Comment to the Editor

Response to Comment by Almeida et al.: Free Area Theories for Lipid Bilayers—Predictive or Not?

ABSTRACT Free area theories for lateral diffusion in lipid bilayers are reviewed and discussed. It has been suggested by Almeida et al. that free area theories yield quantitative predictions for lateral diffusion coefficients of lipids. We investigate the plausibility of this suggestion by first sketching what is to be expected of a quantitative theory with predictive power, and subsequently examining whether existing free area theories comply with these expectations. Our conclusion is that current free area theories for lipid bilayers are not quantitative theories with predictive power. They involve a number of adjustable parameters, all of which are not estimated independently, but derived from fitting the theory to the very data whose behavior the theory is supposed to predict. Further, the interpretation and behavior of some of the parameters are ambiguous. The best example is the so-called activation barrier, whose qualitative behavior with the cholesterol concentration in a DMPC bilayer varies depending on the experimental method used to generate the input data and the exact assumptions made to formulate the theory. Independent determination of the activation barrier from numerical simulations or experiments appears to be very difficult.

INTRODUCTION

In a recent comment to our article (1), Almeida et al. (2) argue that free area models quantitatively describe lipid diffusion in bilayers. They attribute predictive power to such models, stating that ''the free area model actually predicts the correct result for lipid diffusion.''

Simple free area theories such as the one advocated by Almeida et al. (3,2) may be conceptually useful for understanding dynamic processes in lipid bilayers. However, in our opinion these models are not predictive. Concerns about the predictivity and validity of free volume theories to dynamic processes in bilayers have also been expressed by other authors (4–7).

In this reply we explain why free area models for bilayers cannot be considered predictive. We start by discussing what is to be anticipated of models termed quantitative or predictive. An overview of free area and volume theories follows. We subsequently focus on the free area theory by Almeida et al. (3) and explain why we do not deem this model predictive. Finally, we briefly discuss lateral diffusion in phospholipid/cholesterol systems.

WHAT ARE PREDICTIVE MODELS?

Models are tools for interpreting, understanding, and anticipating experimental results. These tools can be roughly divided into two major categories, which we will call generic and specific. Generic models, such as the Rouse and Zimm models for polymer dynamics (8), usually represent a large group of related systems or phenomena, giving us insight into qualitative experimental trends and the underlying mechanisms. They are not, however, expected to give accurate predictions for the actual numerical values extracted from experiments. Specific models, on the contrary, have been tailored to faithfully represent a given system and phenomenon, e.g., the diffusion of dialkyl-adipate plasticizers in poly(vinyl chloride) (9). These models are often intended to be quantitative, i.e., they should predict experimental values with a reasonable accuracy.

Quantitative models with predictive power may or may not contain adjustable parameters. If such parameters are included in the model, the way in which their values are chosen determines whether the model is quantitative and predictive. Let us, e.g., consider a model that describes the behavior of the lateral diffusion coefficient of a lipid in terms of temperature. Further, let us assume that our model contains two or three adjustable parameters.

If the parameter values are derived from, say, numerical calculations and/or independent experiments that do not measure the actual quantity whose behavior we wish to model (the lateral diffusion coefficient as a function of temperature), the theory may have predictive value. Such will not be the case if we perform an experiment on the same system and in the same conditions we wish to model, and then fit the experimental data to our theory. We will certainly obtain values for our parameters, some of which, given that the parameters have well-defined physical interpretations, may even be reasonable. Not surprisingly, a theory, however implausible, with such parameters, will reproduce the original experimental findings—at least if there are enough parameters. Such a model, however, does not give any actual predictions for the behavior of the lateral diffusion coefficient as a function of temperature; even less so if some of the parameters do not have a clear, unique, physical meaning.

