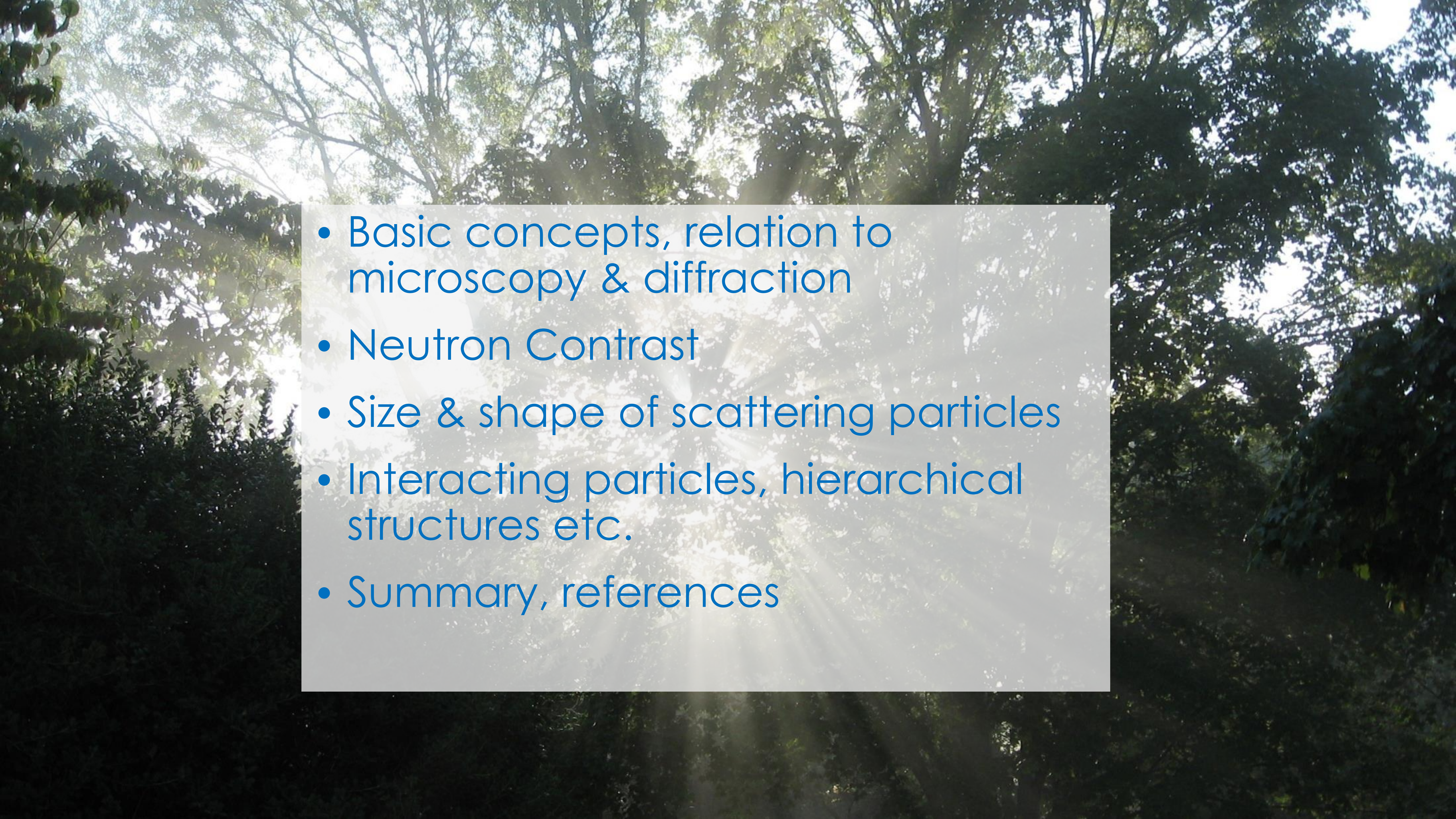
Introduction to Small-Angle Neutron Scattering

Volker S. Urban
Section Head
Large Scale Structures
Neutron Scattering Division

Practical Guide for Biological Small-Angle Neutron Scattering Experiments and Data Analysis
October 9-10, 2024

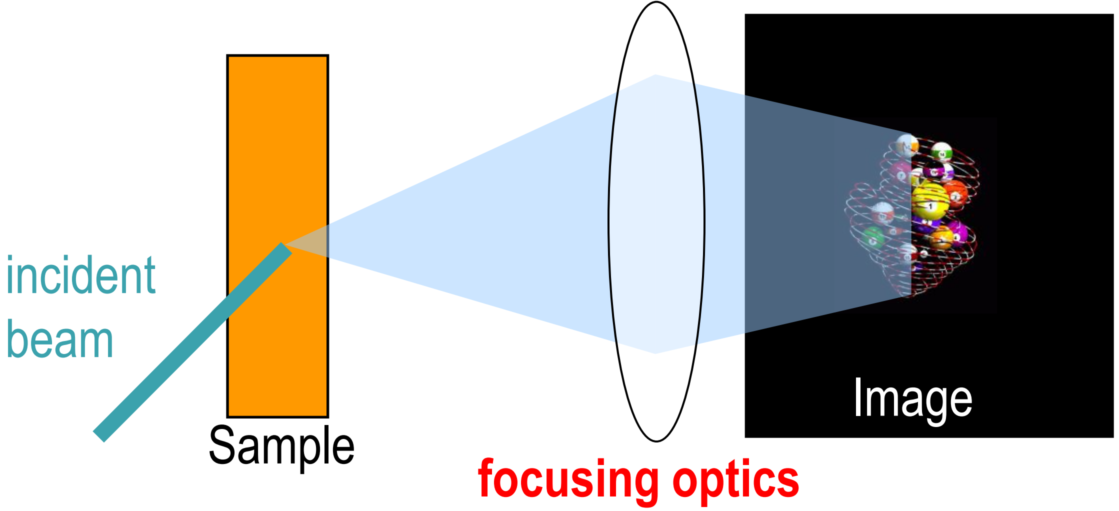


- Basic concepts, relation to microscopy & diffraction
- Neutron Contrast
- Size & shape of scattering particles
- Interacting particles, hierarchical structures etc.
- Summary, references

Small Angle Scattering and Microscopy

- Common features
  - Size range 1nm-1μm
  - Contrast labeling options (stains / isotope labels)
- SANS practical aspects
  - No special sample preparation necessary (such as cryo freezing)
  - Sample environments available for pressure, temperature, magnetic field etc.
  - Non-invasive
  - In-situ, time-resolved
- Fundamental difference
  - “Real space” image with certain resolution
  - Scattering pattern, averaged over volume
- Complementarity

Microscopy : enlarged image



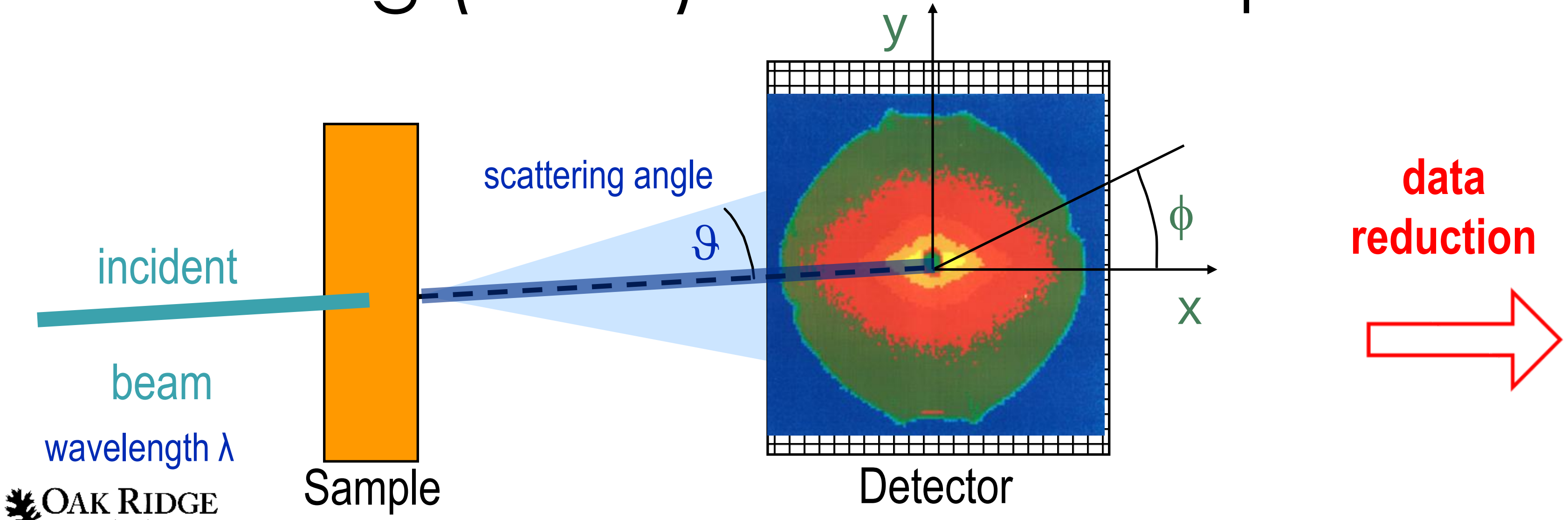
incident beam

Sample

focusing optics

Image

Scattering (SANS): interference pattern



scattering angle ϑ

incident

beam

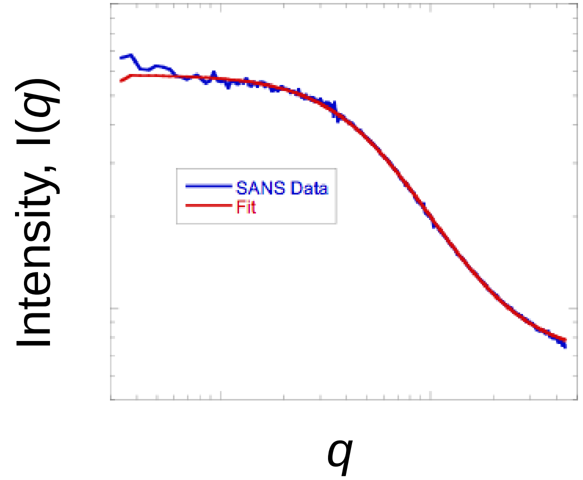
wavelength λ

Sample

Detector

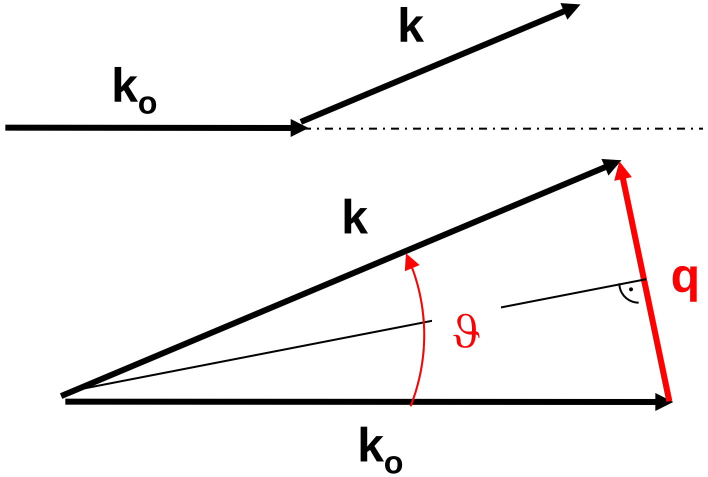
data reduction

“reduced” data



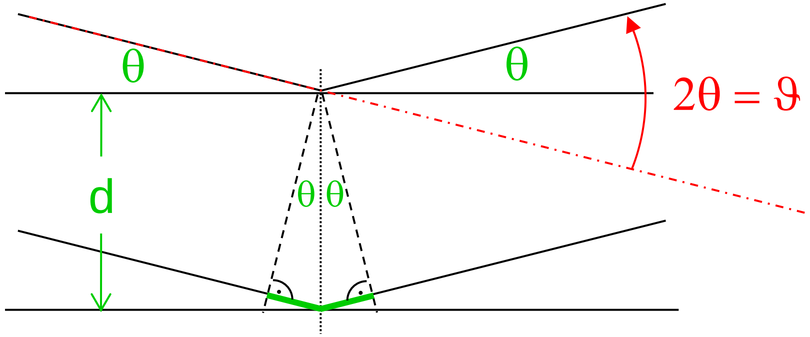
From scattering angle to scattering vector

Wave vector **k**: |k| = k = 2π/λ



$$q = 2k\sin\left(\frac{\vartheta}{2}\right) = \frac{4\pi}{\lambda}\sin\left(\frac{\vartheta}{2}\right)$$

Bragg: waves with wavelength λ reflected by sets of lattice planes



Δ = 2d sin(θ)

if Δ = n λ then reflection, else extinction

$$\frac{1}{d} = \frac{2}{\lambda}\sin\left(\frac{\vartheta}{2}\right)$$

$$\boxed{q = \frac{2\pi}{d}}$$

q in nm^{-1} or Å^{-1}

scattering 'vector', *q*

also known as momentum transfer *Q, q, h, k, s*

Scattering and Diffraction (Crystallography)

- Diffraction from crystals, Scattering from anything else (less ordered)
- Same basic physics: interactions of radiation with matter
  - SAXS/WAXS, SAND/WAND
  - Instruments: resolution vs flux tradeoff (diffraction/scattering)
  - Diffraction needs crystal lattices, scattering does not.
  - Data analysis is very different in most cases.
- At small ***q*** (small angles, large λ): observe nm-sized volume elements, "blobs" NOT atoms
  - Scattering length → scattering length density **SLD**, symbol ρ
  - SA(N)S is sensitive to spatial non-uniformity of SLD: ΔSLD = Contrast → contrast variation!

Small Angle (Neutron) Scattering Intensity

- Interference of wavelets from distribution of nuclei (= structure) adds up to "net scattering" amplitude (Fourier transform of structure).

$$\boxed{I(q)=\left|\int_V(\rho(\vec{r})-\rho_s)e^{-i\vec{q}\bullet\vec{r}}d^3r\right|^2}$$

- Measured intensity *I*(*q*) is the magnitude square of amplitude.

common form:

$$I(q)=\underset{I(0)}{\boxed{\frac{N}{V}(\Delta\rho)^2V_p^2}}P(q)\underset{\text{structure factor}}{S(\vec{q})}\text{ where }\underset{\text{form factor}}{P(q)}=\left|\underset{\text{form factor amplitude}}{F(q)}\right|^2$$

- *I(q)* also is the Fourier transform of the pair correlation function *P*(*r*).
- Incoming waves scatter off individual nuclei according to scattering length ***b*** (can be + or -).

