diffusion-fundamentals

The Open-Access Journal for the Basic Principles of Diffusion Theory, Experiment and Application

Molecules Dancing in Membranes

Ilpo Vattulainen

Laboratory of Physics & Helsinki Institute of Physics, Helsinki University of Technology, Finland,
and
Memphys-Center of Biomembrane Physics, University of Southern Denmark, Denmark

Abstract

In 1828, a botanist named Robert Brown found that small pollen particles suspended in water migrated in an erratic fashion. Later it was realized that the dance Brown observed was essentially a two-dimensional *random walk* driven by thermal fluctuations, thus this *Brownian motion* was more intense at higher temperatures. The pioneering ideas and observations by Brown have inspired people for a long time to think about the fascinating aspects of random walks, and hence of diffusion. In this brief contribution, we consider this topic at complex biological interfaces known as cellular membranes and discuss how the dance of lipids and small molecules can be quantified through experiments and theoretical approaches. Some illustrative examples of diffusion in membrane systems are discussed.

1 Introduction

Cellular membranes are central components of cells [1, 2, 3, 4]. They are essentially thin interfaces composed of two lipid monolayers whose hydrophobic sides are attached to each other such that the contact with water is minimized. The polar head groups of lipids in turn are in the vicinity of water, thus maximizing their contact with the water phase. The schizophrenic nature of lipids due to their water-hating as well as water-loving character is hence the key that leads to the formation of bilayer like structures (see Fig. 1), which serve as models of actual biological membranes.

Despite the fact that the thickness of lipid membranes is about 5 nm, that is, about the size of lipids themselves, membranes have various roles on a cellular level. Lipid membranes regulate the passive flow of ions, water and other small particles across membranes, and they serve as mattresses in which a variety of different proteins are embedded. Such proteins include, for example, protein channels that direct the flow of certain specific ions and water molecules across a membrane. Glycoproteins in turn are related to recognition events, and electron carriers such as ubiquinone migrate inside a membrane, thus acting as signaling molecules. The importance of lipid membranes is further accentuated by their dynamic and heterogeneous nature, which is exemplified by the formation of highly ordered nanosized domains, so-called rafts [6, 7], which have been suggested to act as platforms for a number of membrane proteins. For the same reason, rafts have been suggested to play an important role in cellular processes such as signal transduction and protein sorting.

In membranes, not surprisingly, diffusion is one of the most fascinating processes that

arise from the dynamics of individual biological molecules. If we looked at membranes in molecular detail, we would realize that the motion of molecules in the plane of the membrane can essentially be described as a random walk. This dance plays an important role in a variety of processes, such as the formation of lipid domains and the formation of membrane channels such as Gramicidin A; since Gramicidin A consists of two proteins in different leaflets of a membrane, the formation of the channel is facilitated by diffusion. In more general terms, an understanding of the nature of diffusional processes in living matter is one of the grand challenges. Indeed, diffusion is involved in almost every stage of cellular mechanisms: in the self-assembly of membrane domains, in the transport of DNA and sugars, in intra- and inter-cellular communication, and ultimately in programmed cell death.

Summarizing, lipid membranes are key players in many cellular processes and they essentially regulate or even govern many cellular functions. In many of these cases, the lateral diffusion of, for example, lipid and protein molecules in the plane of the membrane plays an essential role. Our purpose in this short contribution is to consider some of these aspects through illustrative examples.

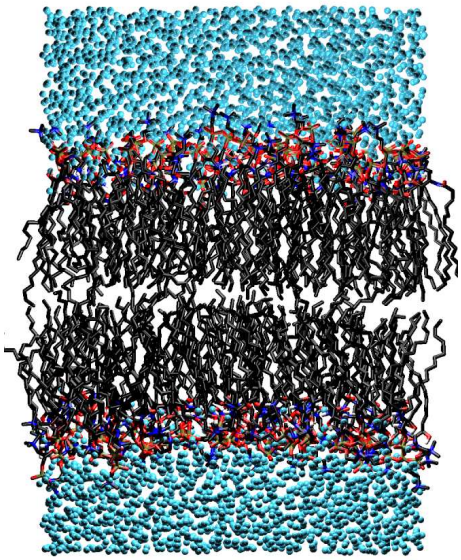

Figure 1: An atomic-level description of a lipid bilayer studied through molecular dynamics simulations [5]. The palmitoyl-sphingomyelin bilayer in the middle is surrounded by water on both sides.

