# An Analysis Of T Cell Activation Using A Simulation Of Cell-To-Cell Contact

by Yves Konigshofer (Chien lab)

## Abstract

T cells encounters with antigen presenting cells (APCs) that express the appropriate combinations of agonist peptides and MHC can result in the formation of immunological synapses. When this happens, different molecules on both the T cell and the APC accumulate at the contact area between the cells. In addition, molecules appear to accumulate at different distances from the middle of the contact area. For instance, the TCR and MHC usually accumulate close to the middle of the contact area while LFA-1 and ICAM-1 accumulate towards the outer edge. In fact, most cell-surface molecules and some intracellular molecules appear to accumulate in and under the contact area. However, why they do this and whether or not this is important for a particular molecule is still frequently unclear.

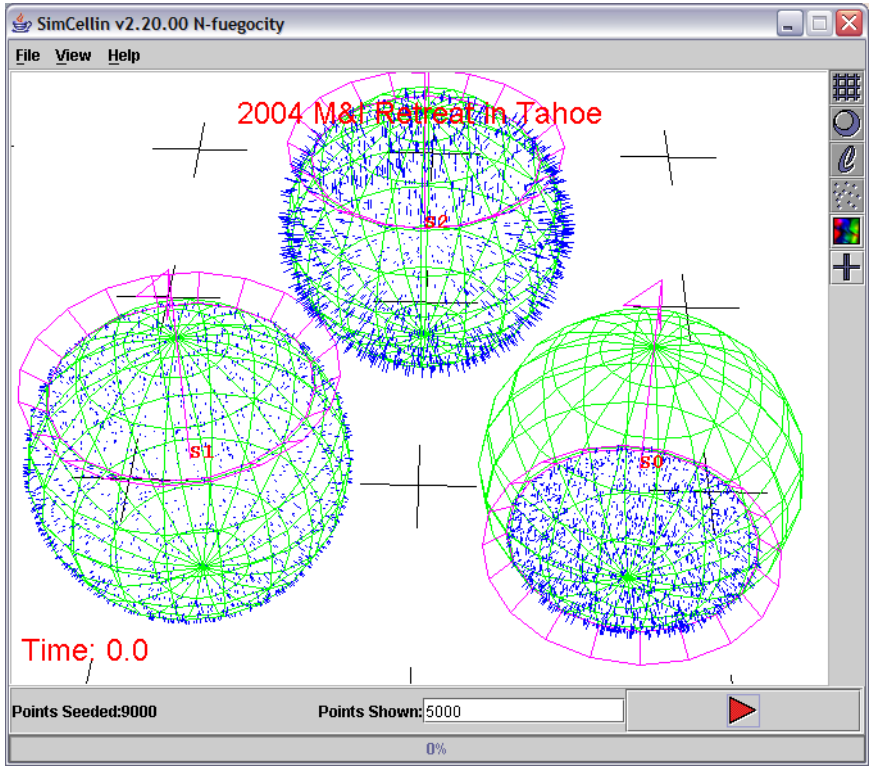
In order to better understand cell-to-cell contact and the events of synapse formation, a Java-based simulation of molecular interactions during cell-to-cell contact was designed that runs on a number of different operating systems. Simulations allow for hypothesis testing under conditions that may be difficult to obtain experimentally. In addition, simulations use known values and equations and, thus, the design and use of simulations often reveals important areas that must still be extensively researched in order to properly design and understand certain experiments.

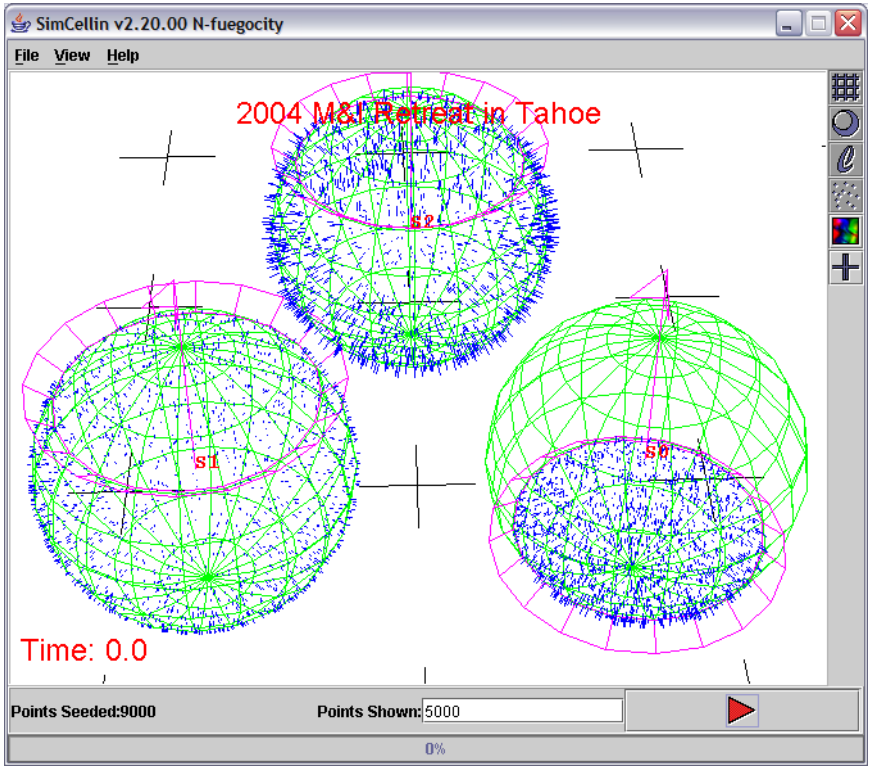
Recently, this simulation has been enhanced to include rates for receptor synthesis, exocytosis, activation, deactivation, endocytosis and destruction. In addition, receptors can now bind to more than one potential ligand, which makes it possible to simulate the interaction between the TCR and different combinations of peptides and MHC.

## Acknowledgements

- Everyone in the Chien lab
- Everyone in the Davis lab, especially
  - Lawren Wu (now at Genentech)
    for TCR-related data
  - Cenk Sumen (now in von Andrian's lab at Harvard)
    for helpful discussions
  - Johannes Huppa
    for helpful discussions
- The leland computer cluster
  for lots and lots of cpu time
  (now if only other people would run their programs with the *nice* command too)

# The Graphical User Interface For SimCellin





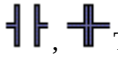
This toggles the display of a grid.


This toggles the display of wireframe spheres. The spheres themselves are green. The vectors pointing to the contact area are represented by a magenta lines from the center of the sphere that end in two triangles that represent two normals that are used for determining the regions that molecules are found in. Spheres are surrounded by magenta rings that represent the borders of the contact areas.


This toggles the display of sphere labels (S0, S1, etc.).


This toggles the display of molecules. The number of molecules that are displayed is specified using the "Points Shown" box. The molecules appear as triangles on the surfaces of the spheres whose height is determined by the height of the molecule or dimer being represented and whose width is determined by the current and last location.


This renders all of the molecules according to their color.


This indicates whether or not the simulation is paused (left) or running (right). This button is used to pause and resume the simulation as the configuration file is executed and as diffusion takes place.


This is indicates whether the simulation is stopped (left), waiting for the user to press the button (middle) or currently running (right). The left image is also displayed when no configuration file is loaded. The middle image is displayed when a configuration file has been loaded and when the user is asked to press the button. The right image is displayed as the configuration file is executed and when molecules are diffusing.

# Accurate Diffusion Coefficients Are Needed But Are Rare

In order to accurately simulate diffusion, one must know the diffusion coefficients of the molecules being simulated. How these are determined is very important. Diffusion coefficients are dependent on viscosities and membrane viscosities are very temperature dependent. In addition, it may not be advisable to stain molecules with fluorescently-labeled antibodies when performing FRAP measurements.

