ESRF	Protrusion modes in single supported lipid bilayers	Experiment number: SC 2748
Beamlin e: BM 32	<b>Date of experiment</b> : from: 08/04/2009 to: 14/04/2009	Date of report: 22/09/2009
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## **Report:**

The physics of fluid membranes is intrinsically a multiscale phenomenon. At the molecular scale, single phospholipids display translational, rotational and vibrational motion. In turn, the collective motion of the membrane at larger length scales reveals the constitutive parameters of the membrane as its elasticity. A wide variety of physical phenomena, like phase transitions, equilibrium and non-equilibrium fluctuations, or destabilization and topological changes involve complex mechanisms in a range of length scales going from nanometer scale to hundreds of microns. In particular, lipid protrusions which are molecular-scale fluctuations of the molecules protruding out of the membrane play a key role in short-range potential and are essential for many biological properties. Nevertheless, this molecular-scale fluctuations are difficult to access experimentally, and have essentially been studied theoretically [1] and numerically [2].

The aim of the experiment was to investigate the cross-over between collective fluctuation modes and the individual protrusion modes. In order to achieve this goal, we extended a recent experimental work [3] to investigate strongly adsorbed or grafted bilayers. Experiments were carried out on single supported bilayer (DSPC and DMPC in both gel and fluid phase

(see figure 1 left)) deposited on silicon substrates. All samples, including bare silicon wafers and first grafted layers, were characterized by specular reflectivity measurements.

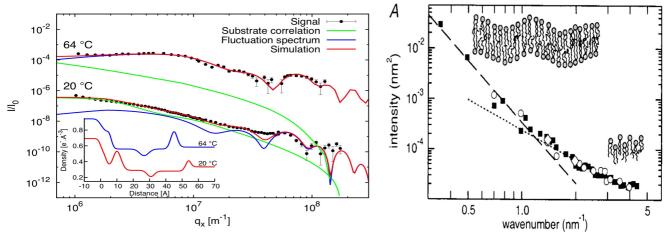


Fig.1: (left) Off-specular reflectivity experiments of a single supported DSPC bilayer in gel an fluid phase and best fit (in insert electronic density profile). (right) Results from numerical simulation showing the cross-over between collective fluctuation modes (small wave number) and individual protrusion modes.

These experiments clearly show that the single bilayer interaction with the silicon substrate is strong enough to suppress the collective fluctuation modes, and we observe a  $q^{-2}$  fluctuation spectrum, associated to a large surface tension ( $\gamma \sim 70 \text{ mN.m-1}$ ). This observation is in good agreement with theoretical paper [2] and recent numerical simulations [1] (see Fig. 1 right). As far as we know, this is the first direct experimental observation of this short wavelength spectrum. We could also investigate the effect of chain length by performing experiments on shorter molecules (DMPC). The interpretation of this experiments is still in progress.

We were also able to record the off-specular scattering from fully charged phospholipid bilayer (100 %). These preliminary experiments show interesting correlated fluctuations of the double bilayer, and a possible effect of charges on protrusion modes.

The beamline BM32 was working well (although the 16-bunch lead to a doubling of the acquisition time, as expected) and the beamtime was used at 100%. Sample preparation was also satisfactory, according to both quantitative (transfer rates reproducibly > 95%) and qualitative criteria.

[1] Lipowsky, R. and Grotehans, S., *Hydration vs. protrusion forces between lipid bilayers*, Europhysic Letters **23**, 599-60 (1993).

[2] Lindahl, E. and Edholm, O., *Mesoscopic Undulations and Thickness Fluctuations in Lipid Bilayers from Molecular Dynamics Simulations*, Biophysical Journal, **79**, 426-433 (2000).

[3] J. Daillant, E. Bellet-Amalric, A. Braslau, T. Charitat, G. Fragneto, F. Graner, S. Mora, F. Rieutord, B. Stidder *Structure and fluctuations of a single floating lipid bilayer*, PNAS **102** 11639-11644 (2005).