Scattering of Neutrons: Basics

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The conceptual experiment and theory is the same for X-rays and neutrons.

The differences are the physics of the interactions of X-rays (electro-magnetic radiation) versus neutrons (neutral particle) with matter.
Neutrons have zero charge and negligible electric dipole and therefore interact with matter via nuclear forces.

Nuclear forces are very short range (a few fermis, where 1 fermi = 10^{-15} m) and the sizes of nuclei are typically 100,000 smaller than the distances between them.

Neutrons can therefore travel long distances in material without being scattered or absorbed, i.e. they are and highly penetrating (to depths of 0.1-0.01 m).

Example: attenuation of low energy neutrons by Al is ~1%/mm compared to >99%/mm for x-rays.
Neutrons are particles that have properties of plane waves.

They have amplitude and phase.
They can be scattered elastically or inelastically

Elastic scattering changes direction but not the magnitude of the wave vector

Inelastic scattering changes both direction and magnitude of the neutron wave vector
It is the elastic, coherent scattering of neutrons that gives rise to small-angle scattering.
Coherent scattering is “in phase” and thus can contribute to small-angle scattering. Incoherent scattering is isotropic and in a small-angle scattering experiment and thus contributes to the background signal and degrades signal to noise.

Coherent scattering essentially describes the scattering of a single neutron from all the nuclei in a sample.

Incoherent scattering involves correlations between the position of an atom at time 0 and the same atom at time t.
The neutron scattering power of an atom is given as $b$ in units of length.

Circular wave scattered by nucleus at the origin is:

$$(-b/r)e^{ikr}$$

$b$ is the scattering length of the nucleus and measures the strength of the neutron-nucleus interaction.

The scattering cross section

$$\sigma = 4\pi b^2$$

..as if $b$ were the radius of the nucleus as seen by the neutron.
Neutron scattering lengths for isotopes of the same element can have very different neutron scattering properties.

**Fig. 22.** Irregular variation of neutron scattering amplitude with atomic weight due to superposition of ‘resonance scattering’ on the slowly increasing ‘potential scattering’; for comparison the regular increase for X-rays is shown. (From Research (London) 7, 257 (1954).)
As nuclei are point scattering centers, neutron scattering lengths show no angular dependence.

**Fig. 16.** X-ray and neutron scattering amplitudes for a potassium atom.
At very short wavelengths and low Q, the X-ray coherent scattering cross-section of an atom with Z electrons is $4\pi(Zr_0)^2$, where $r_0 = \frac{e^2}{m_ec^2} = 0.28 \times 10^{-12}$ cm.

### b values for nuclei typically found in bio-molecules

<table>
<thead>
<tr>
<th>Atom</th>
<th>Nucleus</th>
<th>$(10^{-12}$ cm)</th>
<th>$f_{x\text{-ray}}$ for $\theta = 0$ in electrons (and in units of $10^{-12}$ cm)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>$^1$H</td>
<td>-0.3742</td>
<td>1.000 (0.28)</td>
</tr>
<tr>
<td>Deuterium</td>
<td>$^2$H</td>
<td>0.6671</td>
<td>1.000 (0.28)</td>
</tr>
<tr>
<td>Carbon</td>
<td>$^{12}$C</td>
<td>0.6651</td>
<td>6.000 (1.69)</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>$^{14}$N</td>
<td>0.940</td>
<td>7.000 (1.97)</td>
</tr>
<tr>
<td>Oxygen</td>
<td>$^{16}$O</td>
<td>0.5804</td>
<td>8.000 (2.25)</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>$^{31}$P</td>
<td>0.517</td>
<td>15.000 (4.23)</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Mostly $^{32}$S</td>
<td>0.2847</td>
<td>16.000 (4.5)</td>
</tr>
</tbody>
</table>
\[ I(Q) = \langle \int \Delta \rho \ e^{-i(q \cdot r)} \ dr \rangle^2 \]

where \( \Delta \rho = \rho_{\text{particle}} - \rho_{\text{solvent}} \).

As average scattering length density \( \bar{\rho} \) is simply the average of the sum of the scattering lengths (b)/unit volume.

Because H (\(^1\text{H}\)) and D (\(^2\text{H}\)) have different signs, by manipulating the H/D ratio in a molecule and/or its solvent one can vary the contrast \( \Delta \bar{\rho} \).
Planning a neutron scattering experiment

- Choose your data collection strategy (solvent matching or full contrast variation?)
- Determine how much sample is needed
- Decide which subunit to label
- What deuteration level is needed in the labeling subunit
- See MULCh*

http://www.mmb/usyd.edu.au/NCVWeb/

MULCh

- ModULes for the analysis of neutron Contrast variation data
  - *Contrast*, computes neutron contrasts of the components of a complex
  - *$R_g$*, analyses the contrast dependence of the radius of gyration to yield information relating to the size and disposition of the labelled and unlabeled components in a complex
  - *Compost*, decomposes the contrast variation data into composite scattering functions containing information on the shape of the labeled and unlabeled components and their dispositions
Solvent matching

- Best used when you are interested in the shape of one component in a complex, possibly how it changes upon ligand binding or complex formation.
- Requires enough of the component to be solvent matched to complete a contrast variation series to determine required %D_{2}O (~4 x 200-300 µL, ~5 mg/ml).
- Requires 200-300 µL of the labeled complex at 5-10mg/ml.
Solvent Match Point Determination

![Graph showing solvent match point](image-url)
Deuterated calmodulin (\(^{\text{D}}\)CaM) complexed with the MA protein from HIV-1 measured in 41% D\(_{2}\)O. Only the \(^{\text{D}}\)CaM contributes to the scattering and we can distinguish between a fully extended, collapsed or partially collapsed CaM conformation.
Contrast variation

- To determine the shapes and dispositions of labeled and unlabelled components in a complex
- Requires ≥ 5 x 200-300μL (= 1 – 1.5mL) of your labeled complex at ≥ 5 mg/ml.
- Deuteration level in labeled protein depends upon its size.
  - Smaller components require higher levels of deuteration to be distinguished.
  - Ideally would like to be able to take data at the solvent match points for the labeled and unlabeled components
The host Ca$^{2+}$ receptor calmodulin binds the multifunctional MA protein from HIV-1 and unfolds its N-terminal domain in the presence of Ca$^{2+}$; removal of Ca$^{2+}$ results in dissociation and refolding of MA.

