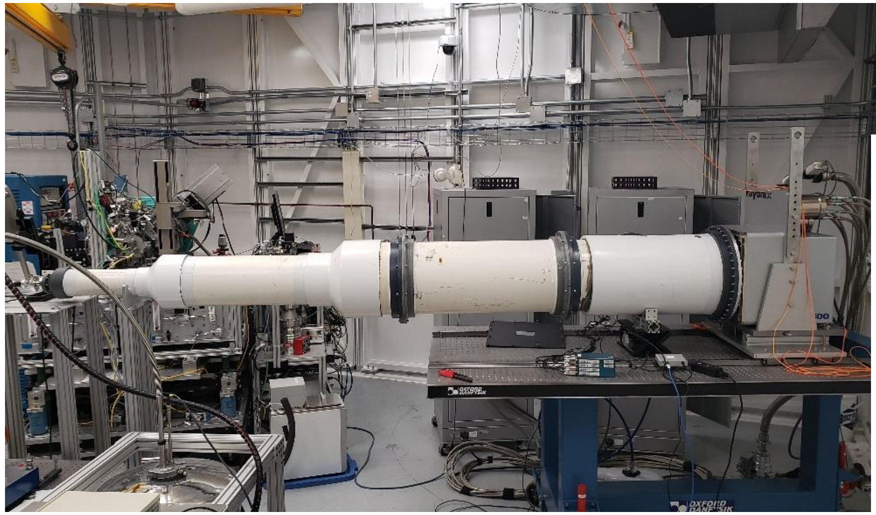
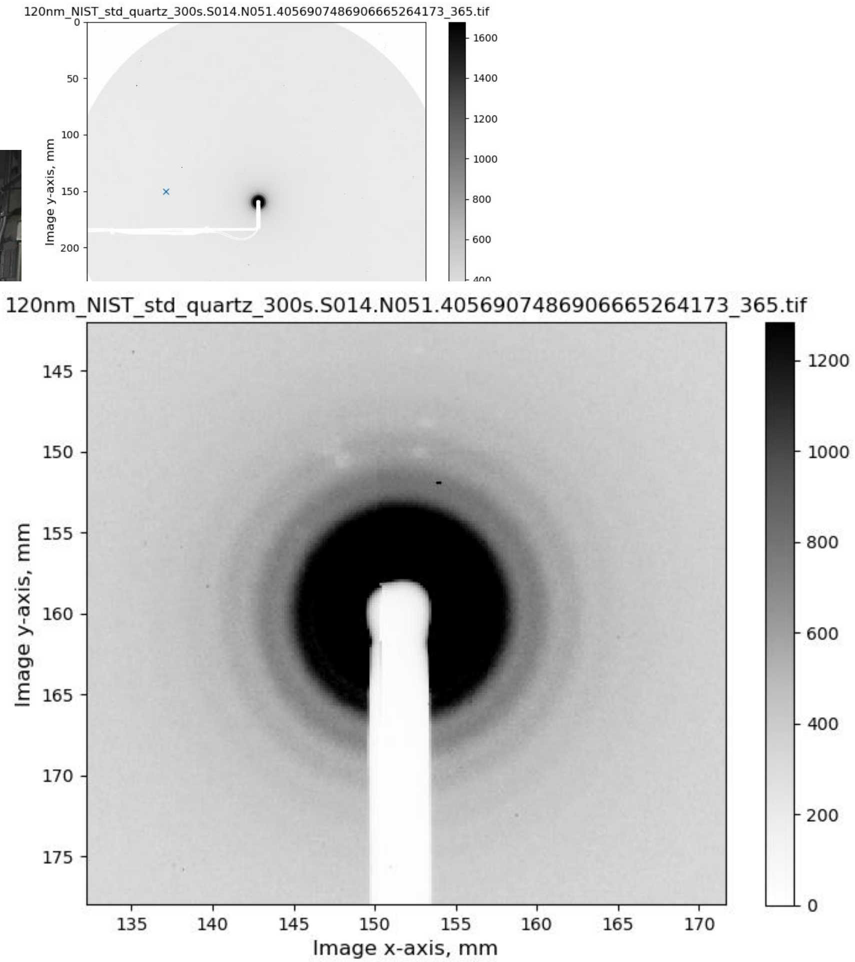
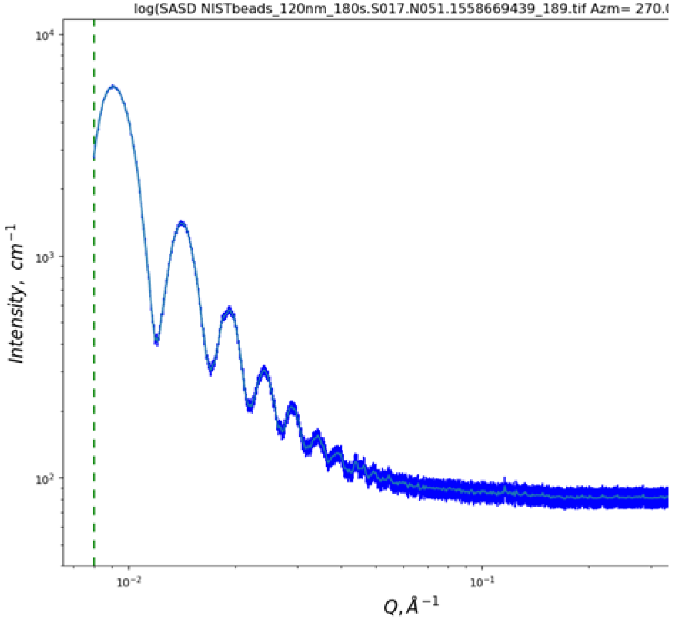
An Introduction to Small Angle X-ray Scattering (SAXS)







Adam Leontowich
CLS XRD school, Friday, August 18, 2023

Canadian Light Source
Centre canadien de rayonnement synchrotron

The small angle X-ray scattering (SAXS) region

The instrumentation and sample prep

Data collection and reduction

Basic data processing with examples from BXDS

Brockhouse X-ray Diffraction and Scattering (BXDS) sector

3 “hard X-ray” beamlines (5 – 100 keV)

JSR JOURNAL OF SYNCHROTRON RADIATION

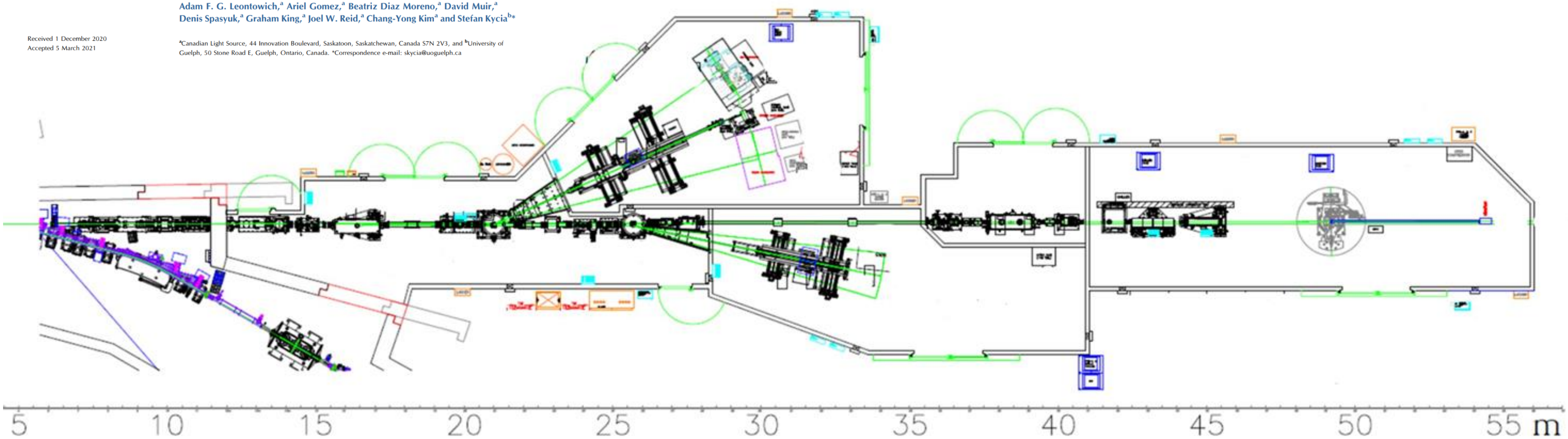
ISSN 1600-5775

The lower energy diffraction and scattering side-bounce beamline for materials science at the Canadian Light Source

Adam F. G. Leontowich,[a] Ariel Gomez,[a] Beatriz Diaz Moreno,[a] David Muir,[a] Denis Spasyuk,[a] Graham King,[a] Joel W. Reid,[a] Chang-Yong Kim[a] and Stefan Kycia[b]*

Received 1 December 2020
Accepted 5 March 2021

[a]Canadian Light Source, 44 Innovation Boulevard, Saskatoon, Saskatchewan, Canada S7N 2V3, and [b]University of Guelph, 50 Stone Road E, Guelph, Ontario, Canada. *Correspondence e-mail: skycia@uoguelph.ca

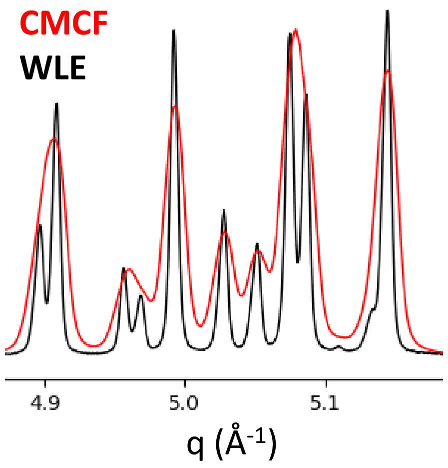


Canadian Light Source
Centre canadien de rayonnement synchrotron

WLE beamline

High-resolution powder diffraction

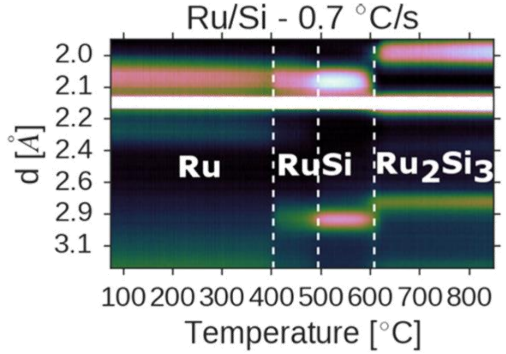
J. Reid, synthetic bone compound



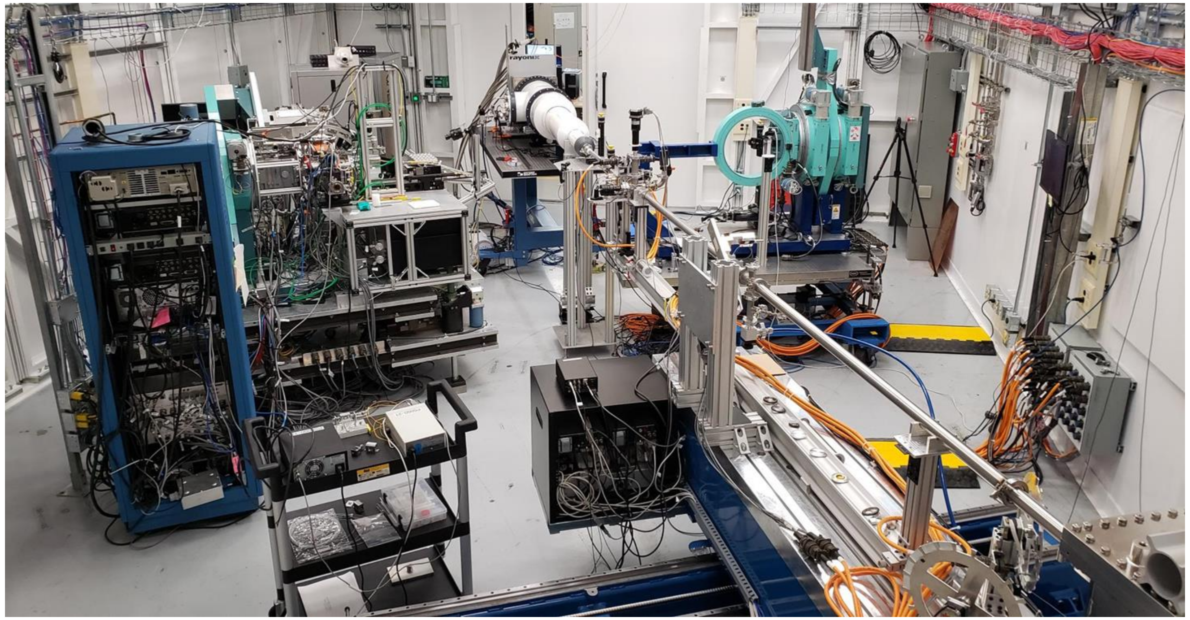
- Peak shape analysis
- Complex mixtures
- Complex structures

IBM (rapid thermal annealing to 1100 °C)

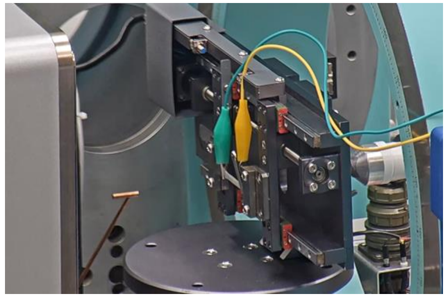
- Thin film studies: XRD, resistivity, roughness, under ultra-high purity gas



S. Dey et al., J. Vac. Sci. Technol. A 35, 03E109 (2017)

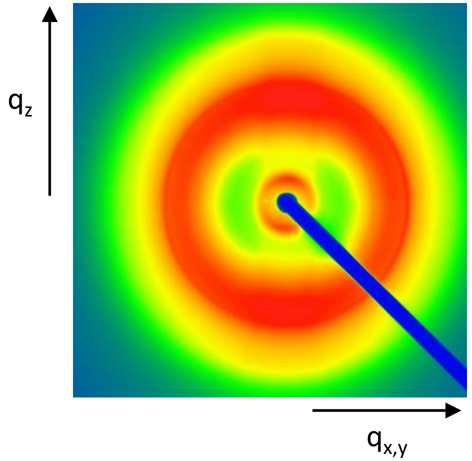


WAXS (XRD with area detector)



Biopolymers for bandages
Youchao Teng, Yimin Wu, U. Waterloo

Measure WAXS, voltage and current while stretching (0 – 100 N)



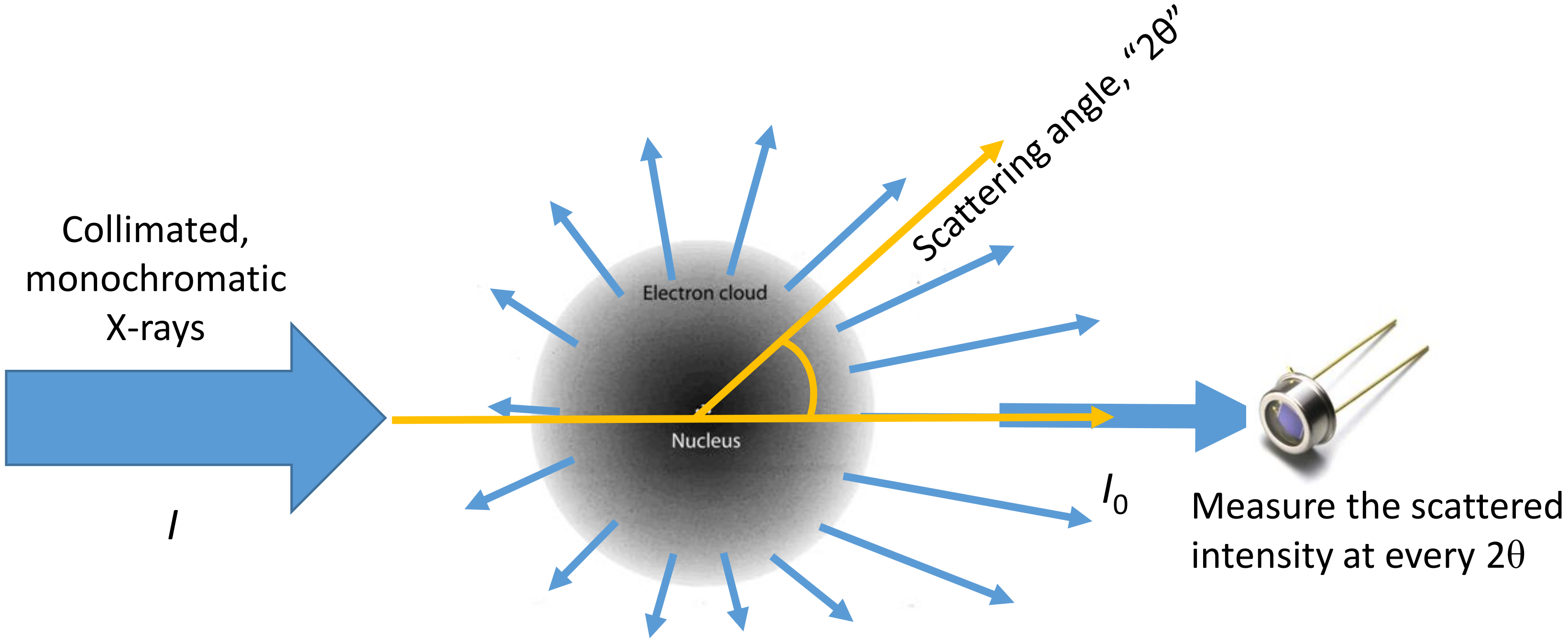
- Texture on surfaces
- Degree of orientation
- % crystallinity
- Speed/in-situ

SAXS

SAXS has been running since February 2021, fairly recent

No dedicated SAXS beamline at CLS

X-ray scattering

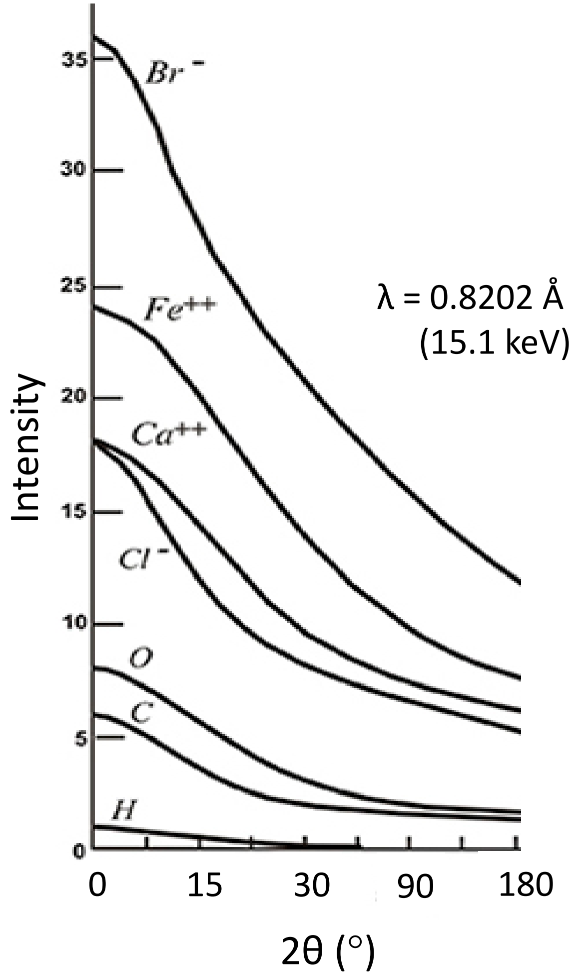


$T = I/I_0 = e^{-(\mu/\rho)\rho t}$

"Elastic" scattering

no wavelength change

Atomic form factors
(or atomic scattering factors)



