Supporting Information

Tear of Lipid Membranes by Nanoparticles

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Supporting Figures



Figure S1. Static light scattering (SLS), small-angle X-ray and neutron scattering experiments (SAXS and SANS) of liposomes, NPs and of mixtures of liposomes and NPs.



Figure S2. Cryo-TEM images of SiO₂ nanoparticles (SM30 Ludox[®]) and DOPC liposomes at nanoparticles per liposome ratio r = 5, at different pH. **A**. Image of the SM30 particles in a brine solution of 10 mM KCl at pH 7, one minute after the nanoparticles have been added to the liposome solution. **B**. Similar conditions after 30 minutes. **C**. Image of the SM30 particles in a brine solution of 10 mM KCl at pH 10, one minute after the nanoparticles have been added to the liposome solution. **D**, **E** and **F**. Similar conditions after 30 minutes.



Figure S3. Cryo-TEM images of SiO₂ nanoparticles (SM30 Ludox[®]) and DOPC liposomes at nanoparticles per liposome ratio r = 5, at pH 5.5. **A**. Image of the SM30 particles in a solution without added salt one minute after the nanoparticles have been added to the liposome solution. **B**. Similar conditions after 30 minutes.



Figure S4. Effect of the number ratio r between the SiO₂ nanoparticles (SM30 Ludox[®]) and DOPC liposomes, in a brine solution of 10 mM KCl at pH 5.5, one minute after the nanoparticles have been added to the liposome solution. Cryo-TEM images of the SM30 particles and DOPC liposomes at **A**. r=5, **B**. r=50 and **C**. r=140.



Figure S5. Effect of the number ratio r between the SiO₂ nanoparticles (SM30 Ludox[®]) and DOPC liposomes, in a brine solution of 10 mM KCl at pH 5.5, one hour after the nanoparticles have been added to the liposome solution. Cryo-TEM images of the SM30 particles and DOPC liposomes at r=50.



Figure S6. Effect of the number ratio r between the SiO₂ nanoparticles (SM30 Ludox[®]) and DOPC liposomes, in a brine solution of 10 mM KCl at pH 5.5, one hour after the nanoparticles have been added to the liposome solution. Cryo-TEM images of the SM30 particles and DOPC liposomes at r=140.



Figure S7. Left panels display autocorrelation functions C(q,t) as a function of time and right panels the corresponding relative distribution of relaxation times recorded at a scattering wave vector $q = 6.84 \times 10^{-3} \text{ nm}^{-1} (\theta = 30^{\circ})$ for free liposomes (•) and for different mixtures of nanoparticles per liposome ratios (A, B) r=280, (C, D) r=140, (E, F) r=50, (G, H) r=5. Analysis realized at different time interval; (\blacktriangle) immediately after preparation, (\triangledown) after 2h, (\blacksquare) after 24h. Dashed lines are given as guides to the eye.



Figure S8. Left panels display the q² dependence with relaxation rate (Γ) and right panels the corresponding q² dependence with diffusion coefficient at scattering vector (q) ranging between 4.6 x 10⁻³ nm⁻¹ and 2.55 x 10⁻² nm⁻¹ for free liposomes (\bullet) and for different mixtures of nanoparticles per liposome ratios (A, B) r=280, (C, D) r=140, (E, F) r=50, (G, H) r=5. Analysis realized at different time interval; (\blacktriangle) immediately after preparation, (\blacktriangledown) after 2h, (\blacksquare) after 24h. Dashed lines are given as guides to the eye.



Figure S9. Total light scattered intensity (I) as a function of the scattering wave vector q at 20°C for free nanoparticles (\blacklozenge), free liposome (\bullet) and for different mixtures of nanoparticles per liposome ratios; (A) r=280, (B) r=140, (C) r=50, (D) r=5. Analysis realized at different time interval; (\blacktriangle) immediately after preparation, (\triangledown) after 2h, (\blacksquare) after 24h. Dashed lines are theoretical expressions of light scattered intensity obtained using Eq.4 (described below in the supporting information, parameters used are listed in table-S1). The schematic representations illustrate the resulting objects after mixing liposomes with SiO₂ nanoparticles.



