Experimental report

27/11/2015

Proposal: 9-12-357 **Council:** 4/2014

Title: Detection of nano-rafts in hybrid lipo-polymersomes

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: PEO17-b-PDMS68-b-PEO17 / DPPC-d62
PDMS25-b-PEO15 / DPPC-d62
PEO12-g-PDMS22-g-PEO12 / DPPC-d62
PDMS70-b-PEO40 / DPPC-d62

Instrument	Requested days	Allocated days	From	To
D33	2	0		
D22	2	0		
D11	3	2	12/12/2014	14/12/2014

Abstract:

Hybrid vesicles made by the self-assembly of both amphiphilic copolymers and phospholipids attracted a lot of interest in the last years. Such assemblies are more advanced vesicular structures than their liposome and polymersome forerunners, as the best from the two systems is integrated in a single hybrid vesicle. They could be used as nano-reactors for enzymatic reactions or artificial biological cell membrane mimics. But the mechanisms governing phase-separation of the membrane into domains analogous to lipid rafts in cells are not known. For giant vesicles (GUV) observed by microscopy, hybrid vesicles either homogeneous (at least at the micrometer scale) or presenting micrometric domains can be obtained, depending on the two control parameters which are: the lipid/polymer ratio and the temperature relatively to the melting temperature (Tm) of lipids. However, no information is available in literature about the membrane structure of lipid/polymer hybrid small unilamellar vesicles (SUV). We aim to elucidate the effects of lipid fluidity, copolymer architecture and block length on the membrane structure of hybrid SUVs and to correlate them with the membrane structure seen on GUVs.

Detection of nano-rafts in hybrid lipo-polymersomes (proposal 9-12-357)

SANS experiments were carried out on D11 at scattering vectors q ranging from 0.0019 to 0.32 $Å^{-1}$ covered by three sample-to-detector distances (d= 1.2,8 and 34m) at the λ=6Å (with a full width at half-maximum value 10%). Contrast variation: different solvents mixtures have been used to match the scattering contribution of different components. The scattering length densities are reported below. In 9%v/v D_2O/H_2O mixture, the scattering length density (SLD) of PDMS polymer is matched and mainly lipid signal is observed. In 81%v/v D_2O/H_2O mixture, the lipid is matched and the scattering originates from polymer. Finally in pure D_2O, the whole polymer/$DPPC_{d62}$ objects are seen, with a weaker contribution of lipids regarding the small difference of SLD with the one of D_2O and its small volume fraction (0.15-0.30 v/v) in the samples. Samples in 1 or 2mm thick quartz cells were thermalized ±1°C. They were first measured at room temperature, then at 47°C, and finally again at 25°C to test reversibility. Raw curves were corrected from the empty cell and other sources using LAMP to obtain SANS curves in cm^{-1}. Different models were used for analysis. Various fits to vesicle, disk or core-shell cylinder form factors were done with SasView. A new model is proposed to describe the scattering of phase separated polymer/lipid vesicles. This hybrid vesicle model is inspired by a previous work on holey vesicles [*L.M. Bergström, S. Skoglund, K. Edwards, J. Eriksson, I. Grillo, Langmuir, 2014, 30, 3928*]. The analytical expression of the form factor employed is:

$$S_{hybrid\ vesicle} = \left(\frac{(\rho_v - \rho_o)\, V_v\, F_{b,\delta_v}\, F_v(q, R_v + \delta_v/2) - (\rho_v - \rho_o) N_d\, (\pi R_d^2\, \delta_v) \quad F_{b,\delta_v}\, F_{h,R_d} + (\rho_d - \rho_o)\, N_d\, V_d\, F_{b,\delta_d}\, F_{h,R_d}}{V_v - N_d\, (\pi R_d^2\, \delta_d) + N_d\, V_d} \right)^2$$

where ρ_v, ρ_d, ρ_o are the scattering length densities of the vesicle (polymer), of disks (lipid) and of the solvent. $F_v(q, R_v + \delta_v/2)$ is the scattering amplitude of an infinitely thin circular shell with radius $R_v + \delta_v/2$, F_{h,R_d} is the scattering amplitude of symmetrical circles with radius R_d. F_{b,δ_v} (F_{b,δ_d}) is the scattering amplitude of a bilayer cross-section of thickness δ_v (δ_d). V_d is the disk volume $V_d = \pi R_d^2\, \delta_d$ and V_v is vesicle volume approximated by $V_v \sim 4\pi (R_v + \delta_v/2)^2\, \delta_v$. For polydisperse hybrid vesicles, this model has 11 fit parameters. Considering our data up to a q value about 0.12$Å^{-1}$ where the scattering generally reaches the incoherent background, the number of data points is N≈ 150. To reasonably describe the main trends governing the phase separation occurring, we reduced the number of fit parameters using following assumptions:

1. Scattering length densities are those of pure compounds (as on the table),
2. The membrane thickness of polymers and of lipids (δ_v, δ_d) are fixed to the values measured for the pure compound vesicles.
3. The volume fraction of lipid is let to vary in a range compatible with independent measurement by gravimetry and phospholipid chemical titration *($\Phi \sim 0.0035$)*.

