Active Patterning on a cell surface : Asters, Bull's eye and Rings

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Meeting on Nucleation, Aggregation and Growth – Bangalore, 2010

Active Matter : an unusual state of matter

Hydrodynamic Equations

Active phases of cortical actin on the cell surface

Nanoscale patterning and dynamics of passive molecules

Anomalous Number Fluctuations of passive molecules on the cell surface

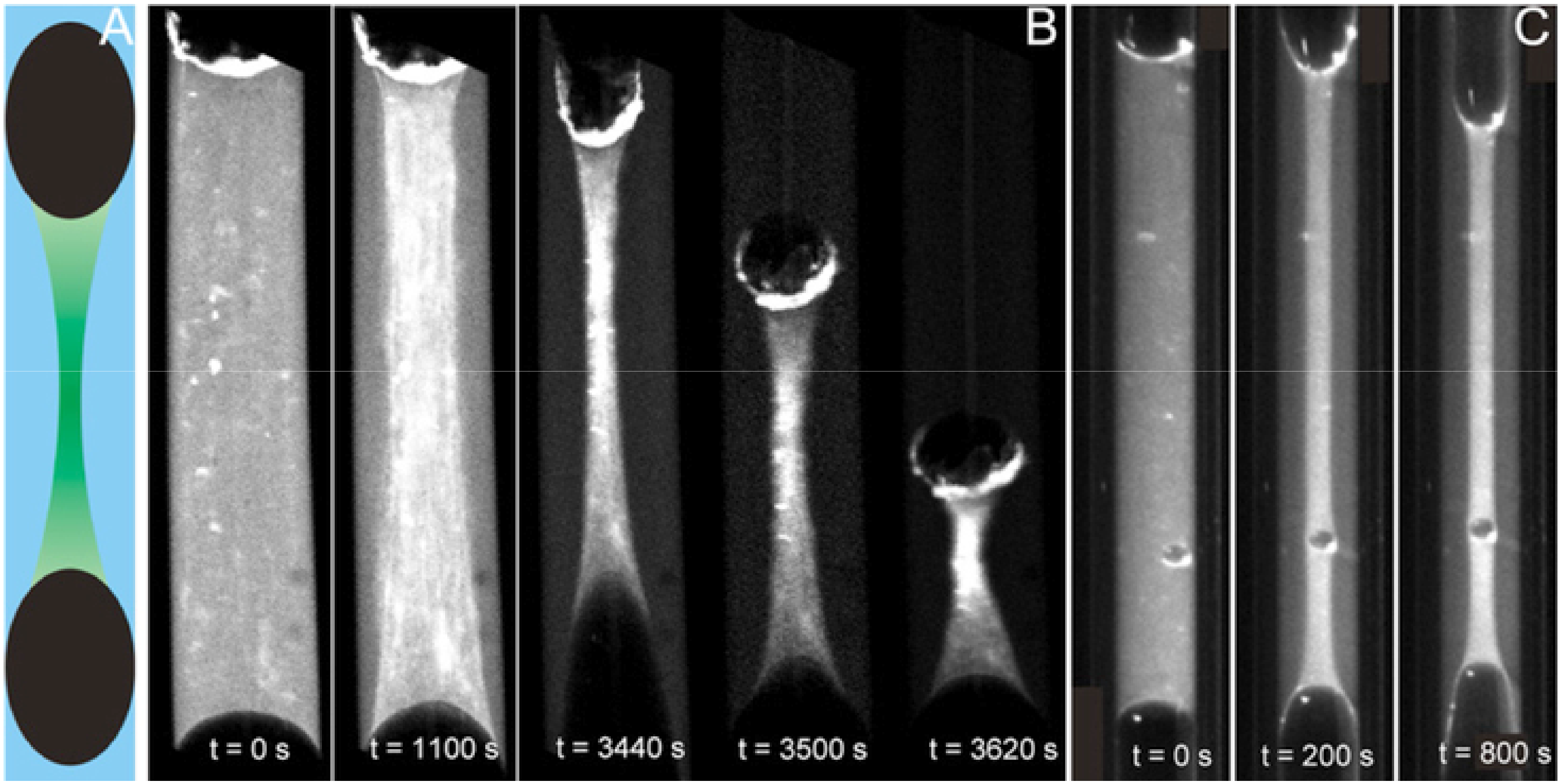
Spatiotemporal regulation of Chemical Reactions : Signaling and Sorting

**Active phase segregation : patterning at the immunological synapse

**Information Cascade

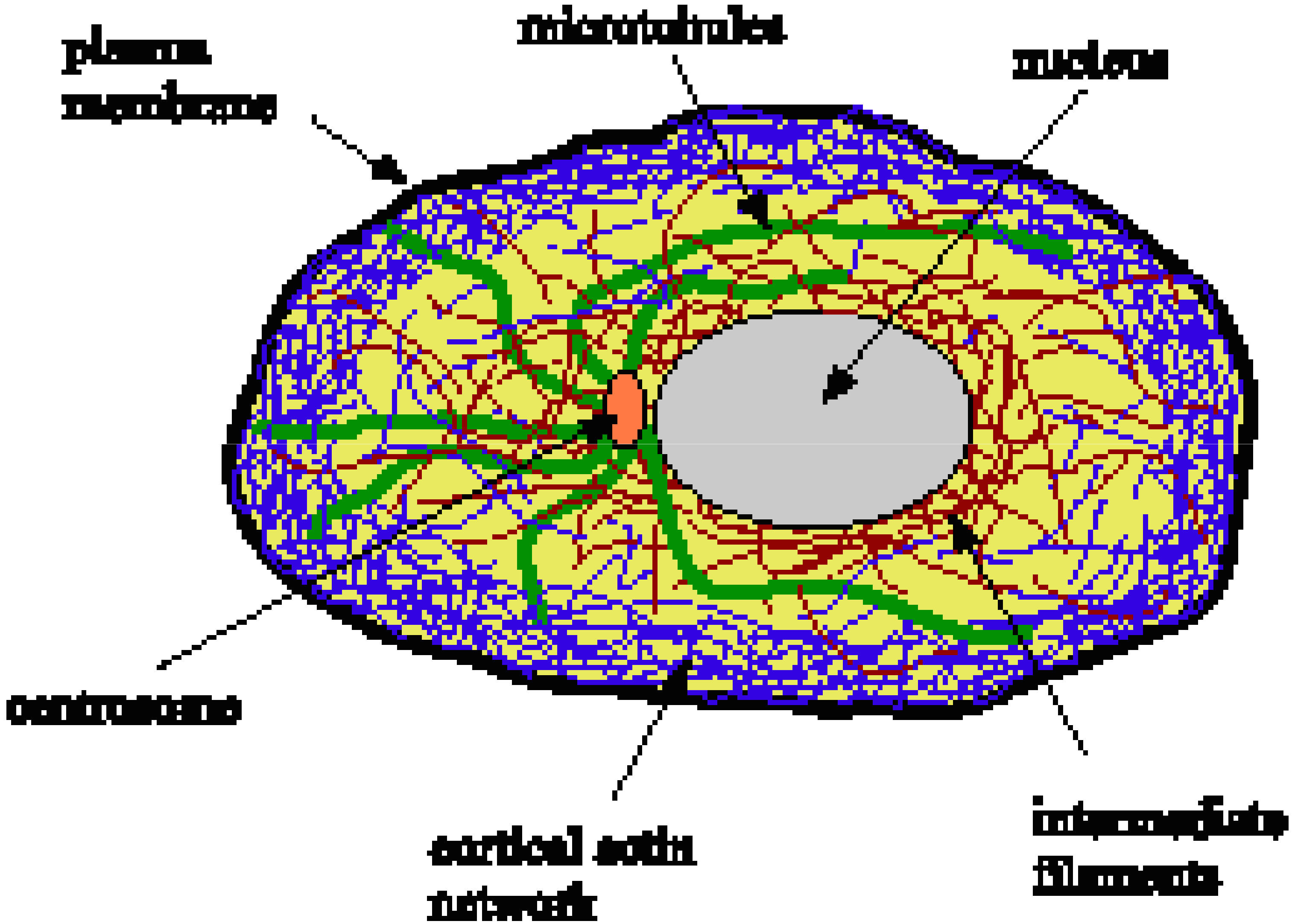
**Slipping of a contractile actin ring on spherical and cylindrical cells

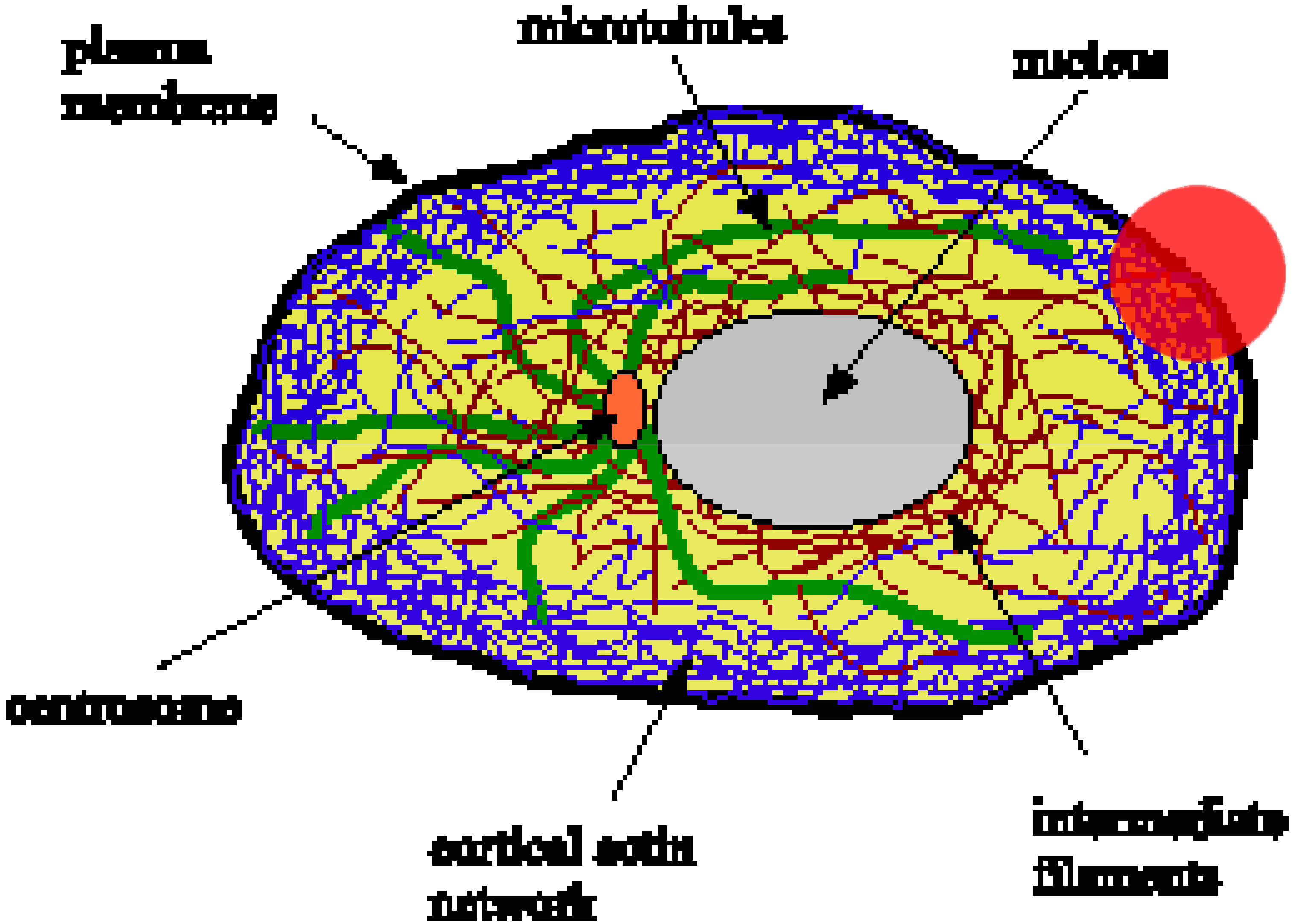
Active Gel Contractility (Force ~ 1 μN, i.e., 100 pN per actin filament)



