



TD SAXS Rénafobis

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Instructions

1) Using Primus, open the file:

01_Mast/mast2_avg.dat

- Is the protein folded ?
- What is the Rg value ?
- What is the MW of the sample?
- What is the Dmax value ?
- Generate a bead models.
- Is the NMR structure is compatible ?
- Can we do more ?
- 2) Using Primus, open the file:

- Look at the PDB file (pymol)
- Can we do more ?
- 3) More difficult files:

03_OthersExamples/

Primus

This program is used to display curves and apply some operation on the curves (scaling, substraction or merging...). It includes also a lot of script to estimate the Radius of Gyration, the Distance Distribution, the Porod Volume and the Molecular Weight.



	File	From	То	Conc.	Ang Scale	I Scale
🔽 aldo	olase_116.dat	1	1077	0.00	1.000	1.000
Active	Curves name	1st and th	last point o e curve	f		

You can load the SAXS curves (click on "File" button). When the data are loaded in the table, the name appears in the "Documents" area.

With the buttons "From" and "To" you can remove respectively the first points or the last points of the curve. It's useful to hide the noisy part of the curve (last points).

Primus



For Kratky representation, you can go to **Plot -> I*s² vs. s (Kratky plot).** Only the first points are necessary (q between 0 and 0.3)



For Rg calculation, you can go to Tools -> Analysis -> Radius of Gyration and modify manually the parameters or use directly the program "AutoRg".



You can evaluate the quality of the fit with the residual plot which represents the distribution of the experimental points around the regression line. You can control also the limit s*Rg max which should be inferior to 1. This limit depends of the geometry of the object : For globular protein, the limit can be increased up to 1.3 whereas for elongated or unfolded protein, the Guinier region is more restricted (less than 1). The first points which correspond to the lowest values of q can be also removed due to large error measurement (data close to the beam stop).

Molecular Weight

For Molecular Weight estimation, you can go to **Tools -> Analysis -> Molecular Weight.** A Guinier Analysis is necessary for the First Step.

Qp		MoW	
q _{max} [A ⁻¹]	0.25964	q _{max} [A ⁻¹]	0.40006
		V [A3]	32648
MW [Da]	28716	MW [Da]	26936
Vc		-Size & Shape	
Vc		Size & Shape	
Vc q _{max} [A ⁻¹]	0.30026	-Size & Shape	
Vc q _{max} [A ⁻¹] Vc	0.30026 298	Size & Shape	

Qp : Based of Porod invariant (only folded protein) MoW : Integration of I(q).q²=f(q) Vc : Integration of I(q).q=f(q) + Rg Size&Shape : From Rg estimation

Bayesian Inference	
MW Estimate [Da]	27550
MW Probability [%]	30.41
Credibility Interval [Da]	[25900, 29250]
Credibility Interval Probability [%]	91.80

Statistic calculation to give an interval of the estimated MW



You need a normalized curve in absolute scale extrapolated to a concentration at 1mg/mL.

Partial Specific Volume and Contrast can be calculated from the 1D sequence



Distance Distribution

The program GNOM is used to determine the autocorrelation function p(r) from the SAXS data and to estimate the Dmax.

Using Primus, click on [Distance Distribution]



At the end, you will obtain a new file called <*.out> containing all informations about the p(r) determination such as parameters defined preliminary in GNOM (nBeg, nEnd, ...), biophysical parameters (Rg, I(0) and Dmax). You will find also in ASCII format, the SAXS curve with the corresponding regularization curve and the p(r) function.

The file <*.out> will be used by ab initio program such as DAMMIF, DAMMIN or GASBOR.

Distance Distribution

To determine a correct value of Dmax, we must proceed by trial and error to find a Rg calculated with GNOM similar to that found with the calculation of Guinier. We start in general with a value of Dmax equal to 4 or 5 times the value of Rg, and decrease gradually the value of the Dmax in order to obtain a smoothed p(r) that cuts the axis of the distance r.







Dammif

The program DAMMIF is dedicated to low resolution shape modeling using a sphere containing beads (with a defined value of electronic density) as initial model.

Mode Selection		
/volumes/THUREAU_AUR/S	oleii/Congres/20170124_Pasteur/InputFiles/Aldolase/aldolase.dat	
Manual colorition of assumption		
• Manual selection of parameters		

Rg and P(r) are necessary to generate a bead model.

