Small Angle Scattering (SAS)

- what is SAS & what can it measure?
- how is it measured?
- sample considerations
- data analysis

Reference Texts

- The SANS Toolbox, B. Hammouda, NIST (available as pdf: http://www.ncnr.nist.gov/staff/hammouda/the_SANS_toolbox.pdf)

- Structure Analysis by SAXS & SANS, L.A. Fegin & D.I. Svergun (1987) (available as pdf: http://www.embl-hamburg.de/biosaxs/reprints/feigin_svergun_1987.pdf)

- Small Angle X-ray Scattering, eds O. Glatter & O. Kratky (1982) (available as pdf from web archives)

What is SAS?

- Coherent, elastic scattering of radiation at small angles – close to the straight-through beam
- Incoherent scattering = background

sample

2θ
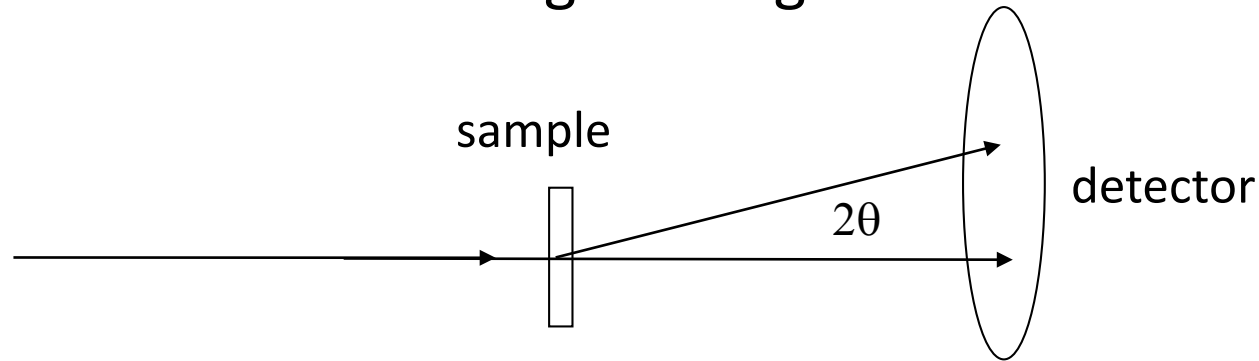
detector
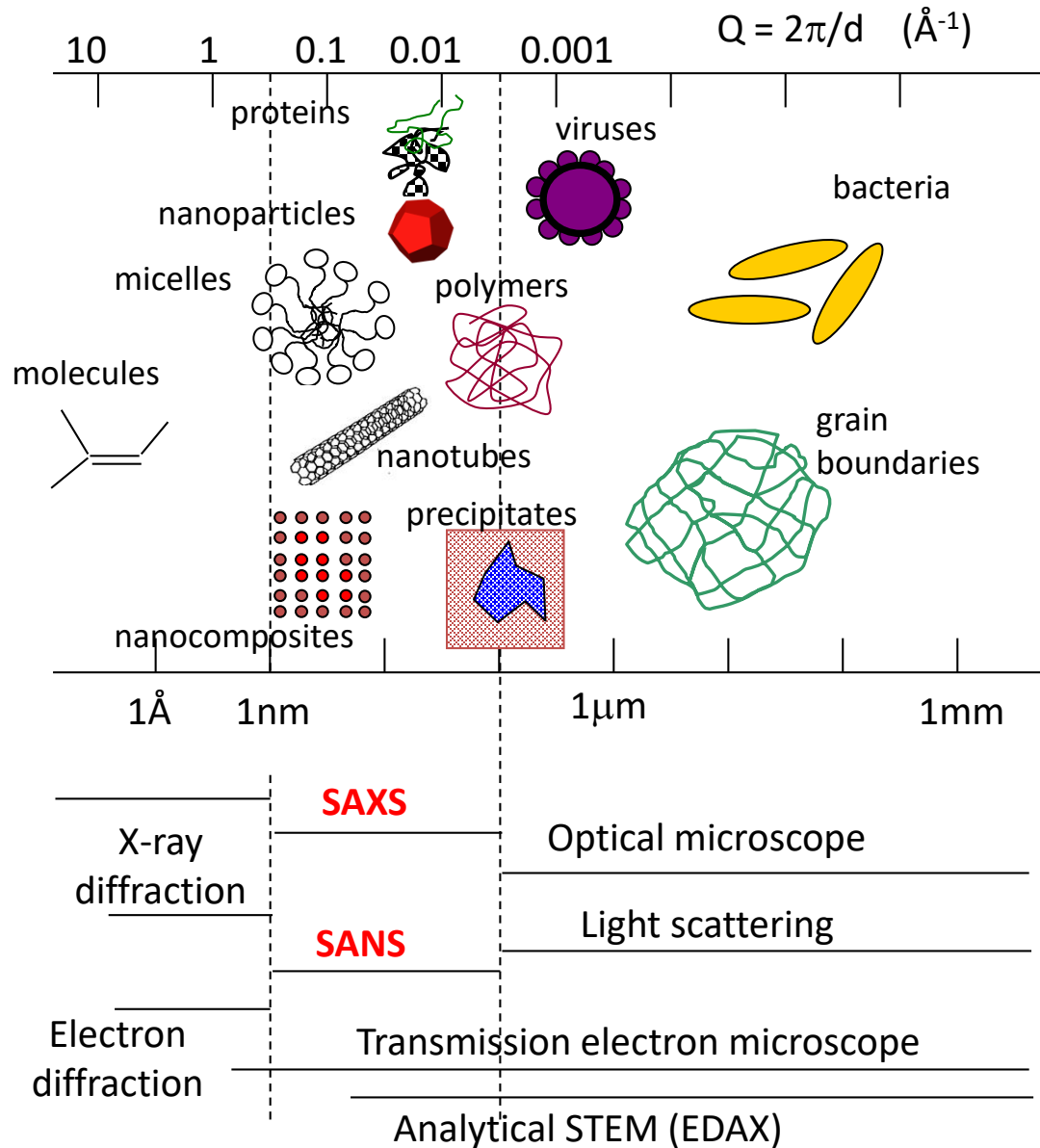
- Typically $0.3 < 2\theta < 5°$ or $Q < 0.5$ Å^{-1}

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

units: Å^{-1}

$$Q = \frac{2\pi}{d}$$

$\Rightarrow$ bigger distance, smaller Q

Size Range Comparisons

$Q = 2\pi/d$ (Å^{-1})

10	1	0.1	0.01	0.001

proteins

viruses

bacteria

nanoparticles

micelles

polymers

molecules

nanotubes

grain boundaries

precipitates

nanocomposites

1Å   1nm   1μm   1mm

X-ray diffraction

SAXS

Optical microscope

Light scattering

SANS

Electron diffraction

Transmission electron microscope

Analytical STEM (EDAX)

SAS Instruments
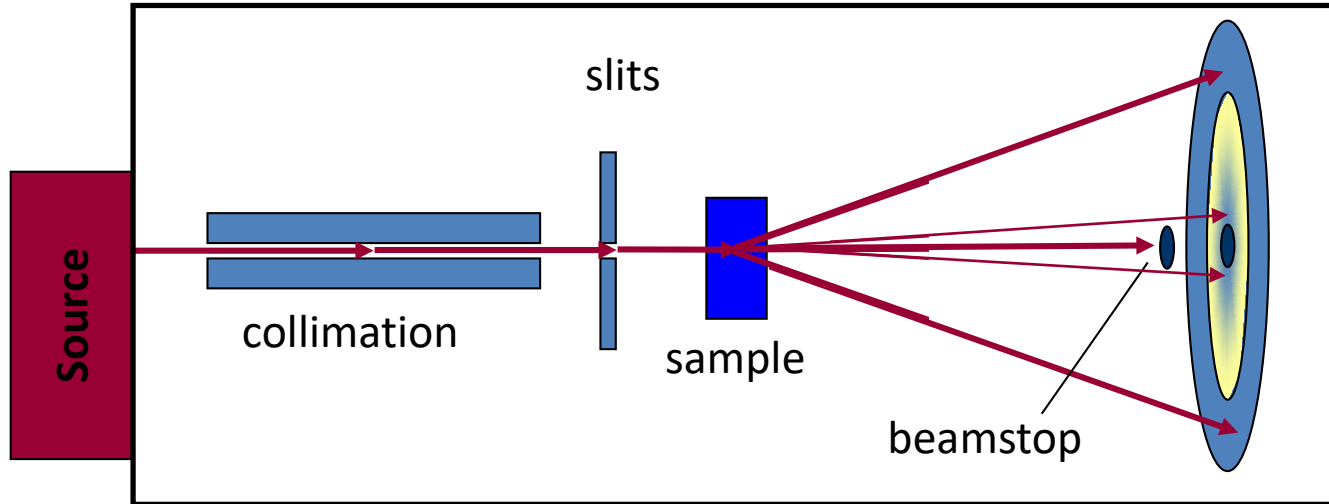
- Neutrons/X-rays must be parallel to each other; "collimated"
- Slit defines shape of beam (circle, square, slit)
- Distance from sample to detector & wavelength determines size range measured
  - Tof – wide simultaneous Q range, lower flux
  - Reactor – smaller Q range, higher flux at short sample-detector distances
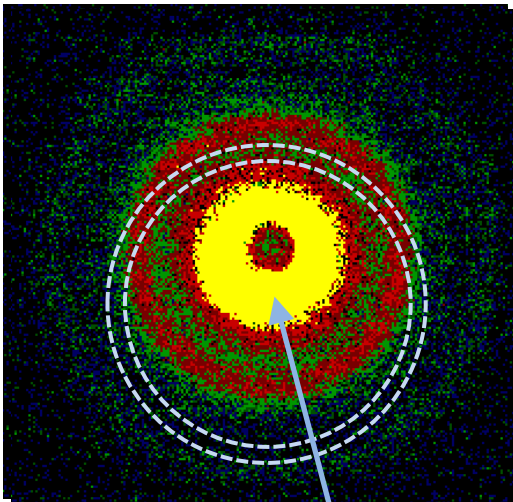
Scattering Patterns: From detector to 1D

- eg diblock copolymers
- Circular 1D average
  - take average over ring
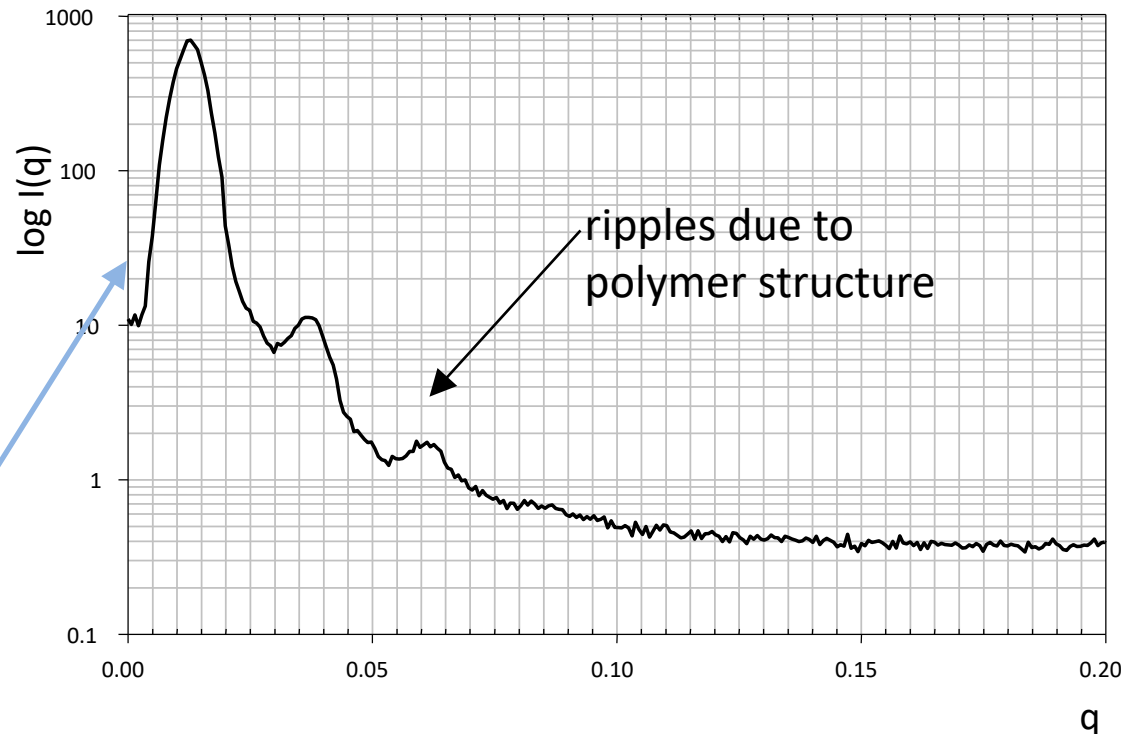 - each ring corresponds to one data point in reduced 1D SAXS data

Real space
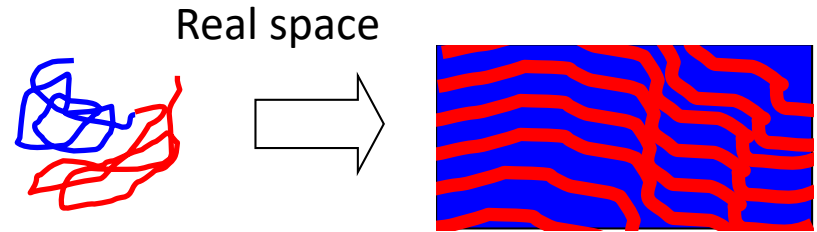
detector

shadow of beamstop

radial average:

ripples due to polymer structure

q

SAS Measurements

- Observed intensity:

$$J(\lambda,\theta) = J_0(\lambda)\Delta\Omega\eta(\lambda)TVI(Q)$$

where:

$J0(\lambda)$ = flux incident on sample

$\Delta\Omega$ = angle covered by detector

η = detector efficiency

T = sample transmission

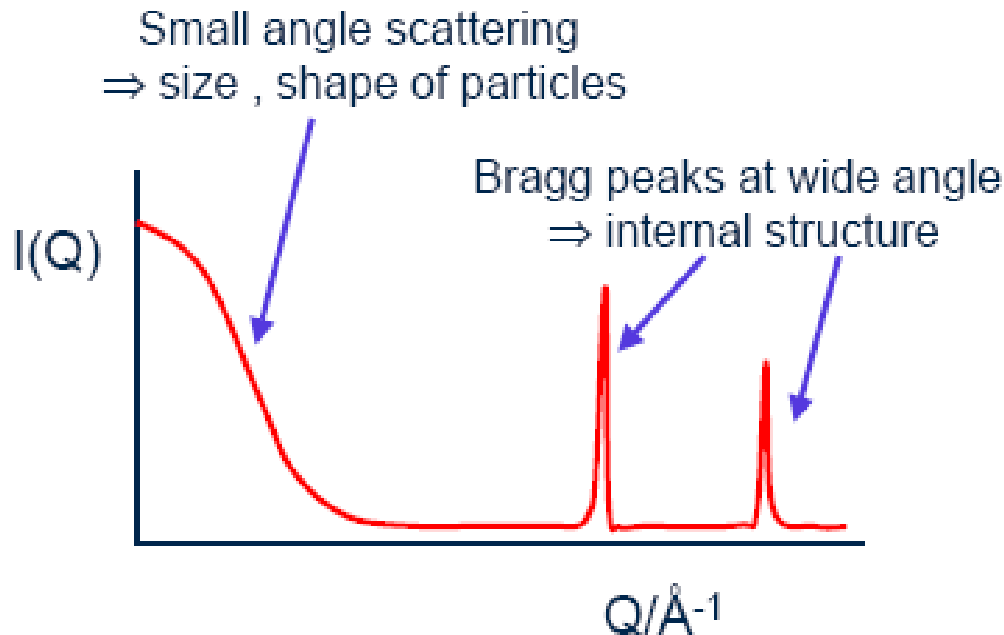V = volume of sample in beam

Can measure all of these
$\Rightarrow$ Used to correct data during data reduction

I(Q) = differential cross section

$\Rightarrow$ contains information about sample

Scattering from Large Structures

- Neutron/X-ray wavelength $\lambda \approx$ space between atoms in crystal
  - $\Rightarrow$ bounce off layers of atoms like light off a mirror
  - $\Rightarrow$ see diffraction peaks at high angles (correspond to atomic positions)
- BUT for larger objects, sees <u>average</u> structure

Small angle scattering
$\Rightarrow$ size , shape of particles

Bragg peaks at wide angle
$\Rightarrow$ internal structure

I(Q)

Q/Å^{-1}

- large structures scatter at small angles

$\Rightarrow$ for techniques using small angles use material properties rather than atomic properties

How Big is "Large"?

