EFFECTS OF SPATIAL VARIATION IN MEMBRANE DIFFUSIBILITY AND SOLUBILITY ON THE LATERAL TRANSPORT OF MEMBRANE COMPONENTS

JOSEF EISINGER AND BERTRAND I. HALPERIN
AT&T Bell Laboratories, Murray Hill, New Jersey 07974; and Harvard University, Cambridge, Massachusetts 02138

ABSTRACT There exist many examples of membrane components (e.g. receptors) accumulating in special domains of cell membranes. We analyze how certain variations in lateral diffusibility and solubility of the membrane would increase the efficiency of transport to these regions. A theorem is derived to show that the mean-time-of capture, $\bar{t}_c$, for particles diffusing to a trap from an annular region surrounding it, is intermediate to the $\bar{t}_c$ values that correspond to the minimum and maximum diffusion coefficients that obtain in this region. An analytical solution for $\bar{t}_c$ as a function of the gradient of diffusivity surrounding a trap is derived for circular geometry. Since local diffusion coefficients can be increased dramatically by reducing the concentration of intra-membrane particles and/or allowing them to form aggregates, such mechanisms could greatly enhance the diffusion-limited transport of particular membrane components to a trap (e.g. coated pit). If the trap is surrounded by an annular region in which the probe particles' partition function is increased, say, by the local segregation of certain phospholipids, $\bar{t}_c$ is shown to vary inversely with the logarithm of the relative partition function. We provide some conjectural examples to illustrate the magnitude of the effects which heterogeneities in diffusibility and solubility may have in biological membranes.

INTRODUCTION

Many functions of the cell are thought to be regulated (or at least affected) by the modulation of membrane fluidity, and specifically by the lateral mobility of glyco-protein receptors. This is manifested most dramatically in the formation of coated pits and endocytosis (1), and in "patch" and "cap" formation (2, 3). It has also been suggested that the lateral diffusion of redox components controls the rate of electron transfer in mitochondrial membranes (4). Synapse formation has been reported to be accompanied by the creation of a high local concentration of acetylcholine receptors (5, 6), possibly by diffusive transport (7). And finally, the intramembrane particle density in the growth cones of neurons has been shown to be very heterogeneous (8). The biological relevance of these and other examples of lateral membrane transport were recently reviewed (9). A number of mechanisms have been proposed to explain capping and may be relevant to other phenomena. They include lipid flow (3), membrane surface waves (10) and the modulation of the attachment of membrane proteins to the cytoskeleton (11, 12). Regardless of the mechanisms that may operate in particular cells, it is logical to consider, as we do in this paper, how the transport of membrane components would be affected by variations in lateral fluidity and solubility from one point to another within a membrane.

It has recently been demonstrated experimentally that the lateral diffusion of lipid analogue probes in a vesicle system is reduced by an order of magnitude by the presence of proteins (13–15). In intact erythrocytes, the local short range (~1–10 nm) diffusion coefficient of such probes has been shown to be at least ten times greater than that measured for long-range diffusion (~1 μm), where the diffusing molecules must thread their way among an archipelago of membrane proteins (16, 17). Quantitative information about this effect was obtained by means of computer simulation of the lateral diffusion of lipid probes in the presence of obstacles, ("obstructed diffusion") which showed that in the long-range limit, the probes move diffusively, i.e., the mean-square diffusion distance is proportional to time (Fig. 1). The effective diffusion coefficient for obstructed diffusion (D), given by the slopes of the curves in Fig. 1 is, however, considerably smaller than the value for free or unobstructed diffusion (D_0), with the ratio D/D_0 depending on the density and size of the obstacles (16) (Fig. 1). Until now these results have been demonstrated only for lipid analogue probes, but the lateral mobility of larger membrane components, e.g. receptor proteins, is expected to be similarly reduced by the presence of obstacles.

Dr. Eisinger's new address is Department of Physiology and Biophysics, Annenberg 21, Mount Sinai School of Medicine, 5th Avenue, and 100th Street, NY, NY 10029. Please address all reprint requests to Dr. Eisinger.