Neutron Contrast – Atomic Scattering Lengths

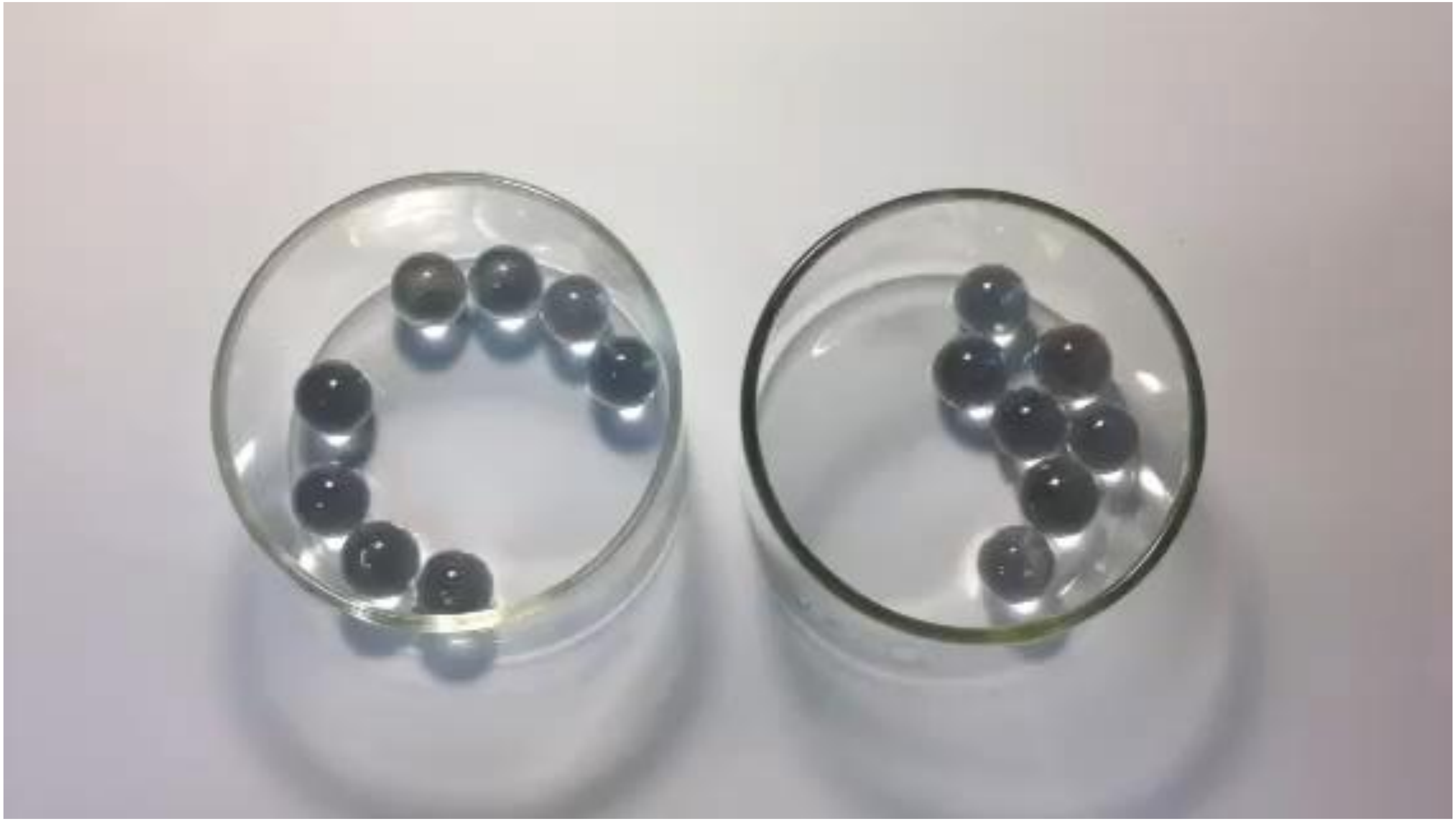
Element	Neutrons (10^{-12} cm)	X-rays (10^{-12} cm)	Electrons	
^{1}H	-0.374	0.28	1	○
^{2}H (D)	0.667	0.28	1	○
C	0.665	1.67	6	●
N	0.940	1.97	7	●
O	0.580	2.25	8	●
P	0.520	4.23	15	●

For Small Angle Scattering

SANS (concept applies also to X-rays)

SL → SLD → ΔSLD

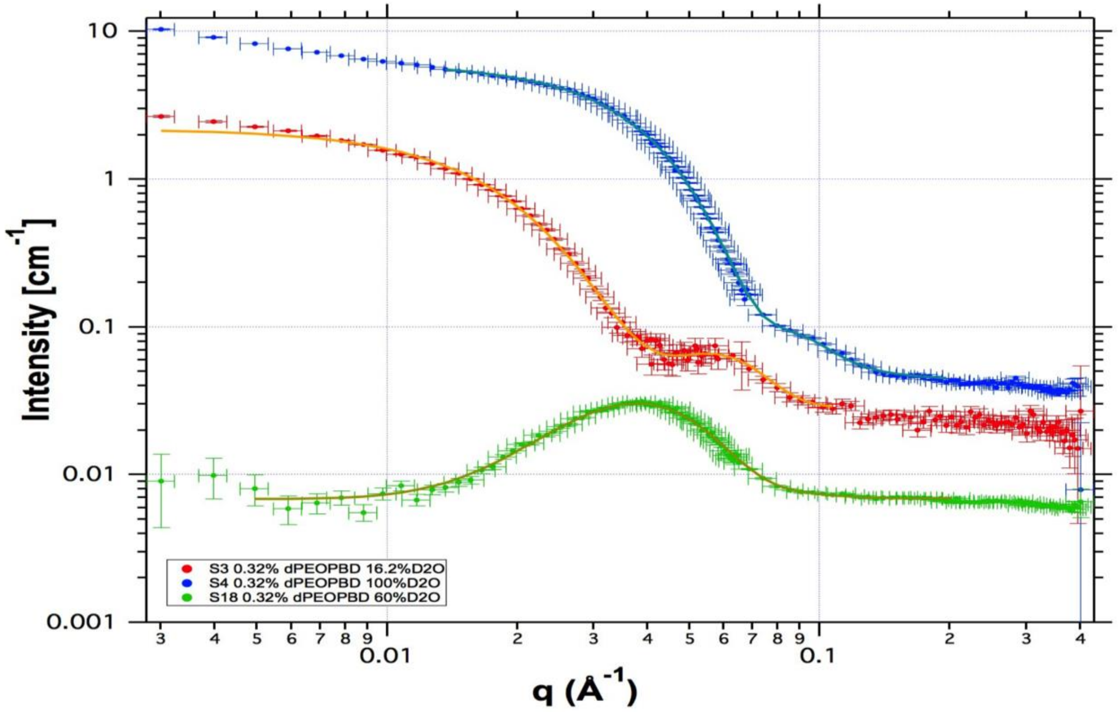
$$\boxed{\bar{\rho} = \frac{1}{V}\sum_{i=1}^{N} b_i}$$

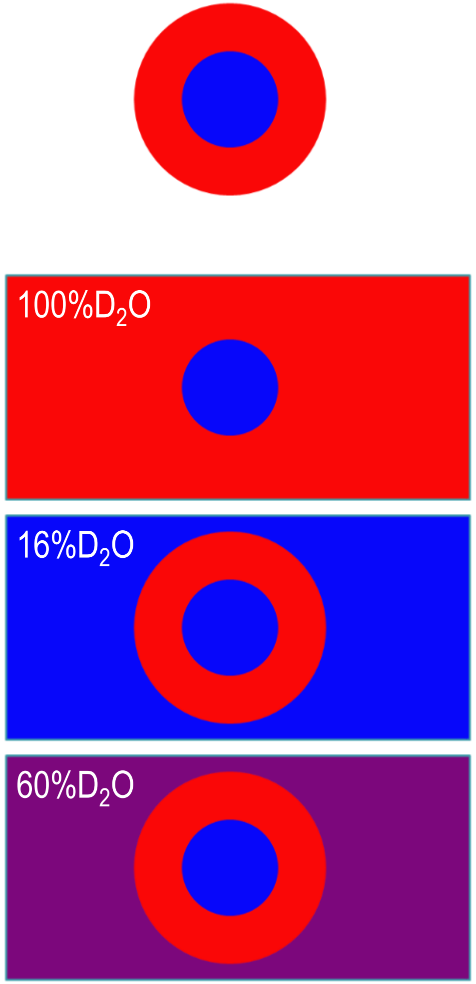


MOV
IE

Increasing SAS information content by neutron contrast variation

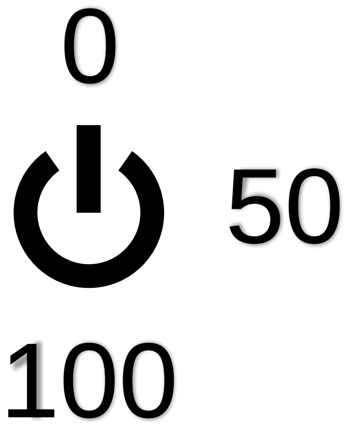
- Block copolymer micelle with deuterated PEO block
- In solution neutrons probe:
  - Core only in 100% D_2O
  - Shell only in 16% D_2O / 84% H2O
  - Core-shell-interference term only in 60% D_2O / 40%H_2O



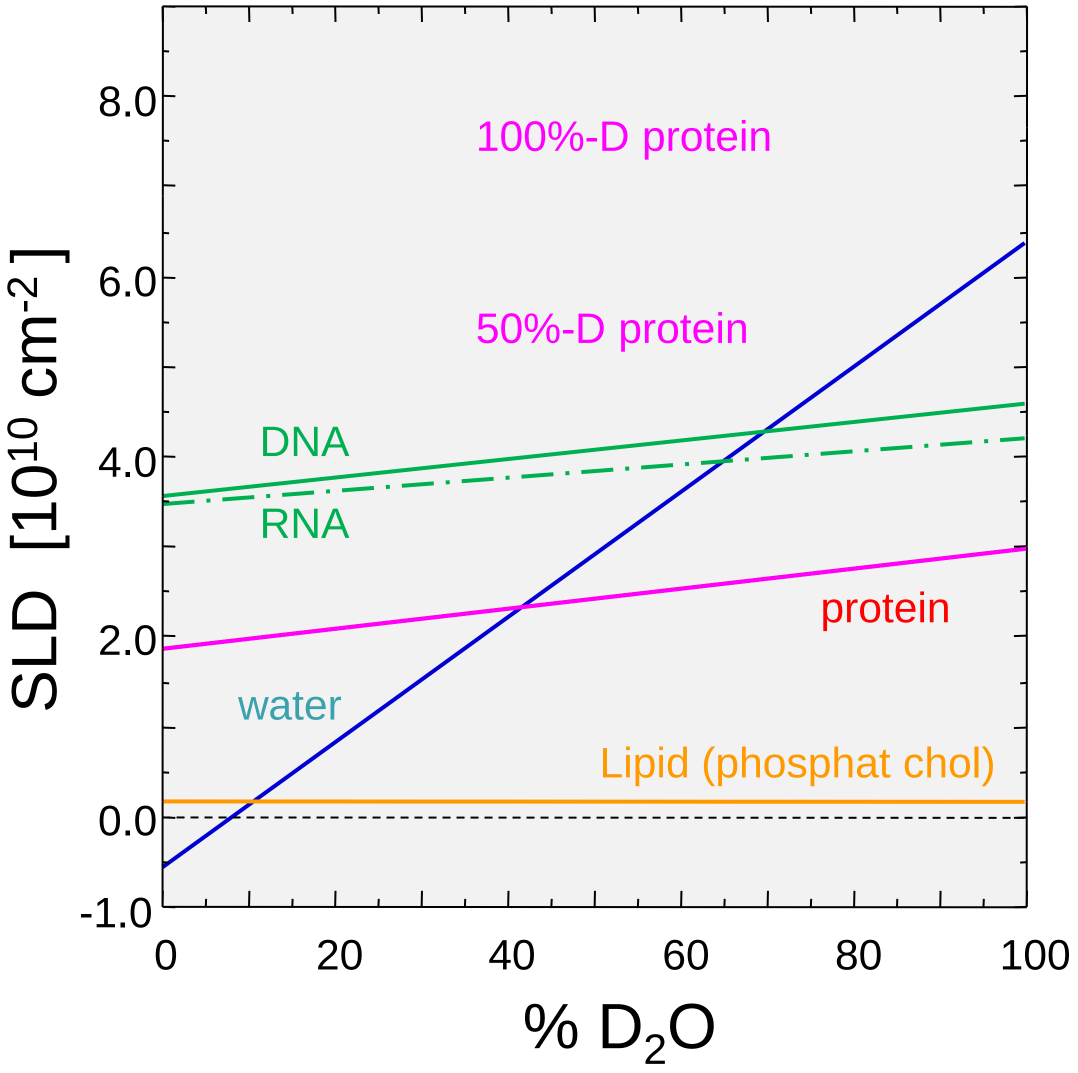


We can tune contrast by specific deuteration

D-labeling tuning knob

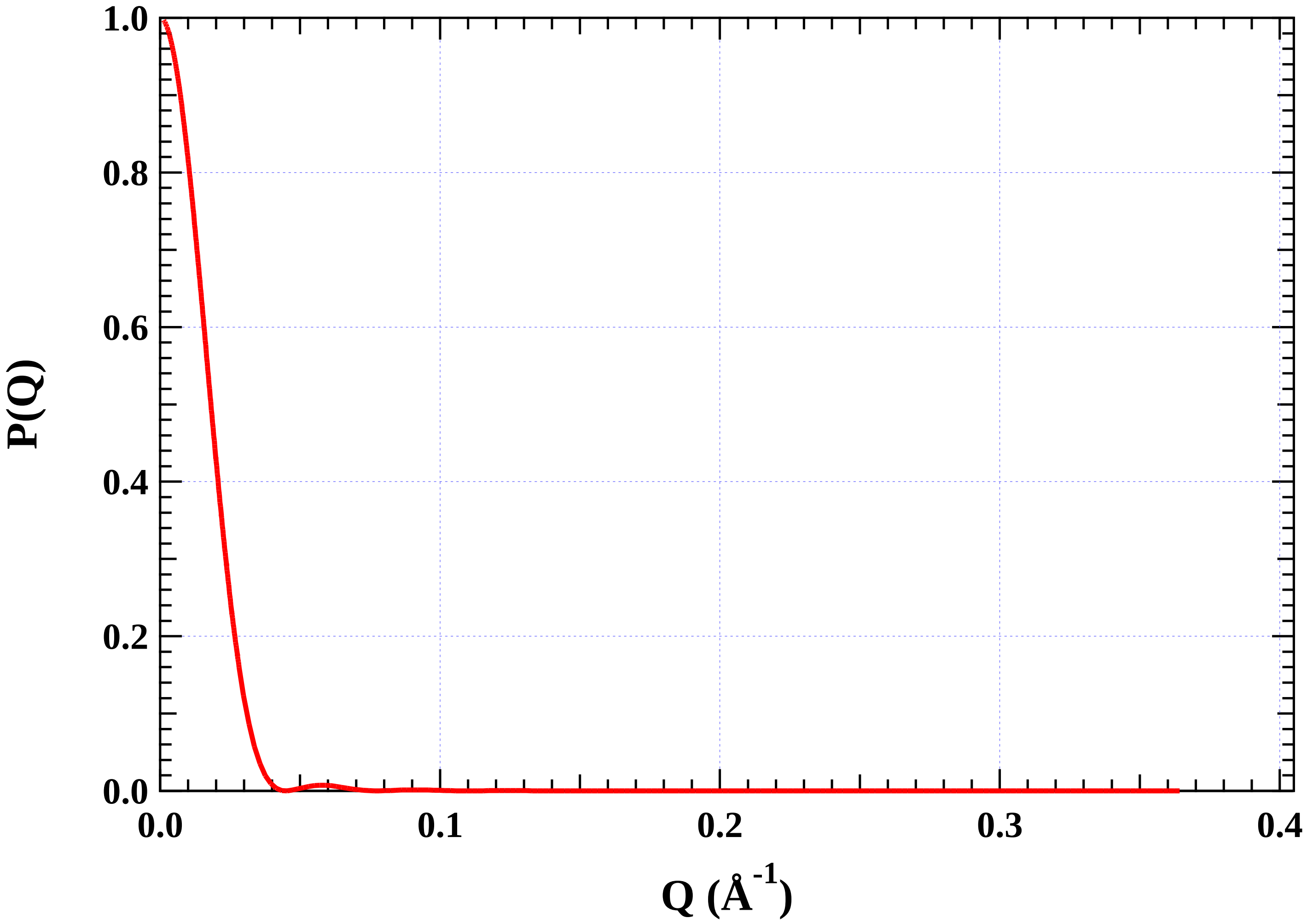


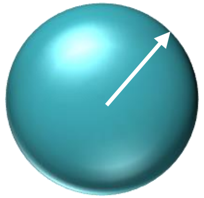
- 100% deuteration is not always what you want
- Proteins, polymers, organic molecules



