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Free Volume Theory Applied to Lateral Diffusion in Langmuir Monolayers: Atomistic Simulations for a Protein-Free Model of Lung Surfactant

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We hereby present a study on lateral diffusion of lipids in Langmuir monolayers. We apply atomistic molecular dynamics simulations to a model system whose composition is consistent with protein-free lung surfactant. Our main focus is on the assessment of the validity of the free volume theory for lateral diffusion and on the interpretation of the cross-sectional area and activation energy parameters appearing in the theory. We find that the diffusion results can be fitted to the description given by the free volume theory, but the interpretation of its parameters is not straightforward. While the cross-sectional area appears to be related to the hard-core cross-sectional area of a lipid, its role in the lateral diffusion process is unclear. Also, the activation energy derived using the free volume theory is different from the activation energy found through Arrhenius analysis, and its physical interpretation remains elusive. Finally, we find that lipid diffusion does not occur via rapid single-particle "jumps". Instead, lipids move in a concerted manner as loosely defined transient clusters, as observed earlier for lipid bilayers.

I. Introduction

The significance of lipid monolayers in life sciences has been overshadowed by the major body of research done on lipid bilayers,[1,2] which are an integral component of cell membranes and a number of other biological entities. Yet, the biological significance of lipid monolayers in, e.g., lung surfactant and human lens membranes is paramount.[2−4] Moreover, Langmuir monolayers are used in a variety of technological applications that range from proteins functioning in supported lipid films to applications in electronic devices.[3,4]

Living matter is in constant motion, driven by thermal fluctuations giving rise to diffusion of molecules. In lipid monolayers, this motion is manifested as lateral diffusion in the plane of a membrane, playing a role in a variety of phenomena such as domain formation, ordering phenomena in membranes covering the eye, and nonequilibrium dynamics of lipids in lung surfactant during the respiratory cycle. Lung surfactant dynamics is particularly important for human health. Lung surfactant is a surface-active mixture of phospholipids, cholesterol, and proteins that create a unique and highly dynamic film separating air and liquids at the alveolar cell surface. Normal lung function requires surfactant that reduces the surface tension to near-zero values. Insufficiently low surface tension at the air−liquid interface (arising, for example, as a consequence of acute inflammation) leads to respiratory distress syndrome. As this condition can be fatal, understanding the lateral dynamics of the lung surfactant lipid components is an issue of medical relevance.

Given these examples, it is somewhat surprising how poorly the diffusion of lipids is understood. While a number of studies have quantified typical lipid diffusion coefficients that characterize the pace of lipid motion in the monolayer plane,[5−8] the mechanism by which lipids migrate along these soft interfaces has remained unknown. Moreover, while monolayers are an example of soft matter driven by thermal fluctuations, the importance of collective density fluctuations in lipid diffusion has not been clarified in monolayers. Overall, the theoretical understanding of lipid diffusion in monolayers is limited.

For lipid bilayers, the understanding of lateral lipid dynamics is somewhat more advanced. It has been proposed that the free volume theory[9] can be used to describe lateral diffusion in lipid bilayers.[10] The free volume theory assumes that lipids diffuse via thermally activated, lateral displacements or "jumps"; these "jumps" occur as often as there is a large enough free volume pocket adjacent to the lipid. Assuming that this idea holds, the lateral diffusion of lipids can be described with an activation barrier and the close-packed cross-sectional area of a lipid in the bilayer plane.

Recently, concerns about the validity of the free volume theory for describing lipid and membrane dynamics have been brought about.[11−15] These largely stem from the fact that the free volume theory was originally developed for colloid-like systems.[9] While

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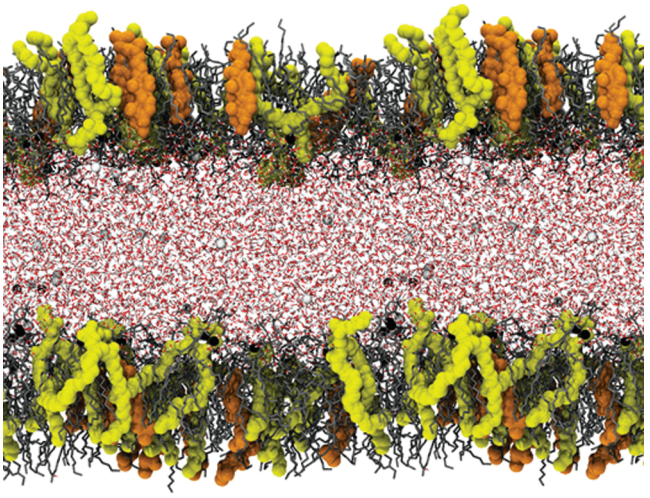

Figure 1. Snapshot of the system with $\langle A \rangle = 56$ Å^2 per lipid using two replicas in the membrane plane. Vacuum slabs below and above the system have been omitted. DPPC and POPC are colored in gray and are shown using the licourice scheme. POPG is colored in yellow and cholesterol in orange, and they both are rendered with the surface scheme. Sodium (white) and chloride (black) ions are shown by the van der Waals scheme. Water is rendered by licourice with hydrogen in white and oxygen in red.

the assumptions made in deriving the free volume theory were justified in systems composed of hard spheres, it is not obvious that they are also appropriate for lipid diffusion, since the cross-sectional area of lipids is not constant[16] and diffusion likely does not take place as single-particle "jumps".[17−19] Instead, recent simulation studies have shown that the diffusion of lipids in fluid bilayers takes place through concerted lipid motions, where tens or possibly hundreds of lipids move in concert as loosely defined clusters,[17,18] and that the lifetime of these dynamically correlated lipid clusters is of the order of a microsecond.[18] Recent quasi-elastic neutron scattering experiments have confirmed the existence of concerted lipid motions in lipid bilayers.[19]

In this article, our objective is to shed light on the diffusion properties of lipids in monolayers. Particular attention is paid to the free volume theory, with an objective to clarify whether it describes lipid diffusion data in monolayers and how the parameters appearing in the free volume theory should be interpreted. For this purpose, we consider the lateral diffusion of lipids in Langmuir monolayers through atomistic molecular dynamics simulations for a model system whose composition is consistent with protein-free lung surfactant. We find that the free volume theory fits the lateral diffusion data reasonably well, but the interpretation of the parameters appearing in the free volume theory is nontrivial.

