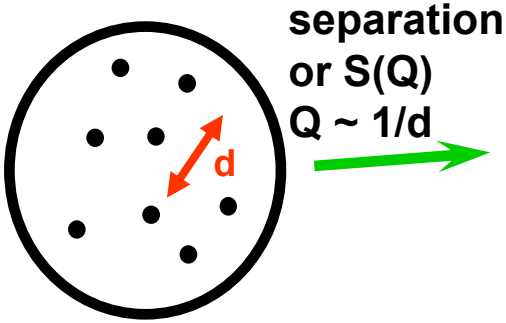
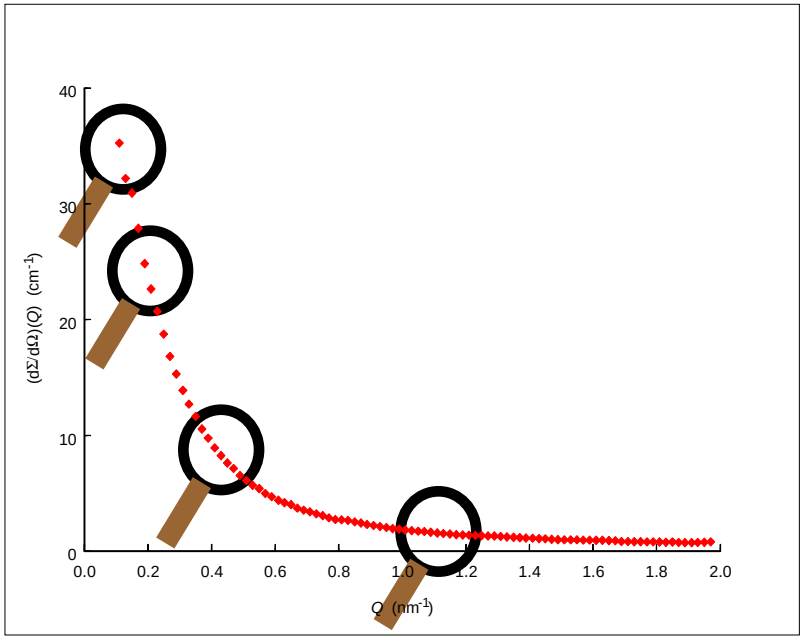
Small Angle Neutron Scattering Part 4
Polymers and Biological systems

(R.K.Heenan, with thanks to Steve King, ISIS, for use of some of his slides)

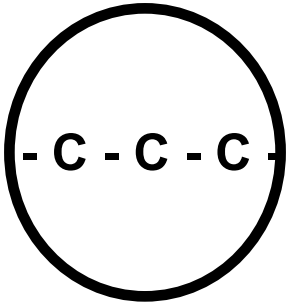
SANS – from a polymer solution - in a "neutron microscope"

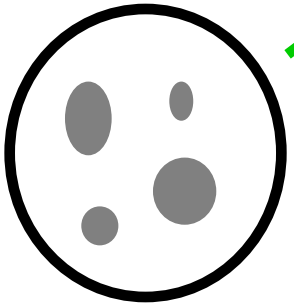


Phase separation or S(Q) Q ~ 1/d

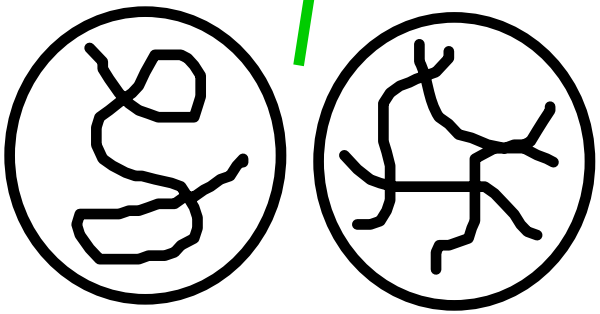


bond lengths need wide-angle diffraction

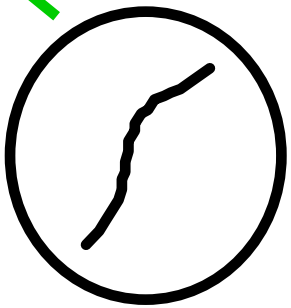




Q ~ 1 / R_G



Q ~ 1 / ζ



Q ~ 1 / l_p =1/persistence length

SANS from polymers

We have already seen the "Debye Gaussian coil" equation. This is strictly only correct for dilute solutions in a "theta solvent" where chain-chain and solvent-chain interactions are equal. (Also works at any concentration in a bulk or melt homo-polymer, as the solvent is the same polymer!)

$$P_1(Q) = 2(e^{-x} + x - 1)/x^2 \qquad \text{Where } x = Q^2R_G^2$$

The Kratky Plot

At high Q values, say $Q > 5R_g^{-1}$ the Debye Form Factor simplifies to:

$$\frac{\partial \Sigma}{\partial \Omega}(Q) \approx \frac{2\,N\,V^2\,(\Delta\rho)^2}{(QR_g)^2}$$

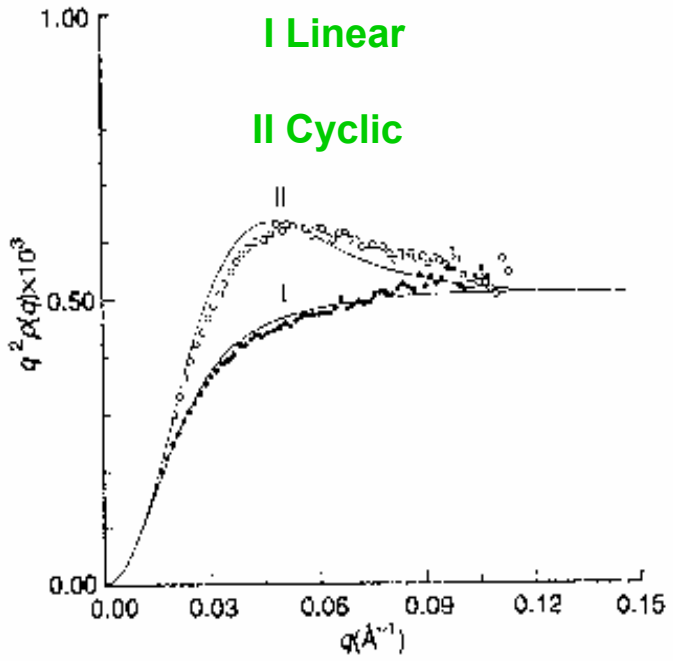
A "Kratky Plot" of $Q^2I(Q)$ against Q, for a *linear* polymer should approach a flat plateau value with increasing Q [assuming any incoherent background has been subtracted!]

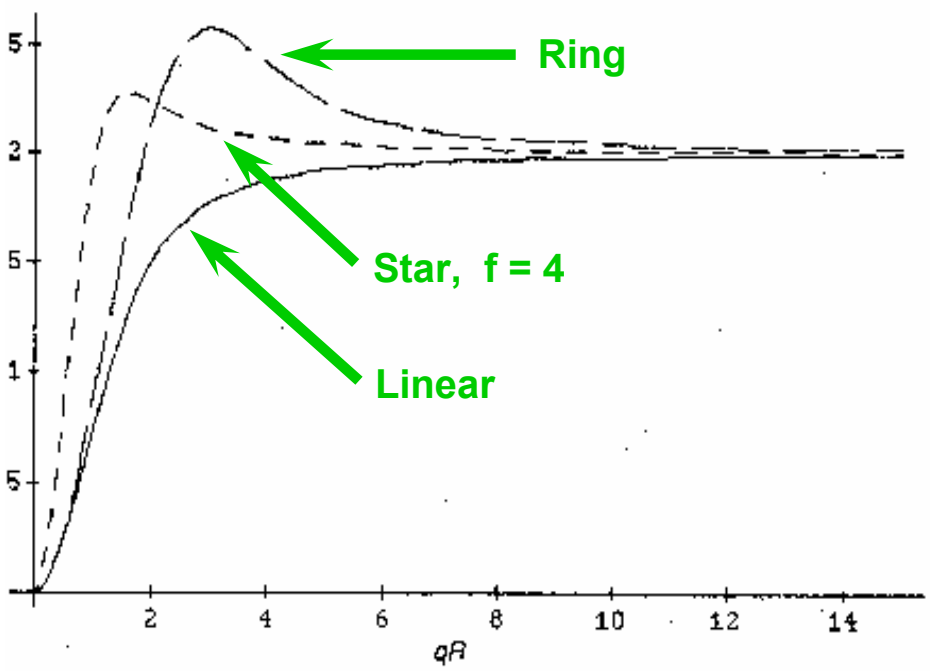
Deviations from this may indicate different (short range) ordering of the polymer segments and may be used to *fingerprint* different types of polymer architecture, such as rings, stars or branched chains.

Further equations are available to directly fit rings or star branched chains.

Kratky Plot $Q^2I(Q)$

PS in d12-cyclohexane
Hadziioannou G; Cotts P M; ten Brinke G; Han C C; Lutz P; Strazielle C; Remp P; Kovacs A J, *Macromol*, (1987), 20, 493



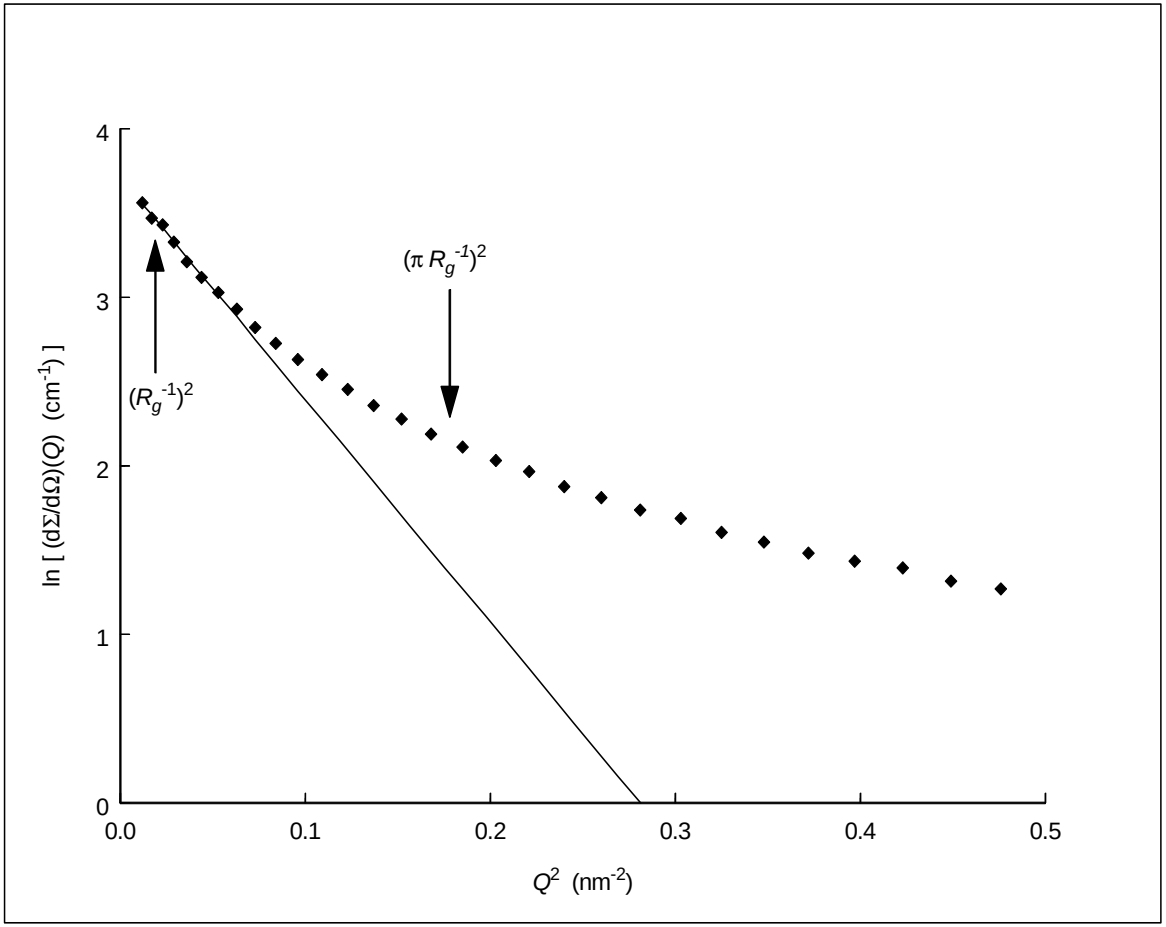


Asymptote approached from above the plateau: ***Branched polymer***
Cyclic polymer

Peak precedes the asymptote: ***Star polymer***

Book: Higgins & Benoit, *Polymers and Neutron Scattering*, 1994.

Example: Guinier plot of polymer blend



The scattering from a homogeneous blend of perdeuterated and hydrogenous polystyrene (M_{dPS} ~ 69200 g mol^{-1}, ϕ_{dPS} ~ 0.2) plotted according to the Guinier approximation. The fit gives M_w ~ 70700 g mol^{-1}, R_g ~ 6.3 nm.

Swollen coils

In a "good solvent" the polymer coil may expand, so that at intermediate Q there is no longer a Q-2 dependence. Instead:

$$P(Q) \propto Q^{-1/\nu}$$

Where in a "good solvent" $\nu = 3/5$ compared to ½ for a theta solvent.

The Zimm Approximation (at higher concentrations)

As the polymer concentration c increases, but is still less than the overlap concentration c*, P(Q) gets pushed down, but instead of using I(Q)=P(Q)S(Q) we have:

$$I(Q) = kcM[P_1(Q) - 2A_2cMP_1(Q)^2 + \ldots] \qquad k = \frac{\Delta\rho^2}{N_A d^2}$$

Where c = concentration, M = molecular weight, d = bulk density, $P_1(Q)$ is the Debye Gaussian coil equation, and A_2 is the "second virial coefficient".

In the Guinier range, at low Q, a "Zimm Plot" of 1/I(Q) against Q^2 gives M and R_G.

$$\frac{1}{I(Q)} \approx \frac{N_A d^2}{(\Delta\rho)^2 cM}\left(\frac{Q^2 R_G{}^2}{3} + 1 + 2A_2cM + \ldots\right)$$

Zimm Plot (at low concentration)

For a DILUTE solution (or in a bulk homopolymer) we can ignore the A_2 term and use a straight line fit to a Zimm plot to get M and R_G

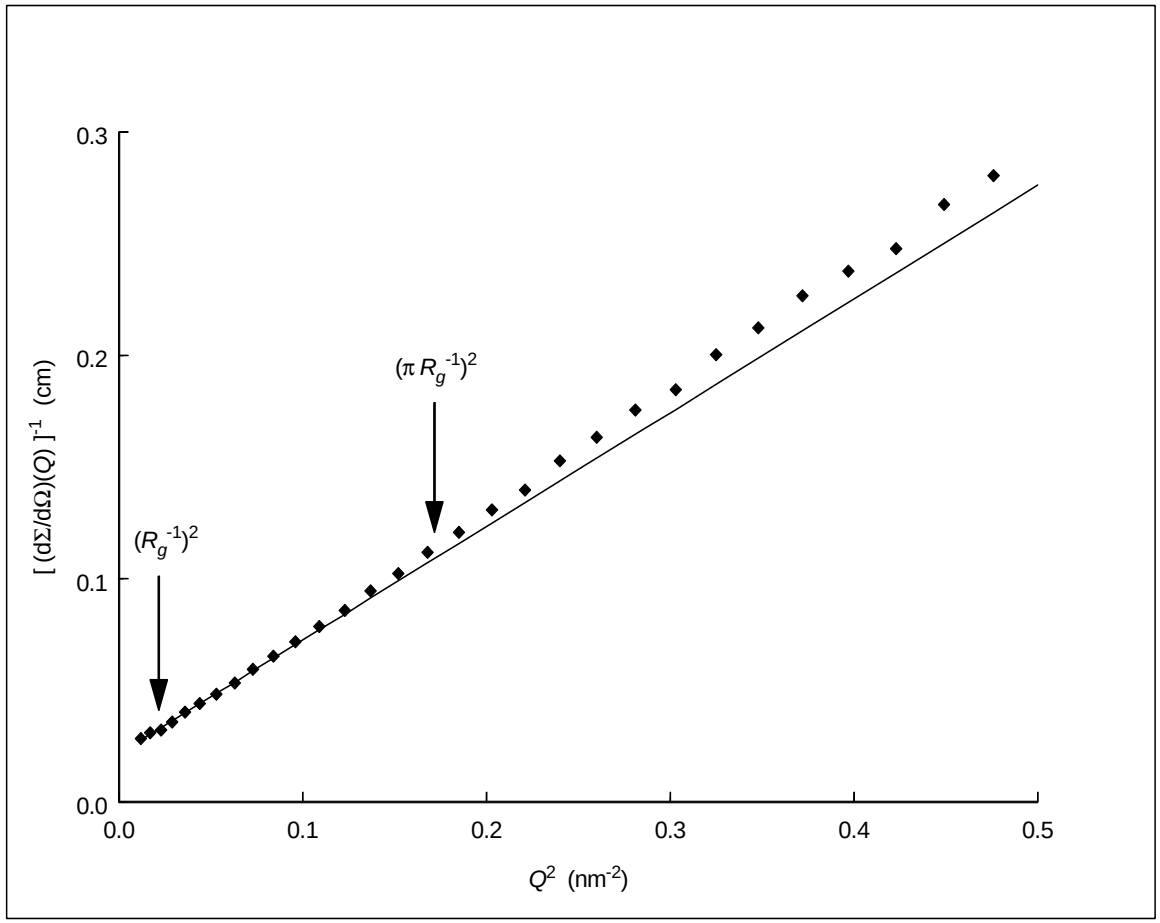
$$intercept = \frac{1}{M} \times \frac{N_A \delta^2}{c(\Delta\rho)^2} = \frac{1}{M} \times \frac{N_A \delta}{\phi(\Delta\rho)^2}$$

$$gradient = \frac{R_g^2}{3} \times intercept$$

[Note that there are a few polymers where D- and H- chains do have different interaction parameters and phase separation my occur.]

Zimm B, *J Chem Phys*, (1948), 16, 1093

Example: Zimm plot, 1/I(Q) against Q^2 of polymer blend



The scattering from the same homogeneous blend of perdeuterated and hydrogenous polystyrene (M_{dPS} ~ 69200 g mol^{-1}, ϕ_{dPS} ~ 0.2) plotted according to the Zimm approximation. The fit gives M_w ~ 72100 g mol^{-1}, R_g ~ 8.4 nm.

