An Introduction to Cell Signaling

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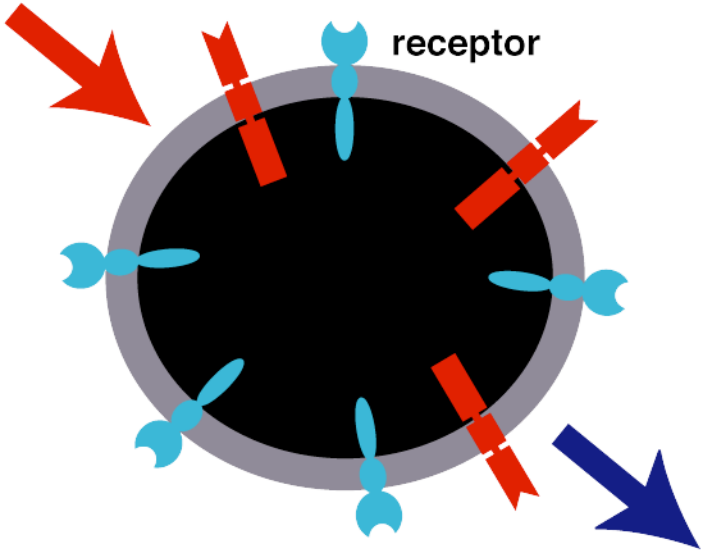
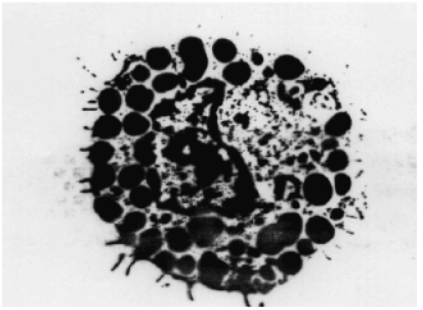
A grand challenge in cell biology: to predict how a cell will respond to a particular stimulus (the cell signaling problem).

Stimulus (pathogen, hormone, growth factor, toxin, etc.)

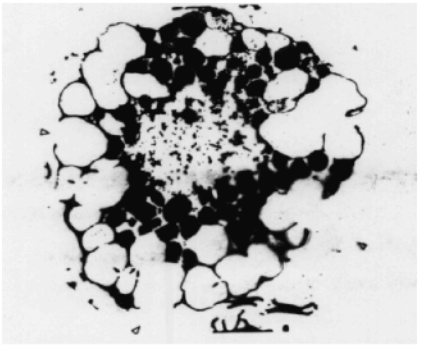


Response (secretion of proteins, directed motion, suicide, cell division, etc.)

Rat Peritoneal Mast Cell



Untreated



Sensitized and exposed to allergen for 3 min

An Introduction to Cell Signaling: Outline

1. **Question: How does the inside of a cell know something is happening on the outside of the cell?**
   - 1a. Receptors
   - 1b. Plasma membrane
   - 1c. Ligands

2. **Answer: The inside of the cells knows when a chemical modification occurs inside the cell.**
   - 2a. Kinases (Src, Syk, Jaks) and phosphatases
   - 2b. Protein domains (SH2, SH3, PH, kinase, etc.)
   - 2c. EGFR and transphosphorylation

3. **The players in a signaling cascade**
   - 3a. Receptors, non-membrane associated adaptors, scaffolding proteins, kinases, phosphatases, STATS, transcriptional factors

4. "General Principals"

4a. Combinatorial Complexity

4b. Serial engagement

4c. Kinetic proofreading

5. An Example of a signaling cascade - FcεRI and release of histamine.

Cells must constantly sense their environment and respond to it.

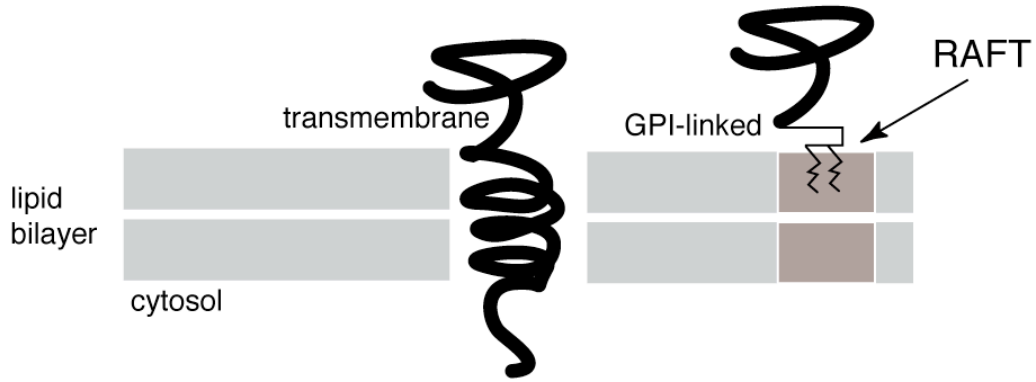
How does the cell do this?

How does the inside of the cell know that something has happened on the outside of the cell?

It all starts with **ligands** binding to mobile **receptors** on the surface of cells.

1. Receptors are specific for one, or a small subset of ligands.

2. Almost all receptors span the membrane one or more times. An important exception are GPI-linked receptors. The GPI anchor binds proteins to the noncytoplasmic surface of the membrane, in specialized lipid domains (RAFTs).



3. Receptors can be a single chain or composed of two or more subunits.

4. Receptors are mobile in the plane of the membrane, but their motion is heterogeneous.

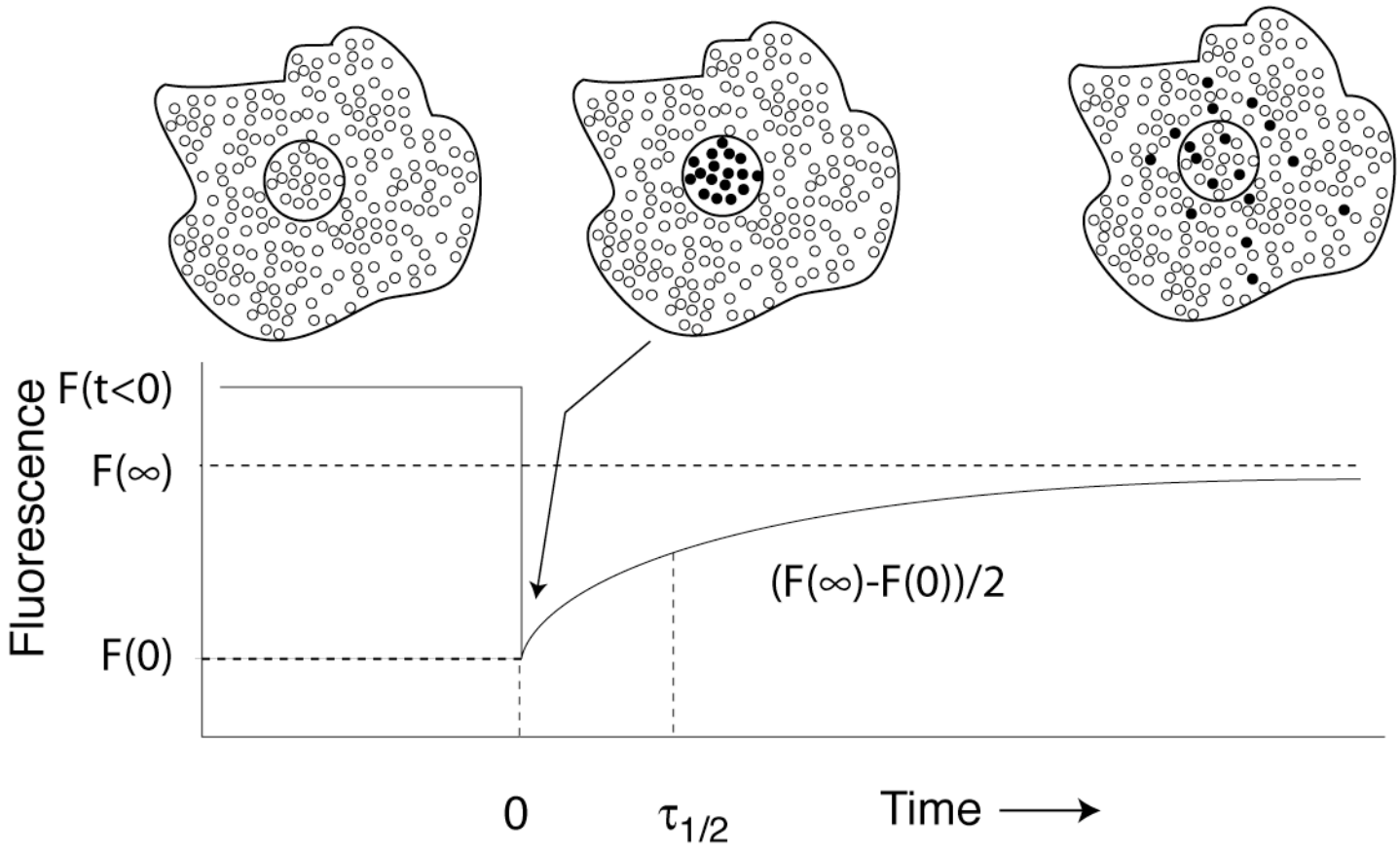
5. Many receptors occur in a soluble form as well as cell associated. Their ectodomains can be shed or secreted.

GPI - glycosylphosphatidylinositol

6. Cell surface receptor populations change (receptor internalization, degradation, cleavage, synthesis) in response to their environment, i.e. ligands that they (and sometimes other receptors) bind.

7. Even in the absence of their ligands, receptors may not be uniformly distributed over the cell membrane.

Fluorescence recovery after photobleaching (FRAP)



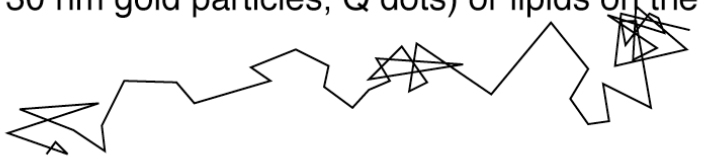
$$\text{Mobile Fraction} = \frac{F(\infty)-F(0)}{F(t<0)-F(0)} \qquad D = \frac{r_o^2\gamma}{4\tau_{1/2}}$$

r_o^2 is the $1/e^2$ radius of the Gaussian profile laser beam used for both photobleaching and measuring fluorescences, and γ is a parameter that depends on the extend of photobleaching, varying slowly from 1.0 to 1.2.

Single Particle Tracking (SPT)

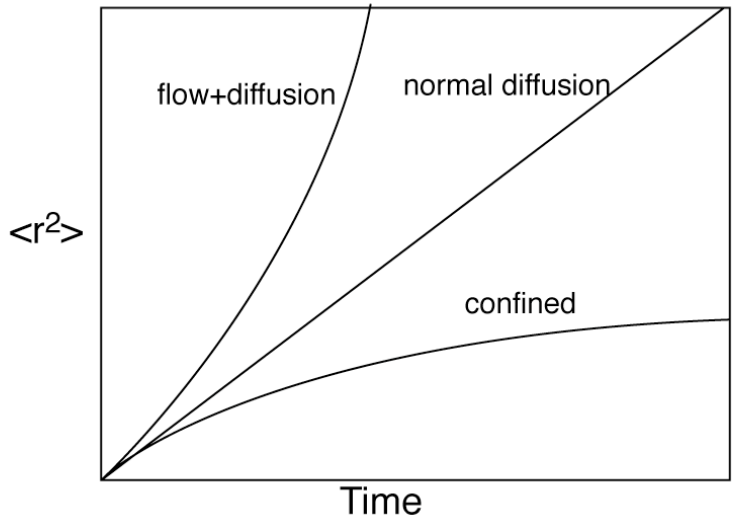
(Saxton and Jacobson (1997). Single-particle tracking: Applications to membrane dynamics. *Ann. Rv. Biophys. Biomol. Struct*. **26**:373-399.)

Computer-enhanced video microscopy is used to tract the motion of labeled proteins (e.g. 30 nm gold particles, Q dots) or lipids on the cell surface.



In an average over many tracks one expects the following for different modes of motion

$\langle r^2 \rangle = 4Dt$	normal diffusion
$\langle r^2 \rangle = 4Dt^a$	anomalous diffusion (a<1)
$\langle r^2 \rangle = 4Dt + (vt)^2$	directed motion plus diffusion
$\langle r^2 \rangle \approx \langle R_c^2 \rangle [1 - A_1 \exp(-4A_2 Dt / \langle r_c^2 \rangle)]$	confined motion



Tracts are often seen that are mixed modes as if a receptor is confined for a while then escapes and diffuses or diffuses and flows, then is confined again, etc. This heterogeneity in motion reflects the heterogeneity of the membrane.

From FRAP experiments:

The mobile fraction ranges from 20-80% for cell surface proteins that span the membrane.

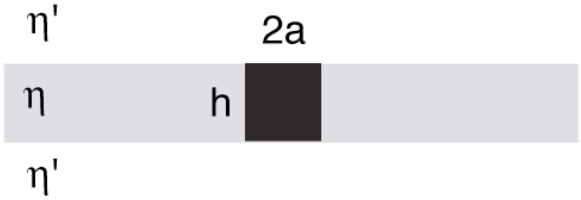
Receptor diffusion coefficients $D = 10^{-9} - 10^{-11}$ cm^2/s

Einstein Relation: $D=k_BT/f$
For a sphere of radius *a* diffusing in 3D the frictional coeff. $f=6\pi\eta a$
$D=k_BT/6\pi\eta a$

The viscosity of a membrane at 36^o C η=1.3 P, which is a little more viscous than olive oil at room temp (η=0.84 P) and about 180 times higher than the viscosity of water at 36^o C (η=0.0071 P).

