

# Lateral diffusion of lipids in complex biological membranes

(lipid-protein interactions/scaled-particle theory/photobleaching-recovery)

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**ABSTRACT** Lateral diffusion of lipids in biological membranes may be influenced by polypeptides, proteins, and other nonlipid membrane constituents. Using concepts from scaled-particle theory, we extend the free-volume model for lipid diffusion to membranes having an arbitrarily large number of components. This theory clarifies the interpretation of the free-volume theory, better reproduces the free-area dependence of lipid lateral diffusion rates, and quantitatively predicts the experimental observation that the lateral diffusion rates of membrane lipids are significantly reduced when proteins or polypeptides are incorporated in the membrane.

Rapid lateral diffusion of lipids in pure monolayer or bilayer systems is well described by "jump" or "hopping" models based on the free-volume diffusion theory of Cohen and Turnbull (1–4). In this model, first applied to biomembrane systems by Galla *et al.* (5), diffusion is considered to be a three-step process in which first a local free volume into which a molecule may diffuse is created by equilibrium density fluctuations, then the diffusing molecule "jumps" into the void, and, finally, another solvent molecule fills the hole created by this diffusional "jump." Although the results of this model agree well with experiments for a number of systems (5–7), the theory is not applicable to complex systems in which the solvent consists of many species having different molecular dimensions. As a result, it cannot be directly applied to membrane systems in which the membrane contains nonlipid species, such as proteins. To describe such systems, Saxton (8) applied effective-medium and continuum-percolation theories.

Effective-medium theory is usually used to estimate the electrical conductivity of a composite material in which particles of one conductivity are embedded within a medium of another conductivity. Because effective-medium theory relies on the solution of self-consistency equations for the electric field within the medium, with which we are not concerned in molecular diffusion, its *a priori* applicability to lipid diffusion is not obvious. The theory predicts total immobilization of diffusing molecules when impermeable domains exceed 50% of the membrane area, and it predicts a linear reduction of diffusion rate with increasing area fraction of impermeable domains below this point. This threshold is different than that predicted by percolation theory.

Percolation theory predicts that at a certain critical permeable area there will be a sudden increase in the conductivity of the membrane and thus of observed diffusion rates. In the vicinity of this critical area the conductivity change shows a power-law dependence; further from this critical area the power-law expression is inappropriate, necessitating another theory, such as the effective-medium theory. The percolation limit depends on the size and shape of diffusing molecules but differs from the limit obtained by effective-

medium theory. To reconcile the theories, Saxton constructs an interpolating polynomial that matches the results of effective-medium theory for infinitely dilute impermeable domains and the results of continuum-percolation theory when impermeable domains occupy 23.2% of the membrane. Although this hybrid theory predicts a decrease in the apparent lipid lateral-diffusion constant when impermeable domains, such as proteins, are present in the membrane, it apparently does not correctly predict the magnitude of the reduction (7).

In this paper I develop a model of lipid diffusion that is based on the free-volume concept. Lateral density fluctuations are treated using a modified scaled-particle theory (9), however, allowing the incorporation of many species of different sizes. When applied to a single-component system, this theory provides insight into the physical interpretation of the phenomenological constant in the original Cohen-Turnbull diffusion theory. In addition, the theory accounts for the reduction of lipid lateral-diffusion rates observed when polypeptides and proteins are incorporated into membranes.

## Theory

We assume that diffusion takes place via the free-volume mechanism (1–5) in three steps: (i) a hole is created in the solvent via lateral density fluctuations; (ii) a diffusing molecule jumps into this hole from an adjoining site; and (iii) the resulting void is filled by diffusion of a solvent molecule. We further assume that the membrane may be approximated by a two-dimensional collection of randomly distributed hard disks. In the spirit of Cohen and Turnbull (1), the average diffusion coefficient is given by

$$D = \int_{R^*}^{\infty} D(R)P(R)dR, \quad [1]$$

where  $P(R)$  is the probability of finding a circular region of the membrane with radius between  $R$  and  $R + dR$  that does not contain any molecules, and  $R^*$  is the radius of the smallest hole into which a lipid molecule can diffuse. The probability of finding such a region is given by

$$P(R) = \exp[-W(R)/kT], \quad [2]$$

where  $W(R)$  is the work required to create a hole of radius  $R$  in the solvent,  $T$  is the absolute temperature, and  $k$  is Boltzmann's constant.

