Small-Angle Neutron Scattering

Gergely Nagy

Neutron Scattering Division

Oak Ridge National Laboratory

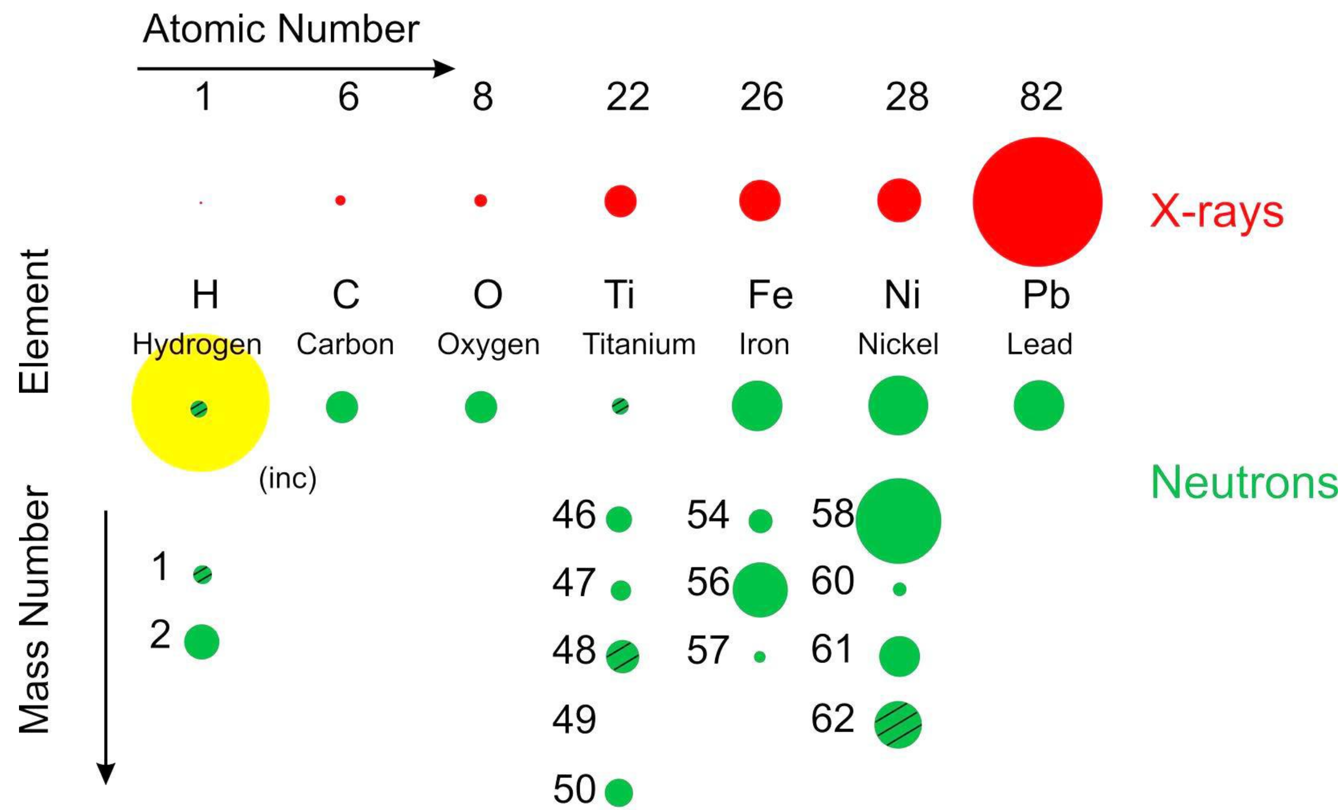
ORNL is managed by UT-Battelle, LLC
for the US Department of Energy

Contents

Neutrons

- Electric neutrality
- Penetrate deeply into matter
- No Coulomb-barrier
- Interaction with the atomic nuclei
- Interaction with different atoms does not depend systematically on atomic number
- Interaction can be very different for isotopes of the same atom (e.g. H/D)
- Non destructive
- Sensitive to magnetic structures

Neutrons
What makes them special for biology?



The diameters of the circles shown scale with the scattering amplitude $f_x(\sin\Theta=0)$ for x rays, and b_{coh}*10 for neutrons. Hatching indicates negative scattering amplitudes.

From M. V. Avdeev presentation at Central European Training School on neutron techniques

Neutrons

- Wavelength – crucial parameter for NS techniques
- Reactors, spallation sources – neutrons with E ~ MeV
- E < 1 keV is required
- Energy distribution can be modified through thermalisation in moderators
- Maxwellian distribution of velocities

$$E = k_B T = \frac{1}{2} m v^2 = \frac{h^2}{2m\lambda^2} = \frac{\hbar^2 k^2}{2m}$$

$$[E] = meV, [T] = K, [v] = \frac{km}{s}, [\lambda] = \text{Å}, [k] = \frac{1}{\text{Å}}$$

$$\lambda = 6.283 \frac{1}{k} = 3.956 \frac{1}{v} = 9.045 \frac{1}{\sqrt{E}} = 30.81 \frac{1}{\sqrt{T}}$$

Neutrons

- Wavelength distribution ⟺ moderator temperature

	Neutron energy	Moderator material and temperature
Cold neutons	$E \leq 10\, meV$	Liquid H_2 or D_2, $T = 20K$
Thermal neutrons	$10\, meV \leq E \leq 100\, meV$	H_2O and D_2O, $T = 290K$
Hot neutrons	$100\, meV \leq E \leq 500\, meV$	Graphite, $T = 2000K$
Epithermal neutrons	$500\, meV \leq E$	

From Lovesey, S. W., 1984. Theory of Neutron Scattering from Condensed Matter. Oxford, Clarendon Press

- SANS – ideally cold neutrons $E \leq 10\, meV$ $\lambda \geq 3\,\text{Å}$ $k \leq 2.2 \frac{1}{\text{Å}}$

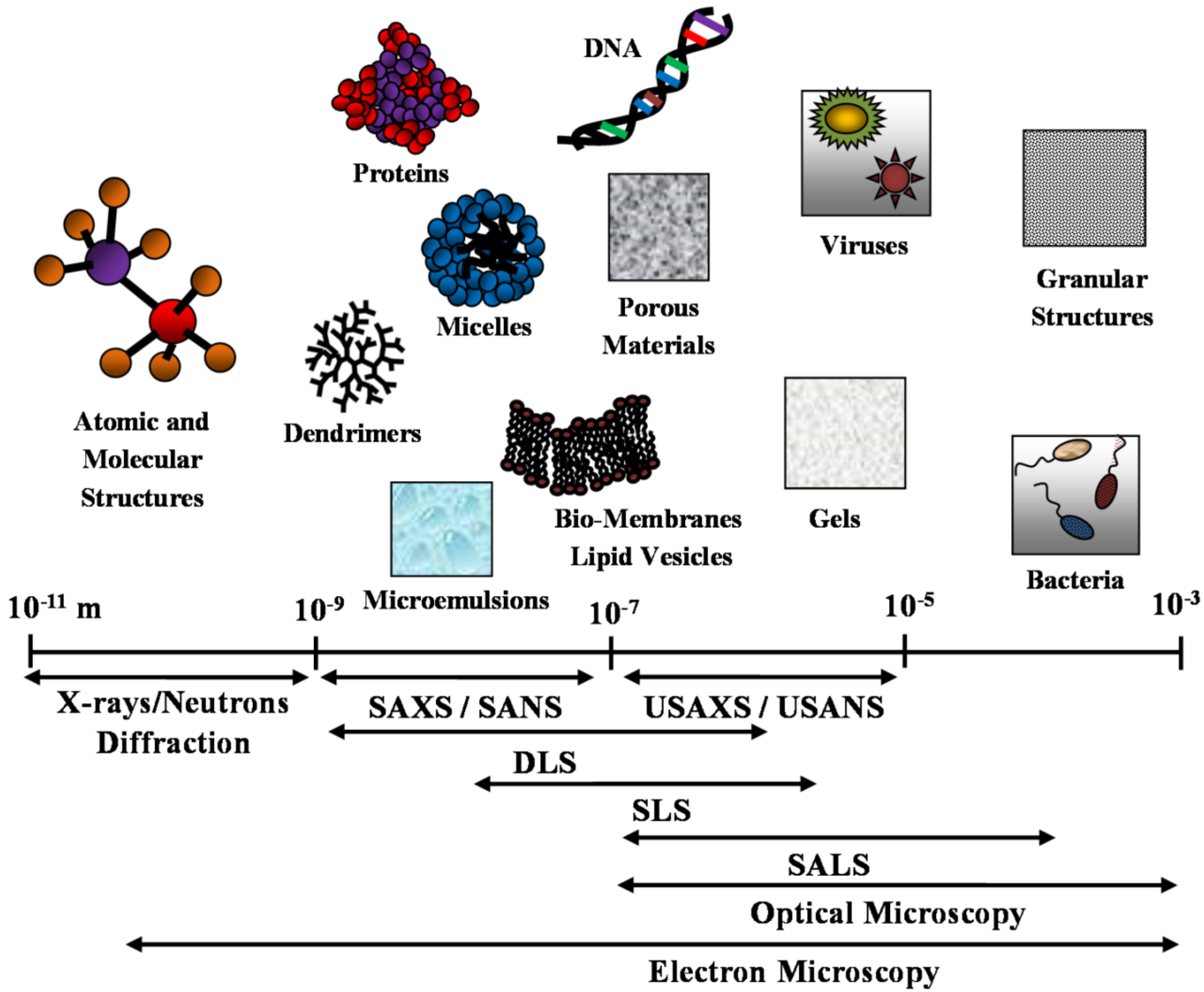
Aim of Small-angle neutron scattering

- "Large" scale structural data: 1-100 nm
  - Shape
  - Size
  - Interactions, organization
  - NO atomic resolution information
- Typically in continuous medium
- Time averaged information
- Often in situ - no specific sample preparation
- Typical objects: Particles, aggregates, etc.
- Objects often randomly oriented
  - 2D detector, but 1D averaged data to reconstruct 3D objects

Aim of Small-angle neutron scattering

- Biology
  - Proteins
  - Model or natural membranes
- Soft matter, food science
  - Colloidal particles
  - Polymers
  - Surfactants
  - Foams
- Geology, mining, construction
  - Porous materials
- Archeology and Arts
  - Ceramics
  - Weapons
  - Sculptures
- Engineering
  - Alloys
  - Irradiated samples
- Magnetic structures
- ...
- …

Aim of Small-angle neutron scattering



From Lombardo et al. 2020 Molecules – Open Access

SAS in Nature

- Sun or moon corona
  - SAS from thin clouds – each corona light ray is scattered by only one droplet or ice crystal
  - smaller objects -> larger corona
  - https://atoptics.co.uk/droplets/corona.htm
  - https://atoptics.co.uk/droplets/cormoon.htm

SAS in Nature

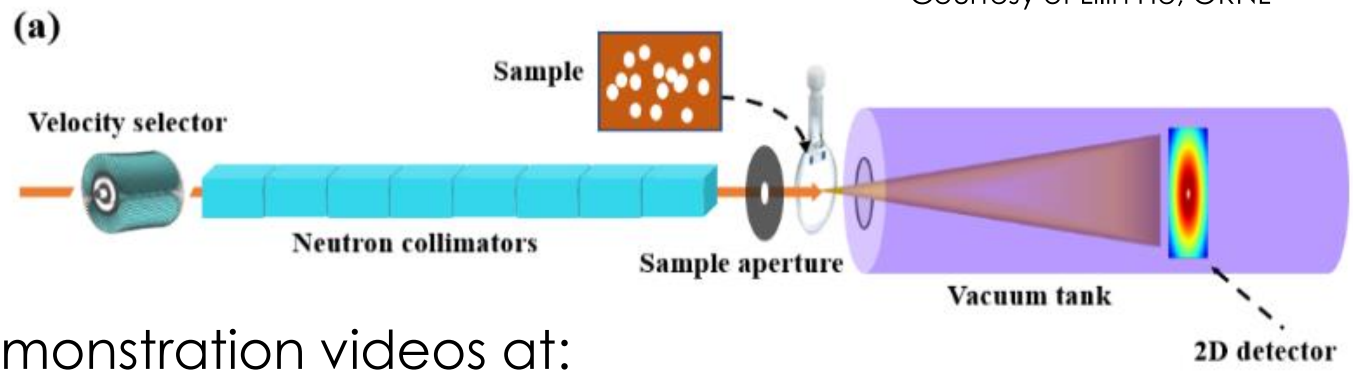
- Pollen corona
  - Non spherical objects with preferred orientation
  - https://atoptics.co.uk/droplets/corim24.htm
  - https://atoptics.co.uk/droplets/pollen1.htm

SANS Instrumentation

SANS Instrument

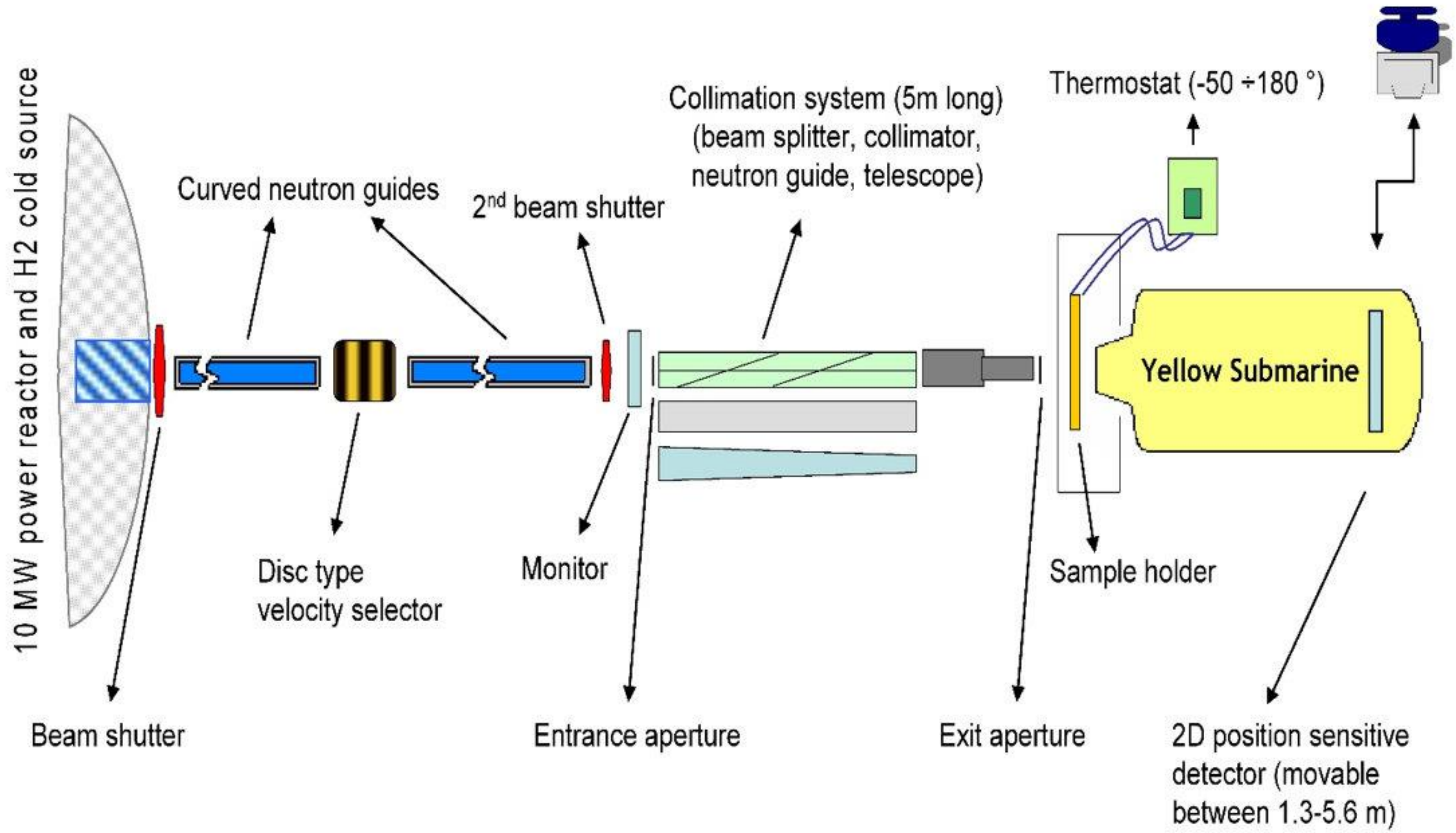
- "Reactor" SANS

Courtesy of Lilin He, ORNL



- Demonstration videos at:
- https://www.ill.eu/fileadmin/user_upload/ILL/3_Users/Instruments/Instruments_list/00_-_LARGE_SCALE_STRUCTURES/D11/html5/D11-principle/D11.html
- What setup should we use?
- A priori information about the sample -> Required q-range, required resolution -> SD, wavelength, collimation, detector offset
- If possible, keep λ constant