The diffusion coefficient (D) of a molecule in solution with a given radius (r) is dependent on the viscosity (η) of the medium it is diffusing through.

$$D = \frac{RT}{6\pi r N \eta} = \frac{kT}{6\pi r \eta}$$

Viscosities of membranes are highly dependent on temperature. For example, the reported viscosities of erythrocyte membranes increase from 0.76 at 37°C to 1.3 P at 30°C to 1.8 P at 25°C to 2.1 P at 20°C to 3.3 P at 10°C and to 5.7 P at 5°C. Thus, a 32°C difference in the temperature can lead to a 7.5-fold difference in the diffusion coefficient. Therefore, diffusion coefficients of membrane-bound proteins must be measured at 37°C. Unfortunately, most are not. In addition, different cell types have different membrane viscosities.

**Weirdness with Thy-1**

The above equation can be solved for the radius of the molecule where η is given in Poise (1 P = 1 g·cm$^{-1}$·s$^{-1}$ = 0.1 kg·m$^{-1}$·s$^{-1}$) and D is given in m$^2$s$^{-1}$.

$$r = \frac{kT}{6\pi\eta D} = \frac{1.38\times10^{-23}\,\frac{kg\times m^2}{s^2\times K}\times 310.15K}{6\pi\times 0.1\eta\,\frac{kg}{m\times s}\times D\,\frac{m^2}{s}} = \frac{2.272\times10^{-21}}{\eta D}m$$

For example, the diffusion coefficient of the 16.9kDa protein Myoglobin in water (η = 0.01 P) is reported to be 1.13x10$^{-10}$ m$^2$s$^{-1}$ and thus has a radius of about 2.0 nm. So far, so good.

Membrane-bound proteins frequently have extracellular, transmembrane and intracellular domains. The diffusion coefficient of the whole molecule can be determined from the diffusion coefficients of the extracellular ($D_E$), transmembrane ($D_T$) and intracellular ($D_I$) domains (derived from the complexed receptor formula in *Coombs D, Kalergis AM, Nathenson SG, Wofsy C, Goldstein B. Nat Immunol. 2002 Oct;3(10):926-31*).

$$D = \frac{D_E D_T D_I}{D_E D_T + D_E D_I + D_T D_I}$$

$$D = \left(\frac{kT}{6\pi}\right)\times\left(\frac{1}{r_E\eta_E + r_T\eta_T + r_I\eta_I}\right)$$

As the membrane viscosity is ~100-fold greater than the viscosities of water and cytoplasm and as the transmembrane domain is not 100-fold smaller than that of the extracellular domain, D should be primarily dependent on the radius of the molecule's transmembrane domain and the viscosity of the membrane.

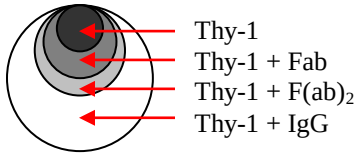
GPI-anchored proteins have some of the fastest reported diffusion coefficients of membrane-bound proteins. One of these, Thy-1, is composed of a single immunoglobulin V-type domain with a mass of only 17.9kDa.

Assuming that immunoglobulin domains have radii of about 2 nm, this would suggest a diffusion coefficient of 1.14 x10$^{-10}$ m$^2$s$^{-1}$ in the absence of a transmembrane domain.

However, the diffusion coefficient of mobile Thy-1 in lymphoma membranes (~50% of total) at room temperature has been reported to be 3.2x10$^{-13}$ m$^2$s$^{-1}$ (*Zhang F, Schmidt WG, Hou Y, Williams AF, Jacobson K. Proc Natl Acad Sci U S A. 1992 Jun 15;89(12):5231-5*). This suggests that the GPI anchor contributes a significant amount of drag. The viscosity of lymphocyte membranes at room temperature has been reported to be about 3 P (*Feinstein MB, Fernandez SM, Sha'afi RI. Biochim Biophys Acta. 1975 Dec 16;413(3):354-70*). This makes it possible to approximate the radius for the GPI anchor as being on the order of 2.4 nm, which is close to the expected size of the extracellular domain.

Thus, Thy-1 diffuses as if its extracellular domain is found inside, not outside, the membrane.

When Thy-1 is detected using Fab, F(ab)$_2$ and whole IgG, diffusion coefficients of 2.0, 1.6 and 1.0x10$^{-13}$ m$^2$s$^{-1}$ are obtained, respectively (*Zhang F et al.*). This yields radii of 3.8, 4.7 and 7.6 nm, respectively.

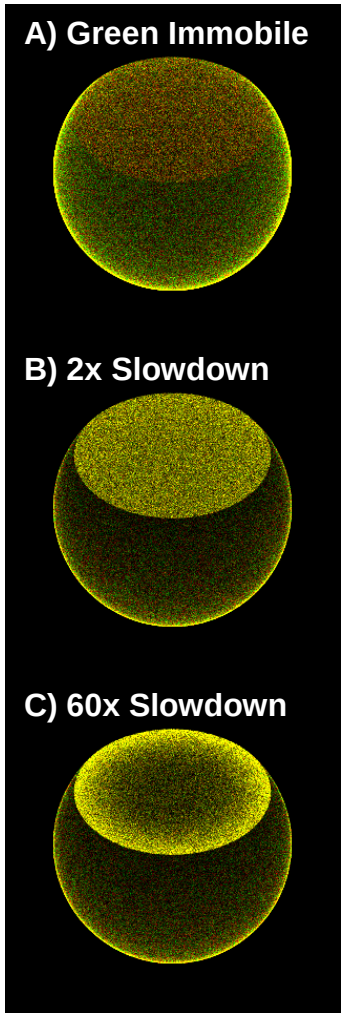


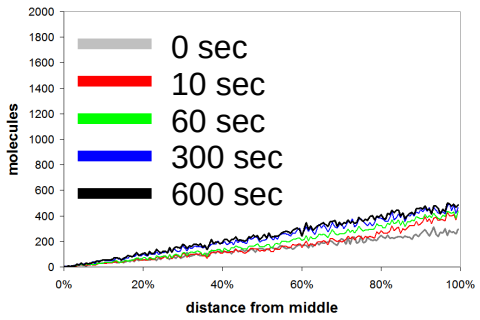

This suggests either that the antibody used to detect Thy-1 (MRC-OX7) is binding to Thy-1 such that it ends up inside the membrane or that there is a dense region just outside the cell membrane that has a similar viscosity to the cell membrane.

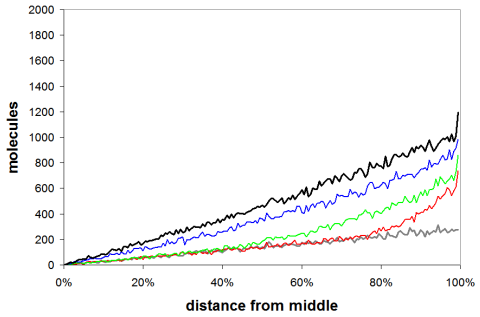
Either way, the use of antibodies (or larger gold beads) to detect proteins may lead to lower than actual diffusion coefficients.

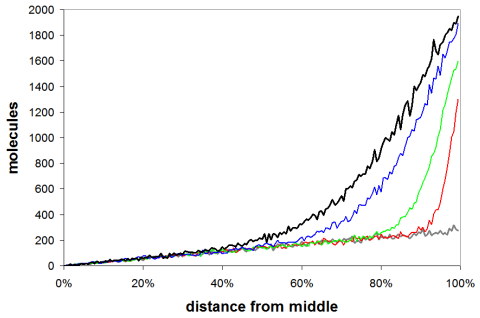
Thus, diffusion coefficients of proteins need to be measured on the cells being studied, at 37°C and with as small a change to the size of the protein as possible.

