Documentation for Slab Functions

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1 Introduction

This file documents the methods used to fit the small angle x-ray scattering from a liposome dispersion to a model radial electron density profile of the liposome. A non-linear least squares fitting package (lmfit) is employed to vary the parameters of describing the electron density profile model to the experimentally measured small angle x-ray scattering data.

2 Experimental Data

Data were taken in transmission geometry through a 1.5±0.1 mm diameter flow capillary. To avoid x-ray damage, data was collected in a set of 40 0.1 s duration exposures. Reference data was also taken with a water filled cell that did not contain liposomes, and with an empty capillary containing only the outer glass and air inside. To produce a final data set the 40 run of liposome data are averaged together and the average of 40 runs of the water is then subtracted. Liposome samples had a nominal concentration of 10 mg/ml. Their size and polydispersity were characterized in advance of the x-ray measurements using dynamic light scattering (DLS). An excel notebook titled "Liposome_Spring2021_Data_Summary.xlxs" contains details from the lab notebook regarding the data sets.

3 Calculation of Scattering Intensity

3.1 scattering cross section for water

The scattering from pure water in the capillary at low angles is due to thermal diffuse scattering. There is also background scattering at very small angles, most likely due to the surface of thee capillary glass. The differential scattering cross section for thermal diffuse scattering is given by:

$$\frac{1}{V}\frac{d\Sigma}{d\Omega} = \rho_e^2 r_0^2 k_B T \chi_T \tag{1}$$

giving

$$N_w = I_t \Lambda \frac{1}{V} \frac{d\Sigma}{d\Omega} d\Omega = I_t \Lambda \rho_e^2 r_0^2 k_B T \chi_T d\Omega \tag{2}$$

Here I_t is the transmitted intensity, χ_T is the isothermal compressibility of water and ρ_e its electron density.

At $T = 23°\text{C}$, $\chi_T = 0.45$ GPa^{-1}, $\rho_e = 335.5e^-/\text{nm}^3$ giving a scattering cross section of $\frac{1}{V}\frac{d\Sigma}{d\Omega} = 1.64 \times 10^{-2}\text{cm}^{-1}$

The values returned from the SAXS are in units of $r_m = C_J N_w / I_t$ with C_j a setup dependent constant. The actual experimental data returns $r_m = n_p/n_t$. Here $n_t = C_t I_t$ is the number of counts measured in the transmitted beam, which is related to the true number of photons in the transmitted beam, I_t via C_t. Similarly n_p is the measured number of counts in a pixel, which is related to the number of photons in a pixel via $n_p = C_p N_w$. Thus $C_j = C_p/C_t$. A rough estimate gives $C_j = 2.2 \times 10^9$, which is within a factor of 4 of the value needed to make the measured cross section equal to the theoretical cross section. This can be improved, by redoing the circular averaging, but indicates things aren't too far off.

Thus we expect

$$\frac{1}{V}\frac{d\Sigma}{d\Omega} = \frac{1}{C_J \Lambda d\Omega}\frac{I}{J_t}$$

Values for the constants are

- The transmitted beam conversion factor $C_J \approx 2.2 \times 10^9$
- The detector solid angle $d\Omega = \left(0.146 \times 10^{-3}/2.261\right)^2 = 4.2 \times 10^{-9}$
- The sample thickness $\Lambda = 0.15$ cm

In addition to the thermal diffuse scattering from water, there will be incoherent scattering due to compton scattering. The compton (or modified scattering) cross section is approximately given by

$$\frac{1}{V}\frac{d\Sigma}{d\Omega} = r_e^2 \rho_e \left[\frac{\Sigma_i n_i \left(Z_i - f_i\right)}{\Sigma_i n_i Z_i}\right]$$

Here f_i is the atomic scattering factor for atom i, n_i is the stochiometry coefficient for atom i and I have assume small angle and low energy so that polarization and quantum effects can be ignored. The dashed green line in fig. 1 shows the expected compton cross section, which agrees reasonably well with the slow rise in the scattering at larger q

The python code for these calculations is in water_diffuse_calc.py in the directory liposomes.

