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TRANSVERSE THZ DYNAMICS OF PHOSPHOLIPID MEMBRANES: A NEUTRON SCATTERING STUDY

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Abstract. The off-plane dynamics in the frequency window from 1 THz (4 meV) to 3 THz (12 meV) in multiple phospholipid bilayers as studied by means of neutron scattering is presented. A weak inelastic signal is observed, indicating that off-plane dynamics is dominated by the *disordered* inter-layer water. A collective mode is detected which shows a low-momentum dispersion similar to that of bulk water, while a more complex dynamics is found on increasing the momentum transfer.

1. Introduction

A consistent and comprehensive picture of the vibrational dynamics of phospholipid bilayers in the THz range is pivotal to frame their role for the membrane functionality. Recent inelastic X-ray scattering (IXS) and inelastic neutron scattering (INS) studies were useful to identify a complex behavior of the *in-plane* dynamics in the THz window (Zhernenkov *et al.* 2016; D'Angelo *et al.* 2018) in 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoyl-sn-glycero-3-phoshatidylcholine (DMPC). These results confirmed the presence of an additional acoustic mode and established the existence of a *transverse phonon gap* in the liquid phase, providing also evidence for an avoided crossing interaction between the acoustic mode and a low-energy optical mode (D'Angelo *et al.* 2018). The IXS and INS observations were found consistent with the prediction of a previous molecular dynamics (MD) simulation (Conti Nibali *et al.* 2014) and as well in agreement with the findings of a Far-Infrared spectroscopic study (D'Angelo *et al.* 2017). As such, in the last few years, the description of collective excitations in lipid bilayers has continuosly changed and enriched by new details, as proof that this field is far from being thoroughly understood.

Here, we report the results of a INS study on the *off-plane* THz dynamics of DMPC lipid bilayers, to be compared to the results obtained for the in-plane dynamics in the same spectral range. This initial investigation of a completely unexplored region provides an interesting indication for the presence of a collective mode much similar to that observed in bulk liquid water (Sette *et al.* 1995; Sacchetti *et al.* 2004; Amann-Winkel *et al.* 2016).

This behavior is of major interest for many systems having biological relevance because the same dynamics is observed in hydration water of proteins (Orecchini *et al.* 2009) and in dry proteins too (Paciaroni *et al.* 2012). More important, the same trend was observed also in hydration water of DNA (Orecchini *et al.* 2012).

2. Samples and methods

Neutron measurements were carried out on both the gel and liquid phases of DMPC lipid bilayers. INS spectra were collected using the Brillouin neutron spectrometer BRISP (Aisa *et al.* 2005) to cover the energy and wave vector transfer region $-20\,\mathrm{meV} \leq \hbar\omega \leq 20\,\mathrm{meV}$ and $0.2\,\text{Å}^{-1} \leq Q \leq 1.5\,\text{Å}^{-1}$. Two temperatures were measured, i.e. $T = 303\,\mathrm{K}$, where the sample is in the liquid crystalline (fluid, L_α) phase, characterized by a high liquid-like disorder of the acyl chains (D'Angelo *et al.* 2008), and $T = 283\,\mathrm{K}$, where the sample is in the highly ordered gel phase $L_{\beta'}$ (Smith *et al.* 1988). The sample, containing 36 mole of $\mathrm{D_2O}$ per lipid mole, was prepared according to the protocol of Hallock *et al.* (2002) to obtain aligned multilayers of DMPC-d54 on thin mica strips. In the model membrane DMPC-d54, 54 out of 72 hydrogen atoms of the acyl chains were substituted by deuterium atoms to enhance the coherent contribution to the INS cross section. The sample was composed of 250 layers with six 80 μm thick ^{10}B spacers inserted to reduce multiple scattering. The sample holder was designed to preserve the correct alignment of the mica strips. The sample was then sealed in a properly shielded aluminum cell with a small water reservoir to maintain the hydration. A temperature stability better than 0.5 K was ensured to keep the sample in gel or liquid phase.

