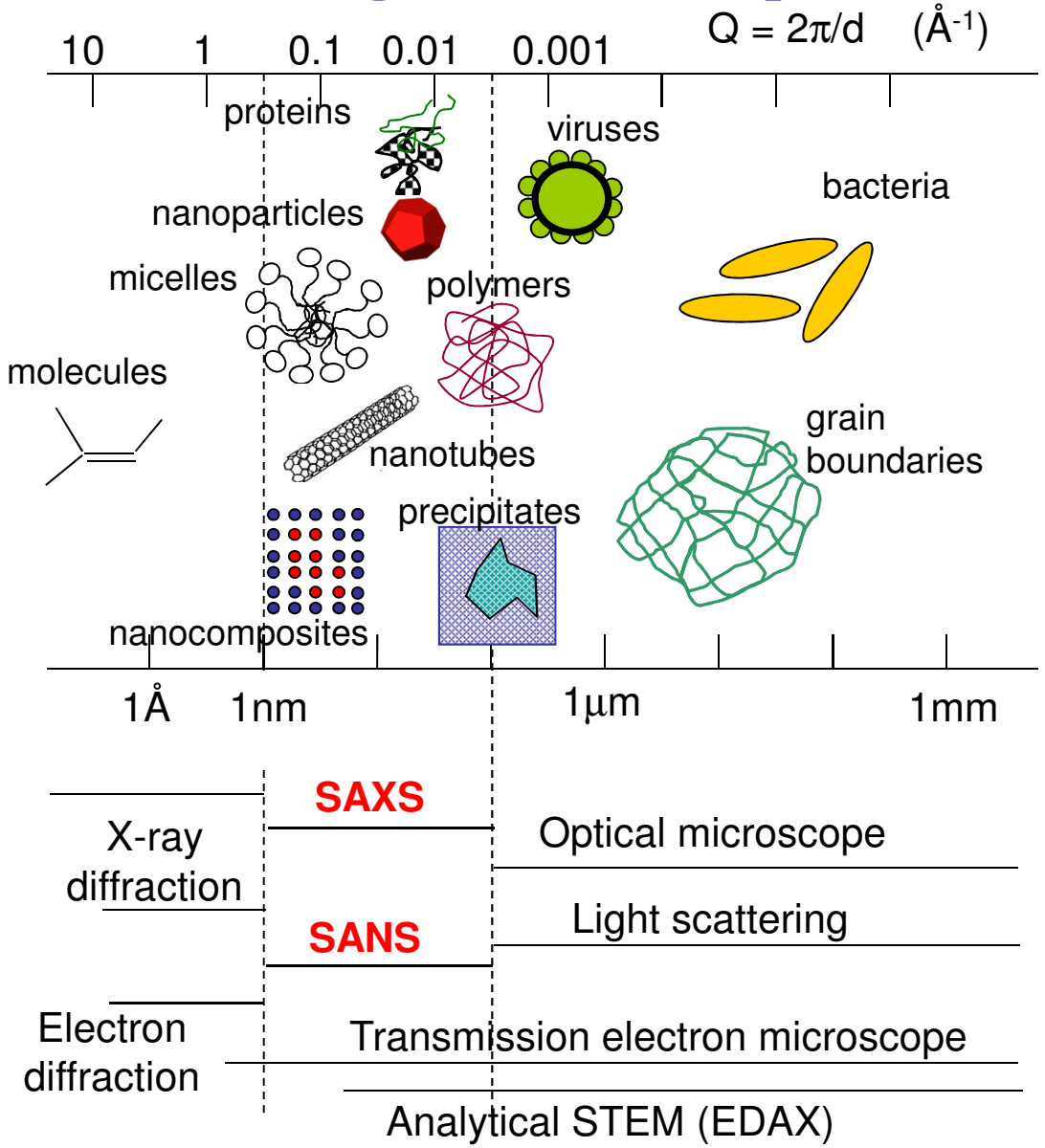
UNIVERSITY OF BATH

SAS Data Analysis – Colloids

Dr Karen Edler

Size Range Comparisons

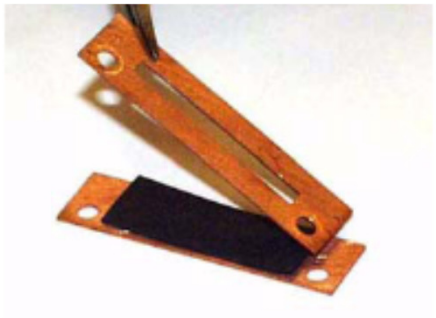


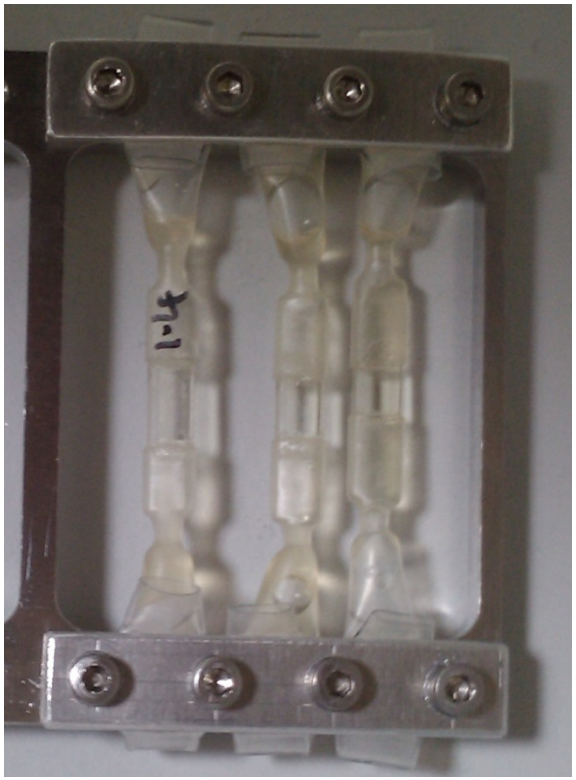
Sample Considerations

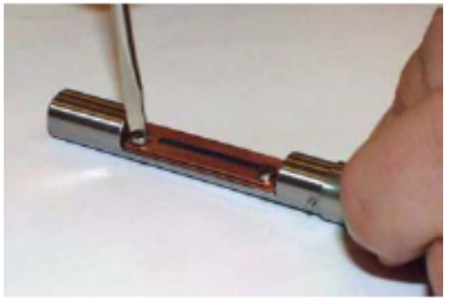
- Solid, liquid, (gas!)
  - air scatters X-rays, so sample often in vacuum
- Thickness
  - multiple scattering
- Concentration
  - structure factor effects
  - minimum concentrations
- Contrast

Sample Holders

- Variety depending on instrument & sample

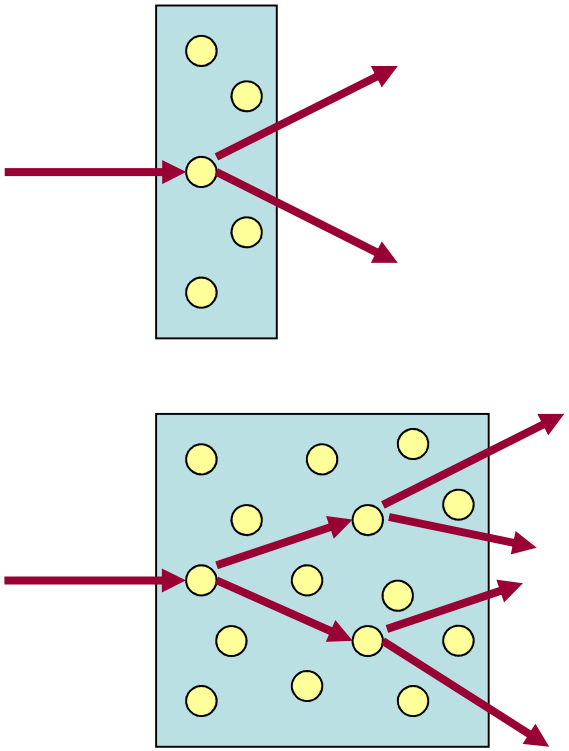


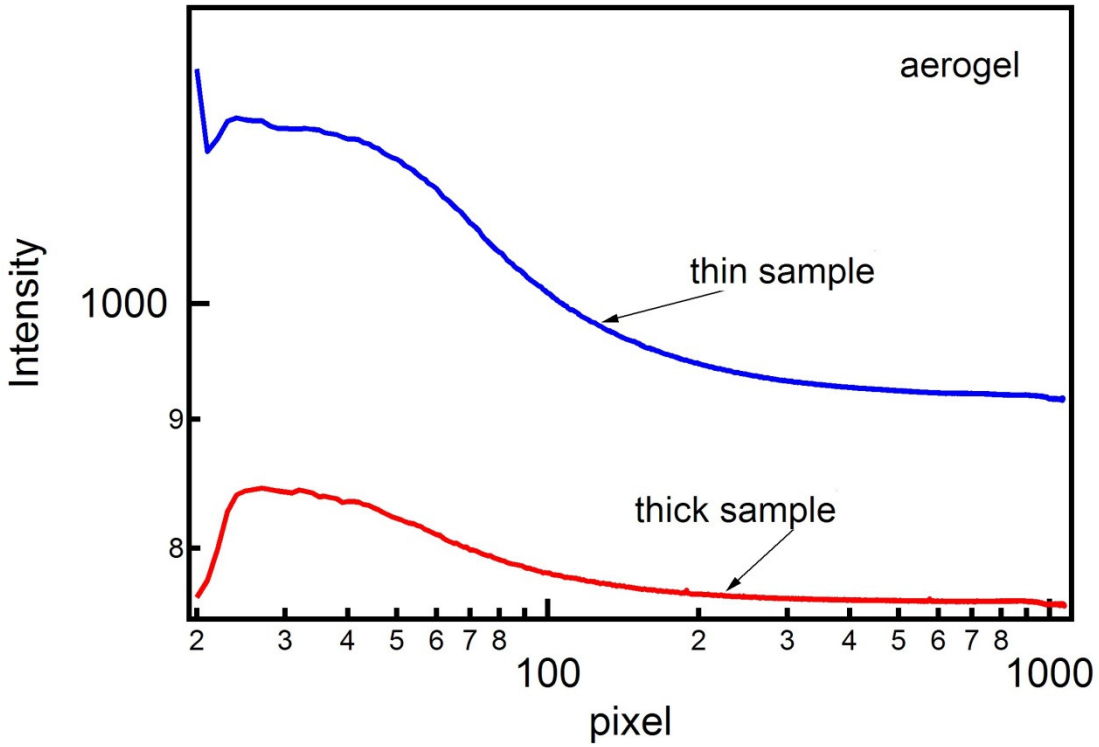




Sample thickness

- Affects transmission (total intensity)
- Also affects shape of curve ⇒ hard to analyse
- Aim for ~70% transmission





Concentration

$$I(Q) \propto N_p V_p$$

- Big particles scatter more (can hide small ones)
- Higher concentration = more signal