Because we have assumed that membrane lipids and proteins may be approximated by randomly distributed hard disks, we may obtain  $W(R)$  from scaled-particle theory (9).

The work  $W(R)$  required to create a cavity of radius  $R$  from which fluid particles are totally excluded is

$$W(R)/kT = -1/kT\{\ln[1 - \pi\sum\rho_i(R_i + R)^2]\} \quad [3]$$

for  $R \leq 0$ . This is an exact result which does not depend upon scaled-particle theory. In the scaled-particle theory, when  $R \geq 0$ ,  $W(R)$  is approximated by a three-term Taylor series expansion around  $R = 0$

$$W(R)/kT = -1/kT\{\ln[1 - \pi\sum\rho_i R_i^2] + 2\pi(\sum\rho_i\gamma_i R_i)R/[1 - \pi\sum\rho_i R_i^2]\} + \pi PR^2, \quad [4]$$

where  $\rho_i$  is the number density of particle  $i$ ,  $R_i$  is the radius of particle  $i$ ,  $\gamma_i$  are semiempirical constants, and  $P$  is the lipid lateral-spreading pressure. The first term of Eq. 4 is an excluded volume term, which corresponds to the exact result from Eq. 3 at  $R = 0$ . The third term in Eq. 4 is a pressure-area term, which is required to satisfy thermodynamic considerations for cavities with radius  $R$  of macroscopic size. The second term of Eq. 4 is the "surface work" required to expand the cavity; Lebowitz *et al.* (9) assume that  $W'(R)$  is continuous across  $R = 0$ , so that  $\gamma_i = 1$ . We relax this assumption, for which there is no *a priori* necessity, to allow greater flexibility in defining the behavior of the lipid membrane without sacrificing either the simple functional form or the essential physical principles underlying the scaled-particle theory. By combining Eqs. 2 and 4 we obtain the probability

$$P(R) = [1 - \pi\sum\rho_i R_i^2]\exp[-2\pi\gamma(\sum\rho_i R_i)R/(1 - \pi\sum\rho_i R_i^2)]\exp(-\pi PR^2/kT) \quad [5]$$

of finding a hole of radius  $R$  at a particular site in the membrane. Molecules can diffuse only when a sufficiently large void has been created. Hence,

$$D(R) = 0 \text{ for } R < R^*, \text{ and} \\ D(R) = D(R^*) = \pi \text{ constant, for } R \geq R^*, \quad [6]$$

where we now assume that  $R^*$  is the effective radius of a lipid molecule. Thus,

$$D = D(R^*) \int_{R^*}^{\infty} P(R) dR. \quad [7]$$

For lateral pressure  $P = 0$  we have, when the probability of finding a hole of radius  $R \geq 0$  is properly normalized to  $1 - \sum\rho_i R_i^2$ ,

$$D = D(R^*)[1 - \pi\sum\rho_i R_i^2]\exp[-2\pi(\sum\gamma_i\rho_i R_i)R^*/(1 - \pi\sum\rho_i R_i^2)]. \quad [8]$$

For molecules whose diffusion is hindered primarily by collisions with other molecules in the lipid bilayer,  $D(R^*)$  is expected to have the form  $D(R^*) = c(2kT/m)^{1/2}$ , where  $c$  is a constant and  $m$  is the mass of the diffusing molecule. For molecules whose motion is hindered by the forces of the aqueous solvent and midplane of the bilayer  $D(R^*) = c kT/f$ , where  $c$  is a constant and  $f$  is a translational friction coefficient (10). The latter expression seems to give a better fit to experimental data and gives a more reasonable limiting value for the diffusion rate in membranes that have low densities. For this analysis, I will assume that  $D(R^*)$  is independent of the molecular composition of the bilayer and present the results in a manner independent of its precise form and origin.