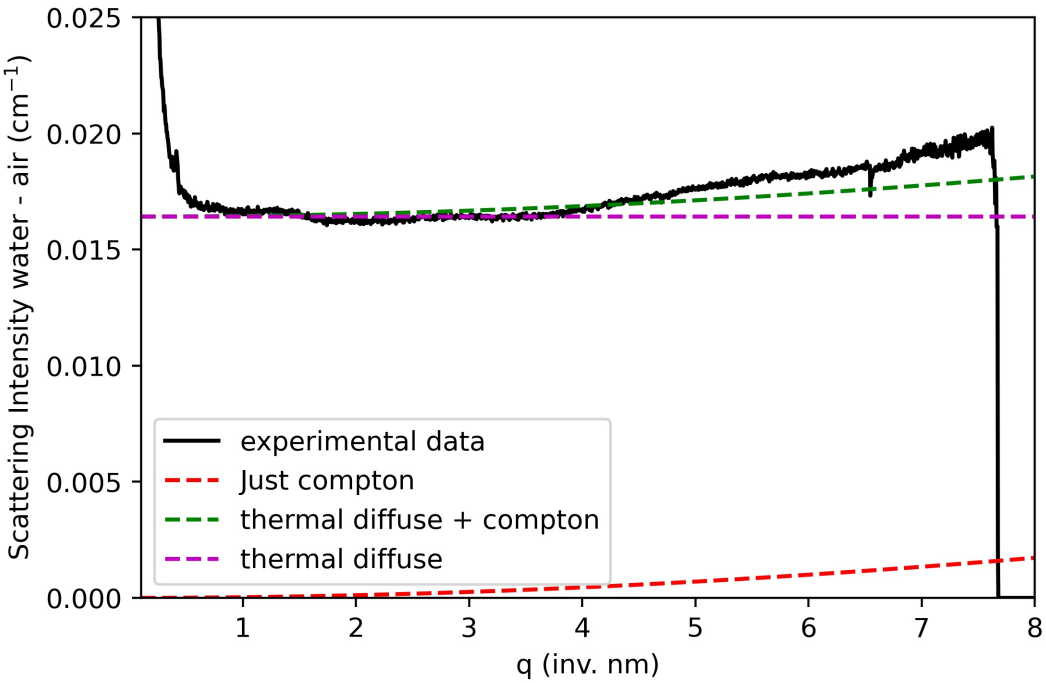

Figure 1: Diffuse scattering from water with empty capillary background subtracted. Data normalized by 0.185 in order to match plateau region of scattering to theoretical cross section for water. Dashed magenta line shows thermal diffuse cross section, while inclusion of the expected compton scattering is shown in the dashed green line.

3.2 Scattering cross section for liposomes

For the liposomes the scattering cross section per unit volume is given by

$$\frac{1}{V}\frac{d\Sigma}{d\Omega} = r_e^2 \rho_n \left|F(q)\right|^2$$

Here ρ_n is the number density of liposomes and $F(q)$ is the liposome form factor.

The number density of liposomes can be related to the mass per unit volume in mg/ml. Let W_l be the average molecular weight of the lipid, S_l be the area density of lipids, and R be the average radius of the liposomes. Then the liposome mass is

$$M_l = W_l \times 2 \times \left(4\pi R^2\right) \times S_l \tag{3}$$

If ρ_m is the mass density of liposomes and ρ_n the number density of liposomes, then

$$\rho_n = \frac{\rho_m}{M_l} \tag{4}$$

Thus

$$\frac{1}{V}\frac{d\Sigma}{d\Omega} = r_e^2 \frac{\rho_m}{8W_l \pi R^2 S_l} \left|F(q)\right|^2 \tag{5}$$

If we have a distribution of liposomes centered on radius R_0 with standard deviation σ_R then the liposome mass is averaged over the distribution of radii

$$M_l = \frac{8W_l \pi S_l}{\sqrt{2\pi\sigma_R^2}} \int_{-\infty}^{\infty} R^2 e^{-(R-R_0)^2/2\sigma_R^2} dR = 8W_l \pi S_l \left(R_0^2 + \sigma_R^2\right) \tag{6}$$

