
Spatial Organization and the Mechanics of Cellular Signal Transduction

Jay T. Groves

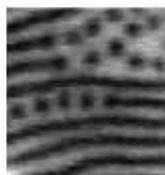
Department of Chemistry
UC Berkeley

Cell Membrane Nanotechnology

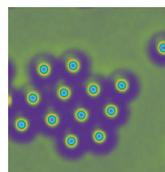


Angewandte
Chemie

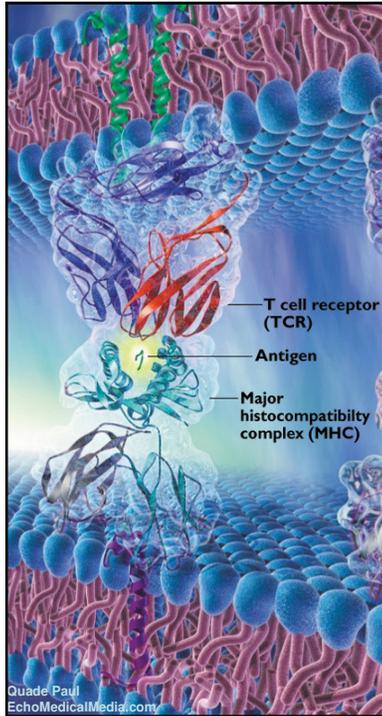
Science **310**: 1191 (2005)
Angew. Chem. Int. Ed. **44**: 3524 (2005)
Sci. STKE **2005**, pe45 (2005)
Nature Chem. Biol. **1**: 283 (2005)
J. Am. Chem. Soc., (2006), **128**: 15354



Phys. Rev. Lett., **96**: 118101 (2006)
Phys. Rev. Lett. **95**: 048101 (2005)
J. Am. Chem. Soc. **127**: 36 (2005)
PNAS **101**: 12798 (2004)



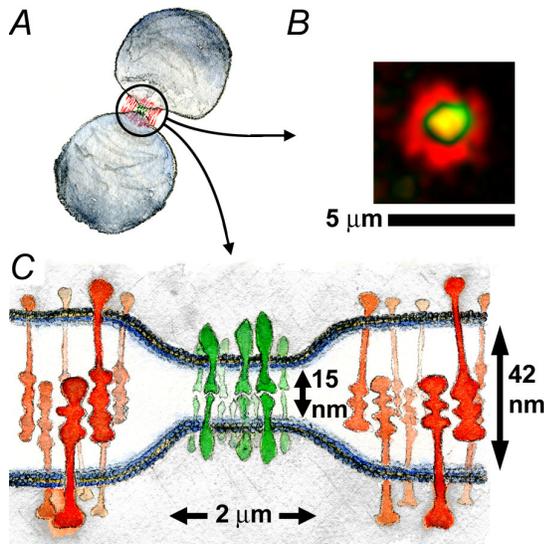
Nature **427**: 139 (2004)
Adv. Mater. **17**: 1477 (2005)
Anal. Chem. **78**: 174 (2006)



T Cell Recognition

- Front line of adaptive immune system
- Detect foreign (e.g. viral) antigen peptides displayed on antigen presenting cell (APC) surface by major histocompatibility complex (MHC)
- T cell receptor (TCR)
Diverse combinatorial array
- Individual proteins well understood
How it works remains unclear

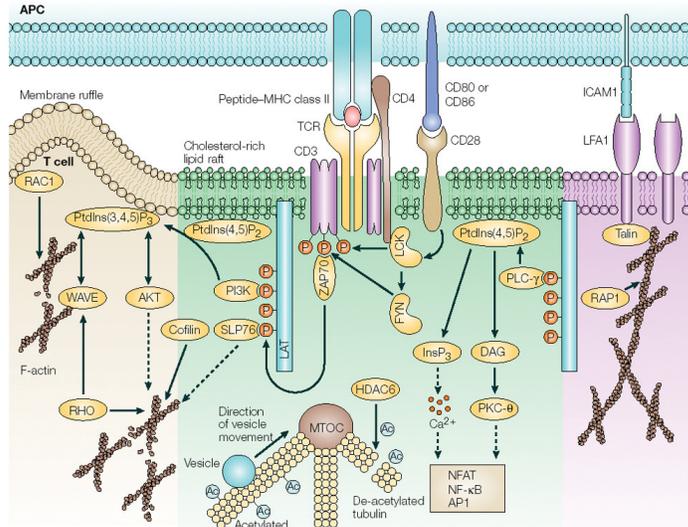
The Immunological Synapse



- Proteins are spatially organized within the junction
- Spatial pattern correlates with activity

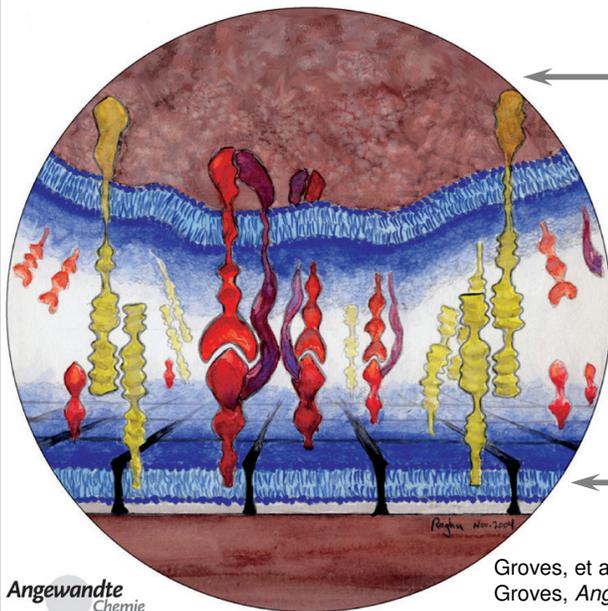
TCR Signaling Reaction Network

How does spatial patterning impact signal transduction?