The article is organized as follows. In section II we describe the models and methods employed in this work. Section III deals with how the lateral diffusion coefficient is defined and also introduces the free volume theory and its assumptions. The simulation results are presented in section IV and discussed in section V, concluding remarks closing the article in section VI.

II. Models and Methods

To model protein-free lung surfactant through atomistic molecular dynamics (MD) simulations, we considered systems composed of two monolayers separated by a water slab (see Figure 1). Each monolayer consisted of 100 lipid molecules with the composition of 60 mol % dipalmitoylphosphatidylcholine (DPPC), 20 mol % palmitoyloleoylphosphatidylcholine (POPC), 10 mol % palmitoyloleoylphosphatidylglycerol (POPG), and 10 mol % cholesterol. These values are consistent with the relative content of saturated, unsaturated, charged, and neutral lipid components observed in lung surfactant.[20,21] The systems were fully hydrated with 7235 water molecules. The systems included 20 sodium counterions to compensate for the negative charges in POPG headgroup and a concentration of 150 mM NaCl.

The simulations were performed in the NVT ensemble, constraining the monolayer area to a fixed value. Periodic boundary conditions were applied in all three directions. For initial configurations, we first constructed a random lipid distribution that was confined to a regular grid. The system structure obtained in this fashion was simulated with a varying monolayer area to generate initial configurations for seven systems with molecular areas (the total area of the monolayer divided by the number of molecules in the monolayer) ranging from $\langle A \rangle = 44$ to 68 Å^2 with a step of 4 Å^2. Larger and smaller systems were also built, but they either underwent pore formation or were unable to relax to a planar configuration during the simulation and are thus not discussed in this work.

The force fields used for lipids followed the Berger description,[22] which is often combined with the SPC (or SPC/E) model for water. Here we used instead the TIP3P model since the Lennard-Jones parameters in the Berger model are based on OPLS-UA, which is often used together with the TIP3P water model. For a pure DPPC monolayer we calculated its dynamic properties with both SPC and TIP3P water models and found essentially no difference. For cholesterol, we used the description of Holtje et al.[23] Salt and counterions were described by the GROMACS force field. In the simulations, temperature was kept at 310 K with the Nose−Hoover thermostat[24] using a time constant of 0.5 ps. Electrostatic interactions were calculated using the particle mesh Ewald (PME) method.[25] Interactions in the reciprocal space were calculated using a fourth-order B-spline interpolation, and the grid spacing was ∼0.12 nm. A cutoff of 1 nm was employed for Lennard-Jones interactions. The neighbor list with a radius of 1 nm was updated every 10 steps. All bonds were constrained using LINCS.[26,27] The simulations were performed with the GROMACS 4 simulation package[28] using a time step of 2 fs.

In all simulations, the systems were simulated for 260 ns. Equilibration was monitored by considering the time dependence of the total energy, which stabilized after about 75 ns. Yet, we consider this time scale to be too short for true equilibration. It is known that the adsorption and desorption events of monovalent ions at the lipid−water interface are slow processes and may slow

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down equilibration to the 100 ns time scale.[29] Further, it is worth stressing that here we deal with a four-component system where the mixing of lipids through lateral diffusion has to be accounted for. Assuming a typical lateral diffusion coefficient of $D = 1 \times 10^{-7}$ cm^2/s, and considering diffusion in the plane of the membrane over a length scale of $L \approx 2.4$ nm (roughly three molecular diameters in the plane of the monolayer), the time scale for local mixing would be $t = L^2/(4D) \approx 120$ ns. Consequently, to avoid significant concerns with respect to mixing of lipids in this many-component system, we considered the first 180 ns of the simulation as the equilibration period and used the last 80 ns of the trajectory for analysis. We emphasize that the simulation time scale is short compared to time scales associated with domain formation. If the lipid composition with a given surface pressure corresponded to a different domain structure, that would not be seen in the simulations. This is currently a general limitation for atomistic membrane simulations.

The above studies at 310 K were complemented with further simulations to consider the temperature dependence of lateral diffusion using the Arrhenius description for this transport coefficient:

$$D = D_0 \exp(-E_{\mathrm{Arrh}}/k_{\mathrm{B}}T) \tag{1}$$

where D_0 is a constant assumed not to depend on T, or its temperature dependence is presumed to be weak. To find the barrier E_{Arrh}, we carried out simulations with the molecular areas of 48 and 68 Å^2 at 292, 301, 320, and 330 K, in addition to the above-described simulations at 310 K. The initial structures in these simulations corresponded to the final structures (after 260 ns) at 310 K for each given area. The simulations were run for at least 100 ns, allowing the systems to equilibrate for 20 ns and using the rest of the trajectory for analysis.

For comparison with the area parameter given by the free volume theory (see next section), we determined the average close-packed cross-sectional area of the lipids using the alternative technique discussed by Falck et al.[16] For each of the lipid types, we computed the average hard-core cross-sectional area profile as slices across the monolayer. As the procedure is described in ref 16 in detail, here we present only the essential features. We map each system configuration on several cubic three-dimensional grids as follows. If a grid point lies within the hard-core radius r_{HC} of an atom belonging to (say) cholesterol molecule, this point is considered occupied, and otherwise empty, on a grid keeping account of cholesterol molecules. Grid points within hard-core radii of atoms belonging to POPC, in turn, will be occupied on a grid characterizing the POPC molecules, and so forth for each molecule and ion type in the system. The hard-core radius r_{HC} has been chosen to be determined by the distance at which the Lennard-Jones interaction of the atomtype with itself equals 10 $k_{\mathrm{B}}T$. The grid spacing was fixed to 0.05 nm in all three dimensions. The grids found in this fashion can be used to view given slices of the monolayers, as they show cross sections of DPPC, other lipids, water (and ions) as well as patches of free area. An illustration of the analysis for the occupied and free-volume regions inside a membrane is given in Figure 8 of ref 16, which also depicts that the above approach has a certain resemblance to tomography. From the grids constructed, we compute total area profiles for the various molecular species, that is, average total areas occupied by the molecules as functions of the distance from the middle of the water phase. In addition, we can calculate free area (volume) profiles, i.e., the amount of free area (volume) (space not occupied by any molecule or ion) as a function of the distance from the middle of the water phase. Summarizing, the analysis averages over the lipids in a membrane and provides an average "hard-core" or close-packed shape of the lipids. Also, it yields the profile of the average free volume across a membrane.

