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Impact of morphology on diffusive dynamics on curved surfaces

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Diffusive processes on nonplanar substrates are deeply relevant for cellular function and transport and increasingly used to probe and characterize the behavior of proteins in membranes. We present analytical and numerical analyses of in-plane diffusion of discrete particles on curved geometries reflecting various generic motifs in biology and explore, in particular, the effect that the shape of the substrate has on the characteristic time scales of diffusive processes. To this end, we consider both collective measures (the relaxation of concentration profiles towards equilibrium) and single-particle measures (escape rates and first passage times of individual diffusing molecules): the first relevant for the correct interpretation of FRAP experiments in curved environments; the second, for single-particle tracking probes. Each of these measures is sensitively affected by the morphology of the substrate, and we find that the exit rate out of a domain is not uniquely set by the size of its boundary, illustrating the general principle we reveal: By varying the shape of a substrate, Nature can control the diffusive time scales in a microenvironment without changing the bare substrate properties.

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1. INTRODUCTION

An estimated 20% to 30% of all cellular proteins are membrane proteins that reside exclusively within the two-dimensional confines of the various lipid bilayers in the cell. This confinement has profound consequences for the functionality, the aggregation behavior, and the modalities and regulation of the transport of these proteins. All these subjects have been the focus of vast amounts of research over the past 50 years (see, for instance, [1] and references therein). More recently, giant strides have been made in this field due to the arrival and perfection of sensitive experimental techniques such as fluorescence recovery after photobleaching (FRAP) [2,3] and single-particle tracking (SPT) [4,5]. Such techniques allow one to observe the behavior of proteins within membranes in unprecedented detail at the level of single proteins and their trajectories as they move around in the membrane, but also offer a coarser view of the evolution of protein concentrations with time.

These experiments reveal that cellular membranes present a highly heterogeneous environment to the diffusing species that inhabit it. Membranes are compartmentalized into so-called microdomains that may originate from a number of distinct mechanisms, including but not limited to the putative (micro)phase separation of membrane lipids into rafts [6], specific protein-protein interactions [7], and cytoskeletal corrals [8]. In this paper, we focus on geometrical compartmentalization, where out-of-plane curvature is used to create relatively isolated microenvironments in the membrane. This is a common motif in cells: Among the many examples of biological systems in which morphology creates small microdomains within a membrane are cristae in mitochondria [9,10], dendritic spines in synapses [11–14], and grana thylakoids in chloroplasts [15,16] (see Fig. 1). As we show, such curved microenvironments serve to retain proteins for increased periods of time in the vicinity of some functional domain, and their shape modulates the diffusive dynamics in and out of the compartment. While our work is motivated by these biological examples, the effect is completely generic and may, conceivably, be used to control diffusion in more general soft matter settings such as Pickering emulsions, colloids in two dimensions, and (microfluidic) particle-laden flows.

The increased precision with which experiments can record individual trajectories as well as the evolution of concentration profiles requires an equally precise theoretical framework to interpret the results, and as we demonstrate, the effects of observing diffusive processes in curved geometries introduce subtle but important phenomena. In the present work, we ask the question how the shape of a membrane alters the relevant time scales and escape rates of particles laterally diffusing on the surface, at both the single-particle level and that of concentration profiles.

Our approach is to combine random walk (RW) simulations with calculations of the so-called mean first passage time (MFPT) to study how diffusive time scales and escape rates are altered by geometrical constraints of the membrane. First, we verify the obvious: that shape in itself cannot retain an elevated concentration of molecules in a certain subdomain indefinitely. Eventually gradients in the concentration vanish. Nonetheless, we find that shape can, and does, affect the temporal evolution of a concentration profile. In general, biological systems where this effect is exploited are characterized by a large functional compartment, which we term a microdomain, separated from the remainder of the membrane by a narrow neck. We find that particle concentrations within such microdomains may remain elevated for increased periods of time, depending on the radius and length of the neck separating the two regions. More specifically, we find that the equilibration time dependence on the neck radius and the length of the neck follows a power law over several decades: \( \tau_{eq} \sim (\text{neck radius})^{-\alpha} \) and \( \tau_{eq} \sim (\text{neck length})^{\eta} \). The (positive) exponents \( \alpha \) and \( \eta \) reflect the precise geometry of the system.

