SANS: Sweedness PhD School
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Data Fitting using SasView

Software Required

Follow the link to the SasView web page and download and install the latest version. SasView has online documentation as well as several tutorials. If you don't yet feel comfortable with the SasView GUI, go through the *Getting Started with SasView* tutorial. The *Basic 1D Data Fitting* tutorial can also be used to familiarize yourself with fitting $I(q)$ vs q data.

Reduced SANS data

This guide shows how to fit the data from Series A and Series B at 1 mg/mL and 100 mg/mL (1lys.sub, 1lysNaCl.sub, 100Lys.sub 100LysNaCl.sub).

1. Fitting the 1 mg/mL Data

Load the 1 mg/mL data for both Series A and Series B into SasView. We will be fitting the data to an ellipsoidal form factor, P(q), and the Hayter-MSA structure factor [1], S'(q), using the decoupling approximation. We will first fit the data to P(q) only and then to P(q)S'(q) to define conditions where S'(q) ≈ 1 for all q values. This will allow us to compare the calculated S'(q) to those obtained at higher concentrations.

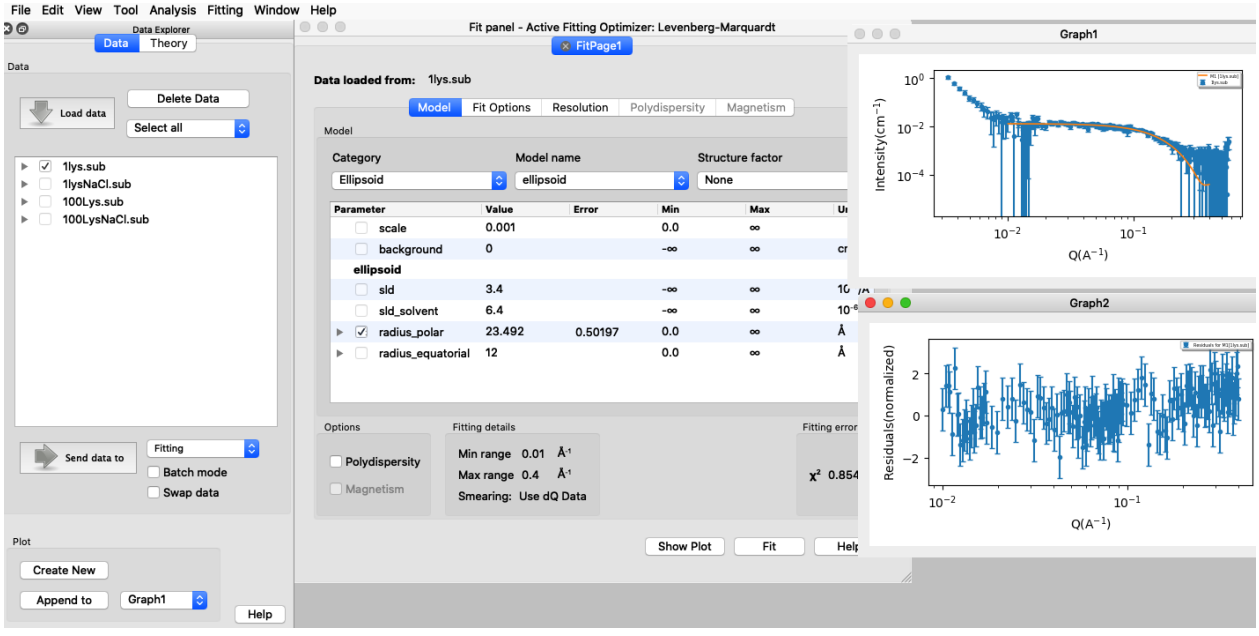
1.1 Ellipsoidal Form Factor

Making sure that only the Series A 1mg/mL Lysozyme data is checked, click on *Send Data to* Fit. Choose ellipsoid as the Model Category and ellipsoid as the Model name. When fitting a model with a large number of parameters, you want to hold as many parameters fixed as possible. In this case, we will fit one parameter, the polar radius. Once we perform the initial fit, we can adjust some of the other parameters to see if we can get a better fit.

