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Monte Carlo simulations of receptor dynamics: Insights into cell signaling

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Summary

Many receptor-level processes involve the diffusion and reaction of receptors with other membrane-localized molecules. Monte Carlo simulation is a powerful technique that allows us to track the motions and discrete reactions of individual receptors, thus simulating receptor dynamics and the early events of signal transduction. In this paper, we discuss simulations of two receptor processes, receptor dimerization and G-protein activation. Our first set of simulations demonstrates how receptor dimerization can create clusters of receptors *via* partner switching and the relevance of this clustering for receptor cross-talk and integrin signaling. Our second set of simulations investigates the activation and desensitization of G-protein coupled receptors when either a single agonist or both an agonist and an antagonist are present. For G-protein coupled receptor systems in the presence of an agonist alone, the dissociation rate constant of agonist is predicted to affect the ratio of G-protein activation to receptor phosphorylation. Similarly, this ratio is affected by the antagonist dissociation rate constant when both agonist and antagonist are present. The relationship of simulation predictions to experimental findings and potential applications of our findings are also discussed.

Introduction

Ligand binding to cell surface receptors initiates a signal transduction cascade. Early models of signaling correlated cell responses with the number of ligand bound receptors by calculating the number of bound receptors at equilibrium, i.e.

$$\frac{R_{\text{bound}}}{R_{\text{total}}} = \frac{[L]}{K_D + [L]}, \tag{1}$$

where K_D is the equilibrium dissociation constant, $[L]$ is the concentration of free ligand, R_{total} is the total number of surface receptors, and R_{bound} is the number of ligand bound surface receptors. Other models used a function based on the quantity $R_{\text{bound}}/R_{\text{total}}$ (Stephenson 1956, Furchgott 1966, Kenakin 1993, Linderman 2000). In other words, the number of receptors bound at equilibrium is taken as the key input the cell detects. More recent studies have suggested that responses may be related to the time course of receptor binding (especially for responses that occur long before equilibrium binding is reached) and that the relationship between bound receptors and responses may be complicated by the dynamics of receptor transitions between active, desensitized, or internalized states (Hoffman *et al.* 1996, Haugh *et al.* 1998, Waller *et al.* 2004).

We believe that receptor dynamics play a key role in signal transduction. Receptor dynamics are loosely defined here as dynamic events that the receptors may participate in, including interactions with other molecules and transitions between various receptor states (e.g., active, inactive, desensitized, internalized) that evolve with time. In other words, a quantitative prediction of cell responses elicited by ligand binding may require knowledge of the timing of events including and in addition to ligand/receptor binding. In this paper, we focus particularly on quantitative models that describe receptor–receptor interactions, receptor–ligand interaction, receptor-G-protein interactions, and/or receptor–receptor kinase interactions.

We use mathematical models of receptor dynamics because the system of interactions between receptors and between receptors and other molecules is often too complicated to understand with intuition alone. These models of receptor dynamics allow us to quantitatively assess the response of the system as key physical parameters are changed. Predictions of the models give insights into signal transduction mechanisms and can be compared with results from experimental systems to suggest new areas for investigation. This iterative process using models and experiments together can help enhance our understanding of cell signaling.

Receptor dynamics can be described with several different mathematical techniques (Lauffenburger & Linderman 1993, Kholodenko *et al.* 2000, Woolf & Linderman 2000, Woolf *et al.* 2001, Haugh 2002, Resat *et al.* 2003). Two common methods are ordinary differential equations (Hoffman *et al.* 1996, Bhalla & Iyengar 1999, Riccobene *et al.* 1999, Faeder *et al.* 2003) and Monte Carlo simulations (Shea *et al.* 1997, Irvine *et al.* 2002, Saxton 2002, Shimizu *et al.* 2003, Woolf & Linderman 2003a). For example, ordinary differential equations can be written to describe the change in concentrations of various molecular species as a function of time or position. However, in this mathematical framework individual members of a species are indistinguishable, which does not allow them to adequately simulate discrete processes. In contrast, the Monte Carlo techniques we describe here can be used to track the spatial location of individual molecules as a function of time.

Monte Carlo methods for tracking receptor diffusion and reaction

Monte Carlo is the name for the technique of solving mathematical problems with random events (Fishman 1996). A random event has more than one possible outcome, while a certain event has only one possible outcome. An example of a random process is diffusion. A molecule will travel in a random direction that cannot be predicted in advance.

Cell surface receptors diffuse in the two dimensional plane of the membrane with a diffusivity of 10^{-11}–10^{-9} cm^2/s (Gennis 1989). Because interactions between molecules on the cell surface (between receptors or between receptors and other membrane molecules) presumably require first the diffusion of the molecules to adjacent locations, our models will require a description of diffusion. The Monte Carlo implementation of diffusion is shown schematically in Figure 1. A single molecule is allowed to move in a random direction; the molecule moves a distance determined by the diffusivity and simulation time step (Figure 1a). This process of picking directions and moving repeats many times for many different molecules and results in a sample of possible paths the molecules could take while diffusing in the cell membrane (Figure 1b). The simulation collects the positions of molecules for many different possible paths and gathers statistics on the movement of molecules.