### Application to Single-Component Membranes

For a single component, Eq. 8 reduces to

$$D = D(R^*)(1 - \pi\rho R^{*2})\exp[-2\pi\gamma\rho R^{*2}/(1 - \pi\rho R^{*2})], \quad [9]$$

where  $R^*$  is the effective radius of the lipid molecule. If we define a hard-core lipid area  $A_{HC} = \pi R^{*2}$ , this further reduces to

$$D = D(R^*)(A_F/A_L)\exp(-2\gamma A_{HC}/A_F), \quad [10]$$

where  $A_{HC}$  is the "hard-core" area of the lipid molecule and  $A_F$  is the free area per lipid molecule, and  $A_L$  is the total area per lipid molecule =  $A_F + A_{HC}$ . The exponential part of this equation has the same form as that used by Galla *et al.* (5),

$$D = D(R^*)\exp(-\gamma A_{HC}/A_F), \quad [11]$$

to fit data on the thermal dependence of lipid lateral-diffusion rate constants. The coefficient  $A_F/A_L$  of Eq. 10 results from properly normalizing the probability of hole formation in the solvent to account for the excluded area of the lipids. This factor was omitted by Cohen and Turnbull (1-4). To fit the thermal data (5), or data on the dependence of lateral diffusion in lipid monolayers as a function of area (11, 12), it is necessary to use  $\gamma < 1$ , whereas scaled-particle theory gives  $\gamma = 1$  if we assume that  $W'(R)$  is continuous at  $R = 0$ .

There are in principle several reasons that  $\gamma = 1$  might not be appropriate: (i) We assume that particles are hard disks, whereas a different shape, such as rod or ellipsoid, might be more appropriate. Scaled-particle theories (13, 14) based on two-dimensional rods and capped rods have effectively larger values for  $\gamma$ , rather than the smaller values necessary to match the experimental values. (ii) We assume that all lipids may be represented by disks of uniform size. It would be more realistic to assume a distribution of sizes for liquid crystalline-phase lipids. Numerical experiments, however, demonstrate that compensating for this only changes  $\gamma$  slightly. (iii) Scaled-particle theory could overestimate the size of the hole required for diffusion, since lipid molecules are very flexible and trans-gauche isomerization occurs rapidly by comparison with diffusion, perhaps allowing lipids to fit into oddly shaped cavities. However, since we assume a minimum hard-disk area that corresponds to that of the headgroup on an all-trans chain, such effects should not be very important. (iv) Scaled-particle theory (with  $\gamma = 1$ ) overestimates the "surface component" (second term) of the work required to create a cavity, because it neglects the ways in which the lipid differs from a hard disk. In particular, it tends to overestimate the change in surface pressure as the lipid membrane is expanded, because it ignores the stabilizing effects of trans-gauche isomerization and van der Waals attraction. We may include the effects of such interactions, which could also be included via one or more additional terms in Eq. 4, within the scaled-particle formalism by allowing  $\gamma \neq 1$ . Decreasing the value of  $\gamma$  decreases the theoretical lateral-spreading pressure at any density and also the rate with which this pressure changes as the density (free area) is varied. Determining the most appropriate value for  $\gamma$  is not within the scope of this theory, but  $\gamma$  should clearly be considerably lower than 1; approximate fitting of the theory to the data of Beck and Peters (11, 12) suggests that a value of  $\approx 0.25$  may be appropriate (see below). Use of  $\gamma < 1$  results in a discontinuity in  $W'(R)$  at  $R = 0$  and hence does not correspond to the original formulation of scaled-particle theory. As stated earlier, there is no *a priori* reason why such a discontinuity cannot exist; it seems reasonable and acceptable in the present context, because it corresponds to a change in the lipid molecule from all-trans to partially gauche.

In the all-trans bilayer, one expects no stabilization due to trans-gauche isomerization, and so the value  $\gamma = 1$  that comes from the original scaled-particle theory seems reasonable. The change of  $\gamma$  cannot be used to completely describe the lipid-bilayer phase transition, however, since it ignores the entropic effects and details of the major structural changes that occur at this phase transition.

Because the excluded volume interactions are better treated in this theory than in the original Cohen-Turnbull theory, we expect this theory to better describe diffusion in single-component lipid systems. Fig. 1 illustrates the agreement between the theory and experimental results on diffusion of dilauroylphosphatidylcholine, for  $\gamma = 0.25$  and  $A_{HC} = 0.425 \text{ nm}^2$  [the values determined by Peters and Beck (11) using the Cohen-Turnbull theory]. Although no attempt was made to adjust the hard-core-excluded area  $A_{HC}$  or  $\gamma$ , the agreement between theory and experiment is better, particularly for low values of  $A_F/A_{HC}$ , for the new theory than for the old.

Comparison of the two theories, moreover, clearly demonstrates that the factor  $\gamma$  in the Cohen-Turnbull theory is not necessary so much to compensate for the overlap of free volume in cell models as to account for the surface energy of cavity formation. It further explains why the  $\gamma$  obtained from lipid systems is significantly lower than that seen in liquids; normal liquids lack the conformational flexibility which gives rise to the cavity-stabilizing effects of surfactant molecules, such as phospholipids. Finally, it explains why the slopes of  $\ln D$  vs.  $1/A_F$  curves differ for different phospholipids with the same headgroup— $\gamma$  is expected to be sensitive to the effects of chain-length differences on trans-gauche isomerization and van der Waals interactions between chains. In the discussion that follows on the effects of polypeptides and proteins on lateral diffusion, we will assume that a value of 0.25 for  $\gamma$ , which reproduces experimental data on monolayer systems (11, 12), is appropriate to both pure lipids and lipid-protein dispersions.

#### Application to Lipid-Protein Membranes

We assume for our calculations that the area occupied by a single lipid molecule does not change on addition of proteins or other impurities. For many liquid crystalline-phase lipid-protein systems, there is ample justification for this assumption. For example, Raman spectra obtained for a number of

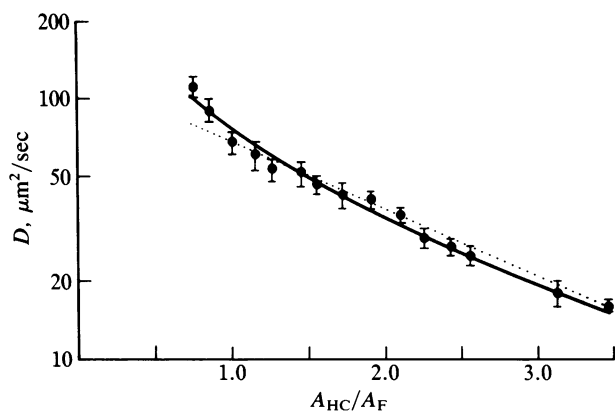


FIG. 1. Theoretical and experimental results on diffusion of lipids in pure dilauroylphosphatidylcholine monolayers. ●, Experimental; ···, best fit of the data (Peters and Beck, ref. 11) to the original free-volume diffusion theory.  $D$ , diffusion rate. At low values of  $A_{HC}/A_F$  the predicted diffusion rate differs from the experimental diffusion rate by nearly 50%. —, Prediction of the present theory, using values of  $A_{HC}$  and  $\gamma$  determined by Peters and Beck. At low values of  $A_{HC}/A_F$ , the agreement of present theory with experimental data is substantially better than that of the original free-volume diffusion theory.

lipid-peptide and lipid-protein systems (15, 16) show no change in lipid C-H stretching region spectra, which reflect both acyl chain trans-gauche isomerization and chain packing (17) when peptides or proteins are incorporated into liquid crystalline-lipid bilayers well above the phase-transition temperature. Although this assumption is probably not universally valid, the theory allows effects of proteins on membrane area to be separated from their direct effects on diffusion. We further assume that for protein and polypeptide species,  $\gamma_i = 1$ , as given by original scaled-particle theory. This assumption also seems reasonable, given the success of hard-sphere virial equations in describing the nonideal solution behavior of concentrated proteins (18, 19). Although the use of a different  $\gamma$  changes the numerical results, it does not change any qualitative conclusions. For purposes of these calculations, we consider only two-component bilayers; the theory should be valid for any number of components, however. We define the lipid as species L with density  $\rho_L$  and hard-core radius  $R_L$ , and we define the protein as species P, with density  $\rho_P$  and radius  $R_P$ . Because the area per lipid  $A_L$  is held constant for each set of calculations, we have

$$A_L = (1 - \pi\rho_P R_P^2)/\rho_L. \quad [12]$$

The lateral pressure  $P$  is zero, because the system is in thermodynamic equilibrium, and the pressure of the membrane is thus balanced by an equal external pressure. This assumption is also appropriate for multilamellar liposomes (20).