Bendix et al, 2008

In-vitro reconstitution : F-actin, α-actinin, myosin II and ATP


plasma membrane
microtubules
nucleus
centrosome
cortical actin network
intermediate filaments



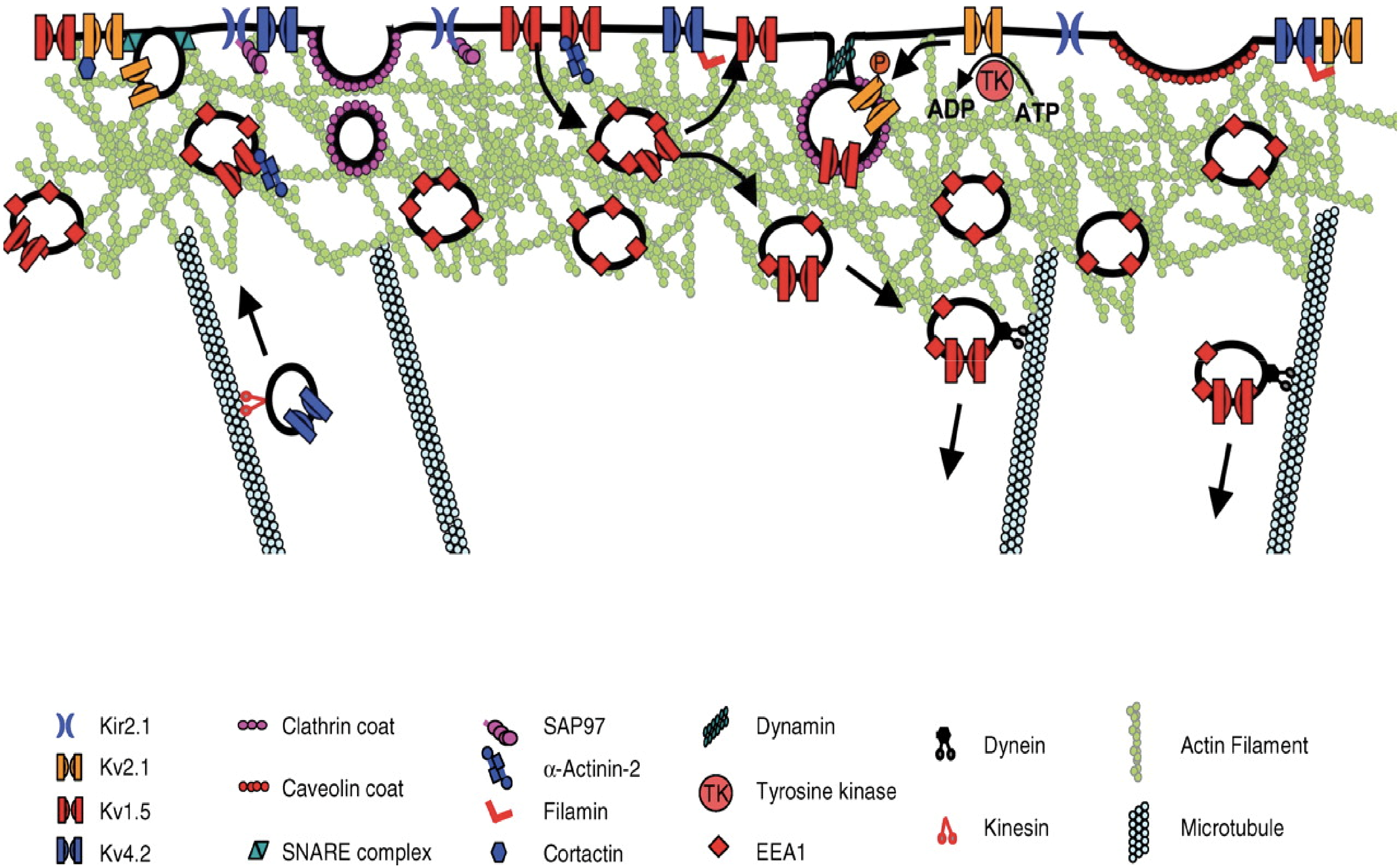
plasma
membrane
microtubules
nucleus
centrosome
cortical actin
network
intermediate
filaments

Meshwork of stiff polymers

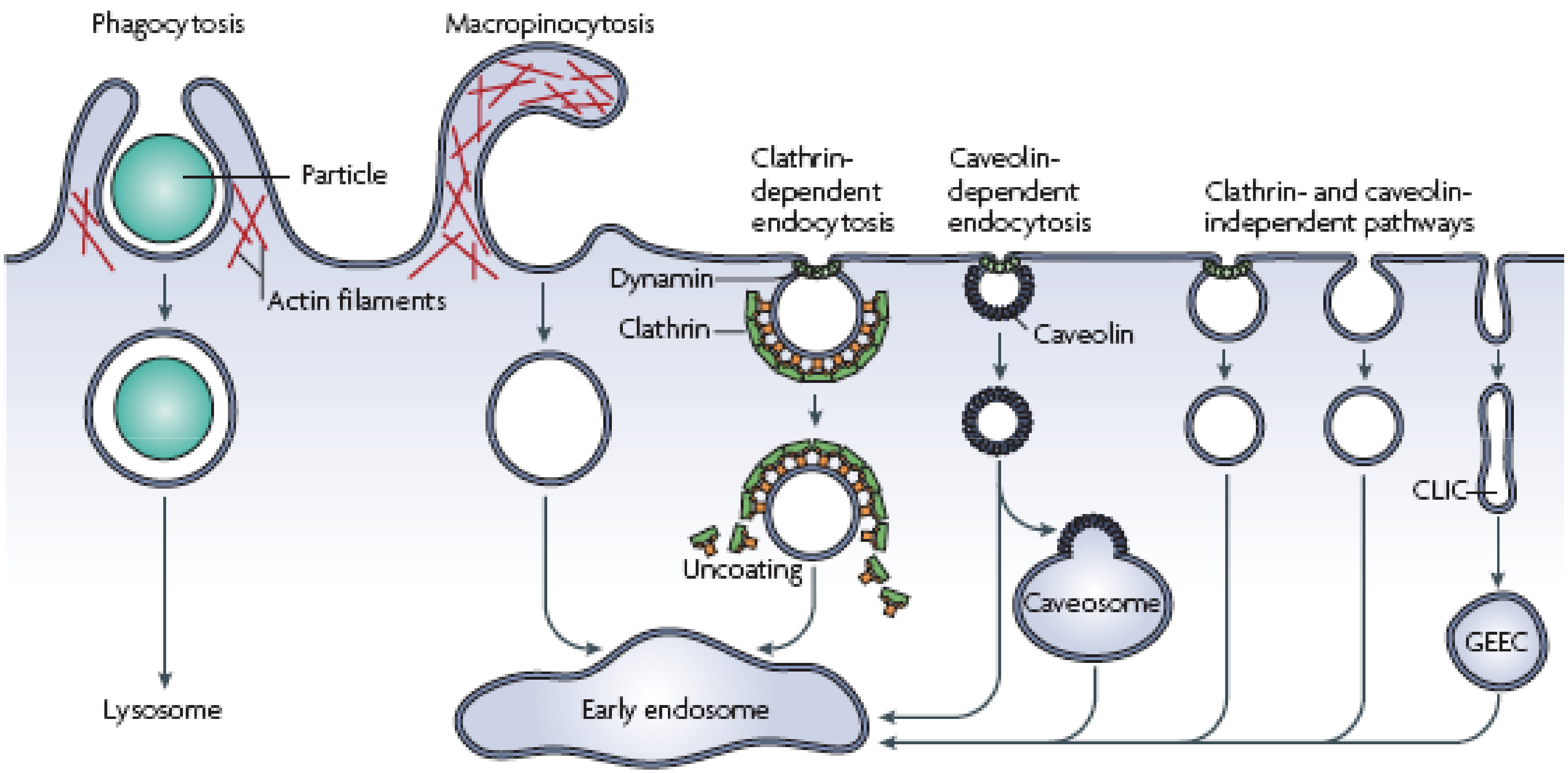
`permanent' and transient crosslinkers (branching and bundling)