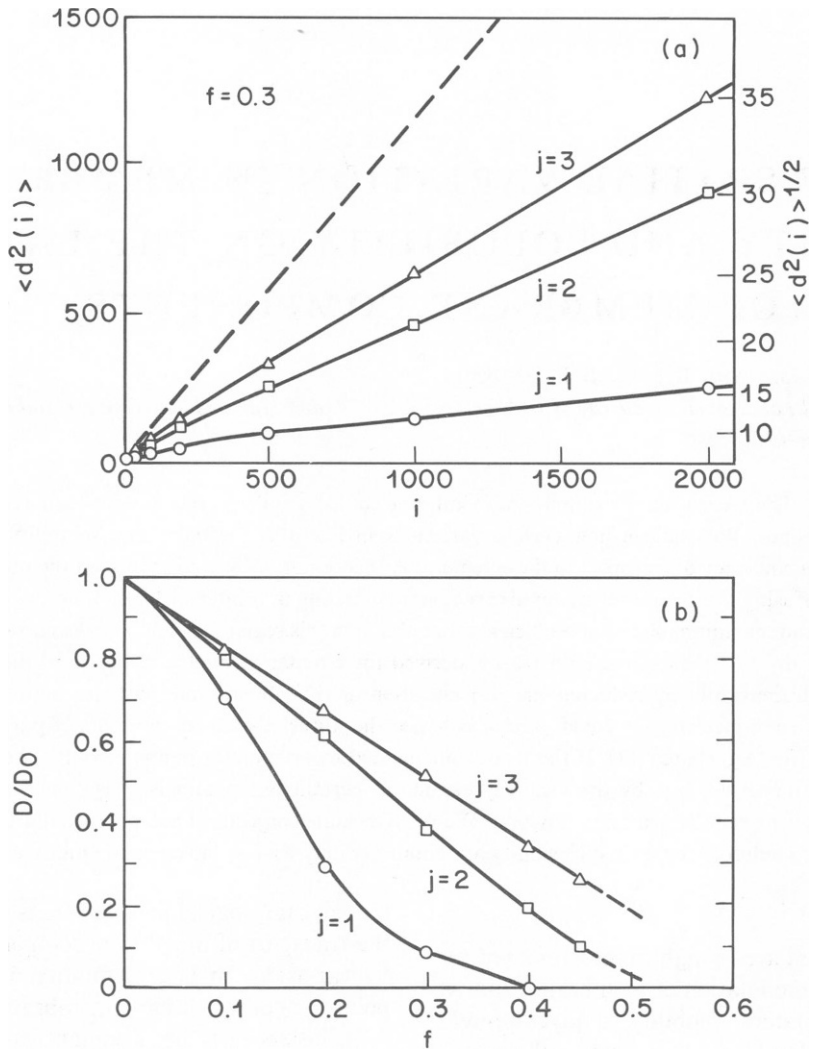

FIGURE 1 Simulation of diffusion in a membrane in the presence of obstacles according to the "milling crowd" model (16). (*a*) The mean square diffusion distance of a lipid analogue probe, $<d^2(i)>$ as a function of i, the number of random exchanges with one of its six nearest neighbors. The dashed curve represents unobstructed diffusion while the remaining three curves are simulations in the presence of random distributions of obstacles. The membrane lipids are assumed to form a regular trigonal array with lattice constant, λ, the obstacles are hexagons with sides $j\lambda$, $j = 1, 2, 3$ and d is measured in units of λ. Note that for large i, $\langle d^2(i)\rangle$ is linear with i. The motion is therefore diffusive and the slope of the line is proportional to the effective diffusion coefficient, D. The fraction of the membrane surface covered by obstacles is $f = 0.3$ in this example. (*b*) The relative diffusion coefficient, D/D_0, as a function of f and j. f is here calculated by using the total area of the hexagonic obstacles with all lipids exterior to the hexagons undergoing spatial exchanges at the same rate and it is seen that obstructed diffusion is inhibited more drastically for the smallest obstacles ($j = 1$). A much weaker dependence on obstacle size is obtained for alternate methods of modeling obstacle area (16).

The following questions suggest themselves: How is the diffusion-limited rate of formation of aggregates (e.g. coated pits, patches) affected by local variation in the density and size of membrane proteins; and is the creation of a protein density gradient in the membrane plane an effective means for modulating the transport of diffusing particles?

To shed light on these questions we have examined the following model. A particle is confined to a region bounded by a closed curve S_2 that is impenetrable to it. Inside S_2 is a smaller closed curve S_1 which is a sink to any particle striking it, as indicated in Fig. 2. (We may think of the particle as a membrane-bound receptor and S_1 as the edge of a receptor aggregate or coated pit that eventually undergoes endocytosis.) At time $t = 0$, the particle is assumed to be at a point $\hat{\mathbf{r}}_0$, somewhere in the region A which is exterior to S_1 and interior to S_2. It then diffuses until captured by S_1, with a diffusion coefficient $D(\hat{\mathbf{r}})$ which is a specified function of the particle's position in region A. We wish to compute the particle's mean-time-of-capture $\bar{t}_c$, averaged with equal weight over all positions $\hat{\mathbf{r}}_0$ in A and will employ $\bar{t}_c$ as a measure of the efficiency of transport.

The problem we consider is therefore a generalization of

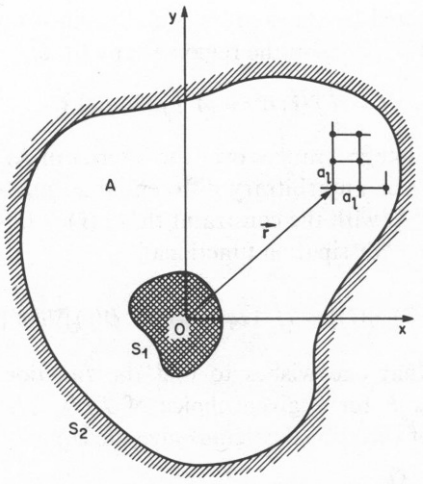

FIGURE 2 Schematic representation of a perfect trap for probe particles, S_1, surrounded by an impermeable boundary S_2. At each point $\hat{\mathbf{r}}$ in the annular region A, between S_1 and S_2 the diffusion coefficient $D(\hat{\mathbf{r}})$, the particle density $\rho(\hat{\mathbf{r}})$ and current density $\hat{\mathbf{j}}(\hat{\mathbf{r}})$ are defined as described in Formulation of the Diffusion Problem.

one of the problems considered by Berg and Purcell (18) and earlier, by Adam and Delbrück (19), where S_1 and S_2 are concentric circles and where D is a constant, independent of position. Needless to say, we shall not be able to derive a simple expression for the general case we are addressing. It is, however, possible to obtain useful information about the dependence of $\bar{t}_c$ on $D(\hat{\mathbf{r}})$. For example, if the function $D(\hat{\mathbf{r}})$ has lower and upper bounds, $D_{\min}$ and $D_{\max}$, so that

$$D_{\min} \leq D(\hat{\mathbf{r}}) \leq D_{\max} \tag{1}$$

for $\hat{\mathbf{r}}$ in A, then $\bar{t}_c$ is in turn bounded according to

$$\frac{K}{D_{\min}} \geq \bar{t}_c \geq \frac{K}{D_{\max}}, \tag{2}$$

where K is a constant which depends only on the geometry of the system. The constant K is defined by the property that if $D(\hat{\mathbf{r}})$ is simply a constant, D, then $\bar{t}_c$ is simply K/D.

The theorem embodied in Eq. 2 is intuitively plausible, and it can be derived using variational methods that are well known particularly in the analysis of electrical conduction problems (20), which are analogous to our diffusion problem. The mathematical proof is given in Formulation of the Diffusion Problem. We remark that the theorem also applies to diffusion on the surface of a sphere, in which case S_1 may enclose an arbitrary small region and A may be taken to be the remainder of the sphere, "exterior" to S_1.

The theorem applies equally to the case where the trap boundary S_1 consists of several disjointed closed curves—i.e. when there are two or more separated traps within the region A.

The effects of spatial variations of $D(\hat{\mathbf{r}})$ are illustrated by considering in more detail the case where S_1 and S_2 are concentric circles and D depends only on the radial distance, $r = |\hat{\mathbf{r}}|$. In this case one finds that $\bar{t}_c$ varies linearly with a certain weighted average of $1/D(r)$, which can be evaluated explicitly for some simple cases. This discussion is given in Circular Geometry. The geometry here may be considered to be an approximation to the situation that occurs when many traps are distributed on the surface of a cell membrane. The circle S_2 is then an approximation to the boundary of the "Voronoi polygon" which is the set of all points on the membrane closer to the trap S_1 than to any other trap on the surface. The area of S_2 is then the area of the cell membrane divided by the number of traps.

As a logical extension of the problems defined above, we also consider the situation where there is a chemical change in the membrane at some instant of time, such that the equilibrium partition coefficient of the probe particle is subsequently a function of position. This could conceivably result from the lipid bilayer adopting a nonuniform distribution of its constituent phospholipids. The gradient of the partition coefficient leads to a driving term in the diffusion equation, so that the probe particle moves preferentially towards regions of higher solubility. In Spatially Varying Partition Coefficient we examine a simple case, where S_1 and S_2 are again concentric circles, (as in Circular Geometry), and the partition coefficient depends only on the distance from the origin, while the diffusion coefficient D is independent of position. It is then possible to show that for an appropriate spatial dependence of the equilibrium partition coefficient, there is a significant decrease in the mean-time-to-capture of the probe particles.