BUT

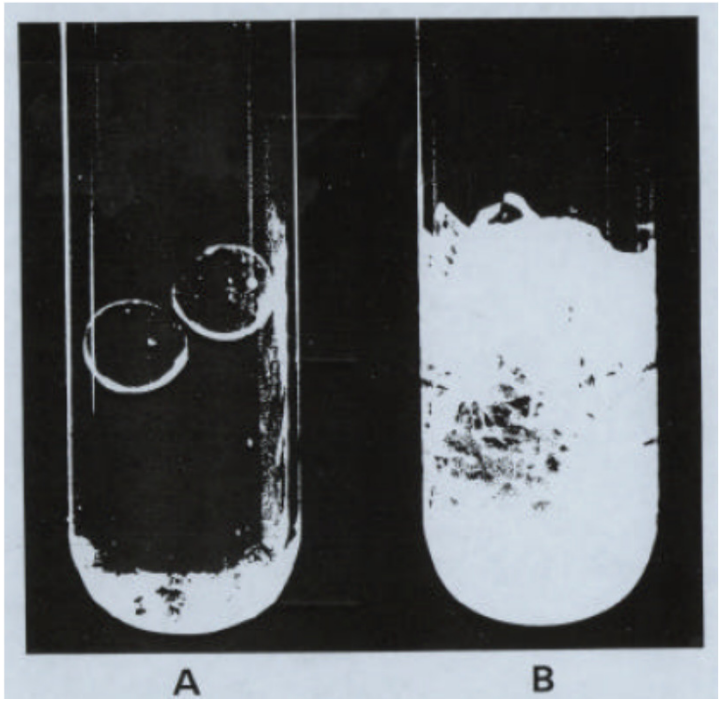
- **Consider detector limits!**
  - Don't burn out your detector…
- High concentration can complicate analysis
  - especially for charged particles (see later)
- Minimum concentration for lab source: ~10mg/ml
  - watch out for highly coloured solutions eg nanoparticles

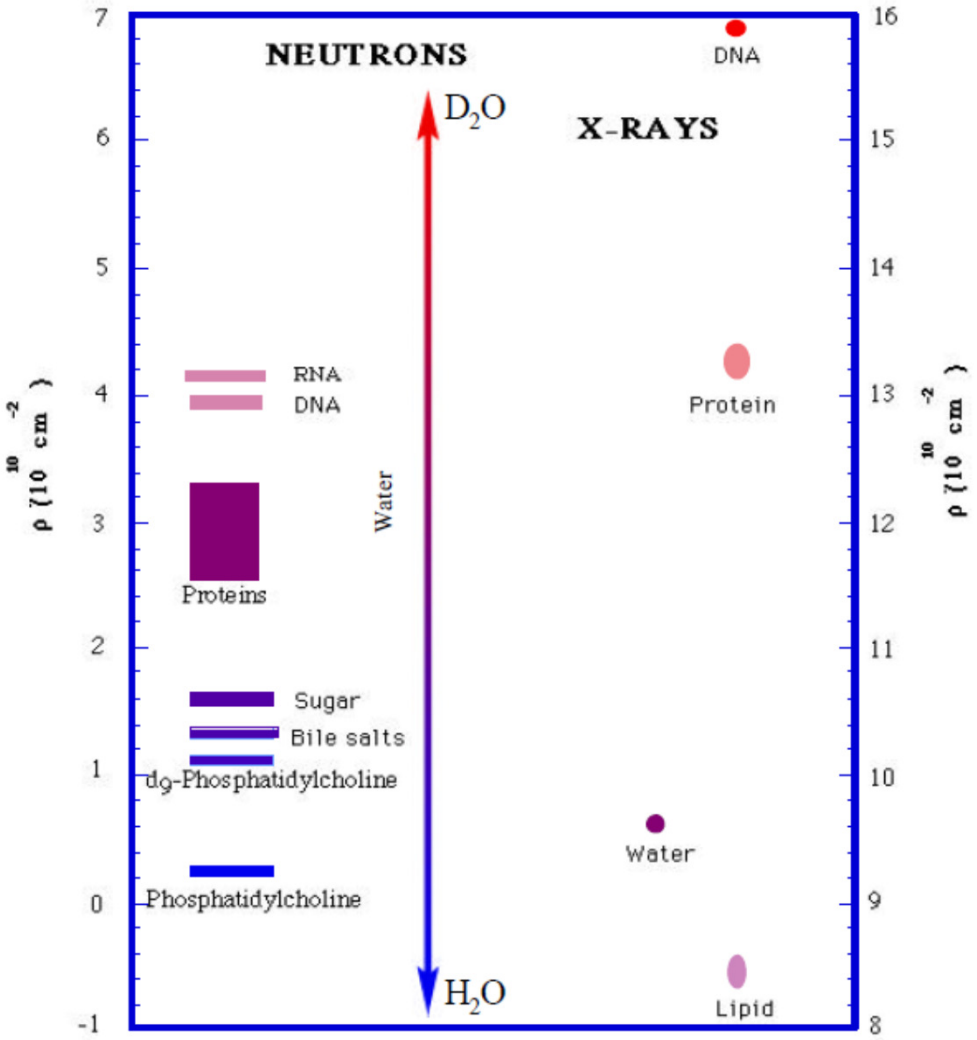
Contrast & Contrast Matching

- 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





* Chart courtesy of Rex Hjelm

Scattering Length Density

- scattering from an object depends on how many electrons there are in unit volume
- use *scattering length density, Nb,* to calculate scattering from molecules:

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

$$= N \sum_i b_i$$

where: b_i = neutrons: scattering length for element, cm

X-rays: b = 2.81×10^{-13} × 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}

Units of *Nb*: cm^{-2}

Will I see scattering?

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

- Scattering depends on <u>difference</u> in scattering length density between two regions
- but also the sample adsorption (also $\propto$ no. of e^-)

Polystyrene spheres $\rho_p = 9.5\times10^{10}$cm

Neutrons
$\rho_s = 1.4\times10^{10}$cm

In water (xrays)	In hexanol (xrays)	In chloroform (xrays)
$\rho_s = 9.41\times10^{10}$cm	$\rho_s = 7.36\times10^{10}$cm	$\rho_s = 1.25\times10^{11}$cm
✓	✓ ✓	✓
$\rho_s = -0.5\times10^{10}$cm	$\rho_s = -0.3\times10^{10}$cm	$\rho_s = 0.8\times10^{10}$cm

SAS Data Analysis

- Simple but not very accurate:
  - Porod slopes
  - Guinier analysis
  - (Zimm plots & Kratky plots – polymers, proteins)
- More helpful, but more complex:
  - fitting models to data
- Most complex (need more data):
  - fitting protein structures
  - monte carlo/simulated annealing methods

Scattered Intensity

- From scattering theory:

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

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

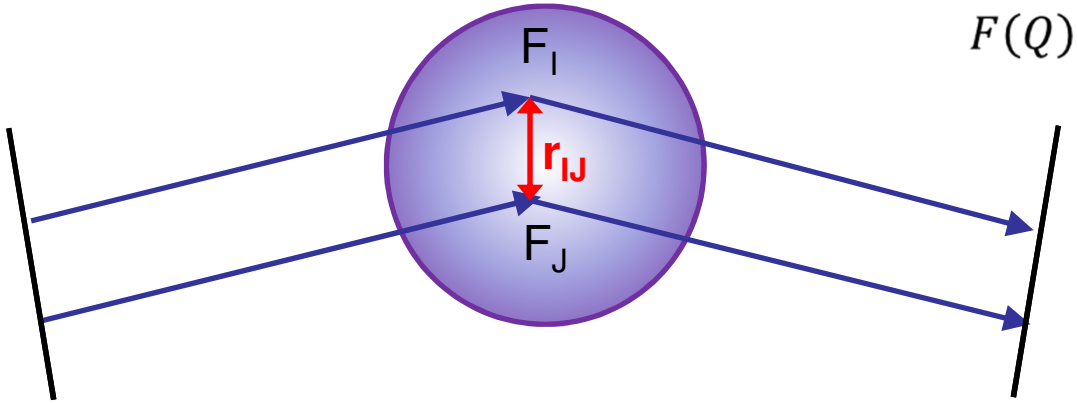
Form Factor = scattering from within same particle
$\Rightarrow$ depends on particle shape

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

Form Factors

- For particular particle shapes can calculate correlation functions
- Need to calculate Fourier transform of the distribution of scattering length density in real space:
  - interference from X-rays scattered from different parts of the same particle
  - Angular part of the scattering gives information on particle shape, size
  - Sum scattering from all scattering centres in particle

    $\Rightarrow$ "form factor"



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

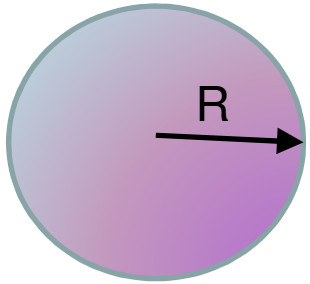
$$= \left| \int_V \rho(\boldsymbol{r})\, e^{i\boldsymbol{q}.\boldsymbol{r}} d\boldsymbol{r} \right|^2$$

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:



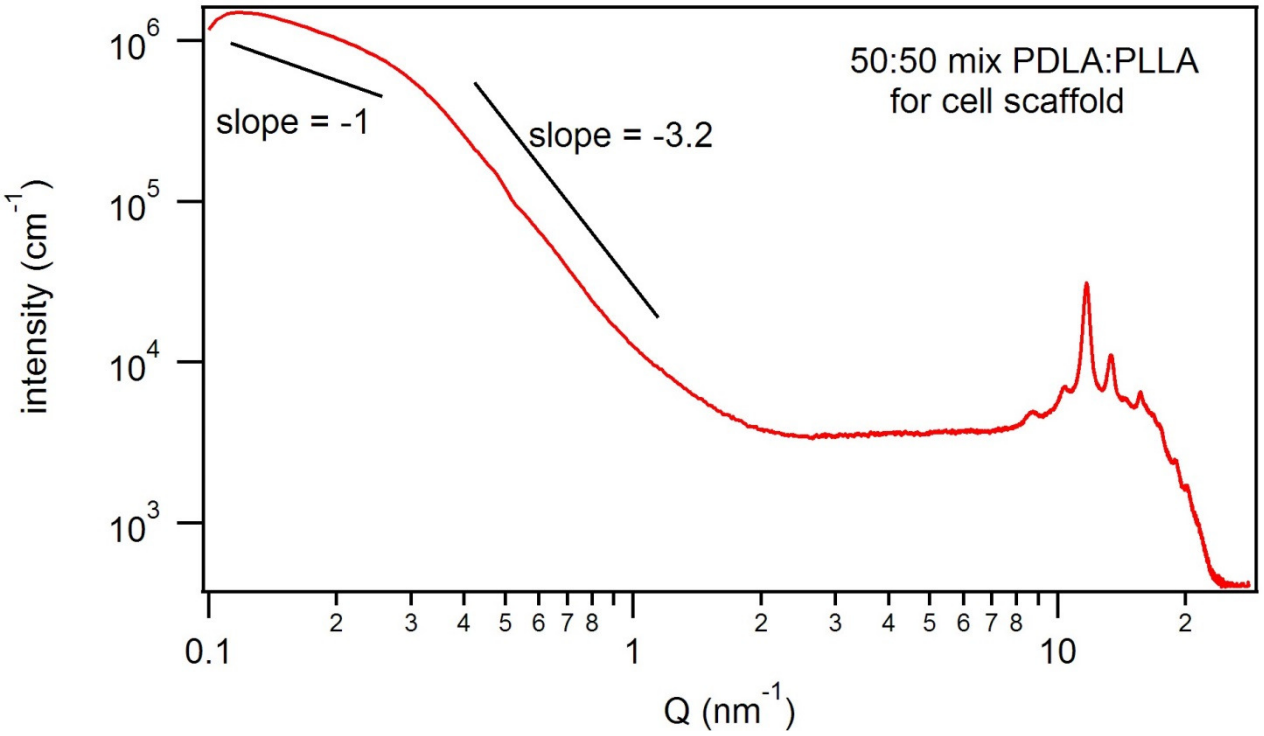
$$\gamma(r) = 1 - \frac{3}{4}\left(\frac{r}{R}\right) + \frac{1}{16}\left(\frac{r}{R}\right)^3$$