2 Lateral diffusion

In general, there are two commonly adapted means to characterize and quantify the diffusive motion of individual particles. Perhaps the most commonly used approach is to follow Einstein's ideas and to define the single-particle (tracer) diffusion coefficient as follows [8],

$$D_{\mathrm{T}} = \lim_{t\to\infty} \frac{1}{2dt} \langle |\vec{r}(t) - \vec{r}(0)|^2 \rangle, \tag{1}$$

where $\vec{r}(t)$ is the position of the *tagged* particle (or its center of mass) at time t, and d is the dimensionality of the diffusion process. Hence for lipids diffusing in the plane of a membrane we have $d = 2$, while for bulk water in a three-dimensional environment the dimensionality is $d = 3$. The angular brackets in Eq. (1) stand for ensemble averaging over independent realizations of the process. For simplicity, here we assume that $\vec{r}(0) = 0$.

The above definition is identical to the original one by Einstein for the motion of Brownian particles in a solvent [9]. Essentially, it defines the diffusion coefficient as the long-time limit of $\langle |\vec{r}(t)|^2 \rangle$, which is the mean-squared displacement for the tagged particle, see Fig. 2.

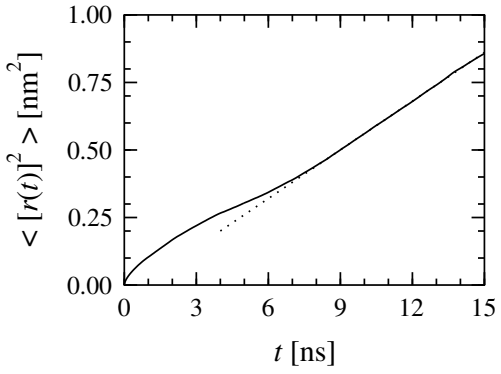

Figure 2: The mean-squared displacement of a dipalmitoylphosphatidylcholine (DPPC) lipid molecule in a DPPC bilayer, determined through atomistic computer simulations [10]. The fit to the long-time behavior is shown by a dashed line.

Another commonly used means to write down the diffusion coefficient is the so-called Green–Kubo equation [8],

$$D_{\mathrm{T}} = \frac{1}{d} \int_0^\infty \mathrm{d}t\, \phi(t), \tag{2}$$

where $\phi(t) \equiv \langle \vec{v}(t) \cdot \vec{v}(0) \rangle$ is the velocity correlation function of a tagged particle (or its center of mass) in terms of its velocity $\vec{v}(t)$ at time t. Thus, here the diffusion coefficient is defined in terms of velocity correlations.

The above two descriptions can be shown to be equivalent and to yield the same diffusion coefficient. However, Eq. (1) is in many cases more convenient for practical purposes and it is commonly employed in both experimental and theoretical (computational) studies. Then all that one has to do is to follow the motion of the particle vs. time t, and to extract the single-particle diffusion coefficient from the long-time behavior of the mean-squared displacement. This seemingly simple task is highly problematic in soft matter systems, however, since the spatial resolution of most experimental techniques does not allow one to track the position of the native particle under consideration. Consequently, means such as probing techniques based on the use of fluorescent markers are commonly employed instead. These techniques facilitate studies of single-particle motion, though the price one has to pay is the uncertainty of probe-induced effects in the diffusion process (see next Section).

