Synchrotron Small-Angle X-Ray Scattering as Universal Instrument of Structural Analysis of Bio and Nanosystems

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Motivation for Small-angle X-ray scattering in structural study

**SAXS** is universal low resolution method for structural analysis

- is applied to solutions, polydisperse systems, gels, fractal systems, multilayered structures, supramolecular structures, nanocomposites, biological complexes, *etc*
- requires neither crystals nor special sample preparation
- is applicable under nearly physiological conditions
- yields complementary information to other structural methods like crystallography, NMR, EM, AFM, *etc*
- permits quantitative analysis of complex systems and kinetic processes
- allows to study structural transitions and conformational changes
Basics of SAXS

X-ray beam is scattered by the bound electrons of the sample.

Concept of contrast

\[ \Delta \rho(\mathbf{r}) = \rho(\mathbf{r}) - \rho_s \]

\( \rho_s \) - electron density of the medium

\( \rho(\mathbf{r}) \) - electron density of the particle

\[ A(s) = \Im[\rho(\mathbf{r})] = \int \Delta \rho(\mathbf{r}) \exp(is\mathbf{r}) \, d\mathbf{r} \]

\( V \)
To extract information about the structure of the objects under study one needs to solve the reciprocal task: using 1D scattering curve $I(s)$ to restore 3D structure.

In general, the solution of the reciprocal tasks is ambiguous.
Problems

Additional information is **ALWAYS** required to resolve or reduce ambiguity of interpretation

3D search model  Trial-and-error  1D scattering data

before  after
Distance distribution function $p(r)$

$I(s) = \int_0^{D_{\text{max}}} p(r) \frac{\sin(sr)}{sr} dr$

Using distance distribution function one can determine size, shape and internal structure of the particle at low (1-10 nm) resolution.

$p(r) = \frac{r^2}{2\pi^2} \int_0^{D_{\text{max}}} s^2 I(s) \frac{\sin sr}{sr} ds$

$p(r) = 0$ at $r > D_{\text{max}}$
Many different programs required for SAXS data interpretation are placed in this portal.

SAS Portal
Software
http://smallangle.org/
Data Processing and Analysis

A program suite

**ATSAS**
**All That SAS**

It allows data reduction, processing and structure analysis using 3 main approaches to the data interpretation:

1. *Ab initio* shape reconstruction;
2. **Rigid body modeling** (method of molecular tectonics);
3. **Hybrid methods**

Major programs running on a PC under Win9x/NT and/or on UNIX workstations are documented and available at:

http://www.embl-hamburg.de/ExternalInfo/Research/Sax
The maximum size $D_{\text{max}}$ is the largest size of the scattering object. It determines the diameter of the search volume, and it is calculated using the size distribution analysis, i.e. from the $p(r)$ function by the program GNOM (Svergun, D. I. J. Appl. Crystallogr. 1992, 25, 495.)
Rigid body refinement (method of molecular tectonics)

- The structures of two subunits are known.
- Arbitrary complex can be constructed by moving and rotating the subunits.
- This operation depends on three Euler rotation angles and three Cartesian shifts.

The process of rotation and moving stops when model curve fits the experimental data.

Petoukhov MV, Svergun DI. Global rigid body modelling of macromolecular complexes against small-angle scattering data. Biophys J. 2005; 89: 1237-1250
Hybrid methods:

Combination of *ab initio* reconstruction and molecular tectonics
Analysis of Flexibility of Multidomain Macromolecules

\[ R_g \text{ и } D_{max} \]

distributions:
1 – random pool,
2 – selected ensemble

Some examples of SAXS application to biological samples:

4 important enzymes involved in metabolic processes in living cells
**Class I fructose-1,6-bisphosphate aldolase (FbaB)**

**FbaB – an enzyme with a previously unknown structure – can associate into decamers where each individual protomer has a core TIM-barrel fold.**

