Regulation of Protein Mobility in Cell Membranes: A Dynamic Corral Model

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ABSTRACT We analyze a two-state stochastic corral model for regulation of protein diffusion in a cell membrane. This model could mimic control of protein transport in the membrane by the cytoskeleton. The dynamic corral acts as a gate which when open permits an otherwise trapped protein to escape to a neighboring corral in the cytoskeletal network. We solve for the escape rate over a wide range of parameters of the model, and compare these results with Monte Carlo simulations. Upon introducing measured values of the model parameters for Band 3 in erythrocyte membranes, we are able to estimate the value for one unknown parameter, the average rate at which the corral closes. The ratio of calculated closing rate to measured opening rate is roughly 100:1, consistent with a gating mechanism whereby protein mobility is regulated by dissociation and reassociation of segments of the cytoskeletal network.

INTRODUCTION

Proteins spanning cell membranes mediate transport of materials and information between the cell and its environment. Early models of the plasma membrane, notably the fluid mosaic model (Singer and Nicolson, 1972), postulated that proteins, homogeneously distributed within the membrane, move by free diffusion in a lipid bilayer, a view in harmony with theories of chemoreception (Berg and Purcell, 1977) that optimally arrange receptors evenly or randomly around the membrane. The picture that protein motion is mediated merely by the homogeneous environment of the lipid bilayer comprising the membrane has, however, been challenged for some time by evidence that transmembrane proteins also interact with heterogeneously distributed membrane lipids and proteins, as well as with proteins in the cytoplasm of the cell. It also appears that such interactions may be closely connected to function (Axelrod, 1983; McCloskey and Poo, 1983; Peters, 1988; Zhang et al., 1993; Winckler et al., 1999). Revision of the fluid mosaic model is currently underway (Jacobson et al., 1995) as experimental information about the interactions regulating protein transport becomes available and theories are developed to interpret measurements.

Though numerous interactions regulate membrane protein transport (Edidin, 1990), the cytoskeleton just below the membrane appears to play a central role in controlling mobility in a variety of cells, such as epithelial, nerve, and red blood cells (Fleming, 1987; Saxton, 1990b; Saxton and Jacobson, 1997; Winckler et al., 1999). The best-studied membrane protein for which cytoskeletal control of motion has been well characterized is Band 3 in erythrocyte membranes. The dense cytoskeletal network in erythrocytes has long been recognized to hinder and mediate transport of membrane proteins (Cherry, 1979; Schindler et al., 1980; Sheetz et al., 1980; Koppel et al., 1981; Sheetz, 1983). This view is strongly supported by experiments on the diffusion of Band 3 in both normal erythrocytes and erythrocytes that are deficient in spectrin, the building block for the cytoskeletal network. Corbett et al. (1994) studied rotational and translational diffusion of Band 3 in normal erythrocytes, and in erythrocytes with genetic disorders that leave the erythrocyte with a much sparser skeletal network. Rotational diffusion of Band 3 was found to be indistinguishable in both classes of cells. Translational diffusion, about two orders of magnitude smaller than predicted by the fluid mosaic model in normal cells, was observed to be about an order of magnitude faster in spectrin-deficient cells than in normal cells. The cytoskeleton affects the motion of membrane proteins in broadly two ways. Membrane proteins may bind to the cytoskeleton, remaining essentially immobile during the period in which they are tethered. For example, $\sim\frac{1}{3}$ of Band 3 binds to the cytoskeletal network via ankyrin at any one time. Unbound transmembrane proteins are still affected by the network, appearing to be temporarily corralled due to steric interactions with segments of the cytoskeletal network (Fig. 1). Such corralled, but unbound, proteins diffuse in the membrane, albeit much more slowly than envisioned by the fluid mosaic model. Sheetz (1983) presented a matrix model for the transport of proteins in erythrocyte membranes, which has since been elaborated on by Tsuji et al. (1986, 1988). The "skeleton fence model," as it is currently called, has been shown experimentally to characterize the control of protein transport by the cytoskeleton in numerous cells (Kusumi and Sako, 1996; Sako et al., 1998).

Evidence supporting the skeleton fence model for regulation of protein transport in cell membranes has been assembled largely by three classes of experiments: fluorescence recovery after photobleaching (FRAP) (Webb et al.,

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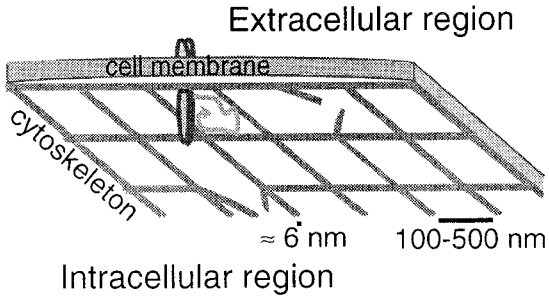

FIGURE 1 Ultraschematic illustration of a mobile transmembrane protein as viewed from under the membrane. The cytoskeleton immediately below the membrane hinders and regulates transport, confining the protein temporarily to a corral, the typical size of which is indicated in the figure. One hypothesis for proteins to move from one corral to a neighbor is for segments of the cytoskeletal network to dissociate and reassociate. We model this two-state process and predict the average time for proteins to escape from a corral. The thickness of the corral can affect the rate of escape. A thickness of 6 nm, representative for the cytoskeleton of erythrocytes, is indicated.

1981; Jacobson et al., 1982); single particle tracking (SPT) (Qian et al., 1991; Saxton and Jacobson, 1997); and experiments with laser tweezers (Edidin et al., 1991; Kusumi et al., 1998). SPT, which monitors the motion of individual or small numbers of proteins at video rates or in some cases faster (Tomishige et al., 1998), provides particularly detailed information about the nature of protein transport in the membrane (Simson et al., 1995; Saxton and Jacobson, 1997). SPT has helped to pin down the sizes of the cytoskeletal regions that temporarily compartmentalize proteins, revealing distinct time and spatial domains for diffusion of mobile proteins. At short times and over regions of order $0.01–0.1\ \mu m^2$, diffusion appears as theoretically expected for a protein in a lipid bilayer. Over longer times and distances, diffusion of mobile proteins is often observed to be one or more orders of magnitude slower (Kusumi et al., 1993; Saxton and Jacobson, 1997). Laser tweezers have been used to move small numbers of proteins up to and beyond the boundaries of corrals (Edidin et al., 1991; Tomishige, 1997; Kusumi et al., 1998), providing further detailed information about the range of corral sizes and of the extent of corral control over the transport of transmembrane proteins. The cytoskeleton itself has been manipulated with laser tweezers (Tomishige et al., 1998), dragging mobile proteins with it, which has lent further support to the cytoskeleton fence model.

