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# Small Angle Scattering from Solutions

SM$^2$ Workshop, Monash University

13th July 2022

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Science. Ingenuity. Sustainability.

# Breakdown

- **Small Angle Scattering of X-rays and Neutrons by Solutions**
  - What can we use small angle scattering to measure?
  - When would you use X-rays or Neutrons?
- **SAS Data Content & Analysis**
  - SAXS information content
  - Methods of data analysis
- **Materials-related SAS Studies in Solution**
  - Colloids & Dispersions
  - Polymers
  - Proteins

# Small Angle Scattering of X-rays and Neutrons by Solutions

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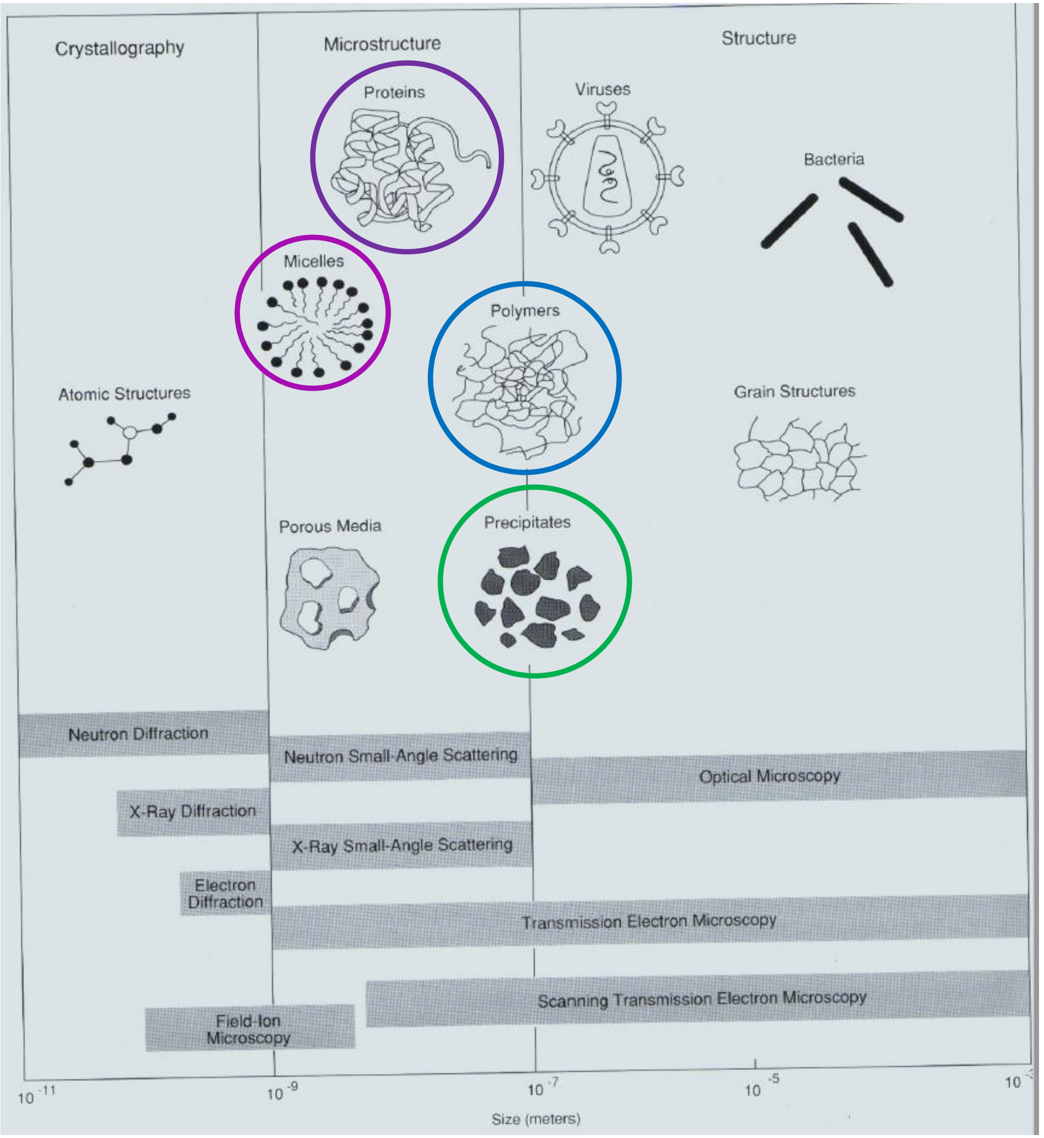
# Small Angle Scattering




**R. Pynn, Neutron Scattering A Primer**

**Small angle scattering provides (typically) low resolution structural information on nm-µm sized particles/films** including but not limited to

Proteins in solution (+ ligands/drugs)

Surfactants/Lipids in solution (colloids/self-assembly)

Polymers in solution or solid state (during polymerisation)

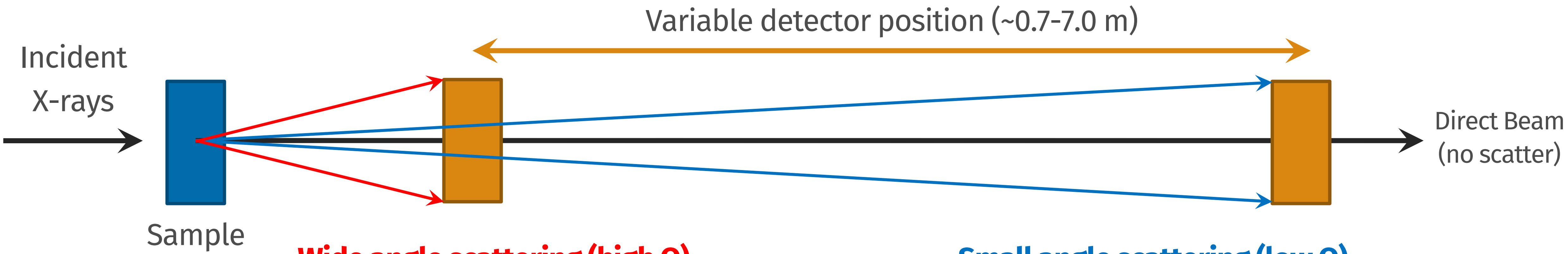
Dispersed particles/gels (drugs, minerals, food systems)

Voids in porous materials

Ions tracks in materials

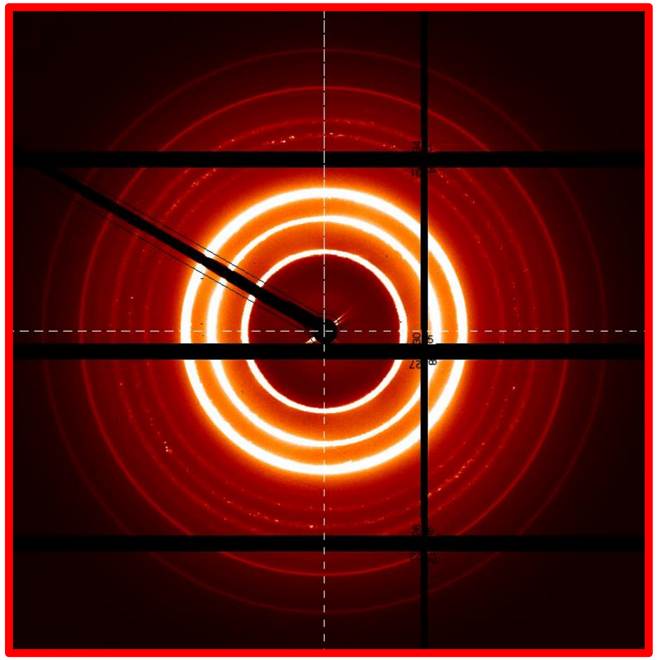
Thin films for electronics (GISAXS/reflectometry)

# Typical SAS Experiment

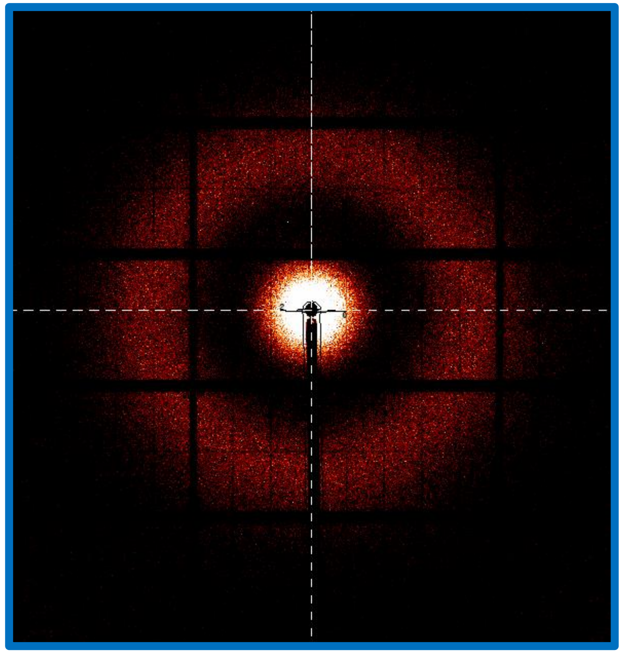



**Wide angle scattering (high Q)**
Å-nm structuring

**Small angle scattering (low Q)**
nm-µm structuring



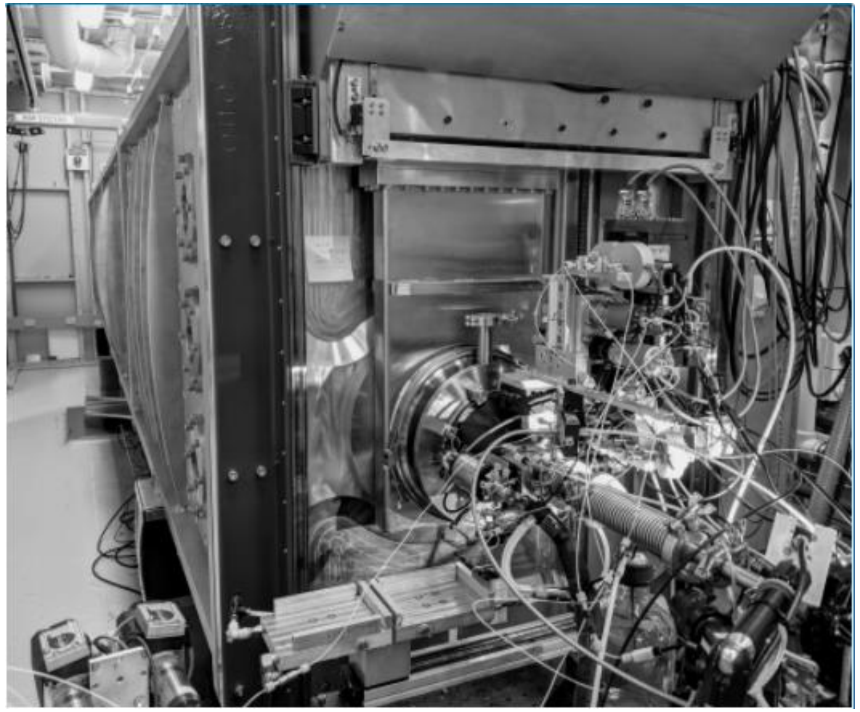
Crystals (diffraction)
Liquid crystals
Small micelles/particles



Aggregate structures
Larger micelles
Vesicles
Macromolecules
Proteins

# National Research Infrastructure in Aus

Australian Synchrotron (ANSTO Clayton, X-rays)



SAXS/WAXS – fully flexible

BioSAXS – geared up for solutions (under commissioning)

OPAL reactor (ANSTO Lucas Heights, neutrons)

QUOKKA – monochromatic SANS

BILBY – TOF-SANS & mono-SANS

KOOKABURRA - USANS

National Deuteration Facility (ANSTO Lucas Heights)

