

# High resolution studies of lipid bilayers

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Fluctuations are vital to biological systems. Average structures, which specify the baseline about which fluctuations occur, are also important. We have found, in studying biomembranes, that taking account of fluctuations in the analysis of data can be an important part for correctly determining average structure. Therefore, there are two important reasons to study fluctuations.

Our x-ray diffraction measurements were carried out on multilamellar vesicles composed of an onion-skin-like packing of layers. Each layer contains a bilayer membrane made of a single type of lipid molecule and a water layer which separates successive membranes. This layer structure gives rise to "low angle" scattering peaks corresponding to the periodicity,  $D$ , of typically 60-70 Å. "Wide angle" peaks measure the in-plane packing of the lipid molecules with typical spacings of 4.2-4.6 Å. Vesicles are of the order of one micron in diameter.

Biomembranes are usually quite fluid. In the biologically relevant  $L_\alpha$  or fluid phase, the hydrocarbon chains of the lipid molecule are disordered and there is only short range order in the intermolecular packing. One observes the well known broad, liquid-like peak in the wide angle region. Nevertheless, in this same phase, the low angle peaks are well separated and quite sharp, revealing highly anisotropic ordering characteristic of smectic liquid-crystalline systems (see Fig. 1).

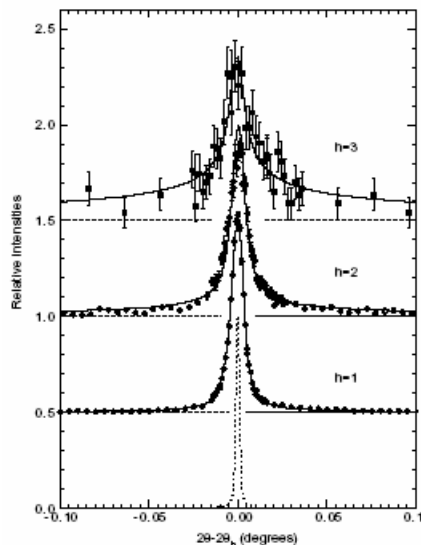
In the lower temperature chain-ordered gel phase, considerable diffuse scattering coexists with a very sharp (20) chain packing peak (see Fig. 2). At CHESS we were able to resolve this sharp peak and found that its width corresponds to a correlation length of 2900 Å, or about 300 molecular spacings [1]. Fig. 2 emphasizes that despite this long ranged order, there is substantial diffuse scattering under the in-plane peaks. It is remarkable that these membranes can simultaneously have large correlation lengths and still have so much diffuse scattering due to disorder and fluctuations.

Our current work at CHESS focuses on those fluctuations that affect the low-angle scattering peaks in Fig. 1. The first kind of fluctuation involve a statistical distribution of  $D$ -spacings along the stack; this is basically

disorder of the second kind as defined by Guinier or paracrystalline theory as developed by Hosemann. However, our experimental results show that the data are inconsistent with this picture. The missing ingredient is undulations consisting of wavelike motions of the membranes. The statistical thermodynamic theory that includes both effects was given by DeGennes, and many of the most important scattering implications were worked out by Caillé, with subsequent experimental verification in liquid crystal physics. This development (for references, see [2]) reveals the key feature that the system has power law correlation functions, so the low-angle peaks, corresponding to the different orders of lamellar reflection, are not Bragg peaks, but have power-law tails. This means that the system is not a crystal with true long range order.

While the preceding conclusion may seem obvious, since these systems are liquid crystals rather than crystals, there are implications in biophysics that have not been recognized. One of the most important uses of x-ray scattering data is to obtain the local, average electron density profile along the normal to the bilayer; determination of this average structure requires the relative intensities of the low angle scattering peaks. Traditional analysis, however, assumes that the system is a one-dimensional crystal and does not account for disorder and fluctuations. This ignores the fact that much of the intensity is pushed out of the central scattering peaks and into the long power law tails where it cannot be measured reliably. Crucially, this effect becomes more pronounced for the higher order peaks so that the measured form factors,  $F(h)$ , are erroneously too small for the higher order reflections. In turn, reconstructed electron density profiles will be incorrect.

