Combining X-ray and Neutron Scattering

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EMBL Hamburg
Birds view of the Grenoble ESRF and ILL research area.

High flux reactor Institute Laue-Langevin ILL

Third generation synchrotron facility ESRF
Small Angle Neutron Scattering SANS
Basic parameters

Neutron reactors:
• ILL Grenoble France
• FRM II Munich Germany
• CARR Beijing China
• HFIR Oak Ridge USA

Spallation sources:
• PSI Villingen Switzerland
• SNS Oak Ridge USA
• ISIS Oxfordshire UK
• ESS Lund Sweden (2019)
Small Angle Neutron Scattering SANS

Neutron reactor

Oak Ridge National Laboratory, Oak Ridge, USA
Small Angle Neutron Scattering SANS

Basic parameters

The neutrons produced by reactors are at too high energy (too high speed, too short wavelength …) for scattering experiments.

They are moderated in a cold source to lower energies.
Small Angle Neutron Scattering SANS
A classical SANS beamline

D11 at the Institute Laue-Langevin ILL Grenoble France
# Small Angle Neutron Scattering (SANS) - Basic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>cold</th>
<th>thermal</th>
<th>hot</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (meV)</td>
<td>1</td>
<td>25</td>
<td>1000</td>
</tr>
<tr>
<td>Temperature (K)</td>
<td>12</td>
<td>290</td>
<td>12000</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>0.9</td>
<td>0.18</td>
<td>0.029</td>
</tr>
<tr>
<td>Velocity (ms⁻¹)</td>
<td>440</td>
<td>2200</td>
<td>14000</td>
</tr>
</tbody>
</table>

Neutron mass: \(1.674 \times 10^{-27} \text{kg}\)
Radiation from Synchrotron Storage Rings

Dipole bending magnet (APS)
Schematic SAS setup
X33 BioSAXS EMBL Hamburg

Beamstop with diode for measurement of transmitted beam

Beamshutter with diode for measurement of incident beam (prior to exposure)

MAR345

1400 mm

1000 mm

Sample cell

s: 0.1 nm$^{-1}$ to 4.3nm$^{-1}$
d: 65 nm to 15 nm
## Small Angle X-ray Scattering (SAXS)

### Basic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Infrared</th>
<th>Ultraviolet</th>
<th>X-rays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (eV)</td>
<td>0.1</td>
<td>4</td>
<td>12 000</td>
</tr>
<tr>
<td>Temperature (K)</td>
<td>1</td>
<td>1000</td>
<td>1 000 000</td>
</tr>
<tr>
<td>Wavelength (nm)</td>
<td>10000</td>
<td>300</td>
<td>0.1</td>
</tr>
<tr>
<td>Velocity (ms⁻¹)</td>
<td>300 000</td>
<td>300 000</td>
<td>300 000</td>
</tr>
</tbody>
</table>
Radiation damage is caused by:

- Beam heating
- Hydroxyl radicals
- Direct bond cracking

Dissoziation of a multi-subunit protein upon X-Ray radiation damage after 10 sec.
Radiation Damage
Hydroxyl radicals

Production of OH radicals by ionizing radiation:

\[
\begin{align*}
\text{H}_2\text{O} & \xrightarrow{\text{hv}} \text{H}_2\text{O}^\cdot + \text{e}^- \\
\text{H}_2\text{O}^\cdot + \text{H}_2\text{O} & \xrightarrow{\text{very fast}} \text{H}_3\text{O}^+ + \text{OH}^\cdot
\end{align*}
\]

5' end

\[
\begin{array}{c}
\text{O} \\
\text{P} \\
\text{O} \\
\text{C} \\
\text{O}
\end{array}
\]

\[
\begin{array}{c}
\text{O} \\
\text{P} \\
\text{O} \\
\text{C} \\
\text{O}
\end{array}
\]

\[
\begin{array}{c}
\text{O} \\
\text{P} \\
\text{O} \\
\text{C} \\
\text{O}
\end{array}
\]

\[
\begin{array}{c}
\text{O} \\
\text{P} \\
\text{O} \\
\text{C} \\
\text{O}
\end{array}
\]

Base

\[
\begin{array}{c}
\text{O} \\
\text{P} \\
\text{O} \\
\text{C} \\
\text{O}
\end{array}
\]

\[
\begin{array}{c}
\text{O} \\
\text{P} \\
\text{O} \\
\text{C} \\
\text{O}
\end{array}
\]

\[
\begin{array}{c}
\text{O} \\
\text{P} \\
\text{O} \\
\text{C} \\
\text{O}
\end{array}
\]

\[
\begin{array}{c}
\text{O} \\
\text{P} \\
\text{O} \\
\text{C} \\
\text{O}
\end{array}
\]

3' end

Attack at the C4'
Radiation Damage
Hydroxyl radicals

\[ 2 \text{H}_2\text{O} \rightarrow \text{OH}^\bullet + \text{H}_3\text{O}^- \]

Hydroxyl radical are attacking hydrogen at the surface of proteins or DNA/RNA.