- Consider H_2O: volume of one molecule = 30Å^2

  radius of one molecule = 2 Å

density

ρ_{av}

R

R=10 Å
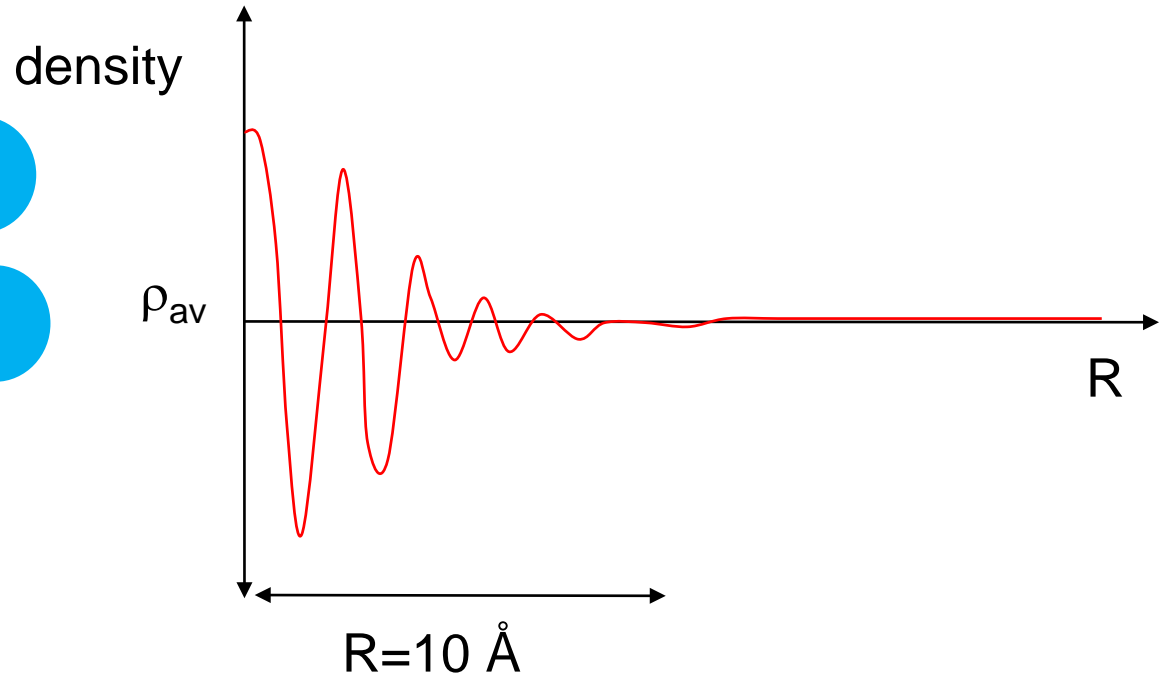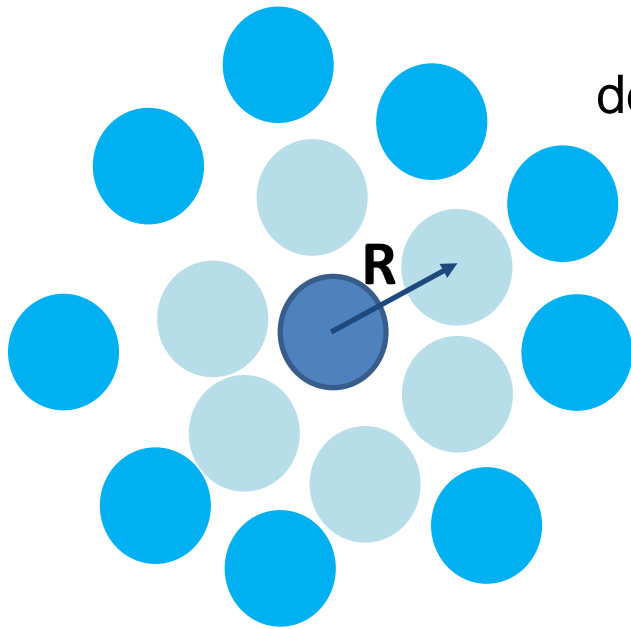
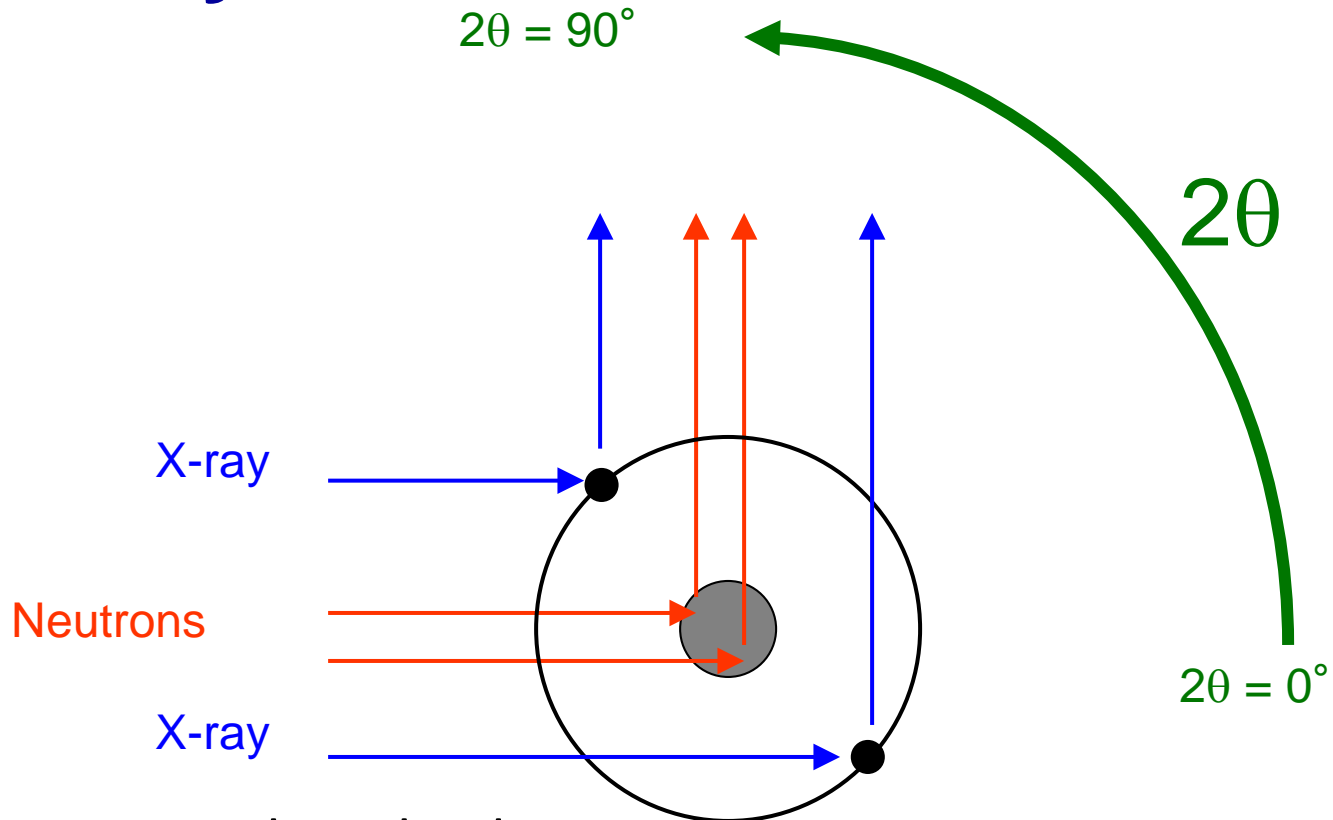$\Rightarrow$ for distances > ~5 molecules, see only average density

$$Q = 2\pi/d$$

- so can use material properties for $Q < {\sim}0.6 \text{ Å}^{-1}$

How X-rays & neutrons are scattered

- atomic scattering length = b

- b = measure of how much a neutron/X-ray interacts with a nucleus/atom (units: 10^{-15} m or 10^{-13} cm)

- b is different for coherent and incoherent scattering
 - *different nuclei have different b*

Neutron vs X-ray Scattering

- Neutrons more penetrating than X-rays (interact less with matter)
- Interaction of neutrons with nuclei depends on isotope
- Interaction of X-rays just depends on number of electrons
- b = scattering length (units Å or cm, normally)
- Scattered intensity measured depends on which isotopes are in sample for neutrons, only on elements for X-rays

X-rays
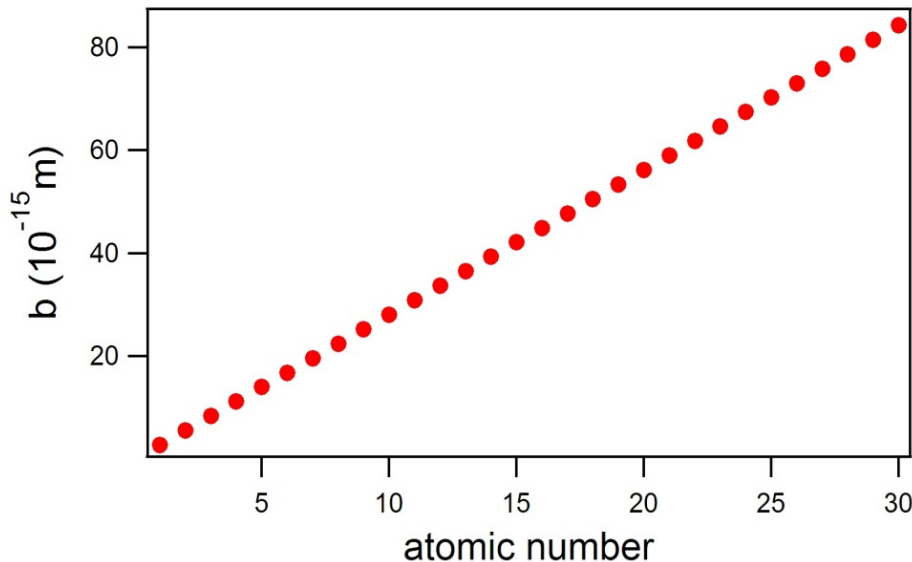
neutrons
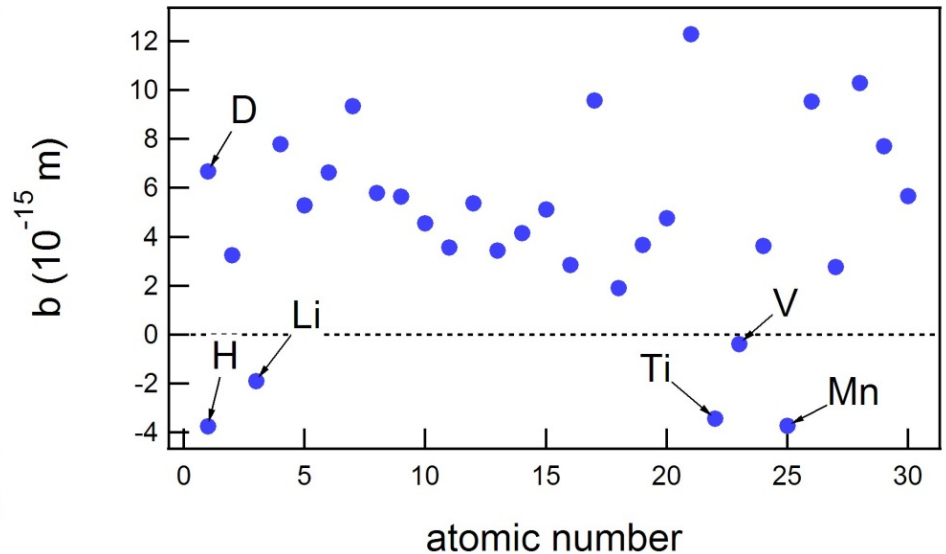
Scattered Intensity

- observed scattered intensity is Fourier Transform of real-space shapes

$$I(Q) = N_p V_p^2 (\rho_p - \rho_s)^2 F(Q) S(Q) + B$$

where: N_p = number of particles

V_p = volume of particle

ρ = scattering length density (of particle/solvent)

B = background

$F(Q)$ = form factor

$S(Q)$ = structure factor

- Sample considerations… (thickness, cell material, absorption etc)

Scattering Length Density

- scattering from an object/material depends on how many electrons or nuclei there are in a unit volume

- use *scattering length density, Nb,* to calculate scattering from molecules:

$$Nb = \frac{N_A \cdot \rho}{MW} \sum_i b_i$$

Units of *Nb*: cm^{-2} or $\mathring{A}^{-2}$

$$= N \sum_i b_i$$

where: b_i = scattering length for element, cm

(for X-rays b = $2.81 \times 10^{-13} \times$ no. of e^- in atom)

ρ = density of compound, g cm^{-3}

N_A = Avogadro's number, mol^{-1}

MW = molecular weight, g mol^{-1}

N = number density of atoms in material, cm^{-3}

NB/ if feeling lazy see: www.ncnr.nist.gov/resources/sldcalc.html

Important Scattering Length Densities

H_2O $b_H = -3.742 \times 10^{-13}$ cm D_2O $b_D = 6.674 \times 10^{-13}$ cm

$b_O = 5.805 \times 10^{-13}$ cm $b_O = 5.805 \times 10^{-13}$ cm

$MW_{H_2O} = 2H+O = 20$ g mol^{-1} $MW_{D_2O} = 2D+O = 18$ g mol^{-1}

$\rho_{H_2O} = 1.0$ g cm^{-3} $\rho_{D_2O} = 1.1$ g cm^{-3}

$$Nb = N \sum_i b_i$$

These have similar number densities of atoms ie number of atoms in 1 cm^3:

N_{H_2O} $= \dfrac{6.022 \times 10^{23} \text{ atoms mol}^{-1} \times 1.0 \text{ g cm}^{-3}}{18 \text{ g mol}^{-1}}$

N_{H_2O} $=3.35 \times 10^{22}$ atoms cm^{-3} N_{D_2O} $=3.31 \times 10^{22}$ atoms cm^{-3}

BUT very different scattering length densities!

$Nb_{H2O} = (2b_H + b_O) \times N_{H2O}$ $Nb_{D2O} = (2b_D + b_O) \times N_{D2O}$

Nb_{H2O} **= -0.562 $\times 10^{10}$ cm^{-2}**   **= 6.34 $\times 10^{10}$ cm^{-2}**

Contrast & Contrast Matching

- Both tubes contain pyrex fibers + borosilicate beads + solvent.

