A Statistical Mechanics Model for Receptor Clustering

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Abstract. We introduce and study a simple lattice statistical mechanics model for the clustering of tumor necrosis factor receptor I (TNFR1). Our model explains clustering under over-expression of the cytoplasmic signal transducer as well as the clustering induced via extracellular ligand binding; also we explain why the loss of transducer leads to a rapid break-up of the clusters. The basic mechanism at work is a first-order (cooperative) phase transition caused by the multimeric binding capability of the receptor-transducer complex. Using cooperativity of this type, the cells are found to have an enhanced sensitivity and robustness. In general, our method can be applied to other receptor-clustering related signaling system.

Key words: Signal transduction, receptor clustering, statistical mechanics

Abbreviations: TNFR1 – Tumor Necrosis Factor Receptor I; TNF_α – Tumor Necrosis Factor; SODD – Silencer of Death Domain; TRADD – TNF Receptor Associated Death Domain; UV – Ultra-Violate; TRAF_2 – TNFR Associated Factor 2; FADD – Fas Associated Death Domain; RIP – Receptor Interacting Protein; JNK – *c-Jun* N terminal Kinase; $\text{NF}\kappa\text{B}$ – Nuclear Factor- κB

1. Introduction

Cell growth, differentiation, migration, and apoptosis are regulated in part by extracellular polypeptide cytokines [1]. Since most peptides are unable to pass through the hydrophobic cell membrane, executing the biological functions requires specific surface receptors to recognize the peptides and thereby transmit the signals. In many cases, it has been observed that clustering of receptors is the essential part of the mechanism whereby proper intracellular signals can be generated. This leads to two fundamental questions: (1) why is the receptor clustering required for signal transduction, and (2) how is the clustering regulated under physiological conditions (i.e., the dose response curve)? Here, via simple modeling, we investigate the second question for one particular example, that of tumor necrosis factor receptor I (TNFR1) signaling.

TNFR1 regulates cell death and survival through its association with a cytoplasmic transducer, ‘TNF receptor associated death domain’ (TRADD) and via the clustering of TRADD-TNFR1 complexes [2]. Specifically, the clustering is accomplished by the multiple self-binding motifs on TRADD (different from its

receptor binding site), Figure 1(a), [3]; thus over-expression of TRADD can lead to spontaneous clustering and constitutive signaling [4, 5]. To prevent this, TNFR1-TRADD association is normally blocked by a cytoplasmic inhibitor, ‘silencer of death domain’ (SODD) which was found to bind to the TNFR1-TRADD binding site [5]. Clustering is restored in the presence of a trimeric extracellular ligand, tumor necrosis factor α (TNF_α) which is capable of oligomerizing three receptors regardless of their cytoplasmic condition [2, 5].

From the biochemical prospective, it is not totally understood how trimerizing TNFR1 can restore the clustering. This behavior clearly requires enhanced TRADD recruitment to liganded receptors. One molecular hypothesis is that trimerizing receptors brings them into close proximity and thus ‘squeezes’ out the bulky inhibitors (molecular weight of SODD = $62KD$, TRADD = $32KD$) [5]. This, however, is inconsistent with the observed time course of SODD-TNFR1 association during ligand treatment and the low TNF-TNFR1 dissociation rate. Normally, TRADD is recruited to (and SODD is released from) receptors after 5 min of ligand treatment. The recruited TRADD is then phosphorylated by activated downstream enzymes and released from TNFR1, followed by an SODD-TNFR1 re-association; this occurs about 10 min after the treatment [5]. Now, the experimental data suggested that TNFR1- TNF_α has a binding lifetime longer than hours [6]. Thus, if trimerizing receptors could block the inhibitor binding, there would be no inhibitor-receptor re-association in 10 min since most receptors would still be di/trimeric.

There is, however, a biophysical property of this receptor system that has not been addressed. The system has an intrinsic cooperativity arising from TRADD self-multimerization capacity. Then, if applying ligand can properly modulate the effective binding between nearest-neighbor TNFR1-TRADD complexes, a first-order phase transition can occur with the surface molecules spontaneously segregated into dilute and dense phases [7]. To see if this idea works, we study the statistical mechanics of TNFR1 clustering, based on a simple lattice model that incorporates the most relevant parameters. Our results show that (a) simple modeling does predict TNFR1 clustering in a relevant parameter range, and (b) using cooperativity of this type, the cells can enhance both their sensitivity and robustness. Finally, to examine the validity of this statistical mechanics approach, we estimate the time scale of system nucleation rate; our finding agrees well with the experimental data.

