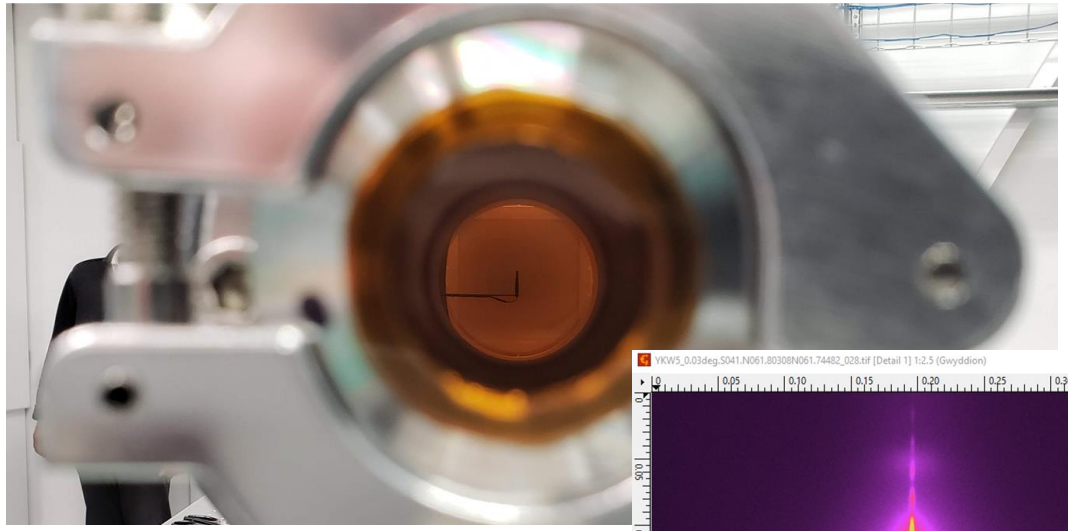
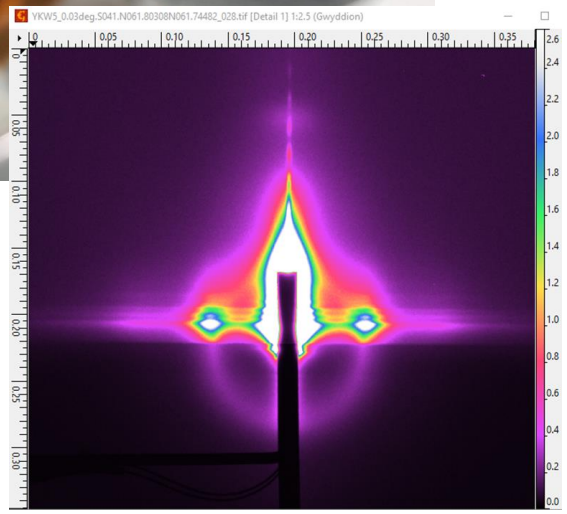
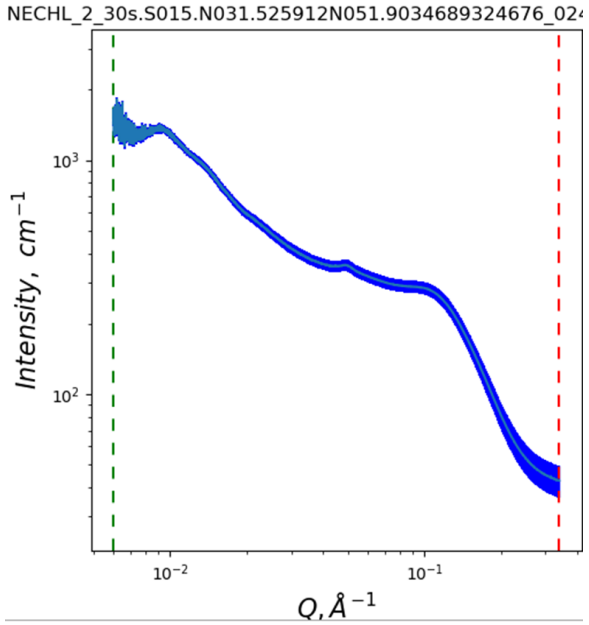
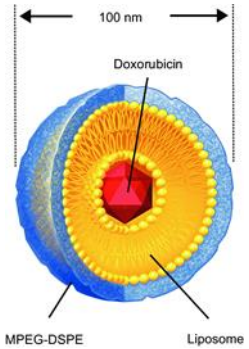
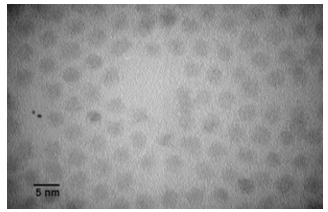
An Introduction to Small Angle X-ray Scattering (SAXS)











CLS XRD school, August 18, 2022

Adam Leontowich
Associate Scientist
Canadian Light Source

Brockhouse X-ray Diffraction and Scattering (BXDS) sector

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

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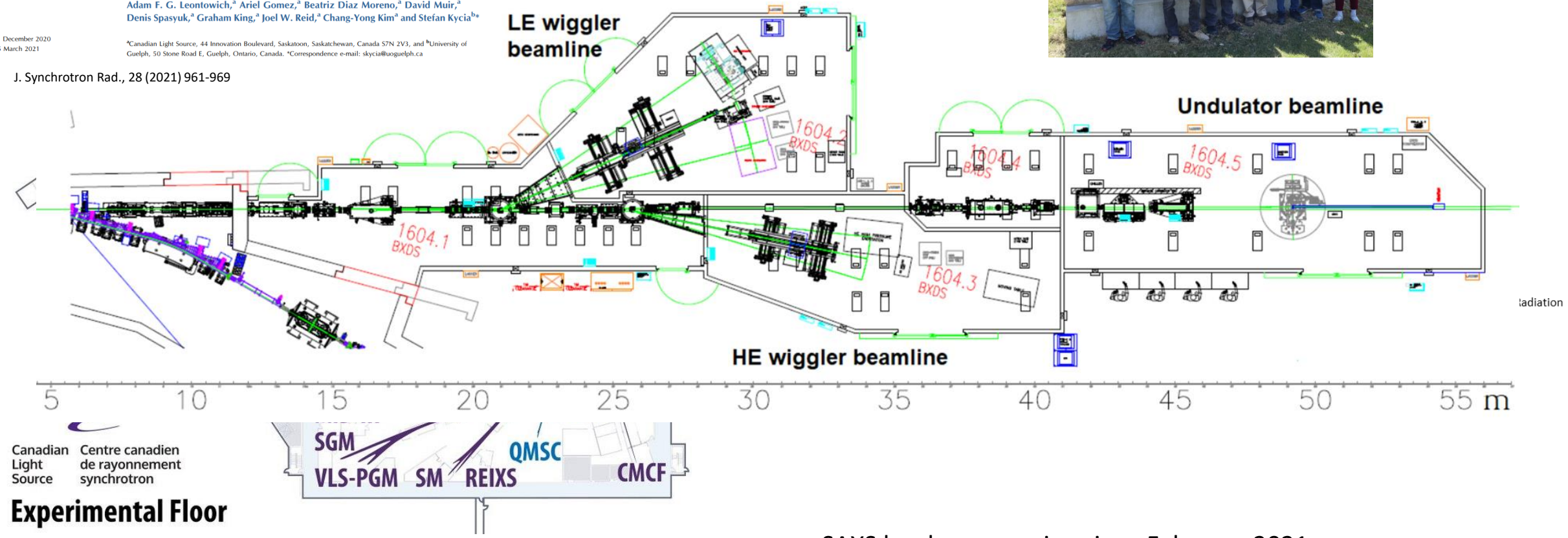
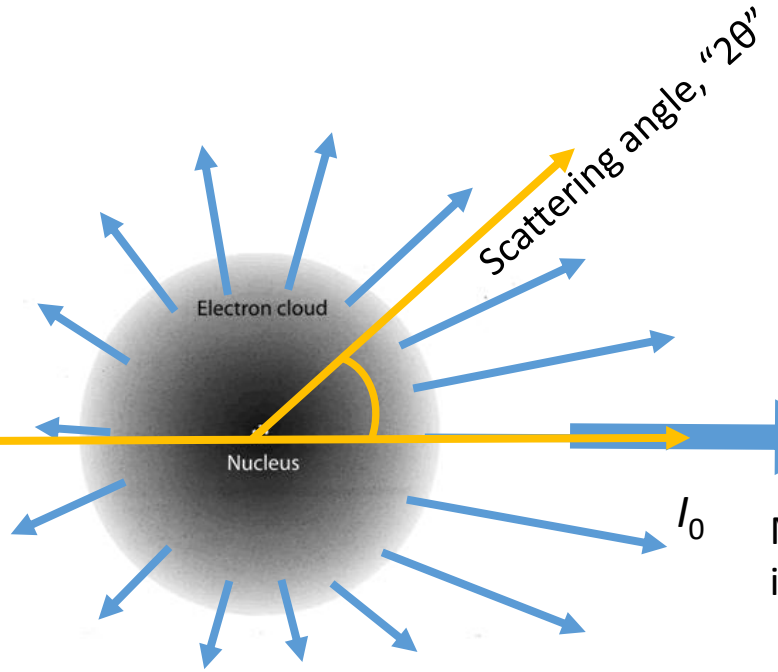


SAXS has been running since February 2021
No dedicated CLS SAXS beamline

X-ray scattering

Collimated, monochromatic X-rays

I



I_0

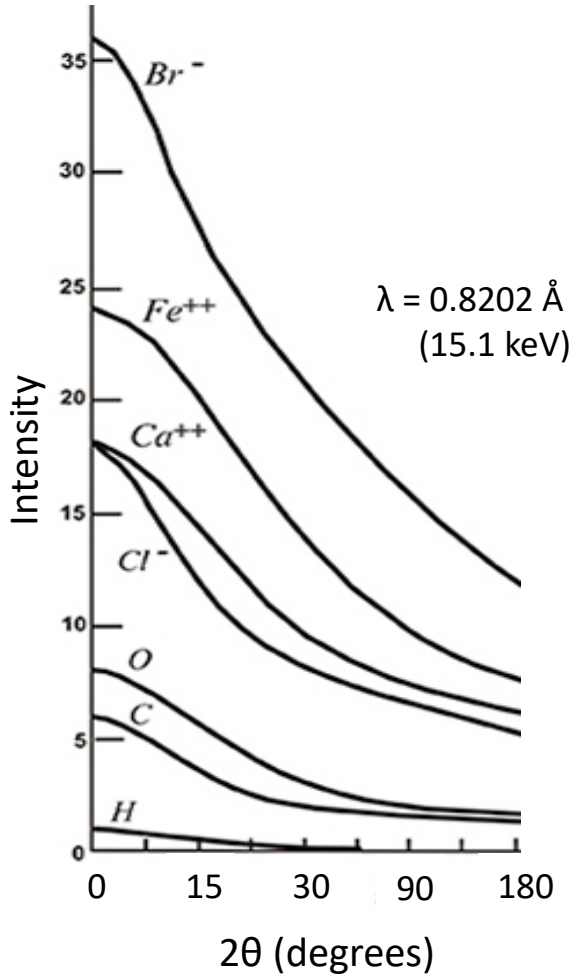
Measure the scattered intensity at every 2θ

Photoabsorption
"Elastic" scattering

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

no wavelength change

Atomic scattering factors



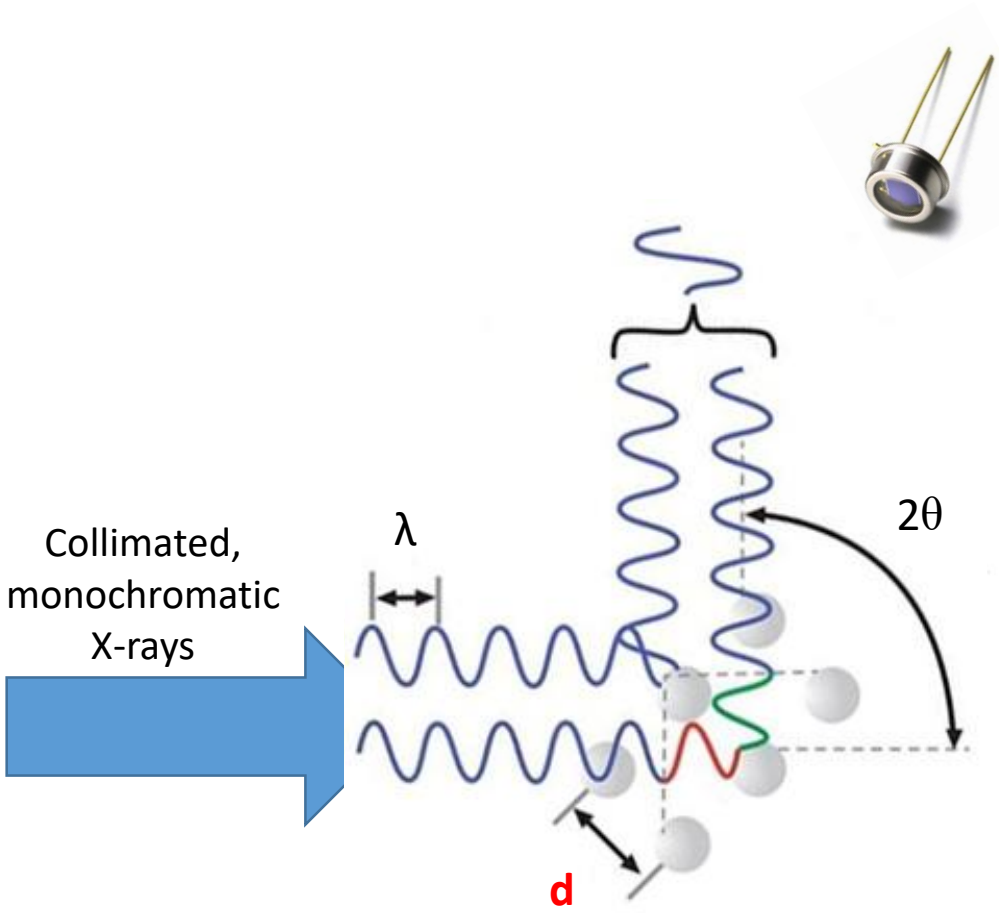
λ = 0.8202 Å
(15.1 keV)

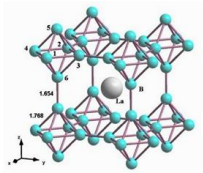
From scattering to diffraction

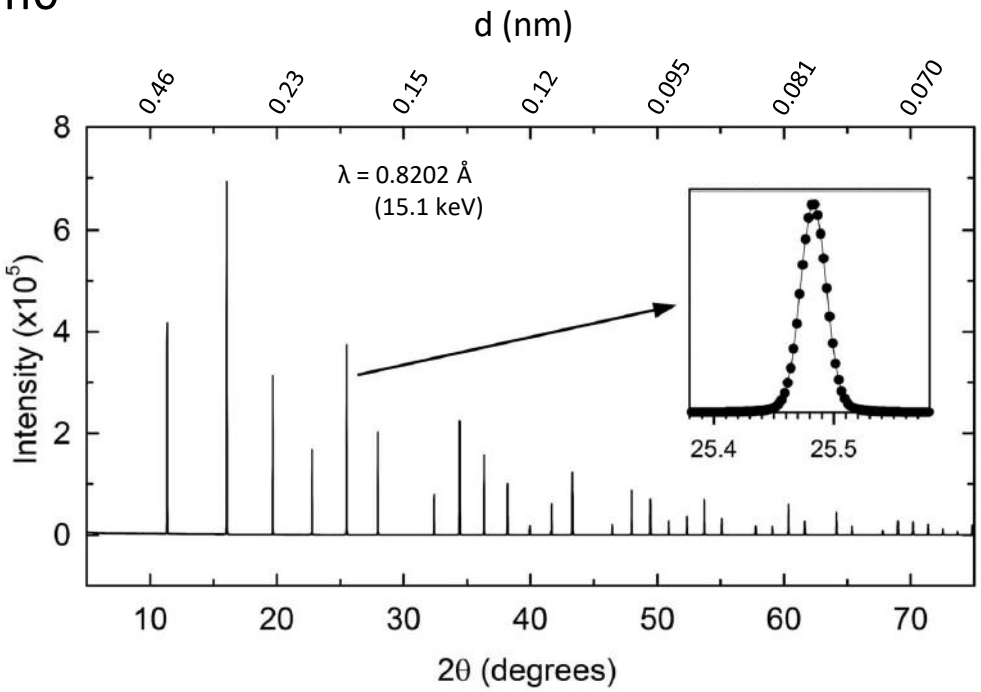
"Bragg" diffraction

Constructive interference channels
scattered intensity into specific directions

$n\lambda = 2d\sin\theta$





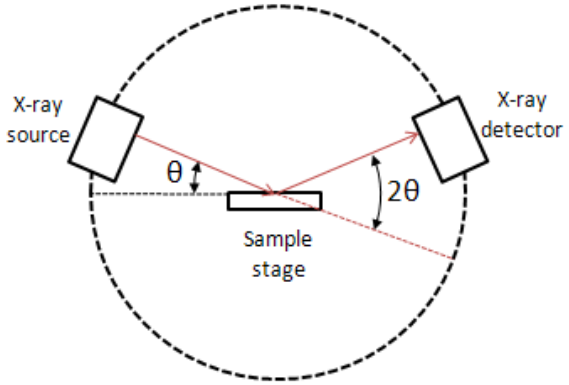


X-ray scattering and diffraction reveals structural order within materials
on the atomic to >150 nm length scale