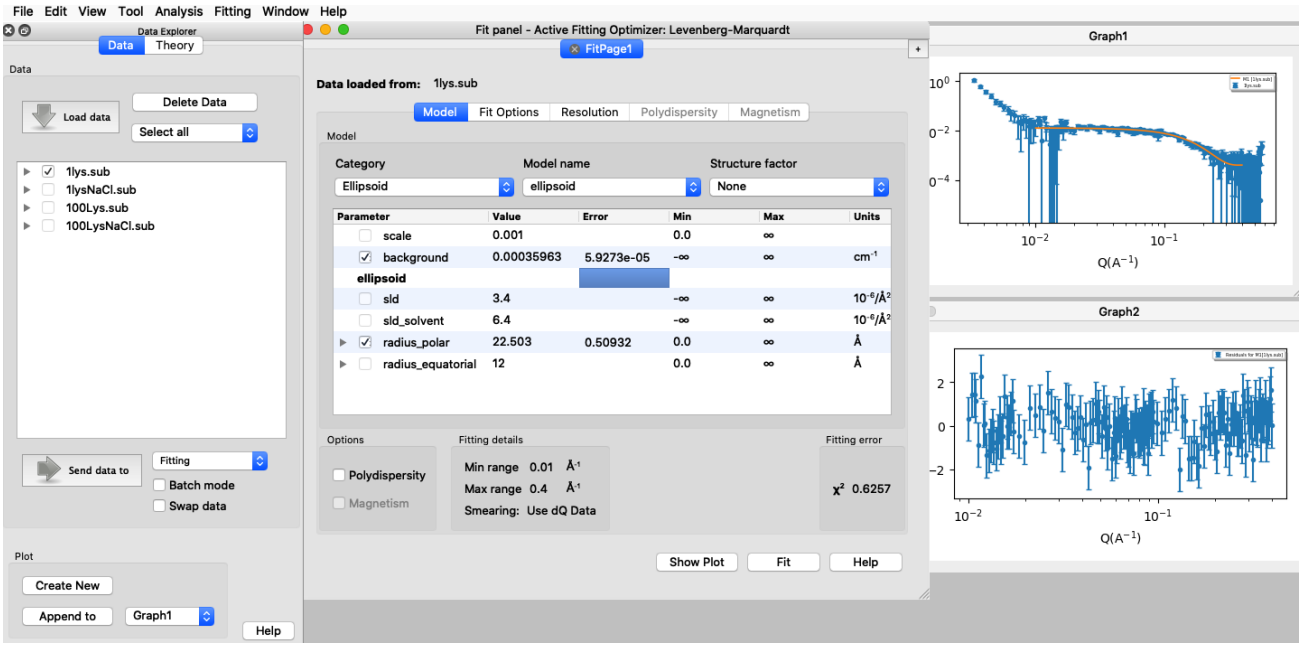
a. The scale parameter should be the volume fraction of lysozyme, since the data are on an absolute scale. The concentration is 1 mg/mL, so the volume fraction – and thus the scale parameter – is 0.001 or 0.1%.
b. The background has been subtracted from the data, so set it to 0.
c. Set the protein SLD to 3.4×10^{-6} Å^{-2} and solvent SLD to 6.4×10^{-6} Å^{-2} (these can be estimated using, for example, the contrast calculator module in the program SASSIE [2, 3]).
d. Start with a polar radius of 20Å and an equatorial radius of 10Å. Click on *Show Plot* at the bottom of the window.
e. Under the *Fit Options* menu, adjust the q range to 0.01 – 0.4 Å^{-1}. Go back to the *Model* menu. Make sure ONLY the polar radius parameter is checked. You can either click on

the check box, or right click on the line containing the parameter and select (or deselect) the parameter for fitting. Then, click the *Fit* button at the bottom of the window.

f. The fit is pretty good, but the ellipsoid is fairly elongated, with an axial ratio > 3:1. This isn't consistent with the structure of lysozyme. Change the equatorial radius to 12Å and do the fit again. Now we have a fit with a more reasonable axial ratio for lysozyme, a lower $|^2$ value and reasonable residuals.



g. You may be able to get a better fit by allowing the background to vary as well. Try it.



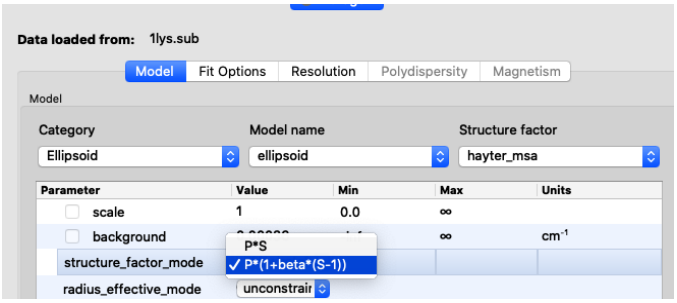
h. Repeat the process for the 1lysNaCl.sub data. Before you start a new fit, it is generally a good idea to make sure you unselect all fits and data on the Data explorer window, and to save your project.

1.2 Ellipsoidal Form Factor and Hayter_MSA Structure Factor

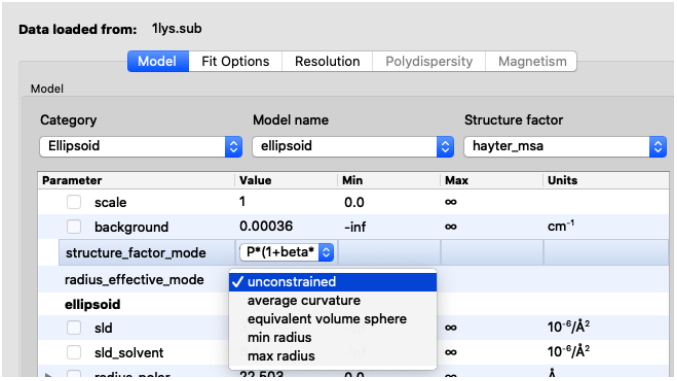
a. Make sure that the 1Lys.sub data is selected on the Data explorer and *Send to: Fitting*. Again choose ellipsoid for both the model Category and Model name. Choose Hayter_MSA for the structure factor. Fill in the ellipsoid parameters with your final

parameters from above. However, this time, set the scale parameter to 1.0 and the *volfraction* parameter to 0.001. (You may have to scroll down to find it.) Since this model specifically has a volume fraction parameter, the scale parameter must be fixed at 1.0.