000	Primus Shape Wizard	
Ab Initio Mod Select t	el Setup ne parameters for modeling.	
Prefix Mode Repetitions Symmetry Anisometry Angular Scale	aldolase fast Speed of the simulated annealing (slow mode better but longer) 5 Image: Number of shape you want to generate (no more 5 for today !!!) P1 Precise the symmetry of the protein (if known) unknown If known, precise the global form of the protein Angstrom Angstrom Image: Average with DAMAVER DAMAVER allows to superimpose and to compare the between them. DAMMIN DAMMIN will recalculate a shape starting from dam necessary here. Usefull to obtain a better « resolution of the protein a better with the between them.	¢ ¢ ¢ ¢ e shapes
	< Back	Commit

Be patient, it will take few minutes/hours depending of your computer. Let's have a break !!!

Dammif



For each repetition asked, a fit is proposed:

- 1) The statistics of Cormap Test have to be correct (green or yellow), otherwise it means that "The hypothesis of similarity of experimental data and shape model could be rejected"
- 2) The Chi square Test is also an other method to validate the models of shape.

By Saving the result:

- You will obtain, a pdb file (-1.pdb) containing the shape composed of dummy residues, a fitting curve (smoothed curve) dam_xx.fit, a fitting curve corresponding to the experimental data dam_xx.fir and a log file with initial parameters and the process of minimization steps for each shape.
- 2) If you have perform an average with damaver, you will obtain also a table contained in the file damsel.log presents a matrix giving a non-deviation standard score (NSD) for each pair of shapes, and gives a classification of the shape. The most representative shape presents the lowest mean value of NSD. Be careful with the damfilt.pdb which represents a filtered shape corresponding to the common part off all shape. This model, in general case, does not fit the SAXS data !
- 3) You can open the pdb files (*-1.pdb) with pymol

Pymol

O O O MacPyMOL	
other usage is specifically prohibited and may constitute a violation of United States and international copyright laws.	Reset Zoom Orient Draw Ray
This Executable Build integrates and extends Open-Source PyMOL 1.5.0.3. Detected OpenGL version 2.0 or greater. Shaders available. Detected GLSL version 1.20. CmdLoad: "/Volumes/THUREAU_AUR/Soleil/Congres/20170124_Pasteur/InputFiles/Aldolas aldolase-4-1.pdb" loaded as "aldolase-4-1".	Unpick Deselect Rock Get View I < Stop Play > MClear
PyMOL>	
	all ASHLC
	aldol As: Show:
	lines as
	ribbon sticks
	cartoon ribbon
	label cartoon
	cell label
	dots pophonded
	spheres dots
	nb_spheres spheres
	mesh nb_spheres
	surface mesh
	organic
	main chain
	side chain
	Mawaa Mada Z-Dut ualanaa
	Buttons L M K wheel
	& Reys Rota Move Mov2 Slab Shft +Box _Box Clip MovS
	Ctrl +/- PkAt Pk1 MvS2 CtSh Sele Orig Clip MovZ
	SnglClk +/- Cent Menu DblClk Menu - PkAt
	Selecting Residues State 1/ 1
PyMOL>_	

- 1) To have a nicer view of bead modelon pymol. Select Show -> As -> Spheres
- 2) Type the command line : set sphere_scale, 2.5

AND / OR

1) alter <name of the object>,vdw=<value in the dammif log file
at dummy atom radius>

- 2) set solvent_radius = 4.3
- 3) Show -> As -> mesh

1) To represente the protein as cartoon: Show -> As -> Cartoon

2) To color some part of a protein (residue 6 to 107), type the command:

color red, resi 6-107

Crysol

CRYSOL is a program for evaluating the solution scattering from macromolecules with known atomic structure and fitting it to experimental scattering curves from Small-Angle X-ray Scattering (SAXS). As an input one can use a PDB file with an X-ray or NMR structure of a protein or a protein-DNA(RNA) complex.



https://dadimodo.synchrotron-soleil.fr/



2018 March, Roudenko O., Thureau A. & Perez J.



SULLEIL 🚲 🎝 INEXT





Pdb + rigid domains definition

Saxs curve