

# The synapse assembly model

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A framework for quantitative analysis of the mechanisms underlying immunological synapse assembly has been recently developed. This model uses partial differential equations to describe the binding interactions of receptors and ligands, with the constraint that they are embedded in apposed deformable membranes linked to a cytoskeletal complex.

The equations that describe the model are (see Table 1 for definitions of symbols):

$$\frac{\partial C_T}{\partial t} = D_T \nabla^2 C_T - k_{on}(z) C_T C_M + k_{off} (I - P) C_{TM} + \bar{V} \cdot \bar{\nabla} C_T \quad [\text{Eqn 1}]$$

$$\frac{\partial C_M}{\partial t} = D_M \nabla^2 C_M - k_{on}(z) C_T C_M + k_{off} C_{TM} \quad [\text{Eqn 2}]$$

$$\frac{\partial C_{TM}}{\partial t} = D_{TM} \left[ \nabla^2 C_{TM} + \frac{1}{k_B T} \bar{\nabla} C_{TM} \cdot \bar{\nabla} \frac{\delta F}{\delta C_{TM}} \right] + k_{on}(z) C_T C_M - k_{off} C_{TM} \quad [\text{Eqn 3}]$$

$$\frac{\partial C_{Ai}}{\partial t} = D_{Ai} \nabla^2 C_{Ai} - k_i(z) C_{Ai} C_{Bi} + k_{-i} C_i \quad [\text{Eqn 4}]$$

$$\frac{\partial C_{Bi}}{\partial t} = D_{Bi} \nabla^2 C_{Bi} - k_i(z) C_{Ai} C_{Bi} + k_{-i} C_i \quad [\text{Eqn 5}]$$

$$\frac{\partial C_i}{\partial t} = D_i \left[ \nabla^2 C_i + \frac{1}{k_B T} \bar{\nabla} C_i \cdot \bar{\nabla} \frac{\delta F}{\delta C_i} \right] + k_i(z) C_{Ai} C_{Bi} - k_{-i} C_i \quad [\text{Eqn 6}]$$

$$\frac{\partial z}{\partial t} = -M \frac{\delta F}{\delta z} + \xi \quad [\text{Eqn 7}]$$

$$F = \frac{\lambda_T}{2} \iint dx dy C_{TM}(x, y, t) [z(x, y, t) - z_{TM}]^2 + \sum_i \frac{\lambda_i}{2} \iint dx dy C_i(x, y, t) [z(x, y, t) - z_i]^2 + \frac{1}{2} \iint dx dy \left[ \gamma (\nabla z)^2 + \kappa (\nabla^2 z)^2 \right] \quad [\text{Eqn 8}]$$

These equations represent the physical processes discussed in the associated article in mathematical terms. The model proposes a specific mechanism for how the short-length scale processes of binding and dissociation, protein mobility and membrane mechanics influence each other, such that large-scale

patterns can emerge. The first six equations represent the spatio-temporal evolution of receptors, ligands and receptor–ligand complexes. Terms proportional to  $\nabla^2 C_j$  in which  $j$  denotes a species, represent membrane protein transport owing to diffusion. In the equation for T-cell receptor (TCR) concentration (Eqn 1), the term  $\bar{V} \cdot \bar{\nabla} C_T$  represents directed transport owing to cytoskeletal motion. Binding kinetics are represented (Eqns 1–6) using the standard mass action law. An important aspect of the model is that the kinetic constants for receptor–ligand association depend on the local intermembrane separation (i.e. membrane shape) because the TCR–peptide–MHC (pMHC) pair and the leukocyte function-associated molecule-1 (LFA-1)–intercellular adhesion molecule-1 (ICAM-1) pair have different topographic sizes. Equation 7 describes the time evolution of membrane shape. This type of Landau–Ginzburg equation is also referred to as potential motion [i.e. it, by itself, attempts to drive the membrane shape to an equilibrium state corresponding to the minimum in the free energy ( $F$ ) of the membrane]. The free energy functional is represented in Eqn 8. The first two terms correspond to the penalties associated with membrane shapes that lead to deformation of the receptor–ligand bonds away from their natural size. The last two terms represent the free energy penalties arising from membrane shape changes that lead to new area creation (proportional to  $\gamma$ ) and high curvature shapes (proportional to  $\kappa$ ). The equations that describe the spatio-temporal evolution of the receptor–ligand complexes contain terms that lead to transport of these species to regions of the membrane where the intermembrane spacing is close to their natural lengths. These terms are proportional to  $\frac{\delta F}{\delta C_i}$ . The system is not necessarily driven to an equilibrium state by Eqns 1–6. It is also important to note that the equations describing the model dissipate energy and in this sense, energy producing cellular processes are required for self-organization of synaptic patterns.

