Cell Acitivation Mediated by Immuno-Receptors

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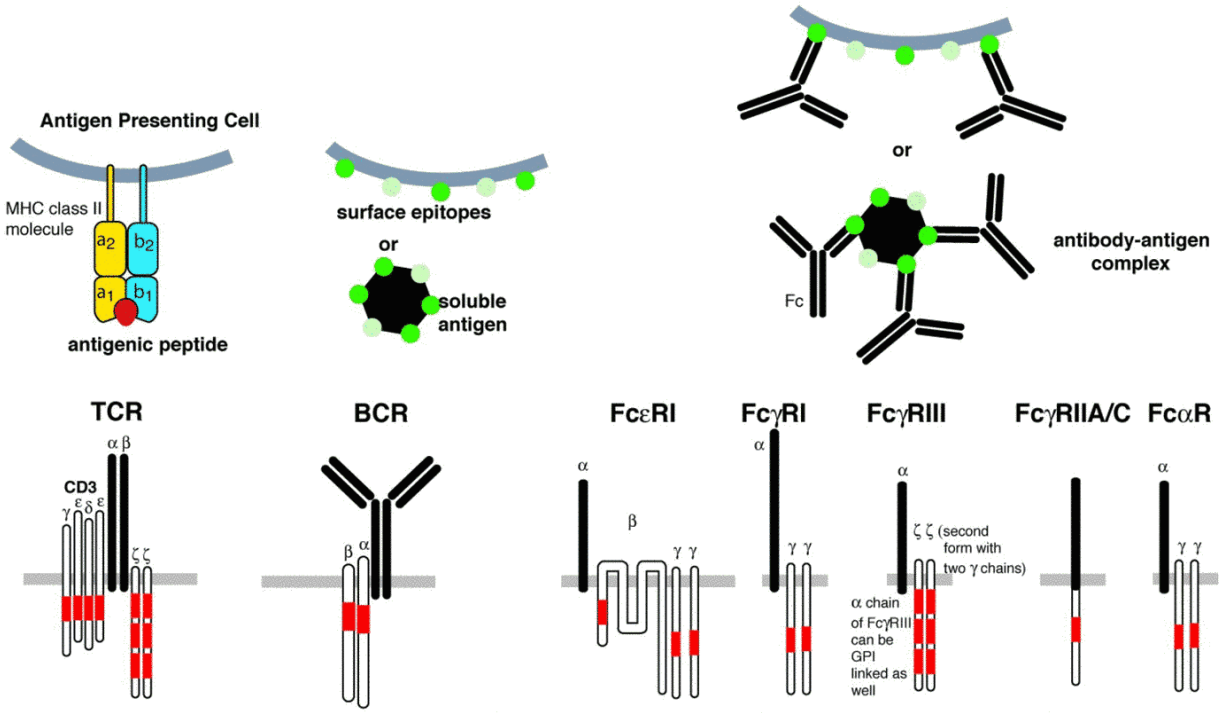
Immuno-Receptors and the Ligands They Recognize



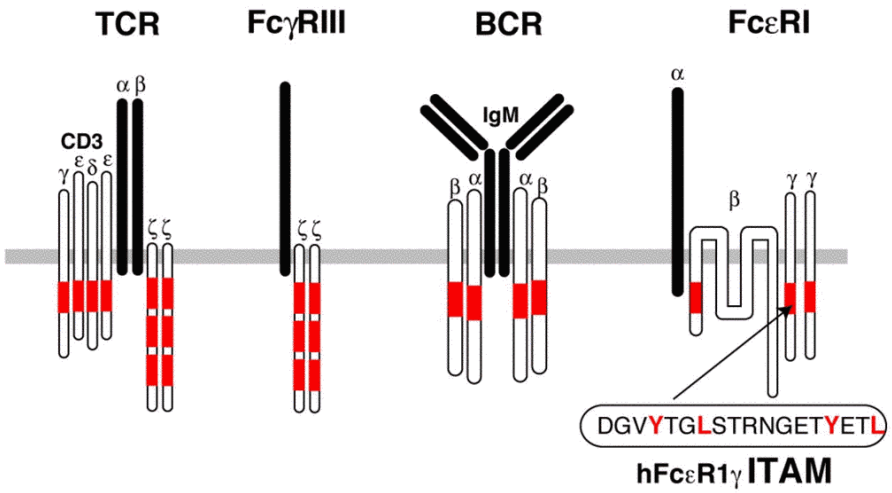
The cell surface receptors that are involved in recognizing (binding) antigenic determinants (things foreign)

The multisubunit immune recognition receptors (MIRR):

1. Have separate binding and signaling subunits;

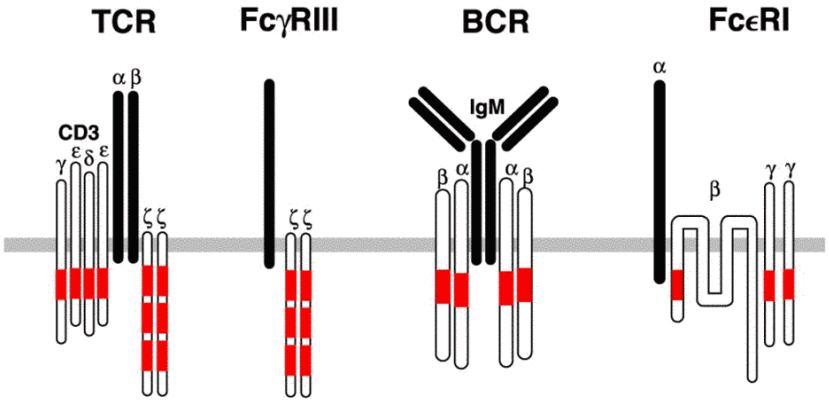
2. The signaling subunits all have one or more protein sequences of the form: **YxxL/IxxxxxxxYxxL/I,** called an **immunoreceptor tyrosine-based activation motif** or **ITAM;** Each ITAM has two tyrosines (**Y**)

3. Have no intrinsic kinase activity - associate with a kinase to initiate signaling.



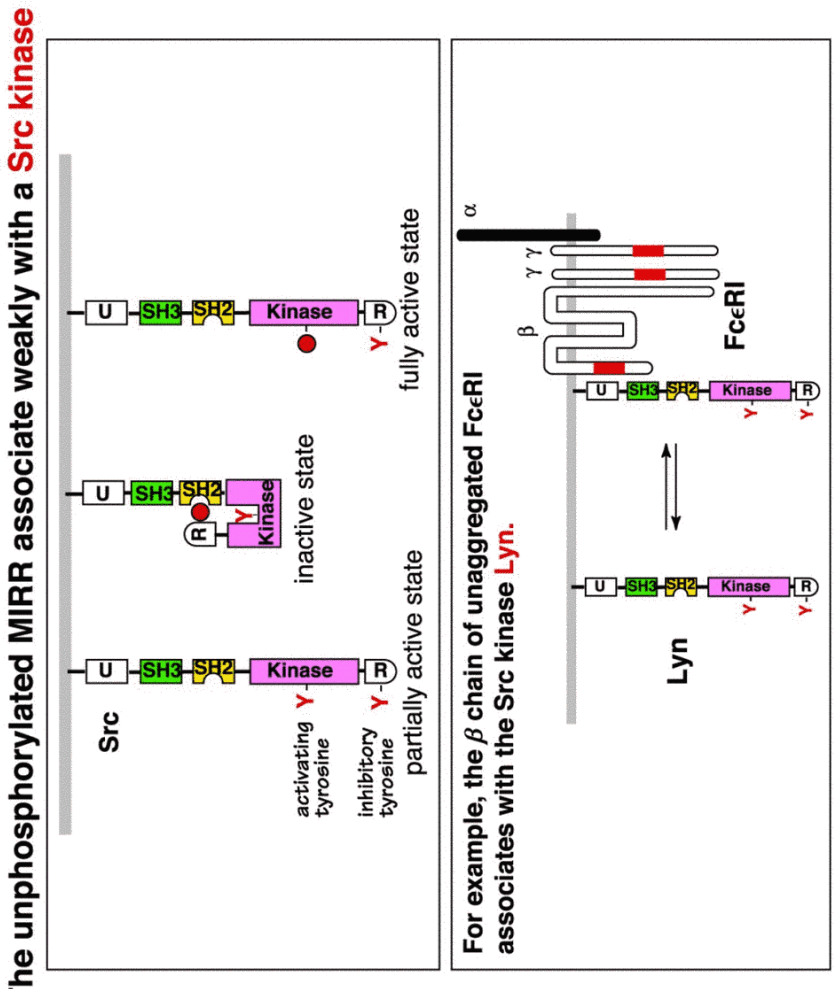
The multisubunit immune recognition receptors (MIRR) :

