Modelling LAT clusters on the surfaces of T cells

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Abstract

Formation of clusters of proteins on the surfaces of cells is essential in some cases for modulating signalling networks within the cell. T cells are an example, where clustering of the adaptor protein LAT is necessary to bring together a large pool of other molecules that initiate further downstream signalling events. This project attempts to understand the mechanism of formation of these LAT clusters by simulating the diffusion, binding and unbinding of individual LAT molecules on a lattice and looking at the characteristics of the clusters as a function of time.

1. INTRODUCTION

Signalling networks in cells involve a variety of molecules. Sometimes, a number of molecules come together within these signalling networks to form clusters. One such example is the clustering of LAT molecules in T cells.

During TCR-pMHC engagement, the kinase Lck bound to the CD4 or CD8 coreceptor gets recruited to the TCR-complex and is activated. Lck phosphorylates the immunotyrosine activation motifs (ITAMs) within the CD3- and zeta-chains of the TCR complex. Doubly phosphorylated ITAMs bind the kinase ZAP-70, which is then activated by phosphorylation, either by other ZAP-70 molecules or Lck. Activated ZAP-70 can then phosphorylate tyrosines on a membrane-bound adaptor molecule called linker for activation of T cells (LAT). LAT contains nine conserved tyrosines that when phosphorylated, bind to the SH2 domains of several other proteins to assemble a signalling complex called the signalosome. The LAT signalosome involves molecules such as Gads, SLP-76, Itk, Grb2, SOS, PLCγ1, Vav, etc. Some of these interactions stabilize the resulting complex (for example, PLCγ1-SLP76 and Grb2-SOS. Grb2-SOS enables the formation of LAT clusters, stabilizing the signalosome further). SOS, which is bound to Grb2, activates Ras (converting RasGDP to RasGTP), which in turn activates Raf and sets off the MAPK (ERK) signalling pathway. The strength of the resulting signal is dependant on the number of molecules recruited to the signalosome, which in turn depends on the size of the LAT clusters. The downstream signalling events include positive feedback loops which cause bistability [1] and can be strongly dependant on the number of activating molecules recruited to the cluster.

A number of experimental studies have been performed in this regard. Bunnell et al[2] found by mutational studies that LAT cluster formation was necessary for T cell activation via the formation of cluster-mediated localization of the proteins Gads and SLP-76. Cluster sizes were found to be about 20-30 μm in diameter, which, if assumed to be fully connected and contain no internal gaps, would contain on the order of a hundred LAT molecules.

Houtman et al [3] showed that LAT clusters are formed through the interactions of proteins LAT, SOS and Grb2. Grb2 molecules contain SH2 and SH3 domains, via which they can interact with other molecules. Each of the two SH2 domains of a Grb2 molecule can bind to a SOS molecule, although

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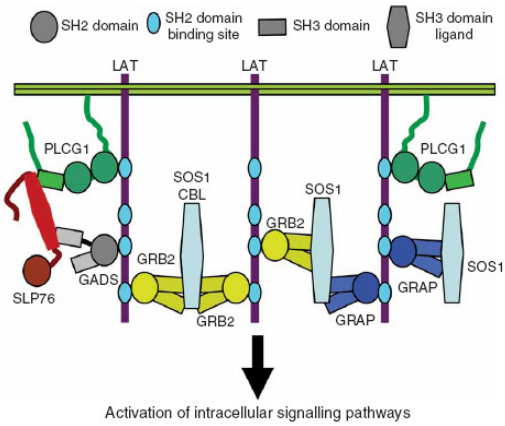
under biological conditions each Grb2 molecule binds to only one SOS molecule. Similarly, while SOS also contains four SH3 binding sites, under biological conditions, each SOS molecule has been found to bind to at most two Grb2 molecules. LAT molecules were shown to bind to up to three Grb2 molecules per LAT, with a ΔH of about 4.5 kcal/mol per bond.

