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Deducing the lateral distribution of proteins in lipid bilayer membranes

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Abstract. We consider four models of the lateral distribution of proteins in lipid bilayer membranes and study the fraction of lipids which are adjacent to at least one protein (adjacent lipids) and how this quantity depends upon protein concentration. The models are *(i)* hard hexagons free to move from one lattice site to another; *(ii)* hard disks moving on a continuum; *(iii)* a mixture of two sizes of "nearly-hard" disks moving on a continuum; *(iv)* a modification of *(ii)*. The hexagons or disks represent proteins, while unoccupied lattice sites or the remainder of the continuum represents lipids. In *(iii)* large disks represent proteins and small disks represent lipids. In *(iv)* some of the continuum between pairs of disks, where packing defects might occur, is not occupied by lipids. We find that an analytical expression for the adjacent lipids (Hoffmann et al. 1981), which is in excellent agreement with the results of the Hexagon model *(i)*, breaks down at a packing density of $f_A \cong 0.805$, and we show by considering the hexagon pair correlation function, that this indicates the onset of random close packing, and that a transition to ordered close packing occurs at $f_A = 0.866$. We thus obtain an operational definition for a "random" distribution of hexagons: distributions of packing densities $\lesssim 0.805$. We show that the Disk model *(ii)* gives results for adjacent lipids that are greater than the Hexagon model and compare these results to the Two Disk model *(iii)* which gives a result substantially less than the Hexagon model (Mountain et al. 1986). We show that the Modified Disk model *(iv)* gives results in essential agreement with the Hexagon model except for $f_A \gtrsim 0.77$. Finally we discuss the general appearance of the "motion restricted" ESR spectrum and conclude that, of these four models, the Modified Disk or the Hexagon models best account for the data. We discuss why this is so with reference to the representation of a 3-dimensional membrane by a 2-dimensional plane.

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Abbreviations: ESR, Electron Spin Resonance

Key words: Lipid bilayers, protein distribution, computer simulation

Introduction

The question of the lateral distribution of proteins in biological membranes is of interest because an understanding of the lateral distribution may throw light on the interaction between proteins and possibly upon the mechanism of their function. In the last decade there has been less than complete agreement concerning the interpretation of experiments which might give indirect information about this. Electron spin resonance (ESR) measurements using nitroxide spin labels attached near the terminal methyl group of lipid molecules forming bilayer membranes in a homogeneous fluid-like phase which contain integral proteins show a change in spectrum characteristic of the labelled hydrocarbon chain becoming "motion restricted". By making use of the assumption that the ESR spectrum is a linear superposition of one due to "free" (i.e. as if in the absence of proteins) lipids and one due to "motion restricted" lipids, the dependence of the fraction of the total spectrum which is "motion restricted" upon protein concentration can be determined. It is generally agreed that the most reliable data is that obtained at "intermediate" protein concentrations, and there is essentially no data available for very low protein concentrations. The interpretation of data by models which one considers to correctly describe the system thus requires that the entire range of data be used to fit the, generally two, parameters of the models. It is because of the unavailability of data for limiting cases, together with scatter in the data, that the same sets of data can be used to deduce different results (Marsh and Watts 1982; Brophy et al. 1984; Silvius et al. 1984; Hoffmann et al. 1981; Pink et al. 1984; Griffith et al. 1986).

This debate has resulted in properties of some models being studied (Laidlaw and Pink 1985; Mountain et al. 1986) and it is the intention of this paper to compare the packing properties of various models. Specifically, we shall study the questions (*a*) To what extent are lattice models of protein distribution in bilayer membranes similar to continuum models? and (*b*) How important are the details of the shapes which are chosen to represent the cross-sections, in the hydrophobic section of bilayers, of integral proteins? The study of cross-sectional shapes has been motivated by recent results (Pink et al. 1987) which seem to be able to discriminate between various shapes for the hydrophilic segment of Ca^{2+} ATPase based upon an analysis of recent data using spin labelled lipids covalently bonded to the proteins (Griffith et al. 1986).

