Experimental report

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Title: Neutron Reflectometry study on therole of the protein corona in the interaction between nanoparticles and lipid bilayers

Research area: Biology

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Samples: Fe3O4
phosphocoline
serum proteins

Instrument	Requested days	Allocated days	From	To
FIGARO	5	0		
D17	5	3	27/04/2015	30/04/2015

Abstract:

Engineered nanoparticles (NPs) found large application in medicine as theranostic materials for the diagnosis and therapy of many diseases. The understanding of the interactions of NPs with cell membranes is of fundamental importance both to tune the efficiency of NPs entry in the cell and limit their cytotoxicity. Neutron reflectometry (NR) is a powerful technique widely used to examine supported lipid bilayers (SLB) morphology and their response to different effectors. We propose the use of this tool to understand the nature of the interaction between NPs and supported lipid bilayers (SLBs), used as model of the cell membrane, in vitro and in a relevant biological environment when NPs are in contact with proteins and form a new biological entity called protein corona NPs (PC).

Experiment N° 9-13-584 **Instrument** D17 **Dates** 27-30/04/2015

Neutron Reflectometry study on the role of the protein corona in the interaction between nanoparticles and lipid bilayers

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Introduction. An effective and safe use of nanoparticles (NPs) for nanomedicine purposes relies on understanding interactions between NPs and cell membrane.[1] The presence of proteins in the environment modify NPs surface by coating it with a protein corona (PC).[2] The PC, a new bio-nano interface, consists of outer layers of proteins loosely bound to the NP (soft corona, SC) and an inner layer strongly attached to it (hard corona, HC). Supported lipid bilayers (SLBs) are good models for biological membrane. The aim of this study was to use neutron reflectometry (NR) to investigate the effect of carboxylated and pegylated NPs on SLB. Moreover, *in situ* and HC NPs were applied to the SLB to elucidate the role of proteins.

Experimental. Carboxylated Fe_3O_4 NPs were prepared according *Sun et al*[3] and coated by poly(maleic anhydride- alt- octadecene). Pegylation of Fe_3O_4 NPs was achieved by Jeffamine M100 (Huntsman). Carboxylated Polystyrene NPs (PS-COOH20) were purchased by Invitrogen. HC NPs were obtained according *Di Silvio et al.*[4] SLBs were formed by collapsing 0.5 mg/ml DOPC liposomes dispersion and they were characterized in three contrasts (D_2O, SMW and H_2O). Afterwards, NPs were injected in the cell and the SLB characterized in four contrasts (D_2O, 4MW, SMW, H_2O). Experiments we carried out at 37°C. Motofit macro on Igor was used as software to fit the data.[5] For fitting the data, SLB was divided in four layers, two hydrophobic tail regions and two polar heads regions, one in contact with the SiO_2 layer of the Si-substrate. In absence of proteins, a model with constrains was applied fixing the minimum value for the area per molecule (APM) to 72 $Å^2$.

Results and Discussion. SLBs were very reproducible and the averaged size was 4.7±0.8 nm 4.6±1.4 nm. The thickness of the hydrophobic tail region was 28.5±0.8 Å. The outer polar head region was 6.3±0.1 Å while the inner headgroup region was 11.6±0.9 Å. The averaged value of tail hydration (ϕ) was less than 2% (1.6±1.4%) meaning that the overall coverage of the silicon chamber was 98% (the complement to 100%).

In Fig.1, the effect of pristine NPs on SLBs is presented. None of the NPs caused SLB disruption. Between the carboxylated NPs (Fe_3O_4 and PS-COOH20), main differences were found at high **Q** (Fig.1a-b), while pegylated NPs caused a compression of the NR profile.
The fitting process revealed that carboxylated NPs caused SLB hydration and swelling. The solvation concerned principally the hydrophobic tails region in the case of PS-COOH20 (ϕ went from 0.3% to 10.7%), while the thickness of the SLB increases of 7%, with the area per molecule (APM) of the outer leaflet going from 72 $Å^2$ to 74.6 $Å^2$. The modelling suggested lipids removal from the SLB.

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[4] Di Silvio, D.; Rigby, N.; Bajka, B; Mayes, A.; Mackie, A; Baldelli Bombelli F.; *Nanoscale* **2015**, *7*, 11980.
[5] Nelson, A. *Journal of Applied Crystallography* **2006**, *39*, 273.