The Zimm Approximation (at higher concentrations)

$$\frac{1}{I(Q)} \approx \frac{1}{k_1 cM}\left[1+\frac{(QR_g)^2}{3}\right]+2A_2k_2c+3A_3k_3c^2+\ldots$$

$$\text{where } k_1=\frac{(\Delta\rho)^2}{N_A d^2} \text{ and } k_2 = \frac{1}{k_1\ c}$$

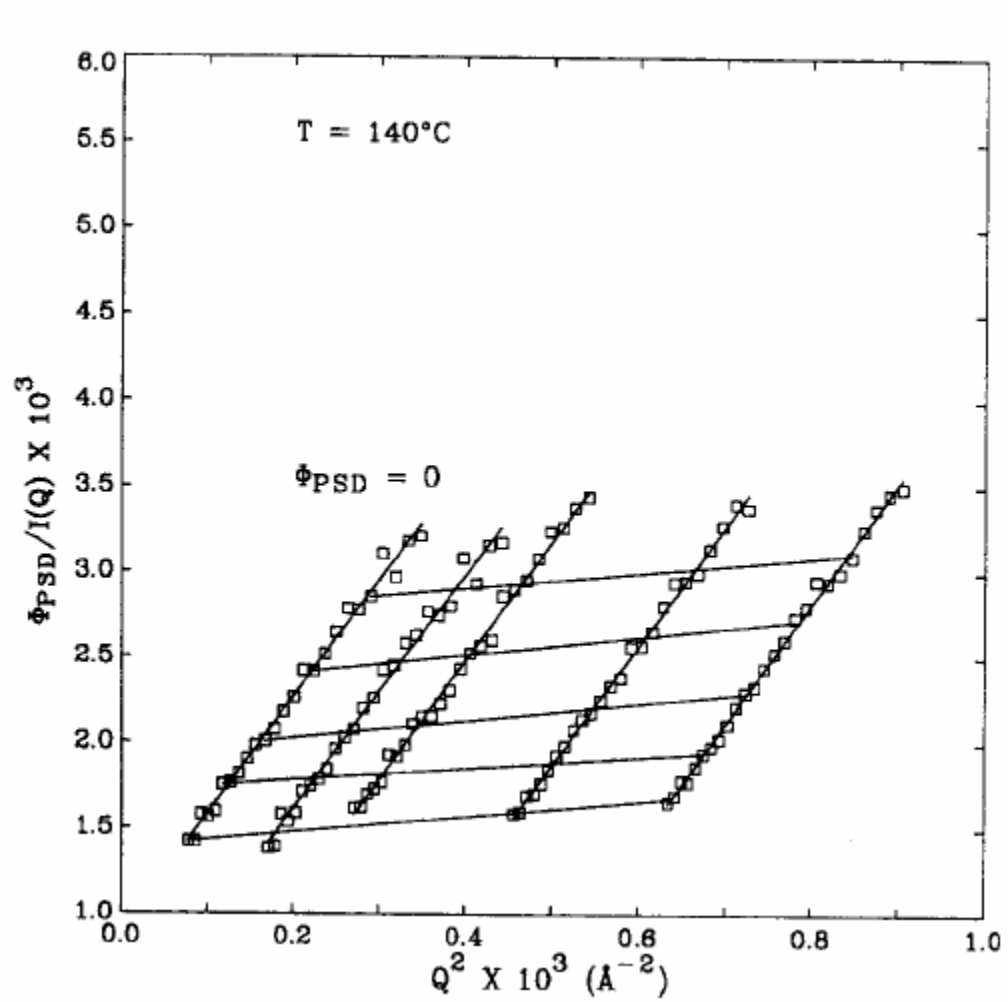
A "classical" Zimm plot has lines of $k_1c/I(Q)$ against $(Q^2 + \alpha\ c)$ for a series of concentrations where α is an arbitrary scaling factor.

Then the second virial coefficient A2 can then be extracted. (Note by extrapolation to zero concentration the higher terms disappear!)

A "Berry Plot" uses the *square root* of both sides.
[Berry G C, *J Chem Phys*, (1966), 44, 4550]

Consult the literature carefully as methods and the interpretation of A2 vary in different circumstances.

Example – a "classical Zimm plot to extract A_2



Standard Zimm plot for deuterated polystyrene/polyvinylmethylether blend (Mw=1.88x10^5 and 3.98x10^5 g/mole respectively) for polystyrene volume fractions 1%, 1.8%, 3.8% and 5.4% at 140°C. Extrapolation of slope and intercept to zero volume gives degree of polymerization for polystyrene and R_G respectively, as well as A_2 (From polymer notes of B.Hammouda, see NIST web site)

The Second Virial Coefficient A_2

$$A_2 = \frac{1}{\overline{V}_s \, \delta^2} \times (\tfrac{1}{2} - \chi)$$

interaction parameter

molar volume of solvent

$$A_2 = \frac{4}{M \, \delta} \times \frac{V_{solvated}}{V_{dry}}$$

degree of solvation

$$A_2 = \frac{N_A}{2 \, M^2} \times V_{ex}$$

excluded volume

$$A_2 = \frac{4 \, N_A}{M^2} \times V_{hs}$$

"hard sphere" volume

Polymers at high Q: Kratky - Porod Worm-like Chain

The scatter from a long, thin rod tends at high Q to a 1/Q , so a $Q^2I(Q)$ Kratky plot for a polymer with a long "persistence length" may not have a plateau after all, but will increase linearly with Q.

At still higher Q, if we have not reached background and the polymer chain has a reasonably large cross sectional radius, then I(Q) may fall off more steeply due to a "cross sectional radius of gyration" R_{Gxs} and the Kratky plot falls off again.

$$I(Q) \approx \frac{\pi c(\Delta\rho)^2}{N_A d^2} \times \frac{M_L}{Q} \times \exp\left(-\frac{Q^2 R_{g,xs}^2}{2}\right)$$

Where M_L is the mass per unit length of the polymer.

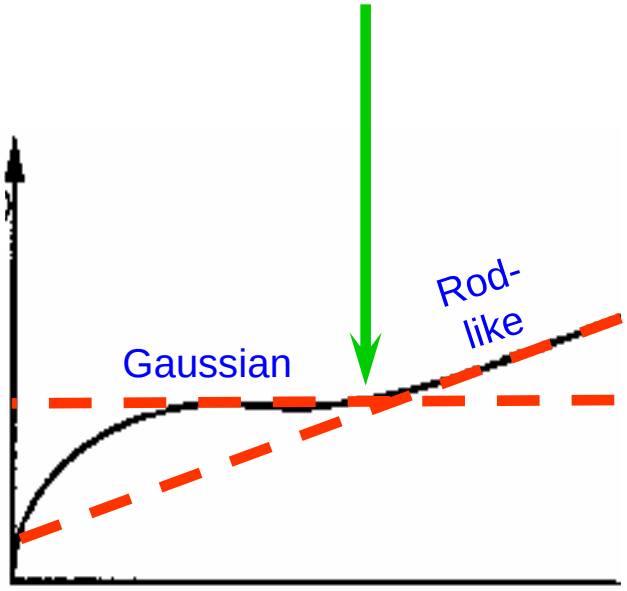
Various mathematical models or ones based on the results of computer simulations are available to attempt to fit the whole Q range from a polymer chain, but it is good to understand the asymptotic limits first using these traditional plots!

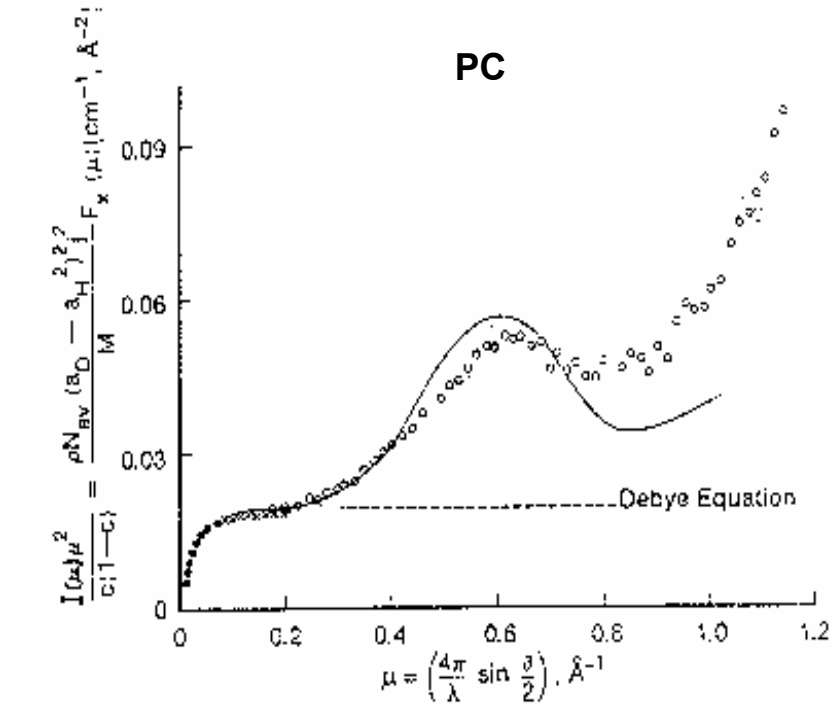
Kratky O; Porod G, *Recl Trav Chim Pays-Bas*, (1949), 68, 1106

Polymers at high Q: Kratky - Porod Worm-like Chain

$$Q_{cross} \approx \frac{12}{\pi\ l_K} = \frac{6}{\pi\ l}$$

Gawrisch, Brereton & Fischer, *Polym. Bull.*, 1981





Statistical chain element length, Kuhn length = 2 x persistence length,

As usual this needs very good background subtraction!

Also see: Higgins & Benoit, *Polymers and Neutron Scattering*, 1994

Polymers (and gels) at high concentrations OR with long range interactions

Above the overlap concentration c* SANS starts to see at smaller Q scattering from a characteristic screening length or mesh size ξ,(in good solvent is "average mesh size" of gel), g(r) follows an "Ornstein-Zernicke law". DeGennes and others propose:

$$I(Q) = 8\pi\phi(1-\phi)(\Delta\rho)^2\xi^3\frac{1}{(1+Q^2\xi^2)}$$

$$\xi = R_g\left(\frac{c}{c*}\right)^{\nu/(1-3\nu)} \qquad c^* = \frac{M}{N_A R_g^3}$$

This shape function is known as a "Lorentizian", and has the same form as a "critical scattering S(Q).

Kratky O; Porod G, *Recl Trav Chim Pays-Bas*, (1949), 68, 1106
Cheung Y W; Stein R S; Wignall G D; Yang H E, *Macromol*, (1993), 26, 5365
Marr D W M, *Macromol*, (1995), 28, 8470
Wignall G D; Alamo R G; Londono J D; Mandelkern L; Kim M H; Lin J S; Brown G M, *Macromol*, (2000), 33, 551

Aside – more Gels and 2 Phase

Often a polymer gel is "lumpy", regions of gel network have "particles" or "density fluctuations" on much longer distance scales.

These may be represented by either adding in an extra "Guinier"

$$I(Q) \propto \exp(-Q^2 \Xi^2)$$

or as Debye-Beuche "density fluctuations" - Lorentzian squared

$$I(Q) = 8\pi\phi(1-\phi)(\Delta\rho)^2 a^3 \frac{1}{(1+Q^2a^2)^2}$$

This is identical to "Debye random two phase model" for g(r) = exp{-r/a} *completely random* pore size distribution of volume fraction ϕ. Note this goes to Q^{-4} Porod scatter at high Q, so it assumes sharp, well defined interfaces – which may not be true for polymers.

E.Hoinkis, p71-241 in "Chemistry and physics of carbon", vol 25, ed. P.A.Thrower, Pub. M.Dekker, New York 1997. [long review of general interest, including porous materials.]

I.Pezron, M.Djabourov & J.Leblond, Polymer 32(1991)3201-3210 [good example of gelatin]

```
T  1 P 10 S  1 C  0 N  1
W  1 K  1 IP 0 MS 1 IY 0 Q -6 R -6    0    0   65   14    3    0    0    0    0
 Lorentz + Debye-Bueche (for gelatin)
Marquardt Steepest descent & least squares, WT=  1.00E-06
          ESD(not reliable) on/off
 1 27  1 I1(0) Lortz
             0.000E+00 0.0E+00 0.0
 2 27  2 ZETA    '
             1.000E+01 0.0E+00 0.0
 3 27  3 I2(0) D-B
             2.057E+03 5.2E+01 1.0
 4 27  4 A       '
             5.333E+01 4.8E-01 1.0
 5  3  1 BKG
             0.737E-02 1.0E-01 1.0
 6  3  2 Q
             0.000E+00 0.0E+00 0.0
 7  3  3 Q**2
             0.000E+00 0.0E+00 0.0
 8 15 11 SMEAR
             0.000E+00 0.0E+00 0.0
 9 15 12 NSIMP
             2.100E+01 0.0E+00 0.0
10 99  1 SCALE
             1.000E+00 0.0E+00 0.0
 1  free 62881    1 to   65 SSE= 1.783E+03
XDWE= 1.929E-04   SSE= 1.783E+03
1.000E-06
```

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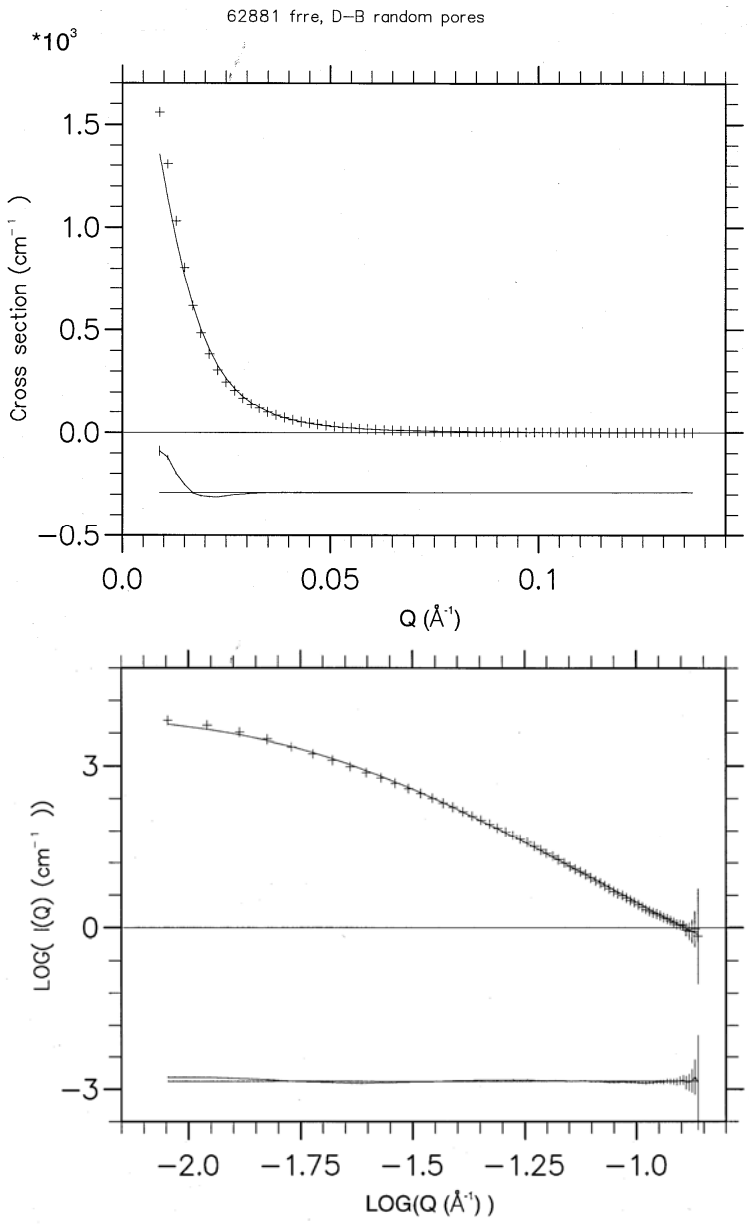


Example chemically etched, "porous" silicon, which approximately follows Debye random 2 phase with a=53 Å

Polymer blends – the RPA approximation

The theory for homopolymer blends (solid or melt) or mixtures involving block copolymers can get quite complicated. The "RPA random phase approximation" (or DeGenne's formula) is usually the starting point.

For an incompressible blend of polymers *a* and *b*:

$$\frac{1}{P(Q)} = \frac{1}{N_a \phi_a P_a(Q)} + \frac{1}{N_b \phi_b P_b(Q)} - 2\chi$$

Where $P_a(Q)$ is the usual Debye Gaussian coil, $\varphi_a + \varphi_b = 1$, and χ is a "Flory-Huggins interaction parameter" – which is of great interest to polymer chemists!

