Biological Small Angle X-ray Scattering: An Introduction to the Experiment and Overview of Guinier Analysis

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Talk Outline

• Brief History of Small Angle Scattering (SAS)
• Intro to SAS
• Overview of a SAS experiment and SAS data processing
• Calculating structural parameters from SAS data
  – Radius of gyration ($R_g)$
• Evaluating SAS data quality from Guinier plots
A Brief History of Small Angle Scattering

- SAS methods were introduced in the 1930s, by André Guinier
- Throughout the 1938 – 1950s, Guinier and others developed SAS fundamentals (inc. Peter Debye, Otto Kratky, Günther Porod)
- First experiments with proteins occurred in the 1950s
- The use of SAS increased with the ‘user friendly’ beamlines that adopted the method.
- Since the mid-1990’s, the number of SAS publications has increased.
SAS Examples in the literature
1957 - catalase

- Small angle X-ray scattering studies of the size, shape, and hydration of catalase
  - $R_g$ – 39.8 Å
  - Maximum linear dimension – 146 Å
  -Avg electron density – 0.425 e-/Å³
  - The shape appears to be that of a slightly elongated figure whose length is about twice the average cross-sectional diameter.
  - ‘Considerable internal water of hydration which swells the molecule’

**SAS Examples in the literature**

**1990s - ab initio modeling from SAS data**

- 1\textsuperscript{st} ab initio shape described the scattering particle by an angular envelope function
- Svergun further adapted method in T7 virus model
  - Good agreement with cryo-EM structure
- Svergun implements particle modeling from densely packed dummy atoms

*Biophysical Journal, Volume 76, Issue 6, 2879-2886, 1 June 1999*
SAS Examples in the literature
2007 – insulin fibrillation

- SAS is now used for a wide range of studies, helped by advanced modeling methods and advanced methodologies, including time resolved studies.
- Additionally, method in SANS provide additional structural information for complexes and ‘mixed’ systems.


Jill Trewhella
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Crystallography versus SAXS

Advantage | Limitation
--- | ---
Atomic structure information | Requires a crystal
Readily available software | Flexible portions may not be seen

The SAXS Experiment

Thomson scattering ➔ Elastic scattering

\[ I_{2\theta} = r_0^2 \frac{1 + \cos^2(2\theta)}{2} \frac{1}{r^2} I_0 \]

X-ray source

Incident Beam

Scattered Beam

Debye formula
Scattering from assembly of electrons

\[ I(q) = \sum_{i=1}^{N} \sum_{j=1}^{N} f_i(q)f_j(q) \frac{\sin(qr_{ij})}{qr_{ij}} \]

\[ r_{ij} = |r_i - r_j| \]

\[ q = k_s - k_i \]

\[ q = \frac{4\pi}{\lambda} \sin\theta \]

\[ |k_s| = |k_i| = \frac{2\pi}{\lambda} = q \]

When X-rays hit the sample, some fraction will pass through the sample while some fraction is absorbed.
Calibrating with a Powder Standard

- Silver behenate (AgBeh)
  - Q-calibration, Beam center

<table>
<thead>
<tr>
<th>Peak Order</th>
<th>d-spacing (Å)</th>
<th>s-value (Å⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d(001)</td>
<td>58.38</td>
<td>10.72</td>
</tr>
<tr>
<td>d(002)</td>
<td>29.17</td>
<td>21.53</td>
</tr>
<tr>
<td>d(003)</td>
<td>19.44</td>
<td>32.31</td>
</tr>
<tr>
<td>d(004)</td>
<td>14.58</td>
<td>43.04</td>
</tr>
</tbody>
</table>

Log average I (counts per m² per sec)

58.32 Å
The SAXS Experiment

$$q = \frac{4\pi \sin \theta}{\lambda}$$

1D avg

Intensity

X-ray source → sample → beam stop

momentum transfer

degrees

$[\text{Å}^{-1}]$

$[\text{Å}^{-1}]$
Limitation of the SAXS Experiment

- Macromolecules are tumbling in solution thus in many different orientations in the beam.
  - Also, there may be many conformations of the molecule in solution.
- As a result, the observed scattering is a spherical average (isotropic)
  - We’ve lost any 3D information
  - We reduce the scattering data to a 1D intensity distribution I(q).
- This loss of information constitutes the most severe limitation of the SAXS method.
The SAXS Experiment

- Collect data for both protein and buffer
  - Must be exactly matched buffer
- Choose a capillary size \( t_{opt} \) best suited for the energy of your beam
- Check transmission factors to correct for any deviations in direct beam intensity
- Collect data for several concentrations
  - Do results agree across all concentrations
  - Are there concentration effects?
- Check data for radiation damage

\[
\frac{1}{\mu_t} = t_{opt}
\]

\( t_{opt} \approx 1 \text{ mm for 8 keV} \)

Srinivas Chakravarthy
Jill Trewhella
Scattering reveals information about molecular shapes

Because macromolecules are generally polymorphic and/or polydisperse, sharp minima are not seen.

\[ \log(I) \]

\[ s, \text{ nm}^{-1} \]

\[ q, \text{ Å}^{-1} \]

\[ \text{apoferritin} \]


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Deriving Structural Parameters and Information from SAXS Data

- **SAXS pattern**
- **Guinier plot**
- **Kratky plot**
- **Pair distribution function**


Javier Pérez

Eddie Snell

Richard Gillilan
Determining $R_g$ from Guinier Plots

- **Guinier Law**
  \[
  \ln[I(q)] \approx -\frac{q^2 R_g^2}{3} + \ln[I(0)]
  \]

  $R_g$ – radius of gyration

  $I(0)$ – forward scattering

- **Plot $\ln I$ vs. $q^2$**
  - $q$-min $< q < 1.3R_g$
  - Slope $\alpha R_g$
  - Check for linearity

Points 9 to 18 fidel = 0.35
sRg limits: 0.700 to 1.28
$R_g = 47.4 \pm 0.614$

$I_0 = 6.047 \pm 5.63 \text{ e}^{-2}$

Comptes Rendus Hebdomadaires Des Seances De L Académie Des Sciences 1938, 206:1374-1376

Guinier Plot above created with primus:

Basics for Interpreting Guinier plots

• What is the $q_{\text{min}}$ in your experimental data?
  – For very large particles, you will need a lower $q_{\text{min}}$ to use the Guinier region for $R_g$ determination.
  – This will also be determined by the number of data points in your 1D profile.