On the theoretical side, the mobility of membrane proteins has been extensively simulated by Saxton (1987; 1989; 1990a,b; 1993; 1995; 1997). While considering a range of traps and obstacles for proteins in membranes, Saxton (1995) has also addressed escape of proteins from corrals. The specific corral model studied by Saxton is akin to standard models of chemical reactions, whereby a particle escapes over an energy barrier that is fixed in time. In this case, the protein diffuses inside the corral until it hits the barrier, at which point it has a fixed probability to escape. Saxton simulated protein dynamics in the corral and determined the mean first passage time out of the corral for a variety of corral sizes, shapes, and escape probabilities. An expression for first mean passage times due to Deutch (1980) for escape over a circular static barrier closely fits results of the simulations. A second model studied by Saxton (1989; 1990a,b) describes hopping among corrals of the "skeleton fence." In one realization, the skeleton fence is static and a percolation network is required for diffusion over the membrane. Since the fraction of the erythrocyte cytoskeleton that is dissociated is far smaller than what would be required for percolation, Saxton suggested that large-scale diffusion could occur only if the skeleton fence were dynamic; for example, if segments of the cytoskeleton could dissociate and reassociate. In a dynamic model, there is no longer any percolation threshold (Druger et al., 1985; Harrison and Zwanzig, 1985), and it is always possible for an object to diffuse globally. The dynamic corral model we investigate here predicts the hopping rate of a protein from one corral to its neighbor in the cytoskeletal network.

In this article we study a dynamic model for protein motion in which the corral is described as a stochastic gate. This picture is related to models of chemical reactions in which escape occurs over an energy barrier that changes in time (Zwanzig, 1990). Dynamical gating models have been applied for some time to the study of ligand-protein binding kinetics (McCammon and Northrup, 1981; Northrup et al., 1982; Szabo et al., 1982; Zwanzig, 1992; Wang and Wolynes, 1993; Eizenberg and Klafter, 1995), in which the binding rate is governed by the accessibility of the binding site, lying inside the protein, to a ligand that has to pass through pockets in the exterior of the protein that are regulated by variation of the protein's conformation. For a protein to escape from a corral, where the cytoskeleton sterically interacts with the cytoplasmic region of the transmembrane protein, the gate can open when a segment of the spectrin network corralling the protein dissociates, as illustrated in Fig. 1. Alternatively, a protein can escape from a corral if the distance between the membrane and cytoskeleton is sufficiently large so that the cytoplasmic portion of the protein can pass between them. This can occur through fluctuations in the distance between the membrane and corral, which can provide a gap large enough for the protein to escape, or through conformational changes in the cytoplasmic portion of the protein. Large-scale simulations of the cytoskeletal network by Boal (1994) and Boal and Boey (1995) have revealed that the barrier-free path for a membrane protein can be regulated by fluctuations in the shape of the cytoskeleton. Recent laser tweezer experiments by Tomishige and Kusumi (1999), in which the network itself was manipulated, have been interpreted to imply spectrin tetramer dissociation/reassociation in the gating process. The dynamic model that we adopt and discuss in this article

has two metastable states, one open and one closed, with random transitions between them, so it is most appropriate for the possible case in which opening and closing of the gate corresponds to dissociation and reassociation of spectrin tetramers. This model bears some resemblance to two-state stochastic models for ion channels (Colquhoun and Hawkes, 1995), with the additional feature here that protein transport in the skeleton fence involves the interplay between diffusion within the corral and the dynamics of the skeleton fence. Results we obtain from our two-state dynamic model, together with available experimental data for Band 3 in erythrocyte membranes, are consistent with a picture in which Band 3 transport is regulated by dissociation/reassociation of the cytoskeleton fence, though we cannot rule out other mechanisms.

In the following section we present the dynamic corral model and theoretical methods used to solve for the escape rate of proteins from the corral. We then briefly describe a Monte Carlo procedure to simulate protein motion in a dynamic corral, which we use to compare with theoretical results. Finally, we present and discuss results for the model, and compare these results with experimental measurements for the mobile fraction of Band 3 in erythrocytes.

THEORY

We consider a dynamic, two-state model for a membrane protein confined to a corral in which we picture the corral as a fluctuating gate. In one state the corral is closed and proteins are trapped, while in the other it is open and proteins diffusing within it can escape. Transitions between these two states are taken to occur randomly. The time during which the gate is closed or open is exponentially distributed with, respectively, mean W_o^{-1} and W_c^{-1}, where W_c and W_o are, respectively, the mean closing and opening rates of the gate. The shape and size of the corral and the diffusion coefficient, D, for the protein's motion within the corral comprise the other parameters of the model. The latter is just the diffusion coefficient for a protein within the lipid bilayer, and has been estimated theoretically by Saffman and Delbrück (1975) to be of the order 10^{-9} cm^2 s^{-1}. The diffusion coefficient due to the lipid bilayer is, in the context of the skeleton fence model, sometimes referred to as D_{micro} (Kusumi et al., 1998), the coefficient for diffusion within the "microscopic" corral region of the membrane, in contrast to D_{macro}, the coefficient for diffusion over lengths of order 1 μm or longer in the membrane. The corral size, D, and D_{macro} have been measured by SPT, FRAP, and with the aid of laser tweezers for various proteins and cells (Saxton and Jacobson, 1997). A suggestive value for W_o has also been reported for erythrocytes (Tomishige, 1997; Tomishige and Kusumi, 1999). We will discuss possible ranges for W_c and W_o below based on conclusions from our model, combined with measured values for the corral dimensions and protein diffusion.

It is often of interest to know that the protein is somewhere inside the corral at a given time. The survival probability, $P(t)$, is the probability that a protein starting in the corral remains there at time t. While calculation of $P(t)$ is generally complicated, we can simplify it significantly by making certain statistical assumptions, detailed below. We can describe $P(t)$ with these assumptions by closely following calculations by Zwanzig (1992) and Eizenberg and Klafter (1995) for ligand-protein binding kinetics involving passage through a fluctuating gate.

Suppose that the concentration of proteins, C, within a corral decays as

$$dC/dt = -K(x_i)C, \tag{1}$$

where x_i is a state of the corral: x_o = open, or x_c = closed. Because the state of the system is changing in time, the rate constant K is time-dependent and given by

$$K(x_o) = k, \tag{2a}$$

$$K(x_c) = 0, \tag{2b}$$

where we define a rate constant, k, for decay of the protein population from an open corral. We calculate k in the Appendix. Justification for a simple open-state rate equation will be provided with results of numerical simulations in the following sections. Transitions between the open and closed states are assumed to be stochastic. If the gate happens to be in state x_o (x_c), the probability that it will remain there at time t after opening (closing) is $W_c \exp(-tW_c)dt$ ($W_o \exp(-tW_o)dt$), where W_c and W_o are the rates to close and open, respectively.

