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Intersecting polymers in lipid bilayers: cliques, static order parameters and lateral diffusion

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We have modelled a macrolipid polymer composed of lipid molecules (monomers) embedded in a lipid bilayer or monolayer and polymerized via their polar groups. Because of fluctuations perpendicular to the plane of the bilayer, the polar region occupied by the polymer chain possesses sufficient space so that the polymer might exhibit 'self-intersection' if its conformational state is projected onto the plane of the bilayer/monolayer. We represent the plane of the bilayer/monolayer by a triangular lattice. Each site can be occupied by a monomer or be empty (and thus occupied by one of the unpolymerizable lipids which make up the bilayer/monolayer). A macrolipid is represented by a sequence of N monomers connected by $N-1$ bonds. Bonds may be either short (connecting nearest neighbour monomers) or long (between second neighbour monomers), in accord with the average properties of the spacers between the polymerized lipids. We have carried out computer simulation of this system using the Carmesin-Kremer bond stretching algorithm. Although no two monomers can occupy the same site, bonds may cross each other. We analyzed the dependence of $\langle R^2 \rangle$ and $\langle R_G^2 \rangle \sim N^{2\nu_c}$ and $\langle N_{sc} \rangle + \langle N_{mc} \rangle \sim N^{2\sigma_c}$, where N_{sc} and N_{mc} are the number of bond-crossings in the same macrolipid ('self-crossing') or in two different macrolipids ('mutual-crossing'). For single macrolipids, we confirmed that $\nu_c = 3/4$ and have found that $\sigma_c \approx 0.52$, which we consider supports that $\sigma_c = 1/2$. For the dense case with monomer concentration, $c = 0.72$, we found that $\nu_c = 1/2$ and that $\sigma_c \approx 0.52$ supports that $\sigma_c = 1/2$. In the semi-dilute regime ($c = 0.2$) we found crossover behaviour, although $\sigma_c = 1/2$. The total number of bond crossings thus scale like N, independent of concentration. We studied the connectivity of the system by calculating the weight averaged cluster, or 'clique', size. Cliques are defined as being composed of all macrolipids which exhibit at least one crossing bond with one other member of the clique. We found that while the average clique contains about two macrolipids at low concentrations, the clique size approaches the maximum possible value at high concentrations if the macrolipids are sufficiently long. In the latter case a transition appears to occur as the macrolipid length increases. This transition occurs at length = 40 when $c = 0.72$. These observations should have experimental consequences for the viscoelastic properties of the system. We defined an order parameter, B, and showed that although B depended upon c, it appeared to be independent of N for both crossing and non-crossing macrolipids. We calculated the dependence of the lateral diffusion coefficient upon c for some values of N and compared it to the case of non-crossing macrolipids and single lipid molecules. We suggest how the existence of crossing bonds might be observed.

Introduction

In recent years it has become possible to construct polymerized quasi-two-dimensional systems and so make available systems of polymers moving in essentially two dimensions. These are polymerized lipid bilayers or monolayers, or lipid bilayers and monolayers into which polymers are embedded [1–3]. Here we are interested in polymers which are composed of lipid molecules linked via their polar groups. These 'macrolipids' have their lipid hydrocarbon chains embedded in the lipid bilayer membrane, or monolayer, which thus acts as a quasi-two-dimensional solvent for the hydrocarbon chain region of the macrolipids, while the polymer backbones, to which the polar groups are attached, run in the water layer or near the membrane/water interface. The molecular spacers separating the lipid polar groups from each other along the length of such a macrolipid can give rise to different physical systems. The polar groups are located at the membrane/water interface, so that if the molecular

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spacers are short ($\sim$ 1 nm) then the physical picture is of a polymer, constrained to lie in two dimensions, at the membrane/water interface [4]. At the other extreme if the molecular spacers between polar groups are very long ($>$ 10 nm) then the physical picture is one of a polymer in a three-dimensional half-space constrained to touch the membrane/water interface at intervals [5]. Apart from their intrinsic interest, the fact that such systems can be constructed makes it of interest to study their properties theoretically.

Theoretical studies of polymers in two dimensions have a long history [6] and in the last decade substantial advances have been made in the study of dense polymeric systems in two dimensions [7]. Here we want to model a class of polymers in two dimensions which, because of the properties of the bilayer or monolayer interface, might possess characteristics not normally encountered in polymer studies. In general, polymer models have recognized the fact of the excluded volume interaction by requiring that two objects cannot occupy the same volume of space, or the same lattice site, simultaneously. Some models, on the other hand, have ignored this requirement completely. In the last decade, studies of 'trails' have relaxed this restriction to permit certain multiple occupancies of lattice sites though they do not permit bonds to overlap [8–13].

Here we present a model of the macrolipids described above, in which the spacers separating the lipid polar groups possess a length of about 1 nm. This is approximately the diameter, $\sim$ 0.75 nm, of a lipid molecule in its fluid state projected onto the plane of the bilayer. The cross section of the spacer itself is assumed to be much less than this. The spacer is assumed to be sufficiently flexible so that the pair of lipids connected by it can be adjacent or as much as about one lipid diameter apart. One consequence of this is that, when the spacer is extended, there is sufficient room for a segment of a macrolipid to pass underneath that spacer bridge between two lipids. This might come about because the lipid bilayer or monolayer is a 'soft' interface, i.e., it exhibits large (on the scale of a C-C bond length) thermal fluctuations perpendicular to the average plane of the membrane. Because of this, sufficient space can be created for one segment of a macrolipid to pass under a spacer belonging to itself or to another macrolipid. The implication of this for a model of such a system is that, viewed as a purely two-dimensional system, some portions of the macrolipid, namely the lipid molecules, must exhibit the excluded volume interaction while other portions of the macrolipid, namely the spacers connecting the lipids, may cross each other.