Friedl et al., *Nat. Rev. Immunol.* 5: 532 (2005)

Hybrid Live Cell - Supported Membrane Synapse



live cell

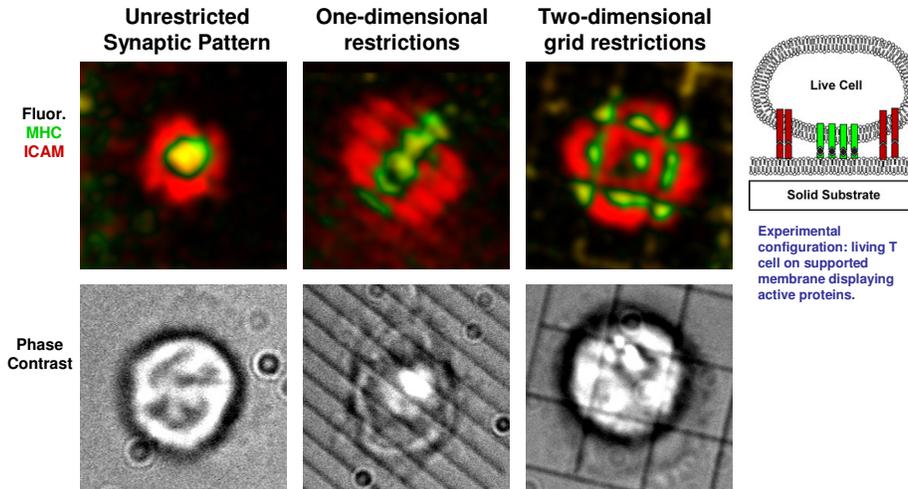
- Supported membrane replaces APC
- Proteins in supported membrane must be mobile

supported membrane

Angewandte Chemie

Groves, et al., *Science* (1997)
Groves, *Angew. Chem. Int. Ed.* 44: 3524 (2005)

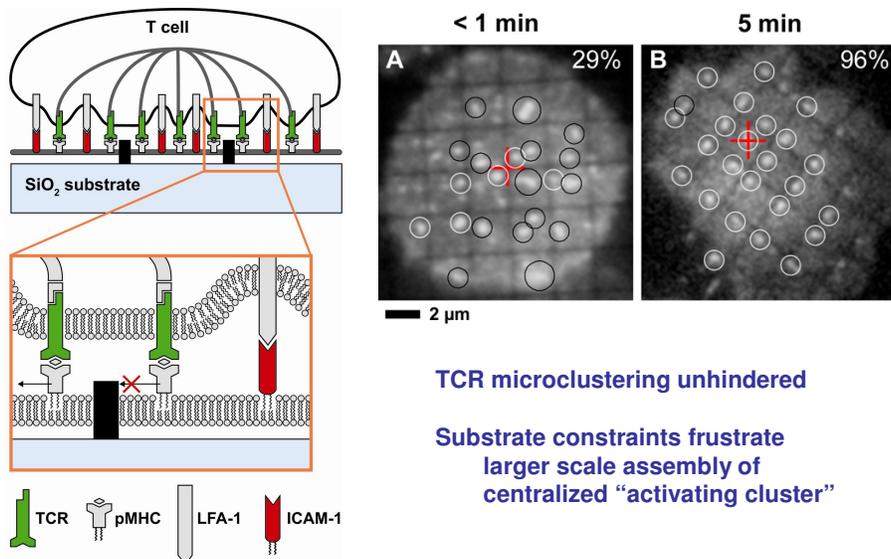
Repatterning the T Cell Immune Synapse



Spatial mutations of the signaling apparatus.

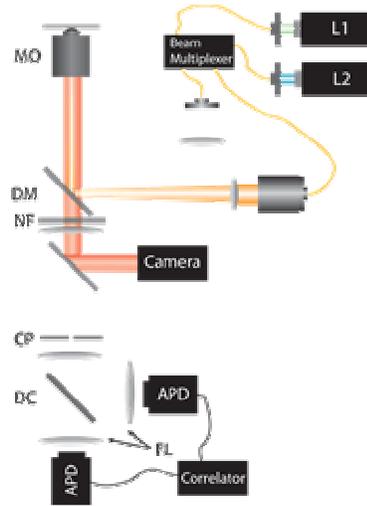
Mossman *et al. Science* 310: p. 1191 (2005)

Repatterning the T Cell Immune Synapse



Mossman *et al. Science* 310: p. 1191 (2005)

Tracking Synapse Assembly

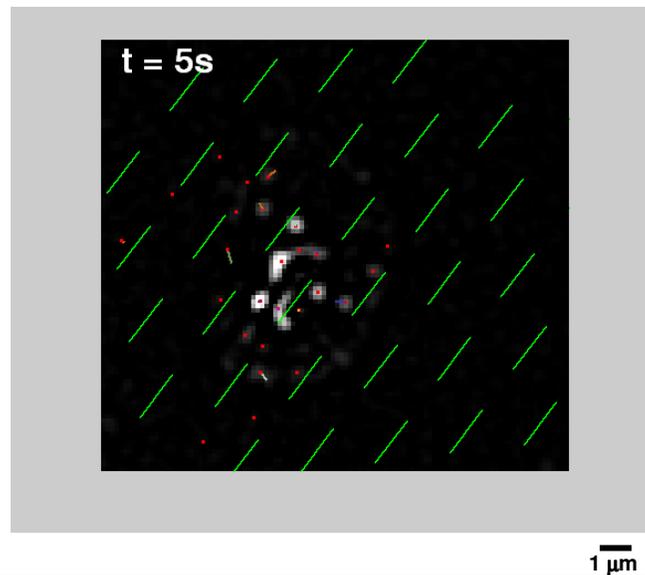


Total internal reflection fluorescence (TIRF)

