ESRF	Experiment title: Interaction of Gold Nanoparticles with Supported Lipid Bilayers	Experiment number: **SC-4350**
Beamline: **ID03**	Date of experiment: from: 26/08/16 to: 30/08/16	Date of report: 20/02/18
Shifts: 9	Local contact(s): Francesco Carlà	*Received at ESRF:*

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Report:

Inorganic nanoparticles are of great relevance for applications in Nanomedicine, both for therapeutic and for diagnostic purposes. However, in spite of the intense research devoted to the development of efficient nanodevices for biomedical applications, their full translation into medical practice is still limited, mainly due to the poor knowledge on the fate of nanomaterials in living organisms. In order to fill this gap, in recent years increasing interest has grown to the study of the interaction of nanoparticles with biologically relevant interfaces [1, 2].

In this context, the aim of the experiment sc-4350 was to obtain structural information on the interaction of nanoparticles with bidimensional lipid membranes, taken as cell membrane model systems, which is a topic of particular interest of our group [3, 4]. Concerning the nanoparticles, we chose gold nanoparticles (AuNPs), which are among the most studied inorganic nanoparticles for applications in Nanomedicine and, thus, of paradigmatic relevance; concerning the lipid membranes, we formed through vesicle fusion supported lipid bilayers (SLBs) with different compositions, mimicking specific features of cell membrane, for instance surface charge and fluidity, in simplified conditions.

As a first X-ray Reflectivity (XRR) and GISAXS experiment on supported lipid bilayers (SLB) we performed, the beamtime was first devoted to: (i) determine the experimental conditions to obtain SLBs of different compositions, with complete surface coverage (this was achieved through *in-situ* vesicle fusion method [5]), (ii) determine XRR reproducibility on different samples and SLBs resistance to radiation damage. In Figure 1 experimental XRR curves acquired for different SLBs of POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) and POPC mixed with POPG (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)) are displayed. As clearly shown in Figure 1A, measurements carried out in different areas of the same samples and on different samples displayed consistent XRR curves; this proves both the reliability of the experimental protocol to obtain SLBs and the intactness of SLBs upon at least three consecutive XRR measurements on the same area (see Figure 1B), before radiation damage occurring. The same approach was adopted to characterize SLBs of different compositions (DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, in fluid phase at r.t.; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine, in gel phase at r.t., DOPC-DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine)-cholesterol, containing raft-like domains).

Once obtained reliable characterization of SLBs, their interaction with different AuNPs was monitored. Particularly interesting experimental results were obtained concerning the interaction of POPG:POPC 1:1 (mol:mol) SLBs with cationic AuNPs (AuNPs@TMA), and of POPC SLBs with anionic Turkevic-Frens-type AuNPs with a citrate coating (AuNPs@ct), which are shown in Figure 2.

Figure 2A shows representative XRR curves of POPG:POPC 1:1 SLB in the absence and upon incubation with AuNPs@TMA. The presence of AuNPs clearly modifies XRR profile with a shift of the oscillations to higher Q values and the concomitant decrease in the oscillation bumps intensity. This effect is consistent with an extremely high interaction energy of AuNPs@TMA with POPG:POPC 1:1 SLB, attributable to a combination of Van der Waals and Electrostatic contributions [4], leading to a partial disruption of the SLB, highlighted through XRR as an overall decrease in the thickness of the SLBs (resulting in the shift of the oscillations to higher Q values) and an increase in its thickness polydispersity (resulting in the decrease of bumps intensity). A manuscript on this topic (interaction of AuNPs@TMA and lipid membrane), combining theoretical and experimental data (in particular XRR data from sc-4350), is currently in preparation.

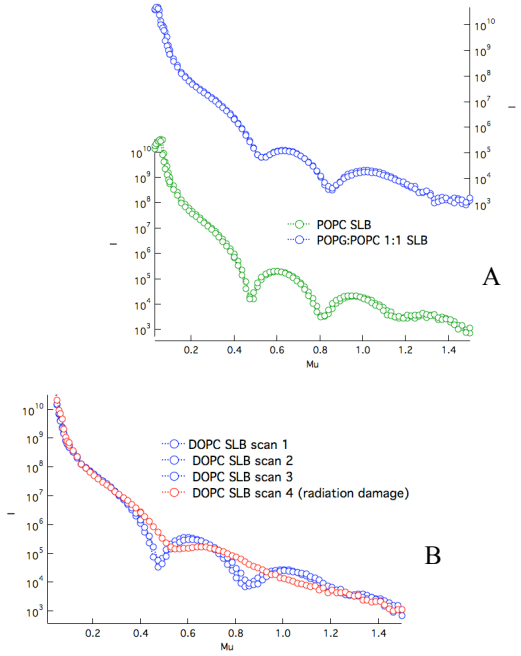

Figure 1: (A) Representative XRR curves of POPC and POPG:POPC 1:1 SLB, taken in different areas of different samples; (B) Representative XRR curves acquired for DOPC in the same area: three XRR curves can be acquired in the same region before radiation damage appears relevantly affecting the shape of the curve.