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BRIEF OVERVIEW OF FREE VOLUME THEORIES

Free volume theories for transport in soft condensed matter emerged some 50 years ago. The free volume model by Cohen and Turnbull (10) relates the tracer (or self) diffusion coefficient D_T of a system of hard spheres to the average free volume per particle v_f. The amount of free volume in a system could, e.g., change as a function of temperature or pressure (10). The foundation of the model is that molecular diffusion proceeds by jumps: a diffusive jump takes place when a free volume greater than a critical volume v^*, formed by redistribution of free volume, opens up next to the diffusing molecule. It is assumed that free volume redistribution is instantaneous and requires no local free energy. Further, the length of a jump is close to the molecular diameter. The tracer diffusion coefficient as a function of free volume can then be expressed as

$$D_T = gd^* u \exp(-\gamma v^*/v_f), \quad (1)$$

where g is a geometrical factor, d^* close to the particle diameter, u the gas kinetic velocity, and γ a parameter introduced to correct for overlap of free volume. This model has been used to describe transport in liquids and glasses, as well as to characterize the glass transition (10,11). In addition to hard sphere fluids, the model has also been applied to describe transport in simple van der Waals fluids and metallic liquids (10).

Since the late 1950s several refinements to this free volume theory have been suggested. Among the first ones was taking into account that the diffusive displacement d would vary with the size of the free volume v available to the diffusant (12), $d = \alpha v$, altering the form of Eq. 1 to

$$D_T = (u\alpha/3)(v^* + v_f/\gamma)\exp(-\gamma v^*/v_f). \quad (2)$$

Another early idea was to assume that in order for the diffusive jump to take place, the diffusant would need enough energy to escape from the influence of its neighbors (13,14). This assumption led to the so-called Macedo-Litovitz hybrid equation, which essentially is Eq. 1 combined with a Boltzmann factor:

$$D_T = D_0 \exp(-\gamma v^*/v_f - E_a/k_B T), \quad (3)$$

where E_a is the so-called activation energy, k_B the Boltzmann constant, T the temperature, and D_0 varies slowly with temperature. The actual need for including an activation energy in the model has been debated (6). The various free volume models for (mainly) simple liquids have been recently reviewed by Liu et al. (15).

Some of the more recent free volume theories have been tailored for more complex systems and phenomena, e.g., molecular diffusion in homogeneous polymer systems. Modified approaches to transport in polymers have been put forward by Vrentas et al. (16–22) and Storey et al. (9,23–26). The diffusing molecules and their surroundings need no longer consist of small spherical entities: large and complex molecular shapes as well as partial displacements, i.e., displacements that are but a fraction of the size of the diffusant have been considered (18,23,25, 26). Although Storey et al. in particular make an encouraging attempt to obtain independent estimates for their parameters, neither the model of Vrentas et al. nor that of Storey et al. is fully independent of data for tracer diffusion. These models, however, need little such input data, and appear to be in decent agreement with experimental (9) and numerical (27) results. They could be termed semipredictive or semiquantitative.

In addition to the other modifications—the activation energy and the more complex diffusing entities—the effect of the dynamics of the surrounding solvent on a diffusing molecule has been discussed (6,7). Assuming that free volume redistribution is instantaneous implies neglecting the memory effects for solvent relaxation over the timescale for the translational motion of the diffusant. This has a profound effect on the qualitative, not to mention the quantitative, behavior of the tracer diffusion coefficient (5,6). For instance, in the case of diffusion of small solutes in lipid bilayers, characteristic timescales for solvent motions, i.e., overall rotational and local isomerization motions of lipid molecules, may well be of the same order of magnitude as those for the translational motions of the solute.

FREE AREA THEORIES

The first attempt to describe lateral diffusion in lipid bilayers using a free area model was by Galla et al. (28). They assumed that lipids are hard rods with well-defined cross-sectional areas, arriving at a model which is a two-dimensional version of the original free volume theory of Cohen and Turnbull (10; see also Eq. 1, this article). MacCarthy and Kozak (29) discussed a similar approach, except that they suggested a reduction of the number of parameters by assuming that $\gamma a^* = a_0$, where a^* is a critical area corresponding to v^* and a_0 is the van der Waals or close-packed area of a lipid. This is likely to be in the correct ballpark (see, e.g., Ref. 30). However, MacCarthy and Kozak did not show that their assumption is generally, quantitatively accurate. Vaz et al. (31) returned to the model of Galla et al., but added a novel feature: the viscous drag-forces a diffusing lipid is likely to experience from the opposing monolayer and the aqueous phase.