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

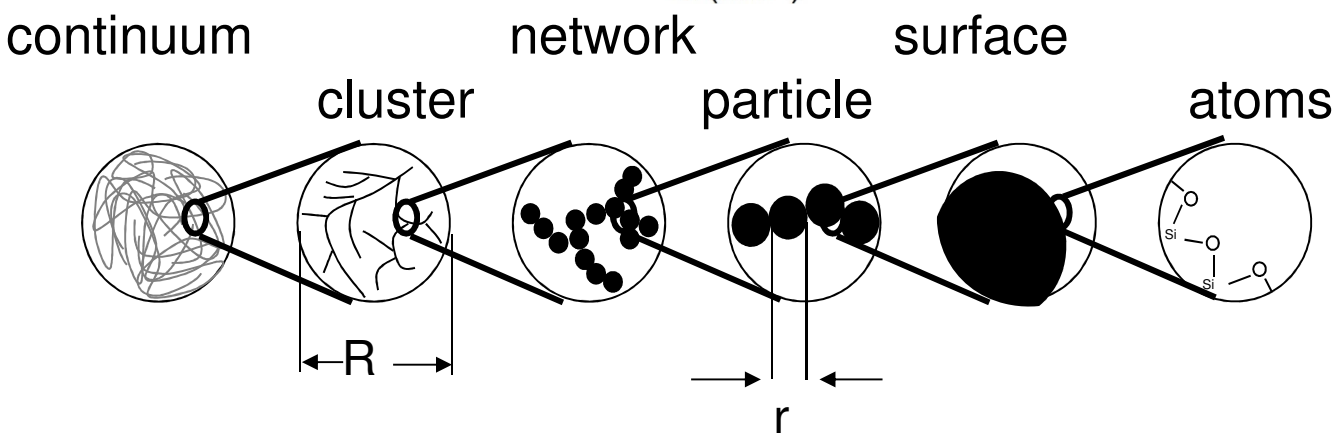
- Integrate by parts three times: $F(qR) \approx \frac{3}{2R^3}\frac{S_p}{V_p}\frac{1}{q^4}$

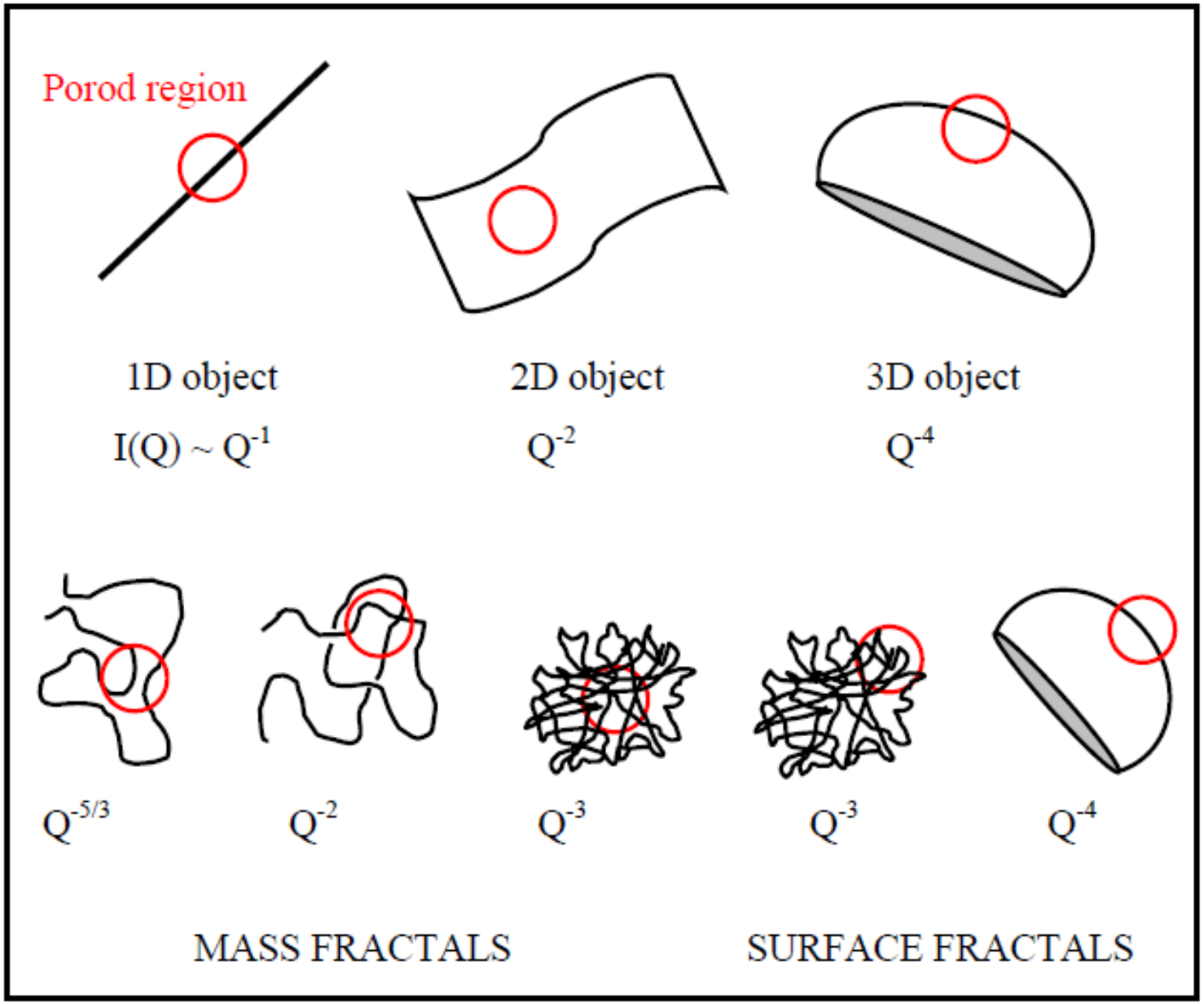
At high scattering angles, for any system with sharp, smooth surfaces: $I(Q) \propto \frac{1}{q^4}$

What can SAS measure?



eg





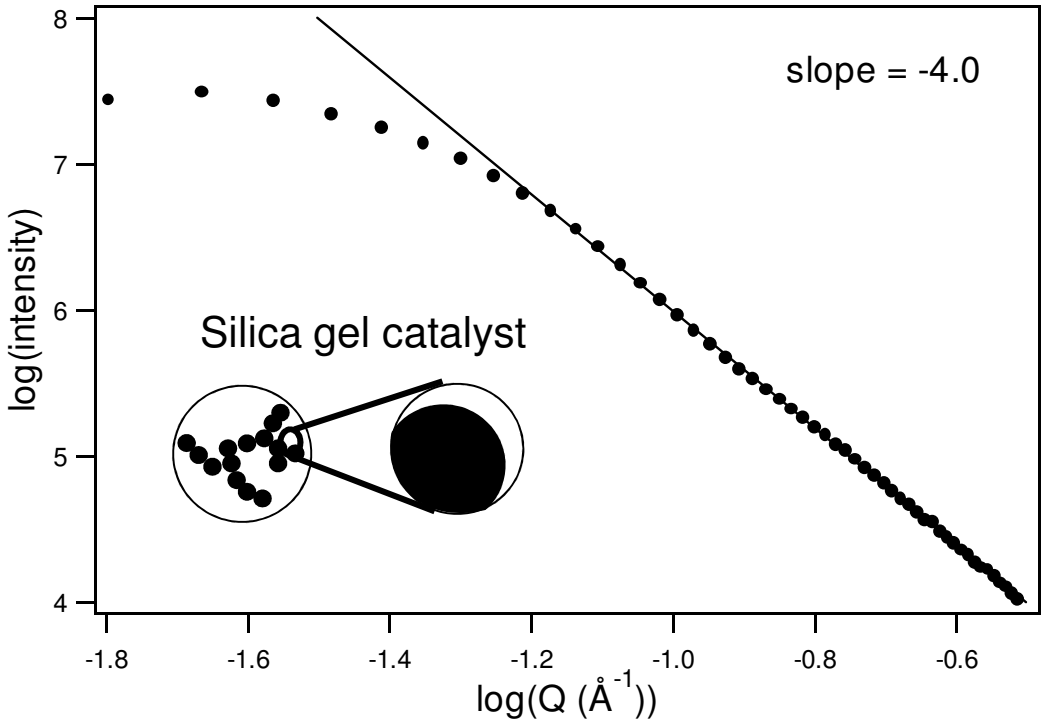
The SANS Toolbox. Boualem Hammouda, NIST

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

Porod Slope

- plot data as $\log_{10}$(intensity) against $\log_{10}$ (Q)
- slope = -D (mass fractal) or slope = $-D_s-6$

  $\Rightarrow$ fractal dimension of particle or particle surface
  - Keep in mind size range you are using!



Analysing Scattered Intensity

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

$$I(Q)=N_p V_p^2 (\rho_p - \rho_s)^2 F(Q)S(Q)+B_{inc}$$

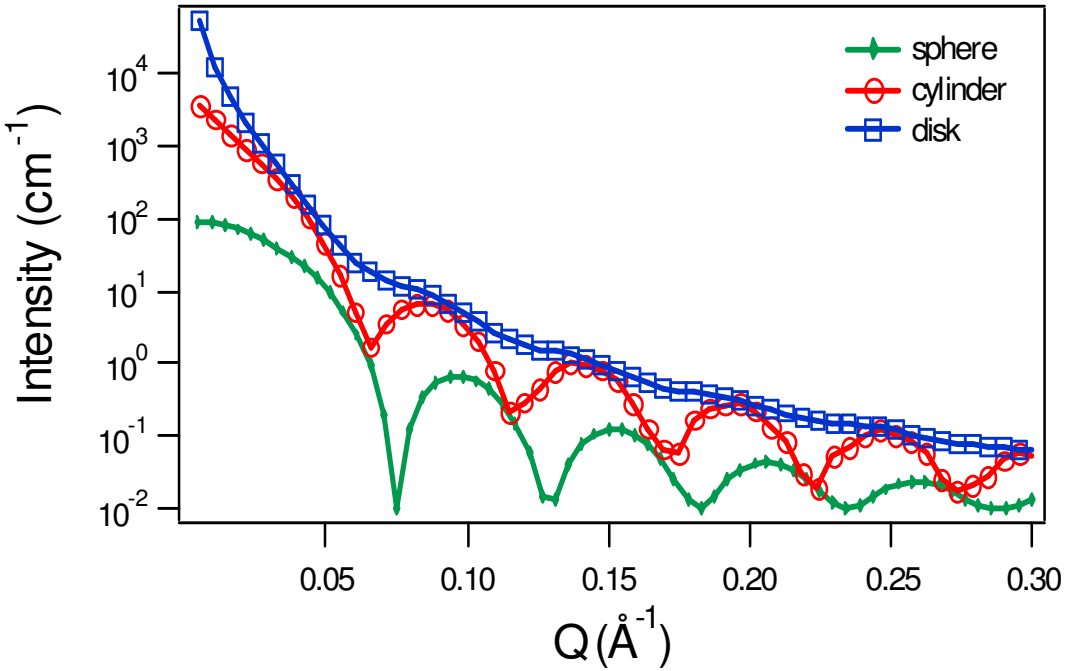
Where: Np = number of particles
Vp = volume of particle
ρ = scattering length density (of particle/solvent)
Binc = incoherent background
F(Q) = form factor
S(Q) = structure factor