2. The Model

2.1. BASIC ASSUMPTIONS

To simplify the model, we have made several assumptions. Our goal is to construct a system Hamiltonian that can be easily simulated and analyzed. Here, we are interested in the short-term behavior (the onset of clustering), and the long-term consequences of clustering (such as downstream signaling, receptor internalization,

Figure 1. (a) The cytoplasmic domain of receptor (TNFR1) can associate with either the inhibitor (SODD) or transducer (TRADD). TRADD has multiple motifs for self association; this allows the clustering of TNFR1-TRADD complex, whereas the binding of SODD prevents clustering. (b) If we assume that on average each recruited TRADD can associate with 4 nearest neighbors, TNF treatment can enhance the cooperativity from 4 to 6.

adaptation/desensitization) will not be included. Thus our first assumption is that the system can reach a ‘quasi-equilibrium’ state via rapid receptor diffusion. This requires the predicted nucleation rate (for the onset of clustering) to be within a reasonable time scale.

Second, we assume that the binding strength between TRADD-TNFR1 complex is much weaker than that between TNFR1 and TNF α ; given the long TNFR1-TNF α binding lifetime, this should be a reasonable approach. Then, in the time scale of interest, the ‘effective molecules’ that allow for clustering will be either solitary TNFR1 monomers or liganded di/trimers.

Third, given the TRADD multiple self-binding capacity, the number of effective nearest neighbors that a solitary TNFR1 can bind to will be different from that of a liganded TNFR1. For instance, if we assume that every TRADD can self-associate with four nearest-neighbor TRADD, when the receptors are liganded to di/trimer, each TNFR1-TRADD complex unit will be able to bind to 6 similar ones, Figure 1(b). Then, the leading effect of ligand binding is to change the number of effective nearest neighbors and hence the system cooperativity. This could of course happen without TNF α ; however, the entropy cost of co-localizing two complexes would be too high without the offsetting ligand binding energy.

Fourth and finally, we treat the cell surface as a lattice with a spacing $a_0 \approx 1nm$; this is the closest that neighboring surface molecule complex (solitary or liganded TNFR1) can get to each other. Then, each lattice site i can be occupied by either zero or one effective molecule, denoted as $n_i = 0$ or 1. Also, we define a state label t_i for the occupying molecule (i.e., $t_i \equiv R_k L_l S_m T_n$ with k, l, m, n as the content number of TNFR1 (R), TNF α (L), SODD (S) & TRADD (T)). Here, we ignore the obvious fact that dimers and trimers are bigger than monomers; this approximation overvalues the entropy of the multimeric state, but certainly can be subsumed as a change in the effective binding energy. Also, we treat separately the cases of clustering caused by ‘over-expression’ of TRADD, or caused by ligand binding.

2.2. THE HAMILTONIAN

Next, we construct the system Hamiltonian. Our first step is to define a linear term

$$H_0(\{n, t\}) = - \sum_i \mu(t_i) n_i \equiv - \sum_i \mu((R_k L_l S_m T_n)_i) n_i \quad (1)$$

with $\mu(t_i)$ as the chemical potential for the state of the occupied site. Explicitly, in the TNF_α -free case, we have

$$\begin{aligned} \mu(R_1 L_0 S_0 T_0) &= \mu_R, & \rightarrow \text{a single free TNFR1} \\ \mu(R_1 L_0 S_1 T_0) &= \mu_R + \mu_S + g_s, & \rightarrow \text{a single TNFR1-SODD complex} \\ \mu(R_1 L_0 S_0 T_1) &= \mu_R + \mu_T + g_T, & \rightarrow \text{a single TNFR1-TRADD complex} \end{aligned}$$

with μ_R, μ_S, μ_T as the molecule chemical potential and $-g_s, -g_T$ as the binding energies.

In the TNF_α -present case, on the other hand, the possible states includes $k \in \{1, 2, 3\}$, corresponding to a solitary, dimeric, or trimeric TNFR1. Obviously, we need $(m + n) \leq k, l \in \{0, 1\}$, and $k > 1$ if $l = 1$ (the liganded case). Overall, we have

$$\begin{aligned} \mu(R_k L_l S_m T_n) &= k\mu_R + l(\mu_L + kg_L) + m(\mu_S + g_S) \\ &+ n(\mu_T + g_T) + f(n)g_E \end{aligned} \quad (2)$$

with μ_L as the ligand chemical potential, and $-g_L, -g_E$ as the TNFR1- TNF_α , TRADD-TRADD binding energies. Here $f(1 \leq n \leq 2) = n - 1, f(3) = 3$ (see Figure 1) corresponds to the number of TRADD self-bindings allowed inside a TNFR1 oligomer.