For eggPC the molecular weight is $W_l \approx 746$. For the area per lipid we will assume the value for DPPC $\frac{1}{S_l} = 0.48$ nm^2 This gives $8W_l \pi S_l = 6.49 \times 10^{-5}$ kg/m^2. If we put in values for $R = 62$nm and $\rho_m = 10$mg/ml we get

$$\frac{1}{V}\frac{d\Sigma}{d\Omega} = 3.2 \times 10^{-12} \left|F(q)\right|^2 \tag{7}$$

See file liposome_normalization.py for details.

4 Model for liposome form factor

4.1 Slab model for Liposomes

Currently we are modeling the liposome profile using a symmetric slab model. The model consists of 2 slabs describing each leaflet of the bilayer. The two slabs represent the head group and the hydrocarbon tails respectively. Each slab is characterized by an electron density (amplitude) (A_H, A_T, A_M) and

a thickness. We parameterize the thickness as the overall thickness of the bilaer (W) and the individual thicness of the headgroup (d_H) and the methyle tail overlap (d_M). There is also a transition region between each slab (a gradual transition from one density to the other) which is characterized by a roughness parameter (sigma). Sigma is fixed to be the same for all slabs. In the center of the bilayer we assume a methyl overlap region with zero density and a narrow width. The density is actually negative the electron density of water, since we take all electron densities as differences from the water backround. This requires seven parameters to describe the layer, 3 thicknesses, 3 amplitudes (we allow the possiblity for the methyl overlap region amplitude to vary for now) and a roughness. In addition, there are 3 other fitting parameters, an overall scale factor (I) which determines the total intensity and the radius (Rs) and standard deviation of the radius (Rsig) of the liposomes. R0 and Rsig should be fixed based on the DLS results. Eventually, we can try to calculate I0 from the concentration of liposomes, but that will require a bit more work and for now we can just treat it as a free parameter. Two types of objects have been defined so far, slabs and profiles. A profile is composed of a list of slabs. Each slab consists of an amplitude, a center position a transition width (sigma), and a name. All lengths are in nm and amplitudes in e^-/nm^3

You can create a slab by inputting the amplitude, center, sigma and name in the slab function, e.g: myslab = slab(15,0,.3,'my first slab')

Note that the slabs go on forever in the z direction (e.g. they are one sided). If you want to make a slab with finite width, you need to have a negative and a positive amplitude with opposite signs, e.g.

s1 = slab(10,-1,.3,'inner half') s2 = slab(-10,-1,.3,'outer half')

These can be combined into a profile using the profile function:

MyfirstProfile = profile([s1,s2])

Note that the profile function takes a single argument which is a list (e.g. [s1,s2]) comprised of the two slabs s1 and s2.

The x-ray scattering amplitude only depends on density differences. Thus, since the whole liposome is surrounded by water, only the difference in electron density relative to water counts, and profile amplitudes should be specified as density differences.

There is an option to include an offset when plotting to the density profile to put the water back in, but that is just for visualization purposes.

Note that the density profile is written in terms of electron density. As an example of this calculation, consider the electron density of water.

$\rho_e = \rho N_A Z/A$. Here $\rho = 997\text{kg/m}^3$, $N_A = 6.022 \times 10^{26}$/kilomole $Z = 10$ and $A = 18.015$ kg/kilomole. Putting this together gives $\rho_e = 3.333 \times 10^{29} e^-/\text{m}^3$ or 333.3 e^-/nm^3.

The function make_F_res(q,R0,sig) calculates the form factor expected from a distribution of liposomes of average radius R0 and standard deviation in radius of sig. The functional form can be calculated analytically which should speed up the fitting process.

There are more free parameters for the individual slabs than are specified in the fitting model. This is because the fitting model constrains the layer to be symmetric and uses the same roughness for all the layers. In principle we can consider asymmetric bilayers later and then make more complicated models using the same slab code as the underlying description.

