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Receptor aggregation by intermembrane interactions: A Monte Carlo study

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Abstract

The lateral organization of receptors on cell surfaces is critically important to their function; many receptors transmit transmembrane signals when redistributed into clusters, while the response of others is potentiated by their aggregation. Cell–cell contact can play a crucial role in receptor aggregation, even when the bonds between receptors on one cell and ligands on the other are monovalent. Monte Carlo simulations on a two-membrane model were carried out to determine whether weak enthalpic interactions among receptors in one membrane, and among ligands in another, can work synergistically to give large-scale clustering when the two membranes are brought into contact. The simulations give support to such a clustering mechanism. In addition, because clustering is a cooperative process akin to a phase separation, individual receptors and ligands may undergo repeated binding and unbinding while in a clustered "phase," and a single ligand could interact with multiple different receptor partners. The results suggest a resolution of the dichotomy between serial triggering and aggregation models of T cell activation.

Keywords: Monte Carlo simulation; Receptor aggregation; T cell; Lattice model; Phase separation

1. Introduction

Ligand-induced receptor clustering is a transmembrane signaling mechanism that is ubiquitous in cell biology. In the prototypical cases (e.g. IgE receptors and other immune receptors), the cytoplasmic tails of the receptors contain enzymatic domains with tyrosine kinase activity, and other enzymatic domains that are activated by tyrosine phosphorylation. In the isolated receptor, the kinase is sterically unable to autophosphorylate the receptor; the clustering of receptors results in transphosphorylation and thus receptor activation [1–3].

Receptor aggregation now appears to be critical not only for those receptors that are directly activated by transphosphorylation, but also for G-protein coupled receptors (GPCR), which have generally been considered to be monomeric. Significant evidence has accumulated that these receptors form clusters or oligomers in the membrane. The formation of these oligomers may be a mechanism for cooperativity in ligand binding and receptor activation, providing a more nonlinear response (better 'switching power') in the GPCR system [4,5].

Because of the great biological significance of receptor clustering, many different theoretical studies have been undertaken to elucidate various mechanisms that can, in principle, give rise to such non-uniform receptor distributions. In the simplest situation, receptors and ligands are multivalent, allowing direct physical (non-covalent) cross-linking to drive receptor redistribution. This is the case with the immunoglobulin E receptors (IgE-R), for example. Even in this 'simple' case, the actual distributions of aggregate sizes, and their kinetics of formation, are complicated and have been the subject of a number of theoretical and experimental studies [6–8]. These analyses are especially important when one considers the fact that the steric constraints on IgE-R transphosphorylation seem to prevent some dimerized receptors from signaling at all [9]; thus, higher-order aggregates are essential to effective signaling.

Regardless of the stoichiometric and kinetic complexity in IgE receptor cross-linking, the mechanism of clustering, at any rate, is straightforward. In other instances, the origin of the physical forces causing receptor aggregation is less clear.

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Of particular importance is the dramatic redistribution of cell surface receptors on T cells, when they are brought into contact with an antigen-presenting bilayer or cell [10]. In this and other cases, the physical mechanism for aggregation has not yet been definitively established. A number of interesting proposals have been put forth regarding high order clustering. For example, it has been suggested that transient dimerization can lead to larger-scale aggregation through a rapid partner-switching mechanism, and Monte Carlo simulations support that concept [11]. An alternative explanation involves membrane fluctuations. When target ligands are membrane-bound, there should be entropic benefits to aggregation of liganded receptors. Each ligand-receptor bond pins the membranes together and reduces the overall entropy of the system. Aggregating the pinning points reduces the receptor entropy, but increases the entropy of much of the membrane, which is now free to undergo thermally driven spatial fluctuations [12–14]. Such a view is supported by experiments conducted more than two decades ago showing that IgE receptors will aggregate when monovalently bound to laterally mobile ligands in a supported lipid bilayer [15]. Similar effects have been observed in Monte Carlo simulation of stiff membrane inclusions, which can entropically phase separate [16].