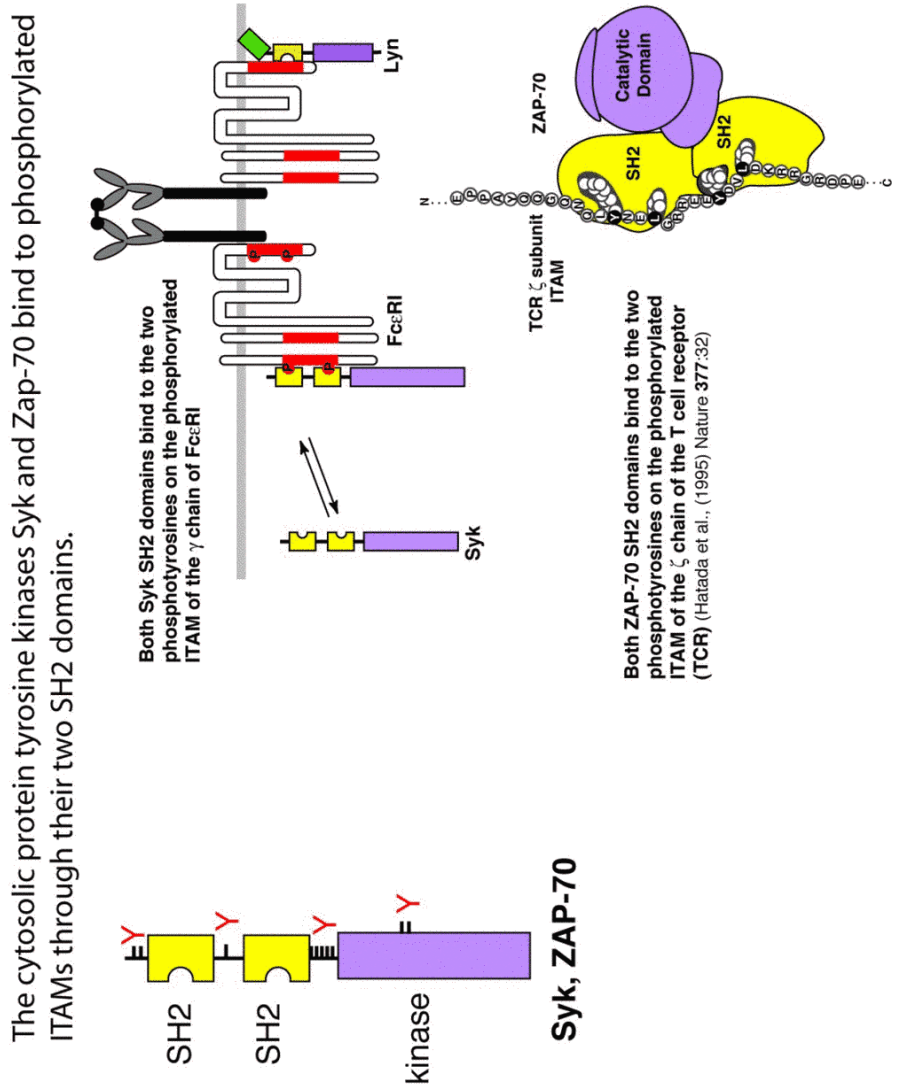
The signaling chains have one or more ITAMS each having two tyrosines.