The free area theory by Almeida et al. (3) is a blend of the free area theory of MacCarthy and Kozak (29) and the Macedo-Litovitz hybrid equation (see Eq. 3). This model should be applicable for lipid bilayers of any composition, as long as the bilayer is in a single well-defined phase. In the spirit of MacCarthy and Kozak (29), Almeida et al. assumed that $\gamma a^* =$

a_0, arriving at an expression for the lateral diffusion coefficient (in units of cm^2/s) as a function of temperature,

$$D_T = 3.224 \times 10^{-5} \times \sqrt{Ta(T)/M}\,\exp[-a_0/a_f(T) - E_a/k_BT], \quad (4)$$

where M is the molar mass of the diffusant (in g/mol), a is the average area per lipid (in $Å^2$), and a_f is the free area per phospholipid.

Using fluorescence recovery after photobleaching, Almeida et al. (3) measured diffusion coefficients as functions of temperature for phospholipid probes in DMPC/cholesterol bilayers above the main phase transition temperature with different cholesterol concentrations χ from 0 to 50 mol %. The authors developed empirical expressions for $a(T)$, separately for $\chi = 0\%$, $\chi = 30\%$, $\chi = 40\%$, and $\chi = 50\%$, which all represent bilayers in a single liquid-disordered or liquid-ordered phase. The free area per phospholipid was calculated by dividing the total free area, i.e., the total area of the bilayer minus the combined close-packed areas of all phospholipid and cholesterol molecules, by the number of *phospholipid* molecules in a monolayer. In other words, no free area was assigned to cholesterol. This is a somewhat arbitrary procedure for a binary mixture where both components are of similar sizes and move at the same characteristic timescale (see below).

The above scheme leaves three adjustable parameters, E_a, a_0, and a_0^{cho}, where a_0 and a_0^{cho} are the close-packed areas of DMPC and cholesterol molecules. Almeida et al. fixed a_0 to 45 $Å^2$ and extracted a_0^{cho} and E_a by fitting Eq. 4 to their (whole) diffusion data. A value of $a_0^{cho} \approx 26.6$ $Å^2$ was said to give the best overall results. Finally, they found $E_a \approx \{2.7, 1.9, 2.1, 2.5\}$ kcal/mol for $\chi = \{0, 30, 40, 50\}\%$, respectively. According to Almeida et al. (2), the minimum of E_a at $\chi = 30\%$ could be an important property of the DMPC/cholesterol system. This choice of parameters yielded good agreement with the original experimental data.

FITTING LATERAL DIFFUSION DATA TO FREE AREA THEORY

Before starting to examine the validity of the assumptions behind Eq. 4, we tested the robustness of the fitting procedure of Almeida et al., examined the behavior of E_a, and investigated the general applicability of Eq. 4. To do so we first extracted Almeida's data from Ref. 3. A simultaneous three-parameter fit to the data with finite χ yielded unphysical values for a_0, E_a, and a_0^{cho}. Hence, we decided to fix one of the parameters to a plausible value. Instead of setting $a_0 = 45$ $Å^2$, we fixed a_0^{cho}. This choice was motivated by our expectation that a_0 may vary with cholesterol concentration. In general one would not expect van der Waals volumes (areas) in a genuinely three-dimensional (two-dimensional) system to vary with either temperature or cholesterol concentration. As bilayers are not truly two-dimensional, a close-packed or van der Waals area of a molecule is somewhat ill-defined (see below), and may change with its tilt or the amount of *gauche* defects in its acyl chains. This is far more likely to happen to phospholipids than cholesterols, since the steroid ring structure of the cholesterol is very compact, and oriented, irrespective of χ, nearly in the direction of the bilayer normal.

