Clustered Diffusion of Integrins

David Lepzelter and Muhammad H. Zaman*
Department of Biomedical Engineering, Boston University, Boston, Massachusetts

ABSTRACT We discuss the diffusion of clusters of integrins (and other similar membrane proteins) on a cell membrane with a cortical cytoskeleton. We argue that protein clusters—in contrast with normal oligomers, which are forced to pass through cytoskeletal barriers all at once—should be treated essentially as many-legged random walkers that can pass through a cytoskeletal barrier by putting one leg at a time through the fence. We present the mathematics that should describe the phenomenon, which result in a two-parameter model of diffusion that should apply to any cluster size. We also perform and discuss numerical simulations of the effect in the erythrocyte model system.

Received for publication 8 September 2010 and in final form 3 November 2010.

*Correspondence: zaman@bu.edu

The cell membrane contains a large number of intramembranous proteins, which carry out functions that allow the cell to interact with its environment (see, e.g., Arnaout et al. (1)). Diffusion of these proteins on the membrane is an important process to cells. Diffusion is involved in localization of signaling molecules, cell adhesion and cell motility via integrins (2), and various related processes, such as signal amplification (3).

Within this phenomenon of lateral diffusion on membranes there is a scientific puzzle that has confounded researchers for over a decade: proteins on artificial membranes diffused quickly, in agreement with the Saffman-Delbrück model, but on real cell membranes diffusion was significantly slower. Although explanations of this effect were proposed (4), the data were not conclusive until single-particle tracking became viable. At this point, it became clear that proteins on real cells were confined in corrals within which they diffused as they would on an artificial membrane. Slower large-scale diffusion occurred because proteins could occasionally jump to a neighboring corral. For membrane proteins, the primary corralling mechanism is thought to be physical blocking of the cytoplasmic tails of membrane proteins by the cortical cytoskeleton (Fig. 1) (5).

Recently, Auth and Gov discussed a model of diffusing membrane proteins on the red blood cell (RBC) membrane (6). Their model was simple but reasonable, and discussed, to a point, the effects of oligomerization on the slowing phenomenon. However, their work focused on the band-3 protein, which is significantly different from the proteins of primary interest in this study: integrins.

We note that integrins are not common on RBCs. However, the well-studied and simple RBC membrane is an ideal model system for the study of membrane protein diffusion. Also, we note that integrin behavior at this level is still sparsely studied. We present a picture of integrin diffusion, mentioning that even if integrins specifically do not demonstrate this behavior, other membrane proteins likely do.

Proteins do not diffuse only as monomers. Even traditional protein oligomers are capable of jumping from corral to corral, though this is known to slow them down significantly. (Indeed, the effect of oligomerization on diffusion was one of the signs that diffusion did not correctly follow the Saffman-Delbrück model (7). Protein oligomerization effectively increases the size of the diffusing particle, making barrier-crossing more difficult.) Protein clustering is different, but similar principles affect the system.

Protein clustering is distinct from standard oligomerization, because integrin (and similar) clusters form around groups of extracellular ligands. Their tails, therefore, are likely not intertwined in a way that would simply increase the effective size of the target for a piece of cytoskeleton to come in contact with. (Large semipermanent clusters are an exception (8).) Rather, they would provide multiple targets separated by however much space is between ligands. We therefore picture macroscopic diffusion of a protein cluster differently from that of a band-3 oligomer. Instead of a single protein or set of proteins hopping across a fence all at once, we propose the analogy of a creature with multiple legs crossing the same fence by putting one leg over it at a time (Fig. 2). This approach makes possible an analytical equation relating cluster diffusion constants.

To determine the effects of integrin-type clustering on membrane protein diffusion, we explore a simple mathematical model. The outline is presented here, with details given in the Supporting Material. We begin with the monomer diffusion rate, D_m, in the absence of a cytoskeleton. This rate should not decrease significantly with the size of the protein (or, therefore, the number of clustered proteins). With a cytoskeleton, the monomer diffusion rate becomes D_1 ($<D_m$) because of blocking by cytoskeletal barriers.

Editor: R. Dean Astumian.

doi: 10.1016/j.bpj.2010.11.007

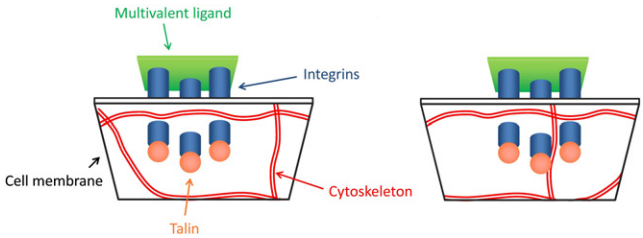

FIGURE 1 Illustration of the cytoskeleton impeding integrin cluster diffusion. Integrin cytoplasmic tails (and attached talin molecules) are physically blocked by cytoskeletal proteins, even without chemical binding. A cluster of integrins will be blocked multiple times by the same proteins.