The structural features of the sample have been discussed previously (D'Angelo *et al.* 2018) starting from the static structure factor $S(Q,0)$. In particular, the sharp diffraction *elastic* peak, already ascribed to the correlations of pairs of adjacent hydrocarbon chains in the lipid bilayer (Rheinstädter *et al.* 2004), is located at $Q = 1.49 \pm 0.02\,\text{Å}^{-1}$ and at $Q = 1.41 \pm 0.02\,\text{Å}^{-1}$ in the gel and in the liquid phase respectively. The first diffraction peak of hydration water (Amann-Winkel *et al.* 2016), which occurs at $Q \simeq 2.0\,\text{Å}^{-1}$ in bulk water, was not detected in the in-plane scattering experiment. This situation suggested a rather disordered structure of water molecules in DMPC-d54 also in comparison with bulk water, as already observed by X-rays (Chen *et al.* 2001).

In the present experiment we were specifically interested in detecting the off-plane dynamics of the oriented membrane. The monochromatic incoming neutron beam, traveling along the z axis, was in the same plane as the lipid bilayers, the x axis was assumed to be on the same plane, while the y axis was perpendicular to this plane. The neutron intensity data, collected by the BRISP detector having 20480 pixels, was analyzed in order to obtain the cross section at a given small volume at the Q_x, Q_y, Q_z position in the momentum space and in a given energy transfer interval $d(\hbar\omega)$. We fixed the path in the reciprocal space along the $Q_x = 0$ direction with variable Q_y and Q_z according to the (energy and momentum) conservation rules and the neutron counts were binned in a sphere of radius $\delta Q = 0.025\,\text{Å}^{-1}$. A sample of the results for the dynamic structure factor $S(\boldsymbol{Q},\hbar\omega)$ is shown in Fig. 1.

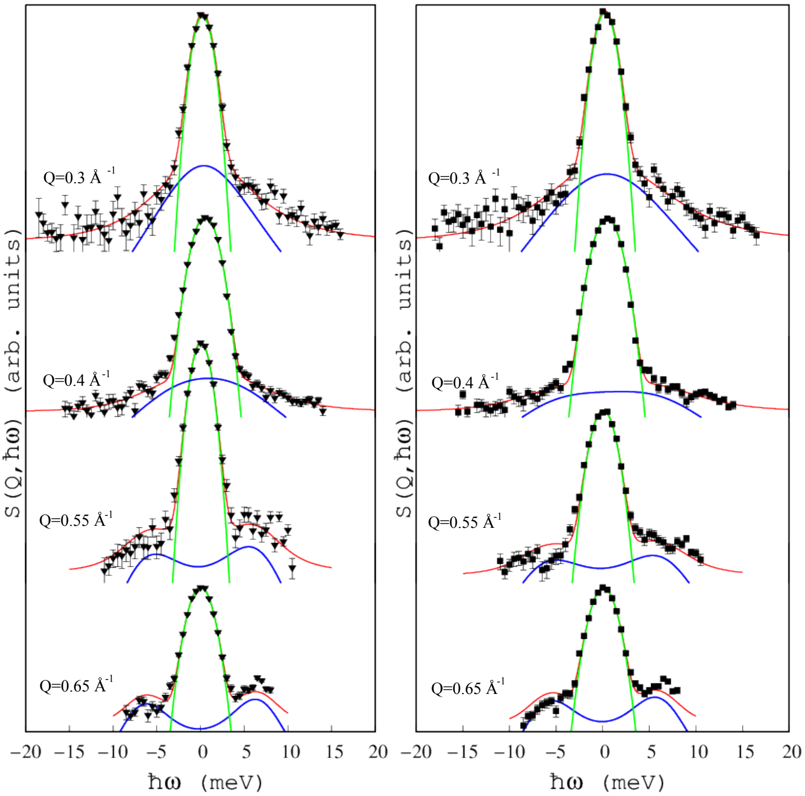

FIGURE 1. Log of the dynamic structure factor $S(Q,\hbar\omega)$ of DMPC-d54 at different Q values. Left side (triangle): gel phase, $T = 283$ K. Right side (square): liquid phase $T = 303$ K. The lines are the components of fitted model.

