Solution Scattering, an Overview

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Organizers:
D. Svergun, EMBL Outstation Hamburg
R. Willumeit, GKSS Research Centre, Geesthacht
case: small-angle scattering from a monodisperse dilute solution of macromolecules

A. Guinier: $I(Q) = C \exp\left(\frac{R^2 Q^2}{3}\right)$

- $R =$ radius of gyration
- $Q = \frac{4\pi}{\lambda} \sin \theta$
- $\lambda =$ wavelength

scattering intensity

few degrees

scattering angle $2\theta$
Für schweres Wasser ergibt sich der viel höhere Wert von $6,34 \cdot 10^{10}$ cm$^{-2}$. Proteine und Ribonucleinsäuren haben Streulängendichten, die in dem von H$_2$O und D$_2$O abgesteckten Intervall liegen (Abbildung 7). Die Werte der Streulängendichte sind deshalb so Anschein „intra-molekularer Phasentrennung“ geben, wie man ihn zuweilen auch bei Blockcopolymeren beobachten kann. Unter diesen Umständen kann man erwarten, daß die Streulängendichte der Komponenten Maul werden beim Zusatz von schwerem Wasser deutlicher in dem Maße, wie die Umrisse verblassen. Die Streulängendichte einer 8 Prozent D$_2$O enthaltenden H$_2$O/D$_2$O-Mischung ist null und entspricht daher der Beschreibung der Katze im Vakuum.
Contrast with macromolecules:

Radiation interacts with atoms and/or their nuclei.

For the present purpose, the strength of interaction is described by the **scattering length** \( b \).

In a volume containing a reasonably large number of atoms it is convenient to define the scattering length density (or **scattering density**) :

\[
\rho = \sum_n b_n / \text{volume}
\]

For a macromolecule in solution:

**Contrast** :

\[
\rho (\mathbf{r}) = \rho (\mathbf{r})_{\text{MM}} - \rho_{\text{solvent}}
\]
Contrast variation

variation of solvent density
(external contrast variation,
or solvent contrast variation)

variation of the scattering density of parts of
a macromolecule
(internal contrast variation)

From real space
to momentum space

\[ A(Q) = \int_V \rho(r) \exp(iQr) \, dv \]

\[ |Q| = 4 \pi \sin \theta / \lambda, \ 2\theta = \text{scattering angle}, \ \lambda = \text{wavelength} \]

\[ A(Q) = U(Q) + x \, V(Q) \]

↑ contrast, in a wide sense

The expression for the intensity \( I(Q) \) may vary. In the simplest case it is given by

\[ I(Q) = |U(Q)|^2 + x \, 2\text{Re}[U(Q)V^*(Q)] + x^2 |V(Q)|^2 \]
For **small-angle scattering** from randomly oriented particles $I(Q)$ is the result of an integration over all orientations of the dissolved particles. The **basic scattering functions** are obtained by variation of $x$.

Contrast variation started with X-ray diffraction from a hemoglobin crystal (Bragg & Perutz, 1952).

Considerable changes of the low order reflections of hemoglobin were observed with the variation of the electron density of the solvent.

→ packing of the hemoglobin molecules in the unit cell

This method is the precursor of multiwavelength anomalous solvent contrast variation (MASC) (Fourme et al. 1995)

$$x = f' + i f''$$

Hence

$$I(Q) = |U(Q)|^2 + f' 2 \text{Re}[U(Q)V^*(Q)] + f'' 2 \text{Im}[U(Q)V^*(Q)]$$

$$+ (f'^2 + f''^2) |V(Q)|^2$$

Note the presence of four basic scattering functions

→ shape and packing of the proteins in the unit cell.
Anomalous small-angle scattering

\[ I(Q) = \int |U(Q)|^2 + f' \cdot 2 \text{Re}[U(Q)V^*(Q)] + (f'^2 + f''^2) |V(Q)|^2 \, d\Omega \]

Example: phosphorus of rRNA of the large ribosomal subunit.

