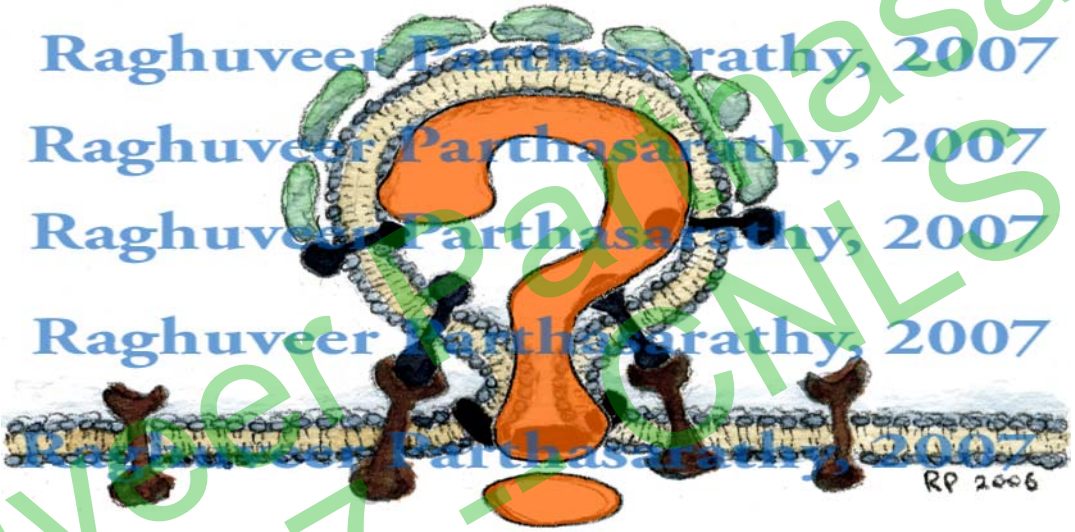
Curvature and spatial organization in biological membranes



Raghuveer Parthasarathy

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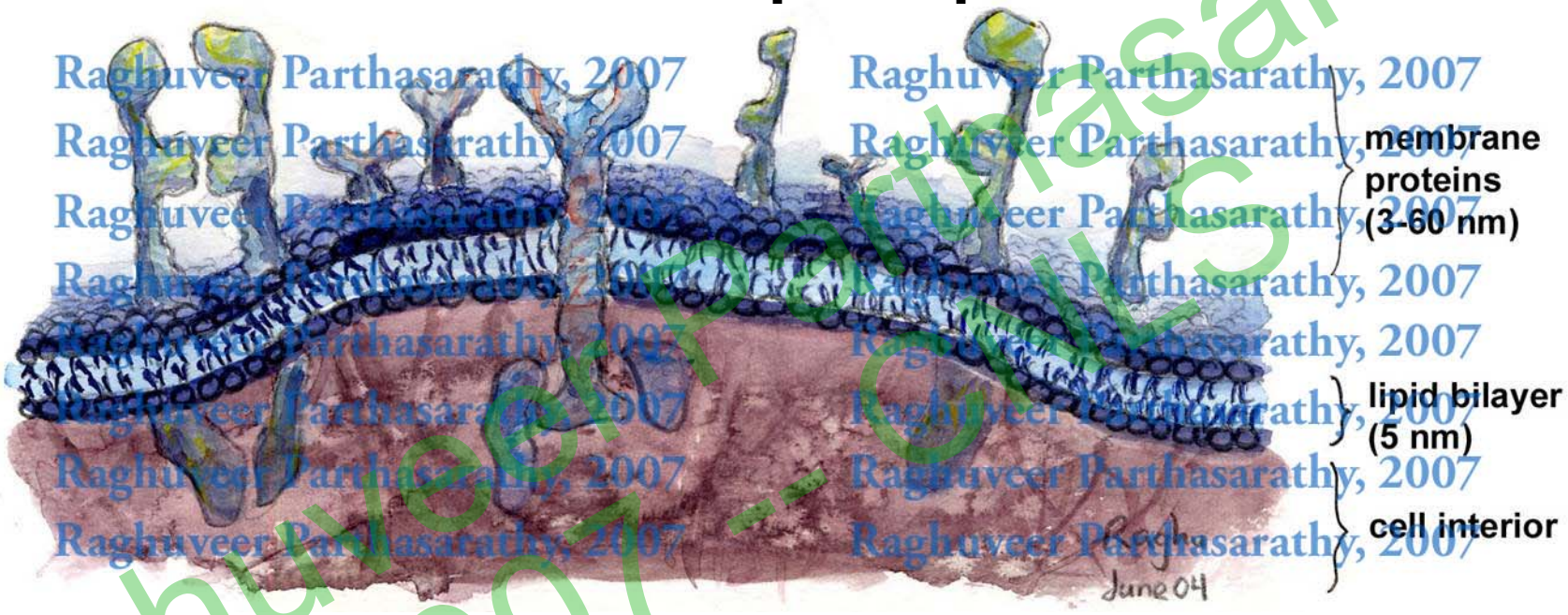
The University of Oregon

See **: R. Parthasarathy and Jay T. Groves, *Soft Matter* 3, 24-33 (2007)**

membrane properties

Cellular membranes: *Active* participants in cell functions



Physical properties → biological consequences

- **2D fluidity**
- **Spatial heterogeneity**
- **Curvature**

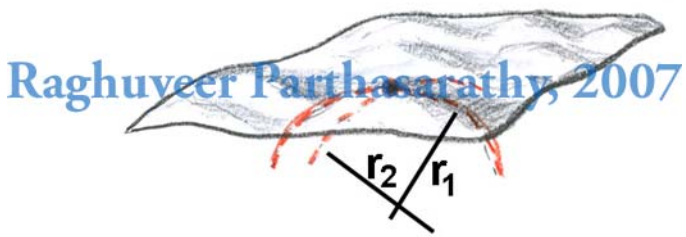
curvature

Membranes bend & curve in a variety of contexts

Proteins & lipids can control curvature
Curvature can control protein & lipid organization

Membrane mechanics* ↔ *membrane biochemistry
Bending → mechanisms for long-range spatial patterning

membrane bending energetics



principle curvatures

$c_1 = 1/r_1$, $c_2 = 1/r_2$

Bending Energy (per unit Area):

$$E_c = (1/2)\, k_c\, (c_1 + c_2 - 2c_0)^2 + k_G\, c_1\, c_2$$

spontaneous curvature: c_0

bending modulus: k_c, **Gaussian modulus:** k_G

membrane bending energetics

$k_c \sim 10^{-19}$ J = 20 $k_B T$

- Difficult, imprecise measurements: micropipette aspiration, observation of thermal fluctuations
- (*New methods: driven fluctuations?*)

k_G? Even more poorly characterized.

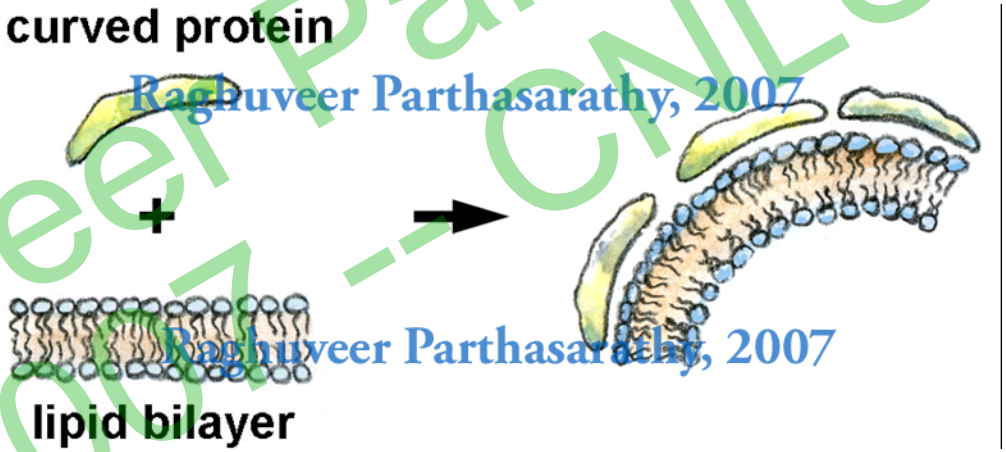
- k_G ?≈ -0.8 k_c – Siegel & Kozlov, *Biophys. J.*, 2004, 87, 366-374.

curvature: short length scales

Curvature at short length scales

- a variety of mechanisms
- lipid, protein shapes are important

e.g.



- qualitatively (not quantitatively) understood

At large length scales, still less is known…

R. Parthasarathy and Jay T. Groves, *Soft Matter* 3, 24-33 (2007) & Refs. therein

curvature at *large* length scales

At large length scales, still less is known about couplings between composition, curvature

Collective properties – different responses to curvature?

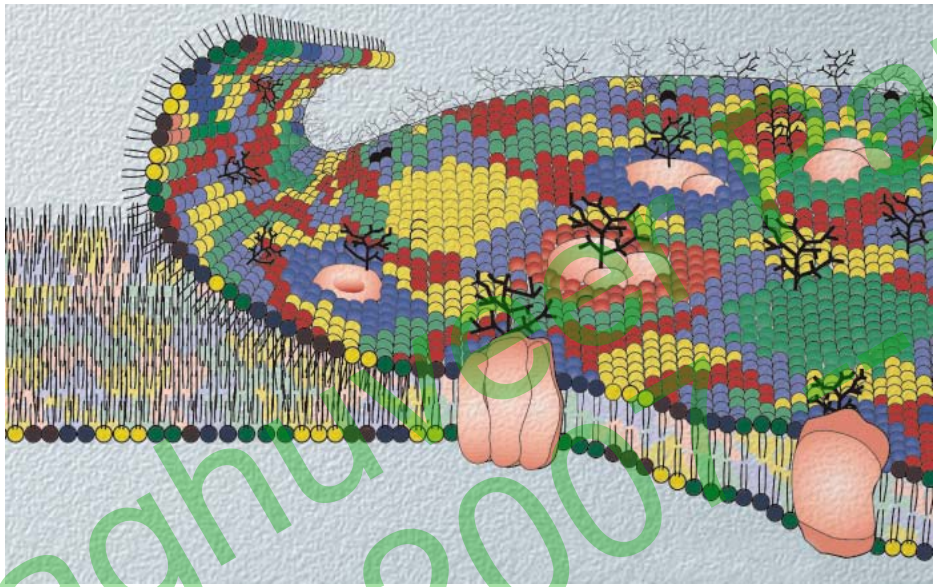
Recent experiments: *Yes*.

curvature and phase separation

Curvature and Phase Separation in Lipid Membranes

membrane microdomains

Cellular membranes are spatially heterogeneous in composition – membrane microdomains:

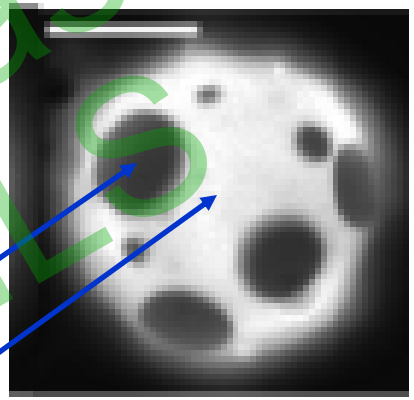


M. Edidin, *Nat. Rev. Mol. Cell Biol.* 4, 414-418 (2003)

See refs cited: R. Parthasarathy and Jay T. Groves, *Soft Matter* 3, 24-33 (2007).

phase separated domains

Cholesterol-dependent phase separation:



Bar = 20 μm

S.L. Veatch & S.L. Keller, *Phys. Rev. Lett.* 89, 268101 (2002)

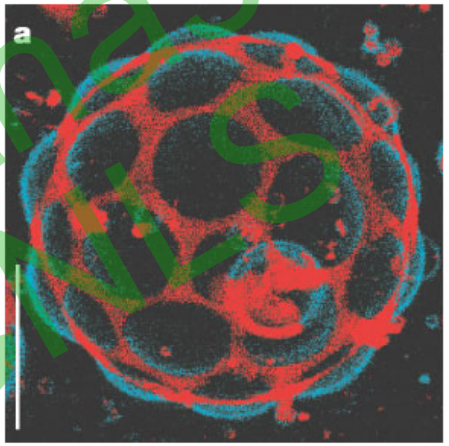
e.g. Ternary mixtures: Saturated lipids (DPPC), unsaturated lipids (DOPC), cholesterol

→ Liquid Ordered (L_o) and Liquid Disordered (L_d) phases

phase separation → curvature

Domains in giant vesicles (Webb[1], Schwille[2], & others)

→ "Bulging," differential curvature



Bar = 5 μm; from [1]

Two mechanisms:

- **differential rigidity**
- **line tension (*relevant*?)**

R. Parthasarathy, 2007

Line tension (alone) → bulging

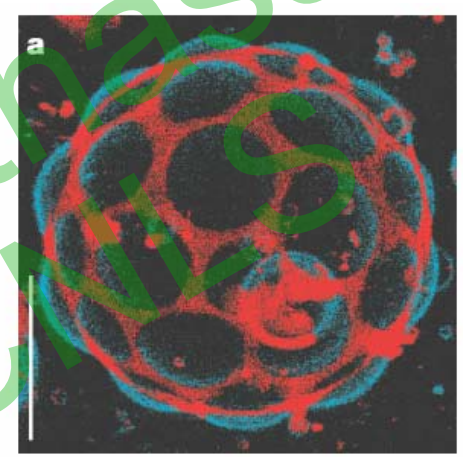
[1] T. Baumgart, S. T. Hess and W. W. Webb, *Nature*, 2003, 425, 821-824.

