MICROSCOPIC SIMULATION OF EPIDERMAL GROWTH FACTOR RECEPTOR DIFFUSION ON CORRALLED MEMBRANE SURFACES

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ABSTRACT

The current understanding of how receptors diffuse and cluster in the plasma membrane is limited. Data from single particle tracking and laser tweezer experiments have suggested that membrane diffusion is affected by the presence of barriers dividing the membrane into corrals. Herein, we have developed a stochastic, spatial model to simulate the effect of corrals on the diffusion of receptors in the plasma membrane. The results of this simulation confirm that a fence barrier (the ratio of the transition probability for diffusion across a boundary to that within a corral) on the order of $10^3 - 10^4$ recreates the experimentally measured difference in diffusivity between artificial and natural plasma membranes. An expression for the macroscopic diffusivity of receptors on corralled membranes is derived to analyze the effects of the corral parameters on diffusion rate. We also examine whether the lattice model is an appropriate description of the plasma membrane and look at three different sets of boundary conditions that describe the barriers. This analysis reveals that diffusion events on the plasma membrane may occur with a single physically relevant length scale.

INTRODUCTION

Signal transduction is typically initiated when a ligand (i.e., Epidermal Growth Factor or Heregulin) binds a receptor (i.e., ErbB1 or ErbB2 respectively). The binding of a ligand to a receptor often leads to receptor dimerization and higher order receptor clustering. The clustered receptors then initiate a signal transduction cascade (including receptor phosphorylation and recruitment of adaptor proteins, kinases, and other signaling proteins), which controls cellular physiology. For example, many signaling pathways lead to the activation of transcription factors controlling genes involved in regulating cell division and differentiation (1; 2). Deregulation of signaling pathways (i.e. ErbB, TNFR) has been shown to be involved in development of the cancer-causing abilities of continuous division, evasion of cell death, angiogenesis, and formation of metastases (3). Specifically, studies of ovarian, cervical, bladder and oesophageal cancers show that patients with increased expression of ErbB1 have lower survival rates than patients with normal ErbB1 expression levels (4).

Receptor dimerization and clustering is critical for the activation of signaling pathways by many growth factor receptors (e.g., ErbB1). The receptor monomers are usually incapable of signaling; it is the dimerization that leads to the phosphorylation events triggering the signaling cascades (such as the Mitogen-Activated Protein Kinase (MAPK) cascade which is activated by
In order to efficiently signal, the receptors must be in a sufficiently high local concentration on the membrane surface for dimerization to occur. The receptor population is concentrated into small regions in the plasma membrane (5). The clustering of receptors thus leads to signal amplification because they are close enough to dimerize and share ligand molecules (6). Due to the importance of receptor interactions in the plasma membrane, understanding the spatial-temporal dynamics of receptor diffusion is critical.

Widely accepted for over thirty years, the fluid mosaic model of the plasma membrane describes the phospholipid bilayer in which globular proteins are suspended and can diffuse freely within the plane of the membrane surface (7). Experimental data, however, yields two observations that are inconsistent with the fluid mosaic model. Diffusion coefficients for proteins in artificial membranes are higher than those in a natural membrane by a factor between 5 and 50 (8). Also, the diffusion rates of receptor dimers are significantly lower than those of receptor monomers (8), even though doubling particle size should have only a small effect on diffusivity (9). Insights into these discrepancies have been provided by recent single particle tracking experiments with 25 µs resolution that have revealed that diffusion does not follow a Brownian motion as earlier experiments with 33 ms resolution indicated (10; 11). Receptors and other membrane protein molecules are trapped within and occasionally hop between compartments (hereafter also termed corrals), which are separated by barriers (hereafter also called fences) (8; 10-17). These compartments range in size from 30 to 230 nm, and the average residence time of a molecule within a compartment ranges from 1 to 17 ms, depending on the cell type (11). It is hypothesized that membrane corrals are formed by interactions between membrane molecules and the cytoskeleton. Fences dividing the corrals are created either by steric hindrance due to the closeness of the actin cytoskeleton to the membrane (membrane-skeleton fence model) (14) or by membrane proteins bound to the cytoskeleton between which diffusing particles must pass in order to diffuse to an adjacent corral (protein picket model) (8). These fences and corrals are possibly the mechanism by which receptors are localized to specific areas of the plasma membrane, and an understanding of how these fences work could aid in designing new cancer therapies.

Due to the importance of the problem, several stochastic Monte Carlo (MC) simulations have been performed to address various aspects of diffusion on corralled plasma membranes. These simulations revealed that diffusion consisting of infrequent inter-compartmental hops can appear as slow Brownian motion if results are analyzed at a low data collection rate (10; 18; 19). Despite this knowledge, a theoretical framework for predicting the diffusion of receptors on a corralled plasma membrane is currently lacking. Several questions still remain unanswered about the meaning of the experimental results. Are the microscopic (or macroscopic) diffusivities of particles on the membrane actually measured by single particle techniques? Is the lattice model used in simulations an accurate representation of the plasma membrane? Can these membrane barriers lead to clustering of receptors? Understanding what factors control the macroscopic diffusivity on corralled membrane surfaces could lead to a more comprehensive analysis of the data.

In this work, we recreate the simulation results of Ritchie et al. (10), and derive an analytical expression for the diffusivity of particles on a corralled surface. We investigate the effect of the lattice constant on the diffusivity both in the simulation and in the derived expression to attempt to find an understanding of how the fences behave. Finally, the theoretical formula is applied to diffusion data from single particle tracking experiments to compare the membrane fences from various cell types.
METHODS

Kinetic Monte Carlo (KMC) Simulation

For the majority of the simulations reported here, the plasma membrane was modeled as a square lattice with a lattice constant, $a$, which was chosen to be 6 nm, following the work of Ritchie et al. (10). The suitability of a lattice model is assessed in Appendix A. Only a small portion of the plasma membrane was modeled, and periodic boundary conditions were used to represent the entire membrane. On a square homogeneous lattice, the diffusivity can be calculated from the following equation (10; 20):

$$ D = a^2 \Gamma_d (1 - \Theta), $$

where $\theta$ is the coverage (fraction of sites occupied by receptors), $a$ is the lattice constant (lattice site-to-site distance) and $\Gamma_d$ is the propensity or transition probability per unit time for diffusion of a receptor in one direction. The propensity is equal to the inverse of the average time step for a given event for a single particle. For the parameters used here ($D=9$ nm$^2$/µs; $a=6$ nm (10)), $\Gamma_d = 0.25$ µs$^{-1}$. The coverage was chosen to be equal to 0.01 for the lattice constant of 6 nm, and the density of receptors was kept constant for simulations with varying lattice constant.

