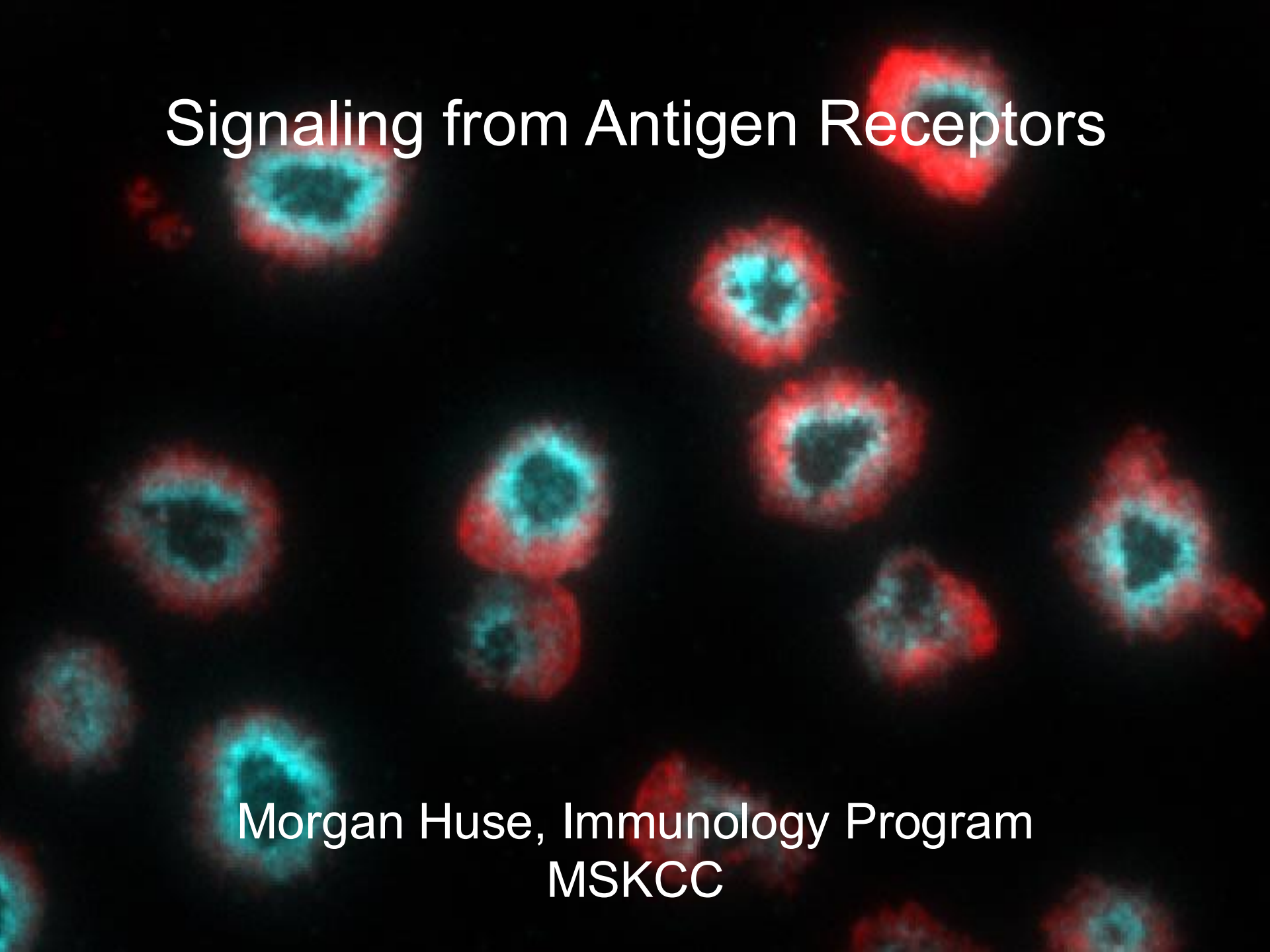
Signaling from Antigen Receptors



Morgan Huse, Immunology Program
MSKCC

How do T cells and B cells recognize antigen?

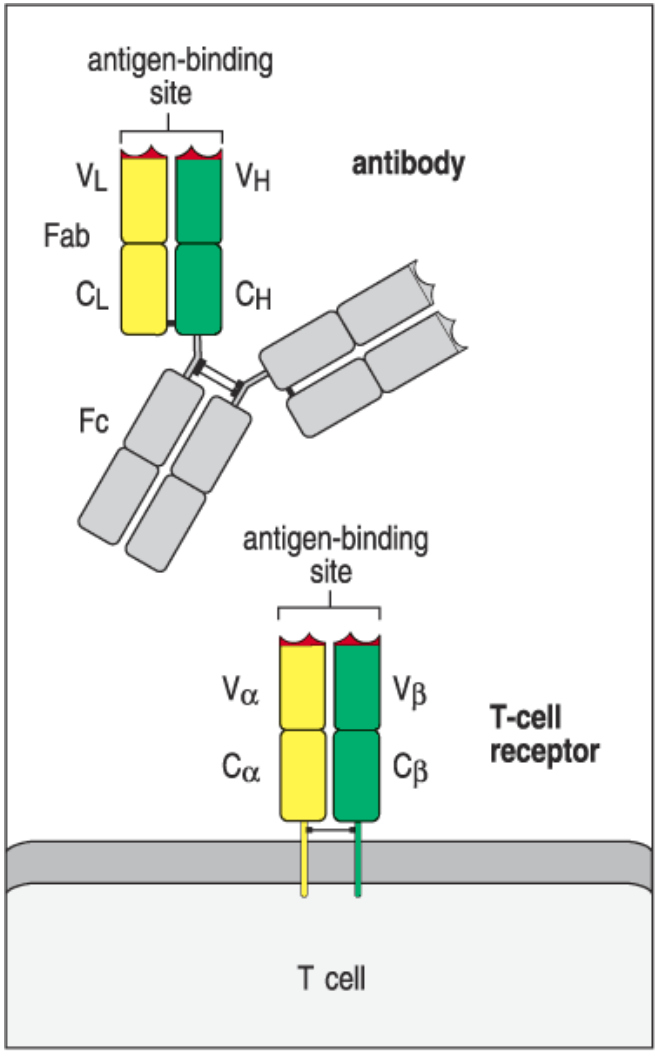
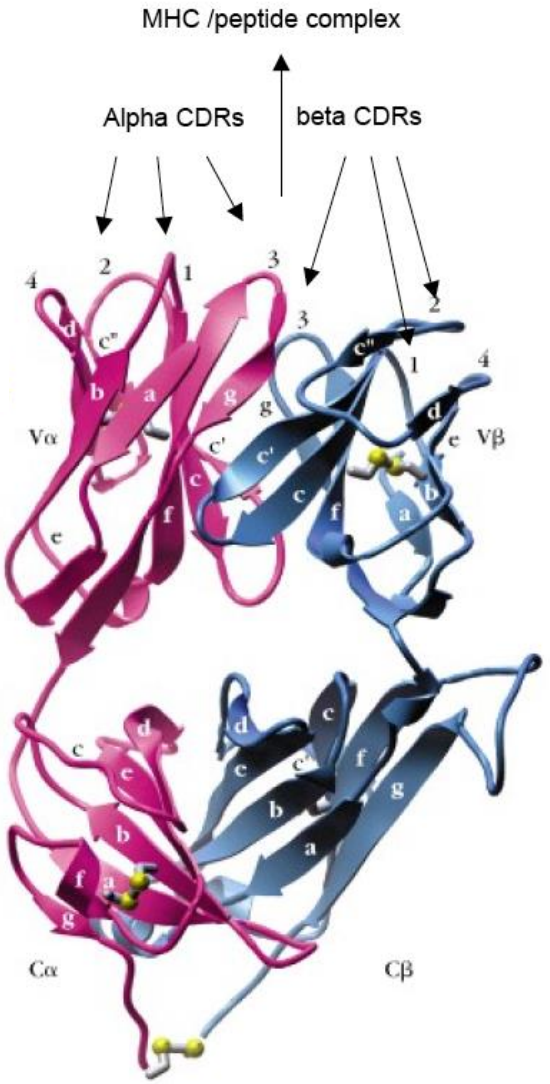
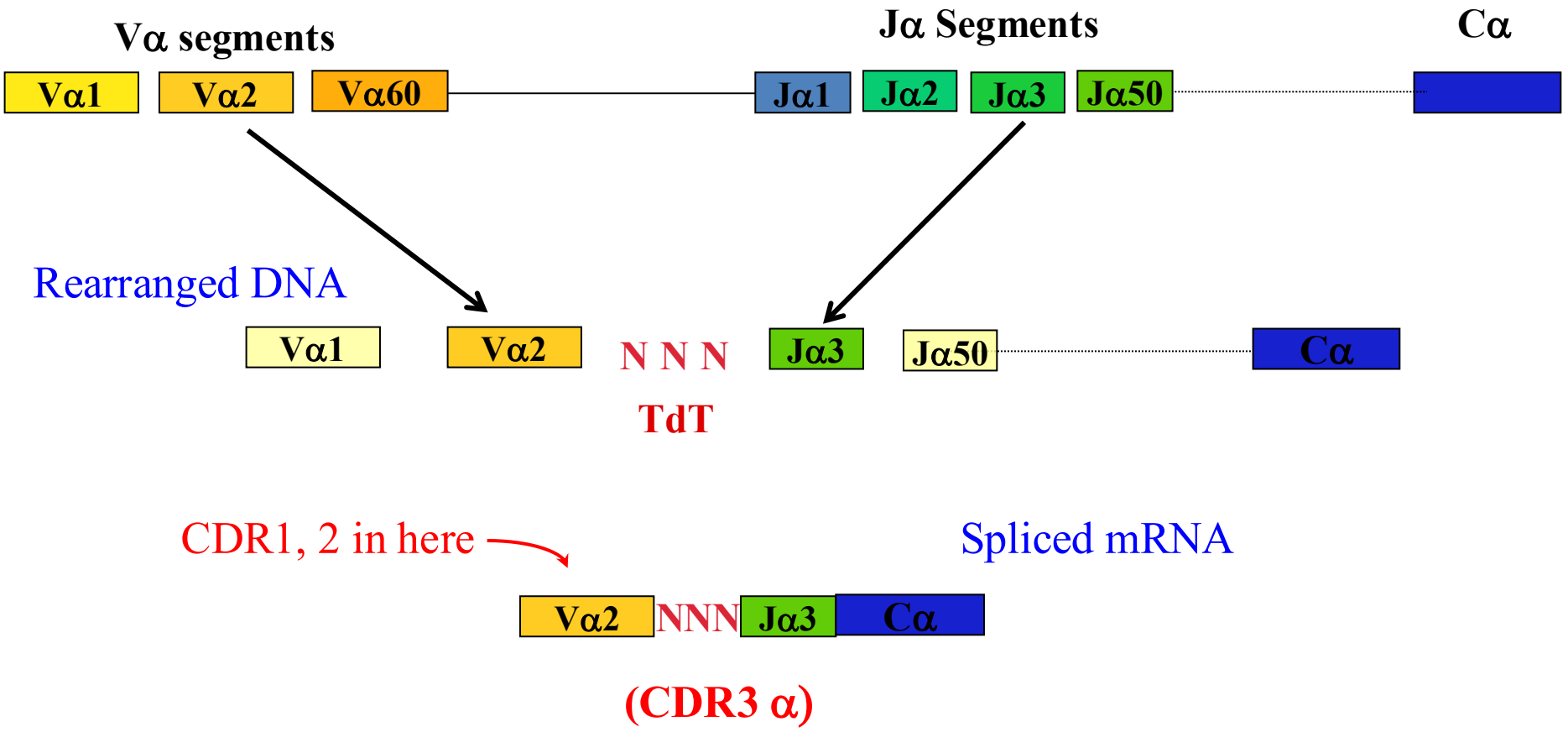


Fig 3.11 © 2001 Garland Science



VDJ recombination generates diversity



Diversity concentrated in CDR3 regions

T cells recognize antigen bound to MHC

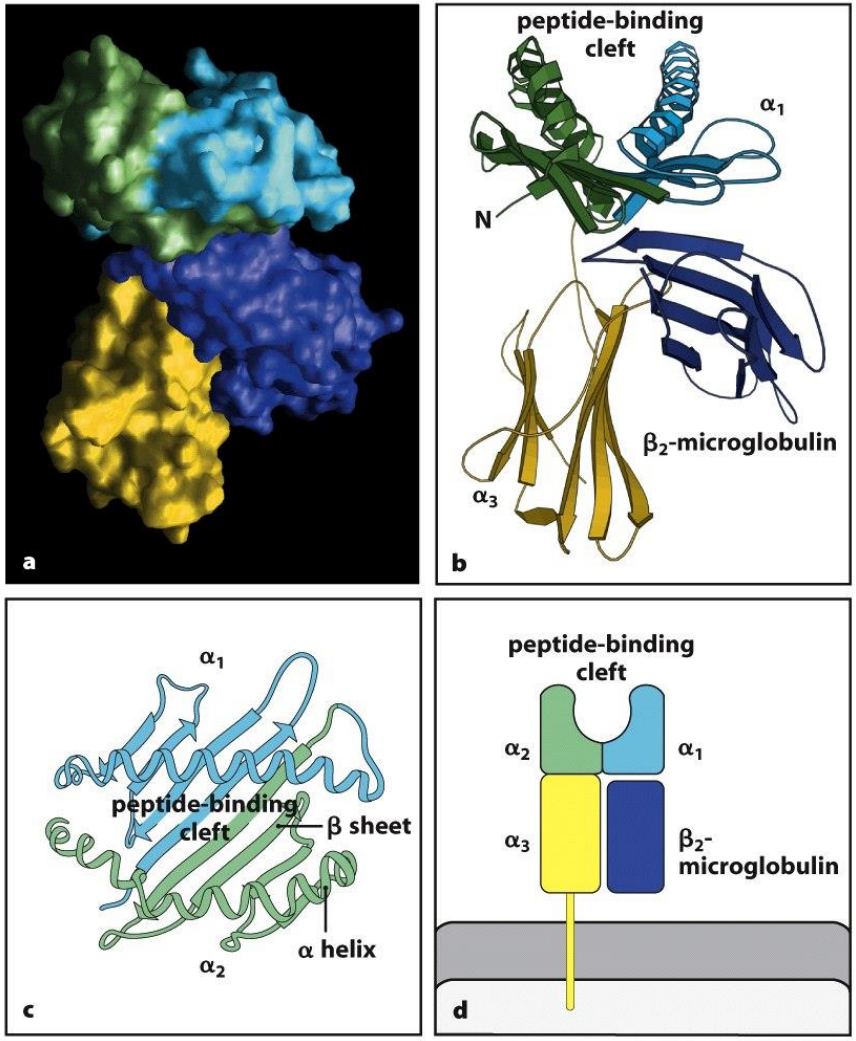
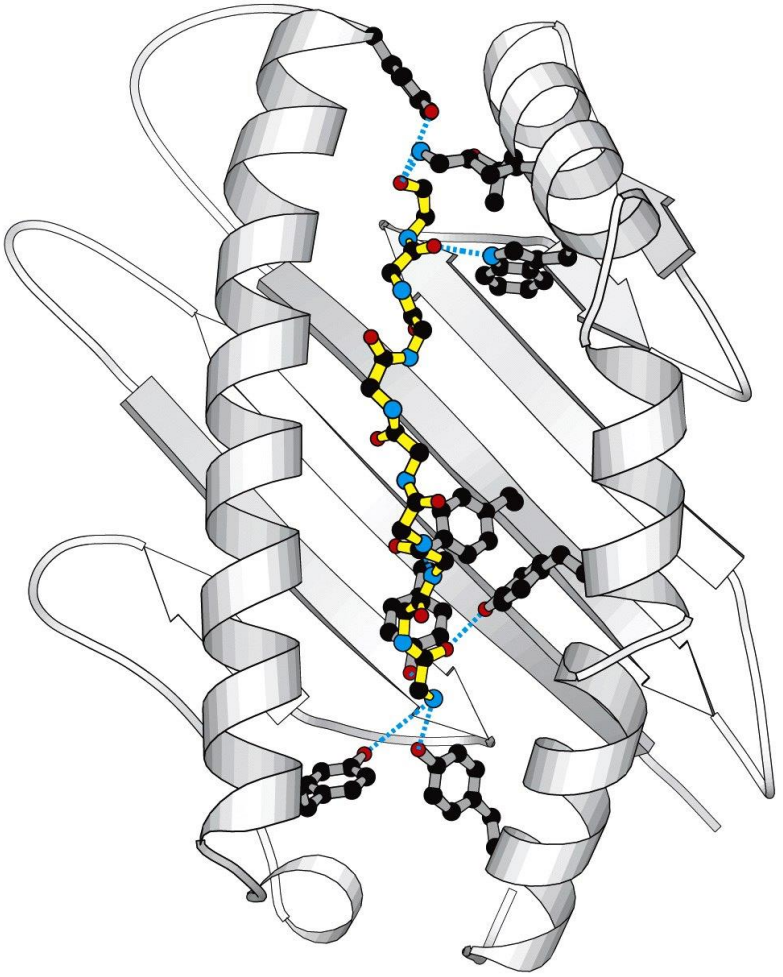


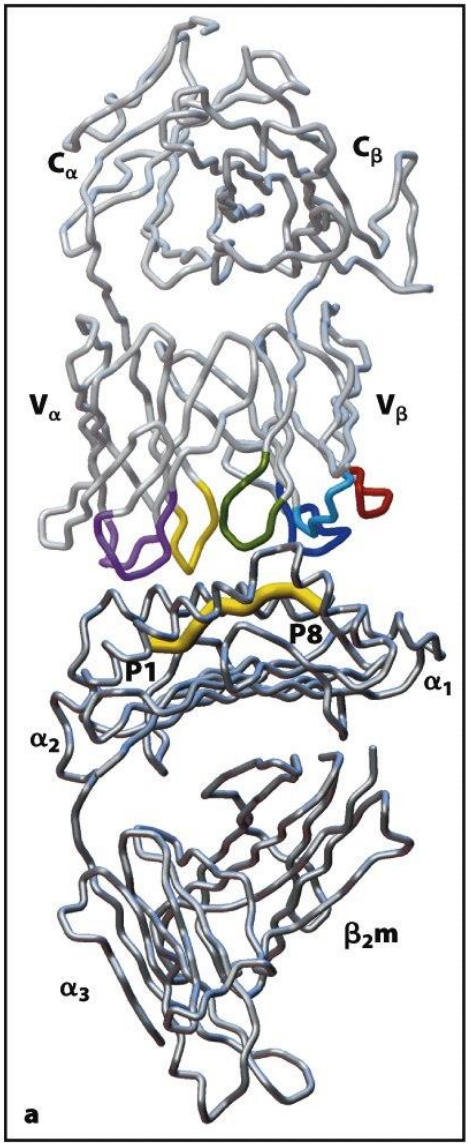
Figure 3-15 Immunobiology, 7ed. (© Garland Science 2008)

Class I MHC



Immunobiology, 7th ed.

TCRs recognize pMHC with a composite interface



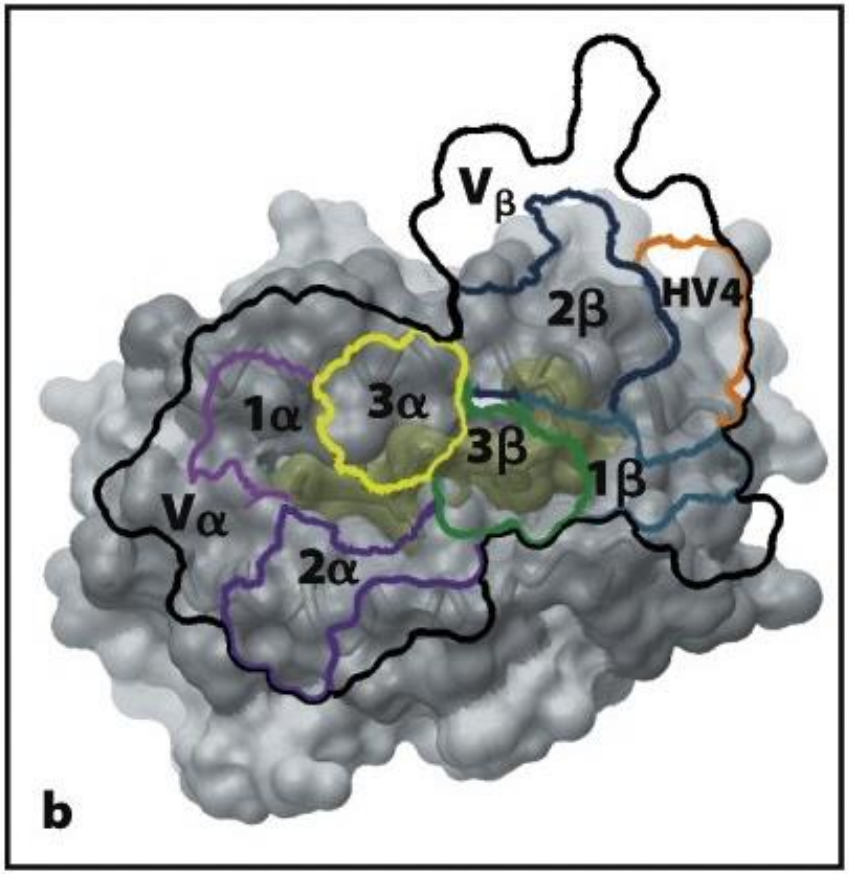


Figure 3-22 Immunobiology, 7ed. (© Garland Scien

The organization of the TCR-CD3 complex

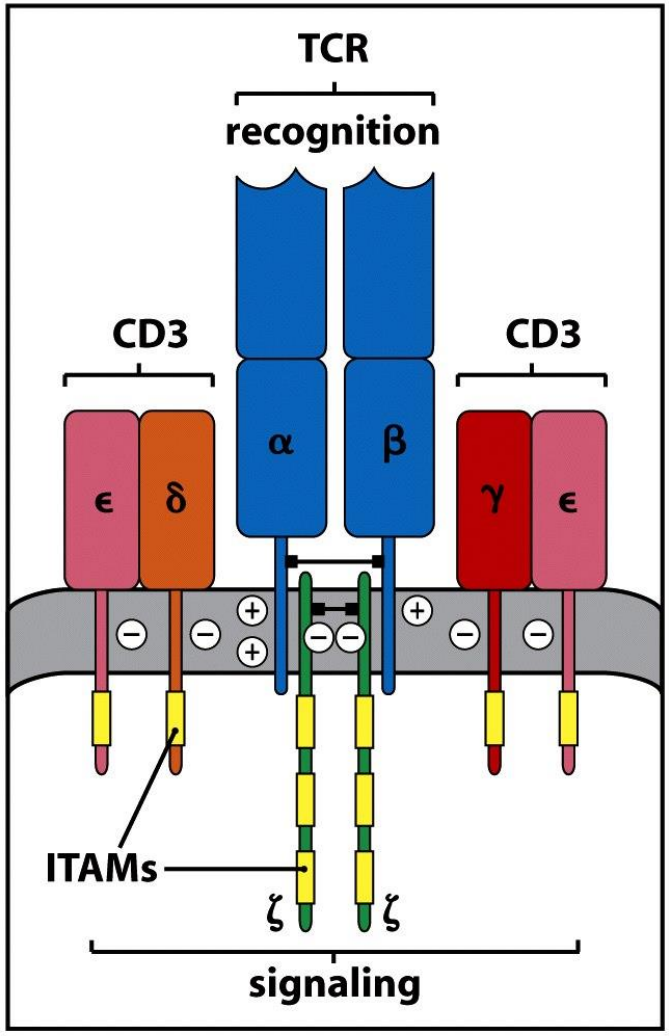


Figure 6-10 Immunobiology, 7ed. (© Garland Science 2008)

pMHC engagement induces ITAM phosphorylation

ITAMs, consensus sequence Y-x-x-(L/I)-x_{6-8}-Y-x-x-(L/I)

Phosphorylated by Src-family kinases (e.g. Lck)

Recruit Syk-family kinases (e.g. Zap70)