Chemical & Bio Deuteration

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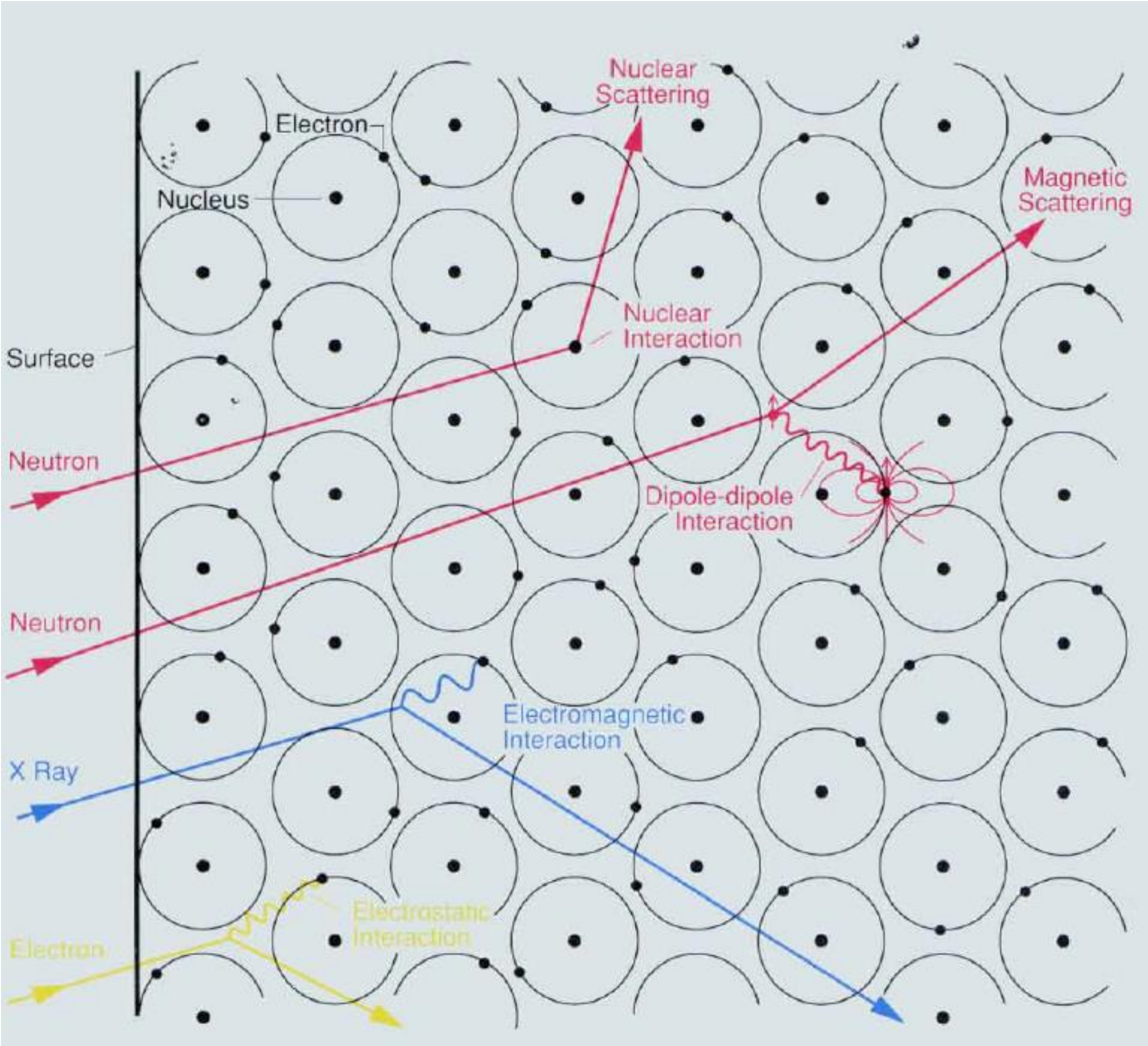
# X-rays or Neutrons?




Roger Pynn, *Neutron Scattering: A Primer*

**Neutrons (spin ½ particles)**

scattered by *nuclei and magnetic moments* (highly penetrating)

scattering power determined by nuclear composition (**isotopes**)

**X-rays (electromagnetic waves)**

scattered by *electrons*

scattering power determined by electron density (**atomic number**)

Contrast in scattering power is key to seeing anything

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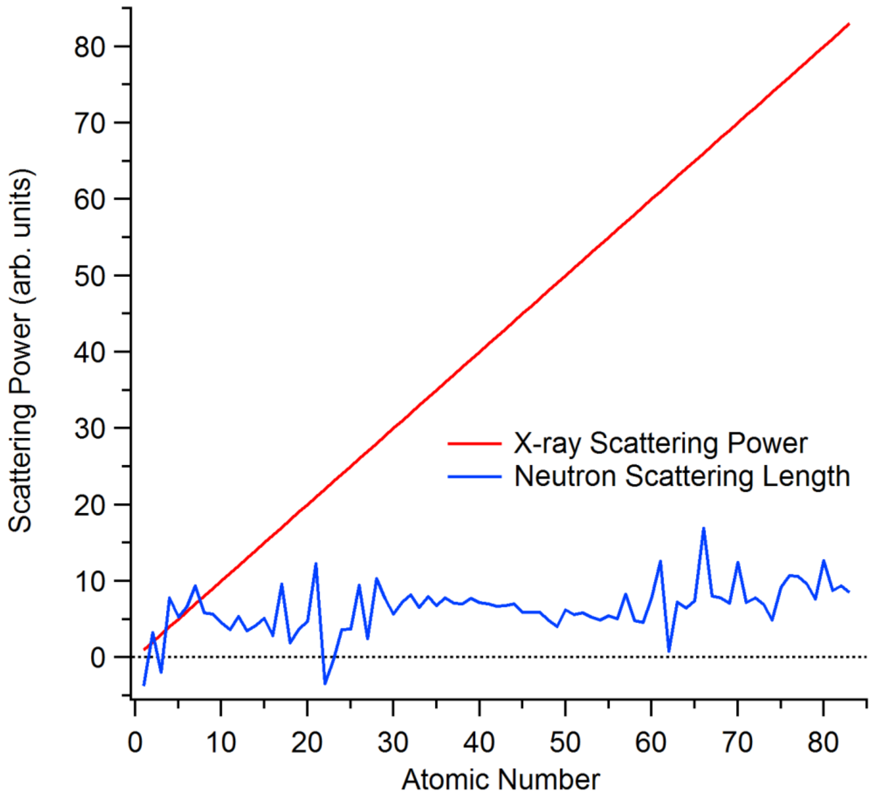
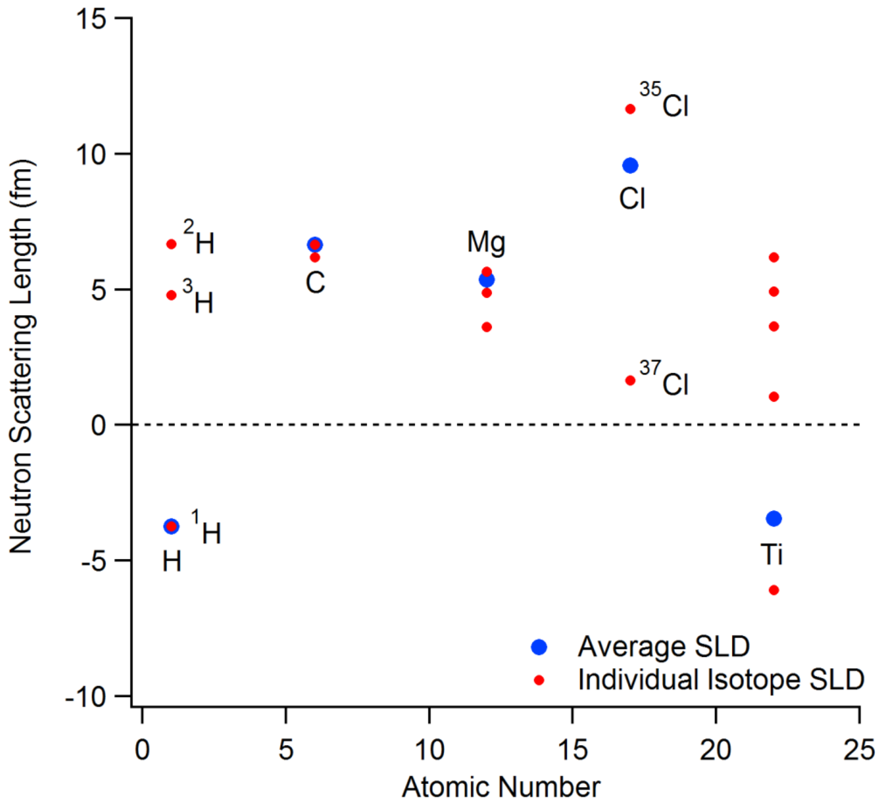
# X-rays or Neutrons? – Contrast




The ability to discern elements close together in atomic number and being able to alter contrast between sample and matrix by **molecular deuteration** are key benefits of neutron scattering

**X-ray** scattering power increases roughly linearly with atomic number

**Neutron** scattering power varies randomly with atomic number

neutron scattering power can be manipulated by **isotopic substitution**

H/D substitution is particularly useful in distinguishing organic compounds

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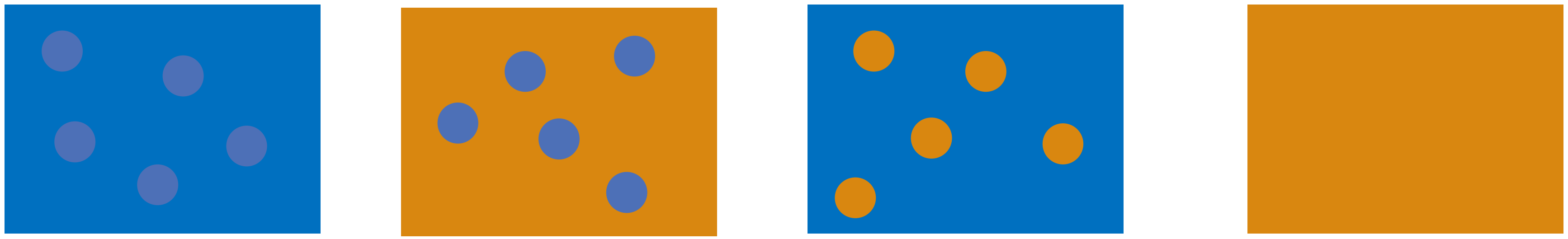
# X-rays or Neutrons? – Contrast

**Detergent particles with/without deuterated tails in water/$D_2O$**

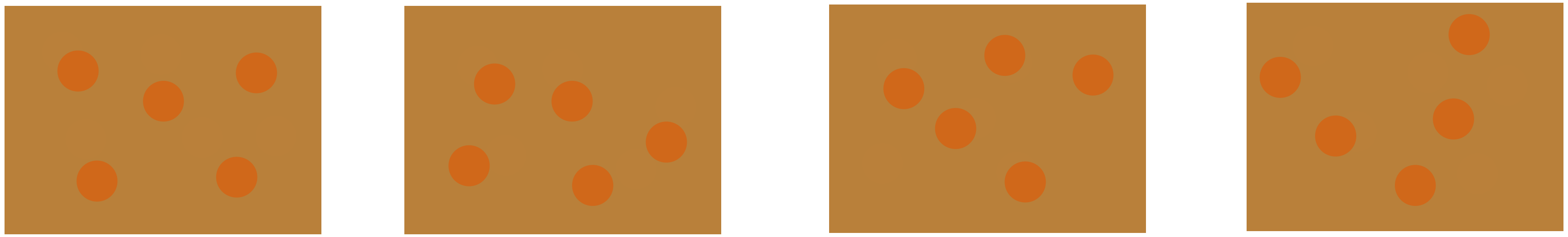
$H_2O$/detergent | $D_2O$/detergent | $H_2O$/d-detergent | $D_2O$/d-detergent



$-0.56 \times 10^{-6}$ — neutron SLD ($Å^{-2}$) — $6.41 \times 10^{-6}$

$2.93 \times 10^{-6}$

deuteration makes a profound difference to neutron contrast but not to X-ray contrast



$6.00 \times 10^{-6}$ — X-ray SLD ($Å^{-2}$) — $13.0 \times 10^{-6}$

$9.47 \times 10^{-6}$

Contrast in scattering power is key to seeing anything

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# X-rays or Neutrons? – Flux

Flux at sample (photons/neutrons $s^{-1}$)

$10^{14}$

$10^{13}$

$10^{12}$

$10^{11}$

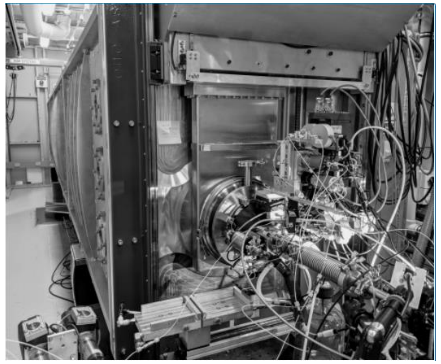
$10^{10}$

$10^{9}$

$10^{8}$

$10^{7}$

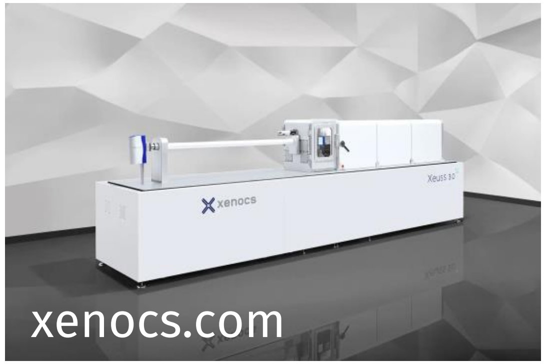
$10^{6}$



measurements on the order of **milliseconds-seconds (fast kinetics)**

Small beam spot, greater potential for sample damage by radiolysis

+




+


measurements on the order of **minutes-hours (slower kinetics)**

Neutron beams typically use a larger beam spot, samples may be activated by a neutron beam

Key benefits of synchrotron X-ray scattering experiments include their rapid nature, allowing access to fast kinetic processes and offering the ability to measure hundreds of samples a day

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# SAS Data Content & Analysis

