Beyond standard BioSAXS

Andrey Gruzinov - Martin Schroer

Solution Scattering from Biological Macromolecules 2020
May 5 — June 2, 2020
BioSAXS

- Weakly scattering samples
- Radiation damage
- Careful sample preparation and handling
- Well characterized before SAXS
- Low amount of sample available
“Standard” experiment

\[ s = k_s - k_i \]

X-ray beam

Sample

Buffer

Detector

Scattering vector
“Non-standard” experiment

\[ S = k_s - k_i \]

Scattering vector

Sample

Detector

X-ray beam

Buffer

2θ
“Non-standard” experiments

- Microfluidic sample environments
- Microfluidics cell for THz illumination and SAXS measurements
- Thermostated capillary holder
- Scanning-SAXS
- CryoSAXS
- Stopped-flow
- Laser setup
- ASAXS
“Non-standard” experiments

Microfluidic sample environments

Microfluidics cell for THz illumination and SAXS measurements

Thermostated capillary holder

Scanning-SAXS

Other…

Stopped-flow

Laser setup

ASAXS
Anomalous small angle X-ray scattering (ASAXS)

- What is ASAXS?
- Why ASAXS?
- How to use ASAXS?
- How to measure ASAXS?

NB! “Semi-standard” experiment
Background Physics of ASAXS

\[ \mu t = \ln \left( \frac{I_0}{I_1} \right) \]

Absorption Edge
Background Physics of ASAXS

Electron density = \( \frac{\text{Number of electrons}}{\text{Volume}} \)

\[ I(s) = \sum_{i=1}^{K} \sum_{j=1}^{K} f_i(s) f_j(s) \frac{\sin(sr_{ij})}{sr_{ij}} \]

Anomalous corrections

\[ f(E) = f_0 + f'(E) + if''(E) \]
Background Physics of ASAXS

Electron density = \( \frac{\text{Number of electrons}}{\text{Volume}} \)

\[
I(s) = \sum_{i=1}^{K} \sum_{j=1}^{K} f_i(s) f_j(s) \frac{\sin(sr_{ij})}{sr_{ij}}
\]

“Effective” number of electrons changes the contrast within the sample.
**Background Physics of ASAXS**

Electron density = \[
\frac{\text{Number of electrons}}{\text{Volume}}
\]

\[
I(s) = \sum_{i=1}^{K} \sum_{j=1}^{K} f_i(s) f_j(s) \frac{\sin(sr_{ij})}{sr_{ij}}
\]

\[
f(E) = f_0 + f'(E) + if''(E)
\]

For Br: 35 e\(^-\) - 8 e\(^-\)

"Effective" charge: 27 e\(^-\) (="Co")
Background Physics of ASAXS

Electron density = \frac{\text{Number of electrons}}{\text{Volume}}

\[ f(E) = f_0 + f'(E) + if''(E) \]

“Effective” number of electrons changes the contrast within the sample.

TTAB micelles


Absorption Edge
How to measure anomalous corrections?

\[ \mu t = \ln \left( \frac{I_0}{I_1} \right) \]

\[ f'' = m_e c \varepsilon_0 E \mu_a / e \hbar \]

\[ f'(E_0) = \frac{1}{2\pi} \int_0^\infty \frac{E f''(E)}{(E_0^2 - E^2)} dE. \]

Kramers-Kronig relations
How to measure anomalous corrections?

- Absorption

\[ \mu t = \ln \left( \frac{I_0}{I_1} \right) \]

\[ f'' = m_e c \varepsilon_0 E \mu_\alpha / e \hbar \]

\[ f'(E_0) = \frac{2}{\pi} \int_0^\infty \frac{[E f''(E)]/(E_0^2 - E^2)]}{E} \, dE. \]

Kramers-Kronig relations

**CHOOCH**

http://chooch.sourceforge.net


**NB!:** This concept is used for MAD phasing in MX (Taylor GL. 2010)
Absorption Edges of different Elements

http://skuld.bmsc.washington.edu/scatter/AS_periodic.html
Absorption Edges of different Elements

Table 1-1. Electron binding energies, in electron volts, for the elements V to Ag in their natural forms.

