

# Thermodynamics of clustered and unclustered receptor systems in cell adhesion

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## Abstract

The thermodynamics of cell adhesion are regulated by the conformation, energetics and dynamics of adhesion receptors. In addition, clustering of these receptors regulates adhesion as well as downstream signaling. In this Letter, we present an integrated model incorporating Monte Carlo simulations and a mean field approach to study the effect of receptor clustering on free energy of cell adhesion. The Monte Carlo simulation incorporates receptor diffusion and dimerization and provides input for the mean field computation of free energy of adhesion in a receptor–ligand–solvent system. Our results show that cell adhesion is regulated by high co-operativity and synergy between receptor density and interaction energy.

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## 1. Introduction

Receptor–ligand binding is an important mechanism in maintenance of cellular homeostasis. The binding of extra-cellular matrix ligands to trans-membrane integrins triggers many cellular processes, e.g. signal transduction, cell proliferation, differentiation, migration, etc [1–8]. A key feature of receptor activity is the formation of aggregates or clusters [1,9–12]. These clusters initiate focal adhesions and trigger numerous signaling pathways. A quantitative description of cell adhesion therefore requires understanding the effect of clustering on cell–matrix interactions.

Receptor–ligand interactions in cellular systems have been studied through a variety of computational and experimental methods [13–24]. However, the thermodynamic aspects of cell adhesion, mediated by receptor clustering,

have been elusive. It is not clear in what regimes clustering leads to decrease in free energy of interaction and under what circumstances receptor clustering has negligible or even a negative effect on binding. In order to answer this question, we have developed a framework integrating a lattice free Monte Carlo model with a previously described mean field theory for cell adhesion [25]. Application of a lattice free model allows us to simulate receptor diffusion on cell membranes of any curvature and hence has applications to cells embedded in matrices. The Monte Carlo simulation provides equilibrium distribution of receptors that serves as an input in the mean field cell–matrix interaction model. The mean field model incorporates receptor and ligand conformations, entropic repulsion effects, as well as long- and short-range interactions of receptors and ECM molecules. The outcome of our model is a direct comparison of the free energy of adhesion in clustered and unclustered systems under varying receptor concentrations and cell–substrate interaction energies. The results of the model provide a quantitative description of the regimes where clustering provides a clear advantage in cell adhesion and regimes where cell adhesion does not depend upon receptor clustering.

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## 2. Method

Our system of interest is illustrated in Fig. 1. The two plates are parallel to each other, with receptors tethered from the top one, ligands and solvent molecules embedded in between. Both plates are assumed to be planar and impenetrable. We apply a 2-step method to calculate the free energies of adhesion. First, we use a Monte Carlo routine to calculate the equilibrium distribution of receptors and cluster sizes. Second, we input the results derived from the first step and calculate the free energy for clustered and unclustered systems.

### 2.1. Monte Carlo simulation to find distribution of receptor cluster

Cell adhesion receptors are simulated by a coarse-grained method incorporating amino groups,  $C_{\alpha}$ 's and carboxyl groups in amino acid backbone and uses hard-spheres for side chains. The dihedral angles are chosen randomly from allowed basins in the phi/psi map of amino acids. The binding and interaction region of the chain is assumed to be the last 20 amino acids in the 200 amino acid receptors. The receptors can only bind when the binding region of two chains are within a certain interaction distance. This coarse-grained assumption is based on numerous studies of protein–protein interaction and binding that suggest that binding and aggregation occurring only when two protein molecules are in the correct conformation.  $10^6$  unique conformations of the receptor molecules are generated using our coarse-grained simulations.