From scattering to diffraction

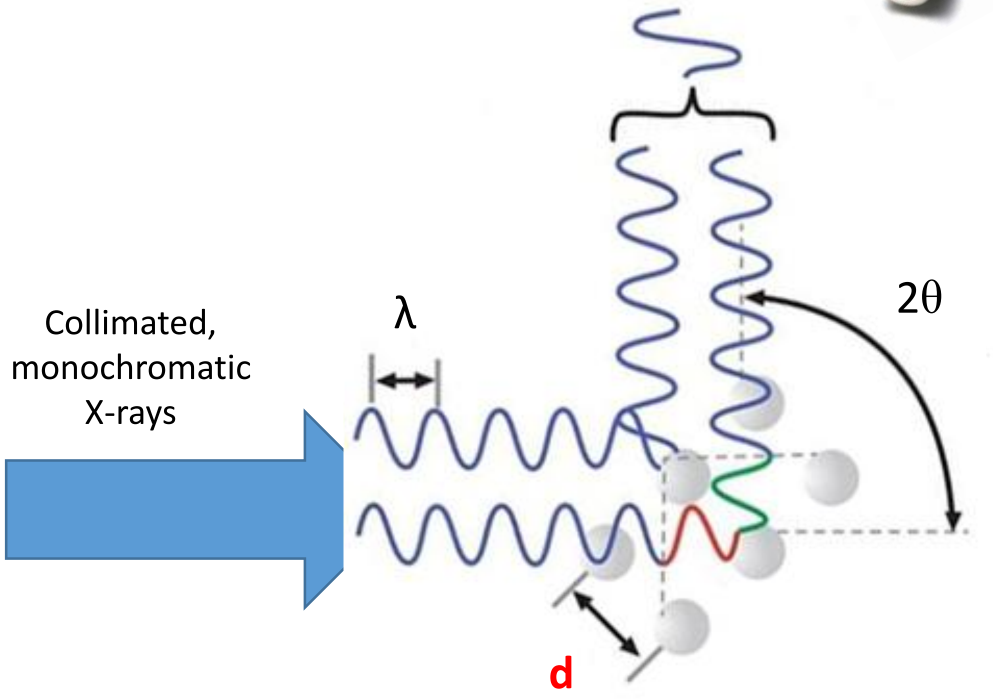
“Bragg” diffraction

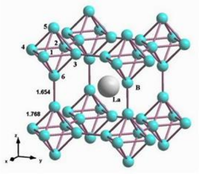
$n\lambda = 2\mathbf{d}\sin\theta$ **2θ** $q = (4\pi\sin\theta)/\lambda$

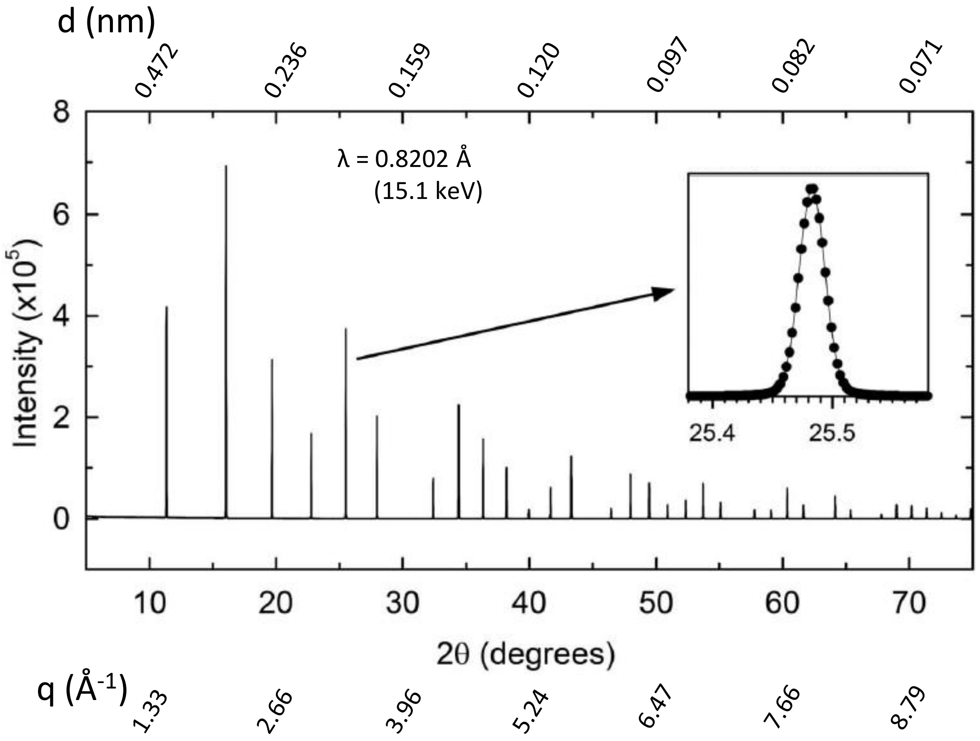
d ↔ **q**

$q = 2\pi/d$

Constructive interference channels
·ttered intensity into specific directions



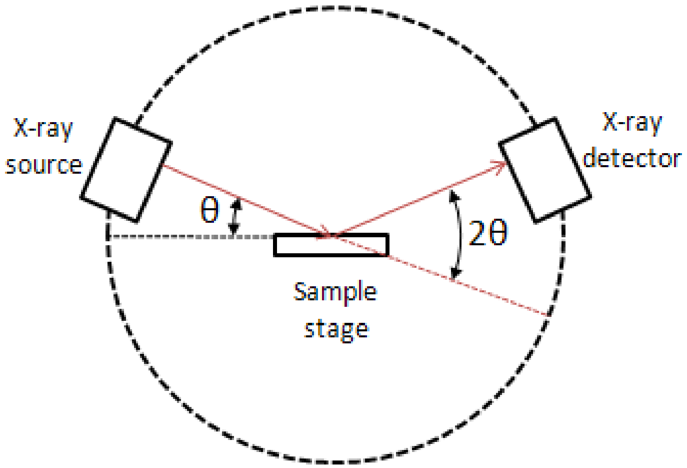


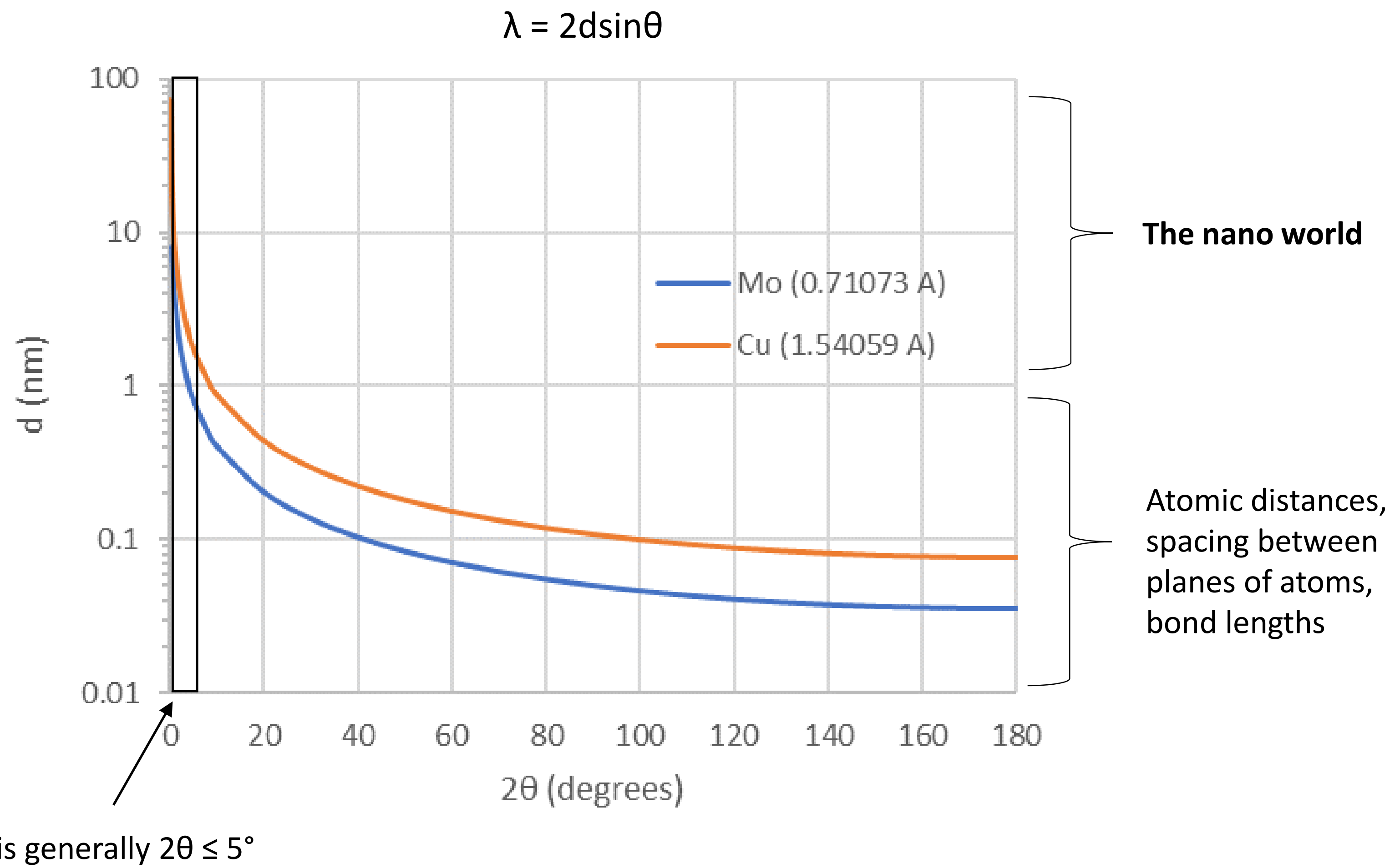


X-ray scattering and diffraction reveals structural order within materials
on the atomic to >150 nm length scale

What is small angle X-ray scattering (SAXS)?

Starting from powder diffraction…

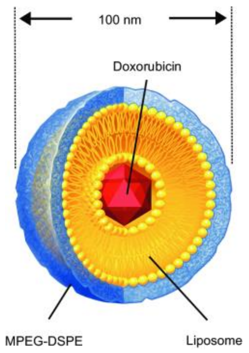




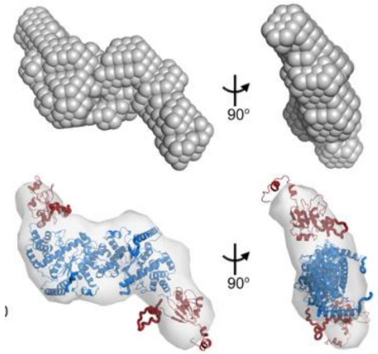
Probe relatively big things (1 - 150 nm) by measuring elastic X-ray scattering at small angles

Applications

Drug delivery, Pharmaceuticals

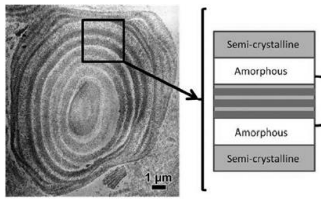


Proteins



define the global shape and conformation in solution

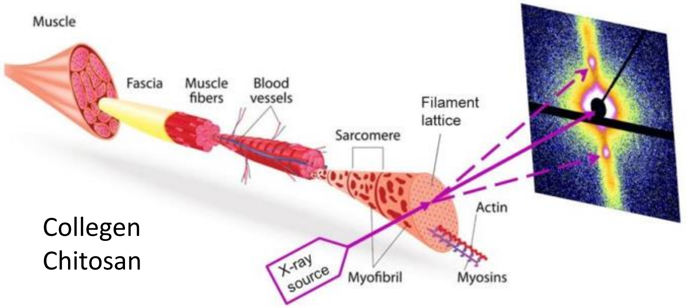
Food science



S. Perez and E. Bertoft, *Starch* **2010**, *62*, 389 - 420

Micelles, emulsions

Biomacromolecules



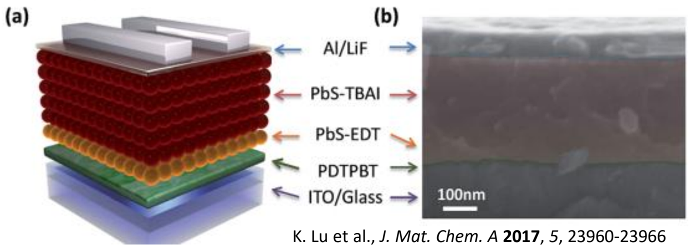
Collegen
Chitosan

https://www.techexplorist.com/scientists-investigated-pork-fillet-x-ray-light/11572/

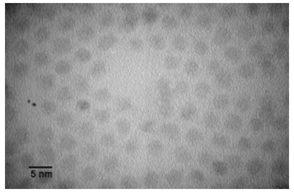
1) Packing, ordering
2) Size, size distribution
3) Shape

Geology

All kinds of nanoparticles

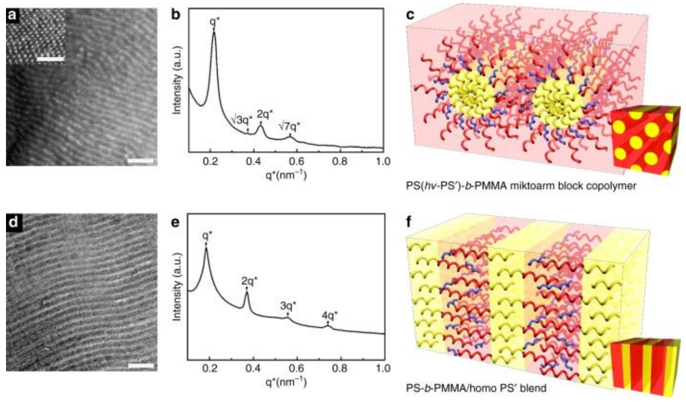


K. Lu et al., *J. Mat. Chem. A* **2017**, *5*, 23960-23966



M. Yuan et al., Adv. Mater. 2014, 26, 3513–3519

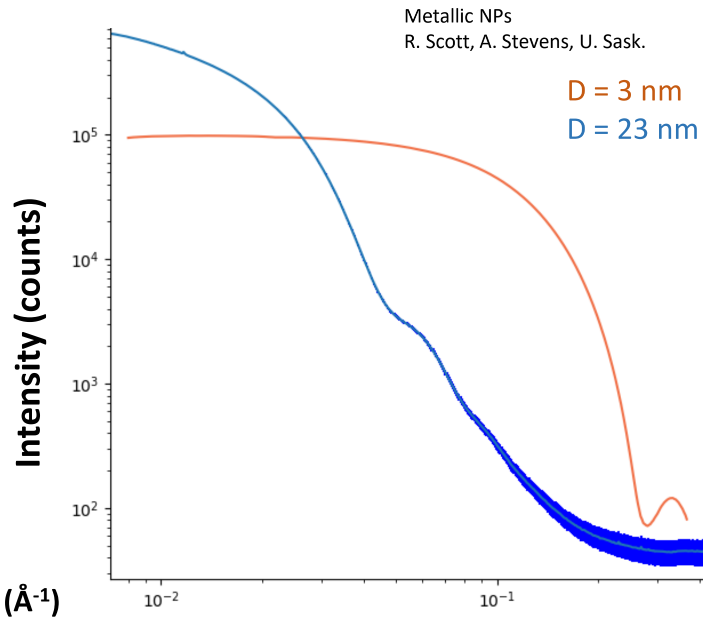
Polymers



Nature Comm. 8, 1765 (2017)

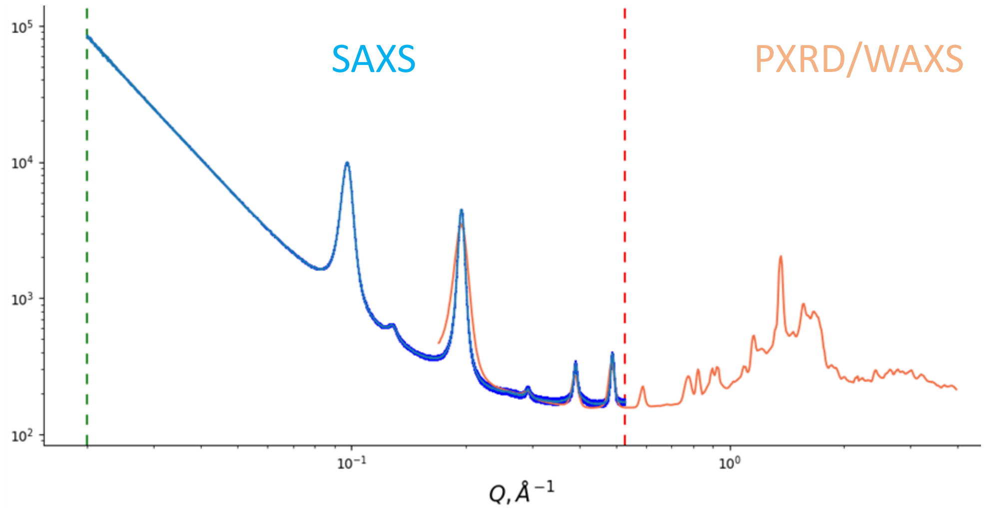
Canadian Light Source
Centre canadien de rayonnement synchrotron

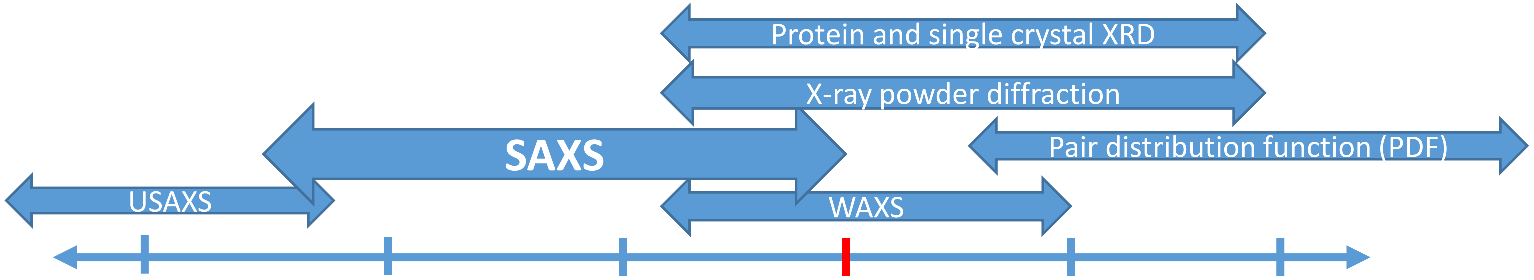
How is SAXS related to other X-ray diffraction techniques?