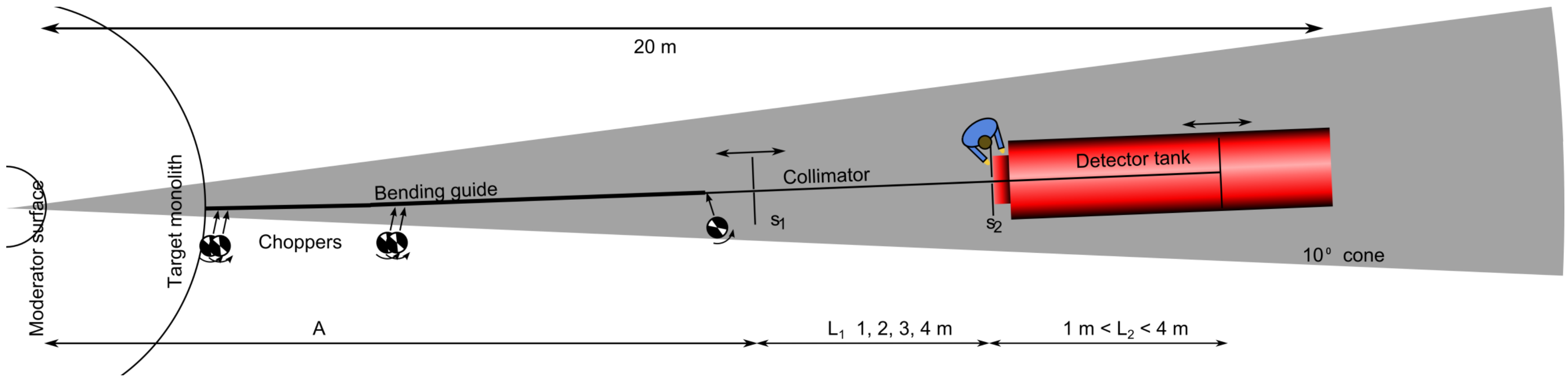
Yellow Submarine



From www.bnc.hu

SANS Instrument at a Spallation Source

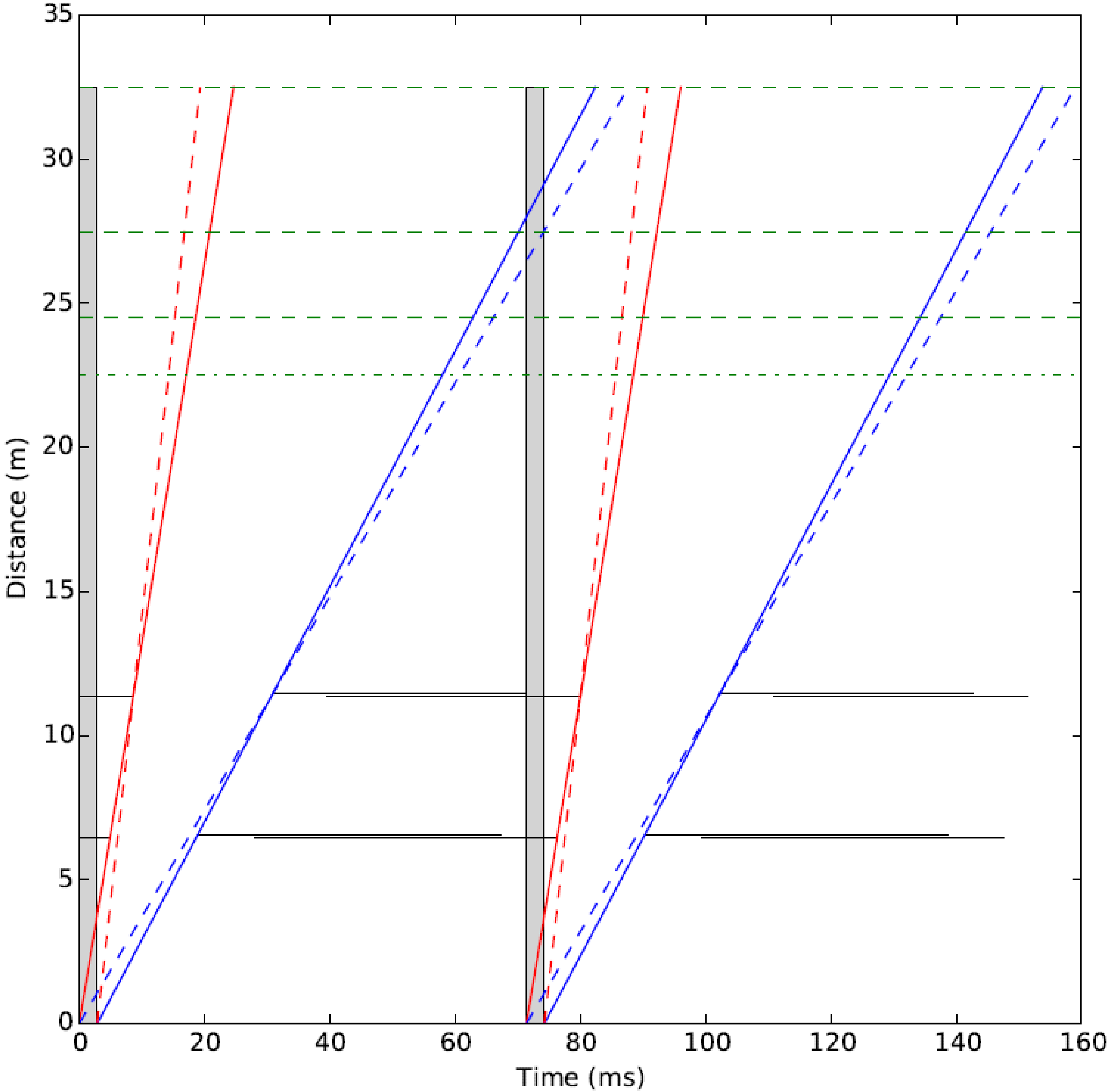
- ESS Proposed Compact SANS Instrument for Small Sample Volumes



From Klenø, K. et al. Instrument Construction Proposal

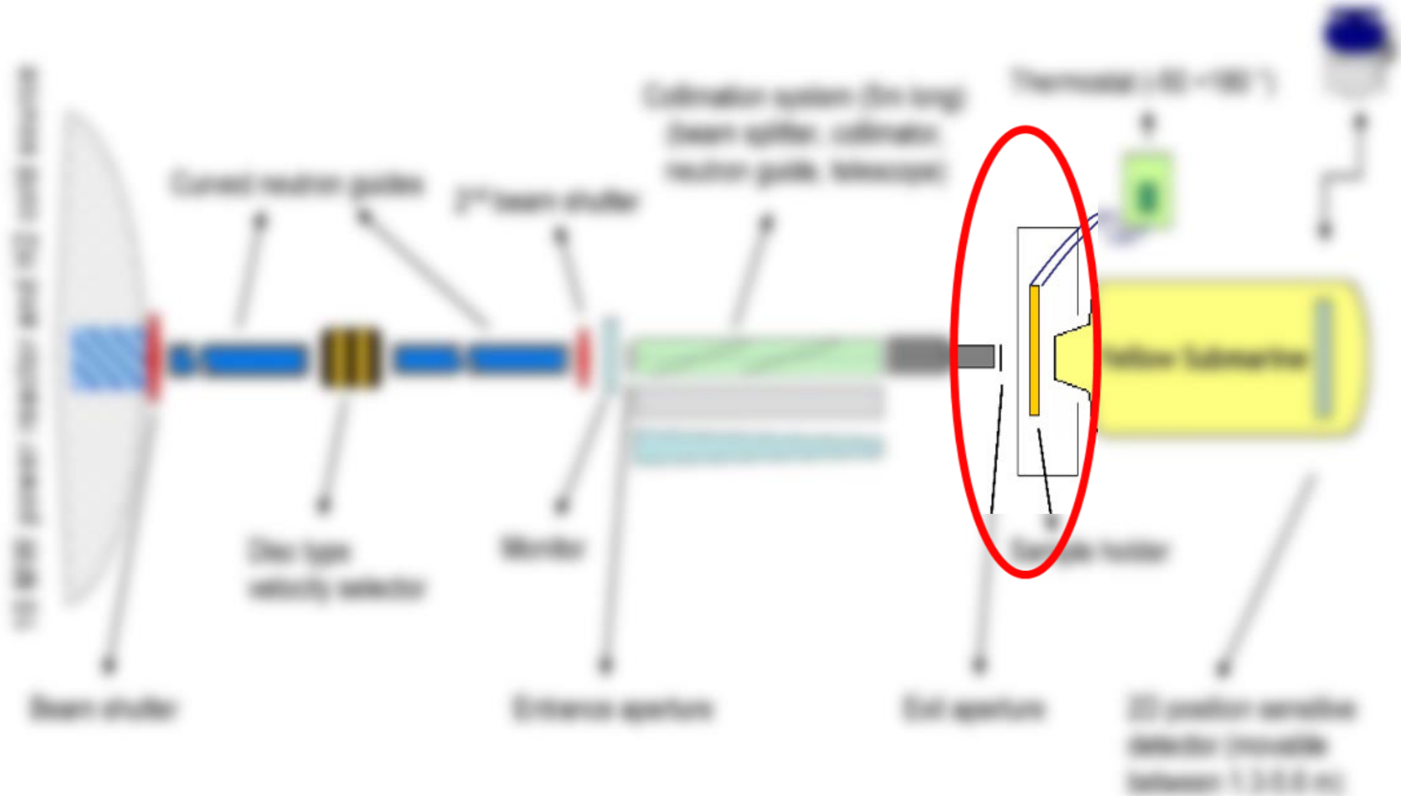
- Neutron pulses – simultaneous use of a wide wavelength range – large dynamic range in scattering vector with single setup
  - Ideal for the study of time-dependent processes
- Time of detection ⟺ time-of-flight ⟺ speed ⟺ wavelength
- Time-distance diagram

T vs D graph



35
30
25
20
15
10
5
0
Distance (m)
0
20
40
60
80
100
120
140
160
Time (ms)

Sample environment

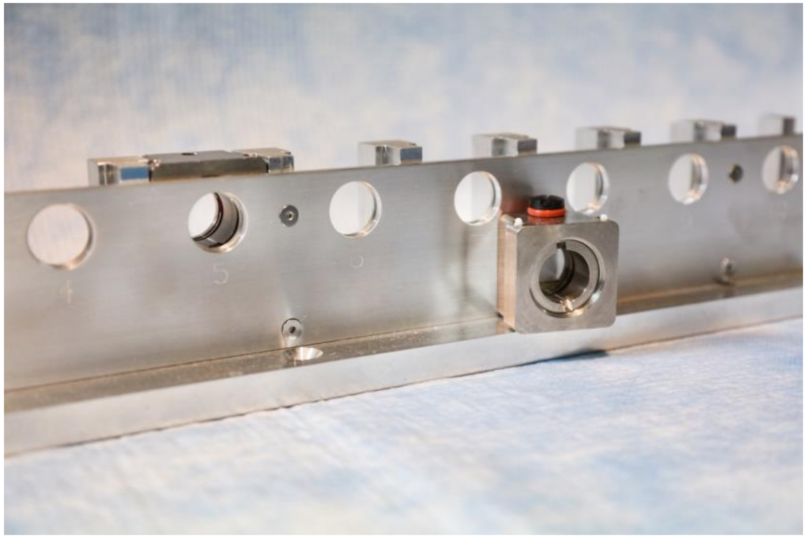


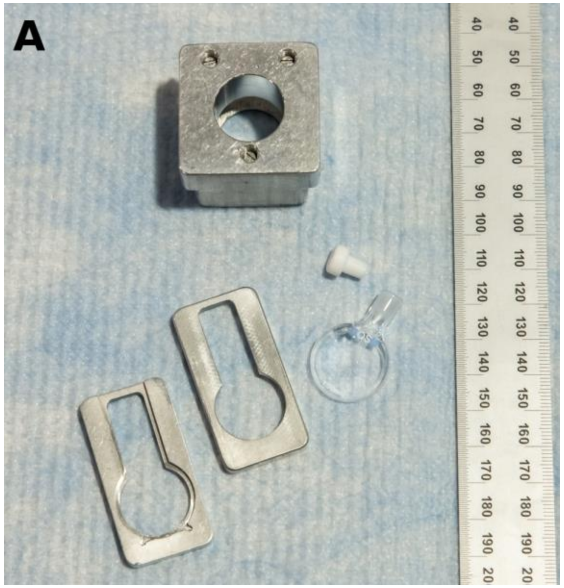
From www.bnc.hu

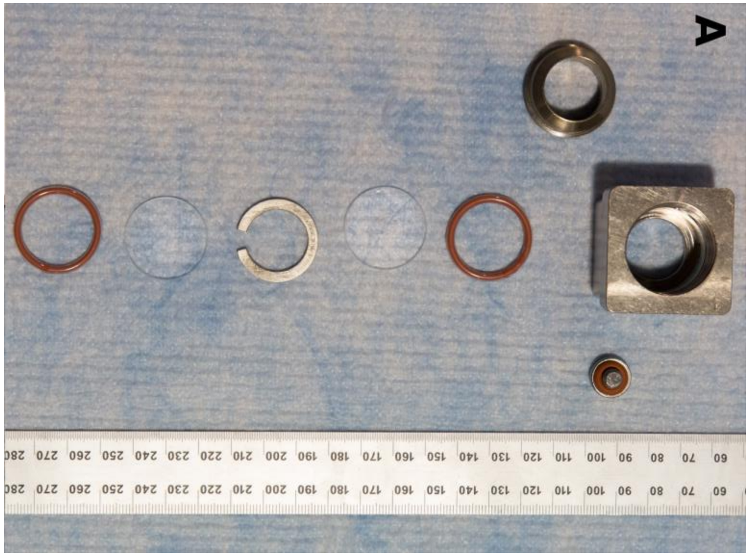
- Low neutron cross-section for various structural materials
- Versatile

