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PAPER

Short range ballistic motion in fluid lipid bilayers studied by quasi-elastic neutron scattering†

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Diffusion is the primary mechanism for movement of lipids and proteins in the lateral direction of a biological membrane. In this paper we have used quasi-elastic neutron scattering to examine the diffusion process of lipid molecules in fluid DMPC membranes. We found that the motion over length scales greater than the lipid diameter could be characterized as a continuous diffusion process, with a diffusion coefficient of $D = 64 \times 10^{-12}$ m^2/s. The continuous diffusion model has been successfully used in the past to describe the motion of lipid over long length scales. However, the focus of this measurement was to determine how the character of the molecular motion changes on length scales shorter than the nearest neighbour distance. At very short length scales (<2.37 Å), we see first experimental evidence for a short-range flow-like ballistic motion.

1 Introduction

Diffusion is not only the primary mechanism for proteins to move through the lipid matrix, but it also plays an important role in the formation of various macromolecular structures, such as lipid rafts and nanoenvironments for proteins.

It is commonly accepted that the Brownian motion of lipid molecules over long length scales (length scales larger than the nearest neighbour distance of the lipid molecule) is characterized by a continuous diffusion process.[1–5] On very short length scales, the motion of the molecules is often modeled as a "rattling in the cage" motion,[6,7] as shown in Fig. 1b), where the molecule is constraint in its local environment for a certain time before leaving the cage. This model has been used successfully to fit, in particular, diffusion determined by neutron scattering experiments.[8] However, our understanding of molecular diffusion in membranes has been challenged by new results from experiments and simulations. From computer simulations, sub-diffusive and ballistic regimes were observed on very short time scales and, respective, small distances.[9] It has also been reported that lipids move coherently in loosely bound clusters, rather than as independent molecules.[10,11] A "hopping" diffusion of lipids into nearest neighbor sites was reported in single supported bilayers.[12] Recently, it has been suggested that there is also a flow-like component to the motion of the lipid molecules over long length scales.[13] While continuous diffusion can be pictured as the Brownian motion of individual lipid molecules, a flow-like motion involves the coherent movement of several lipid molecules, as pictured in Fig. 1c). If a flow like motion is present, it should be apparent at all length scales, and be particularly

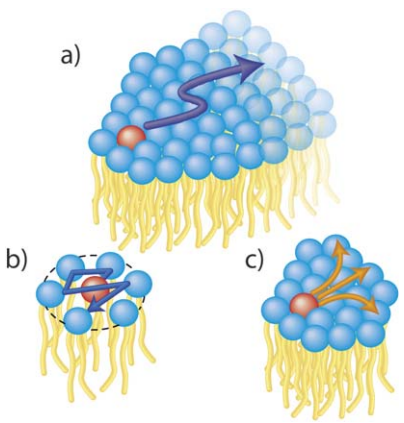

Fig. 1 Models depicting the motion of lipid molecules at different length scales. The motion at longer length scales is characterized by continuous diffusion (a). Previously accepted models suggest a "rattling in the cage" ballistic motion (b) at short length scales, while recent measurements show evidence for a flow-like ballistic motion (c).

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pronounced at shorter length scales where the continuous diffusive motion is no longer dominant.

Quasi-elastic neutron scattering is a powerful tool for observing short range lipid dynamics at time scales on the order of pico-nanoseconds, enabling the characterization of this type of motion. We measured lipid diffusion in a phospholipid model membrane over distances from 1.3 Å to 22 Å using quasi-elastic neutron scattering on the backscattering spectrometer IN13.[14] With typical lipid distances of about 8 Å, the experiments cover motions of the lipid molecules from about 1/5 to almost 3 times the lipid-lipid distance. While we observe the well established continuous diffusion at large distances, we find experimental evidence for a ballistic motion of the lipids at distances smaller than 2.37 Å. The velocity of the lipids (1.12 m s^{-1}) is significantly smaller than their thermal velocity (87 m s^{-1}), thus indicating a flow-like ballistic motion at small length scales.