[2] K. Bacia, P. Schwille and T. Kurzchalia, *PNAS*, 2005, 102, 3272-3277.

phase separation → curvature

Domains in giant vesicles (Webb[1], Schwille[2], & others) →

"Bulging," differential curvature

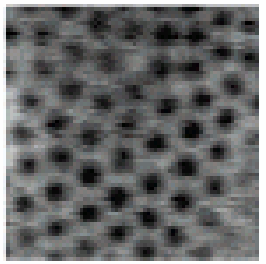


Bar = 5 μm;

from [1]

Strange sterol dependence [2]

Long-range domain ordering [3] ⟶



5 μm

[1] T. Baumgart, S. T. Hess and W. W. Webb, *Nature*, 2003, 425, 821-824.

[2] K. Bacia, P. Schwille and T. Kurzchalia, *PNAS*, 2005, 102, 3272-3277.

[3] S. Rozovsky, Y. Kaizuka and J. T. Groves, *JACS.*, 2005, 127, 36-37.

curvature → phase separation

Converse: Can curvature control domain organization?!

How is phase separation spatially organized?

Quantitative experiments linking curvature and chemical composition require:

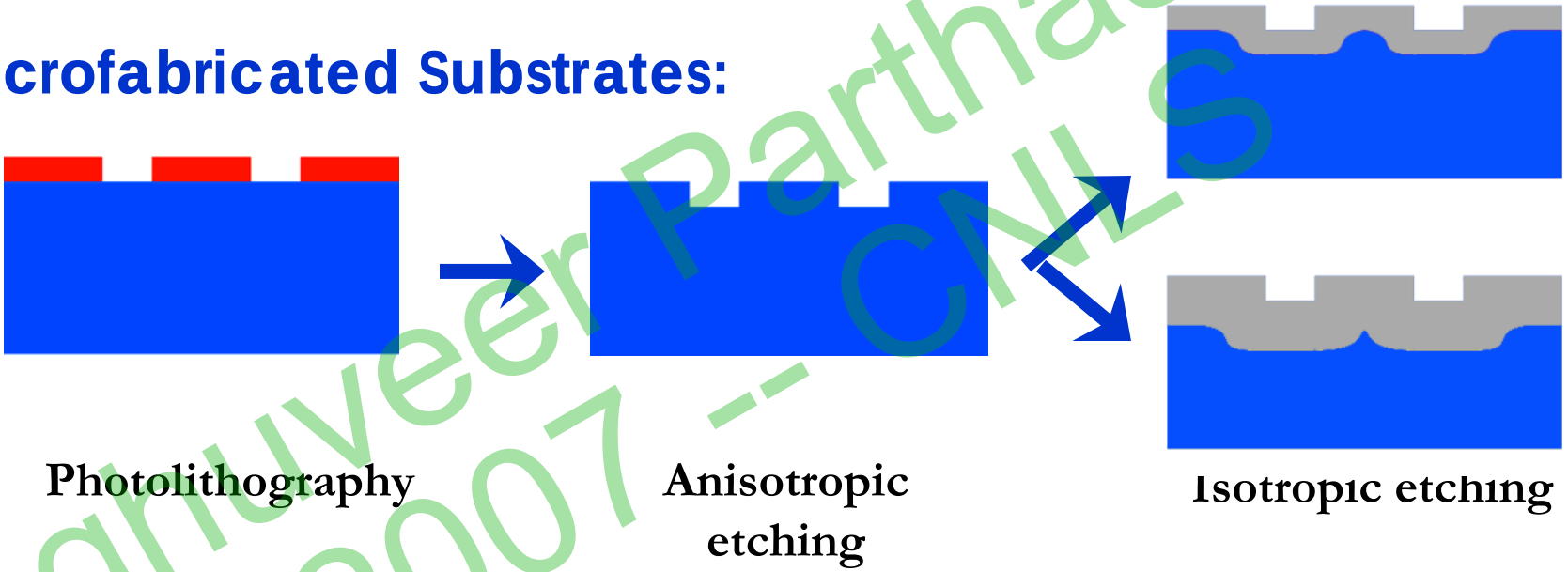
- **Membranes with well-understood phase behavior**
- **Specific mechanical deformations**

R. Parthasarathy, C. Yu and J. T. Groves, *Langmuir*, 2006, 22, 5095-5099

substrate-controlled curvature

Goal: imposing specific curvatures onto phase-separated lipid membranes

Microfabricated Substrates:



Controlled etching → controlled curvature

Measure by AFM

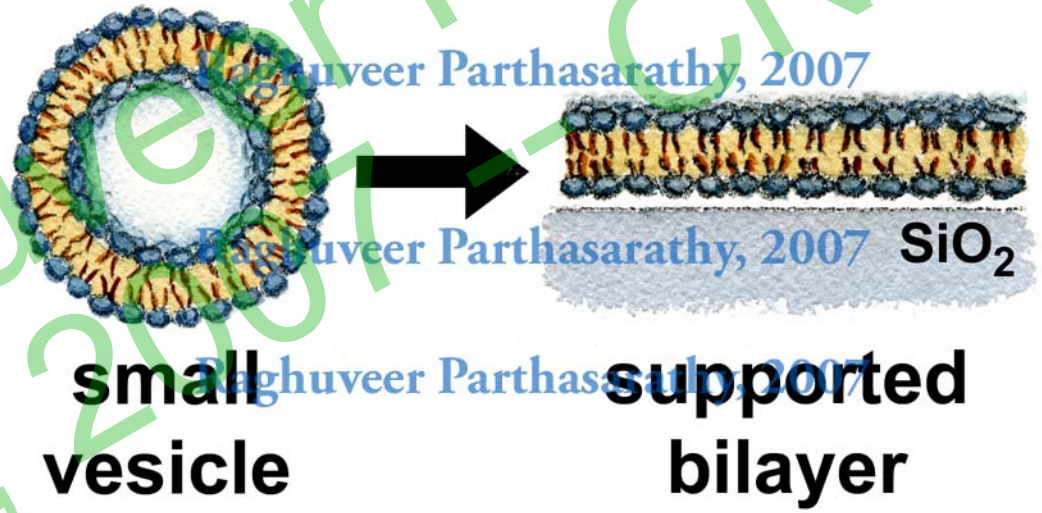
Range: flat to r ≈ 100nm

double membrane system (1)

Double membrane system

Lower membrane:

- formed by vesicle fusion
- spatially uniform (~DMPC)



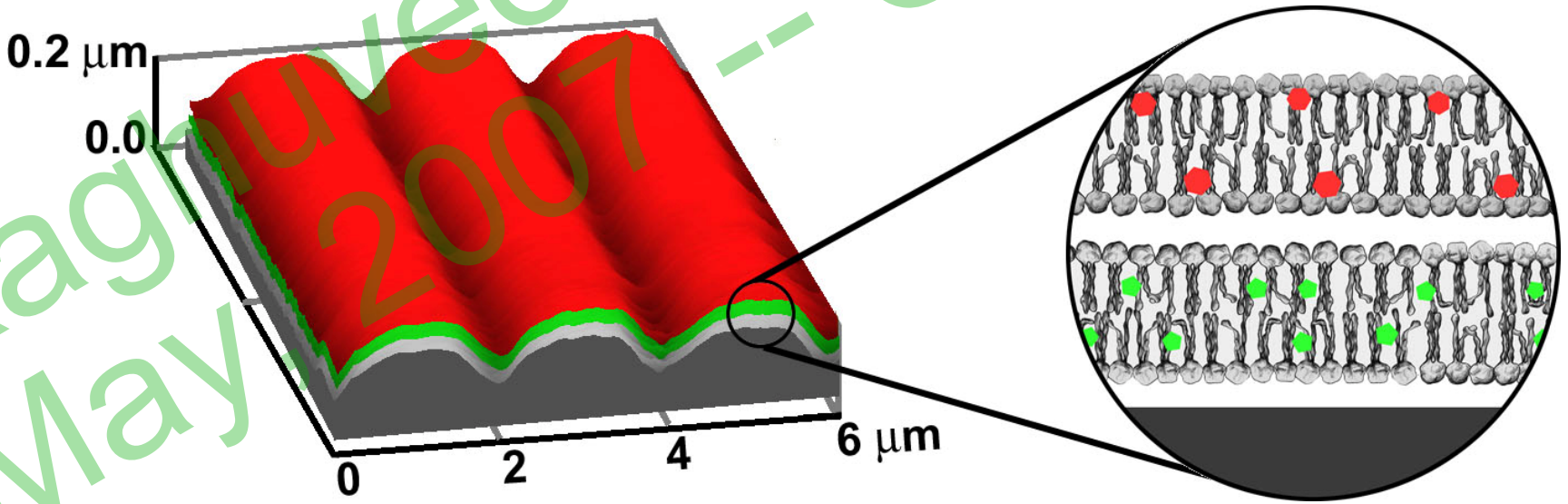
Fluidity unaffected by substrate topography (isotropic, same *D*)

double membrane system

Double membrane system

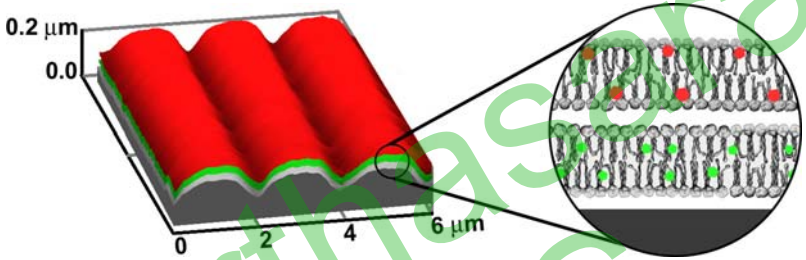
***Upper membrane*:**

- **formed by giant vesicle rupture**
- **phase separation**
- **decoupled from substrate – *important***