Sphere

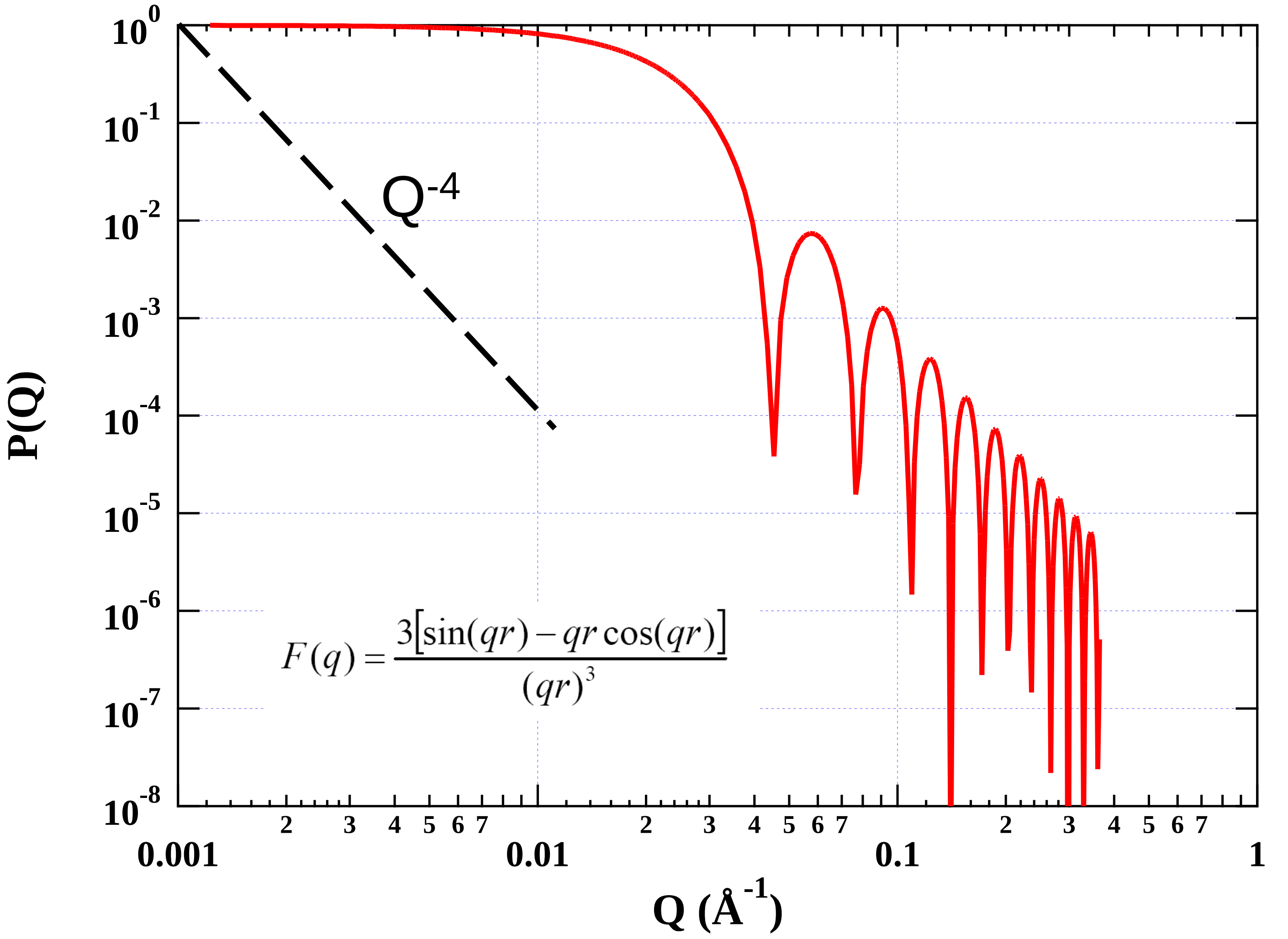
precisely: monodisperse sphere of uniform density with sharp and smooth surface





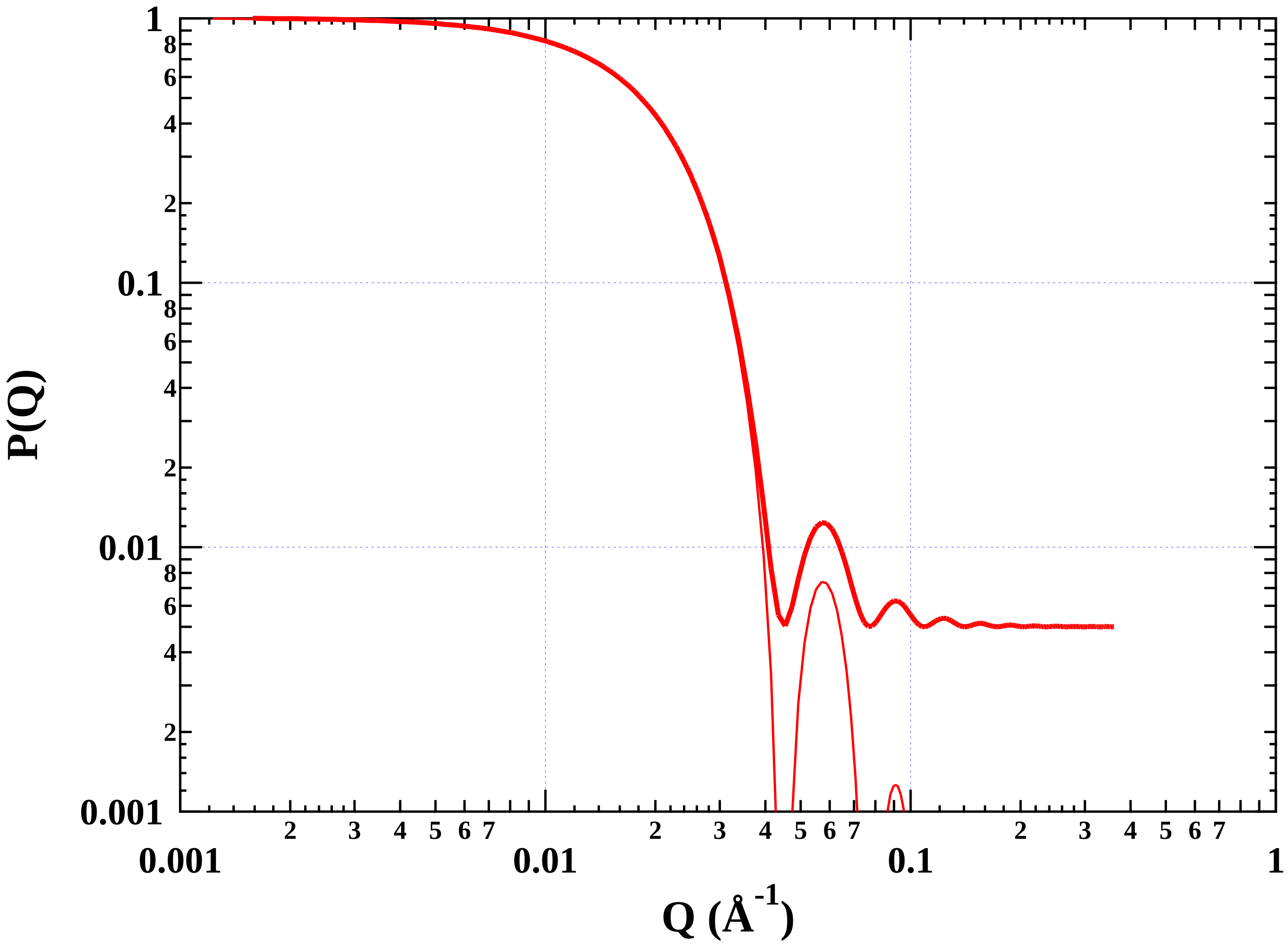
100 Å
radius

Sphere



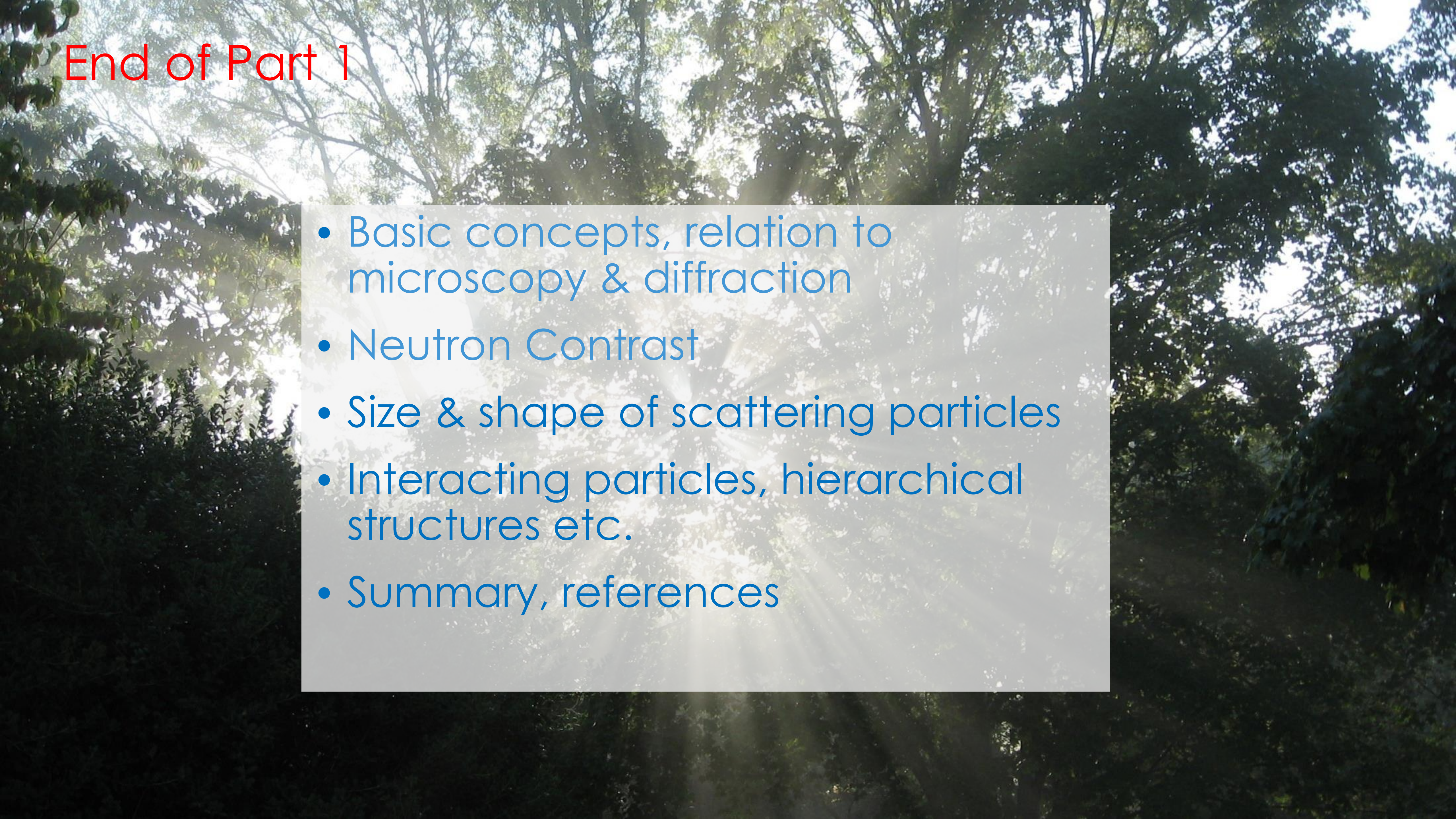
$$F(q) = \frac{3[\sin(qr) - qr\cos(qr)]}{(qr)^3}$$

In practice: sphere + constant background



Some Notes on Background Subtraction

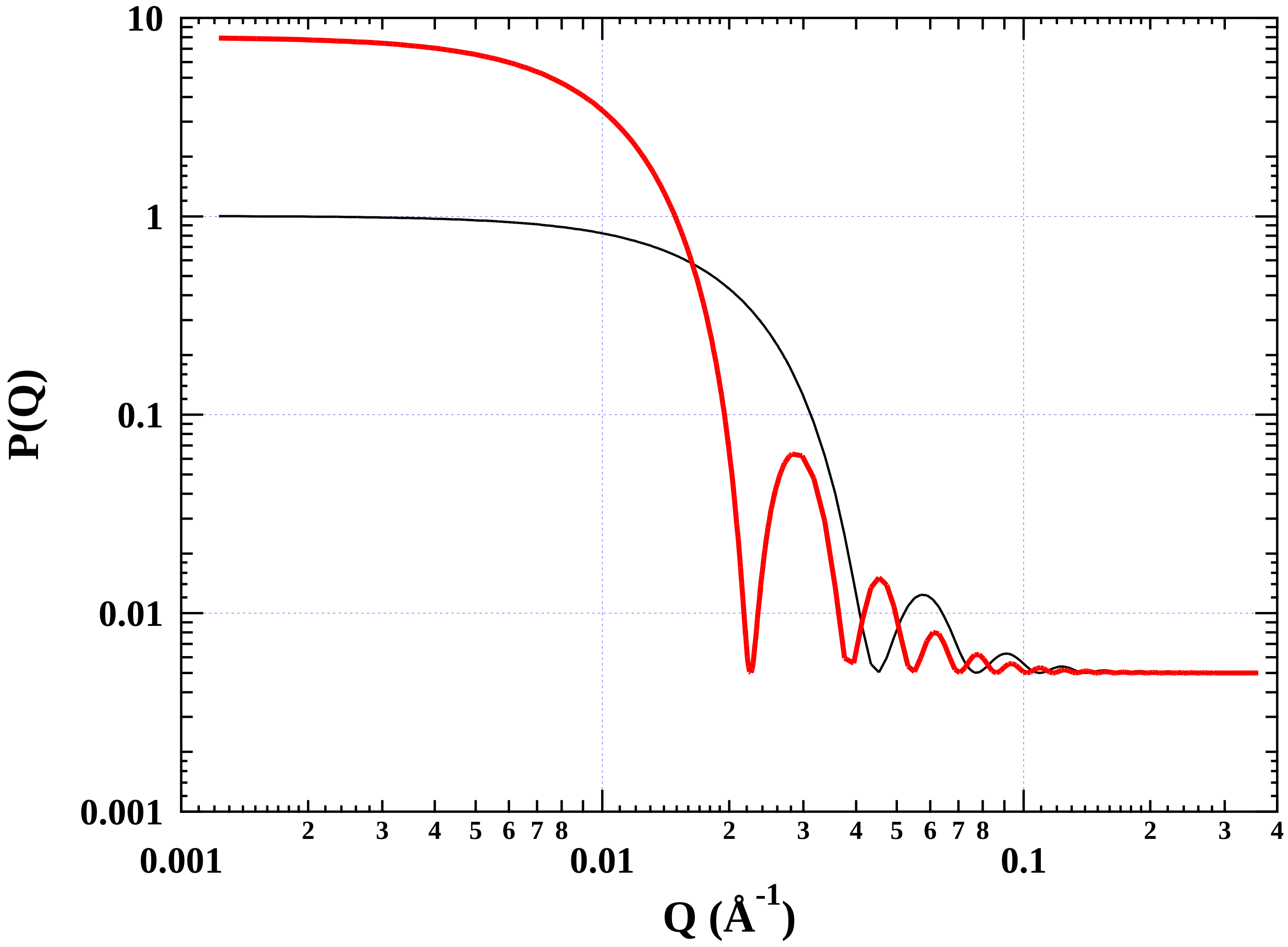
- A crucial step in data reduction/analysis
- Different sources of background (ambient, cell, intrinsic to sample) often require different treatments.
- *Coherent s*cattering intensity drops over orders of magnitude with q (log scale)*, incoherent* background is constant.
- *More* hydrogen means more *incoherent* background, this can influence the choice of contrast. (Less background is better.)