Figure S10. Schematic representation of model used in theoretical expression (Eq- S4) of light scattering intensity for free liposomes in the absence (A) and in the presence (B) of SiO₂ nanoparticles.

Supporting Methods

1. Sample preparation

1.1 Preparation of DOPC LUVs

a- Cryo-TEM characterization. A solution of DOPC in chloroform/MeOH was evaporated in a rotary evaporator to obtain a thin film. This film was hydrated at room temperature with deionized water (resistivity higher than 18 Ω) containing 10 mM of KCl and homogenized by vigorous stirring to yield 10 mg.mL⁻¹ concentrated dispersions of vesicles. Suspensions of liposomes without added KCl where equally prepared. The resulting coarse dispersion was submitted to sonication (Vibracell 75042, 15 mm titanium probe, 16 min, 50% pulse). The resulting transparent dispersion was heated at 40 °C for 1hour in order to anneal defects that could have been produced by sonication.

b- Light scattering measurement. Liposomes were prepared by the film rehydration process followed by extrusion through a polycarbonate membrane. Briefly, a solution (100 mg.mL⁻¹) of DOPC in chloroform/MeOH (3v:1v) was prepared, then a thin film of lipid was formed by evaporation of solvents under reduce pressure. The thin film was then hydrated in aqueous solution of 10 mM of KCl under gentle agitation during 2 hours. Afterwards, LUVs (1 mg.mL⁻¹) were obtained by extrusion (21 times) through a polycarbonate filter with pore sizes of 100 nm at room temperature.

1.2 Preparation of DOPC/SiO2 nanoparticles mixtures

a- Cryo-TEM characterization. Microscope images from mixtures with nanoparticle per liposome ratio r=5 where prepared from aqueous dispersions (10 mM of KCl) of SiO₂ nanoparticles (3 mg.mL⁻¹) mixed with liposomal suspensions of DOPC (10 mg.mL⁻¹) giving a final concentration of 8.33 mg.mL⁻¹ of DOPC liposomes. Other ratios r where prepared from the same initial concentrations of nanoparticles and liposomes, with the proportions of NP dispersions and liposome suspensions required to achieved the target ratios.

b- Light scattering measurements. In order to prepare mixtures with four nanoparticle per liposome ratios (r), r= 5, 50, 140 and 280, aqueous dispersions (10 mM of KCl) of SiO₂ nanoparticles (diameter of 10 nm) with appropriate concentrations were added separately to the suspension of liposomes (0.12 g.L⁻¹) described above. The final liposome concentration in mixtures was kept constant (0.06 g.L⁻¹) for all ratios. Samples were analyzed immediately after preparation and at 2 and 24 hours.

2. Cryo-Transmission Electron Microscopy (cryo-TEM).

Images were obtained a vitrified sample, at room temperature. The vitrification of the samples was carried out in a homemade vitrification system. The chamber was held at 22 °C and the relative humidity at 80%. A 5 μ l drop of the sample was deposited onto a lacey carbon film covered grid (Ted Pella) rendered hydrophilic using an ELMO glow discharge unit (Cordouan Technologies). The grid is automatically blotted to form a thin film which is plunged in liquid ethane hold at -190°C by liquid nitrogen. In that way a vitrified film is obtained in which the native structure of the vesicles is preserved. The grid was mounted onto a cryo holder (Gatan 626) and observed under low dose conditions in a Tecnai G2 microscope (FEI) at 200 kV. Images were acquired using an Eagle slow scan CCD camera (FEI).

