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Theoretical comparison of the self diffusion and mutual diffusion of interacting membrane proteins

(lateral diffusion/fluorescence photobleaching/*in situ* electrophoresis)

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ABSTRACT **Self diffusion and mutual diffusion in two-dimensional membrane systems are analyzed. It is shown that interprotein interactions can produce markedly different density-dependent changes in the diffusion coefficients describing these two processes; the qualitative differences are illustrated by using a theoretical formalism valid for dilute solutions. Results are obtained for three analytical potentials: hard-core repulsions, soft repulsions, and soft repulsions with weak attractions. Self diffusion is inhibited by all three interactions. In contrast, mutual diffusion is inhibited by attractions but is enhanced by repulsions. It is shown that such interaction-dependent differences in self diffusion and mutual diffusion could underlie, among other things, the disparity in protein diffusion coefficients extracted from fluorescence recovery after photobleaching and postelectrophoresis relaxation data.**

The lateral diffusion of membrane proteins has been extensively studied (1–8). A considerable body of experimental data has established rates of protein diffusion in both natural membranes and well-characterized model bilayer systems. The experimental work has helped to identify the physical variables (e.g., membrane viscosity, temperature, protein geometry, and protein concentration) that influence diffusion and has amply demonstrated the biological importance of protein mobility. Theoretical descriptions of two-dimensional diffusion (9, 10) have also served to define those properties of the bilayer that most profoundly affect the diffusion of proteins. Similarly, recent computer simulation studies have shown that interprotein interactions modulate membrane protein mobility (11–13).

In this paper we analyze the interaction dependence of two important types of diffusional phenomena in biomembranes: self diffusion and mutual diffusion. We focus on these two processes because each is manifest experimentally and each underlies fundamental biological phenomena. Self diffusion is perhaps most easily explained in the context of a thought experiment. Imagine a uniform system in which a single solute molecule is labeled. Brownian forces will cause this molecule to undergo some mean-squared displacement $\langle r^2 \rangle$ in a time t. In two dimensions, the self-diffusion coefficient, D^s, is then defined by the relationship $\langle r^2 \rangle = 4D^s t$. Self diffusion is monitored by fluorescence recovery after photobleaching (FRAP; refs. 14–17). Moreover, certain biological processes [e.g., visual transduction (18) and mitochondrial bioenergetics (19)] rely on self-diffusive motion to bring about the requisite intermolecular contacts.

Mutual diffusion, on the other hand, refers to the relaxation of fluctuations or gradients in protein concentration. A mutual diffusion coefficient, D^m, can be defined, for example, by Fick's laws. An understanding of mutual diffusion will help us to appreciate protein movement toward coated pits (theoretical discussions include ref. 20 and references therein) and the disassembly of structures such as gap junctions (21). The mutual diffusion coefficient can be experimentally determined by monitoring postelectrophoresis relaxation (PER; refs. 22 and 23).

At infinite dilution, the self- and mutual-diffusion coefficients have the same value, D_0. This "bare" diffusion coefficient is given, within the confines of one model, by the Saffman–Delbrück equation (9). However, at nonzero lateral protein densities, membrane proteins interact through mutual excluded volume and sometimes through longer-ranged potentials (24–27). These interactions can produce markedly different density-dependent changes in D^s and D^m.

Here the aim is to understand the qualitative effects that interactions can have on protein diffusion; hence, we present a relatively simple theory and numerical data that describe dilute systems containing a single protein species. For the sake of clarity, we will temporarily neglect hydrodynamic interactions between protein molecules; the interested reader is referred to the three-dimensional literature (28). We study three analytical potentials[¶]—hard-core repulsions, soft repulsions, and soft repulsions with weak superimposed attractions—that illustrate the richness of possible interaction-modified diffusive behavior. All three potentials inhibit self diffusion. In contrast, mutual diffusion is slowed by attractions and enhanced by repulsions. We conclude by showing that interaction-induced differences in self diffusion and mutual diffusion could underlie the observed disparity (23) in diffusion coefficients determined by FRAP and PER.

THEORY

In this section we analyze two-dimensional self diffusion and mutual diffusion; a similar discussion of three-dimensional diffusion has been presented by Ohtsuki and Okano (30) and Felderhof (31). The development will rely on the concept of the distribution function and ideas that are central to the theory of simple fluids (32, 33).

