Lipid Bilayers, NMR Relaxation, and Computer Simulations

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Received September 17, 2001

ABSTRACT

Brownian and molecular dynamics simulations of a lipid bilayer are described, and the calculated frequency-dependent ^{13}C NMR T_1 relaxation times are compared with experiment. A consistent model emerges. Through fast internal motions, individual lipids average themselves into relatively cylindrical shapes on the 100 ps time scale and "wobble" in a cone-like potential on the nanosecond time scale. These motions take place in a highly fluid environment, much like a liquid alkane. Lateral diffusion of the lipids is on a significantly longer time scale because of restrictions at the bilayer/water interface, not because the interior of the bilayer is highly viscous.

Introduction

It is now possible using molecular dynamics (MD) to simulate pure lipid bilayers on the 10−100 ns time scale. These bilayers will typically contain approximately 100 lipids, described to at least the level of individual heavy atoms, and hydrating water (about 30 waters/lipid). A snapshot from one such simulation is shown in Figure 1. Based on the accuracy of such simulations, studies of bilayers with imbedded proteins and complex mixtures of lipids have begun in earnest. Given that in the early 1990s a 170 ps simulation of a 72 lipid/water system required *6 months* of computer time,[1] and that in the early 1980s only much simpler models[2] could be simulated with MD, the progress is most gratifying.

Many different groups have made significant contributions to the present state of affairs.[3−6] This Account details work which began around 1982 as one of us, R.W.P., was completing his thesis research in the group of Martin Karplus. The initial simulations were *not* MD. Rather, Brownian dynamics (BD) simulations were used to develop a relatively simple model for chain dynamics to help resolve a controversy involving the NMR relaxation of the chains. Later we used this model to generate a starting configuration for an MD simulation and have recently carried out a much longer MD simulation partly to test the validity of our original assumptions. In each step, we modeled bilayers consisting of the simple lipid dipalmitoyl phosphatidylcholine (DPPC). As illustrated in Figure 2, DPPC consists of two acyl chains and a zwitterionic headgroup. DPPC is plentiful in biological membranes, forms stable bilayers when pure, and has been well-studied experimentally. Consequently, good data have been available for refining parameters and testing models. We begin with the original problem in NMR relaxation, the motivation for a computer simulation, and the thinking that went into choosing the particular computer model. Then we discuss three different simulations: Brownian dynamics of a single chain in a mean field (simulation I);[7,8] a 170 ps MD of 72 fully hydrated lipids (simulation II);[1] a new 20 ns MD of the preceding system (simulation III, and the source of Figure 1). We conclude with a discussion of some interesting research directions.

NMR Relaxation and Order Parameters

In 1983 Brown and co-workers published the results of meticulous measurements of the ^{13}C NMR T_1 relaxation for carbons in the acyl chains of DPPC bilayers.[9] Upon analyzing data taken at seven different magnetic field strengths, they developed a new model of chain relaxation. In this model the ^{13}C relaxation time for a carbon j is given by

$$(1/NT_1)_j = B_j\,\omega^{-1/2} + \tau_j \qquad (1)$$

where ω is the Larmor frequency, N is the number of protons bound to the carbon, and τ_j is a frequency independent component of the relaxation. Frequency independent terms arise when $\tau\omega^2 \ll 1$, so for the spectrometer frequencies in the range of 60−500 MHz used to fit eq 1, τ must be less than several hundred picoseconds. Gauche/trans isomerization of the chain dihedral angles lead to relaxation times on the tens of picoseconds time scale in liquid alkanes,[10] and Brown and co-workers proposed that these motions were responsible for the fast relaxation times. Frequency dependence implies that some motions are at least on the nanosecond time scale; i.e., $(\tau\omega)^2 \approx 1$. Frequency dependence is common for proteins[11] and had been expected to arise for lipids in a bilayer due to a restricted rotation (or

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10.1021/ar0100529 CCC: $22.00
Published on Web 04/09/2002