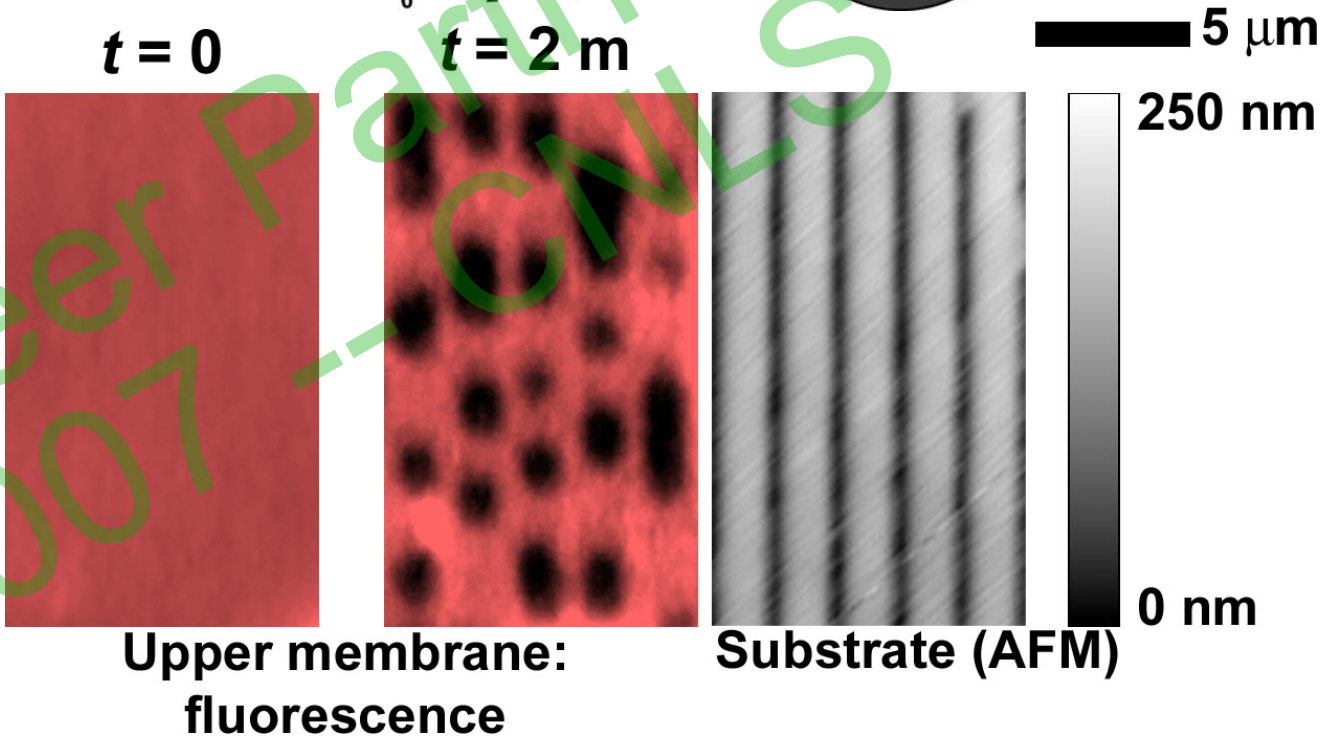


R. Parthasarathy, C. Yu and J. T. Groves, *Langmuir*, 2006, 22, 5095-5099

curvature guides phase separation



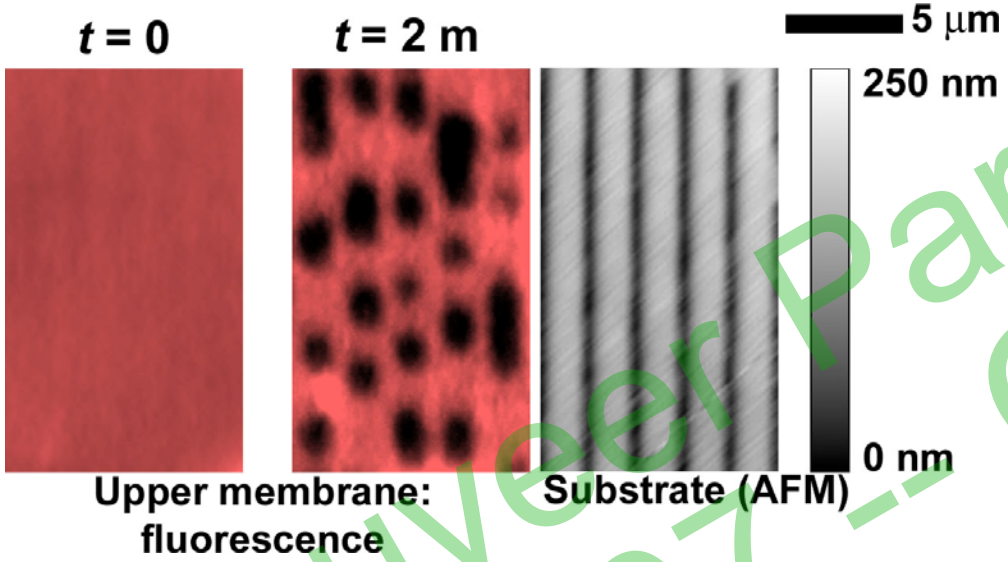
L_o domains **align with** and **elongate along** topographic plateaus!

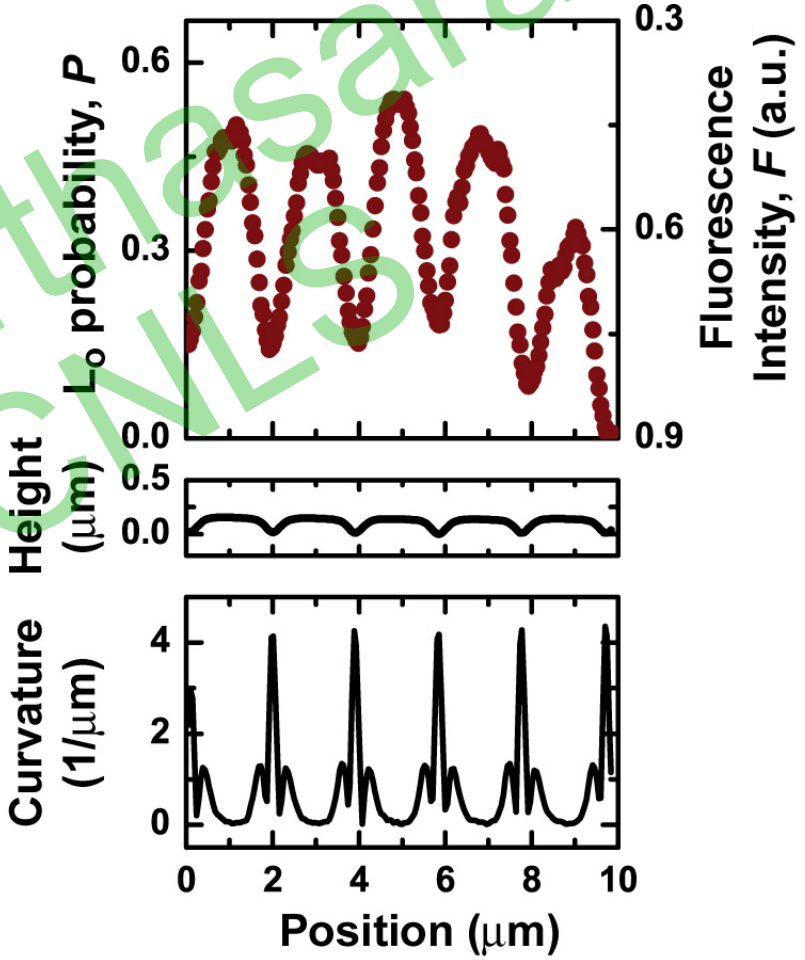


FRET: contact between membranes

R. Parthasarathy, C. Yu and J. T. Groves, *Langmuir*, 2006, 22, 5095-5099

curvature guides phase separation





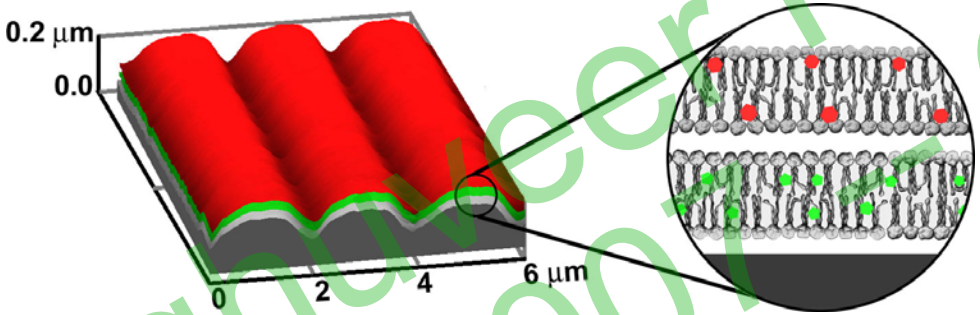
L_o domain positions controlled by the topography – preference for low curvature regions

What does this tell us?

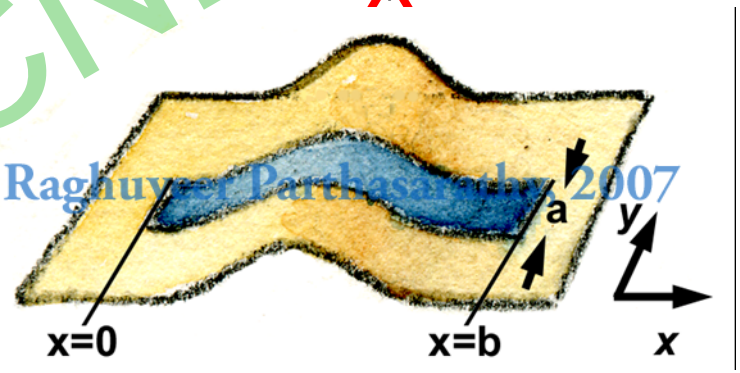
1D curvature

Substrate-induced curvature

- **Quantitative**
- **Highlights particular deformation modes**



line tension ~~→~~ curvature



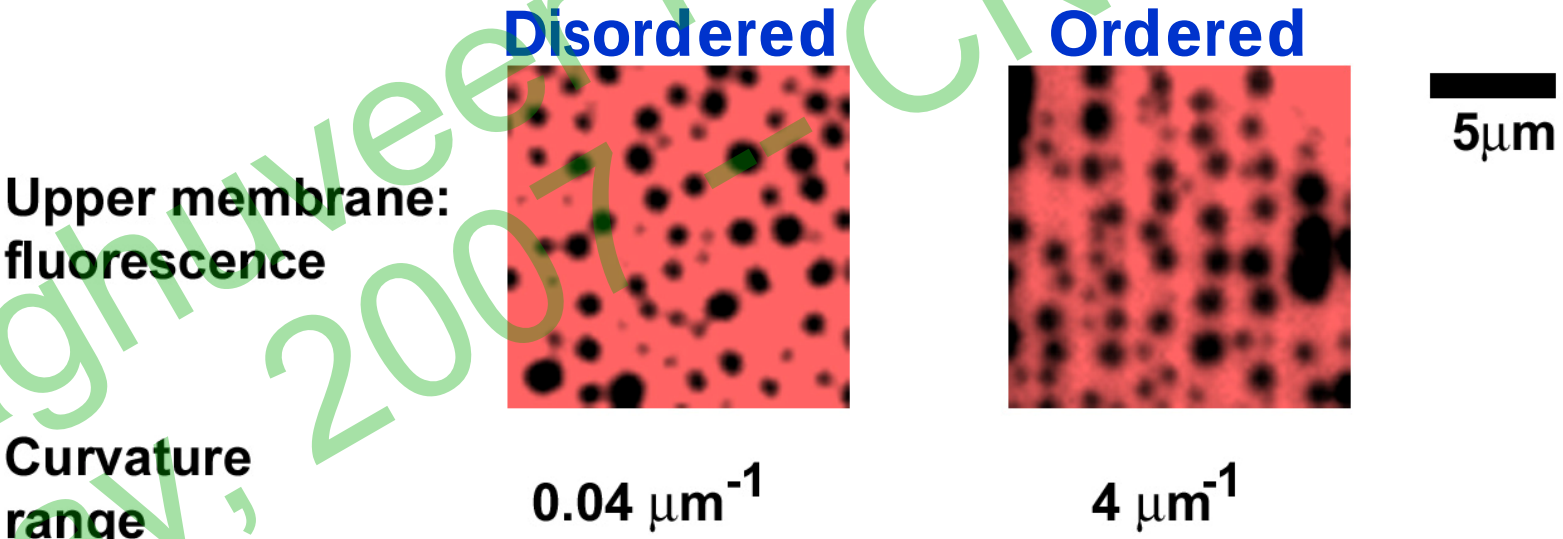
One-dimensional curvature → line tension irrelevant; only bending rigidity differences matter