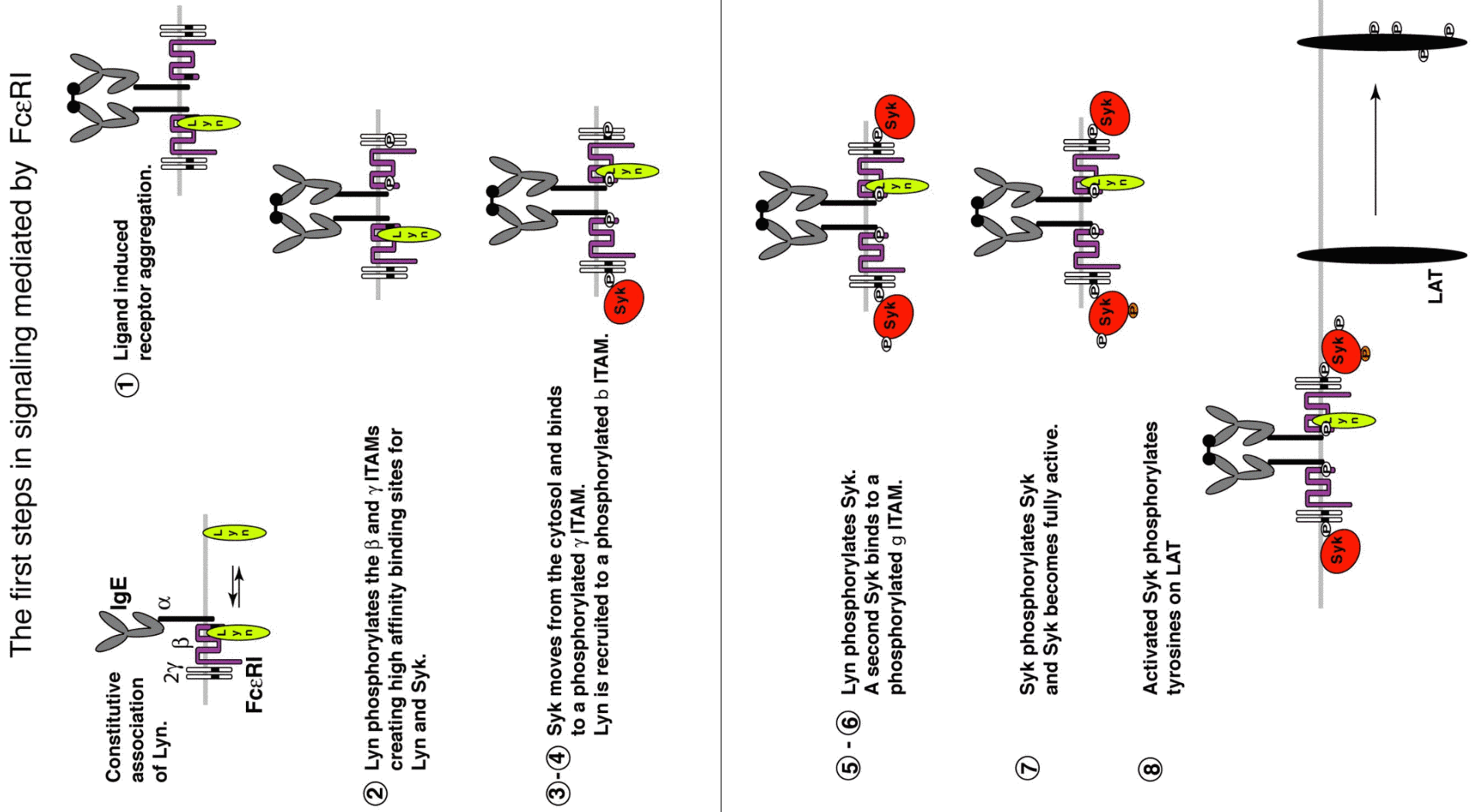


Receptor	ITAMs	States of phosphorylation in an aggregate of two receptors
TCR	10	$2^{40} \sim 1.1\times10^{12}$
FcγRIII (ζ form)	6	$2^{24} \sim 1.7\times10^{7}$
BCR	4	$2^{16} = 65536$
FcεRI	3	$2^{12} = 4096$

A detailed mathematical model will not be a roadmap where one inch equals one inch.


The unphosphorylated MIRR associate weakly with a Src kinase
Src
U
SH3
SH2
Kinase
R
Y
activating tyrosine
inhibitory tyrosine
partially active state
inactive state
fully active state
For example, the β chain of unaggregated FcεRI associates with the Src kinase Lyn.
α
β
γ γ
FcεRI
Lyn


The cytosolic protein tyrosine kinases Syk and Zap-70 bind to phosphorylated ITAMs through their two SH2 domains.
SH2
SH2
kinase
Syk, ZAP-70
Y
Both Syk SH2 domains bind to the two phosphotyrosines on the phosphorylated ITAM of the γ chain of FcεRI
FcεRI
Lyn
Syk
TCR ζ subunit ITAM
ZAP-70
Catalytic Domain
SH2
SH2
N
C
Both ZAP-70 SH2 domains bind to the two phosphotyrosines on the phosphorylated ITAM of the ζ chain of the T cell receptor (TCR) (Hatada et al., (1995) Nature 377:32)


The first steps in signaling mediated by FcεRI
Constitutive association of Lyn.
IgE
α
β
2γ
FcεRI
Lyn
① Ligand induced receptor aggregation.
② Lyn phosphorylates the β and γ ITAMs creating high affinity binding sites for Lyn and Syk.
③-④ Syk moves from the cytosol and binds to a phosphorylated γ ITAM. Lyn is recruited to a phosphorylated b ITAM.
Syk
⑤ - ⑥ Lyn phosphorylates Syk. A second Syk binds to a phosphorylated g ITAM.
⑦ Syk phosphorylates Syk and Syk becomes fully active.
⑧ Activated Syk phosphorylates tyrosines on LAT
LAT

1. All immune recognition receptors have at least one ITAM in their cytoplasmic domains.

2. Upon receptor aggregation, tyrosines within the ITAMs become phosphorylated.

3. Src protein tyrosine kinases (Lyn, Fyn, Lck, etc.), attached to the inner leaflet of the plasma membrane, initiate ITAM tyrosine phosphorylation.

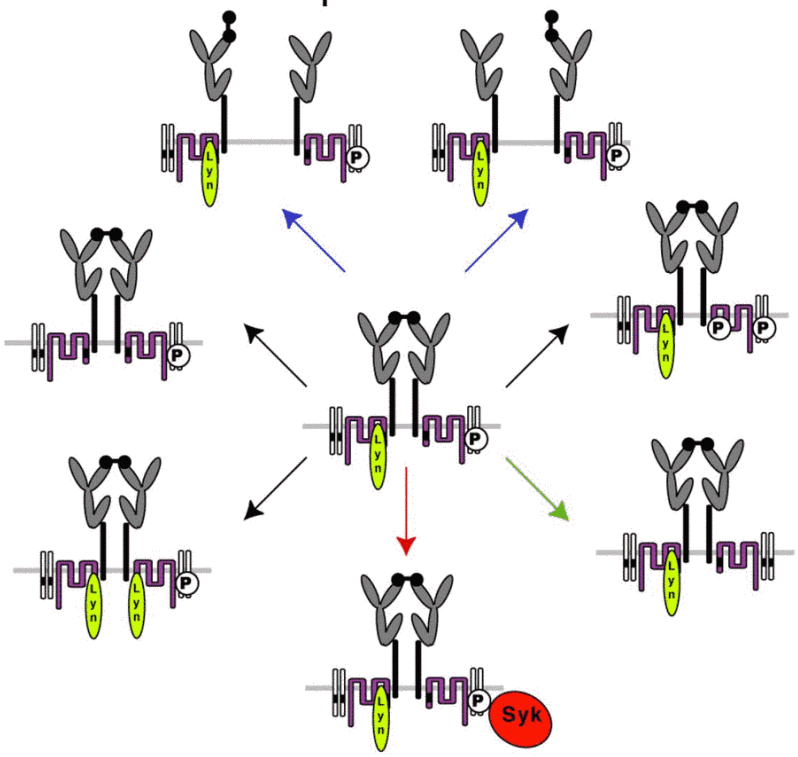
4. Syk protein tyrosine kinases (Syk, Zap-70) bind to phosphorylated ITAMS. In this way, Syk, Zap-70 or both are recruited from the cytosol to the membrane.

5. Once activated, Syk and Zap-70 phosphorylate other proteins, such as LAT, that act as scaffolds for signaling molecules to bind to.

6. Breakup of receptor aggregates results in rapid (seconds) dephosphorylation. Within an aggregate, phosphorylation and dephosphorylation are in constant competition.

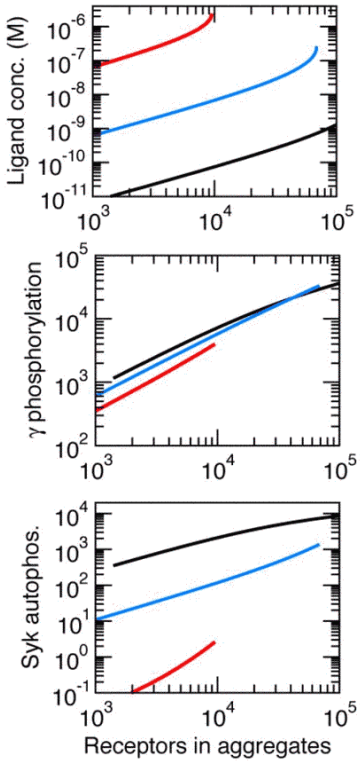
The cytoplasmic domains of the receptors and other scaffolding proteins are sites for the coalescence of kinases, phosphatases and adapters. The structures that form are ephemeral with components going on and off rapidly. **The success of their construction depends on the lifetime of the receptor-ligand bond.**

A signaling cascade is not a linear chain of chemical reactions but a complex chemical network.