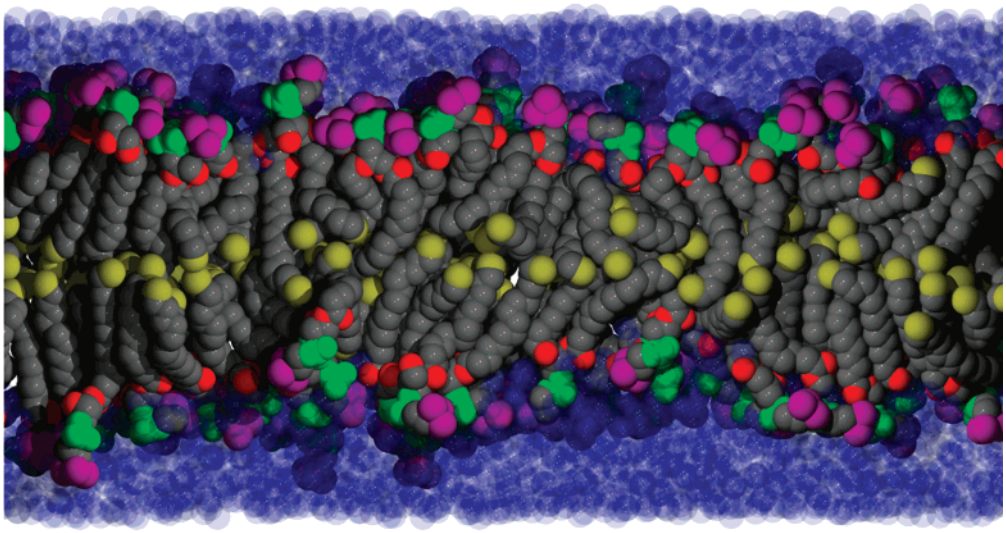

FIGURE 1. Slab from the 20 ns trajectory frame from simulation III. Atoms and atom groups are colored as follows: yellow, chain terminal methyl; gray, chain methylene and glycerol carbons; red, carbonyl and ester oxygen; green, phosphate; pink, choline; transparent blue, water oxygen. To increase the number of different lipids in the figure while maintaining clarity, a diagonal was taken across three periodic cells and any lipid chain within a predetermined width was included along with its headgroup; sufficient waters were included to duplicate the water/methylene density in the simulation.

pendulum-like motion) of the lipid long axis,[12] later formulated as "wobbling in a cone".[13] However, the $\omega^{-1/2}$ dependence explicitly specified in eq 1 is characteristic of director fluctuations of nematic liquid crystals,[14] and Brown argued that long-range collective motions such as splay and twist of the entire bilayer were responsible for the observed frequency dependent relaxation. This was controversial because the time scale of the collective motions was expected to be significantly slower, bilayers are smectic liquid crystals, where the frequency dependence of collective motions is ω^{-1}, and the model appeared to discount the contribution of single molecule motions (including wobble) to the relaxation.

A related problem involved the deuterium order parameter, S_{CD}. In bilayers, this quantity is obtained from NMR of deuterated lipids[15] and is given by the average

$$S_{CD} = \frac{1}{2}\langle 3\cos^2\beta - 1\rangle \qquad (2)$$

where β is the angle of the CD vector with respect to the bilayer normal. When the CD vector is uniformly distributed, $\langle\cos^2\beta\rangle = 1/3$, and $S_{CD} = 0$; when the chains are all-trans and there is no wobble, $|S_{CD}| = 0.5$. Experimentally, $|S_{CD}| \approx 0.2$ for the top and middle parts of the chains of DPPC,[15] consistent with the partial order. The order parameter contains contributions from motions that change β and are averaged out on the deuterium NMR time scale, about 10^{-5} s. When these motions are independent, the observed order parameter can be approximated as the product of order parameters for each. This means that S_{CD} can be interpreted in many different ways. Alternatively stated, dramatically different distributions of chain conformations can lead to the same values of S_{CD}.

In principle, computer simulation is the ideal tool for resolving the preceding uncertainties. Deuterium order parameters can be calculated as a time average over a

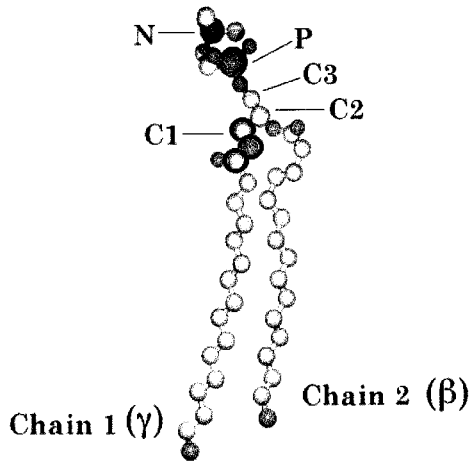

FIGURE 2. Heavy atoms of dipalmitoyl phosphatidylcholine (DPPC), with carbon and heteroatoms in light and dark gray, respectively, and key atoms and chains labeled. The model used for simulation I included the 16 carbons of chain 1 and C1 and O1 of the glycerol group, with the top three atoms in the system (highlighted by rings) fixed.

trajectory from eq 2 and their individual components approximately resolved into components. The ^{13}C T_1 relaxation is also straightforward to calculate from a dynamics trajectory. Assuming that relaxation is due to dipolar interactions between the ^{13}C nucleus and its attached protons,[13]

$$\frac{1}{NT_1} = \frac{1}{10}\left(\frac{\hbar\gamma_C\gamma_H}{r_{C-H}^3}\right)^2 [J(\omega_H - \omega_C) + 3J(\omega_C) + 6J(\omega_H + \omega_C)] \qquad (3)$$

where $\hbar$ is Plank's constant divided by 2π, r_{C-H} is the effective C−H bond length, γ_H, γ_C, ω_H, and ω_C are the gyromagnetic ratios and Larmor frequencies, respectively, of the ^{13}C and ^{1}H nuclei; $\omega_C = \gamma_C H$ and $\omega_H = \gamma_H H$, where