In addition to the dynamics of diffusively relaxing concentration profiles, characterized by \( \tau_{eq} \), we consider the escape dynamics of individual particles in the same microdomains,
characterized by the MFPT for crossing the microdomain boundary. Again, we recover a power-law dependence of the escape time through the boundary of the system on the neck radius: \( t_{\text{MFPT}} \sim (\text{neck radius})^\lambda \), with an exponent \( \lambda \) that is identical to that of the concentration equilibration time, but with a different prefactor. We also consider the distribution of escape times and find that it features an exponential tail: \( P(t) \sim \exp(-t/t_{\text{tail}}) \), where \( t_{\text{tail}} \) is, within our numerical accuracy, equal to \( t_{\text{MFPT}} \). Thus, the residence time of those particles that dwell in the microdomain the longest, like the relaxation time and the MFPT, depends on the size of the neck in power-law fashion.

By what precise mechanism does the shape of the membrane regulate the equilibration dynamics of concentration profiles, as well as the exit dynamics of single particles? To answer this question we isolate the effect of shape and study exit dynamics keeping the curvature of the membrane connecting to it. At this constant boundary size, we demonstrate that in out-of-equilibrium situations the characteristic time scale for particles leaving the domain may change over several orders of magnitude, depending on the morphology of the system. That is, the time scales and escape rates through a boundary do not depend solely on the size of the boundary itself but, rather, are determined by an interplay between the size of the boundary and the available area around the absorbing boundary.

This paper is organized as follows: Section II presents the model system and methods we use to simulate and calculate the relevant time scales associated with lateral diffusion of particles on a curved substrate. In Sec. III we quantify the effect of shape on the relaxation time scale of concentration gradients. Section IV presents results on the signatures of shape on individual particle traces. In Sec. V we present the governing principles behind the morphological effects on diffusion and report an—at first sight—counterintuitive morphology-induced asymmetry. In Sec. VI we summarize our findings, state the principal conclusions of our work, and present an outlook for further research.

FIG. 2. (Color online) The two geometries we consider throughout this paper: (a) two planar surfaces connected by a narrow neck with radius \( d \) and height \( h \) (shape A) and (b) a funnel-like geometry connecting an enclosed head domain (which we also refer to as the top of the funnel) to the surrounding membrane (shape B). See text for parameter values.

II. METHODS

A. Model system

To study how the morphology affects the time scales of in-plane diffusion we study two distinct systems, shown in Fig. 2, A and B. The first system, to which we refer as A, consists of two large planar surfaces, separated by a tubular neck with radius \( d_1 \) and height \( h_1 \). The second system, to which we refer as B, consists of a bulbous head compartment, connected to the remainder of the system by a funnel-like neck of radius \( d_2 \). The axisymmetric funnel-like geometry of system B is parametrized as

\[
\begin{align*}
x(u,v) &= R \sin u \cos v, \\
y(u,v) &= R \sin u \sin v, \\
z(u,v) &= h_2 - \frac{R \cos u}{A u},
\end{align*}
\]

where \( R \) is the maximal radial distance of the surface representing the largest radius of the funnel, \( h_2 \) a measure for the height of the funnel, and \( A \) a shape parameter which is large for systems with a narrow neck and small for systems with a wider neck. In Appendix A, we calculate the corresponding metric and Christoffel symbols of this geometry. These shapes are connected to a flat membrane with outer radius \( R_{\text{ext}} \) in A and \( R_{\text{ext}} \) in B.

These two systems are chosen to represent the two most widely encountered geometries in cellular membranes: two regions connected by a slinder neck, where one of these two regions is an extended plane, and the other is either an extended planar region too (as happens, for instance, in cristae connected to the inner membrane of mitochondria or the stacked grana in thylakoids) or a smaller, bulbous compartment (as is the case in dendritic spines and membrane pits). We do not claim that these geometries are represented with exact precision by the sample geometries used here; our interest is in the general functioning of relevant generic shape motifs.

B. Random walk simulation

In this section we present the RW simulation methodology that we use throughout this work. The aim of this method is to simulate the in-plane Brownian motion of the particles on a curved surface. The method we use to account for the curved shape in the context of RW simulations is extensively
discussed in [19]; we now briefly summarize our algorithm for computing such RW traces. We simulate RWs as trajectories composed of fixed length steps in random directions on a curved geometry with in-plane coordinates \( u \) and \( v \). On a curved surface, both selecting a random direction and traveling a fixed distance in this chosen direction require some attention. A true random directional unit vector \( \vec{w} \) of unit length is chosen subject to the constraint \( |\vec{w}|^2 = g_{uu}(w^u)^2 + g_{vv}(w^v)^2 = 1 \), where \( g_{ij} \) are the elements of the metric (for rotationally symmetric systems \( g_{ij} = 0 \) if \( i \neq j \)), which we explicitly calculate in Appendix A for geometry B. The length of this random vector in local coordinates \( (u, v) \) is thus \( |\vec{w}| = \cos(r_n)/\sqrt{g_{uu}} \) and \( |\vec{w}| = \sin(r_n)/\sqrt{g_{vv}} \), where \( r_n \) is a number picked randomly, between 0 and \( 2\pi \).

