Solution SAXS in Grenoble

What can be done and how to do it

Adam Round



Contents

- What is Solution SAS
- What can you learn from solution SAS
 - Basics of what can be obtained
 - Analytical analysis
 - Modelling
 - Examples
- How to collect data
 - Automation
 - Data collection and analysis



Solution scattering data collection



Thanks to A. Kikhney for this slide

Experimental Equipment: The Basics







Source of Radiation Neutrons / X-rays

Sample Holder

Detector



Calibration

Conversion 2D to 1D

All pixel positions with equal 20 are averaged

θ converted to s based on
 known beamline parameters
 for geometry and λ

Data are scaled for intensity Based on measurement of intensity of direct beam

Performed automatically by Beamline software based on calibration by LC's



EMB

10,000

1,000

100

10



Intensity is proportional to: The number of interactions between



Scattered particles (Neutrons/X-rays) Scattering object (Biological Macromolecule)







 \mathbf{O}























Scattering intensity

Is proportional to: The number of interactions between Scattered particles (Neutrons/X-rays) and Scattering object (Biological Macromolecule)

> **Therefore if:** Neutron/X-ray flux is normalised

> > And

The observed scattering intensity is corrected for the number of scatterers (proportional to concentration)

The resulting measured intensity is proportional to the <u>size</u> and contrast of the scattering particles under investigation

Warning! Concentration measurements might not be accurate



Intensity Calibration





Radius of Gyration and Zero Angle Intensity





Porod Analysis in Primus gives excluded

volume





Distance Distribution P(r) Function

Calculated by GNOM give the Dmax and the input file required for Ab-initio modeling



Indirect Fourier Transform!



1D curve and P(r) Function are effected by shape



Indirect Fourier Transform!



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 scattering which shows structural effects (find optimum conditions – monitor behaviour)
- Dynamic investigations under physiological conditions: Conformational changes with temperature, pH, binding etc.



Form Factor fitting of basic shapes

- 1. Homogeneous sphere
- 2. Spherical shell
- 3. Spherical concentric shells
- 4. Particles consisting of spherical subunits
- 5. Ellipsoid of revolution
- 6. Tri-axial ellipsoid
- 7. Cube and rectangular parallelepipedons
- 8. Truncated octahedra
- 9. Faceted Sphere
- 10. Cube with terraces
- 11. Cylinder
- 12. Cylinder with elliptical cross section
- 13. Toroid
- 14. Infinitely thin rod
- 15. Infinitely thin circular disk





Form Factor fitting of polymers

- 1. Flexible polymers with Gaussian statistics
- 2. Polydisperse flexible polymers with Gaussian statistics
- 3. Flexible ring polymers with Gaussian statistics
- 4. Flexible self-avoiding polymers
- 5. Polydisperse flexible self-avoiding polymers
- 6. Semi-flexible polymers without self-avoidance
- 7. Semi-flexible polymers with self-avoidance:
- 8. Polyelectrolyte Semi-flexible polymers with self-avoidance:
- 9. Star polymer with Gaussian statistics
- 10. Polydisperse star polymer with Gaussian statistics
- 11. Regular star-burst polymer (dendrimer) with Gaussian statistics
- 12. Regular comb polymer with Gaussian statistics
- 13. Arbitrarily branched polymers with Gaussian statistics
- 14. Arbitrarily branched semi-flexible polymers
- 15. Arbitrarily branched self-avoiding polymers



EMB

Adapted from talk of JS Pederson

Combining Form Factors



Self-assembling peptides forming nanodiscs





S. R. Midtgaard, M. C. Pedersen et al. Soft Matter, 2014, 10, 738-752



Ab-initio Modelling

Modelling of particles 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 particles 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 Different phases





MONSA

Ab-initio Example



SAXS data + crystal and NMR structures show the Nterminal domains of mouse cMyBP-C form an extended structure with a defined disposition of the modules

(C)



However mixing with actin results in a dramatic increase in scattering signal due to the formation of a large, rod-shaped assembly

