

Small Angle X-ray Scattering (SAXS)

1st AOFSRR Synchrotron School

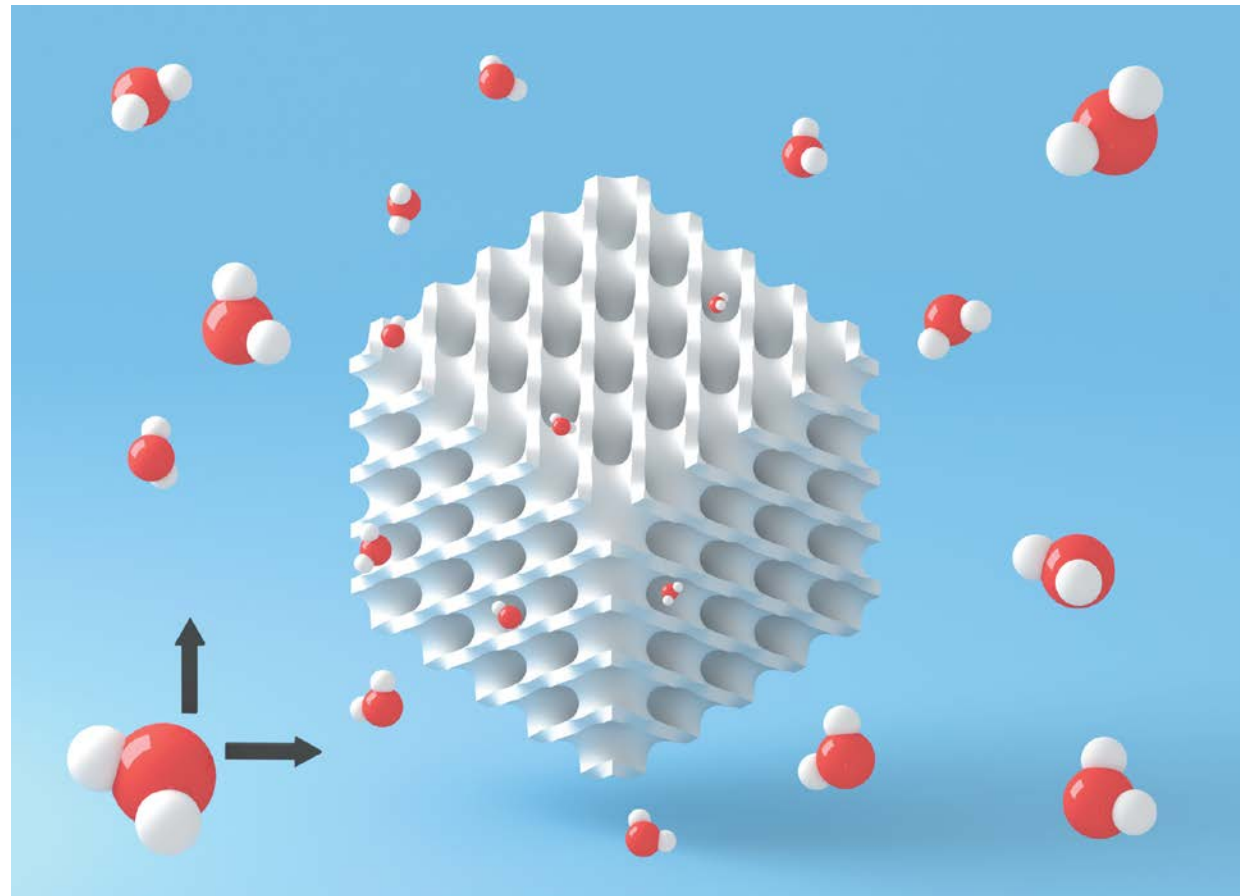
Charlotte Conn

RMIT University



Overview

- **SAXS Overview**
- **SAXS beamline set-up**
- **SAXS Analysis**
- **SAXD Analysis**
- **Case Study: In meso crystallisation of membrane proteins**



My Scattering Background

BA/MSci University of
Cambridge
(1998-2002)

- SANS – ILL (Grenoble, France)

PhD Imperial College London
(2002-2006)

- SAXS – ESRF (Grenoble, France)
- Daresbury Laboratory

Post-doc/Research Scientist
CSIRO (Melbourne)
(2006-2013)

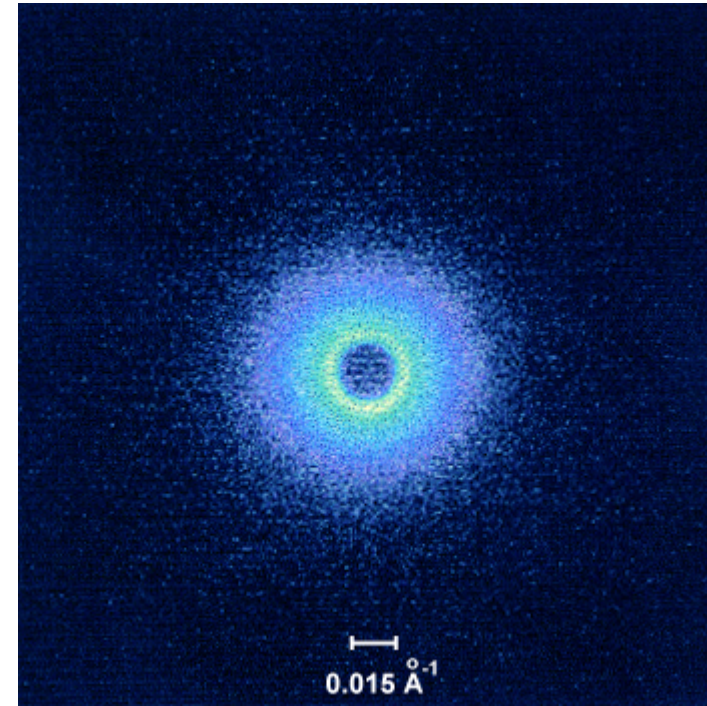
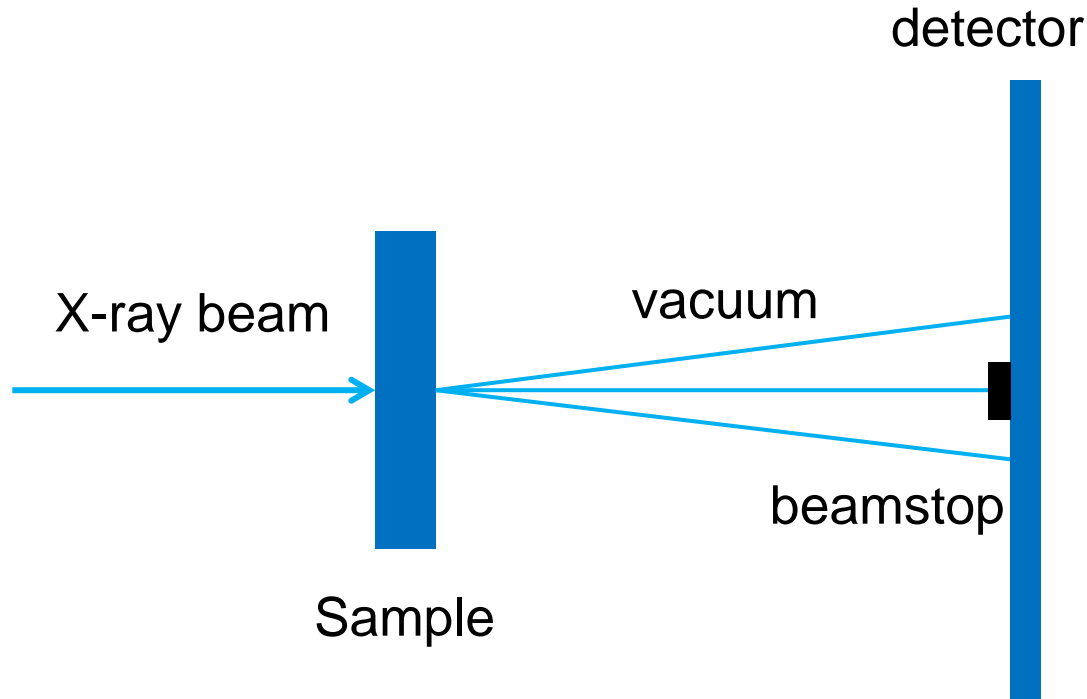
- SAXS – APS – Chicago
- SAXS – Australian Synchrotron

Senor Lecturer RMIT
University
(2013 – present)

- Chair of the Program Advisory Committee for the SAXS/WAXS beamline 2014-2016
- SANS – Bragg Institute (Sydney, Australia)

SAXS Overview

Small angle X-ray Scattering

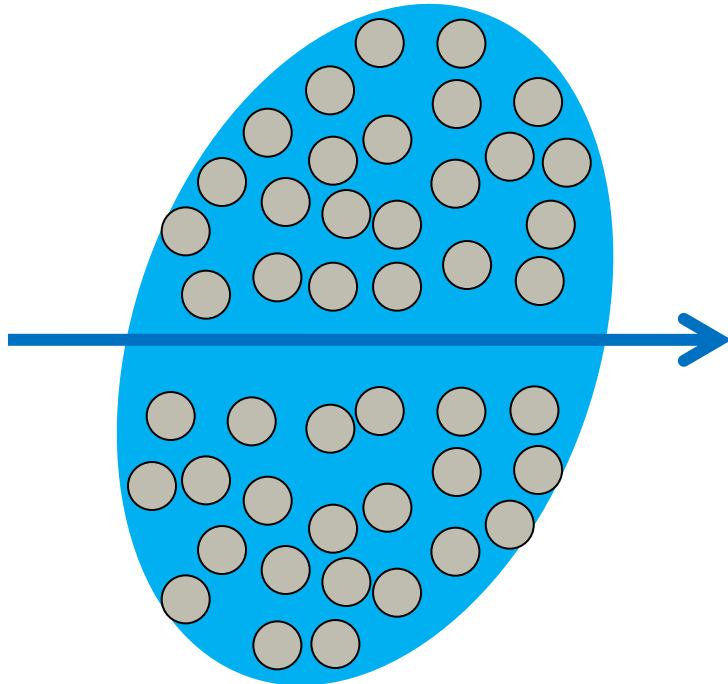


<http://www.physics.queensu.ca/>

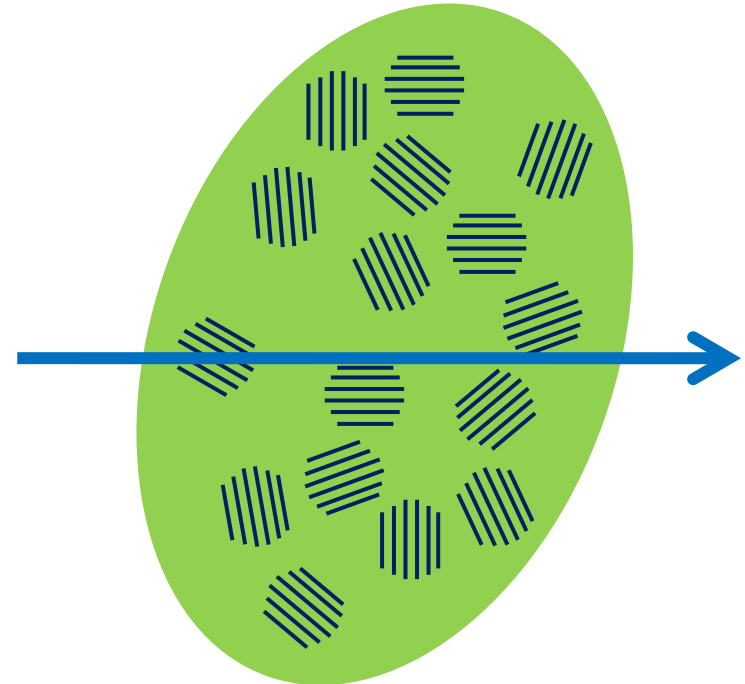
- Elastic scattering of x-rays
- Recorded at very low angles (typically $0.1 - 10^\circ$)
- SAXS typically covers up to 1° while WAXS covers the angular range $5 - 60^\circ$

Types of SAXS

Scattering from particles in solution – SAXS – 1-25nm

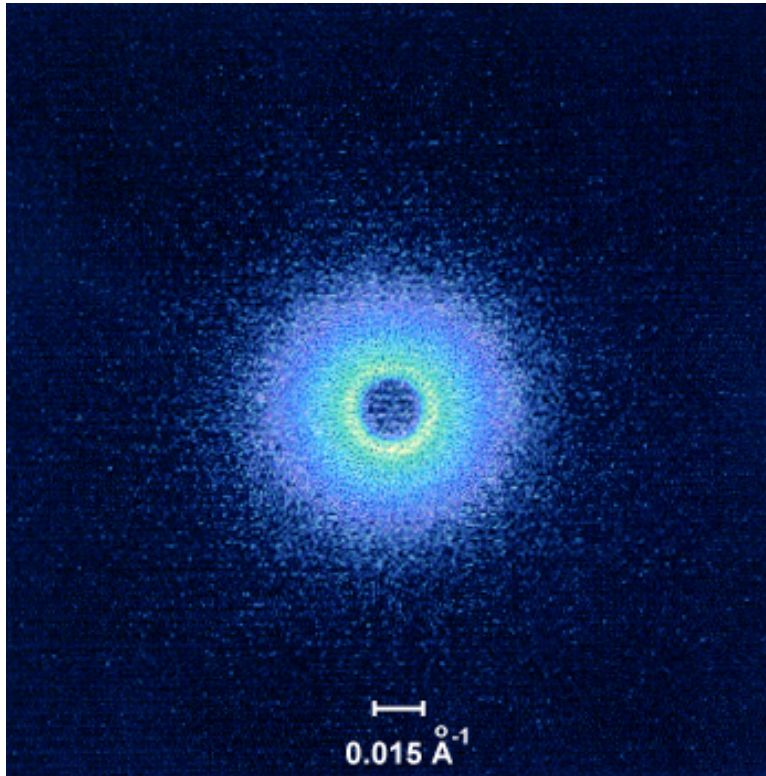


Diffraction from ordered or partially ordered materials – SAXD - < 150nm

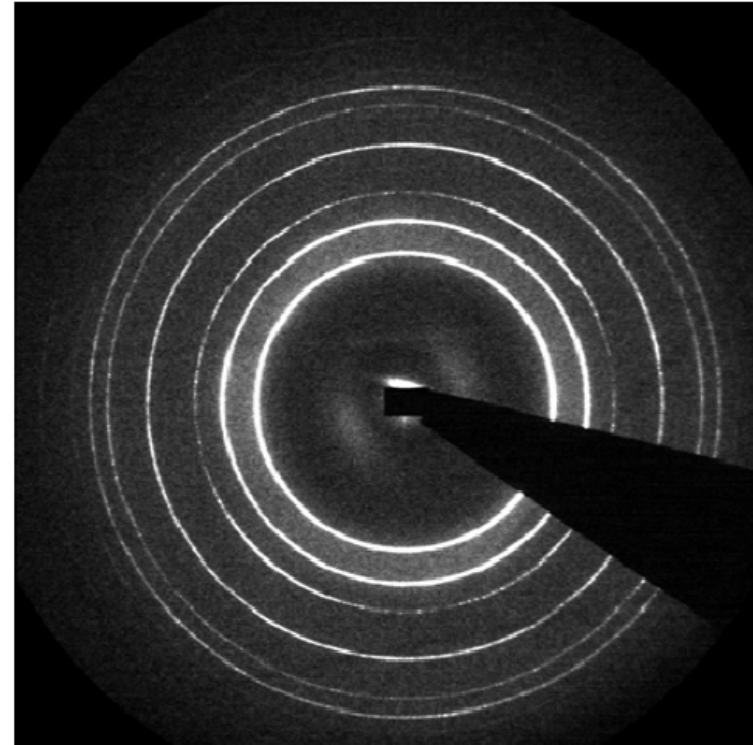


Types of SAXS

Scattering from particles in solution

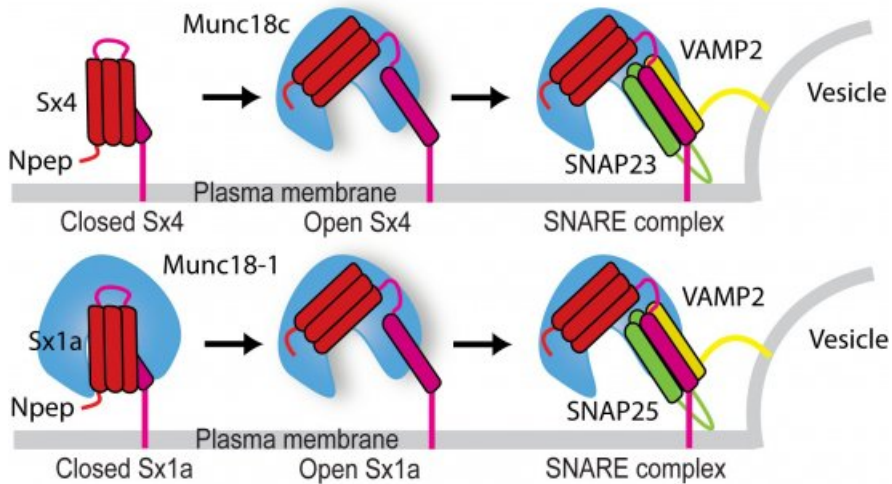


Diffraction from ordered or partially ordered materials



What is synchrotron SAXS used for?