The organization of the TCR-CD3 complex

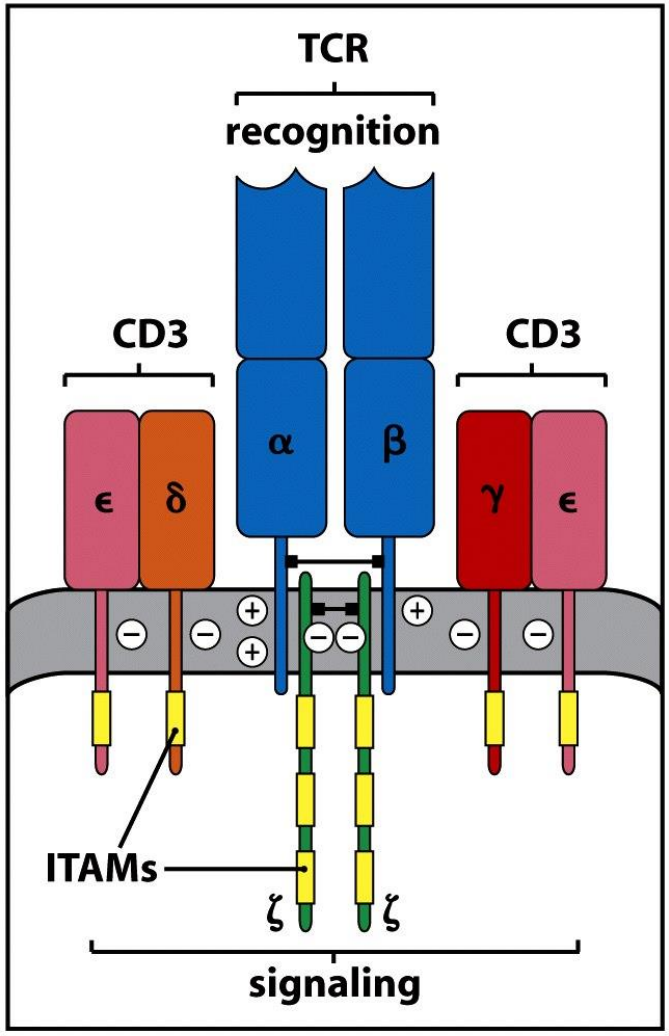
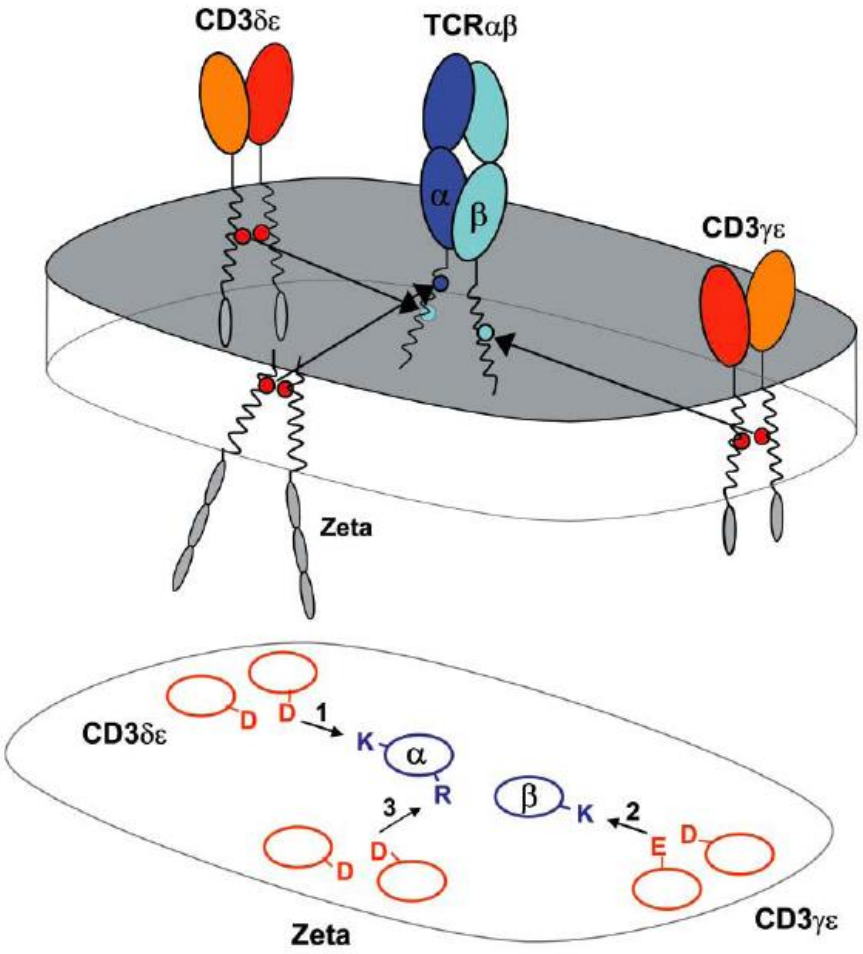
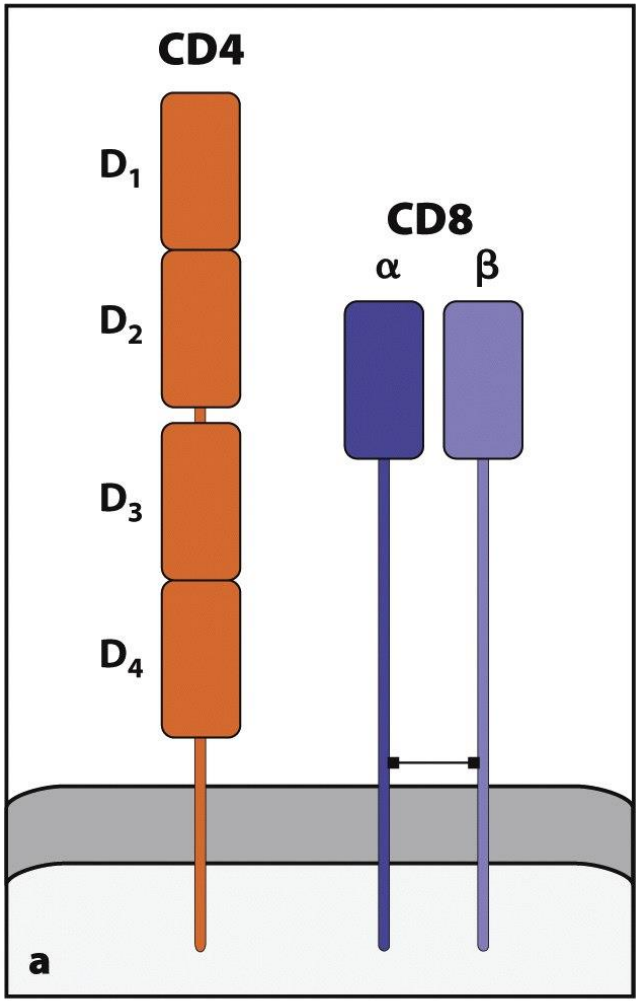


Figure 6-10 Immunobiology, 7ed. (© Garland Science 2008)



Call et al., 2002

TCR binds to pMHC in conjunction with CD4 or CD8



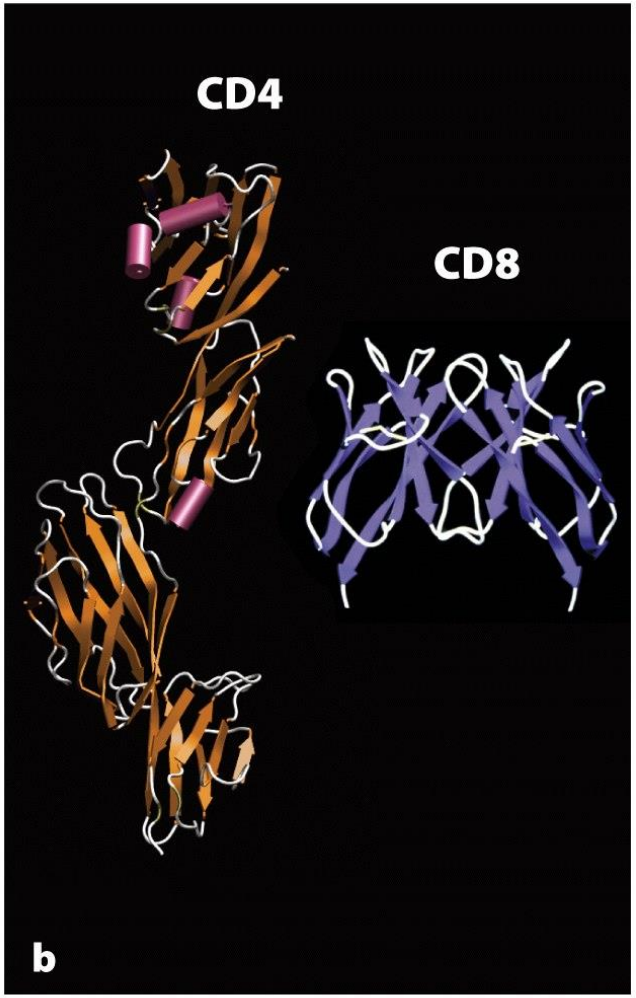


Figure 3-24 Immunobiology, 7ed. (© Garland Science 2008)

CD8 is a homodimer, and binds with higher affinity than CD4 (μM versus mM). Both potentiate TCR signaling, but not required for it.

TCR binds to pMHC in conjunction with CD4 or CD8

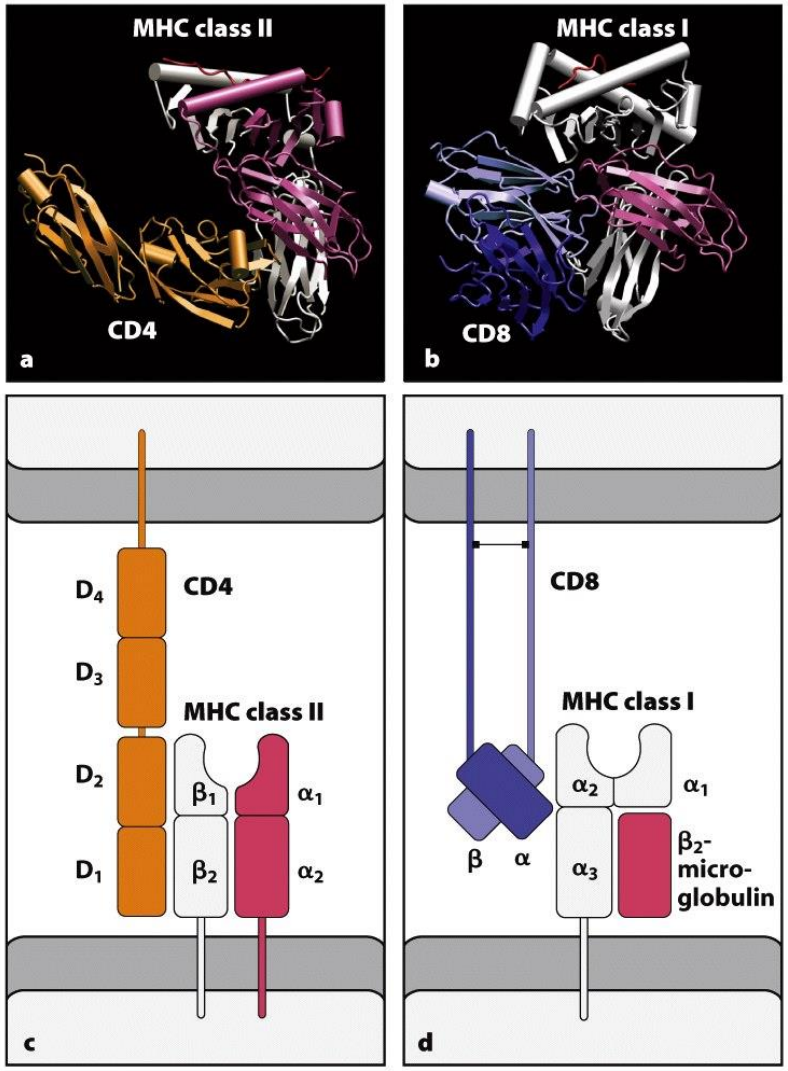


Figure 3-25 Immunobiology, 7ed. (© Garland Science 2008)

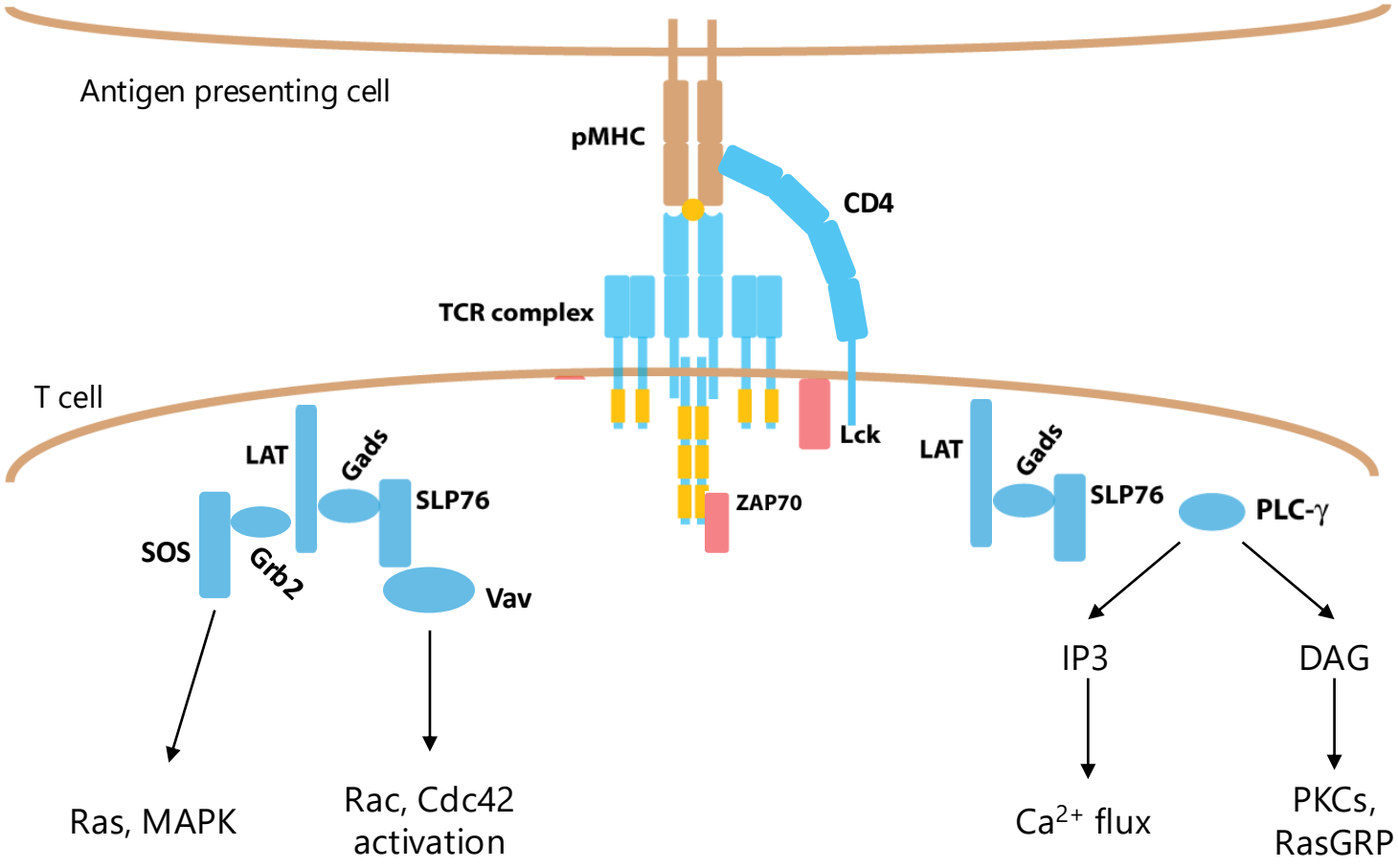
CD4 and CD8 bind to analogous portions of the MHC molecules.

They potentiateTCR signaling by:

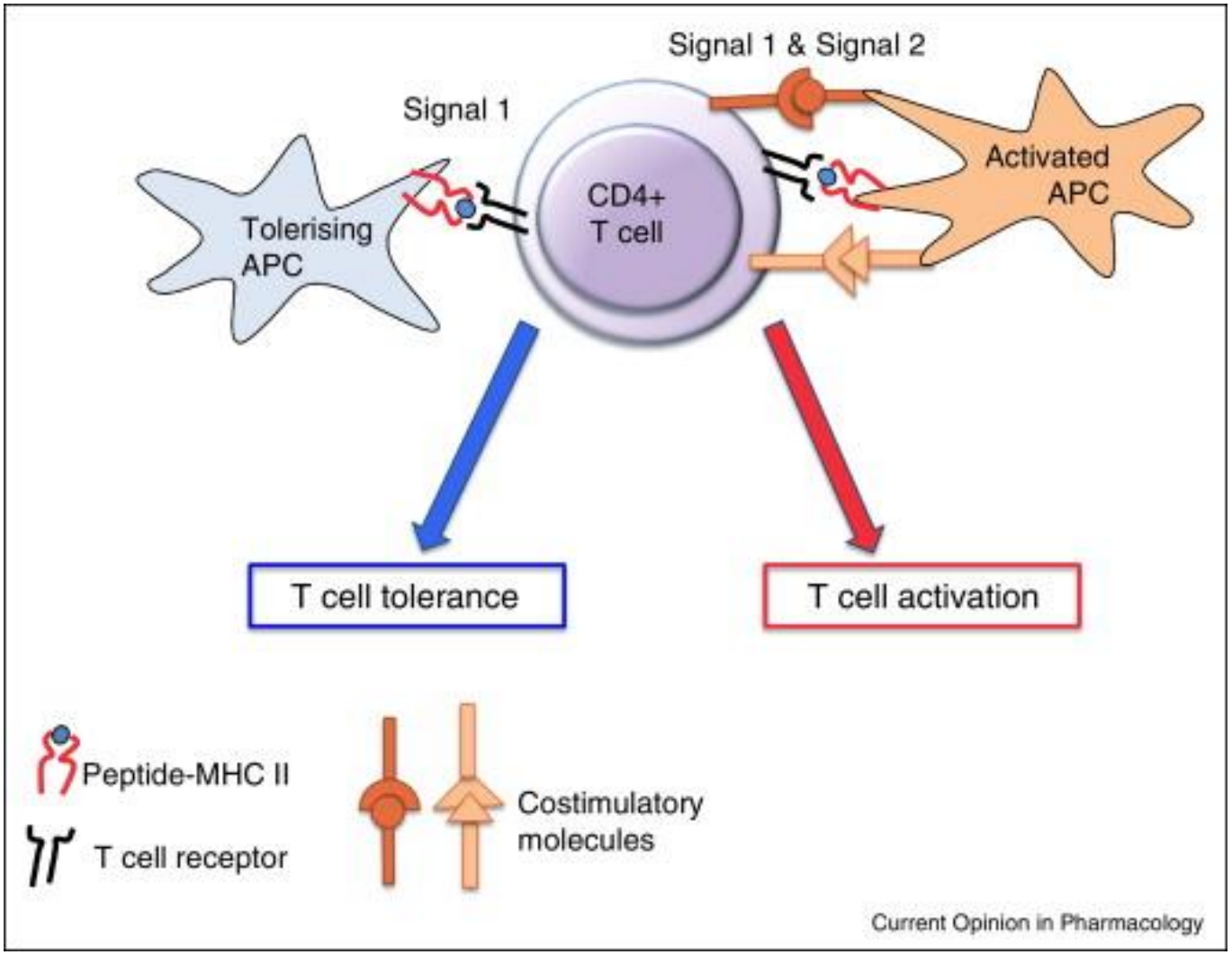
1) Enhancing strength of interaction.

2) Recruitment of Lck kinase activity (both CD4 and CD8 associate with Lck).

Early TCR signaling



The costimulation concept

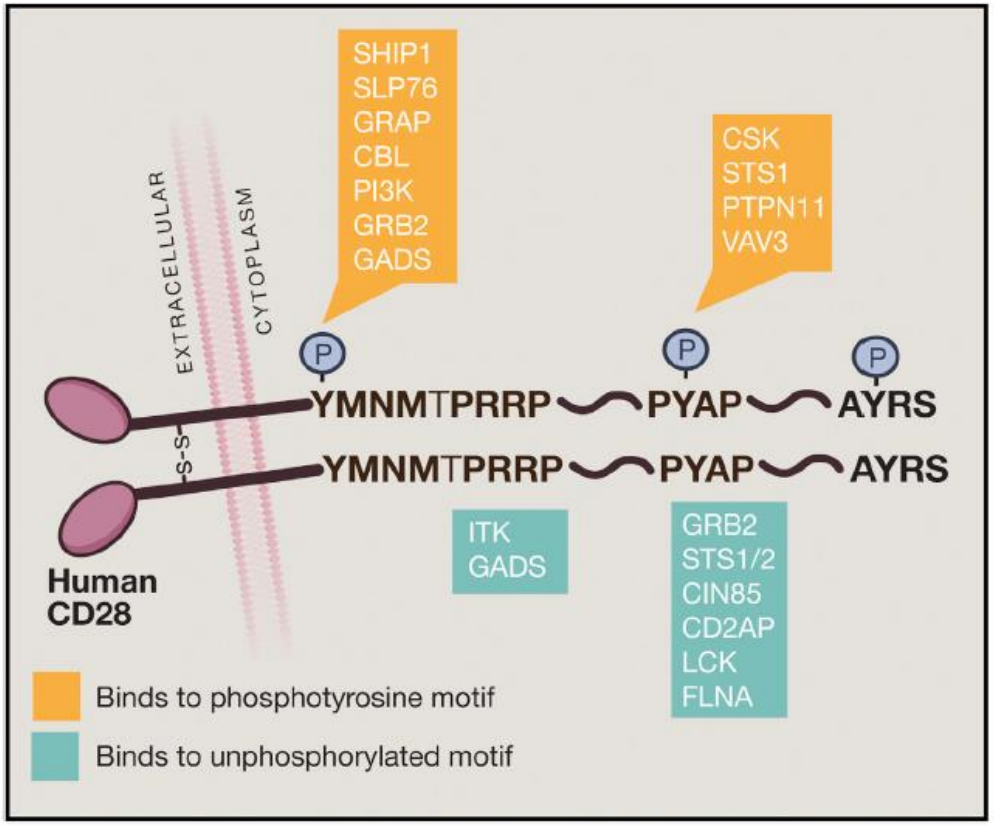


Important conceptual note.

Costimulatory molecules should not induce signals or responses on their own.

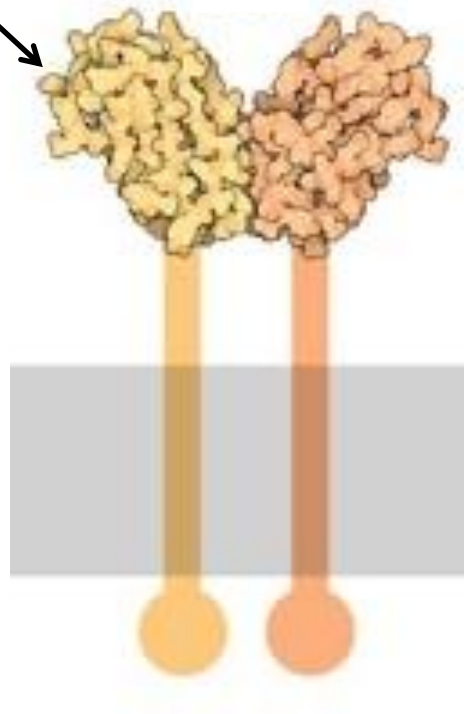
CD28 architecture

Ligands: B7.1 (CD80), B7.2 (CD86)



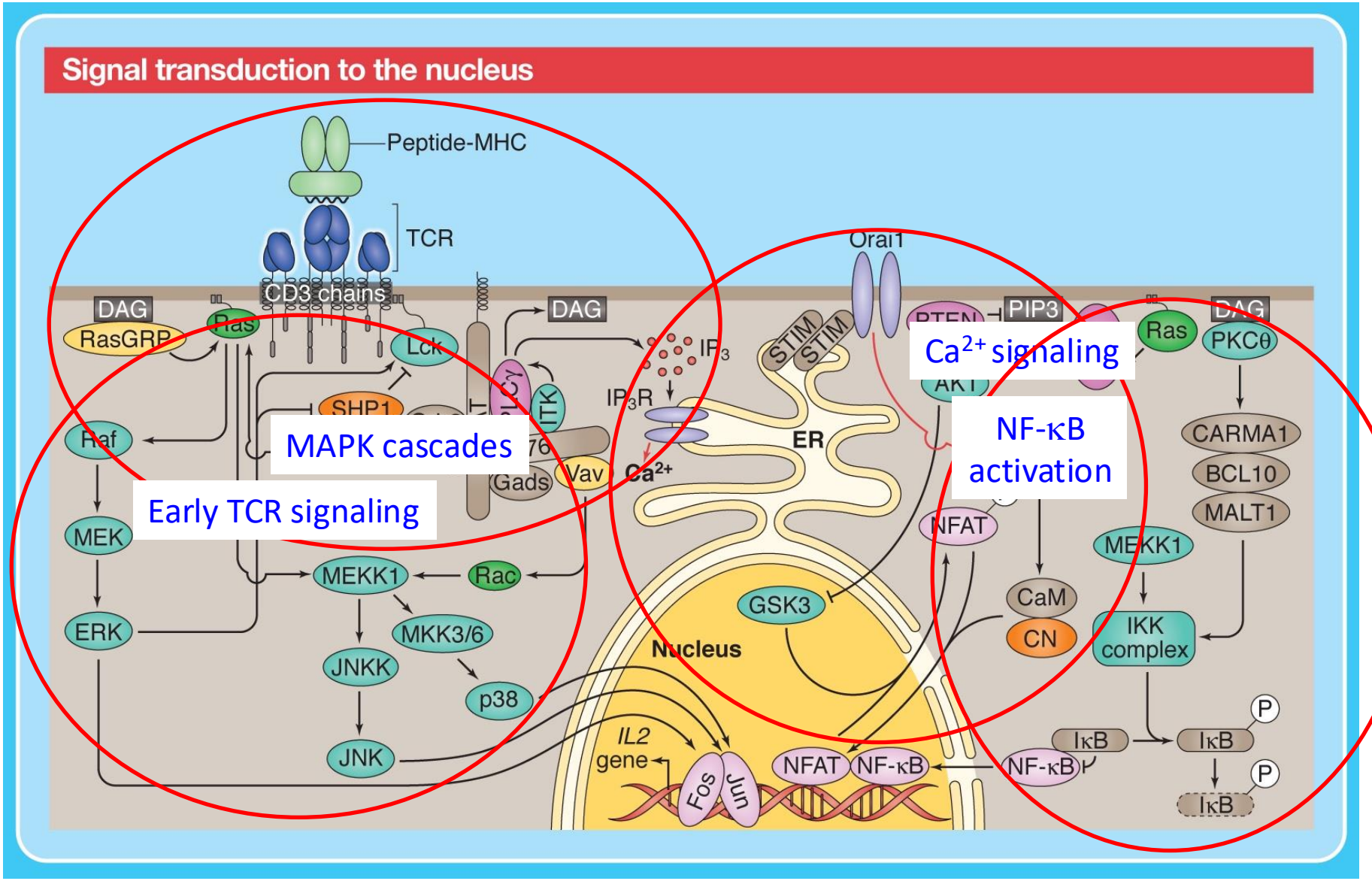
Esensten et al., 2016

Ligand binding



rcsb.org

Signal transduction to the nucleus



Huse, 2009

Lecture topics

1) How does ligand binding induce TCR activation?