(Also, Gaussian curvature = 0)

critical curvature

A critical membrane curvature $c^* = 0.8 \pm 0.2\ \mu m^{-1}$ is necessary to spatially organize the phases

Substrates with curvature range 0 to *c*:



R. Parthasarathy, C. Yu and J. T. Groves, *Langmuir*, 2006, 22, 5095-5099

rigidity difference of membrane phases

Measurement of c* allows determination of the difference in bending rigidity between phases ($\Delta\kappa$):

Difference in bending energy $E_b = A\,(\Delta\kappa/2)\,c^2$

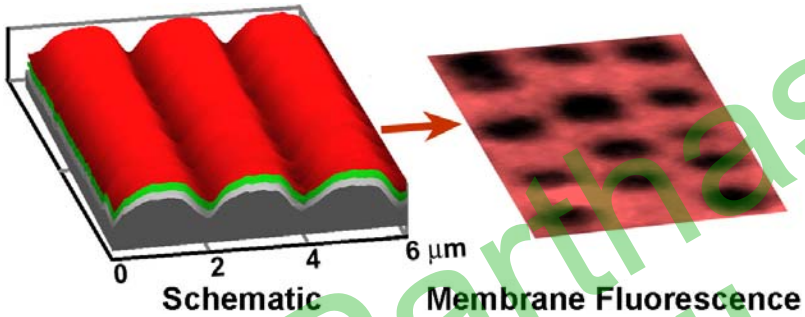
must exceed thermal energy, k_BT:

$$A\,(\Delta\kappa/2)\,c^{*2} = k_BT$$

$$\rightarrow \quad \Delta\kappa = 1.2 \pm 0.6 \times 10^{-20}\ \text{J (with } A = 1\ \mu\text{m)}$$

In cells, $A \approx 0.01\ \mu m^2$, so $r^* = 1/c^* = 100$ nm, curvatures sharper than this should affect local composition!

conclusions (part 1)



Conclusions

- **Curvature , beyond a critical value, can direct the spatial organization of lipid domains**
- **Response to (1D) curvature allows extraction of membrane mechanical properties ($\Delta\kappa$)**

***Future*: composition, protein sorting, kinetics, other 2D materials**

inter-membrane junctions

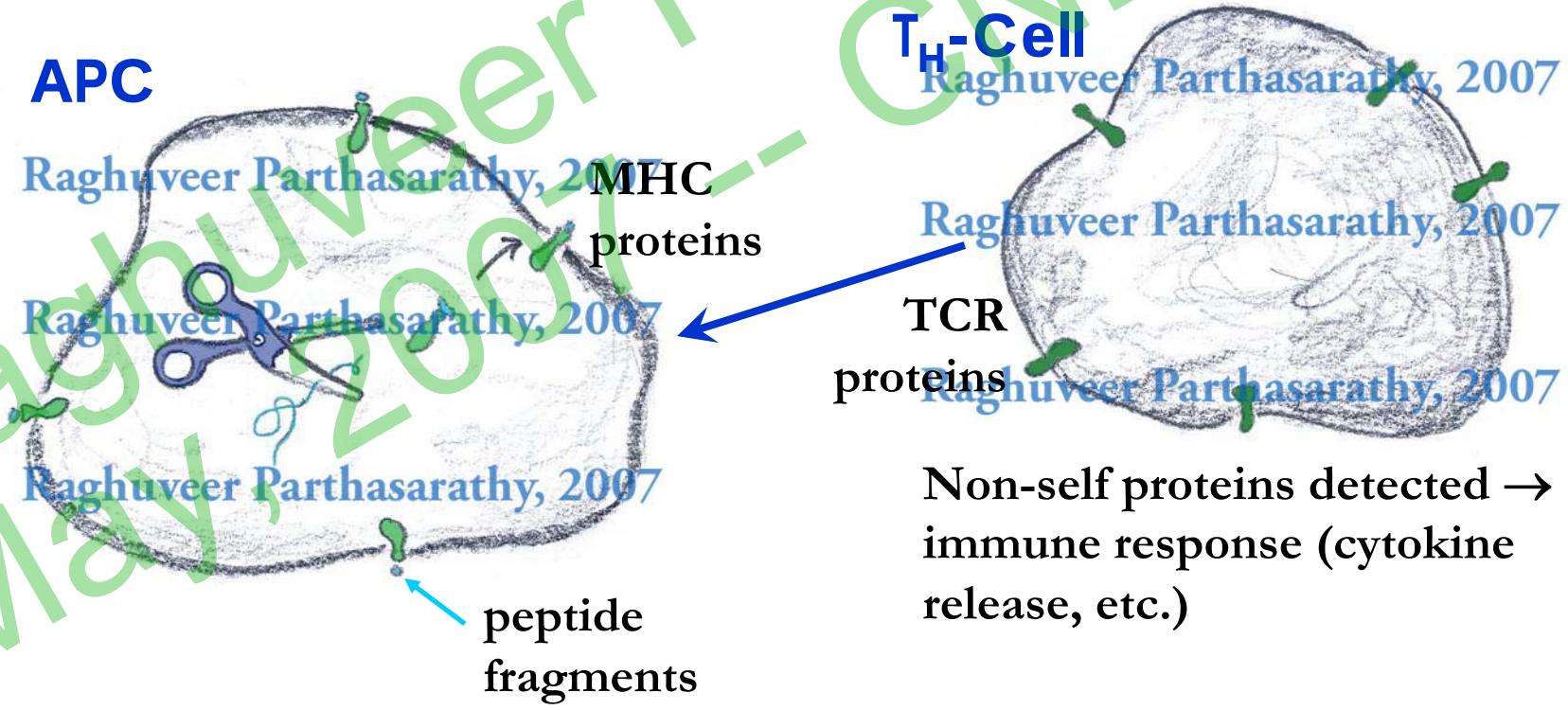
Another class of phenomena involving membrane topography...

Membrane Mechanics at Inter-Membrane Junctions

the immunological synapse

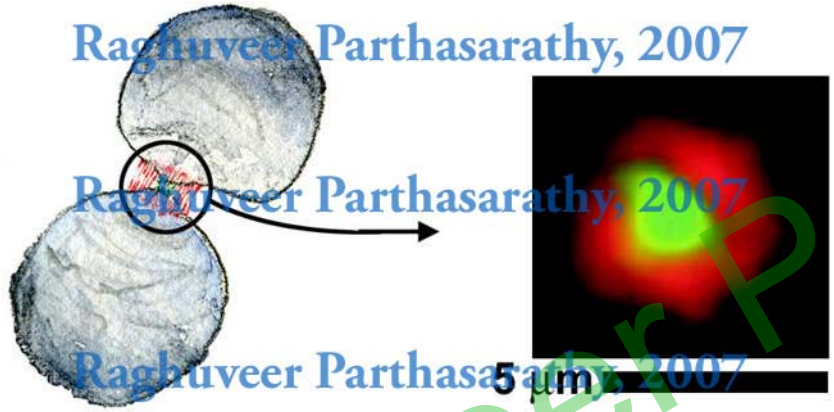
Communication at inter-cellular contacts

The immunological synapse between helper T-cells and Antigen-Presenting Cells (APCs)



the immunological synapse

The **immunological synapse**



***Green (center)*: signaling proteins (TCR / MHC)**

***Red (ring)*: Adhesion proteins (LFA / ICAM)**

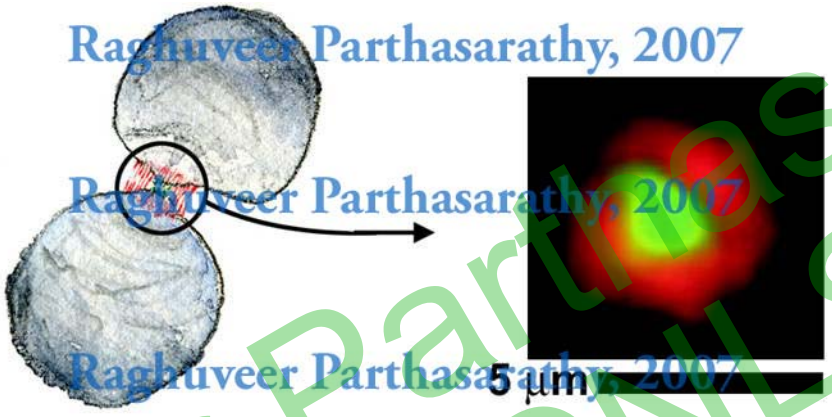
Long-range spatial organization!

Correlated with T-cell activation.

How is it controlled?...

Data from A. Grakoui, ... M. L. Dustin, *Science*, 1999, 285, 221-227.

driving the immunological synapse



What drives protein motions?

(1) "Active" cytoskeletal forces pulling TCR proteins

- **Actin depolymerization inhibits synapse formation**
- **Tracking of TCR clusters shows directed motion [1]**

(2) "Physical," membrane-mediated forces...

[1] K. Mossman and J. Groves, *Chem. Soc. Rev.*, 2007, 36, 46-54;
K. Mossman *et al. Science* 2005, 310, 1191-1193.

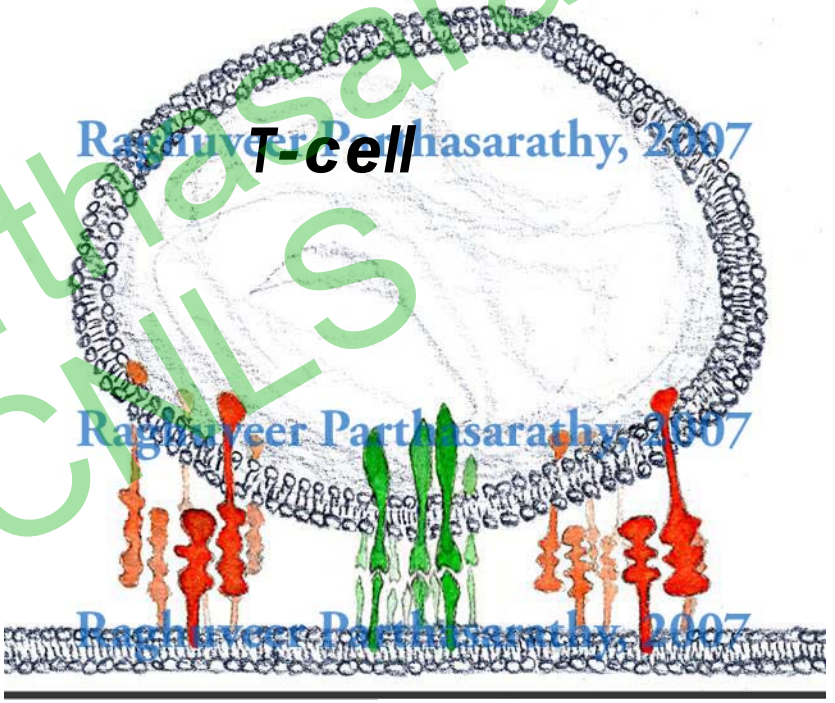
driving the immunological synapse

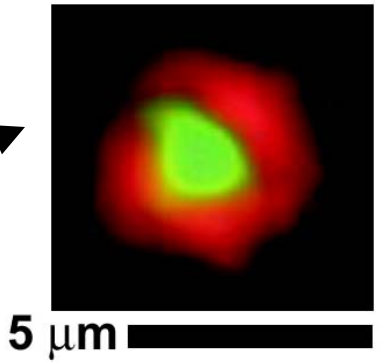
(2) Physical, membrane-mediated forces

- **APC isn't necessary:**

  T-cell / supported bilayer synapse! [1] MHC, ICAM at bilayer

(also, substrates with patterned barriers! [2])





[1] A. Grakoui, ... M. L. Dustin, *Science*, 1999, 285, 221-227.