DNA/RNA is very sensitive to this attack and one single hydroxyl radical cleaves the DNA/RNA.
Small Angle Neutron Scattering SANS
Contrast variation technique

<table>
<thead>
<tr>
<th>atomic number</th>
<th>Element or Isotope</th>
<th>$b_{\text{coh}}$ for neutrons</th>
<th>$f_x$ for X-rays</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H</td>
<td>-0.374</td>
<td>0.28</td>
</tr>
<tr>
<td>1</td>
<td>D</td>
<td>0.667</td>
<td>0.28</td>
</tr>
<tr>
<td>6</td>
<td>C</td>
<td>0.665</td>
<td>1.69</td>
</tr>
<tr>
<td>6</td>
<td>$^{13}$C</td>
<td>0.600</td>
<td>1.69</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>0.940</td>
<td>1.97</td>
</tr>
<tr>
<td>8</td>
<td>O</td>
<td>0.580</td>
<td>2.25</td>
</tr>
<tr>
<td>8</td>
<td>$^{17}$O</td>
<td>0.578</td>
<td>2.25</td>
</tr>
<tr>
<td>12</td>
<td>Mg</td>
<td>0.530</td>
<td>3.38</td>
</tr>
<tr>
<td>15</td>
<td>P</td>
<td>0.510</td>
<td>4.23</td>
</tr>
<tr>
<td>16</td>
<td>S</td>
<td>0.285</td>
<td>4.50</td>
</tr>
<tr>
<td>19</td>
<td>K</td>
<td>0.370</td>
<td>5.30</td>
</tr>
</tbody>
</table>
Small Angle Neutron Scattering (SANS) is a contrast variation technique.

From the table one can derive the following:

- Neutrons are more sensitive to light atoms such as hydrogen or deuterium as X-rays.
- There is a large difference in the biological relevant atom hydrogen and its isotope deuterium.
- The b-factor does not increase with the atomic number as for X-rays.
Small Angle Neutron Scattering SANS
Contrast variation technique

\[ \rho [10^{10} \text{m}^{-1} \text{cm}^2] \]

- D-labelled protein
- DNA/RNA
- H-protein
- Buffer

fraction D\textsubscript{2}O in buffer [%]
Small Angle Neutron Scattering SANS
Contrast variation technique

\( \rho_{\text{Buffer}} = \rho_{\text{Protein}} \)
protonated (=native)
protein in 40%

\( \rho_{\text{Buffer}} \neq \rho_{\text{Protein}} \)
deuterated protein in 40%

Upon complex formation the proteins undergo conformational changes

Mixed re-constituted complexes of d-labeled and native protein
The Chaperonin folding machinery

**main chaperonin GroEL**
- two heptameric rings
- 800 kDa MW
- hollow cylinder
- binds denatured protein and facilitate the refolding

**co chaperonin GroES**
- heptameric dome
- 70 kDa MW
- bind to one end of the GroEL cylinder and close the cavity like a lid
GroEL/GroES complex bead modeling
The in-situ structures
GroEL/GroES complex

Rigid body model based on the in situ structures

$Ab\ inito$ bead model

EMBO Global Exchange Lecture
Beijing 28th April to 6th May 2011
Intermolecular distances
Stuhrmann plot

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

Center of mass to center of mass distance determined by the Stuhrmann plot

Small Angle Neutron Scattering SANS
Contrast variation technique

The distance of the two peaks reflects the inner molecular distance between the two visible proteins in e.g. 40% D2O buffer solution.

Center to Center distance

Center to Center distance

EMBO Global Exchange Lecture

Beijing  28th April to 6th May 2011
Combining SAXS and SANS
Theoretical background

One scattering data set:
„Black and White“ ab inito model building, with only one the contrast solvent – particle.