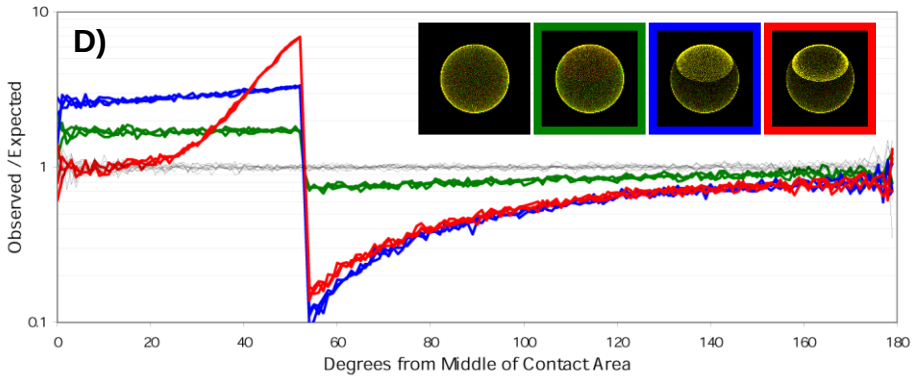
# Whichever Way Molecules Bind, They Always Form A "Synapse"




Ever-increasing numbers of proteins are being found to accumulate at the synapse. Is this a special characteristic of some proteins? Probably not…

150,000 red molecules were randomly seeded onto one cell and 150,000 green molecules were seeded onto another cell. Both cells are represented using the same 5 μm radius sphere with a flat 4 μm radius contact area. Mobile free molecules had diffusion coefficients of $3.0x10^{-14}$ $m^2s^{-1}$ while bound complexes were either (A) immobile or had diffusion coefficients of (B) $1.5x10^{-14}$ $m^2s^{-}1$ or (C) $0.5x10^{-15}$ $m^2s^{-1}$. At 0, 10, 60, 300 and 600 seconds, the distribution of red molecules inside of the contact area was analyzed. The affinities of the molecules for each other were based on the activated LFA-1 interaction with ICAM-1 ($k_{on}$ = $2.0x10^5$ $M^{-1}s^{-1}$; $k_{off}$ = 0.1 $s^{-1}$ (*Tominaga Y, Kita Y, Satoh A, Asai S, Kato K, Ishikawa K, Horiuchi T, Takashi T. J Immunol. 1998 Oct 15;161(8):4016-22*)). The confinement distance inside the contact area was set to 2 nm.

A) A simulation of situations where one molecule is immobile; such as when an adhesion molecule is anchored to the cytoskeleton. Green molecules and their complexes were simulated as being immobile. In this case, red molecules initially accumulated at the edge of the contact area at 10 seconds but further accumulation was minor and distributed over the whole contact area.

B) A simulation of situations where both molecules are equally mobile and their complexes have a diffusion coefficient that is half that of the monomers. In this case, red and green molecules initially accumulate at the edge of the contact area. This accumulation is stronger than when one molecule is immobile. At later timepoints, the accumulation evens out over the surface of the contact area. Because bound molecules cannot leave the contact area while bound, because both molecules diffuse at equal rates and thus enter the contact area at equal rates and because both molecules bind each other well, this leads to an area just outside the contact area where molecules are scarce.

C) A simulation of situations where both molecules are equally mobile but their complexes have 60-fold lower diffusion coefficients; such as when receptor activation leads to anchoring to the cytoskeleton. This situation is similar to (B) but leads to a stronger accumulation at the edge of the contact area.

D) The simulations in A-C were rerun using a curved as opposed to flat contact area. After 600 seconds, the distribution of molecules from the middle of the contact area to the opposite end of the cell (180 degrees away) was analyzed and compared to the expected number of molecules. The edge of the contact area (event horizon) is readily apparent as molecules are enriched inside of the contact area and are scarce just outside it.

# The Equations For Simulating Release, Diffusion and Binding

**Release**

The probability, $p_{rel}$, that a complex will dissociate in a given amount of time is calculated using the standard formula for first-order reactions:

$$p_{rel} = 1 - e^{-k_{off} \times t}$$

A random number between 0 and 1 is generated and, if this random number is smaller than $p_{rel}$, the complex will dissociate.

**Diffusion**

In two-dimensional diffusion, the distance molecules diffuse in each dimension has a normal distribution. The average squared distance moved is the product of 4, the diffusion coefficient and the time being simulated. Thus, in order to simulate diffusion, two random numbers, $x_a$ and $x_b$, with Gaussian distributions, means of 0 and standard deviations of 1 are generated. These numbers are then used with the following equation in order to obtain the distance, $d_{diff}$, that a given molecule with a diffusion coefficient of D will diffuse in a random direction in a given amount of time.

$$d_{diff} = \sqrt{\left(x_a^{\,2} + x_b^{\,2}\right) \times 2 \times D \times t}$$

**Binding**

Tracking the locations and interactions of individual molecules presents a computational challenge as each molecule could potentially interact with each other molecule. In order to get around this n-body problem at the expense of some precision, binding is calculated based on the concentrations of molecules in particular regions of the contact area. The rate of binding of a receptor to different types of ligands can be represented as follows where A is the concentration of the receptor, $B_1$, $B_2$ and $B_n$ are the concentrations of the ligands and $k_1$, $k_2$ and $k_n$ are the rate constants for the binding of the receptor to particular ligands. For a given final rate, the amount of receptor that binds to a particular ligand is proportional to the product of the rate constant for binding to that ligand and the concentration of that ligand divided by the sum of all products of rate constants and ligand concentrations. This leads to the following equation for determining the total amount, x, of initially unbound receptor A that will be bound to a ligand after a given amount of time, t. At that time, the concentration of unbound A will be A – x.

$$\frac{dx}{dt} = (A - x)\left(B_1 - \frac{k_1 B_1}{k_1 B_1 + k_2 B_2 + \ldots + k_n B_n} x\right) + (A - x)\left(B_2 - \frac{k_2 B_2}{k_1 B_1 + k_2 B_2 + \ldots + k_n B_n} x\right) + \ldots + (A - x)\left(B_n - \frac{k_n B_n}{k_1 B_1 + k_2 B_2 + \ldots + k_n B_n} x\right)$$

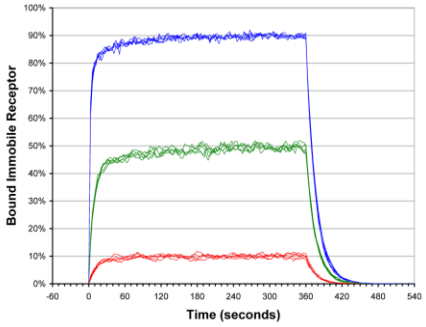
This can be integrated to obtain the probability, x/A, that any initially unbound receptor A will be bound to a ligand during the time being simulated. The probability that receptor A will be bound to the particular ligand $B_i$ is obtained by multiplying x/A by the rate of binding to $B_i$ divided by the rate of binding to any ligand.

$$\frac{x}{A} = \frac{\left(\sum_{i=1}^{n} B_i k_i\right)^2 \times \left(1 - e^{\frac{t \times \left(A \times \sum_{i=1}^{n} B_i k_i^2 - \left(\sum_{i=1}^{n} B_i k_i\right)^2\right)}{\sum_{i=1}^{n} B_i k_i}}\right)}{\left(\sum_{i=1}^{n} B_i k_i\right)^2 - A \times \left(e^{\frac{t \times \left(A \times \sum_{i=1}^{n} B_i k_i^2 - \left(\sum_{i=1}^{n} B_i k_i\right)^2\right)}{\sum_{i=1}^{n} B_i k_i}}\right) \times \sum_{i=1}^{n} B_i k_i^2} \qquad p_{bind} = 1 - \sqrt{1 - \frac{x}{A}}$$

The simulation sequentially calculates release, diffusion and binding for each molecule and receptor/ligand complex. If a molecule ends up forming a receptor/ligand complex by binding to another molecule, the other molecule is no longer treated separately and both molecules are treated as a complex for the duration of the interaction. Due to the sequential nature of the simulation, if a molecule does not end up binding another molecule, this other molecule still has the chance to bind to the initial molecule once it is its turn to undergo diffusion and binding. Thus, the above probability, x/A, is too large when simulating binding sequentially for all molecules. The correct probability, $p_{bind}$, is obtained as follows:

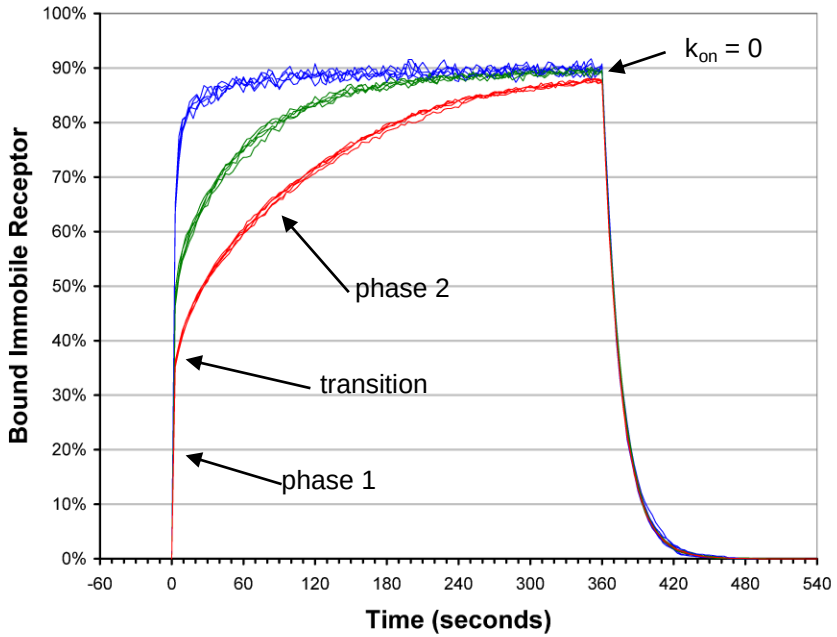
**The binding and release formulas lead to proper equilibrium binding**

The binding of two molecules was simulated using spheres where the contact area was $1/6^{th}$ of the surface area (cell radius = 5 μm; contact area radius = 4 μm; $k_{on} = 1.57 \times 10^3\ M^{-1}s^{-1}$; $k_{off} = 0.063\ s^{-1}$; $D = 1 \times 10^{-13}\ m^2 s^{-1}$). 10000 immobile molecules were seeded over the surface of one cell. 10167, 10833 or 11500 mobile molecules were then seeded over the surface of the other cell and confinement distances of 12.3, 1.37 and 0.15 nm were used such that, at equilibrium, 10, 50 or 90% of immobile molecules inside the contact area should be bound, respectively. After 360 seconds the on-rates were set to zero so that the molecules could no longer associate with one another.




The 10, 50 and 90% targets were obtained.

# The Two Phases Of Binding And The Importance Of Diffusion



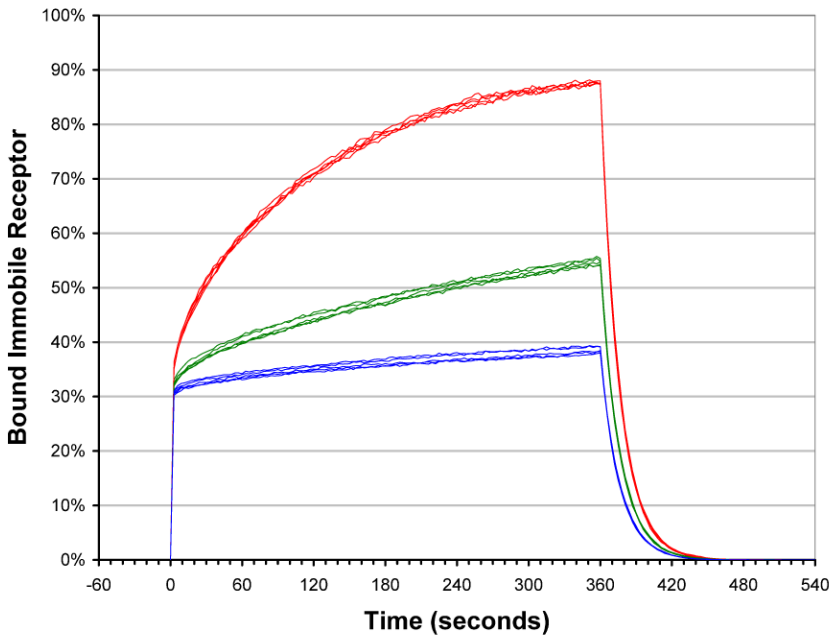

**Receptor binding takes place in two phases**

The binding of two molecules was simulated using spheres where the contact area was 1/6$^{th}$ of the surface area (cell radius = 5 μm; contact area radius = 4 μm; $k_{on}$ = 1.57x10$^{3}$ M$^{-1}$s$^{-1}$; $k_{off}$ = 0.063 s$^{-1}$; D = 1x10$^{-13}$ m$^{2}$s$^{-1}$). Confinement distances were chosen such that 90% of immobile molecules should end up being bound at equilibrium. Binding was allowed for 360 seconds.

10000 (blue), 30000 (green) or 60000 (red) immobile molecules served as the ligands for 11500, 14500 and 19000 mobile molecules, respectively. Each condition was simulated five times.

These results show that equilibrium levels of binding are more quickly approached when the amount of mobile molecules is in excess to the amount of immobile molecules. However, the actual time required to reach equilibrium should be the same in all cases.

In addition, binding occurs in two phases. First, the molecules inside the contact area have a chance to associate. This occurs in the first 10 seconds and is shown by the initially rapid increase in binding to 30%. Additional molecules are then bound in the second phase as more mobile molecules diffuse into the contact area. It is assumed that membrane spacing does not change.




**The diffusion coefficient is important in the second phase of binding**

The binding of two molecules was simulated using spheres where the contact area was 1/6$^{th}$ of the surface area (cell radius = 5 μm; contact area radius = 4 μm; $k_{on}$ = 1.57x10$^{3}$ M$^{-1}$s$^{-1}$; $k_{off}$ = 0.063 s$^{-1}$). A confinement distances was chosen such that 90% of immobile molecules should end up being bound at equilibrium. Binding was allowed for 360 seconds.

60000 immobile molecules served as the ligands for 19000 mobile molecules that had diffusion coefficients of (red) 1x10$^{-13}$, (green) 1x10$^{-14}$ or (blue) 1x10$^{-15}$ m$^{2}$s$^{-1}$. Each condition was simulated five times.