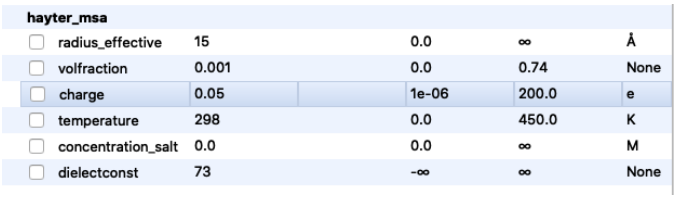
b. Set the structure_factor_mode (from the pull-down menu) to the Beta approximation.



c. Set the radius_effective_mode to unconstrained so that we can vary it later. However, for now, we will set it to the equivalent sphere radius using the ellipsoid parameters that we fit above, i.e., r^3 = 12 x 12 x 22.5 $Å^3$, or r ≈ 15Å.

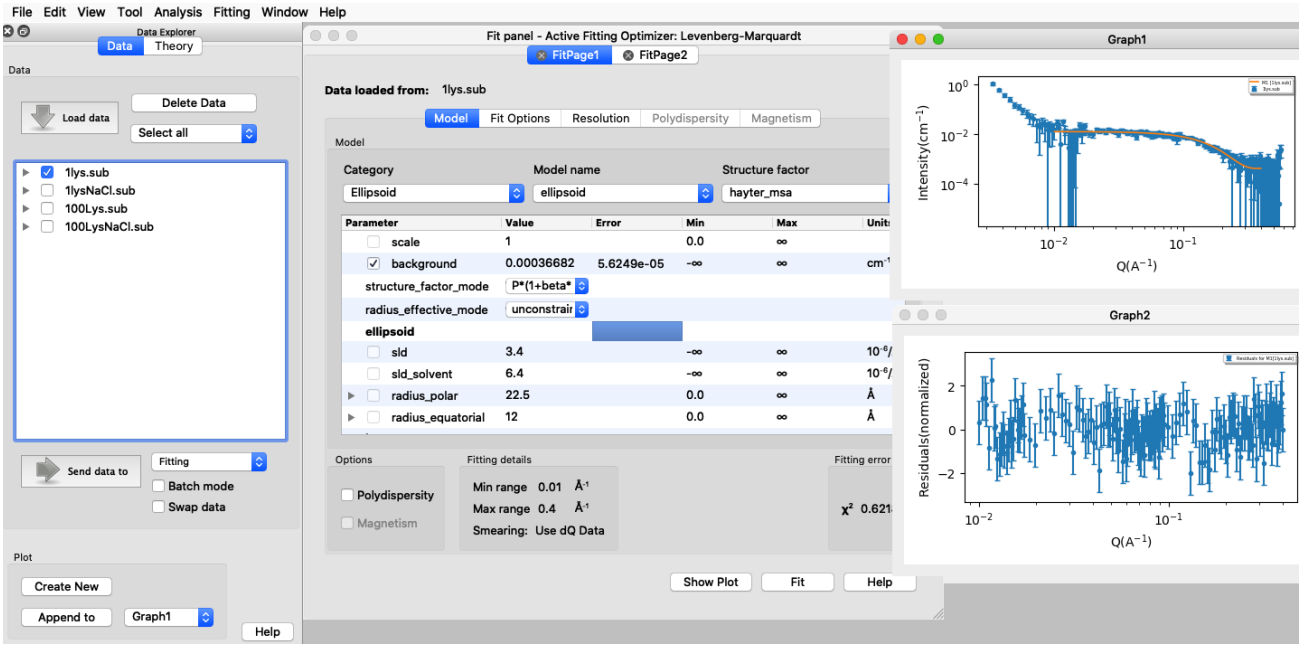


d. Set the Hayter_MSA parameters as shown below. This structure factor is not designed for dilute solutions. However, if we set the charge to a small value such as 0.05e, we will obtain a S′(q) that is ≈ 1 for all q values for comparison to those calculated for more concentrated samples.