Models

Here we shall consider three models of protein distribution in the plane of a lipid bilayer membrane, calculate packing properties of them, and compare them with reference to data from ESR measurements. We repeat that we are concerned with modelling a lipid bilayer membrane in a single homogeneous (on a sufficiently large scale) fluid-like phase. The *Hexagon model* represents the plane of a lipid bilayer by a triangular lattice and the cross-section of an integral protein by a hexagon. Hexagon centres and vertices lie on lattice sites and those sites not occupied by hexagons are occupied by lipid hydrocarbon chains or molecules. A site may not be occupied by more than one hexagon. On a finite-size lattice there is thus a finite number of ways of distributing the hexagons on the lattice and lipids fill all the area not occupied by hexagons (Pink et al. 1984). The *Disk model* represents a protein cross-section by a hard disk which can occupy any position on a plane subject to the constraint that two disks may not overlap. The area not occupied by disks is assumed to be occupied by lipids. The *Two-Disk model* represents a protein cross-section by a "nearly-hard" disk and represents a lipid molecule by a "nearly-hard" disc of a different radius. The (repulsive) interaction between the disks is of the two-body exponential form

$$\phi(r) = \varepsilon \mathrm{e}^{-\alpha(r-\sigma)}. \tag{1}$$

A choice of a large value for α gives the interaction its "nearly-hard" character (Mountain et al. 1986). Here we shall report on computer simulations of the distributions associated with the first two models and compare them with molecular dynamics simulations of the third model. The quantities which we calculate are related to those relevant to the analysis of ESR experiments.

In their analysis of ESR data, Hoffmann et al. (1981) assumed that (*a*) following earlier workers in this field, the ESR spectrum, $S(\omega)$, is to a good approximation given by

$$n S(\omega) = S_f(\omega)(n - n_a) + S_i(\omega)\, n_a, \tag{2}$$

where S_f and S_i are spectra characteristic of a "free" spin labelled lipid and a "motion restricted" labelled lipid, n is the total number of labelled lipids and n_a is the number of "motion restricted" labelled lipids; (*b*) the labelled lipids are distributed in the plane of the bilayer like the unlabelled lipids and that those labelled lipids adjacent to the hydrophobic core of at least one integral protein contribute entirely to the "motion restricted" spectrum, while those not so located contribute entirely to the "free" spectrum. The number, n_a, is thus the average number of lipids adjacent to the hydrophobic core of the integral proteins. We therefore need to calculate $p_\alpha(M, c)$, the probability that a labelled lipid chain is not adjacent to any integral protein, so that

$$S(\omega) = S_f(\omega)\, p_\alpha(M, c) + S_i(\omega)(1 - p_\alpha(M, c)). \tag{3}$$

Here α is a label referring to the model used, $\alpha = \mathrm{H}$ (hexagon), D (Disk) or TD (Two-Disk), c is the protein concentration in mole fraction and M is a set of parameters describing the protein shape.

The quantity $p_\alpha(M, c)$ depends upon multi-particle correlations. Although the hard-disk system has been studied extensively (e.g. Wood 1970; Hoover et al. 1979; Erpenbeck and Wood 1982; Berryman 1983), this quantity, $p_{\mathrm{D}}(M, c)$, seems not to have been studied. Similarly, the hard hexagon model has been studied extensively by Baxter and his co-workers (e.g. Baxter 1981; Baxter and Pearce 1982; Pearce and Baxter 1984 and other references therein) but the question of $p_{\mathrm{H}}(M, c)$ has not arisen. The hard hexagons studied by Baxter may have edge sites in common, unlike the model referred to here as the Hexagon Model. The calculation of $p_{\mathrm{TD}}(M, c)$ for the Two-Disk model was performed by Mountain et al. (1986) for the purpose of comparing with the Hexagon model.