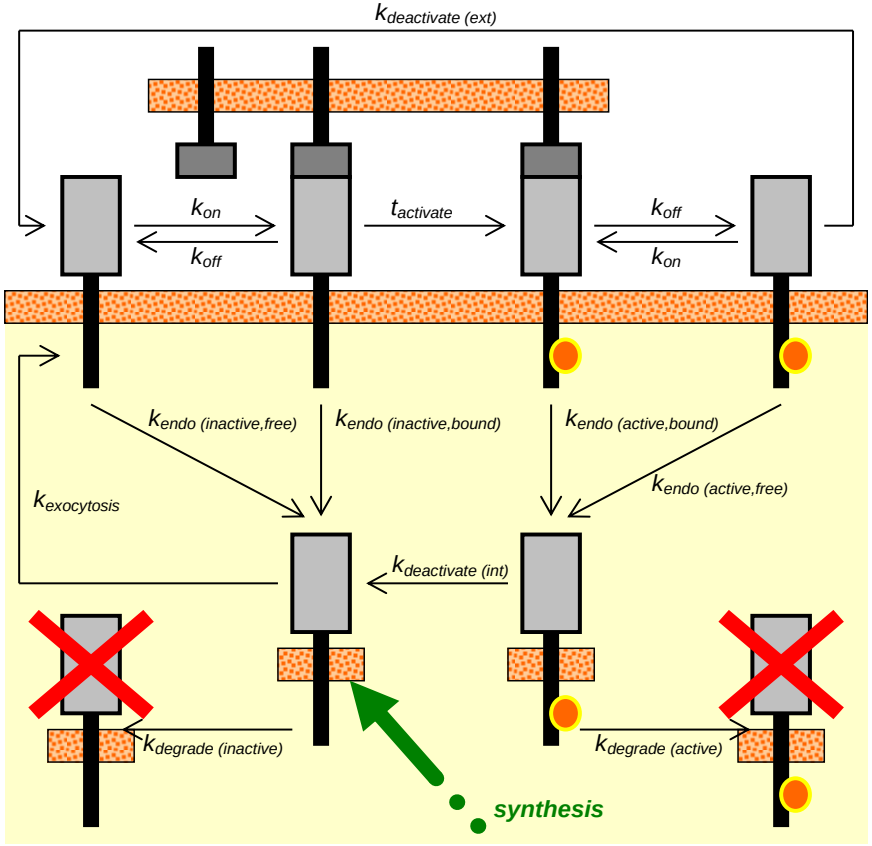
These results show that approximately 30% of immobile molecules are bound in the first phase due to the mobile molecules that are initially present in the contact area. Thereafter, additional immobile molecules are bound as equilibrium (90% target) is slowly attained. The rate at which equilibrium is attained is dependent on the diffusion coefficients of the mobile molecules.

# Simulating Receptor Dynamics

Initially, the simulation allocates space for all potential molecules. These molecules are then either randomly seeded onto the surfaces of spheres or treated as being internalized or degraded. They are then put through iterations where a specified period of time is simulated. The shorter this period of time is, the more accurate the simulation becomes. Ideally, this time should be chosen such that molecules do not diffuse more than one region away in the contact area and do not all release and bind in each period of time. All rates use first-order rate constants except for $k_{on}$, which relies on second-order rate constants. In addition, receptor activation occurs after a fixed period of time and molecule synthesis does not rely on a random number to determine whether or not it should take place.

When using first-order rate constants, the following formula is used (the binding equation is on a separate page) to determine the probability that the event will take place. A random number between 0 and 1 is then generated and the event takes place if this number is lower than the calculated probability.

$$p = 1 - e^{-k \times t}$$




**The sequence of events for each molecule**

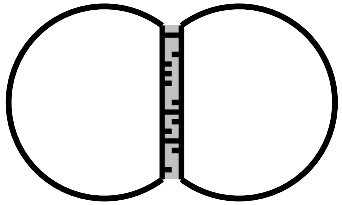
The simulation starts with the first molecule in a long list of molecules and puts it through the following process. If more than one thread is used (e.g. on multi-processor machines) then the list is divided evenly among threads and the starting molecules are chosen accordingly. Once the last molecule is reached, this sequence starts anew at the first molecule until a certain amount of total time is simulated.

- Molecules that are in a complex where they are the binding partner (i.e. the other molecule initiated the binding event) or that are currently internal or degraded are skipped.
- Molecules that are part of a complex and are not the binding partner attempt to release from the binding partner using the specified $k_{off}$.
- Molecules that are still part of a complex (i.e. they did not release) have their bound time increased. If this bound time or the bound time of their partners exceeds a specified amount, $t_{activate}$, they and/or their partners become activated.
- Molecules attempt to internalize based on the applicable $k_{endo}$ (active/inactive, bound/free). When bound molecules internalize, this dissociates the complex and the other molecule is freed.
- External free molecules that are in an activated state attempt to deactivate based on the specified $k_{deactivate(ext)}$.
- Molecules then move and/or diffuse and, if bound, their binding partner is moved to the same location in the contact area.
- Molecules that are not bound and are in the contact area then attempt to bind to other molecules inside the same region of the contact area based on the specified $k_{on}$ values.
- Internal molecules that are in an activated state attempt to deactivate based on the specified $k_{deactivate(int)}$.
- Internal molecules that are in an inactive state attempt to externalize based on the specified $k_{exocytosis}$. During exocytosis, molecules can either randomly appear anywhere on the surface of a sphere or can be biased to appear a certain distance from the middle of the contact area with a normal distribution.
- Internal molecules that are in an inactive state and did not externalize may be degraded based of the specified $k_{degrade(inactive)}$.
- Internal molecules that are in an activated state may be degraded based on the specified $k_{degrade(active)}$.
- Degraded molecules are resynthesized if the current rate of synthesis (a fixed number per unit of time) dictates that additional molecules still need to be added. If a molecule is currently in a degraded state and molecules still need to be synthesized, then the molecule will be resynthesized.

# Basics About The Simulation

**The Simulation, in a nutshell**

The simulation is based on a model of two cells that touch each other at a contact area. These two cells are represented by one or more spheres that each contain a representation of the contact area. The contact area is divided into smaller regions. Molecules are found on the spheres and those inside the same region of the contact area can interact with each other. Molecules diffuse on the spheres and can also move towards a target. Concentrations of molecules are determined by using the area of each region and a confinement distance. On-rates for molecule binding are adjusted based on the membrane separations in the regions.

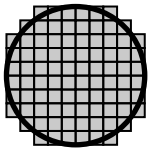


**Cells**

Cells are represented by one or more spheres. For each sphere, it is possible to specify the location of the sphere, the radius of the sphere, the orientation of the contact area, the mean distance from the middle of the contact area for targets of directed molecule movement, the standard deviation of this distance and whether or not the sphere is larger or smaller than a hemisphere. Given that two cells are represented by one or more spheres, this raises the question of why one should represent two cells using more than two spheres. The answer is that this is a simulation of molecular interactions and images usually only have three color channels: red, green and blue. This makes it difficult to tell more than three different types of molecules apart on the same sphere. Thus, in order to visualize the locations of a large number of different molecules and in order to be able to tell them apart, molecules can be assigned to different spheres.

**Contact Area**

It is possible to specify the radius of the contact area, which must be smaller or equal to the radii of all spheres. In order to use experimentally-determined on-rates, the contact area is divided into smaller regions. It is possible to specify the maximum number of molecules that can be found inside each region on a cell and the number and height of virtual immobile bound molecules that contribute to the separation of membranes inside each region.



**Molecules**

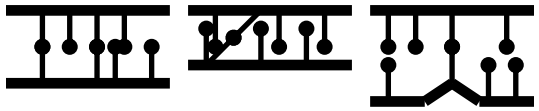
For each type of molecule, it is possible to specify a large number of parameters. These include the name, the sphere it is located on, the color used to represent it when free, bound and activated, the height, the heights of all complexes formed with this type of molecule, the standard deviation for entering regions in an unbound state when the membranes are spaced closer together than the height of the molecule, the standard deviation for entering regions in a complexed state when the membranes are not separated by the height of the complex, the on-rates for forming potential complexes, the standard deviations of on-rate adjustments when the membranes are not separated by the heights of potential complexes, the confinement distance for adjusting on-rates, the off-rates for dissociating complexes, the velocity by which the unbound molecule moves towards a target, the velocities by which complexes move towards a target, the diffusion coefficient when unbound, the diffusion coefficients for complexes, the fraction that is initially intracellular, the fraction that is initially degraded, the rate of resynthesis, the target for exocytosis, the standard deviation of exocytosis, the rate of exocytosis, the rate of endocytosis when unbound and resting, the rate of endocytosis when unbound and activated, the rates of endocytosis when complexed and resting, the rates of endocytosis when complexed and activated, the rate of degradation when resting and intracellular, the rate of degradation when activated and intracellular, the rate of deactivation when extracellular, the rate of deactivation when intracellular and the duration of constant binding needed for activation.

**Confinement Distance**

On-rates are typically determined experimentally for volume and not area concentrations. In order to use on-rates determined for volume concentrations, the area concentrations are divided by so-called confinement distances. While the separation of membranes in the contact area between two interacting cells typically ranges between 15 and 40 nm, the confinement distance has sometimes been reported to be as small as 2 nm (or orders of magnitude larger). This may be because, unlike in solution, molecules in membranes are oriented towards each other (or are separated such that they cannot bind).