Next, we approximate the geodesic curve, parameterized by the arc length \( s \) [19], and apply a second-order approximation of the tangential plane,

\[
\vec{r}(s + ds) = \vec{r}(s) + \frac{d\vec{r}(s)}{ds}ds + \frac{1}{2} \frac{d^2\vec{r}(s)}{ds^2}ds^2,
\]

(2)

where the first derivative is the unit tangent vector \( \vec{w} \) and the second-order correction is obtained by solving the local geodesic equation for the surface

\[
\frac{d^2\vec{r}}{ds^2} = -\Gamma^i_{kl} \frac{dr^i}{ds} dk^l,
\]

(3)

with \( \Gamma^i_{kl} \) the Christoffel symbols of the surface and \( r^i \) the \( i \) component of \( \vec{r} \). We adopt the Einstein summation convention: summation over repeated indices is implied, i.e., \( a_i b^i = \sum_i (a_i b^i) \) [20]. Rewriting this to the parametrization \((u, v)\), a step of size \( \lambda \) [i.e., \( |\vec{w}|^2 = g_{uu}(w^u)^2 + g_{vv}(w^v)^2 = \lambda^2 \)] in these local coordinates is achieved by a shift in coordinates \( \Delta \vec{w} = (\Delta u, \Delta v) = (u_{q+1} - u_q, v_{q+1} - v_q) \), where the index \( q \) labels the discrete iteration step number, and

\[
\Delta u = w^u\lambda - \frac{1}{2} \Gamma^u_{uv}(w^v)^2\lambda^2 - \Gamma^u_{uu}(w^u)^2\lambda^2 - \frac{1}{2} \Gamma^u_{uv}(w^v)^2\lambda^2,
\]

\[
\Delta v = w^v\lambda - \frac{1}{2} \Gamma^v_{uv}(w^v)^2\lambda^2 - \Gamma^v_{uu}(w^u)^2\lambda^2 - \frac{1}{2} \Gamma^v_{uv}(w^v)^2\lambda^2.
\]

(4)

The resultant diffusive motion is, by construction, locally Brownian (\( \langle x^2 \rangle = 4Dt \)) to second order in the curvature. In our simulation, we measure, among others, the average number of steps \( \langle N \rangle \) of length \( \lambda \) that it takes to arrive at a boundary. This quantity can be related to the effective mean escape time using the standard diffusive definition

\[
\tau_{\text{escape}} = \frac{\langle N \rangle \lambda^2}{4D},
\]

(5)

where \( D \) is the diffusion constant. This implies that in RW simulations, the effective time step \( \delta t \) and the step length \( \lambda \) should be related by the diffusion coefficient: \( \delta t = \lambda^2/(4D) \). In the remainder of this paper we use a step size \( \lambda = 0.005 \mu m \) (this choice is motivated in Appendix B). For a diffusion coefficient \( D = 0.1 \mu m^2/s \), this requires a time step of \( 6.25 \times 10^{-8} \). Thus, when we report an escape time of 100 s, this corresponds to \( 1.6 \times 10^8 \) steps. The results reported here all reflect the fact that even for uniform and identical values of \( D \), the quantity \( \langle N \rangle \) is strongly influenced by the shape of the substrate: this is the mechanism for the regulation of diffusive dynamics in curved geometries. We show in Sec. IV that this mean escape time, obtained from simulations, equals the theoretically calculated MFPT (see next section) for those situations where an analytical computation is possible; we use the numerical values in cases where an analytical answer is not available. It should be noted that in both geometry A and geometry B, the connection between the funnel and the plane and between the cylinder and the plain is continuous but nonsmooth. This is solved by truncating any step that takes the trajectory outside of the domain in which it originated to the point of intersection between the domains. Such steps are therefore shorter than \( \lambda \). We have verified that this method yields the same results, for our choice of \( \lambda \), as methods in which the remaining part of the step is rotated onto the second domain (a method that yields steps of length \( \lambda \) but is computationally more involved).

C. Mean first passage time

To analyze the effects of geometry and curvature on the confinement of particles theoretically, we calculate the MFPT. This MFPT may be considered a characteristic time scale for diffusion in a system with absorbing boundaries. Numerous previous sources consider this quantity to characterize time scales and have calculated the MFPT analytically for geometries such as membrane patches, spheres, and various other surfaces of revolution [21–30].