Q (Å⁻¹)		
d (nm)	62.8	6.28
2θ (°)	0.108	1.08

- Intensity vs. Q
- log-log plot





λ = 0.819 Å
(15.1 keV)

Q (Å⁻¹)	0.000603	0.00603	0.134	0.67	5.87	9.87
d (nm)	1042	104	4.69	0.938	0.106	0.0637
2θ (°)	0.0045	0.045	1	5	45	80

Nano-scale

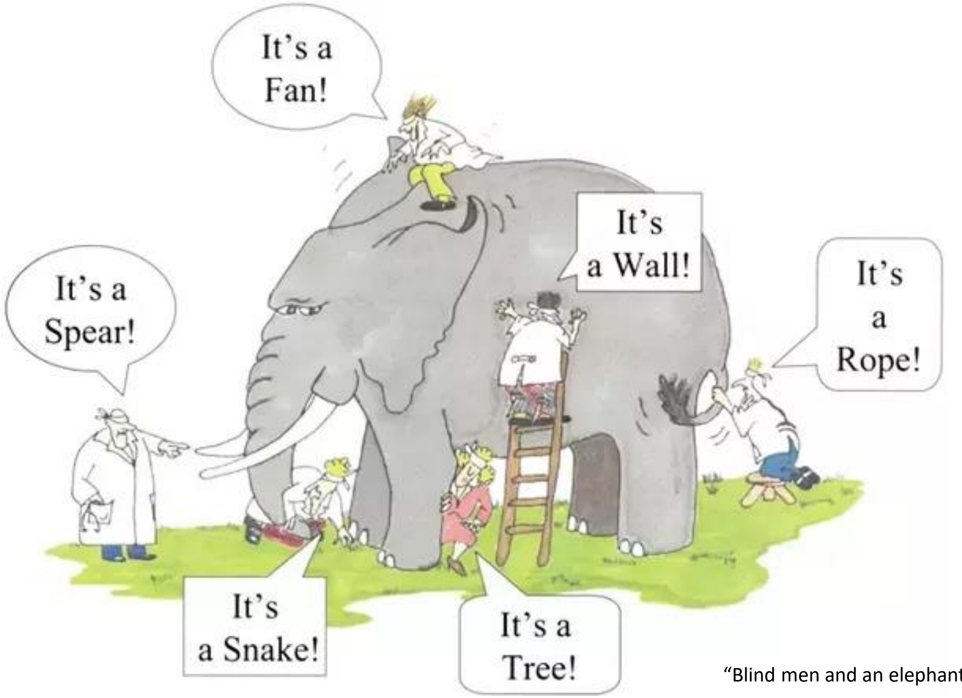
Inter-molecular distances, packing arrangement, particle shape, size,

Atomic-scale

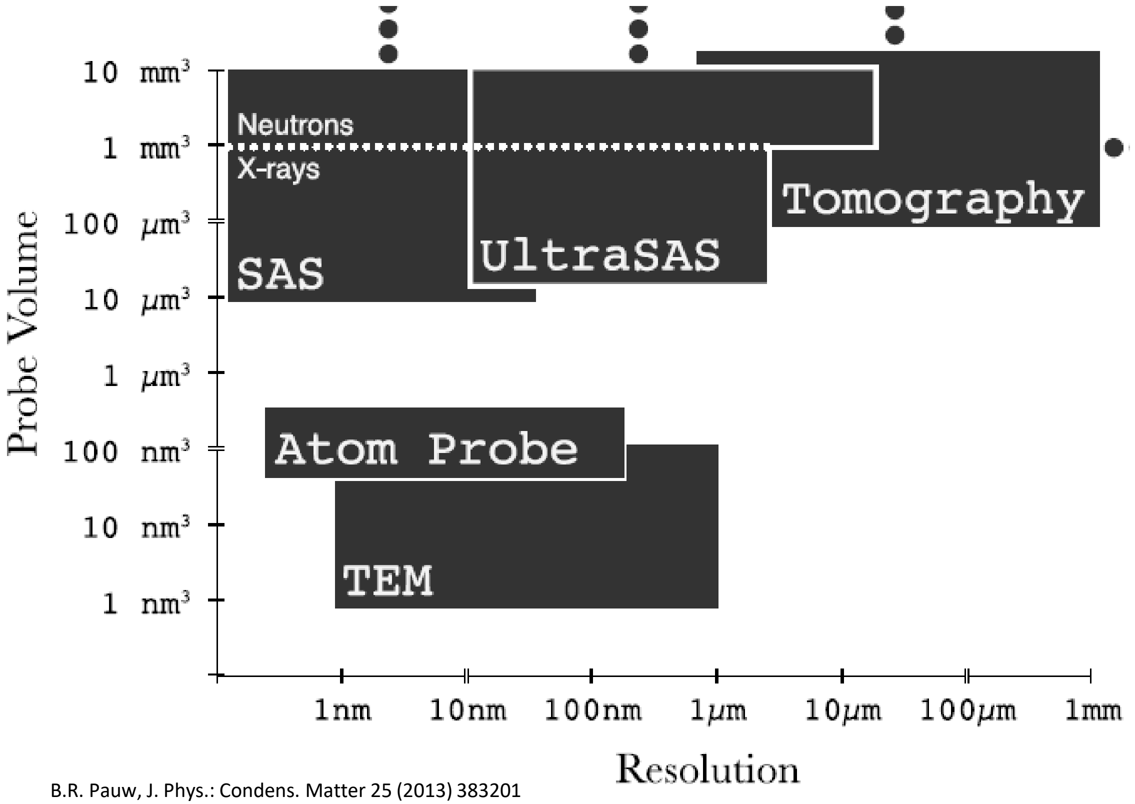
Inter-atomic distances, bond lengths, nearest neighbor atoms
Crystal structures with atomic resolution

Canadian Light Source
Centre canadien de rayonnement synchrotron

How is SAXS complementary to microscopy?



"Blind men and an elephant"



B.R. Pauw, J. Phys.: Condens. Matter 25 (2013) 383201

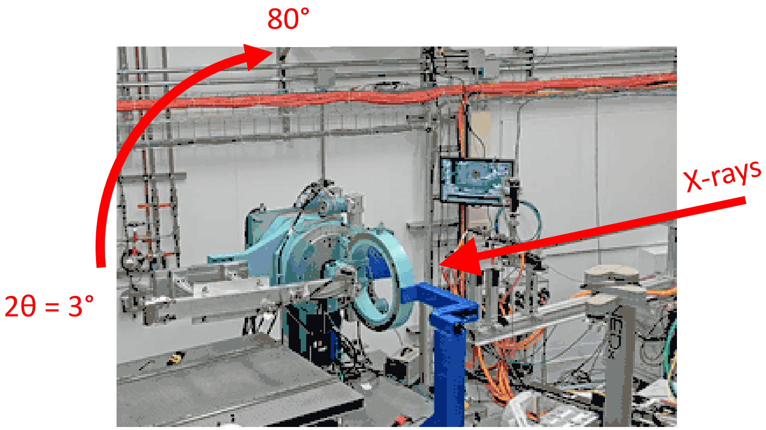
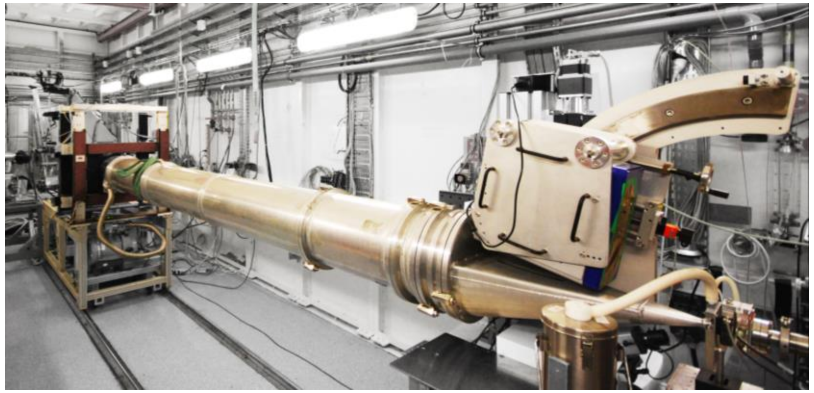
- FOV decreases as resolution increases
- Observe a small fraction of the complete sample at a time
- Challenging sample prep at smaller size

- SAXS provides nanoscale information averaged over the beam volume (~mm^3)
- Complementary info: shape, folding/unfolding, assembled state in solution

Canadian Light Source
Centre canadien de rayonnement synchrotron

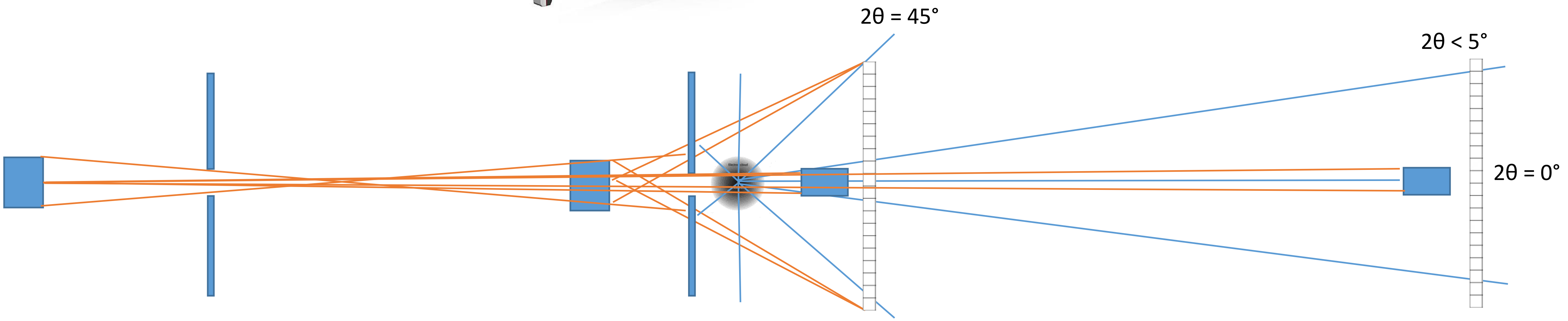
The instrumentation and sample prep

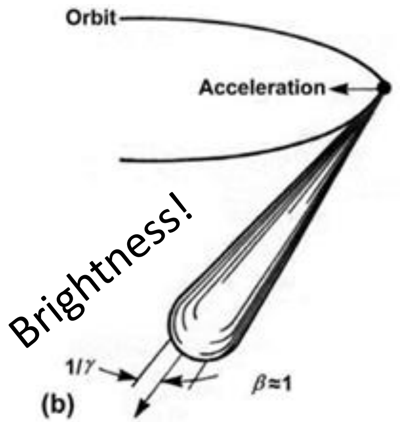
How to measure small angle X-ray scattering?

	PXRD	SAXS
Lab source		
Synchrotron		ESRF - BM26 

1) Special instrumentation required for SAXS
2) SAXS instruments are long!

SAXS instrumentation basics





- More flux (better SNR), weakly scattering objects (organics/biology)
- Better q resolution
- Time resolved, SAXS mapping
- Choice of many wavelengths

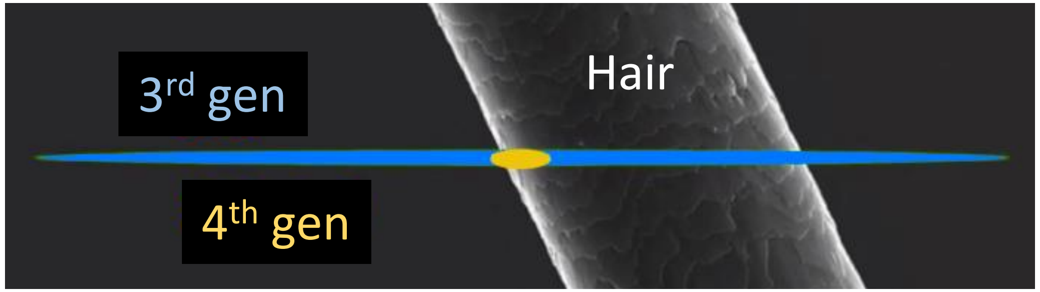
- Highly collimated hard X-ray beam (multiple slits)
- Large area detector with beamstop
- Space for >2 m sample to detector distance
- Some ability to change detector distance (SAXS/WAXS)
- All or most components in vacuum

State-of-the-art instrumentation

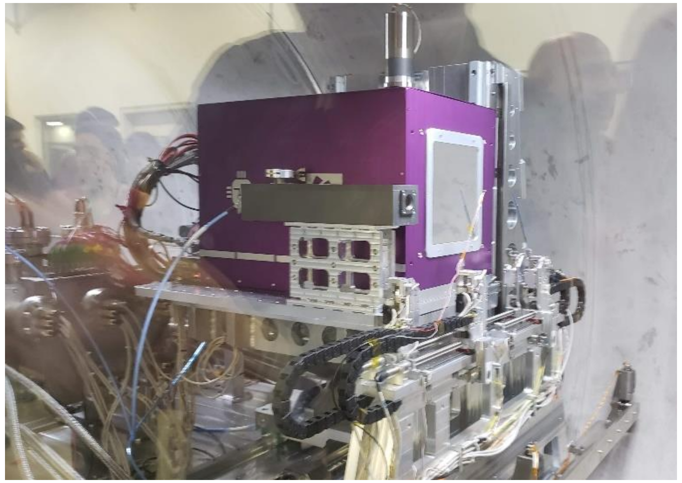
CATERETÊ beamline @ SIRIUS
Campinas, Brazil

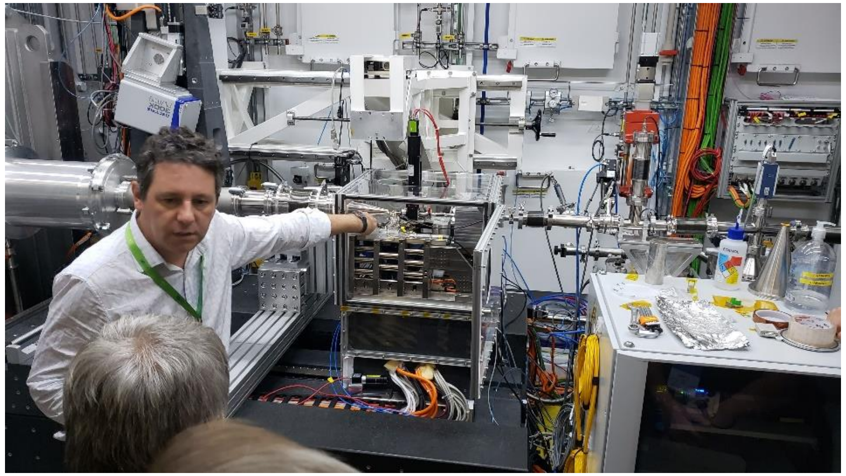
Dedicated SAXS beamline, new for 2022

- 4th gen synchrotron, 88 m source to sample
- Modern large area detector with beamstop
- 0 - 28 m sample to detector distance
- All components in vacuum, no windows from source to detector



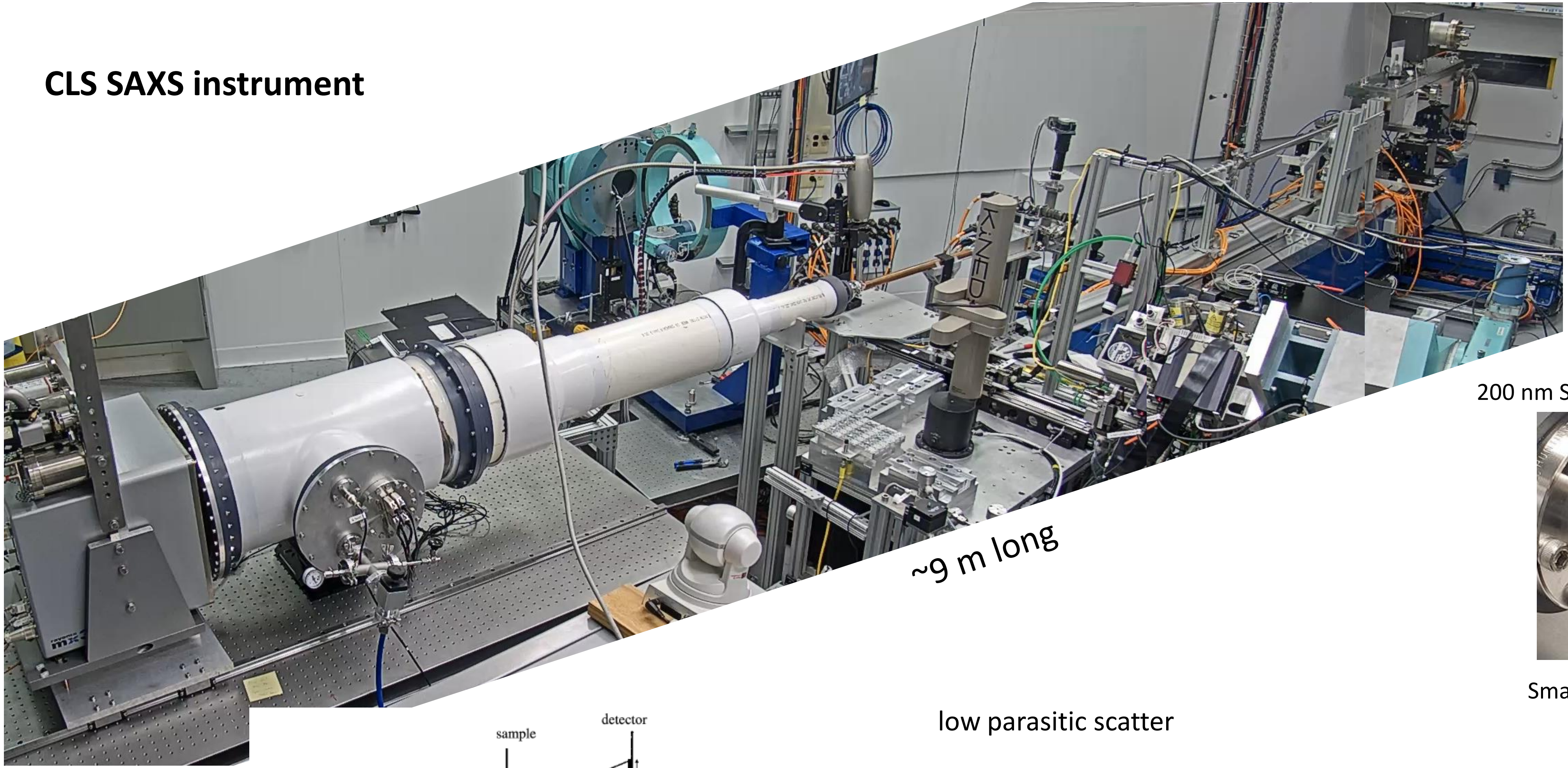
P. R. Willmott, 2022





Canadian Light Source   Centre canadien de rayonnement synchrotron

CLS SAXS instrument



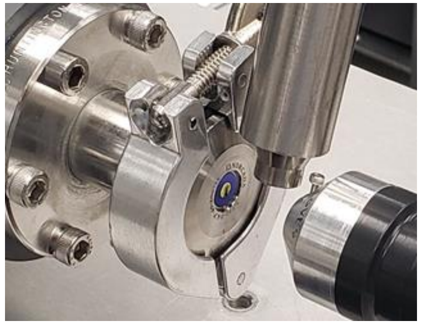
~9 m long

1) Beam defining slit
+6.1 m
(1.00 mm x 1.00 mm)

2) Anti-scatter slit
+0.9 m
(0.25 mm V x 0.50 mm H)

200 nm SiN window

3) Guard slit (0.80 mm)



Small air gap for sample (~35 mm)

3 pinhole layout

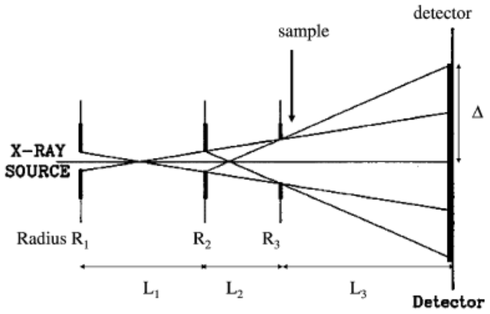
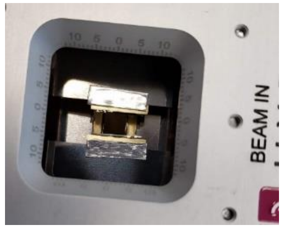
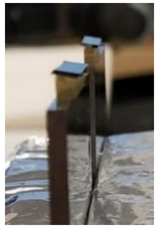


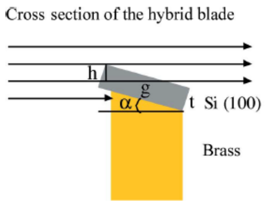
Figure 4
Schematic layout of a SAXS pinhole camera.

