Effects of Quenched and Annealed Macromolecular Crowding Elements on a Simple Model for Signaling in T Lymphocytes

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Received: July 28, 2005; In Final Form: November 21, 2005

Biochemical reactions in cells occur in an environment that is crowded in the sense that various macromolecular species and organelles occupy much of the space. The effects of molecular crowding on biochemical reactions have usually been studied in the past in a spatially homogeneous environment. However, signal transduction in cells is often initiated by the binding of receptors and ligands in two apposed cell membranes, and the pertinent biochemical reactions occur in a spatially inhomogeneous environment. We have studied the effects of crowding on biochemical reactions that involve both membrane proteins and cytosolic molecules by investigating a simplified version of signaling in T lymphocytes using a Monte Carlo algorithm. We find that, if signal transduction occurs on time scales that are slow compared to the motility of the molecules and organelles that constitute the crowding elements, the effects of crowding are qualitatively the same as in a homogeneous three-dimensional (3D) medium. In contrast, if signal transduction occurs on a time scale that is much faster than the time over which the crowding elements move, then the effects of varying the extent of crowding are very different when reactions occur in both 2- and 3D space. We discuss these differences and their origin. Since many signaling reactions are fast, our results may be useful for diverse situations in cell biology.

1. Introduction

A large fraction of the total volume of a cell is occupied by different types of macromolecules.[1,2] As no single species occurs at high concentrations, the interior of the cell is termed "crowded" rather than "concentrated". Various biochemical reactions (for example, those involved in signal transduction) occur in this crowded environment. Even though many of the macromolecules and organelles do not participate in a particular cascade of reactions, the fact that they occupy space can have a dramatic effect on reaction thermodynamics and kinetics. Specifically, crowding leads to an increase in the activity coefficients that represent nonideal behavior of the solution in which the reactions take place. This, in turn, results in an increase in the rates of forward reactions and a concomitant change in the equilibrium product distribution. However, crowding also leads to a decrease in the mobility of various species, and this has an inhibitory effect on chemical reactions. These competing effects can lead to an optimal fractional occupancy (or crowding) that maximizes the rate and equilibrium amount of product formation. There have been several excellent reviews over the past few years describing these effects.[3−7]

Despite the fact that crowding is a feature common to all types of cells, its effects on the efficacy of biochemical reactions have not been extensively studied. With some exceptions, biochemical experiments have been carried out in dilute solutions.[8] The exceptions have shown that crowding can have a dramatic effect on the reaction rate and equilibrium characteristics of protein (re)folding, association and aggregation, the action of chaperones,[3−6] and ultrasensitivity.[9−12]

The problem of diffusion in the presence of randomly placed obstacles has been studied extensively using theory and computation, and conditions that lead to anomalous diffusion have been determined.[13−16] Theoretical studies and computer simulations have also been carried out to examine biochemical reactions in crowded environments comprised of chemically inert immobile objects.[7,17−20] Some of these studies were carried out on two-dimensional (2D) lattice models using Michaelis−Menten reaction schemes that were not specific for a particular biological system. Schnell and Turner[7] and Berry[17] illustrated that, under certain conditions, crowding leads to a breakdown in the law of mass action and power-law approximation. Their simulations show that, in inhomogeneous media, reactions follow fractal-like kinetics and there is anomalous diffusion and mixing of the biochemical species. The deterministic approach often used so successfully in modeling reactions in simple, homogeneous, in vitro conditions is sometimes inadequate for modeling reactions in a crowded molecular environment. For example, under certain conditions, assumptions and approximations such as the quasi-steady-state approximation break down.[7,17,18] Furthermore, numerical simulations where the available volume was varied[21] showed how variations in available space can be used to tune the thermodynamic activity of reacting species.

Bray and co-workers have developed a computational algorithm to study signaling processes that is based on Smoluchoski equations and have used it to obtain important insights into signal transduction in *Escheri coli* chemotaxis.[19,20] In this context, they have examined the effects of randomly placed immobile obstacles that occupy 41% of the available volume.

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10.1021/jp054191+ CCC: $33.50
Published on Web 01/12/2006

For the conditions of their simulations, they find that there is a 2-fold decrease in the diffusion coefficient of a key signaling molecule compared to the situation where there are no obstacles. They also report that in parts of the cell where signaling molecules are concentrated, crowding accelerates the response (because of a higher chemical potential), while in others the local concentration of signaling molecules is reduced because crowding agents hinder diffusion and this delays cellular response.