Anomalous dispersion at wavelengths near the K-edge of phosphorus \((\lambda = 5.76 \text{ Å})\) (M. Hütsch, 1995)

Variation of anomalous scattering length with \(\lambda\): 16 e.u.  
(1 e.u. = scattering length of one electron = 0.28E-12 cm)

Local concentration of P: up to 3 M/l

→ in situ structure of rRNA at low resolution
In the mid sixties, solvent contrast variation was used in small-angle scattering. The electron density was varied by addition of glycerol or sugar. Variation of the radius of gyration with contrast in myoglobin.

With the advent of high flux neutron sources, contrast variation is almost entirely done by exchange of the hydrogen isotope $^{1}\text{H}$ by $^{2}\text{H}$ (Deuterium).

- High abundance: $70 \text{ H} / \text{nm}^3$ (110 M/l)
- Large change of scattering length: $0.69 \times 10^{-12} \text{ cm}$ (corresponds to the scattering length of 2.5 electrons in X-ray scattering).

Applications of solvent contrast variation were numerous:

Ribosomes, Chromatin, RNA polymerase, Viruses, Lipoproteins

The constituents of these particles are

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→ low resolution architecture (quaternary structure)
Examples of **internal contrast variation**:

**Ribosomes:**

The positions of all 21 proteins of the small ribosomal subunit were found by **triangulation between specific deuterated ribosomal proteins** (D.M. Engelman & P.B. Moore, W. Hoppe).

The method of **triple isomorphous replacement** uses fully and partially deuterated proteins as labels (I. Serdyuk).

It resembles in some respects a method which uses the change of the scattering length of hydrogen with proton spin polarisation in polarised neutron small-angle scattering.

\[ x = P \quad (= \text{proton spin polarisation}) \]

The intensity of polarised neutron small-angle scattering is

\[ I_{\uparrow \uparrow}(Q) = \int |U(Q)|^2 + P 2\text{Re}[U(Q)V^*(Q)] + P^2 |V(Q)|^2 \, d\Omega \]

if the direction proton spins coincides with that of the neutron spins

\[ I_{\downarrow \uparrow}(Q) = \int |U(Q)|^2 - P 2\text{Re}[U(Q)V^*(Q)] + P^2 |V(Q)|^2 \, d\Omega \]
The method of proton spin contrast variation was used to determine the in situ structure of the tRNAs and of the mRNA, i.e. the position and orientation of these molecules inside the functional ribosome.

Largest variation of the scattering length with the polarisation of the proton spins:

\(-0.56 \pm 1.456 \times 10^{-12} \text{ cm}\)

Corresponding to 10 electron units in X-ray scattering.

Best results are obtained with particles which have been deuterated. Only the regions of interest are left protiated. For biological macromolecules a mixture of deuterated glycerol and heavy water is a convenient solvent.

The deuterons are polarised as well, although to an extent which is much lower than that of the protons: Their scattering length changes as well:

\((0.66 \pm 0.27 \times 10^{-12} \text{ cm})\)
Neutron scattering from hydrogen:

The neutron and the nucleus of $^1\text{H}$ (=proton) have a spin $\frac{1}{2}$.

There are two different channels of neutron-proton interaction:
- one for the total spin $\frac{1}{2} + \frac{1}{2} = 1$ with $b^+$ (3 substates)
- one for the total spin $\frac{1}{2} - \frac{1}{2} = 0$ with $b^-$ (1 substate)

The effective length $b$ of coherent scattering is the weighted average of $b^+$ and $b^-$. With $b^+ = 1.083 \times 10^{-12}$ cm and $b^- = -4.74 \times 10^{-12}$ cm we obtain

$$b = \frac{3}{4} b^+ + \frac{1}{4} b^- = -0.374 \times 10^{-12} \text{ cm for } ^1\text{H}$$

A similar calculation yields for $^2\text{H}$ (=Deuterium)
$$b = +0.66 \times 10^{-12} \text{ cm for } \text{D}$$

This difference is the basis for practically all application of isotopic methods of contrast variation in biology and soft condensed matter research in general.

Incoherent scattering:

$$\sigma_{\text{incoh.}} = \frac{3\pi}{4} (b^+ - b^-)^2 = 79.9 \times 10^{-24} \text{ cm}^2 \text{ for } ^1\text{H}$$

$$\sigma_{\text{incoh.}} = 2.0 \times 10^{-24} \text{ cm}^2 \text{ for } ^2\text{H}$$

Incoherent scattering from $^1\text{H}$ is reduced by deuteration
Polarised neutron scattering from polarised nuclear spins of hydrogen:

Both the neutron and the nucleus of $^1$H (=proton) have a spin $\frac{1}{2}$.