Form Factor = scattering from within same particle
⇒ depends on particle shape

Structure Factor = scattering from different particles
⇒ depends on interactions between particles

Form Factors

- depend on shape of particle
- for dilute solutions S(Q) = 1 and so I(Q) ∝ F(Q)



General form of F(Q):

$$F(Q)=\frac{1}{V_p^2}\left|\int_0^{V_p}\exp[if(Q\alpha)]dV_p\right|$$

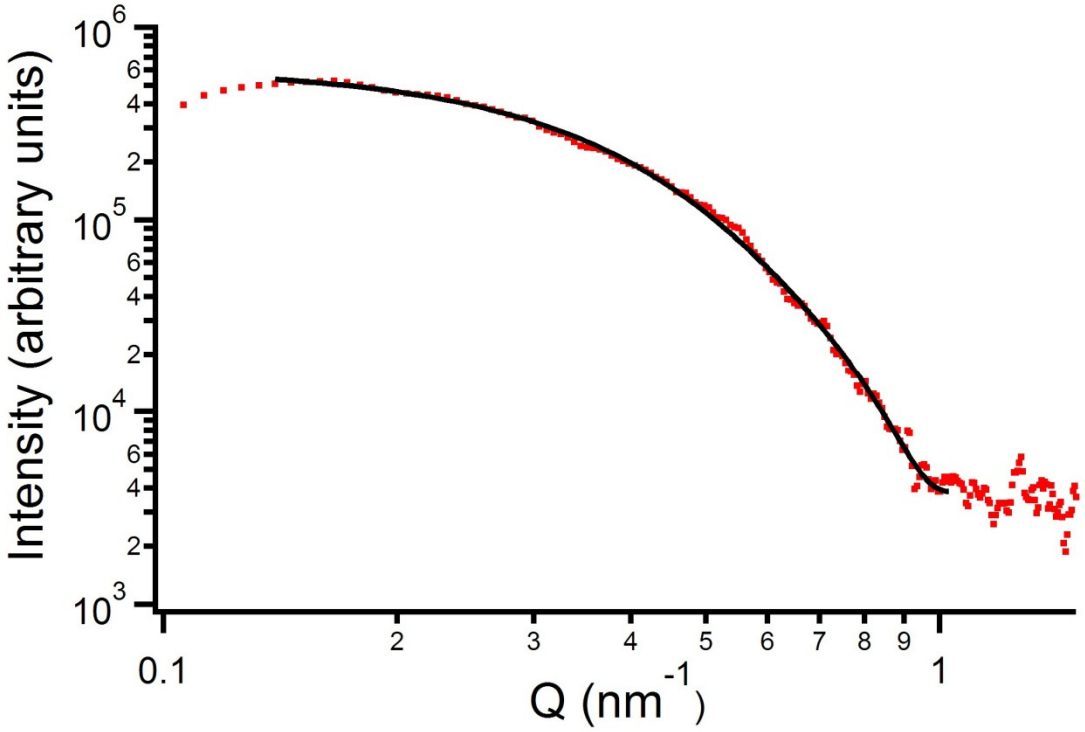
where
α = shape parameter
eg radius of gyration

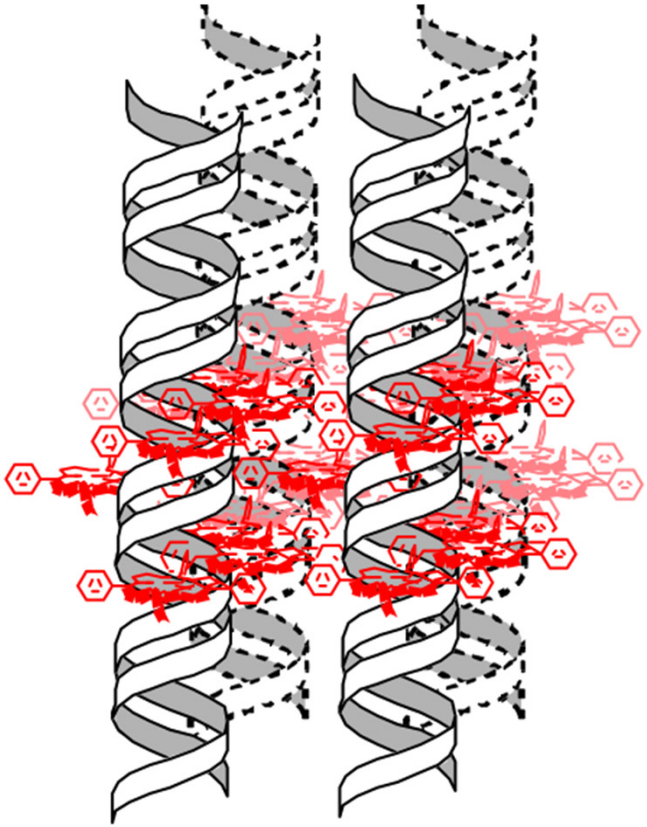
can work out F(Q) exactly for some shapes
eg sphere, radius R_p:

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

DNA Complexes in Solution

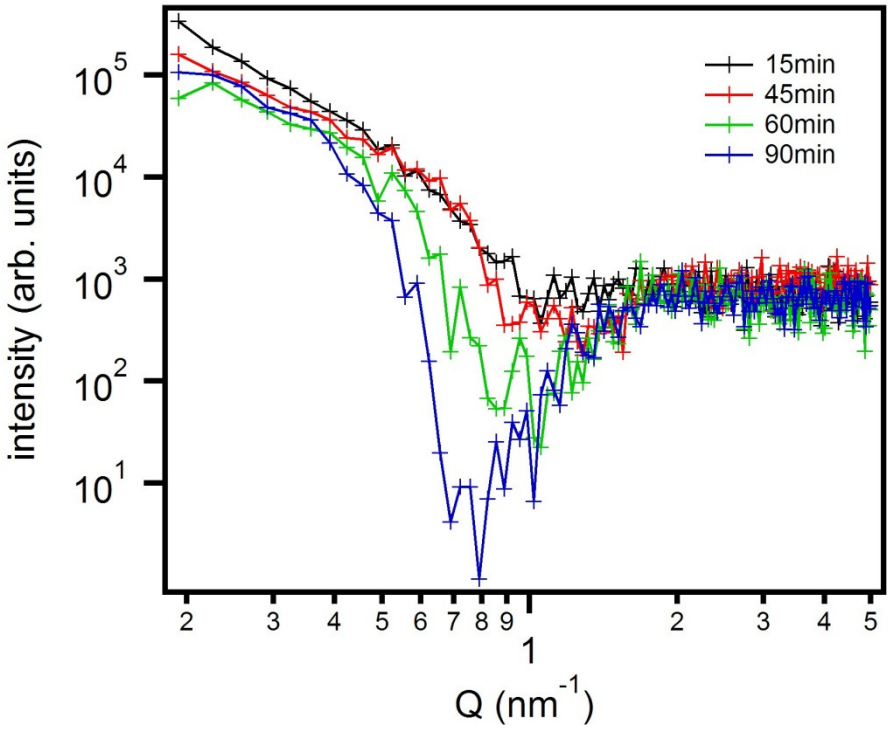
- Prepared by Dr Eugen Stulz (Southampton) & Dr Cameron Neylon (ISIS)
- Porphyrin complexes intercalated in DNA
- 12 hr exposure, 50μM solution

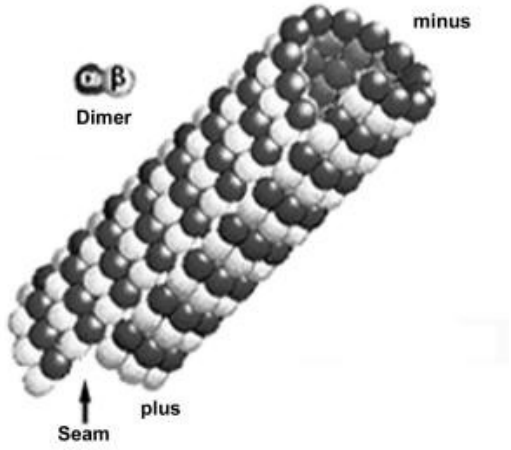




Tubulin

- With Niels Galjart, Erasmus MC, Rotterdam
- 5mg/ml solution in buffer BRB80, 15min exposures



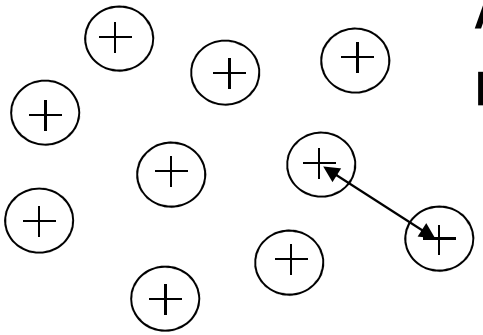


Initial scattering fits to cylinder, radius ~6nm, length ~30nm

Not yet able to model later scattering!

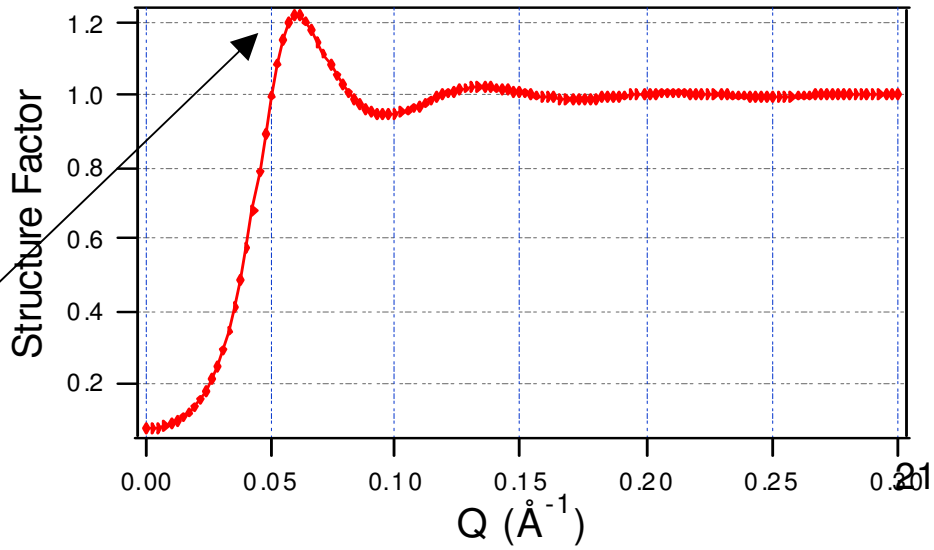
Structure Factors

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



Average distance between nearest neighbours relatively constant
= “correlation distance”

Position of first maximum related to correlation distance



Concentration effects

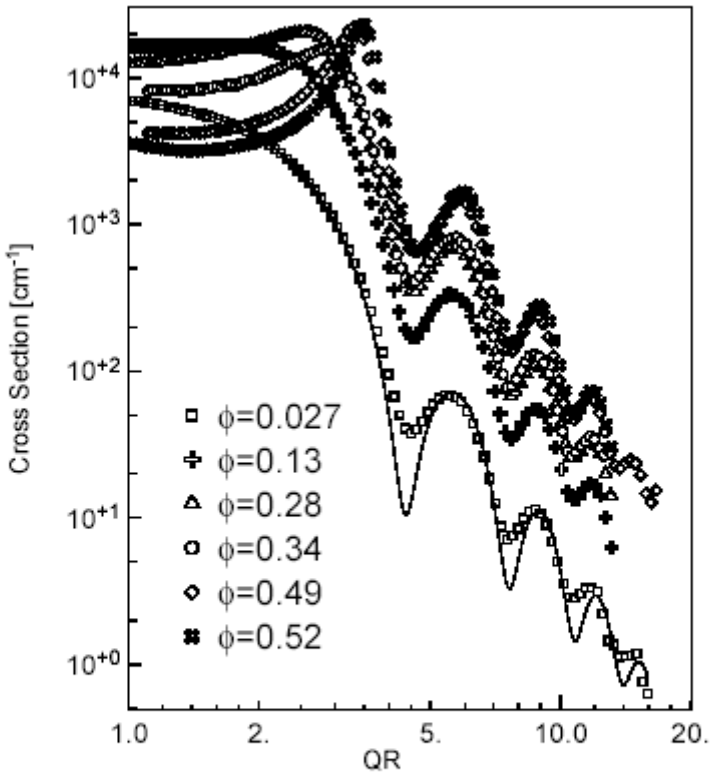


Figure 1: Cross-section for several different volume fractions of PS spheres in glycerol vs. *QR*.