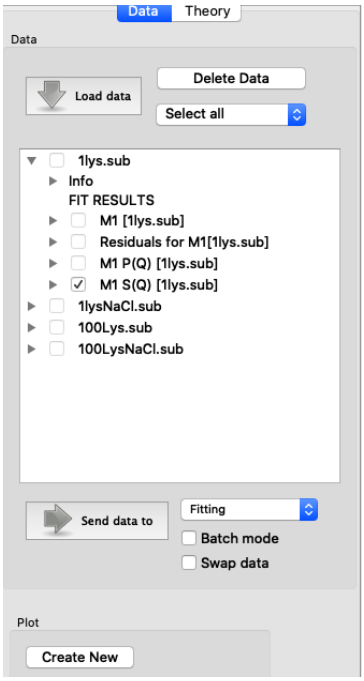


e. Click on *Show Plot* at the bottom of the window once you have set everything up.

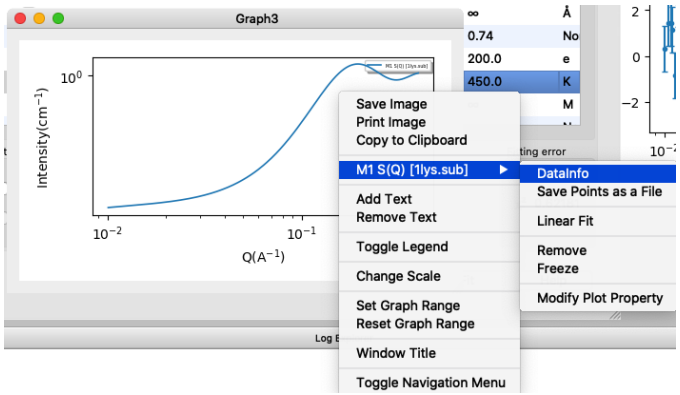
f. Fit the data with background as the only fitting parameter. Make sure that you adjust the q range to 0.01 – 0.4 $Å^{-1}$.



g. Now we can have a look at S'(q). Expand the 1lys.sub entry in the Data Explorer and make sure that only S(Q) is checked as shown below.

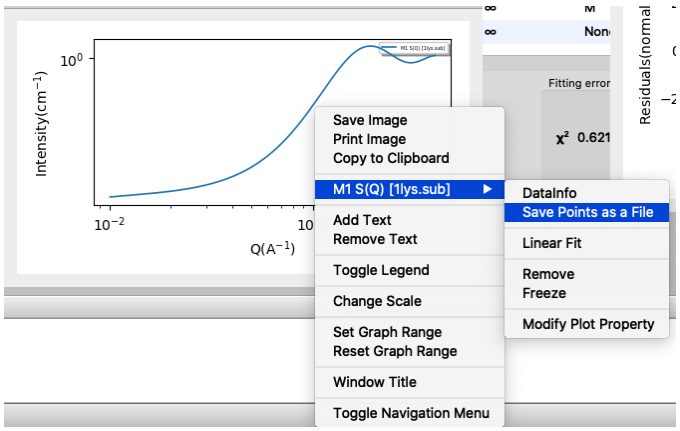


h. Click the *Create New* button at the bottom to obtain a plot of S'(q).



It doesn't look like S'(q) = 1. However, if we examine the actual values in the plot (by right-clicking on the plot and selecting 'DataInfo' we can see that the values are > 0.99 for all q. This will be good enough for comparison to S'(q) at higher concentrations.

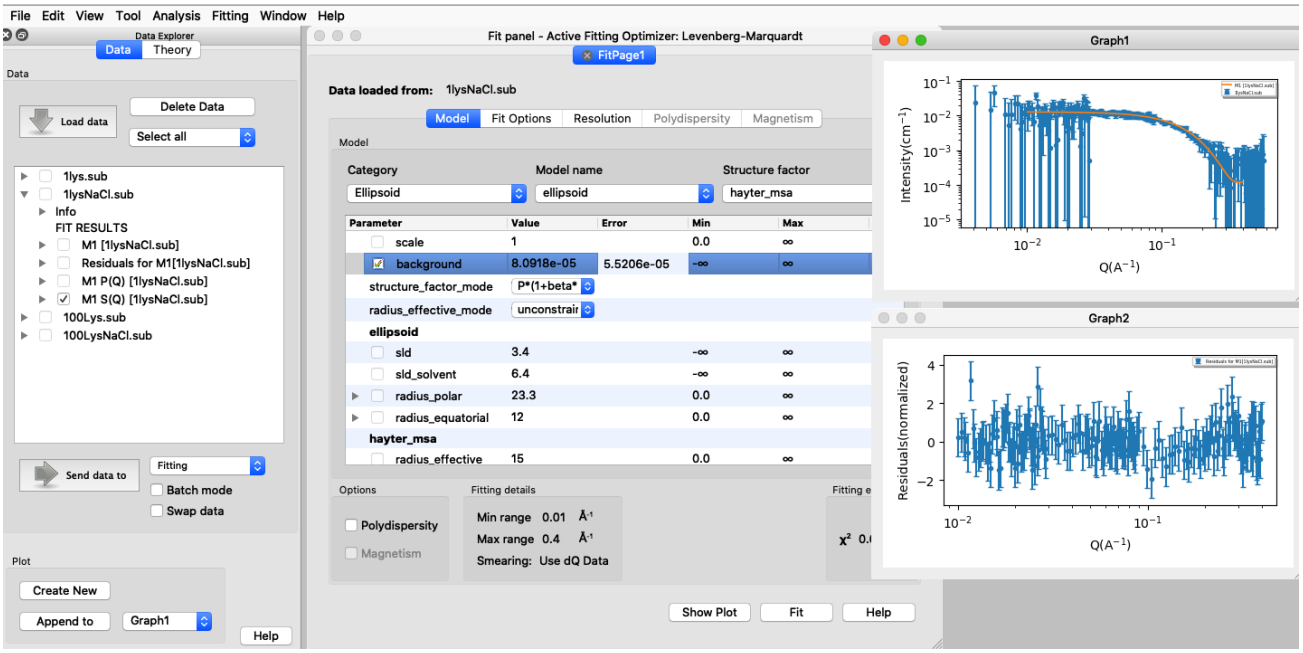
i. Save the S'(q) curve by selecting 'Save Points as a File'.