<table>
<thead>
<tr>
<th>Element</th>
<th>K 1s</th>
<th>L1 2s</th>
<th>L2 2p1/2</th>
<th>L3 2p3/2</th>
<th>M1 3s</th>
<th>M2 3p1/2</th>
<th>M3 3p3/2</th>
<th>M4 3d3/2</th>
<th>M5 3d5/2</th>
<th>N1 4s</th>
<th>N2 4p1/2</th>
<th>N3 4p3/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>5465</td>
<td>626.7†</td>
<td>519.8†</td>
<td>512.1†</td>
<td>66.3†</td>
<td>37.2†</td>
<td>37.2†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>5989</td>
<td>696.0†</td>
<td>583.8†</td>
<td>574.1†</td>
<td>74.1†</td>
<td>42.2†</td>
<td>42.2†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mn</td>
<td>6539</td>
<td>769.1†</td>
<td>649.9†</td>
<td>638.7†</td>
<td>82.3†</td>
<td>47.2†</td>
<td>47.2†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>7112</td>
<td>844.6†</td>
<td>719.9†</td>
<td>706.8†</td>
<td>91.3†</td>
<td>52.7†</td>
<td>52.7†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co</td>
<td>7709</td>
<td>925.1†</td>
<td>793.2†</td>
<td>778.1†</td>
<td>101.0†</td>
<td>58.9†</td>
<td>59.9†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>8333</td>
<td>1008.6†</td>
<td>870.0†</td>
<td>852.7†</td>
<td>110.8†</td>
<td>68.0†</td>
<td>66.2†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>8979</td>
<td>1096.7†</td>
<td>952.3†</td>
<td>932.7†</td>
<td>122.5†</td>
<td>77.3†</td>
<td>75.1†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>9659</td>
<td>1196.2*</td>
<td>1044.9*</td>
<td>1021.8*</td>
<td>139.8*</td>
<td>91.4*</td>
<td>88.6*</td>
<td>10.2*</td>
<td>10.1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ga</td>
<td>10367</td>
<td>1299.0*b</td>
<td>1143.2†</td>
<td>1116.4†</td>
<td>159.5†</td>
<td>103.5†</td>
<td>100.0†</td>
<td>18.7†</td>
<td>18.7†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ge</td>
<td>11103</td>
<td>1414.6*b</td>
<td>1248.1*b</td>
<td>1217.0*b</td>
<td>180.1*</td>
<td>124.9*</td>
<td>120.8*</td>
<td>29.8</td>
<td>29.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As</td>
<td>11867</td>
<td>1527.0*b</td>
<td>1359.1*b</td>
<td>1323.6*b</td>
<td>204.7*</td>
<td>146.2*</td>
<td>141.2*</td>
<td>41.7*</td>
<td>41.7*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>12658</td>
<td>1652.0*b</td>
<td>1474.3*b</td>
<td>1433.9*b</td>
<td>229.6*</td>
<td>166.5*</td>
<td>160.7*</td>
<td>55.5*</td>
<td>54.6*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Br</td>
<td>13474</td>
<td>1782*</td>
<td>1596*</td>
<td>1550*</td>
<td>257*</td>
<td>189*</td>
<td>182*</td>
<td>70*</td>
<td>69*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kr</td>
<td>14326</td>
<td>1921</td>
<td>1730.9*</td>
<td>1678.4*</td>
<td>292.8*</td>
<td>222.2*</td>
<td>214.4*</td>
<td>95.0*</td>
<td>93.8*</td>
<td>27.5*</td>
<td>14.1*</td>
<td>14.1*</td>
</tr>
<tr>
<td>Rb</td>
<td>15200</td>
<td>2065</td>
<td>1864</td>
<td>1804</td>
<td>326.7*</td>
<td>248.7*</td>
<td>239.1*</td>
<td>113.0*</td>
<td>112*</td>
<td>30.5*</td>
<td>16.3*</td>
<td>15.3*</td>
</tr>
<tr>
<td>Sr</td>
<td>16105</td>
<td>2216</td>
<td>2007</td>
<td>1940</td>
<td>358.7*</td>
<td>280.3*</td>
<td>270.0*</td>
<td>136.0*</td>
<td>134.2*</td>
<td>38.9*</td>
<td>21.3</td>
<td>20.1*</td>
</tr>
<tr>
<td>Y</td>
<td>17038</td>
<td>2373</td>
<td>2156</td>
<td>2080</td>
<td>392.0*</td>
<td>310.6*</td>
<td>298.8*</td>
<td>157.7*</td>
<td>155.8*</td>
<td>43.8*</td>
<td>24.4*</td>
<td>23.1*</td>
</tr>
<tr>
<td>Zr</td>
<td>17998</td>
<td>2532</td>
<td>2307</td>
<td>2223</td>
<td>430.3*</td>
<td>343.5*</td>
<td>329.8*</td>
<td>181.1*</td>
<td>178.8*</td>
<td>50.6*</td>
<td>28.5*</td>
<td>27.1*</td>
</tr>
<tr>
<td>Nb</td>
<td>18986</td>
<td>2698</td>
<td>2465</td>
<td>2371</td>
<td>466.6*</td>
<td>376.1*</td>
<td>360.6*</td>
<td>205.0*</td>
<td>202.3*</td>
<td>56.4*</td>
<td>32.6*</td>
<td>30.8*</td>
</tr>
<tr>
<td>Mo</td>
<td>20000</td>
<td>2866</td>
<td>2625</td>
<td>2520</td>
<td>506.3*</td>
<td>411.6*</td>
<td>394.0*</td>
<td>231.1*</td>
<td>227.9*</td>
<td>63.2*</td>
<td>37.6*</td>
<td>35.5*</td>
</tr>
<tr>
<td>Tc</td>
<td>21044</td>
<td>3043</td>
<td>2793</td>
<td>2677</td>
<td>544*</td>
<td>447.6</td>
<td>417.7</td>
<td>257.6</td>
<td>253.9*</td>
<td>69.5*</td>
<td>42.3*</td>
<td>39.9*</td>
</tr>
<tr>
<td>Ru</td>
<td>22117</td>
<td>3224</td>
<td>2967</td>
<td>2838</td>
<td>586.1*</td>
<td>483.5*</td>
<td>461.4*</td>
<td>284.2*</td>
<td>280.0*</td>
<td>75.0*</td>
<td>46.3*</td>
<td>43.2*</td>
</tr>
<tr>
<td>Rh</td>
<td>23220</td>
<td>3412</td>
<td>3146</td>
<td>3004</td>
<td>628.1†</td>
<td>521.3†</td>
<td>496.5†</td>
<td>311.9†</td>
<td>307.2†</td>
<td>81.4*</td>
<td>50.5†</td>
<td>47.3†</td>
</tr>
<tr>
<td>Pd</td>
<td>24350</td>
<td>3604</td>
<td>3330</td>
<td>3173</td>
<td>671.6†</td>
<td>559.9†</td>
<td>532.3†</td>
<td>340.5†</td>
<td>335.2†</td>
<td>87.1*b</td>
<td>55.7*a</td>
<td>50.9†</td>
</tr>
<tr>
<td>Ag</td>
<td>25514</td>
<td>3806</td>
<td>3524</td>
<td>3351</td>
<td>719.0†</td>
<td>603.8†</td>
<td>573.0†</td>
<td>374.0†</td>
<td>368.3</td>
<td>97.0†</td>
<td>63.7†</td>
<td>58.3†</td>
</tr>
</tbody>
</table>

