Experimental Report

Proposal: **9-13-481** **Council:** 10/2012

Title: Room-Temperature Ionic Liquids Interacting with a POPC Phospholipid Bilayer: a Structural and Dynamical Study

This proposal is a new proposal

Researh Area: Biology

Main proposer: **BENEDETTO Antonio**

Experimental Team: BENEDETTO Antonio

Local Contact: GONZALEZ Miguel Angel
WATKINS Erik

Samples: Room--Temperature Ionic Liquids: [bmim][Cl], [bmim][PF6], and [bmim][Tf2N]
POPC Phospholipid Membrane

Instrument	Req. Days	All. Days	From	To
D16	2	2	15/07/2013	17/07/2013
FIGARO	3	2	10/05/2013	12/05/2013

Abstract:

The aim of this proposal is to study the effect of adding different Ionic Liquids (ILs) to a POPC phospholipid membrane. Recent experiments (fluorescence spectroscopy and AFM) and MD simulations have shown that ILs can be incorporated into POPC bilayers, modifying their properties and undermining their stability. Those results have important implications concerning the toxicity of ILs and merit further experimental verification. We propose to investigate the structural and the dynamical properties of the system POPC+water(+IL) for three prototypical ILs ([bmim][Cl], [bmim][PF6], [bmim][Tf2N]). Neutron diffraction and reflectivity will allow determining the changes occurring in the membrane structure when different ILs are added, while the dynamical properties will be determined by QENS using a combination of a TOF and a backscattering spectrometers; using lipids with deuterated chains and deuterated water we will focus on the determination of the head group motion. The results will serve to validate the force fields employed in the simulations, as well as to identify the role of the solvent (water) in the evolution of the bilayer structure and stability upon the addition of ILs.

Study of Room-Temperature Ionic Liquid Interacting with a Phospholipid Bilayer

Work-Team: Antonio Benedetto*, Miguel A. Gonzalez, Giovanna Fragneto, Erik Watkins, Pietro Ballone

The main aim of the experiment is the characterization of the interaction between a water solution of Room-Temperature Ionic-Liquid (RTIL) and a phospholipid bilayer. The obtained results could have important implications concerning the toxicity of RTILs when released into the environment, and could also point to pharmacology and biotechnological applications. Furthermore, in comparison to the experiments available up to now, the obtained results provide for the first time the access to the microscopic details on the RTIL-phospholipid interaction.

The di-myristoyl-phosphatidyl-choline (h-DMPC, cat. n. 850345P) and the d-DMPC with perduterated hydrocarbon chains and tails (DMPC-d_{67}, cat. n. 860348P) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL), whereas the RTILs [bmim][Cl], [bmim][PF6], and [bmim][NTf2] were purchased from Sigma-Aldrich.

For better characterize the RTIL-phospholipid bilayer interaction, we have studied the system DMPC+water(+RTIL) under eight different contrasts. More specifically, Case I: DMPC-d_{67} in D_2O; Case II: DMPC-d_{67} in a 66/34 wt/wt D_2O/H_2O mixture (called 4MW); Case III: DMPC-d_{67} in a 38/62 wt/wt D_2O/H_2O mixture approximately contrast matched to silicon (SMW); Case IV: DMPC-d_{67} in H_2O; Case V: DMPC in D_2O; Case VI: DMPC in a 66/34 wt/wt D_2O/H_2O mixture; Case VII: DMPC in a 38/62 wt/wt D_2O/H_2O mixture approximately contrast matched to silicon (SMW); Case VIII: DMPC in H_2O.

The neutron reflectivity experiment on FIGARO allowed us to determine the changes occurring in the bilayer structure and composition after the interaction with the water solution of RTIL. By using H/D contrast both on lipid and solvent, it was possible to characterize the above-mentioned changes at the molecular level. In particular, the experiment gave access to the density profile of the fully hydrated bilayer (i) **before**, (ii) **during**, and (iii) **after** the interaction with the RTIL.

As a result, we have found that the RTIL penetrates, in a no reversible way, into the phospholipid bilayer that still remains well structured (with a small increment in the bilayer thickness), and account for about 2% of their volume, at a concentration of 0.075 ± 0.015 RTIL molecules per phospholipid molecule. This result is in good agreement with MD forecast.

Furthermore, the diffraction experiment carried out on D16 on multi-bilyer in interaction with three different RTILs, i.e. [bmim][Cl], [bmim][PF6], and [bmim][NTf2], confirms that (i) the bilayer main structure is not drastically modifies by the addition of the RTIL, and that (ii) the RTIL-phospholipid interaction drastically increases above the gel-fluid phase transition temperature of the membrane.

The work was supported by the European Union under a Marie Curie Intra-European Fellowship for Career Development (IEF) within the 7th European Community Framework Programme (grant HYDRA n. 301463).