H is the field strength. $C_2(t)$ is the second rank reorientational correlation function and $J(\omega)$ its spectral density

$$C_2(t) = \langle P_2(\hat{\mu}(0)\cdot\hat{\mu}(t))\rangle \tag{4}$$

$$J(\omega) = \int_0^\infty C_2(t)\cos(\omega t)\,dt \tag{5}$$

where P_2 is the second-order Legendre polynomial and $\hat{\mu}(t)$ is the unit vector along the C−H bond direction at time t. While the preceding equations might appear formidable at first glance, calculation of the appropriate correlation functions requires only a small fraction of the time required to generate the trajectory, and the remaining effort mostly involves keeping units straight. Hence, all one needs to do is carry out a sufficiently long trajectory of a sufficiently large and sufficiently detailed DPPC bilayer, calculate the T_1 relaxation times, and compare with experiment. If the results agree, the details of the trajectory could then be analyzed to reveal the origin of the motions responsible for the frequency dependence.

Planning the Simulation

One must ask what the phrase "a sufficiently long trajectory of a sufficiently large and sufficiently detailed DPPC bilayer" means. This is an important exercise to carry out before starting any MD simulation, and the answer dictates the strategy.

We begin with "sufficiently detailed" without concern, at first, to practicality. The NMR relaxation data were from carbons of the acyl chains of the lipids and included contributions from motions most likely associated with torsional motions. Consequently, a model with detail to at least the level of individual carbons is required. This decision to include individual carbons can lead to very large systems with many particles. Referring to Figure 2, DPPC has 50 heavy atoms and 80 hydrogens. The 30 hydrating waters add another 90 atoms, for a total of 220 atoms/lipid (including hydrogens). Hence, even a modest 6 × 6 grid of lipids on each leaflet (72 lipids total) and water is a system of almost 17 000 particles (10 000 if lipid hydrogens are not included).

This next issue is time scale. The variance, σ^2, in a correlation function with relaxation time τ from a trajectory of length T_{run} (and $T_{run} \gg \tau$) is given by[16]

$$\sigma^2[C(t)] \approx \frac{2\tau C(0)^2}{nT_{run}} \tag{6}$$

where n is the number of independent particles for which correlation functions have been evaluated,

$$\tau = \int_0^\infty \frac{C(t) - C_\infty}{C(0) - C_\infty}\,dt \tag{7}$$

and C_∞ is the long-time (or plateau) value of $C(t)$. Equation 6 also provides a reasonable estimate for the statistical error in the relaxation time itself. For the bilayer system of interest τ is in the nanosecond range. This implies that if the system contains 100 independent lipids, a trajectory of at least 2 ns is required to limit the statistical error to 10% (the approximate error in the T_1 measurements); for a single lipid system, or if one assumes that the lipids are not independent, the trajectory must be at least 200 ns for a 10% statistical error.

The question of a "sufficiently large bilayer" is somewhat more difficult to answer. If collective motions are to be explicitly simulated, then a rather sizable patch on the order of 1000 Å/side must be considered.[17] Since the surface area per lipid in a fluid-phase DPPC bilayer is about 64 Å^2,[18] the preceding bilayer patch would contain approximately 30 000 lipids.

To summarize, we have just argued that a trajectory of at least 2 ns must be generated for a system of as many as 30 000 lipids (6.6 million particles) in order to test eq 1 in a completely brute force manner. While such a simulation is useful to ponder, it was out of the question in 1982 (and still is). Several hundred particles was a loose upper limit.

There are two ways to reduce the number of particles in the system: reduce the complexity of the lipids or reduce the number of lipids. Had the research begun in a different group, the former route might have been taken. Lipids can be described as individual cylinders or ellipsoids tethered at the surface to model a bilayer. A simulation of this model could have been used to test the hypothesis that single molecule "wobble" is a greater contributor to the NMR relaxation than collective motions. Contributions from the fast motions could not be determined, however, and questions as to whether the result was an artifact of the parametrization would arise.

Research in the Karplus group at that time was centered on atomic level systems. What is more, an analysis of NMR relaxation of alkanes based on BD simulations had just been published,[19] and R.W.P. had just finished a comparison of different stochastic simulation methods on butane.[20] Hence, both environment and personal experience led to the idea of eliminating lipids, rather than simplifying them. The following subsection describes the simulation and analysis of the 18 particle model we adopted, a single acyl chain in a mean field.