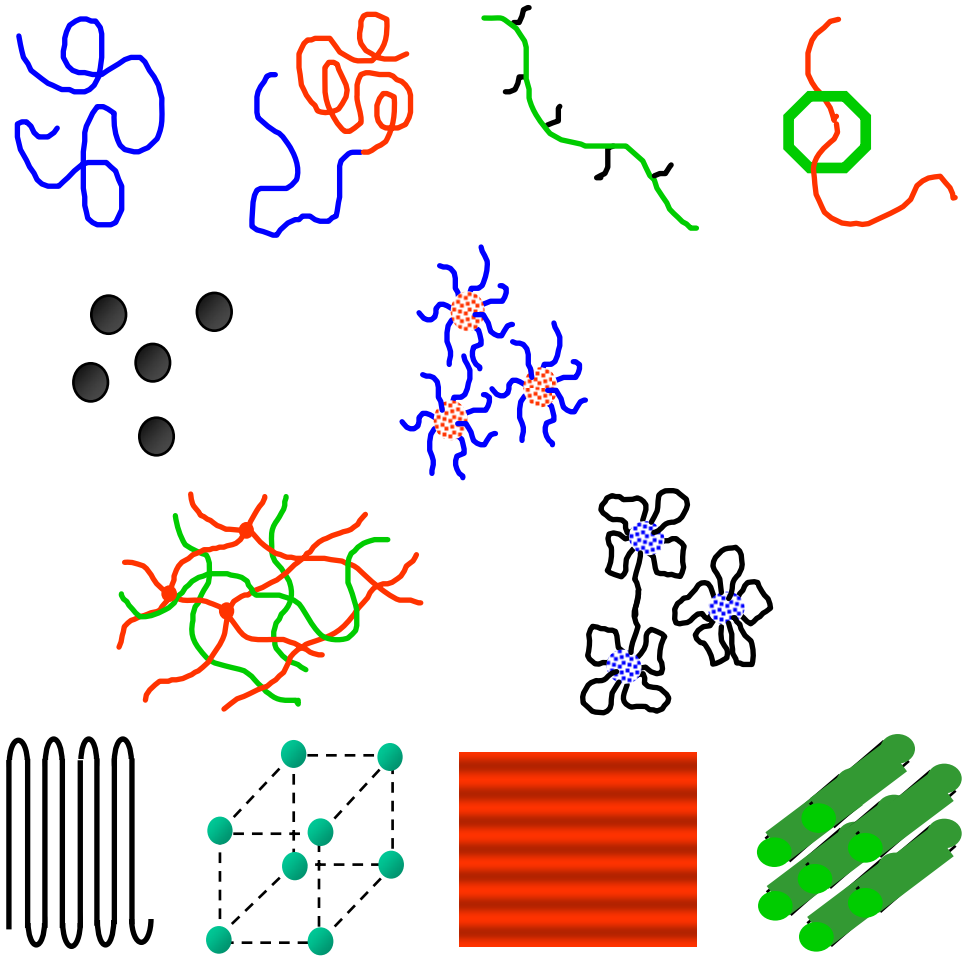
BEWARE there are various ways of representing the terms here, sometimes involving monomer volumes and polymerisation index, sometimes with P(Q) per unit volume etc etc, so check the literature carefully!

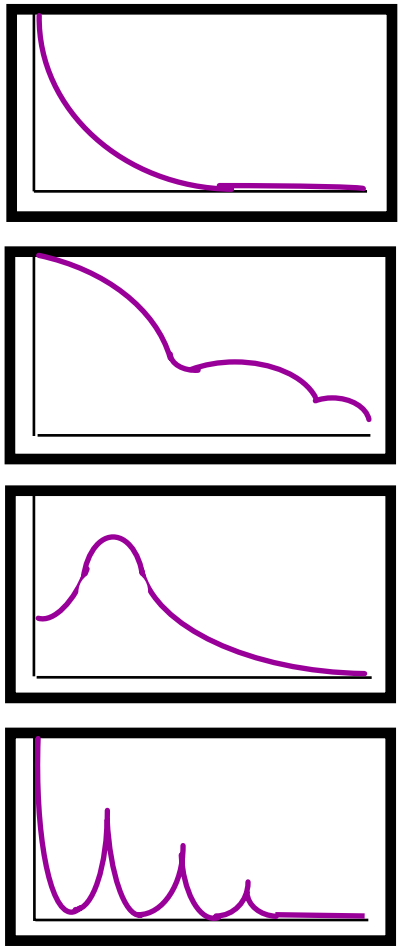
The equations are more complicated for compressible systems, where χ becomes a function of Q, and can be expanded for more phases.

More complex polymers

- As well there being many branching types for homopolymers, there are many different types of "block copolymer" which combine different chemical functionalities. [P(Q) general architectures: D.J.Read, Macromolecules 31(1998)899-911]
- In solution many block copolymers will aggregate into micelles just like surfactants, we have seen SANS from some of these already. The distinction between "surfactant" and "polymer" behaviour is often blurred.
- A block copolymer can be used as a "compatibiliser" between two different polymer types. So a sample may have competition between several interacting polymer types. Selective deuteration and SANS are key to understanding their structures.
- "Liquid crystalline polymers", with more or less ordered side chains show a wide variety of structures.
- Charged polymers "ionomers" may be chemical or biological in origin (e.g. proteins), and again require modifications of the scattering equations.
- SANS and neutron reflection are also very powerful to look at polymers at interfaces.

Characteristic Scattering Patterns ??





Its so complicated ! - Where do I begin ??

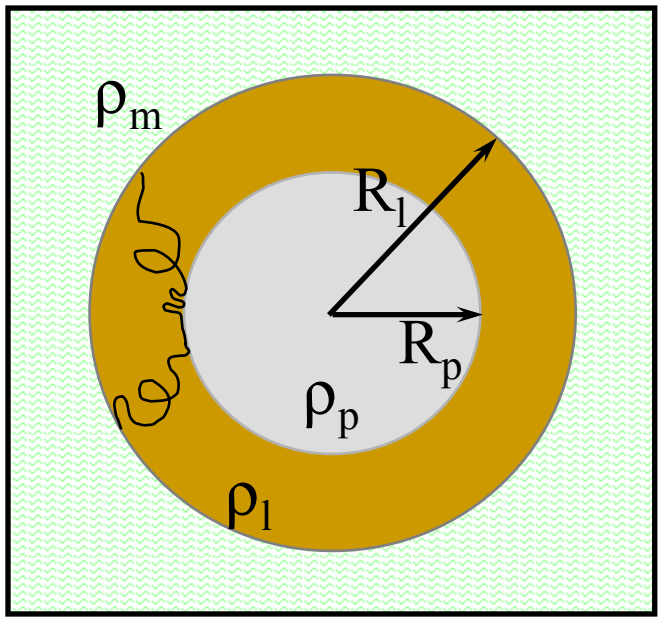
- It is often hard to know which model to use (especially if the data go approximately as Q^{-2}.
- Some times the structure is not what we “expect”.
- Checking power laws from a log-log plot is often useful.
- Fitting simple models such as spheres, rods, ellipsoids, discs will give an idea of the size and shape of scattering.
- Changing the sample composition, molecular weight, temperature etc. will provide clues.
- Contrast variation , often of the solvent, is very powerful.
- Start with a simple system that has been well characterised by other methods.
- Read the scientific literature on similar systems, ask a friendly instrument scientist for advice, check the web.

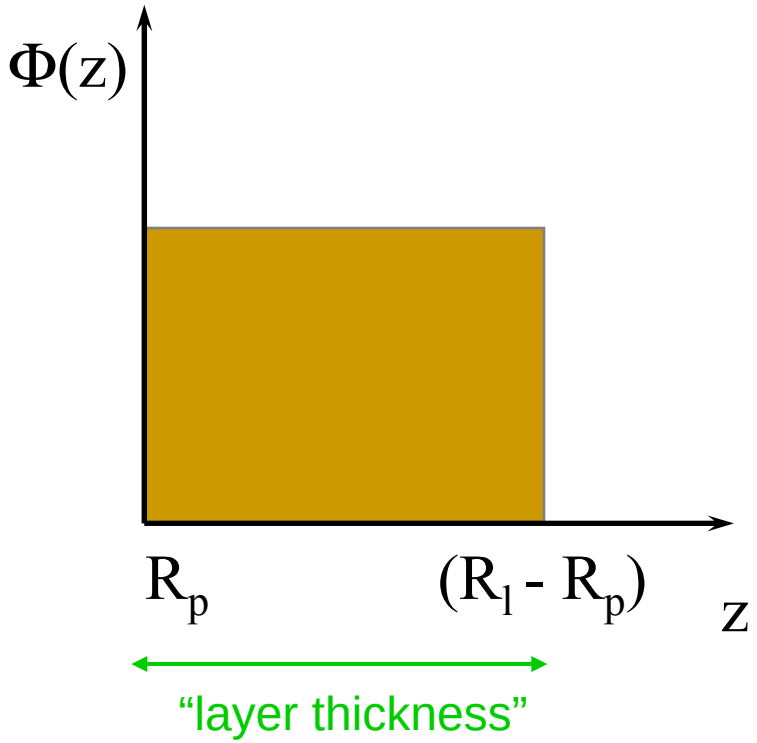
Polymers at surfaces

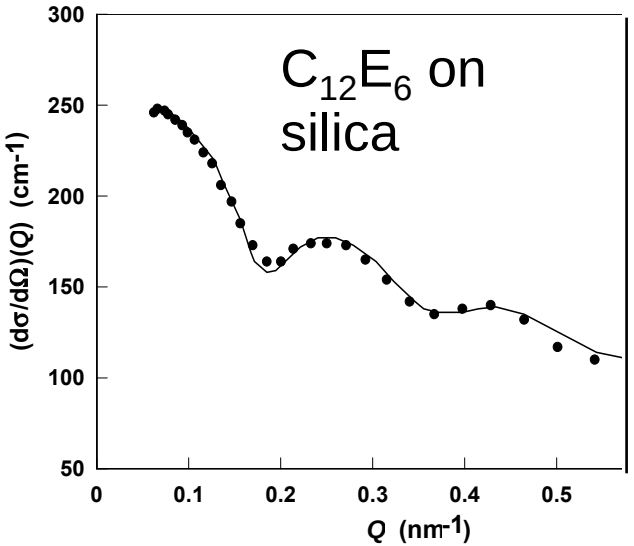
- In a previous talk we saw that SANS is sensitive to density profiles at a spherical shell surface, especially if the core particle can be contrast matched to the solvent.

- Polymers are often used as steric stabilisers for liquid droplets or solid particles. Understanding the structure of this layer is important to improve product performance – such as paint, or drug delivery systems or artificial blood.

The spherical "Core-Shell" Model



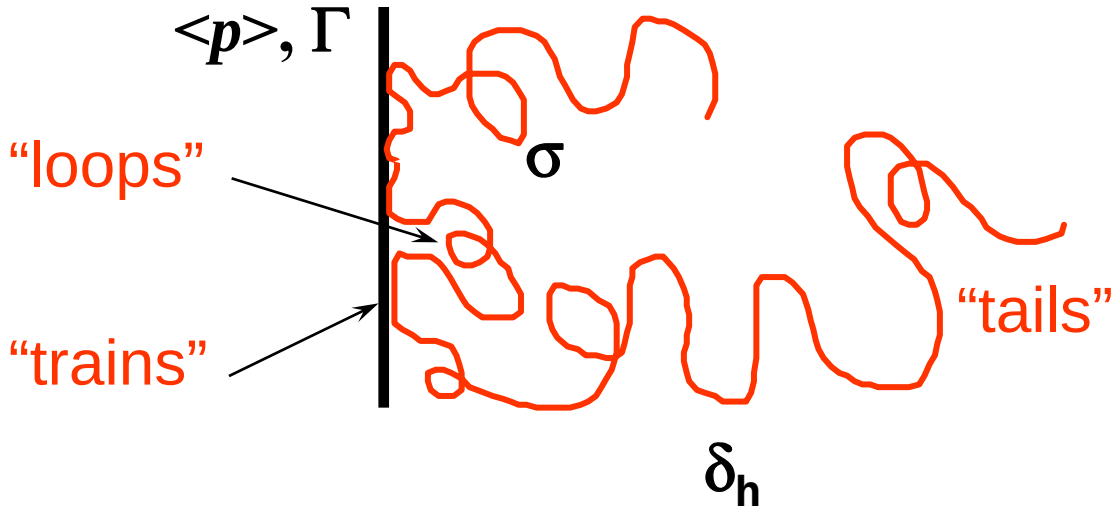




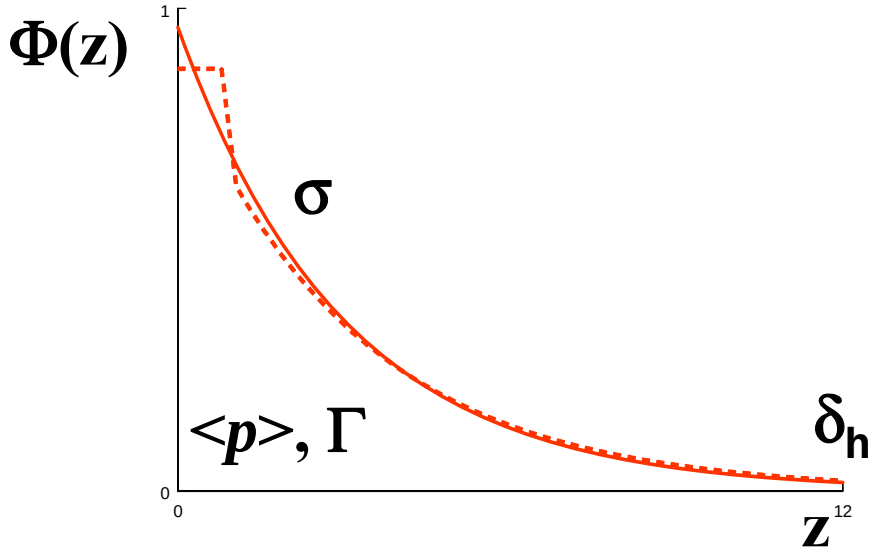
This is the method we have seen previously, using the exact equations for core/shell spheres, integrated over polydispersity. This approach still works for large particles if the adsorbed layer is reasonably uniform and homogeneous

Describing Adsorbed Layers (I)

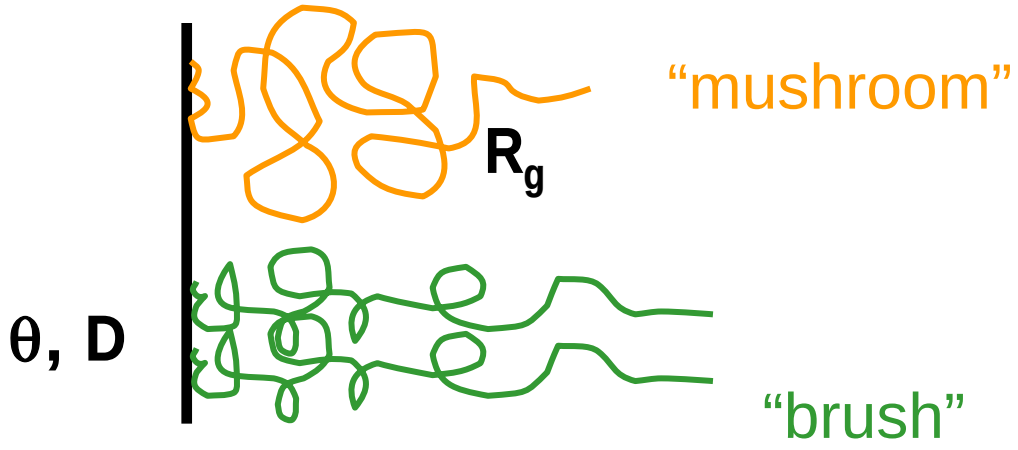
Depending on the nature of adsorption of the polymer to the interface and the polymer/solvent interaction, polymer scientists predict a variety of density profiles:



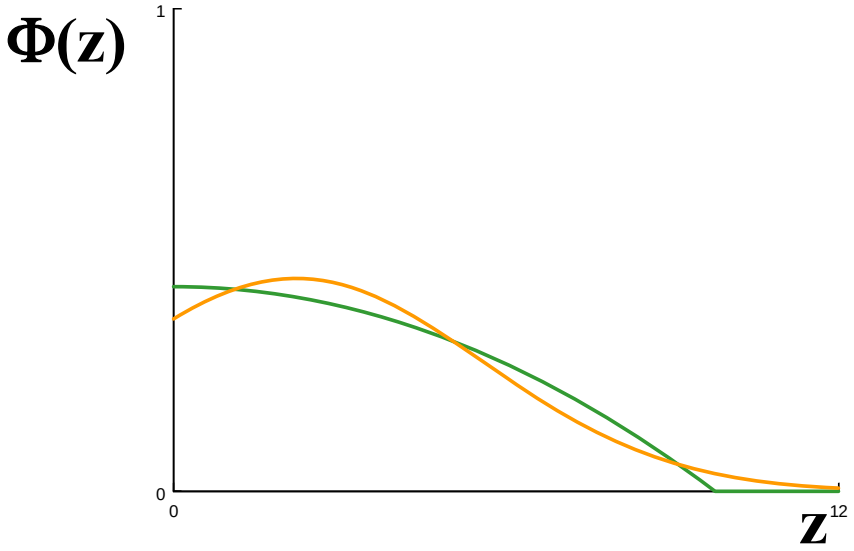
Exponential
&
Block+Exponential



Describing Adsorbed Layers (II)



Gaussian
&
Parabola



SANS from Polymers at "flat" surfaces

For dilute, LARGE droplets Crowley *et al.*, Auvray *et al.* Show how to simplify I(Q) and identify the important terms.

