# Collective Dynamics in Fully Hydrated Phospholipid Bilayers Studied by Inelastic X-Ray Scattering

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The short wavelength density fluctuation of DLPC (dilaurylphosphatidylcholine) bilayers close to full hydration has been studied by the inelastic x-ray scattering technique below and above the main transition temperature. The analysis based on a generalized three effective eigenmode theory allows us to construct the dispersion relation of the high frequency sound mode for the first time. The marked softening of the excitation near $k = 14$ nm$^{-1}$, corresponding to the lipid chain-chain correlation peak in the structure factor, in the $L_\alpha$ phase implies prevalent occurrences of short-wavelength in-plane motions of lipid chains that might be of importance for transportation of small molecules across membranes.

Phospholipids are amphiphilic molecules of biological interest, consisting of a phosphoryl choline group as the polar head and a bulky, hydrophobic double chain of acyl hydrocarbons in the tail region. They are known to readily self-assemble into ordered bilayers or multilamellar vesicles in the environment of water. Fully hydrated phospholipid bilayers have been employed as a model system in many biophysical studies to provide insight into the structure and function of biological membranes. The structures of phospholipid bilayers have been studied by a variety of methods such as differential scanning calorimetry [1], H$^2$ nuclear magnetic resonance [2], x-ray and neutron diffractions [3–5], and computer molecular dynamics simulations [6]. For the single particle dynamics, the diffusion and rotation of lipid molecules in bilayers have been studied by ESR [7], NMR [8], excimer [9], and by fluorescence recovery after photobleaching [10]. A recent incoherent quasielastic neutron scattering experiment [11] measured the confined diffusion of lipid molecules in oriented DPPC bilayers by time of flight and backscattering neutron spectroscopy. Also, the undulatory low frequency excitations of highly ordered stacks of chain-deuterated lipid bilayers at a high hydration level have been studied by coherent neutron spin-echo spectroscopy [12]. However, studies of the in-plain collective dynamics of lipid chains are scarce so far. Knowledge of collective density fluctuation of hydrocarbon chains in lipid bilayers is essential for understanding some aspects of biological functions of natural membranes. For example, it has been conjectured that the formation of gauche-trans-gauche kinks due to the collective thermal motion of hydrocarbon chains provides a possibility for the trans-membrane transport of small molecules such as water or ions [13].

In this Letter, we report on a study of fully hydrated DLPC (dilaurylphosphatidylcholine) bilayers by coherent inelastic x-ray scattering (IXS). Since x rays are scattered by fluctuation of electron density in the medium, the IXS probes directly the collective dynamics of atomic density fluctuations. The analysis of high resolution IXS spectra shows that some characteristic features of the dispersion relation of the propagating in-plane density fluctuation of hydrocarbon chains are similar to but enhanced compared to that of simple liquids.

The DLPC bilayer sample close to full hydration was measured at two temperatures, $T = 269$ and 294 K. Since the main transition temperature of a fully hydrated DLPC bilayer is at about 272 K [14], these two temperatures studied correspond to the gel phase ($L_{\beta'}$) and the liquid crystalline phase ($L_\alpha$) of the bilayer. The nearly full-hydrated DLPC has water content of about 20 wt % (corresponding to about ten water molecules per headgroup). The sample was a 0.5 mm thick flat layer deposited on a horizontal flat quartz surface and was believed to be in a state of partially oriented multilamellar vesicles which is a lyotropic smectic liquid crystal at room temperature.

The IXS experiment was carried out at a very high-resolution IXS beam line (BL21-ID 16) at the European Synchrotron Radiation Facilities [15]. The undulator x-ray source was premonochromated by a Si(111) double crystal monochromator, followed by a high energy resolution backscattering monochromator (temperature controlled and scanned), operating at a higher-order Si(11 11 11) Bragg reflection (x-ray energy 21.748 KeV). The scattered photons were collected by a grooved spherical silicon crystal analyzer of 10 cm diameter, operating at the same Bragg back reflections (in a Rowland geometry). The net energy resolution was measured by an elastic scattering of a plastic sample at the maximum of its structure factor. This gives a Gaussian shape energy resolution function of 1.5 meV in width (FWHM). The x-ray beam size at the sample was 0.15 mm (high) $\times$ 0.35 mm (wide). The incident x-ray beam enters horizontally, parallel to the plane of the sample and the scattered beam is collected also in the horizontal direction so that the scattering plane coincides

with the plane of the sample. The scattering vector $k$ is largely in the plane of lipid bilayers. The path length of the x-ray beam in the sample is about 1 cm.