We consider here an alternative, simple enthalpic mechanism for the aggregation of receptors (such as T cell receptors) by monovalent ligands presented on a companion cell membrane. The mechanism is based on the fact that aggregation is highly non-linearly dependent on intermolecular interaction strength: below a threshold interaction energy, the distribution of membrane proteins or receptors will appear to be quite random, while above the threshold, large-scale aggregation occurs as a two-dimensional phase separation. If weak attractive interactions exist among receptors on one membrane, and among ligands on the other, the receptor-ligand binding might then "sum" those interactions to give large ligand-receptor clusters. This study confirms this expectation, and provides an indication of what interaction strengths would be necessary for the mechanism to be effective. These enthalpic contributions, perhaps acting in concert with various kinetic or entropic mechanisms, could cause dramatic switching from a dispersed to an aggregated receptor distribution. The inherent non-linearity of the physical process of phase separation can thus provide a powerful switching mechanism in biological systems.

2. Methods

Cell membranes were modeled using a square lattice of n sites ($n=10^4$, typically) with periodic boundary conditions. Each site on the lattice represented a possible protein location, and each protein's 'size' was one lattice site. At each time step of the algorithm, n randomly chosen sites in the lattice were selected for possible update. If the selected site, s_0, contained a protein, then an attempt was made to move the protein to a randomly chosen adjacent (non-diagonal) site. In accord with the Metropolis Monte Carlo algorithm, the move was automatically accepted if it resulted in a decreased energy for the system, and was accepted with a probability of

$$P = e^{-\Delta E/k_B T} \tag{1}$$

if the move increased the energy by ΔE. (k_B=Boltzmann constant, T=absolute temperature). If the target site was already occupied, then no move was made. The energy of the system was taken to be proportional to the number of protein–protein (non-diagonal) contacts in each state; thus, the change in energy is

$$\Delta E = -\varepsilon k_B T\left(N_{pp,s1} - N_{pp,s0}\right) \tag{2}$$

where $N_{pp,s}$ is the number of contacts when the protein is at site s, and ε is a dimensionless energy.

Intermembrane interactions were modeled by placing two membranes together to form a 2-ply space. Proteins were placed on both membranes. Proteins were restricted to one membrane in their motions, but the energy of the system was lowered when proteins in the two membranes 'overlapped', i.e. when two proteins share the same lattice site coordinates within their respective membranes. An additional dimensionless energy parameter, ε_x is used to characterize the favorability of intermembrane contact.

It is important to recognize that Monte Carlo simulations of this sort are neither capable of nor intended for representing the dynamics of membrane protein systems. To properly represent dynamics, all the physical mechanisms that can result in changes in microstate must be included, and must be properly weighted with appropriate activation energies and attempt frequencies [17]. The "moves" in our Monte Carlo algorithm may superficially mimic protein diffusion, but could just as easily (and correctly) have been chosen to be non-local exchanges with no physical interpretation. It is important that the moves in the algorithm satisfy the 'detailed balance' for the transition probabilities,

$$\frac{P_{i\to j}}{P_{j\to i}} = \exp\left[-\Delta E_{ij}/k_B T\right] \tag{3}$$

which is guaranteed by the Metropolis rule [18,19]. The Monte Carlo moves merely ensure that the configuration space is fully sampled, and that configurations with lower energy are appropriately weighted by the Boltzmann factor. The steady-state macroscopic properties of the Monte Carlo ensemble then correspond to the thermodynamic equilibrium state of the system.

Each experiment consisted of 15,000 iterations on a 100×100 lattice with periodic boundary conditions. During each iteration, 10,000 randomly chosen sites were updated. Random site selection prevented artifacts that could have been caused by update precedence. 15,000 iterations were generally found to be sufficient for the system to reach a steady state; cases where steady state was not reached are noted below. In some experiments, the aggregation fractions or cluster numbers for identical runs were averaged together to reduce statistical noise, which can be large at small protein densities.

3. Results

A number of simple MC simulations were first conducted, in order to verify that thermodynamically expected behavior is reproduced in a simple system. In the first simulation, proteins were allowed to diffuse on a square lattice, in the presence of attractive protein–protein interactions. These interactions are modeled by the addition of a free energy term that favors protein–protein contacts. As discussed in Methods, protein–protein contact was interpreted as occupancy of adjacent, but not diagonal, sites on the lattice. When the interaction energy is highly favorable, the system will phase separate, giving rise to large (infinite, in the thermodynamic limit) protein clusters. The phase separation of such a lattice gas has been extensively studied. The phase behavior can be most clearly seen by starting from a fully clustered initial state, and running the Monte Carlo simulation to determine whether dissociation occurs; above a critical interaction energy, the phase separated state persists indefinitely.