ATP , Motors

Polymerisation-depolymerisation

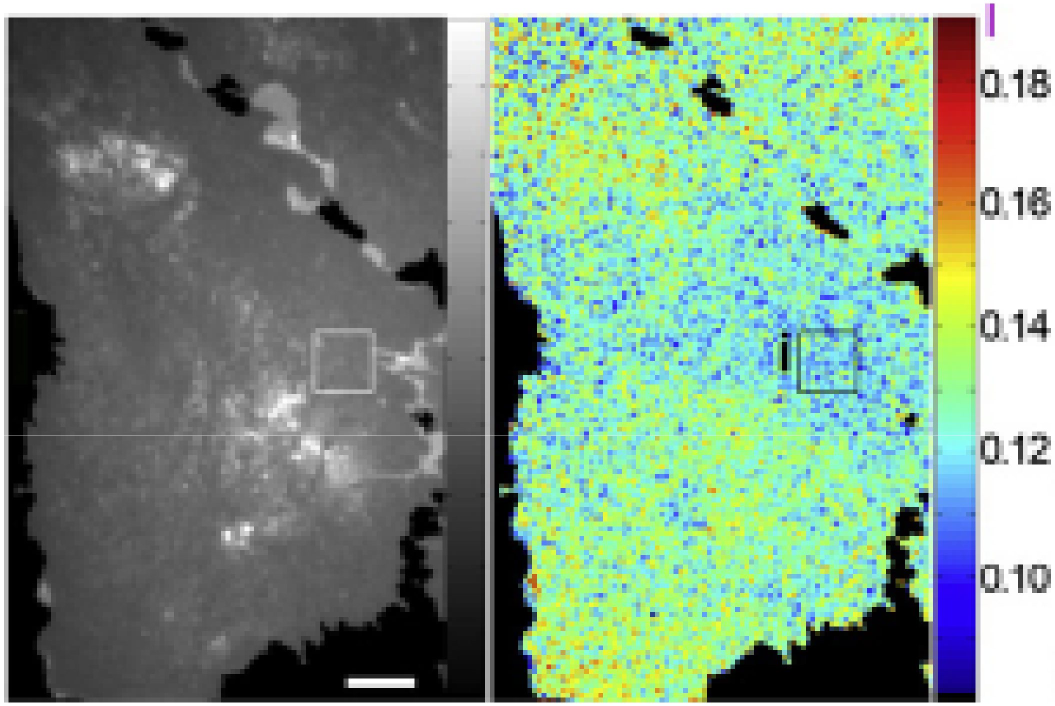


Non Clathrin Mediated Endocytic Pathways

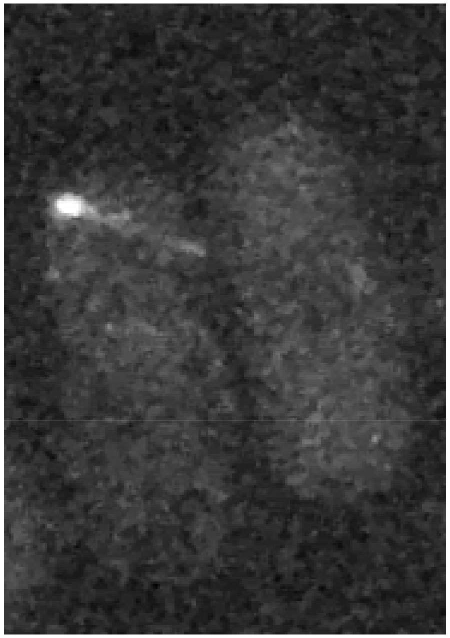


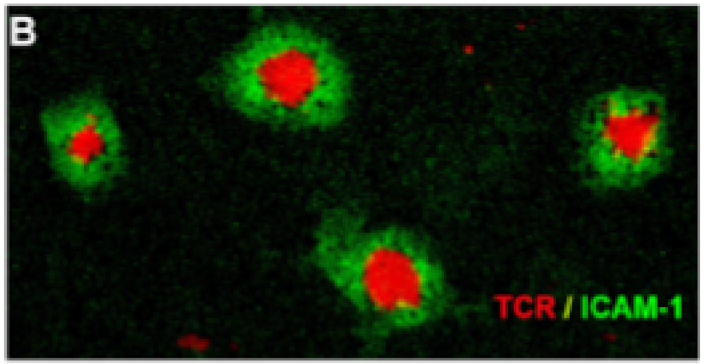
Mayor and Pagano, Nat. Cell Biol. 2007

Active Gel Contractility gives rise to STRESSES and CURRENTS
Resulting hydrodynamic equations : (in)stabilities, patterning and fluctuations



Nanoscale Organization of Cell Surface (GPI) Proteins
Mayor lab, Bangalore

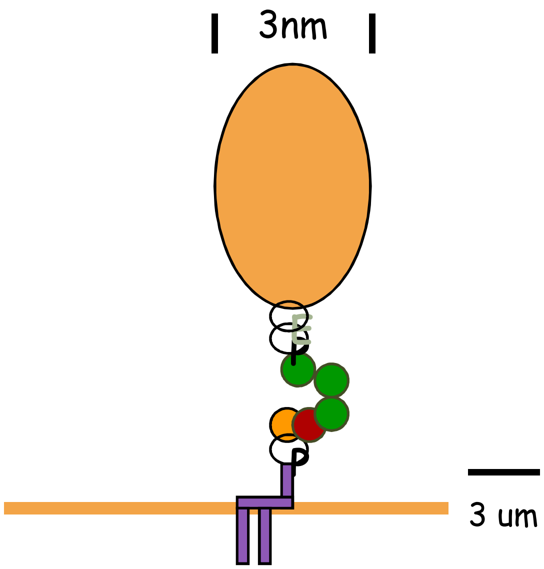

Sliding Contractile Rings in fission Yeast
Balasubramanian lab, Singapore

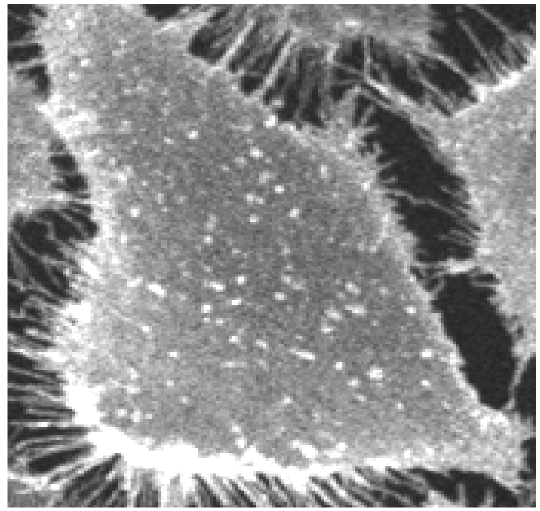


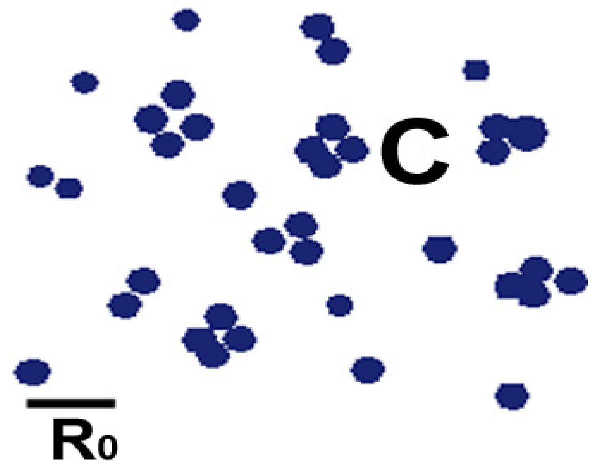
Bulls-eye patterning in Immunological synapse formation
R. Vale lab, UCSF

Organization and Regulation of Lipid Tethered Proteins

e.g., GPI-anchored proteins, Ras-signaling proteins, glycolipids







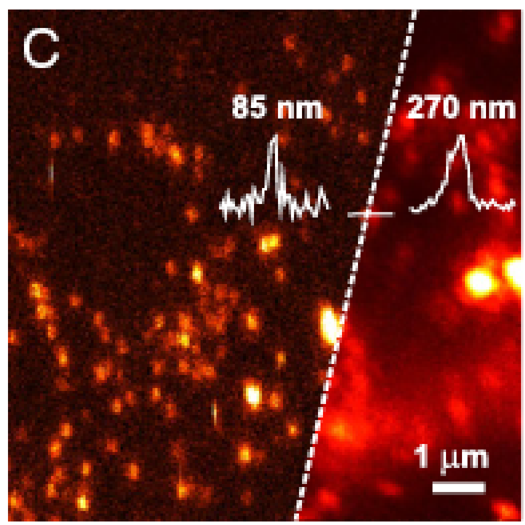
~ 40 % GPI-anchored proteins in dense nanoclusters < 5 molecules

GPI-anchored Proteins
(> 10% of all membrane proteins :
300 functionally diverse proteins)

Mayor and Rao, Traffic (2004)

Sharma et al, Cell 2004

Goswami et al, Cell 2009



Garcia-Parajo et al, PNAS 2009

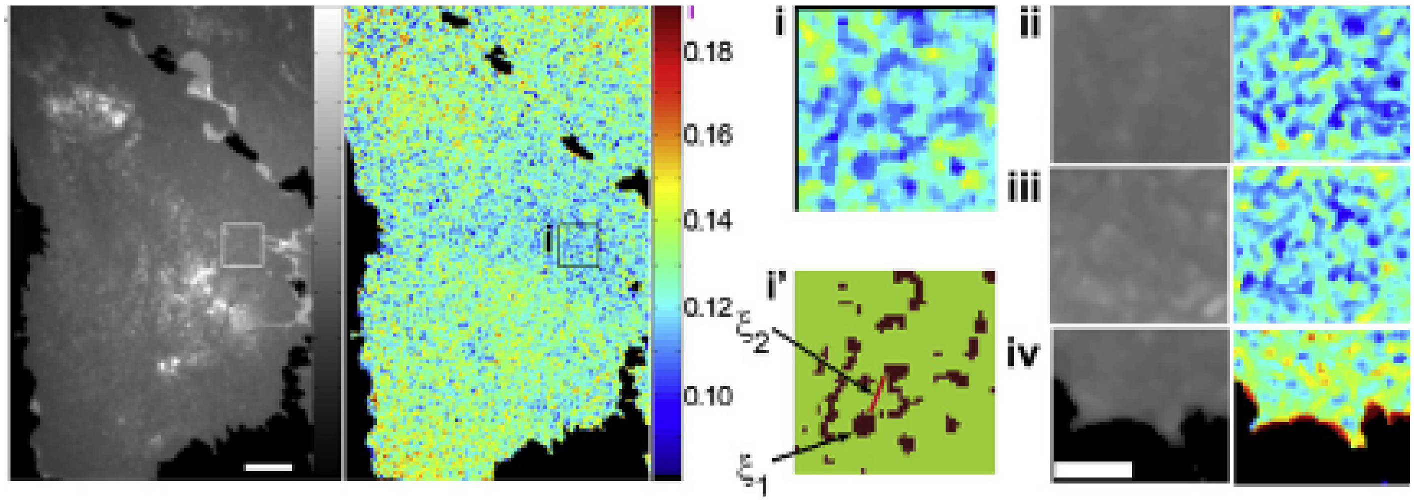
Low FRET Anisotropy

↓

High nanocluster concentration

Nano Clusters of GPI-Proteins on Flat Taut regions of cell surface

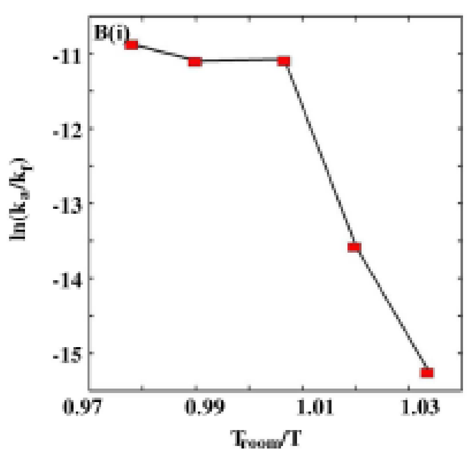
Acto-Myosin Activity regulates Clustering and Dynamics



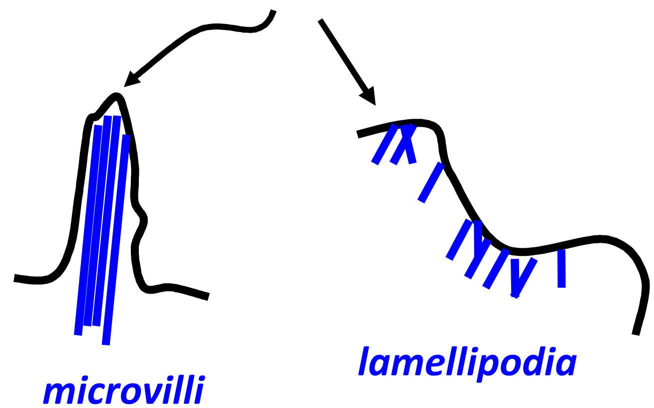
Low Anisotropy = High nanocluster concentration