Apart from fixing a_0^{cho} instead of a_0, we closely followed the fitting procedure of Almeida et al., ignoring the data for $\chi = 30\%$ at $T > 38°C$. The best match to the experimental data was obtained with $a_0^{cho} \approx 27$ $Å^2$, and our fits are shown in Fig. 1. Our values for a_0 and E_a with $\chi = \{0, 30, 40, 50\}\%$ were $E_a \approx \{2.7, 1.9, 2.1, 2.3\}$ kcal/mol, in respective order, and $a_0 \approx 45$ $Å^2$ for all values of χ. Concluding, a fit of Almeida's data to Eq. 4 is robust, and the match to the experimental data is good.

One should, however, not put too much weight on the exact parameter values. Assuming, in contrast to Ref. 3, that the free area is divided equally between DMPCs and cholesterols and modifying Eq. 4 accordingly—this is no less plausible than assigning all free area to DMPCs—yields $E_a \approx \{2.7, 1.2, 1.0, 1.0\}$ kcal/mol and $a_0 \approx \{45, 42, 41, 40\}$ $Å^2$ with $\chi = \{0, 30, 40, 50\}\%$, respectively. The match to the experimental data is, again, good. Note that E_a now appears to decrease with χ.

We also tried to fit Eq. 4 to the DMPC/cholesterol data of Filippov et al. from Table 1 in Ref. 32. These data were obtained using pulsed-field gradient ^1H NMR and are for $\chi = 0\%$ and $\chi = 33\%$, which is close enough to $\chi = 30\%$ for us to use the expression for $a(T)$ derived for $\chi = 30\%$ by Almeida et al. The data for $\chi = 33\%$ are very much like the data by Almeida et al. for $\chi = 30\%$, whereas the two sets of data for pure DMPC differ from each other. Reasons for the discrepancy are discussed by Filippov et al. (33), possible explanations being the different timescales probed by fluorescence recovery after photobleaching and pulsed-field gradient ^1H NMR, as well as differences in the water content. Our fit to the data of Filippov et al. is shown in Fig. 2. The match is excellent, with $E_a \approx \{1.9, 2.0\}$ kcal/mol and $a_0 \approx \{47, 44\}$ $Å^2$ for $\chi = \{0, 33\}\%$, respectively. In contrast to the behavior of

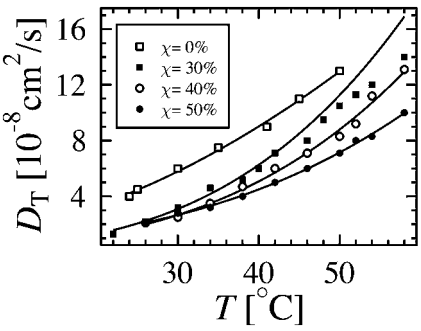

FIGURE 1 Lateral diffusion coefficients as functions of temperature in DMPC/cholesterol bilayer systems with different cholesterol concentrations χ. The circles and squares represent experimental data extracted from Ref. 3, whereas the solid lines are our fits to the data (see text).

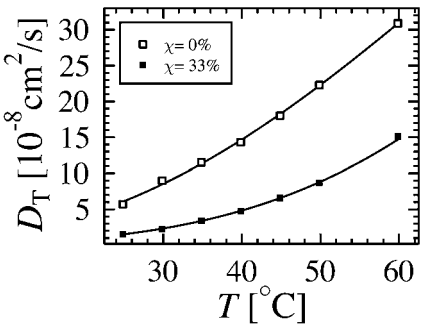

FIGURE 2 Lateral diffusion coefficients as functions of temperature in DMPC/cholesterol bilayer systems with different cholesterol concentrations χ. The squares represent experimental data extracted from Ref. 32, whereas the solid lines are our fits to the data (see text).

the activation barrier obtained from the data of Almeida et al. (see above), there is no minimum for E_a at $\chi \approx 30\%$.