Simulation I: A Single Chain

Reducing a system from many thousands of particles to 18 seems extreme. However, recall the familiar diffusion equation for a distribution of noninteracting particles:[21]

$$\frac{\partial c(x,t)}{\partial x} = D\frac{\partial^2 c(x,t)}{\partial t^2} \tag{8}$$

where $c(x,t)$ is the concentration of particles at position x and time t and D is the diffusion constant of the individual particles. The interactions between the particles and the solvent are satisfactorily described by the single parameter D; i.e., the solvent degrees of freedom have been removed. Equation 8 can be generalized to include forces acting on the particles and inertial terms, as in the Fokker−Planck (FP) equation.[21] The solutions of the diffusion and FP

equations are distributions. An alternative is to write an equation for the trajectory for an *individual* particle, generate many trajectories, and then average to obtain the distribution and other time averages. The single particle analogue of the FP equation is the Langevin equation:[21,22]

$$m\frac{d^2x(t)}{dt^2} = F(t) - m\gamma\frac{dx(t)}{dt} + R(t) \qquad (9)$$

where m is the particle mass, x the position, γ the collision frequency, F the systematic force, and R the random force. The average value of the random force is zero, and its variance is related to the friction constant by the fluctuation−dissipation theorem. As the particle moves, it is buffeted by random forces and damped by frictional forces. This combined action models the dynamic effects of solvent. At high collision frequency motion is diffusive, like a random walk; trajectories can be effectively generated by algorithms denoted *diffusive* or *Brownian* dynamics. In the limit $\gamma = 0$, the Langevin equation reverts to Newton's equation, and trajectories become ballistic; this is the province of molecular dynamics. Part of the art of doing Langevin dynamics involves assigning a value of γ that adequately models solvent damping.[22,23]

In addition to damping the motion of a solute, the solvent may also impose structure. Returning to the "one-chain" model of the bilayer, if only the interatomic forces (as arise from Lennard-Jones, dihedral, and bond angle interactions) are applied, the chain would show no preferred orientation. However, bilayer chains are loosely aligned along the bilayer normal, as is clear from their order parameters. Hence, an additional set of terms was required to model the *structural* effects of the solvent, which in this case was the rest of the bilayer. These were adopted from the mean field model of Marcelja,[24] who had generalized the Maier−Saupe treatment of rigid nematic liquid crystals to flexible chains. The parameters of the mean field were tuned to yield the experimental deuterium order parameters of the chain carbons via a method similar to that of Schindler and Seelig.[25] Last, as shown in Figure 2, the chain carbonyl carbon, O1, and C1 of the glycerol were constrained. Consequently, whole lipid motions, such as wobble and axial rotation, or collective motions were not explicitly simulated.

In essence, the "18 carbon model" contains only gauche/trans isomerization and bending (i.e., internal single chain motions), so the simulation could only directly demonstrate the effects of these dynamics. Conversely, because there were so few particles in the system, it was possible to simulate for 660 ns, long enough for over 39 000 transitions to occur. This ensured that the reorientational correlation functions of the CH vectors could be evaluated with high precision.

The results of the BD simulations are described extensively in the original papers[7,8] and several reviews.[22,26,27] Now we concentrate on the original question: are collective motions required to explain the observed frequency dependence?

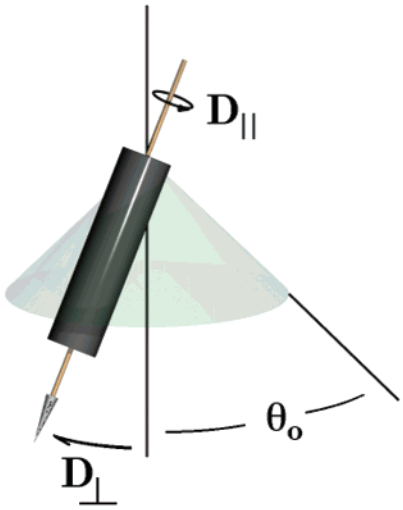

FIGURE 3. Schematic representation of a cylinder "wobbling in a cone" with a cone angle θ_0. Diffusion constants $D_{||}$ and $D_\perp$ describe rotation about the cylinder axis and of the cylinder axis, respectively.

Importantly, T_1 times calculated directly from the trajectory showed *no* frequency dependence for any reasonable value of the collision frequency, and hence could not reproduce the experimental data. This showed, as Brown and co-workers had proposed, that the internal motions could plausibly account for the fast relaxation, but not the "slow" motion.

The most natural route when starting with a single chain model is to include the effects of the single lipid motions axial rotation and wobble. To do this, one first assumes that the chain motions rapidly average each lipid into roughly cylindrical shape. As sketched in Figure 3, the "cylinder" then independently axially rotates and wobbles with diffusion constants $D_{||}$ and $D_\perp$, respectively. Because the wobble is restricted to a cone-like region, there is a "tilt" order parameter for the long axis vector given by

$$S_T = \frac{1}{2}\langle 3\cos^2\theta - 1\rangle \qquad (10)$$

where θ is the angle between the long axis vector and the bilayer normal. Assuming that each chain carbon has an order parameter S_j associated only with internal dynamics, the deuterium order parameter is $(S_{CD})_j = S_jS_T$. Then, using the methods developed by Szabo,[28] the effects of the whole lipid motions were factored into the correlation functions for the internal motions (simulated by BD) and new T_1's calculated with a range of parameters $D_{||}$, $D_\perp$, S_T, and, as before, γ.