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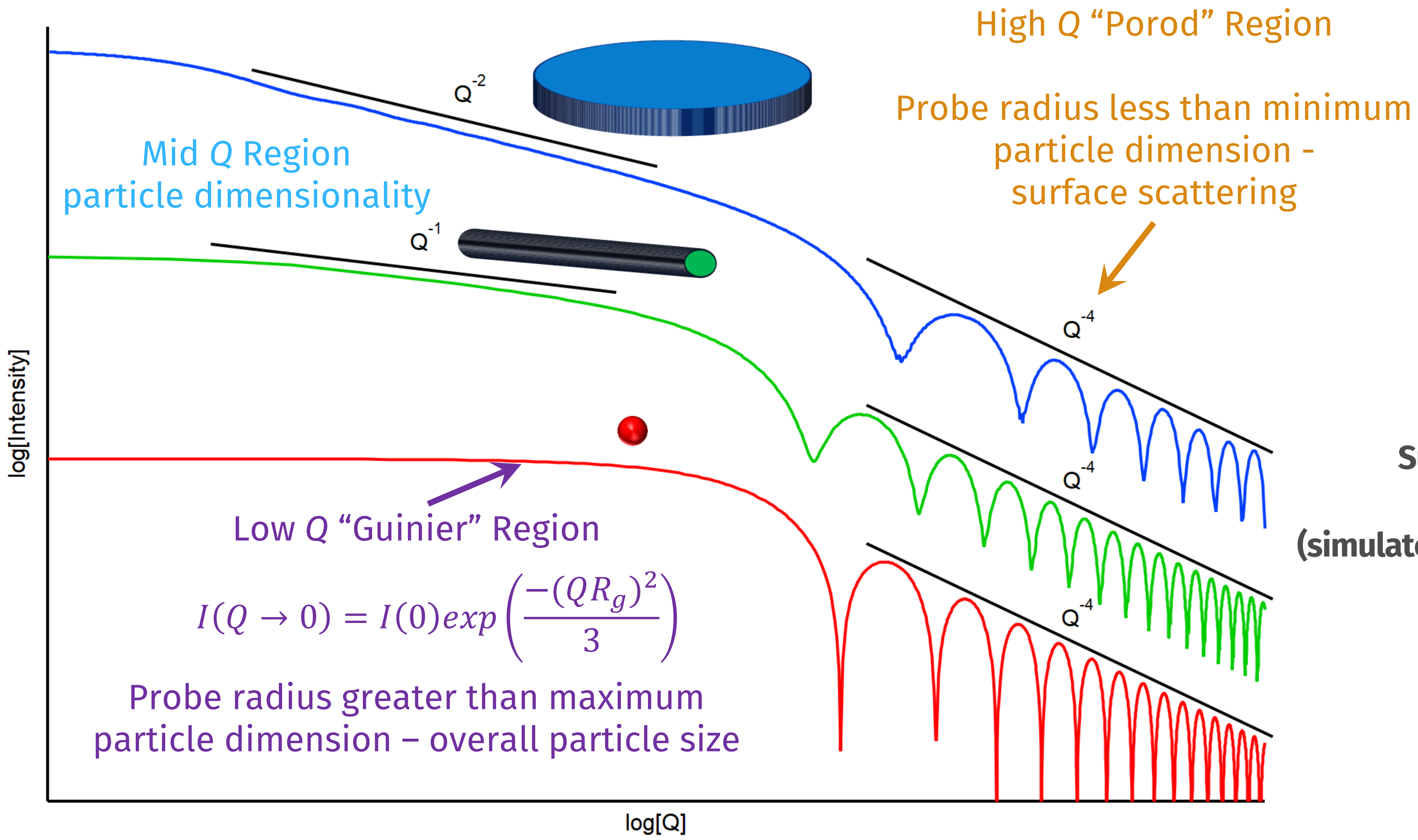
# Typical Data Content – Particle Structure




**Particle size**

**Particle shape**

**Surface roughness**

**(simulated data for ideal particle shapes)**

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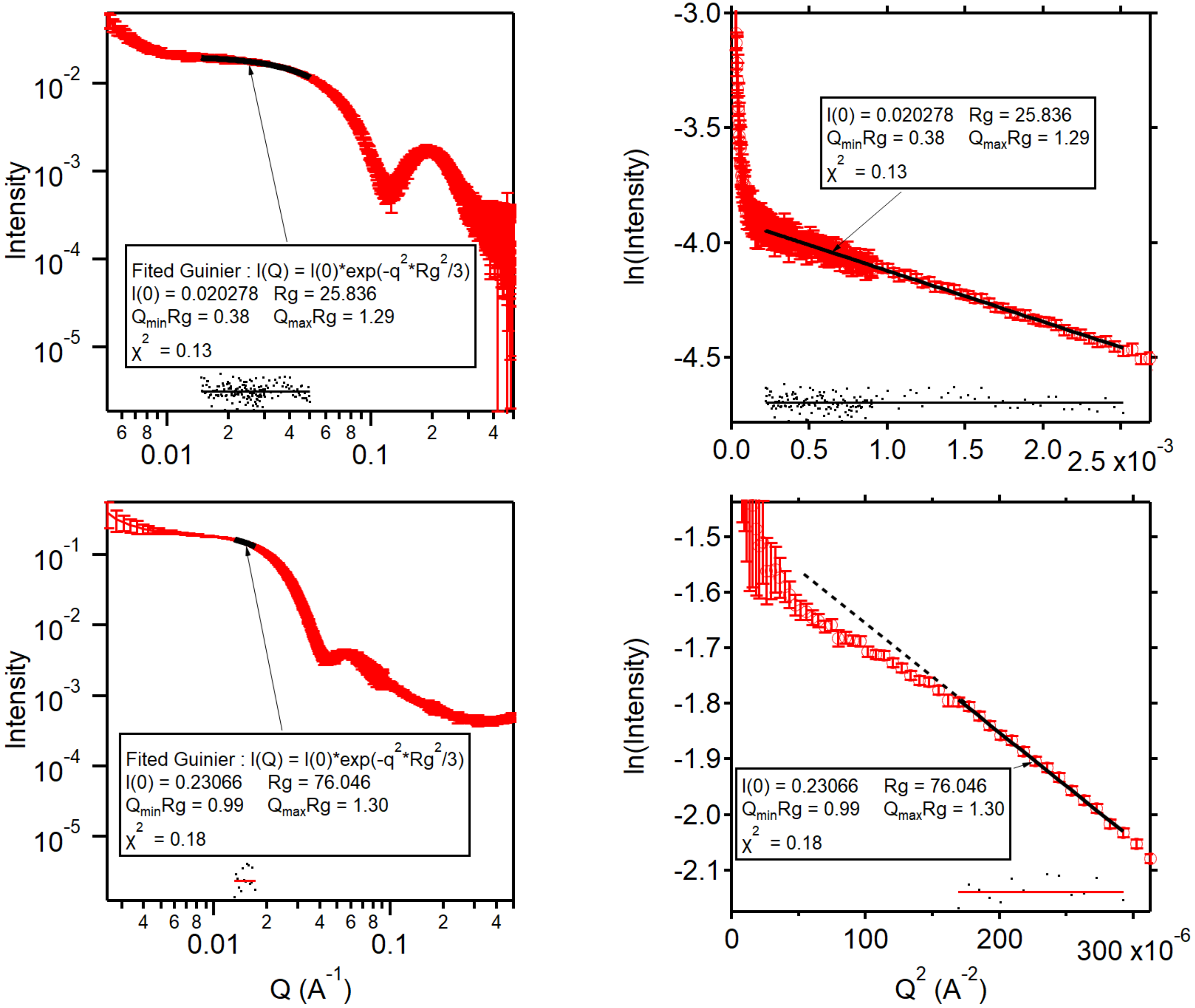
# Typical Data Content – Guinier Fitting




**Affords the radius of gyration ($R_g$) of the electron density in the particle (SLD for neutrons)**

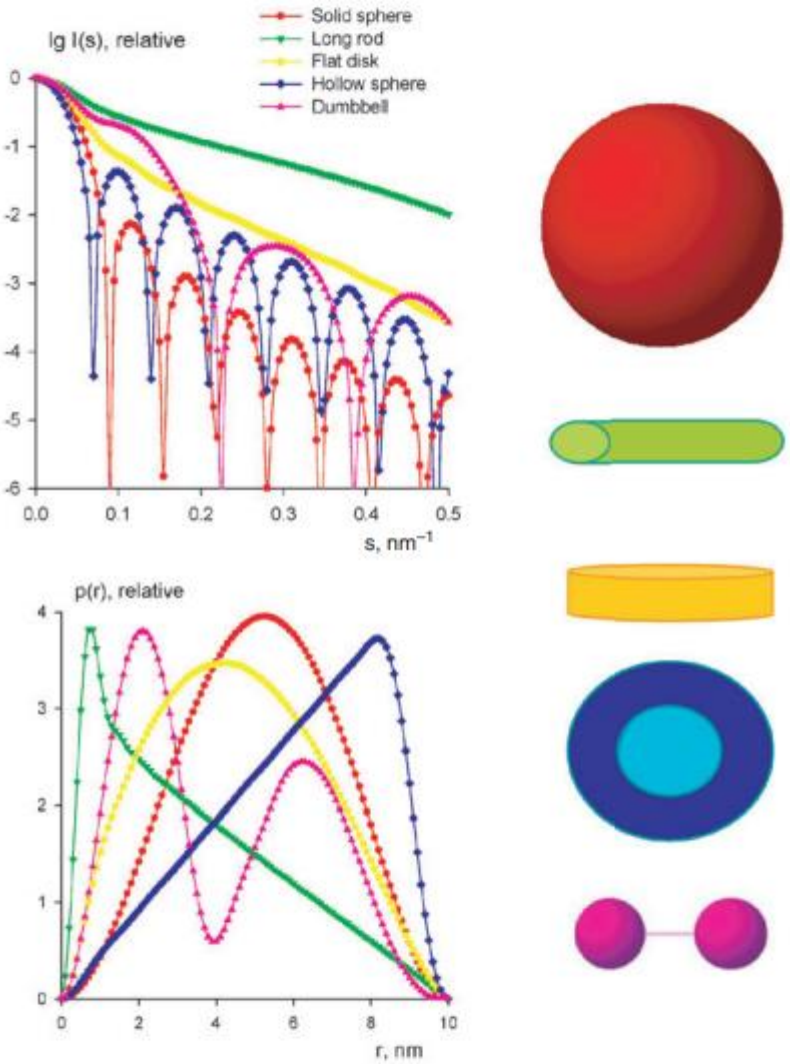
**Gives a measure of overall particle size**

**Only valid at low *q* ($qR_g$ < 1.3 for spherical particles)**

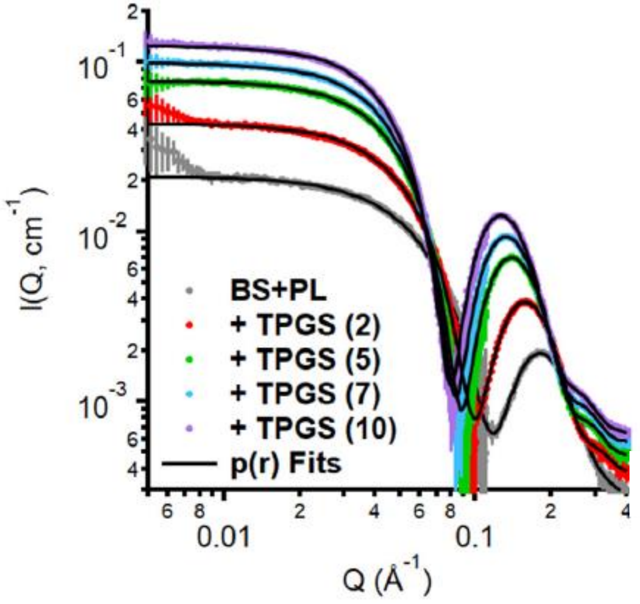
**Can provide a measure of aggregation/interparticle interactions (structure factor)**

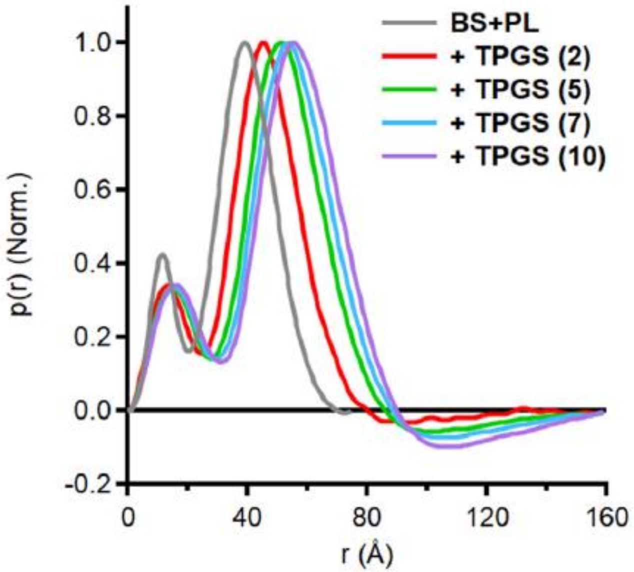
***Data processing/visualisation in IRENA macros (IgorPro)**

# Typical Data Content – PDDF




**Svergun & Koch, *Rep. Prog. Phys.* 2003, 66, 1735-1782**




**Usually determined by indirect Fourier transformation (IFT) of the measured scattering curve**

**Gives the probability function that you will be within the particle at any given distance**

**Can be used to determine the maximum dimension of the particle, the shape of the PDDF can also give you an idea of the shape of the particle**

