Investigations on the melting and bending modulus of polymer grafted bilayers using dissipative particle dynamics

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(Received 30 March 2010; accepted 7 July 2010; published online 30 September 2010)

Understanding the influence of polymer grafted bilayers on the physicochemical properties of lipid membranes is important while developing liposomal based drug delivery systems. The melting characteristics and bending moduli of polymer grafted bilayers are investigated using dissipative particle dynamics simulations as a function of the amount of grafted polymer and lipid tail length. Simulations are carried out using a modified Andersen barostat, whereby the membrane is maintained in a tensionless state. For lipids made up of four to six tail beads, the transition from the low temperature L_β phase to the L_α phase is lowered only above a grafting fraction of $G_f=0.12$ for polymers made up of 20 beads. Below $G_f=0.12$ small changes are observed only for the HT₄ bilayer. The bending modulus of the bilayers is obtained as a function of G_f from a Fourier analysis of the height fluctuations. Using the theory developed by Marsh *et al.* [Biochim. Biophys. Acta **1615**, 33 (2003)] for polymer grafted membranes, the contributions to the bending modulus due to changes arising from the grafted polymer and bilayer thinning are partitioned. The contributions to the changes in κ from bilayer thinning were found to lie within 11% for the lipids with four to six tail beads, increasing to 15% for the lipids containing nine tail beads. The changes in the area stretch modulus were also assessed and were found to have a small influence on the overall contribution from membrane thinning. The increase in the area per head group of the lipids was found to be consistent with the scalings predicted by self-consistent mean field results.

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I. INTRODUCTION

In liposomal based drug delivery systems, drugs are encapsulated in the aqueous core region of the liposome or confined between the hydrocarbon chains of the lipid molecules. Freestanding liposomes suffer from poor *in vitro* stability due to aggregation, while *in vivo*, they are exposed to body fluids and can undergo fusion with the cell membrane. Polymers grafted on the lipid membrane overcome these drawbacks improving *in vivo* circulation times.^{1,2} Studying the effect of polymers grafted onto lipid bilayers is of primary importance in understanding and developing liposomal based drug delivery systems.¹ Chemical modification of the grafted polymer offers the possibility of creating site specific carriers for both delivery as well as diagnostic purposes. Since liposomes are of the order of 80–200 nm,¹ in diameter, they can be effectively modeled as infinite bilayers. In addition to liposome stabilization, polymers play an important role in the synthesis of supported membranes where the bilayer rests on a polymer cushion tethered to a solid substrate.^{3–5}

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Polymer-supported membranes have shown remarkable stability in air and have the potential to be used for *in vitro* biosensor applications.⁵ Grafted polymers influence both the thermodynamics as well as the mechanical properties of the lipid bilayer. Liposomes are most effectively formed and function when the lipid bilayer is in the L_α phase and mechanical deformation, particularly under flow, is influenced by the modulus of the membrane. In this paper we investigate the influence of polymer grafting on the melting transition and the bending modulus of the lipid membrane using dissipative particle dynamics.

In addition to the effects associated with steric stabilization, polymers in the brush regime induce lateral interactions, which modify the properties of the host membrane. Thus the presence of the polymer directly influences the bilayer thickness and area per head group.^{6–8} These changes influence the mechanical properties, such as the area stretch modulus (k_A) and bending modulus (κ) of the membrane. Theoretical analysis⁹ suggests that the area stretch modulus decreases with addition of polymer. If a_h is the area per head group, the polymer contribution to the area stretch modulus scales as $a_h^{5/3}$ based on self-consistent scaling theory and as $a_h^{4/3}$ based on mean field theory.^{6,9} The bending modulus scales as $n_p^3 \rho^m$, where n_p is the degree of polymerization, ρ is the grafting density of polymer, and the exponent, $m=7/3$ from self-consistent mean field theory^{10–12} and $m=5/2$ from the de Gennes mean field theory.¹³ The analysis, which extends the theory to polymer grafted bilayers,⁶ additively includes the contribution from the lipids, which scales as $k_A d^2$, where d is the membrane thickness and k_A represents the altered stretch modulus due to the presence of polymer. The scaling contributions to κ from n_p and ρ are similar to the earlier theories. For low degrees of polymerization ($n_p < 8$), the decrease in k_A dominates and κ is marginally lowered with increasing polymer density.⁶

Although there have been a number of molecular dynamics simulations, which compare the scaling of polymer brushes on hard surfaces with predictions from the theory,^{14–16} the scaling results for polymer grafted lipid membranes have not been examined using molecular dynamics simulations. In a Monte Carlo simulation on a constant area membrane represented by a Helfrich Hamiltonian with a single quadratic curvature term and a finitely extensible nonlinear elastic potential for the polymers,¹⁷ the N^3 scaling for κ was captured extremely well, and scaling results for κ on the grafting density were captured asymptotically at high grafting densities.¹⁷ Since the scaling theory predictions were only captured at the higher grafting densities, the value of the exponents from different theories could not be differentiated.