Saffman and Delbruck (1975) PNAS, 72:3111

$D = (k_BT/4\pi\eta h)(-\gamma + \ln\theta)$ $\quad \theta = \eta h/\eta' a$ $\quad \gamma = 0.5772$



η = membrane viscosity

Predicts weak dependence on radius of the cylinder embedded in the membrane and **D** of about 10^{-8}-10^{-9} cm^2/s.

Receptor densities on cell surfaces and the mean nearest neighbor distance

Diameter of a lymphocyte (a Jurkat T cell) $2a = 12.4 \pm 1.2\ \mu m$

Surface area $A_{cell} = 800\ \mu m^2$

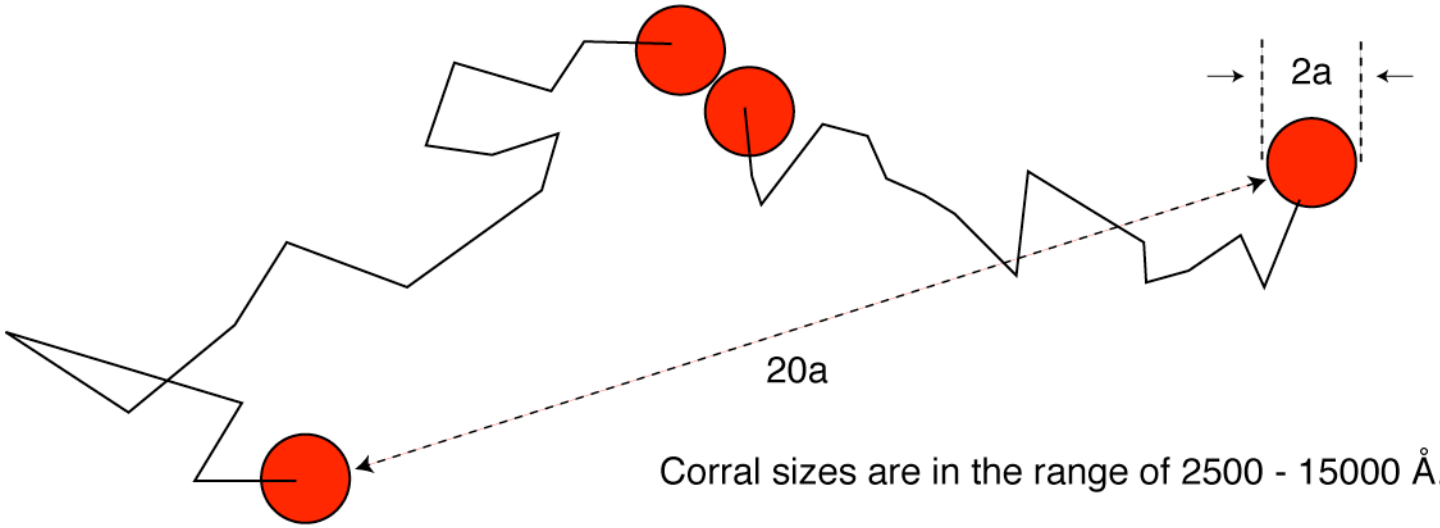
For a cell with 500,000 receptors (about the number of T cell receptors on a Jurkat cell) the receptor density $\rho = 62.5$ receptors/μm

In 2 dimensions (surface of a cell) the mean nearest neighbor distance if receptors are distributed randomly $\langle d_{nn} \rangle = 1/(2\sqrt{\rho}) = 0.06\ \mu m = 630\ \text{Å}$

For a million receptors $\langle d_{nn} \rangle = 0.014\ \mu m = 140\ \text{Å}$

Although no single population of receptors covers more than 1% of the cell surface, the total surface protein could cover as much as 45%.
(Ryan et al., (1988) Molecular crowding on the cell surface, *Science* **239**, 61-64)

If two receptors with diffusion coeff. $D=5x10^{-10}$ cm^2/s are in the vicinity of each other how long does it take them to separate due to diffusion? We'll say the receptors are separated when the distance between them is100 Å, about ten receptor diameters.



Corral sizes are in the range of 2500 - 15000 Å.

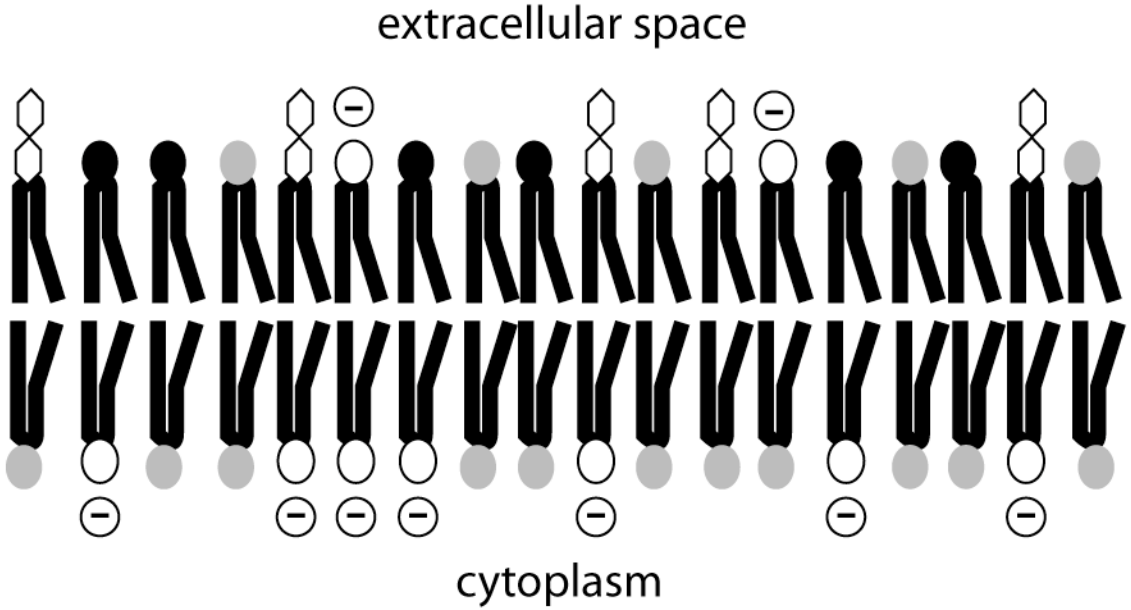
$$t = r^2/4(2D) = (1x10^{-6}\ cm)^2/(8x5x10^{-10}\ cm^2/s) = 2.5x10^{-4}\ s$$

Receptors separate rapidly if there is nothing holding them together..

Fluid-Mosaic Model of the Plasma Membrane (Singer and Nicolson (1972) *Science* **175**, 720-731): Singer and Nicolson proposed that the plasma membrane is a 2D solution of proteins in a viscous lipid bilayer.

1. The lipid bilayer is asymmetric in lipid composition and charge.

Lipid bilayer composed mainly of phospholipids, cholesterol and glycolipids.
All are amphipathic having a hydrophilic (polar) qand a hydrophobic (nonpolar) end.



phosphatidylserine carries a net negative charge at physiological pH

cholesterol

polar head group
rigid planar structure

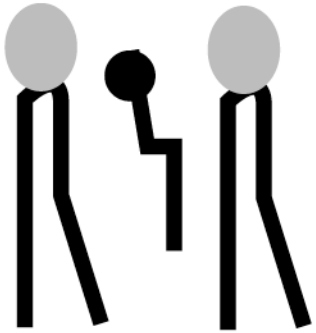
nonpolar hyrocarbon
tale

phospholipid

polar head group

nonpolar hyrocarbon
tale

2. Cholesterol makes lipid bilayers less fluid.

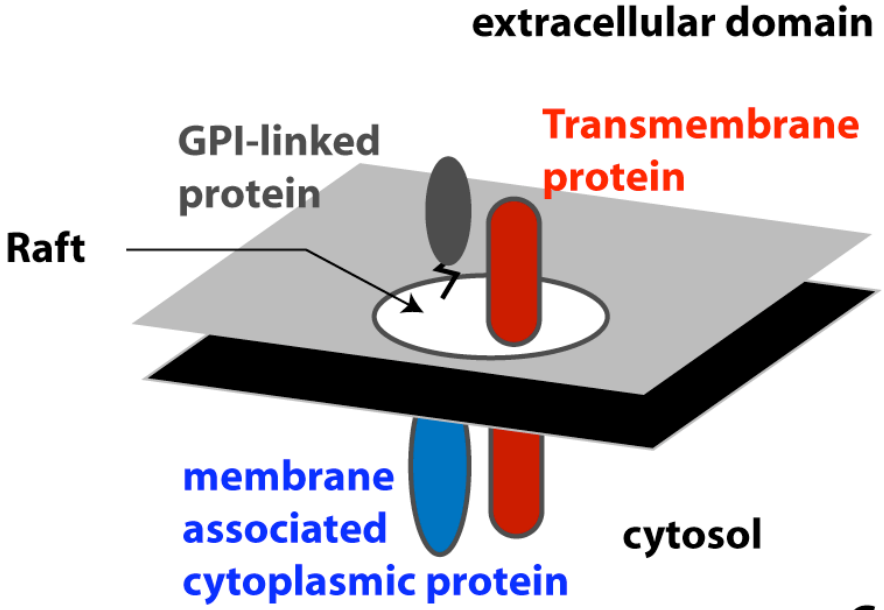


cholesterol
stiffened region

Specialized Membrane Domains

Rafts: regions of specialized lipid composition (enriched in cholesterol, sphingomyelin and gangliosides) and specialized protein composition (enriched in GPI-linked proteins, certain Src kinases, certain scafold proteins and others.) Also called GEMs (glycolipid enriched membranes), DIGs (detergent insoluble glycolipid-enriched domains) and DRMs (detergent resistant membranes).

Rafts play an important role in cell signaling.



$r = 26 \pm 13$ nm (~ 3500 lipid molecules per raft)

$D_{raft} = 3.9 \times 10^{-8}$ cm^2/s

lifetime ~ 1 min

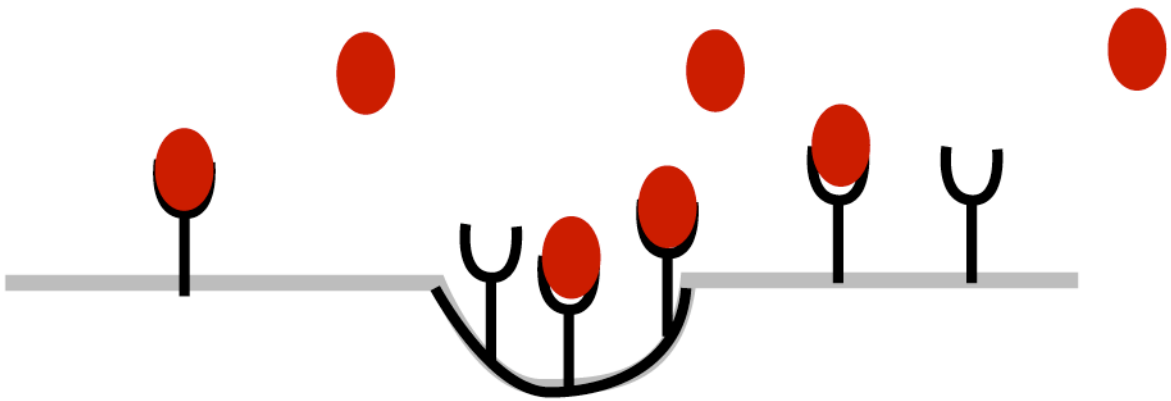
Pralle et al. (2000) *J. Cell Biol.* **148**,997-1008

Great variation in lifetime and size estimates

A large fraction, as much as 50 % of membrane lipid can be in rafts. Varies from cell type to cell type.

Specialized Membrane Domains (continued)

Coated Pits: Dynamic structures that internalize receptors and membrane and are central to the process of receptor mediated endocytosis.



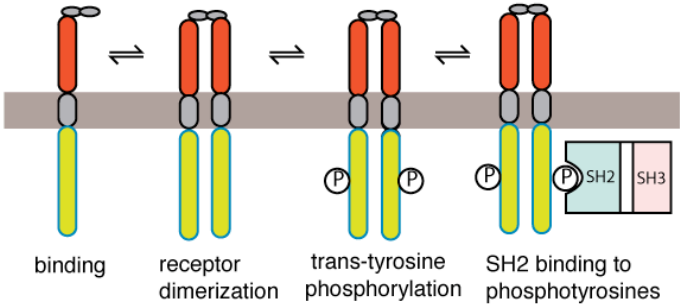
coated pit (clathrin and other coat-associated proteins)

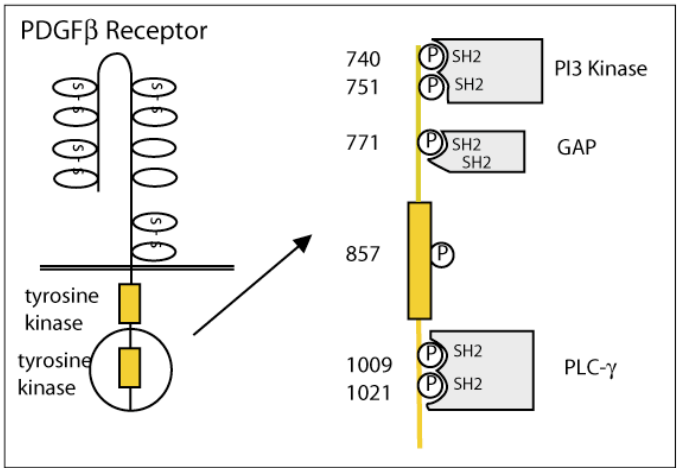
For many receptor systems ligand induced receptor aggregation - bringing the cytoplasmic domains of receptors in proximity and holding them together for seconds to minutes – is required for signaling initiation.