To simulate systems with barriers at regularly spaced intervals, a fence was added surrounding a simulated space representing a corral. Diffusion across the periodic boundary was given a lower propensity of occurrence than normal diffusion. The ratio of propensities for diffusion within a corral ($\Gamma_d$) and across a boundary ($\Gamma_f$) is termed the fence barrier, $R_b$:

$$ R_b = \frac{\Gamma_d}{\Gamma_f}. $$

A large fence barrier (e.g., $10^9$) indicates a strong fence, where receptors very rarely escape from their initial corral, whereas a smaller fence barrier (e.g., $10^1$) indicates a weak fence. At the limit of $\Gamma_d/\Gamma_f=1$, there is no fence (the mesh is completely homogeneous). For most simulations, a fence barrier value of $10^3$ was used, corresponding to the hop probability of 0.0008 used by Ritchie et al. (10).

The propensities for each event must be normalized to a $\Gamma_{\text{max}}$ value, such that the total probability of an event occurring for any given receptor at any given site is less than or equal to one. In the case of only diffusion, $\Gamma_{\text{max}} = 4 \times \Gamma_d$. In this algorithm, the probability of a specific event ($P_j$) is equal to:

$$ P_j = \frac{\Gamma_j}{\Gamma_{\text{max}}}, \Gamma_j = \begin{cases} \Gamma_d, & \text{non-crossing a fence} \\ \Gamma_f, & \text{crossing a fence} \end{cases}. $$

We use a modified null-event algorithm for the KMC simulations (21; 22) which is briefly described next. For each iteration, a receptor is selected at random. A random number (from a uniform distribution) between zero and one is used to select an event, in this case a direction for diffusion. If the random number is between 0 and $P_1 = \Gamma_1/\Gamma_{\text{max}}$, then direction 1 is selected; if the random number falls between $P_1 = \Gamma_1/\Gamma_{\text{max}}$ and $P_1 + P_2 = (\Gamma_1 + \Gamma_2)/\Gamma_{\text{max}}$, direction 2 is selected; and so on for all four possible directions for diffusion. In this way, the probability of a receptor diffusing in a given direction is weighted by the propensity of the given diffusive event. If the site adjacent to the selected receptor in the randomly chosen direction is empty, the receptor is moved and the clock incremented by an average time step of (the actual time increment is given by an exponential distribution):
\[
\Delta t = \frac{1}{\Gamma_{\text{total}}} ,
\]

(4)

where,

\[
\Gamma_{\text{total}} = \sum_{i=1}^{n} \sum_{j=1}^{4} \Gamma_{j,i} (1 - \Theta_{j,i}) .
\]

(5)

For our simulations, \(\Gamma_{j,i}\) is either \(\Gamma_d\) or \(\Gamma_t\) depending on whether or not receptor \(j\) would cross a boundary by diffusing in the \(i^{th}\) direction, \(n\) is the number of receptors, and \(\theta_{j,i}\) is the occupancy of the \(i^{th}\) neighbor site of receptor \(j\) (\(\theta_{j,i} = 0\) if empty, \(\theta_{j,i} = 1\) if full). If the adjacent site is full or the random number is greater than the total of all the probability values, the event ends with no movement of receptors and the clock is not incremented (i.e., a null-event occurs). After the execution, or not, of an event, a new receptor is selected, and the process repeats.

**Diffusivity Calculations**

Locations of all receptors were recorded at regular intervals chosen as the resolution of the simulation, similar to time resolution of experimental data (8; 10; 11; 17). From these positions, the mean squared displacement (MSD) was calculated and averaged over all particles and starting times. The diffusivity is given by the following equation (23):

\[
D = \frac{1}{4} \lim_{t \to \infty} \frac{\langle x^2 \rangle}{t} .
\]

(6)

The diffusivity was calculated by fitting the 2\(^{nd}\) through 4\(^{th}\) points on the MSD vs. \(t\) plot to a straight line, similar to the \(D_{2.4}\) described by Kusumi, et al. (24). In the time scale of long simulations, the slope reached a constant value, which was assumed to be equal to the infinite time limit of MSD/\(t\). Therefore, the long time scale (macroscopic) diffusivity was calculated by dividing the slope by 4.

**EFFECT OF BARRIERS ON DIFFUSIVE BEHAVIOR OF MEMBRANE RECEPTORS**

Simulations of 100 receptors (1\% coverage) diffusing within a 600 nm \(\times\) 600 nm corral with a lattice constant of 6 nm, were run for fence barriers \((R_b)\) between \(10^9\) and 1. The MSD for these simulations are shown in Figure 4. In the absence of a fence, the MSD varies linearly with time. An increase in \(R_b\) causes the MSD to decrease from this linear limit. In the limit of a zero probability of crossing a boundary \((R_b \to \infty)\), the MSD reaches a maximum value and remains constant thereafter, making the diffusivity equal to zero.

At short times, the diffusivity is relatively unaffected by the presence of the barriers, as shown near the origin of Figure 4. On the other hand, the barriers decrease the long time diffusivity by several orders of magnitude. Figure 5 shows the trends in both the short and long time diffusivity calculated from the slopes in Figure 4. The short time diffusivity changes only slightly with changes in the fence barrier, while the long time diffusivity decreases as the fence barrier increases. At short times, the receptors diffuse within the corral, and do not interact with the barriers. As a result, the short-time diffusivity is close to the microscopic value of 9 nm\(^2\)/\(\mu\)s. At an \(R_b\) value of \(10^3\), the long time diffusivity is 0.80 nm\(^2\)/\(\mu\)s, a factor of approximately 11 lower than the microscopic value. This ratio falls within the experimentally determined range of 5-50 for the difference between diffusivities on natural and artificial membranes (8). At very large values of \(R_b\), the diffusivity is very low, and there are too few intercompartment hops in the simulation time used to accurately calculate the exact value. As a result, it appears that the diffusivity plateaus for \(R_b>10^9\), but as discussed later, this results from poor sampling.
The KMC simulations of corralled diffusion recreate the appearance of Brownian motion at low frame rates, as was found experimentally (8; 10; 11; 17), even though the diffusion is not Brownian. Figure 6a shows the trajectory results from a simulation of a single receptor particle with a time resolution of 33 ms (corresponding to a video rate experimental resolution) over a course of approximately one second; the motion of the particle appears Brownian. Figure 6b shows the results of the same simulation with a resolution of 25 µs. The hop-diffusion behavior of the receptor is evident from the boxes (representing corrals) created by the trajectory.

As evident from the fact that not every “box” in Figure 6 is full, the particle does not visit every site within a corral before moving to another corral, making it difficult to determine the exact size and shape of the corrals. This is also true in experimental systems (17). However, there is a relationship between the number of sites visited in a corral and the residence time (18). Because of these complications, the area visited by a receptor in between intercompartment jumps must be assumed to be a lower limit on the size of a corral, not the actual size of the corral.

**EFFECT OF RECORDING RESOLUTION ON ESTIMATED DIFFUSIVITY**

Current experimental techniques limit the time resolution of single particle tracking to 25 µs. Murase et al. measured a diffusivity of ~5 nm²/µs for particles diffusing within 200 nm corrals and estimated the diffusivity in liposomes to be ~10 nm²/µs (11). Therefore, even at a 25 µs resolution, the measured short-term diffusivity appears to be approximately half of the true value. This introduces the question of what the measured short and long time diffusivities really mean and whether, as experimental techniques improve, the true microscopic diffusivity of particles on a corralled membrane surface can ever be experimentally measured.