Polymer grafted membranes are representative of a mesoscale system and the typical length and time scales associated with these systems limit their study with conventional atomistic molecular dynamics. In this regard dissipative particle dynamics (DPD) has emerged as a robust coarse grained simulation method and has been used extensively to study a wide variety of soft matter systems.^{18–22} In particular, DPD has been used to study behavior of polymers grafted onto surfaces^{23,24} and to investigate the influence of solvent quality on polymer brush morphology.²⁵ In the work by Pal and Seidel,²⁴ the scaling for the brush length with grafting density was found to agree well with the theoretical predictions. In a recent study we investigated the influence of polymer grafting on the melting transition of lipid bilayers⁸ and compared the changes in the melting transition and areal expansion with scaling theories for grafted membrane systems. Simulations were carried out using a modified Andersen barostat to obtain tensionless membranes made up of single tailed lipids, with polymers in the brush regime. The simulations were found to capture the areal expansion and lowering of the gel to liquid crystalline transition due to grafting, and the corresponding scalings were found to follow the self-consistent mean field results. In this paper we expand our earlier analysis⁸ and study the influence of grafting on the melting of smaller chain lipids and determine the bending modulus as a function of the grafting fraction using dissipative particle dynamics. We also analyze the changes to the bending modulus and partition the contributions arising from bilayer thinning and the presence of grafted polymer.

II. MODEL DETAILS

A. Dissipative particle dynamics

The governing equations of motion for a DPD simulation are standard, we however briefly present them here for completeness.^{26,27} The interactions are assumed to be pairwise and consist of conservative, dissipative, and random forces. The forces acting between a pair of particles i and j are

$$\mathbf{F}_{ij} = \mathbf{F}_{ij}^C + \mathbf{F}_{ij}^D + \mathbf{F}_{ij}^R, \quad (1)$$

where the dissipative force

$$\mathbf{F}_{ij}^D = -\gamma w_D(r_{ij})[\mathbf{e}_{ij} \cdot \mathbf{v}_{ij}]\mathbf{e}_{ij} \quad (2)$$

and the random force

$$\mathbf{F}_{ij}^R = \alpha w_R(r_{ij})\mathbf{e}_{ij}\theta_{ij}. \quad (3)$$

In the above equations, $\mathbf{r}_{ij} = \mathbf{r}_i - \mathbf{r}_j$, the distance between two particles, $r_{ij} = |\mathbf{r}_{ij}|$. The unit vector $\mathbf{e}_{ij} = \mathbf{r}_{ij}/r_{ij}$ and the relative velocity, $\mathbf{v}_{ij} = \mathbf{v}_i - \mathbf{v}_j$. γ and α are the strengths of dissipative and random forces, respectively, and θ_{ij} is the random force with zero mean and unit variance. It is a Gaussian white noise term satisfying the following conditions:

$$\theta_{ij} = \theta_{ji},$$

$$\langle \theta_{ij}(t) \rangle = 0, \quad (4)$$

$$\langle \theta_{ij}(t) \theta_{kl}(\tau) \rangle = (\delta_{ik}\delta_{jl} + \delta_{il}\delta_{jk})\delta(t - \tau).$$

In order to preserve Boltzmann statistics in the canonical ensemble, the relationship between the weight functions, $w_D(r) = [w_R(r)]^2$, results in the following fluctuation-dissipation relation.²⁶

$$k_B T = \frac{\alpha^2}{2\gamma}, \quad (5)$$

where $k_B T$ is the Boltzmann constant and T is the temperature.

Following the original prescription of the method, the weight functions used in Eqs. (2) and (3) are^{26,27}

$$w_R = \sqrt{w_D} = \begin{cases} 1 - r, & r \leq 1 \\ 0, & r > 1, \end{cases} \quad (6)$$

where r is scaled by σ , which is the cutoff radius for the interaction and defines an appropriate length scale for the DPD simulation. The conservative force between DPD beads is soft repulsive, and

$$\mathbf{F}_{ij}^C = \begin{cases} a_{ij}(1 - r)\mathbf{e}_{ij}, & r \leq 1 \\ 0, & r > 1. \end{cases} \quad (7)$$

The corresponding potential to $\mathbf{F}_{ij}^C$ is

TABLE I. Bonded interaction parameters.

	Lipid	Polymer
k_s	100.0	20.0
r_0	0.7	0.5
k_θ	10.0	...
θ_0	180°	...

$$u(r_{ij}) = \begin{cases} \frac{a_{ij}}{2}(1-r)^2, & r \leq 1 \\ 0, & r > 1. \end{cases} \quad (8)$$

In the above formulation and in the following text all units are reduced with respect to the energy $\epsilon=1$ where the reduced temperature, $T^*=k_B T/\epsilon$, size $\sigma=1$ and mass is unity. In what follows, $T^* \equiv T$.

B. Simulation details: Lipid and polymer models

The lipids consist of a single head and n tail beads (denoted as HT_n) with the membrane model based on the model used by Kranenburg *et al.*²⁰ for investigating melting of single tail lipid bilayers. The melting transition is investigated for lipids with $n=4$ and 6 and results for the bending modulus are carried out for the $n=9$ systems as well. Two lipid beads are connected by a harmonic potential,

$$U_{\text{bond}} = \frac{k_s}{2}(r_{ij} - r_0)^2, \quad (9)$$

where k_s is the spring constant and r_0 denotes the equilibrium distance between the two beads. A bond bending potential is introduced between two adjacent bonds (connecting three consecutive beads), which is

$$U_\theta = \frac{k_\theta}{2}(\theta - \theta_0)^2, \quad (10)$$

where k_θ is the bending spring constant and θ_0 is the equilibrium angle between two adjacent bonds. The values of the interaction parameters for the bonded interactions are given in Table I.