Finally, we wish to mention that in this article we focus on the lateral dynamics in this model system. As for structural aspects, the pressure−area isotherm has been found to be in reasonable agreement with previous studies for simpler model systems,[30] though a direct comparison is not possible. The results concerning the structural properties will be discussed elsewhere (Javanainen et al., work in progress).

III. Formulation of Lateral Diffusion and Free Volume Theory

A. Definition of Lateral Diffusion Coefficient. Diffusion of single particles is often described in terms of the mean-squared displacement

$$\mathrm{MSD}(t) \equiv \langle [\mathbf{r}_i(t+t') - \mathbf{r}_i(t')]^2 \rangle \tag{2}$$

where $\mathbf{r}(t)$ is the position of the particle i at time t. The angle brackets denote averaging over all particles of a given type as well as averaging over all time origins t'. The diffusion coefficient D describing the stochastic motion of a particle in a random-walk-like manner is then defined as

$$D \equiv \lim_{t\to\infty} \frac{1}{2dt} \mathrm{MSD}(t) \tag{3}$$

where d is the dimensionality of diffusion. For lateral diffusion $d = 2$. In order to have a well-defined diffusion coefficient, one must find $\mathrm{MSD}(t) \sim t^{\alpha}$ with $\alpha = 1$. This condition is satisfied at long times. Meanwhile, at short times the diffusion is subdiffusive, and the motion along the membrane normal direction also plays a role via protrusions and undulations[31] which imply that lipid motion is not truly two-dimensional.

In simulations of membrane systems, the membrane position may fluctuate with respect to the water phase. Consequently, the motion of individual lipids has to be computed with respect to the motion of the membrane's center of mass.[32]

B. Brief Overview of Free Volume Theories. Free volume theory, presented in 1959 by Cohen and Turnbull,[9] is a model for describing diffusion in a liquid environment. It was originally developed for colloids, more specifically for hard spheres, but it has later been further developed and extended to describe diffusion also in lipid membranes.[10,33−37]

The free volume theory suggests that diffusion occurs via jumps[33−35] where the diffusing particle moves in a short period of time a distance close to its own size. The theory connects the diffusion coefficient with the average free volume available for the

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diffusing particle to undergo diffusive motion. The relation is given as

$$D = A \exp(-\gamma v^*/v_f) \quad (4)$$

where v_f is the free volume available and v^* is called critical volume, that is, it represents the minimum volume of the void required for the jump. The parameter A is a constant, and γ is a numerical factor which accounts for possible overlap of free volume ($0.5 < \gamma < 1$).[9]

The free volume theory was further developed by Macedo et al.,[36] who proposed that free energy should also be taken into account for a diffusive jump to overcome possible energetic barriers. The equation for the diffusion coefficient is then written as

$$D = D' p(v) p(E) \quad (5)$$

where $p(v)$ is the probability for finding a sufficiently large void next to the diffusing particle and $p(E)$ represents the probability that the diffusing particle has enough free energy to release itself from the interactions with neighboring molecules. The expression $p(E)$ follows the Boltzmann distribution, and D' is a constant.

More recent studies have extended the free volume theory to describe diffusion in lipid bilayers.[10,33−35,37] As there are quite a few slightly different versions of the free volume theory, we focus here on one of the most recent ones.[10] It is also worth pointing out that, as in lipid bilayers one deals with diffusion in two dimensions, it is common to speak about the free area theory where the quantity of interest are area fluctuations in the plane of a lipid membrane. Another relevant remark is that while the free volume in a membrane is distributed in small pieces, its total amount inside a membrane is rather substantial.[38,39] It has been found, for example, that there is sufficient free volume around the water−membrane interfacial region to enable fast rotations of non-native molecules such as fluorescent probes.[40,41]

Almeida et al.[10] applied the free volume theory to lipids in a planar membrane. They assumed that the problem is essentially two-dimensional, and therefore the critical volume can be replaced with a critical area parameter a^*. For diffusion in a plane, a^* describes the area above which lateral diffusion becomes possible. Following the results of MacCarthy and Kozak,[34] Almeida et al. considered that $\gamma a^* = a_0$, where a_0 is now the cross-sectional close-packed area of the lipid. The expression they used is thus given as

$$D = \frac{\delta}{2\sqrt{2}} \sqrt{\frac{k_B T}{m}} \exp\left[\frac{-a_0}{a(T)-a_0} - \frac{E_a}{k_B T}\right] \quad (6)$$

where δ is the distance between cages, k_B the Boltzmann constant, $a(T)$ the area per lipid at temperature T, and E_a the activation energy of a diffusion jump. The theory considers lipids as homogeneous rods that all span the same height in the membrane. The lipids rattle in a cage formed by the surrounding lipids until they are able to jump a distance close to their own size to the neighboring cage. The jump is assumed to happen at thermal speed (corresponding to thermal energy),[10] meaning that for a lipid it would take of the order of 10 ps to move the distance of about 1 nm. By noticing that δ in a tight-packed cubic lattice is just the square root of the area per lipid and by plugging the numerical values of the constants into eq 6, one obtains a useable formula for the diffusion coefficient

$$D = 3.224 \times 10^{-5} \sqrt{\frac{Ta(T)}{M}} \exp\left[\frac{-a_0}{a(T)-a_0} - \frac{E_a}{k_B T}\right] \quad (7)$$

where M is the molar mass given in units of g/mol. The values for the areas are given in Å^2.

When the free volume theory is applied in practice, the measured lateral diffusion data is fitted to the above description, yielding the close-packed cross-sectional area of a lipid a_0 and the activation energy E_a.

