BioSAXS @ ESRF

Life after ID14eh3

Current status and future possibilities for BM29

Adam Round
Contents

• BioSAXS on ID14eh3
• Current Status of BM29
  • Data collection
    • BioSAXS sample changer
    • Online SEC
  • ISPyB
    • Sample preparation
    • Experimental logging
    • Data reduction and processing
    • Analysis and Interpretation

• Future possibilities for BM29
Idealised Solution SAS Experiment

Black Box

Solution Scattering Data from Protein of Interest
Experimental Procedure

- Clean
- Water
- Detergent
- Water
- Dry
- Load New Sample/Buffer
- Interlock
- Measure
2nd generation SC (evaluation setup)
2nd generation SC @ ID14eh3

Developed by EMBL-GR, HH and ESRF

- In use since September 2010 at ESRF
- Sister units at: P12@PETRAIII B21@Diamond
- Sample capacity up to 3x96 well plates from 0.2 to 2 mL
- Pipetting and mixing enables remote data collection
SC development

2007
- Manual sample handling
- EMBL-HH 1st gen SC X33@DORIS

2009
- Evaluation Setup ID14-3@ESRF

2010
- 2nd Gen SC ID14-3@ESRF

2012
- 2nd Gen SC BM29@ESRF
- 2nd Gen SC BM29@ESRF P12@PETRAIII B21@Diamond

Sample Volume
Cleaning time
Total cycle time

Reliability
Confidence
Throughput

Now
Data collection protocols on BM29:

**Additives**
- No strict limitations but best to minimise where possible to avoid complications
- Recommended
  - \(< 0.5 \text{ M salt}\)
  - \(< 5\% \text{ glycerol}\)

**Sample Volume**
- Minimum 10 µL per exposure
  - 30 µL recommended
- Minimum 3 concentrations required per construct
  - Approx. 1-20 mg/mL
- Plus buffer measurement for background subtractions

**Temperature**
- Independent temperature regulation for
  - Storage 4-40 degrees C
  - Measurement 4-60 degrees C

**Exposure Time**
- Standard starting time (10 s)
  - Easily modifiable in case of SNR or Radiation issues

**Summary**
- Users recommended to bring total volume of 100 µL of stock (Ideally > 10 mg/mL) solution per construct (plus approx. 1 ml buffer for dilutions/background measurements)
Automated data collection

Beamline data acquisition interface initially used to define samples and experiments

Effective but:
Time consuming
Error Prone
No logging
Improved feedback for experimental preparation
Improved feedback for experimental preparation

**Define Measurements**

Define only the macromolecule's measurement you want to make. This wizard will add buffers' measurement needed for substraction automatically.

<table>
<thead>
<tr>
<th>Single Measurement</th>
<th>Concentration Series</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macromolecules:</td>
<td>Buffer:</td>
</tr>
<tr>
<td>PGK</td>
<td>ATP</td>
</tr>
</tbody>
</table>

**How many unknown concentrations do you have?:**

<table>
<thead>
<tr>
<th>Exposure Temp.:</th>
<th>Vol. To Load (μl):</th>
<th>Transmission (%)</th>
<th>Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>50</td>
<td>100</td>
<td>✓</td>
</tr>
</tbody>
</table>

**Wait Time:**

| Viscosity: |
| low |

**Add**

**Measurements**

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Parameters</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macromo.</strong></td>
<td>Conc. (mg/m)</td>
<td>Buffer</td>
</tr>
<tr>
<td>PGK 1.000</td>
<td>1.000</td>
<td>AMP</td>
</tr>
<tr>
<td>PGK 2.000</td>
<td>2.000</td>
<td>AMP</td>
</tr>
<tr>
<td>PGK 3.000</td>
<td>3.000</td>
<td>AMP</td>
</tr>
<tr>
<td>PGK 1.000</td>
<td>1.000</td>
<td>ATP</td>
</tr>
<tr>
<td>PGK 2.000</td>
<td>2.000</td>
<td>ATP</td>
</tr>
<tr>
<td>PGK 3.000</td>
<td>3.000</td>
<td>ATP</td>
</tr>
</tbody>
</table>
### Estimation of required Volume

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Estimated Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>300.00 µl</td>
</tr>
<tr>
<td>PGK + ATP</td>
<td>150.00 µl</td>
</tr>
<tr>
<td>PGK + common buffer</td>
<td>150.00 µl</td>
</tr>
<tr>
<td>PGK + p38buffer</td>
<td>150.00 µl</td>
</tr>
<tr>
<td>common buffer</td>
<td>300.00 µl</td>
</tr>
<tr>
<td>p38buffer</td>
<td>300.00 µl</td>
</tr>
</tbody>
</table>

**Go to Shipment**
Improved feedback for experimental preparation

<table>
<thead>
<tr>
<th>PGK</th>
<th>ATP</th>
<th>common buffer</th>
<th>p38buffer</th>
<th>1.m Deep Well</th>
<th>2.m 4 x (8 + 3) Block</th>
<th>3.m 96 Well plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGK</td>
<td>1.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>1.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>1.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>1.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>2.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>2.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td>C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>2.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>2.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>3.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>3.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td>3.000 mg/ml</td>
<td>50.00 µl</td>
<td>2</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PGK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Improved feedback with ISPyB
Automatic acquisition = big data

Preliminary analysis is required to obtain feedback on sample behaviour and data quality to ensure experimental aims are met.

Without automation significant experimental time can be lost to data reduction.

