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Planning a SANS experiment with protein lipid interactions

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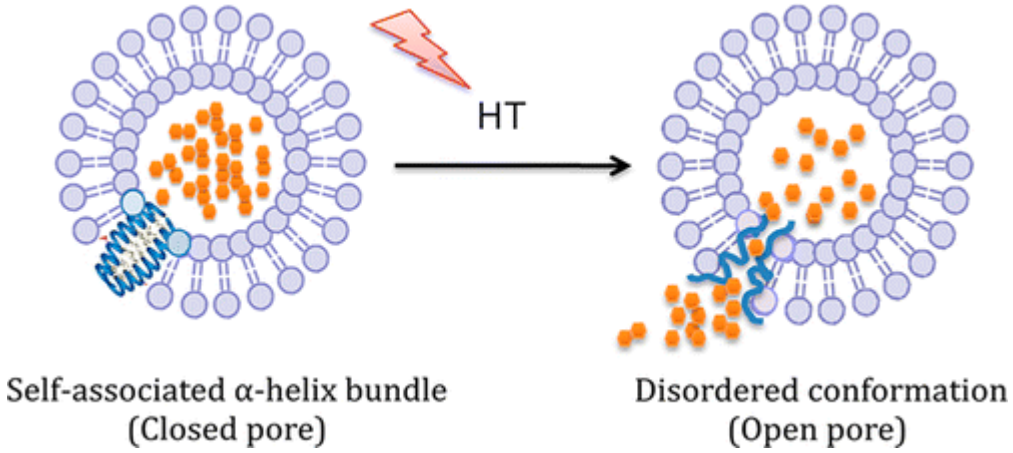
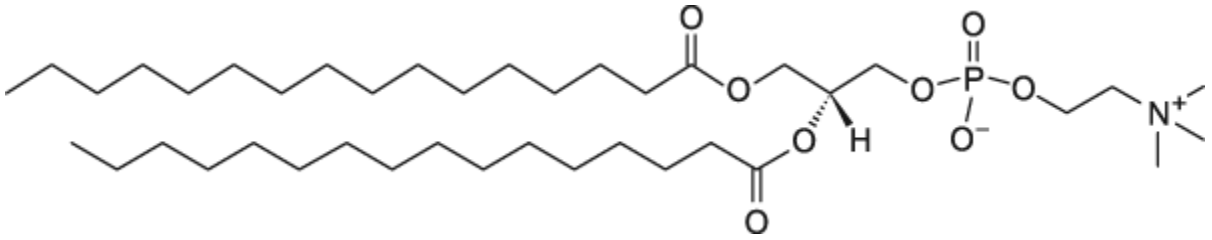
Potential inspiration for SANS experiment planning



Z.S. Al-Ahmady et al. ACS Nano 2012, 6, 10, 9335–9346

Studying protein / peptide interactions with DPPC vesicles



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

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 200µL, particle concentration of 2 x 10^{13} particles / mL)

$$\#\ \text{moles} = \frac{mass}{mw}$$

$$\#\ \text{moles} = \text{concentration} \times \text{volume}$$

$$\text{sphere surface area} = 4\pi r^2$$

Calculate the lipid concentration for the SANS experiment	
# particles / cuvette	4E+12
# lipids / particle	
Vesicle surface area (nm^2)	7,85E+03
# DPPC / vesicle	2,45E+04
lipids per sample	
# DPPC / sample	9,82E+16
DPPC conc (mM)	8,15E-01
DPPC conc (mg/mL)	5,98E-01
mass DPPC / sample (g)	1,20E-04

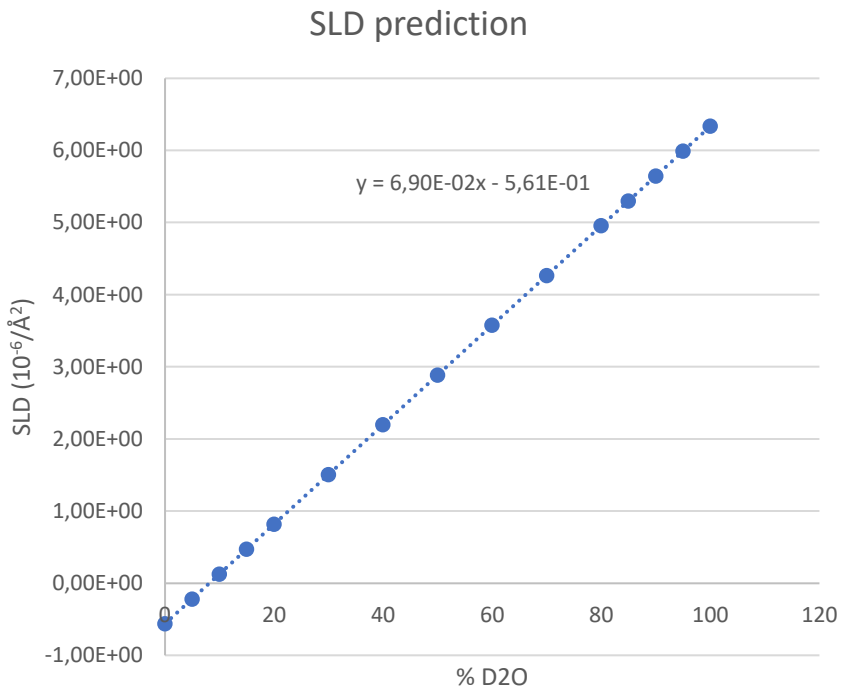
Considerations
- Peptide / protein concentration required for detectable scattering
- Lipid : peptide / protein ratio

