Soft matter

Horizontal reflectometer FIGARO

Lipid flip-flop mechanism: loss of asymmetry in a model membrane system

Lipid translocation in membranes is a crucial process in biological science still far from being understood and characterised. 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 hypothesised to be promoted by the action of specific enzymes and by retentive mechanisms that trap lipids in one leaflet of the bilayer. The experiment performed showed that for a reconstituted system, where traps and enzymes were not present, it is not possible to keep the original asymmetric structural composition once all the lipids are in the fluid phase.

The nature of the process, its timescale and the necessary condition for its activation are not only far from being understood in natural cell membranes but also in simpler model systems. For example several discrepancies about the occurrence of the process and its characteristic time scale are present in the literature [3,4]. With the experiment performed on the neutron reflectometer FIGARO we provided a clear interpretation of the structural conditions necessary to activate this process in a model membrane system. Among all the available techniques neutron reflectivity is a unique tool for the investigation of the absolute composition and the relative location of the molecules within the bilayer and at its interfaces. Using Langmuir-Blodgett and Langmuir Schaefer deposition techniques (made available at the PSCM* facility of the ILL) we could prepare

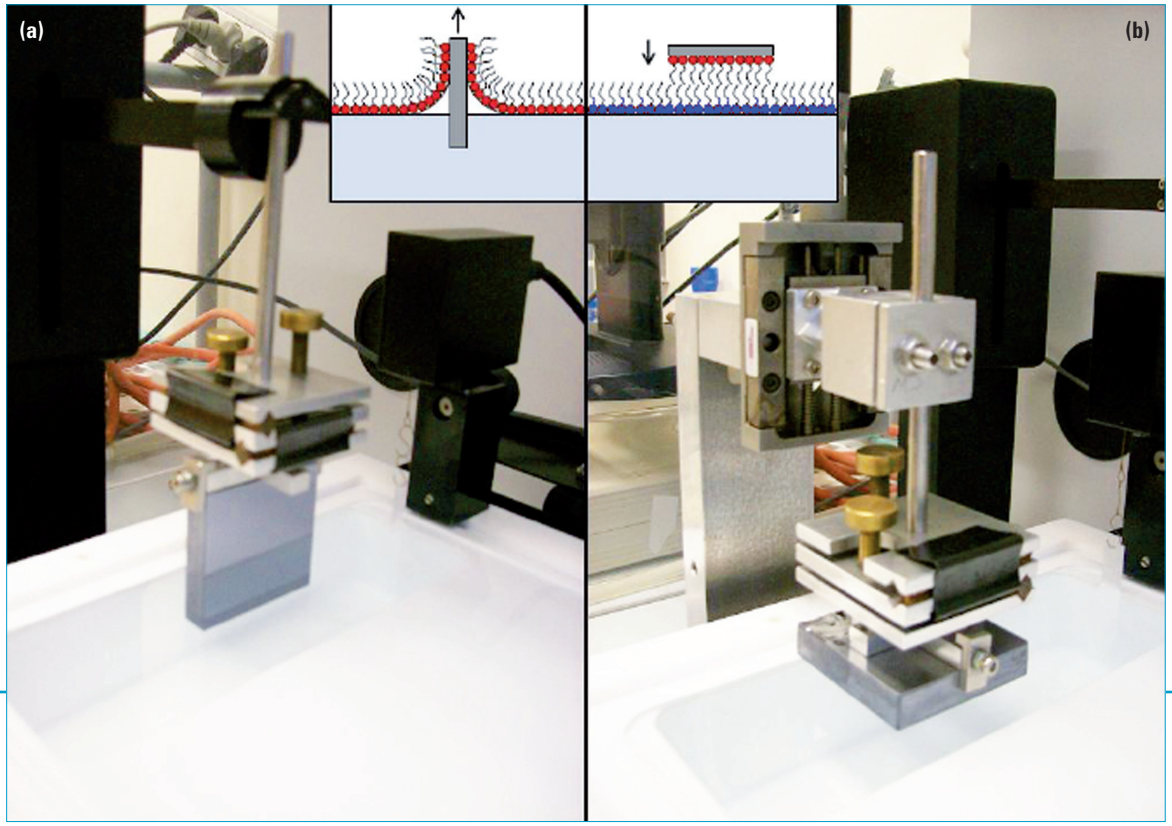

Figure 1: Langmuir-Blodgett **(a)** and Langmuir-Schaefer **(b)** stages used to produce the asymmetric starting bilayer. The NIMA trough is part of the PSCM equipment.

an asymmetric lipid bilayer with deuterated phospholipids in the gel phase and hydrogenated ones in the fluid phase in the inner and outer leaflet, respectively. The preparation steps are summarized in **figure 1**. In both phases the lipid molecules are constrained to the two dimensional plane of the membrane, but in liquid phase the molecules diffuse freely within this plane, being the packing of the molecules less ordered. The transition temperature between the two phases is indicated as melting point and this is different for different lipid types.