J. Appl. Cryst. (2004). 37, 369-380

low parasitic scatter







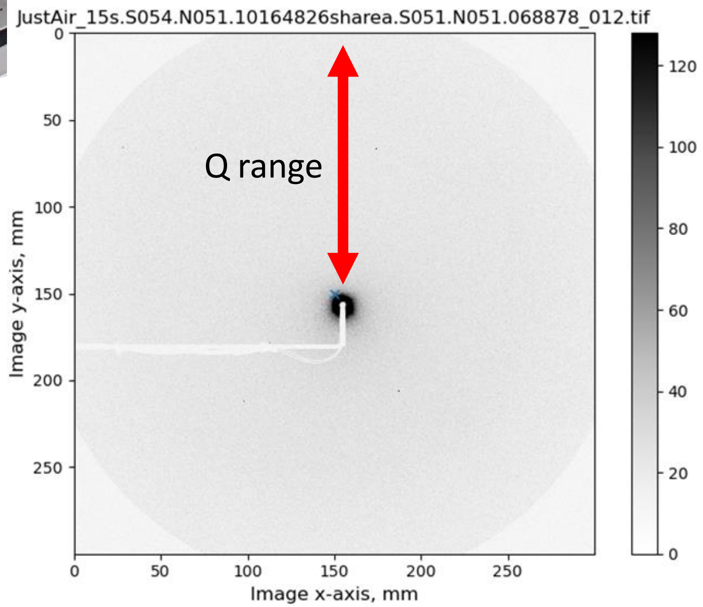
Y. Li et al., J. Appl. Cryst. (2008). 41, 1134–1139
N.M. Kirby et al., J. Appl. Cryst. 46, 1670–1680 (2013)

Canadian Light Source
Centre canadien de rayonnement synchrotron

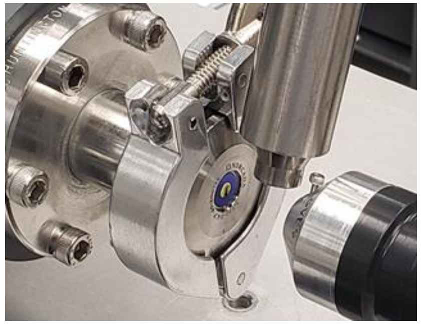
CLS SAXS instrument

λ = 1.26 Å (9.85 keV)
0.658 Å (18.8 keV)

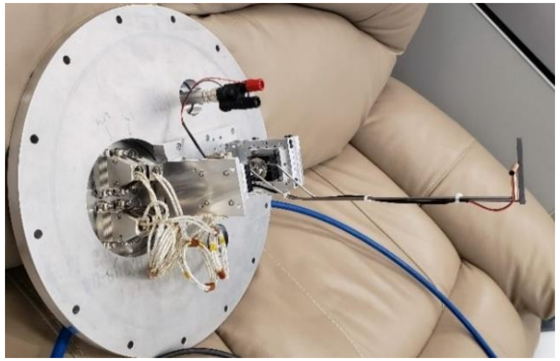
Q ~ 0.005 – 0.5 $Å^{-1}$
d ~ 125 – 1.25 nm
2θ ~ 0.05 – 3.7°



200 nm SiN window
3) Guard slit (0.80 mm)



Small air gap for sample (~35 mm)



Vacuum tube (≤ 10-2 Torr)
Beamstop with photodiode
-2.0 m
(4.0 mm)

13.5” Kapton window (125 µm)
~2100 pounds

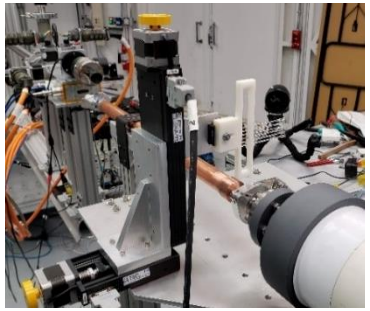
Canadian Light Source
Centre canadien de rayonnement synchrotron

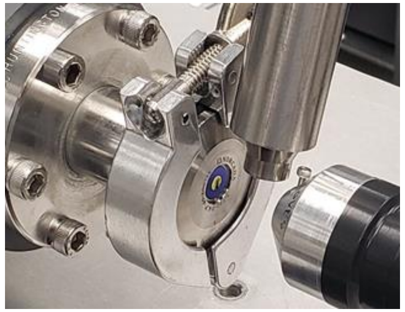
Sample preparation and mounting

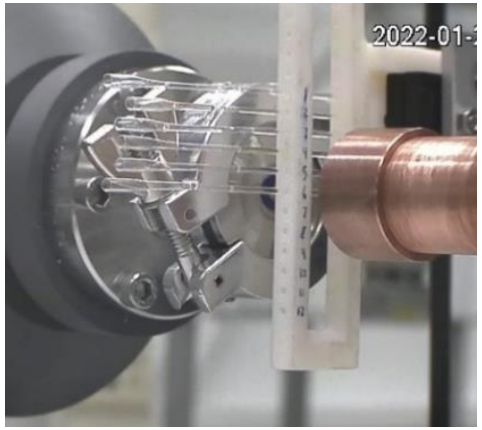
Transmission

Freestanding,
Between Scotch tape

Capillaries: 1.0 to 1.5 mm diameter quartz,
borosilicate glass







-193 – +226ºC
>1000 °C

Grazing incidence

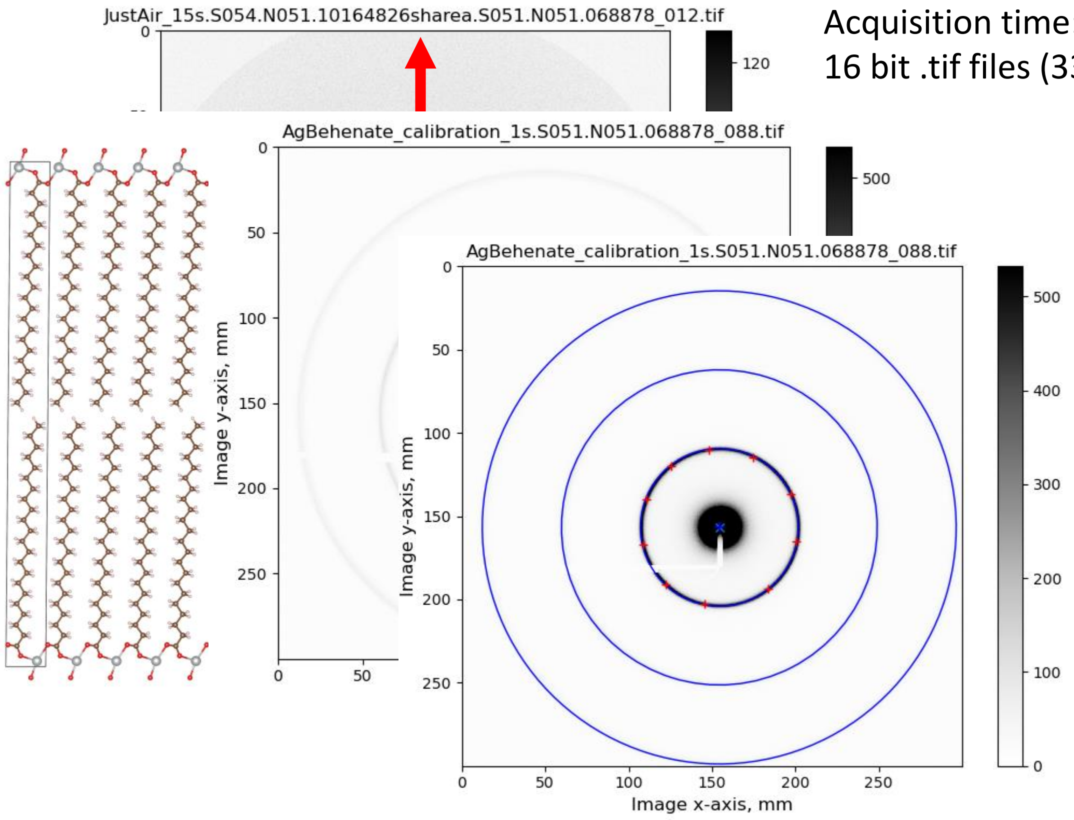
Thin film sample on Si wafers,
or glass slides

Calculate the transmission of your sample… 1/e is ideal
Bring blanks
Bring a sample where you know what to expect
Please buy your own capillaries

Data collection and reduction

Detector acquisition and calibration

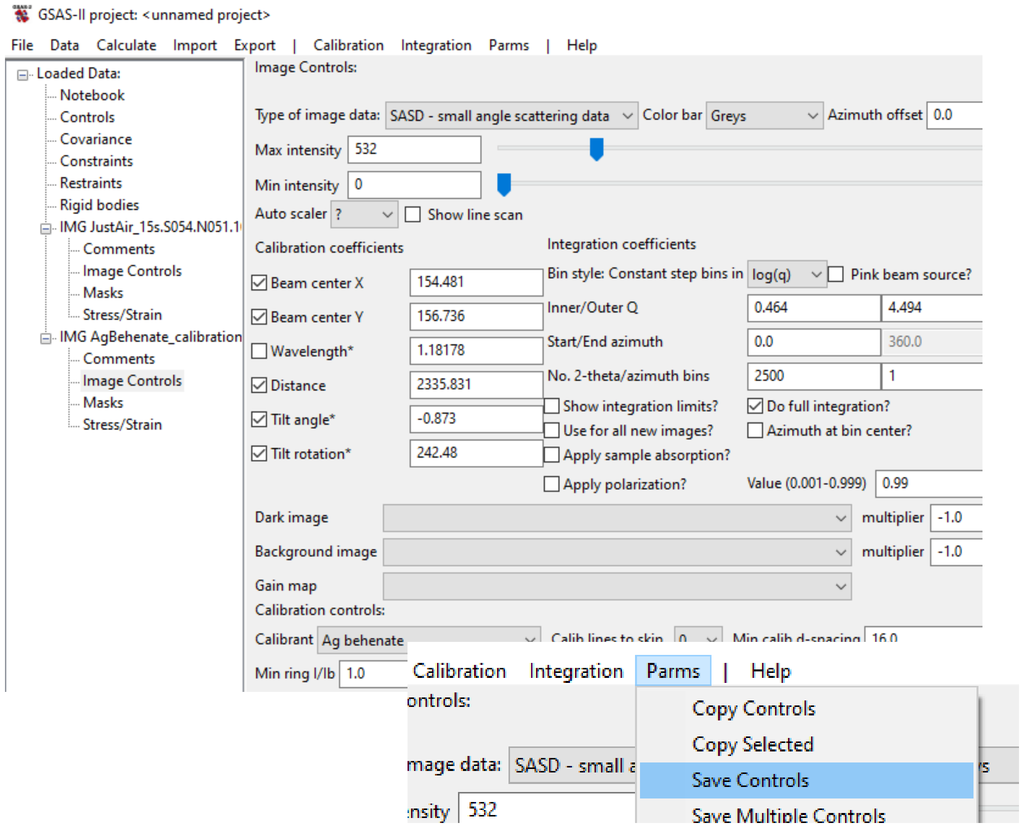
During your beamtime, note the wavelength, the approximate detector distance, and detector details



Acquisition time: 1 s - 90 s
16 bit .tif files (33 MB)

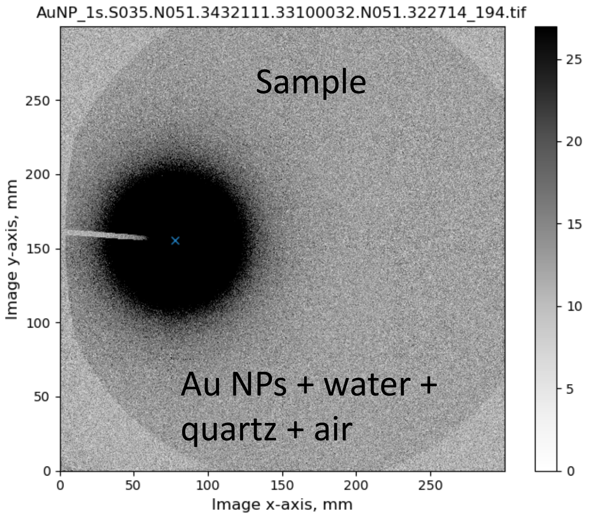
Ring	q (A-1)	d (nm)
1	0.1076	5.839
2	0.2152	2.920
3	0.3228	1.946
4	0.4304	1.460
5	0.5380	1.168

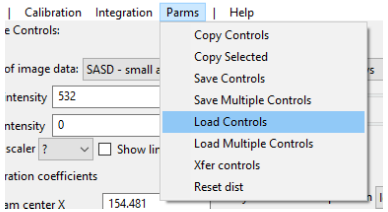
GSAS-II demo, “Calibration of an area detector”



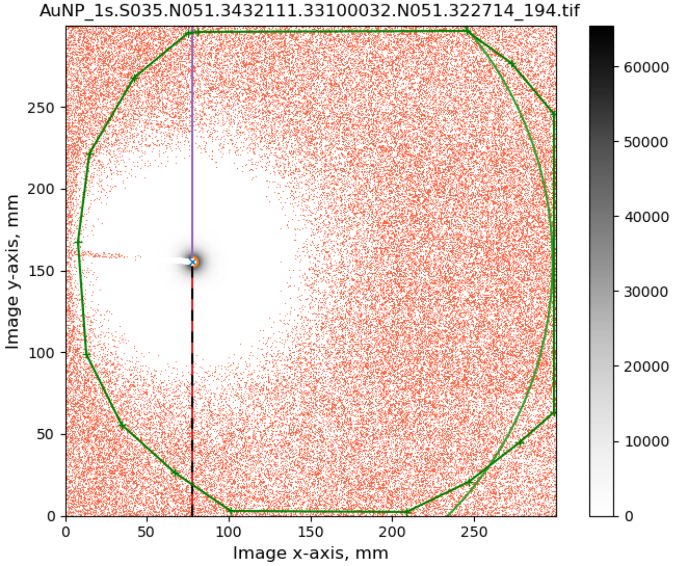
Canadian Light Source
Centre canadien de rayonnement synchrotron

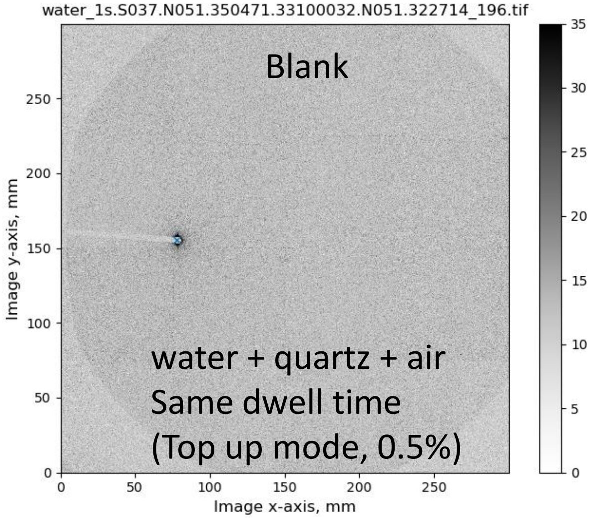
Data correction, and reduction to 1D plot