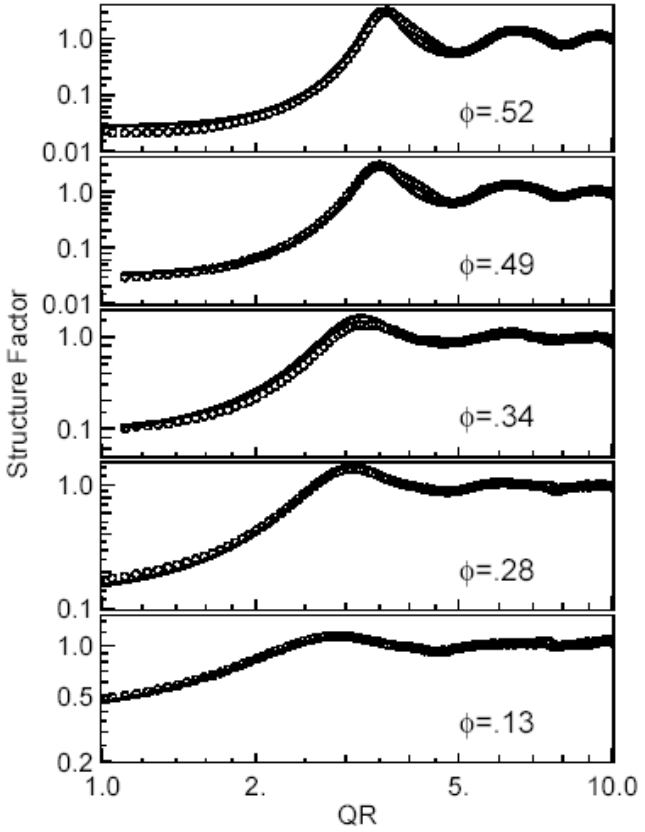


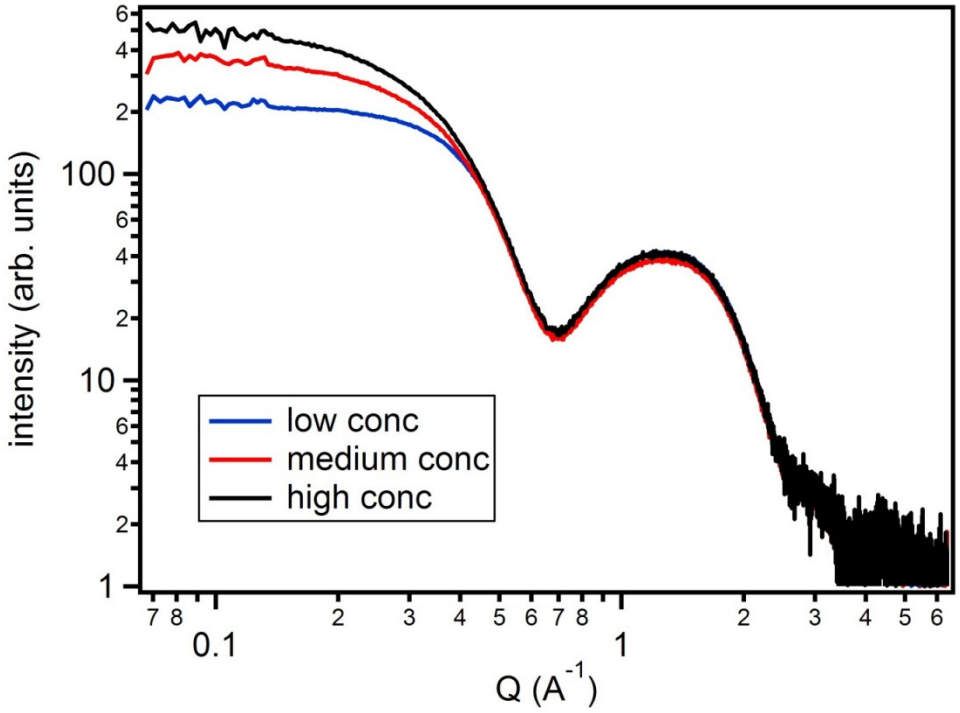
Figure 2: Measured and model structure factors, $S(Q)$, (circles and dashed lines, respectively) vs. *QR* for PS spheres in glycerol.

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

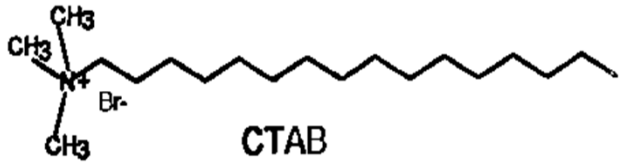


- Normalised for concentration

SAS Data Analysis - Fitting

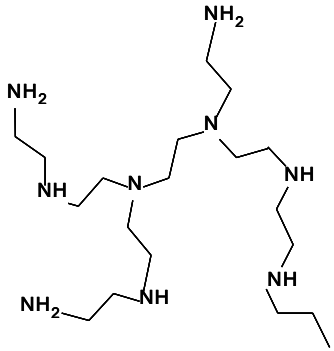
SAXS on PEI/CTAB Solutions

cetyltrimethylammonium bromide

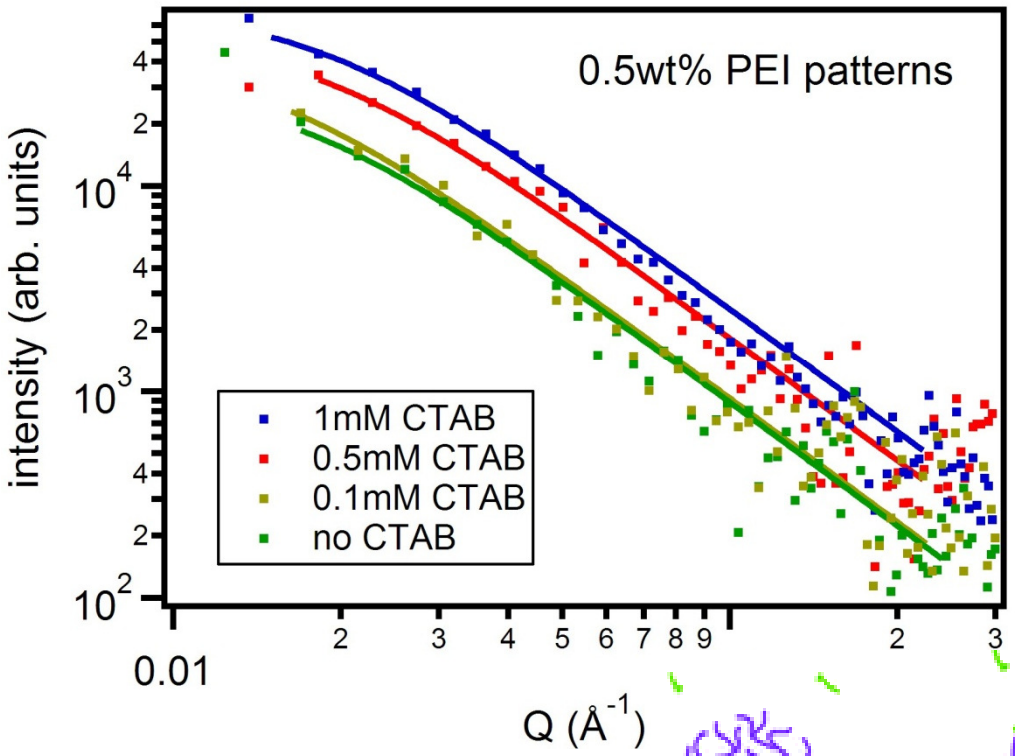


CTAB

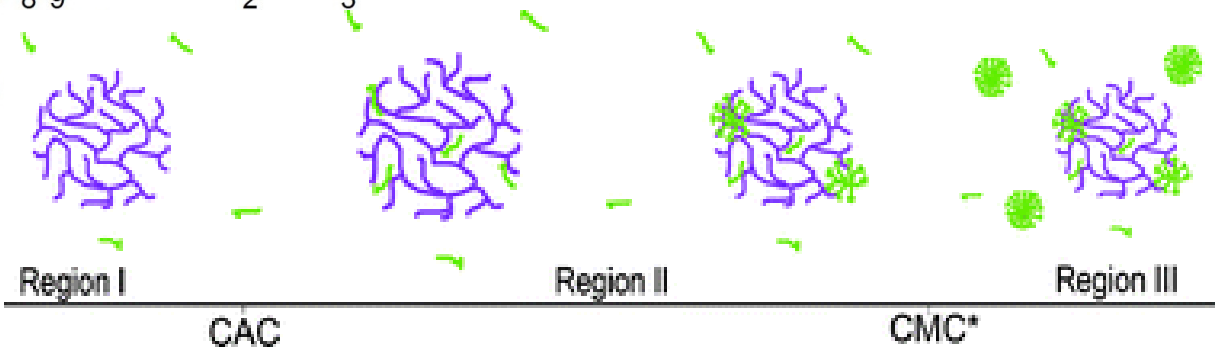
polyethylenimine (PEI)



MW 750000
branched



- Fitted to function for a gaussian coil polymer chain in dilute solution
- Polymer radius of gyration swells as surfactants bind
- Then shrinks as micelles form



