Resolving hydrated lipid bilayer structures– A novel technique to characterize wet 2D crystals

R. Ziblat, K. Kjaer1,2 L. Leiserowitz3 and L. Addadi

Department of Structural Biology, Weizmann Institute of Science, 76100 Rehovot, Israel
1 Max-Planck Institute of Colloids and Interfaces, D-14476 Potsdam / Golm, Germany
2 Niels Bohr Institute, University of Copenhagen, DK-2100 Copenhagen, Denmark
3 Department of Materials and Interfaces, Weizmann Institute of Science, 76100 Rehovot, Israel,

Cell membrane lipid bilayers are currently thought to consist of different phases and different domains at the nanometer scale. These domains (also known to some as ‘lipid rafts’) differ in composition, structure, stability, and consequently in properties and function. They selectively incorporate or exclude specific proteins, and thereby fulfill an important function in cell activity and signaling. Understanding the rules that govern membrane dynamics and structure is thus crucial to understanding cell biology.

Grazing incidence X-ray diffraction (GIXD) has been used for studying the structure of two-dimensional (2D) lipid monolayers at the air-water interface. Membranes are, however, composed of two opposing leaflets, i.e. of two juxtaposed monolayers, sandwiched between water. It is conceivable that not only lateral interactions within the layer, but also interactions between the hydrophobic tails of the two leaflets influence their packing, i.e. the two leaflets may correlate. It has been argued that order in one leaflet might, in some cases, impose order on the other. The structure of the bilayer may thus be different from that of the corresponding monolayer. In order to guarantee preservation of the structural integrity of a membrane bilayer with hydrophobic interior and hydrophilic external surfaces, wetting on both sides of the bilayer is most probably required. Failing to fulfill this requirement would inevitably raise doubts on the validity of the determined structures. However, due to the strong X-ray background scattering contribution of liquid water, until now GIXD experiments have usually been reported on single (or odd number of) lipid films which are dry at the side of the impinging X-ray beam, or stacks of many superimposed bilayers. We have developed a technique, which enables GIXD measurements on single hydrated bilayers, in a state more similar to that in cells. It does so by maintaining the sample under high humidity close to the dew point, which results in a thin condensed water layer on top of the bilayer. This method results in a strong diffraction signal, as can be seen in Fig 1.

Comparison between the ceramide (Cer) bilayer and the Cer monolayer shows small differences, mainly in the coherence length of the diffracting domain. However, comparison between bilayer and mono-layer of cholesterol:Cer (Ch:Cer) mixtures (Fig 2) show that domain sizes of the mixed phase differ greatly. In addition, the Ch:Cer monolayer withholds up to two phases as can be expected from the Gibbs phase role, however the bilayer system has an additional phase. Both of these differences are a direct result of the opposing leaflets correlation.
Figure 1: Diffracted intensity signal from (a) Cer C16 hydrated bilayer deposited on a Si wafer and (b) Cer C16 monolayer at the water/He interface on a Langmuir trough. The images were produced unmodified from the raw data. In each image, black represents the highest intensity. (c) Bottom: Bragg peak from Cer bilayer deposited at pressure of 35 mN/m compared with the Bragg peaks from Cer monolayers at ~0 and 35 mN/m (top and middle, respectively). Bragg peaks were fitted with Voight functions (solid lines) (d) Bottom: Bragg rods from Cer bilayer compared with the Bragg rods from Cer monolayers at ~0 and 35 mN/m (top and middle, respectively).

Figure 2: Diffraction peaks of ceramide and cholesterol composite bilayers at varying ratios. The monolayer of Ch:Cer at ratio of 60:40 is at a homogenous mixed phase, and has a diffraction peak at qxy=1.28. For comparison, the diffraction signal of the Ch:Cer 60:40 monolayer is shown at the top.