Non-crystalline structural biology



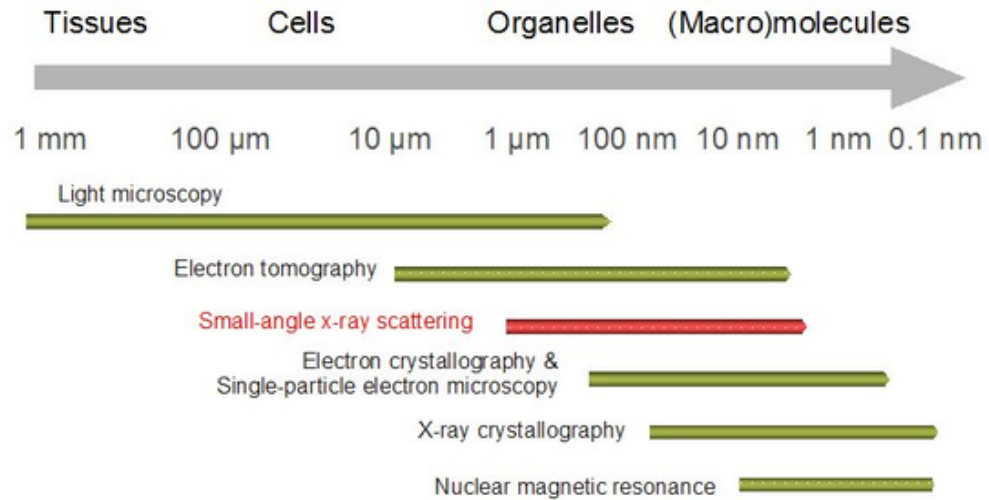
Interactions between a SNARE protein (Syntaxin) and its regulatory protein partner (Munc18)
Jenny Martin (UQ)

Soft condensed matter



Structure of milk fats
Ben Boyd (Monash)

SAXS length scales



Comparison to other characterisation techniques

Technique	Range	Volumes	Comments
Optical Microscopy	1 μm – 1000 μm	Very Small, thin	Poor statistics, slow
Electron Microscopy (TEM)	<1 nm – 1000 nm	Very Small, thin	Poor statistics, slow, high resolution, preparation artefacts
Electron Microscopy (SEM)	1 nm – 1000 mm	Surface only	Analysis tricky, preparation artefacts (?)
Dynamic Light Scattering	1 nm to 10 μm	Large, suspensions	Fast, low resolution
Static Light Scattering	100 nm to 10 μm	Large, suspensions	Slow, High resolution
Small Angle X-Ray Scattering	< 1 nm – 100 nm	Large	Fast, Low resolution
Small Angle Neutron Scattering	< 1 nm – 100 nm	Large	Slow, Low resolution, Sample preparation can be difficult

Advantages and Disadvantages of SAXS

Advantages

- Samples need not be crystalline
- Minimum of sample preparation required
- Measurement is usually non-destructive
- Can deal with larger macromolecules than NMR (>30000-40000)
- Able to measure various samples e.g. powder, liquid, solution

Disadvantages

- Spatial averaging occurs due to random orientation of dissolved or partially ordered samples – leads to a loss of information
- Radiation damage is possible for less robust samples e.g. proteins
- Scattered intensity is weak for commonly used systems. If synchrotron radiation is required, these facilities are often heavily oversubscribed.

Industry applications

- Pharmaceutical Industry
- Food industry
- Detergents
- Personal care products
- Polymers
- Medical diagnostics



<http://csabusiness.com>



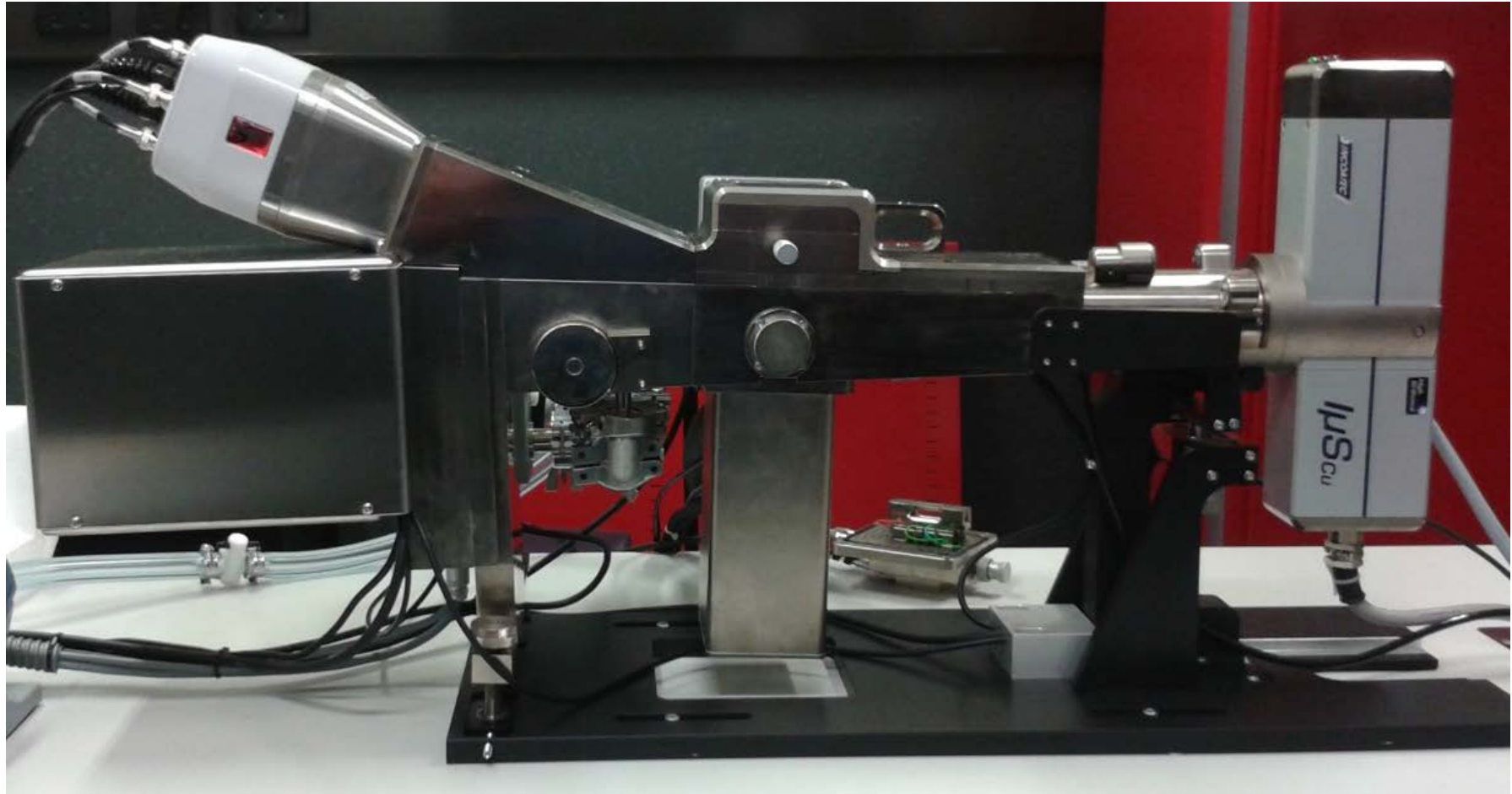
<http://www.eurmscfood.nl>



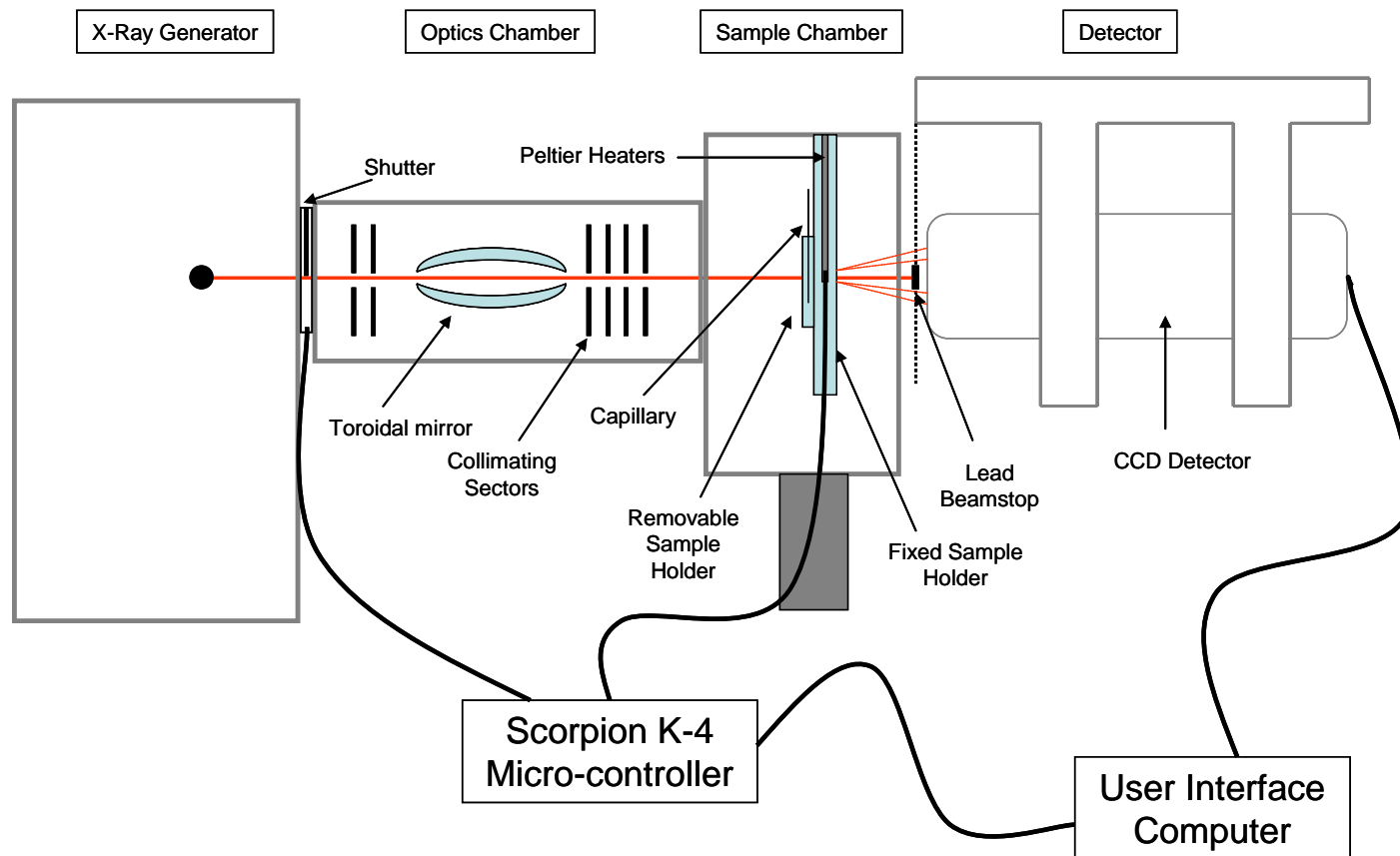
<http://www.schaefer-ph.com>

SAXS Beamline Set-up

Benchtop SAXS beamline (RMIT)



Benchtop SAXS beamline

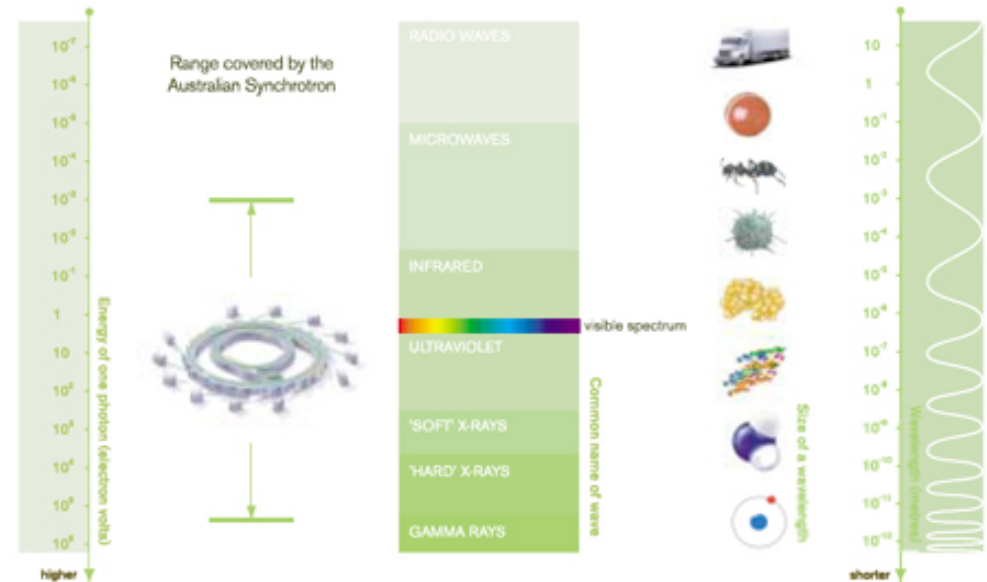
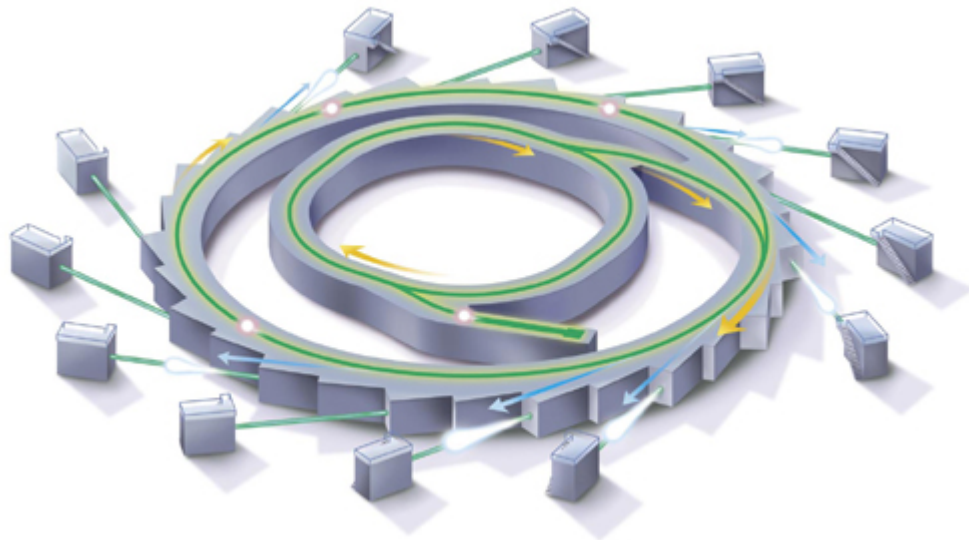


Benchtop SAXS beamline (RMIT)