Flat Regions Enriched in Nanoclusters

Dynamic Clusters
Lifetime ~ 0.1 s



Deficient in nanoclusters



Mayor and Rao, Traffic (2004)

Sharma et al, Cell 2004

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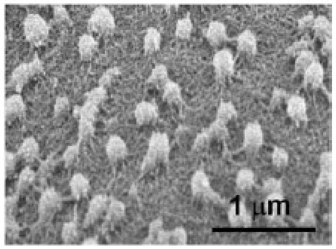
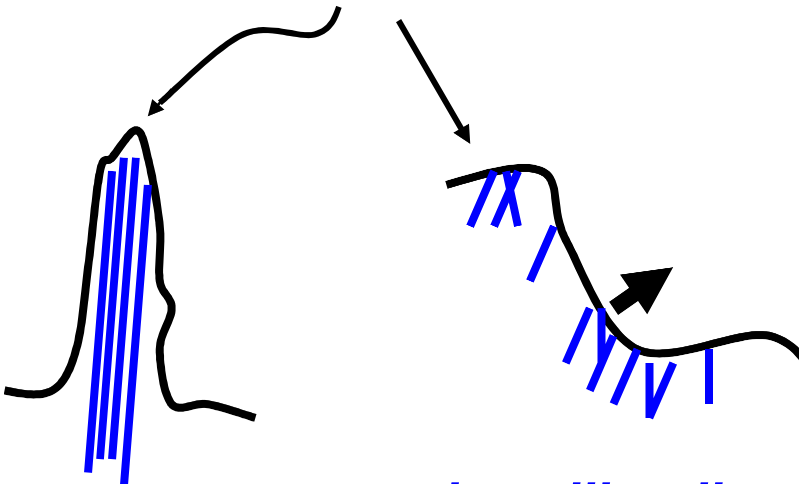


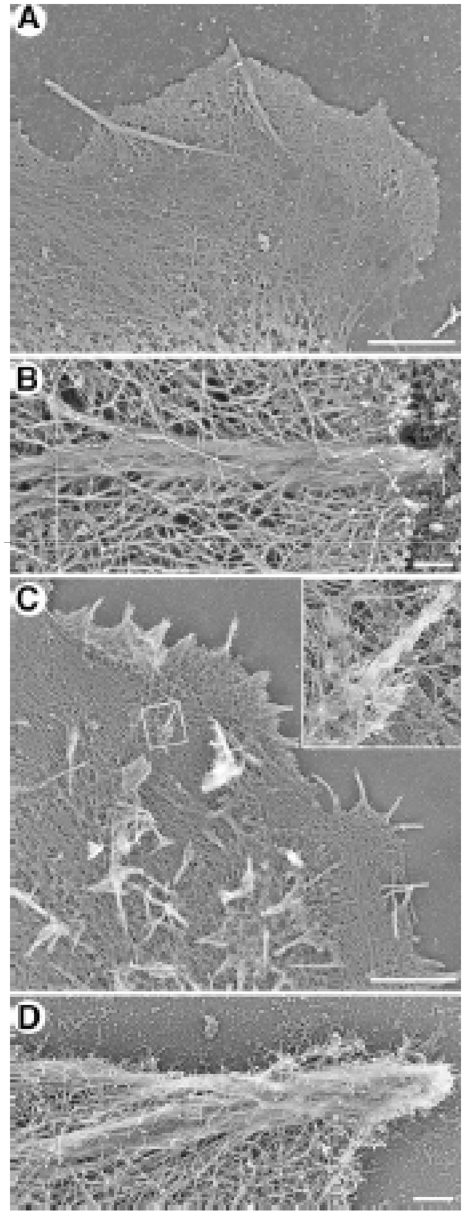
Actin dependent Active Stresses

Deficient in nanoclusters



microvilli

lamellipodia



EM image of the actin-based membrane skeleton

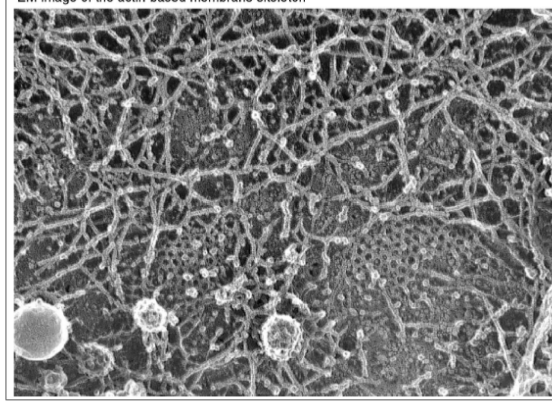


Figure 24. The membrane skeleton is largely comprised of actin. The thin white bands seen on the filaments in this high magnification image indicate that the membrane skeleton in close proximity to the lipid bilayer is comprised of actin filaments.

enriched in nanoclusters

Normal stresses

shape deformation

composition (via spontaneous curvature)

Tangential stresses

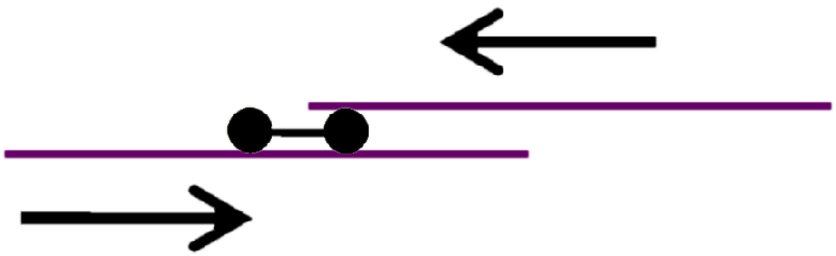
composition

shape deformation

Vertical actin

Horizontal actin

ACTIVE MATTER



Acto – Myosin Filaments

Consume Chem Energy (ATP)

↓

Cyclical Conformation Change

↓

Movement

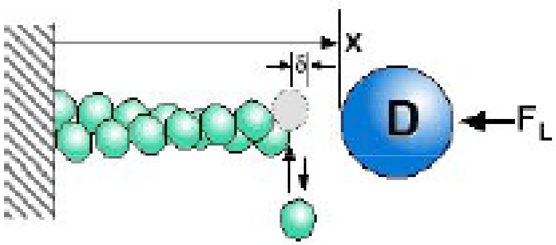
Toner, Tu
Ramaswamy, MR, Hatwalne, Simha
Prost, Joanny, Julicher, Kruse, Voituriez
Marchetti, Liverpool, Baskaran

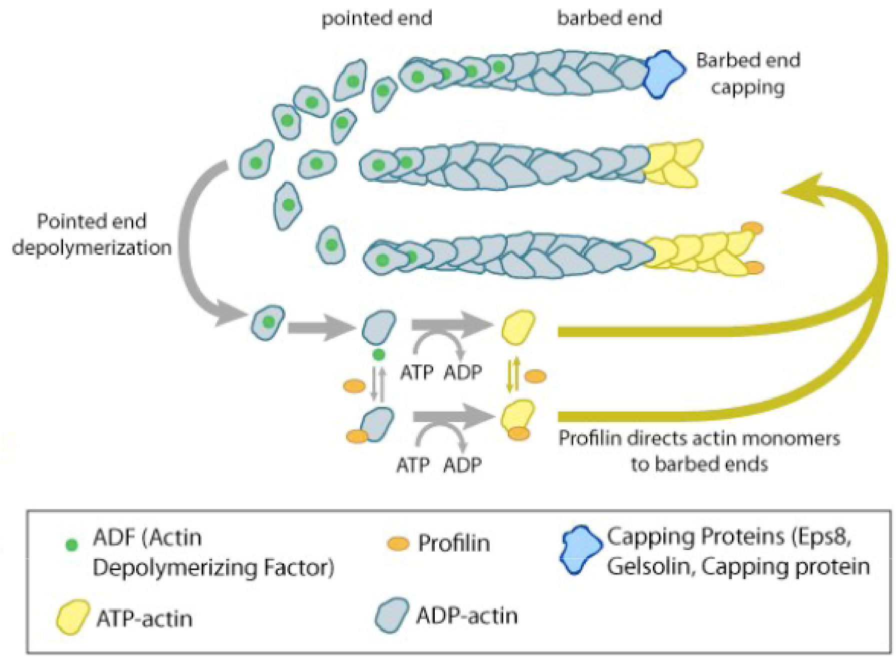
…. many others

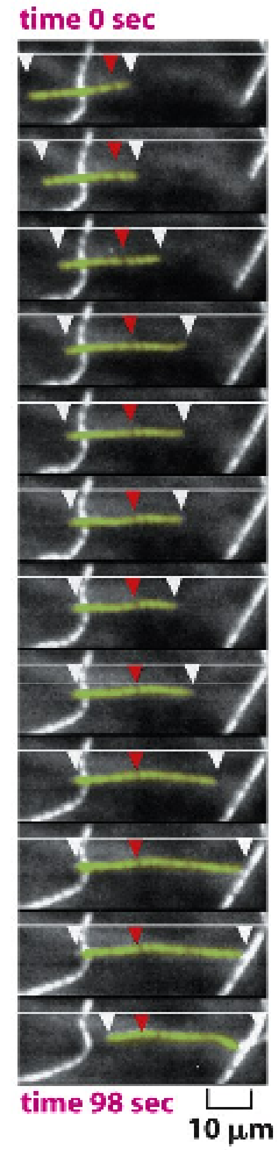
Simha R. A. and Ramaswamy S., *Phys. Rev. Lett.*, 89 (2002) 058101.
Liverpool T. B. and Marchetti M. C., *Phys. Rev. Lett.*, 90 (2003) 138102; *Europhys. Lett.*, 69 (2005) 846 cond-mat/0607285; Ahmadi A. *et al.*, cond-mat/0607287 and 0507590.
Voituriez R., Joanny J.-F. and Prost J., *Phys. Rev. Lett.*, 96 (2006) 028102.
Hatwalne Y., Ramaswamy S., Rao M. and Aditi Simha R., *Phys. Rev. Lett.*, 92 (2004) 118101.