The polymer molecule consists of 20 beads denoted as AB_{19} . Consecutive beads on the polymer molecule are held together using the spring potential given in Eq. (9). The repulsion parameters used for the different species are given in Table II. The interaction parameters between the polymer bead and the solvent are such that the polymers are in a good solvent, in order to model hydrophilic polymers such as polyethylene glycol. The water (W) and lipid interaction parameter are similar to those used in previous DPD simulations of lipid bilayers.^{20,21} The polymer

TABLE II. Repulsion parameter a_{ij} of various beads. W represents the water bead. H and T represent the hydrophilic head and hydrophobic tail bead of the lipid, respectively. A and B represent the polymer beads.

a	W	H	T	A	B
W	25.0	15.0	80.0	25.0	15.0
H	15.0	15.0	80.0	-15.0	25.0
T	80.0	80.0	25.0	80.0	80.0
A	25.0	-15.0	80.0	80.0	80.0
B	15.0	25.0	80.0	80.0	80.0

is grafted to the head group (H) of the lipid molecules through the terminal bead A and the A-H interaction parameter is attractive (Table II). The amount of polymer in the system is measured in terms of the grafting fraction,

$$G_f = \frac{N_p}{N_l}, \quad (11)$$

where N_p represents the total number of polymer molecules and N_l is the total number of lipid molecules in the membrane. G_f was varied from 0 to 0.2 in this study.

Melting in the bilayer was monitored using the orientational order parameter,

$$s = \frac{1}{2} \langle 3 \cos^2 \theta - 1 \rangle, \quad (12)$$

where θ is the angle between the vector obtained by joining the first and last beads of the lipid with the bilayer normal.

The density profile was obtained by dividing the volume into 200 bins parallel to the bilayer surface. The density of a particular species i is

$$\rho_i(z) = \left\langle \frac{N_i(z + \Delta z/2, z - \Delta z/2)}{A \Delta z} \right\rangle, \quad (13)$$

where N_i is the number of particles of species i , A is the area of the bin of thickness Δz , and the brackets denote a time average. The membrane thickness d is defined as the distance between the peak positions of the head groups in the corresponding density distribution.

For the melting transition studies, polymer grafting is carried out in a manner similar to that used in our earlier work.⁸ A sphere of given radius, r_A , is drawn from bead A of the polymer. The head beads H of the lipid that lie in a distance $r < r_A$ are attached with a harmonic potential [Eq. (9)] with a spring constant of 20.0. A value of $r_A = 0.7$ was used and the A bead of the polymer was found to coordinate (on average) with 4 H beads. We have also carried out simulations for $r_A = 0.1$ and $k = 1.0$ and 100.0 to test the influence of the grafting method for $G_f = 0.2$ with 200 lipids. We found that the main transition temperature was unaffected in this range of parameters. For evaluating the bending modulus a different mode of attachment was used. Here the polymer bead (A) was chemically grafted to the head (H) group of the lipid bilayer using a harmonic potential with spring constant $k = 200$. In order to determine the influence of the grafting method on the melting transition, simulations were carried out for the chemically grafted HT₆ system at $G_f = 0.16$ and 0.09. Although some small differences are observed around the main melting transition, the comparison shown in Fig. 1 indicates that the transition temperature is unaffected by the method of grafting in this range of G_f values. Examination of the area per head group and bilayer thickness as a function of temperature did not reveal any changes either. For all simulations, the bilayer is maintained in a tensionless state using a modified Andersen barostat.⁸ Maintaining a zero tension across the bilayer is important while studying the melting transition and becomes crucial while determining the low q scalings while computing the bending modulus of the membrane.

The DPD simulations were carried out with 800 lipid molecules and 54 224 total particles in a tensionless state. A systematic comparison was also carried out for the melting transition using a 200 lipid system and the results were found to be unchanged. While determining the bending modulus a bilayer of 3200 lipids and a total of 216 896 particles were used. In all cases a preassembled bilayer was used as the initial configuration. The equations of motion are integrated using the modified velocity-Verlet algorithm²⁷ with $\Delta t = 0.01$ and a value of 23 was used for the tangential pressure components. At this pressure the corresponding solvent density is 3.0 at a temperature of 1.0. The details of the algorithm can be found in our earlier work.⁸ A heating protocol was used for the simulations, in which the bilayer is assembled at $T = 0.2$ and heated successively up to the maximum temperature. We also carried out a few cooling runs where we successively cooled the membrane from the L_α phase to a temperature below the main transition. The data shown in Fig. 2 did not show any evidence of hysteresis.

FIG. 1. Comparison of two different methods for polymer grafting. In method 1, a sphere of radius, $r_A=0.7$, is drawn from bead A of the polymer. The head beads H of the lipid that lie at a distance $r < r_A$ are attached with a harmonic potential [Eq. (9)] with a spring constant of 20.0. In method 2, the polymer bead (A) is chemically grafted to the head (H) group of the lipid bilayer using a harmonic potential with spring constant $k=200$. Method 1 was primarily used to study the melting transition, whereas method 2 was used for the study of elastic properties. The method of grafting does not effect the main transition temperature, although small differences in the values of the order parameter are observed around the melting transition.

FIG. 2. Comparison of two different simulation protocols. In the heating protocol, a preassembled bilayer is used and heated successively from $T=0.2$ up to the maximum temperature. In the cooling protocol, the bilayer is assembled at higher temperatures (above the melting transition) and cooled successively. Both protocols show the same transition temperature and hysteresis effects were not observed.