In the next section we shall describe a model of this system and investigate some of the properties of both a dilute and a dense system of this kind. The question in which we are interested is, to what extent does one macrolipid penetrate the space of another and what are the experimental consequences of such effects. Recent computer simulation studies on dense systems of polymers confirmed that, in two dimensions, polymers define a relatively compact area from which other polymers are excluded [7]. That is, the areas of phase space available for polymers to move in two dimensions does not permit the intertwining of any two polymers [6,7,14]. Our intention here is to see to what extent this is maintained if restricted macrolipid (i.e., polymer bond) crossing is permitted.

Here we shall study the statics of single macrolipids and dense systems of them. We shall define an order parameter and see how quantities such as the radius of gyration and number of crossing bonds depend upon degree of polymerization, i.e., the polymer length. We shall then model the lateral diffusion of macrolipids, attempt to predict its dependence upon polymer length and concentration and say to what extent measurements of diffusion can distinguish between such crossing and non-crossing systems.

The model

We consider one half of a lipid bilayer, or a monolayer, composed of two kinds of lipids: lipid molecules which are not part of a polymer, and polymerized lipids which form macrolipids as described above. Fig. 1 shows an idealization of this system. Here (Figs. 1A

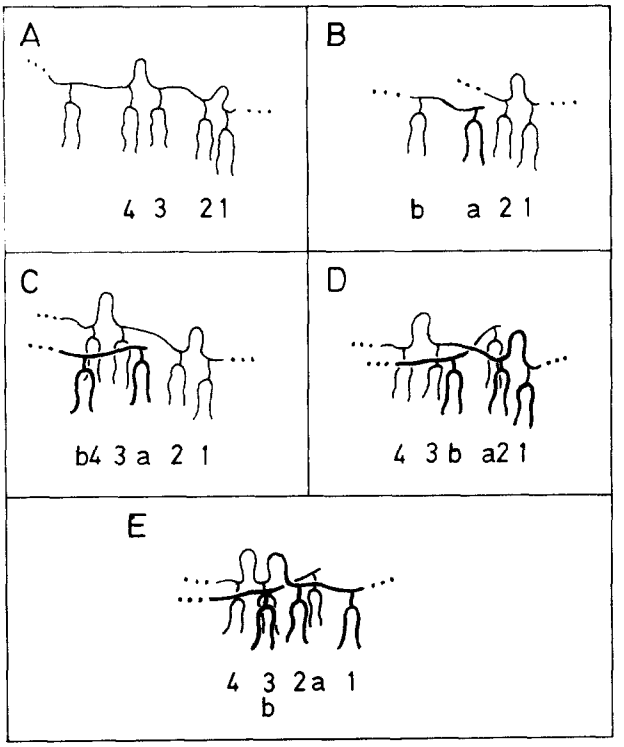

Fig. 1. Examples of bond crossing by polymerized lipids in one half of a planar lipid bilayer. In B, a-b cannot pass between 1 and 2, but they can pass between 2 and 3 in C, which they have done in D. In E, 2 has moved closer to 3 and a-b cannot move.

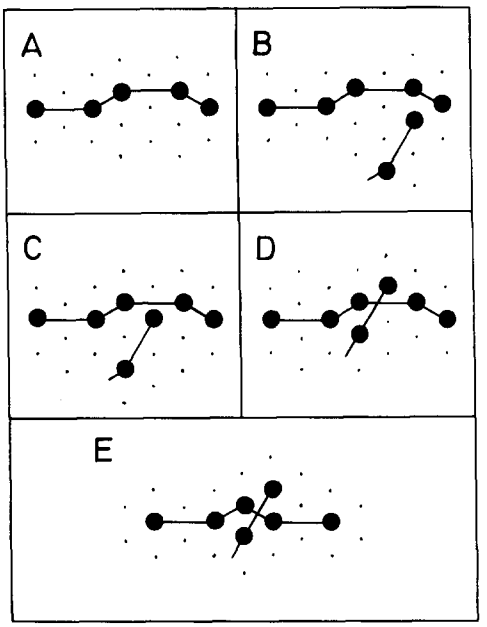

Fig. 2. The representation of the examples of Fig. 1, on a triangular lattice. Polymerized lipids (filled circles) move between nearest neighbour sites. Bonds extend between nearest, or second nearest, neighbours.

and B) four successive lipids along a polymer backbone have been labelled 1,...,4, while two other (adjacent) lipids are labelled a and b. It is understood that the remainder of the plane of one half of the lipid bilayer is filled with unpolymerizable lipid molecules. The intent of this is to illustrate how spacer crossing can occur. In Fig. 1B lipid a cannot pass between 1 and 2, because the spacer connecting them is not extended and they are adjacent. In Fig. 1C, however, there is sufficient space for a to pass between 2 and 3, and this can be achieved via the thermal fluctuations of the plane of the membrane. This is shown to have occurred in Fig. 1D. In Fig. 1E, lipid 2 has moved to become a nearest neighbour of 3 thereby preventing lipid b from moving towards lipid a. We will refer to loops such as that between 3 and 4 as short bonds and extended spacers, such as that between a and b, as long bonds.

In order to model this system, we represent the plane of the bilayer by a triangular lattice, each site of which can be occupied by a lipid molecule (Fig. 2). The backbone of the polymer is indicated by lines joining lipid molecules and we assume that adjacent lipid molecules along the polymer may be either nearest neighbours and joined by a short bond, or second neighbours and joined by a long bond. Sites which are not occupied by polymerized lipids are understood to be occupied by unpolymerized lipid molecules. Figs. 2A–E shows the representation of the examples of Figs. 1A–E. Here, polymerized lipids are represented by filled circles at lattice sites and the bonds connecting them are represented by heavy lines. We shall refer to the filled circles as monomers. This figure is not intended to show the process by which the two polymers intersect: Clearly a number of steps are needed for the system to develop from the configuration of Fig. 2B to that of Fig. 2D. However, the step from the latter to Fig. 2E should be clear since it involves only the movement of the lipid labelled 2 in Fig. 1.