Simulations at varying time scales were carried out to estimate the potential capabilities of higher resolution techniques. The diffusivity at a given resolution was calculated from the slope of MSD at the 2nd through 4th time points, as done by Murase et al. (11). Figure 7 shows the calculated diffusivities from these simulations for three different corral sizes.

All three curves exhibit the interesting characteristic of an asymptotic limit at each end. At very short time scales, the measured diffusivity value is relatively constant and of the same order of magnitude as the microscopic diffusivity because the receptors are unaffected by the presence of fences at these short time scales. In the transition region, the receptors are moderately affected by the fences. The “bouncing” of receptors back into their initial corrals decreases the rate of displacement from their initial positions. At long time scales, the receptors interact with the fences multiple times and the effective motion appears as a hop mechanism with a macroscopic diffusivity.

As expected, results from simulations taken at low resolution yield calculated diffusivities between 0.070 and 0.78 nm²/µs (depending on the corral size), 1 or 2 orders of magnitude lower than the microscopic diffusivity of 9 nm²/µs (see Table 1 for calculated diffusivity values). At these slow video rates, the diffusivity measured corresponds roughly to the macroscopic (infinite time) value (this is not exactly the case for large corrals as Figure 7 indicates). At the fast resolution rate of 25 µs, calculated diffusivities are between 0.46 and 7.1 nm²/µs, on the same order of magnitude as the microscopic self-diffusivity, supporting experimental evidence mentioned above. Interestingly, as the time resolution is improved, the diffusivity approaches an asymptotic limit less than the value of the microscopic diffusivity (see inset of Figure 7). Therefore, no matter how much the data collection rate for single particle
tracking improves, the measured diffusivity will be slightly less than the actual microscopic diffusivity. The reason for this is addressed below.

The short-time scale limit is closer to the microscopic diffusivity for larger corrals than smaller corrals. This is expected because as corrals become smaller, receptor movements more frequently sample the corral boundaries. Figure 8 shows the calculated diffusivity from simulation resolutions of 0.1 and 25 µs and varying corral sizes. As corrals become large, the diffusivity approaches the microscopic diffusivity of 9 nm²/µs. The agreement between simulation and experimental data is fairly good.

AN ANALYTICAL EXPRESSION FOR THE MACROSCOPIC DIFFUSIVITY

In the simulations, there is a three order of magnitude difference between $\Gamma_d$ and $\Gamma_f$ for $R_b = 10^3$ (10). Thus, for every inter-compartmental hop, there are many events where a receptor is held in its corral by the boundary and even more events where a receptor merely diffuses from one mesh site to another within the corral. Therefore, most of the computational time is spent on fast events, and only a few slow events (hops over the fences) actually occur. As a result, it takes several hours to obtain results for diffusion of a single receptor over a time period of ten seconds. It is clear that in order to efficiently simulate these systems, i.e., to treat the separation of time scales, coarse-graining is necessary. Coarse graining will also be needed for incorporating more complexity, such as reactions, into the model and simulating an area of the cell surface larger than a few corrals. Therefore, a coarse-grained propensity must be derived which yields the same diffusivity results as the microscopic lattice simulations. An analytically derived expression for the coarse-grained diffusivity will also enable easy extraction of information from experimental data and a better understanding of the dependence of diffusivity on parameters, such as the corral size.

In our simulations, a coarse-grained lattice site was defined as the collection of all $(q^2)$ microscopic lattice sites within a single corral. Several methods of coarse-graining on a two dimensional lattice exist. A probability-weighted Monte Carlo (22) determines the probability of leaving a coarse site by adding the propensities of all possible events, and finding the fraction of events that lead to a coarse-grained event. The coarse-grained diffusion propensity can also be calculated by an equation derived by Chatterjee (20) using non-equilibrium statistical mechanics coarse-graining theory for a uniformly coarse-grained lattice. Another method is to treat the boundary as a partially permeable membrane (25). While all of these methods lead to a reasonable expression for the macroscopic diffusivity in some suitable limit, the equation derived using non-equilibrium statistical mechanics coarse-graining theory (20) gave the closest match to simulation results, and will be discussed here.

The transition probability for diffusion along the x-axis for a uniformly coarse-grained lattice from region (corral) $k$ to region (corral) $l$ is (20):

$$\Gamma_{\text{coarse}}(k \rightarrow l) = \frac{\Gamma_x}{2q^2}e^{-\beta \eta_k(1 - \Theta_l)},$$

where $\eta_k$ equals the number of receptors in region $k$, $q$ is the number of microscopic sites along one side of a corral, $\Gamma_x$ is the propensity for diffusion from one microscopic site to a neighboring site, and $\Theta_l$ equals the coverage within region $l$, defined as the number of receptors in the region divided by the number of microscopic lattice sites. The exponential term is an energetic term representing the activation energy required for a particle to move between adjacent energy minima, and is ignored because the plasma membrane does not have energy barriers between
lattice sites. Assuming a constant coverage from one corral to another (in the equilibrium limit), the expression simplifies to:

$$\Gamma_{\text{coarse}} = \frac{N\Gamma}{2q^2}(1-\Theta) = \frac{N}{2}\frac{1}{\tau}(1-\Theta),$$  \hspace{1cm} (8)

where $N$ is the number of receptors in a single corral. The time scale for a single receptor to diffuse from one coarse lattice site to another depends on the coarseness and the transition probability as follows:

$$\tau \propto \frac{q^2}{\Gamma_{x}}.$$  \hspace{1cm} (9)

This expression applies to a uniform surface with no corrals or fences. To move from the center of one coarse region to the center of another, a receptor must make take $q$ steps in one direction. In a system with a fence at every coarse grain boundary, $(q-1)$ of these steps are within a corral, and one is a jump across a barrier. This must be incorporated into an expression for $\tau$ as a function of $q$, $\Gamma_i$, and $\Gamma_d$ in such a way that the original expression is retained in the limit of $\Gamma_f=\Gamma_d$.

