Molecular Dynamics Simulation of Lipid Reorientation at Bilayer Edges

Peter M. Kasson*† and Vijay S. Pande†‡
*Medical Scientist Training Program, †Biophysics Program, and ‡Department of Chemistry, Stanford University, Stanford, California

ABSTRACT Understanding cellular membrane processes is critical for the study of events such as viral entry, neurotransmitter exocytosis, and immune activation. Supported lipid bilayers are commonly used to model these membrane processes experimentally. Despite the relative simplicity of such a system, many important structural and dynamic parameters are not experimentally observable with current techniques. Computational approaches allow the development of a high-resolution model of bilayer processes. We have performed molecular dynamics simulations of dimyristoylphosphatidylcholine (DMPC) bilayers to model the creation of bilayer gaps—a common process in bilayer patterning—and to analyze their structure and dynamics. We propose a model for gap formation in which the bilayer edges form metastable micelle-like structures on a nanosecond timescale. Molecules near edges structurally resemble lipids in ungapped bilayers but undergo small-scale motions more rapidly. These data suggest that lipids may undergo rapid local rearrangements during membrane fusion, facilitating the formation of fusion intermediates thought key to the infection cycle of viruses such as influenza, Ebola, and HIV.

INTRODUCTION

Phospholipid bilayers form the barriers that define and partition all living cells. They are also the medium in which many cellular signaling proteins reside. Many polar substances such as neurotransmitters are transported in and out of cells by membrane budding and fusion (for recent studies of synaptic vesicle fusion see Aravanis et al., 2003; Gandhi and Stevens, 2003); these processes are also employed for the entry and release of enveloped viruses (reviewed in Tamm et al., 2003; Colman and Lawrence, 2003; Jahn and Sudhof, 1999). Membrane organization and fusion are thus critical for cellular survival as well as viral infection. To study the dynamics of lipid bilayers under perturbation in more detail, we have chosen to simulate the process of edge formation. Our system is a direct computational analog of a process used in biofabrication, and, as we will discuss, it may have relevance to the biological process of membrane fusion as well.

In most biological systems, lipid bilayers form closed continuous surfaces and therefore have no edges. However, recent advances in techniques for the micropatterning of synthetic bilayers on glass or polymer surfaces allow the creation of discrete, water-bounded patches of bilayer (Groves et al., 1997; Hovis and Boxer, 2000; Kung et al., 2000). A specially prepared poly(dimethylsiloxane) stamp is micropatterned with a series of ridges and depressions as described in Hovis and Boxer (2000). This stamp is then pressed upon the bilayer and removed, pulling with it both bilayer leaflets wherever the ridges contact the bilayer. This process allows the creation of a set of discrete, patterned bilayer regions that can then be used for a number of applications (Kam and Boxer, 2000; Kung et al., 2000). Although the patterned bilayer patches undergo a slight area expansion, they are then stable for weeks at room temperature. These patches do have edges, the stability of which is critical for many biological and industrial applications of supported bilayers. The structure of bilayer edges created in this manner is not amenable to study by most conventional techniques—no experimental approach attempted to date has succeeded. We have therefore undertaken a series of molecular dynamics simulations to generate a theoretical model of the behavior of such bilayer boundaries.

We have chosen to simulate fluid-phase planar bilayers using two- and three-dimensional periodic unit cells in a manner similar to that used by a number of other investigators (Feller and Pastor, 1996; Smondyrev and Berkowitz, 1999; Tu et al., 1995; van der Ploeg and Berendsen, 1982; Berger et al., 1997; Tieleman and Berendsen, 1996; Egberts et al., 1994). Dimyristoylphosphatidylcholine (DMPC) molecules were represented using a unified-atom model with explicit water, and bilayer motions were simulated using molecular dynamics. After allowing our simulated bilayer to equilibrate, we deleted a strip of lipid molecules to form a gap analogous to that created experimentally by blotting. We then simulated the motions of these gapped bilayers for 2–7 ns, using three different starting states and two independent molecular dynamics runs for each state.

METHODS

Molecular dynamics simulations were run using NAMD v2.1 (Nelson et al., 1996; Kalé et al., 1999) on clusters of four or eight dual-processor Linux systems and on a Cray T3E supercomputer at the National Energy Research Scientific Computing Center (NERSC, Berkeley, CA). The force field was CHARMM19 (Neria et al., 1996), extended for lipids based on DMPC structural data (Vanderkooi, 1991). The parameter set used to model DMPC is given in the Supplementary Material. Water molecules were represented using the TIP3 model (Jorgensen et al., 1983).