We note that we are modeling a two-dimensional projection of diffusive motion in a three-dimensional membrane. Hence, membrane protein diffusion is associated with three-dimensional momentum transfer and should be free from any anomalies that arise in true two-dimensional systems.

Self Diffusion. Here we establish a relationship between the density and interaction potential of a system and the normalized two-dimensional self-diffusion coefficient, D^s/D_0. We begin by stating that the particles in the system move under the influence of Brownian, viscous drag, and interprotein forces. In addition, it will be assumed for analytical rea-

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Abbreviations: FRAP, fluorescence recovery after photobleaching; PER, postelectrophoresis relaxation.

[¶]In previous work (27) we determined the potential that characterizes the interactions between proteins in mouse liver gap junction at high (native) lateral densities. Since it is possible that the gap-junction force varies with density (29), we defer analysis of diffusion in the presence of the gap-junction potential.

sons that one particular particle, denoted 1, is subjected to an oscillatory external force, $F_0\hat{e}_0\exp(i\omega t)$. Therefore, the motion of particle 1 is "driven" and can be characterized by computing the mobility, $\mu(\omega)$, defined by

$$\langle \mathbf{v}_1 \rangle = \mu(\omega)\mathrm{F}_0\hat{e}_0 e^{i\omega t}, \quad [1]$$

where $\langle \mathbf{v}_1 \rangle$ is the average velocity of particle 1.

A self-diffusion coefficient D^s describing the purely diffusive or "undriven" motion of a particle in the system can be extracted from $\mu(\omega)$ as follows (34):

$$D^s = \lim_{\omega \to 0} k_B T \mu(\omega). \quad [2]$$

Here k_B is Boltzmann's constant and T is the temperature.

The mobility and hence D^s can be calculated once $\langle \mathbf{v}_1 \rangle$ is known. An expression for $\langle \mathbf{v}_1 \rangle$ follows directly from the Langevin equation of motion for particle 1:

$$m \frac{d\mathbf{v}_1}{dt} = -f_s \mathbf{v}_1 + F_0\hat{e}_0 e^{i\omega t} - \sum_{j>1} \nabla_1 u(r_{1j}) + \mathbf{F}_{\mathrm{Brown}}(t). \quad [3]$$

Here m is the mass of a protein molecule, f_s is the friction factor, $u(r_{1j})$ is a pair-wise additive interaction potential that is summed over other molecules j, and $\mathbf{F}_{\mathrm{Brown}}(t)$ is the random Brownian force. If the inertial term in Eq. 3 is neglected, $\langle \mathbf{v}_1 \rangle$ takes the form

$$\langle \mathbf{v}_1 \rangle = \frac{1}{f_s}\left[F_0\hat{e}_0 e^{i\omega t} - \frac{1}{\rho}\int \nabla_1 u(r) P_2(\mathbf{r}, t) d\mathbf{r}\right], \quad [4]$$

where $\mathbf{r} = \mathbf{r}_2 - \mathbf{r}_1$ (see Fig. 1), and $P_2(\mathbf{r}, t)$ is the time-dependent two particle distribution function. In obtaining Eq. 4, we have invoked the fact that the average of the Brownian force is zero. To proceed with the calculation of $\langle \mathbf{v}_1 \rangle$, $P_2(\mathbf{r}, t)$ must be known. The thrust of this discussion will center on the method for determining this distribution function.

With the neglect of inertial effects, the two-particle distribution function will satisfy a generalized diffusion equation,

$$\frac{\partial P_2(\mathbf{r}, t)}{\partial t} = \nabla \cdot \mathbf{J}^s, \quad [5]$$

where the flux, $\mathbf{J}^s$, is given by

$$\mathbf{J}^s = D_0\{[2\nabla_r + 2\beta\nabla_r u(r)]P_2(\mathbf{r}, t) + F_0\beta\hat{e}_0 P_2(\mathbf{r}, t)e^{i\omega t}\}. \quad [6]$$

Here $\beta \equiv 1/k_B T$. Eqs. 5 and 6 follow from the N-particle Smoluchowski equation upon integration over particle positions, $\mathbf{r}_i$, $i > 2$, and conversion to relative coordinates (35). We have neglected a term in $\mathbf{J}^s$ that depends on the three-particle distribution function because, in a dilute solution, the probability of finding three particles in proximity is small.