## Choosing parameter values

The mechanics of the deformable membranes of interest are those wherein an intact cytoskeleton is connected to the lipid bilayer (e.g. through proteins, such as Talin). Experiments have shown that mutant cells not expressing Talin have values of the tension and bending rigidity that are 75% smaller than those of wild-type cells [1]. A decrease in these important membrane characteristics is also observed for cells that do not express cortexillin, a protein that interconnects the actin filaments [1]. The results reported in the associated article correspond to parameters for wild-type cells [1]. However, we have carried out calculations for a wide range of  $\kappa$  and  $\gamma$ . For TCR–pMHC binding kinetics corresponding to the peptide MCC88–103 and the 2B4 TCR [2,3], variations of the tension by an order of magnitude

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Table 1. Equation symbols

Symbol	Quantity
F	Free energy
$C_T$	TCR concentration in T-cell membrane
$C_M$	MHC-peptide concentration in supported membrane
$C_{TM}$	Concentration of TCR-MHC-peptide complex
$C_{Ai}$	LFA-1 concentration in T-cell membrane
$C_{Bi}$	ICAM-1 concentration in supported membrane
$C_i$	Concentration of LFA-1-ICAM-1 complex
$k_{on}$	On rate for TCR-MHC-peptide binding
$k_{off}$	Off rate for TCR-MHC-peptide binding
$k_i$	On rate for LFA-1-ICAM-1 binding
$k_{-i}$	Off rate for LFA-1-ICAM-1 binding
$D_j$	Diffusion coefficient of the $j^{\text{th}}$ protein complex
Z	Local intermembrane separation
$Z_j$	Natural length of $j^{\text{th}}$ protein complex
t	Time
$\gamma$	Interfacial tension of cell membrane
$\kappa$	Bending rigidity of cell membrane
$\zeta$	Thermal noise
$k_B T$	Thermal energy at temperature T
M	Phenomenological constant for membrane response to free energy changes
$\lambda_j$	Curvature of binding energy well for the $j^{\text{th}}$ protein complex
V	Speed of directed TCR transport

around the value used in the calculations described enable a mature immunological synapse to form. Variation of the bending rigidity by as much as a factor of three still enables synapse formation. If these parameters are too high (i.e. the membrane is too stiff) synaptic patterns do not form. For certain values of the tension and bending rigidity below the optimal range, synaptic patterns do form but in others many exotic non-synaptic patterns are found (e.g. when  $\kappa = 30$  and  $\gamma = 0.2$ , roughly 10% of the value for wild-type cells, double ring patterns are predicted). The parameters describing the cell mechanics are determined by the nature of the cell membrane, the cytoskeleton and the proteins that connect these two cellular sub-structures. Therefore, synaptic pattern formation is dependent on membrane mechanics that are based on regulated membrane-cytoskeletal interactions.

Measured values of the on and off rates for the interaction of LFA-1 and ICAM-1 are used (see associated article). When TCRs are crosslinked with soluble antibodies, the collective avidity of LFA-1 molecules transiently increases the strength of adhesion (increased avidity) [4]. Under conditions of immunological synapse formation with antigen presenting cells (APCs) or planar bilayers, the induction of high LFA-1 avidity is sustained over long periods of time [2,5]. In our current model the affinity range displayed by LFA-1 does not change in resting or activated cells, rather, the organization of interactions and their number changes, giving rise to adhesion and synapse formation. A distribution of LFA-1 affinities can, however, be easily incorporated in the model.

ICAM-1 and pMHC are known to be mobile on APCs [6,7]. Regulation of the lateral mobility of these

proteins by cytoskeletal interactions augments some T-cell responses, but is not essential for T-cell activation [6]. They are definitely mobile in the supported bilayer experiments [2], and the measured values of the diffusion coefficients that we use are of the order of  $1 \mu\text{m}^2 \text{s}^{-1}$ , which is typical of GPI-linked proteins in supported bilayers (specific values [8,9]).