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

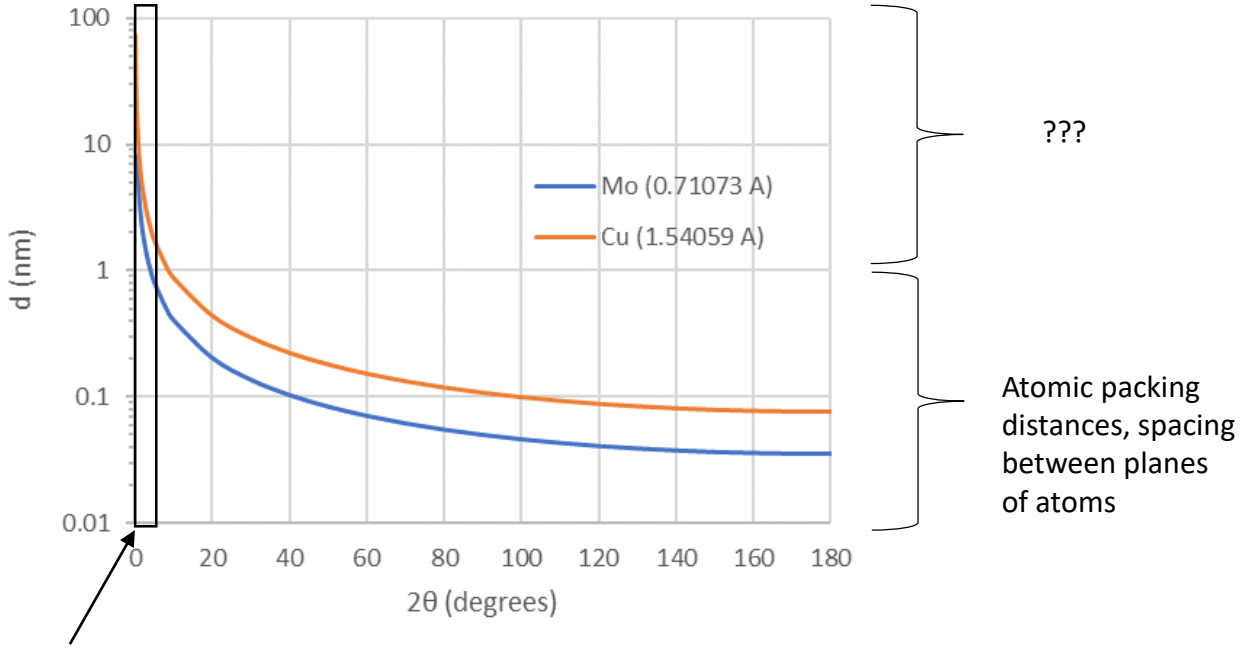
Starting from powder diffraction…

λ = 1.54059 Å (Cu Kα)
0.71073 Å (Mo Kα)



Reveal structural order within materials

$$n\lambda = 2d\sin\theta$$

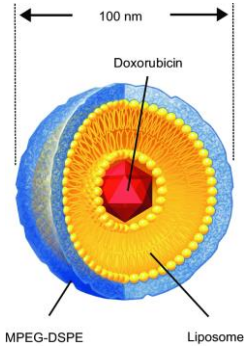


SAXS is generally 2θ ≤ 5°

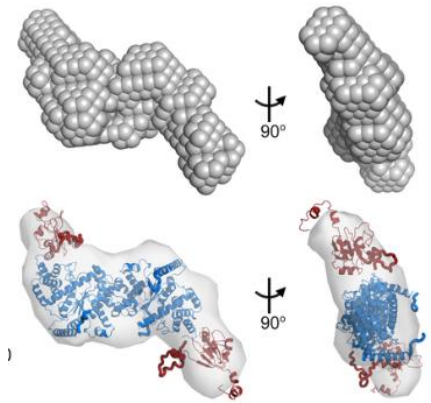
Measuring small X-ray scattering angles to probe relatively big things (1 - 150 nm)

Applications

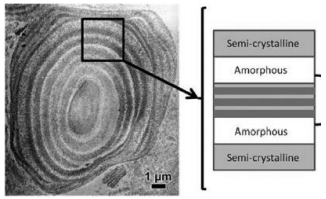
Drug delivery, Pharmaceuticals



Proteins

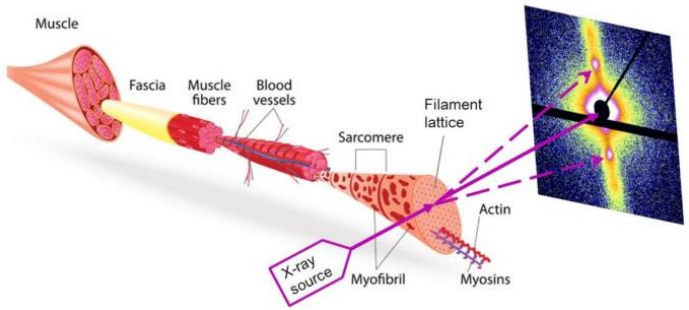


Food science



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

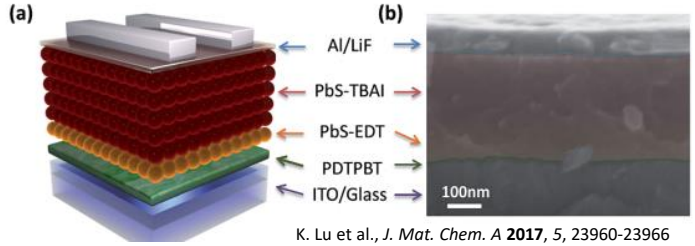
Biomacromolecules



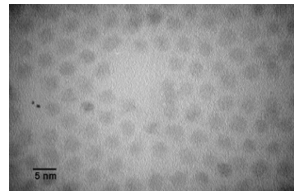
https://www.techexplorist.com/scientists-investigated-pork-fillet-x-ray-light/11572/

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

All kinds of nanoparticles

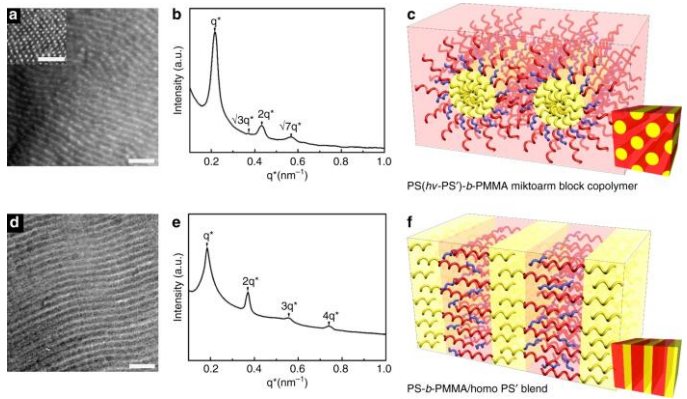


K. Lu et al., *J. Mat. Chem. A* **2017**, *5*, 23960-23966



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

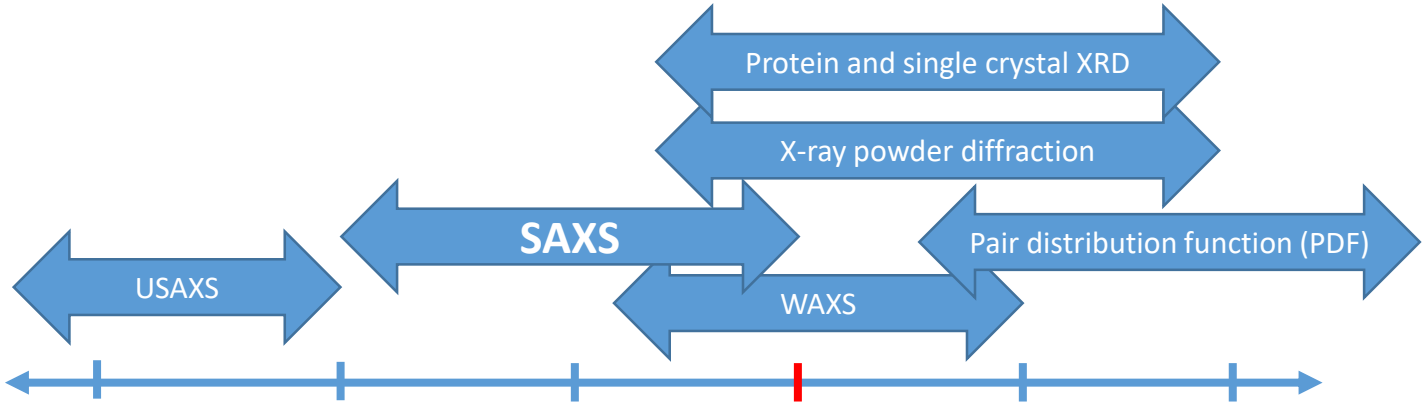
Polymers



Nature Comm. 8, 1765 (2017)

Canadian Light Source | Centre canadien de rayonnement synchrotron

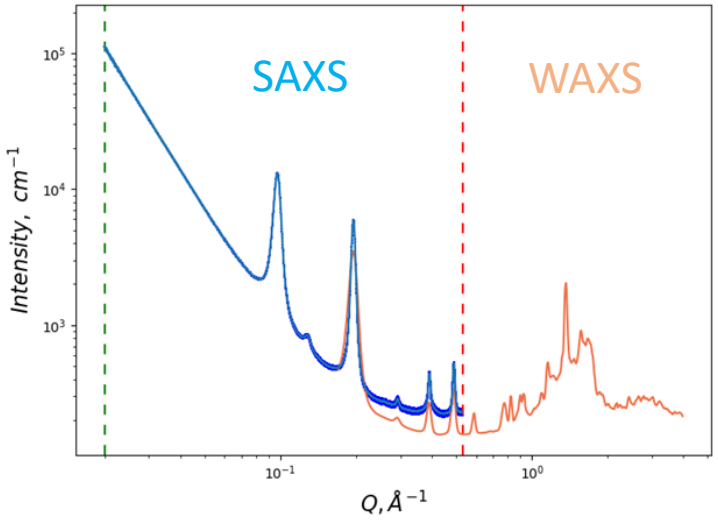
How is SAXS related to other X-ray diffraction techniques?



0.0045	0.045	1	5	45	80	**2θ (deg)**
0.0000785	0.000785	0.0174	0.0873	0.785	1.4	**2θ (radians)**
1042	104	4.69	0.938	0.106	0.0637	**d (nm)**
0.000603	0.00603	0.134	0.67	5.87	9.87	**q (Å^{-1})**

λ = 0.819 Å (15.1 keV)

Lindt chocolate sample

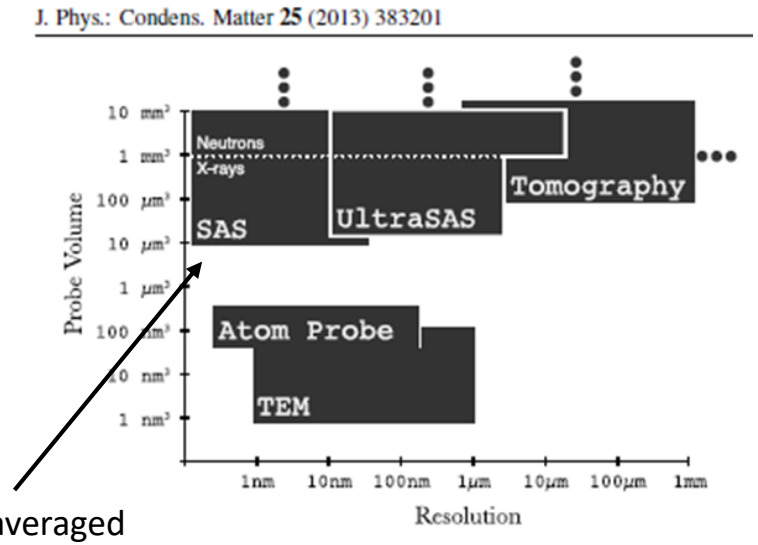


Nano-scale

Inter-molecular distances
Whole particle shape, size, packing arrangement
Macromolecules, polymers, superlattices

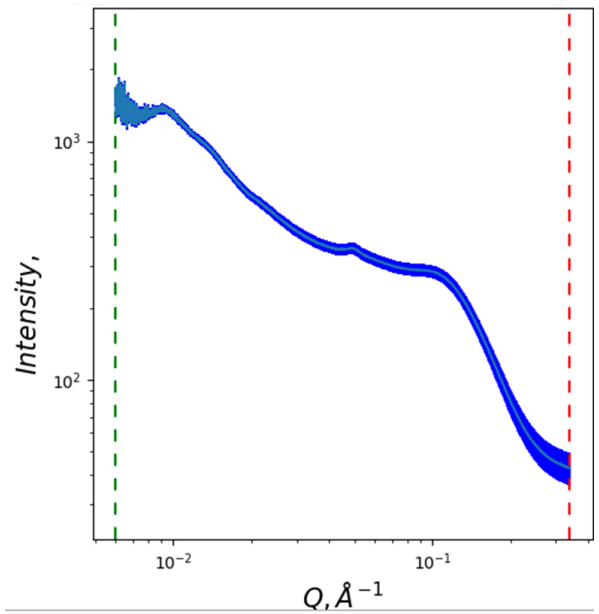
Atomic-scale

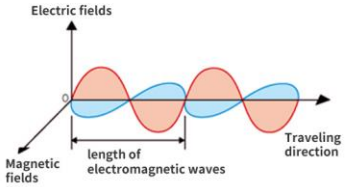
Inter-atomic distances, bond lengths
Nearest neighbor atoms
Crystal structures with atomic resolution
Molecular packing



Nanoscale information averaged over the beam foot print (mm^2)

What is q?





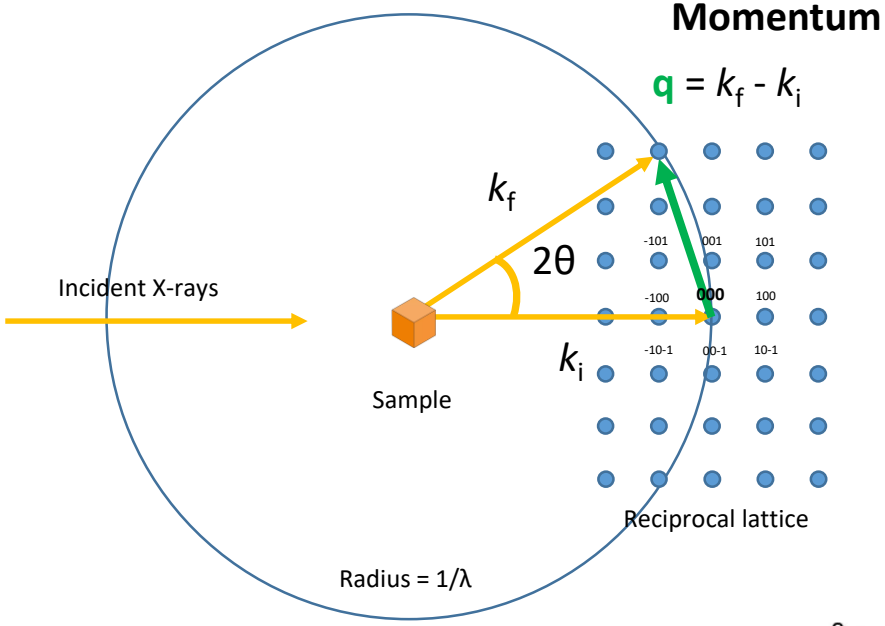
p = 2πħ/λ p is a vector

p increases as *λ* decreases (higher energy)

p = kħ *k* is wavenumber, *k = 2π/λ*

k = ħ/p

Ewald Sphere



Momentum transfer

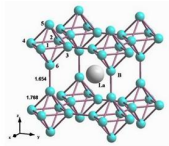
$\mathbf{q} = k_f - k_i$

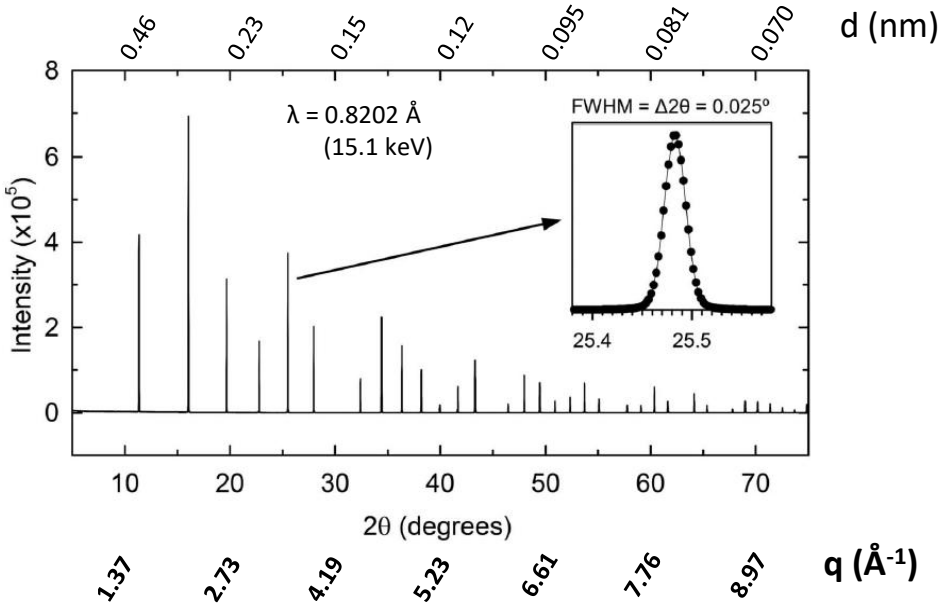
Important relationships

$q = (4\pi\sin\theta)/\lambda$

$q = 2\pi/d$

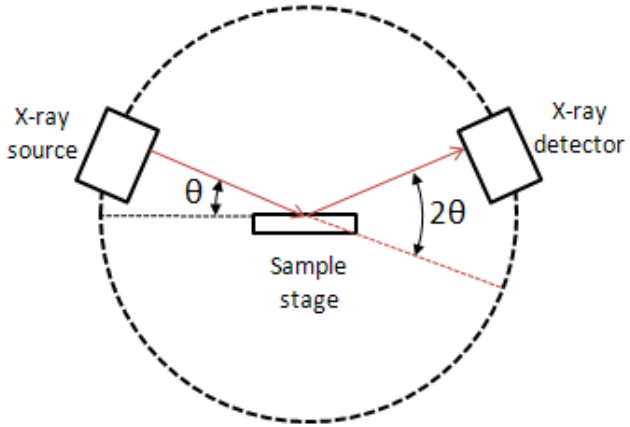
$n\lambda = 2d\sin\theta$





How to measure small angle X-ray scattering?