The model is intended to represent the bilayer or monolayer at a temperature, T, sufficiently greater than the gel-fluid transition temperature, T_m, of the lipid hydrocarbon chains so that the system is in a homogeneous phase.

Computer simulation

We shall study the system described here using computer simulation. One Monte Carlo (MC) step will consist of visiting each monomer once and only once in a random sequence, and attempting to move each monomer by one lattice constant in a randomly chosen direction. Two monomers may not occupy the same site, and bonds are constrained to be either short (between nearest neighbour monomers) or long (between second neighbour monomers). In moving monomers from one site to a nearest neighbour site, we are modelling the displacement undergone by a lipid in one time increment. If the monomer moves to an empty site then a polymerized lipid has exchanged positions with an unpolymerized one. The exchange of two monomers represents the exchange of two polymerized lipids. We shall, however, ignore processes which involve the exchange of two monomers. The reason is that essentially all such processes either result in bonds which stretch beyond the maximum allowed length of $\sqrt{3}$ (in units of the lattice constant) or else represent unphysical processes such as the physical motion of one macrolipid either through another one or through a part of itself. The only exception appears to be, in some cases, the movement of segments of macromolecules along the directions in which the backbones are locally oriented. In order to make our simulations faster, we shall ignore all such processes while realizing that we shall be excluding a small number of possible movements. It is clear that the freedom for a bond to be short or long as the monomers move, is analogous to the 'bond-stretching' model used by Carmesin and Kremer [7] to study densely packed polymers in two dimensions. The requirement, used there, that bonds do not cross, is now relaxed: bonds may cross, as shown in Fig. 2, as long as the restrictions listed above are not violated.

We shall model lateral diffusion as has been done successfully elsewhere [15–17]. Because we are concerned with temperatures, $T > T_m$, the dominant forces acting on the system are random. Further, there is only one time-scale in the region of 10^{-6} s, namely that

characteristic of the time taken for a single lipid molecule to move through a distance equal to its diameter. This can be seen by considering that, with a single-lipid diffusion coefficient, $D_0 \approx 8\ \mu m^2/s$, and a lattice constant equal to the approximate effective diameter of a lipid molecule, of 0.7 nm, the increment of time equivalent to one Monte Carlo step is $\sim 10^{-7}$ s.

We shall write $\langle R^2 \rangle \sim N^{2\nu_c}$, $\langle R_G^2 \rangle \sim N^{2\nu_c}$ and $\langle N_c \rangle \equiv \langle N_{sc} \rangle + \langle N_{mc} \rangle \sim N^{2\sigma_c}$, where $\langle R^2 \rangle$ is the average end-to-end length squared of a macrolipid, $\langle R_G^2 \rangle$ is the average radius of gyration squared, and $\langle N_{sc} \rangle$ and $\langle N_{mc} \rangle$ are the average numbers of bond-crossings involving the same macrolipid (sc, self-crossing) or different macrolipids (mc, mutual crossing). We shall also compute $\langle r_{mon}^2 \rangle_M$ the average distance (squared) moved by all the monomers making up the macrolipids, in M Monte Carlo steps. The lateral diffusion coefficient, D, is then

$$D(N,c) = 4\langle r_{mon}^2 \rangle_M / M \tag{1}$$

where we recognize that D might depend upon both the number of (lipid) monomers in a macrolipid, N, and the total monomer concentration, c.

We will define an order parameter, B,

$$B = \tfrac{1}{2}((((\langle B_l \rangle - \langle B_s \rangle)/L) + 1) \tag{2}$$

where $\langle B_l \rangle$ and $\langle B_s \rangle$ are the average numbers of long and short bonds, and L is the total number of bonds/macrolipid. We shall study how these quantities depend upon macrolipid concentration and L. The former will be defined as the fraction of all lipid molecules which make up the macrolipids.

In a system which exhibits the gelation of unit objects each of mass M_1, the viscosity depends upon the weight-averaged molecular weight, M_w, defined to be

$$M_w = M_1 \sum_{n=1} n^2 P(n) / \sum_{n=1} nP(n) \tag{3}$$

where $P(n)$ is the number of clusters or 'cliques' containing n unit objects (e.g., Ref. 18). In order to see whether any effect might be observed in a measurement of shear viscosity, we shall calculate the weight averaged clique size, defined by Eqn. 3, where a clique is defined as being composed of all macrolipids which exhibit at least one crossing bond with one other member of the clique. We shall calculate the average value of M_w, $\langle M_w \rangle$, as well as the distribution of the macromolecules into cliques. This will be seen by plotting

$$D_w(n) = M_1 n^2 P(n) / \sum_{n=1} nP(n) \tag{4}$$

as a function of n. Since it is the crossing of bonds which might give rise to viscous effects, our definition of a clique, to be composed of all macrolipids which exhibit at least one crossing bond with one other member of the clique, is reasonable. Each unit object is thus a single macrolipid and so M_1 corresponds to the degree of polymerization, N, the number of monomers per macrolipid. In our calculation, the maximum possible value of Eqn. 3 is the total number of monomers so that if we divide Eqn. 3 by the size of the lattice then the upper bound is the monomer concentration c.

Some results are already known. From the study of trails one knows that, in the case of a single polymer, $\nu_c = 3/4$, which is the same result as that for non-crossing polymers in two dimensions. It can also be shown that $\sigma_c = 1/2$ for single polymers (Whittington, S., private communication). We shall present results for these cases so as to confirm that our simulations are sufficiently correctly sampling the space of macrolipid conformations.