Given that our experimental model is a lipid bilayer rather than a multilayer, we chose to use two dimensionally periodic boundary conditions.

Submitted June 13, 2003, and accepted for publication February 6, 2004.

Address reprint requests to Vijay S. Pande, Tel.: 650-723-3660; Fax: 650-725-0259; E-mail: pande@stanford.edu.

0006-3495/04/06/3744/06 $2.00

doi: 10.1529/biophysj.103.029652

Three dimensionally periodic conditions (approximating a DMPC multilayer) were run on ungapped bilayer models and yielded equivalent results in terms of stability, bilayer width, and lipid motion, suggesting that the system is stable on the timescale considered in this work.

Two simulation runs were performed for each set of starting parameters (ungapped bilayer, gapped bilayer, gapped bilayer with water filled in, and long gapped bilayer), with each run spanning at least 2 ns. Simulations of up to 7 ns in length were used to test the structural stability of both the simulation conditions and the micelle-like structures formed by gapped bilayers and for long timescale measurements. Independence between simulation runs was achieved via separate randomization of starting velocities and random seed variation.

The ungapped bilayer starting state was constructed as follows. Coordinates for DMPC were obtained from previously computed and reported energy minimization of crystal structures (Pearson and Pascher, 1979; Vanderkooi, 1991). A total of 64 DMPC molecules per leaflet were spaced on a square 60- × 60-Å lattice at an area per headgroup of 56.25 $Å^2$, consistent with experimental values (Nagle and Tristram-Nagle, 2000). The remainder of the 60- × 60- × 60-Å cubic unit cell was filled with preequilibrated water molecules at 1 g/cm^3, resulting in full hydration with a ratio of >90 water molecules per lipid molecule.

To construct the gapped bilayer, an ungapped bilayer simulation was run for 250 ps to allow the DMPC chains to equilibrate. At this point, all lipid molecules within 7 Å of an *x-z* plane along the center of the bilayer were deleted, and the simulation was restarted at time 0. The water-filled gapped bilayer was constructed similarly except that water was also deleted and then reinserted from an equilibrated box. To form the long gapped bilayer, lipids from the gapped bilayer and ungapped bilayer unit cells were joined end to end and the remainder of the 120- × 60- × 60-Å unit cell filled with water. The ungapped and short gapped bilayers thus have 128 lipid molecules, whereas the long gapped bilayers have 256 lipid molecules.

The following additional simulation conditions were chosen and tested for equivalence against other conditions commonly used. Equivalence was assessed via bilayer structural parameters in ungapped simulations and also by gap formation behavior. Simulations were run at a constant temperature of 300 K (NVT ensemble), at which DMPC bilayers exist in a fluid phase (Blume, 1979; Koynova and Caffrey, 1998). Constant pressure simulations at 1 bar (NPT ensemble) using NAMD's Nosé-Hoover implementation (Nosé, 1984; Hoover, 1985) with fixed cross sectional area yielded equivalent results. Time step sizes of 2 fs were used, with bond lengths for all water molecules fixed using the SHAKE algorithm (Ryckaert et al., 1977). Time steps of 1 fs without SHAKE yielded equivalent results. Cutoff values for Coulomb and van der Waals interactions were set to 11 Å. Particle mesh Ewald electrostatic interaction modeling (Darden et al., 1993) was used for the simulations reported here; similar effects on bilayer gap formation and dynamics were observed with traditional electrostatic cutoffs.

RESULTS

All gapped bilayers underwent similar structural rearrangement at their edges, forming micellized structures (Fig. 1). Micellization was apparent after ~1.5 ns, and the micelle-like structures remained stable over the course of the longest run performed (~7 ns). Membrane curvature extended to the box boundary in the short gapped simulation, but the long