C. Assumptions in Free Volume Theory for Lateral Diffusion. For the discussion in this article, it is useful to consider the limitations of the current free volume theories for lateral diffusion. The main assumptions of the free volume model of Almeida et al.[10] are as follows:[15] (1) For describing diffusion in membranes, a lipid is considered as a hard rod with a well-defined close-packed area a_0, which is independent of temperature and composition of the membrane. (2) Diffusion proceeds via jumps. During a jump, a whole lipid moves a distance close to its own diameter in a short interval of time. (3) A lipid may jump when it is given a patch of free area larger than a_0 next to it. (4) Changes in free volume (area) distribution occur faster (on a much faster time scale) than the translational motion of lipids and do not require local free energy. (5) The lipid needs to overcome an activation barrier, i.e., break loose from the interactions with its nearest neighbors. This requires an activation energy E_a. The activation energy also incorporates the interactions with the aqueous phase. The validity of some of these assumptions has been questioned in the literature. We summarize here the issues related to three of these assumptions, namely the cross-sectional area, the activation barrier, and the diffusion mechanism.

While the free volume theory regards membranes as homogeneous in the direction of the membrane normal, they are actually heterogeneous.[12,16,42] The average close-packed area of a phospholipid and the average free area per lipid vary considerably along the membrane normal.[11,12,16,39,43,44] Also, this close-packed area profile and free area distribution change with lipid composition[16] and are likely to be temperature dependent, too. For the reasons above, the assumption of a well-defined and constant cross-sectional area of a lipid seems not well justified for lipid membranes.

The activation energy E_a describes the barrier needed to break loose from the interactions with the lipid's nearest neighbors. This view is largely similar to the interpretation of the effective activation barrier E_{Arrh} extracted from the very commonly used Arrhenius description. Given this idea, are these two activation barriers related? More generally, how should E_a be interpreted in terms of the interactions in a membrane?

The free volume theory assumes that particle motion takes place via "jumps".[33−35] While this is possible for hard-sphere systems like colloids, it is not clear whether this assumption is appropriate for lipids in membranes. Simulation studies by Essmann and Berkowitz,[45] Moore et al.,[46] and Voth et al.[47]

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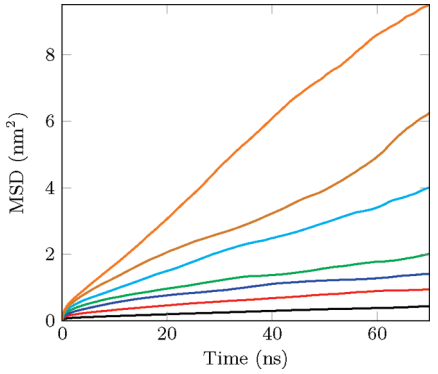

Figure 2. Mean-squared displacement data for DPPC. The different curves stand for an area of 44 Å^2 (black), 48 Å^2 (red), 52 Å^2 (blue), 56 Å^2 (green), 60 Å^2 (cyan), 64 Å^2 (brown), and 68 Å^2 (orange).

Table 1. Long-Time Diffusion Coefficients for the Lipids (in Units of 1×10^{-7} cm^2/s)[a]

A/lipid (Å^2)	D_{average}	D_{DPPC}	D_{POPC}	D_{POPG}	$D_{\text{cholesterol}}$
44	0.146	0.143	0.142	0.177	0.117
48	0.386	0.378	0.367	0.363	0.582
52	0.697	0.649	0.805	0.560	1.014
56	0.740	0.687	0.858	0.596	1.069
60	1.312	1.258	1.278	1.039	2.523
64	1.777	1.702	1.862	1.370	3.040
68	2.838	2.760	2.978	2.286	4.137

[a] The error bars are less than 10%. They were calculated by estimating the maximum error, which was determined by the difference of the diffusion coefficients in the x and y directions (on the monolayer plane).

suggest that jumps do not dominate lateral diffusion in bilayers but instead collective fluctuations[47] are prevalent. Simulations by Falck et al.[17] and Apajalahti et al.[18] also provided evidence for concerted lipid motions, where lipids move as transient lipid clusters. The same view has been given by recent experimental data.[19] Considering these results, if there are no jumps, then how should the area parameter a_0 and the activation energy E_a be interpreted?

In the rest of this paper we try to answer the questions above. In particular, we investigate the physical interpretation for the area parameter and the activation barrier, and we also consider the mechanism of lipid diffusion. Lipid monolayers provide interesting test systems for studying the free volume theory since the total area of the system can be varied systematically and in a controlled way, both in experiments and in simulations.

IV. Results

A. Free Volume Theory Fitted to Lateral Diffusion Data. The mean-squared displacement of DPPC with varying area per molecule is shown in Figure 2. The regime of normal diffusion where MSD(t) $\sim t^1$ emerges at times of about 10−20 ns, depending on the area per lipid. The diffusion coefficients determined from the long-time limit of the mean-squared displacement are given in Table 1. The results indicate that the diffusion coefficients for all lipid types increase for increasing area per molecule, as expected.

The diffusion coefficients were fitted to eq 7. Here, the activation energy of a diffusion jump (E_a), one of the parameters in the free volume theory, was considered to be independent of the compression state of the system. The area of a cholesterol molecule was fixed to a value of 30 Å^2. This assumption seems reasonable because cholesterol has a smooth and rigid structure, with a well-defined cross-sectional area.[16] Under these conditions,

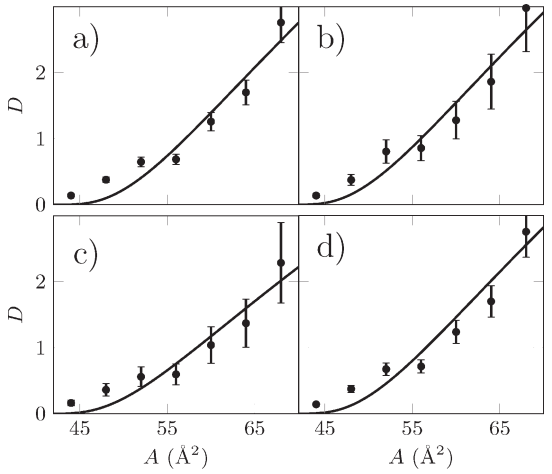

Figure 3. Free volume theory fits (full line) to the calculated diffusion coefficients (crosses): (a) DPPC, (b) POPC, (c) POPG, and (d) average over all phospholipids. Diffusion coefficients are given in units of 10^{-7} cm^2/s.