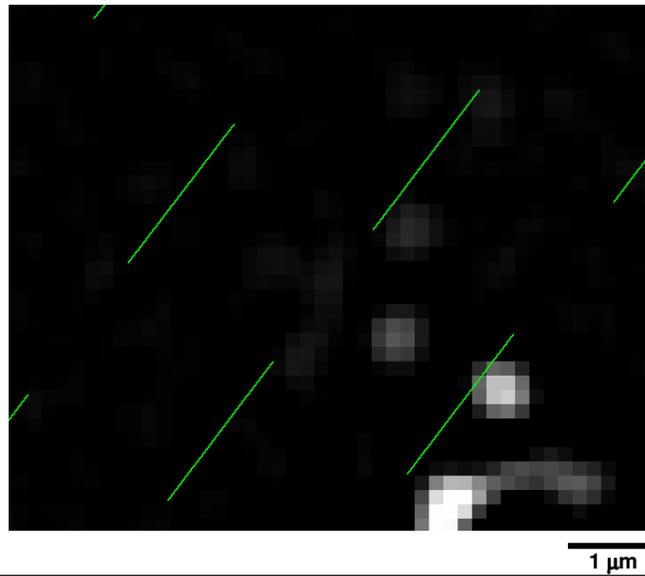
- Single molecule resolution
- Flow tracking algorithm
- Automated statistical analysis of protein movement

DeMond *et al.*

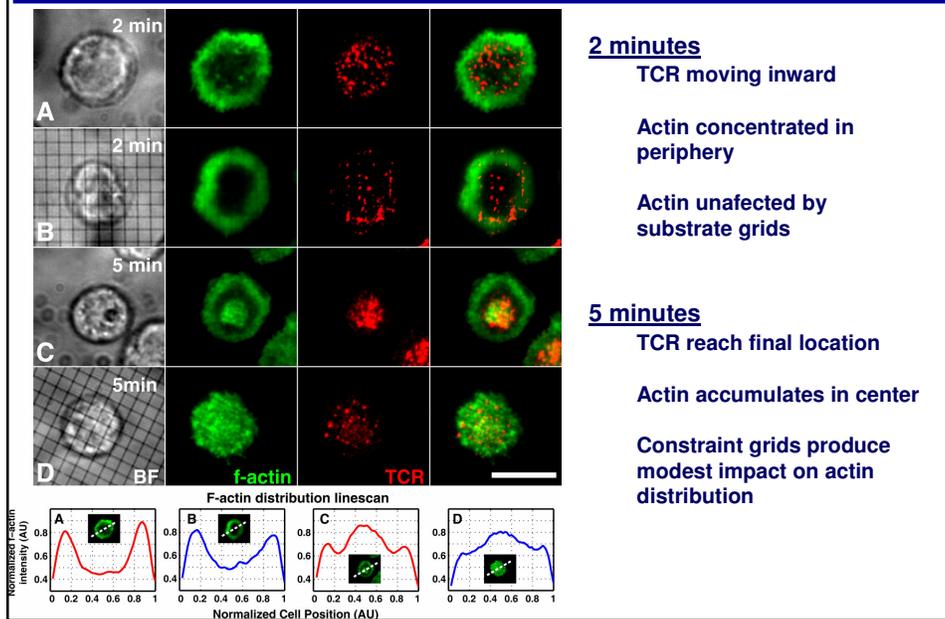
Tracking Synapse Assembly (TIRF)



Tracking Synapse Assembly (TIRF)



Actin cytoskeleton drives TCR motion



Two Stages of Synapse Assembly

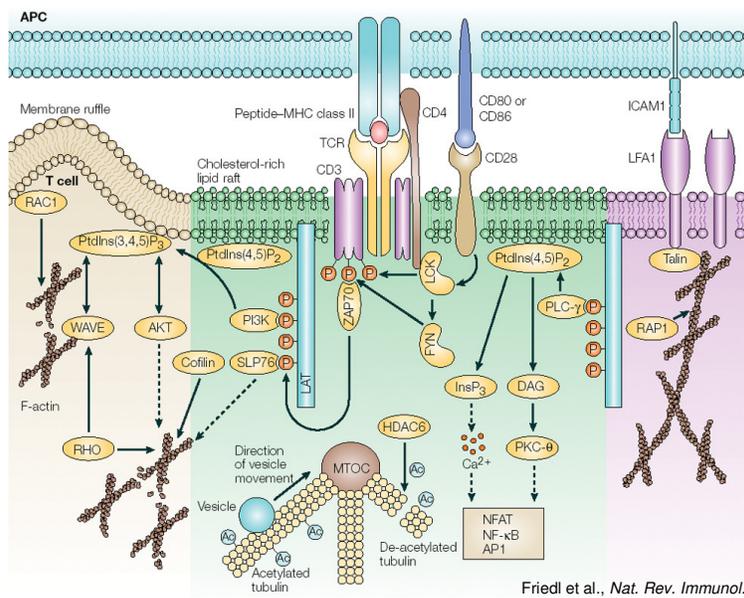
Stage 1: TCR microcluster formation

appears diffusive
unaffected by constraint pattern down to 1 μm

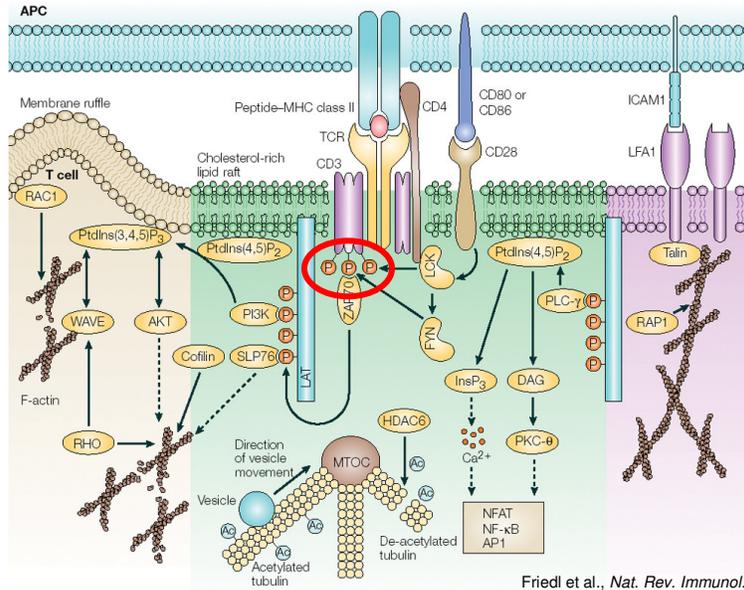
Stage 2: Cluster transport into mature synaptic pattern

directed transport
driven by actin cytoskeleton (blocked by latrunculin)
cluster transport is redirected by constraint pattern