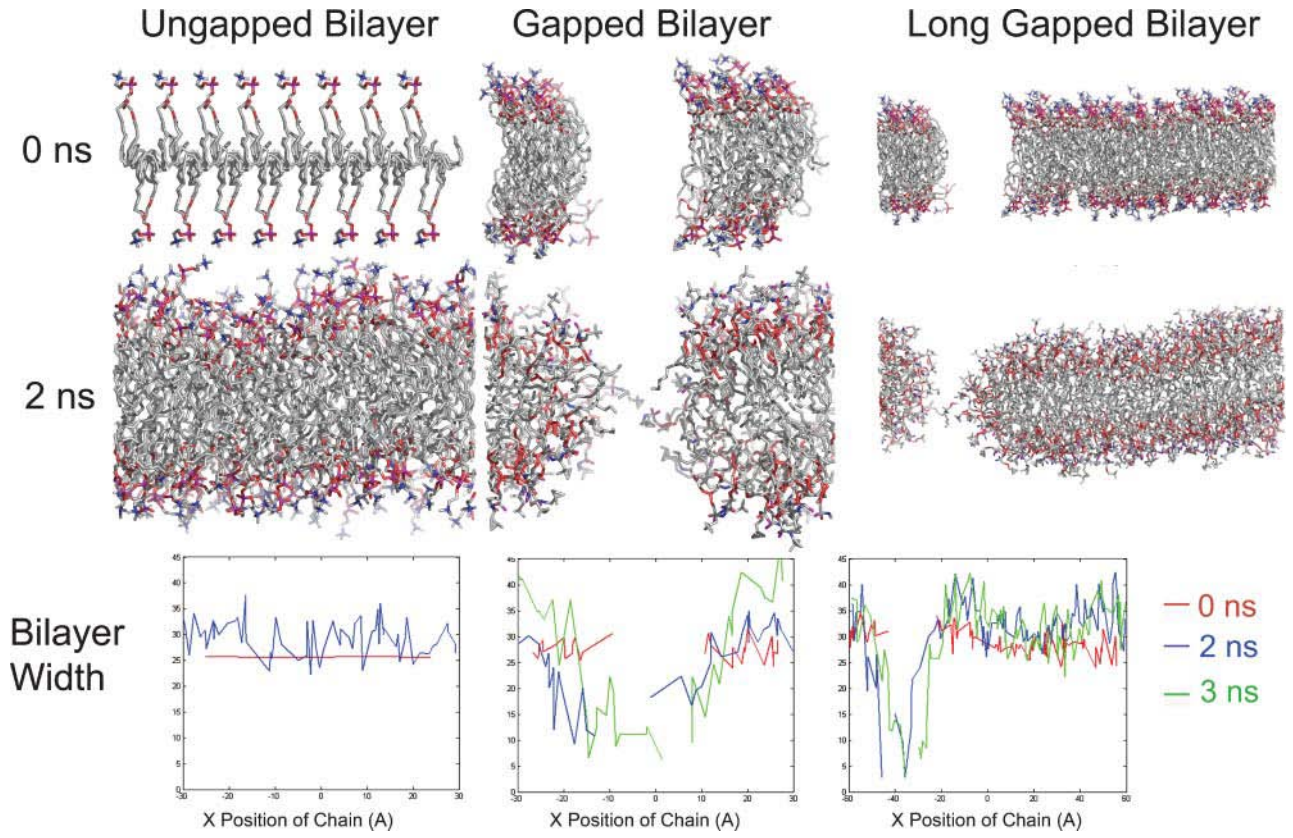

FIGURE 1 Time course for simulated bilayers. Snapshots are displayed from three simulations: an ungapped DMPC bilayer, a gapped bilayer, and a longer gapped bilayer, at 0 and 2 ns. Bilayer width is plotted as distance between phosphate groups on opposite bilayer leaflets. Micellization at gap boundaries is clearly visible.

gapped simulations showed this to be a local effect, as the membrane regained its flat structure farther from the gap. Water-bounded edges appeared to attain stability by simply reorienting so as to present a consistently polar surface at the lipid-water interface, protecting the nonpolar hydrophobic tail groups.

Characterization of simulated bilayers

The results of our structural and dynamic analyses on the ungapped bilayer simulations support the applicability of these simulations to physical DMPC bilayers. The mean bilayer width, as measured by phosphate-phosphate distance perpendicular to the bilayer, was 29.7 Å, which agrees well with the analogous experimental value (analysis by Nagle and Tristram-Nagle, 2000).

Segmental carbon-deuterium (S_{CD}) bond order parameters obtained via NMR experiments on deuterated bilayers are another important tool for monitoring lipid membrane structure. S_{CD} measurements may be calculated based on carbon coordinates in the lipid tail (Essex et al., 1994) and provide information on the order at different portions of the hydrocarbon tail. As shown in Fig. 2, our calculated values agree well with those observed experimentally for DMPC, suggesting that the simulated bilayers are structurally similar to those used for the experimental measurements. The experimental data used as a reference standard did not include an order parameter for carbon 4, so the validity of the seemingly anomalous value at that point remains unknown. However, based on other reported data (Trouard et al., 1999), we would expect this to be a simulation artifact.

