

Applications of Small Angle Scattering

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National School on Neutron and X-ray Scattering

- 
- **Applications – is SAS for you?**
 - **Comparison with microscopy and diffraction**
 - **Basic concepts of the technique**
 - **SANS instrumentation**
 - **Planning a SAS experiment and data reduction**
 - **SAS data analysis and interpretation**

SAS of x-rays, neutrons, laser light

- **SAXS & SANS: structural features 1nm-1 μ m**
 - precision can be better than 1 Å
- **X-rays**
 - Rotating anode / sealed tube: ~ 1/2 M\$
 - **Synchrotron: high flux, very small beams**
- **Neutrons**
 - **Isotope contrast, high penetration, magnetic contrast**
- **Laser Light scattering**
 - Bench top technique, static and dynamic
- **Applications in ...**
 - Important for polymers, soft materials, (biology)
 - Particulate and non-particulate
 - Pretty much anything **1nm-1 μ m**



...really
anything?

SAS applications A to Z

Alzheimer's disease, aerogel, alloys

Bio-macromolecular assemblies, bone

Colloids, complex fluids, catalysts

Detergents, dairy (casein micelles)

Earth science, emulsions

Fluid adsorption in nanopores, fuel cells,
food science (chocolate)

Gelation, green solvents

High pressure, high temperature...,
hydrogen storage, helium bubble growth
in fusion reactors

Implants (UHDPE)

Jelly

Kinetics (e.g. of polymerization or protein
folding), keratin

Liquid Crystals

Magnetic flux lines,
materials science

Nano-anything

Orientational order

Polymers, phase behavior, porosity

Quantum dots (GISAXS)

Rubber, ribosome

Soft matter, surfactants, switchgrass

Time-resolved, thermodynamics

Uranium separation


Vesicles, virus

Wine science

Xylose isomerase

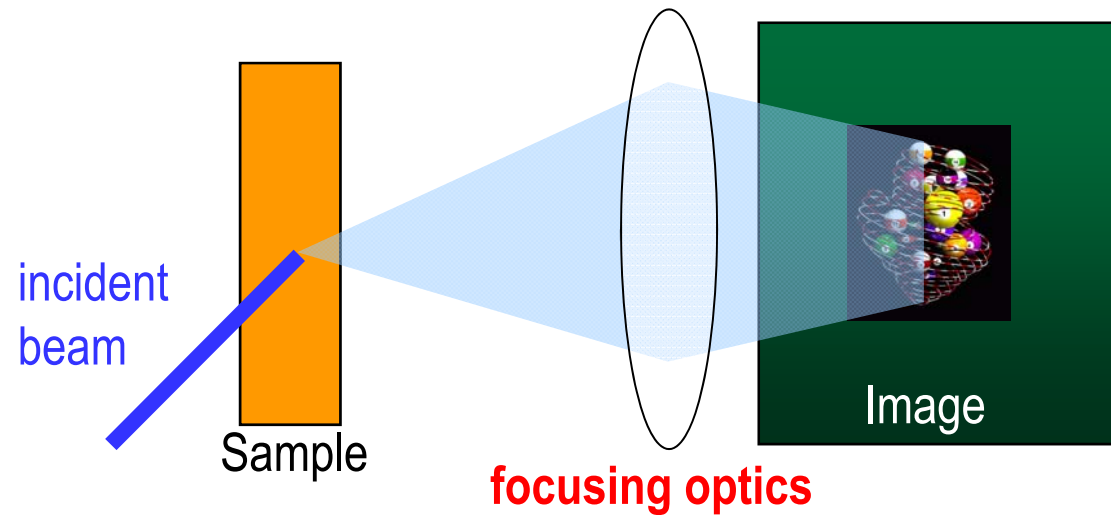
Yttrium-stabilized zirconia (YSZ)

Zeolites

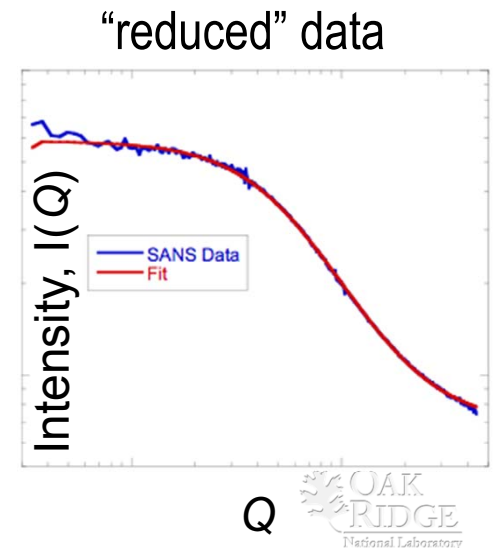
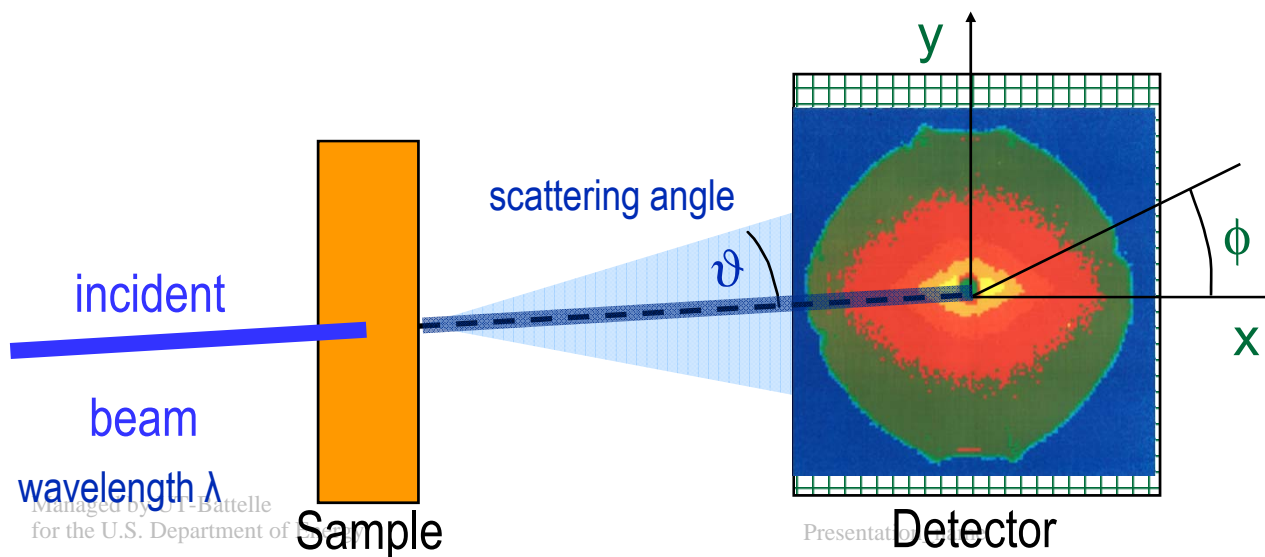


But what
about SEM,
TEM, AFM
...?

Microscopy : enlarged image



SAS : interference pattern

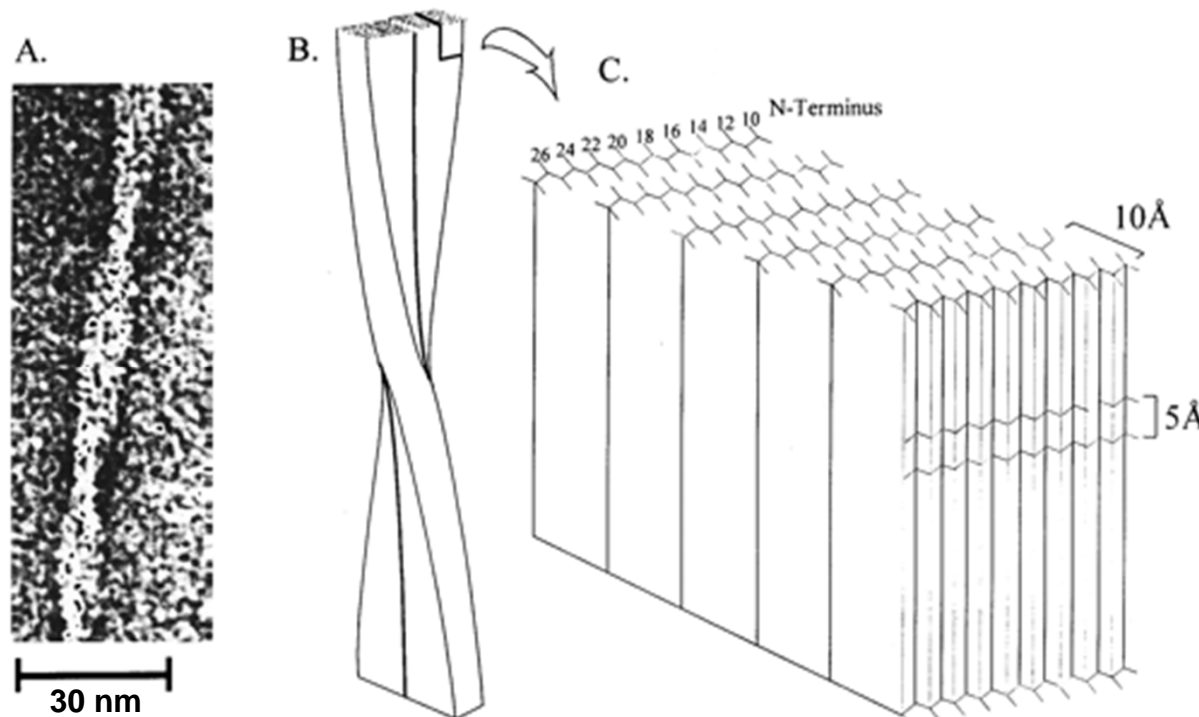


Small Angle Scattering and Microscopy

- **Common features**
 - Size range 1nm-1 μ m
 - Contrast labeling options (stains / isotope labels)
- **SAS practical aspects**
 - No special sample preparation such as cryo-microtome
 - Sample environments control (p, T, H)
 - Non-invasive (exception: radiation damage in synchrotron beam)
 - In-situ, time-resolved
- **Fundamental difference**
 - “Real space” image with certain resolution
 - Scattering pattern, averaged over volume
- **Complementarity**

Alzheimer's Disease – β -Amyloid

- Among 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

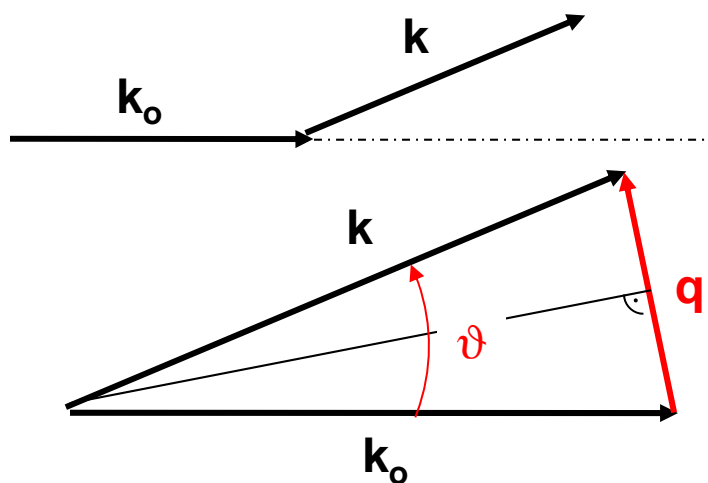
- **NMR**
 - β -fold
- **SANS**
 - Fiber shape
 - Diameter
 - 6 sheet stack
- **EM**
 - Overall morphology
 - Twist

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 (D) / flux (S)
 - Diffraction needs crystals, scattering does not.
 - Analysis?!
- At **small Q** (small angles, large λ): observe nm-sized volume elements, **“blobs” NOT atoms**
 - Scattering length \rightarrow scattering length density (SLD)
 - SAS is sensitive to spatial non-uniformity of SLD:
 $\Delta\text{SLD} = \text{Contrast}$ \rightarrow contrast variation!

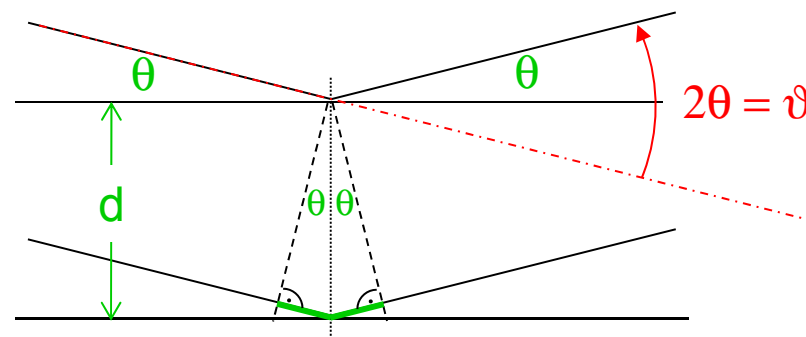
Scattering Vector, q or momentum transfer, Q , h , k , s

Wave vector \mathbf{k} : $|\mathbf{k}| = k = 2\pi/\lambda$



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

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



$$\Delta = 2d \sin(\theta)$$

if $\Delta = n \lambda$ then reflection, else extinction

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

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

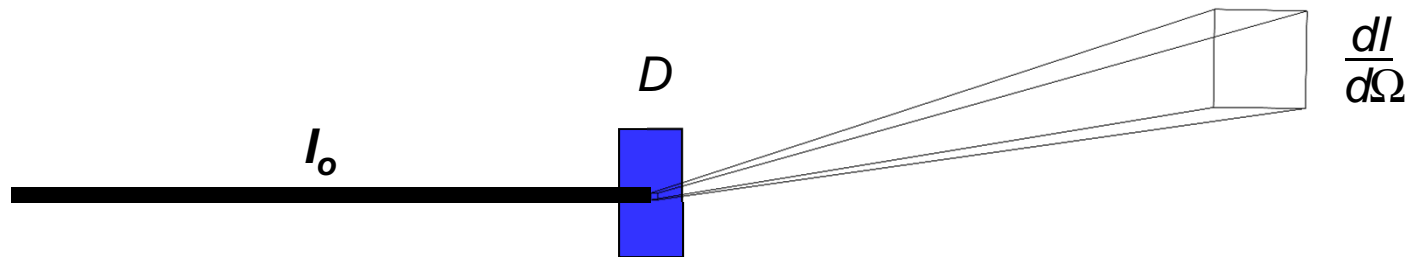
q in nm^{-1} or \AA^{-1}

Neutron Scattering Intensity

- Incoming waves scatter off individual nuclei according to scattering length **b** (can be + or -).
- Interference of wavelets from distribution of nuclei (= structure) adds up to “net scattering” amplitude (Fourier transform of structure).
- Measured intensity is the magnitude square of amplitude.
- Measured intensity is also the Fourier transform of pair correlation function $P(r)$.