Eq. 5 may be solved by using perturbation-theory techniques. Before the external force is applied to particle 1, the protein distribution is governed by the equilibrium radial distribution function $g(r)$. Therefore, when the external field is turned on, we assume that the perturbed molecular distribution takes the form

$$P_2(\mathbf{r}, t) = \rho^2 g(r)[1 + \gamma p(r, \omega)\cos\theta e^{i\omega t}], \quad [7]$$

where γ is an expansion parameter that characterizes the strength of the external force (35), $p(r, \omega)$ is the radial perturbation, and $\cos\theta$, the cosine of the angle between $\mathbf{r}$ and $\hat{e}_0$, dictates the angular dependence of $P_2(\mathbf{r}, t)$.

If the perturbation expression for P_2 is inserted into Eq. 5 and the coefficients of γ^1 are equated {the γ^0 term yields $g(r) = \exp[-\beta u(r)]$}, one obtains an equation for $p(r, \omega)$. It is apparent from Eq. 2 that we are interested in the limit $\omega \to 0$; in this limit, $p(r, \omega = 0) \equiv p(r)$ satisfies the equation

$$\nabla_r \cdot \{[\nabla_r + \beta\nabla_r u(r)]g(r)p(r)\cos\theta\} = -\cos\theta \frac{dg}{dr}. \quad [8]$$

The expression under the divergence in Eq. 8 is simply $g(r)\nabla_r[p(r)\cos\theta]$. Upon writing the vector operators in two-dimensional polar coordinates, we diverge from the previous three-dimensional analysis (31) and obtain a perturbation equation valid in two dimensions

$$r^2 g \frac{d^2 p}{dr^2} + \left(r^2 \frac{dg}{dr} + rg\right)\frac{dp}{dr} - gp = -r^2 \frac{dg}{dr} \quad [9]$$

Eq. 9 is solved numerically (see *Methods*) subject to boundary conditions, which state that the radial perturbation approaches zero as r becomes very large,

$$\lim_{r \to \infty} p(r) = 0, \quad [10a]$$

and that the flux $\mathbf{J}^s$, vanishes when two particles "collide,"

$$\frac{dp}{dr} = -1. \quad [10b]$$

The implementation of Eq. 10 is discussed under *Methods*.

Once $p(r)$ is known, the diffusion coefficient is easily determined. It follows from Eqs. 1, 2, 4, and 7 that

$$\frac{D^s(\rho)}{D_0} = 1 + \frac{\rho\beta\pi}{2}\int_0^\infty \frac{du(r)}{dr} p(r)g(r)r dr. \quad [11]$$

Mutual Diffusion. Here we investigate the interaction and density dependence of the mutual-diffusion coefficient, D^m, in dilute solutions. The calculation of an interaction-modified D^m will proceed by analogy with standard discussions of mutual diffusion. These standard treatments do not take into account interparticle interactions and hence are strictly valid only at infinite solute dilution. It is usually assumed that a gradient in concentration or in the one-particle distribution function, $P_1(\mathbf{r}_1, t)$, exists in the membrane and that the flux is

$$\mathbf{J}^m = D_0\nabla P_1(\mathbf{r}_1, t). \quad [12]$$

We use the same notation, D_0, for the bare self- and mutual-diffusion coefficients because at infinite solute dilution, these quantities should be identical. Our problem is then to identify the analogue of Eq. 12 that is valid at nonzero densities, where D_0 is replaced by a generalized D^m.

Ohtsuki and Okano (30) and Felderhof (31) have discussed this problem in three dimensions; in their treatments, the

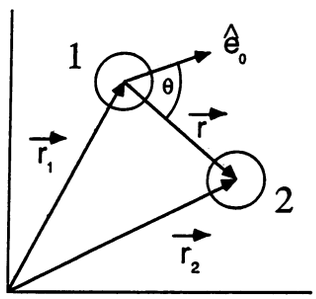

FIG. 1. Coordinate system used in the evaluation of diffusion coefficients.