The MFPT \( W \) is generally obtained by solving the following differential equation:

\[
\nabla^2 W = -1/D,
\]

(6)

where \( D \) is the two-dimensional diffusion constant and \( \nabla^2 \) is the Laplace-Beltrami operator, which, for non–trivially curved surfaces such as those we consider (geometry B) here, may also be calculated analytically [31]. Assuming rotational symmetry, the Laplace-Beltrami operator can easily be calculated from the metric and Christoffel symbols that we provide in Appendix A:

\[
\nabla^2_n = \nabla_i \nabla^i = g^{ij} \frac{\partial^2} {\partial x^i \partial x^j} - g^{ij} \Gamma^k_{ij} \frac{\partial} {\partial x^k},
\]

(7)

where \( g^{ij} \) are the Christoffel symbols and \( g^{ij} \) the components of the inverse metric defined by \( g^{ij} = g_{ij} \). We solve the equation for the resultant MFPT [Eq. (6)] numerically for geometry B and compare it to to the escape time obtained from the RW simulations presented in the previous section.

III. IMPACT OF MORPHOLOGY ON THE RELAXATION OF CONCENTRATION PROFILES

To determine how the shape affects the time scales of lateral diffusion in morphological constricted environments we perform RW simulations in the manner discussed in the previous section. We do this for two distinct geometries, shown in Fig. 2, left and right. For these two systems, we calculate the temporal evolution of the density in the two indicated domains, 1 and 2 (Fig. 2, left and right), by releasing 5000 noninteracting particles at a distance 1 \( \mu m \) from the center of the disk in A and at the top of the funnel (i.e., the leftmost part of shape B in Fig. 2, right). (We consider, for the time being, a low-density regime for the diffusing species. We are aware that this may

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FIG. 3. (Color online) (a) Temporal evolution of the density in domains 1 and 2 of shape B for various neck radii ($d_2 = 0.1, 0.05,$ and $0.025 \mu m$) after the release of 5000 particles in domain 1. (b) Neck length dependence of the equilibration time $\tau_{eq}$ for shape A, given a fixed neck radius $d_1 = 0.5 \mu m$. We find that $\tau_{eq} \sim h^{-0.8}$ (c,d) Equilibration time $\tau_{eq}$ as a function of the radius of the constriction $d$. Decreasing the size of the neck increases the equilibration time in power-law fashion for both geometries: planar-planar $d_1, \tau \sim d_1^{-0.85}$, and funnel $d_2, \tau \sim d_2^{-0.75}$ In (c) we have also plotted [filled (red) circles] the dependence of the equilibrium time on the neck radius $\tau \sim d_1^{-1.73}$, keeping the total surface area of the neck constant ($d_1 \times h_1 = 0.5 \mu m^2$).

not reflect cellular conditions in all cases, and in follow-up work we address the effects of molecular crowding in curved membranes.) We find that the density equilibrates to a uniform value, as it should (to avoid all particles exiting the system, for this measurement we place a reflecting boundary at the edge of the system, leading to a conserved number of particles). This reflects the fact that the morphology of a system alone cannot retain density gradients; eventually a uniform equilibrium must be reached. However, we show in Fig. 3(a) that the time it takes to reach equilibrium depends on the shape of the domains. We focus initially on the role of the radius of the constriction, separating the two large structures, as shown in Fig. 3(a); we vary the neck radius in geometry B using values of $d = 0.1$ (red line), $0.05$ (blue line), and $0.025 \mu m$ (black line). To isolate the effect of the neck, we keep the diffusion coefficient fixed at $0.1 \mu m^2/s$ and use $R = 1 \mu m, h_2 = 4 \mu m$, and $R_{eq} = 1 \mu m$ in all cases considered.

To quantify the ability of a shape to retain an elevated concentration of particles in a certain domain we calculate the equilibration time $\tau_{eq}$, which we define as the time it takes for the difference in concentration to reach 99% of its equilibrium value across domains 1 and 2, for geometries A and B. We extract $\tau_{eq}$ as a function of the height of the system $h$ for system A and as a function of the radius of the constriction $d_1$, separating the two domains for systems A and B, where for A we also consider the case where the total surface area of the neck remains constant such that a decreasing neck radius is compensated by an increased neck length: $h_1 \times d_1 = 0.5 \mu m^2$ (system A: $h_1 = 1 \mu m$, radius of the neck $d_1 = 0.5 \mu m$, and $R_{eq} = 2 \mu m$ fixed; system B: $R = 1 \mu m, h_2 = 4 \mu m$, and $R_{eq} = 1 \mu m$ fixed). We demonstrate that this system exhibits power-law dependencies of the equilibration time both on the length of the constriction $\tau_{eq} \sim h^{0.8}$ [see Fig. 3(b)] and on the neck radius: Figs. 3(c) and 3(d) show scaling for both systems $\tau_{eq} \sim d^{-\lambda}$. The exponent $\lambda$ for the equilibration time $\tau_{eq}$ for system A equals 0.85 for a fixed neck length and 1.73 for a constant area, and that for system B equals 0.75.