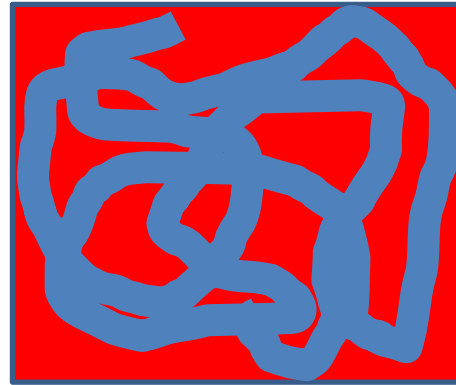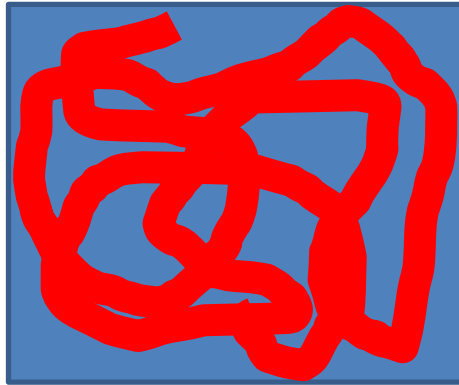(A) solvent refractive index matched to pyrex fibres

(B) solvent index different from both beads & fibers – scattering from fibers dominates
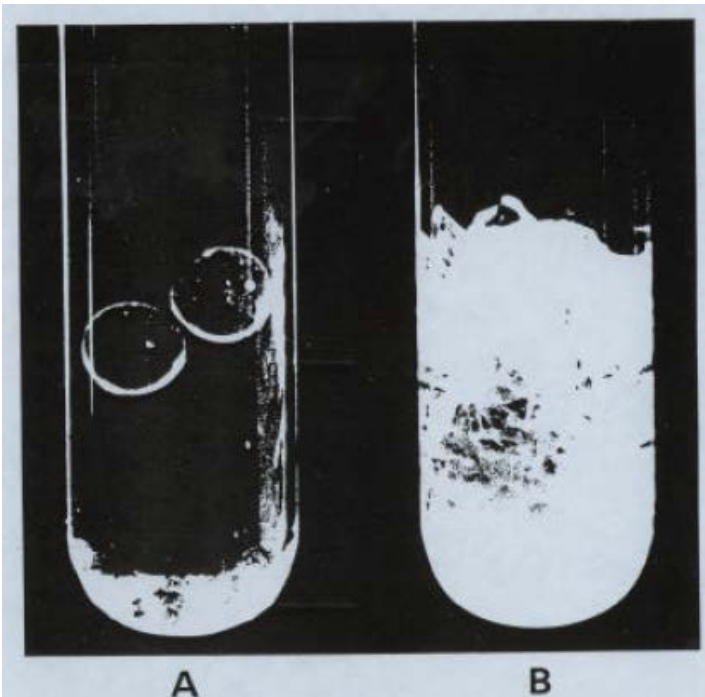
Similarly, there must be a difference between object and surrounding to measure scattering

$$I(Q) \propto (\rho_p - \rho_s)^2$$

Babinet's Principle


- These two structures give the same scattering

$$I(Q) \propto (\rho_p - \rho_s)^2$$

- Contrast is relative
- Loss of phase information i.e.: is $\rho_1 > \rho_2$?
- Very important in multi-phase systems
  - ➢ Solve by use of multiple contrasts using SANS!

    (for X-rays = anomalous scattering)

Scattering ∝ "Contrast"

- objects and solvent have different scattering length densities (SLD)

- Intensity ∝ SLD difference between solvent & particle

- in water for neutrons can manipulate solvent ρ by using mixture of H_2O and D_2O

- When solvent and object have same SLD they are said to be "contrast matched"
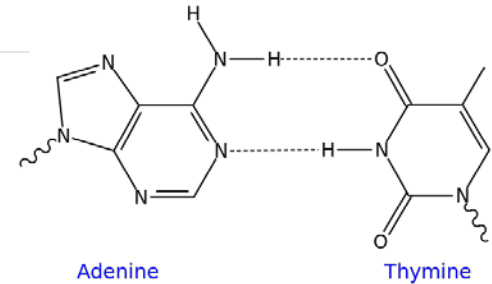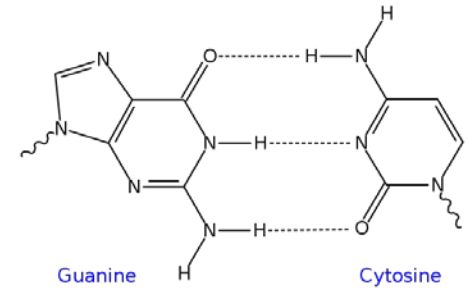
Example: silica spheres in water
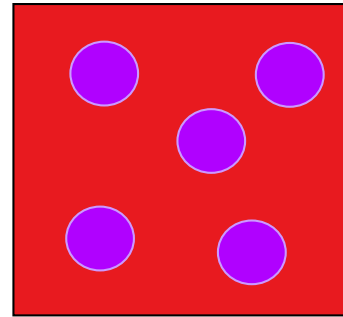
30% D_2O in H_2O
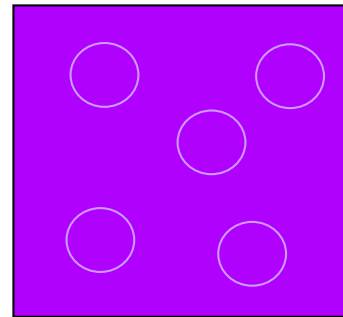
59% D_2O in H_2O
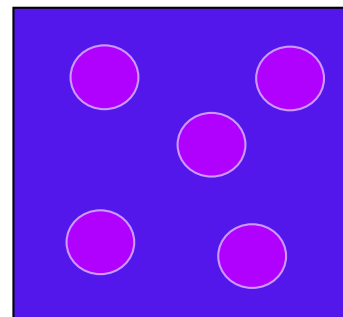
95% D_2O in H_2O

Predicting Contrast Match Point

- By calculating the SLD can predict %D_2O where the scattering signal will be zero
- BUT if have exchangeable hydrogens in the structure the SLD will vary with %D_2O

Guanine — Cytosine
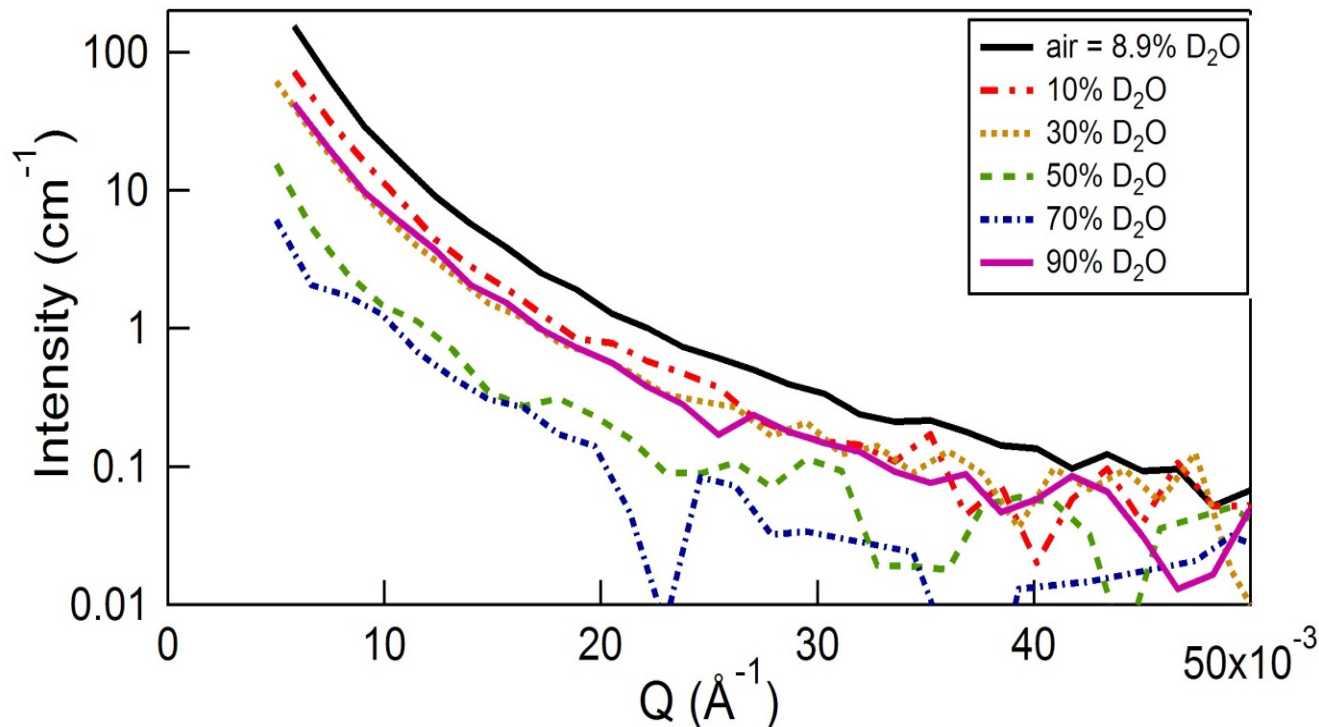

Adenine — Thymine
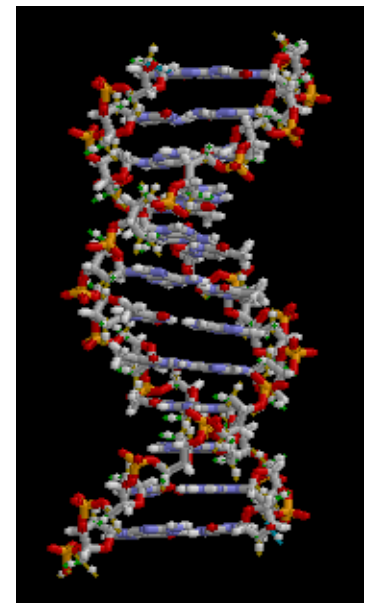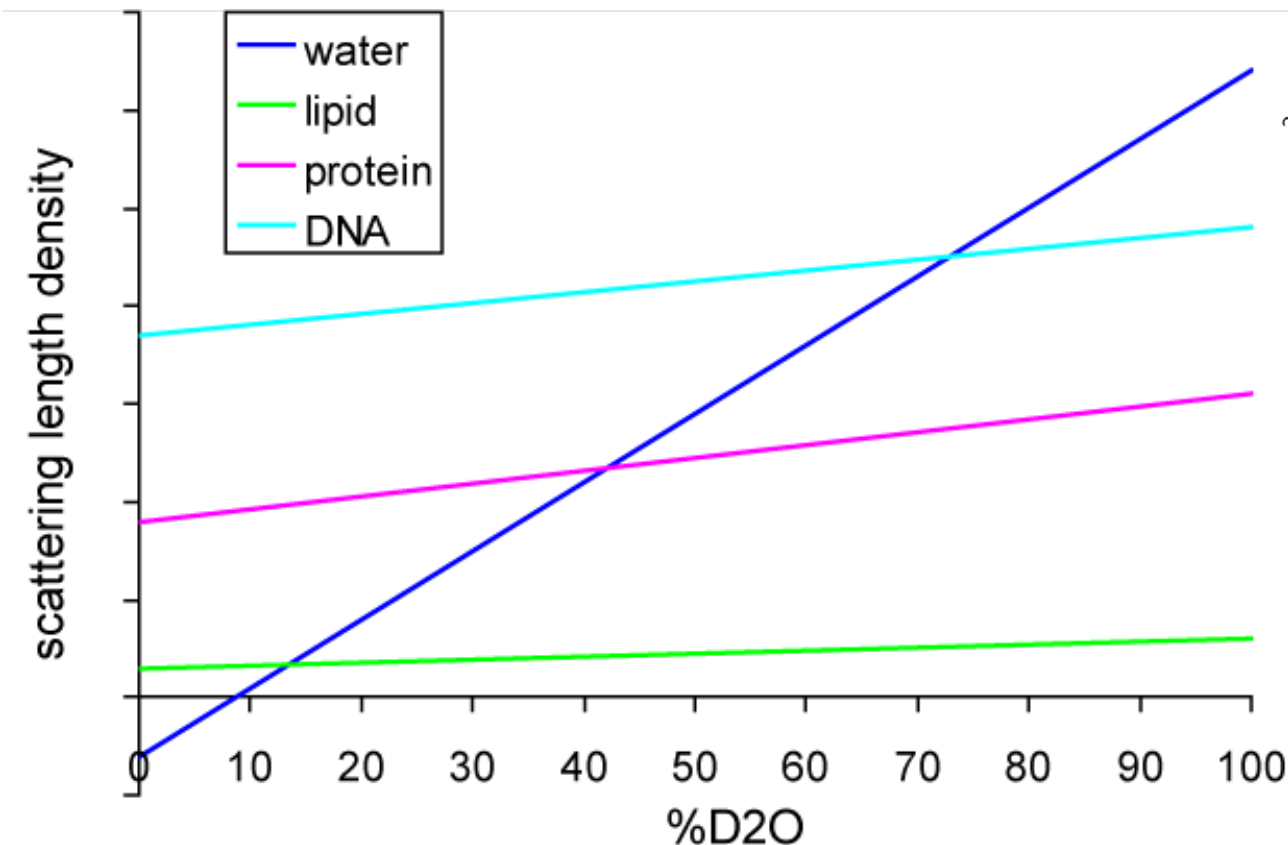
Neutron "Contrast" Series

- intensity of scattering depends on difference between particle and solution.

$$I(Q) \propto (\rho_{particle} - \rho_{solution})^2$$

- measure scattering at a series of solution contrasts
- extrapolate scattering to Q = 0 and measure I_0
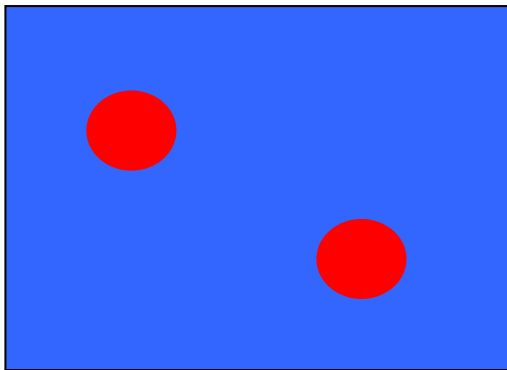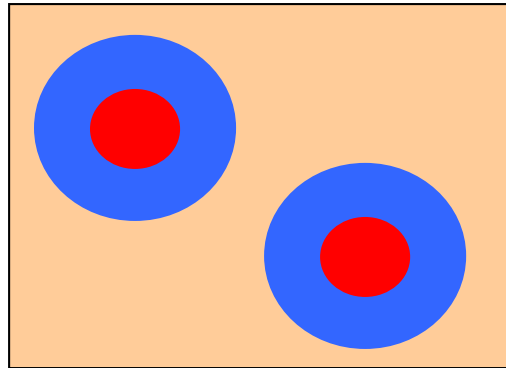
Contrast Match Point
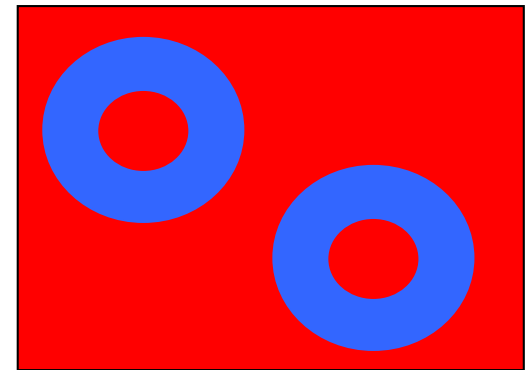
- Plot as $\sqrt{I_0}$ vs $[D_2O]$
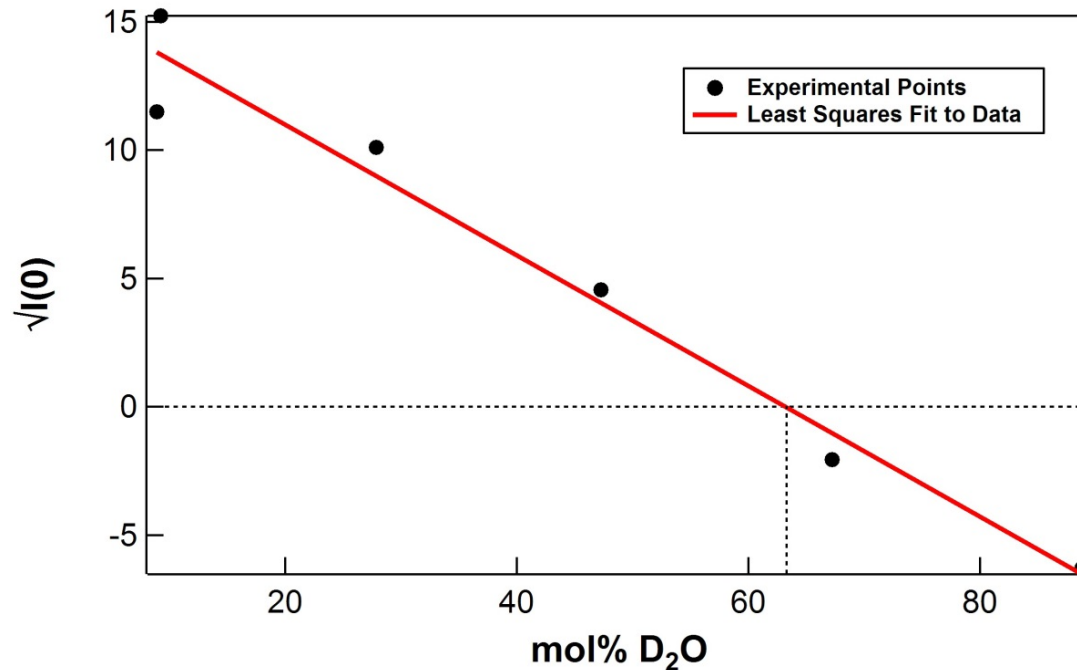
- Place where line cuts zero is where the solution has the same scattering length density as the particle
  $\Rightarrow$ *contrast matched*