Monte Carlo models can also simulate reactions between membrane molecules (Figure 1c). Monte Carlo models are particularly useful for modeling reactions in which diffusion plays an important role in determining the overall rate of reaction (termed "diffusion-controlled" or "partially diffusion-controlled" reactions) because of the ability to track the spatial location of molecules. Monte Carlo models are also useful for modeling events in which the stochastic (probabilistic) nature of reactions has an impact on the outcome. In other words, when the number of reaction events per time is small, the events must be modeled discretely. Many conditions can cause the number of reaction events to be small; two examples are when the concentrations of reactants are small (e.g., G-protein activation and receptor phosphorylation) and when the reaction between molecules lasts for relatively long periods of time, thus blocking the binding sites from interacting with other molecules (e.g., receptor dimerization). Stochastic reactions can only be adequately simulated with discrete methods (Sander 2000). The four key reactions of interest in this paper – receptor dimerization, receptor binding to immobilized ligand, receptor activation of G-proteins, and receptor phosphorylation – contain stochastic elements (Shea *et al.* 1997, Woolf & Linderman 2003) and it is likely that they are diffusion-controlled or partially diffusion-controlled (Lauffenburger & Linderman 1993, Mahama & Linderman 1994, Broday 2000). In fact, our interest in accurately describing these discrete, diffusion-controlled reactions contributed greatly to our decision to use the simulation techniques described here.

We note that reactions between membrane receptors and molecules in solution also occur and could be

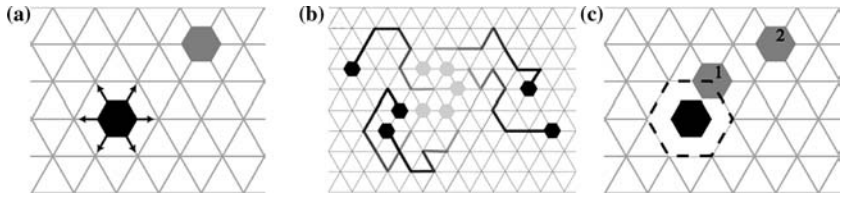

Figure 1. Monte Carlo simulation of molecules diffusing and reacting on a two dimensional surface. (a) Molecules are randomly placed on a lattice. Then one molecule is randomly chosen and a direction for movement is randomly chosen, with each direction given an equal probability of being chosen. The molecule moves to the new location unless it is occupied. (b) After a molecule has moved many times, one path that the molecule could take will be described. One possible outcome for a system with five molecules is shown. The simulation result is an aggregate of many possible paths. (c) Reactions between two molecules are allowed if the chosen molecule is close enough to a molecule of the appropriate species. Here, black and gray molecules can react and molecules have a non-zero probability of reacting when they are within an interaction radius (black dotted line), typically equal to the radius of a molecule. For example, the black molecule can react with molecule 1, but not molecule 2.

described by Monte Carlo or other probabilistic techniques (Franks *et al*. 2002, Lee *et al*. 2003). However, in most cases the rates of these reactions are expected to be dominated by intrinsic reaction kinetics and not diffusion (Lauffenburger & Linderman 1993) and thus there is less need to track the motions of individual molecules; simply using the measured association and dissociation rate constants should be sufficient.

Monte Carlo simulations can be more intuitive to understand than other modeling methods. As the diffusion example illustrates, Monte Carlo simulations mimic events at the molecular level by tracking each molecule's position and state as a function of time. The simulation copies the processes that occur *in vivo* according to the probability of the event, which is based on experimental observations. The inputs and model parameters are molecular properties such as the diffusivity and binding rate constants and other quantities such as the number of molecules of a particular species, quantities that have been measured (or presumably could be) and have a direct connection to the physical system. Simulation outputs can include the fraction of molecules in a particular state or the spatial distribution of molecules (e.g., mean separation distance or average cluster size). Comparing these results to experimental observations can be straight-forward when experimental data are available because these quantities have an intuitive meaning for the system.

In this paper, we show examples of how Monte Carlo simulations can be used to examine two different aspects of receptor dynamics (Figure 2). First, a model of receptor dimerization is described and the application of this model to receptor cross-talk and cell adhesion is discussed. Second, a model of activation and desensitization of G-protein coupled receptors (GPCR) is described and then applied to a system with a single agonist and a system with an agonist and an antagonist.

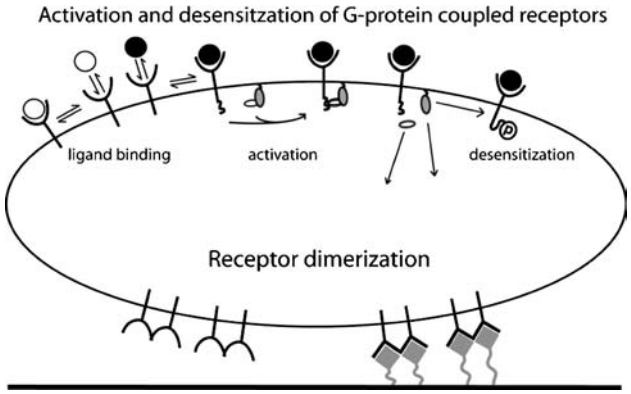

Figure 2. The two systems we will focus on in this paper; (1) receptor dimerization applied to receptor cross-talk and cell adhesion, (2) GPCR activation and desensitization following the binding of an agonist in the absence or presence of an antagonist.

Receptor dimerization: models and results

Receptor dimerization and cross-talk

Many receptors are known to dimerize, and dimerization has been suggested to influence cell signaling although the mechanisms for this are not clear (Hebert & Bourier 1998, Gomes *et al*. 2001, Li *et al*. 2001, Rios *et al*. 2001, Laplantine *et al*. 2002, Myou *et al*. 2002, Li *et al*. 2003). Such dimerization is likely to be at least partly diffusion-controlled (Broday 2000, Woolf & Linderman 2003) in other words, the ability of receptors to diffuse toward (or away) from each other will be critical in determining the dynamics of dimer formation.