**Membrane Separation**

When a molecule of type A on one cell binds to a molecule of type B on another cell, the membranes close to those molecules are spaced so that additional A to B binding is favored. As more A to B binding takes place, this membrane spacing may be reinforced. On the other hand, other molecules whose complexes are taller or shorter than A/B complexes may not bind as readily as their interacting regions may not be optimally spaced. In order to determine the membrane spacing in a region, the simulation averages the heights of all complexes that are found in that region and can also include virtual immobile complexes that do not undergo release, diffusion and binding. In order to penalize interactions between two molecules that are not optimally spaced, on-rates are reduced if membranes are not separated by the distance that is spanned by the potential complex. In addition, the spacing of membranes affects whether molecules and complexes can enter regions (e.g. some may be too tall).



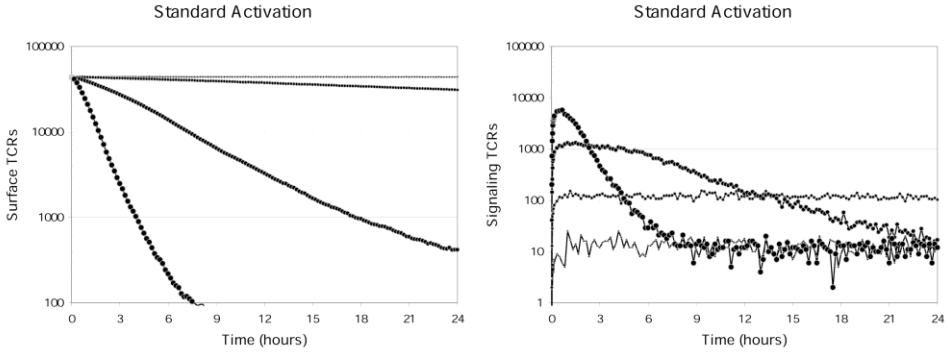
# How Ligand Numbers, Directed Movement And CD2 May Be Important For T Cell Activation

**Simulation parameters**

5 μm radius cells with a 4 μm radius contact area. 50,000 TCRs that can bind to 50, 500, 5,000 or 25,000 peptide/MHC complexes with on-rates of $1.57x10^3$ $M^{-1}s^{-1}$ and off-rates of 0.063 $s^{-1}$ and 25,000 peptide/MHC complexes with on-rates of $1.0x10^3$ $M^{-1}s^{-1}$ and off-rates of 2.0 $s^{-1}$. TCRs can move to the contact area at 0.05 $\mu m \cdot s^{-1}$ and TCR/MHC complexes also actively move at that rate. TCRs are given a very slow lateral diffusion coefficient of $5.0x10^{-15}$ $m^2s^{-1}$. TCRs are set to activate if bound continuously for 20 seconds. 0.0167 TCRs are synthesized per second. 13% of TCRs start out as being internal. The rate constant for TCR externalization is 0.00167 $s^{-1}$. The rate constant for resting and activated TCR internalization is 0.00025 and 0.00197 $s^{-1}$, respectively. The rate constant for TCR deactivation is 0.01155 $s^{-1}$. In addition, there are 50,000 LFA-1, 50,000 ICAM-1, 14,000 CD2 and 50,000 CD48 molecules. CD2 and CD2/CD48 complexes also move to the synapse at a rate of 0.05 $\mu m \cdot s^{-1}$.

**Analysis of TCR surface levels and TCR activation**

Experimentally, T cell activation leads to a downregulation of TCR levels if there are enough ligands. Surface TCR levels over 24 hours of simulated time using 10 second intervals were analyzed as well as numbers of activated TCRs.

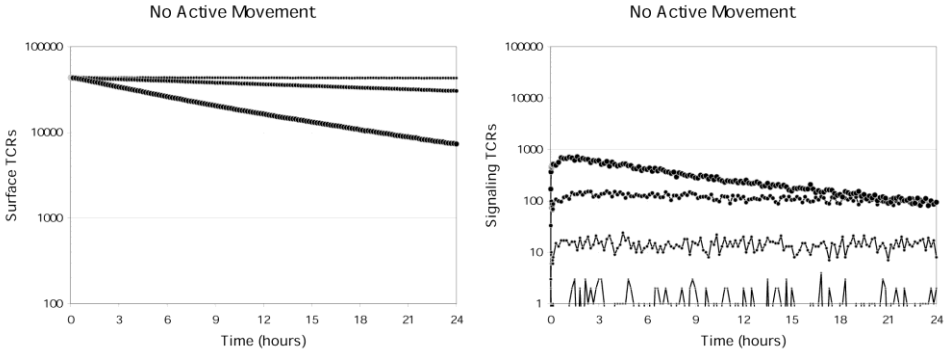



The large dots represent the surface and signaling TCR levels when 25,000 agonist peptide/MHC complexes are present on the APC. The increasingly smaller dots represent 5,000, 500 and 50 agonist peptide/MHC complexes, respectively.

Thus, the presence of a large number of agonist peptides leads to a large number of signaling TCRs but this only lasts for 6 hours. On the other hand, lower numbers of agonist peptide/MHC complexes lead to prolonged signaling at lower levels.

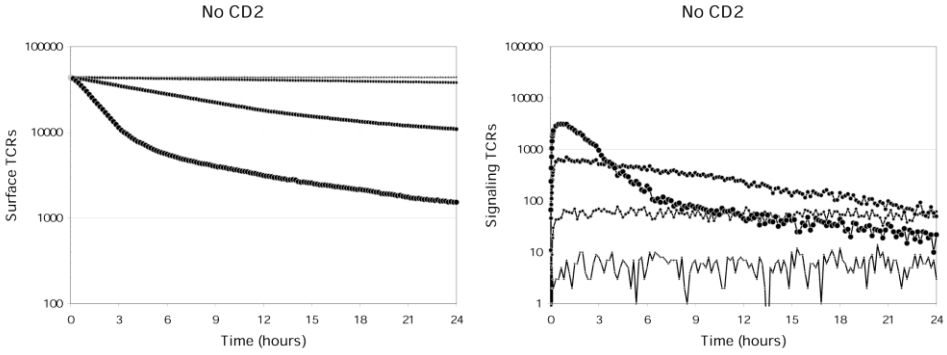
**Directed movement is important**

TCRs and CD2 accumulate at the synapse in part through their association with CD2-AP that moves towards the synapse. When there is no directed movement, the simulation predicts that there should be less TCR degradation and lower levels of TCR signaling.