**Can also indicate interparticle interactions**

**Clulow *et al. Int. J. Pharm.* 2020, 588, 119762**

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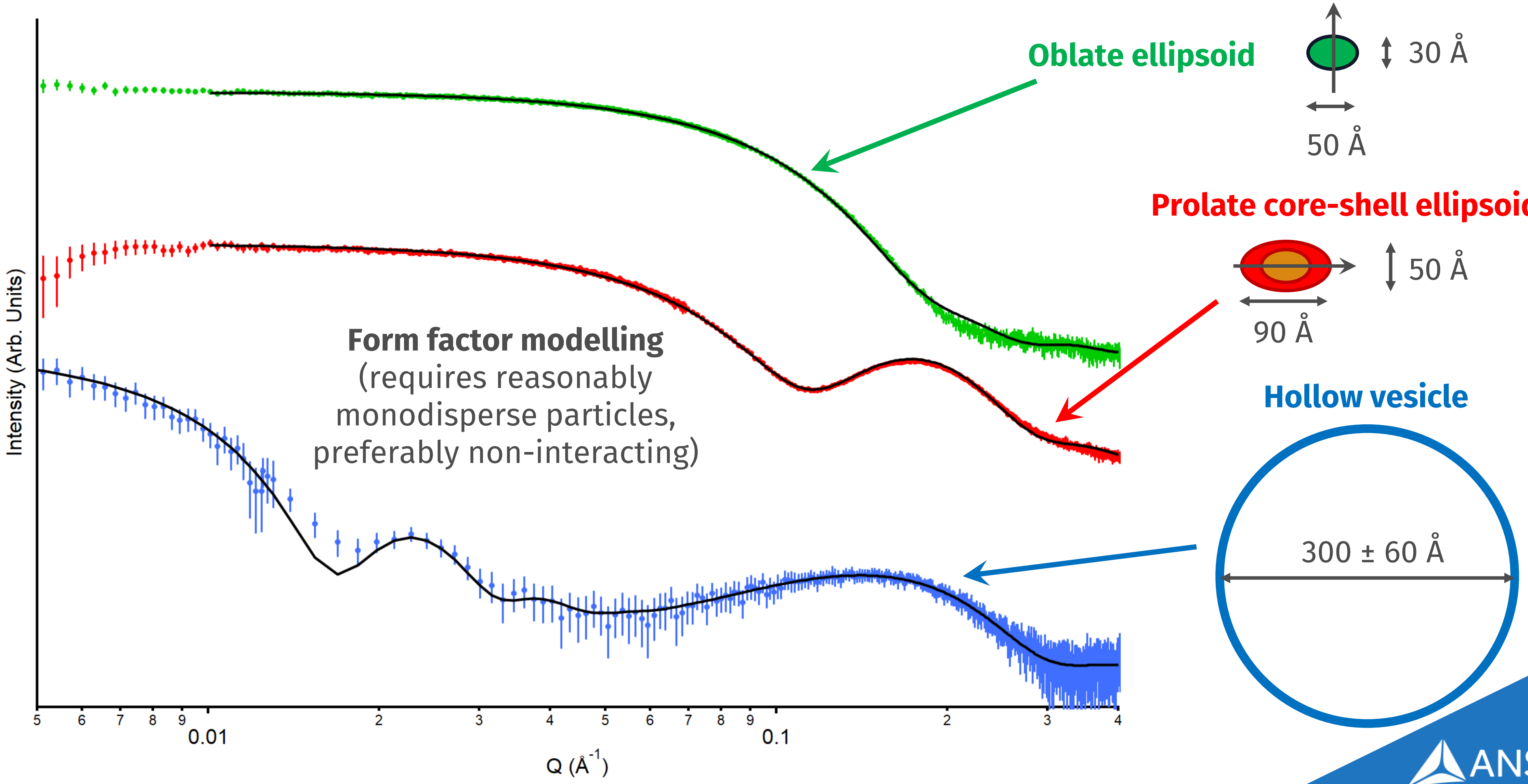
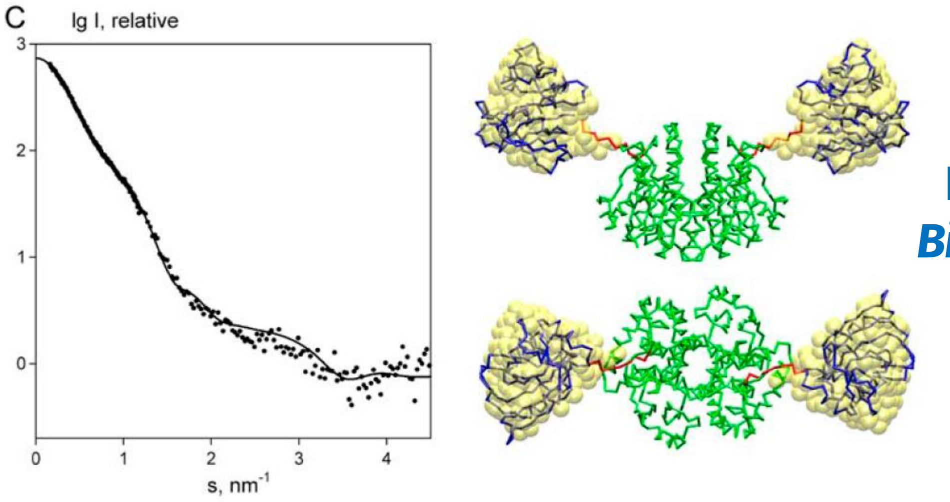
# Typical Data Content – Particle Structure



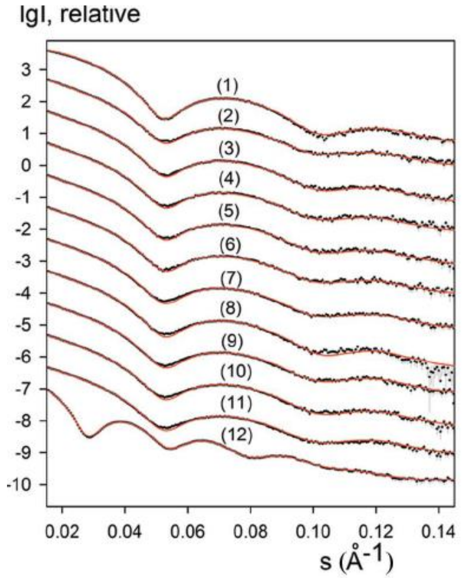
# Typical Data Content – Protein Structure

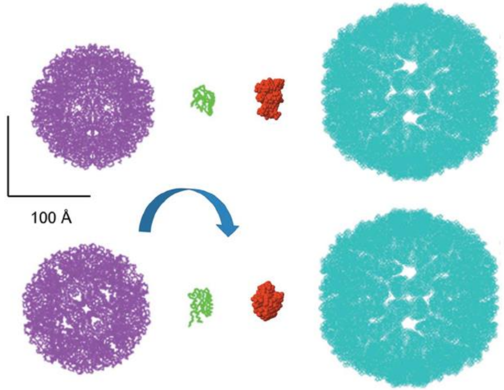
## Rigid body/bead ab initio modelling




**Petoukhov *et al.* *Biophys J.* 2005, 89, 1237-1250**

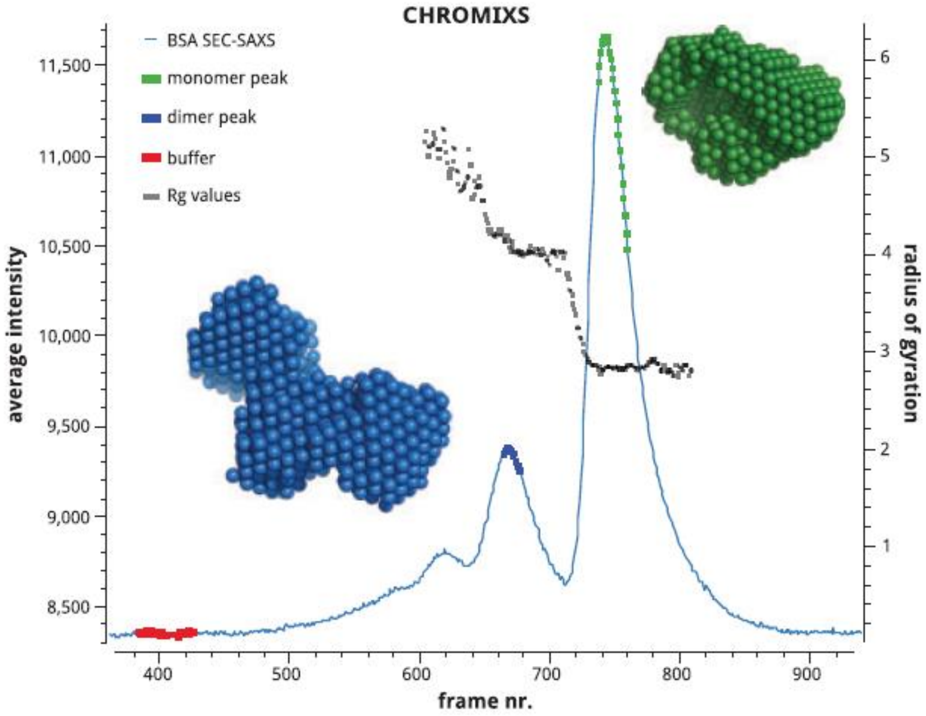
## Intermediates and oligomers in evolving mixtures




**Konarev *et al.* *IUCrJ* 2018, 5, 402-409**

## SEC-SAXS




**Panjkovich *et al.* *Bioinformatics* 2018, 34 (11), 1944-1946**

**ATSAS 3.0 Overview**

**Manalastas-Cantos *et al.* *J. Appl. Crystallogr.* 2021, 54, 343-355**

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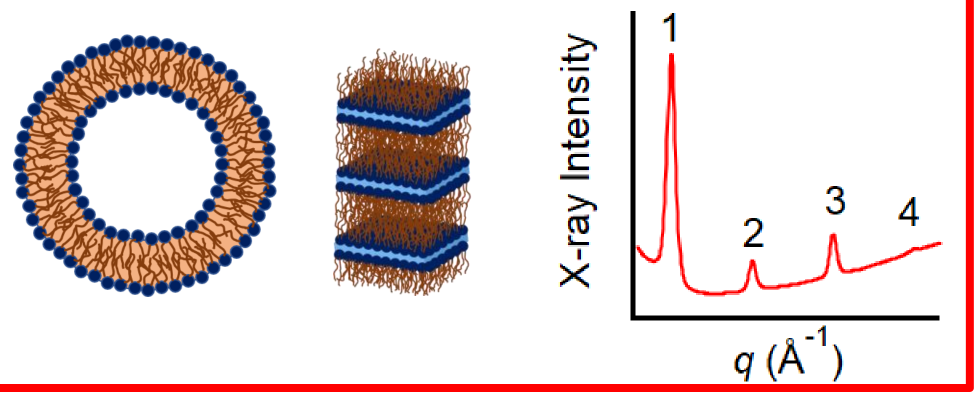
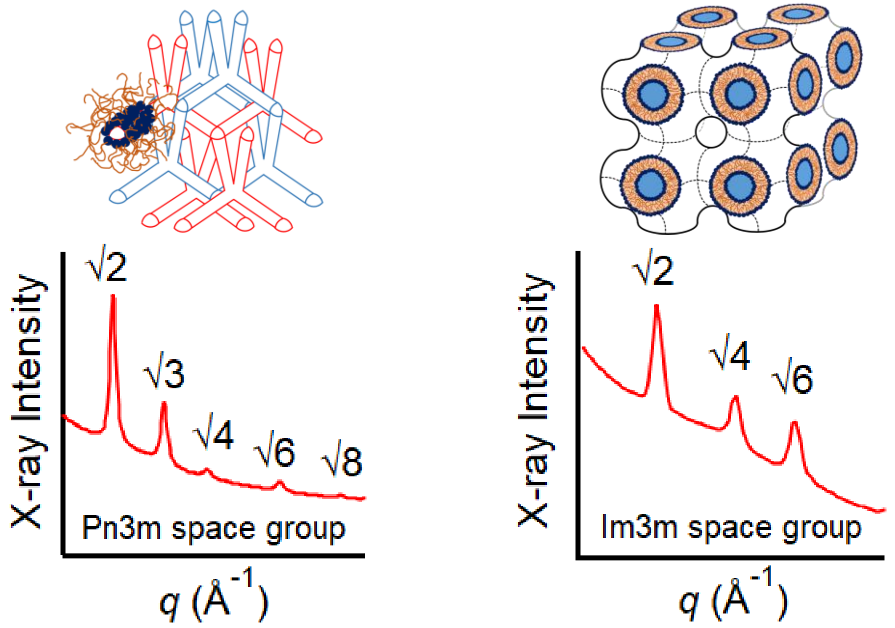
# Typical Data Content – Mesophases

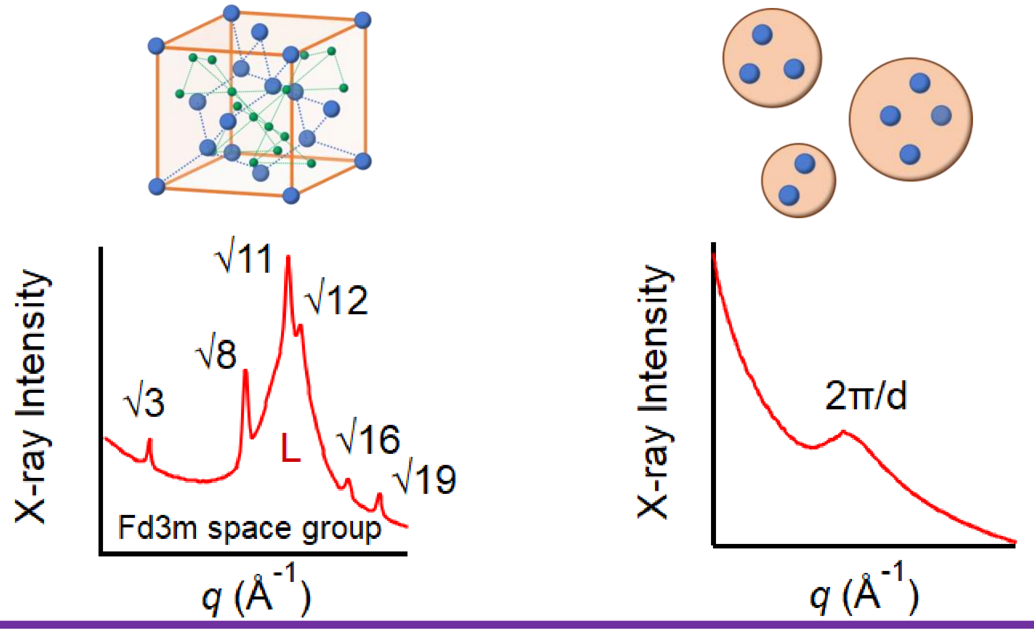
## Lamellar ($L_\alpha$)