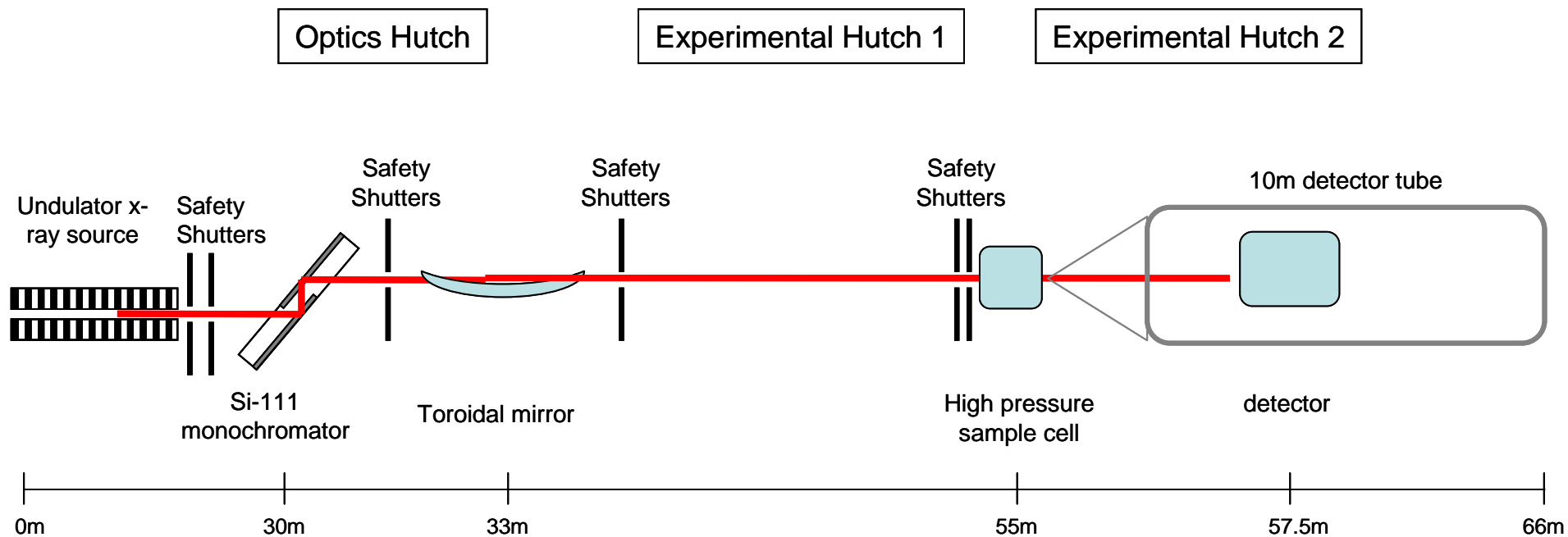
Component	Type	Characteristics
X-Ray Source	Bruker 1 μ S, air cooled	50 W, Cu Ka, $\lambda = 1.54 \text{ \AA}$
X-Ray Optics	Multilayer Montel focussing optics and 2D-Kratky collimator	FWHM < 250 μ m
Beam stops	Full elimination (W-blade) Semitransparent (Ni-blade)	Electronically micro-controlled. For beam measurement
Resolution and q range	q_{\min} (HR mode) SAXS range WAXS range	0.004 \AA^{-1} Up to $7^\circ 2\theta \sim 0.6 \text{ \AA}^{-1}$ $19^\circ - 26^\circ 2\theta \sim 0.8 - 1.7 \text{ \AA}^{-1}$
SAXS Detector	Pilatus 100k 2D solid state pixel detector. Pixelated CMOS-based silicon sensors.	Active area 83.8 x 33.5 mm ² pixel size 172x172 μ m ² 20 bit counter depth/pixel Dynamic range $\sim 10^6$ /s/pixel
WAXS Detector	VÅNTEC-1 gas detector	Active length 50 mm 1500 channels
Focusing Modes	1. HF-high flux 2. S-standard 3. HR-high resolution	10^8 photons/s $q_{\min} 4 \times 10^{-3} \text{ \AA}^{-1}$

Synchrotron SAXS

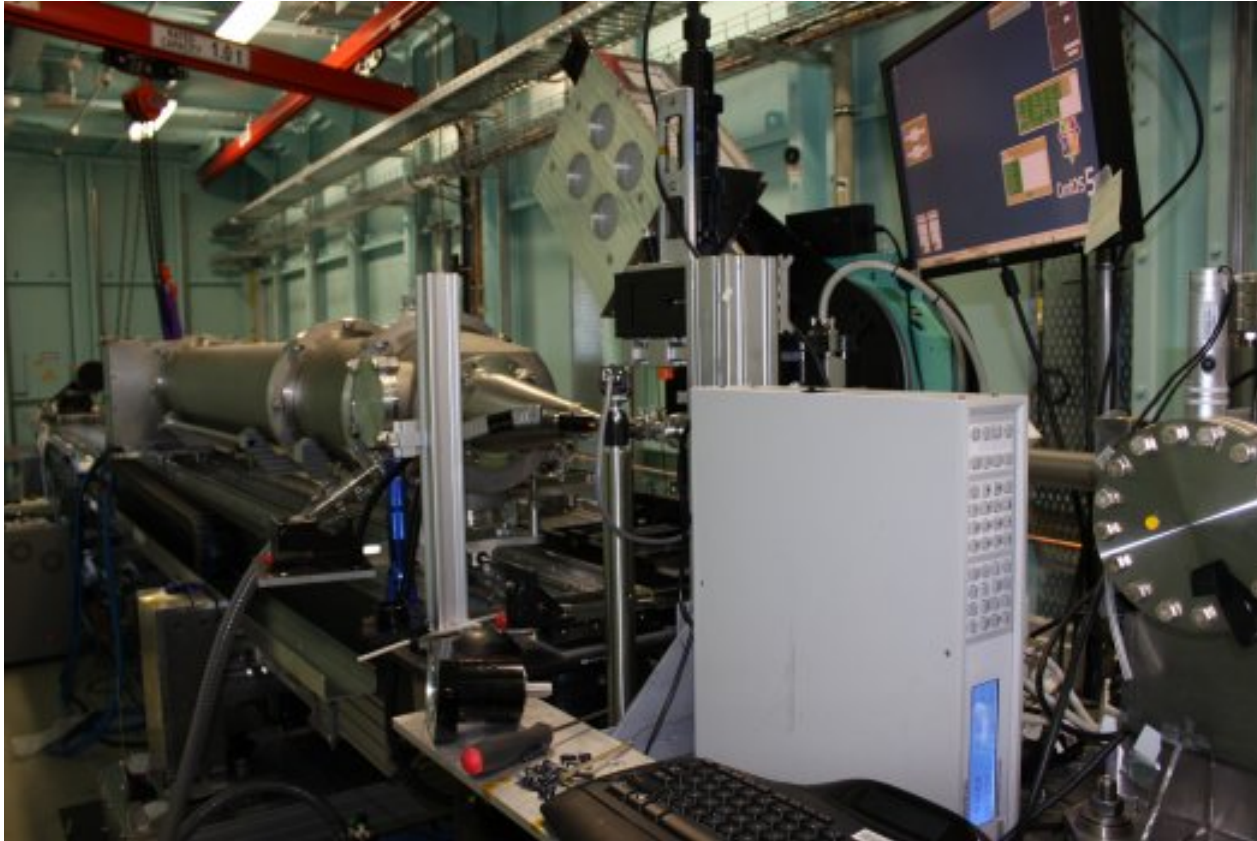
A synchrotron accelerates electrons to almost the speed of light.
As the electrons are deflected through magnetic fields they create extremely bright EM radiation.
Can be generated across the range of the EM spectrum from x-rays to IR
The radiation is channelled down beamlines to experimental workstations.



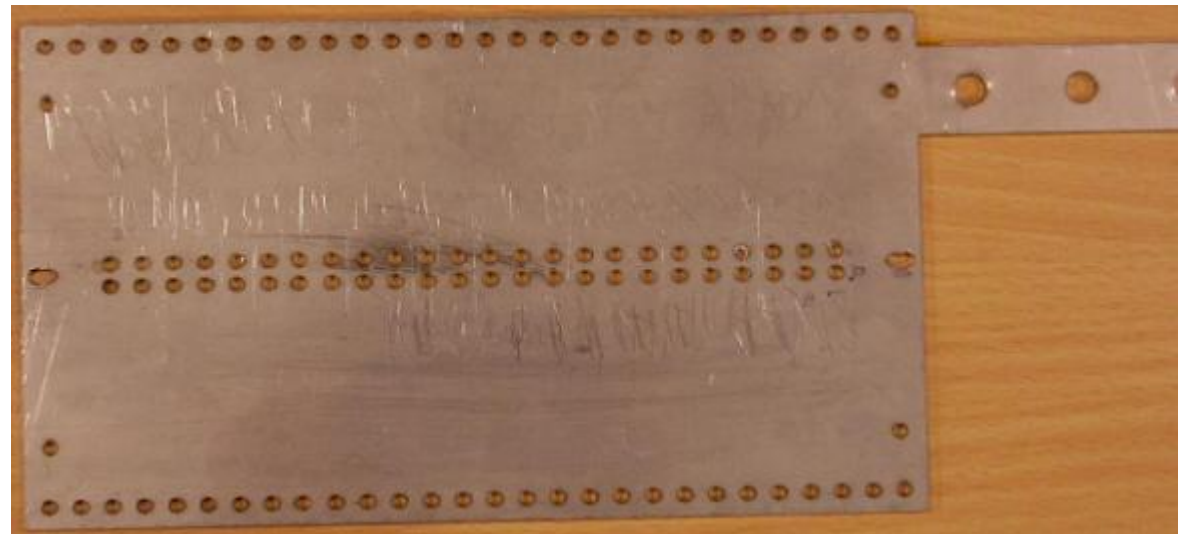
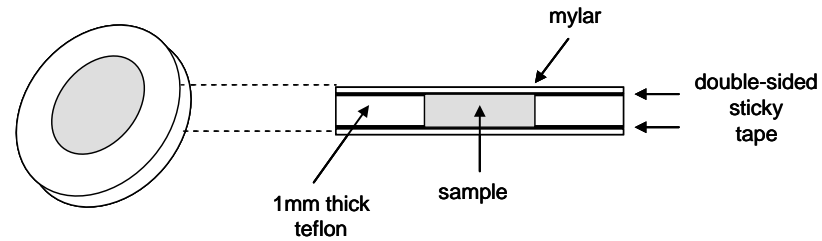
Synchrotron SAXS/WAXS beamline (ID02 at ESRF)



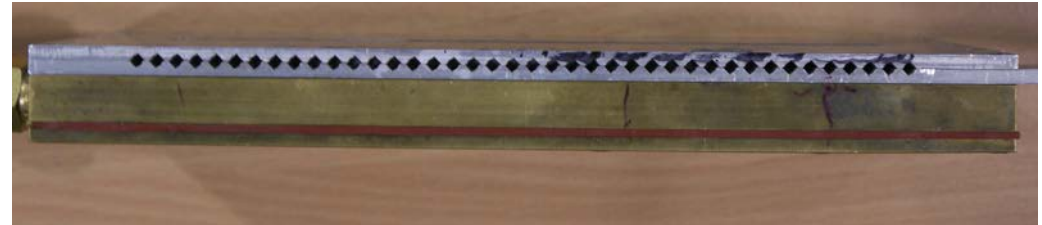
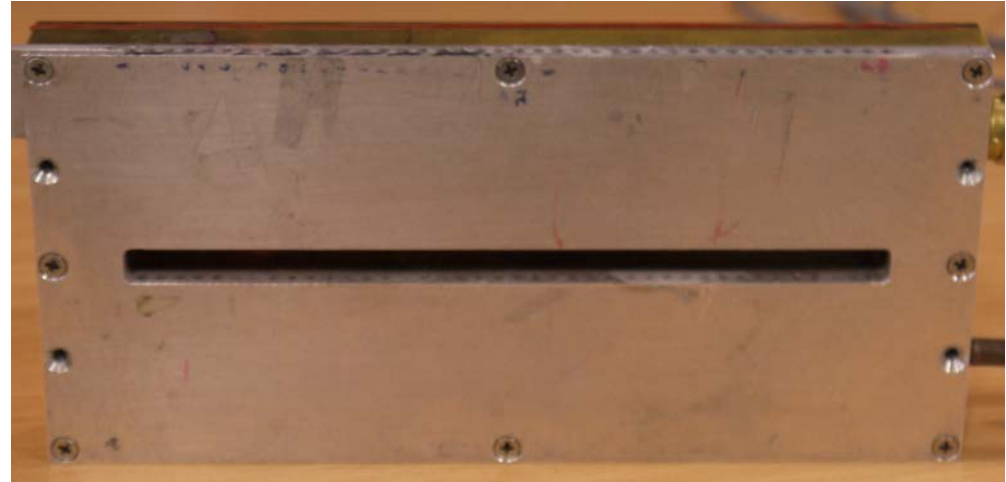
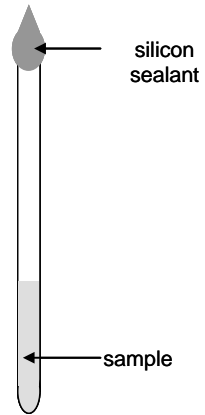
Synchrotron SAXS/WAXS Beamline at the Australian Synchrotron



Typical sample holders for SAXS – gel or solid samples



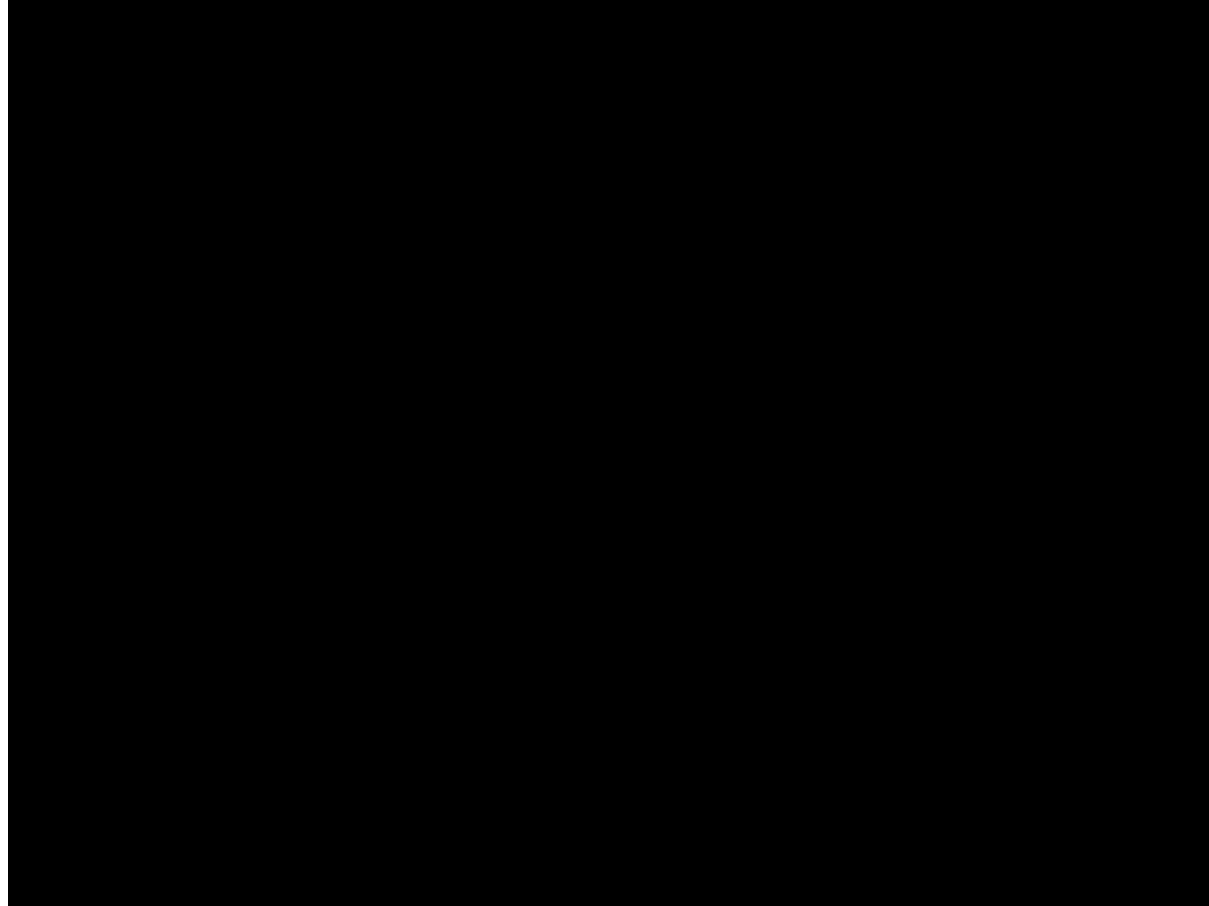
Typical sample holders for SAXS – liquid samples