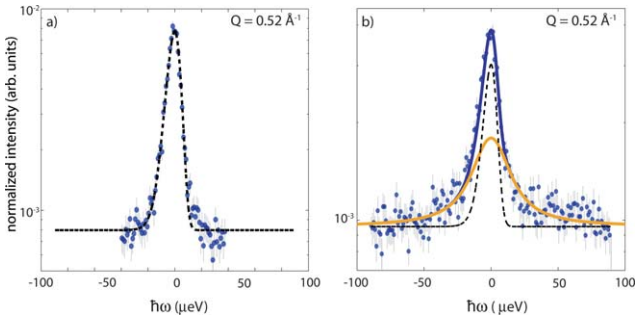

Fig. 2 Typical data analysis used for low Q values. a) Vanadium resolution with asymmetric Gaussian fit (black). b) Lipid sample at 310 K fit with the asymmetric resolution function (black) and a Lorentzian function convoluted with the resolution (orange). The total fit is shown in blue. The deconvoluted FWHM of the Lorentzian at this Q value was determined to be 27.5 μeV.

2 Experimental details

Multi-lamellar bilayers of the model system 1,2-dimyristoyl-*sn*-glycero-3-phoshatidylcholine (DMPC) were prepared on Si wafers and hydrated with heavy water. By using selective deuteration, the experiment was mainly sensitive to the incoherent scattering of diffusing lipid molecules.[15]

The DMPC powder was dissolved in a 3 : 1 chloroform-trifluoroethanol mixture following a protocol described by Ding and co-workers.[16] The dissolution was kept at −20 °C overnight. Measuring the in-plane motions of the lipids in the membrane required the use of highly oriented samples. This was achieved by preparing the lipids on silicon wafers. The wafers had a thickness of ∼380 μm and Si(111) orientation.

The lipid solution was sprayed onto wafers, producing a system of highly oriented stacked membranes. After the deposition, the wafers were dried over silica gel for 2 days in a desiccator. The sample was rehydrated from pure D_2O at 40 °C to achieve full hydration (corresponding to at least 12 water molecules per lipid[17]), resulting in a lamellar spacing of d ∼ 59.3 Å as measured by a θ–2θ scan.[18]

After rehydration six wafers were stacked together in order to achieve a total amount of about ∼100 mg lipid. This amount of sample is needed to achieve sufficiently high statistics in a reasonable measuring time. The total sample thickness of the six wafers with the deposited DMPC was 3 mm. The sample cell was sealed using indium wire and the weight of the sample was monitored before and after the experiment, with no change observed.

The experiment was performed at the Collaborative Research Group (CRG) thermal neutron backscattering spectrometer IN13 at the high flux reactor of the Institut Laue-Langevin (ILL), Grenoble.[14] This instrument is unique in its capability of accessing very high Q-values, corresponding to very small distances in the bilayer sample, in combination with a high dynamic range of about −100 to 100 μeV. The incident wavelength was $\lambda = 2.23$ Å, with an incident neutron energy of about 16 meV. This setup yields a uniquely broad range of momentum transfers, Q (0.28 $Å^{-1} < Q < 4.9$ $Å^{-1}$), corresponding to distances 1.3 < d < 22 Å. The 8 μeV elastic energy resolution corresponds to a 0.10 ns time scale. The experiment was therefore sensitive to pico-nanosecond diffusive motions of lipid molecules over a wide range of distances. A detailed description of the instrument and selected applications in the field of biophysics can be found in Natali *et al.*[14]

The lipid sample as well as a sample holder containing six cleaned silicon wafer for background subtraction, were measured in an energy range of ±90 μeV. The instrumental resolution was determined by measuring a 2 mm vanadium sample in the range of ±40 μeV as shown in Fig. 2. The incident energy of the neutrons was varied by changing the temperature of the crystal monochromator of the instrument. This procedure introduces a small asymmetry in the resolution function which will be discussed in more detail in the data analysis section. All measured samples were aligned at an angle of 135° with respect to the incident neutron beam, with the scattering vector Q in the plane of the bilayers (Q_{‖}). Transmission of the sample was measured and found to be in the order of 93%, so multiple scattering effects were not taken into consideration for the data treatment. All scans were measured at 310 K, in the fluid phase of the DMPC bilayers.