Once we generate unique conformations of receptors, we allow the receptors to undergo random walk and dimerize/monomerize until the equilibrium state is reached, whereupon cluster sizes are counted. While previous studies have shown that that binding itself is initiated by atypical conformations that arise due to fluctuations rather than equilibrated average conformations [26], the purpose of our study in this Letter is not to model the dynamics of how cell adhesion is initiated, but to study at equilibrium, the

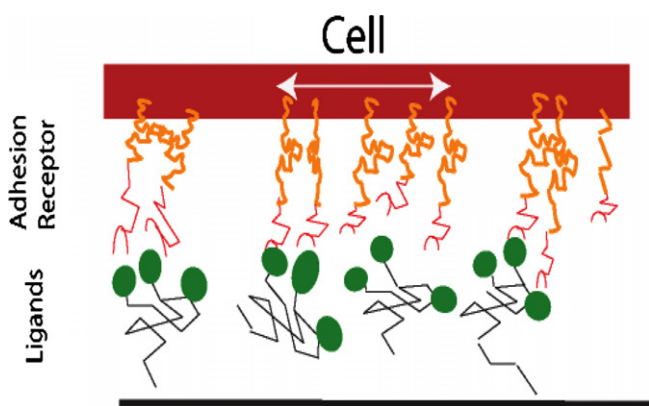


Fig. 1. A cartoon depicts the system of interest. Receptors are tethered on the top plate and are 200 monomers long. 20mer RGD ligands can bind to multiple receptors in the cluster.

free energy features of the receptor–ligand–solvent system. In this regard, previous computational and experimental studies (Ref. [11] and references therein) have shown that only under circumstances where ligands are clustered or are islanded, the binding of receptors to ligands has big effect on receptors clustering. On the other hand, it has been observed that when the ligands are homogeneously distributed, as is the case with the current study, the effect of ligand–receptor binding on overall clustering is minimal. Our lattice free model to calculate cluster sizes is based upon previous lattice based models [11] with a number of critical improvements. Firstly, our receptors are chosen from the conformation space and hence are not identical. Secondly, we have used a lattice free model that does not suffer from unrealistic constraints of a lattice model.

After the generation of unique conformations, we allow the receptors to undergo random walk without any change in conformations. We sample a large number of conformations (on the order of  $10^6$ ) to represent the set of possible conformations of receptors. Since during binding the receptors do not undergo significant conformational changes, but rather experience small perturbations in conformations, our approach to simulate a large number of receptor configurations and then freezing them for free energy calculations allows us to capture realistic experimental scenarios. Two receptors can bind if their interaction regions are within the interaction distance. The excluded volume constraint is executed by the calculation of centers-of-masses distances of the receptors and the interacting regions to ensure that steric constraints of binding are satisfied. At each time step, each receptor chooses from one of the following three options: dimerization with a neighboring receptor, monomerization, or diffusion one step of length  $l$  with respective probabilities  $P_{\text{dimer}}$ ,  $P_{\text{mono}}$ , and  $P_{\text{move}}$ . If more than one receptor is within the interaction distance of a monomer, the partner is chosen randomly. A converse process is carried out for monomerization of the receptor from its partners. Lastly if a receptor is in a monomeric state and chooses to diffuse, it picks up a random direction and diffuses one step of length  $l$ . If a receptor is a dimer, it can either approach of move away from its partner by length  $l$  or move together with its partner by a distance  $l/2$ . The estimate of the probabilities  $P_{\text{dimer}}$ ,  $P_{\text{mono}}$ , and  $P_{\text{move}}$  is based on assumption of these processes as Poisson processes with small time step  $\Delta t$ , e.g.

$$P_{\text{mono}} = \exp(-k_{\text{mono}}\Delta t) \sum_{n=1}^{\infty} \frac{(k_{\text{mono}}\Delta t)^n}{n!}$$

$$= 1 - \exp(-k_{\text{mono}}\Delta t) \approx k_{\text{mono}}\Delta t \quad (1)$$

