Lipid Dynamics Experiments

You are a PhD student working on a project on the signalling lipid 1-stearoyl-2-arachidonoyl-sn-glycerol, 18:0-20:4 DG. It allosterically activates PKC and other proteins that affect cell growth, development, survival, apoptosis, carcinogenesis and metastasis. It activates transient receptor potential channels 3 and 6 that regulates the intracellular free calcium levels. Your thesis involves identifying how this lipid interacts with Ca+ ions in a cell membrane. Following some structural studies you are asked to investigate whether whether it is feasible to use neutron spectroscopy to study the flexibility of these lipids.

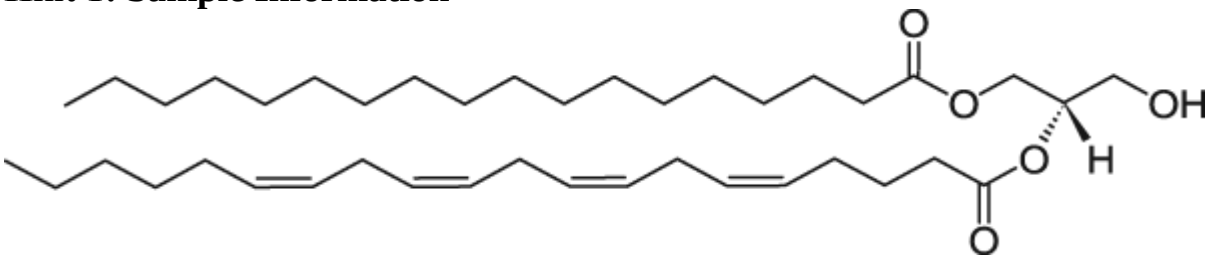
You have been asked to prepare a solution of DG at 0.5mg/ml with and without Ca+ for experiments and to plan an experimental campaign.

Consider what steps to take next. What would you need to consider when preparing for this experiment?

Hint 1: What steps to follow to answer the questions

1. Need to think about what timescales of the motions you want to look at
2. Need to think of what instruments can achieve this
3. What samples will you measure? (contrast)
4. What is the state of your sample? (containers)
5. Calculate scattering signal of the experiment
6. Calculate sample thickness
7. Temperatures

Hint 1: Sample Information



Molecular formula of repeat unit: $C_{41}H_{72}O_5$
Melting temperature, T_m = 32-37 C
Density, ρ = 1.1 g cm^{-3}

D_2O, density ρ = 1.1 g cm^{-3}
D_2O, viscosity η=10^{-3} kg $m^{-1}s^{-1}$

Hint 2: Sample Scattering

To decide the sample thickness, we need to understand what is happening in the sample. For neutron transmission, the universal law of attenuation of radiation passing through matter (**Beer-Lambert Law**) is valid.

$$I = I_0 \exp(-\Sigma_T \tau) \qquad \text{Eq. O1}$$

The ratio between the emerging neutron flux I and the incident flux I_0 is called the transmission. For a typical QENS experiment, we want to keep the transmission at 90% to avoid multiple scattering effects (why?).

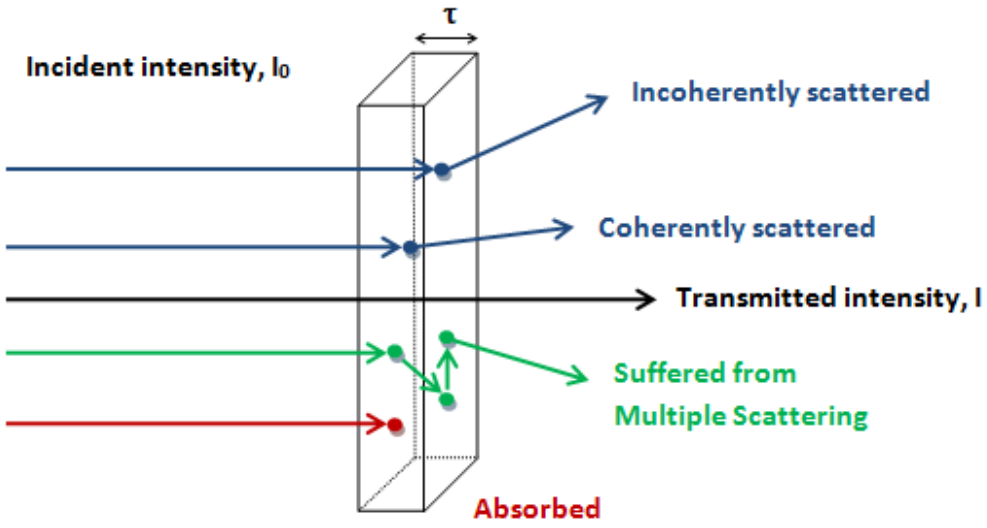


Figure 2. *Sketch of possible interaction mechanisms between neutron and atom*

Σ_T is the **macroscopic scattering cross-section** and is sum of the three scattering interactions, incoherent scattering, coherent scattering and absorption = $\Sigma_{inc} + \Sigma_{coh} + \Sigma_{abs}$, and depends on the number of atoms in the sample. So that we can write:

$$\Sigma_T = N\sigma_T = N(\sigma_{inc} + \sigma_{coh} + \sigma_{abs}) = \frac{\rho N_A}{M_W}(\sigma_{inc} + \sigma_{coh} + \sigma_{abs}) \qquad \text{Eq. O2}$$