Next, we add an interaction term into the Hamiltonian. This takes the general form

$$H_{int}(\{n, t\}) = -\frac{1}{2} \sum_{ij} J_{ij} a(t_i, t_j) n_i n_j \quad (3)$$

with $J_{ij} = 1$ if i, j are nearest-neighbors and 0 otherwise. Here $a(t_i, t_j)$ is a ‘state’-dependent function indicating the nearest-neighbor interaction. Since the cooperativity arises from the capacity of TRADD self-multimerization, the simplest form of $a(t_i, t_j)$ is the product $a(t_i, t_j) = g_E \theta(t_i) \theta(t_j)$. In the TNF free case, we have $\theta(t_i) = 1$ if the site has a TNFR1-TRADD complex. In the TNF present case, however, we are interested in the molecular concentration (i.e., [TRADD], [SODD]) range where spontaneous clustering does not occur. This implies that the molecules responsible for clustering are liganded TNFR1 oligomers with at least two TRADD associated. Thus $\theta(t_i = R_k L_l S_m T_n) = 1$ if $n \geq 2$ and 0 otherwise.

3. Simulations

To see if our model can generate clustering, we perform a Metropolis Monte Carlo simulation. First, in the absence of TNF, we assume that each TNFR1-TRADD

complex can bind to four similar ones (i.e., the system cooperativity $D = 4$ as illustrated in Figure 1(a)), and perform simulations on an 100×100 square lattice with periodic boundary condition. The jumping probability for a surface molecule to move to another lattice site and the probability for surface molecules to react and change their type/states are determined by the Hamiltonian and obey detailed balance [7]. In our simulation, clusters are not allowed to move collectively, which might not be the case in reality.

In the ligand-absence case, the ensemble for a TNFR1 occupying a single lattice i will be

$$\begin{aligned} & e^{\beta\mu_R} \left\{ \underbrace{1, e^{\beta(\mu_S+g_S)}}_{\text{subensemble not effective for clustering}}, e^{\beta[\mu_T+g_T+g_E \sum_j J_{ij} n_j \theta(t_j)]} \right\} \\ \rightarrow & e^{\beta\left(\mu_R + \frac{1}{\beta} \ln[1+e^{\beta(\mu_S+g_S)}]\right)} \left\{ \underbrace{1}_{\text{subensemble not effective for clustering}}, \left[\frac{e^{\beta(\mu_T+g_T)}}{1+e^{\beta(\mu_S+g_S)}} \right] e^{\beta g_E \sum_j J_{ij} n_j \theta(t_j)} \right\} \end{aligned}$$

where we have separated the subensemble not effective for clustering into $\{\dots\}$. Obviously, aside from the ‘shifted’ μ_R (which can be fixed by the given receptor density anyway), the relevant parameters that switch TNFR1 between non/effective states are g_E and the ratio $B = e^{\beta(\mu_T+g_T)} / [1 + e^{\beta(\mu_S+g_S)}]$.

Since $-g_E$ is the binding energy between each TRADD self-binding motif, as a simple estimate, we might take $g_E \approx 4k_B T$, roughly the energy of a single hydrogen bond at room temperature. The crucial B factor is related to the molecular concentrations and dissociation constants via

$$\begin{aligned} [\text{TNFR1}^{(m)}]_{eq} [\text{TRADD}]_{eq} &= K_d^{\text{TRADD}} [\text{TNFR1}^{(m)} \bullet \text{TRADD}]_{eq} \\ [\text{TNFR1}^{(m)}]_{eq} [\text{SODD}]_{eq} &= K_d^{\text{SODD}} [\text{TNFR1}^{(m)} \bullet \text{SODD}]_{eq} \end{aligned}$$

Here the notation ‘TNFR1^(m)’ means TNFR1 molecules distributed on the membrane, and the brackets $[\dots]_{eq}$ indicates the equilibrium concentration of the respective molecule. In detail, we have

$$\begin{aligned} e^{\beta(\mu_S+g_S)} &= [\text{SODD}] [K_d^{\text{SODD}}]^{-1}, \quad e^{\beta(\mu_T+g_T)} = [\text{TRADD}] [K_d^{\text{TRADD}}]^{-1} \\ B &= \frac{[\text{TRADD}] / K_d^{\text{TRADD}}}{1 + [\text{SODD}] / K_d^{\text{SODD}}} \end{aligned} \quad (4)$$

Obviously a larger B factor implies a higher transducer to inhibitor concentration ratio. From Figure 2, we immediately see that for a given receptor density, tuning the B factor moves the system from a non-clustering to a clustering phase. Thus our model can predict spontaneous clustering as seen in experiment [4, 5].

