Experimental report

16/02/2017

Proposal: 9-13-597 **Council:** 4/2015

Title: Effects of Gold Nanoparticles on the Elasticity of Model Lipid Membranes: a Neutron Spin Echo Study

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: gold nanoparticles
POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) vesicles

Instrument	Requested days	Allocated days	From	To
D11	0	1	04/11/2015	05/11/2015
IN15	7	0		
D22	1	0		
D33	0	0		

Abstract:

Gold nanoparticles (AuNPs) have been extensively investigated in the past years, for the development of smart and multifunctional devices for theranostics, specific targeted drug delivery or biosensing. In parallel, there has been increasing necessity to evaluate their interaction with biological interfaces, such as lipid membranes, still far from being completely understood.
We propose to use NSE to study the effect of four types of nanoparticles (cationic gold nanorods and nanospheres, anionic gold nanoparticles as-synthesized and coated by a protein corona) on the membrane fluidity of POPC liposomes, to provide an overview of the possible structural and functional factors that determine their effect on membranes.
In particular, the effects on membrane fluidity will be assessed with the Zilman-Granek model and correlated with the results already obtained with Fluorescence Correlation Spectroscopy on the same stystems.

Effects of Gold Nanoparticles on the Elasticity of Model Lipid Membranes: a Neutron Spin Echo Study

Thanks to their optical properties, facile synthetic routes and easy surface functionalization, gold nanoparticles (AuNPs) have been extensively investigated in the past years, for the development of smart and multifunctional devices for theranostics, specific targeted drug delivery or biosensing.[1,2] In parallel, there has been increasing necessity to evaluate their interaction with biological interfaces to evaluate possible toxic effects and improve their efficacy. Cell membranes are among the key barriers that NPs-based therapeutics encounter in living systems. The high surface energy of NPs is a main factor governing the interaction with lipid membranes, causing different effects, from clusterization of the NPs onto the lipid membrane, to modifications of the membrane structure arising from formation of raft-like domains.[3–7] However, the interaction between NPs and cell membranes is still far from being completely understood.

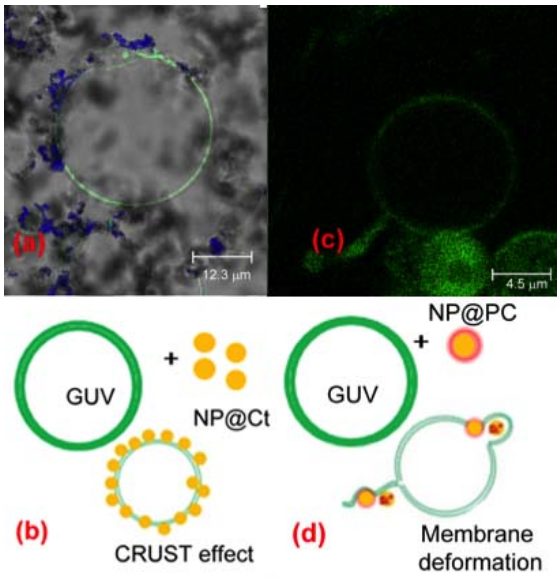

Figure 1. NPs-GUVs- interaction from Laser Scanning Confocal Microscopy (LSCM): (a,b) AuNPs@Ct (citrated)–GUVs and (c,d) AuNPs@PC (protein corona-covered)–GUVs: (a) GUV fluorescence (green), AuNPs scattering (blue) superimposed to the transmission image; (c) GUV fluorescence (green); (b,d) schemes representing respectively AuNPs@Ct-GUVs

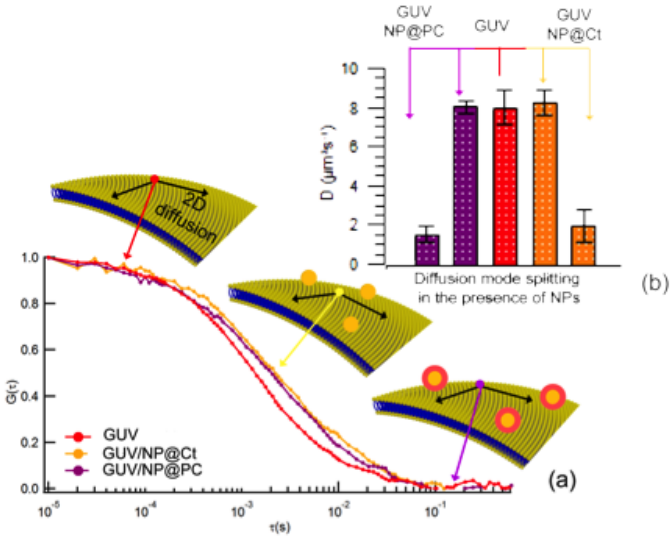

Figure 2 Diffusion of a lipid probe in the GUV bilayer after interaction with NPs. (a) Representative normalized (a, lines and markers) FCS curves acquired for bare GUVs (red) and on GUVs after incubation with NP@Ct (yellow) and NP@PC (purple). (b) In the presence of NPs, together with unperturbed diffusion, both for GUV–NP@Ct (yellow) and GUV–NP@PC (purple), a bimodal diffusion emerges, with the faster diffusion coefficient consistent with unperturbed diffusion and a slower component identical within experimental uncertainty for both NP types.

