Inline purification methods

Joint application SAXS & FPLC & Light Scattering (SEC-SAXS/SLS)

Melissa Graewert
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The odd one out
What is SEC-SAXS, why does one need this?
What information is gained with light scattering?

How is SEC-SAXS done?
- Experiment and data analysis
- Sasha: Chromixs
- Experiment

X-ray beam

$I(s)$
- Experiment

X-ray beam

1 %
0.1 %
0.01 %
pure
What is SEC-SAXS/SLS, why does one need this?

- Polydisperse samples

- Aggregates

- Intrinsic property of the sample e.g. monomer-dimer-oligomer equilibrium or incomplete formation of complexes
Monomer (65 kDa)

Dimer (122 kDa)
research papers

Liquid-chromatography-coupled SAXS for accurate sizing of aggregating proteins

Elizabeth Mathew, Ahmed Mirza and Nick Menhart*

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Combined sampler robot and high-performance liquid chromatography: a fully automated system for biological small-angle X-ray scattering experiments at the Synchrotron SOLEIL SWING beamline

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Information extracted from the elution profile of an initially polydisperse solution of commercial BSA. Column: SHODEX 402.5-4F. Flow rate: 150 µl min$^{-1}$. Injected volume: 5 µl at 88.8 g l$^{-1}$. (a) Chromatogram of the elution profile. The complex profile indicates the presence of several species. The main peak, at 17.04 min, corresponds to BSA monomer.
The application of hybrid pixel detectors for in-house SAXS instrumentation with a view to combined chromatographic operation

Gareth S. A. Wright, a,‡ Hyun Chul Lee, a,‡ Clemens Schulze-Briesè, b J. Günter Grossmann, a Richard W. Strange a and S. Samar Hasnain a,‡

The SAXS instrument at the Barkla Laboratory of Biophysics. The set-up includes a Dectris PILATUS 300K-20Hz detector, three pin-hole optics and Rigaku FR-E+ Superbright X-ray generator.
- SEC-SAXS mode
- SEC-SAXS/MALLS mode
Light absorbance: $\sim c, \varepsilon$
Light absorbance: $\sim c, \varepsilon$

Refraction: $\sim c, \frac{dn}{dc}$

dual cell, deflection design
Light absorbance: \( \sim c, \varepsilon \)

Scattering: \( \sim c, \text{dn/dc, MW} \)

Refraction: \( \sim c, \text{dn/dc} \)
- SEC-SAXS/MALLS mode
X-ray scattering:

\[ \text{MW}_{\text{dimer}} = 131\, \text{kD} \]
\[ \text{MW}_{\text{monomer}} = 60\, \text{kD} \]
\[ \text{RG}_{\text{dimer}} = 4.1\, \text{nm} \]
\[ \text{RG}_{\text{monomer}} = 2.8\, \text{nm} \]
X-ray scattering:

A) Comparison of theoretical to experimental data

B) Transforming data into real space

C) Ab initio reconstruction

DAMMIN

DAMMIF

GASBOR

SASBDB – BSA Monomer, SASDF99
SASBDB – BSA Dimer, SASDFR8

MW_{dimer} = 131k
RG_{dimer} = 4.1nm

MW_{monomer} = 60 kD
RG_{monomer} = 2.8nm
Static & Dynamic Laser Light Scattering:

MW\textsubscript{dimer} = 132kD
RH\textsubscript{dimer} = 4.8nm

MW\textsubscript{monomer} = 64kD
RH\textsubscript{monomer} = 3.6nm

X-ray scattering:

MW\textsubscript{dimer} = 131kD
RG\textsubscript{dimer} = 4.1nm

MW\textsubscript{monomer} = 60 kD
RG\textsubscript{monomer} = 2.8nm
Automated Pipeline for Purification, Biophysical and X-Ray Analysis of Biomacromolecular Solutions

Melissa A. Graewert¹, Daniel Franke¹, Cy M. Jeffries¹, Clement E. Blanchet¹, Darja Ruskule¹, Katja Kuhle³, Antje Flieger³, Bernd Schäfer³, Bernd Tartsch³, Rob Meijers⁴ & Dmitri I. Svergun¹
- **M.O.S.E.S. (Microsplitting for **Online Separation**, Extended characterization and SAXS analysis)**

Phospholipase B of *Legionella pneumophila* (Lpn PlaB)
- **M.O.S.E.S.** (Microsplitting for **Online Separation**, Extended characterization and SAXS analysis)
$\text{MW}_{\text{RALS}} = 230 \pm 15 \text{ kD}$

$\text{MW}_{I(0)} = 225 \pm 15 \text{ kD}$

$\text{MW}_{\text{Vol}} = 170 \pm 30 \text{ kD}$

$\text{MW}_{\text{DAMMIF}} = 203 \pm 30 \text{ kD}$

$\text{MW}_{\text{SEC}} \sim 100 \text{ kD}$
MW_{RALS} = 230 \pm 15 \text{ kD}

MW_{l(0)} = 225 \pm 15 \text{ kD}

MW_{Vol} = 170 \pm 30 \text{ kD}

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MW_{Vol} = 170 \pm 30 \text{ kD}

MW_{DAMMIF} = 203 \pm 30 \text{ kD}

MW_{SEC} \sim 100 \text{ kD}
Simultaneous Data Collection
**M.O.S.E.S. (Microsplitting for Online Separation, Extended characterization and SAXS analysis)**

Electron micrograph of *Legionella pneumophila* www.wikimedia.org

- Lipolytic active monomeric PlaB
- Activation Via dimeric state
- Self protection through inactive tetrameric PlaB

- Host pathogen
Batch mode or SEC-SAXS mode

- solubilized protein vs free micelles
- aggregate vs monodisperse sample
- oligomer vs monomer
- complex vs subunits
What is **SEC-SAXS/SLS**, why does one need this?

- Alternative strategy to study (moderately) polydisperse samples

How is **SEC-SAXS** done

- Required sample amounts: at least 50 ul of > 5mg/ml
- Sufficient buffer
- Optimize your SEC run
- If possible collect batch sample as well

- Check for radiation damage, add 3% glycerol (if feasible)