When the initial state is unclustered, the computer time (i.e. Monte Carlo iterations) required to reach a fully phase separated state can be prohibitive. The reason for this is kinetic, not thermodynamic: the algorithm does not provide any mechanism for the collective motion of small aggregates, and thus the coalescence of small aggregates is remarkably slow. (It is difficult to incorporate aggregate motion without biasing the simulation toward aggregation. To prevent bias, for any MC step or action incorporated into the model, the time-reversed step must also be allowed. Collective cluster motion allows for cluster fusion by contact; therefore, cluster fragmentation would also need to be included). This unrealistically slow kinetic behavior illustrates the well-known fact that, as noted above, Monte Carlo simulations give correct equilibrium behavior but, in the absence of a complete set of realistic transition probabilities, do not give correct kinetic behavior.

This study is concerned with equilibrium, rather than kinetics. Correct equilibrium states will be achieved (eventually) provided the transitions between states allow a sampling of all microstates, and that the transitions occur bi-directionally with probabilities proportional to the Monte Carlo–Boltzmann factor when energy increases, and to unity when energy decreases.

Fig. 1 shows the phase behavior the single membrane model, at a density of 0.10 proteins per lattice site. Starting from a fully phase separated protein distribution, the simulation was run until equilibrium was reached. The spatial distribution of the proteins in the resulting lattice was characterized by the protein–protein correlation function, which was then fit to an exponential decay.

The results are consistent with well-established lattice gas simulations [20]. As the interprotein attractive interaction (energy ε, in units of k_BT) increases, the correlation length increases as

$$\xi \propto \frac{1}{\varepsilon_c - \varepsilon} \tag{4}$$

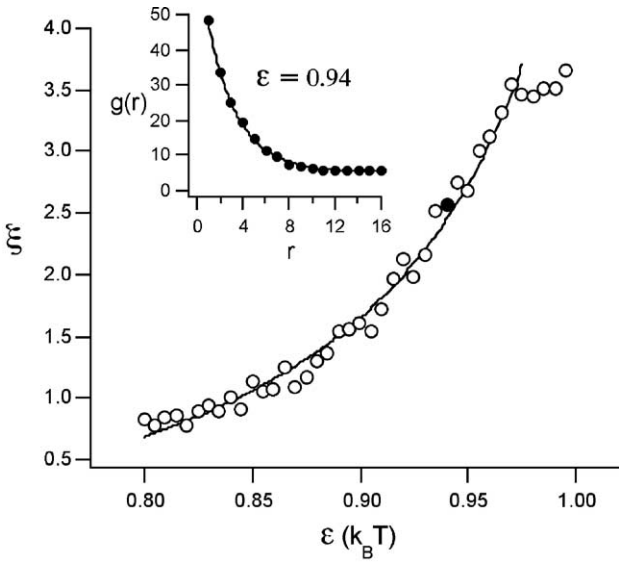

Fig. 1. The correlation length of protein distribution as a function of the protein interaction energy, for a density of 0.1 protein per lattice site. A typical correlation function, $g(r)$, is shown in the inset, for an interaction energy $\varepsilon = 0.94$. The correlation function is well fit by an exponential decay; the characteristic decay length is ξ, which is plotted as a function of the interaction energy in the main graph. The correlation length increases as $|\xi - \xi_c|^{-1}$, shown as the fitting curve. In finite simulations, the correlation length cannot diverge, but begins to saturate when the interaction energy is close to the phase separation point; points near phase separation were excluded from the fit.

where ε_c is the critical interaction energy at the onset of phase separation. In any finite model, the correlation length cannot diverge, but reaches a plateau dictated by the system size. The result for the 100×100 lattice is shown in the figure. The deviations caused by the finite system are apparent at the upper right portion of the graph.