The time scale is split into two components: one component corresponding to diffusion within a corral and the other component corresponding to diffusion across the boundary from one corral to another. To accomplish this, one factor of $q$ (representing the size of the corral in the direction of the fence hop) is separated into $(q-1)$ and 1, while the other factor of $q$ (representing the size of the corral in the perpendicular direction) is left alone. The following expression meets the aforementioned requirements:

$$\tau \propto \frac{q(q-1)}{\Gamma_d} + \frac{q}{\Gamma_f} = \frac{\Gamma_f q(q-1)+\Gamma_d q}{\Gamma_d \Gamma_f}.$$  \hspace{1cm} (10)

Note that in the limit of $\Gamma_f=\Gamma_d$, the original expression for the time scale of diffusion on a uniform coarse-grained surface is recovered. Using this expression, the transition probability for diffusion from one corral to another is calculated from Eq. 8:

$$\Gamma_{\text{coarse}} = \frac{\frac{1}{2}\Gamma_d \Gamma_f}{\Gamma_f q(q-1)+\Gamma_d q} \times N(1-\Theta).$$  \hspace{1cm} (11)

The general equation for the diffusivity on a lattice is:

$$D = (aq)^2 \Gamma(1-\Theta).$$  \hspace{1cm} (12)

Inserting the expression for $\Gamma_{\text{coarse}}$ into Eq. 12 yields:

$$D_{\text{coarse}} = \frac{a^2 q\Gamma_f \Gamma_d (1-\Theta)}{\Gamma_f (q-1)+\Gamma_d}.$$  \hspace{1cm} (13)

This equation can be rearranged to express $D_{\text{coarse}}$ in terms of the microscopic diffusivity instead of the microscopic transition probability. Eq. 14 is the result of such a rearrangement:

$$D_{\text{coarse}} = \frac{D_{\text{micro}} q}{(q-1)+R_b},$$  \hspace{1cm} (14)

where $D_{\text{micro}} = a^2 \Gamma_d (1-\Theta)$ is the microscopic diffusivity and $R_b$ is the fence barrier. The corralled diffusivity varies directly with the microscopic diffusivity, and in the limit of very large $q$, approaches $D_{\text{micro}}$. In other words, if the barriers are sufficiently far apart, diffusion is practically unaffected by barriers.
Figure 9 compares the values for the diffusivity calculated using Eq. 13 to values obtained using KMC simulations. The corral size \((q)\), coverage \((\Theta)\), frequency of diffusion \((\Gamma_d)\), and frequency of jumping over a fence \((\Gamma_f)\) were each varied to determine if the theoretical expression gives the correct dependency of diffusivity on each parameter. The results show an almost exact correlation between the theoretical values and the simulation values.

The derived analytical expression for the macroscopic diffusivity shows that the diffusivity varies with \(R_b^{-1}\) when \(R_b \gg q\). In the \(R_b\) range of interest, there is an order of magnitude or more difference between \(R_b\) and \(q\), so this limit applies. The solid line in Figure 5 shows the theoretical result for long time diffusivity decreasing with increasing \(R_b\). The theoretical result is indistinguishable from the simulation at small to moderate fence barriers. The deviation between simulation and theory for large values of \(R_b\) is caused by the poor statistics (infrequent jumping over barriers) of the simulation. The derived expression (Eq. 14) accurately explains the previously determined macroscopic diffusivity.

This expression holds only for large time scales (e.g., \(>10,000\) µs). This is because the coarse-graining averages together events occurring at short times scales into a lumped diffusion event. This theoretical expression therefore does not capture the short time scale microscopic and transitional diffusivities seen in Figure 7.

**EFFECT OF LATTICE CONSTANT ON DIFFUSIVITY**

The expression for coarse-grained diffusivity derived above includes the parameter \(q\), which is the number of lattice sites along the edge of a corral. This parameter depends on both the length of a corral and the length of a single lattice site \((q=L/a, \text{where } L \text{ is the corral size, and } a \text{ is the lattice constant})\). Kusumi’s group chose a lattice constant of 6 nm so that the time step for a diffusive move would be 1 µs (10). However, the effect of the lattice constant on diffusivity is unclear.

A characteristic of the simulation results is the difference between the microscopic diffusivity value and the diffusivity calculated from the short time simulation results (see inset in Figure 7). The smallest corrals contain only a few points and finite size effects can be important. For example, a lattice constant of 6 nm means that a 42 nm corral is modeled as a 7×7 square of lattice sites.

To determine whether the expression’s dependence on the lattice constant is an artifact of the derivation or an actual representation of the simulation, simulations were run varying the lattice constant from its nominal value of 6 nm down to 0.01 nm. Diffusion propensities were recalculated from Eq. 1 for these simulations. The propensity for diffusion across a corral boundary \((\Gamma_i)\) was chosen to keep \(R_b\) constant at \(10^3\). Therefore, the probability of a receptor next to a boundary jumping over rather than diffusing back into the same corral is the same for all simulations. Physically, this represents a fence with the same width as a lattice site, where the propensity of diffusing across it depends on its width. Diffusivity values calculated from these simulations are shown in Figure 10. The results from short times indicate that decreasing the lattice constant does indeed bring the diffusivity closer to the microscopic limit of 9 nm²/µs. The long time diffusivity is highly dependent on the lattice constant. The theoretical and simulated diffusivity values are given in Table 2.

Our simulation results are in good agreement with the theoretical diffusivity. As the lattice constant decreases, the diffusivity increases. In the limit of \(a \rightarrow 0 \ (q \rightarrow \infty)\), \(D_{\text{coarse}}\) approaches the microscopic diffusivity:
\[
\lim_{q \to \infty} D_{\text{coarse}} = \lim_{q \to \infty} \frac{D_{\text{micro}} q}{(q-1) + R_b} = \frac{D_{\text{micro}} q}{q} = D_{\text{micro}}. \tag{15}
\]

This is because a hop from one corral to another is \((q-1)\) hops within a corral, and 1 hop across a corral boundary. As the lattice constant decreases, \(q\) becomes large, and the time for the many hops within a corral dominates over the time required for the single boundary hop. This occurs even though a boundary hop takes orders of magnitude longer than a single hop within a corral.

The dependence of the diffusivity on the lattice constant leads to the question of whether the lattice model accurately describes the plasma membrane system. Another model for the corrals is described in Appendix B, where the propensity of crossing a fence is kept constant (a fixed time scale for diffusion across a fence) with changes in lattice constant, and \(R_b\) is allowed to change. Off-lattice simulations, described in Appendix A, yield results matching those of lattice-based simulations for properly scaled values of time steps/lattice constant. Comparisons of the fence models in the main text and Appendix B with the off-lattice model in Appendix A possibly indicate that the plasma membrane has a physically relevant length scale over which particles diffuse. Rather than a membrane protein or phospholipid being able to diffuse to any point on the surface of the membrane, the membrane consists of a lattice of likely positions. Diffusion behavior on the plasma membrane could take the form of phospholipids of finite size exchanging places with each other (26). Because the membrane is crowded with phospholipids, the phospholipids themselves form a sort of lattice structure on the surface of the membrane. While this lattice may be somewhat fluid because the phospholipids are free to diffuse, it is no less capable of defining the length of individual diffusive events. A third lattice model for diffusion simulations is described in Appendix C. In this model, the probability of a receptor at a fence boundary crossing into the next corral is proportional to the lattice constant. This method successfully models a macroscopic diffusivity that is independent of the lattice constant. However, it is unclear whether this is a biologically relevant model, or simply a mathematical trick.

**ANALYSIS OF EXPERIMENTAL DATA**

The analytical expression for the macroscopic diffusivity derived above was shown to closely describe the dynamics of the lattice-based simulation. In order to determine what insights can be gained from this analytical expression, it was applied to the experimental data obtained by Kusumi’s group. Table 3 shows experimental data for the diffusivity and average compartment size in various cell types obtained by single particle tracking techniques (11). Assuming a constant microscopic diffusivity allowed the calculation of \(\Gamma_f\). This assumption implies that the structure of the plasma membranes of different cell types is similar except for the corrals. \(\Gamma_f\) was calculated for each cell type’s diffusivity and compartment size according to Eq. 13 and are given in Table 3.