Upon averaging Eq. 1 over all stochastic trajectories, we can express the probability of finding a protein inside the corral as $P(t) = P_c(t) + P_o(t)$, where P_c and P_o are, respectively, the survival probabilities in the closed and open states. Then

$$d\mathbf{P}/dt = -\mathbf{L}\,\mathbf{P}(t); \qquad \mathbf{P}(t) = \begin{pmatrix} P_c(t) \\ P_o(t) \end{pmatrix}, \tag{3}$$

where

$$\mathbf{L} = \begin{pmatrix} W_o & -W_c \\ -W_o & W_c + k \end{pmatrix}. \tag{4}$$

The solution to Eqs. 3 and 4 is

$$P(t) = c_+ e^{-\mu_+ t} + c_- e^{-\mu_- t}, \tag{5}$$

where

$$c_+ = \frac{1}{2} - \frac{\frac{1}{2}(W_o + W_c) + k\left(\frac{1}{2} - \frac{W_o}{W_o + W_c}\right)}{\sqrt{(W_o + W_c + k)^2 - 4kW_o}}, \tag{6a}$$

$$c_- = 1 - c_+, \tag{6b}$$

$$\mu_{\pm} = \frac{W_c + W_o + k}{2}\left(1 \pm \sqrt{1 - \frac{4kW_o}{(W_o + W_c + k)^2}}\right). \qquad (6c)$$

We see, given that we can justify an open-state rate equation with rate constant k, that the survival probability for proteins in a corral decays biexponentially; at longer times Eq. 5 reduces essentially to single-exponential decay. For the range of parameters typically representative for cells, $W_c \gg W_o$. Then, after only a very brief transient period, decay is simply exponential with $c_- \approx 1$ and rate $\mu = \mu_-$.

In calculating the survival probability, $P(t)$, we assumed that when the corral is open we can describe the open-state survival probability, $P_o(t)$, by $dP_o/dt = -kP_o$. The open-state rate constant, k, is derived in the Appendix for a square corral, and its variation with the parameters of the model and its influence on μ are discussed in the following sections. Our calculation of k is simplified greatly upon introducing the convenient and, as we shall see, reasonable assumption that, between opening events, the corral is closed sufficiently long for proteins inside it to equilibrate. When the corral reopens, a protein can then be found with equal probability anywhere inside the corral. Given a circular corral of radius R, or a square corral of half-length R, the characteristic diffusion time within the corral, $\tau_D = R^2/D$, is the time for a protein to move anywhere within the corral, and can be used as an estimate for the reequilibration time. We will justify this reequilibration approximation below with reference to available experimental data for the diffusion of membrane proteins.

In summary, two approximations have gone into our calculation of μ: 1) we have assumed that the survival probability when the gate is open can be described using a single rate constant, k, when in fact the proteins are diffusing out of the open corral; and 2) we have assumed that the gate is closed long enough for the proteins inside the corral to lie anywhere within it with equal probability at the time it reopens. The second assumption can be justified for sufficiently small W_o. The first can also be justified if W_c^{-1} is so small that $P_o(t)$ changes little until the corral closes. It is important to check the validity of both approximations in our calculation for the escape rate, and we do this by simulating protein escape from a stochastic two-state corral.

Numerical calculations

As a check on the theoretical predictions for our two-state dynamic corral model, we have computed escape rates from a corral directly by Monte Carlo simulations. We compute the rate of escape from either a circular or square corral superimposed on a square lattice, on which the protein moves randomly from one site to a nearest-neighbor at each time step. The radius of the circular corral or half-width of the square is given by the parameter R. For two-dimensional diffusion modeled by our simulations, $s^2 = 4D\,\delta t$, where s is the distance between lattice points and δt is a time step. As parameters for our model we have chosen $R = 60$ nm for a square corral and $D = 5 \cdot 10^{-9}$ cm^2 s^{-1}, both representative values for Band 3 in erythrocyte membranes (Tomishige et al., 1998). We take the lattice spacing for the square grid on which proteins diffuse in our simulations to be 2 nm, so that 60 lattice points lie within the length of a square corral. We chose this grid size since somewhat denser grids with smaller lattice spacings did not affect our results significantly. So that the areas within the square and circular corrals are the same, we take $R_{circle} = R\sqrt{4/\pi}$ for our Monte Carlo simulations using circular corrals. Given a 2-nm lattice spacing and our chosen value for D, we have that each time step, δt, corresponds to $2 \cdot 10^{-6}$ s.

We introduce a given number of proteins into the corral initially, and follow their survival inside the corral over the simulation. Given a closing rate, W_c, and opening rate, W_o, the fraction of time the corral is open over the length of the simulation is $f_o = W_o/(W_c + W_o)$. Randomly choosing a corral to be initially open with probability f_o, or closed with probability $f_c = 1 - f_o$, the probability that the corral will change its state at a given time step is $\delta t\, W_c$ and $\delta t\, W_o$, respectively. We take both $\delta t\, W_c$ and $\delta t\, W_o$ to be much smaller than 1, which can in general always be satisfied with a sufficiently small lattice spacing, as it is for our particular grid selection.

If the corral happens to be closed when a protein attempts to escape, the protein is reflected back to the lattice point from which it attempted to leave. If the corral is open, the protein is allowed to escape and continues to diffuse, walking randomly to nearest-neighbor sites at each time step. The protein can return to the corral as long as the gate is still open, but is removed from the simulation if it lies outside the corral and the gate is closed. We find that removing proteins from the simulation after the corral closes has little effect on the escape rate if the corral is closed at least as long as the characteristic diffusion time, τ_D.