generalization of Eq. **12** is determined as follows. The diffusion equation for $P_1(\mathbf{r}_1, t)$ is

$$\frac{\partial P_1(\mathbf{r}_1, t)}{\partial t} = D_0 \nabla_1 \cdot \left[\nabla_1 P_1(\mathbf{r}_1, t) + \beta \int \nabla_1 u(\mathbf{r}) P_2(\mathbf{r}_1, \mathbf{r}_2, t) d\mathbf{r}_2 \right]. \quad [13]$$

Therefore, the flux that enters into the calculation of D^m is

$$\mathbf{J}^m = D_0 \left[\nabla_1 P_1 + \beta \int \nabla_1 u(r) P_2 d\mathbf{r}_2 \right]. \quad [14]$$

If Eqs. **12** and **14** are compared, it is evident that we can identify an interaction-modified mutual-diffusion coefficient if **14** is recast into a form in which the flux is directly proportional to $\nabla P_1(\mathbf{r}_1, t)$. To do this, we need to calculate P_1 and P_2 when the gradient is present. For a uniform system, $P_1 = \rho$, the density of the system, and $P_2 = \rho^2 g(r)$. However, the imposition of a gradient serves to change the distribution of protein and the functions P_1 and P_2. The gradient is, therefore, the analogue of the external field in the self-diffusion problem, and we proceed, as we did in the earlier discussion, with a perturbation analysis of its effect on distribution.

We will allow the gradient to produce small deviations from the equilibrium particle configuration. Moreover, if it is assumed that the distribution functions vary slowly on the microscopic time scale, we may neglect the temporal variation in P_1 and P_2 and write

$$P_1(\mathbf{r}_1) = \rho + p_1(\mathbf{r}_1), \quad [15a]$$

where the gradient is taken to have the form

$$p_1(\mathbf{r}_1) = \boldsymbol{\chi} \cdot \mathbf{r}_1. \quad [15b]$$

We also write

$$P_2(\mathbf{r}_1, \mathbf{r}_2) = P_1(\mathbf{r}_1) P_1(\mathbf{r}_2) G(\mathbf{r}_1, \mathbf{r}_2). \quad [16]$$

In Eq. **16** the function $G(\mathbf{r}_1, \mathbf{r}_2)$ is the sum of the equilibrium radial distribution function $g(r) = \exp[-\beta u(r)]$ and a perturbation term $g(\mathbf{r}_1, \mathbf{r}_2)$.

Our goal now is to determine the relationship between the flux and the perturbation functions. If Eqs. **15** and **16** are inserted into the flux expression **14** and terms up to first order in density are retained, it is found that

$$\mathbf{J}^m = D_0 \left[\nabla_1 p_1(\mathbf{r}_1) + \beta\rho \int \nabla_1 u(r) p_1(\mathbf{r}_2) g(r) d\mathbf{r}_2 \right]. \quad [17]$$

Note that when $\rho \equiv 0$, Eqs. **17**, **15a**, and **12** yield $D^m = D_0$. Therefore, these generalized expressions reduce to the standard relationships when the solute is infinitely dilute.

To complete the calculation of D^m, the gradient and integral expressions appearing in Eq. **17** must be evaluated for our particular choice of the perturbation p_1. We have $\nabla_1 p_1 = \nabla_1(\boldsymbol{\chi} \cdot \mathbf{r}_1) = \boldsymbol{\chi}$. The integral in Eq. **17** can also be related to the gradient parameter χ. At this point, we diverge from the discussion that has been presented previously by the three-dimensional theorists and derive expressions that apply to a two-dimensional membrane. We find

$$\int \nabla_1 u(r) p_1(\mathbf{r}_2) g(r) d\mathbf{r}_2 = -\pi\chi \int_0^\infty \frac{du(r)}{dr} r^2 g(r) dr. \quad [18]$$

The generalized two-dimensional diffusion coefficient can now be identified. Note that Eq. **15a** implies that $\nabla P_1(\mathbf{r}_1) = \nabla p_1(\mathbf{r}_1) = \boldsymbol{\chi}$. Eqs. **17** and **18** therefore show that

$$\mathbf{J}^m = D_0 \left[1 - \pi\beta\rho \int_0^\infty \frac{du(r)}{dr} r^2 g(r) dr \right] \nabla P_1(\mathbf{r}_1). \quad [19]$$

By analogy with Eq. **12**, the proportionality factor relating the flux to the gradient in Eq. **19** is just the generalized diffusion coefficient. Thus,

$$\frac{D^m(\rho)}{D_0} = 1 - \pi\rho\beta \int_0^\infty \frac{du(r)}{dr} r^2 g(r) dr. \quad [20]$$

A similar result has been written down by Phillies (14).