Solvent/	SLD (10^{10} cm^{-2})
D_2O/ H_2O 9%v/v	0.0665
D_2O/ H_2O 81%v/v	5.03
H_2O	-0.56
D_2O	6.40
PDMS	0.064
$DPPC_{d62}$ 20°C	5.84
$DPPC_{d62}$ 50°C	5.42

Thus 5 parameters allows describing the phase separation occurring in the hybrid vesicles: $R_v, \sigma_{R_v}, N_d, R_d, \sigma_{R_d}$. Data fitting was performed with Matlab® using nonlinear least squares curve-fit. Throughout all the fits with Matlab® and SasView, corrections were made for instrumental smearing.

Pure compounds.

All the SANS curves of solutions of pure compounds display the characteristic q^{-2} decreasing in a wide intermediate q range: they are well fitted with the polydisperse vesicle model (see Figure 1).

SANS curves of pure block copolymers (● PDMS-g-(PEO)$_2$, o PEO-b-PDMS$_{1.5K}$-b-PEO + PEO-b-PDMS$_{3K}$-b-PEO ◢ PEO-b-PDMS$_{5K}$-b-PEO shifted for a sake of clarity). Lines are the best fits to the vesicle model. The grafted copolymer PDMS-g-(PEO)$_2$ forms well-defined vesicles of inner radius R_v~35nm and membrane thickness δ_v=5.6nm. Triblock copolymers PEO-b-PDMS-b-PEO also form vesicles, either much larger or smaller but all very disperse in size. However, membrane thickness can be accurately determined: from 5.5nm to 11.7nm when increasing copolymer molecular weight. When increasing the temperature from 20°C to 47°C, these morphologies remain. DPPC$_{d62}$vesicles are also rather well defined, R_v~30nm σ_{Rv}~0.3 but their membrane thickness decreases from δ_v= 4.3nm at 20°C down to 3.1nm at 47°C, in agreement with the values determined by volumetry,[1] respectively 4.57nm at 20°C for DPPC in the gel L_β phase and 3.51nm at 50°C in the fluid L_α phase. Although the global size of pure vesicles cannot be well controlled, their membrane thickness well correlates with the copolymer block length. Only the lipid membrane thickness changes with temperature.

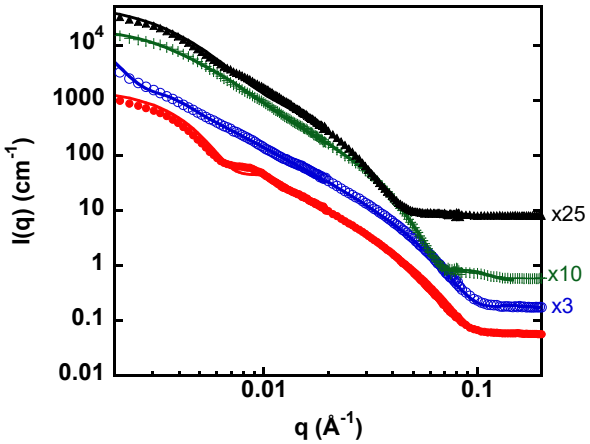


Figure 1: *SANS curves of pure ● PDMS-g-(PEO)$_2$, o PEO-b-PDMS$_{1.5K}$-b-PEO + PEO-b-PDMS$_{3K}$-b-PEO ◢ PEO-b-PDMS$_{5K}$-b-PEO. Lines are the best fits to the vesicle model.*