From this, we conclude that an elevated concentration in one of the subdomains, separated by a slender neck from the rest of the system, may be retained for significantly longer times if the neck separating the two domains is narrow (and thus highly curved in one direction). Clearly, this phenomenon serves to compartmentalize functionality in several biological systems as mentioned before: Diffusing species remain localized longer and are contained within effectively isolated compartments created by curvature, rather than physical boundaries.

IV. IMPACT OF MORPHOLOGY ON THE EXIT DYNAMICS OF SINGLE PARTICLES

We now turn to the manifestations of morphology in single-particle behavior. Single particles exhibit similar morphological dependencies as do concentration profiles, as one observable is directly linked to the other. However, in membrane protein systems it is generally not obvious or even warranted to speak of concentrations, as some proteins exist at very low copy numbers and coarse graining is ill advised. In single-particle tracking, likewise, the behavior of one or a few distinct diffusing entities is studied and the question of how such traces are affected by local and global geometry is of immediate relevance. Since most curved geometries in cell biology serve to retain functional proteins within some microdomain, we focus on measures associated with the escape from a region. This question has received considerable attention in the last couple of years, motivated particularly by the context of dendritic spines [24,28]. For these spines, the neck has been shown to be an important barrier for diffusion: increasingly so when it becomes longer or narrower.

To focus, again, on the isolated effect of the neck radius we assume shape B with a fixed height and a constant radius of the head [see Fig. 4(a)]. Values chosen were a height of $4 \mu m$, head radius of $1 \mu m$, and diffusion constant of $D = 0.1 \mu m^2/s$. We vary the radius of the neck $d_2$ by varying the shape parameter $A$ in Eq. (6). As mentioned earlier, we calculate the probability density function (pdf) of escape times, and the MFPT $\tau_{MFPT} = \langle \tau \rangle_{pdf}$. In Fig. 4(c) we graph the computed values $\tau_{MFPT}$ as a function of the neck radius for particles released at the top of the funnel [i.e., the leftmost part of shape B in Fig. 2, left], represented by the filled circles and for a homogeneous concentration of particles (filled triangles). It can be seen that for the release at the top, the solid line, which represents direct computation through Eq. (6), perfectly coincides. Decreasing the size of the neck leads to a divergent increase in the MFPT (as it should; the MFPT becomes infinite in the limit of zero neck diameter), and does so in the same fashion as the equilibration time computed in Sec. III: $\tau \sim d_2^{-0.75}$ [see Fig. 4(d)].

From the distribution of escape times in Fig. 4(b), we note that upon a decrease in the size of the neck, the width of the
distribution increases significantly. If we now plot these data for various radii $d_2$ on a log-linear scale, we find that for long times the tail of the distribution scales exponentially: $\text{pdf}(\tau) \sim \exp(-\tau/\tau_{\text{tail}})$. Direct comparison, furthermore, yields that $\tau_{\text{tail}}$ is equal to $\tau_{\text{MFPT}}$. Thus, not only does the MFPT scale with neck radius, but also the fraction of particles that remains inside the domain does too. In Appendix C we explore the distribution and its dependence on neck size further. The exponential tail of the distribution is characteristic for so-called narrow-escape systems [30,32], of which ours is an example. The single exponent here is noteworthy: We speculate that it may be relevant in light of the small copy number of some proteins or in systems where only very few proteins suffice to ensure functionality. A suitably chosen shape can ensure that a minimal occupancy is statistically retained over very long times.

It also means that one must be careful with numerical simulations: for reliable averaging it is essential to observe a sufficient number of very long-time events. If one does not, for instance, by averaging over too few particles, $\tau_{\text{MFPT}}$ is systematically underestimated. By observing the convergence of the MFPT as a function of the sample volume we have determined the appropriate number of events to sample in our simulations.

FIG. 4. (a) Probability density function (pdf) of the escape times for various neck radii (Full line: $d_2 = 0.02 \, \mu m$ dotted line: $d_2 = 0.03 \, \mu m$, dashed line: $d_2 = 0.12 \, \mu m$, where we assume shape $B$ (Fig. 2), see main text for the parameter values. Decreasing the size of the neck is seen to increase the breadth of the distribution considerably. (b) Log-linear plots (See Appendix III) reveal that tail of the distribution follows $\text{pdf}(\tau) \sim \exp(-\tau/\tau_{\text{tail}})$, where moreover $\tau_{\text{tail}} = \tau_{\text{MFPT}} \sim d_2^{-0.75}$. (c) Mean First Passage Time $\tau_{\text{MFPT}}$ as function of the neck radius $d_2$, measured in simulations for particles starting at the top of the funnel (dots) and a homogeneous distribution of the particles across the surface (triangles). In agreement with [29], we find identical scaling but slightly lower values for the distributed case. The escape time for the particles starting at the top show exact agreement with the MFPT obtained by direct computation (see Methods section). (d) Log-log plot of $\tau_{\text{MFPT}}$ revealing power-law behavior over 2 decades. The exponent is $\lambda = -0.75$.