j. Repeat the process for the 1lysNaCl.sub data, setting the concentration_salt parameter to 0.15 M.

k. Save your S'(q) plot!

hayter_msa				
radius_effective	15	0.0	∞	Å
volfraction	0.001	0.0	0.74	None
charge	0.05	1e-06	200.0	e
temperature	298	0.0	450.0	K
concentration_salt	0.15	0.0	∞	M
dielectconst	73	-∞	∞	None



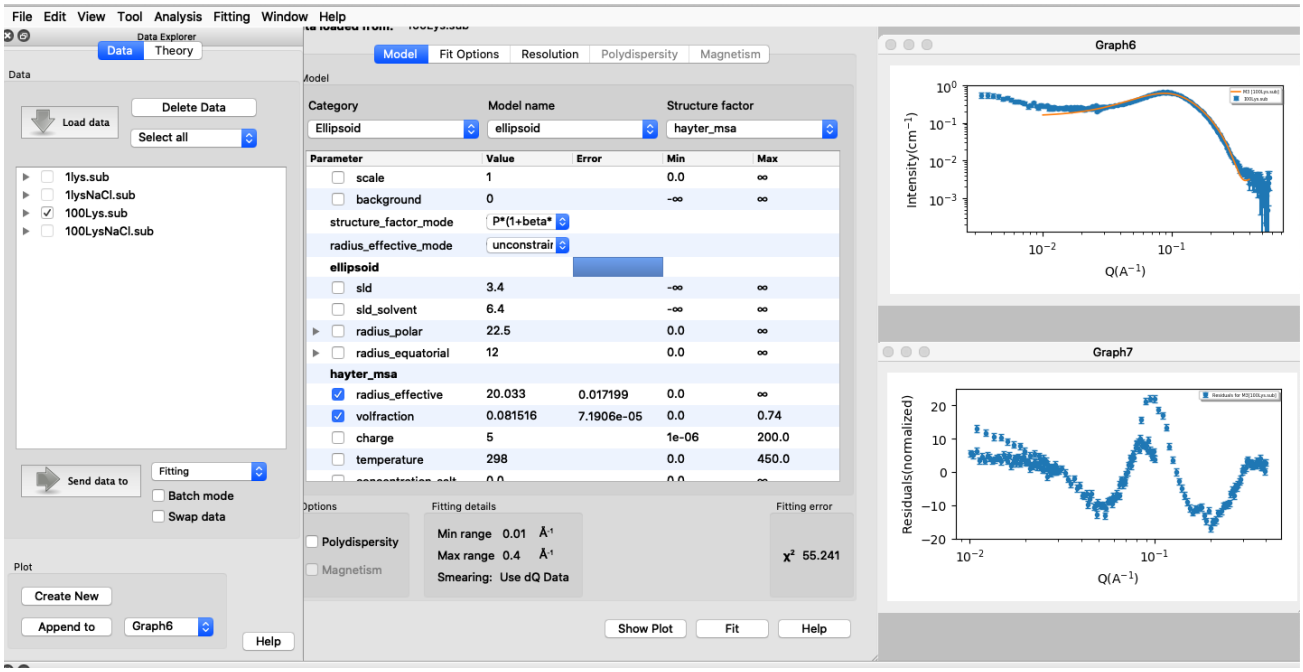
2. Fitting the 100 mg/mL data

Before you start a new fit, it is generally a good idea to make sure you unselect all fits and data on the Data explorer window, and to save your project.