Sample environment

- Linear sample changer





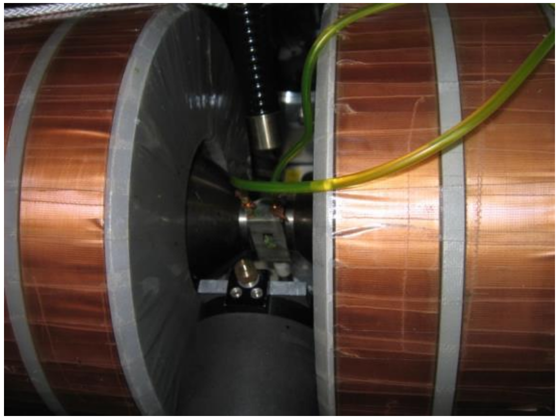


From https://neutrons.ornl.gov/eqsans/gallery

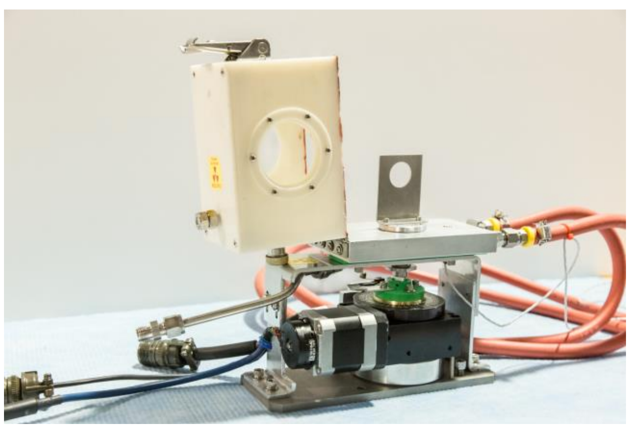
Sample environment

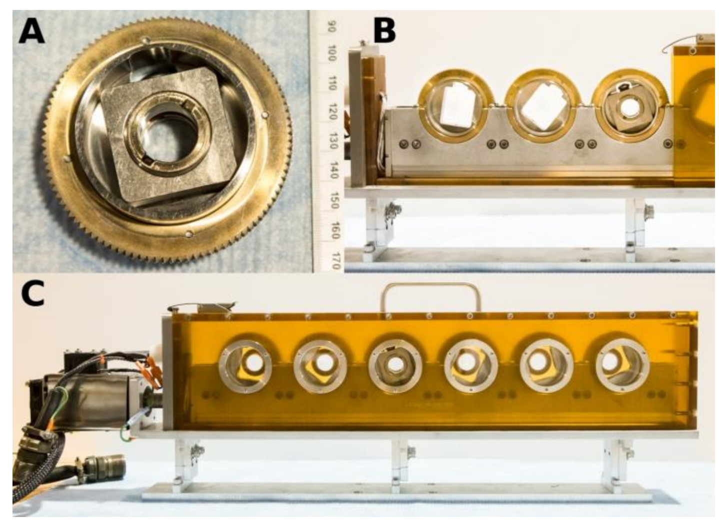
- Linear sample changer

Sample environment



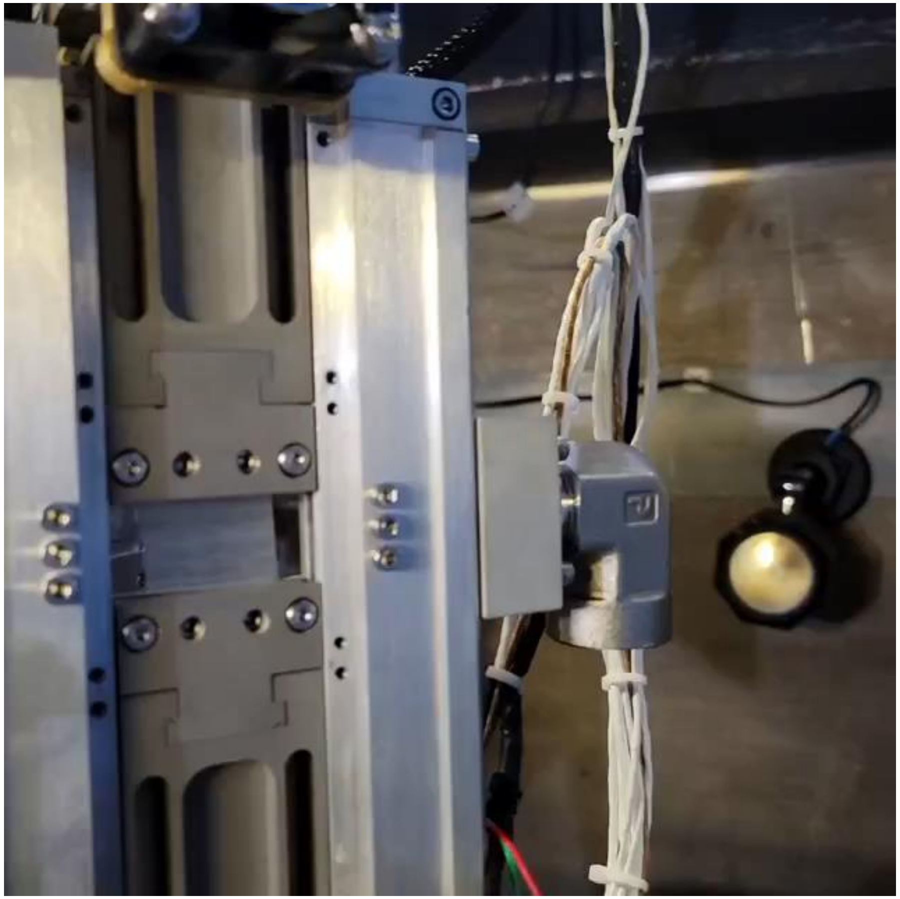
See Ünnep et al. 2014 Plant Physiology and Biochemistry





From neutrons.ornl.gov

Sample environment – tensile stage



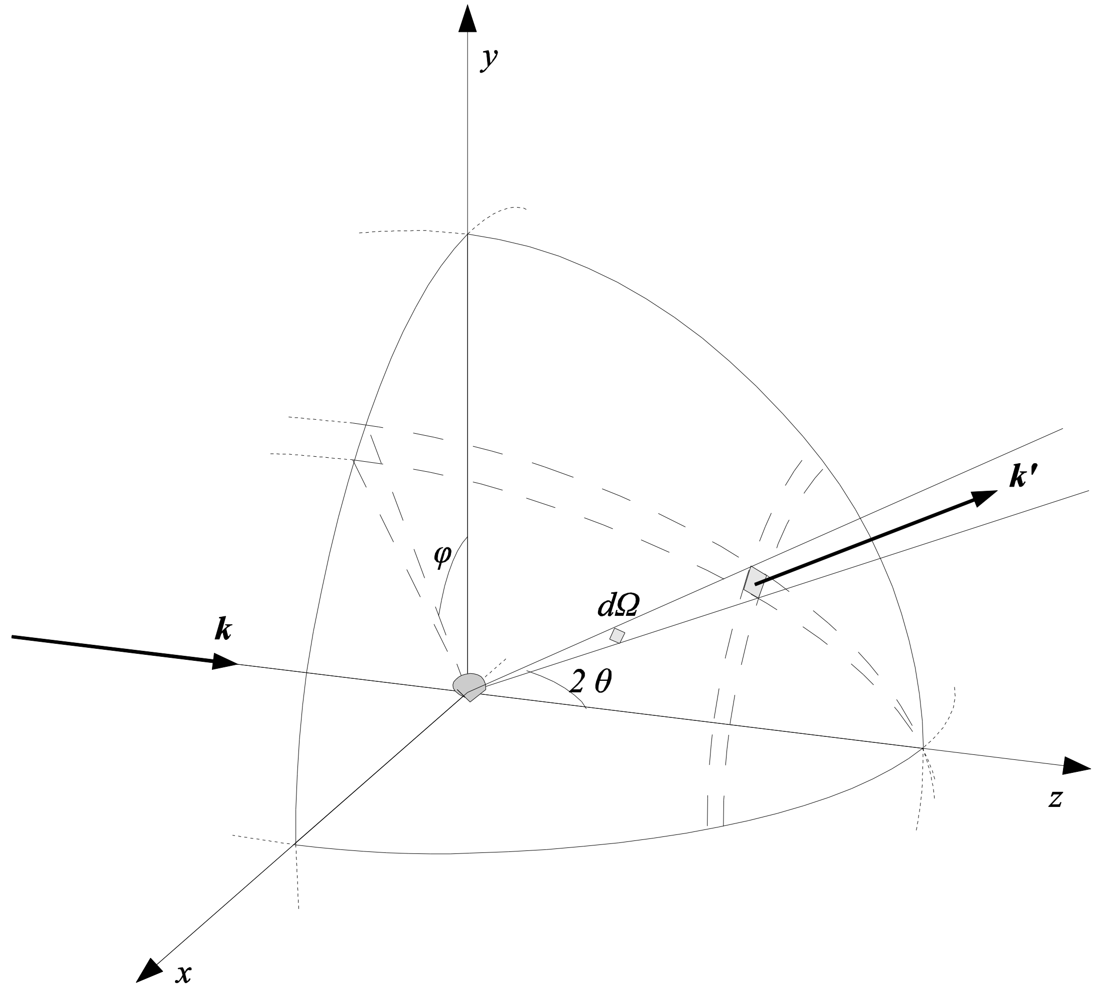
From neutrons.ornl.gov

SANS
Basic Theory

Scattering process

$$\frac{d^2\sigma_s}{d\Omega dE'} = \frac{N(\theta, \varphi, E')}{\Phi d\Omega dE'}$$

$$|\mathbf{k}| = \frac{2\pi}{\lambda}$$



From Nagy 2011

Scattering process

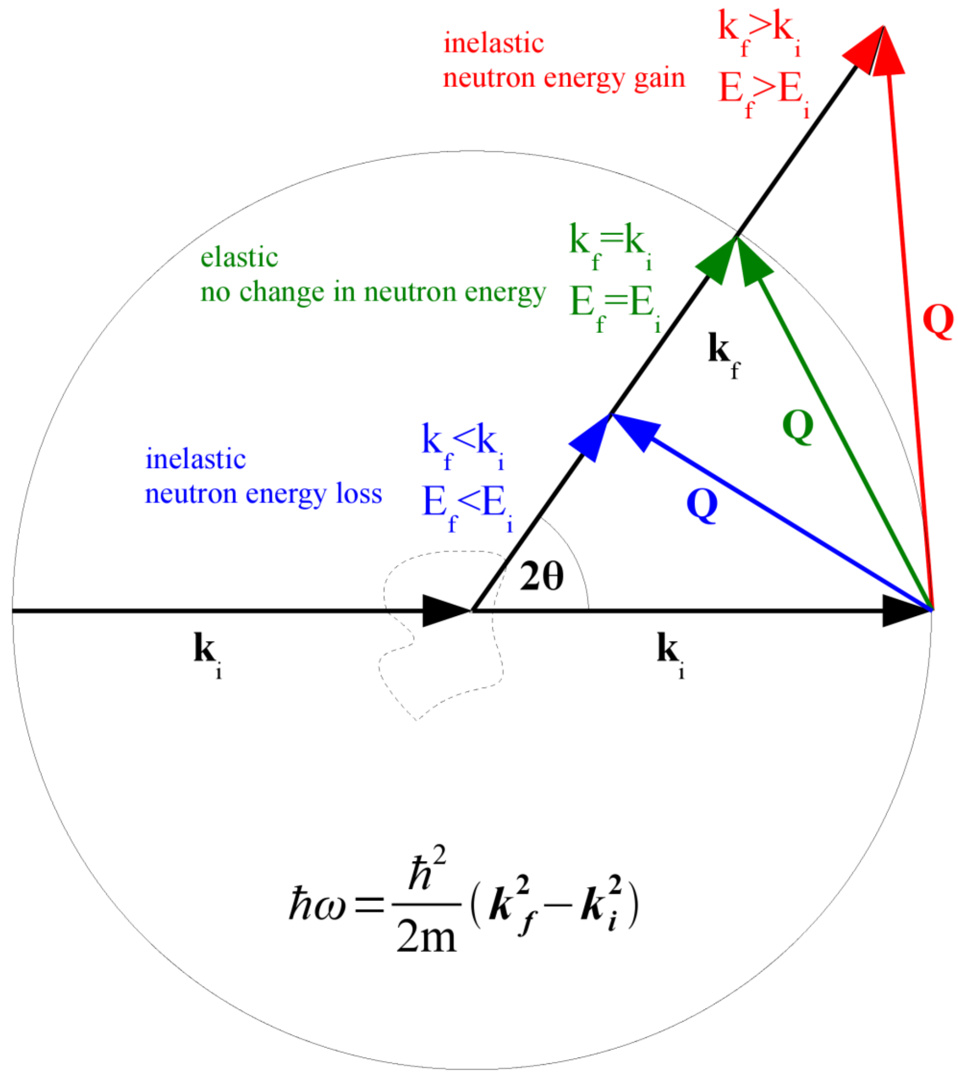
$$\frac{d^2\sigma_s}{d\Omega dE'} = \frac{N(\theta, \varphi, E')}{\Phi d\Omega dE'}$$

$$|\mathbf{k}| = \frac{2\pi}{\lambda}$$

$$\mathbf{Q} = \mathbf{k}' - \mathbf{k}$$

If $|\mathbf{k}'| = |\mathbf{k}|$

$$Q = \frac{4\pi \sin\theta}{\lambda}$$



Contrast

- Differential scattering cross-section $\frac{d\sigma_s}{d\Omega} = \left| \sum_j b_j e^{i\mathbf{QR}_j} \right|^2$
- Minimal d-spacing determinable in an experiment $\Delta x \approx \frac{2\pi}{Q_{\max}}$
- When Δx >> atomic distances -> can be considered as continuum
- Scattering length density – SLD $\rho(\mathbf{R}) = \frac{\sum_k b_k}{v}$

  (sum of the scattering lengths of the atoms in the volume element)
- $\frac{d\sigma_s}{d\Omega} = \left| \int_V \rho(\boldsymbol{R}) e^{i\boldsymbol{QR}} d^3R \right|^2$
- Solvent with ρ_0 SLD -> $\frac{d\sigma_s}{d\Omega} = \rho_0^2 \left| \int_V e^{i\boldsymbol{QR}} d^3R \right|^2 = const \times \delta(Q)$
- Solvent with ρ_0 SLD and particles with particles $\rho(\mathbf{R})$
- $\frac{d\sigma_s}{d\Omega} = \left| \int_V (\rho(\boldsymbol{R}) - \rho_0) e^{i\boldsymbol{QR}} d^3R \right|^2$

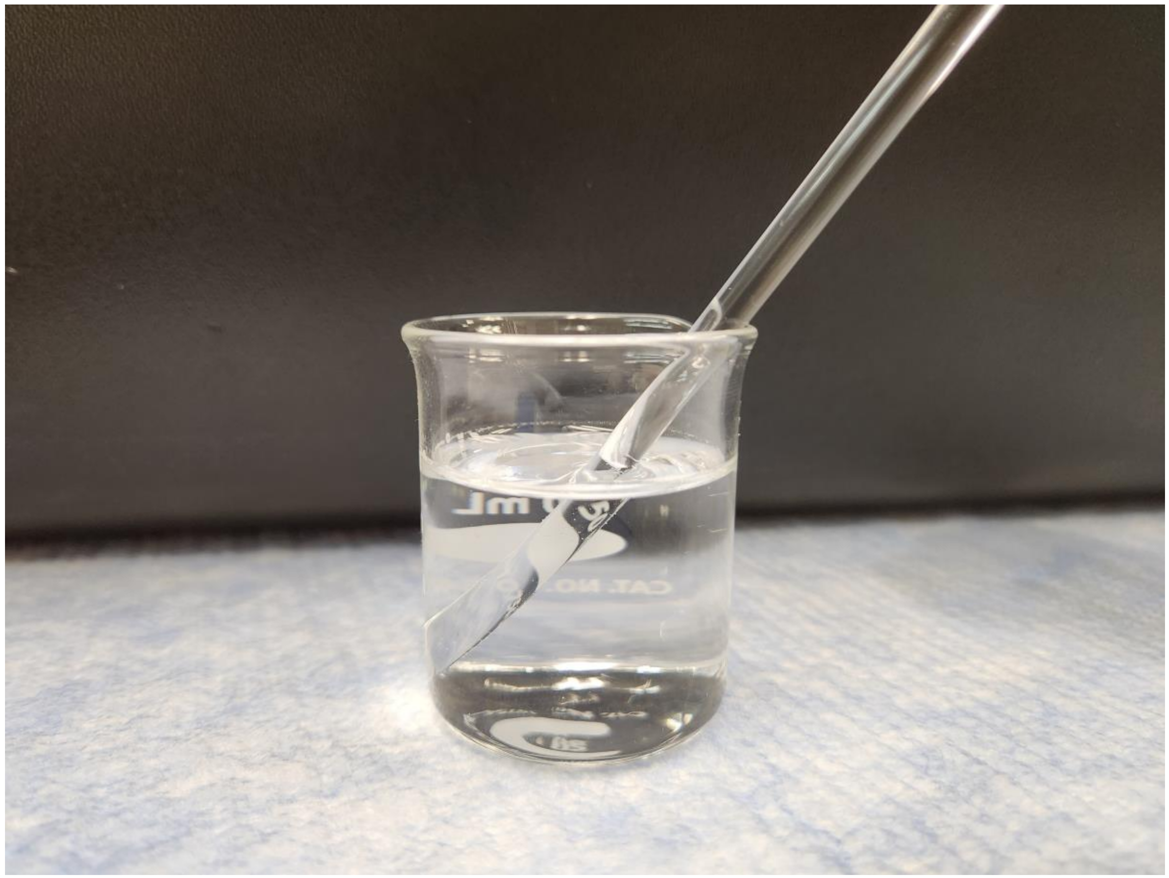
Contrast

$\rho(\mathbf{R}) - \rho_0$

Contrast

- Refractive index:

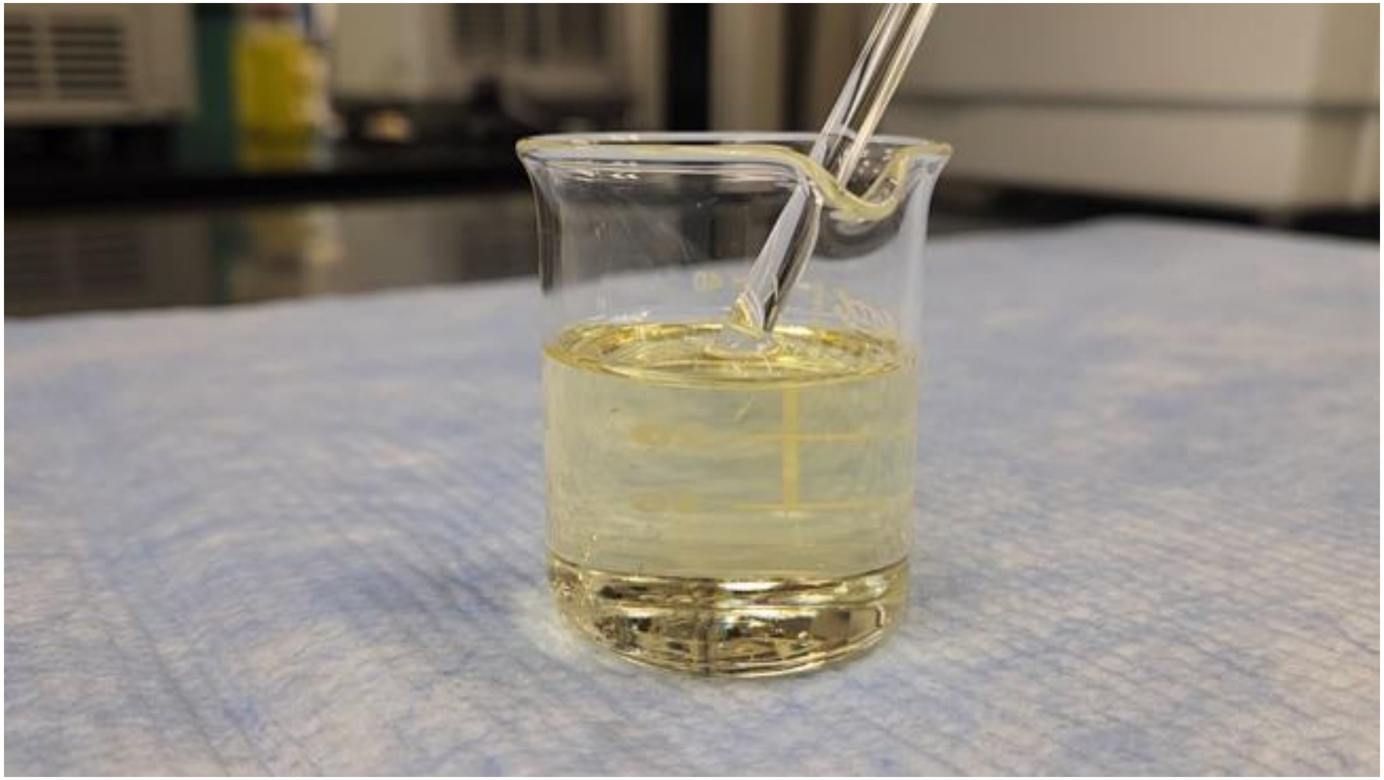
air ~ 1.0003, water ~ 1.33, glass ~ 1.5



Contrast

- Refractive index:

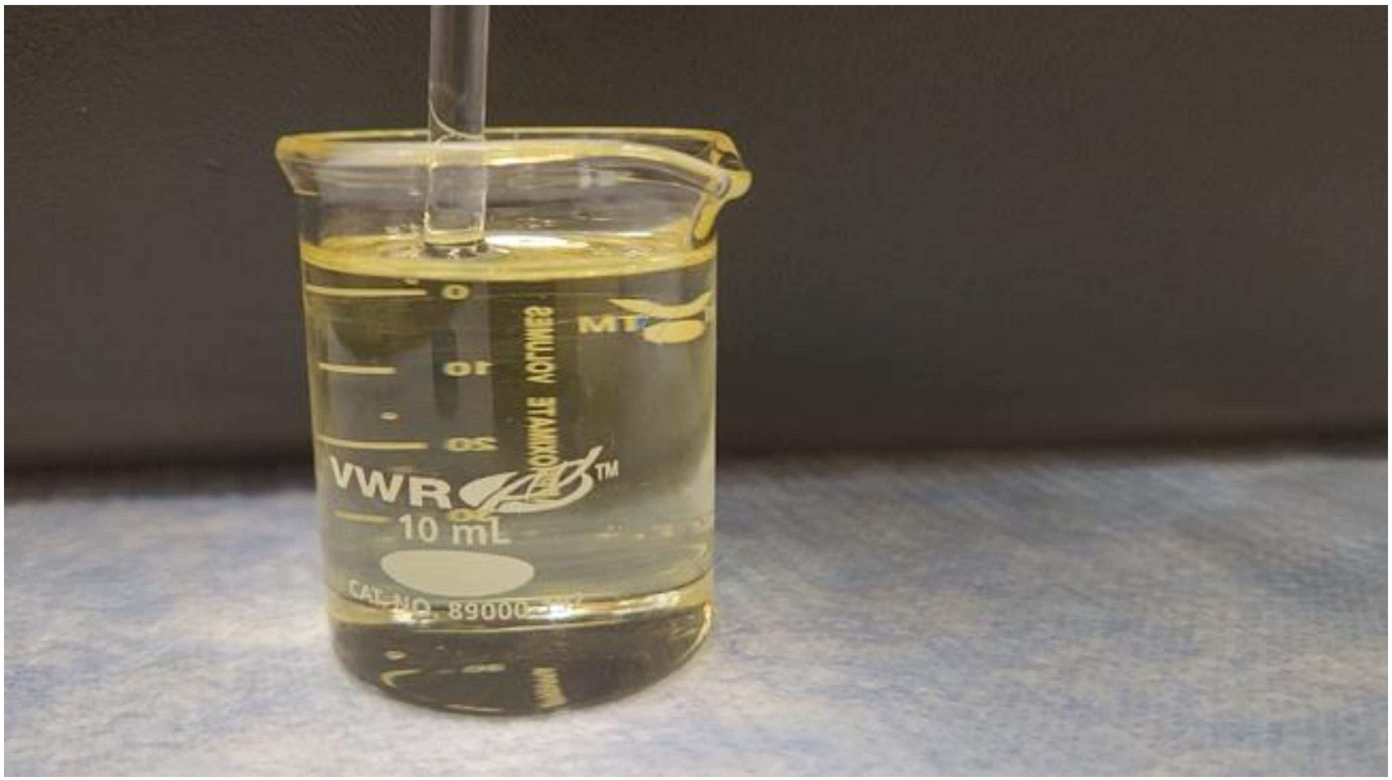
air ~ 1.0003, water ~ 1.33, glass ~ 1.5, vegetable oil ~ 1.47



Contrast

- Refractive index:

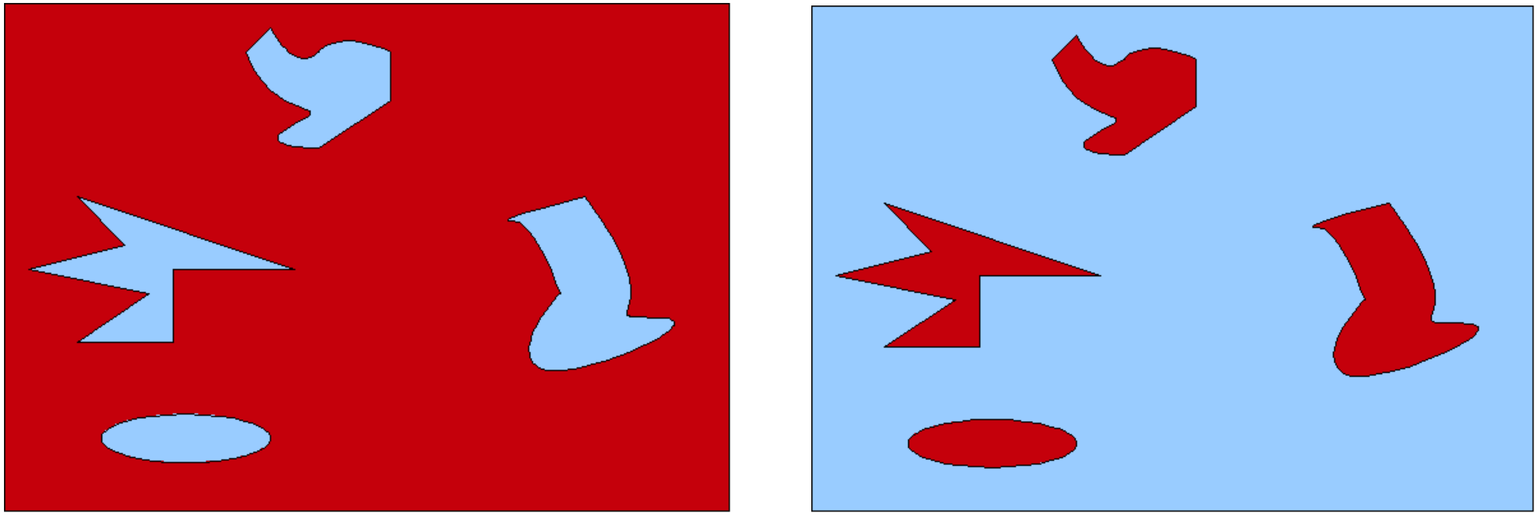
air ~ 1.0003, water ~ 1.33, glass ~ 1.5, vegetable oil ~ 1.47



Babinet's principle

$$\frac{d\sigma_s}{d\Omega} = \left| \int_V \left(\rho(\boldsymbol{R}) - \rho_0 \right) e^{i\boldsymbol{QR}} d^3 R \right|^2$$

- Identical scattering signal (apart from forward and incoherent scattering)



Two phase statistically isotropic system

- Uncorrelated particles
- Scattering length densities ρ_1 and ρ_0 and $\Delta\rho = \rho_1 - \rho_0$
- $\frac{d\sigma_s}{d\Omega} = \left| \int_V (\rho(\mathbf{R}) - \rho_0) e^{i\mathbf{QR}} d^3R \right|^2$ simplifies to $\frac{d\sigma_s}{d\Omega} = \left| \int_{V_1} \Delta\rho e^{iQR} dR \right|^2$
- Scattering intensity/unit volume $I(Q) = \frac{\Delta\rho^2}{V} \left| \int_{V_1} e^{iQR} dR \right|^2$
- For a set of N_p identical particles

$$I(Q) = \frac{\Delta\rho^2}{V} N_p \left\langle \left| \int_{V_p} e^{iQR} dR \right|^2 \right\rangle = \frac{V_p^{\ 2}}{V} \Delta\rho^2 N_p \frac{1}{V_p^{\ 2}} \left\langle \left| \int_{V_p} e^{iQR} dR \right|^2 \right\rangle = \Phi V_p \Delta\rho^2 P(Q)$$

$\Phi = \frac{N_p V_p}{V}$ – volume fraction of the particles

$P(Q)$ – particle form factor

Particle Form Factors - Examples

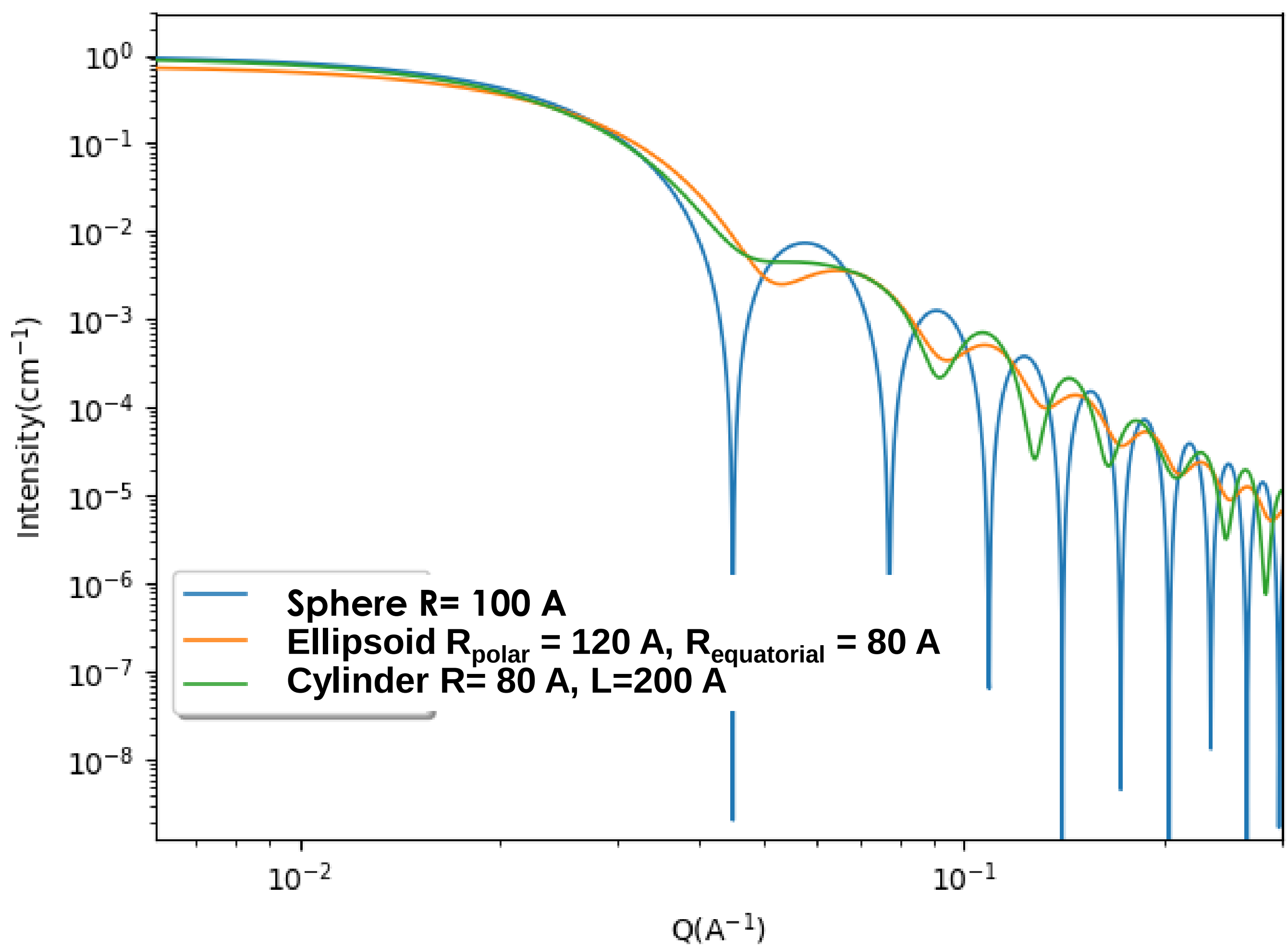
- Sphere with radius R $$P = \left[3 \frac{\sin(QR) - (QR)\cos(QR)}{(QR)^3} \right]^2$$
- Ellipsoid of revolution $$P = \int_0^{\pi/2} P_{sphere}(Q, R(r,\varepsilon,\alpha)) \sin\alpha \, d\alpha$$
  where $R(r,\varepsilon,\alpha) = r\sqrt{\sin^2\alpha + \varepsilon^2\cos^2\alpha}$
- Cylinder $$P = \int_0^{\pi/2} \frac{\sin^2(QL\cos\alpha)}{(QL\cos\alpha)^2} \frac{4J_1^2(QR\sin\alpha)}{(QL\sin\alpha)^2} \sin\alpha \, d\alpha$$

- This work benefited from the use of the **SasView** application, originally developed under NSF award DMR-0520547. **SasView** contains code developed with funding from the European Union’s Horizon 2020 research and innovation programme under the SINE2020 project, grant agreement No 654000.