Many receptors including the growth factor receptors,the cytokine receptors, the immune response receptors and the killer cell inhibitory receptors, initiate signaling through the following steps:

1. Ligand binding
2. Ligand induced receptor aggregation
3. Phosphorylation of tyrosines on cytoplasmic domains of the receptor
4. Binding of signaling molecules containing SH2 domains to the phosphorylated tyrosines

Example: **Early Events in Growth Factor Signal Transduction**





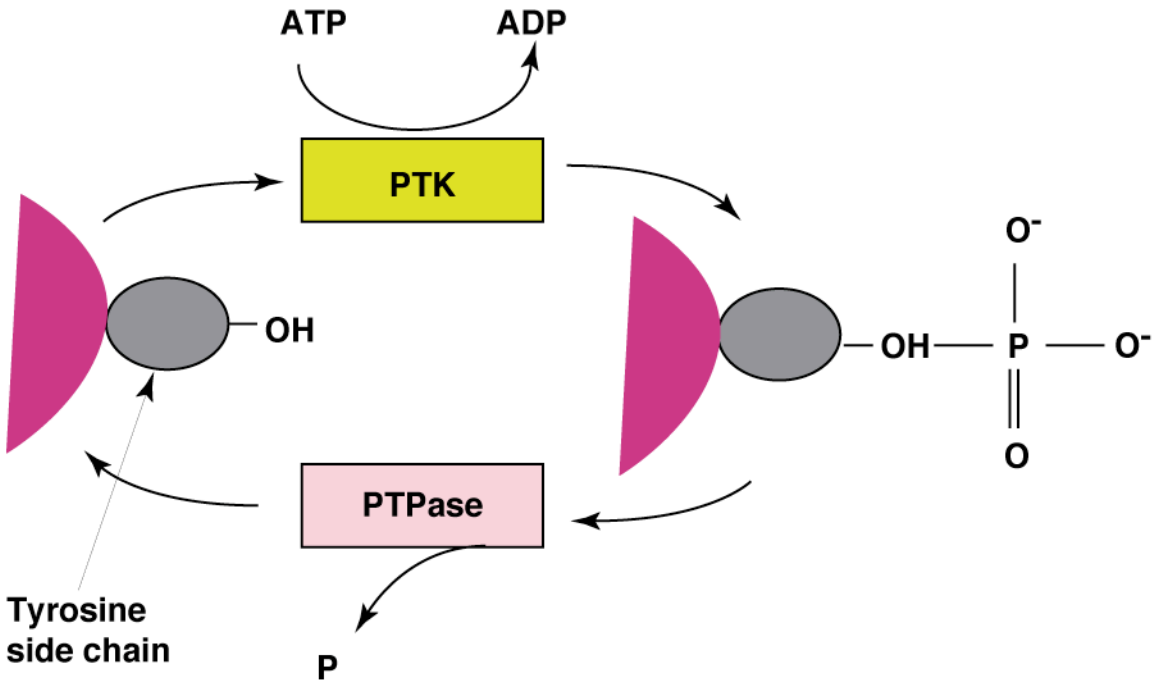
Proteins can be phosphorylated on three classes of amino acids:

1. tyrosine
2. serine or threonine
3. histidine

Only tyrosine and serine/threonine phosphorylation occurs in signaling in the immune system.

Protein tyrosine kinases (PTK) transfer a phosphate group from an ATP molecule to a hydroxyl group on a tyrosine side chain of a protein.

Protein tyrosine phosphatases (PTPase) remove phosphates from tyrosines.



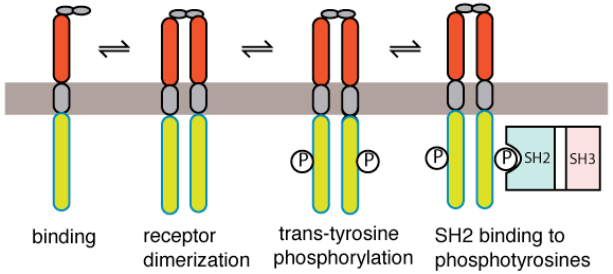
Introduction

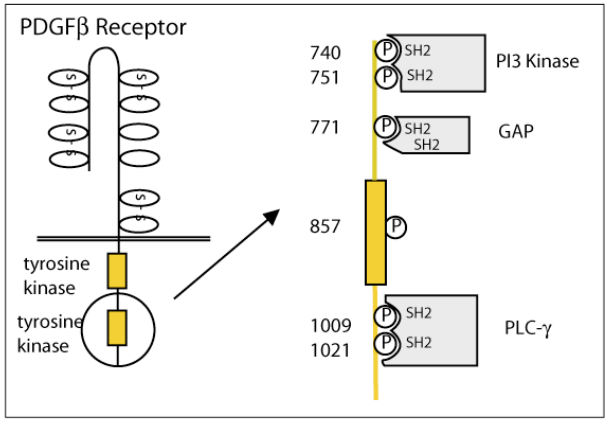
1. Transmitting information across the cell membrane by juxtaposing the cytoplasmic tails of receptors is a ubiquitous signaling mechanism.

2. The growth factor, cytokine, and immune recognition receptors all initiate signaling through ligand induced receptor aggregation.

3. By converting cytoplasmic domains of the receptor to phosphorylated forms, the cell "senses" the external ligand and initiates a signaling cascade. The phosphorylation of aggregated receptors reflects a dynamic balance between the action of kinase and phosphatases.

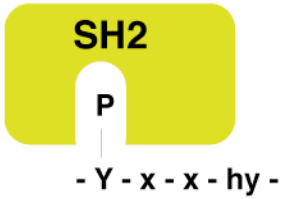
Example: **Early Events in Growth Factor Signal Transduction**



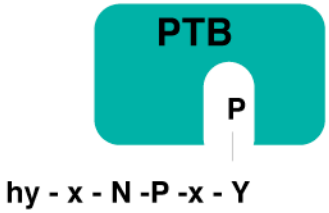


4. If an aggregate breaks up, phosphatases rapildly dephosphorylate the receptor and the chemical modification is erased.

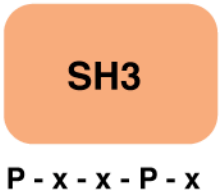
The domains of a signaling molecules determine what molecules it can associate with and what enzymatic activity it possess.



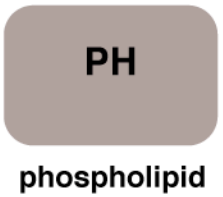
Src homology 2 (SH2) domain: recognizes short phosphotyrosine motifs.



pTyr-binding (PTB) domain: recognizes phosphotyrosine motifs in which pTyr is preceded by residues from a β turn.



Src homology 3 (SH3) domain: binds to proline rich motifs.

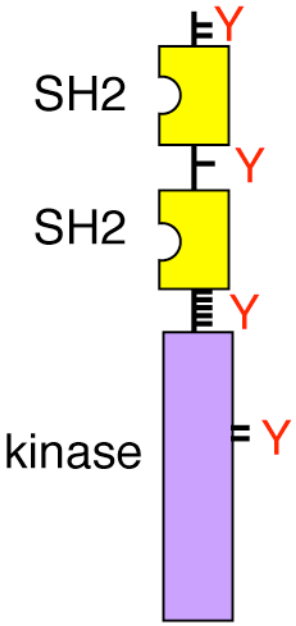


Pleckstrin homology (PH) domain: binds to charged head groups of specific polyphosphoinositides.

Proteins that are enzymes possess kinase or phosphatase domains.

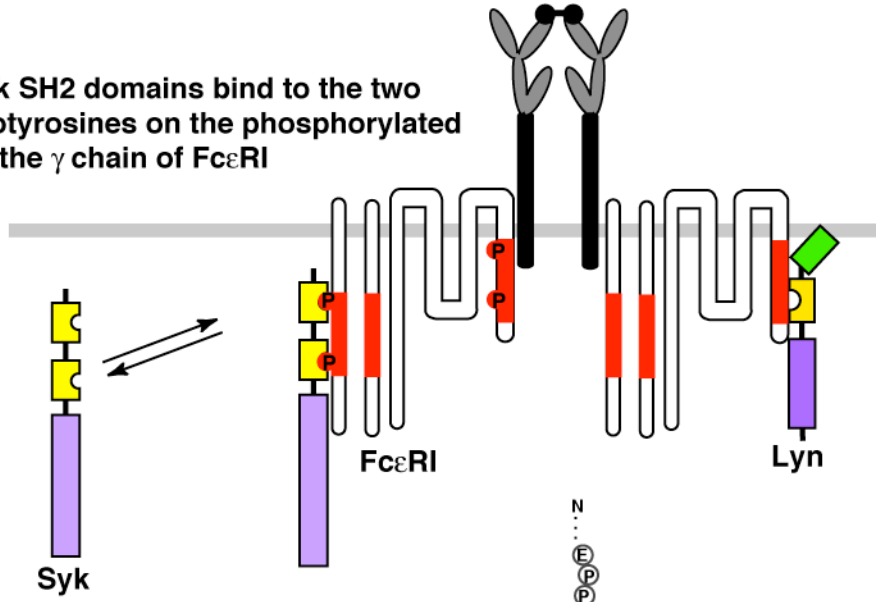
Pawson and Scott (1997). Signaling through scaffold, anchoring, and adapter proteins. Science, **278**: 2075 - 2080

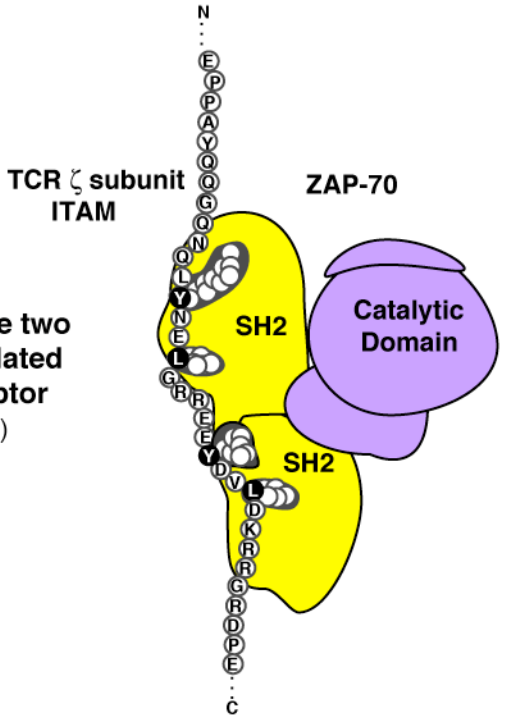
The cytosolic protein tyrosine kinases Syk and Zap-70 bind to phosphorylated ITAMs through their two SH2 domains.



Syk, ZAP-70

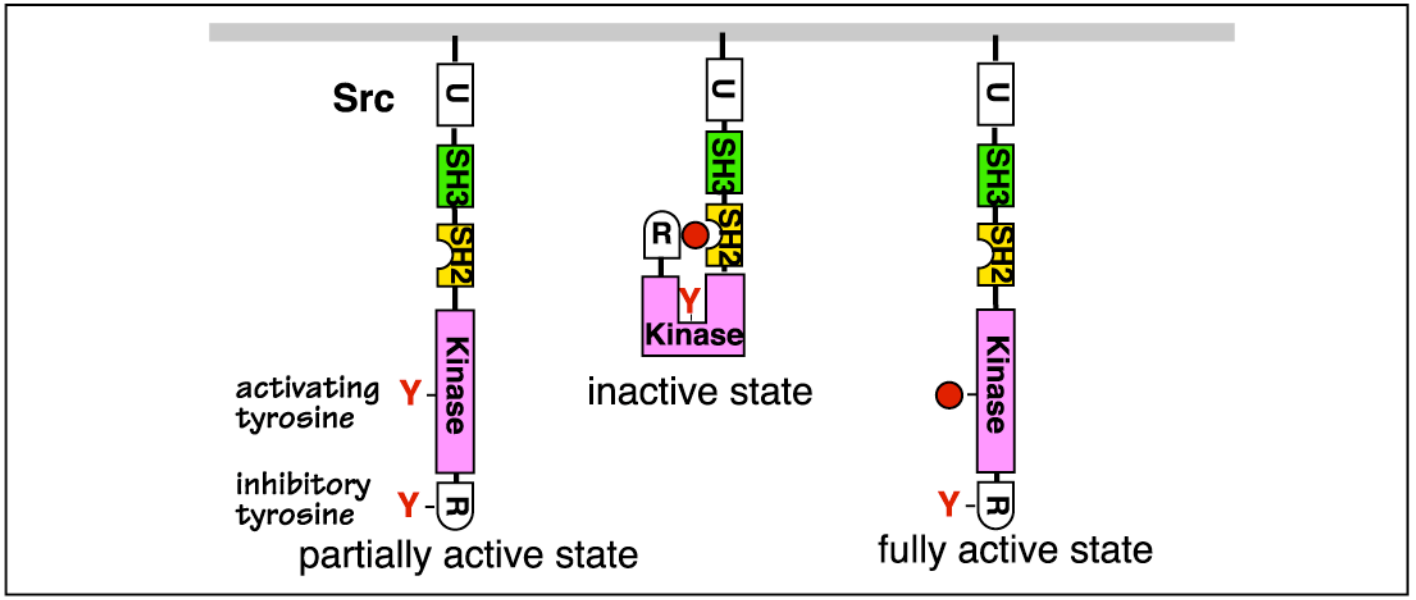
Both Syk SH2 domains bind to the two phosphotyrosines on the phosphorylated ITAM of the γ chain of FcεRI