Multiple scattering functions with data sets from SANS contrast variation:
„Colored“ ab inito model building with multiple contrasts
This approach works as well with rigid body modelling.
Combining SAXS and SANS
Protein-Protein complexes

Complex formed by the receptor tyrosine kinesine Met and the Listeria monocytogenes Invasion Protein InlB.

The Met extracellular region consists of six domains:
The N-terminal Sema domain is followed by a small cysteine-rich PSI domain and four immunoglobulin (Ig)-like Ig domains.

InlB: 630 AS with a leucine rich repeat region with binds with high affinity to Met.
Combining SAXS and SANS
Complex of Met Receptor tyrosine kinase and Invasion Protein InlB

<table>
<thead>
<tr>
<th>Constructs/experiments</th>
<th>X-rays</th>
<th>0% D$_2$O</th>
<th>35% D$_2$O</th>
<th>50% D$_2$O</th>
<th>60% D$_2$O</th>
<th>81% D$_2$O</th>
<th>100% D$_2$O</th>
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</thead>
<tbody>
<tr>
<td>Protonated protein</td>
<td>2.82</td>
<td>2.285</td>
<td>0.275</td>
<td>-0.586</td>
<td>-1.160</td>
<td>-2.309</td>
<td>-3.457</td>
</tr>
<tr>
<td>50% deuterated protein</td>
<td>2.82</td>
<td>4.652</td>
<td>2.643</td>
<td>1.781</td>
<td>1.207</td>
<td>0.059</td>
<td>-1.090</td>
</tr>
<tr>
<td>Perdeuterated protein</td>
<td>2.82</td>
<td>7.020</td>
<td>5.010</td>
<td>4.149</td>
<td>3.575</td>
<td>2.426</td>
<td>1.278</td>
</tr>
</tbody>
</table>

List of measurements

<p>| | | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protonated InlB$_{321}$</td>
<td>1.6 (1)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Met$_{967}$</td>
<td>1.4 (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Met$<em>{967}$ + protonated InlB$</em>{321}$</td>
<td>1.1 (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Met$_{638}$</td>
<td>1.9 (4)</td>
<td>0.9 (33)</td>
<td>-</td>
<td>0.9 (34)</td>
<td>-</td>
<td>-</td>
<td>5.0 (32)</td>
</tr>
<tr>
<td>Met$_{928}$</td>
<td>2.0 (5)</td>
<td>0.8 (31)</td>
<td>1.8 (30)</td>
<td>-</td>
<td>1.1 (29)</td>
<td>2.0 (28)</td>
<td></td>
</tr>
<tr>
<td>Met$<em>{638}$ + protonated InlB$</em>{321}$</td>
<td>2.8 (6)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Met$<em>{928}$ + protonated InlB$</em>{321}$</td>
<td>2.1 (7)</td>
<td>1.0 (27)</td>
<td>0.5 (24)</td>
<td>-</td>
<td>1.7 (9)</td>
<td>-</td>
<td>2.6 (23)</td>
</tr>
<tr>
<td>Met$<em>{638}$ + 50% deuterated InlB$</em>{321}$</td>
<td>-</td>
<td>1.1 (25)</td>
<td>0.5 (24)</td>
<td>-</td>
<td>1.7 (9)</td>
<td>-</td>
<td>2.6 (23)</td>
</tr>
<tr>
<td>Met$<em>{928}$ + 50% deuterated InlB$</em>{321}$</td>
<td>-</td>
<td>1.5 (22)</td>
<td>0.7 (21)</td>
<td>0.6 (20)</td>
<td>-</td>
<td>1.9 (19)</td>
<td>3.1 (18)</td>
</tr>
<tr>
<td>Met$<em>{638}$ + perdeuterated InlB$</em>{321}$</td>
<td>-</td>
<td>1.7 (17)</td>
<td>1.1 (16)</td>
<td>-</td>
<td>1.0 (8)</td>
<td>-</td>
<td>2.5 (15)</td>
</tr>
<tr>
<td>Met$<em>{928}$ + perdeuterated InlB$</em>{321}$</td>
<td>-</td>
<td>1.6 (14)</td>
<td>1.4 (13)</td>
<td>1.4 (12)</td>
<td>1.3 (35)</td>
<td>4.1 (11)</td>
<td>4.8 (10)</td>
</tr>
</tbody>
</table>
Combining SAXS and SANS
Complex of Met Receptor tyrosine kinase and Invasion Protein InlB

Met – InlB protein complex:

Rigid body refinement of the existing high resolution structures.