In Examples and Discussion, finally, we provide a few conjectural examples of how the results might affect transport in biological membranes. These examples are of course limited by our imperfect knowledge of membrane structure and dynamics, and in particular of the topological heterogeneity that may prevail in membranes.

FORMULATION OF THE DIFFUSION PROBLEM

The diffusion problem is conveniently formulated in terms of the probability density $\rho(\hat{\mathbf{r}})$ for finding a particle at point $\hat{\mathbf{r}}$, at a specified time t. One must also define a probability current $\hat{\mathbf{j}}(\hat{\mathbf{r}})$, which is the net rate of flow of particles at point $\hat{\mathbf{r}}$, or equivalently, $\hat{\mathbf{j}}(\hat{\mathbf{r}})$ is the mean velocity of a particle at point $\hat{\mathbf{r}}$, multiplied by the probability density $\rho(\hat{\mathbf{r}})$. Then the diffusion equation is

$$\hat{\mathbf{j}}(\hat{\mathbf{r}}) = -D(\hat{\mathbf{r}})\,\hat{\nabla}\,\rho(\hat{\mathbf{r}}), \tag{3}$$

where $D(\hat{\mathbf{r}})$ is the diffusion coefficient at point $\hat{\mathbf{r}}$. Conservation of particle number requires that

$$\frac{\partial \rho(\hat{\mathbf{r}})}{\partial t} = -\hat{\nabla} \cdot \hat{\mathbf{j}}(\hat{\mathbf{r}}) \tag{4}$$

at all points in the interior of the sample. At an impenetra-

ble boundary one employs the condition that the component of $\hat{\mathbf{j}}$ normal to the boundary must vanish. In view of Eq. 3, this is equivalent to insisting that $\hat{\nabla}\rho(\hat{\mathbf{r}})$ have a zero normal component.

If a finite, connected region is completely surrounded by an impenetrable boundary, then we expect that regardless of the initial conditions, the density $\rho(\hat{\mathbf{r}})$ will approach a unique, time-independent limit at sufficiently long times. If we set $\partial\rho/\partial t = 0$, in Eq. 4, we see that the equations will be satisfied if we have $\rho(\hat{\mathbf{r}})$ = constant, independent of $\hat{\mathbf{r}}$. In particular, this means $\hat{\mathbf{j}}(\hat{\mathbf{r}}) = 0$ everywhere, so that Eq. 3 and the boundary conditions are automatically satisfied. It can be shown that $\rho(\hat{\mathbf{r}})$ = constant is the time-independent solution for this geometry.

The fact that the equilibrium solution of our equations has constant $\rho(\hat{\mathbf{r}})$, even if $D(\hat{\mathbf{r}})$ varies from point to point, was built into Eq. 3 in order to reflect the following microscopic assumption: that the variations in the membrane composition that are responsible for the spatial variation of the diffusion coefficient do not appreciably affect the relative solubility of the probe particle in different portions of the membrane. We adhere to this assumption throughout this section.

The problem described in the introduction requires that in addition to the external boundary S_2, there exists an absorbing boundary at S_1. The boundary condition at S_1 is that $\rho(\hat{\mathbf{r}}) = 0$, as any particle striking the boundary is immediately removed from future consideration. In this case, the number of particles in the system is not conserved, but gradually decays to zero, as time goes on.

To calculate the mean-time-to-capture $\bar{t}_c$, we may start with a uniform distribution, $\rho(\hat{\mathbf{r}})$ = constant, at $t = 0$, and solve the time-dependent Eqs. 3 and 4, with appropriate boundary conditions, to obtain the value of $\rho(\hat{\mathbf{r}})$ at all times $t > 0$. An equivalent, but simpler method is to imagine a steady-state problem in which, say, one particle per second is placed in the system at a random position in the region A, and is allowed to diffuse until captured at S_1. It is then not difficult to see that the mean number of particles in the system at any time is precisely equal to $\bar{t}_c$, the mean-time-to-capture, in seconds, of the individual particles that have been added to the system. In this formulation, one is looking for a time-independent solution for $\rho(\hat{\mathbf{r}})$, when there is a distributed source of particles in the interior of the region, as well as a sink at the boundary S_1. In this case, Eq. 4 must be replaced by

$$\frac{\partial\rho(\hat{\mathbf{r}})}{\partial t} = -\hat{\nabla}\cdot\hat{\mathbf{j}}(\hat{\mathbf{r}}) + q(\hat{\mathbf{r}}) = 0, \tag{5}$$

where $q(\hat{\mathbf{r}})$ is the source density. In our case, $q(\hat{\mathbf{r}})$ is a constant and is given by

$$q(\hat{\mathbf{r}}) = \frac{1}{A}, \tag{6}$$

where A is used here to denote the area of region A. The diffusion Eq. 3 and the boundary conditions at S_1 and S_2 are unaffected by the sources. Finally, since $\bar{t}_c$ equals the number of particles in the region at any time,

$$\bar{t}_c = \int\rho(\hat{\mathbf{r}})\,d^2r = A\int\rho(\hat{\mathbf{r}})\,q(\hat{\mathbf{r}})\,d^2r, \tag{7}$$

where the integral ranges over the interior of A.

Let $\eta(\hat{\mathbf{r}})$ be an arbitrary differentiable function of $\hat{\mathbf{r}}$ in the region A, with the constraint that $\eta(\hat{\mathbf{r}}) = 0$ for $\hat{\mathbf{r}}$ on S_1. We define a "dissipation functional"

$$F[D(\hat{\mathbf{r}}),\eta(\hat{\mathbf{r}})] \equiv \int_A [2q(\hat{\mathbf{r}})\eta(\hat{\mathbf{r}}) - D(\hat{\mathbf{r}})\,|\hat{\nabla}\eta(\hat{\mathbf{r}})|^2]d^2r. \tag{8}$$