3.1. Intramembrane aggregation

Below the critical interaction energy, the system does not phase separate, but proteins are clearly non-randomly distributed. At very low interaction energies and protein densities, it is reasonable to expect that the number of free, monomeric (non-clustered) proteins will obey a law of mass action:

$$K = \frac{[d]}{[m]^2} \tag{5}$$

based on an association reaction $m + m \rightarrow d$ in which two monomers bind to form a dimer. To reach this limit, it is important that trimers, tetramers, and higher order aggregates be present only at very low concentrations.

Fig. 2 shows that the model behavior does reproduce this expected result. The equilibrium fraction of clustered proteins is plotted as a function of protein density, for several subcritical values of the interaction energy. In the absence of any interaction energy, the probability of a protein having a near neighbor is

$$P_{nn} = 1 - (1 - \rho)^4 \tag{6}$$

where ρ is the density of proteins (expressed as the fraction of filled sites). The experimental data were therefore fit to a sum

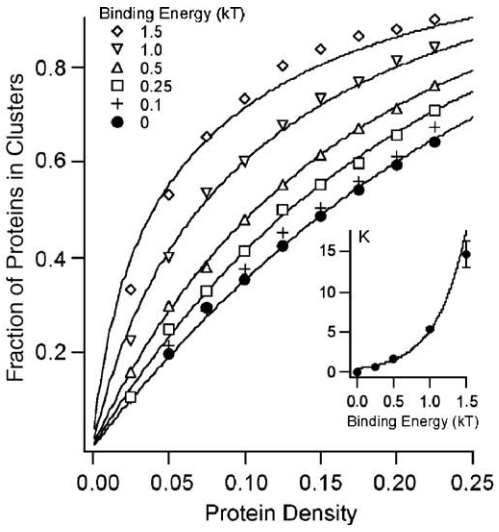

Fig. 2. Fraction of proteins in clusters (dimers or higher aggregates), as a function of protein density, for various attractive interaction energies. The clustered fraction was fit to a background level of statistical aggregation, plus a mass action term (a dimerization equilibrium). The dimerization constant K is plotted in the inset versus the binding energy, and shows the expected exponential dependence. Exact correspondence is not expected, because higher order aggregation is possible. Data from 100×100 lattice run for 10,000 iterations.

of this "background" level of aggregation, plus a mass action term applied to the remaining proteins. The fit is quite good, as can be seen in the figure. Some discrepancy is to be expected, since the aggregation reaction is not limited to dimer formation.

3.2. Intermembrane dimerization

When two membranes are apposed, it is necessary to specify two distinct interaction energies: the intramembrane interaction and an energy of interaction between proteins in different membranes. To further check our model results, a number of simulations were run in which the intramembrane protein binding energy was zero, while the intermembrane protein binding energy was varied, Fig. 3. As expected, there is no phase separation behavior in this case: since the interaction can only lead to the formation of intermembrane dimers, no cooperative phenomena occur. As shown in the figure, the fraction of protein dimers is well fit by a law of mass action, if an additional term for random dimers is included. (Even without any interaction energy, a protein in one membrane will sometimes be directly across from a protein in the apposed membrane. The probability for this occurrence is equal to the protein density, expressed as the fraction of occupied sites).

Although an intermembrane interaction, by itself, cannot cause large-scale protein aggregation or phase separation, it is reasonable to hypothesize that intermembrane interactions could potentiate the effects of weak, *intramembrane* protein attractions. The conditions under which such large scale aggregation can occur is the principal subject of this study.

Although correlation length is the most commonly used parameter in quantifying the approach to phase separation, it poses special difficulties in finite-size computer models. As seen in Fig. 1, the correlation length cannot diverge in a finite system. (Rigorously speaking, of course, finite systems cannot undergo phase changes). As the interaction energy approaches a value that would give phase separation in an infinite system, the correlation function for the finite system begins to deviate from the theoretical functional form, and thus even the notion of "correlation length" becomes ambiguous. To circumvent these difficulties, we have chosen to use a metric for protein aggregation based on the average size of protein clusters, similar to the approach taken by Woolf and Linderman [11]. In the phase separated state, there will be only one (infinite) cluster, while in a highly random state, the number of clusters will be nearly the same as the number of proteins. The reciprocal of the number of clusters, $1/N_C$ thus varies between ~0 for a homogeneous protein distribution, to 1 for a fully "phase separated" system. This metric has the advantage that it emphasizes the large-scale aggregation, while being little affected by formation of transient dimers or trimers. The disadvantage is that $1/N_C$ can fluctuate wildly when N_C is small. This can be readily overcome by simply taking an ensemble average over many Monte Carlo runs, however.