4.2 Calculation of the form factor

The liposome form factor is given by:

$$|F(q)|^2 = \left| \frac{4\pi}{q} \int_0^\infty \Delta\rho(r) r \sin(qr)\, dr \right|^2$$

We are approximating $\rho(r)$ by a series of slabs. In particular, the transition in density for each slab is given by

$$\frac{d\Delta\rho_i(r)}{dr} = \frac{A_i}{\sqrt{2\pi\sigma_i^2}} \exp\left[-\frac{(r - R - r_i)^2}{2\sigma_i^2} \right]$$

Here r_i is the position relative to the liposome wall center, and R is the radius of the liposome (distance from center of liposome to center of wall). Since its easier to work with the derivative $\frac{d\Delta\rho_i(r)}{dr}$ we can integrate by parts and throw away the boundary terms, since the profile is zero at both $r = 0$ and $r = \infty$:

$$\frac{4\pi}{q} \int_0^\infty \Delta\rho(r) r \sin(qr)\, dr = \frac{4\pi}{q^2} \int_0^\infty \frac{d\Delta\rho_i(r)}{dr} \left[\frac{1}{q} \sin(qr) - r\cos(qr) \right] dr$$

This can be further simplified by recognizing that $\frac{d\Delta\rho_i(r)}{dr}$ is zero sufficiently far from the liposome wall so that the integral can be extended to infinity. We can then change variable to $y_i = q(R + r_i)$, $x + y_i = qr$ and $\epsilon_i = q\sigma_i$, This gives:

$$\frac{4\pi A_i}{q^3\sqrt{2\pi\epsilon_i^2}} \int_{-\infty}^\infty \exp\left(-\frac{x^2}{2\epsilon_i^2} \right) \left[\sin(x + y) - (x + y_i)\cos(x + y_i) \right] dx$$

We can expand $\cos(x + y_i) = \cos(x)\cos(y_i) - \sin(x)\sin(y_i)$ and $\sin(x + y_i) = \sin(x)\cos(y_i) + \cos(x)\sin(y_i)$. Since $\exp(-x^2/2\epsilon_i^2)$ is an even function, the odd

terms in x give zero, leaving:

$$\frac{4\pi A_i}{q^3\sqrt{2\pi\epsilon_i^2}}\int_{-\infty}^{\infty}\exp\left(-x^2/2\epsilon_i^2\right)\left\{\cos(x)\left[\sin(y_i)-y_i\cos(y_i)\right]+x\sin(x)\sin(y_i)\right\}dx \tag{8}$$

The term in $x\sin(x)$ can be simplified via integration by parts (again throwing away the boundary terms)

$$\int\exp\left(-x^2/2\epsilon_i^2\right)x\sin(x)\sin(y_i)dx=-\int\epsilon_i^2\exp\left(-x^2/2\epsilon_i^2\right)\cos(x)\sin(y_i)dx \tag{9}$$

So that the integral becomes:

$$\frac{4\pi A_i}{q^3\sqrt{2\pi\epsilon_i^2}}\int_{-\infty}^{\infty}\exp\left(-x^2/2\epsilon_i^2\right)\cos(x)\left[\sin(y_i)\left(1-\epsilon_i^2\right)-y\cos(y_i)\right]dx \tag{10}$$

The cosine transform is given by:

$$\int_{-\infty}^{\infty}\exp\left(-x^2/2\epsilon_i^2\right)\cos(x)dx=\sqrt{2\pi\epsilon_i^2}e^{-\epsilon_i^2/2}$$

This gives the final result

$$f_i(q)=\frac{4\pi A_i}{q^3}\left[\sin(y_i)\left(1-\epsilon_i^2\right)-y_i\cos(y_i)\right]e^{-\epsilon_i^2/2} \tag{11}$$

with $F(q)=\left|\sum_i f_i(q)\right|^2$

5 Distribution of Radii

There is typically a distribution of liposome radii. Let us assume the probability density of liposomes with radius R is given by:

$$n(R)=\frac{1}{\sqrt{2\pi\sigma_R^2}}\exp\left[-\left(R-R_0\right)^2/2\sigma_R^2\right] \tag{12}$$