**CD2 is important**

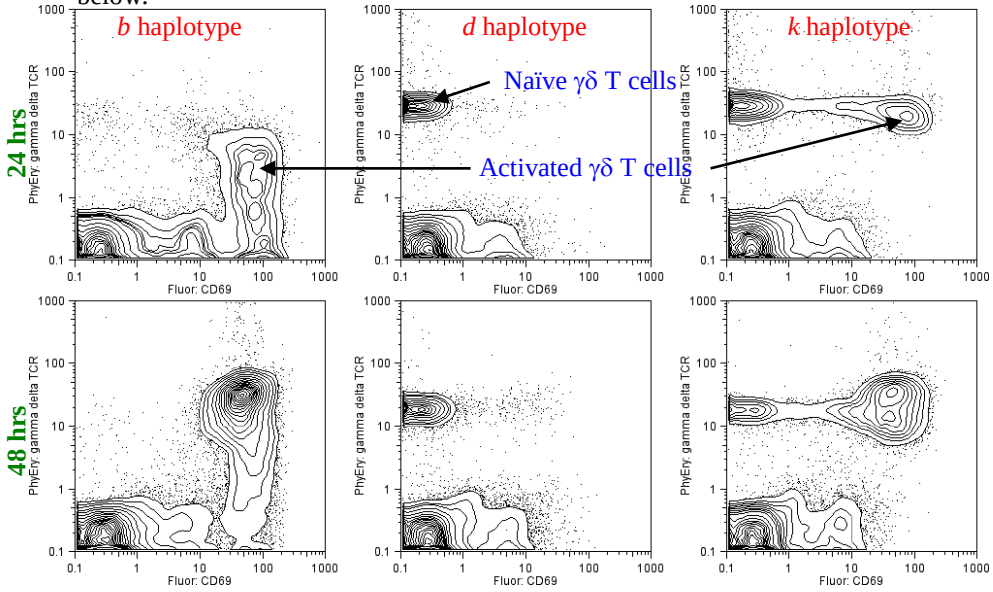
The CD2/CD48 complex spans a distance similar to that of a TCR/MHC complex. CD2 has a reasonably high affinity for CD48 where the on-rate is a fast $1.0x10^5$ $M^{-1}s^{-1}$ but the off-rate is a very fast 6.0 $s^{-1}$ (i.e. the half life of the interaction is only a fraction of a second). Thus, the CD2/CD48 interaction may be involved in temporarily spacing the membranes optimally for TCR/MHC binding. In order to explore the important of CD2 on TCR levels and signaling, the simulation was run in the absence of CD2 and predicts that there should be less endocytosis (something observed when CD2-deficient intraepithelial lymphocytes are compared to CD2-expressing splenic T cells).



# The Case Of The Missing γδ T Cells

**The Experimental System**

The G8 γδ TCR recognizes the MHC class I-like molecules T10 and T22 of the *b* haplotype as well as the T10 molecule of the *k* haplotype. It does not recognize the T10 molecule of the *d* haplotype that is found in BALB/c mice. When γδ T cells from G8 BALB/c mice are co-incubated with splenocytes from BALB/c mice that express only the *d* haplotype, the γδ T cells do not activate. On the other hand, when they are co-incubated with splenocytes from BALB/c mice that are congenic for either the *b* or *k* haplotypes, they activate as is evidenced by their expression of CD69. In addition, when T10/T22 levels are high, as is the case with the *b* haplotype, γδ TCR levels can drop so severely that it is no longer possible to identify some activated γδ T cells. This is shown below.




**The Observation**

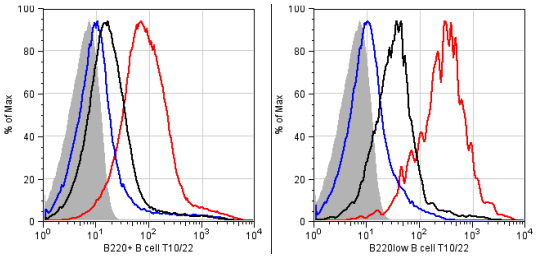
When splenocytes expressing the T10/T22 molecules of the *b* haplotype are used for the co-cultures, sorted CD69-negative γδ TCR-negative cells and sorted CD69-positive γδ TCR-negative cells still contain transcripts for the γδ TCR.

**The Question**

Could there be γδ T cells among the γδ TCR-negative cells that are either CD69 negative or CD69 positive?

**B cells express a range of T10/T22 levels**

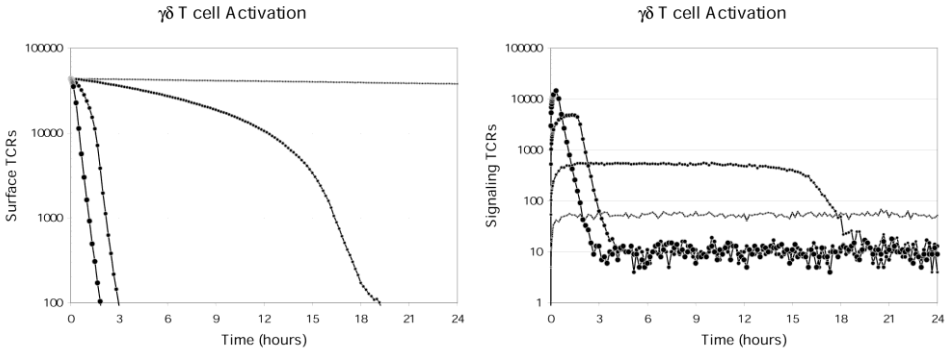
T10/T22 staining was analyzed on cells from BALB/c (blue; *d*), C57Bl/6 (black; *b*) and B.10BR mice (red; *unknown*). B cells from BALB/c mice that are congenic for the *k* haplotype (not shown) stain similar to B cells from BALB/c mice.




Thus, B cells express a range of T10/T22. The majority express levels within a 1 log range. However, there are also outliers that express more than 1 log more T10/T22 than the majority of B cells.

**A simulation of γδ TCR levels and signaling**

24 hours of γδ T cell activation were simulated. Predicted surface γδ TCR levels and numbers of activated γδ TCRs were analyzed for conditions where 25,000 (large dots), 5,000, 500 and 50 (smallest dots) T10/T22 of the *b* haplotype were present on B cells.




**The Answer: Yes, γδ T cells may lose their TCR expression within 3 hours**

The majority of γδ T cells activated experimentally by T10/T22 of the *b* haplotype exhibit TCR downregulation that is similar to what is predicted for 500 ligands. Thus, some B cells (1 log higher T10/T22 levels) may lead to a result that is similar to the 5,000 ligand plot. This would lead to less than 3 hours of signaling, which may not be enough for full activation and CD69 expression. It would also lead to undetectable γδ TCR levels.

# The Reason For Null Peptide/MHC Accumulation At The Synapse

**The Observation**

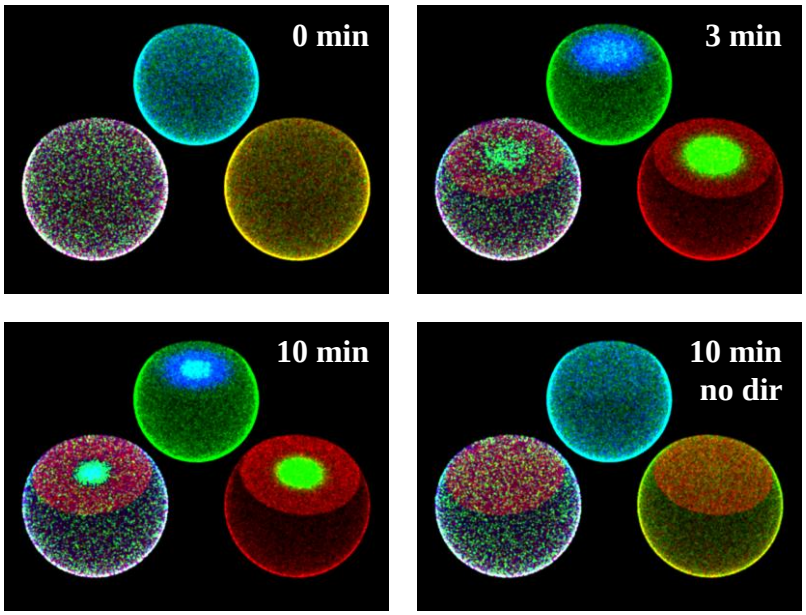
When agonist peptide/MHC complexes accumulate in the synapse, there is also an accumulation of unrelated null peptide/MHC complexes.

**The Question**

Is this accumulation indicative of a novel clustering process such as MHC dimers or does it occur because of something much more mundane.

**A simulation of TCR activation**

50,000 TCRs were allowed to bind to 5,000 agonist peptide/MHCs and 50,000 null peptide/MHCs in the presence of LFA-1, ICAM-1, CD2 and CD48. There was active movement of the TCR and CD2 towards the synapse. 10 minutes were simulated in 0.1 second intervals.