Next, we consider the ligand-presence case. To perform the simulations, from Equation (2), we need two additional parameters, i.e., the ligand chemical potential μ_L and binding energy $-g_L$. Using the procedure introduced in our previous work [7], we can connect these parameters with real experimental variables through the

Figure 2. The presence of TNFR1-TRADD clustering at (A) high inhibitor or (B) transducer concentrations in the absence of TNF_α . Here $\langle n \rangle$ is the given receptor density, and $B = \frac{[\text{TRADD}]/K_d^{\text{TRADD}}}{1 + [\text{SODD}]/K_d^{\text{SODD}}}$ are fixed quantities during the Metropolis Monte Carlo simulation (4×10^8 steps). The open diamond represent free TNFR1 or TNFR1 associated with SODD, and a filled square indicates a TNFR1-TRADD complex.

formula

$$\begin{aligned} [\text{TNFR1}^{(m)}]_{eq} [\text{TNF}_\alpha] &= K_d^{\text{TNF}} [\text{TNF}_\alpha \bullet \text{TNFR1}^{(m)}]_{eq} \\ \rightarrow e^{\beta(\mu_L + g_L)} &= [\text{TNF}] [K_d^{\text{TNF}}]^{-1} \end{aligned} \quad (5)$$

Treating the ligand as an ideal gas [7] allows us to find an estimate of the ligand binding energy

$$g_L = k_B T \ln \left[\left(\frac{2\pi m_{\text{TNF}} k_B T}{h^2} \right)^{\frac{3}{2}} / K_d^{\text{TNF}} \right] \approx 60 k_B T, \quad (6)$$

with m_{TNF} as the mass of TNF_α [7]. This estimate is far larger than the order of g_E and thus supports our assumption that binding between TNFR1-TRADD

Figure 3. The presence of TNFR1-TRADD clustering at **(C)** moderate and **(D)** low B conditions with the presence of TNF_α . Here the receptor density $\langle n \rangle$, ligand concentration $[TNF_\alpha]$ and the B factor are fixed quantities. The open diamond represent unliganded free TNFR1 or TNFR1-SODD complex, a filled square indicates a TNFR1-TRADD complex, the filled/open circles indicate liganded TNFR1 with/out TRADD associated, and the filled/open triangles indicate TNFR1 trimer with/out TRADD associated. Note that **(C)** has the same cytoplasmic parameters as **(A)**, but clustering can occur with the presence of ligand.

complex is much weaker than that between TNFR1 & TNF_α . In general, we can take $15 < g_L/k_B T < 60$, i.e. 3 to 10 times the value of g_E ; this entire range yields similar numerical results.

Then, we perform the simulation on a 100×100 triangular lattice, assuming the cooperativity $D = 6$ caused by ligand binding (Figure 1(b)). In the simulation, the TNF_α concentration is a fixed quantity (consistent with the experimental procedure [5]) and the monomeric TNFR1s are allowed to update their un/liganded states according to the TNF_α binding energy and concentration, whereas the liganded TNFR1 oligomers are not allowed to dissociate; given the long binding lifetime, this is a reasonable constraint. Thus most TNFR1 will be driven into oligomeric structures after long term simulation.

In the presence of ligand, we found that the same parameters that previously caused no clustering (Figure 2A) now can induce clustering (Figure 3C). At the very low [TRADD] (small B factor), however, TNF_α treatment is not sufficient as the SODD binding dominates (Figure 3D). Thus, if the downstream components of the signaling cascade are activated by clustering, one can imagine a ‘dynamical’ process progressing from Figure 3C to Figure 3D, i.e., the TNFR1-associated TRADD are phosphorylated and dissociated, followed by a SODD re-association and break-up of the existent of clusters, while most TNFR1s remain trimeric. This is consistent with the experimental result [5] and confirms that our model can capture the basic physics of TNFR1 clustering. To have a more quantitative view, we next utilize a mean-field approach to analyze the conditions under which clustering can take place.