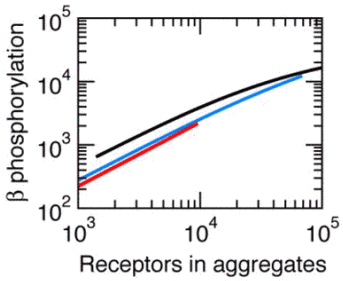
Recruitment of Syk to phosphorylated γ ITAM

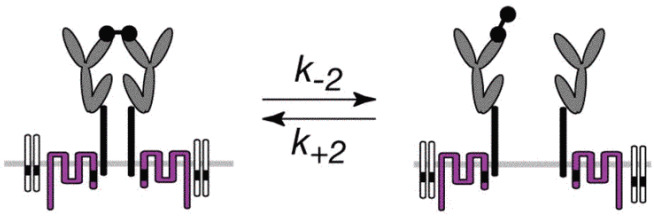
A 354 state model for the initial signaling events mediated by FcεRI exhibits **kinetic proofreading** (Faeder et al., 2003, J. Immunol. 170: 3769) as have been observed (Torigoe et al., 1998, Science 281:568).



$k_{+1} = 1 \times 10^6\ M^{-1}s^{-1}$

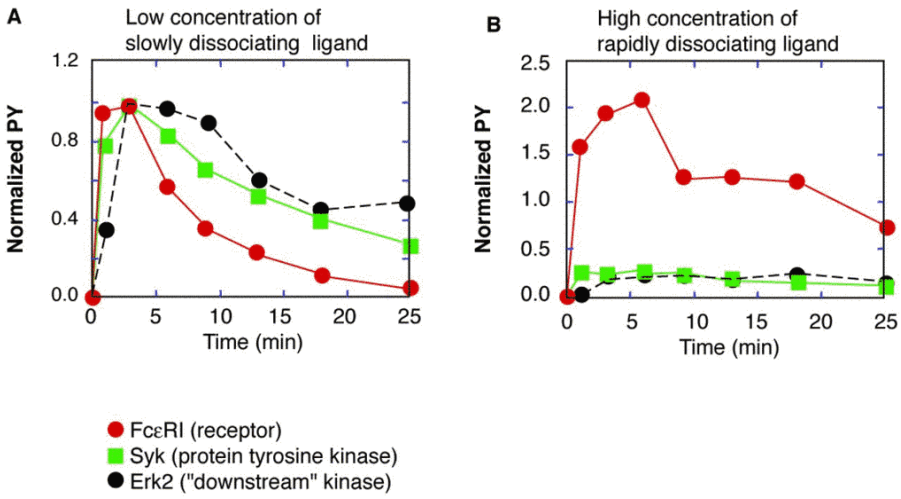
$k_{-1} = k_{-2} = 0.05\ s^{-1}$, $0.5\ s^{-1}$ and $5.0\ s^{-1}$





Evidence for kinetic proofreading in mast cell responses to two ligands

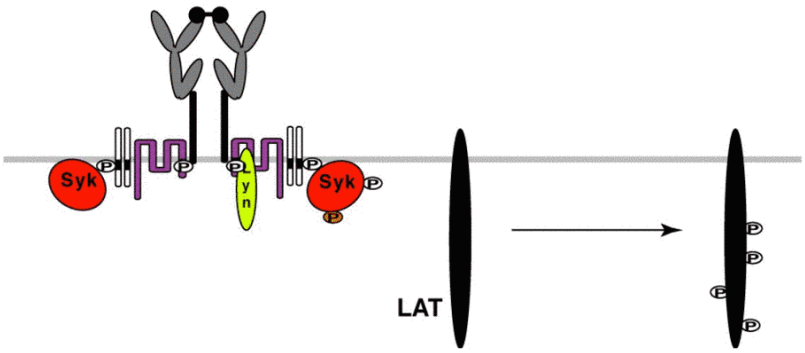
Time course of phosphorylation of tyrosines on several proteins
(Torigoe, Inman & Metzger. 1998. *Science* 281:568-572)



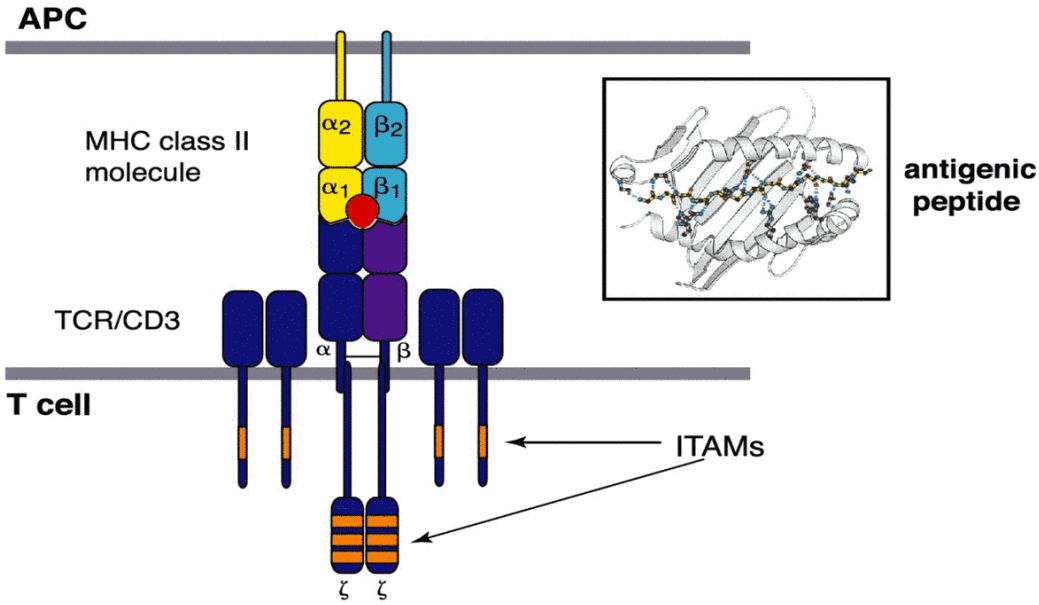
If Syk doesn't form a long lived complex with LAT then there should be no kinetic proofreading beyond LAT.

If there is kinetic proofreading beyond LAT, then the present picture of how Syk and LAT interact is wrong.

⑧ **Activated Syk phosphorylates tyrosines on LAT**



Peptide-MHC on antigen presenting cells (**APC**) binds to T cell receptors (**TCR**). Binding is rapidly followed by phosphorylation of tyrosines on the receptor (**TCR/CD3**) subunits in regions known as immunoreceptor tyrosine-based activation motifs (**ITAMs**).

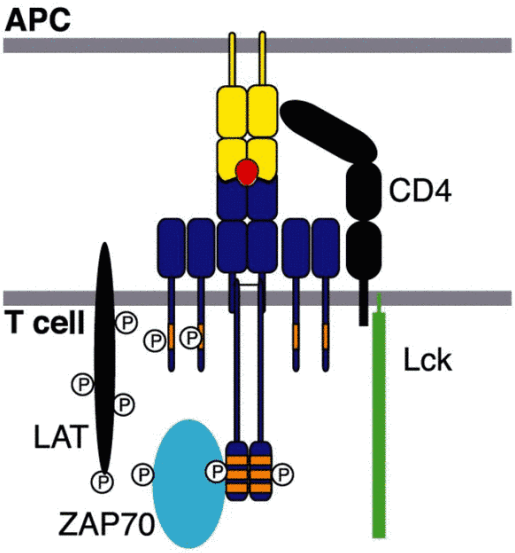


Upon binding of Peptide-MHC to T cell receptors (TCR) a rapid series of biochemical events occurs.