In a previous study[8] Giant Unilamellar Vesicles (GUVs) were challenged with NPs and observed through Confocal Microscopy and Fluorescence Correlation Spectroscopy; while as-synthesized nanoparticles, characterized by a high surface energy, form a "crust" on the bilayers (Figure 1 a, b), the protein corona-passivated NPs cause conformational stresses on the lipid membrane, with formation of bubbles and tubules (Figure 1c, d). Surprisingly, (Figure 2) the effect of the nanoparticles on the membrane at the molecular level is similar for the two kinds of NPs, i.e. a decrease of fluidity, possibly due to the formation of small rigid raft-like domains in the contact regions between the particles and the membrane.

In April 2015 we made a NSE proposal at ILL for seven days beamtime on IN15 (with in addition one day SANS beamtime in order to characterize the samples before the NSE) to study the effect of gold nanoparticles with

different coatings (anionic gold nanoparticles as-synthesized and coated by a protein corona) on the membrane fluidity of POPC liposomes. We had one day SANS beamtime allocated at D11 in November 2015 in order to fully characterize the samples in view of possible NSE experiments. Thanks to SANS beamtime we were able to make a full characterization of bare POPC liposomes in two contrasts (pure D2O and AuMW, a mixture of D2O and H2O matching gold contrast) and to measure the SANS spectra of liposomes incubated with different concentrations of as-synthesized citrated gold nanoparticles in the absence and in the presence of a protein corona shell.

Figure 3a displays the SANS curve obtained for the liposomes in D2O. The curves measured in D2O and AuMW were analyzed according to a poly-core-shell model, allowing to estimate liposomes' diameter (around 45 nm), shell thickness (3.5 nm) and polydispersity (0.25) of the samples. Figure 3b displays representative SANS curves obtained for the liposomes in D2O, in the presence of citrated gold nanoparticles in different concentrations: 1:1 and 4:1 with respect to liposomes and of protein-corona-coated gold nanoparticles.

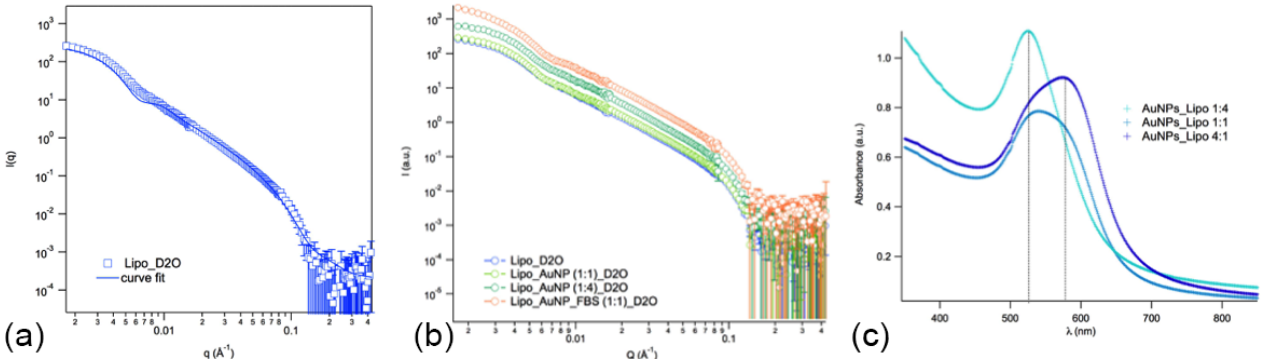

Figure 3. (a) Experimental SANS curves measured for liposomes in D2O and curve fit of the data according to a poly-core-shell model; (b) Experimental SANS curves measured for bare liposomes (blue line and markers) and for liposomes in the presence of AuNP@Ct 1:1 (light green line and markers) and 4:1 (dark green line and markers) with respect to liposomes' concentration; (c) UV-vis absorbance of citrated gold nanoparticles (AuNPs) in the presence of an excess of liposomes (AuNPs_Lipo 1:4), of an equal quantity of liposomes with respect to AuNPs (AuNPs_Lipo 1:1), in the presence of an excess of AuNPs with respect to liposomes (AuNPs_Lipo 4:1). Clearly, a red shift of the plasmonic peak of AuNPs is detected, from the expected maximum at 520 nm, attributable to a clusterization of the gold nanoparticles upon interaction with the liposomal membrane.

Clearly, no major differences can be detected in the SANS profiles acquired for POPC liposomes in the absence and in the presence of nanoparticles. However, a UV-vis spectroscopy investigation on the same samples (see Figure 3c) highlights that in the very same experimental conditions, liposomes provoke a clusterization of the nanoparticles, that can be detected as a shift of the plasmonic peak of citrated gold nanoparticles at 520 nm. Thus, a clear interaction between the nanoparticles and the lipid membrane of liposomes provokes the clusterization of the nanoparticles, probably on the liposomal surface.

Bibliography:1-E. C. Dreaden et al. *Chem. Soc. Rev.*, 2012, **41**, 2740; 2-D. A. Giljohann et al. *Angew. Chemie*, 2010, **49**, 3280–94.; 3-J. Lin et al. *ACS Nano*, 2010, **4**, 5421–5429.; 4-B. Wanget al. *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 18171–18175; 5-S. Li et al., *Soft Matter*, 2013, **9**, 4969; 6-T. Yue, X. Wang, F. Huang and X. Zhang, *Nanoscale*, 2013, **5**, 9888–96; 7-S. Tatur et al., *Langmuir*, 2013, **29**, 6606–6614; 8-