Typical sample holders for SAXS – multi-well plates

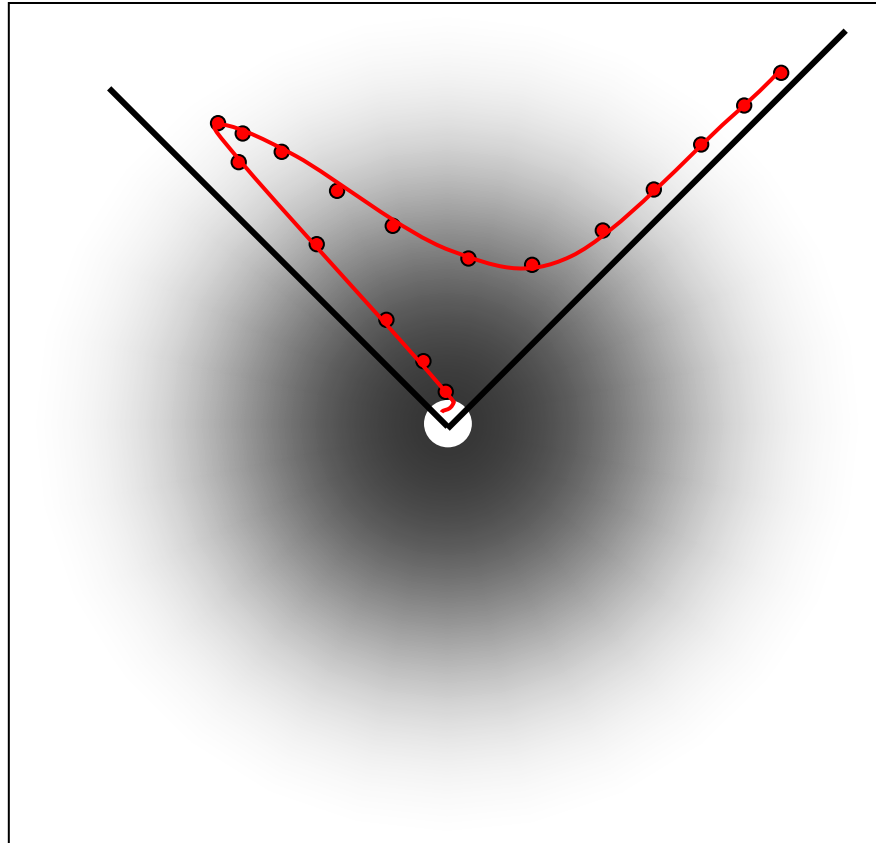


Typical sample holders for SAXS – auto-loader for protein samples



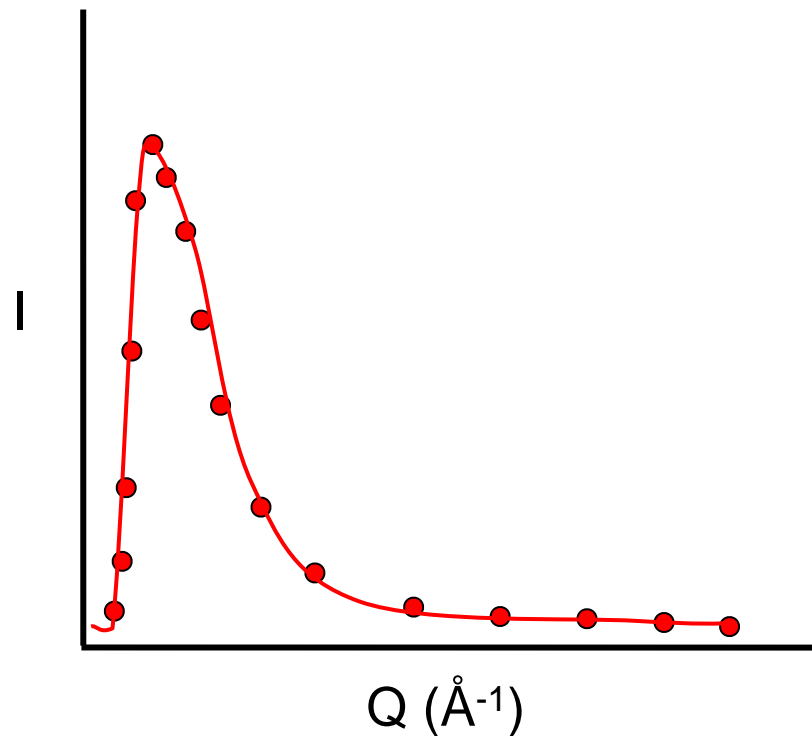
SAXS Analysis

SAXS on protein samples



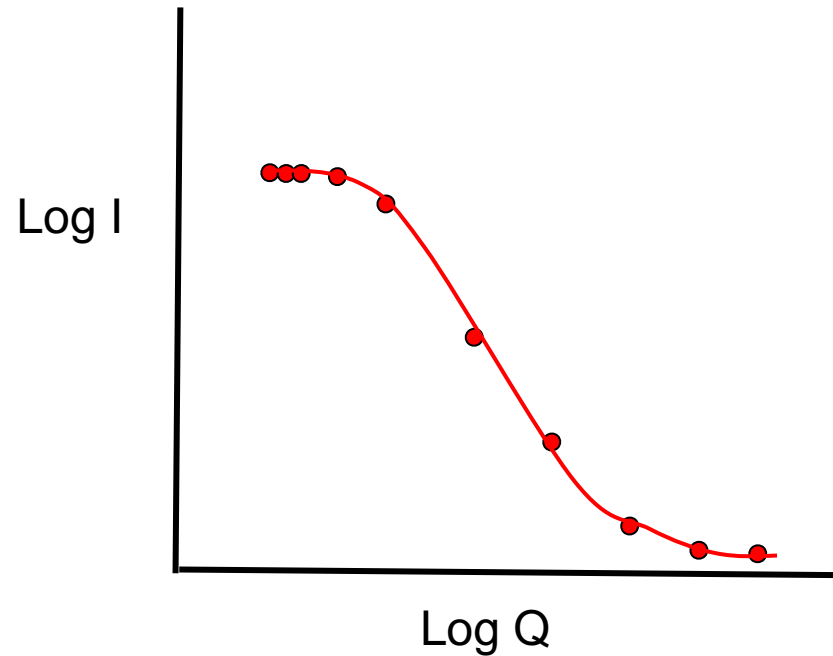
SAXS on protein samples

The Y axis shows scattered intensity (I) and is generally in arbitrary units though careful calibration of the SAXS instrument can allow an absolute measure of scattered intensity in photons.



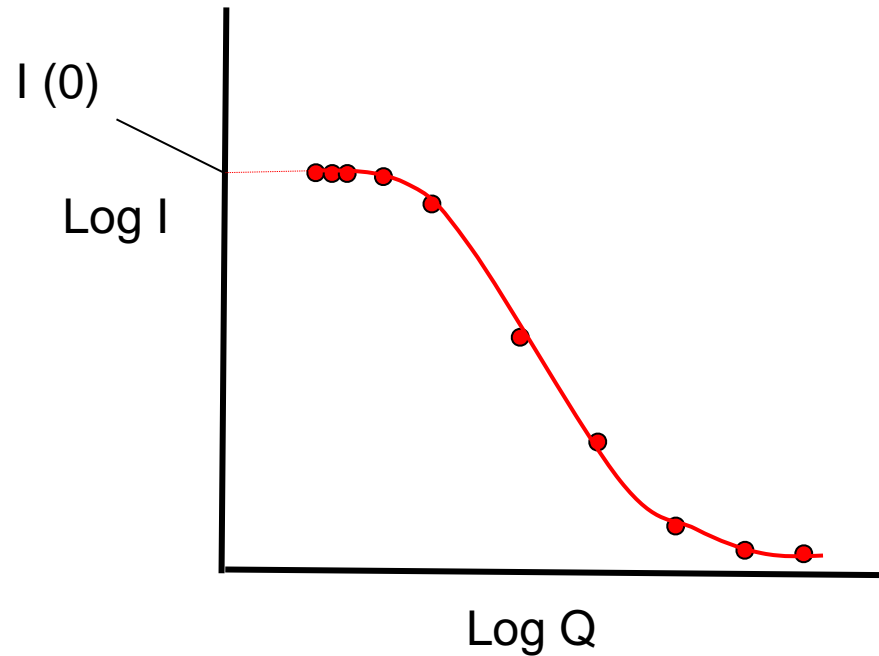
The measure 'Q' is often used instead of a simple scattering angle (θ). Q is related to angle by the formula $Q = 4\pi \cdot \sin(\theta) / \lambda$ and uses the units \AA^{-1} or nm^{-1} .

SAXS on protein samples



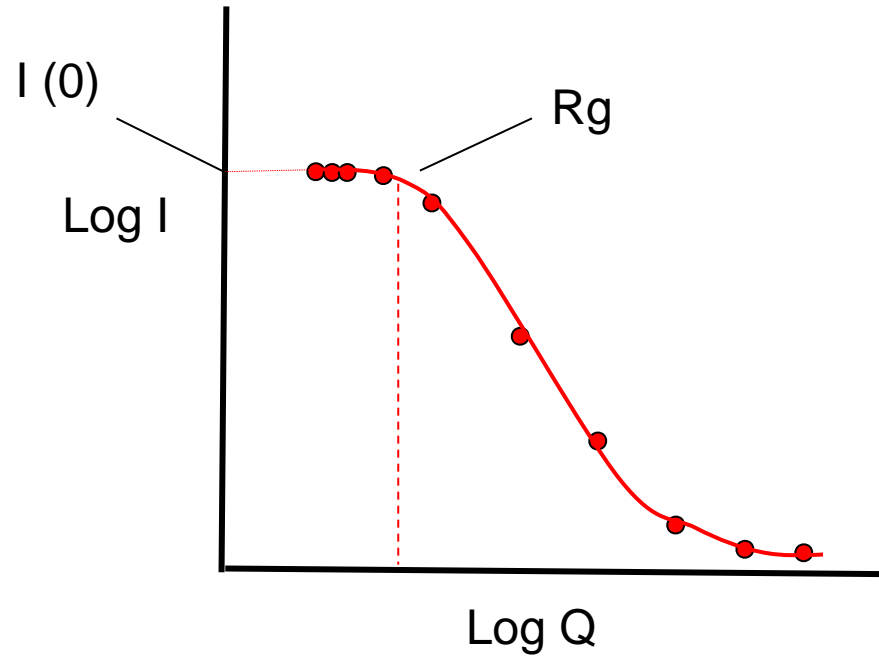
When displaying SAXS data the background shadow is generally blocked out and the data displayed on a log/log scale

SAXS on protein samples



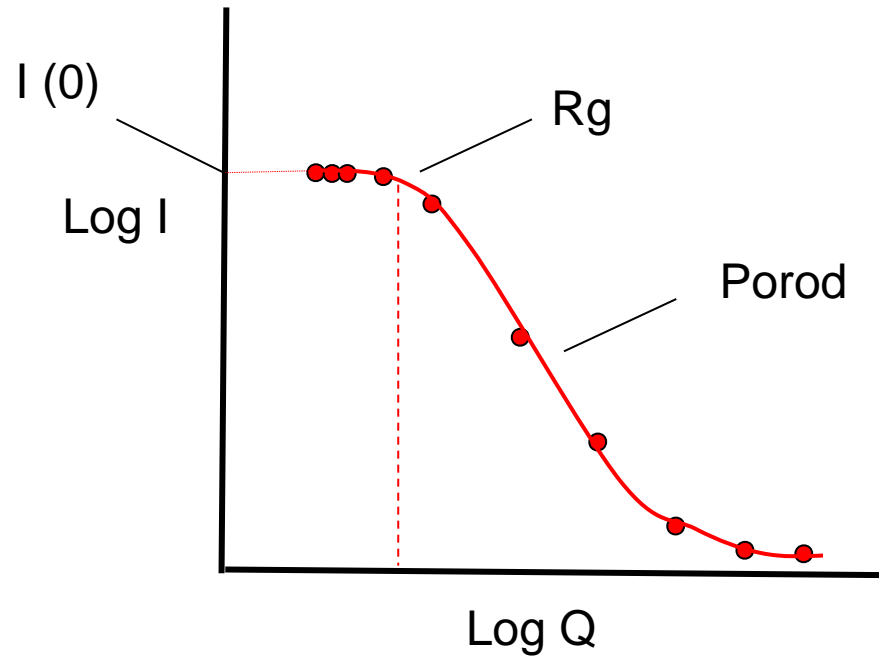
The scattering intensity at zero degrees ($I(0)$) can be estimated by extrapolating back from the plateau region at low Q . This is proportional to the particle size.

SAXS on protein samples



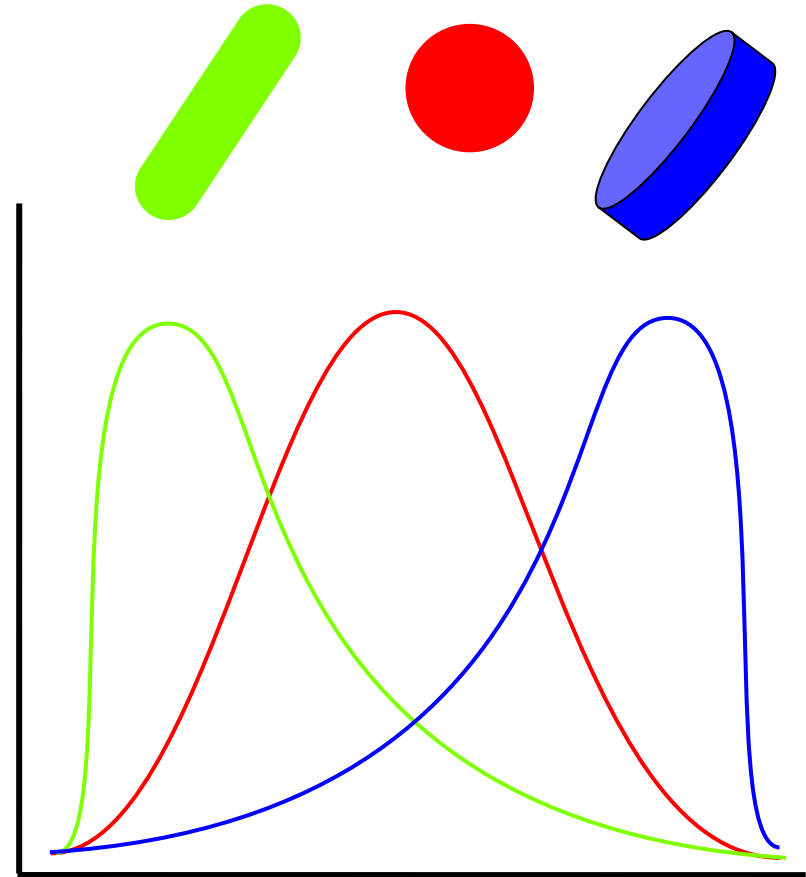
The inflection point of the curve or 'Guinier knee' is proportional to the radius of gyration (R_g) of the particle.

SAXS on protein samples



The porod region relates to the surface of the protein. It slopes away at a gradient of 1×10^{-2} in a well ordered protein. With increasing disorder the slope approaches 1×10^{-4} .

Finally, a fourier transform of the scattering curve gives $P(r)$, a function that represents a distribution of length vectors within a particle.



$P(r)$ is no longer in reciprocal units (ie \AA^{-1}) and gives a good qualitative idea of particle shape.