C. Bending modulus

The bending modulus is evaluated using a Fourier analysis of the height fluctuations.²⁸ The starting point is the free energy of a bilayer which is given by

$$F = \int \left(\gamma + \frac{\kappa}{2}(c_1 + c_2 - c_0)^2 + \bar{\kappa}c_1c_2 \right) dA, \quad (14)$$

where γ is the interfacial tension, κ is the bending modulus, and $\bar{\kappa}$ is the saddle-splay modulus. c_1 and c_2 represent the local principal curvatures, c_0 is the spontaneous curvature, and the integration is carried out over the entire membrane area A . Expressing the curvature in terms of a deviation from the midplane and noting that $\bar{\kappa}=0$, Eq. (14) in a Monge representation reduces to

$$E = \int (\gamma + \kappa(\nabla^2 h)^2) dx dy \sqrt{1 + (\nabla h)^2}, \quad (15)$$

where h is the local deviation from the midplane. Retaining terms up to quadratic order and using Fourier transforms along with the equipartition theorem, Eq. (15) reduces to

$$S(q) = \frac{k_B T}{\kappa q^4 + \gamma q^2}, \quad (16)$$

where $S(q) = \langle |h(q)|^2 A \rangle$. The equation indicates that the undulatory modes will dominate over stretching modes in the small q limit, provided that γ is sufficiently small. For wave numbers smaller than $q_c = \sqrt{\gamma/\kappa}$, the stretching modes become important. Hence, it is imperative to have a tensionless bilayer in order to extract the bending modulus. For wave numbers smaller than $q_0 = 2\pi/d$, the continuum theory is no longer valid and for tensionless membranes, protrusion modes begin to dominate over the undulatory modes.²⁹ The bending modulus can be obtained from the intercept of the log-log plot of $S(q)$ versus q . While computing the bending modulus, the simulation temperature was 1.5 to ensure that all the systems were in the L_α phase. In addition to the HT₄ and HT₆ systems we also compute κ for the HT₉ system. Equilibration consisted of 50 000 steps, and particle coordinates were collected every 50 steps over the next 950 000 steps for computing time averaged properties.

The surfaces, $z_u(x, y)$ and $z_l(x, y)$, formed by the headgroups on the upper and lower leaflets, respectively, are determined by a Delaunay triangulation, where the position of each of the head-group atoms is assigned to the vertices of a triangle. The method is easy to implement and does not involve surface fitting parameters.^{28,30} Interpolation of this triangulated surface on the regular $n_x \times n_y$ grid was carried out to obtain the surfaces $z_u(x, y)$ and $z_l(x, y)$. The surface $h(x, y) = \{[z_u(x, y) + z_l(x, y)]/2\} - z_0$, where z_0 is the mean position of the interface for a given configuration defined as $z_0 = (1/N_l) \sum_{i=1}^{N_l} z_{h,i}$, where $z_{h,i}$ is the z coordinate of the head group of the i th lipid. Fast Fourier transforms were performed on $h(x, y)$ to obtain the spectral intensities,^{31,32} $h(q)$. The time averaged magnitude of these intensities along with the ensemble averaged box area, A , is used to obtain the bending modulus from Eq. (16).

III. RESULTS

A. Influence of grafted polymer on bilayer melting transition

Figure 3(a) shows the order parameter, s , as a function of temperature for different grafting fractions and the corresponding membrane thickness changes are illustrated in Fig. 3(b) for HT₆ bilayer. The order parameter, s , provides the first indication of the structural changes that occur in the bilayer as the temperature is raised. At lower temperatures, the lipid chains are oriented along the normal to the bilayer headgroup plane and the bilayer is in the L_β phase. This low temperature structure is illustrated in the snapshot shown in Fig. 4(a) for $G_f=0.16$. The drop in s , as the temperature is raised, indicates that the bilayer is undergoing a melting transition to the L_α phase [see snapshot in Fig. 4(b)]. Although a small decrease in the values of s is observed, the melting

FIG. 3. (a) Order parameter, s , (b) membrane thickness, d , and (c) area per head group, a_h , as a function of temperature and grafting fractions, G_f , for the HT_6 bilayer. (a) The drop in the order parameter locates the main phase transition from the L_β to L_α phase. (b) The corresponding membrane thickness and (c) area per head group also show a sharp change at the onset of the transition. The melting transitions are observed only above $G_f=0.12$. Below $G_f=0.12$, the polymer only weakly effects the membrane.

FIG. 4. Snapshots of the lipid bilayer illustrating both the surfactant and grafted polymer configurations for the HT₆ system at $G_f=0.16$. (a) At low temperatures, the chains are highly ordered (L_β phase). (b) At high temperature, the disorder in the chain is clearly visible (L_α phase). The transition from the L_β to the L_α phase occurs at $T=0.7$.

temperature is relatively unaffected for $0.0 \leq G_f \leq 0.12$. At $G_f=0.16$, a downward shift in the melting temperature is observed, and the onset of melting shifts from $T=0.8$ to $T=0.7$. In addition to the order parameter we monitored both the membrane thickness, d , as well as the area per head group, a_h , which are shown in Figs. 3(b) and 3(c), respectively. The largest value of d is obtained for the L_β phase. As the temperature is raised, a_h begins to increase as the lipids begin to sample a larger available volume, which in turn leads to a drop in the membrane thickness. The decrease in the membrane thickness across the transition compensates for the increased hydrophobic core volume of the lipid tails and the increase in area per head group due to chain melting.³³ At higher temperatures the lipids are in a highly interdigitated state. These changes are clearly observed in the snapshots (Fig. 4). Both the variations of a_h and d with temperature are consistent with the changes observed with the order parameter, indicating that changes to the melting temperature occur only above $G_f=0.12$. The presence of grafted polymer increases the lateral pressure in the membrane, which leads to an increase in a_h , lowering the transition temperature.

