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Membrane Proteins Diffuse as Dynamic Complexes with Lipids

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Biological membranes govern a large number of cellular functions. While the structural aspects of membranes are still being explored in terms of the lipid raft model,[1,2] the understanding of membrane dynamics[3] is more limited. One of the relevant dynamical membrane processes is lateral diffusion of lipids and proteins, involved in a variety of phenomena including domain formation, protein sorting, and formation of membrane protein complexes. Recent studies have highlighted the importance of collective phenomena in lateral diffusion, as lipids have been found to undergo diffusion in a concerted fashion as loosely defined transient clusters.[4−6] Meanwhile, the diffusion of membrane proteins has remained one of the greatest unclear issues in membrane dynamics.[7−9]

Here, we consider the lateral diffusion of membrane proteins and show its intriguing complexity. We find that proteins diffuse in a concerted manner with numerous lipids around them. Our findings highlight the prominent role of lipid−protein interactions and suggest that in cell membranes there are only a few if any lipids with no coupling to membrane proteins.

We performed atomistic molecular dynamics simulations for a single Kv1.2 voltage-gated ion channel in a lipid bilayer of 910 POPC molecules. Related studies for Kv1.2 structure and dynamics have been discussed elsewhere.[10] Two simulations were carried out with GROMACS[11] for 500 ns at a temperature of 310 K. In addition, we performed coarse-grained (CG) simulations with the MARTINI force field[12] for two other systems: a single LacY protein in a POPC bilayer and a WALP23 dimer in a DPPC bilayer. To highlight the generality of the observations, we also performed simulations of two-dimensional (2D) Lennard−Jones (LJ) systems. Diffusion of lipids was analyzed with respect to the protein, and its rotational motion was found not to affect the conclusions. Details of the simulation methodology and additional results are given in the Supporting Information (SI).

We find several intriguing related phenomena; see Figure 1. The protein and the neighboring lipids form a transient complex: approximately 50−100 lipids move laterally together with the protein. On average, they move significantly more slowly than the other lipids. Below, we quantify and discuss these aspects in detail.

The layer of lipids in direct contact with Kv1.2 is affected most strongly, but a substantial slowing-down effect can be observed up to a distance of 5−6 nm from the protein center of mass (COM), i.e., approximately 1−2 nm away from the outermost surface of the protein. Weaker effects are observed up to 8 nm (∼4 nm from the lipid−protein interface). For lipids around Kv1.2, the S_{CD} order

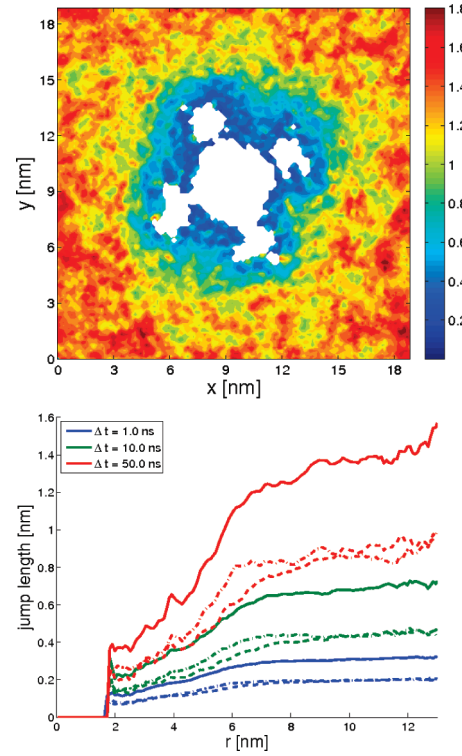

Figure 1. Mean lateral displacements of lipids in the intracellular leaflet over different time scales Δt. Top: the 2D displacement distribution $P(x,y)$ over $\Delta t = 50$ ns, with the protein COM centered in the box. Bottom: the radial average of $P(x,y)$ as a function of distance from the protein COM. The total averages (solid) are shown together with the radial (dashed) and tangential (dot-dashed) components.

parameter is disturbed up to ∼5−6 nm from the protein COM (see SI); the dynamical protein−lipid complex is larger in size than this structurally altered membrane region.

To quantify diffusion in different regions, we computed the lateral diffusion coefficients based on lateral displacement distributions over a range of time scales (see SI). The lipids can be divided into two groups: protein non-neighbors ($r > 7$ nm from protein COM) and neighbors ($r < 3$ nm), with distinctly different diffusion coefficients of $D \approx 90 \times 10^{-9}$ cm^2/s and $D \approx 6 \times 10^{-9}$ cm^2/s, respectively. The former diffusion coefficient is typical of unperturbed lipid bilayers in the liquid-disordered phase,[13] whereas the latter is close to the value calculated for the protein in this study, $D_{prot} \approx 3 \times 10^{-9}$ cm^2/s. Annular lipids diffused very slowly but are not bound to the protein.