Concentration series to determine particle size (for monodisperse solutions)

SAXS from particles e.g. proteins in solution can be used to determine particle shape
The scattered intensity can be fitted to models for different shapes e.g. sphere, flat, rod-type particles...

$$I(q) = P(q)S(q)$$

We determine the particle shape from the form factor $P(q)$
The structure factor $S(q) = 1$ at infinite dilution

A concentration series is therefore generally run and extrapolated to infinite dilution i.e. scattered intensity from a single protein

SAXS Analysis Programs

Software for data reduction and visualisation

Scatterbrain (Australian Synchrotron)

Software for model fitting

Software for the analysis of biomolecular and fibre systems

Software for peak fitting and correlation function analysis

For an exhaustive list see:

<http://smallangle.org/content/software>

Example SAXS Analysis Program

For experimental small-angle scattering data files PRIMUS can:

- average, subtract and merge data
- extrapolate to zero concentration and curve fitting
- evaluate the integral parameters from Guinier and Porod plots such as radius of gyration (for globular, flat and rod-type particles), Porod's volume, zero intensity and molecular weight.

P.V.Konarev, V.V.Volkov, A.V.Sokolova, M.H.J.Koch and D. I. Svergun (2003). PRIMUS - a Windows-PC based system for small-angle scattering data analysis. *J Appl Cryst.* 36, 1277-1282.

SAXS for Proteins: Pros and Cons

Pros

- Solution based method for measuring structure at nm resolution.
- Can give dynamic data with < 1 second resolution
- Gives averaged data for polydispersed systems
- Very powerful for comparison of samples

Cons

- Can be challenging to interpret
- Relatively information poor
- Hard to deal with uncertainties

Popular bioSAXS applications (from Diamond website)

Flexible proteins:

- As there is no need for crystals, protein SAXS is suitable for use with flexible proteins and proteins that have proved challenging to crystallise.
- Protein SAXS can also be used to screen buffer conditions to monitor folding and for domain structure analysis to determine suitability for crystallography.

Macromolecular complexes

- The solution molecular weight of a protein or protein complex can be determined from protein SAXS which can be used to determine oligomerisation state.
- Multi-domain proteins can be characterised using data from subcomponents of a modular protein or complex.

Ligand binding

- SAXS is extremely sensitive to overall shape of macromolecules in solution and provides powerful tool to investigate conformational changes associated with ligand binding.
- Structures can be validated by comparing bioSAXS to crystallographic data.

SAXS vs Crystallography

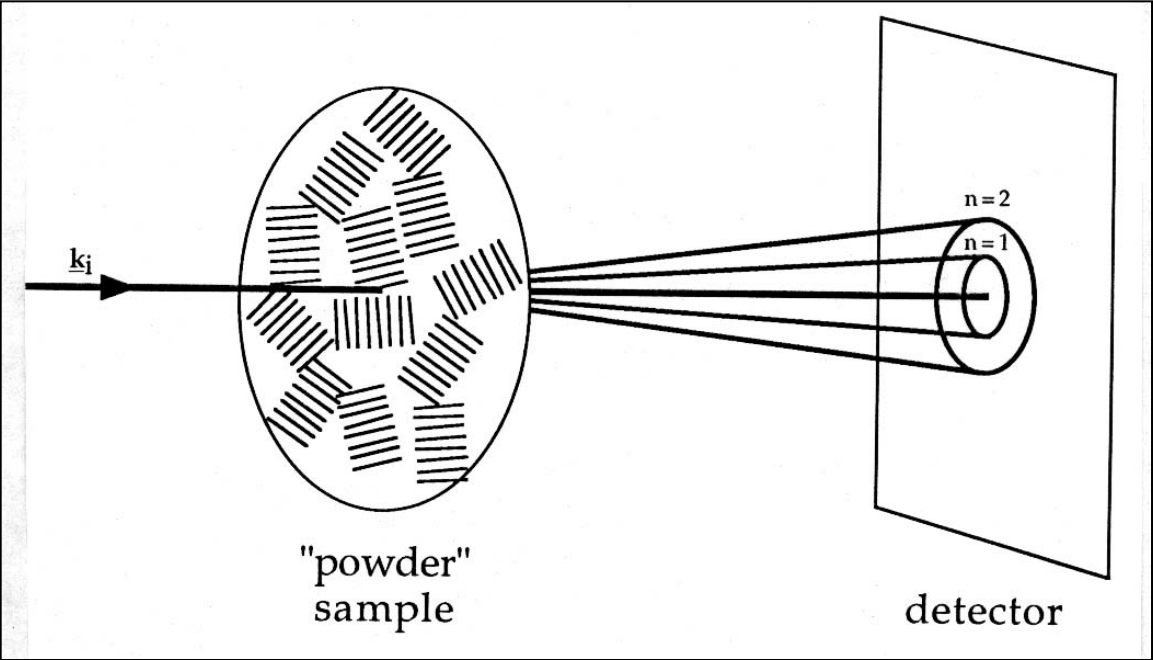
SAXS is complimentary to crystallography giving:

- Solution structure
- Oligomerisation state
- Dynamics/kinetics
- Comparative measurements (conformational change)
- Validation of computational models
- Characterisation of protein samples

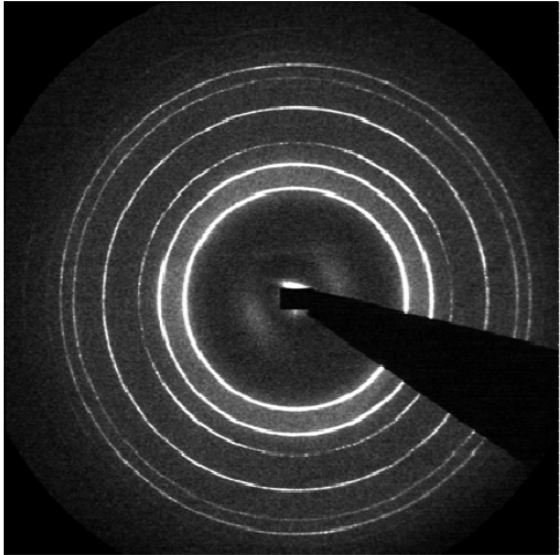
SAXS is information poor and needs careful validation and critical assessment.

SAXD Analysis

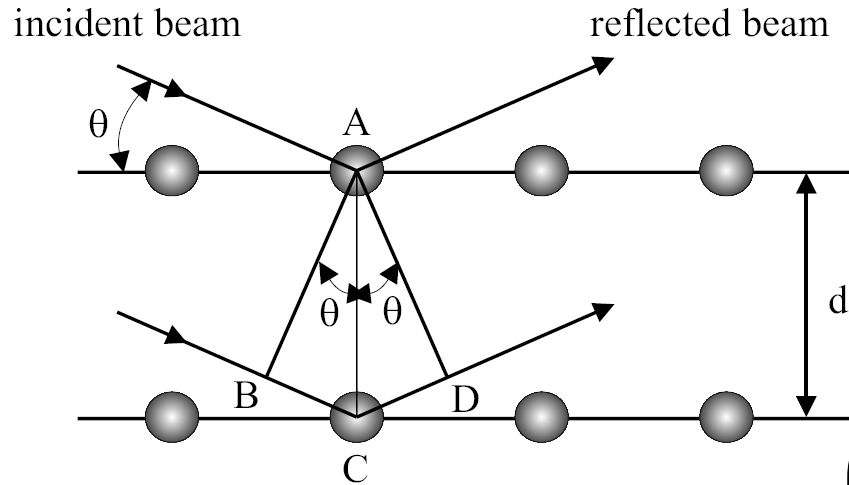
Small Angle X-ray Diffraction of ordered materials



Pn3m					
(110)	(111)	(200)	(211)	(220)	(221)
$\sqrt{2}$	$\sqrt{3}$	$\sqrt{4}$	$\sqrt{6}$	$\sqrt{8}$	$\sqrt{9}$



The Bragg Equation



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

where:

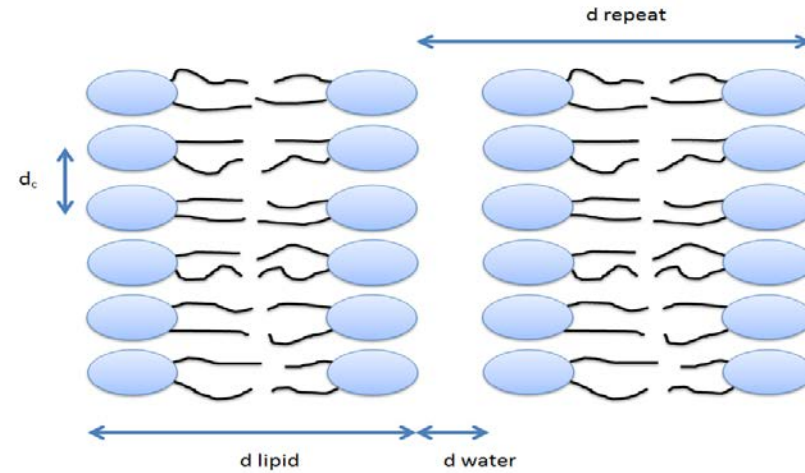
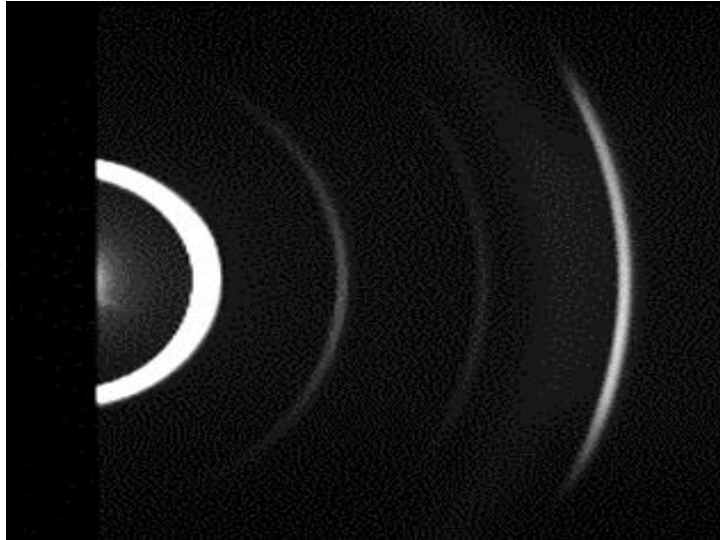
n is the order of the reflection,

λ the wavelength,

d the spacing between the parallel planes, and

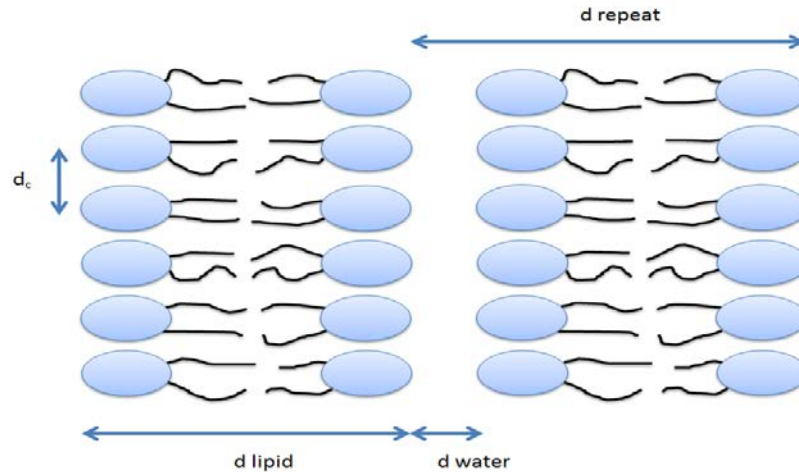
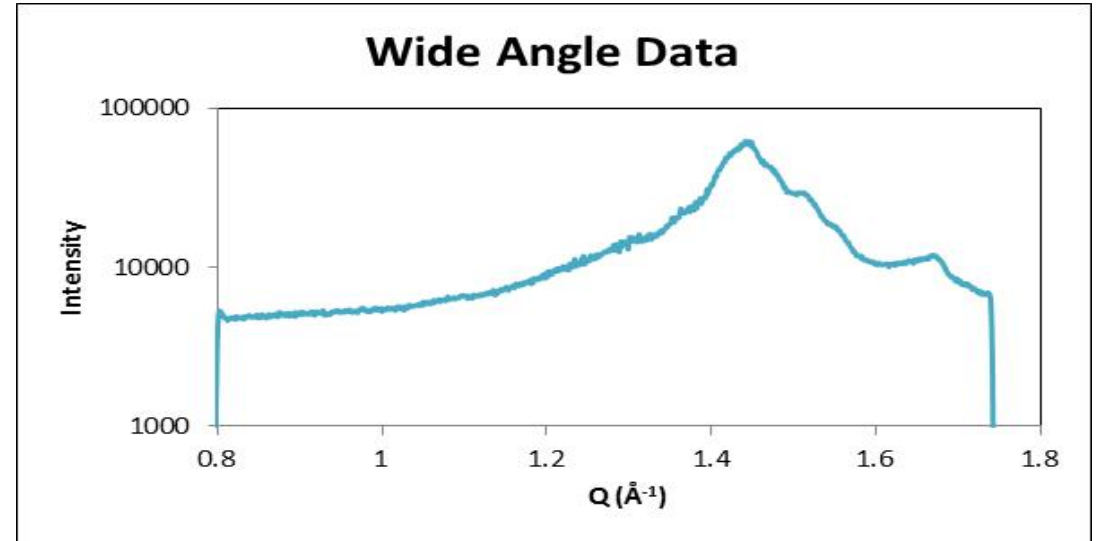
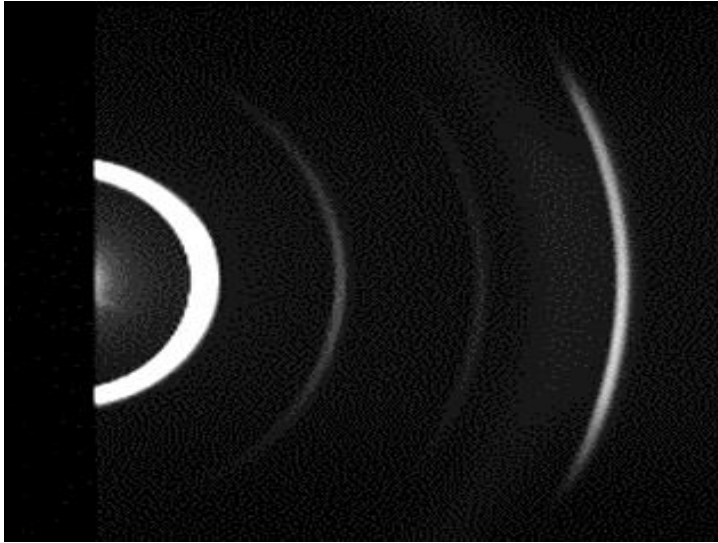
θ the angle of incidence.