$$\frac{\partial \Sigma}{\partial \Omega}(Q) = N_p P(Q) + B$$

$$P(Q) = \left[\underbrace{(\rho_p - \rho_m) F_p(Q)}_{\text{intra-}particle\text{ form factor}} + \underbrace{(\rho_l - \rho_m) F_l(Q)}_{\text{intra-}layer\text{ form factor}} \right]^2$$

p particle

l layer

m medium

$$P(Q) = \left[(\rho_p - \rho_m)^2 F_p(Q)^2 \right] + \qquad I(Q)_{pp}$$

$$\left[2(\rho_p - \rho_m)(\rho_l - \rho_m) F_p(Q) F_l(Q) \right] + \qquad I(Q)_{pl}$$

$$\left[(\rho_l - \rho_m)^2 F_l(Q)^2 \right] \qquad I(Q)_{ll}$$

At *exact* match these go to zero

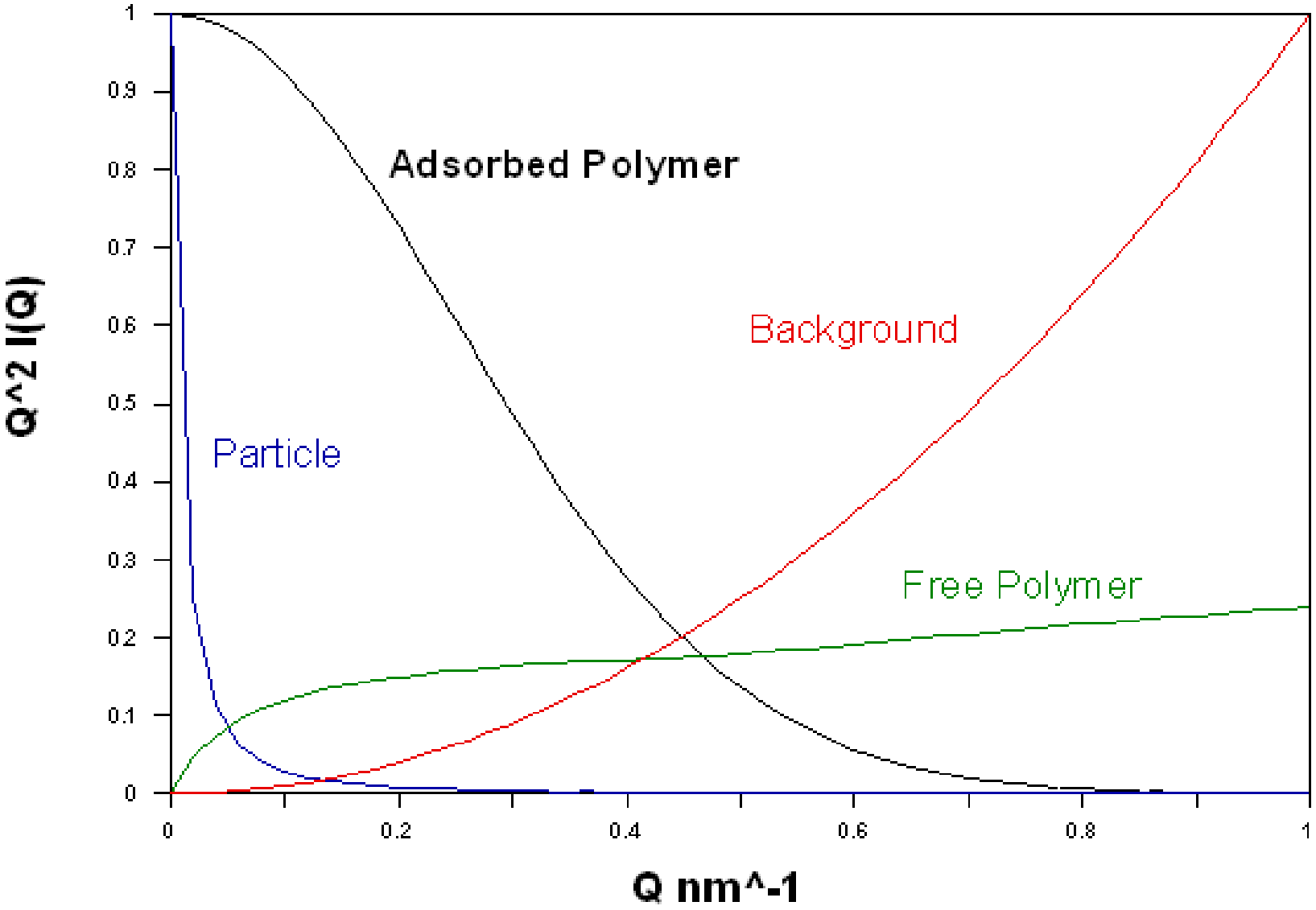
At an exact match the density profile normal to the (approximately flat) surface can be determined by a Fourier transform method. Since this still requires some smoothing and extrapolation at small Q and high Q, and a real sample may not be exactly at match, is still better to model fit the SANS data:

Cosgrove T; Crowley T L; Vincent B; Barnett K G; Tadros Th F, *Faraday Symp of the Chem Soc*, (1981), 16.

Auvray L; de Gennes P G, *Europhys Lett*, (1986), 2, 647

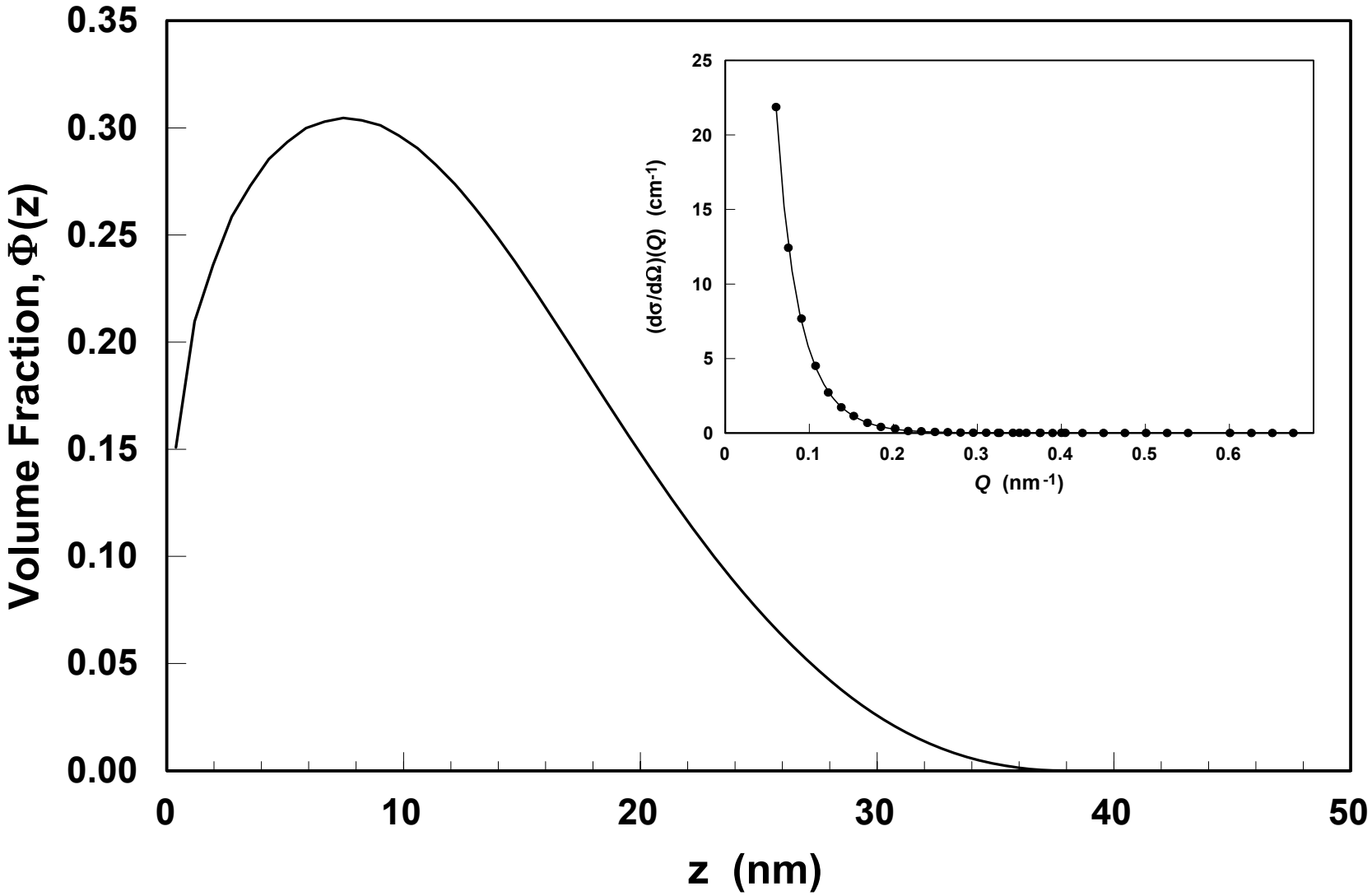
Auvray L; Cotton J P, *Macromol*, (1987), 20, 202

Contributions to the Scattering (almost at match)

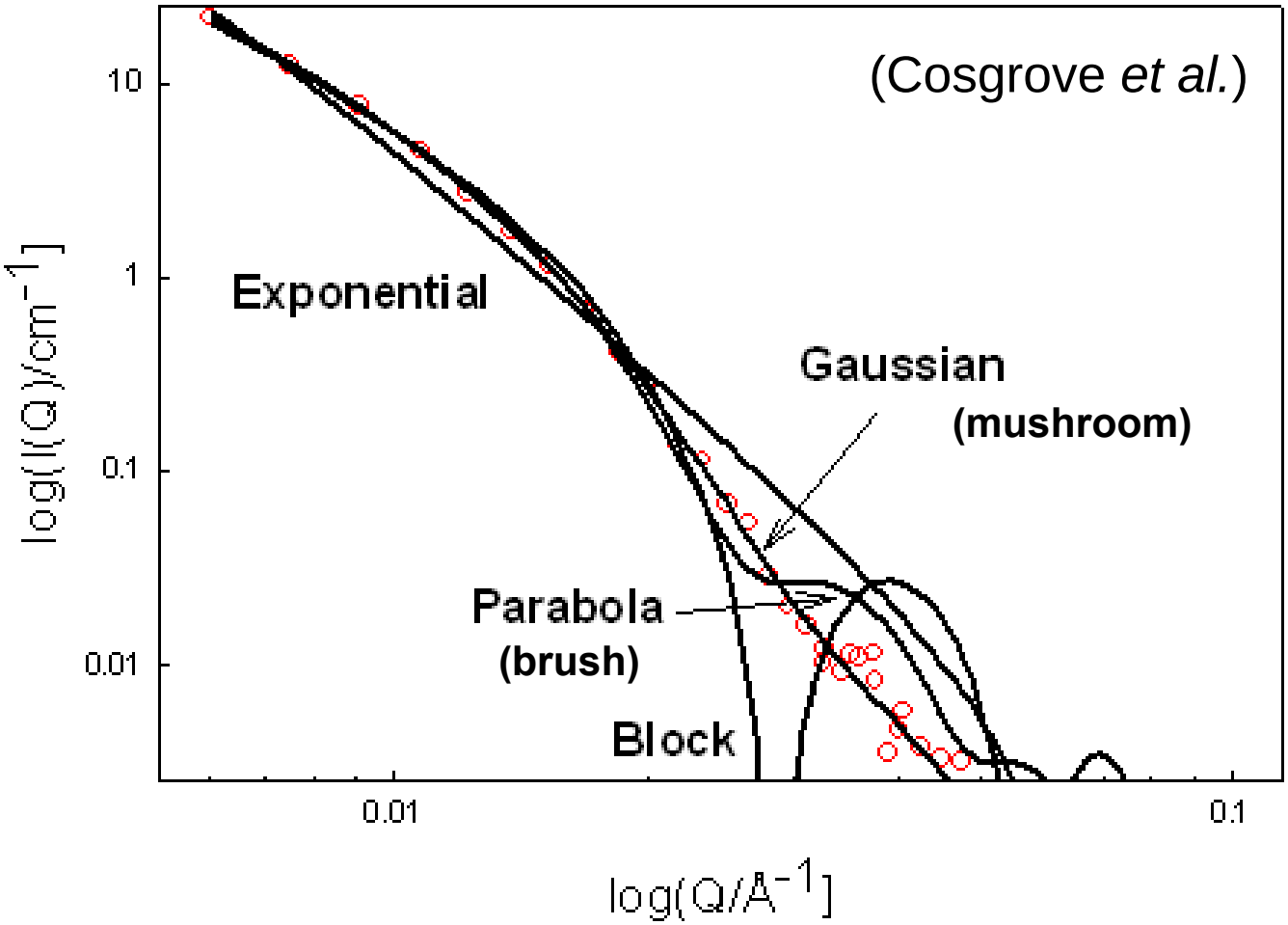


Example of "mushroom" – polystyrene grafted onto SiO_2 in DMF, by Fourier transformation, using the Crowley method.

(Cosgrove *et al.*)



Example - Profile fitting SANS data, same PS-g-SiO2



The layer term $I(Q)_{ll}$

For a general layer profile φ(z)

$$I_{LL} \rightarrow 2\pi S(\rho_L - \rho_M)^2 \left[\frac{1}{Q^2} \left| \int_o^t \phi(z) e^{iQz} dz \right|^2 + \bar{I}_{LL} \right]$$

Where S is the Porod surface $N4\pi R^2$ for the bare particle, and $\bar{I}_{LL}$ is a term for "concentration fluctuations". Ignoring the fluctuations (for which see specialist papers!) this may be roughly approximated by:

$$I_{ll}(Q) \approx (\rho_l - \rho_m)^2 \frac{2\pi S M^2}{Q^2} \exp\left(-Q^2\sigma^2\right) \quad \textbf{where}$$

$$\sigma^2 = \left\langle z^2 \right\rangle - \left\langle z \right\rangle^2$$

$$\left\langle z^n \right\rangle = M^{-1} \int_0^t \Phi(z) z^n dz$$

$$M = \int_0^t \Phi(z) dz = \frac{\Gamma}{d}$$

σ is the root mean square (rms) thickness of of the layer.
Note M is a normalisation constant (NOT molecular weight),
Γ is adsorbed amount (g/cm^2),
d the bulk density (g/cm^3).
Beware different authors use different symbols here !

For a uniform layer of thickness t

$$I_{LL} \approx \frac{8\pi S}{Q^4} \sin^2\left(\frac{Qt}{2}\right)(\rho_L - \rho_M)^2 \approx \frac{2\pi S t^2}{Q^2}(\rho_L - \rho_M)^2$$

[Recent example: J.C.Marshall et.al. Langmuir 2004(20)4480-4488 & references therein.]

Example: Polymer density profiles from SANS on "artificial blood"

Architecture of "bio-compatible" tri-block copolymer affects the bio-distribution of injected colloidal particles! Why? How?