- Phosphatase exclusion
- TCR conformational change
- The kinetic proofreading model

2) How does receptor activation induce downstream signaling?

- Liquid-ordered signaling microclusters
- Lipid second messengers
- Calcium signaling

3) How does interfacial architecture influence T cell activation and effector responses?

- Microvilli and ligand search
- Actin dynamics and mechanotransduction
- Directional secretion

Lecture topics

1) How does ligand binding induce TCR activation?

- Phosphatase exclusion
- TCR conformational change
- The kinetic proofreading model

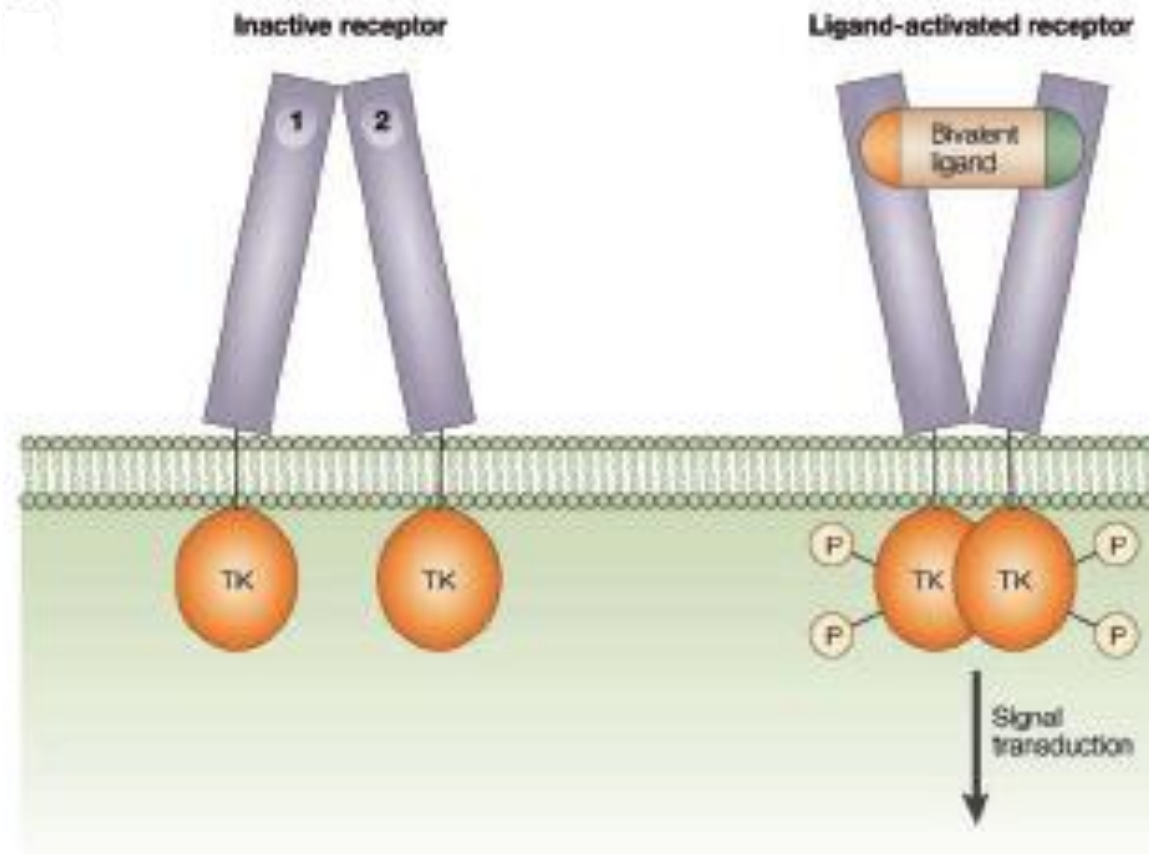
2) How does receptor activation induce downstream signaling?

- Liquid-ordered signaling microclusters
- Lipid second messengers
- Calcium signaling

3) How does interfacial architecture influence T cell activation and effector responses?

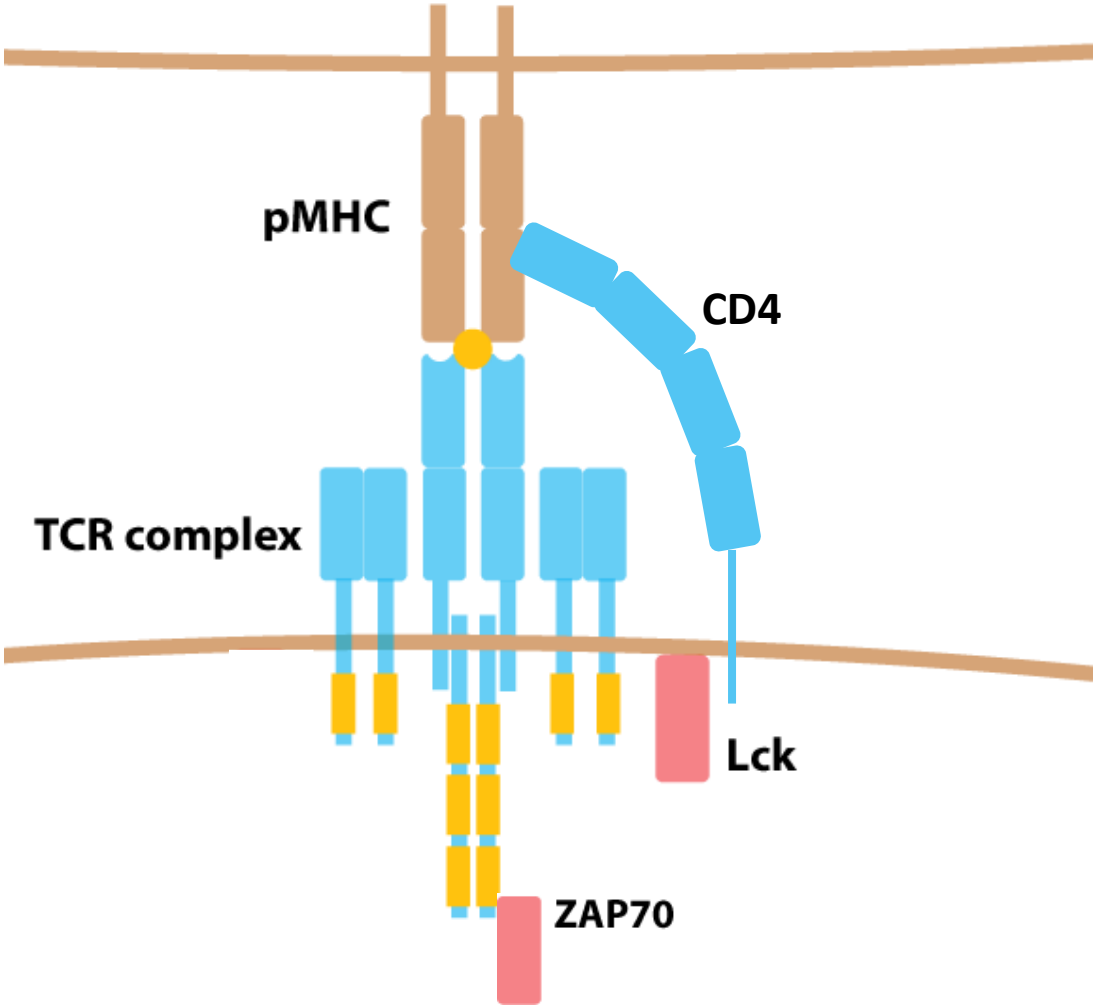
- Microvilli and ligand search
- Actin dynamics and mechanotransduction
- Directional secretion

Receptor tyrosine kinase signaling



DeMeyts and Whittaker, 2002

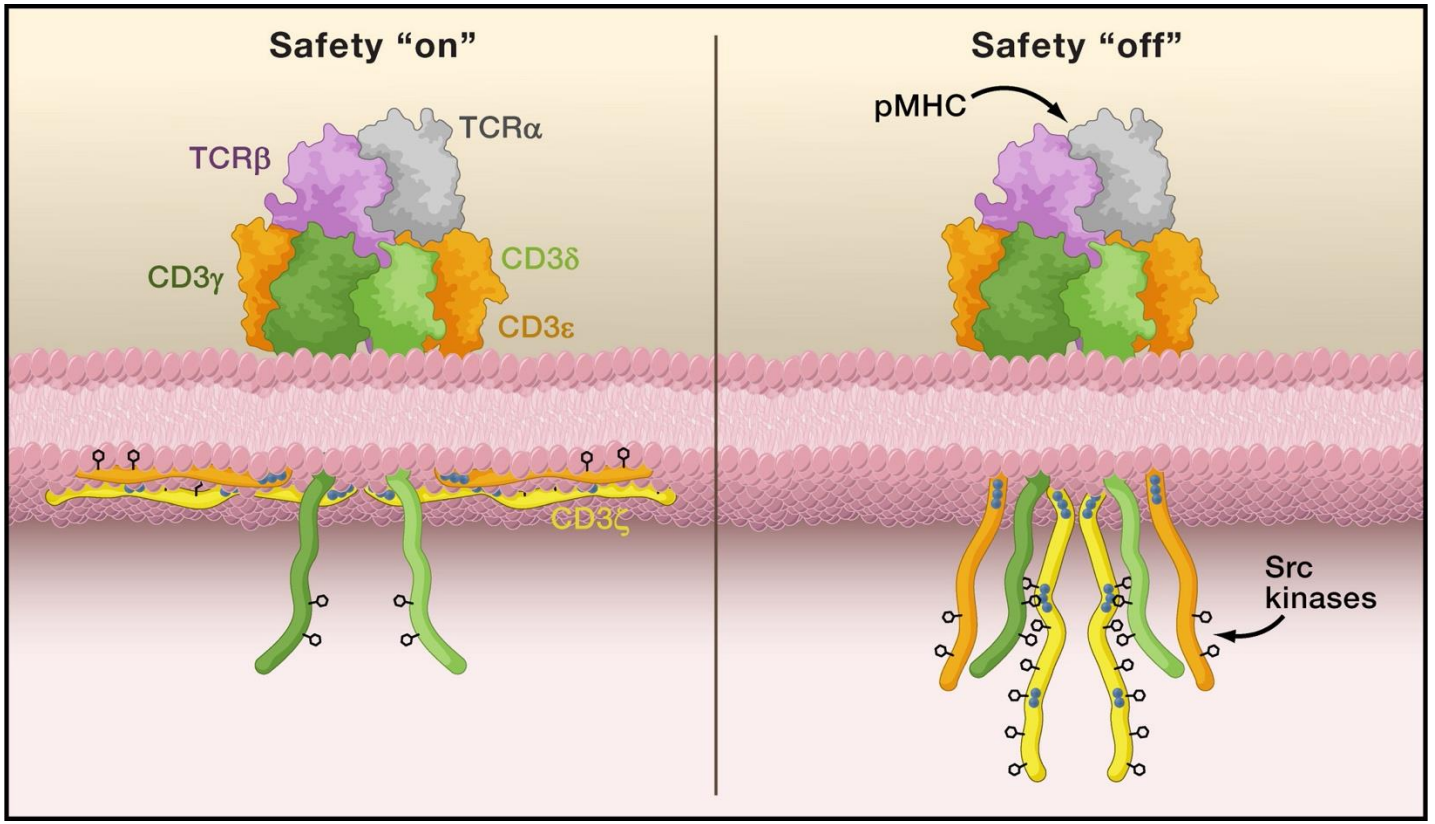
The old model for TCR activation in presence of CD4



However, CD4/CD8 not required for TCR signaling. So how to signal through a monovalent receptor?

TCR conformational change

pMHC binding induces conformational change, exposing cytoplasmic signaling motifs



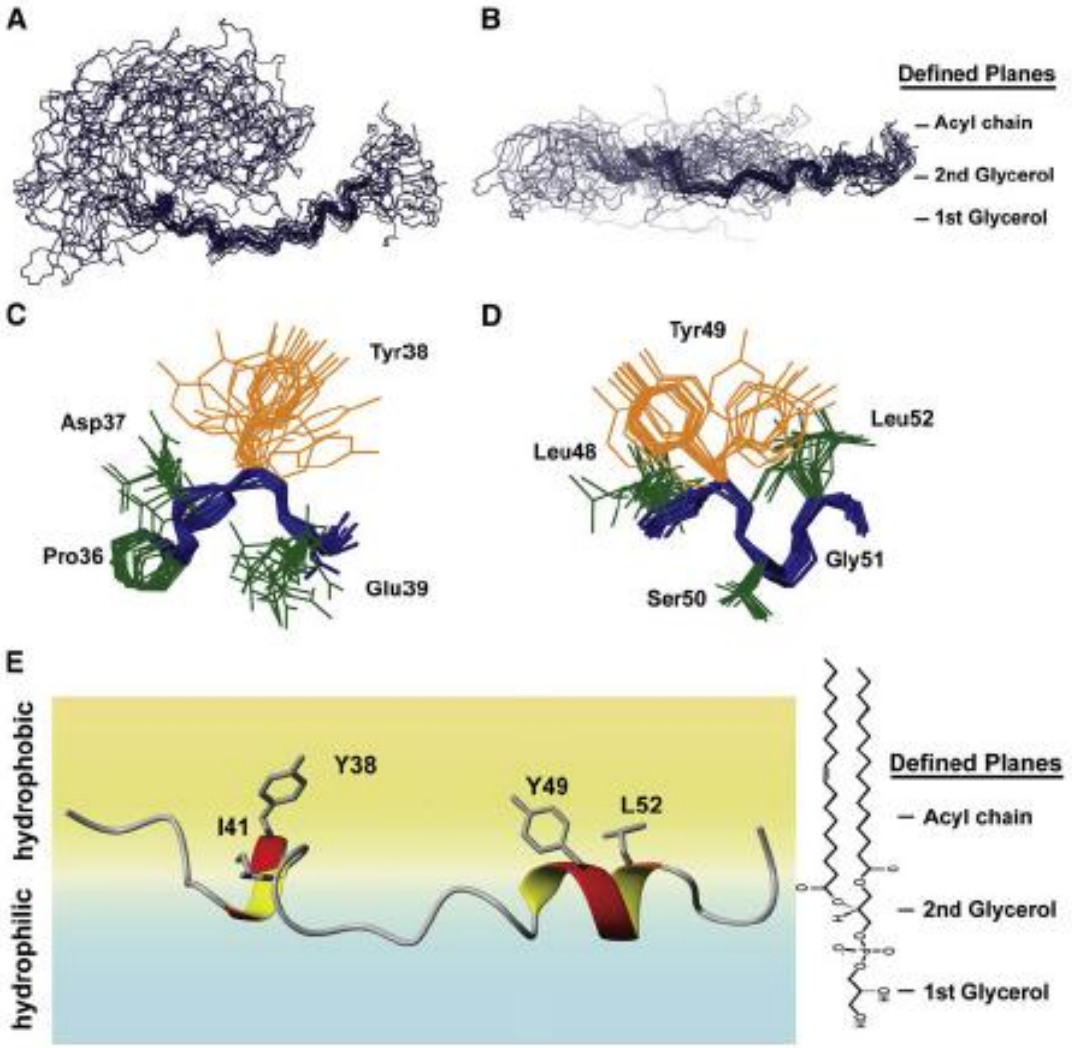
Kuhns and Davis, 2008

CD3 tails associate with the plasma membrane

NMR structure in the presence of lipid bicelles.

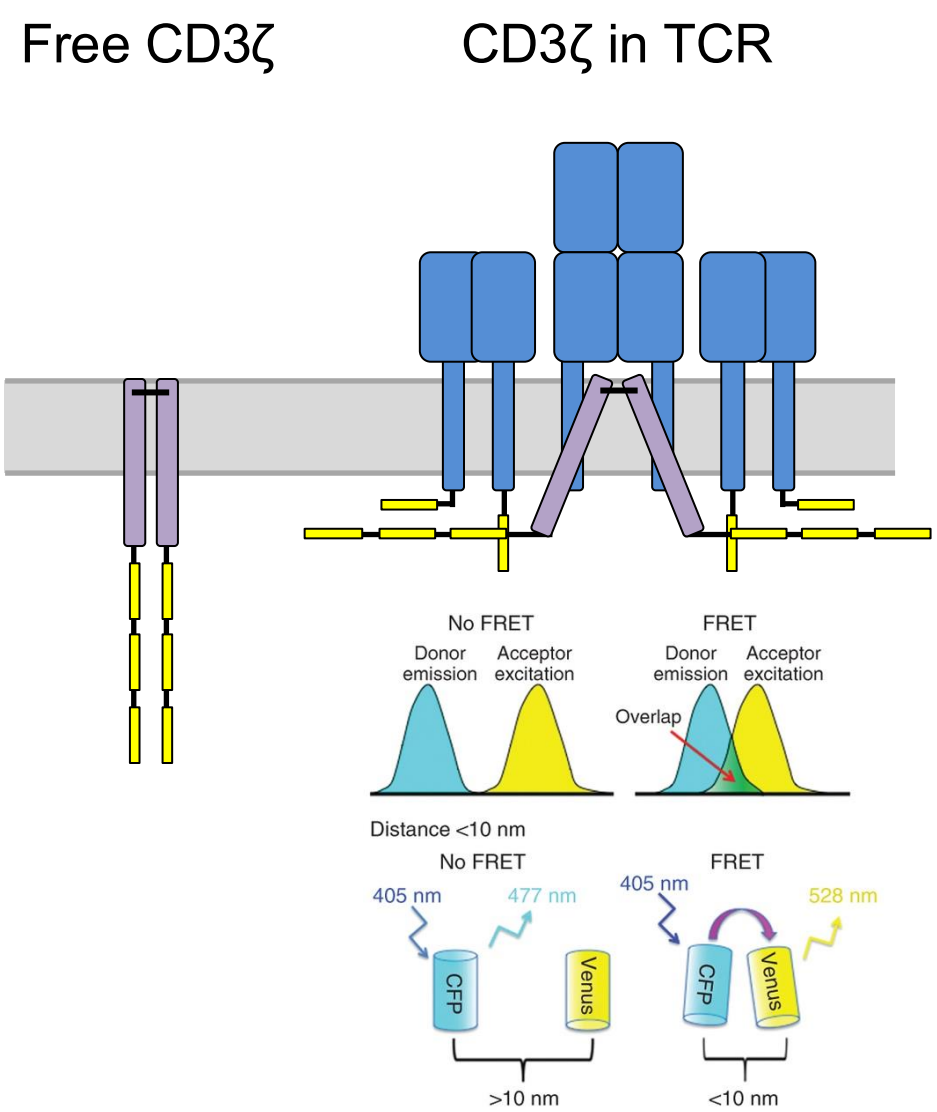
Tyrosines are buried in membrane.

Both CD3ε and CD3ζ do this.