Suppose that one wishes to find the function $\eta(\hat{\mathbf{r}})$ that maximizes F for a given choice of $D(\hat{\mathbf{r}})$. The standard methods of variational calculus give the equation

$$\frac{\delta F}{\delta\eta\,(r)} = 2\{q(\hat{\mathbf{r}}) + \hat{\nabla}\cdot[D\,(\hat{\mathbf{r}})\,\hat{\nabla}\,\eta(\hat{\mathbf{r}})]\} = 0, \tag{9}$$

with the boundary condition that the normal derivative of η must vanish at the boundary S_2. These equations are just the conditions satisfied by the density $\rho(\hat{\mathbf{r}})$ [cf. Eqs. 3 and 5] so it is clear that $\rho(\hat{\mathbf{r}})$ is itself the function that maximizes F. In other words

$$F[D(\hat{\mathbf{r}}),\rho(\hat{\mathbf{r}})] \geq F[D(\hat{\mathbf{r}}),\eta(\hat{\mathbf{r}})], \tag{10}$$

for any differentiable function $\eta(\hat{\mathbf{r}})$ which vanishes on S_1. It follows from the definition Eq. 8 that for fixed $\eta(\hat{\mathbf{r}})$, F is a monotonically decreasing function of $D(\hat{\mathbf{r}})$, and therefore

$$F[D_{\min},\eta(\hat{\mathbf{r}})] \geq F[D(\hat{\mathbf{r}}),\eta(\hat{\mathbf{r}})] \geq F[D_{\max},\eta(\hat{\mathbf{r}})], \tag{11}$$

when $D_{\min}$ and $D_{\max}$ are the minimum and maximum values of $D(\hat{\mathbf{r}})$, respectively. Also, if we apply an integration by parts to the second term in Eq. 8, and take into account Eq. 7 and the differential equation Eq. 9 satisfied by $\rho(\hat{\mathbf{r}})$, we find

$$\bar{t}_c = AF[D(\hat{\mathbf{r}}),\rho(\hat{\mathbf{r}})]. \tag{12}$$

Let $\rho_{\max}(\hat{\mathbf{r}})$ be the function $\eta(\hat{\mathbf{r}})$ that maximizes $F[D_{\max},\eta(\hat{\mathbf{r}})]$ and let $\rho_{\min}(\hat{\mathbf{r}})$ be the function that maximizes $F[D_{\min},\eta(\hat{\mathbf{r}})]$. Then from the above inequalities,

$$F[D_{\min},\rho_{\min}] \geq F[D_{\min},\rho] \geq F[D,\rho] \geq F[D,\rho_{\max}] \geq F[D_{\max},\rho_{\max}]. \tag{13}$$

Therefore, we have

$$\bar{t}_c(D_{\min}) \geq \bar{t}_c \geq \bar{t}_c(D_{\max}). \tag{14}$$

It is clear that if $D(\hat{\mathbf{r}})$ is independent of $\hat{\mathbf{r}}$, the density $\rho(\hat{\mathbf{r}})$ and the mean-time-to-capture $\bar{t}_c$ are each inversely proportional to the value of D. Thus the inequality (Eq. 14) is equivalent to the inequality (Eq. 2) stated in the introduction.[1]

[1]Actually, it is possible to set rigorous bounds to the diffusion coefficient in an inhomogeneous medium which are much more restrictive than the ones considered here. See for example, A. Hashin and S. Shtrikman (1962), *J. Appl. Phys.* 33: 3125, where the case of a material with varying magnetic susceptibility is discussed.

CIRCULAR GEOMETRY

Mathematics

In this section, we consider the case where S_1 and S_2 are the circles $r = a$, and $r = b$, respectively, with $a < b$, and $D(\hat{\mathbf{r}})$ depends only on the distance r from the origin. Clearly, $\rho(\hat{\mathbf{r}})$ will also depend only on the distance from the origin, in this case, and the vector $\hat{\mathbf{j}}(\hat{\mathbf{r}})$ is purely in the radial direction. Eqs. 3, 5 and 6 may then be written, in circular coordinates

$$\frac{1}{r}\frac{d}{dr}\left[r\,D(r)\frac{d\rho}{dr}\right] = \frac{-1}{A}\,, \tag{15}$$

where $A = \pi(b^2 - a^2)$. Integrating twice one obtains the results

$$rD(r)\frac{d\rho(r)}{dr} = \frac{1}{2A}(b^2 - r^2), \tag{15a}$$

$$\rho(r) = \int_a^r ds\,\frac{(b^2 - s^2)}{2AsD(s)}\,. \tag{15b}$$

The constants of integration in these equations are determined by the conditions $d\rho/dr = 0$ at $r = b$ and $\rho = 0$ at $r = a$. Finally, we obtain from Eqs. 7 and 15b

$$\begin{aligned}\bar{t}_c &= \frac{\pi}{A}\int_a^b r dr \int_a^r ds\,\frac{(b^2 - s^2)}{sD(s)} \\ &= \frac{\pi}{A}\int_a^b ds\,\frac{(b^2 - s^2)}{sD(s)}\int_s^b r dr \\ &= \frac{1}{(b^2 - a^2)}\int_a^b \frac{ds}{2D(s)}\,\frac{(b^2 - s^2)^2}{s}\,.\end{aligned} \tag{16}$$

In going from the first line to the second in Eq. 16, we have interchanged the order of r and s integrations; in the third line, we have carried out the inner integration.

It is useful to rewrite Eq. 16 in the form

$$\bar{t}_c = \int_a^b \frac{r dr}{2D(r)} f(r), \tag{17}$$

where

$$f(r) \equiv \frac{(b^2 - r^2)^2}{(b^2 - a^2)r^2}\,. \tag{18}$$

If the spatially varying factor f were not present in the integrand, then the mean-time-to-capture $\bar{t}_c$ would be simply determined by the arithmetic average of $[D(r)]^{-1}$, for points within the annulus A. The weighting factor f varies monotonically, however, from a maximum at $r = a$ to a minimum value of zero, at $r = b$. Thus the actual value of $\bar{t}_c$ is most sensitive to the value of $[D(r)]^{-1}$ at points close to the sink at $r = a$, where the diffusion current density is highest. This is evident in Fig. 3, which shows the form factor $f(r/b)$ as a function of the dimensionless radial distance. This means that the diffusion coefficient close to the trap radius a is weighted heavily compared with the remaining area A. The curve shown in Fig. 3 was calculated for $b/a = 20$, but it differs insignificantly from that for $b/a = 10$ and 5 for b/r greater than 0.1 and 0.2, respectively.