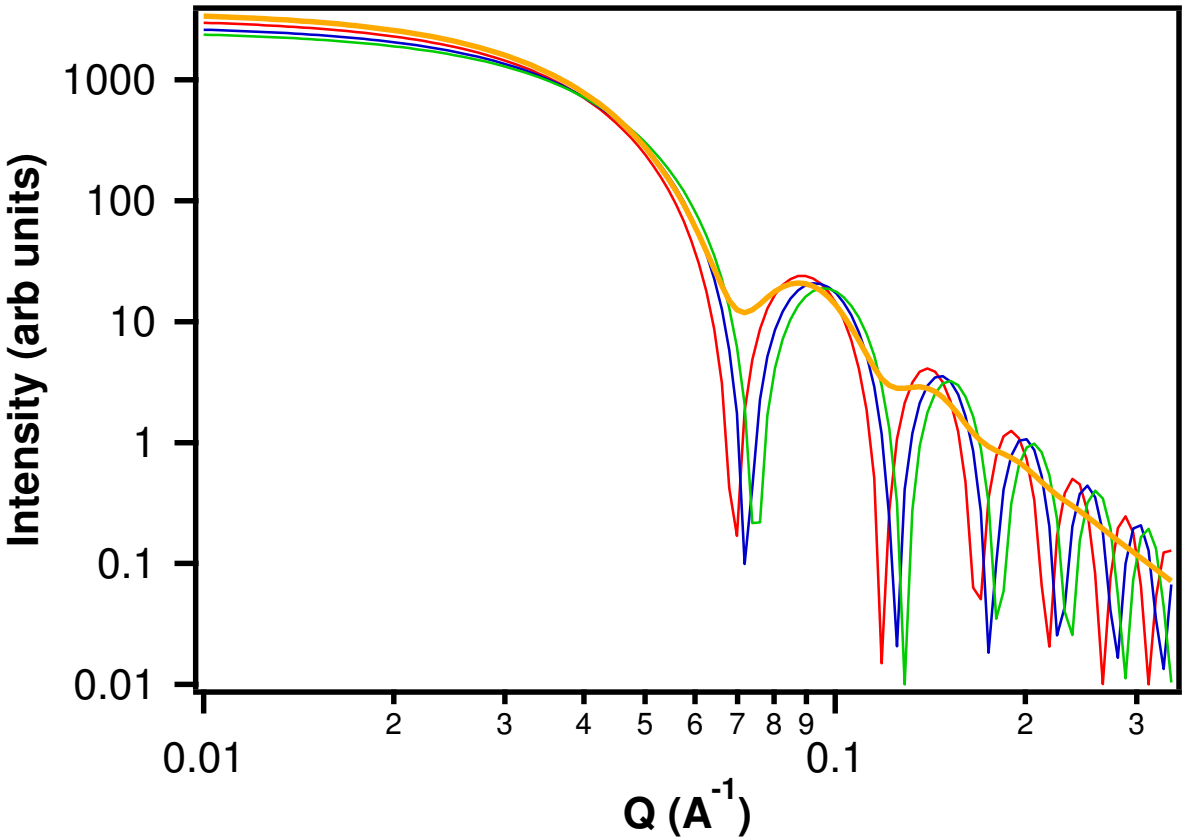
Soft Matter, **2**, 747 (2007)

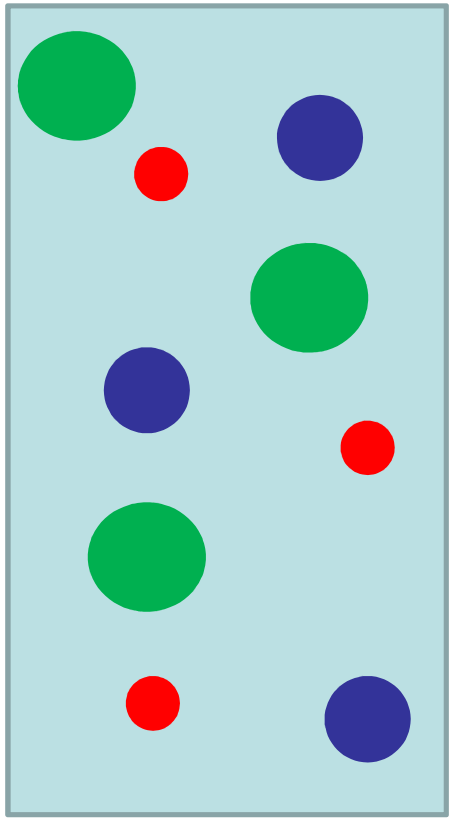
Instrumental Smearing Effects

- Calculations of models assumes point radiation source
- In reality beam might be 1x1mm or even 1x10mm (lab source)
- Need to account for beam shape/size
- Can either “desmear” data
  - Issues with removing some of the information from your sample scattering
  - Problematic for rod-like scatterers
- Or smear the model
  - Slows down fitting