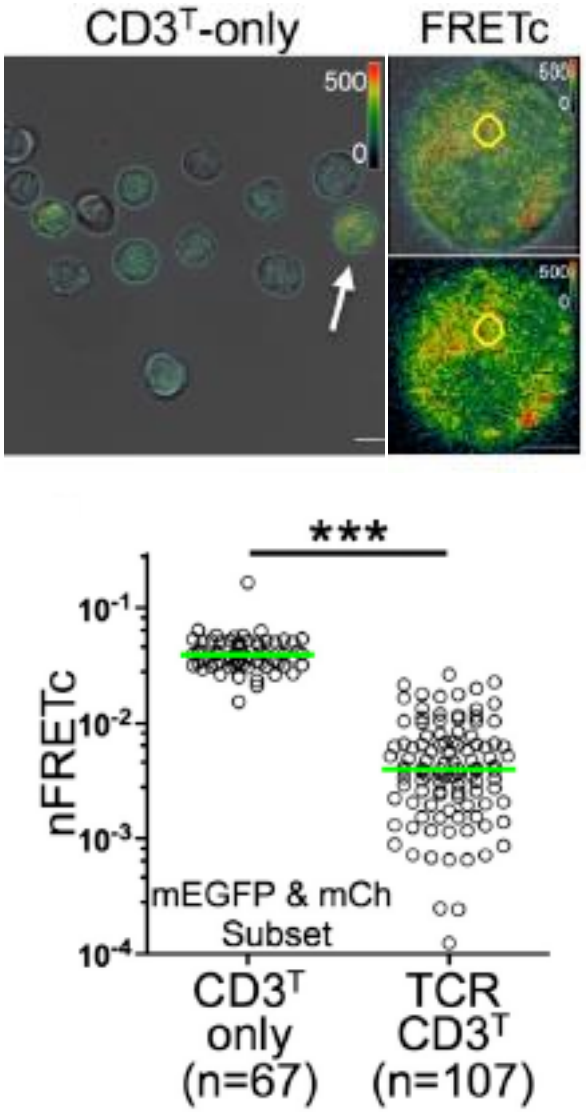


Xu et al., 2008
Aivazian and Stern, 2000

FRET-based detection of conformational change

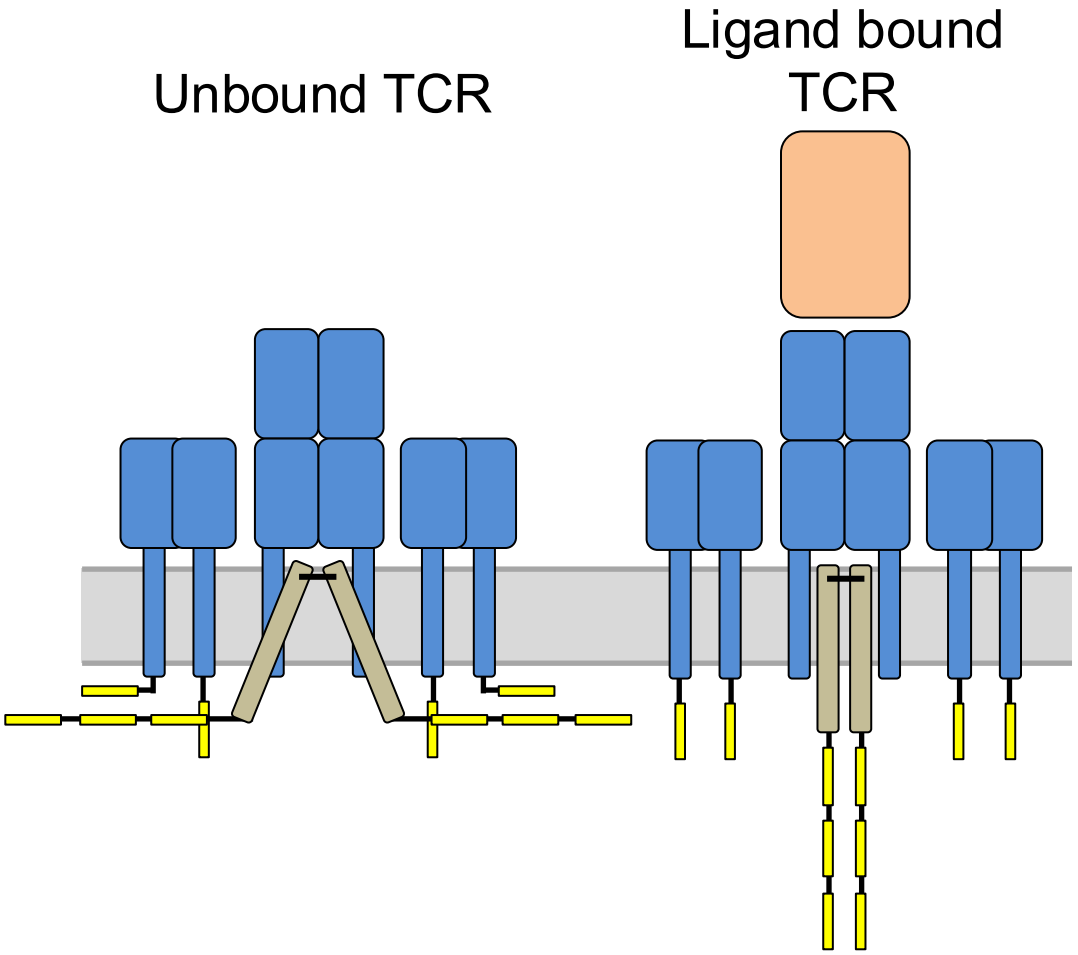


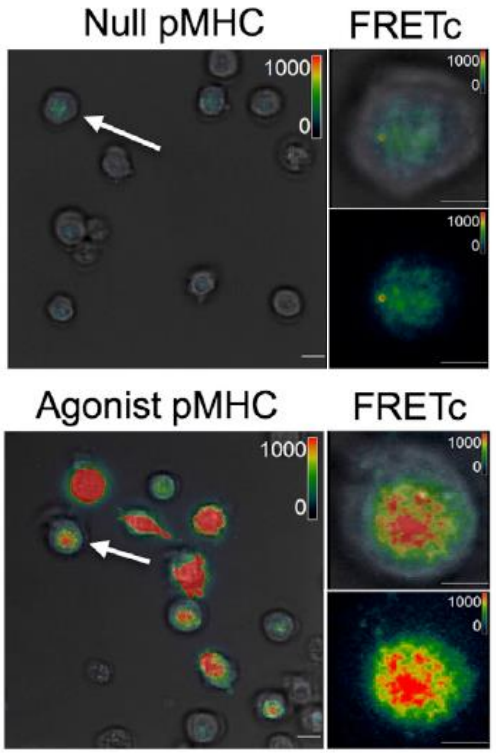
Broussard et al., 2013

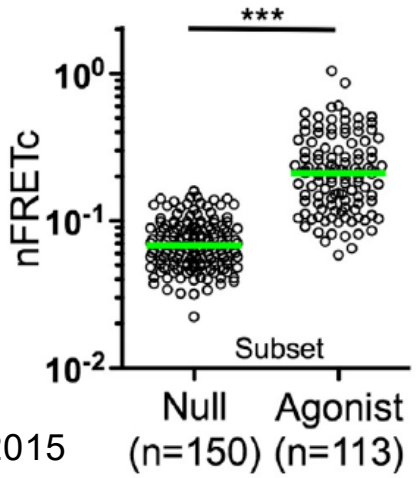


Lee et al., 2015

FRET-based detection of conformational change



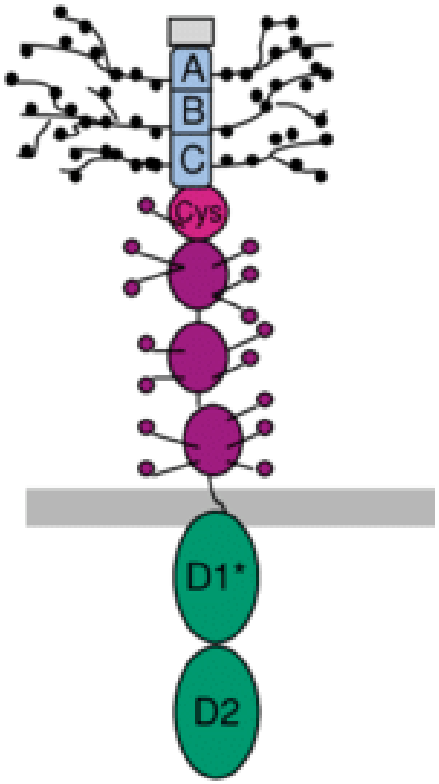


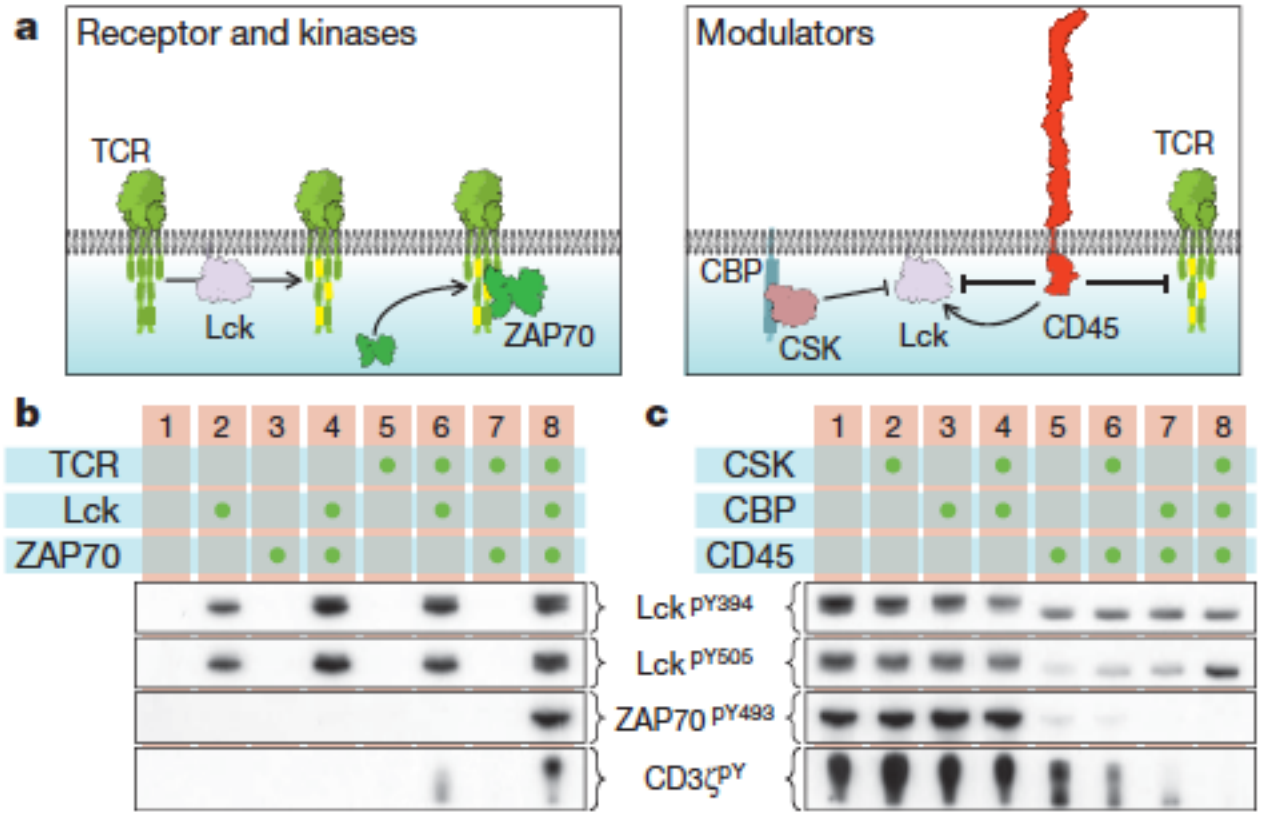


Lee et al., 2015

CD45 is necessary for restraining TCR signaling

CD45 -Tyrosine phosphatase with large extracellular domain



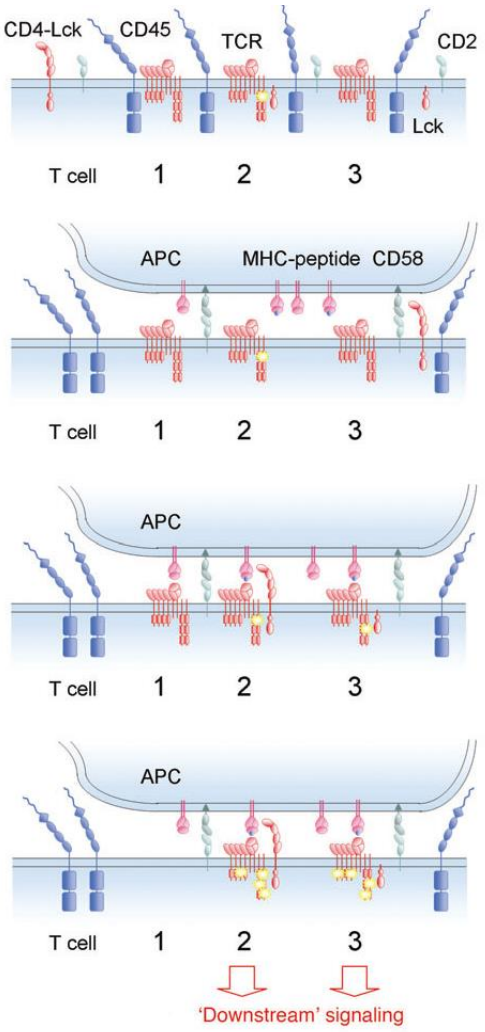


James et al., 2012

Reconstitution of TCR signaling in HEK-293 cells.

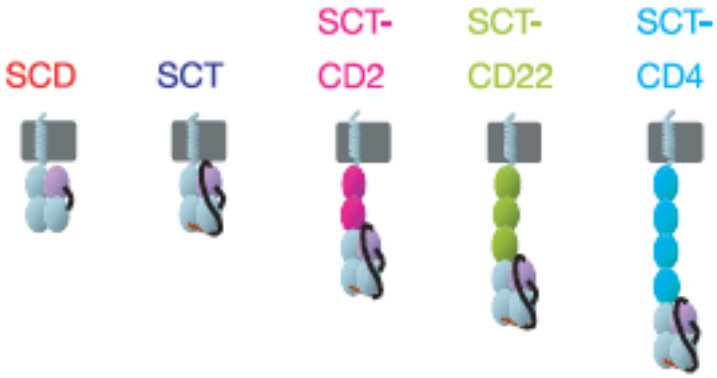
The kinetic segregation model

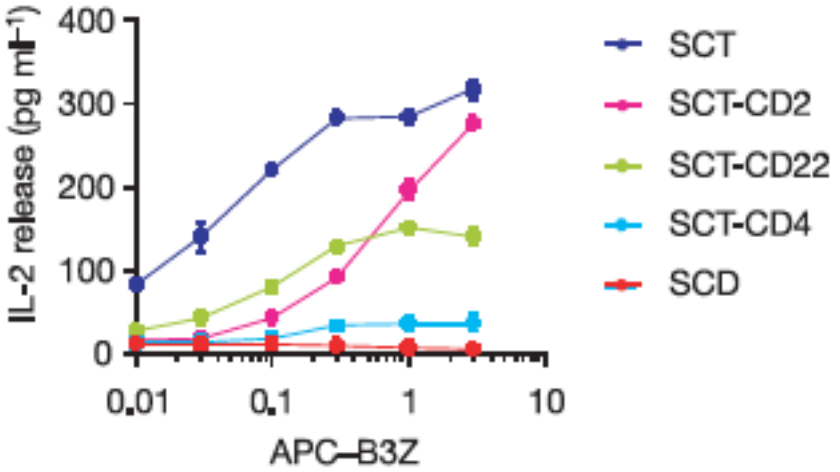
Molecules segregate on the basis of size. Inhibitory phosphatases (like CD45) move away from activating receptors.

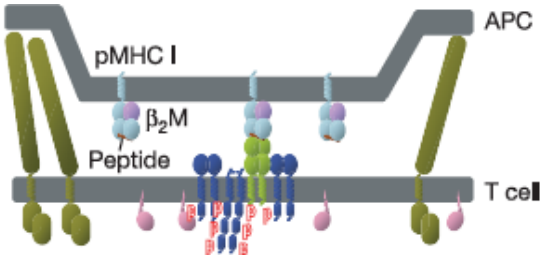


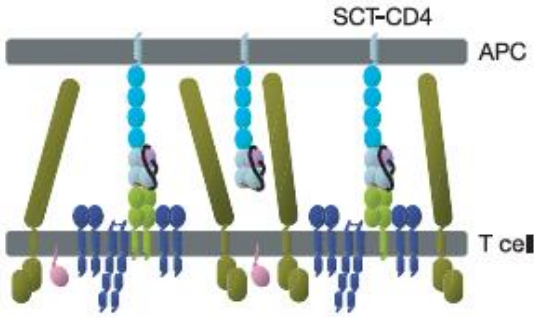
Davis and van der Merwe, 2006

Increasing pMHC size inhibits TCR signaling



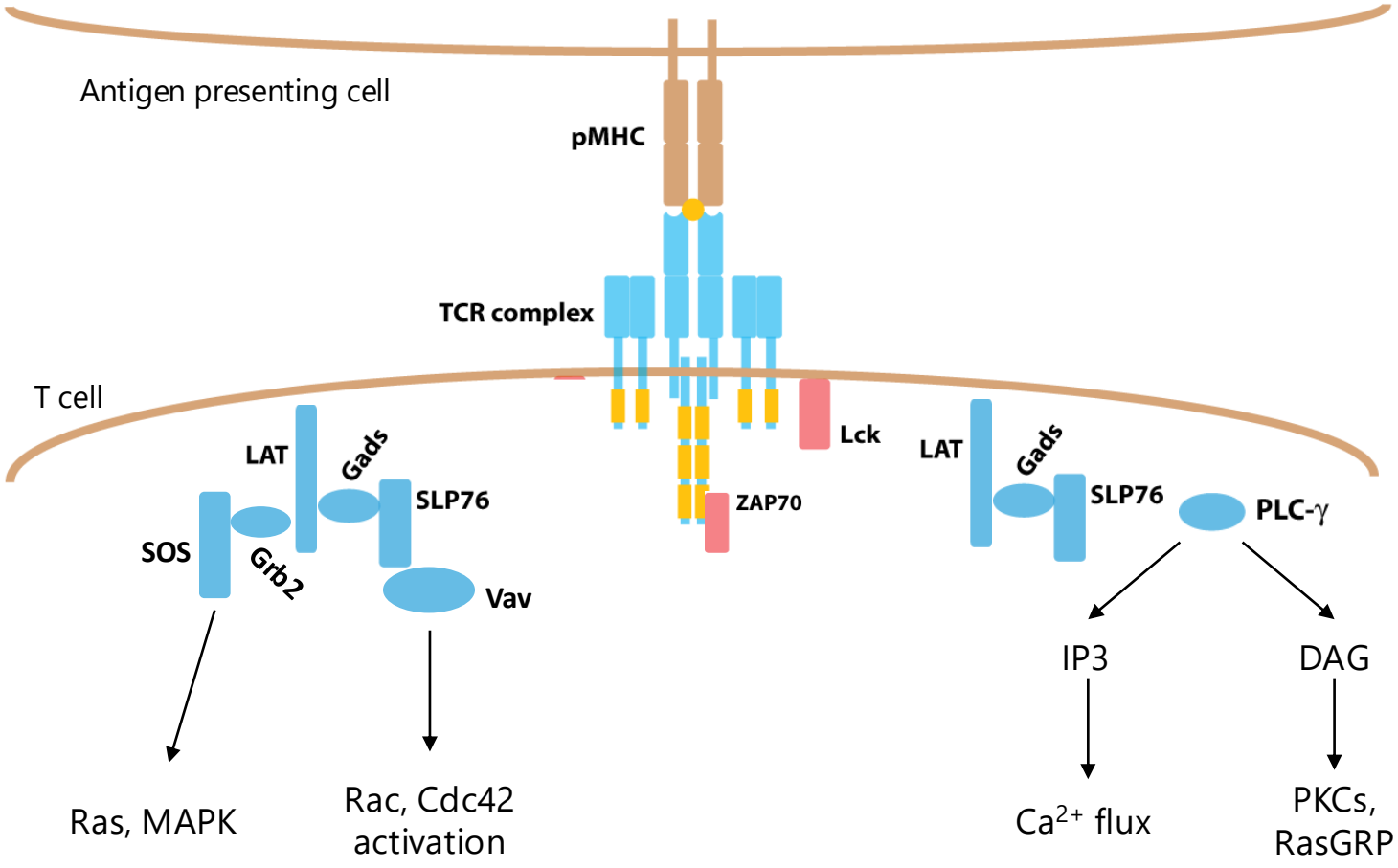






Choudhuri et al., 2005

Early TCR signaling has many steps



How is pathway structure related to output?

1) Amplification

2) Regulation

3) Crosstalk

4) Discrimination

TCR signaling is highly discriminatory

	Proliferation	Cytokine Secretion	Killing
Agonist	+++	+++	+++
Partial Agonist	-	++	+++
Null	-	-	-

			K_D	k_{off}	Half-time
42.12	OVA/K^b	Strong agonist	6.5	0.02	24.5
42.12	OVA E1/K^b	Weak agonist	22.6	0.068	7.3
42.12	V-OVA/K^b	Antagonist	29.8	0.039	12.9
42.12	OVA R4/K^b	Antagonist	57.1	0.146	3.4
42.12	OVA K4/K^b	Null	>360	>0.2	<2.5

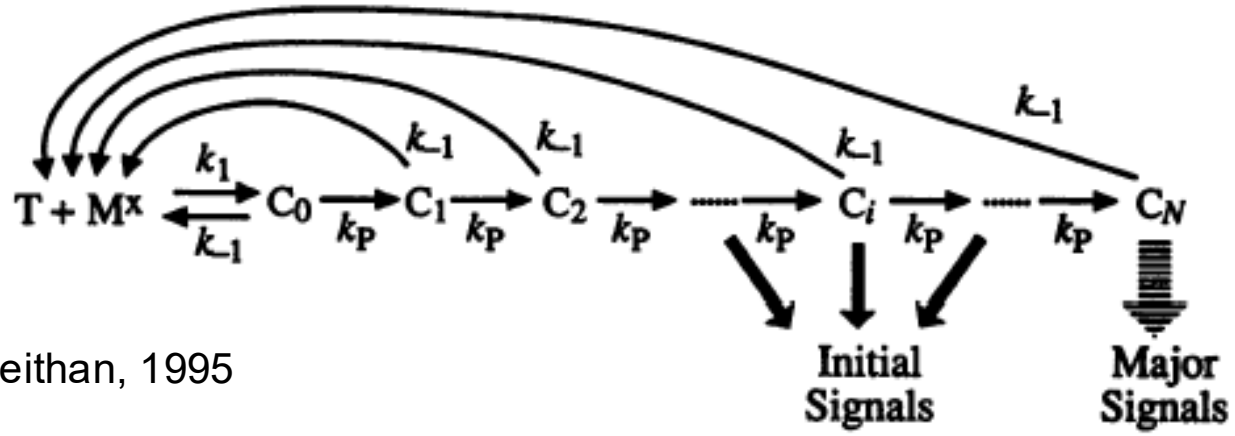
Davis et al., 1998

A relationship between affinity and/or lifetime and potency.

The kinetic proofreading model

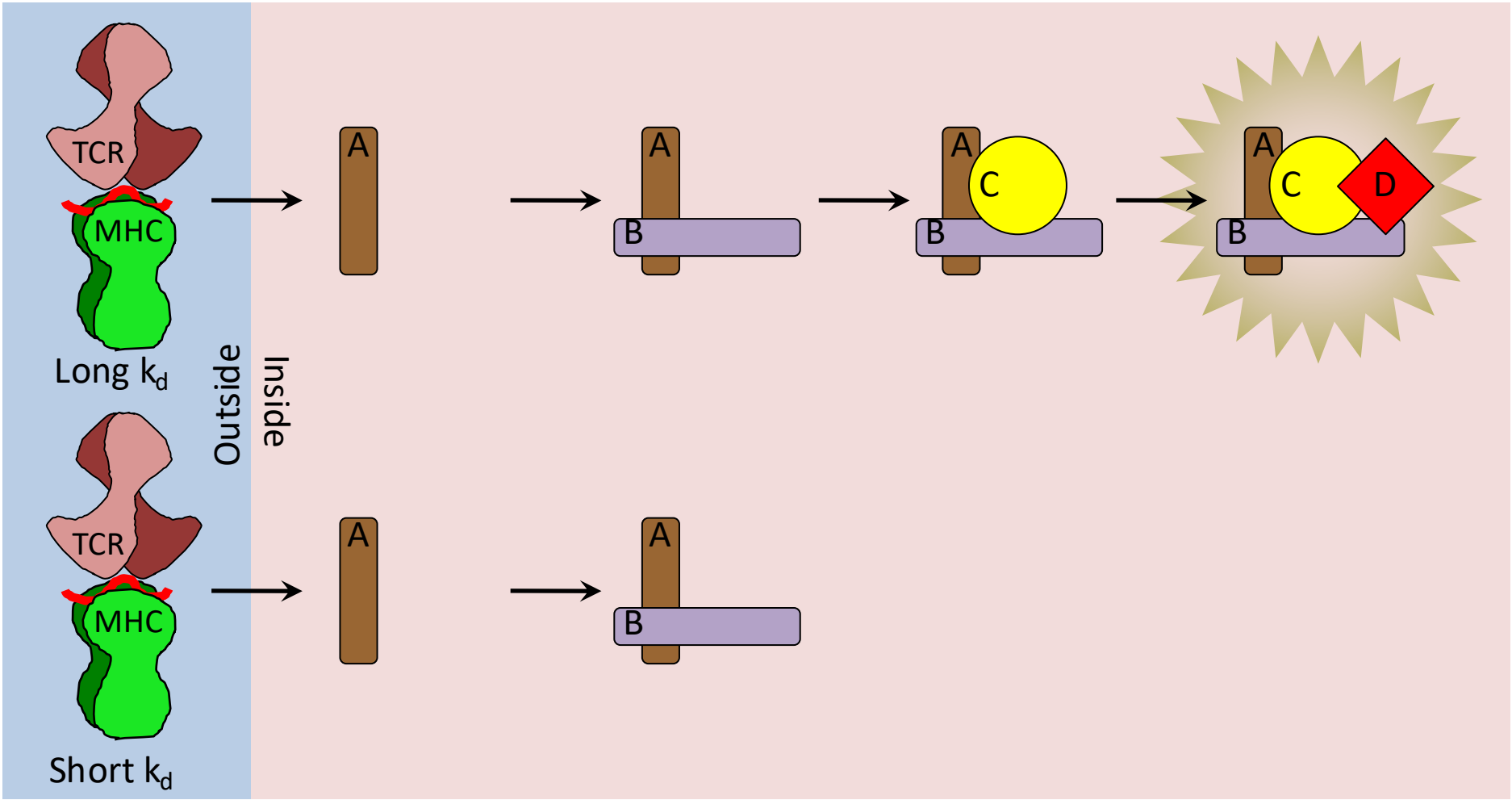
A way to link lifetime of the pMHC-TCR interaction with downstream responses.

Also fits with the multicomponent nature of the TCR signaling cascade.



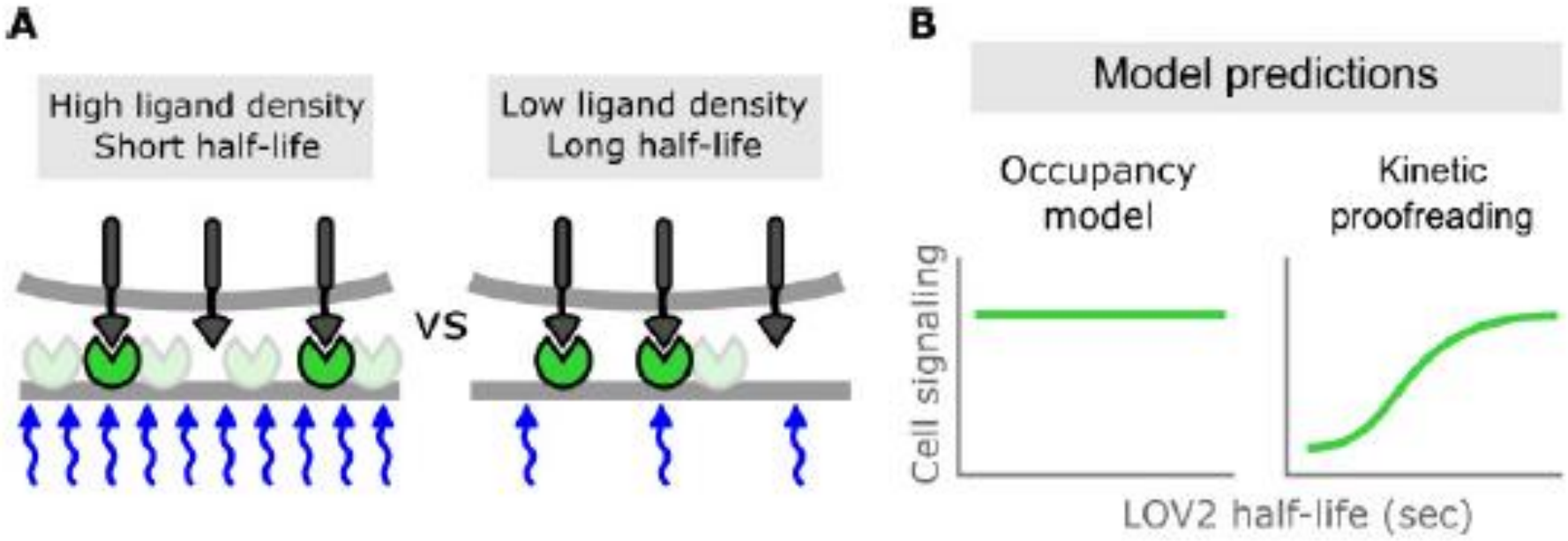
McKeithan, 1995

The kinetic proofreading model



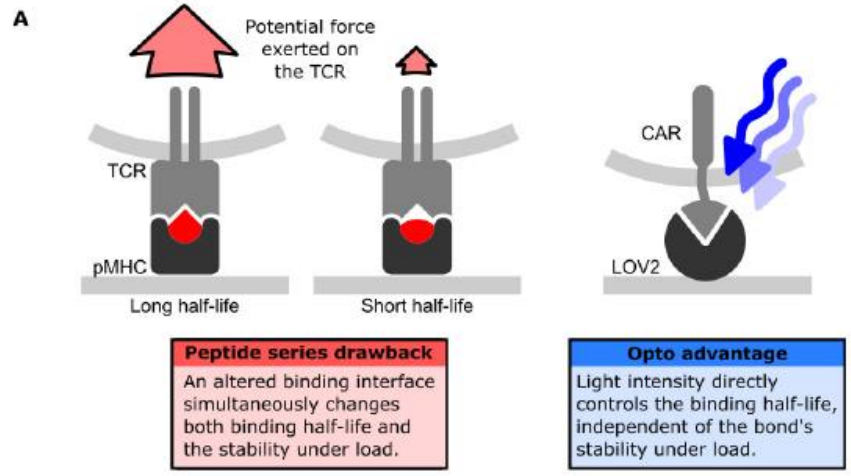
Prediction: Magnitude of activation depends on dwell-time, not occupancy

