

Small Angle Neutron Scattering

Studies in Life Science & Biomaterials

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Outline

- What is a SANS experiment and when to use it
- The SANS scattering process:
 - Different contributions to scattering
 - Scattering length densities and Contrast
 - Structure factors
 - Form Factors
- SANS data analysis
 - Model dependent
 - Model independent
- SANS Data quality and recommendations when planning a SANS experiment

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Neutrons in Life Sciences and Biomaterials

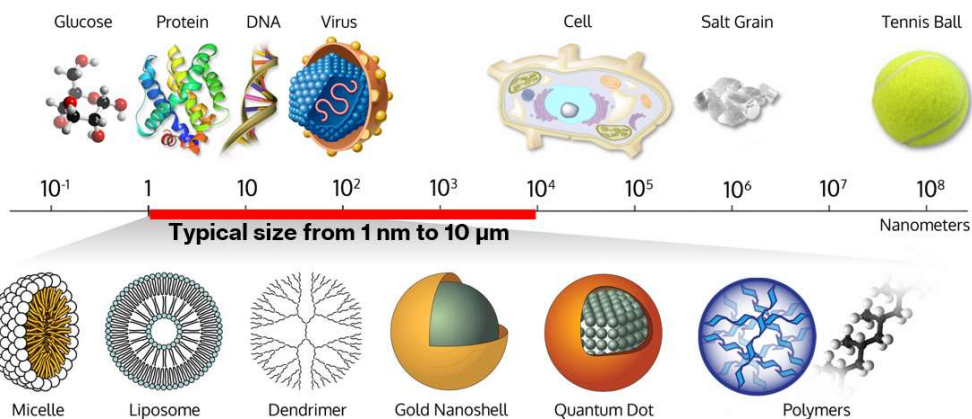


Image source: www.wichlab.com/research



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Small Angle Scattering (SAS):

Complementary probes

| | | |
|------------------------|---|--------------------|
| X-rays (SAXS) | $0.6 \text{ \AA} < \lambda < 2 \text{ \AA}$ | (Electrons) |
| Neutrons (SANS) | $1 \text{ \AA} < \lambda < 20 \text{ \AA}$ | (Nuclei) |
| Light (LS) | $4000 \text{ \AA} < \lambda < 7000 \text{ \AA}$ | (Refractive index) |

The nature of the radiation determines:

- Sample environment that can be applied
- Length scales that can be probed
- Nature of the information that can be obtained

Information from SAS:

- Size
- Shape
- Molecular weight
- Particle/Pore size distribution
- Interaction
- Fractal Dimension

Phenomena:

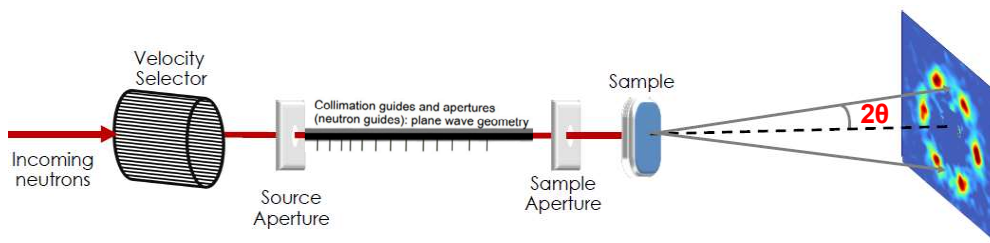
- Self Assembly
- Phase transition
- Conformational change
- Thermodynamics/kinetics



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Small Angle Neutron Scattering (SANS) Experiments

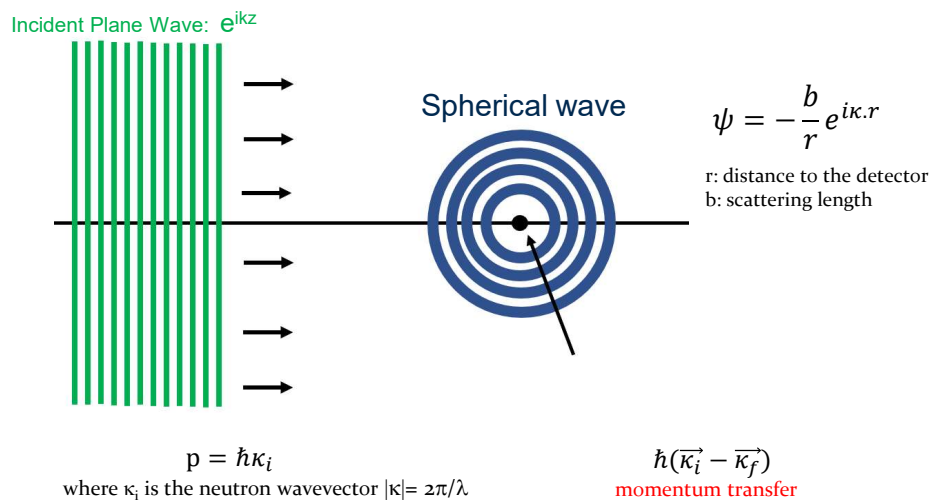


SANS probes structure on a scale d , by measuring the static scattering function $I(q)$.