Various properties of LAT molecules and clusters were obtained by Douglass et al [4]. By tagging LAT molecules with GFP and using single-molecule imaging techniques, they were able to determine diffusion coefficients of individual LAT molecules. They found that LAT molecules did not perform the perceived diffusive motion until the T cells were activated, and activated LAT-GFP molecules had diffusion coefficients on the order of 0.5 $\mu m^2/s$. They also observed that larger clusters were much slower and less likely to diffuse, but could exchange molecules.



Balagopalan et al [5] showed that these clusters were formed quickly (time scale of a few seconds) and lasted for around 10 minutes, after which they were degraded by the action of the protein c-Cbl.

2. THE MEAN-FIELD ANALYTIC MODEL

The partition function for the system is:

$$Z = \sum_{configurations} e^{-\frac{E_{bond} \cdot N_{bonds}}{k \cdot T}}$$

The variable N_{bonds} denotes the total number of LAT-LAT bonds in the configuration, restricted to constant total mass, and E denotes the energy of such a bond.

We can approximate the system's statistics by partially ignoring the entropic contribution to the probability distribution, as described below. The analysis follows the derivation of statistics from a grand canonical ensemble.

The reaction network between the LAT clusters (n-mers) is in general complicated: any n-mer can break up into various combinations of other n-mers, and can be formed from a variety of combinations of n-mers. In equilibrium, however, the principle of detailed balance suggests that the following subnetwork must be independently equilibrated:

$$c_1 \leftrightarrow c_2 \leftrightarrow c_3 \leftrightarrow \ldots c_{n-1} \leftrightarrow c_n \leftrightarrow c_{n+1} \leftrightarrow \ldots \leftrightarrow c_\infty$$

where the forward reactions consume a monomer and the reverse reactions liberate a monomer. That is, each reaction is shorthand for:

$$c_{n-1} + c_1 \leftrightarrow c_n$$

If we assume that any of the n components of a dissociating n-mer can fall off, the forward and reverse reaction rates are, respectively, for each reaction:

$$r_f^n = k_f \cdot c_{n-1} \cdot c_1$$
$$r_r^n = n \cdot k_r \cdot c_n$$

(The case that the activity of an n-mer binding a monomer is n times its concentration can be treated analogously, with similar results.)

The equilibrium constant can be approximated as (for $r_f = r_r$ or each n):

$$K = \frac{k_f}{k_r} = \frac{n \cdot c_n}{c_{n-1} \cdot c_1}$$

The constant K will be independent of n if the entropic differences between reactions involving different cluster sizes are neglected. The entropic contributions should tend to favor the monomeric state, so the statistics derived should represent upper bounds on the population distribution.

This leads to the recursion relation for c_n in equilibrium:

$$c_n = \frac{K^{n-1} \cdot c_1^n}{n!} = \frac{1}{K} \cdot \frac{(K \cdot c_1)^n}{n!}$$

The mass balance in the system requires:

$$c_T = \sum_{n=1}^{\infty} n \cdot c_n = c_1 \cdot \exp\left(K \cdot c_1\right)$$

where c_T is the initial charge of monomer (LAT) in the system. The summation should technically terminate at the largest possible cluster; this difficulty is neglected in this analysis.

The average cluster size, in terms of c_1 is:

$$N_{av} = \frac{\sum_{n=1}^{\infty} n \cdot c_n}{\sum_{n=1}^{\infty} c_n} = \frac{Kc_1 \cdot e^{Kc_1}}{e^{Kc_1} - 1} = \frac{c_T \cdot K}{e^{Kc_1} - 1}$$

The following relations can be used to explore finite upper bounds and the possibility that the activity of an n-mer depends on its size:

$$r_f^n = k_f \cdot (n-1)\, c_{n-1} \cdot c_1$$

$$r_r^n = k_r \cdot n \cdot c_n$$

Neglecting the difference between n-1 and n, the predictions are:

$$\frac{c_1}{\left(1 - K \cdot c_1\right)^2} = c_T$$

$$N_{av} = \frac{1}{1 - K \cdot c_1}$$

For both models, the predicted average cluster size for the estimated values of K and c_T, as described in the introduction, are about 1, in equilibrium.