Where
N = number density (cm^{-3})
ρ = mass density ($g\ cm^{-3}$)
N_A = Avogadro's number = 6.022×10^{23} (mol^{-1})
M_w = molecular weight ($g\ mol^{-1}$)
σ_{inc} = incoherent scattering x-section (cm^2)
σ_{coh} = coherent scattering x-section (cm^2)
σ_{abs} = absorption x-section (cm^2)
1 barn, b = 1×10^{-24} cm^2

Table 1. *(from [http://ncnr.nist.gov/resources/n-lengths/)](http://ncnr.nist.gov/resources/n-lengths/)*

Element	σ_{inc} (b)	σ_{coh} (b)	σ_{abs} (b) @ λ=1.8Å	M_w ($g.mol^{-1}$)
C	0.001	5.551	0.0035	12.011
H	80.27	1.7583	0.3326	1.0079
D	2.05	5.592	0.000519	2.0158
O	0.0008	4.232	0.00019	15.999
Ca+	0.05	2.78	0.43	40.078

Hint 3: Details of the IRIS Spectrometer

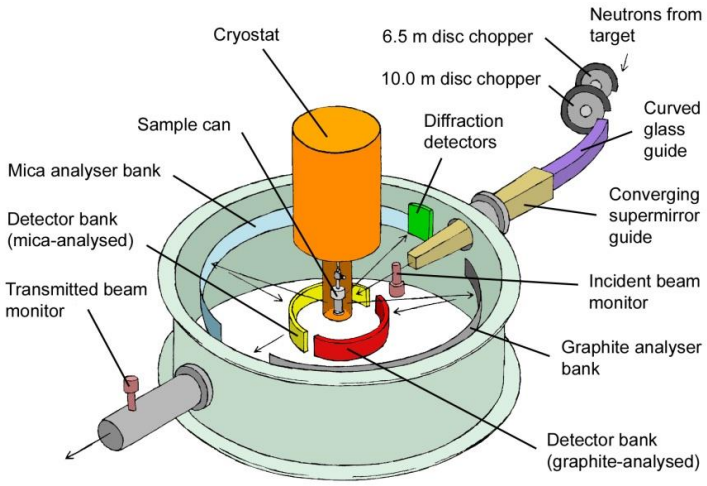


Figure 3. *Sketch of the IRIS Spectrometer at ISIS, UK*

Q range with PG002 analyser reflection is 0.42 to 1.85 $Å^{-1}$
Energy resolution is 17.5 μeV and typical energy window is +/- 0.4 meV

In general, annular geometry is preferred so that the scattering path is almost constant at all detector angles. In this way we avoid blind spots and having to do absorption corrections to the data. Sometimes it is not practical and flat geometry has to be used. In this case choose either 45 or 135° orientations, to either concentrate on high Q or low Q regimes.

Hint 4: Details for Neutron Spin Echo experiment

A necessary step is to do a SANS experiment to determine what we can see: shape fluctuations of bending rigidity.

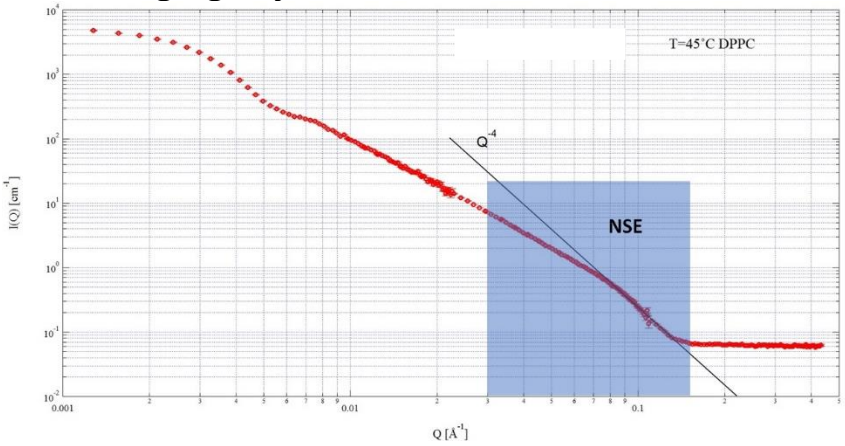


Fig. 5. Example SANS pattern

The vesicle is too big for the shape fluctuation to be observed in the NSE Q range (1/500 $Å^{-1}$=0.002 $Å^{-1}$). The region probed by NSE roughly follows a Porod law with exponent -4. Deviations from the Porod law behaviour are related to polydispersity and, possibly, multilamellarity. Hence, the dynamics probed is related to the surface of the vesicles. NSE will probe the bending surface dynamics.

Following the Zilman-Granek theory the Intermediate Scattering Function of the bending fluctuations can be modeled as:

$$\frac{I(Q,t)}{I(Q,0)} = exp\left[-(\Gamma_b t)^{2/3}\right]$$

And

$$\frac{\Gamma_b}{Q^3} \approx 0.0069\sqrt{\frac{k_B T}{\kappa}}\frac{k_B T}{\eta}$$

Combining the two equations:

$$\frac{I(Q,t)}{I(Q,0)} = exp\left[-\left(0.0069\sqrt{\frac{k_B T}{\kappa}}\frac{k_B T}{\eta}\right)^{2/3} Q^2 t^{\frac{2}{3}}\right]$$

Measuring I(Q,t)/I(Q,0) versus Q^2 allows you to compute the bending modulus κ which is of the order of 50 k_BT.

If you use tail deuterated lipids, you could also probe thickness fluctuations of the bilayer. The relevant Q-value will be the lengthscale which matches the inverse of the bilayer thickness.