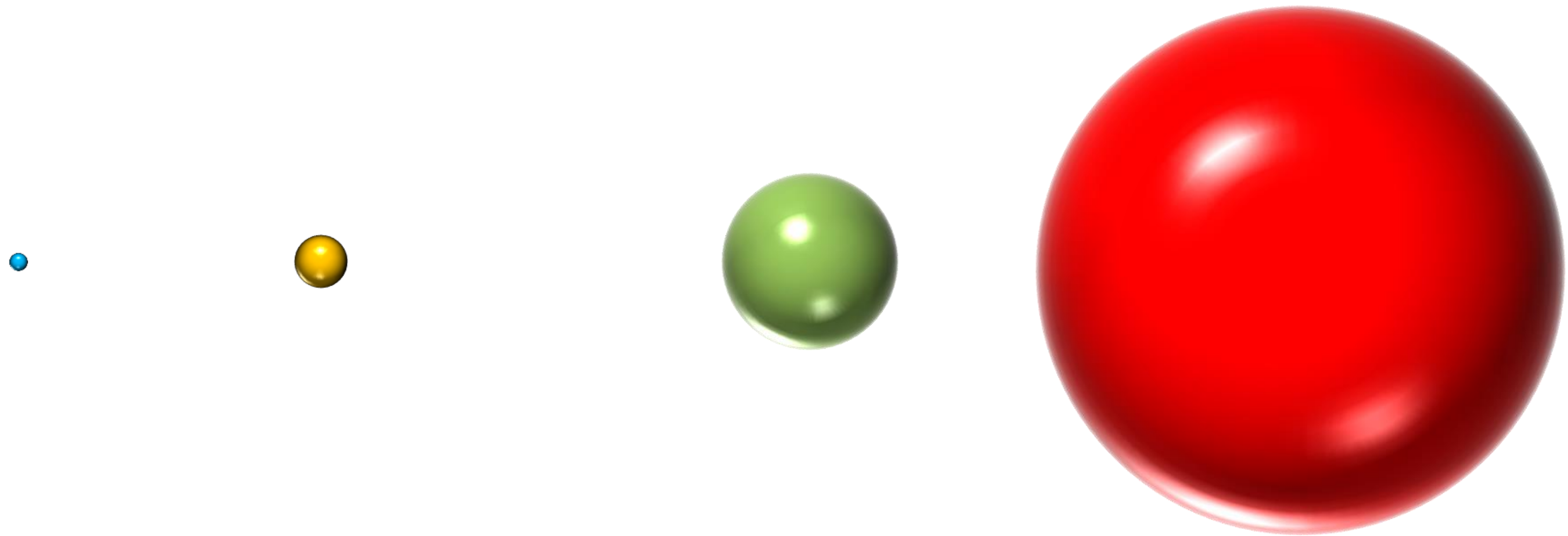
- M. Doucet et al. SasView Version 5.0.4, Zenodo, DOI:10.5281/zenodo.4467703

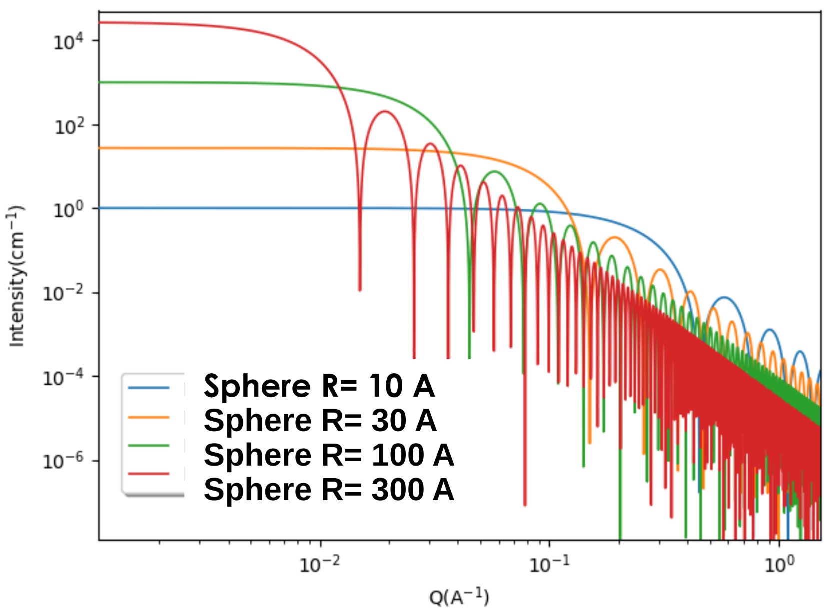
Particle Form Factors - Examples

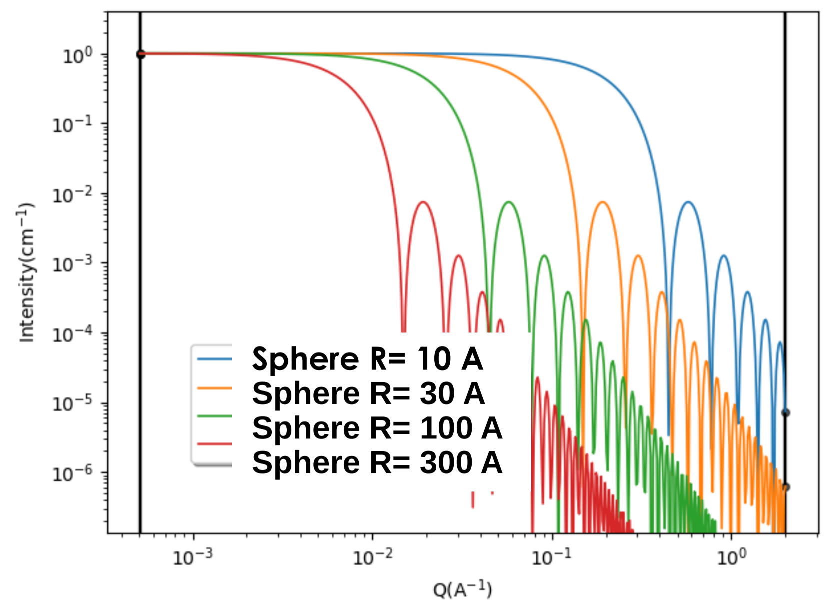
- Spher
- Ellipsc
- Cylinc



Guinier law




Intensity(cm⁻¹)
Q(A⁻¹)
Sphere R= 10 A
Sphere R= 30 A
Sphere R= 100 A
Sphere R= 300 A


Intensity(cm⁻¹)
Q(A⁻¹)
Sphere R= 10 A
Sphere R= 30 A
Sphere R= 100 A
Sphere R= 300 A

Guinier law

- Average contrast to solvent $\bar{\rho}$
- For small Q values $I(Q) = \left| \int_V (\rho(\mathbf{R}) - \rho_0) \left(1 + i\mathbf{QR} - \frac{1}{2}(\mathbf{QR})^2 + \ldots \right) d^3R \right|^2$
  - presume center of gravity of volume and $\rho(\mathbf{R})$ coincide

$$I(Q) = (\bar{\rho}V)^2 \left(1 - \frac{1}{3} \frac{\mathbf{Q}^2}{\bar{\rho}V} \int_V R^2 (\rho(\mathbf{R}) - \rho_0) d^3R \right)$$

$\frac{1}{\bar{\rho}V} \int_V R^2 (\rho(\mathbf{R}) - \rho_0) d^3R = R_G{}^2$ and R_G is the radius of giration

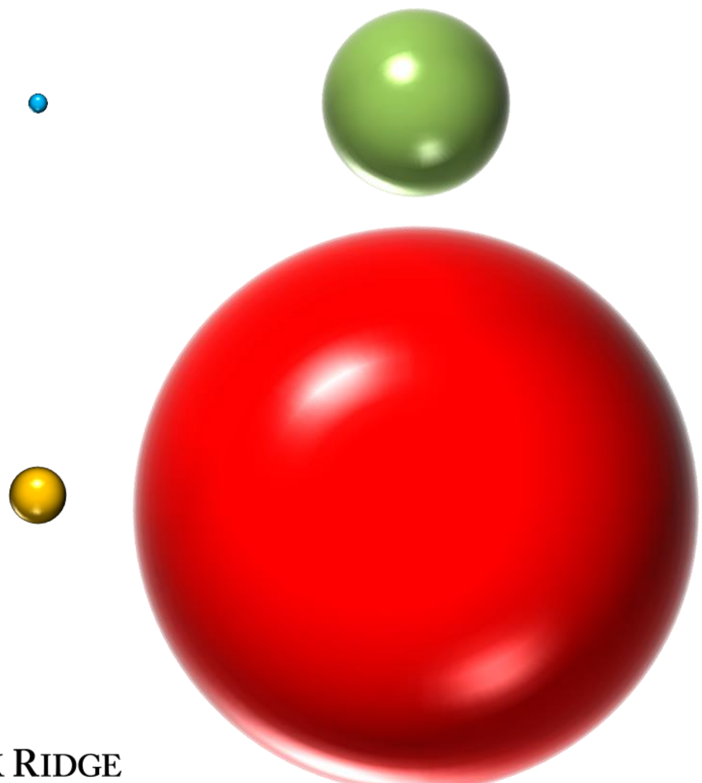
- Guinier law $I(Q) = I_0 \exp\left(-\frac{R_g^2 Q^2}{3} \right)$

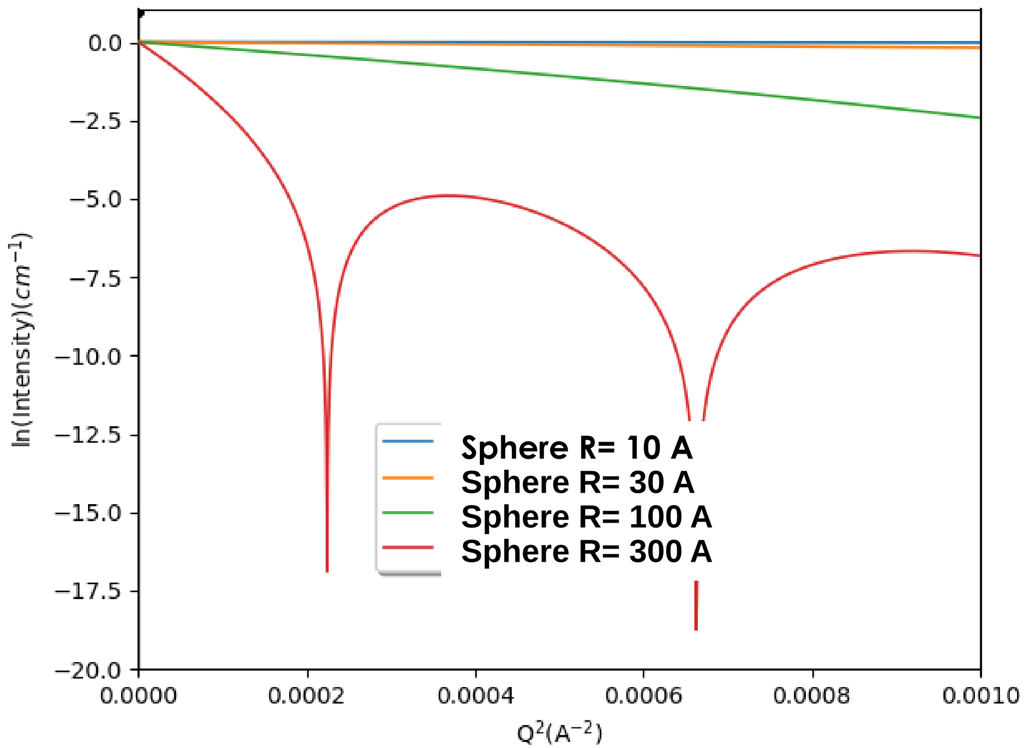
- ln(I) vs Q[2] plot $QR < 1.3$
- Validity: low Q

For details see e.g. Jacrot 1976 Rep. Prog. Phys. 39

Guinier law

- For small Q values $QR < 1.3$
- Guinier law $$I(Q) = I_0 \exp\left(-\frac{R_g^2 Q^2}{3}\right)$$
- ln(I) vs Q^2 plot

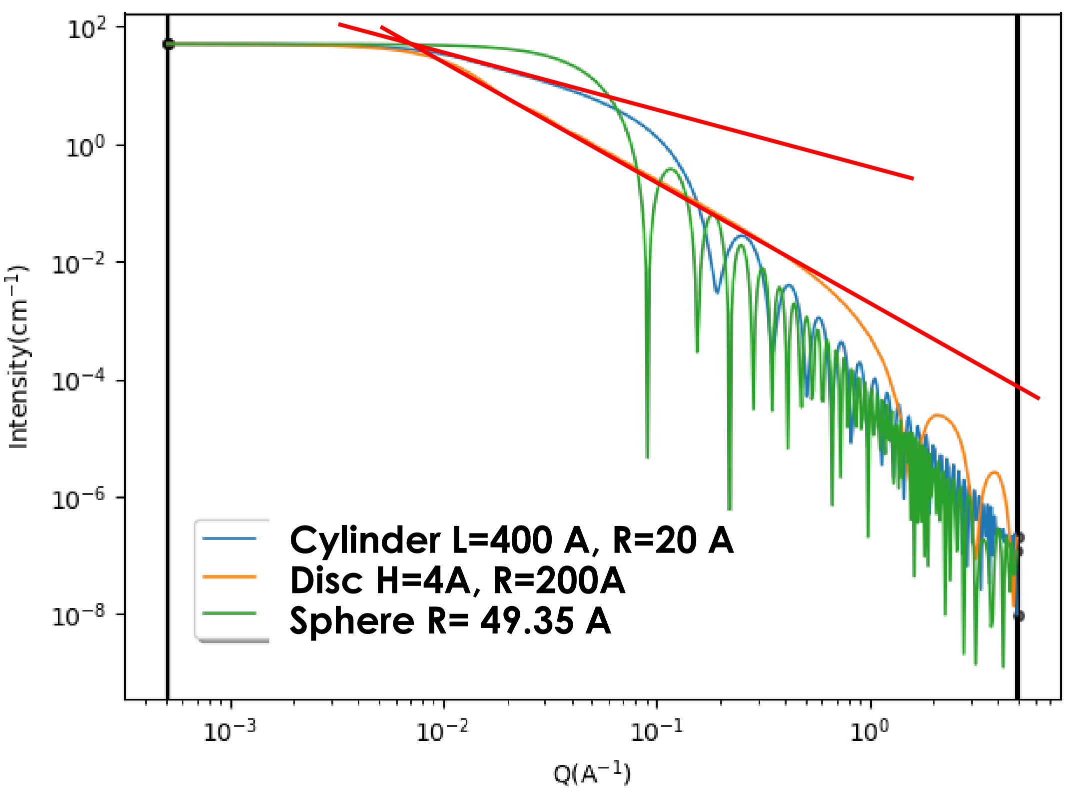




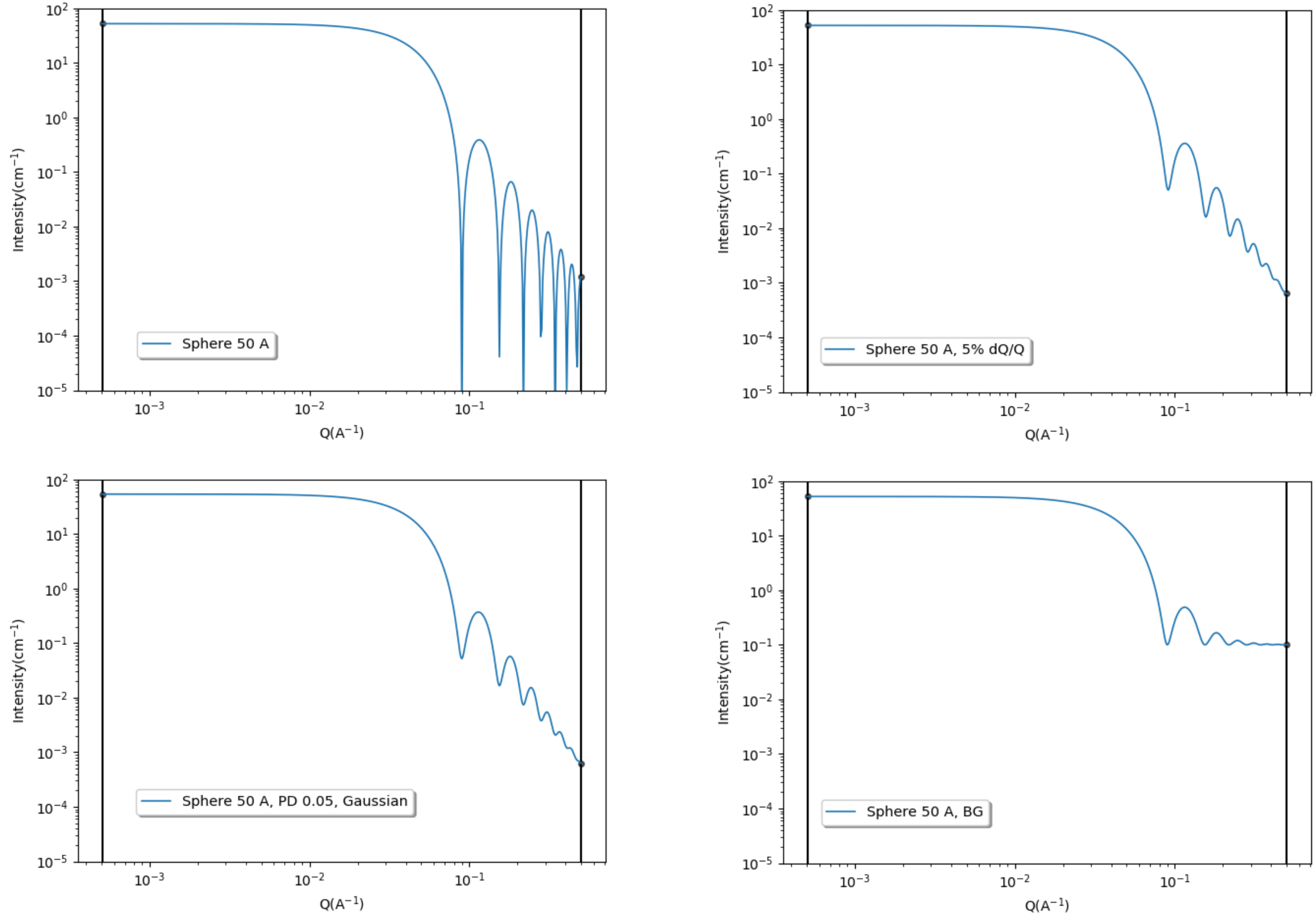
For details see e.g. Jacrot 1976 Rep. Prog. Phys. 39