3. THE HEXAGONAL LATTICE MODEL: EFFECT OF BINDING/UNBINDING RATES

A. Model description

To test the effect of the binding and unbinding rates on the growth of LAT clusters, we performed Monte-Carlo simulations on a hexagonal lattice such that each LAT molecule can form 3 bonds with its neighbours (which mimics the physics of the problem). An approximation was made in assuming that only monomers were permitted to perform random walk on the lattice.

A range of the k_{on}/k_{off} ratio has been examined. Invariably, the resulted populations were dominated by dimers and trimers, with small numbers of monomers and larger clusters (up to 15-mers observed). The size and number of clusters of size greater than 3 are dependent on the ratio of association and dissociation rates.

B. Results

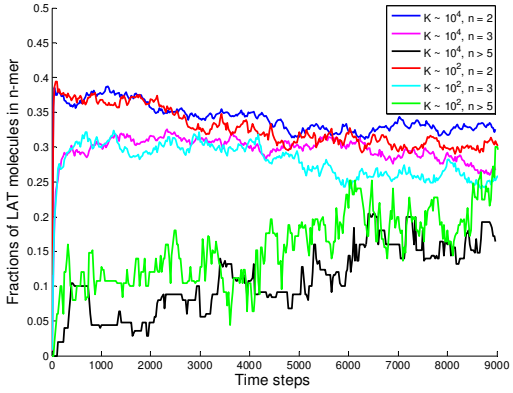


Figure 1: Fractions of LAT molecules in clusters of different sizes over time. K is the ratio of association rate over dissociation rate, n is the cluster size. The curves represent the mean value of 50 trajectories, each with 250 LAT molecules on a 50*50 hexagonal lattice.

Since the clusters are not permitted to move, once a dimer forms, its members are frozen in the dimeric / trimeric states for prolonged periods of time, thus depleting the system's reservoir of LAT molecules, rendering further enlargement of the existing clusters difficult. Only through dissociation and subsequent diffusion can a molecule escape the frozen dimer state. Yet because of the high ratio of binding-to-unbinding rate, dissociation only occurs very infrequently, and the dissociated molecules frequently re-join the cluster before they random-walk away.

Therefore it is not surprising that by slightly increasing the dissociation propensity, we can obtain larger clusters. Figure 1 compares the average fractional population of LAT molecules in n-mer states at different times. It appears that the fractions of dimers and trimers very rapidly reach a high level, then gradually declines as the number of larger n-mers slowly increase. At a lower ratio of binding-to-unbinding propensity, the fractions of n-mers, n>5, increase slightly more. These subtle effects disappear when the ratio is made further smaller, (e.g. *K~10*), in which case excessively rapid dissociation hinders the formation of stable large clusters.

4. THE SQUARE LATTICE MODEL: EFFECT OF CLUSTER DIFFUSION

A. Model Description

To test the effect of diffusion of clusters on the characteristics of cluster formation, we performed Monte Carlo simulations on a square lattice. The diffusion rate of monomers was taken from the work by Douglass et al [4]; the diffusion rate was assumed to be proportional to the inverse of the cluster size. This simulation was done with physical times; a Gillespie algorithm [6] was set up to determine the next reaction and next reaction time. The list of reactions was generated as all possible diffusive moves and all physically possible debinding steps (the binding rate, k_{on}, was assumed to be infinity). The rate of debinding was related to the number of bonds that had to be broken as:

$$k = k_0 e^{-\frac{n_b E_b}{RT}}$$

where n_b represents the number of bonds broken and E_b represents the average bond energy. The simulation was performed on a 25 x 25 square lattice with 5% monomer coverage. In this model, each LAT molecule can bind up to four other LAT molecules; the physical constraint of three bonds per LAT molecule has not been included.

We varied k_0, which controls the kinetics of debinding in the problem, and observed the growth of clusters as a function of real time.