That intermembrane interaction can potentiate large-scale protein clustering is readily seen, as shown in Fig. 4. Shown in Fig. 4a is the protein distribution in a membrane with an intramembrane protein interaction energy of 0.6 k_BT, well below the energy needed to cause large-scale clustering (~2.0 k_BT, at this protein density). Although the number of transient small clusters (mainly dimers and trimers) is slightly higher than with no interaction, the protein distribution in this membrane is largely homogeneous. When two such membranes are apposed, and an intermembrane protein interaction energy of 5.0 k_BT is included, the equilibrium distribution becomes highly clustered, as shown in Fig. 4b.

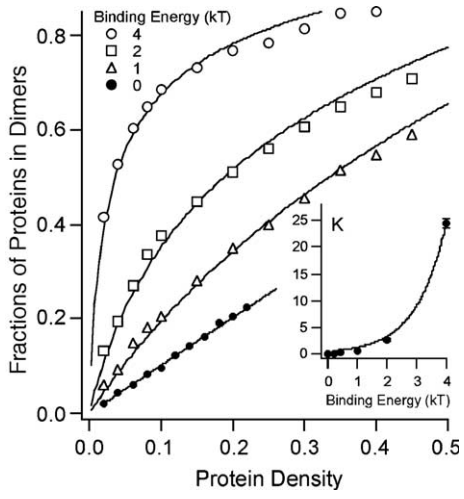

Fig. 3. Fraction of proteins in *intermembrane* dimers as a function of protein density in both membranes. At zero interaction energy, the fraction of dimers is the same as the protein density, as expected for random associations. The dimer concentration can be well fit by the sum of the background association, plus a mass action term. The dimerization constant for the mass action term is well fit to an exponential in binding energy, inset.

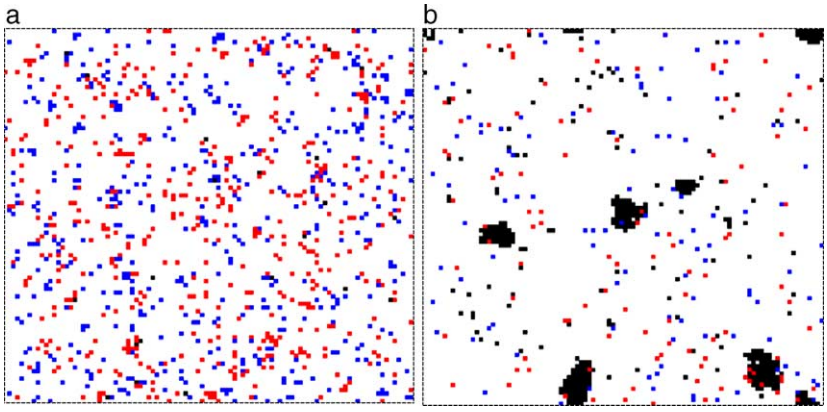

Fig. 4. (a) left: 100×100 double lattice at step 6500 with a protein concentration of 0.05 on each lattice. Intra-lattice protein interaction energy is 0.6 k_BT and the intermembrane interaction energy is zero. At this interaction energy, large clusters are never observed. (b) right: The same lattice configuration as in (a) but with an added intermembrane interaction energy of 5.0 k_BT. Note the formation of large clusters as a consequence of the added intermembrane interaction.

A complete map of the two-membrane aggregation behavior is shown in Fig. 5, in which the aggregation metric, $1/N_C$, is plotted versus the intramembrane protein interaction energy (ε, in k_BT) and the intermembrane protein interaction (ε_x). The blue regions of the plotted surface indicate the largest clustering; the red regions the least. As might be expected, for very strong intermembrane interactions, the threshold *intramembrane* protein interaction energy for large-scale aggregation is reduced by about half. (This suggests that the entropic opposition to aggregation is not significantly larger in the eightfold-coordinated lattice than in the fourfold-coordinated lattice). Somewhat surprisingly, even for rather weak intermembrane interactions (i.e. ~4 k_BT per protein pair), there is still a strong tendency toward aggregation.