In earlier analyses of ESR spectra an analytic expression was written for $f_{\mathrm{H}}(M, c) = 1 - p_{\mathrm{H}}(M, c)$

$$f_{\mathrm{H}}(M, c) = 1 - (1 - c)^M + \Delta f_{\mathrm{H}}(M, c)$$
$$c = N_{\mathrm{H}}/(N_{\mathrm{H}} + N - n_{\mathrm{H}} N_{\mathrm{H}}), \tag{4}$$

where N_{H} is the number of hexagons, N is the total number of lattice sites and n_{H} is the number of lattice sites occupied by a hexagon (Hoffmann et al. 1981 [where Δf_{H} was omitted, see Laidlaw and Pink 1985]). M is the number of lattice sites adjacent to an isolated hexagon. It was shown by computer simulation that Δf_{H} is smaller than $\sim 2.3\%$ of $1 - (1 - c)^M$ for the range of c for which the distribution of hexagons can

be considered as "random". This point will be studied here. It should be noted that an expression similar to (4), with $\Delta f = 0$, was used by Holcomb and Rehr (1969) in their study of percolation in semiconductors (see also Hammersley and Handscomb 1964).

In the next section we shall study the packing of hard hexagons and define more precisely what is meant by a "random" distribution. We shall calculate $f_D(M,c) = 1 - p_D(M,c)$ compare it to $f_H(M,c)$ and compare both of them to published values of $f_{TD}(M,c) = 1 - p_{TD}(M,c)$. We shall describe what may be thought of as a realistic modification of the calculation of $f_D(M,c)$ and compare it to $f_H(M,c)$. Finally we shall refer to published ESR data and try to suggest which of these models might best describe lipid-protein bilayers. It might be noted that the so-called "annulus" model is not being considered here. This is because, in that case, the exact result is known, $f_A(M,c) = Mc/(1-c)$. Our omission should not be taken as indicating anything other than that this paper is concerned only with comparing the three models described above.

The simulation methods used in the Hexagon model have been described elsewhere (Pink et al. 1984). In the case of the Disk model, the calculation of $f_D(M,c)$ proceeds as follows: N_D hard disks, each of radius 1, are distributed on a square of side L with periodic boundary conditions. One Monte Carlo step consists of selecting all disks, once and only once, in a random sequence and attempting to move them in random directions by randomly chosen distances selected from the range 0 to s. Disks may be moved as long as they would not overlap other disks. At the end of each step the total area enclosed by rings of width lying between radius 1 and $1+w$ from the centre of each disk, with overlapping ring areas counted only once, is counted. This area is averaged over a sufficient number of Monte Carlo steps after the system has been initialized and expressed as a fraction of the total area not covered by disks. This fraction is $f_D(M,c)$.

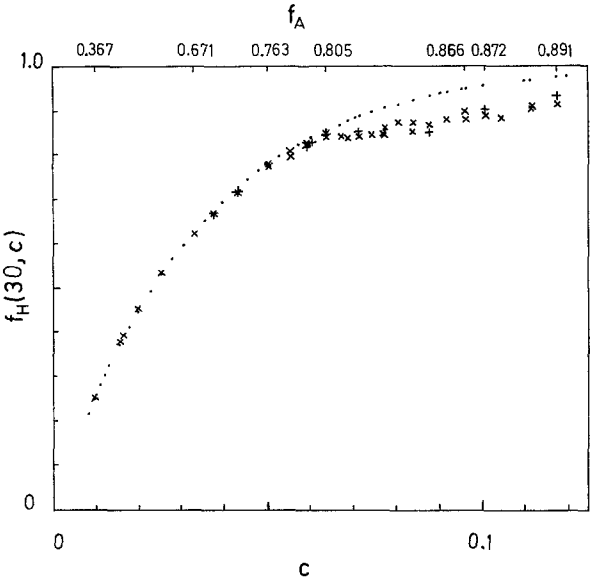

Fig. 1. The Hexagon Model. $f_H(M,c)$ for $M = 30$ as a function of hexagon concentration (c). The results of simulations performed on triangular lattices of 10^4 sites are shown by $\times$ and $+$. The *dots* show $1-(1-c)^{30}$