Fence barriers are all between 100 and \(10^3\), corresponding to the hop probability of 0.0008 used by Kusumi’s group (10). Since the fence barriers are all of the same order of magnitude, it is concluded that the corrals are created by the same mechanism in all the cell types tested. The differences in the cytoskeletal structure of the different cell types yields different compartment sizes due to actin filaments being closer together or farther apart; however, the fences themselves are probably similar in structure.

Given the dependence of the diffusivity on the lattice constant, the effect of the lattice on the analysis of Kusumi’s experimental data was investigated. The calculated values of \(R_b\) for three values of the lattice constant are given in Table 4. The fence ratio, \(R_b\), is seen to be
inversely proportional to the lattice constant, \( a \). Since the fence barrier is equal to the average number of times a molecule bounces off a barrier before passing through one, decreasing the lattice constant in simulations causes the receptors to hit and bounce off the barriers more often. If the fence barrier, \( R_b \), has a physically meaningful value, there must also be a physically relevant value for the lattice constant, \( a \), in order for the lattice model of the plasma membrane to be meaningful.

CONCLUSIONS

In this work, a stochastic, spatial model was developed to describe the corralled diffusion behavior of epidermal growth factor receptors on the plasma membrane. These simulations confirm the hypothesis that corrals created by the actin cytoskeleton (or membrane proteins bound to it) can rationalize the difference between diffusivities in natural cell membranes and those in artificial membranes.

A theoretical expression for the diffusivity of corralled particles on a membrane surface as a function of corral size, microscopic diffusivity and fence barrier was derived using coarse-graining principles. This expression allows the prediction of macroscopic diffusivities at various values of the parameters, and an estimation of the fence barrier from experimental data of various cell types. Since similar values for the fence barrier were calculated for all cell types tested, it can be concluded that the physical basis of these fences is probably universal. What differs between cell lines is the structure of the overall cytoskeleton within the cell, and the distance between its filaments. This leads to the conclusion that cytoskeletal design is not only important for defining the structure of the cell as a whole, but also for controlling the diffusion of molecules on its surface.

The lattice constant has a great effect on the magnitude of the macroscopic diffusivity and the fence barrier. Both on- and off-lattice (Appendix A) models with constant fence barrier, \( R_b \), and on-lattice models with constant time scale of a fence hop \( \Gamma_f^{-1} \) (Appendix B) showed a strong dependence of the macroscopic diffusivity on the lattice constant. This could indicate that the plasma membrane actually behaves as a lattice with nearly regularly spaced sites. The milling crowd model suggests that membrane particles diffuse by exchanging places with one another (26) and supports the idea of a lattice. This would mean that all diffusive moves are on the length scale of a single phospholipid molecule (approximately 0.5 nm). Though the constant crossing probability per lattice size model yields a diffusivity independent of the lattice constant, further work is required to determine whether this model is biologically relevant.

In summary, our simulations support the idea of discrete EGFR diffusive moves of a certain characteristic length scale rather than a continuum diffusion mechanism. While the exact nature of a fence is still unclear, it appears that the probability of moving across fences is \( 10^3-10^4 \) lower than moving within corrals. Further work, though, is needed to elucidate details of the diffusion and the fence structures.

Appendix A: COMPARISON OF ON-LATTICE AND OFF-LATTICE SIMULATIONS

Since lattice simulations revealed that the lattice constant affects the macroscopic diffusivity, an off-lattice simulation was used to assess whether this behavior is a peculiarity of the lattice model. An algorithm for two-dimensional diffusion on a surface with regularly spaced fences was developed based on work by Higham (27) on Brownian motion (continuous in space and discrete in time).
Continuous-Space Algorithm

Higham’s simulation method for Brownian motion uses a fixed time step for each event. Then, the displacement during that time step is normally distributed with a mean value of zero (27). The variance of the displacement is calculated from the microscopic diffusivity of the particles. Eq. 6 gives the relationship between the MSD of a particle as a function of time and its diffusivity. Rearranged, the mean square displacement is equal to:

\[ MSD = 4Dt \]  

(16)

Since diffusion is isotropic, the displacement can therefore be separated into its components as follows:

\[ MSD = \langle \Delta X^2 \rangle + \langle \Delta Y^2 \rangle = 4 \cdot D \cdot dt \]  

(17)

Since the x and y displacements have the same distribution,

\[ \langle \Delta X^2 \rangle = 2 \cdot D \cdot dt \]  

(18)

For a distribution with mean zero, the expected value of \( x^2 \) is equal to the variance of the distribution. Therefore, the standard deviation is given by:

\[ \sigma = \sqrt{2 \cdot D \cdot dt} \]  

(19)

Simplified, the position of a receptor based on its previous position in one dimension is given by:

\[ X_{n+1} = X_n + (2D \cdot dt)^{1/2} N(0,1) \]  

(20)

where \( N(0,1) \) is the normal distribution with zero mean and variance 1.

Receptors are initially placed at random positions within a single corral using a uniform random number generator. In these off-lattice simulations, receptors are not affected by the presence of other receptors. Multiple receptors are used to obtain statistics.

During each time step, each receptor is moved once. For each move, two normal random numbers are generated with mean zero and variance as described above. These values determine the displacements in the x and y directions. If one of the random values is greater than the length of a corral (occurs for less than 0.001% of events for 42 nm corrals if \( dt < 6.1 \mu s \)), the displacement in the corresponding direction is set equal to the length of a corral. The randomly generated displacements are added to the position of the receptor. If no fence is encountered in this displacement, the new coordinates of the receptors are saved, and the simulation moves to the next receptor.

When a receptor reaches a fence, the probability of jumping over it is \( P_{\text{cross}} \), and the probability for bouncing away from it is equal to \( 1-P_{\text{cross}} \). During a time step in which a particle encounters a wall, it moves a distance \( (2D \times dt)^{1/2} N(0,1) \) with \( \text{Prob.}=P_{\text{cross}} \) corresponding to moving through the fence into another corral, or it moves a distance \( X_{\text{wall}}-X_n \) towards the wall and a distance \( (2D \times dt)^{1/2} N(0,1)-(X_{\text{wall}}-X_n) \) back away from the wall with \( \text{Prob.}=1-P_{\text{cross}} \), corresponding to bouncing off the fence and back into its current corral. Displacements in the x and y directions are treated independently, so a receptor could encounter two walls in a given time step.