The lipid tail density distributions for the HT₆ bilayer are illustrated in Fig. 5. At $G_f=0.09$ the density distributions are similar to those observed for membranes in the absence of polymer. At lower temperatures, polymer does not influence the ordering in the lipid tails and some layering of solvent adjacent to the bilayer is observed. At $T=0.7$, which is in the region prior to melting, the intensity of the density peaks is lower [Fig. 5(b)] for $G_f=0.16$ when compared with $G_f=0.09$ [Fig. 5(e)]. At $T=1.0$ both bilayers are in the L_α phase and both systems are at similar levels of disorder as indicated by the intensity of the peaks. Small differences are observed in the central regions of the bilayer where the presence of polymer has induced a slightly more disordered interior. Note that at $T=1.0$, a_h has increased from 0.68 at $G_f=0.09$ to 0.8 at $G_f=0.16$. The resulting increase in free volume is partly compensated by the decrease in membrane thickness, d , leading to a more interdigitated state. The degree of solvent penetration however is relatively unchanged with G_f as seen in the density distributions.

The data for the HT₄ system are given in Fig. 6. The qualitative trends are similar to those

FIG. 5. Lipid bead density profiles along the bilayer normal, z , for the HT₆ bilayers at various temperatures for grafting fraction [(a)–(c)] $G_f=0.16$ and [(d)–(f)] $G_f=0.09$. Dashed lines represent the water density profile. [(a) and (d)] The bilayer structures at low temperature are similar for both grafting fractions. [(b) and (e)] The lipid density peaks are lowered with an increase in polymer concentration as the temperature increases. [(c) and (f)] In the L_α phase, the level of disorder and the extent of water penetration are similar for both values of G_f .

observed for the HT₆ system. However the effect of grafting is stronger for the HT₄ system and the magnitude of changes induced in s , d , and a_h is larger. The changes at $G_f=0.16$ are particularly significant. Since the enthalpy for chain melting in the HT₄ system is lower, the transition temperature is decreased to a greater extent when compared with the HT₆ system. The transition temperature (identified as the temperature at which a sharp change in the order parameter is observed) for the HT₆ system undergoes a downward shift of 0.1 for a G_f change from 0 to 0.16. The corresponding downward shift is nearly doubled for the HT₄ system. At $G_f=0.16$, a drop in the order parameter occurs at $T=0.5$ and the transformation to the L_α phase is more gradual when compared with the relatively sharp transformation observed for the HT₆ system (Fig. 3). This gradual change is observed not only in the order parameter, s , but also in the a_h as well as d variations with temperature. The influence of polymer is observed at a lower temperature for the HT₄ system. The smaller chain length allows for lateral expansion as revealed in the a_h data at lower temperatures. Since the temperature is low, there is insufficient thermal energy to cause chain melting and the drop in the order parameter is not as sharp as observed for the HT₆ system, where the changes occur at a higher temperature. Hence the complete transformation to the L_α phase occurs over a broadened temperature range for the HT₄ bilayer.

The density distributions for the HT₄ system at two different G_f values are shown in Fig. 7. The influence of polymer is clearly seen at $T=0.7$, where the density distributions at $G_f=0.16$ [Fig. 7(b)] indicate an interdigitated bilayer, whereas the corresponding distributions at $G_f=0.09$ [Fig. 7(e)] show a significantly more ordered bilayer. At $G_f=0.16$ the extent of disorder induced in the membrane is nearly complete at $T=0.7$ and small changes are observed as the membrane is further heated to $T=1.0$ [Fig. 7(c)]. The solvent density distribution reveals that solvent penetration occurs to a similar extent for both the HT₄ and HT₆ bilayers. The above results for the HT₄ and HT₆ bilayers present an expansion of our earlier results⁸ to include data at $G_f=0.12$ as well as the changes in the membrane thickness as a function of grafting fraction.

Figure 8 illustrates the polymer and solvent density distributions for various cases at $T=1.5$. These density distributions correspond to the situation where the polymer is chemically grafted to the lipid molecule. The height fluctuations of these systems were used to evaluate the bending modulus in the following section. In all cases a drop in the solvent density is observed in the region adjacent to the bilayer. This decrease in density is attributed to the association of solvent with the polymer as well as penetration of the solvent into the bilayer. The decrease in density is

FIG. 6. (a) Order parameter, s , (b) membrane thickness, d , and (c) area per head group, a_h , as a function of temperature and grafting fractions, G_f for the HT_4 bilayer. In comparison with the HT_4 bilayer grafted polymer is seen to have a stronger effect on the membrane properties. At $G_f=0.16$, the transition is shifted downward in comparison with the HT_4 system and changes in the membrane properties occur over a broader temperature window.

FIG. 7. Lipid bead density profiles along the bilayer normal, z , for HT₄ bilayers at various temperatures for grafting fraction [(a)–(c)] $G_f=0.16$ and [(d)–(f)] $G_f=0.09$. Dashed lines: solvent density profiles.

larger as the temperature is increased. Away from the bilayer headgroups, the solvent density attains a constant value. Similar trends were observed in the density distributions illustrated in Figs. 5 and 7. These trends have also been observed in the simulations of polymer free membranes by Kranenburg and Smit.²⁰ The deviation from a bulk solvent density of 3.0 is the smallest in the absence of polymer and increases as the amount of polymer is added. Figure 8 illustrates that there is sufficient solvent beyond the polymer to prevent spurious interactions between polymers on the opposite leaflet due to periodicity in the direction normal to the bilayer plane.