1. Tyrosines on T cell receptor subunits are rapidly phosphorylated.

2. These phosphotyrosines act as docking sites for other molecules that participate in the signaling cascade.

3. A scaffolding of molecules builts up about the receptor cytoplasmic domains.

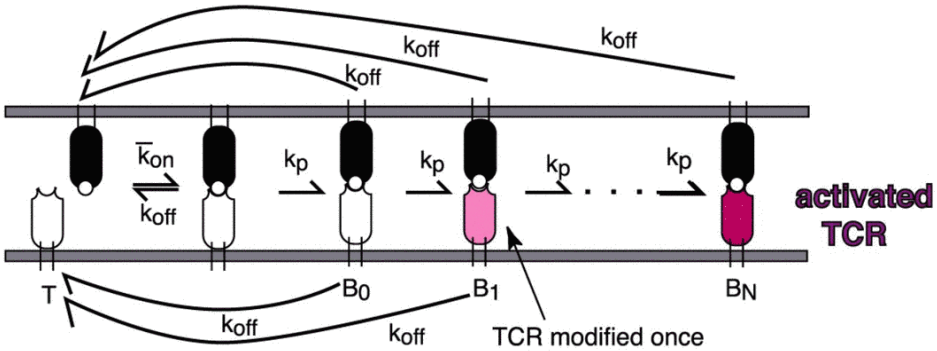


To try to understand how the binding properties of the peptide-MHC for the TCR influence the activation of the T cell, McKeithan (1995, PNAS, 92:5042) introduced the **kinetic proofreading model.**

The kinetic proofreading model replaces the complex chemistry of the signaling cascade but captures one key feature: that a series of events, e.g., the building of a scaffolding about the receptor, is required if a TCR is to become activated.

McKeithan's Kinetic Proofreading Model

$\bar{k}_{on}$ and k_{off} are the rate constants for TCR-peptide-MHC bond formation.
$k_{off} = \ln 2/t_{1/2}$

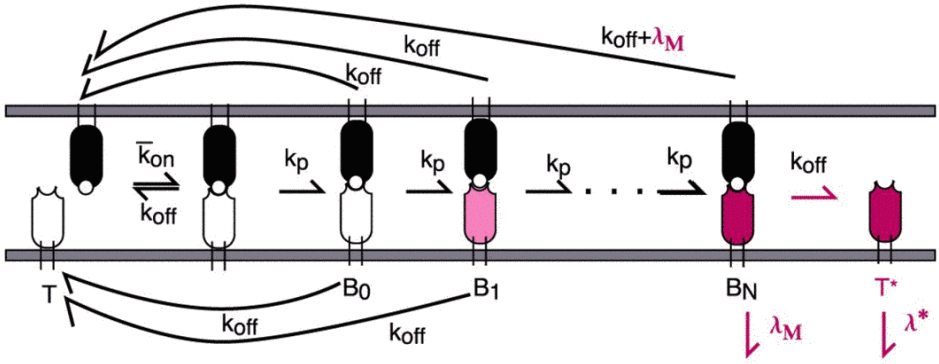


1. For the response of interest the true chemical cascade is replaced by a series of irreversible reactions.

2. A TCR becomes activated after undergoing N modifications, each with rate constant k_p.

3. When a bound TCR dissociates it reverts to its basal state.

Extending McKeithan's model to include TCR internalization

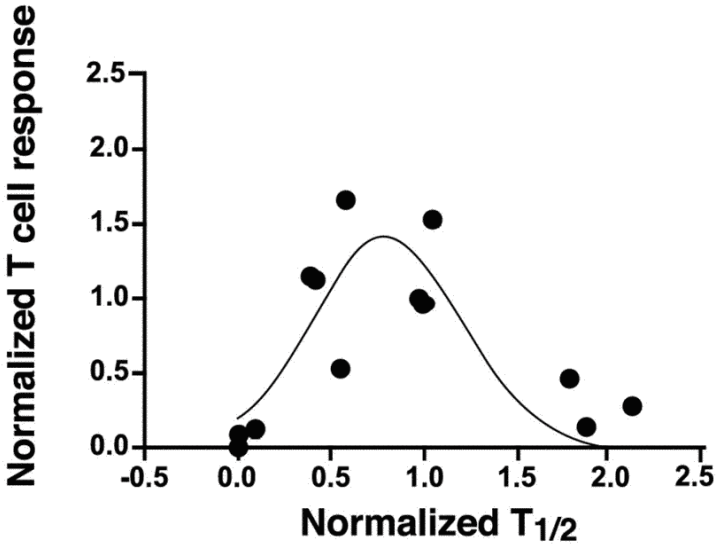


A bound TCR can be internalized with rate constant λ_M freeing a peptide-MHC.

A TCR that has been fully modified remains active after dissociation (T^*). It is internalized at a rate λ^*.

Correlation between the half-life (T1/2) of the TCR-peptide-MHC bond and T cell activation.

Kalergis et al. (2001) Nature Immunol. **2**: 229-234

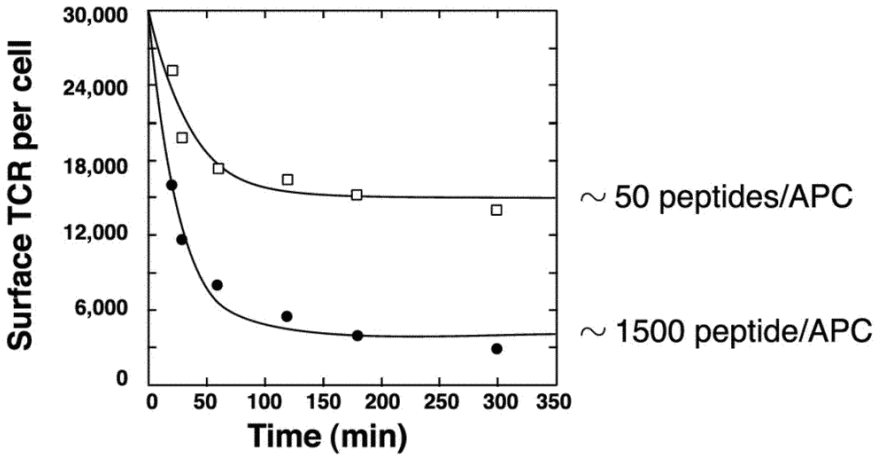


(Normalization = observed response/WT response)

Serial Engagement

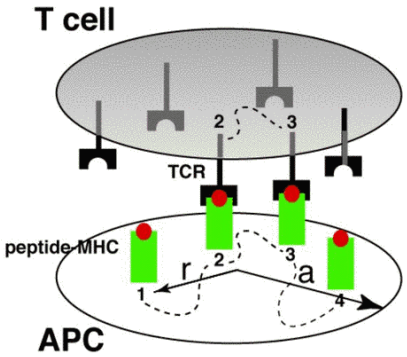
Valitutti et al. (1995), Serial triggering of many T-cell receptors by a few peptide-MHC complexes. **376**: 148 - 152

APC with as few as 100 peptide-MHCs can trigger the internalization of thousands of TCRs -- "a single complex can serially engage and trigger up to ~ 200 TCRs."



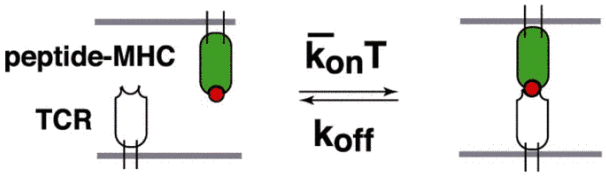
Serial Engagement

An unbound peptide-MHC (1) binds to a TCR (2), activates it (2-3), dissociates (3), and moves on (4) to bind another TCR, ...