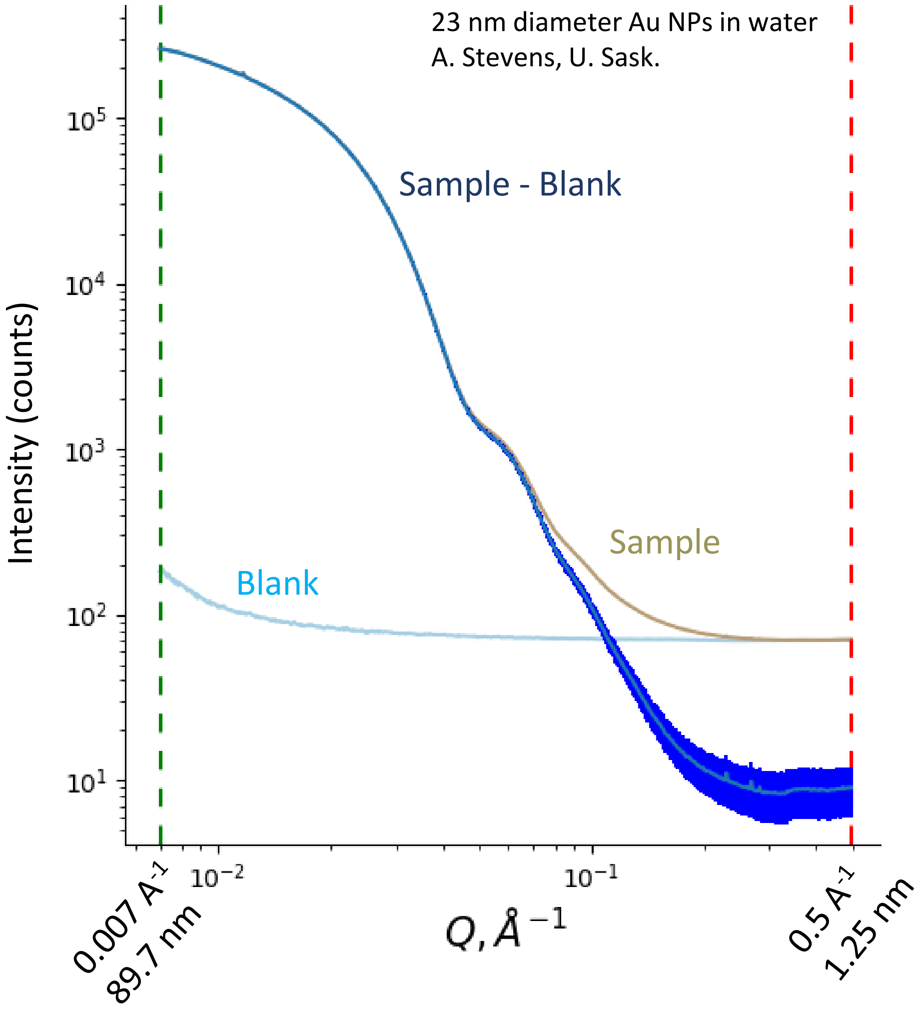




Subtract blank







Proper units of intensity are cm^{-1}
IF data are scaled to absolute intensity

5 tips for a great data collection experience

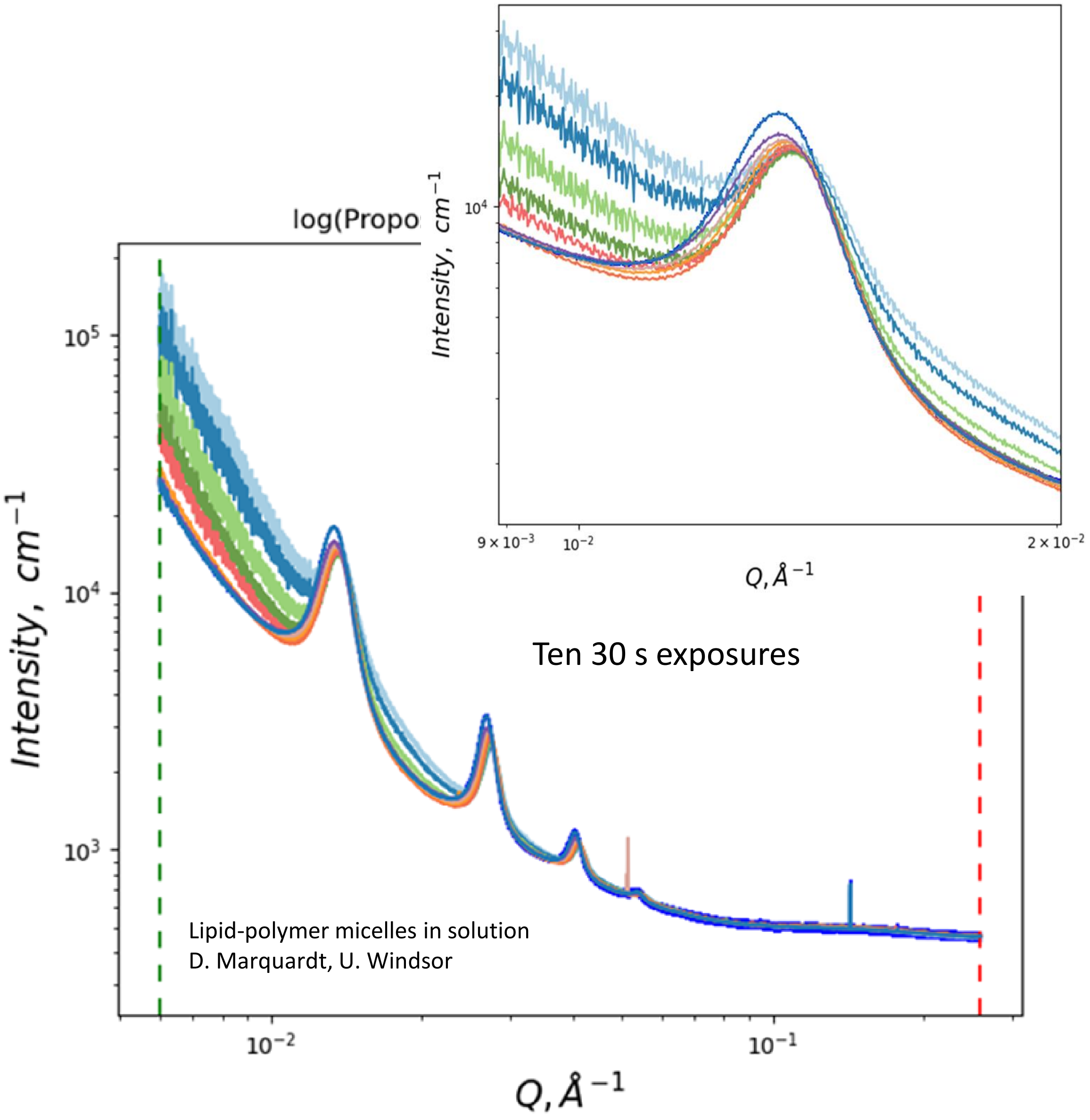
1) It’s your experiment.

2) Get in contact ~2 weeks in advance.

3) Know what q range you want, and include it in your proposal. We can tune the endstation to your problem.

4) Know what your data should look like. Find published examples, or bring a known good sample.

5) **Chose quality over quantity**: Check for radiation damage at the start, work up data as you go along, many blank measurements, priority list



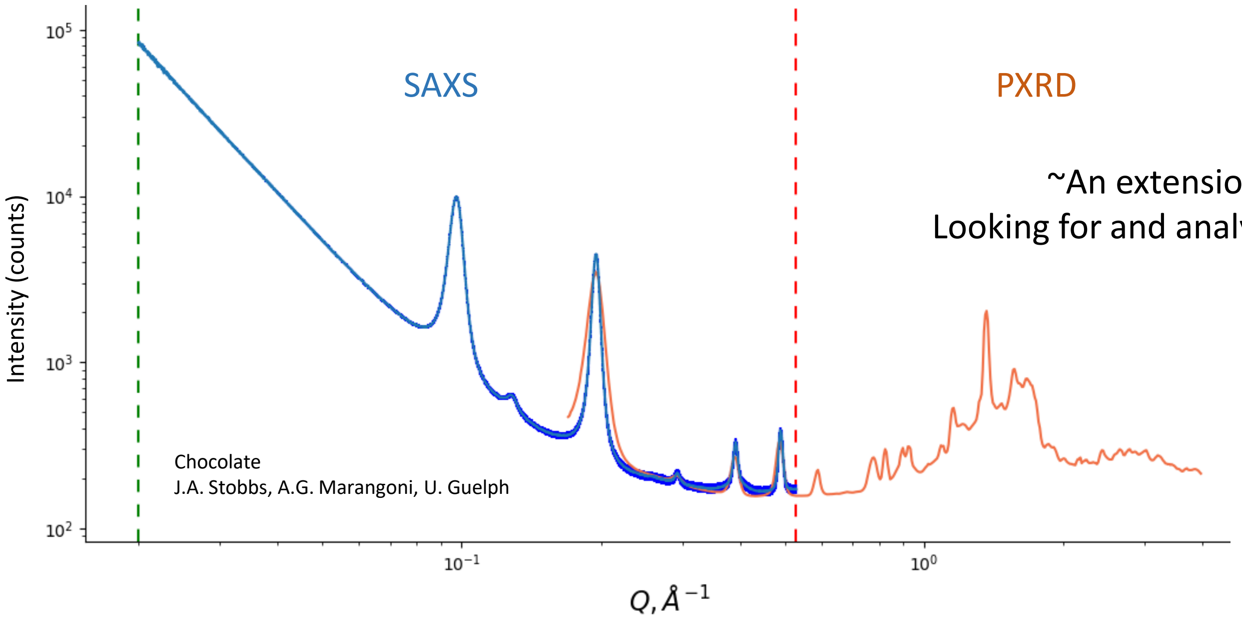
Data analysis with examples

Origins of the SAXS signal

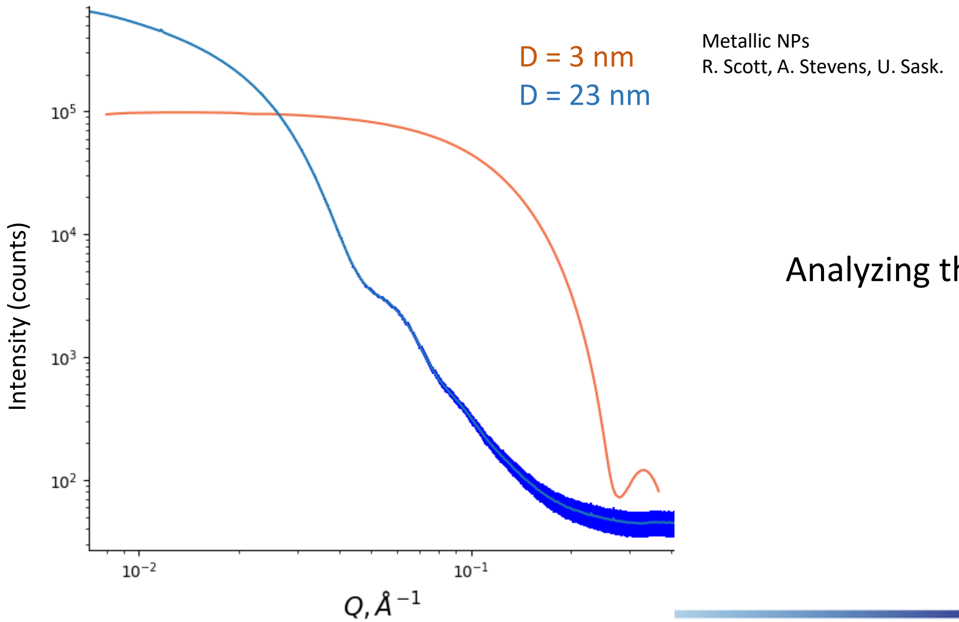
$$I(Q) = S(Q) \times P(Q)$$

S(Q) is the **structure** factor
- *Inter*-particle interferences
- High concentrations (>5% vol.)
- Ordering/packing of particles

P(Q) is the **form** factor
- *Intra*-particle interferences
- Low concentration (dilute limit)
- Size/polydispersity/shape of particles



~An extension of PXRD
Looking for and analyzing Bragg peaks



Analyzing the whole curve

Canadian Light Source Centre canadien de rayonnement synchrotron

Phospholipid reverse micelle formation in model cocoa butter: Evidence for in-situ seeding as a route to Form V polymorph in chocolate

J.A. Stobbs, S.M. Ghazani, E. Pensini, A.F.G. Leontowich, B. Barlow, and **Alejandro G. Marangoni** (*in preparation*)

Dept. Food Science
University of Guelph

The nanostructure of chocolate is manipulated using time and energy-intensive "tempering"

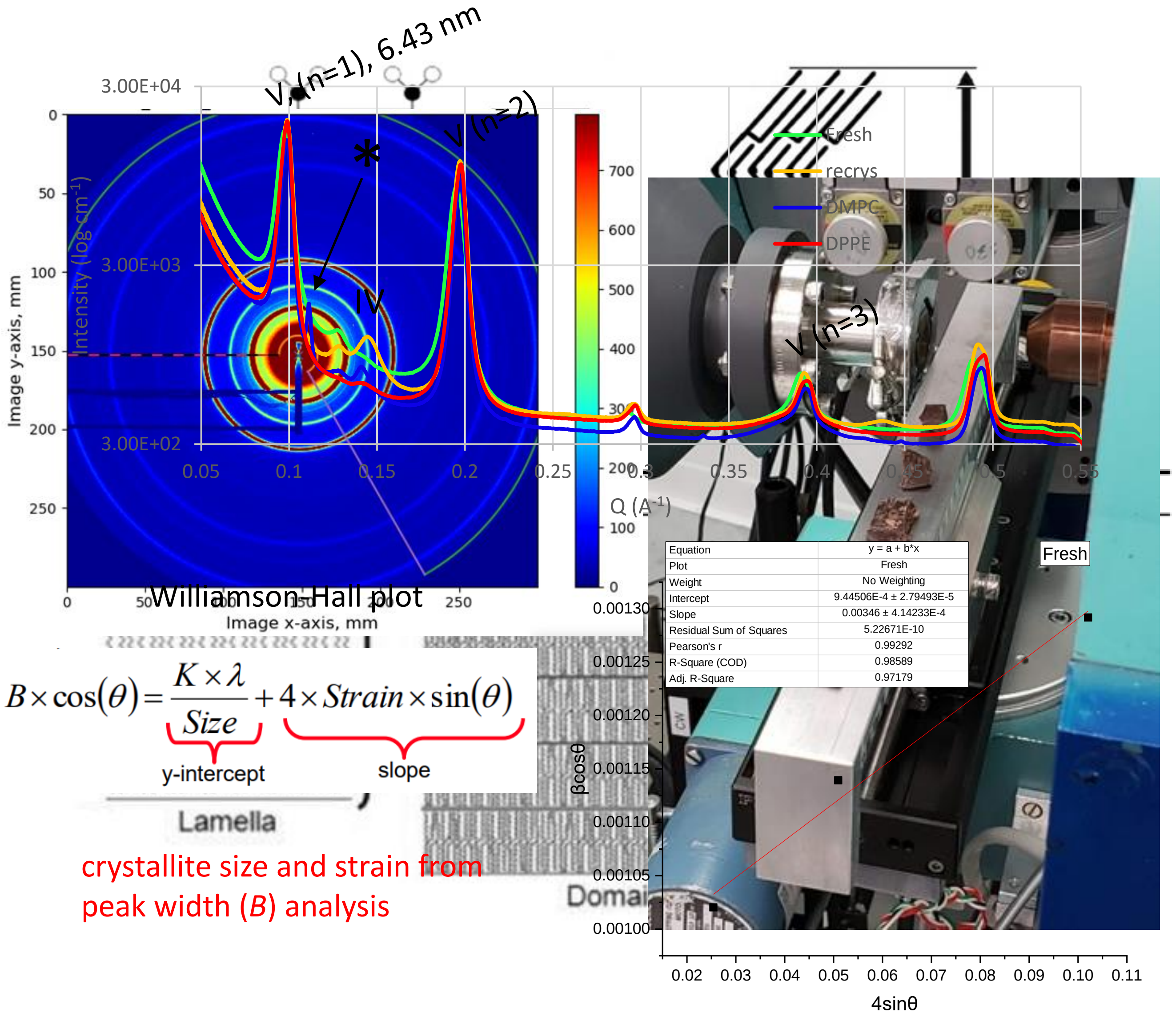
Guides crystallization of cocoa butter to "polymorph V"

Tempering of cocoa butter and chocolate using minor lipidic components

Jay Chen[1], Saeed M. Ghazani[1], Jarvis A. Stobbs[1,2] & Alejandro G. Marangoni[1]

NATURE COMMUNICATIONS | (2021)12:5018

Why does it work?



$$B \times \cos(\theta) = \underbrace{\frac{K \times \lambda}{Size}}_{\text{y-intercept}} + \underbrace{4 \times Strain \times \sin(\theta)}_{\text{slope}}$$

crystallite size and strain from peak width (B) analysis

Canadian Light Source
Centre canadien de rayonnement synchrotron

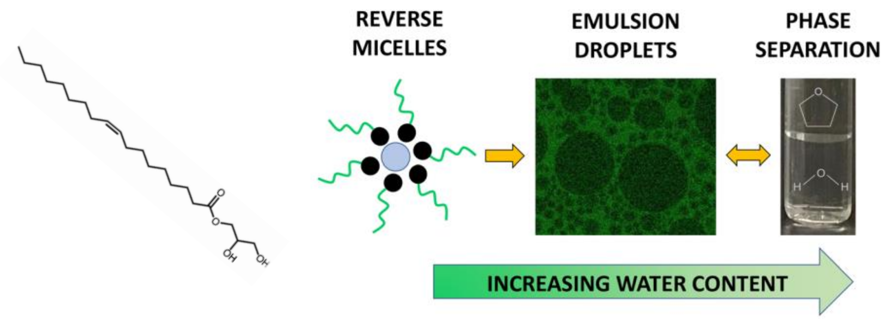
Environmental science: Waste water treatment

School of Engineering
University of Guelph

L. Earnden, A.G. Marangoni, T. Laredo, J. Stobbs, **Erica Pensini**

Scientific Reports **12**, 15832 (2022)
J. Molecular Liquids **367**, 120551 (2022)
Physics of Fluids **34**, 097119 (2022)

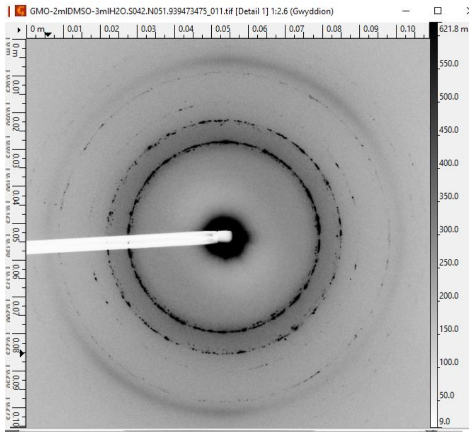
Separating solvents (THF, DMSO, DMF and acetonitrile) and metals from waste water using amphiphiles, emulsifiers and surfactants

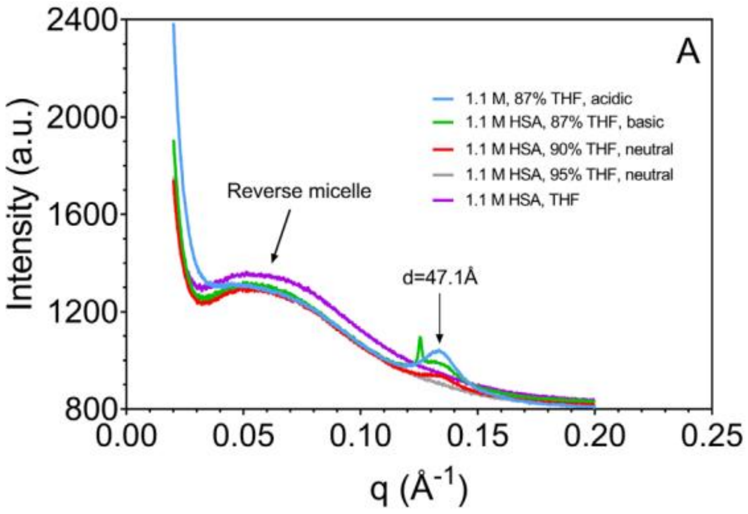


Mechanism of solvent separation?

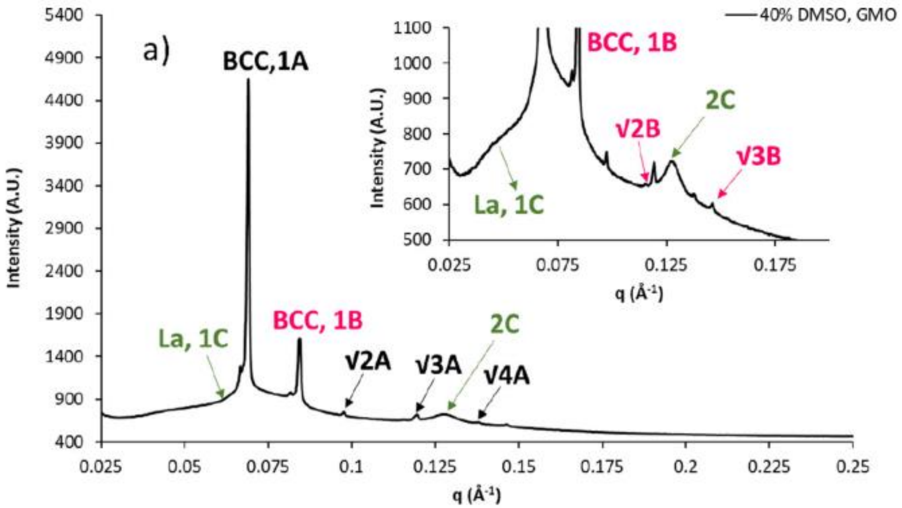
1.1 M HSA, 87% THF, acidic pH		ξ (Å)	d (Å)	d/x
a_2	1.158	19.5	86.0	4.4
c_1	−98.92			
c_2	18 245			

Powder diffraction ... ??