- Can use this to find the density of the particle

Neutron "Contrast" for Complex Objects

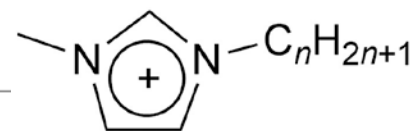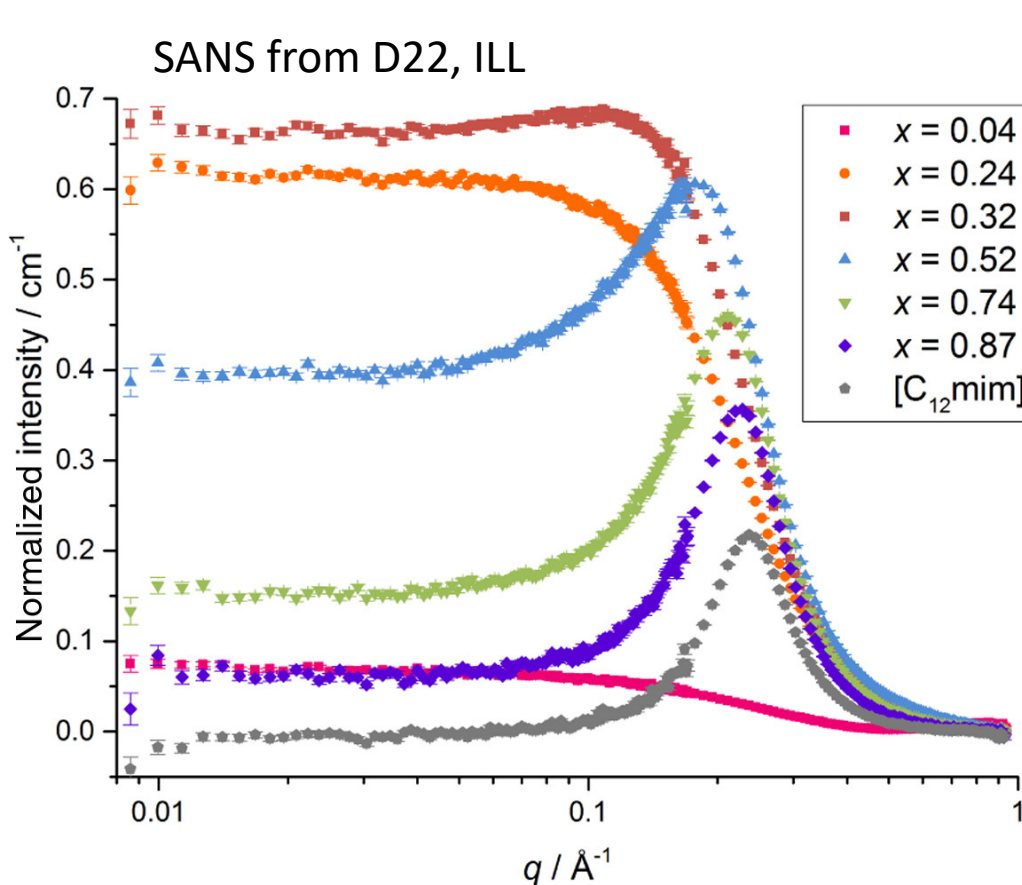- contrast matching allows us to "remove" scattering from parts of an object

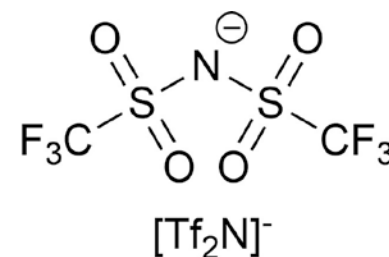

"shell-contrast"
$\Rightarrow$ see only core

"core-contrast"
$\Rightarrow$ see only shell

Using Contrast in SANS

- Deuteration can highlight structure in low contrast systems for SANS measurements.

SANS from D22, ILL

$(n = 2)$ [C$_2$mim]$^+$

$(n = 12)$ [C$_{12}$mim]$^+$

[Tf$_2$N]$^-$

Legend:
- $x = 0.04$
- $x = 0.24$
- $x = 0.32$
- $x = 0.52$
- $x = 0.74$
- $x = 0.87$
- [C$_{12}$mim][Tf$_2$N]

- Solutions of [dC$_{12}$mim]$^+$ in [C$_2$mim]$^+$[Tf$_2$N]$^-$

Segregation in Ionic Liquids

- Deuterated C_{12} chains on $[C_{12}mim]^+$ allowed mesostructure with changing $[C_{12}mim]^+$ concentration to be determined
  - Low concentrations, fitted to elliptical model,
  - High concentrations, fitted to bicontinuous network
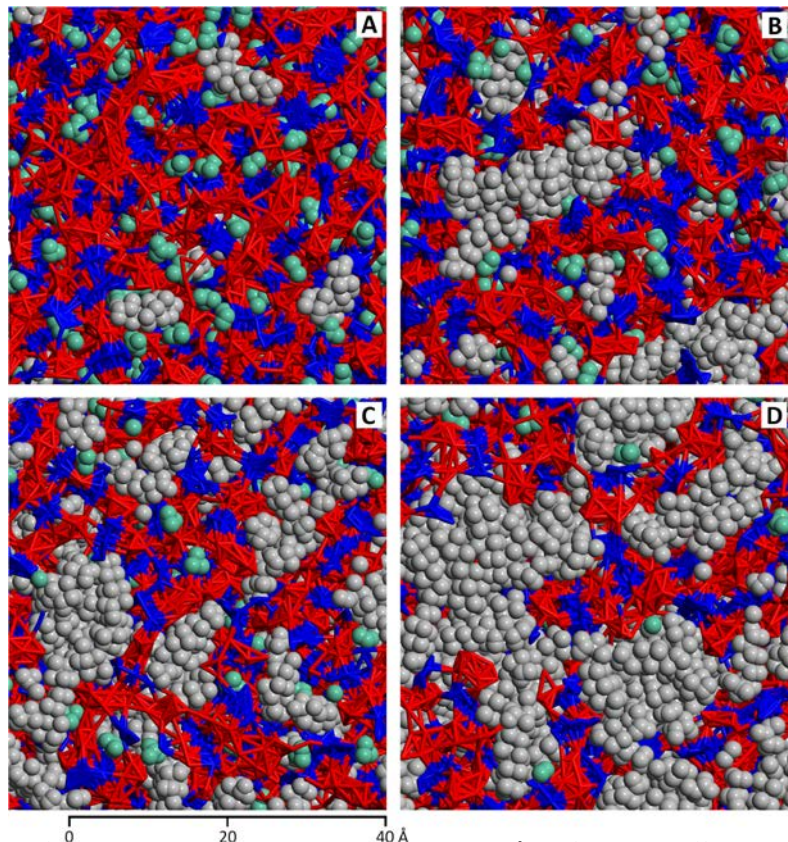
  - SANS fitting compared to molecular dynamics simulations

Nanosegregation between the polar network (red/blue mesh) and nonpolar domains (grey and green beads) in $[C_2mim]_{1-x}[C_{12}mim]_x[Tf_2N]$ (a) $x = 0.04$, (b) $x = 0.24$, (c) $x = 0.52$, and (d) $x = 0.87$.

Scattered Intensity

- For concentrated solutions:

$$I(Q) = N_p V_p^2 (\rho_p - \rho_s)^2 F(Q)S(Q) + B$$

where: N_p = number of particles

V_p = volume of particle

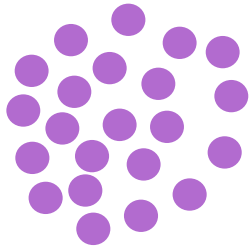ρ = scattering length density (of particle/solvent)

B = background

$F(Q)$ = form factor

$S(Q)$ = structure factor

Form Factor = scattering from within same particle
$\Rightarrow$ depends on particle shape

Structure Factor = scattering from different particles
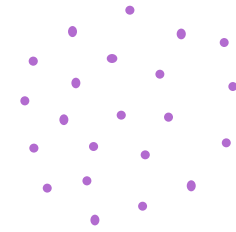$\Rightarrow$ depends on interactions between particles

Solution of particles

Solution   =   Motif   *   Lattice

$I(c,Q)$ (protein, micelle, nanoparticle) $S(c,Q)$

$F(0,Q)$

Form factor   Structure factor
of the particle   of the particle

c = concentration

SAS Data Analysis

- Simple but not very accurate:
  - ➢ Porod slopes
  - ➢ Guinier analysis

- More helpful, but more complex:
  - ➢ fitting models to data

- Most complex (need more data):
  - ➢ fitting protein structures using crystal structures
  - ➢ monte carlo/simulated annealing methods

Scattering from Independent Particles

- Scattered intensity per unit volume of sample
  - ➢ arises from spatial distribution of regions with different scattering length density

$$I(q) = \frac{d\sigma}{d\Omega} = \frac{1}{V}\left|\int_V \rho(\boldsymbol{r})\, e^{i\boldsymbol{q}.\boldsymbol{r}} d\boldsymbol{r}\right|^2$$

V_p, ρ_p

V, ρ_s
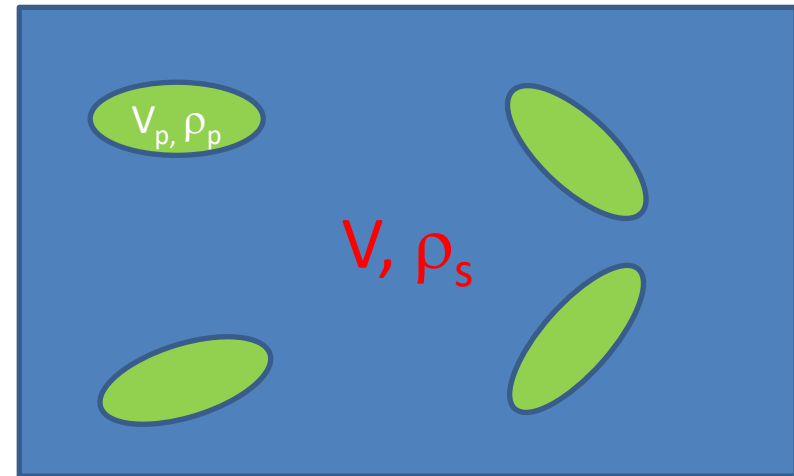
- For identical particles:

$$I(q) = \frac{N}{V}(\rho_p - \rho_s)^2 V_p^2 \left\langle \frac{1}{V_p}\left|\int_{particle} e^{i\boldsymbol{q}.\boldsymbol{r}}\, d\boldsymbol{r}\right|^2\right\rangle$$

Particle form factor, F(Q)

Dilute Randomly Ordered Uniform Particles

- scattering from independent particles:

$$I(q) = \frac{N}{V}(\rho_p - \rho_s)^2 V_p^2 \left\langle \frac{1}{V_p} \left| \int_{particle} e^{i\boldsymbol{q}.\boldsymbol{r}} \, d\boldsymbol{r} \right|^2 \right\rangle$$

- Assume: i) system is isotropic, then $\left\langle e^{-iqr} \right\rangle = \frac{\sin(qr)}{qr}$

  ii) no long range order, so no correlations between two widely separated particles

$$I(q) = (\rho_p - \rho_s)^2 V_p \int_0^\infty \gamma(r) \frac{\sin(qr)}{qr} 4\pi r^2 dr$$

$\gamma(r)$ = correlation function within particle

P(r)=$4\pi r^2\gamma$(r) is the probability of finding two points in the particle separated by r

Porod's Law

- Start with form factor:

$$F(q) = \frac{1}{V_p} \int_0^\infty \gamma(r) \frac{\sin(qr)}{qr} 4\pi r^2 dr$$

- Now consider radial pair correlation function for sphere, with sharp edges, radius R:

$$\gamma(r) = 1 - \frac{3}{4}\left(\frac{r}{R}\right) + \frac{1}{16}\left(\frac{r}{R}\right)^3$$

$$F(qR) = \frac{1}{V_p} \int_0^\infty \left[1 - \frac{3}{4}\left(\frac{r}{R}\right) + \frac{1}{16}\left(\frac{r}{R}\right)^3\right] \frac{\sin(qr)}{qr} 4\pi r^2 dr$$
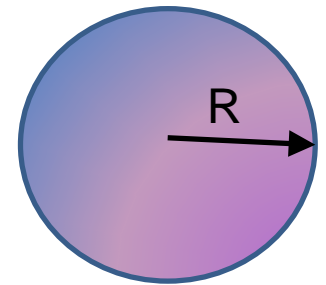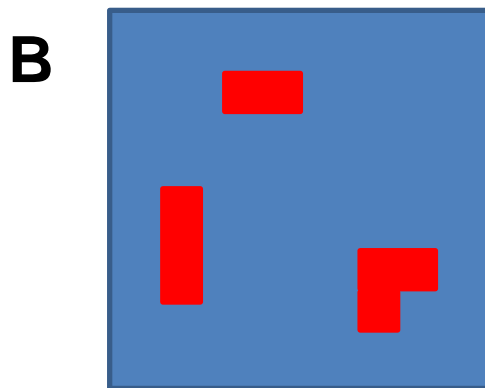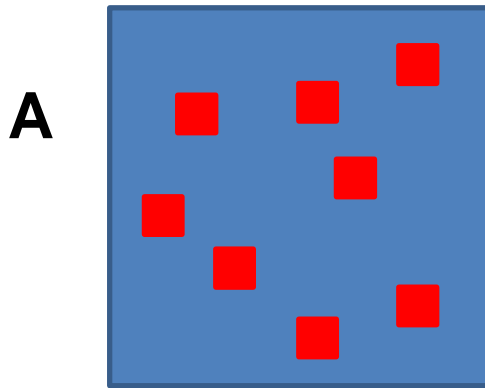
- Integrate by parts three times:

$$F(qR) \approx \frac{3}{2R^3} \frac{S_p}{V_p} \frac{1}{q^4}$$

At high scattering angles, for any system with sharp, smooth surfaces: $I(Q) \propto \frac{1}{q^4}$