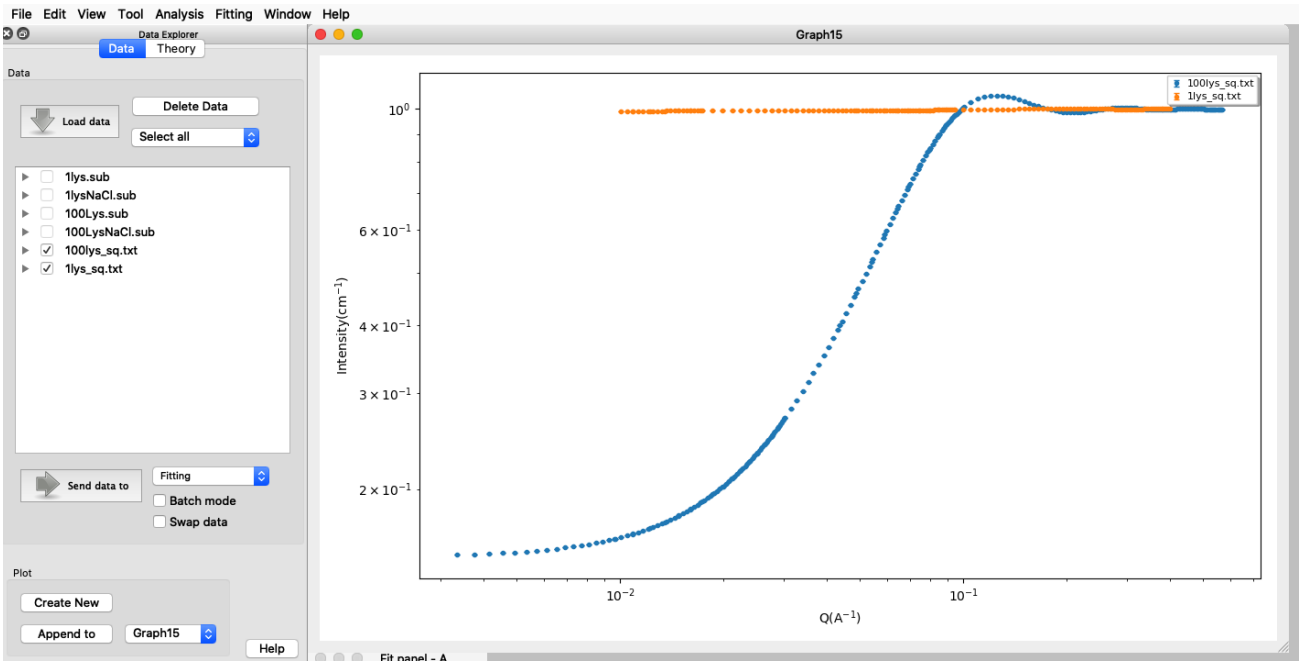
a. Load the 100Lys.sub data and set up the fitting parameters for an ellipsoid model with a Hayter_MSA structure factor. Set the parameters as you did for the 1 mg/mL samples, except now the volfraction parameter should be 0.1 and the charge can be set to 5. Set the background to 0 to start. Make sure to adjust the q range to 0.01 – 0.4 $Å^{-1}$.

b. Click on *Show Plot* to see the plots. Try fitting the data only allowing the radius_effective parameter to vary. How is the fit? You can probably get a little better fit by allowing the volfraction parameter to vary as well. Try it.

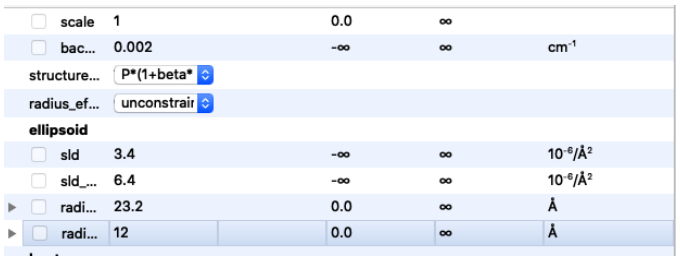
hayter_msa				
☑ radius_effective	20.033	0.017199	0.0	∞
☑ volfraction	0.081516	7.1906e-05	0.0	0.74
☐ charge	5		1e-06	200.0
☐ temperature	298		0.0	450.0
☐ concentration_salt	0.0		0.0	∞
☐ dielectconst	73		-∞	∞