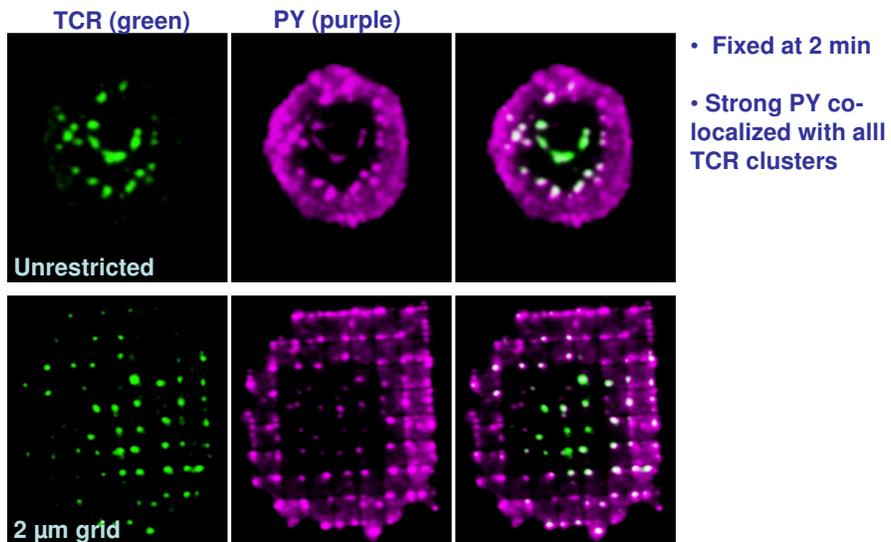
Mapping Intracellular Signaling



Phosphotyrosine Signaling

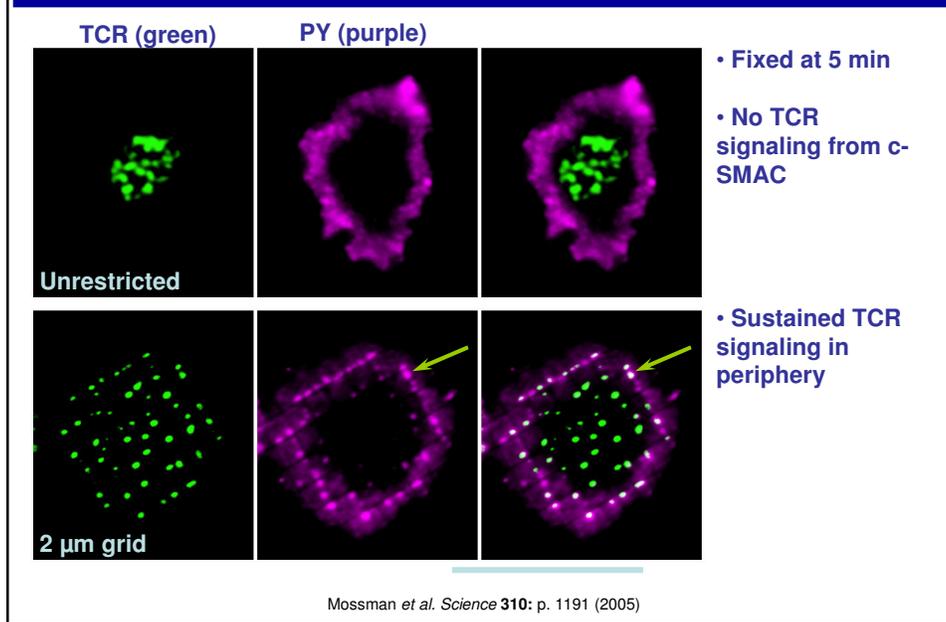


Phosphotyrosine Signaling

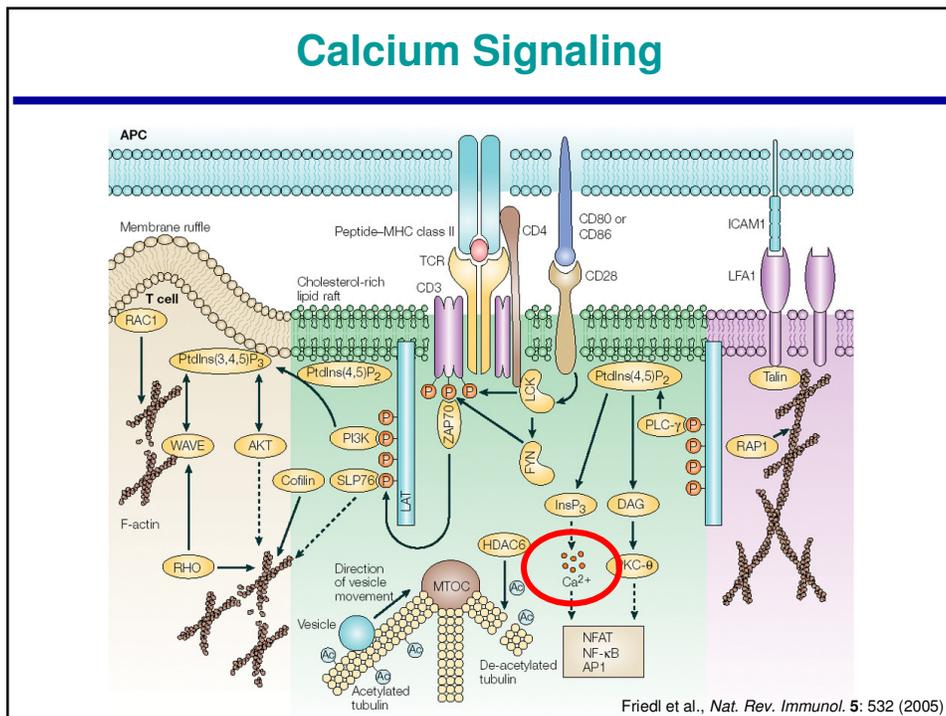


Mossman et al. *Science* 310: p. 1191 (2005)