Taylor et al., Biophys. J. 103, 1-9, 2012
KinA$_2$-2$^D$Sda complex experiment

- Measure sample and solvent blanks at each contrast point (use a broad range of D$_2$O concentrations)
- Subtract solvent blank data from sample
- Sample to low-$q$ with sufficient frequency to determine large distances accurately (min. 15-20 points in the Guinier region)
- Measure to high enough $q$ to aid in checking background subtraction ($q = 0.45$ Å$^{-1}$)
- $q = 0.01 - 0.45$ is typical range for 10-150 kDa particles, usually requires two detector positions
Use $R_g$ (from MULCh) for Sturhman analysis

$$R_{obs}^2 = R_m^2 + \frac{\alpha}{\Delta \rho} - \frac{\beta}{\Delta \rho^2}$$

- $R_H = 25.40 \text{ Å}$
- $R_D = 25.3 \text{ Å}$
- $D = 27.0 \text{ Å}$

Sign of $\alpha$ indicates whether the higher scattering density object is more toward outside (+) or inside (-)
Use Compost (from MULCh) to solve for $I(q)_{11}, I(q)_{22}, I(q)_{12}$

$$I(q) = \Delta \rho_1^2 I_{11}(q) + \Delta \rho_2^2 I_{22}(q) + \Delta \rho_1 \Delta \rho_2 I_{12}(q)$$
MONSA
3D shape restoration
Use SASREF7 to do rigid body refinement of the components against the scattering data (if you have pdb files for components)

\[ \chi^2 = 1.27 \]
\[ \chi^2 = 0.97 \]
\[ \chi^2 = 0.63 \]
\[ \chi^2 = 0.56 \]
\[ \chi^2 = 0.76 \]
\[ \chi^2 = 0.92 \]
\[ \chi^2 = 1.12 \]

Whitten et al. (2007) *J. Mol. Biol.* 368, 407-420
Bacterial histidine kinase (KinA) and its protein inhibitors (Sda and KipI): Neutrons reveal inhibitors bind at the base of the dimerisation domain that connects to the sensor domains.

Whitten et al., *J. Mol. Biol.* 368, 407, 2007
..and the relationship between the Kip I inhibitor and its regulatory binding partner Kip A

cMyBP-C in Muscle Contraction

- cMyBP-C plays **structural** and **regulatory** roles in striated muscle sarcomeres. However, the specific details of how it interacts with actin and myosin are unclear.
SAXS data + crystal and NMR structures of individual modules show the N-terminal domains of mouse cMyBP-C form an extended structure with a defined disposition of the modules.

SANS data show regulatory cMyBP-C domains (mouse) stabilise F-actin

Whitten et al. (2008) *PNAS* 105, 18360
SAXS data show significant species differences

Correlation between % Pro/Ala composition in the C0-C1 linker and heart rate from different organisms (Shaffer and Harris (2009) J. Muscle Res. Cell Motil. 30:303-306)

Jeffries et al., J. Mol. Biol. 414, 735-748, 2011
2D reconstruction of human C0C1-actin assembly from neutron contrast series consistent with C0 binding with a flexible and extended P/A$_L$

Lu et al., *J. Mol. Biol.*, 413, 908-913, 2011
Predictable Deuteration of Recombinant Proteins Expressed in *Escherichia coli*

Barbara Leiting,¹ Frank Marsilio, and John F. O’Connell

The described protocols allow the deuteration content in recombinant proteins to be predicted.

Incorporation of deuterium up to 86% of the chemically Non-exchangeable protons can be obtained by using D₂O as the deuterium source. Complete deuteration can only be obtained by addition of perdeuterated carbon source (glucose or glycerol).

Use mass spec to determine deuteration levels.
Contrast variation in biomolecules can take advantage of the fortuitous fact that the major bio-molecular constituents of have mean scattering length densities that are distinct and lie between the values for pure D$_2$O and pure H$_2$O.
DNA and protein have inherent differences in scattering density that can be used in neutron contrast variation experiments.
Jacques & Trehewella (2010) “Small-angle Scattering for Structural Biology; Expanding the Frontier While Avoiding the Pitfalls,” Protein Science 19, 642-657


Publication guidelines for structural modelling of small-angle scattering data from biomolecules in solution
Neutron scattering sample cells

- Helma quartz cells (high precision path-length, suprasil) – need lots of them!
- Banjo-style (280 μL per 1 mm path length) or rectangular (170 μL per 1 mm path length) cells can be used
- Path lengths are only good to 1%, so good idea to measure sample and solvent background in the same cell if practical, but experiment logistics may prohibit that, so calibrate cells?
- High incoherent scattering for \(^1\text{H}\) means you always want \(\leq 1\text{mm} \ ^1\text{H}_2\text{O}\) in the neutron beam to avoid multiple scattering