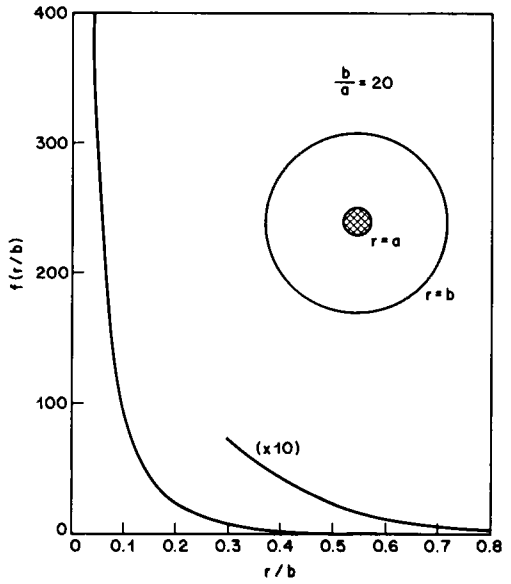

FIGURE 3 The spatially varying factor $f(r)$, as defined by Eqs. 17 and 18, for circular geometry with $b/a = 20$. f is here plotted as a function of the dimensionless radial distance r/b. Curves for $b/a = 10$ and 5 are almost identical to this curve for $r \geq 0.1$ and 0.2, respectively.

Discussion

The results of Formulation of the Diffusion Problem and Circular Geometry: Mathematics allow us to formulate and to answer several questions about the mean-time-to-capture for a membrane with variable $D(\hat{\mathbf{r}})$.

Suppose that each point of a cell membrane is free to choose any diffusion coefficient it wishes, in a limited range $D_{min} < D(r) < D_{max}$. Then if the cell wishes to minimize the mean-time-to-capture $\bar{t}_c$, it should choose $D(\hat{\mathbf{r}}) = D_{max}$ at all points in the region A, and the mean-time-to-capture is directly proportional to the value of D^{-1}_{max}.

Now suppose that in addition to the constraint $D_{min} < D(\hat{\mathbf{r}}) < D_{max}$, it is required that the average value of $[D(r)]^{-1}$ in the region A be equal to some specified value $\bar{D}^{-1}$, where $D_{min} < \bar{D} < D_{max}$. How can the membrane minimize the mean-time-to-capture in this case? It is clear from the analysis of Circular Geometry: Mathematics that the value of $D(\hat{\mathbf{r}})$ should be as large as possible close to the trap, while paying the price of a small value of $D(\hat{\mathbf{r}})$ far from the trap. In the case of circular symmetry this situation is achieved by the following geometry:

$$D(r) = \begin{cases} D_{max} & \text{for } a < r < c \\ D_{min} & \text{for } c < r < b, \end{cases} \tag{19}$$

where c is determined by

$$\frac{1}{D_{max}}(c^2 - a^2) + \frac{1}{D_{min}}(b^2 - c^2) = \frac{1}{\bar{D}}(b^2 - a^2). \quad (20)$$

Now from Eq. 12 we find that $\bar{t}_c$ is given by

$$\bar{t}_c = \frac{1}{(b^2 - a^2)}\left\{\frac{1}{D_{max}}\left[\frac{b^4}{2}\ln\left(\frac{c}{a}\right) - \frac{b^2(c^2 - a^2)}{2} + \frac{(c^4 - a^4)}{8}\right] + \frac{1}{D_{min}}\left[\frac{b^4}{2}\ln\left(\frac{b}{c}\right) - \frac{b^2(b^2 - c^2)}{2} + \frac{(b^4 - c^4)}{8}\right]\right\}. \quad (21)$$

The dependence of $\bar{t}_c$ on the cross-over radius c is illustrated in Fig. 4 for dimensions and diffusion coefficients that are relevant to cell membranes.

Note that if we set $D_{max} = \infty$ in Eq. 16, keeping the cross-over radius c a constant, the mean time to capture is given by the expression

$$\bar{t}_c = \frac{1}{2(b^2 - a^2)}\frac{1}{D_{min}}\left[b^4\ln\left(\frac{b}{c}\right) - \frac{3}{4}b^4 + b^2c^2 - \frac{c^4}{4}\right]. \quad (22)$$

The conditions under which this relationship applies are equivalent to equating c to a, the radius of the trapping center (sink) and setting the diffusion coefficient in the region $a < r < b$ equal to a constant D. Eq. 22 may then be simplified to

$$\bar{t}_c = \frac{1}{D}\left[\frac{b^4}{2(b^2 - a^2)}\ln\frac{b}{a} - \frac{3b^2 - a^2}{8}\right], \quad (23)$$

which is identical to Eq. B4 in Berg and Purcell's analysis (18) of the mean-capture-time for a constant diffusion coefficient and is illustrated graphically in Fig. 5. Note that when $b \gg a$, Eq. 23 approaches (18)

$$\bar{t}_c = \frac{b^2}{2D}\left(\ln\frac{b}{a} - \frac{3}{4}\right). \quad (24)$$

SPATIALLY VARYING PARTITION COEFFICIENT

In this section we consider how the diffusion of a probe particle is affected if its relative solubility varies from one point to another in the membrane. In this situation, Eq. 3 for the diffusion current must be replaced by

$$j(\hat{\mathbf{r}}) = -D(\hat{\mathbf{r}})\left[\hat{\nabla}\rho(\hat{\mathbf{r}}) + \rho(\hat{\mathbf{r}})\hat{\nabla}\phi(\hat{\mathbf{r}})\right], \quad (25)$$

where $\phi(\hat{\mathbf{r}})$ is a specified function of position. Now, for a closed system, with no source or sink of particles, the equilibrium solution of the diffusion equation has $\hat{\mathbf{j}} = 0$ at all points and is given by

$$\rho_{eq}(\hat{\mathbf{r}}) = \rho_0 e^{-\phi(\hat{\mathbf{r}})}, \quad (26)$$

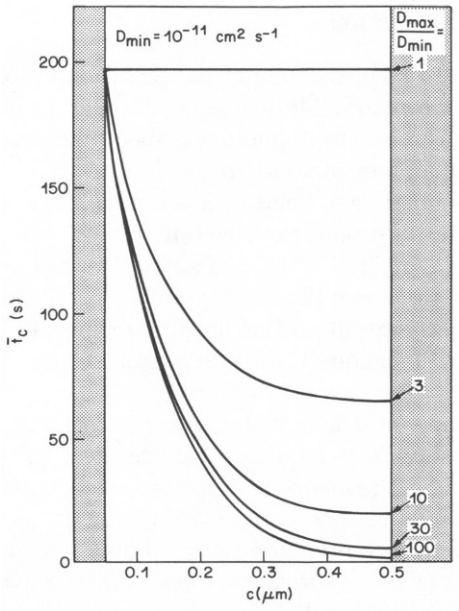

FIGURE 4 Mean-time-to-capture $\bar{t}_c$, according to Eq. 21, for particles initally distributed uniformly in the annular region $a < r < b$, where the diffusion coefficient has a value $D_{min} = 10^{-11}\text{cm}^2\text{s}^{-1}$ in the region $c < r < b$ and a larger value (D_{max}) in the region $a < r < c$. The ratio D_{max}/D_{min} is indicated for each curve, while $a = 0.05$ μm and $b = 0.5$ μm. These curves may be used to estimate the time-to-capture for other diffusivities, since $\bar{t}_c$ varies inversely with D_{min}, if the ratio D_{max}/D_{min} is held fixed.