In order to obtain reliable values for D it is necessary to know how the longest relaxation times for macrolipid motion depends upon the length of the macrolipid, L. We must know this in order to ensure that we are in the regime in which $\langle r_{mon}^2 \rangle_M$ is proportional to M, the number of Monte Carlo steps. From the Rouse model one expects to get the following dependence of the Rouse relaxation time, τ_R, upon L [19],

$$\tau_R \sim L^{5/2} \tag{5}$$

for a self-avoiding walk (SAW) in two dimensions, which corresponds to the non-crossing case. For such a (non-crossing) polymer, if a simulation is run for $M > \tau_R$ then it is found that $\langle r_{mon}^2 \rangle_M \sim M$. Accordingly, we must carry out the simulations for $M > \tau_R$ while monitoring the dependence of $\langle r_{mon}^2 \rangle_M$ upon M until this dependence becomes linear.

Results

We first present the results for single macrolipids in order to check that we obtain $\nu_c = 3/4$. We then present the results for finite concentrations and finally we show the results for the diffusion coefficient for both the dilute and dense cases. In all cases we used periodic boundary conditions.

Single macrolipids

We used lattices ranging in size from $(50)^2$ to $(150)^2$, so that the single polymers did not 'see' themselves as a consequence of the periodic boundary conditions. The starting configuration was initialized for 10^5 Monte Carlo steps (MCS) per monomer, after which averaging was carried out for 10^4 MCS/monomer. After the first

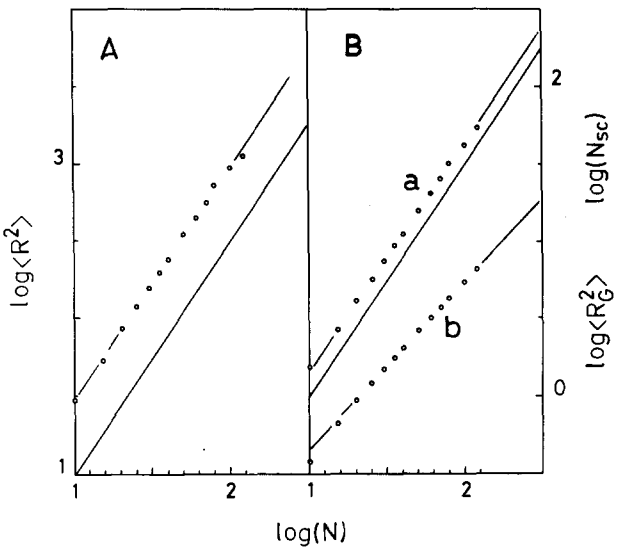

Fig. 3. Single polymer. A: $\log\langle R^2\rangle$ vs. $\log(N)$. B: $\log\langle R_G^2\rangle$ (a) and $\log(N_{sc})$ (b) vs. $\log(N)$. In both, the straight line indicates a slope of 3/2. The slope of $\log(N_{sc})$ is ~1.04.

such case was run, the system was allowed to run for a further 10^4 MCS/monomer, during which no averaging was carried out but which was treated as a second initialization process, using as a starting configuration the final configuration of the previous case. After this, averaging was carried out for 10^4 MCS/monomer. A total of 199 such cases were performed which, with the first case, yielded a total of 200. Statistics were kept on the average distance moved by the monomers, the fraction of attempted moves per monomer that were successful, and the fractions of long and short bonds. We saw no reasons to think that the results are biased.

Fig. 3 shows plots of $\log\langle R^2\rangle$ (Fig. 3A), and $\log\langle R_G^2\rangle$ and $\log(N_{sc})$ (Fig. 3B) as functions of $\log(N)$, together with straight lines which show a slope of 3/2. It can be seen that $\nu_c = 3/4$ and that, with a slope of 1.49, the exponent of $\langle R_G^2\rangle$ is also 3/4. This result thus coincides with that of a SAW where no bond-crossing is permitted, in accord with what is known about trails (above). The slope of $\log\langle N_{sc}\rangle$, is ~1.04 which gives $\sigma_c \approx 0.52$, which is essentially in accord with what is expected (above).

Semi-dilute and dense cases

Fig. 4 shows the dependence of $\langle R_G^2\rangle$ upon chain length, for $N = 20$, 30 and 40, for single macrolipids (a) and for monomer concentrations $c = 0.2$ (b), and $c = 0.72$ (c). Also shown are lines with slopes of 3/2 and 1. Fig. 4A shows data for cases of crossing macrolipids while Fig. 4B shows corresponding data for cases in which the macrolipids are not allowed to cross, viz. SAWs. It can be seen, in both cases, that, for single macrolipids, the slope is 3/2, that for high concentrations of macrolipids, the slope is 1 and that there is a cross-over regime in the neighbourhood of $c = 0.2$. These results are in accord with what has been found for SAWs and trails, though we are not aware that high concentrations of trails have been studied. The result that $\nu_c \to 1/2$ at $c = 0.72$ shows that at sufficiently high concentrations the dependence of $\langle R_G^2\rangle$ upon polymer length scales like that of ideal chains [6,18]. It should be noted that the mean field arguments which implies that this should occur, are valid in three dimensions but not obviously true in two dimensions. We are in the process of computing higher moments of the chain conformations, $\langle R_G^{2n}\rangle$, in order to see whether crossing and non-crossing macrolipids scale similarly at high concentrations. Examples of longer macrolipids, with $N = 80$, and $c = 0.2$ and 0.72 are shown in Fig. 5 (A and B), where the self- and mutual-crossings can be seen. It should be noted that the polymers are not obviously confined to their own volume of space, to the exclusion of other polymers, [7], but that they can extend substantially into the space of other polymers. This can be seen more clearly in Fig. 5C which shows a blown-up section of Fig. 5B. Although we expect such an occurrence, one could not be sure a priori that a sufficient number of paths existed in phase space so as to permit substantial mutual-crossings of bonds.