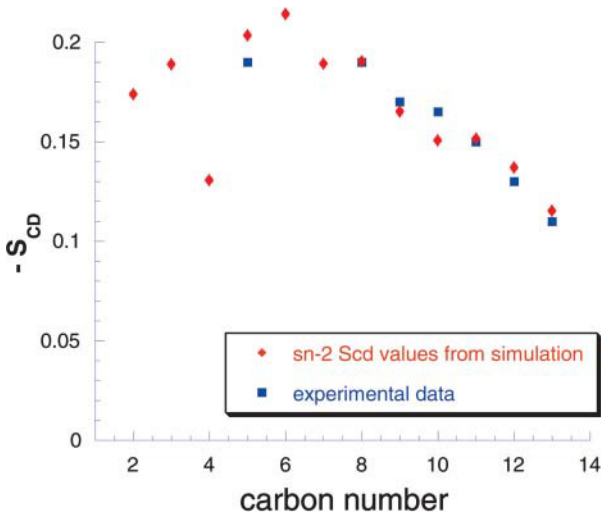

FIGURE 2 Carbon-deuterium bond order parameters for simulated bilayer. Bond order parameters are derived from carbon backbone vectors in the molecular dynamics data as previously described (Seelig and Seelig, 1974; Essex et al., 1994). Calculated values agree well with the analogous experimental values for the *sn*-2 chain as determined by NMR on ^{2}H-DMPC (Boden et al., 1991).

Characterization of simulated bilayer edges

We performed several additional analyses of the gapped and ungapped bilayer simulations to generate our theoretical model for bilayer edges. Reorientational correlation functions for lipid hydrocarbon tails have been studied both experimentally and by simulation as a means of probing internal flexibility (Brown et al., 1983; De Loof et al., 1991; Pastor et al., 1988; Venable et al., 1993). As in these studies, we calculated correlation functions as $<P_2(\mu_0 \cdot \mu(t))>$, where P_2 is the second order Legendre polynomial and μ are unit vectors along the C–C bond. Correlation functions were measured over 3-ns windows at 10-ps offsets; the results are shown in Fig. 3. These correlation functions yield information on fast timescale rearrangements, slow (nanosecond and longer) timescale rearrangements, and lipid order. We measured reorientation using biexponential fits analogous to those for ^{13}C and ^{2}H NMR relaxation measurements of lipid tail dynamics (Brown et al., 1983), fitting the correlation functions to equations of the form $a_1 \times \exp(-t/T_{fast}) + a_2 \times \exp(-t/T_{slow}) + S_{CC}^2$. It has been shown that the S_{CC} measurements thus obtained are related to S_{CD} order parameters by the equation $S_{CC}^k + S_{CC}^{k+1} = -2S_{CD}^k$ (Douliez et al., 1995). Fast timescale relaxations were observed on the order of 100 ps, and slow relaxations were observed on the order of 1 ns (Table 1), both consistent with previous observations (Venable et al., 1993; Brown et al., 1983; Koubi et al., 2001). The relaxation times generated show gapped bilayer lipids to have consistently faster reorientation over both fast and slow timescales as well as lower S_{CC} order parameters, reflecting an increase in both

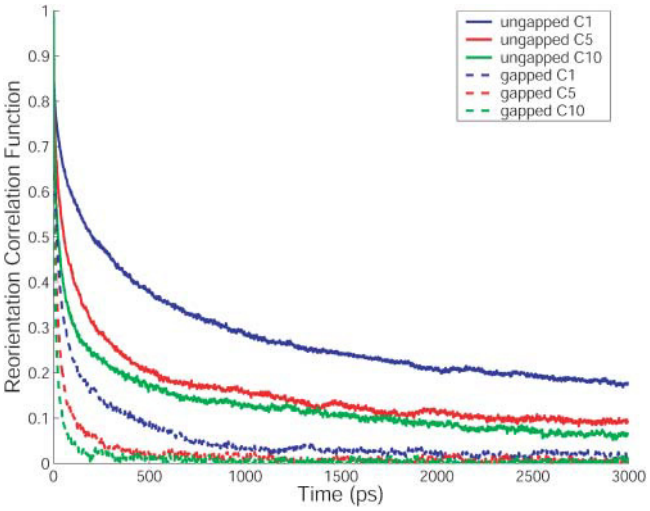

FIGURE 3 Internal flexibility of hydrocarbon chains in gapped and ungapped bilayers. Plotted are reorientation correlation functions for tail group carbons 1, 5, and 10 in gapped and ungapped DMPC bilayer simulations. Starting at 2 ns simulation time, correlation functions were computed over 3-ns windows at offsets of 10 ps. Data plotted represent the mean of these offset correlation functions. The faster decay of the gapped bilayer functions indicates greater internal flexibility near bilayer edges.

static and dynamic disorder. Both relaxation times decreased farther from the headgroup. S^2_{CC} parameters also decreased farther from the headgroup as expected, but due to fit error could not be compared accurately with the directly measured S_{CD} parameters for ungapped bilayers. However, we consider the large and significant difference between S_{CC} parameters in the gapped and ungapped bilayers to be physically as well as statistically ($p < 10^{-6}$) significant.