Online analysis is essential to help users work better at the beamline.
Data Processing: ATSAS tools in EDNA

- **Image Processing**
  - Radial Integration PyFAI
  - Frame merging and Radiation damage detection

- **1D data reduction**
  - Compare buffers to determine the "Best"
  - Subtract "Best" Buffer from protein curve

- **Curve reduction**
  - Group all protein curves from same construct
  - Compare curves

- **Curve Analysis**
  - AutoRg
  - DATGNOM
  - DAMMIF

- 1D curve
- Protein Curve
- Idealized curve Indication of quality (similarity of all curves)
- Ab-initio Models Model independent Parameters
## Improved feedback with ISPyB

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>Concentration</th>
<th>Scattering</th>
<th>Frames (Averaged/Total)</th>
<th>Guinier</th>
<th>Gnom</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>taHEFD33</td>
<td>14.00 mg/ml</td>
<td></td>
<td></td>
<td>4.75 nm</td>
<td>90.78</td>
<td>154.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.51</td>
<td>4.94</td>
<td>77.1 - 102.8</td>
</tr>
<tr>
<td>taHEFD38</td>
<td>7.00 mg/ml</td>
<td></td>
<td></td>
<td>3.97 nm</td>
<td>71.21</td>
<td>112.54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
<td>3.91</td>
<td>56.3 - 75.0</td>
</tr>
<tr>
<td>taHEFD33</td>
<td>3.50 mg/ml</td>
<td></td>
<td></td>
<td>3.37 nm</td>
<td>59.53</td>
<td>95.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.53</td>
<td>3.44</td>
<td>47.6 - 63.6</td>
</tr>
<tr>
<td>taHEFD33</td>
<td>1.25 mg/ml</td>
<td></td>
<td></td>
<td>3.23 nm</td>
<td>78.16</td>
<td>90.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.59</td>
<td>3.26</td>
<td>45.2 - 60.2</td>
</tr>
<tr>
<td>taHEFD33</td>
<td>0.61 mg/ml</td>
<td></td>
<td></td>
<td>3.16 nm</td>
<td>78.86</td>
<td>84.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.75</td>
<td>3.20</td>
<td>42.2 - 59.2</td>
</tr>
</tbody>
</table>
Improved feedback with ISPyB
Improved feedback with ISPyB
Improved feedback with ISPyB
Summary of SC experiments

Easy, fast, reliable, fully automated

Online results and immediate feedback for better experiments

Pipetting and mixing enable remote experiments

Sample characterisation and preparation are key

Mixtures, buffer mismatches, aggregation will destroy any chance of finding an answer

Online purification and biophysical characterisation have been implemented to help overcome these issues
Addition of Online SEC
Addition of Online SEC

4-way valve
Fast automatic switching between sample changer and HPLC modes

Mounted in Fridge
Operation at 4 °C or 20 °C
SEC data collection protocols:

Additives
- No strict limitations but best to minimise where possible to avoid complications
- Recommended
  - < 0.5 M salt
  - < 5% glycerol

Sample Volume
- Minimum 50 µL per injection
  - 100 µL recommended
  - Approx. 5 mg/mL
- Plus 0.5 L buffer per injection and equilibration

Temperature
- SEC operation at 4 or 20 degrees C

Exposure Time
- Standard 1 FPS (5 FPS max)
  - S200 column ~1 hour
  - Increase column ~10 mins

Summary
- Users recommended to bring own column(s)!
  - 100 µL of stock protein per run
  - minimum 1 L buffer

Buffers can be prepared onsite in EMBL user support lab upon request
Online SEC in ISPyB

Automatic output of 1D files:
Merged buffer
Subtractions
Merged file
(Found using peak of I0 and matching Rg)
Online SEC in ISPyB
Online SEC

Biophysical characterization

Summary of online SEC experiments

Easy, automated switching to maximise efficiency

Online results and immediate feedback for better experiments

Online sample characterisation
  DLS
  RALS
  UV
  RI

Mixtures can be separated

Buffer mismatches can be overcome

Aggregation can be removed
Future of online SEC experiments

Online SEC is increasing in popularity and requires improved efficiency

New columns!
   Superdex 200 increase columns
   enable injections every 10 minutes

Offline system for equilibrating columns in parallel

Upgraded online system with multiple columns
   Multiplexing

Automation of:
   Injection sequences
   Buffer switching
   Data acquisition based on peak sensing?
Current developments

ISPyB

Ongoing addition of logic for crosschecking
Intuitive feedback on data quality within GUI
Automated optimisation of data acquisition
Link to HTX CRIMS database
Automated sample preparation

Smaller beam (scatterless pinhole)
Optimised for smaller capillaries
Minimise sample volume requirements
Enable microfluidics

Redesign SEU
SC compatible Microfluidics
SC compatible Microfluidics
SC compatible Microfluidics

Potential use in:

- Mixing studies
- Time resolved measurements (seconds)
- Dynamic screening of buffer conditions
Future

Redesign SEU

<table>
<thead>
<tr>
<th>New multicolour light source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photo activated / sensitive</td>
</tr>
<tr>
<td>Wide angle scattering</td>
</tr>
<tr>
<td>Extended s range</td>
</tr>
<tr>
<td>Additional information, in-line</td>
</tr>
<tr>
<td>DLS</td>
</tr>
<tr>
<td>Spectroscopy?</td>
</tr>
<tr>
<td>UV, fluorescence, etc.</td>
</tr>
</tbody>
</table>

Facilitate Microfluidics

On axis camera / lighting

Modular design? (increased flexibility)
Summary of current operation

Automation of data acquisition and analysis for both Static (SC) and online SEC (HPLC) gives:

- Reliability
- Confidence
- Independence
- High Throughput
- Efficiency

Easy switching between experiments is required to maintain efficiency and reliability

Frequent training courses in data acquisition and analysis (EMBO, Hercules, BAG training)
SAS validation Workshop
Commitment to Data Quality and integrity

28 Experts in Neutron and X-ray scattering
From 15 institutes in 7 different countries
Acknowledgments