SAXD on ordered materials



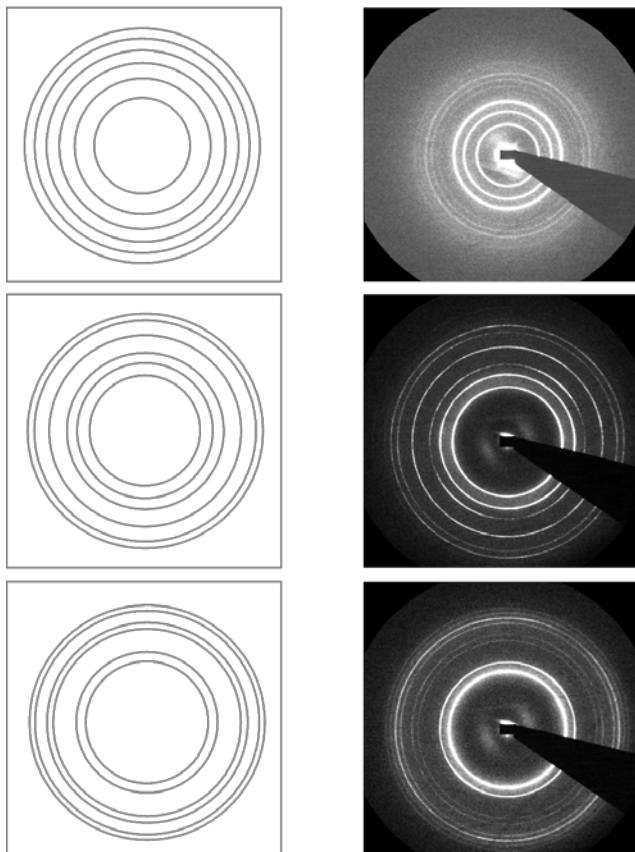
Results from fully hydrated 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)

Wide-angle X-ray Scattering



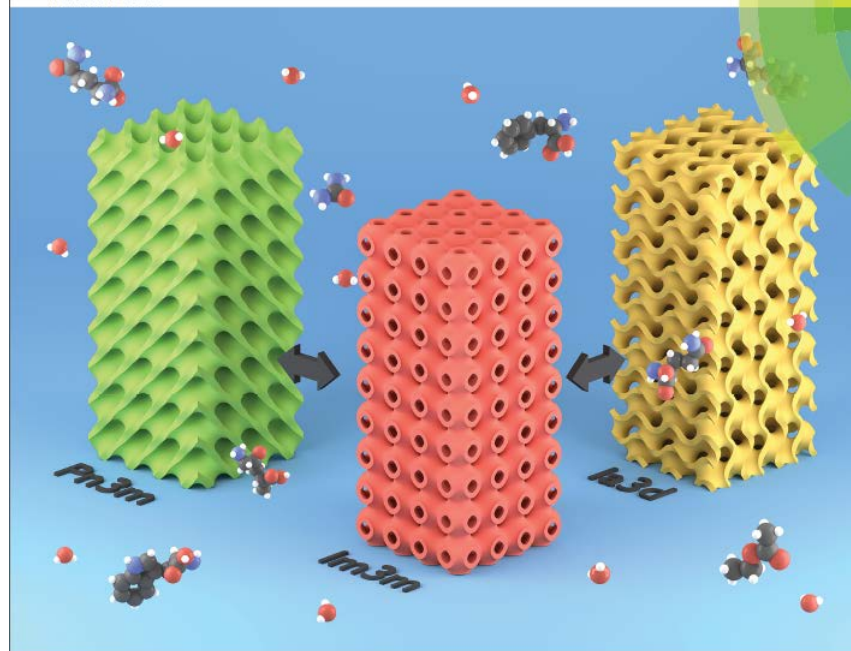
Results from fully hydrated 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)

SAXD on ordered materials



Chem Soc Rev

Chemical Society Reviews
rsc.li/chem-soc-rev

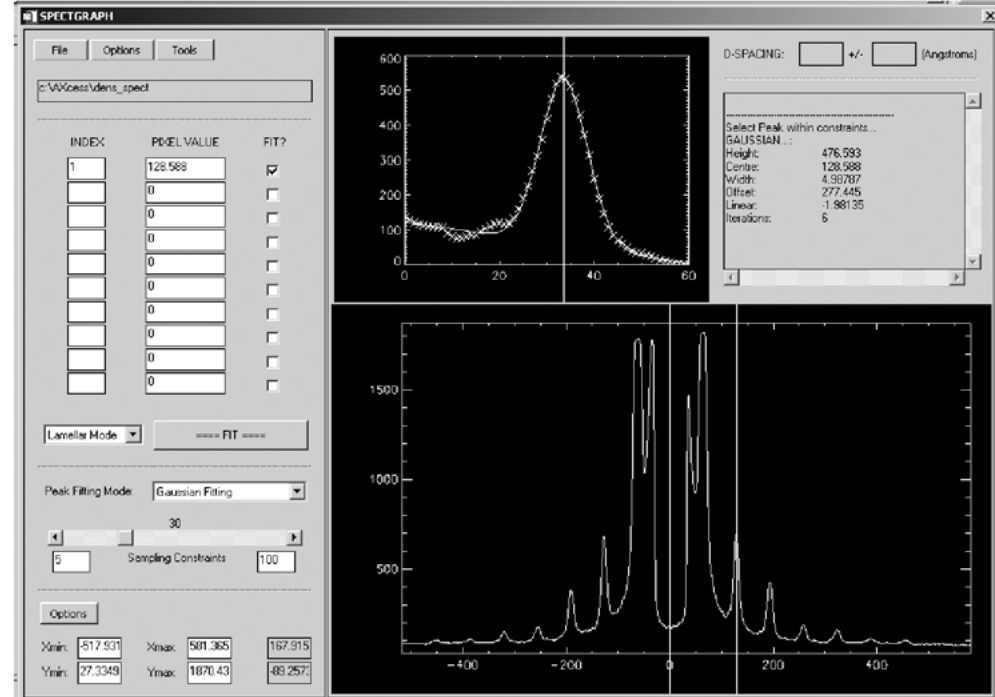
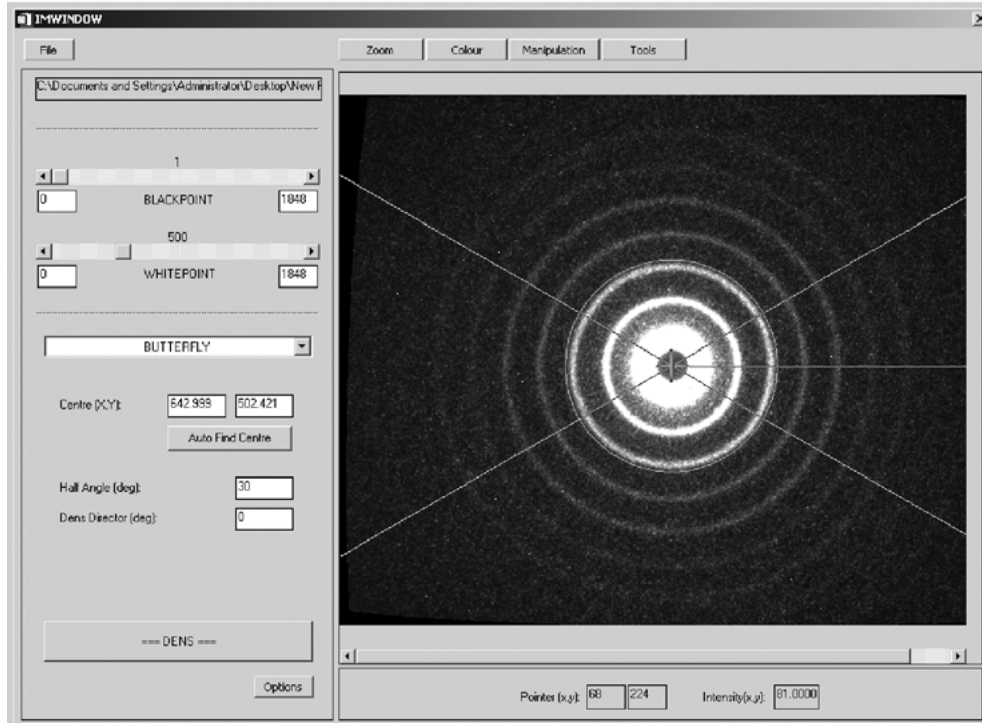


ISSN 0306-0012



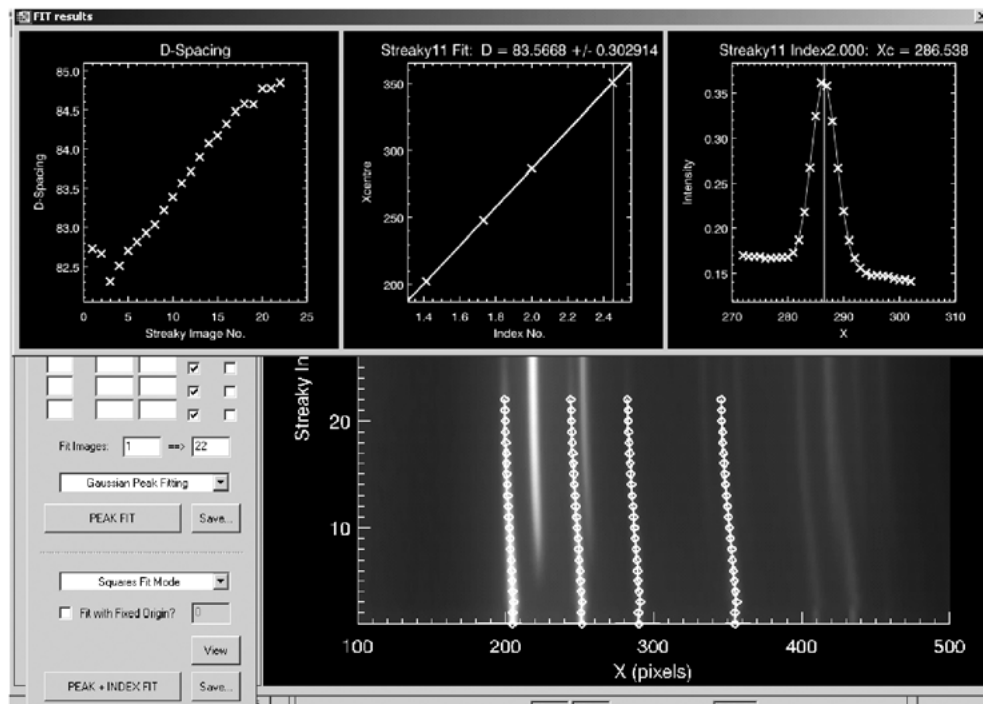
REVIEW ARTICLE
Charlotte E. Conn, Calum J. Drummond et al.
Lyotropic liquid crystal engineering moving beyond binary compositional space – ordered nanostructured amphiphile self-assembly materials by design

SAXD Analysis - AXcess



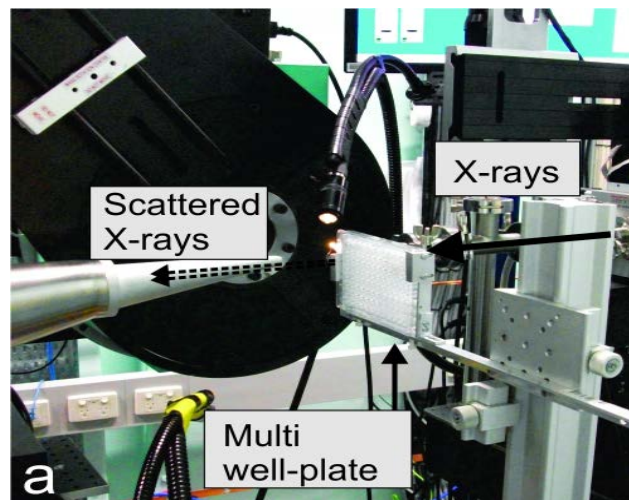
Seddon, J. M.; et al. *Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences* **2006**, 364, (1847), 2635-2655.

SAXD Analysis - AXcess



Seddon, J. M.; et al. *Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences* **2006**, 364, (1847), 2635-2655.

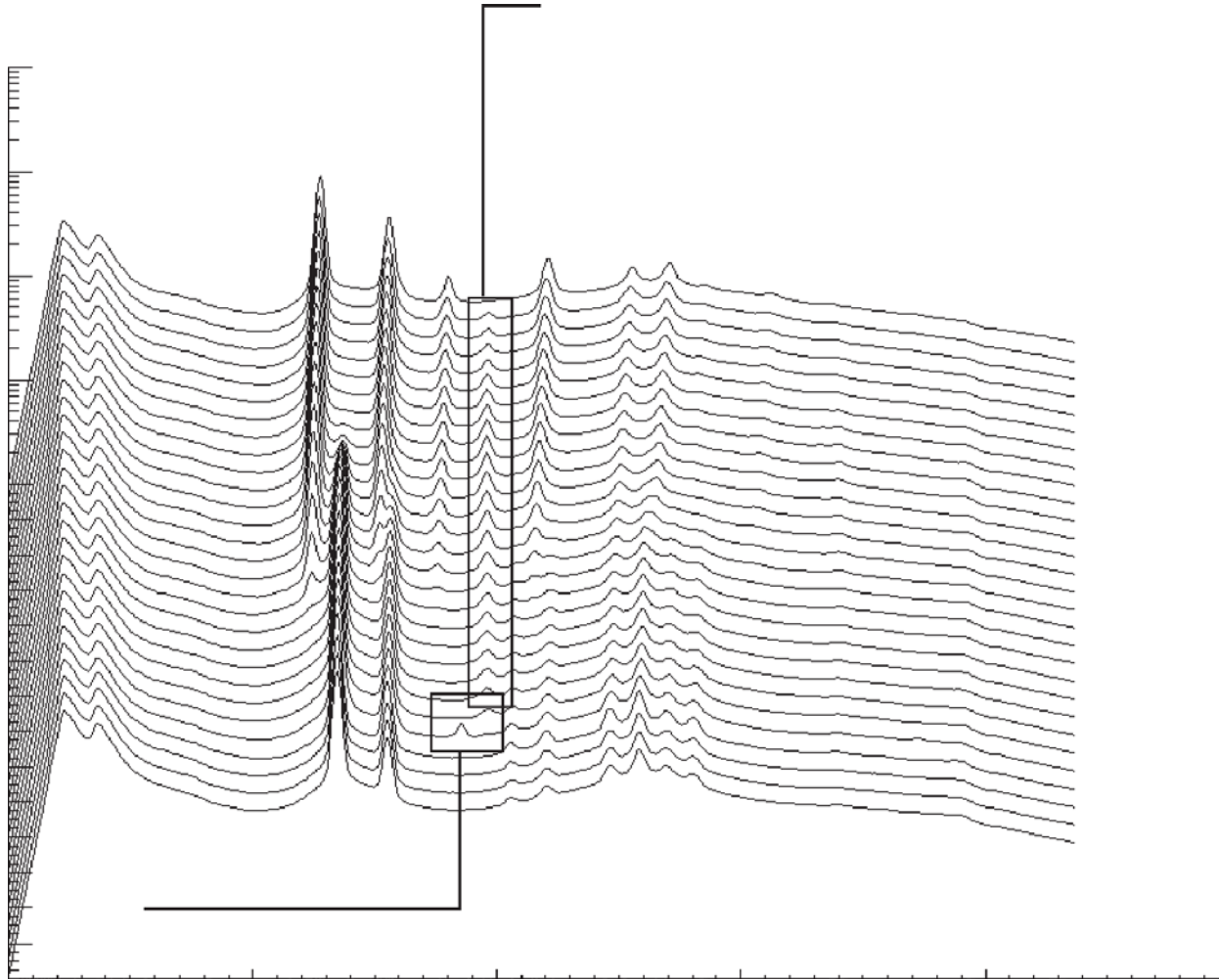
High-throughput SAXS characterisation



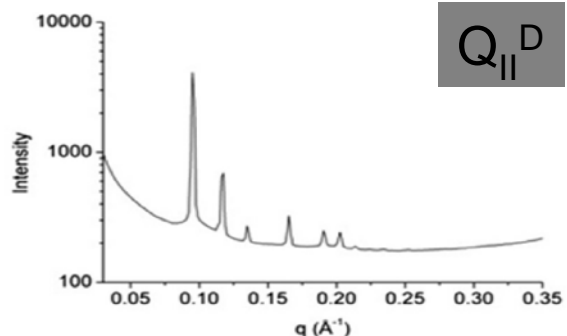
SAXS/WAXS beamline
(Australian Synchrotron)

- Fast (200 samples in 10 minutes)
- Fully automated
- Reproducible
- Temperature-control (-5 - 70°C)

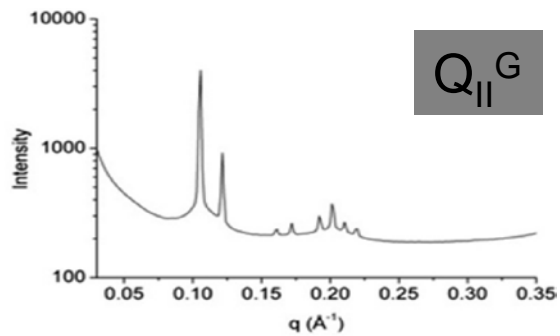
High-throughput SAXS Analysis



High-throughput SAXS analysis



Q_{II}^D



Q_{II}^G

The screenshot shows a software interface for SAXS analysis. The main window displays the following information:

- Space Group : pn3m_0.
- Lattice Parameter : $8.3386566 \pm 0.000918033$ nm.
- Chi Squared : $8.01040e-007$.
- FTEST : 26941.1.