Calculate the SLD of DPPC and d62DPPC and use these to calculate the theoretical contrast match points (% D_2O) of the DPPC vesicles

- Calculate the SLD of H_2O and D_2O mixtures (10% intervals) and plot the H_2O:D_2O SLD as a function of % D_2O

- Fit a straight line to the H_2O:D_2O ratios and use the equation to calculate the % D_2O required to contrast match DPPC and d62DPPC i.e. the % D_2O required to match the DPPC and d62DPPC SLDs

% H_2O	% D_2O	SLD (10^{-6}/Å^2)
0	100	6,34E+00
5	95	5,99E+00
10	90	5,65E+00
15	85	5,30E+00
20	80	4,96E+00
30	70	4,27E+00
40	60	3,58E+00
50	50	2,89E+00
60	40	2,20E+00
70	30	1,51E+00
80	20	8,18E-01
85	15	4,73E-01
90	10	1,29E-01
95	5	-2,16E-01
100	0	-5,61E-01



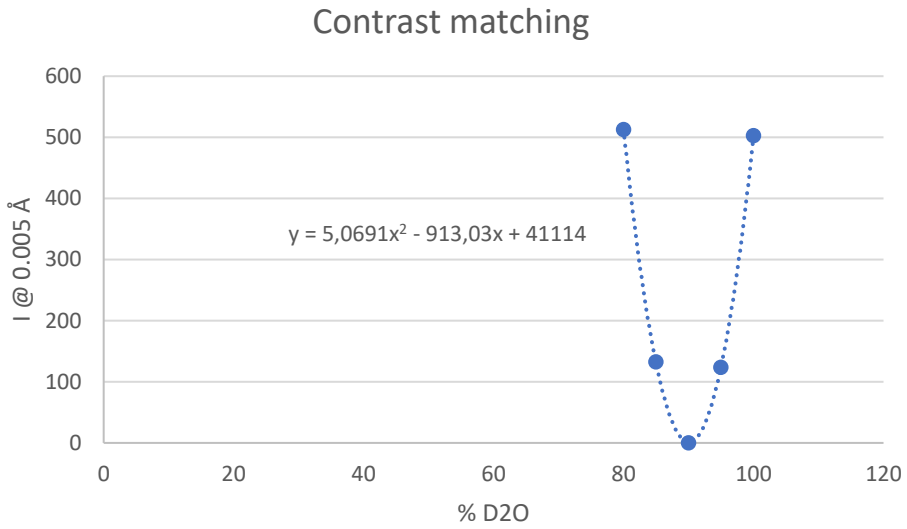
Molecule	Density (g / cm^3)	SLD (10^{-6}/Å^2)	% D2O contrast match
C40H80NO8P	0,9235	0,209	11,2
C40H18NO8PD62	0,9235	4,702	76,3
C40H80NO8P	1	0,226	11,4
C40H18NO8PD62	1	5,091	81,9
C40H80NO8P	1,11	0,251	11,8
C40H18NO8PD62	1,11	5,651	90,0

Example table showing how SLD varies with density

Using the simulated SANS data provided, calculate the experimental contrast match point of 50nm DPPC vesicles.

- Plot the Intensity at q = 0.005 $Å^{-1}$ as a function of % D_2O
- Fit the data and find the % D_2O at which the scattering is a minimum

% D_2O	I at 0.005 Å
100	502,294
95	123,381
90	0,0020736
85	132,27
80	512,627



Differentiate to get minimum	
dy/dx = 10,1382x - 913,03	
at dy/dx = 0, find x	90,06

Note: Simulated SANS data generated using a density = 1.11 g / cm^3, bilayer thickness 47.8 Å

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 uA per sample, for d62DPPC you need 40 uA per sample and that you have a beam current of 40 uA/h.

Potential experiment sections

- Concentration screening
- Hydrogenous samples
- Contrast matching
- Deuterated lipid at lipid contrast match point
- Deuterated lipid at protein contrast match point

Note: Data collection above and below the T_m

Potential supplementary experiments

- DLS
- SAXS of lipid and lipid + protein / peptide
- DSC of samples
- Dye release assays (if applicable)