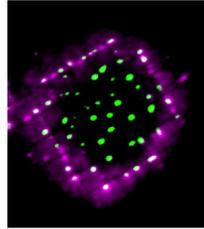
Phosphotyrosine Signaling



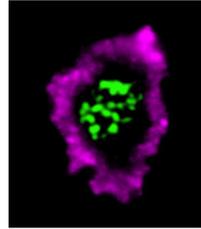
Calcium Signaling



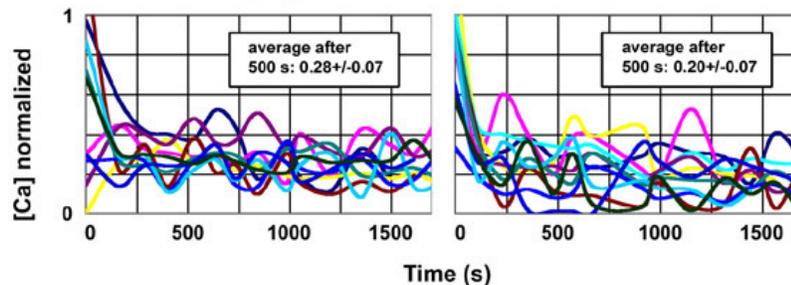
Calcium Signaling



Cells on 2 μm grids



Cells off grids



Mossman *et al. Science* 310: p. 1191 (2005)

Summary: Synaptic Pattern Formation

Biological Conclusions

- Distinctive patterns correlate with different types of signaling activity.
- Can causality be established?
- Cytoskeleton is a key regulator of pattern
Mechanism of cytoskeletal signal regulation: spatial translocation

Technological Conclusions

- Patterned supported membrane substrates enable molecular-scale dissection of live signaling processes
- Spatial mutations of chemical signaling apparatus

Molecular Dissection of a Signaling Cluster

High antigen density

- T cells signal regardless of spatial configuration or cluster size (1, 2, and 5 micron grids)
- Radial position of cluster correlates with duration of signal
- central supramolecular **activating** cluster (cSMAC) formation is a **deactivating** process

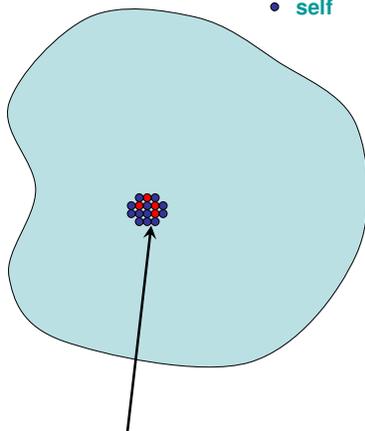
Low antigen hypothesis

- TCR microclusters are amplifiers required for sensitivity to low antigen density.

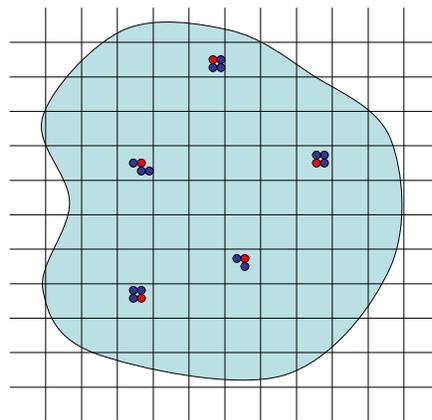
Molecular Dissection of a Signaling Cluster

At low antigen density, less than one per corral is possible

- antigen
- self

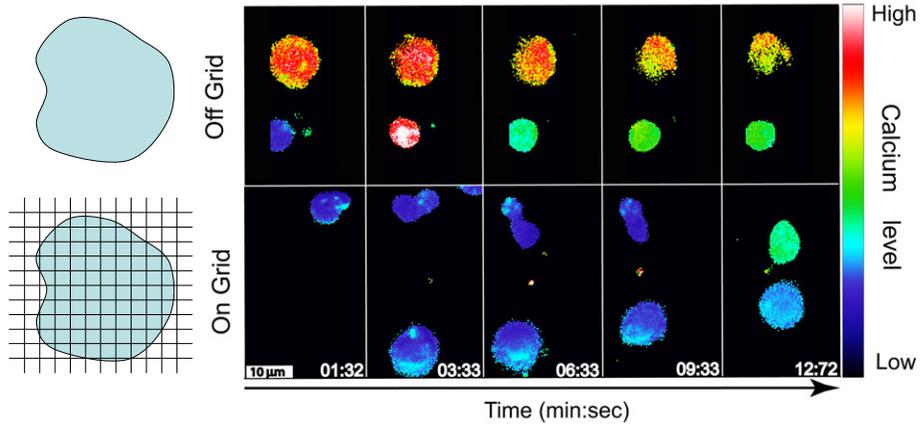


Multiple antigens in one TCR cluster



One antigen per TCR cluster

Molecular Dissection of a Signaling Cluster



Long-range cooperativity observed at low antigen density

1 antigen pMHC per μm^2
Poisson distributed in $1 \times 1 \mu\text{m}$ grid elements
100 per μm^2 overall pMHC (same as high antigen experiments)