X-ray scattering amplitude of an atom is proportional to the atomic form factor which is a $k$-dependent quantity and its magnitude is equal to the atomic number $Z$ of the atom at $k = 0$. The IXS spectral intensity is then contributed mainly by the partial dynamic structure factors between pairs of oxygen atoms in the hydration water and that of carbon atoms in the hydrocarbon chains of DLPC, due to their higher atomic numbers and larger number densities. In order to find which atom contributes dominantly in the IXS spectra, we measured also the structure factor by x ray. The structure factor at a particular $k$ determines the total spectral area in the dynamic structure factor at that $k$ value and a peak in the structure factor implies the importance of the corresponding dynamic structure factor at that $k$ value. The diffraction peak arising from correlations of two adjacent hydrocarbon chains in the bilayer is located at about $k = 14$ nm$^{-1}$. It is clearly visible in the measured structure factor, but we do not see the first diffraction peak (O-O correlation peak) of the hydration water which should be located at about $k = 20$ nm$^{-1}$. This may be due to the fact that hydrated water molecules are in a state of less order than the hydrocarbon chains of DLPC molecules constituting the bilayer. The higher degree of order among the hydrocarbon chains gives a much larger partial structure factor of C-C than that of O-O in the hydrated water. We may then conclude that the total dynamic structure factor is largely dominated by the partial dynamic structure factor of C-C.

The IXS spectra were analyzed by a generalized three effective eigenmode theory (GTEE) we formulated recently by extending a TEE model formulated previously by de Schepper and co-workers for simple liquids to supramolecular liquids [16,17]. The GTEE model is an approximate dynamical theory governing a set of slow, collective dynamic variables of a supramolecular liquid. We identify this set of variables as the three atomic form factor weighted quasiconserved quantities, namely, the number density, the longitudinal current, and the energy density. In the GTEE model, the normalized dynamic structure factor $S(k, \omega)/S(k) = \mathrm{Re}\{S(k, z = i\omega)\}/\pi$ can be written as [16]

$$S(k, z) = \left[ z + \frac{f_{un}^2(k)}{z + z_u(k) + \frac{f_{uT}^2(k)}{z + z_T(k)}} \right]^{-1} \quad (1)$$

where $f_{un}(k) = kv_0(k)/\sqrt{S(k)}$, $v_0(k)$ is the generalized $k$-dependent thermal velocity with the atomic form factor included [18], and $S(k)$ is the form factor weighted generalized structure factor. Besides $f_{un}(k)$, there are three unknown quantities, treated as fitting parameters in our analysis, $z_u(k)$, $z_T(k)$, and $f_{uT}(k)$. They are cross terms arising from coupling of the longitudinal current and energy density. The GTEE theory can be considered as the solution of a generalized hydrodynamic equation,

where the hydrodynamic variables have been extended to $k$-dependent quantities and the exact second moment of the dynamic structure factor has been incorporated into the solution through the factor $f_{un}^2(k)$. In the hydrodynamic limit $k \to 0$, for monoatomic fluid, the three unknown quantities have values given by [17] $z_u(k) = \phi k^2$, $z_T(k) = \gamma D_T k^2$, $f_{uT}(k) = kc_s\sqrt{(\gamma - 1)/\gamma}$, where $c_s = v_0[\gamma/S(0)]^{1/2}$ is the adiabatic speed of sound, $\gamma = c_p/c_v$ is the ratio of the specific heat per unit mass at constant pressure and volume, $\phi$ is the kinematic longitudinal viscosity, and $D_T$ is the thermal diffusivity.