In this paper, we study the effects of different extents of molecular crowding on signal transduction that is triggered by the binding of receptors and ligands that are expressed on the surfaces of apposed cells. Thus, the signaling reactions occur in a spatially inhomogeneous environment wherein some reactions are confined to the 2D membrane while others take place in the cytosol. We ask two questions: (1) Are the effects of molecular crowding different when signaling occurs in spatially organized structures? Specifically, we examine how the effects of molecular crowding depend on the fraction of the volume that is occupied by the crowding agents. To our knowledge, this has not been studied before in a spatially inhomogeneous system. (2) Do the effects of molecular crowding depend on whether the inert objects that lead to crowding are mobile or quenched in place over the time scale over which signal transduction occurs? Past studies of the differences between quenched and mobile crowding elements have been restricted to understanding the effects of this variable on diffusion alone,[13,16] rather than on the entire process of signal transduction in a spatially inhomogeneous medium. Other studies of the effects of quenched crowding elements in a spatially inhomogeneous medium have been restricted to a single value of the fractional crowding.[19] To address these questions, we study a specific biological system that provides an example of signal transduction initiated by receptor−ligand binding.

T lymphocytes (T-cells) are the orchestrators of the adaptive immune response. They have evolved to react to pathogens that have invaded cells. Specialized cells, called antigen presenting cells (APCs), display molecular signatures of the pathogen on their surface. Peptide fragments from proteins derived from the pathogen can bind to a protein coded for by the major histocompatibility gene complex. These peptide−major histocompatibility complex (pMHC) molecules are the molecular signatures of the pathogen displayed on APC surfaces. T-cell receptors (TCRs) are proteins expressed on the surface of T-cells that can bind pMHC molecules on APCs. The binding of TCRs to pMHC molecules initiates signaling cascades that involve membrane-bound species and cytoplasmic molecules and can ultimately lead to the activation of the adaptive immune response.[22] The binding of TCRs to pMHC molecules can also lead to the formation of a spatially organized motif in the intercellular junction called the immunological synapse.[23−25] The synapse is characterized by a central accumulation of TCRs and pMHC molecules. Previous computational studies of T-cell signaling have not considered the effects of molecular crowding.[26−29] Here, we describe results obtained from Monte Carlo simulations studying the effects of crowding on a simplified representation of signaling in T-cells. We study situations where the inert particles that result in a crowded environment are mobile as well as immobile and find qualitative differences between these scenarios. For signals that are propagated on a time scale that is short compared to that associated with the motion of the crowding elements, the crowding elements can be considered immobile. For slower signals, the crowding agents are mobile. Therefore, our results imply that the effects of macromolecular crowding on signal transduction could be different depending upon whether we are considering fast or slow signal transduction. Although our results are specific for a simplified representation of signaling in T-cells, it is worth noting that fast and slow signal transduction pathways initiated by receptor−ligand binding at a membrane are ubiquitous in cell biology and so our results may be relevant more broadly.

2. Models and Methods

Each protein in our system is represented as a particle on a 3D lattice. These particles have three characteristics: color (species), spin state (+1 if phosphorylated, otherwise 0), and bound state (some species can be bound to multiple species while others cannot make bonds). Only a single species may occupy a lattice site at a time. When species are bound in a complex, they will move together until these bonds are broken. The 3D grid is $101 \times 100 \times 100$ (see Figure 1) and finite (i.e., there are no periodic boundary conditions). The $x = 0$ plane represents the APC membrane, and only pMHC molecules appear here. The $x = 1$ plane is the T-cell membrane, and only TCRs appear in this plane. The $x = 2$ plane is the first cytosolic layer, and since the Src family kinase (Lck) is associated with the cytoplasmic tail of the coreceptor CD4 that lives in the membrane, Lck is confined to this layer. The same is true for the adapter molecule, linker for activation of T-cells (LAT). TCR is modeled as being composed of two lattice sites so it lives in both the T-cell membrane ($x = 1$) and the membrane-associated region ($x = 2$). Cytosolic species that can diffuse freely, such as Grb2-related adaptor (GADS), ζ-associated protein 70 (Zap70), SH2 domain-containing leukocyte protein of 76 kDa (SLP-76), phospholipase C (PLCγ), and phosphotases (Pho), can occupy sites in the 3D lattice without constraints. We have two reporters for downstream signal strength, DS1 and DS2. The intensity of signaling and efficacy of signal transduction will be measured by the number of active DS1 and DS2 molecules. Of course, we also have varying numbers of inert particles that can occupy sites and are distributed randomly on the lattice.