V. IMPACT OF MORPHOLOGY ON ASYMMETRIC DIFFUSIVE DYNAMICS

The central finding of the previous two sections is that shape, quantified in our case by the dimension of the restrictive passage between two domains, affects both the equilibration of concentrations and the behavior of single particles. An objection that one might raise is that, obviously, as the radius of the passage is decreased there is simply a smaller exit, less likely to be found by particles. We now show, however, that even when the passage remains the same size, but the shape in its vicinity changes, the MFPT is directly affected. To this end, we consider the funnel-like geometry, with an absorbing boundary at the top and one at the bottom. Both boundaries—the one at the top and the one at the bottom—are circular, and both have the same size. We release particles at different locations on the funnel surface and record the fraction of particles that escape through the top compared to those that escape through the bottom of the funnel, as a function of the height at which we release the particles (see Fig. 5, where we have assumed a fixed shape with height $h_2 = 4 \, \mu m$, a constant head radius of $R = 1 \, \mu m$, and a radius of the boundary of 0.06 $\mu m$). We find that, when particles are released at a height $0.25$ times the total height of the funnel, there is an equal probability of their escaping through the top or through the bottom exit. When released halfway up the funnel, only $25\%$ of the particles exit through the base of the neck. Thus, the asymmetry of the shape is directly reflected in an asymmetric relaxation: the shape controls and, in effect, guides the particles to a target region of interest.

If, however, we do distribute particles homogeneously over the surface there is still an asymmetry: eventually, all particles must leak away through one of the exits and the manner in which they do so distributed over the two exits is again asymmetric. We measure the fraction that escapes through the bottom and through the top as a function of the shape, for different neck radii and thus for various sizes of the absorbing boundaries (still, of course, identical at the top and bottom). We...

FIG. 5. (Color online) (a) The fraction of particles that escape through the boundary at the neck compared to these escaping through the boundary at the top $N_{\text{bot}}/N_{\text{top}}$ as function of the height of release for a fixed shape (see parameter values in the main text). (b) The fraction of particles that escape through the boundary at the neck compared to these escaping through the boundary at the top $N_{\text{bot}}/N_{\text{top}}$ as function of overall shape of the membrane, varying from tubular where obviously $N_{\text{bot}}/N_{\text{top}} = 1$ to funnel-like where $N_{\text{bot}}/N_{\text{top}} \ll 1$. The surface in (b) is prepared such that the particles were homogeneously distributed over the surface.
use shapes ranging from a cylinder, where we expect a 50:50 ratio in escape rate, to a funnel with a thin neck, where we expect to recover the asymmetry discussed earlier. In Fig. 5(b) we show, for 1000 receptors, this fraction as a function of the size of the neck and thus the size of the absorbing boundaries given a system with height $h_2 = 4 \mu m$, a constant head radius of $R = 1 \mu m$, and a diffusion constant of $0.1 \mu m^2/s$. This figure shows that for decreasing neck size an asymmetry occurs between exit at the top and exit at the bottom. This phenomenon suggests that, by varying the shape of the surrounding substrate, one can, in principle, regulate capture at functional domains and fluxes through boundaries. This somewhat counterintuitive effect is not determined by the size of the absorbing boundary but, rather, by the available surface area in the vicinity of the absorbing boundary.

The discrepancy in the escape times and probabilities of leaving the funnel through the neck versus through a hole at the top, evident in both the simulations and theory, can be understood by considering the difference in available surface within one characteristic diffusion length from the absorbing boundary. This available surface around the absorbing boundary at the top of the shape is larger than that at the base of the neck. To quantify the effect, we break the nonstandard geometry of a funnel into two more intuitive geometries: a cylindrical surface with a total area of $A_{cyl} = 2\pi R_0 h_0$, with $R_0$ the radius of the cylinder and $h$ the height of the cylinder; and a flat disk with an inner radius $R_0$ and outer radius $R_1$ and a total surface of $A_{\text{disk}} = \pi(R_1^2 - R_0^2)$. For a fair comparison, we set the two areas to be equal: $A_{\text{disk}} = A_{cyl}$. The two geometries are shown in Fig. 6. On these two equally sized surfaces with reflecting boundaries at $z = h, z = 0$ (cylinder) and $R_{\text{out}}$ and $R_0$ (disk), we simulate 1000 random walking particles until their density has equilibrated and is homogeneous across the entire surface, where we have used a diffusion constant of $0.1 \mu m^2/s$. After this equilibration, the inner radius of the disk and the boundary $h = 0$ at the base of the cylinder are changed to absorbing boundaries, and we measure the mean escape time of the receptors through the absorbing boundaries. In Fig. 6 (filled black circles) we show the ratio of the two mean escape times $\tau_{\text{disk}}/\tau_{\text{cylinder}}$ and find that, assuming a constant $R_1$, decreasing $R_0$, and also increasing $h$ (to ensure area conservation), strongly decreases the fraction $\tau_{\text{disk}}/\tau_{\text{cylinder}}$.