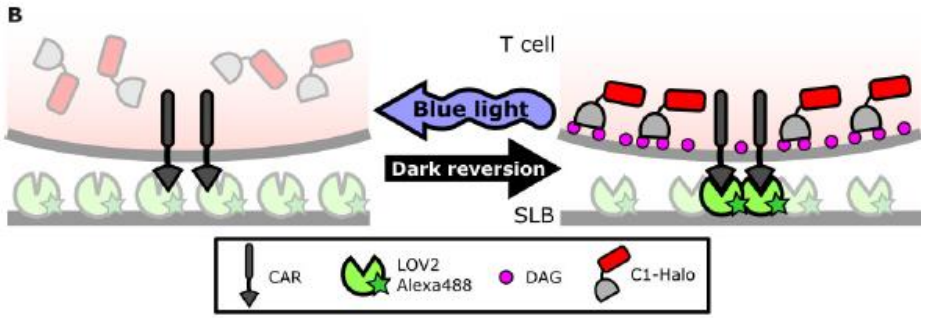
Dwell-time versus occupancy

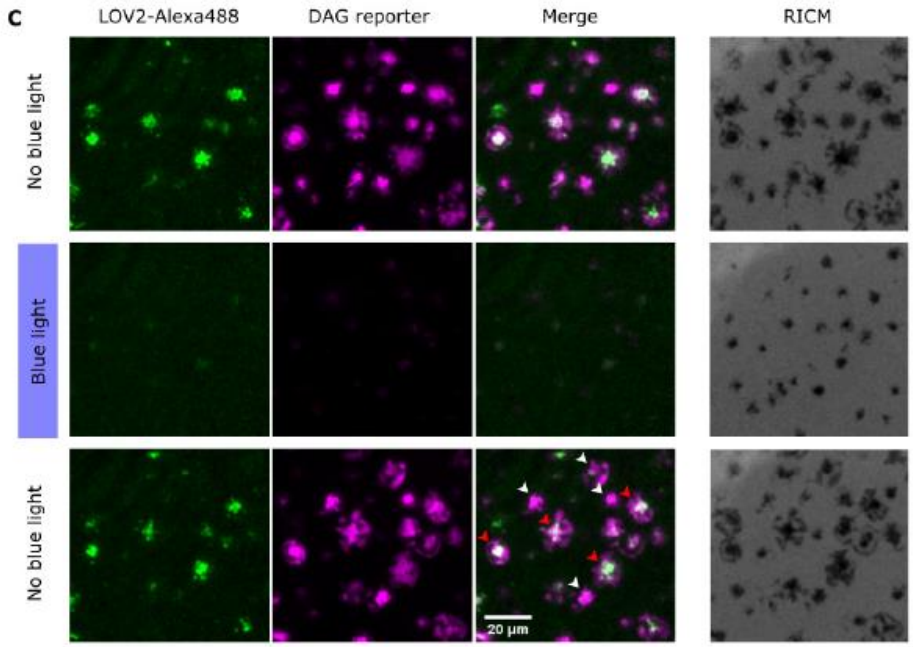


Tischer and Weiner, 2019

Optogenetic assessment of kinetic proofreading

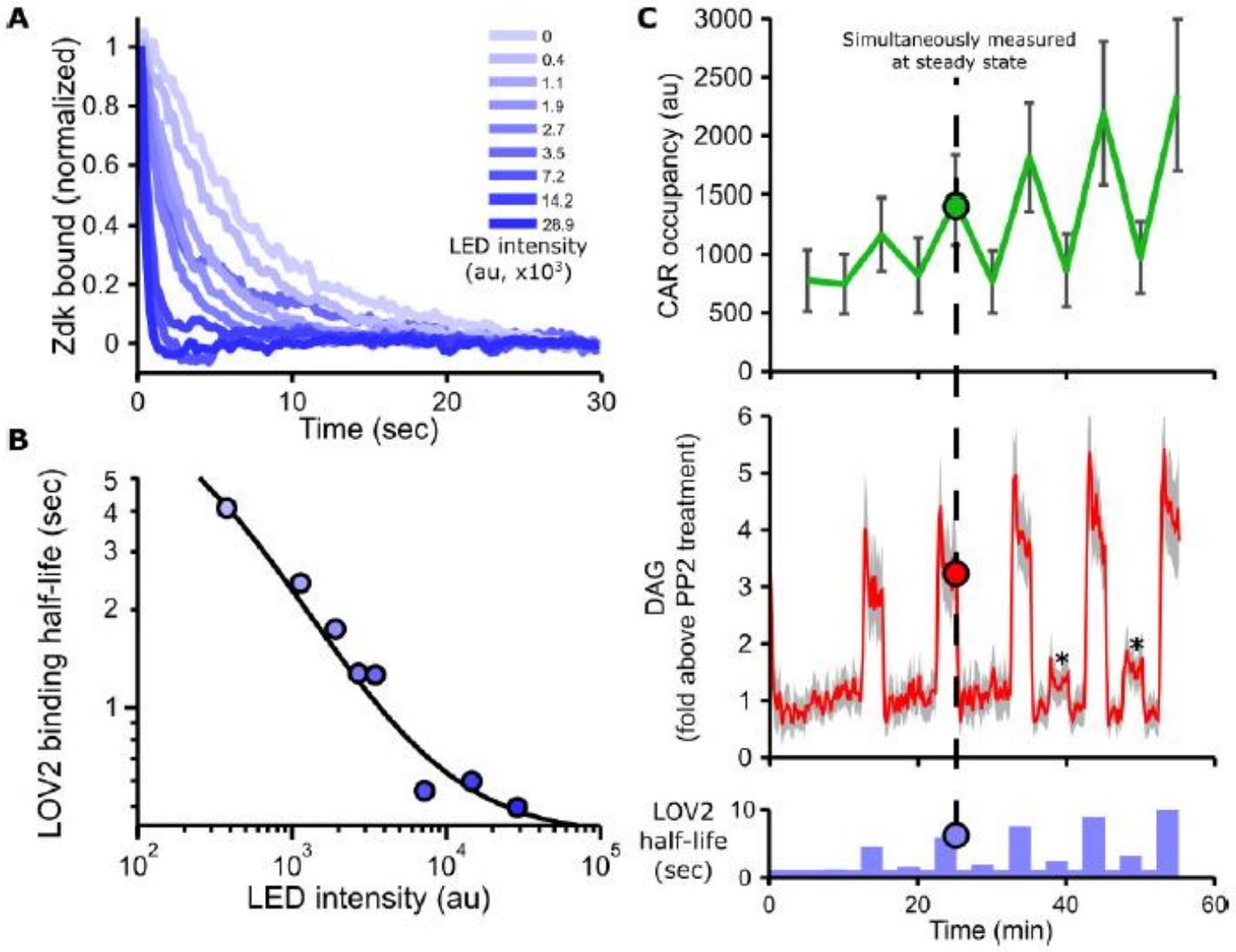






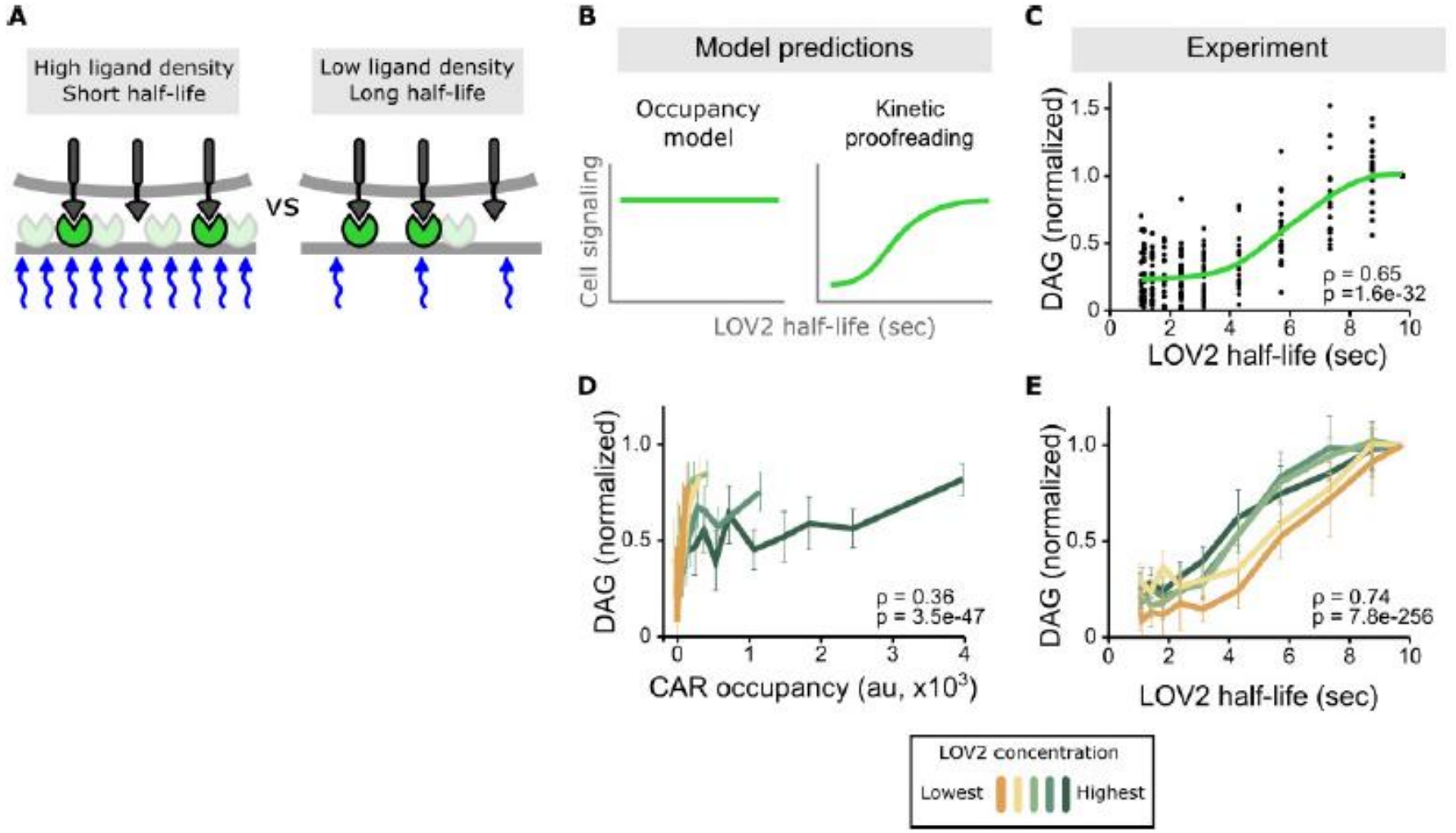
Tischer and Weiner, 2019

Optogenetic assessment of kinetic proofreading



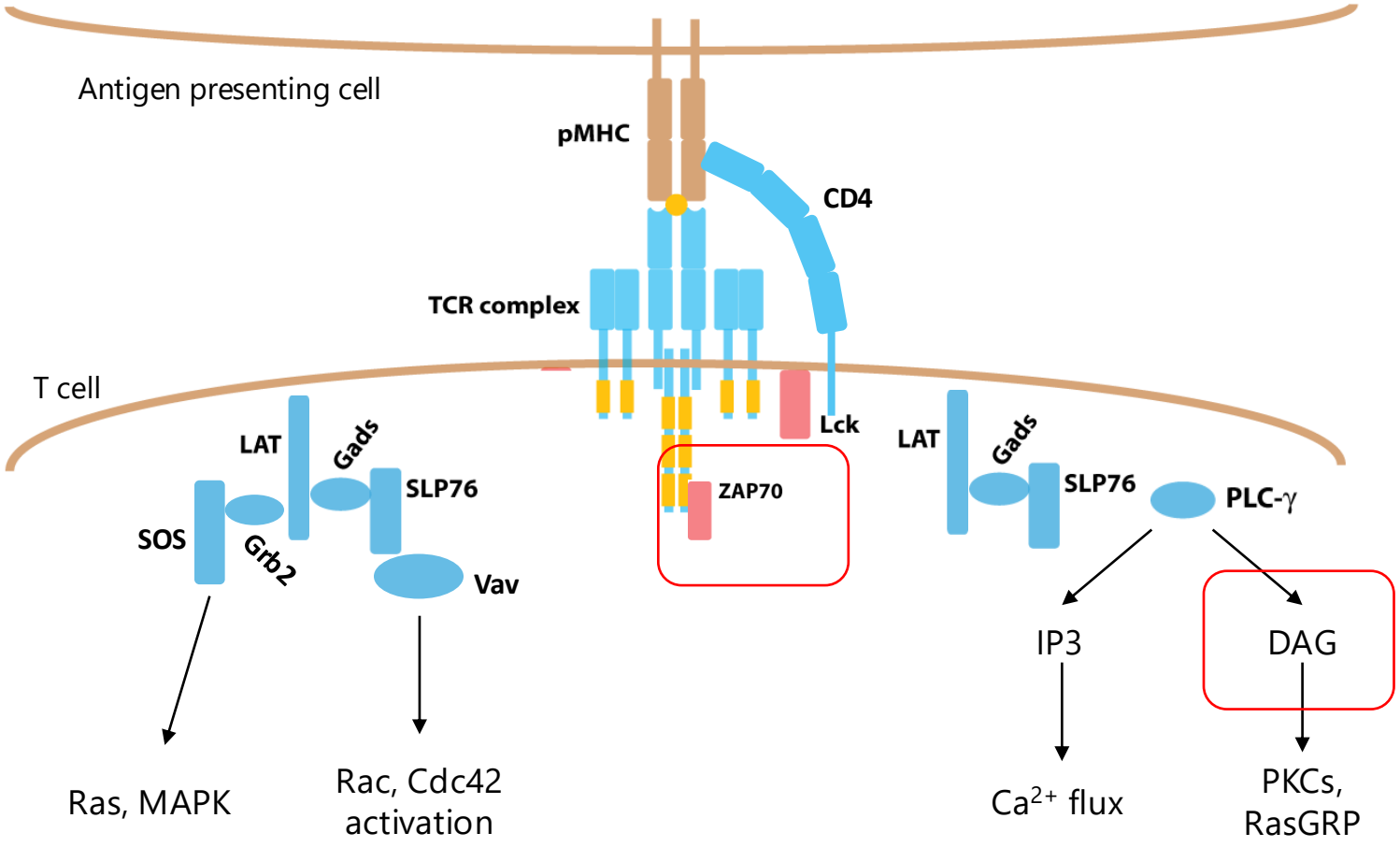
Tischer and Weiner, 2019

Optogenetic assessment of kinetic proofreading

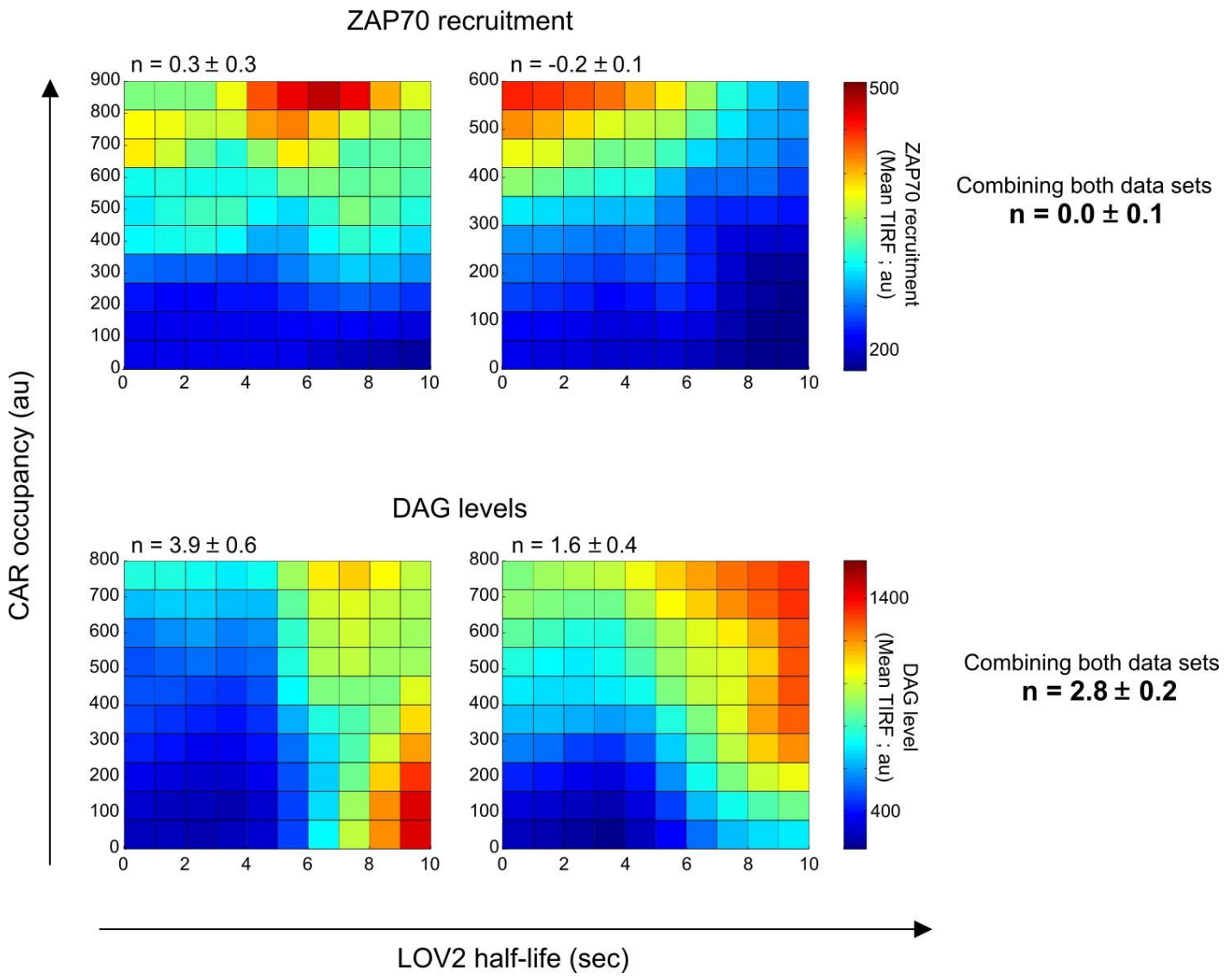


Tischer and Weiner, 2019

Early TCR signaling



What is the proofreading step?



Tischer and Weiner, 2019

Lecture topics

1) How does ligand binding induce TCR activation?

- Phosphatase exclusion
- TCR conformational change
- The kinetic proofreading model

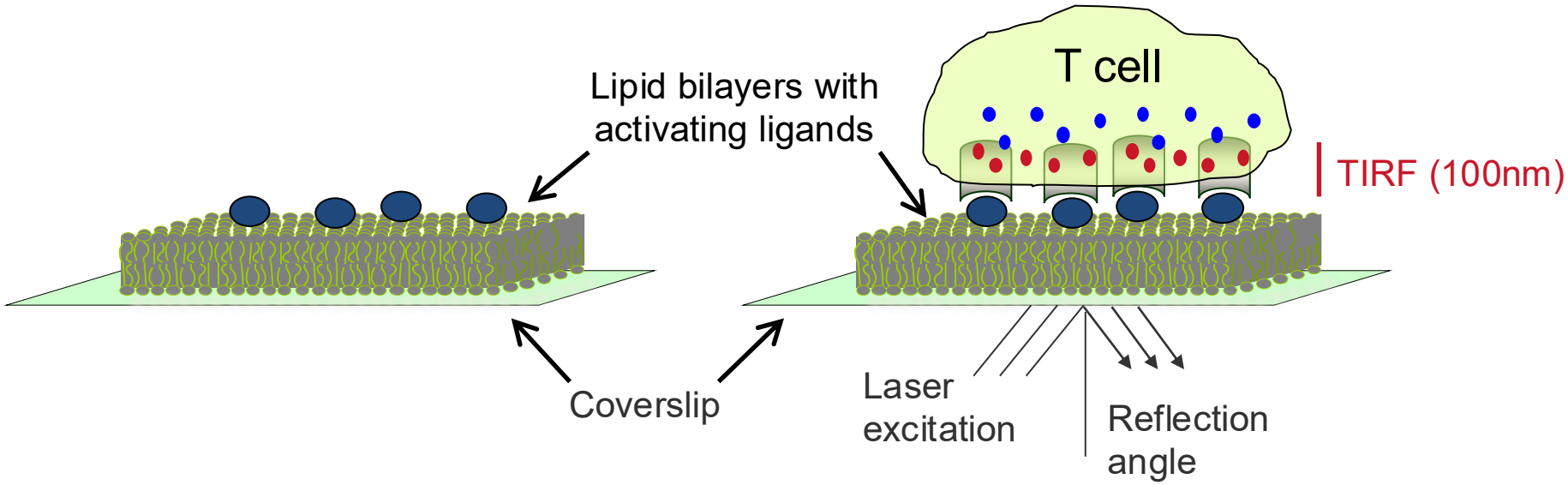
2) How does receptor activation induce downstream signaling?

- Liquid-ordered signaling microclusters
- Lipid second messengers
- Calcium signaling

3) How does interfacial architecture influence T cell activation and effector responses?

- Microvilli and ligand search
- Actin dynamics and mechanotransduction
- Directional secretion

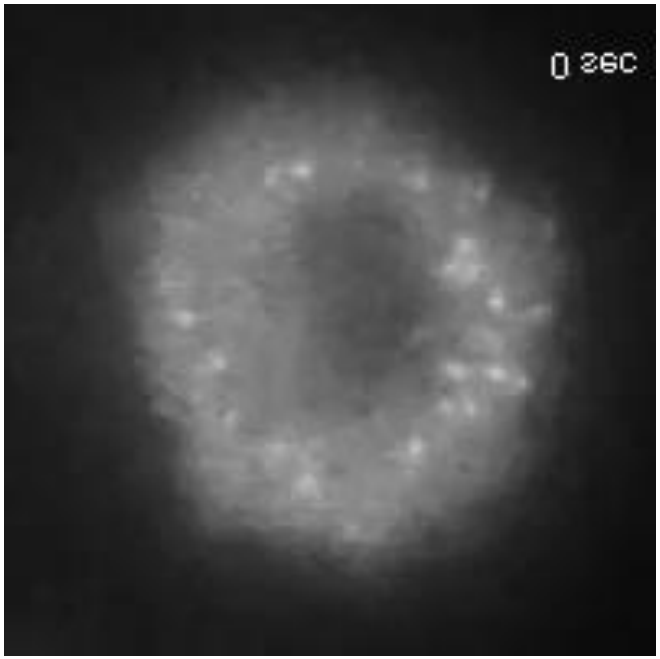
Synapse imaging by TIRF microscopy



TCR proximal signaling proteins form microclusters

T cell landing on stimulatory bilayer

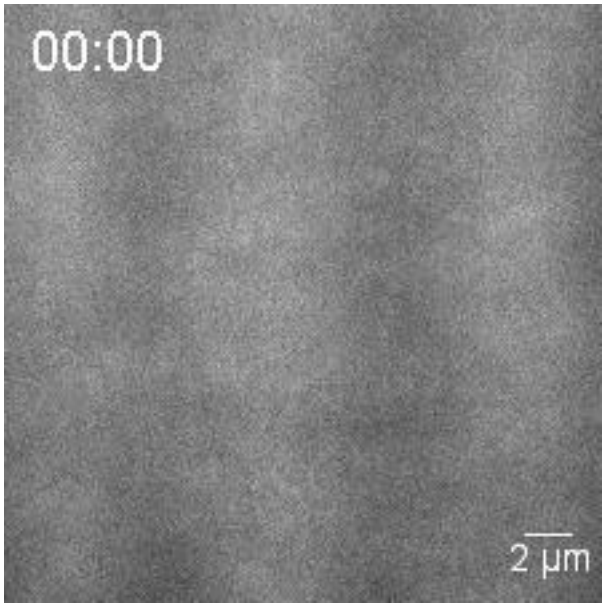
Slp76 clusters



Yokosuka et al., 2005

In vitro: pLat on bilayer
Add Grb2 and Sos (binding partners)
Then add phosphatase.

Lat clusters

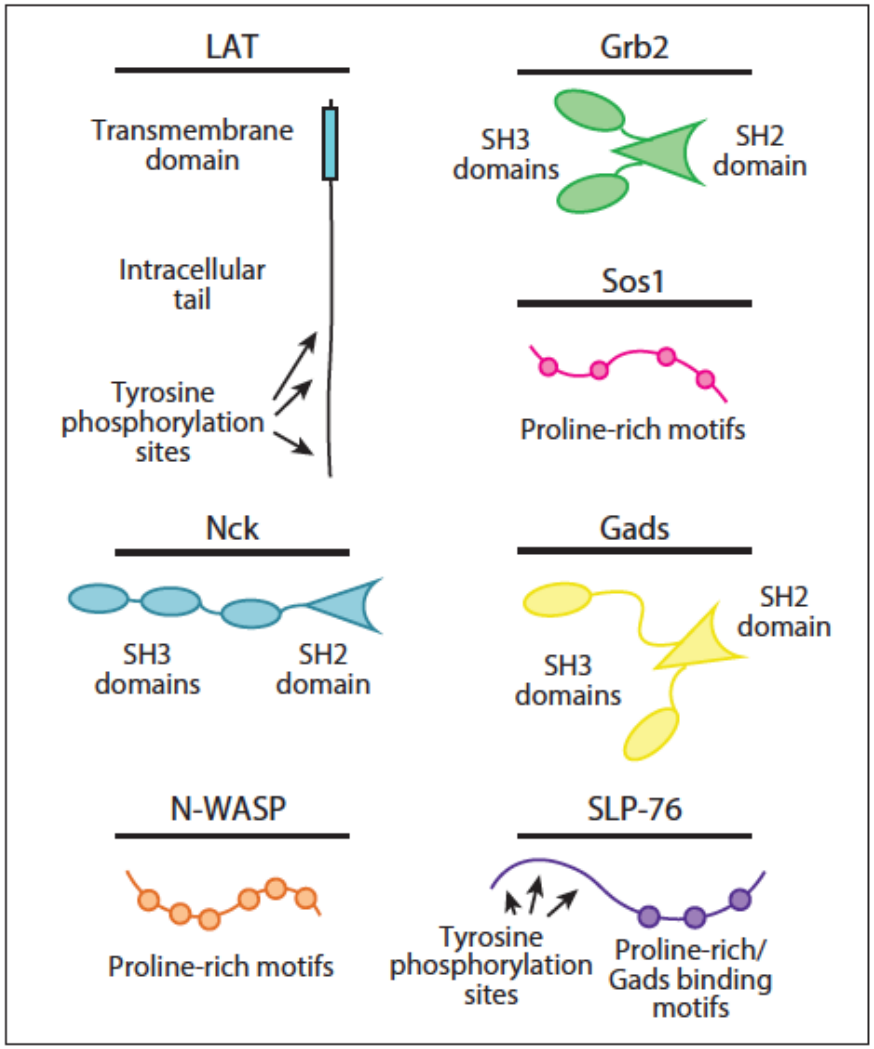


Su et al., 2016

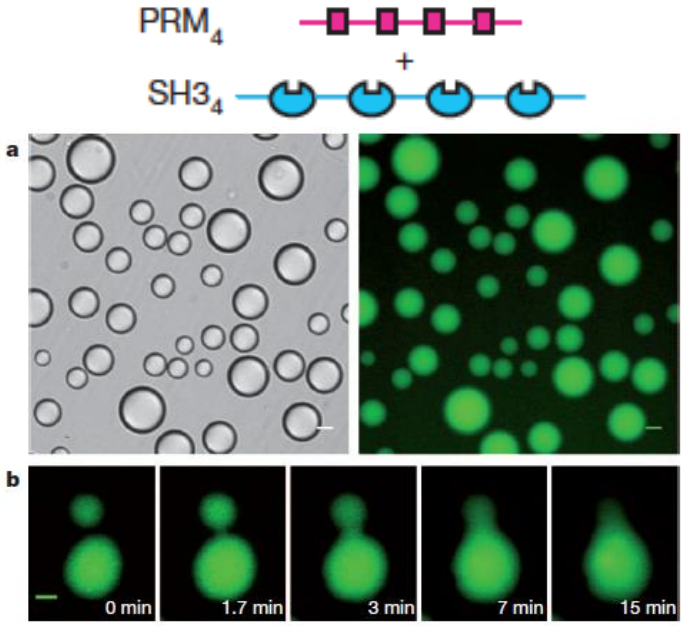
Clustering behavior a property of the constituent molecules.