Fractal dimensions

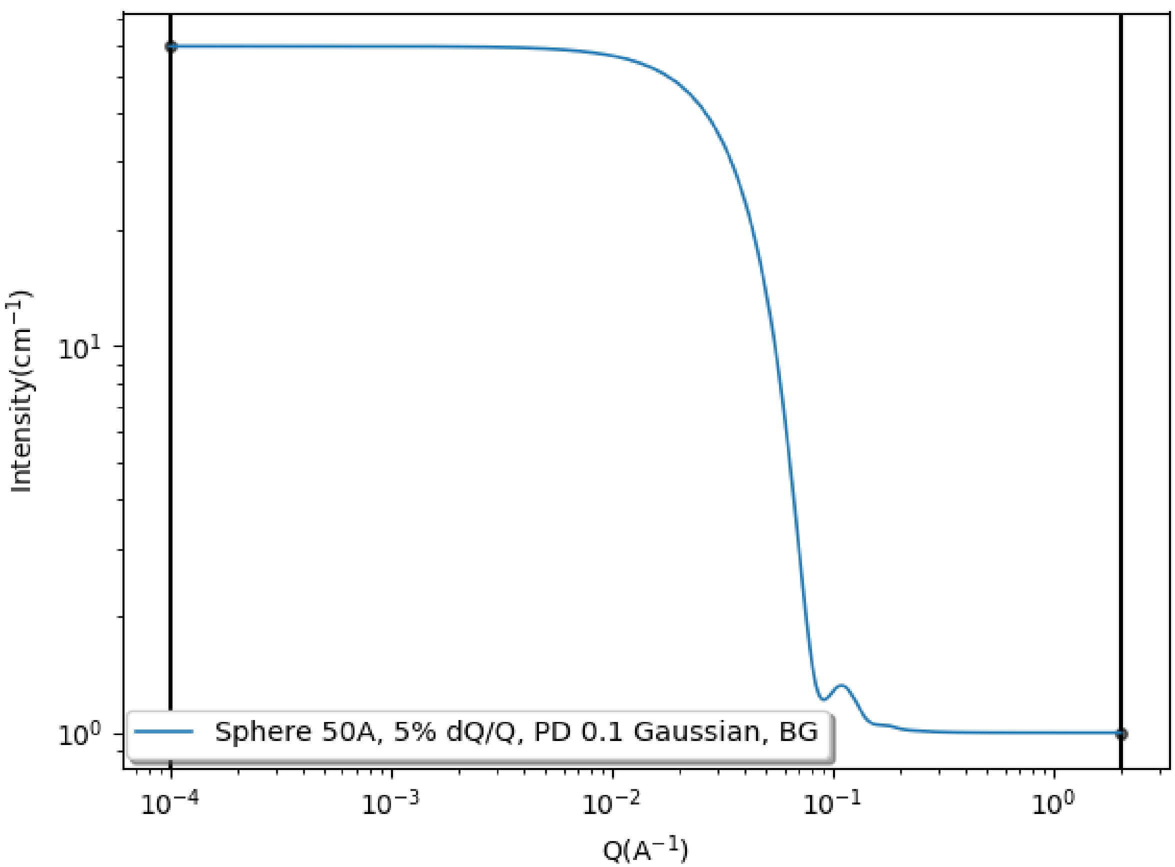
- Mass fractals $M \propto R^D$ $I(Q) \propto Q^{-D}$
  - long elongated objects Q^{-1}
  - 2 D objects Q^{-2}
- Surface fractals $I(Q) \propto Q^{6-D}$
  - For smooth surfaces Q^{-4}
  - Rough fractal interfaces $Q^{-x}, 3 < x < 4$
  - Diffuse interfaces $Q^{-x}, x > 4$



Sphere – 50 A
Resolution, polydispersity, background



Sphere – 50 A
Resolution, polydispersity, background



Structure factor

$$I(Q) = \frac{1}{V}\left|\int_V \rho(\mathbf{R}) e^{i\mathbf{QR}} d^3R\right|^2$$

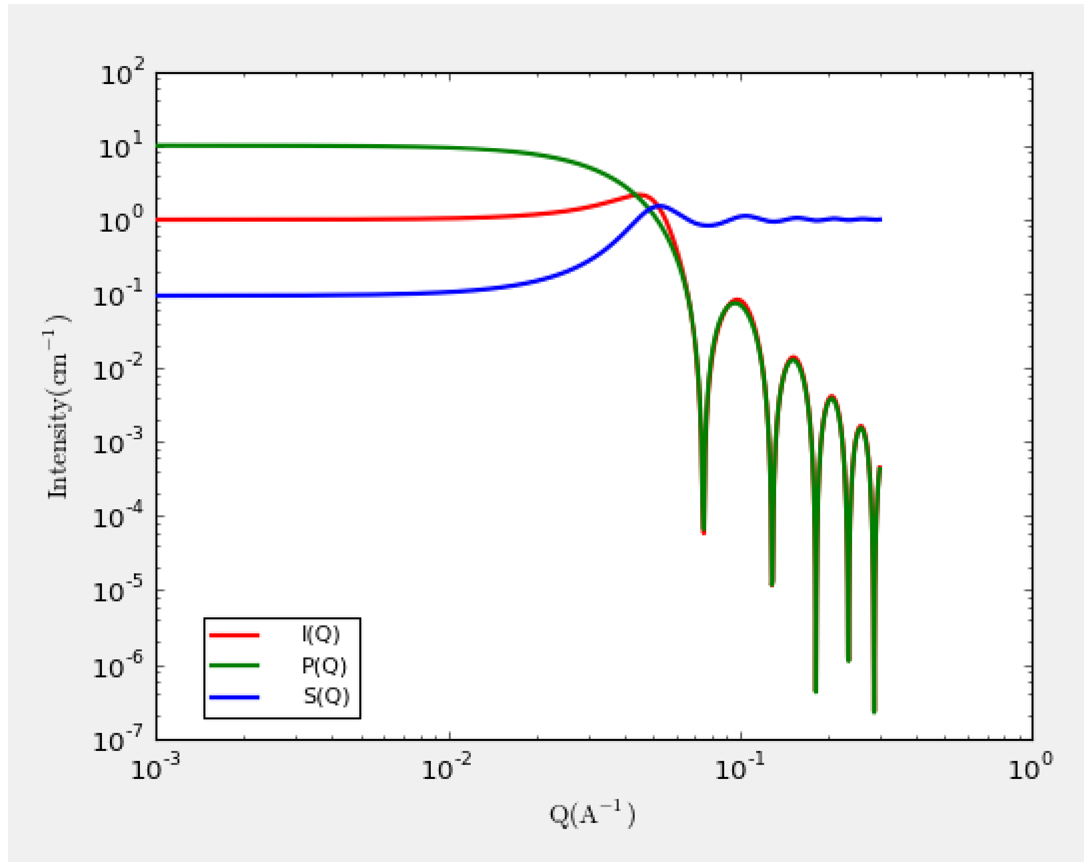
- Correlated particles, N identical particles, at particle i $\mathbf{R} = \mathbf{R}_i + \mathbf{u}$
- $I(Q) = \frac{N_p}{V}\left\langle \frac{1}{N_p}\sum_{i=1}^{N}\sum_{j=1}^{N} e^{i\mathbf{Q}(\mathbf{R_i}-\mathbf{R_j})}\left|\int_{V_p}\rho(u)e^{iQu}du\right|^2\right\rangle$ where 2nd term $\propto P(Q)$
- Structure factor: $S(Q) = \left\langle \frac{1}{N_p}\sum_{i=1}^{N}\sum_{j=1}^{N} e^{i\mathbf{Q}(\mathbf{R_i}-\mathbf{R_j})}\right\rangle = 1 + \left\langle \frac{1}{N_p}\sum_{i=1}^{N}\sum_{j\neq i}^{N} e^{i\mathbf{Q}(\mathbf{R_i}-\mathbf{R_j})}\right\rangle$

$$I(Q) = \Phi V_p \Delta\rho^2 P(Q) S(Q)$$

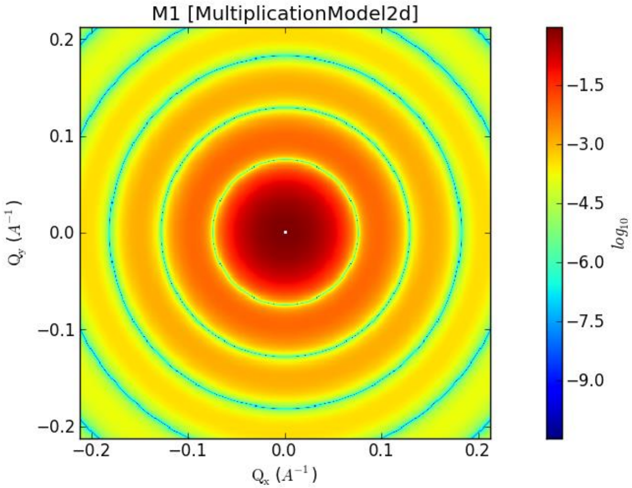
For details see e.g. I. Grillo, Small-Angle Neutron Scattering and Applications in Soft Condensed Matter in Soft-Matter Characterization (Ed.: R. Borsali, R. Pecora) 2008 Springer

Structure factor

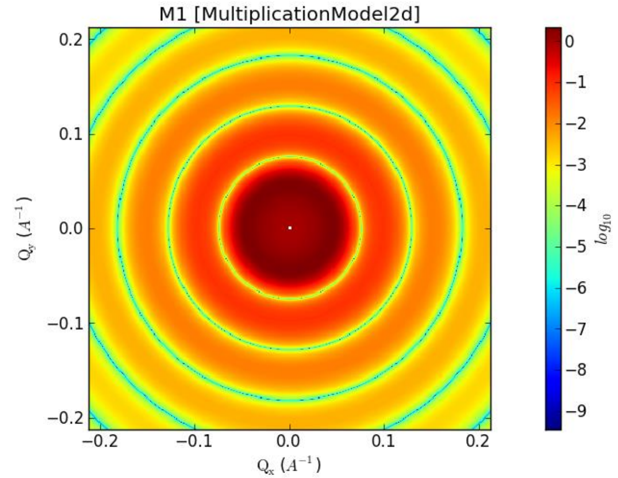
- Hard sphere interaction
- Interparticle correlation
- Particle size



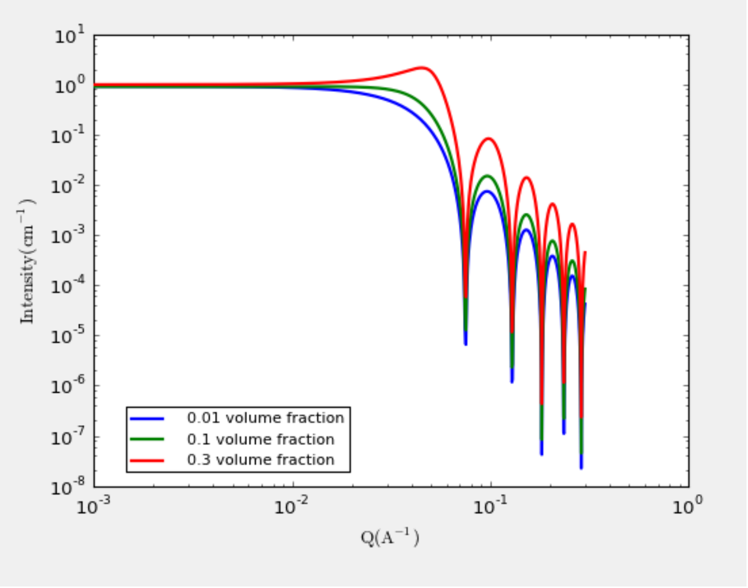
Structure factor



0.01 volume fraction



0.3 volume fraction

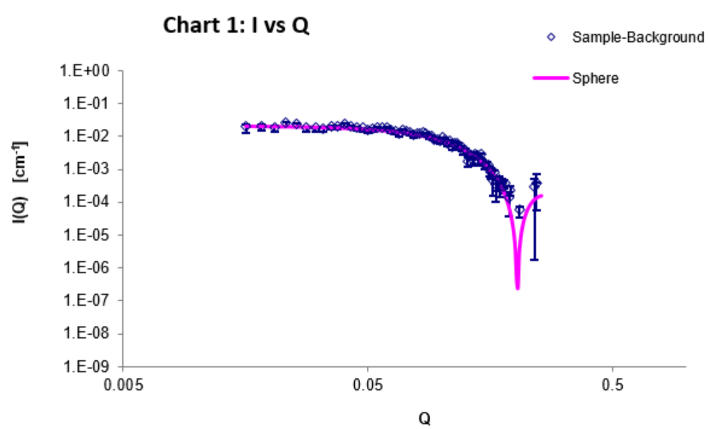


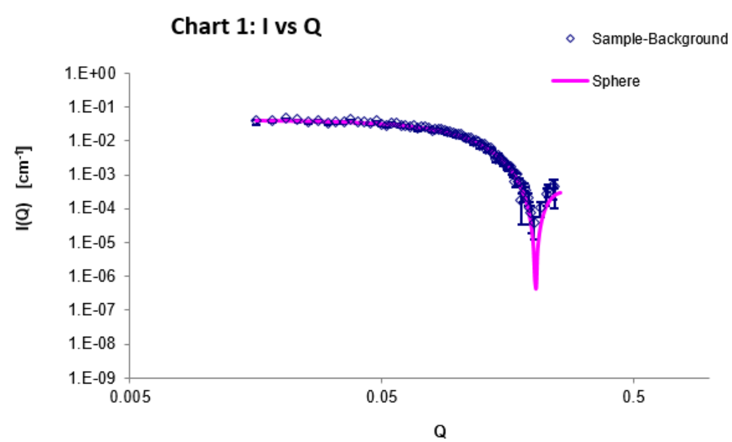
SANS
for Biology and Soft Matter

Neutrons
What makes them special for biology?

[] = 10^{-12} cm	^{1}H	^{2}H	^{12}C	^{14}N	^{16}O	^{32}S	^{31}P
Coherent	-0.3741	0.6671	0.6651	0.937	0.5803	0.2804	0.513
Incoherent	2.5274	0.404		0.2			0.02

Adapted from Jeffries et al. 2016 Nat Protoc based on data in Sears VF. Neutron scattering lengths and cross sections. Neutron News. 1992;3:26–37.