The latter approach (Eq. (2)) is most useful in theoretical descriptions since essentially all transport coefficients can be described in terms of the same framework, that is, as an integral over a time correlation function for some dynamical variable. In modeling studies, in particular, the Green–Kubo approach provides many assets. First, the time at which the integral converges provides an estimate of the characteristic time at which the motion becomes diffusive: above this time scale, presumably, the mean-squared displacement in Eq. (1) is linear in time and hence Eq. (1) can be used to yield the diffusion coefficient. Second, the short-time behavior of the velocity correlation function provides important insight into memory effects that highlight the complexity of particle motion at short times. For example, as shown in Fig. 3, at short times the velocity autocorrelation function (vacf) is positive which means that the particle diffuses in the same direction as at time $t = 0$. For intermediate times the vacf is negative, hence in this case the particle is moving in the opposite direction compared to time $t = 0$. At long times, the vacf decays towards zero.

Here we focus on single-particle diffusion in lipid membranes. For this purpose, both of the above two approaches work well, though Eq. (1) is perhaps more convenient and can rather easily be employed to study the lateral diffusion of lipids, integral proteins and other small molecules in the plane of the membrane, or inside it. Below we demonstrate some of these processes by a few topical and relevant examples.

3 Single-particle diffusion in lipid membranes

3.1 Techniques used to measure single-particle diffusion

3.1.1 Experiments.

As illustrated by its name, the single-particle diffusion process refers to a case where *one follows the motion of a tagged individual particle.* This seemingly easy task gives rise to some problematic issues in experiments, where it is exceedingly difficult to keep track of the positions and velocities of nanometer-sized molecules in time. Hence, many techniques are based on labeling lipid molecules by attaching bulky hydrophobic molecules such as pyrene to their acyl chains or a colloidal particle (typically about 40 nm in diameter) to the head group by proper anti-body functionalization. The attached molecule has typically

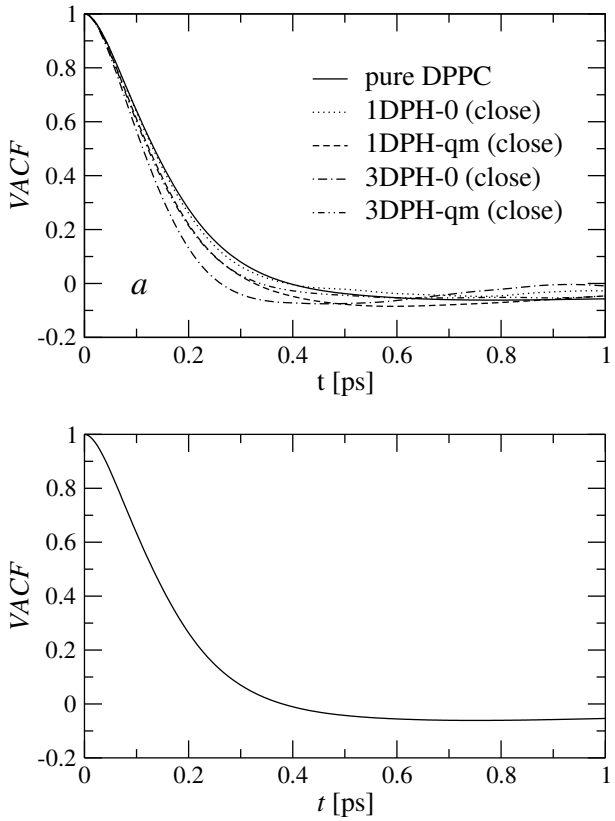

Figure 3: Velocity autocorrelation function of DPPC molecules in a one-component lipid membrane versus time t, at times of the order of 1 ps [J. Repakova et al., unpublished]. Note the short-time decay and the region of negative values at intermediate times.

some property that allows one to follow its position, fluorescent labeling being probably the most commonly used technique. Then, one can employ *single-particle tracking* to follow the trajectories of individual labeled molecules in bilayers by computer-enhanced video microscopy, and analyze a large number of traces to determine the lateral tracer diffusion coefficient using Eq. (1).