RESULTS AND DISCUSSION

Protein distribution in a corral

In Fig. 2 we plot the radial concentration profile, $C(r, t)$, i.e., the concentration of proteins a distance r from the center of a dynamic circular corral at time t. We have calculated $C(r, t)$ to illustrate that the distribution of proteins remains essentially flat within the corral for a range of relevant parameters. Proteins are taken initially from a flat distribution within the corral, and we then compute $C(r, t)$ at discrete grid points inside and outside the corral, where $C(r, t)$ is propagated by the diffusion equation in each of the two states (Zwanzig, 1990). We have chosen a corral radius of $R = 60$ nm, $D = 5 \cdot 10^{-9}$ cm^2 s^{-1}, and $W_o = 10$ s^{-1}; these parameter values are representative for Band 3 in erythrocytes. An absorbing boundary is placed at $r = 120$

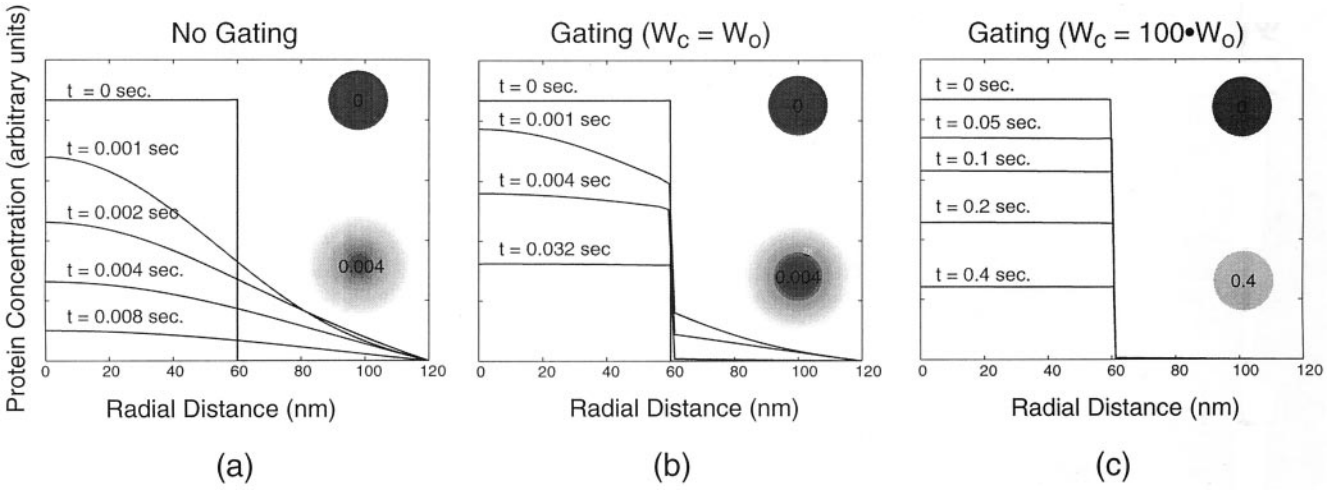

FIGURE 2 Radial concentration profiles are plotted at various times for three different values of the closing rate. We have chosen $D = 5 \cdot 10^{-9}$ cm^2 s^{-1}, $R = 60$ nm, and $W_o = 10$ s^{-1}, representative values for Band 3 in erythrocyte membranes. The proteins are initially equidistributed within the corral. We have placed an absorbing boundary at 120 nm, far enough away to have little effect on escape from the circular corral. In (*a*) the corral remains open and concentration profiles for normal diffusion are observed. In (*b*) and (*c*), where $W_c = W_o$ and $W_c = 100W_o$, respectively, a flat distribution of proteins is observed at all but very short times.

nm, far beyond the gate but nevertheless apparent in the radial profiles plotted in the figure. In Fig. 2 *a* the gate opens at $t = 0$, and remains open for the length of the calculation. Here we see simple and unobstructed diffusion (apart from artifacts due to the absorbing boundary at $R = 120$ nm). For the results plotted in Fig. 2, *b* and *c*, we have used $W_c = W_o$ and $W_c = 100W_o$, respectively. The latter closing rate is of the order of what it might actually be in erythrocytes, as discussed below. For the slower closing rate, shown in Fig. 2 *b*, we observe that the concentration of proteins near the edge of the corral is briefly lower than it is in the center; after this transient period the distribution within the corral is flat. When the gate closes more rapidly, as in Fig. 2 *c*, the distribution appears flat at all times plotted, lending credibility to our assumption that proteins within the corral are equidistributed. Deviations at very short times will be seen to have a negligible effect on our calculation of the escape rate from a corral.

Escape rate from a corral

We turn now to the decay of the survival probability of a protein in a two-state dynamic corral. We begin by looking first at results from Monte Carlo simulations of protein diffusion in and escape from a corral. We have run the simulations on a square lattice using both a square and circular corral for comparison. For each simulation we begin with 10 proteins placed randomly inside the corral, and monitor their survival inside the corral over the length of the simulation, as described above. In Fig. 3 we plot results for $P(t)$, where we have averaged the results over 10,000 runs. The diffusion coefficient, D, and the half-width, R, of the square corral are $5 \cdot 10^{-9}$ cm^2 s^{-1} and 60 nm, respectively. Various opening and closing rates are indicated in Fig. 3. The results are plotted as ln $P(t)$ versus time, together with the theoretical predictions of Eqs. 5 and 6. We observe that, regardless of corral shape and over the range of parameters plotted, escape of proteins from a dynamic corral is well-described by single-exponential decay to within fluctuations in the numerical results. Only at very short times and when W_c is not very different from W_o

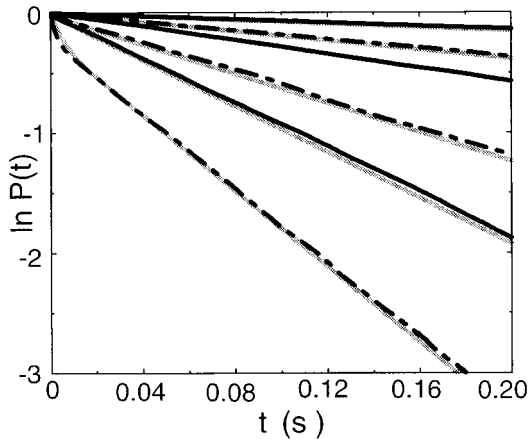

FIGURE 3 Results from Monte Carlo simulations for ln $P(t)$ are plotted. Broken curves are results from simulations with circular corrals, while solid curves are results for square corrals. Gray curves are the theoretical results of Eqs. 5 and 6. The half-width of the square corral is $R = 60$ nm and $D = 5 \cdot 10^{-9}$ cm^2 s^{-1}. The areas of the square and circular corrals are the same. From top to bottom, W_o (s^{-1}) and W_c (s^{-1}) are, respectively: 5, 5120; 5, 640; 10, 1280; 10, 160; 20, 320; 20, 40.

is biexponential decay apparent. Escape from both square and circular corrals is seen to be well-described by a theory for squares. That corral shape should have little effect on the escape rate is consistent with Saxton's (1995) results for escape from a static corral, for which computed mean first passage times for escape from corrals with a wide variety of shapes were found to be nearly shape-independent.

