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Collective dynamics in phospholipid bilayers investigated by inelastic neutron scattering: exploring the dynamics of biological membranes with neutrons

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Abstract

We present the first inelastic neutron scattering study of the short wavelength dynamics in a phospholipid bilayer. We show that inelastic neutron scattering using a triple-axis spectrometer at the high-flux reactor of the ILL yields the necessary resolution and signal to determine the dynamics of model membranes. The results can quantitatively be compared to recent Molecular Dynamics simulations. Reflectivity, in-plane correlations and the corresponding dynamics can be measured simultaneously to gain a maximum amount of information. With this technique, complete dispersion relations can be measured with a high-energy resolution. Structure and dynamics in phospholipid bilayers, and the relation between them, can be studied on a molecular length scale.

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Motions in biological membranes span a wide range of length and time scales [1]. Short wavelength density fluctuations in the plane of the bilayer (as opposed to bilayer bending modes) are likely to play a key role in the transport of small molecules across the bilayer [2], but have received little experimental attention. While the structure of lipid bilayers has been the object of several studies in the last decades, dynamical properties even of simple model systems such as 1,2-dimyristoyl-sn-glycero-3-phoshatidylcholine (DMPC) remain largely unknown. A more quantitative understanding of short range molecular motions in a lipid bilayer is of fundamental interest in membrane biophysics. Experimental results can be linked to modern Molecular Dynamics (MD) simulations [3]. In this context, Chen et al. have recently presented a seminal measurement of the dispersion relation of the acyl chain fluctuations in lipids [4] by inelastic X-ray scattering (IXS). As an important result, Chen et al. found a minimum in the dispersion relation at about $Q_0 = 1.4$ Å^{-1}, the maximum of the static

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structure factor $S(Q_{xy})$. This soft mode was hypothetically linked to transport phenomena within and across the bilayer. Inelastic neutron scattering using a triple-axis spectrometer should offer a much better energy resolution and avoid the danger of radiation damages in the sample, but has to date not been applied to study the dynamics of lipid bilayers. A further important advantage of INS over IXS is the fact that different parts of the bilayer can be probed by use of selective deuteration. We carried out the first inelastic neutron scattering experiments in model membranes on the cold triple-axis spectrometer IN12 at the high-flux reactor at the ILL. In this study we used fully deuterated chains DMPC-d54 (Avanti lipids, Alabama) in D_2O to minimize incoherent background. We report on experimental details and show examples of neutron diffraction and inelastic neutron scattering in deuterated DMPC bilayers.

From a solution of DMPC-d54 (deuterated 1,2-dimyristoyl-sn-glycero-3-phoshatidylcholine) in TFE/chloroform (1:1) with a concentration of typically 40 mg/ml, we prepared highly oriented membrane stacks on 2″ silicon wafers (thickness 300 μm) [5]. Ten of these wafers were stacked on top of and aligned with respect to each other to create a 'sandwich sample' consisting of several thousands of highly oriented lipid bilayers with a total mass of about 300 mg of deuterated DMPC. Aluminum spacers between the substrates allow to let the heavy water vapor in between the wafers to hydrate the bilayers. In rocking scans we find a mosaicity of the sandwich of $\Delta\omega = 0.6^\circ$.

The inset in Fig. 1 shows a schematic of a triple-axis spectrometer. By varying the three axes of the instrument, the axes of rotation of the monochromator, the sample and the analyzer, the wavevectors k_i and k_f and the energies E_i and E_f of the incident and the scattered beam can be determined. The accessible $(\mathbf{Q}, \Omega)$ range for a fixed energy of the scattered beam E_f of 10 meV is shown in Fig. 1. It is limited by the range of incident neutron energies offered by the neutron guide as well as by mechanical restrictions of the spectrometer. The instrumental energy resolution in this configuration is $\Delta\Omega = 500$ μeV. By choosing smaller incident energies and energy transfers the energy resolution can be enhanced. By rotating the sample the scattering vector $\mathbf{Q}$ can be placed either within the plane of the membranes (Q_{xy}) to measure inter-chain correlations or perpendicular to it (Q_z) to measure reflectivity (see inset in Fig. 4). The use of a triple-axis spectrometer thus offers the unique possibility to measure reflectivity, the static structure factor $S(Q_{xy})$ in the plane of the membranes and in plane dynamics ($S(Q_{xy}, \Omega)$) on the same instrument in the same run. To control