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

Absolute Intensity / Scattering Cross Section – cm^{-1} ?



$$\frac{dI}{d\Omega} = TI_o D \frac{d\Sigma}{d\Omega} \quad \longleftrightarrow \quad \frac{d\Sigma}{d\Omega} = \frac{1}{TI_o D} \frac{dI}{d\Omega} \quad [\text{cm}^{-1}\text{sterad}^{-1}]$$

$dI/d\Omega$ = Scattered intensity per solid angle

I_o = Primary beam intensity

T = Transmission (x-ray absorption, incoherent neutron scattering)

D = Thickness

$d\Sigma/d\Omega$ = Scattering **cross section per unit volume** [$\text{cm}^{-1}\text{sterad}^{-1}$]

Comparing SAXS and SANS

- **SAXS & SANS**

- nm scale structural analysis ($\sim 1\text{nm}$ - $1\mu\text{m}$)
- Non-destructive (radiation damage in synchrotron SAXS can be an issue)
- In-situ






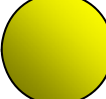
- **Synchrotron X-rays**

- High throughput
- Time-resolution (ms – ps)
- Tiny beams – microfocus: e.g. scanning of cells

- **Neutrons**

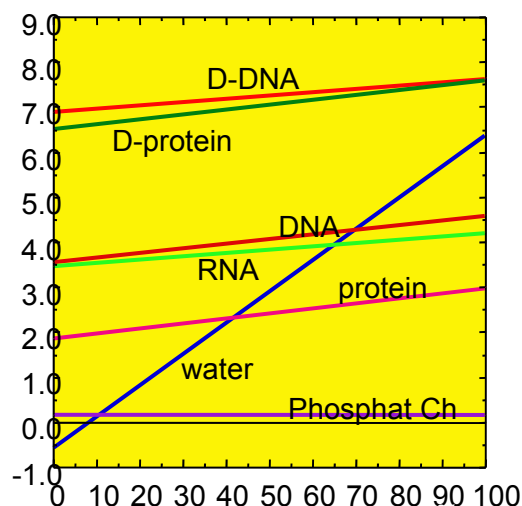
- ‘see’ light atoms: polymers, biology, soft condensed matter, hydrogen in metals
- **Isotope labeling**
- High penetration
 - bulky specimens, e.g. residual stress in motor block
 - complicated environments (P,T), e.g. ^4He cryostat
- Magnetic contrast
- No radiation damage

Contrast – Atomic Scattering Lengths

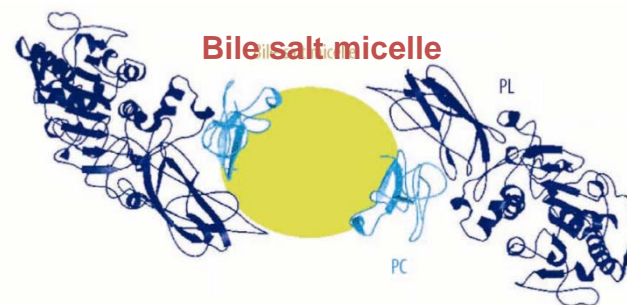
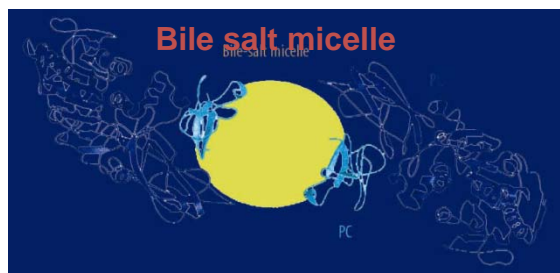
Element	Neutrons (10^{-12} cm)	X-rays (10^{-12} cm)	Electrons
^1H	-0.374	0.28	1 
^2H (D)	0.667	0.28	1 
C	0.665	1.67	6 
N	0.940	1.97	7 
O	0.580	2.25	8 
P	0.520	4.23	15 

For SAS: $\text{SL} \rightarrow \text{SLD} \rightarrow \underline{\Delta\text{SLD}}$

SANS – Contrast Variation



**D₂O/H₂O
contrast variation**



Directed Assembly of Hollow Polymer Nanocapsules



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Scientific Achievement

Structural information obtained from small-angle neutron scattering helped investigate the synthesis process of polymer nanocapsules that were templated from surfactant micelles.

Significance and Impact

Spontaneous formation of predominantly unilamellar vesicles loaded with monomers without the need for further processing, such as extrusion, coupled with the use of inexpensive materials, greatly simplifies the synthesis of hollow nanocapsules, versatile platforms with diverse applications including drug delivery, nanoreactors, and nanosensors.

Research Details

- Structural details obtained from neutron scattering support the observation that capsule monomers stabilize vesicle structures.
- Both concurrent-loaded and diffusion-loaded surfactant/monomer vesicles produce hollow polymer nanocapsules upon the polymerization of monomers.

M. Kim et al., *Langmuir* 30(24), 2014.



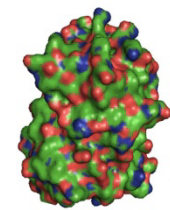
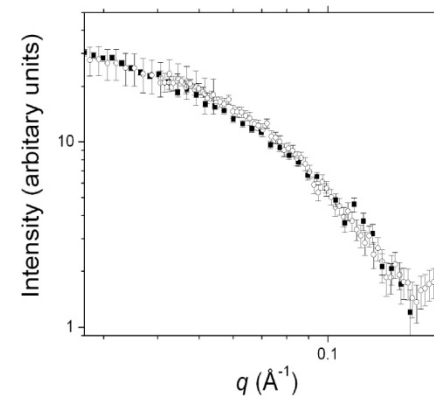
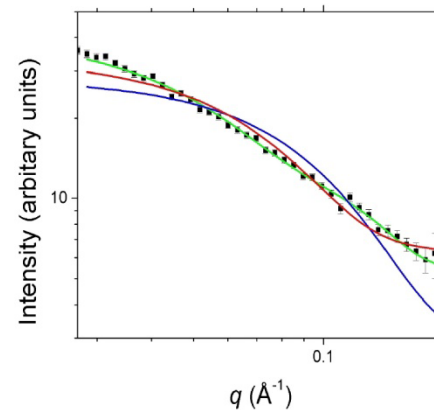
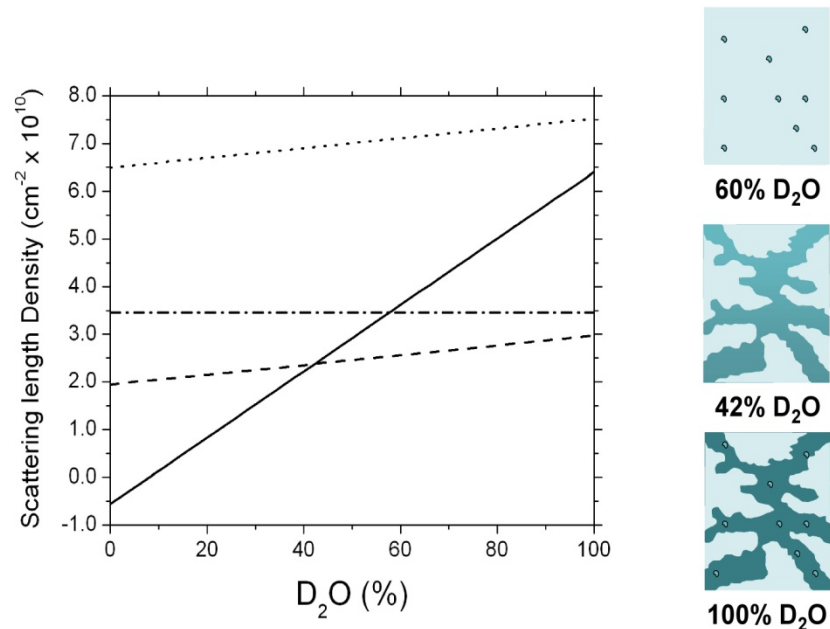
U.S. DEPARTMENT OF
ENERGY

Office of
Science

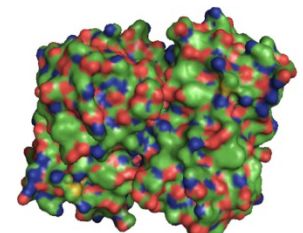
**OAK
RIDGE**
National Laboratory

Visualizing Proteins in Inorganic Hydrogels

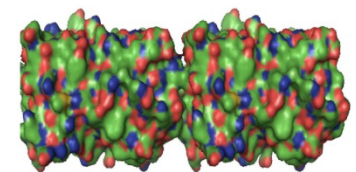
- Entrapment of bio-macromolecular assemblies: bio-composite, biomimetic, bio-inspired for catalysts, sensors, functional materials – for example light harvesting antenna complexes for solar energy
- SANS shows that green fluorescent protein, an enzyme with potential applications in energy transfer and sensor development, is homogeneously dispersed in a silica gel matrix as a functional end-to-end dimer.
- **SANS with contrast variation shows structure of proteins in a complex gel matrix**



monomer



parallel dimer

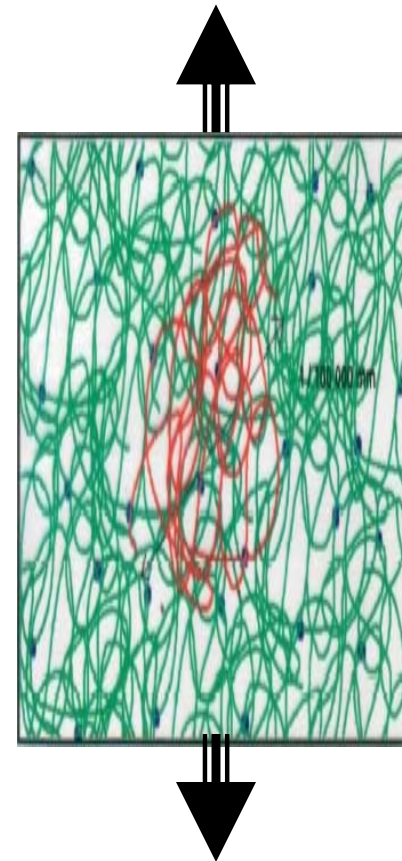
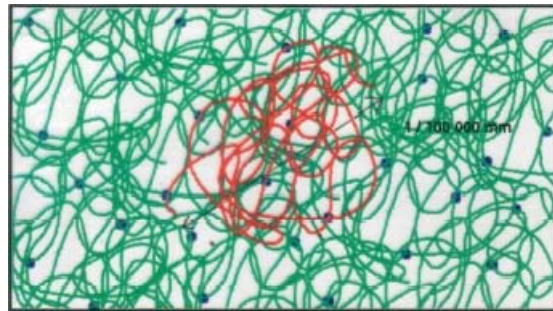


end-to-end dimer

Luo, G., Zhang, Q., Del Castillo, A. R., Urban, V. and O'Neill, H., *ACS Appl. Mater. Interfaces* **1**: 2262-2268 (2009).

Rubber (Polymer Network)

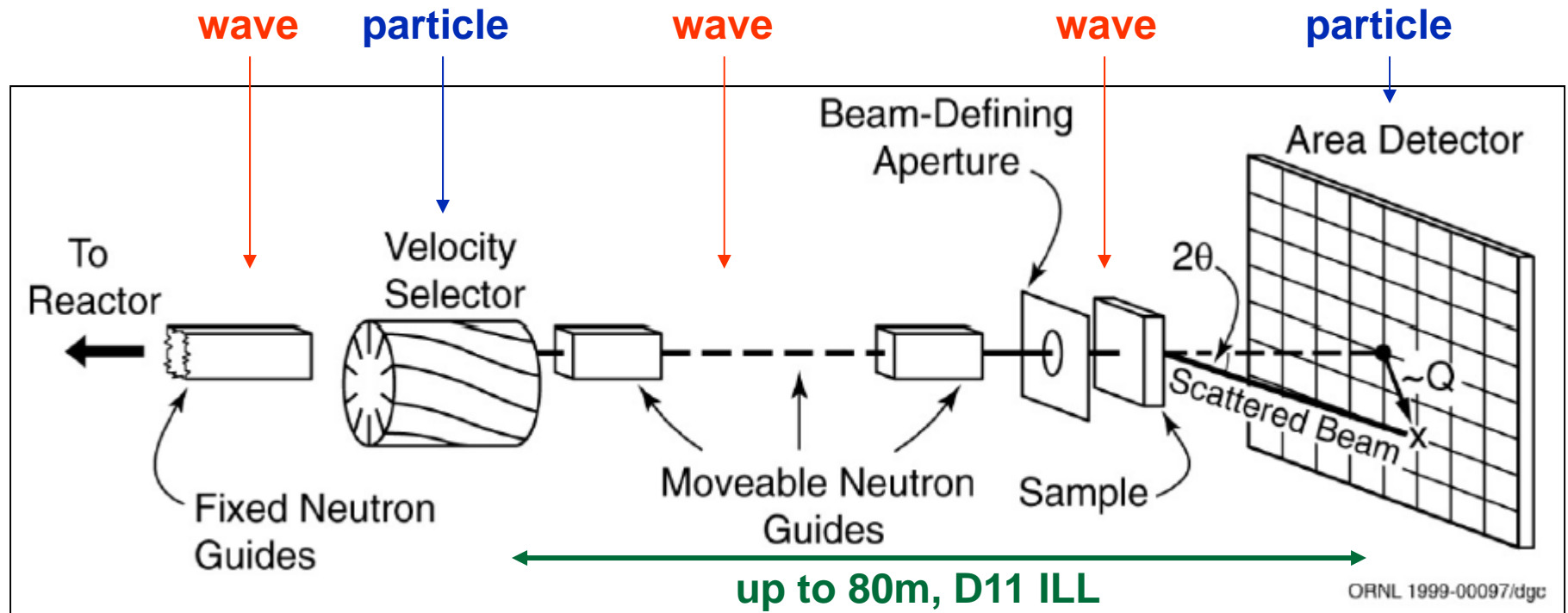
- Unique mechanical properties – “liquid” on local scale but long range structure memory
- Economic importance – Tires



?