Polydispersity

- “smears out” sharp features in pattern

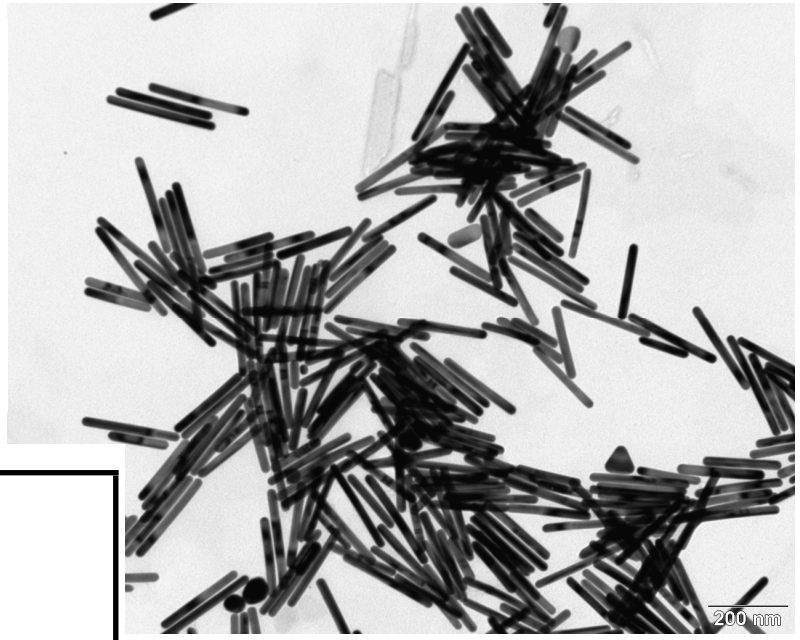


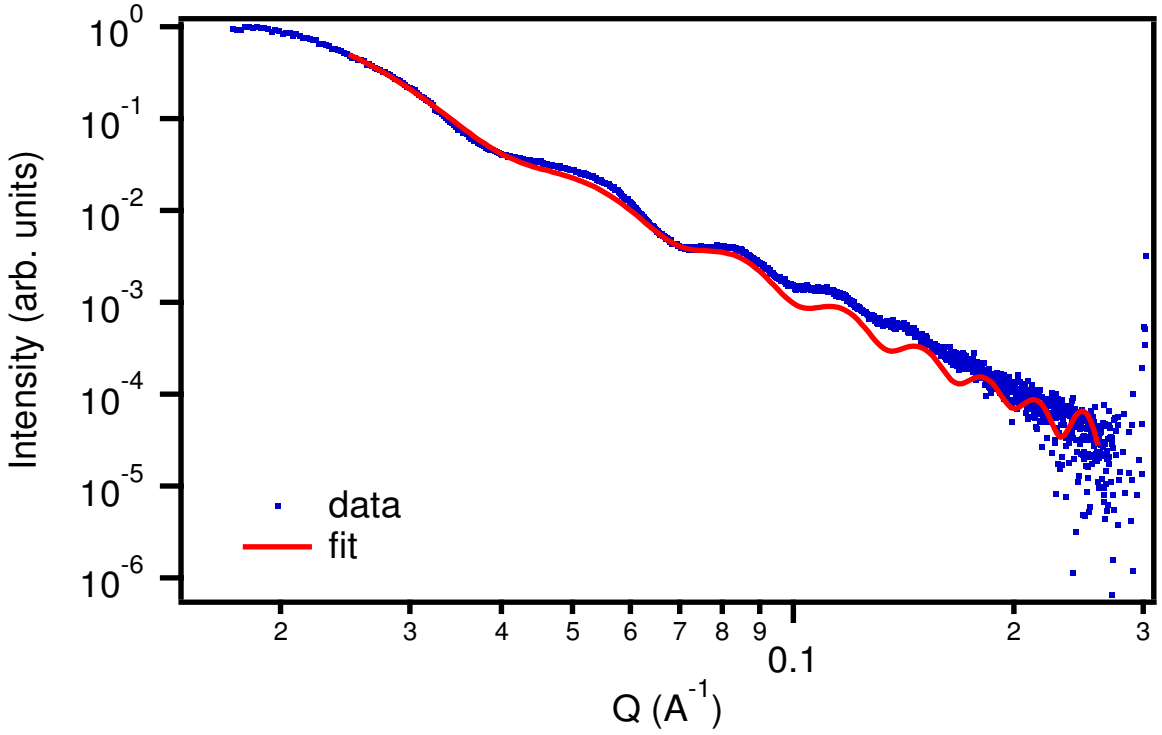


Au Nanorods

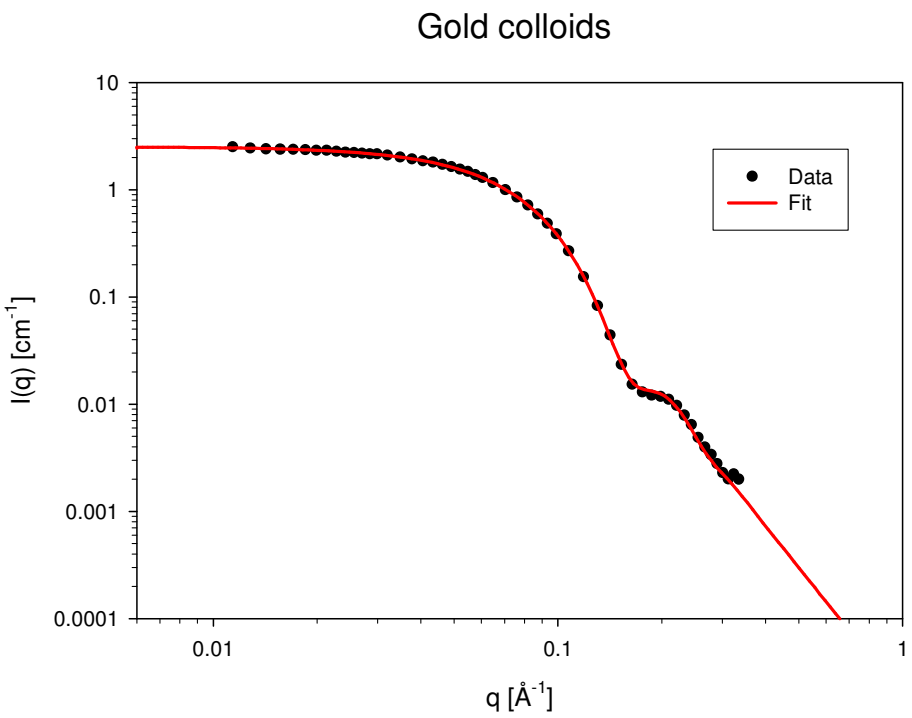
Fitted to charged cylinders

- Radius 80Å
- Length 190Å
- Polydispersity 0.29

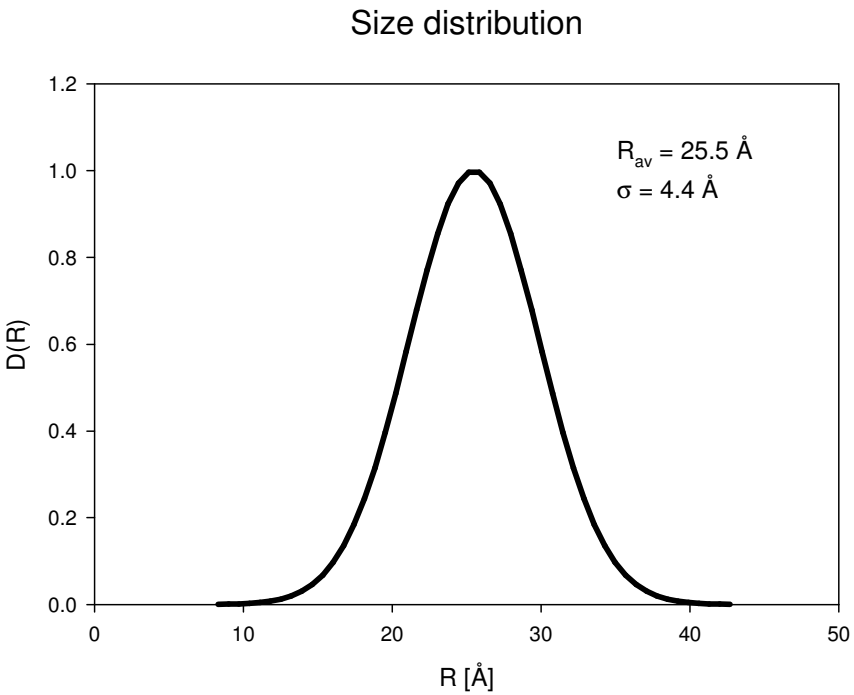


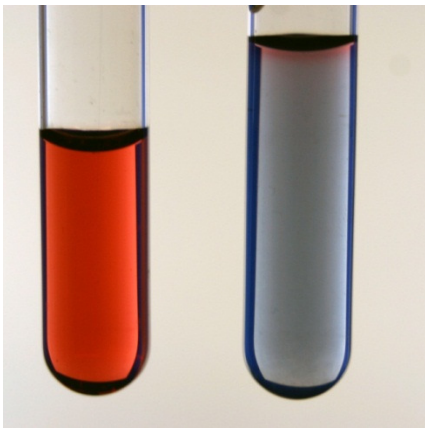


Gold Colloid



The spherical gold colloidal particles coated with thiols can be dissolved in an organic solvent like toluene



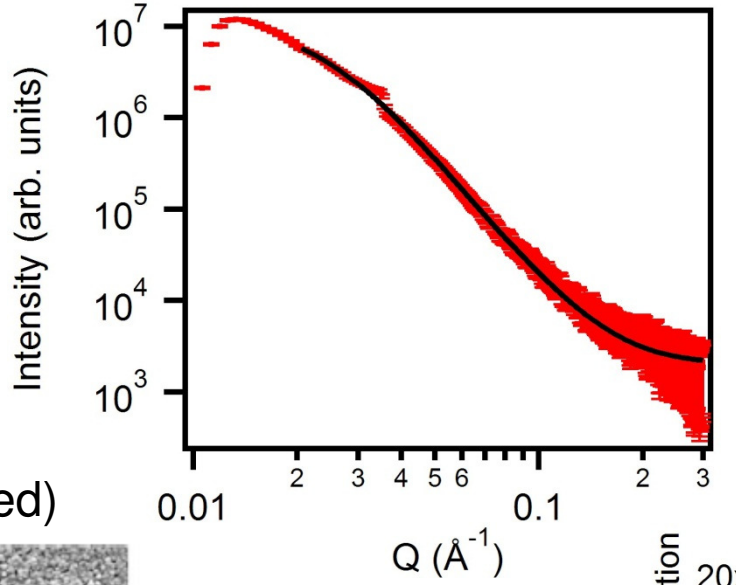


Dr Nick Terrill, Diamond, Small Angle Scattering

Carbon Nanoparticles

- Group of Dr Frank Marken

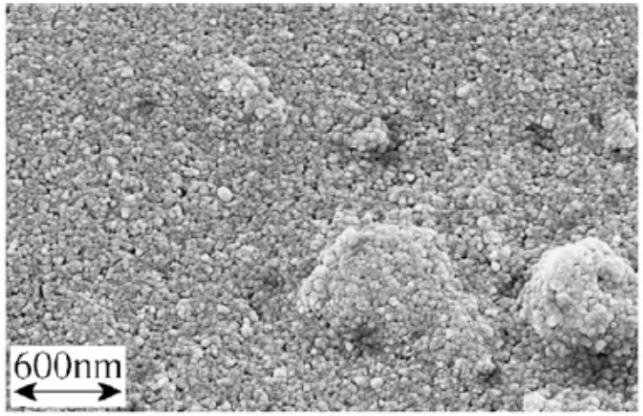
Carbon nanoparticles
6wt% in water,
20 min exposure

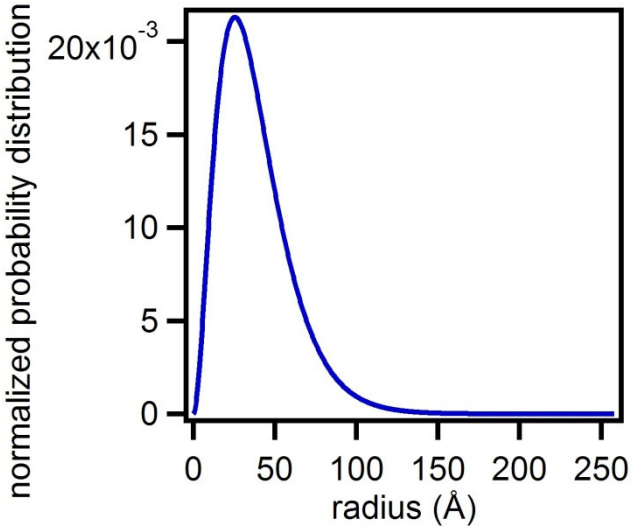


Fitted to model of sphere with Schulz polydispersity in the radius.

Radius = 38±2Å

SEM image (Au coated)





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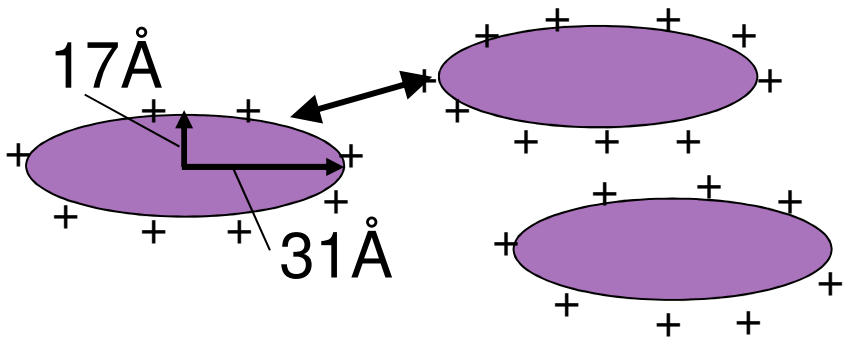
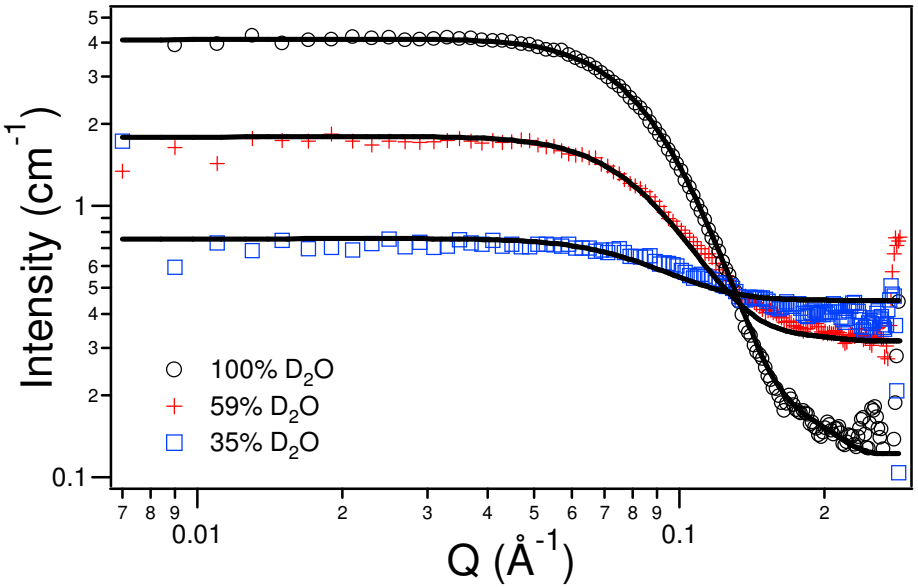
Fitting SANS Data

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

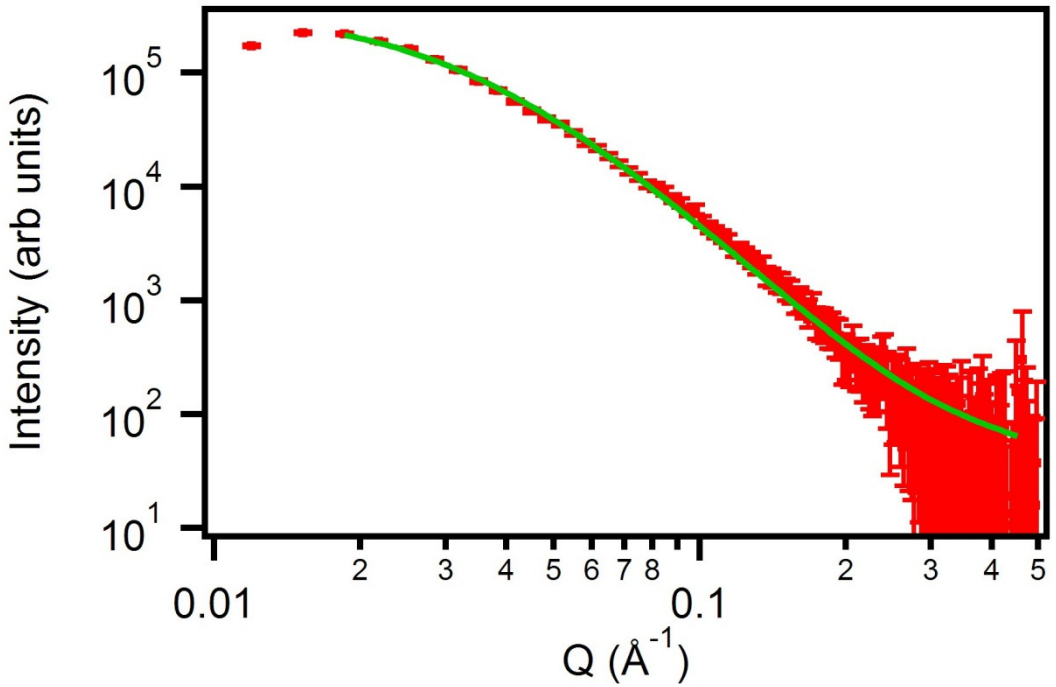




- Fit using ellipse + structure factor for charged objects which repel each other
- Use three contrasts to help pin down shape and size accurately

Silica Aerogels

- Gels made from SiO_2 in acidic water, supercritically dried.
- Very strong scatterers!
- With Mike Grogan, Physics (Uni. of Bath)
- Applications in fibre optics