Thanks to J Trewhella for these slides



Whitten, Jeffries et al. (2008) PNAS 105, 18360

Ab-initio Example



Thanks to J Trewhella for these slides

Whitten, Jeffries et al. (2008) PNAS 105, 18360



Thanks to J Trewhella for these slides

Whitten, Jeffries et al. (2008) PNAS 105, 18360



0.81

Ab-initio Example



Thanks to J Trewhella for these slides

EMBL

Whitten, Jeffries et al. (2008) PNAS 105, 18360

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

Sample Preparation

In solution SAXS we observe the Average

Average

Monodisperse



Mixture









Online SEC in ISPyB





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											
Define Measur Define only buffers' m	rements y the macr easureme	omolecul ent neede	e's m d for	easuremen substractio	nt you want to m on automatical	iake. This w ly.	izard will a	dd					
Single Measu	rement	Concent	tratio	n Series									
Macromolecul How many un Exposure. Ter	les: PGK know con np.: 4	centratio	✓ ns do	you have?:	Buffer: : Vol. To Load (АМР 3 µl): 50	×]	Tra	unsmissio	on 100	~	
Wait Time:	Time: 0				Viscosity:	low Add	low 🗸). w:			
Measurements	5												
Specimen							Paramete	rs					
Macromo.	Conc	. (mg/ml)		Buffer	Exp. Temp.	Vol. Load	Trans.	Wait T.	Flow	Viscosity	Comments		
PGK	1.00	00		AMP	4.00 c	50.00 μι	100 %		yes	low			REMOVE
PGK	2.00	00		AMP	4 00 c	50 00 ut	100 %		ves	low			BEHOVE

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	
PGK 1.000 AMP 4.00 c 50.00 μl 100 % yes low	REMOVE
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