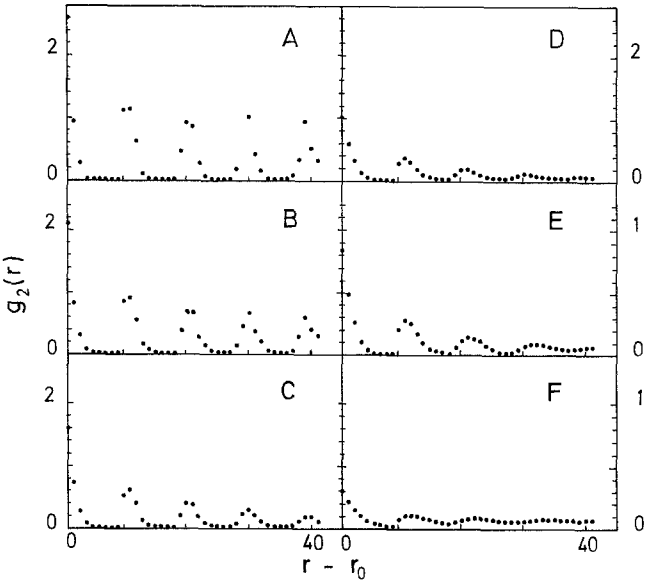

Fig. 2 A–F. The Hexagon Model. The pair correlation function $g_2(r)$ for r lying along one of the six-fold symmetry axes in a direction from the centre to a vertex of a hexagon. Simulations were carried out on triangular lattices of 10^4 sites for $M = 30$. The value of r_0 is 9 lattice constants. **A:** $f_A = 0.878$; **B:** $f_A = 0.866$; **C:** $f_A = 0.860$; **D:** $f_A = 0.817$; **E:** $f_A = 0.793$; **F:** $f_A = 0.671$

Results

Figure 1 shows $f_H(M,c)$ as a function of c for $M = 30$. The simulations were carried out on lattices of 10^4 sites and involved between 80 and 146 hexagons. More than 5000 Monte Carlo steps were used for initialization while averaging was carried out over 20000 steps. We define the fractional area covered by hexagons to be

$$f_A = n_H N_H / N, \tag{5}$$

where n_H, N_H and N were defined in Eq. (4). It can be seen that the results of the simulations are in excellent agreement with the expression $1-(1-c)^{30}$ (Pink et al. 1984) up to a fractional area covered by hexagons of $f_A \cong 0.805$ which corresponds here to 132 hexagons. Above this value the simulation differs markedly from $1-(1-c)^{30}$. Figure 2 shows what is happening. Here we plot the pair correlation function $g_2(r)$ which is defined as the probability that the centre of a hexagon

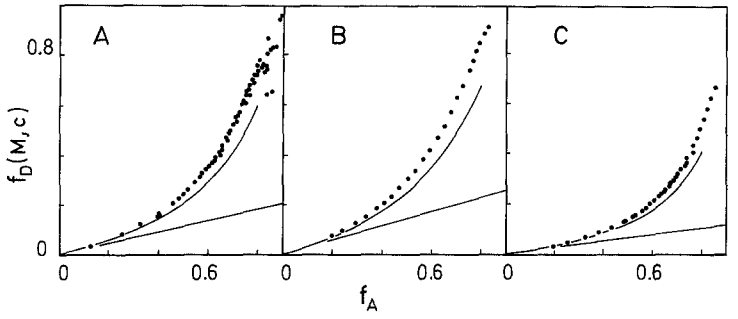

Fig. 3 A–C. The Disk model. $f_D(M,c)$ as a function of f_A for disks of radius 1.0. **A:** $w = 0.125$; **B:** $w = 0.1538$; **C:** $w = 0.0723$. Simulations (•) were carried out using between 30 and 100 disks. The curved lines are $1-(1-c)^M$ for $M = 53.4$ (**A**), $M = 44.1$ (**B**) and $M = 90.1$ (**C**) which are the value appropriate to each case. The *straight line* indicates the slope at $c = 0$.