Agreement with experiment was excellent. The best fit for the model was obtained for $\gamma = 52$ ps^{-1}, $D_\perp = 2 \times 10^8$ s^{-1}, $D_{||} \approx 2.5 \times 10^{10}$ s^{-1}, and $S_T = 0.54$; Figure 4 is a plot of the results for the average of carbons 4−13. Somewhat improved results were obtained when S_T was allowed to vary slightly for each carbon ($0.53 \le S_T \le 0.67$), leading to $\gamma = 47$ ps^{-1}, $D_\perp = 1.3 \times 10^8$ s^{-1}, and $D_{||} \approx 1.3 \times 10^{10}$ s^{-1}. This showed that chain and single lipid motions can explain the data in the MHz regime, and thereby provide an alternative to Brown's collective model. It does not

imply that large-scale collective motions are not present. Rather, our model implies that they would occur on a much longer time scale.

It is important to relate these parameters with other, perhaps more familiar, treatments and physical properties. First, as may be deduced from hydrodynamic modeling,[22,23] the value of γ is in the centipoise range, implying that the interior of the bilayer is a medium of relatively low viscosity (e.g., the viscosities of decane and hexadecane at 50 °C are 0.62 and 1.87 cP, respectively). This agrees with the conclusions of Overton who argued in 1895 that the bilayer interior was much like a liquid alkane.[29] It is not consistent with the treatment of Saffman and Delbruck,[30] who modeled the bilayer as a slab of uniform viscosity surrounded by water. In this model, the small lateral diffusion constants observed for lipids and proteins in membranes imply that the viscosity of the membrane interior is 1−2 P (about that of glycerin at 20 °C).

The meaning of S_T may be pictured within the "cone model".[13] In this model a cylinder can wobble freely within the confines of a cone of half-angle θ_0 (Figure 3), which is related to S_T by

$$S_T = \frac{1}{2}\cos\theta_0(1+\cos\theta_0) \tag{11}$$

While a real lipid is obviously not restricted by a hard conical wall, $\theta_0 = 49°$, consistent with $S_T = 0.54$, is a physically reasonable value.

The relaxation of a vector in an ordering potential is more complicated than in isotropic media,[21] so relating $D_\perp$ to a single relaxation time is not trivial. We may use the three-exponential approximation developed by Szabo,[28] written below in terms of averages over powers of $z = \cos\theta$:

$$C_2(t) = \left[\frac{3}{2}\langle z^2\rangle - \frac{1}{2}\right]^2 + \frac{9}{4}[\langle z^4\rangle - \langle z^2\rangle^2] \times \exp\left\{-4D_\perp t\left[\frac{\langle z^2\rangle - \langle z^4\rangle}{\langle z^4\rangle - \langle z^2\rangle^2}\right]\right\} + 3[\langle z^2\rangle - \langle z^4\rangle] \times \exp\left\{-D_\perp t\left[\frac{1-3\langle z^2\rangle + 4\langle z^4\rangle}{\langle z^2\rangle - \langle z^4\rangle}\right]\right\} + \frac{3}{4}[1 - 2\langle z^2\rangle + \langle z^4\rangle] \times \exp\left\{-4D_\perp t\left[\frac{1-\langle z^4\rangle}{1-2\langle z^2\rangle + \langle z^4\rangle}\right]\right\} \tag{12}$$

For a given S_T, $\langle z^2\rangle$ is obtained by eq 10, and $\langle z^4\rangle$ from the definition of the fourth order Legendre polynomial P_4 and the relation of $\langle P_4\rangle$ and S_T in the cone model:[13]

$$\langle P_4\rangle = (1/8)(28\langle z^4\rangle - 30\langle z^2\rangle + 3) = (S_T/8)(28S_T + 1 - 7(1+8S_T)^{1/2}) \tag{13}$$

For the values $D_\perp = 2 \times 10^8$ s^{-1} and $S_T = 0.54$, the relaxation in eq 12 is dominated by a single term with a decay constant of 0.97 ns, i.e., the nanosecond relaxation expected to lead to frequency dependence. The parameters for the improved fit noted above yield a dominant decay time of 1.3 ns.

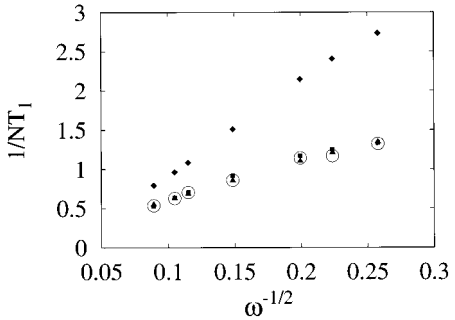

FIGURE 4. $1/T_1$ vs $\omega^{-1/2}$ of carbons 4−13 (averaged) for experiment (open circles), model-free fits to experiment (triangles), BD including wobble and axial rotation (squares), and the 20 ns MD simulation III (diamonds).