To study the effect of receptor density, we study the effect of four different receptor concentrations,  $1000 \mu\text{m}^{-2}$ ,  $2000 \mu\text{m}^{-2}$ ,  $4000 \mu\text{m}^{-2}$  and  $8000 \mu\text{m}^{-2}$ . The discretized time step  $\Delta t$  is set to  $10^{-6}$  s. We assume  $P_{\text{dimer}} = 0.1$ ,  $P_{\text{mono}} = 10^{-3}$ ,  $P_{\text{move}} = 0.1$  which is consistent with previous lattice based simulations of clustering and aggregation. In real simulation, we used the normalized

probabilities with total steps equal to  $10^4$ . We used the following two-fold equilibrium criteria: (1) the cluster-size distribution is unchanged within tolerance; (2) the number of dimers is unchanged within tolerance. The cluster size of a receptor and receptor distribution is determined by counting all receptors within the interaction distance of receptor, which is set to be 12 nm. Simulations are carried out multiple times to ensure that our results are not affected by initial positions of the receptors.

## 2.2. Calculations of free energy

In order to calculate the free energy, we define an interaction region between the cell membrane and virtual plate 60 nm away from the cell. The space between the cell and this virtual plate is the region of interest for our calculations. The choice of 60 nm long interaction region is based on the fact that nearly all of the receptors (~90%) are located are within 60 nm from the cell. Therefore, in our model the interaction region is defined as the region between 0 and 60 nm from the top plate. The ligands for receptors are 20 repeating units of RGD peptide. The conformations of these ligands are also computed by coarse-grained dynamics, where the phi/psi angle distributions are derived from allowed phi/psi values for the three amino acids. The 20mer RGD peptides are distributed randomly in the interaction region. Since each RGD unit can bind to a receptor, presence of up to 20 units allow for multiple binding of receptors to a single ligand. The number of bonds is determined by the cluster size of the receptors. The larger the cluster size, the more receptor–ligand bonds form. We adopt an ensemble average method which takes the cluster size distribution derived from the Monte Carlo simulations as an input. For example, if the percentage of appearance of cluster sizes is  $\{p_i\}$ , where  $i$  denotes the cluster size, and the number of bonds formed with receptors are  $\{b_i\}$ , the ensemble average of the receptor–ligand bond number is given by

$$\bar{b} = \sum_i p_i b_i \quad (2)$$

We utilize a previously described method to minimize the free energy of the system to calculate the probability distribution function (PDF) of receptors and ligands as well as the spatial dependent osmotic pressure [25,27–30]. The free energy of the system of interest can be described by the following relationship:

$$F = k_B T N_R \sum_{\{\alpha\}} P_{R\alpha} \ln P_{R\alpha} + k_B T N_P \sum_{\{\beta\}} P_{P\beta} \ln P_{P\beta} + \frac{k_B T A}{v} \int dz \varphi_s(z) \ln \varphi_s(z) - e_{r-l} \bar{b} N_P \sum_{\{\beta\}} P_{P\beta} \delta(\beta) - e_{r-r} f_{r-r} N_R \quad (3)$$

where  $k_B$  is the Boltzmann constant,  $T$  is the temperature of the system,  $N_R$ ,  $N_P$  is the total number of receptor and ligand molecules, respectively.  $P_{R\alpha}$ ,  $P_{P\beta}$  is the probability

of finding receptors or RGD proteins in the  $\alpha$ th or  $\beta$ th conformation, separately,  $A$  is the area of any of the two plates and  $v$  is the Van der Waals volume of solvent. Our model assumes that the volume of one segment of receptor or RGD protein is equal to  $v$ . This assumption is based on previous studies and models of cell adhesion.  $\varphi_s(z)$  is the volume fraction of solvent in the layer ( $z$ ,  $z + dz$ ).  $e_{r-l}$  and  $e_{r-r}$  are the binding energies of one receptor–ligand pair and receptor–receptor pair, individually. For simplicity, we assume  $e_{r-l} = e_{r-r} = e$ .  $\delta(\beta)$  is 1, where the interaction segments of RGD falls into the interaction region, and 0 otherwise.  $f_{r-r}$  is the fraction of receptor–receptor bonds over  $N_R$ , which is derived from the Monte Carlo simulation. Dividing Eq. (3) by  $k_B T A$  yields:

$$f = \frac{F}{k_B T A} = \sigma \sum_{\{\alpha\}} P_{R\alpha} \ln P_{R\alpha} + r_P \sigma \sum_{\{\beta\}} P_{P\beta} \ln P_{P\beta} + v^{-1} \int dz \varphi_s(z) \ln \varphi_s(z) - r_P \sigma \bar{b} e' \sum_{\{\beta\}} P_{P\beta} \delta(\beta) - e' f_{r-r} \sigma, \quad (4)$$

where  $\sigma = \frac{N_R}{A}$  is the surface coverage of receptor.  $r_P = \frac{N_P}{N_R}$  is the ratio of protein to receptor.  $e' = \frac{e}{k_B}$  is dimensionless binding energy. Short-term repulsion among segments of chain molecules and solvents is accounted by solvent incompressibility. In the layer ( $z$ ,  $z + dz$ ), it is of the form:

$$\langle \varphi_R(z) \rangle + \langle \varphi_P(z) \rangle + \varphi_s(z) = 1, \quad (5)$$

where  $\varphi$ 's are volume fractions, e.g.,

$$\langle \varphi_R(z) \rangle = \frac{N_R \langle n_R(z) \rangle dz v}{A dz} = \sigma v \sum_{\{\alpha\}} P_{R\alpha} n_{R\alpha}(z) \quad (6)$$

$\langle n_R(z) \rangle$  is the average number of segments of receptor in the layer ( $z$ ,  $z + dz$ ).  $n_{R\alpha}(z)$  is the number density of segments of the  $\alpha$ th conformation of receptor, which we derive from the conformational sampling. Now the constraint equation can be written as

$$\sigma v \sum_{\{\alpha\}} P_{R\alpha} n_{R\alpha}(z) + r_P \sigma v \sum_{\{\beta\}} P_{P\beta} n_{P\beta}(z) + \varphi_s(z) = 1 \quad (7)$$

We minimize Eq. (4) with respect to two pdf's  $P_{R\alpha}$  and  $P_{P\beta}$  and solvent fraction  $\varphi_s(z)$ . Taking into consideration the packing constraint, Eq. (5), we introduce a Lagrangian multiplier  $x(z)$ , which is the dimensionless osmotic pressure. The minimization results in

$$P_{R\alpha} = \frac{1}{g_R} \exp \left( - \int dz x(z) n_{R\alpha}(z) \right) \quad (8)$$

$$P_{P\beta} = \frac{1}{g_P} \exp \left( - \int dz x(z) n_{P\beta}(z) + \bar{b} e' \delta(\beta) \right) \quad (9)$$

$$\varphi_s(z) = \exp(-x(z)) \quad (10)$$

$$\sigma v \sum_{\{\alpha\}} P_{R\alpha} n_{R\alpha}(z) + r_P \sigma v \sum_{\{\beta\}} P_{P\beta} n_{P\beta}(z) + \exp(-x(z)) = 1 \quad (11)$$

in which,  $g_R = \sum_{\{z\}} \exp(-\int dz x(z)n_{Rz}(z))$  is the normalization factor (partition function) for receptor, and similarly,  $g_P$ . Taking surface coverage of receptors  $\sigma$ , volume  $v$ , average bond number  $\bar{b}$ , binding energy  $e'$ , the ratio of protein to receptor  $r_P$ , pdf's  $P_{Rz}$  and  $P_{P\beta}$ , and the distribution of ligands  $\delta(\beta)$  as inputs, Eqs. (8), (9), and (11) form a closed set.