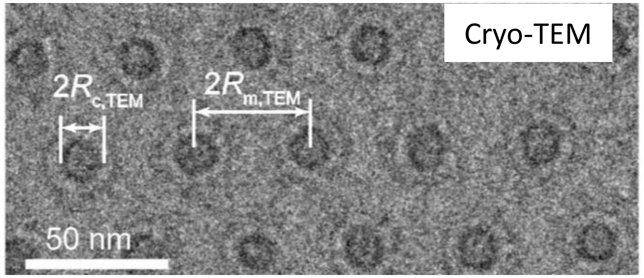




Symmetry	Peak position ratio
BCC (Im3m)	√2, √3, √4 ...



Reverse micelle lattice

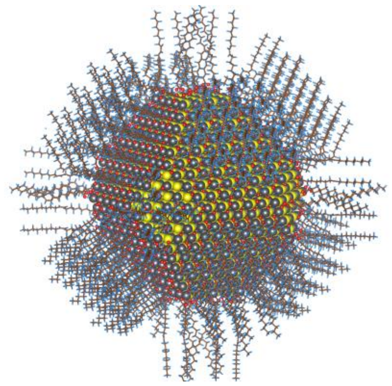


L. Chen et al., *PNAS* **115**, 7218-7223 (2018)

Canadian Light Source
Centre canadien de rayonnement synchrotron

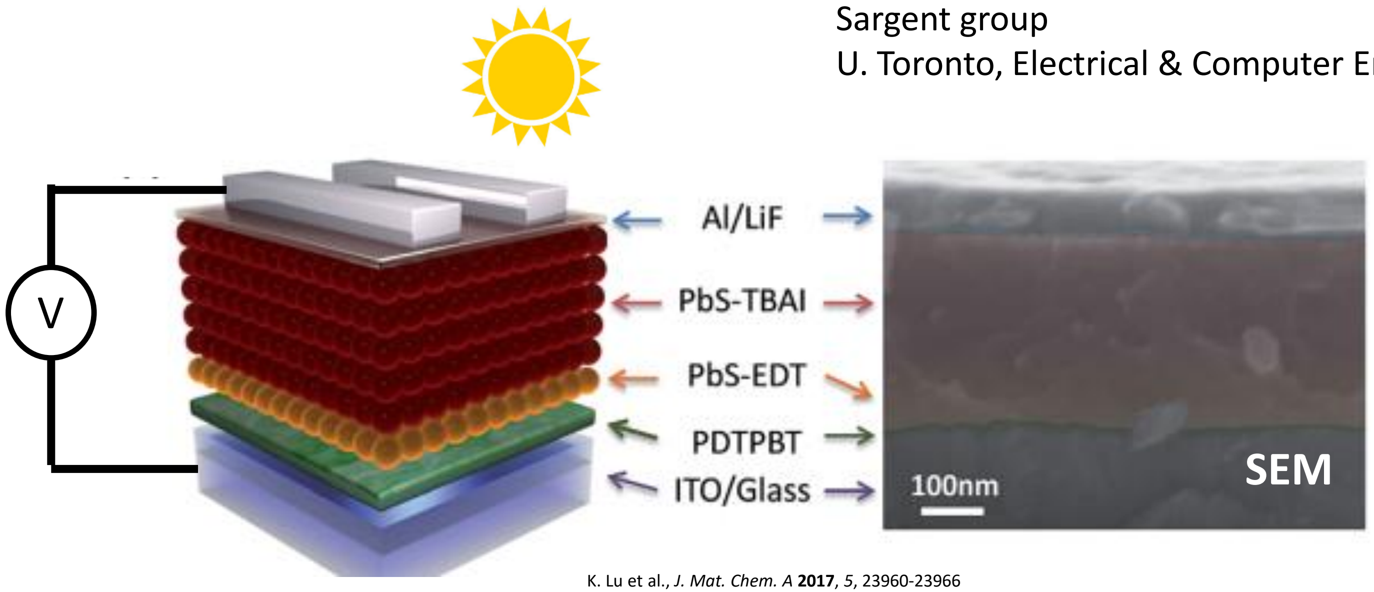
Energy materials: Quantum dot solar cells

Sargent group
U. Toronto, Electrical & Computer Engineering



PbS
$CsPb(Br/I)_3$
InAs

https://www.olcf.ornl.gov/2015/05/05/demystifying-quantum-dot-conundrums/

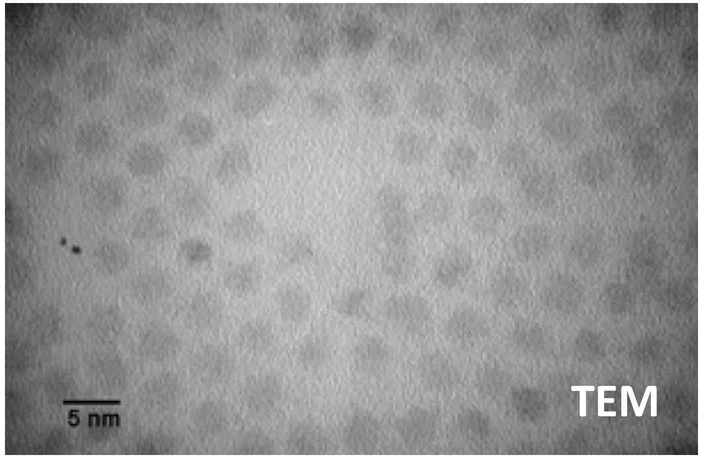


K. Lu et al., *J. Mat. Chem. A* **2017**, *5*, 23960-23966

QD films deposited layer-by-layer using spin coating
Top electrodes deposited using thermal evaporation

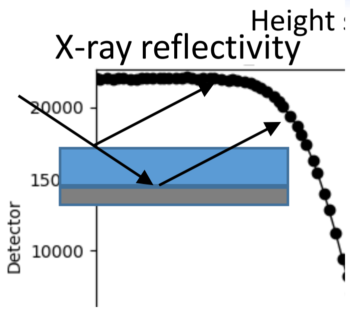
Efficient exciton generation
Size-based, tune-able band gap

18.1% record efficiency in 2022
Performance improved by tuning:
- Particle size distribution or polydispersity
- How particles pack and the interparticle distance



M. Yuan et al., Adv. Mater. 2014, 26, 3513–3519

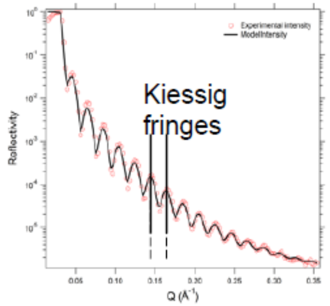
Control the size and packing by:
- Ligand exchanges cycles, centrifuge to remove largest QDs
- Rinsing steps during buildup
- Spin speeds, solvents, additives

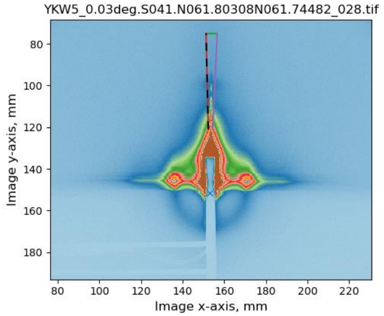


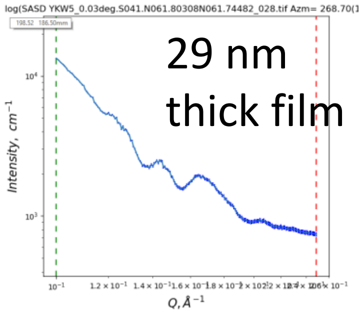
Simple estimation of film thickness

Fringes with uniform spacing

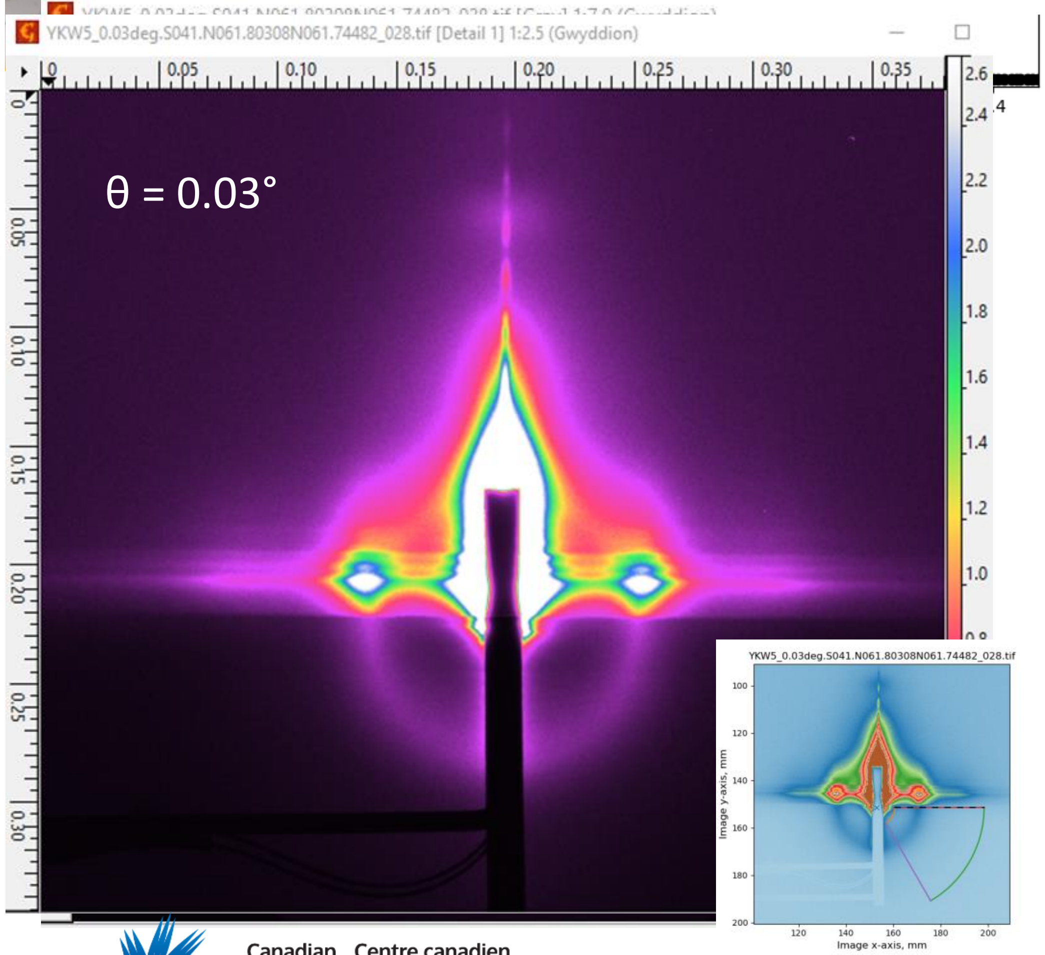
Thickness of the layer : $t = \dfrac{2\pi}{\Delta q_z}$

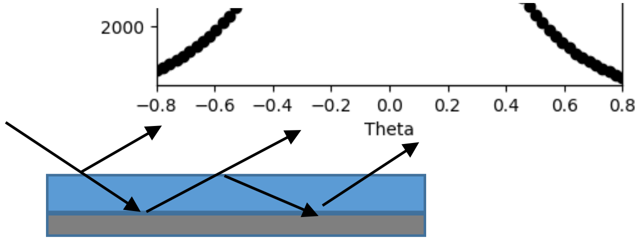






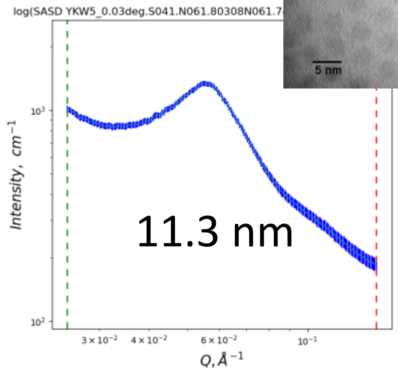
- Reflectivity gives film thickness

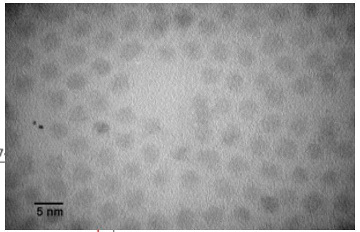


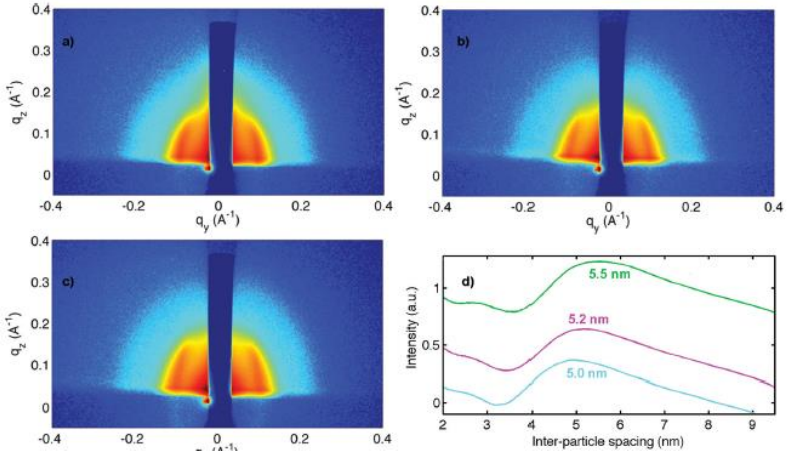


- Position gives interparticle distance
- Peak width gives size distribution
- Average over relatively huge area

- Indicates very flat, high quality film
- Adjusting grazing angle can give depth information





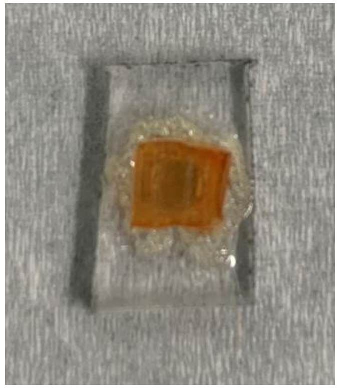


M. Yuan et al., Adv. Mater. 2014, 26, 3513–3519

I(Q) = S(Q) × P(Q)

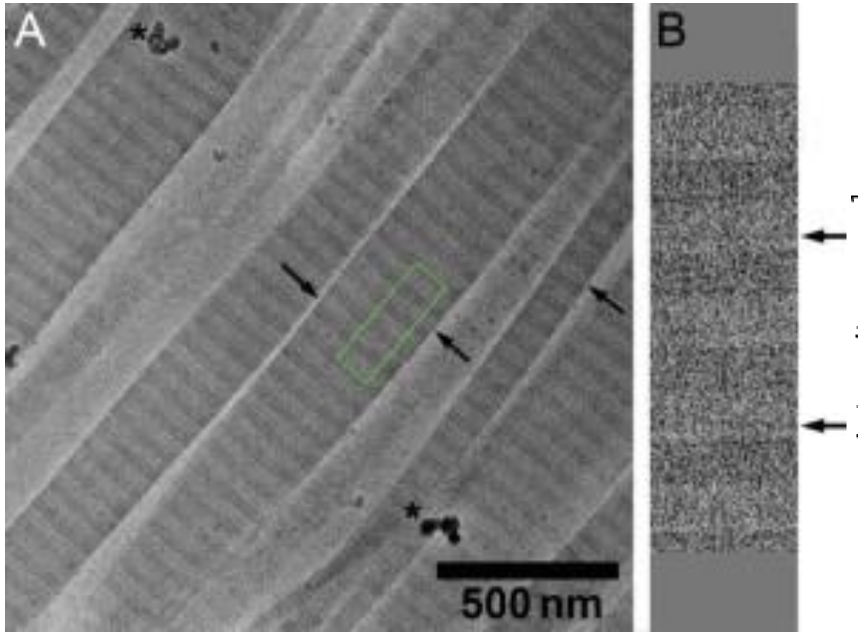
S(Q) is the **structure** factor
- *Inter*-particle interferences
- High concentrations (>5% vol.)
- Ordering/packing of particles