$$2\theta \approx \frac{\lambda}{d} \approx \frac{6 \text{ \AA}}{60 \text{ to } 1000 \text{ \AA}} \quad 2\theta \approx 0.3^\circ \text{ to } 5^\circ$$

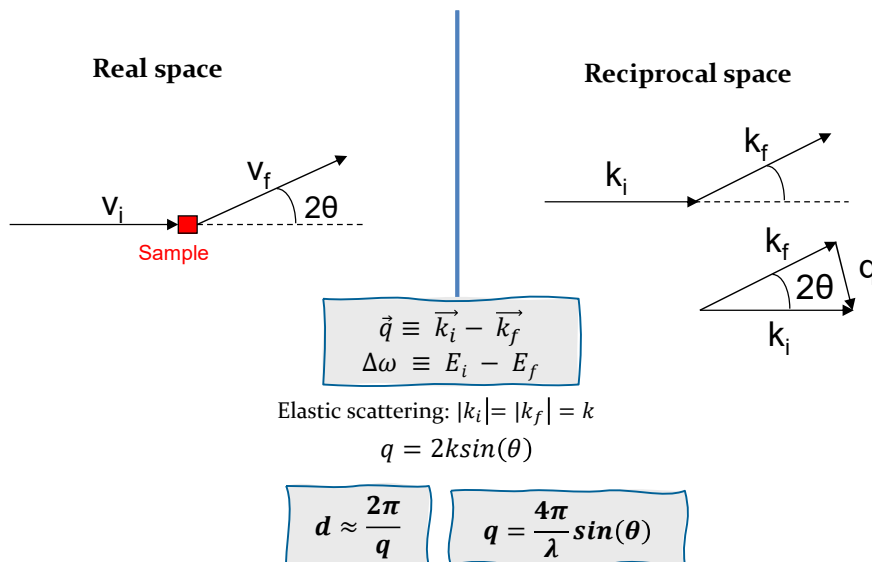
Small Angle Neutron Scattering (SANS)

The scattering process: there is a momentum transfer from the neutron to the scatterer

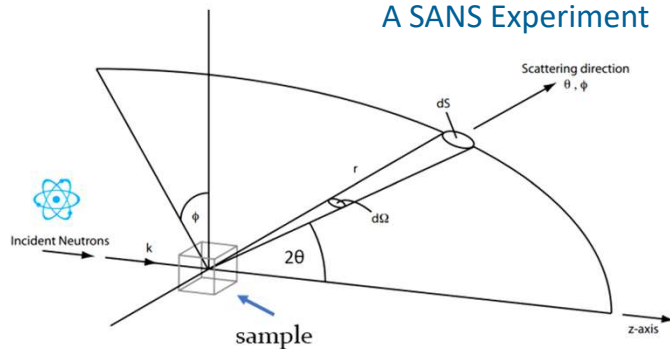


Small Angle Neutron Scattering (SANS)

The scattering process: there is a momentum transfer $\hbar q$ from the neutron to the scatterer



A SANS Experiment



Beam flux
 $= nr$ incident neutron/(s cm²)
 $= v |\psi_i|^2 = v$

Solid angle $= dS/r^2$

Differential scattering cross section

$$\frac{d\sigma}{d\Omega} = \frac{\text{neutrons scattered into } d\Omega/s}{\text{Flux } d\Omega} = \frac{v dS |\psi_i|^2}{v d\Omega} = b^2$$

Total scattering cross section σ (microscopic cross section; what we measure)

$$\sigma = \int \frac{d\sigma}{d\Omega} d\Omega = 4\pi |b|^2 \quad \text{in units of area (cm}^2\text{); 1 barn} = 10^{-24} \text{ cm}^2$$

Small Angle Neutron Scattering (SANS)

Reduced data

Macroscopic cross section Σ (total scattering normalized by sample volume)

$$\Sigma = \frac{\sigma}{V} \quad \text{in absolute units of cm}^{-1} \quad \text{relating what we measure to information about the sample}$$

$$\frac{d\Sigma}{d\Omega}(q) = \frac{N}{V} \frac{d\sigma}{d\Omega}(q) = \frac{1}{V} \left| \int \overbrace{\rho(\mathbf{r}) e^{i\mathbf{q}\cdot\mathbf{r}} d\mathbf{r}}^{\text{Fourier transform of the SLD distribution}} \right|^2 \quad \text{Rayleigh-Gans Eq.}$$

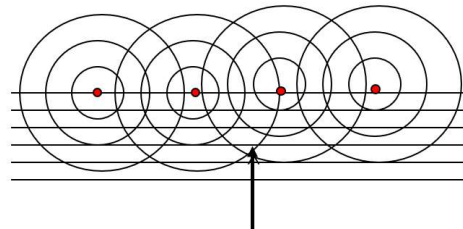
$$\rho = \frac{\sum_i^N b_i}{V} = \bar{\rho} + \Delta\rho(r) \quad \text{N atoms occupying a volume V}$$

