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

Diffusion is the primary mechanism for the movement of lipids and proteins in a biological membrane. It is important in the formation of various macromolecular structures, such as lipid rafts. The commonly accepted theory for diffusion in membranes suggests that the molecules undergo continuous Brownian diffusion at long length scales, with a "rattling-in-the-cage" motion at short length scales, as shown in **figure 1**. However, this model has recently been challenged by experimental and simulation results. It has been observed that lipids move in loosely bound clusters rather than as individual molecules [1,2], and that there is a flow-like component to long range lipid diffusion [3]. Ballistic and sub-diffusive regimes have been observed in molecular dynamics simulations [4,5].

Diffusion is mainly studied by two experimental methods: fluorescence techniques and incoherent quasi-elastic neutron scattering. The two techniques access distinctly different length scales, resulting in a “blind spot” at mesoscopic distances. We note that the diffusion coefficients measured by these two techniques often differ by as much as orders of magnitude. The mechanism for diffusion, therefore, seems to depend on the length scale at which it is observed. The blind spot in the mesoscopic range will hopefully be closed in the future using high energy resolution lamor precession techniques performed with spin-echo spectrometers.

To extend the window of length scales and investigate the motion of lipid molecules at very short distances, we used the unique capabilities of the IN13 thermal backscattering spectrometer. IN13 provides access to an exceptionally large Q range, covering length scales from 1.3 to 31 Å ($0.2\,Å^{-1} < Q < 5\,Å^{-1}$). We used IN13 to study lipid diffusion at length scales smaller than a typical lipid-lipid distance in fluid bilayers. The aim of the experiment was to prove the validity of the Brownian diffusion model down to very small length scales. We chose a stacked model membrane system (DMPC) for this study and analysed the quasi-elastic neutron scattering response of the lipid molecules. Membranes were prepared as solid-supported, multi-lamellar membrane stacks on silicon wafers. Protonated lipids were hydrated by heavy water, so that the experiments were sensitive to the incoherent signal of the lipids. To increase the scattering signal, several wafers with thousands of highly oriented membranes were stacked. The membranes were studied in their physiologically relevant fluid state, at high temperature (T=30 °C) and full hydration.

The width of the quasi-elastic energy response (full width at half maximum, FWHM) is shown in **figure 2**. If a particle diffuses via random Brownian motion, the time evolution of its displacement can be written as $\sigma = \sqrt{2Dt}$, and the quasi-elastic energy broadening has a Lorentzian shape, which demonstrates

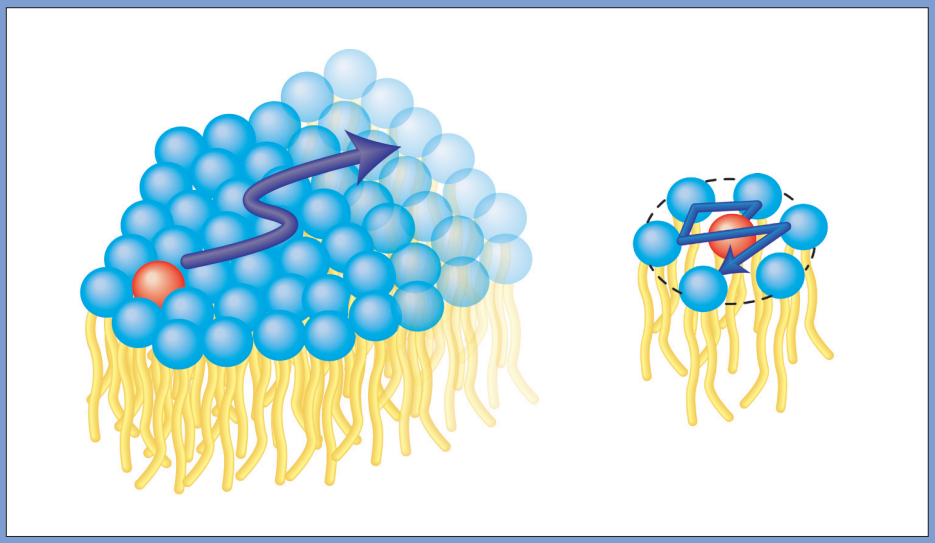

Figure 1: *Continuous Brownian diffusion at long length scales. The early stage of this motion is often modeled as a "rattling-in-the-cage" motion.*

Backscattering spectrometer IN13

Despite intense efforts, there is still no coherent model which describes the motions of lipid molecules from less than lipid-lipid to macroscopic distances. The aim of our experiment was to prove the validity of the Brownian diffusion model down to very small length scales. During our investigation, we observed, for the first time, the transition from Brownian to ballistic motion of lipids in a fluid lipid membrane and demonstrated that the early stage of lipid motion is the result of a flow-like ballistic motion rather than the previously accepted "rattling-in-the-cage".