As in the lattice simulations, positions are recorded at pre-determined time intervals and used to calculate the MSD over the time frame of the simulation. The slope of the MSD provides the diffusivity of the receptors.
Comparison Between on- and off-Lattice Simulation Results

Off-lattice simulations of diffusion of receptors on the corralled surface were run. Values of the time step were chosen so that the results could be directly compared to those of the lattice simulations. Results can be compared under the condition that the average distance traveled in a single time step is the same. In the off-lattice simulation, the average distance traveled during the time step $dt$ is equal to:

$$
\sqrt{(2 \cdot D \cdot dt)^2 + (2 \cdot D \cdot dt)^2} = \sqrt{4 \cdot D \cdot dt} = 2\sqrt{D \cdot dt}
$$

In the on-lattice simulations, the distance traveled by a receptor in a single event is equal to $a$. Therefore, simulations where $a = 2\sqrt{D \cdot dt}$ can be compared to each other. This correlation can also be calculated from the equation for diffusivity on a lattice, $D = a^2 \Gamma_d (1 - \Theta)$. If the coverage term is ignored (valid since the coverage is $\sim 0.01$), solving for the lattice constant gives:

$$
a = \sqrt{D / \Gamma_d}
$$

(21)

In this case, $\Gamma_d$ is equal to the inverse of the time scale for diffusion over a distance $a$ in each of the four possible directions. Therefore, the time scale for diffusion in a single direction is equal to $(4\Gamma_d)^{-1}$. So, the lattice constant and time scale for a single event are related by the following expression:

$$
a = \sqrt{D / \Gamma_d} = \sqrt{D / (4dt)^{-1}} = \sqrt{4D \cdot dt} = 2\sqrt{D \cdot dt}
$$

(22)

This is the same expression derived above for the average step size for the off-lattice simulations.

In the lattice simulations, the fence barrier was used to define the probability of a receptor crossing a fence rather than remaining in its current corral. If a receptor is at a site next to a fence, the probability that the receptor leaves the corral and crosses the boundary is equal to $\Gamma_f / (\Gamma_d + \Gamma_f)$, and the probability that the receptor remains in the same corral is equal to $\Gamma_d / (\Gamma_d + \Gamma_f)$. Since $\Gamma_f$ is 2-3 orders of magnitude less than $\Gamma_d$, the probability of a receptor at a boundary crossing the fence can be simplified to $P_{\text{cross}} = \Gamma_f / \Gamma_d = R^{-1}_b$.

In the off-lattice simulations, the probability of a receptor that encounters a fence crossing the fence is equal to $P_{\text{cross}}$, an input to the simulation. On- and off-lattice simulations can represent the same strength of the fence if the parameters are such that $P_{\text{cross}}$ (off-lattice probability) is equal to $R^{-1}_b$ (on-lattice fence barrier).

The diffusivities calculated from the off-lattice simulations are plotted together with the previously given results from lattice simulations in Figure 11. All of these results are for a corral size of 42 nm and a fence barrier of $10^3$. The off-lattice results match those of the lattice simulations for corresponding $a$ and $dt$ values. Also, decreasing the time step for a single event, just like decreasing the lattice constant, increases the value for the macroscopic diffusivity.

Decreasing the time step for Brownian dynamics corresponds to decreasing the lattice constant for lattice random walk. Again, the number of steps needed to reach the fence increases, but the probability, $P_{\text{cross}}$, of jumping over the wall is kept constant. Since the time to move from one fence to the next remains constant, whereas $dt$ for jumping across a fence decreases, the effect of fences on diffusivity also decreases. These simulation results show that discretizing in time rather than space does not change the effect of the step size on the calculated diffusivity.
Appendix B: AN ALTERNATIVE FENCE MODEL OF CONSTANT HOPPING TIME

In the above simulations, it has been assumed that the fence barrier remains constant. Another possibility is that the time scale for a fence hop is constant and independent of diffusion events within the corral. In this case, there would be an inherent length scale of the fence (its width), and the probability of crossing a barrier should depend on the lattice constant.

In order to implement this different concept of the time scale for diffusing across the fence, the propensity for diffusing within a corral is changed according to the lattice constant while the propensity for diffusing across a boundary is kept constant.

Given that $q = L/a$, $R_b = \Gamma_d/\Gamma_f$, and $\Gamma_d = D_{\text{micro}}/a^2$, Eq. 14 can be rewritten as:

$$D_{\text{coarse}} = \frac{D_{\text{micro}} La \Gamma_f}{(L-a) a \Gamma_f + D_{\text{micro}}}$$

(24)

In the limit of $a \to 0$, the numerator of this expression approaches zero while the denominator approaches $D_{\text{micro}}$. Therefore, the coarse-grained diffusivity approaches the microscopic diffusivity for constant $R_b$ or zero for constant $\Gamma_f$.

Lattice-based simulations were carried out with varying lattice constants and a constant value of $\Gamma_f$. Parameter values were chosen such that the simulations with a lattice constant 6 nm are the same as those described earlier. The results from lattice-based simulations, where the propensity for crossing a barrier is held constant while changing the lattice constant, are shown in Figure 12.

As in other simulations, the diffusivity at short time scales is close to the value of the microscopic diffusivity of 9 nm$^2$/µs. The diffusivity approaches a macroscopic value asymptotically at long time scales. Unlike the results for constant $R_b$, as the lattice constant is decreased, the macroscopic diffusivity decreases and approaches zero. The simulation results shown in Figure 12 indicate that the derived expression for macroscopic diffusivity is accurate in its description of the system’s behavior at limiting values of the lattice constant. The results from this alternate model together with the model presented in the paper indicate that a characteristic length scale should exist in these systems or possibly another physical model for fences is needed.

Appendix C: AN ALTERNATIVE FENCE MODEL OF CONSTANT CROSSING PROBABILITY PER LATTICE SIZE

In the previous sections, two models of hopping were studied. The first model included a constant fence barrier model, which gave a diffusivity approaching the microscopic diffusivity as the lattice constant became small. The second model employed a constant propensity for fence hopping, which gave a diffusivity approaching zero as the lattice constant became small. In this appendix, we consider an alternative model in which, a variable $\alpha$ equal to:

$$\alpha = \frac{P_{\text{cross}}}{a}$$

(25)

is held constant. Here $P_{\text{cross}}$ is the probability of a receptor at a fence boundary crossing into the next corral and $a$ is the lattice constant. By definition, one has

$$P_{\text{cross}} = \frac{\Gamma_f}{\Gamma_f + \Gamma_d}.$$  

(26)
A constant value of $\alpha$ means that the probability of a receptor crossing a fence into another corral is proportional to the size of the microscopic lattice sites. This scaling makes sense because the time between microscopic moves decreases with the lattice constant. The specifics of the scaling are herein chosen so that one gets lattice constant independence in the limit where the lattice constant tends to zero. So, the smaller the distance between lattice sites, the more times a receptor will alternate between being next to the fence and being one lattice site away.

**Comparison to Other Models**

In the constant fence barrier model, $R_b (\Gamma_d/\Gamma_f)$ is held constant. At the $R_b$ value of interest ($10^3$ for $a = 6$ nm), one has:

$$P_{\text{cross}} = \frac{\Gamma_f}{\Gamma_f + \Gamma_d} \approx \frac{\Gamma_f}{\Gamma_d} = \frac{1}{R_b}$$

(27)

So, in our previous model, holding $R_b$ constant is the same as holding $P_{\text{cross}}$ constant. In the new model, $\alpha$ is inversely proportional to the lattice constant. This implies that as the lattice constant becomes small, $P_{\text{cross}}$ is disproportionately large.