Based on the evidence so far, we are rather skeptical that the behavior of E_a should be taken very seriously, also because the actual interpretation of the parameter is unclear (see below).

Thus far, by adjusting the parameter values in Eq. 4, we have achieved a good match with experimental data. However, situations where the match is poorer also exist. As an example we considered data for pyrene-labeled PCs in pure DMPC monolayers from Merkel et al. (30). The data are diffusion coefficients as functions of area per lipid measured at $T = 20$°C, and have been obtained using a kinetic excimer probe technique.

We are aware that the free area theory of Almeida et al. (3) is for lateral diffusion in *bilayers*. However, MacCarthy and Kozak (29), whose free area theory is closely related to that of Almeida et al., use theirs to examine lateral diffusion in both bilayers and monolayers. Indeed, assuming that free area theories are valid descriptions of diffusion in bilayers, one might expect them to be applicable to monolayers in the expanded and condensed (34) phases. In these phases lateral diffusion of a lipid could proceed as in bilayers, i.e., by jumps between cages formed by other lipids. In the gaseous phase (34), i.e., when lipids are far enough apart that they exert little force on another, this will not necessarily be the case, and we would therefore not expect the free area theory to be applicable.

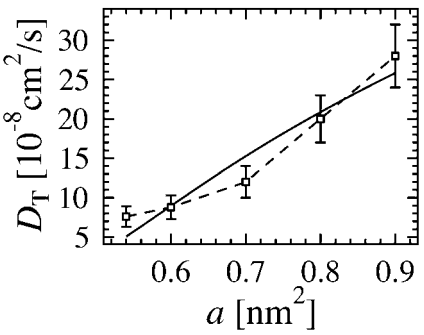

FIGURE 3 Lateral diffusion coefficients as functions of area per lipid in DMPC monolayer at $T = 20$°C. The squares represent experimental data extracted from Ref. 30, whereas the solid lines are our fits to the data (see text). The dashed line is to guide the eye.

Our fit to the data of Merkel et al., using Eq. 4, is shown in Fig. 3. The two measurements corresponding to the highest areas per lipid (see Ref. 30) have been omitted from our fit and are not shown in Fig. 3. This is because the monolayer is probably in the gaseous phase. As for fitting, irrespective of the values of E_a and a_0, the experimental trend cannot be reproduced in a completely satisfactory manner.

CHALLENGES FOR FREE VOLUME THEORIES FOR LIPID DIFFUSION

The above examples cast doubt on the quantitative significance of simple free area theories and suggest that the behavior of parameter values derived from fitting Eq. 4 to experimental data may or may not have a physical interpretation. As explained above, one might instead obtain independent estimates for the model parameters and subsequently compare the predictions of a free area theory with experimental data for tracer diffusion. We believe this is not easy. Indeed, it may be unfeasible, since the physical interpretation of parameters such as E_a is ambiguous (see the discussion below).

Free volume is likely to play a role in dynamic processes in lipid bi- and monolayers (5,35–39). Understanding and quantifying the role of free volume would be useful. However, the relation between a given transport coefficient and the distribution of free volume is not obvious (1,4,5), and formulating a free volume theory for any kind of transport may be difficult. In the following we will highlight the limitations of the current free area theories for lateral diffusion in bilayers.

The main assumptions of the free area model of Almeida et al. (3) are as follows:

1. A lipid is a hard rod with a well-defined close-packed area a_0, which is independent of, e.g., temperature or cholesterol concentration.
2. Diffusion proceeds by jumps where a whole lipid moves a distance close to its own diameter in a short interval of time.
3. A lipid may jump, given a patch of free area larger than a_0 next to it.
4. Free area distribution occurs at a much faster timescale than the translational motion of lipids and does 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 is described by an activation energy E_a. The activation energy also incorporates the interactions with the aqueous phase and the opposing monolayer.