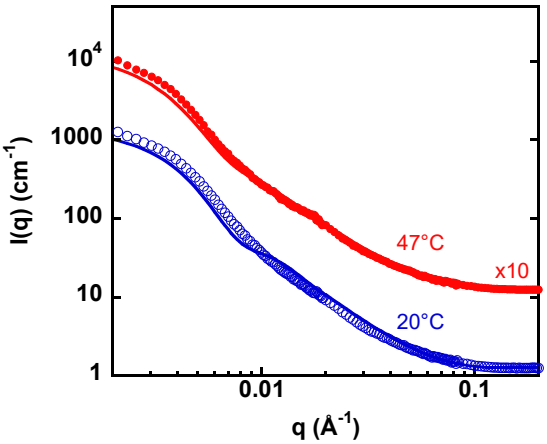
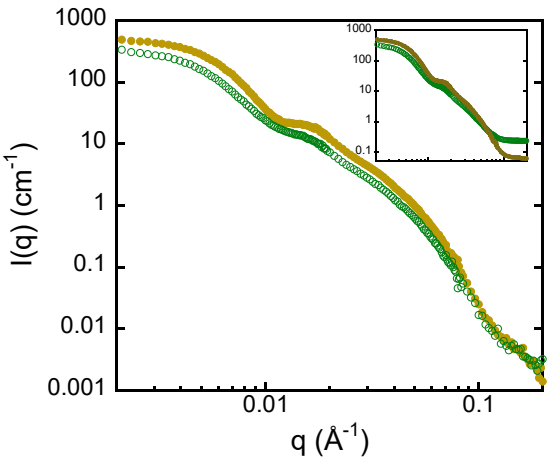


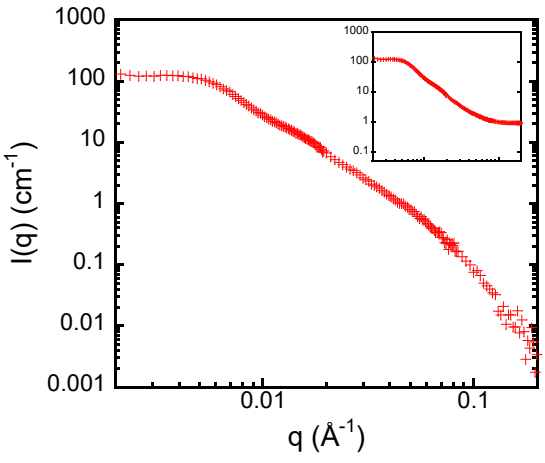
Figure 2: SANS curves of DPPC$_{d62}$ in H_2O at room temperature and at 47°C. Lines are the best fits to the vesicle model.

Mixed systems: Copolymers / DPPC$_{d62}$

Contrast matching method was used to measure either the whole mixed system in full contrast (pure D_2O), or the lipids by matching the polymer (lipid contrast) or the copolymers by matching lipid (polymer contrast). All samples contained deuterated DPPD$_{d62}$ to have the same interaction parameter χ between the copolymers and lipids and produce the same phase separation. Three series at different lipid fractions were measured: 0.15, 0.21, 0.30 v/v. So, the studied systems were mainly composed of copolymers. Three temperature series were performed: first at 20°C where the lipid is in the gel phase, then at 47°C where it is in the fluid phase and then back at 25°C. Curves obtained in polymer contrast and full contrast resemble each other: they display a damped oscillation, around 0.01Å^{-1}, assigned to a spherical shape of polydisperse scattering objects and at intermediate range a kind of q^{-2} decreasing generally ascribed to flat vesicle membranes (Figure 3a). The curves in full contrast are nevertheless slightly higher. This is not surprising since first the volume fraction of DPPC$_{d62}$ is small (0.15 up to 0.3) and second its scattering length density 5.8 10^{10} cm^{-2} is close to the one of D_2O, 6.4 10^{10} cm^{-2}, i.e. its contrast in pure D_2O is small. The scattering is thus dominated by polymers. The ratio of intensities of full contrast and polymer contrast is in fair agreement with the ratio of contrasts and only little changes in the scattering are visible at high q. In lipid contrast (Figure 3b), different features are observed depending on the systems: the scattering signal looks like neither disk-like lipid domains nor large pure liposomes (Figure 2). The signal at low q indicates large fluctuations, indicating that lipids could be located all over the vesicles and not only located in small disk-like domains. Regarding temperature, curves obtained at 25°C after heating at 47°C above the gel-to-fluid transition are perfectly superimposed to the ones measured at 20°C before the heating. For all the samples, the structural changes observed are thus perfectly reversible.