Starting from powder diffraction…

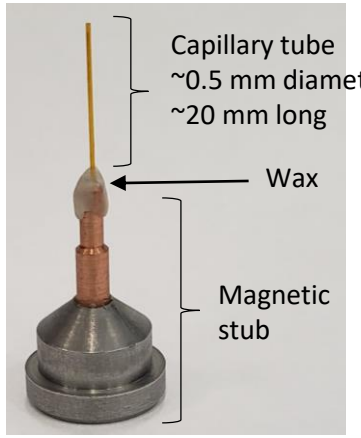


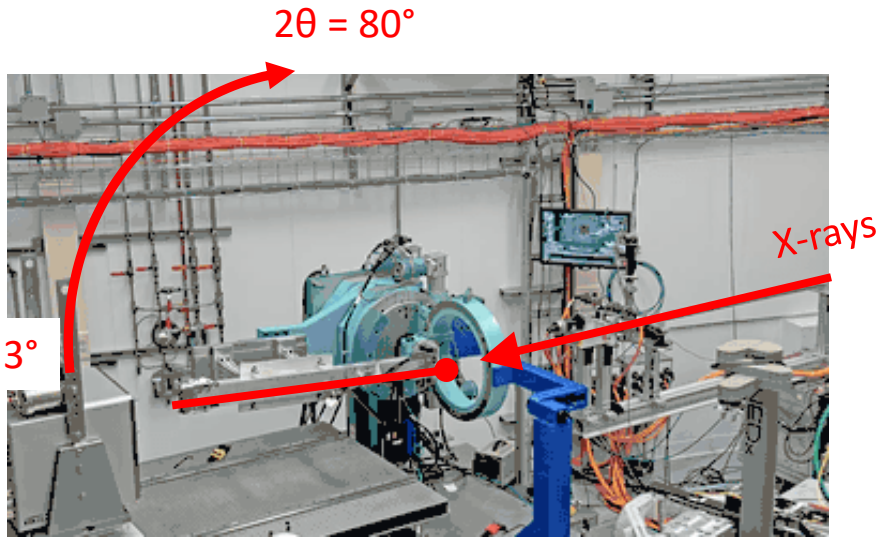
$n\lambda = 2d\sin\theta$

There are also lab-source SAXS instruments
Why do it at a synchrotron?

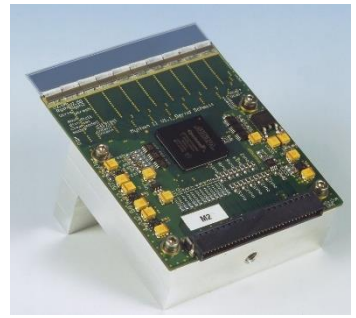
- More flux (better SNR)
- Weakly scattering objects (organics/biology)
- Flexible setups, in-situ experiments
- Choice of many wavelengths
- SAXS mapping

Powder X-ray diffraction (PXRD)

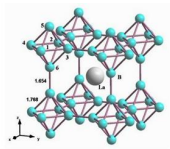


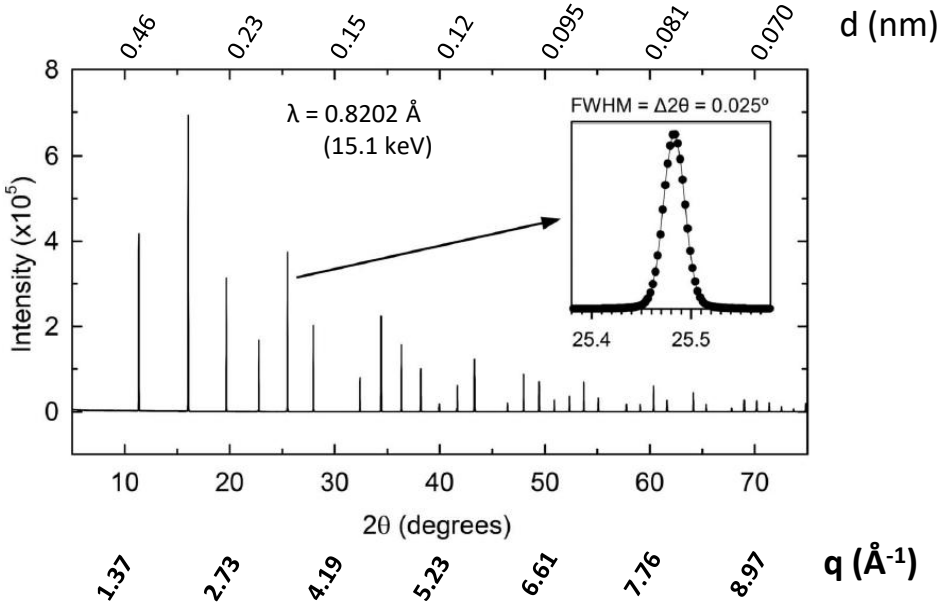


Linear strip detector
1280 strips
50 µm x 8 mm



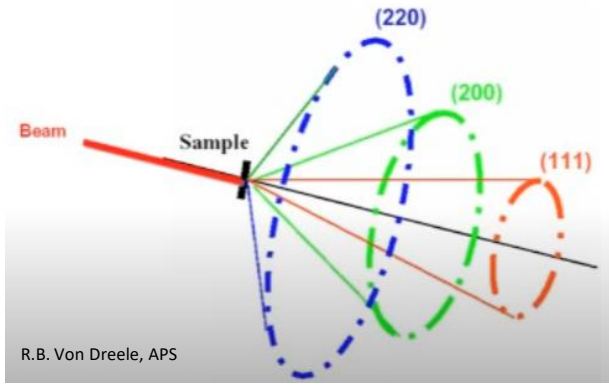
8 detectors and a robot in 2023





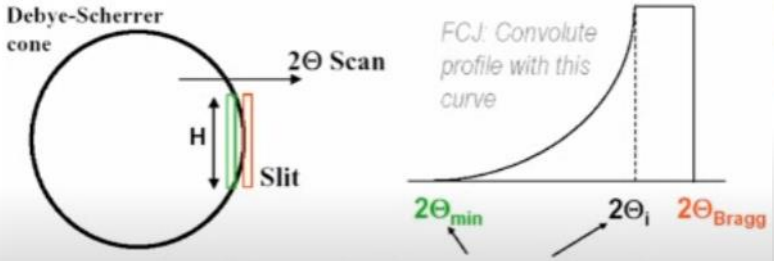
Why not just move the PXRD detector to low angle?

Axial divergence – Low angle peak asymmetry



Axial Divergence (Low Angle Asymmetry)

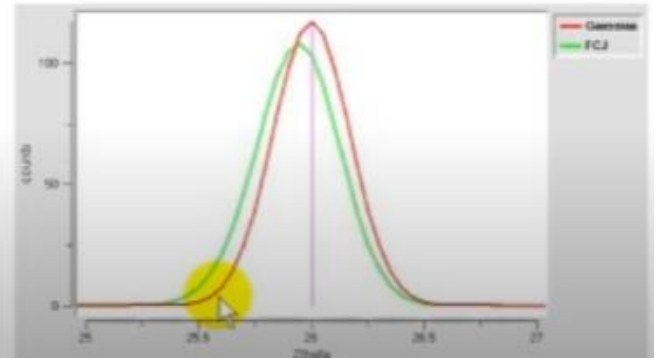
Work of Finger, Cox & Jephcoat, based on derivations by van Laar & Yelon



Depend on slit & sample "heights" wrt diffr. radius
H/L & S/L - parameters in function
(typically 0.005 - 0.020)

- The Finger-Cox-Jephcoat correctly models the effective shift of the peak due to axial divergence.

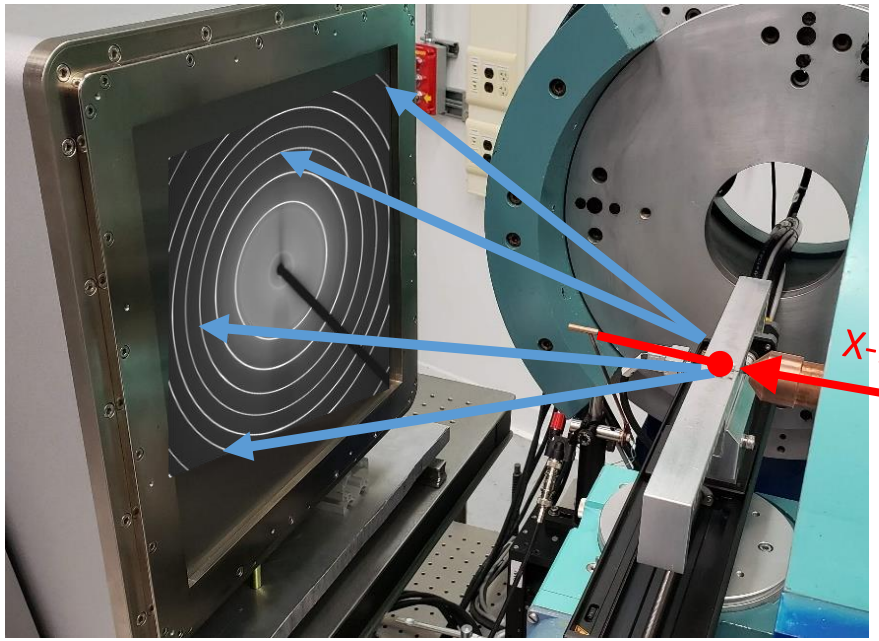
Note: the "competition," the split Pearson VII (empirical), does not model this effect at all!



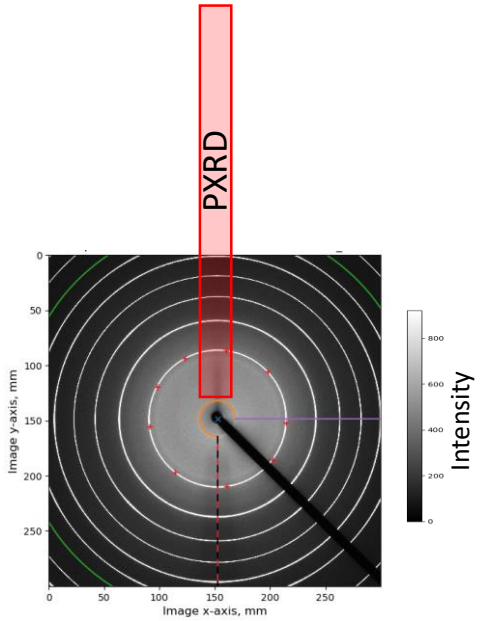
https://www.youtube.com/watch?v=SIz6Ng6UzAw

Need something different…

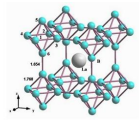
Wide angle X-ray scattering (WAXS)



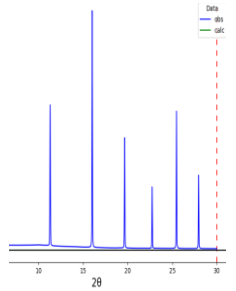
Rayonix MX300 CCD
Small, square pixels
73.242 um x 73.242 um
(4096 x 4096)

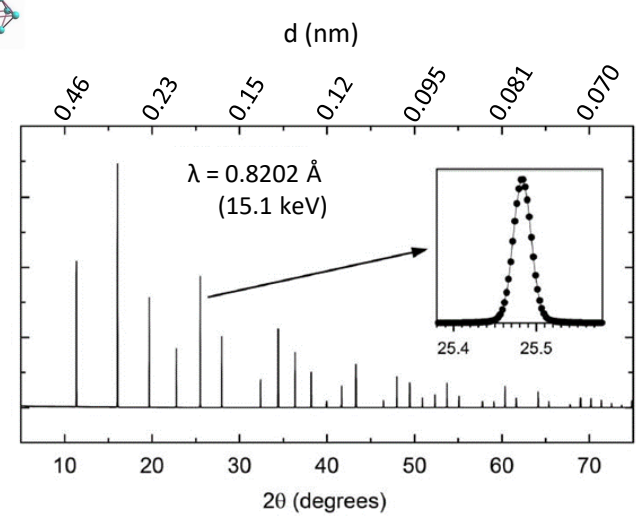


WAXS



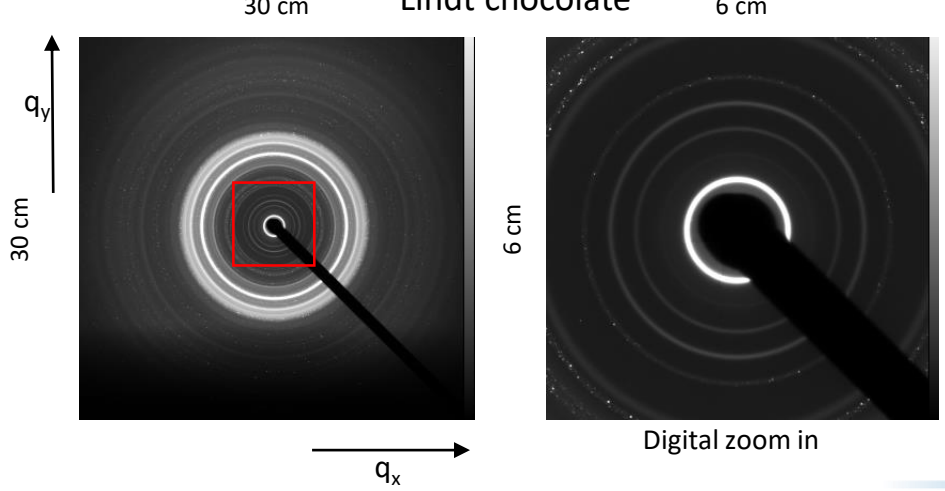
Azimuthal or radial integration



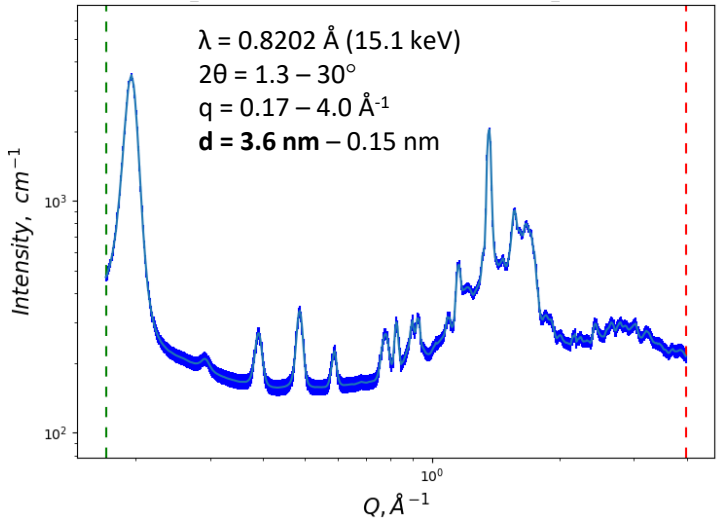


PXRD

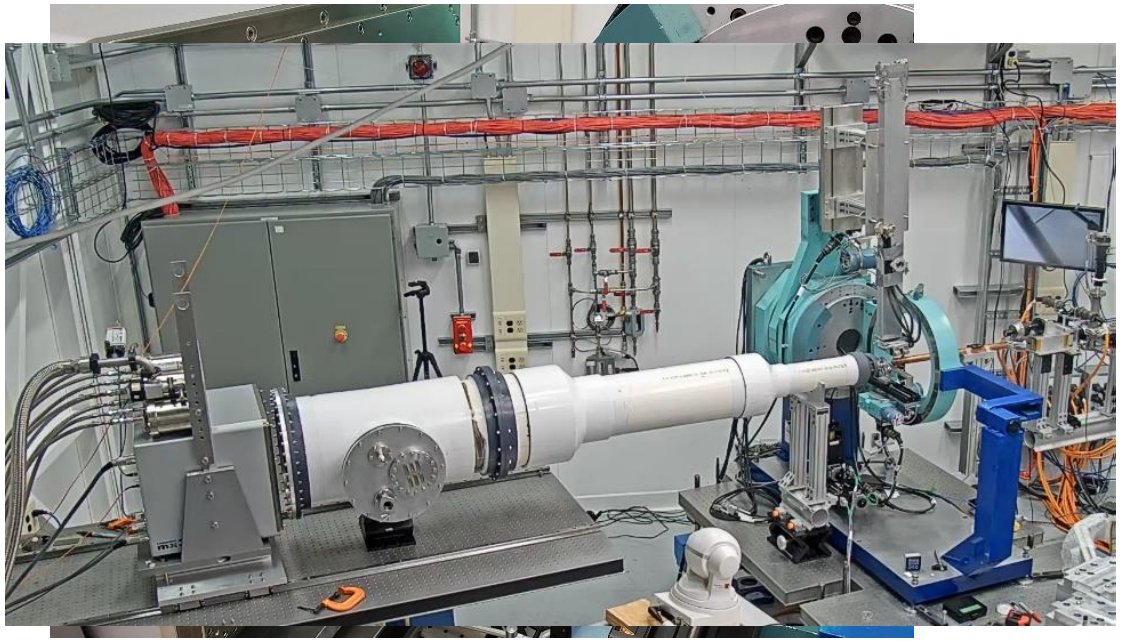
Lindt chocolate



Digital zoom in



Small angle X-ray scattering (SAXS)

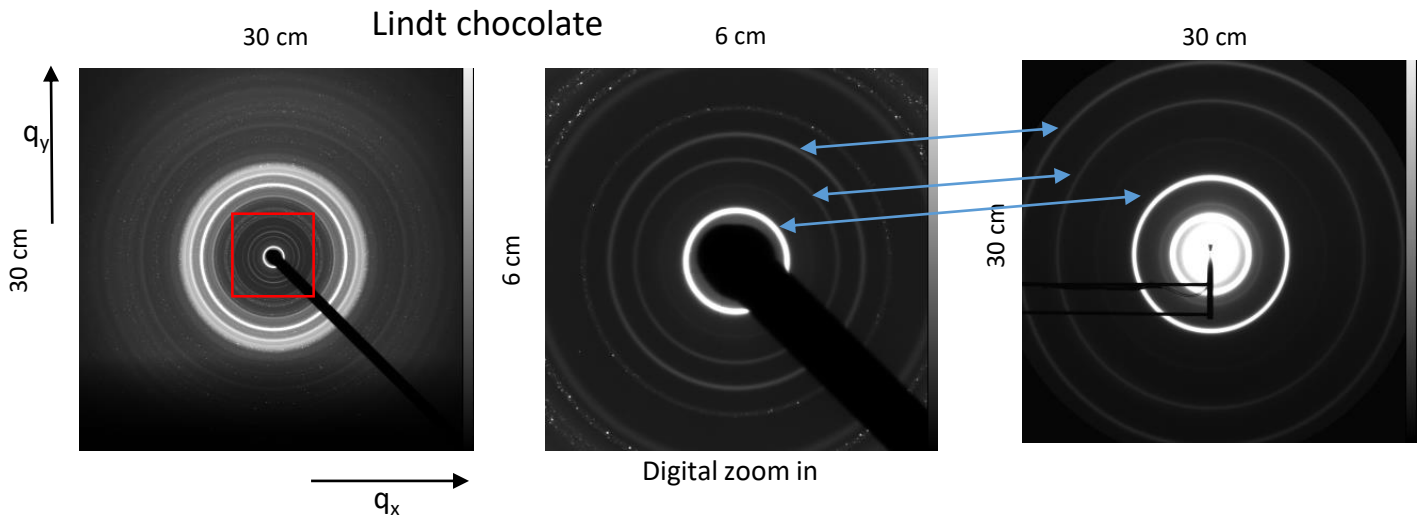


λ = 0.8202 Å (15.1 keV)
D = ~0.3 m

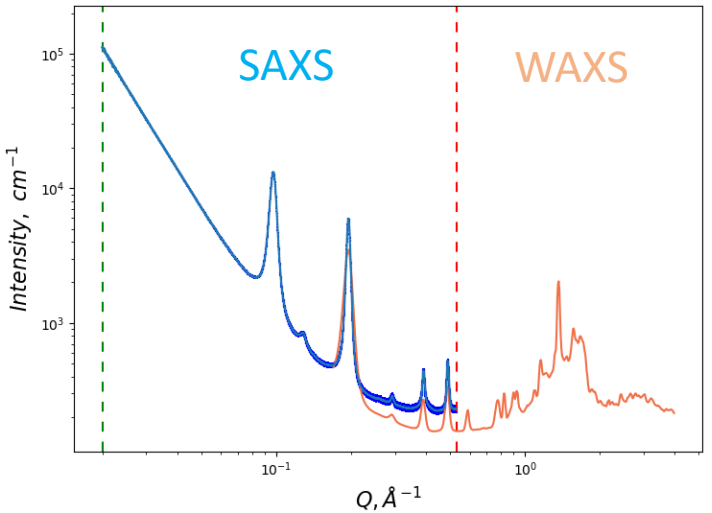
2θ = 1.3 – 30°
q = 0.17 – 4.0 Å^{-1}
d = 3.6 nm – 0.15 nm

λ = 0.8202 Å (15.1 keV)
D = ~2.3 m

2θ = 0.057 – 3.4°
q = 0.008 – 0.45 Å^{-1}
d = 80 nm – 1.4 nm



"SAXS/WAXS"



Fancy SAXS setups

The further back you can get the detector the better!

