

Studying peptide interactions with DPPC vesicles

Exercise to be performed on 16 June 2021

DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) is often the predominant lipid component in temperature responsive liposome systems. With a T_m of 41°C it is a useful candidate for the design of temperature responsive pharmaceuticals. The binding of peptides / proteins / enzymes to lipid vesicles is often impacted by the phase state of the lipid mixture e.g. fluid or gel. In this exercise we will design and plan a SANS experiment to look at the temperature dependent association of a model peptide with 50nm DPPC vesicles using hydrogenous and deuterated DPPC.

1. Using the physical parameters for DPPC provided in Table 1, calculate the concentration (mg/mL, mM) and mass of DPPC required for each neutron sample. (Hint: Assume that the DPPC vesicle is a sphere, neutron sample volume 200 μ L, particle concentration of 2×10^{13} particles / mL)
2. Calculate the SLD of DPPC and d62DPPC and use these to calculate the theoretical contrast match points (% D₂O) of the DPPC vesicles.
 - Calculate the SLD of H₂O and D₂O mixtures (10% intervals) and plot a graph of H₂O:D₂O SLD as a function of % D₂O
 - Fit a straight line to the H₂O:D₂O ratios and use the equation to calculate the % D₂O required to contrast match DPPC and d62DPPC i.e. the % D₂O required to match the DPPC and d62DPPC SLDs.
3. Using the simulated SANS data provided, calculate the experimental contrast match point of 50nm DPPC vesicles.
 - Plot the Intensity at $q = 0.005 \text{ \AA}^{-1}$ as a function of % D₂O
 - Fit the data and find the % D₂O at which the scattering is a minimum
4. Outline an experimental plan (i.e. which samples you would run) to study peptide binding as a function of temperature using SANS and contrast matching. You can assume that for DPPC you need 13 μ A per sample, for d62DPPC you need 40 μ A per sample and that you have a beam current of 40 μ A/h.

Resources

- Folder containing simulated data for 50 nm DPPC vesicles. These have been generated using the SaSView vesicle model using a PD ratio of 0.1.
 - o DPPC at 20, 50°C in 100% D₂O
 - o d62DPPC at 20°C in 80, 85, 90, 95, 100% D₂O
- Lipid SLD can be calculated here: <https://www.ncnr.nist.gov/resources/activation/>

DPPC chemical formula	C40H80NO8P
d62DPPC chemical formula	C40H18NO8PD62
DPPC mw	734,039
DPPC Area per Lipid @50°C (nm ² /molecule)	0,64
DPPC bilayer thickness @20°C (nm)	4,78
DPPC bilayer thickness @50°C (nm)	3,85

Table 1. DPPC physical parameters. Data sourced from www.avantilipids.com and J.F. Nagle et al. Biochim Biophys Acta. 1469(3), 159–195, 2000