Experimental Report

Proposal: **DIR-125** **Council:** 4/2014

Title: Characterization of the interaction between functionalized nanoparticles and lipid bilayers

This proposal is a new proposal

Researh Area:

Main proposer: **NYLANDER Tommy**

Experimental Team: NYLANDER Tommy
VITIELLO Giuseppe
FRAGNETO Giovanna

Local Contact: DENNISON Andrew
BARKER Robert

Samples: CHOLESTEROL
Silicon Blocks
Functionalized Fe3O4-Au nanoparticle dispersion
Palmitoyl oleoyl phosphatidylcholine
Palmitoyloleoylphosphatidylglycerol

Instrument	Req. Days	All. Days	From	To
D17	2	2	29/09/2014	01/10/2014

Abstract:

Characterization of the Interaction between Lipid Bilayers and Functionalized Nanoparticles

Introduction.

Fe_3O_4 nanoparticles (MNPs) exhibit superparamagnetic properties responsible for influencing transversal proton relaxation time (T2). Aiming at improving the nanoparticle, systems combining Fe_3O_4 iron oxide with other inorganic and organic components are now widely used, and many examples can be found in the literature. Recently, we have optimized a novel functionalization strategy, based on the use of phospholipids, known biocompatible molecules. In particular, MNPs were synthesized trough the thermal decomposition method, coated with oleic acid and oleylamine, used as stabilizing agents in order to control nanoparticle clustering. The functionalization protocol we introduced exploits the hydrophobic layer composed by oleic acid and oleylamine to introduce a second amphiphilic layer. We tested this functionalization strategy for several amphiphilic molecules, differing for the structure of both the nonpolar and polar portion. In order to investigate preliminary aspects of the nanoparticle behaviour within biological systems, we performed Neutron Reflectivity (NR) experiments aiming at the characterization of the interaction between our functionalized nanoparticles and lipid bilayers, mimicking a cellular membrane.[2] The cellular membrane represents the most external cellular component and it is extremely relevant to investigate the effects of the interaction with the functionalized Fe_3O_4 MNPs. Thus, we prepared two different kinds of lipid bilayers, using 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (POPG) in the presence and in the absence of cholesterol (CHOL). Two differently functionalized Fe_3O_4 MNPs were prepared with the biocompatible phospholipid, 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (18LPC) (named 18LPC/MNPs) and the cationic surfactant cetyl trimethylammonium bromide (CTAB) (named CTAB/MNPs).

Experimental section.

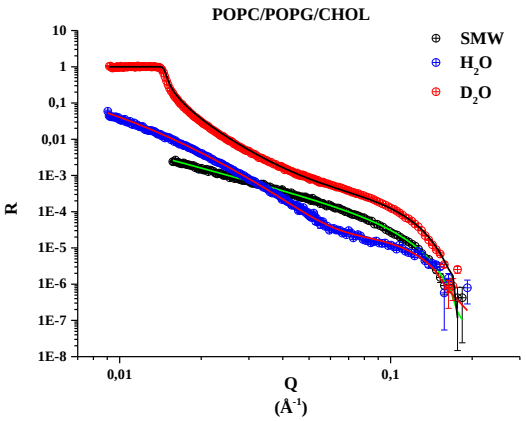

Figure 1: *Reflectivity data and fitting curves for the POPC/POPG/CHOL bilayer in the three different solvents.*

Neutron reflectivity measurements were performed on the D17 reflectometer. The first set of data was collected in order to characterize the two different bilayers composed by POPC/POPG (90:10 molar ratio) and POPC/POPG/CHOL (65:10:25 molar ratio). The bilayers were prepared through vesicle adsorption on a silicon support. In particular, single unilamellar vesicle suspensions in D_2O were prepared at 0.5mg/ml total lipid concentration, and injected into the NR cells. After 30 min, the lipid excess was washed out by pumping D_2O into the cells. [3,4] The subsequent NR measurements, performed using

three different contrasts (D_2O, H_2O and Silicon Match Water (SMW)), confirmed the formation of a single bilayer on the silicon surface. The collected data were treated as arising by a stack of layers with different scattering length density and thickness depending on the composition (Figure1). According to the Slab model based on Parrat formalism, the bilayer was divided in three different regions corresponding to lipid head-group layer, the hydrophobic lipid tails layer and again the lipid head-group layer. The presence of cholesterol within the bilayer was detected and characterized by means of the variation in the scattering length density ($\Delta\rho$) of both the lipid head-group layer, $\left|\Delta\rho^{head-group}\right| = 0.2 \cdot 10^{-6}\text{Å}^{-2}$, and the lipid tails region, $\left|\Delta\rho^{tails}\right| = 0.1 \cdot 10^{-6}\text{Å}^{-2}$.

After the characterization of the two bilayers, the D_2O suspension of functionalized MNPs was injected. NR measurements were performed right after nanoparticle injection, after 6h, and after several washing steps, in order to test the stability of the interaction between the nanoparticles and the bilayer before proceeding with the collection of the data corresponding to the other two contrasts. In Figure 2 the data collected for the bilayer, containing 25% mol:mol cholesterol, in the presence of CTAB/MNPs, are reported as a representative example of the investigated systems.