The specific signaling pathway that we simulate is shown in Figure 2. It is a highly simplified version of the T-cell signaling pathway.[22,30] Examples of features that have been ignored are as follows. Downstream signaling involving the MAPK pathway, activation of G proteins such as Rac, and calcium signaling is not treated explicitly.[22,31−35] Feedback loops that influence the activity of Lck are not considered.[36] TCRs that are highly phosphorylated are ubiquitinated and, hence, targeted for degradation. We ignore TCR downregulation, and so, the T-cell signaling pathway that we simulate has an equilibrium state as long as stimulatory pMHC molecules are present. This is not accurate because TCR downregulation makes T-cell signaling an inherently nonequilibrium process. Nevertheless, the pathway that we simulate is an adequate representation of membrane-proximal signaling in T-cells and should enable us to study how molecular crowding affects T-cell signaling at early time points.

To start the simulation, all species are randomly placed given the constraints described earlier. The number of each species is in Table 1. Similar to Lee et al.,[27] the number of TCRs was chosen based on experimental measurements[24] assuming each lattice unit corresponds to 10 nm (roughly the size of a typical globular protein). The surface of an APC expresses numerous pMHC molecules where the peptide is derived from endogenous proteins and only a few molecules with the peptide component derived from foreign proteins. The latter are stimulatory and,

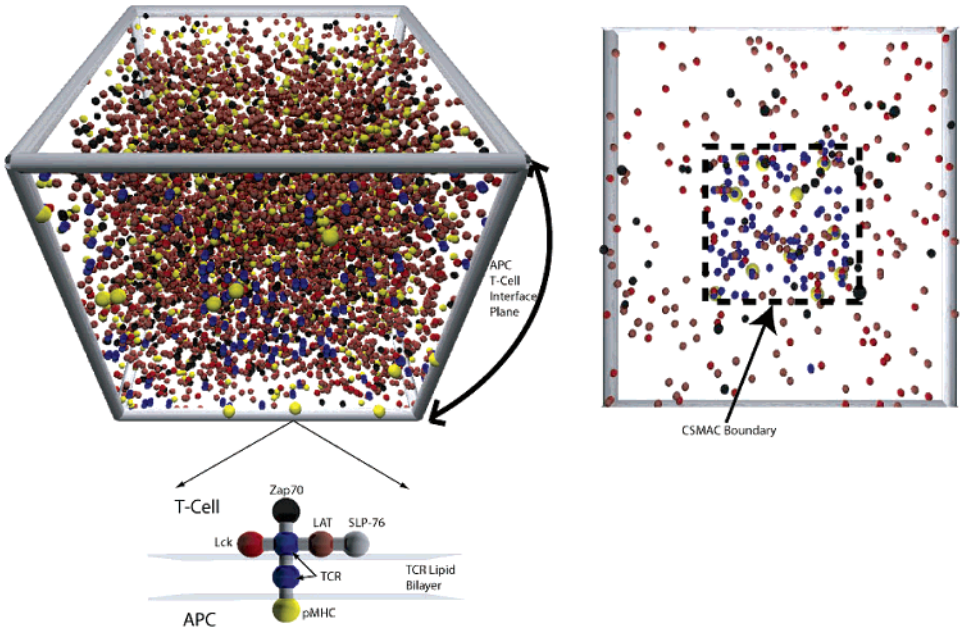

Figure 1. 3D lattice Monte Carlo simulations took place on the 101 × 100 × 100 grid shown above. The front face of the cube is the APC T-cell interface (where pMHC and all other associated species are relegated). TCRs can make bonds across this interface with pMHC while the rest of the pathway propagates from there (see inset above). The above right image shows an example when the CSMAC is mimicked such that TCR and pMHC are forced into the center region (40 × 40 square region).