This large difference can be understood as follows: First, consider the mean distance to the absorbing boundary, which is $\bar{h} = A_{cyl}/(4\pi R_0)$ for the cylinder and $\bar{R}^2 = 0.5(R_1^2 + R_0^2)$ for the plane. Next, we calculate the MFPT for a particle starting at this average distance from the absorbing boundary. In Fig. 6(d) we demonstrate that quantitatively this argument indeed accurately reproduces the marked difference in escape times observed for the funnel-like geometry in Fig. 5. Rewriting slightly the equations for $\bar{R}$ and $\bar{h}$ above, and assuming equal areas and equal exit sizes, we find that

$$\left(\frac{\bar{R}}{\bar{h}}\right)^2 = 4 \left(\frac{R_0}{h}\right)^2 + 4 \left(\frac{R_0}{h}\right).$$

Thus, in the thin neck limit ($R_0 \ll h$) we are primarily interested in $\bar{h} \gg \bar{R}$. In short, it takes a lot longer to escape from a thin cylinder than it does from a plane, even for equal total areas, and even for equal exit sizes. This is the effect

\[ \text{FIG. 6. (Color online) The local geometry around the exits of the funnel-like geometry (a) is a planar surface (b) at the top, with a hole in the center of radius } R_0, \text{ but is a cylinder (c) with radius } R_0 \text{ at the bottom. We fix the total surface area and the size of the exit, and compute the average escape time: (b) right-hand side panels show a marked difference in escape time between the two geometries. (d) shows the fraction of escape times as calculated in (b) and (c), from which we see that, depending on the size of the hole, the escape through the circular hole may be up to an order of magnitude faster than escape through the base of the cylinder.} \]

of shape in its simplest form, and clearly determined by the global geometry, which dictates that a particle is, on average, closer to the boundary on the disk than it is on the cylinder.

\section{VI. Conclusions}

We have analyzed how the morphology of a membrane within which particles diffuse regulates time scales and fluxes through the boundaries of domains for some biologically relevant geometries. In general, these microdomains serve to retain gradients in particle concentrations for increased time scales. While this regulation by shape is certainly not the only regulatory mechanism, and we do not compare its magnitude directly to other factors at play, the highly curved geometries in several biological systems, like dendritic spines in synapses, cristae in mitochondria, and the thylakoid membrane (which all share a single generic design), strongly suggests that the shape is, indeed, directly used to control diffusive behavior.

By quantifying the dynamics of particles escaping through a narrow neck we have found a general power-law dependence for both the equilibration time scale of a population of particles and the single-particle characteristic time scales of escape through the boundary of a domain such as the MFPT and the escape time. These diffusive time scales depend, in general, on the neck radius and the length of the neck following a power law over several decades: $\tau_{\text{eq}} \sim (\text{neck radius})^{-\lambda}$ and
\( \tau_{\text{eq}} \sim (\text{neck length})^{\nu} \), where the (positive) exponents \( \lambda \) and \( \eta \) reflect the precise geometry of the system.

In addition to summarizing the statistics of the first passage process by its mean, we have also determined the distribution of timescales. This confirms that for our system the tail of the distribution is single exponential, \( \text{pdf}(\tau) \sim \exp(-\tau/\tau_{\text{MFPT}}) \), as previously observed in cylindrical and circular membrane patches \([29,30,32]\). For systems where a low, but obviously nonzero concentration of proteins is required for functionality, we speculate that this mechanism helps keep a small quantity of these proteins around the functional domain for very long times.

In addition, we find that the morphology of a substrate may regulate not only the characteristic escape times but also the escape rates. This is illustrated by considering a funnel-like geometry and observing that, depending on the shape, absorption rates at the top of the funnel may be up to an order of magnitude higher compared to those at the base, even though the two absorbing structures are of identical size. We have shown that the crucial geometric quantity is the available surface in the vicinity of the absorbing boundary, which directly determines the characteristic escape time of the particles—larger available surfaces leading to smaller average distances between particles and boundary.