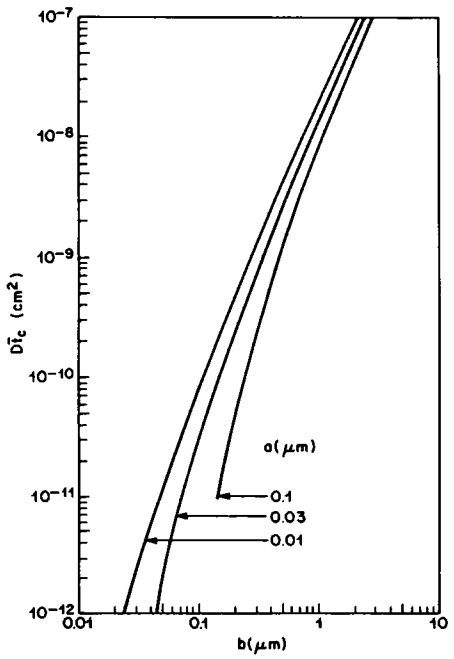

FIGURE 5 The mean-capture-time in seconds for particles diffusing from an annular region ($a < r < b$), where the diffusion coefficient D is constant, to a trap ($r = a$). The product $D\bar{t}_c$ is shown in cm^2, with a and b in μm (c.f. Eq. 23. E.g., with $D = 10^{-10}$ cm^{2}s^{-1}, $a = 0.03$ μm and $b = 0.3$ μm, $\bar{t}_c = 100$ s.

where ρ_0 is any constant. Thus we may make the identification

$$\phi(\hat{\mathbf{r}}) \equiv -\ln(\rho_{eq}(\hat{\mathbf{r}})/\rho_0) = -\ln K(\hat{\mathbf{r}}), \tag{27}$$

where $K(\hat{\mathbf{r}})$ is the equilibrium partition coefficient of the probe molecule, in the membrane at point $\hat{\mathbf{r}}$, relative to some arbitrary point where $\phi = 0$. Clearly, we may add any position-independent constant to $\phi(\hat{\mathbf{r}})$, or multiply $K(\hat{\mathbf{r}})$ by any such constant, with no effect on the diffusion current (c.f. Eq. 25).

As in the previous sections, we consider the situation where the probe particle is located, at $t = 0$, at a random point $\hat{\mathbf{r}}_0$ in the region A, with equal probability for all points. (This is the equilibrium solution if we assume ϕ to be independent of position, for times $t < 0$.) At time $t = 0$, we change the membrane structure to give a new spatially varying $\phi(\hat{\mathbf{r}})$, and we begin to trap any particle that reaches the inner curve S_1. We again wish to calculate the mean-time-to capture $\bar{t}_c$.

As in the previous sections, we may compute $\bar{t}_c$ by using Eqs. 5–7, together with the conditions $\rho(\hat{\mathbf{r}}) = 0$, at the boundary S_1, and the condition that the normal component of $\hat{\mathbf{j}}(\hat{\mathbf{r}})$ should vanish at the boundary S_2.

For simplicity, we assume that diffusion coefficient is a constant D, and as in Circular Geometry, we assume circular symmetry, so that S_1 and S_2 are the circles $r = a$ and $r = b$, respectively, and $\phi(\hat{\mathbf{r}})$ depends only on the radius r. Then Eqs. 5, 6, and 25 become

$$\frac{1}{r}\frac{d}{dr}\left[r\frac{d\rho}{dr} + r\rho\frac{d\phi}{dr}\right] = \frac{-1}{AD}. \tag{28}$$

Integrating once, we find

$$D\left[\frac{d\rho}{dr} + \rho\frac{d\phi}{dr}\right] = \frac{1}{2Ar}[b^2 - r^2], \tag{29}$$

which may be compared with Eq. 15a of Circular Geometry. The right hand side of this equation gives the explicit r-dependence of the particle current $\hat{\mathbf{j}}$, which is the same here as in Circular Geometry. The explicit solution of Eq. 29 is given by

$$\rho(r) = \frac{e^{-\phi(r)}}{2AD}\int_a^r ds\, e^{\phi(s)}\frac{b^2 - s^2}{s}. \tag{30}$$

As an application of this analysis, we may pose the following problem. Suppose that the cell membrane can vary its properties at each point so as to achieve any value of $\phi(r)$ it wishes, in a limited range.

$$\phi_{min} \le \phi(r) \le \phi_{max}. \tag{31}$$

How should it choose $\phi(r)$ if it wishes to minimize the mean-time-to capture, as given by Eqs.7 and 30?

The optimization problem stated above can be solved by means of the calculus of variations, as discussed in the Appendix. Since the exact solution given there is somewhat complicated, however, we present here a simple approximate solution, which will illustrate the essential physics more clearly.

It is apparent, from Eq. 25, that the mean-time-to-capture is reduced, if the term $-\rho\nabla\phi$ drives particles inward toward the trap. Hence, we wish to set $\phi = \phi_{max}$ at the outer boundary ($r = b$), and $\phi = \phi_{min}$ at $r = a$, with a smooth, presumably monotonic variation of $\phi(r)$ in between. As a simple trial function, we may choose $\phi(r) = \phi_{min}$, for a range of r extending from the inner radius a up to a value c, and then choose $\phi(r)$ in the range $c < r < b$ in such a fashion that the density $\rho(r)$ is a constant in this range. Then from Eq. 28, we see that, in the outer range

$$\phi = \phi_{max} - \frac{1}{2AD\rho_0}\left[b^2\ln\left(\frac{b}{r}\right) - \frac{b^2}{2} + \frac{r^2}{2}\right], \tag{32}$$

where ρ_0 is the constant value of $\rho(r)$. Requiring $\phi = \phi_{min}$ at $r = c$, gives

$$\rho_0 = \frac{1}{2AD\,\Delta\phi}\left[b^2\ln\left(\frac{b}{c}\right) - \frac{b^2}{c} + \frac{c^2}{2}\right], \tag{33}$$

where

$$\Delta\phi = \phi_{max} - \phi_{min}. \tag{34}$$

In the inner range,where $\phi(r)$ is a constant, the variation of $\rho(r)$ is given, according to Eq. 30 by

$$\rho(r) = \frac{1}{2AD}\left[b^2\ln\left(\frac{r}{a}\right) + \frac{a^2}{2} - \frac{r^2}{2}\right]. \tag{35}$$

The value of c is then determined by equating Eqs. 33 and 35 at $r = c$.