The results plotted in Fig. 3 indicate that the survival probability decays exponentially, as we already expected from Eqs. 5 and 6 which, after a very brief time, describe the escape of proteins from a dynamic corral as

$$P(t) \approx \exp(-\mu t), \tag{7}$$

where

$$\mu = \frac{W_c + W_o + k}{2}\left(1 - \sqrt{1 - \frac{4kW_o}{(W_o + W_c + k)^2}}\right), \tag{8}$$

which is μ_- defined by Eq. 6c. A protein's escape rate clearly depends on the rates at which the corral opens and closes, W_o and W_c, respectively, and on the open-state rate constant k, which contains the influence of the other parameters of our model, i.e., the corral size and the diffusion coefficient, D.

Our calculation of k is presented in the Appendix. We have assumed there that the corral is closed sufficiently long between opening events for the proteins inside it to equilibrate, so that each time the corral opens a protein can be found anywhere within the corral with equal probability, as illustrated by the profiles plotted in Fig. 2 *c*. With an equiprobable initial protein distribution, we calculate the fraction of proteins remaining within the corral during the period, t, in which it is open. The open-state rate constant, k, in Eq. 8 is an average over all open periods, so we average $k(t)$ over an exponential distribution of t. The resulting average open-state rate constant, $k = k(W_c)$, then depends on W_c, corral size, and D.

The open-state rate constant k for a square corral, derived in the Appendix, is given by Eq. A3 in terms of one numerical integral, which we compute to obtain μ in general. In the important limiting case where the gate closes rapidly, i.e., $W_c \gg \mathrm{DR}^{-2}$, where R is the half-width of the square corral, our expression for k simplifies to

$$k = W_c^{1/2} D^{1/2} R^{-1}, \qquad W_c \gg DR^{-2}. \tag{9}$$

Eq. 9 for k can be easily understood in terms of the short time, W_c^{-1}, during which proteins can leave the corral when the gate is open. Then essentially only proteins within a length $l \sim \sqrt{DW_c^{-1}}$ of the edge of the corral will escape, a part of the corral that we refer to as the "transition region." If all proteins within the transition region of length l from the edge of the corral escape when the gate closes, a fraction $1 - 2l/R$ of proteins that were in the corral when it opened still remain, where we ignore contributions of order l^2. If W_c^{-1} is small, $P_o(W_c^{-1}) \approx P_o(0)(1 - 2l/R)$, so that the survival probability can be approximated by an exponential. In this case, $k = 2lW_c/R \sim \sqrt{DW_c}/R$. Comparing with Eq. 9, we see that the length of the transition region is $l = \frac{1}{2}\sqrt{DW_c^{-1}}$ in the limit of fast W_c. The open-state rate constant, k, is simply the product of the rate to close and the relative size of the transition region to the size of the corral; k increases with increasing closing rates, since it takes longer for proteins in a larger transition region to diffuse out of the corral.

The escape rate, μ, given by Eq. 8, takes on two limiting forms that depend on the relative sizes of k, W_c, and W_o. When the rates of closing and opening are both much faster than the rate of escape from an open corral,

$$\mu = k\frac{W_o}{(W_o + W_c)}, \qquad W_c, W_o \gg k, \tag{10}$$

which is just the probability that the gate is open times the rate of leaving an open corral. Since the average closing rate is much greater than k in this limit, k appearing in Eq. 10 is given by Eq. 9. If the corral is typically closed longer than it is open,

$$\mu = \frac{W_o D^{1/2}}{RW_c^{1/2}}, \qquad W_c \gg W_o \gg k. \tag{11}$$

The escape rate is then simply the rate to open times the fraction of proteins in the transition region of the corral. In the limit where k is much larger than both the rate to open and close,

$$\mu = W_o, \qquad W_c, W_o \ll k, \tag{12}$$

so that the rate at which the gate opens is rate-limiting. In the slow-gating limit, Eq. 12, the escape rate is independent of the size of the corral. Since for our assumptions to hold W_c is typically greater than W_o, the crossover from the limiting regimes of Eqs. 10 and 12 can be seen from Eq. 8 to occur where $k \approx W_c$. To estimate the location of this crossover, we note that $k \approx W_c$ when $W_c \approx DR^{-2}$. The crossover from slow to fast gating thus occurs where $W_c^{-1} \sim R^2/D = \tau_D$, the diffusion time, corresponding to an open period sufficiently long for the transition region to encompass the whole corral.

To assess the validity of the assumptions that underly our prediction for the escape rate of membrane proteins from dynamic corrals, we have compared the escape rate, μ, given by Eq. 8 with results of Monte Carlo simulations. We have chosen two corral shapes for our simulations: a square corral, for which our expression for k is derived, and a circular corral whose area is the same as the square's. We plot the results of our simulations in Fig. 4 together with μ calculated using Eq. 8. The opening rates, W_o, used in the simulations are 5, 10, and 20 s^{-1}; the closing rates, W_c, range from 1 to 10^6 s^{-1}. The diffusion coefficient, D, and the half-width, R, of the square corral are $5 \cdot 10^{-9}$ cm^2 s^{-1} and 60 nm, respectively. The opening rates we have chosen

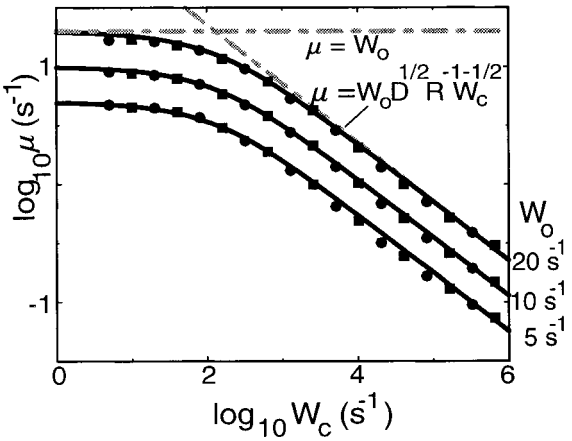

FIGURE 4 Average escape rate, μ, of a protein from a dynamic corral. Curves are results from calculations using Eqs. 8 and A3. Results from simulations using square and circular corrals are plotted as squares and circles, respectively. D and R are the same as those used in Fig. 3. Values of W_o and W_c are indicated in the figure.

for our simulations are also plausible values for the opening rates of the "skeleton fence" that temporarily compartmentalizes membrane proteins (see below). For these choices of D, R, and W_o, the values of W_c over which we plot results in Fig. 4 span a range in which the escape rate is almost completely controlled by the rate of opening, i.e., $\mu \approx W_o$ for small W_c; to the large W_c regime where μ is given by Eq. 11. Each of these regimes is indicated in the figure. The crossover from one regime to the other occurs where $W_c \sim D/R^2$, which for the chosen R and D is $W_c \sim 100\ s^{-1}$. We observe in Fig. 4 that the crossover indeed lies around this value. To obtain good statistics, the simulations were run with 10 proteins initially inside the corral and an ensemble of 10^4 corrals. As in Fig. 3, single-exponential decay was observed at all but very short times. The results plotted in Fig. 4 were obtained by a linear fit to the computed ln $P(t)$, where the short-time contribution was excluded. Reasonable agreement between theory and results of the numerical simulations using both square and circular corrals is seen over the complete range of parameters plotted in Fig. 4.