These assumptions imply that bilayers are regarded as homogeneous in the direction of the bilayer normal, and therefore effectively two-dimensional, whereas, in fact, they

are quite heterogeneous (1,5,40). The average close-packed area of a phospholipid and the average free area per lipid vary significantly with the distance z from the bilayer center (1,4,5,41,42). Further, the close-packed and free areas as functions of z, i.e., $a_0(z)$ and $a_f(z)$, change with cholesterol concentration (1) and are likely to change with temperature as well. To understand why this is, we should recall that lipids may be tilted with respect to the bilayer normal, their acyl chains may contain *gauche* defects, and their headgroups may assume various orientations, etc. As these properties vary with temperature and cholesterol concentration (43,44), and as they should certainly influence the average cross-sectional area at a certain distance from the bilayer center, it is plausible that $a_0(z)$ and $a_f(z)$ vary with T and χ. Concluding, neither the average close-packed cross-sectional area of a lipid nor the free area per lipid is well-defined or constant, not even in the average sense.

Another problem with the definition of a_f arises in mixtures: how should the total free area (or volume) be divided between the molecular species constituting the mixture? Almeida et al. (3) distribute the free area among DMPCs only (see above), whereas Vrentas and Duda (18) assign an equal amount of free volume to each monomeric segment, irrespective of the species the segments belong to.

The activation energy E_a appears to be the third potential problem. Exactly what does it describe? In particular, what is its relation to the effective apparent activation barrier E_{app} extracted from an Arrhenius description? Further, could it be estimated directly from computations or experiments?

The Arrhenius description is universally applied for different kinds of activated processes, e.g., lateral diffusion in bilayers (28,32,33,45,46). Here the behavior of the lateral diffusion coefficient is expected to depend on temperature as $D_T \sim \exp(-E_{app}/k_BT)$, where E_{app} is an (effective) apparent activation barrier. The interpretation given to E_{app} is very similar to the interpretation of E_a in Almeida et al. (3). Filippov et al. (32) extracted Arrhenius barriers for DMPC/cholesterol systems above the main phase transition temperature T_m, finding that $E_{app} \approx \{7, 12\}$ kcal/mol for $\chi = \{0, 33\}\%$, respectively (see also Ref. 33). In the one-phase regions and sufficiently far from T_m, $\ln D_T$ indeed appears to be linearly proportional to $1/T$, as expected of a process of Arrhenius type. For comparison, our analysis based on Eq. 4 (see above), using the data of Filippov et al., resulted in $E_a \approx \{1.9, 2.0\}$ kcal/mol for $\chi = \{0, 33\}\%$, in respective order. It seems evident that the Arrhenius barrier E_{app} is not related to E_a in any simple fashion.

It also seems unlikely that an activation barrier could be extracted from the intermolecular interactions by studying an ensemble of bilayers. A related problem in adatom diffusion on metal surfaces has been addressed by Vattulainen et al. (47). The local configuration around a lipid fluctuates strongly, as does indeed the conformation of the lipid itself (see below). One would therefore also expect the instantaneous activation barrier for an individual jump to fluctuate. Hence, the effective activation barrier must be a complicated average over a complex and broad distribution of instantaneous activation barriers. Further, as typical lipid conformations change with T and χ, one should expect the interaction of a lipid with its surroundings to change, altering the distribution of instantaneous activation barriers.

As we continue to venture beyond the mean-field level, the situation becomes more and more complicated. An individual phospholipid has many more degrees of freedom than the two taken into account in free area theories, and it may therefore assume a number of conformations (see, e.g., Ref. 48). These complex shapes have not been addressed in free area theories. Does the whole lipid always move as a single unit or could its motion proceed segment by segment? Are jumps smaller than the size of the lipid possible? What happens in the surrounding medium during a jump, e.g., how does the local three-dimensional distribution of free volume (5,42,49) affect the process? Is a two-dimensional description adequate in the first place? Should one pay attention to the dynamics and timescales of free volume redistribution? Indeed, does the lateral diffusion in bilayers actually proceed by jumps, and what actually constitutes a jump?