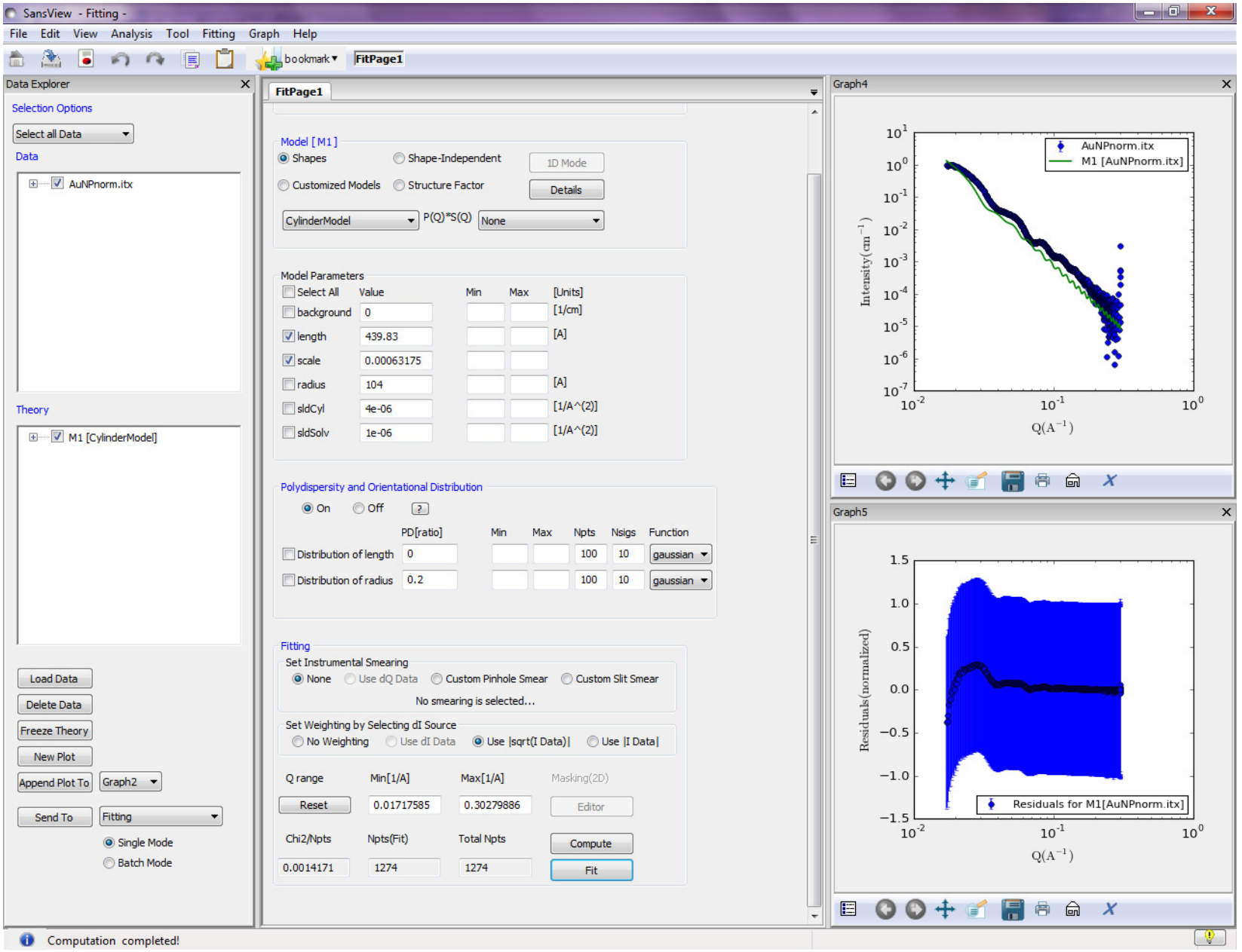
Model by Teixeira assumes fractal aggregate of spherical building blocks:

Block radius: 3Å
Fractal Dimension: 2.96
Correlation length: 29Å

DANSE SANSView software

- Designed for fitting neutron data but can also be used (with care) for X-ray data
- Includes reflectivity analysis
- Available from: http://danse.chem.utk.edu/sansview.html

Fitting Software – SANSView



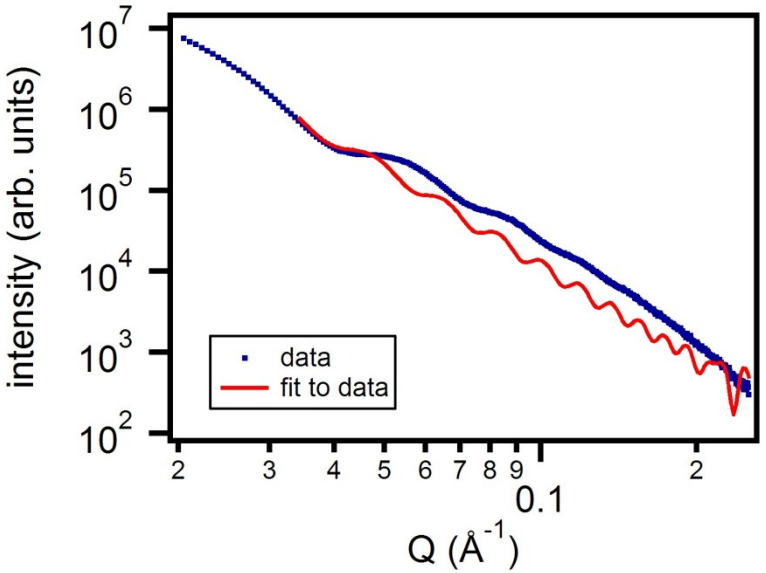
Other Free SAS Software

- Library of available software at:

  http://www.small-angle.ac.uk/small-angle/Software.html

Fitting Tips

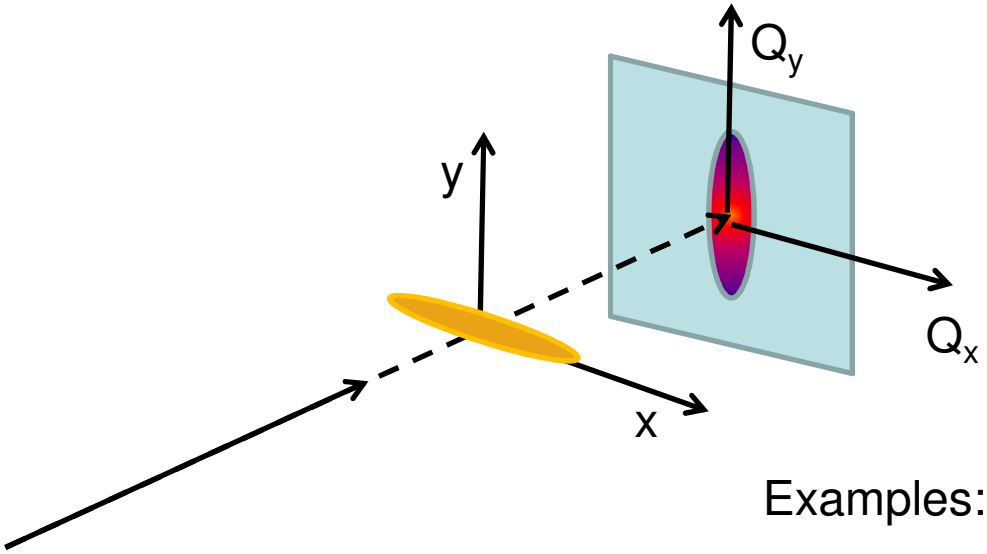
- Models have lots of variables
  - set as many as possible to known values!
- Initially set reasonable values for unknowns
- Fit only 2 variables at a time until are close to good fit
- Check χ^2 – should get smaller as fit improves
- Don't trust significant figures
  - look at how fit changes as you alter values to get errors
- USE COMMON SENSE!



volume fraction	0.00513365
radius (A)	84.3844
length (A)	222.573
SLD cylinder (A^{-2})	0.000123
SLD solvent (A^{-2})	9.39e-06
charge	20
movalent salt (M)	0.0182752
Temperature (K)	298
dielectric const	78
incoh. bkg (cm^{-1})	3

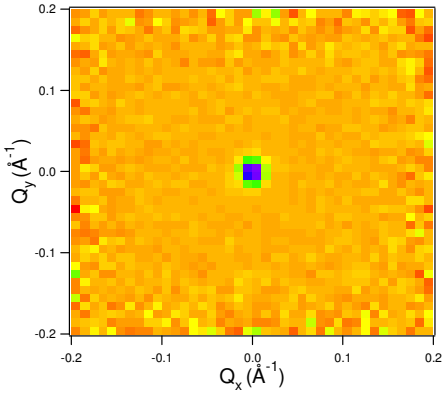
Effects of Sample Alignment

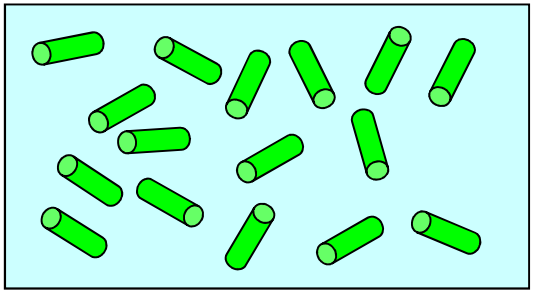
- Scattering no longer circular
- Form areas of high intensity perpendicular to direction of alignment



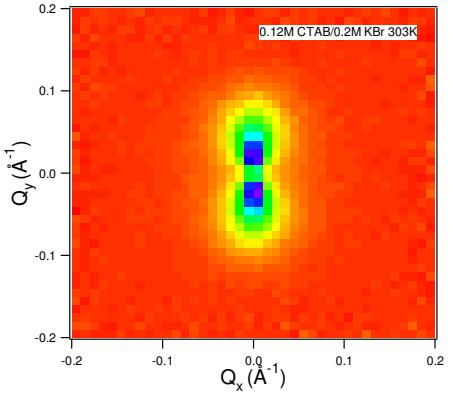
Examples: shear, flow
magnetic alignment

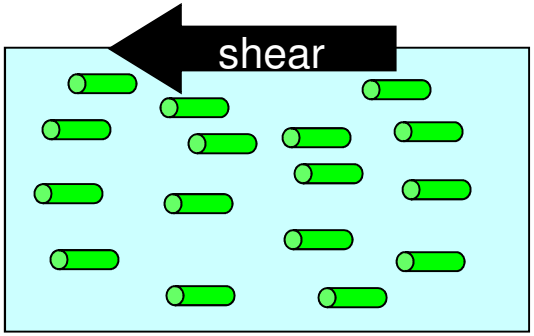
Isotropic vs Nonisotropic Structures



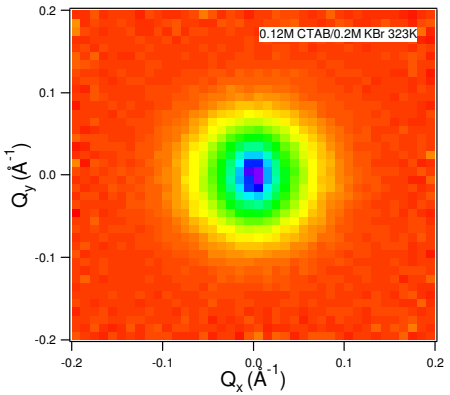


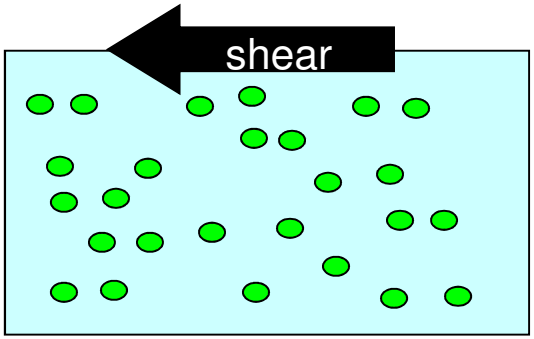
No shear
⇒Isotropic solution





Shear
⇒ aligned micelles





Shear + higher T
⇒ isotropic again