METHODS

In this section we present the basic systems that were analyzed and describe the methods used to obtain self- and mutual-diffusion coefficients.

The Potentials. We studied diffusion in the presence of two types of potential: 6-4 and hard core. The potentials and associated distribution functions are shown in Fig. 2. A general 6-4 potential is defined according to

$$u_{64}(r) \equiv \frac{27}{4} k_B T [(\sigma/r)^6 - (\sigma/r)^4]. \quad [21]$$

This potential crosses zero at $r = \sigma$ and attains its minimum value, $-k_B T$, at $r = (3/2)^{1/2}\sigma \equiv r_0$. The associated force is repulsive for $r < r_0$ and attractive for $r > r_0$.

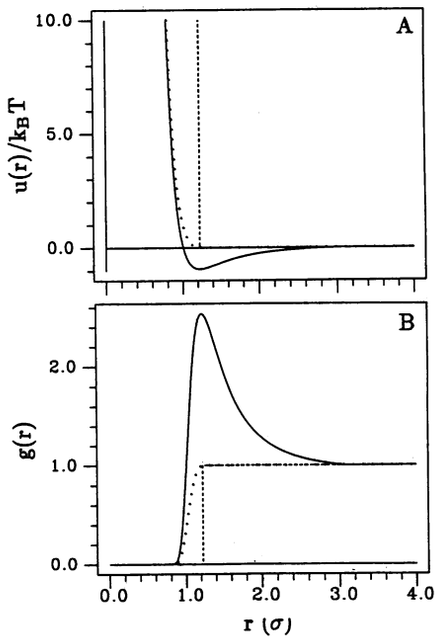

FIG. 2. Analytical potentials and radial distribution functions used in the calculation of diffusion coefficients. (*A*) Potentials A (—) and R (···) and the hard-core interaction (---) defined in Eqs. **22–24**. The value of d_{HC} associated with the hard-core potential is arbitrarily shown equal to r_0; only the product $\pi\rho(d_{HC})^2/4 \equiv f_A$ determines the rate of change of the diffusion coefficient. (*B*) Radial distribution functions associated with the potentials in *A*. These distribution functions were determined from the analytical expression $g(r) \equiv \exp[-u(r)/k_B T]$, which is valid in the dilute limit. The radial distribution function gives a measure of the probability of finding a second particle a distance r from a given central particle. A value of $g = 1$ indicates that there is a uniform likelihood of locating particle 2; values <1 (diminished probability) are associated in the dilute limit with $u > 0$, while values >1 (enhanced probability) are associated with $u < 0$.

To maintain consistency with published work (29), we defined a 6-4 potential with attractions and repulsions (fluid A) by truncating the 6-4 potential at $r = 2.5r_0$ and shifting it up slightly to maintain continuity

$$u_A(r) = \begin{cases} u_{64}(r) - u_{64}(2.5r_0) & r \le 2.5r_0 \\ 0 & r > 2.5r_0. \end{cases} \quad [22]$$

The force was not changed by this process, except that $du_A/dr = 0$ for $r \ge 2.5r_0$.

A closely related, purely repulsive interaction (fluid R) was derived from the 6-4 potential by using the Weeks–Chandler–Andersen decomposition (36) [i.e., by subtracting from Eq. **21** the value $u_{64}(r_0)$ and then setting the potential equal to zero beyond r_0]:

$$u_R(r) = \begin{cases} u_{64}(r) - u_{64}(r_0) & r \le r_0 \\ 0 & r > r_0. \end{cases} \quad [23]$$

The repulsive force is identical in fluids R and A; however, there are no interactions in Eq. **23** at separations that correspond to the attractive component of Eq. **22**.

The repulsive component in the A and R potentials is meant qualitatively to model the rather soft excluded-volume repulsions that might arise from interactions between proteins that are soft or deformable. The attractive component likewise represents qualitatively the case of fairly long-ranged attractions (26).

We also analyzed a hard-core repulsion.

$$u_{HC}(r) = \begin{cases} \infty & r \le d_{HC} \\ 0 & r > d_{HC}. \end{cases} \quad [24]$$

Here d_{HC} is the hard-core diameter of the diffusing particles. The associated force is a delta function centered on d_{HC}.

Diffusion coefficients for the 6-4 potential are formulated in terms of a reduced particle density, $\rho^* = \rho\sigma^2$. Results for the hard-disk potential are expressed as a function of area fraction, f_A, of protein coverage for reasons made clear in *Results and Discussion*. A (unique) hard-disk diameter cannot be associated with the soft 6-4 potentials.

Analysis of the 6-4 Potentials. The 6-4 self-diffusion coefficients were calculated numerically from Eqs. **9–11**. The differential equation, **9**, that determines $p(r)$ was converted into a system of linear equations that coupled together values of p at discrete points r_i, following standard techniques (37). Derivatives of $p(r)$ were replaced by weighted differences based on a five-point quartic fit (38). Boundary conditions in Eq. **10** were imposed by replacing the edge equations generated from Eq. **9** by the linearized boundary conditions. Eq. **10a** was implemented for a (large) value of r at which $du(r)/dr$ is zero. Similarly, Eq. **10b** was imposed for a (small) value of r at which $g(r) \approx 0$. The result of these manipulations was a matrix equation representing Eqs. **9** and **10** with $p(r)$ as its unique solution. Eq. **11** was then evaluated by using Simpson's rule (39).

The mutual-diffusion coefficient is given by Eq. **20**. The integral was evaluated by using Simpson's rule.

Analysis of the Hard-Disk Potential. The hard-core self- and mutual-diffusion coefficients were calculated analytically. We consider the calculation of D^s. For the excluded volume interaction, $g_{HC}(r < d_{HC}) = 0$ and $g_{HC}(r > d_{HC}) = 1$. It follows that the dilute hard-core perturbation equation reads (for $r > d_{HC}$)

$$r^2 \frac{d^2p}{dr^2} + r\frac{dp}{dr} - p = 0. \quad [25]$$

Expression **25** is just Euler's equation (40) and, therefore, the solution for $p(r)$, which satisfies the boundary condition at $r = \infty$, is $p(r) = C/r$. The constant C is fixed by the boundary condition in Eq. **10b**. We find $C = (d_{HC})^2$. If the hard-core perturbation $p(r) = (d_{HC})^2/r$ (valid for $r > d_{HC}$) is inserted into Eq. **11** and the quantity $-\beta g(r)du/dr = dg/dr$ is replaced by $\delta(r - d_{HC})$, one finds $D^s = 1 - 2f_A$. This result, for the hard-core interaction, was previously obtained by Ackerson and Fleishman (41).

The mutual-diffusion coefficient is also easily evaluated analytically. The result is given in the next section.

RESULTS AND DISCUSSION

Interpretation of Results. In the *Theory* section, we demonstrated that, for a dilute system, the self- and mutual-diffusion coefficients are linear functions of density. The precise functional form was determined for two related long-ranged interactions and a hard-core potential. The data are summarized in Table 1. Comparison with results obtained from a more general theory (to be published elsewhere) shows that these formulae are accurate to within about 5% if $\rho^* \le 0.2$ (fluid A and fluid R) and $f_A \le 0.2$ (hard core).

We see that interparticle interactions act to slow self diffusion in all three fluids; however, the effect is slightly more pronounced in fluid A than in the closely related fluid R. At nonzero solute concentrations, strong contact repulsions inhibit self diffusion by blocking potential paths, thereby making particle movement more circuitous. Attractions also inhibit self diffusion because they effectively tether particles into less mobile aggregates.

The three potentials induce markedly different changes in the mutual-diffusion coefficient. Mutual diffusion was retarded in fluid A but was enhanced in fluid R and the hard-core liquid. Repulsions serve to push neighboring particles apart; regions of high concentration tend, therefore, to dissipate more quickly than they would in the absence of interaction. In contrast, the attractions in fluid A hold particles together and so retard large-scale separations.

In general, the theoretical expressions that determine the density and potential dependence of D^s are not particularly susceptible to qualitative analysis. The formalism is complex because the perturbation $p(r)$ must first be computed, and then $p(r)$ and du/dr must be used together to find D^s/D_0. Therefore, the details of both $p(r)$ and the force combine to determine the nature of the modulation.

