Monte Carlo Cellular Automaton Simulation in Biomedical Science: Heterodimerization of Receptors

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Abstract: It has become well known that simulation can be used to investigate complex biomedical systems in situations where traditional methodologies are difficult or too costly to employ. Once the model, constructed to represent important aspects of the system under evaluation, has been validated, it may be used to investigate the effects of differences in the system inputs, changes in initial conditions, or its environment, and alterations in the system structure. Many recent advances in technology have greatly enhanced the power and expressiveness of simulation. We give an example where Monte Carlo cellular automaton simulation is employed to study heterodimerization of receptor proteins.

Key-Words: Monte Carlo simulation, heterodimerization, membrane receptors, signal transduction

1. Introduction

Some signaling molecules such as neurotransmitters, cytokines, growth factors, although all of which are called receptor ligands, are unable to permeate the hydrophobic cell membrane. Cells receive such information from an external environment through a class of proteins known as receptors which are located on the surface membrane. To initiate intracellular signal, binding of ligands to receptors is needed [1].

Many types of receptors such as GABA, neurotransmitter gamma-aminobutyric acid receptors, and Dopamine receptors, are activated by ligand binding individually, but some of them are activated after ligand-induced dimerization or oligomerization [2-3]. Moreover, the investigations of intracellular signal transduction pathways have revealed that the activities of several components in these pathways are also regulated by dimerization.

There are several examples in which activation of receptors are invoked after ligand-induced dimerization. The EFG receptor was the first protein-tyrosine kinase receptor to be shown to dimerize after ligand binding. The receptor for platelet-derived growth factor (PDGF), PDGF is classified as a receptor tyrosine kinase (RTK), which is the other type of cell surface receptors dimerized upon activation by PDGF [3].

The essential roles of ligand binding are different in different systems. In the case of stem cell factor (SCF) receptors, the bridging between two receptors, involving epitopes located outside the ligand-binding domains, are important for stabilization of receptor dimers. The ligand binding may work closely with related ligands and provide docking sites for downstream signal transduction molecules in heterodimeric complexes between ErbB2 and ErbB3 or ErbB4 [4]. In insulin-like growth factor 1 (IGF-1) receptor family, ligand binding does not induce receptor dimerization, but presumably causes a conformational alteration in the preformed dimeric receptor, which leads to receptor activation [3].

Receptor clustering due to an interaction between nearest-neighbor receptors appears to be a cooperative process in a statistical mechanics point of view [2]. Recently, based on the thermodynamics model for receptor clustering, the lattice Hamiltonian of receptor dynamics was proposed in [2, 5]. Their ideas can be a prototype model to simulate the dimerization process among receptors induced by ligand binding.

We believe that the dimerization process of receptors induced by ligand binding is similar to a process described by an aggregation limited diffusion [6] plus the Ising model [7] in which the reaction of each particle is governed by a lattice gas Hamiltonian. Moreover, the scaling law which is a good theory to describe a diffusion process should
still be applicable throughout the dimerization of receptors.

In order to examine these hypotheses further, we have written computer programs that simulate the diffusion and association of receptors over a two-dimensional membrane. The program is based on a simple random walk of receptor molecules over a fixed lattice. The interaction and dynamics of these particles is in the form of the lattice Hamiltonian which is proposed by Guo et al. [2]. A Metropolis algorithm [8] is used to determine which configurations are lower in free energy and therefore favored. We used this program to examine the formation of two-dimensional clusters in a defined area of surface membrane containing receptor molecules of different types (unliganded receptors and liganded receptors). In particular, we measured the number of dimers throughout the dynamics and try to define the power law that governs the process.

2. Model of Dimerization Process
To simplify the model for the dimerization process, we have made several assumptions. Our first step toward the ultimate goal is to provide a system of Hamiltonian that can be easily simulated and possesses the common characteristics of all dimerization processes of receptors induced by ligand binding.

This work is based on a thermodynamics model of receptors which was proposed by Guo et al. in 1999 [2]. According to this work, the dimerization of receptors is the result of ligand binding. After binding of a ligand to an unliganded receptor, a free receptor, the state of the receptor is changed to that with a lower energy level, and it is able to be induced to form a heterodimer with other unliganded receptors. Therefore, the total number of all species of interest in our simulation is three: a liganded receptor, an unliganded receptor, and a free space.

We focus our attention on the heterodimerization process only (schematically presented in Fig. 1). For the sake of simplicity, each receptor will be affected by the nearest neighbor receptors, called a short range interaction. Here, a clustering of receptors can be explained by means of a simple lattice Hamiltonian:

\[ H = H_0 + H_I \]  

where \( H_0 \) is the potential energy of the receptor in each lattice site, and \( H_I \) is the interaction Hamiltonian between two receptors.

\[ \mu \] is the interaction Hamiltonian in the form

\[ H_{\mu} = \sum_{i} \mu(t_i) n_i \]  \( \mu(U) = \mu_{R} + \mu_{L} + g_{L} \) . The summation is over all lattice sites.