PS latices —*rapid uptake*→ Liver

PS latices → + Tetronic 908 → Liver

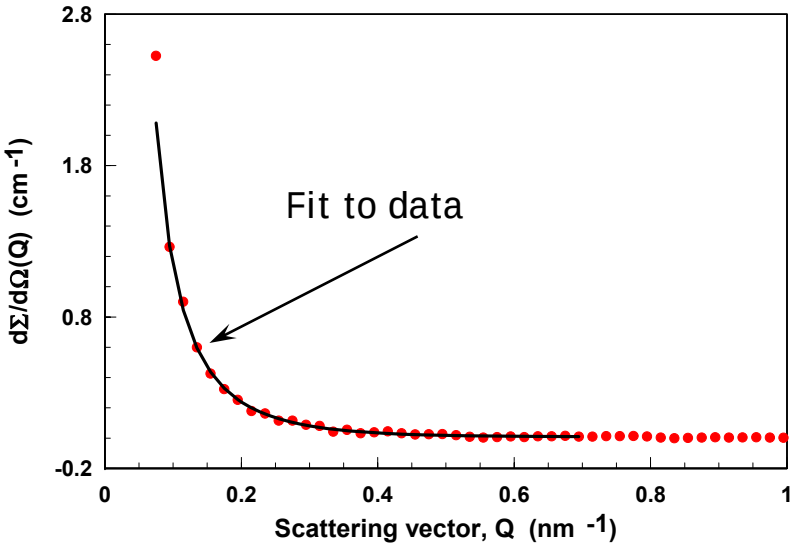
longer circulatory lifetime

PS latices → + Pluronic F127 → Bone Marrow

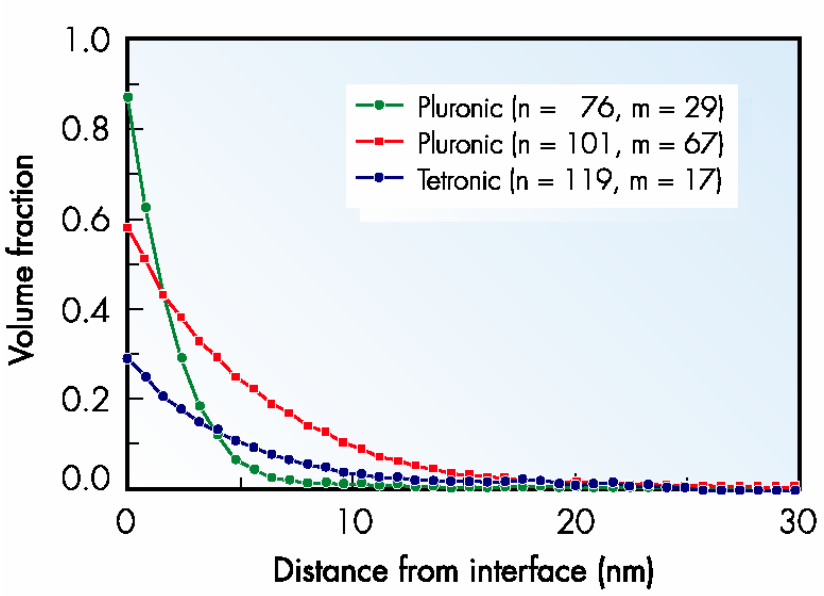
Poloxamer 407 = **Pluronic 127**

Illum *et al*, Life Sciences, 1987

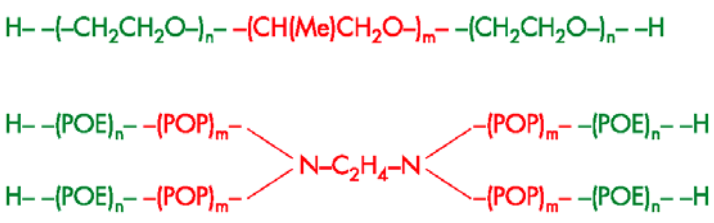
Pluronic F68 (n=76, m=29)
fit to SANS

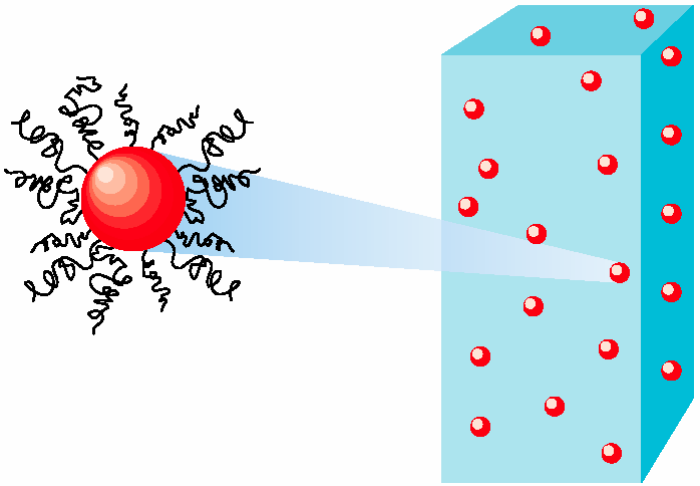


Real space density profiles

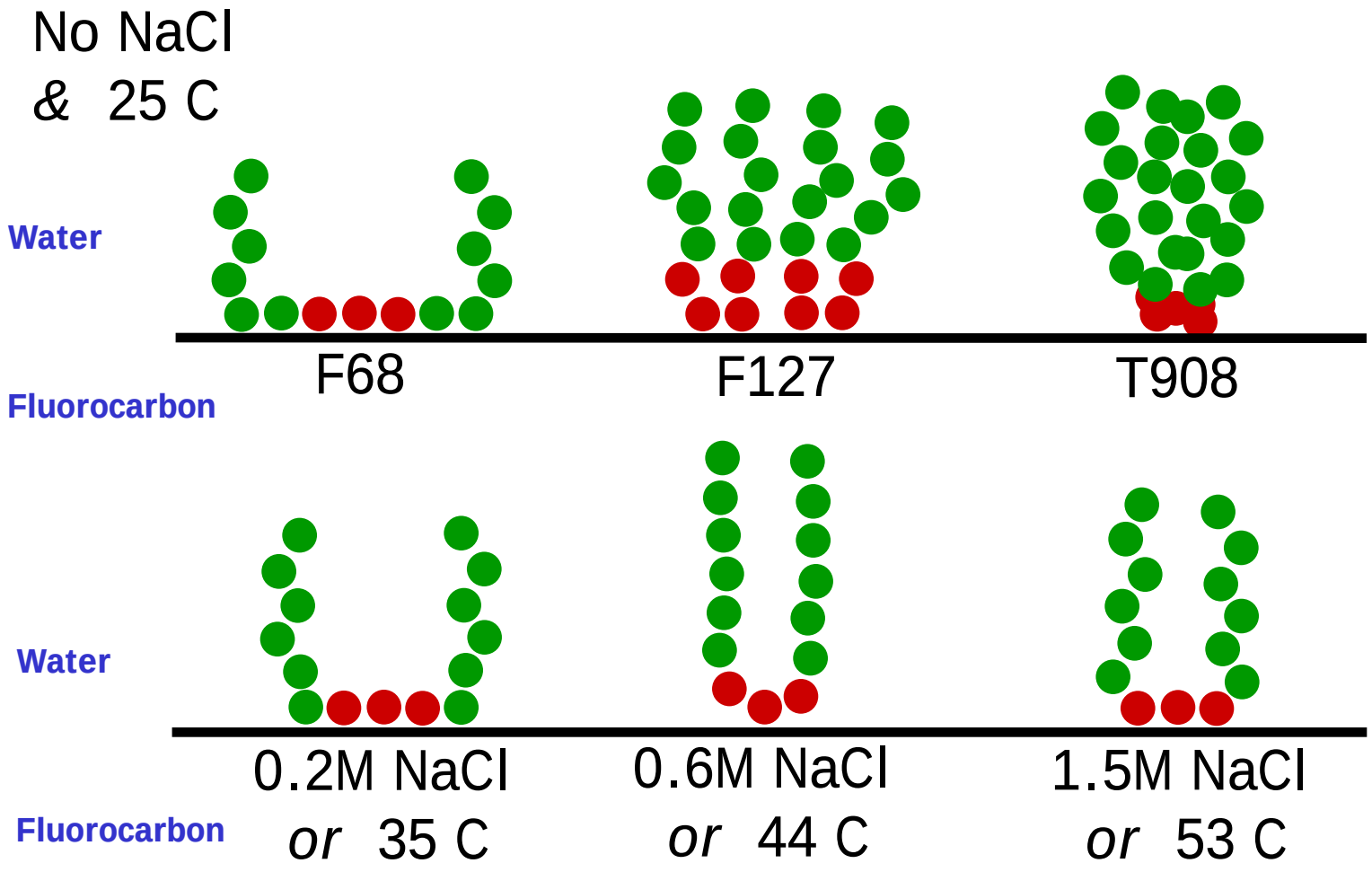


Emulsion (large particles) with oil phase contrast matched to water – SANS only sees thin interface!





Schematic results



Salt and temperature ought to dehydrate and collapse EO tails, *but* binding at water/oil interface also changes – so it would be hard to guess what happens!

C.Washington, S.M.King & R.K.Heenan, J.Phys.Chem. 100(1996)7603-7609.

IFT "Indirect Fourier Transform" Methods (– link to "biology")

- **In theory it is possible to Fourier transform SAS data to recover a radial distribution function g(r). To do so effectively requires some way to extrapolate missing data at high and low Q.**

$$I(Q) = 4\pi N \int_0^\infty g(r) \frac{\sin(Qr)}{Qr} dr \qquad g(r) = \frac{1}{2\pi^2 N} \int_0^\infty I(Q) Qr \sin(Qr) dQ$$

- **A "direct transform" of I(Q), as above, is not reliable. The "indirect transform" involves first fitting the data with a sum of smooth "basis functions" such as splines, that do behave well at high and low Q. The basis functions are then transformed to get g(r).**

- **This is most reliably achieved for well defined systems such as the "polymer layer at match" or for "dilute, monodisperse biomolecules". In the biomolecule case a "maximum dimension" of the molecule may be a useful constraint.**

- **Prof. Glatter has tried in recent years to make more extensive use of supposedly "model independent" IFT methods – however there are usually some implicit assumptions in such methods and they do not always work as well as direct fitting with a scientifically plausible model.**

Example - IFT methods comparing bovine IgG1 with IgG2

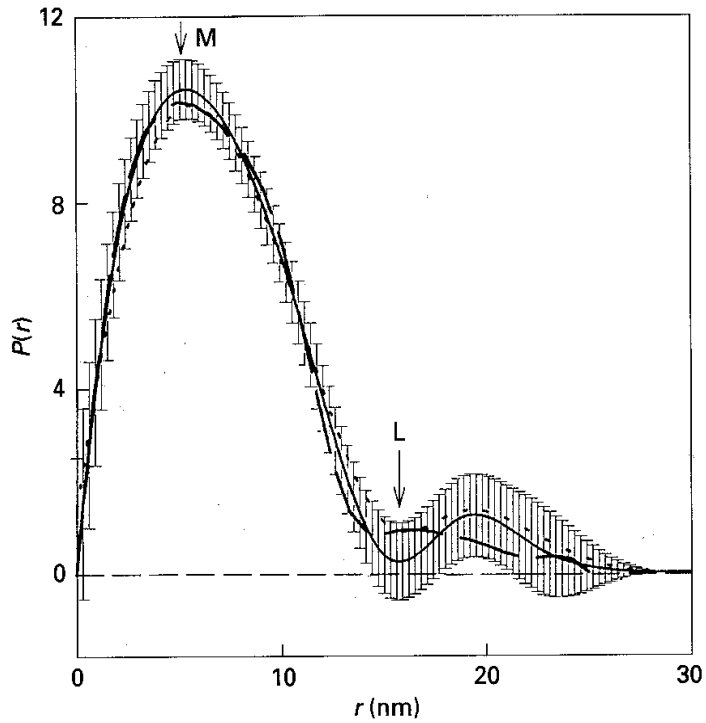


Figure 2 Neutron distance distribution functions $P(r)$ for bovine IgG1 and IgG2

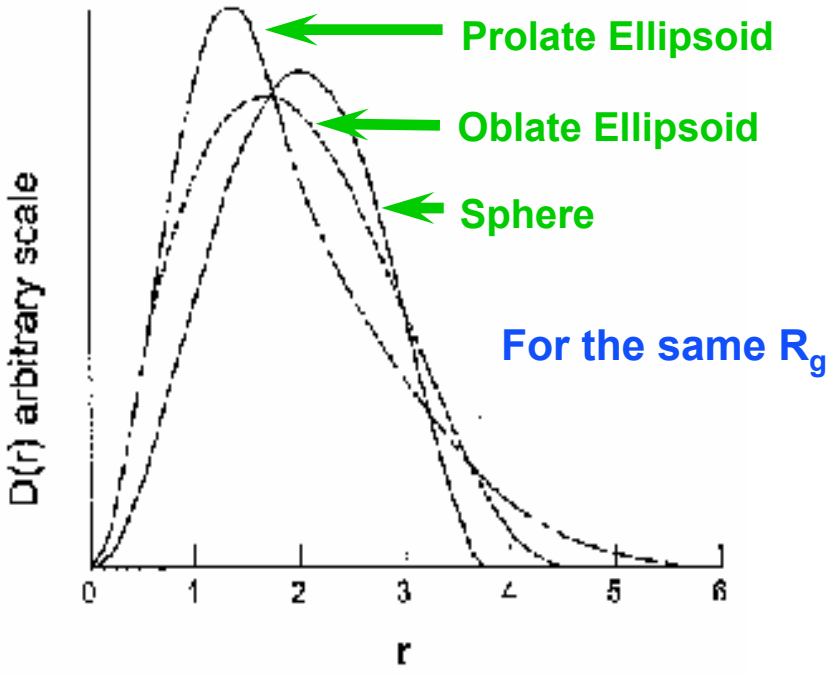
The continuous line with error bars corresponds to $P(r)$ of IgG1 using ITP-91, and the dotted line to IgG2. The dashed line corresponds to $P(r)$ for IgG1 calculated from GNOM. The maximum M in $P(r)$ occurs at 5.4–5.7 nm, and the maximum dimension was determined to be 15.6 ± 2.1 nm.

***g(r)* is the sum of a set of "basis functions" - e.g. cubic splines - chosen by prior knowledge or even by trial & error to give the best fit I(Q) to the data with the fewest oscillations**

- can include instrument resolution
- difficult to estimate errors

Glatter O, *J Appl Cryst*, (1977), 10, 415
Glatter O, *J Appl Cryst*, (1979), 12, 166
Moore P B, *J Appl Cryst*, (1980), 13, 168
Svergun D I; Semenyuk A V; Feigin L A, *Acta Cryst*, (1988). A44, 244

ρ(r) for some different particle shapes



$$R_g^2 = \frac{\int_0^\infty D(r)\, r^2\, dr}{2\int_0^\infty D(r)\, dr}$$

Computer Simulation Methods – usually for biomolecules

Can work very well for monodisperse biomolecules – all are the same size and shape – particularly if a number of different contrast are used to highlight parts of the structure (protein/DNA/carbohydrate etc) and if some parts have "known" structures from crystallography.

"Debye spheres modelling"

Simulated annealing ("dummy atom") methods

See the web pages of Dimitri Svergun's group at EMBL Hamburg:
www.embl-hamburg.de/ExternalInfo/Research/Sax/index.html

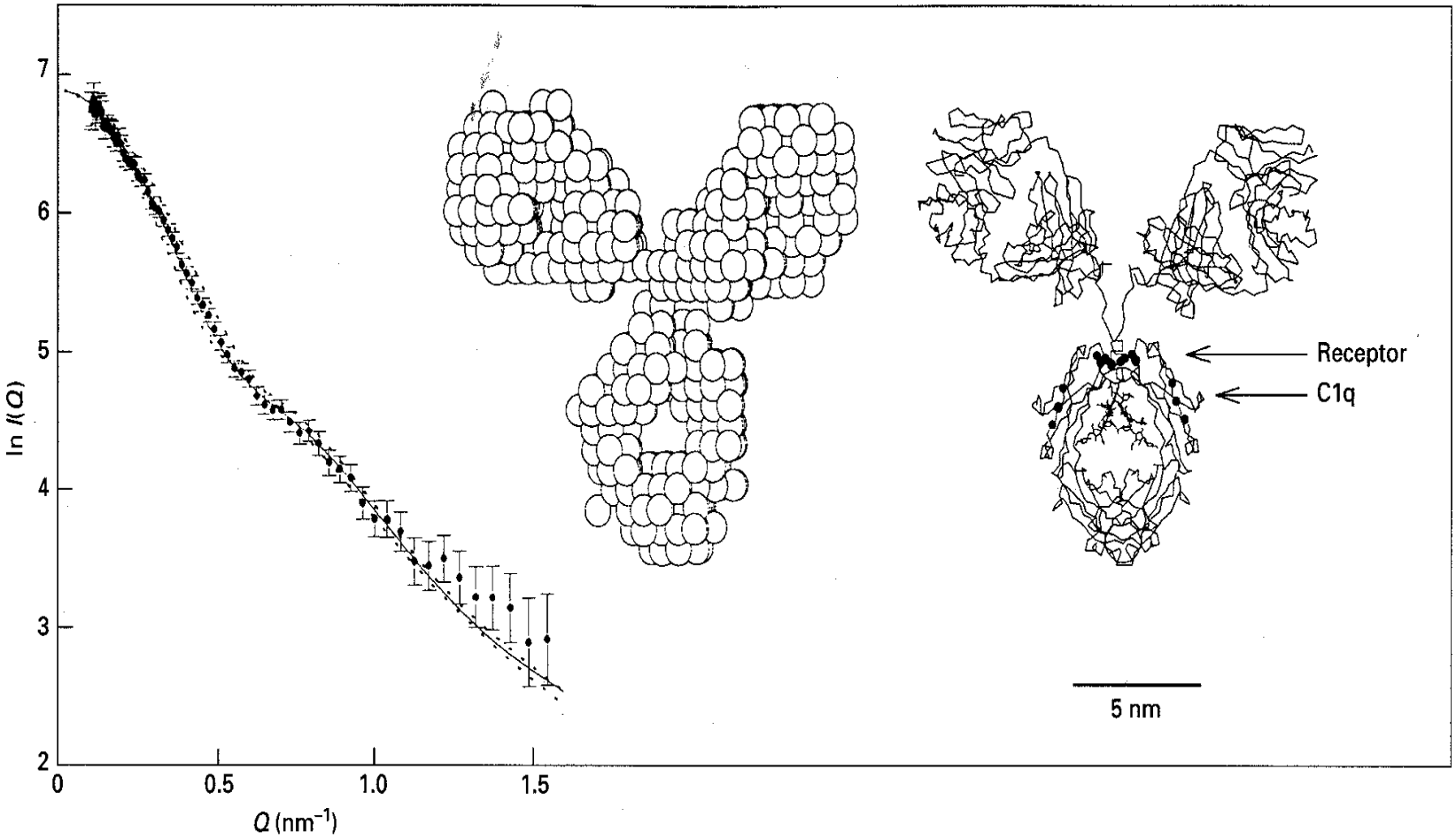
Debye Spheres Modelling

- **Build a "low resolution" structural model: represent each CH_2, or repeat unit, or amino acid residue, by a sphere of appropriate radius and scattering density.**
- **Calculate the scattering from all pairs of spheres:**

$$\frac{\partial \Sigma}{\partial \Omega}(Q) = \sum_{i=1}^{N} \sum_{j=1}^{N} P_i(Q,R) P_j(Q,R) \left[Sin(Qr_{ij}) / Qr_{ij} \right]$$

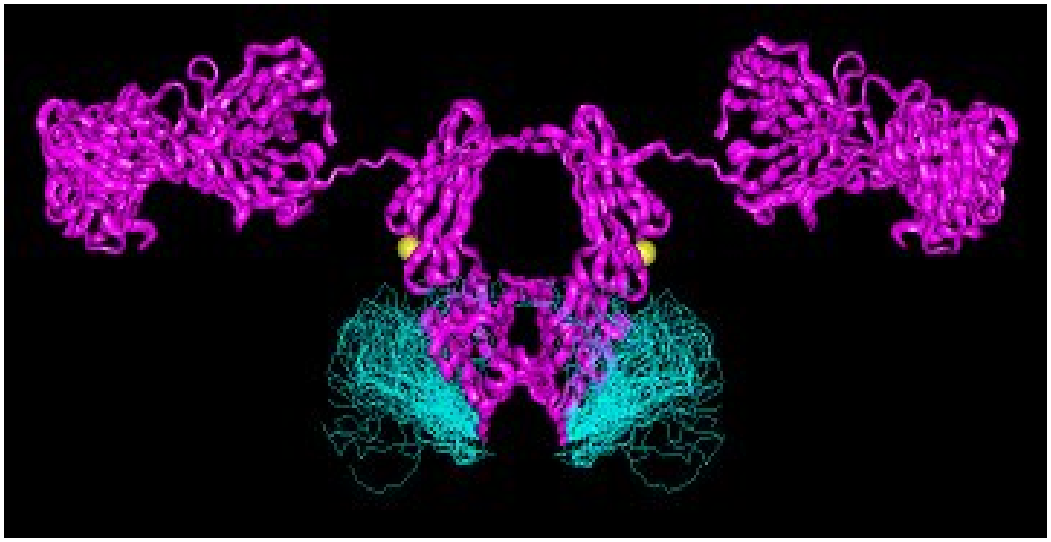
- **Search all likely conformations of the model structure, whilst maintaining "connectivity" to find best fits.**
- **(Note a very similar method is used for size and shape determination from Analytical Ultracentrifuge (AUC) data.)**

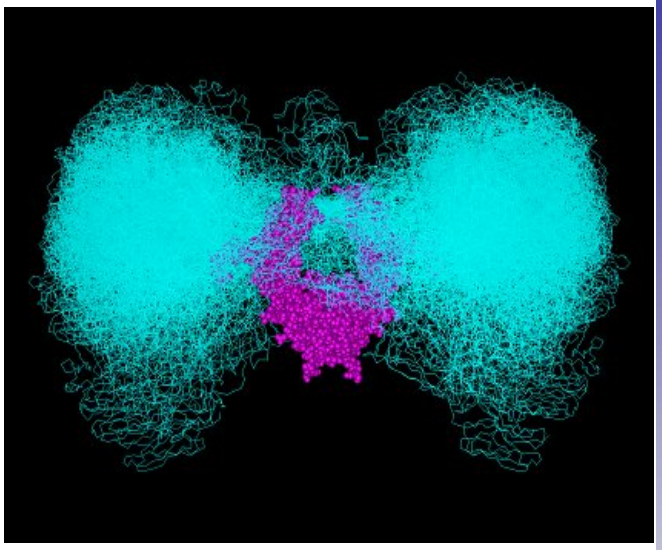
Example - Debye Spheres Modelling on IgG

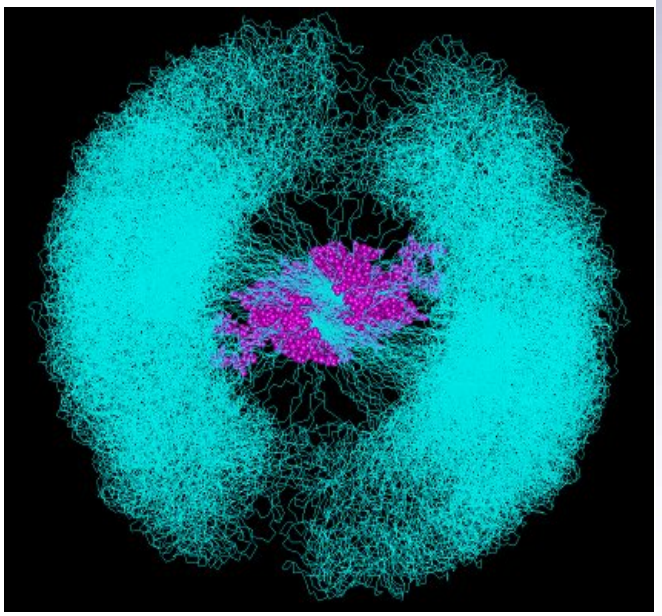


From: Mayans, Coadwell, Beale, Symons & Perkins (1995)