Putting all the terms together we get

$$\begin{aligned}I\propto\frac{16\pi^2}{q^6\sqrt{2\pi\sigma_R^2}}\sum_{i,j}A_ie^{-\epsilon_i^2/2}A_je^{-\epsilon_j^2/2}\\ \int_{-\infty}^{\infty}\exp\left[-\left(R-R_0\right)^2/2\sigma_R^2\right]\left[\sin(y_i)\sin(y_j)\left(1-\epsilon_i^2\right)\left(1-\epsilon_j^2\right)-\right.\\ \left.2\sin(y_i)\cos(y_j)y_j\left(1-\epsilon_i^2\right)+\cos(y_i)y_i\cos(y_j)y_j\right]dR\end{aligned} \tag{13}$$

Change variables to $z = q(R - R_0)$, $\phi = q\sigma_R$, $w_i = y_i - z = q(R_0 + r_i)$, and $\delta_i = \left(1 - \epsilon_i^2\right)$. This gives:

$$I \propto \frac{16\pi^2}{q^6\sqrt{2\pi\phi^2}} \sum_{i,j} A_i e^{-\epsilon_i^2/2} A_j e^{-\epsilon_j^2/2} \int_{-\infty}^{\infty} \exp\left[-z^2/2\phi^2\right] [\sin(z+w_i)\sin(z+w_j)\delta_i\delta_j$$
$$-2\sin(z+w_i)\cos(z+w_j)(z+w_i)\delta_j + \cos(z+w_i)\cos(z+w_j)(z+w_i)(z+w_j)]dz \tag{14}$$

Each of these three terms can be simplified by expanding the trig functions and discarding terms odd in z.

- First term

$$\cos(w_i)\cos(w_j)\,\delta_i\delta_j - \cos(w_i+w_j)\cos^2(z)\,\delta_i\delta_j$$

- Second term

$$2\cos(w_i)\sin(w_j)w_j\delta_i - \sin(2z)\cos(w_i+w_j)z\delta_i - 2\cos^2(z)\sin(w_i+w_j)w_j\delta_i \tag{15}$$

- Third term

$$[\cos^2(z)\cos(w_i+w_j)+\sin(w_i)\sin(w_j)](z^2+w_iw_j) - \sin(2z)\sin(w_i+w_j)zw_i \tag{16}$$

Collecting terms in similar z simplifies to:

$$I \propto \frac{16\pi^2}{q^6\sqrt{2\pi\phi^2}} \sum_{i,j} A_i e^{-\epsilon_i^2/2} A_j e^{-\epsilon_j^2/2} \int_{-\infty}^{\infty} e^{-z^2/2\phi^2} \left(C_1 + C_2\cos(z)^2 + C_3 z^2\cos^2(z) + C_4 z\sin(2z) + c_5 z^2\right) dz$$

Here we have

- C_1

$$\cos(w_i)\cos(w_j)\delta_i\delta_j + 2\cos(w_i)\sin(w_j)\delta_i w_j + \sin(w_i)\sin(w_j)w_iw_j$$

- C_2

$$\cos(w_i+w_j)(w_iw_j - \delta_i\delta_j) - 2\sin(w_i+w_j)\delta_i w_j$$

- C_3

$$\cos(w_i+w_j)$$

- C_4

$$-\cos(w_i+w_j)\delta_i - \sin(w_i+w_j)w_i$$

- C_5

$$\sin(w_i)\sin(w_j)$$

Evaluating the integrals gives

$$\frac{16\pi^2}{q^6} \sum_{i,j} A_i e^{-\epsilon_i^2/2} A_j e^{-\epsilon_j^2/2} \{C_1 + \frac{1}{2}(1+e^{-2\phi^2})C_2 + \frac{\phi^2}{2}[1+(1-4\phi^2)e^{-2\phi^2}]C_3 + 2\phi^2 e^{-2\phi^2}C_4 + \phi^2 C_5\}$$