Porod Scattering

- Slope at high q the same
- But point where slope changes depends on particle dimensions
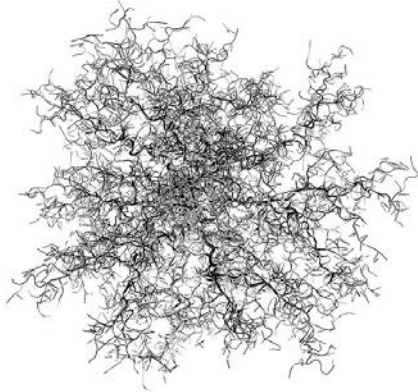

A

B

10% red / 90% blue in each square
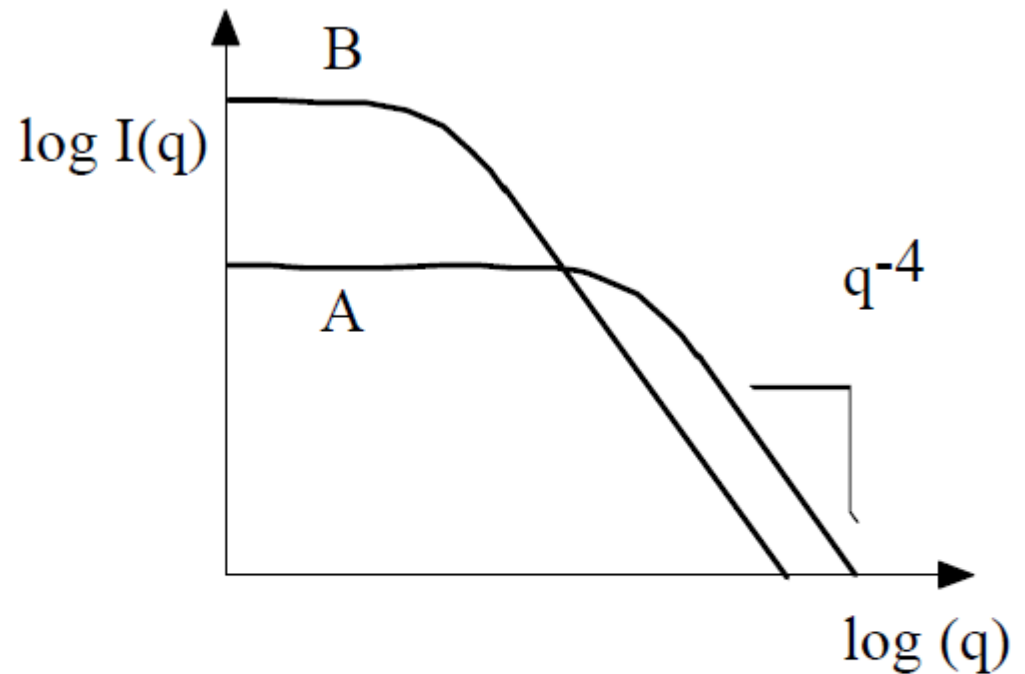
*Glatter & Kratky pp. 30-1.

Fractal Systems

- Fractals are systems that are self-similar as you change scale

Diffusion-limited aggregation in 3 dimensions
(Paul Bourke,
http://local.wasp.uwa.edu.au/~pbourke/fractals/dla3d/)

- For a Mass Fractal the number of particles within a sphere radius R is proportional to R^D where D = fractal dimension
- Thus:

  $4\pi R^2 \gamma(R)dR$ = number of particles between distance R and R+dR

  $\qquad = cR^{D-1}dR$

Fractal Systems Continued…

- So for a Mass Fractal:

$$F(Q) = \int dR\, e^{iQR} \gamma(R) = \frac{2\pi}{Q} \int dR\, .\, R\sin(QR).\left(\frac{c}{4\pi}\right) R^{D-3}$$

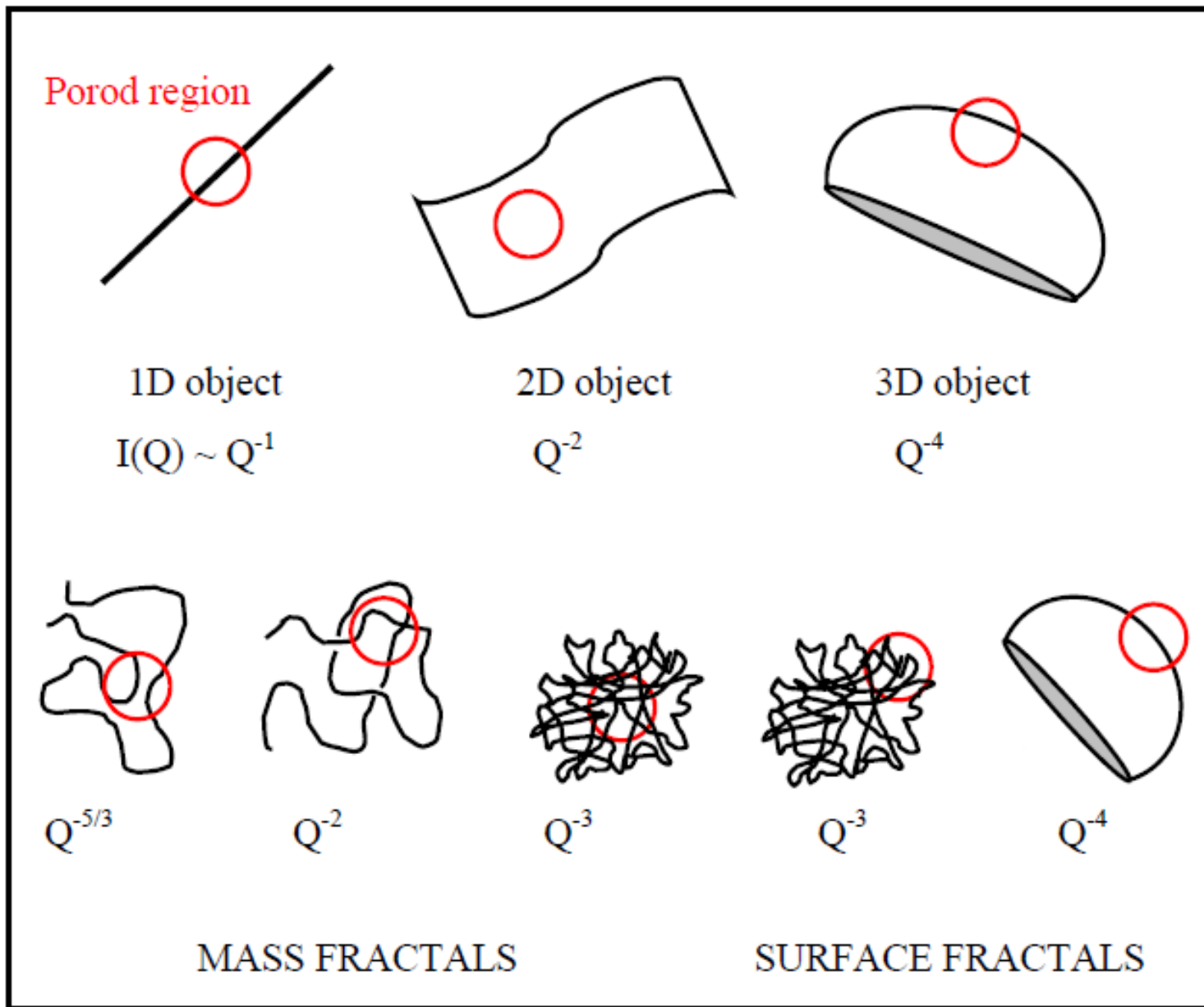$$= \frac{c}{2}\frac{1}{Q^D} \int dx\, .\, x^{D-2}.\sin x = \boxed{\frac{constant}{Q^D}}$$
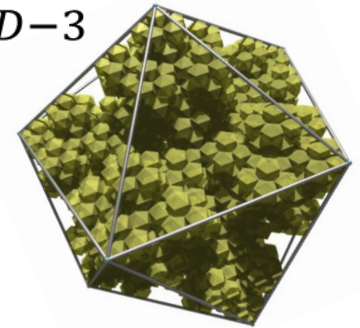
Paul Bourke

- For a fractal surface can show that $F(Q) = \frac{constant}{Q^{6-D}}$ (this reduces to the Porod Law for smooth surfaces of dimension 2)
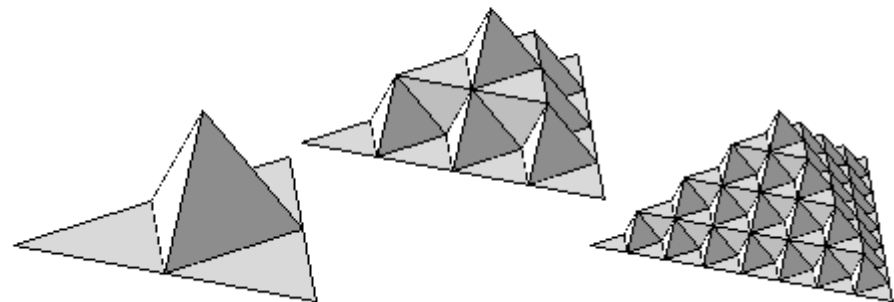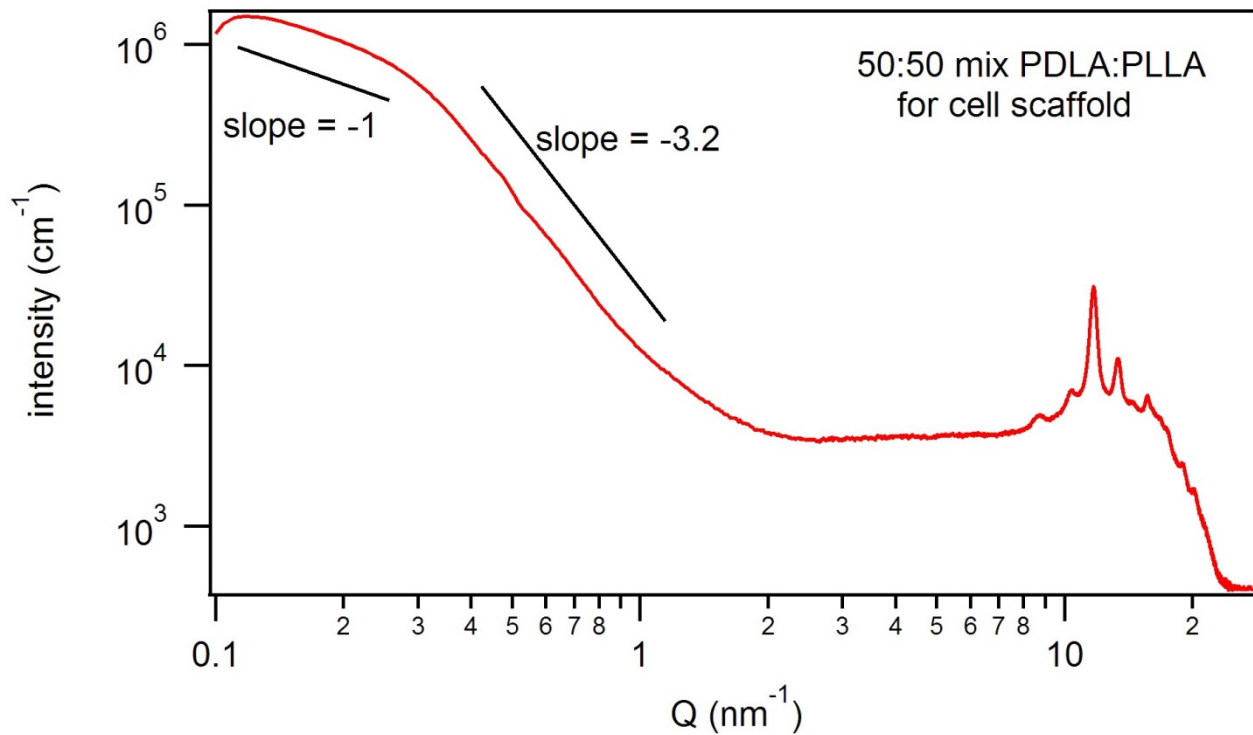
First stages of Koch (triangle) surface
(Robert Dickau)

Porod region

1D object
$I(Q) \sim Q^{-1}$

2D object
Q^{-2}

3D object
Q^{-4}

$Q^{-5/3}$

Q^{-2}

Q^{-3}

Q^{-3}

Q^{-4}

MASS FRACTALS

SURFACE FRACTALS

The SANS Toolbox. Boualem Hammouda, NIST

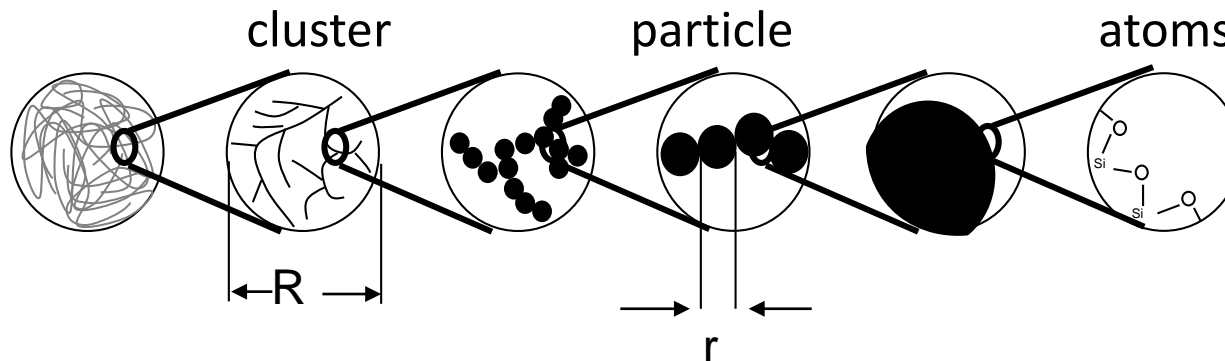http://www.ncnr.nist.gov/staff/hammouda/the_SANS_toolbox.pdf

Porod Slopes & Structures

intensity (cm^{-1}) vs Q (nm^{-1})

slope = -1

slope = -3.2

50:50 mix PDLA:PLLA
for cell scaffold

NB/ SAXS data,
seldom measure such
a wide Q range in
SANS

eg
continuum   network   surface

cluster   particle   atoms

←R→   r

Form Factors

- Form factors are the sum of scattering from every point inside a particle

$$F(Q) = \sum_I \sum_J \left\langle \left| \frac{\sin(Qr_{IJ})}{Qr_{IJ}} \right| \right\rangle_{orientations}$$