SAXS / SANS & Molecular Modelling





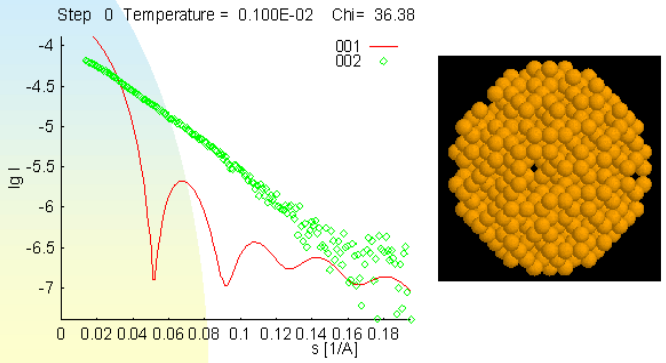


The superposition of 104 best-fit molecular models (from 12000 conformations optimised by molecular dynamics, based on crystal structure coordinates) gives an idea of the solution conformation of human Immunoglobin IgA1

From: Boehm, Woof, Kerr & Perkins
J. Mol. Biol., (1999), *286*, 1421

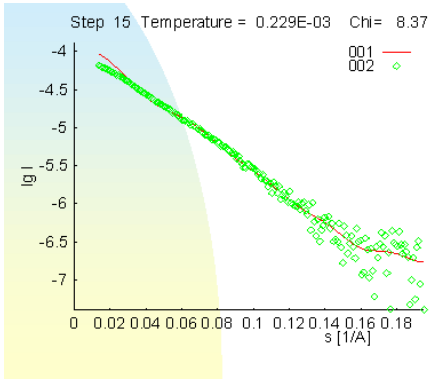
Simulated Annealing Methods (I)

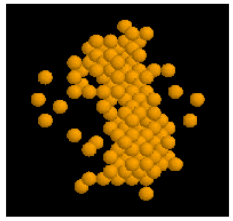
S1 shape reconstruction



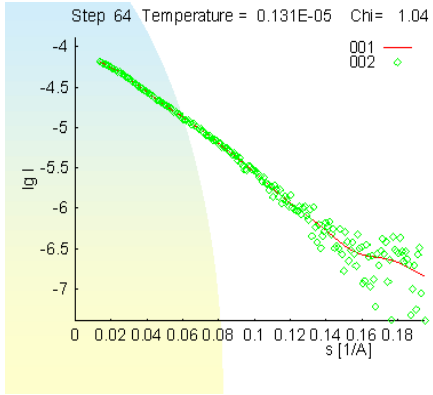
"DAMMIN" Svergun *et al,* spheres are added or removed at random, but with high penalties for "non-connected" or "non-smooth" models. Useful for a genuinely "unknown" particle.

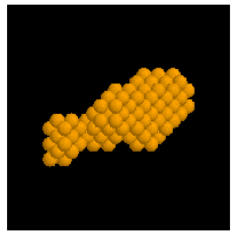
S1 shape reconstruction





S1 shape reconstruction



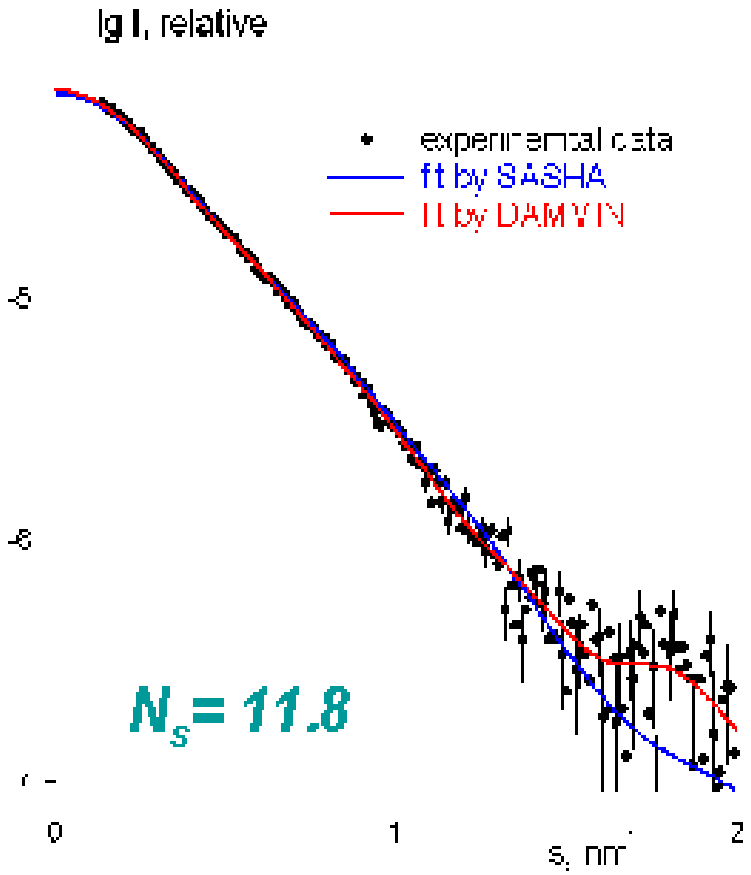


"SASHA" Svergun *et al,* is an older method using 3d "spherical harmonic" basis functions to fit a smooth shape. Useful for a genuinely "blob shaped" particle, as elongations or projections add too many functions.

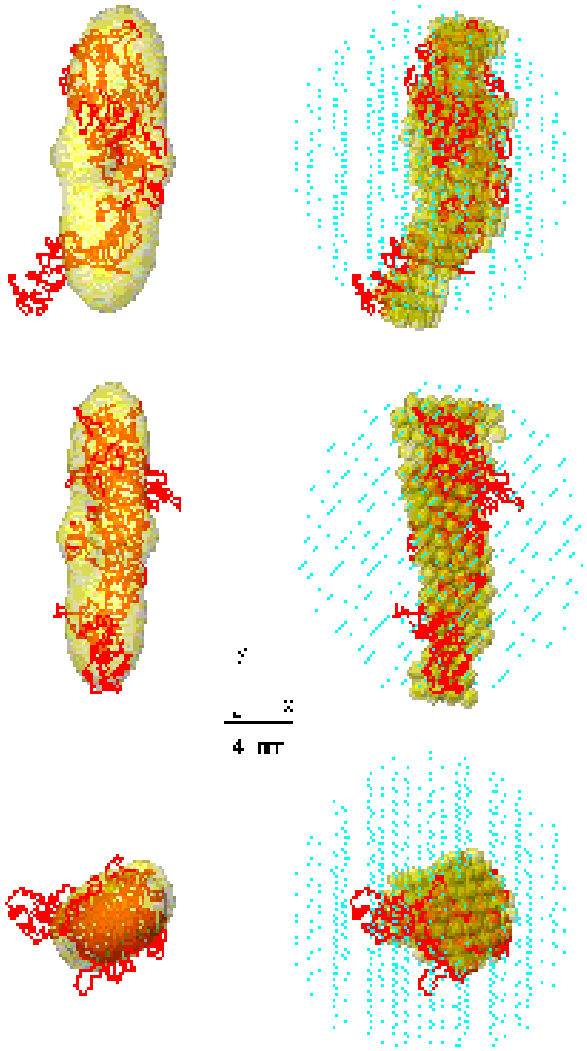
Simulated Annealing Methods (II) – test on "known" crystal structure

S1 shapes restored by *SASHA and DAMMIN*

Red: atomic model



$N_s = 11.8$



Number of parameters: M=19 M=2729

SANS on "biomolecules" – general comments

As we have seen, SAS can be very powerful for monodisperse (all the same size and shape) molecules.

Svergun's group have a wide range of programs where more or less constraints, such as known crystal structures of fragments, may be included.

SANS is quite sensitive to any large aggregates due to the ΦV term in I(Q)

SANS can work at < 1mg/ml in most cases (but can be hard in H_2O due to incoherent background from H).

SANS on "biomolecules" – contrast variation

Contrast variation is very useful for multi-component systems. (e.g. where protein, nucleic acid, carbohydrate etc are combined) and / or where specific deuteration is worthwhile.

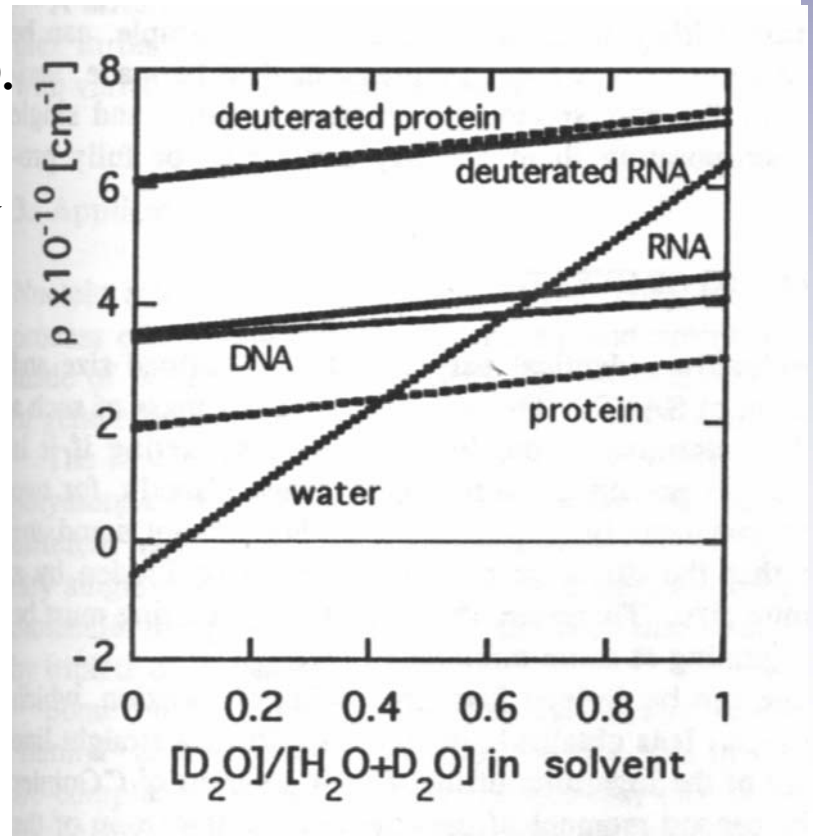
Help with deuteration is available from the ILL-EMBL deuteration laboratory.

The simplest two contrasts are to use neutrons in D_2O and then X-rays in H_2O. In this case X-rays are more sensitive to hydration shell of "dense water", so may give slightly larger R_G.

Very detail models have to allow for the hydration shell.

H_2O/D_2O contrast variation must allow for H-D exchange.

E.g. protein matches at ~40% D_2O



Fibre Diffraction (I)

- Though many macromolecules cannot, or will not, crystallise - they may form oriented fibres (or can be *induced* to form fibres); e.g., DNA, nylon
- More common with X-rays than neutrons (but contrast variation …)
- Fibres have helical symmetry (c.f. 3-dimensional symmetry of crystals)
- Fibre diffraction patterns may be:

Crystalline - fibres pack to form crystallites with a common crystallographic axis (*c*-axis); e.g. A-DNA, (PTFE is at best only 90% crystalline)

Non- / Semi- crystalline - fibres have a common crystallographic axis but are randomly packed; e.g., B-DNA, nylon, PET

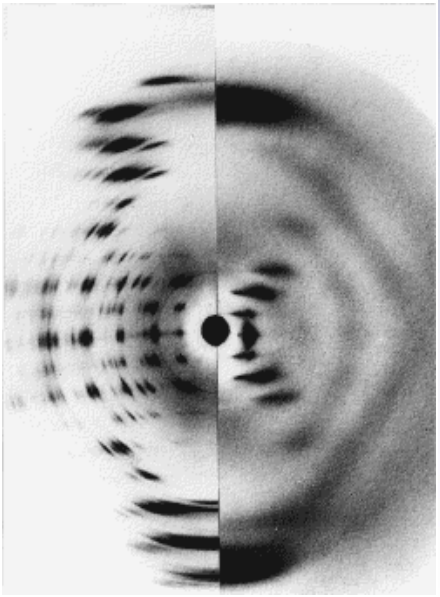
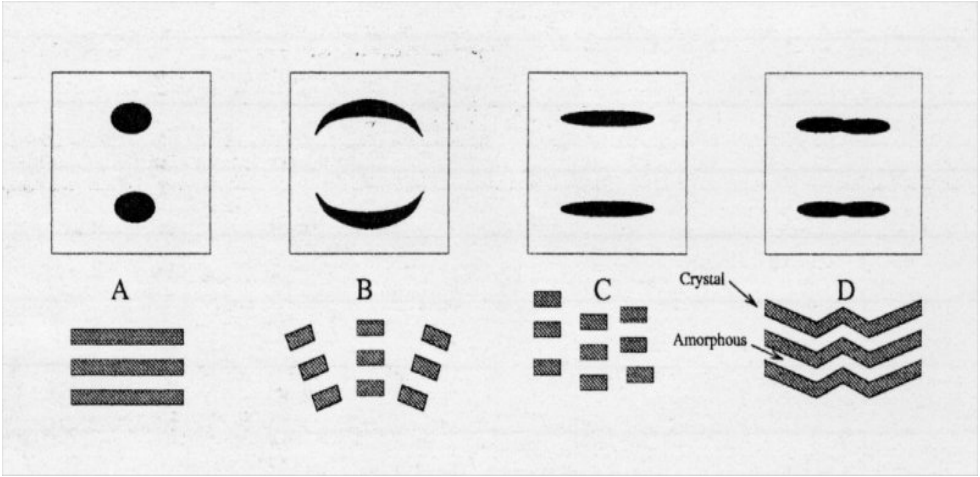


Image from Ken Holmes, MPI Heidelberg

Fibre Diffraction (II)



Goschel & Urban, (1995)

Matyi & Crist, (1978)

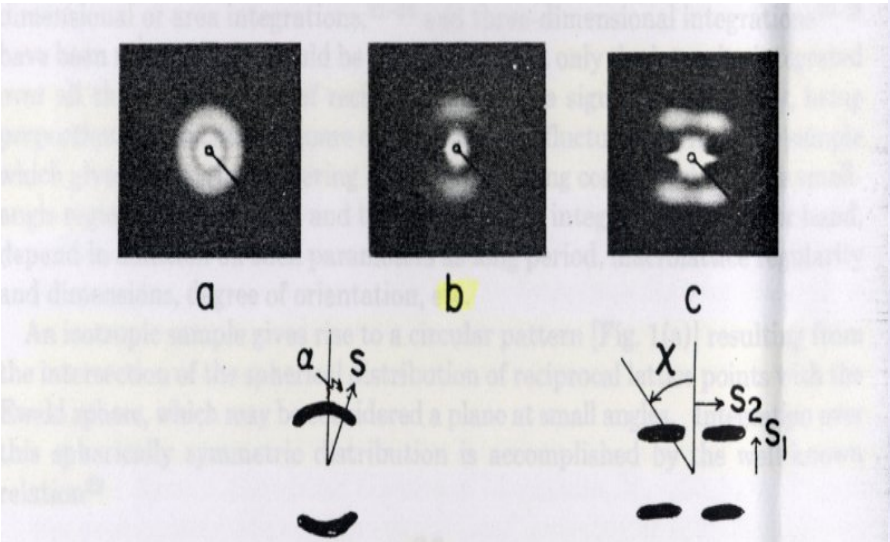


Fig. 1. SAXS patterns of nylon 6: (a) isotropic pattern, (b) incompletely oriented (arced) pattern, (c) well-oriented four-point pattern. The orientation direction (fiber axis) is vertical.