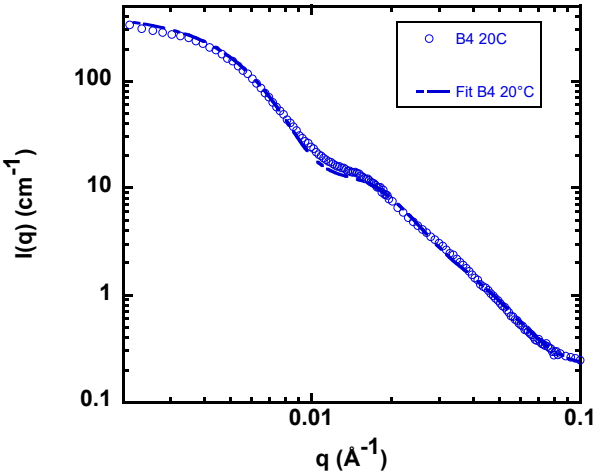


a)



b)

Figure 3: SANS signal of PEO-b-PDMS$_{1.5k}$-b-PEO / DPPC$_{d62}$ mixtures at 20°C, at a volume fraction f_d=0.15: (a) in full contrast (●), in polymer contrast (○), and (b) in lipid contrast (+). Insets are SANS curves before subtraction showing the incoherent components.



PEO-b-PDMS$_{1.5k}$-b-PEO / DPPC$_{d62}$

Figure 4a: Hybrid vesicle fit R_v =20.5nm σ_{Rv}=0.32 N_d =8.9 R_d =1.6nm σ_{Rd} = 0.9

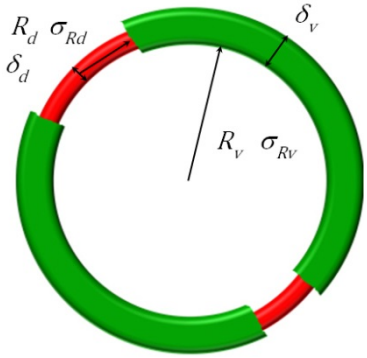
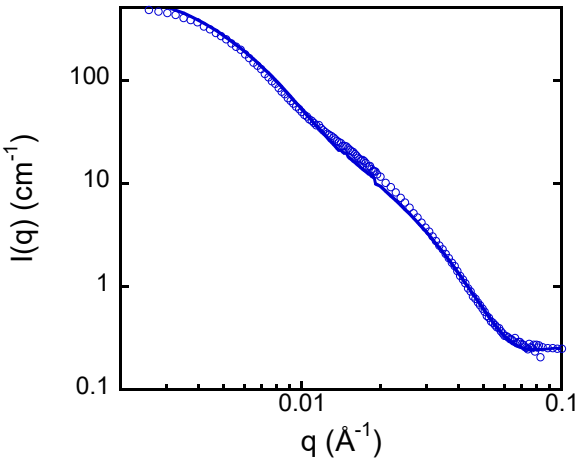
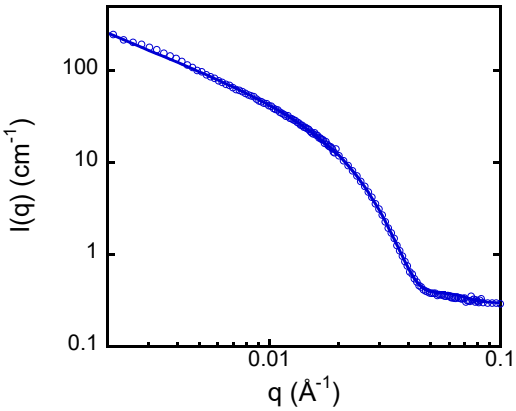


Figure 4b: Scheme of the hybrid vesicle model: a vesicle with inner radius R_v (distribution σ_{R_v}) and thickness δ_v, containing N_d disks of radius R_d (distribution σ_{R_d}) and thickness δ_d.



PEO-b-PDMS$_{3k}$-b-PEO / DPPC$_{d62}$

Figure 4c: Vesicle fit: R_v =12.4nm σ_{Rv}=0.52 δ_v =8.3nm $\sigma_{\delta v}$ = 0.07



PEO-b-PDMS$_{5k}$-b-PEO / DPPC$_{d62}$

Figure 4d: Cylinder fit: R_i =7.7nm σ_{Ri}=0.16 (infinite length)

To conclude, these SANS experiments on D11 evidenced a rich nano-structuration of hybrid lipo-polymersome membranes and are currently correlated with independent studies by other techniques (FRET, NSE, NMR relaxometry…) in order to draw a global picture.

[1] *J.F. Nagle and M.C. Wiener Biochimica et Biophysica Acta, 942 (1988) 1-10; J. F. Nagle, S.Tristram-Nagle Biochimica et Biophysica Acta 1469 (2000) 159-195*