FIG. 8. Polymer bead density profiles along the bilayer normal, z , at $T=1.5$. The numbers correspond to the polymer beads with the first bead (1) chemically grafted to the headgroups. The other polymer beads and lipid beads are not shown for clarity. The dashed line corresponds to the solvent density profile, indicated on the right y-axis. The solvent density drops in the region adjacent to the bilayer headgroups due to penetration into the headgroup region as well as association with the grafted polymer.

FIG. 9. Frequency spectra, $S(q)$, for various lipid-polymer systems at $T=1.5$. The solid line corresponds to the q^{-4} fit, which is extremely well captured in each case. The dashed line corresponds to the -2 scaling for the protrusion modes. These modes are not clearly resolved in all cases.

B. Bending modulus

The bending modulus, κ , is obtained from the q^{-4} scaling determined from the Fourier transform of the height fluctuations as discussed earlier. All the membranes are in a tensionless state by virtue of the barostat,⁸ and the κ values are determined for membranes in the L_α phase at a temperature of $T=1.5$. To prevent desorption of the polymer from the membrane the bending modulus was computed using polymers chemically grafted to the head group as described earlier. This method of grafting is also similar to the polymer grafted lipids that are used in the experiments. The $S(q)$ versus q data for some representative cases for the HT₄ and HT₆ systems are illustrated in Fig. 9. In all cases the low q scalings are captured extremely well and the system sizes are large enough to reliably extract the bending modulus. Figures 9(a) and 9(c) show the data for the HT₄ and HT₆ bilayers at $G_f=0.0$. For the HT₄ bilayer, the membrane thickness d as determined from the distance between the headgroup peak positions is 4.3. This distance corresponds to a value of $q_0=2\pi/d=1.48$, above which protrusion modes are expected to dominate³⁴ the spectral response ($q>q_0$). This can be observed by a deviation from the q^{-4} scaling in Fig. 9(a). However, the q^{-2} scalings which are evidence of the protrusion modes^{29,34} are not clearly captured in our simulations. This is particularly true in the presence of polymer [Figs. 9(b) and 9(d)]. These modes have been attributed to collective lipid protrusions which would have to be sufficiently long lived in order to be captured in a simulation and have been observed in simulations of bilayers with united atom models³⁴ as well as in the spectral analysis of monolayers.²⁸ However, in a spectral analysis of the height fluctuations in a DPD simulation of the two tailed lipid models by Sugii *et al.*,³⁰ the scalings for the protrusion modes were not clearly captured. Since the -4 scalings that capture the bending modes shift to lower values of q , the number of points available for obtaining this scaling decrease as the membrane thickness increases.

The effect of polymers on the elastic properties of lipid membranes has been analyzed using mean field and scaling theories.^{6,9,12} The presence of polymer increases the area per head group, a_h , which decreases the area stretch modulus, k_A , as well as the membrane thickness, d . The combined effect of the polymer on the bending modulus of the membrane can be expressed as⁶

$$\kappa = \frac{k_A(G_f, a_h)}{\alpha} d^2 + \beta \left(\frac{G_f}{a_h} \right)^p. \quad (17)$$

In the above expression, the area stretch modulus, k_A , is a function of both G_f and a_h , d is the membrane thickness, α is a proportionality constant, $p=5/2$ in the mean field theory, and $7/3$ in the self-consistent field theory. Since the temperature, polymer length, and monomer size are held fixed in our simulations, β is a constant for a given value of p . The first term on the right hand side of Eq. (17) represents the influence of the bending modulus from a continuum analysis.³⁵ An increase in G_f increases k_A due to the elastic contribution from the brush alone, and the increase in a_h due to the presence of the polymer lowers k_A . These opposing effects have been shown to partially compensate each other to some extent, resulting in a weak dependence of k_A due to the grafted polymer.⁶ The relative insensitivity of k_A to G_f in the low to intermediate grafting regime has also been observed in micropipet experiments with polymer grafted lipid vesicles.³⁶ The changes in k_A further diminish when the degree of polymerization is small and G_f is low, as is the case for our simulations. In what follows, we are only interested in estimating the relative contributions from each of the two terms on the right hand side of Eq. (17). For convenience of notation we set $k_A = \epsilon k_{A0}$, where k_{A0} is the bare membrane expansion modulus, and $0 < \epsilon$ represents the change due to grafting. For the low values of G_f and degree of polymerization, $n_p=20$ used in our study, ϵ is estimated to range between 0.8 and 1.2 from the analysis for monolayers and bilayers by Marsh.⁶ Noting that the bare membrane modulus

$$\kappa_0 = k_{A0} d_0^2 / \alpha, \quad (18)$$

Eq. (17) can be rewritten as

$$\frac{\Delta \kappa}{\kappa_0} = \left(\frac{\epsilon d^2}{d_0^2} - 1 \right) + \frac{\beta}{\kappa_0} \left(\frac{G_f}{a_h} \right)^p, \quad (19)$$