λ = 1.1817 Å (10.5 keV)
D = ~2.3 m

$2\theta = 0.057 – 3.4^{o}$
$q = 0.005 – 0.32\ Å^{-1}$
d = 118 nm – 2.0 nm

λ = 1.1817 Å (10.5 keV)
D = ~4.0 m

$2\theta = 0.029 – 2.1^{o}$
$q = 0.0027 – 0.20\ Å^{-1}$
d = 230 nm – 3.1 nm

SAPUCAIA, Sirius, Brazil
Dedicated SAXS beamline, June 2023

14 m long x 2 m tall tube

Canadian Light Source | Centre canadien de rayonnement synchrotron

CLS SAXS instrument

Need low divergence …

3 pinhole layout

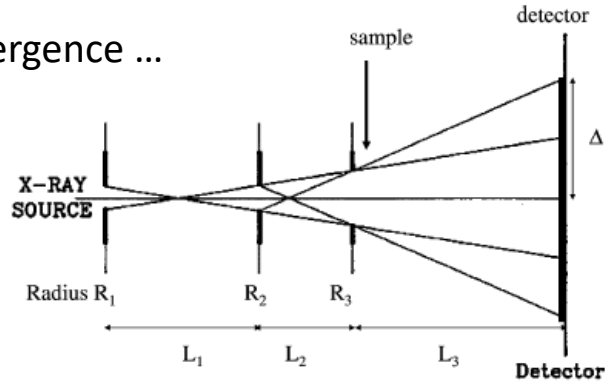
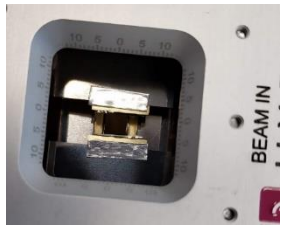
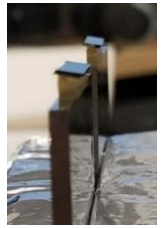


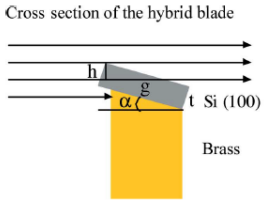
Figure 4
Schematic layout of a SAXS pinhole camera.

J. Appl. Cry

… and low parasitic scatter

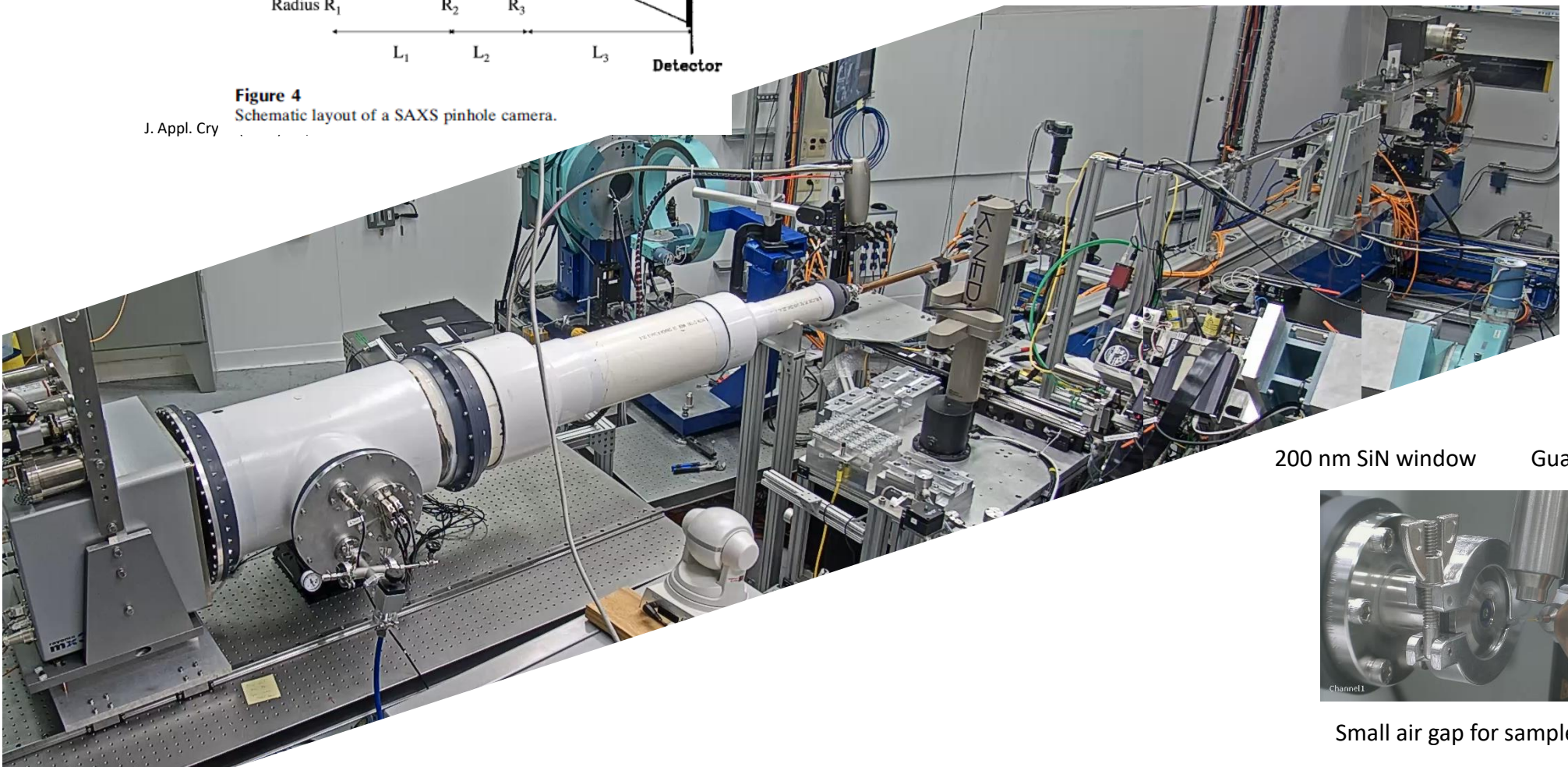






Y. Li et al., J. Appl. Cryst. (2008). 41, 1134–1139
N.M. Kirby et al., J. Appl. Cryst. 46, 1670–1680 (2013)

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



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

200 nm SiN window

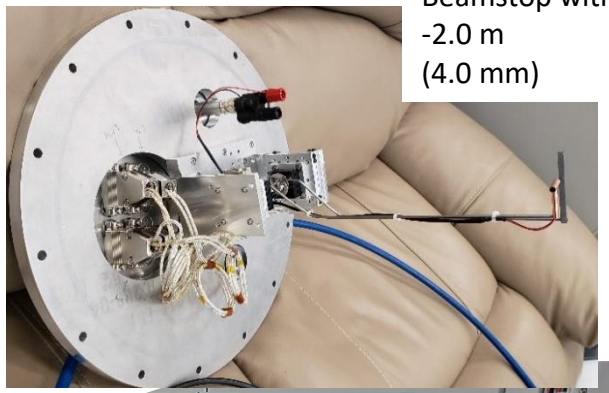
Guard slit (0.80 mm)

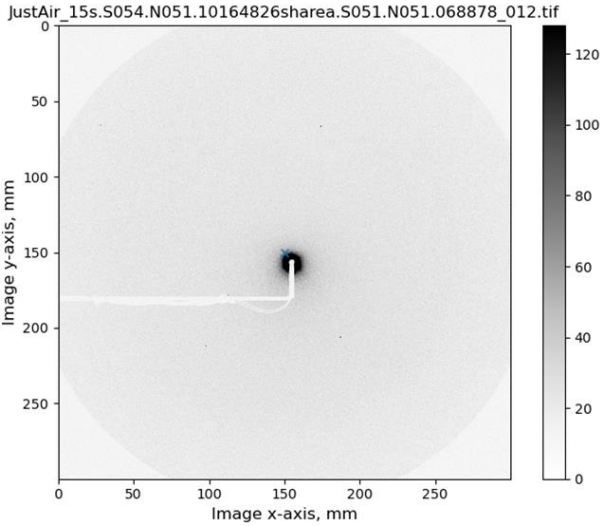
Small air gap for sample (~35 mm)

CLS SAXS instrument

Vacuum tube (≤ 10-2 Torr)
13.5” Kapton window (125 µm)
~2100 pounds

Beamstop with photodiode
-2.0 m
(4.0 mm)





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

~8.5 m long

200 nm SiN window

Guard slit (0.80 mm)

Small gap (~35 mm)

λ = 1.1817 Å (10.5 keV)
D = ~2.3 m

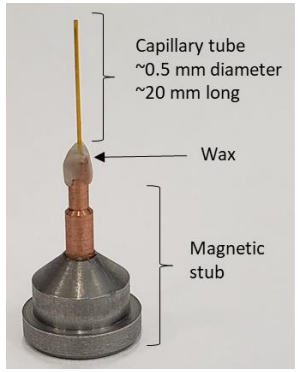
2θ = 0.057 – 3.4°
q = 0.005 – 0.32 Å^{-1}
d = 118 nm – 2.0 nm

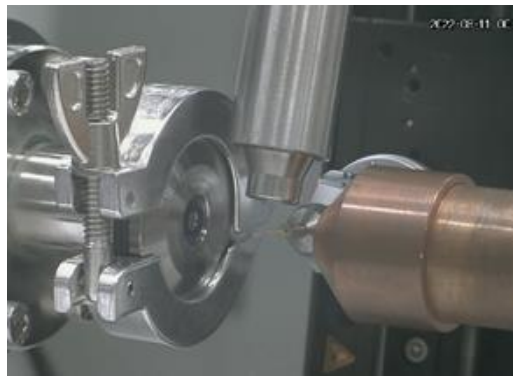
Sample preparation, data collection, and data work up

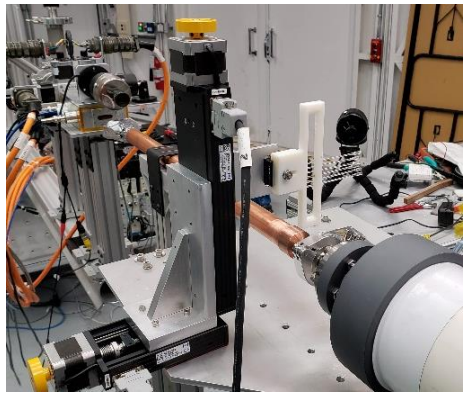
Sample preparation and mounting

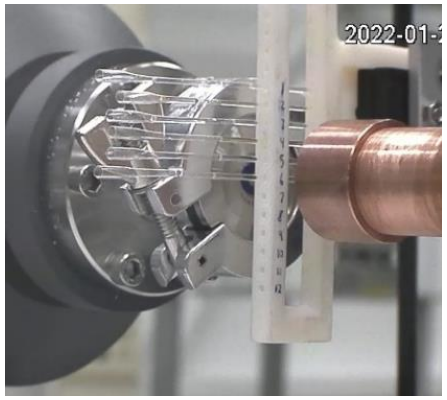
Transmission

Capillaries: 0.3 to 1.5 mm diameter Kapton, quartz, borosilicate glass









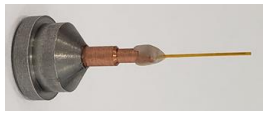
Freestanding,
Between Kapton tape

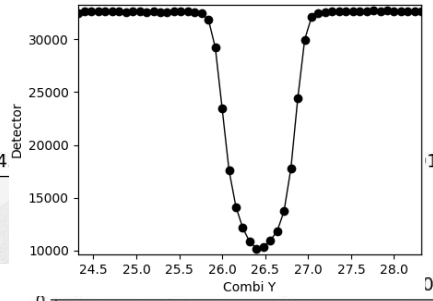
Grazing incidence

Thin film sample on Si wafers, or glass slides

Liquids: Solutions need to be dilute (1 - 10 mg/mL, ~10 mmol) to learn about the particle size and shape
Bring a range of concentrations

Detector acquisition and calibration

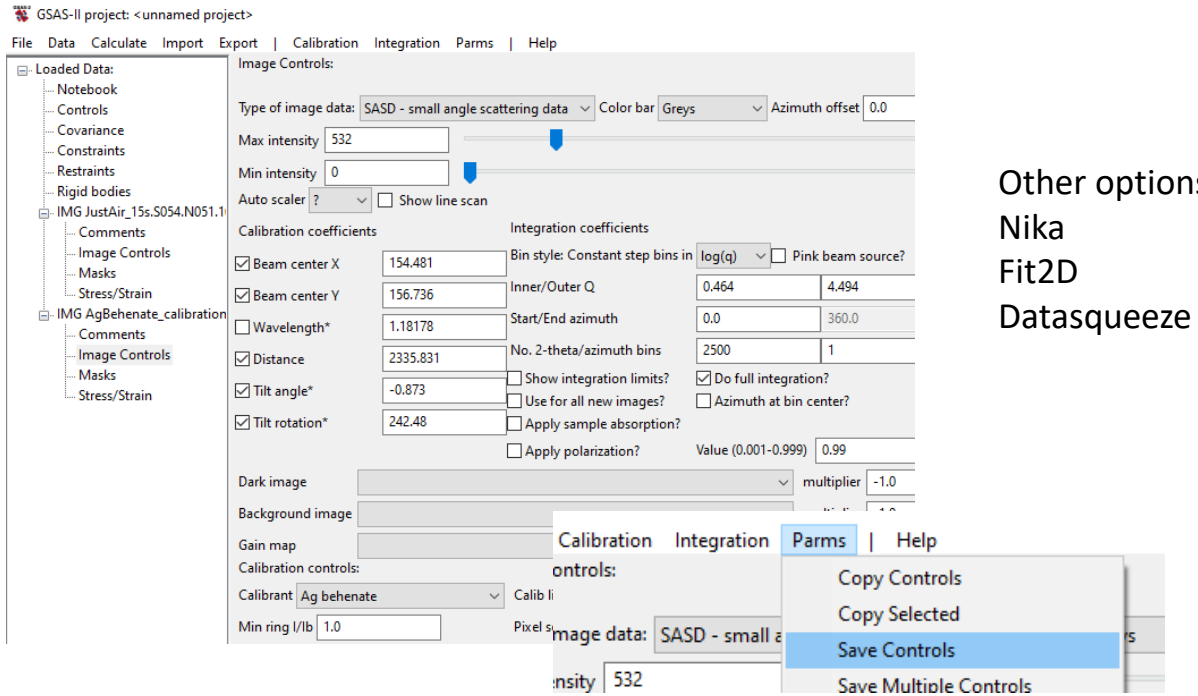




Acquisition time: 1 s - 90 s
16 bit .tif files (33 MB)

Note during your beamtime!!!
The wavelength (1.18178 Å)
The approximate detector distance (2350 mm)

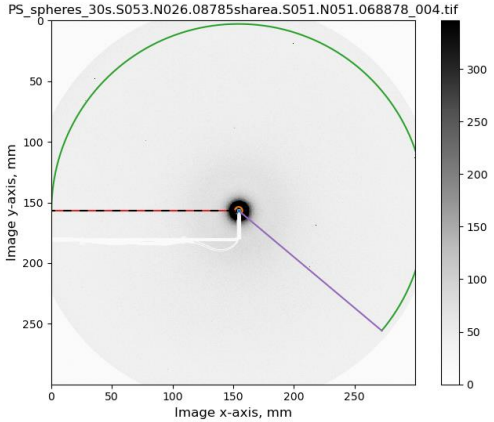
GSAS-II demo, “Calibration of an area detector”

