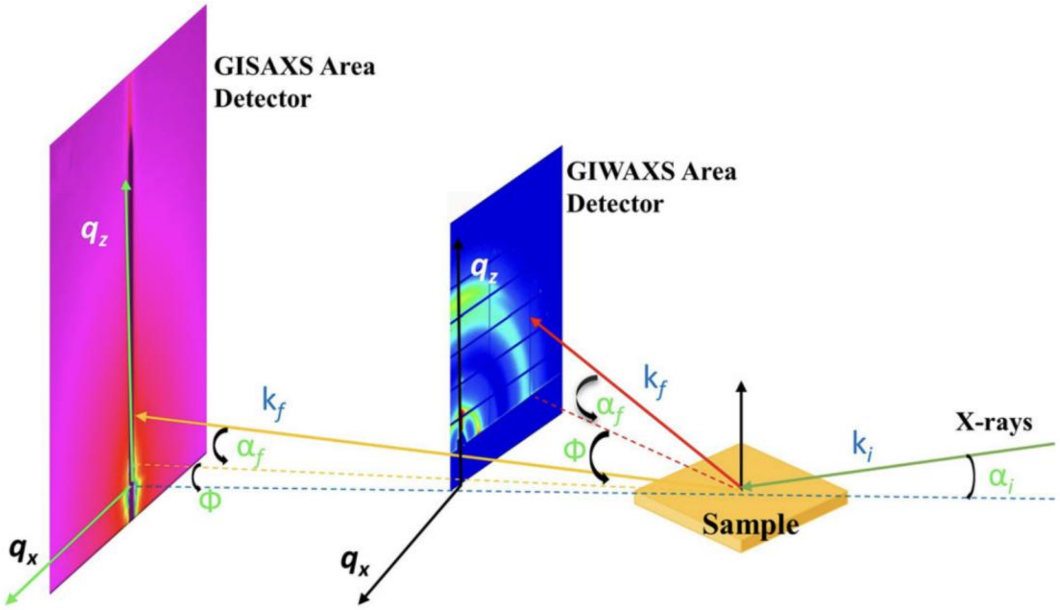
2D WAXS diffractogram of vertically positioned Chs-ChNC-MF at 600, 700, 800 and 900 µl/min (from left to right)

Full-width half maximum of the azimuthal peak

Orientation is calculated by azimuthal intensity distributions.

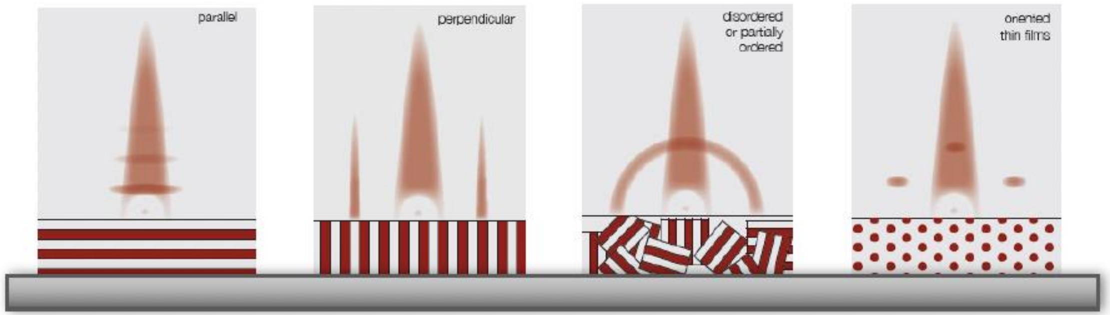
Grazing-incidence small- & wide-angle X-ray scattering (GISAXS & GIWAXS)

GISAXS:
- Domain size
- Domain shape
- Phase separation
- Phase purity



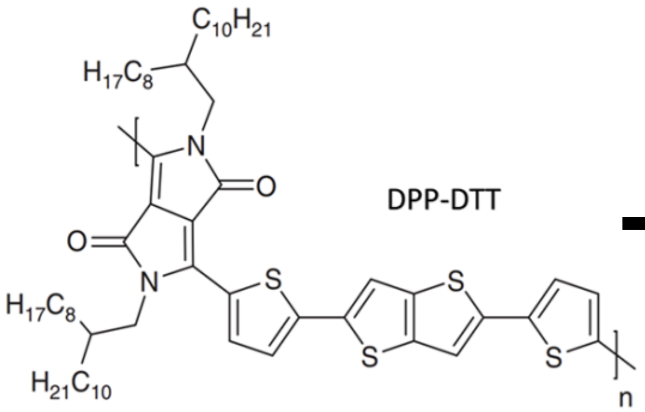
Sample holder is tilted between 0.05-0.5º