Fig. 2 illustrates the effects on the diffusion constant of incorporating a protein of radius  $R_P = 10 R_L$  in the membrane. There is a reduction in the lipid diffusion rate at all lipid areas, with the most dramatic decrease occurring at small free areas. The effects of proteins are more pronounced at higher protein concentrations and for proteins with larger radii. This is further illustrated in Fig. 3 A and B respectively for tightly packed and more loosely dispersed lipids. For a given protein concentration and radius, the fractional reduction in diffusion rate from the value in pure lipid is independent of  $\gamma_L$ , although the absolute magnitude of the diffusion rate is not.

The magnitudes of the reductions of lipid diffusion rates are similar to those observed experimentally in dimyristoylphos-

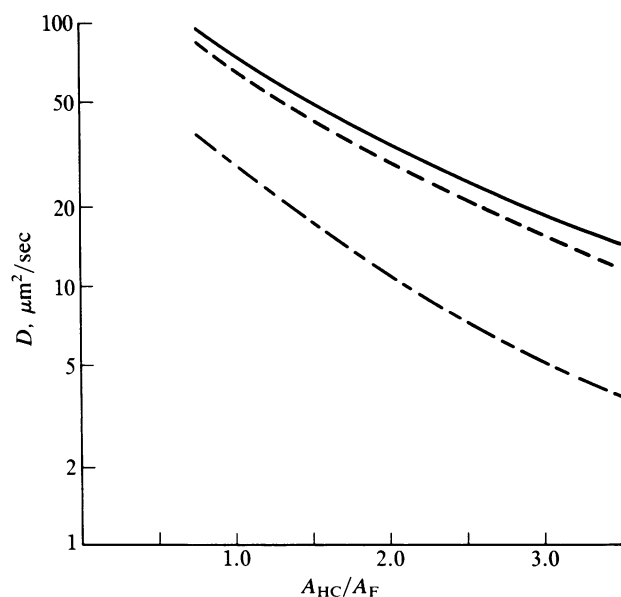


FIG. 2. Theoretical predictions on the reduction of lipid diffusion rates ( $D$ ) for various concentrations of a protein with protein radius  $R_P$  equal to  $10 \times$  the lipid radius  $R_L$ . —, Pure lipid; ---,  $\rho_P = 2.5 \times 10^{-4}/\text{nm}^2$ ; - · - ·,  $\rho_P = 5 \times 10^{-5}/\text{nm}^2$ .

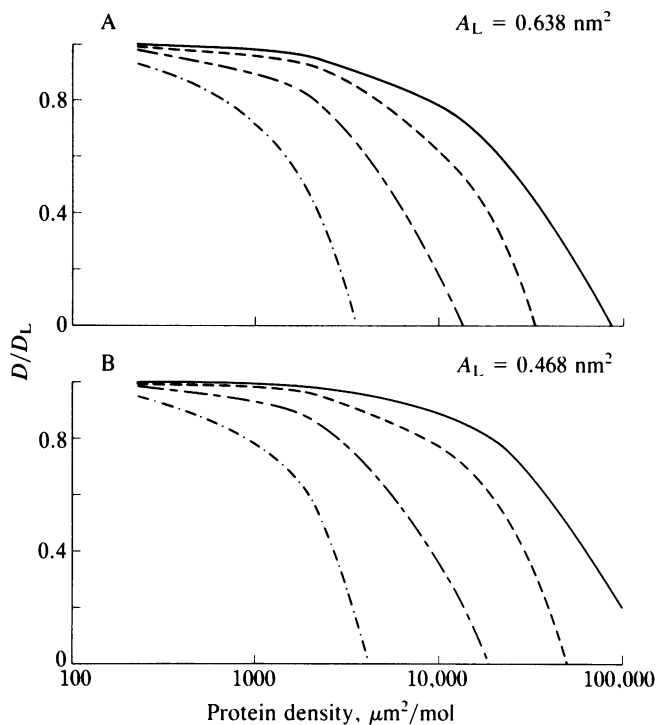


FIG. 3. Theoretical predictions of the reduction of lipid lateral-diffusion rates resulting from incorporation of proteins of various sizes as a function of protein density for tightly packed (A,  $A_L = 0.47 \text{ nm}^2$ ) and loosely packed (B,  $A_L = 0.64 \text{ nm}^2$ ) membranes. Lipids are assumed to have a cross-sectional area  $A_{HC} = 0.425 \text{ nm}^2$ . Proteins are assumed to have areas 9 (—), 25 (---), 100 (---), and 400 (---) times that of the lipid.