Next, we assume that clustered proteins are attached to each other in ways that do not involve their cytoplasmic domains, and with enough space around them that their cytoplasmic tails are distinct from each other (estimated distance $\geq$ diameter of cytoplasmic domain + width of cytoskeletal barrier, 0 in the ideal, but finite in reality). In such an instance, they should not directly interfere with each other's passage through the barriers. However, if they are still bound together in the extracellular domain, they will not diffuse freely while at least one protein is on each side of a cytoskeletal barrier. Instead, they will continuously diffuse against the barrier until the entire cluster has gone to one side of it or the other. The rate at which these bound proteins pass through the barrier once constrained to be near it will be D_2, where $D_2 > D_1$.

We now consider the main slowing effect for a normal cluster, the chance that the cluster will hop back over the fence in the direction from which it came. The mean free path length of the cluster should be much smaller than the size of even a single membrane protein or cytoskeleton molecule, so a cluster's component proteins on either side of the fence may hop to the other in history-independent fashion. This should happen only for those closest to the barrier, however: one or two on each side, in all likelihood. We first examine the case with one on each side.

The process begins with a cluster having passed a single protein through the cytoskeletal barrier. The cluster is considered to have passed through the barrier if and only if all of its component proteins are on the other side of the barrier. In a similar way, it will be considered to have failed if all its component proteins are on the original side of the barrier.

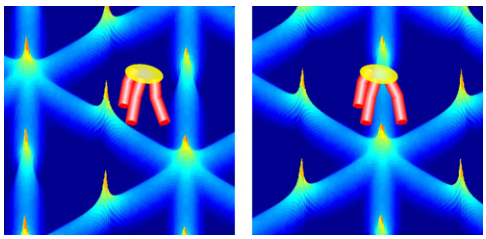

FIGURE 2 A different view of the cluster-diffusion issue, specific to red blood cells. Energy barriers due to spectrin pressure are pictured, with overlaid representations of proteins and multivalent ligands.

What, then, are the chances of successfully crossing? If there is only one possibly hopping protein on either side at any given time, the probability that the cluster will go forward is ½, and the probability that it will go backward is also ½. This amounts to a random walk: the fence takes a random one-dimensional walk through the cluster.

The probabilities of a cluster of n proteins crossing are represented by a square matrix, H, of dimension $n + 1$. The elements of this matrix are given by $H(i,j) = \delta_{i,j+1} + \delta_{i,j-1}/2$ except in the first and last columns, which contain only zeros. The probability of eventual passage is in the matrix $P = H + H^2 + H^3 + H^4 + \ldots$

Specifically, the probability that the cluster will fully pass through the barrier is the matrix element $P\ (n + 1, 2)$, and the probability that it fails to do so is $P\ (1, 2)$. The end result is simple; the probability of the cluster fully passing through the barrier is $1/n$.

In addition, we can calculate the total amount of time taken by the cluster to pass through the cytoskeletal fence. The time will be equal to the number of protein crossings after the first multiplied by the amount of time it takes for a single protein to cross (τ). The end result is the total amount of time spent on the act of crossing per successful crossing, or $\tau(n^2 - n)$.

When we define $D_2 = A/\tau$, where A is a fit-parameter area related to corral size, the macroscopic diffusion rate becomes

$$D = \frac{D_1}{n}\left(\frac{1}{(D_1/D_2)(n-1) + 1}\right). \quad (1)$$

This equation was derived assuming that the cluster was purely linear (i.e., one protein on either side diffusing against the barrier at any given time). This will not always be the case, of course. It would not be unreasonable for there to be two proteins on either side of the barrier, each of which could jump across independently (though simple geometric arguments can show that more than two should be unusual).

A calculated correction for this gives a roughly 10% decrease in simple clusters with at least three proteins. Precise approximations of this correction would require some knowledge of clustering geometry.

We note that our equation for D corresponds to previous measurements of similar quantities. Specifically, it was found that for gold nanoparticles with four to five proteins bound separately to them, diffusion constants in measurements decreased by a factor of ~4–5 (M. Edidin, The Johns Hopkins University, personal communication, 2010). This corresponds well to $D_2 \gg D_1$.

In our attempts to test the analytical equation above, we used methods from Auth and Gov (6) to simulate protein diffusion on a corralled membrane via Metropolis Monte Carlo. We adapted them to allow for multiple proteins in a single diffusing cluster, and to allow the movement to properly represent a rotating protein cluster. The interaction volume of an integrin with a spectrin molecule was estimated

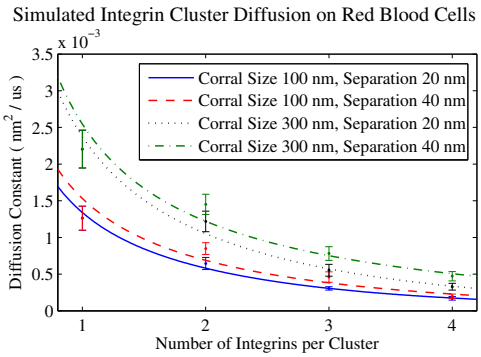

FIGURE 3 Calculated effective diffusion constants from simulated diffusion of clustered integrins. Normal RBC is a triangular corral 100 nm on a side. Stretched refers to a geometrical manipulation in which each side is made to be 300 nm but the cytoskeletal barrier is not weakened or made wider.

as 7400 nm^3, using the combined cytoplasmic regions of a β-integrin and a talin molecule from Anthis et al. (9). We estimated the free diffusion constant, D_m, of an integrin as that of a band-3 protein (0.25 $nm^2/\mu s$). The size of each side of the triangular RBC corral was 100 nm, except where otherwise noted. Other parameters were as in Auth and Gov (6).