There are two different channels of neutron-proton interaction:
- one for the total spin $\frac{1}{2} + \frac{1}{2} = 1$ with $b^+$ (3 substates)
- one for the total spin $\frac{1}{2} - \frac{1}{2} = 0$ with $b^-$ (1 substate)

If the neutron spins are polarised in the same direction as the proton spins then the length $b$ of coherent scattering is

$$b(\uparrow\uparrow) = b^+ = 1.083 \times 10^{-12} \text{ cm}$$

If the neutron spins are polarised antiparallel to the direction of the proton spins then the length $b$ of coherent scattering is

$$b(\uparrow\downarrow) = \frac{(b^+ + b^-)}{2} = -1.83 \times 10^{-12} \text{ cm}$$

$$b(H) = [-0.374 \pm 1.456 P(H)] \times 10^{-12} \text{ cm}$$

A similar calculation yields for $^2$H (=Deuterium)

$$b(\uparrow\uparrow) = 0.93 \times 10^{-12} \text{ cm}$$

$$b(\uparrow\downarrow) = 0.39 \times 10^{-12} \text{ cm}$$

$$b(D) = [0.667 \pm 0.27 P(D)] \times 10^{-12} \text{ cm}$$

Incoherent scattering:

$$\sigma_{(\text{incoh.})} = \pi (b^+ - b^-)^2 \left( \frac{3}{4} \pm \frac{1}{2}P - \frac{1}{4} P^2 \right)$$

Incoherent scattering from $^1$H is reduced by deuteration
..and, even more easily, by polarisation of the proton spins in direction of neutron spin polarisation
How to polarize proton spins

by 'brute force'

How to polarize proton spins
by 'dynamical nuclear spin polarisation' (DNP)

In the presence of convenient paramagnetic centres, all nuclear spins are dynamically polarised by irradiation of 2.8 mm microwaves in a field of 3.5 Tesla, at temperatures $T \leq 1$ K.

The polarisation is monitored by NMR:
The protons near the paramagnetic centre usually remain unobserved.

The direction of proton spin polarisation can be reversed by the method of adiabatic fast passage (AFP). If the rf scan is restricted to frequencies of the central peak, then only the protons of the bulk will change their polarisation direction.
In dilute paramagnets, DNP is described by the 'well resolved solid effect':

Nuclear polarisation....
- develops first near the paramagnetic centres
- and then diffuses into the bulk.

At very low concentration of radicals (or paramagnetic centres in general) the effect of DNP cannot be observed by NMR.
Is DNP is confined to the magnetically perturbed region near the paramagnetic centre?

Tyrosyl doped catalase is such an example.

C < 0.5 mM/l ( < 1.8E+17 e⁻ /cm³)

Preparation:

A small amount of peroxy acetic acid is added to a solution of bovine liver catalase.
A greenish color develops, which at T = 4°C turns into dark red after one minute. This is an indication that the tyrosyl is formed (it is not the color of the tyrosyl radical!).
EPR suggests the conversion of TYR369 into the radical state with a yield of up to 80%.
Time resolved polarised neutron scattering was measured in time slots of 50 milliseconds at the instrument D22 of the Institut Laue Langevin.

The direction of proton spin polarisation was changed each 5 seconds. For a fast change of the microwave frequency, two microwave sources tuned to slightly different frequencies were run in parallel, and used in an alternating mode.