where $\Delta \kappa = \kappa - \kappa_0$, and the two terms on the right hand side of Eq. (19) will henceforth be referred to as $\Delta \kappa_I$ and $\Delta \kappa_{II}$, respectively. Since we have evaluated the membrane thickness d as a function of G_f , Eq. (19) can be used to estimate the relative contribution to changes in κ from changes in d by explicitly evaluating $\Delta \kappa_I$. This term is always negative when $0 < \epsilon \leq 1$ and corresponds to a net decrease in the bending modulus contribution from $\Delta \kappa_I$, both due to a decrease in d as well as a decrease in k_A over the polymer free bilayer. If $\epsilon > 1$ then the first term can be either positive or negative depending on the ratio of d/d_0 . Since $\Delta \kappa/\kappa_0$ [Eq. (19)] is known from the simulation, $\Delta \kappa_{II}$, which represents the effect of polymer contributions explicitly to the changes in the bending modulus, can be obtained as a difference. Using the values of d and the values of $\Delta \kappa/\kappa_0$ obtained from the Fourier analysis, we evaluated the contribution from $\Delta \kappa_I$ and $\Delta \kappa_{II}$. In the first part of the analysis we set $\epsilon=1.0$. In this case, membrane thinning by a reduction in d has a negative contribution to $\Delta \kappa$ and opposes the increase in κ from the grafted polymer as reflected in $\Delta \kappa_{II}$ in Eq. (19). The changes in membrane thickness are illustrated in Fig. 10 as a function of G_f . The decrease in d is larger as the tail length is increased with a 25% change for the HT₉ system. Figure 11(a) illustrates the contributions from $\Delta \kappa_I$ and $\Delta \kappa_{II}$ to the overall change in the bending modulus as a function of G_f . The contributions from changes in d lower the overall bending modulus of the membrane as the grafting fraction is increased. This decrease is not sensitive to the bare membrane thickness, d_0 , of the membrane and the data for $\Delta \kappa_I$ from the different systems collapse onto a single curve in this scaled representation. The polymer contribution to the relative change in the bending modulus is reflected in $\Delta \kappa_{II}$, where the largest relative changes occur for the thinner membranes. The results indicate that the dominant contribution to changes in κ is from the grafted polymer. As one would expect from physical considerations, the largest contributions from changes in d are observed at the highest values of G_f . Hence for moderate grafting densities, the mechanical properties of the grafted membrane are only weakly influenced by changes induced in the membrane itself and the dominant contributions arise from the presence of polymer. Note that

FIG. 10. Relative change in the membrane thickness as a function of the grafting fraction G_f . The membrane thickness $d_0=4.38$, 5.91, and 8.07 for HT₄, HT₆, and HT₉, respectively.

this effect would be further weighted toward the polymer as the length of the polymer chain is increased provided ϵ remained close to unity.

In order to evaluate ϵ , values of k_A as a function of G_f are required. However the evaluation of k_A is not entirely straightforward as illustrated in several molecular dynamics simulations of bilayer membranes.^{29,37,38} The values of k_A are a strong function of the lateral system size, decreasing with an increase in the system size. This decrease is attributed to the coupling of long wavelength undulation modes to the areal compression. Thus smaller system sizes minimize this coupling and the projected area is expected to be closer to the true area. Smaller system sizes, however, have a stronger influence from periodicity. We also observed a similar trend where the k_A values obtained from areal fluctuations for the HT₄ polymer free membrane were 9.36, 7.61, and 5.83 for systems containing 200, 800, and 3200 lipids, respectively. Given this uncertainty in computing k_A directly, we estimate the contributions to the bending modulus from the changes in k_A using the analysis developed by Marsh *et al.*³⁹ Based on a free energy proposed by Israelachvili⁴⁰ for a tensionless polymer free membrane, the expression for the stretch modulus which incorporates the effect of grafted polymer (in reduced units) is³⁹

$$k_A = k_{A0} \left(\frac{a_{h0}}{a_h} \right)^2 + 2m(1+m)Tb^{2m}n_p \left(\frac{G_f}{a_h} \right)^{(m+1)}. \quad (20)$$

The first term on the right hand side of Eq. (20) represents contributions from changes in a_h and the stretch modulus decreases as the square of a_h . The second term on the right hand side of Eq. (20) represents contributions from the polymer. In the above expression the exponent $m=5/6$ in the scaling theories and $m=2/3$ for the mean field theory. In DPD units corresponding to the conditions of this study, $T=1.5$ is the reduced temperature, $b=1$ is the reduced size of the monomer, and $n_p=20$ is the degree of polymerization. Since the right hand side of Eq. (20) is known exactly, the value of ϵ can be directly estimated. A value of $n_p=20$ maps into a polymer of molecular weight 840 if one assumes that each bead consists of three methylene beads. For HT₄, we used $k_{A0}=9.36$ and for the HT₉ system we used a value of 9.32 obtained from NVT simulations. In Fig. 11 the influence of varying ϵ on the two contributions $\Delta\kappa_I$ and $\Delta\kappa_{II}$ is illustrated. For the HT₄ system, $\epsilon=1.048$ ($G_f=0.04$), 1.12 (0.09), and 1.19 (0.16), and for the HT₉ system ϵ

FIG. 11. Partitioning contributions to the overall change in the bending modulus from membrane thinning, $\Delta\kappa_I$, and from the polymer $\Delta\kappa_{II}$ [see Eq. (19)] as a function of grafting fraction for HT_4 , HT_6 , and HT_9 . (a) The stretch modulus was assumed to be a constant and $\epsilon=1$. In (b) the values of ϵ obtained from Eq. (20) were used. The bending modulus of the grafted membrane is only weakly influenced by changes induced in the membrane itself ($\Delta\kappa_I$) and the dominant contributions arise from the presence of polymer ($\Delta\kappa_{II}$). Solid lines: $\Delta\kappa_I$; dashed lines: $\Delta\kappa_{II}$.