- Blend “normal” H- and some % D-polyisoprene
- Cross-link to form rubber network
- Stretch rubber sample in the SANS beam and collect data

Layout of a SANS instrument



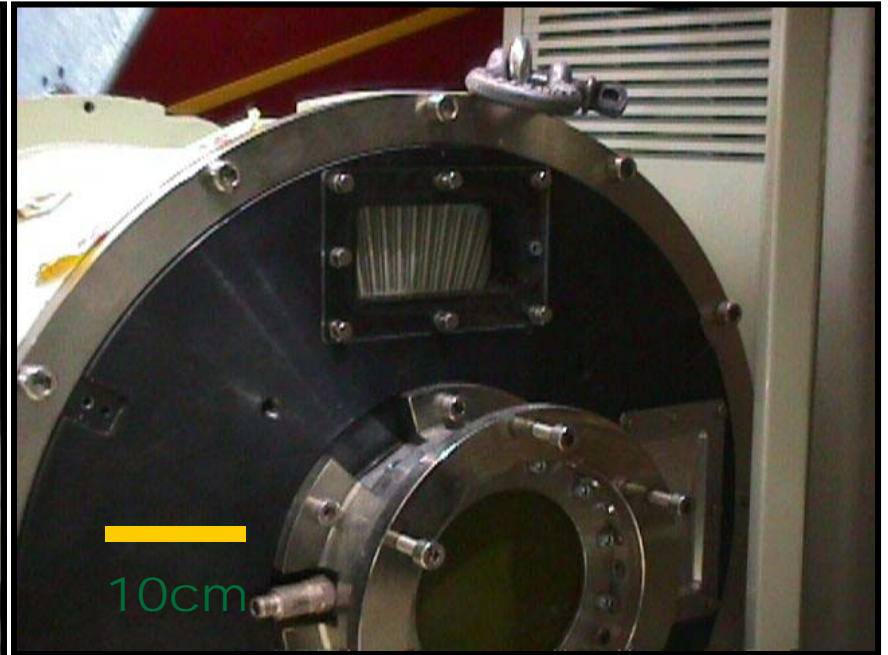
Typical layout at a continuous (reactor) source

***particle** and **wave** characteristics of neutrons are utilized*

SANS guide hall (HFIR)
a few years ago



Monochromator – Velocity Selector *neutron wavelength – neutron momentum*



De Broglie: $\lambda = \frac{h}{p} = \frac{h}{mv}$

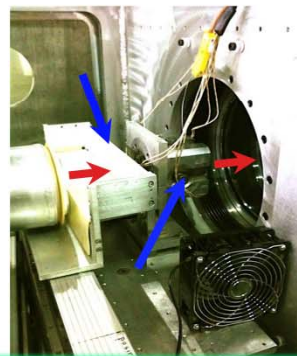
	Cold	Thermal
T (K)	20	300
v (m/s)	574	2224
E (meV)	1.7	25.9
λ (Å)	6.89	1.78

Sample Environments (Bio-SANS examples)

- 'Tumbler' Rotating sample changer



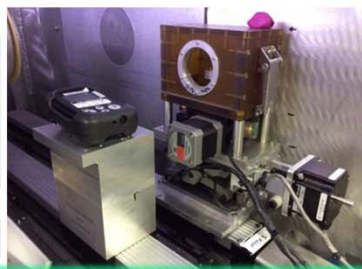
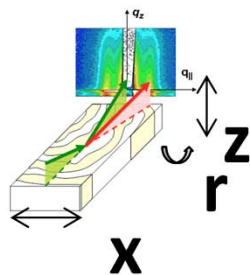
- In-situ Dilute Acid Pretreatment Cell for biomass



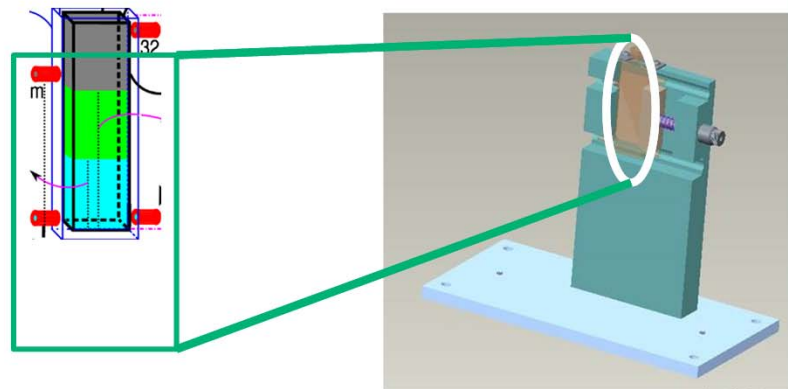
McHugh Cell



- RH-controlled Cell and Adjustable Sample Stage (LDRD)



- Flow cell for multi-phase microemulsions (LDRD)



Practical Considerations for SAS

Plan your experiment well!

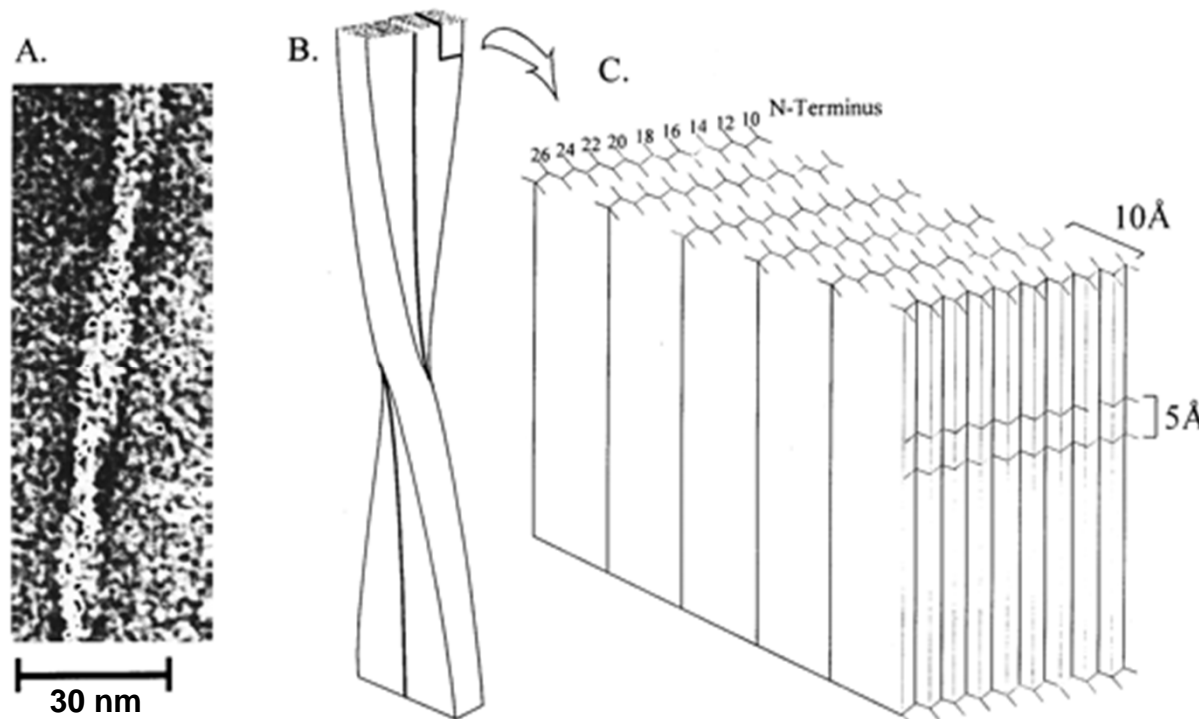
- What Q-range would I like, and what must I have?
- For how long should I measure my samples? – *counting statistics, sample size ($\sim 10 \times 10 \times 1 \text{ mm}^3$)*
 - *SANS measurement times vary: min to hrs is typical*
 - *Typical sample volume: 0.1 – 0.5 mL*
 - *Concentration for solution SANS $\sim 0.1\text{-}1\%$ (1 – 10 mg/ml)*
- How will I correct for backgrounds?
- How can I optimize my sample quality?
- Less is often more: Do fewer things but those do right! (especially with neutrons)
- **Ask your local contact / instrument scientist for advice well ahead of time!**

Data Reduction, Processing, Correction

- Normalization to monitor or time
 - Backgrounds
 - Transmission
 - Azimuthal averaging
 - Absolute intensity scale (cm^{-1})
-
- Measure and subtract background very carefully!
 - Do the absolute intensity calibration – it's worth the extra effort!

Alzheimer's Disease – β -Amyloid

- Among leading causes of death
- Miss-folded peptides form hierarchical ordered fibril structures & plaques
- Structure established using synthetic model peptides and complimentary methods NMR, SANS, EM



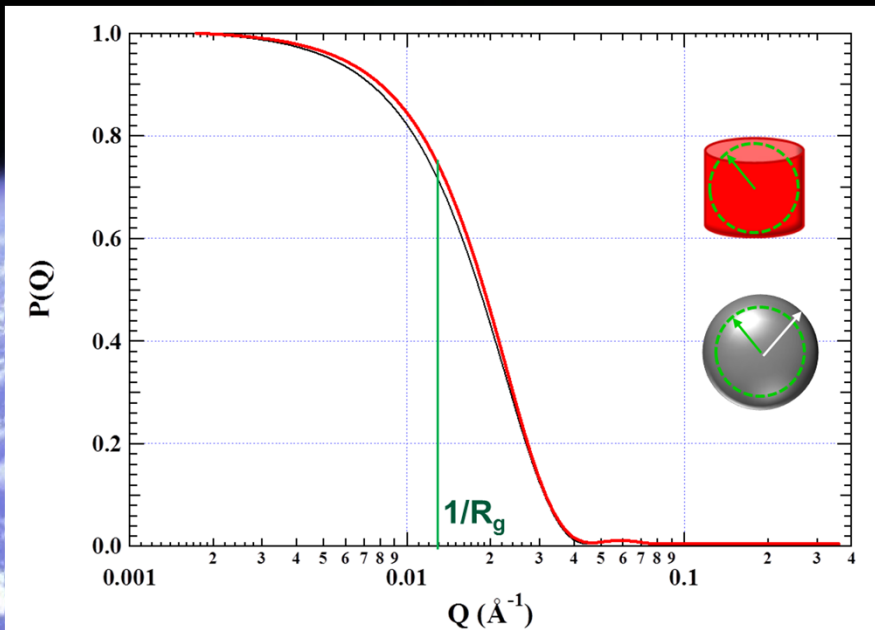
- **NMR**
 - β -fold
- **SANS**
 - Fiber
 - Diameter
 - **6 sheet stack**
- **EM**
 - Overall morphology
 - Twist

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

SAS Analysis –

A spacewalk of sorts
Fourier, Q , reciprocal space

*how to get your bearings...
baby steps*



Bruce McCandless II took the first untethered space walk in February 1984. Here we see him from Challenger, floating above Earth.

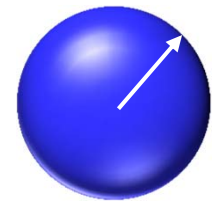
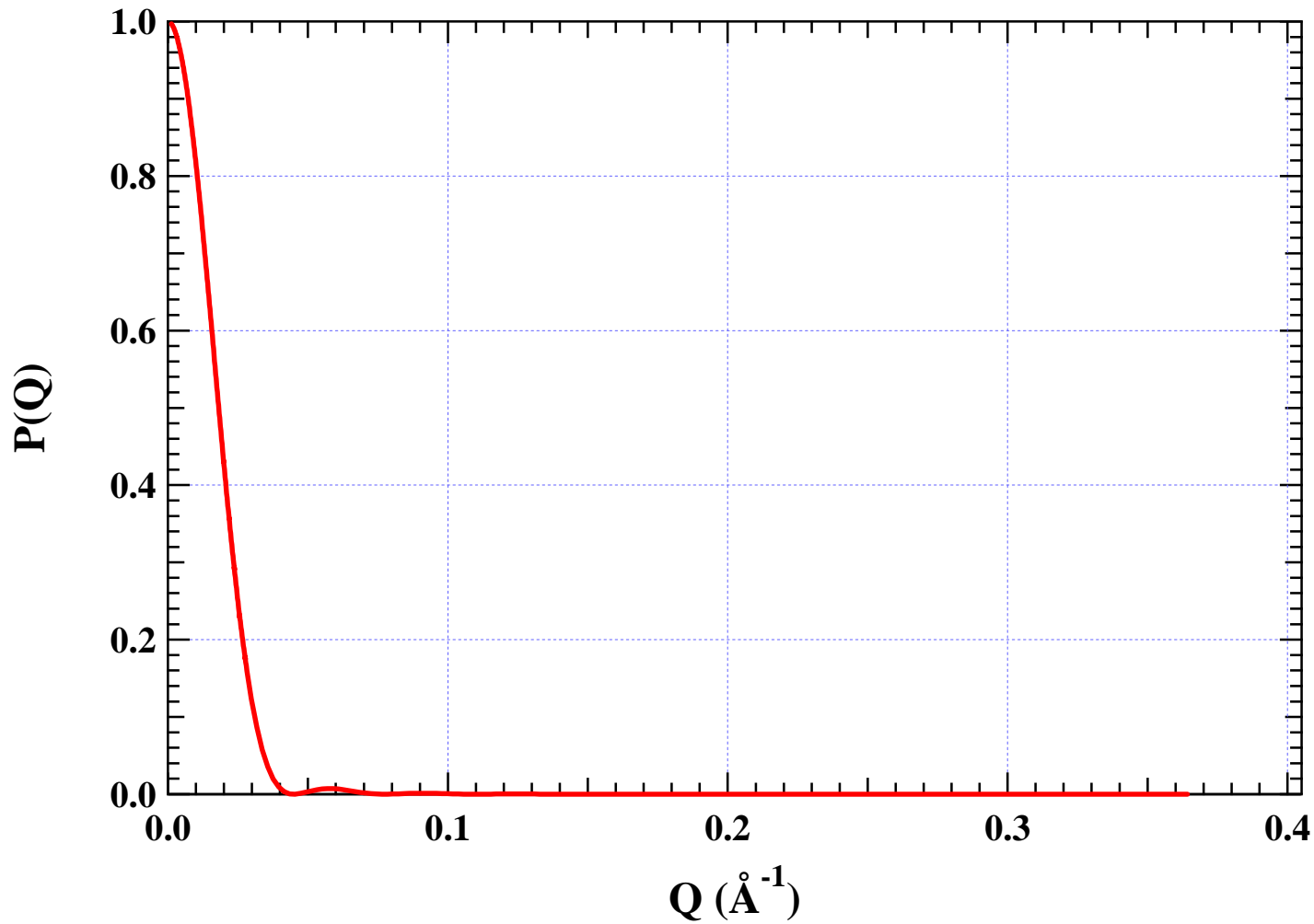
Carl Meade and Mark Lee rehearse spacewalk contingency plans in 1994



Ed White, the first American to walk in space, hangs out during the Gemini 4 mission. He's attached to the craft by both umbilical and tether lines.