 Besides the chemical potential affecting the Hamiltonian, the dimerization between receptors also changes the Hamiltonian. One might write an interaction Hamiltonian in the form

\[ H_I = -\sum_{i<j} J_{ij} \alpha(t_i, t_j) n_i n_j \]  

We further assume a short range interaction. Here, a nearest neighbor condition is to set \( J_{ij} = 1 \) only when \( <i,j> \) is the nearest neighbor site and \( J_{ij} = 0 \) otherwise.

The function \( \alpha(t_i, t_j) \) represents how each receptor interacts to other receptors or how much energy is required for dimerization. It is clear that this function should depend on the state of a receptor. For example \( a(U, U) \) is the energy between an unliganded receptor and an unliganded receptor, \( a(U, L) = a(L, U) \) is energy between an unliganded receptor and a liganded receptor, and \( a(L, L) \) represents the energy between a liganded receptor and a liganded receptor.

![Fig. 1. Schematic representation of different forms of dimeric complexes of typical receptors after ligand binding. (a) a homodimeric complex; (b) a heterodimeric complex of two receptor subunits.](image-url)
The functions $a(U,L)$ and $a(L,U)$ can be viewed as bond energy between these two receptors. In this simple model of dimerization, we are particularly interested in dimerization between different types of receptors. Moreover, the dimer arises via weak interaction (one or two hydrogen bonds). We will set the effective bond strength $g_e$ in the order of $g_e \approx 3k_B T$ [5].

In the ligand inducing dimerization, we assume that the dimerization between similar types of receptors will rarely be formed. For the sake of simplicity, we set $a(U,U) = a(L,L) = - g_e$. An example of these receptors is rhodopsin which prefers to form a dimer only in some arrangements [9]. In eq. (3), we can rewrite the interaction Hamiltonian as

$$H_I = \sum_{\langle ij \rangle} J_{ij} g_e n_i n_j \tau_i \tau_j$$

where the summation is over all lattice sites. We further define the states of receptors:

$$\tau_i = \begin{cases} 1 & \text{for } t_i = L \\ 0 & \text{for } t_i = U \end{cases}$$

The full form of our Hamiltonian can be written as

$$H_{\mu}(\mu_1, \mu_2) = \sum_{\mu} \mu(0) \mu(1) \tau_i \sim \sum_{\langle ij \rangle} J_{ij} g_e n_i n_j \tau_i \tau_j$$

### 3. Monte Carlo Simulation

Cell membranes were modeled using a square lattice of $n$ sites with periodic boundary condition, used to reduce the finite size effect. In general, cell membrane contains many kinds of particles which may move around the membrane. At the equilibrium state, any part of the membrane should have a constant flux of particles. Therefore, the periodic boundary condition is appropriate for this system. Each site on the lattice represents a possible receptor location, and each receptor’s size is one lattice site. No two molecules are allowed to occupy the same lattice site. To illustrate a simple statistical model of receptor dimerization, we performed Monte Carlo simulations with the conventional Metropolis algorithm [8]. For the sake of simplicity, the number of receptors was assumed to be conserved throughout the time evolution.

Receptors were initially distributed according to a given distribution function (random distribution or rectangular distribution), but became associated as their independent random movements brought them into contact. At each time step of the algorithm, $n$ randomly chosen sites in the lattice were selected for possible update. If the selected site, $s_j$, contained a receptor, then an attempt was made to randomly bring the receptor to a new nearest neighbor site, $s_j$. In accordance with the Metropolis Monte Carlo algorithm [8], the move is automatically accepted if it results in a decreased energy for the system, and is accepted with a probability of

$$P = \min[1, e^{-\beta \Delta H}]$$

where $\beta \equiv \frac{1}{k_B T}$, $k_B$ is the Boltzmann constant, and $T$ is the absolute temperature. If the target site was already occupied, then no move was made. The difference of the Hamiltonian could be calculated as

$$\Delta H = E_{\mu_2} - E_{\mu_1}$$

which is guaranteed by the Metropolis rule. The steady state of macroscopic properties of the Monte Carlo ensemble then corresponds to the thermodynamics equilibrium state of the system. We might write a pseudo algorithm for this simulation as consisting of the following few steps.

1. **Initialize each parameter**
2. **Monte Carlo step-loop.**
3. **Trial loop.** In each trial, a particle will be randomly chosen to offer a chance of moving to a new position.