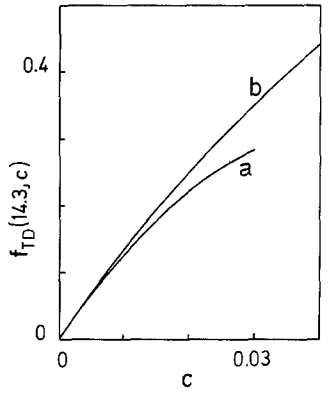

Fig. 4. The Two Disk model. (*a*) $f_{TD}(M,c)$ for the case $M = 14.3$ as a function of large disk concentration, (*c*) (Mountain et al. 1986). (*b*) $1-(1-c)^{14\,3}$

will be found at r relative to the centre of another hexagon located at the origin. We chose r to be along one of the 6-fold symmetry axes in order to obtain as many maxima as possible. It can be seen that g_2 changes between $f_A = 0.8662$ and $f_A = 0.8601$ (corresponding to 142 and 141 hexagons respectively). This probably reflects a transition from an ordered close packed structure to random close packing (e.g. Berryman 1983). It can be seen that the positions of the peaks remain unchanged through this transition but that their width at half height increases. At $f_A = 0.8174$ (134 hexagons) the peak positions are unchanged, but this has altered at $f_A = 0.7930$ (130 hexagons) and at $f_A = 0.6710$ the fourth peak at ~ 33 units can barely be detected. The transition at $f_A \sim 0.805$ is, therefore, probably from the random close packed phase to what we would consider to be a "random" distribution as referred to in the last section.

In Fig. 3 we compare $f_D(M,c)$ with $f_H(M,c)$ in order to see what changes occur if we represent the protein cross-sections by hard disks. Here we have chosen to plot the results as a function of f_A which, for disks, is defined to be

$$f_A = \pi N_D / L^2, \tag{6}$$

where N_D is the number of disks and L is the length of the side of the square on which they move. Simulations with the disks used between 30 and 100 disks with initialization between 2000 and 8000 steps, and runs over which averaging was done involving between 4500 and 50000 steps. We found no significant variations due to initialization or runs. We chose values of w and s (width of "adjacent" lipid layer and range from which a protein distance move was randomly selected): $w = 0.125$, $s = 0.04$; $w = 0.1538$, $s = 0.04$; $w = 0.0723$, $s = 0.02$. We used

$$(1-c)^M = \left[\frac{n_H(1-f_A)}{1+(n_H-1)(1-f_A)}\right]^M \tag{7}$$

and calculated M and n_H, for a disk, from the number of disks of diameter w which fit around and cover a disk of radius 1. We found that $M = 53.4$, $n_H = 232.2$; $M = 44.1$, $n_H = 153.4$; $M = 90.1$, $n_H = 694$ respectively for the three cases considered. The curves of $1-(1-c)^M$ are plotted in Fig. 3 and it is seen that they differ substantially from the simulation results for the hard disks. It should be noted in Fig. 3 A that we have shown the results of many simulation using different size squares and different numbers of Monte Carlo steps. There we can see that large scatter in the simulation results occur for $f_A \gtrsim 0.82$. If we take this as indicating the onset of random close packing, as it appears to in the Hexagon model, then our result of f_A lying between 0.81 and 0.82 is in accord with the results of other workers (see, e.g., Berryman 1983).

That $f_D(M,c) \geqq f_H(M,c)$ is not surprising because even at the highest density of disks, the surface of the square is not completely covered. When two disks approach each other there is an area between them that is considered "adjacent" no matter how close they are, which does not occur in the case of two hexagons on lattice. Because of this and because it seems unlikely that lipid molecules can be so distorted so as to fit into any available space, the Disk model seems unsatisfactory for the description of proteins distributed in a lipid bilayer.

In Fig. 4 we compare the results of the Two Disk model with $1-(1-c)^M$ for the case $M = 14.3$. Using molecular dynamics Mountain et al. (1986) calculated $f_{TD}(M,c)$ and, for a particular definition of what is a lipid "adjacent" to a protein, fitted it to the curve

$14.3\,c - 160\,c^2$. As they point out it is clear that the difference between $f_{TD}(M,c)$ and $f_H(M,c)$ is substantial, with $f_{TD}(M,c) \leqq f_H(M,c)$.