Using a two-state dynamic corral model, we predict the average escape rate, μ, in terms of the dynamic properties and size of a single corral of the membrane. In SPT or FRAP experiments information is provided about the diffusion coefficient, D_{macro}, for mobile proteins over larger regions of the membrane of the cell. Values for D_{macro} have been typically observed to be one or more orders of magnitude smaller than D, where the latter has been measured by SPT over length scales smaller than and on the order of the corral size (Saxton and Jacobson, 1997; Kusumi et al., 1998). The membrane consists of a meshwork of corrals of varying size, shape, and gating dynamics. While corral shape seems to have only a small influence on the escape rate, as our simulations using square and circular corrals indicate, corral size and dynamics strongly affect escape and thus D_{macro}. We can estimate D_{macro} in terms of the corral size and protein escape rate calculated above as $D_{macro} \approx \langle R^2 \mu(R) \rangle$, where the brackets denote an average over the membrane. For example, the median D_{macro} measured in SPT experiments on Band 3 in erythrocyte membranes is $6.6 \cdot 10^{-11}\ cm^2\ s^{-1}$ (Tomishige et al., 1997), from which, together with the median R of 55 nm, an average escape rate $2.2\ s^{-1}$ can be deduced. [Tomishige et al. (1997, 1998) report a hopping rate of $2.8\ s^{-1}$ based on these values for D_{macro} and R, but assuming elliptical corrals.]

The extent to which D_{macro} is regulated by the dynamic cytoskeleton fence depends on the average corral opening and closing rates, W_o and W_c, respectively, as well as D and R. We can understand the range of effects these parameters have on D_{macro} by turning to the limiting expressions for μ, given by Eqs. 10–12. When the gating rates are slow, $D_{macro} = \langle R^2 \rangle W_o$. In this slow-gating limit D_{macro} is related only to the rate at which the corral opens and its size. If both W_o and W_c are sufficiently fast, μ is given by Eq. 11 and $D_{macro} = \langle R \rangle W_o D^{1/2} W_c^{-1/2}$. In this fast-gating regime, D_{macro} is expressed as the product of the opening rate, W_o, and R^2 times the fraction of proteins lying in the transition region of the corral, averaged over the corrals of the cytoskeleton fence. In this limit, D_{macro} increases linearly with R. Thus, when gating is fast, corral size has a more modest effect on D_{macro} than when gating is slow. This is due to the fact that for given fast opening and closing rates, the fraction of proteins escaping from the corral decreases with increasing R, since the relative size of the transition region to corral area varies as R^{-1}. We shall see below that the faster-gating limit, where D_{macro} increases linearly with R, more nearly describes Band 3 in erythrocytes than does the slow-gating limit.

Finite size of proteins

Our calculations of the escape rate of a membrane protein from a dynamic corral have thus far neglected the finite width of both the cytoskeleton that corrals the protein and the cytoplasmic region of the membrane protein that interacts with the corral. As a result of the finite thicknesses of the corral and trapped protein, each a few nanometers, there is a minimum distance, r, that the protein must traverse when the gate is open before it actually escapes. This distance would be about half the sum of the thicknesses of the protein and barrier. For example, the diameter of the spectrin cytoskeleton in erythrocytes is ~6 nm (Boal and Boey, 1995), as indicated in Fig. 1, while the diameter of the cytoplasmic region of Band 3 is ~2–3 nm (Tomishige, 1997), so that $r \approx 4$ nm. Band 3 must therefore move laterally at least 4 nm to escape from an open corral before the gate closes.

The minimum protein traversal distance, r, due to finite thicknesses influences the open-state rate constant, k. We

can easily understand this influence for the case where the closing rate is fast, and k is given by Eq. 9 when $r = 0$. The transition region, whose $r = 0$ length is $l = \frac{1}{2}\sqrt{DW_c^{-1}}$ from the edge of the corral when W_c is fast, shrinks to $l - r \approx \frac{1}{2}\sqrt{DW_c^{-1}} - r$, which clearly limits how large W_c can be before proteins are trapped. For example, for Band 3 in erythrocyte membranes, where $r \approx 4$ nm and $D \approx 5 \cdot 10^{-9}$ cm^2 s^{-1}, W_c should be no greater than $\approx 10^4$ s^{-1}. Faster closing rates, within the framework of our dynamic corral model, would essentially permanently confine Band 3 inside the corral.

In the Appendix we modify our expression for k to account for finite r. In terms of this modified k, we plot the escape rate, μ, in Fig. 5 where we observe that, as expected, μ drops precipitously when the closing rate, W_c, is sufficiently large. This rapid drop reflects the $\exp(-r^2W_c/4D)$ probability of a protein diffusing the required minimum distance r during the very short time the gate is open. Fig. 5 indicates that W_c cannot, as anticipated above, be faster than $\approx 10^4$ s^{-1} for erythrocytes. Since the size of the transition region depends only on D, r, and W_c, a limit of $W_c \approx 10^4$ s^{-1} should be quite typical for cells if the cytoskeleton is regulating the lateral motion of membrane proteins.