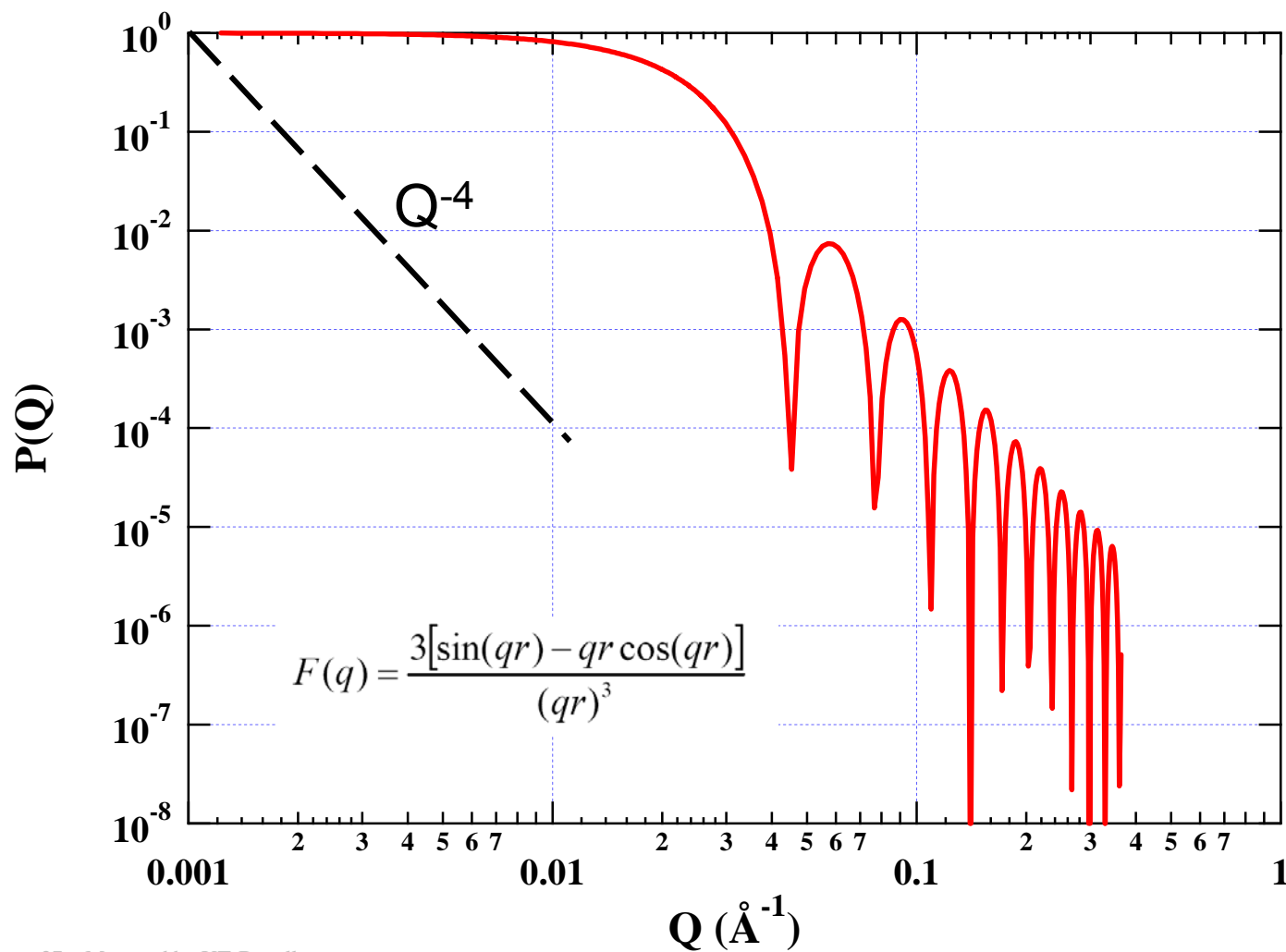
Sphere

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

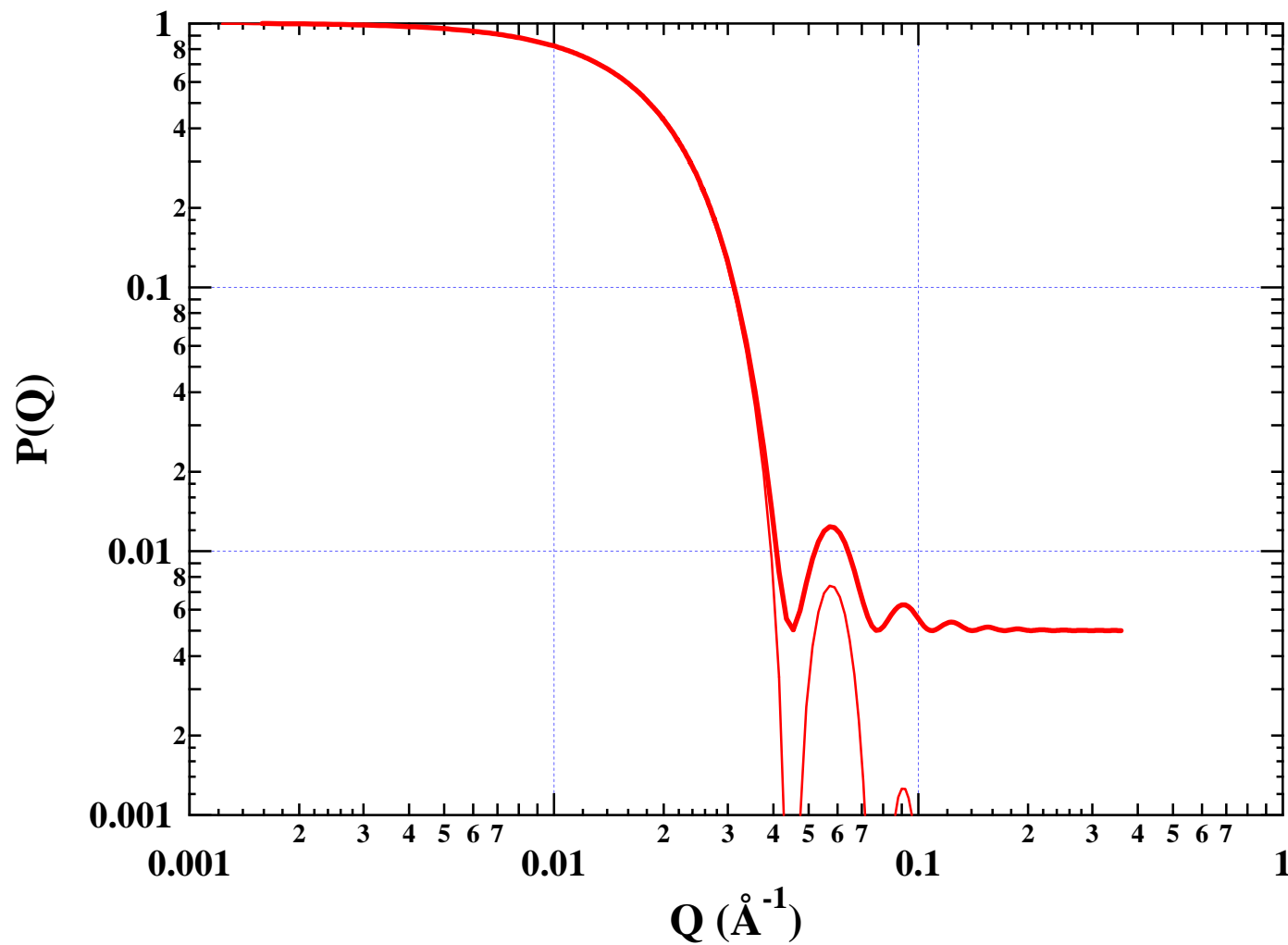


100 Å
radius

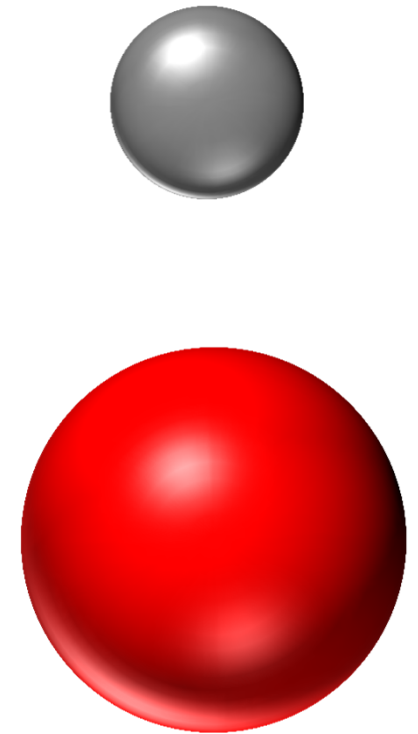
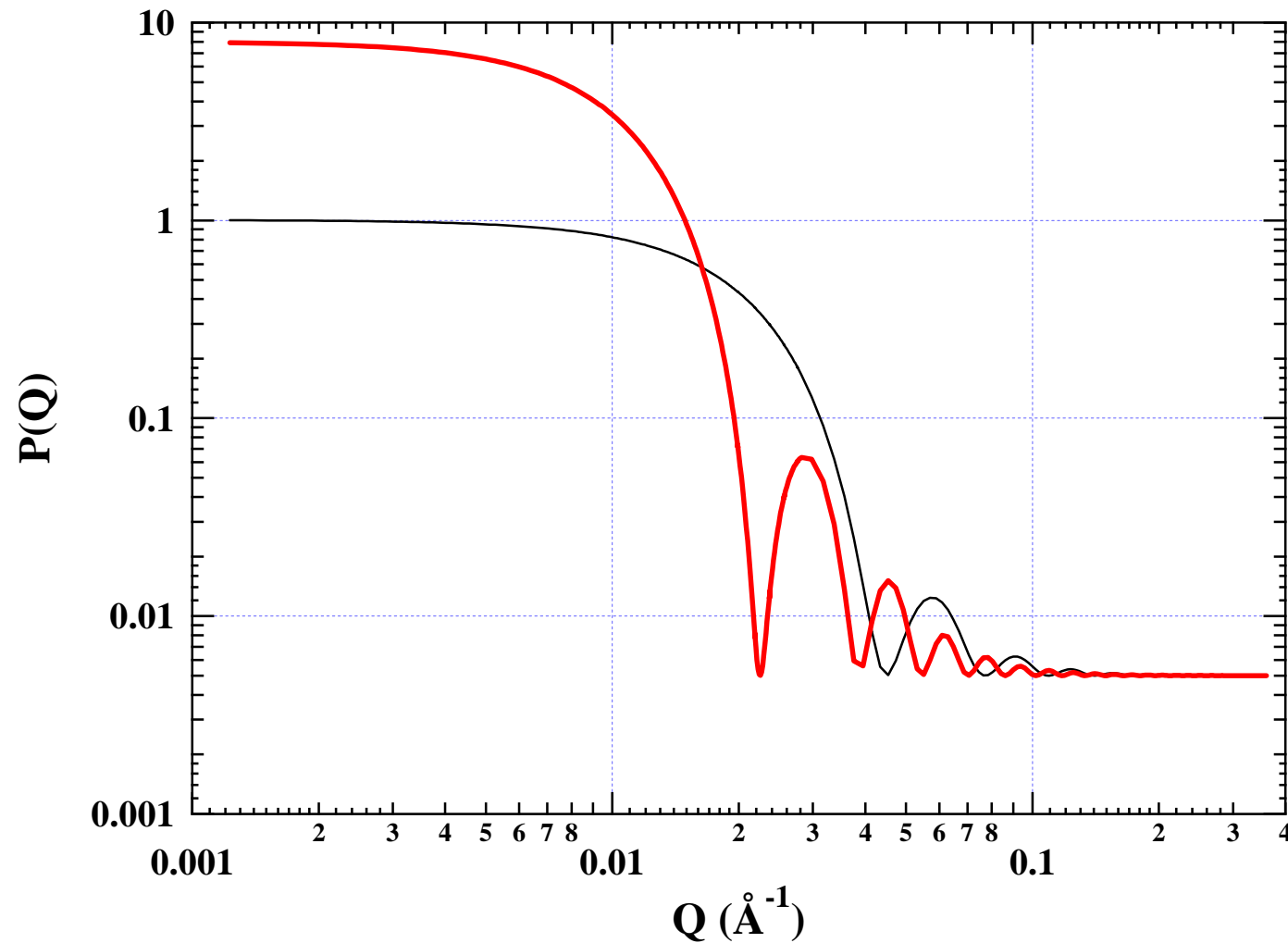
Sphere



