Mutual diffusion of interacting membrane proteins

James R. Abney,* Bethe A. Scalettar,‡ and John C. Owicki*,§
*Cell and Molecular Biology Division and ‡Chemical Biodynamics Division, Lawrence Berkeley Laboratory, Berkeley, California 94720; and §Department of Biophysics and Medical Physics, University of California, Berkeley, California 94720

ABSTRACT The generalized Stokes-Einstein equation is used, together with the two-dimensional pressure equation, to analyze mutual diffusion in concentrated membrane systems. These equations can be used to investigate the role that both direct and hydrodynamic interactions play in determining diffusive behavior. Here only direct interactions are explicitly incorporated into the theory at high densities; however, both direct and hydrodynamic interactions are analyzed for some dilute solutions. We look at diffusion in the presence of weak attractions, soft repulsions, and hard-core repulsions. It is found that, at low densities, attractions retard mutual diffusion while repulsions enhance it. Mechanistically, attractions tend to tether particles together and oppose the dissipation of gradients or fluctuations in concentration, while repulsions provide a driving force that pushes particles apart. At higher concentrations, changes in the structure of the fluid enhance mutual diffusion even in the presence of attractions. It is shown that the theoretical description of postelectrophoresis relaxation and fluorescence correlation spectroscopy experiments must be modified if interacting systems are studied. The effects of interactions on mutual diffusion coefficients have probably already been seen in postelectrophoresis relaxation experiments.

INTRODUCTION

The lateral mobility of membrane proteins affects many biological processes and has, therefore, been the subject of intensive experimental study (Axelrod, 1983; Petersen, 1984; McCloskey and Poo, 1984; Edidin, 1987). The physics of protein diffusion is probably best understood, however, from a theoretical perspective. From theoretical considerations we can establish a mathematical relationship between protein mobility and physical properties of the protein and lipid bilayer.

It was shortly after the introduction of the fluid-mosaic model (Singer and Nicolson, 1972) that an expression for the protein diffusion coefficient was first obtained, from hydrodynamic fluid theory (Saffman and Delbrück, 1975; Saffman, 1976; Wiegel, 1980; Hughes et al., 1981). The most familiar hydrodynamic result, known as the "Saffman-Delbrück equation," was then subjected to experimental test and found to provide a good description of protein diffusion in simple reconstituted systems containing a low lateral density of protein (Vaz et al., 1984; Clegg and Vaz, 1985). However, we now know that, as the concentration of protein is increased to biologically relevant levels and the proteins begin to interact significantly, the theoretical description of two-dimensional diffusion must be generalized.

For example, our previous theoretical work (Scalettar et al., 1988) demonstrated that two classes of diffusional phenomena, self and mutual diffusion, must be distinguished when the protein molecules interact. Self diffusion describes the random Brownian motion of an individual protein; the self-diffusion coefficient, D^s, is a coefficient of proportionality between the mean-squared displacement of the protein and the time. Mutual diffusion, on the other hand, refers to the relaxation of gradients or fluctuations in protein concentration; a mutual-diffusion coefficient, D^m, may be defined mathematically through the generalized Stokes-Einstein relationship, Fick's laws, and so on. When the protein concentration is nonzero, these two diffusion coefficients are in general different. We recently discussed the nature of the self-diffusion coefficient in Abney et al. (1989); here, we focus on mutual diffusion.

Mutual diffusion is frequently manifest in biological systems and in experimental data. Biologically, gradients in membrane protein concentration may be created near regions of protein insertion or depletion, such as coated pits (Eisinger and Halperin, 1986), in regions of active growth, such as the developing axon (Small et al., 1984), or after the disassembly of membrane structures, such as gap junctions (Lane and Swales, 1980). Experimentally, macroscopic concentration fluctuations are established

Drs. Abney and Scalettar's present address is Laboratories for Cell Biology, Department of Cell Biology and Anatomy, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599.

Dr. Owicki's present address is Molecular Devices Corporation, 4700 Bohannon Drive, Menlo Park, California, 94025.

Address correspondence to Dr. Abney in North Carolina.

0006-3495/89/08/315/12 $2.00

and allowed to decay in postelectrophoresis relaxation (PER) experiments (Poo, 1981; Young et al., 1984), while microscopic gradients (concentration fluctuations) are monitored in fluorescence correlation spectroscopy (FCS) experiments (Elson and Magde, 1974; Magde et al., 1974).

Here, we study the effects that direct (e.g., hard-core and electrostatic) interactions have on mutual diffusion in dense two-dimensional systems. We also analyze the influence of protein-induced changes in lipid flow (hydrodynamics) and conformation (viscosity). Although the light-scattering community has extensively discussed the effects that direct and hydrodynamic interactions have on three-dimensional mutual diffusion, relatively few works (Phillies, 1975; Ackerson and Fleishman, 1982; Scalettar et al., 1988) have addressed the analogous two-dimensional problems. This paper, in particular, represents the first study of two-dimensional mutual diffusion in dense systems, such as biomembranes.

The paper may be outlined as follows. In the Theory section, we derive an expression that describes the effects of direct interparticle interactions on the mutual-diffusion coefficient. Under Methods, we describe the model interprotein interactions that we analyzed and the techniques used to obtain numerical solutions to the theoretical equations. Our numerical data are presented under Results. Finally, in the Discussion, we analyze the effects that attractions, repulsions, hydrodynamics, and protein-induced changes in lipid conformation have on the mutual-diffusion coefficient. We also discuss interaction-generalized descriptions of macroscopic and microscopic gradient-diffusion experiments, such as PER and FCS, and analyze the available experimental data.

2. THEORY

We will analyze mutual diffusion in a two-component system in which there is no volume change upon mixing. For such a system, the relaxation of gradients in the concentrations of species 1 and 2 can be described by flux equations of the form $\mathbf{J}_i = -D_i \nabla C_i$ $(i = 1, 2)$, where J_i, C_i, and D_i are, respectively, the flux, concentration, and diffusion coefficient associated with component i. (The significance of this flux equation is discussed further in Section 5.7.) Moreover, because we are assuming that there is no volume change upon mixing, a net flux of species 1 must in some sense be compensated for by movement of species 2; this statement implies that $D_1 = D_2 \equiv D^m$ (Gosting, 1956). The following discussion focuses on the single mutual-diffusion coefficient, D^m, that then describes the relaxation to equilibrium in the two-component system as a whole.

In our discussion, we will assume that diffusion in the absence of interaction (i.e., at infinite dilution) can be described by D_0, a bare-diffusion coefficient. D_0 can be determined theoretically from the Saffman-Delbrück equation and can, in principal, be measured experimentally by monitoring the motion of a single protein alone in a membrane. The goal then is to calculate the magnitude of the interaction-modified mutual-diffusion coefficient relative to D_0.

We are fundamentally interested in lateral diffusion, and so model only the two-dimensional projection of protein motion. It is well known that calculations of a strictly two-dimensional bare-diffusion coefficient are often plagued by anomalies. However, these anomalies are not manifest in a more realistic de novo calculation of D_0 (Saffman and Delbrück, 1975). Our analysis of interaction effects also does not manifest these difficulties.

The interaction dependence of $D^m(\rho)$ can be analyzed by invoking any of several appropriate physical models. For example, if one starts with the kinetic theory of liquids and introduces an interaction-dependent term into the flux equation given above, one can obtain, to first order in density, an expression for the interaction dependence of the mutual-diffusion coefficient (Phillies, 1974; Felderhof, 1978). We have previously adopted this method in our analysis of mutual diffusion in dilute membrane systems (Scalettar et al., 1988). One can also take a less microscopic viewpoint and formulate (an equivalent) thermodynamic description of the generalized mutual-diffusion problem. We will adopt this latter, complementary approach in the current work.