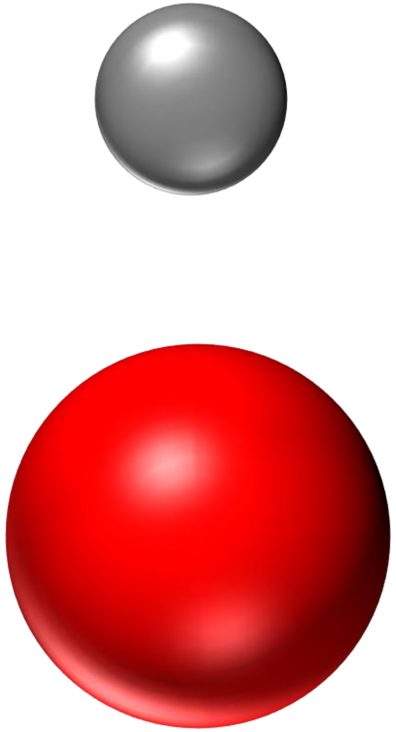


End of Part 1

- Basic concepts, relation to microscopy & diffraction
- Neutron Contrast
- Size & shape of scattering particles
- Interacting particles, hierarchical structures etc.
- Summary, references

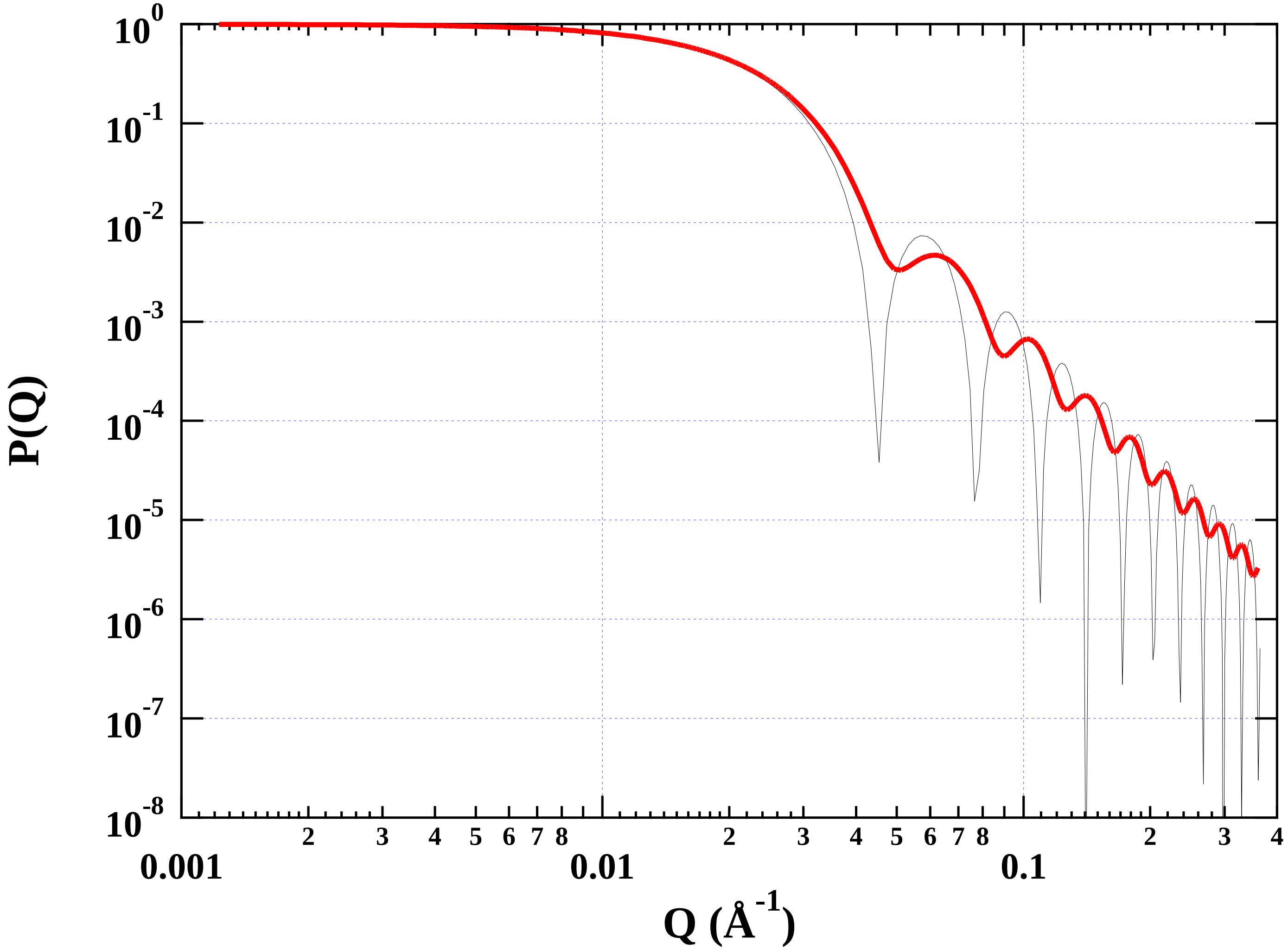
Spheres of different sizes

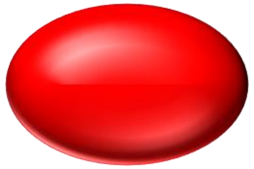




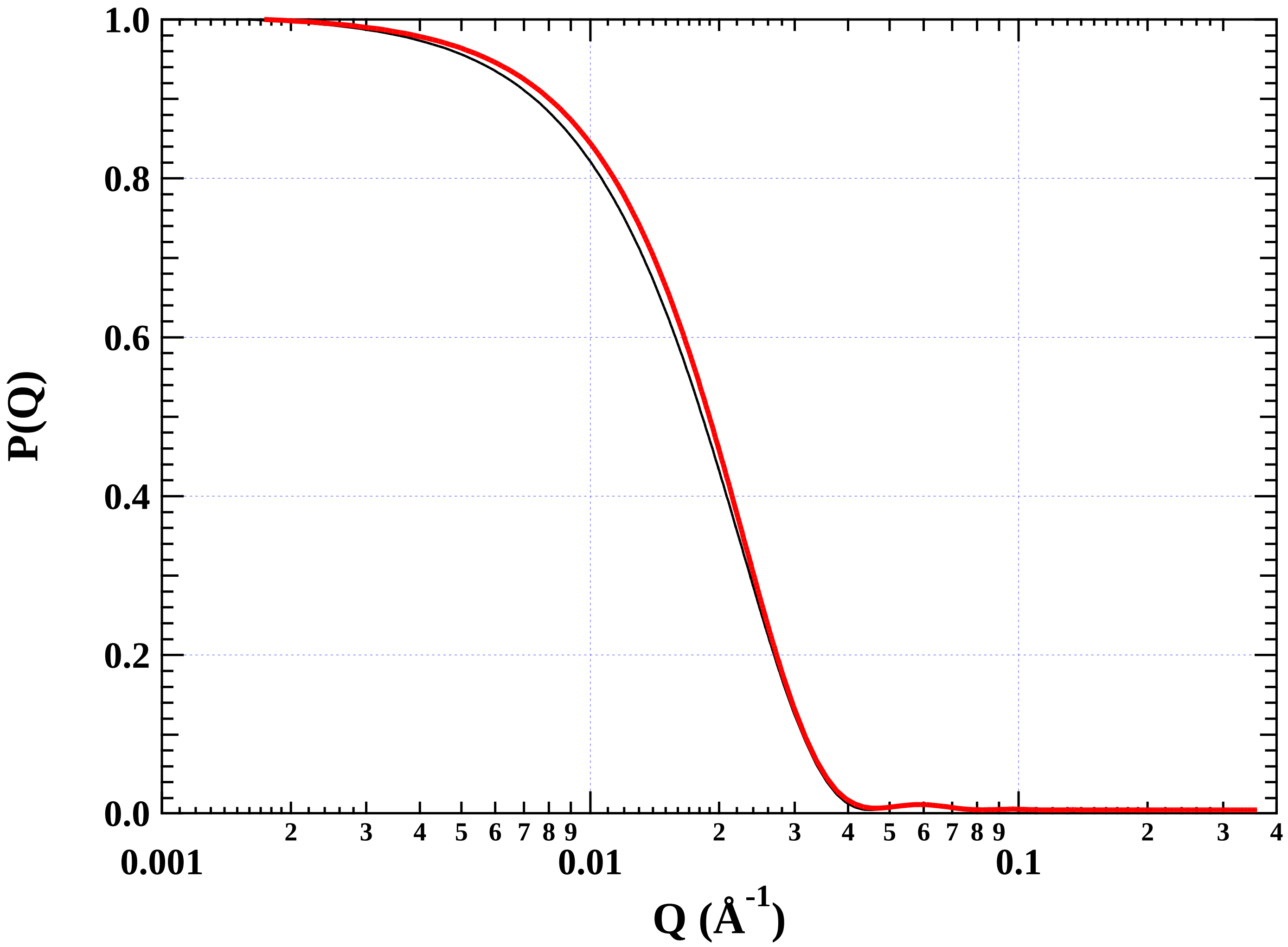
Ellipsoid

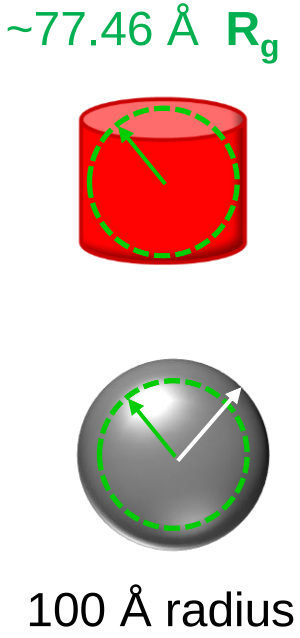
aspect ratio 1.5





Circular Cylinder -*with same Rg as the sphere*





Radius of Gyration, R_g = rms average distance from center of *scattering mass*

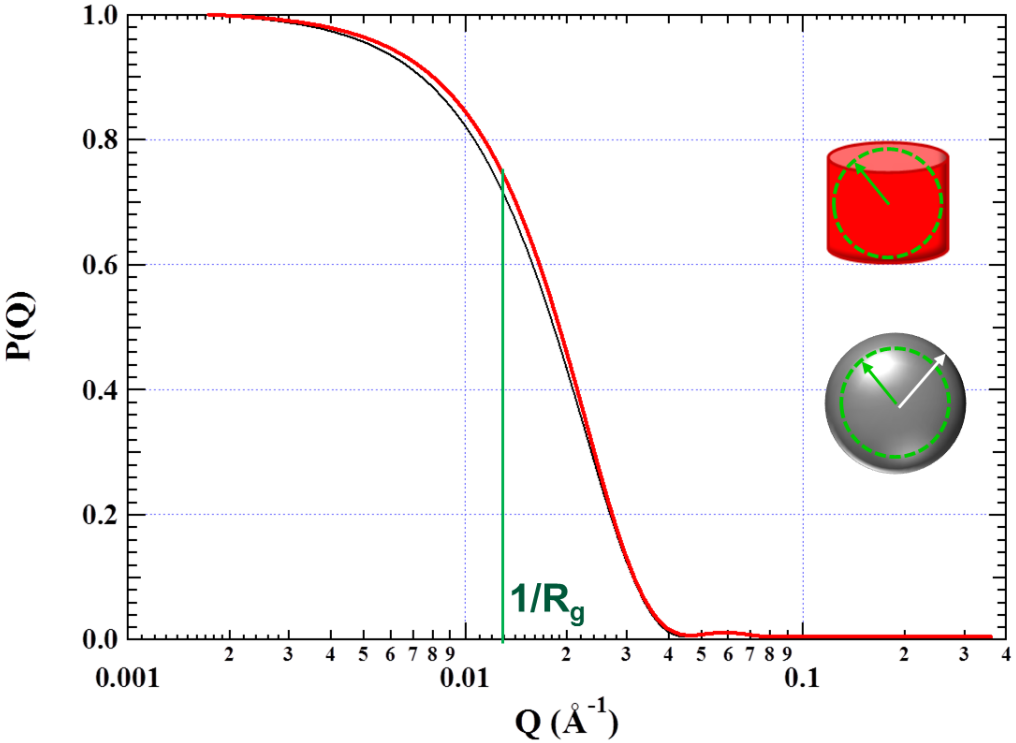
Guinier Analysis

size of any kind of object

- At small enough Q anything that could reasonably be considered a discrete object follows Guinier's approximation.