The above approach is appealing but suffers from the fact that labeling inevitably changes the properties of the molecule being studied and also perturbs the membrane around it. Consequently, the diffusion characteristics of the labeled molecule may be very different from those of non-labeled parent molecules, thus rendering the interpreting of experimental diffusion data more difficult. Though this problem is usually not major (for studies of probe-induced perturbations in lipid bilayers, see Ref. [44]) there is reason to keep it in mind.

In principle, all other experimental approaches are based on following some collective property of the whole membrane system, such as the decay rate of density fluctuations, instead of the single-particle positions in time. Nevertheless, a variety of different techniques such as fluorescence recovery after photobleaching (FRAP) [11, 12], nuclear magnetic resonance (NMR) [13], and (incoherent) quasi-elastic neutron scattering (QENS) [14, 15, 16, 17] have been used to obtain information of single-molecule motion in membranes. The time and length scales probed by these techniques differ substantially, for which reason they are often characterized as either microscopic (or perhaps nano-scale) or macroscopic methods.

3.1.2 Computer simulations.

Alternatively, one can employ atomistic or coarse-grained simulation techniques to model membrane systems in sufficient detail. The atomic-scale classical molecular dynamics (MD) approach [18], in particular, has developed in recent years to a versatile method for studies of lipid and protein dynamics in lipid membranes [19, 20, 21, 22]. This approach provides one with a classical but detailed description of the system, and can yield plenty of relevant information of the structure and dynamics of membranes at the atomic level. Nevertheless, the price one has to pay is the computational cost. At present, state-of-the-art MD simulations are for about $128-1024$ lipid molecules in a bilayer plus about $20-50$ water molecules per lipid to account for hydration. This allows one to study the system over a time scale of about 100 ns. Although this may sound rather extensive, the resulting length as well as time scales are actually rather modest. In a fluid phase, the lateral diffusion coefficient is $D_{\mathrm{T}} \approx 10^{-7}\,\mathrm{cm}^2\,/\,\mathrm{s}$, which implies that over a time scale of $\delta t = 100\,\mathrm{ns}$ the diffusion length $\ell_D = \sqrt{4\,D_{\mathrm{T}}\,\delta t}$ is about 2 nm. That is, if a tagged molecule diffuses for 100 ns, its average distance from the initial position will be about 2 nm which is roughly three times its size in the bilayer plane. In single-component bilayers this is not a problem, but in many-component membranes characterized by domain formation, the incomplete mixing will be problematic since the true long-time limit (see Eq. (1)) is not achieved. What is needed, therefore, is some way to model these complex systems over larger length and time scales, and that is done by coarse-graining atomistic approaches to a less detailed level.

Coarse-grained simulation techniques have recently been used in membrane systems, including studies of both structural and dynamical aspects. Roughly speaking, coarse-grained approaches can be classified into methods that fully account for hydrodynamic interactions (such as dissipative particle dynamics), and those which consider membranes as dissipative interfaces without momentum conservation (such as Monte Carlo and Brownian Dynamics). Here we do not discuss these methods in further detail. For comprehensive descriptions of these approaches, see Refs. [23, 24].

3.2 Influence of cholesterol on lateral diffusion

Cholesterol, see Fig. 4, is by far the most commonly found sterol. It is a major component of animal cell plasma membranes, comprising up to 50 mol % of the total lipid content [25]. As shown by many studies, cholesterol plays a significant role in a cell membrane [26]. It has a variety of notable effects on the physical properties of lipid bilayers, including an increase in the bulk bending modulus of bilayers containing cholesterol [27], changes in the orientational ordering of phospholipid hydrocarbon chains [2], and changes in the packing and void distributions inside a membrane [28]. Additionally, cholesterol has a strong influence on the dynamical properties of lipid bilayers.