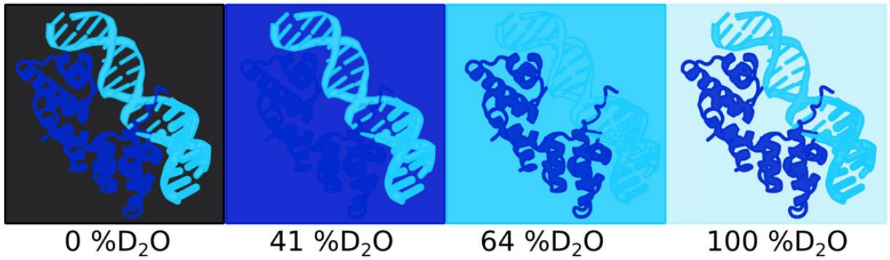


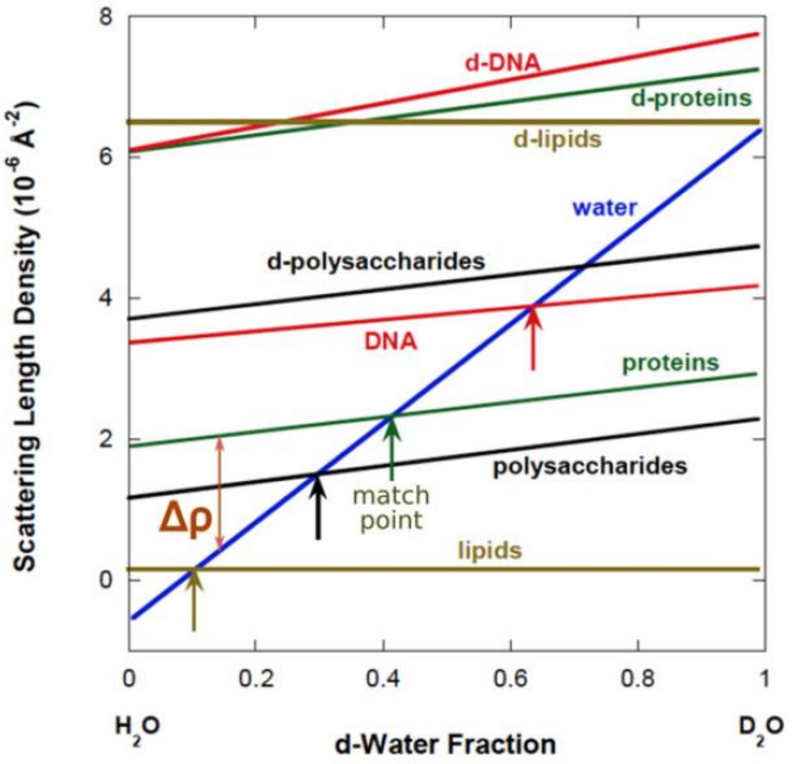


Retrieved from code of Volker Urban (ORNL) for the Bio-SANS instrument

Contrast Variation in Biology

- Often hydrogenated sample in H_2O/D_2O mixture
- Complex macromolecules
- Subunits with different SLD
- Partial deuteration
- Contrast matching
- Exchangeable hydrogens
- Alcohol, acid, base



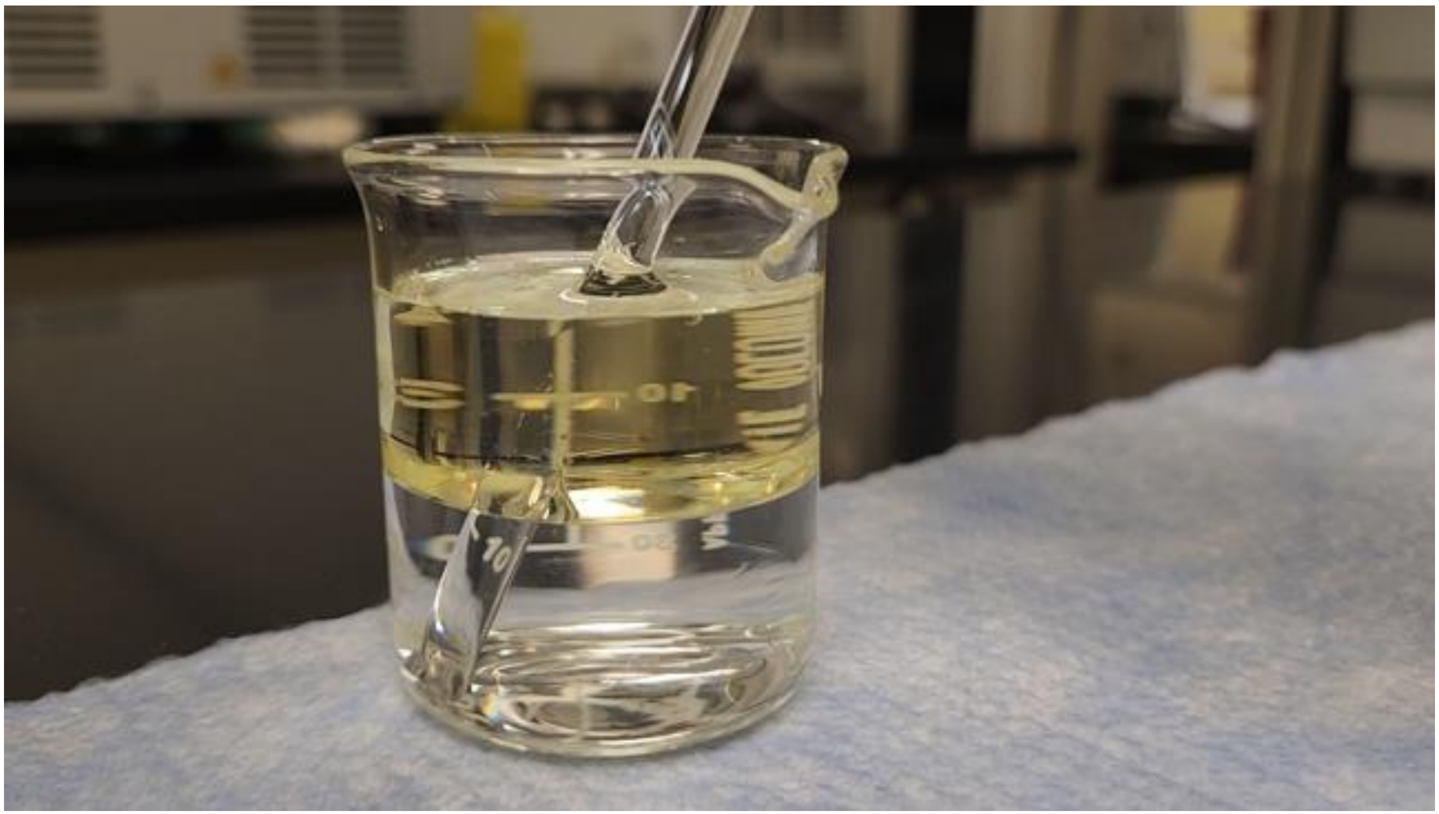


From Castellanos et al. 2017 Comp Struct Biotech J Open Access

Contrast

- Refractive index:

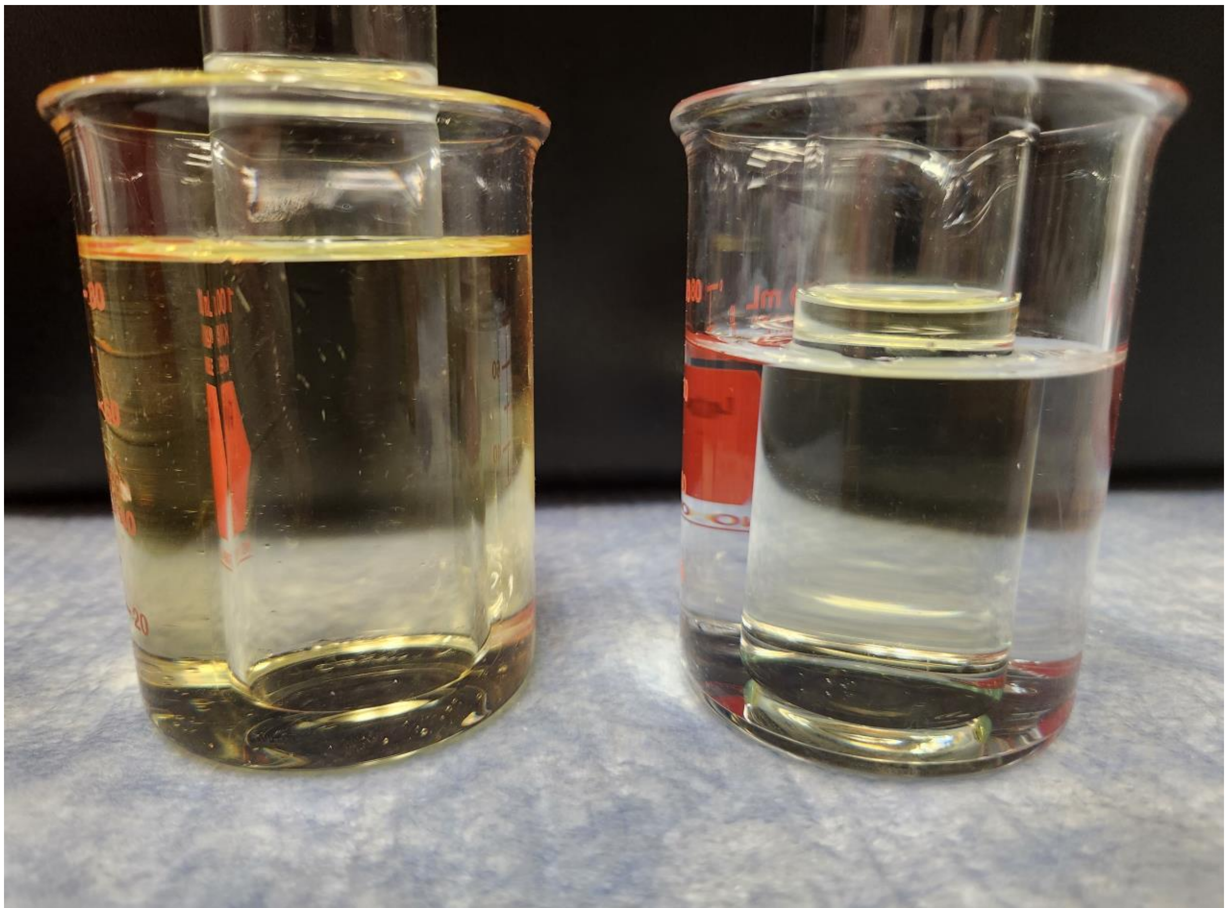
air ~ 1.0003, water ~ 1.33, glass ~ 1.5, vegetable oil ~ 1.47



Contrast

- Refractive index:

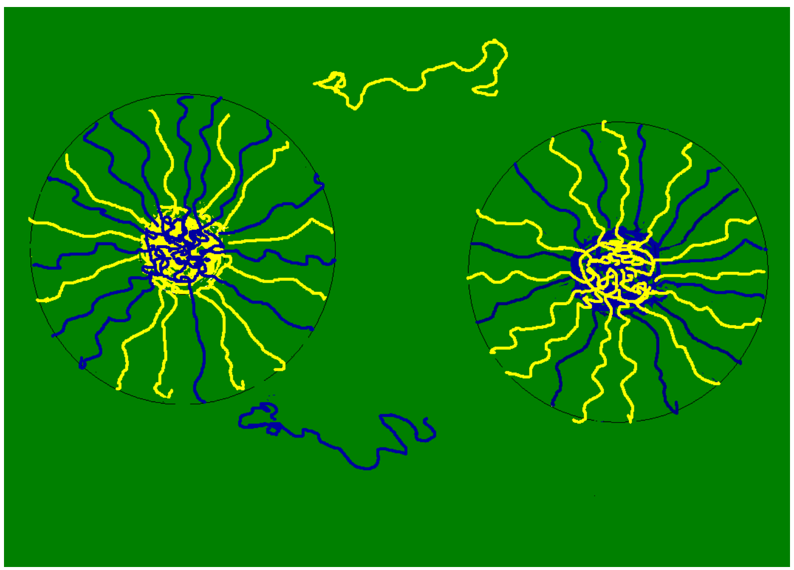
air ~ 1.0003, water ~ 1.33, glass ~ 1.5, vegetable oil ~ 1.47

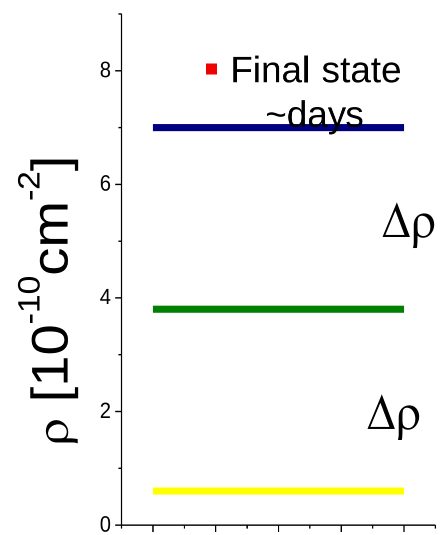


OAK RIDGE National Laboratory

SANS kinetics

- Equilibrium chain exchange kinetics of block copolimer micelles
  – poly(ethylene-alt-propylene)–poly(ethylene oxide) (PEP–PEO) in water=N;N-dimethylformamide (DMF)
  – Chain exchange -> contrast lost -> Intensity drops





Courtesy of Joachim Kohlbrecher (PSI, Switzerland)
for further details see Lund, R. et al. 2006. Macromolecules

Small-angle Scattering Demonstration

- Hair thickness determination with laser pointer

http://www.lookingatnothing.com/index.php/archives/178

- Computer SAS experiment

http://www.lookingatnothing.com/index.php/archives/991

- Example images

https://bitbucket.org/toQDuj/liveft/src/master/

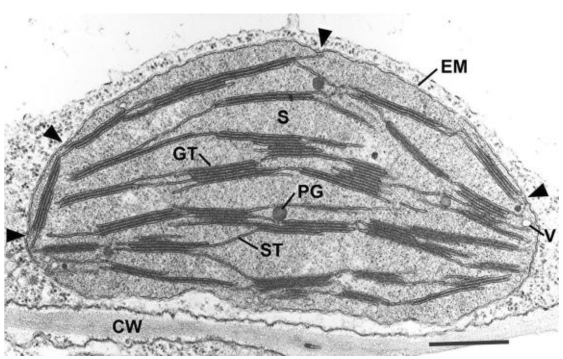
- Looking At Nothing; Brian Richard Pauw, NIMS, Japan
  - Now: Bundesanstalt für Materialforschung und -prüfung: Berlin, Berlin, DE
- Wifi: ......

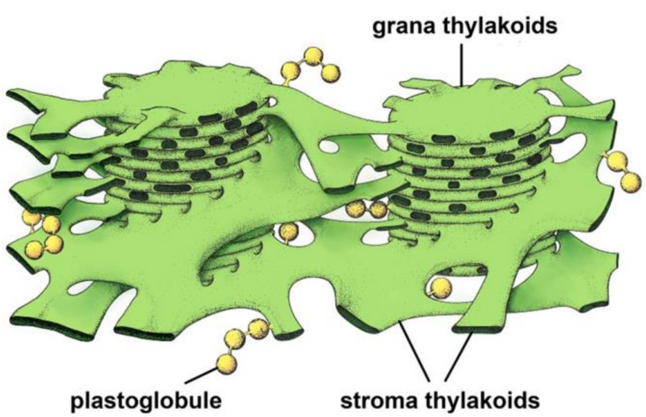
Fourier Camera
amahta
4,8 ★

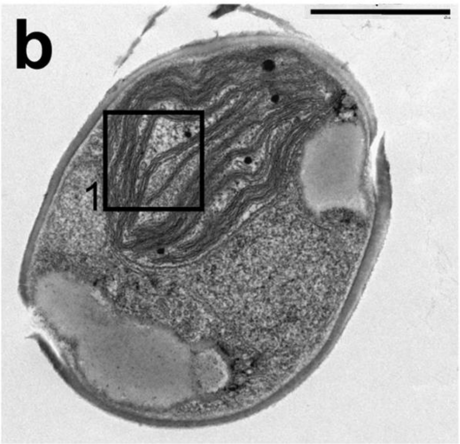
FFT Camera
Remy Horton

Thylakoid membrane structure

- Large variations in different organisms
- Lateral heterogeneity, granum – stroma separation and quasihelical structure in higher plants
- Stacks of several membranes in unicellular organisms (e.g. diatom)
- Ideal targets for diffraction studies







From Staehelin and Paolillo, Photosynthesis Research, 2020

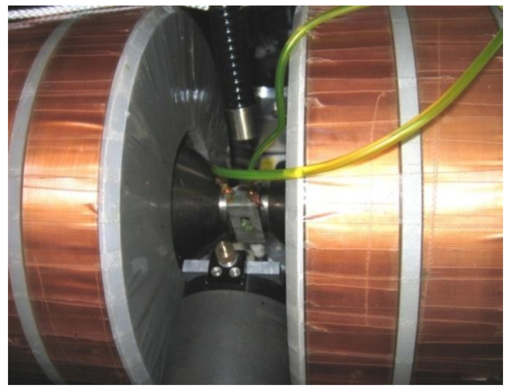
Bína, D. et al. Sci Rep 6, 25583 (2016)

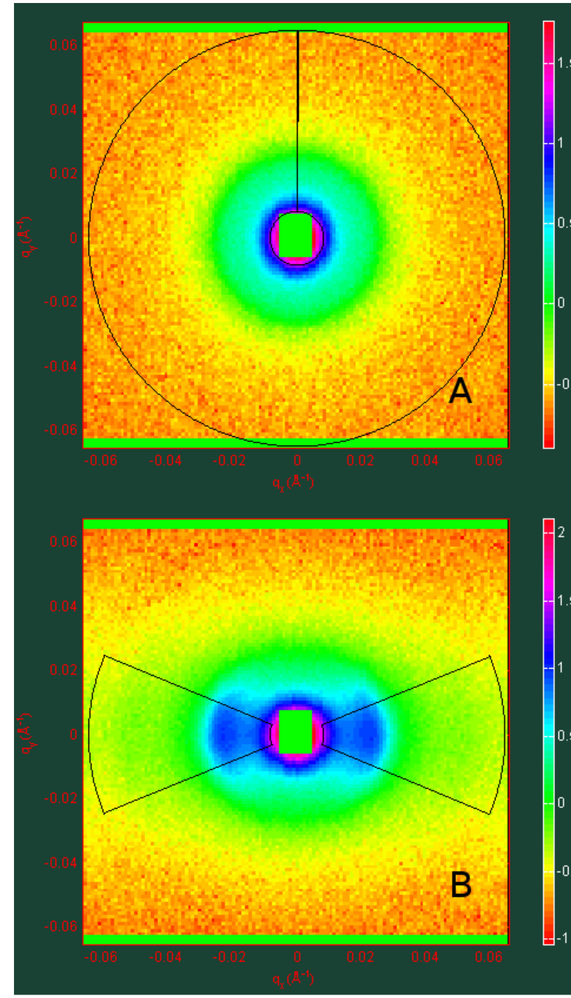
What questions can be answered with NS?