In practice: sphere + constant background

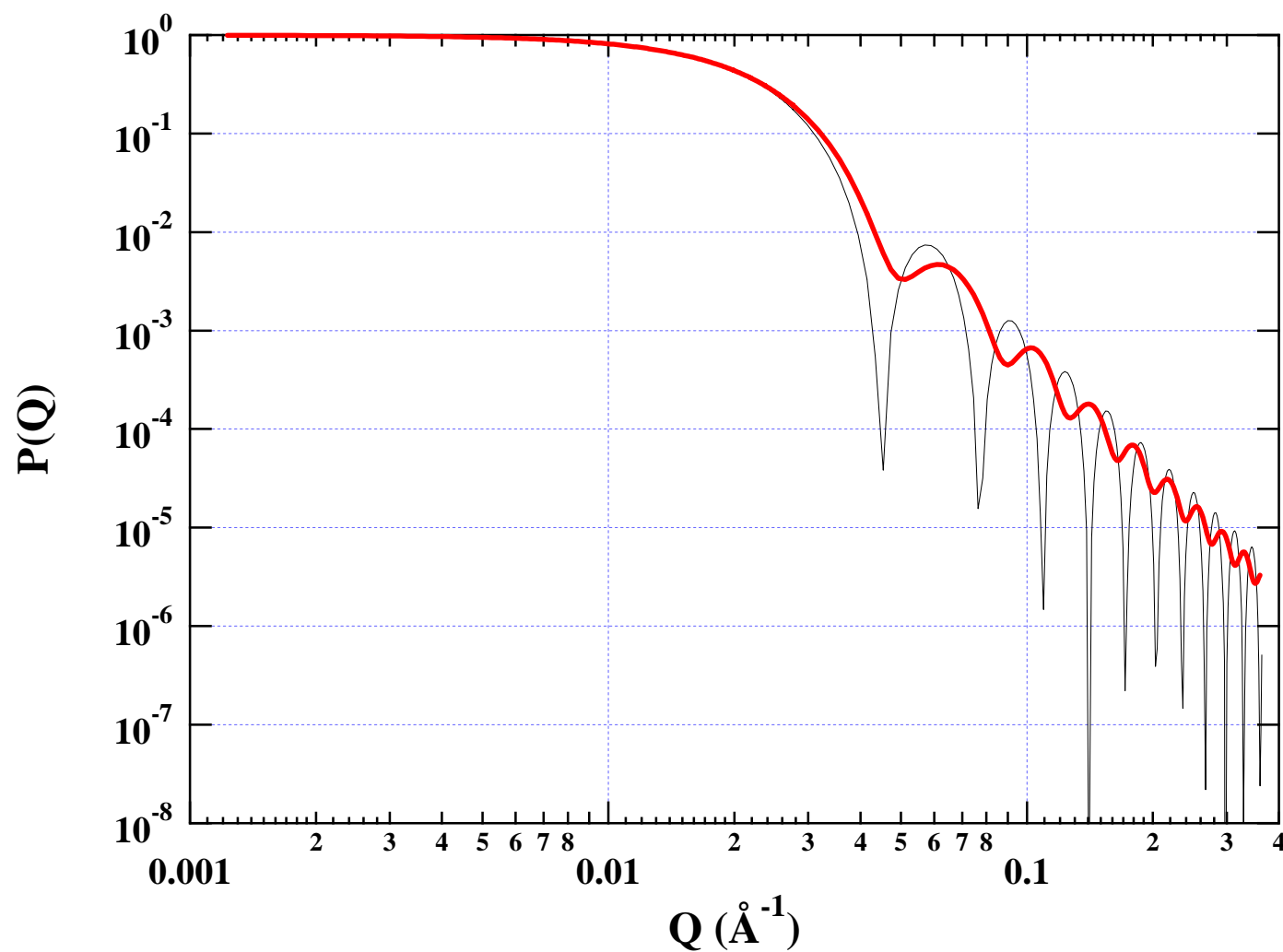


Spheres of different sizes

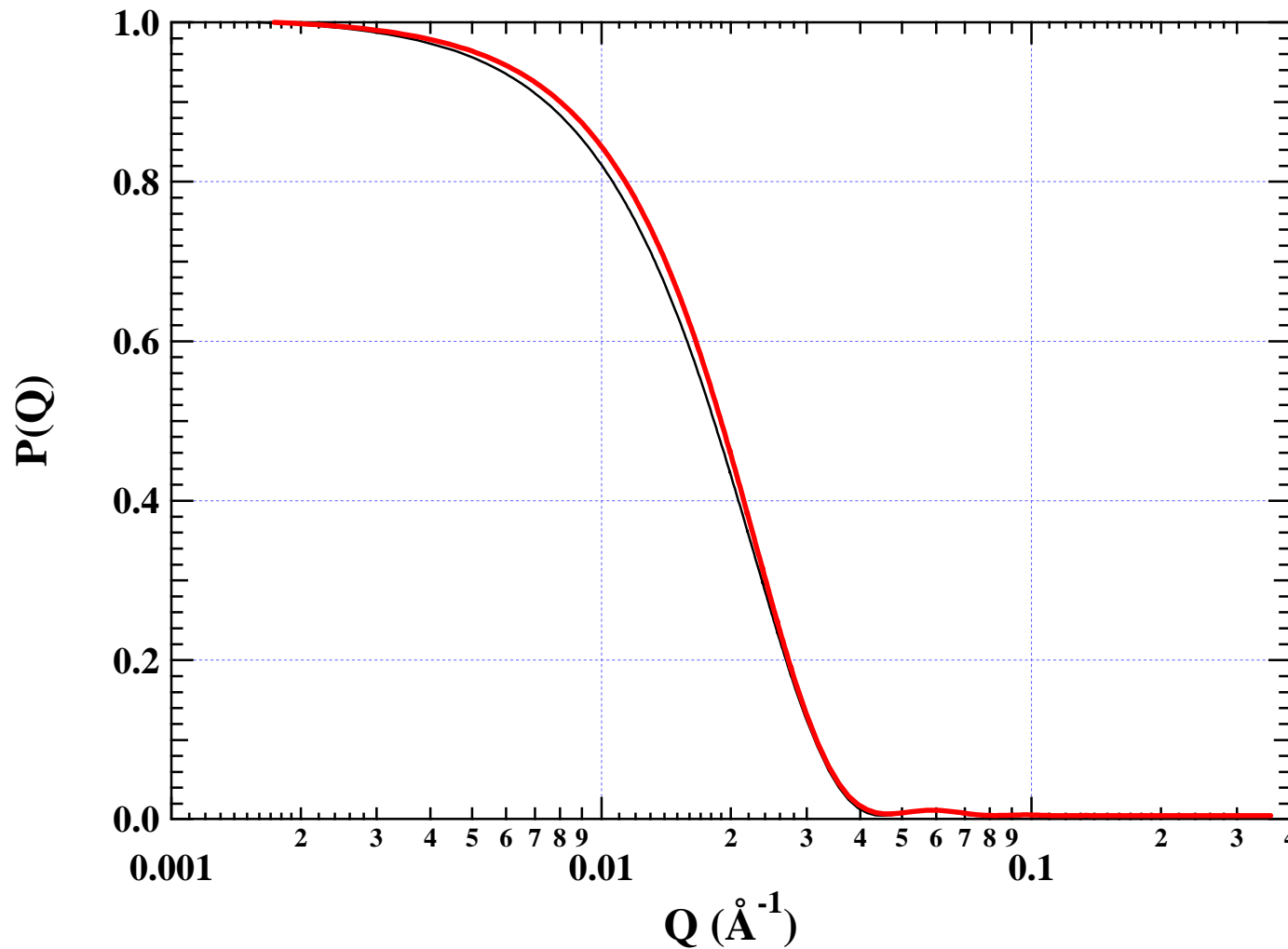


Ellipsoid

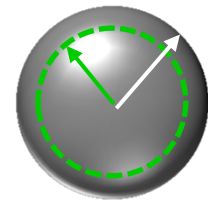
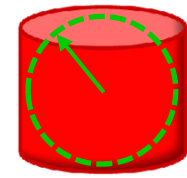
aspect ratio 1.5



Circular Cylinder - *with same R_g as the sphere*



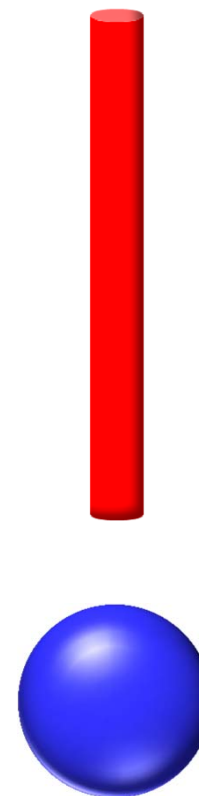
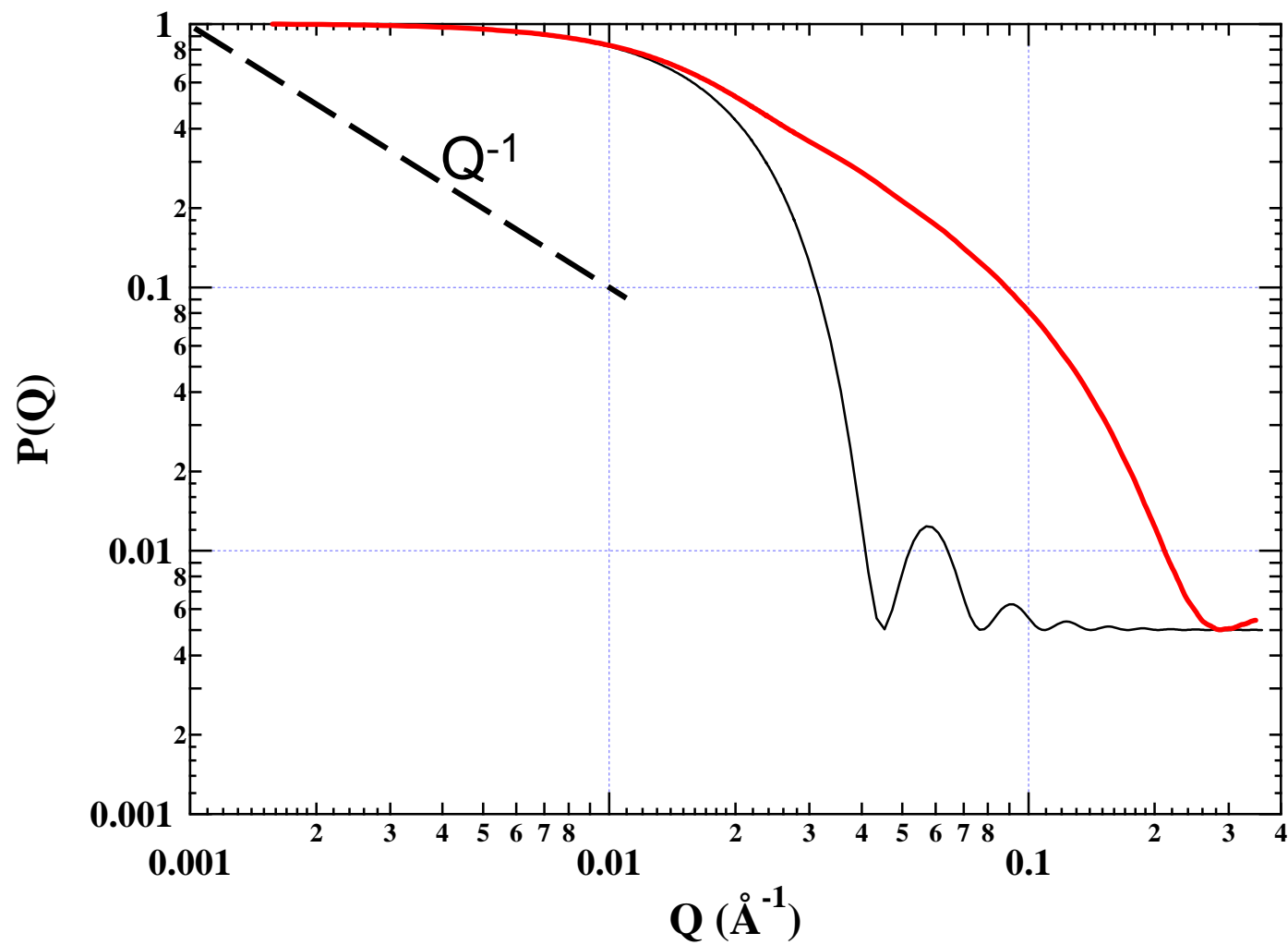
$\sim 77.46 \text{ \AA } R_g$



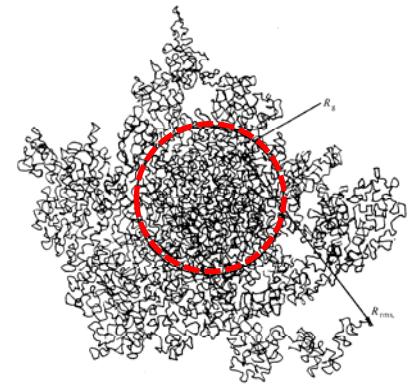
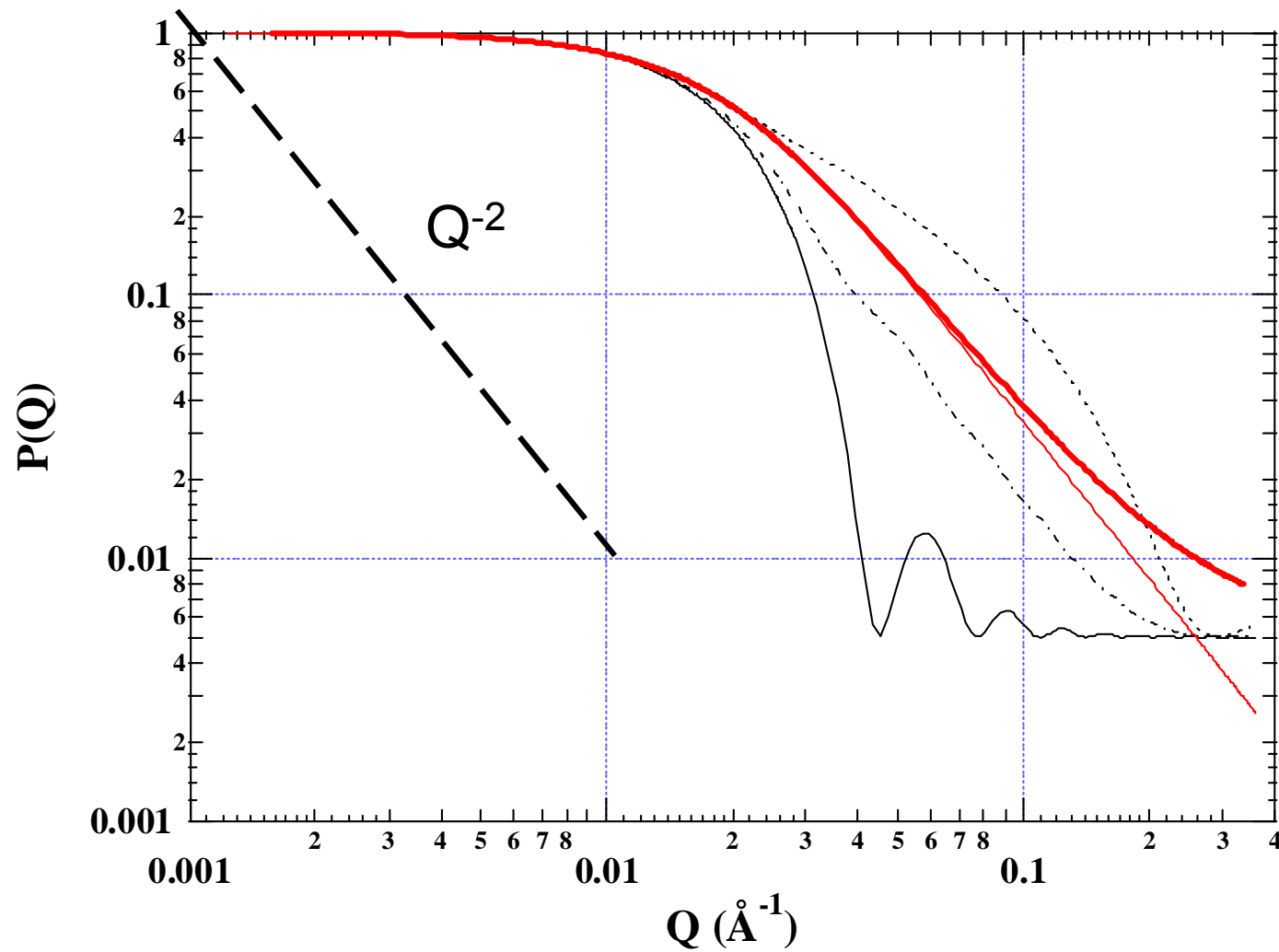
100 Å radius