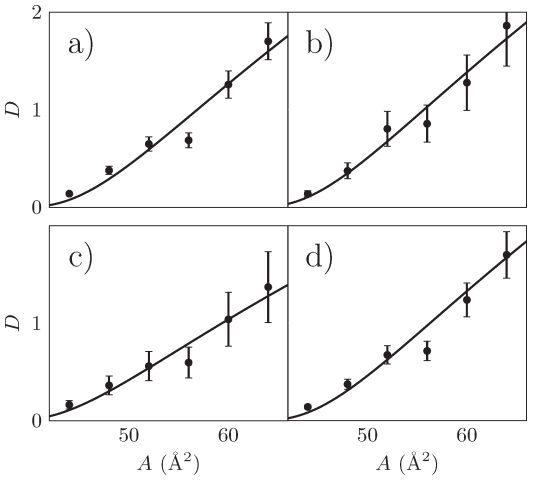

Figure 4. Free volume theory fits (full line) to the calculated diffusion coefficients (crosses), without the data for the largest system (A = 68 Å^2): (a) DPPC, (b) POPC, (c) POPG, and (d) average over all all phospholipids. Diffusion coefficients are given in units of 10^{-7} cm^2/s.

the fitting process allowed us to extract the two parameters that are central to the free volume theory, namely a_0 and E_a. Additional studies with cholesterol's cross-sectional area different from 30 Å^2 (between 25 and 35 Å^2) indicated that this had only a marginal effect on the results below: the activation barrier remained unchanged and a_0 varied less than 0.5 Å^2.

The fitting procedure was first performed on the data of all seven systems, resulting in the fit shown in Figure 3. It is clear that the data and the free volume theory do not match particularly well in this case. Especially the data of the system with the largest area per molecule deviates from the description of the free area theory. This is expected since the system with the largest area indicated formation of membrane pores, which speed up diffusion compared to a defect-free monolayer. For this reason we also performed another fit leaving out the most expanded monolayer. Fits obtained in this manner were found to follow the diffusion data better (see Figure 4).

The parameters extracted from both fits (a_0, E_a) are given in Table 2. The area parameter is about 35 Å^2, and the activation

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Table 2. Parameters Obtained through Fitting to the Free Volume Theory

	all points		largest area left out	
lipid	a_0 (Å^2)	E_a (kJ/mol)	a_0 (Å^2)	E_a (kJ/mol)
DPPC	40.11	13.37	35.95	14.74
POPC	39.21	13.33	35.38	14.59
POPG	39.32	14.03	34.47	15.55
phospholipids	39.70	14.21	35.84	15.47

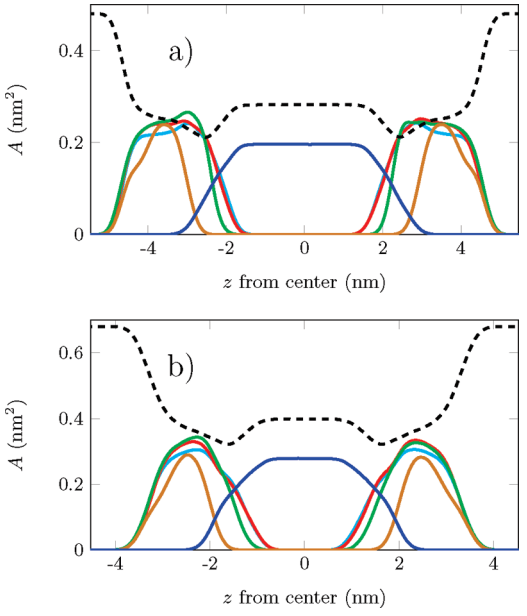

Figure 5. Close-packed cross-sectional area profiles for the lipids as functions of distance from the middle of the water phase: (a) $\langle A \rangle = 48$ Å^2 and (b) $\langle A \rangle = 68$ Å^2. Color codes are as follows: water (dark blue), POPC (red), DPPC (light blue), POPG (green), cholesterol (brown), free volume (dashed black). Note that here results are shown for the two different monolayers facing the water phase (see Figure 1). The minor differences in the almost symmetric profiles (with respect to $z = 0$) indicate that the errors are of the order of a few percent.

barrier is ~15 kJ/mol. There is only minor variation among the parameter values for the different lipid types.

B. Cross-Sectional Closed-Packed Area Profile of Lipids. The cross-sectional area of lipids in a membrane is conceptually difficult to define, unless one considers rigid molecules such as cholesterol, or very ordered phases such as the gel state. The difficulty arises from the highly flexible conformations of phospholipid acyl chains. The conformations of individual phospholipids change rapidly in time, and the distribution of possible conformations is very broad.

To get more insight into this issue, we determined the average close-packed cross-sectional area of the lipids using the technique discussed by Falck et al.[16] (see Models and Methods). The results in Figure 5 show that the cross-sectional area is not constant but varies rather strongly along membrane normal direction. The cross-sectional area is the largest close to the headgroup region, as the packing of lipids is the highest in this part of the membrane. As we need some operational definition for the close-packed area, we decide to consider the largest values of the area profiles in Figure 5 and use them as the close-packed cross-sectional area for the lipids discussed here.

Figure 5 shows that the close-packed cross-sectional area of cholesterol is about 0.25 Å^2 at $\langle A \rangle = 48$ Å^2 and about 0.30 Å^2 at

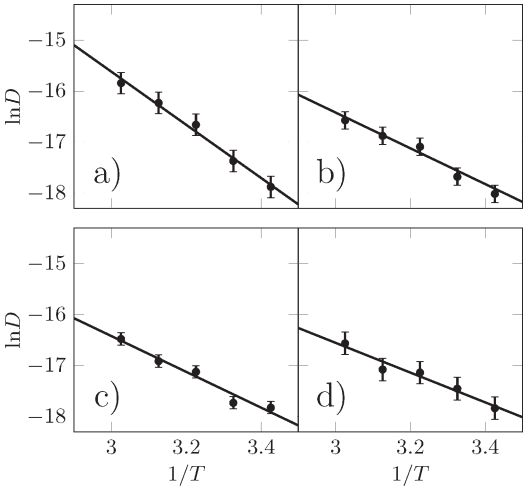

Figure 6. Arrhenius equation fits to the calculated diffusion data for the system with $A = 48$ Å^2/lipid. Inverse temperatures are given in units of 10^{-3} K^{-1}. (a) Cholesterol, (b) DPPC, (c) POPC, and (d) POPG.