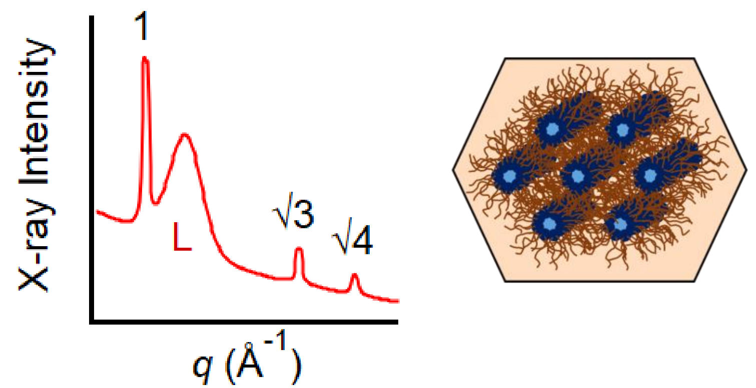

## Inverse Bicontinuous Cubic ($V_2$)




## Inverse Micellar Cubic ($I_2$) and Disordered Micellar ($L_2$)




## Inverse Hexagonal ($H_2$)

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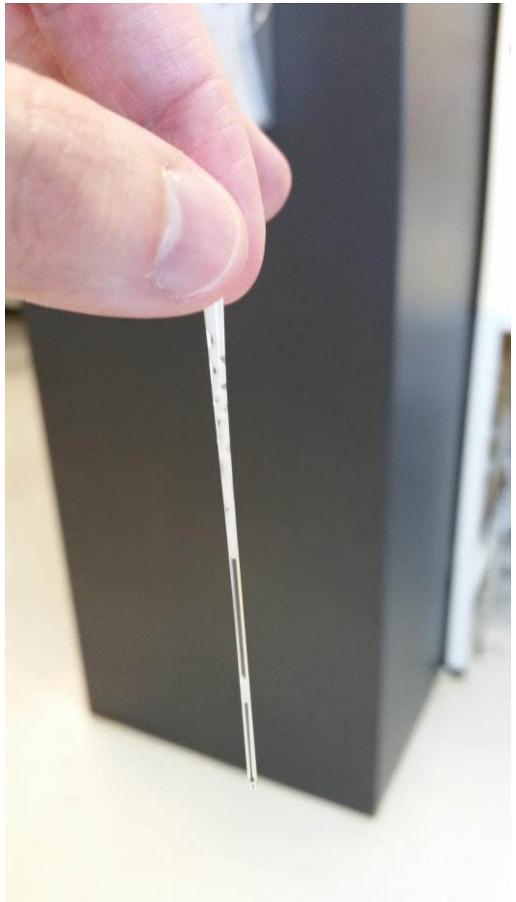
# Materials-relevant SAS Studies in Solution

# Simplest Solution Measurement

Load a suitable sample cell with your solution, place it in the beam, measure, clean/dispose of cell



1.5 mm diameter capillary (X-ray)

(Note that this sample has partially phase separated)

Hellma/Banjo cell (neutron)

Picture from ORNL (https://neutrons.ornl.gov/gpsans/sample-environments)

# Solution SAXS Environments

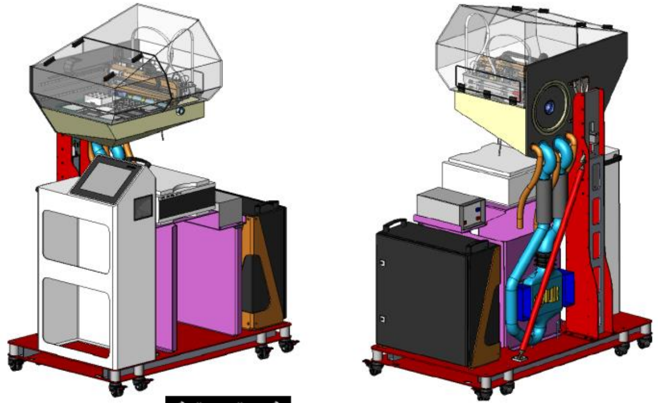
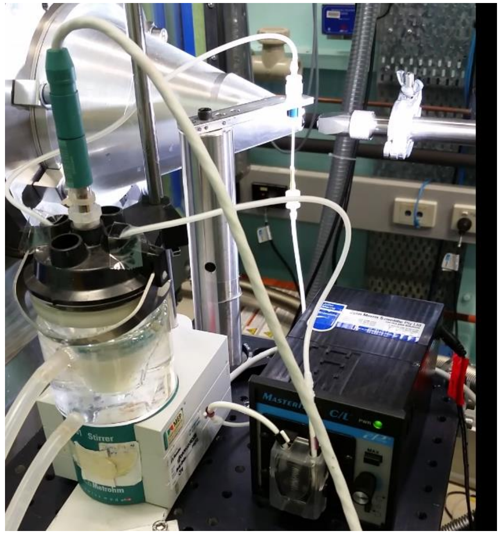


Image curtesy of



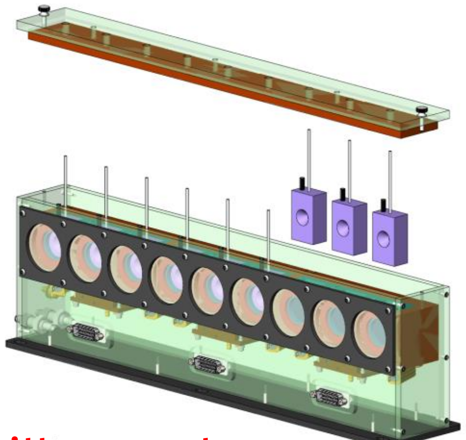
*In situ* flowthrough

Size exclusion chromatography

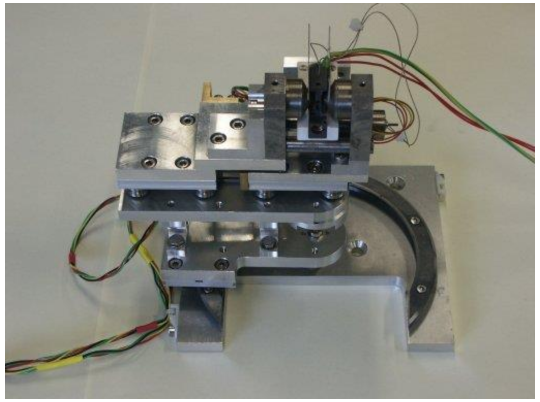
“Coflow” sheath flow sample environment

N. Kirby, et. al. *Acta Cryst. D 2016,* D72 1254-1266 & T.M. Ryan, et al. *J. Appl. Crystallogr.* 2018, 51, 97-111

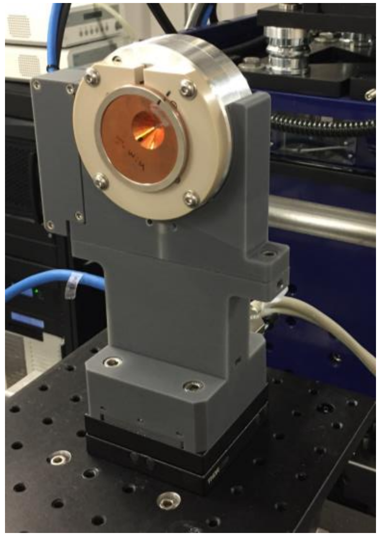
Linkam hotstage
(−196-350 °C)



Capillary rack
(5-90 °C)

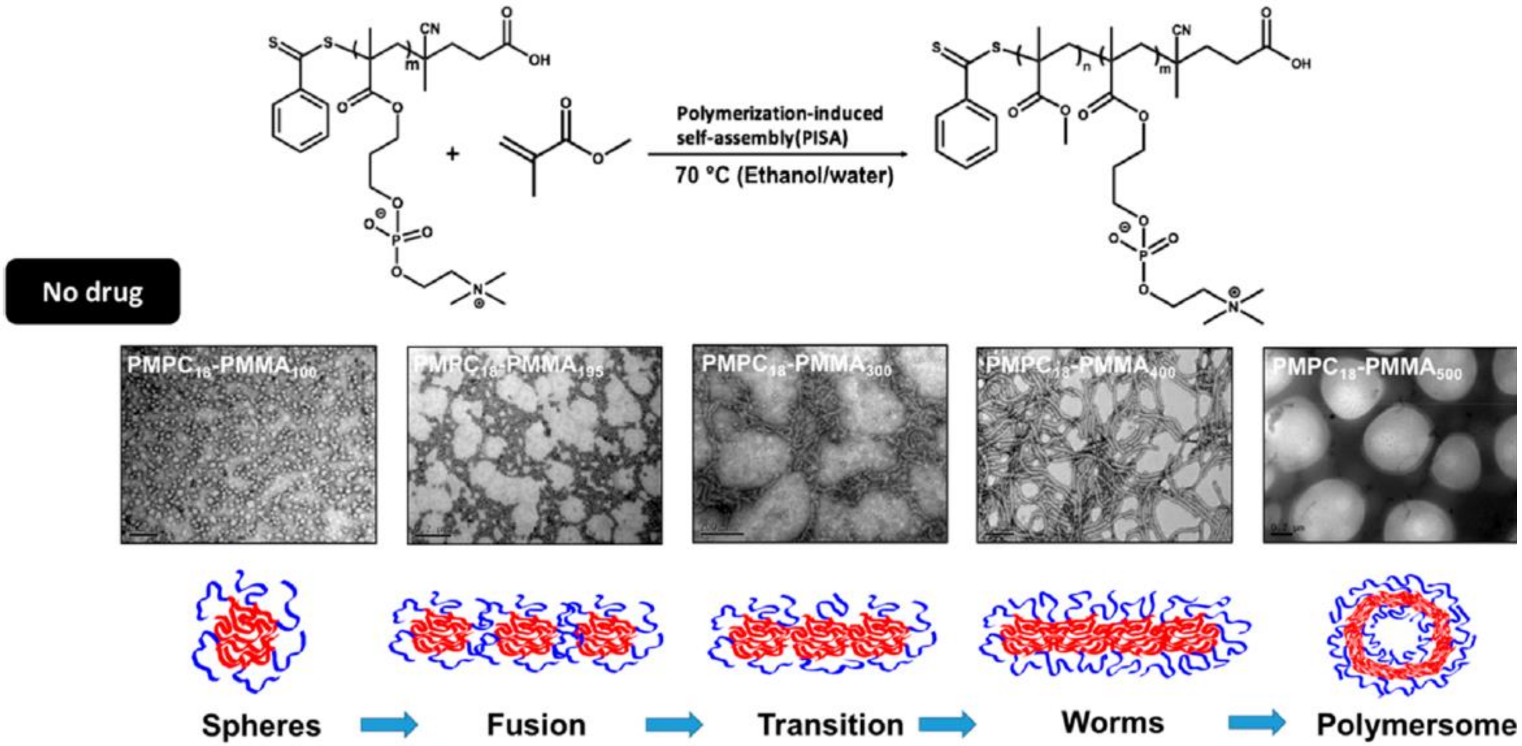


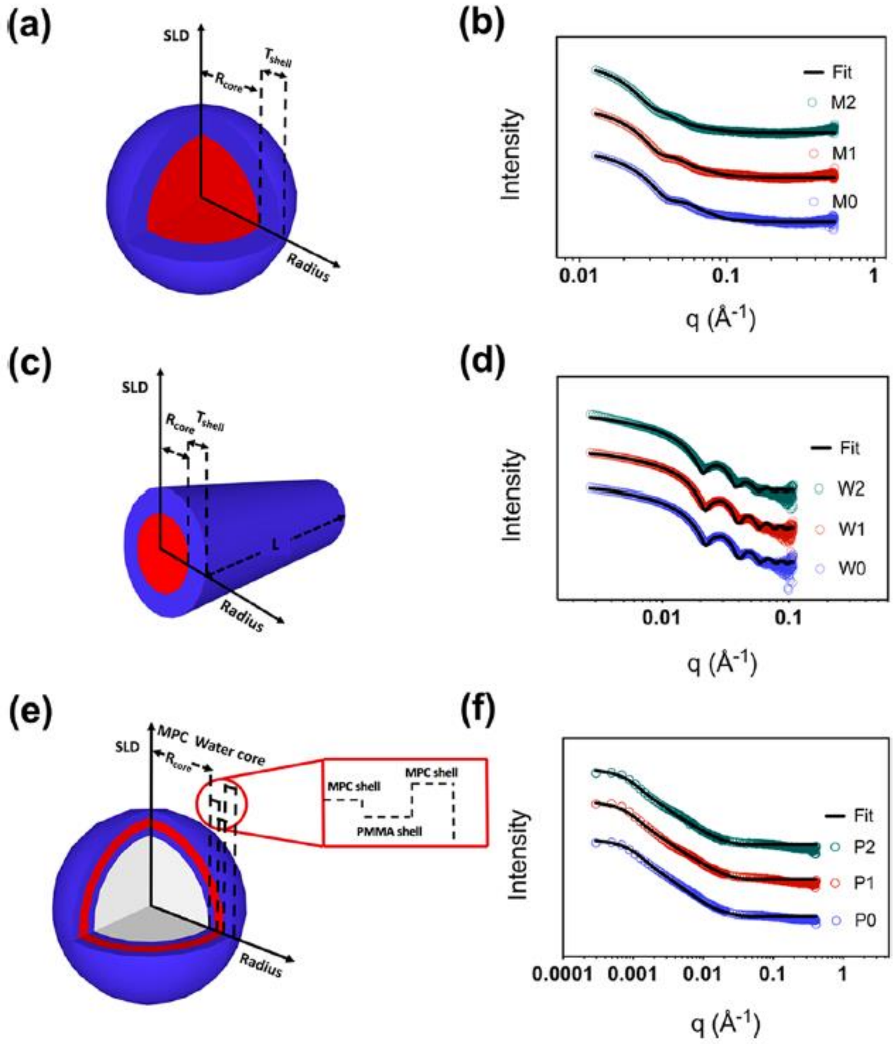
Magnet Arrays
(BM26 @ ESRF)