Microclusters are phase separated droplets

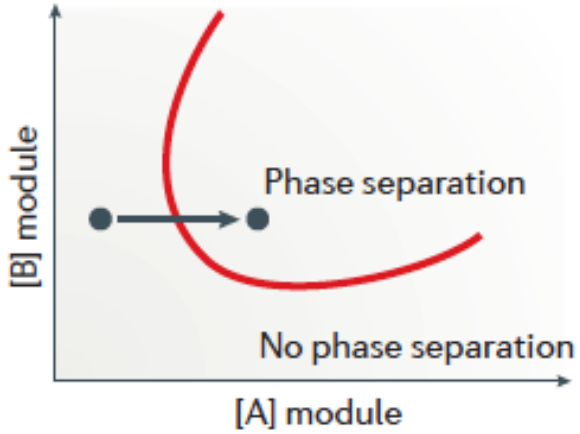
TCR signaling proteins



Case et al., 2019

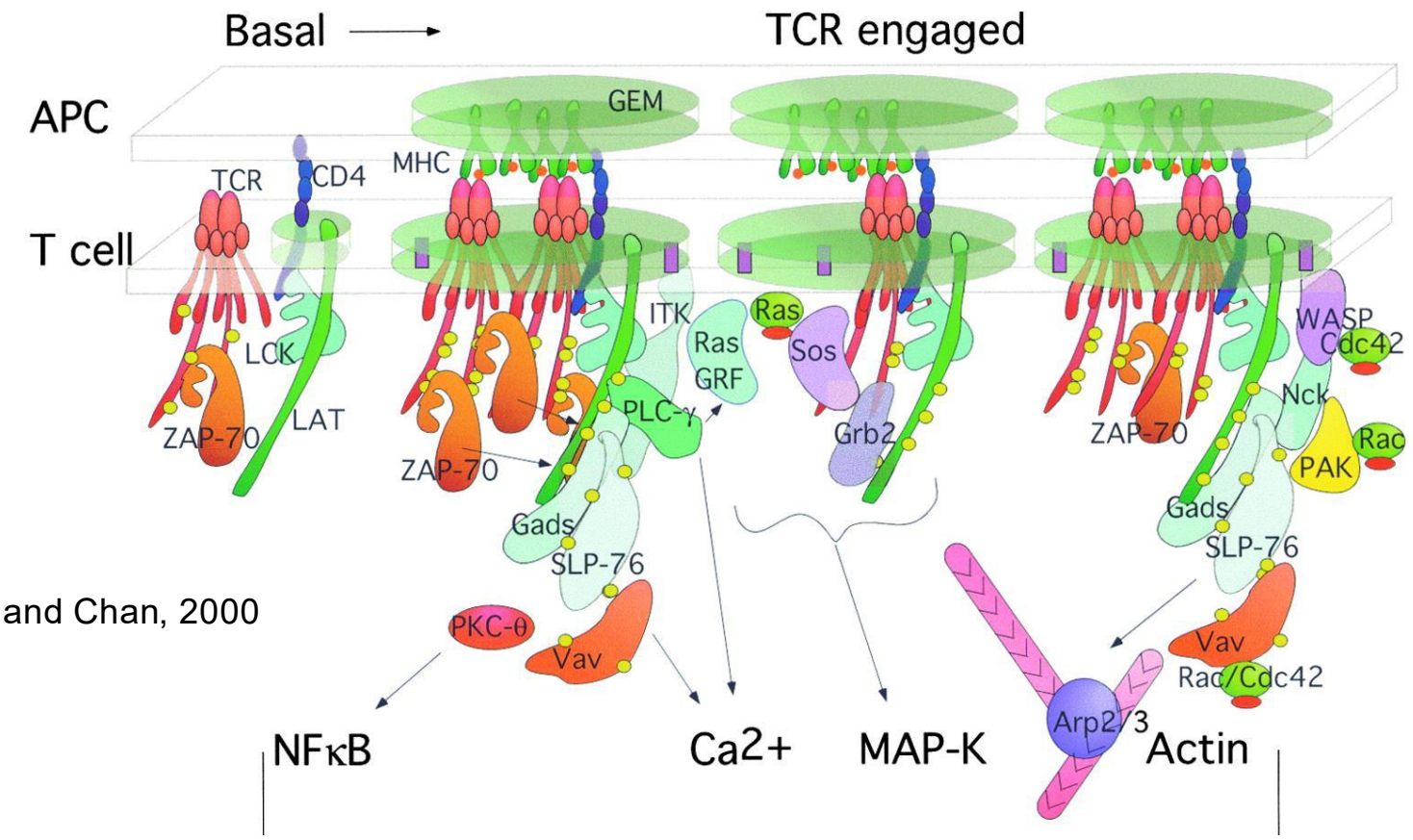


Li et al., 2012



Banani et al., 2017

Microcluster architecture



Dustin and Chan, 2000

Cooperative assemblies nucleated by protein-protein interactions help receptor modules overcome background phosphatase activity.

Structurally heterogeneous, composed of molecules with multiple protein-protein interaction domains.

Advantages of phase separation

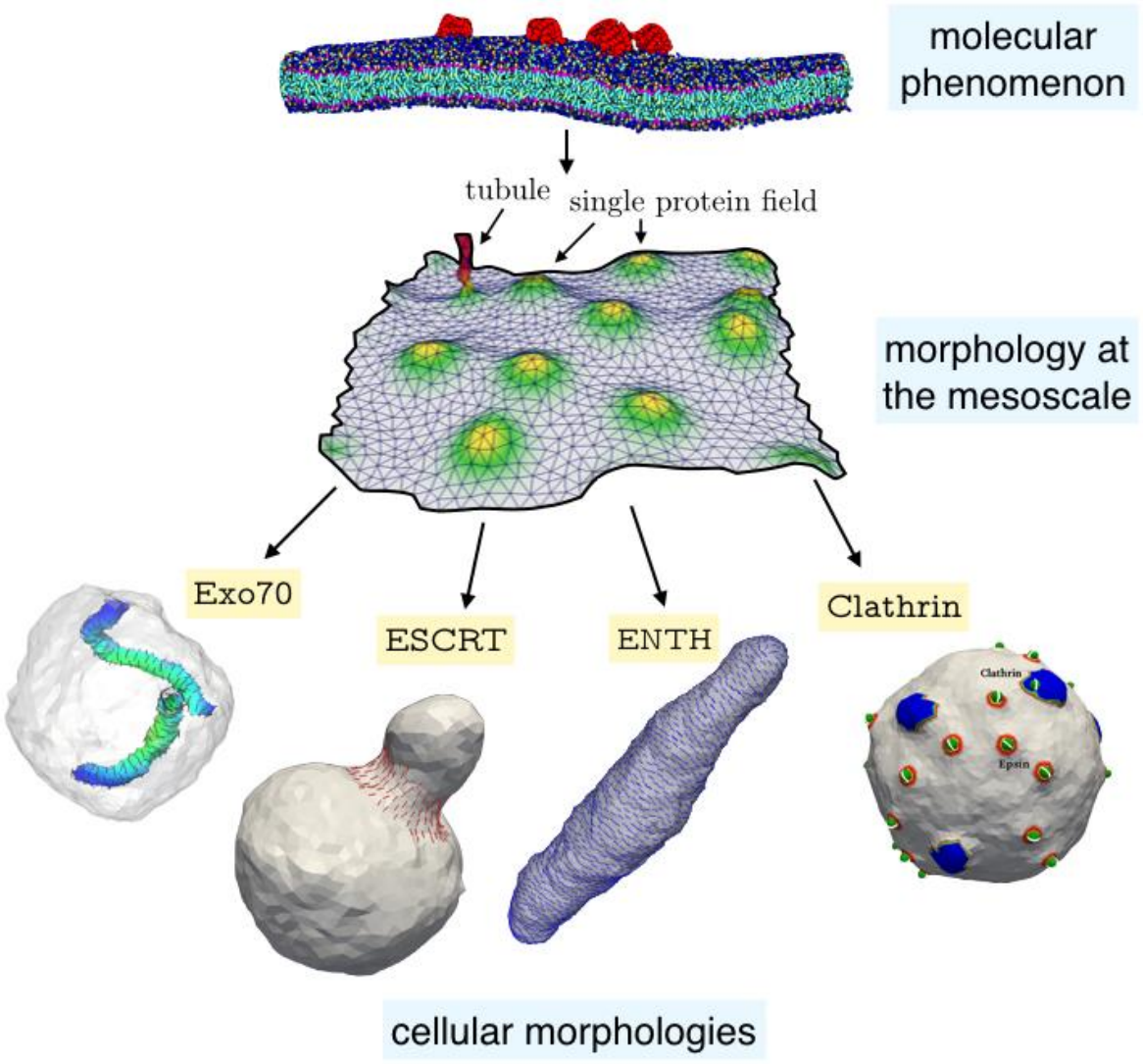
1) Increased enzyme and substrate concentration in an environment that promotes mixing.

2) Promotes selectivity by excluding unrelated proteins.

3) Architectural flexibility accommodates heterogeneity.

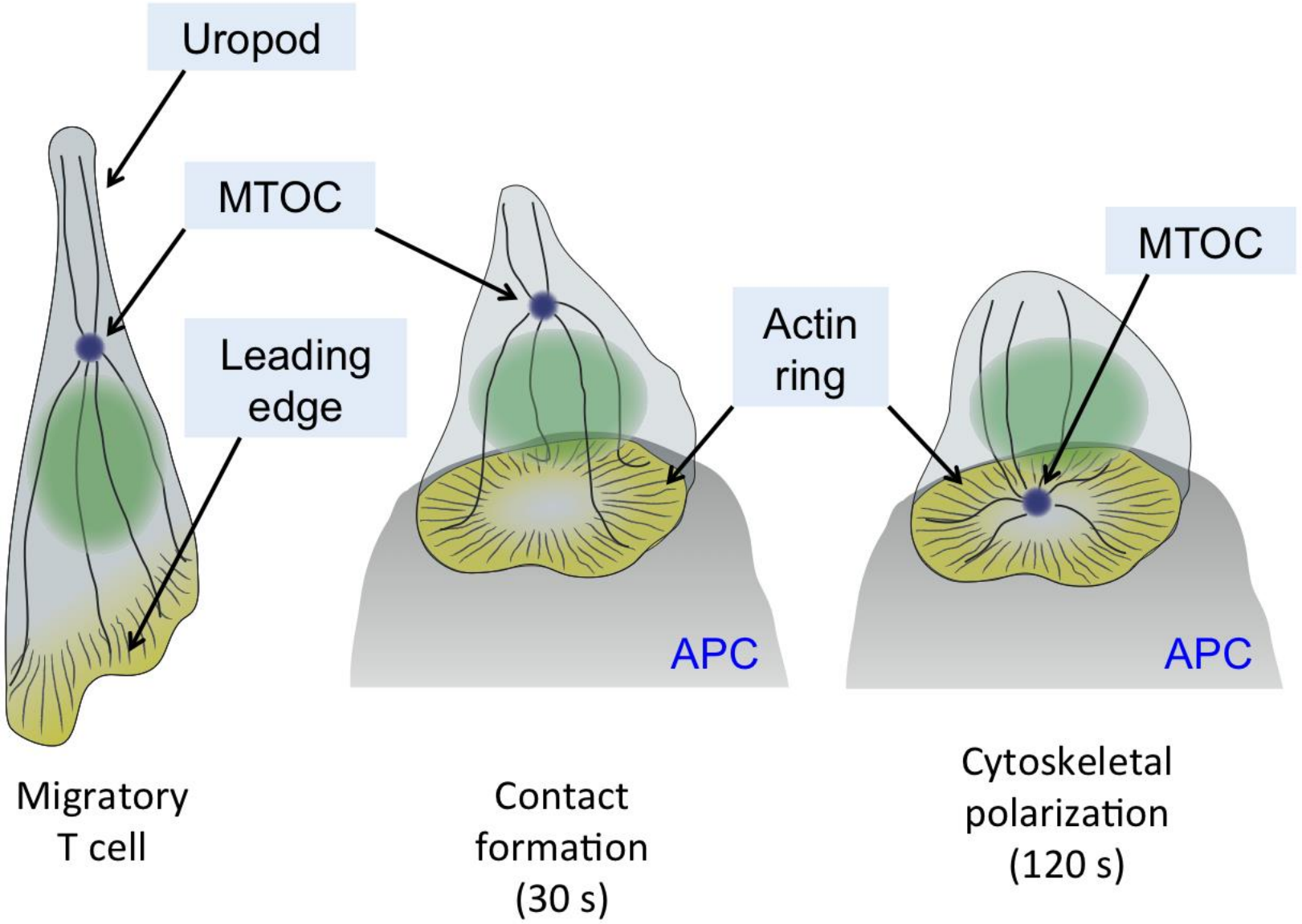
4) Sharp phase transitions (change in stoichiometry or state of one component) makes process fast and regulatable.

Converting signals from molecular to cellular scale



Ramakrishnan Natesan

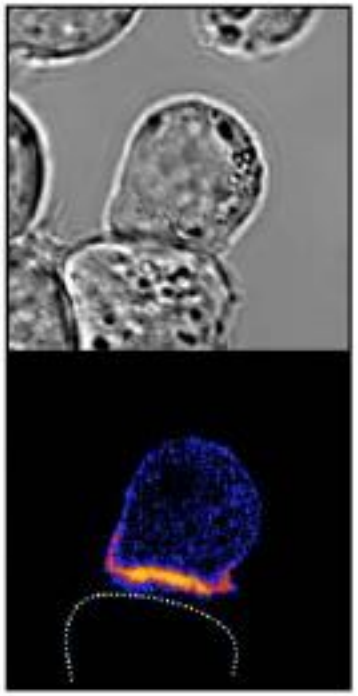
TCR activation induces morphological change



Lipid second messenger gradients within T cells

DIC

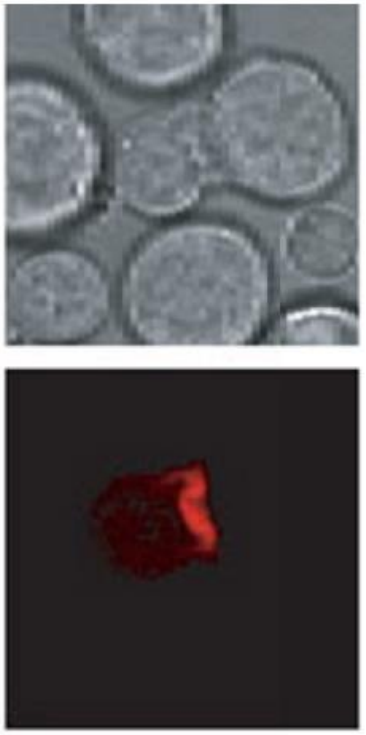
PKD-C1-GFP



Spitaler et al., 2006

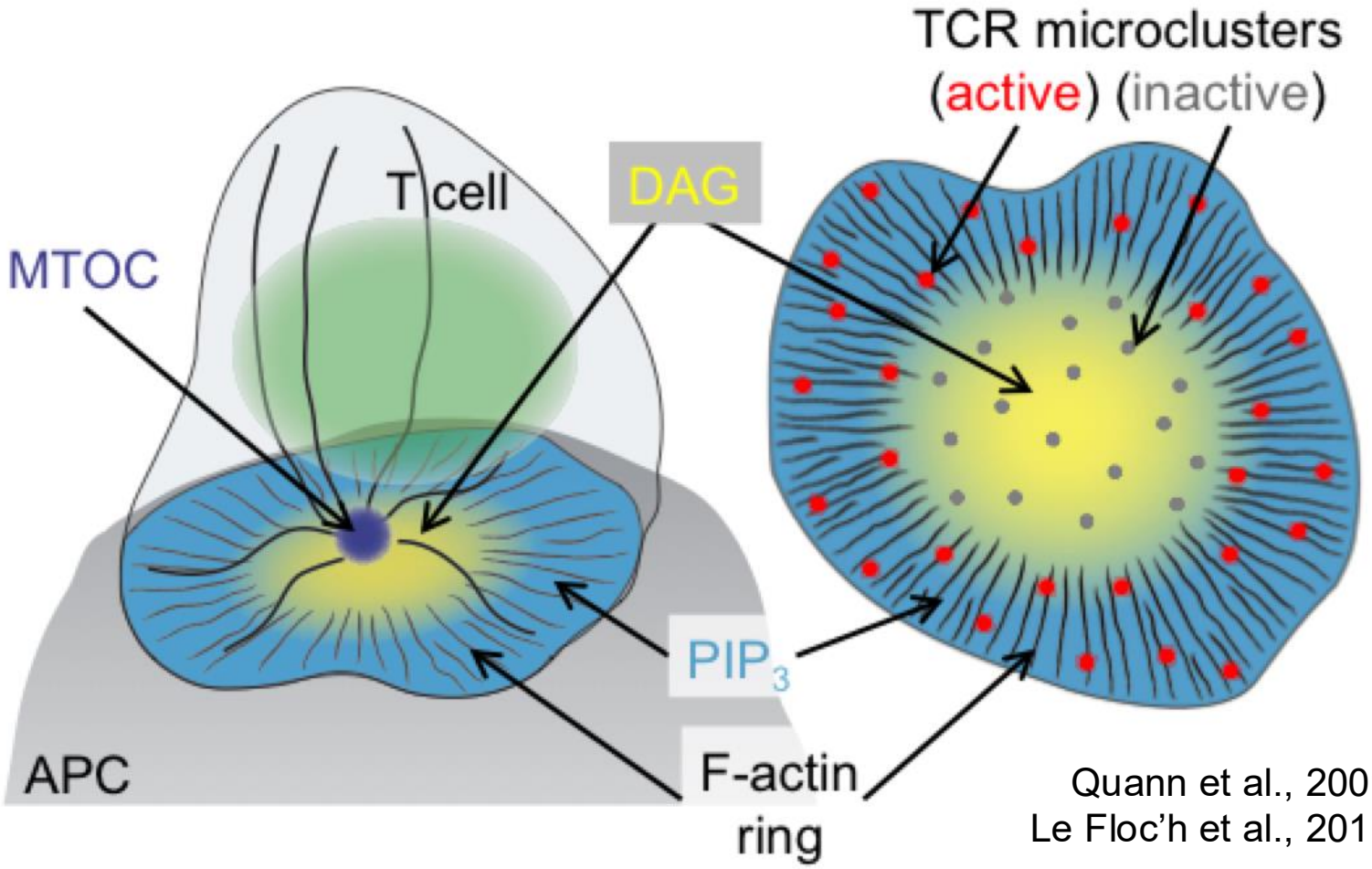
DIC

AKT-YFP



Huppa et al., 2003

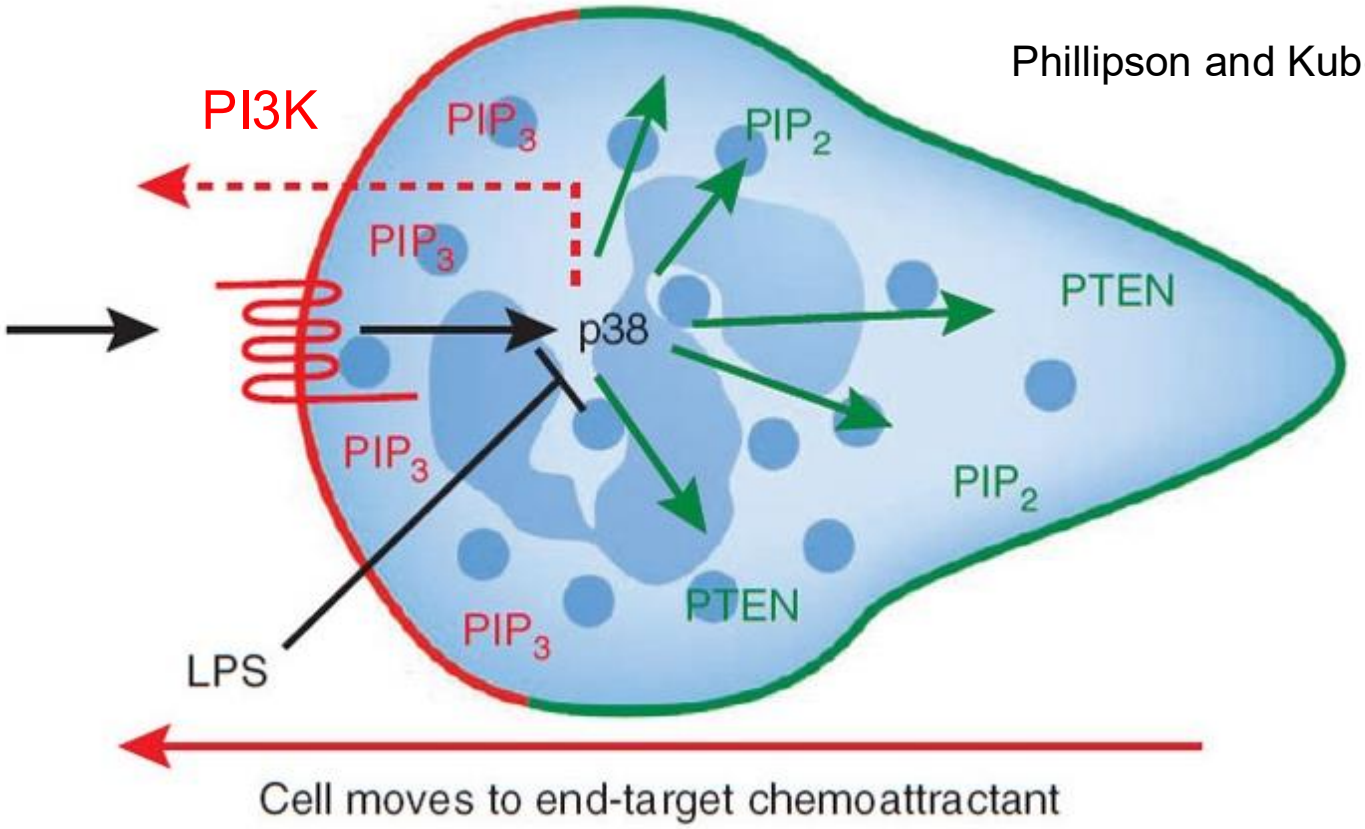
Patterning by lipid second messengers



Quann et al., 2009
Le Floc'h et al., 2013

Lipids gradients spread signals from protein complexes to the local cellular neighborhood.

Maintenance of lipid gradients



Phillipson and Kubes, 2011

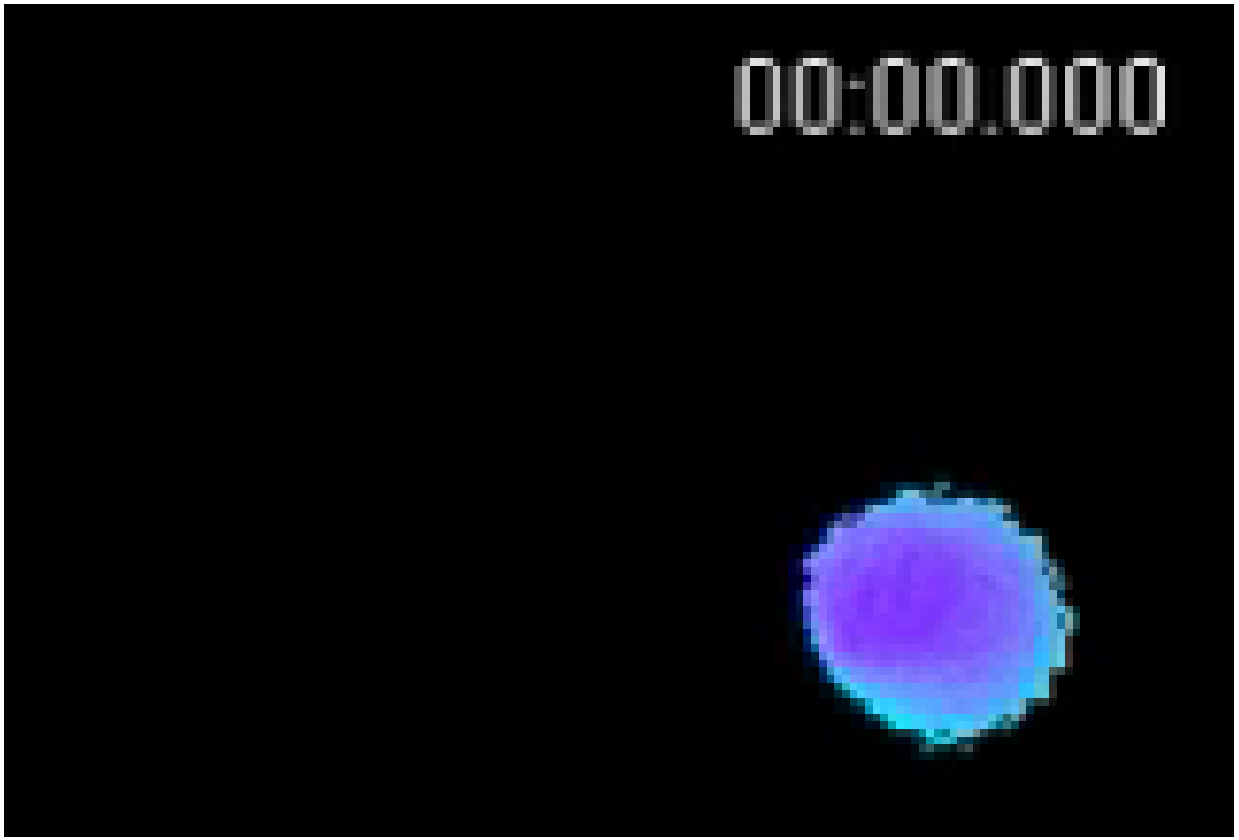
Coupled production and destruction constrains the scope of lipid diffusion.