Finally, Fig. 5 shows a modification of the Disk model: When the centres of two disks are closer than $2+2w$, their regions of "adjacent" lipids overlap. In this modification we omit the area indicated as black in Fig. 5 A, i.e., we assume that lipids will not distort themselves to fill this space. This modification does assume that lipids fill the space away from the disks so that packing defects are not present in a fluid lipid bilayer except for the regions adjacent to two proteins which are close together. For such a pair of disks of radius 1, with their centres separated by a distance d, the area associated with "adjacent" lipids is

$$A(r) = 2A_0 - A_1 - A_2, \quad 2 + w \leqq d < 2 + 2w$$
$$A(r) = 2A_0 - A_1 - A_2 - A_3, \quad 2 \leqq d < 2 + w$$
$$A_0 = \pi w(2+w)$$
$$A_1 = 2(1+w)^2 \sin^{-1}\left[\frac{\sqrt{(1+w)^2 - d^2/4}}{1+w}\right] - d\sqrt{(1+w)^2 - d^2/4} \tag{8}$$
$$A_2 = w(2+w)\sin^{-1}\left[\frac{\sqrt{(1+w)^2 - d^2/4}}{1+w}\right]$$
$$A_3 = w(2+w)\sin^{-1}\left[\frac{\sqrt{1-(d^2 - w(2+w))^2/4d^2}}{1+w}\right].$$

The results of the Modified Disk model are shown in Fig. 5 B for $w = 0.125$ and $s = 0.04$, and a comparison is made with the Hexagon model with $M = 53.4$, $n_H = 232.2$. It can be seen that the two models are in essential agreement up to $f_A \approx 0.76$ but that for higher values of f_A, $f_{MD}(M,c) < f_H(M,c)$.

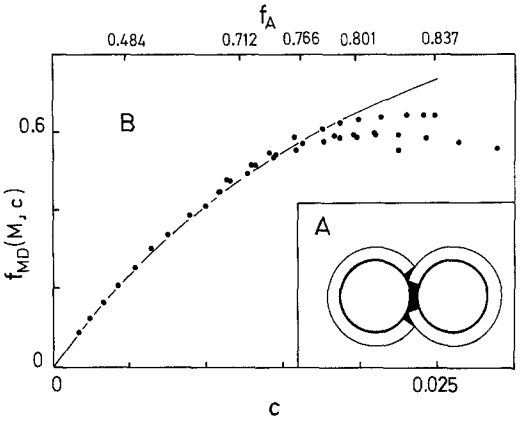

Fig. 5 A and B. The Modified Disk model. **A:** The heavy circles represent two disks of radius 1 each while the light circles represent the layer of adjacent lipids of width w. The modification assumes that the black region is unfilled by lipids. **B:** Simulation results for $f_{MD}(M,c)$ with $w = 0.125$ as a function of c (•). The solid line is $1-(1-c)^M$ for $M = 53.4$ which is the value appropriate to this case.

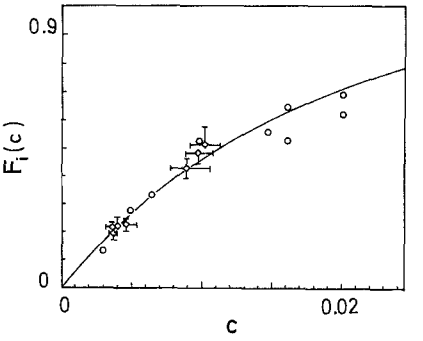

Fig. 6. ESR data for Cytochrome c Oxidase of Knowles et al. 1979 (o), and Griffith et al. 1982 (◇). Data show the fraction of the spectrum which is due to "motion restricted" lipids, $F_i(c)$, as a function of protein concentration (c), in mole fraction. The solid line is the prediction of the Hexagon model with 31 molecules able to surround an isolated such protein in each half of the bilayer (Pink et al. 1987) were it was assumed that it represents a Cytochrome c Oxidase dimer

Conclusions

Figure 6 shows ESR data of Knowles et al. (1979) and Griffith et al. (1986) for Cytochrome c Oxidase. It gives the dependence of that fraction of labelled lipids which yield a "motion restricted" spectrum as a function of protein concentration in mole fraction, which we denote by $F_i(c)$. Studies of other systems yield similar results, e.g. Brophy et al. (1984). Also shown in this figure is $1-(1-c)^M$ for an appropriate value of M simply in order to provide a basis for a comparison of the different models. It is clear that, if one accepts the data at high concentrations, the Hexagon model appears to account for the data. It should be remembered that the interpretation of ESR data is based not only upon a particular model of lipid-protein membranes, but also upon the two assumptions (*a*) and (*b*) leading to Eq. (3). This statement is true not only of the models studied here but also of the socalled "annulus" model. Those who discard the high-concentration data argue that the value of $F_i(c)$ at high values of c is smaller than it should be because of artifacts in the system (private comments to DAP). It is, therefore, generally agreed that the high-c values of $F_i(c)$ should not lie below those values shown in Fig. 6.