The Ig-like domains were kept flexible to allow refinement of the overall structure in respect to the SAXS and SANS data.

DNA-Protein Complex Modeling

Homology modeling

Ab initio modeling consider a homogenous scattering density. For proteins this is nearly always fulfilled, but not for protein-DNA/RNA complexes!

SASREF modeling with homology models. Lucky case palindormic DNA!
Combined SAXS – SANS
Protein-RNA complex

Exportin-t is a vertebrate nuclear export receptor for tRNAs that binds tRNA cooperatively with GTP-loaded Ran

Ran (structure known)  Exportin-t  t-RNA (structure known)
(tentative homology model)
Combined SAXS – SANS
Protein-RNA complex

**X-ray scattering**

- From ternary complex, Ran, tRNA 3 curves

**Neutron scattering**

- Ternary complex with protonated Ran
  in 0, 40, 55, 75, 100% D$_2$O 5 curves

- Ternary complex with deuterated Ran
  in 0, 40, 55, 70, 100% D$_2$O 5 curves

*TOTAL 13 curves*
Combined SAXS – SANS
Protein-RNA complex

High resolution models of the components docked into the three-phase ab initio model of the complex based on X-ray and neutron scattering from selectively deuterated particles
Combined SAXS – SANS
Protein – DNA complexes

Nuclear hormone receptors (NHRs) heterodimers (RXR–RAR, PPAR–RXR and RXR–VDR) in complex with DNA

Table 1 SAXS and SANS data

<table>
<thead>
<tr>
<th>Complexes</th>
<th>D$_2$O/H$_2$O (%)</th>
<th>$R_g$ (Å)</th>
<th>$R_g$ (Å)</th>
<th>$D_{max}$ (Å)</th>
<th>MM (MM$_{seg}$) (kDa)</th>
<th>$\chi_D$</th>
<th>$\chi_R$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RXR$\Delta$AB–RAR$\Delta$ABF</td>
<td>0</td>
<td>35 ± 0.7</td>
<td>38 ± 1</td>
<td>130 ± 10</td>
<td>70 ± 10 (76)</td>
<td>1.05</td>
<td>1.16</td>
</tr>
<tr>
<td>RXR$\Delta$AB–RAR$\Delta$AB</td>
<td>0</td>
<td>36 ± 0.5</td>
<td>38 ± 1</td>
<td>130 ± 10</td>
<td>75 ± 10 (80)</td>
<td>1.08</td>
<td>1.18</td>
</tr>
<tr>
<td>RXR$\Delta$AB–RAR$\Delta$ABF–DR5</td>
<td>0</td>
<td>39 ± 0.5</td>
<td>38 ± 1</td>
<td>140 ± 10</td>
<td>80 ± 10 (87)</td>
<td>1.10</td>
<td>1.15</td>
</tr>
<tr>
<td>RXR$\Delta$AB–RAR$\Delta$AB–DR5</td>
<td>0</td>
<td>42 ± 0.5</td>
<td>44 ± 0.5</td>
<td>150 ± 10</td>
<td>95 ± 15 (91)</td>
<td>1.08</td>
<td>1.21</td>
</tr>
<tr>
<td>dRXR$\Delta$AB–RXR$\Delta$AB–DR5</td>
<td>0</td>
<td>43 ± 0.5</td>
<td>44 ± 0.5</td>
<td>150 ± 10</td>
<td>95 ± 15 (91)</td>
<td>1.08</td>
<td>1.21</td>
</tr>
<tr>
<td>RXR$\Delta$AB–RAR$\Delta$AB–DR5</td>
<td>0.7</td>
<td>38 ± 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dRXR$\Delta$AB–RXR$\Delta$AB–DR5</td>
<td>0.95</td>
<td>35 ± 0.6 (35)$^a$</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>RAR$\Delta$AB–RXR$\Delta$AB–DR1</td>
<td></td>
<td>44 ± 0.5</td>
<td></td>
<td>150 ± 10</td>
<td>95 ± 10 (90)</td>
<td>1.04</td>
<td>1.49</td>
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<tr>
<td>RXR$\Delta$AB–RAR$\Delta$AB–Med1</td>
<td></td>
<td>45 ± 1</td>
<td></td>
<td>150 ± 15</td>
<td>100 ± 15 (102)</td>
<td>1.09</td>
<td>1.22</td>
</tr>
<tr>
<td>RXR$\Delta$AB–RAR$\Delta$AB–DR5–Med1$^b$</td>
<td></td>
<td>52 ± 0.5</td>
<td></td>
<td>170 ± 15</td>
<td>130 ± 25 (113)</td>
<td>1.21</td>
<td>–</td>
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<tr>
<td>RXR$\Delta$ABm–RAR$\Delta$AB–DR5–Med1</td>
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<td>50 ± 0.5</td>
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<td>170 ± 10</td>
<td>120 ± 15 (113)</td>
<td>1.03</td>
<td>2.17</td>
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<td>RAR$\Delta$AB–RXR$\Delta$AB–antagonist–DR1–Med1</td>
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<td>46 ± 1</td>
<td></td>
<td>150 ± 10</td>
<td>95 ± 10 (90)</td>
<td>1.25</td>
<td>–</td>
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<tr>
<td>RXR$\Delta$AB–VDR</td>
<td></td>
<td>36 ± 0.5</td>
<td></td>
<td>130 ± 10</td>
<td>75 ± 10 (80)</td>
<td>0.98</td>
<td>1.23</td>
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<td>RXR$\Delta$AB–VDR–DR3</td>
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<td>41 ± 0.5</td>
<td></td>
<td>140 ± 10</td>
<td>85 ± 15 (91)</td>
<td>1.00</td>
<td>1.08</td>
</tr>
<tr>
<td>PPAR$\gamma$AB–RXR$\Delta$AB–DR1</td>
<td></td>
<td>39 ± 0.5</td>
<td></td>
<td>140 ± 10</td>
<td>88 ± 15 (93)</td>
<td>1.05</td>
<td>1.4</td>
</tr>
<tr>
<td>PPAR$\gamma$–RXR$\Delta$AB–DR1</td>
<td></td>
<td>44 ± 0.5</td>
<td></td>
<td>160 ± 10</td>
<td>106 ± 15 (111)</td>
<td>1.64</td>
<td>–</td>
</tr>
<tr>
<td>PPAR$\gamma$–RXR$\Delta$–DR1</td>
<td></td>
<td>52 ± 0.5</td>
<td></td>
<td>180 ± 10</td>
<td>170 ± 15 (130)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Combined SAXS – SANS
Protein – DNA complexes
Combined SAXS – SANS
Protein – DNA complexes

