Experimental Report

02/05/2013

Proposal: **8-02-674** **Council:** 10/2012

Title: Mimic natural membranes: the role of asymmetry in lipid bilayers

This proposal is a new proposal

Researh Area: Physics

Main proposer: **GERELLI Yuri**

Experimental Team: FRAGNETO Giovanna
GERELLI Yuri
PORCAR Lionel

Local Contact: FRAGNETO Giovanna

Samples: Syntetic lipids

Instrument	Req. Days	All. Days	From	To
FIGARO User-supplied	3	3	08/03/2013	11/03/2013

Abstract:

Cell membrane are usually characterized by a cmpositional asymmetry betwen inner and outer leaflet. This asymmerty is not just due do the location of membrane proteins but also to that of lipid molecules. In the case of plasma membrane its outer part is mainly composed by zwitterionic phosphatidylcholine while the inner one by negatively charged phosphatidylserine. The main difficulty in mimic such a structural inhomogeneities is to prevent the spontaneous transmisgration of lipid molecules between the two leaflet of the deposited bilayer. To achieve this we propose to exploit the stabilizing role of electrostatic interaction between negatively charged lipids (PS) and the sapphire substrate which bears a positive charge. The results will help in developing a stable asymmetric deposition to mimic cell membrane.

In nature the lipid distribution across the inner and outer leaflet of cell membranes is asymmetric[1] and this asymmetry plays a prominent role during cell fusion, activation of the coagulation processes, recognition and removal of apoptotic cell corpses by macrophages.[2] Lipid asymmetry in natural membranes is hypothesized to be promoted by the action of specific enzymes and by retentive mechanisms that trap lipids in one leaflet of the bilayer.[3] In absence of these enzymes any induced asymmetry is spontaneously lost and this process is commonly called lipid flip-flop. From a biophysical point of view several studies about the characteristics of the process in model systems were conducted showing that the process is still far from being well understood and characterized. In the present experiment we combines the potentialities of neutron reflectometry together with the approach derived by Nakano and coworkers.[4] A solid supported lipid bilayer made of hydrogenated lipids was formed in D_2O by vesicle fusion method; subsequently it was exposed to a solution containing vesicles composed by the same molecules but in deuterated form. Lipid molecules form the vesicles replaced, following a certain kinetics, those originally composing the adsorbed bilayer. By following this exchange at different temperatures and concentration it was possible to determine the thermodynamic properties of the process.

The structural characterization of the adsorbed bilayer was performed in three different media namely D_2O, H_2O and $4MW$. Kinetics measurements were performed by acquiring the reflectivity signal of the supported bilayer exposed to a D_2O vesicle solution till the typical curve of a completely exchanged bilayer was found. Typical concentrations of the vesicles solution were 1 mg/ml and 5 mg/ml. The acquisition time for a single run was adjusted according to the time-dependence of the kinetics i.e. using short acquisition times at the beginning (2.5 min) and longer ones (10 min) towards the end of the process. To extract thermodynamic information, kinetics measurement were recorded at 4 temperatures (48 – 57 and 68 °C) i.e. always with bilayer and vesicles in the liquid phase. An example of the kinetics runs is given in Figure 1 where the changes in $R(Q)$ upon lipid exchange are quite evident. The main result from the experiment was in fact the quantity of exchanged lipids in the original matrix. Its time evolution, for different temperatures and concentrations is given in Figure 2.

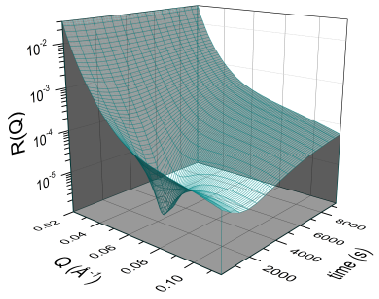

Figure 1: **Time evolution of the reflectivity curves during a typical D–to–H kinetics. Data were actually taken from the 57°C experiment; the experimental curves were smoothed for a better graphical representation.**

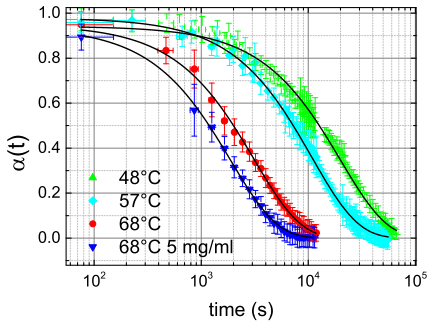

Figure 2: **Content of deuterated molecules in the adsorbed bilayer as a function of time at different temperatures as described in the legend. Continuous line are fits according to single exponential decay law.**

References

[1] P. F. Devaux, *Biochemistry*, 1991, **30**, 1163–1173.

[2] B. Fadeel and D. Xue, *Critical reviews in biochemistry and molecular biology*, 2009, **44**, 264–277.

[3] D. L. Daleke, *Journal of lipid research*, 2003, **44**, 233–242.

[4] M. Nakano, M. Fukuda, T. Kudo, H. Endo and T. Handa, *Phys. Rev. Lett.*, 2007, **98**, 238101.