- Small angle scattering is the result of inhomogenities in **scattering length density (SLD)**
- Differential cross-section proportional to Amplitude² (phase information lost)

Small Angle Neutron Scattering (SANS)

The scattering process & the orders of magnitude

| | |
|---|---|
| Wavelength | 10^{-10} m |
| Inter-atomic distances: | 10^{-10} m |
| Atomic nuclei: | 10^{-14} m |
| (Scattering cross section) ^{1/2} : | 10^{-14} m |
| Velocity of the neutrons: | 10^{-10} m/ps |
| Neutron flux at the sample position: | 10^7 n.cm ⁻² s ⁻¹ |



Assumptions:

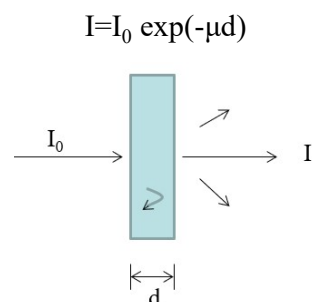
- some neutrons pass through the sample undeviated
- neutrons undergo single scattering events (negligible multiple scattering) – kinematic app.
- incident beam is not distorted significantly by the medium.
- nuclei are considered fixed at their position in the solution: the cross-sections are listed for different nuclei as *bound cross sections*

Total Scattering in SANS

$$\sigma = \sigma_{coh}(q) + \sigma_{inc} + \sigma_{abs}$$

| | | |
|---|--|---|
| coherent: depends on structure q-dependent (S) | incoherent: 'noise' or 'background' T-dependent (N) | absorption: Reduces S/N Wavelength dependent |
|---|--|---|

| | b_{coh} (fm) | σ_{coh} (barns) | σ_{inc} (barns) | σ_{abs} (barns) |
|----------------|-------------------|---------------------------|---------------------------|---------------------------|
| ¹ H | -3.741 | 1.8 | 80.3 | 0.3 |
| ² D | 6.671 | 5.6 | 2.1 | 0.0 |
| B | 5.304 | 3.5 | 1.7 | 767.0 |
| C | 6.646 | 5.6 | 0.0 | 0.0 |
| Ti | -3.438 | 1.5 | 2.9 | 6.1 |
| V | -0.382 | 0.0 | 5.1 | 5.1 |
| Cd | 5.130 | 3.3 | 2.5 | 2520.5 |



<https://www.ncnr.nist.gov/resources/n-lengths/>

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Total Scattering in SANS

Neutrons are penetrating radiation

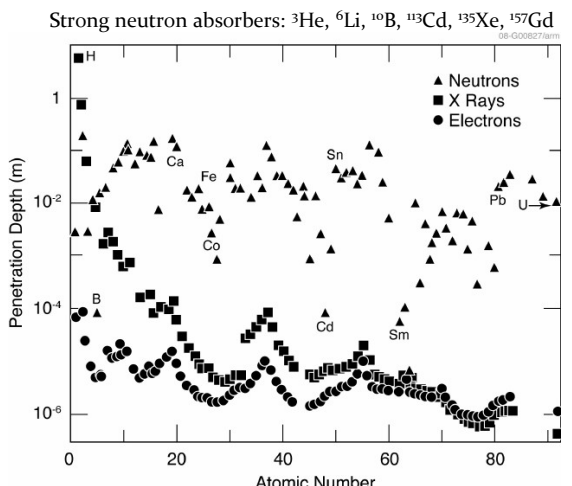


Image from: Pynn R. (2009) Neutron Scattering—A Non-destructive Microscope for Seeing Inside Matter. In: Liang L., Rinaldi R., Schober H. (eds) Neutron Applications in Earth, Energy and Environmental Sciences. Neutron Scattering Applications and Techniques. Springer, Boston, MA.



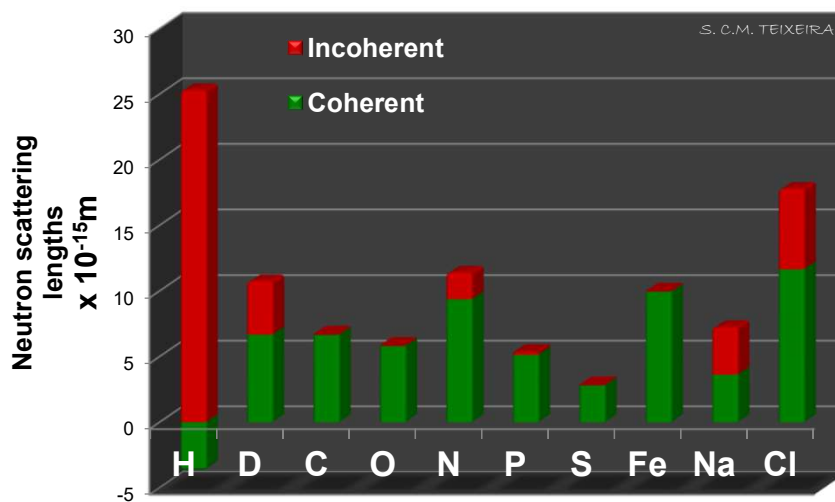
Neutron image: Daniel Hussey/NIST

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Small Angle Neutron Scattering (SANS)