- Simplify to the integral

- Scattering pattern calculated from the Fourier transform of the real-space density distribution
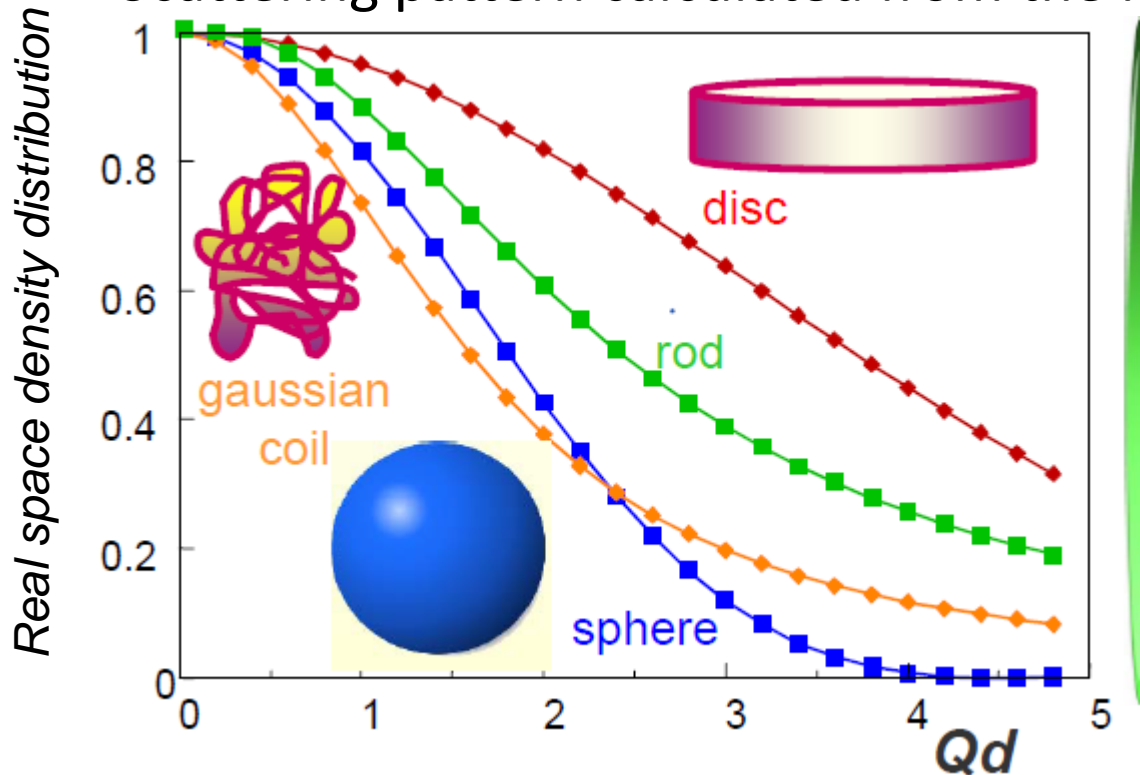
- Pattern for most shapes must be solved analytically

- Some simple shapes can be solved directly

Simple Analysis - Guinier Approximation

- Assume particle is a sphere in dilute solution

$$F(Q) = \left[\frac{3(\sin(QR_p) - QR_p \cos(QR_p))}{(QR_p)^3} \right]^2$$

R_p = radius of sphere

- Measure scattering at very low angles so that

$$R_g Q \lesssim 1$$

- Use mathematical expansion of *F(Q)*

$$F(Q) = 1 - \left(\frac{Q^2 R_g^2}{3} \right) + O()^5 \dots$$

- Write in logarithmic form ➜

Guinier Plots

- at low concentrations and small values of Q, can write intensity as:

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

- so plot of ln(I) against Q^2 will have slope = $\dfrac{-R_g^2}{3}$
- only valid for $R_g Q \leq 1$

Radius of Gyration – depends on particle shape
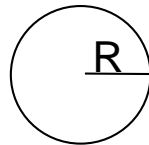
- Sphere $\quad R_g^2 = \dfrac{3}{5}R^2$

What do we mean by "R_g"?

Radius of gyration:

R_g^2 is the average squared distance of the scatterers from the centre of the object
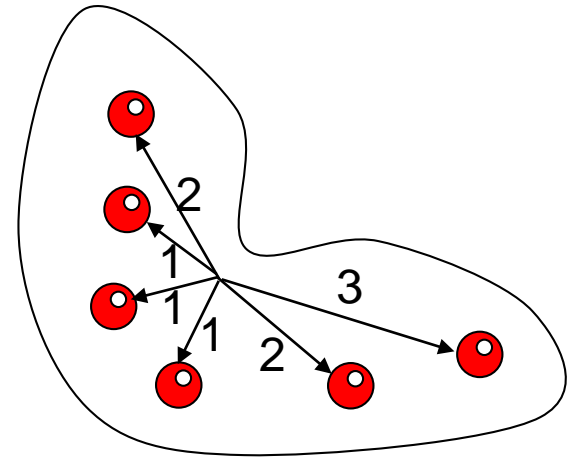
$$R_g^2 = (1^2 + 1^2 + 1^2 + 2^2 + 2^2 + 3^2)/6 = 20/6$$

$$R_g = \sqrt{3.333} = 1.82$$

Radius of Gyration – depends on particle shape

- Sphere $R_g^2 = \dfrac{3}{5}R^2$

- Ellipse $R_g^2 = \dfrac{a^2 + b^2}{4}$

- Cylinder $R_g^2 = \dfrac{R^2}{2} + \dfrac{h}{12}$
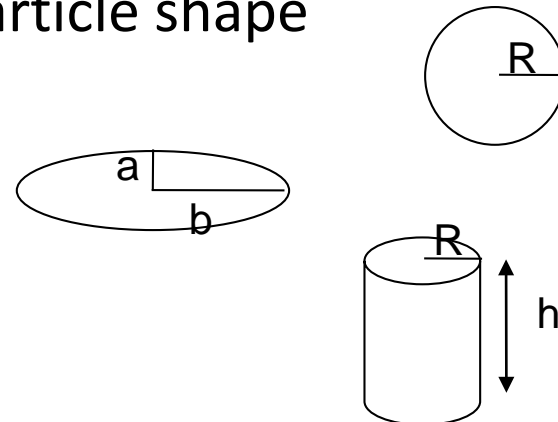
Guinier Plot Example

- ## Polymerised surfactant micelles

Large Scale Structures, ISIS Annual Report, 1999-2000 http://www.isis.rl.ac.uk/isis2000/science/largescale.htm

Q (Å^{-1})	Q^2 (Å^{-2}) ×10^{-3}	Intensity (cm^{-1})	ln(intensity)
0.032	1.03	0.127	-2.064
0.050	2.51	0.113	-2.183
0.070	4.87	0.106	-2.245
0.081	6.56	0.096	-2.341
0.095	9.03	0.087	-2.441
0.104	10.81	0.080	-2.528
0.115	13.23	0.073	-2.618
0.123	15.13	0.063	-2.769
0.129	16.64	0.062	-2.789
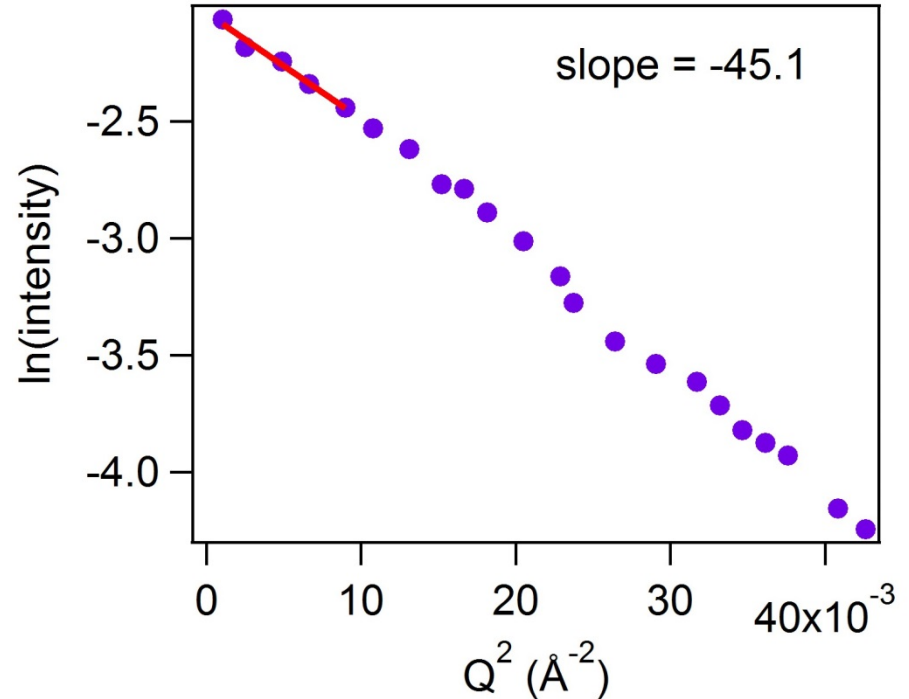
Slope = $\dfrac{-R_g^2}{3}$ = -45.1 Å

so: R_g = 11.6 Å

Check validity: $R_g \times Q_{max}$ = 11.6×0.095 = 1.1   OK

More Complex: Fitting Scattering

- observed scattered intensity is Fourier Transform of real-space shapes

$$I(Q)=N_p V_p^2 (\rho_p - \rho_s)^2 F(Q)S(Q)+B$$

where: N_p = number of particles

V_p = volume of particle

ρ = scattering length density (of particle/solvent)

B = background

$F(Q)$ = form factor

$S(Q)$ = structure factor

Form Factor = scattering from within same particle

$\Rightarrow$ depends on particle shape

Structure Factor = scattering from different particles

$\Rightarrow$ depends on interactions between particles

Form Factors

- depend on shape of particle

- for dilute solutions S(Q) = 1 and so I(Q) $\propto$ F(Q)

- General form of F(Q):

$$F(q) = \frac{1}{V_p} \int_0^\infty \gamma(r) \frac{\sin(qr)}{qr} 4\pi r^2 dr$$
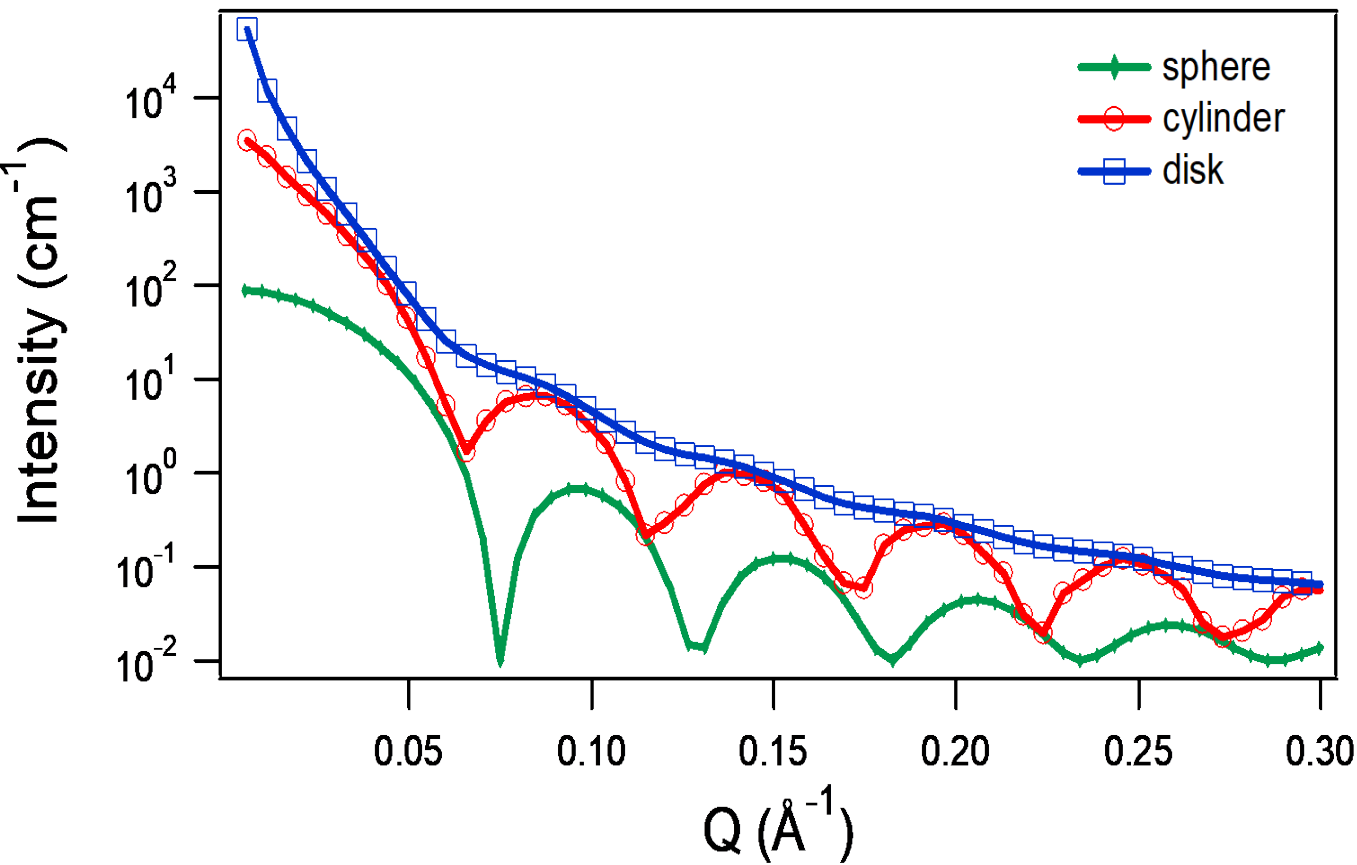
where
r = shape parameter
eg radius of gyration

Polydispersity

- "smears out" sharp features in pattern
- "smearing" can also be due to poor Q resolution or beam shape (correct for this during data reduction)
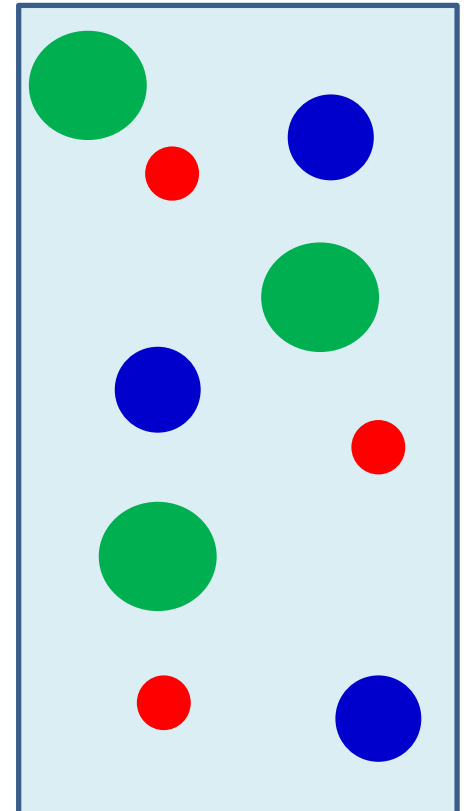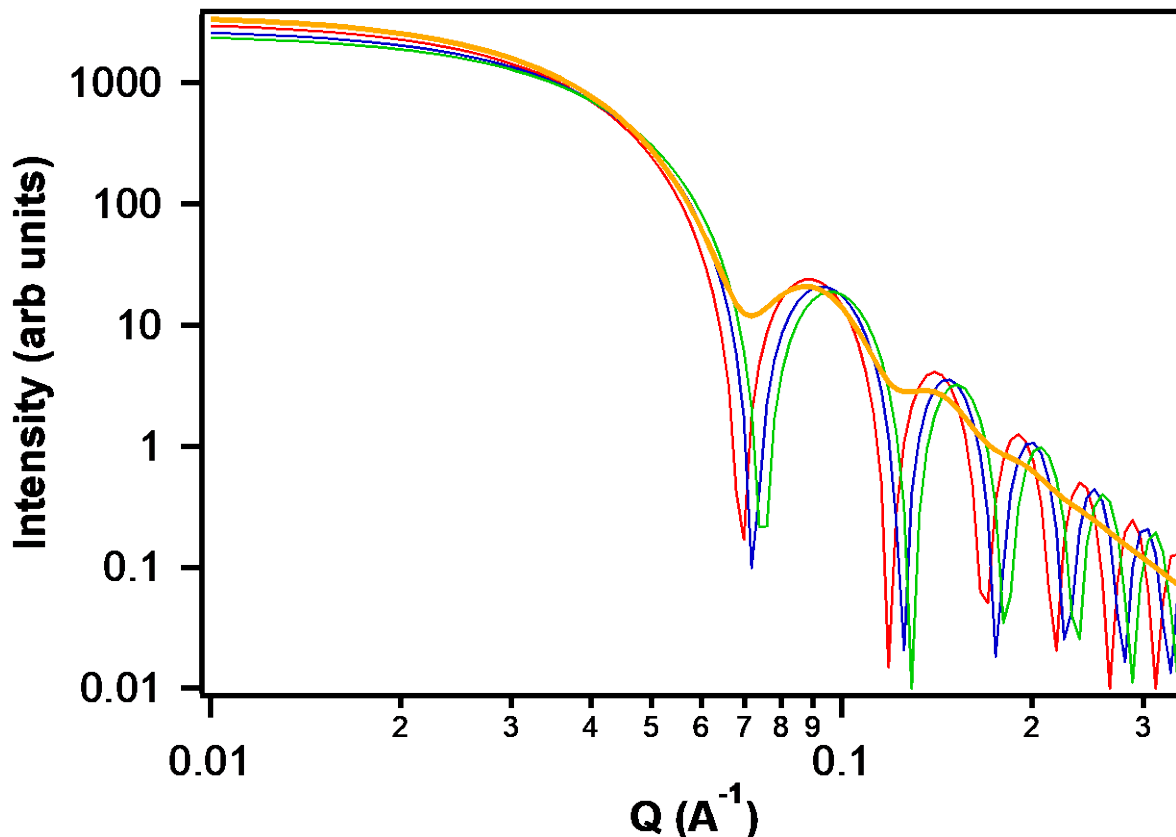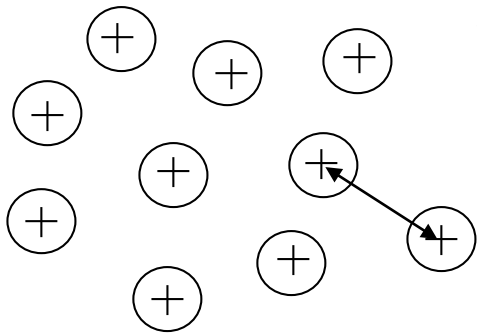
Au Nanorods