We have recently used Monte Carlo simulations to follow receptor dimerization on the cell membrane (Brinkerhoff & Linderman 2004, Woolf & Linderman 2003). In these simulations, receptors are free to diffuse on a two dimensional surface representing the cell membrane. When two receptors are close to each other, they are allowed to form a dimer with a probability related to the dimerization rate constant k_{dimer}. Once a receptor dimer is formed, it may diffuse as such or may dissociate to form two individual receptors with a probability related to the monomerization rate constant k_{mono}. Receptor dimers are not allowed to bind to additional receptors; there is a specific protein–protein interaction between two monomers to form the dimer and additional binding is not possible. In the cases we will discuss here, simulations start as a random arrangement of monomers. Once the simulations reach equilibrium, data in the form of snapshots of the molecules are collected over a time period much greater than the time to reach equilibrium. From these snapshots the average cluster size is measured. Receptors are counted as members of a cluster if they are separated by less than one receptor radius (termed the interaction radius, 3 nm for GPCRs and 6 nm for integrin receptors) from another receptor in the cluster; note then that clusters may in general be a mix of nearby dimers and monomers. The simulation results are the combination of the equilibrium data from many different random starting configurations.

A key prediction of these simulations is that under appropriate conditions, receptor dimerization can drive the formation of larger clusters of receptors on the cell membrane. In other words, the receptors are able to self-organize when dimerization is allowed. This clustering uses a partner switching mechanism (Figure 3a). Partner switching allows multiple molecules to effectively share a single bond and occurs when the proteins diffuse slowly but form and break dimerization bonds quickly. Under these conditions, multiple proteins can form a cluster of proteins larger than a dimer pair. The clustering of molecules can be directly

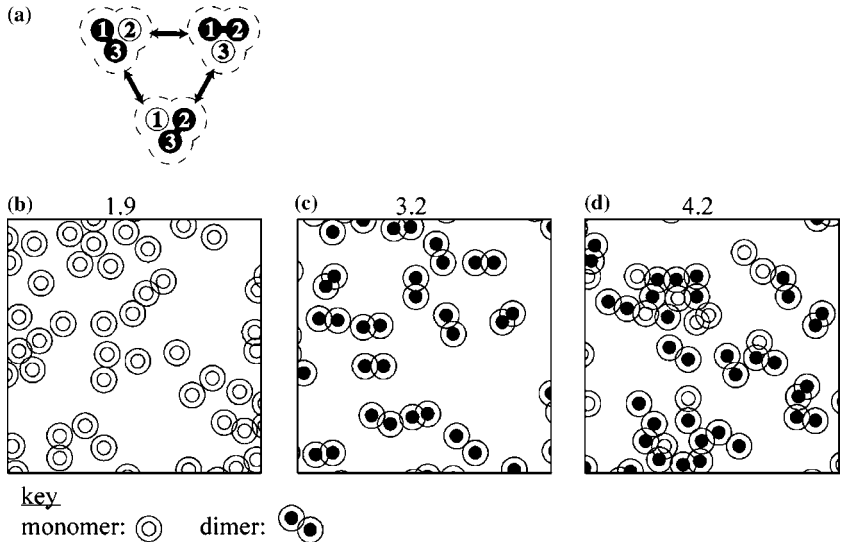

Figure 3. Protein dimerization can generate larger clusters via diffusion-limited partner switching. (a) A schematic of partner switching allows molecules to effectively share a single dimer bond and form a cluster. The rates of dimerization and monomerization must be fast relative to the rate of diffusion for partner switching to be significant. (b–d) Single snapshots of receptor clustering from Monte Carlo simulations show the effect of different values of the dimerization and monomerization rate constants. The receptor is shown as a solid circle for dimers and as an open circle for monomers; two receptors cannot overlap this radius. The interaction radius is shown by the outer circle, when two receptors overlap at this distance they are within the same cluster and can form a dimer, but may also remain as monomers. The average cluster size, calculated from many snapshots, is also shown. (b) When no dimerization allowed, the receptors are randomly distributed and the average cluster size is only 1.9. (c) When dimerization is strongly favored, the receptors are primarily found as dimer pairs and these pairs are randomly distributed ($k_{dimer} = 10^6$/s, $k_{mono} = 10^3$/s, diffusivity $= 10^{-9}$ cm^2/s), increasing the average cluster size to 3.2. (d) Only when dimerization is weak can larger clusters form from an intermingling of dimers and monomers ($k_{dimer} = 10^6$/s, $k_{mono} = 10^5$/s, diffusivity $= 10^{-9}$ cm^2/s), further increasing the cluster size to 4.2.

observed from a snapshot of the simulation results. This snapshot can give an intuitive understanding of the system. When dimerization is not allowed, the receptors are spread randomly on the surface (Figure 3b). When dimerization is heavily favored, the formation of bonds occurs quickly, but the breakage of bonds occurs slowly and the receptors form many tightly bound dimers (Figure 3c). Clustering due to partner switching only takes place when the rates of dimerization and monomerization are fast and relatively balanced (Figure 3d).

While snapshots of simulation results are illuminating, it is also useful to obtain more quantitative measures of clustering by calculating various quantities from the snapshots and then averaging over thousands of snapshots. For the parameter sets used in Figure 3, the average cluster size and the cluster size distribution were calculated. When no dimerization occurs, the average cluster size is 1.9 receptors per cluster. This quantity is greater than 1 because some receptors by chance alone are close enough to be considered a cluster. Analysis of the cluster size distribution shows that more than 85% of molecules are in clusters of size 5 or smaller. When simulation parameters are changed to strongly favor dimerization (large k_{dimer}, small k_{mono}), the average cluster size is increased to 3.2 receptors per cluster. The molecules are able to dimerize and occasionally two dimer pairs will be close enough to be considered a cluster, increasing the average cluster size above 2.0. The cluster size distribution shows that 65% of the clusters are of size 5 or smaller. Most interestingly, when weak dimerization is allowed, partner switching is likely and the average cluster size is increased still further to 4.2 receptors per cluster, and only 50% of the receptors are now found in clusters of size 5 or smaller. While the rate constants for dimerization and monomerization of GPCRs have not been directly measured, our estimates (Woolf & Linderman 2003, Brinkerhoff & Linderman 2004) indicate that GPCR dimerization occurs at rates that allow significant partner switching and can create large clusters of receptors in the cell.