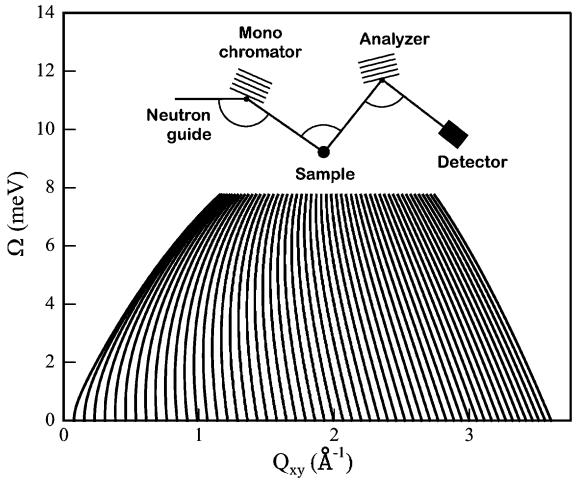

Fig. 1. Accessible (Q, Ω) range for a fixed energy of the scattered beam of $E_f = 10$ meV. In the inset, a schematic of a triple-axis spectrometer is shown.

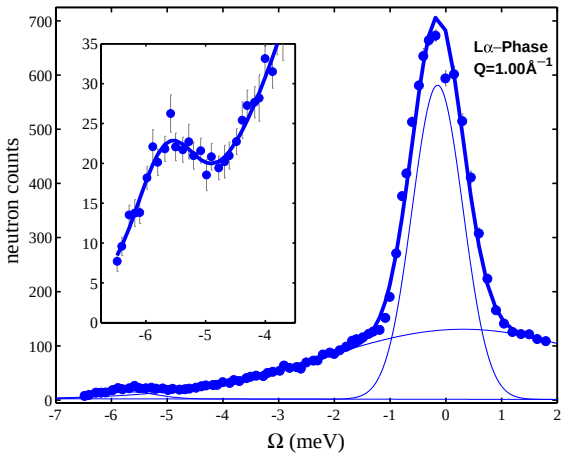

Fig. 2. Energy scan at $Q = 1.0$ Å^{-1} and $T = 30^\circ$C. The inset shows the excitation of the DMPC-layers in magnification. Solid lines are guides to the eye.

temperature and the degree of hydration during the experiment the sample was kept in a humidity chamber. Inside the chamber there was a heavy water reservoir, thermally isolated against the sample. By adjusting the temperature of the chamber and independently the temperature of the reservoir using two Haake bath controllers, the humidity inside can be controlled. Close to the sample, a humidity sensor and a platinum resistor were installed. IN12 is equipped with a vertically focusing graphite monochromator and a vertically and horizontally curved graphite analyzer. The flux at the sample position is of the order of 5×10^6 n/(cm^2 s). For the inelastic measurements the counting times were about five minutes per point. Fig. 2 shows an energy-scan at $Q_{xy} = 1.0$ Å^{-1} and $T = 30°$C, in the fluid phase of the DMPC bilayer. The signal basically consists of a sharp central peak and a broad contribution, centered at $\Omega = 0$ meV. The solid lines are guides to the eye. At $\Omega = -5.5$ meV, the inelastic signal of the membrane is observed in form of a pronounced peak with a width (FWHM) of about $\Delta\Omega = 1$ meV. Fig. 3 shows the corresponding dispersion relation in the fluid $L\alpha$ phase at $T = 30°$C as measured by several constant-Q scans. The measurements can quantitatively be compared to