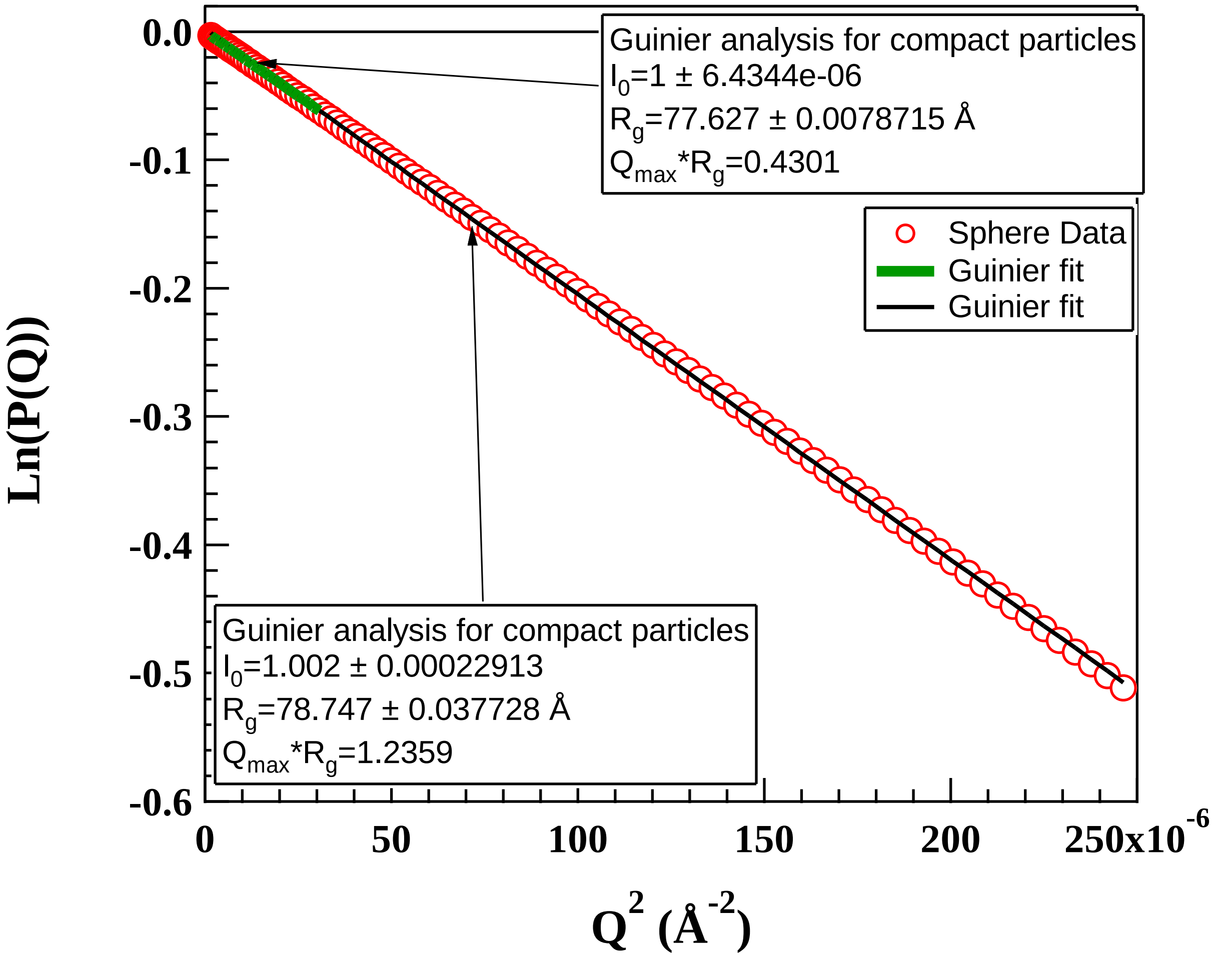
$$\ln[I(q)] \propto q^2 R_g^2/3 \quad qR_g < 1; \quad \text{sphere}: R = \sqrt{\frac{5}{3}} R_g$$

- Modified Guinier approximations exist to determine cross sectional radius of rods or thickness of sheets



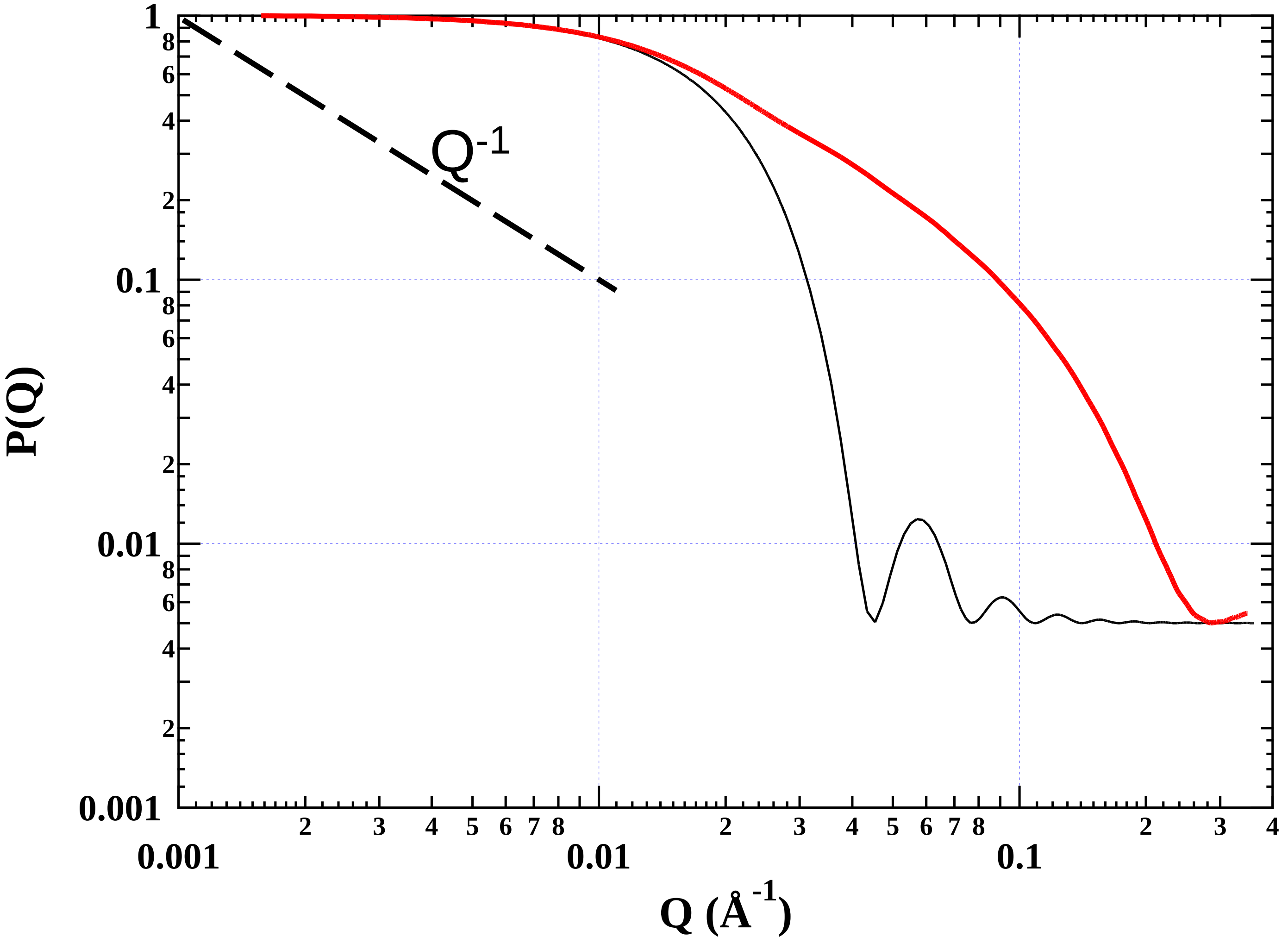
Guinier Analysis

size of any kind of object

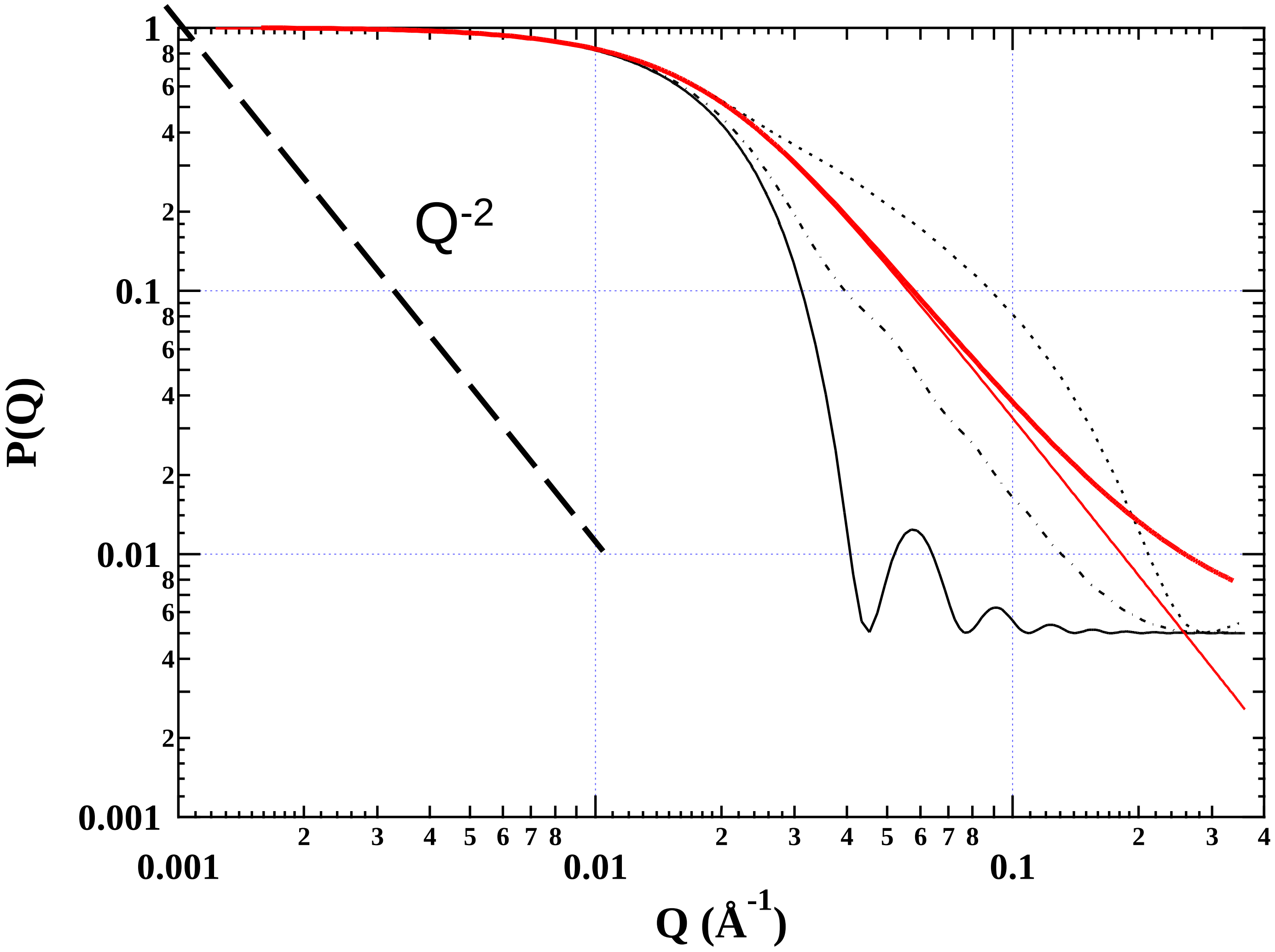


Precise R_g is 77.46 Å

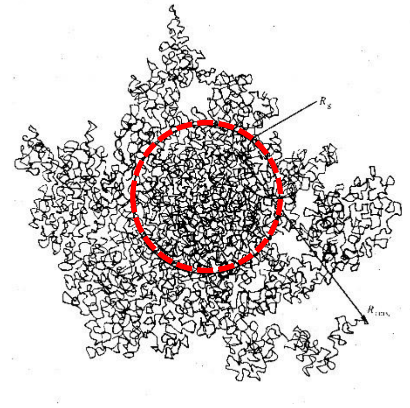
“Long & thin” cylinder



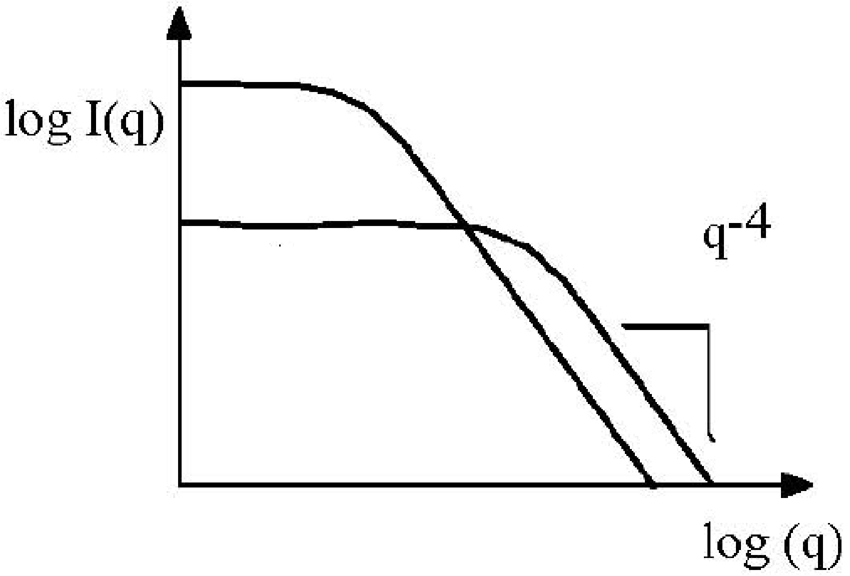
Polymer coil



$$I(q)=\frac{2I(0)}{\left(q^2R_g^2\right)^2}\left(q^2R_g^2-1-e^{-q^2R_g^2}\right)$$



Surface Scattering - *Porod*



At large q:

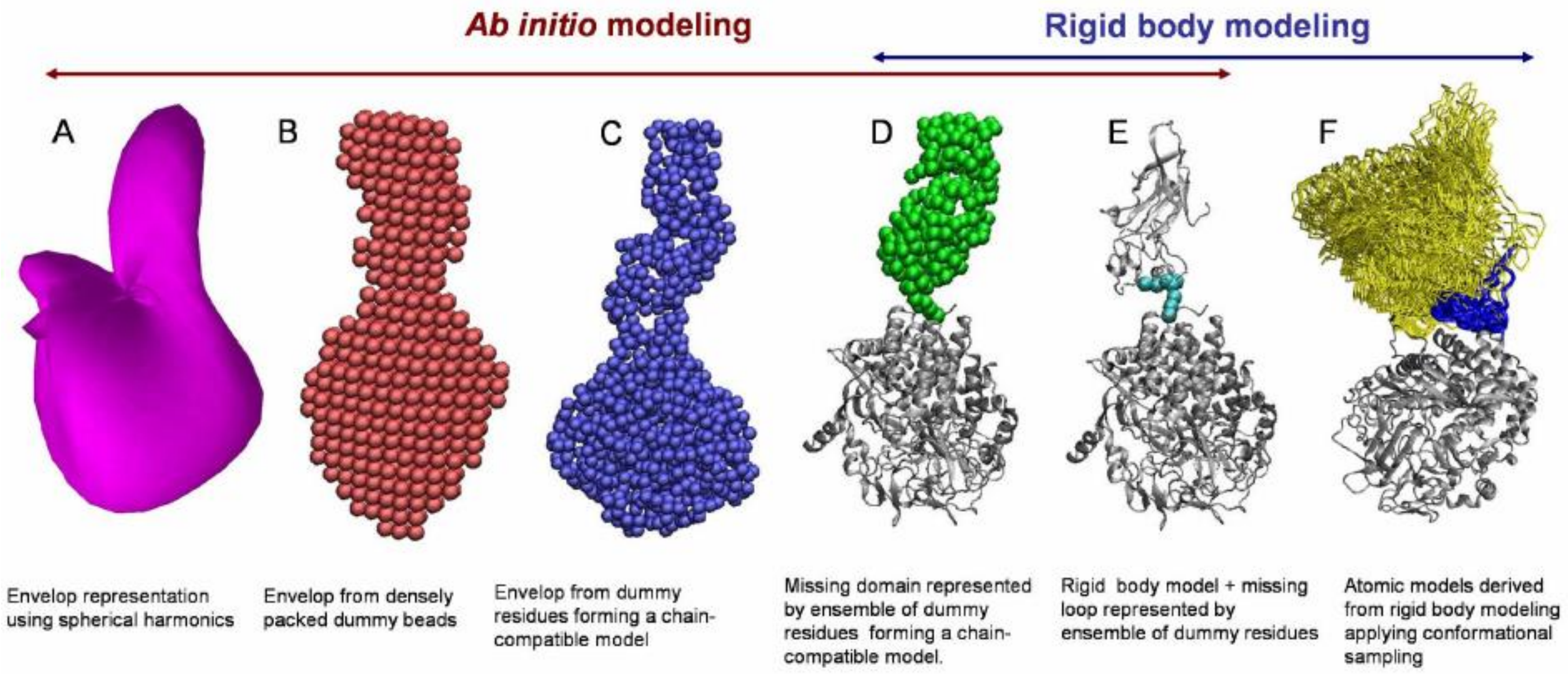
$$I(q) \propto q^{-4}$$

Specific Surface Area, S_V

$$\lim_{q \to \infty} I(q) = 2\pi S_V |\Delta\rho|^2 q^{-4}$$

But, fractal rough interfaces: Q^{-x} , $3 < x < 4$
Diffuse interfaces: $x > 4$

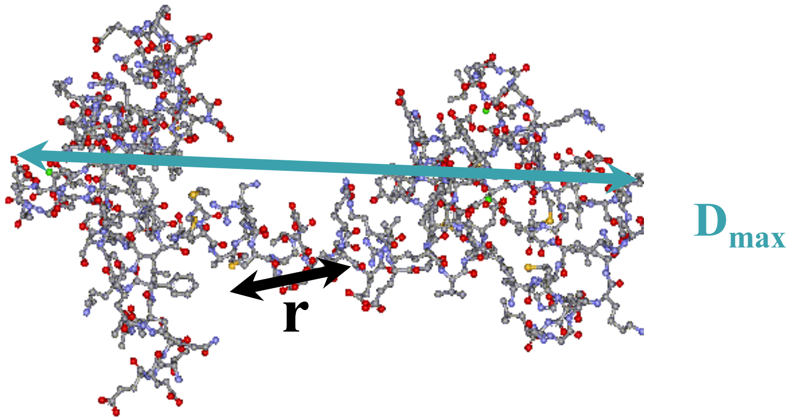
SAS Form Factor Modeling
used in structural biology

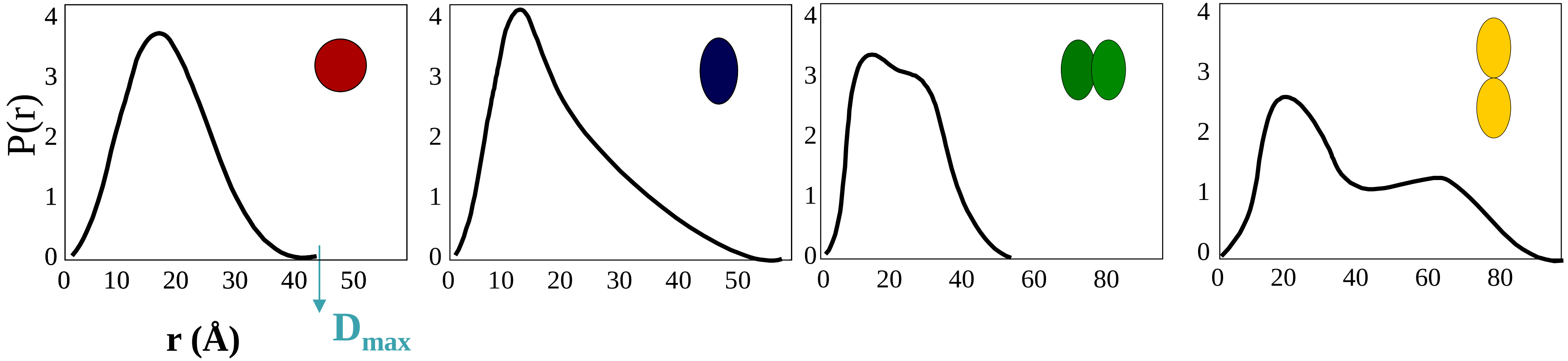


- Spherical Harmonics (Svergun, Stuhrmann, Grossman …)
- Aggregates of Spheres (Svergun, Doniach, Chacón, Heller …)
- Sets of High-resolution Structures (Svergun, Heller, Grishaev, Gabel …)
- Simple Shapes and Custom Approaches (Henderson, Zhao, Gregurick, Heller …)

Pair correlation function and shape

P(r) : inverse Fourier transform of scattering function : Probability of finding a vector of length r between scattering centers within the scattering particle.



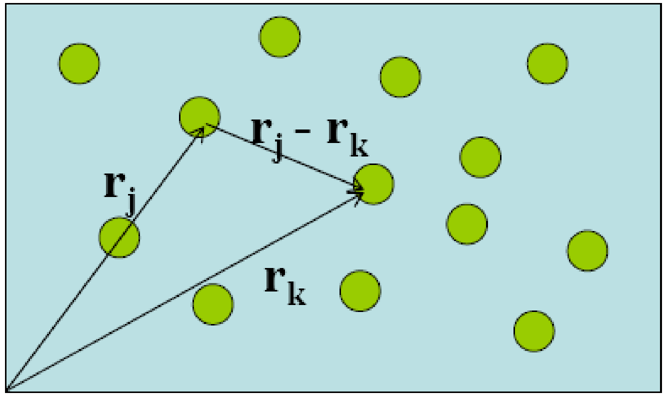


Shape : Modeled as a uniform density distribution that best fits the scattering data.

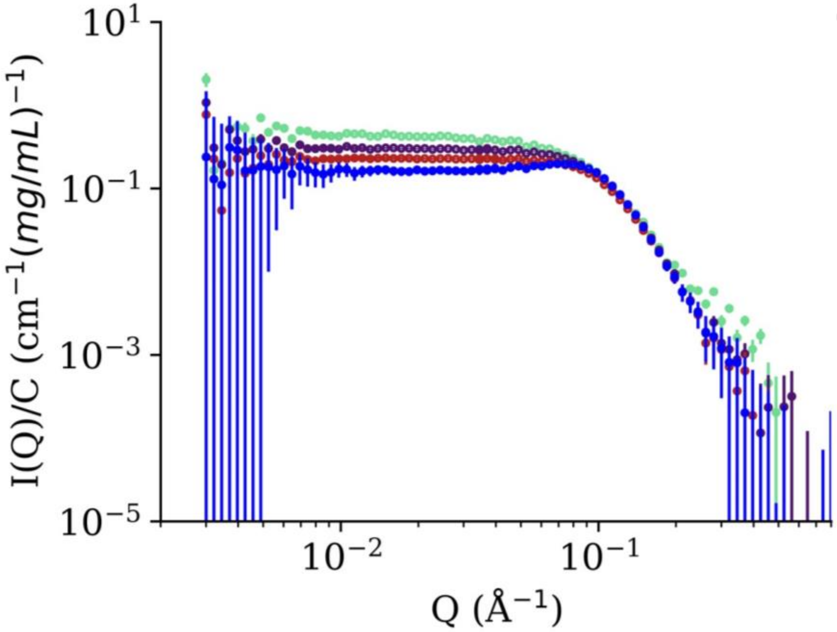
Interparticle Structure Factor $S(Q)$

$$I(q) = \frac{N}{V}(\Delta\rho)^2 V_p^2 P(q) S(\vec{q})$$

$$S(\vec{q}) = 1 + \left\langle \sum_{k=1}^{N} \sum_{\substack{j=1 \\ j \neq k}}^{N} e^{i\vec{q}\cdot(\vec{r}_k - \vec{r}_j)} \right\rangle$$



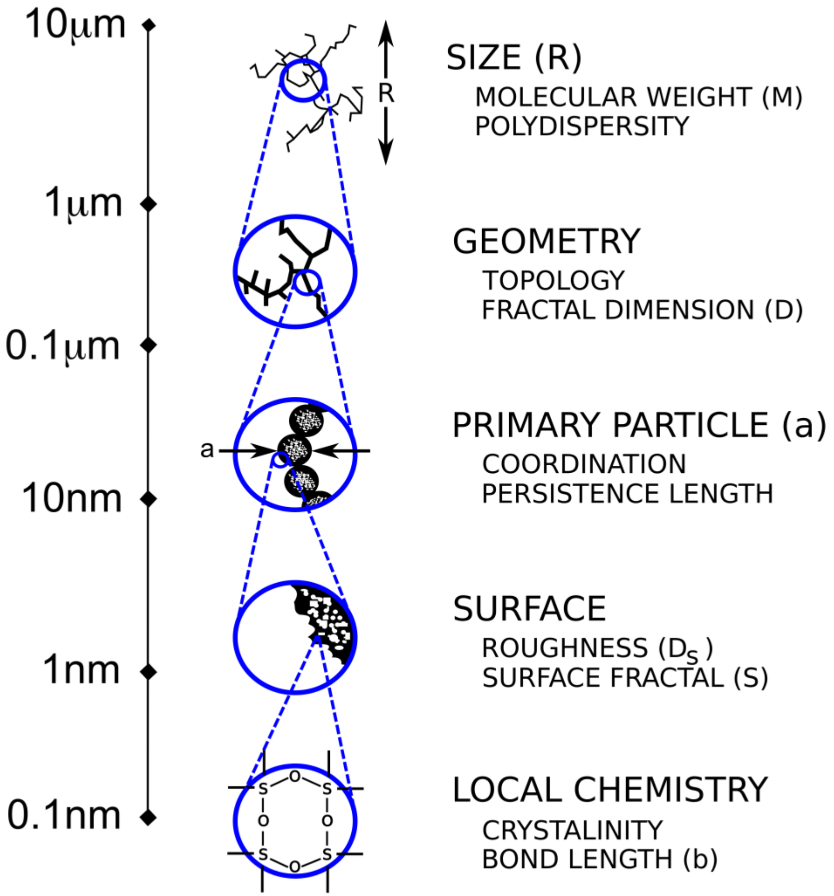
I(q) is modulated by interference effects between radiation scattered by different scattering bodies.

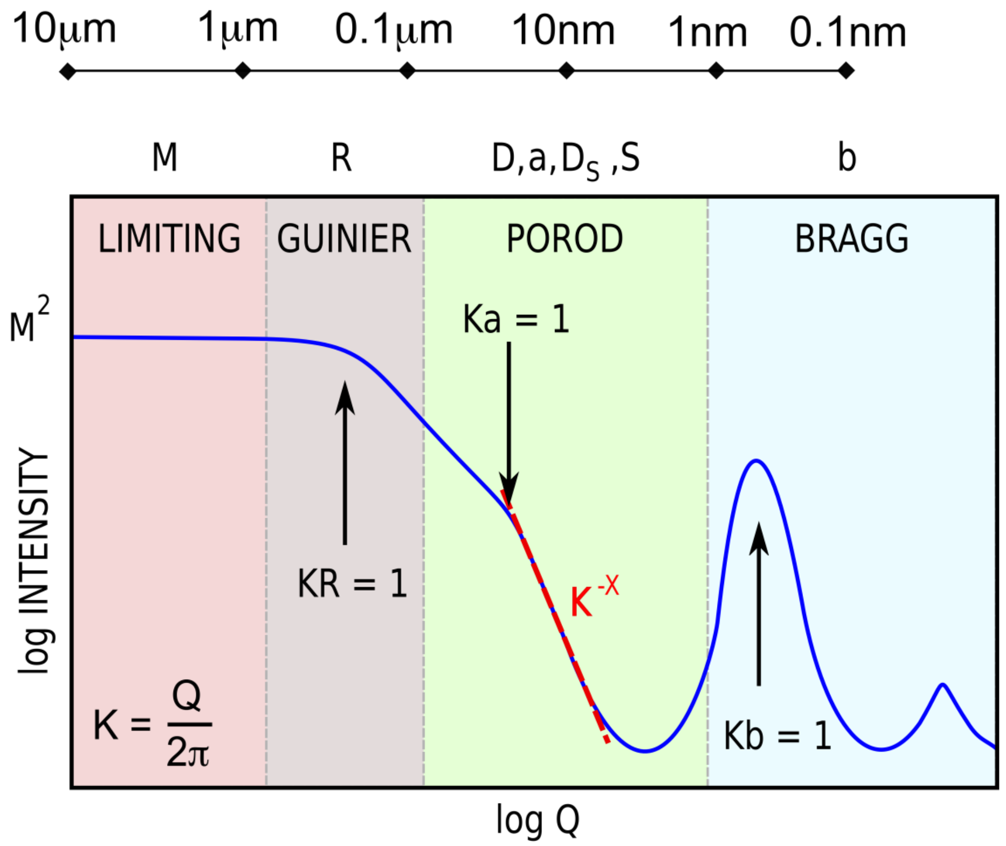


S(q) examples: hard sphere potential, sticky sphere, screened coulomb etc.

S(q)·P(q) is not always valid and useful!

Structural Hierarchy (particulate)





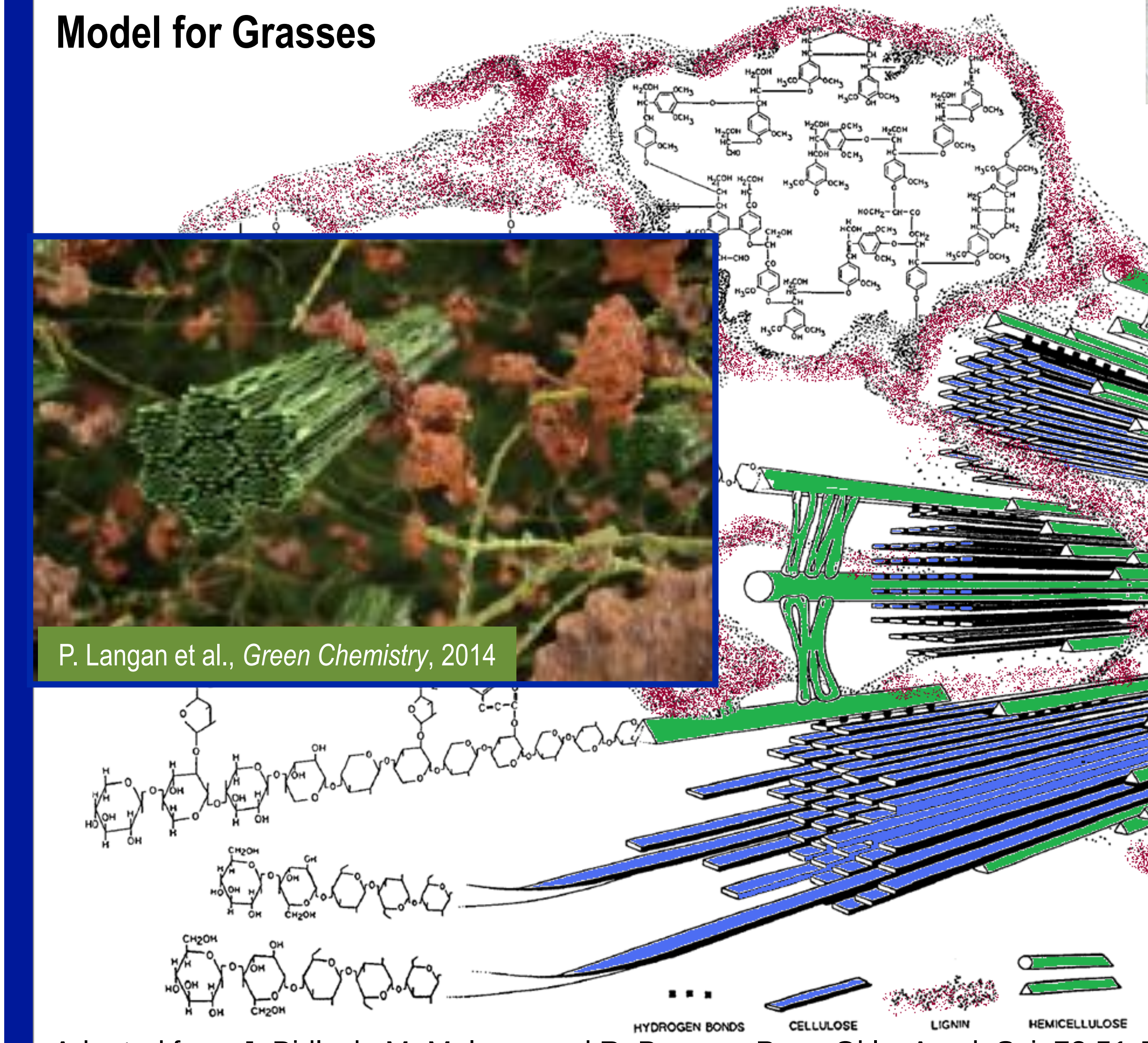
Adapted from DW Schaefer *MRS Symposium Proceeding* 1987

Structural information viewed on five length scales. Structural features at larger length scales are observed at smaller Q.