The role of such clustering in GPCR signaling is unknown. One possibility is that dimerization may dramatically affect receptor crosstalk, and our simulations can be used to investigate that possibility. One type of cross-talk occurs when two receptor species share a common effector (e.g., a G-protein). This type of cross-talk is presumably a function of the distance separating the receptor species. In other words, if the two species are found in close proximity to each other, sharing a common effector is more likely than if the two species are spatially segregated (shown

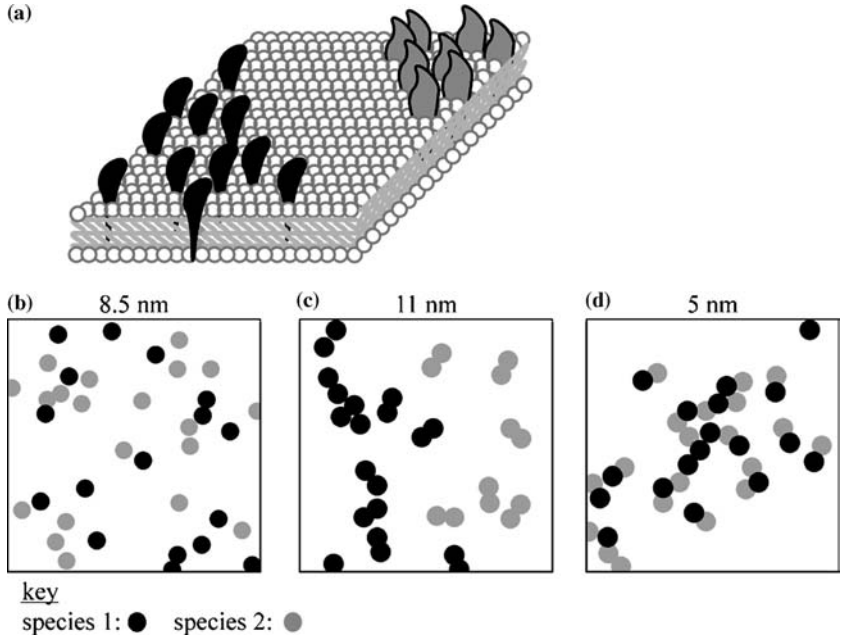

Figure 4. Protein dimerization can influence cross-talk between two receptor species. (a) Homo-dimerization of two species (one black, the other gray) of receptors can change the organization of receptors on the surface. When the receptors are clustered into different regions of the cell, the species are isolated from each other and cross-talk between the receptors can be reduced. (b) When no dimerization is allowed, the two species are well-mixed and the separation distance is 8.5 nm. (c) When homo-dimerization is allowed, large homogeneous receptor clusters are created. The separation distance between the two species is increased to 11 nm, minimizing cross-talk. This effect is also shown in Fig 4a. (d) When receptors can hetero-dimerize, large heterogeneous clusters are created. The separation distance between the two species is only 5 nm, the two species are closer together than when no dimerization is allowed. By minimizing the separation distance between the two different types of receptors, cross-talk is presumably increased. Parameters: diffusivity = 10^{-11} cm^2/s, concentration of species A = species B = 100/μm^2, for dimerizing species $k_{\text{dimer}} = 10^6$/s, $k_{\text{mono}} = 10^3$/s.

schematically in Figure 4a). To examine this possibility, we have performed Monte Carlo simulations of receptor dimerization with two receptor species present (Woolf & Linderman 2003, Woolf & Linderman 2004). Snapshots of these simulations are shown in Figure 4. The degree of segregation of the two receptor species is quantified by calculating the average shortest separation distance between receptors of different types. In other words, for each receptor of species A the distance to the nearest receptor of species B is calculated, and these distances are averaged over all receptors of species A; the same calculation is also done with the species identities reversed. When receptors cannot dimerize at all, the two receptor species appear well-mixed (Figure 4b) and the average shortest separation distance is 8.5 nm (for the simulation using parameter values given in the legend to Figure 4). When receptors can homodimerize, large homogenous clusters form (Figure 4c) and the average shortest separation distance is increased (11 nm). In this case, each receptor species is colocalized in a homogeneous cluster and because diffusion is slow, each cluster would only have access to the local pool of G-proteins. This association of receptor clusters with spatially segregated pools of G-proteins would reduce cross-talk because receptors would be unable to deplete another receptor's pool of G-proteins. Such a lack of cross-talk has been reported in one system (Graeser & Neubig 1993). Finally, receptor heterodimerization (Figure 4d) reduces the average separation distance to 5 nm. In this case, cross-talk would be expected because the receptor species share common pools of G-proteins. Thus, receptor homo- and heterodimerization are predicted to modulate receptor crosstalk and influence cell signaling. Further, because for some receptor types ligand binding is known to influence a receptor's ability to dimerize (Rodriguez-Frade *et al*. 1999, Rocheville *et al*. 2000), this modulation may be ligand-dependent.

The predictions from simulations of receptor dimerization are, as described above, snapshots of receptor positions, average cluster sizes and cluster distributions, and distances between molecules. It will be important to compare these predictions with experimental measurements. Although such numbers are not now readily available from experiment, it is likely that these will be in the not too distant future. For example, techniques such as fluorescence energy transfer (FRET) or cryo-AFM might be used to gain information on receptor clustering and distances between receptors (Angers *et al*. 2000, Cornea *et al*. 2001, Liang *et al*. 2003). In addition, experiments examining crosstalk in the presence of various ligands can be interpreted through this new lens of dimerization

interactions (Woolf & Linderman 2004), and new simulations and experiments suggested. Thus we expect that an interplay between modeling and experimental work will be used to determine the role that receptor dimerization plays in signaling.