~An extension of PXRD
Looking for and analyzing Bragg peaks

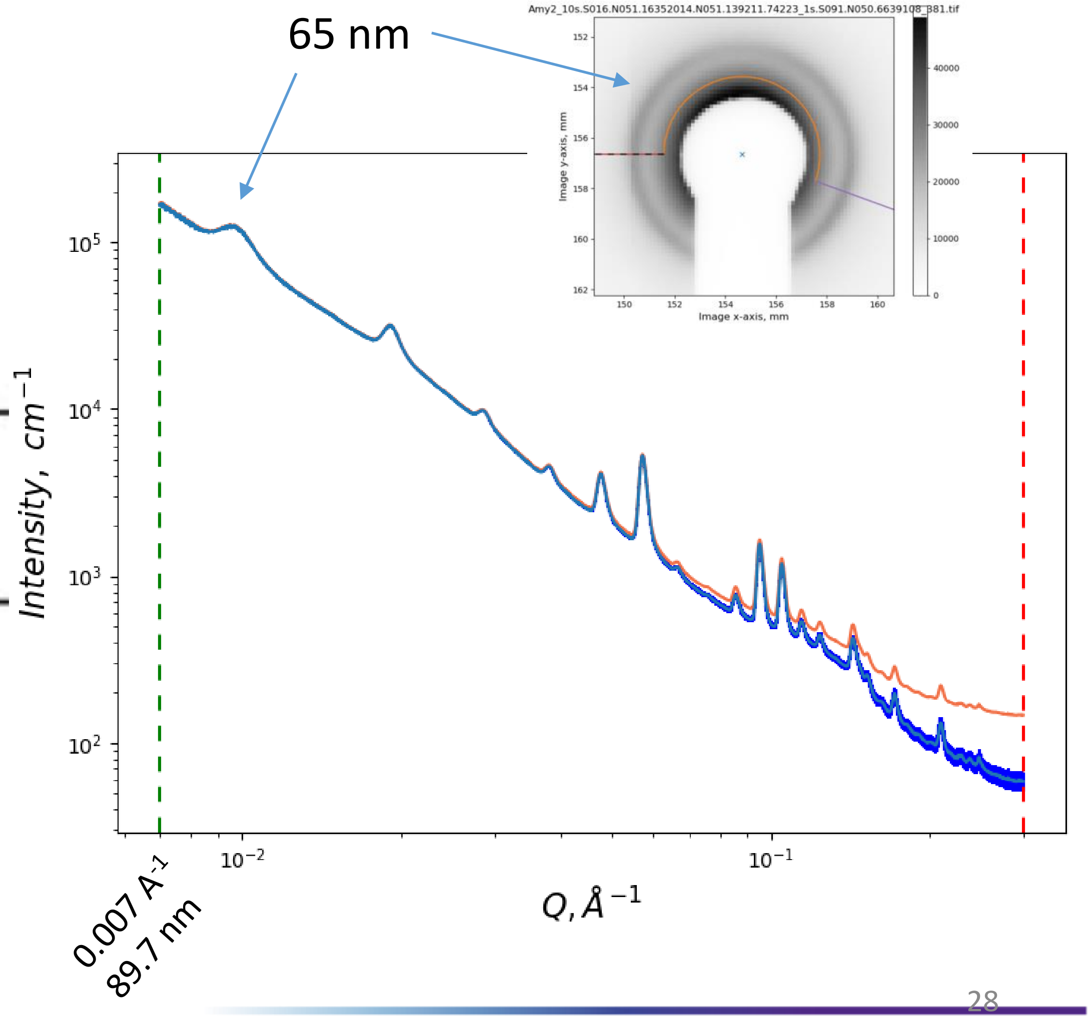


Sea cucumber dermis
M. Harrington, McGill

Type 1 collagen fibril gaps



https://doi.org/10.1016/B978-0-12-416617-2.00009-6

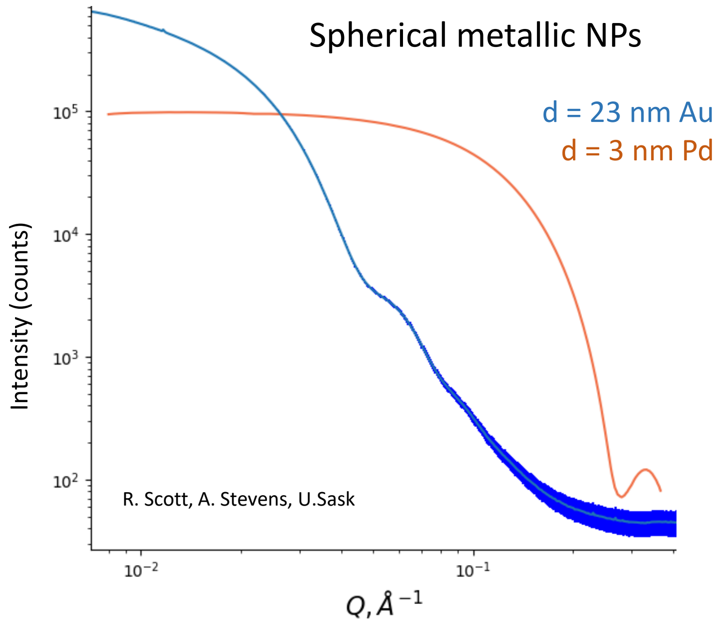


Canadian Light Source Centre canadien de rayonnement synchrotron

Origins of the SAXS signal

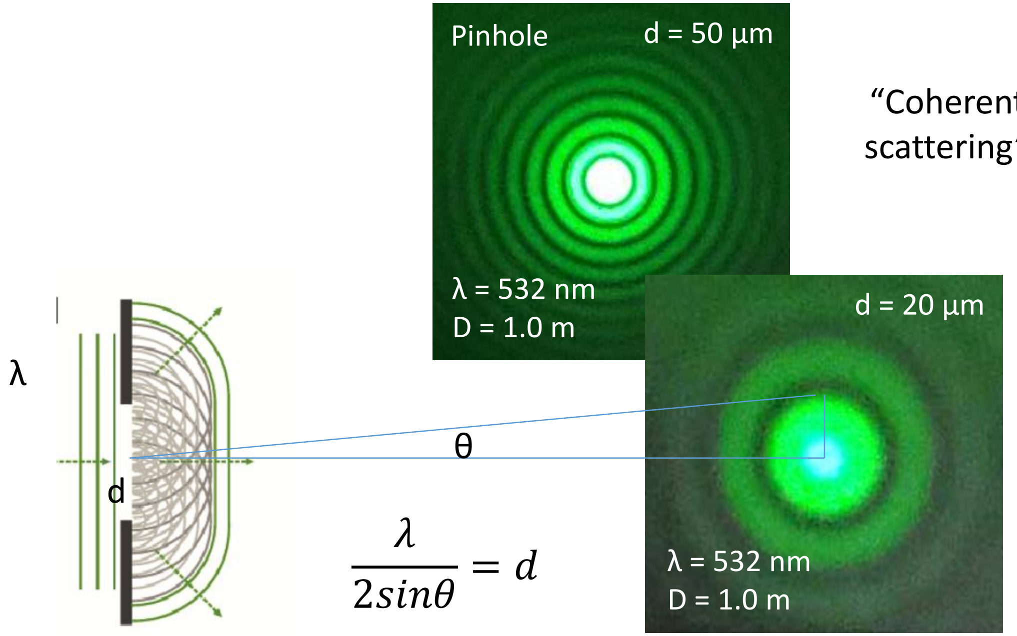
$$I(Q) = S(Q) \times P(Q)$$

P(Q) is the **form** factor
- *Intra*-particle interferences
- Low concentration (dilute limit)
- Size, shape, polydispersity of particles



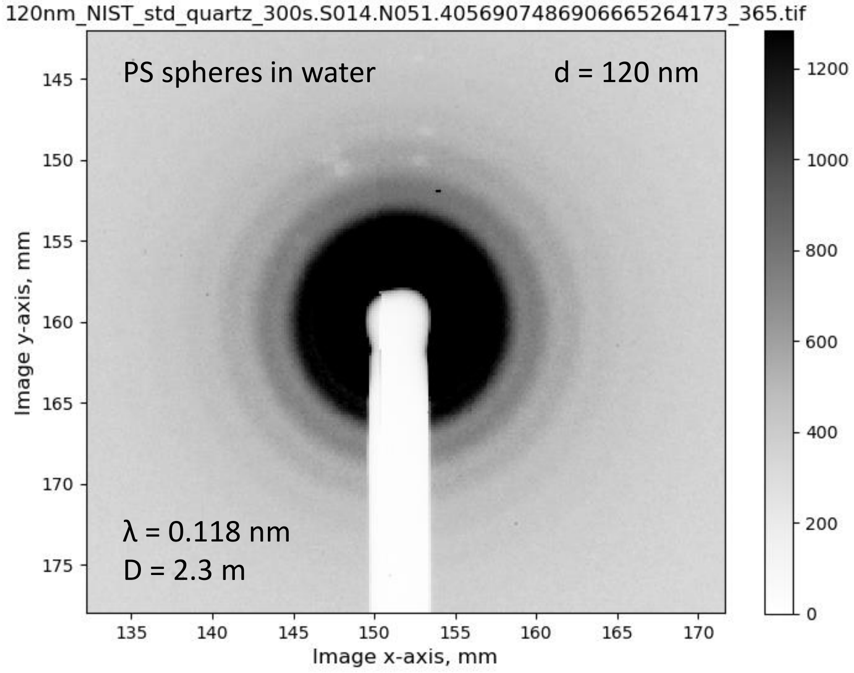
Sample prep is critical !!!

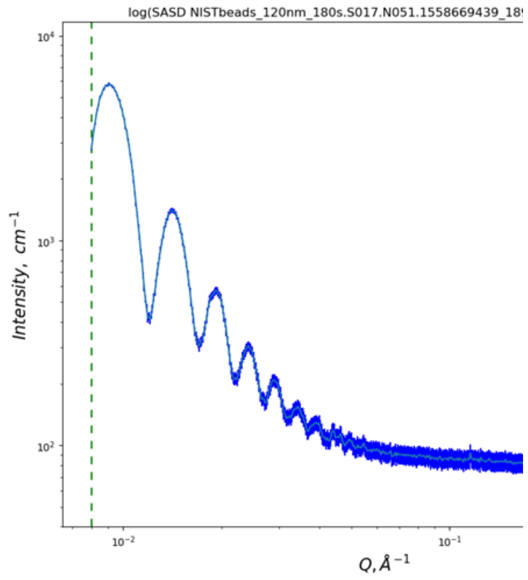
1. Dilute (~0.5 – 10 mg/mL) no interparticle interactions
2. Pure
3. Monodisperse



$$\frac{\lambda}{2sin\theta} = d$$

“Coherent scattering”



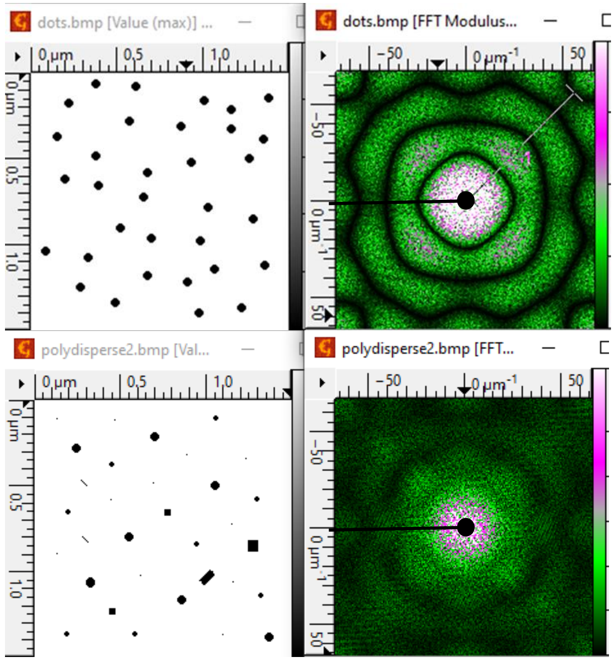


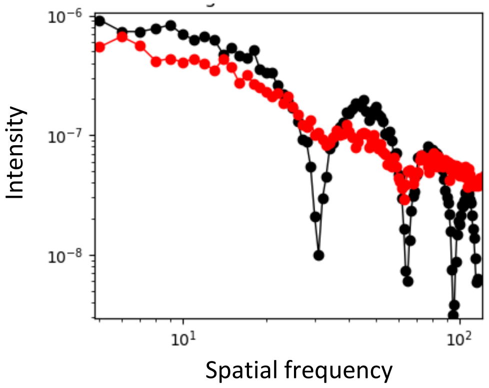
Canadian Light Source | Centre canadien de rayonnement synchrotron

But there are limits!

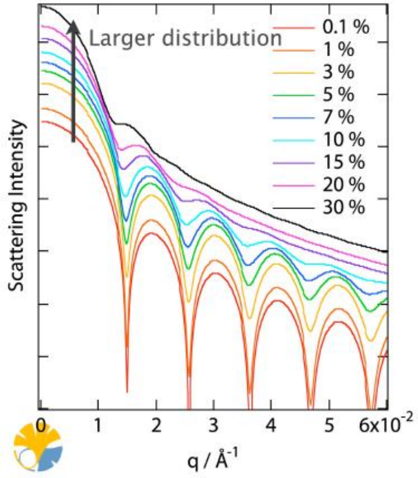
Solutions must be monodisperse, and pure

Simulated sample → FFT → Simulated SAXS detector image





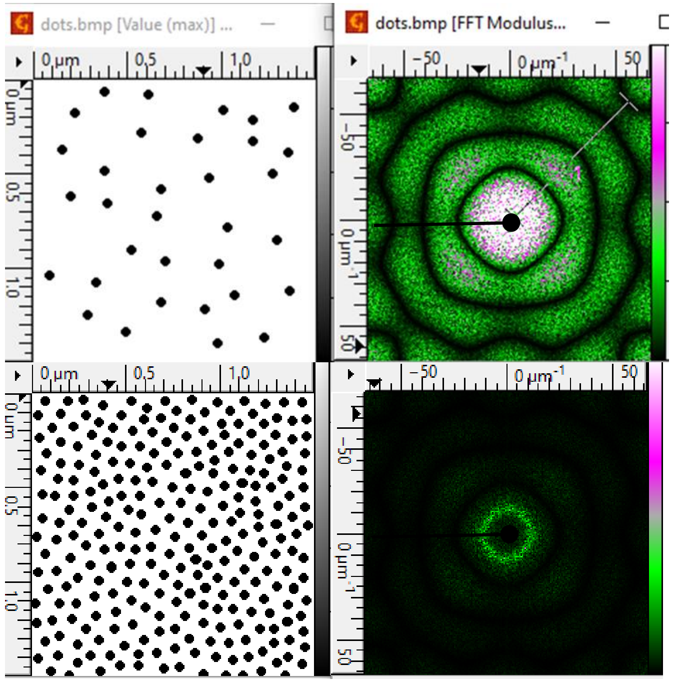
Polydispersity

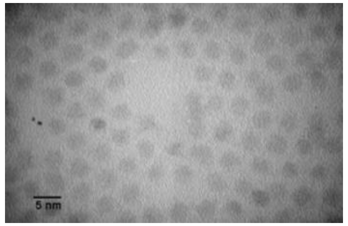


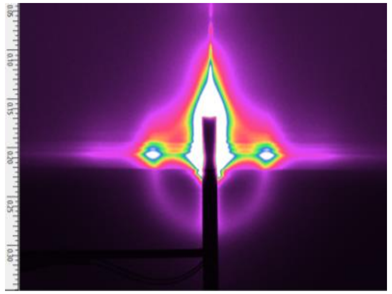
Form factor oscillations smear out when size polydispersity is ≥15%

Solutions must be dilute: No interparticle effects

~1 – 10 mg/mL… Bring many concentrations







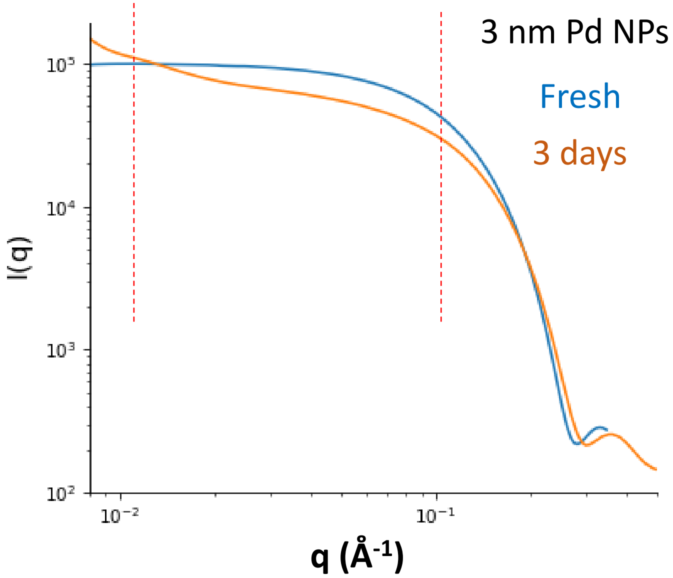
M. Yuan et al., Adv. Mater. 2014, 26, 3513–3519

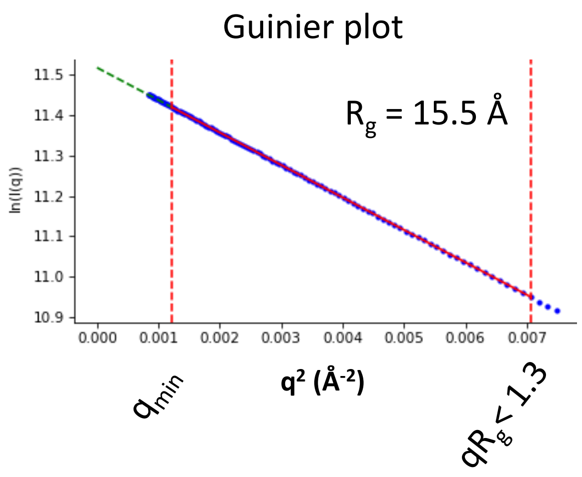
Canadian Light Source | Centre canadien de rayonnement synchrotron

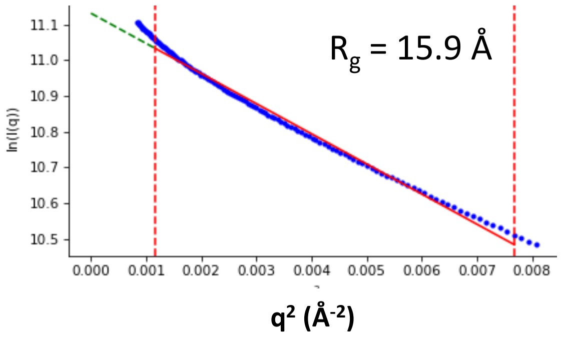
Form factor data analysis: Catalytic nanoparticles

Guinier's approximation: $I(q) \approx I(0)e^{-q^2 R_g^2/3}$

R_g: Radius of gyration
I(0): Intensity at q=0







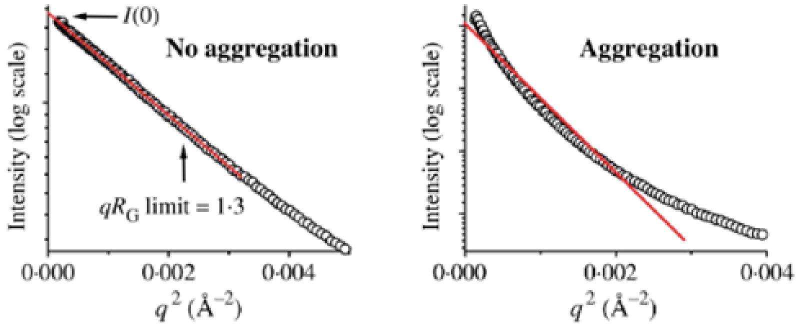
Rg is the square root of the average squared distance of each scatterer from the particle center

RMS distance of the objects parts from its center of mass

- Particle radius

Require no assumption about the shape or internal structure of the particle

- Sample quality: non-linearity can indicate aggregation, repulsion, radiation damage

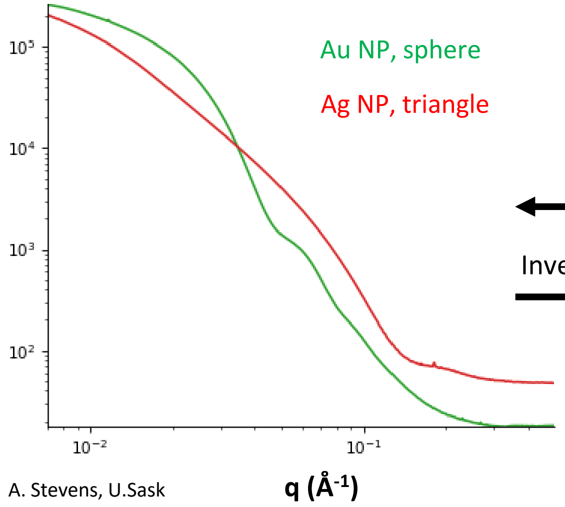


D. Putnam et al. Quart. Rev. Biophys. 40, 191-285 (2007)

Canadian Light Source
Centre canadien de rayonnement synchrotron

Form factor data analysis: Catalytic nanoparticles

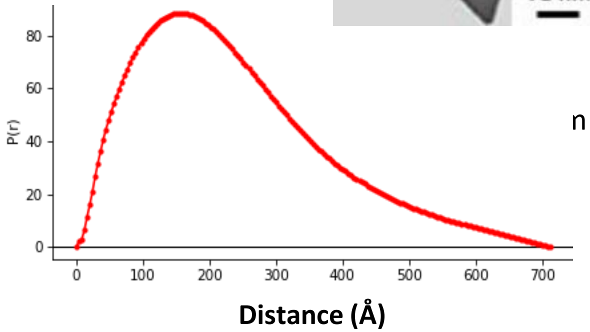
Pair distance distribution function (PDDF)