### Table: Sample Metrics

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_g$, nm</th>
<th>$D_{max}$, nm</th>
<th>MM$_{d[0]}$, kDa</th>
<th>MM$_{Porod}$, Kda</th>
<th>$R_{g\ crist}$*, nm</th>
<th>MM$_{aa}$, kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>FbaB</td>
<td>4.4±0.1</td>
<td>12.7±0.6</td>
<td>340±20</td>
<td>335±15</td>
<td>4.4</td>
<td>339.14</td>
</tr>
</tbody>
</table>

*R$_{g\ crist}$ for decamer of archaea FbaB class I from *Thermoproteus tenax* (homologue) (PDB ID: 1OJX)
Inorganic pyrophosphatase (PPase)

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_g$, nm</th>
<th>$D_{max}$, nm</th>
<th>MM$_{I(0)}$, kDa</th>
<th>MM$_{Porod}$, kDa</th>
<th>$R_g$ cryst, nm</th>
<th>MM$_{aa}$, kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPase</td>
<td>2.8±0.1</td>
<td>7.7 ±0.5</td>
<td>130±10</td>
<td>116±10</td>
<td>2.9</td>
<td>117.28</td>
</tr>
</tbody>
</table>

The main difference between the crystal and solution conformations appears to be a rotational shift in the orientation of the individual PPase subunits. The overall low-resolution shape fits the experimental curve very well and are spatially superimposable with the rigid body model.
The ability of KduI to form mixtures of different oligomeric species is an important property of the protein that contributes to regulating enzymatic activity. The different oligomeric forms differ in their catalytic efficiency, as it has been established for a number of other allosteric enzymes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>( R_g ), nm</th>
<th>( D_{max} ), nm</th>
<th>( MM_I(0) ), kDa</th>
<th>( MM_{Porod} ), kDa</th>
<th>( R_g ) cryst, nm</th>
<th>( MM_{aa} ), kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>KduI</td>
<td>6.2±0.1</td>
<td>-</td>
<td>183±10</td>
<td>182±10</td>
<td>3.9</td>
<td>187.35</td>
</tr>
</tbody>
</table>
Glutamate decarboxylase (GadA)

**Condition of the sample preparation**
The composition of the buffer:
1. 100 mM Na-acetate, \textbf{10 mM NaCl}, 1 mM DTT, pH 4.6.
2. 100 mM Na-acetate, 1 mM DTT, pH 4.6.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_g$, nm</th>
<th>$\text{MM}_{I(0)}$, kDa</th>
<th>$\text{MM}_{\text{Porod}}$, kDa</th>
<th>$R_g \text{ cryst}$, nm</th>
<th>$\text{MM}_{\text{aa}}$, kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>GadA</td>
<td>4.8±0.1</td>
<td>249±15</td>
<td>252±15</td>
<td>4.2</td>
<td>316</td>
</tr>
<tr>
<td>GadA, low salt</td>
<td>4.4±0.1</td>
<td>260±15</td>
<td>265±15</td>
<td>4.2</td>
<td>316</td>
</tr>
</tbody>
</table>
Glutamate decarboxylase (GadA)

\[ \chi^2 = 1.4 \]

Volume fraction of hexamers 60%

Volume faction of dimers 40%

PDB: 1XEY

SASBDB: SASDB33
Glutamate decarboxylase (GadA)

Volume fraction of hexamers 80%
Volume fraction of dimers 20%

Comparison of hexamer structures: crystal structure, and modeled by program SASREFMX

IMPORTANT: All structural rearrangements in solution observed by us could be crucial for revealing new binding sites that form additional protein-protein interfaces for modulating enzyme activity within cells.
Small-Angle Scattering Biological Data Bank

http://www.sasbdb.org/browse-dissemination/

All obtained structures were placed in SASBDB

Investigations were mostly performed by L. Dadinova (postgraduate student):
"Essentially, all models are wrong, but some are useful”

George Edward Pelham Box, British statistician
I invite you to see more examples of SAXS application during the poster session today: posters 18, 20, 21, 22 and 47
The main conclusion

SAXS and advanced SAXS data analysis methods can be employed to systematically characterize structure of different complicated nanosized systems which can be used in biology and medicine.
Thanks for your attention!