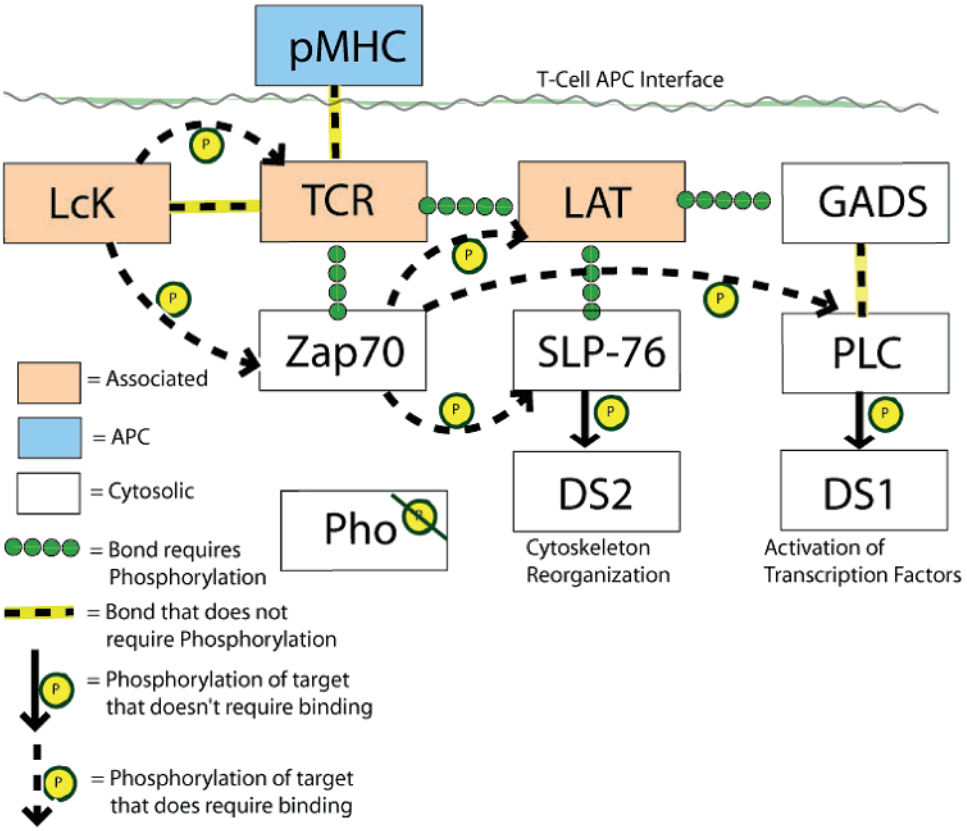

Figure 2. Cartoon of the T-cell signaling pathway. pMHC and associated species are relegated to the APC side and T-cell side of the APC T-cell interface, respectively. All other cytosolic species can diffuse freely in the grid. Bonds that require the binding species to be phoshporlyated are represented by beads. Those that do not require phosphorlyation are represented by the thick dashed lines. Phosphorlyation (or spin flipping) may also require a bond to be made prior to the flip as determined by the solid (does not require bond) and dashed (does require bond) arrows. Phosphatase (represented by Pho above) can dephosphorylate (flip down) any species. The two down stream factor species (DS1 and DS2) are measured as the outputs of the system. In the real system, there are several downstream molecules that eventually lead to cytoskeleton reorganization and the activation of a transcription factor, but for simplicity, our model ends the reaction pathway here.

hence, called agonists. We are using a small number of pMHC molecules because we only wish to represent the agonists. Signaling due to cooperativity between agonist pMHC molecules and some part of the endogenous pMHC repertoire has recently been shown to be very important for signal amplification and the sensitive detection of pathogens by T-cells,[26,37−39] but for

TABLE 1: 12 pMHCs Have Shown to Be Sufficient to Illicit a Sustained T-cell Response while the Number of Other Species Used during the Simulations Were Chosen Such that They Were Not Rate Limiting

species	location	no.	species	location	no.
MHC	integral	12	SLP-76	cytosolic	1200
TCR	associated	120	GADS	cytosolic	1200
LcK	associated	120	DS1/2	cytosolic	1200
LAT	associated	120	Pho	cytosolic	varied
ZAP70	cytosolic	1200	inert	cytosolic	varied
PLCγ	cytosolic	1200			

the purposes of obtaining a qualitative understanding of the role of molecular crowding on the T-cell signaling pathway, we do not consider such a detailed model. Since synergy between endogenous and pathogen-derived pMHC molecules should be more important at small dosages of pathogen-derived (agonist) pMHC molecules, our results are relevant to situations where agonist concentration is high (as in experiments where pMHC molecules are presented on a supported lipid bilayer).[24] The number of remaining species is large enough to prevent them from being rate limiting.

A metropolis Monte Carlo (MC) method is used to follow the spatiotemporal evolution of the system.[40] There are several types of MC moves: displacement, bond forming\breaking, and changes in internal state (spin flips). One of these actions is chosen with equal probability during each MC step. This choice is convenient, but others are not expected to influence qualitative results. If displacement is chosen, then a random particle\complex and direction (2D for those particles that are in the membrane or membrane-associated and 3D for cytosolic species) are chosen. If this move does not violate excluded volume and grid boundary conditions, then it is accepted.