Typical components of biological solutions

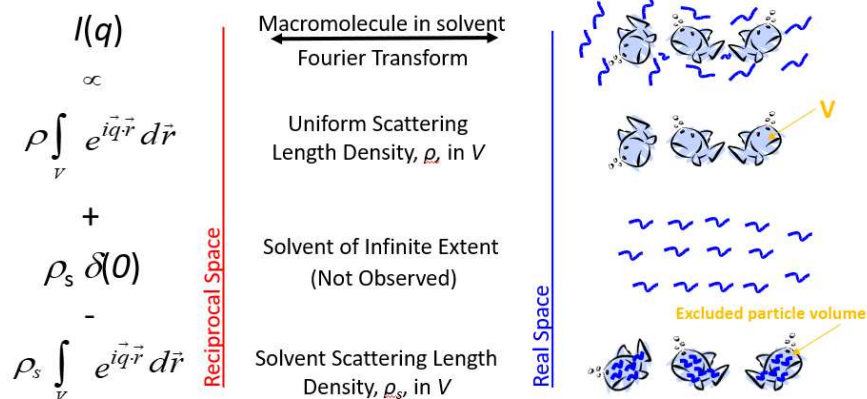


Data from the Special Feature section of neutron scattering lengths and cross sections of the elements and their isotopes in *Neutron News*, Vol. 3, No. 3, 1992, pp. 29-37.

Small Angle Neutron Scattering (SANS)

Since SANS does not provide information on the length scale of atomic bonds, the scattering interaction is described as a function of a uniform SLD for the whole molecule within the molecular volume V :

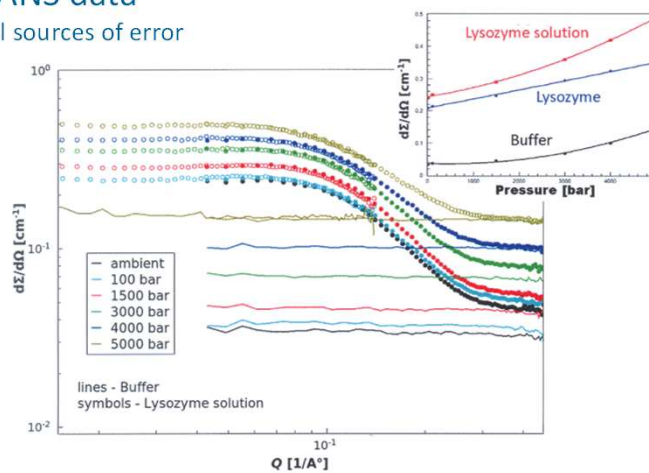
$$I(\vec{q}) = \rho^2 V \left| \frac{1}{V} \int_V e^{i\vec{q}\cdot\vec{r}} d\vec{r} \right|^2 = \rho^2 V |\mathbf{F}(\vec{q})|^2$$



SANS data

Typical sources of error

For a thorough discussion on background subtraction: Rubinson et al. (2008); J. Appl. Cryst. 41, 456-465



- Buffer mismatch
- Air bubbles
- Incoh. & multiple scattering
- Incorrect data scaling

Image from: Radulescu et al. (2016). J. Vis. Exp. (118), e54639, doi:10.3791/54639



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Contrast in SANS

In a solvent: $I(\vec{q}) = (\rho - \rho_{solv})^2 V |F(\vec{q})|^2$ $\Delta\rho^2 = \text{SLD contrast}$

For N molecules with a density n (N/V), averaged over random orientations:

$$I(q) = n \Delta\rho^2 V^2 \langle |F(q)|^2 \rangle$$

scattered
intensity

particle
volume

form
factor

structure
factor

$$I(q) = \phi V \Delta\rho^2 P(q) S(q)$$

particle volume
fraction

scattering length
density contrast

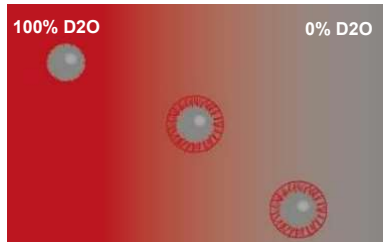


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Contrast in SANS

$$\rho = \frac{\sum b_i}{V}$$

b_i = scattering length
 V = molecular volume

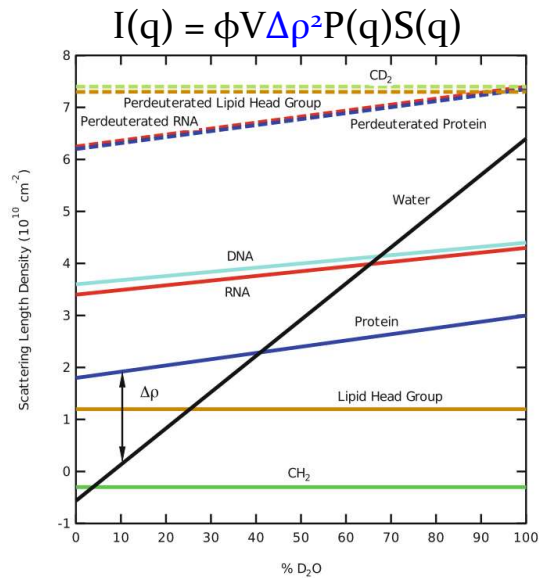


Scattering lengths (cm^2) of elements:

<https://www.ncnr.nist.gov/resources/n-lengths/>

Scattering length density (cm^{-2}) calculator:

<https://www.ncnr.nist.gov/resources/activation/>



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SANS for concentrated solutions

Structure factors

$$\frac{d\Sigma}{d\Omega}(q) = n (\rho_{\text{Protein}} - \rho_{\text{Solvent}})^2 V_{\text{protein}}^2 P(q) S(q) + B$$

For an **isotropic system**, where interactions are **uniform** across the sample solution and **do not depend** on the orientation of the molecule, the structure factor can be written as:

$$S(q) = 1 + 4\pi n \int [g(r) - 1] \frac{\sin qr}{qr} r^2 dr$$

Radial distribution function: governed by the interaction potential.

$$\frac{d\Sigma}{d\Omega}(q) = n \Delta \rho^2 V_{\text{protein}}^2 P(q) S'(q) + B$$
 (decoupling approximation)

$$S'(q) = 1 + \beta(q) [S(q) - 1]$$

$$\beta(q) = \frac{|\langle F(q) \rangle|^2}{\langle |F(q)|^2 \rangle}$$

Monodisperse systems: $\beta = 1$

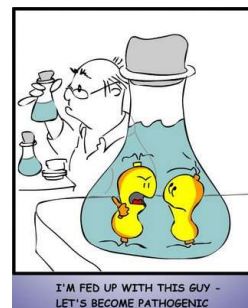


Photo by Unknown Author, licensed under [CC BY-NC-ND](https://creativecommons.org/licenses/by-nc-nd/4.0/)

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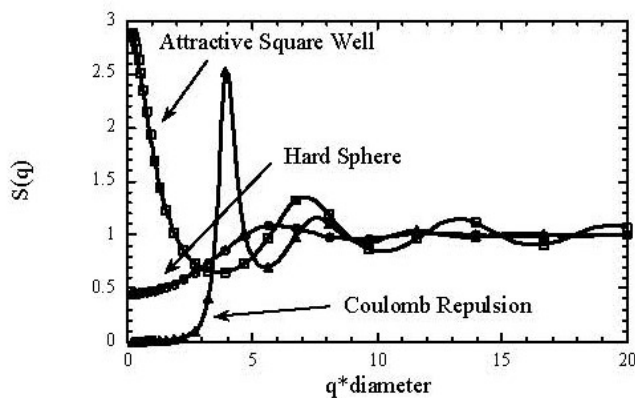
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SANS for concentrated solutions

Structure factors and statistical thermodynamics

$$S(q=0) = k_B T \left(\frac{\partial C_m}{\partial \pi} \right)_T \approx \frac{1}{(1 + 2A_2 M_w C + \dots)}$$

Osmotic Compressibility
 C_m = monomer density distribution
 A_2 = second virial Coefficient
 C = concentration



Attractive \Rightarrow More compressible
 Repulsive \Rightarrow Less compressible

Teixeira (2019). High pressure SANS for food studies. *Cur. Op. Coll. Inter.Science* 42, 99-109.

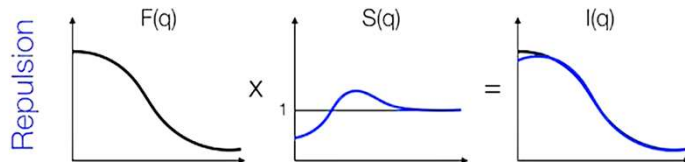
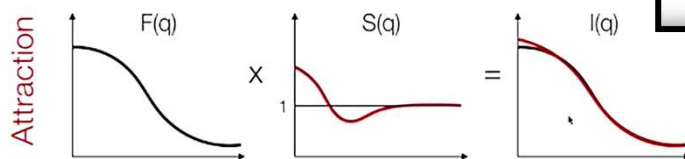
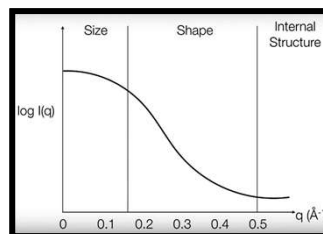


SANS for concentrated solutions

Structure factors

$$I(q) = \phi V \Delta \rho^2 P(q) S'(q)$$

In the dilute regime, $S(q)$ can usually be considered ~ 1 (but we don't assume, we verify).