Table 1. Fast Correlation Times τ_j and Amplitudes A_j^2 of the Slow Decay Time for Selected Carbons from Model-Free Fits (Equation 14) to the T_1 Data (τ_S = 1830 ps), τ from Reorientational Correlation Functions of Simulation II, and Results from a Carbon-by-Carbon Model-Free Fit to the Calculated $1/NT_1$ of Simulation III[a]

	model-free fit to expt		simulation II	simulation III		
carbon	τ_j	A_j^2	τ	τ_j	$(\tau_S)_j$	A_j^2
2	41.4	0.0615	48	62	3500	0.176
3	32.3	0.0396	43	49	1700	0.132
4−13	20.4	0.0350	23	27	2300	0.0758
14	9.5	0.0123	8.5	12	1900	0.0320
15	6.8	0.0068	6.8	9.4	2300	0.0185

[a] All relaxation times in picoseconds.

Only the value of $D_\parallel \approx 2 \times 10^{10}$ s^{-1} is suspect, as a ratio $D_\parallel/D_\perp \approx 100$ is too high for a cylinder the approximate shape of a lipid. This implies that the fast axial averaging is probably coming from motions not included in the single chain simulation and does not correspond to the simple model of rotation of a rigid cylinder.

As a final topic of this subsection we review a very simple treatment of the data by Szabo[31] using "model-free" formalism.[11] Here the relevant correlation function is written for each carbon j as the sum of fast decay constants τ_j and a common slow time, τ_S:

$$C_j(t) = (1 - A_j^2)\exp(-t/\tau_j) + A_j^2\exp(-t/\tau_S) \tag{14}$$

A fit to the data yielded $\tau_S = 1.83$ ns and the fast decay times and amplitudes listed in Table 1. As shown in Figure 4 for the average of carbons 4−13 (see ref 8 or 31 for a complete comparison), the fit is excellent, and highly linear even though there is no explicit $\omega^{-1/2}$ dependence in eq 14. The value of τ_S is comparable to that extracted from the BD simulations, and a simple derivation (see the appendix of ref 8) yields the link to S_T

$$A_j^2 = \frac{(1 - S_T^2)(S_{CD}^2)_j}{S_T^2} \tag{15}$$

Inserting the experimental deuterium order parameters[15] and amplitudes from Table 1 and averaging yield $S_T = 0.66$.

To summarize, we were able to calculate accurately NMR T_1 values for a DPPC bilayer using a Brownian dynamics simulation of a single lipid chain in a mean field, analytic theory, and four adjustable parameters. A model-free fit to the data yields results consistent with the preceding approach and will be used to provide important checks of the molecular dynamics simulations to be discussed in the following two subsections.

Simulation II: 72 Fully Hydrated Lipids (170 ps)

A skeptical reader might comment that Brownian dynamics and mean fields are approximate methods, that the analytic model contains numerous assumptions, and that the number of adjustable parameters is unacceptably large.

At least partly to answer such criticisms, we decided to simulate a bilayer with molecular dynamics. There are no comparable adjustable parameters or reliance on the mean field potential because *all* of the lipids in a patch are explicitly included. As already noted, the most serious limitation is the huge amount of computer time required to simulate a system for a sufficient length of time. By the early 1990s, however, 100 ps simulations on sizable systems were possible. While such a simulation would not probe collective motions of the bilayer or overall single lipid motions, it would be sufficient for determining if our conclusions regarding internal dynamics were correct.

Before such a simulation could be undertaken, however, reliable potential energy parameters and initial configurations were required. The need for the former is obvious: inaccuracies in the potentials could have a large impact on the isomerization rates and, thereby, the fast relaxation times. These were provided by a massive effort by MacKerell and co-workers.[32] The need for a good initial configuration is less obvious. In principle, initial conditions in molecular dynamics simulations dissipate during equilibration assuming that the rest of the methodology is correct. Therefore, one should be able to start with *any* configuration. In practice, equilibration might take longer than the amount of computer time available. As already noted, the bilayer contains a nanosecond relaxation time, so it was essential to begin with a state fairly close to equilibrium. To do this, we generated single lipid configurations consistent with the experimental order parameters,[33] with the balance of internal and overall disorder determined from the BD simulations just described. These configurations were then packed into a bilayer geometry using our best estimates of interlayer spacing and surface area per lipid. The MD simulation consisting of 20 ps of equilibration system followed by 170 ps of production required approximately 6 months of computer time on what was then a state-of-the-art IBM 3090 with an array processor.

As listed in Table 1, relaxation times evaluated from the 0−50 ps interval of $C_2(t)$ obtained from the simulation showed a remarkable similarity to the those deduced by Szabo in his earlier fit to the data. (While a rigorous comparison requires an estimate of A_j^2 from the trajectory and subsequent scaling by $(1 - A_j^2)^{-1}$, the correction is small.) A simulation of hexadecane was also carried out. Relaxation times averaged for C3 and C14 of hexadecane were 8.7 ps and for C2 and C15 were 6.5 ps, almost identical to the those of C14 and C15 of DPPC (Table 1). Hence, an important conclusion of the BD study, that the bilayer interior is similar to a liquid alkane, was borne out by these MD simulations. Later simulations of a series of liquid alkanes[34] showed that isomerization rates are virtually independent of chain length. This is consistent with a notion of a similar "microviscosity" for alkane environments.