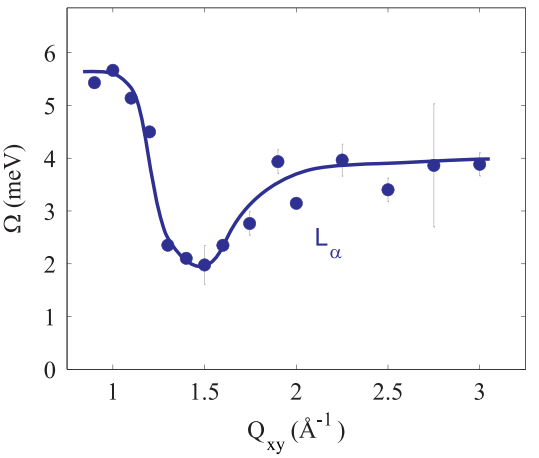

Fig. 3. Dispersion relation in the fluid phase of the lipid bilayer. Note the minimum at the maximum of the static structure factor $S(Q_{xy})$ shown in Fig. 4.

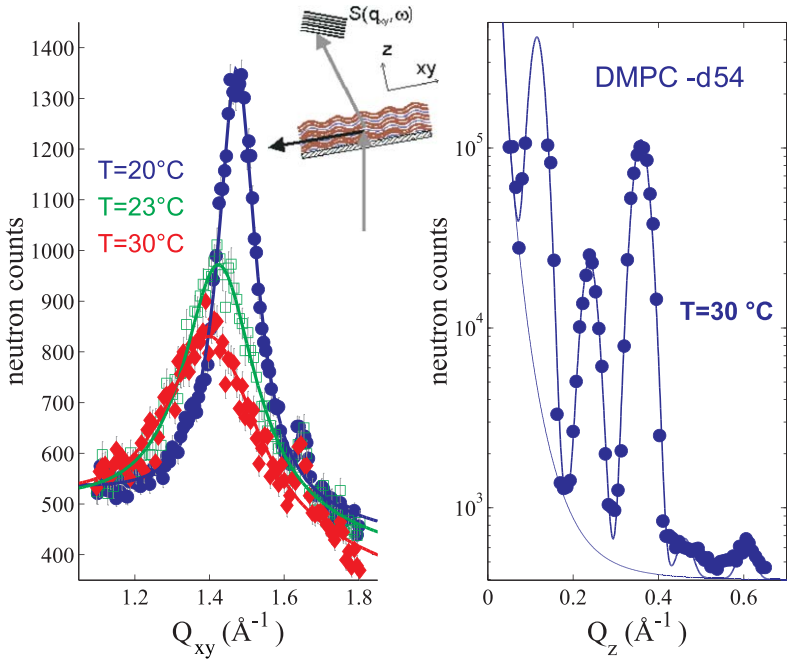

Fig. 4. The inter-acyl-chain correlation peak (left) and reflectivity (right). The inset schematically shows the orientation of the sample with respect to the incoming beam for the in-plane measurements. Solid lines are fits after the Lorentzian- (chain-peak) and Gaussian model (reflectivity).

corresponding Molecular Dynamics simulations by Tarek et al. [3]. Fig. 4 shows the corresponding inter-acyl-chain peak for temperatures below and above the gel–liquid phase transition (phase transition temperature of the deuterated compound $T_c = 21.5°C$) and the reflectivity at $T = 30°C$. When heating from the more rigid gel-phase into the fluid phase, the inter-chain peak broadens drastically (decreasing correlation length ξ_{xy}) and the peak center shifts to smaller Q_{xy}-values (larger average next-neighbor distance and smaller packing density). The reflectivity shows five well-developed Bragg peaks which positions yield the distance of the layers in z-direction. By Fourier transformation, the z-profile of the bilayer can be modelled. At $T = 30°C$ we find a next-neighbor distance of the acyl chains of $d_{xy} = 4.5$ Å and a correlation length of $\xi_{xy} = 11$ Å. From the reflectivity there is a distance between the bilayers of $d_z = 55$ Å. The combination of diffraction and inelastic measurements leads to a complete picture of structure and dynamics of model membranes on a molecular length scale. This will be especially useful in temperature-dependent measurements in the range of the gel–liquid phase transition to study the development of structure and dynamics and the relation between them.

Acknowledgements

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