![Diagram showing time slots and DNP states](image-url)
The results of time-resolved polarised neutron scattering from dynamically polarised proton spin targets were obtained by a collaborating team consisting of

Ben van den Brandt
Patrick Hautle
Joachim Kohlbrecher
Ton Konter
Salvatore Mango  Paul-Scherrer-Institut, Villigen, Schweiz

Hans Glättli
Edouard Leymarie   CEA, Saclay, France

Jaques Gaillard   CEA, Grenoble

Eric Fanchon
Hélène Jouve
Richard Kahn     Institut de Biologie Structurale Jean-Pierre Ebel, CEA/CNRS, Grenoble, France

Heinrich Stuhrmann  GKSS and  IBS, Grenoble, France

Oliver Zimmer     Technische Universität München, Germany

Isabelle Grillo
Roland May     Institut Laue-Langevin, Grenoble, France
EPR of the tyrosyl radical

**positive** nuclear spin polarisation

**negative** nuclear spin polarisation

97.3 GHz
(2.8 mm microwaves)
neutron small-angle scattering of a frozen solution of catalase
-- measured
--- calculated
The polarisation is driven to more negative values during the first 5 seconds and then to more positive values during the following 5 seconds. Repetition: many thousand times.
Internal relaxation:

**Space and time resolved proton spin polarisation diffusion in tyrosyl doped catalase, negative DNP**

![Graph showing density vs. radius for different times](image1)

**Space and time resolved proton spin polarisation diffusion in tyrosyl doped catalase, positive DNP**

![Graph showing density vs. radius for different times](image2)
Scattering from catalase, in vacuo

\[ I(Q) = \sum_{l=0}^{L} \sum_{m=-l}^{l} |A_{lm}(Q)|^2 \]

\[ \sum_{m=1}^{L} |A_{lm}(Q)|^2 \]
Data analysis:

I(Q,t) from time resolved neutron scattering

\[ \frac{d I(Q,t)}{dt} \]

\( \to \) this eliminates the scattering which does not vary with time

find relaxation times \( \tau_n \):

\[ \frac{d I(Q,t)}{dt} = \sum_n G_n(Q) \exp(-t/\tau_n) \quad n=1,2,3 \]

Describe \( G_n(Q) \) by a polynomial:

\[ G_n(Q) = (a_{n,0} + a_{n,1}Q^2 + \ldots a_{n,6} Q^{12}) \exp(-bQ^2) \]

Small-angle scattering:

Monopole approximation (neglect higher multipoles)

The time dependent amplitude then is

\[ nF_{00}(Q) \equiv G_n(Q) / I_{00}(Q)_{\text{catalase}} \]

Fourier transform to real space:

\[ n\rho_{oo}(r) = \int nF_{oo}(Q) \sin(Qr)/Qr \, Q^2 \, dQ \]

Time dependent radial density of catalase

\[ \frac{d \rho(r,t)}{dt} = \sum_n n\rho_{00}(r) \exp(-c_n \, t) \]
catalase: x,z plane:
the tyrosyls are inside a sphere of 15 A
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Ways of contrast variation

1) X-ray and neutron scattering:

Solvent density variation by addition of low molecular compounds
Methods of isomorphous replacement.

Resonant scattering
(Multi-wavelength Anomalous Solvent Contrast, (MASC) if applied to solvent)

Neutron scattering:

Isotopic substitution, mostly deuteration
- static experiments (deuteration of solvent, specific labelling of the macromolecule)
- kinetic of isotope exchange (H/D exchange with solvent)

Polarized neutron scattering:
(absence of neutron polarisation weakens the signal)

Nuclear spin polarisation
- static experiments, often with 'pre-contrasting' by deuteration
- dynamic experiments: creation and of selectively polarized proton spin domains and their internal relaxation near paramagnetic centres.
Contrast with macromolecules:

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For the present purpose, the strength of interaction is described by the **scattering length** $b$.

In a volume containing a reasonably large number of atoms it is convenient to define the scattering length density (or **scattering density**):

$$\rho = \sum_n b_n / \text{volume}$$

For a macromolecule in solution:

**Contrast**: $\rho(\mathbf{r}) := \rho(\mathbf{r})_{\text{MM}} - \rho_{\text{solvent}}$

**Internal contrast**:

$$\rho = \rho(x,y,z) = \rho(\mathbf{r}).$$

as opposed to

**External contrast** (or solvent dependent contrast)
If the contrast is made to vary, then we have

**Contrast variation**

variation of solvent density
(external contrast variation, or solvent contrast variation)

variation of the scattering density of parts of a macromolecule
(internal contrast variation)

**From real space to momentum space**

\[ A(Q) = \int_V \rho(r) \exp(iQr) \, dv \]

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corresponding to \textbf{10 electron units} in X-ray scattering

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