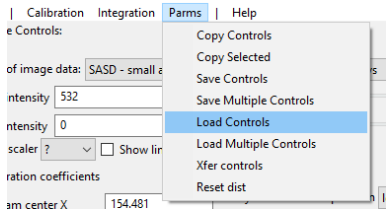


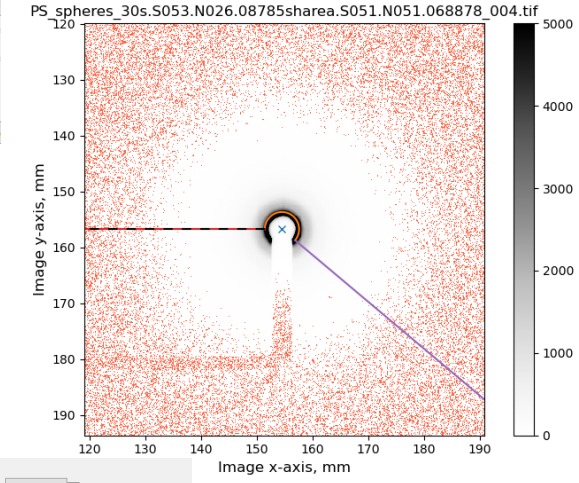
Other options:
Nika
Fit2D
Datasqueeze

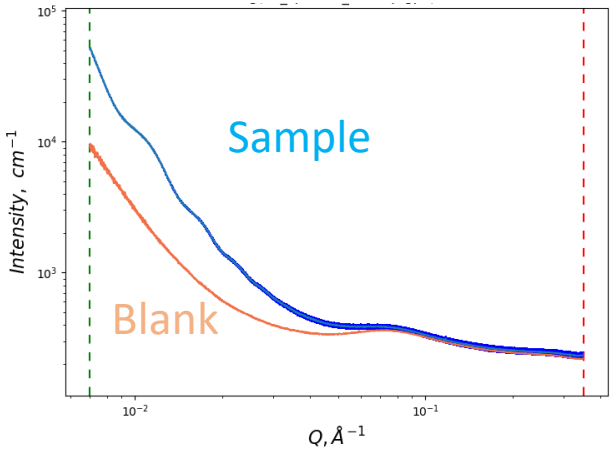
Ring	q (A-1)	d (nm)
1	0.1076	5.839
2	0.2152	2.920
3	0.3228	1.946
4	0.4304	1.460
5	0.5380	1.168

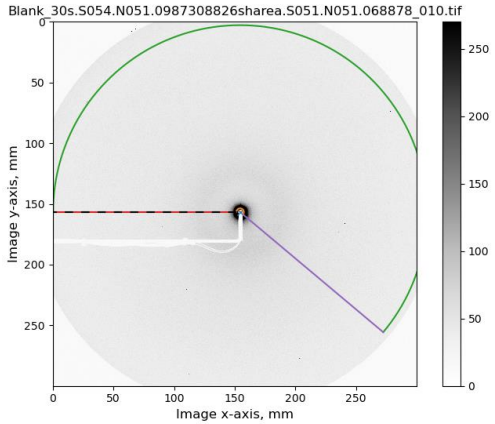
Data correction, and reduction to 1D



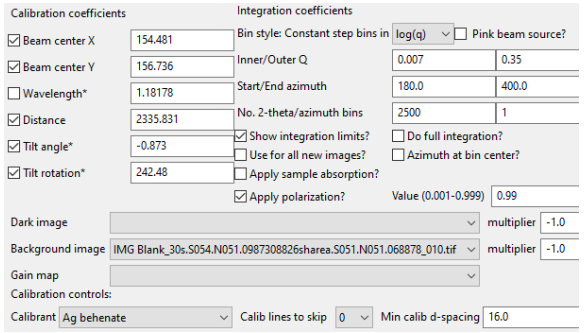


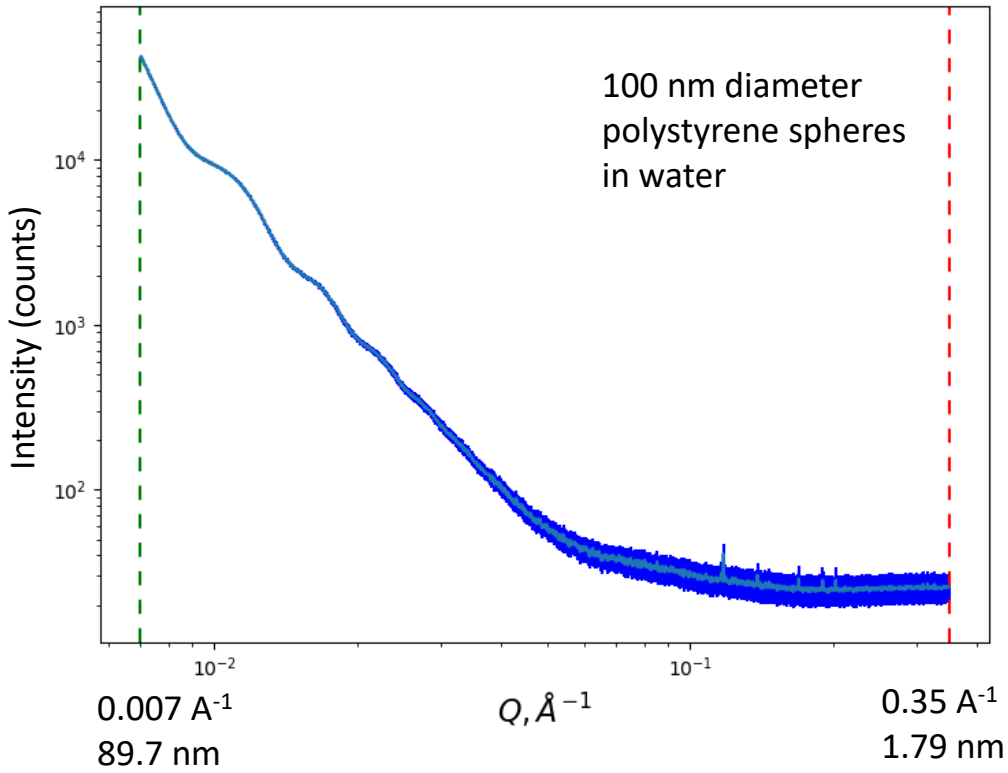






Subtract blank
(equivalent time)





- Top up mode (0.5%)
- Raw detector images are corrected for dark current, flat-field, and spatial distortion

Data analysis with examples

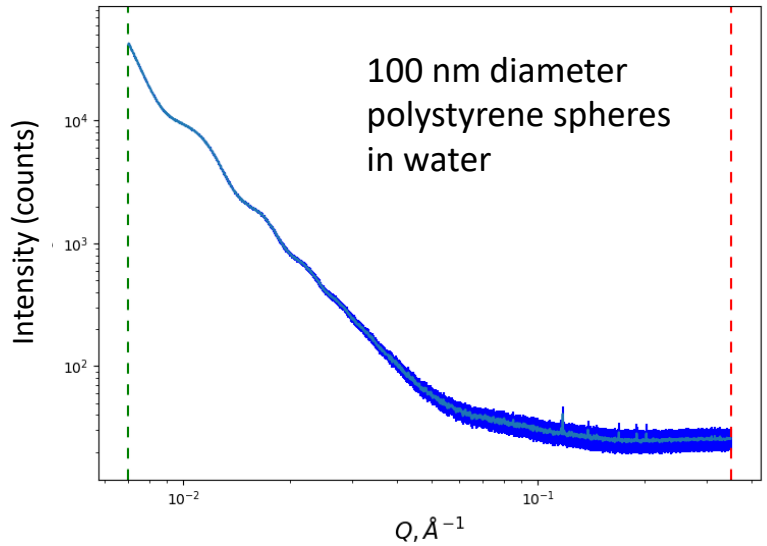
Origins of the SAXS signal

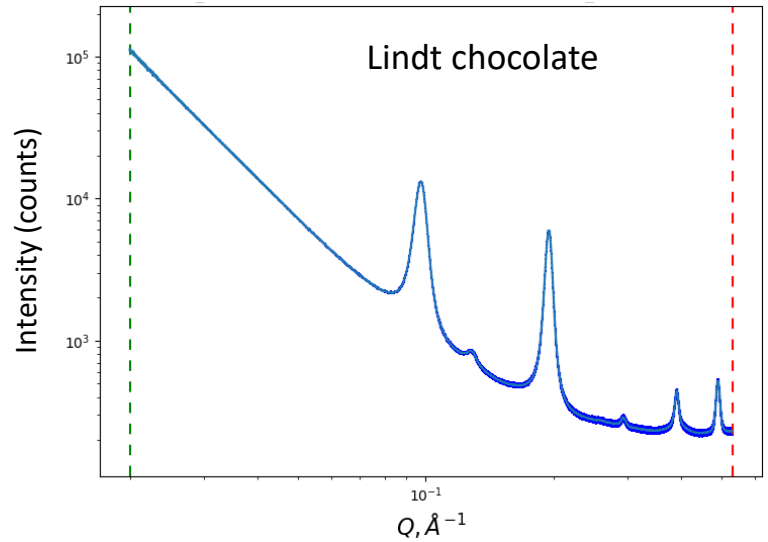
$$I(Q) = P(Q) \times S(Q)$$

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

S(Q) is the **structure** factor
- *Inter*particle interferences
- Correlation distances between particles
- High concentrations
- Ordering/packing of particles

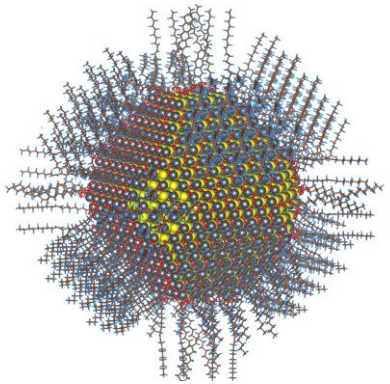
Looking for and analyzing Bragg peaks
An extension of PXRD





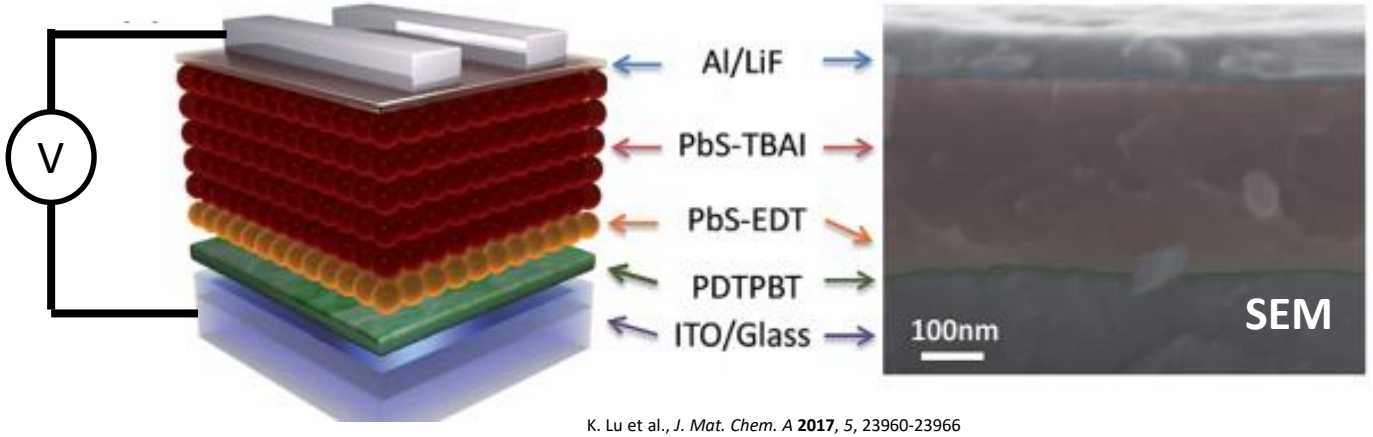
Energy materials: Quantum dot solar cells

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/



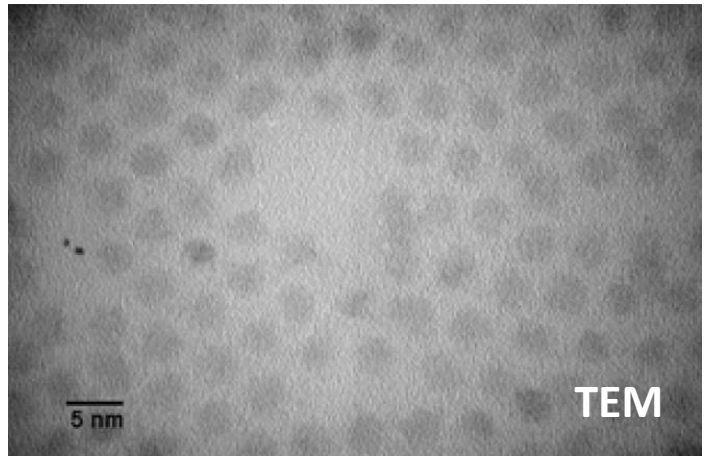
K. Lu et al., *J. Mat. Chem. A* **2017**, *5*, 23960-23966

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

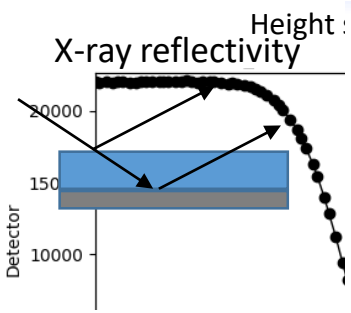
Efficient exciton generation
Size-based, tune-able band gap

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

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



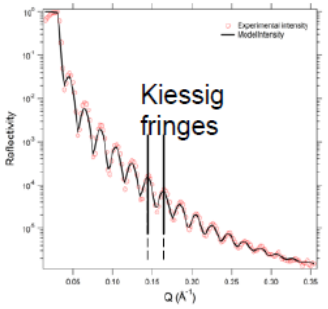
M. Yuan et al., Adv. Mater. 2014, 26, 3513–3519

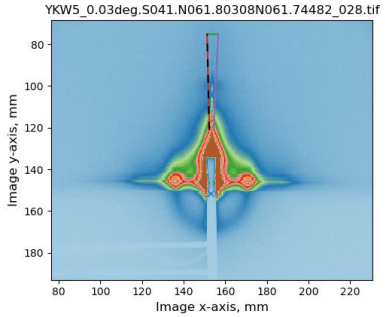


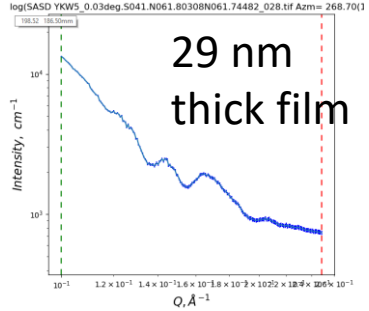
Simple estimation of film thickness

Fringes with uniform spacing

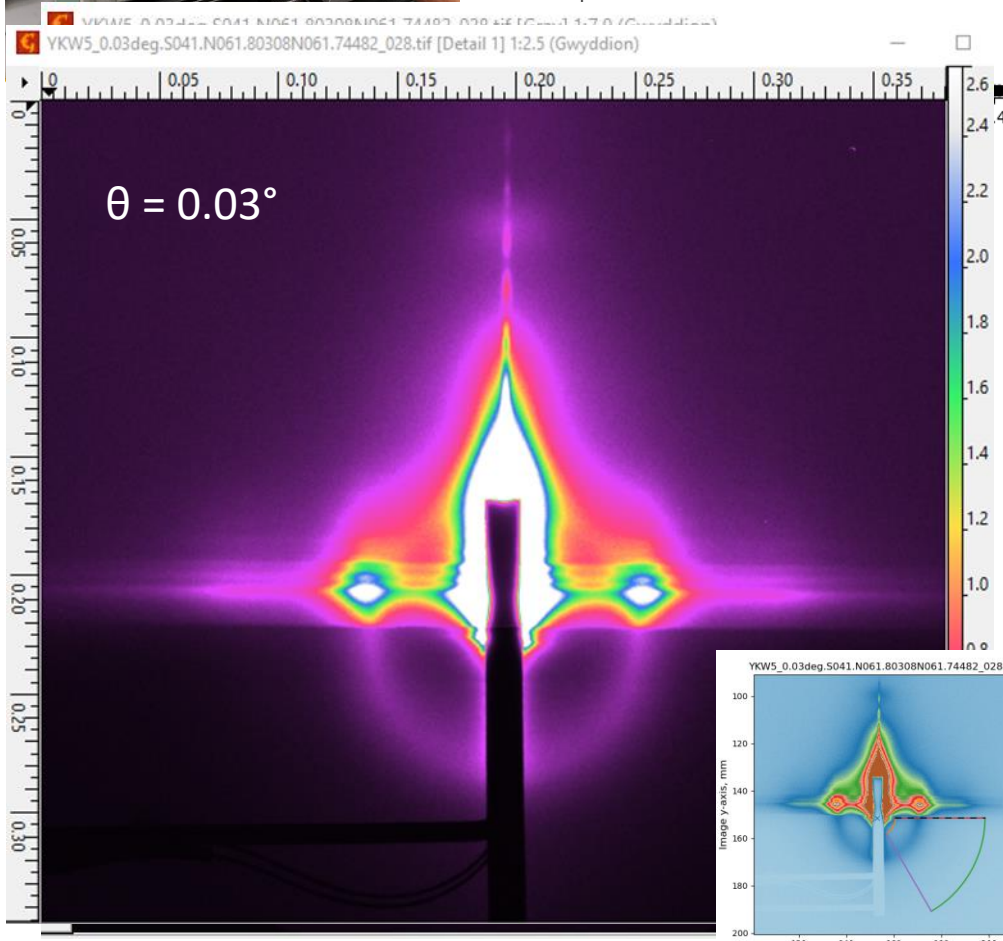
Thickness of the layer : $t = \frac{2\pi}{\Delta q_z}$