If making or breaking a bond is the chosen move, then a particle is randomly picked. If the particle is currently bound, then the change in energy associated with breaking of the bond, $\Delta E = 5k_bT$ for all bonds, is used to determine the outcome. The bond will break if a random number uniformly distributed from zero to one is less than $(-\Delta E/k_bT)$. If the chosen particle is not bound, then a random direction is chosen. If there is a compatible species (see Figure 2 for compatibilities) in the chosen direction, then a bond is made. Thus, we assume that forming a bond has a negligible barrier. This is often not true, and Lee et al.[27] simulated a detailed T-cell signaling pathway with barriers in both directions. However, we use this approximation since it makes the simulations computationally more tractable and is not expected to change any of the qualitative results. Several bonds can only be made after one of the species has been phosphorylated, that is, spin $= +1$ (see Figure 2). Each species can only be bound to one compatible species of each type. It should be noted that it is thought that SLP-76 cannot bind to LAT until both PLCγ and GADS are bound.[32] This binding rule would not have changed the general results of this paper, and so we do not include this constraint.

When a spin flip is the chosen move, again a species is picked randomly. The rules that determine when a spin can be flipped depend on the particular species that is being considered. Some species must be bound to another species in order to change the spin state. For example, Lck can phosphorylate TCR (flip its spin to $+1$), but only after its active form is bound to TCR (the dashed arrows in Figure 2 represent spin flips that require a bond first with that species). Similarly, Lck can phosphorylate Zap70 (flip its spin to $+1$) but only when Lck and ZAP70 are bound to TCR. Zap70, in turn, can phosphorylate several species but they must all satisfy certain conditions. Thus, many reactions that constitute the T-cell signaling pathway (and, indeed other signal transduction pathways) can be mapped onto dynamic facilitated spin models.[41−45] In these models, a spin can flip only when its neighboring spins satisfy certain conditions, and these constraints lead to many interesting dynamic characteristics. This is similar to the fact that spin flips corresponding to the reactions described above can only occur when neighboring species satisfy certain conditions. It will be interesting in the future to examine if certain general features of signal transduction networks can be deduced by studying a formal mapping to facilitated spin models.

LcK can also autophosphorylate. If its spin state is 0 and a random number uniformly distributed from zero to one is less than $\exp(-\Delta E/k_bT)$, with $\Delta E = 10k_bT$, LcK will flip to $+1$. Given this choice of parameters, at low crowding concentrations, approximately 85% of the LcK are active while rising to about 95% at high crowding concentrations. Our treatment of the regulation of Lck activity does not include well-known reactions. For example, Lck activates its own inhibitor, SHP1, a phosphatase that deactivates Lck by dephosphorylating the Y394 site. This is prevented by phosphorylation of the S59 site on Lck by a downstream signaling product, ERK. We ignore these details[36] that may be important for signal transduction in T-cells as it will perhaps not influence the qualitative results pertinent to the effects of macromolecular crowding.

Other moves that correspond to spin flips do not require that a species be bound to others. For example, species such as DS1 and DS2 can flip spin without being bound to PLCγ or SLP-76, respectively (solid arrows in Figure 2). Pho represents a generic phosphatase that can dephosphorylate any species and is the only species that will flip spins down from $+1$ to 0.

During T-cell recognition of antigen, a supramolecular structure called the immunological synapse forms in the junction between the T-cells and APCs.[24,25] A particular feature of the synapse is that there is a central accumulation of TCR and pMHC in an area that is several micrometers in size. This central cluster of TCRs is called the CSMAC. The signaling function of the CSMAC remains unresolved, but several insights have emerged recently that suggest that it serves to regulate signaling and receptor degradation.[27,46,47] We wanted to examine whether the effects of molecular crowding on signaling are different if the CSMAC forms compared to the situation where a spatially organized motif does not form. This cannot be studied comprehensively in our model because we ignore receptor degradation, which has been considered to be a key feature of CSMAC function.[27] Nevertheless, we can study the effects of crowding when certain species are spatially localized in a specific place as in the CSMAC. The formation of the CSMAC is known to be mediated by membrane and cytoskeletal forces that are connected to signaling processes.[39,48−51] In this study, we model formation of the CSMAC in a very approximate way that ignores connections to signaling processes. The CSMAC is taken to be a 40×40 square on the $x = 0,1$ plane. The CSMAC does not have a square shape, but since our interest is in understanding qualitative features of molecular crowding on signaling in a spatially organized motif, we believe that this simplification is unimportant. Once a TCR or pMHC molecule enters this region, there is an energetic penalty, ΔE_{csmac}, of $5k_bT$ for diffusing across the boundary that defines the CSMAC. If a TCR or pMHC is chosen to diffuse across the CSMAC boundary, then only if a random number uniformly distributed between zero to one is less than $(-\Delta E_{csmac}/k_bT)$ is the move accepted. This leads to accumulation of TCR, pMHC, and other signaling molecules in the CSMAC.