Rossenova *et al.*

Antigen Specificity

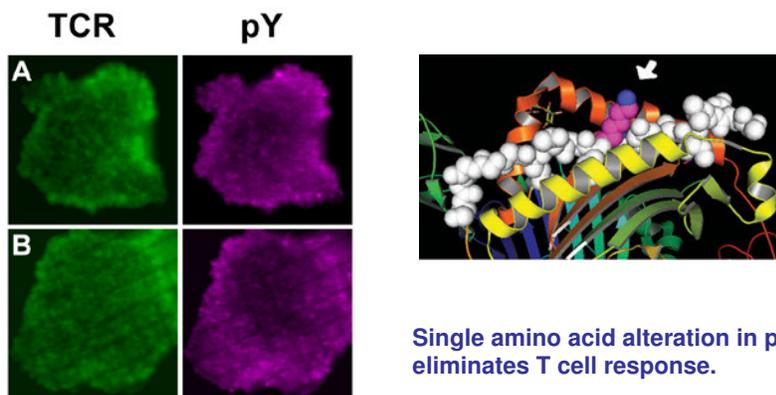
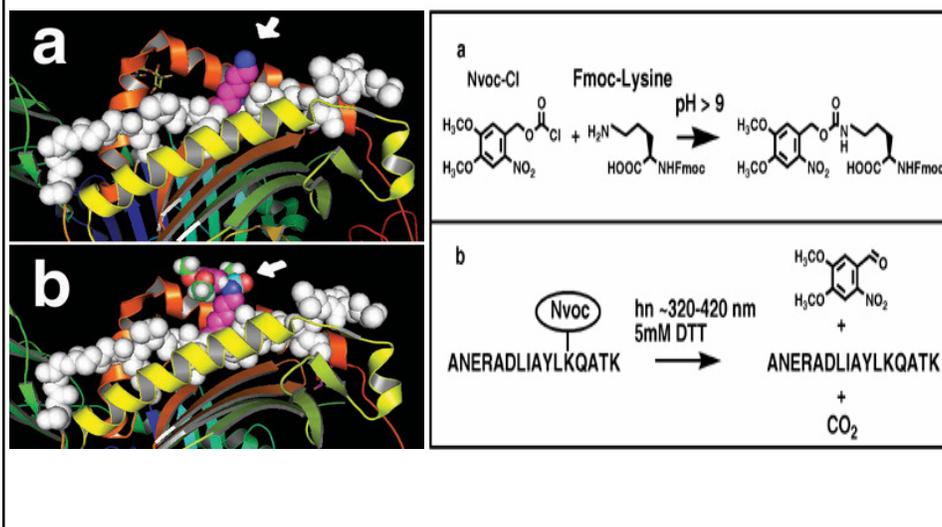
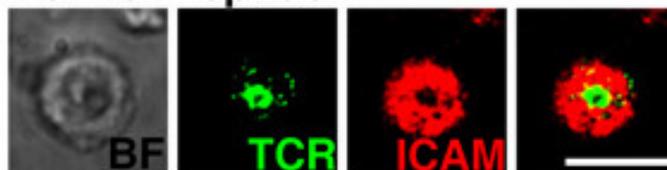


Photo-Activated Peptide Antigen

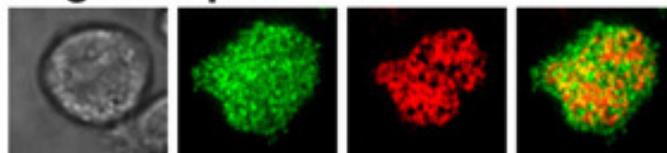


Caged Peptide Antigen

Normal Peptide

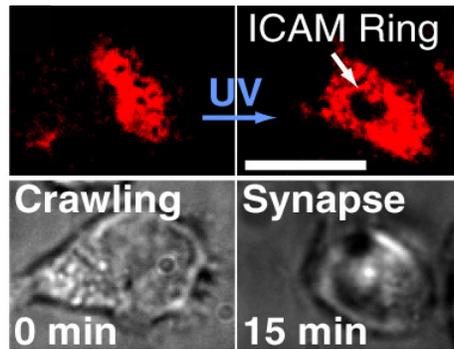


Caged Peptide



DeMond and Groves, *J. Am. Chem. Soc.*, (2006), **128**: 15354

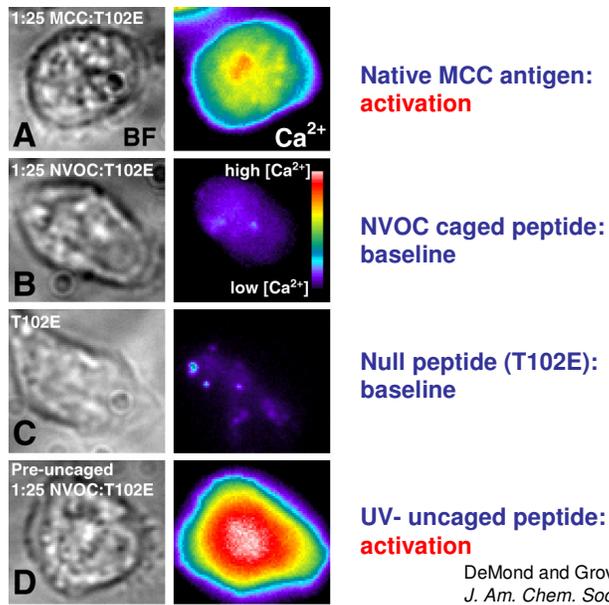
In vivo Photolithography



UV-uncaged antigen peptide is recognized by T cells

DeMond and Groves, *J. Am. Chem. Soc.*, (2006), **128**: 15354

Calcium Signaling



DeMond and Groves,
J. Am. Chem. Soc., (2006), **128**: 15354

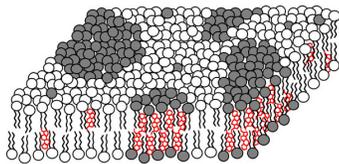
Membrane Forces

What are the physical mechanisms of spatial pattern formation?