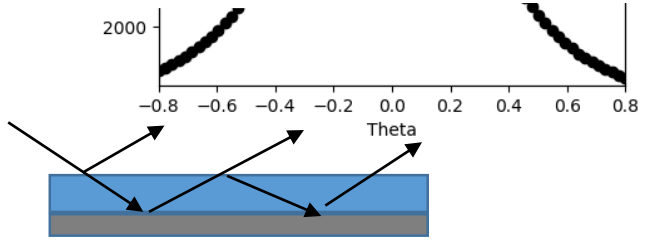






- Reflectivity gives film thickness

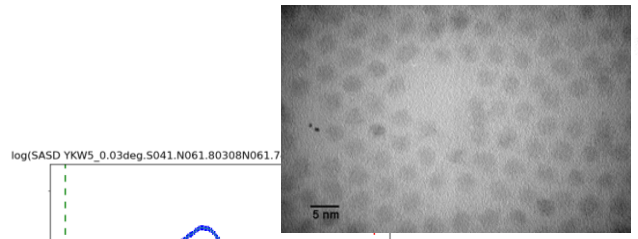


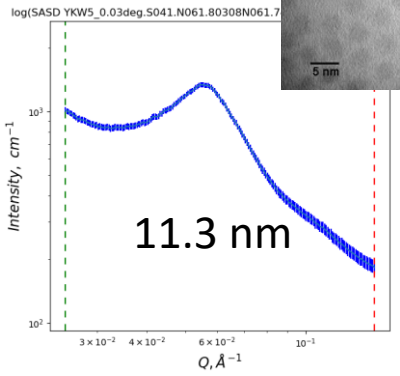


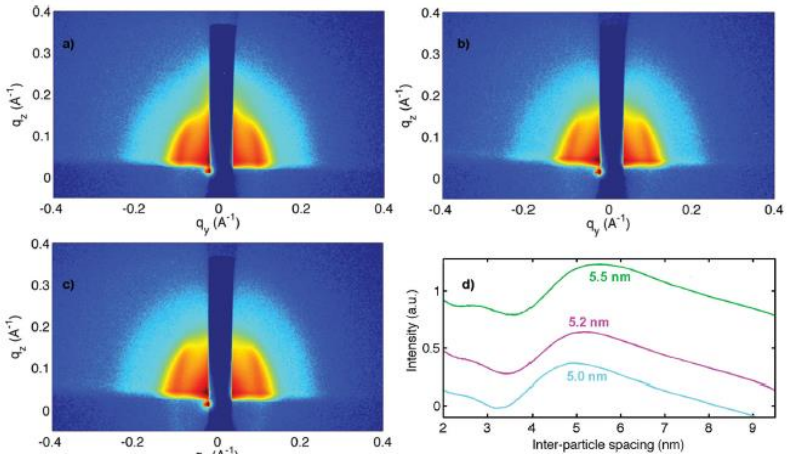
Waveguide modes

- 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



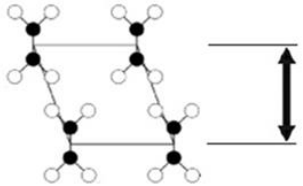


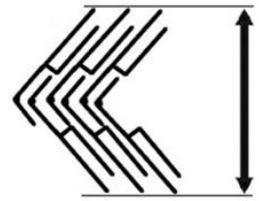


M. Yuan et al., Adv. Mater. 2014, 26, 3513–3519

Food science: Better chocolate without tempering

Alejandro Marangoni, Jarvis Stobbs
University of Guelph
Dept. Food Science





Short Spacing **Long Spacing**

The nanostructure of chocolate is manipulated by chocolate makers using time and energy-intensive “tempering”

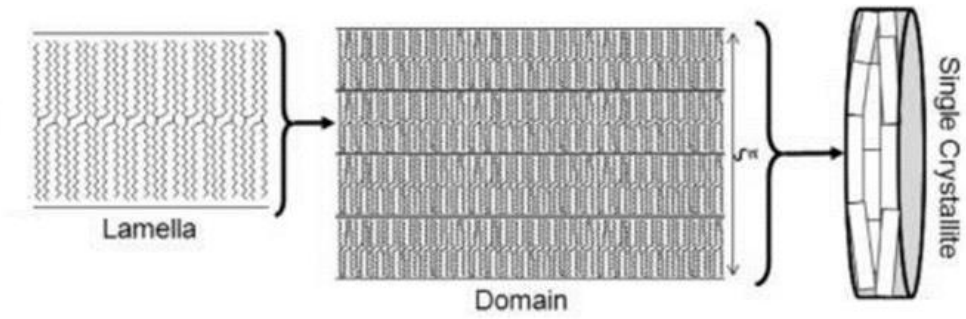
Tempering guides crystallization of cocoa butter to the desirable “polymorph V” phase

Polymorph	I	II	III	IV	V	VI
Long Spacing (A°)	55.1	49	49	45	63.8	64.1

Tempering of cocoa butter and chocolate using minor lipidic components

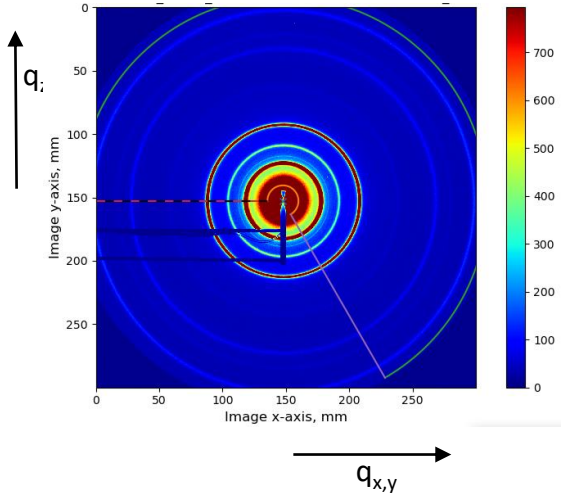
Jay Chen[1], Saeed M. Ghazani[1], Jarvis A. Stobbs[1,2] & Alejandro G. Marangoni[1]✉

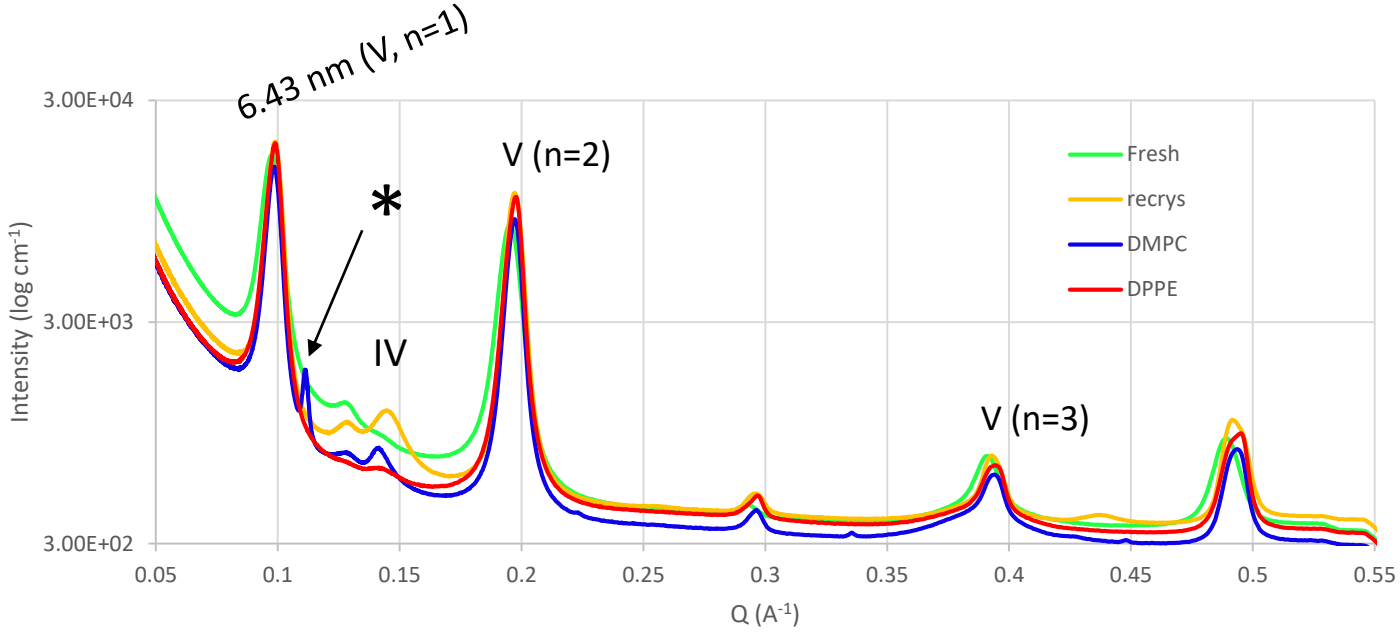
NATURE COMMUNICATIONS | (2021)12:5018

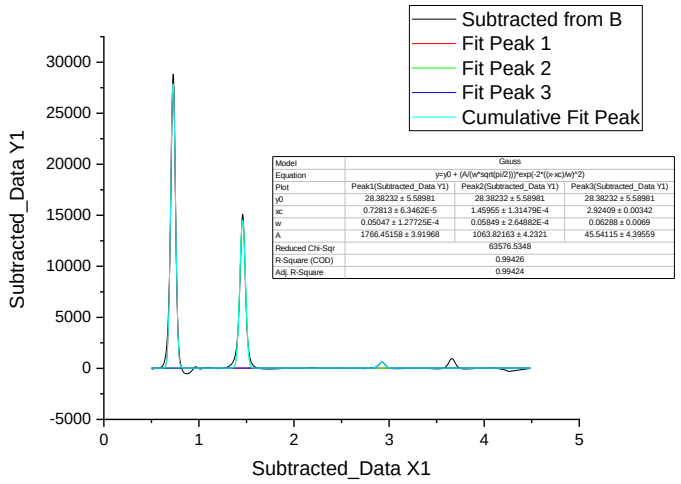


Why does it work? Can SAXS help?

Food science: Better chocolate without tempering





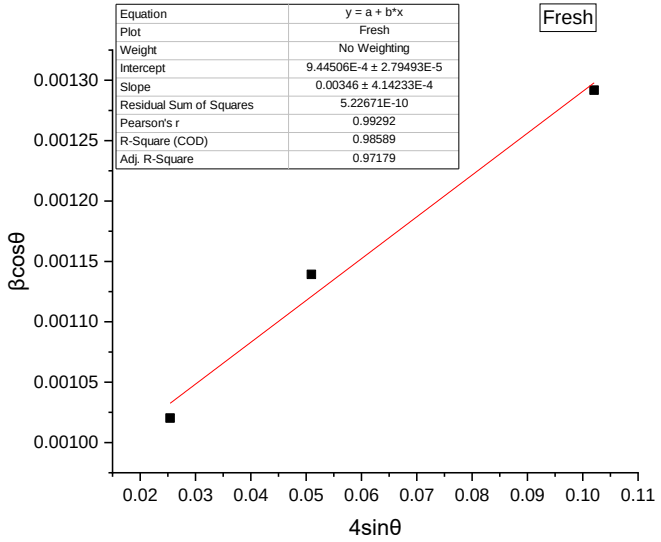


Model	Gauss		
Equation	y=y0 + (A/(w*sqrt(pi/2)))*exp(-2*((x-xc)/w)^2)		
Plot	Peak1(Subtracted_Data Y1)	Peak2(Subtracted_Data Y1)	Peak3(Subtracted_Data Y1)
y0	28.38232 ± 5.58981	28.38232 ± 5.58981	28.38232 ± 5.58981
xc	0.72813 ± 6.3462E-5	1.45955 ± 1.31479E-4	2.92409 ± 0.00342
w	0.05047 ± 1.27725E-4	0.05849 ± 2.64882E-4	0.06288 ± 0.0069
A	1766.45158 ± 3.91968	1063.82163 ± 4.2321	45.54115 ± 4.39559
Reduced Chi-Sqr	63576.5348		
R-Square (COD)	0.99426		
Adj. R-Square	0.99424		

Williamson-Hall plot

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

crystallite size and strain from peak width (*B*) analysis

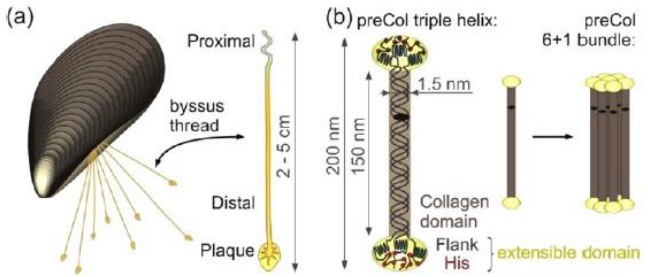


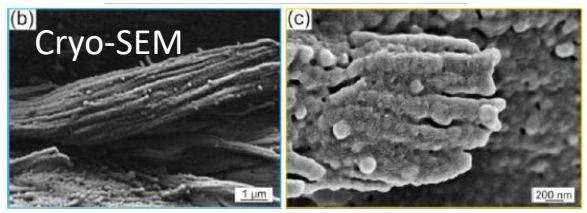
Equation	y = a + b*x
Plot	Fresh
Weight	No Weighting
Intercept	9.44506E-4 ± 2.79493E-5
Slope	0.00346 ± 4.14233E-4
Residual Sum of Squares	5.22671E-10
Pearson's r	0.99292
R-Square (COD)	0.98589
Adj. R-Square	0.97179

Biology and Polymer science: Self-healing polymers

Harrington group
McGill, Chemistry

Mussel byssal threads





Ability to stretch and heal, but are composed almost entirely of proteins.
How? Does healing depend on aspects of the thread structure?

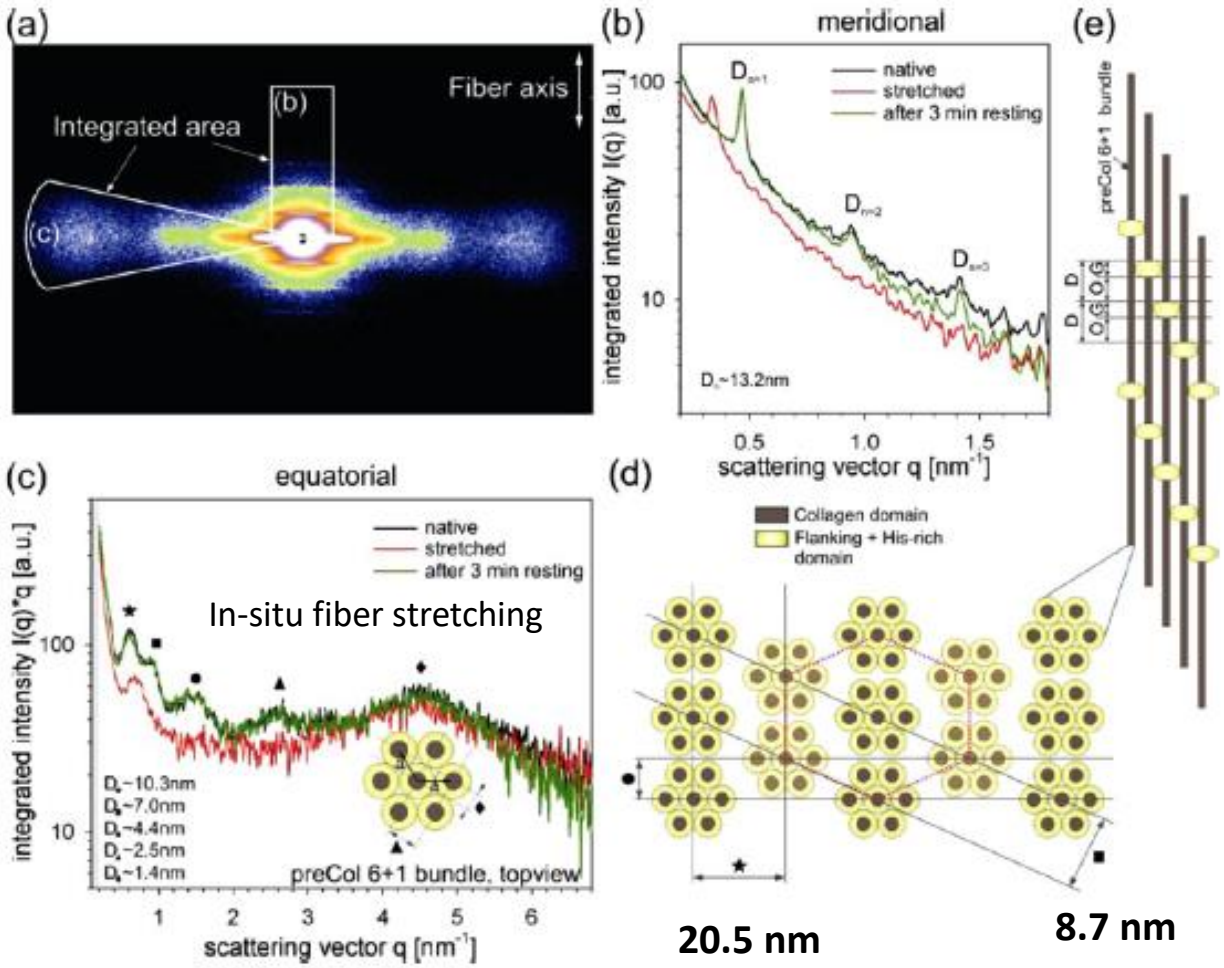
- Orientation

- Resolve the partially crystalline structure

Symmetry	Peak position ratio
Lamellar	1, 2, 3, 4, 5 …
Cubic	1, √2, √3, 2, √5 …
Hexagonal	1, √3, 2, √7, 3 …

Framework of highly ordered tightly folded protein domains

It can spring back on stress release, bringing sacrificial binding and crosslinking sites back into register: “Self-repair”

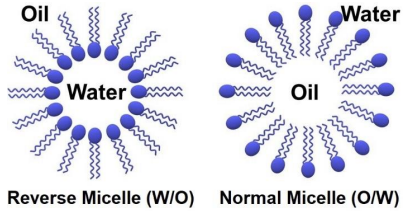


S. Krauss et al., Self-Repair of a Biological Fiber Guided by an Ordered Elastic Framework. Biomacromolecules 2013, 14, 1520−1528

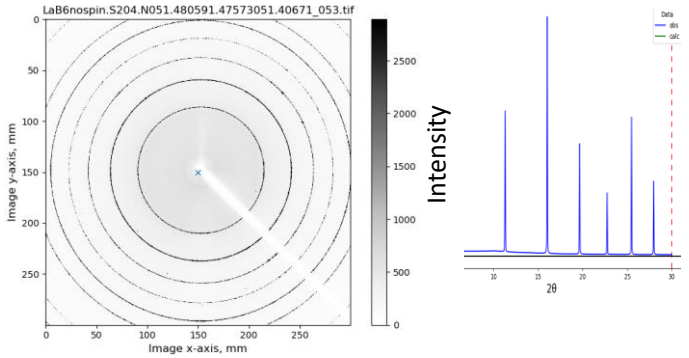
Environmental science: Waste water treatment