The theoretical dynamic structure factor is then multiplied by the Bose factor (the detailed balance factor) and convoluted with the energy resolution function before compared with experimental spectra which have been normalized to have unit area. All the experimental spectra have been fitted very well by the theory. The three poles of $S(k, z)$ are $\Gamma_h(k)$, $\Gamma_s(k) \pm i\Omega_s(k)$, respectively, where $\Gamma_h(k)$ is the relaxation rate of the central nonpropagating mode, $\Gamma_s(k)$ is the damping, and $\Omega_s(k)$ is the frequency of the high frequency sound mode (propagating density fluctuation). These three physical parameters are functions of the four fitting parameters: $f_{un}(k)$, $z_u(k)$, $f_{uT}(k)$, and $z_T(k)$.

Figure 1 shows $k$ dependence of the frequency of high frequency sound (the dispersion relation) and the sound damping (inset). The sound frequencies increase linearly at small $k$, with a sound speed of about 2580 m/s. In contrast, the IXS of bulk water at room temperature gives the high frequency sound speed of 3200 m/s [15]. When $k$ increases to 6 nm$^{-1}$, which is about halfway to the peak position of the structure factor (14 nm$^{-1}$), the sound frequencies start to decrease as $k$ increases. The second upward swing of the dispersion curve commences just after

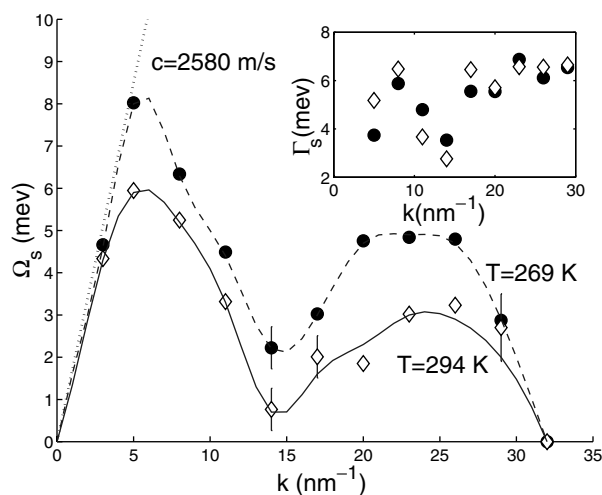

FIG. 1. The high frequency sound dispersion relation (symbols) and damping (inset) of fully hydrated DLPC at two different temperatures $T = 269$ K (solid circles) and $T = 294$ K (diamonds). The lines are drawn only to guide the eyes. The initial slopes of the dispersion relations give the high frequency sound speed indicated in the figure.

$k = 14$ nm$^{-1}$ thus forming a valley at the peak position of the structure factor. The data points at the largest $k$ value with zero sound frequencies imply that there are no more sound propagation at this $k$ value, and the three poles in $S(k, z)$ all become real. In this case, the dynamic structure factor consists of three Lorentzians centered at $\omega = 0$ with different relaxation rates. We note that the existence of the valley is similar to but more pronounced than that in simple liquids. The examples are the gap in the dispersion curves in liquid argon [19], the Lennard-Jones fluid [20], and the dips in liquid cesium [21] and liquid He$^4$ [22]. One can make a qualitative argument as to why the dispersion relation has a dip at the peak of the structure factor. One knows that the second moment of dynamic structure factor is given by [23] $\langle \omega^2 \rangle = k^2 v_0^2(k)/S(k)$, assuming that the dynamic structure factor consists of a sharp triplet with a central peak at $\omega = 0$ and symmetrically shifted side peaks located at $\pm\Omega$. Then the second moment is dominated by the shifted peaks and is approximately equal to $2\Omega^2$. Hence, we have an approximate relation that the sound frequency $\Omega = kv_0(k)/\sqrt{2S(k)}$, inversely proportional to the square root of the structure factor. This argument although plausible is, however, not quatitatively accurate exept for the position of the minimum. This is because the argument does not take into account finite damping of the central and side peaks. For the lipid bilayer, the dip in the dispersion relation is actually deeper in the liquid crystal phase than in the gel phase, while the experimental structure factor peak is lower in the former than in the latter as can be seen from Fig. 3. Likewise, in simple liquids, the magnitude of the dip varies from liquid to liquid without quantitative theoretical understanding so far.