Receptor dimerization and cell adhesion

Cell adhesion is mediated by adhesion receptors, particularly integrins. Integrins bound to immobilized ligands form a mechanical attachment between the cellular cytoskeleton and the cell's external environment, and clustering of integrins is necessary to initiate the signaling cascade that creates this attachment (Miyamoto *et al*. 1995). Integrin clustering can be caused by binding to multivalent ligands (Koo *et al*. 2002, Rowley *et al*. 2002). An additional physiological mechanism for generating integrin clusters (>2 integrins) is integrin dimerization. Integrin dimerization has been observed, but, as in the previous example, the role of dimerization in integrin function is unclear (Li *et al*. 2001, Laplantine *et al*. 2002, Myou *et al*. 2002, Li *et al*. 2003). However, based on the results described above, we suspect that integrin dimerization may play an important role in organizing integrin receptors during adhesion.

We have developed Monte Carlo simulations to follow both the dimerization of integrins and the binding of integrins to immobilized ligands in order to elucidate the interplay between integrin dimerization and specific arrangements of ligands on integrin clustering (Brinkerhoff & Linderman 2004). Our results are shown schematically in Figure 5. We first investigated the effects of dimerization and ligand binding separately. Monte Carlo simulations of integrin dimerization without ligand binding show that clusters of integrins can form (similar to the clusters in Figure 3). For the parameters representative of integrin receptors and used in our simulations, the calculated average cluster size is 3.1 integrins per cluster. To assess the impact of ligand organization, we simulated integrin binding to immobilized ligand in the absence of dimerization. When integrins bind to randomly arranged ligands the integrins are also randomly distributed and the average cluster size is 1.5 integrins per cluster, similar to the cluster size when integrins diffuse freely without ligand binding or dimerization. When integrins bind to ligands arranged into "islands" (to mimic a multivalent ligand by creating regions of high ligand concentration separated by regions devoid of ligand), the average cluster size is increased to 4.4.

Finally, we simulated integrins that both dimerize and bind to ligand. When dimerizing integrins bind to randomly arranged ligand the average cluster size is 4.8 integrins per cluster. The cluster size is limited by the competition of two effects: dimerization acts to cluster the integrins but the randomly spread ligand acts to spread the integrins over the surface. In contrast, when ligands are placed in islands with an optimal spacing of ligands, dimerization and ligand binding effects are synergistic and the average cluster size is increased further to 9.7 integrins per cluster. This optimal spacing is approximately equal to 15 nm, an intermediate distance between the maximum distance at which dimerization can occur (21 nm) and the minimum distance between two ligands that are both available to bind integrins (9 nm). Thus, the simulations can be used to predict optimal ligand arrangements to increase integrin clustering, possibly affecting cell adhesion/signaling. Experiments to test these predictions can be done by patterning ligand surfaces with a technique such as soft lithography (Shim *et al*. 2003), or by covering a surface with a controlled mixture of polymer molecules containing a specific density of ligand and unligated polymer molecules (Koo *et al*. 2002, Rowley *et al*. 2002).

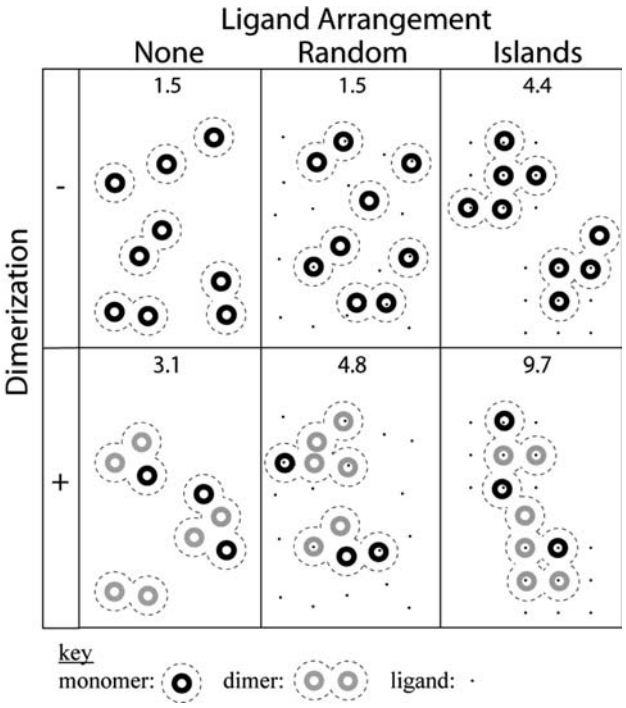

Figure 5. The dimerization of integrins and arrangement of ligand can compete or cooperate for integrin clustering and ligand binding. This is a schematic representation of integrin clustering and ligand binding for combinations of integrin dimerization and ligand arrangement. Each combination of dimerization and ligand conditions shows a schematic description of simulation results and the average cluster size calculated from simulations. The largest integrin clusters are formed when the integrins can dimerize and the ligand is arranged into islands. Parameters: diffusivity $= 10^{-11}$ cm^2/s, $k_{dimer} = 10^5$/s, $k_{mono} = 10^3$/s, $k_{bind} = 10^{-7}$ cm^2/s, $k_{unbind} = 1$/s, ligand density $1047/\mu$m^2, 9 ligands/island.

Activation and desensitization of G-Protein coupled receptors: models and results

Agonist-induced Activation and Desensitization

Agonist–receptor binding can trigger both activation and desensitization. For GPCRs, the initial phases of the activation and desensitization pathways occur primarily at the cell membrane and are shown in Figure 6. Agonist binding is associated with the active receptor conformation and leads to G-protein activation. The active G-protein initiates intracellular pathways leading to responses (e.g., *via* Ca^{2+} or cAMP) but, importantly, also initiates a desensitization pathway (Krupnick & Benoric 1998). The $G_{\beta\gamma}$ subunit recruits a receptor kinase to the membrane. Once attached to the membrane, the receptor kinase can phosphorylate receptors and in doing so target them for arrestin binding and eventual internalization.