phatidylcholine (Myr<sub>2</sub>-PtdCho) membranes reconstituted with bacteriorhodopsin. Peters and Cherry (21) report a 60% reduction in dioctadecyloxatricarbocyanine diffusion rates when bacteriorhodopsin is incorporated in Myr<sub>2</sub>-PtdCho membranes at a 1:70 mol ratio. If we assume a protein radius  $R_P$  of 1.8 nm (22), an area/lipid ( $A_L$ ) of 0.51 (computed using thermal expansion data for Myr<sub>2</sub>-PtdCho cited in ref. 10), and a protein/lipid ratio of 1:65, the theory yields a diffusion constant that is 42% that of the diffusion constant in pure lipid membranes. The excellent agreement between the theoretical prediction and the experimental results suggests that the scaled-particle diffusion model provides a reasonable description of the effects of proteins on lipid diffusion in membranes.

The magnitudes of the reductions in lipid diffusion rates predicted by this theory are similar to those predicted by Saxton's theory (8). Thus, like Saxton's theory, the scaled-particle diffusion model fails to predict the magnitude of the difference in diffusion rates between fibroblast plasma membrane and bilayers composed of pure fibroblast lipids (23). This disagreement between the theoretical predictions and the experimental data may result from the fact that the freeze-fracture experiments used to estimate protein concentrations may not detect all relevant membrane proteins (7) or could easily result from a slight reduction in the area per lipid ( $A_L$ ) in intact fibroblast plasma membranes as compared with bilayers created from fibroblast lipids.

## Discussion

In this paper I have presented a model for lipid diffusion in complex biological membranes. Diffusion is assumed to proceed by "hopping" of molecules into vacancies formed by lateral-density fluctuations. The probability of vacancy formation is calculated using concepts derived from scaled-

particle theory. For single component membranes, the theory has the same functional form as the free-volume diffusion theory of Turnbull and Cohen (1–4), except for a pre-exponential factor. This factor is required to properly normalize the probability of finding a hole at a particular site in the membrane; its inclusion substantially improves the agreement between theory and experimental data on lipid lateral diffusion obtained from pure lipid monolayers. The theory predicts that incorporation of proteins into lipid membranes will reduce the lipid diffusion rate and accurately predicts the amount by which the lipid diffusion rate is reduced by incorporating bacteriorhodopsin into Myr<sub>2</sub>-PtdCho membranes. Although the magnitudes of the predictions of this theory are similar to those obtained using Saxton's theory (8), the scaled-particle diffusion model is conceptually simpler and is applicable to arbitrarily complex membranes. When the formula incorporates experimentally accessible molecular dimensions, it readily accounts for changes in lipid structure that affect diffusion. Because the calculations may be done on a hand calculator or represented by a computer program requiring fewer than 20 lines of FORTRAN code, it should prove easy to apply to the analysis of experimental data.

Two specific predictions should be useful in testing the theory. First, at a given concentration of a given protein, the reduction in lipid lateral-diffusion rate should be greater for more condensed liquid crystalline-phase membranes than for less condensed liquid crystalline-phase lipids; data presented by Peters and Cherry (21) for Myr<sub>2</sub>-PtdCho-bacteriorhodopsin membranes support this prediction. Second, for lipids with a given hard-core area and a specific free area per lipid, the reduction in the lipid diffusion rate resulting from incorporation of a given amount of a given protein should be independent of the precise chemical structure of the lipid. I am unaware of any data that test this prediction. If this prediction can be confirmed experimentally, the modified free-volume diffusion theory should prove valuable in describing diffusion in reconstituted lipid-protein systems.

**Note Added in Proof.** Eisinger *et al.* (24) recently published a model for lipid diffusion in complex membranes. In this model, impermeable domains such as proteins are spatially fixed, unlike the model developed in this paper. Several differences in the relative effects of small and large protein molecules on lipid diffusion result.

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