To solve the above equations, we follow the method of Szleifer and co-workers to discretize the space between the two surfaces into a series of layers [31]. We then iterate these equations to arrive at a final solution. In the following equations, subscripts  $i$  and  $j$  is used to denote discrete layers at different heights. The equations now have the following form:

$$P_{Rz} = \frac{1}{g_R} \exp\left(-\sum_j x(j)n_{Rz}(j)\right) \quad (12)$$

$$P_{P\beta} = \frac{1}{g_P} \exp\left(\bar{b}e'\delta(\beta) - \sum_j x(j)n_{P\beta}(j)\right) \quad (13)$$

$$\phi \sum_{\{z\}} P_{Rz} n_{Rz}(i) + r_P \phi \sum_{\{\beta\}} P_{P\beta} n_{P\beta}(i) + \exp(-x(i)) = 1, \quad (14)$$

where  $\phi = \frac{N_R}{A\delta/v}$  are surface fraction of receptors,  $\delta$  is the width of one layer.

In this Letter, we study the effect of initial concentration of receptors as well as interaction energy between receptors and ligands on adhesion in clustered and unclustered systems. By comparing the overall free energy with these two variables, our model can quantify the regimes where receptor clustering dominates adhesion and regimes where clustering provides no benefit in overall adhesion free energy.

### 3. Results and discussion

We first study the distribution of cluster sizes as a function of initial receptor concentration. Fig. 2 shows the distributions of four different receptor concentrations,  $1000 \mu\text{m}^{-2}$ ,  $2000 \mu\text{m}^{-2}$ ,  $4000 \mu\text{m}^{-2}$  and  $8000 \mu\text{m}^{-2}$ . The average cluster size increases with increase in initial concentration of the receptors, and shows that at the receptor concentration  $1000 \mu\text{m}^{-2}$ , most receptors exist in the form of monomer. As the receptor concentration increases, so does the cluster size, with a Poisson like distribution.

Fig. 3 shows the free energy advantage for receptor clustering as a function of receptor concentration and interaction energy. We note that the change in free energy between clustered and unclustered systems varies non-linearly with increase in receptor concentration. For low interaction energies, the free energy change is mainly linear, but becomes highly non-linear at higher receptor concentrations. Our results indicate that for weakly interacting systems, such as interaction of receptors with matrices or ligands with poor binding, increase in clustering provides

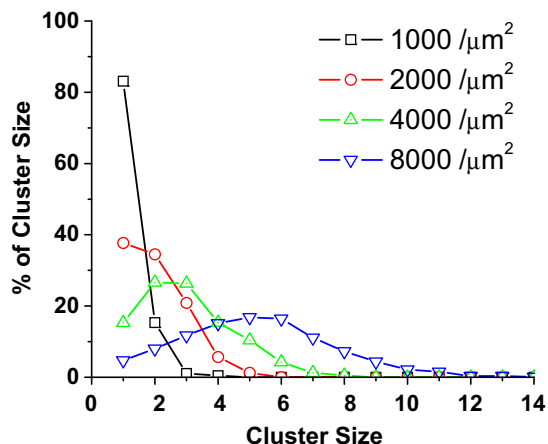


Fig. 2. The cluster size distribution for receptor concentration  $1000 \mu\text{m}^{-2}$ ,  $2000 \mu\text{m}^{-2}$ ,  $4000 \mu\text{m}^{-2}$  and  $8000 \mu\text{m}^{-2}$ .

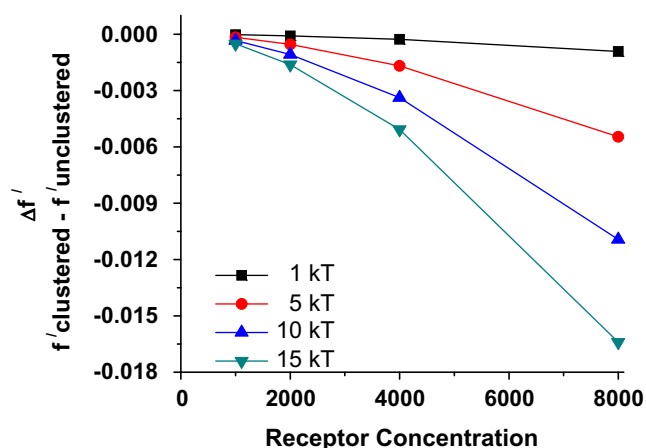


Fig. 3. The free energy difference for clustered and unclustered systems as a function of receptor concentration.  $\Delta f'$  is the dimensionless free energy difference, in unit  $(F/k_B T)(v/A\delta)$ . The results indicate that the stability of the system is a non-linear function of receptor concentration and interaction energy. At higher interaction energies, the system is highly non-linear as opposed to weakly interacting systems, which show negligible dependence on clustering.

no significant advantage in the overall stability of the system. On the other hand, for highly interacting systems, a smaller increase in receptor concentration can lead to greater stability of the system.