G-protein activation and receptor phosphorylation processes stem from the same initial event (agonist–receptor binding) and therefore might be expected to be linearly related. However, experimental evidence from the μ-opioid and dopamine D_{1A} receptor systems suggest that G-protein activation and receptor phosphorylation are not linearly related (Yu *et al.* 1997, Lewis *et al.* 1998, Zhang *et al.* 1998), while data from the β_2-adrenergic system show such a linear relationship (Benovic *et al.* 1988). If one could identify ligand-specific parameters that influence the relationship between activation and desensitization, this might allow the design of agonists that maximize activation and minimize desensitization.

Mathematical models of the GPCR activation and desensitization processes can be used to decipher the relationship between activation and desensitization in different systems. To first study the activation process alone, we developed Monte Carlo simulations that included agonist binding, G-protein activation, hydrolysis of GTP by the G_α subunit, and recombination of the G_α-GDP and $G_{\beta\gamma}$ subunits (Mahama & Linderman 1994). Our simulations demonstrated that the agonist dissociation rate constant $k_{\text{off-ag}}$ can significantly affect the amount of G-protein activation when cases of *equal receptor occupancy* are compared. At one extreme, a very tightly binding agonist (very small dissociation rate constant $k_{\text{off-ag}}$) binds receptors. The resulting agonist–receptor complex is long-lived and thus activates nearly all the nearby "local" G-proteins. In other words, diffusion of receptors and G-proteins is slow enough ($D = 10^{-10}$ cm^2/sec) that the supply of "activate-able" G-proteins is depleted near these agonist–receptor complexes. As $k_{\text{off-ag}}$ is increased, the agonist–receptor complex produces more activation *per bound receptor* than when $k_{\text{off-ag}}$ is small. This is because the agonist–receptor complex lifetime is decreased, agonists rapidly move among free receptors on the cell surface, and the depletion of "activate-able" G-proteins near any one receptor is decreased. For example, if 25% of the receptors are bound by agonist, our Monte Carlo simulations predict that G-protein activation can be increased up to 2.5-fold when $k_{\text{off-ag}}$ is large compared to when $k_{\text{off-ag}}$ is small (Mahama & Linderman 1994). This prediction is consistent with data in the α_1-adrenergic and β-adrenergic receptor systems (Mahama & Linderman 1995, 1993).

With an understanding of how agonist properties (in particular the agonist–receptor dissociation rate constant $k_{\text{off-ag}}$) may influence G-protein activation, we next turned to receptor phosphorylation, an early step in the desensitization pathway. Because receptor phosphorylation is initiated by receptor activation, it should be affected by $k_{\text{off-ag}}$ as well. We assume that unoccupied receptors cannot be phosphorylated (Bunemann *et al.* 1999). We also included in our Monte Carlo simulations the ability of different agonists to bias the receptor into the active state, an effect captured in the ligand-specific conformational selectivity factor α (Woolf & Linderman 2003a). In Figure 7a, the increase in activation with increasing values of $k_{\text{off-ag}}$ is shown, as described above. Also shown is the increase in receptor phosphorylation, expected because activated G-proteins recruit the kinases that perform the phosphorylation. However, increasing $k_{\text{off-ag}}$ does not increase G-protein activation and receptor phosphorylation proportionally; this is best seen by calculating the ratio of G-protein activation to receptor phosphorylation (termed GARP). At an intermediate value of $k_{\text{off-ag}}$ the GARP value is a maximum (Figure 7b). The explanation for this result involves the interplay between

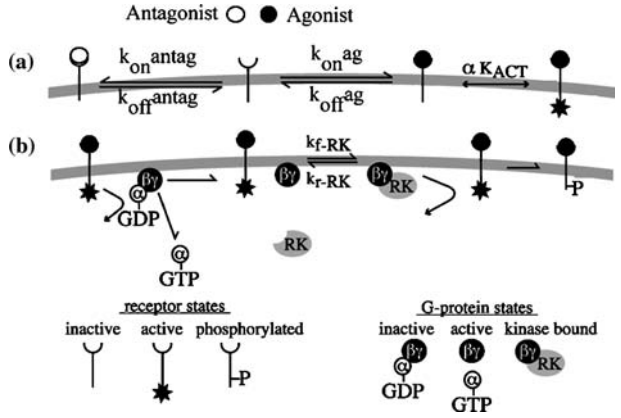

Figure 6. Processes that affect G-protein activation and receptor desensitization. (a) Receptors bind to agonist or antagonist according to the concentration and binding kinetics of each ligand. Antagonist-bound receptors are blocked from any further action (G-protein activation or receptor phosphorylation) agonist-bound receptors can assume an active conformation. (b) Receptors in an active state bind to and activate a G-protein, splitting it into G_α and $G_{\beta\gamma}$ subunits. The $G_{\beta\gamma}$ subunit can recruit receptor kinase to the membrane, leading to receptor phosphorylation and ultimately desensitization.