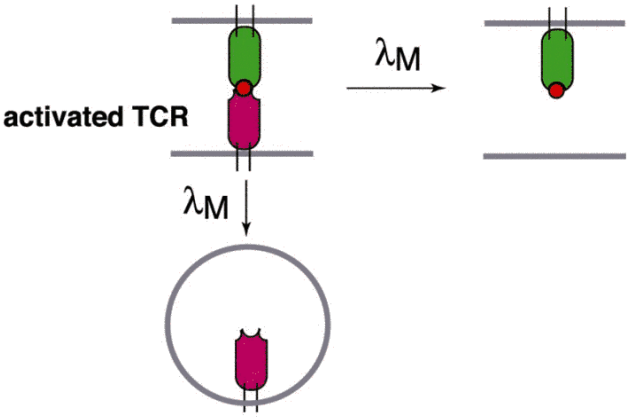
The time the TCR stays bound must be long enough for the TCR to undergo the full set of modifications required for activation (kinetic proofreading) (Wofsy et al., 2001. Calculations show substantial serial engagement of T cell receptors, Biophys. J. 80:606.)

In the contact area



It is usually assumed that only the rate constants for binding and the TCR concentration (T) influence serial engagement.

However, if bound activated TCR can be internalized, the rate of internalization will also influence serial engagement (the hitting rate).



Initial rates of TCR activation and internalization at low peptide concentration

By low peptide concentration we mean there are few peptides on the APC (100 or less) and many TCR (30,000) on the T cell so peptides act independently -- peptides don't compete for TCR.

By initial we mean for an initial period when the TCR concentrations in the contact region are still uniform.

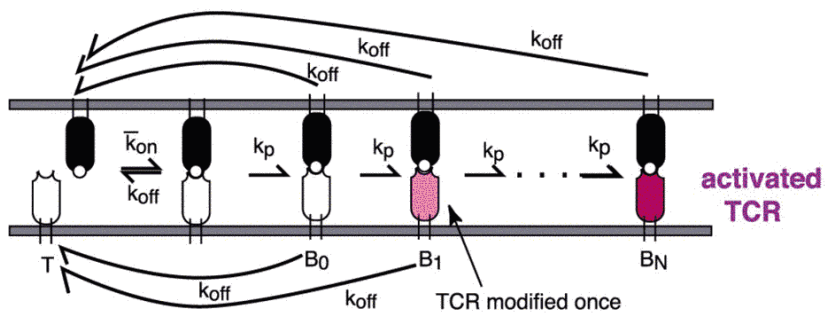
serial engagement — kinetic proofreading

activation rate= (hits/s) x (fraction activated)

internalization rate=(hits/s) x (fraction internalized)

hits/s =1/(mean time between hits)

A hit is a peptide-MHC binding to a TCR



Case 1. In the absence of TCR internalization

serial engagement

$$\text{hits/s} = \left[\frac{1}{k_{off}} + \frac{1}{\bar{k}_{on}T}\right]^{-1}$$

kinetic proofreading

$$\text{fraction activated} = \left(\frac{k_p}{k_p + k_{off}}\right)^N$$

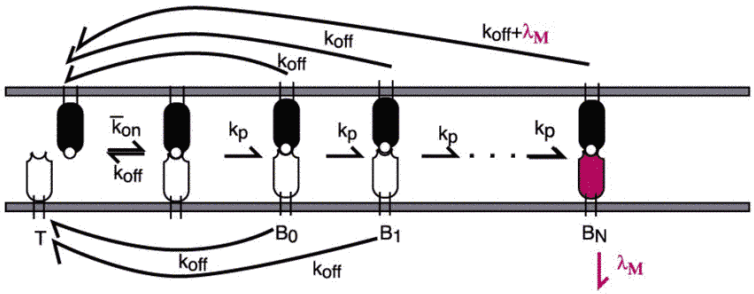
activation rate= (hits/s) x (fraction activated)= $\left(\frac{k_{off}\bar{K}T}{1+\bar{K}T}\right)\left(\frac{k_p}{k_p + k_{off}}\right)^N$

internalization rate=(hits/s) x (fraction internalized) = 0

Result: As a function of k_{off}, the activation rate has a maximum.

When $\bar{K}T >> 1$ $\quad k_{off}^{max} = k_p/(N-1)$

Case 2. Only bound, activated TCR are subject to internalization



$$\text{hits/s} = \left[\frac{1}{k_{off}}\left(1-\left(\frac{k_p}{k_p+k_{off}}\right)^N\left(\frac{\lambda_M}{k_{off}+\lambda_M}\right)\right)+\frac{1}{\bar{k}_{on}T}\right]^{-1}$$

$$\text{fraction activated} = \left(\frac{k_p}{k_p+k_{off}}\right)^N$$

$$\text{fraction internalized} = \left(\frac{k_p}{k_p+k_{off}}\right)^N\left(\frac{\lambda_M}{k_{off}+\lambda_M}\right)$$

Results: As a function of k_{off}:

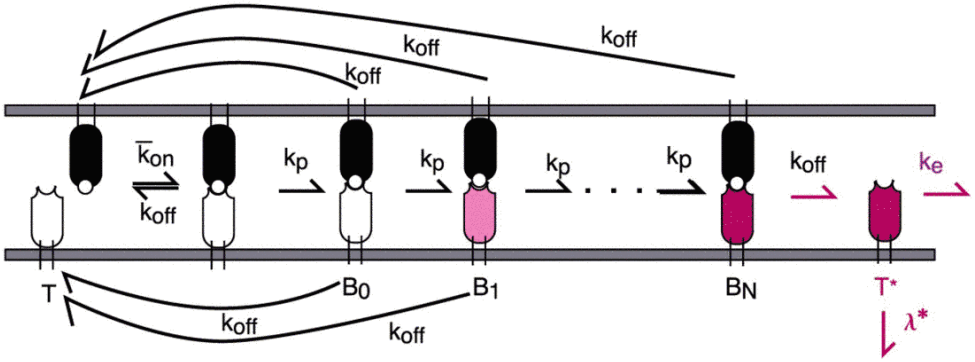
1. The internalization rate is a decreasing function of k_{off} .

2. The activation rate goes through a maximum only if

$$\lambda_M^2 < \left(\frac{1}{\bar{k}_{on}T}\times\frac{N}{k_p}+\frac{N(N-1)}{2k_p^2}\right)^{-1}$$

Otherwise the activation rate decreases monotonically.

Case 3. Activated TCR subject to internalization only after dissociation



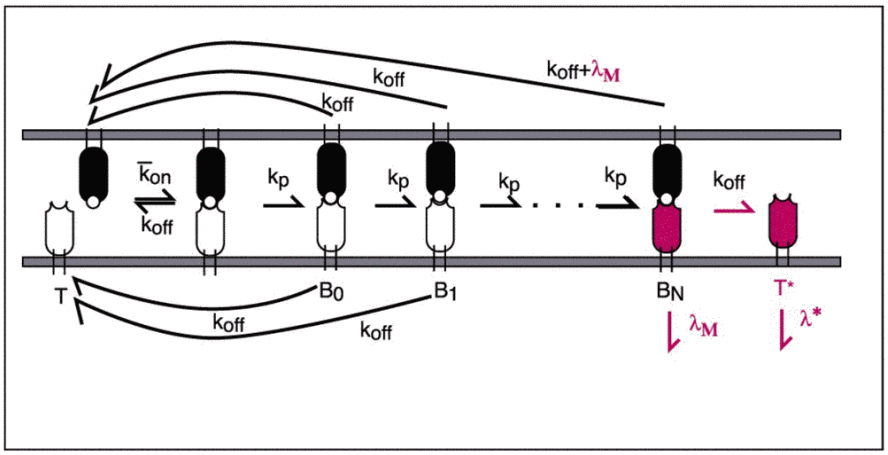
k_e = rate of escape from contact region

$$\text{activation rate} = \left(\frac{\lambda_M}{k_{off}+\lambda_M}\right)\left(\frac{k_p}{k_p+k_{off}}\right)^N$$

$$\text{internalization rate} = \left(\frac{\lambda_M}{k_{off}+\lambda_M}\right)\left(\frac{k_p}{k_p+k_{off}}\right)^N\left(\frac{\lambda^*}{k_e+\lambda^*}\right)$$