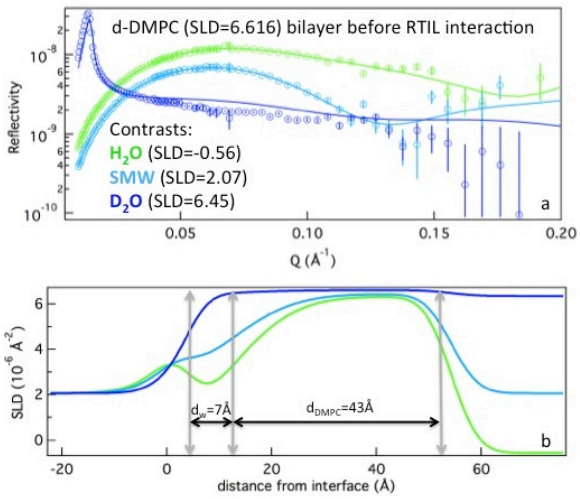

Fig. 1 – (a) Reflectivity data as a function of the momentum transfer Q, and (b) scattering length density profile for the d-DMPC bilayer at T=31C (i.e. fluid phase) **before** the interaction with the [bmim][Cl] room temperature ionic liquid. Data for three different H/D solvent contrasts have been collected and are here shown together with the fit curves. The solid lines are best fits to the data.

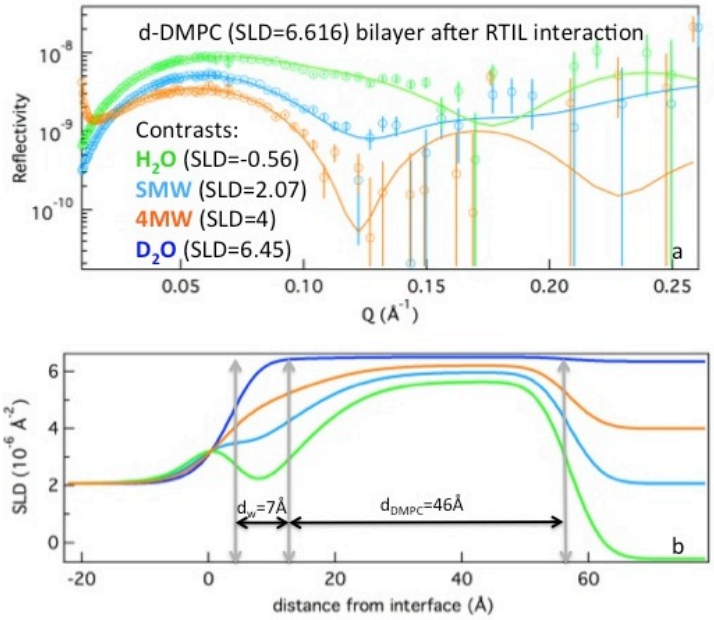

Fig. 2 – (a) Reflectivity data as a function of the momentum transfer Q, and (b) scattering length density profile for the d-DMPC bilayer at T=31C (i.e. fluid phase) **after** the interaction with the [bmim][Cl] room temperature ionic liquid. Data for three different H/D solvent contrasts have been collected and are here shown together with the fit curves. The solid lines are best fits to the data.

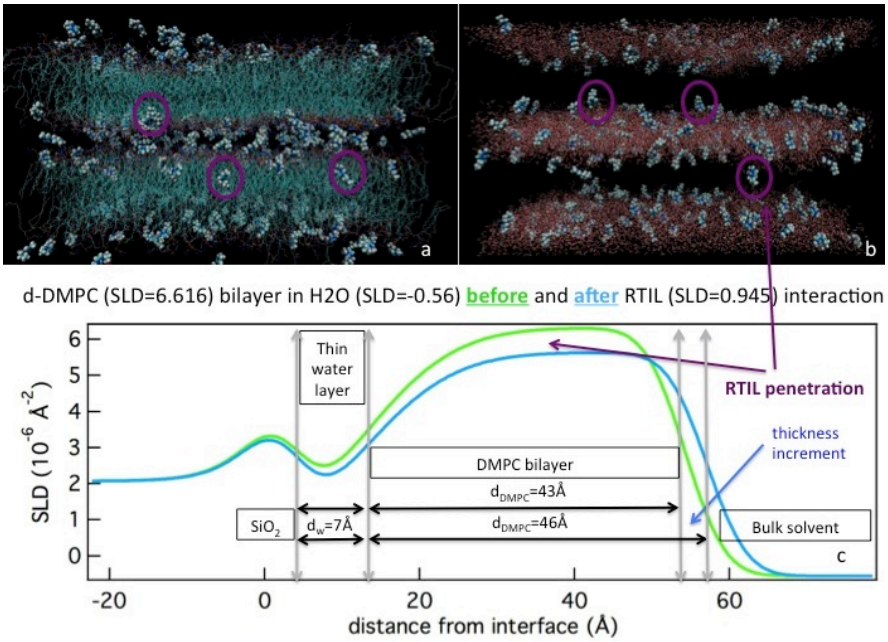

Fig. 3 – Molecular Dynamics simulation screenshot at 70ns of POPC bilayer in interaction with [bmim][Cl]; (a) water and Cl are not shown, (b) phospholipid bilayer and Cl are not shown. Some of the penetrated bmim ions are highlighted into violet circles. As it can be seen the RTIL penetrate the bilayer without affected their main structure. (c) The experimental results: the comparison between the SLD profile of the phospholipid bilayer system before (green line) and after (blue line) the interaction with the RTIL; the only contrast d-DMPC in H_2O is here shown for a better understanding. As it can be seen, the differences in the two profiles highlight the presence of RTIL that penetrates into the bilayer, as well as a small increment in the bilayer thickness. More specifically, it was possible to determine the amount of RTIL incorporated into the membrane; it results of about 1.8%, which corresponds to 0.075 ± 0.015 RTIL per phospholipid. This result is in good agreement with the MD predictions.