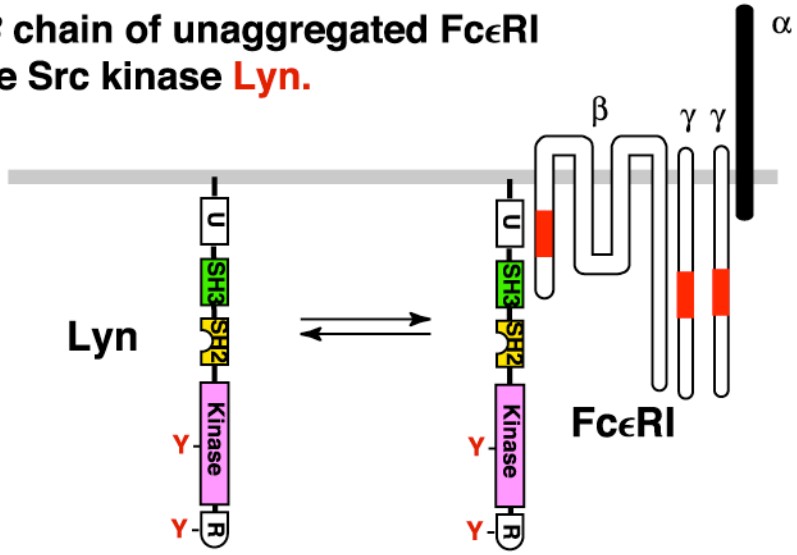


Both ZAP-70 SH2 domains bind to the two phosphotyrosines on the phosphorylated ITAM of the ζ chain of the T cell receptor (TCR) (Hatada et al., (1995) Nature **377**:32)

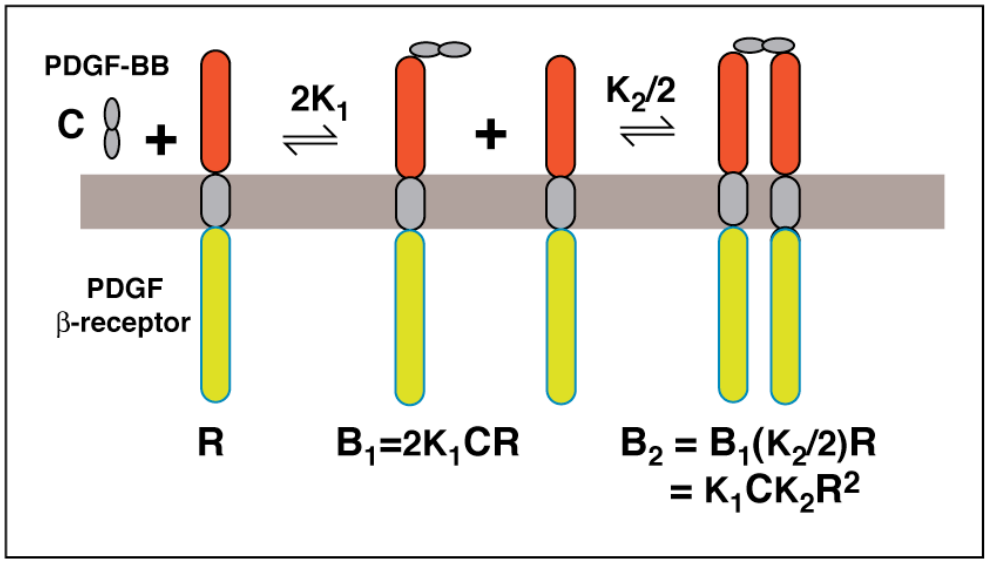
The unphosphorylated MIRR associate weakly with a Src kinase



For example, the β chain of unaggregated FcεRI associates with the Src kinase Lyn.



At equilibrium:



Conservation of receptors - ignoring any internalization

1. $R_T = R + B_1 + 2B_2 = R + 2K_1CR + 2K_1CK_2R^2$

2. $1 = r(1 + c) + ck_2r^2$

where: $c = 2K_1C$, $r=R/R_T$ and $k_2 = K_2R_T$

$w = r(1+c) =$ fraction of unaggregated receptor

$$\delta = \frac{k_2 c}{(1+c)^2}$$

3. $1 = w + \delta w^2$

$$1 = w + \delta w^2$$

w = r(1+c) = fraction of unaggregated receptor

$$\delta = \frac{k_2 c}{(1+c)^2}$$

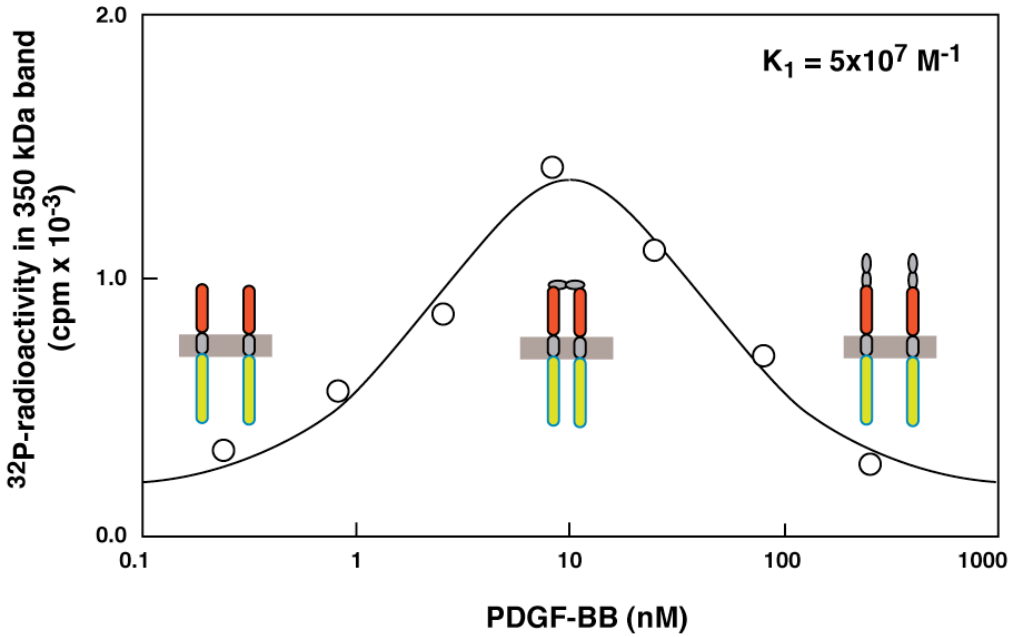
Receptor agg. maximal when **(1-w)** maximal, i.e., when **dw/dc** = 0.

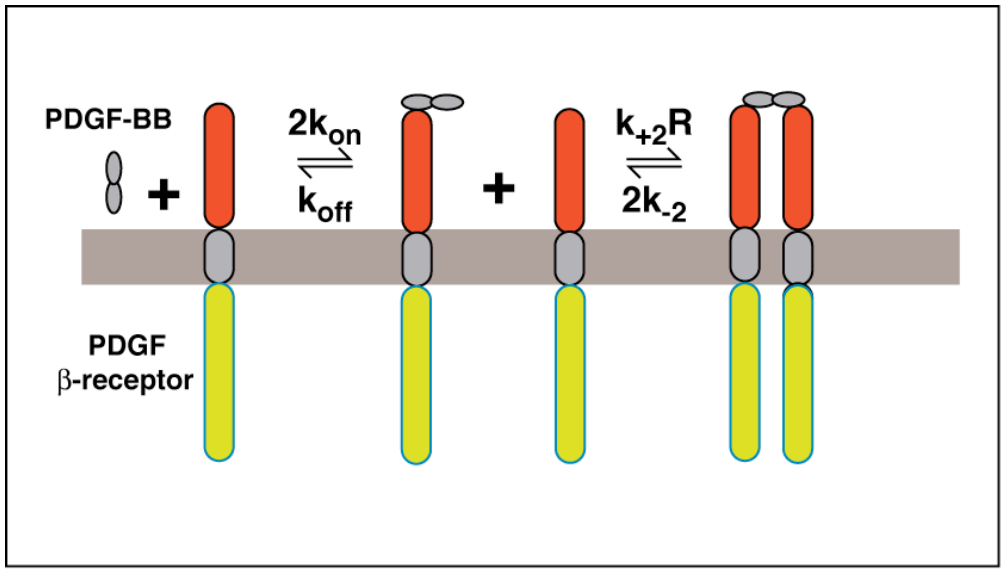
$$0 = (dw/dc)(1 + 2\delta w) + (d\delta/dc)w^2$$

Receptor agg. maximal when **d**δ**/dc=0,** i.e., when

$$c_{max} = 1$$

$$C_{max} = 1/(2K_1)$$





The average lifetimes of a singly and doubly bound ligand are:

$$\langle t_1 \rangle = \frac{1}{k_{off}} + \frac{k_{+2}R}{2k_{off}^2}$$

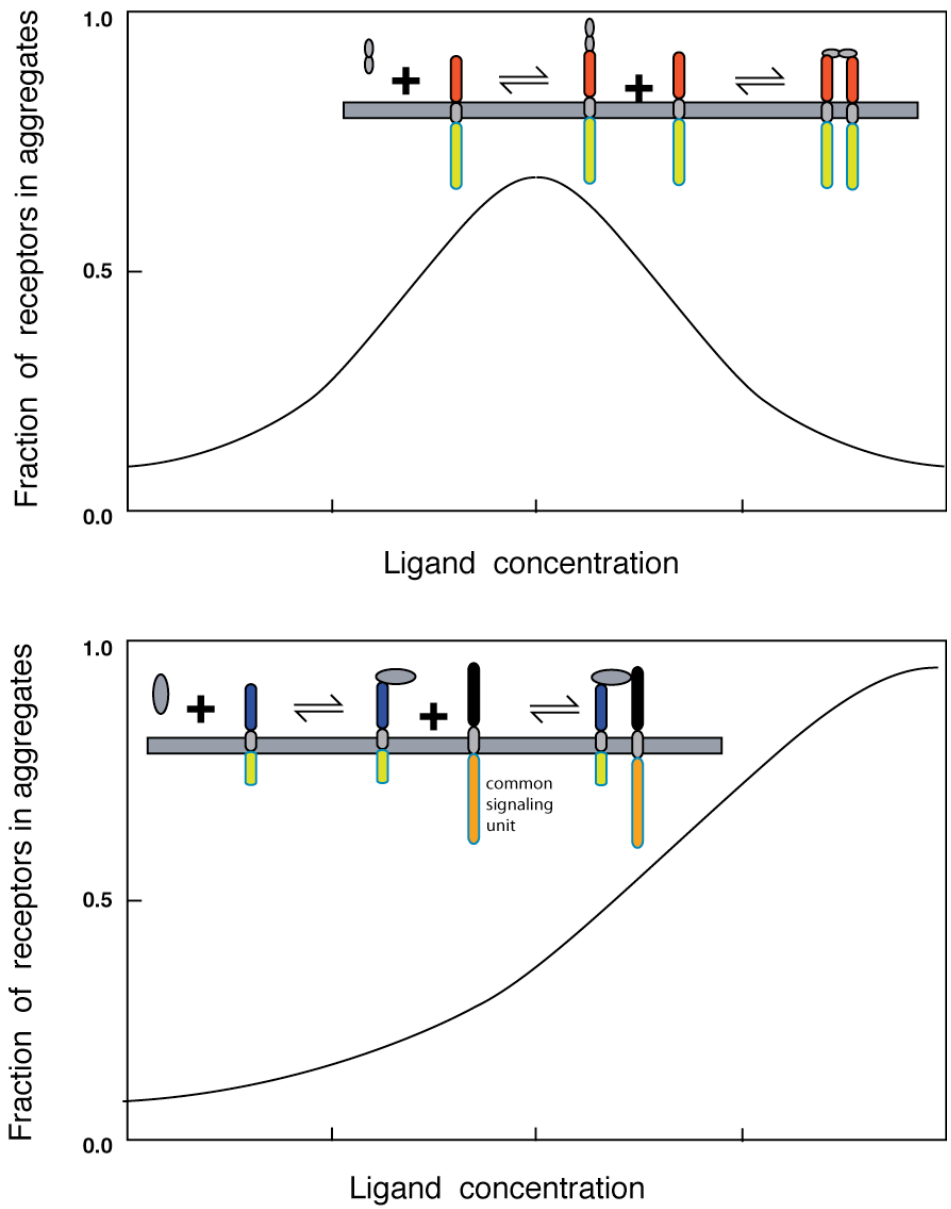
A single ligand may interact with many different receptors

$$\langle t_2 \rangle = \frac{1}{2k_{off}} + \frac{1}{k_{off}} + \frac{k_{+2}R}{2k_{off}^2}$$