Fitted to charged cylinders
- Radius 80Å
- Length 190Å
- Polydispersity 0.29
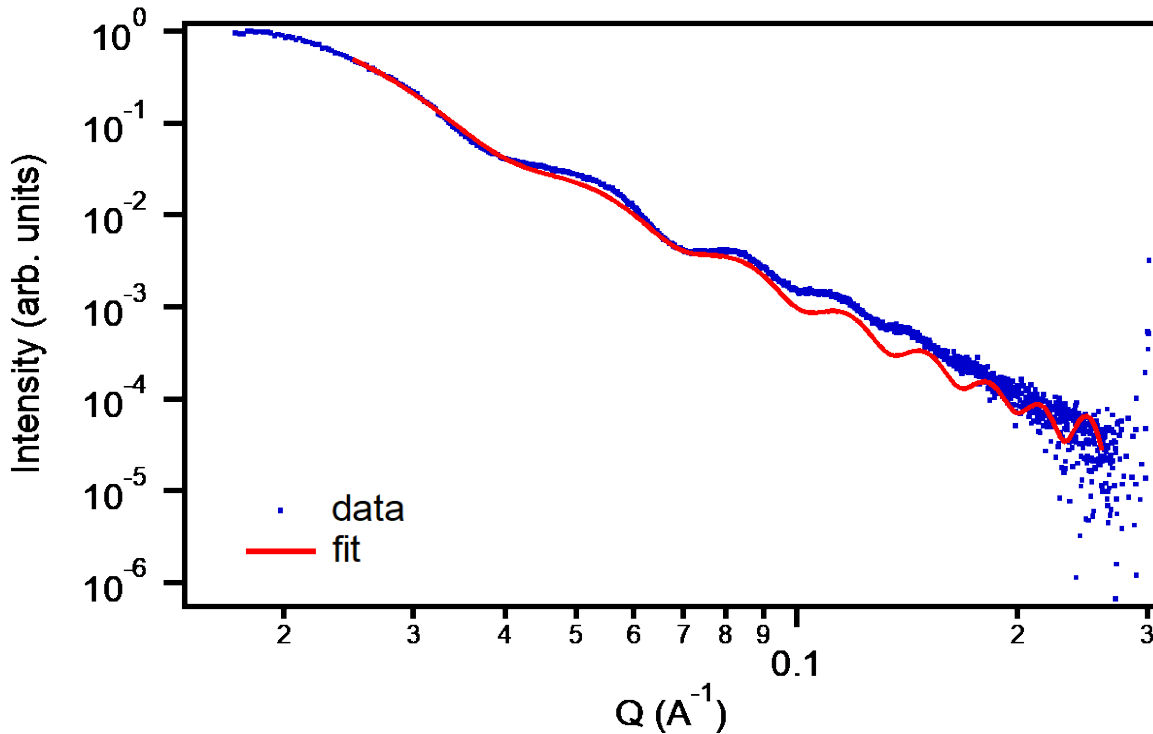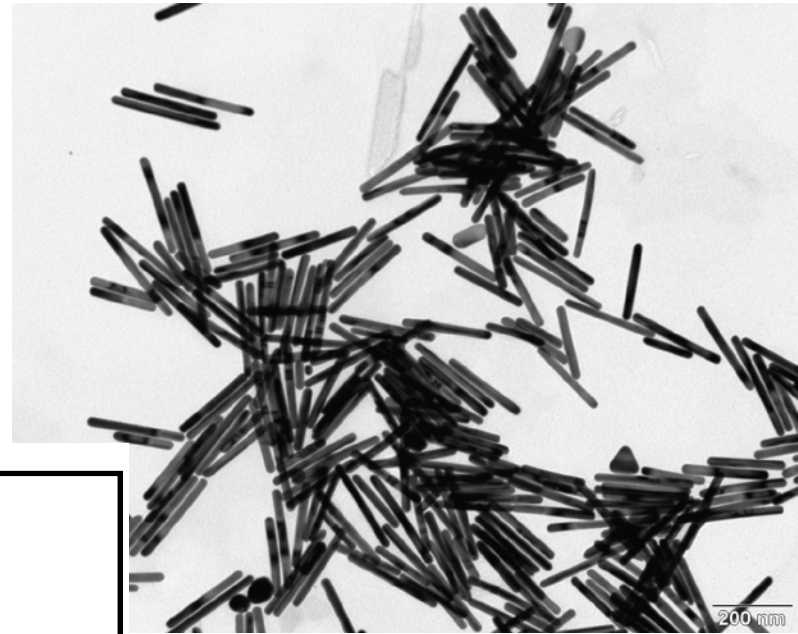
Structure Factors

- for dilute solutions $S(Q) = 1$
- particle interactions will affect the way they are distributed in space $\Rightarrow$ changes scattering
- for charged spheres:

Average distance between nearest neighbours relatively constant = "correlation distance"

Position of first maximum related to correlation distance
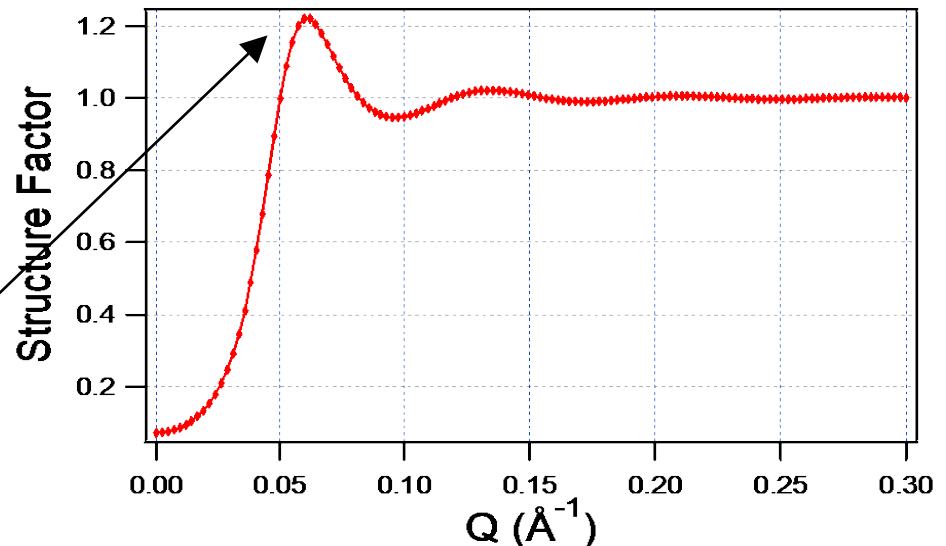
Concentration effects
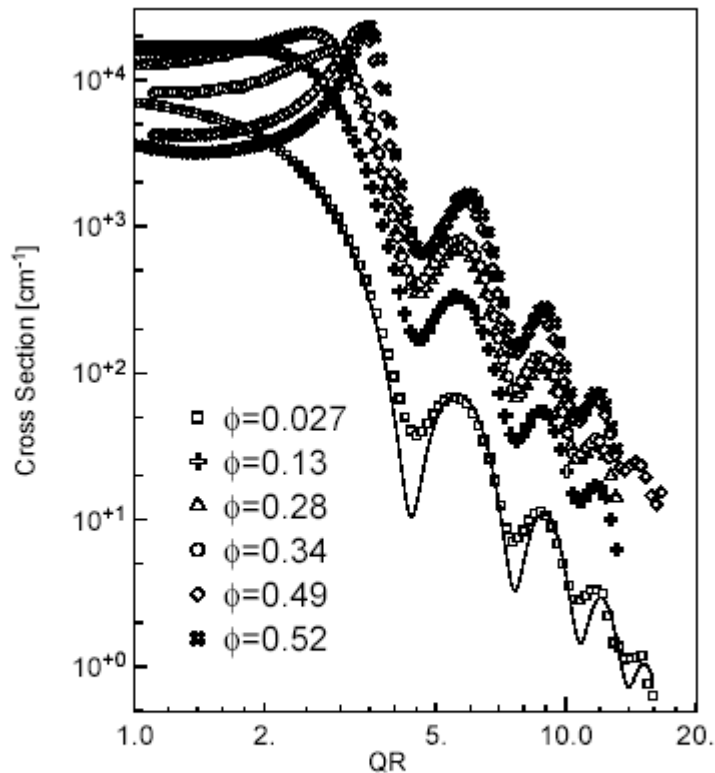
Figure 1: Cross-section for several different volume fractions of PS spheres in glycerol vs. QR.
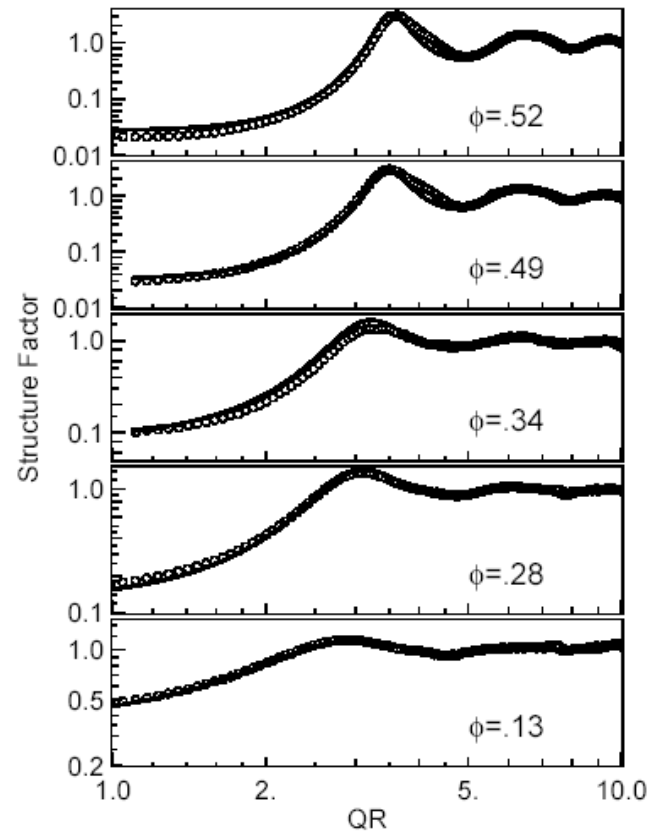
Figure 2: Measured and model structure factors, S(Q), (circles and dashed lines, respectively) vs. QR for PS spheres in glycerol.

Small Angle X-ray Scattering Study of a Hard-Sphere Suspension: Concentrated Polystyrene Latex Spheres in Glycerol

L. B. Lurio[1], D. Lumma[1], A. R. Sandy[1], M. A. Borthwick[1], P. Falus[1], S. G. J. Mochrie[1], J. F. Pelletier[2], M. Sutton[2], Lynne Regan[3], A. Malik[4] and G. B. Stephenson[4]

Combining F(Q) & S(Q)

- In most cases when fitting will need to include both form and structure factor

- Can tell by taking concentration series

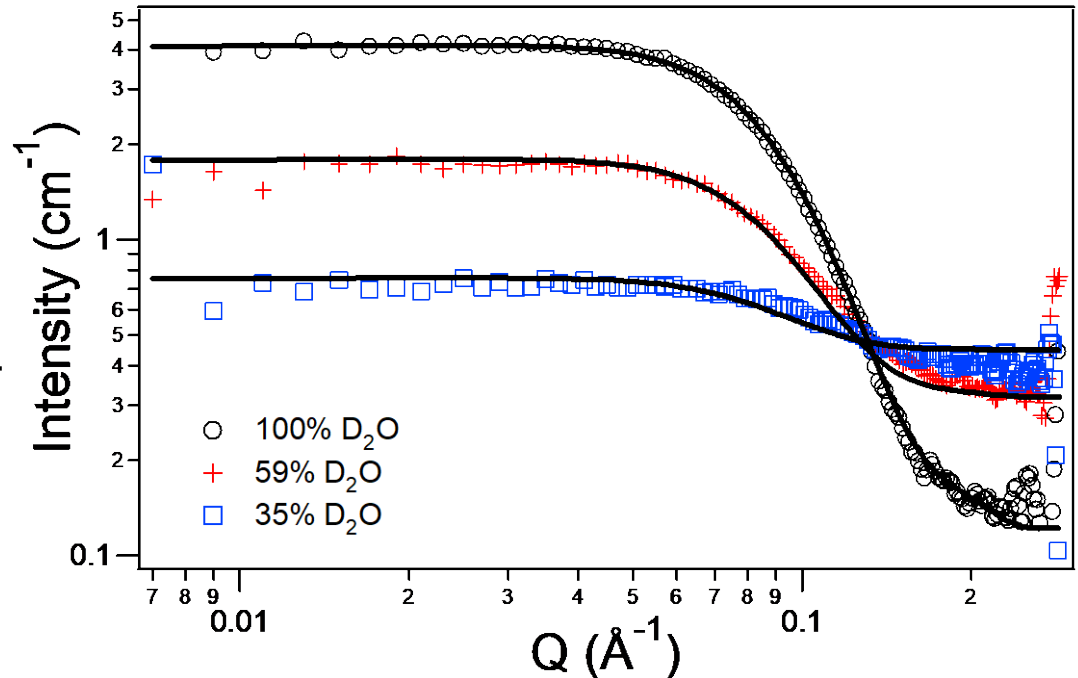 - ➢ if shape of scattering doesn't change when sample is diluted then S(Q) = 1
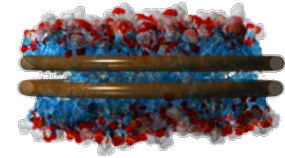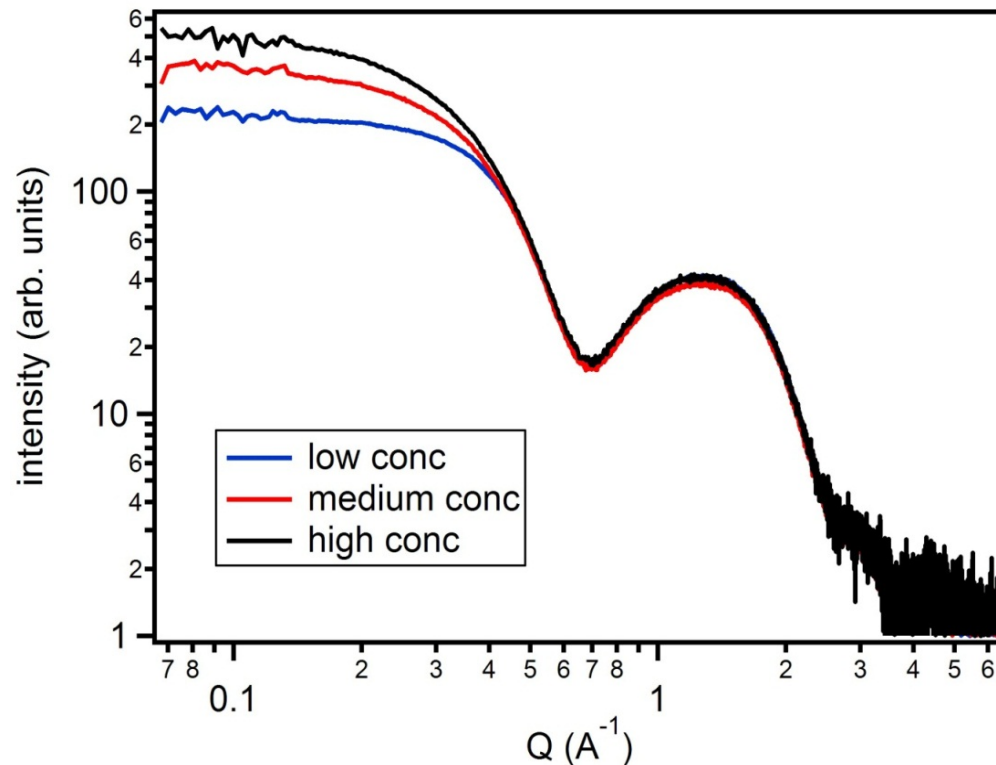
- Polymer-lipid discs
- Normalised for concentration