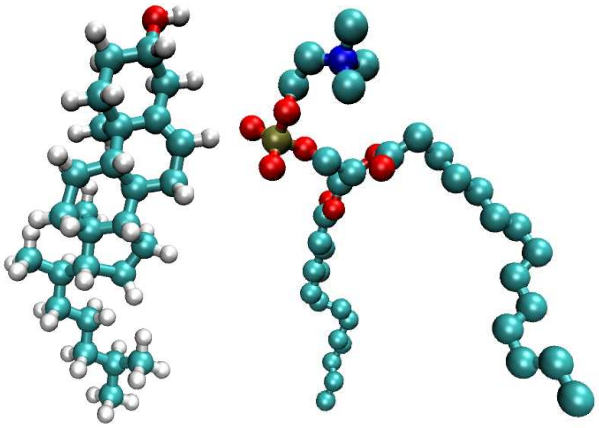

Figure 4: Molecular structures of cholesterol (left) and DMPC (dimyristoylphosphatidylcholine, right). DMPC is one of the most commonly studied saturated phospholipids; here shown in united-atom representation without explicit hydrogens.

Experimental studies [29, 30] have shown that cholesterol affects the rate of lateral diffusion. We will consider these studies shortly, but let us first have a look at diffusion results for a coarse-grained model of a phospholipid-cholesterol mixture.

The details of the coarse-grained model are discussed in Refs. [31, 32]; here we simply note that it describes lipids and cholesterol molecules in a membrane as two-dimensional discs interacting via short-range interactions. Lipids are further described as molecules having two possible states for hydrocarbon chains: a disordered and a fully ordered one. Despite its simplicity, the model yields a phase diagram which is qualitatively fully consistent with experimental findings for phosphatidylcholine-cholesterol bilayer mixtures, see Fig. 5 (top). The phase diagram shows that there are three main phases: the liquid-disordered (**ld**, fluid) phase at small cholesterol concentrations above the main phase transition temperature T_{M}, the low-temperature solid-ordered (**so**) phase below T_{M}, and the liquid-ordered (**lo**) phase at high cholesterol concentrations. Additionally, there are coexistence regions at intermediate cholesterol fractions.

Figure 5 (bottom) illustrates that in the liquid-disordered (fluid) phase above T_{M}, an increase in cholesterol concentration leads to a reduction in the diffusion coefficient. This decrease in D_{T} is closely related to enhanced ordering of lipid hydrocarbon chains due to cholesterol, and to reduced free volume inside a bilayer [33]. Hence, the dynamics is coupled to changes in membrane structure.

In the solid-ordered phase below T_{M}, on the other hand, an increasing cholesterol concentration enhances the diffusion rate since cholesterol perturbs the solid-like order, which is prominent in a neat lipid bilayer in the absence of cholesterol. It is plausible that this leads to an increasing free volume, though, to our knowledge, detailed studies of this issue have not been carried out.

The above findings are in line with experimental findings. For example, in a recent study by Filippov et al. [30], the authors employed NMR to gauge lateral diffusion in various two-component membrane mixtures including cholesterol. They found that in the fluid-phase the lateral diffusion coefficient reduced as the concentration of cholesterol was increased, in agreement with the results presented in Fig. 5.

Though the details vary to some extent from one system to another, the qualitative trends are largely similar in many cases. The main message here is that cholesterol changes a variety of physical (static) properties of membranes, and these changes play a major role in diffusion behavior. Recent atomistic simulations, which can clarify details of diffusion processes in great detail, are consistent with the above conclusions [33, 34].