In Fig. 4 we see that f_{TD} departs more and more from f_H after they have risen to a value of $\lesssim 0.2$, for that particular calculation ($M = 14.3$). If this behaviour is maintained as M gets bigger then it is clear that the Two-Disk model will not describe the dependence of $F_i(c)$ upon c, and therefore might not be a good model for a lipid-protein membrane.

The principal objection to the Hexagon model is not that it does not explain the data – it is, in fact, remarkably successful – but that *(i)* it is a lattice model whereas one expects a lipid-protein bilayer to be modelled by molecules moving in a continuum, and *(ii)* it represents the lipids as occupying all sites not occupied by hexagons. The objection to the Disk model is a stronger form of the second one: That the lipids fill all of the space not occupied by Disks no matter how small, or of what shape, that space is. The Hexagon model on a lattice only allows a lipid in if there is enough space for a complete lipid. However when the Disk model is modified, as in Fig. 5, to allow spaces between disks not to be filled completely the model then gives the same result as the Hexagon model up to $f_A \approx 0.76$. It can be argued that, in the Modified Disk model, we overestimate the extent of packing defects between pairs of disks because it is plausible that real lipids would change the conformation to occupy some of this space. This, however, is a refinement that is not worth considering in such a simple model.

We studied the packing properties of the Hexagon model and found that it appears to display properties similar to those of hard disks: A transition to random close packing at $f_A \approx 0.805$, and to ordered close packing at $f_A \approx 0.86$. This lattice model, therefore, behaves in this respect like a continuum model.

It appears then, that there is some evidence that a lipid bilayer membrane in a homogeneous fluid-like phase, containing integral proteins which have such a structure that protein-protein "contacts" are possible, contains lipid packing defects predominantly in a neighbourhood between pairs of proteins, and little in other regions of the bilayer. If this is so then one can partly understand why the Hexagon model appears to be acceptable: The exclusion of fractions of lipids between pairs of hexagons is equivalent to the creation of packing defects. At very high concentrations, however, these packing defects might become so numerous that the Hexagon model is not longer valid. This, however, occurs above $f_A \approx 0.76$, where $f_{MD}(M, c) < f_H(M, c)$. An inspection of Fig. 6 actually suggests just this behaviour of the data of Knowles et al. (1979) for $c > 0.015$, though we can make no stronger claim other than it is suggestive.

Finally, it should be realized that these models all represent a three-dimensional bilayer by an idealized projection onto a two-dimensional surface. A good model might represent the lipid hydrocarbon chains and the surfaces of the proteins correctly by space-filling structures which can change their conformations and positions. Molecular dynamics simulations would involve at least 10 proteins and several hundred lipids. The problem would have a number of time-scales. Such a simulation would possibly settle all the questions discussed here and would take a long time to carry out unless it could be simulated on a vector computer possibly running at between 10^3 and 10^4 Megaflops, or an equivalent system. However, the hydrocarbon chains of the lipids are not extended but are twisted polymers which may be, to some extent, intertwined. They change conformations on the same timescale as thery move laterally by a few Ångstroms. One might therefore expect to find that when one projects the hydrocarbon chains onto the two-dimensional bilayer plane, the surface is indeed covered, and no packing defects arise except possibly in the neighbourhood of proteins. It is because of this that we feel that the Two-Disk model, as described here, is possibly not a good model for a lipid bilayer membrane in a homogeneous fluid-like phase and that such a membrane is better described by a Modified Disk model which has lipid packing defects between pairs of disks, or by the Hexagon model for which the calculations are most easily performed.

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