Shear cells

# Polymeric Nanoparticles



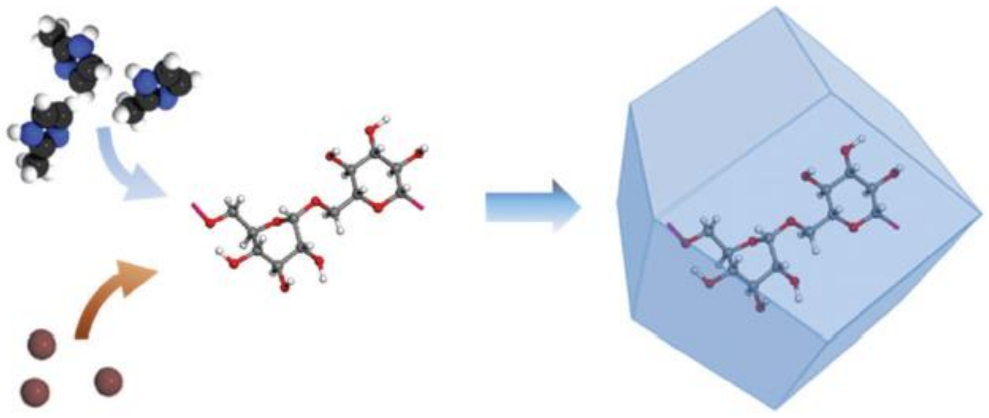




Polymerisation-induced self-assembly, increasing the length of the PMMA block changes the aggregate structures and consequently curcumin loading/uptake

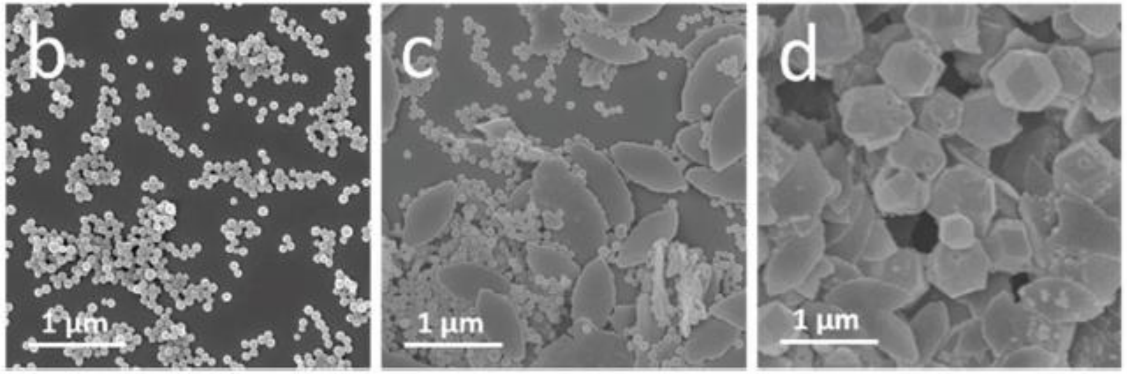
PISA also studied in a time-resolved *in situ* study on SAXS/WAXS

Cao *et al.*, *ACS Appl. Mater. Interfaces* 2020, 12, 30221-30233

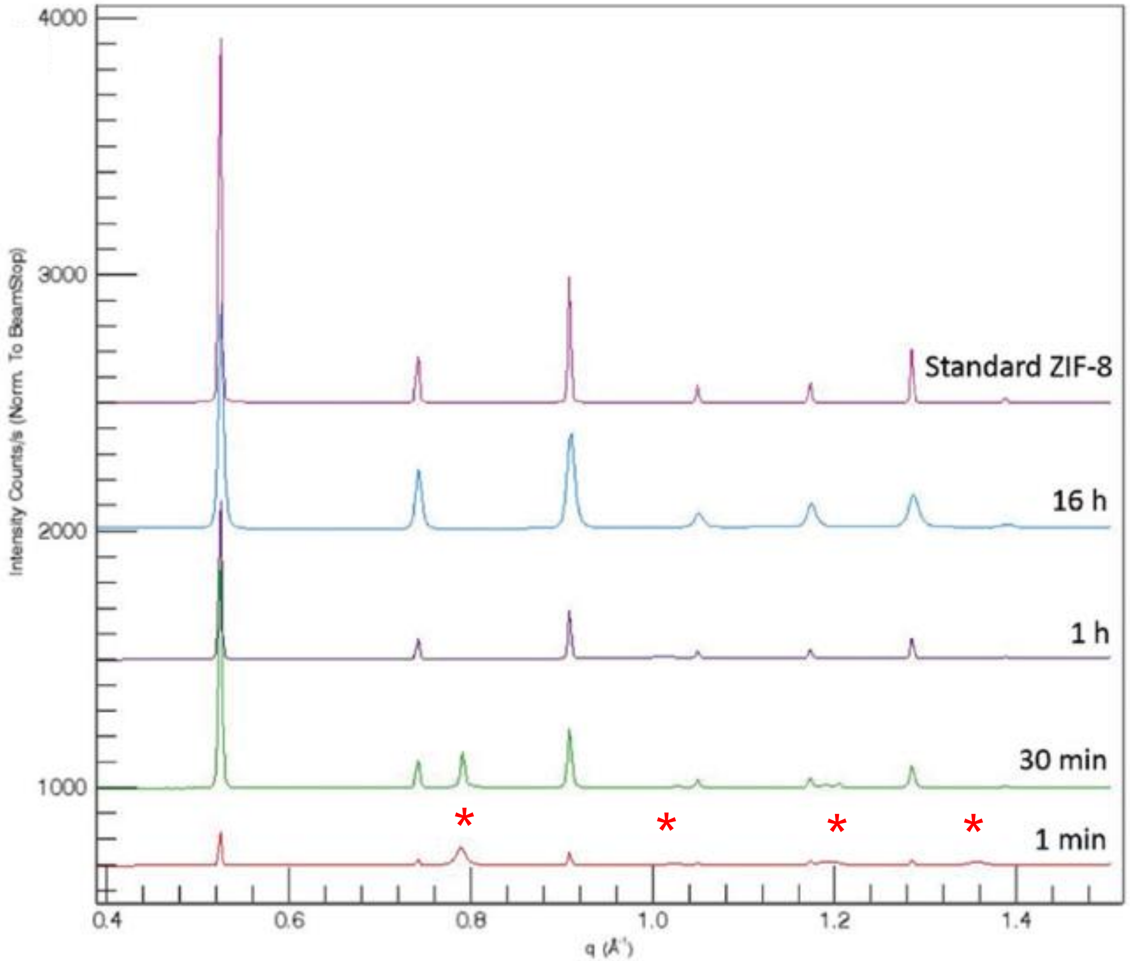
# Mineralisation & MOF Formation



Templating the formation of metal-organic-frameworks around biological substrates e.g., polysaccharides (or inside plants)




1 min 30 min 16 h




Initial crystal structure is not the standard crystal structure (*)

Liang *et al.*, *Chem. Commun.* 2017, 53, 1249-1252

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# Nanoparticles & Stimuli – Magnetism

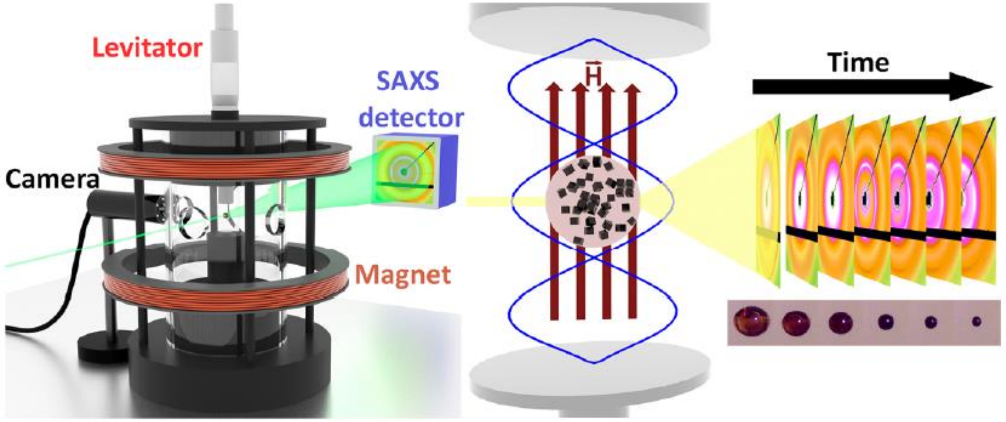
Self-assembly of cubic, iron-oxide nanoparticles may be studied by time-resolved SAXS

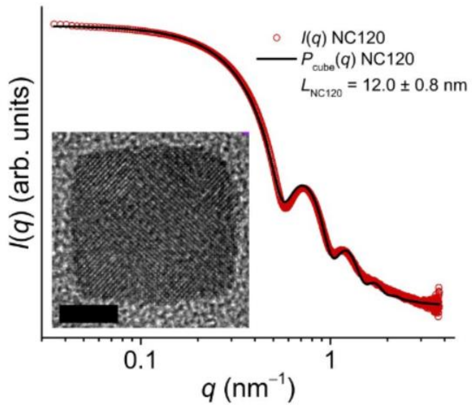
Particles were suspended in custom acoustic levitation setup and then exposed to a weak magnetic field

Several hundred frames were collected over 2 minutes with 0.5 s time resolution

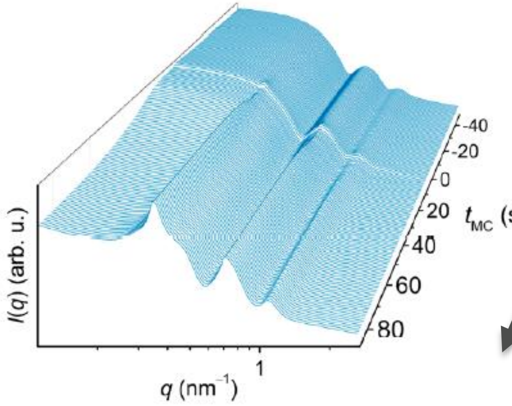
Results show self-assembly happens in two phases:

- ➢ Initially, cubes assemble into mesocrystal cuboids
- ➢ Afterwards, the cuboids form long mesocrystal "fibers"