How mobile TCRs and LFA-1 are in the T-cell membrane is known less precisely. There is some evidence that the diffusion coefficient of a TCR might be as low as  $0.01 \mu\text{m}^2 \text{s}^{-1}$  [10]. Furthermore, the fractional mobility of a TCR displaying this mobility was small [2]. However, a TCR is known to acquire higher mobilities after T-cell polarization and during activation [11–13] and diffusion coefficients ranging from  $0.1$ – $1 \mu\text{m}^2 \text{s}^{-1}$  have been reported for mobile proteins on cell surfaces [14]. There is evidence that cytoskeletal motion owing to myosin motor proteins could be the microscopic mechanism that regulates TCR mobility during synapse formation [15]. If this mechanism leads to a directed or convected flow of the TCR rather than isotropic diffusion, then the term proportional to V in Eqn 1 is necessary. Values for the velocity of the order of  $3 \mu\text{m} \text{min}^{-1}$  have been estimated [15] but the fraction of molecules that are involved in this type of transport has not been reported. Thus, rather than introduce another fitting parameter into the equations, to study intrinsic synaptic pattern forming tendencies an isotropic diffusion coefficient is used. Importantly, to match the time scales over which different protein patterns evolve in the experiments [2], the highest observed value of protein diffusivity on cell surfaces ( $\sim 1 \mu\text{m}^2 \text{s}^{-1}$ ) was used [16]. We emphasize that, in our view, cytoskeletal motion and other cellular processes provide a microscopic mechanism for receptor protein mobility and set the values of the relevant phenomenological parameters.

#### Further details on parameter values used

For LFA-1 and ICAM-1 the measured values  $K_d = k_{-i}/k_i = 0.3 \mu\text{m}^2 \text{molecules}^{-1}$ , and  $k_{off} = 0.1 \text{s}^{-1}$  [17,18] were used and the binding kinetics for TCR-pMHC were varied. We used measured values of the tension and bending rigidity for *Dictyostelium discoideum* cells [1]:  $= 3.1 \pm 1.4 \mu\text{N m}^{-1}$  (used 3.1) and  $= 391 \pm 156 k_B T$  (used 400). The natural lengths of complexes are:  $Z_{\text{TCR-pMHC}} = 15 \text{ nm}$  [19–21];  $Z_{\text{LFA-1-ICAM-1}} = 40 \text{ nm}$  [19]. The data on three-dimensional (3D)  $K_d$  was converted to 2D values using the measured value of  $1.2 \text{ nm}$  [2]. For  $D_{\text{ICAM-1}}$  (or CD54) in the bilayer the measured value for a similarly GPI-linked CD58  $= 0.59 \mu\text{m}^2 \text{s}^{-1}$  [22] was used. Typical diffusion coefficients of GPI-linked proteins, including MHC, in supported bilayers are of the order  $1 \mu\text{m}^2 \text{s}^{-1}$  [8]; this value for  $D_{\text{pMHC}}$  was used. A discussion of how mobile TCRs and LFA-1 are in the cell membrane is provided in the associated article. Calculations have been carried out [16] for a range of values of  $D_{\text{TCR}}$  and  $D_{\text{LFA-1}}$  ( $0.01, 0.1, 1.0 \mu\text{m}^2 \text{s}^{-1}$ ). Here

we use  $1.0 \mu\text{m}^2 \text{s}^{-1}$ .  $P = \exp[-k_{\text{off}}\tau]$ ;  $\tau$  is estimated to be 2–5 s [23]; we have used 5 s because this is the value used by Qi *et al.*, and provides a good representation of experimental data [2]. Other values of this parameter would lead to quantitative but not qualitative differences. TCR density is  $200 \text{ molecules } \mu\text{m}^{-2}$ . For the curvature of the binding wells ( $\lambda_c$ ) we used  $50 k_B T \mu\text{m}^{-2}$  and  $M = 4 \times 10^{-5} \mu\text{m}^4 \text{s}^{-1} (k_B T)^{-1}$ ; changing these values by factors of two or three does not affect qualitative results [16]. Studies using various dosages of pMHC and ICAM-1 have been carried out using the model. The results reported here are for pMHC and ICAM-1 loadings of 20 and 40 molecules  $\mu\text{m}^{-2}$ , respectively, which provides a good representation of the experimental data [2,16]. Two types of experiments, micropipette experiments [24] and fluorescence methods [2], have been used to obtain the dependence of 2D  $K_d$  on  $z$ . The results are presented in

terms of a confinement thickness and the two types of experiments yield very different results (confinement thickness ranging from 5 nm to 1 cm). We use a Gaussian distribution centered around  $z = z_j$  for  $k_{\text{on}}(z)$ ; calculations have been carried out with the widths of the distribution equal to 5 nm and 13 nm.

#### Numerical solution method

The model equations were solved numerically using a straightforward finite difference scheme. Initially, the membrane proteins were distributed uniformly on the membranes and no flux boundary conditions were imposed far away from the edge of the contact region. Also, the upper membrane was initially given a parabolic shape and the intermembrane distance was held constant far from the edge of the contact region. The results are insensitive to  $\pm 5 \text{ nm}$  changes in the initial intermembrane spacing.