Simulation III: 72 Fully Hydrated Lipids (20 ns)

Several years had to pass before sufficient computer time became available to investigate the character of the nanosecond relaxation time. During this time there were numerous improvements to the simulation methods,[4−6] including a reworking of the potential energy parameters.[35] Results from a 10 ns trajectory of a DPPC bilayer compared very favorably to dipolar NOE measurements and a variety of other structural data,[36] and it was decided for this Account to extend this simulation for another 10 ns in order to look for direct evidence of wobble and axial rotation.

A difficulty in this sort of analysis is that while wobble and axial rotation are well-defined for a rigid cylinder (Figure 3), they might not be for a flexible molecule like DPPC. Hence, one must try to define a vector that is relatively independent of the internal motions while sensitive to the given overall rotation, keeping in mind that such a vector might not exist when coupling is very large. The natural candidate for the long axis is the corresponding eigenvector of the moment of inertia tensor of the lipid. This notion is supported in a qualitative way by Figure 5. In this series 20 equally spaced configurations of a single lipid are depicted directly (panel a), and then translated so that the C2 carbons of the glycerol are superimposed (panel b). An "umbrella"-like arrangement of the lipids and their eigenvectors (panel c) consistent with wobbling is clear. Last, panel d shows the lipids rotated so that the eigenvectors and bilayer normal coincide. With the exception of a part of a single tail, the ensemble of lipids is cylindrical. For a quantitative determination, the $C_2(t)$ of the long-axis vector was calculated for all of the lipids and averaged (Figure 6). A two-exponential fit to the function yields a dominant decay time of 1.4 ns ($D_\perp = 1 \times 10^8$ s^{-1}) and an order parameter $S_T = 0.66$ (a cone angle of 41°). These are close to the values $\tau_S = 1.83$ ns and $S_T = 0.66$ obtained from the model-free fit to the data, and $D_\perp = 1 \times 10^8$ s^{-1} and $S_T = 0.54$ obtained from the BD study.

Interestingly, the short-axis vectors of the moment of inertia tensor are much less well behaved. Here internal motions of the chains cause frequent interchange of the eigenvectors, and, consequently, it is not possible to

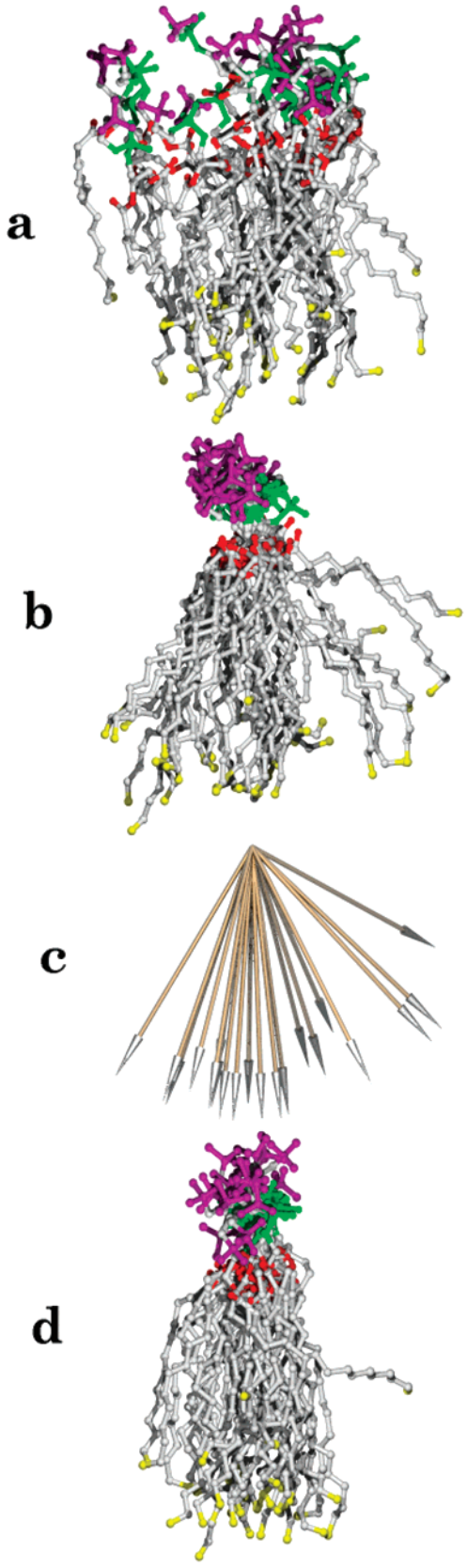

FIGURE 5. Configurations spaced 2 ns apart of a single lipid from simulation III: (a) direct from simulation, including translational displacements; (b) translated so that the C2 carbons of the glycerol are superimposed, but not rotated; (c) eigenvectors of long axis for lipids in panel b; (d) lipids rotated so that the eigenvectors shown in panel c and bilayer normal coincide.