3 Data analysis

Fig. 2a) depicts the instrumental energy resolution of the IN13 spectrometer as determined from a Vanadium sample, which is usually well described by a Gaussian peak shape. To accommodate the slightly asymmetric resolution function, the resolution was fit with an asymmetric Gaussian function. The asymmetry was introduced by incorporating an exponential function into one side of the Gaussian peak[19] to get

$$y = y_o + H(\hbar\omega_o - \hbar\omega)Ae^{-\left(\frac{(\hbar\omega-\hbar\omega_o)^2}{2\sigma^2+\tau|\hbar\omega-\hbar\omega_o|}\right)} + H(\hbar\omega-\hbar\omega_o)Ae^{-\left(\frac{(\hbar\omega-\hbar\omega_o)^2}{2\sigma^2}\right)}, \quad (1)$$

where A is the amplitude, ω_o the elastic energy peak position, σ the Gaussian standard deviation, H is a Heaviside function, and τ the asymmetry parameter. For $\tau = 0$, a Gaussian peak function

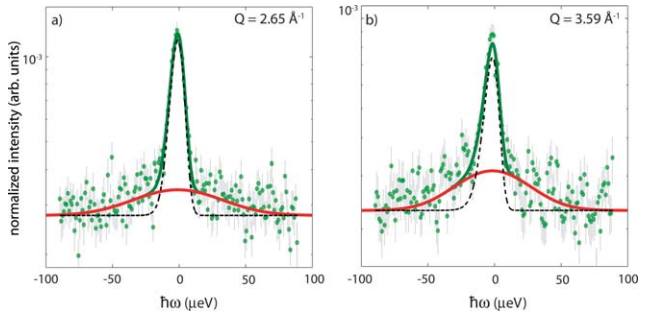

Fig. 3 Exemplary high Q data fit with the resolution function (black) and a Gaussian quasi-elastic broadening (red), see text for details. The total fit is shown in green. a) $Q = 2.65$ Å^{-1} b) $Q = 3.59$ Å^{-1}. While the quasi-elastic broadening is larger at $Q = 2.65$ Å^{-1} as compared to $Q = 0.52$ Å^{-1} in Fig. 2b), the broadening at higher Q (3.59 Å^{-1}) becomes significantly smaller again. FWHM for all measured Q are shown in Fig. 4.

is obtained. The energy resolution in Fig. 2a) is slightly asymmetric, with a τ value of 3.29.

The scattering obtained from the lipid sample is described by a narrow central component, corresponding to the instrumental resolution (~8 μeV), a constant background, and a quasi-elastic broadening due to relaxational dynamics of the lipid molecules. Two different functions were used to fit the quasi-elastic broadening, depending on the Q range.

Typical data obtained at low Q-values are shown in Fig. 2b). The intensity was normalized to Vanadium to correct for detector efficiency. For the low Q values ($Q < 2.65$ Å^{-1}) the broadening (25–72 μeV) was fit with a Lorentzian function, convoluted with the Gaussian instrumental resolution. It should be noted that the FWHM of the Lorentzian functions quoted in the paper are the deconvoluted values.

Exemplary data from the high Q scans are shown in Fig. 3. Because the neutron flux of the instrument is significantly lower at high Q values, these scans had significantly lower statistics than the small Q scans. There was a distinct change in the trend of the FWHM of the Lorentzian functions when fitting the high Q values ($Q > 2.65$ Å^{-1}). After a detailed data analysis (which is provided in the Electronic Supplementary Information†), we concluded that the high Q values were better fit with a Gaussian function rather than a Lorentzian.

Fig. 4 displays the FWHM for all measured Q-values as function of Q^2. In this plot the FWHM of the low Q data ($Q < 2.65$ Å^{-1}), fit with a Lorentzian function (blue), scale linearly with Q^2, a behaviour which is indicative of continuous diffusion. The broadening for higher Q-values was found to drop from about 70 μeV to 40 μeV and then raise again to 60 μeV. The FWHM of the Gaussian functions used to fit the high Q data (green) were found to fall on a square root curve§. The relationship between the Q scaling of the FWHM and the character of motion will be discussed in more detail in the following section. Note that we cannot exclude additional faster dynamics outside of the energy window of the IN13 instrument.

§ The Gaussian standard deviation σ is related to the FWHM of the Gaussian peak by $\mathrm{FWHM}_G = 2\sqrt{2ln2}\sigma$.