GIWAXS:
- Crystallinity
- Crystal orientation
- Crystal size
- Interlayer spacing



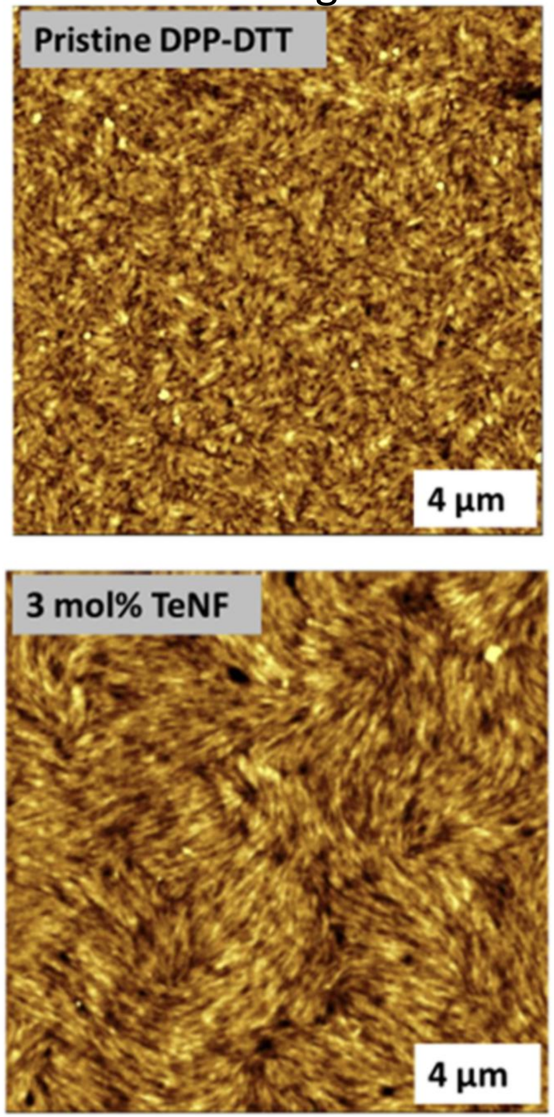
A. Mahmood and J.-L. Wang *Sol. RRL* **2020**, *4*, 2000337

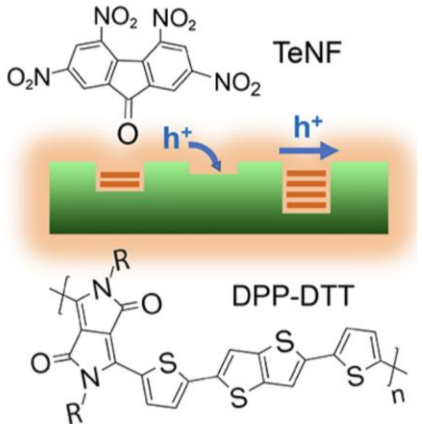
Effect of doping nitromolecules on thin film: AFM



Polymer

AFM images





2,4,5,7-tetranitrofluorenone (TeNF)