Murthy & Orts, (1994)

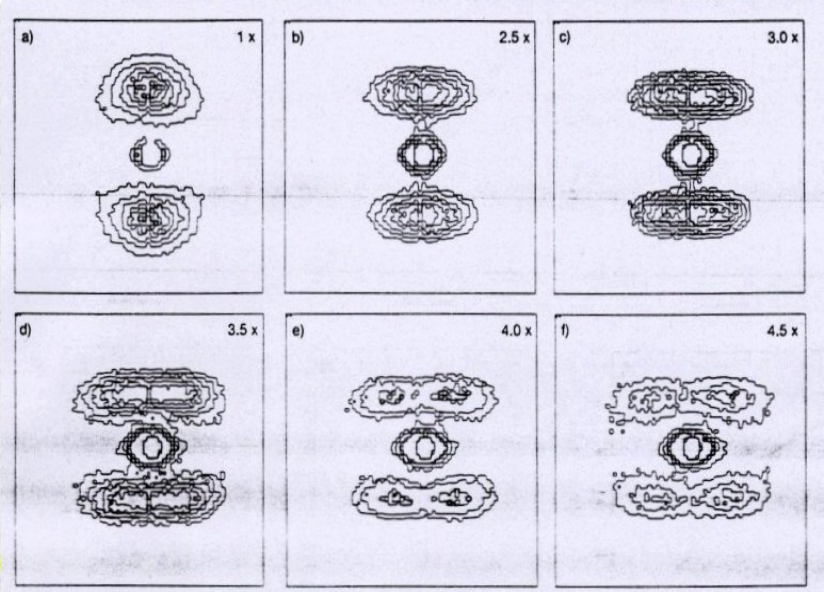
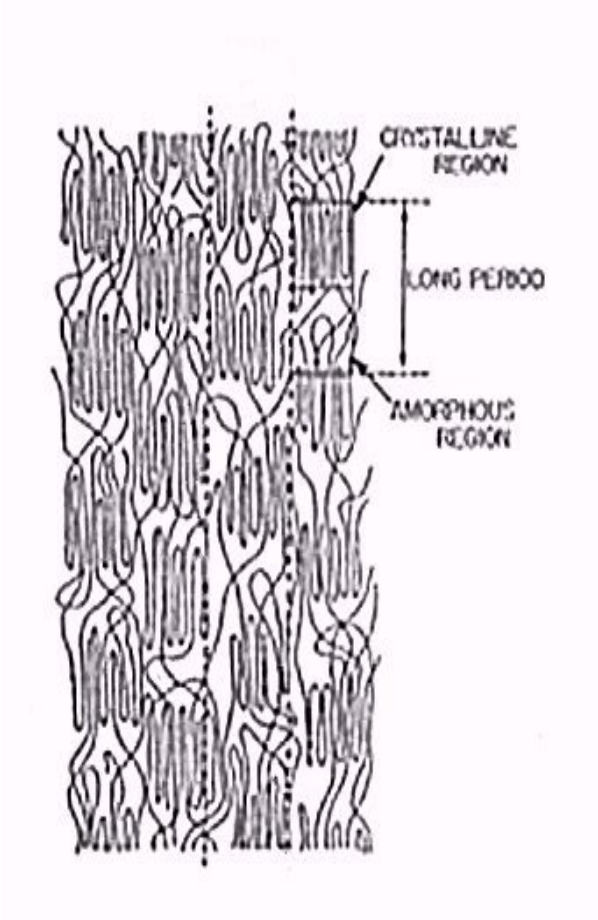


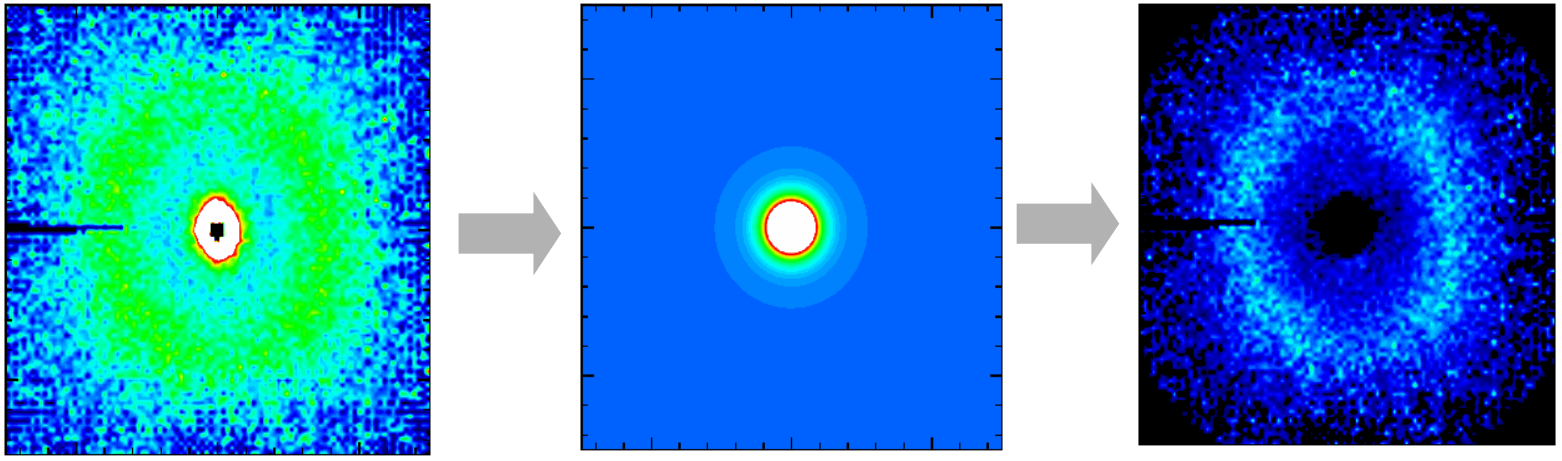
Figure 1. Intensity contour plots of the SANS data for fibers with draw ratios from 1× to 4.5×

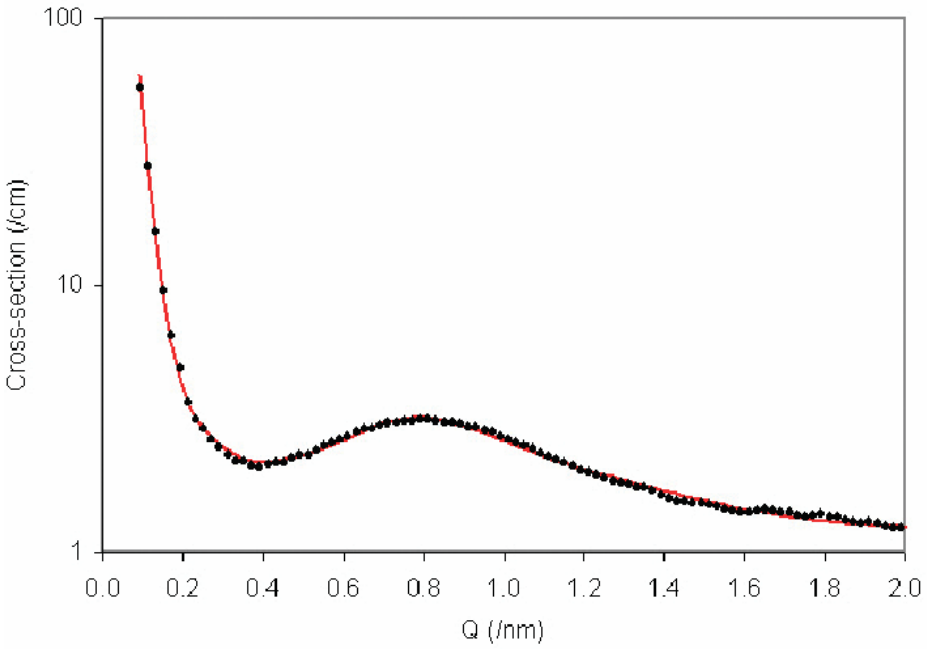
Fibre Diffraction (III)

- Most fibre-forming synthetic polymers are *semi-crystalline*
- Important to subtract the amorphous contribution before analysis:
  - by eye!
  - by measuring a totally amorphous analogue
  - by measuring the semi-crystalline polymer above its T_m
  - (by using an empirical function for the form of the amorphous scattering)



SANS From Hydrated Nylon Fibres

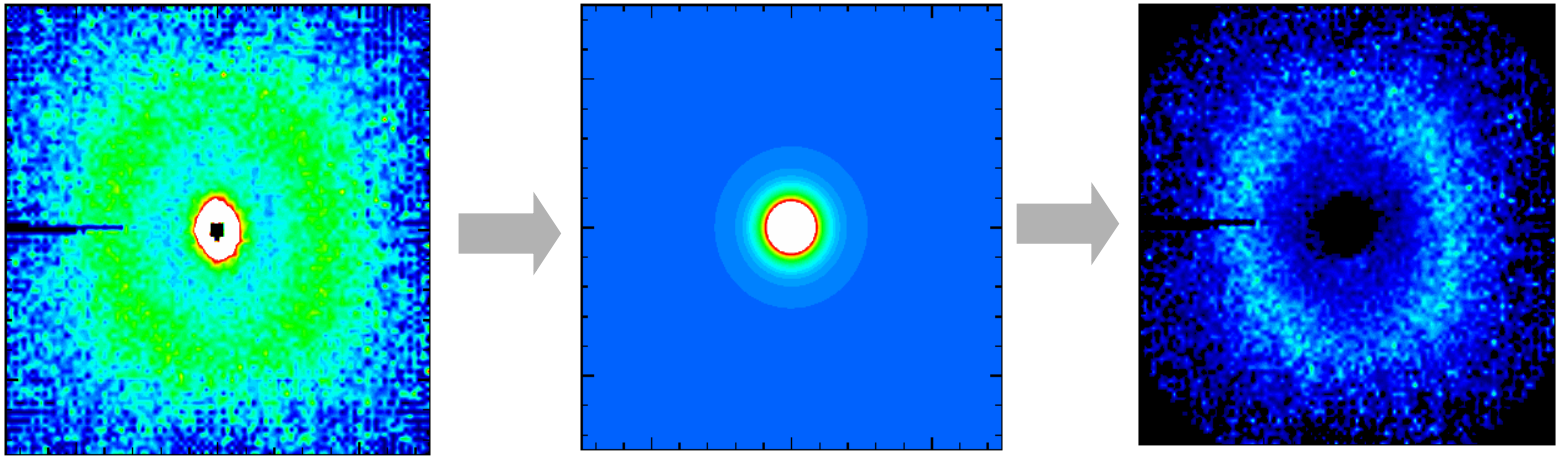


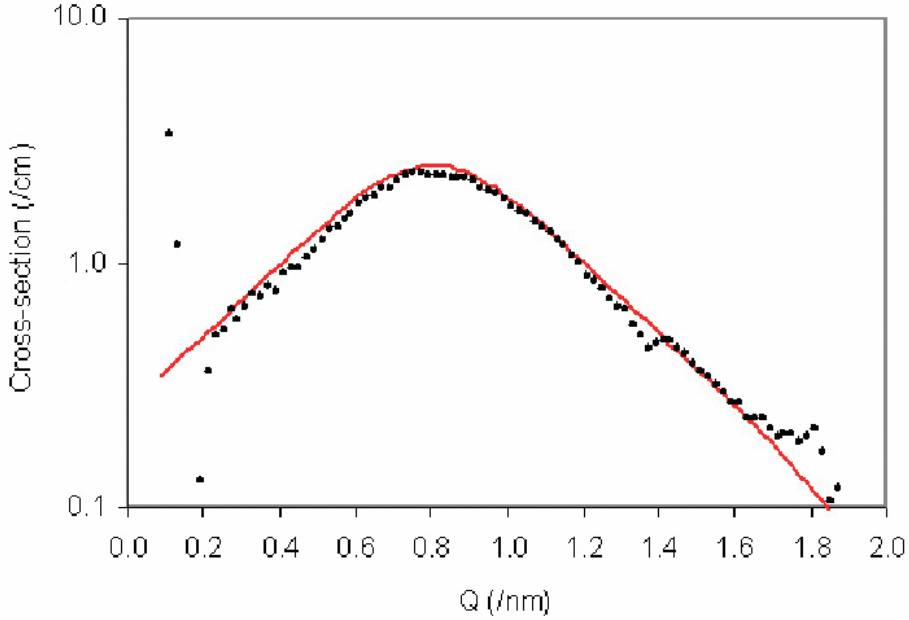


Degraded fibre in 11% D_2SO_4, D_2O, paracrystal plus Q^{-4} fit

S.M.King, D.G.Bucknall & R.K.Heenan, *Fibre Diffraction Review* 12(2004)41-49

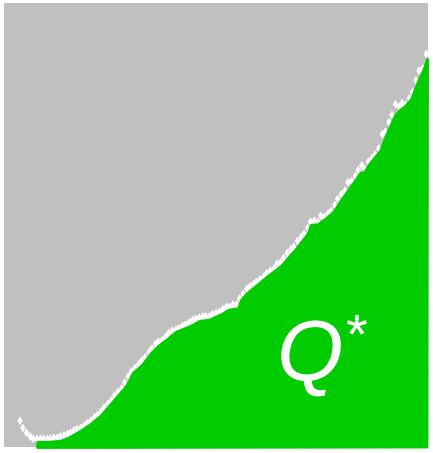
SANS From Hydrated Nylon Fibres

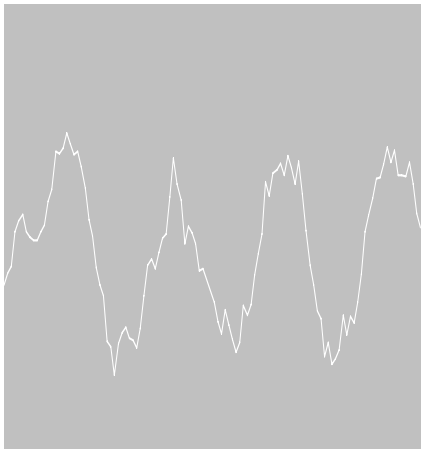


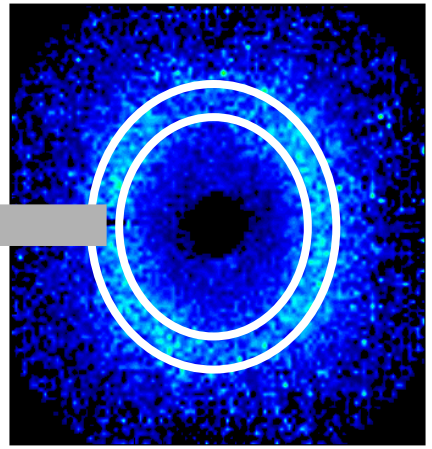


Static fibre in D2O, after subtraction of Q-3.9 background, with Cauchy peak fit (d=8.6nm)

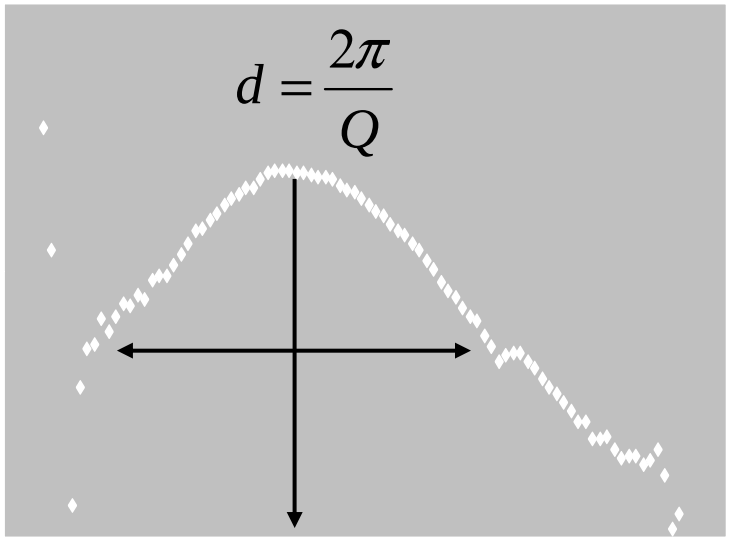
SANS From Hydrated Nylon Fibres







x Q^2



Peak width may give information about the distribution of *d* values.

(See Debye-Scherrer equation)

The (Porod) Invariant, Q^*

$$Q^* = \int_0^\infty \frac{\partial \Sigma}{\partial \Omega}(Q) \times Q^2 \, dQ$$

$$= 2\pi^2 V \phi_1 \phi_2 (\Delta\rho)^2$$

Uses:

- relates "total amount of scatter" to composition and volume fractions

  $\varphi_1 + \varphi_2 = 1$ (there is a version for 3 or more phases)

- determining the phase composition or degree of hydration
- evaluating the degree of crystallinity of a semi-crystalline polymer, *x* :

$$x = \frac{Q^*_{xtal}}{Q^*}$$

Limitations:

- need to know the scattering length density of the phases
- crystalline phase appreciably altered by imperfections, defects, etc
- Need a wide Q range to integrate over (smooth the data at high Q ?)

See: Roe, *Methods of X-ray and Neutron Scattering in Polymer Science*, 2000.

Structure Correlation Functions (I)

- **A Fourier transform method for characterising INFINITE "lamellar" stack morphologies – either oriented in one direction or randomly oriented.**
- **The correlation function is the Fourier transform of the scattering in *one*, G_1 , or *three*, G_3 , dimensions - Note: $G_1(0)=1$ & $G_3(0)=1$**
- **Can interpret $G_1(R)$ as the probability that a rod of length *R* moving through the sample will encounter equal scattering length density at both ends**
- **Thus, any regularly repeating spacing generates a peak in $G_1(R)$**

$$\Gamma_1(R) = \frac{\int_0^\infty Q^2 \frac{d\Sigma}{d\Omega}(Q)\, Cos(QR)\, dQ}{Q^*}$$

$$\Gamma_3(R) = \frac{\int_0^\infty Q^2 \frac{d\Sigma}{d\Omega}(Q) \frac{Sin(QR)}{QR}\, dQ}{Q^*}$$

Structure Correlation Functions (II)

Regular d-spacing

Irregular d-spacing

Irregular d-spacing
+
thickness variations

Irregular d-spacing
+
diffuse boundaries

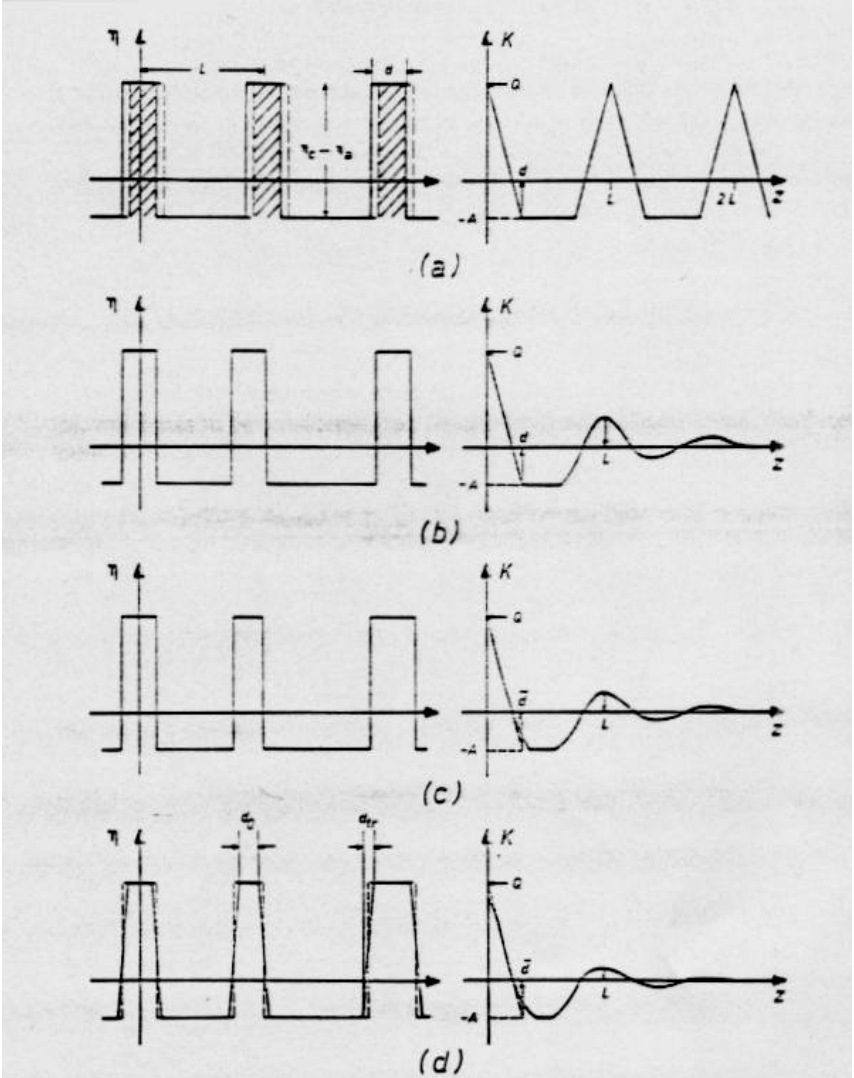
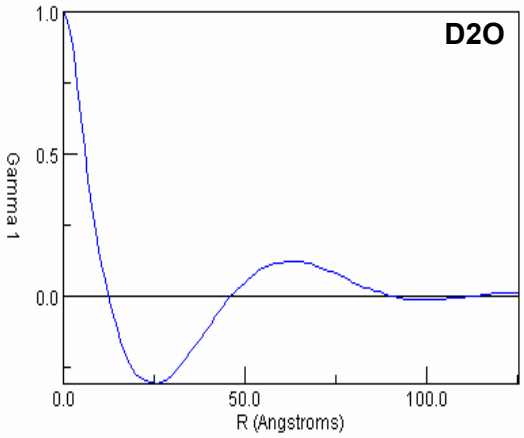


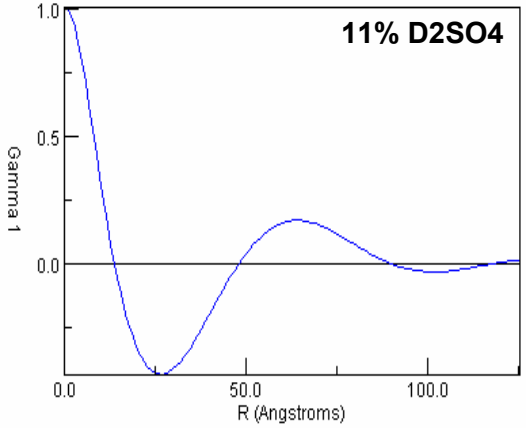
Fig. 1. Electron density distribution $\eta(z)$ and the related correlation function $K(z)$ for lamellar systems of different regularity. (a) Periodic two-phase system. (b) Effect of long-spacing variations. (c) Effect of additional thickness fluctuations. (d) Effect of introduction of diffuse phase boundaries.