4. Analytical Approach

4.1. MEAN-FIELD APPROACH

The partition function for this model equals

$$Z = \sum_{\{n_i, t_i\}} e^{-\beta[H_0(\{n, t\}) + H_{int}(\{n, t\})]} \quad (7)$$

where $\sum_{\{n_i, t_i\}}$ means ensemble summation over the different configurations as detailed earlier. To proceed, we introduce an auxiliary Gaussian field [7, 8] to decouple the quadratic term in H_{int} into a linear term such that we can sum over the single site ensembles independently. This yields

$$Z = C \int \mathcal{D}\phi e^{-\frac{\beta}{2} g_E \sum_{ij} J_{ij} \phi_i \phi_j + \sum_i \Psi_i(\{\phi\})} \quad (8)$$

Here, $\mathcal{D}\phi = \prod_i d\phi_i$ with ϕ ranged from $-\infty$ to $+\infty$, and C is a normalization constant which does not affect the thermodynamic properties of the partition function.

For the case of no ligand present, we have

$$\Psi_i(\{\phi\}) = \ln [1 + e^{\beta\mu_R} (1 + e^{\beta(\mu_S + g_S)} + e^{\beta(\mu_T + g_T)} e^{\beta g_E J_{ij} \phi_j})] \quad (9)$$

Defining the effective chemical potential for the order parameter ϕ via

$$e^{\beta\mu_\phi} = \frac{e^{\beta(\mu_R + \mu_T + g_T)}}{1 + e^{\beta\mu_R} (1 + e^{\beta(\mu_S + g_S)})} \quad (10)$$

we can simplify $\Psi_i(\{\phi\}) \rightarrow \ln[1 + e^{\beta(\mu_\phi + g_E \sum_j J_{ij} \phi_j)}]$. We now approximate the partition function by assuming that the dominant contribution arises near a spatially-uniform saddle-point. This leads to the self-consistent mean-field equation

$$\phi^* = \frac{e^{\beta(\mu_\phi + g_E D\phi^*)}}{1 + e^{\beta(\mu_\phi + g_E D\phi^*)}} \quad (11)$$

Here, $D = \sum_j J_{ij}$ is the number of nearest neighbors which we take as 4 in this TNF $_{\alpha}$ -free case. Similarly, one can find an expression for the mean receptor density in terms of the derivative of the effective free energy with respect to the chemical potential μ_R ,

$$\langle n \rangle = \frac{x(1 + Be^{\beta g_E D \phi^*})}{1 + x(1 + Be^{\beta g_E D \phi^*})} \quad (12)$$

where $x = e^{\beta \mu_R} [1 + e^{\beta(\mu_S + g_S)}]$ and B is defined in Equation (4). Then, for a fixed receptor density, the two unknowns ϕ^* and x can be determined by solving Equation (11), (12).

Next, we proceed to the model with TNF present. Again, we introduce a field ϕ to decouple the quadratic term. Working this out explicitly, the partition function becomes

$$\Psi_i(\{\phi\}) = \ln \left[1 + \sum_{k=1}^3 \alpha_k x^k + \sum_{k=2}^3 \gamma_k x^k e^{\beta g_E \sum_{ij} J_{ij} \phi_j} \right] \quad (13)$$

where the coefficients are given by $\alpha_k = (kB + 1)(\delta_{k0} + e^{\beta[\mu_L + (k-1)g_L]})$, $\gamma_2 = B^2 e^{\beta(\mu_L + 2g_L + g_E)}$, $\gamma_3 = B^2 e^{\beta(\mu_L + 3g_L + g_E)} [3 + B e^{2\beta g_E}]$, and x and B are defined previously. Just as the TNF $_{\alpha}$ free case, we introduce a ϕ chemical potential, here via $e^{\beta \mu_{\phi}} = \sum_{k=2}^3 \gamma_k x^k / [1 + \sum_{k=1}^3 \alpha_k x^k]$. Up to a constant, we find that $H(\{\phi\})$ is reduced to the same expression as that given above for the ligand-free case. So, the mean-field equations remain the same, and only the parameter μ_{ϕ} gets redefined. And, because of the nature of the liganded receptor, the coordination neighbor D is now set to 6.

4.2. PHASE DIAGRAMS

Using this mean-field approach, we can find the phase diagram by solving a pair of coupled equations for two unknowns; the order parameter ϕ^* and x , the variables simply related to the receptor density and chemical potential.