The mutual-diffusion formula is more transparent: the sign of the integrand in Eq. **20** is determined by the sign of the interaction force. If the force is purely repulsive, mutual diffusion is always enhanced. If the force is purely attractive, mutual diffusion is always inhibited. The effect of a force containing both repulsive and attractive components is determined by the details of the integration.

Comparison with Other Studies of Self Diffusion. Our analytical expression for D^s/D_0 in hard-disk systems can be compared with previous studies (11, 13) of the self diffusion of hard hexagons on a lattice. These latter results depend only on the area fraction occupied by protein and at low densities are fit to within 5% by the relationship $D^s(f_A) = 1 -$

Table 1. Equations for diffusion coefficients

System	D^s/D_0	D^m/D_0
Fluid A	$1 - 1.68\rho^*$	$1 - 6.20\rho^*$
Fluid R	$1 - 1.48\rho^*$	$1 + 3.34\rho^*$
Hard core	$1 - 2f_A$	$1 + 4f_A$

These equations describe the density dependence of the self- and mutual-diffusion coefficients. Results for fluids A and R are given in terms of the reduced density of particles, while results for the hard-core interaction are expressed as a function of the area fraction of protein.

$2.1187 f_A$ (13); this fit compares favorably with our formula, $1 - 2f_A$.

The density dependence of D^s has been monitored for the membrane proteins bacteriorhodopsin (3, 42) and gramicidin (43). The experimental data clearly demonstrate that D^s is indeed a decreasing function of concentration. However, the scatter in the data at low lateral densities makes it difficult quantitatively to compare our theory and experiment.

Implications for the Experimental Determination of Diffusion Coefficients. We noted in the Introduction that both self and mutual diffusion are biologically important and are, therefore, the subject of extensive experimental study. However, the interpretation attached to the experimental data is not completely unambiguous. For example, the recent review article by Young *et al.* (23) compared diffusion coefficients determined by FRAP and PER. Results were presented for three proteins: concanavalin A receptors and acetylcholine receptors from *Xenopus* muscle cells and low-density lipoprotein receptors on human fibroblasts. In each case the diffusion coefficients obtained from PER measurements were significantly larger than the corresponding values obtained with the FRAP technique (5–20 times larger for the concanavalin A receptors, 50 times larger for the acetylcholine receptors, and 100 times larger for the low density lipoprotein receptors). In contrast, diffusion coefficients for Fcε receptors on rat basophilic leukemia cells determined by the two techniques did not differ significantly (44). It has been suggested that the typically smaller FRAP diffusion coefficients could reflect a retarding interaction between the FRAP label and the extracellular glycocalyx. The existence and magnitude of such an effect would depend on the particular label and cell surface characteristics.

It is also possible that interprotein interactions give rise to the differences noted above. We have mentioned that PER monitors mutual diffusion, while FRAP measures self diffusion. For particles that interact, even through simple excluded volume forces, D^s and D^m will differ and hence D_{FRAP} will not, in general, be the same as D_{PER}. Moreover, to date, the interactions between (studied) membrane proteins have been shown to be predominantly repulsive in character (24, 27); hence, we would predict that the diffusion coefficient extracted from a FRAP experiment should be smaller than that obtained from PER ($D_{FRAP}/D_0 < 1$, while $D_{PER}/D_0 > 1$). Other forms of potential could give rise to other behavior (this could be the case for the Fcε receptors).

The magnitude of an interaction-mediated modulation is probably sufficient to explain the observed differences. Data from Pink (11), Saxton (13), and our generalized analysis of the hard-core interaction (data not shown) indicate that $D^s(f_A = 0.5)/D_0 \approx 1/4$. We have also found (data not shown) that $D^m(0.5)/D_0 \approx 12$ for the hard-core fluid. Both of these results hold when hydrodynamic effects and protein-induced lipid perturbations are neglected. Such differences are qualitatively consistent with the experimental data.

Finally, we mention that Small *et al.* (45) monitored the mutual diffusion of proteins along developing bullfrog olfactory axons and found that the measured diffusion coefficients were larger than typical FRAP protein diffusion coefficients. The authors postulated that differences between self and mutual diffusion could underlie this observation, although they did not treat this point quantitatively.

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