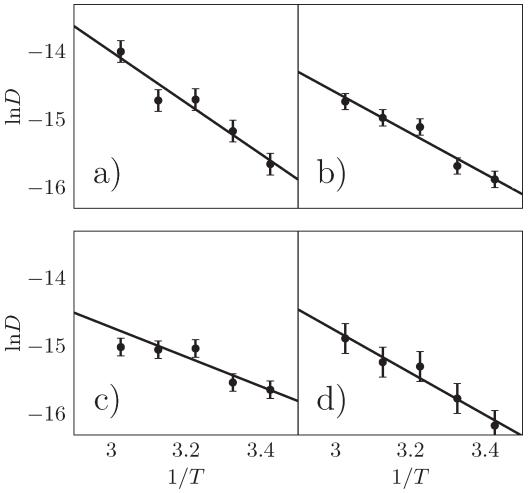

Figure 7. Arrhenius equation fits to the calculated diffusion data for the system with $A = 68$ Å^2/lipid. Inverse temperatures are given in units of 10^{-3} K^{-1}. (a) Cholesterol, (b) DPPC, (c) POPC, and (d) POPG.

$\langle A \rangle = 68$ Å^2. The figure of 0.25 Å^2 found in the highly compressed case is consistent with the steric profile measured by Rothman and Engelman, who found the cross-sectional area of cholesterol to have a plateau around 25 Å^2.[48] This confirms that the present analysis is valid, providing insight for the close-packed cross-sectional area profile. For other lipids, the cross-sectional areas are larger, as expected, and range between 0.25 and 0.28 Å^2 at $\langle A \rangle = 48$ Å^2 and between 0.32 and 0.35 Å^2 at $\langle A \rangle = 68$ Å^2. The close-packed cross-sectional areas are smallest for cholesterol and increase in the order of DPPC, POPC, and POPG.

C. Activation Barrier and Diffusion Mechanism. The interpretation of the activation barrier E_a in the free volume theory also calls for attention. To better understand its nature, we compared its value to the diffusion barrier determined from the Arrhenius analysis. The results in Figures 6 and 7 highlight that the Arrhenius form describes the temperature dependence of lateral diffusion well over the given temperature window.

(48) Rothman, J. E.; Engelman, D. M. *Nat. New Biol.* **1972**, *237*, 42–44.

Table 3. Arrhenius Barriers (kJ/mol) for the Two Different Systems with Area per Lipid of 48 and 68 Å^2

lipid	48 Å^2	68 Å^2
all	30.76	23.65
cholesterol	43.34	31.31
DPPC	30.66	24.95
POPC	29.18	18.04
POPG	24.29	25.79

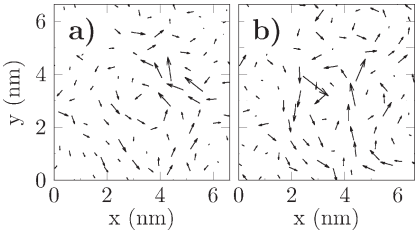

Figure 8. Diffusion patterns for a system with an area per molecule equal to 40 Å^2. The displacements are shown with respect to the same initial structure. The given positions are in units of nanometers. The displacement time intervals are (a) 20 ns and (b) 40 ns.

Table 3 shows that the Arrhenius barriers found through this analysis are about 30 kJ/mol at 48 Å^2 and 24 kJ/mol at 68 Å^2.

One of the underlying assumptions in the free volume theory for lipid diffusion is that lipids migrate in terms of "jumps" from one cage to another. We considered this aspect by following the motions of all the lipids in the studied systems. During these analyses, we found no indication for single-particle "jumps", where a lipid would have rapidly moved a distance comparable to its own size, while other lipids around it would have remained in their own cages.

A pictorial representation of lipid diffusion is given in Figure 8, showing in-plane lateral displacements for time intervals of 20 and 40 ns. The cooperative nature of lipid diffusion at this length scale is evident. Lipids diffuse in a concerted manner as loosely defined dynamic clusters. The motion gives rise to diffusion patterns which closely resemble those observed in fluid single-component and many-component lipid bilayers.[17,18]

V. Discussion

A. Diffusion Coefficients. The values of diffusion coefficients shown in Table 1 are in line with earlier simulations and experiments. Baoukina et al. have calculated the diffusion coefficients for a coarse-grained single-component (DPPC) monolayer in the liquid-expanded (LE) and liquid-condensed (LC) phases at 300 K.[30] They found values of $(2.4 \pm 0.1) \times 10^{-7}$ and $(3.3 \pm 0.1) \times 10^{-7}$ cm^2/s with areas per molecule of 57 and 63 Å^2, respectively. Our present results indicate diffusion coefficients about 2−3 times smaller. The discrepancy is minor and can be explained by the difference in the composition between the simulated systems. Indeed, the present systems contain cholesterol, which is known to slow down diffusion. In fluidlike two-component bilayers the slowing down has been observed to take place by a factor of about 2−3,[10,49] while in membranes whose composition is consistent with pulmonary surfactant, de la Serna et al. found cholesterol to slow down diffusion by about 10−15%.[50]

Comparison to Langmuir monolayer experiments is more difficult to carry out. Diffusion in Langmuir monolayers is often measured using lipid-linked probe particles whose sizes range from about 30 to 100 nm,[6] and they inevitably have some role to play in the dynamics of the tagged lipid. Another common concern is the collective drift of a monolayer, which complicates the analysis of diffusion. Forstner et al.[6] made a considerable effort to rule out many of such undesired effects in a study of lateral diffusion for a DMPC monolayer at room temperature. They found a diffusion coefficient of about 1×10^{-8} cm^2/s for a wide range of surface pressures from about 1 to 30 mN/m. This diffusion coefficient is considerably smaller than the ones we have observed. However, it is not clear why the diffusion coefficients found in ref 6 do not depend on the area per molecule, as one would expect. Gudmand et al. have very recently used fluorescence correlation spectroscopy to measure lateral diffusion in a DMPC monolayer at 295 K.[5] They found[5] $D \approx 4 \times 10^{-7}$ cm^2/s at 70 Å^2, about 2.5×10^{-7} cm^2/s at 60 Å^2, and 1.5×10^{-7} cm^2/s at 55 Å^2. These are roughly 2 times larger than our results, but considering the presence of cholesterol in our monolayer, the agreement seems very reasonable. Remarkably, lateral diffusion coefficients found in those experiments increase monotonously with increasing surface area (i.e., decreasing surface pressure), in line with our observations.