One important structural problem that arises in our model for micellization of bilayer edges is the hydrocarbon tail packing. Standard packing or interdigitation measurements are difficult to evaluate at bilayer edges because of the curved structures formed. However, carbon density profiles computed at different distances from the gap in the long gapped bilayer simulation (shown in Supplementary Material) do not show an increase in density near the gap, suggesting that tail packing does not become more dense in the vicinity of the gap. Our calculations do not show a significant difference in the length of phospholipid tail group vectors between gapped and ungapped bilayers (Fig. 4 *a*). Furthermore, no correlation was observed between tail group length and distance from the bilayer edge in gapped bilayer simulations (Fig. 4 *b*). Our simulations of gapped bilayers show a very slight increase in bilayer width near the edges. In long gapped bilayer simulations, the width increases near the edge but returns to normal values farther away, suggesting that this is indeed an edge-induced effect rather than a simulation artifact. The increase in width falls within the normal range of fluctuation about the mean width in the ungapped bilayer control (mean 29.7 Å, standard deviation 3.94 Å in the control; mean 32.3 Å in the gapped bilayer). However, this bulging of bilayer ends may help solve the packing problem created by micellization.

Because the bilayer surface is both curved and changing in our edge creation simulations, these bilayer edges present a special challenge for the measurement of any metrics that rely on assumptions about the shape of the bilayer surface. For instance, S_{CD} parameters become extremely difficult to calculate directly, since they rely on a bilayer normal for each phospholipid at each time point considered. To gain approximate data regarding lateral diffusion rates at the edges, we have measured the three-dimensional, rather than the two-dimensional, mean-squared displacement for phospholipid headgroups in both gapped and ungapped bilayer simulations. The motions thus measured ignore the anisotropic nature of lipid diffusion at bilayer edges, yet the motion of phosphate headgroups near simulated bilayer edges is ~10 times faster than in ungapped simulations (see Fig. 5).