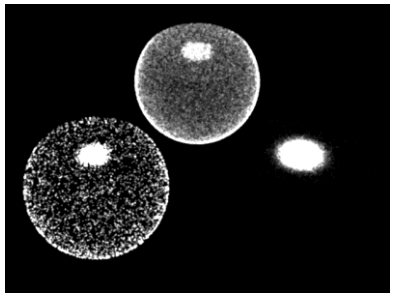

The upper middle sphere shows CD2 and CD48 in blue and green, respectively. The lower left sphere shows ICAM-1 in red, agonist peptide/MHC in green and null peptide/MHC in blue. The lower right sphere shows LFA-1 in red and the αβ TCR in green.

The upper left, upper right and lower left panels show the 0, 3 and 10 minute results of the simulation. The lower right panel shows that active TCR and CD2 movement is required to form a central cluster.

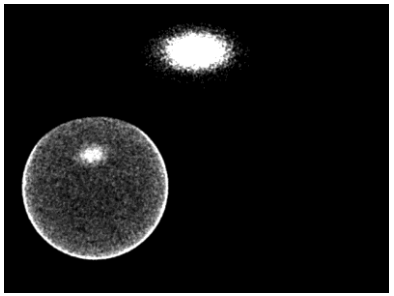
**Null Peptide/MHC clusters in the simulation**

The green and blue channels of the 10 minute result were analyzed further to clearly reveal the locations of CD48, agonist peptide/MHC and TCR (green) and CD2 and null peptide/MHC (blue).

CD48, agonist peptide/MHC and TCR



CD2 and null peptide/MHC

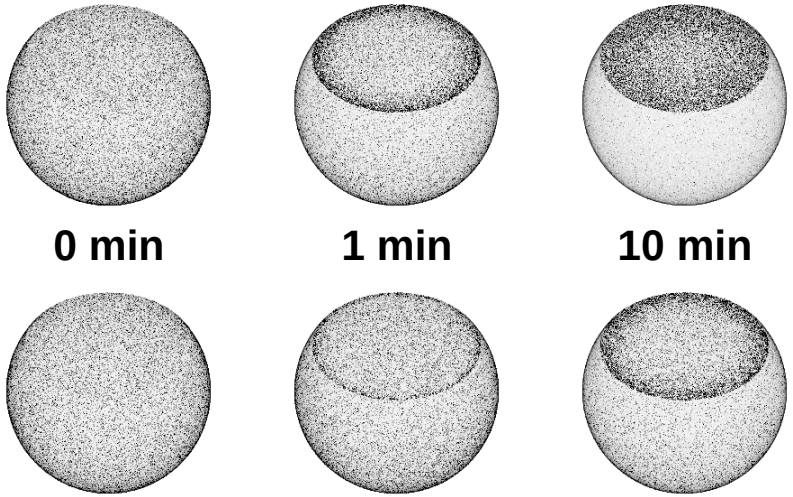


**The Answer: TCR accumulation drives MHC accumulation**

TCR movement towards the synapse is needed for the formation of the ICAM-1/LFA-1 ring and the central TCR cluster. High concentrations of TCR are then obtained and drive the formation of TCR/agonist peptide/MHC and TCR/null peptide/MHC complexes. While TCR/null peptide/MHC complexes are short-lived, they keep forming and dissociating. The large amount of TCR compared to agonist peptide/MHC ensures that not all TCRs end up bound to agonist peptide/MHCs and thus also form complexes with null peptide/MHCs.

# Diffusion, Accumulation And LFA-1 Activation

$D = 3x10^{-13}\ m^2s^{-1}$



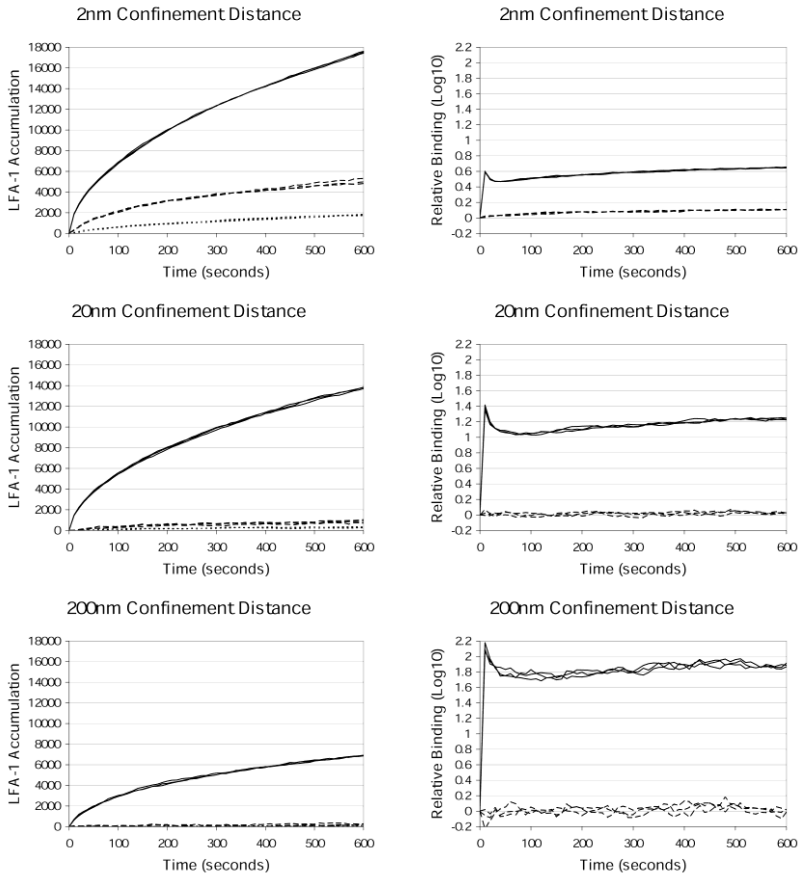

$D = 3x10^{-14}\ m^2s^{-1}$

**The diffusion coefficient affects accumulation**

The binding of 15,000 mobile molecules to 300,000 immobile molecules was simulated using spheres where the contact area was 1/6$^{th}$ of the surface area and using on-rates and off-rates that were similar to those of activated LFA-1 binding ICAM-1 (cell radius = 5 μm; contact area radius = 4 μm; $k_{on} = 2.0x10^5$ $M^{-1}s^{-1}$; $k_{off} = 0.1\ s^{-1}$; confinement distance = 2nm).

When a 10-fold lower diffusion coefficient was chosen, accumulation took about 10 times longer.

While this result is obvious, bound molecules affect membrane spacing. As faster moving molecules can accumulate faster, complexes of these faster moving molecules will more rapidly affect membrane spacing to favor the binding of additional molecules that span the same distance as the complex. As activation may change the mobility of some membrane proteins and as different types of cells have different membrane viscosities, this may lead to physiologically-relevant differences in accumulation patterns and synapse formation.




**Activated LFA-1 binds well under suboptimal conditions**

LFA-1 activation has been reported to lead to a higher affinity ($k_{on} = 2.0x10^5$ $M^{-1}s^{-1}$ vs. 367 $M^{-1}s^{-1}$ and $k_{off} = 0.1\ s^{-1}$ vs. 0.033 $s^{-1}$) for ICAM-1 and increased mobility ($D = 2.9x10^{-14}$ $m^2s^{-1}$ vs. $2.3x10^{-15}$ $m^2s^{-1}$). The confinement distance was either 2 (optimal), 20 or 200 nm (suboptimal). 600 seconds of binding were simulated and LFA-1 accumulation inside the synapse was analyzed (left) under conditions where LFA-1 bound and diffused as if it was activated (solid lines), bound and diffused as if it was resting (short dashes) or bound as if it was resting but diffused as if it was activated (long dashes). In addition, the amount of bound fast-moving activated LFA-1 (solid lines) and bound fast-moving resting LFA-1 (long dashes) was compared to the amount of bound slow-moving resting LFA-1 (right). Thus, the activation of LFA-1 allows it to bind well under suboptimal conditions (a 100-fold increase in confinement distance only reduces accumulation 3-fold).