SANS data: Form Factors

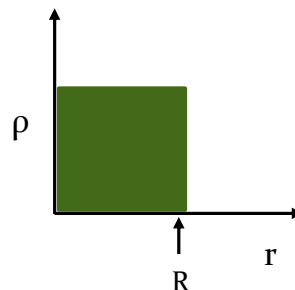
P(q) related to the Fourier transform of the real space density distribution

$$I(q) = \phi V \Delta \rho^2 P(q) S'(q)$$

Real space

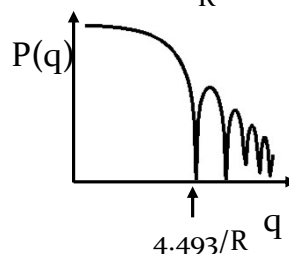


$$\rho(r) = \begin{cases} \rho_o & r < R \\ 0 & r > R \end{cases}$$



Fourier space: normalized form factor

$$P(Q) = \frac{|\int \rho(r) e^{-iQr} dr|^2}{|\int \rho(r) dr|^2} \rightarrow P(Q) = \left[\frac{3(\sin QR - QR \cos QR)}{(QR)^3} \right]^2$$



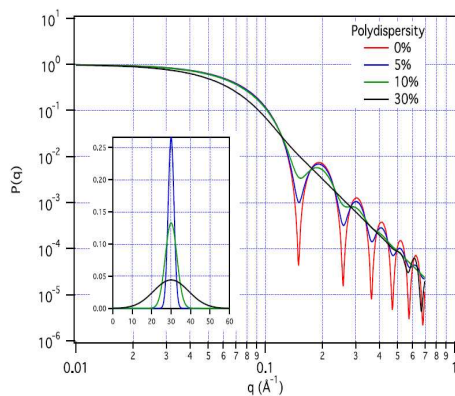
adapted from R-J. Roe 'Methods of X-ray and Neutron Scattering in Polymer Science'



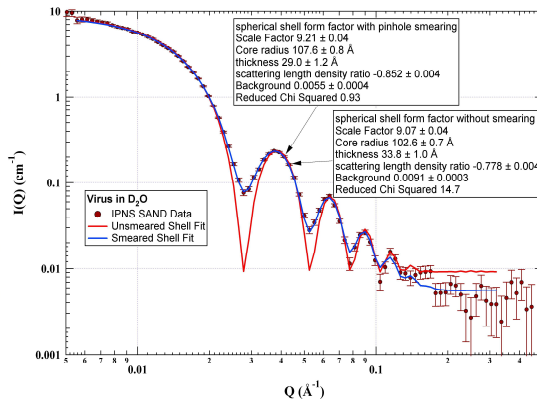
SANS data: Form Factors

Models & Polydispersity

Spherical shell



Sample polydispersity



Instrumental smearing



(Model independent) Analysis of SANS data

Dilute, low q regime: Guinier

$$\frac{d\Sigma}{d\Omega}(q) = n \Delta\rho^2 V_{protein}^2 P(q) S'(q) + B$$

$$S'(q) = 1 + \beta(q)[S(q) - 1]$$



As $q \rightarrow 0$: $I(q) = I(0) \exp\left(-q^2 \frac{R_g^2}{3}\right)$

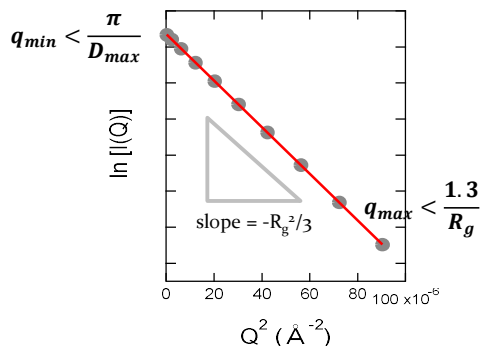
Valid for: $q R_g \lesssim 1.3$ (globular proteins)

$$\ln I(q) = \ln I(0) - q^2 \frac{R_g^2}{3}$$

For dilute solutions, $\beta = 0$ and $S'(q) = S(q) \approx 1$.

$$I(0) = n \Delta\rho^2 V^2 = \frac{CN_A}{M_w} \Delta\rho^2 \left(\frac{\bar{v}M_w}{N_A}\right)^2$$

For dilute solutions of N identical particles, where \bar{v} is the specific vol. (cm^3/g). For low q, where uniform density approx. is valid. N: both R_g and $I(0)$ depend strongly on the contrast.