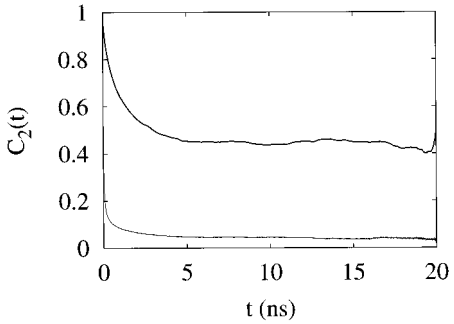

FIGURE 6. Reorientational correlation function for the lipid long-axis vector (heavy solid line), averaged over all lipids, and CH vector (light solid line), averaged over carbons 4−13 of all lipids from simulation III.

characterize axial rotation in this manner. It is probable that the high value of $D_{||}$ obtained from our original treatment reflects chain−chain dynamics and not axial rotation of the lipid. The MD simulations in this case help to show where the simple rigid body model breaks down.

$C_2(t)$ for the CH vectors of C4−C13 is included in Figure 6. The correlation function has reached its plateau value, indicating convergence, and the T_1's were calculated. Results for the set C4−C13, included in Figure 4 and representative of the other carbons, are mixed: the frequency dependence is linear but overestimates experiment. For a simple analysis, the calculated $1/NT_1$ were fit using the model-free formalism on a carbon-by-carbon basis (i.e., with a different slow decay time $(\tau_S)_j$ for each carbon). As listed in Table 1, the fast decays are comparable to those from experiment and simulation II. Scaling by $(1 - A_j^2)^{-1}$ as discussed earlier leads to even closer agreement between the simulations. With the exception of C2, the slow decays are in the 2 ns range, close to the 1.83 ns obtained in the model-free fit to experiment, and in the range of the decay time of the wobble vector. The A_j^2's, however, are considerably higher than those obtained from experiment and lead to the exaggerated frequency dependence. The origin of this is not yet clear.

Summary and Outlook

It is easy to argue that computer simulations have enormous potential. Computer hardware is progressively becoming faster and cheaper, and innovative solutions to software problems are routine. With regard to molecular dynamics simulations of biological macromolecules and assemblies such as membranes, it is reasonable to believe that the intricacies of small molecule and ion binding and transport, protein−protein interaction, and even membrane rafts will successfully be simulated in the next 10 years. It is obvious, nonetheless, that such simulations need to be carried out with care, or the results will be little more than "pretty pictures".

We have reviewed in this Account one path in our joint research. Motivated by the unusual frequency dependent ^{13}C NMR T_1 relaxation data obtained by Brown and coworkers, we carried out Brownian dynamics simulations on a lipid chain in a mean field which modeled the

remainder of the bilayer. Using these simulations, theory, and fits to the experimental data, we developed a simple model of lipid dynamics. Individual lipids average themselves into a relatively cylindrical shape through internal motions on the 100 ps time scale and "wobble" in a cone-like potential on the nanosecond time scale. These motions take place in an environment much like a liquid alkane, consistent with the notion that the interior of membranes have a low microviscosity. Molecular dynamics simulations have largely confirmed this model. The fast relaxation times agree with experiment and thereby support the notion that the viscosity of the bilayer interior is low, and the 20 ns MD simulation III confirms the presence of the wobble. Simulation results not discussed here indicate that motion in the headgroup region is highly restricted,[37] consistent with our assertion that the slow lateral diffusion of the lipids and proteins is primarily modulated by hydrogen bond and electrostatic interactions at the bilayer/water interface.

The predicted ^{13}C T_1 frequency dependence from simulation III is significantly larger than experiment. This could indicate a problem with the potential energy parameters, despite excellent agreement with experiment for most structural and other dynamic properties. The disagreement may also arise from differences between multilayer systems (as simulated) and the high-curvature vesicles used for the experiments. Simulations of highly curved systems and revisions of the parameter set will be carried out to resolve this question.

After a satisfactory explanation of the T_1 data is obtained, an investigation into the complex details of lipid dynamics may commence. Simulations on the 10−20 ns time scale will be sufficient for studying most internal motions, including scissoring of the chains and other potential departures from the simple "wobble" description. Trajectories on the 100−1000 ns time scale will be required to follow lateral diffusion of lipids.[38] Larger systems will be required to discern collective undulations. Simulations on such systems would enable further tests of the present model (excellent multiparameter fits do not guarantee its correctness) and help resolve controversies associated with the effective surface tensions in small systems. Other challenging areas in simulations of lipid structure and dynamics include the following: the evaluation of free energy changes of bilayer compression and expansion and the related calculation of surface area of both simple and complex membranes; reorganization of lipids in membranes with imbedded peptides, proteins, and cholesterol; the effects of ions, electric fields, and osmotic gradients; complex lipids including gangliosides and phosphoinositides (e.g., PIP2); and large scale rearrangements such as fusion. There are many interesting things to do.

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AR0100529