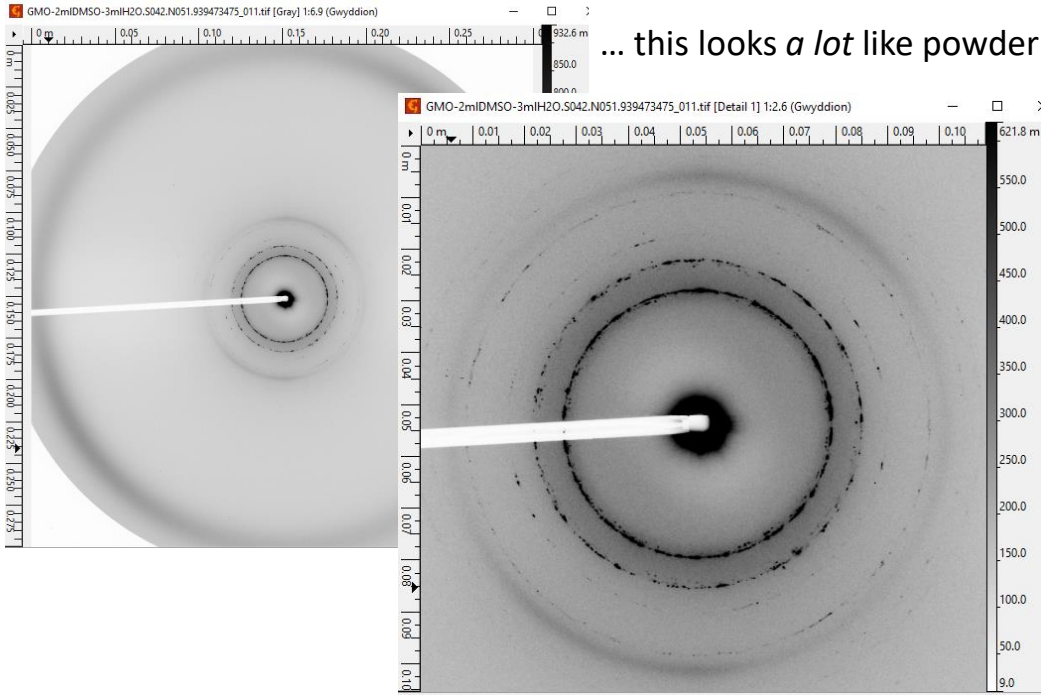
Pensini group
U. Guelph, Engineering

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

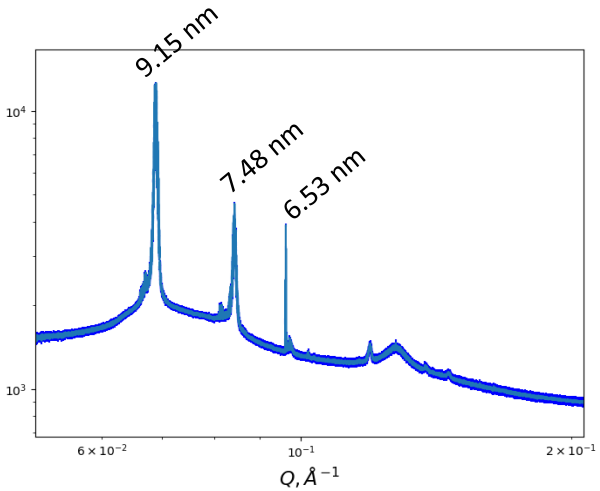


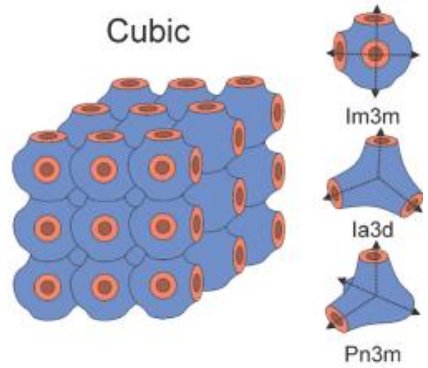
LaB6, typical PXRD standard





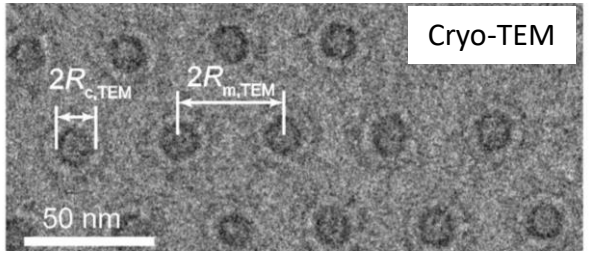
… this looks *a lot* like powder diffraction … ??





bioRxiv: https://doi.org/10.1101/791848

Symmetry	Peak position ratio
Cubic, Pn3m	√2, √3, √4 …



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

Self-assembly of amphiphiles into cubic reverse micelle lattice … behaviour correlates with enhanced solvent separation

Reverse micelle squishy lattice

Canadian Light Source / Centre canadien de rayonnement synchrotron

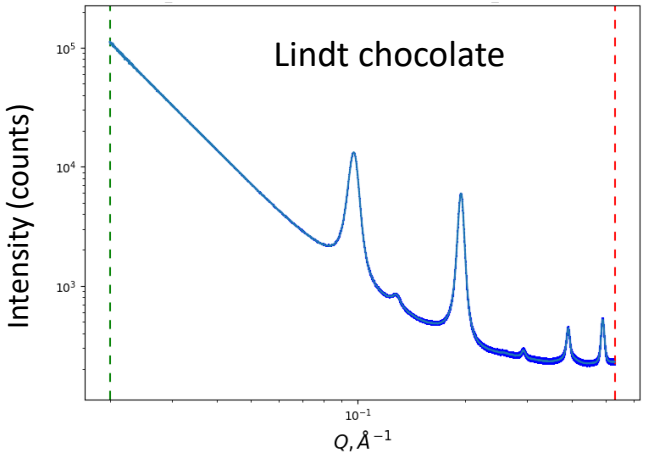
Origins of the SAXS signal

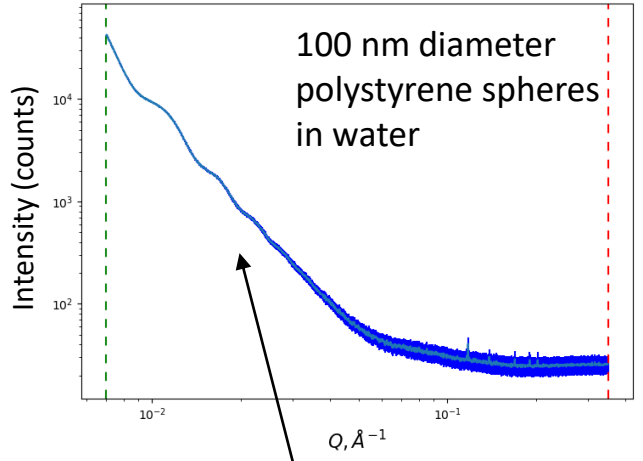
$$I(Q) = P(Q) \times S(Q)$$

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

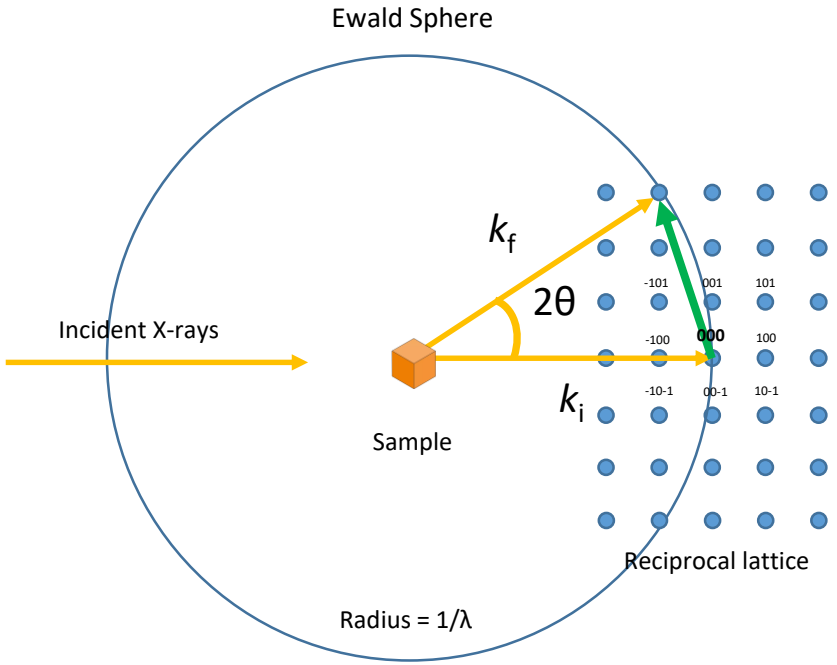
S(Q) is the **structure** factor
- *Inter*particle interferences
- Correlation distances between particles
- High concentrations
- Ordering/packing of particles

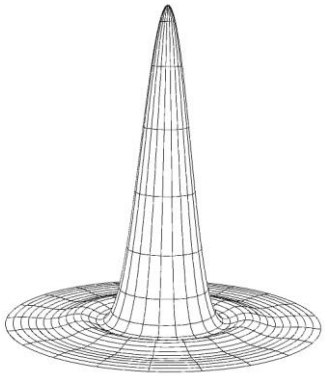
~~Looking for and analyzing Bragg peaks~~
~~An extension of PXRD~~





Consider this as the [000] peak



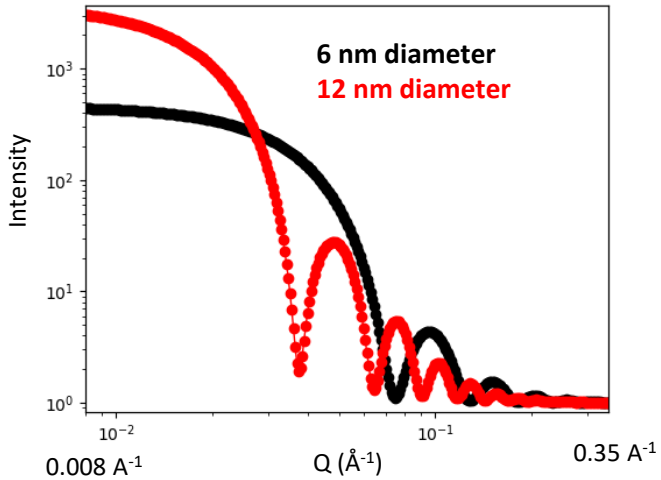


Origins of the SAXS signal: Form factor

SAXS pattern is a Fourier transform of the sample

Consider this as the [000] peak…

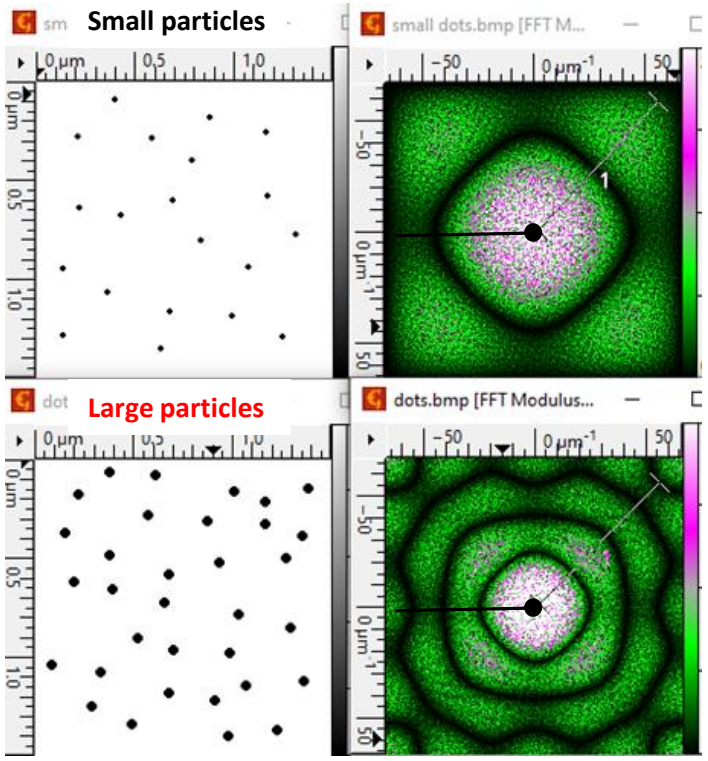
Simulated SAXS patterns, spherical particles, 1% polydispersity

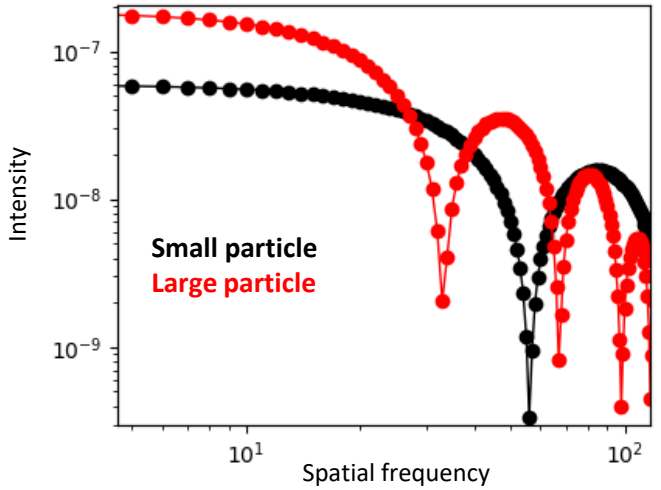


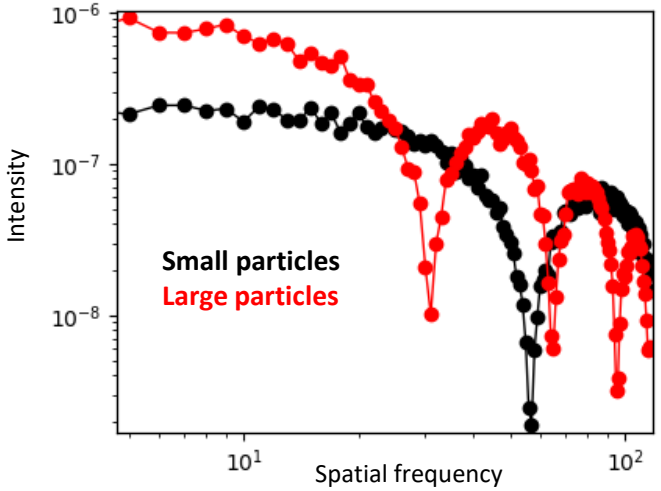
Small particles = Broad [000] peak
Large particle = Narrow [000] peak **(particle size via Guinier)**

Relationship to Scherrer equation explained in
Morelhao and Kycia, Acta Cryst. A, https://doi.org/10.1107/S2053273322007215

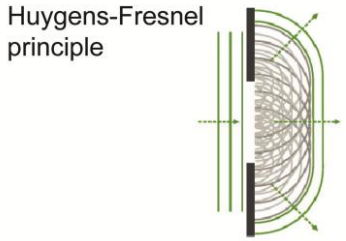
Simulated sample → FFT → Simulated SAXS detector image

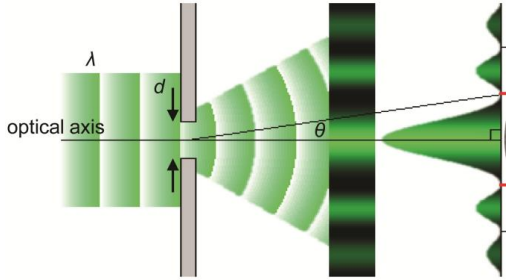




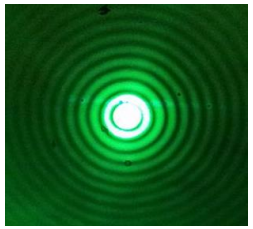


What are the oscillations?



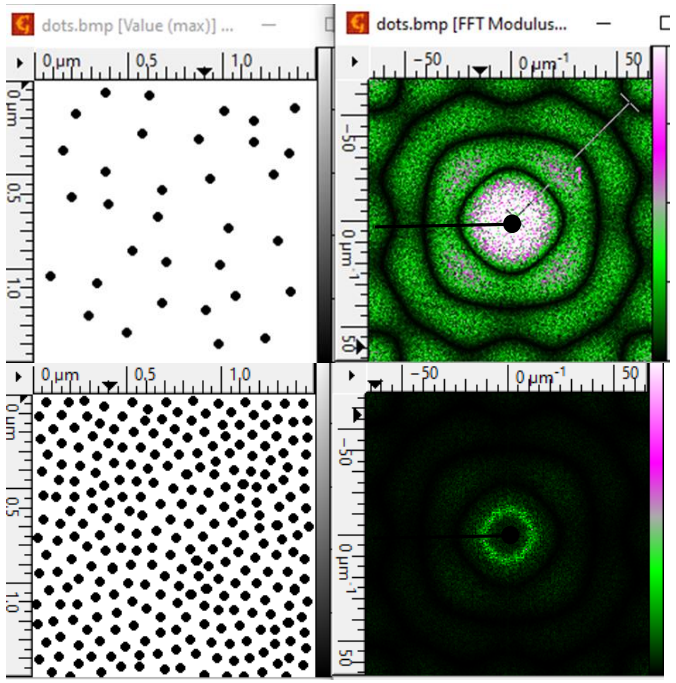


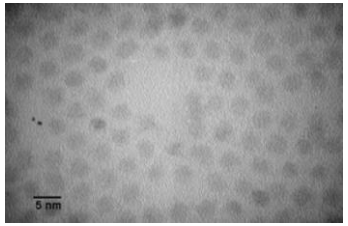
Babinet's principle



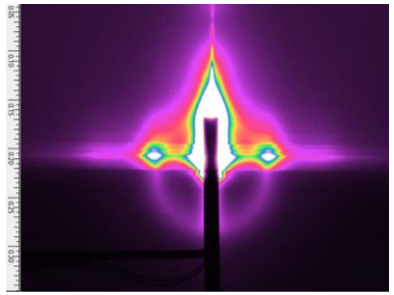
But there are limits!