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

"Long & thin" cylinder



Polymer coil



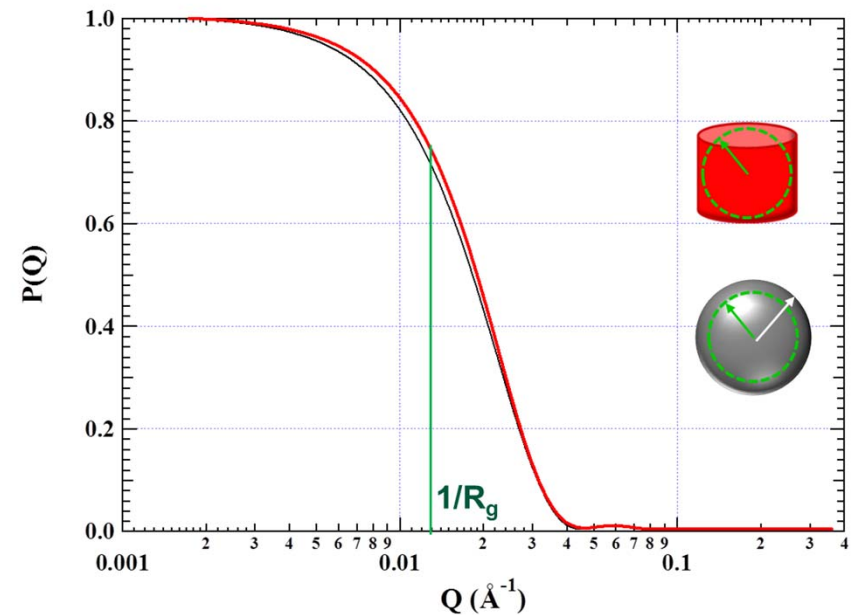
Guinier Analysis

size of any kind of object

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

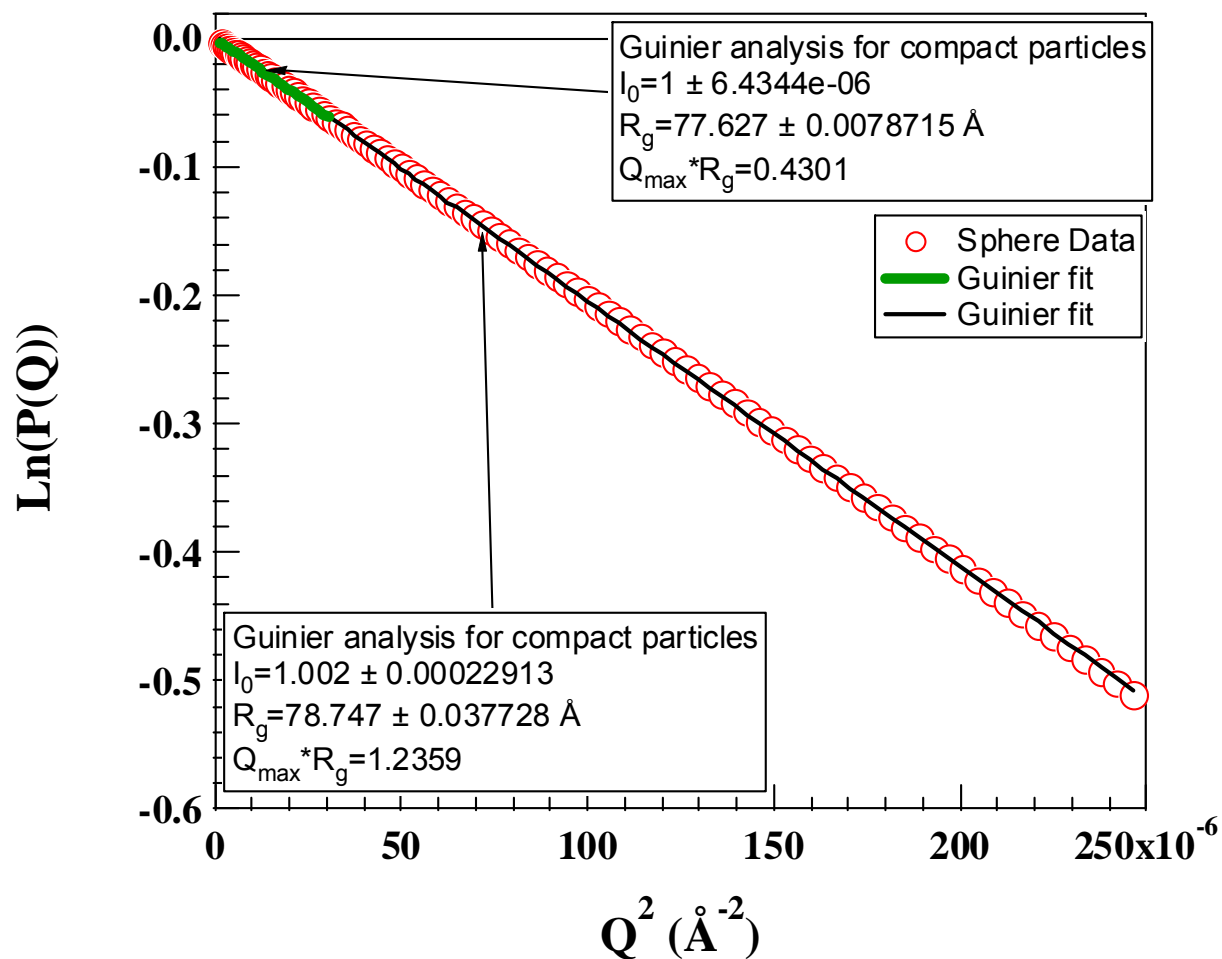
$$\ln[I(q)] \propto q^2 R_g^2 / 3 \quad qR_g < 1; \quad \text{sphere : } R = \sqrt{\frac{5}{3}} R_g$$

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



Guinier Analysis

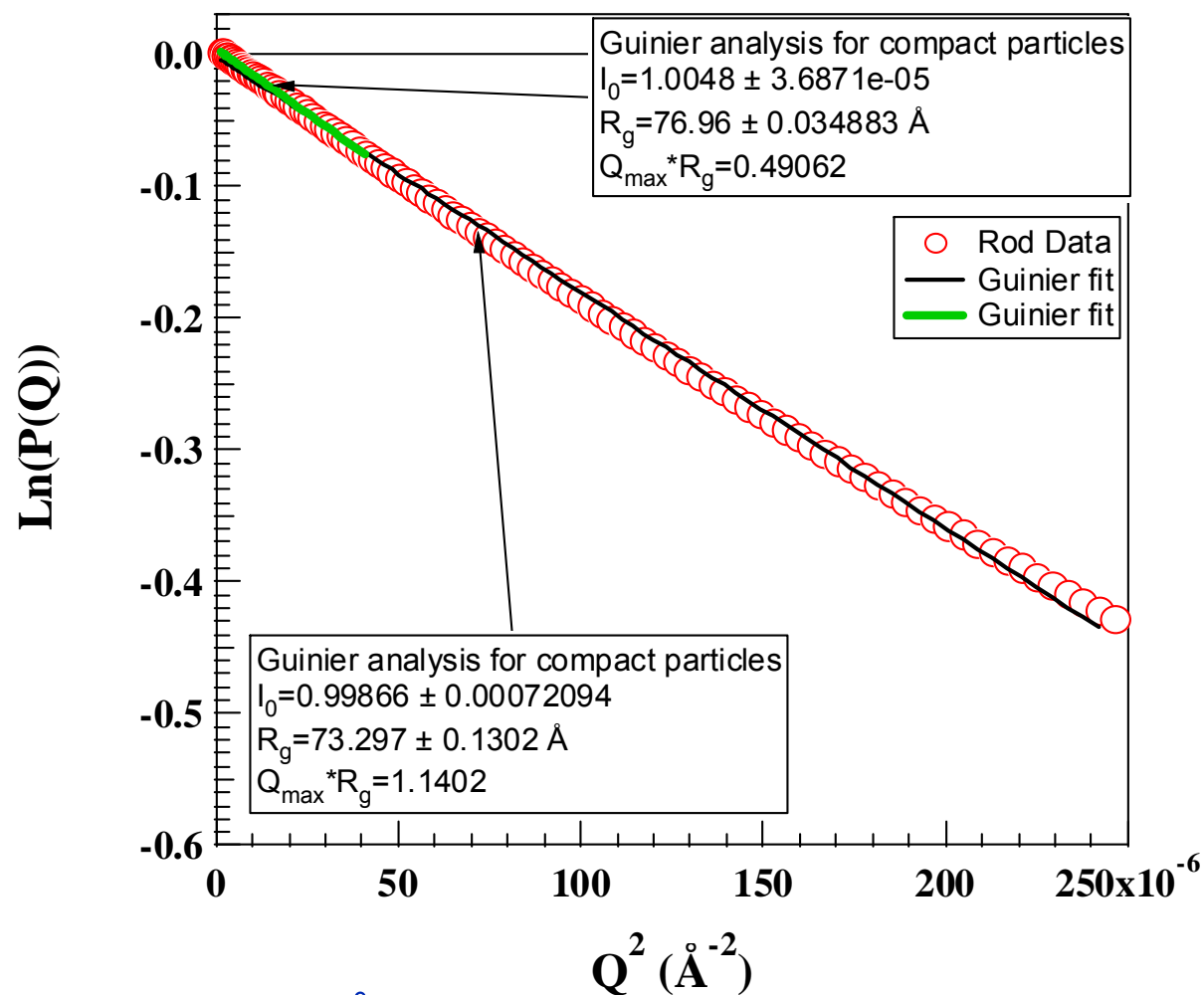
size of any kind of object



Precise R_g is 77.46 \AA

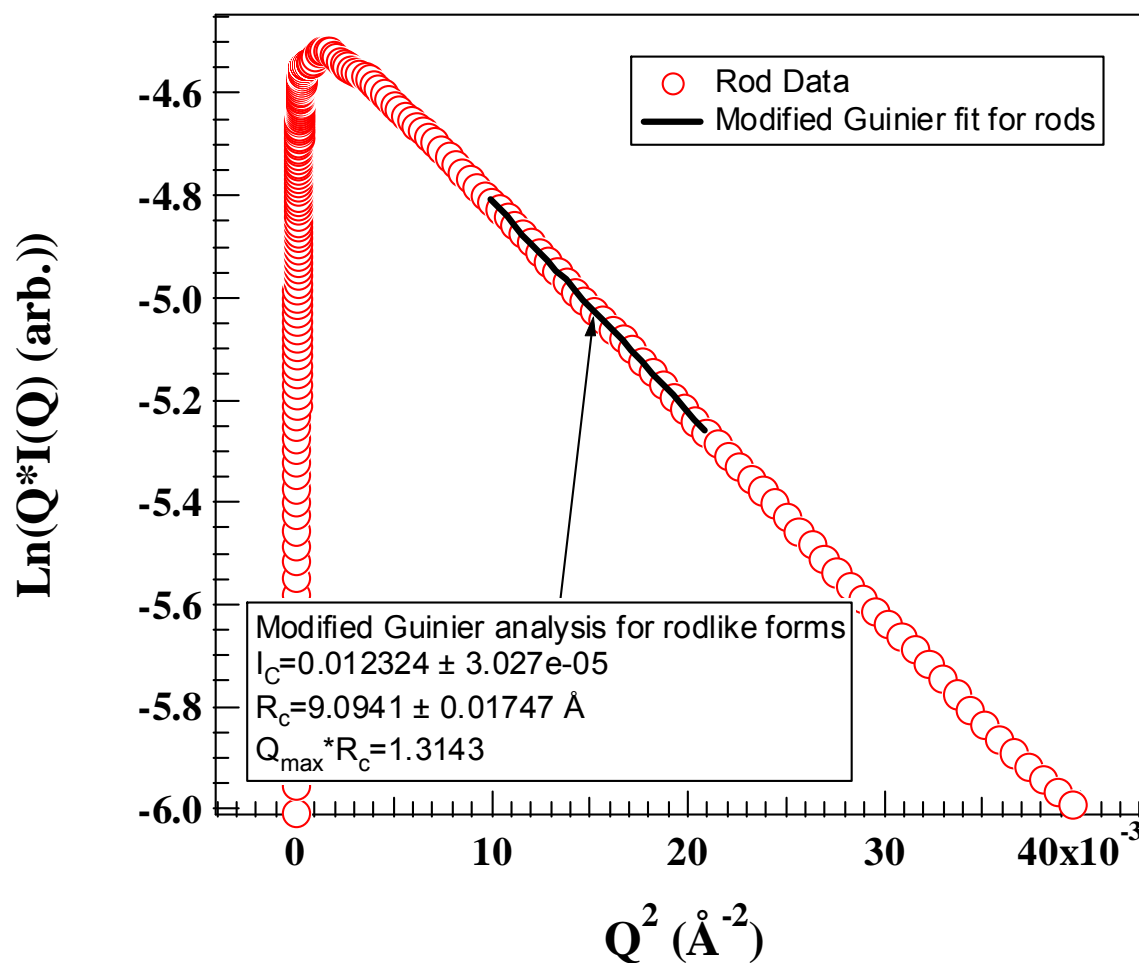
Guinier Analysis

size of any kind of object



Precise R_g is 77.46 \AA

Modified Guinier Analysis *for object extended in 1 dimension*

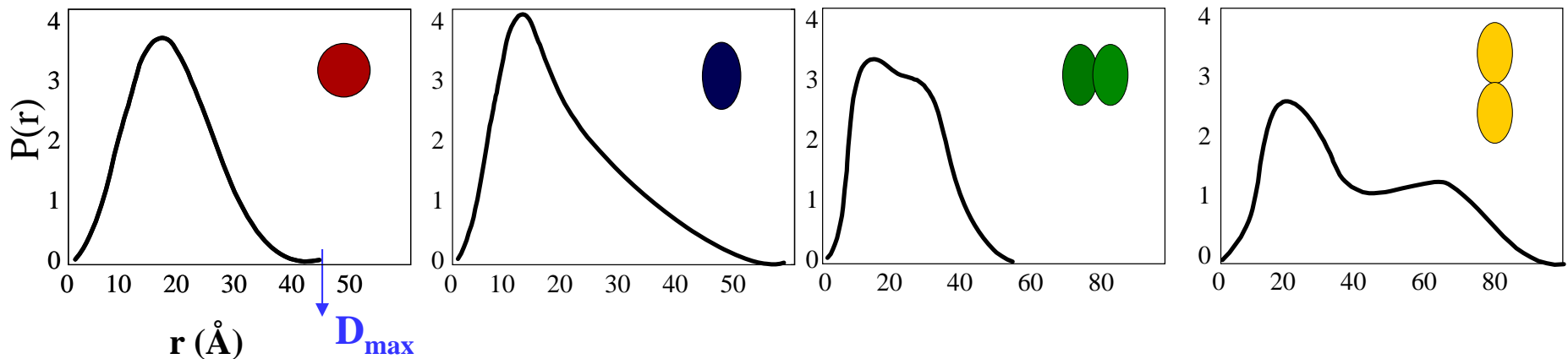
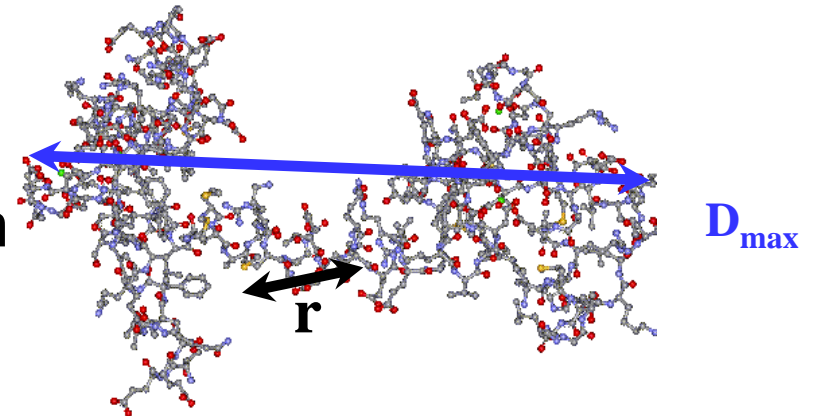