Collapse buffer Specimen Collapse buffer Collapse buffer Collapse buffer Collapse buffer Macromo. Conc. (mg/m) Buffer Exp. Temp. Vol. Load Trans. Wait T. Flow Viscosity Time Comments Image: Specimen 0.03 20.00 c 100.0 100 % yes Low DOHE 66.47.07 mb buffer Image: Specimen 0.03 20.00 c 100.0 100 % yes Low DOHE 66.48.23 pm buffer Image: Specimen 0.03 20.00 c 100.0 100 % yes Low DOHE 06.49.41 pm buffer Image: Specimen 0.03 20.00 c 100.0 100 % yes Low DOHE 06.52.59 pm buffer Image: Specimen 0.33 20.00 c 100.0 100 % yes Low DOHE 0652.40 pm buffer Image: Specimen 0.53 20.00 c 100.0 100	Overv	iew	M	leas	uren	nent	s	An	alysi	s	10	View	er																						
Image: Speciment Speciment Exp. Temp. Vol. Load Trans. Wat T. Flow Viscosity Status Time Comments Image: Macromo. Conc. (mg/m) Butfer Exp. Temp. Vol. Load Trans. Wat T. Flow Viscosity OHE 06149 23 pm Littler Image: Macromo. D33 20.00 c 100.0 100 % yes Low DOHE 06149 23 pm Littler Image: Macromo. D33 20.00 c 100.0 100 % yes Low DOHE 06149 23 pm Littler Distler Image: Macromo. 06149 23 pm Littler Distler Image: Macromo. 06149 23 pm Littler Distler Image: Macromo. Distler Image: Macromo. Distler Distler Image: Macromo. Distler Distler		Collapse buffers																																	
Macromo. Conc. (mg/mt) Buffer Exp. Temp. Vol. Load Trans. Wat T. Flow Viscoss Status Itme Continents ta4EFD33 14.000 D33 20.00 c 100 % yes Low D0HE 0647.07 pm buffer ta4EFD33 14.000 D33 20.00 c 100 % yes Low D0HE 0649.41 pm buffer ta4EFD33 7.000 D33 20.00 c 100 % yes Low D0HE 0649.41 pm buffer ta4EFD33 7.000 D33 20.00 c 100 % yes Low D0HE 065954 pm [J1ahef d33 truncation] ta4EFD33 3.500 D33 20.00 c 100 % yes Low D0HE 0659.54 pm [J1ahef d33 truncation] ta4EFD33 1.250 D33 20.00 c 100 % yes Low D0HE 0659.54 pm [J1ahef d33 truncation] ta4EFD33					ę	Speci	imen							Parameters																					
Image: marker D33 20.00 c 100 mm 100 mm yes Low D0HE 0647:07 pm jutfer Image: marker D33 14.000 D33 20.00 c 150 mm 100 mm yes Low D0HE 0649:12 pm [1]tabel d33 truncation Image: marker D33 7.000 D33 20.00 c 100 mm yes Low D0HE 0649:12 pm 101tfer 100 mm yes Low D0HE 0649:12 pm 101tfer 100 mm yes Low D0HE 0650:54 pm [2]tabel d33 truncation Image: marker D33 3,500 Image: marker D33 20.00 c 100 mm yes Low D0HE 0652:9 pm Ig1tabel d33 truncation Image: marker D33 3,500 Image: marker D33 20.00 c 100 mm 100 mm yes Low D0HE 0655:62 pm Ig1tabel d33 truncation Image: marker D33 1,250 Image: marker D33 20.00 c 100 mm 100 mm yes Low D0HE 0655:62 pm Ig1tabel d33 truncation Image: marker D33 0,610 Image: marker D33 20.00 c 100 mm		Macromo. Conc. (mg/ml) Buffer			Exp. Temp. Vol. Load Trans. Wait T. Flow Viscosity						iy S	tatus	5 1	ime			Col	nmer	nts																
Image: tableFD33 14,000 D33 20.00 c 150.0 100 % yes Low DONE 0648.23 pm [1] tabef d33 runcation Image: tableFD33 7,000 D33 20.00 c 100.0 100 % yes Low DONE 0648.23 pm [2] tabef d33 runcation Image: tableFD33 7,000 D33 20.00 c 100.0 100 % yes Low DONE 065.95 pm buffer Image: tableFD33 3,500 D33 20.00 c 100.0 100 % yes Low DONE 0652.95 pm buffer Image: tableFD33 3,500 D33 20.00 c 100.0 100 % yes Low DONE 0654.40 pm buffer Image: tableFD33 1,250 D33 20.00 c 100.0 100 % yes Low DONE 0655.56 pm [4] tabef d33 runcation Image: tableFD33 0,610 D33 20.00 c 100.0 100 % yes Low DONE 0655.56 pm [5] tabef d33 runcation Image: tableFD33 0,610 D33 20.00 c <										D33	3		20.0	0 с	1	.00.0		100	96			yes		Low	D	ONE	0	6:47	:07 p	om	but	fer			
Image: Control in the term of term of the term of term		taHE	FD33		1,	4.00	00			D33	3		20.0	0 с	1	.50.0		100	96			yes		Low	D	ONE	0	6:48	:23 p	om	[1]	tahet	f d33	trunc	ation
Image: tallEFD33 7.000 Image: D33 20.00 c 90.00 µl 100 % yes Low D0HE 0650.54 µm [2] tallef d33 truncation Image: Tallef D33 3.500 Image: Tallef D33 20.00 c 100 0, 100 % yes Low D0HE 0650.54 µm Lighter d33 truncation Image: Tallef D33 3.500 Image: Tallef D33 20.00 c 90.00 µl 100 % yes Low D0HE 0653.25 µm [3] tallef d33 truncation Image: Tallef D33 1.250 Image: Tallef D33 20.00 c 90.00 µl 100 % yes Low D0HE 0655.56 µm [4] tallef d33 truncation Image: Tallef D33 1.250 Image: Tallef D33 20.00 c 100 0, 100 % yes Low D0HE 0655.56 µm [4] tallef d33 truncation Image: Tallef B4EFD33 0.610 Image: Tallef D33 20.00 c 100 0, 100 % yes Low D0HE 0655.28 µm [5] tallef d33 truncation Image: Tallef D33 0.610 Image: Tallef D33 20.00 c 100 0, 100 % yes Low D0HE 0659.43 µm<										D33	3		20.0	0 с	1	.00.0		100	96			yes		Low	D	ONE	0	6:49	:41 p	om	but	fer			
Image: mark to make the mark to mark tothand to mark to mark to mark to mark to mark to mark t		taHE	FD33		7.	000)			D33	3		20.0	0 с	9	0.00	μ	100	96			yes		Low	D	ONE	0	6:50	:54 p	om	[2]	tahet	f d33	trunc	ation
Image: table FD33 3,500 Image: table FD33 20,00 90,00 µµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµµ										D33	3		20.0	0 с	1	.00.0		100	96			yes		Low	D	ONE	0	6:52	:09 p	om	but	fer			
Image: Construct of the c		taHE	FD33		З.	500)			D33	3		20.0	0 с	g	0.00	μ	100	96			yes		Low	D	ONE	0	6:53	:25 p	om	[3]	tahei	f d33	trunc	ation
tableFD33 1.250 D33 20.00 c 90.00 µl 100 % yes Low D0HE 06:55:56 pm [4] tablef d33 truncation tableFD33 0.610 D33 20.00 c 100.0 100 % yes Low D0HE 06:55:56 pm [5] tablef d33 truncation tableFD33 0.610 D33 20.00 c 100.0 100 % yes Low D0HE 06:52:28 pm [5] tablef d33 truncation C D33 20.00 c 100.0 100 % yes Low D0HE 06:59:43 pm buffer C D33 20.00 c 100.0 100 % yes Low D0HE 06:59:43 pm buffer C Participan D33 20.00 c 100.0 100 % yes Low D0HE 06:59:43 pm buffer C Participan D33 20.00 c 0.0.0 100 % yes Low D0HE 06:59:43 pm buffer 1 Participan D3 2-4 x (8 + 3) Block A 0 0 0 0<0 0 0<0 0 0<0 0 0<0 0 0<0 0										D33	3		20.0	0 с	1	.00.0		100	96			yes		Low	D	ONE	0	6:54	:40 p	om	but	fer			
Image: Control (Control (Contro) (Control (Control (Contro) (taHE	FD33		1.	250)			D33	3		20.0	0 с	g	0.00	μΙ	100	96			yes		Low	D	ONE	0	6:55	:56 p	om	[4]	tahei	f d33	trund	ation
Image: HerD33 0.610 D33 20.00 c 90.00 µl 100 % yes Low DONE 06.58:28 pm [5] tahef d33 truncation Image: Constraint of the table of table o										D33	3		20.0	0 с	1	.00.0		100	96			yes		Low	D	ONE	0	6:57	':11 p	om	but	fer			
Image: Control in the part of the p		taHE	FD33		0.	610)			D33	3		20.0	0 с	9	0.00	μΙ	100	96			yes		Low	D	ONE	0	6:58	:28 p	om	[5]	tahei	f d33	trunc	ation
I-Deep Well I-OPE										D33	3		20.0	0 с	1	.00.0		100	96			yes		Low	D	ONE	0	6:59	:43 p	om	but	fer			
I Deep Well I O O																																			
Pready 1 - Dep Well 2 - 4 x (8 + 3) Block 3 - 9 C Well plate A 0 </th <th></th>																																			
1. Deep Well 2. 4 x (8 + 3) Block 3. 96 Well plate A O	٢	Ready	/																																
A O	1 - De	ep W	ell										2-4×	(8+	3)B	lock								3 - 96 W	/ell r	late									
0 0	A 0	0	0	0	0	0	0	0	0	0	0	0	A 0	0	0	0	0	0	0	0	0	0	0	A O	0	0	0	0	0	0	0	0	0	0	0
0 0	вО	0	0	0	0	0	0	0	0	0	0	0												вО	0	0	0	0	0	0	0	0	0	0	0
0 0	° 0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	с О -	0	0	0	0	0	0	0	0	0	0	0
1 1	D 0	0	0	0	0	0	0	0	0	0	0	0		Ũ	Ũ	Ũ	Ũ	Ũ	Ũ	Ũ	Ŭ	0	Ŭ	DO	0	0	0	0	0	0	0	0	0	0	0
0 0	вO	0	0	0	0	0	0	0	0	0	0	0		_	_	_	_	-	_	_	~	~	~	ЕO	0	0	0	0	0	0	0	0	0	0	0
0 0	F O	0	0	0	0	0	0	0	0	0	0	0	c O	0	0	0	0	0	0	0	0	0	0	FО	0	0	0	0	0	0	0	0	0	0	0
	в О	0	0	0	0	0	0	0	0	0	0	0												вО	0	0	0	0	0	0	0	0	0	0	0
	нQ	Q	Q	Q	Q	Q	ρ	Q	Q	õ	Ö	ō	D P	2	9	9	<mark>.</mark>	o	ρ	P	9	Q	Õ	нQ	Q	Q	Q	Q	Q	ρ	ρ	Q	Q	Ö	Ō