Figure 1 also highlights that the component of lipid movement tangential to the protein surface is larger than the radial one. This

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arises from geometric factors: for lateral displacements comparable to the distance to the protein surface, movement toward the protein surface is less likely than in the tangential direction. To quantify the directional correlations between the movements of the protein and the lipids, we computed the 2D lateral displacement correlation map[5] (Figure 2). The directions of lipid motion are strongly correlated with those of protein motion within a distance of approximately 5−6 nm. At larger distances, the correlation remains positive in front of and behind the protein but becomes negative on the sides. Similar features have been observed earlier for pure lipid bilayers,[5] three-dimensional LJ fluids,[14] and supercooled liquids.[15] These analogies suggest concerted diffusion for a dynamical complex consisting of a colloid (protein) and neighboring solvent particles (lipids) to be a general phenomenon in soft matter.

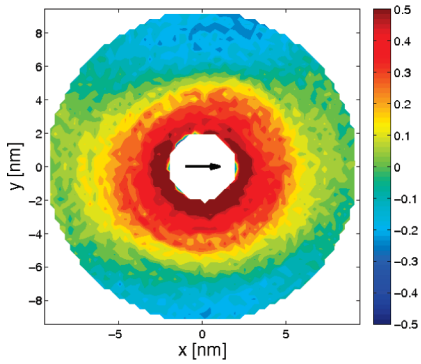

Figure 2. 2D lateral displacement correlation plot, averaged over the whole trajectory. For each 10 ns interval, the protein displacement vector was centered in the box, pointing to the $+x$ direction. The cosine value between each protein and lipid displacement vector over a given 10 ns interval was binned into the (midpoint) location of the lipid vector. The protein is moving in the middle to the $+x$ direction.

We compared the findings for Kv1.2 with two CG simulations of membranes with LacY and WALP23. The data on all three systems agree qualitatively. Finite-size effects were considered for Kv1.2 and LacY, with no change in conclusions. The protein diffusion coefficients are in reasonable agreement with experiments for the largest systems we studied (see SI).

In every system, lipids in the proximity of the protein have diffusion coefficients similar to (but slightly higher than) those of the protein, indicating that they are diffusing together with the protein. The most significant slowing down effect extends to a few nanometers from the outermost surface of the protein. Both tangential and radial components of lipid displacements are reduced in all systems, the radial component being slowed down slightly more.

To better understand the physical origin of the observed effects, we simulated a highly simplified system of 2D LJ discs modeling the protein (big disk) and lipids (small discs) via both LJ and hard-wall interactions. Despite its simplicity, this model yields similar correlations as in atomistic and CG simulations (see SI). We find that the reduction of the radial component is a general "wall effect" due to blocking of particle motion.[16] The slowing down of the tangential component of lipids is related to the interaction with the protein surface, as the lipids in its vicinity move at the same pace as the protein surface. The same holds for more distant lipid layers with respect to the layer closer to the protein. This phenomenon, common in fluid dynamics, appears to be caused mainly by surface roughness. The slowing down effect is strongest for Kv1.2 with well-defined lipid binding sites within the grooves between neighboring voltage-sensor domains[17] and becomes weaker for smoother and less complex proteins. For completely smooth LJ and hard disk systems, the tangential motions of lipids close to the protein are even faster than those in the bulk.

The results give rise to a number of ideas and questions. The number of lipids in the dynamic protein−lipid complex is here ∼50−100 for Kv1.2. For comparison, biological membranes are usually crowded, membrane proteins occupying ∼30% of the total surface area,[18] the lipid/protein ratio being roughly 50.[19] Consequently, our results suggest that there are no "free" bulk lipids in biological membranes. Further, while membrane protein crystal structures often include a few lipids,[20] those lipids are apparently just the tip of the iceberg, as the actual protein−lipid complex is considerably larger due to the many lipids included in the dynamical complex.

The size of the complex plays a role also in theories for lateral diffusion of membrane proteins. First, as the theories include the protein radius as one of the key variables,[7−9] the present results suggest that the protein radius is determined by the effective size of the dynamical lipid−protein complex. Second, the theories for protein diffusion also include membrane viscosity η. Our data for lipid diffusion indicate that D depends on distance from the protein, and hence the Einstein relation $D \sim 1/\eta$ highlights that the protein senses a viscosity that differs from the one in a protein-free membrane. While the theories need η as input, it is evident that this cannot be measured from pure lipid membranes.

One of the grand challenges in membrane biophysics is to understand the dynamics of lipids and proteins in cell membranes. Our results highlight that these are not two separate issues but have to be considered together.

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Supporting Information Available: Simulation protocol, additional results, and studies of finite size effects. This material is available free of charge via the Internet at http://pubs.acs.org.

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