3. Results and discussion

The first information we derived from the data is the dynamic structure factor at zero energy transfer $S(\boldsymbol{Q},0)$ as a function of the perpendicular momentum transfer Q_y which is reported in Fig. 2. The data give an information on the *static* structure of the system in a time scale defined by the energy resolution of the experiment which is fairly broad in the present experiment. A sharp peak is observed at $Q = 0.134 \pm 0.002$ Å^{-1} and $Q = 0.137 \pm 0.005$ Å^{-1} for the gel and the liquid phase respectively. These peaks can be attributed to the diffraction from the coherent structure of the large number of stacked bilayers and correspond to the inter-layer spacing equal to 46.9 ± 2 Å and 45.9 ± 2 Å at the two temperatures. The data provide an evidence for the expected contraction of the inter-layer distance when the system structure evolves from the gel phase to the liquid phase. In principle the peak width is a measure of the coherence length of the system layers but in the present experiment the result

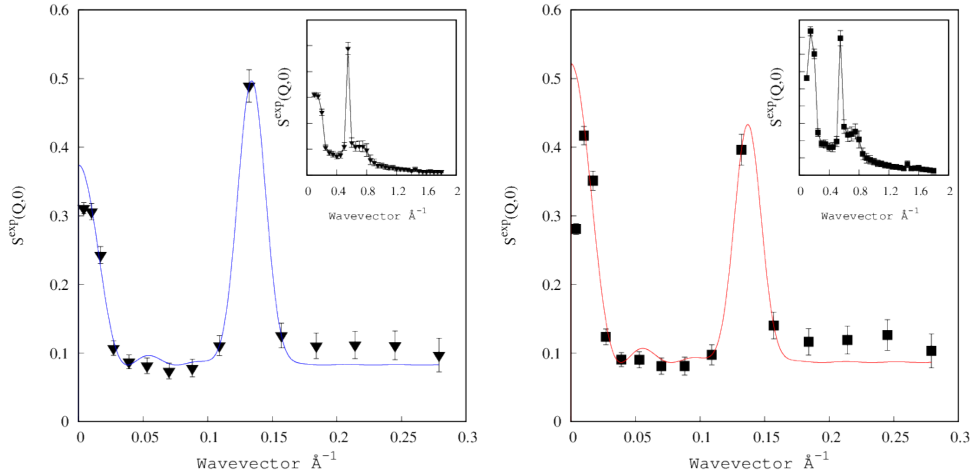

FIGURE 2. Experimental elastic structure factor $S^{exp}(Q,0)$ as a function of Q_y in the low momentum region (see text). Left side: (triangle) gel phase, $T = 283\,\mathrm{K}$. Right side (square): liquid phase $T = 303\,\mathrm{K}$. The continuous lines are best fit to a simple model for the elastic scattering plus a Gaussian function describing the diffraction peak. In the inset the static structure factor $S(Q) = \int_{-\infty}^{+\infty} S(Q,\omega)\,\mathrm{d}\omega$ is plotted on a wider Q range but with lower resolution, showing the presence of a higher peak.

is resolution limited, therefore we can just state that this coherent length is in excess of about 1000 Å.

As to the dynamic structure factor as a function of the energy, we analyzed the spectra using a simplified model based on the presence of a single collective mode in the energy and momentum window explored by the experiment. The model is given by the equation:

$$\begin{aligned} S^{\mathrm{mod}}(Q,\hbar\omega) =& A_{\mathrm{e}}(Q)\delta(\hbar\omega) + \\ &+ [n(\hbar\omega)+1]A_{\mathrm{i}}(Q)\frac{\Gamma(Q)\omega}{[\omega^2-\omega_{\mathrm{o}}(Q)^2]^2+[\Gamma(Q)\omega]^2} \end{aligned} \tag{1}$$

where $n(\hbar\omega)$ is the Bose factor, $\hbar\omega_o(Q)$ is the energy of the mode, $\hbar\Gamma(Q)$ is the damping of the mode and $A_{\mathrm{e}}(Q)$ and $A_{\mathrm{i}}(Q)$ are the elastic and inelastic amplitudes respectively.

As already done for what concerns the in-plane dynamics, we apply the simple and physically sound assumption that also the *off-plane* dynamic structure factor has a negligible dependence on the exact $\boldsymbol{Q}$ direction, therefore we studied the experimental $S(Q,\omega)$ as a function of $Q = \sqrt{Q_y^2 + Q_z^2}$. The contribution of the Q_z in-plane component, along the incoming neutron beam, is fairly small at small energy transfer and increases on increasing the energy. Considering the weakness of the inelastic signal we believe that such an approximation is adequate to obtain a reasonable guess about the off-plane dynamics.