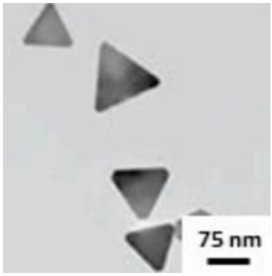


A. Stevens, U.Sask

FT

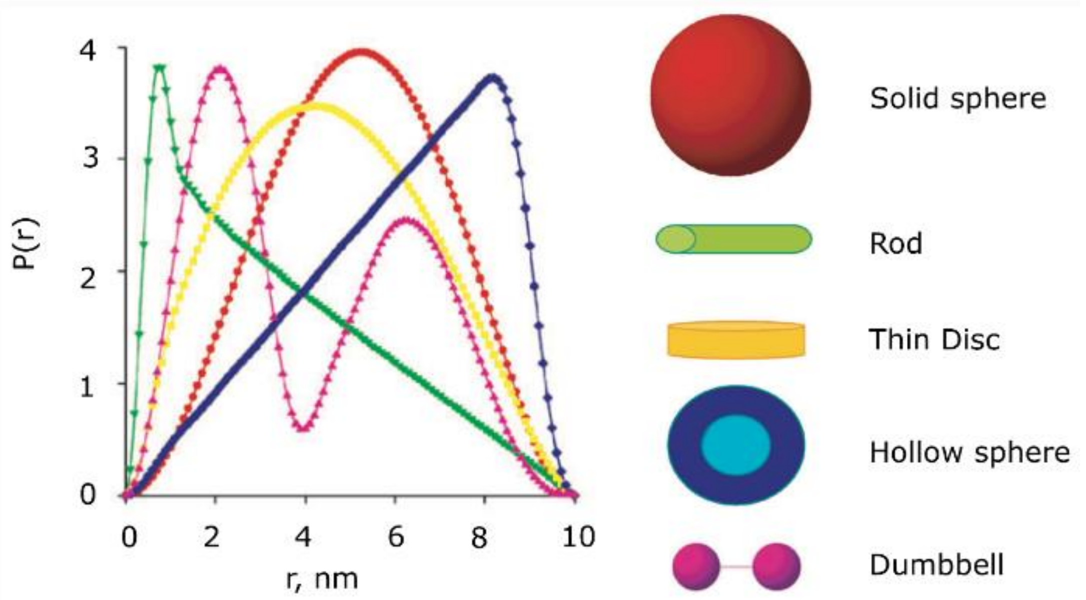
Inverse FT





n

Histogram of atom-atom distances within a single particle



D. Svergun, M.H.J. Koch, Rep. Prog. Phys. 66 1735 (2003)

D_{max} - Maximum dimension of the particle

Shape of the P(r) function is related to the shape of the particle, with low spatial resolution

Canadian Light Source   Centre canadien de rayonnement synchrotron

Optimized Chitosan-Based Nanoemulsion Improves Luteolin Release

C. Diedrich, I.C. Zittlau, N.M. Khalil, A.F.G. Leontowich, R.A. de Freitas, **Ildiko Badea**, R.M. Mainardes

Pharmaceutics **15**, 1592 (2023)

College of Pharmacy and Nutrition
U. Saskatchewan

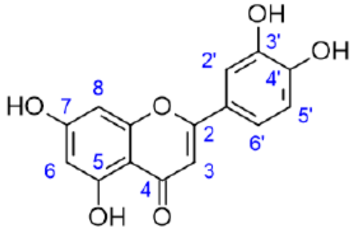
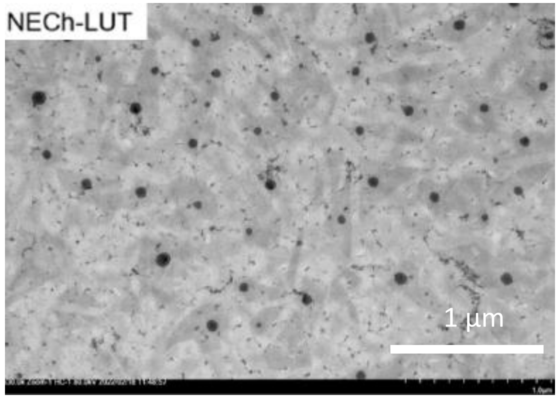
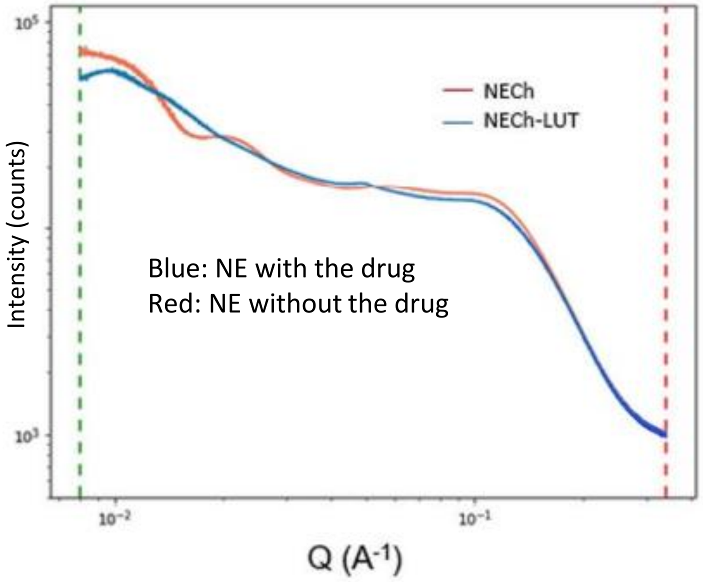


Figure 1. Luteolin structure.

Anti-oxidant, -inflammatory, -tumor, -viral
Poor absorption after oral administration, limited water solubility

Nano-encapsulation might improve the solubility of luteolin?

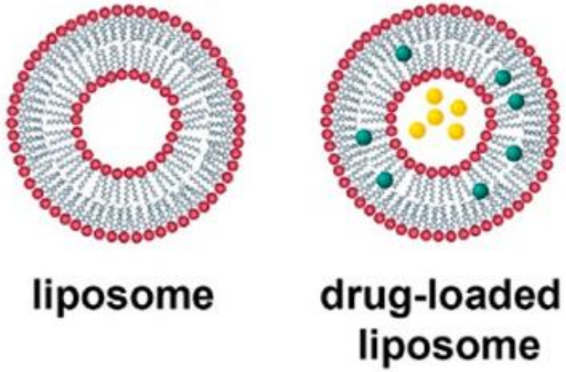




Blue: NE with the drug
Red: NE without the drug

Diameter: 20 – 200 nm
Bilayer: ~5 nm

Delivery vehicle of the COVID-19 mRNA vaccines by Moderna and Pfizer/BioNTech



The most reasonable fit was obtained modeling two spherical droplets

d = 27.0 ± 2.6 nm	Liposomes
d = 1.4 ± 0.26 nm	Tween 20 micelles

Where is luteolin?

Oil phase: Luteolin, oleic acid, with ethylene glycol and Tween 20 as surfactants
Aqueous: Chitosan solubilized in 0.25% acetic acid.

1. **Dilute (~0.5 – 10 mg/mL), no interparticle interactions**
2. **Pure**
3. **Monodisperse**

Sample prep is critical !!!

Drop aqueous into oil while sonicating

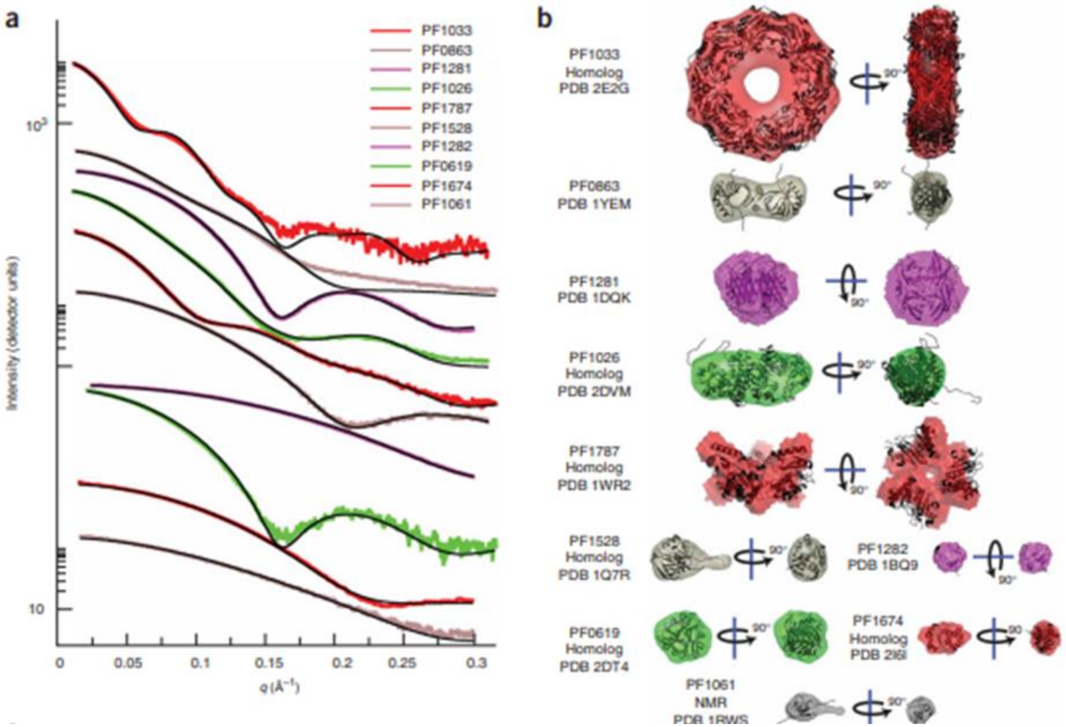
Canadian Light Source
Centre canadien de rayonnement synchrotron

Proteins in solution

Aid structure determination
- Size and low resolution shape
- Molecular weight (>10%) to verify the oligomeric state

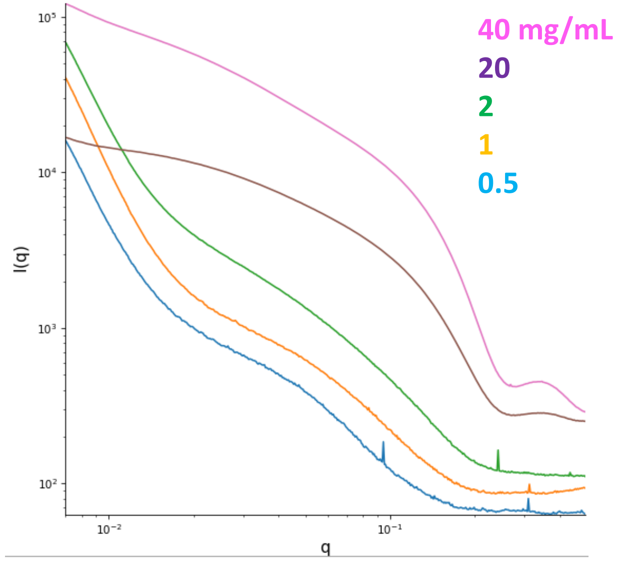
Studies of crystallization (nucleation vs. precipitation)

Challenging !!
- Limited amounts of material
- Weakly scattering
- Radiation damage

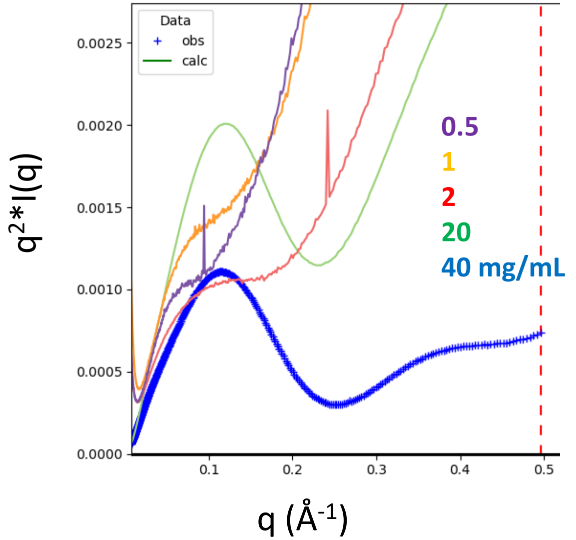


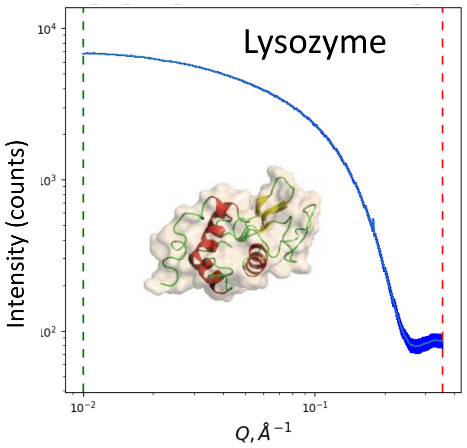
G.L. Hura et al., *Nature Methods* **6**(8), 606 (2009)

Limit of detection?

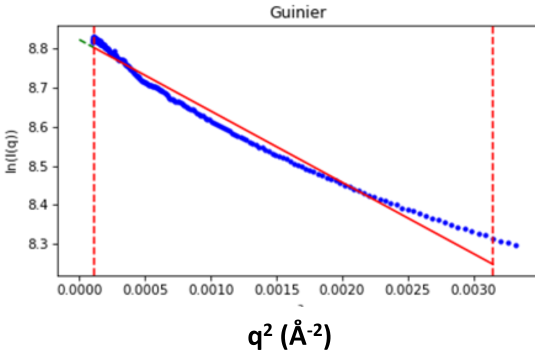


Kratky plots (degree of unfolding)





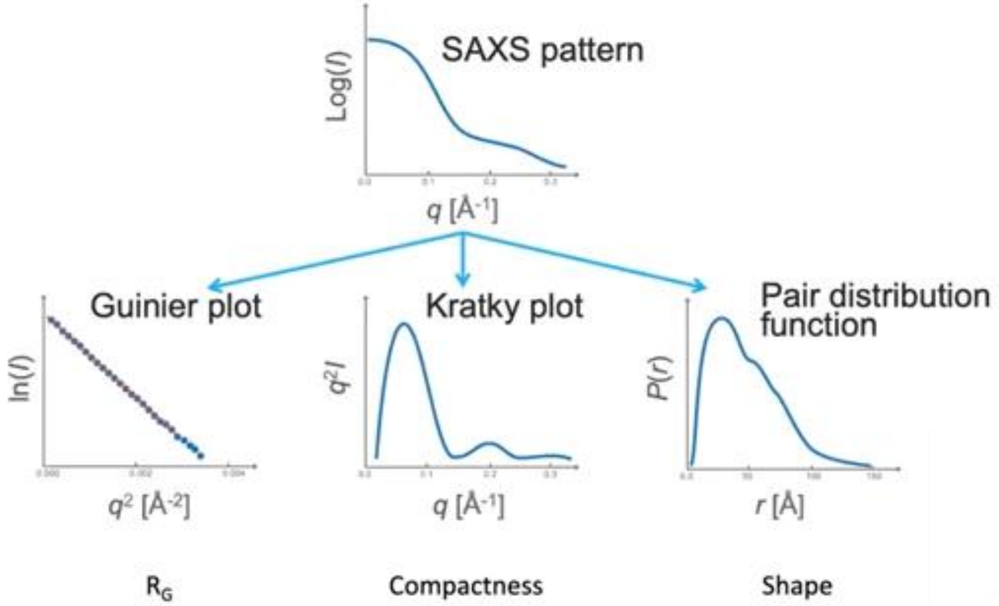
April 2023



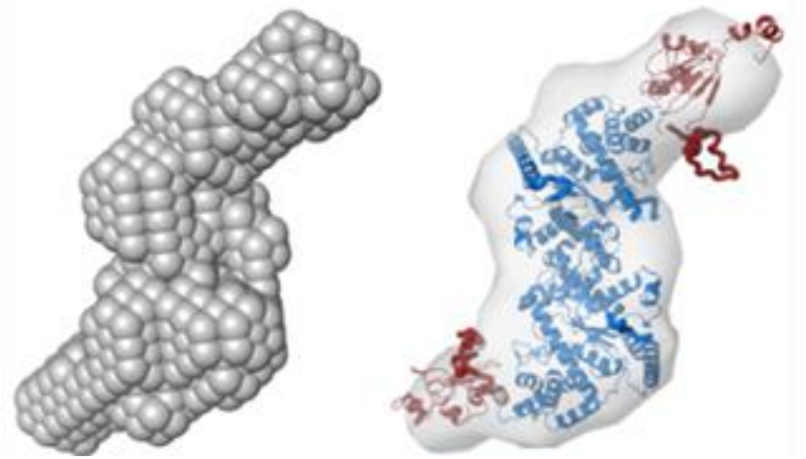
Canadian Light Source
Centre canadien de rayonnement synchrotron

I(Q) = S(Q) × P(Q)

P(Q) is the **form** factor
- *Intra*-particle interferences
- Low concentration (dilute limit)
- Size/polydispersity/shape of particles



These provide boundary conditions for modelling programs



ATSAS package
DAMMIF
Irena

SAXS combined with crystallography and computation 263

Table 5. *SAXS parameters for data validation and interpretation*

Parameters	Assessment
Experimental	
q-range	Range must be suitable through the entire spatial resolution required for determined models
Guinier plot	Non-linear behavior indicates aggregation or inappropriate q-range
R_G	Consistency of extracted R_G with multiple methods (Table 1) increases confidence in not only R_G but also assigned D_{max}
$I(0)$	Should correlate with molecular weight and concentration
D_{max}	Proper description of the range of D_{max} for well behaved $P(r)$ functions
$P(r)$	High frequency oscillations or discontinuities in $P(r)$ may indicate problematic Fourier transform process

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

Summary

The CLS has a SAXS endstation available and our user community is growing!

Probe relatively big things (1 - 150 nm) with X-rays by measuring elastic scattering at small angles

Can learn about 1) packing, 2) particle size, and 3) particle shape, but generally not all 3 at the same time

Limited structural information

Complementary information, that should be supported with supplementary techniques such as microscopy

Further reading

Basic SAXS concepts

H. Schnablegger, Y. Singh, “The SAXS Guide: Getting Acquainted With the Principles”, Anton Parr, Austria. (2017)

Making a good measurement

B.R. Pauw, “Everything SAXS: small-angle scattering pattern collection and correction” J. Phys.: Condens. Matter 25 (2013) 383201

Basic data work up

J.B. Hopkins, R.E. Gillilan, S. Skou
https://bioxtas-raw.readthedocs.io/en/latest/saxs_tutorial.html

BioSAXS, data workup, introduction to structure modelling

C.D. Putnam, M. Hammel, G.L. Hura, J.A. Tainer, “X-ray solution scattering (SAXS) combined with crystallography and computation: defining accurate macromolecular structures, conformations and assemblies in solution”
Quarterly Reviews of Biophysics 40, 3 (2007), pp. 191–285.

The Brockhouse Sector Team

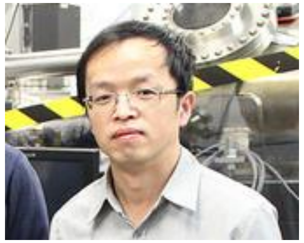
Stefan Kycia
Professor, U. Guelph
Beamteam Leader

Al Rahemtulla

Chang-Yong Kim

Narayan Appathurai

Beatriz Diaz Moreno
Beamline Responsible
Beatriz.Moreno@lightsource.ca



Feizhou He
Mat. Chem. Sci. Manager

Joel Reid

Graham King

Adam Leontowich
Adam.Leontowich@lightsource.ca

https://brockhouse.lightsource.ca

Call for General User Proposals closes August 23, 2023 at noon (Sask. time)

Canadian Light Source Centre canadien de rayonnement synchrotron