Combined SAXS – SANS
Shape determination of membrane proteins

Membrane proteins do often not solubilize and precipitate in buffer because of the hydrophobic membrane anchor part.

They can stabilized with lipids.

With SANS the scattering of the lipid can be matched out and the shape of the entire protein determined.
Combined SAXS – SANS
Shape determination of membrane proteins

Experimental SANS data from the membrane protein **IntegrinIIb3** in buffer containing 16% D\textsubscript{2}O, the contrast matching point of phospholipids.
Combined SAXS – SANS
Shape determination of membrane proteins

Fitting of SAXS models or high resolution MX/NMR structures into the SANS envelope.

A. Nogales, C. García, J. Perez, P. Callow, T. A. Ezquerra, and J. Gonzalez-Rodríguez; JBC VOL. 285, NO. 2, pp. 1023–1031, January 8, 2010
Combined SAXS – SANS SAS study on micelles

Goyal & Aswal 2006
Combined SAXS – SANS

SAS study on micelles

CTAC:
Cetlytrimethyl ammonium Chloride

Structure factor peak at 0.6 nm\(^{-1}\) is determined by the intermolecular distance and hence method independent.

The small shoulder at 1 nm\(^{-1}\) arises from scattering of the condensed chloride counter ions around the micelles.
Combined SAXS – SANS
SAS study on micelles

**CTAC:**
Cetytrimethyl ammonium Bromide

Structure factor peak at 0.6 nm\(^{-1}\) is determined by the intermolecular distance of the micelle sphere and hence method independent.

The second peak at 1 nm\(^{-1}\) arises from the strong scattering of the Bromide counter ion layer around the micelles.
Combined SAXS – SANS

Conclusions

• SANS and SAXS are complementary methods
• SANS is a sensitive method for hydrogens, while SAXS is sensitive for heavy atoms
• Contrast variation method is a powerful tool and increase the information content especially for investigations on Protein-DNA complexes
• Neutrons do not produce radiation damage to biological samples