Figure 4 shows how we use graphical intersection to determine ϕ^* and μ_{ϕ} . For the case at hand, we have chosen $\langle n \rangle = 0.1$, $\beta g_E = 4$, and $D = 4$. Notice that we have a van der Waals loop in the solution diagram of Equation (11). In detail, this means that there exist two spinodal points $\phi_{s\pm}$ at which the solution branch determining the chemical potential μ_{ϕ} become unstable. There also exist two binodal points, labeled $\phi_{b\pm}$ which correspond to places at which the different solutions for the chemical potential (the two possible phases of the system) have equal free energy. From the saddle-point equations, we find $\phi_{s\pm} = (1/2) \pm \sqrt{(1/4) - (1/\beta g_E D)}$ and $\phi_{b\pm} = (1/2) \pm \xi$ with $\xi = 2 \tanh[g_E D \xi / 2]$. This implies that the van der Waals loop exists only if $g_E \geq g_E^{min} = 4k_B T / D$, i.e., there is a minimal TRADD self-binding strength that allows for clustering.

In Figure 4, we notice that the solution curve of the second equation intersects the first curve past the lower binodal curve. This means that the system will attempt

Figure 4. The mean-field solution for Equation (11) (the solid curve) and Equation (12) (the dashed curve). Here the density is given by $\langle n \rangle = 0.1$. The uniform solution given by the intersection of the two curves lies above the binodal point ϕ_{b-} . Thus the system is metastable and can segregate into a dilute phase and a dense phase (the long-dashed curve) corresponding to $\langle n \rangle = 0.999979$. Note that the solid curve ends at the point $\phi^{(u)}$ which satisfies $Be^{\beta g_E D \phi^{(u)}} [1/\phi^{(u)} - 1] = 1$ (and hence $x = \infty$, as one can see from Equation (11)).

(via nucleation) to spontaneously segregate into a dilute and a dense phase so as to generate the lowest overall free energy. The last curve shown verifies that the solution curve for $\langle n \rangle = 0.999979$ intersects the upper branch of the van der Waals loop at exactly the same value of μ_ϕ , showing that these two phases can coexist.

Consequently, the system becomes metastable if the receptor density is large enough such that the saddle point solution ϕ^* is located between the binodal ϕ_{b-} and spinodal ϕ_{s-} points. This requires a minimal receptor density $\langle n \rangle_{min}$ at a given set of transducer, inhibitor concentrations. For the ligand-free case, from Equation (11), (12) we have

$$\langle n \rangle_{min} = n_c + \left[e^{\frac{\beta g_E D}{2}} + e^{\beta g_E D \left(\frac{1}{2} - \xi \right)} \right] B^{-1}, \quad \text{with } n_c = \frac{e^{-\beta g_E D \xi}}{1 + e^{-\beta g_E D \xi}} \quad (14)$$

where ξ and the B factor are defined as before. Likewise, the minimal receptor density in the presence of ligand can be computed. The consequences of these results are shown in Figure 5.

Figure 5. (a): The dependence of the (dimensionless) minimal receptor density $\langle n \rangle_{min}$ on transducer, inhibitor concentrations (determined by the B factor). The solid curve is for the ligand-independent and the dashed curve is for the ligand-induced case. In general, the dashed curve will depend on $[TNF_{\alpha}]$, here fixed at $[TNF_{\alpha}] = K_d^{TNF}$. **(b):** The receptor density corresponding to the two binodal points at given strength of the B factor (related to $[TRADD]$, $[SODD]$), as a function of the TNF_{α} concentration. The upper curve corresponds to ϕ_{b+} and the lower one is related to ϕ_{b-} . Here $a_0 \approx 1nm$.

What can we learn from these phase diagrams? First, the existence of a first-order phase transition has an immediate implication that the cells use an ‘all-or-none’ reaction to execute the programmed death or proliferation. Second, compared to Figure 5(a) (the constitutive signaling), the deep-‘well’-like phase boundaries in Figure 5(b) indicates that the variation of ligand at small (moderate) concentration ranges can cause a strong (weak) response in ligand-induced clustering. Obviously, this gives the cells a sensitivity and a robustness; both are required for functional biology [9].

Also, these diagrams reveal qualitatively how the signaling responds to a change in any of the molecular concentrations. For instance, it is known that the receptor density can be down-regulated by antibody treatment and the active, unphosphorylated TRADD can be increased by applying osmotic pressure [10]. Then, assuming a simple scaling between the osmotic pressure overload ΔP and the increment of active TRADD, $\Delta[\text{TRADD}] \sim [\Delta P]^\gamma$ with $\gamma > 0$, we find from Equation (14) a power law divergence $\Delta P^* \sim [n - n_c]^{-\gamma}$ for the pressure necessary to induce clustering, when the receptor density n is down-regulated to n_c . Likewise, there are many other ways to modulate the molecular concentrations including over-expressing (suppressing) SODD, TRADD by mRNA (antisense RNA), & UV irradiation [4, 5, 10], and our results can be directly tested by experiment.