Figs. 6 and 7 show plots of the distribution $\langle n^2P(n)/\Sigma mP(m)\rangle$ as functions of the numbers of macrolipids in the cliques, for $c = 0.2$ (Fig. 6) and $c = 0.72$ (Fig. 7). In Fig. 6 it can be seen that the distribution is peaked at $n = 1$ except for the case of $N = 100$ where the peak occurs at $n = 2$. Cliques are thus small, comprising less than about 5 macrolipids when $c = 0.2$. The shear viscosity of this system should differ little from that of a system which exhibits no bond intersection. Fig. 7, on the other hand, shows the change in behaviour from broad distributions when $N = 20$ and 30, to one which exhibits a broad peak at

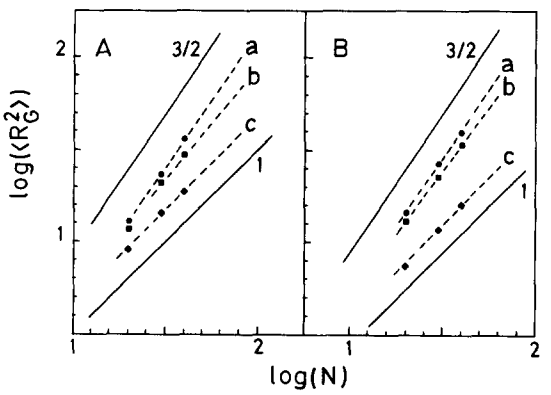

Fig. 4. Log $\langle R_G^2\rangle$ vs. $\log(N)$ for macrolipids with crossing (A) and non-crossing (B) bonds for various concentrations. (a) single macrolipids, (b) $c = 0.2$ and (c) $c = 0.72$. The two solid lines indicate slopes of 3/2 and 1 as indicated.

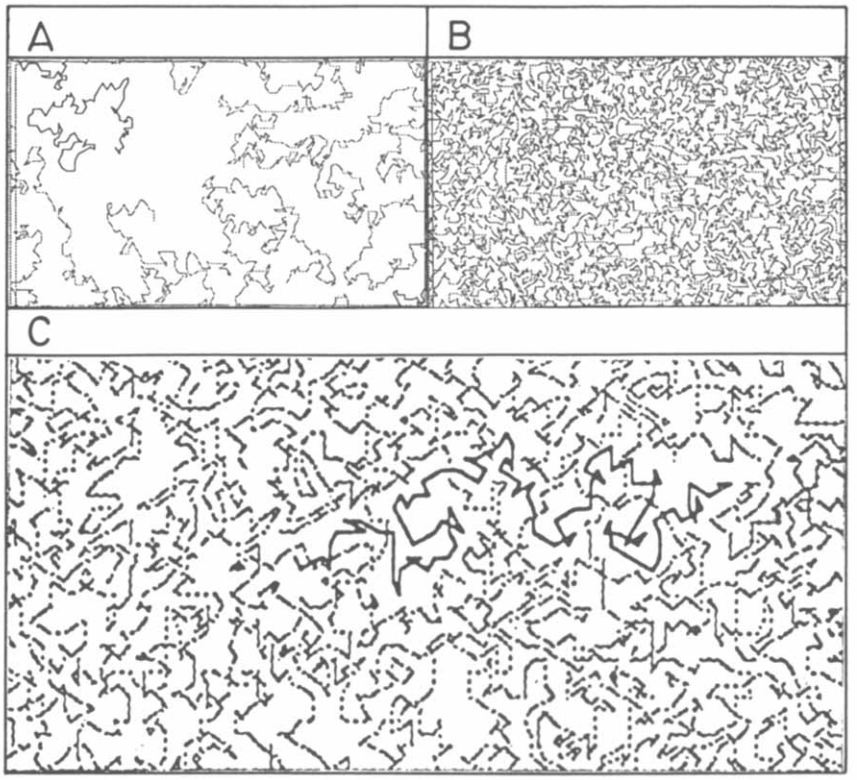

Fig. 5. Sample polymer distributions for polymers with $N = 80$ monomers. (A) $c = 0.2$. (B) $c = 0.72$. (C) Expanded area of $c = 0.72$ to exhibit crossing bonds.

large cliques when $N = 40$, to a sharp peak at clique size = 135 when $N = 50$ (for which the largest possible clique contains 144 macrolipids), through a progressively narrowing distribution as N increases and a shifting of the maximum towards the largest possible n-values, and finally, when $N = 100$, to a sharp distribution in which the largest possible clique (size = 72) is by far the most probable. The abrupt change as N increases from 30 to 50 is indicative of a transition from a partially-connected to a fully-connected system, and this should be reflected in the shear viscosity. Fig. 8 shows $\langle NM_w \rangle$ as a function of N and there we can see the transition at $N \approx 40$ in the case of $c = 0.72$. Note that a value of 7200 is the upper bound on $\langle NM_w \rangle$ at this concentration. This should be compared with the case of $c = 0.20$ where $\langle NM_w \rangle$ remains small and exhibits no transition.

Fig. 9 shows a plot of the average total number of crossings per polymer per MC step, $\langle N_{sc} + N_{mc} \rangle$, for $c = 0$, $c = 0.2$ and $c = 0.72$. It can be seen that in all cases the slope is ~ 1.04 so that $\sigma_c \approx 0.52$, independent of c. Also shown is a slope of 1.0, and we consider that our results support the conclusion that we have confirmed that $\sigma_c = 1/2$, independent of concentration.