Overview N	Measurements	Analysis 1D Viewe	r										
Maaramalaaula	Concentration	Septtering			Guinie	r			Gnom		Porod		
Macromolecule	concentration	scattering	Frames (Averagedrio(a))	Rg (nm)	Points	Quality (%)	l(0)	Rg (nm)	Total	Dmax (nm)	Volume (nm ³)	MM (kD) Vol. est	
•													
taHEFD33	14.00 mg/ml		D33 (10 of 10)	4.75 nm	19 - 37 (18)	83.95	90.78	4.94 nm	0.51	24.09	154.27	77.1 - 102.8	
			taHEFD33 (10 of 10)				±0.88492						
			D33 (1 of 10)										
		4111grand											
taHEFD33	7.00 mg/ml		D33 (1 of 10)	3.97 nm	12 - 42 (30)	92.14	71.21 ±4.1859e-2	3.91 nm	0.44	13.90	112.54	56.3 - 75.0	
			taHEFD33 (5 of 10)										
		1	033 (50110)										
taHEFD33	3.50 mg/ml	- Million - Mill	D33 (5 of 10)	3.37 nm	50 - 77 (27)	72.77	59.53 ±6.55654	3.44 nm	0.53	11.81	95.25	47.6 - 63.5	
	_		taHEFD33 (10 of 10)										
			D33 (10 of 10)										
taHEFD33	1.25 mg/ml		D33 (10 of 10)	3.23 nm	40 - 82 (42)	78.58	78.16	3.26 nm	0.59	10.81	90.31	45.2 - 60.2	
			taHEFD33 (10 of 10)				10.023446-2						
		1.1.1	D33 (10 of 10)										
+=UEED22	0.61	· · · · · · · · · · · ·			27 79 (51)	86.16	70.00	2.20	0.75	11.06	04.05	42.2 56.2	
taHEFD33	U.DI mg/ml		D33 (10 of 10)	3.10 nm	21-10(21)	00.10	78.80 ±9.98563	3.20 nm	0.75	11.06	04.30	42.2 - 20.2	
		. Malaria	D33 (10 of 10)										