Scattering analysis that describes hierarchical structures: Mass Fractal (Teixeira), Unified Fit (Beaucage) *combine power law scattering ranges with R_g transitions*

OAK RIDGE National Laboratory

“BIOMESS”



P. Langan et al., *Green Chemistry*, 2014

Adapted from J. Bidlack, M. Malone, and R. Benson. Proc. Okla. Acad. Sci. 72:51-56 (1992)

Why neutrons?

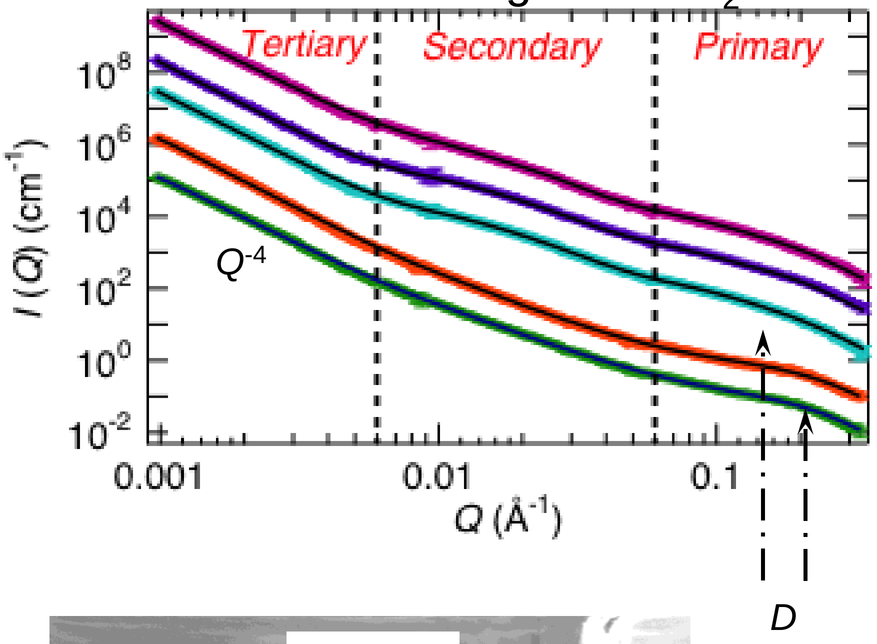
- Use D_2O for contrast
- Observe changes over time in pressure reaction cell with SANS

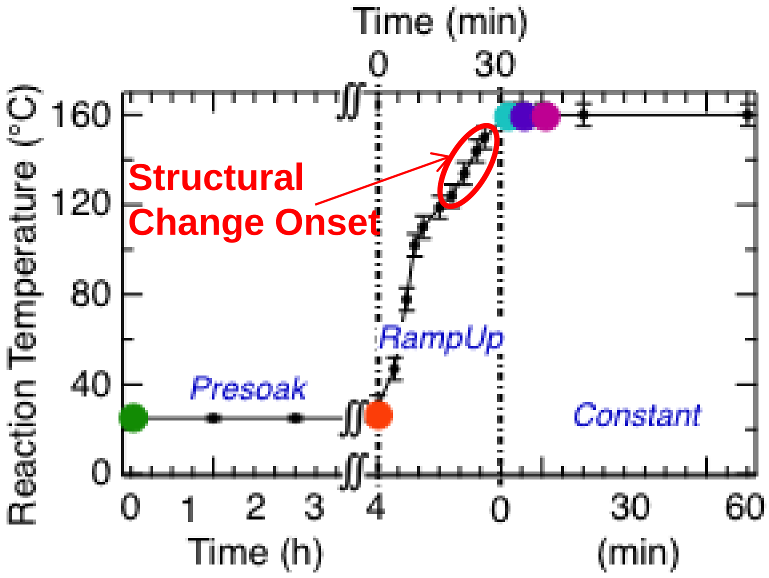
Outcome

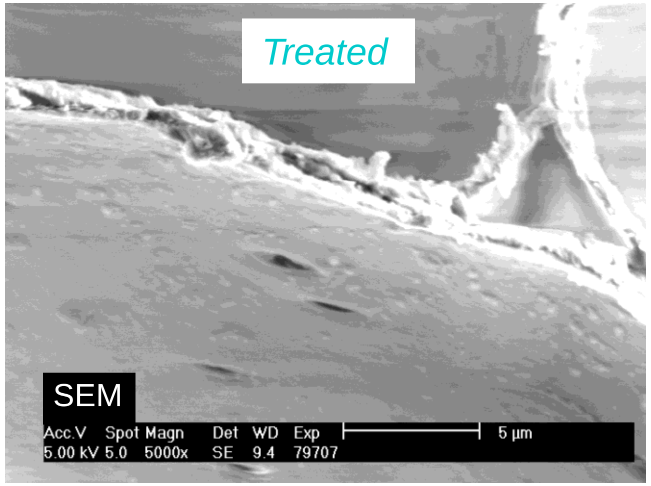
New understanding of what processes actually happen during industrial pretreatment.

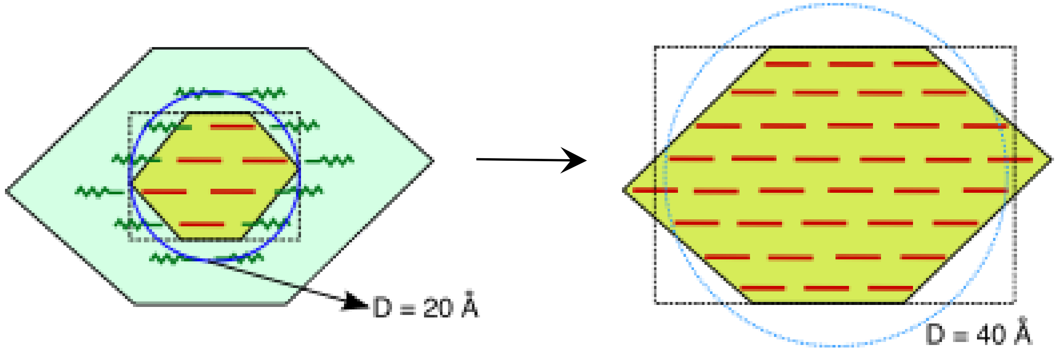
Dilute Acid Pretreatment of Switchgrass

SANS of Switchgrass in D_2O









Elementary Cellulose Fibril
Cross-sectional View

Pingali et al., *Biomacromolecules* 2010

SANS Summary

- Applications are in the nm to µm range and otherwise only limited by imagination.
- SANS does not see atoms, but larger, interesting features over many length scales.
- Precision of structural parameters such as R_g can be 1Å or better.
- SANS is used alone, but often complementary to other methods, such as microscopy, NMR.
- Scattering is similar to diffraction but does not require crystals.
- Data analysis is application dependent, using a diverse set of approximations, models and software.

SA(N)S Reference Suggestions

- Guinier, A., Fournet, G. 1955. Small-Angle Scattering of X-rays. John Wiley & Sons, New York.
  The classical work on small-angle scattering. Even though focused on x-rays, much of the theory and data interpretation apply just as well to neutrons.
- Pedersen, J. S., 1997. Analysis of small-angle scattering data from colloids and polymer solutions: modeling and least-squares fitting. *Adv. Colloid Interface Sci.* 70:171-210.
  Contains a comprehensive list of form factors and structure factors that are used for interpreting small-angle scattering data.
- Urban, V. S., 2012. Small-Angle Neutron Scattering. In: *Characterization of Materials*, edited by Elton N. Kaufmann. Copyright 2012 John Wiley & Sons, Inc.
  A concise introduction to theory and practical considerations of Small-Angle Neutron Scattering.
- Chaudhuri, Muñoz, Qian, Urban (Editors), 2017. Biological Small Angle Scattering: Techniques, Strategies and Tips. Springer.

SA(N)S References continued

- Roe, R. J. 2000. Methods of X-Ray and Neutron Scattering in Polymer Science. Oxford University Press, New York and Oxford.

  Even though focused on polymers, this book gives a very thorough account on the basic scientific principles of small-angle scattering in a fashion that is accessible to non-expert scatterers.

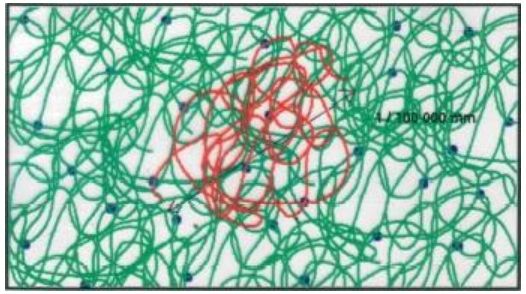
- Higgins, J. S., and Benoît, H. C. 1994. Neutron Scattering from Polymers. Clarendon Press, Oxford.

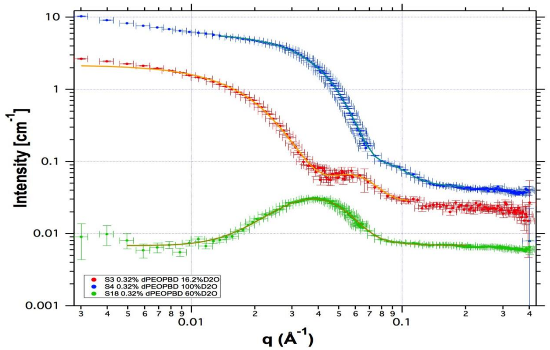
  A comprehensive description on neutron scattering and in particular small angle neutron scattering. Even though focused on polymers, the book is very useful for anyone interested in small angle neutron scattering.

- Lectures from the National School on Neutron and X-Ray Scattering: http://neutrons.ornl.gov/nxs
- PROBING NANOSCALE STRUCTURES – THE SANS TOOLBOX, by Boualem Hammouda (NIST): https://www.ncnr.nist.gov/staff/hammouda/the_SANS_toolbox.pdf
- DOE BER Structural Biology Portal - https://berstructuralbioportal.org/

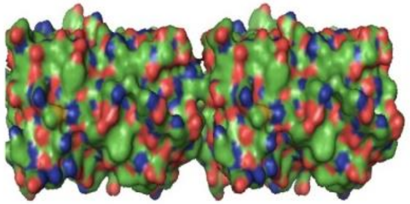
Questions?

Cases for using neutron contrast

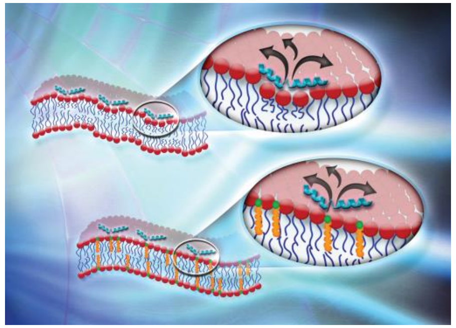


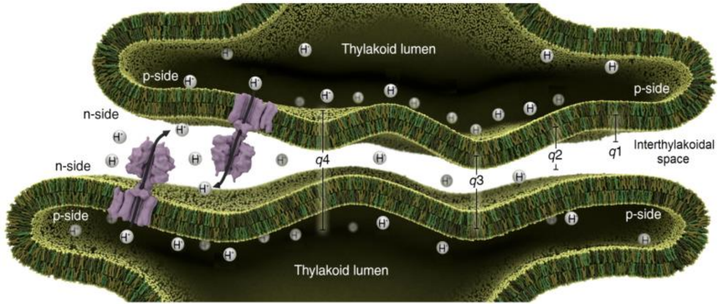


1. Zero (0) natural electron density contrast
2. Reducing degeneracy / increasing information content of SAS data
3. Overcoming extremely low signal to noise "needles in haystacks"
4. Zooming in on specific features in very complex systems – biomass, live cells
5. Combination with other valuable neutron traits: fine energy resolution, no radiation damage, high penetration/in situ



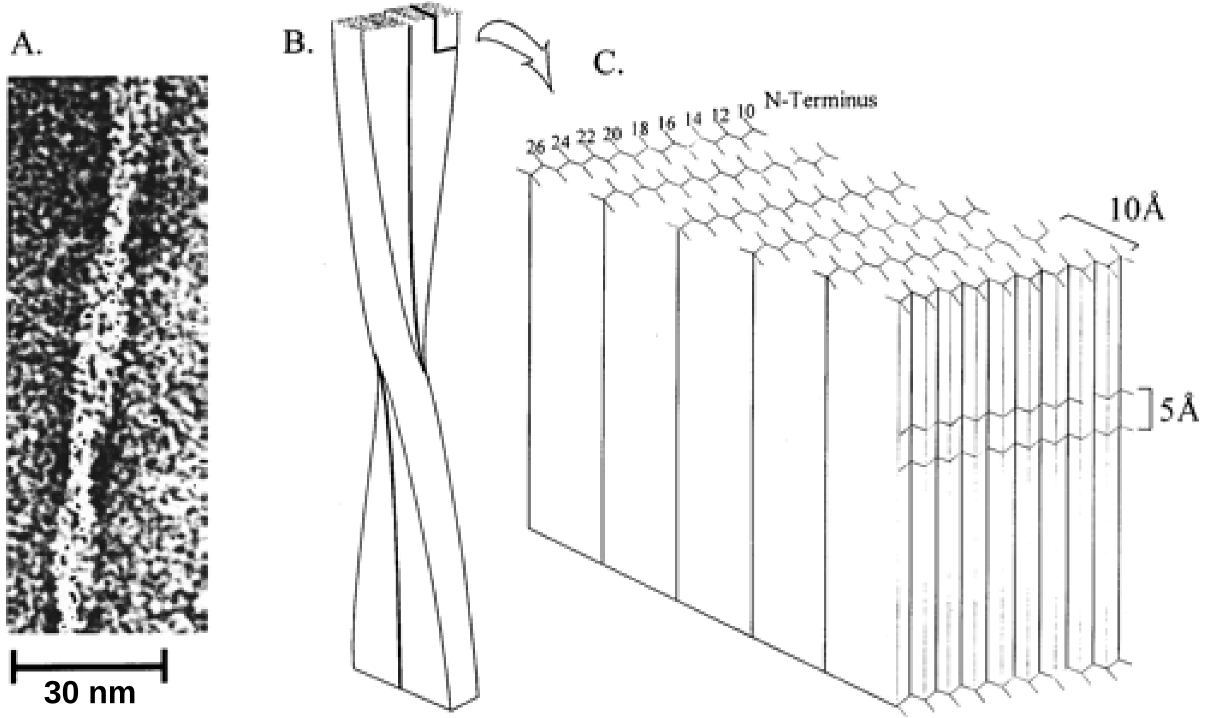
end-to-end dimer





Complementary methods example: β-Amyloid

- Alzheimer's DiseaseAmong leading causes of death
- Miss-folded peptides form hierarchical ordered fibril structures & plaques
- Structure established using synthetic model peptides and complementary methods NMR, SANS, EM