SPT and laser tweezer experiments have to date provided most directly values for R, D, and μ, the latter obtained by observing the diffusion of mobile proteins over the cell membrane, as discussed above. Results for our model and the measured values of R, D, and μ can help us pin down W_c and W_o. The effective limit on W_c which we have calculated also imposes an effective range of possible opening rates, W_o. When both the opening and closing rates of the corral

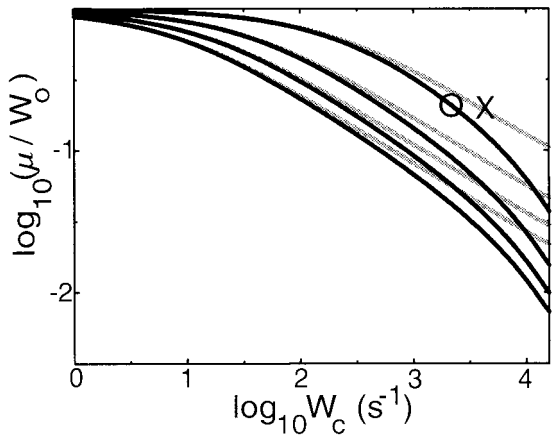

FIGURE 5 Effects of finite thickness of the corral and protein on the escape rate are shown. Log(μ/W_o) is plotted against log(W_c) for $D = 5 \cdot 10^{-9}$ cm^2 s^{-1} and, from top to bottom, $R = 60$, 140, 220, and 300 nm. The thick, black curves were calculated accounting for a half-thickness of $r = 4$ nm; the thin, gray curves were calculated for $r = 0$. Estimates for the closing rate based on the measured escape rate, opening rate and corral size (Tomishige, 1997; Tomishige et al., 1998; Tomishige and Kusumi, 1999) are indicated with an O, accounting for finite thickness, and X, neglecting this effect.

are fast, the escape rate, given by Eq. 11, is $\mu = W_o D^{1/2}/RW_c^{1/2}$. Upon measuring μ, R, and D, and since the maximum $W_c \approx 10^4$ s^{-1}, an effective lower limit on W_o can be determined. For example, for erythrocytes, μ, R, and D have been reported to be 2.8 s^{-1}, 55 nm, and $5.3 \cdot 10^{-9}$ cm^2 s^{-1}, respectively (Tomishige, 1997; Tomishige et al., 1998). The maximum opening rate, given the experimentally measured rates, would be $W_o \approx 30$ s^{-1}. When, however, the gate opens slowly, $\mu = W_o$. Since for erythrocytes $\mu \approx 2$–3 s^{-1}, the average corral opening rates, W_o, would range between ~ 3 and 30 s^{-1}. Thus the observed thickness and widths of the corrals and proteins, D, and the observed escape rate, together with results from our model, limit the range of values of W_o to only about an order of magnitude. We note that the diffusion time within the corral, $\tau_D = R^2/D$, is ≈ 0.007 s, and much less than the smallest value of W_o^{-1} in this range. Thus our assumption of reequilibration of proteins prior to opening appears fully justified.

In addition to measuring D, R, and μ for Band 3 in erythrocytes, Tomishige (1997) also reports measurements of a corral opening rate of ~ 14.3 s^{-1}. This result was deduced by dragging a gold bead attached to Band 3 with laser tweezers at various rates to determine the barrier free path (BFP), by which it could be determined if the bead was dragged a distance of one or more corrals. A dragging rate of ~ 14 s^{-1} per corral apparently dramatically increased the BFP. Still, for a given dragging rate a distribution of BFPs would be expected (Edidin et al., 1991). In the absence of BFP distributions we can at best take the reported opening rate to be suggestive. Nevertheless, it is reassuring that this opening rate lies within the range consistent with the measured values for R, D, and μ. Taking $W_o \approx 14$ s^{-1}, we can estimate W_c from Fig. 5, where we find $W_c \approx 2 \cdot 10^3$ s^{-1}. We note that $W_c \approx 4.5 \cdot 10^3$ s^{-1} if we neglect the effect of finite thickness in our calculations.

CONCLUDING REMARKS

A variety of experimental studies of protein motion in cell membranes indicates that free diffusion of transmembrane proteins is hindered by the cytoskeletal network directly below the plasma membrane (Jacobson et al., 1995; Saxton and Jacobson, 1997). A skeleton fence model, whereby proteins are temporarily corralled to regions of order 0.01–0.1 μm^2 before moving over to a neighboring region, has been proposed and supported by recent single particle tracking (SPT) and laser tweezer studies on numerous proteins and cells (Sako and Kusumi, 1995; Saxton and Jacobson, 1997; Kusumi et al., 1998). The current, if only tentative, picture confines proteins to cytoskeletal corrals until a conformational change in the corral or protein structure, or position of the membrane with respect to the cytoskeleton, allows the protein to move within the network and over the membrane. Motivated by this description, we have studied a

simple dynamic corral model for the lateral diffusion of transmembrane proteins.

In the dynamic model examined here, the corral fluctuates between two metastable states, one of which traps the protein while the other allows it to escape. These states could be, for example, the associated spectrin tetramer on the one hand, where the integrity of the cytoskeletal corral is maintained and the protein confined, and the dissociated dimer state on the other, in which the corral is open. This mechanism has for some time been suggested to regulate the lateral motion of proteins in cell membranes (Tsuji et al., 1986, 1988; Tomishige, 1997; Tomishige and Kusumi, 1999). For this model, we find that the rate of closing controls the size of the region within the corral from which proteins can escape, which we refer to as the transition region, while the rate of opening controls the rate at which proteins escape once there. The overall escape rate is then given by the product of the opening rate and the probability of lying within the transition region. Using measured values for D_{macro}, R, and W_{o} for Band 3 in erythrocytes, we have been able to calculate W_{c}.

Anywhere from $<$1% (Sheetz, 1983) to $\sim$5% (Liu et al., 1981; Palek and Lux, 1983) of spectrin is believed to be in the dissociated dimer state at any one time. Thus our estimate that W_{c}:W_{o} is $\sim$140:1, so that just under 1% of corrals would be open at any one time, is consistent with the hypothesis that dissociation/reassociation of spectrin tetramers is responsible for gating. This possibility could be explored further by SPT and laser tweezer experiments on cells for which the spectrin content and fraction of spectrin dimers is different from normal cells. In this case, R, W_{o}, and W_{c} would presumably change; if the former two could be measured, as they have been in normal erythrocytes, then W_{c} could be calculated and the ratio W_{o}:W_{c} checked for consistency. Since we observe in Fig. 5 that the thickness of the cytoskeleton appears to affect the escape rate, modest changes in the size and dynamics of the cytoskeletal network could have a sizable effect on D_{macro}.

We must bear in mind, however, that the available body of experimental data by no means rules out alternative mechanisms for intercompartmental transport. SPT experiments on cleaved Band 3 (Tomishige et al., 1998), where the cytoplasmic portion of Band 3 is largely removed, reveal D_{macro} to be about six times larger than for normal Band 3, though still an order of magnitude smaller than D_{micro}. Thus fluctuations in the distance between the cytoskeleton and the membrane, or protein conformational changes, may be the operative gating mechanism, at least to some degree. An interesting alternative dynamic corral model appropriate for this picture would describe the dynamic corral in terms of a "gap" whose motion diffuses according to the thermal fluctuations of the membrane, cytoskeleton, or protein. In this model, the corral would open when the gap between the membrane protein and cytoskeleton reaches a value large enough for the protein to escape. A diffusive gate model has been proposed and analyzed in the context of ligand-protein binding kinetics (Zwanzig, 1992; Wang and Wolynes, 1993; Eizenberg and Klafter, 1995).