Polymerization - Depolymerization

treadmilling

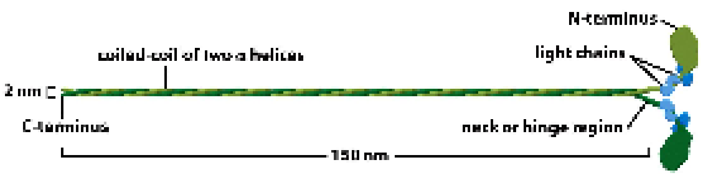


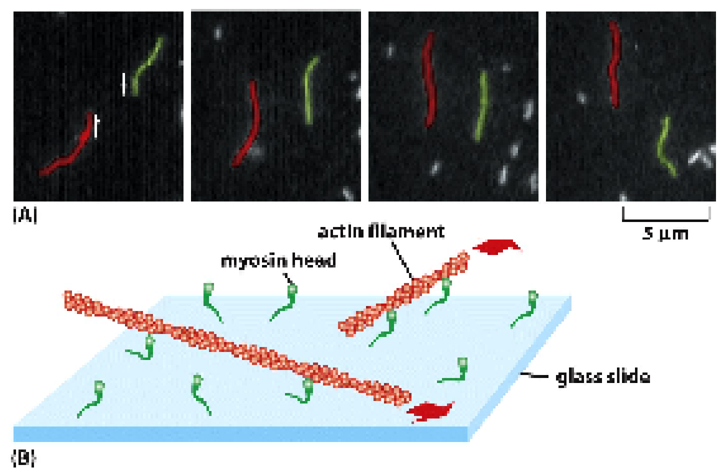




Acto-Myosin Contractility

Relative rotations, sliding, translation





$$\int_\Omega \mathbf{F}(r) = 0$$

Active Forces are Internal Forces

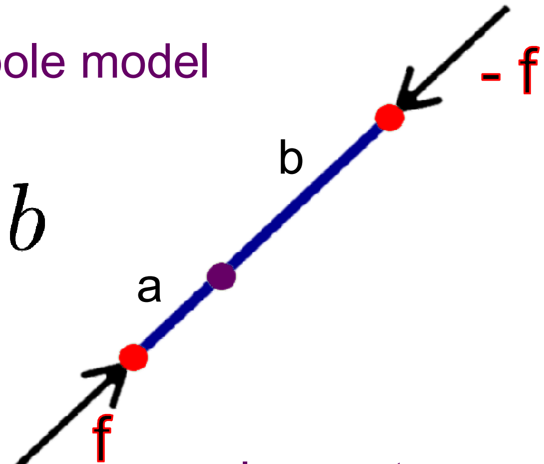
$$\Longrightarrow \mathbf{F} = \nabla \cdot \sigma$$

Momentum Conservation implies

Active stresslets

Point-force dipole model

$$a \neq b$$



$$\sigma_{ij} \propto Q_{ij}$$

$$\mathbf{Q} \equiv S\left(\mathbf{nn} - n^2(1/d)\mathbf{I}\right)$$

polar, vector

$$\mathbf{n}_\alpha = n_0 \mathbf{N}_\alpha$$ (relative velocity)

Hatwalne et al, PRL 2004

2 – component Active Hydrodynamics

HYDRODYNAMIC VARIABLES

(vector) orientation or relative velocity $\mathbf{n}(\mathbf{x},t)$ (Broken Symmetry)

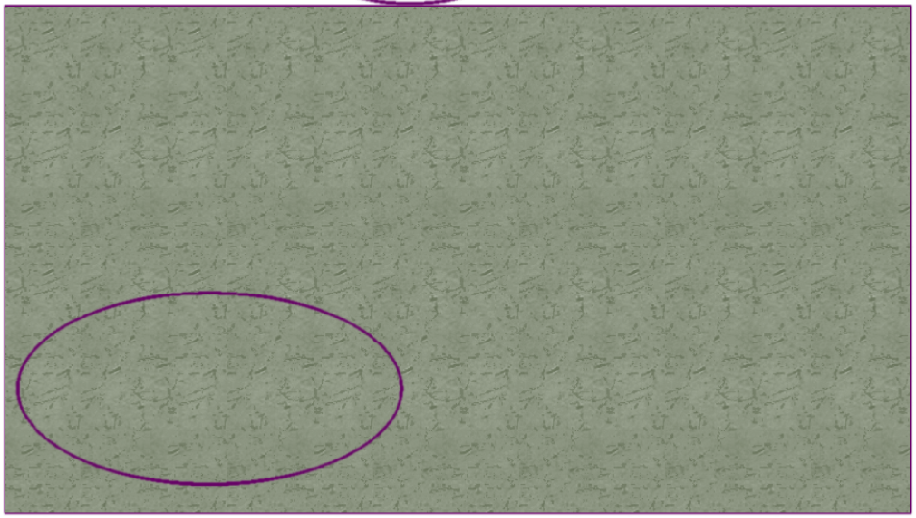
Filament concentration $c(\mathbf{x},t)$ (Conservation)

Hydrodynamic Velocity $\mathbf{v}(\mathbf{x},t)$

$$D_t\mathbf{n} + \lambda\left(\mathbf{n}\cdot\nabla\right)\mathbf{n} = \Lambda\nabla\mathbf{v}\cdot\mathbf{n} - \zeta\nabla c + K\nabla^2\mathbf{n} + \alpha\mathbf{n} + \beta|\mathbf{n}|^2\mathbf{n}$$

$$D_t c = -\nabla\cdot\left(c v_0\mathbf{n} - D_f\nabla c\right)$$

Force Balance



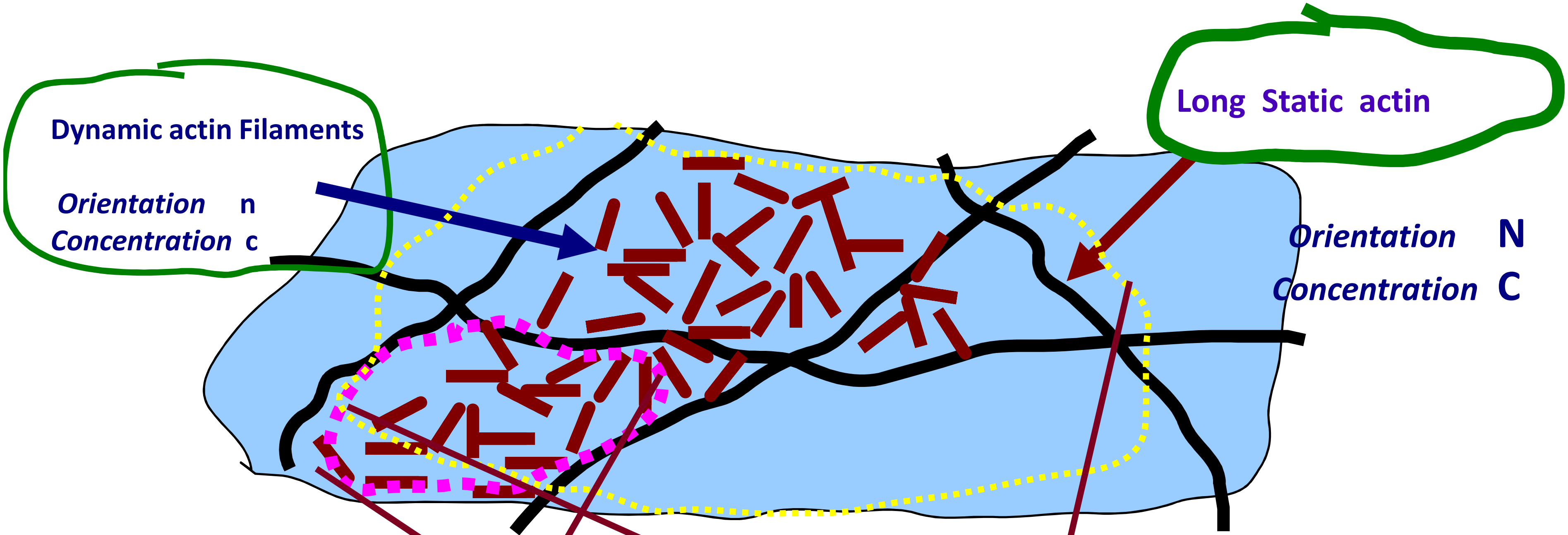
Note :

Active terms not derivable from free energy

Detailed Balance violations

Active Composite Cell Surface : Membrane + cortical actin

Kripa, G., S. Ghosh et al (under review)



Boundary (anchoring) condition

Quenched random anchoring

EM image of the actin-based membrane skeleton

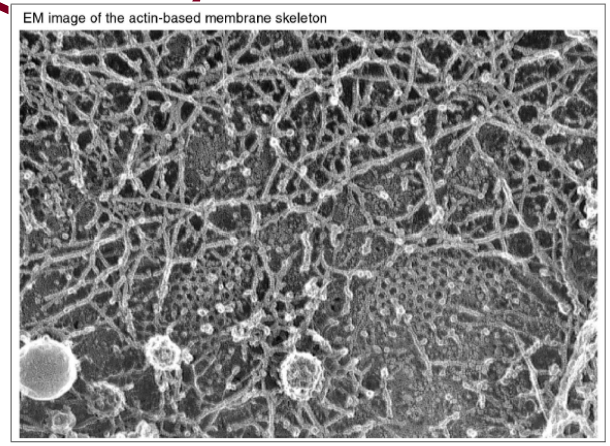
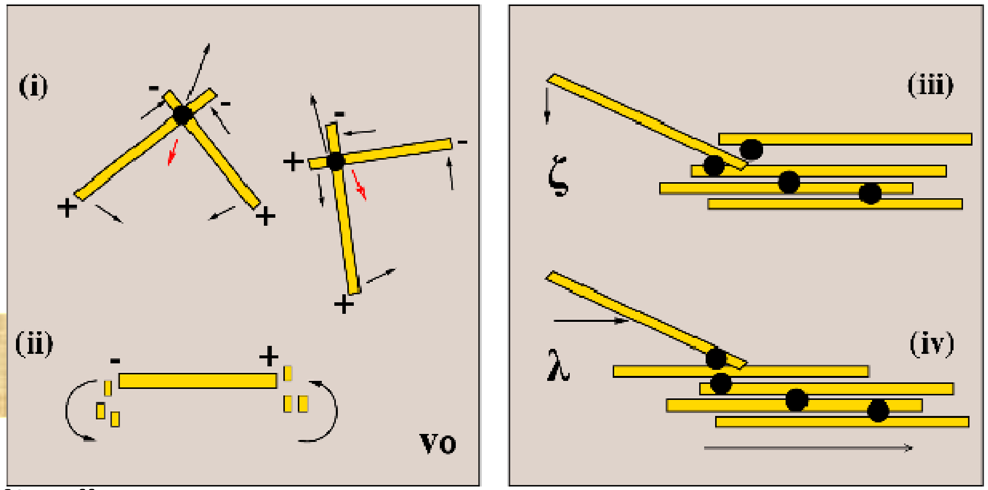


Figure 24. The membrane skeleton is largely comprised of actin. The thin white bands seen on the filaments in this high magnification image indicate that the membrane skeleton in close proximity to the lipid bilayer is comprised of actin filaments.

$$\Gamma = \mu/\xi^2$$

Local frictional damping

Active Forces and Currents : Dynamics of Polar Actin Filaments on cell surface



Filament orientation

Poly/Depoly "Treadmilling"