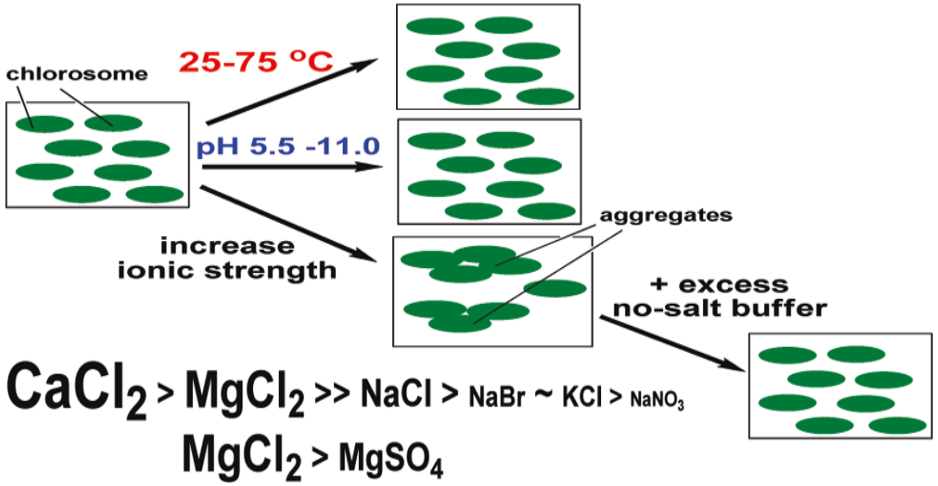


T.S. Burkoth et al. *J. Am. Chem. Soc.* **2000,** *122*, 7883-7889

- **NMR**
  - **β-fold**
- **SANS**
  - **Fiber shape**
  - **Diameter**
  - **6 sheet stack**
- **EM**
  - **Overall morphology**
  - **Twist**

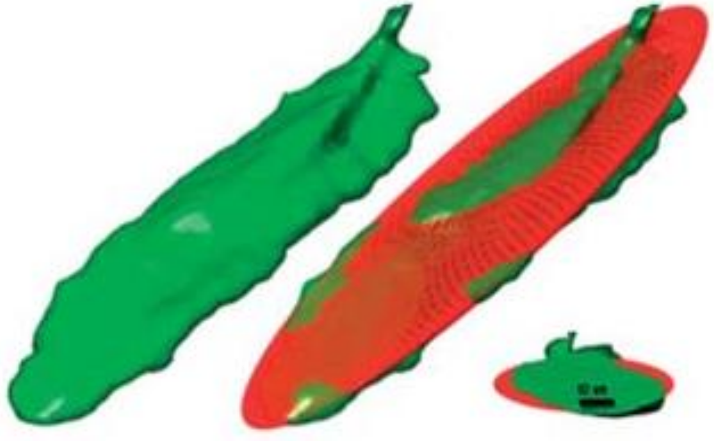
Ellipsoid example: Chlorosomes stable under harsh conditions including entrapment in silica sol-gel

Neutron Scattering probes chlorosome under range of temperature, pH and salt conditions



Remarkable stability of the chlorosome, reversible association in high ionic strength

K.-H. Tang, L. Zhu, V. S. Urban, A. M. Collins, P. Biswas, and R. E. Blankenship, “Temperature and Ionic Strength Effects on the Chlorosome Light-Harvesting Antenna Complex,” *Langmuir* 27 (8), 4816–4828(2011).

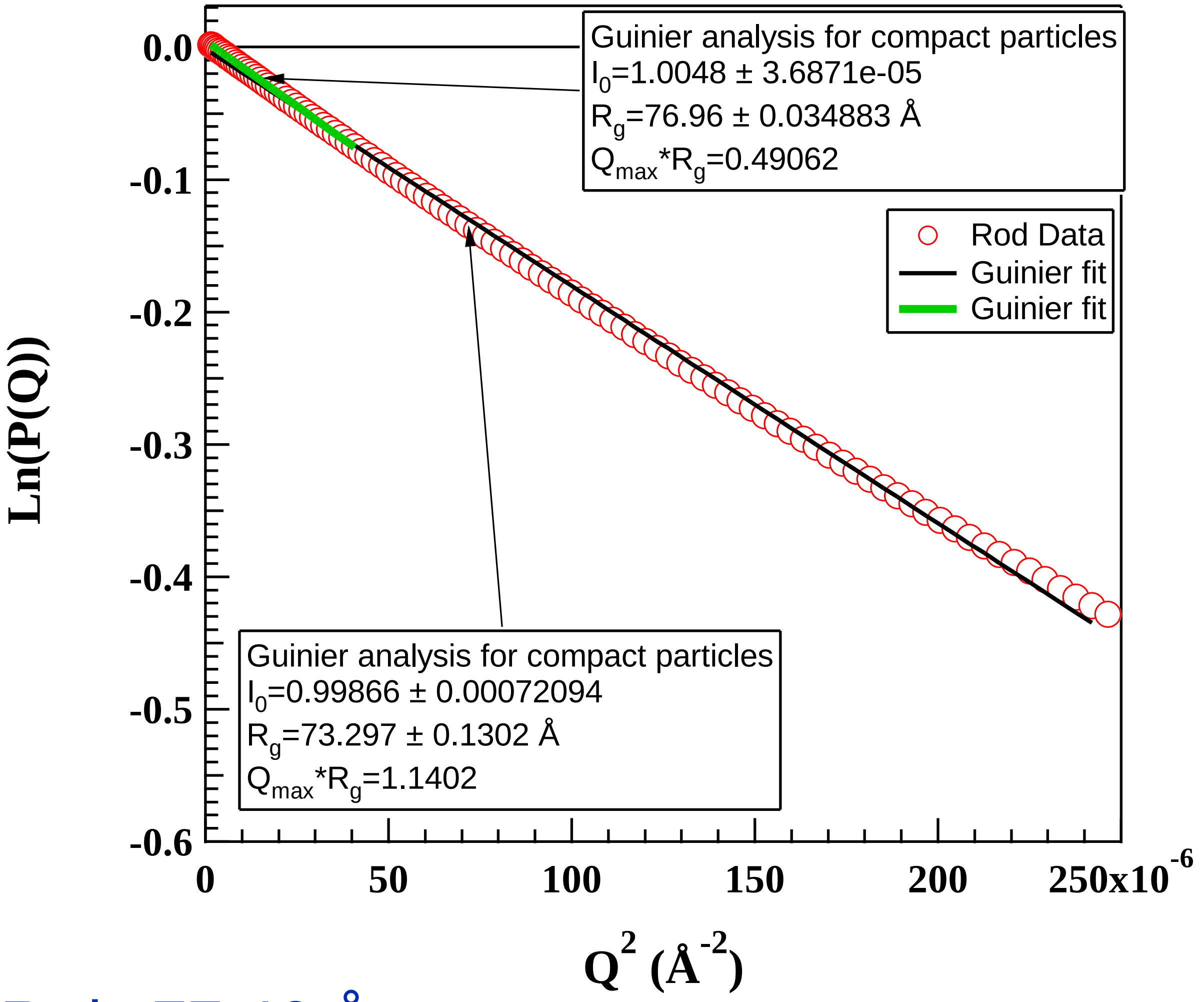


C. aurantiacus chlorosome (green) and model ellipsoid form (red) calculated from neutron scattering data of chlorosome entrapped in silica gel. Green volume reconstructed from electron density map of free chlorosome reported by Psencik et al.

W.B. O'Dell, K.J. Beatty, J.K.H. Tang, R.E. Blankenship, V.S. Urban and H. O'Neill, “Sol-gel entrapped light harvesting antennas: immobilization and stabilization of chlorosomes for energy harvesting,” *J. Mater. Chem.* 22(42), 22582-22591 (2012).

Guinier Analysis

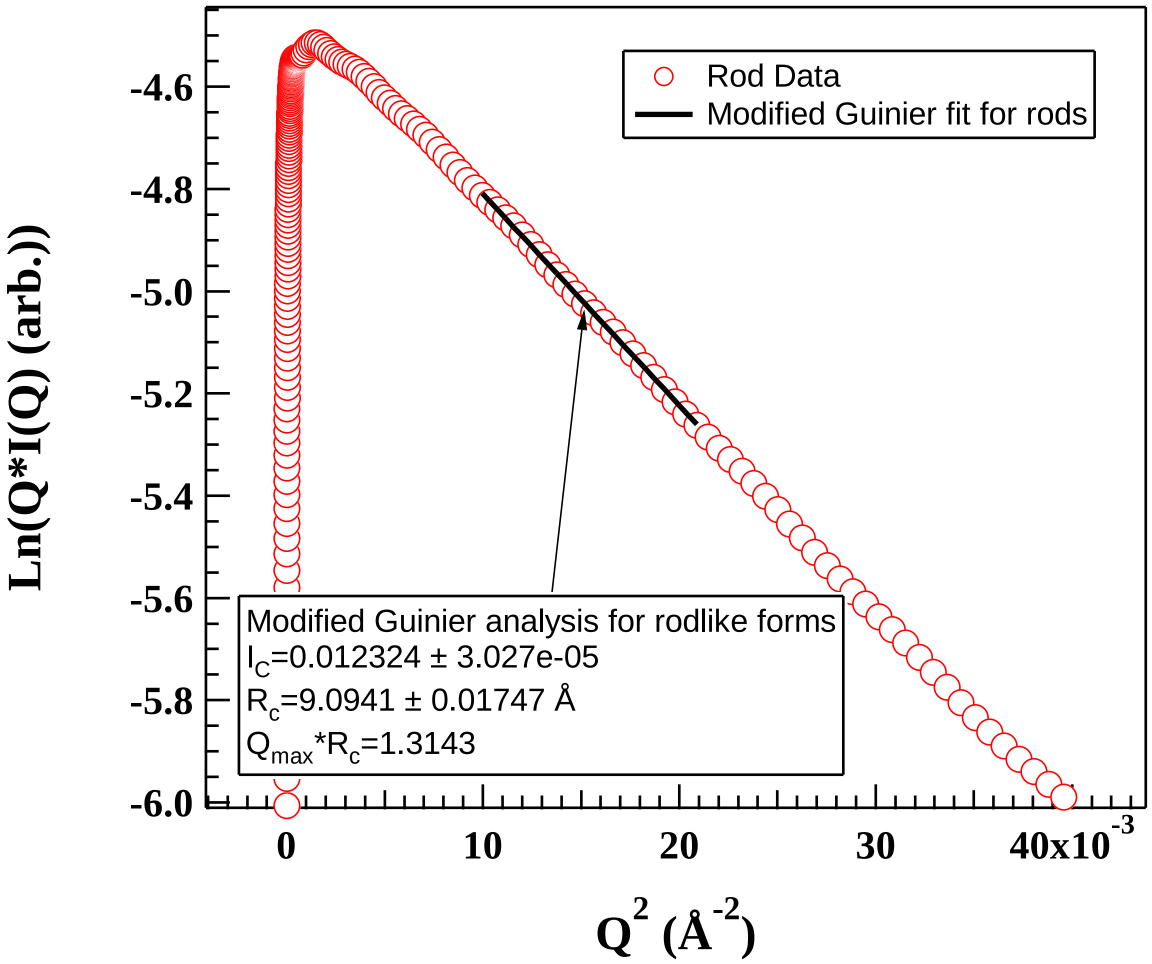
size of any kind of object



Precise R_g is 77.46 Å

Modified Guinier Analysis

for object extended in 1 dimension

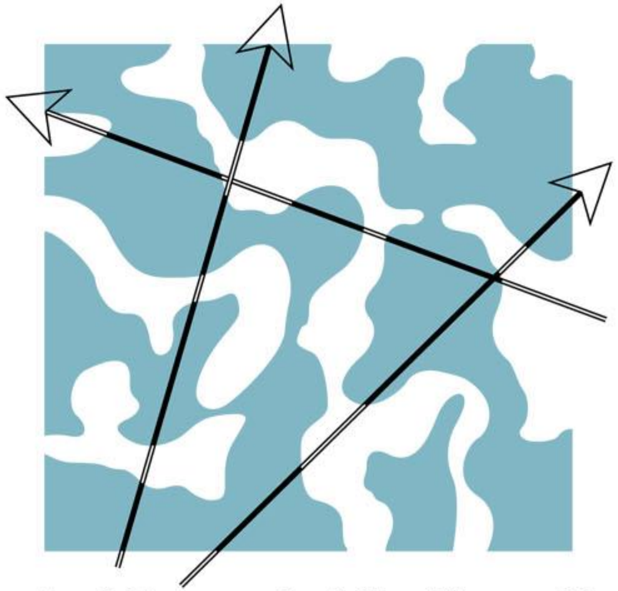


Rod radius = √2 * R_c = 12.9 Å, exact radius = 13.3 Å

A similar approach exists for thickness of (2d) sheet-like structure.

Non-particulate Scattering

Debye Bueche Model for Two-Phase System, Each with Random Shape, Uniform Electron or Scattering Length Density and Sharp Boundaries



Physical Concept of the Mean Chord or Inhomogeneity Length

Mean Chord Intercepts:

$$L_1 = \frac{a}{\phi}$$

$$L_2 = \frac{a}{(1-\phi)}$$

The fluctuations in scattering power at two points A and B, distance r apart, can be characterized by $\gamma(r) \langle\eta^2\rangle_{AV} = \langle\eta_A\eta_B\rangle_{AV}$. For random two phase system: $\gamma(r) = e^{-r/a}$

$$\frac{d\Sigma}{d\Omega}(Q) = \frac{A}{[1 + Q^2a^2]^2}$$

J. Appl.Cryst., 28, 679 (1957)

ORNL-DWG 92M-9485

Facility Acknowledgment Statement

- A portion of neutron scattering research presented as examples in this introduction used resources at the High Flux Isotope Reactor or Spallation Neutron Source, DOE Office of Science User Facilities, operated by the Oak Ridge National Laboratory.
- The Bio-SANS of the Center for Structural Molecular Biology at the High Flux Isotope Reactor is supported by the Office of Biological and Environmental Research of the U.S. DOE.