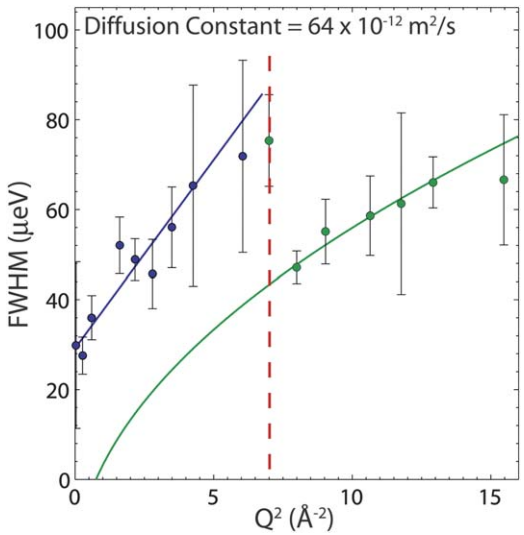

Fig. 4 FWHM of the quasi-elastic broadening plotted as function of Q^2. The linear Q^2 relationship at low Q (blue) is indicative of continuous diffusion. The high Q data (green) show a quasi-elastic broadening described by a Gaussian peak shape. The corresponding FWHM scale linearly with Q. The corresponding fit (green) displays as square root when plotted against Q^2. The transition between continuous and ballistic diffusion is observed at $Q = 2.65$ Å^{-1}, marked by the red dashed line.

4 Discussion

There are different models used to describe diffusion.[8] For a particle diffusing via random Brownian motion, the displacement of the particle can be characterized as a function of time,

$$\sigma_d = \sqrt{2Dt} \tag{2}$$

where D is the translational diffusion coefficient of the system. With this characteristic length scale, one can define a self time-dependent pair-correlation function for incoherent scattering,

$$F_d(r,t) = (4Dt)^{-\frac{3}{2}} exp\left(\frac{-r^2}{4Dt}\right), \tag{3}$$

which is a solution of Fick's law,

$$\frac{\partial F_d(r,t)}{\partial t} = D\nabla^2 F_d(r,t). \tag{4}$$

This results in an intermediate scattering function which decays exponentially,

$$I_d(Q,t) = \exp(-Q^2 Dt), \tag{5}$$

and can be Fourier transformed to give a Lorentzian incoherent scattering function

$$S_d(Q,\omega) = \frac{1}{\pi\hbar}\left(\frac{Q^2 D}{(Q^2 D)^2 + \omega^2}\right). \tag{6}$$

Thus, quasi-elastic scattering which exhibits itself as Lorentzian broadening with a FWHM that has a Q^2 dependence, is indicative of a continuous diffusion process ($FWHM_L = 2\hbar DQ^2$). This behavior was observed for the low Q values, shown in Fig. 4 as

the data fit with a blue line. From this linear fit a diffusion coefficient could be extracted and was found to be $D = 64 \times 10^{-12}$ m^2/s. This value is comparable to diffusion coefficients quoted in the literature for similar systems.[1,4,12,13,20] We note that the linear fit in Fig. 4 does not pass through the origin, as one would expect, and the offset is larger than the instrumental resolution. This effect is often observed in the literature, however no consistent explanation has been offered.

Taking advantage of the unique high Q-range accessible on the IN13, it was found that the data for Q values >2.65 Å^{-1} no longer agreed with the continuous diffusion model and the broadening is better fit with a Gaussian curve. This feature is indicative of a change in character of the lipid motion.

In the case of a ballistic motion, the displacement of the particle can be written as a function of time, simply defined by its velocity,

$$\sigma_b = vt, \tag{7}$$

which will change the form of the self pair-correlation function,

$$F_b(r,t) = \left(2\pi v^2 t^2\right)^{-\frac{3}{2}} exp\left(\frac{-r^2}{2v^2t^2}\right). \tag{8}$$

The spacial Fourier transform of this now gives an intermediate scattering function which is a Gaussian,

$$I_b(Q,t) = exp\left(\frac{-Q^2v^2t^2}{2}\right). \tag{9}$$

Thus, the incoherent scattering function will also have a Gaussian form,

$$S_b(Q,\omega) = \frac{1}{\sqrt{2\pi}Qv\hbar} exp\left(\frac{-\omega^2}{2Q^2v^2}\right). \tag{10}$$

In this new model, the velocity of the particle can be extracted from the linear Q dependence of the FWHM of the Gaussian function ($FWHM_G = 2\sqrt{2ln2}\hbar vQ$). This fit is shown as the green curve in Fig. 4, which displays as a square root when plotted against Q^2. The data suggest a transition from continuous diffusion to ballistic diffusion at $Q = 2.65$ Å^{-1}, corresponding to a length scale of 2.37 Å, about 1/3 of the typical lipid-lipid distance. If this ballistic motion is driven by thermal energies, neglecting friction, the expected velocity of the molecule should be that of a free particle, and thus dictated by the thermal energy as given from the equipartition theorem $\left(k_BT = \frac{1}{2}mv^2\right)$. Using the mass of a single lipid molecule (1.1258×10^{-24} kg) results in a velocity of $v_{thermal} = 87$ m s^{-1} at $T = 310$ K. From the fit in Fig. 4, the experimentally determined velocity was $v_{exp} = 1.12$ m s^{-1}.