Acto-Myosin Contractility

$$\underbrace{\partial_t \mathbf{n} + \lambda(\mathbf{n}\cdot\nabla)\mathbf{n}}_{\text{active advection}} \;=\; K_1\nabla^2\mathbf{n} + K_2\nabla(\nabla\cdot\mathbf{n})$$

$$\underbrace{-\zeta\nabla c + A\mathbf{n} - B|\mathbf{n}|^2\mathbf{n}}_{\text{actomyosin contractility}}$$

more c , more myosin binding

$$+\ldots\ldots + \mathbf{f}_n$$

Active noise

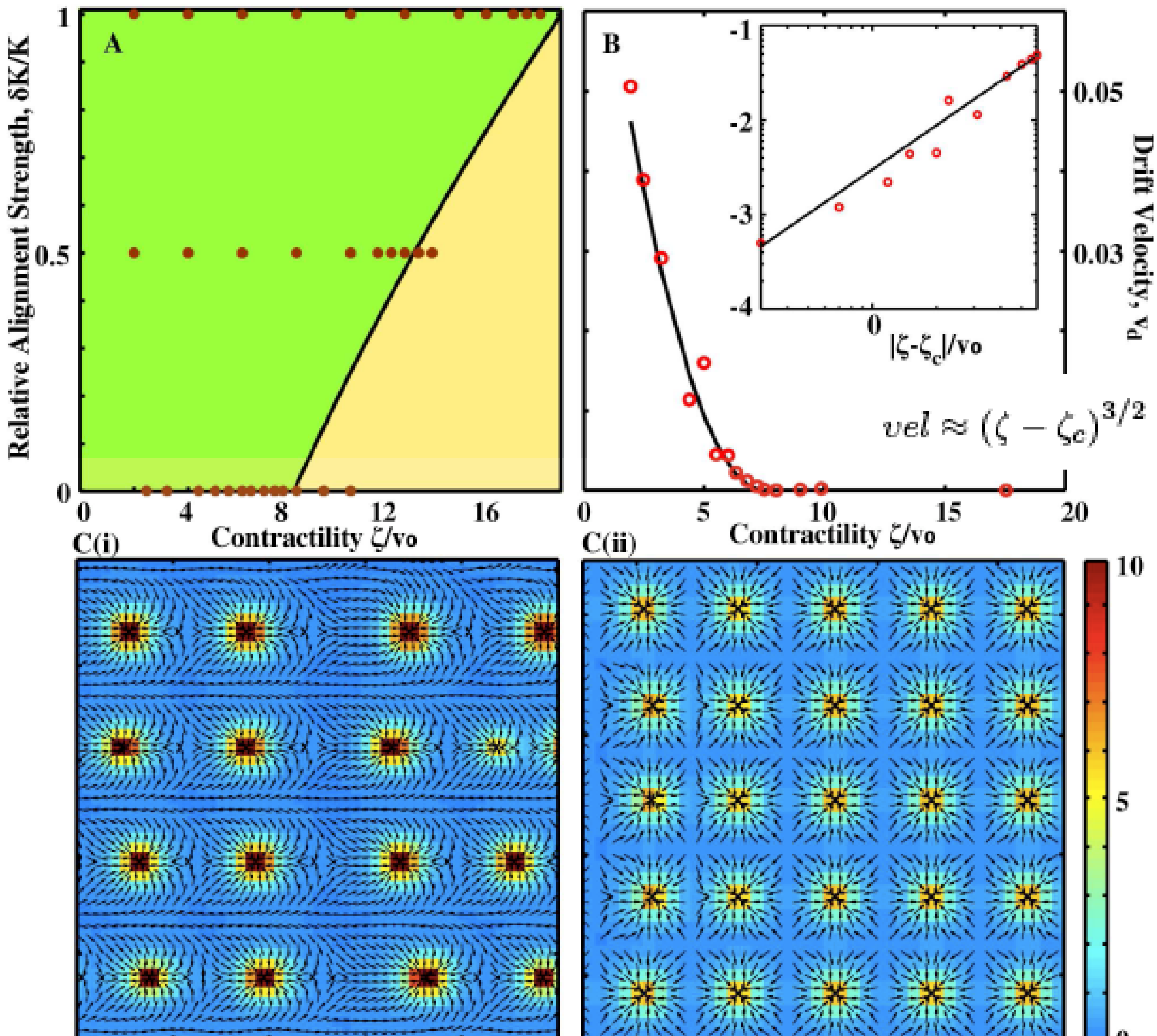
$\propto T_a$ active temperature

Filament concentration

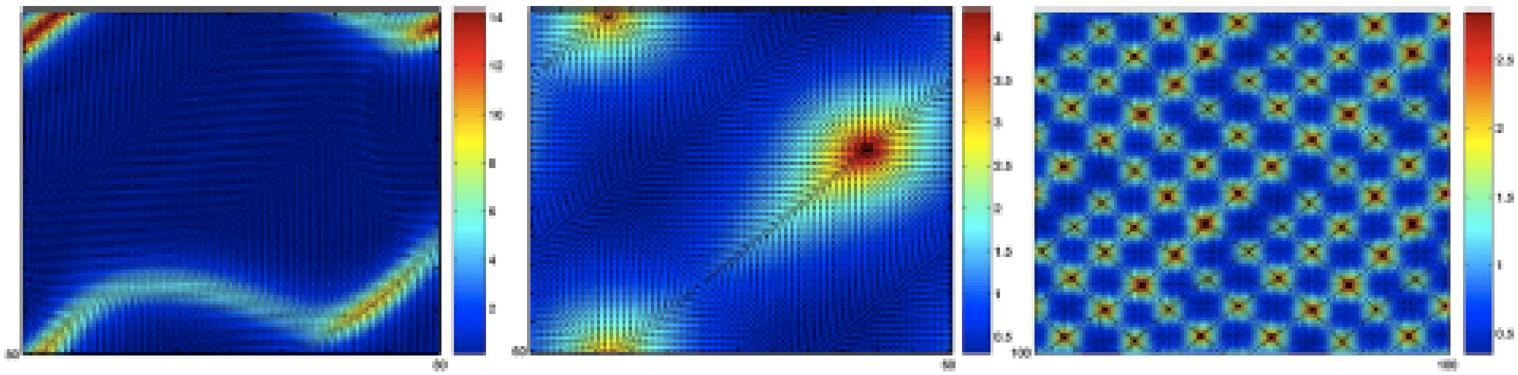
$$\partial_t c = -\nabla\cdot\mathbf{J}$$

$$\mathbf{J} = v_0 c\mathbf{n} - D_f\nabla c + \mathbf{f}_c$$

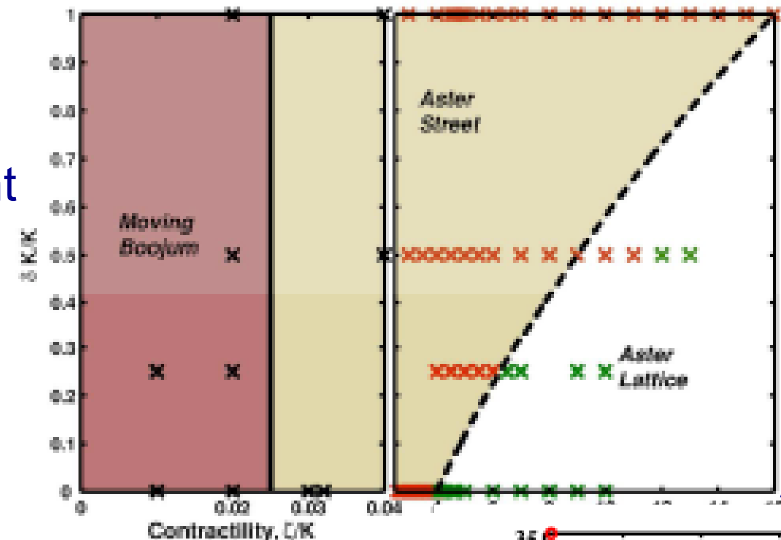
Steady State Patterns : Inward Pointing Asters



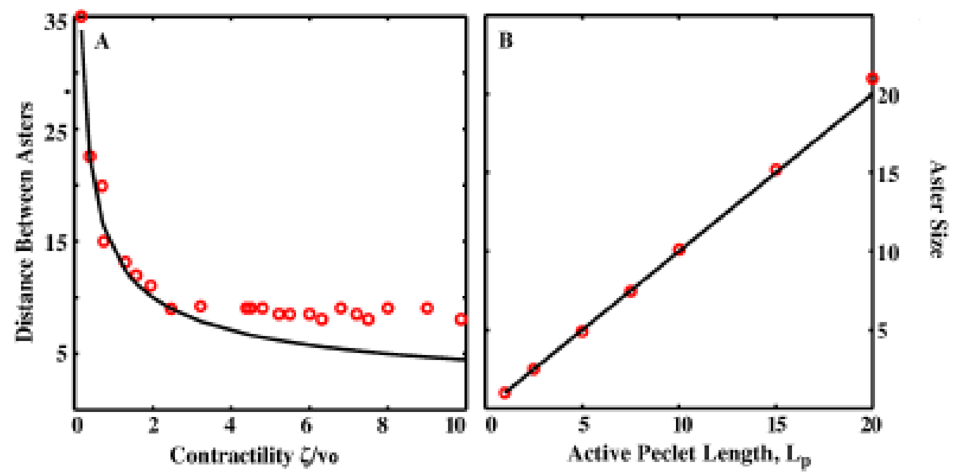
Kripa, G., S. Ghosh et al (under review)