Rod radius = $\sqrt{2} * R_C = 12.9 \text{ \AA}$, exact radius = 13.3 \AA

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

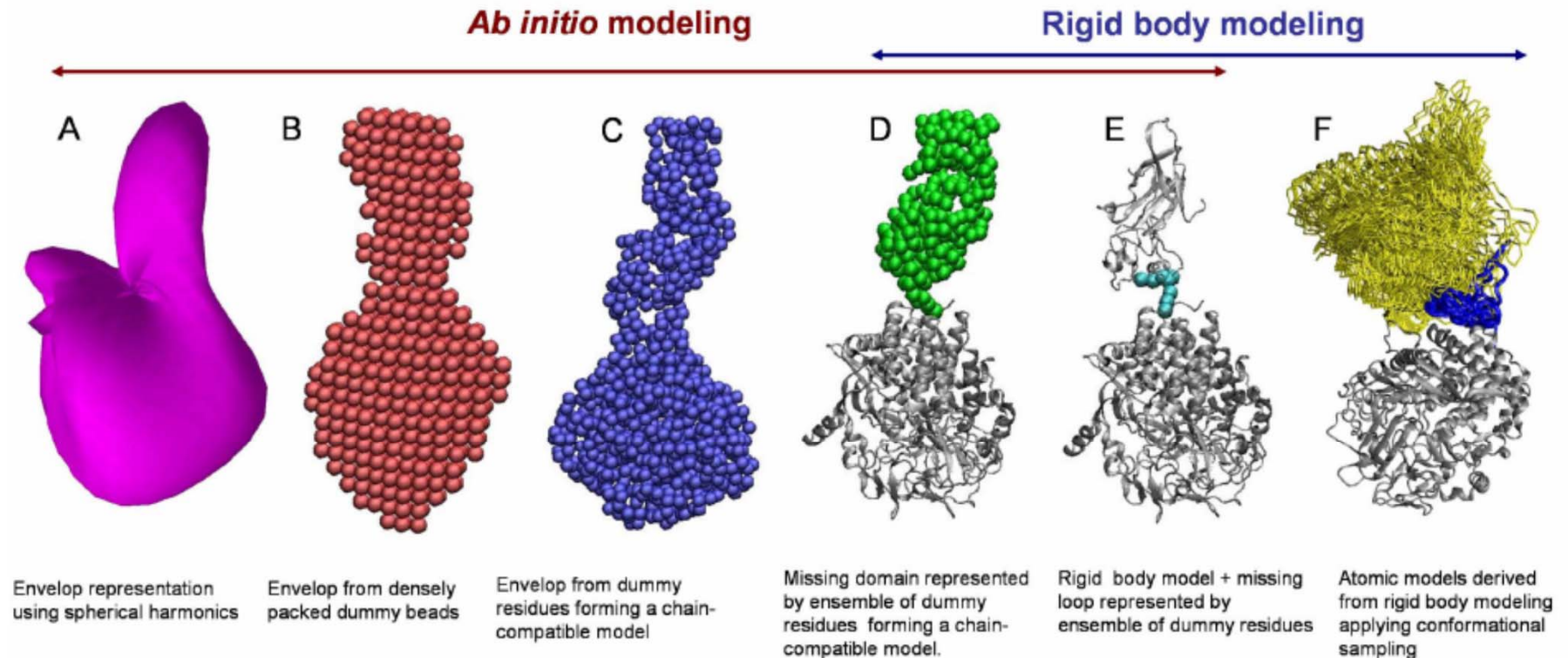
Pair correlation function and shape

$P(r)$: 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.

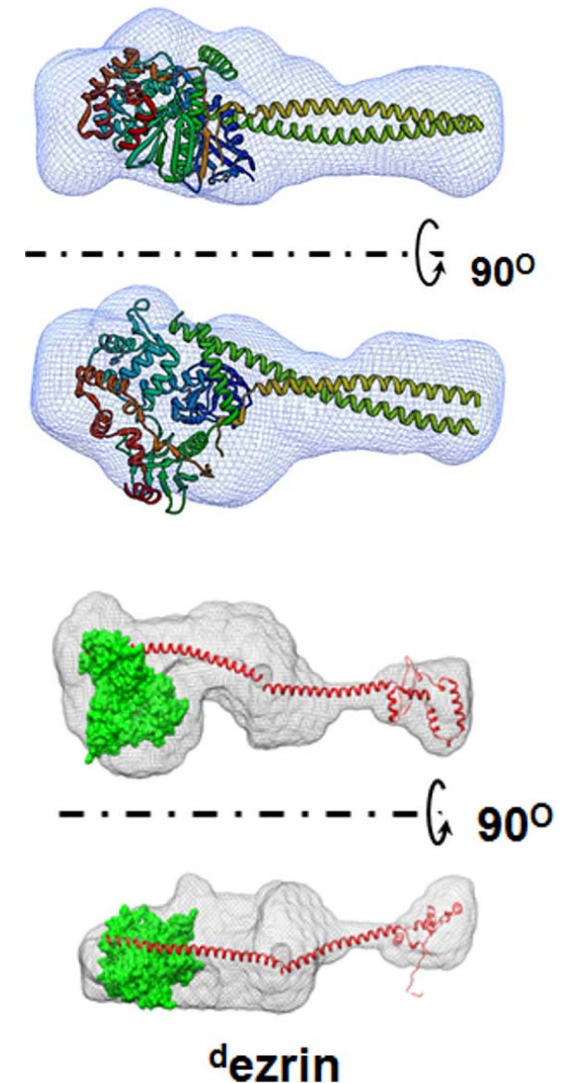
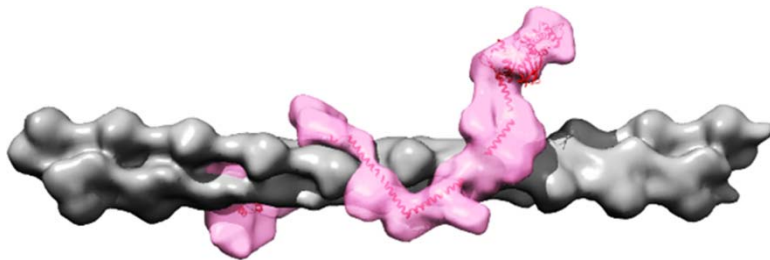
SAS Form Factor Modeling *often used in biology*



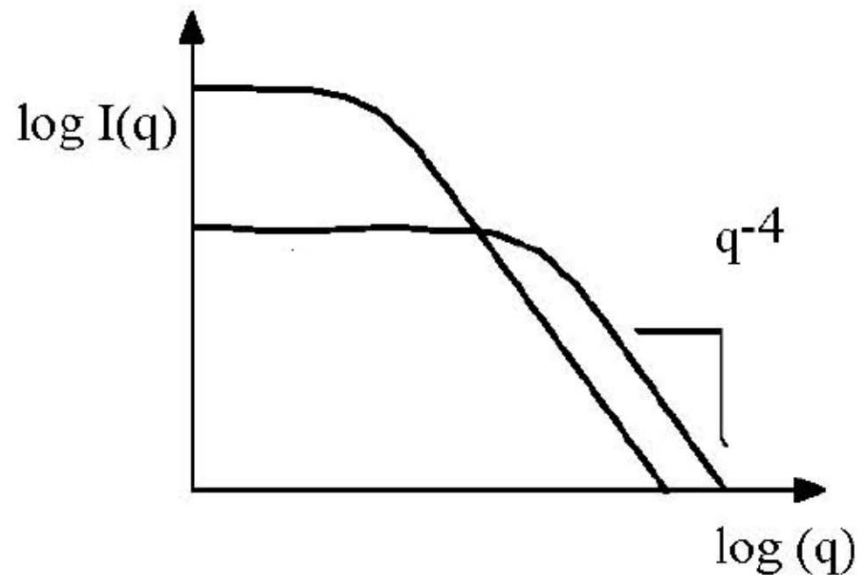
- Spherical Harmonics (Svergun, Stuhrmann, Grossman ...)
- Aggregates of Spheres (Svergun, Doniach, Chacón, Heller ...)
- Sets of High-resolution Structures (Svergun, Heller, Grishaev, Gabel ...)
- Simple Shapes and Custom Approaches (Henderson, Zhao, Gregurick, Heller ...)

Example: Protein Complex

- Open Conformation of Ezrin Bound to Phosphatidylinositol 4,5-Bisphosphate and to F-actin Revealed by SANS
- Ezrin is an adapter protein localized at the interface between cell membrane and actin cytoskeleton, regulating a variety of cellular functions.
- D-labeling of ezrin and contrast variation allowed observation of
 - conformational change upon signaling lipid (PIP2) binding
 - influence of mutation on conformational change
 - ezrin on F-actin: extensive contacts with the filament



Surface Scattering - *Porod*



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

Specific Surface Area, S_v

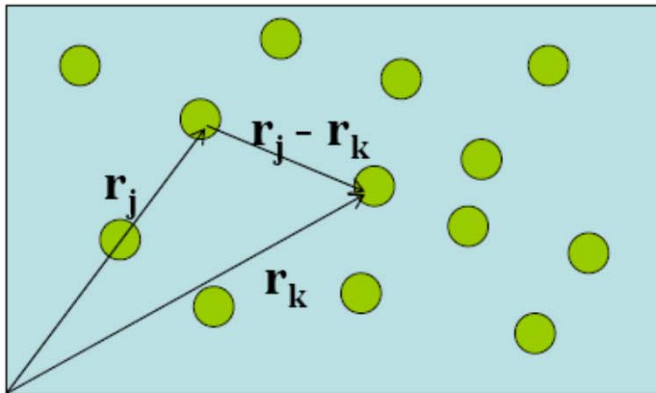
$$\lim_{q \rightarrow \infty} I(q) = 2\pi S_v |\Delta\rho|^2 q^{-4}$$

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

Interparticle Structure Factor $S(Q)$

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

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

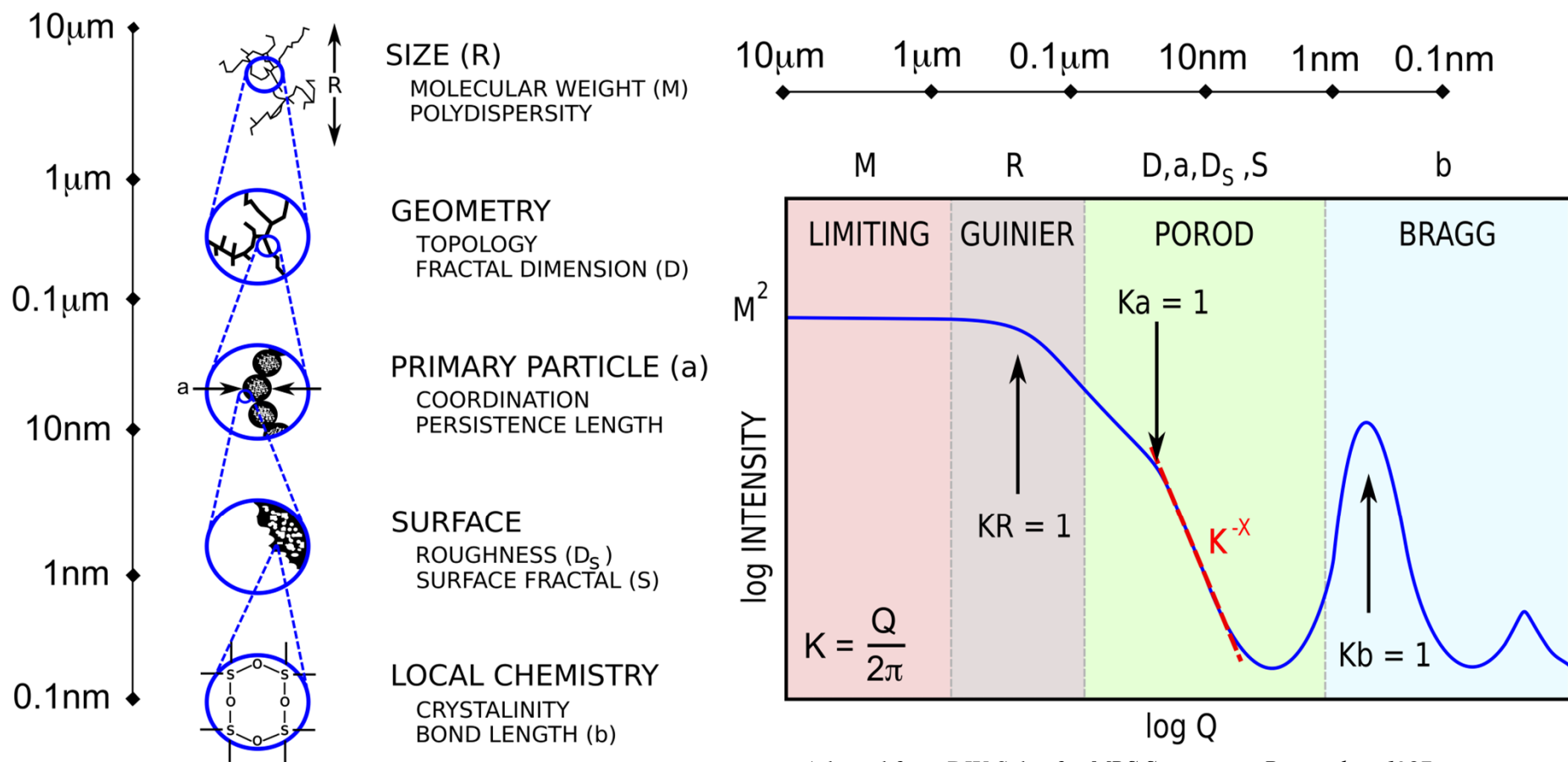


$I(q)$ is modulated by interference effects between radiation scattered by different scattering bodies.

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

$S(q) \cdot P(q)$ is not always valid and useful!