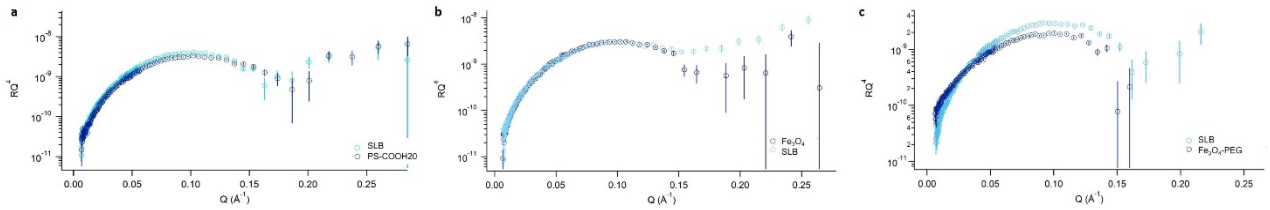

Figure 1 *Neutron reflectivity profiles for SLBs before (light blue circles) and after NPs injection (dark blue circles). A) Carboxylated polystyrene NPs; B) carboxylated magneite NPs; C) pegylated magnetite NPs. The contrast presented is SMW. Plots are in RQ^4 vs Q.*

Carboxylated Fe_3O_4 NPs induced solvation of the tail regions, as in the previous case. The overall increase of the SLB thickness was the 16%. The biggest difference involved the outer polar heads whose thickness and hydration doubled keeping constant the APM. We could speculate that carboxylated Fe_3O_4 NPs exert a stronger influence over the outer leaflet compared to PS-COOH20 with significant modification of the head tilt angle, as consequence of the different surface chemistry characterizing Fe_3O_4 NPs respect to PS NPs. For the latter, such alterations could happen but they were not as evident as here. Pegylated Fe_3O_4 NPs, on the contrary, induced slight dehydration of the polar heads and shrinking of the SLB. This effect is ascribed to the cumbersome hydration shell that surrounds the hydrophilic PEG chains and hinders interactions with the membrane.

Proteins effect on SLB was studied comparing the behaviour of proteins alone, *in situ* PS-COOH20 and HC PS-COOH20. Comparing original SLB, effects of proteins and of *in situ* NPs an increasing hydration of the tail regions was pointed out (Fig. 2a-b-c respectively in which SLDs profiles were derived fitting raw data). Fitting parameters showed that the addition of FBS induced a slight dehydration of the outer head groups (ϕ goes from 35% to 27%), while for *in situ* NPs, the tail and the outer polar head regions exhibited a higher hydration which was reflected by the splitting of the curves in the range 25-45 Å in Fig. 2c. We can ascribe SLB modifications to lipid-protein exchange with NP PCs. HC NPs induced only a small perturbation of the outer polar head (Fig.2d). We can speculate that since the HC proteins constitute a resistant and stable shell around NPs characterized by low total surface free energy, any interaction with the SLB would be unfavourable and hence very limited. This result compared to FBS and *in situ* NPs data could suggest that the presence of a soft corona could in fact have a role on the NP interaction with lipid membranes.

SLB perturbations were small but significant. SLBs might be too resistant to evidence alterations due to NPs. Future works involve the use of different membrane models and NPs.

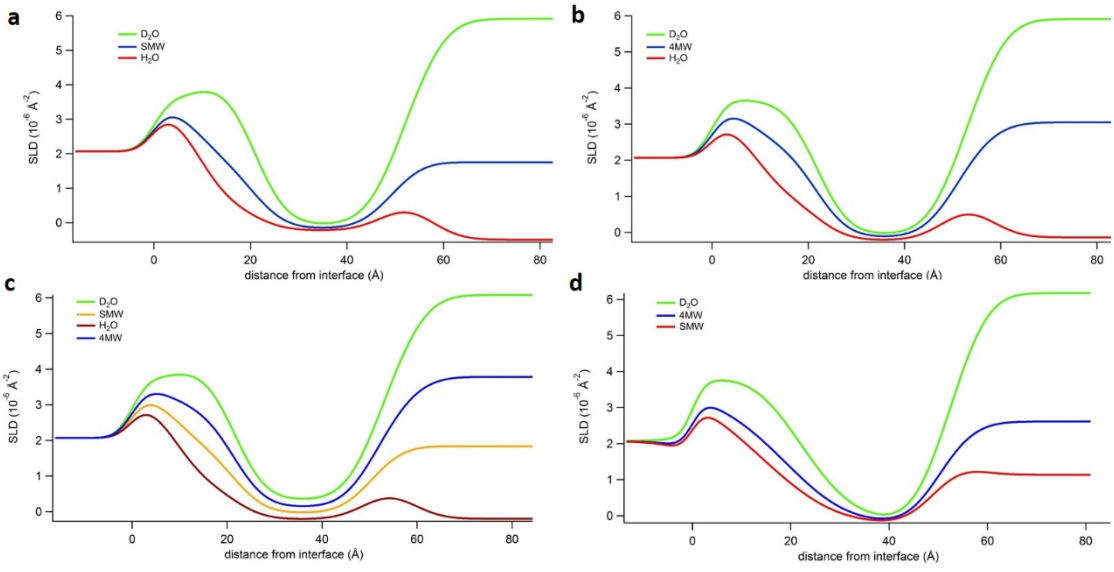

Figure 2 *Scattering length densities by distance from the interface derived from fitting NR raw data. A) original SLB; B) 55% FBS proteins; C) in situ PS-COOH20; D) HC PS-COOH20.*