Figure 1 shows that there is a significant difference in high frequency sound dispersion relations at the two temperatures studied. The sound frequencies in the gel phase ($T = 269$ K) are higher than those in the liquid crystalline phase ($T = 294$ K). This temperature dependence of the collective movements of hydrocarbon chains can also be observed directly from the IXS spectra at $T = 269$ and $T = 294$ K (Fig. 2). The thick solid lines in the figure are the result of the fitting (chi square is of the order of unity). In both cases in the energy loss side, the sound modes can be observed as shifted peaks from the raw data. At the gel phase ($T = 269$ K), the hydrocarbon chains are ordered, the dynamics is solidlike. The sound frequencies are higher and the sound mode is less damped, but in the liquid crystalline phase, the hydrocarbon chains are in a state of disordered liquid; the increase of viscosity increases the sound damping. This is the first experimental observation from collective dynamics that the hydrocarbon chains melt when temperature increases and crosses the main transition temperature.

This order-disorder transition can also be observed in the measured x-ray structure factor (lines in Fig. 3). The structure factor of gel phase shows a much sharper peak at 15 nm$^{-1}$. Figure 3 serves as one of the verifications of the GTEE analysis of the IXS spectra. We can compare the
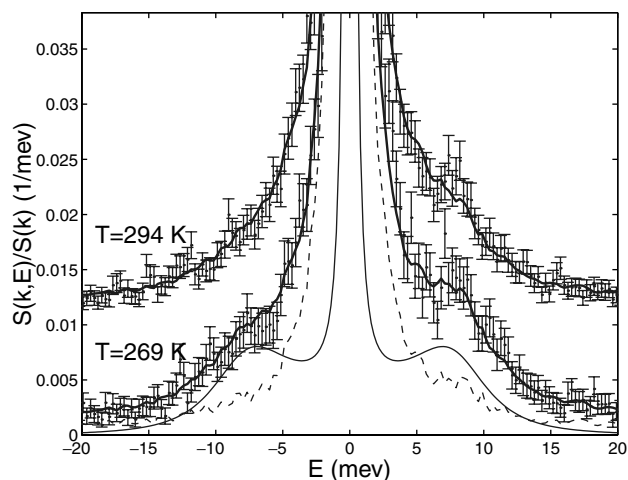


FIG. 2. The IXS spectra of fully hydrated DLPC at $k = 5$ nm$^{-1}$ at $T = 269$ and 294 K. The thick solid lines are the fits by the GTEE theory in absolute scale. The thin solid line is the theoretical dynamic structure factor at $T = 269$ K, and the dashed line is the energy resolution function. The full height of the central peak of IXS spectra is about 0.35 in the $y$ scale.

experimentally measured $S(k)$ (lines) with the calculated structure factor from a fitted parameter (symbols). One can calculate the $S(k)$ by the equation: $S(k) = [kv_0(k)/f_{\mathrm{un}}(k)]^2$, where the generalized thermal velocity $v_0(k)$ is calculated according to $v_0(k) = v_0 f(k)$, where $f(k)$ is the atomic form factor of carbon and $v_0 = \sqrt{k_B T/m_c}$ is the thermal velocity of the carbon atom. The experimentally determined $S(k)$ is obtained from x-ray scattering measurements without energy analysis. In order to compare them at the same scale, the experimental $S(k)$ were multiplied by an appropriate constant factor. One can readily see that the two curves obtained through different methods exhibit significant similarities. We note that the experimental
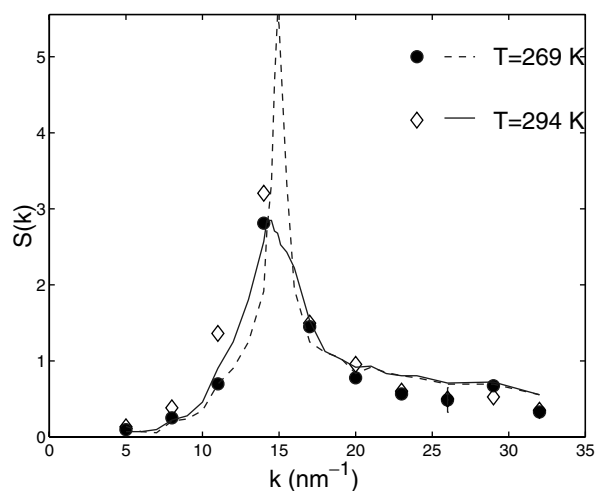