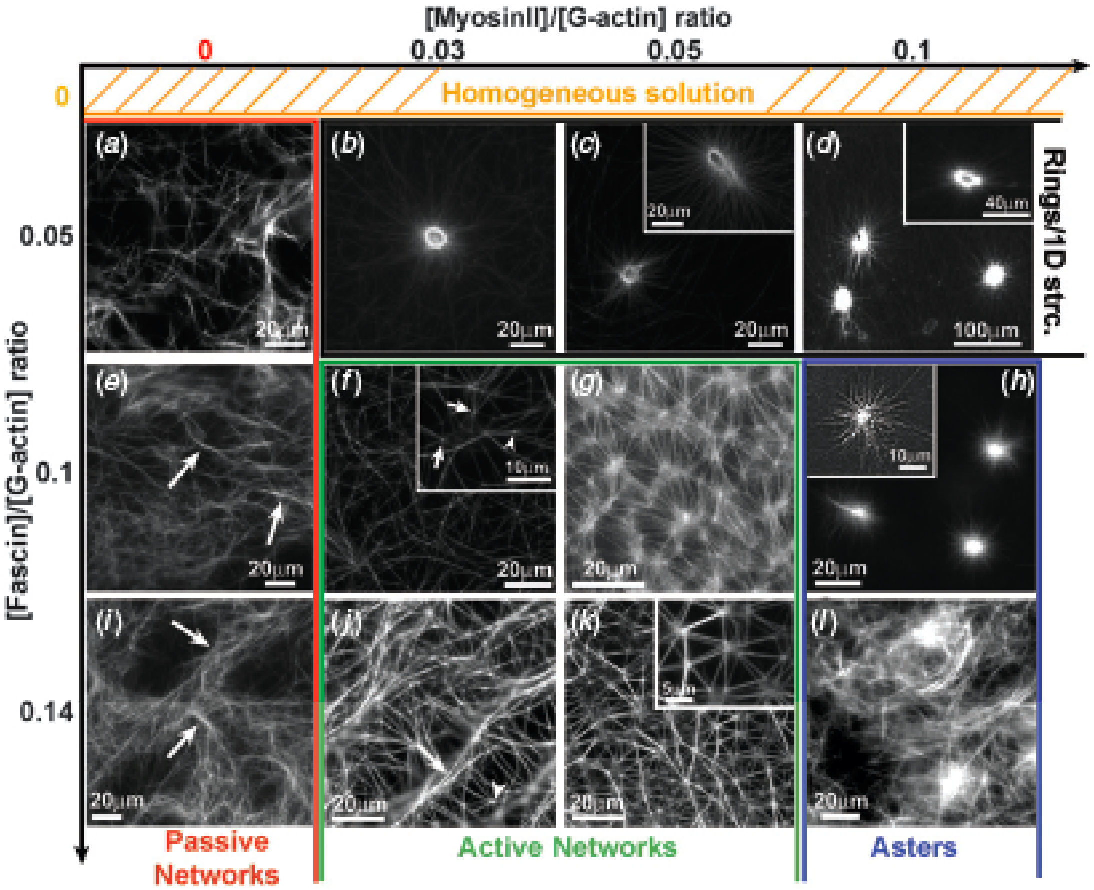
Relative Alignment

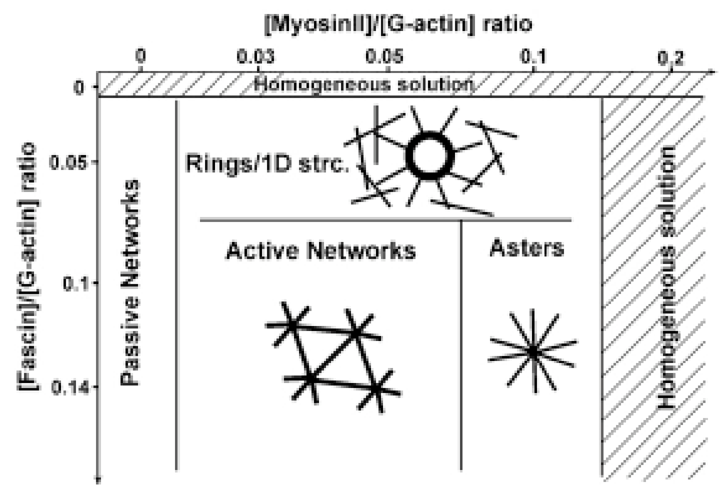


Actomyosin Contractility



In-vitro Reconstitution sees Asters at high activity

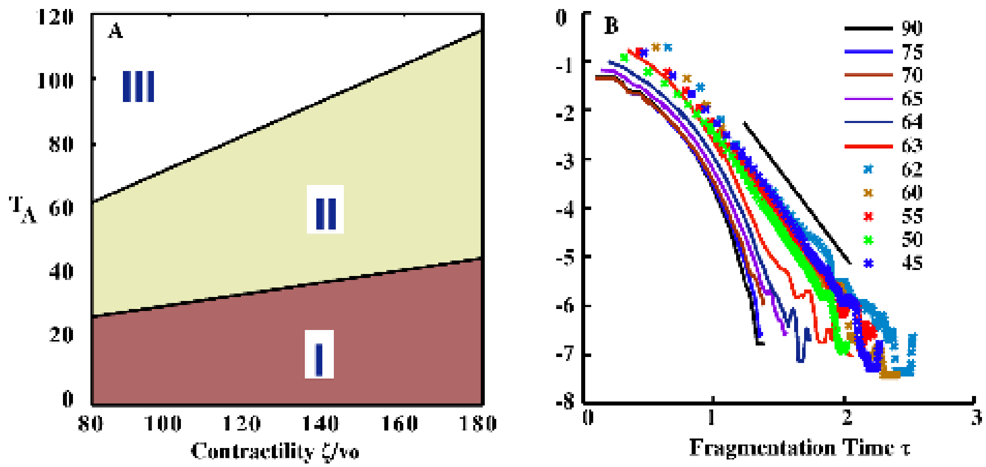




F Backouche[1], L Haviv[1], D Groswasser[2] and A Bernheim-Groswasser[1]

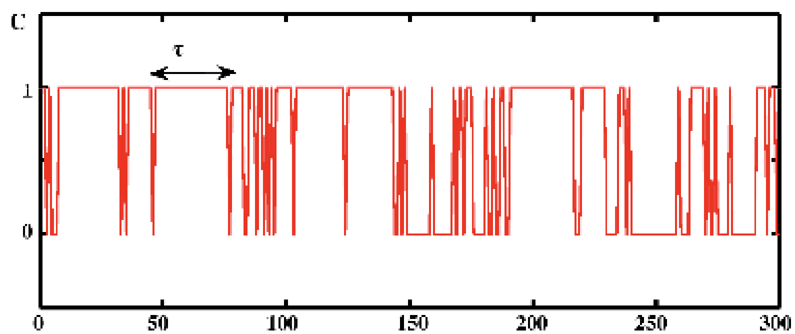
Phys. Biol. 3 (2006) 264–273

Dynamics in steady state : Active Temperature > 0



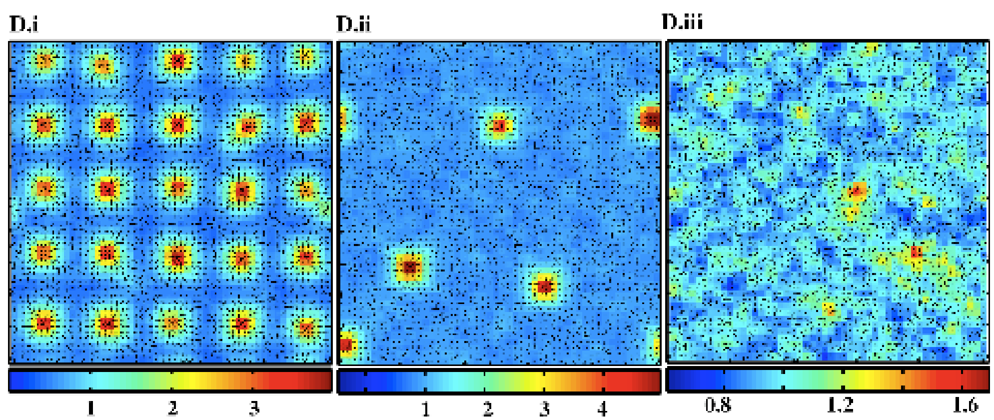
Aster

$$c\,|\nabla\cdot\mathbf{n}|$$



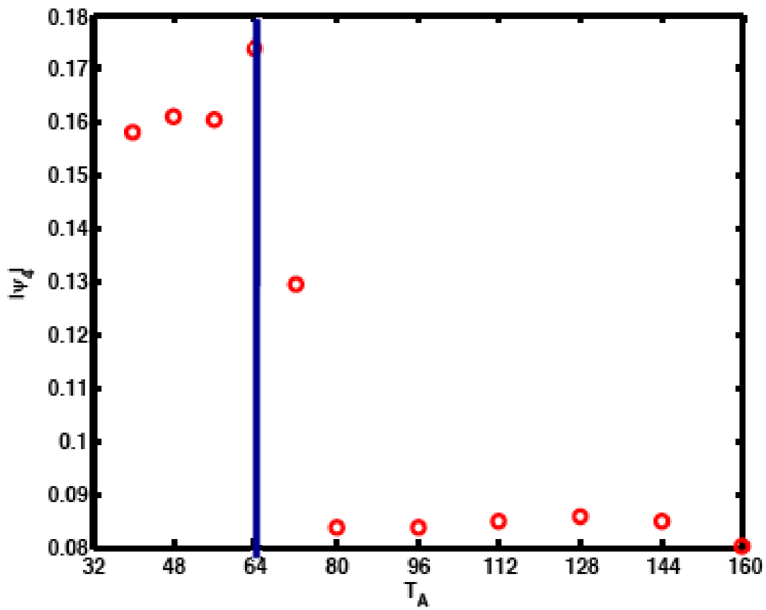
time

``Phonons''

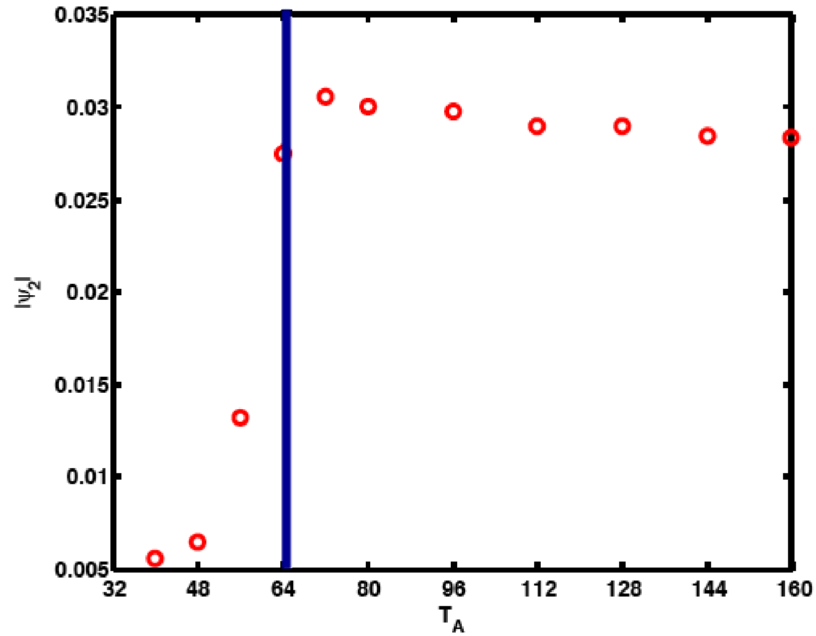


``Remodeling''

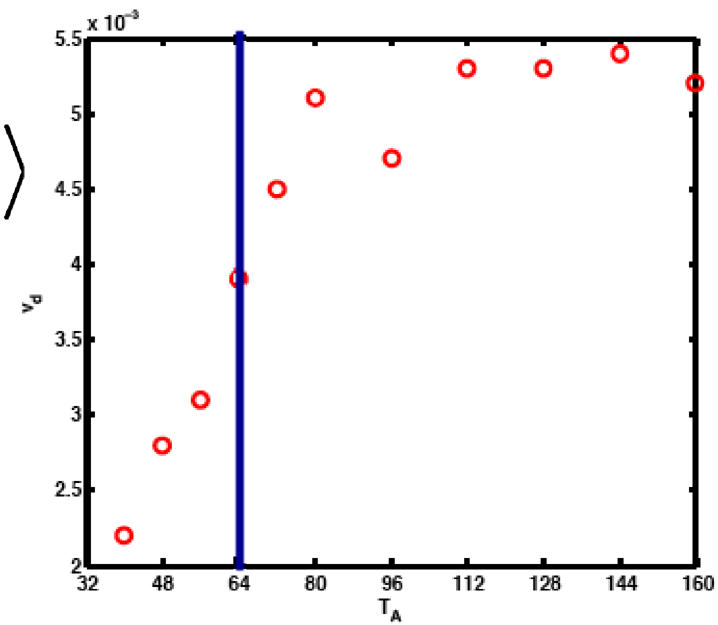
$$\Psi_4 = \langle e^{i4\theta} \rangle$$