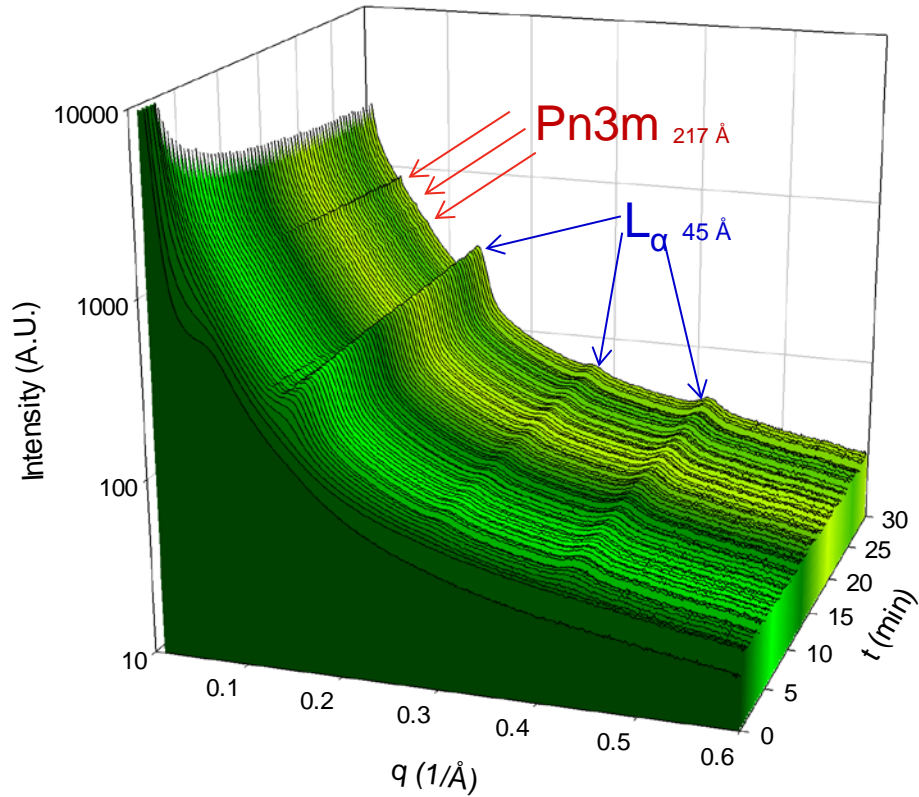
A table titled "Total No. Peaks Found : 8." is visible, showing the following data:

Space Group	Peaks Matched	Accessible Peaks	Lattice Parameter	Chi Squared
pn3m_0	5	5	83.386566	8.01040e-007
pn3m_0	5	5	83.777507	1.18721e-006
Pa_0	3	3	68.114703	6.67553e-007
I_0	2	2	58.823905	0.000000
ia3d_0	1	5	-1	-1

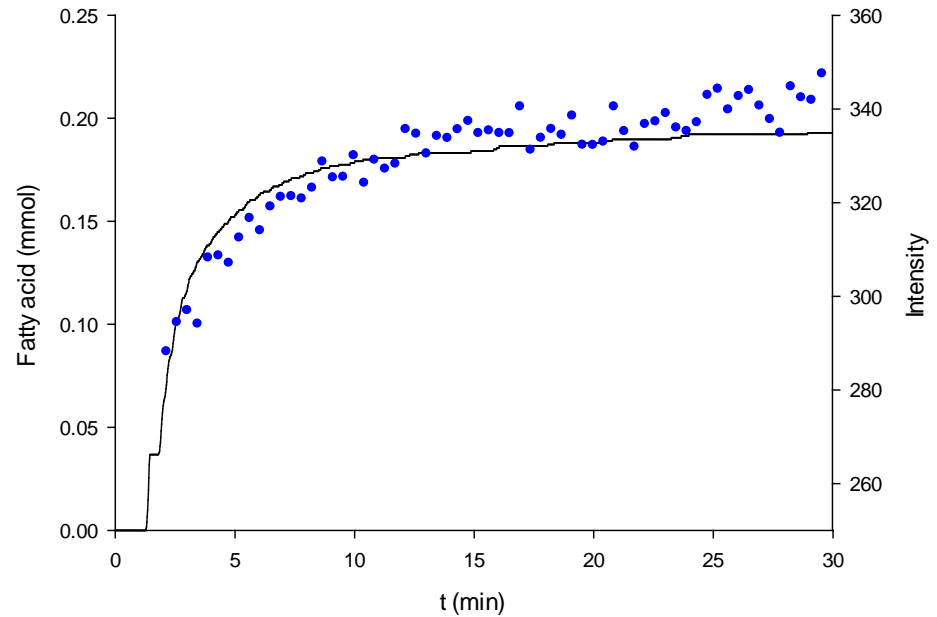
The interface also includes a list of data files (e.g., M985_20C_C9G2_0001.dat) and a "Plot" window showing a zoomed-in view of the peaks with a red line fit.

Stephen.mudie@synchrotron.org.au

Time-resolved SAXS (Prof. Ben Boyd)



Correlation between digestion kinetics and composition



Synchrotron SAXS for Materials

Advantages

- ❖ Highly intense
- ❖ Provides more structural information
- ❖ Shorter exposure time
- ❖ Can provide information on more weakly scattering samples
- ❖ Can be used for time-resolved studies

Disadvantages

- ❖ Highly intense – radiation damage
- ❖ Can be difficult to get beamtime
- ❖ Timings are constrained

How do I get beamtime?

The Australian Synchrotron holds proposal rounds three times per year

Need to submit an application via an online portal

Applications are assessed by expert reviewers in the field on three main areas:

- Scientific Quality of the Proposal
 - National Benefit
 - Experience of Participants, and outcome of previous Australian Synchrotron Experiments:
 - The need to use Synchrotron Radiation for this research
- (Try it on a benchtop SAXS first!)



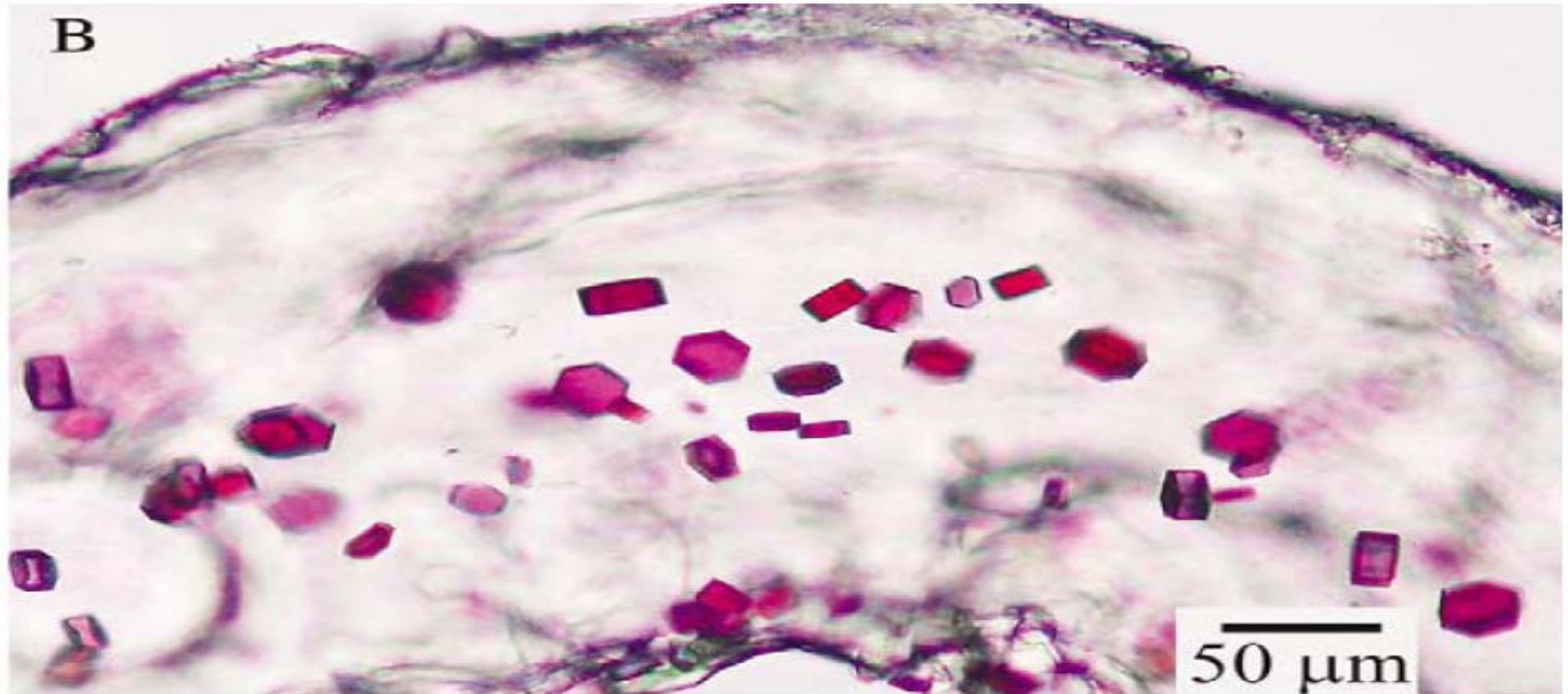
Why do I need Synchrotron SAXS?

- When scattering from the sample is very weak
 - Synchrotron provides high flux, quick acquisition for weak samples
 - Lab instrument limited by signal to noise
- Where specific properties are required
 - Tuneable wavelength
 - Variable q range (more “sizes”)
 - Need high quality peak resolution
 - Studying fast kinetic effects.
- When you have many variables to probe
 - Lab SAXS 4 – 8 hrs per sample, Synchrotron a few seconds per sample
 - Eg. Exploring a 3 phase system in 10% increments requires 36 samples. Less than a minute on Synchrotron, minimum 12 days on a lab SAXS
 - Add Temperature to this and times required quickly increase.

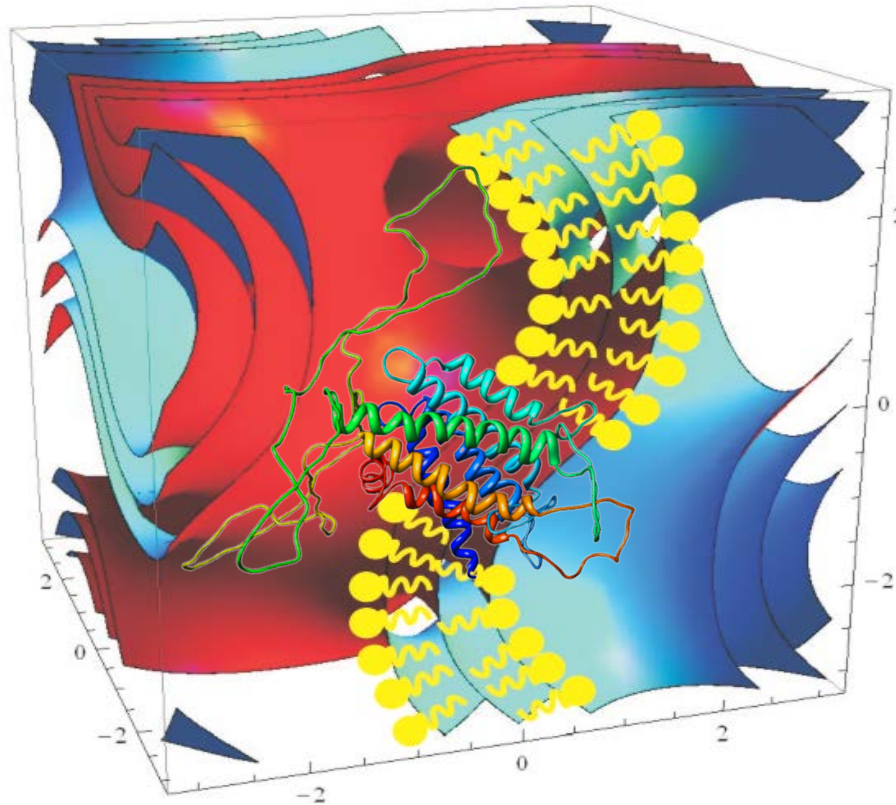
Case Study

In meso crystallisation of membrane proteins

In Meso crystallisation of membrane proteins



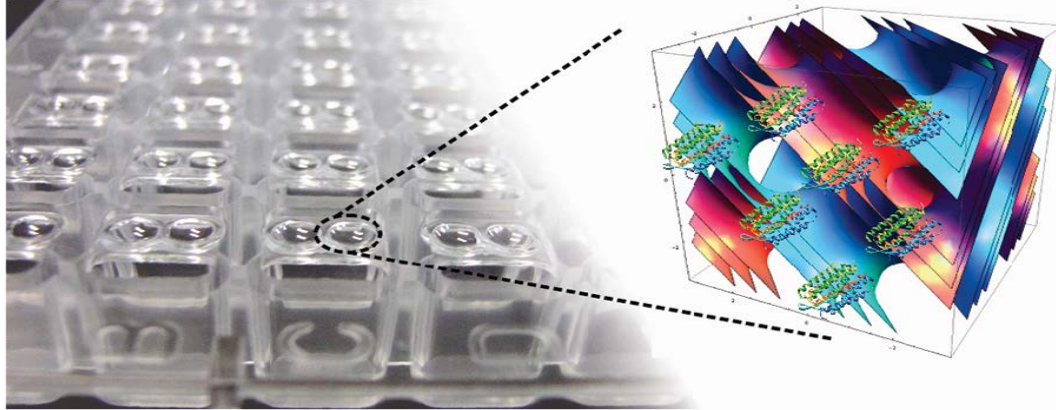
In meso crystallisation



- Viscoelastic properties similar to biological membranes
- Able to incorporate high protein loading
- Protein can diffuse across the plane of the bilayer

Conn, C. E.; Darmanin, C.; Sagnella, S. M.; Mulet, X.; Greaves, T. L.; Varghese, J. N.; Drummond, C. J., *Soft Matter* **2010**, 6, (19), 4828-4837.
Conn, C. E.; Darmanin, C.; Sagnella, S. M.; Mulet, X.; Greaves, T. L.; Varghese, J. N.; Drummond, C. J., *Soft Matter* **2010**, 6, (19), 4838-4846

Typical *in meso* crystallisation trial



	1	2	3	4	5	6	7	8	9	10	11	12
A	PEG 1500					PEG 6000						
B												
C												
D												
E												
F	PEG 3350											
G												
H												

PEG molecular weight

	1	2	3	4	5	6	7	8	9	10	11	12
A										5		
B	4	5	6	7	8	9						
C												
D												
E												
F												
G	6.5											
H	7.5											
	8.5											

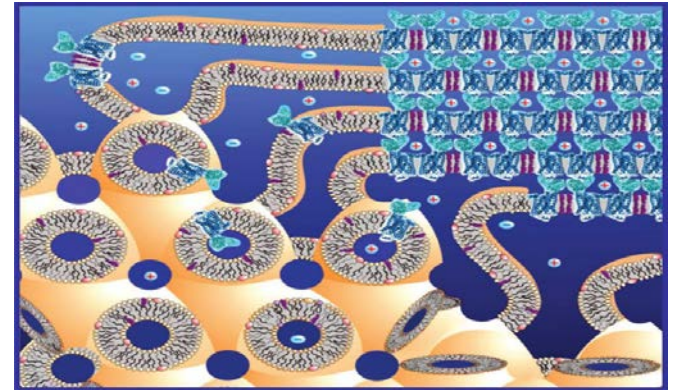
pH

	1	2	3	4	5	6	7	8	9	10	11	12	
A							NaCl	NH ₄ Cl	LiCl	MgCl ₂	CdCl ₂	ZnCl ₂	
B													
C													
D													
E													
F	NaF	NaBr	NaI	KSCN	NaNO ₃	HCOONa	CH ₃ COONa	Na ₂ SO ₄	KNa[CH(OH)(COO)] ₂	Na ₂ H/Na ₂ PO ₄	C ₂ H ₃ (OH)(COO) ₂ Na ₃	CH ₃ (COO) ₂ Na ₂	
G													

Salt

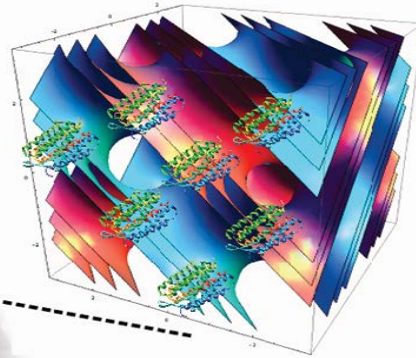
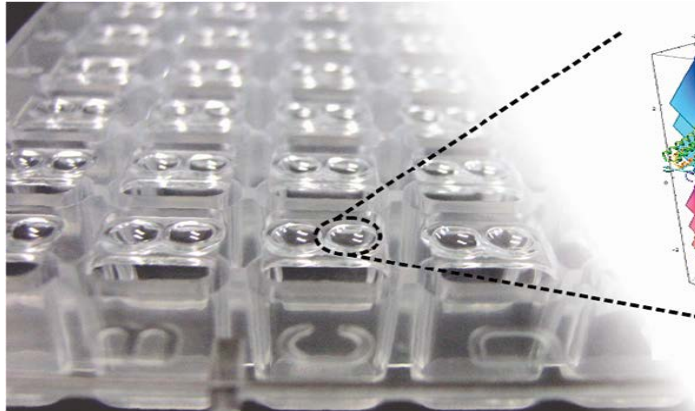
	1	2	3	4	5	6	7	8	9	10	11	12
A	Succinate phosphate glycine						Sodium acetate					
B	Malonate imidazole borate						MES					
C	Propionate cacodylate-bis tris propane						HEPES					
D	Malate MES-tris						tris					
E												
F												
G	Bis-tris propane											
H												

Buffer



*Caffrey, M. (2008) Crystal Growth & Design 8(12): 4244-4254.