The last question is very relevant, since the free area model is based on the assumption that lateral diffusion proceeds by jumps. Large-scale, detailed studies of mechanisms of lateral diffusion in bilayers do not yet exist, but computational studies by Essmann and Berkowitz (50) and Moore et al. (51) suggest that jumps do not necessarily completely dominate lateral diffusion in bilayers. Diffusion could be reminiscent of that of an ideal fluid on a two-dimensional surface (50) or a combination of jumps and liquidlike two-dimensional diffusion (51). More studies into diffusion mechanisms in bilayers are clearly warranted.

LATERAL DIFFUSION IN PC/CHOLESTEROL BILAYERS

Although free area theories may be useful for understanding lateral diffusion in bilayers at a conceptual level, they do not seem to be feasible for quantitative predictions. What about classical atomic-level molecular dynamics (MD) simulations? Our MD data suggest that the lateral tracer diffusion coefficient of DPPC in DPPC/cholesterol bilayers well above the main transition decreases by approximately an order of magnitude upon increasing the cholesterol concentration from 0 to 30% (1). The decrease of D_T with χ is monotonous.

Experimental studies (3,32,33,45,52–54) of PC/cholesterol systems above the main transition indicate that the reduction should be more modest, between a factor of 2 and 4. Some results point at monotonous behavior (32,33,45, 53,54), others at a plateau in the liquid-disordered phase (3,52,54). Results for, e.g., DMPC/cholesterol vary depending on the experimental technique used to measure lateral diffusion coefficients (3,33,54). Recent studies of lateral

diffusion in ternary systems have shown similar differences (55,56). A probable and well-known (32,57) reason for these apparent discrepancies is that different techniques measure diffusion at different time- and lengthscales. Analogously, as pointed out by Almeida et al. (2), the comparatively short timescales currently within reach of MD simulations may be largely responsible for the difficulties in comparing the results of MD simulations with those obtained by macroscopic experimental techniques. Another possibility is that the system sizes within reach of current MD simulations could complicate the comparison.

Force fields for MD simulations are under constant development (see, e.g., Ref. 58 for a recent review). Current force fields, although semi-empirical, are already fairly generic and transferable. In addition, they produce results that are in satisfactory agreement with each other and experimental data. It is fair to say that MD simulations provide valuable insight into trends and mechanisms in complex biological systems. As for being quantitative tools with predictive power, they are not bad, and are constantly improving.

CONCLUDING REMARKS

In this reply we have argued that current free area models for lateral diffusion of lipids in bilayers cannot be considered quantitative theories with predictive power. Free area models (see, e.g., Eq. 4) involve adjustable parameters whose interpretation is ambiguous. Finding independent, accurate estimates for these parameters is difficult or beyond the scope of current numerical and experimental methods. Notably, parameter values obtained by fitting lateral diffusion coefficients as functions of temperature to Eq. 4 cannot be considered such independent estimates: it is to be expected that adjusting the parameter values such that they conform to a given input data will, when inserted in Eq. 4, yield something very close to the original input. This does not mean the model is predictive.

We have also discussed the basic premises of current free area theories. Most important, free area theories include the assumption that diffusion proceeds by jumps, where a whole lipid rapidly moves from a cage formed by its neighbors to an nearby, empty molecular cage, i.e., free area. The length of a jump is always comparable to the diameter of the lipid, and the dynamics of free area redistribution is considered instantaneous. Free area theories are strictly two-dimensional mean-field descriptions that ignore the three-dimensional conformations and internal degrees of freedom of lipids. Nor do they take into account the complex distribution of free volume. It is, in our opinion, possible that some of these assumptions and approximations are rather too strong. Removing or relieving them is expected to have an impact on the final form of the theory.

Finally, we are not of the opinion that descriptions that relate dynamic processes, packing of lipids, and free volume would be either impossible to achieve or useless. First of all, free volume theories already offer us a feasible qualitative model for dynamic processes in bilayers. This is a major achievement as such. Further, existing free volume theories might be a good starting point for further model development.

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