3.Dynamic and static light scattering (SLS and DLS)

Experiments were performed with a multibit, multitau, full digital ALV/CG-3 correlator in combination with a Spectra-Physics laser (22 mW HeNe linear polarized laser operating at wavelength of λ = 632.8 nm) and a thermostat bath controller (20 °C). Measurements were made at angles θ ranging between 20° and 150° with corresponding scattering vectors q ranging from q=4.6 x 10⁻² nm⁻¹ to 2.55 x 10⁻¹ nm⁻¹. The autocorrelation functions g₁(t) were analyzed in terms of relaxation time (τ) distribution (Eq-S1) according to the REPES method (see examples at θ =30°, Fig. S7). Hydrodynamic radius (R_H) was estimated using the Stokes-Einstein relation (Eq-S2), where D₀ is diffusion coefficient obtained from the relaxation rate (Γ) (see Fig. S6), K_B is the Boltzmann constant (1.38 10⁻²³ J.K⁻¹), T is experimental temperature (20°C) and η_s is the viscosity of the solvent (water, 1 Pa.s).

$$g_1(t) = \int A_{(\tau)} \exp\left(\frac{-t}{\tau}\right) d\tau$$
 Eq-S1

$$R_H = \frac{\kappa_B T}{6\pi \eta_s D_0}$$
Eq-S2

For each angle θ , rayleigh ratio (R $_{\theta}$) of samples, recorded by SLS measurements was obtained using (Eq–S3) as shown in fig. S7. Where I_{solution}, I_{water}, and I_{toluene} are the intensities scattered by the solution, water (solvent) and toluene (reference), respectively. n_{water} = 1.33 and n_{toluene} = 1.496 are the refractive indexes, and R_{toluene} = 1.35 ×10⁻⁵ cm⁻¹ is the Rayleigh ratio of toluene for a wavelength λ = 632.8 nm.

$$R_{\theta} = \frac{I_{solution}(\theta) - I_{water}(\theta)}{I_{toluene}(\theta)} \times \left(\frac{n_{water}}{n_{toluene}}\right)^2 \times R_{toluene}$$
Eq-S3

The large optical contrast between liposome $(n_{DOPC} = 1.52)$, SiO₂ nanoparticles $(n_{SiO_2} = 1.45)$ and water $(n_{H_2O} = 1.33)$ is favorable to study the interaction process of nanoparticles with liposomes. To this aim, the Rayleigh ratio R₀ was fitted by a modified Rayleigh-Gans-Debye approximation (RGDA) of the total scattered intensity *I* for a coated sphere using (Eq-S4) [1,2,3], where $f = 1 - \frac{t}{R}$, with R the radius of vesicle and t the thickness of lipid bilayer as schematically depicted in Fig. S8-A; $x = R \times q$, with q the scattering vector; $j_1(x)$ is the first-order spherical Bessel function; $m_1 = \frac{n_m}{n_w}$ with n_w and n_m the refractive indexes of aqueous medium (1.33) and membrane, respectively. For free liposomes, n_m is estimated to be of 1.52 while in case of the nanoparticles/liposomes mixture, the membrane refractive index n_m was estimated from the refractive indexes of the SiO₂ nanoparticles (n_{NPS}=1.45) and of the lipids (n_{lipid} 1.52) using the mass fraction of each component (y_{NPs} of silica nanoparticle and lipid (y_{NPs}) (Eq-S5).

$$I = \left((m_1 - 1) \left(\frac{3 \times j_1(x)}{x} - f^3 \times \frac{3 \times j_1(f \times x)}{f \times x} \right) \right)^2$$
Eq-S4

3.1 DOPC/SiO2 interaction:

In order to study the interaction of DOPC liposomes (R_h = 58 nm) with small SiO₂ nanoparticles (R_h = 5 nm), four aqueous SiO₂ nanoparticle solutions were mixed separately with a diluted aqueous liposome solution with a constant concentration of 0.12 g.L⁻¹ as described above. Four mixtures with different nanoparticle per liposome ratio r were then prepared; high ratio (r=280), intermediate ratios (r= 50, 140) and low ratio (r=5).