The non-linear observations of Fig. 3 allows us to answer an interesting and potentially useful question. Is clustering beneficial for adhesion, and if so, in what regimes is clustering most efficient? The answer to this question is depicted in Fig. 4. The figure shows 3D plot of the difference in free energy between the clustered and unclustered systems for various receptor concentrations and interaction energies. The figure suggests that at low interaction energies, up to  $5k_B T$ , clustering has no effect, irrespective of the cluster size or the initial concentration. The figure also suggests that at receptor concentrations less than  $4000 \mu\text{m}^{-2}$ , clustering provides no advantage over

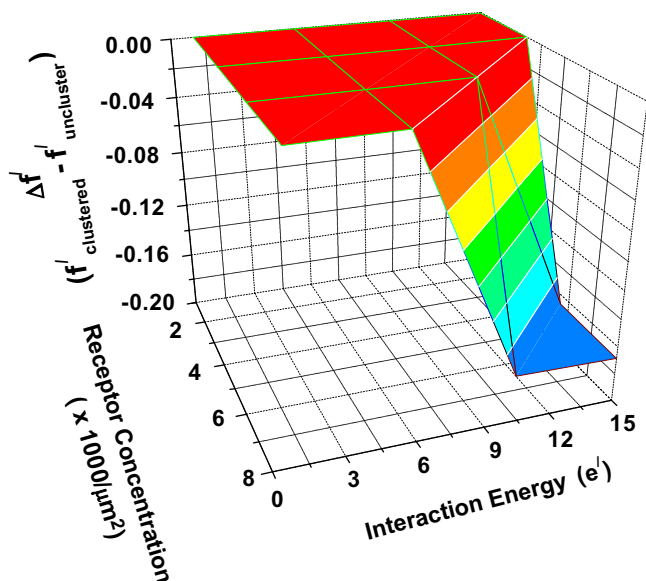


Fig. 4. The change in free energy between clustered and unclustered systems is shown as a function of receptor concentration and interaction energies. Only energies greater than  $10k_B T$  and receptor concentrations higher than  $8000 \mu\text{m}^{-2}$  result in decrease in the  $\Delta f'$ .

unclustered systems, regardless of interaction energy. Only at cluster sizes of  $4000 \mu\text{m}^{-2}$  and above, and interaction energies greater than  $10k_B T$ , clustering of receptors results in increase in adhesion. While interaction energies over  $10k_B T$  are not unusual in biological systems, receptor concentration is often modulated by external or internal signaling and inhibitors. Our results show that both interaction energies or receptor concentrations alone are not sufficient in yielding a significant advantage and while both of these conditions are necessary, they are not sufficient by themselves. We note that by keeping energies greater than  $10k_B T$ , but decreasing receptor density by a factor of two (from  $8000 \mu\text{m}^{-2}$  to  $4000 \mu\text{m}^{-2}$ ) would result in an unstable regime of the free energy plot, where clustering provides no benefit. Similarly, large clusters by themselves are not particularly useful at interaction energies of  $10k_B T$  or less, as they provide no significant benefit in overall adhesion.

In summary, we have developed an integrated model of receptor clustering and adhesion to computationally inves-

tigate the role of adhesion in cell adhesion. Our method combines Monte Carlo studies of receptor diffusion and dimerization as well as mean field calculation of adhesion free energy. Our results show the synergy between interaction energy and cluster size in determining the free energy landscape of adhesion and will useful information about the complex receptor–ECM interactions.

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