“10 keV” (Zn)

“12 keV” (Se)
Anomalous small angle X-ray scattering (ASAXS)

• What is ASAXS?

• Why ASAXS?

• How to use ASAXS?

• How to measure ASAXS?
Exploiting the ability of proteins to bind metals

Essential trace elements:
- Copper (Cu)
- Zinc (Zn)
- Manganese (Mn)
- Molybdenum (Mo)
- Chrome (Cr)
- Selenium (Se)
- Cobalt (Co)
- Fluorine (F)
- Silicon (Si)
- Iron (Fe)
- Iodine (I)

Minerals (bulk elements):
- Magnesium (Mg)
- Calcium (Ca)
- Potassium (K)
- Sodium (Na)
- Chlorine (Cl)
- Phosphorus (P)
- Sulfur (S)

The elements used as cofactors by enzymes. (Waldron et al., 2009).

Irving-Williams series for the metal-protein complexes:
(Irving & Williams, 1953)
Why ASAXS?

<table>
<thead>
<tr>
<th>Element</th>
<th>Ratio of total entries, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>9,94</td>
</tr>
<tr>
<td>Se</td>
<td>5,99</td>
</tr>
<tr>
<td>Fe</td>
<td>5,69</td>
</tr>
<tr>
<td>Mn</td>
<td>2,24</td>
</tr>
<tr>
<td>Br</td>
<td>1,17</td>
</tr>
</tbody>
</table>

~ 25 % of total number of entries

https://www.rcsb.org
Complications of ASAXS on biological samples

• Weak scattering from particles themselves
• Even weaker (~1%) anomalous scattering contribution
• Radiation damage
Complications of ASAXS on biological samples

- Weak scattering from particles themselves
- Even weaker (~1%) anomalous scattering contribution
- Radiation damage
Anomalous small angle X-ray scattering (ASAXS)

• What is ASAXS?

• Why ASAXS?

• How to use ASAXS?

• How to measure ASAXS?
How to work out element-specific scattering?
How to work out element-specific scattering?
How to work out element-specific scattering?
How to work out element-specific scattering?

Non-anomalous contribution

Cross-term

Anomalous contribution

\[ I(q, E) = F_0^2(q) + 2f'(E)F_0(q)v(q) + [f''^2(E) + f'''^2(E)]v^2(q) \]

Sztucki, M, E Di Cola, and T Narayanan. 2011
How to work out element-specific scattering?


How to work out element-specific scattering?