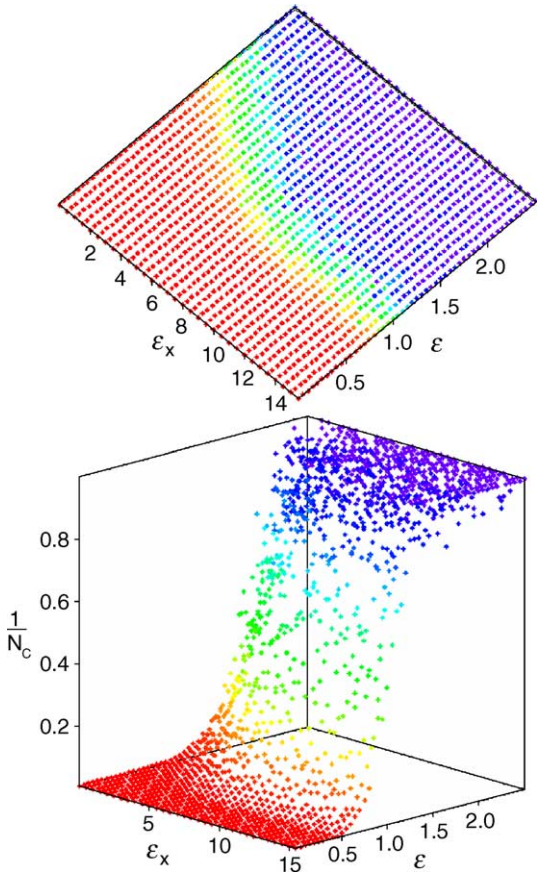

Fig. 5. The reciprocal of the mean number of clusters (per membrane) in the two-membrane model, as a function of the intra- and intermembrane protein interaction energies. Each of the two 100×100 square lattices hosted 500 proteins, a density of 0.05. (Bottom) a 3D plot; (Top) same data, viewed from above. Color coding helps to identify the range of parameters that give strong clustering: blue and purple colors correspond to fewer than 2 clusters per membrane in the ensemble. The model was run for 15,000 iterations. To reduce the statistical variation, $1/N_C$ was averaged over 5 runs.

The quasi-exponential dependence on the cross-membrane protein interaction energy suggests that a temporally averaged or "mean field" approach might be used to predict the behavior in these systems. We can consider the effective interprotein interaction to be the time-weighted average of ε (when the protein has no partner in the apposing membrane) and 2ε (when it is partnered; for a symmetric system). The ratio of the fraction of time when it is unpartnered to the

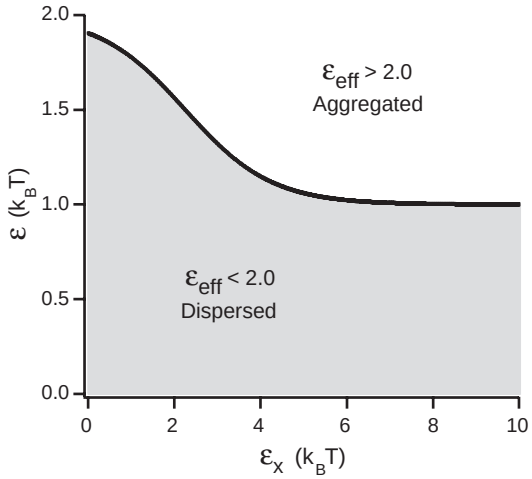

Fig. 6. The strengths of the intra-(ε) and intermembrane (ε_x) protein interaction energies (in k_BT) required to give an effective interprotein interaction of 2 k_BT, according to a simple "mean field" estimate. 2 k_BT is the threshold for receptor aggregation at this concentration (5%). The line should be compared with the boundary between aggregated (blue) and dispersed (red) protein phases in Fig. 5, Top.