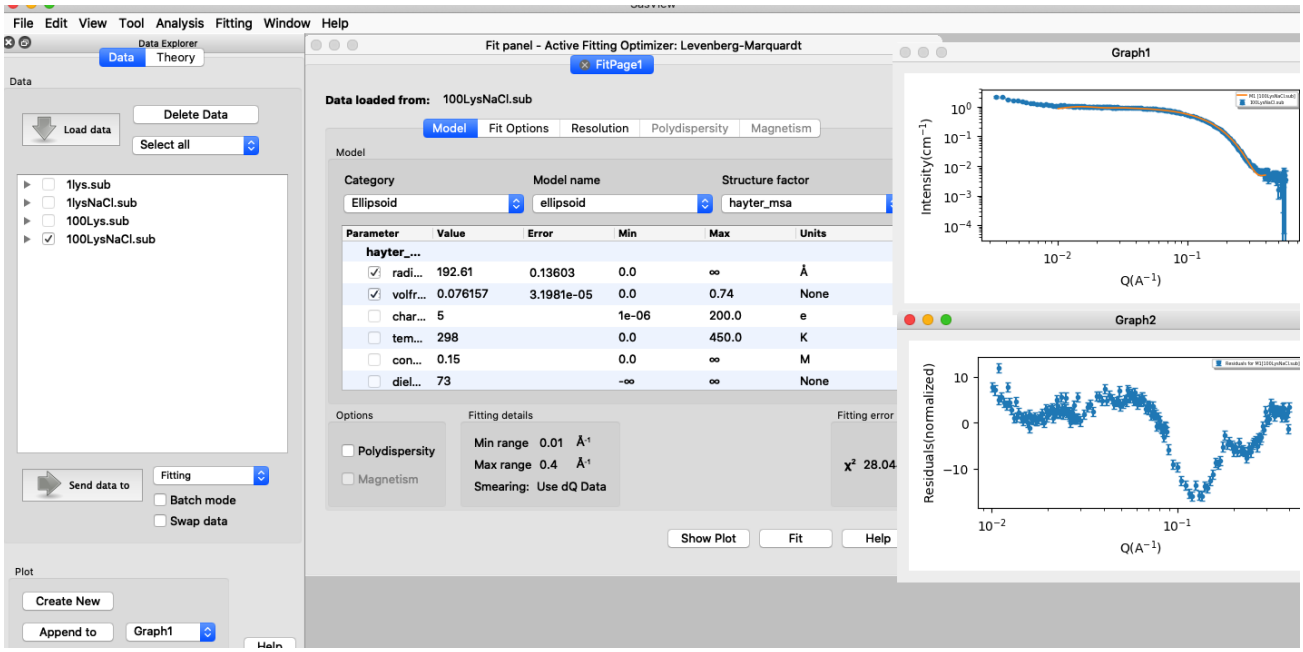


c. Do you get a better fit by allowing the background to vary?
d. Try increasing the charge parameter to 10. How does this affect the shape of the interaction peak? Set it back to 5 and fit the data again to get your final fit.
e. Create a plot with only S'(q) and save your curve.
f. Load both the 1 mg/mL and 100 mg/mL S'(q) curves and send them both to the same new plot. Make sure that ONLY the two file names are checked. You may have to drill down into the folders to uncheck unwanted curves.

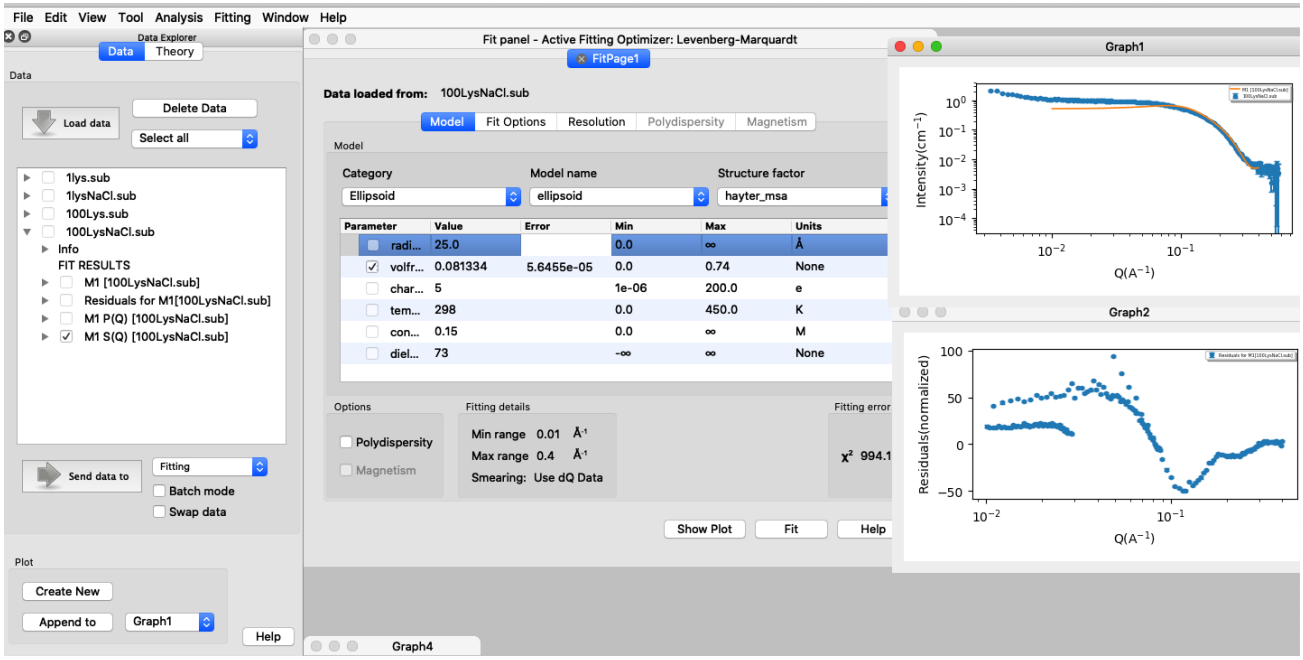


g. Repeat the fitting process for the 100LysNaCl.sub data. What happens when you let both the volume fraction and radius_effective vary? What happens to S'(q)?