Using data from CHESS, we have tackled this question using the Caillé type of theory [2] which had not previously been applied to this type of biophysical question. We found that the



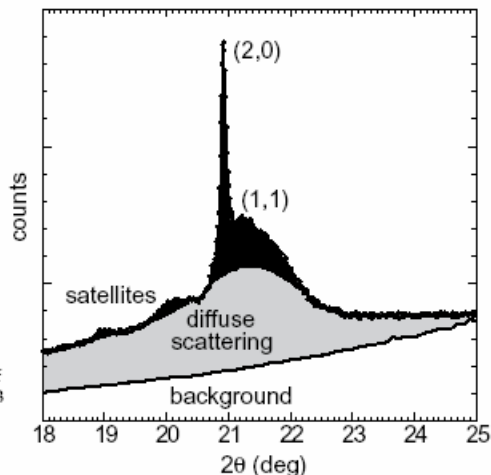
(Figure 1) High resolution low angle scattering from multilamellar DPPC bilayers at 60°C. Three scattering peaks,  $h = 1-3$ , are plotted versus  $2\theta - 2\theta_0$ . Since  $2\theta \approx h$  in degrees, these peaks are well separated. The heights of the peaks have been normalized to facilitate comparison of peak shapes. The background level for each peak is shown by a dashed line. The dotted line shows our in-plane resolution. The solid lines show the fits using the theory in [2].

original Caillé theory predicts a huge error. However, we improved an approximation employed by Caillé and found a much more modest, but still significant, correction that will be important for obtaining accurate lipid bilayer structure [2].

To obtain the corrected intensities experimentally requires careful measurement of the shapes of the scattering peaks. From the shapes, one learns about the fluctuations. In particular, the Caillé fluctuation parameter that governs the power law behavior of the scattering tails involves the elastic moduli that govern the undulatory and compressional fluctuations. The fluctuation parameter obtained from the peak shapes also yields the factors by which successive scattering peaks are attenuated by the fluctuations and we can correct the observed intensities in order to extract the form factors,  $F(h)$ . In the end, we gain knowledge of both the average structure, in the form of the local electron density profile, and the amplitude of long wavelength fluctuations allowed by this structure [2].

To measure peak shapes quantitatively requires the high resolution/high intensity combination that is available at the F3 station and that is not available with rotating anode sources. The F3 station double bounce monochromator was tuned to 1.2147 Å x-rays, the scattering angles were selected by a Si analyzer crystal, and the intensity was measured by a scintillation detector. In-plane resolution was measured to be  $2 \times 10^{-4}$  Å<sup>-1</sup> and this successfully resolved the intrinsic peak shapes by about a factor of 5. Although out-of-plane resolution was only 0.012 Å<sup>-1</sup>, this is easily taken into account in the analysis. Getting the background low enough to measure low in-

(Figure 2) Two-dimensional Braggrod scattering from DPPC bilayers at 20°C (after [1]). These in-plane, wide angle peaks are indexed on a nearly hexagonal, centered rectangular lattice. The (2,0) is sharp and the (1,1) is broad because the hydrocarbon chains are tilted relative to the membrane plane about the (2,0) direction.

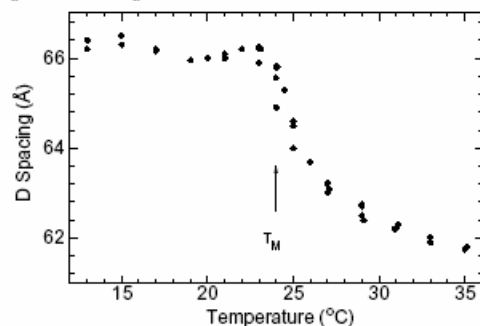


tensities in the tails of the peaks required extension of the 2θ arm in the F3 station. While this was not a huge instrumental challenge, it did require

several trips and considerable assistance from CHESS Staff Scientist Randy Headrick, our on-site collaborator, to get it all right. Typical data are shown in Fig. 1, along with our theoretical fit.

Our first application was to lipid bilayers near the transition into the  $L_{\alpha}$  phase. As a function of temperature the D-spacing has an anomalous change that suggests critical fluctuations, as indicated in Fig. 3. One model suggested that these would be fluctuations in the water spacing due to increased undulations in the membranes; this would require the Caillé fluctuation parameter to change substantially with temperature which would dramatically change the size of the power law tails. The temperature dependence of our peak shapes do not, however, support this model [3].

The successful model has the bilayer itself, not the water spacing changing thickness anomalously near the melting temperature  $T_M$ . Our data for the membrane form factors strongly support this interpretation [3].



(Figure 3) Lamellar D-spacing versus temperature for DPPC bilayers (after [3]).

This model suggests that there is another kind of fluctuation that plays the most important role in structural changes near the transition, namely, fluctuations in the thickness of the membrane brought about by critical freezing of the rotameric degrees of freedom in the hydrocarbon chains. Of course, the interlamellar fluctuations still play their usual role and we observe that the Caillé fluctuation parameter is strongly affected by moderate dehydration of the water layer between membranes.

Perhaps not surprisingly, there are many kinds of fluctuations in complex biological materials such as biomembranes. High resolution x-ray scattering, coupled with careful theoretical analysis, is an important way to elucidate them and, at the same time, to determine average structure.

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3. R. Zhang, W. Sun, S. Tristram-Nagle, R. Headrick, R. M. Suter and J. F. Nagle, Critical Fluctuations in Membranes. *Phys. Rev. Lett.* 74, 2832-2835 (1995).