The experimentally determined velocity is about two orders of magnitude slower than the thermal velocity. The primary mechanism for short range motion of lipids is thus too slow for a purely thermal free particle motion, and is, rather, the result of a flow-like process.

The initial movement of a lipid molecule in a tightly packed fluid state most likely requires the re-arrangement of the nearest neighbour molecules. Diffusion of lipid molecules at distances smaller than a lipid diameter could then be pictured as a flow-like process, which involves the coherent motion of several lipid molecules. This picture is in agreement with previously reported results on possible motional coherence in fluid phospholipid bilayers.[11] Here lipids were reported to fluctuate as coherently coupled, ~30 Å large patches rather than individual molecules.

The ballistic regime was observed in the data in Fig. 4 for length scales less then 2.37 Å. Our data can be compared to results from Molecular Dynamics (MD) simulations by Flenner *et al.* who studied lipid diffusion over a wide range of time scales (10^{-16} s to 10^{-6} s).[9] At very short timescales, *i.e.* very early stages of the diffusion process (10^{-14} s), a ballistic diffusion regime was observed. This regime was followed by a sub-diffusive regime and lead into the well known continuous diffusion process at longer times. Considering typical diffusion constants of $\sim 10^{-11}$ m^2 s^{-1}, the simulated ballistic regime corresponds to length scales of only ~0.003 Å, and thus occurs at a significantly shorter length scale than found in this experiment. We argue that the MD simulations qualitatively describe lipid diffusion over a broad range of time scales; however, due to imperfections in the force fields, discrepancies in area per lipids used in the simulations, as well as insufficient sampling, the absolute values obtained by Flenner *et al.* may differ from our experimental results. While we find experimental evidence for a ballistic regime, determining a possible sub-diffusive behavior would require fitting non-Lorentzian peak shapes to the quasi elastic data. The unavoidable statistical error of the data in Fig. 2 and 3, unfortunately, did not allow for the unambiguous determination small deviations from a purely Lorentzian peak shape. The existence of a possible sub-diffusive regime can experimentally not be confirmed at this point and will be addressed in future experiments.

While we observe a flow-like motion at very small length scales, long distance flow-like diffusion has been reported recently.[13] We note that data with a very small statistical error were needed for the Bayesian data analysis to detect a small Gaussian broadening of the instrumental resolution. The instrument used in our study was optimized to study diffusion at very high Q values. The data quality (as shown in Fig. 2b) did not allow the same kind of analysis to determine a possible flow-like diffusion over long length scales. We note that the low Q data in Fig. 4 can be reasonably fit using the continuous Brownian diffusion model. This model is also widely used in the literature to determine diffusion coefficients from neutron scattering experiments. It can, therefore, be speculated that flow and continuous lipid diffusion possibly coexist in fluid membranes at long distances. Our data presents the first experimental evidence that flow-like diffusion dominates at small distances of up to about 1/3 of a lipid-lipid distance. Strong interactions between the lipids may eventually lead to a change in character of the motion to a continuous diffusion process.

5 Conclusions

We studied pico-nanosecond diffusion of lipid molecules in the fluid phase of DMPC phospholipid model membranes using quasi-elastic incoherent neutron scattering experiments. Using unique neutron instrumentation, the measurements covered lateral length scales from 1.3 to 22 Å, 1/5 to about 3 lipid-lipid distances. At large distances, we observe continuous diffusion with a diffusion coefficient of $D = 64 \times 10^{-12}$ m^2 s^{-1}, in excellent

agreement to values reported in the literature. For distances smaller than 2.37 Å, the character of the diffusion changes from continuous to ballistic diffusion with a velocity of 1.12 m s^{-1}, much smaller than the thermal velocity. Motion of lipid molecules at very small distances was, therefore, characterized as a flow-like ballistic motion.

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