Because system requires active maintenance, it can be disassembled quickly.

Diffusible agents propagate signals downstream

Fura-2

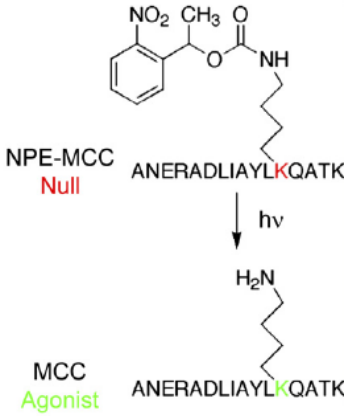
Ca^{2+} dye



T cells landing on surface coated with pMHC

Speed of Ca^{2+} response

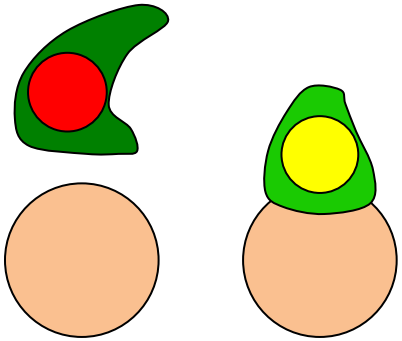
Photoactivatable agonist



Photoactivation of Ca^{2+} influx in 6 s

Huse et al., 2007

NFAT-based Ca^{2+} sensing



In living tissue!

Marangoni et al., 2013

Lecture topics

1) How does ligand binding induce TCR activation?

- Phosphatase exclusion
- TCR conformational change
- The kinetic proofreading model

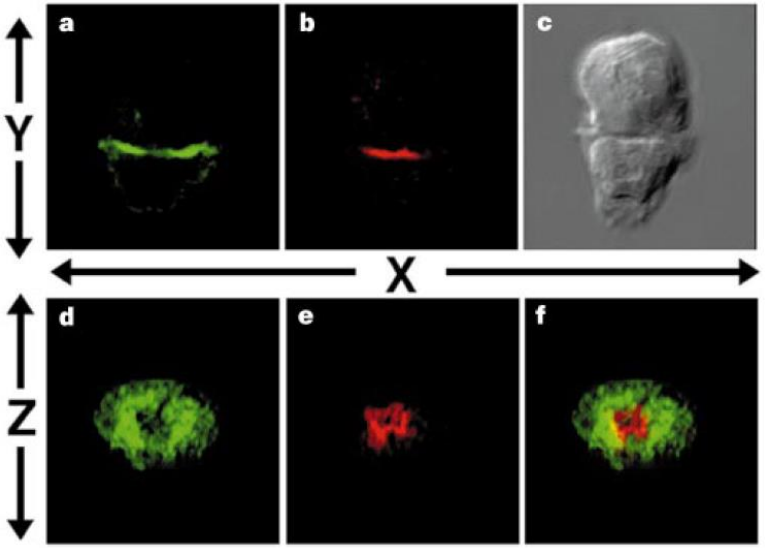
2) How does receptor activation induce downstream signaling?

- Liquid-ordered signaling microclusters
- Lipid second messengers
- Calcium signaling

3) How does interfacial architecture influence T cell activation and effector responses?

- Microvilli and ligand search
- Actin dynamics and mechanotransduction
- Directional secretion

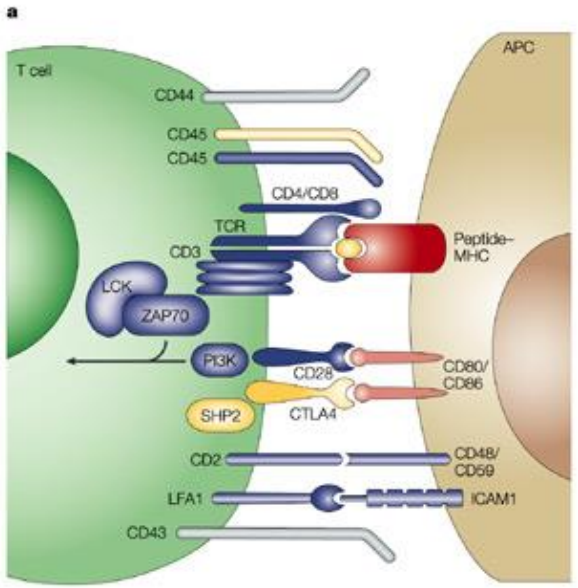
TCR signaling occurs at a cell-cell interface

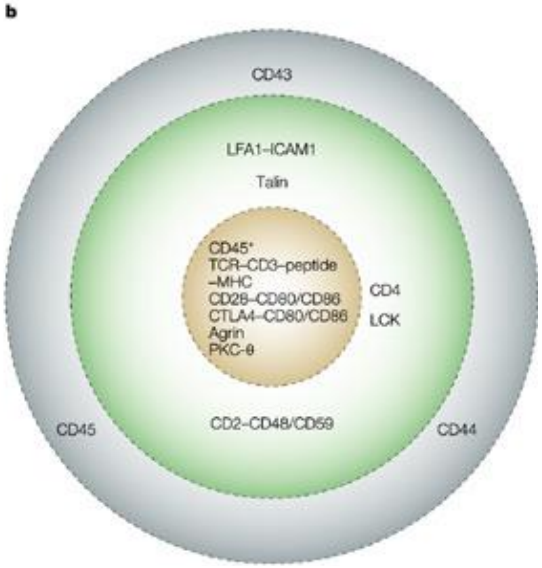


Green: Talin

Red: PKCθ

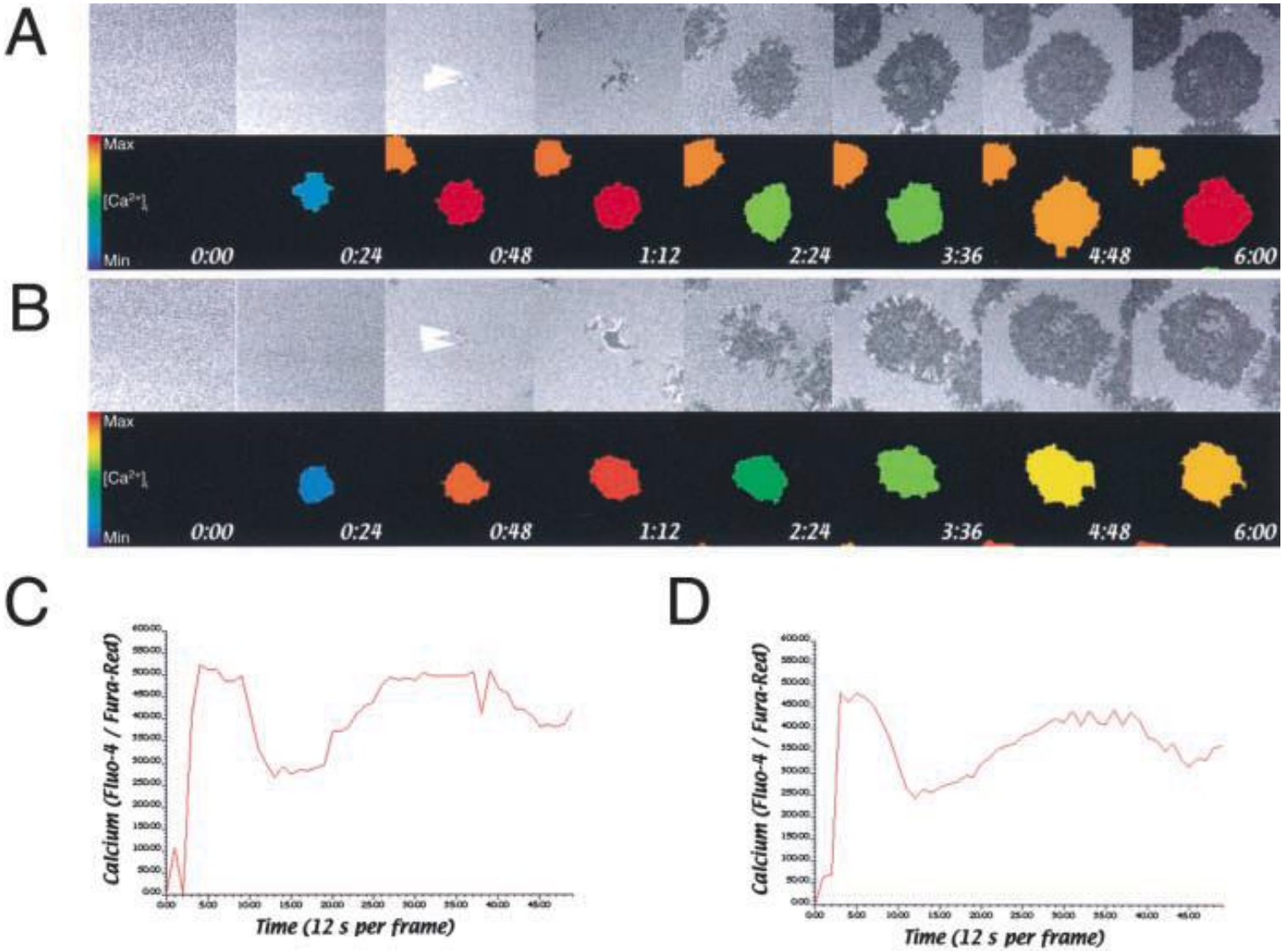
Monks et al., 1998





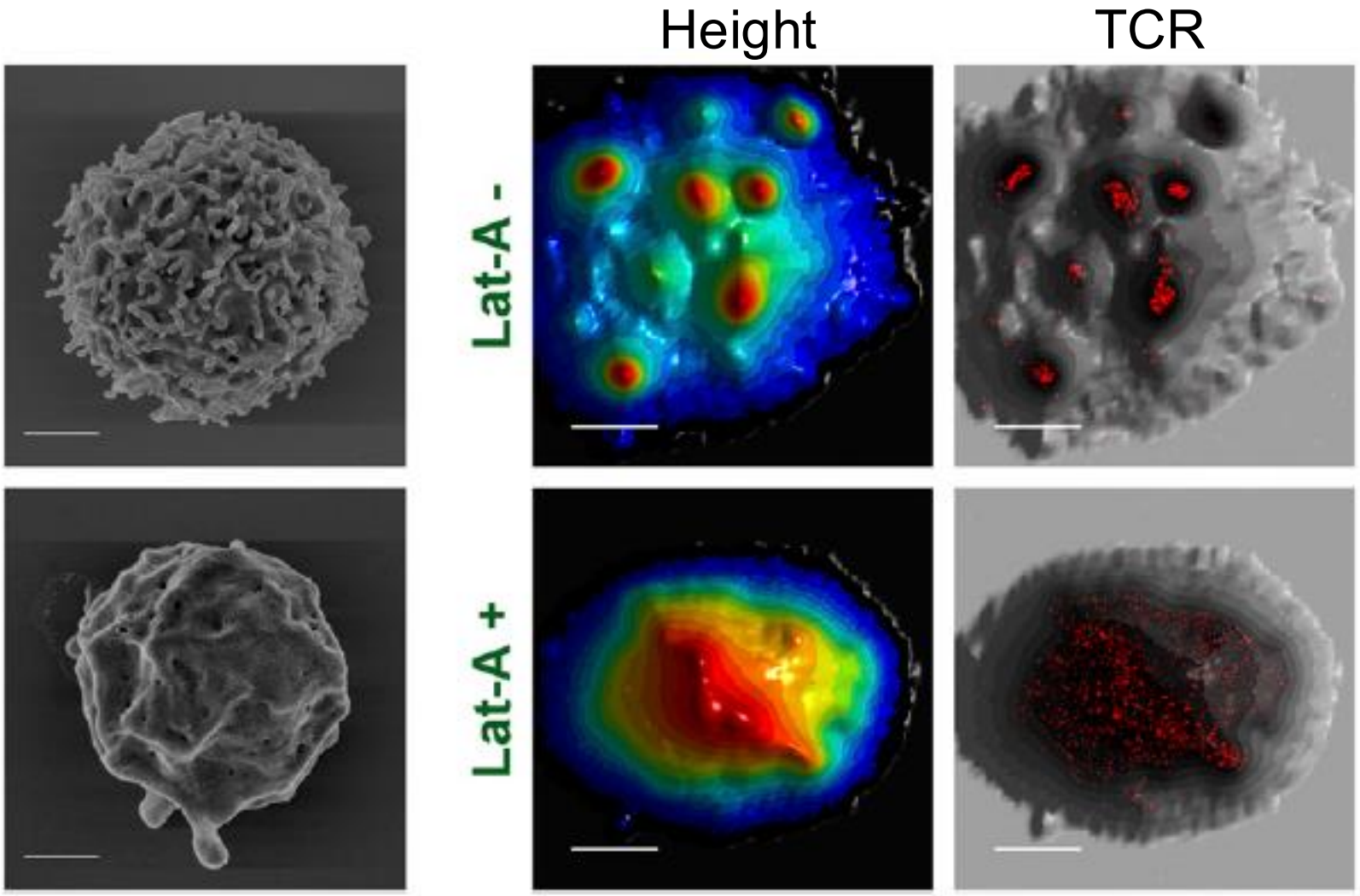
Huppa and Davis, 2003

The synapse is the product of early signaling



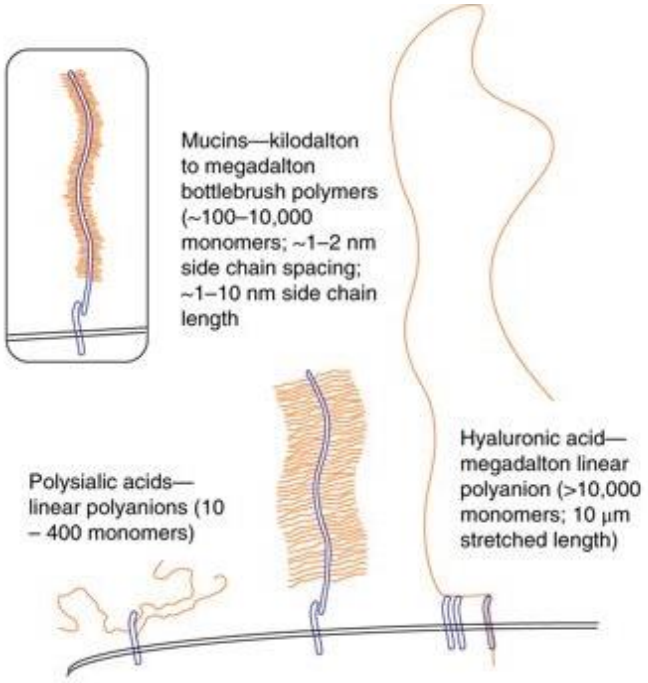
Bunnell et al., 2002

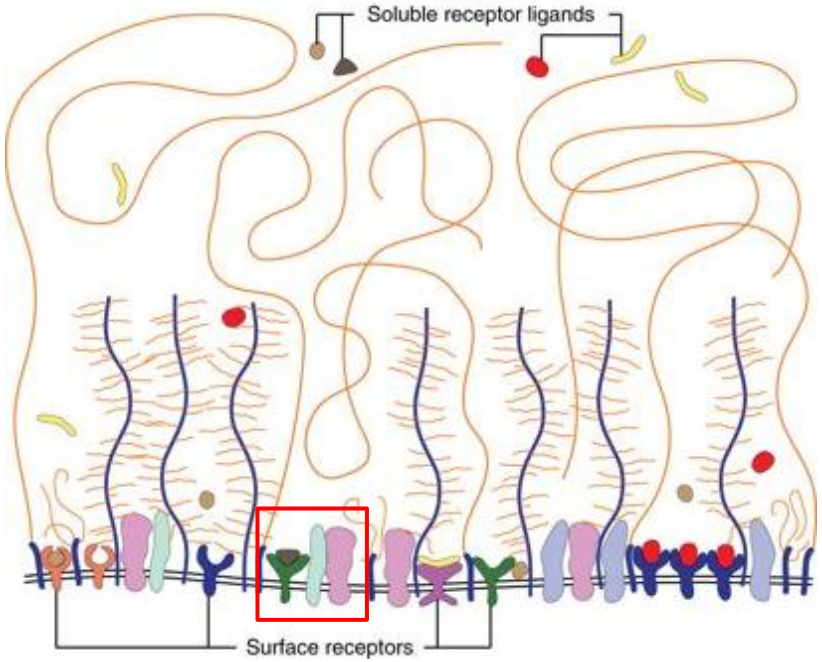
T cells are covered in sensory microvilli