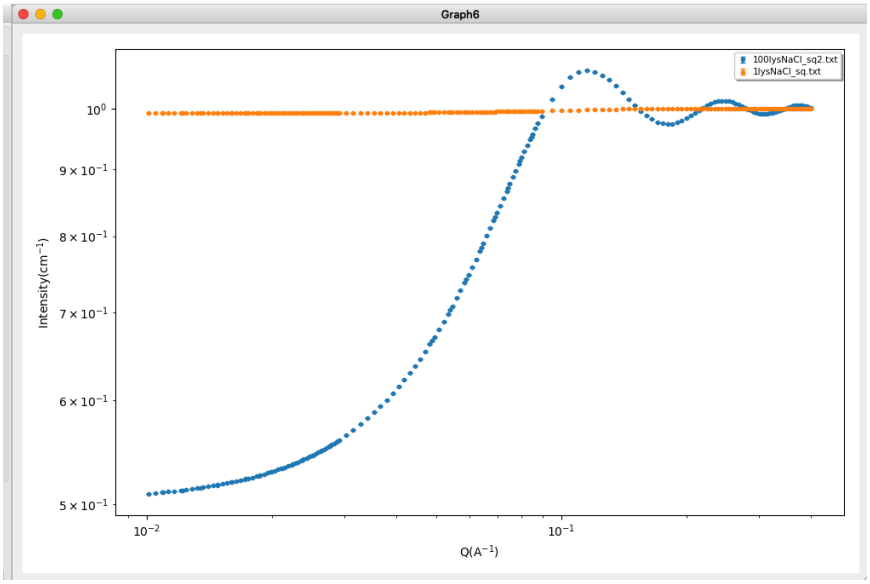


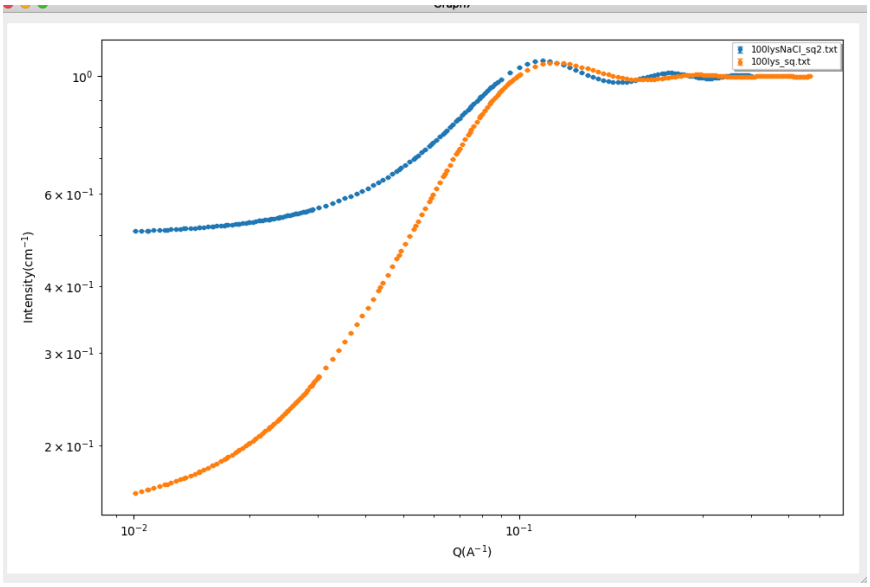
h. Set the radius to a FIXED value between 20 – 30 Å and fit the data again. Can you get a good fit to the data? In what q range is the fit failing? What is a reason why that might be?



i. Save your S’(q) plot.

j. Compare the S’(q) plot with that from the 1lysNaCl.sub data and with the 100Lys.sub data without NaCl. You can make two separate plots.





SUGGESTED DISCUSSION

A. Why are the fitted volume fractions less than 0.1 for the 100 mg/mL data?

B. What would be a better choice for the structure factor for the higher concentration data in 150 mM NaCl, where both attractive and repulsive forces could be in play?

C. Why didn't we choose the hard sphere structure factor for the 0 mM NaCl case?

DISCLAIMER

Certain software is identified to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the software identified are necessarily the best available for the purpose.

REFERENCES

[1] J.B. Hayter, J. Penfold, An analytic structure factor for macroion solutions, Molecular Physics 42(1) (1981) 109-118.

[2] J.E. Curtis, S. Raghunandan, H. Nanda, S. Krueger, SASSIE: A program to study intrinsically disordered biological molecules and macromolecular ensembles using experimental scattering restraints, Computer Physics Communications 183(2) (2012) 382-389.

[3] J. Curtis, Sassie-web: https://sassie-web.chem.utk.edu/sassie2/.