1D Scattering Curves Visualizer × Criteria ~ List ۳ 7 File 🔺 6 taHEFD33_044_sub.dat taHEFD33_044_sub.out 5 taHEFD33_045_00001.dat taHEFD33_045_00002.dat taHEFD33_045_00003.dat 4 taHEFD33_045_00004.dat taHEFD33_045_00005.dat 3 taHEFD33_045_00006.dat 2 taHEFD33 045 00007.dat taHEFD33_045_00008.dat taHEFD33_045_00009.dat 1 taHEFD33_045_00010.dat March taHEFD33_045_ave.dat 0 taHEFD33_045_ave.dat taHEFD33_046_00001.dat -1 taHEFD33_046_00002.dat taHEFD33_046_00003.dat -2 taHEFD33_046_00004.dat taHEFD33_046_00005.dat -3 taHEFD33_046_00006.dat ⊡ -4 Macromolecules + Tree Ð -5 Save Cancel



USR





Idealised Solution SAS Experiment



Neutron or X-ray source



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

		surements Analysis 181	News								
	Macromolecule	Concentration Scattering	Frames (Averaged/Total)	(luinier			Gnom			Pored
1			Rgi	nm) Points	Qualty (14)	1(0)	Rg (nm)	Total	Dinax (nm)	Volume (nm3	 MM (kD) Vol. est.
	LEIL LE	14.00 mg/ml		5 nm 19 - 37 (18)	83.95	90.78 ±6.89192	4.94 nm	0.51	24.09	154.27	77.1-102.8
	H46703	7.00 mg/ml	033 (1 of 30) 3.9 suffEF033 (5 of 30) 023 (5 of 30)	7 m 12 · 42 (30)	92.14	71.21 ±4.3959+2	3.91 am	0.44	13.90	112.54	56.3 - 75.0
	COLOR	3.50 mg/ml		7 m 50 - 77 (27)	72.77	59.53 ±6.55654	3.44 nm	0.53	11.81	95.25	47.6 - 63.5
1	Trafford	125 mg/ml	033 (30 of 33) suHEF033 (30 of 33) 033 (30 of 33)	40 · 82 (42)	78.58	78.16 #8.02344+-2	3.26 nm	0.59	10.81	90.31	452-602
	w#753	0.61 mg/rel	203 (10 of 18) 3.1 twieF003 (20 of 18) 003 (20 of 18)	inn 27 - 78 (51)	86.35	78.86 +9.99563	3.20	0.75	11.06	84.35	42.2 - 56.2
	11										



Automated data acquisition

With feedback



Acknowledgments