   3.1 **Choose a particle randomly.**
   3.2 **Evaluate Hamiltonian $H_1$ of the selected particle.**
   3.3 **Offer a new position randomly which is close to the current position of the chosen receptor.** The chosen receptor is allowed to move if the new position is free.
   3.4 **Assume that the chosen receptor moves to the new position already.** Then evaluate Hamiltonian $H_2$.
   3.5 **Calculate a transition probability of the event in step 3.3 by means of the Metropolis algorithm.**

The transition probability is defined by

$$P = \min[1, e^{-\beta \Delta H}]$$

3.6 **Pick a number from uniform random number generator.** If the random number is greater than the probability $P = \min[1, e^{-\beta \Delta H}]$, the chosen receptor is allowed to move to the new position. If it is not greater than $P$, the event is rejected.
4. **Repeat step 3, the trial loop, up the number of particles in the system.**
5. **Repeat steps 2 and 3, the Monte Carlo step-loop, until the system reaches a steady state.**
Throughout our simulation, we are interested in the heterodimerization of receptors. We consider the evolution of the system as it undergoes dimerization process. We qualitatively monitor the temporal evolution of a dimering parameter (or disordering parameter),

\[ A = \sum_i \sum_j (1 - \delta(n_i, n_j)) J_{ij} \]  

(8)

where \( J_{ij} = 1 \) only when \( i, j \) are nearest neighbors and is 0 otherwise, and the summation is done over all lattices \( i \) and \( j \). The delta function \( \delta(n_i, n_j) = 1 \) if \( n_j \) is the same as \( n_i \), and \( \delta(n_i, n_j) = 0 \) otherwise.

First, both types of receptors are located at different lattice sites with periodic boundary condition. In the simulation, a filled circle and an open circle represent a liganded receptor and an unliganded receptor, respectively. A particle will be randomly selected in each trial to offer a chance of moving to a new position.

If a chosen particle lives without other receptors in its nearest neighborhood, it moves like a free diffusion. The unliganded receptor in Fig. 2a, which is denoted by the filled circle, is offering an opportunity to move to the right. Clearly, the Hamiltonian of this particle, \( H_1 \), is equal to \( H_0 \). If it has already moved to the new lattice site (Fig. 2b), the new Hamiltonian, \( H_2 \), must be evaluated and is equal to \( H_0 \). After that, a transition probability \( P = \min[1, e^{-\beta \Delta}] \) will be calculated. In this case, one sees that \( P = 1 \) which means that the particle will definitely move to the lattice site on the right as in Fig. 2b.

4. Results and Discussion

Throughout, a number of simple Monte Carlo simulations were first conducted in order to verify that thermodynamically expected behavior is reproduced in a simple system and two-dimensional aggregates are produced. All simulations were performed with two types of receptors, liganded receptors (L) and unliganded receptors (U), on a square lattice with the periodic boundary condition.

Fig. 3. Sequence of snapshots showing the dimerization process of a \( 80 \times 80 \) system with \([C_L] = [C_U] = 5\% \). The filled and opened squares represent liganded receptors and unliganded receptors, respectively, and the white region denotes the free space. The configurations were recorded at \( 0, 10^2, 10^4 \), and \( 10^7 \) MCS.

Fig. 2e shows an unliganded receptor, an open circle, surrounded by three liganded receptors, filled circles. This simulation considers only the short range interaction; therefore, the interaction between the farthest filled circle particle and the open circle particle is omitted. The Hamiltonian of the open circle particle is equal to \( H_i = H_0 + 3g_e \). If it is moving to the right, Fig. 2f, the new Hamiltonian will be equal to \( H_s = H_0 + g_e \). The lower energy renders the particle with the tendency to move to the right with the transition probability \( P = \min[1, e^{-\beta \Delta}] \).

Fig. 3. shows the result of a typical simulation. Both receptors were initially distributed at random positions (Fig. 3a.), but became associated as their independent random movements brought them into contact. After \( 10^7 \) steps (Fig. 3c.), many dimers had formed. As the simulation...
continued, the number of dimers or clusters seemed to slightly fluctuate. We assessed the extent of dimerization by counting the number of broken bonds, a term used as a dimering parameter (Fig. 4., open circles corresponding to AB-Bonds). Other measurements were also made such as the number of similar bonds, bonds between the same species (Fig. 4., filled circles), the mean cluster size, and the number of monomeric molecules.