Fig. S9 displays the scattered intensity as a function of the scattering vector for liposome solutions without nanoparticles and with nanoparticles at four ratios. In the case of the liposome solution, the scattered intensity was well fitted by theoretical expression described above (Eq. S4) using parameters listed in table-S1. Fig. S9 shows also that immediately after mixing nanoparticles with liposomes the light scattering intensity becomes, for all solutions, different from the sum of the single components (liposomes and nanoparticles), indicating the presence of rapid and strong interactions between SiO₂ nanoparticles and liposomes. In addition, compared to the scattering intensity of liposomes, a shift of values and deviation of shapes are observed after adding nanoparticles depending on the nanoparticles per liposome ratio used, indicating the presence of different interaction processes as discussed below.

3.2 Case-1: High ratio of nanoparticles per liposome (r=280):

Fig.S7-A shows the relaxation functions C(q,t) as a function of time for liposomes before and after mixing with nanoparticles at r = 280. A small shift of the relaxation function is observed in comparison to the free liposomes - see fig. S7-A - indicating a small change of the diffusion coefficient of the scattering objects as depicted also in figs. S8-A,B. The diffusion coefficients of the liposome-nanoparticle mixtures are smaller than that of the liposomes, indicating a small increase of hydrodynamic radius R_h , 58 ± 3 nm in the absence and 66.6 ± 3 nm in the presence of nanoparticles.

Fig.S9-A shows the light scattering intensity as a function of the scattering vector q for liposomes before and after adding nanoparticles at r = 280. The shape of the scattering function is preserved indicating a conservation of the vesicular morphology of liposomes upon addition of the nanoparticles, and therefore shows that there is no cluster formation. In addition, a significant rising of scattered intensity is observed for the resulting objects comparing to free liposomes, indicating strong interaction between liposomes and nanoparticles as described above. Landfester et.al. [2] have observed similar phenomenon after adding SiO₂ nanoparticles having higher hydrodynamic radii (14, 25, 36 and 57 nm) to PDMS-b-PMOXA polymersomes. Authors have associated such increase of light scattering to increasing values of inner polymersome refractive index due to nanoparticles incorporation into the interior of polymersomes by endocytosis mechanism [2]. This mechanism is unlikely in our case given that nanoparticle crossing of the membrane requires membrane consumption leading to vesicle shrinkage [2], while figs S8-A,B show that in our case the size of resulting objects is almost similar to pure liposomes. The increase of the scattered intensity observed in fig. S9-A can be associated to the adsorption and saturation of liposomes surface by nanoparticles leading to hybrid liposomes as schematically described in fig. S10A. By considering such scenario (see fig. S10-B), the scattering intensity of the resulting objects could be fitted well with the theoretical expression (Eq-S4) using a higher thickness (12.5 \pm 0.15 nm) and modified refractive index of hybrid membrane (1.466) as listed in table-S1.

3.3 Case-2: intermediate ratios of nanoparticles per liposome (r= 50 and 140):

Figs.S9-B,C show the light scattered intensity as a function of the scattering vector q for liposomes before and after adding nanoparticles at r = 140 and r = 50. In both cases, an increase of scattered intensity is observed after mixing liposomes with nanoparticles, indicating a strong interaction of liposomes with nanoparticles as described above. For these ratios the shape of the scattering intensity are significantly modified by the addition of nanoparticles, the resulting objects are modified comparing to the free liposomes, indicating change of vesicular morphology and therefore does not allow fitting light scattering by the theoretical expression (Eq-4) as it previously made at r= 280. Such deviations are associated to cluster formation as schematically presented in figs. S9-B,C, which is corroborated by the rise of the resulting objects sizes depicted in figs. S8-C,D and figs. S8-E,F. For both ratios, a white solid is formed after 24h mixing. Cluster formation could be attributed to the presence of bridges formed by nanoparticles between the membranes of different liposomes given the strong attraction between DOPC and SiO₂ nanoparticles, and to the low coverage of liposome surfaces by nanoparticles given the lower r used here.

3.3 Case-3: low ratio of nanoparticles per liposome (r= 5):

Fig.S7-G shows the relaxation function C(q,t) as a function of time for liposomes before and after mixing with nanoparticles at low nanoparticle per liposome ratio (r=5). No shift of the relaxation function is noticed after adding the nanoparticles, indicating that the resulting size of the objects remain similar to the ones of bare liposomes as it is demonstrated by the diffusion coefficients D(q) shown in figs. S8-G,H. However, unlike observations made above at high ratios (r=280, 140 and 50), fig. S9-D shows that when a small amount of nanoparticles is added to the liposomes (r=5), the light scattered by the suspension decreases immediately, from its initial value after mixing. Such phenomenon is consistent with the partial destruction of liposome exposed to a small number of nanoparticles, which causes a reduction of the liposome destruction also leads to nanoparticles wrapped by a lipid bilayer, this new species do not contribute enough to the signal to be detected. It is also important to stress that, despite the decreasing values scattered light intensity, the shapes of the scattered functions do not change, indicating that the vesicles remaining in solution are preserved, without the formation of clusters.

Light scattering experiments thus confirm the scenario presented in the main text of the paper. At high nanoparticles per liposome ratio, hybrid nanoparticles/liposomes objects are produced by adhesion of nanoparticles on liposomes surface. At intermediate ratios, cluster formation is observed. However, when low amount of nanoparticles is mixed with liposomes, partial liposome destruction occurs and wrapped nanoparticles by lipid bilayer are produced.

		n_w^a	$n_m{}^b$	R ^c	T ^d
Free liposomes		1.33	1.52	58nm	5nm
r=280	Immediately after mixing	1.33	1.466	60nm	11nm
	2 hours	1.33	1.466	60nm	12.5nm
	24 hours	1.33	1.466	60nm	14nm

Table-1 Parameters used in theoretical expression (Eq. 4) of light scattering intensity for free liposomes and mixture at nanoparticles per liposome ratio of 280.

^aRefractive index of water. ^b Refractive index of membrane. ^c Hydrodynamic radius of vesicle. ^d Thickness of vesicle membrane.

4. Small angle X-ray and neutron scattering (SAXS-SANS)

Small angle X-ray scattering experiments have been performed with a Rigaku microfocus rotating anode generator (Micromax-007 HF) operating at 40kV and 30 mA (Cu-K radiation, λ = 1.54 Å). The X-ray beam was monochromatized and focused with a confocal Max-Flux Optics developed by Osmics, Inc. together with a three pinholes collimation system. Scattered intensity was measured with a 2D multiwire detector located at 0.81 m from the sample. This configuration allowed q vectors to be investigated in the range 0.01 Å⁻¹< q <0.33 Å⁻¹ (q is defined as q=4 π / λ sin (θ /2) where λ is the wavelength of the incoming beam and θ is the scattering angle). The suspensions were held in calibrated mica cells of 1 mm thickness. Scattering patterns were corrected according to the usual procedures. Data were radially integrated, corrected for electronic background, detector efficiency, empty cell scattering and transmission. Scattering from the pure solvent was measured separately and removed from the sample measurements. Intensity was converted into absolute scale using calibrated Lupolen as a standard. Measurements have been performed on pure NP and DOPC suspensions as well as on DOPC-NP mixtures.

Small angle neutron scattering experiments have been performed two weeks after sample preparation at the Orphée neutron facility of LLB-CEA Saclay on the PAXY spectrometer with an anisotropic two-dimensional detector. Dispersions of SiO₂ NPs (2.8 g.L⁻¹) mixed with DOPC liposomes (48 g.L⁻¹) at a number ratio r=5 where prepared in two solvent compositions: in pure D₂O, of neutron scattering length density $SLD(D_2O)=6.40\times10^{-6}$ Å⁻², where the neutron scattering signal originates both from silica, $SLD(SiO_2)=3.32\times10^{-6}$ Å⁻², and from DOPC, $SLD(DOPC)=0.30\times10^{-6}$ Å⁻², and in a D₂O:H₂O mixture (56:44 v/v) matching the neutron scattering length density of silica, in which the signal arises from the phospholipids only. Three beamline configurations were used to cover overlapping scattering vector (q) ranges of $1.92\times10^{-3} - 2.84\times10^{-2}$, $1.05\times10^{-2} - 0.154$, and $3.19\times10^{-2} - 0.427$ Å⁻¹, with the following values of sample-to-detector distance D and neutron wavelength λ : D=7 m and λ =15 Å, D=3 m and λ =6 Å, D=1 m and λ =6 Å. The scattering intensity curves were divided by the transmission factor and subtracted from the incoherent background, before normalizing by the flat signal of a cuvette filled with light water to correct the detector efficiency, yielding the absolute intensity in cm⁻¹.

Nanoparticles suspensions

NPs have been characterized in very dilute suspensions i.e. in a concentration range where only their form factor contributes to the scattered intensity (the inter-particle structure factor S(q)=1)). Analysis with a spherical model (homogeneous scattering length density ρ =18.65 10¹⁰ cm⁻²) reveals clear deviations from a pure monodisperse system. A model of polydisperse spheres forming very small clusters (program SASview) has been used to determine the geometrical properties of the NP. The mean diameter of the spherical subunits is 9 nm (σ =0.25) while clusters contain two elementary spherical particles. This description is in accordance with cryo-TEM measurement that leads to a typical size of the order of 12 nm, in between the average diameter of one spherical subunits (9 nm) and the average length of the NP doublets (18 nm).

Nanoparticles – DOPC suspensions

SAXS experiments were performed on the same suspension as measured by cryo-TEM while SANS measurements was done at much higher concentration and shorter delay after preparation (two weeks). After two weeks, the typical form factor of multilamelar vesicles $(q^{-2}$ dependence and characteristic Bragg peaks) is still observable on the SANS curves. Neutron scattering overlaps with SLS at low q (Guinier plateau) and is compatible with the SAXS curve of pure lipids at high q (Bragg peaks at q=0.1 and 0.2 Å⁻¹). Strong scattering (very similar to that of pure NPs suspensions) is observed by SAXS in the low qregion. Furthermore, a large shoulder appears around 0.1Å⁻¹ that matches the scattering of pure DOPC suspensions (if we omit Bragg peaks). The most important point is that Bragg peaks (initially present for pure DOPC suspensions) disappear in SAXS measurements (i.e. after four months) while it is still present on SANS measurements after two weeks. The destruction of multi-lamellar vesicles observed after four months evidences the large impact of the NPs on the organisation of the DOPC membranes at long times. SAXS curves cannot be explained on the basis of a single form factor associated to "decorated" NPs (core-shell organisation: NP + adsorbed DOPC layer). More likely, free unilamellar vesicles are still present in the suspension and are responsible for the large shoulder around 0.1 Å⁻¹. The system contains a large fraction of unilamellar vesicles (no Bragg peak). Due to the large Xray scattering length density of the SiO₂ NPs with respect to the solvent (but also to the DOPC molecules), their contribution dominates the scattered intensity at low q. The adsorbed DOPC layer only contributes at larger q. Unfortunately this contribution is hidden by the large shoulder observed above 0.07Å⁻¹ that originates from pure and free DOPC vesicles. Therefore, from the scattering experiments, the exact nature of the adsorbed layer (between monolayer or bilayer) cannot be determined.

Supporting References

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