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 information 1nm-1µm

• X-rays
  – Rotating anode / sealed tube: ~ 400 k$
  – 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-1µm

…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-1μm
  – Contrast labeling options (stains / isotope labels)

• SAS practical aspects
  – No special sample preparation such as cryo-microtome
  – Sample environments control (p, T, H)
  – Non-destructive (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

- 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 \( \lambda \)): 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 \( k \):** \(|k| = k = \frac{2\pi}{\lambda}\)

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

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

\[
d = \frac{2\pi}{q}
\]

\( q \) in nm\(^{-1}\) or Å\(^{-1}\)

**Bragg:** waves with wavelength \( \lambda \) reflected by sets of lattice planes

\[
\Delta = 2d \sin(\theta)
\]

if \( \Delta = n \lambda \) then reflection, else extinction
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 $P(r)$.

$$I(q) = \left| \int_V (\rho(\vec{r}) - \rho_s) e^{-i\vec{q} \cdot \vec{r}} \, d^3 r \right|^2$$
Absolute Intensity / Scattering Cross Section – cm⁻¹?

\[
\frac{dI}{d\Omega} = TI_0 D \frac{d\Sigma}{d\Omega}
\]

\[
\frac{d\Sigma}{d\Omega} = \frac{1}{TI_0 D} \frac{dI}{d\Omega}
\] [cm⁻¹sterad⁻¹]

- \(dI/d\Omega\) = Scattered intensity per solid angle
- \(I_0\) = Primary beam intensity
- \(T\) = Transmission (x-ray absorption, incoherent neutron scattering)
- \(D\) = Thickness
- \(d\Sigma/d\Omega\) = Scattering cross section per unit volume [cm⁻¹sterad⁻¹]
Comparing SAXS and SANS

• SAXS & SANS
  – nm scale structural analysis (~1nm-1μm)
  – 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

<table>
<thead>
<tr>
<th>Element</th>
<th>Neutrons (10^{-12} cm)</th>
<th>X-rays (10^{-12} cm)</th>
<th>Electrons</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>-0.374</td>
<td>0.28</td>
<td>1</td>
</tr>
<tr>
<td>$^2$H (D)</td>
<td>0.667</td>
<td>0.28</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>0.665</td>
<td>1.67</td>
<td>6</td>
</tr>
<tr>
<td>N</td>
<td>0.940</td>
<td>1.97</td>
<td>7</td>
</tr>
<tr>
<td>O</td>
<td>0.580</td>
<td>2.25</td>
<td>8</td>
</tr>
<tr>
<td>P</td>
<td>0.520</td>
<td>4.23</td>
<td>15</td>
</tr>
</tbody>
</table>

For SAS: $SL \rightarrow SLD \rightarrow \Delta SLD$
SANS – Contrast Variation

D$_2$O/H$_2$O contrast variation

Bile salt micelle

Phosphat Ch
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**

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 – wave proof machine”
SANS guide hall (HFIR) a few years ago
Monochromator – Velocity Selector

neutron wavelength – neutron momentum

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

<table>
<thead>
<tr>
<th></th>
<th>Cold</th>
<th>Thermal</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (K)</td>
<td>20</td>
<td>300</td>
</tr>
<tr>
<td>(v) (m/s)</td>
<td>574</td>
<td>2224</td>
</tr>
<tr>
<td>E (meV)</td>
<td>1.7</td>
<td>25.9</td>
</tr>
<tr>
<td>(\lambda) (Å)</td>
<td>6.89</td>
<td>1.78</td>
</tr>
</tbody>
</table>
Practical Considerations at SANS and SAXS User Facilities

• 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 (~ 10 x 10 x 1 mm³)

• 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 calibration – it’s worth the extra effort!
Alzheimer’s Disease – $\beta$-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**
  - $\beta$-fold
- **SANS**
  - Fiber
  - Diameter
  - 6 sheet stack
- **EM**
  - Overall morphology
  - Twist

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.

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

![Graph of P(Q) vs. Q (Å⁻¹) with a 100 Å radius sphere](image)
Sphere

$$F(q) = \frac{3[\sin(qr) - qr \cos(qr)]}{(qr)^3}$$
In practice: sphere + constant background
Spheres of different sizes
Ellipsoid
aspect ratio 1.5
Circular Cylinder -*with same $R_g$ as the sphere*

$$\sim 77.46 \, \text{Å} \quad R_g$$

Radius of Gyration, $R_g$ = rms average distance from center of *scattering mass*
“Long & thin” cylinder
Polymer coil

\[ P(Q) \]

\[ Q^{-2} \]
Guinier Analysis
size of any kind of object

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

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

• Modified Guinier approximations exist to determine cross sectional radius of rods or thickness of sheets.
Guinier Analysis
size of any kind of object

Guinier analysis for compact particles
\[ I_0 = 1 \pm 6.4344 \times 10^{-6} \]
\[ R_g = 77.627 \pm 0.0078715 \text{ Å} \]
\[ Q_{\text{max}}^2 R_g = 0.4301 \]

Guinier analysis for compact particles
\[ I_0 = 1.002 \pm 0.00022913 \]
\[ R_g = 78.747 \pm 0.037728 \text{ Å} \]
\[ Q_{\text{max}}^2 R_g = 1.2359 \]

Precise \( R_g \) is 77.46 Å
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 = $\sqrt{2} \times R_c = 12.9 \, \text{Å}$, exact radius = 13.3 Å

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 of great use 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

Jayasundar et al., JBC 2012
Surface Scattering - *Porod*

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

Specific Surface Area, \( S_V \)

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

But, fractal rough interfaces: \( Q^{-x} \), \( 3 < x < 4 \)
Interparticle Structure Factor $S(Q)$

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

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

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

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

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

- 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

Adapted from DW Schaefer MRS Symposium Proceeding 1987
Example: Biomass to Cellulosic Ethanol
“grass to gas”

Feedstock
- Poplar
- Switchgrass

Hydrolysis
- Cellulase enzyme
  - 4 X 14 nm

Pretreatment
- Cell wall
  - 100 nm

Fermentation
- Microbes ferment sugars to ethanol, which is then separated from the mix of ethanol, water, microbes, and residue and purified through distillation.

The challenge: structural complexity!

Adapted from DOE GTL image gallery (genomics.energy.gov)
“BIOMESS”

Model for Grasses

Why neutrons?
- Use D$_2$O 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 D₂O

Structural Change Onset

Elementary Cellulose Fibril
Cross-sectional View

Pingali et al., *Biomacromolecules* 2010
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: \( \gamma(r) = e^{-r/a} \)

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

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

  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.

  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.

  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.

  Contains a comprehensive list of form factors and structure factors that are used for interpreting small-angle scattering data.