$=1.046(G_f=0.04)$, 1.204 (0.16), and 1.236 (0.2). The corresponding values of $a_h=0.7609(G_f=0.0)$, 0.7597 (0.04), 0.7830 (0.09), and 0.8726 (0.16) for the HT_4 system and $a_h=0.7918(G_f=0.0)$, 0.7899 (0.04), 0.8936 (0.16), and 0.9592 (0.2) for the HT_9 system. Since $\epsilon>1$ the values of $\Delta\kappa_I$ show positive deviations at intermediate grafting fractions. Hence the values of $\Delta\kappa_{II}$ are

FIG. 12. The bending modulus κ as a function of grafting fraction G_f for three different lipids: HT₄, HT₆, and HT₉. The bending modulus increases with the grafting fraction in all cases; however the increase is smaller than that predicted by scaling theories.

lowered when the changes in k_A due to changes in G_f and a_h are explicitly included. However the percentage contributions to the overall change in the bending modulus are similar to that observed with $\epsilon=1.0$. We note that the values of k_A obtained from areal fluctuations for the HT₉ system varied between 15.279 and 13.65 for the 200 and 3200 lipid systems and did not show the typical decrease with system size that was observed for the shorter lipids. A larger value of k_A in the analysis presented above only lowers the deviation of ϵ from unity.

Figure 12 illustrates the changes in the bending modulus κ as a function of grafting fraction G_f for HT₄ and HT₆ as well as HT₉. κ is seen to increase even for the lowest G_f values investigated. We attempted to fit our data to the power law scalings for G_f ; however, in all cases the increase in κ with G_f was lower than that predicted by the theory. Additional simulations with longer polymers and higher grafting fractions might be required to test these scalings.¹⁷ Similar deviations from theory were observed for κ values obtained in low grafting, micropipet experiments of Evans and Rawicz,³⁶ and the scalings regimes were only approached asymptotically at high grafting fractions in the work by Laradji.¹⁷

C. Areal expansion

The presence of polymer leads to a lateral expansion of the membrane and here, we compare our DPD simulation results with the predictions for scaling theories for polymer grafted membranes. In the region of small areal changes, scaling and mean field theories predict that the areal expansion, Δa_h , scales as³⁹

$$\frac{\Delta a_h}{a_{ho}} \approx mT(G_f/a_{ho})^{m+1}, \quad (21)$$

where $\Delta a_h = a_h - a_{ho}$, a_{ho} is the head group area in the absence of grafted polymer, and m is the scaling exponent which is 5/6 for the scaling theory and 2/3 for the mean field theory. Figure 13 illustrates that the data obtained from the DPD results fit the scaling theory predictions with $m = 5/6$ reasonably well. The typical standard deviations are within the symbol sizes. Since the changes in a_h occur only at the higher temperatures, we tested the theory for bilayers in the L_α

FIG. 13. Increase in the bilayer area per head group [Eq. (21)] as a function of $(G_f/a_{ho})^{1+m}$. The data are in reasonable agreement with the scaling theory, where $m=5/6$. Circles: $T=1.0$; triangles: $T=0.9$; solid symbols are for HT₄ bilayer whereas open symbols are for HT₆ bilayer.

phase. The data in Fig. 13 illustrate that the percentage a_h deviations from polymer free bilayers are within the range typically observed in experiments.⁷

IV. SUMMARY AND CONCLUSION

The melting transition and bending moduli of polymer grafted bilayers have been investigated using DPD simulations for lipid bilayers. Simulations are carried out using a modified Andersen barostat and the bilayers are maintained in a tensionless state. The increase in the area per head group and decrease in melting transition as the grafting fraction (G_f) is increased are in qualitative agreement with experimental observations. For the HT₆ [one head (H) bead and six tail (T) beads] lipids, the melting transition is unaltered for grafting fractions, G_f , up to 0.12, whereas changes, although small, are observed for the HT₄ system. The decrease in the melting transition above $G_f=0.12$ is greater for the HT₄ bilayer when compared with the HT₆ bilayer. The increase in area per head group, as a function of the grafting fraction, is in reasonably good agreement with the scaling theory results for grafted lipid membranes.

The bending moduli are evaluated from the Fourier transform of the height fluctuations using a Delaunay triangulation representation of the surface formed by the headgroups. Using results from continuum and scaling theories, we estimate the percentage contribution to the overall bending modulus from the changes in the membrane thickness. If the membrane stretch modulus is assumed to be independent of the grafting fraction, a decrease in membrane thickness lowers the bending modulus. However the corresponding contribution from bilayer thinning is typically within 10% of the overall change in the bending modulus for the HT₄ and HT₆ bilayers and increases to about 15% for the longer chain HT₉ lipids. Incorporating changes in the stretch modulus did not significantly change these findings. For the range of grafting fractions investigated in this study the changes in the bending modulus as a function of G_f were lower than that predicted from scaling and mean field theories. The length of the polymer chains range from about two to five times the number of tail beads in the lipid molecule and the maximum value of $G_f=0.2$. Additional simulations with longer polymers and higher grafting fractions are likely to be required in order to make a comparison with the scaling results. The results presented in this paper indicate that DPD simulations are able to capture the essential features that are present in these

complex polymer-lipid systems. However in order to make quantitative comparison with experiments a systematic coarse graining procedure with parameter estimation is clearly required.

ACKNOWLEDGMENTS

We would like to acknowledge Prabal Maiti and V. Kumaran for several useful discussions during the progress of this work. The snapshots are prepared using visual molecular dynamics (VMD).⁴¹ One of us, K.G.A., would like to thank Nicholas Spencer, Department of Materials at the ETH Zurich for the hospitality and support extended during his sabbatical. We are also grateful to the referee's for their critical and careful reading of the manuscript.

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