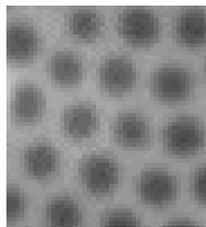
Membrane curvature and bending generate important driving forces.

Topography of Membrane Rafts

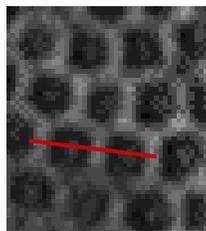
Phase separated membrane domains create topography



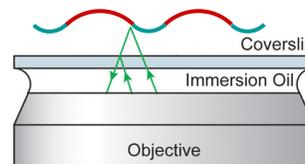
- DOPC:Chol:SM
- Coexisting fluid phases



- Fluorescence



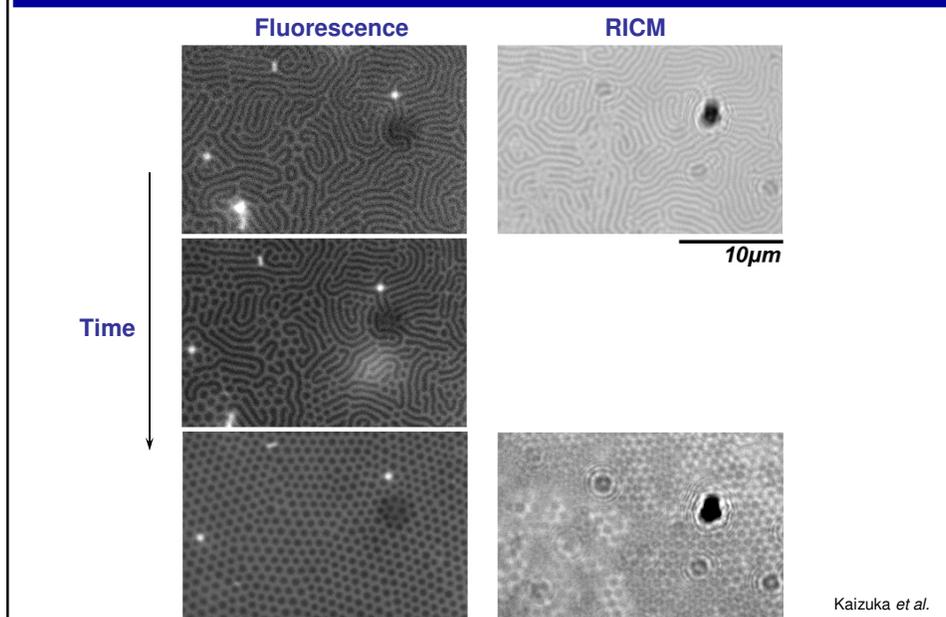
- Reflection Interference Contrast Microscopy



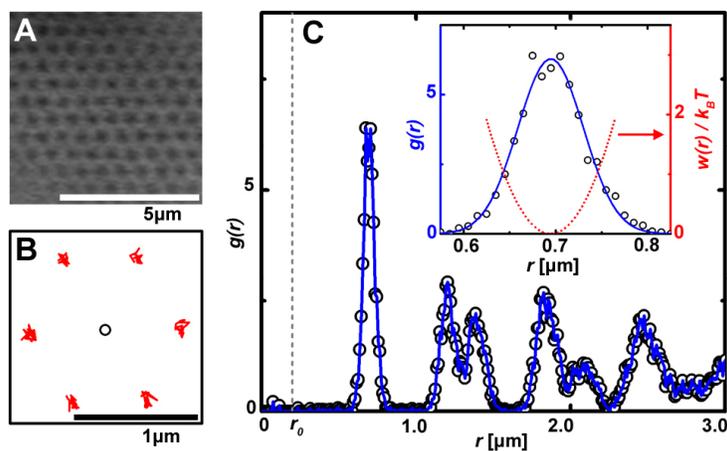
5 μm

Kaizuka *et al.*

Bending-Mediated Superstructure



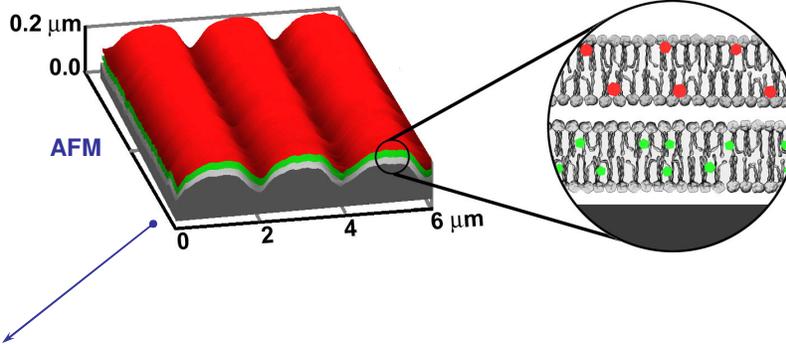
Bending-Mediated Forces



- Image analysis yields pair distribution function, $g(r)$
- Curvature of potential of mean force, $w''(r) = 790 \text{ kT}/\mu\text{m}^2$