Einstein was the first to show that, in an inhomogeneous system, the mutual-diffusion coefficient is related to the osmotic pressure, Π, and the mutual-friction coefficient, f^m, as follows (Pusey and Tough, 1985)

$$D^m(\rho) = \frac{1}{f^m(\rho)} \frac{\partial \Pi(\rho)}{\partial \rho}. \tag{1}$$

In this generalized Stokes-Einstein equation, ρ is the number density of proteins. This result is not, perhaps, the most familiar of the Einstein diffusion relationships; however, it can be shown to be equivalent to the well known Einstein formula, $D^m = k_B T / f^m$, if one notes that, for noninteracting systems, $\Pi = \rho k_B T$. Here k_B is Boltzmann's constant and T is the absolute temperature. Note also that we have emphasized the mutual character of the friction coefficient by putting a superscript m on f. Such notation serves as a reminder of the fact that mutual- and self-friction coefficients can differ just as the mutual- and self-diffusion coefficients do (Altenberger and Tirrell, 1984).

Eq. 1 connecting D^m and Π is valid even for interacting systems. This point has been extensively discussed by theorists who are interested in three-dimensional diffusional phenomena (Phillies, 1974; Ohtsuki, 1983; Kops-

Werkhoven et al., 1983; Pusey and Tough, 1985). Moreover, in these analyses of three-dimensional diffusion, it is generally assumed that the osmotic pressure is modified by direct interparticle interactions and that the density dependence of the friction coefficient is primarily due to the effects of hydrodynamic interactions. We can thus analyze these two interactions separately. (We note, however, that the possibility that direct interactions contribute to $f^m(\rho)$ has been discussed [Phillies, 1981].) In the development that follows, we focus our attention on the contribution that direct interparticle interactions make to D^m. Mathematically, we set $f^m(\rho) \equiv f^0$, a constant; hydrodynamic effects are discussed semi-quantitatively in Section 5.4.

It has been shown (Braun et al., 1987) that the osmotic pressure associated with a two-dimensional system of particles that interact through a pair-wise additive potential $u(r)$ is given by the expression

$$\frac{\Pi(\rho)}{k_B T} = \rho - \frac{\rho^2}{4k_B T}\int_0^\infty r\frac{du(r)}{dr}g(r,\rho)2\pi r\,dr. \quad (2)$$

In obtaining Eq. 2, it is assumed that the distribution of particles in the fluid is described by the radial distribution function, $g(r, \rho)$; recall that $g(r, \rho)$ defines the relative probability of finding a second particle at a distance r from a given central particle (e.g., Hill, 1956; McQuarrie, 1976; Braun et al., 1987). In the rest of the paper, we follow standard convention and set $g(r, \rho) \equiv g(r)$.

If we now use Eq. 2 to evaluate the derivative appearing in Eq. 1 and replace $f^m(\rho)$ by f^0, we find that

$$D^m(\rho) = \frac{1}{f^0}\frac{\partial\Pi(\rho)}{\partial\rho} = \frac{k_B T}{f^0} \cdot \left\{1 - \pi\rho\beta\int_0^\infty \frac{du(r)}{dr}\left[g(r) + \frac{1}{2}\rho\frac{\partial g(r)}{\partial\rho}\right]r^2\,dr\right\}. \quad (3)$$

Here $\beta \equiv 1/k_B T$ and the ratio $k_B T/f^0 \equiv D_0$; thus, as $\rho \to 0$, $D^m(\rho)$ reduces, as it should, to D_0.

We can now isolate the effects that direct interactions have on the mutual-diffusion coefficient as the ratio

$$\frac{D^m(\rho)}{D_0} = 1 - \pi\rho\beta\int_0^\infty \frac{du(r)}{dr}\left[g(r) + \frac{1}{2}\rho\frac{\partial g(r)}{\partial\rho}\right]r^2\,dr. \quad (4)$$

Note also that Ohtsuki (1983) has shown that the three-dimensional analogue of Eq. 4 may be obtained from arguments founded in the more microscopic formalism of kinetic theory.

In the dilute limit, the radial distribution function can be approximated by the density-independent analytical expression $g(r) = \exp[-\beta u(r)]$. In this limit, the mutual-diffusion coefficient is given by

$$\frac{D^m(\rho)}{D_0} = 1 - \pi\rho\beta\int_0^\infty \frac{du(r)}{dr}e^{-\beta u(r)}r^2\,dr. \quad (5)$$

This result was previously derived from microscopic arguments in Scalettar et al. (1988).

3. METHODS

In this section, we will describe, in order, (*a*) our sources of data, (*b*) the natural units associated with each data set, (*c*) the determination of $g(r)$ and $\partial g(r)/\partial\rho$, and (*d*) the solution of Eq. 4 for D^m/D_0.

3.1. Sources of data

Data were obtained from Monte-Carlo generated configurations of particles interacting through specified analytical potentials. The potentials defined below serve as models for the effects of both repulsive and attractive interactions on the mutual-diffusion coefficient. Note that we have not presented results for the gap junction potential analyzed in Abney et al. (1989) because we cannot directly obtain values for $\partial g(r)/\partial\rho$: the force and distribution functions for this system are strictly known and valid only at the in vivo densities.

3.1.1. 6-4 potentials

Two, related 6-4 potentials were chosen as models for proteins that interact through soft repulsions at small separations and either through weak attractions, or not at all, at larger separations. In a real membrane system, soft repulsions might arise if there is some deformability at contact; weak, long-ranged attractions could arise from a lipid-mediated protein-protein interaction (Abney and Owicki, 1985).

A general 6-4 potential was defined as

$$u_{64}(r) \equiv \frac{27}{4}\epsilon[(\sigma/r)^6 - (\sigma/r)^4]. \quad (6)$$

This potential crosses zero at $r = \sigma$ and reaches its minimum value, $-\epsilon$, at $r = r_0 = (3/2)^{1/2}\sigma$. See Fig. 1. Differentiation yields the 6-4 pair force, which is repulsive for $r < r_0$ and attractive for $r > r_0$.

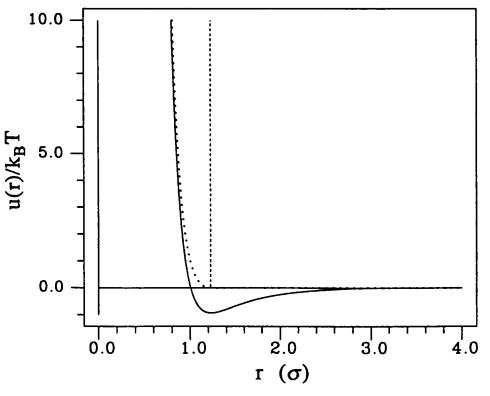

FIGURE 1 Potentials used in the calculation of diffusion coefficients. Three analytical potentials were analyzed: hard core (– – –), fluid A (——), and fluid R (· · · · ·). To facilitate comparison between the functional forms for these potentials, we have arbitrarily set the hard-core diameter d_{HC} equal to r_0. It is shown in the text that only the product $\pi\rho d_{HC}^2/4 \equiv f_A$ determines the rate of change of the diffusion coefficient.