Saxton (1995) has investigated protein escape from a corral that could be described as static. Protein escape in this model occurs with a certain probability every time the protein enters a transition region at the edge of the corral. If the escape probability from the transition region is much less than 1, as it would typically be for the lateral diffusion of proteins in cell membranes (Saxton, 1995), then the escape rate for proteins from static corrals can be described as the product of the escape probability from the transition region and the attempt frequency, i.e., the average rate of entering the transition region from the rest of the corral. The size of the transition region would sensibly be about the thickness of the cytoskeletal segment that has to be overcome for the protein to escape; then what is left to determine the escape rate is the probability that a protein can push its way through to the other side of the barrier.

There is only indirect evidence, such as effects of temperature on the barrier free path (Edidin et al., 1991), to support a dynamic cytoskeleton fence model over a static one, such as that studied by Saxton (1995) for the regulation of diffusion of membrane proteins. Deciding between a dynamic or static barrier for the cytoskeleton fence model requires going beyond calculation of the average rate of escape. To distinguish between these pictures, we need to consider the fluctuations in the escape rate, which we address in a future study.

APPENDIX

Our calculation of the survival probability of a protein in a dynamic corral, Eqs. 1–6, requires a rate constant, k, for escape from an open corral. We calculate the open-state rate constant for a square corral assuming proteins within the corral are equidistributed when the corral opens. We choose a square corral for convenience, since the number of proteins within it at a given time is simply the product of the number within two one-dimensional corrals. We thus first solve for the escape rate from the ends of an open one-dimensional corral, assuming proteins to be equidistributed inside it when it opens. The corral spans a length from $-R$ to R.

We assume that the number of proteins, $N(t)$, inside a corral that has remained open a period t has decayed exponentially,

$$N(t) = N(0)e^{-\mathrm{kt}}. \tag{A1}$$

Then averaging $N(t)$ over the distribution of open times,

$$P_{\mathrm{o}}(W_{\mathrm{c}}^{-1}) = W_{\mathrm{c}} \int_0^{\infty} dt\, e^{-(\mathrm{k}+\mathrm{W_c})\mathrm{t}}, \tag{A2}$$

gives the rate constant, k, in terms of $P_{\mathrm{o}}(W_{\mathrm{c}}^{-1}) = \langle N(W_{\mathrm{c}}^{-1})/N(0)\rangle$, the open-state survival probability at the closing time W_{c}^{-1}.

$$k = W_{\mathrm{c}} \frac{(1 - P_{\mathrm{o}}(W_{\mathrm{c}}^{-1}))}{P_{\mathrm{o}}(W_{\mathrm{c}}^{-1})}. \tag{A3}$$

If we assume that the proteins are found with equal probability anywhere in the corral when the gate opens, the number of proteins within a one-dimensional corral at time t is

$$N^{1D}(t) = \frac{N^{1D}(0)}{2R}\int_{-R}^{R} dL \int_{-(L+R)}^{R-L} dx W(x, t), \qquad (A4)$$

where $W(x, t)$ is the probability that a protein at time t has moved a distance x from where it started (van Kampen, 1981)

$$W(x, t) = \frac{1}{2(\pi Dt)^{1/2}} e^{-x^2/4Dt}. \qquad (A5)$$

Solving for $N^{1D}(t)$ we find

$$N^{1D}(t)/N^{1D}(0) = \Phi\left(\frac{R}{\sqrt{Dt}}\right) + \left(\frac{Dt}{\pi}\right)^{1/2} R^{-1}(e^{-R^2/Dt} - 1), \qquad (A6)$$

where $\Phi(x) = 2/\sqrt{\pi}\int_0^x ds\, e^{-s^2}$ is the error function.

To obtain k using Eq. A3, we need to first average $N(t)/N(0)$ over the distribution of open times, t. For the one-dimensional corral, $P_o^{1D}(W_c^{-1}) = W_c \int_0^\infty dt\, e^{-W_c t}[N^{1D}(t)/N^{1D}(0)]$. Inserting Eq. A6 and solving the integral (Gradshteyn and Ryzhik, 1980; Abramowitz and Stegun, 1965) we have for the survival probability at time W_c^{-1}

$$P_o^{1D}(W_c^{-1}) = 1 + \frac{D^{1/2}}{2W_c^{1/2}R}(e^{-2RW_c^{1/2}/D^{1/2}} - 1), \qquad (A7)$$

Inserting Eq. A7 into A3, we obtain k for a one-dimensional corral.

$N(t)/N(0)$ for a square corral is the square of $N^{1D}(t)/N^{1D}(0)$. The open-state survival probability at time W_c^{-1} for the square corral is found by integrating

$$P_o(W_c^{-1}) = W_c \int_0^\infty dt\, e^{-W_c t}\left(\frac{N^{1D}(t)}{N^{1D}(0)}\right)^2. \qquad (A8)$$

Inserting Eq. A6 into A8, we solve Eq. A8 numerically and introduce the result for $P_o(W_c^{-1})$ into Eq. A3 to obtain k.

Finally, it is straightforward to generalize our calculation of k to the case of a corral and proteins with finite thicknesses. To account for this important size effect on the escape rate, we change the limits of integration in Eq. A4 to account for the extra distance r that the protein must traverse in order to escape before the gate closes. Then

$$N^{1D}(t) = \frac{N^{1D}(0)}{2(R-r)}\int_{-(R-r)}^{R-r} dL \int_{-(L+R)}^{R-L} dx\, W(x, t), \qquad (A9)$$

for which we find, using Eq. A5,

$$N^{1D}(t)/N^{1D}(0) = \frac{1}{2(R-r)}\left((2R-r)\Phi\left(\frac{2R-r}{2\sqrt{Dt}}\right) + 2\left(\frac{Dt}{\pi}\right)^{1/2}(e^{-(2R-r)^2/4Dt} - 1)\right). \qquad (A10)$$

We solve for the open-state survival probability at time W_c^{-1}, $P_o(W_c^{-1})$, by numerical integration of Eq. A8 using Eq. A10 for $N^{1D}(t)/N^{1D}(0)$. We then insert this result into Eq. A3 to obtain k for the more general case where the corral and proteins have finite thicknesses.

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