fraction of time when is it partnered is proportional to the Boltzmann factor,

$$\frac{\overline{t_u}}{\overline{t_p}} = \frac{1-c}{c} e^{-\varepsilon_x} \tag{7}$$

where c is the concentration of partners (expressed as a fraction of occupied sites). The effective interprotein interaction for the symmetric system is then:

$$\varepsilon_{\text{eff}} = \frac{\varepsilon\overline{t_u} + 2\varepsilon\overline{t_p}}{\overline{t_u} + \overline{t_p}} = \frac{(1-c)e^{-\varepsilon_x} + 2c}{(1-c)e^{-\varepsilon_x} + c}\varepsilon$$

Fig. 6 shows the locus of points for which the ε_{eff} is 2, for comparison with the Monte Carlo data of Fig. 5. Though the qualitative agreement is good, the "mean field" calculation somewhat overestimates the effectiveness of the intermembrane protein interaction in driving aggregation. At 4 k_BT, the interactions in the separate membranes have essentially complete additivity, while the Monte Carlo results indicate 6–7 k_BT is required.

4. Discussion and conclusions

Aggregation of cell surface proteins plays an important role in cellular signaling. In some cases, the aggregation of cell surface receptors can be directly driven by binding of multivalent ligands. But in an increasing number of examples, aggregation of cell surface receptors can be induced by monovalent ligands, especially if the ligands are membrane bound (on an artificial membrane or on a neighboring cell). In these cases, aggregation may be caused by ligand-induced conformational changes, or by the entropy of the fluctuating membrane itself. As we have shown here, large-scale aggregation by monovalent ligands can also occur if the ligands and receptors each exhibit non-ideal behavior in their respective membranes, i.e. if there are weak attractive interaction in each membrane that can effectively add together when ligand-receptor binding occurs. Most notably, not only the intramembrane interactions, but also the cross-membrane interaction can be rather weak and still cause aggregation. A ligand-receptor interaction of 4 k_BT, equivalent to the energy of about 4 hydrogen bonds, is nearly strong enough to give an effective interaction as strong as the sum of the in-membrane interaction energies. These results demonstrate a third possible mechanism for cellular signaling through protein aggregation—a weak enthalpically driven aggregation.

The different mechanisms for monovalent aggregation are not exclusive, of course, and conformational, entropic, and enthalpic mechanisms may share the burden. It is noteworthy that in an important immune response, T cell activation, there is significant experimental evidence that weak attractive forces do exist between receptors [21]. Moreover, the MHC ligand has been shown to exhibit anomalous diffusion on the cell surface [22], reflecting non-ideal solution behavior, and there is evidence that TCR–MHC complexes oligomerize to some extent in solution [23]. The MHC protein is known to associate with the CD4 coreceptor on T cells; CD4 dimerizes at high concentration [24]. With all of these auxiliary weak interactions, it should not be surprising that even when T cell receptor MHC binding is very transient, large-scale aggregation is generally observed with triggering peptides. The cooperative effects among cell membrane proteins are just beginning to be understood on a fundamental level; these cooperative effects make possible biological mechanisms that may be critical to cell behavior.

The importance of relatively weak cell–cell interactions has been noted by other researchers as well [25]. Cell adhesion molecules, which are also important in T cell–APC interactions, can have off-rates of $>1\ s^{-1}$. Our modeling suggests that cooperative effects of many weak interactions can lead to an effective phase separation in biological signaling, which switches the cell from an unstimulated to a stimulated state.

Lastly, we note that a phase separation process, driven by multiple, cooperative weak interactions could help to explain an important observation in T cell stimulation: individual MHC–peptides appear to be capable of activating multiple receptors, via "serial triggering" [26,27]. Phase separation does not require strong or long-lasting interactions between TCR and their ligands; rather, the interaction need only push the total interaction energy over a threshold. The rapid off-rate of the TCR–ligand interaction can allow the ligand to bind multiple receptors through an intermembrane partner-switching mechanism (similar in spirit to the in-membrane partner switching discussed by Woolf and Linderman [11]). Importantly, such "serial triggering" does not imply that ligand engagement force a conformational change in the receptor. Thus, serial triggering and receptor aggregation are not incompatible mechanisms of T cell activation, but may arise naturally from the cooperative nature of the interactions.

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