BioSAS in Grenoble

What can be done and how to do it

Adam Round



Contents

- What can you learn from solution BioSAS
 - Basics of what can be obtained
 - Extending interpretation using complementary information
 - Examples
- How to collect data
 - Automation
 - Data collection and analysis
 - Feedback



Idealised Solution SAS Experiment



Neutron or X-ray source



What can we learn from solution SAXS

- Model independent parameters:
- Size! Rg, Dmax, Volume and MM estimates. Basic shape (Extended or Globular)
- Behaviour in different buffer conditions:
- To assess
 - effects of different buffer formulations
 - if interparticle effects or flexibility could be preventing crystallisation (find optimum conditions)
- Dynamic investigations under physiological conditions: Conformational changes with temperature, pH, binding etc.



What can be done if you know more!



Experimental X-ray scattering of the PYR1 protein in solution in the presence of 1mM (+) ABA.

Scattering curves for possible ensembles were calculated.

Only the curve for ensembles AB/CD produced a good fit to the experimental data (χ =0.72)

SAXS demonstrated that the AB ensemble corresponds to the biologically relevant form found under physiological conditions.



J Santiago et al. Nature 462, 665-668 (3 December 2009)

Functional study of Phosphoglycerate Kinase (PGK)

- Catalyses 7th step in Glycolosis
- It activates L-nucleoside analogue drugs in the treatment of AIDS and Hepititis
- Implicated in oncogenesis
- Drug target for obligate anaerobic pathogens



Phosphoglycerate Kinase (PGK)



Cliff, et al. (2010) J. Am. Chem. Soc., 132, 6507-6516

SAXS analysis of PGK: APO (open) from significantly more open



DEN refinement used to allow flexibility in the linkers to overcome clashes and provide an atomic model



SAXS analysis of PGK: Shows larger domain movement than expected



50° rotation from open to closed



SAXS analysis of PGK: Shows the domain movement during catalysis





Refinement of Rigid Domains



DEN refinement using SAXS and MX data allows visualization of domain movement



Adding Missing Linkers

Modelling of proteins from high resolution fragments/constructs replacing missing portions BUNCH or CORAL



High resolution structure from X-ray crystallography showed missing portions due to disorder

Questions:

is it hexamer or dodecamer in solution?

Where are the missing residues



Ab-initio Modelling

Modelling of proteins with no prior information Build complex models from uniform blocks



like LEGO but we use Dummy Atoms

Acts as a placeholder for, but does not resemble, a real atom

Occupies a known position in space

Has a known scattering pattern (Solvent or Particle)



Ab-initio Modelling

Modelling of proteins with no prior information Build complex models from uniform blocks



Must be Compact

Must NOT be disconnected

Must NOT be Loose



Ab-initio Modelling

With multiple data sets multiphase models can be produced

Points in the search grid can be assigned to Protein 1 Protein 2 or solvent





MONSA



SAXS analysis of yeast Arc1p-complex

P(r) Plot

- 1) MetRS:Arc1p:GluRS + tRNA
- 2) MetRS:Arc1p:GluRS
- 3) GluRS:Arc1p
- 4) MetRS:Arc1p
- 5) GluRS
- 6) MetRS
- 7) Arc1p





SAXS analysis of yeast Arc1p-complex

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- 2) MetRS:Arc1p:GluRS
- 3) GluRS:Arc1p
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- 7) Arc1p



SAXS analysis of yeast Arc1p-complex





SAXS analysis of yeast Arc1p-complex





Idealised Solution SAS Experiment



Neutron or X-ray source























Idealised Solution SAS Experiment



Neutron or X-ray source



Experimental Procedure





<u>Clean</u> Water Detergent Water Dry

Load New Sample/Buffer

Interlock Measure



SC development

Sample Volume Cleaning time Total cycle time





Reliability

Confidence

Sample Preparation

In solution SAXS we observe the Average

Average

Monodisperse



Mixture







Shape reconstruction requires: **MONODISPERSE!**

samples in solution

Pure protein (>90%)! In only 1 oligomeric state! NO aggregation! Free from interparticle effects!