Let us consider the limit where $b \gg a$, and $\Delta\phi$ is large compared with 1. In this case, we need only consider the logarithmic terms in Eqs. 33 and 35. We then find

$$\ln c \approx \frac{\ln b + \Delta\phi \ln a}{1 + \Delta\phi}, \tag{36}$$

$$\bar{t}_c \approx \rho_0 A \approx \frac{b^2\ln(b/a)}{2D(1 + \Delta\phi)}. \tag{37}$$

Within the approximations stated above, we see that the mean-time-to-capture is reduced by the factor $(1 + \Delta\phi)^{-1}$, compared to its value for the case of constant ϕ, which was given by Eq. 24. Since $\Delta\phi$ is the logarithm of the ratio of the equilibrium partition coefficients at the inner and outer edges of region A, it is clear that the reduction factor can have only a modest value, unless the variation of partition coefficient is itself extremely large. This feature is also present in the exact solution of the optimization problem.

EXAMPLES AND DISCUSSION

We have discussed the effects of spatial variations in diffusibility and solubility of membrane components on their diffusion-limited mean-time-of-capture. In this sec-

tion we estimate the magnitude of these effects for biological membranes. For the purposes of this discussion we will assume that the lifetime of the locus where the diffusing particle aggregate is long compared to the capture times. In general, the mean-capture-time of particles diffusing to, say, a coated pit, will be a function of the coated pit lifetime (21, 22, 23).

An obvious question that arises is whether a diffusibility gradient centered around a target area (e.g. a sink) would notably increase the rate with which probe particles are concentrated there. According to the theorem proved in Formulation of the Diffusion Problem, the effective diffusion coefficient in the gradient-region is between D_{min} and D_{max}(Eqs. 2 and 14). We also demonstrate in Circular Geometry: Discussion that the optimum solution (i.e. minimum $\bar{t}_c$) obtains when the particle sink is surrounded by an annular area in which the diffusion coefficient is enhanced maximally by some mechanism (c.f. Eqs. 19–24), say, by drastically lowering the concentration of proteins or, alternately by aggregating the proteins to form larger obstructions (c.f. Fig. 1). Random walk simulations and experimental results suggest that D for a small protein might well increase by an order of magnitude if this occurred, say from 10^{-11}cm^2 s^{-1} to 10^{-10} cm^2 s^{-1}. If the sink radius is 0.03 μm and the annular ring were 0.05 μm in width, the mean-time-of-capture of particles randomly situated in the annular region would then according to Eq. 24 or Fig. 5, drop from 1.4 to 0.14 s. Similarly if the annular ring from which particles diffuse to the same trap were 0.1 μm in width, $\bar{t}_c$ would be lowered from 7 to 0.7 s.

The effect of a spatially varying diffusion is illustrated in Fig. 4 for an annular geometry, with $a = 0.05$ μm and $b = 0.5$ μm, dimensions that are of the right order of magnitude for the transport of, say, low density lipoprotein receptors to coated pits on human fibroblasts (21, 24). Consider as an example the case where $D_{max} = 10^{-10}$ cm^2 s^{-1} and $D_{min} = 10^{-11}$cm^2 s^{-1} and where a circle of radius $c = 0.2$ μm separates the annular regions where the diffusion coefficients have these values. The mean-time to capture is then reduced by a factor of 3.5 relative to the case of $D = D_{min}$ everywhere, even though the region where $D = D_{max}$ occupies only 15% of the total area between the trap and the outer boundary.

It was demonstrated in Spatially Varying Partition Coefficient that only drastic heterogeneity in the membranes partition function could affect trapping rates appreciably. While phase segregation has been reported for various model membrane systems, this is less likely to occur in the rich mixture of phospholipids that characterizes real membranes. However, local segregation of particular lipid may conceivably be induced by, say, cytoskeletal involvement. The mean-time-of-capture then varies logarithmically with the ratio of the local partition coefficients, in accordance with Eqs. 27, 37, and A13. While the present study emphasizes the trapping of membrane components in circular geometry, the analysis of how the efficiency of diffusive transport towards a trap increases with diffusibility, and solubility also applies to quasi-linear membrane structures. For example, the concentration of acetylcholine receptors in synaptic regions may well be facilitated in this manner (5–7).

The advent of increasingly sensitive microscope imaging systems (24) and dynamic fluorescence probes make it possible to investigate detailed features of fluidity in intact cells. Such studies could provide information on stratagems, including the ones discussed here, which the cell employs for selective lateral membrane transport and may provide a deeper understanding of membrane structure and dynamics.

We thank K. A. Jacobson for valuable discussions.

Work at Harvard has been supported in part by National Science Foundation grant DMR 82-07431.

Received for publication 15 November 1985 and in final form 20 March 1986.

APPENDIX

The Optimization Problem

For the problem defined in Spatially Varying Partition Coefficient, we wish to minimize the expression for $\bar{t}_c$, given by Eqs. 7 and 30, subject to the constraint (Eq. 31). Let us define

$$g(r) \equiv (b^2 - r^2)^{1/2}, \tag{A1}$$

$$h(r) \equiv g(r)/r, \tag{A2}$$

$$y(r) \equiv h(r)e^{\phi(r)}. \tag{A3}$$

Then

$$\bar{t}_c = \frac{\pi}{AD}\int_a^b dr \frac{g(r)}{y(r)} \int_a^r ds\, g(s)\, y(s). \tag{A4}$$

Now at each point r we must have one of three conditions: (a) $\phi = \phi_{min}$, or (b) $\phi = \phi_{max}$, or (c) $\delta t_c/\delta\phi(r) = 0$. Conditions ($c$) leads to the integral equation

$$y(r) \int_r^b \frac{g(s)ds}{y(s)} = \frac{1}{y(r)} \int_a^r g(s)y(s)ds, \tag{A5}$$

which must be satisfied at all points for which $\phi_{min} < \phi(r) < \phi_{max}$.