• Sample-to-detector position
• Pixel size

$$q_{\text{min}} < \frac{\pi}{d_{\text{max}}}$$

$d_{\text{max}} = \text{see table}$

<table>
<thead>
<tr>
<th>$d_{\text{max}}$ (Å)</th>
<th>$q_{\text{min}}$</th>
<th>$r_{\text{max}}$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.0628</td>
<td>25</td>
</tr>
<tr>
<td>100</td>
<td>0.0314</td>
<td>50</td>
</tr>
<tr>
<td>200</td>
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<td>100</td>
</tr>
<tr>
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<td>400</td>
</tr>
<tr>
<td>1000</td>
<td>0.00314</td>
<td>500</td>
</tr>
</tbody>
</table>
Basics for Interpreting Guinier plots

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  – For very large particles, you will need a lower $q_{\text{min}}$ to use the Guinier region for $R_g$ determination.
  – This will also be determined by the number of data points in your 1D profile.

• The $q_{\text{max}}$ will depend on the shape of your molecule.
  – For spherical particles, $q_{\text{max}} < 1.3*R_g$
  – For elongated particles, $q_{\text{max}} < 0.8*R_g$

• The Guinier also provides $I(0)$, which is proportional to the # of electrons in the scattering particle (MW).
Guinier Plot Examples

- Deviant Guinier Plots aren’t necessarily ‘bad’ as they tell you something about the state of the macromolecule in solution.

  - BSA Example

    - Determine the $R_g$ at several concentrations.
      - Are there concentration effects?

    - BSA
      1) aggregated
      2) ‘good’ data
      3) inter-particle repulsion

Using \( R_g \) to detect changes in structure

SAXS data collected for an RNA with at varying [RNA] and two [MgCl\(_2\)]

<table>
<thead>
<tr>
<th>[RNA]</th>
<th>[MgCl(_2)]</th>
<th>Guinier ( R_g ) (Å) (error)</th>
<th>GNOM ( R_g ) (Å) (error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 mM</td>
<td>--</td>
<td>17.7 (0.01)</td>
<td>18.5 (3.3)</td>
</tr>
<tr>
<td>0.5 mM</td>
<td>--</td>
<td>18.4 (0.2)</td>
<td>18.6 (1.7)</td>
</tr>
<tr>
<td>0.1 mM</td>
<td>--</td>
<td>18.8 (0.6)</td>
<td>19.4 (0.3)</td>
</tr>
<tr>
<td>0.5 mM</td>
<td>100 mM</td>
<td>26.01 (0.2)</td>
<td>27.5 (2.5)</td>
</tr>
</tbody>
</table>
SAXS Scattering Contributions

X-ray scattering contains information on both particle shape and the particle interactions:

\[ I(c, s) = I(0, s) \times S(c, s) \]

- Analyze the protein shape from the form factor, \( I(0, s) \)

- Analyze the protein distribution from the solution structure factor, \( S(c, s) \)

Images adapted from Journal of Crystal Growth 168 (1996) 28-39
SAXS Scattering Contributions

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Images adapted from Journal of Crystal Growth 168 (1996) 28-39
Impact of Radiation Damage on SAXS Data: Aggregation

Repulsive Interactions detected by SAXS

• Varying $[\text{MgCl}_2]$ for an RNA sample in low ionic strength buffer
• Particle repulsion increases as $[\text{MgCl}_2]$ decreases
• Example in the literature:
  – RNA/DNA studies
  – Protein-protein interactions
  – Crystallization (??)

Studying Interparticle Interactions

- Interparticle interactions are useful for understanding how macromolecules interact in solution. For these purposes, one can extract information about these interactions by collecting at different buffer conditions (i.e. diff. ionic strength)

\[ I(c, s) = I(0, s) \times S(c, s) \rightarrow S(c, s) = \frac{I(c, s)}{I(0, s)} \]

- From the solution structure factor, one can calculate the second virial coefficient:

\[ \frac{1}{S(c, 0)} = 1 + 2MA_2c \]

- To reach the ‘crystallization slot’, one can follow the recommended crystallization recipe:
  - Start far away from precipitation (usually in repulsive conditions)
  - Tune towards attractive conditions
Linear Guiniers $\neq$ Monodispersity

- Scattering if from molecule(s) in all orientations and conformations.
- SAXS can still provide information these ‘mixed’ systems
  \[ I_{total} = x_1 I_1 + x_2 I_2 + x_n I_n \]
  - \( x_n \) – molar fraction of component \( n \)
  - \( I_1 \) – scattering intensity of component \( n \)
- SVD analysis is helpful to identify the total # of scattering components and other programs can calculate volume fractions and scattering profiles of mixtures\(^1\)
- In cases like these, models should be deemed with lots of skepticism and validated by other methods.

\(^1\) J Appl Crystallogr 36: 1277–1282
Linear Guiniers ≠ Monodispersity

• Scattering if from molecule(s) in all orientations and conformations.
ありがとうございます

Thank You