- Static structural characterization of each system
- Capitalize on the possibility for in vivo experiments
- Effect of stressors on the macroorganisation of thylakoid membranes – correlation with photosynthetic processes
  - Illumination under a wide range of light conditions
  - pH variation
  - Heavy metal ions
  - Trace elements
  - Effect of osmoticums and ions involved in membrane stacking

SANS of plant thylakoids

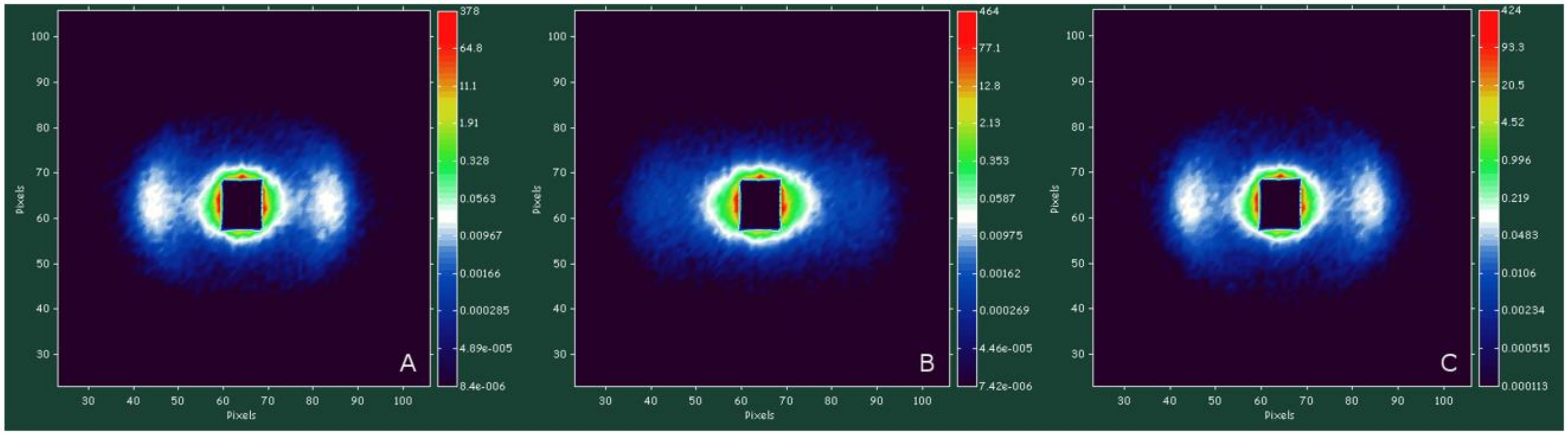
- Suspension, no fixation
- Statistically averaged information
- Magnetic orientation
- 2D scattering signal
- Sectorial averaging
- Instruments: D22, D11 (ILL), SANS I & II (PSI), Yellow Submarine (BNC), KWS II (JCNS, FRM II), EQ-SANS (SNS, ORNL)



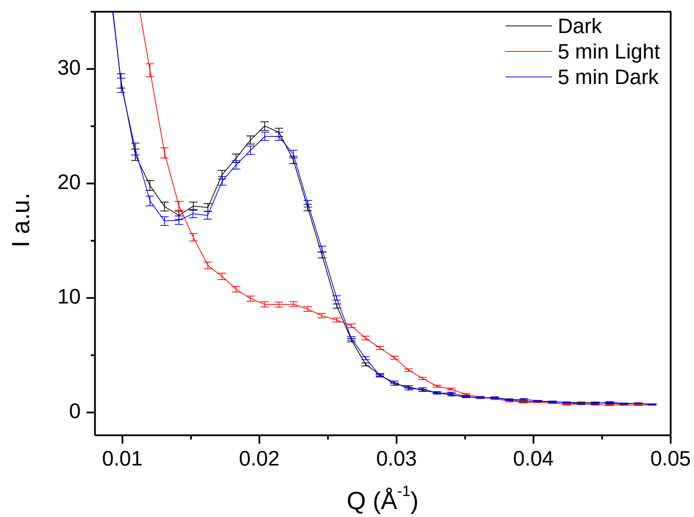


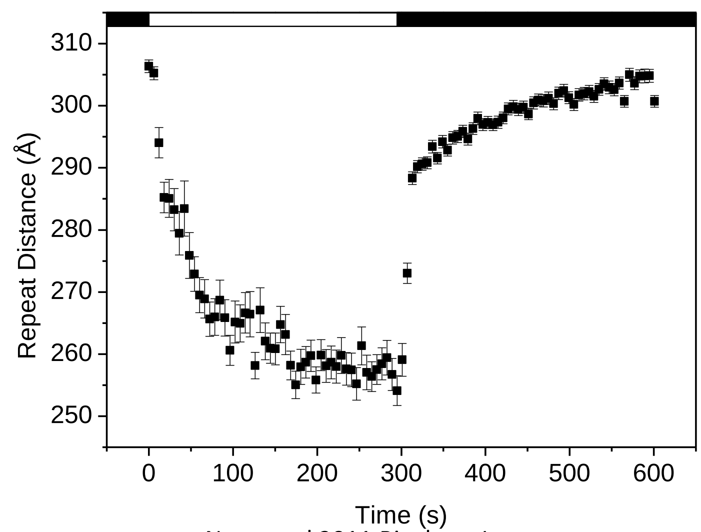
Nagy 2011 PhD Thesis

Illumination



dark **5 min light** **5 min dark**

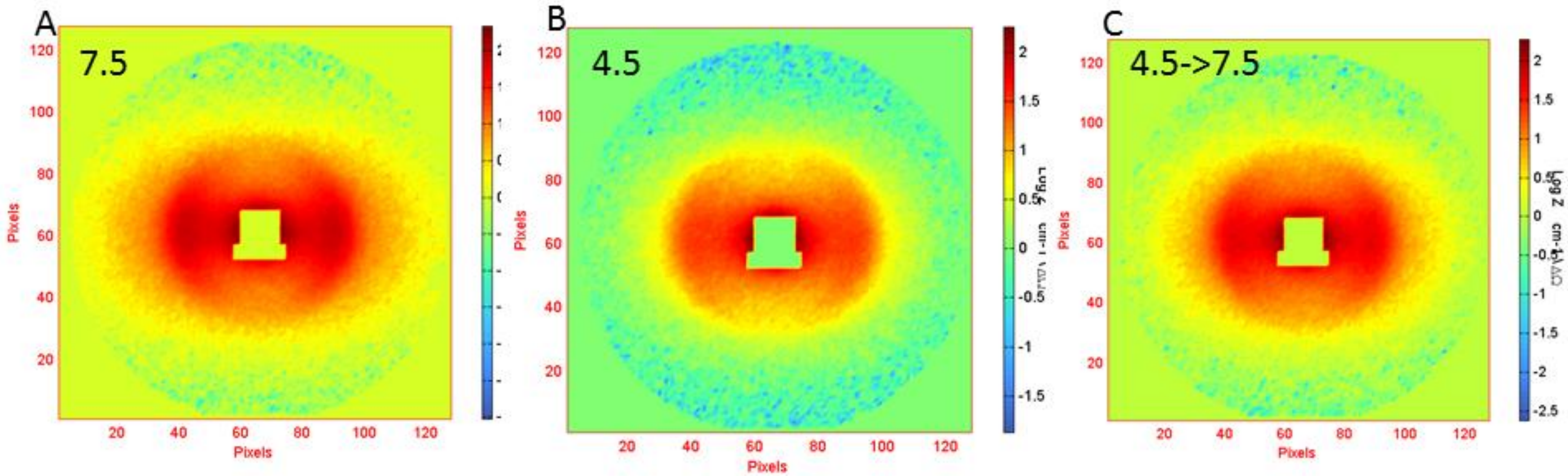




Nagy et al 2011 Biochem J

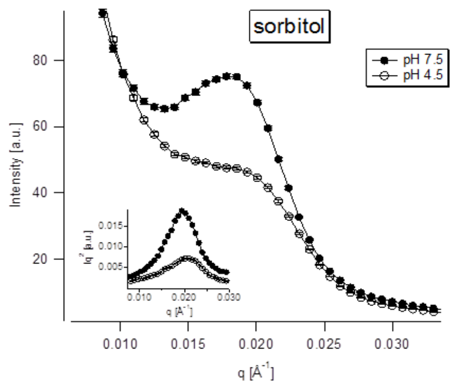
Oak Ridge National Laboratory

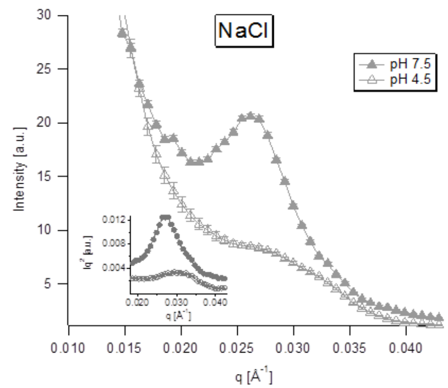
pH variation

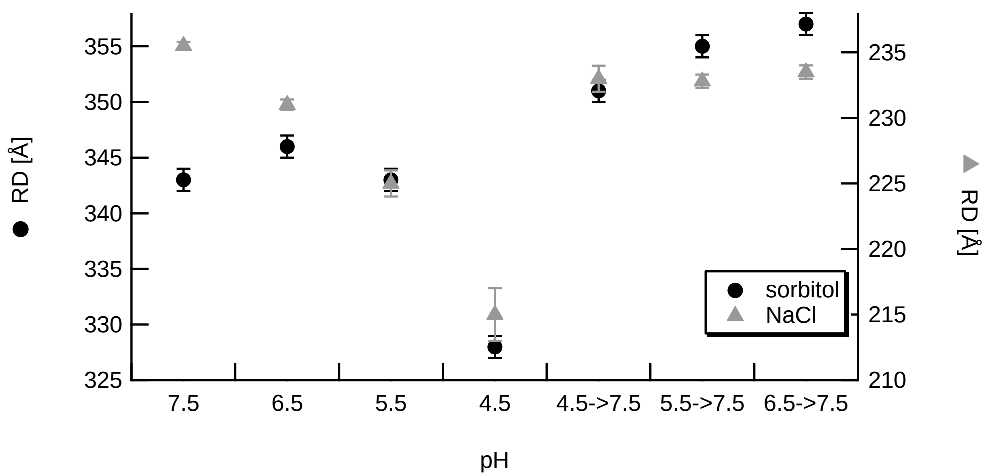


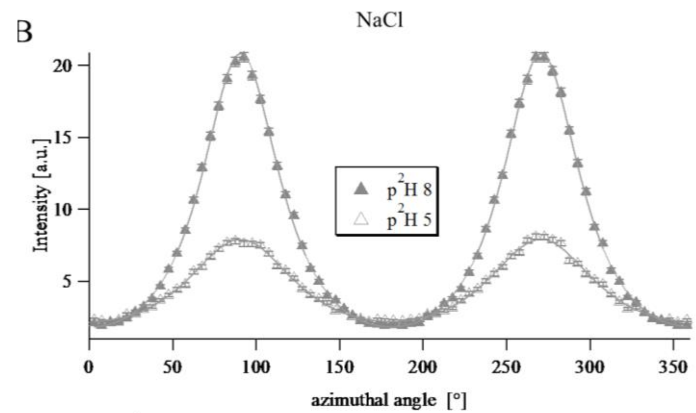
Unnep et al. 2017 BBA

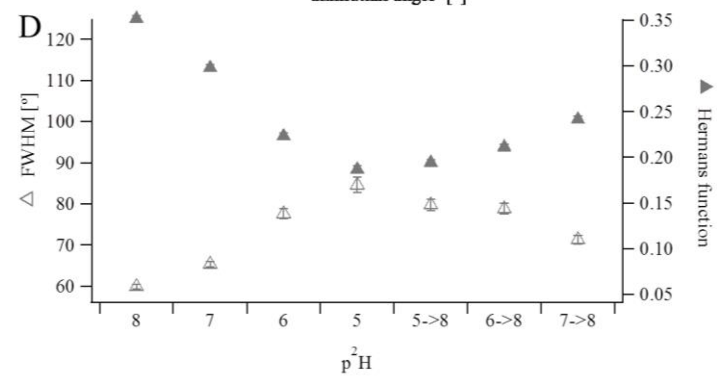
pH variation











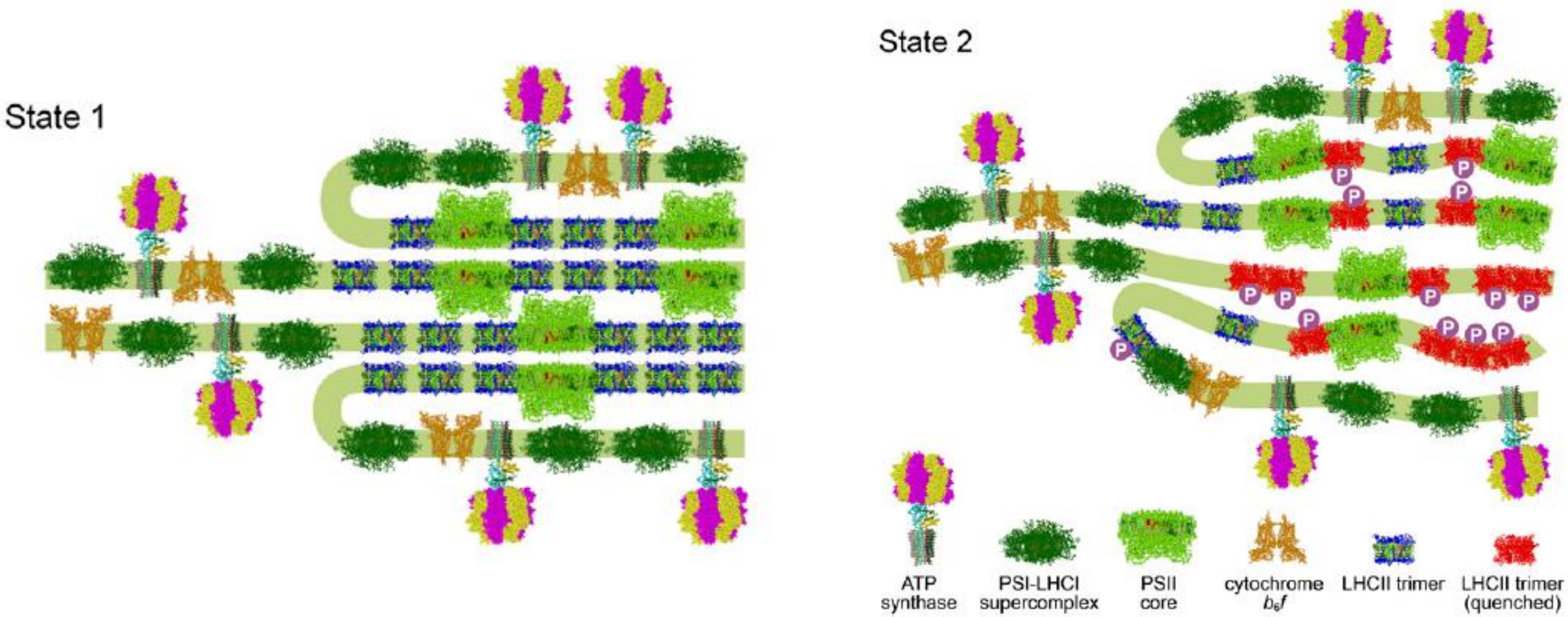
Unnep et al. 2017 BBA

Study of intact leaves

- Monstera deliciosa – climbing rainforest vine

Unnep et al. 2020 Open Biology

State transition in intact algal cells



Nagy et al. 2014 PNAS

Acknowledgements

- Hugh O'Neill, Laura Stingaciu
- Katalin Solymosi, Richard Hembrom
- Győző Garab, Ottó Zsíros, Milán Szabó, Sai Divya Kanna, Bettina Ughy
- Renáta Ünnep, Márton Markó
- Bence Fehér
- Giovanni Finazzi
- Jun Minagawa
- Dorthe Posselt
- Lionel Porcar

Acknowledgements

- A portion of this research used resources at the Spallation Neutron Source, a DOE Office of Science User Facility operated by the Oak Ridge National Laboratory.

Thank you for your attention!

Questions?