For computational economy during Monte-Carlo simulations, the 6-4 potential was truncated at $r = 2.5r_0 = 3.0619\sigma$; it was also shifted up slightly (by $-u_{64}(2.5\ r_0) \approx 0.07\epsilon$) to maintain continuity. This gave a fluid with repulsions and attractions (fluid A)

$$u_A(r) = \begin{cases} u_{64}(r) - u_{64}(2.5\ r_0) & r < 2.5\ r_0 \\ 0 & r \geq 2.5\ r_0. \end{cases} \quad (7)$$

The force is unaltered by the truncation out to $r = 2.5r_0$; beyond this the force is zero.

A similar procedure was used to generate a purely repulsive fluid (fluid R). Here, the 6-4 potential was truncated at $r = r_0 = 1.2247\sigma$ and shifted upward by ϵ:

$$u_R(r) = \begin{cases} u_{64}(r) - u_{64}(r_0) & r < r_0 \\ 0 & r \geq r_0. \end{cases} \quad (8)$$

This truncation preserves the repulsive component of the 6-4 force (Chandler et al., 1983), while eliminating the attractions.

3.1.2. Excluded-volume interaction

We also analyzed a model system in which the proteins experience an infinite repulsive interaction at some contact separation but do not interact over an extended range. Many of the qualitative features of interaction-dependent diffusion emerge from study of this simple hard-core or hard-disk interaction (Scalettar et al., 1988).

The excluded-volume interaction was defined as

$$u_{HC}(r) \equiv \begin{cases} \infty & r \leq d_{HC} \\ 0 & r > d_{HC}. \end{cases} \quad (9)$$

This potential describes the interaction between two particles with hard-core diameters d_{HC}. See Fig. 1. The associated force is a delta function centered on $r = d_{HC}$.

3.2. A discussion of units

The theoretical equations, 1–5, are written in terms of number density, i.e., the number of particles per unit area. However, results for the two classes of potentials discussed above are presented in terms of more standard units. For the hard-core potential, D^m/D_0 is given as a function of area fraction, $f_A = \pi\rho d_{HC}^2/4$, of protein coverage. For the 6-4 potentials, a unique area fraction cannot be assigned; the standard unit of concentration is the reduced density, ρ^*, defined by $\rho^* \equiv \rho\sigma^2$, where σ is given in Eq. 6.

3.3. Computation of $g(r)$ and $\partial g(r)/\partial\rho$

3.3.1. The 6-4 potentials

Equilibrium particle configurations corresponding to the 6-4 potentials were generated for 256 particles in a square patch using the standard Metropolis et al. (1953) Monte-Carlo algorithm. Simulations were run 2,000–3,000 cycles, where one cycle corresponds to one (sequentially) attempted movement of every particle. The radial distribution function was computed from particle configurations as an average over discrete bins of width Δ_r; we approximated the real values of the function by these averaged values. Beginning with an equilibrated sample, averaging was performed every ten cycles over the entire length of the simulation. Data were collected from a lower cutoff of $r = 0$ to an upper cutoff of $r = 4\sigma$. Detailed descriptions of the algorithms have been previously described in Braun et al. (1987), Abney (1987), and Abney et al. (1989).

Derivatives $\partial g(r)/\partial\rho$ were computed for the 6-4 potentials using values of $g(r)$ at three evenly-spaced values of ρ^*, separated by Δ_{ρ^*}, using Savitzky-Golay fits (Savitzky and Golay, 1964).

In the dilute limit, no simulations were required: the radial distribution function and its density derivative were computed analytically from the relationship $g(r) = \exp[-\beta u(r)]$. However, to maintain consistency with the numerical calculations at higher densities, we still reported values and performed subsequent numerical operations at discrete values of r.

3.3.2. Excluded-volume interaction

For the hard-disk fluid, we required $g(r)$ and $\partial g(r)/\partial\rho$ only at contact. These quantities were obtained for arbitrary densities, without simulations, from a Padé approximant to the hard-disk pressure equation (Ree and Hoover, 1967; McQuarrie, 1976). Specifically, the symmetric (3, 3) Padé approximant allowed us to find an analytical expression for $g(d_{HC})$. Note that this Padé approximant is not an exact expression, but rather an excellent approximation based on an evaluation of the first six hard-disk virial coefficients. We began with the relationship

$$\frac{\Pi(\rho)}{\rho k_B T} - 1 = \frac{b\rho - 0.202080(b\rho)^2 + 0.005589(b\rho)^3}{1 - 0.984085b\rho + 0.242916(b\rho)^2} = \frac{\pi d_{HC}^2\rho}{2} g(d_{HC}), \quad (10)$$

where $b \equiv (1/2)\pi d_{HC}^2$. Converting to units of area fraction and rearranging yielded

$$g(d_{HC}) = \frac{1 - 0.404160 f_A + 0.022356 f_A^2}{1 - 1.968170 f_A + 0.971664 f_A^2}. \quad (11)$$

Appropriate differentiation of this expression gave $\partial g(d_{HC})/\partial f_A$. We note that it is also possible to obtain $\partial\Pi(\rho)/\partial\rho$ directly from Eq. 10 without solving for $g(d_{HC})$.

3.4. Determination of diffusion coefficients

The calculated functions $g(r)$ and $\partial g(r)/\partial\rho$ and the analytical potentials were used to compute the ratio $D^m(\rho)/D_0$ (see Eq. 4). For the 6-4 potentials, the integral was computed numerically using Simpson's Rule (Bevington, 1969). For the excluded-volume interaction, integration over the delta function force gave

$$\frac{D^m(f_A)}{D_0} = 1 + 4f_A\left[g(d_{HC}) + \frac{1}{2}f_A\frac{\partial g(d_{HC})}{\partial f_A}\right]. \quad (12)$$

Equations describing the density dependence of the mutual-diffusion coefficient in the dilute limit were previously derived and reported in Scalettar et al. (1988). These dilute formulae are given in the Results section so that they may be compared with the numerical results obtained from the generalized expression.

4. RESULTS

We present results for the 6-4 potentials (fluid A and fluid R) and the hard-disk potential at a variety of densities.

4.1. 6-4 results

Our results for the 6-4 potentials correspond to a choice of $\epsilon = k_B T$; thus, the depth of the attractive well in fluid A is one $k_B T$.

Fluid A and fluid R were simulated at thirteen reduced densities up to $\rho^* = 0.8$. Radial distribution functions are plotted for these fluids at $\rho^* = 0.0$, 0.3, and 0.8 in Fig. 2. Diffusion coefficients for both potentials are presented together in Fig. 3. To obtain these results, we set $\Delta_r = 0.05$ and used a three-point central Savitzky-Golay fit to find $\partial g(r)/\partial \rho$ (Savitzky and Golay, 1964).

These diffusion data should be contrasted with the predictions of the dilute theory:

$$D_A^m(\rho^*)/D_0 = 1 - 6.20\rho^* \quad \text{Fluid A} \tag{13a}$$

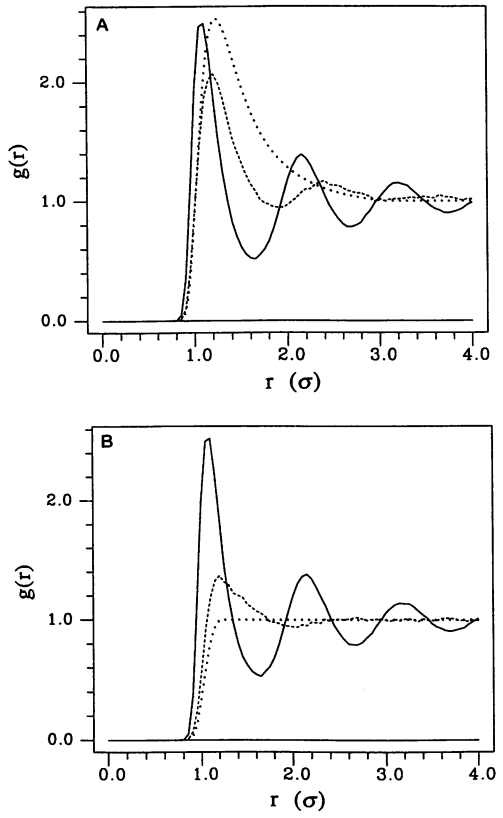

FIGURE 2 Density dependence of the radial distribution functions for fluids A and R. Panel *A* shows $g(r)$ for fluid A at $\rho^* = 0.0$ (· · · · ·), 0.3 (– – –), and 0.8 (——). Panel *B* shows these same functions for fluid R, using the same notation. The radial distribution function gives a probabilistic measure of finding a second particle at a distance r from a given first particle. At small separations, the probability is zero. At large separations, the probability is uniform and normalized to one. At intermediate separations, and with details depending on the potential and the density as shown, the probability oscillates above and below one in indication of shells of enhanced and diminished occupancy, respectively.

$$D_R^m(\rho^*)/D_0 = 1 + 3.34\rho^* \quad \text{Fluid R.} \tag{13b}$$

The linear relationships in Eq. 13, a and b, are also shown in Fig. 3. The dilute theory agrees with the more general formalism at small (reduced) densities, but underestimates D^m/D_0 as ρ^* increases. The disagreement is particularly pronounced for fluid A; for this fluid the dilute result predicts $D^m/D_0 < 1$ when, in fact, at high densities $D^m/D_0 > 1$.

4.2. Excluded-volume results

The density-dependence of the hard-disk diffusion coefficient is displayed in Fig. 4. To first order in area fraction, the following analytical relationship holds

$$D_{HC}^m(f_A)/D_0 = 1 + 4f_A. \tag{14}$$

This result is also shown in Fig. 4. The analytical dilute equation and the numerical results agree exactly for small values of f_A (quantitative comparison not shown); this agreement serves as a check on the accuracy of aspects of our numerical work. At higher concentrations, the dilute expression underestimates the value of the hard-disk mutual-diffusion coefficient.

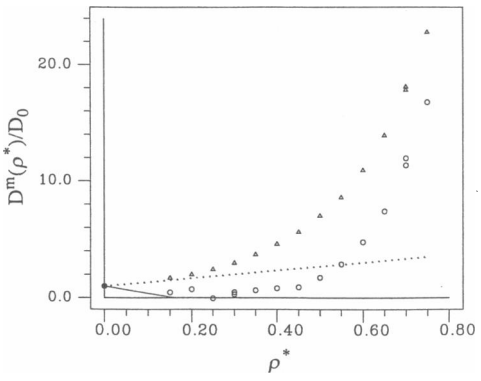

FIGURE 3 Mutual-diffusion coefficients for fluids A (O) and R (△). Error estimates were obtained for two reduced densities, $\rho^* = 0.30$ and 0.70, from identical analysis of independent sets of Monte-Carlo simulations. For both fluids at each of these densities, two points are plotted. Predictions of the dilute theory, Eqs. 13, a and b, for fluid A (——) and fluid R (· · · · ·) are extrapolated to higher densities as references. Relative errors in the data increase as the density decreases. This is because at low densities there is much less structure in the fluid and so the distribution functions are correspondingly noisier. We compensated for this effect to some extent by running the simulations for more cycles at low densities. Note also that, because we used central three-point fits (with $\Delta_{\rho^*} = 0.05$) to find $\partial g(r)/\partial \rho$, an analysis of our simulations at thirteen evenly-spaced values of ρ yielded values of the diffusion coefficient at only eleven densities.

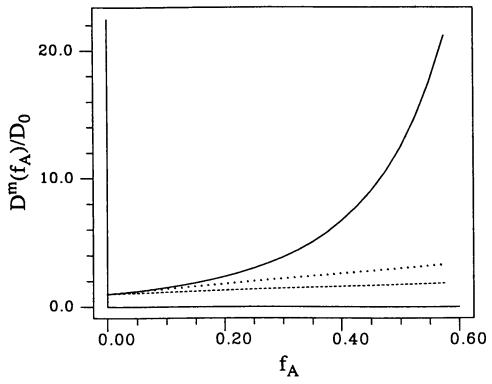

FIGURE 4 Mutual-diffusion coefficients for the hard-core fluid. The general results (——) were obtained analytically from Eqs. 11 and 12. The dilute results (· · · · ·) were derived from Eq. 14 and extrapolated to higher densities as a reference. Finally, an expression incorporating both direct and hydrodynamic interactions, valid in the dilute limit (see Section 5.4), is also plotted (– – –); it too has been extended to higher densities as a reference.

5. DISCUSSION

5.1. Analysis of 6-4 results

We have obtained mutual-diffusion coefficients for fluids A and R at a variety of densities, as shown in Fig. 3. We were interested in comparing the effects of repulsions and long-ranged, weak attractions on diffusive behavior in single-phase systems; hence, we chose $\epsilon = k_B T$ for all simulations (see Section 5.1. of Abney et al. [1989] for more details).

The mutual-diffusion coefficients corresponding to these two potentials display strikingly different density dependences. In fluid R, D^m/D_0 is always greater than unity and grows monotonically with increasing density. In fluid A, on the other hand, this ratio is less than unity if the solution is dilute; however, as the density of the system increases, the interactions in fluid A also begin to enhance mutual diffusion and $D^m/D_0 > 1$. These results show that repulsions and attractions have profound and different effects on the mutual-diffusion coefficient.

If Eq. 4 is examined in detail, a mechanistic rationale for the mutual-diffusion results emerges. Clearly, there are three quantities that are influencing the interaction dependence of D^m: the force, $-du(r)/dr$, and two functions that are related to the structure of the fluid, $g(r)$ and $\partial g(r)/\partial\rho$. If the system is dilute, an integral over the product of two of these quantities, $-du(r)/dr$ and $g(r)$, dictates the interaction dependence of D^m/D_0 (see Eq. 5). Moreover, because the radial distribution function is always positive, the sign of the force determines the magnitude of D^m/D_0. If the interaction is purely repulsive, this ratio is greater than unity, and if the force is purely attractive, it is less than unity. These latter statements hold independent of the detailed characteristics of the interprotein interaction. If the potential is a composite of attractions and repulsions, the results are more complicated. For example, the diffusion coefficient of fluid A is dominated by the attractions, at low densities, because the particles sit preferentially on the attractive component of the interprotein potential (see Fig. 2 in this paper and Fig. 4 in Braun et al. [1987]).

The dilute results are intuitively reasonable. Repulsions tend to push particles apart and accelerate the dissipation of fluctuations or gradients. Conversely, attractions tend to tether particles together and thereby retard the relaxation to the equilibrium state.

What can be said about the behavior of the mutual diffusion coefficient as the density of the solution increases? Because the average interparticle spacing decreases as the density increases, the strong repulsions that are felt by closely packed particles in fluid A must necessarily begin to influence diffusive behavior in this liquid at higher densities. Hence, our low density result, Eq. 5, would lead us to expect that mutual diffusion accelerates in both fluids, A and R, as the density of the solution gets higher. Is this prediction actually in accord with results obtained from our generalized expression, Eq. 4?

Our generalized analysis of mutual diffusion shows that a new quantity, $\partial g(r)/\partial\rho$, also begins to affect the interaction dependence of D^m as the solution becomes less dilute. Moreover, our numerical data indicate that, at high densities, this new term in fact dominates the low density part of Eq. 4. Despite this fact, the prediction made above is correct; at high densities, mutual diffusion does in fact accelerate in both fluids A and R. The reason is as follows.

We have mentioned that as the density of the system increases, the probability of finding particles in proximity increases; therefore, for large ρ and small r, $\partial g(r)/\partial\rho$ is positive and large (see Fig. 2). Moreover, the force becomes very large and positive as the particles approach one another and hence small values of r make dominant contributions to the integral that determines the interaction dependence of D^m. We, therefore, again are led to conclude that the repulsions will dictate the behavior of D^m and that mutual diffusion will accelerate in fluids A and R at high density.

5.2. Analysis of excluded-volume results

We have seen that long-ranged interprotein interactions can markedly influence two-dimensional mutual diffusion. The excluded-volume results obtained here also show that D^m will exhibit interaction-induced changes

even if the membrane protein molecules interact only at contact. Note that mutual diffusion is accelerated in the hard-disk fluid and that the physical and mathematical analysis of fluid R given above is also applicable to the hard-core interaction. Finally, we note that the generalized and dilute hard-disk results agree for low values of f_A.

5.3. Viscosity considerations: perturbation of lipid conformation

We have previously noted that the self-diffusive motion of membrane proteins will be influenced by direct forces and by protein-induced perturbations of lipid conformation and flow (Abney et al., 1989). These same three interactions also affect the mutual-diffusion coefficient. The discussion thus far has been directed only at analysis of the influence that direct forces have on D^m. We now look briefly at conformation and flow effects.

Protein-induced perturbation of lipid conformation (including changes in headgroup and chain orientation and dynamics) probably causes changes in bilayer viscosity. Viscosity, in turn, is a major determinant of the bare-diffusion coefficient of protein molecules. This implies that the denominator in Eq. 4 may not be constant. We have previously suggested that one can account for the effect that lipid perturbation has on the normalized self-diffusion coefficient, D^s/D_0, simply by rescaling the value of the bare-diffusion coefficient, D_0. This same procedure can be applied to the calculation of D^m/D_0. It then follows that increases in viscosity will decrease the mutual-diffusion coefficient and that decreases in viscosity will increase D^m. See Abney et al. (1989) for a more complete discussion.

A protein-induced change in lipid conformation could also alter the interprotein force (Abney and Owicki, 1985). Such density-dependent changes can be incorporated into the analysis of mutual diffusion simply by using the correct expression for the force when evaluating the pertinent equations.

5.4. Hydrodynamic considerations: perturbation of lipid flow

The last interaction that we want to discuss is the hydrodynamic interaction. We have already noted that hydrodynamic forces arise because the solute perturbs the flow of solvent. This perturbation manifests itself in the magnitude of the mutual-friction coefficient and, in turn, in the value of the mutual-diffusion coefficient (see Eq. 1). For repulsively interacting molecules in three dimensions, the mutual-friction coefficient increases when hydrodynamic interactions are important (Pusey and Tough, 1985). Therefore, if the direct force is repulsive, the hydrodynamic interactions push D^m/D_0 back toward unity. In fact, in dense three-dimensional systems that interact through short-ranged repulsions, the effects of the two types of force are so nearly compensatory that $D^m/D_0 \approx 1$ (Pusey and Tough, 1985; Schurr and Schmitz, 1986).

Here we discuss two ways of analyzing the effects that hydrodynamic interactions have on membrane protein diffusion. In the dilute limit one can rigorously incorporate hydrodynamic-interaction tensors into the formalism presented in Scalettar et al. (1988). We then find, within the confines of one model, that the two-dimensional hard-disk mutual-diffusion coefficient is given by the relationship $D^m/D_0 = 1 + 1.5 f_A$ (details to be presented elsewhere); see Fig. 4. This result should be contrasted with the expression that embodies only the direct effects of the hard-disk force, $D^m/D_0 = 1 + 4 f_A$. It is apparent that, in the dilute limit, the hydrodynamic and direct forces produce somewhat compensatory changes in the two-dimensional mutual-diffusion coefficient, as they do in three dimensions. However, in dilute two-dimensional solutions the hydrodynamic and direct interactions do not seem to counteract one another as markedly as they do in three-dimensional systems.

One can also proceed with a semi-empirical analysis of the effects that hydrodynamic interactions have on D^m. Experimental data obtained from three-dimensional systems often indicate that $f^s \approx f^m$ (Phillies, 1984); here f^s is the self-friction coefficient. Hence one can insert an experimentally measured (FRAP) self-friction coefficient into the generalized Stokes-Einstein relationship and thereby hope empirically to incorporate hydrodynamic effects into the formalism. (We are loathe to use our theoretical self-diffusion data to calculate f^s because these data, to date, do not embody hydrodynamic interactions.) Because the experimental f^s increases with density, the qualitative conclusion that one draws from the semi-empirical analysis is the same as that noted above.

5.5. Mutual diffusion and reference frames

We would like to comment briefly on the form of Eq. 1. When one is analyzing mutual diffusion, it is important to specify the frame of reference with respect to which the quantities are measured. We have implicitly used a laboratory-fixed reference frame in our discussion. However, we refer the reader extensively to the three-dimensional literature, in which a solvent-fixed frame of reference is often introduced (see Phillies [1974] and Schurr [1982], among others). (Note that there is, of course, net solvent flow in the macroscopic mutual-diffusion problem, and so one can define a solvent-fixed

frame in which the solvent flux is zero.) In the three-dimensional literature one often finds a modified form of Eq. 1, which reads

$$D^m(\rho) = \frac{1 - f_V}{\xi^m(\rho)} \frac{\partial \Pi(\rho)}{\partial \rho}, \tag{15}$$

where f_V denotes the volume fraction occupied by the solute (i.e., the three-dimensional analogue of f_A, the area fraction) and $\xi^m(\rho)$ is a mutual-friction coefficient measured with respect to a solvent-fixed frame. These two forms of the generalized Stokes-Einstein relationship, Eqs. 1 and 15, are equivalent (Kops-Werkhoven et al., 1983; Pusey and Tough, 1985); the differences in them simply reflect differences in the choice of reference frame for the friction coefficient.

5.6. Relationship to the compressibility equation

We have noted that one can calculate $D^m(\rho)/D_0$ from $\partial \Pi(\rho)/\partial \rho$. Here we began with an expression for $\Pi(\rho)$, which we then differentiated. It is also possible to find $\partial \Pi(\rho)/\partial \rho$ directly from the compressibility equation without evaluating any derivatives (Ben-Naim, 1974; McQuarrie, 1976). We find

$$\frac{\partial \Pi(\rho)}{\partial \rho} = \frac{k_B T}{1 + \rho \int_0^\infty [g(r) - 1] 2\pi r \, dr} \equiv \frac{1}{\rho \kappa_T}, \tag{16}$$

where κ_T is the isothermal compressibility. This expression is superficially simpler than the one that we have used because it depends explicitly only on ρ and $g(r)$, while Eq. 4 also depends on $\partial g(r)/\partial \rho$ and $du(r)/dr$. (Note, of course, that $g(r)$ is a function of the interparticle potential and thus $u(r)$ does appear implicitly in Eq. 16.) The compressibility equation is also of greater generality than Eq. 4 because its derivation does not rest on assumptions about the nature of the interparticle potential (e.g., pair-wise additivity, radial symmetry, etc.).

However, under conditions in which pair-wise additivity obtains, both expressions should (in principle) yield the same result. This statement is true at all densities but is especially easily verified at low densities where Eq. 16 can be shown analytically to reduce to Eq. 5. Despite this equivalence, we feel that the approach we have taken may be better suited to the task at hand, for at least two reasons.

The first reason is pedagogical: Eq. 4 allows one more easily to deduce the sign of the interaction-dependent contribution to the diffusion coefficient, as we saw in Section 5.1.

The second reason is numerical: the difference $[g(r) - 1]$ is weighted by $2\pi r dr$ in Eq. 16 and thus is very sensitive to small fluctuations or errors in $g(r)$ at large separations. Random fluctuations arise due to the small number of particles in the systems under study (membranes or Monte Carlo). These fluctuations are apparent in an analysis of our data (results not shown). Systematic errors can also arise due to the finite sample size and reflect more fundamental theoretical issues. For example, the compressibility equation, which describes number fluctuations, is derived in the grand canonical ensemble. Most Monte Carlo, on the other hand, is performed in the canonical ensemble, while micrograph distribution functions are difficult unambiguously to assign to a particular ensemble. Moreover, the radial distribution function is different in different ensembles. In particular, $g(r) \rightarrow 1 - \rho k_B T \kappa_T / N$ for large r in the canonical ensemble, while $g(r) \rightarrow 1$ for large r in the grand canonical ensemble (Hill, 1956; Lebowitz and Percus, 1961; Ben-Naim, 1974); here N is the number of particles in the system. Only the grand canonical $g(r)$ can properly be used in Eq. 16 for finite N; an erroneous "tail" in an alternative distribution function would significantly alter the integral over $[g(r) - 1]$. We note that these difficulties vanish at large N as they must.

In contrast, our expression involves $g(r)$ and not $[g(r) - 1]$. Thus a small error in $g(r)$ produces only a small error in the integral in Eq. 4. In addition, our integrand is only nonzero at small separations (where $u(r) \neq 0$) and so is completely insensitive to the noise or errors in $g(r)$ that are manifest at large r. Finally, the pressure equation is derived in the canonical ensemble and so is consistent with most Monte Carlo.

To conclude this discussion, we note that the compressibility equation is central to the analysis of diffusion data obtained from light scattering studies of three-dimensional systems. This is because $S(0)$, the zero wave-vector limit of the static structure factor calculated from a dynamic light scattering experiment is closely related to the compressibility.

5.7. Implications for Fick's laws

Thus far, we have presented a quasi-thermodynamic or generalized Stokes-Einstein analysis of interaction-dependent mutual diffusion. However, as we mentioned, mutual diffusion can also be addressed from the viewpoint of kinetic theory. The equivalence of these two approaches has been demonstrated (Phillies, 1974; Kops-Werkhoven et al., 1983; Ohtsuki, 1983).

In this section, we would like to present a brief description of the kinetic or generalized-diffusion formalism because the diffusion equations that are central to that approach are commonly utilized in theoretical descriptions of membrane diffusion. In the standard analyses, the

flux **J**, is written

$$\mathbf{J} = -D\nabla\rho + \frac{D}{k_B T}F\rho. \tag{17}$$

Here D is a (density-independent) diffusion coefficient and F is an applied force, usually electrostatic in origin; most typically this force appears in the flux equations that describe electrophoresis experiments.

Particle conservation dictates that the flux satisfy a diffusion equation of the form

$$\frac{\partial\rho}{\partial t} = -\nabla\cdot\mathbf{J} = D\left[\nabla^2\rho - \frac{1}{k_B T}\nabla\cdot F\rho\right], \tag{18}$$

where D has been pulled out of the divergence. Eqs. 17 and 18 are commonly known as Fick's first and second laws for diffusion with drift, respectively.

We have shown (Scalettar et al., 1988) that in the presence of interactions the form of the flux in Eq. 17 must be modified; this result has also been obtained in analyses of gradient diffusion and light scattering in three dimensions (Phillies, 1974; Ohtsuki, 1983). Specifically, the appropriate form of the generalized flux is

$$\mathbf{J}^m = -D^m(\rho)\nabla\rho + \frac{D_0}{k_B T}F\rho. \tag{19}$$

Here the density-dependent mutual-diffusion coefficient, $D^m(\rho)$, is identical to that derived from thermodynamic arguments in the Theory section and discussed throughout this paper. In the kinetic approach, the introduction of a density-dependent diffusion coefficient does not alter the basic form of Eq. 17. Note that the external term is not influenced by the interactions.

The generalized diffusion equation is now written

$$\frac{\partial\rho}{\partial t} = D_0\nabla\cdot\left[\frac{D^m(\rho)}{D_0}\nabla\rho - \frac{F}{k_B T}\rho\right]. \tag{20}$$

It is no longer possible to pull the full diffusion coefficient outside the divergence because the concentration dependence of D^m gives it an implicit spatial dependence.

5.8. Implications for the experimental determination of diffusion coefficients

At least two of the standard techniques used to monitor the lateral diffusion of membrane proteins reflect mutual diffusion. These are PER and FCS. In this section, we would like to discuss pertinent generalizations of the theories of PER and FCS experiments.

There is a very straightforward way, using the results of the previous section, to generalize the previous theoretical analyses of PER. In a PER experiment, an electric field that is applied to a (cell) membrane creates a concentration gradient by electrophoresing charged proteins toward one pole. The equilibrium distribution that obtains in the presence of the field can be found by setting the flux in Eq. 19 equal to zero. Similarly, the decay of the gradient after the field is turned off is described by the diffusion equation, Eq. 20, when $F = 0$. In both cases, a form for the diffusion coefficient appropriate to charged molecules must be obtained. In a future paper, we will solve these problems for the standard spherical geometry and with a more appropriate diffusion equation that will show how the interparticle interactions modify the results of PER experiments.

The theory of the FCS experiment must also be generalized if the molecules in the system are interacting. In an FCS experiment, one monitors fluctuations in the number of molecules that occupy an open region of a sample. These number (or concentration) fluctuations arise because molecules continually diffuse in and out of the region of sample under observation. It is well known that for ideal (i.e., noninteracting) solutions the fluctuation autocorrelation function, $G(\tau)$, monitored in an FCS experiment, is given by

$$G(\tau) = \frac{G(0)}{(1 + 4D_0\tau/\omega_0^2)}. \tag{21}$$

Here τ is a time parameter and ω_0 characterizes the size of the region of the sample under observation.

Phillies (1975) has shown that this equation must be generalized when the particles interact. We do not present his general result; however, we do display two limiting forms of his equation that are mathematically simple and of particular interest here. Phillies found that if essentially all of the proteins are labeled, $G(\tau)$ is

$$G(\tau) = \frac{G(0)}{(1 + 4D^m(\rho)\tau/\omega_0^2)}. \tag{22}$$

In contrast, if only a small fraction of the proteins have been tagged, the expression is

$$G(\tau) = \frac{G(0)}{(1 + 4D^s(\rho)\tau/\omega_0^2)}. \tag{23}$$

Hence, in the former case, an FCS experiment will yield the mutual-diffusion coefficient; in the latter case, however, the experiment will yield the self-diffusion coefficient. Note that the density-dependent, mutual- and self-diffusion coefficients appearing in Eqs. 22 and 23 are precisely those that we discuss here and in Abney et al. (1989).

5.9. Comparison with self diffusion

In a previous paper (Scalettar et al., 1988), we presented theories of mutual and self diffusion in dilute two-

dimensional systems. Since that time, we have extended the theories (in Abney et al. [1989] and in this paper); they are now also applicable to concentrated solutions. Our new results support the basic qualitative conclusions described in Scalettar et al. (1988). The new data presented here are also better suited for comparison with experimental results, which typically do not refer to truly dilute systems.

For both kinds of diffusion and for all potentials analyzed, we have found that the predictions of the dilute theory coincide with the results obtained from the generalized formalism at lower densities. We emphasize the relationship between the theoretical predictions of the dilute and generalized theories because our initial modeling of the effects of hydrodynamic interactions and protein-induced changes in viscosity are being carried out in the dilute limit.

We can also compare the theoretical expressions for the mutual- and self-diffusion coefficients. Here, both the derivation and final expression are simpler for D^m. While D^m was derived from simple thermodynamic arguments involving pairs of proteins, D^s was obtained using a perturbation approach and involves three-particle information. The programming and data collection necessary to get the diffusion coefficient were also proportionally simpler for D^m. However, we note that because the computation of D^m requires simulations at multiple densities to calculate $\partial g(r)/\partial\rho$, the computational expenses to obtain the two diffusion coefficients are similar.

Finally, we note that, in the case of the ubiquitous excluded-volume interaction, analytical summaries of the data are now available for both self- (Saxton [1987], Eq. 12) and mutual- (this paper, Eqs. 11 and 12) diffusion coefficients. Both results describe diffusion under the same conditions, neglecting hydrodynamic interactions and protein-induced changes in lipid conformation.

5.10. Comparison with experiment

The density dependences of the self-diffusion coefficients of gramicidin, bacteriorhodopsin, and antibodies tethered to lipid hapten at the membrane surface have been experimentally inferred from fluorescence recovery after photobleaching (FRAP) experiments. Therefore, theoretical predictions concerning this diffusion coefficient can directly be compared with experiment (see Abney et al. [1989] for references and further discussion).

The density dependence of the two-dimensional mutual-diffusion coefficient has not been the subject of systematic experimental study. This reflects limitations in the applicability and interpretation of the relevant experimental approaches. PER results must be analyzed in terms of Eqs. 19 and 20 using values of D^m that reflect the range of densities found in the concentration gradient. To date, however, these experiments have been interpreted only within the framework of a single, in some sense, "averaged" diffusion coefficient. FCS, on the other hand, which can be used to measure the mutual-diffusion coefficient at a single density, has not been systematically applied to membrane systems. Thus, a precise comparison of our predictions with experimental data is not possible, and we must be content with a more inferential analysis.

We have already taken one such inferential approach in Scalettar et al. (1988). There we noted that, if the protein molecules interact repulsively, the mutual-diffusion coefficient should be larger than the self-diffusion coefficient. The 6-4 results presented here imply that this statement may also be true at sufficiently high densities even if the proteins interact attractively. Thus, if we compare self- and mutual- (or FRAP and, for example, PER) diffusion coefficients for systems of identical proteins, the ratio D^m/D^s should be greater than one. As noted in Scalettar et al. (1988), such an effect is indeed observed for a variety of systems, although exceptions exist.

A similar set of ideas was set forth previously by Small et al. (1984) when they analyzed diffusion along a concentration gradient in developing bullfrog olfactory axons. The authors noted that the diffusion coefficients they obtained for this system were larger than those typically measured by FRAP experiments. Their results were qualitatively interpreted within the framework of a thermodynamic formalism, known as Darken's Relation (Darken, 1948); this relationship has been used to explain the disparity between self and mutual diffusion in binary alloys containing vacancies.

More recently, Ryan et al. (1988) analyzed the equilibrium spatial distribution of proteins on cells in an applied electric field. These experiments suggest that interactions between the proteins play a major role in dictating their equilibrium distribution within the field. The authors note that the gradient of chemical potential associated with such interactions would serve to enhance lateral diffusion relative to that of an isolated membrane protein. This observation is in agreement with our analysis of the effects that repulsive interparticle interactions have on the mutual-diffusion coefficient. The authors present an apparent paradox involving differences between their experimental observation and theoretical predictions which suggest that (excluded-volume) interactions should inhibit diffusion (their reference 21). We feel that this paradox may be resolved by noting that the works cited by Ryan et al. (1988) contain analyses of self diffusion, for which a different interaction dependence is to be expected.

The above set of experiments strongly suggests that interactions are having an observable effect on measured

mutual-diffusion coefficients. However, precise comparisons between theory and experiment (and especially the separation of direct interactions, hydrodynamics, and viscosity effects) must await more systematic experimental studies.

6. CONCLUSIONS

The most significant conclusion of Abney et al. (1989) was that the self diffusion of membrane proteins should be viewed as the diffusion of interacting particles. Here, we extend this conclusion to the process of mutual diffusion. We have demonstrated that interactions between proteins are sufficient to produce a fewfold modulation in the mutual-diffusion coefficient. The sign of this modulation depends critically on the nature of the interaction. Pure repulsions cause the mutual-diffusion coefficient to increase with density. In composite potentials, attractions cause D^m to decrease at low densities, while repulsions reverse this trend at high densities. Note that the high density results are especially relevant to biological systems where proteins are found under very crowded conditions.

We have also demonstrated that, in dilute solutions, hydrodynamic interactions serve somewhat to offset the modulation produced by direct hard-disk forces.

The effects of interactions on the mutual-diffusion coefficient have probably been observed experimentally; this statement is based on the fact that systematic differences are often observed between FRAP (self) and PER (mutual) diffusion coefficients. However, a systematic set of experiments will have to be performed before we can compare the predicted density dependence of the mutual-diffusion coefficient with real data.

Finally, we note that most biological membranes contain more that one species of protein. In addition, there may or may not be obstacles in the form of anchored proteins and extramembranous structures. Future work must bridge the gap between our single protein analysis and these more complicated systems.

We are pleased to acknowledge helpful conversations on various aspects of diffusion with Andrezj Altenberger, Daniel Axelrod, Aaron Kantor, Daniel Kivelson, George Phillies, Karl Runge, Tim Ryan, Michael Saxton, Richard Scalettar, Michael Schurr, Nancy Thompson, Matthew Tirrell, and Watt Webb.

This work was funded in part by National Institutes of Health grant AI-22860 to John Owicki. James Abney and Bethe Scalettar are supported by appointments to the Alexander Hollaender Distinguished Postdoctoral Fellowship Program supported by the United States Department of Energy, Office of Health and Environmental Research, and administered by Oak Ridge Associated Universities.

Received for publication 27 September 1988 and in final form 7 April 1989.

REFERENCES

Abney, J. R. 1987. Protein-Protein Interactions in Membranes. Ph.D. Thesis, University of California, Berkeley, CA.

Abney, J. R., and J. C. Owicki. 1985. Theories of protein-lipid and protein-protein interactions in membranes. *In* Progress in Protein-Lipid Interactions. A. Watts and J. J. H. H. M. de Pont, editors. Elsevier North-Holland Biomedical Press, Amsterdam. 1–59.

Abney, J. R., B. A. Scalettar, and J. C. Owicki. 1989. Self diffusion of interacting membrane proteins. *Biophys. J.* 55:817–833.

Ackerson, B. J., and L. Fleishman. 1982. Correlations for dilute hard core suspensions. *J. Chem. Phys.* 76:2675–2679.

Altenberger, A. R., and M. Tirrell. 1984. Friction coefficients in self-diffusion, velocity sedimentation, and mutual diffusion. *J. Polym. Sci. Polym. Phys. Ed.* 22:909–910.

Axelrod, D. 1983. Lateral motion of membrane proteins and biological function. *J. Membr. Biol.* 75:1–10.

Ben-Naim, B. 1974. Water and Aqueous Solutions: Introduction to a Molecular Theory. Plenum Publishing Corp., New York.

Bevington, P. R. 1969. Data Reduction and Error Analysis for the Physical Sciences. McGraw-Hill Book Co., New York.

Braun, J., J. R. Abney, and J. C. Owicki. 1987. Lateral interactions among membrane proteins: valid estimates based on freeze-fracture electron microscopy. *Biophys. J.* 52:427–439.

Chandler, D., J. D. Weeks, and H. C. Andersen. 1983. Van der Waals picture of liquids, solids, and phase transformations. *Science (Wash., DC).* 220:787–794.

Clegg, R. M., and W. L. C. Vaz. 1985. Translational diffusion of proteins and lipids in artificial bilayer membranes: a comparison of experiment with theory. *In* Progress in Protein-Lipid Interactions. A. Watts and J. J. H. H. M de Pont, editors. Elsevier North-Holland Biomedical Press, Amsterdam. 173–229.

Darken, L. S. 1948. Diffusion, mobility, and their interrelation through free energy in binary metallic systems. *Trans. AIME (Am. Inst. Min. Metall. Eng.).* 175:184–201.

Edidin, M. 1987. Rotational and lateral diffusion of membrane proteins and lipids: phenomena and function. *Curr. Top. Membr. Transp.* 29:91–127.

Eisinger, J., and B. I. Halperin. 1986. Effects of spatial variation in membrane diffusibility and solubility on the lateral transport of membrane components. *Biophys. J.* 50:513–521.

Elson, E. L., and D. Magde. 1974. Fluorescence correlation spectroscopy. I. Conceptual basis and theory. *Biopolymers.* 13:1–27.

Felderhof, B. U. 1978. Diffusion of interacting Brownian particles. *J. Phys. A: Math. Gen.* 11:929–937.

Gosting, L. J. 1956. Measurement and interpretation of diffusion coefficients of proteins. *Adv. Protein Chem.* XI:429–554.

Hill, T. L. 1956. Statistical Mechanics. McGraw-Hill Book Co., New York.

Hughes, B. D., B. A. Pailthorpe, and L. R. White. 1981. The translational and rotational drag on a cylinder moving in a membrane. *J. Fluid Mech.* 110:349–372.

Kops-Werkhoven, M. M., A. Vrij, and H. N. W. Lekkerkerker. 1983. On the relation between diffusion, sedimentation, and friction. *J. Chem. Phys.* 78:2760–2763.

Lane, N. J., and L. S. Swales. 1980. Dispersal of junctional particles, not internalization, during the in vivo disappearance of gap junctions. *Cell.* 19:579–586.

Lebowitz, J. L., and J. K. Percus. 1961. Long-range correlations in a

closed system with applications to nonuniform fluids. *Phys. Rev.* 122:1675–1691.

Magde, D., E. L. Elson, and W. W. Webb. 1974. Fluorescence correlation spectroscopy. II. An experimental realization. *Biopolymers.* 13:29–61.

McCloskey, M., and M.-m. Poo. 1984. Protein diffusion in cell membranes: some biological implications. *Int. Rev. Cytol.* 87:19–81.

McQuarrie, D. A. 1976. Statistical Mechanics. Harper and Row, New York.

Metropolis, N., A. Rosenbluth, M. Rosenbluth, A. Teller, and E. Teller. 1953. Equations of state calculations by fast computing machines. *J. Chem. Phys.* 27:720–733.

Ohtsuki, T. 1983. Generalized diffusion equation of interacting Brownian particles. *Chem. Phys. Lett.* 98:121–124.

Petersen, N. O. 1984. Diffusion and aggregation in biological membranes. *Can. J. Biochem. Cell Biol.* 62:1158–1166.

Phillies, G. D. J. 1974. Effects of intermacromolecular interactions on diffusion, I. *J. Chem. Phys.* 60:976–982.

Phillies, G. D. J. 1975. Fluorescence correlation spectroscopy and nonideal solutions. *Biopolymers.* 14:499–508.

Phillies, G. D. J. 1981. Contribution of nonhydrodynamic interactions to the concentration dependence of the friction factor of the mutual diffusion coefficient. *J. Chem. Phys.* 74:2436–2440.

Phillies, G. D. J. 1984. Why does the generalized Stokes-Einstein equation work? *Macromolecules.* 17:2050–2055.

Poo, M.-m. 1981. In situ electrophoresis of membrane components. *Annu. Rev. Biophys. Bioeng.* 10:245–276.

Pusey, P. N., and R. J. A. Tough. 1985. Particle interactions. *In* Dynamic Light Scattering: Applications of Photon Correlation Spectroscopy. R. Pecora, editor. Plenum Publishing Corp., New York. 85–179.

Ree, F. H., and W. G. Hoover. 1967. Seventh viral coefficients for hard spheres and hard disks. *J. Chem. Phys.* 46:4181–4197.

Ryan, T. A., J. Myers, D. Holowka, B. Baird, and W. W. Webb. 1988. Molecular crowding on the cell surface. *Science (Wash., DC).* 239:61–64.

Saffman, P. G. 1976. Brownian motion in thin sheets of viscous fluid. *J. Fluid Mech.* 73:593–602.

Saffman, P. G., and M. Delbrück. 1975. Brownian motion in biological membranes. *Proc. Natl. Acad. Sci. USA.* 72:3111–3113.

Savitzky, A., and M. J. E. Golay. 1964. Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.* 36:1627–1639.

Saxton, M. J. 1987. Lateral diffusion in an archipelago: the effect of mobile obstacles. *Biophys. J.* 52:989–997.

Scalettar, B. A., J. R. Abney, and J. C. Owicki. 1988. Theoretical comparison of the self diffusion and mutual diffusion of interacting membrane proteins. *Proc. Natl. Acad. Sci. USA.* 85:6726–6730.

Schurr, J. M. 1982. The thermodynamic driving force in mutual diffusion of hard spheres. *Chem. Phys.* 65:217–223.

Schurr, J. M., and J. S. Schmitz. 1986. Dynamic light scattering studies of biopolymers: effects of charge, shape and flexibility. *Ann. Rev. Phys. Chem.* 37:271–305.

Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science (Wash., DC).* 175:720–731.

Small, R. K., M. Blank, R. Ghez, and K. H. Pfenninger. 1984. Components of the plasma membrane of growing axons. II. Diffusion of membrane complexes. *J. Cell Biol.* 98:1434–1443.

Vaz, W. L. C., F. Goodsaid-Zalduondo, and K. Jacobson. 1984. Lateral diffusion of lipids and proteins in bilayer membranes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 174:199–207.

Wiegel, F. W. 1980. Fluid Flow Through Porous Macromolecular Systems. Lecture Notes in Physics. Vol. 121. Springer-Verlag New York, Inc., NY.

Young, S. H., M. McCloskey, and M.-m. Poo. 1984. Migration of cell surface receptors induced by extracellular electric fields: theory and applications. *In* The Receptors. P. M. Conn, editor. Academic Press, Inc., New York. 511–539