CRYSOL Modeling

```
crysol -en 13474 -el Br *.pdb
```

- **-el** Absorbing element (for ASAXS)
- **-en** Energy (eV), absorption edge (for ASAXS)
- **-ff** File (.csv) with correction factors (for ASAXS)
  format: Energy(eV),fp,fpp

---

**Element 35: Br**

<table>
<thead>
<tr>
<th>Edge</th>
<th>keV</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>13.4737</td>
<td>0.9202</td>
</tr>
<tr>
<td>L-I</td>
<td>1.7820</td>
<td>6.9576</td>
</tr>
<tr>
<td>L-II</td>
<td>1.5960</td>
<td>7.7684</td>
</tr>
<tr>
<td>L-III</td>
<td>1.5499</td>
<td>7.9995</td>
</tr>
</tbody>
</table>

Scattering factors as a function of energy:

- [data file Br.dat](http://skuld.bmsc.washington.edu/scatter/AS_periodic.html)
Changes in Radius of gyration (ASAXS)

\[ R_g = \frac{\int_0^{D_{\text{max}}} r^2 p(r) \, dr}{2 \int_0^{D_{\text{max}}} p(r) \, dr} \]
Changes in Radius of gyration (ASAXS)

\[ R_g = \frac{\int_0^{D_{max}} r^2 p(r)}{2 \int_0^{D_{max}} p(r)} \]
Changes in Radius of gyration (ASAXS)

Frog M-ferritin with cobalt
PDB ID 3KA4

Small Increase of $R_g$ is due to anomalous scattering

Co atoms


CRYSOL Modeling
Anomalous Dispersion of Small-Angle Scattering of Horse-Spleen Ferritin at the Iron K Absorption Edge

BY H. B. STUHRMANN

European Molecular Biology Laboratory, EMBL Outstation at DESY, Notkestrasse 85, D-2000 Hamburg 52, Federal Republic of Germany

(Received 6 May 1980; accepted 7 July 1980)
How it all started?

Fig. 9. Schematic electron density profiles of ferritin and DNA in CaCl₂ solutions. Near the L₂₃ absorption edge of Ca the scattering density decreases from the upper to the lower edge of the black stripes denoting the extreme variations of resonant scattering densities.


How it all started?

“The anomalous scattering $I_a(h)$ of the 4 iron atoms of haemoglobin is not yet accessible, whereas the crossterm $I_{aa}(h)$ could be measured in a synchrotron radiation experiment (Stuhrmann & Notbohm, 1981).”

…”However, the precision of the measurements then has to be improved by one order of magnitude. This will not be entirely impossible in the future”.


“Based on a Gaussian model for the protein electron density, the mean distance from the terbiums to the protein center of mass is determined to be 13.2 Å and is consistent with crystallographic results.”

“Our results demonstrate the usefulness of terbium as an anomalous scattering label and provide criteria to help establish anomalous scattering as a reliable structural technique for proteins in solution.”
Counting Ions around DNA with Anomalous Small-Angle X-ray Scattering

\[ I(q, E) = a f'(E)^2 + b f'(E) + c \]


Absolute Intramolecular Distance Measurements with Angstrom-Resolution
Absolute Intramolecular Distance Measurements with Angstrom-Resolution
Absolute Intramolecular Distance Measurements with Angstrom-Resolution
Absolute Intramolecular Distance Measurements with Angstrom-Resolution

Absolute Intramolecular Distance Measurements with Angstrom-Resolution

Anomalous small angle X-ray scattering (ASAXS)

• What is ASAXS?

• Why ASAXS?

• How to use ASAXS?

• How to measure ASAXS?
Plan your trip to the synchrotron

https://photon-science.desy.de/
Experimental scheme

Sample preparation

• Prepare water
• Prepare solution with target ions
• Prepare BSA + buffer
• Measure “empty cell-water-BSA-Lysozyme-sample” far from the edge.

NB! High concentrations are required
Experimental scheme

Sample preparation
- Prepare water
- Prepare solution with target ions
- Prepare BSA + buffer
- Measure “empty cell-water-BSA-Lysozyme-sample” far from the edge.
- Change energy to the absorption edge
- Measure absorption spectra of the target ions in solution/sample
- Correct energy offset
- Calculate anomalous corrections and target energies
- Change energy to the target point(s)
- Measure “empty cell-water-BSA-Lysozyme-sample 1-sample 2 …”

Beamline setup
- BioSAXS pipeline automatic data reduction procedure

Sample measurement:

Data reduction
Preparing the experiment

Energies to measure: 13383, 13429, 13457, 13466, 13469, 13471 eV

Determined edge position: 13471 (-3 eV)
Keep an eye to the fluorescence

Increased background due to fluorescence
Experiment run
Summary

• ASAXS is a variation of standard SAXS technique

• ASAXS is technically demanding but can provide element-specific information about your sample

• ASAXS can be modeled using computational tools

• New challenging projects from users are highly welcome

https://www.embl-hamburg.de/biosaxs/