Simulated integrin clusters had a number of integrins ranging from 1 to 4; as calculation time grew exponentially with cluster size, more than four would have required significant additional time. Cluster conformations were random, with distance between integrins ~20 nm in some simulations and ~40 nm in others. We simulated diffusion for at least 300 of each kind of cluster, allowing each of them to diffuse for 100 s of simulated time. Then we subjected the data to error calculations via the bias-corrected bootstrap method, which finds confidence intervals without assuming a Gaussian distribution, in MATLAB. (Measurements of D, being measurements of square distance traveled, are not expected to be Gaussian.)

All our simulation results are shown in Fig. 3. The first set, which we believe to be representative of a standard diffusing integrin cluster, has mean separations of 20 nm between adjacent integrins and a 100-nm corral. These simulation results are given with 95% confidence bounds.

Another set of simulations represented a standard RBC with a less dense diffusing cluster, with adjacent integrins separated by ~40 nm. A third set of simulations had a geometrically stretched RBC surface (in which each cytoskeletal barrier was made longer by a factor of 3, but the width and height of the barrier were left unchanged; this is nonphysical, as explained in the Supporting Material, but the steady trends are instructive). The fourth set of simulations combined a stretched RBC with a more separated cluster.

The simulation results we observe do not perfectly match Eq. 1. Indeed, all of the data seem to have the same basic pattern, with a high $n = 2$ point. This implies at least one significant correction factor to the equation, which we discuss in detail in the Supporting Material. These may include geometry-of-cluster effects. There may also be lengthscale-of-cluster effects: a larger cluster (in length dimensions, as opposed to number of integrins) could increase the chances of interacting with a cytoskeletal barrier, slightly increasing the effective value of D_1. Further, increased spacing between integrins should make more accurate the assumption that no more than one integrin interacts with a barrier at any given time. These effects would seem to be at least part of the reason for the increase in diffusion constant when separation between integrins is increased from 20 to 40 nm (Fig. 3).

Together, the analytical equation and the simulation results, with implied correction factors, are an important step in studying the slowed diffusion of collections of proteins seen in experimental work on similar systems (7). Diffusion is clearly important to clustering. Clustering, vital to integrin function (2), is also important to diffusion. A better understanding of these intertwined phenomena could give rise to more accurate models of those parts of cell function that depend on integrins and similar proteins, including signaling and cell motility. As these issues are quite significant to both medicine and biological science in general, we feel that further work on model verification is important.

SUPPORTING MATERIAL

Additional text and two movies are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(10)01375-5.

ACKNOWLEDGMENTS

We thank an anonymous reviewer for very insightful comments, and gratefully acknowledge the support of National Institutes of Health Grant 1 RC2 CA147925.

REFERENCES and FOOTNOTES

1. Arnaout, M. A., B. Mahalingam, and J.-P. Xiong. 2005. Integrin structure, allostery, and bidirectional signaling. *Annu. Rev. Cell Dev. Biol.* 21:381–410.
2. Miyamoto, S., H. Teramoto, …, K. M. Yamada. 1995. Integrin function: molecular hierarchies of cytoskeletal and signaling molecules. *J. Cell Biol.* 131:791–805.
3. Lamb, T. D. 1994. Stochastic simulation of activation in the G-protein cascade of phototransduction. *Biophys. J.* 67:1439–1454.
4. Jacobson, K., A. Ishihara, and R. Inman. 1987. Lateral diffusion of proteins in membranes. *Annu. Rev. Physiol.* 49:163–175.
5. Kusumi, A., C. Nakada, …, T. Fujiwara. 2005. Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned fluid: high-speed single-molecule tracking of membrane molecules. *Annu. Rev. Biophys. Biomol. Struct.* 34:351–378.
6. Auth, T., and N. S. Gov. 2009. Diffusion in a fluid membrane with a flexible cortical cytoskeleton. *Biophys. J.* 96:818–830.
7. Iino, R., I. Koyama, and A. Kusumi. 2001. Single molecule imaging of green fluorescent proteins in living cells: E-cadherin forms oligomers on the free cell surface. *Biophys. J.* 80:2667–2677.
8. Wozniak, M. A., K. Modzelewska, …, P. J. Keely. 2004. Focal adhesion regulation of cell behavior. *Biochim. Biophys. Acta.* 1692:103–119.
9. Anthis, N. J., K. L. Wegener, …, I. D. Campbell. 2009. The structure of an integrin/talin complex reveals the basis of inside-out signal transduction. *EMBO J.* 28:3623–3632.