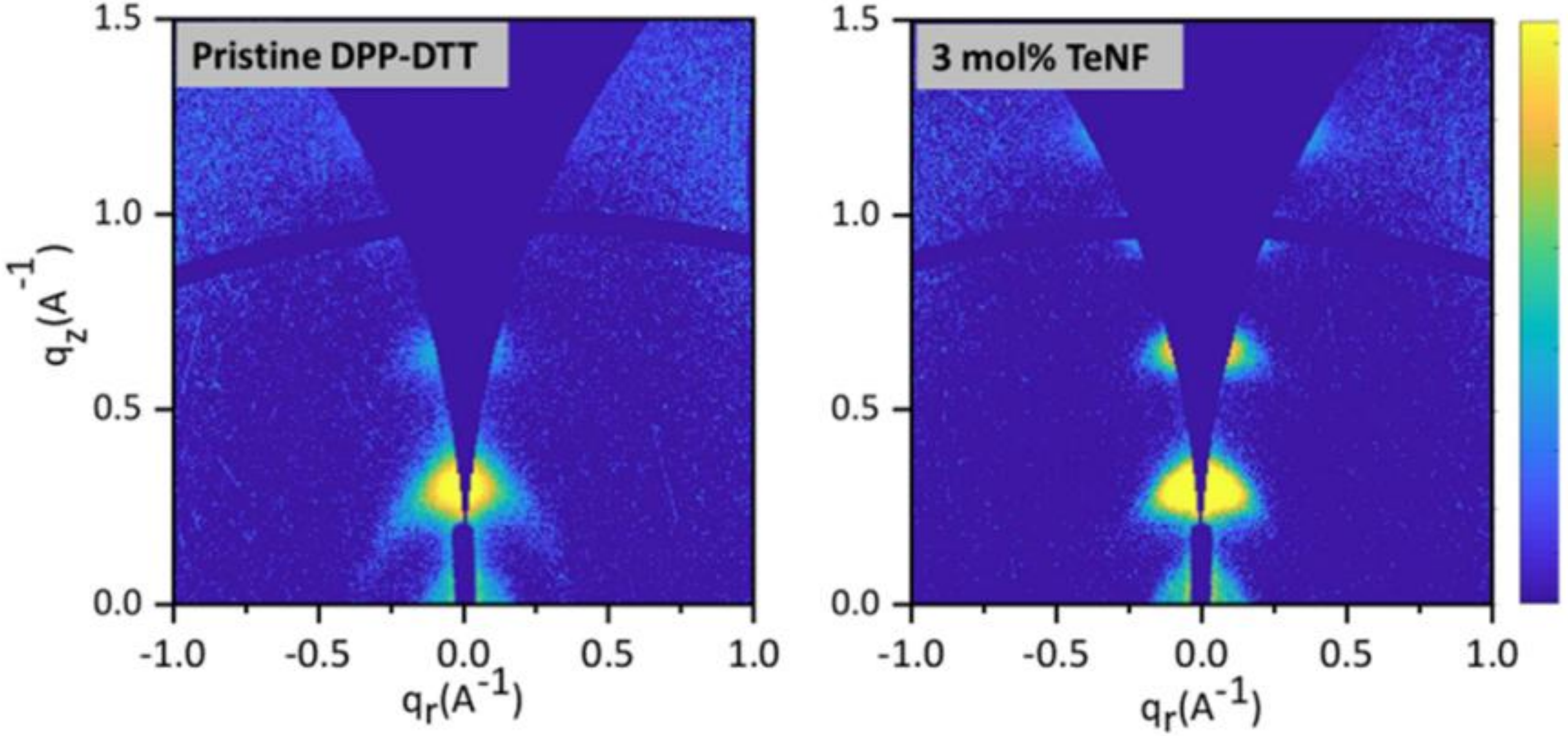
Doped transistor (thin film)

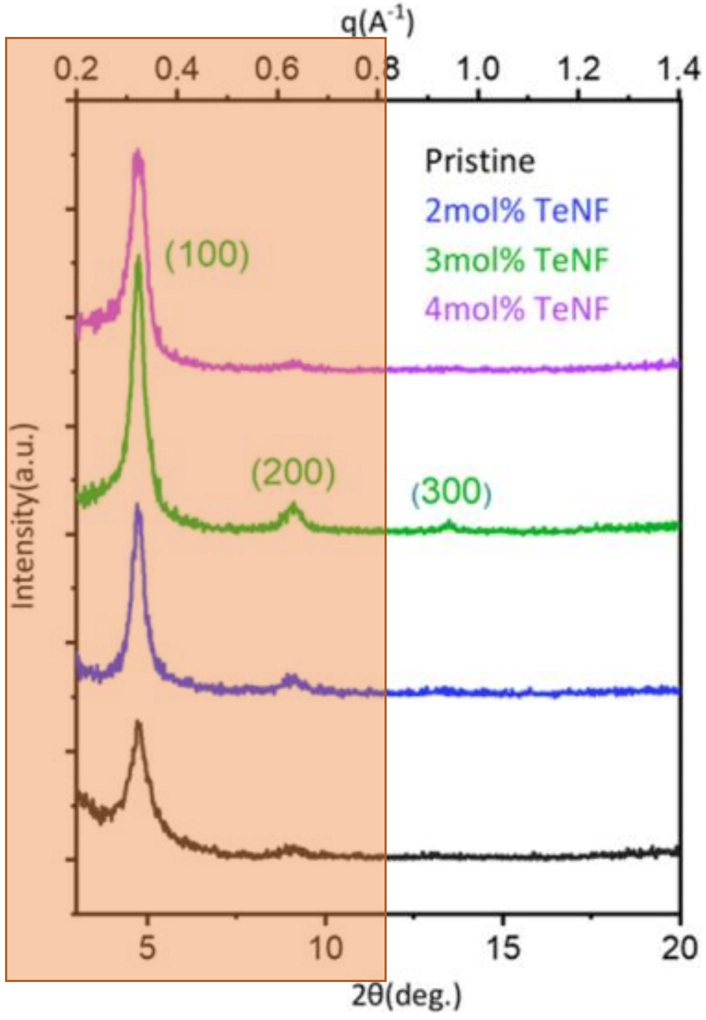
Che *et al. Chem. Mater.* **2021**, *33*, 2937−2947