3.3 Influence of phase behavior

Diffusion in membranes is strongly dependent on phase behavior. As a practical example one may think of diffusion in a single-component bilayer in the vicinity of the main phase transition temperature T_{M} separating the solid-ordered phase from the liquid-disordered phase. As the temperature is changed by a few degrees from $T < T_{\mathrm{M}}$ to $T > T_{\mathrm{M}}$, the system goes through a sudden change from a "frozen and ordered" to a liquid-like bilayer, and similarly the diffusion rate changes abruptly from 10^{-16} – $10^{-10}\,\mathrm{cm}^2\,/\,\mathrm{s}$ to about 10^{-8} – $10^{-6}\,\mathrm{cm}^2\,/\,\mathrm{s}$ [35, 36]. The wide range of diffusion coefficients reflect, in part, differences in systems studied, and, in part, the fact that different experimental techniques yield different diffusion coefficients due to varying length and time scales probed by the techniques under consideration. Notably, while the diffusion coefficient in the solid-ordered phase is

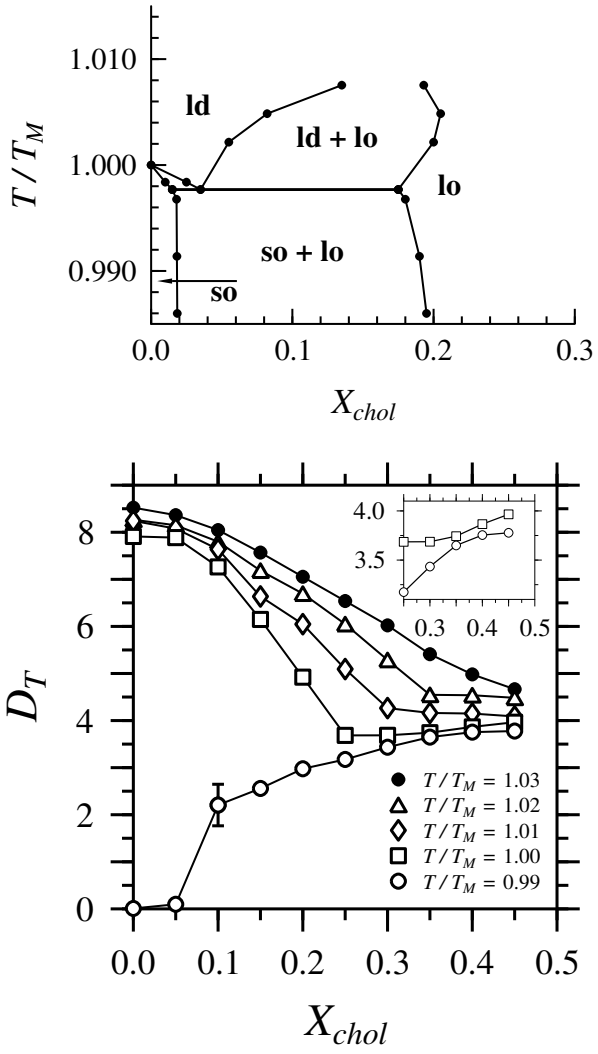

Figure 5: *Top:* Phase diagram for the lipid-cholesterol system, in the plane defined by the temperature (T) and cholesterol concentration ($X_{\rm chol}$) in a coarse-grained model [31, 32]. Here, $T_{\rm M}$ is the transition temperature for the pure lipid system between the high-temperature liquid-disordered (**ld**) phase and the low-temperature solid-ordered (**so**) phase. The lines connecting the data points represent boundaries of coexistence regions between the **so**, **ld** and the liquid-ordered (**lo**) phases. *Bottom*: Lateral diffusion coefficient for single-particle motion, $D_{\rm T}$, for lipids in a cholesterol-phospholipid mixture based on simulations for a coarse-grained model [32]. Results are shown for various temperatures T and cholesterol molar concentrations $X_{\rm chol}$.

exceptionally small, it is not zero. Hence the low-temperature results show that the system is not crystalline but rather gel-like. To demonstrate typical scales of random walks in the gel phase, let us assume that the diffusion coefficient is 1×10^{-13} cm^2 / s and that the particle diffuses over a period of two days. This time scale roughly corresponds to the lifetime of a cell. Then, the diffusion length is about 2.6 μm. In other words, if a lipid resided in a gel-phase domain over the full lifetime of a cell, its average position from its starting point would change by about 4000 molecular diameters in the bilayer plane. Not much. In the fluid phase for a diffusion coefficient of 1×10^{-7} cm^2 / s, however, the diffusion length is about 2.6 mm. Without any doubt, phase behavior may hence have a major effect on lateral diffusion.