Typical high-throughput crystallisation trial



+

pH

4	5	6	7	8	9	5
						6
						7
						8
						6.5
						7.5
						8.5

Polyethylene Glycol (molecular weight)

PEG (1500)	PEG (6000)
PEG (3350)	

Salt additive

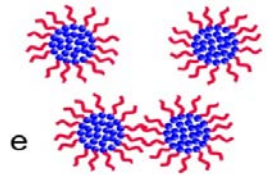
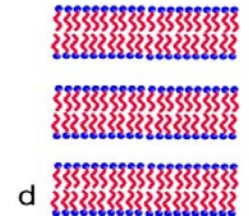
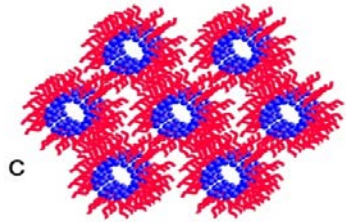
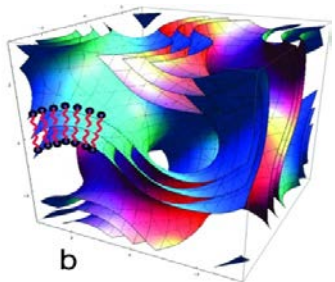
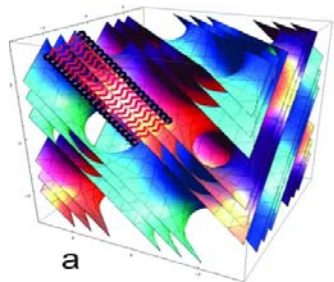
										NaCl	NH ₄ Cl	LiCl	MgCl ₂	CaCl ₂	ZnCl ₂
NaF	NaBr	NaI	KSCN	NaNO ₃	HCOONa	CH ₃ COONa	Na ₂ SO ₄	Na ₂ HPO ₄	Na ₂ H ₂ P ₂ O ₇	C ₂ H ₃ (OH)(COO) ₂ Na	CH ₃ (COO) ₂ Na				

Buffer salt

SPG	Sodium Acetate
MIB	MES
PCB	HEPES
MMT	tris
Bis-tris propane	

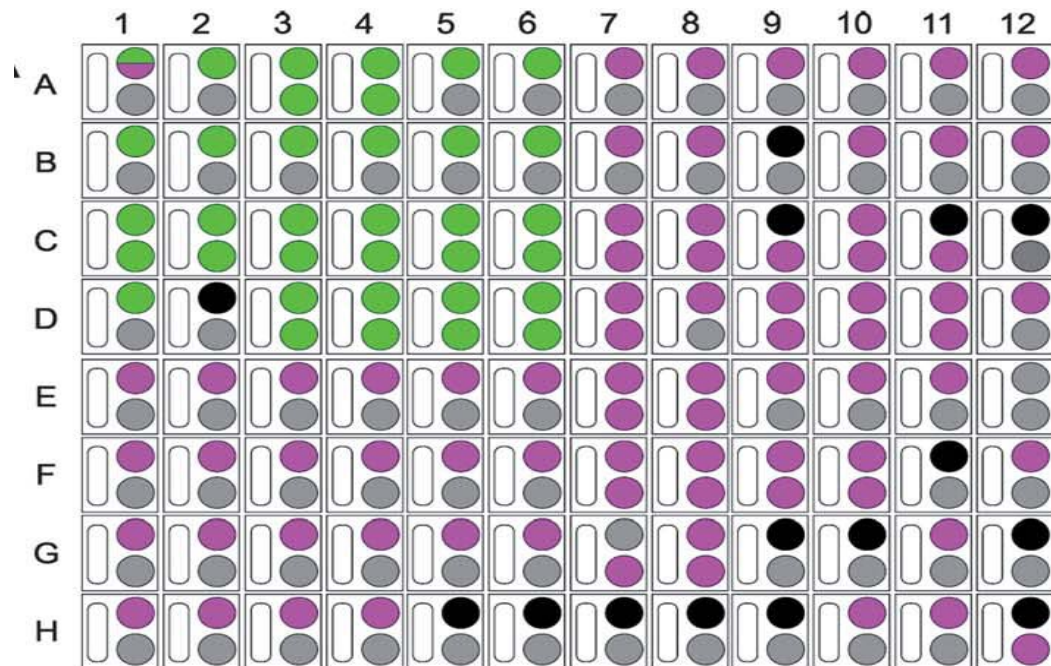


Crystallisation screen

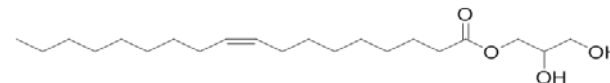


Effect of crystallisation screen components

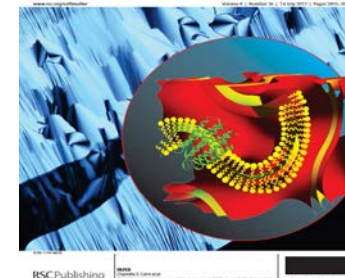
PACT Crystallisation Screen



Monolein



Soft Matter

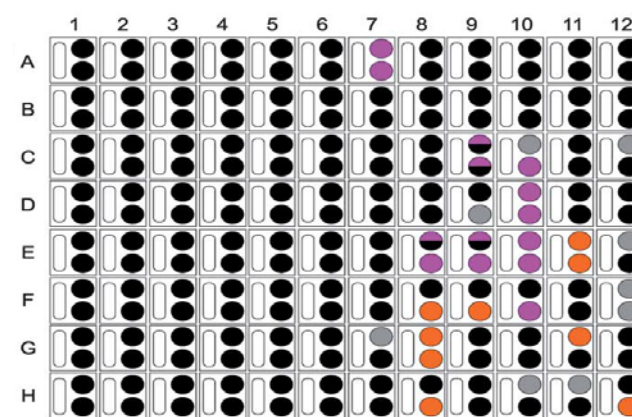
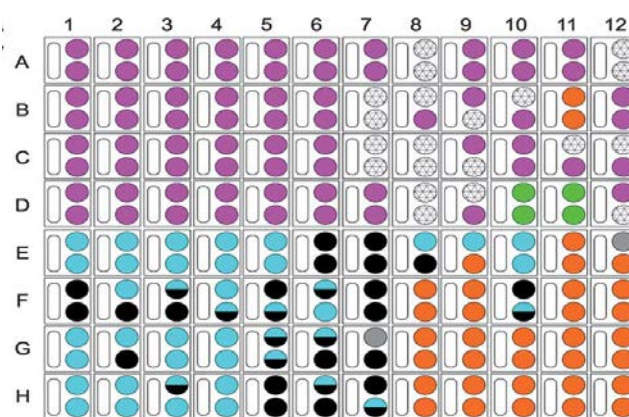
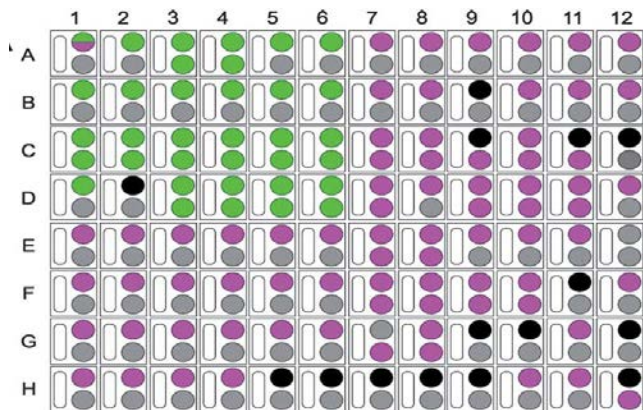


Conn, C. E.; Darmanin, C.; Mulet, X.; Hawley, A.; Drummond, C. J., Effect of lipid architecture on cubic phase susceptibility to crystallisation screens *Soft Matter* **2012** 8 (26), 6884 – 6896

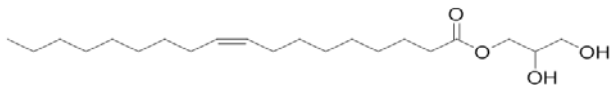
Effect of different lipids



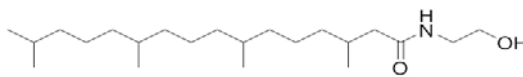
PACT Crystallisation Screen



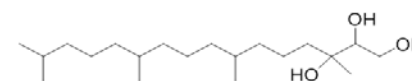
Monoolein



Phytanoyl
monoethanolamide



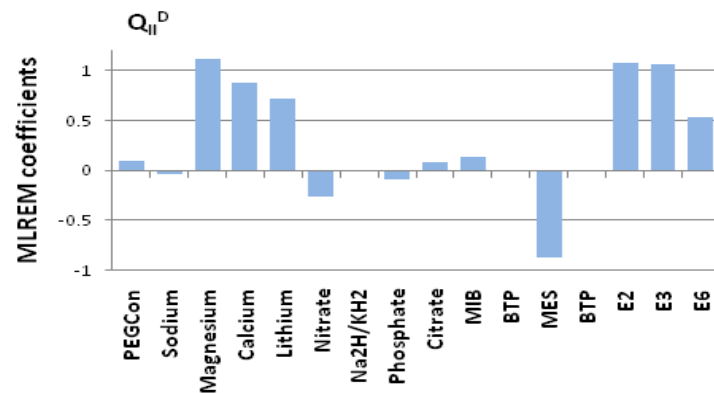
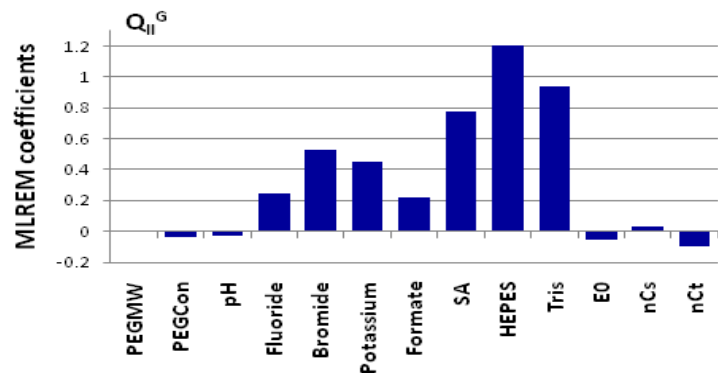
Phytantriol



How does the nanostructure of the cubic phase affect crystal growth? QSPR Modelling

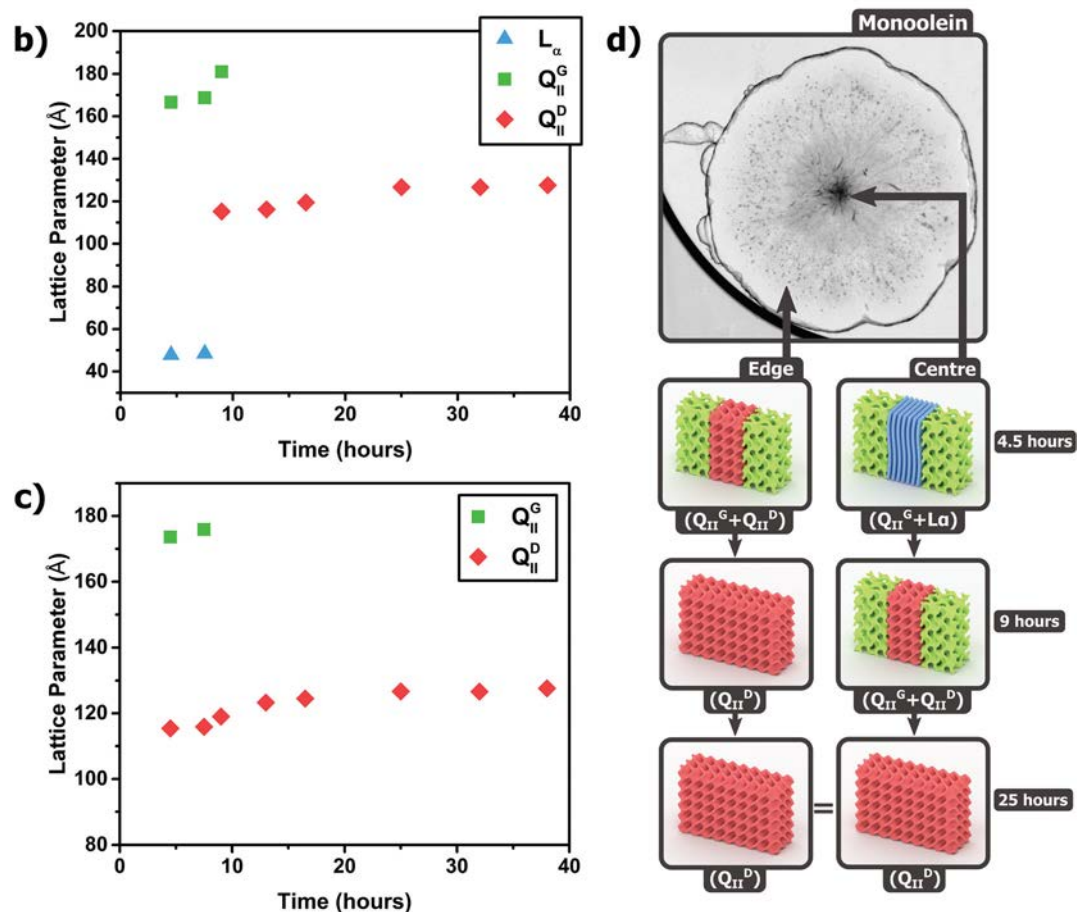
Used a Bayesian regularised neural network to generate quantitative structure-property relationships (QSPR) between

- components of the screen
- molecular characteristics of the lipid and the mesophase structure.



Le, T.; Conn, C. E.; Burden, F. R.; Winkler, D. A., Predicting the effect of lipid structure on mesophase formation during in meso crystallisation. *Crystal Growth & Design* **2013**, 13 (7) 3126-3137
Le, T.; Conn, C. E.; Burden, F. R.; Winkler, D. A., Computational modelling and prediction of the complex time-dependent phase behaviour of lyotropic liquid crystals under in meso crystallization conditions. *Crystal Growth & Design* **2013**, 13 (7) 1267-1276

In situ, time-resolved investigation of crystal growth



Zabara et al. *Nanoscale*, 2017, 9, 754

Thank you!

With thanks to Nathan Cowieson
and Matt Taylor

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