Experimental Report

Proposal: **9-13-489** **Council:** 10/2012

Title: Nucleolipid Anionic Membranes: structural characterization and interaction with Nucleic Acids

This proposal is resubmission of: 9-13-442

Researh Area: Chemistry

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Samples: ss-DNA (from Deoxyribonucleic acid sodium salt from calf thymus, type I hyghly polymerized) l
Polyuridylic acid potassium salt
POPG (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt))
50-dT (Deoxyribothymidine)
POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine)
D-POPC (1-palmitoyl(d31)-2-oleoyl-sn-glycero-3-phosphocholine)
POP-Ade (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphatidyladenosine)

Instrument	Req. Days	All. Days	From	To
FIGARO	4	4	24/05/2013	28/05/2013

Abstract:

PLN (phosphatidylnucleosides) are phospholipid-based anionic nucleolipids, where a nucleoside is enzymatically attached to the
polar head of a lecithin substituting a choline headgroup. In the past years several studies have highlighted that the microstructure of
their self-assemblies depends markedly on the kind of nucleic base due to inter polar-head attractive interactions and, moreover,
that PLN self assemblies provide negatively charged interfaces decorated with nucleic motifs that can complex complementary
nucleic acid strands using, as the driving force, molecular recognition instead of electrostatic interactions. Neutron reflectometry
technique can allow a specific investigation on the interaction between nucleolipids' membranes and different (i.e. characterized by
different length and nucleobase sequence) single strand nucleic acids, allowing hence to better comprehend the molecular

Nucleolipid Anionic Membranes: structural characterization and interaction with Nucleic Acids

The aim of the neutron reflectometry experiment was to characterized bilayers containing the nucleolipid POP-Ade (*1-palmitoyl, 2-oleoyl-phosphatidyl-adenosine*) mixed in different percentages with the zwitterionic lipid POPC (*1-palmitoyl-2-oleoyl-phosphatidylcholine*) and to investigate the interaction between POP-Ade:POPC mixed membranes and nucleic acids.

In the previous neutron reflectometry experiment performed at ILL, we were able to determine the best experimental conditions in order to obtain SLBs from POP-Ade:POPC mixtures with a high percentage of support coverage, while just a few data were collected concerning the interaction between the nucleolipid bilayers and nucleic acids. In this experiment we better characterized POP-Ade:POPC mixed bilayers, by acquiring experimental data on both hydrogenated and deuterated lipid membranes and, moreover, we investigated the interaction between POP-Ade:POPC mixed bilayers and different nucleic acids, in terms of nucleobases composition, length, single or double stranded arrangement. In addition, POP-Ade systems were compared to POPC mixed membranes where the anionic nucleolipid POP-Ade was substituted by the anionic commercial phospholipid POPG (*1-Palmitoyl-2-oleoyl-sn-phosphatidyl-glycerol*), characterized by mononegative polar headgroup, as POP-Ade, but without molecular recognition capabilities.

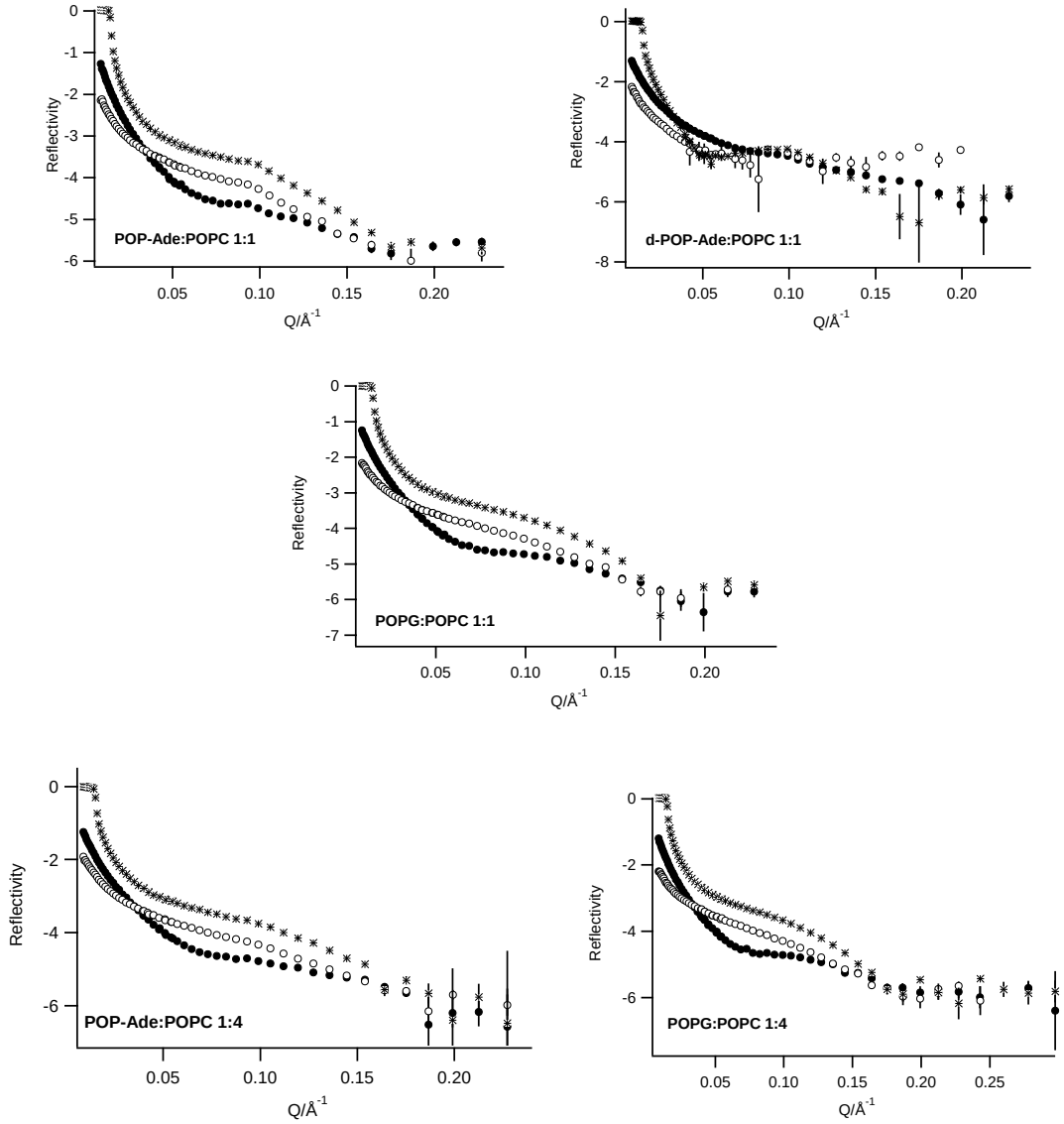

Figure 1. POP-Ade:POPC 1:1 and 1:4 mol:mol, d-POP-Ade:POPC 1:1 mol/mol, POPG:POPC 1:1 and 1:4 mol:mol experimental reflectivity curves measured in contrast variation (H2O, SiMW, D2O).

The experimental curves acquired for all the mixed membranes in the presence of $CaCl_2$ 15mM are reported in Figure 1. All the curves were then analyzed through MOTOFIT (A. Nelson *Journal of Applied Crystallography,* 39,

273-276, 2006), considering a 5 layers model, with a first SiO_2 layer (which thickness and roughness were determined by a previous characterization of each silicon block in D_2O), a second layer composed by the solvent, a third by lipid membranes' polar headgroups, a fourth by lipid chains and a fifth again by lipid membranes' polar headgroups. SLD of respectively lipid polar headgroups and chains were calculated by estimating lipid molecular density from phosphatidylcholine submolecular fragment volumes determined by Armen et al. through molecular dynamic simulations (Armen, R. S.; Uitto, O. D.; Feller, S. E. *Biophysical Journal* **1998**, *75*, 734.). The results were consistent with the previous neutron reflectometry experiment carried out at ILL, confirming thus the reliability of the hypothesized structure of both POP-Ade:POPC and POPG:POPC mixed bilayers obtained through the vesicle fusion technique.

It was then investigated the interaction between POP-Ade:POPC mixed lipid membranes and different nucleic acids in the presence of $CaCl_2$ 15mM. Figure 2, 3, 4 display the neutron reflectometry curves acquired respectively for the naked bilayers (black markers) and for the same samples after incubation with different nucleic acids, in the presence of $CaCl_2$ 15mM (red markers).

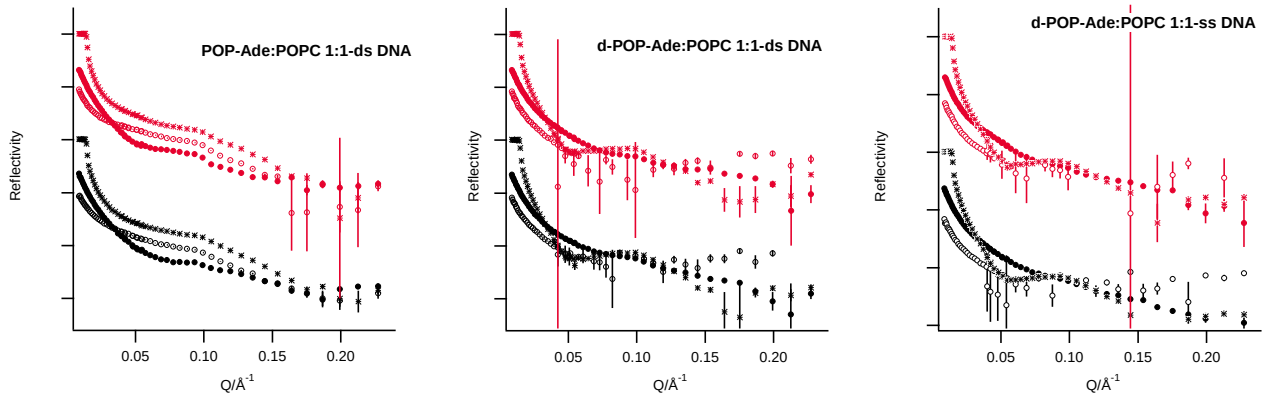

Figure 2. POP-Ade:POPC 1:1 and d-POP-Ade:POPC 1:1 mol/mol, experimental reflectivity curves measured in contrast variation (H2O, SiMW, D2O) for naked bilayers (black markers) and for the same samples in the presence of single stranded (ss DNA) or double stranded (ds DNA) highly polymerized Calf Thymus DNA (red markers).

Figure 2 displays the experimental data acquired concerning the interaction between POP-Ade:POPC 1:1 membranes with double stranded or single stranded highly polymerized DNA. Even if in both cases only slight variations can be identified in the reflectivity curves, these effect are stronger for the interaction with ds-DNA and, consistently with what observed in our QCM studies on the same systems, this can be attributed to the formation of a layer of DNA interacting with the membrane with the contribution of both POP-Ade polar headgroup molecular recognition capability and Ca^{2+} bridges.

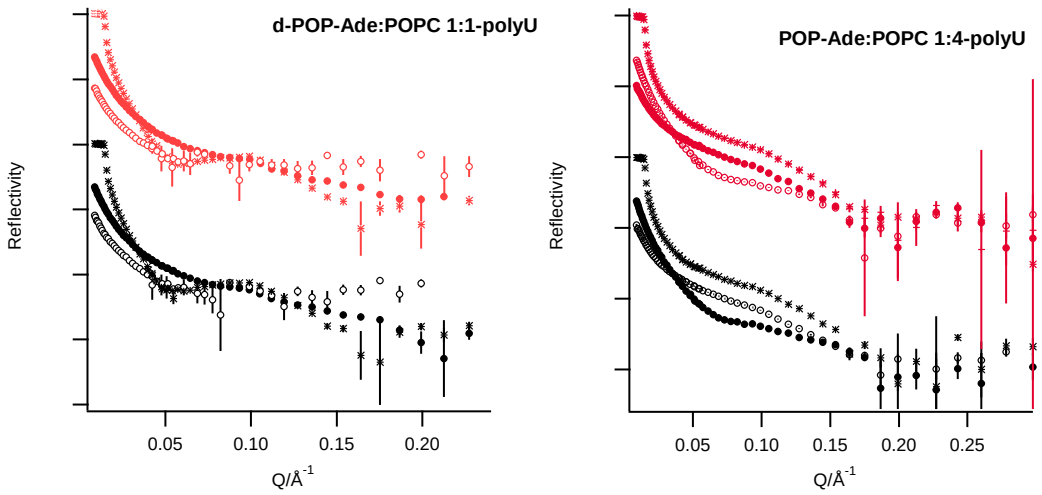

Figure 3. d-POP-Ade:POPC 1:1 and POP-Ade:POPC 1:4 mol/mol, experimental reflectivity curves measured in contrast variation (H2O, SiMW, D2O) for naked bilayers (black markers) and for the same samples in the presence of single stranded polyuridylic acid (polyU) (red markers).

Figure 4 compares the ability of POP-Ade:POPC mixed membranes to interact with highly polymerized polyuridylic acid, when different amounts of POP-Ade are present in the lipid bilayer. The experimental data show that the interaction with polyU is favored in the presence of a higher percentage of POP-Ade with respect to POPC within the membrane, effect that can be consistently related to a specific interaction between POP-Ade polar headgroup and polyU monomers, which increases the affinity of the polynucleotide for the lipid membrane.

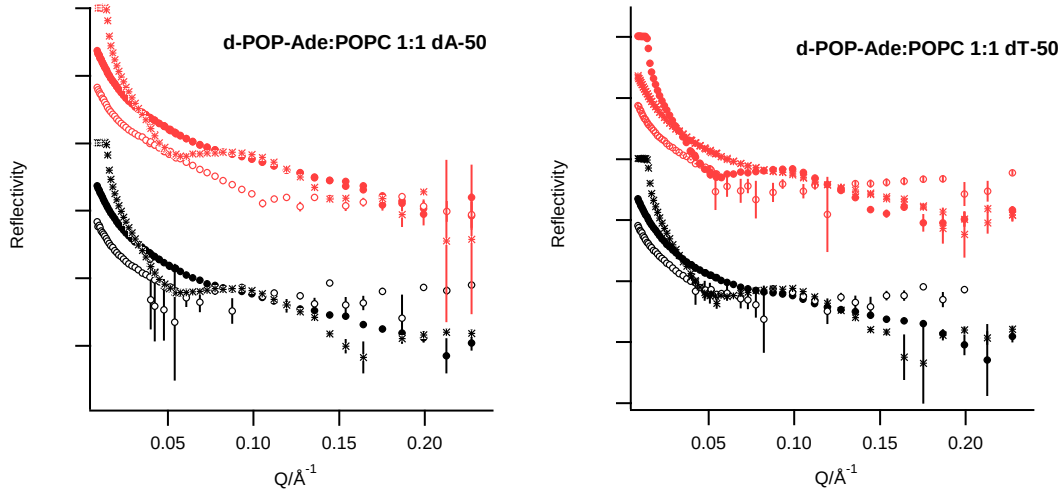

Figure 4. d-POP-Ade:POPC 1:1 experimental reflectivity curves measured in contrast variation (H2O, SiMW, D2O) for naked bilayers (black markers) and for the same samples in the presence of single stranded short oligomers (with 50 nucleobases) made of a repetition of adenosine monomers (50-dA) or thymidine monomers (50-dT) (red markers).

Finally, figure 4 compares the experimental data obtained for POP-Ade:POPC 1:1 mixed membranes incubated respectively with 50-dA and 50-dT, being each monomer composing the last oligomer (50-dT) complementary to POP-Ade polar headgroup, according to Watson-Crick model, unlike 50-dA. In this case the variations in the experimental curves are found stronger for POP-Ade:POPC-50-dT samples with respect to POP-Ade:POPC-50-dA samples, compared to the curves obtained for naked bilayers. This effect can be thus attributed to a better affinity of POP-Ade:POPC mixed membranes for 50-dT with respect to 50-dA, confirming thus both the ability of POP-Ade assemblies to specifically interact with nucleic acids, and the specificity of this interaction.