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Model Independent Analysis of SANS data

Dilute, intermediate-high q regime: Porod

In the Porod q range we are probing sizes smaller than the scattering objects. Even if SANS is a low resolution technique, it can probe local structure



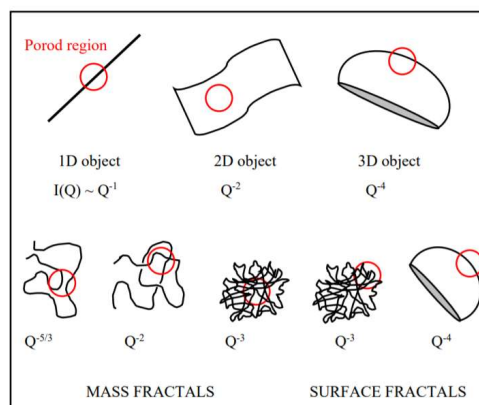
$$I(q) = \frac{A}{q^n} + B$$

OR

$$\log[I(q) - B] = \log A - n \log q$$

n tells us something about the shape of our sample >

local structure \rightarrow 'fractal dimension'



From the SANS toolbox by Boualem Hammouda

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Model Independent Analysis of SANS data

Dilute, intermediate-high q regime: Porod

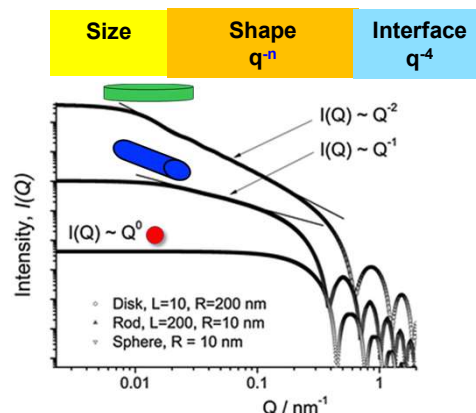
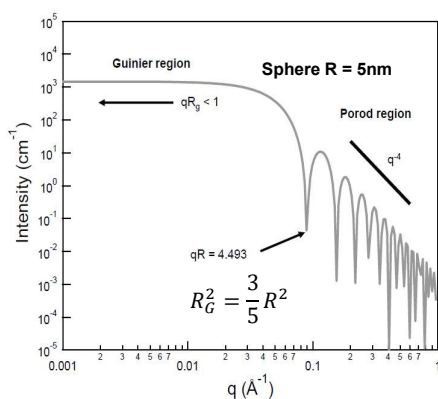
For $q \gg 1/D$, $I(q) \propto q^{-4}$



Porod's Law (form factor for a sphere)

$$F(q) = \frac{3}{2R^3} \left(\frac{S_P}{V_P} \right) \frac{1}{q^4}$$

surface to volume ratio



Adapted from Hammouda (2010). J. Appl. Cryst. 43, 716–719

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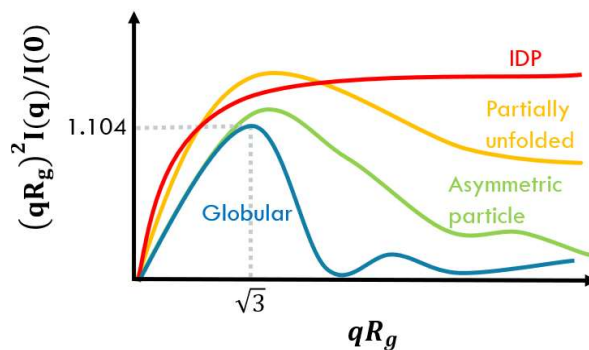
Model Independent Analysis of SANS data

Kratky plot: $(q^2 I)$ as a function of q

$$I(q) = I(0) \exp\left(-q^2 \frac{R_g^2}{3}\right)$$

multiplying both sides by $(qR_g)^2$ and dividing by $I(0)$ to normalize by mass and concentration

$$\frac{I(q)}{I(0)} (qR_g)^2 = (qR_g)^2 \exp\left(-q^2 \frac{R_g^2}{3}\right)$$



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SANS for different shapes of molecules

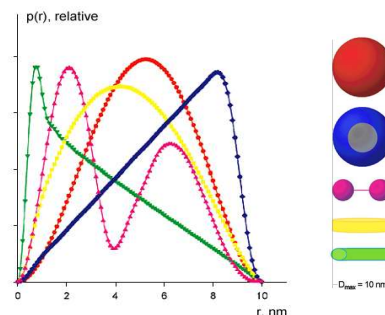
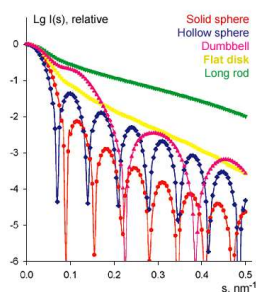
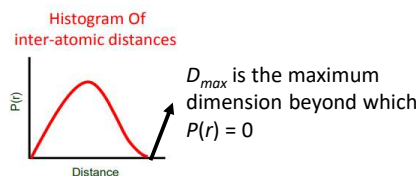
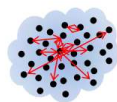
Distance distribution function $P(r)$

$P(r)$ is a radial Patterson function that describes the probable frequency of interatomic vector lengths r within a molecule, where the R_G is the second moment of the $P(r)$ function, and $I(0)$ is the zeroth moment (area under $P(r)$)

$$I(q) = 4\pi V \int_0^{D_{max}} P(r) \frac{\sin(qr)}{qr} dr$$

↓ Indirect Fourier Transf.