Structural Hierarchy (particulate)

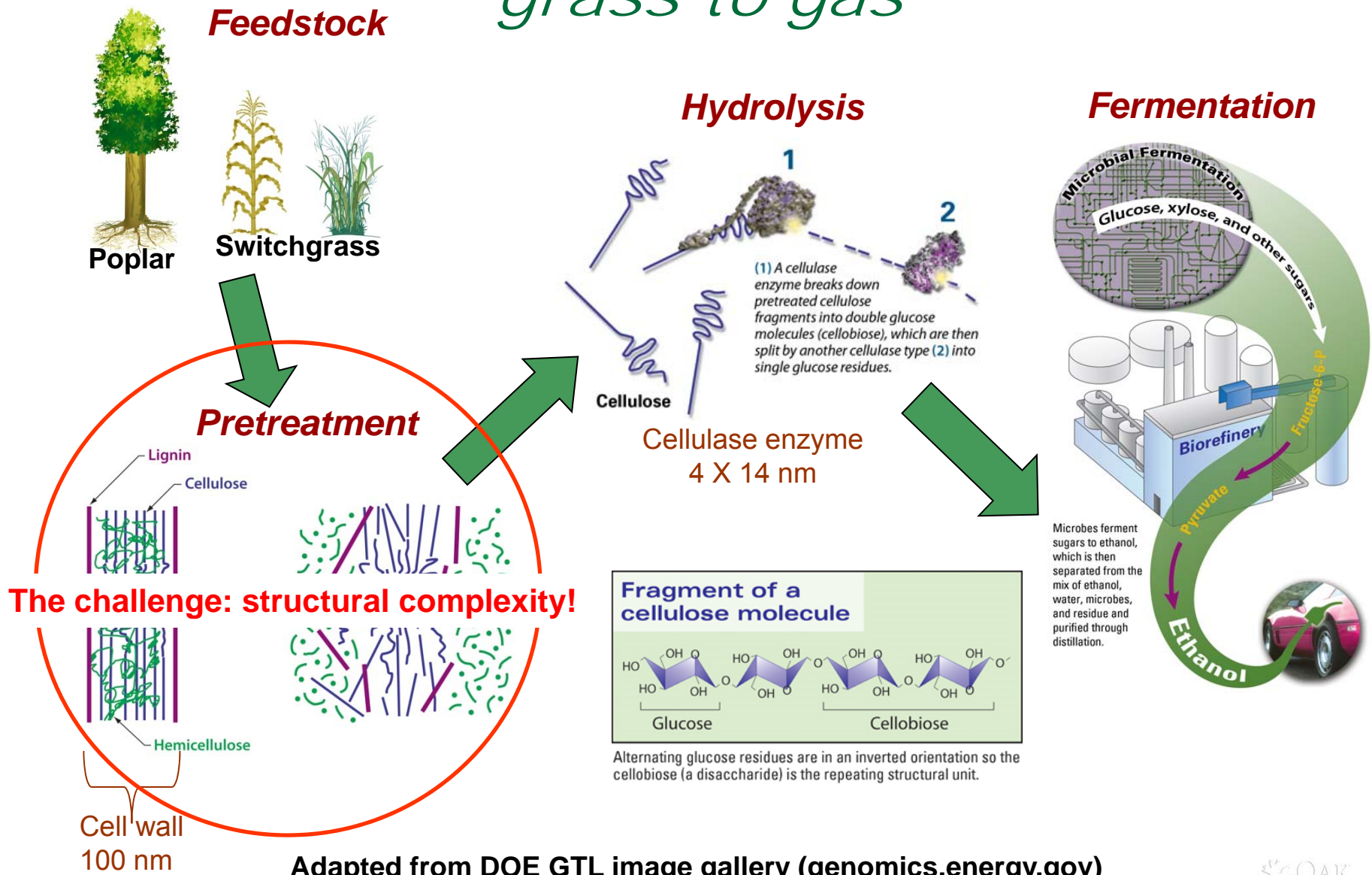


Adapted from DW Schaefer *MRS Symposium Proceeding* 1987

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

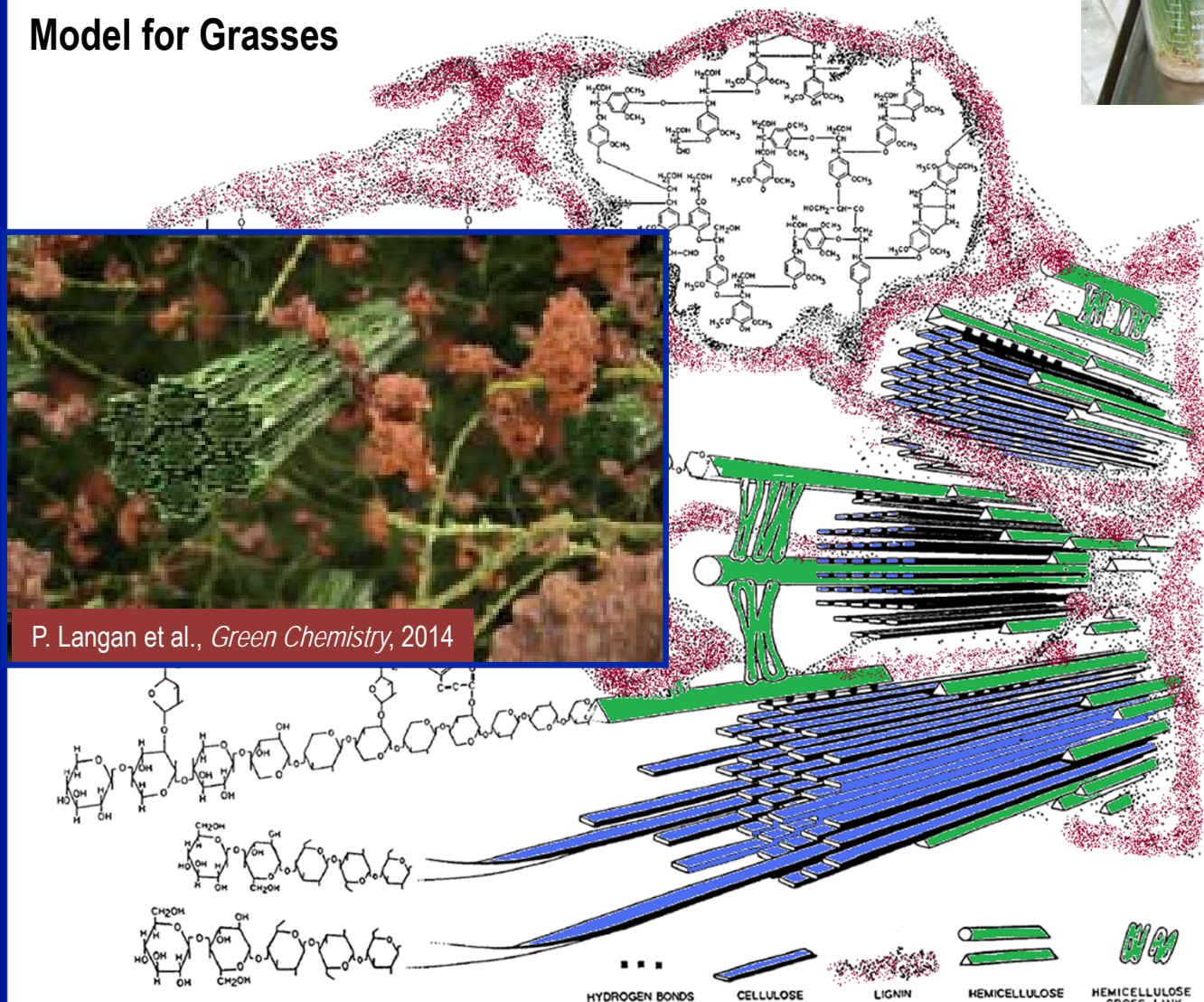
Example: Biomass to Cellulosic Ethanol *"grass to gas"*



"BIOMESS"



Model for Grasses



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

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

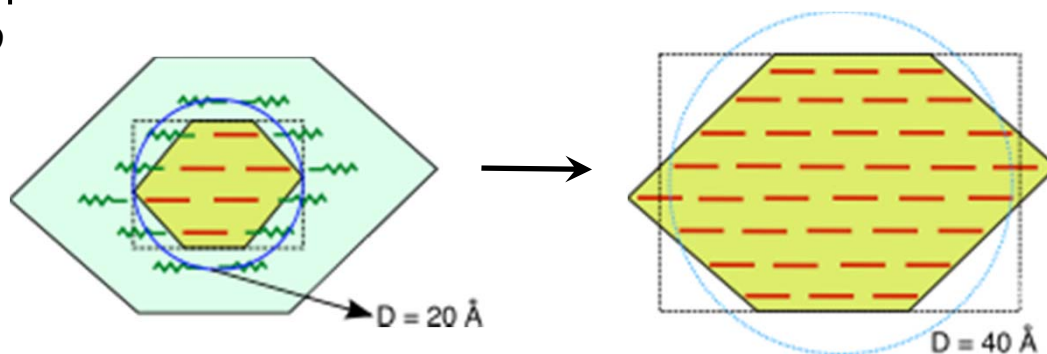
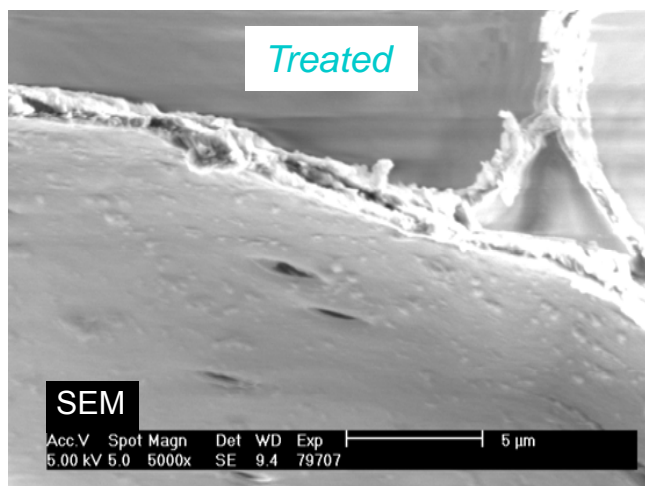
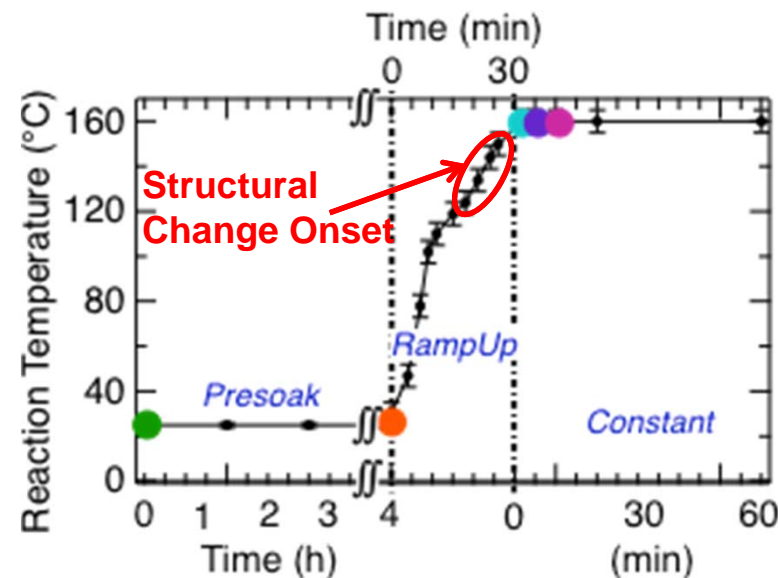
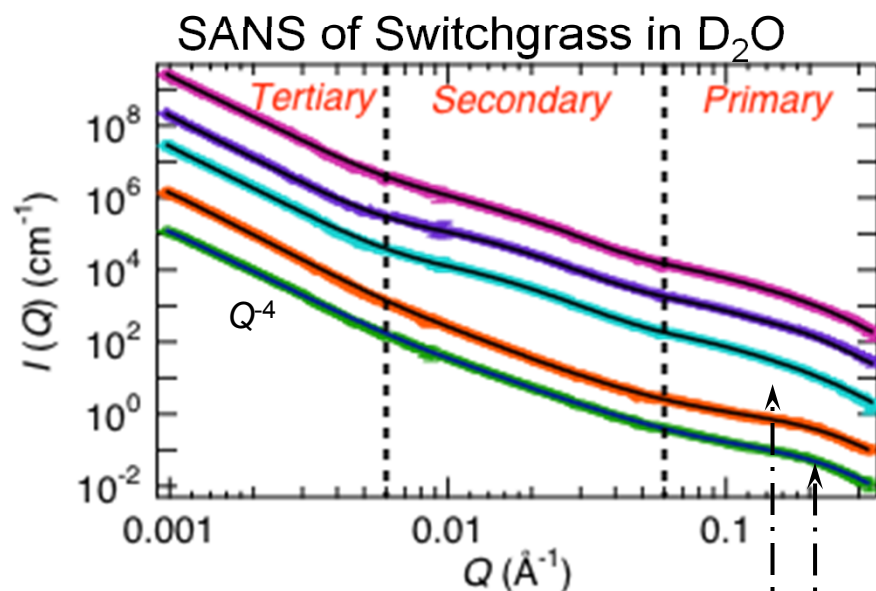
Why neutrons?

- Use D₂O for **contrast**
- Observe changes over time in pressure reaction cell with SANS

Outcome

New understanding of what processes actually happen during industrial pretreatment.

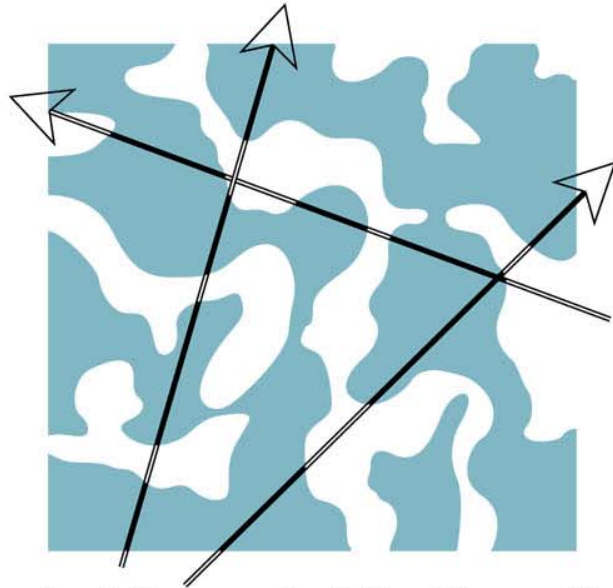
Dilute Acid Pretreatment of Switchgrass



Elementary Cellulose Fibril
Cross-sectional View

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: $\gamma(r) = e^{-r/a}$

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

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

SAS Summary

- SAS applications are in the nm to μm range and otherwise only limited by imagination.
- SAS 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.
- SAS is used alone, but often complementary to other methods, such as microscopy, NMR.
- Scattering is similar to diffraction but does not require crystals.
- SAS data analysis is application dependent, using a diverse set of approximations, models and software.

SAS Literature 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.

- 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.

- 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.