FIG. 3. The calculated $S(k)$ (in absolute scale) from the fitted second moment of the dynamic structure factor $f_{\mathrm{un}}^2(k)$ at $T = 269$ K (sold circles) and at $T = 294$ K (diamonds) are compared with the structure factors (in arbitrary scale) measured by x-ray diffraction at $T = 269$ K (dashed line) and at $T = 294$ K (solid line).
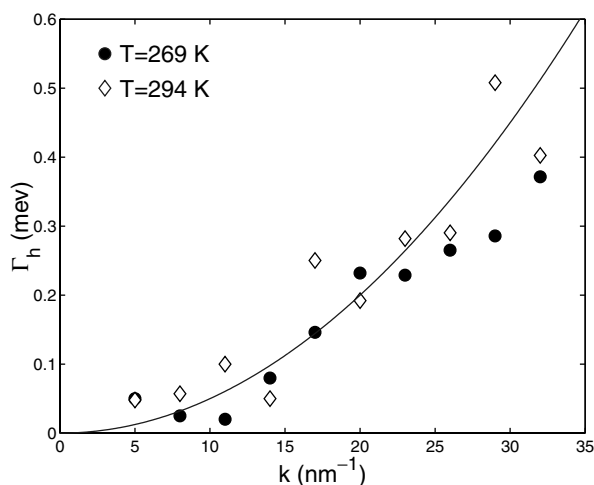
FIG. 4.   The $k$-dependent relaxation rate of the central peak of the dynamic structure factor at the two temperatures. The solid line is the relation $\alpha k^2$, with $\alpha = 7.5 \times 10^{-6}$ cm$^2$/s.

structure factor gives a sharper peak at $k = 15$ nm$^{-1}$ than our calculated one from analysis of the IXS spectra for the case of $T = 269$ K. This is due to an unfortunate lack of the measured inelastic scattering spectrum at $k = 15$ nm$^{-1}$.

Figure 4 shows $k$ dependence of the relaxation rate $\Gamma_h(k)$ of the central peak (the nonpropagating mode) of the dynamic structure factor. There is no significant difference in relaxation rates in the two phases in the measured $k$ range. The relaxation rate has a quadratic dependence on $k$ with a constant thermal diffusivity of $7.5 \times 10^{-6}$ cm$^2$/s.

In conclusion, we used a newly developed high-resolution IXS technique to study the collective dynamics of the fully hydrated DLPC phospholipid bilayer. The collective movements of hydrocarbon chains show a significant temperature dependence due to hydrocarbon chain melting. The GTEE model analysis of IXS spectra allows us to construct the dispersion relation of the high frequency sound. We found a novel feature that the frequencies of the propagating density oscillations show a pronounced minimum at the wavelength corresponding to the two neighboring interchain distances. The fact that this minimum frequency in the $L_\alpha$ phase is less than 1 meV may have theoretical implication on the possible mechanism for passage of water molecules through a functioning bilayer. Although we do not have a theory for permeation of water molecules through the lipid bilayer, it is our feeling that an appropriate theory would have to involve the large amplitude, slow in-plane density fluctuations. In this regard the low frequency interlipid collective oscillations along with the whole dispersion relation should play a key role in this theory. An immediate interest of this discovery is to see whether computer molecular dynamics simulation of hydrated DLPC bilayers can reproduce the measured dispersion relation given in Fig. 1.

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