This study was concerned with the kinetics, rather than the equilibrium state. From Fig. 4., we focused on the intermediate regime, but the simulation was still performed until the time step of $10^7$ at which the system had reached the steady state. The numbers of both types of bonds are constant beyond this time step. To obtain the smooth curves and small fluctuations, the simulations under typical conditions were repeated several times. We then used these averaged results in our analysis. The straight reference line which appears in the second regime (I) has slope $0.252 \pm 0.001$ and the saturation curve in the last regime (S) yields to the number of the broken bonds at equilibrium state which is $252 \pm 5$. We also found that the crossover time $t_L$ is $353 \pm 5$.

Fig. 4. Plot of the broken bond (AB-Bond) and the similar bond (AA-Bond) against Monte Carlo Step (MCS) for a $80 \times 80$ system with concentration of liganded and unliganded receptors: $[L]=[U]=5\%$. We observe the emergence of an early region (E), an intermediate region (I), and a late or saturation region (S). The line of AB-Bonds appears almost symmetrical to that of AA-Bonds.

The formation of the number of broken bonds is influenced by a large number of factors, and it is almost impossible to identify all of them. Nevertheless, we might initially guess that the number of broken bonds mainly depends on four quantities: the lattice size $L$, the concentration of liganded receptors $C_L$, the concentration of unliganded receptors $C_U$, and time $t$. It is not unreasonable to expect that there is a basic law which determines the number of broken bonds in terms of these factors. In the fractal concepts [10], the relationship between the number of the broken bonds $A(L,C_L,C_U,t)$ and the time step in the intermediate regime can be assumed as

$$A(L,C_L,C_U,t) \sim t^\beta$$

(9)

The exponent $\beta$, which we call a dimering exponent, characterizes the time-dependent dynamics of the heterodimerization process. However, the dynamics of the system does not only depend on a time step $t$, but also conditional on the lattice size $L$.

We also performed a series of simulations in which the equilibrium state was measured for different starting parameters, especially the system size. We varied the system size by letting $L \times L = 40 \times 40, 60 \times 80, 80 \times 80$, and $200 \times 200$. Even though the system size changes, the concentrations of liganded receptors and unliganded receptors are fixed: $[C_L]=[C_U]=5\%$. The other parameters and conditions are similar to those in the previous section.

Fig. 5. Data on the number of heterodimer (AB-Bond) versus Monte Carlo Step at the lattice sizes $40 \times 40, 60 \times 60, 80 \times 80$, and $200 \times 200$.

From the plots in Fig. 5 of the broken bonds against Monte Carlo steps, for four sizes of the lattice, it may be seen that all curves display similar behaviors. They go through a relaxation period in the first regime (E). Then the numbers of broken
bonds grow steadily. Finally they become constant in time.

<table>
<thead>
<tr>
<th>Lattice size</th>
<th>Saturated no. of broken bonds</th>
<th>Dimering exponent ($\beta$)</th>
<th>Crossover time ($t_L$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>61.01</td>
<td>0.246 ± 0.003</td>
<td>392.39</td>
</tr>
<tr>
<td>60</td>
<td>140.27</td>
<td>0.248 ± 0.002</td>
<td>384.98</td>
</tr>
<tr>
<td>80</td>
<td>251.53</td>
<td>0.252 ± 0.001</td>
<td>353.19</td>
</tr>
<tr>
<td>200</td>
<td>1581.30</td>
<td>0.247 ± 0.001</td>
<td>375.96</td>
</tr>
</tbody>
</table>

Table 1. Parameter values from the simulation data shown in Fig. 5, giving the saturated number of the broken bonds, the saturated exponent ($\beta$), and the crossover time ($t_L$).

When the system reached the equilibrium state, the number of broken bonds depending on the system size could be expressed as [10]

$$A(L, C_L, C_U; t) \sim L^{\alpha}$$  \hspace{1cm} (10)

where $\alpha$ was the disordering exponent. According Fig. 5., the saturated exponent $\alpha$ was found to be 2.010 ± 0.004 for all the sizes considered.

The simulation results and the power law assumption Eq. (9) and (10) can be combined into a finite-size scaling expression of the form

$$A(L, C_L, C_U; t) \sim L^{\alpha} f\left(\frac{t}{t_L}\right)$$  \hspace{1cm} (11)

where $f(x)$ is a scaling function defined by

$$f(x) = \begin{cases} 
  x^\alpha & \text{for } x << 1, \\
  \text{constant} & \text{for } x >> 1,
\end{cases}$$

Fig. 6. The data in Fig. 5. re-plotted with $L^{\alpha} f(t/t_L)$ . Here, $\alpha = 2.0$ and $t_L = 370$ have been used to collapse all of the data points onto a single curve.

To test this assumption, we plotted $A(L, C_L, C_U; t)/L^{\alpha}$ against $t/t_L$ for several values of the system size. Fig. 6 shows that, with the chosen parameter values, the data points are collected along a single curve, supporting the validity of the scaling assumption in (11).

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References: