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Report:

The experimental study of membrane-membrane or membrane-protein interactions in biophysics depends upon the fabrication and characterization of systems having well-controlled physical properties. A "free" molecular bilayer of phospholipid molecules deposited on top of a strongly adsorbed identical bilayer on a super-polished silicon block was studied *in situ* in an aqueous environment by diffuse synchrotron X-ray scattering and reflectivity measurements. The reported run was a continuation of the program concerning previous synchrotron measurements (32-2-76 and 32-2-89) as well as complementary neutron reflectivity measurements at ILL.

Much higher quality bilayer samples were fabricated by the use of the phospholipid molecule DSPC ($L-\alpha$ Di-steayrl phosphatidyl-choline) for which Langmuir-Blodgett transfer ratios close to unity were achieved. The diffuse scattering results confirm the quality of the samples where a much smaller contribution of the characteristic signal arising from small-scale holes in the bilayer was observed. A total of 23 different scattering curves were obtained through the optimization of the acquisition time, the choice of samples and of sample temperatures around the bilayer fluid-gel transition temperature. Both diffuse scattering and reflectivity data were measured for the bare silicon-oxide/silicon substrate, the strongly adsorbed phospholipid single bilayer as well as three double-bilayers samples (under both light and heavy water - for comparison with the neutron studies). We were able to follow the results in "real-time" and to adjust in consequence the conditions, in particular the choice of temperatures, zeroing-in around the phase transition. The samples were all of excellent quality and the experimental program was followed without a hitch, much due to the preparations but also thanks to the excellent contribution of François Rieutord both during the experiments and through scientific discussions.

The substrates used were of exceptional quality with measured rms roughnesses $< 0.1\ nm$; the very weak diffuse scattering from the bare silicon-oxide/silicon block was measured, and this signal was shown to be negligible compared to the scattering from the fluctuating and imperfect phospholipid bilayer.

The effects of the fluid-gel transition of the bilayer can be clearly seen in both the specular reflectivity measurements as well as in the grazing-incidence diffuse scattering spectra. The fluctuations of the two interfaces : water-hydrophilic head groups of the second "free" phospholipid bilayer are found to be coupled and the temperature-dependent characteristic distance between the two bilayers is obtained.

The specular reflectivity measurements yield first-order structural information that correctly simulate the grazing-incidence diffuse scattering interferences. The neutron results, although containing much less detail and limited to a much smaller range of wavevector transfer, yield valuable information as to the underlying physics of the phase transition. It is interesting to note that differences of structure were measured in the X-ray experiments comparing identical samples under light and heavy water, leading to a re-examination of the neutron reflectivity analysis. We believe the differences are due to the very high sensibility of the phospholipid head group interactions to the presence of ions in the aqueous phase.

The following figure illustrates for one double-bilayer sample a comparison between calculated and measured diffuse scattering spectra, using reasonable physical values for the model.