B. Results

Conceptually, for an infinite value of k_{on}, the non-entropic equilibrium favours a single cluster containing all of the system's LAT molecules. Accordingly, the simulations (Figure 2) show that, as time proceeds, all of the LAT molecules are eventually in large clusters.

As expected, the system approaches the steady state more quickly when the debinding rate is slow. There does not appear to be a regime in which debinding speeds the equilibrium, presumably because clustered LAT molecules can themselves diffuse in this model; their slower motion is compensated by the larger eventual agglomeration.

The time scales for clustering (order one second in the simulations) are on the order of the experimental time scales. Since the debinding rate is experimentally unknown, however, the correspondence can be contrived.

When the diffusion rate is large relative to the debinding rate (R<1), clusters quickly encounter each other and clustered LAT molecule quickly acquire more than one bonded neighbour. Since the additional bonds greatly stabilize the cluster, the dynamics are insensitive to the debinding rate for R<1 (unpublished work).

The typical large clusters formed in the simulation were not dense in LAT, giving rise to clusters with large effective surface areas than would be predicted under a close-packed assumption (Figure 3).

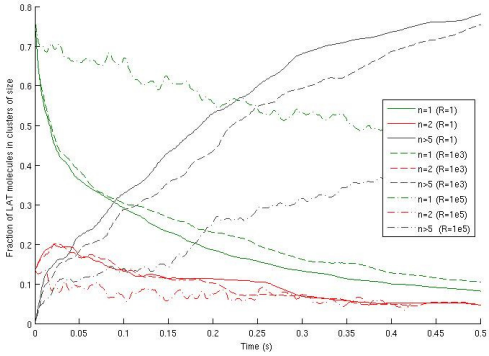


Figure 2: Distribution of LAT molecules in polydisperse clusters for different ratios, R, of the monomer base debinding rate to the monomer diffusion rate. The curves represent the mean values of 30 trajectories, each with 30 LAT molecules placed on a 25*25 square lattice, with periodic boundary conditions.

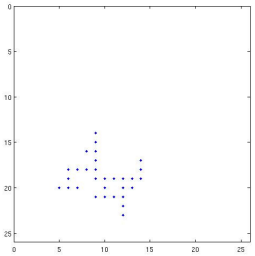


Figure 3: Typical large cluster. The cluster is not dense in LAT.

5. CONCLUDING REMARKS

Consistent with the analytical equilibrium prediction, average cluster sizes in the simulations remained small, dominated by dimmers and trimers at most unless the equilibrium constant was made to greatly favour the formation of clusters. Note that in the equilibrium analysis, entropic effects that would have favoured the monomeric and dimeric states were neglected. Thus, in the range of parameters reported, dimeric and monomeric forms are likely to

be the most common. How, then, can one reconcile the experimental observations that indicate clusters of size of the order of one hundred LAT molecules?

Although it is possible that the true equilibrium constant favors the formation of clusters more than predicted, we can make several other speculations. First, the cluster formation may have been driven, at least partially, by some non-equilibrium process, such as active transport mechanisms, in addition to the passive diffusion. Simulation results showed that when diffusional limitations were severe (only monomers can diffuse), there appears to be an optimal rate of debinding with respect to large n-mer formation. When the diffusional limitations were removed by allowing n-mers to diffuse at a rate inversely proportional to their size, the optimum disappeared. Thus active transport mechanisms can facilitate the formation of large clusters. Secondly, it suggests that perhaps the large clusters observed experimentally are in fact a population of smaller n-mers localized in each other's vicinity, without being inter-connected by stable bonds. Alternatively, as shown by the simulation results, if a large cluster indeed forms, it may be quite sparse, in which case the total area of the cluster outlined by fluorescence does not scale proportionately with the number of LAT molecules in the cluster. Since the signalling efficacy ultimately depends on the number of LAT constituents in a cluster, rather than its physical size or area of coverage, more experimental approaches are needed to complement the current imaging techniques.

6. REFERENCES

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