Table I shows the order parameter for four macrolipids for three concentrations. For three of the cases a comparison with the non-crossing cases can be made. It can be seen that B is greater for the crossing cases (x) than for the non-crossing cases (nox), and that this becomes more pronounced as c increases. In the case of non-crossing polymers the effect of 'bond-compression' in reducing B has been studied by Wittmann et al. [20]. This shows that the ability for bonds to cross permits more long bonds to occur on the average. It can also be seen that B appears to be nearly independent of N for a given concentration. We will comment

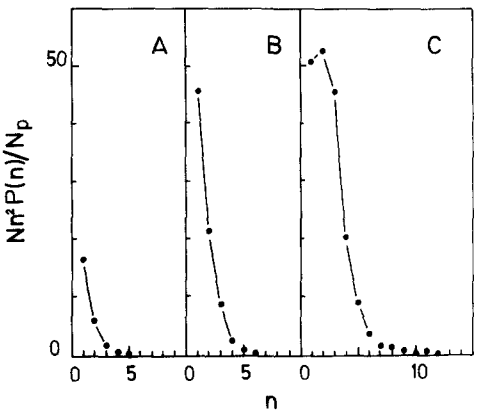

Fig. 6. $Nn^2P(n)/N_p$ as a function of n for $c = 0.2$ where N_p is the number of macrolipids. (A) $N = 20$. (B) $N = 60$. (C) $N = 100$. The solid lines are to aid the eye.

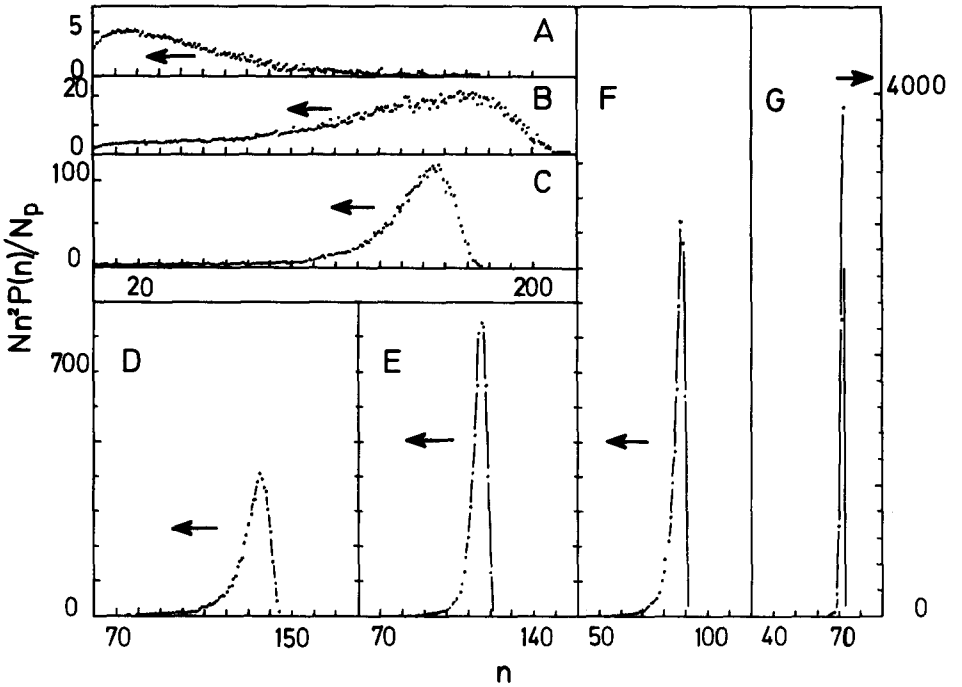

Fig. 7. $Nn^2P(n)/N_p$ as a function of n for $c = 0.72$ where N_p is the number of macrolipids. (A) $N = 20$. (B) $N = 30$. (C) $N = 40$. (D) $N = 50$. (E) $N = 60$. (F) $N = 80$. (G) $N = 100$. The solid lines are to aid the eye.

upon this, as a possible means of detecting crossing macrolipids, in the next section.

Fig. 10 shows a plot of $(\langle N_{sc}\rangle + \langle N_{mc}\rangle)/L$, i.e., the average total number of bond-crossings per bond (in each polymer) for the case $N = 80$, as a function of c. Also shown is a plot of $\langle N_{sc}\rangle/L$, as well as $\langle N_{mc}\rangle/\langle N_{sc}\rangle$ for comparison. A measurement of one of them might be possible if the bonds contain fluorescent labels such that, for example, an excimer can be formed only if the bonds are very close together. Alternatively, the proximity, through bond-crossing, of appropriate nuclei located on different bonds might be detected by the effects of dipole–dipole interactions upon nuclear magnetic resonance spectra. The result that a dense system of macrolipids are physically cross-linked (via crossing bonds as we model here), should have experimental consequences. In particular, the elastic contribution to the viscoelastic properties of such a system should be high at frequencies proportional to the inverse of those times which are characteristic of the uncrossing of crossed macrolipids, i.e., the average time needed for two crossed macrolipids to become uncrossed.

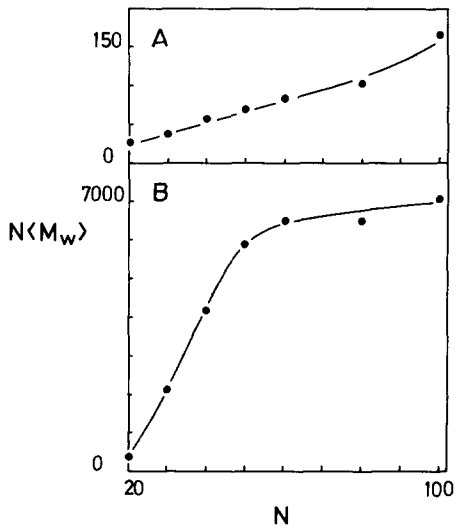

Fig. 8. $N\langle M_w\rangle$ as a function of N for $c = 0.20$ (A) and $c = 0.72$ (B). The solid lines are to aid the eye.