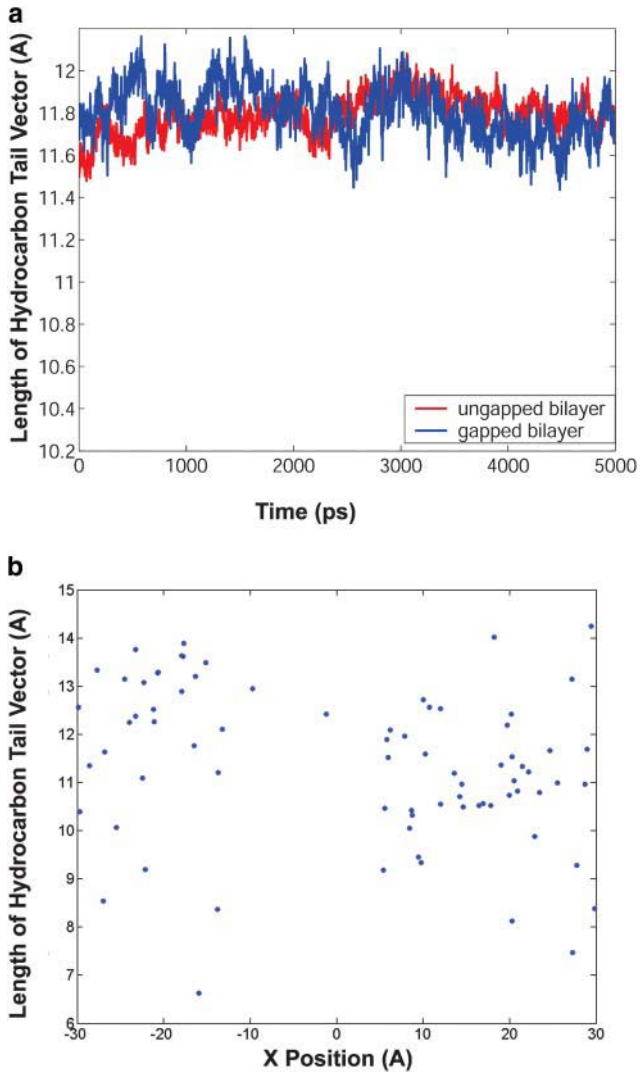

FIGURE 4 Length of hydrocarbon tails at bilayer edges. Shown in *a* is a comparison of hydrocarbon tail group length in gapped and ungapped bilayer simulations. The mean gapped length is 11.80 Å, not detectably different from mean ungapped length of 11.79 Å. In *b*, tail group length is plotted as a function of *x* position in the gapped bilayer after 2 ns simulation.

DISCUSSION

Based on our simulation data, we propose a model for water-bounded phospholipid bilayer edges in which the molecules near the edge reorient to form a micelle-like structure. Such a structure protects the hydrophobic interior of the bilayer from exposure to water while keeping the hydrophilic headgroups in contact with solution. Our simulations suggest that lipids near the edge may remain structurally similar to those in intact bilayers, although tail group static disorder increases. However, we note several changes in the dynamics of lipids at bilayer edges. As both fast timescale hydrocarbon rearrangements and phospholipid headgroup motions increase near the edges, we postulate that the edges are more fluid that the rest of the bilayer. We believe that this increase in fluidity is not the result of a phase change, as there is a continuous rather than a discrete transition in fluidity from gapped to ungapped bilayer in the 120-Å simulations. We predict the micellized bilayer edges to be metastable but energetically strained.

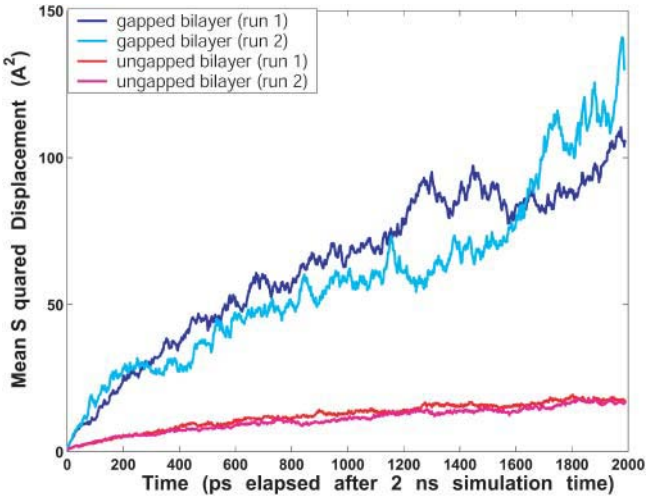

FIGURE 5 Rates of headgroup motion in gapped and ungapped bilayers. Headgroup motion is measured as the mean-squared displacement of phosphate atoms. A start point of 2 ns was chosen for measurement of relative headgroup motion to exclude the gross bilayer rearrangements resulting from gap creation. Runs 1 and 2 refer to independent simulations run under identical conditions but different random number seeding.

This last prediction is well supported by several pieces of experimental data. Bilayer edges formed by blotting undergo slight area expansion but then remain stationary and stable for weeks (Hovis and Boxer, 2000). Although normal bilayers (supported or physiological) do not spontaneously undergo fusion at measurable rates, phospholipid vesicles containing fluorescent dyes preferentially fuse at the bilayer edges created by blotting (S. Boxer, Stanford University, personal communication, 2000). If enough such vesicles are added, the gaps can be entirely filled.

Membrane fusion is thought to proceed via stalk-like intermediates with high local curvature (Yang and Huang, 2002). Our simulations of bilayer edges provide another example of highly curved metastable bilayer structures. The predicted dynamics of bilayer edges may therefore be relevant to membrane fusion. We would therefore postulate that individual lipid molecules in fusion intermediates would have canonical structure but increased dynamic motion.

TABLE 1 Relaxation times computed for reorientation of lipid hydrocarbon tails

Measurement	Carbon no.	Value for ungapped bilayer	Value for gapped bilayer
T_{fast} (ps)	1	83.0	22.5
	5	73.2	14.3
	10	39.6	4.43
	13	1.43	0.707
T_{slow} (ps)	1	843	329
	5	752	157
	10	838	47.7
	13	309	28.4
S^2_{CC}	1	0.17	0.021
	5	0.090	0.0085
	10	0.063	0.0049
	13	0.029	0.0017

Values were computed by fitting unconstrained curves of the form $a_1 \times \exp(-t/T_{fast}) + a_2 \times \exp(-t/T_{slow}) + S^2_{CC}$ to the data plotted in Fig. 3. The gapped bilayer simulation shows consistently faster relaxation times than the ungapped bilayer, suggesting an increase in the flexibility of lipid hydrocarbon tails near bilayer edges.