[2] Mossman *et al. Science* 2005, 310, 1191-1193.

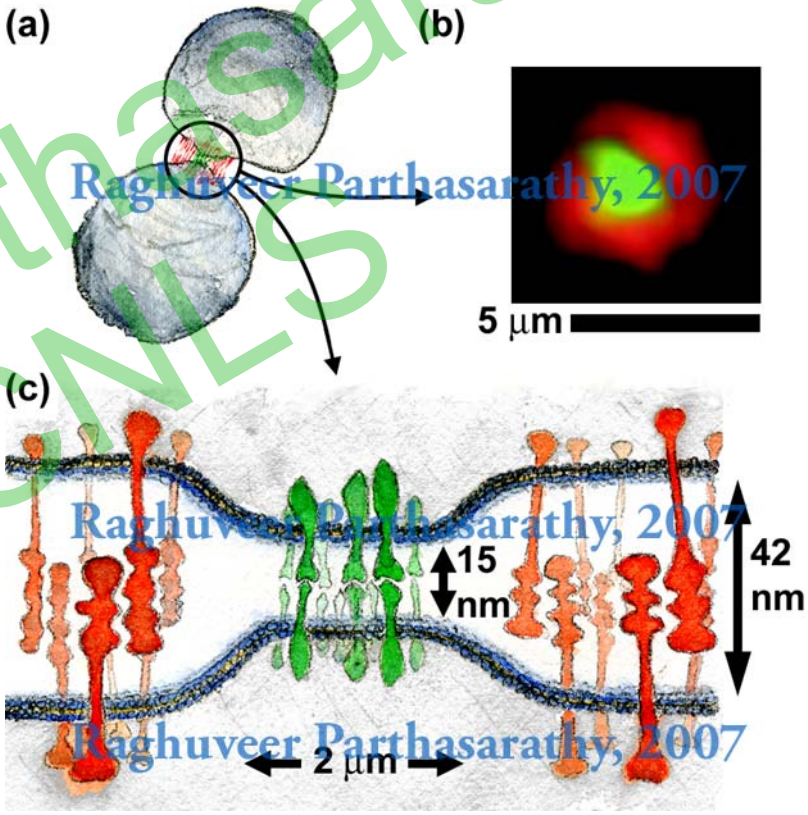
the immunological synapse

(2) Physical, membrane-mediated forces

- **APC isn't necessary**
- **Synapse topography itself suggests physical mechanisms**

  modeling*: passive mechanisms alone → synapse

  ***experiments*...**



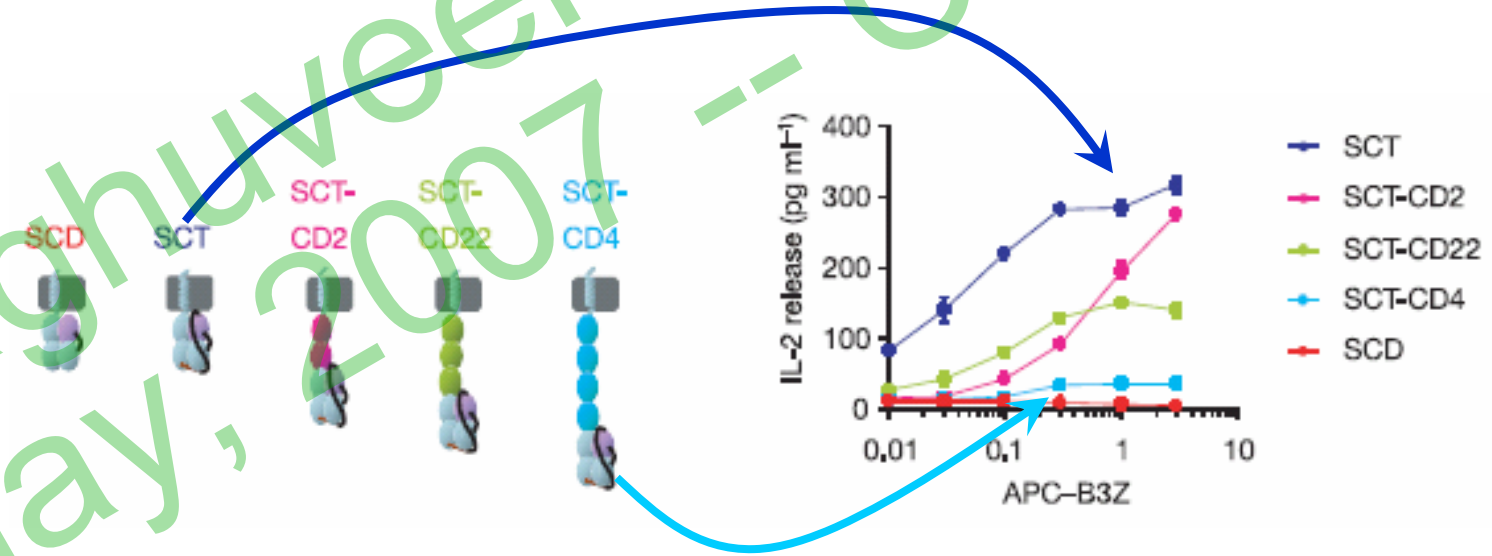
* See refs cited: R. Parthasarathy and Jay T. Groves, *Soft Matter* 3, 24-33 (2007).

T-cell experiments: engineered MHC

Engineered MHC proteins:*

Longer MHC →

- **reduced T-cell triggering (less cytokine production)**
- **less exclusion of large proteins (CD45) from the synapse center – *normally pushed aside by TCR/MHC?***



* K. Choudhuri , ... P. A. van der Merwe, *Nature*, 2005, 436, 578-582

T-cell experiments: patterned substrates

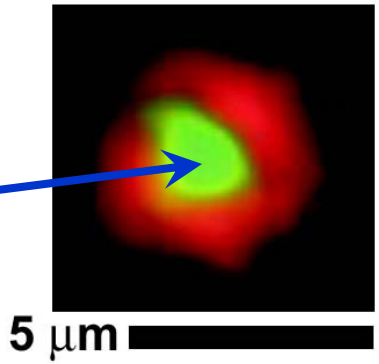
T-cells + Bilayers with MHC, ICAM

unpatterned substrates:

solid substrate

Next slide:

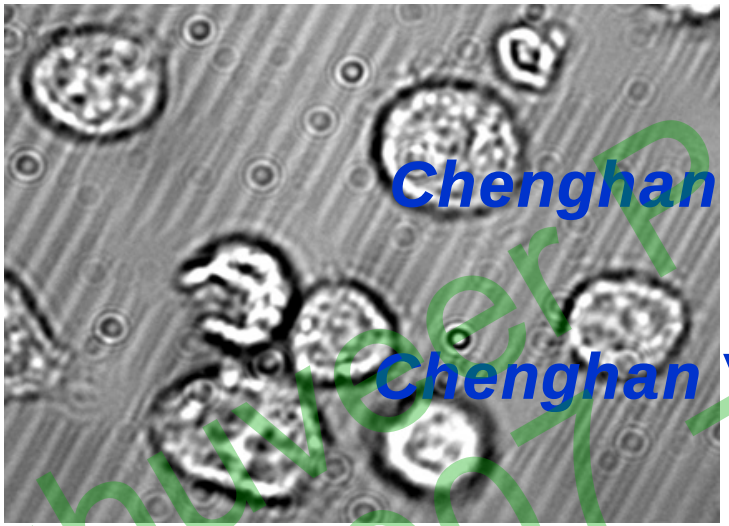
(green) TCR on T-Cell



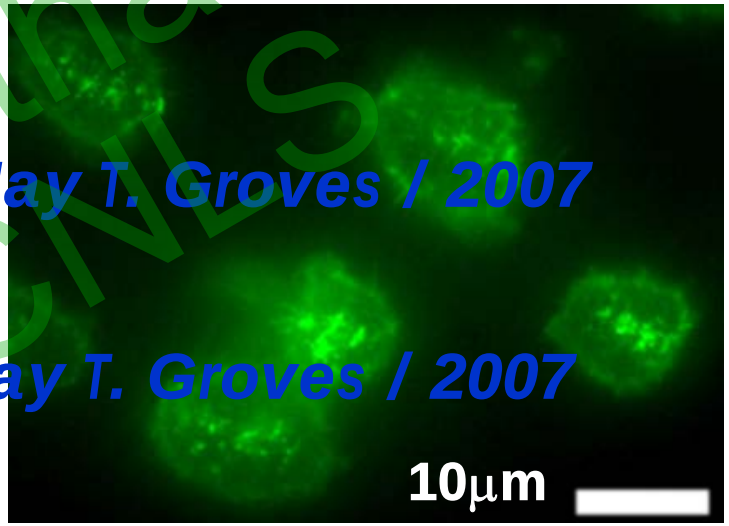
T-cell experiments: patterned substrates

T-cells + Bilayers with MHC, ICAM on topographically patterned substrates:

BrightField



TCR – on the T-cells



Chenghan Yu / Jay T. Groves / 2007

Chenghan Yu / Jay T. Groves / 2007

Topographic control of protein distribution: TCR at plateaus

Subtle patterning (250 nm height, <4 μm^{-1} curvature) → strong influence on protein organization!

(Substrate curvature does NOT influence diffusion)

***Chenghan Yu* – preliminary data**

perspectives

Topographic patterning: influence on cell signaling?

Other synapses

- **Other immunological synapses: cytotoxic T-cells, natural killer cells, "naive" helper T-cells**
- **"Virological synapses"**
- **Neural synapses**
- **Others?**

Modeling – greater specificity needed

***Experimental Model systems*: Cell-free junctions...**

cell-free inter-membrane junctions

To characterize passive modes of protein organization:

cell-free inter-membrane junctions

Control / measure composition, mobility, topography, etc.

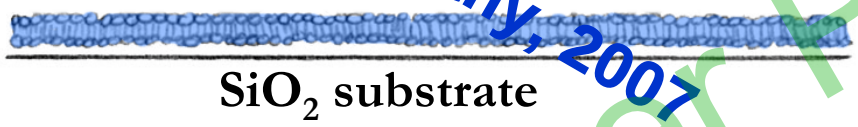
→ ***What sorts of structures can self-assemble? How?***

Pioneering work: Sackmann et al.*

Our setup*...