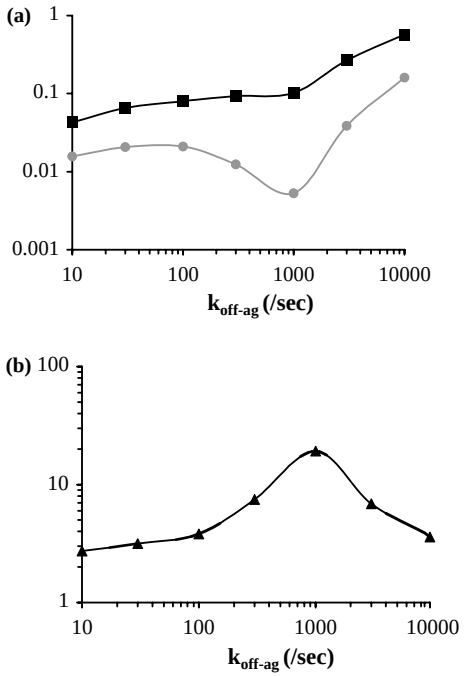

Figure 7. Predicted effects of the agonist dissociation rate constant, $k_{off\text{-}ag}$, on the initial rates of G-protein activation and receptor phosphorylation (or their ratio, termed GARP) when a single agonist ligand is present. (a) Increasing the $k_{off\text{-}ag}$, the agonist dissociation rate constant, increases both the rate of G-protein activation and receptor phosphorylation when *cases of equal receptor occupancy are compared.* (b) The GARP value is calculated by taking the ratio of data in (a) to show the relationship between the GARP value and the $k_{off\text{-}ag}$. GARP is a maximum at intermediate values of $k_{off\text{-}ag}$. Parameters: diffusivity $=10^{-11}$ cm^2/s, receptor concentration = 1000/cell, G-protein concentration = 33,000/cell, $k_{on\text{-}ag} = 10^7$/M·sec, 2.5% of receptors occupied by agonist and active 10% of the time.

receptor occupancy by agonist and the arrival of receptor kinases (for the same number of agonist–receptor complexes). In a possible scenario, agonist may bind to receptor, cause G-protein activation, and dissociate before the arrival of a receptor kinase, leaving the receptor unable to be phosphorylated. Thus activation of G-protein occurs without receptor phosphorylation. This event is most likely to occur when $k_{off\text{-}ag}$ is at an intermediate value: if $k_{off\text{-}ag}$ is small, the agonist is likely still bound when the kinase arrives, and if $k_{off\text{-}ag}$ is large, agonist may not only dissociate but also rebind before the kinase arrives. The surprising implication of these calculations is that drugs might be designed not only to enhance activation but also to minimize desensitization (i.e. to maximize the GARP value), and that this could be accomplished by altering the agonist-receptor dissociation rate constant $k_{off\text{-}ag}$.

Effect of antagonist on agonist-induced activation and desensitization

In the previous section, we described how a single agonist ligand can influence activation and desensitization. However, *in vivo* multiple ligands can compete for receptor binding. How would competition between an antagonist drug and an endogenous agonist ligand affect receptor activation and desensitization? To address this question, we performed simulations using the model described above with both an agonist and antagonist present.

Note first that in previous Monte Carlo simulations of G-protein activation alone, we have shown that a slowly dissociating antagonist has two effects on agonist-induced activation (Mahama & Linderman 1995). First, and obviously, the antagonist competes with the agonist for receptor binding sites. Second, and less obviously, the amount of activation produced *per agonist-occupied receptor* is reduced. This is because diffusion of G-proteins in the membrane is slow, and thus G-proteins near antagonist-bound receptors are not readily accessible to agonist-bound receptors. For example, for parameters representative of the α_1-adrenergic receptor system, when 25% of receptors are occupied by agonist and no antagonist is present, G-protein activation is 20% higher than when 25% are bound to agonist and 60% are bound to antagonist and 40% higher than when 25% are bound to agonist and 75% are bound to antagonist (Mahama & Linderman 1995).

We now turn to simulations that include both G-protein activation and receptor phosphorylation, in order to predict the GARP value when both an agonist and an antagonist are present. In particular, we have begun to explore the effect of the antagonist dissociation rate constant, $k_{off\text{-}antag}$. We assume that antagonist-occupied receptors, unlike agonist-occupied receptors, cannot be phosphorylated by receptor kinases (see Figure 6). The agonist association and dissociation rate constants are held fixed, and the antagonist dissociation rate constant, $k_{off\text{-}ag}$ is varied (but the antagonist association rate constant, $k_{on\text{-}antag}$ is held fixed by varying the concentration of the antagonist). In these simulations, antagonist occupies 59% of the receptors, agonist occupies 2.5%, and the remaining 38.5% of the receptors are unbound when equilibrium binding is reached. G-protein activation increases for increasing values of $k_{off\text{-}antag}$, when cases of equal receptor occupancy by agonist are compared. As $k_{off\text{-}antag}$ is increased, antagonist dissociates from receptors more frequently, so agonist has more opportunities to access those previously antagonist-bound receptors and their nearby G-proteins. Receptor phosphorylation also increases with increasing values of $k_{off\text{-}antag}$, because the presence of increasing numbers of activated G-proteins allows for increased membrane recruitment of receptor kinase.

Interestingly, our simulations again show that G-protein activation and receptor phosphorylation do not increase proportionally with changes in $k_{off\text{-}antag}$, as can been seen from the GARP value (Figure 8b). The GARP value has a maximum for intermediate values of