5. The Nucleation Rate

Next, we test the validity of statistical mechanics approach of this type by estimating the time scale of nucleation. Experimentally, measuring a precise clustering rate on living cells for a cooperative signaling system of this type (TNF and other growth factor receptor families) is rather a big challenge. The available methods to date are direct observation by immuno-staining [10], electron microscopy [11], fluorescence imaging [12], or indirect measurement from the receptor chemical modification (phosphorylation & adaptor association, e.g.) provided that these reactions are fast after clustering [5, 13]. Unfortunately, none of them can provide explicit data; all is known is that the order of magnitude of the clustering rate is estimated to lie in the range of $0.2 \sim 1 \text{ min}^{-1}$ [5, 10, 11, 12, 13].

Here, as the receptor system is pushed into the metastable part of the phase diagram (i.e. between the binodal and spinodal points), a nucleation process [14] will take place, driving the system from a relatively dilute uniform phase to coexisting dilute & dense phases. The rate of this process J_{nuc} is determined by the fact that the system must pass through a spatially inhomogeneous transition state, the droplet of nucleation. Typically, $J_{nuc} \approx \nu e^{-\Delta F}$ with ΔF as the free energy cost (as compared to the metastable initial phase) and ν as the growth rate of this droplet.

To find out ΔF , we need to determine the transition structure (droplet); this can be done via finding an inhomogeneous solution of the equation which results from varying the free energy functional. This equation takes the form

$$a_0^2 \nabla^2 \phi = \frac{\ln \left[\frac{\phi}{1-\phi} \right]}{\beta g_E D} - \phi - \frac{\mu_\phi}{g_E D} \quad (15)$$

where ∇^2 is the Laplacian which in polar coordinates becomes $\frac{d^2}{dr^2} + \frac{1}{r} \frac{d}{dr}$. Here μ_ϕ is the chemical potential fixed by the ‘homogeneous’ metastable state $\beta\mu_\phi = \ln \left[\frac{\phi_{meta}}{1-\phi_{meta}} \right] - \beta g_E D \phi_{meta}$.

Equation (15) can be solved numerically. As $r \rightarrow \infty$ we have $\phi_{r \rightarrow \infty} \approx \phi_{meta} + \delta\phi e^{-\lambda r}$; from Equation (15), we find $\lambda^2 = [\beta g_E D \phi_{meta} (1 - \phi_{meta})]^{-1} - 1$. Since λ^2 must be greater than zero, this implies that the metastable $\phi_{meta} < \phi_{s-}$, as expected. On the other hand, near the origin $\phi(r) \approx \phi_0 + \frac{1}{4}\kappa r^2$, with $\kappa = \frac{1}{\beta g_E D} \ln \left[\frac{\phi_0}{1-\phi_0} \right] - \phi_0 - \frac{\mu_\phi}{g_E D}$. Requiring this to be less than zero leads to $\phi_{meta} < \phi_0 < \phi_{(dense)}$. Thus, we have two unknowns, ϕ_0 and $\delta\phi$, which can be fixed by integrating Equation (15) from both the $r = 0, r = \infty$ ends and matching $\phi, d\phi/dr$ in between.

The droplet profile is plotted in Figure 6(a)A. In general, the droplet becomes more and more flattened as ϕ_{meta} approaches the spinodal point ϕ_{s-} . If we denote the location of the peak in the free energy density as r_m , we find that $r_m \rightarrow \infty$ as $\phi_{meta} \rightarrow \phi_{s-}$, Figure 6(a)C, manifesting the onset of a long wavelength instability for this type of calculation. Also, the free energy barrier $\Delta F \equiv \int d^2r [f(\phi) - f^{MF}(\phi_{meta})]$ is shown in Figure 6(a)D. Clearly, when $\phi_{meta} \rightarrow \phi_{s-}$, $\Delta F \rightarrow 0$, and when $\phi_{meta} \rightarrow \phi_{b-}$, $\Delta F \rightarrow \infty$.