* See refs cited: R. Parthasarathy and Jay T. Groves, *Soft Matter* 3, 24-33 (2007).

inter-membrane junctions: setup



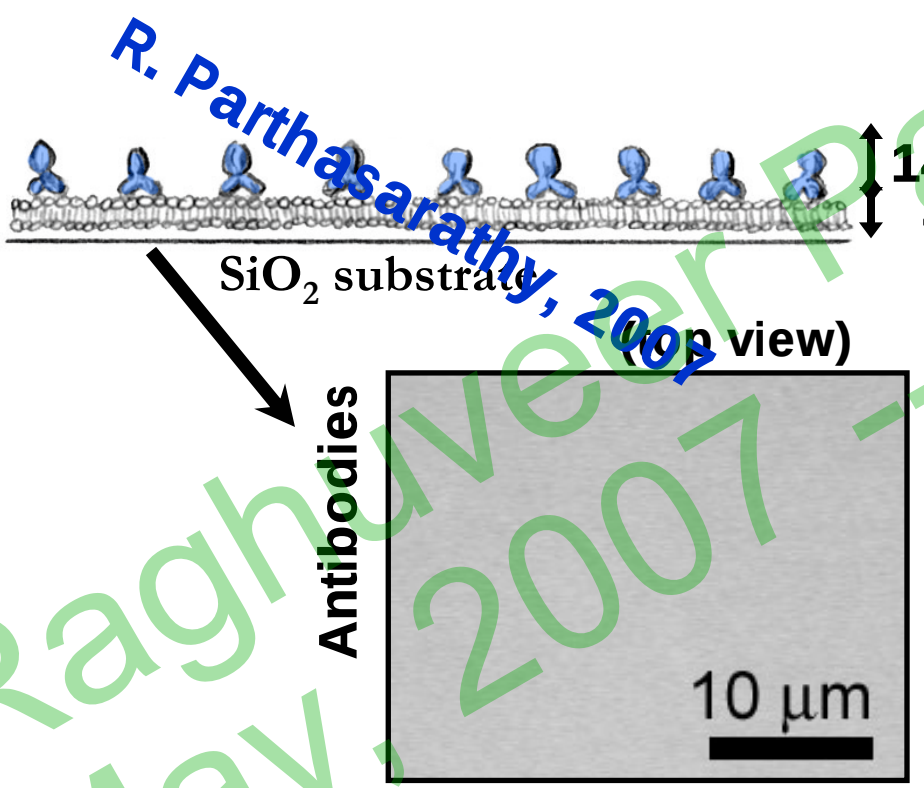
Setup:

- **Supported lipid bilayer**

4 nm [1% biotin-headgroups]

[Not to scale] [All in aqueous solution]

inter-membrane junctions: setup



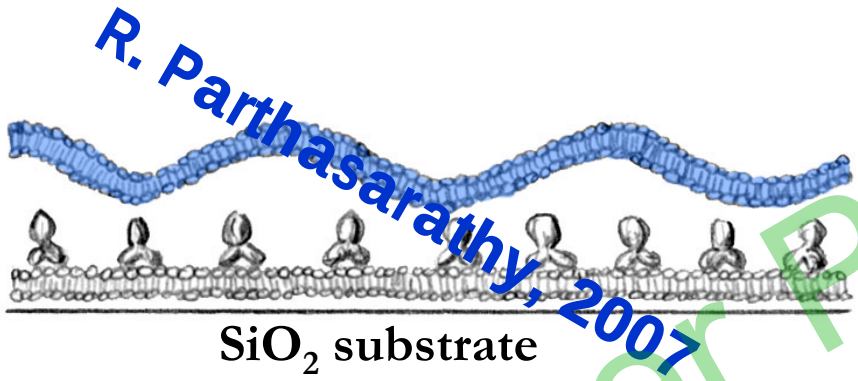
R. Parthasarathy, 2007

Setup:

- **Supported lipid bilayer**
  [1% biotin-headgroups]
- **Peripheral proteins**
  [Anti-biotin antibodies]

proteins (mobile, uniformly distributed)

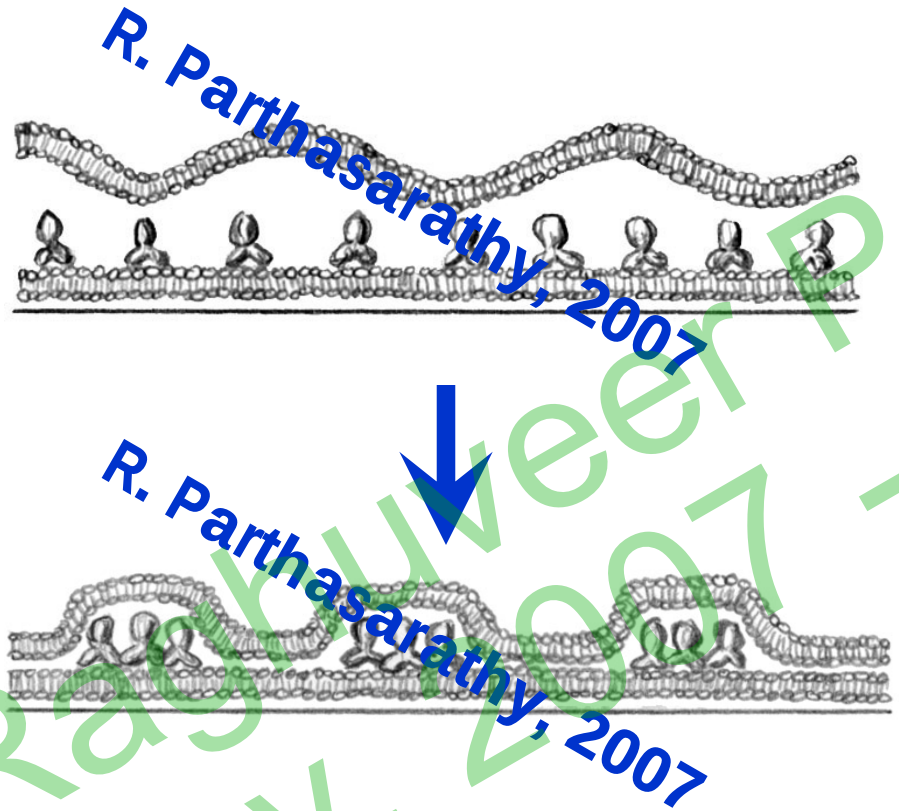
inter-membrane junctions: setup



Setup:

- **Supported lipid bilayer**
[1% biotin-headgroups]
- **Peripheral proteins**
[Anti-biotin antibodies]
- **Upper membrane:**
ruptured giant vesicle

inter-membrane junctions



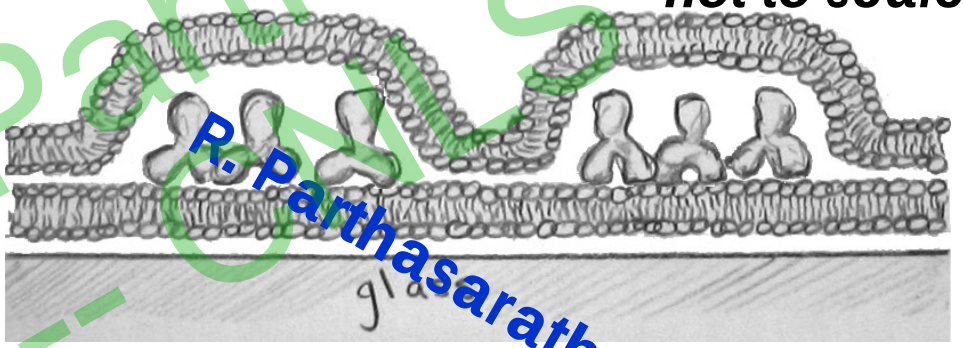
Upon junction formation,
protein reorganization

R. Parthasarathy and J. T. Groves, *PNAS*, 2004, 101, 12798-12803.
R. Parthasarathy and J. T. Groves, *J. Phys. Chem. B*, 2006, 110, 8513-8516

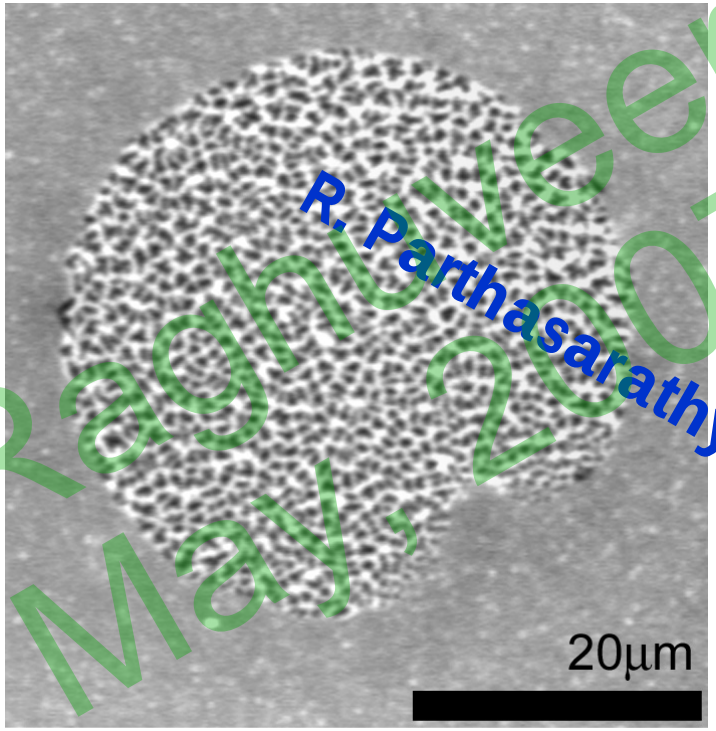
protein patterns

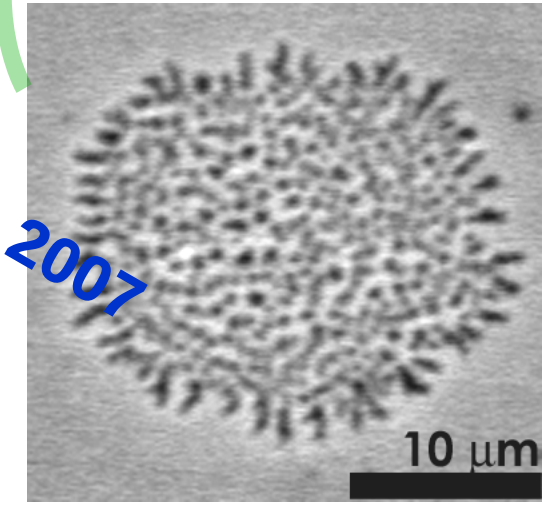
patterns **Adhesion of the second membrane leads to reorganization of the proteins**

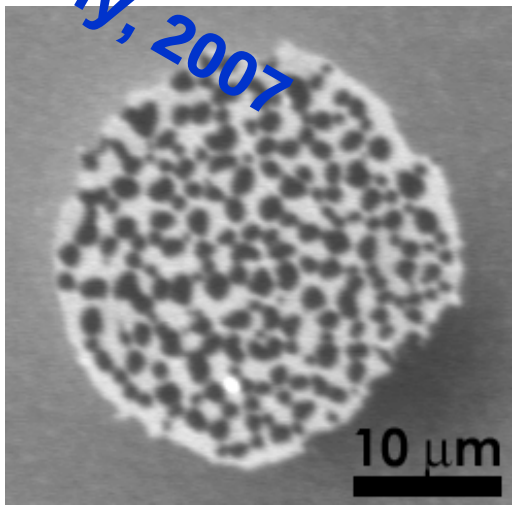
not to scale



Antibodies **(top view)**



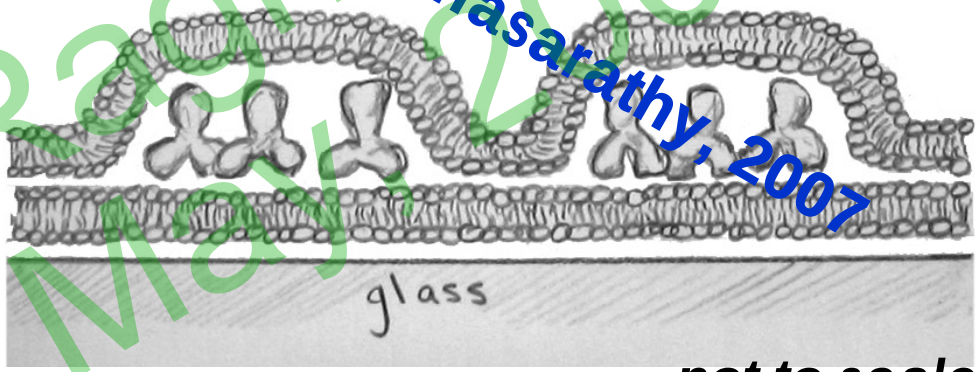




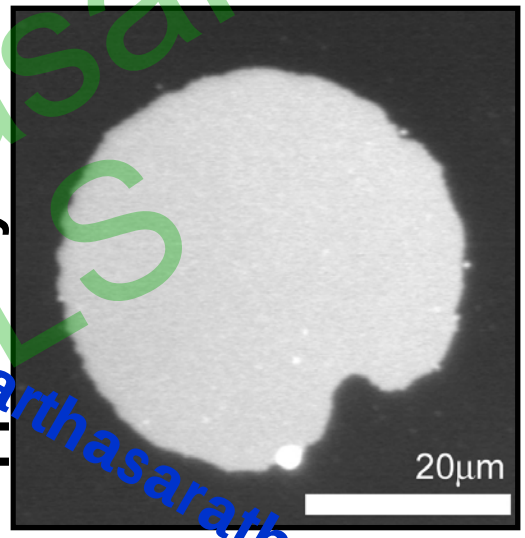
imaging: fluorescence

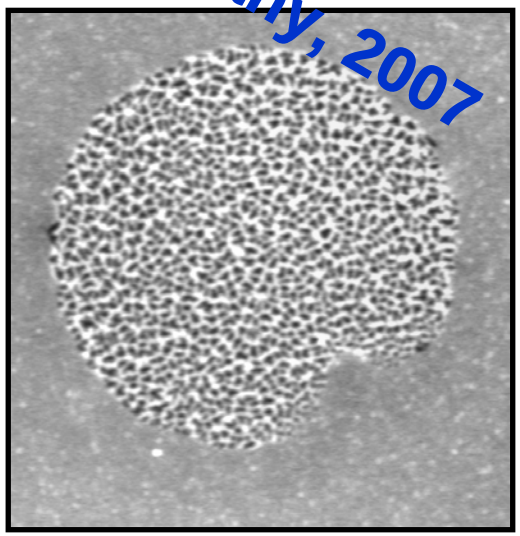
Simple fluorescence microscopy:
lateral organization of proteins, lipids