Crossing both of the phase transition temperatures activates the flip-flop process resulting in a structural rearrangement of the molecules toward complete mixing.
The reflectivity data and the resulting structural profiles are compared in **figure 2 (a)** panels for starting deposition, **(b)** ones for the final structure).

The temperature dependence of the reflectivity signal indicates that the asymmetry is maintained when at least one side of the bilayer is composed by lipids in the gel phase; the molecules mix completely when the melting point of both the lipid species is crossed [5].

Natural membranes are commonly composed by lipid in the fluid phase together with an asymmetric distribution of lipid species between the two leaflets. The experiment performed clearly demonstrates that is not possible to keep the asymmetric structural composition in a model bilayer once all the lipids are in the fluid phase. Therefore more complex systems have to be developed in order to mimic properly a natural asymmetric membrane.

** Partnership for Soft Condensed Matter, see p. 107.*

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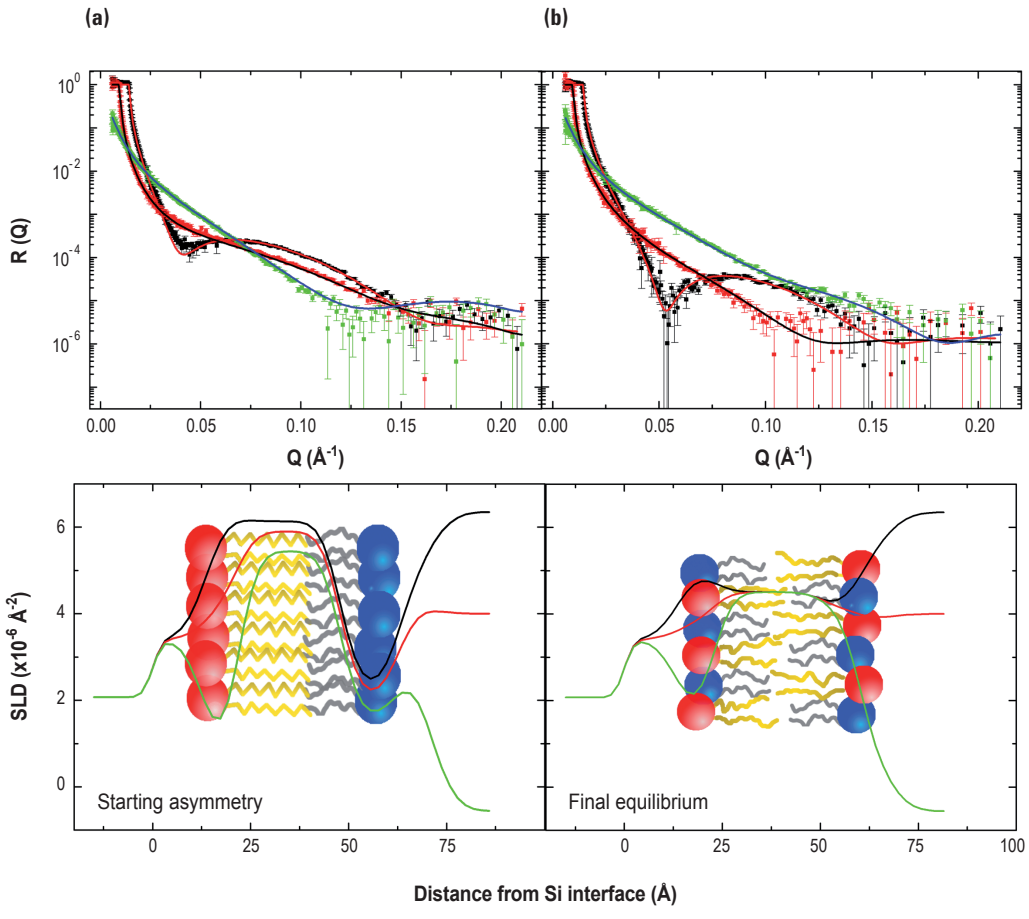

Figure 2: Reflectivity curves measured in different solvents before **(a)** and after **(b)** the phase transition. The resulting scattering length density profiles are shown in the lower panels. A pictorial sketch of the corresponding structures is reported as well.