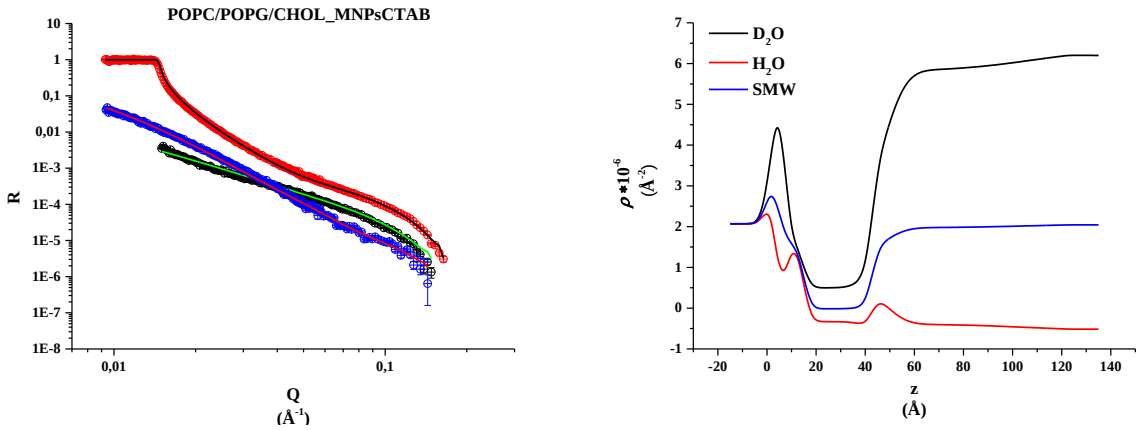

Figure 2: *Reflectivity data and fitting curves (left and scattering length density profiles (right)) for the POPC/POPG/CHOL bilayer with CTAB/MNPs in the three different solvents.*

The best fit was identified considering an additional nanoparticle layer positioned on the top of the external head-group region of the lipid bilayer. The nanoparticle layer resulted to have a high level of hydration, indicating a low density of MNPs on the bilayer surface. The presence of the MNPs on the bilayer induced the variation in the $\rho^{head-group}$, mostly in the external layer ($\left|\Delta\rho^{head-group}\right| = 0.2 \cdot 10^{-6}\text{Å}^{-2}$), and the variation of both ρ^{tails} and hydrophobic region thickness,($\left|\Delta\rho^{tail}\right| = 0.1 \cdot 10^{-6}\text{Å}^{-2}$, $\Delta d = 2$ Å). Indeed, as it is shown in the scattering length density profiles (Figure 2), after MNPs injection, the bilayer is no more symmetric.

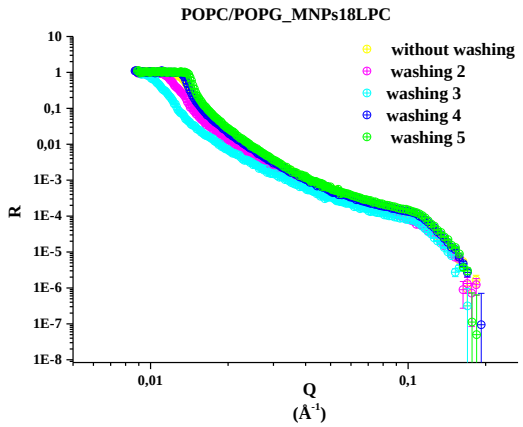

Figure 3: *Reflectivity data for the POPC/POPG bilayer with 18LPC/MNPs in D_2O, after different washing steps as indicated in the legend.*

Furthermore, comparing the result obtained for the bilayer composed by POPC/POPG and the one also containing cholesterol, the same model resulted to be appropriate for data treatment, indicating that in both cases an extra layer of MNPs on bilayer surface is formed after nanoparticle injection. However, a slightly larger effect on bilayer parameters variation is detected when cholesterol is present, suggesting a more extended interaction between the bilayer and the functionalized MNPs. Similar results were obtained when the MNPs were functionalized with 18LPC instead of CTAB.

In the case of the system represented by the POPC/POPG bilayer in the presence of 18LPC/MNPs a very different and unusual behavior was observed. In all the other cases the pumping step necessary for solvent exchange, or washing steps, didn't perturb the system. However, in the case of POPC/POPG bilayer with 18LPC/MNPs, a progressive shift in the critical angle for the curve referring to D_2O solvent was observed. In particular, as shown in Figure 3 it resulted that the critical angle with the first D_2O washing step moved to lower Q values, but after the third washing cycle it moved back to the expected position. This observation certainly suggests that the D_2O pumping step induces a modification in the system structure. However, since the same behavior was not observed in all the other samples, it is not clear if a real effect occurred or if the obtained results were determined by the specific experimental conditions. Thus, the system requires further investigation that will be proposed in a future NR proposal.