In the case of the constant fence hop propensity model, $\Gamma_f$ is held constant. The variable $\alpha$ can be estimated from:

$$\alpha = \frac{P_{\text{cross}}}{a} = \frac{1}{a} \cdot \frac{\Gamma_f}{\Gamma_f + \Gamma_d} \approx \frac{1}{a} \cdot \frac{\Gamma_f}{\Gamma_d} = \frac{1}{a} \cdot \frac{\Gamma_f}{a D_{\text{micro}}/a^2} \cdot \frac{D_{\text{micro}}}{\Gamma_f}$$

(28)

Since $D_{\text{micro}}$ is constant and $\Gamma_f$ is being held constant, $\alpha$ is proportional to the lattice constant. Therefore, $\alpha$ decreases as the lattice constant decreases, and the diffusivity would be expected to decrease to zero as the lattice size becomes infinitely small, in agreement with our simulations.

**Parameter Selection**

The starting point for these simulations was a system with a lattice constant of 6 nm, a corral size of 42 nm, a microscopic diffusivity of 9 nm$^2$/µs, and a fence barrier of $10^3$. This leads to a $\Gamma_d$ of 0.25 µs$^{-1}$ and a $\Gamma_f$ of 0.00025 µs$^{-1}$. The value of $\alpha$ calculated from these parameters is 0.000167 nm$^{-1}$ and is hereafter used so mapping with the other models is possible.

For each value of the lattice constant $a$, $\Gamma_d$ and $\Gamma_f$ are recalculated. The value of $\Gamma_d$ is defined from the microscopic diffusivity and the lattice constant:

$$\Gamma_d = \frac{D_{\text{micro}}}{4a^2}$$

(29)

$\Gamma_f$ is then calculated using Eqs. 25 and 26:

$$\Gamma_f = \frac{\Gamma_f a a}{1 - \alpha a}$$

(30)

This expression was used to select the value of $\Gamma_f$ for the simulations described below. Note that as the lattice constant changes, $\Gamma_d$ and $\Gamma_f$ vary according to Eqs. 29 and 30, respectively. The fence barrier in this case decreases with an increasing lattice constant as shown in Figure 13. However, for the changes in the lattice constant considered here, the fence barrier is within the range considered in the rest of the paper.

**Results**

In order to assess whether this model actually yields diffusivity results independent of the lattice constant, simulations were done at decreasing lattice constants with 42 nm corrals and a
simulation space being a square of 100 by 100 corrals with a total of 4900 receptors. This is equivalent to 1% coverage in the 6 nm lattice constant system. Diffusivities given are averaged over 100 runs.

Simulations were first run for a total time of 0.1 µs, and a data collection resolution of 0.01 µs. The results are shown in Figure 14. A series of t-test analyses indicate that the diffusivity at each lattice constant is significantly different (P-value < 0.01). As the lattice constant decreases, the calculated diffusivity increases toward the microscopic value.

Similar simulations were run with a total time of 250 µs and a 25 µs resolution, equivalent to the resolution of fast rate single-particle tracking techniques. These results are shown in Figure 15. At this time resolution, there is not a statistically significant difference between the diffusivities for lattice constants between 0.6 nm and 3 nm (a t-test yields P-values greater than 0.1).

From these results, it is concluded that deviations from the microscopic diffusivity in extremely short-time simulations are a consequence of finite size effects (large lattice constant with respect to the corral size). For longer time intervals, the diffusivity is fairly unaffected from the lattice constant. Mathematically, this new model can give a coarse diffusivity that is lattice constant independent as one passes to the continuum limit. It indicates that the boundary condition crossing the fence is an important issue that needs further work; for example, the model’s relevance to biology is not fully clear.

**Coarse Diffusivity**

These results are compared to the previously derived expression for the coarse diffusivity on a corralled surface. Inserting the expression for the propensity for a receptor to jump across a fence in terms of \( \Gamma_d \), \( \alpha \), and \( a \), the expression for the diffusivity is equal to:

\[
D_{\text{coarse}} = \frac{D_{\text{micro}}L\alpha}{L\alpha - a\alpha + 1 - a\alpha} = \frac{D_{\text{micro}}L\alpha}{L\alpha - 2a\alpha + 1}
\]  

(31)

In this expression, the lattice constant appears only in the denominator. In the limit of \( a<<L \), the dependence of diffusivity on the lattice constant becomes negligible. This relation also explains the results in Figure 15 that the diffusivity with a lattice constant of 6 nm is slightly higher than the other diffusivities (with a lattice constant of 6 nm, and a corral size of 42 nm, the lattice constant is less than an order of magnitude smaller than the corral, and the limit of \( a<<L \) does not apply).

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FIGURE CAPTIONS

Figure 4: Mean square displacement curves for simulations of receptors diffusing on a membrane with a lattice of 600 nm × 600 nm corrals and fence barriers ranging from 1 to 10^9. Open circles: R_b=1 (no fence); Open Squares: R_b=10; Open Diamonds: R_b=100; Closed Circles: R_b=10^3; Closed Squares: R_b=10^4; other curves with higher R_b’s are indistinguishable from each other on this plot. Increasing the fence ratio (R_b) decreases the MSD at moderate to long times.

Figure 5: Long and short time diffusivities for simulations at various fence barrier values and a corral size of 600 nm × 600 nm. Short time diffusivities are calculated at the first ~10^3 µs, and long time diffusivities are calculated from simulation data collected between 2×10^4 and 1×10^5 µs. The theoretical diffusivity is obtained from Eq. 13. The deviation between the long time diffusivity and the theoretical diffusivity is due to poor statistics of the KMC for large fence barriers.

Figure 6: Single particle trajectories from simulations with a resolution of (a) 33 ms and (b) 25 µs over a period of 1 second. Corral size = 240 nm × 240 nm and a barrier height of R_b = 10^3. The diffusion in (a) appears Brownian, while (b) reveals the corralled behavior.

Figure 7: Initial calculated diffusivity from points 2-4 taken at interval indicated. Average is over 10 simulations of 10,000 corrals with 1% coverage of receptors and a fence barrier value of R_b = 10^3, D_{micro} = 9 nm^2/µs. Open symbols and lines are diffusivities calculated from simulation results. Circles are from 42 nm corrals; squares are from 120 nm corrals; and diamonds are from 240 nm corrals. Black triangles are data from (11) for the measured diffusivity of particles on FRSK cells (average compartment size = 41 nm) at resolutions of 25 µs (fast resolution) and 33 ms (video rate). The inset is a blowup (in linear scale) of the short-term diffusivity.

Figure 8: Diffusivity calculated from simulation points 2-4 at given resolution for receptors with a self-diffusivity of 9 nm^2/µs diffusing on a lattice of corrals with a fence barrier of 10^3 R_b. Diamonds (green) are experimental data from (11) measured by single particle tracking with a resolution of 25 µs.