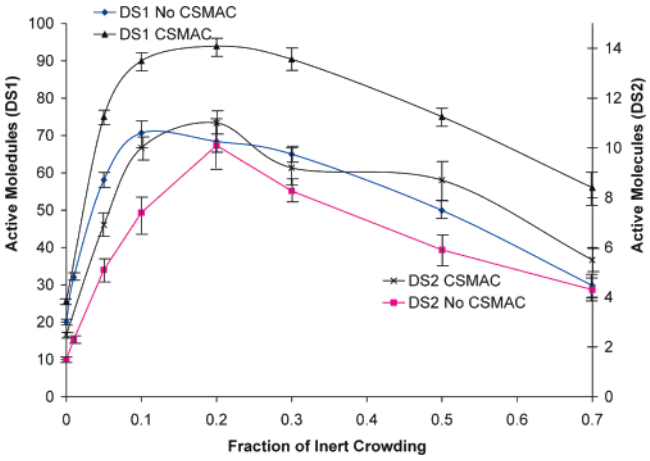

Figure 3. Equilibrium output of the signaling pathway as a function of the fraction of inert mobile objects in the system with and without a CSMAC. We used the activation of the final two species in the pathway shown in Figure 2 (DS1 and DS2) as a metric to determine how the pathway is affected by crowding. DS1 and DS2 have different interactions along the pathway leading to the differences in their equilibrium amplitudes. The maximum output is ~10−30% which is similar to the crowding density found in the cytoplasm.

We have made a number of other approximations to simulate the effects of molecular crowding on the T-cell signaling pathway. The signaling molecules cannot rotate, and there is no bonding direction specificity (bonds can be made on any side of a species). We have not incorporated all the active regions of each molecule.

2. Results and Discussion

We will use the number of activated DS1 and DS2 molecules (see Figure 2) as a metric to determine how T-cell signaling is affected by crowding. Since we do not include receptor downregulation, the system reaches equilibrium and we use the number of activated DS1 and DS2 molecules at equilibrium as a measure of signal strength. Our results may be considered to be relevant to time scales shorter than the time over which signaling attenuates because of the downregulation of triggered receptors. Furthermore, the qualitative results would not be different if we considered time scales that precede equilibrium in our system. However, since we are carrying out MC simulations, choosing a particular "time" to compare signal strength for different conditions is a bit ambiguous, so we chose to compare results at equilibrium. As noted in the Introduction, our main goal is to understand whether the dependence of signal output on the extent of molecular crowding is different in a spatially heterogeneous environment (typical of signaling in cells) compared to homogeneous systems[3,17] that have been studied previously. In particular, we want to understand whether it matters if the crowding agents are "quenched" or "annealed" over the time scale over which signal transduction occurs.

Consider first the situation where the inert objects that constitute the crowded environment are mobile. Figure 3 shows the signal strength for both DS1 and DS2 species as a function of the fraction of inert mobile objects in the system with and without a CSMAC. These results are averaged over at least six runs of 1×10^7 MC passes each (where MC passes are defined as the quotient of MC steps and total number of particles in the simulation).

The addition of an inert crowding agent has a dramatic effect on the amplitude of the pathway outputs. There can be roughly a 3- to 4-fold increase in signal strength upon the addition of molecular crowding agents. The main point to take away from Figure 3 is that, when the inert objects are mobile, the qualitative effects of molecular crowding on signal transduction in a spatially heterogeneous system are not different from those which have been described earlier.[3−6] The signal amplitude goes through a maximum as the degree of crowding is varied, and the maximum occurs when the fractional occupancy of crowding elements is between 10 and 30%, which roughly corresponds to the ranges of molecular crowding found in the cytoplasm of a cell.[1,2] For low levels of crowding elements, as crowding increases, entropic effects result in a larger effective chemical potential for the reactive species. This enhances the probabilities (rates) of the occurrence of chemical reactions (spin flips and bond formation). Our simulations show that this is particularly true for the binding of the cytosolic species (e.g., Zap70, SLP-76, and GADS) to the membrane-associated molecules and the number of these types of complexes increases dramatically when the fraction of inert objects is in the 10−30% range. For low volume fractions of inert objects, this effect dominates over the reduction in the rate of diffusion of reactive species in a crowded environment.[13−16,19] As crowding continues to increase, however, decrease in the frequency of "collisions" between reactive species due to the lower diffusion coefficient dominates and this results in an overall decrease in signal strength. Within our approximations (e.g., ignoring receptor degradation), molecular crowding does not have qualitatively different effects if CSMAC formation is allowed.