the well-known quadratic behaviour $FWHM_L = 2\hbar DQ^2$. If a particle moves ballistically, $\sigma_b = vt$, the broadening has a Gaussian peak shape and the width follows $FWHM_B = 2\sqrt{2\ln 2}\,\hbar vQ$ (please refer to the original article [6] for more details). We found that at longer length scales the lipid motion can be characterised by continuous diffusion with a diffusion constant of 64 x 10^{-12} m^2/s, in excellent agreement with published values. We note that the linear fit of the Brownian diffusion in **figure 2** is not a line through the origin, as is often observed in the literature. This may point to a general shortcoming in our models for microscopic and mesoscopic diffusion in membranes. At very short length scales, however, there is a distinct change in the character of the motion. The energy broadening is no longer well described by a Lorentzian peak shape is better fit using Gaussian profiles. We therefore observe a transition from Brownian to ballistic lipid diffusion at a length scale of about 2.5 Å. From the fit, the velocity of the lipid molecule is determined to be about 1.2 m/s. This is about two orders of magnitude slower than the thermal velocity at 30 °C of about 90 m/s, which can be calculated from the equipartition theorem $k_BT = \frac{1}{2}mv^2$.

Rather than observing a thermally driven "rattling-in-the-cage" motion, we see evidence of a flow-like motion, as pictured in **figure 3**. The flow velocity was found to be significantly smaller than the thermal velocity. Viscosity and friction are essentially macroscopic properties based microscopically on short-time diffusion. However, ballistic diffusion happens before hydrodynamic interactions set in. This type of motion may therefore be impacted by mechanisms such as nano-friction and nano-viscosity.

This is the first observation of a transition from Brownian to ballistic motion in a biological material. The transition occurs at a length scale of about 2.5 Å, 1/3 of the lipid-lipid distance. Future experiments will address the question as to whether the transition is abrupt, as it appears in the data in **figure 3**, or whether the two regimes are separated by a sub-diffusive regime.

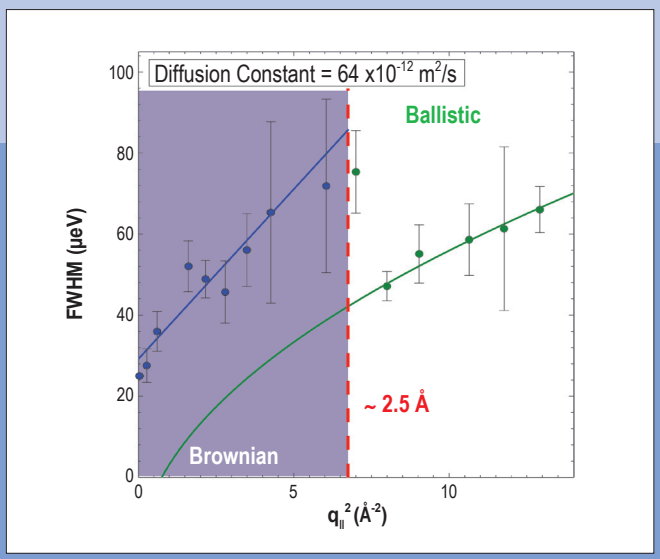

Figure 2: *FWHM of the quasi-elastic broadening plotted as function of Q^2. The linear Q^2 relationship at low Q (blue) is indicative of continuous diffusion. The high Q data (green) show a quasi-elastic broadening described by a Gaussian peak shape, with a corresponding FWHM that scales linearly with Q.*
The fit of the high Q data (green) displays as a square root when plotted against Q^2. The transition between continuous and ballistic diffusion is observed at Q=2.5 $Å^{-1}$, marked by the red dashed line.

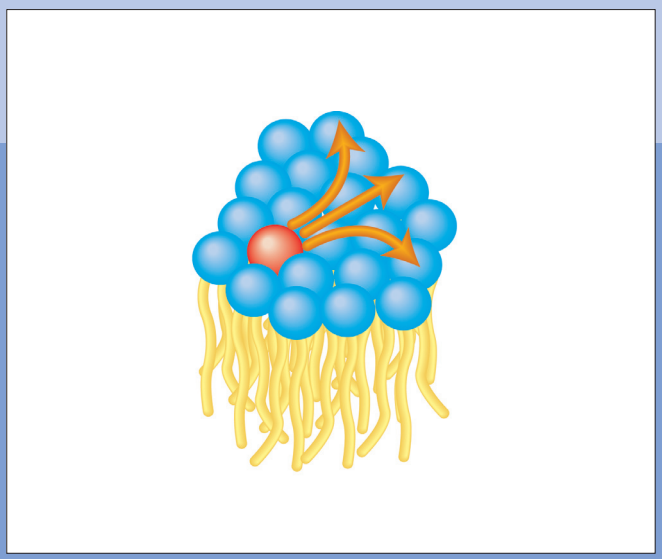

Figure 3: *Flow-like motion of lipid molecules at short length scales.*