Before going to the beamline users are encouraged to use:

HPLC/FPLC purification MALS/DLS Analytical ultra centrifugation



Online SEC GH3-12





open closed

 $\Delta R_{G} = 0.35 \text{ nm}$



Round A, Brown E, Kapp U, Westfall CS, Jez JM, Zubieta C. (2013) Acta D

Online SEC GH3-12





open closed

 $\Delta R_{G} = 0.35 \text{ nm}$

Aggregation! Standard experiment gives no answer



Round A, Brown E, Kapp U, Westfall CS, Jez JM, Zubieta C. (2013) Acta D

Current status of BM29: New online SEC



Malvern currently in place but will be moved to prep lab



Shimadzu installed over Easter and is already available to users







Online SEC in ISPyB





Online SEC









Biophysical characterization



Online SEC





ATP/AMP: closed state APO: flexibility

Round A, Brown E, Kapp U, Westfall CS, Jez JM, Zubieta C. (2013) Acta D



Current status of data collection:

Temperature

- Data acquisition between 4 and 60 C
- SEC operation at 4 or 20 degrees C

Exposure Time

- Standard 1 FPS (10 frames for Static)
 - S200 column ~1 hour (3600 frames)
 - Increase column ~10 mins (600 frames)

Sample Volume

- Minimum recommended 30 µL per measurement
 - Approx. 5 mg/mL
 - 100 μL stock recommended
 - for static and SEC





Automated valve

To switch between SEC and Static modes

Gives users control Safe and reliable switching Maximises efficiency cleaning between SEC runs



SC compatible Microfluidics: First results

Proof of Principle achieved



















Experimental preparation and efficient use of beamtime facilitated through ISPyB



Improved feedback for experimental preparation

OSAXS Experime	ent Design	er											
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50.00 µl 100 %

yes

low

4.00 c

3.000

AMP

PGK



REMOVE

Improved feedback for experimental preparation

IIOSAXS Experiment Designer

×

Define Measurements

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

Single Measurem	ent Concentration Series				
Macromolecules:	PGK 👻	Buffer:	ATP 💌		
How many unknow	v concentrations do you have?	:	3		
Exposure. Temp.:	4	Vol. To Load (µl):	50	Transmission (%):	100
Wait Time:	0	Viscosity:	Iow 🗸	Flow:	
			Add		

Specimen Parameters Comments Macromo. Conc. (mg/ml) Buffer Exp. Temp. Vol. Load Trans. Wait T. Flow Viscosity	
Macromo. Conc. (mg/ml) Buffer Exp. Temp. Vol. Load Trans. Wait T. Flow Viscosity	
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PGK 2.000 AMP 4.00 c 50.00 μl 100 % yes low	REMOVE
PGK 3.000 AMP 4.00 c 50.00 μl 100 % yes low	REMOVE
PGK 1.000 ATP 4.00 c 50.00 μl 100 % yes low	REMOVE
PGK 2.000 ATP 4.00 c 50.00 μl 100 % yes low	REMOVE
PGK 3.000 ATP 4.00 c 50.00 μl 100 % yes low	REMOVE



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USR





Automated processing is being extended:

Additional information such as PDB's of possible conformations



Created by CRYSOL v. 28 on 23-Dec-2014 11:06:02

Batch: crysol -err -cst /tmp/biosaxsworkflows/2014-12-23_11-06-01/pdb3n9g.pdb /data/pyarch/bm29/opd29/2604/1d/Data_050_sub.dat Data file name: /data/pyarch/bm29/opd29/2604/1d/Data_050_sub.dat Model: /tmp/biosaxsworkflow Rg: 24.90 Run: 00 Chi: 14.01



Automated processing is being extended:

Additional information such as PDB's of possible conformations

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ILINE HELP: To be updated			
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The black box should not be scary It should be reasuring that it is accessible



Home source



The black box should not be scary It should be reasuring that it is accessible







Automated data acquisition

With feedback





Acknowledgments