Thus, the results shown in Figure 3 demonstrate that the effects of molecular crowding on signaling pathways wherein the relevant biochemical reactions involve both membrane-associated and cytosolic species are no different than that in a homogeneous 3D medium.

We next examined whether this was also true when the inert objects that constitute the crowded environment are immobile. In other words, now the disordered distribution of crowding agents is quenched (as in ref 19), rather than annealed. Cells contain many organelles and macromolecular complexes that may not move much in the time scale associated with early signaling events such as that being considered in Figure 2. For example, calcium flux in T-cells occurs in less than a minute after stimulation. So, studying the effects of quenched crowding agents is a biologically relevant situation to study.

Figure 4 shows results for the same signaling outputs as Figure 3 but now as a function of the fraction of inert immobile objects. These results are obtained by averaging at least six runs of 5×10^6 MC passes each after equilibration. The results are quite different compared to the case where the inert objects are mobile. There is no maximum in signal strength, but instead, there is a plateau region followed by a sharp decrease. When there is a small to moderate number of inert immobile objects (~0−30%), the signal output is nearly constant. When the density of immobile objects is large (>40% with CSMAC, >60% with no CSMAC), signaling attenuates rapidly.

To understand this result, note that the signaling pathway that we simulate is one where most of the biochemical reactions occur in the membrane-proximal region. The addition of immobile or mobile inert objects increases the chemical potential of the reacting species and should have an enhancing effect on the reactions (vide supra). However, when many of the reactions occur in the membrane-proximal region and involve membrane-associated molecules, and the inert objects are immobile, there is a competing effect. The presence of quenched particles in a 2D manifold results in a more significant reduction in collision frequency between associating and reacting membrane proteins

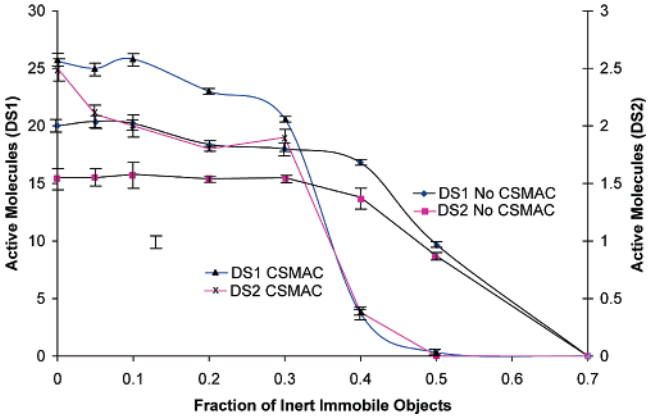

Figure 4. Equilibrium output of the signaling pathway as a function of the fraction of inert immobile objects in the system with and without a CSMAC. These results are averaged equilibrium conditions for at least six runs of 5×10^6 MC waves each. The results are dramatically different than what was found in the mobile case shown in Figure 3.

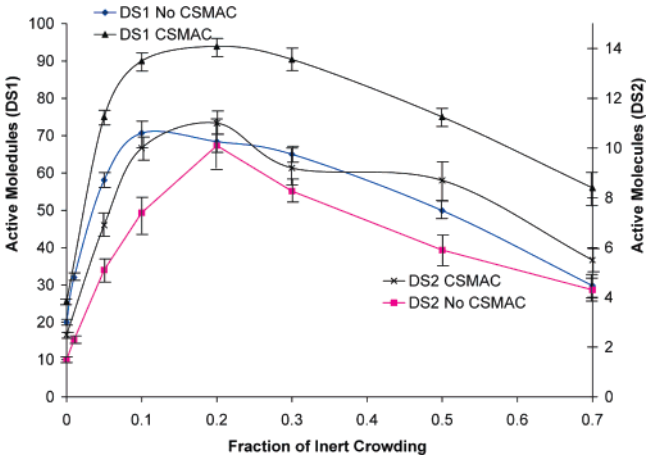

Figure 5. To prevent the surface blocking caused by immobile objects, we placed all of the immobile objects randomly in the grid but this time at least 10 grid units away from the membrane surface. Equilibrium output of the signaling pathway as a function of the fraction of inert immobile objects in the system with and without a CSMAC. These results are averaged equilibrium conditions for at least six runs of 5×10^6 MC waves each. Qualitatively, this behavior is similar to what was found in the mobile case.