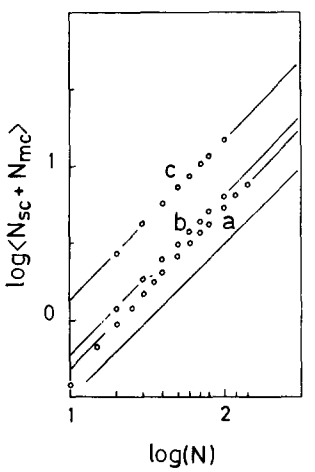

Fig. 9. The total number of bond crossings, $\log\langle N_{sc} + N_{mc}\rangle$ vs. $\log(N)$ for three concentrations. a: $c = 0$; b: $c = 0.2$; c: $c = 0.72$. In all cases the slope is 1.04. Also shown in a slope of 1.

TABLE I

Order parameter, B (Eqn. 2), for crossing (x) and non-crossing (nox) macrolipids as functions of degree of polymerization (N) and concentration (c)

Standard deviations are typically ± 0.01.

N	Single polymer		c = 0.20		c = 0.72	
	x	nox	x	nox	x	nox
20	0.52	0.51	0.52	0.49	0.48	0.38
30	0.52	0.51	0.52	0.49	0.48	0.38
40	0.53	0.51	0.52	0.49	0.48	0.38
80	0.53	–	0.52	–	0.48	–

Finally, Fig. 11 shows the calculated lateral diffusion coefficient for macrolipids of length 20 and 30 monomers, for three concentrations. In these cases we used $(30)^2$ and $(40)^2$ lattices for single macrolipids, and $(100)^2$ lattices for higher concentrations. In order to find the regime where $\langle r^2_{mon}\rangle_M \sim M$, we ran these simulations from 500 to 30 000 or 35 000 Monte Carlo steps per monomer. From the definition of D in Eqn. 1, it can be seen that the diffusion coefficient for single unpolymerized monomers, i.e., single lipid molecules, is $D = 4$ on the scale shown here. The values of D for crossing macrolipids are about two orders of magnitude lower. It can be seen that these values are lower than those for non-crossing macrolipids (SAWs) by a factor of ~ 0.6. It should be realized that there are two factors which determine D for crossing macrolipids: The availability of more pathways for monomers to move because of permitted bond-crossing, and the restriction on this movement via the entanglement of macrolipids due to this crossing of bonds. Our results

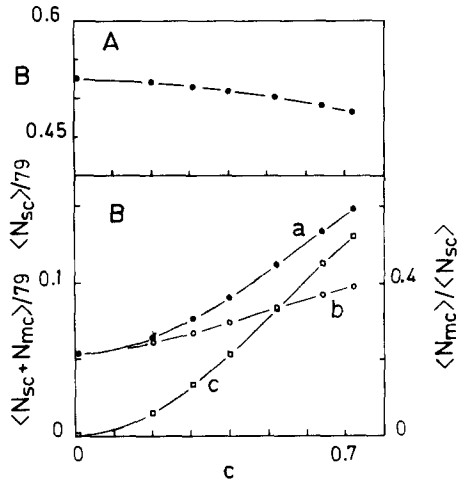

Fig. 10. (A) Order parameter, B, as a function of concentration for $N = 80$. (B) Bond crossings as a function of concentration for $N = 80$. Bond crossings per polymer bond, $\langle N_{sc} + N_{mc}\rangle/79$ (a), $\langle N_{sc}\rangle/79$ (b), and the ratio $\langle N_{mc}\rangle/\langle N_{sc}\rangle$ (c).

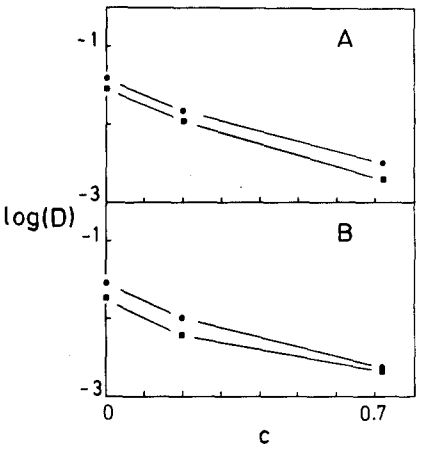

Fig. 11. Diffusion coefficient, $\log(D)$, as a function of concentration, c, for crossing (■) and non-crossing (●) macrolipids. (A) $N = 20$. (B) $N = 30$. Single unpolymerized monomers have $\log(D) = 0.602$.

show that both effects are similar but that the second, which tends to reduce D, has more effect.

Discussion and Conclusions

We have modelled macrolipids, polymers in which the monomers are lipid molecules, embedded in the plane of a lipid bilayer or monolayer. We have assumed that the molecular spacers separating successive monomers along the polymer backbone are short (~ 1 nm) and are flexible and located in the water region. We have studied the consequences of bond crossing which might be permitted because of thermal fluctuations of the interface in a direction perpendicular to the average plane of the bilayer. We have used computer simulation to study this system because of its complexity. We have assumed that $T > T_m$, and that there is no phase separation in such a 2-component lipid bilayer when the polymerizable lipids are not polymerized. In addition to the conformational states of the polymer backbone, each molecular spacer possesses two states, long and short 'bonds', which represent extended and 'twisted' states of the spacer. We have defined and computed an order parameter, B, involving the average number of long and short bonds and we have calculated the diffusion coefficients, D, for cases in which macrolipids may or may not exhibit crossing bonds. We have studied these systems as functions of the number of lipids per macrolipid, N, and polymerizable lipid concentration, c.