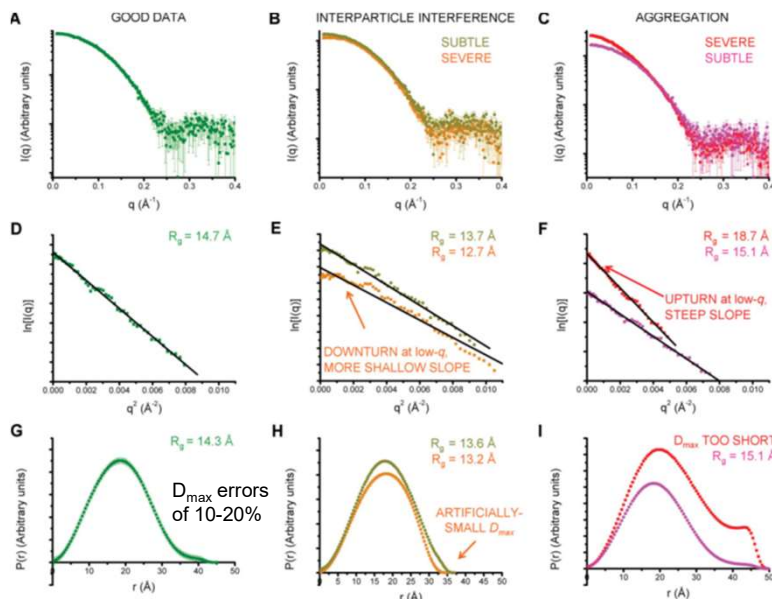
$$P(r) = \frac{r}{2\pi^2} \int_0^\infty I(q) q \sin(qr) dq$$



Images adapted from from Svergun & Koch; Rep. Prog. Phys. 66 (2003) 1735-1782, Fig. 5 (c)IOP Publishing Ltd.

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SANS data quality



Garbage in...

SANS is a volume weighted technique. Larger aggregates have larger volumes, even a small amount contributes to the whole q range (even if more apparent at low q).



Adapted from Jacques and Trehwella, 2010 Protein Science Review.

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SANS Experiments

Ask the right questions

Sample concentration

Verify!(A_{280} and A_{260}). Common sources of error:

- Low % of chromophores leads to errors >10%
- Solvent blanks (DTT oxidation different between sample and solvent)

High concentrations for better S/N... but measure a concentration screening to detect interparticle interference and type.

Typical amounts of sample required: 0.5-1 mL at 1-10 mg/ml → at low contrast conditions higher concentrations may be needed

Polydispersity

Use complementary techniques to ensure minimal aggregation or polydispersity (MALS, SEC, DLS).

Check sample purity (SDS-PAGE, MS,...)

pH/pD effects

Solubility of most biological molecules is reduced in D_2O

Sample environment

What temperature, pressure, pH and precision will you need?

How long is the sample stable for?

If using pressure cells, larger volumes may be needed.



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Planning an experiment

Software suggestions

The contrast of each component in a sample can be calculated from the chemical composition and the stoichiometry of the complex, as a function of the solvent composition.

- **MULCh**: Two-component complexes: contrast variation
[Whitten et al. (2008), *J Appl Crystallogr* **41**, 222-226]
<http://smb-research.smb.usyd.edu.au/NCVWeb/>
- **Contrast Calculator module of SASSIE**: Unlimited # of components & estimate of $I(0)$ as a function of % D_2O
[Curtis et al. (2012), *Comp. Phys. Comm.* **183**, 382-389]
<http://sassie-web.chem.utk.edu/sassiez>
- **SasCalc module of SASSIE** and **Cryson**: Calculate the theoretical SANS curve if a starting structure is available (PDB file)
[Svergun et al. (1998), *Proc. Natl. Acad. Sci. USA* **95**, 2267-2272]
<https://www.embl-hamburg.de/biosaxs/cryson.html>



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OUTLINE

- **SANS experiment and when to use it**

In situ measurements & Contrast

- **SANS scattering process**

$$I(q) = \phi V \Delta \rho^2 P(q) S'(q) + B$$

$$S'(q) = 1 + \beta(q)[S(q) - 1]$$

- **SANS data analyses**

Guinier, P(r), Kratky

$$S(q) = 1 + 4\pi n \int [g(r) - 1] \frac{\sin qr}{qr} r^2 dr$$

- **SANS Data quality**

- Plan your experiment
- Labelling
- Polydispersity

SANS, VSANS, USANS instruments at NIST

✉ susanat@udel.edu



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