Rozovsky, Kaizuka, & Groves *J. Am. Chem. Soc.* (2005) 127: p. 36

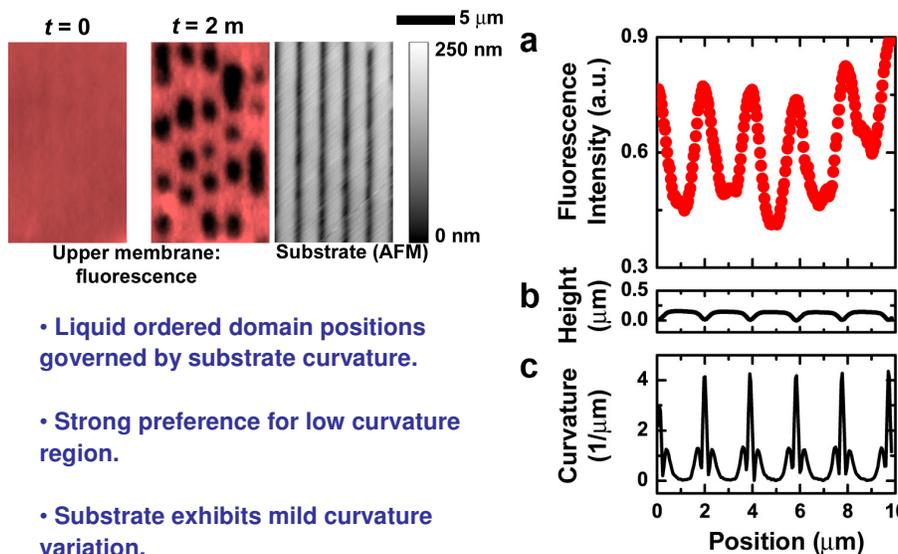
Substrate-Imposed Curvature



- Fused quartz wafer
- Anisotropic oxide etch (CF_4 and CHF_3)
- Isotropic wet etch (HF)
- Upper membrane exhibits normal miscibility phase transitions

Parthasarathy, Yu, & Groves *Langmuir* 22: 5095, 2006

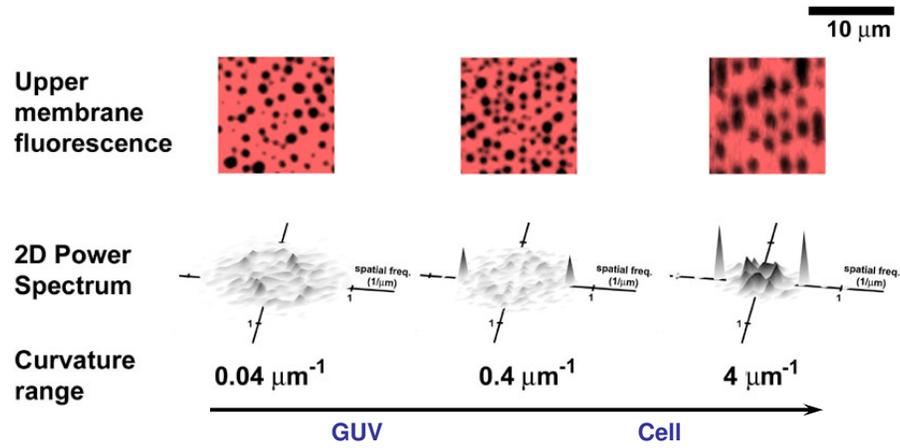
Curvature-Mediated Domain Organization



- Liquid ordered domain positions governed by substrate curvature.
- Strong preference for low curvature region.
- Substrate exhibits mild curvature variation.

Parthasarathy, Yu, & Groves *Langmuir* 22: 5095, 2006

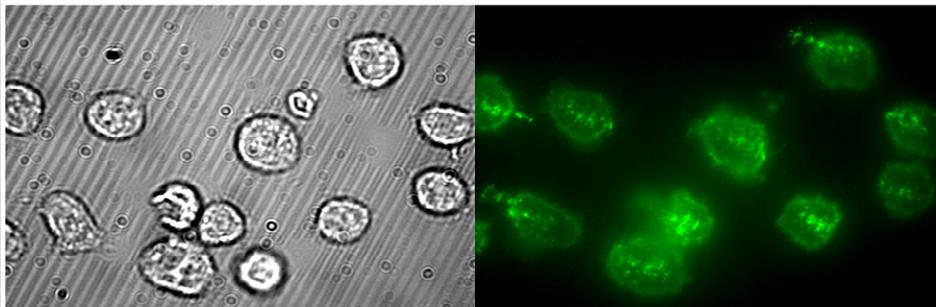
Curvature-Mediated Domain Organization



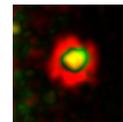
- Threshold curvature differential for ordering between 0.04 and $0.4 \mu\text{m}^{-1}$
- Combination of kinetic and thermodynamic influences

Parthasarathy, Yu, & Groves *Langmuir* 22: 5095, 2006

Live Synapse Repatterning by Curvature



- Substrate curvature does NOT influence diffusion

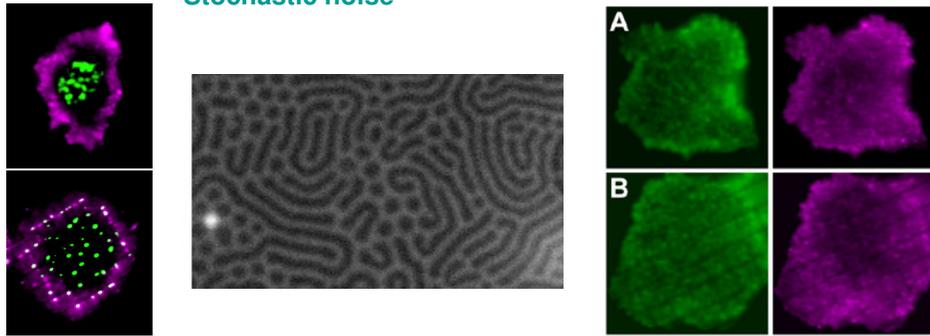


Unpatterned substrate

Outlook

Dynamical manipulation of living chemical networks

- Mechanical perturbation
- *In vivo* photolithography
- Control cell signaling molecule-by-molecule
- Spatial/mechanical regulation of signal transduction
- Stochastic noise



The Group

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Prof. Mark Davis (Stanford)

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Award in the Biomedical Sciences

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Searle Scholar's Award

Hellman Faculty Award

NSF CAREER

DOE NSET

DOE Biomolecular Materials

NuvoMetrix Inc.

Center Participation

CPIMA NSF (Stanford)