Figure 9: Parity plot comparing diffusivities obtained by simulation and calculated theoretically by the coarse-grained method. Results are averaged over 100,000 iterations for a single corral with periodic boundary conditions. Except for parameter being varied, parameters are as follows: corral size = 240 nm × 240 nm, coverage = 0.01, Γ_d =0.25, Γ_f = 0.00025. Circles show variation in corral size (120 × 120 to 600 × 600 nm^2); squares show variation in coverage (0.01 to 0.1); diamonds show variation in Γ_d (0.0005 to 0.25); and X’s show variation in Γ_f (0.25×10^-6 to 0.25).

Figure 10: Diffusivity calculated at various time scales from simulations of receptors diffusing over 42 nm corrals with a fence barrier of R_b = 10^3 on a lattice of 0.1, 1, and 6 nm sites.

Figure 11: Diffusivity calculated from simulations of receptors diffusing on a surface with 42 nm corrals having an R_b value of 10^3. Symbols are results from lattice simulations with varying lattice constants, and the lines are the results from off-lattice simulations with different time steps. A time step of 1 µs, 0.0278 µs, and 0.000278 µs corresponds to an average step size of 6 nm, 1 nm, and 0.1 nm, respectively.

Figure 12: Diffusivities calculated from lattice-based simulations of receptors diffusing on a 42 nm corral with a constant propensity for crossing a barrier of 2.5×10^{-4} with D_{micro}=9 nm^2/µs.

Figure 1: Variation of the fence barrier (Γ_d/Γ_f) with the lattice constant. Γ_d is calculated from Eq. 29 and Γ_f is calculated from Eq. 30.

Figure 2: Diffusivity calculated at a time resolution of 0.01 µs vs. lattice constant. Error bars are standard deviations (over 100 samples of 4900 receptors).

Figure 3: Diffusivity calculated at time resolution of 25 µs vs. lattice constant. At this timescale, the diffusivity is independent of the lattice constant. Error bars are standard deviations (over 100 samples of 4900 receptors).
Table 1: Short time (0.01 and 25 µs resolution) and long time (1000 µs) resolution diffusivity values from simulations with corral sizes 42, 120, and 240 nm.

<table>
<thead>
<tr>
<th>Corral Size (nm)</th>
<th>0.01 µs resolution D (nm²/µs)</th>
<th>25 µs resolution D (nm²/µs)</th>
<th>1000 µs resolution D (nm²/µs)</th>
<th>33 ms resolution D (nm²/µs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>42</td>
<td>7.6</td>
<td>0.46</td>
<td>0.070</td>
<td>0.063</td>
</tr>
<tr>
<td>120</td>
<td>8.5</td>
<td>5.4</td>
<td>0.18</td>
<td>0.17</td>
</tr>
<tr>
<td>240</td>
<td>8.6</td>
<td>7.1</td>
<td>0.78</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Table 2: Theoretical diffusivity calculated from Eq. 13 and simulation diffusivity with a resolution of 1000 µs for a 42 nm corral length and a fence barrier of 10³.

<table>
<thead>
<tr>
<th>Lattice Constant, a (nm)</th>
<th>q=L/a (L=42nm)</th>
<th>Theoretical Diffusivity (nm²/µs)</th>
<th>Simulation Diffusivity (nm²/µs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>7</td>
<td>0.0626</td>
<td>0.0623</td>
</tr>
<tr>
<td>1</td>
<td>42</td>
<td>0.363</td>
<td>0.361</td>
</tr>
<tr>
<td>0.1</td>
<td>420</td>
<td>2.66</td>
<td>2.65</td>
</tr>
<tr>
<td>0.01</td>
<td>4200</td>
<td>7.27</td>
<td>4.99</td>
</tr>
</tbody>
</table>

Table 3: Diffusivity and compartment size data from Murase et al. (2004). A lattice constant of 6 nm and a microscopic diffusivity of 9 nm²/µs are used. Γ_f was obtained using the coarse-grained diffusivity, Eq. 13. The average fence barrier is equal to 3.57×10².

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Diffusivity (nm²/µs)</th>
<th>Compartment Size (nm)</th>
<th>Γ_f × 10⁴ (1/µs)</th>
<th>Fence Barrier, R_b</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRK</td>
<td>1.1</td>
<td>230</td>
<td>9.05</td>
<td>276</td>
</tr>
<tr>
<td>T24</td>
<td>0.17</td>
<td>110</td>
<td>2.62</td>
<td>953</td>
</tr>
<tr>
<td>HeLa</td>
<td>0.21</td>
<td>68</td>
<td>5.26</td>
<td>475</td>
</tr>
<tr>
<td>Hek293</td>
<td>0.38</td>
<td>68</td>
<td>9.69</td>
<td>258</td>
</tr>
<tr>
<td>PtK2</td>
<td>0.48</td>
<td>43</td>
<td>19.50</td>
<td>128</td>
</tr>
<tr>
<td>FRSK</td>
<td>0.19</td>
<td>41</td>
<td>7.87</td>
<td>318</td>
</tr>
<tr>
<td>HEPA-OVA</td>
<td>0.21</td>
<td>36</td>
<td>9.92</td>
<td>252</td>
</tr>
<tr>
<td>CHO-B1</td>
<td>0.24</td>
<td>32</td>
<td>12.78</td>
<td>196</td>
</tr>
</tbody>
</table>

Table 4: Values of the fence barrier (R_b) calculated from experimental data (11), using the coarse-grained expression for diffusivity, Eq. 13, for several values of the lattice constant.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>a=6 nm</th>
<th>a=1 nm</th>
<th>a=0.1 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRK</td>
<td>276</td>
<td>1650</td>
<td>16500</td>
</tr>
<tr>
<td>T24</td>
<td>953</td>
<td>5710</td>
<td>57100</td>
</tr>
<tr>
<td>HeLa</td>
<td>475</td>
<td>2850</td>
<td>28500</td>
</tr>
<tr>
<td>Hek293</td>
<td>258</td>
<td>1540</td>
<td>15400</td>
</tr>
<tr>
<td>PtK2</td>
<td>128</td>
<td>764</td>
<td>7630</td>
</tr>
<tr>
<td>FRSK</td>
<td>318</td>
<td>1900</td>
<td>19000</td>
</tr>
<tr>
<td>HEPA-OVA</td>
<td>252</td>
<td>1510</td>
<td>15100</td>
</tr>
<tr>
<td>CHO-B1</td>
<td>196</td>
<td>1170</td>
<td>11700</td>
</tr>
</tbody>
</table>
Figure 4
Short Time Diffusivity
Long Time Diffusivity
Theoretical Diffusivity

Figure 5
Figure 6
Figure 7
Figure 8
Corral Size (120 nm - 600 nm)

$\varnothing$ (0.01 - 1)

$\Gamma_d$ (0.00025 - 0.25)

$\Gamma_f$ (2.5*10^{-6} - 0.25)

$X = Y$

Figure 9
Figure 10
Figure 11
Figure 12

Diffusivity (nm²/µs)

Time Scale (µs)

- a = 6 nm
- a = 2 nm
- a = 1 nm
- a = 0.6 nm
- a = 0.1 nm
- a = 0.01 nm
Figure 14
Figure 15