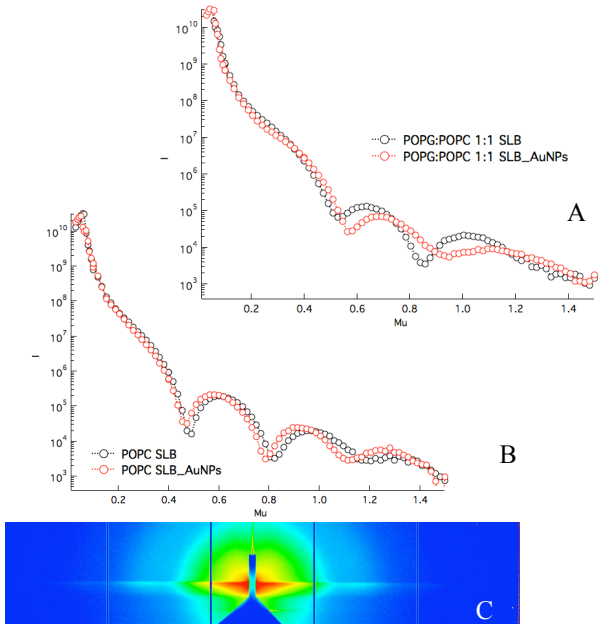

Figure 2: (A) Representative XRR curves of POPG:POPC 1:1 SLB before (black lines and markers) and after (red lines and markers) incubation with AuNPs@TMA; (B) Representative XRR curves of POPC SLB before (black lines and markers) and after (red lines and markers) incubation with AuNPs@ct; (C) Representative GISAXS plot of POPC SLB incubated with AuNPs@ct.

Figure 2 (B, C) shows representative XRR curves of POPC SLB in the absence and upon incubation with AuNPs@ct and the corresponding GISAXS. The experimental XRR curves highlight, in the presence of AuNPs, a slight variation of the curve oscillations, consistent with a slight impact of AuNPs@ct on the overall structure of the SLB; interestingly, GISAXS data measured on the very same sample show a Qy peak (Figure 2C), which is absent both for POPC SLB and for the AuNPs@ct incubated with the bare silicon support (without SLB). Thus, from GISAXS, a clear adhesion and possibly a 2D clusterization of AuNPs on the SLB, templated by the SLB itself, is apparent. These interesting, yet counterintuitive results, are particularly relevant: they provide a decisive experimental proof on the interaction of AuNPs@ct with POPC lipid membrane on a short lengthscale and on a bi-dimensional geometry, complementing Small Angle Neutron and X-ray Scattering, Dynamic Light Scattering and UV-vis spectroscopy investigation on POPC vesicles interacting with AuNPs@ct, and ultimately allowing us to hypothesize an interaction model, which will contribute to the understanding of the main phenomena related to nano-bio interfaces. A manuscript on this topic (interaction of AuNPs@ct and lipid membranes arranged in 2D and 3D assemblies), where the results from the experiment sc-4350 will be included and thoroughly discussed, is currently in preparation.

In addition, preliminary data were acquired on SLBs of different compositions, interacting with superparamagnetic iron oxide nanoparticles (SPIONs) with a gold coating.

Overall, the experimental data will contribute to the improvement of the current knowledge on the interaction of inorganic nanoparticles with lipid interfaces.

References:

[1] W.C.W. Chan, Nanomedicine 2.0, Acc. Chem. Res. 50 (2017) 627–632; **[2]** C.J. Murphy, A.M. Vartanian, F.M. Geiger, R.J. Hamers, J. Pedersen, Q. Cui, et al., ACS Cent. Sci. 1 (2015) 117–123; **[3]** C Montis, D Maiolo, I Alessandri, P Bergese, D Berti Nanoscale (2014) 6 (12), 6452-6457; **[4]** C Montis, V Generini, G Boccalini, P Bergese, D Bani, D Berti Journal of colloid and interface science 516 (2018), 284-294; **[5]** C Montis, Y Gerelli, G Fragneto, T Nylander, P Baglioni, D Berti Colloids and Surfaces B: Biointerfaces 137 (2016), 203-213