B. Free Volume Theory Fits Diffusion Data. The fits shown in Figures 3 and 4 indicate that the free volume theory can be used to describe the diffusion data, if one concentrates on the condensed phase at high surface pressure. Deviations from the theory are evident at the lowest surface pressure, which is characterized by formation of pores. Clearly the inherent assumptions in the free volume theory are broken in those conditions, as larger free volume regions emerge and facilitate diffusion. This topic was recently discussed by Falck et al. in the context of lipid bilayers.[15] Merkel et al. did lipid monolayer experiments and also concluded[51] that the free volume theory was not valid in the regime where the monolayer was in the gas phase characterized by considerable pores in the membrane. In a more recent study by Gudmand et al.,[5] the authors studied a DMPC Langmuir monolayer for areas per molecule from about 50 to 95 Å^2. They observed their data to fit the free volume theory well.

We notice that the formation of pores in monolayer simulations at relatively small surface areas is an artifact due to the unrealistic surface tension of a simulated water−vapor interface. At 25 °C the surface tension of water is ∼72 mN/m, while the TIP3P water model yields only about 48 mN/m (with simulation conditions as specified in the Models and Methods section). As a result, the energy cost of a water−vapor interface is underestimated by the model. This, in turn, allows the formation of pores in the lipid monolayer. Another consequence of this artifact is the underestimation of the total surface tension at the interfaces. Despite these limitations, the properties and the behavior of lipid monolayers in simulations are realistic as long as the simulated systems do not have water−vapor interfaces.

C. Cross-Sectional Area in Free Volume Theory. The cross-sectional area and activation energy obtained from our simulations (see Table 2) are very similar for all phosholipids in our system, so we focus here on DPPC as an example. The cross-sectional area a_0 is found to be 36.0 Å^2, and the activation energy turns out to be 14.7 kJ/mol. How realistic are these numbers, and how do they compare to the other measures we have determined by the other analysis techniques?

The value we obtained for DPPC's cross-sectional close-packed area parameter a_0 = 36.0 Å^2 is seemingly realistic, but are there means to validate it? According to the free volume theory, the critical area $a^* = a_0/\gamma$ should describe the smallest

(49) Filippov, A.; Oradd, G.; Lindblom, G. *Biophys. J.* **2003**, *84*, 3079–3086.
(50) de la Serna, J. B.; Oradd, G.; Bagatolli, L. A.; Simonsen, A. C.; Marsh, D.; Lindblom, G.; Perez-Gil, J. *Biophys. J.* **2009**, *97*, 1381–1389.
(51) Merkel, R.; Sackmann, E. *J. Phys. Chem.* **1994**, *98*, 4428–4442.

possible area that renders a diffusion jump possible. Given that values of γ range between 0.5 and 1,[9] the critical area a^* would be 36−72 Å^2. Intuitively, this value should be almost identical to the area per lipid in the gel phase, where lipids diffuse but do so very slowly due to the very small amount of free volume. For DPPC the area per lipid in the gel phase has been found to be 47.2 ± 0.5 and 47.9 Å^2,[52,53] suggesting that a^* and hence also a_0 are realistic.

More insight is given by the analysis we carried out using the technique discussed by Falck et al.[16] For DPPC, the average close-packed cross-sectional area is about 25 Å^2 in the highly compressed system ($\langle A \rangle$ = 48 Å^2) and about 32 Å^2 in the fluid monolayer ($\langle A \rangle$ = 68 Å^2). In the case of $\langle A \rangle$ = 68 Å^2, the results for DPPC are in good agreement with those in ref 16 for a DPPC−cholesterol bilayer with a small cholesterol concentration.

As the free volume theory assumes lipids to be like rods with a well-defined shape, and with a constant a_0 that does not depend on surface pressure, the logical choice is to compare the smallest close-packed cross-sectional area with a_0. The result in this highly compressed case, 25 Å^2, is somewhat smaller than the value given by the fit to the free volume theory, 36.0 Å^2. The difference between the two may be even larger, as the value we have used for the close-packed cross-sectional area is the maximum in the area profile (see Figure 5). Nonetheless, given the uncertainty in fitting, and the fact that the analysis is based on averaging over the specific shapes of individual lipids, this number is in reasonable agreement with the value of 36.0 Å^2 that results from the free volume theory fit.

For other phospholipids, the results and conclusions are essentially similar to those found for DPPC. The largest areas are found for POPG, which yields a closed-packed cross-sectional area of about 28 Å^2 at $\langle A \rangle$ = 48 Å^2 and about 35 Å^2 at $\langle A \rangle$ = 68 Å^2. The fit of the POPG diffusion data to the free volume theory yields a_0 = 34.5 Å^2.

The results indicate that the cross-sectional area a_0 obtained by fitting D (using the free volume theory) is approximately similar to the cross-sectional area calculated directly from the simulation data, although the agreement is not quantitative. The differences are about 5−40%. Given the uncertainties in the fitting procedure, the agreement is reasonable.

D. Activation Barrier in Free Volume Theory. How about the interpretation of the activation energy E_a? We compared the values of E_a obtained via the free volume theory (Table 2) with the Arrhenius diffusion barriers E_{Arrh} (Table 3), obtained by analyzing simulations at different temperatures. The Arrhenius description can generally be applied to different kinds of activated processes, including lateral diffusion in lipid membranes.[33,49,54−56]

We find that E_a depends only weakly on the type of lipid, the largest differences being about 7%. For Arrhenius barriers the differences between the lipids are more considerable, the largest ones being about 80%.

Let us first consider the Arrhenius barriers since comparison to experimental data is then easier to make. Overall for all the lipids, the results are consistent with typical Arrhenius diffusion barriers of about 27−31 kJ/mol measured by NMR in fluid single-component lipid bilayers in the same temperature range.[49] The figures we found for E_{Arrh} also seem reasonable: 18−31 kJ/mol correspond to about 7−13 k_BT at physiological temperature. For comparison, hydrogen bonds are typically about 5−8 k_BT, and as they are one of the most common interaction types in lipid−water systems, the Arrhenius barrier values seem appropriate.