If $k_{off} = k_{-2}$, then:

$$\langle t_1 \rangle = \frac{1}{k_{off}} \left(1 + K_2R/2\right)$$

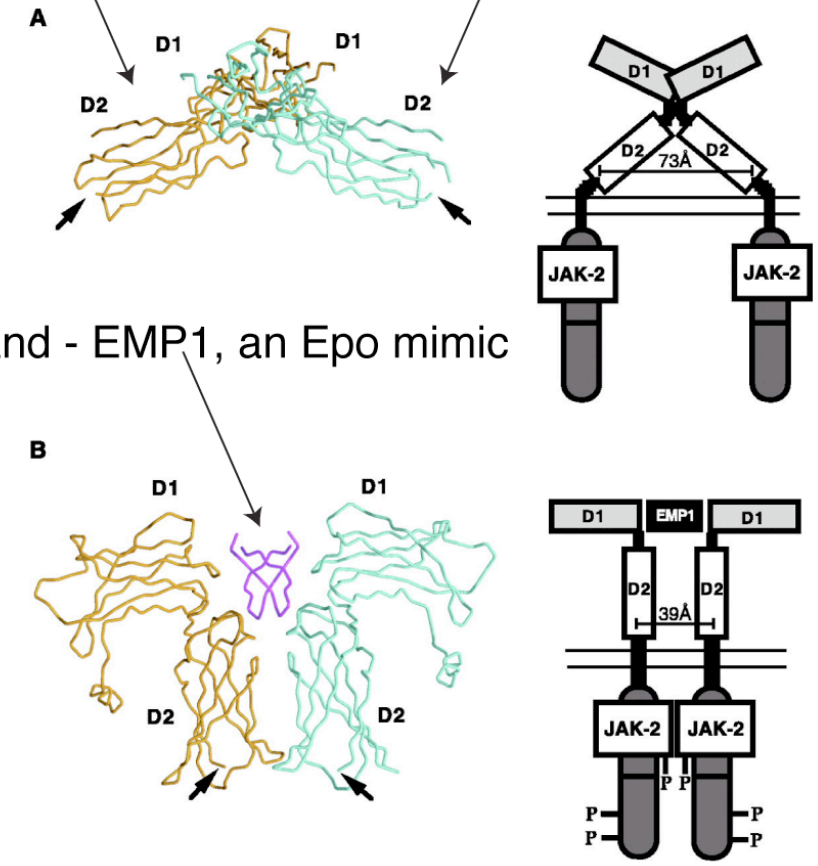
$$\langle t_2 \rangle = \frac{1}{k_{off}} \left(1.5 + K_2R/2\right)$$


1.0
0.5
0.0
Fraction of receptors in aggregates
Ligand concentration
common
signaling
unit
1.0
0.5
0.0
Fraction of receptors in aggregates
Ligand concentration

- Different ligands induce receptor aggregation in different ways.

Extracellular domin of Epo receptor (EBP)

no ligand

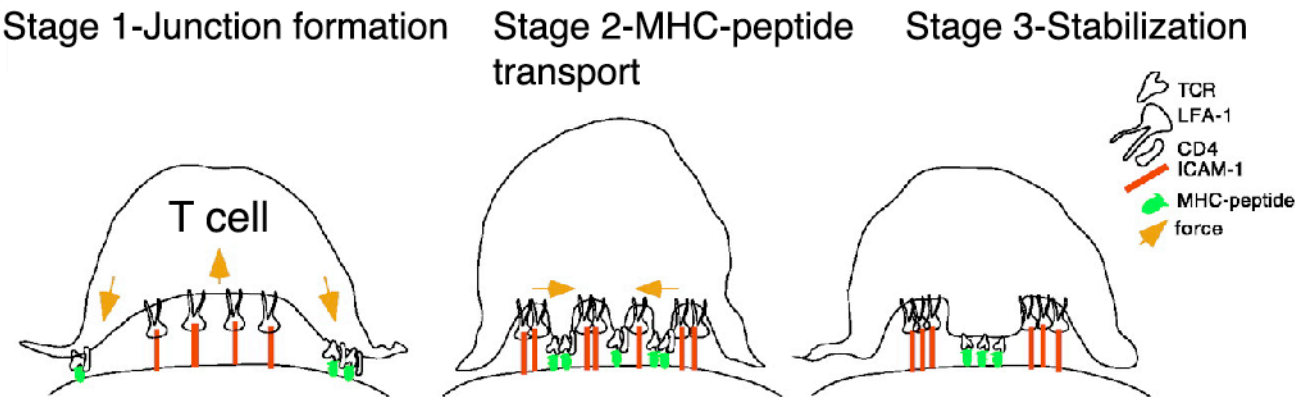


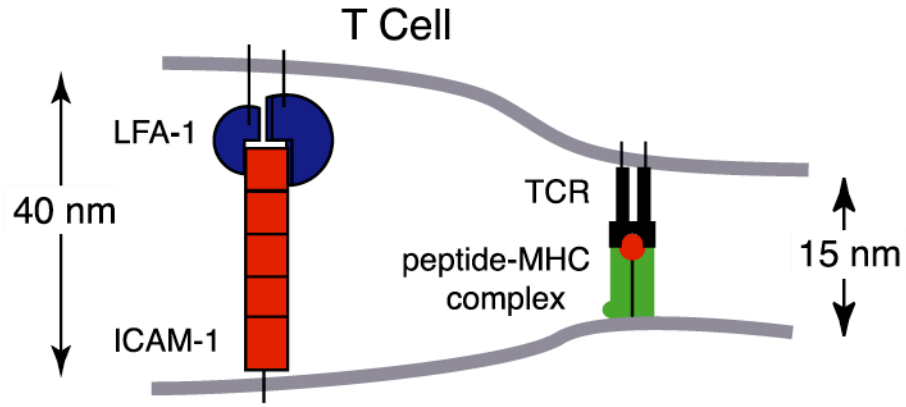
EBP-EBP receptor contact residues are similar
to those used in binding EMP1

Livnah et al. (1999)

- All the examples we have considered so far involve soluble ligands but often cell signaling is triggered by one cell binding to another cell. The “ligand” is now a surface with multiple binding sites.

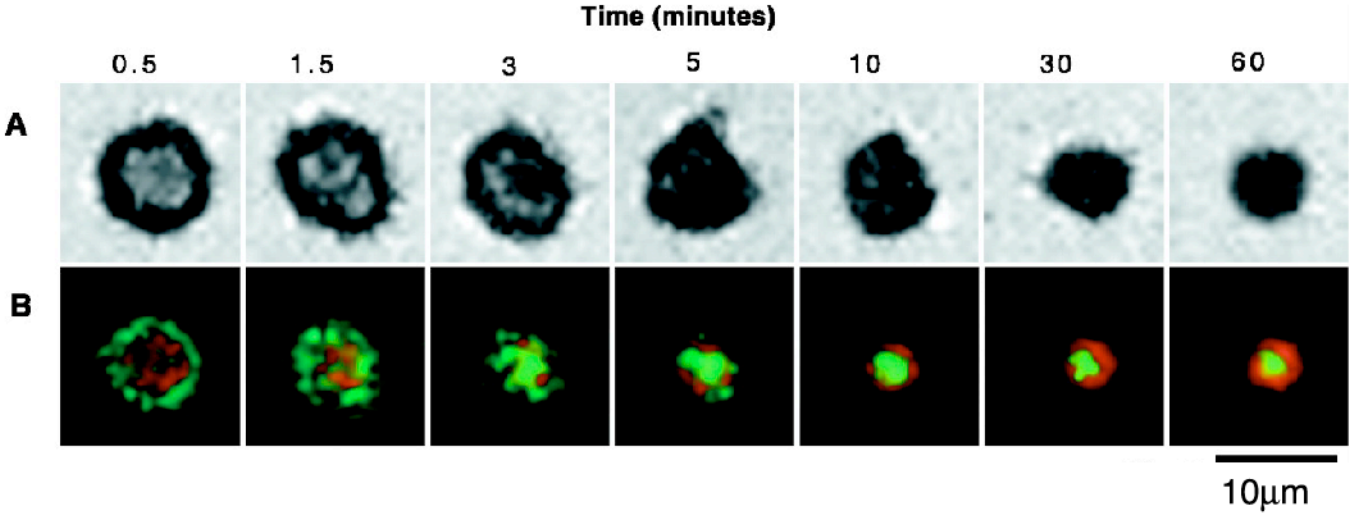
Grakoui et al. (1999), **285**, 221-227





The Immunological Synapse

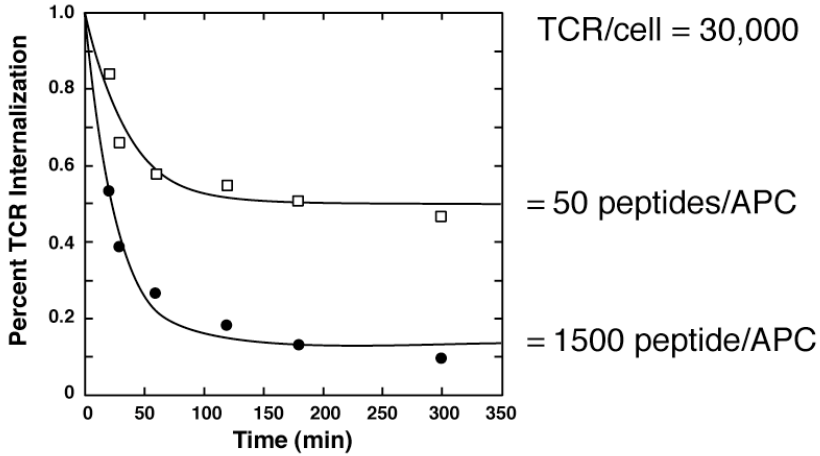
Grakoui et al. (1999) The Immunological Synapse: A molecular machine controlling T cell activation. **285**, 221-227



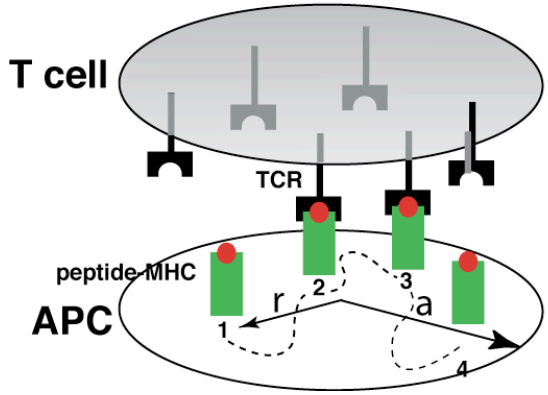
Serial Engagement

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

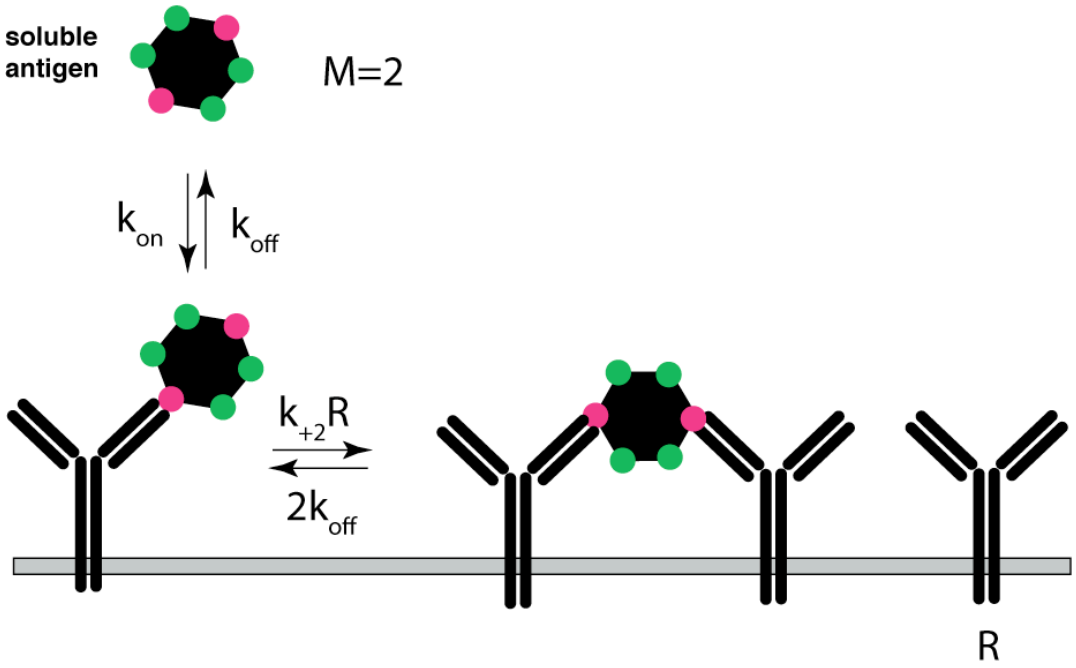
APC with as few as 100 peptide-MHC can trigger the internalization of thousands of TCRs -- " a single complex can serially engage and trigger up to 200 TCRs."



A peptide-MHC binds to a TCR (2), activates it (2-3), dissociates (3), binds to another TCR, ...



It is possible for a **mulivalent Ligand** to **serial engage** and activate many more receptors than its valence before dissociating from the cell surface.