Combining F(Q) & S(Q)

- Use computer programs to combine form factor and structure factor:

17Å

31Å
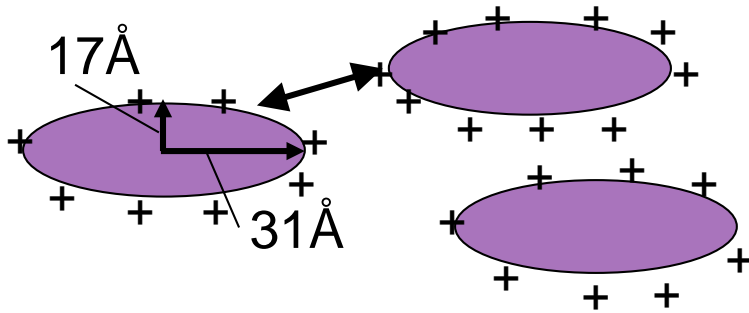
Brennan, Roser, Mann, Edler,
Chem. Mater. **2002,** *14*, 4292

Intensity (cm^{-1}) vs Q (Å^{-1})

○ 100% D$_2$O
+ 59% D$_2$O
□ 35% D$_2$O

- Fit using ellipse + structure factor for charged objects which repel each other $\Rightarrow$ many parameters!
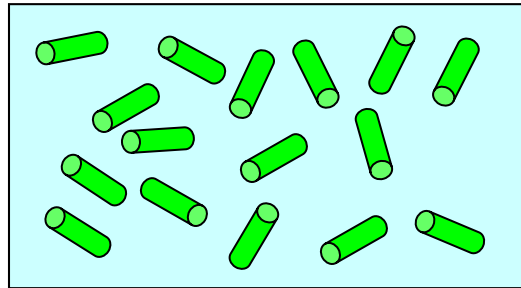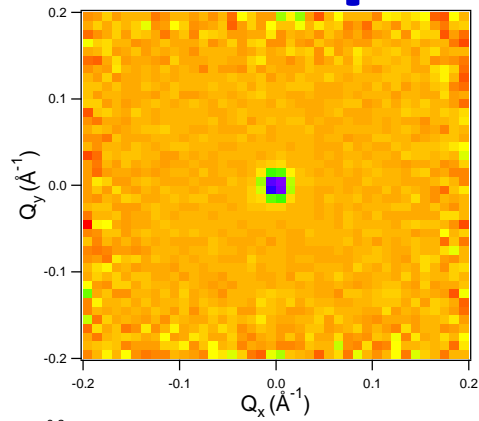- Use three contrasts to help pin down shape and size accurately

Effects of Sample Alignment

- Scattering no longer circular
- Form areas of high intensity perpendicular to direction of alignment
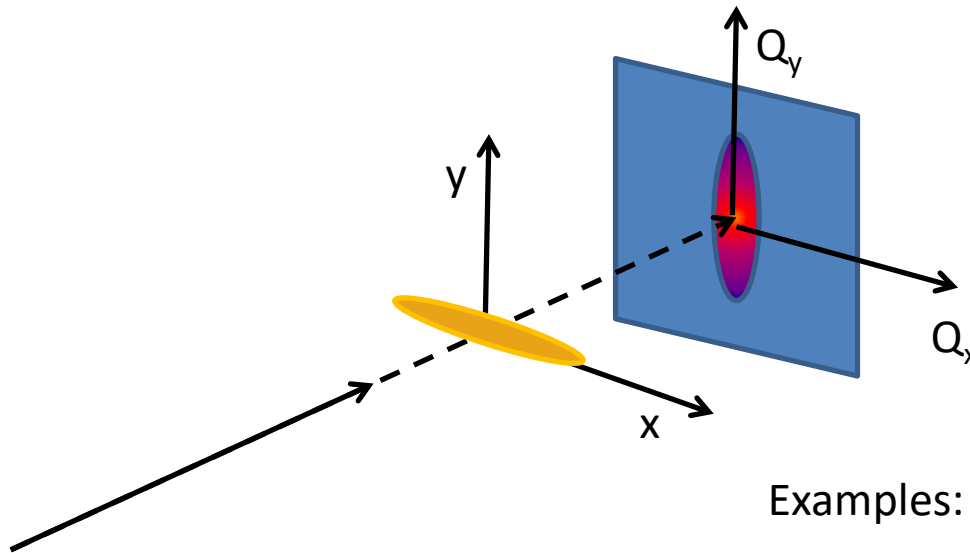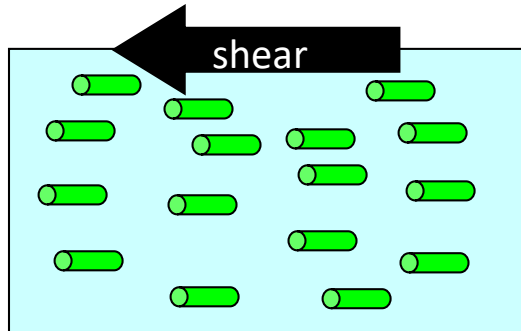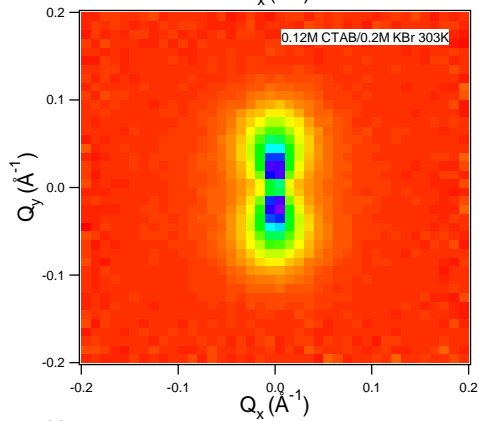

Examples: shear, flow
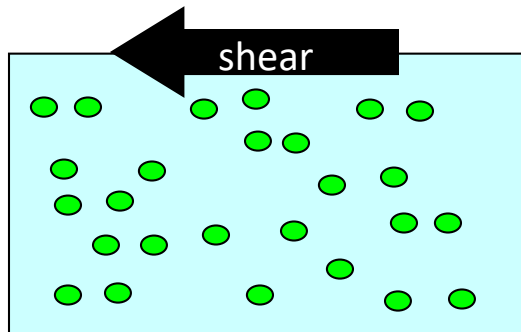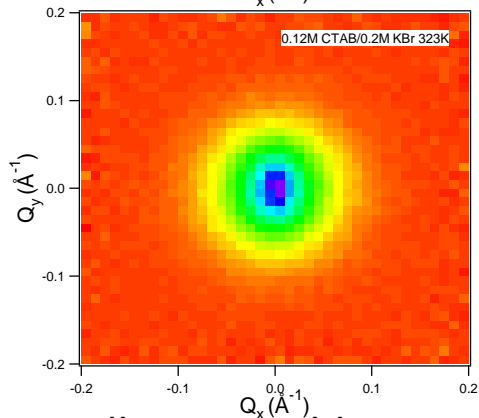   magnetic alignment

Isotropic vs Nonisotropic Structures

No shear
$\Rightarrow$ Isotropic solution

Shear
$\Rightarrow$ aligned micelles

Shear + higher T
$\Rightarrow$ isotropic again

0.12M CTAB/0.2M KBr 303K

0.12M CTAB/0.2M KBr 323K

Edler, Reynolds, Brown, Slawecki, White, *J. Chem. Soc., Faraday Trans.* **1998,** *94*(9) 1287

Fourier Inversion Techniques

- Scattering from dilute, uniform, independent particles
- Assuming i) system is isotropic, then $\langle e^{-iQr} \rangle = \dfrac{\sin(Qr)}{Qr}$

  ii) no long range order, so no correlations between two widely separated particles

$$I(Q) = I_e(Q)(\rho_p - \rho_s)^2 V_p \int_0^\infty \gamma(r) \frac{\sin(Qr)}{Qr} 4\pi r^2 dr$$
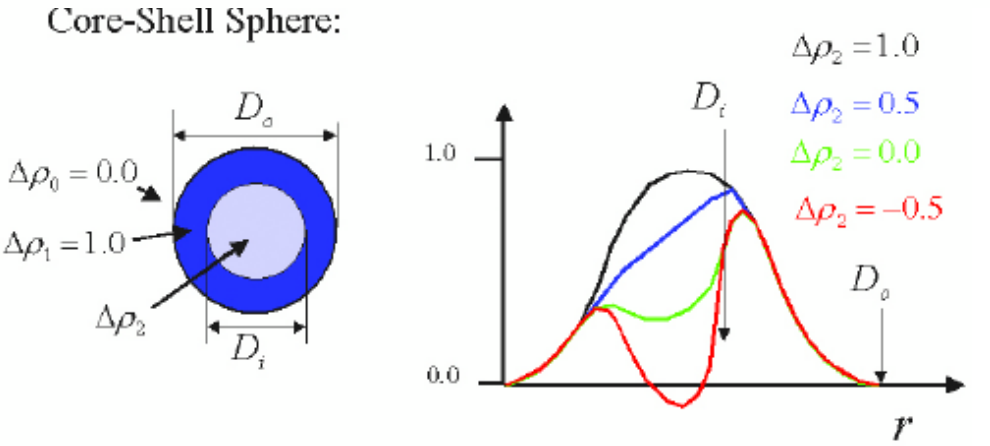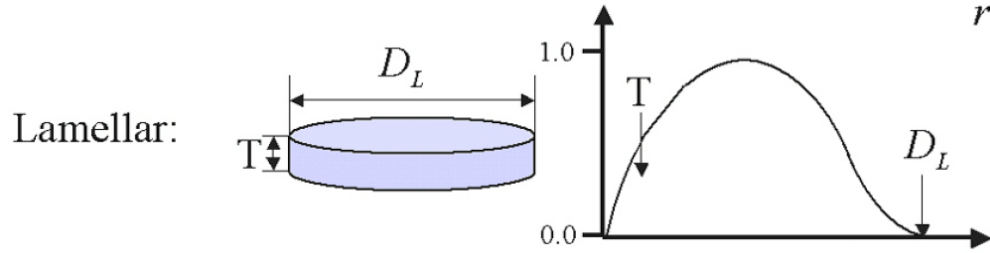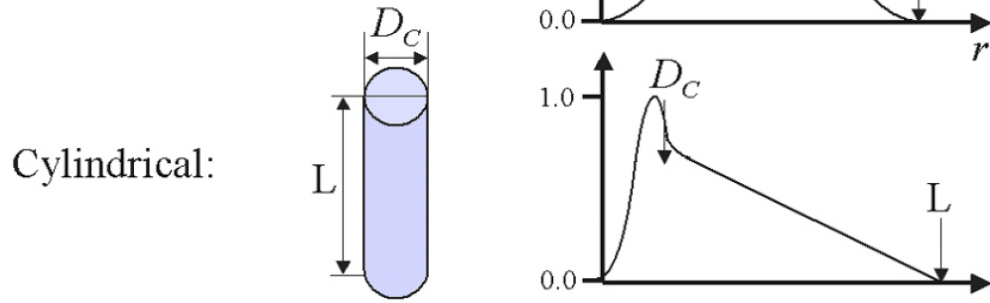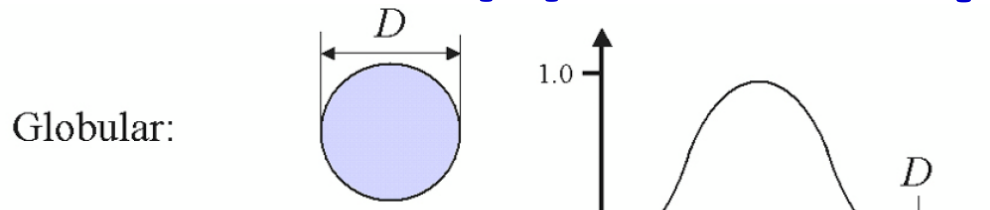
$\gamma(r)$ = correlation function

P(r)=$4\pi r^2\gamma$(r) is the probability of finding two points in the particle separated by r

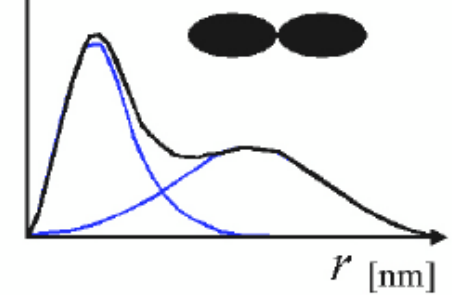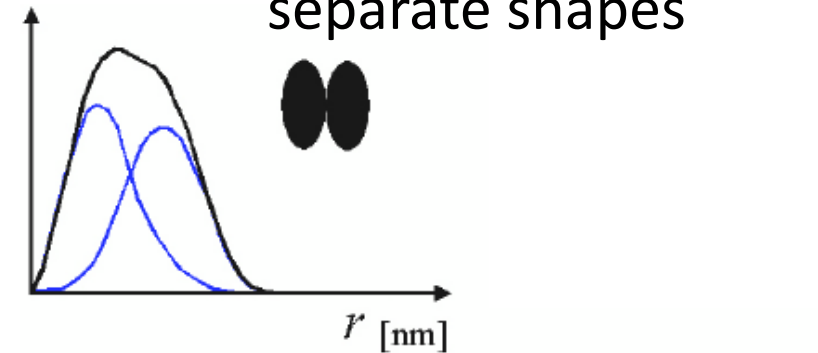- If can measure I(Q) over big enough range can take inverse Fourier transform to find P(r):

$$P(r) = 4\pi r^2\gamma(r) = \frac{2}{\pi} \int QI(Q)\sin(Qr)\,dQ$$

P(r) for Simple Shapes

Globular:

Cylindrical:

Lamellar:

Core-Shell Sphere:

- Note: P(r) can be ambiguous if have polydisperse samples

Aggregates = sum of separate shapes

Free SANS Fitting Software

SASView software

- Designed for fitting neutron data but can also be used (with care) for X-ray data
- Available from: http://www.sasview.org/

OR library of other available software at:

http://smallangle.org/content/software