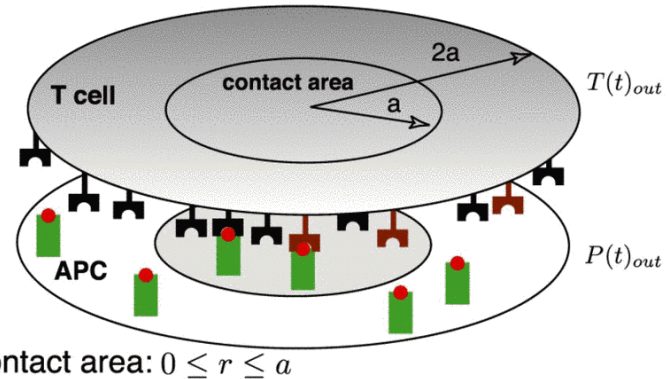
Result: As a function of k_{off}, both the activation and internalization rates have maxima.

When $\bar{K}T >> 1$ $\quad k_{off}^{max} = k_p/(N-1)$



Activated TCR subject to internalization	Internalization	Activation
only bound (B_N)	**no max**	condition
only dissociated (T*)	max	max
both bound (B_N) and dissociated (T*)	condition	condition

Monomer Model for TCR internalization in the immunological synapse

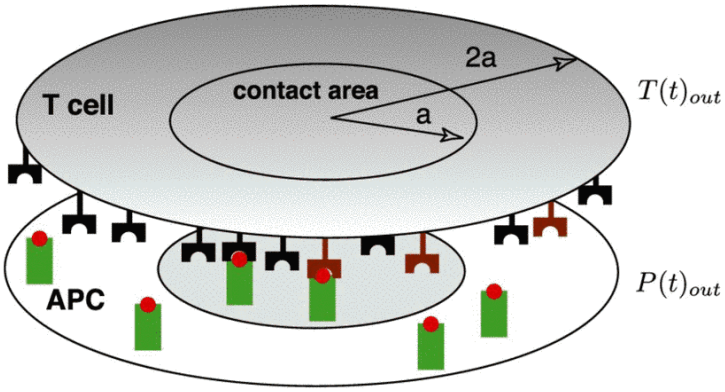


Equations in the contact area: $0 \le r \le a$

$$
\begin{aligned}
\frac{\partial T}{\partial t} &= D_T \nabla^2 T - k_{\mathrm{on}} T P + k_{\mathrm{off}} \sum_1^{N-1} B i \\
\frac{\partial P}{\partial t} &= D_P \nabla^2 P - k_{\mathrm{on}} T P - k_{\mathrm{on}} T^* P + k_{\mathrm{off}} \sum_1^{N} B_i + \lambda_M B_N \\
\frac{\partial B_1}{\partial t} &= D_B \nabla^2 B_1 + k_{\mathrm{on}} T P - k_{\mathrm{off}} B_1 - k_p B_1 \\
\frac{\partial B_i}{\partial t} &= D_B \nabla^2 B_i + k_p B_{i-1} - k_{\mathrm{off}} B_i - k_p B_i \qquad (i = 2 \ldots N-1) \\
\frac{\partial B_N}{\partial t} &= D_B \nabla^2 B_N + k_p B_{N-1} - k_{\mathrm{off}} B_N + k_{\mathrm{on}} T^* P - \lambda_M B_N \\
\frac{\partial T^*}{\partial t} &= D_T \nabla^2 T^* - k_{\mathrm{on}} T^* P + k_{\mathrm{off}} B_N - \lambda^* T^* \\
\frac{dT_i}{dt} &= \int_{\mathrm{inner}} (\lambda^* T^* + \lambda_M B_N) dA
\end{aligned}
$$

plus boundary conditions

Equations in the transition region: $a \leq r \leq 2a$



Diffusion of free peptide, free activated and inactivated TCR, and decay and internalization of activated TCR

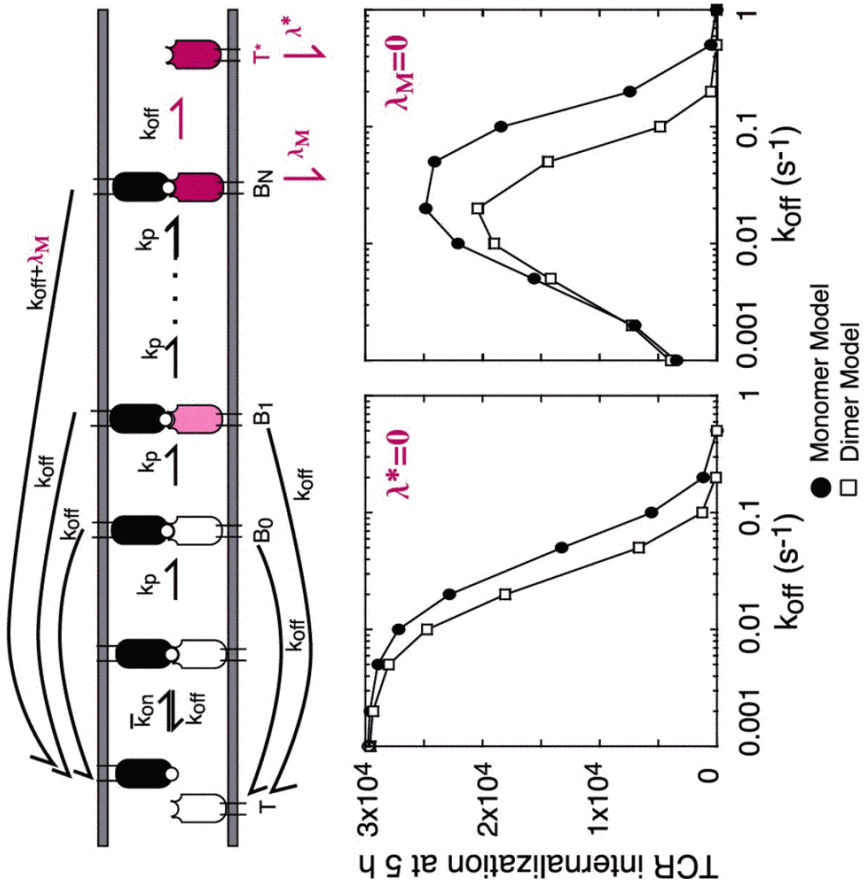
$$\begin{aligned}
\frac{\partial T}{\partial t} &= D_T \nabla^2 T + \mu T^* \\
\frac{\partial T^*}{\partial t} &= D_T \nabla^2 T^* - \mu T^* - \lambda^* T^* \\
\frac{\partial P}{\partial t} &= D_P \nabla^2 P
\end{aligned}$$