Prof. Dmytro Perepichka

Dr. Pegah Ghamari

The effect of doping on the thin film morphology: GIWAXS



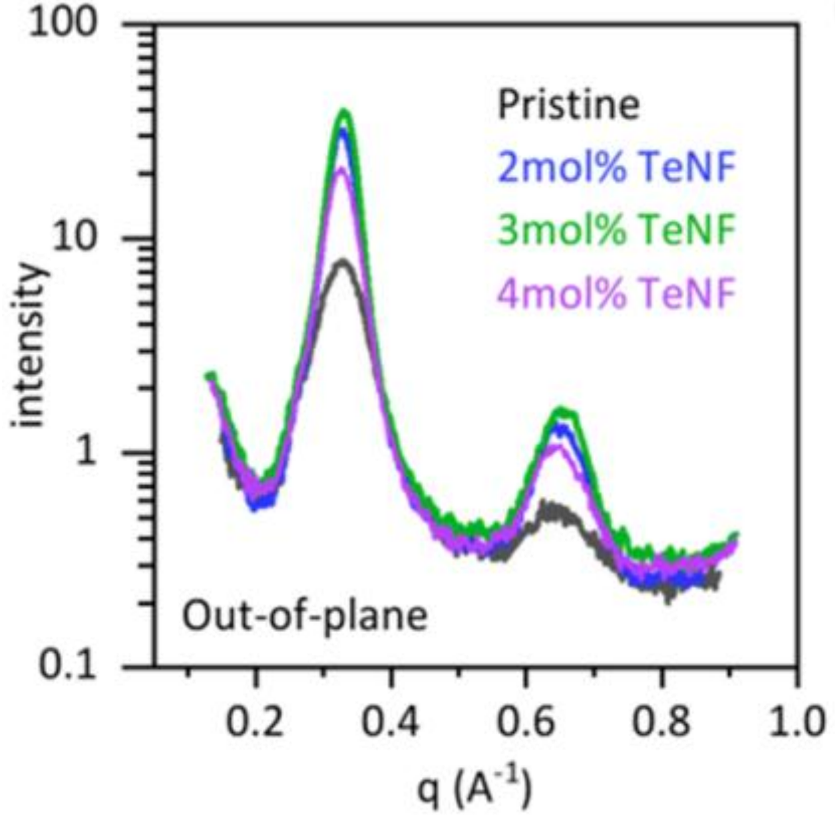


Out-of-plane GIWAXS patterns extracted along the q_z direction

Che *et al*. *Chem. Mater.* **2021**, *33*, 2937−2947

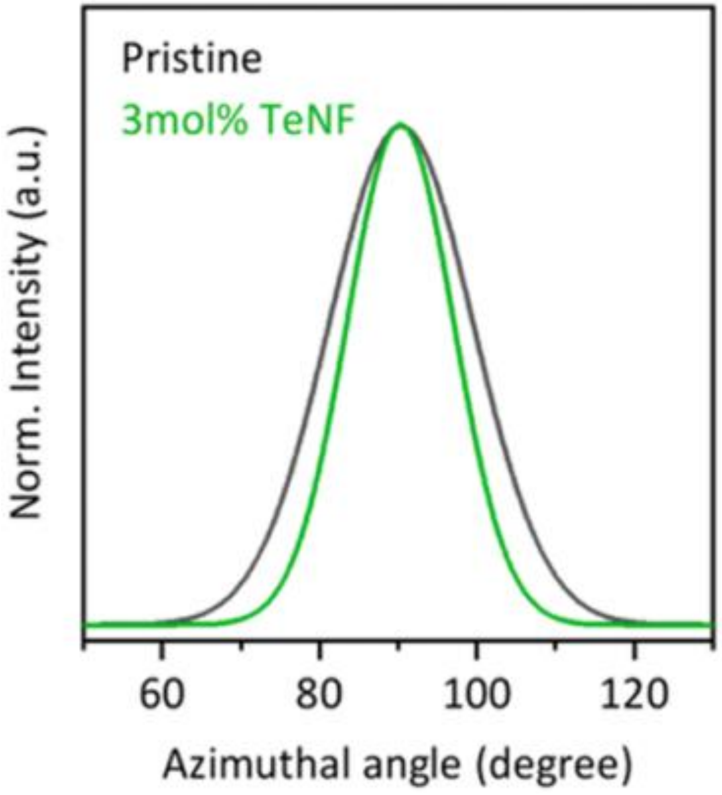
The effect of doping on the thin film morphology: GIWAXS

Selected region



An increase in crystallinity was achieved by adding 3mol% TeNF

Azimuthal angle to determine size and shape



A higher degree of parallelism

Che *et al*. *Chem. Mater.* **2021**, *33*, 2937−2947

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Perpichka’s lab
Andrews’s lab
Robert’s lab
Howarth’s lab

Thank you