3.4 Influence of unsaturation

Many cellular membranes are rich in lipids characterized by their polyunsaturated nature. For example, phospholipids containing docosahexaenoic acid (with six double bonds) constitute about 50 % of the retinal rod outer segment [37], and about 20 % of lipids in human erythrocytes are PLPC with two double bonds (PLPC stands for 1-palmitoyl-2-linoleyl-*sn*-glycero-3-phosphatidylcholine) [38]. From nutritional studies, it is well known that polyunsaturated lipids are important in the development of brain function [39].

Membranes comprised of polyunsaturated lipids are highly fluid-like due to low temperatures for the main phase transition. Since the level of polyunsaturation varies a lot, ranging from one to six double bonds along the chain, it is natural to ask how the dynamics, and the diffusion rate of lipids depends on unsaturation. Rather surprisingly, this problem has been addressed only very recently. In recent NMR studies Dustman et al. found [40] that the diffusion coefficient increased with increasing unsaturation. At the same time, for monounsaturated lipids they observed a decrease in lateral diffusion rates with increasing chain length. For the purpose of comparison, recent simulation studies by Ollila et al. [41] have suggested that D_{T} decreases for an increasing chain length in the case of polyunsaturated lipids. Studies by Niemelä et al. [42] for one-component sphingomyelin bilayers have shown similar behavior for an increasing chain length, though effects observed were minor.

Overall, it seems plausible that an increasing unsaturation enhances lateral diffusion rates. It remains to be seen whether these trends are related to changes in packing (increasing area per molecule in the bilayer plane) or free volume inside a membrane.

3.5 Diffusion of small molecules inside membranes

Let us briefly discuss the diffusion of small molecules inside lipid bilayers. As a specific example, consider diphenylhexatriene (DPH) molecules inside a DPPC bilayer in Fig. 6. DPH is one of the most commonly used fluorescent probes in studies of membrane fluidity and phase behavior. Due to its hydrophobic nature, it prefers to be accommodated in the hydrophobic region close to the hydrocarbon chains of lipid molecules [43]. A careful analysis of its dynamic behavior reveals that DPH diffuses almost at the same rate as the lipids in a membrane, the diffusion coefficient of DPH being slightly larger [44]. Similar

findings have been made for ubiquinone [45], which acts as a charge carrier inside a bilayer, though in this case the diffusion rate was found to depend on the location of the molecule in a bilayer. This is consistent with earlier studies of benzene diffusion inside a DMPC bilayer [46, 47]. Thus, it seems likely that the diffusion rates of small molecules inside lipid bilayers depend on the size and density of voids, which are greatest in the bilayer center, and smallest (usually) close to the highly packed head group region. Nevertheless, a number of molecules prefer to be accommodated close to the acyl chain region due to attractive van der Waals interactions, or close to the membrane-water interface if they are substantially polar or charged.

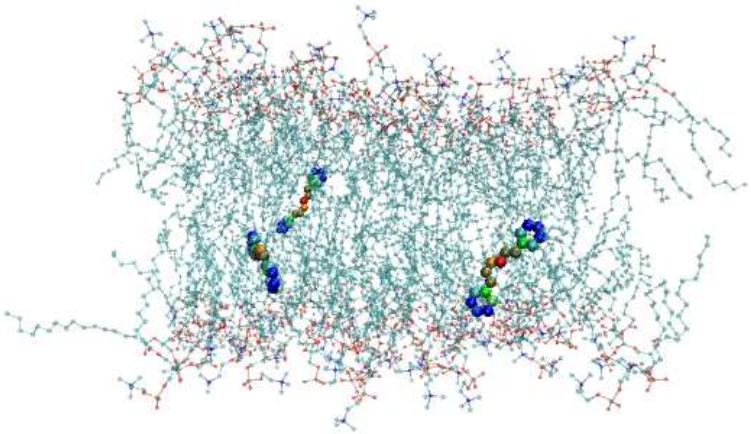

Figure 6: DPPC bilayer together with three diphenylhexatriene (DPH) fluorescent probes freely embedded inside a membrane [43]. DPPC molecules are shown as transparent to clarify the presentation. For the same reason, water is not shown.