plus boundary conditions

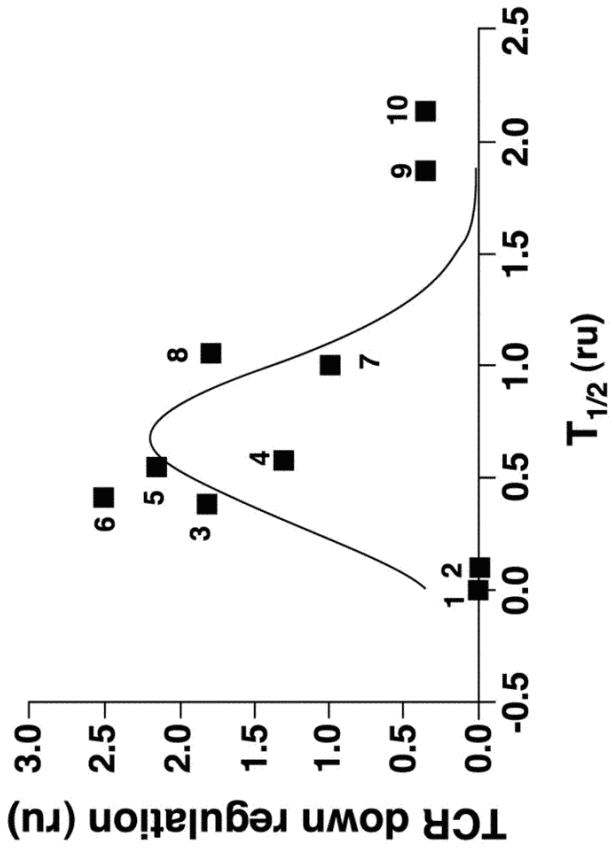
Note that we assume the existence of a stable circular region of contact between T cell and APC. Not all rate constants for TCR-peptide MHC binding will lead to a stable immunological synapse with a disk-like contact area (Qi et al., 2001, Synaptic pattern formation during cellular recognition, PNAS, **98**: 6548 -6553).

Since we allow the rate constants to vary over many orders of magnitude it is possible that some values may not lead to stable synapse formation.

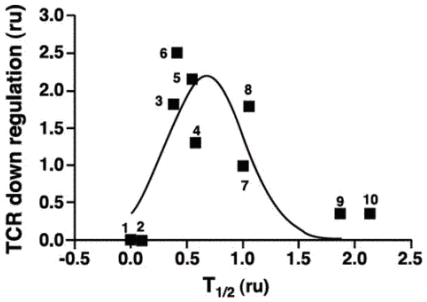
Model Predictions



There is an optimal half-life (T1/2) of the TCR-peptide-MHC bond for TCR internalization.

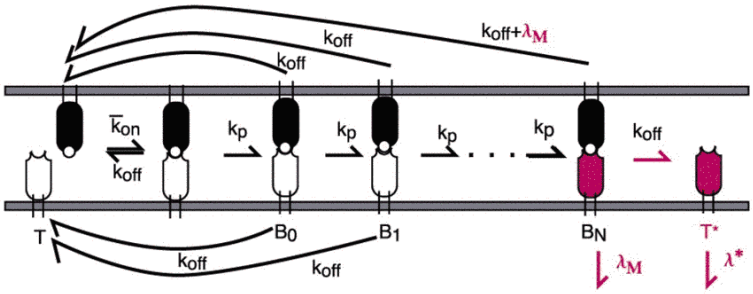


(Normalization = observed TCR down regulation /WT TCR down regulation)



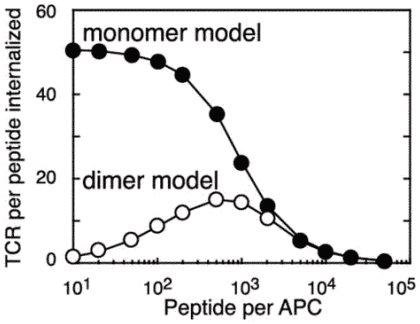
TCR down regulation goes through a maximum. This implies that the trade-off between serial engagement and kinetic proofreading results in an optimal range of dissociation rate constants for the TCR-peptide-MHC bond.

This only occurs if activated TCR remain active and subject to internalization for a period after peptide-MHC dissociation (T*).

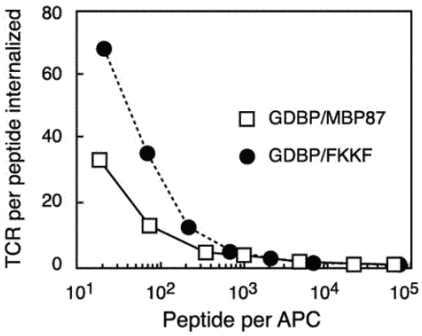


Relationship between TCR downregulation and amount of peptide on the APC.

Model predictions



Experiment
(Itoh et al., 1999,
J. Immunol. **162**:2073)



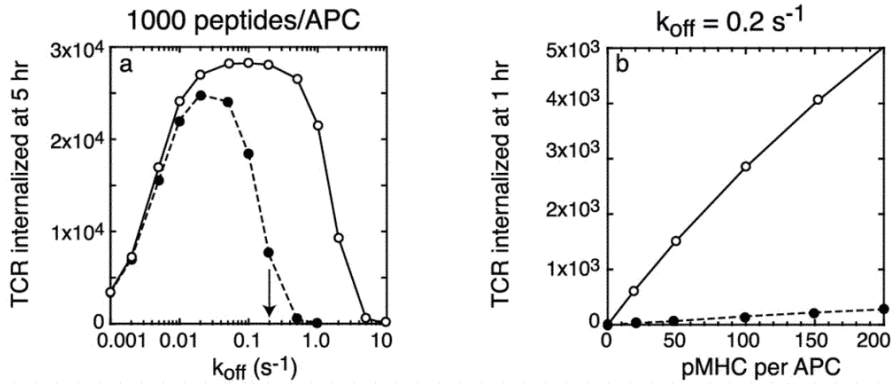
The data do not exhibit behavior expected for low peptide densities. Self-peptides may also be down-regulating TCRs.

T cell response to viral infection "mature" in the sense that the viral peptide concentration required to achieve 50% of maximum interferon-γ production decreases with time since infection (Slifka and Whitton, 2001, Nature Immunol., 2: 711)

Maturation occurs with:

1. no detected change in TCR-peptide binding.
2. increased Lck expression, i.e., viral-specific T cells express more Lck than naive T cells.

T cell maturation in the Kinetic Proofreading Model appears to correspond to increasing the rate constant for modification, kp. (● k_p=0.25 s^{-1}, ○ k_p=2.50 s^{-1})



Conclusions

1. **TCR internalization, like TCR activation, goes through a maximum as a function of the dissociation rate constant (or equivalently, the half-life) of the TCR-peptide-MHC bond.**

2. The trade-off between serial engagement and kinetic proofreading **can** result in an optimal range of dissociation rate constants for the TCR-peptide-MHC bond, **but only if activated TCR remain active and subject to internalization for a period after the peptide-MHC dissociates.**

3. Plots of TCR internalization per petide vs peptide concentration are predicted to have different behavior at low peptide density, depending on whether or not bound TCR must aggregate for the modification steps in the activation pathway to proceed. **Experiments that had been cited as evidence against the requirement for TCR aggregation are inconclusive because low enough peptide densities were not achieved.**

4. T cell responses to viral infection mature without change in the binding affinity of TCR for peptide-MHC but with an increase in the concentration of the initiating enzyme Lck. **Kinetic proofreading predicts such maturation if it is assumed that the rate constant for modification, k_p, increases after viral infection.**