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org.

We thank the National Energy Research Scientific Computing Center at Lawrence Berkeley National Laboratory and the Biocomputation Core Facility at Stanford University for providing supercomputer time, P. Kuhn for technical advice and assistance, and A. Brunger, S. Boxer, J. Hovis, H. McConnell, A. Radhakrishnan, O. Troyanskaya, and T. Wandless for many helpful discussions.

P.K. was supported by a National Defense Science and Engineering Graduate Fellowship.

REFERENCES

Aravanis, A. M., J. L. Pyle, and R. W. Tsien. 2003. Single synaptic vesicles fusing transiently and successively without loss of identity. *Nature*. 423:643–647.

Berger, O., O. Edholm, and F. Jahnig. 1997. Molecular dynamics simulations of a fluid bilayer of dipalmitoylphosphatidylcholine at full hydration, constant pressure, and constant temperature. *Biophys. J.* 72: 2002–2013.

Blume, A. 1979. Comparative study of the phase transitions of phospholipid bilayers and monolayers. *Biochim. Biophys. Acta*. 557: 32–44.

Boden, N., S. A. Jones, and F. Sixl. 1991. On the use of deuterium nuclear magnetic resonance as a probe of chain packing in lipid bilayers. *Biochemistry*. 30:2146–2155.

Brown, M. F., A. A. Ribeiro, and G. D. Williams. 1983. New view of lipid bilayer dynamics from 2H and 13C NMR relaxation time measurements. *Proc. Natl. Acad. Sci. USA*. 80:4325–4329.

Colman, P. M., and M. C. Lawrence. 2003. The structural biology of type I viral membrane fusion. *Nat. Rev. Mol. Cell Biol*. 4:309–319.

Darden, T., D. York, and L. Pedersen. 1993. Particle mesh Ewald—an n·log(n) method for Ewald sums in large systems. *J. Chem. Phys*. 98:10089–10092.

De Loof, H., S. C. Harvey, J. P. Segrest, and R. W. Pastor. 1991. Mean field stochastic boundary molecular dynamics simulation of a phospholipid in a membrane. *Biochemistry*. 30:2099–2113.

Douliez, J. P., A. Leonard, and E. J. Dufourc. 1995. Restatement of order parameters in biomembranes: calculation of C-C bond order parameters from C-D quadrupolar splittings. *Biophys. J.* 68:1727–1739.

Egberts, E., S. J. Marrink, and H. J. C. Berendsen. 1994. Molecular dynamics simulation of a phospholipid membrane. *Eur. Biophys. J.* 22: 423–436.

Essex, J. W., M. M. Hann, and W. G. Richards. 1994. Molecular dynamics simulation of a hydrated phospholipid bilayer. *Philos. Trans. Biol. Sci*. 344:239–260.

Feller, S. E., and R. W. Pastor. 1996. On simulating lipid bilayers with an applied surface tension: periodic boundary conditions and undulations. *Biophys. J.* 71:1350–1355.

Gandhi, S. P., and C. F. Stevens. 2003. Three modes of synaptic vesicular recycling revealed by single-vesicle imaging. *Nature*. 423:607–613.

Groves, J. T., N. Ulman, and S. G. Boxer. 1997. Micropatterning fluid lipid bilayers on solid supports. *Science*. 275:651–653.

Hoover, W. G. 1985. Canonical dynamics—equilibrium phase-space distributions. *Phys. Rev. A*. 31:1695–1697.

Hovis, J. S., and S. G. Boxer. 2000. Patterning barriers to lateral diffusion in supported lipid bilayer membranes by blotting and stamping. *Langmuir*. 16:894–897.

Jahn, R., and T. C. Sudhof. 1999. Membrane fusion and exocytosis. *Annu. Rev. Biochem.* 68:863–911.

Jorgensen, W. L., J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein. 1983. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys*. 79:926–935.

Kalé, L., R. Skeel, M. Bhandarkar, R. Brunner, A. Gursoy, N. Krawetz, J. Phillips, A. Shinozaki, K. Varadarajan, and K. Schulten. 1999. NAMD2: greater scalability for parallel molecular dynamics. *J. Comp. Phys.* 151:283–312.