The largest Arrhenius barrier is found for cholesterol, characterizing that its rate of change is the largest among the lipids considered for increasing T. The Arrhenius barriers further increase as the membrane becomes more packed. This is also consistent with experiments, as Filippov et al. found for cholesterol-rich PC membranes Arrhenius diffusion barriers of about 31−64 kJ/mol,[49] where the largest values were observed in densely packed cholesterol−sphingomyelin systems.

The Arrhenius barriers are higher (by a factor of about 2) than the activation barriers predicted by the free volume theory. In the context of lipid bilayers, Falck et al. also found substantial discrepancies between the Arrhenius diffusion barrier and the activation energy associated with the free volume theory.[15]

The Arrhenius barrier is an average over a distribution of instantaneous activation barriers. The barrier found via the Arrhenius analysis is expected to reflect some effective energy barrier related to the rate limiting step in the diffusion process. It is tempting to assume that the interpretation of E_a in the free volume theory is the same, but apparently this is not the case. Currently, we are unaware of other physical variables describing the thermally activated nature of diffusion that could provide an interpretation for E_a. The physical meaning of the activation energy in the free volume theory remains therefore unclear.

E. Diffusion Mechanism. Finally, let us discuss the diffusion mechanism. When the free volume theory is applied to describe diffusion in lipid bilayers and monolayers, it is rather commonly assumed that the diffusion of lipids takes place as "jumps",[33−35] where the particle moves a distance of its own size rapidly at thermal speed:[10] a lipid rattles for long times in its cage, where it is surrounded by other molecules, and only occasionally it undergoes a rapid jump over a distance of its own linear size in the membrane plane from one cage to another. In our analysis, we did not observe any of such jumps. Instead, the simulation results indicate the diffusion of lipids to take place through concerted lipid movements with tens of lipids moving in unison as loosely defined lipid clusters. These observations are largely similar to those found in lipid bilayers,[17,18,57] and recent quasi-elastic neutron scattering experiments for lipid bilayers are also consistent with this view.[19]

This observation enforces one to reconsider the interpretation and the role of a_0 in the diffusion process. Diffusion of lipids takes place in terms of transient lipid clusters where the cluster diffuses as a whole, and the diffusion of lipids cannot be described as rapid motion of single lipids in a slowly changing environment. Therefore, given that there are no true single-particle displacements that would require local free area pockets of the same size as a_0, the role of the single-particle close-packed cross-sectional area in the lateral diffusion process is unclear.

VI. Concluding Remarks

In this article, we have considered lateral diffusion of lipids in a protein-free model for the lung surfactant. In particular, we have studied the validity of the free volume theory for diffusion in Langmuir monolayers and the interpretation of its parameters.

The applicability of the free volume theory has been discussed previously for lipid bilayers[15,58] and to some extent also for

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(53) Tristram-Nagle, S.; Zhang, R.; Suter, R. M.; Worthington, C. R.; Sun, W. J.; Nagle, J. F. *Biophys. J.* **1993**, *64*, 1097–1109.
(54) Filippov, A.; Oradd, G.; Lindblom, G. *Langmuir* **2003**, *19*, 6397–6400.
(55) Cullis, P. R. *FEBS Lett.* **1976**, *70*, 223–228.
(56) Shin, Y.-K.; Freed, J. H. *Biophys. J.* **1989**, *55*, 537–550.
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(58) Almeida, P. F. F.; Vaz, W. L. C.; Thompson, T. E. *Biophys. J.* **2005**, *88*(6), 4434–4438.

experimental studies of monolayers.[5] With the present study we considered its validity for lipid monolayers in the liquid-condensed and liquid-expanded phases. We found that the free volume theory describes the diffusion data reasonably well, if one concentrates on the highly packed phases. Deviations from the theory are evident in the loosely packed systems characterized by membrane pores, which inevitably facilitate and speed up diffusion. This finding implies that the free volume theory is not particularly useful for describing diffusion in the gas phase. However, even in closely packed phases, there is reason to ask what can we learn from the free volume theory, assuming that it describes realistically the diffusion process. Our present results indicate that the parameters extracted from the fits of the diffusion data to the free volume theory are not easy to interpret.

The values we obtained for the critical area $a^* = a_0/\gamma$ are consistent with the area per lipid in the gel phase. However, this view has to be taken with caution, since the numerical correction factor γ accounting for possible overlap of free volume ranges from 0.5 to 1, and hence also a^* embraces a broad distribution of areas from 36 to 72 Å^2. A more direct comparison of a_0 with the "hard-core" cross-sectional area profile[16] showed that a_0 differs from the hard-core size of a lipid, the quantitative difference being about 5−40%. Given the uncertainty in fitting and analysis, this difference is acceptable. The cross-sectional area parameter a_0 thus may have potential for describing the close-packed size of a lipid.

The interpretation of the activation energy E_a in the free volume theory is more problematic. A careful comparison to the Arrhenius diffusion barrier showed that the activation barrier of the free volume theory and the Arrhenius diffusion barrier are not directly related. While the free volume theory yields $E_a \approx 15$ kJ/mol, the Arrhenius analysis gives $E_{Arrh} \approx 25$ kJ/mol for DPPC and POPG. Moreover, while the activation barriers E_a are almost identical for all the four lipid types we have considered, the Arrhenius diffusion barriers range from 18 to 43 kJ/mol. We conclude that the physical meaning of the activation energy in the free volume theory is unclear.

Another point that has been part of the free volume theory since its derivation for colloidal systems is the assumption of diffusion taking place through jumps. While this assumption of jumplike motions is typically not mentioned in the extensions of free volume theories for membranes, it is unavoidably part of them. Our results for lipid monolayers have shown that, instead of single-particle jumps from one cage to another, lipids diffuse in terms of concerted motions where numerous lipids move in concert as a dynamical cluster. Clearly, there is reason to consider revising the free volume theory to account for this collective mechanism.

Summarizing, the present results are in line with previous studies,[5,10,51] showing that the free volume theory can be parametrized to describe lateral diffusion data for lipids, at least approximately. The more difficult task is to interpret the parameters in a physically meaningful way. There is clearly work to be done to clarify the remaining issues and to develop novel theoretical descriptions that also account for the concerted nature of diffusion.

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