To obtain the growth rate ν , we use the Becker-Doering theory, in which the growth rate $\nu \approx \phi_{meta}/\bar{t}_c$ where ϕ_{meta} is the density of metastable state molecule surrounding the droplet and $\bar{t}_c$ is the mean ‘capture’ time for wandering molecules to adhere to the droplet [15]. For a large system size L , we obtain

$$J_{nuc} = \frac{\phi_{meta}}{2D_m} \ln[L/R_c] e^{-\Delta F} \quad (16)$$

where $D_m \approx 10^{-8} \text{ cm}^2/\text{sec}$ is the membrane diffusion constant and R_c is the effective cluster size defined via $\pi R_c^2 [\phi_{(dense)} - \phi_{meta}] \equiv \int d^2r [\phi(r) - \phi_{meta}]$.

Finally, to have a qualitative range of the nucleation rate, we assume that in physiological condition, the receptor density is just below the binodal point for the ligand-absent case, so as to give the system a maximal sensitivity. Then, from Figure 5B, we get that the corresponding TNFR1 densities $\langle n \rangle$ are roughly 5×10^{-1} , 5×10^{-2} , 5×10^{-3} for the B factor 5×10^{-3} , 10^{-2} , 10^{-1} , respectively. Interestingly, from Figure 6(b), we found that the nucleation rate for these three values are almost in the same range ($J_{nuc} \approx 10^{-2} \sim 1 \text{ min}^{-1}$). This quantity is very close to the experimentally estimate ($0.2 \sim 1 \text{ min}^{-1}$) in TNFR1 [11, 5] and other receptor system [12, 13], and thus confirms the validity of our model. Also, at the maximal sensitivity level, the lack of dependence of the nucleation rate on the details of parameters (the system size and cytoplasmic conditions such as the B factor, e.g.) endows the system with a desired robustness.

6. Discussion

We have presented a lattice statistical mechanics model for TNFR1 clustering. Our basic idea is simple. The interaction between receptors can lead to a first-order

Figure 6. (a): (A) The calculated droplet density and (B) corresponding free energy density profiles in different metastable states. The droplet size R_c corresponding to an effective cluster and the free energy barrier are also estimated in (C) and (D), respectively. (b): The calculated nucleation rate at molecular unit, with different system sizes ($\equiv (La_0)^2 \times nm^2$, indicated by the solid, dashed, and long-dashed curves) and B factors. Here the B factor (i.e., the transducer, inhibitor concentration ratio) is defined in Equation (4). Clearly, the system size does not greatly affect the nucleation rate. Here $[TNF_\alpha] = K_d^{TNF} = 10$ ng/ml is set as the usual application dosage [10].

phase transition with a discontinuous jump in the receptor density as a function of the receptor, transducer, inhibitor densities, and/or the ligand concentration. Turning this around, this implies that the receptor system will spontaneously phase separate for some range of parameters. Thus our model successfully explains how ligand-induced TNFR1 clustering can take place. Given that a cooperative clustering mechanism of this type has been widely adopted by other families of growth factor signaling [1], we expect that our modeling can be easily extended to other systems. Then, two very questions that should be addressed are (a) what is the physiological role of ligand treatment in functional biology? and (b) what design principles might have been used, during evolution, for the cells to acquire the advantage of clustering?

In the current system, it is known that clustering TRADD-TNFR1 complex can recruit adaptors such as TRAF₂, FADD, and RIP, which in turn can activate downstream elements including JNK, NF κ B, and caspase [2], leading to gene expressions for the cell death or proliferation. Recently, one of the adaptors, TRAF₂, was found to self-associate into a trimer [16]. This might shed a light on the possible purpose for clustering, i.e., that clustering TNFR1-TRADD complex provides a molecular scaffold to facilitate signaling.

From the phase diagrams, our results indicate that compared to the constitutive one, the ligand-regulated signaling has a rather larger sensitivity (robustness) in small (moderate) ligand concentration. Also, at the level of maximal sensitivity, our calculations on the nucleation rate show an independence on the details of cellular parameters. This again provides the cells a robustness. Such a ‘digital’ (all-or-none) response is different from the ‘continuous’ one seen in some type of sensory such as amoeba chemotaxis [17] in which an ability to continuously monitor the relative change of stimuli is necessary. But, 2-state-like behavior is also popular in small-scale biochemistry such as functional protein folding and the corresponding chemical reactions. Perhaps after long-term evolution, the cells have developed receptor clustering as a ‘large-scale’ digital machinery to execute an ‘all-or-none’ response, such as programmed cell death or cell proliferation, in an analog world.

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