Our findings are relevant for the interpretation of FRAP data: The increased recovery time after photobleaching in a complex morphology may not originate uniquely from physically distinct local properties such as an elevated viscosity, coupling to the underlying cytoskeleton, and direct protein–protein interactions: we show that, even absent all these effects, differential fluorescent recovery may signal a nontrivial curved morphology may not originate uniquely from phys-}

**APPENDIX A: METRIC AND CHISTOFFEL SYMBOLS FOR SYSTEM B**

In this Appendix we calculate the metric and the corresponding Christoffel symbols for geometries A and B, as described in Sec. II. The metric of system A (see Fig. 2) has the following two nonzero elements:

\[
g_{uv} = \begin{pmatrix} 1 & 0 \\ 0 & R^2 \end{pmatrix}
\]

(A1)

For this cylindrical surface all the Christoffel symbols \( \Gamma^k_{ij} \) vanish. The metric of system B has the following two nonzero elements,

\[
g_{uv} = \begin{pmatrix} R^2(\cos^2 u + \frac{\cos u + u \sin u}{A^u})^2 & 0 \\ 0 & R^2 \sin^2 u \end{pmatrix}
\]

(A2)

from which we can calculate the Christoffel symbols \( \Gamma^k_{ij} \):

\[
\Gamma^u_{uu} = \frac{1}{\tan u},
\]

\[
\Gamma^u_{uu} = -2 \sin u \cos u + \frac{2 \cos u(\cos u + u \sin u)}{A^u} - \frac{2(\cos u + u \sin u)^2}{A^u},
\]

\[
\Gamma^v_{uu} = \frac{\cos u \sin u}{\cos^2 u + \frac{\cos u + u \sin u}{A^u}}.
\]

(A3)

Using this, we can calculate the Laplace-Beltrami operator from Eq. (7).

**APPENDIX B: DETERMINATION OF THE STEP SIZE \( \lambda \)**

In pure RW simulations, the step length is generally irrelevant because of the self-similar behavior expected for long traces. As long as it is chosen in correct proportion to the time step and the diffusion constant, according to Eq. (5), the long-time results should not change. In curved geometries, however, there are intrinsic length scales related to an order of magnitude higher compared to those at the base, even though the two absorbing structures are of identical size. We have shown that the crucial geometric quantity is the available surface in the vicinity of the absorbing boundary, which directly determines the characteristic escape time of the particles—larger available surfaces leading to smaller average distances between particles and boundary.

Our findings are relevant for the interpretation of FRAP data: The increased recovery time after photobleaching in a complex morphology may not originate uniquely from physically distinct local properties such as an elevated viscosity, coupling to the underlying cytoskeleton, and direct protein–protein interactions: we show that, even absent all these effects, differential fluorescent recovery may signal a nontrivial curved morphology may not originate uniquely from phys-}

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FIG. 8. The tail of the distribution of escape times for various neck radii $d$, for a type-B shape. We find perfect single-exponential behavior; $\tau_{\text{tail}} = d_2^{-0.35}$. Moreover, $\tau_{\text{tail}}$ is exactly equal to the Mean First Passage Time, represented by the solid line in the inset (see parameter values in the main text).

to the curvature of the substrate which warrant slightly more care: Too large of a step size renders the simulation insensitive to shape features smaller than this step size. For this reason, we choose a step size that is considerably smaller than the smallest radius of curvature present in any of the systems we study. This, too, requires a caveat: Since the composite shapes (type A as well as type B) consist of subdomains that are not smoothly joined to each other, the smallest radius of curvature is technically 0. We deal with this discontinuity as described in Sec. II but have run the same simulation for a variety of step sizes to determine the appropriate choice. As we show in Fig. 7, below a value of $\lambda = 0.02 \mu m$, the measured escape times saturate. In all simulations presented in this paper, we have chosen $\lambda = 0.005 \mu m$, well within this converged regime.

APPENDIX C: DISTRIBUTION OF ESCAPE TIMES

As pointed out in the text, the distribution of escape times depends on the geometry; in our case, varied by varying the size of the smallest constriction between two regions. In this Appendix we analyze the neck size dependence of the tail of the distribution of escape times (see parameter values in Sec. III). We simulate the type B shape and measure the tail of the escape time for various neck radii $d$. As shown in Fig. 8 we find that the tail is exponential, $\text{pdf}(\tau) \sim \exp(-\tau/\tau_{\text{tail}})$, where $\tau_{\text{tail}}$ perfectly coincides with the MFPT $\tau_{\text{MFPT}}$, so that $\tau_{\text{MFPT}} = \tau_{\text{tail}}$.