	$k_{+2}R = 1$		$k_{+2}R=10$	
M=2	N	$k_{off}t_N$	N	$k_{off}t_N$
	1	1.50	1	6.00
	2	2.00	2	6.50
M=3	1	2.33	1	44.3
	2	3.00	2	46.5
	3	3.33	3	46.8
M=5	1	6.20	1	3.22×10^3
	2	7.50	2	3.30×10^3
	3	8.03	3	3.31×10^3
	4	8.33	4	3.31×10^3
	5	8.53	5	3.31×10^3

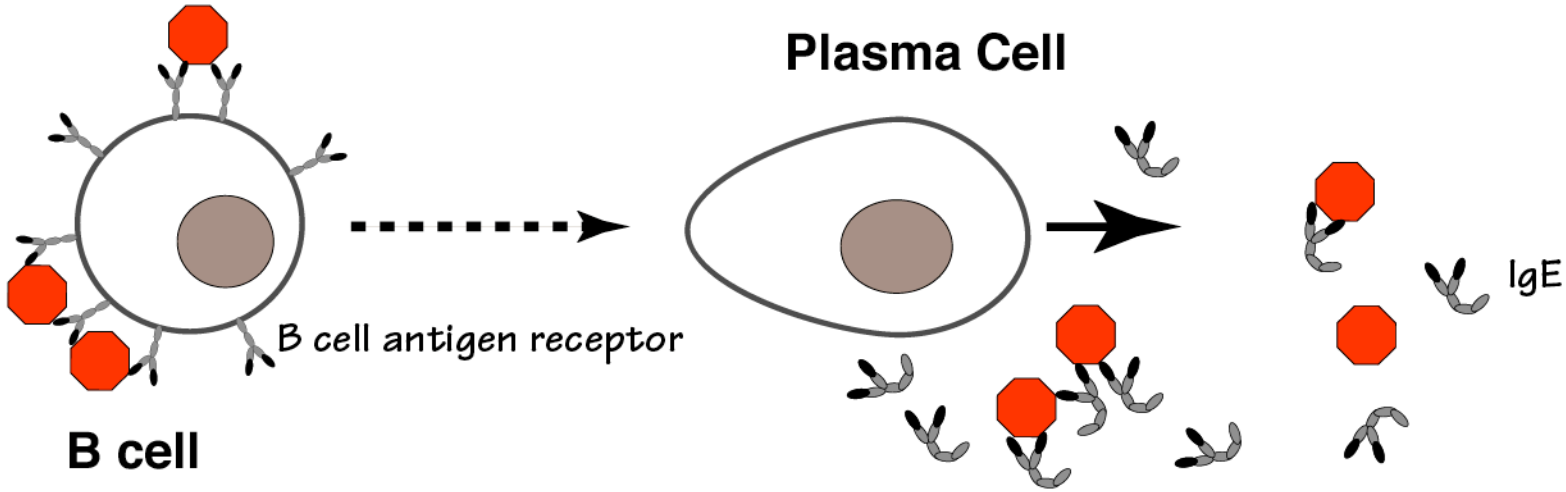
$$t_2 = 1/(2k_{off}) + 1/k_{off} + k_{+2}R/(2k_{off})^2$$

$$t_1 = 1/k_{off} + k_{+2}R/(2k_{off})^2$$

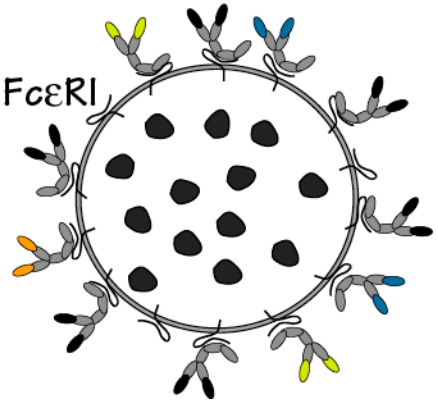
- An example we study,the high affinity receptor for IgE, FcεRI, a key player in allergic reactions.

First Exposure to Allergen ( )

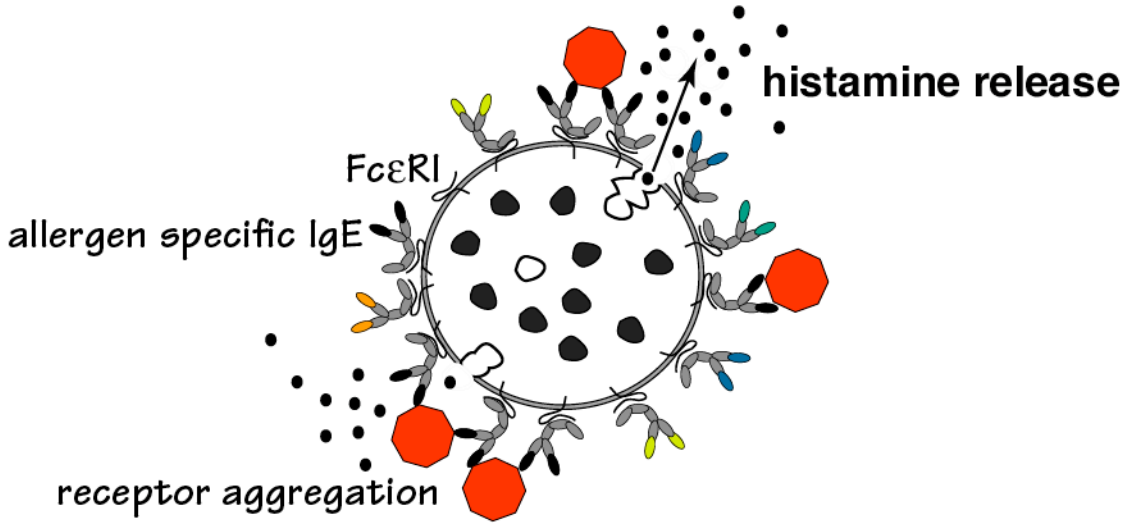
1. Allergen-specific IgE antibody ( ) produced



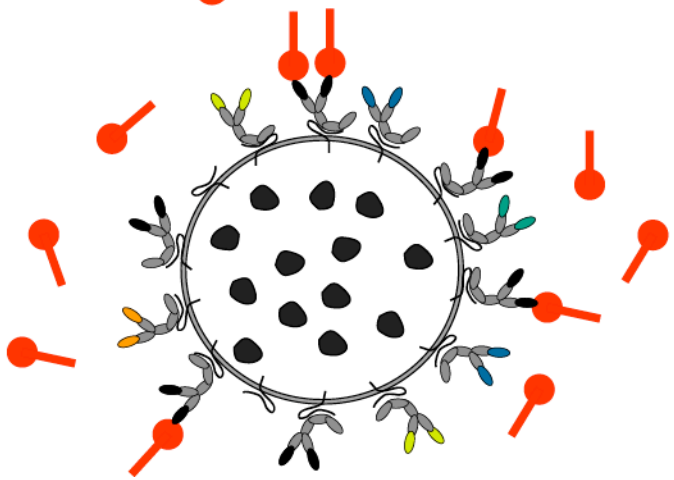
2. IgE binds to receptors (FcεRI) on mast cells and basophils



3. Subsequent exposure to allergen (●) causes basophils and/or mast cells to release histamine and other mediators of anaphylaxsis.

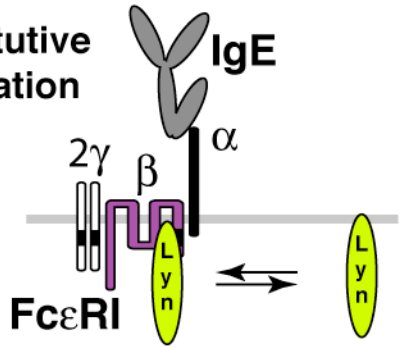


Binding is not enough --- receptor aggregation is essential for histamine release. Monovalent ligands (⊥) do not trigger degranulation.

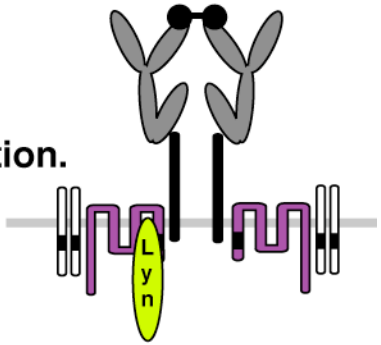


The first steps in signaling mediated by FcεRI

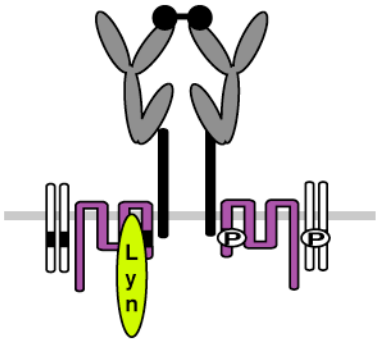
Constitutive association of Lyn.



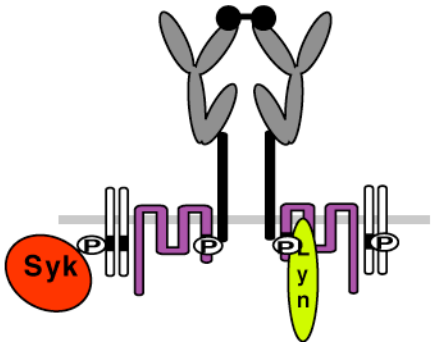
① Ligand induced receptor aggregation.



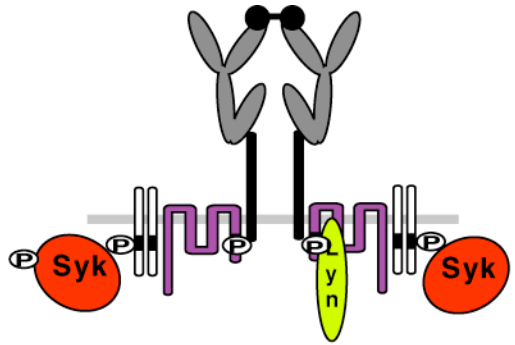
② Lyn phosphorylates the β and γ ITAMs creating high affinity binding sites for Lyn and Syk.



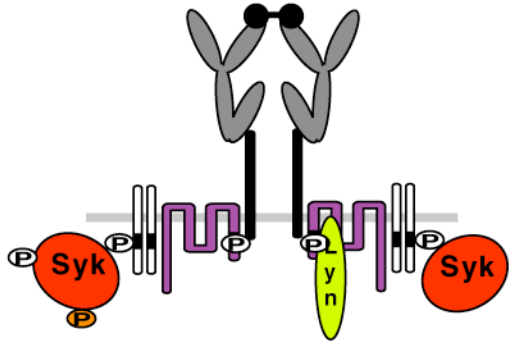
③-④ Syk moves from the cytosol and binds to a phosphorylated γ ITAM.
Lyn is recruited to a phosphorylated b ITAM.



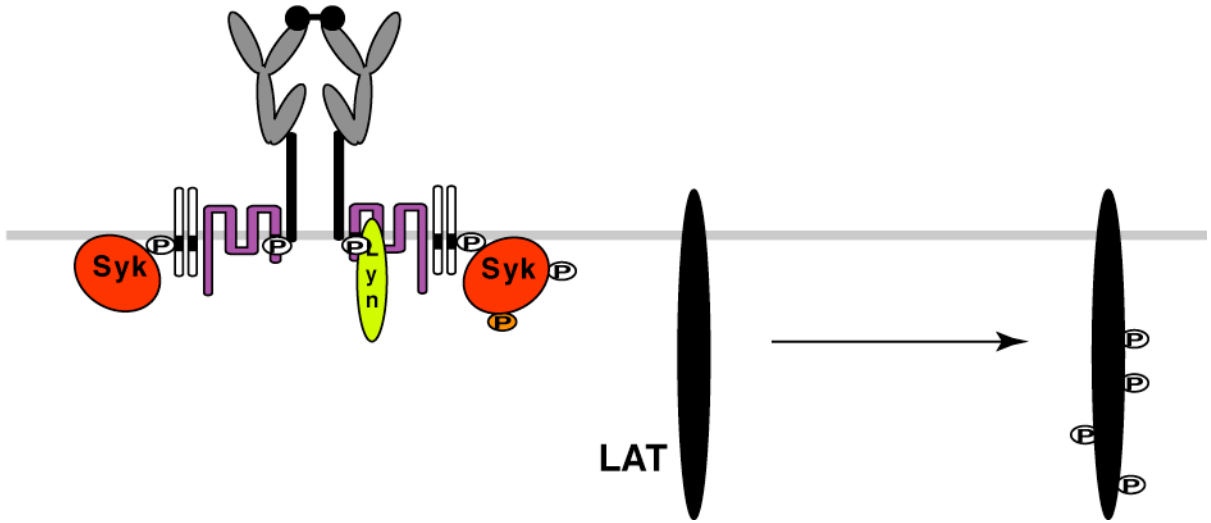
⑤ - ⑥ **Lyn phosphorylates Syk. A second Syk binds to a phosphorylated g ITAM.**



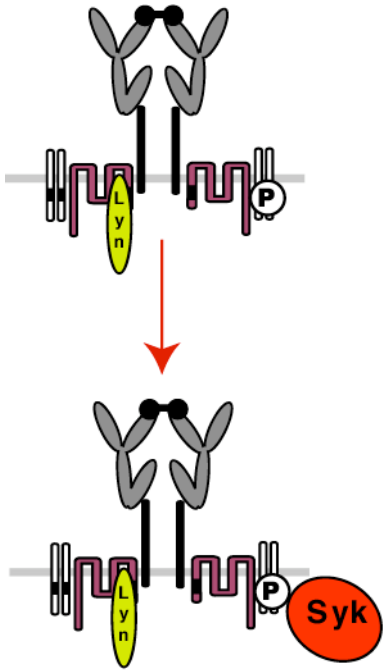
⑦ **Syk phosphorylates Syk and Syk becomes fully active.**