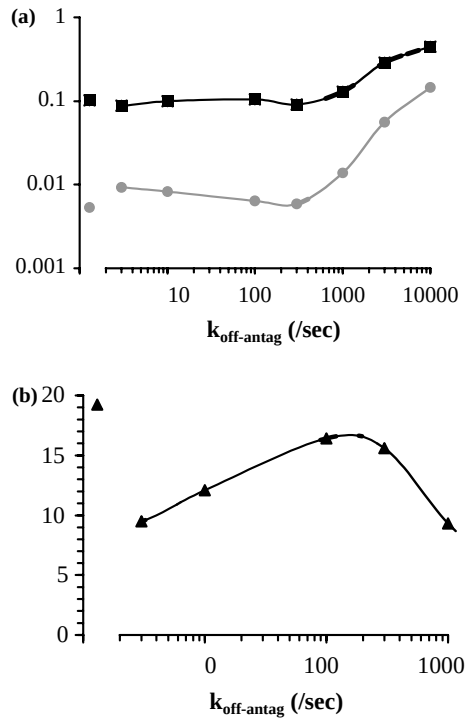

Figure 8. Predicted effects of antagonist dissociation rate constant, $k_{off\text{-}antag}$, on the initial rates of G-protein activation and receptor phosphorylation produced by an agonist. The agonist's properties are held constant at the same conditions as Figure 7, with $k_{off\text{-}ag}$ = 1000/s, shown here for comparison as "No antag". (a) The initial rates of G-protein activation and receptor phosphorylation increase with increases in $k_{off\text{-}antag}$. (b) The GARP value is calculated by taking the ratio of data in (a). The GARP value is a maximum at intermediate values of $k_{off\text{-}antag}$. Parameters: diffusivity = 10^{-11} cm^2/s, receptor concentration = 1000/cell, G-protein concentration = 33,000/cell, $k_{on\text{-}ag}$ = 10^7/M·s, 2.5% of receptors occupied by agonist and active 10% of the time, $k_{on\text{-}antag}$ = 10^8/M·s, 59% receptors occupied by antagonist.

$k_{off\text{-}antag}$. Again, the explanation for this result involves the interplay between receptor occupancy and arrival of receptor kinases. The presence of an antagonist reduces the ability of agonist to rebind to a receptor, especially for the case shown (much higher occupancy of receptors by antagonist than agonist). In a possible scenario, agonist binds to receptor, and G-proteins nearby are activated. Agonist then dissociates, but by the time receptor kinase arrives the receptor is either antagonist-bound or unoccupied and the receptor cannot be phosphorylated. Under these conditions, the amount of G-protein activation is high and receptor phosphorylation is low, so the GARP ratio is a maximum. At small values of $k_{off\text{-}antag}$, the antagonist dissociates from receptors only infrequently, the agonist is not able to move among receptors, and the GARP ratio is low. At large values of $k_{off\text{-}antag}$, antagonist dissociates from the receptor many times during the lifetime of the receptor kinase and thus increases the number of opportunities an agonist has to bind that receptor and allow receptor phosphorylation. Under these conditions, both the amount of G-protein activation and receptor phosphorylation are high and the GARP ratio is low. Similar to the case of a single agonist alone, when an agonist and antagonist are present intermediate values of $k_{off\text{-}antag}$ allow maximal GARP ratios.

A better understanding of the effect of competition between ligands on receptor activation and phosphorylation may have application to the dopamine D_2 receptor. In this system, the endogenous agonist is dopamine and antagonists for the receptor are administered as antipsychotic drugs (Kapur & Seeman 2001). It has recently been observed that the actions of two classes of such drugs, termed "typical" and "atypical" antipsychotics, correlate with the affinity of the antagonist for the receptor and the antagonist dissociation constant, $k_{off\text{-}antag}$ (Seeman 2002). Atypical drugs (which have fewer extrapyramidal signs and symptoms) have a lower affinity and larger $k_{off\text{-}antag}$ for the receptor than dopamine, while the typical drugs have a higher affinity and smaller $k_{off\text{-}antag}$ than dopamine. Our simulations show that differences in $k_{off\text{-}antag}$ could result in different values of GARP, which may contribute to the differences seen clinically.

Thus, it is important to understand the consequence of competition between multiple ligands to understand the impact a drug will have *in vivo*. Monte Carlo modeling may help to direct the search for an antagonist that can minimize agonist-induced receptor desensitization while maximizing signaling or efficacy. Typically, antagonist drugs are designed with a high affinity for receptor (low $k_{off\text{-}antag}$). However, these simulations predict that an intermediate value of the antagonist dissociation rate constant $k_{off\text{-}antag}$ may produce more desirable antagonists.

Discussion

Here we have described several models of signal transduction systems to demonstrate the usefulness of Monte Carlo simulations in modeling receptor dynamics with an eye toward understanding cell signaling. We chose to use Monte Carlo simulations because the events we modeled rely on diffusion-controlled, discrete reactions. One of the systems we investigated was receptor dimerization. Our method can also be used to analyze more complicated dimerization interactions in larger systems and to guide the interpretation of experimental measures of signaling (Woolf & Linderman 2004). These simulations can also predict ligand patterns that could be used in designing biomimetic materials to manipulate cell adhesion. In addition, we also used Monte Carlo simulations to predict the effect of agonist and antagonist dissociate rate constants, $k_{off\text{-}ag}$ and $k_{off\text{-}antag}$, on two cell signaling events, G-protein activation and receptor phosphorylation.

The predictions of our simulations may find application in drug design for many GPCR systems.

Other receptor dynamics systems that have diffusion-limited and/or stochastic reactions could be modeled using Monte Carlo simulations. One example is the assembly of signaling complexes. The formation of signaling complexes provides a level of organization of signal transduction molecules (receptors, effectors and second messengers) that can provide a dynamic system to concentrate reactants and allow integration and cross-talk of cellular signals (Bray 1998). Signaling complexes have important roles in bacterial chemotaxis (Stock & Surette 1996), T-cell activation (Berridge 1997) and the formation of focal contacts in cell adhesion (Jockusch *et al*. 1995).

Finally, although not the focus of this paper, model validation through experiment is essential. With the growing capabilities of molecular biologists to alter the structures of receptors, ligands, and effectors, there is the opportunity to directly test many of our models by intentionally modifying key kinetic rate constants and other system parameters. New methods for studying the locations of molecules on the cell surface, together with kinetic analysis of receptor systems, will create more situations where models will be useful and even essential to understanding the role that receptor dynamics play in cell signaling processes.

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