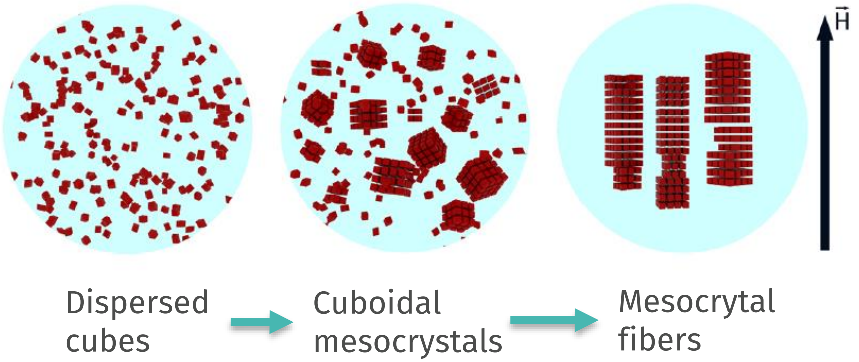



Iron oxide nanoparticle cubic form-factor




Time-resolved SAXS can study formation mechanics of self-assembled structures




**P03@PETRA III**

**Kapuscinski *et al. Nano Lett.* 2020, 20, 7359**

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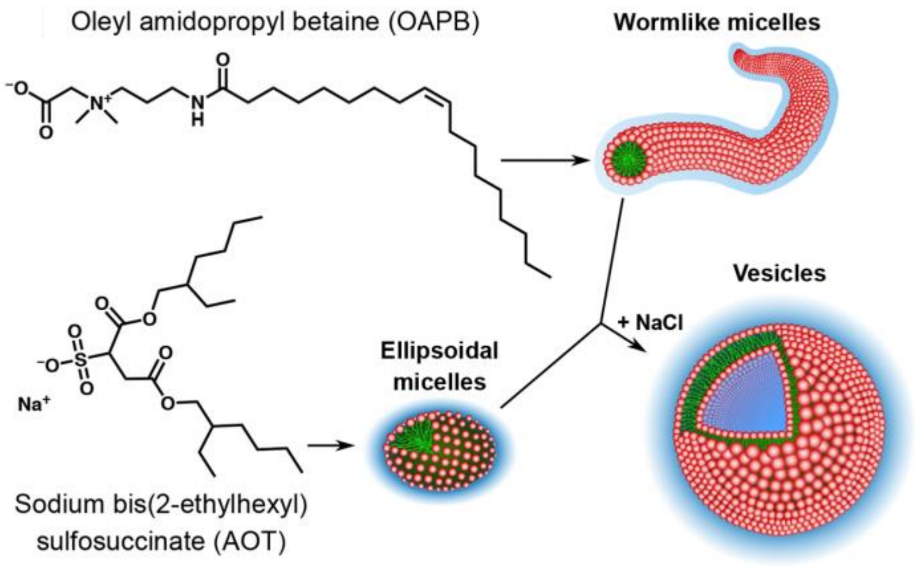
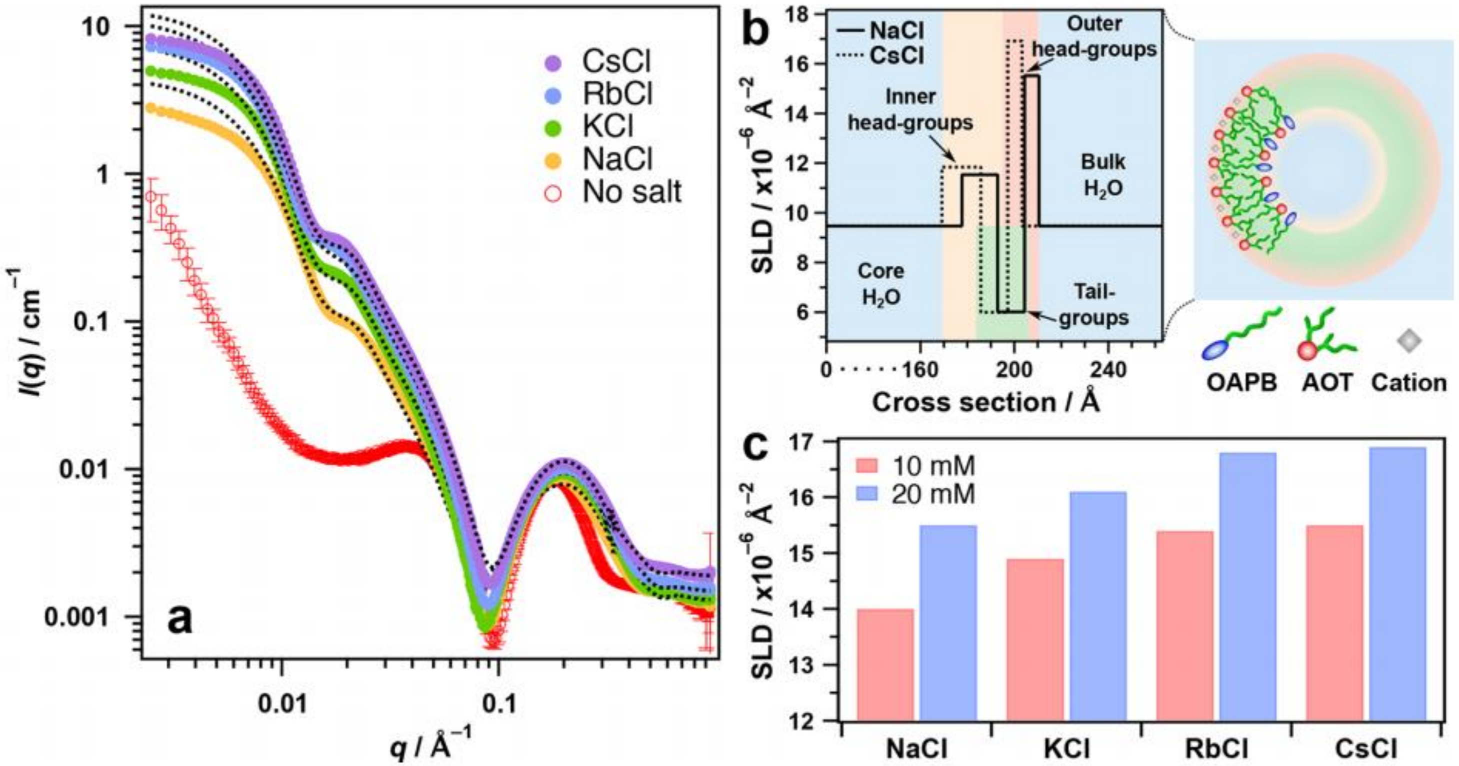
# Complex Surfactant Structuring




Co-refinement of SANS and SAXS data reveals the complex self-assembly behaviour of mixed surfactant systems




Solution SAXS data (from SAXS/WAXS) revealed the localisation of alkali metal ions with anionic surfactant at the vesicle surface

*McCoy et al. Biomacromolecules* 2020, 21, 4569-4579

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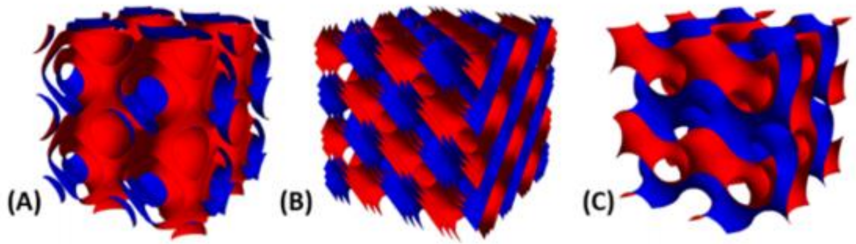
# Lipid Mesophase Interactions

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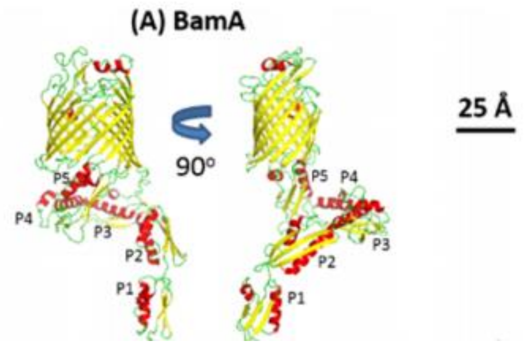
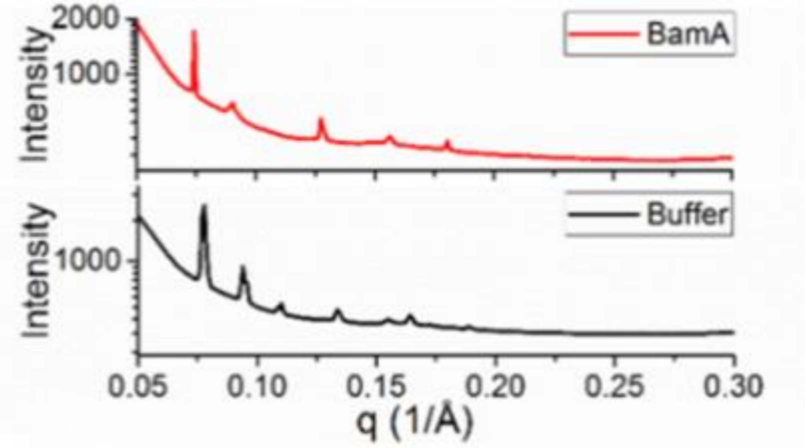
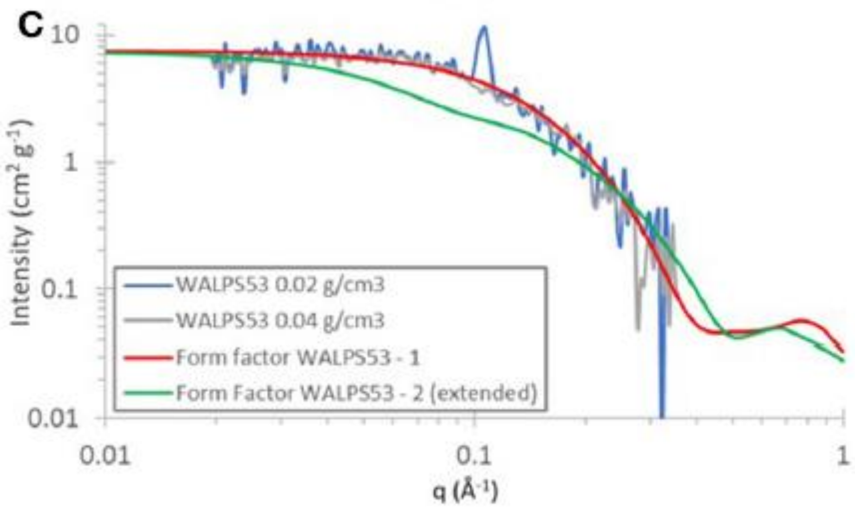
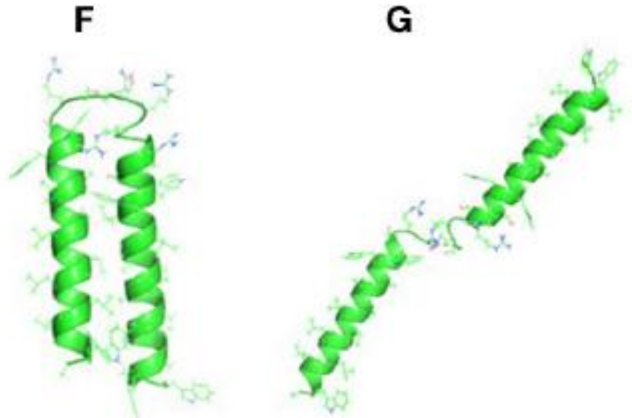



Changes in lipid mesophase structure and lattice parameter measured by **SAXS**




**SANS** with contrast matching can be used to determine the corresponding protein structures *in meso* by matching out the lipid membranes

van't Hag *et al.*, *Langmuir* 2016, 32, 12442-12452 & Conn *et al. Frontiers Chem.* 2021, 8, 619470

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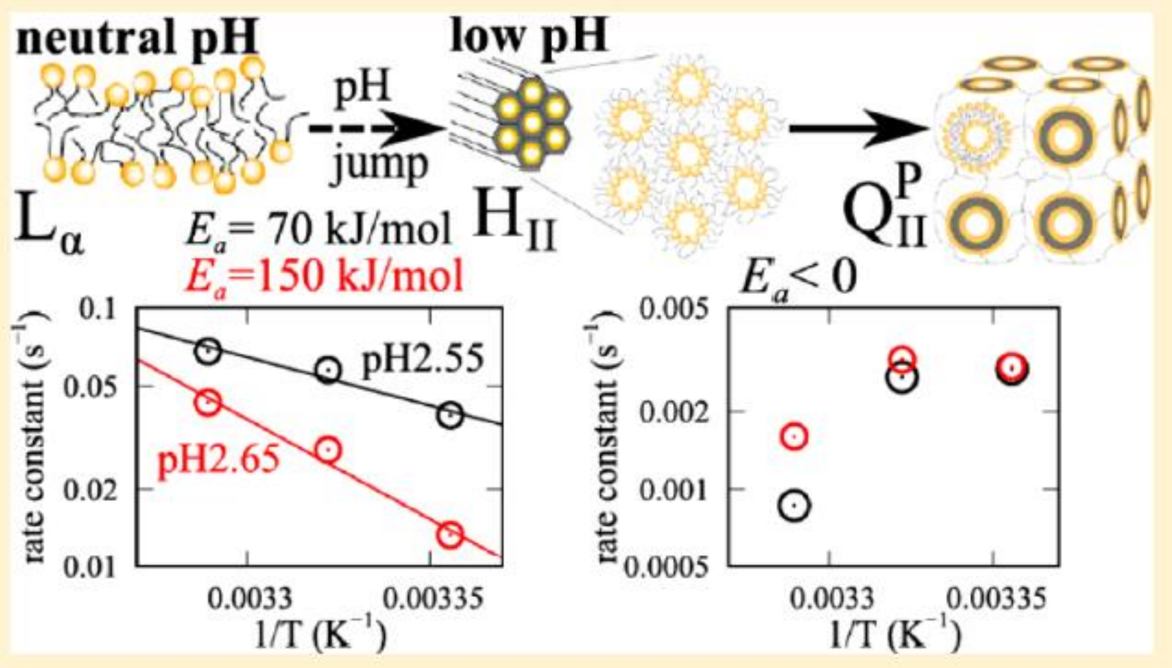
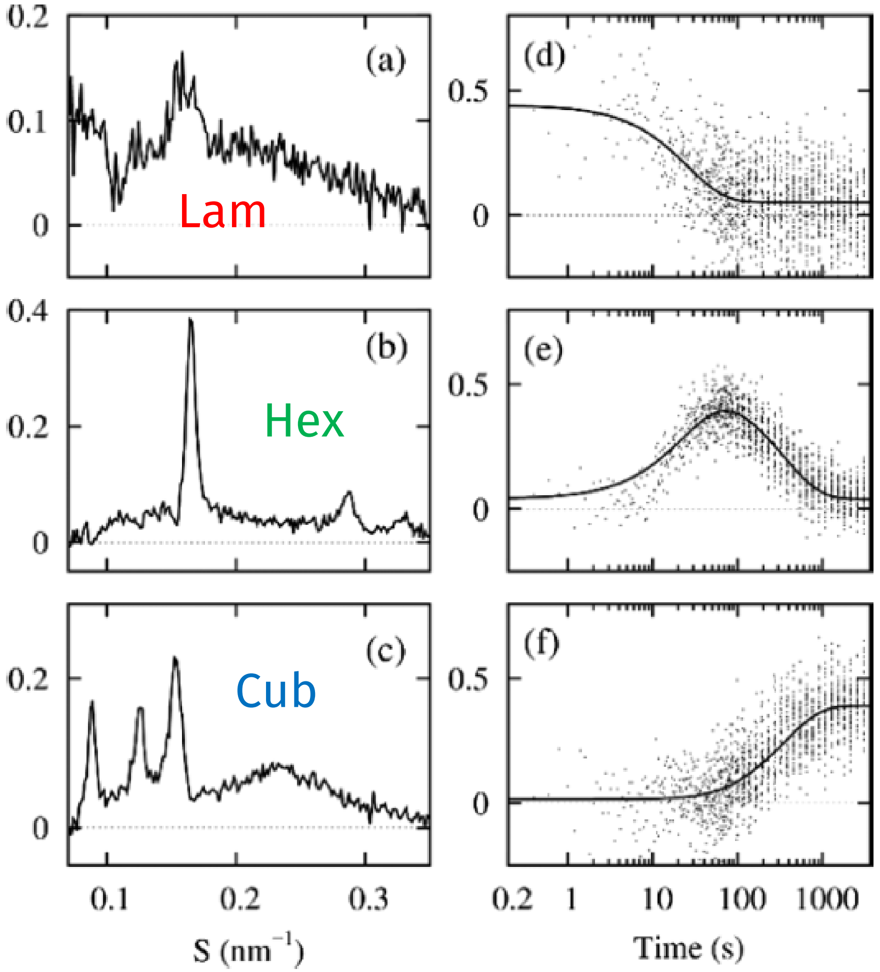
# Fast Time-resolved SAXS (Stopped Flow)