⑧ **Activated Syk phosphorylates tyrosines on LAT**

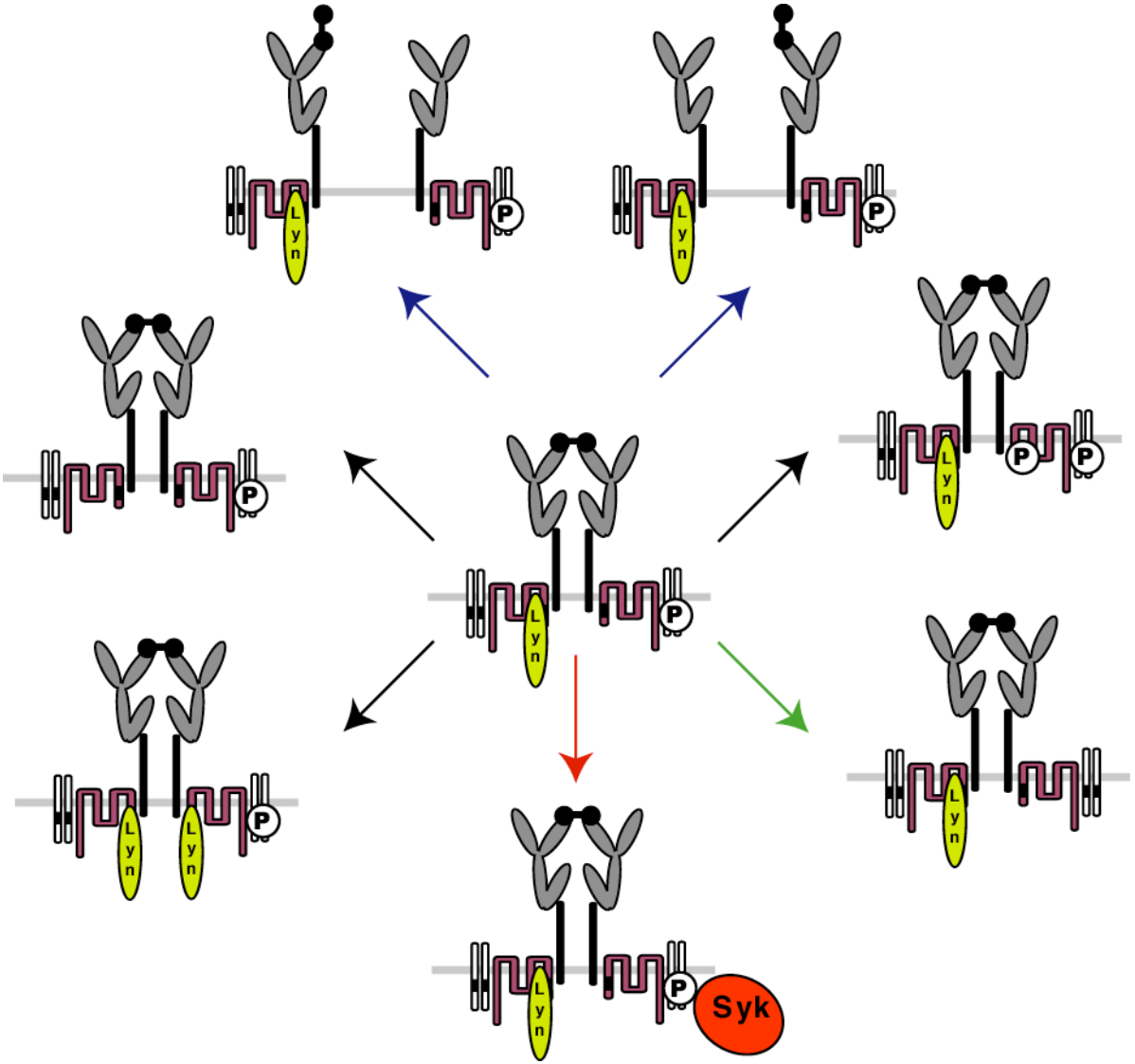


I've described the signaling events as if they were a linear chain (a path) but they aren't.



Recruitment of Syk to phosphorylated γ ITAM

Possible next steps after Lyn phosphorylates γ ITAM



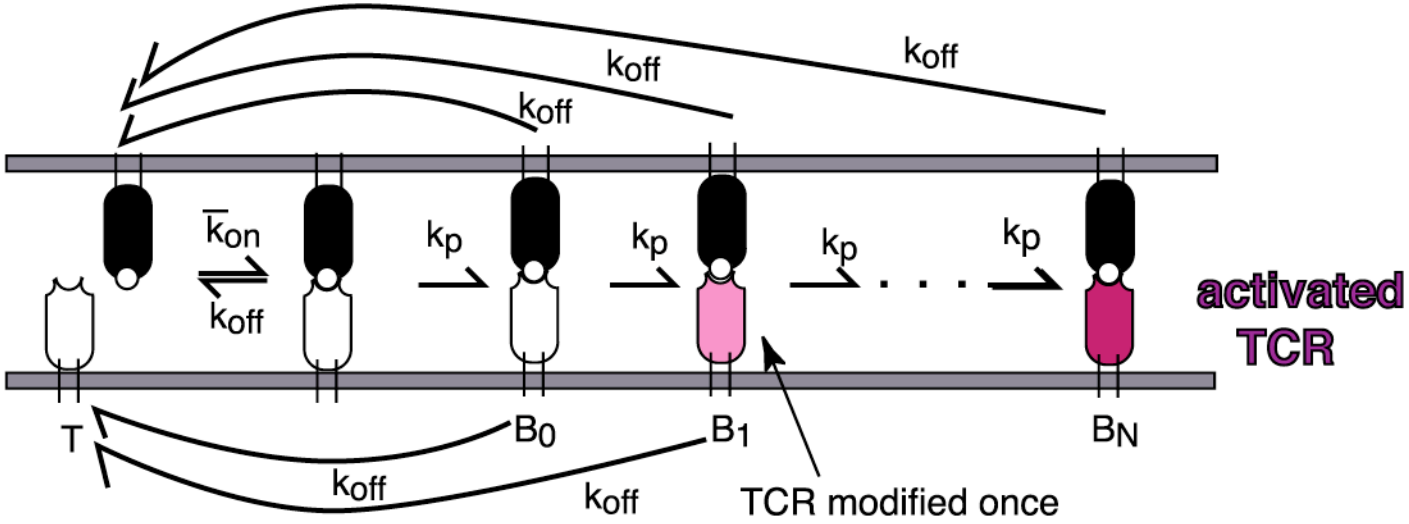
Recruitment of Syk to phosphorylated γ ITAM

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 a key feature: that a series of events (e.g., phosphorylations, the building of a scaffolding about the receptor) is required if a TCR is to become activated.

McKeithan's Kinetic Proofreading Model

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

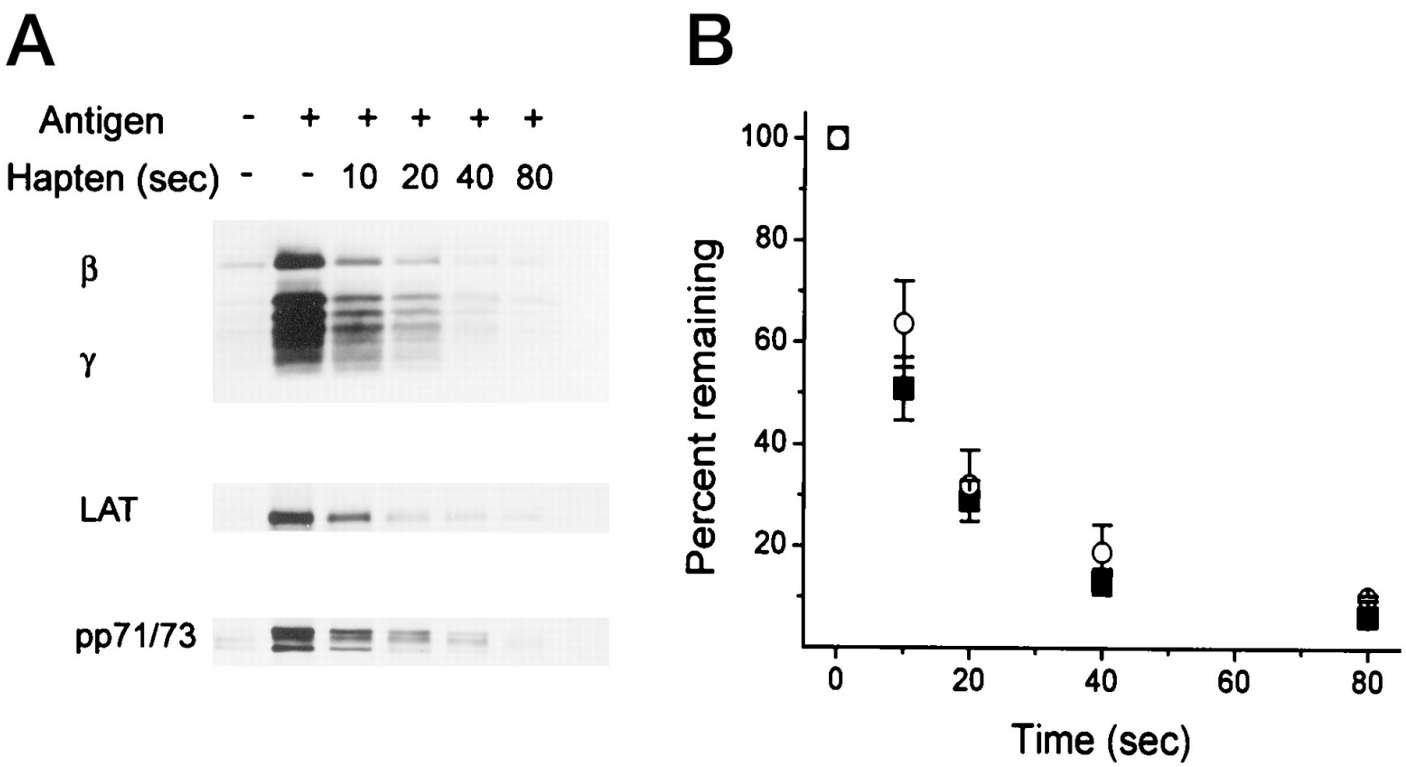


1. For the response of interest the true chemical cascade is replaced by a series of irreversible reactions.

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

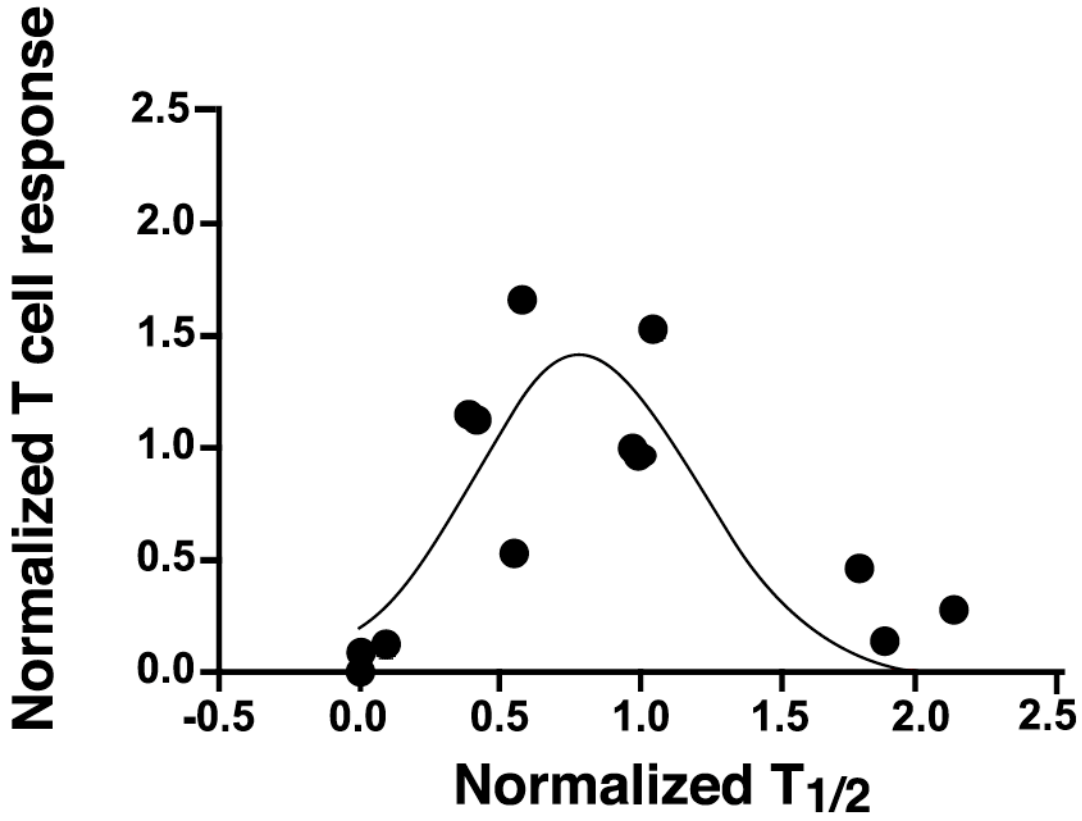
3. When a bound TCR dissociates it reverts to its basal state.