Kam, L., and S. G. Boxer. 2000. Formation of supported lipid bilayer composition arrays by controlled mixing and surface capture. *J. Am. Chem. Soc*. 122:12901–12902.

Koubi, L., M. Tarek, S. Bandyopadhyay, M. L. Klein, and D. Scharf. 2001. Membrane structural perturbations caused by anesthetics and nonimmobilizers: a molecular dynamics investigation. *Biophys. J*. 81:3339–3345.

Koynova, R., and M. Caffrey. 1998. Phases and phase transitions of the phosphatidylcholines. *Biochim. Biophys. Acta*. 1376:91–145.

Kung, L. A., L. Kam, J. S. Hovis, and S. G. Boxer. 2000. Patterning hybrid surfaces of proteins and supported lipid bilayers. *Langmuir*. 16:6773–6776.

Moore, P. B., C. F. Lopez, and M. L. Klein. 2001. Dynamical properties of a hydrated lipid bilayer from a multinanosecond molecular dynamics simulation. *Biophys. J*. 81:2484–2494.

Nagle, J. F., and S. Tristram-Nagle. 2000. Structure of lipid bilayers. *Biochim. Biophys. Acta*. 1469:159–195.

Nelson, M. T., W. Humphrey, A. Gursoy, A. Dalke, L. V. Kale, R. D. Skeel, and K. Schulten. 1996. NAMD: a parallel, object oriented molecular dynamics program. *Int. J. Supercomp. Applic. High Perf. Comp*. 10:251–268.

Neria, E., S. Fischer, and M. Karplus. 1996. Simulation of activation free energies in molecular systems. *J. Chem. Phys*. 105:1902–1921.

Nosé, S. 1984. A unified formulation of the constant temperature molecular dynamics methods. *J. Chem. Phys*. 81:511–519.

Pastor, R. W., R. M. Venable, and M. Karplus. 1988. Brownian dynamics simulation of a lipid chain in a membrane bilayer. *J. Chem. Phys*. 89:1112–1127.

Pearson, R. H., and I. Pascher. 1979. The molecular structure of lecithin dihydrate. *Nature*. 281:499–501.

Ryckaert, J. P., G. Ciccotti, and H. J. C. Berendsen. 1977. Numerical integration of cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. *J. Comp. Phys*. 23:327–341.

Seelig, A., and J. Seelig. 1974. The dynamic structure of fatty acyl chains in a phospholipid bilayer measured by deuterium magnetic resonance. *Biochemistry*. 13:4839–4845.

Smondyrev, A. M., and M. L. Berkowitz. 1999. Structure of dipalmitoylphosphatidylcholine/cholesterol bilayer at low and high cholesterol concentrations: molecular dynamics simulation. *Biophys. J*. 77:2075–2089.

Tamm, L. K., J. Crane, and V. Kiessling. 2003. Membrane fusion: a structural perspective on the interplay of lipids and proteins. *Curr. Opin. Struct. Biol*. 13:453–466.

Tieleman, D. P., and H. J. C. Berendsen. 1996. Molecular dynamics simulations of a fully hydrated dipalmitoyl phosphatidylcholine bilayer with different macroscopic boundary conditions and parameters. *J. Chem. Phys*. 105:4871–4880.

Trouard, T. P., A. A. Nevzorov, T. M. Alam, C. Job, J. Zajicek, and M. F. Brown. 1999. Influence of cholesterol on dynamics of dimyristoylphosphatidylcholine bilayers as studied by deuterium NMR relaxation. *J. Chem. Phys*. 110:8802–8818.

Tu, K., D. J. Tobias, and M. L. Klein. 1995. Constant pressure and temperature molecular dynamics simulation of a fully hydrated liquid crystal phase dipalmitoylphosphatidylcholine bilayer. *Biophys. J*. 69: 2558–2562.

van der Ploeg, P., and H. J. C. Berendsen. 1982. Molecular dynamics simulation of a bilayer membrane. *J. Chem. Phys*. 76:3271–3276.

Vanderkooi, G. 1991. Multibilayer structure of dimyristoylphosphatidylcholine dihydrate as determined by energy minimization. *Biochemistry*. 30:10760–10768.

Venable, R. M., Y. Zhang, B. J. Hardy, and R. W. Pastor. 1993. Molecular dynamics simulations of a lipid bilayer and of hexadecane: an investigation of membrane fluidity. *Science*. 262:223–226.

Yang, L., and H. W. Huang. 2002. Observation of a membrane fusion intermediate structure. *Science*. 297:1877–1879.