#### References

- 1 Simson, R. *et al.* (1998) Membrane bending modulus and adhesion energy of wild-type and mutant cells of *Dictyostelium* lacking talin or cortaxillins. *Biophys. J.* 74, 514–522
- 2 Grakoui, A. *et al.* (1999) The immunological synapse: a molecular machine controlling T cell activation. *Science* 285, 221–227
- 3 Wülfing, C. *et al.* (2002) Costimulation and endogenous MHC ligands contribute to T-cell recognition. *Nat. Immunol.* 3, 42–47
- 4 Dustin, M.L. *et al.* (1998) A novel adapter protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. *Cell* 94, 667–677
- 5 Monks, C.R. *et al.* (1998) Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature* 395, 82–86
- 6 Wade, W.F. *et al.* (1995) Class II cytoplasmic and transmembrane domains are not required for class II-mediated B cell spreading. *Immunol. Lett.* 44, 67–74
- 7 Wülfing, C. *et al.* (1998) Visualizing the dynamics of T-cell activation: intracellular adhesion molecule 1 migrates rapidly to the T-cell/B-cell interface and acts to sustain calcium levels. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6302–6307
- 8 Fein, M. *et al.* (1993) Lateral mobility of lipid analogues and GPI-anchored proteins in supported bilayers determined by fluorescent bead tracking. *J. Membr. Biol.* 135, 83–92
- 9 Groves, J.T. *et al.* (1996) Electrical manipulation of glycan-phosphatidyl inositol-tethered proteins in planar supported bilayers. *Biophys. J.* 71, 2716–2723
- 10 Sloan-Lancaster, J. *et al.* (1998) ZAP-70 association with T-cell receptor  $\zeta$  (TCR $\zeta$ ): fluorescence imaging of dynamic changes upon cellular stimulation. *J. Cell Biol.* 143, 613–624
- 11 Kucik, D.F. *et al.* (1996) Adhesion activating phorbol ester increases the mobility of leukocyte integrin LFA-1 in cultured lymphocytes. *J. Clin. Invest.* 97, 2139–2144
- 12 Montixi, C. *et al.* (1998) Engagement of T-cell receptor triggers its recruitment to low-density detergent-insoluble membrane domains. *EMBO J.* 17, 5334–5348
- 13 Xavier, R. *et al.* (1998) Membrane compartmentation is required for efficient T-cell activation. *Immunity* 8, 723–732
- 14 Jacobson, K.A. *et al.* (1997) Cellular determinants of the lateral mobility of neural cell adhesion molecules. *Biochim. Biophys. Acta* 1330, 138–144
- 15 Wülfing, C. and Davis, M.M. (1998) A receptor/cytoskeletal movement triggered by costimulation during T-cell activation. *Science* 282, 2266–2269
- 16 Qi, S.Y. *et al.* (2001) Synaptic pattern formation during cellular recognition. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6548–6553
- 17 Tominaga, Y. *et al.* (1998) Affinity and kinetic analysis of the molecular interaction of ICAM-1 and leukocyte function-associated antigen-1. *J. Immunol.* 161, 4016–4022
- 18 Labadia, M.E. *et al.* (1998) Molecular regulation of the interaction between leukocyte function-associated antigen-1 and soluble ICAM-1 by divalent metal cations. *J. Immunol.* 161, 836–842
- 19 Shaw, A.S. and Dustin, M.L. (1997) Making the T-cell receptor go the distance: a topological view of T-cell activation. *Immunity* 6, 361–369
- 20 Garcia, K.C. *et al.* (1996) An  $\alpha\beta$  T-cell receptor structure at 2.5 Å resolution and its orientation in the TCR–MHC complex. *Science* 274, 209–219
- 21 Garboczi, D.N. *et al.* (1996) Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. *Nature* 384, 134–141
- 22 Dustin, M.L. *et al.* (1996) Visualization of CD2 interaction with LFA-3 and determination of the two-dimensional dissociation constant for adhesion receptors in a contact area. *J. Cell Biol.* 132, 465–474
- 23 Valitutti, S. *et al.* (1997) Degradation of T-cell receptor (TCR)-CD3- $\zeta$  complexes after antigenic stimulation. *J. Exp. Med.* 185, 1859–1864
- 24 Zhu, C. (2000) Kinetics and mechanics of cell adhesion. *J. Biomech.* 33, 23–33

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