Jung et al., 2016

TCRs must penetrate glycocalyx

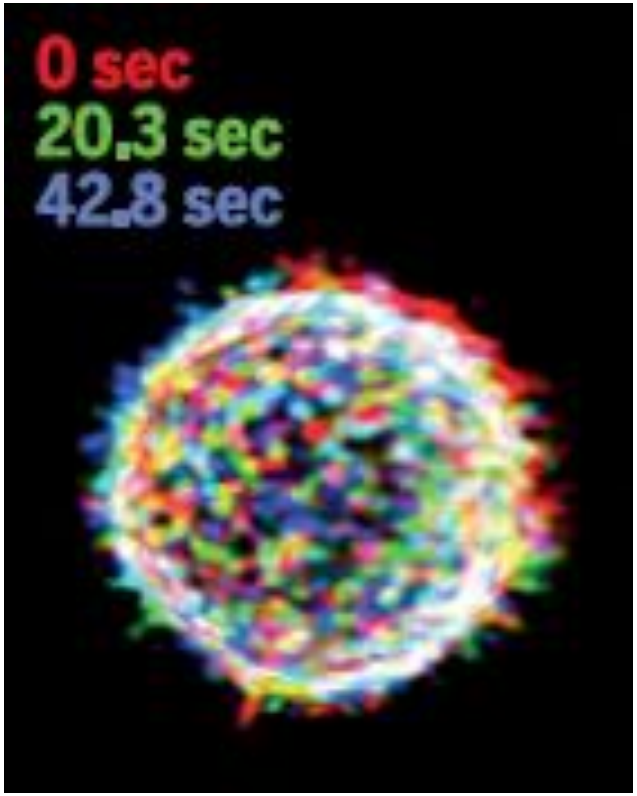


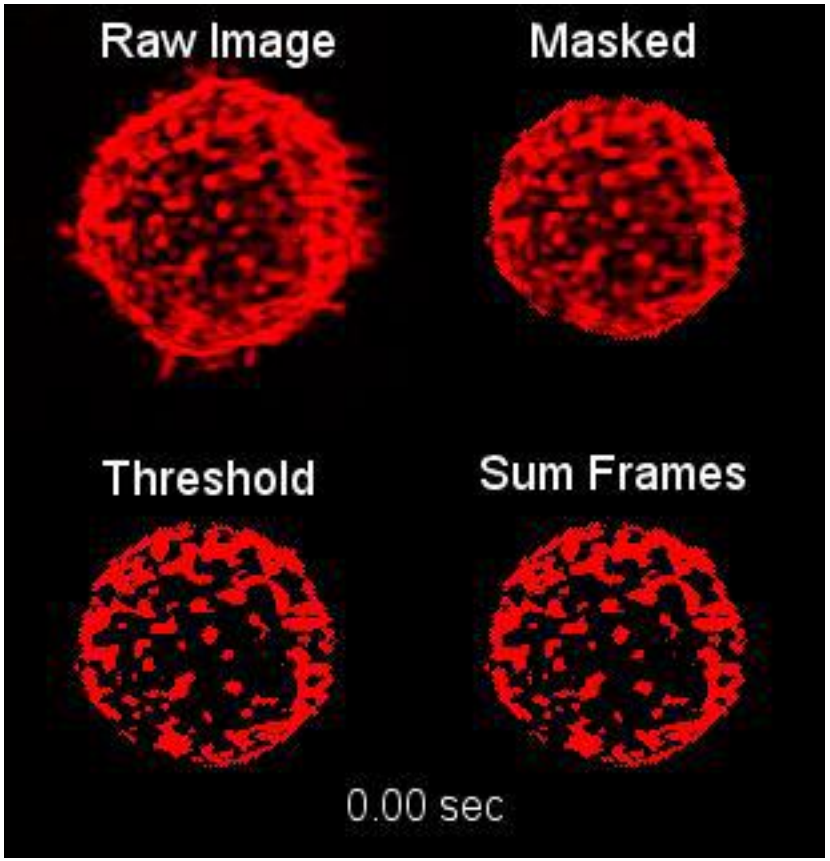


Kuo et al., 2018

T cells use microvilli to survey

Lattice light-sheet imaging of T cell microvilli

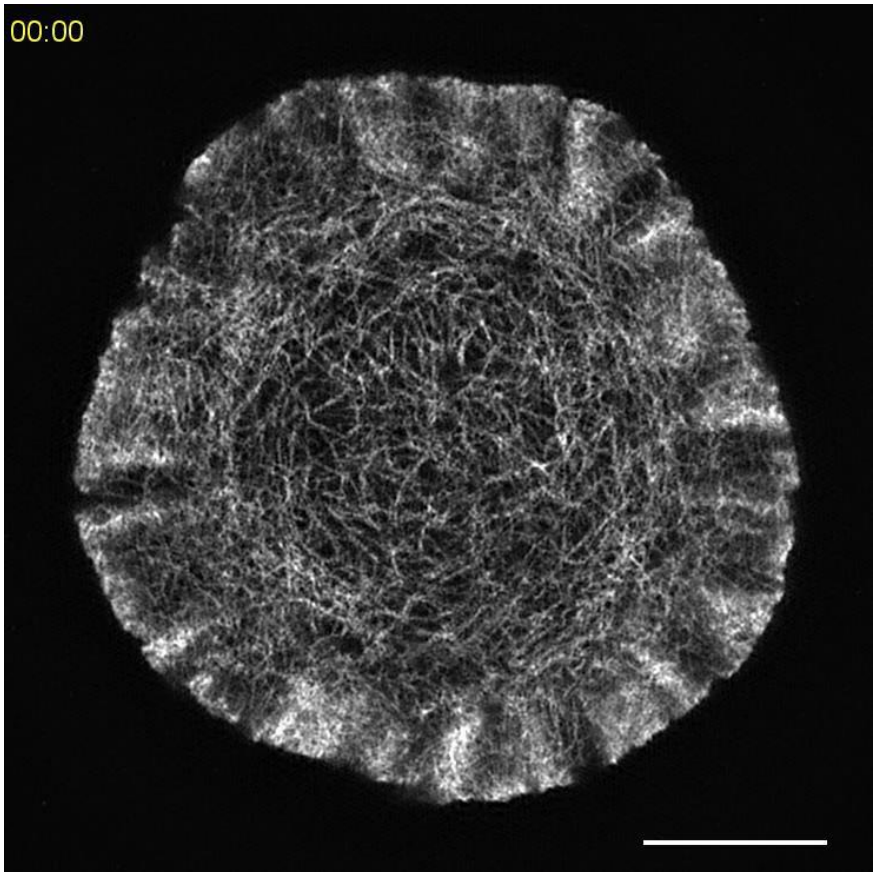




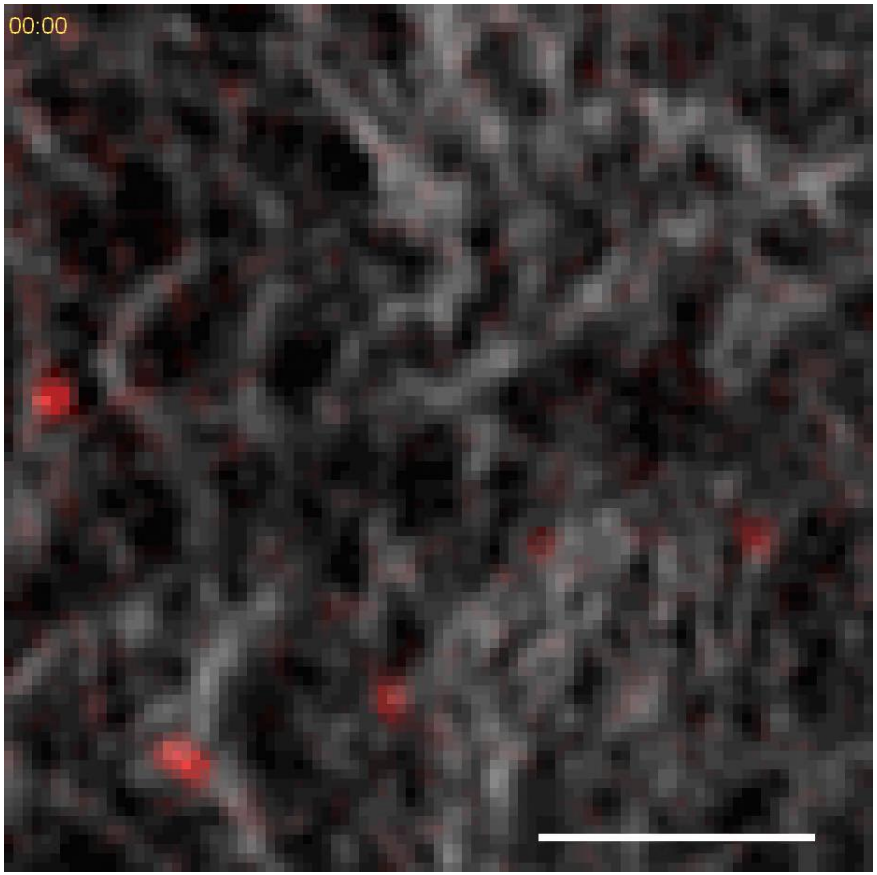
Cai et al., 2017

Mechanotransduction at the immunological synapse

Actin



Actin TCR



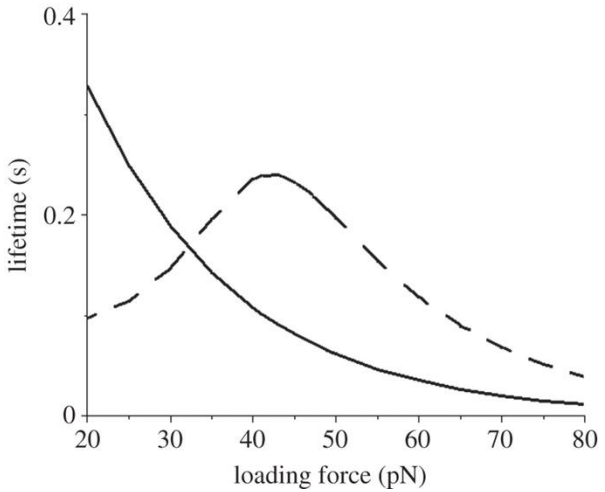
TIRF-SIM imaging

Murugesan et al, 2016

Mechanical enhancement of kinetic proofreading

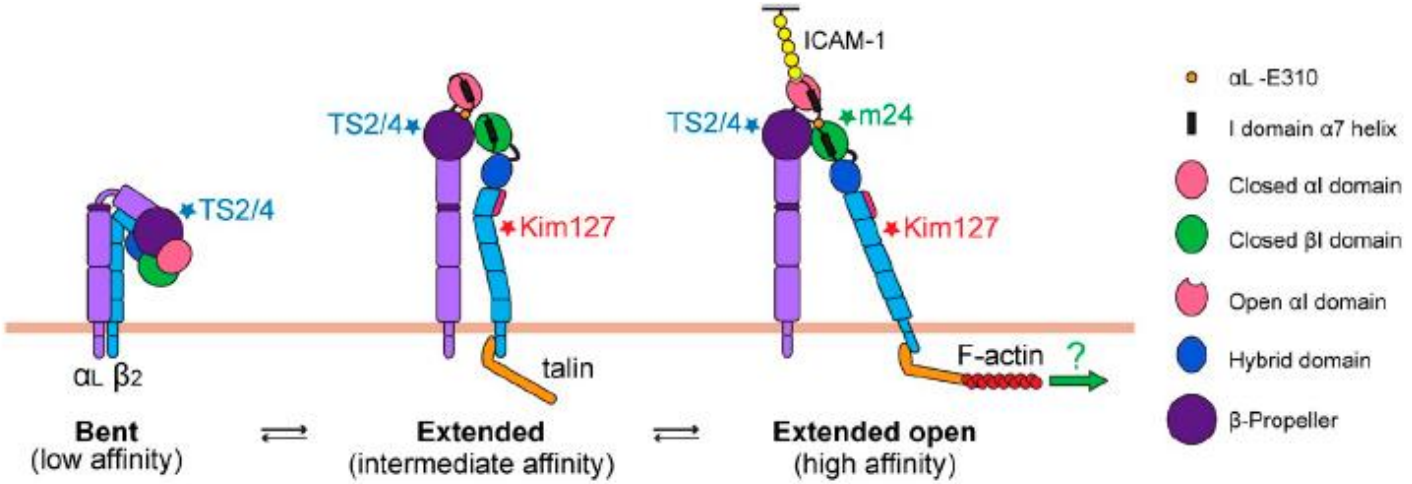
Catch bond – strength increases until optimal applied force.

Slip bond – strength decreased under applied force.



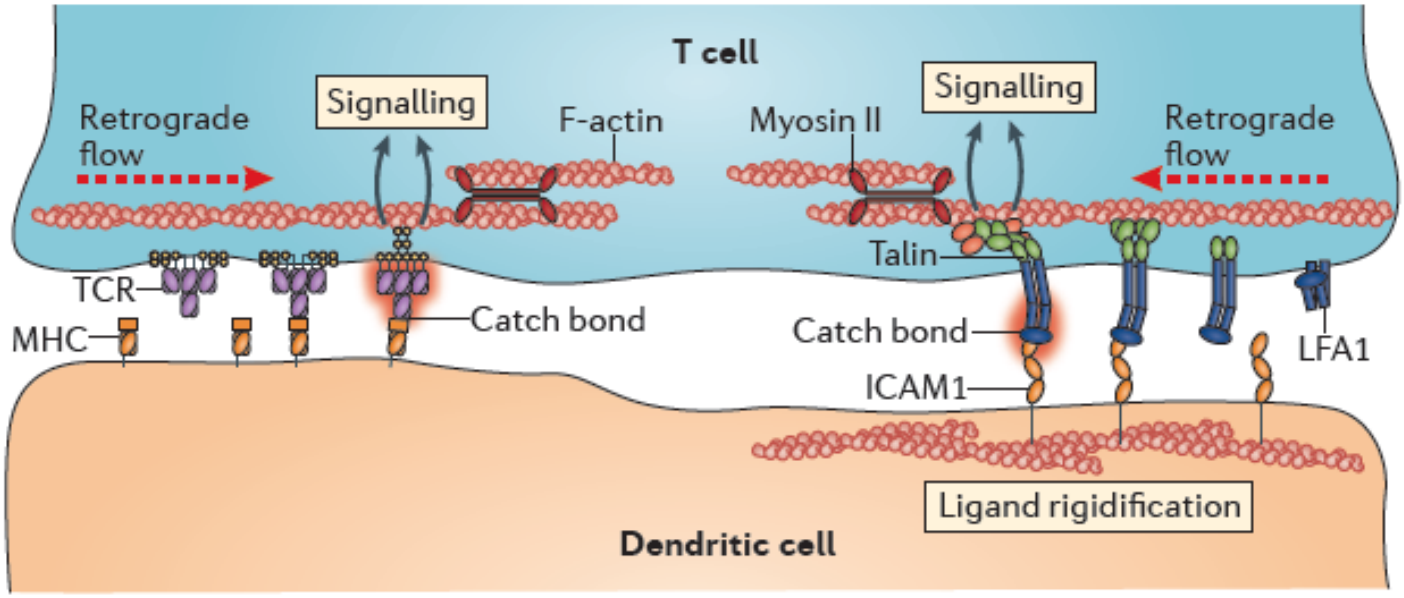
Sun et al., 2011

LFA1 is forms catch bonds with ICAM



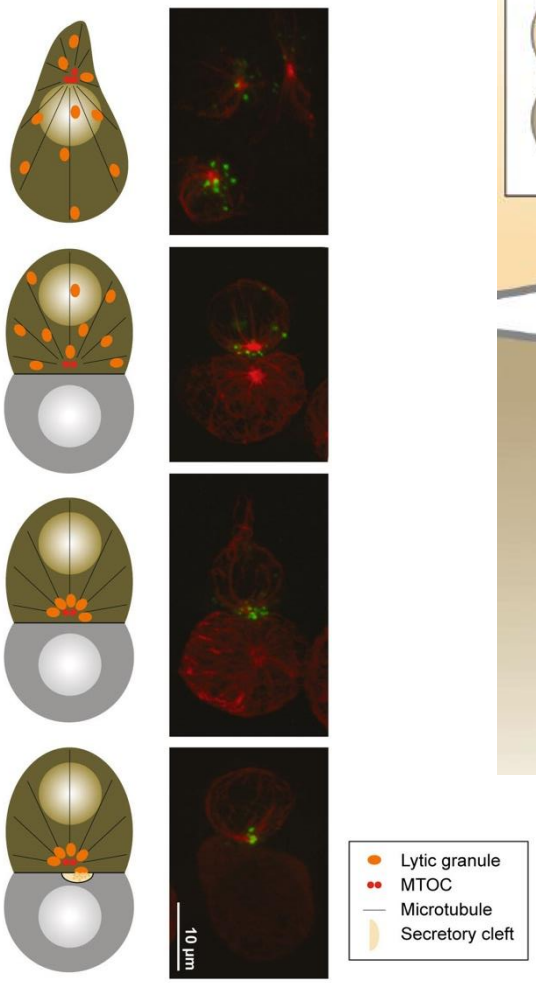
Comrie et al., 2015

Microcluster motility mechanotransduction

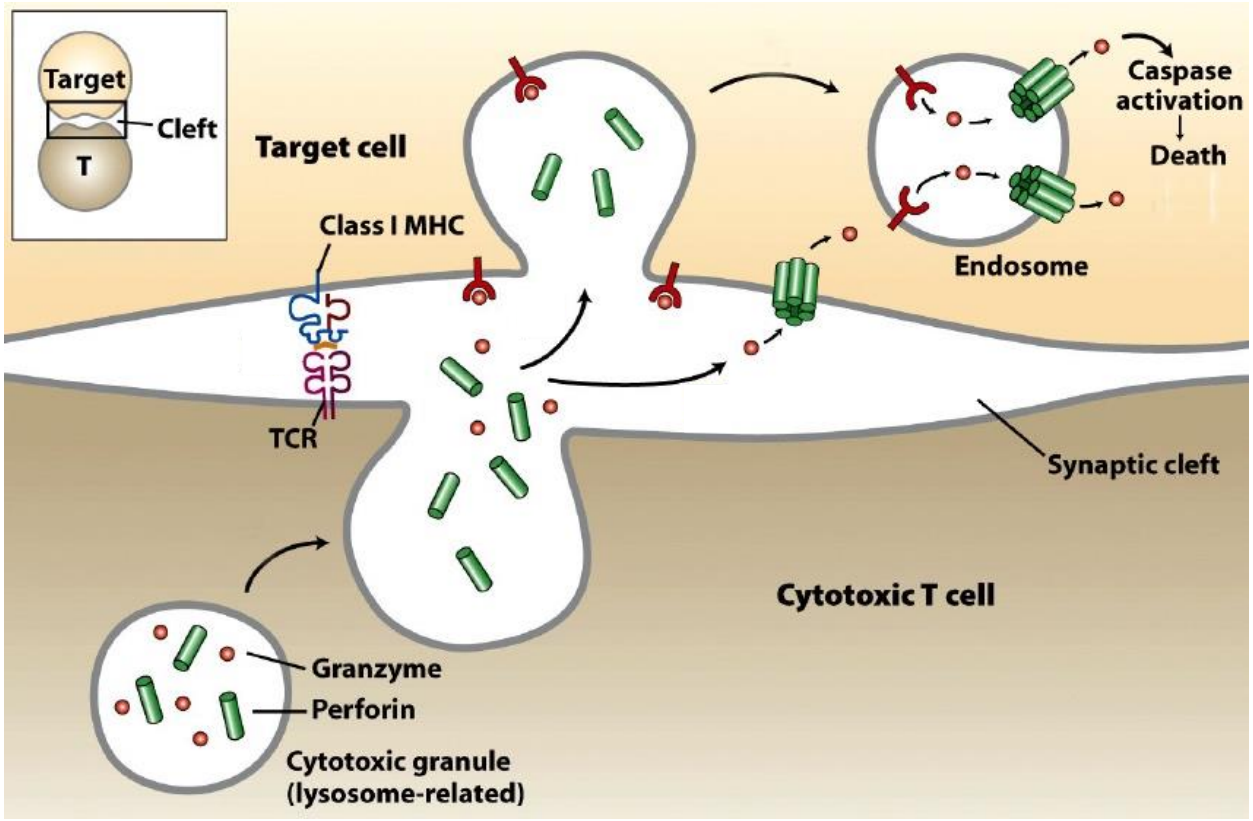


Huse, 2017

CTLs kill by releasing perforin and granzyme



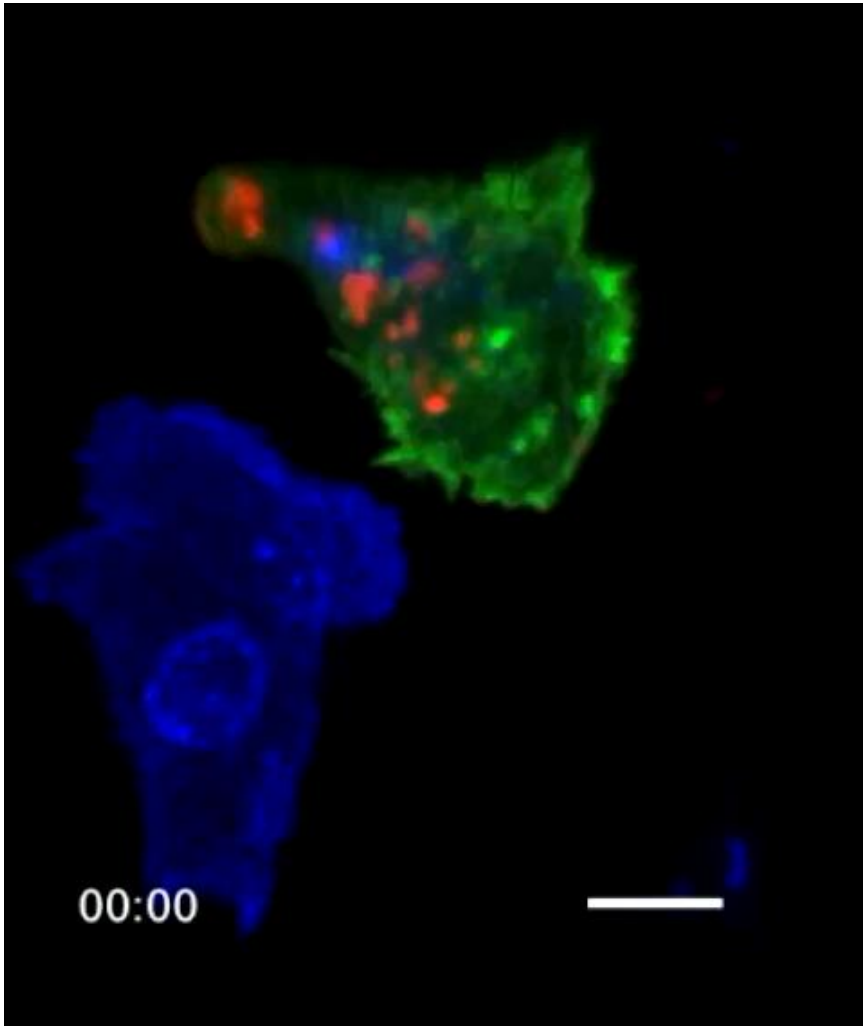
Stinchcombe et al., 2007



Adapted from StudyBlue, Iowa State University

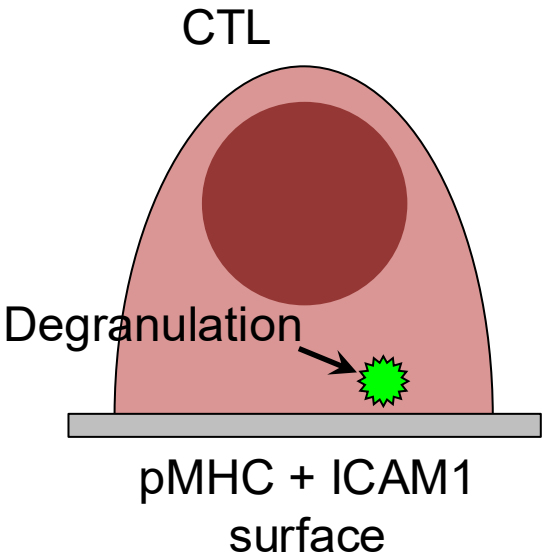
Centrosome polarization and degranulation

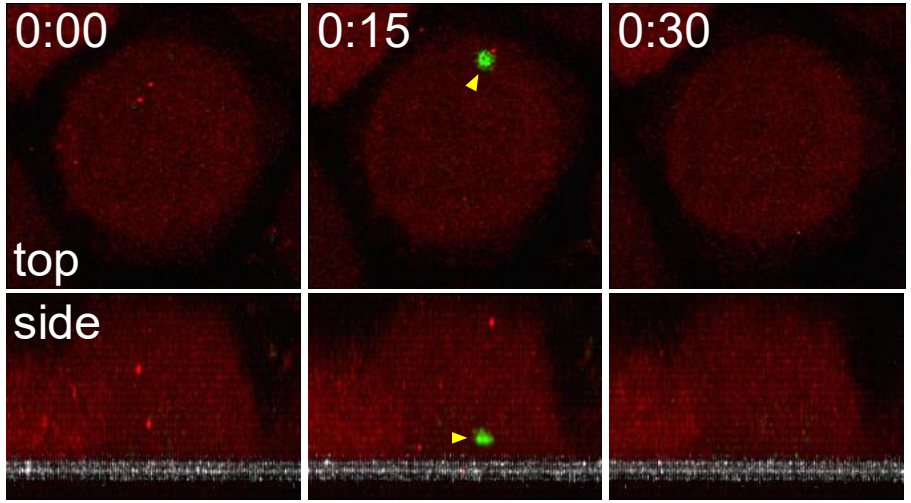
Centrosome Lytic granules

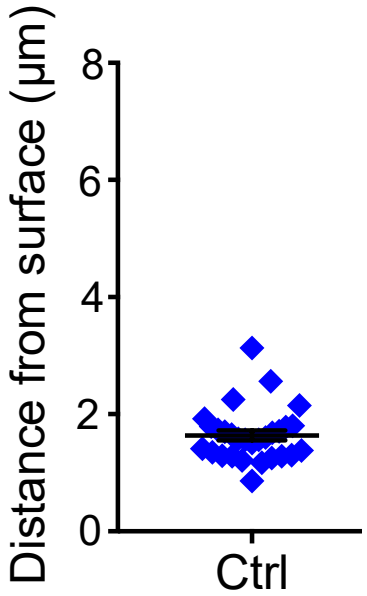


Ritter et al., *Immunity*, 2015

Polarized degranulation by CTLs



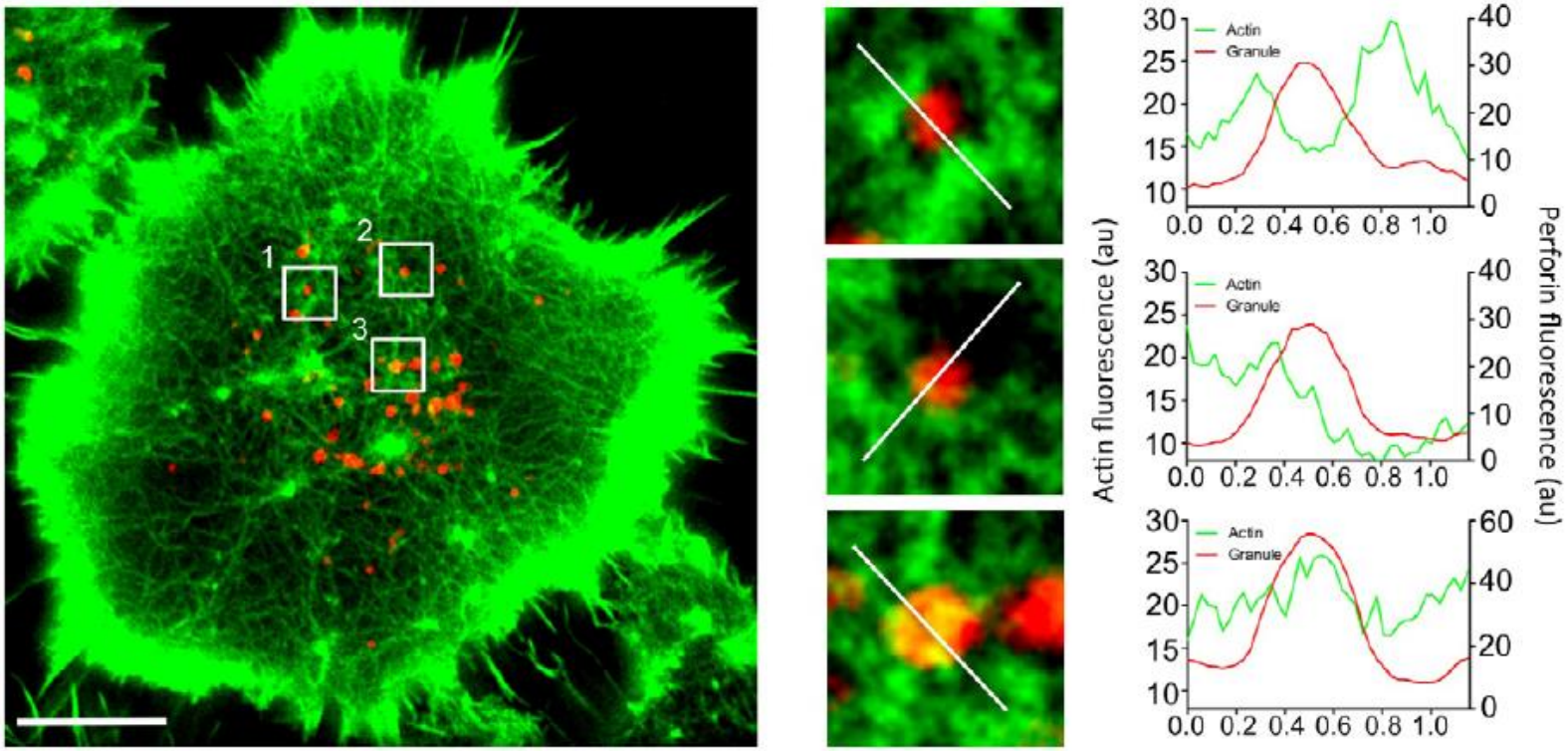




If not microtubules, then what?

Tamzalit et al., *PNAS*, 2020

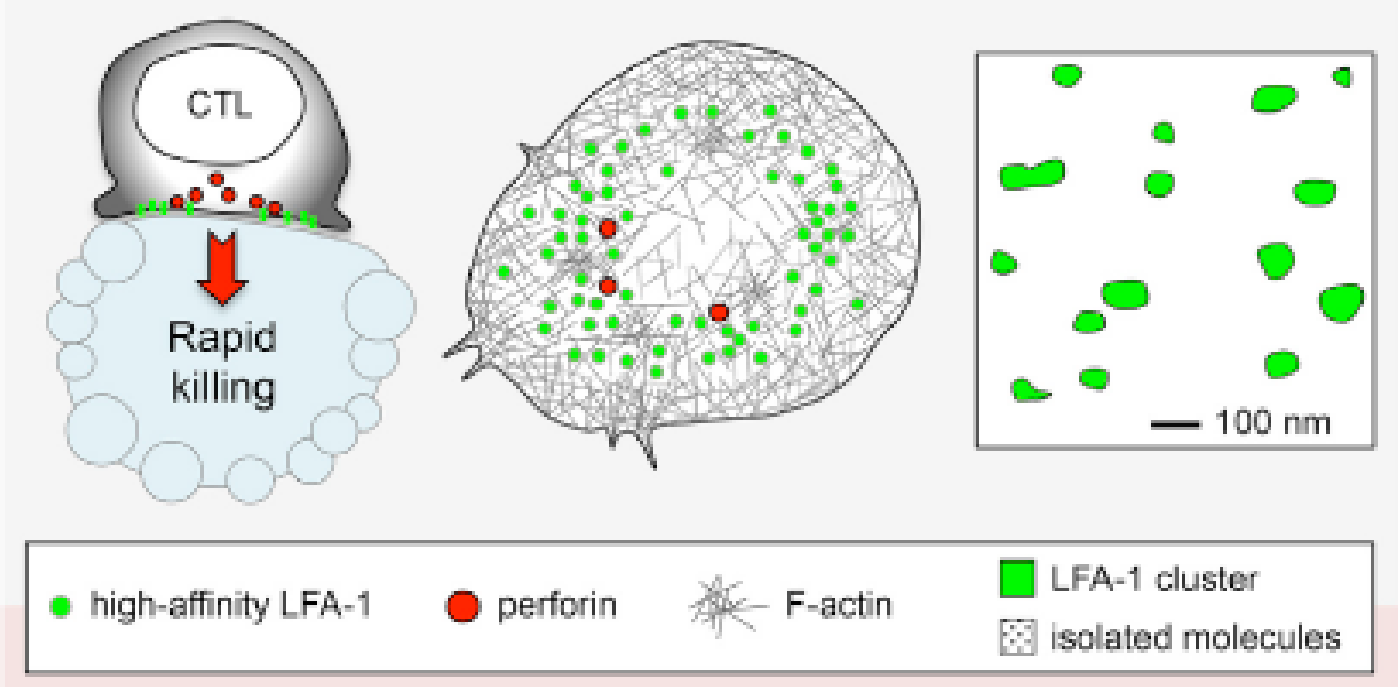
Degranulation requires F-actin clearance



Super-resolution STED imaging

Rak et al., *PLoS Bio*, 2011

Degranulation requires mechanically active integrins



Houmadi et al., *Cell Rep.,* 2018