Rearrangement of lipid mesophases in response to rapid change in pH upon mixing with acidic buffer

Lamallar to hexagonal to bicontinuous cubic transition




Allows access to rapid process kinetics
(200 ms – 9 s exposures)

**Oka *et al.* *Langmuir* 2017, 33, 12487-12496**

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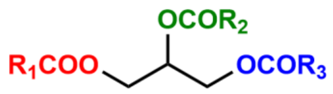
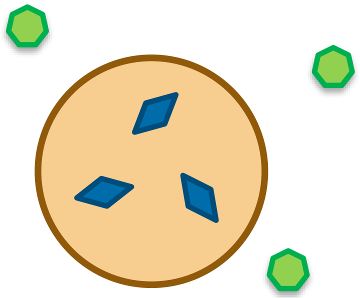
# Lipid Digestion & Drug Delivery



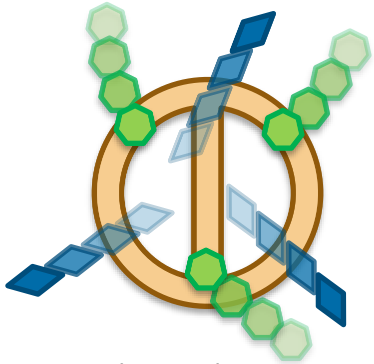

Lipids begin as apolar triglycerides



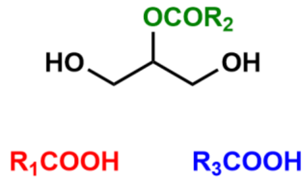
Encapsulated/Dispersed bioactives

Digestion

Change of lipid nanostructure

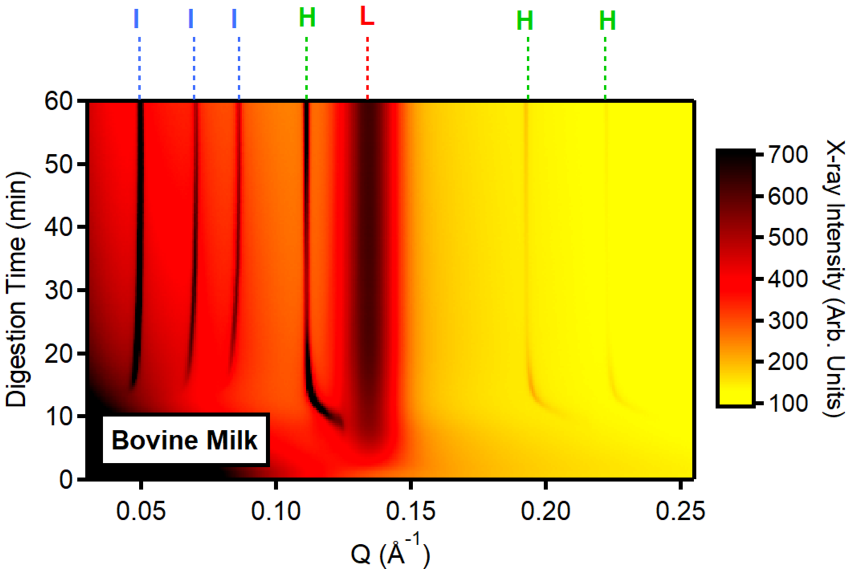


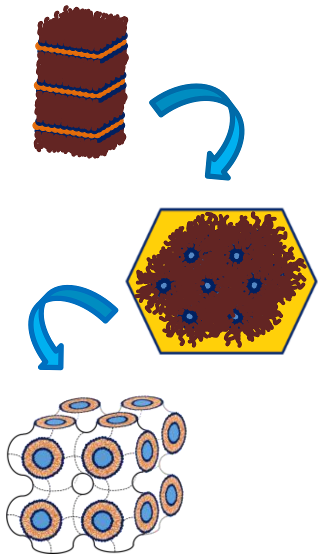
Bioactive release/solubilisation




Digestion products are amphiphilic and can assemble themselves into liquid crystalline mesophases

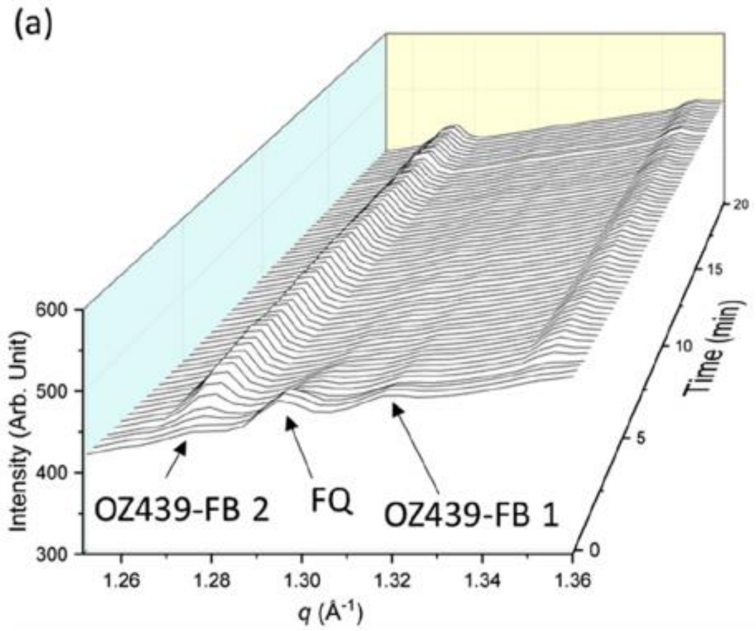
## SAXS – evolution of lipid mesophases



Clulow *et al.*, *Food Hydrocolloids* 2021, 110, 106126

## WAXS – evolution of co-administered drug crystals




**Salim *et al.*, *Mol. Pharmaceutics* 2019, 16, 1658-1668**

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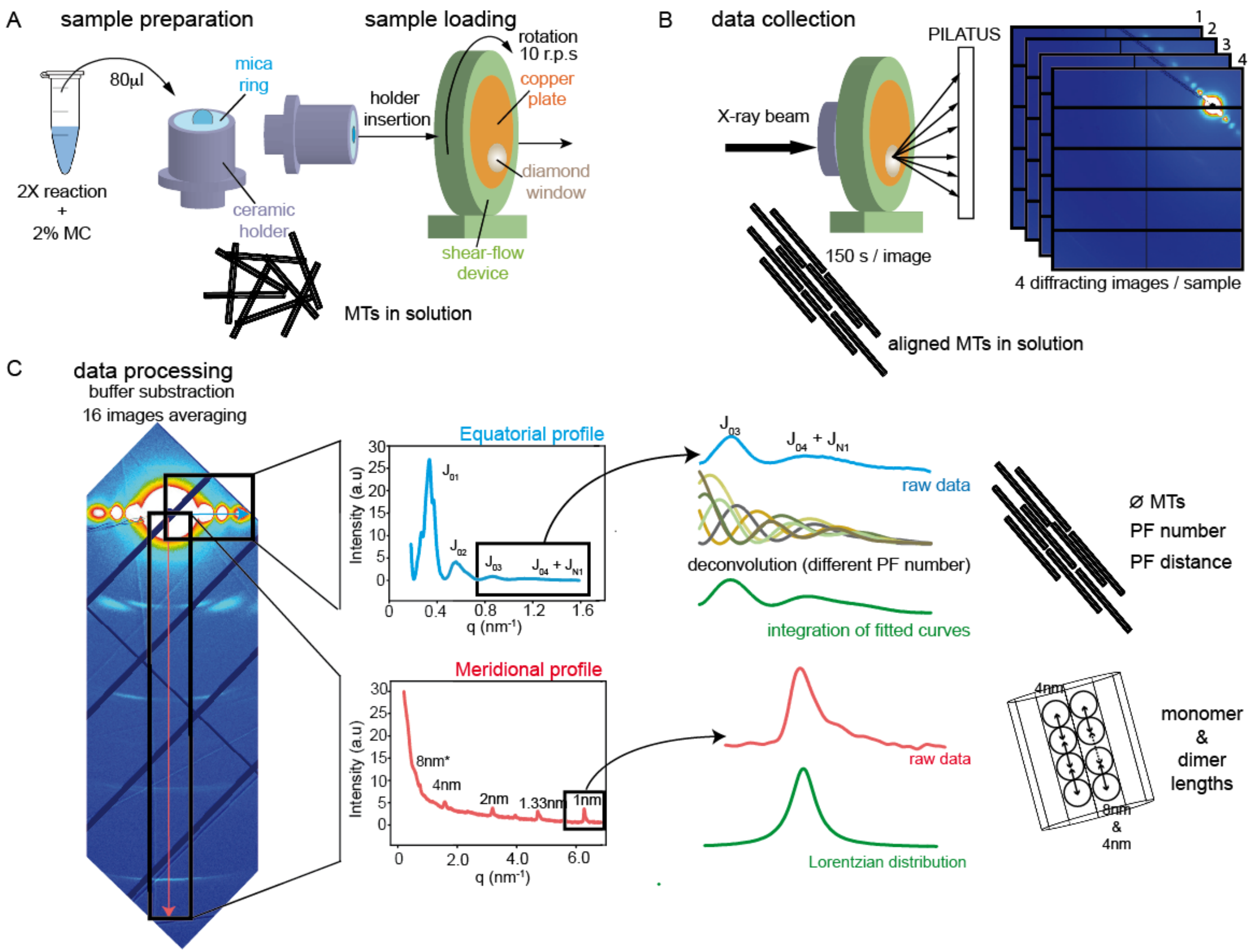
# Microtubule Assembly and Alignment

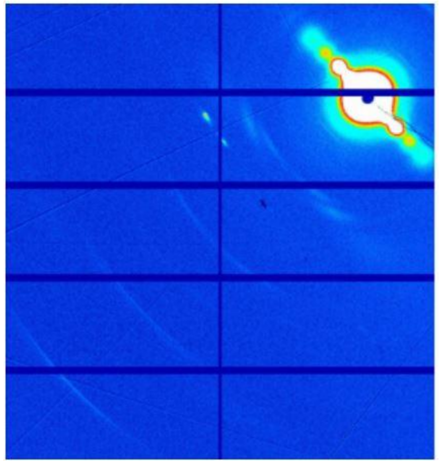


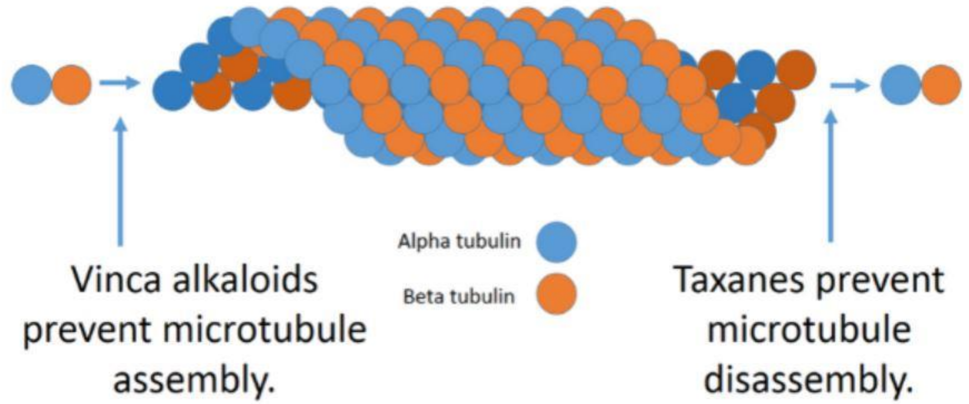

Estévez-Gallego *et al. eLife* 2020, 9, e50155

Taxanes convert regions of perturbed microtubule growth into rescue sites

Induces apoptosis in cancerous cells

**Anti-cancer drug design**






Rai *et al. Nat. Mater.* 2020, 19 (3), 355-365

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## Software Packages Used Throughout:

SASView (form factor modelling, p(r) function, shape-independent analysis)
IRENA (data processing/stitching, Shape-independent analysis, size distribution)
ATSAS (data processing/stitching, p(r) function, protein analysis, irregular shapes, comparison to high-resolution data)

There are other to choose from e.g., McSAS.

# Open Forum (Over to you)