compared to a situation where the molecules are free to move in three-dimensions or when the crowding agents are mobile. This is because the quenched inert objects can form regions where certain membrane-bound molecules are trapped and into which it is very difficult for other membrane-bound molecules to enter. This is not as significant an issue when the inert objects are mobile because these traps dissolve on the time scale over which the signaling reactions occur. The trapping effect is more pronounced when key reactions involve membrane-associated molecules because quenched obstacles in two-dimensions result in a far greater reduction in mobility for species that can only move in two-dimensions compared to the equivalent 3D situation. This is because in the latter situation there are more ways for the mobile molecules to escape from regions with a high concentration of molecular crowding agents. We believe that the greater reduction in mobility in two-dimensions when there are quenched obstacles cancels the enhanced rates of reaction due to increases in the chemical potential due to molecular crowding, and hence, there is no increase in signaling efficacy with fractional occupancy of inert objects in the 0−30% range. It is important to note the very important effect of dimensionality.

There is a weak decline in signal strength as fractional occupancy increases from 0 to 30%. We believe that, in this range, the decline is weak because sufficiently large regions that are free of crowding agents still exist on the membrane surface to allow a number of membrane-proximal signaling molecules to exist in each trap and 3D diffusion of the cytosolic species is not severely hindered. The sharp decline in signal amplitude, when the fractional occupancy of crowding agents is larger, is most likely due to the sharp reduction of diffusion in three-dimensions for the cytosolic species when the fraction of inert objects that constitute the quenched random medium exceeds a threshold. In fact, it could be related to a percolation transition.[52] The percolation transition for a system of hard-sphere obstacles is at about the range in which signal output declines sharply in Figure 4 for the case when there is no CSMAC formation. The decline in signal strength is more pronounced and occurs at a smaller fractional occupancy of inert immobile objects when the CSMAC forms. This is because the CSMAC concentrates TCR and pMHC in a particular region of the surface, and so signaling requires that other molecules must diffuse to this particular region for efficient signaling. This is more difficult than diffusion to multiple regions containing TCR and pMHC, and so signal attenuation sets in at a smaller fractional occupancy of inert immobile objects.

To test these ideas, we carried out simulations where immobile objects where placed randomly everywhere except in a region that was closer than or equal to 10 lattice units from the membrane plane. This prevents the formation of 2D traps on the membrane surface, such as that described above, and should therefore restore the qualitative behavior characteristic of cases where the inert objects are mobile. Figure 5 shows that this is precisely what happens, and a peak in signal intensity appears again at an optimal fractional occupancy of quenched inert objects. The results shown in Figures 4 and 5 vividly show that, when molecular crowding occurs due to immobile cellular components, the effects of varying its extent on signal transduction that is initiated at the membrane surface are quite different from that in homogeneous media[17] or when the inert objects are mobile.

It is worth remarking that maximal signaling occurs at a higher fractional occupancy in Figure 5 compared to the situation where there are uniformly distributed mobile objects (Figure 2). This is because, since there is no molecular crowding in the membrane-proximal region, cytosolic species are driven to this region. This enhances the rates of the reactions more than if mobile inert objects existed in the membrane-proximal region. Furthermore, the effects of reduced diffusion also set in at higher fractional occupancy when inert objects are excluded from the membrane-proximal region. Thus, the deleterious effects of a reduction in mobility begin to dominate over enhancement of chemical potentials of reacting species in a more crowded environment (compared to the case where mobile objects are uniformly distributed).

We have studied the effects of molecular crowding on a typical signal transduction network that is triggered by the binding of receptors and ligands on two apposed cell membranes (a simplified model for T-cell signaling in response to antigen stimulation). We have attempted to understand whether the effects of varying the extent of molecular crowding are different when biochemical reactions occur in a spatially inhomogeneous medium. We have also focused attention on whether there is a difference between situations where signal transduction occurs on time scales slower than that over which the crowding agents

move and vice versa. We find that, if the macromolecular species and organelles that result in crowding are mobile, the qualitative effects of crowding on a biochemical network where the reactions occur in a spatially inhomogeneous medium are not different from that in a homogeneous environment. In contrast, if the elements leading to crowding move on time scales much slower than the time scale over which the signaling reactions occur, then the effects of molecular crowding are dramatically different in a spatially inhomogeneous medium. We show that this is because immobile crowding agents present on a 2D manifold reduce the mobility and the frequency of collisions between membrane-associated molecules much more dramatically compared to situations where the crowding agents are mobile or the signaling molecules can move in 3D space. Since signal transduction often occurs on time scales faster than that associated with the motion of macromolecules and organelles, we hope that our results will motivate further theoretical and experimental studies on the potentially different effects of the extent of crowding on fast and slow signal transduction events.

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