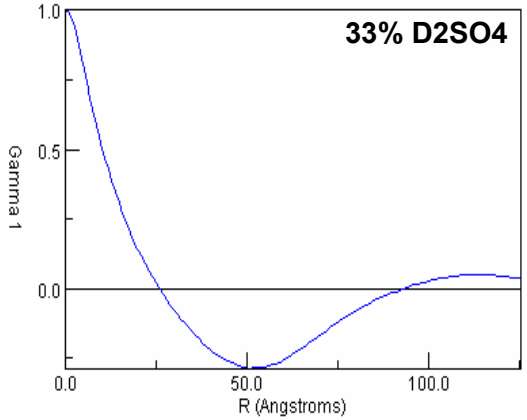
Strobl & Schneider, 1980

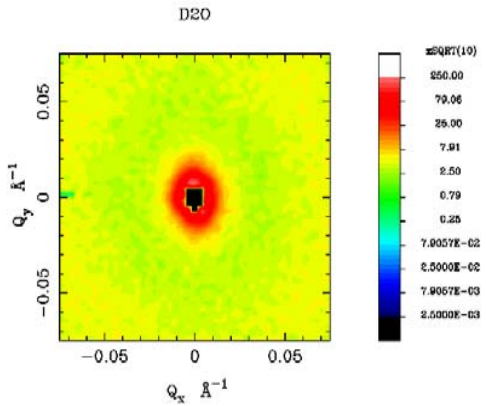
Structure Correlation Functions (III)

Acid-induced degradation of nylon-6 (a polyamide)









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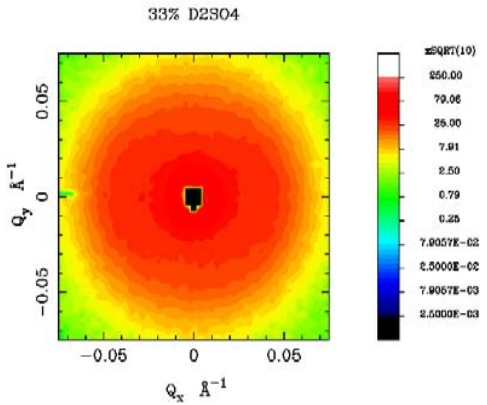
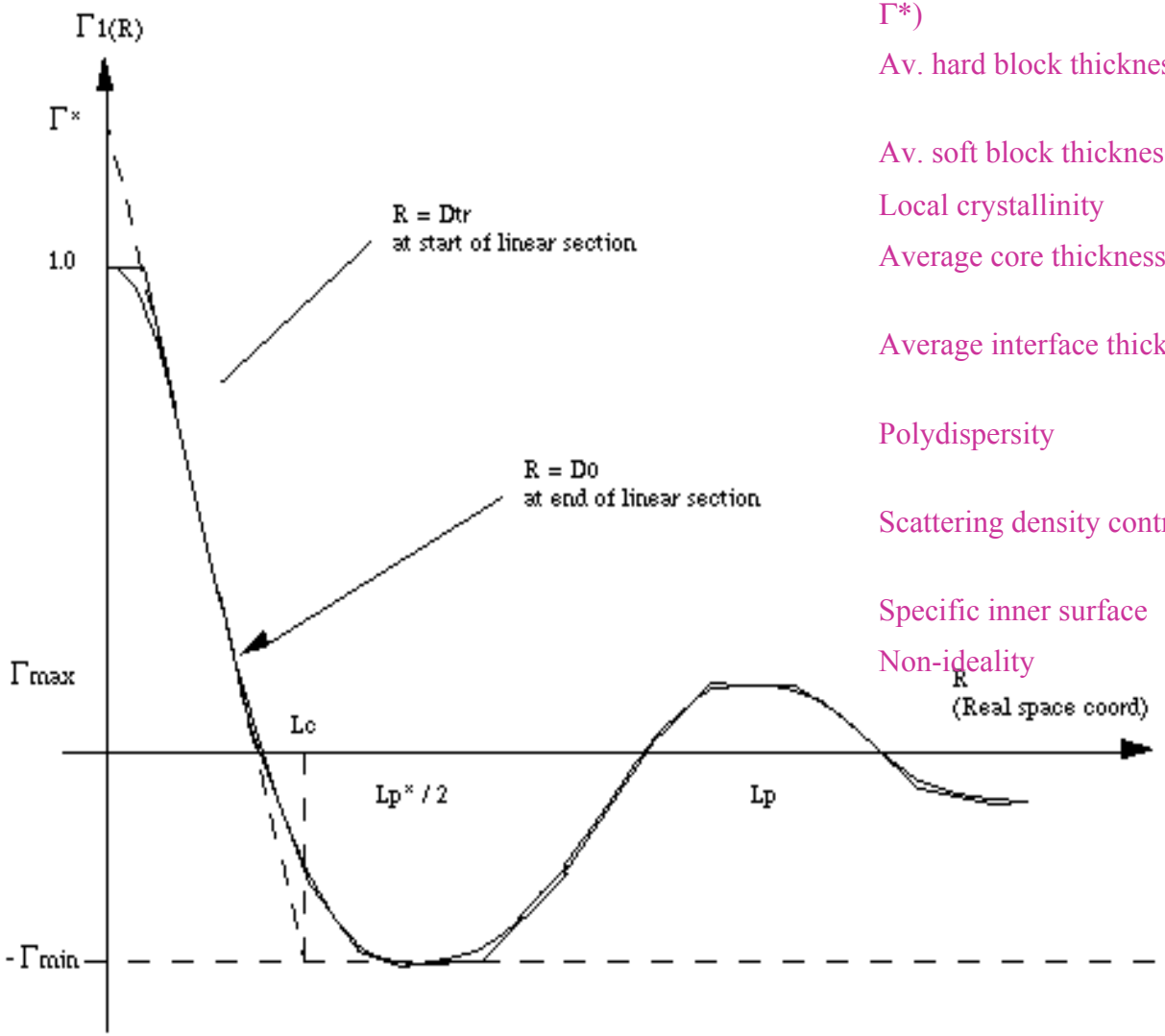
King, Bucknall & Heenan, *Fibre Diffraction Review* (2004)



Structure Correlation Functions (IV)



Parameter	Symbol	Measurement
Long period	L_p	As in diagram
Bulk crystallinity Γ*)	Φ	Γ_{min} / (Γ_{min} +
Av. hard block thickness	L_c	As in diagram
Av. soft block thickness	L_a	L_p - L_c
Local crystallinity	Φ_l	L_c / L_p
Average core thickness	D_0	As in diagram
Average interface thickness	D_{tr}	As in diagram
Polydispersity		Γ_{min} / Γ_{max}
Scattering density contrast	Δρ	Q^* / Φ (1-Φ)
Specific inner surface		2Φ / L_c
Non-ideality		$(L_p - L_p^*)^2 / L_p^2$

"CORFUNC"
CCP13

CCP13

CCP13 is the **Collaborative Computational Project for Fibre and** (latterly) **Polymer Diffraction** established on 1st January 1992. It is supported by the BBSRC and EPSRC.

CCP13 exists to focus the development of software for the analysis of data from a variety of samples exhibiting order and/or preferential alignment.

The CCP13 program suite is **free.**

The programs run under a number of Unix operating systems **including Linux**. Several programs **also run under Windows NT/2000/XP**.

Programs:

- File conversion utilities

- Preliminary analysis of fibre diffraction patterns
- Integration of fibre diffraction pattern intensities
- Fourier-Bessel smoothing of layer lines

- Image space to reciprocal space transformation

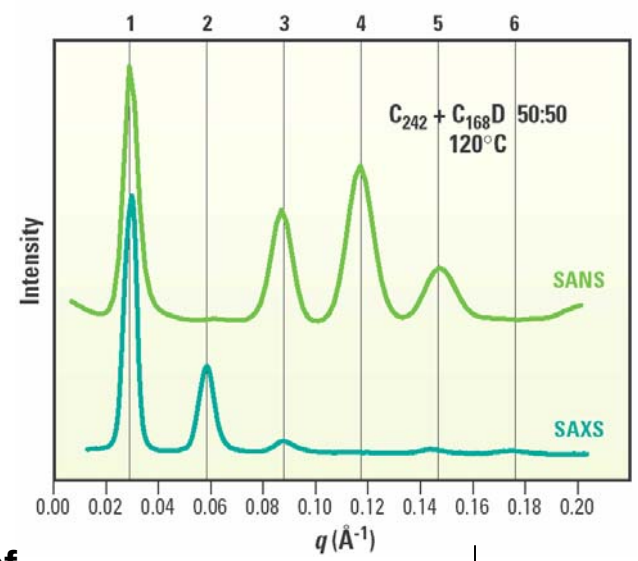
- Peak fitting
- Correlation function analysis

http://www.ccp13.ac.uk

Example: Fourier transform methods applied to lamellar mixed alkanes

XB Zeng, G Ungar, SJ Spells (Sheffield/Sheffield Hallam); synthesis by G.M.Brooke, C.Farran, A.Harden (Durham)

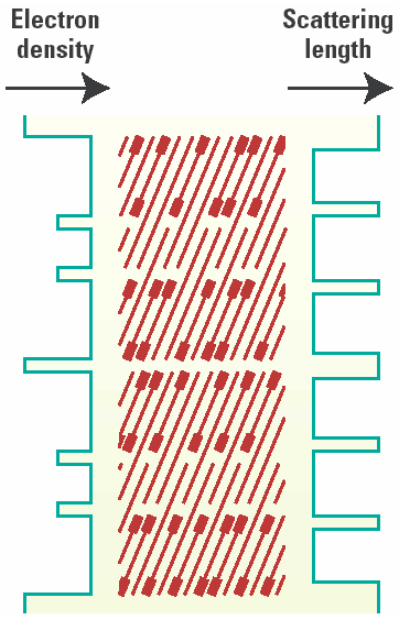
1:1 Mixed lamellar crystals of monodisperse $C_{242}H_{486}$ and $C_{167}H_{288}D_{49}$ with C_{12} terminals deuterated

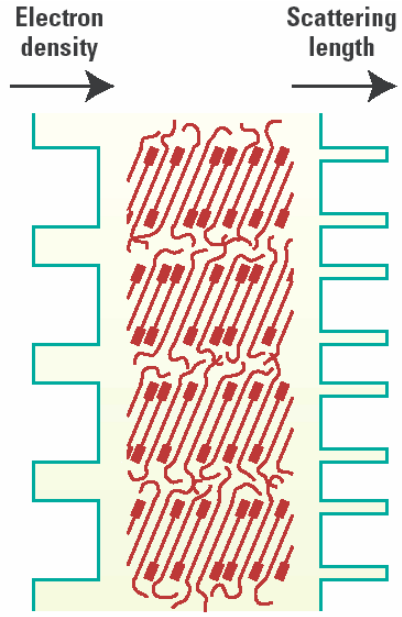


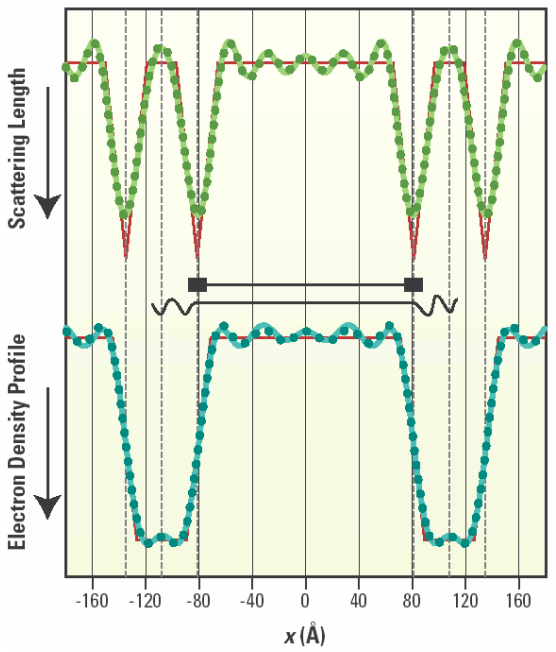
Triple layer superlattice
T < 100°C

Semi crystalline
T > 100°C
amorphous, loose tails of long molecules, D – tails also in interface

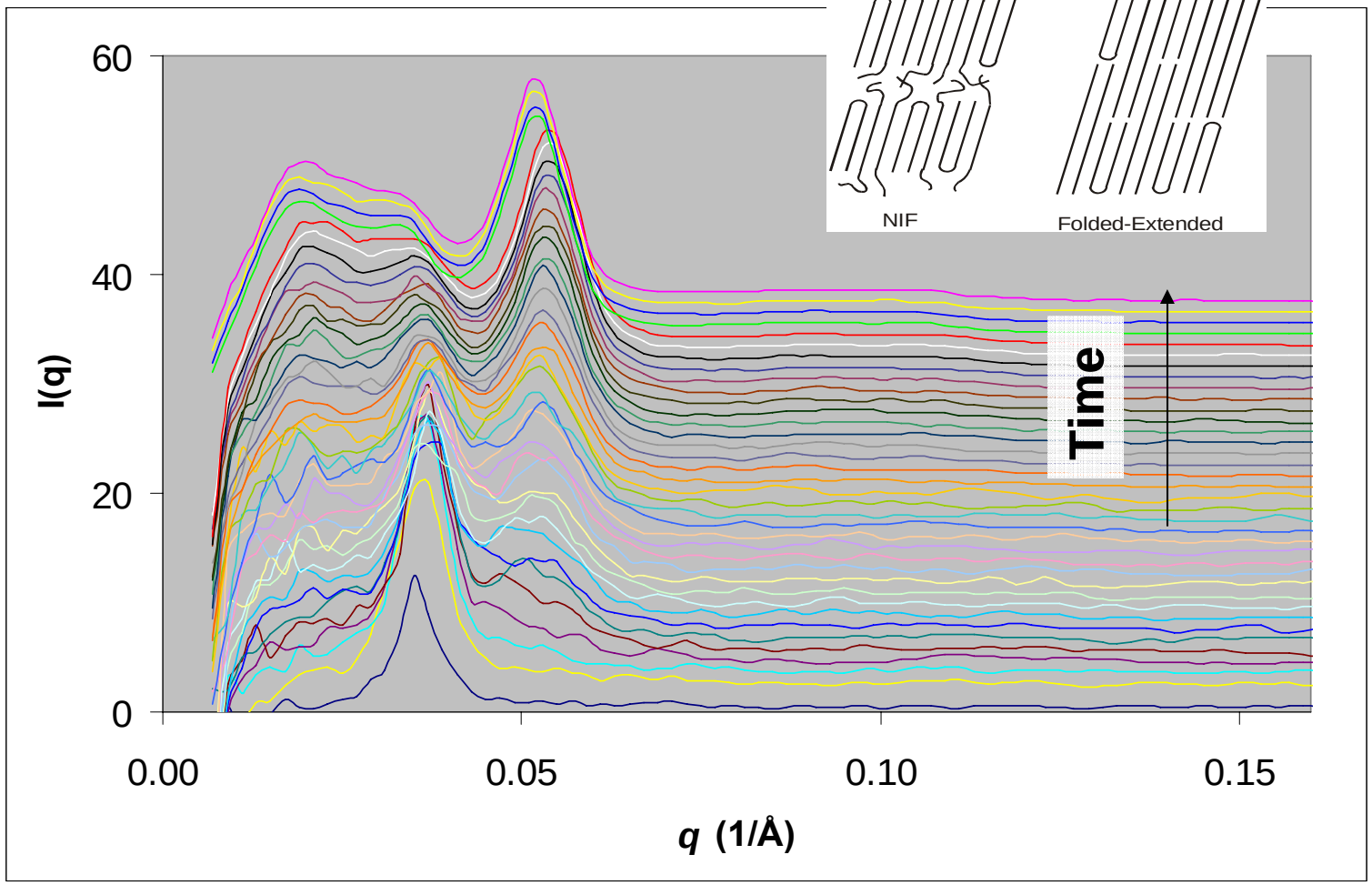
Fourier transform







Example: Alkane crystallisation. **Temperature jump of C_{216} n-alkane with C_{12} terminals deuterated. 20 x 10 sec runs, 19 x 60 sec. (Data from LOQ at ISIS, our TS-2 could do 0.5 sec and expand Q range)**



Semi-crystalline Non Integer Folded to almost fully crystalline Folded-Extended.