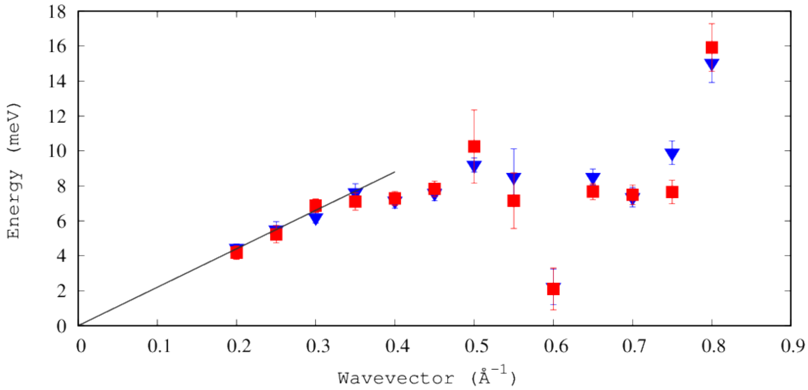

FIGURE 3. Dispersion curves of the collective mode as a function of Q (see text). Gel phase, $T = 283$ K, blue triangles. Liquid phase $T = 303$ K, red squares. A linear fit at low Q shows the dispersion relation of the collective acoustic mode.

The simplified model $S^{\mathrm{mod}}(Q,\hbar\omega)$ is appropriate to fit the experimental data as it is shown in Fig. 1. The most straightforward information is provided by dispersion curves, i.e. the mode energies $\hbar\omega_0(Q)$ as a function of the momentum transfer Q. As shown in Fig. 3 the dispersion relation does not exhibit a clear temperature dependence differently from the case of in-plane dispersion (D'Angelo *et al.* 2018) where the velocities show a temperature dependence on passing from the gel (2330 ± 200 m/s) to the liquid (1810 ± 200 m/s) phase. Furthermore the linear trend observed at small Q corresponds to a propagation velocity of 3350 ± 150 m/s, higher than that observed for in-plane dynamics. In addition, the intensity of the inelastic contribution to the off-plane dynamic structure factor appears to be smaller than in the case of in-plane scattering, with an estimated ratio of the inelastic contribution to the elastic one ($Q < 0.5$ Å^{-1}) of about 1.5 % in the present case at variance with the 2.5 % for the in-plane scattering. Considering these results and the fact that the collective mode velocity is quite similar to that found in bulk water (Sacchetti *et al.* 2004), it is rather tempting to attribute a large part of the off-plane inelastic scattering in the energy window 2 meV $< \hbar\omega <$ 10 meV to the *disordered* water we found to be present in the sample.

As to the damping factor it is worth of note that it shows a Q-squared dependence, a behaviour similar to those already observed in dry proteins (Paciaroni *et al.* 2012), and protein (Orecchini *et al.* 2009) and DNA (Cornicchi *et al.* 2011) hydration water (data not shown). This trend is probably related to the presence of different intra-molecular localized modes.

On increasing the momentum transfer, the dispersion relation is no more linear and a more *crystal-like* trend is observed. In particular a deep minimum is visible at about 0.6 Å^{-1} where the integrated static structure factor shown in the inset of Fig. 2 has a well defined peak. Such a peak corresponds to a characteristic distance of about 11 Å to be related to the inter-layer region where the disordered water can be located (Vishnyakov *et al.* 2017). No further guess can be made from the present data because the rather high Q is related to a mixture of contributions from in-plane and off-plane scattering.

4. Conclusions

In conclusion, we have observed the off-plane dynamics of DMPC in an energy range from 4 meV (1 THz) to 12 meV (3 THz) in a sample where a very good level of coherence of the lipid bilayers is obtained, in the presence of a significant amount of water. The off-plane proper lipid dynamics seems to be largely absent from the present data, suggesting that it is located at much lower energy like in the case of *corrugation modes* (Seitter *et al.* 2000), or other diffusion-like modes (Wanderlingh *et al.* 2014, 2017). Of course, also additional faster modes mainly located above the experimental energy window are expected to be present. As a consequence, the present 3D experiment can open a way to the study of the dynamics of water located in the inter-layer region and its interaction with the lipid bilayer itself, an effect having a basic role in the membrane behavior. The observed dispersion relation, which mimic that of water at low momentum, on increasing Q, shows a behavior which should be related to a complex interaction between water and membrane giving rise to avoided crossing with (partially invisible) membrane modes (Voneshen *et al.* 2013). The mode-mode interaction is very relevant because it allows for observing even transverse modes which cannot be observed in scattering experiments where only longitudinal density fluctuations are visible (Zanatta *et al.* 2013; Zanatta *et al.* 2015).

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