Solutions must be dilute: No interparticle effects





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



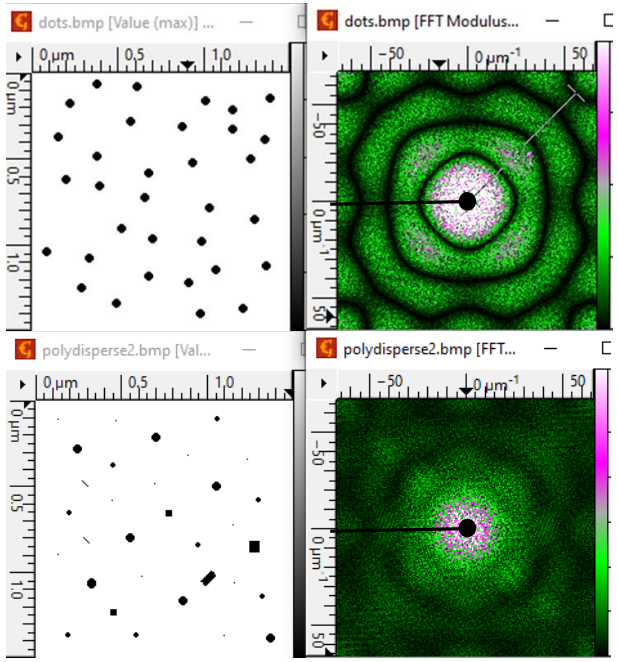
Rule of thumb:
~1 – 10 mg/mL

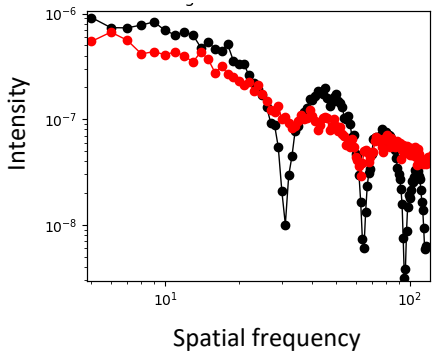
Bring many concentrations

Solutions must be monodisperse, and pure: No [000] peak overlap

Simulated sample

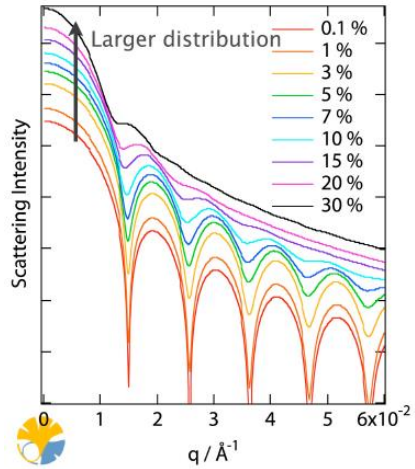
Simulated SAXS detector image





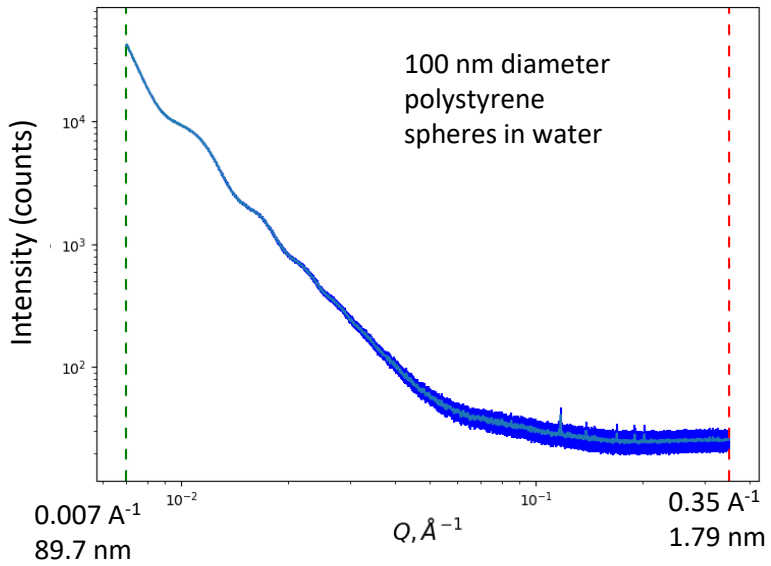
Rule of thumb: Form factor oscillations smear out when size polydispersity is ≥15%

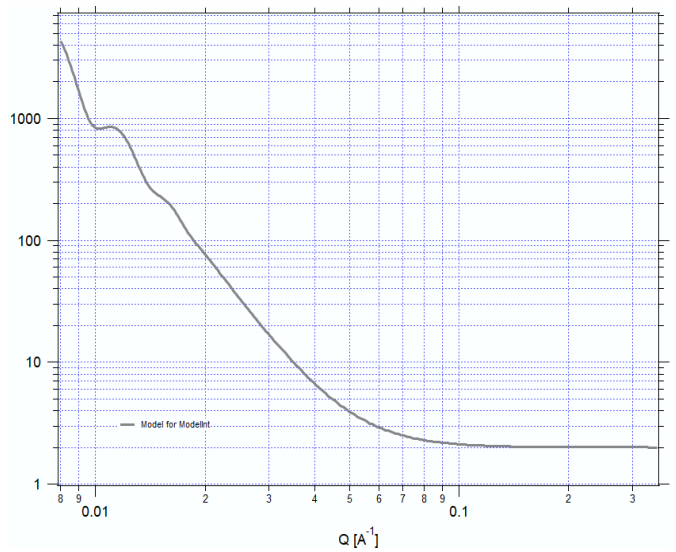
Polydispersity

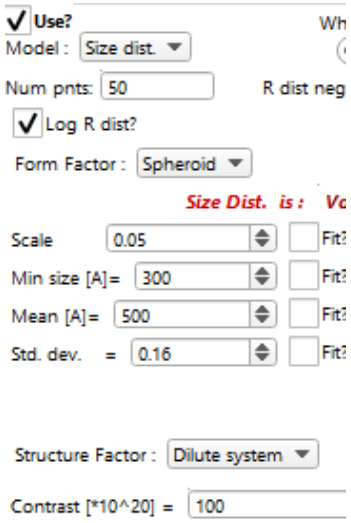


Form factor analysis for particle size and shape

Modeling and whole curve fitting







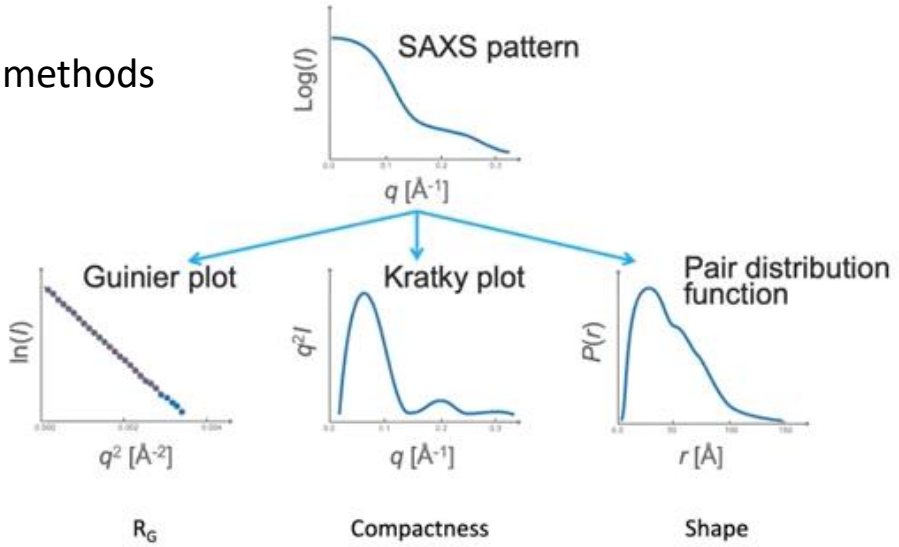
Irena and Nika macros from APS
IgorPro is $1095 USD

SASFit
ATSAS package

J. Ilavsky, P.R. Jemian, Irena: tool suite for modeling and analysis of small-angle scattering, J. Appl. Cryst. (2009). 42, 347–353

Data linearization and plotting methods

Guinier plots
Porod plots
Kratky plots
Debye–Buche plots



All have important conditions to follow

Often useful for rough analysis, but not the final answer

Human health: Nano drug delivery

Badea group
U. Sask, Pharmacy

Successful reprogramming of cellular protein production through mRNA delivered by functionalized lipid nanoparticles

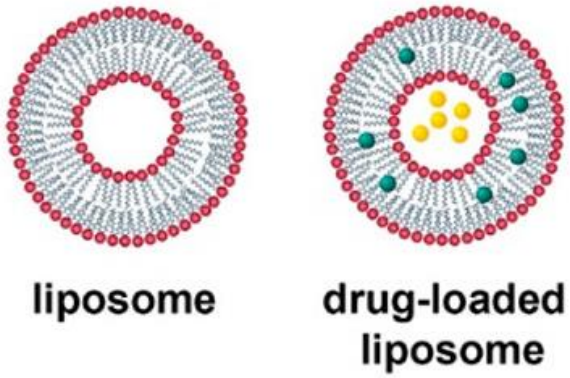
Marianna Yanez Arteta[a,1], Tomas Kjellman[a], Stefano Bartesaghi[b], Simonetta Wallin[b], Xiaoqiu Wu[a], Alexander J. Kvist[c], Aleksandra Dabkowska[a], Noémi Székely[d], Aurel Radulescu[d], Johan Bergenholtz[e], and Lennart Lindfors[a,1]

[a]Pharmaceutical Sciences iMed Biotech Unit, AstraZeneca R&D Gothenburg, 431 83 Mölndal, Sweden; [b]Cardiovascular and Metabolic Diseases iMed Bioscience, AstraZeneca R&D Gothenburg, 431 83 Mölndal, Sweden; [c]Discovery Science iMed Bioscience, AstraZeneca R&D Gothenburg, 431 83 Mölndal, Sweden; [d]Outstation at Maier-Leibnitz Zentrum, Jülich Centre for Neutrons Science, 857 47 Garching, Germany; and [e]Department of Chemistry and Molecular Biology, University of Gothenburg, 412 96 Gothenburg, Sweden

Edited by David A. Weitz, Harvard University, Cambridge, MA, and approved March 5, 2018 (received for review November 28, 2017)

Diameter: 20 – 1000 nm

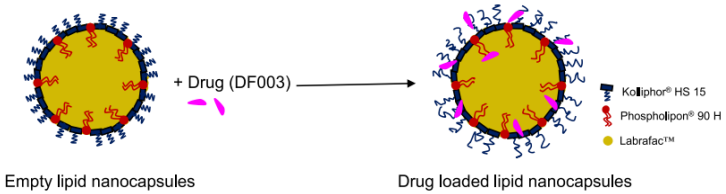
Bilayer: ~5 nm



LNPs are the delivery vehicle in the COVID-19 messenger RNA (mRNA) vaccines by Pfizer/BioNTech and Moderna

Lipid nanocapsules

D. Urimi et al., *Mol. Pharmaceutics* **19**, 1068−1077 (2022)



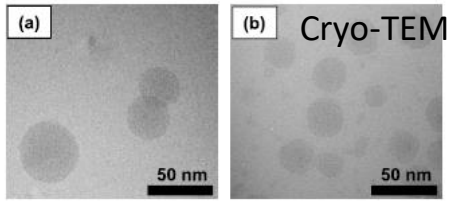
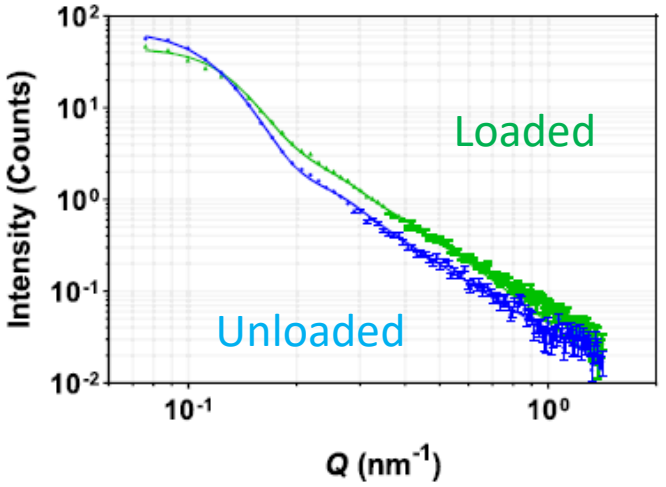


Figure 1. Cryogenic transmission electron micrographs of drug-loaded (a) h-LNCs and (b) d-LNCs.



core-shell model fits better than plain sphere model

Table 3. Structural Information about the LNCs as Determined by SAXS, SANS, and DLS Techniques

parameter	unloaded LNCs	DF003-loaded LNCs
P(*r*) analysis of small-angle X-ray scattering (SAXS) data		
R_g (nm)	17.3 ± 0.09	15.8 ± 0.05
D_{max} (nm)	49 ± 0.5	46 ± 0.5
shape model analysis of small-angle X-ray scattering (SAXS) data		
core radius (nm)	21.7 ± 0.2	18.5 ± 0.2
shell thickness (nm)	2.6 ± 0.1	3.6 ± 0.1
total radius (nm)	24.3 ± 0.3	22.1 ± 0.3
volume fraction[a]	0.092	0.092
polydispersity	0.20	0.35
χ^2	2.4	3.7
small-angle neutron scattering (SANS)		
core radius (nm)	20.0 ± 0.9	20.2 ± 0.6
shell thickness (nm)	≤1.5	~2 ± 0.5
total radius (nm)	21.5 ± 0.9	22.2 ± 1.1
shell hydration (%)	50	70
volume fraction[b]	0.009	0.009
polydispersity	0.20	0.25
SLD of shell	3.3	4.5
dynamic light scattering (DLS)		
hydrodynamic radius (nm)	30.0 ± 1.0	36.0 ± 1.0
polydispersity	0.04 ± 0.02	0.07 ± 0.01
zeta potential (mV)	−3.7 ± 1.6	−13.6 ± 0.8

Human health: Proteins and biomacromolecules

SAXS can reveal a low resolution macromolecule shape

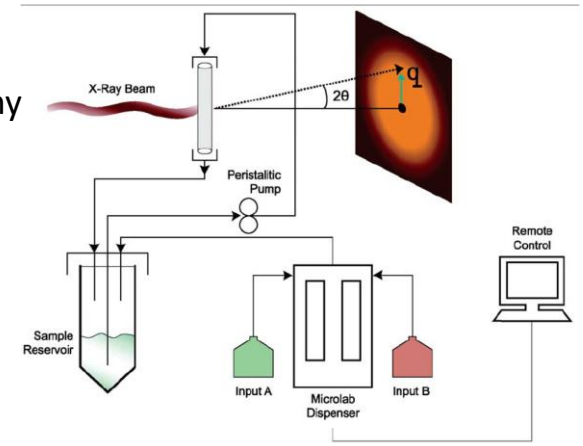
This can aid in solving the structure at a higher spatial resolution

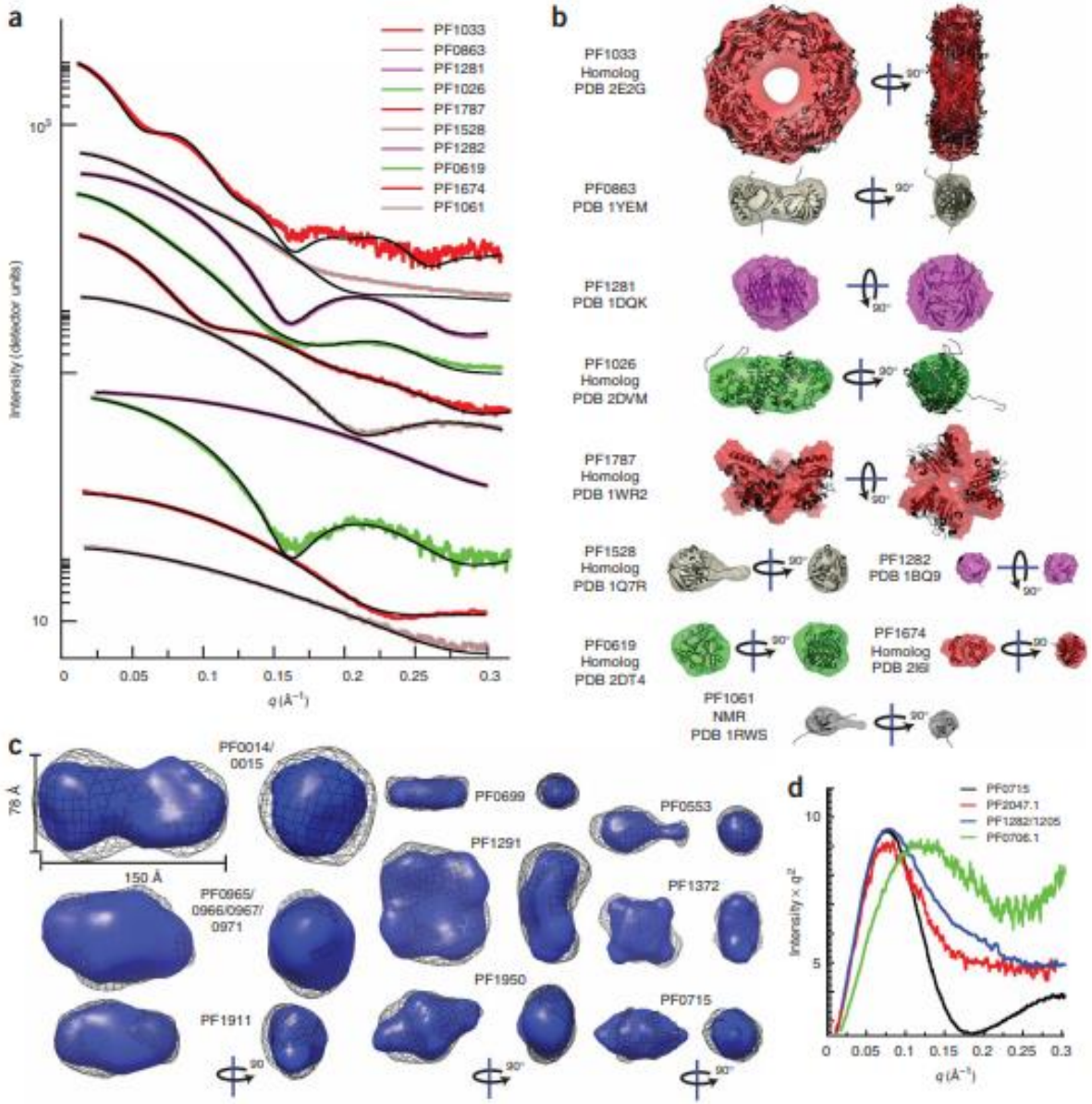
Very challenging sample type
- Limited amounts of material, low concentrations
- Weakly scattering
- Radiation damage

Several Bio-SAXS beamlines dedicated to proteins and biomacromolecules

Flow cell + size exclusion chromatography

We will try it soon





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

Canadian Light Source | Centre canadien de rayonnement synchrotron

Summary

The CLS has a SAXS endstation available for users

Measuring small scattering angles to probe relatively big things (1 - 150 nm) with X-rays

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

The Brockhouse Sector Team

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Professor, U. Guelph
Beamteam Leader

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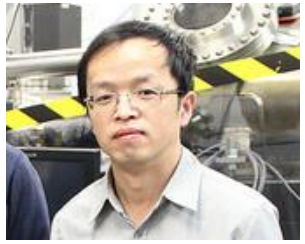

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Call for General User Proposals closes August 24 at noon (Sask. time)