As a final issue, let us consider how the molecules actually diffuse, i. e., what is the diffusion mechanism by which they move from one place to another. Though this issue is not well understood, there are some cases studied through atomistic simulations. Using the above DPH as an example, Fig. 7 depicts the motion of a selected DPH molecule in the plane of the membrane (from above). We find that there is a well-defined process, where DPH jumps from one site to another such that the jump length corresponds (roughly) to an average molecular size in the plane of the membrane. This suggests that DPH spends most of its time by standing in a "cage", being surrounded by a few lipid molecules, and every now and then it performs a jump from one void to a neighboring one. The time scales are well separated: the jump process takes place over a time scale of about $200-300$ ps, while the number of jumps observed was about $1-2$ per probe molecule during a time scale of 40 ns. Hence, the events are rare processes, but it is fascinating to think about this diffusion

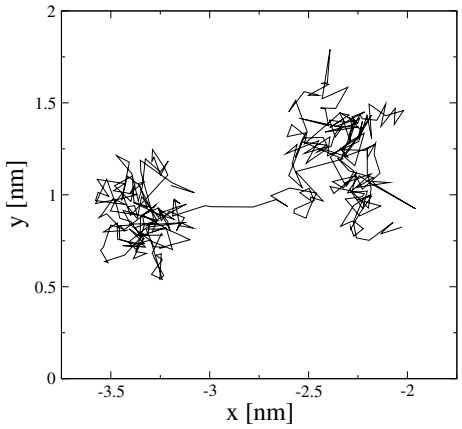

Figure 7: Picture showing the motion of the center of mass of DPH in the plane of the membrane (from above). The route demonstrates one jump event where DPH travels a distance of about 1.0 nm [43].

process as a series of events which gives rise to a “dance”, which after all reminds us of the usual random walk.

4 Concluding remarks

In this brief contribution, we have found that the lateral diffusion of lipids and small molecules largely depends on a variety of molecular features as well as thermodynamic conditions. The complexity of membranes further accentuates this fact, since membranes are actually characterized by hundreds of different lipid molecules.

The phase behavior, in particular, has a prominent influence on lateral diffusion. Lateral diffusion coefficients for lipids in the liquid-disordered (fluid) phase are typically of the order of $10^{-7}\,\mathrm{cm}^2\,/\,\mathrm{s}$, while in ordered domains such as rafts (characterized by large amounts of cholesterol and sphingomyelin) the diffusion is slower by a factor of about 10. In a solid-ordered (gel) phase, in turn, the diffusion coefficient is several orders of magnitude smaller. To get some perspective on the differences between these cases, we may think of how fast we are able to walk along a field compared to swimming in water, and further to swimming in syrup. Without any doubt, there is a major difference.

Lateral diffusion is involved in a variety of exciting cellular processes. Yet, despite extensive studies, many of the fascinating problems related to the dynamics of biomolecules in membranes are still unresolved. We hope that the examples discussed above serve as a guide to new ideas regarding the role of diffusion in cellular functions, and that there will be people who are willing to tackle those issues with great interest.

5 Acknowledgments

I would like to thank many of my colleagues, and especially E. Falck, J. Repakova, J. M. Polson and O. G. Mouritsen for useful discussions over the years. This work has, in part, been supported by the Academy of Finland through its Center of Excellence Program and Academy of Finland Grant No. 80246.

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