Let us introduce the function

$$x(r) \equiv \int_o^r g(s)ds. \tag{A6}$$

By means of a series of manipulations, it can be shown that Eq. A5 implies the following differential equation in the region where $\phi_{min} < \phi(r) < \phi_{max}$:

$$\frac{d^2y}{dx^2} = \left(\frac{dy}{dx}\right)^2 \Big/ y. \tag{A7}$$

The general solution of this equation is

$$y(r) = Ce^{Bx(r)}, \tag{A8}$$

where C and B are arbitrary constants. In order for the integral Eq. A5 to be satisfied however, it is also necessary for two additional conditions to be

satisfied. Specifically, if $c_1 < r < c_2$ is the region where $\phi_{min} < \phi(r) < \phi_{max}$, we must have

$$\frac{1}{B}h(c_1) = \int_a^{c_1} h(r)g(r)\mathrm{d}r, \tag{A9}$$

$$\frac{1}{Bh(c_2)} = \int_{c_2}^{b} \frac{g(r)\mathrm{d}r}{h(r).} \tag{A10}$$

Using Eqs. A3, A6, and A8, the condition $\phi(c_2) - \phi(c_1) = \Delta\phi$ becomes

$$B = \frac{\Delta\phi + \ln[h(c_2)/h(c_1)]}{\int_{c_1}^{c_2} g(r)\mathrm{d}r}. \tag{A11}$$

The integrals in Eqs. A9–A11 can all be expressed in terms of elementary functions, and the result is three coupled transcendental equations that must be solved for the quantities B, c_1, and c_2. We can then use Eq. A4 and our explicit expressions for $y(r)$ to calculate the quantity $\bar{t}_c$.

The above equations simplify considerably in the limit where $\Delta\phi \to \infty$, while b/a is held fixed. Then we find $c_1 \approx a$, $c_2 \approx b, B \approx \Delta\phi / \int_a^b g(r)\mathrm{d}r$, and

$$\bar{t}_c \approx \frac{\pi\left(\int_a^b g(r)\,\mathrm{d}r\right)^2}{AD\,\Delta\phi}. \tag{A12}$$

If $a/b \ll 1$, then Eq. A12 simplifies further to

$$\bar{t}_c \approx \left(\frac{\pi}{4}\right)^2 \frac{b^2}{D\,\Delta\phi.} \tag{A13}$$

It should be cautioned, however, that Eq. A13 is not valid if $\Delta\phi$ is large, but fixed, while $a/b \to 0$.

REFERENCES

1. Nicolson, G. L. 1976. Transmembrane control of the receptors on normal and tumor cells. I. Cytoplasmic influence over cell surface components. *Biochim. Biophys. Acta.* 457:57–108.
2. Taylor, R. B., W. P. H. Duffus, M. C. Raff, and S. de Petris. 1971. Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by anti-immunoglobulin antibody. *Nature New Biol.* 233:225–229.
3. Bretscher, M. S. 1984. Endocytosis: relation to capping and cell locomotion. *Science* (*Wash. DC*). 224:681–686.
4. Gupte, S., E. S. Wu, L. Hoechli, K. A. Jacobson, A. E. Sowers, and C. R. Hackenbrock. 1984. Relationship between lateral diffusion, collision frequency and electron transfer of mitochondrial inner membrane oxidation-reduction components. *Proc. Natl. Acad. Sci. USA.*81:2606–2610.
5. Barnard, E. A., J. Wiekowski, and T. H. Chiu. 1971. Cholinergic receptor molecules and cholinesterase molecules at mouse skeletal muscle junctions. *Nature* (*Lond.*). 234:207–209.
6. Fertuck, H. C., and M. M. Salpeter. 1974. Localization of acetylcholine receptors by I^{125}-labeled α-bungarotoxin binding at mouse motor endplates. *Proc. Natl. Acad. Sci. USA.*71:1376–1378.
7. Edwards, C., and H. L. Frisch. 1976. A Model for the localization of acetylcholine receptors at the muscle endplate. *J. Neurobiol.* 7:377–381.
8. Small, R. K. 1985. Membrane specializations of neuritic growth cones in vivo: a quantitative IMP analysis. *J. Neurosci. Res.* 13:39–53.
9. Axelrod, D. 1983. Lateral motion of membrane proteins and biological function. *J. Membr. Biol.*75:1–10.
10. Oliver, J. M., and R. D. Berlin. 1982. Mechanisms that regulate the structural and functional architecture of cell surfaces. *Int. Rev. Cytol.* 74:55–94.
11. Edelman, G. 1976. Surface modulation in cell recognition and cell growth. *Science* (*Wash. DC*). 192:218–226.
12. Heath, J. P. 1983. Direct evidence for microfilament-mediated capping of surface receptors on crawling fibroblasts. *Nature* (*Lond.*). 302:532–534.
13. Peters, R., and R. J. Cherry. 1982. Lateral and rotational diffusion of bacteriorhodopsin in lipid bilayers: experimental test of the Saffman-Delbrück Equations. *Proc. Natl. Acad. Sci. USA.* 79:4317–4321.
14. Vaz, W. L. C., F. Goodsaid-Zalduondo, and K. A. Jacobson. 1984. Lateral diffusion of lipids and proteins in bilayer membranes. *FEBS* (*Fed. Eur. Biochem. Soc.*) *Lett.* 174:199–207.
15. Sowers, A. E., and C. R. Hackenbrock. 1985. Variation in protein lateral diffusion coefficients is related to variation in protein concentration found in mitochondrial inner membranes. *Biochim. Biophys. Acta.* 821:85–90.
16. Eisinger, J., J. Flores, and W. P. Petersen. 1986. A milling crowd model for local and long-range obstructed lateral diffusion-mobility of excimeric probes in the membrane of intact erythrocytes. *Biophys. J.* 49:987–1001.
17. Bloom, J. A., and W. W. Webb. 1983. Lipid diffusibility in the intact erythrocyte membrane. *Biophys. J.* 42:295–305.
18. Berg, H. C., and E. M. Purcell. 1977. Physics of chemoreception. *Biophys. J.*20:193–219.
19. Adam, G., and M. Delbrück. 1968. Reduction of dimensionality in biological diffusion processes. *In* Structural Chemistry and Molecular Biology. A. Rich and N. Davidson, editors. W. H. Freeman and Co., San Francisco. 198–215.
20. Jeans, J. H. 1966. The Mathematical Theory of Electricity and Magnetism. 5th Edition, Cambridge University Press. 324.
21. Goldstein, B., R. Griego, and C. Wofsy, 1984. "Diffusion-limited forward rate constants in two dimensions" *Biophys. J.* 46:573–586.
22. Keizer, J., J. Ramirez, and E. Peacock-Lopez. 1985. The effect of diffusion on the binding of membrane-bound receptors to coated pits. *Biophys. J.* 47:79–88 226.
23. Goldstein, J. L., R. G. W. Anderson, and M. Brown. 1979. "Coated Pits, Coated Vesicles and Receptor-mediated Endocytosis" *Nature* (*Lond.*). 279:679–685.
24. Kapitza, H. G., G. McGregor, and K. A. Jacobson. 1985. Direct measurement of lateral transport in membranes by using time-resolved spatial photometry. *Proc. Natl. Acad. Sci. USA.*82:4122–4126.