finite upper bilayer defines the intermembrane junction area



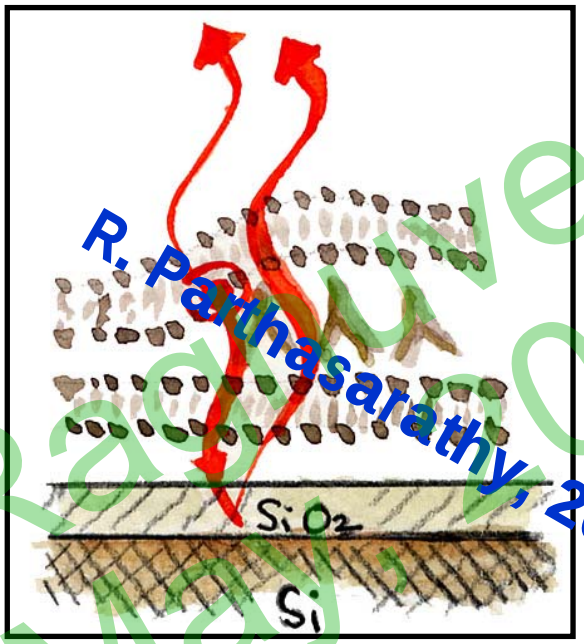
not to scale



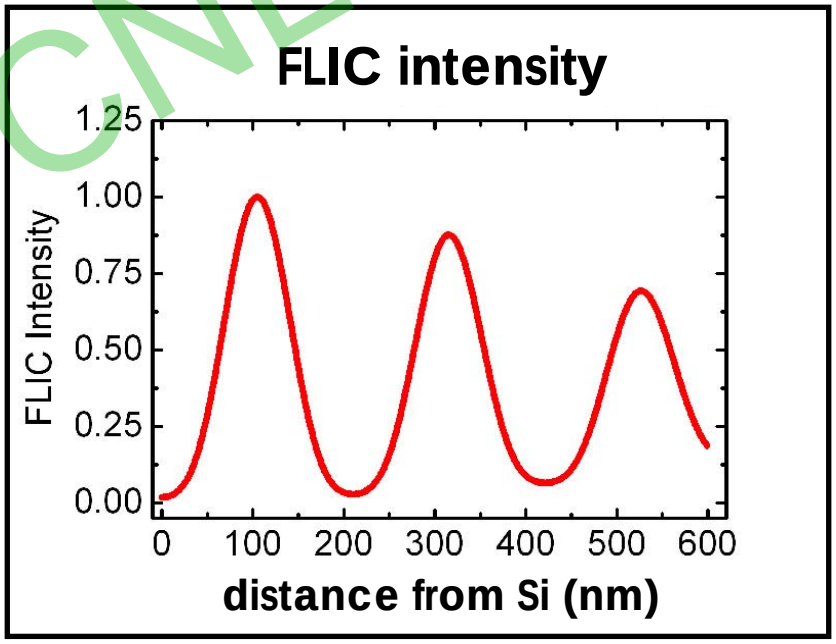


imaging: *FLIC*

- **FLIC (fluorescence interference contrast microscopy): topographic information in the few to hundreds of nm range (Fromherz *et al.*, 1990's)**
- **Interference → intensity maps topography**



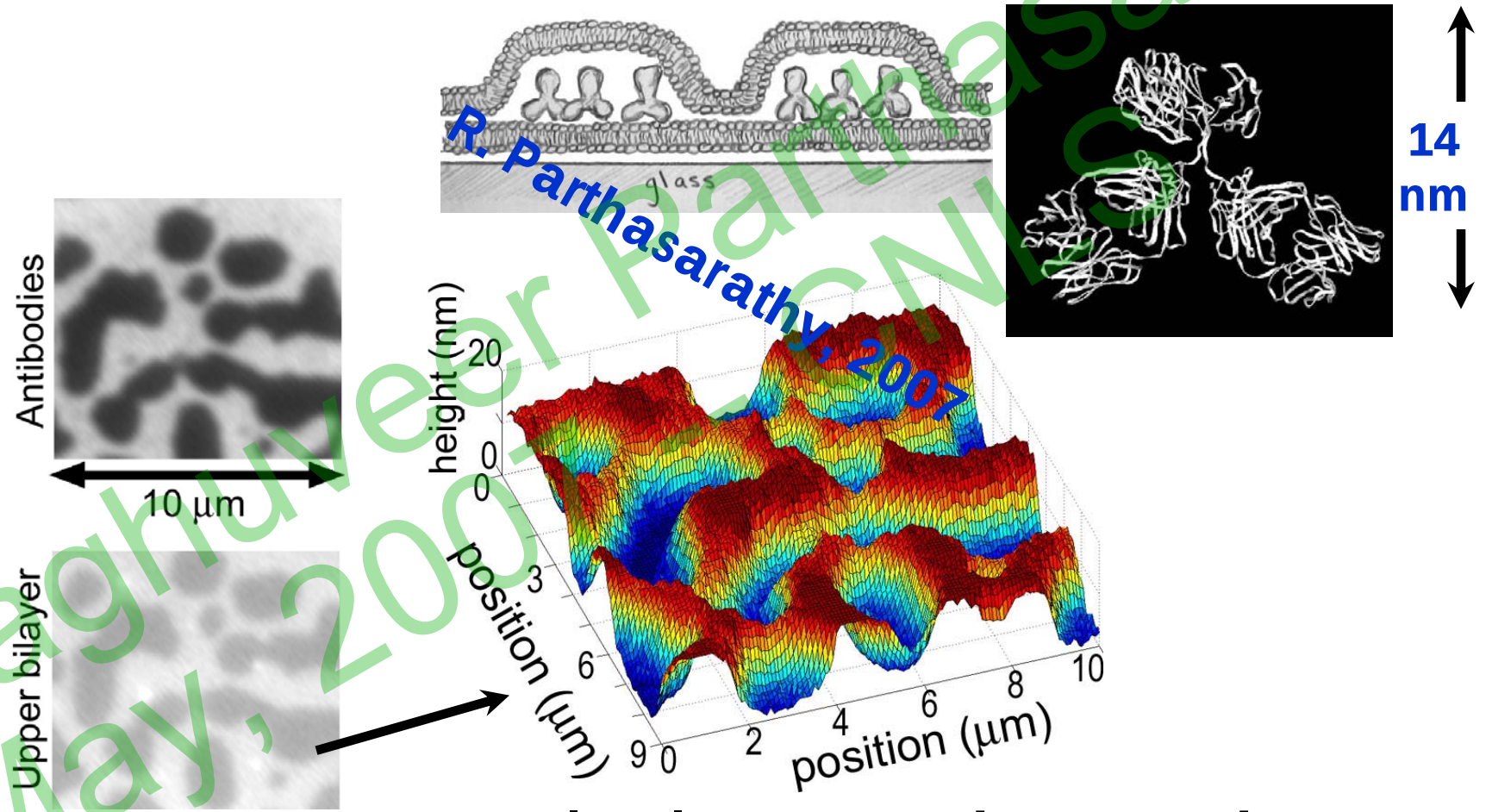
(optics)*



*** R. Parthasarathy and J. T. Groves, *Cell Biochem. Biophys.* 41: 391-414 (2004)]**

structure and imaging: *FLIC*

FLIC imaging → membrane topography, protein orientation



R. Parthasarathy, 2007

Also: lower membrane probes → *FRET*

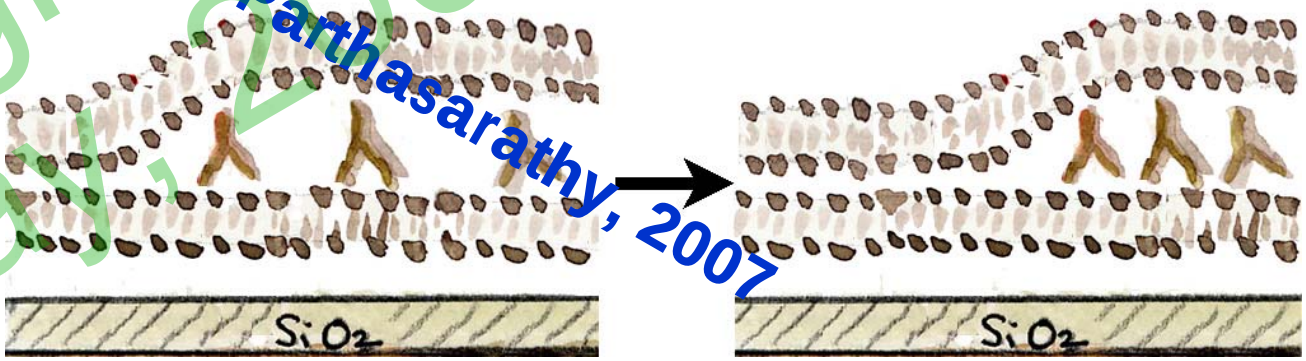
R. Parthasarathy and J. T. Groves, *PNAS*, 2004, 101, 12798-12803.

patterns: mechanisms

Protein reorganization is driven by:

bilayer-bilayer adhesion + protein mobility

- **adhesion is strong — pushing proteins aside**
- **but rapid — not enough time for global expulsion**



patterns: mechanisms

Micron length scale is set by:

membrane rigidity + protein mobility

- **upper membrane fluctuations as junction forms – timescale τ_m a function of wavelength, λ; bending modulus, κ_c**
- **protein motion over distance λ – timescale τ_p a function of mobility, membrane adhesion energy**

To couple, need $\tau_m(\lambda) > \tau_p(\lambda)$.

Satisfied for $\lambda > 1\ \mu m$!

R. Parthasarathy and J. T. Groves, *PNAS*, 2004, 101, 12798-12803.
R. Parthasarathy and J. T. Groves, *J. Phys. Chem. B*, 2006, 110, 8513-8516

outlook

Despite similarities of scale, shape, cell-free systems are so far too simple (compared to cellular synapses)

Needed: greater complexity; "real" adhesion proteins; control of adhesion strength, protein sizes!

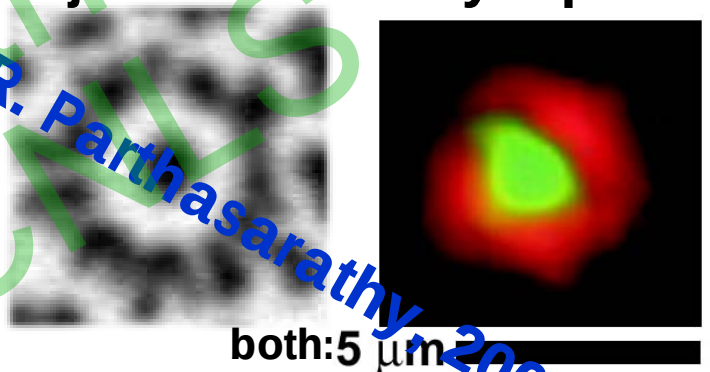
→? an understanding of the range of structures that can self-assemble at inter-membrane junctions.

More physical puzzles...

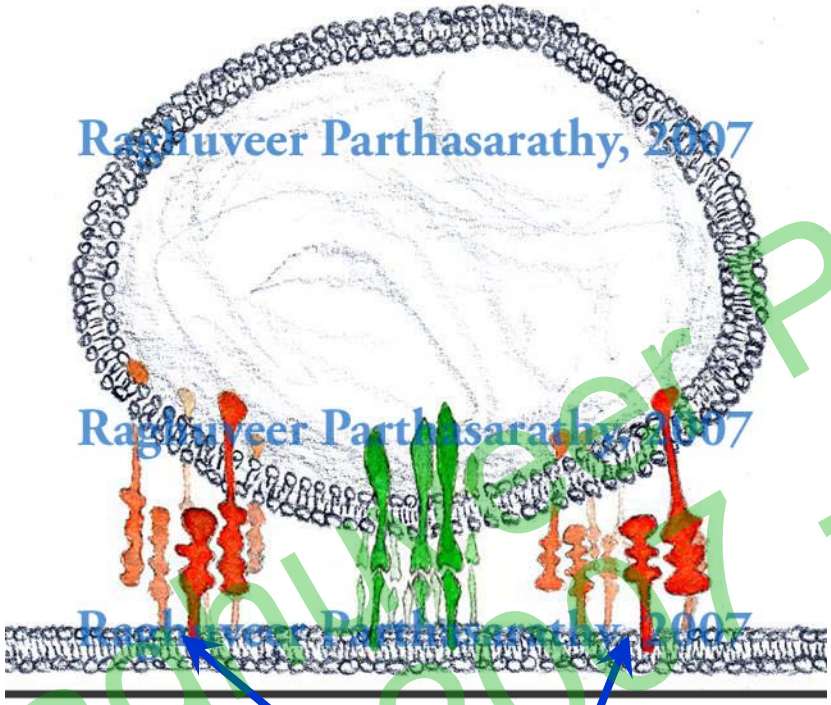
proteins at

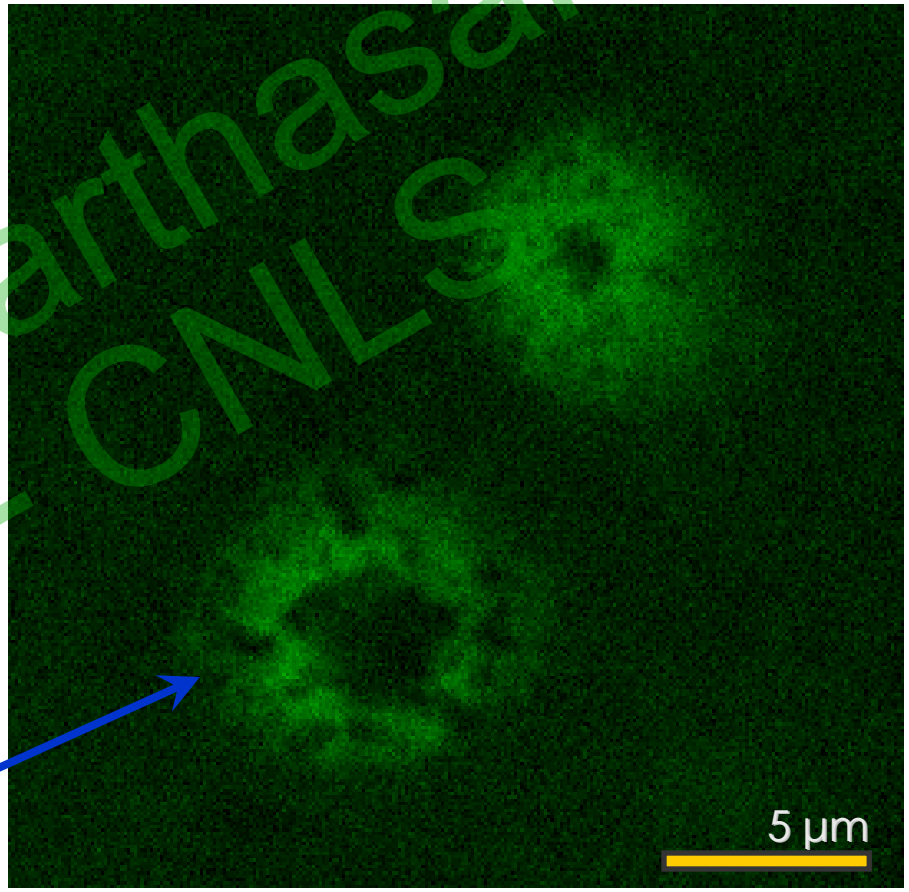
cell-free junction

T-cell/bilayer synapse



Immune Synapse: “holes” amid ICAM



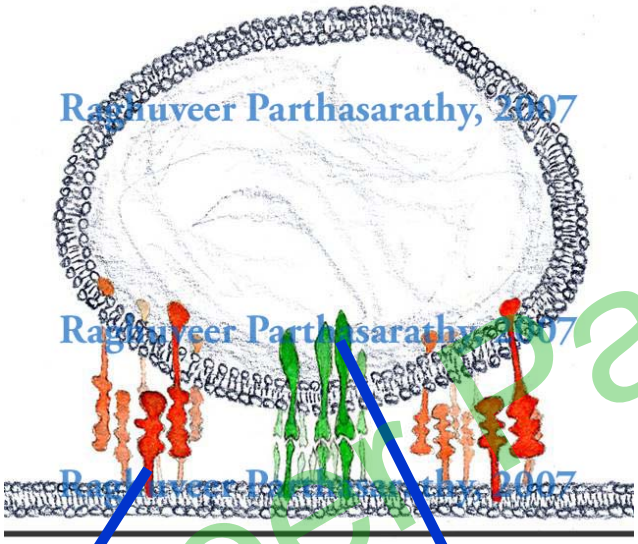


ICAM-YFP (Adhesion protein)

10s between frames

preliminary data from Jeffrey A. Nye

Immune Synapse: “holes” amid ICAM

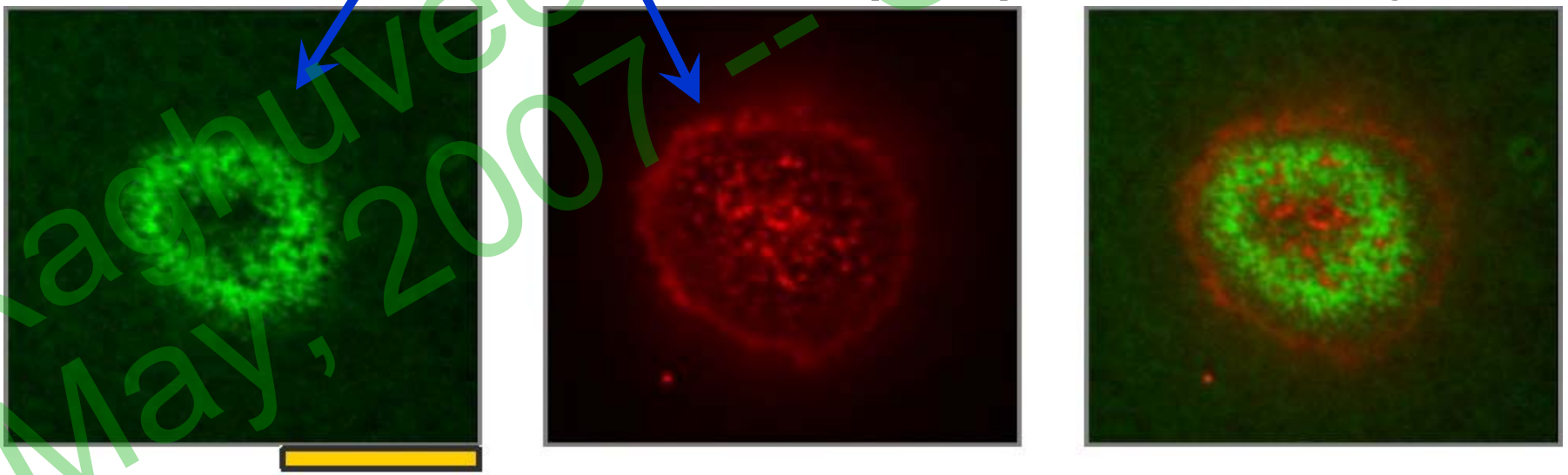


ICAM (bilayer)

TCR (T-cell)

preliminary data from Jeffrey A. Nye

Overlay



“Holes” ↔ TCR clusters

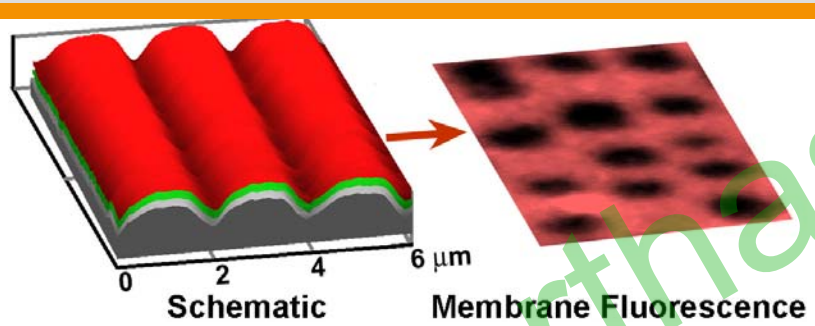
Immune Synapse: “holes” amid ICAM

preliminary data from
Jeffrey A. Nye

“Holes” ↔ TCR clusters – why? ?

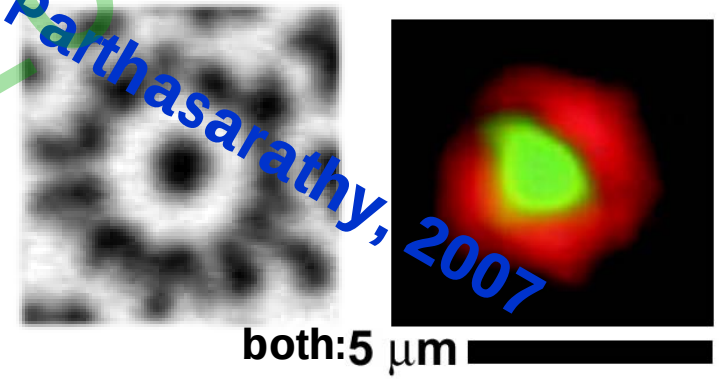
- dense TCR pushing proteins aside?
- topography: smaller TCR not permitting larger ICAM (like cell-free junctions?)

conclusions



At cellular membranes: ***chemistry + mechanics***

- **Curvature ↔ spatial organization of membrane molecules –** ***interfaces between "hard" & "soft" matter***
- **Membrane mechanics → long-range spatial organization –** ***cellular, cell-free, and, "hybrid" junctions***



acknowledgements

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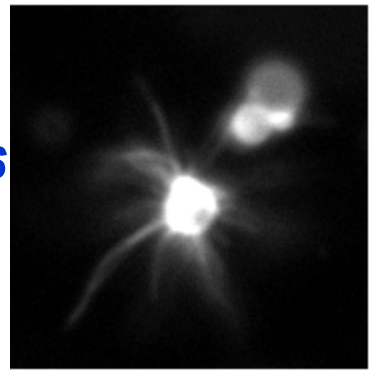
T-Cell Synapses:* Kaspar Mossman, Jeff Nye, Chenghan Yu, Boryana Rossenova; *Prof. Mike Dustin (NYU)

U. of Oregon

Driven Membrane Fluctuations

Curvature generation by vesicle trafficking proteins

etc.: *http://physics.uoregon.edu/~raghu*



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