Our results show that:

(i) For single polymers, in both cases, $\langle R^2\rangle$ and $\langle R^2_G\rangle \sim N^{2\nu_c}$ with $\nu_c = 3/4$. This is in accord with what is known about self avoiding walks (SAWs) and trails.

(ii) For semi-dilute ($c = 0.2$) and dense cases ($c = 0.72$) we again find that $\langle R^2_G\rangle \sim N^{2\nu_c}$. For $c = 0.72$ we

find that $\nu_c = 1/2$ for both cases as in the case of ideal chains. We have made comments about this result. The result that $\nu_c = 1/2$ for crossing polymers has not, we believe, been reported before. We find that $c = 0.2$ is in the cross-over regime between SAW-like behaviour (where $\nu_c = 3/4$) and ideal chain behaviour (where $\nu_c = 1/2$).

(iii) We have found that the total number of bond crossings, $\langle N_{sc} \rangle + \langle N_{mc} \rangle$, scales like $N^{2\sigma_c}$ with $\sigma_c \approx 0.52$ independent of concentration. We consider that this suggests that $\sigma_c = 1/2$.

(iv) We have found that the weight averaged clique size, as a function of the number of lipids per macrolipid, N, exhibits a transition, in the case of $c = 0.72$, around $N = 40$ from a disconnected distribution of macrolipids to a connected distribution. In the latter, essentially all macrolipids are found in the same clique. Such an effect does not occur for $c = 0.2$, at least up to $N = 100$. For macrolipids with $N > 40$ when $c = 0.72$ there should be observable effects in the shear viscosity which should not appear when $c = 0.2$.

(v) We have found that the static order parameters, B (Eqn. 2), for both the crossing and non-crossing cases, appear to be independent of the number of lipids per macrolipid, N, but depend upon the concentration, c. This reflects the fact that in the dense case, short bonds will be favoured over long bonds. The value of B for the crossing case is larger than that for the non-crossing case, at fixed c, again because, while the non-crossing macrolipids are confined to 'their' area of the plane [6,7], the crossing macrolipids can enter the space of other macrolipids and, thus, possess a larger number of long bonds.

(vi) We computed $D(N,c)$ for both crossing and non-crossing macrolipids for $N = 20$ and 30. For crossing macrolipids, we found that $\log(D(20,0)) \approx -1.25$ ($\log(D(30,0) \approx -1.45$), $\log(D(20,0.2)) \approx -1.65$ ($\log(D(30,0.2)) \approx -1.95$) and $\log(D(20,0.72)) \approx -2.40$ ($\log(D(30,0.72)) \approx -2.40$). The values of D for crossing macrolipids should be compared to those for non-crossing macrolipids which have values between ~ 1.2 and 1.8 higher than the corresponding values for the crossing cases. These numbers should be compared to that appropriate to an unpolymerized lipid monomer which would possess a value of $\log(D) \approx 0.602$ on the scale that we are using. The absolute values can be obtained by multiplying all the D-values given here by D_0, the value appropriate to a single lipid molecule, with $D_0 \approx 2\ \mu m^2/s$.

There are three quantities that we have calculated, all of which might be used to distinguish between crossing and non-crossing polymers. The lateral diffusion coefficient could be obtained using fluorescent probes and the number of crossing bonds might be observed via the perturbation using nuclear magnetic resonance of appropriately-labelled nuclei in the bonds. The order parameter might be deduced spectroscopically via bands which are sensitive to the conformations of the molecular spacers linking two adjacent monomers along a macrolipid. Finally, for sufficiently high monomer concentration, c, the shear viscosity should exhibit differences from the case when macrolipids do not permit bond crossing.

We have found that, in this model, there are only a small number of bond crossings per macrolipid: For a single macrolipid this number ranges from ~ 0.9 ($N = 20$) to ~ 2 ($N = 40$) and ~ 4 ($N = 80$). For higher concentrations, the total number per macrolipid is, for $c = 0.2$, ~ 1.2 ($N = 20$) to ~ 2.5 ($N = 40$) and ~ 5 ($N = 80$), while for $c = 0.72$ we found ~ 2.7 ($N = 20$), ~ 5.7 ($N = 40$) and ~ 11.7 ($N = 80$). This is probably due to the shortness of a long bond: only one other bond can cross it, at any one time, and the entropy of a single chain militates against a state in which many self crossings occur.

There are a number of questions which arise concerning polymers in two dimensions which permit bond-crossing of the kind described here. The first is what is the effect of making the bonds longer? If the bonds are very long then, as mentioned above, this corresponds to a polymer in three dimensions constrained to touch the bilayer at points which are determined by the euclidean length of the bonds and constraints concerning the excluded volume of the polymers. A question which arises is, how does the component of the radius of gyration in the plane of the bilayer, $\langle R^2_{Gll} \rangle$, depend upon the number of monomers, N. A second question pertains to the effect of temperature. If the polymer bridges which connect the lipid monomers of a macrolipid possess an appropriate sequence then, for a sufficiently low temperature they will be adsorbed at the bilayer/water interface, while for a sufficiently high temperature they will extend into the water region. This is because of the competition between the (attractive) energy of adsorption and the higher entropy associated with conformations which extend into the water region. What, then, are the effects of the interface-binding transition and under what conditions could it be cooperative? This second question is in the process of being studied.

Note added in proof: (Received 13 July 1993)

Equation 1 should contain a factor of $1/4$ and not 4, as it does. The only effect of this is to change the scale of $\log(D)$ in Fig. 11, and the numbers in Conclusions (vi), by subtracting $\log(16) = 1.204$. Thus, single unpolymerized monomers will have $\log(D) = -0.602$ instead of 0.602 and on the ordinate, -3 and -1 become -4.204 and -2.204. No conclusions of the paper are affected.

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