Rates of protein dephosphorylation after addition of hapten



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

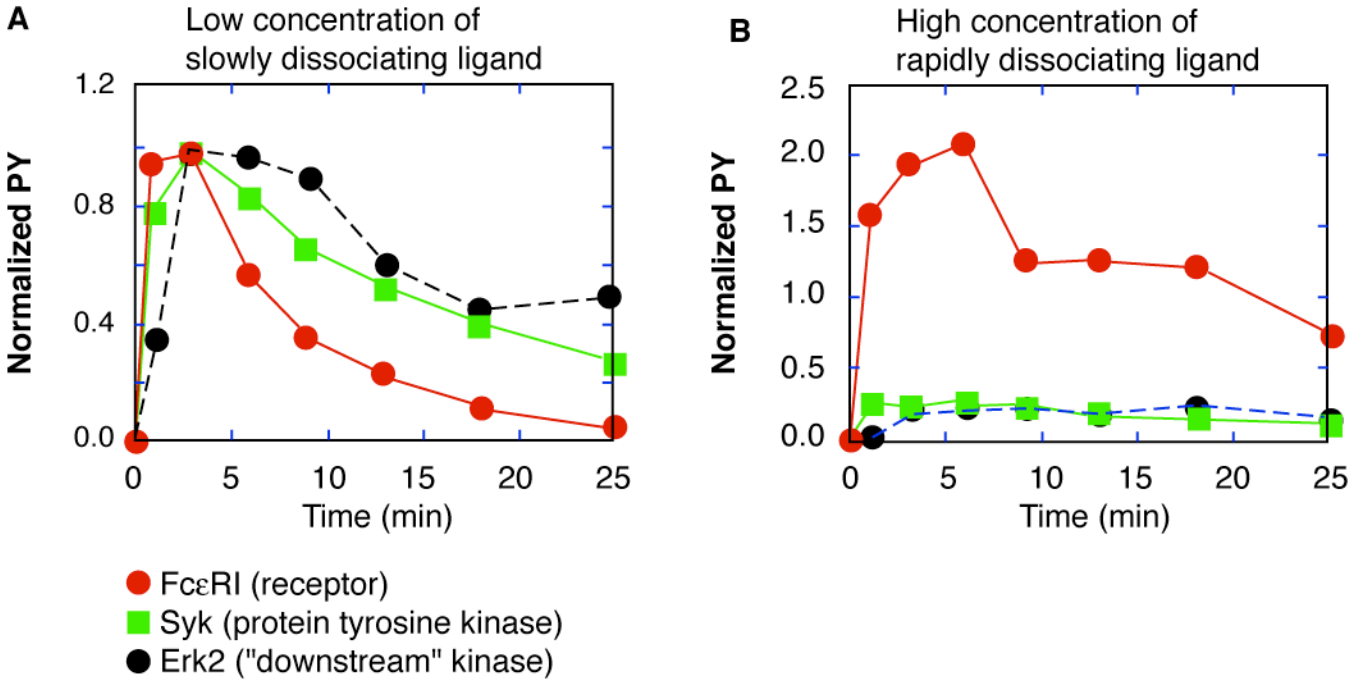
Kalergis et al. (2001) Nature Immunol. **2**: 229-234



(Normalization = observed response/WT response)

Evidence for kinetic proofreading in mast cell responses to two ligands

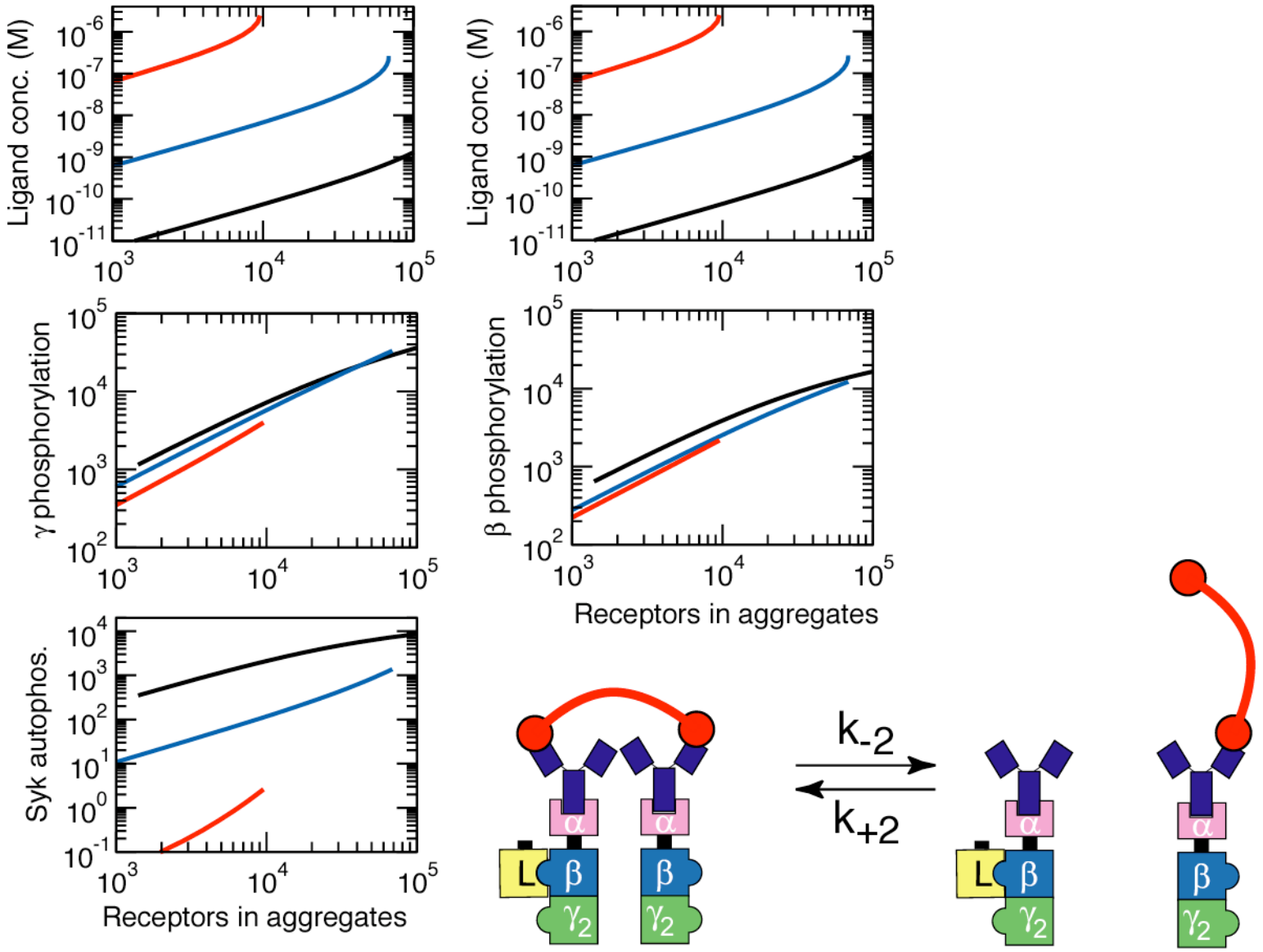
Time course of phosphorylation of tyrosines on several proteins
(Torigoe, Inman & Metzger. 1998. *Science* 281:568-572)



Knowing the average time course of the phosphorylation of the receptor, or even the of the individual ITAMs, is not sufficient to predict downstream events.

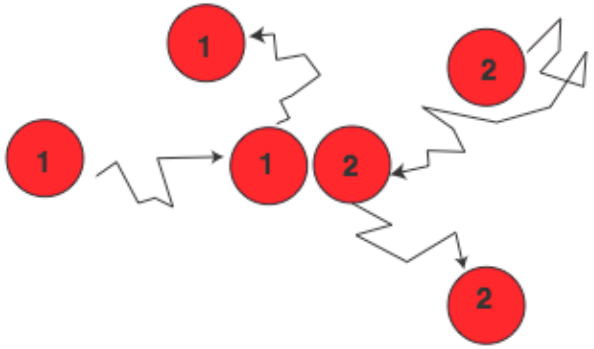
The model exhibits kinetic proofreading

$k_{+1} = 1 \times 10^6\ M^{-1}s^{-1}$ $\quad$ $k_{-1} = k_{-2} = 0.05\ s^{-1}$, 0.5 s^{-1} and 5.0 s^{-1}

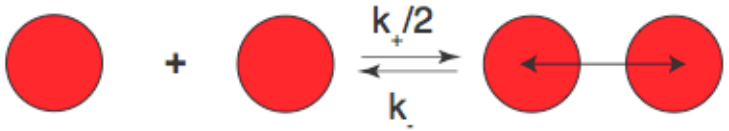


In the basal state (no ligand present) a number of proteins are phosphorylated.

On Rat Basophilic Leukemia (RBL) cells 0.62 % of FcεRI or about 2,500 receptors are phosphorylated in the absence of ligand (Torigoe et al., 2001, *Biochem*. **40**:4016). Why isn't their constant signaling and histamine release?



Spontaneous dimer formation in 2 dimensions of discs of radius a **moving radomly on a surface**

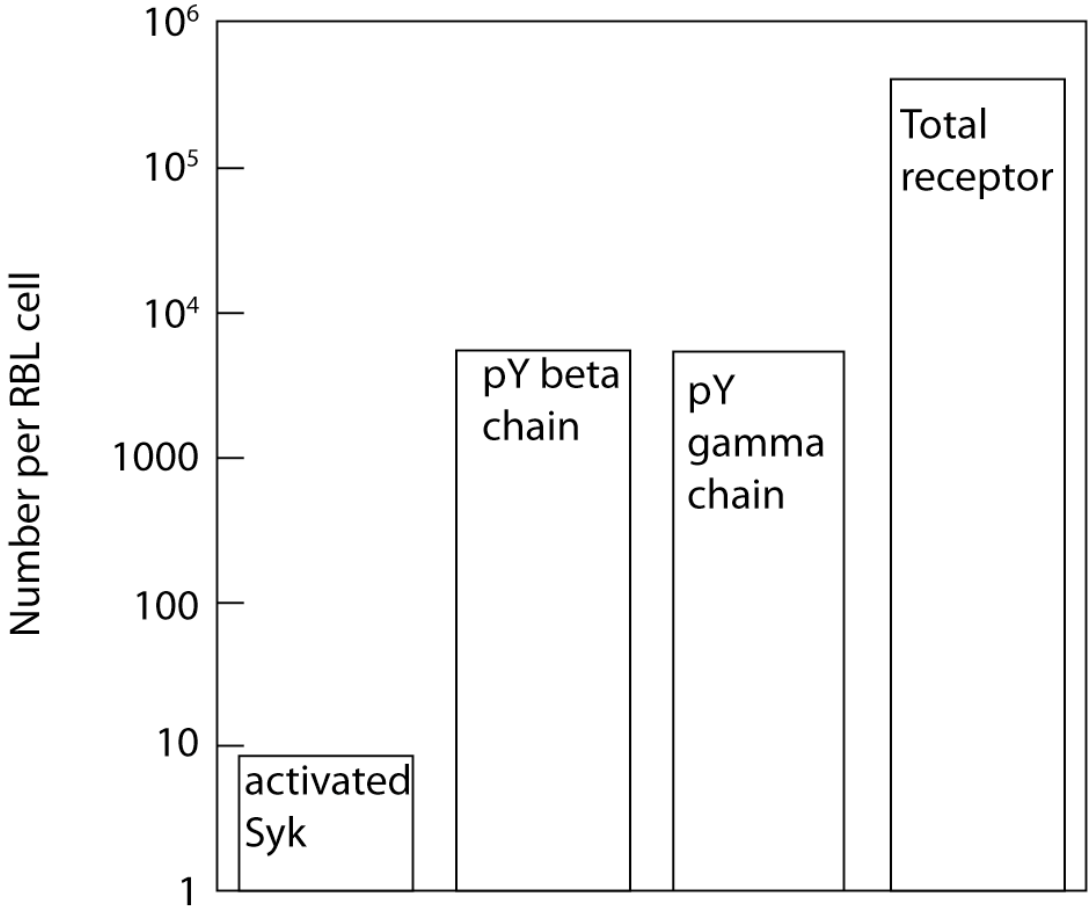


$dD/dt = k_+ R^2/2 - k_- D$

$D=K_+ R^2/2$ where $K_+ = \pi d^2(1 - (2a/d)^2)$

For d=400 nm, $k_+ = 2.19 \times 10^{-9}$ cm^2/s and $k_- = 6677$/s, predicts about 6600 FcεRI in dimers.

Model prediction of basal phosphorylation: Although 5400 receptors are phosphorylated (1.35%) because of kinetic proofreading only 9 Syk molecules are activated.



Kinetic proofreading explains why mobile receptors on the plasma membrane don't trigger cells in the basal state.

A Review

Transmitting information across the cell membrane by juxtaposing the cytoplasmic tails of receptors is a ubiquitous signaling mechanism.

By converting cytoplasmic domains of the receptor to phosphorylated forms, the cell “senses” the external ligand and initiates a signaling cascade. The phosphorylation of aggregated receptors reflects a dynamic balance between the action of kinase and phosphatases.

If an aggregate breaks up, phosphatases rapildly dephosphorylate the receptor and the chemical modification is erased.

Kinetic Proofreading and Serial Engagement

Kinetic proofreading - a series of events, e.g., building a scaffoldings about the receptor and additional transmembrane proteins, chemical modifications, is required for cell activation.

Kinetic proofreading applies to a single receptor. It increases discrimination at the expense of sensitivity to a specific stimuli. It prevents false responses.

Kinetic proofreading offers an explanation why basal phosphorylation of receptors does not lead to a cellular response.

Serial engagement - a property of multivalent antigens (ligands or cells) - can dramatically increases the number of receptors that are activated.

The combination of kinetic proofreading and serial engagement can result in a window of dissociation rate constants that can activate a cell.