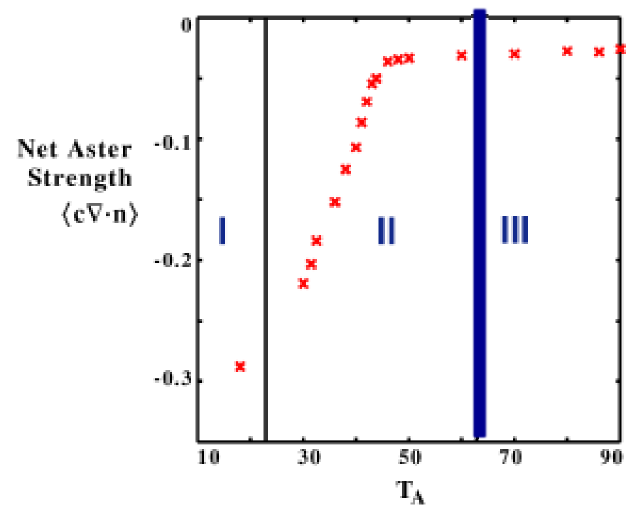


$$\Psi_2 = \langle e^{i2\theta} \rangle$$

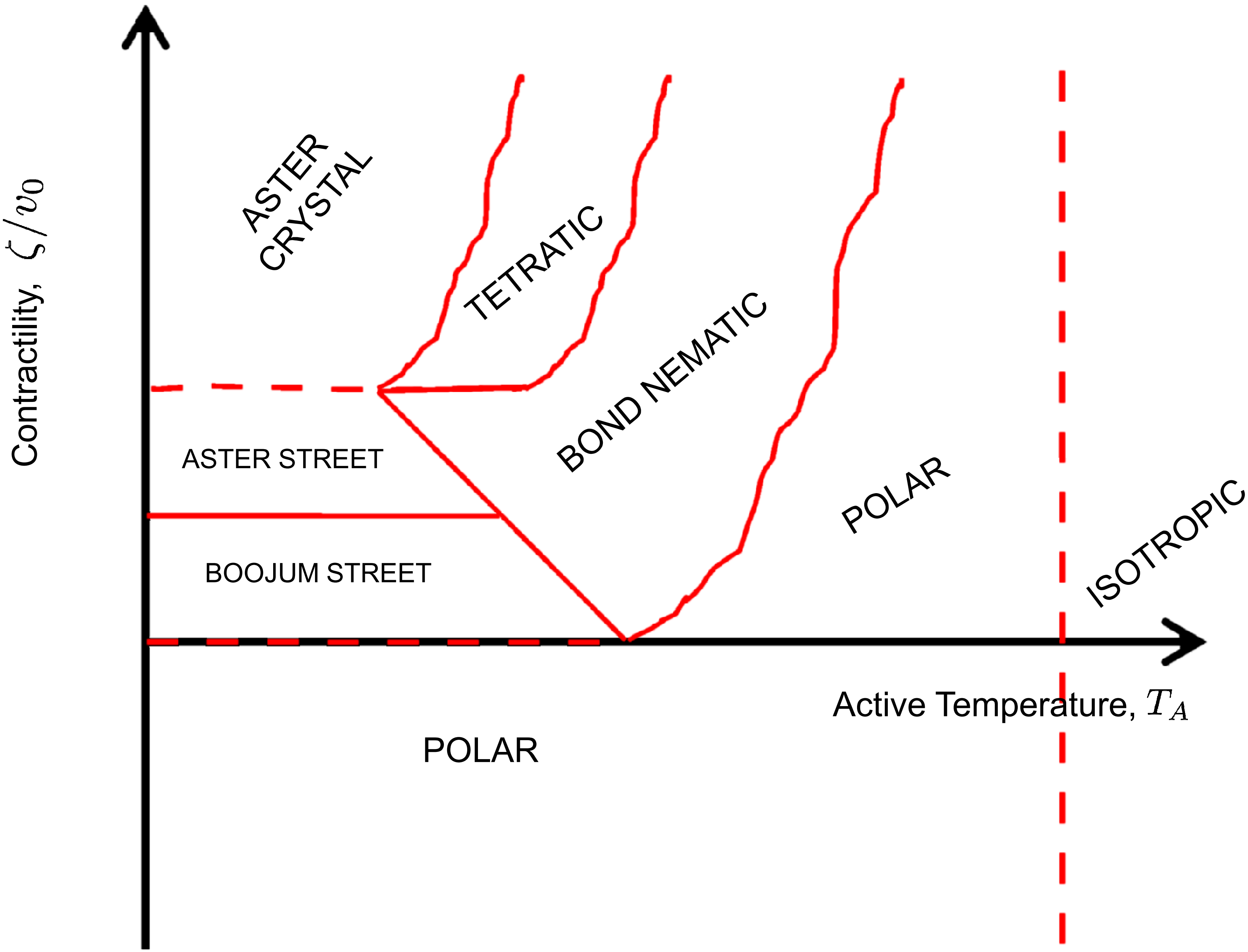


$$v_d = \langle c\mathbf{n} \cdot \hat{x} \rangle$$





Order Parameters



Contractility, ζ/v_0
ASTER CRYSTAL
TETRATIC
BOND NEMATIC
ASTER STREET
BOOJUM STREET
POLAR
ISOTROPIC
Active Temperature, T_A
POLAR

3 Classes of cell surface molecules:

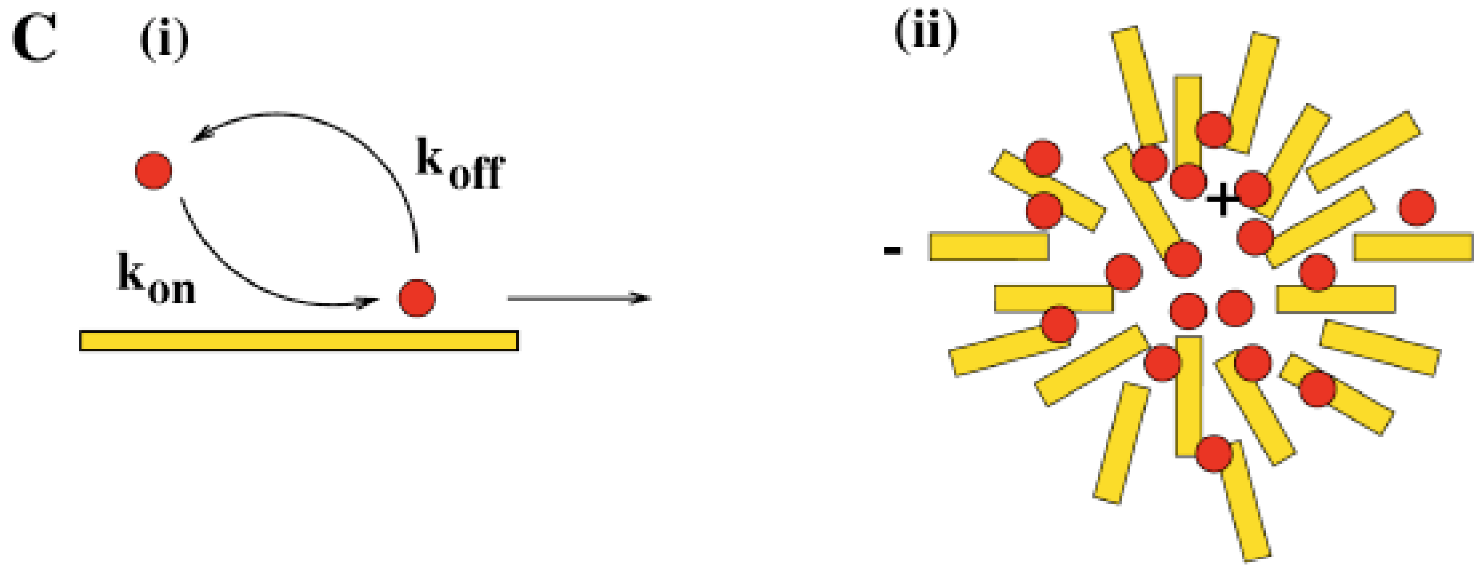
- INERT   does not interact with dynamic actin, e.g. short chain lipids

- PASSIVE   interacts with dynamic actin, but does not remodel it, e.g. GPI-Protein

- ACTIVE   interacts with dynamic actin, and remodels it, e.g., Integrin receptors

Kripa, G., S. Ghosh et al (under review)

Passive Cell Surface Molecules (GPI-AP) : interact with active actin but do not affect it



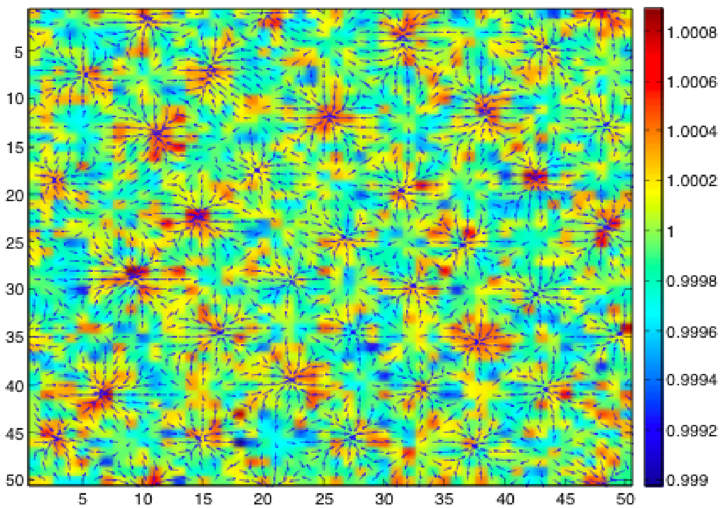
$$\partial_t \rho = -\nabla \cdot \mathbf{J}_\rho$$

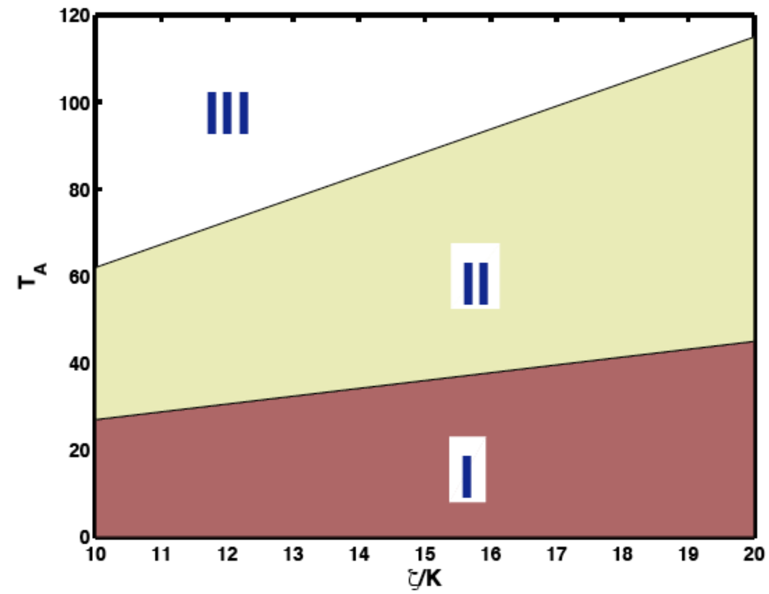
$$\mathbf{J}_\rho = \Phi(t)\rho c\mathbf{n} - D(1 - \Phi(t))\nabla\rho + \mathbf{f}$$

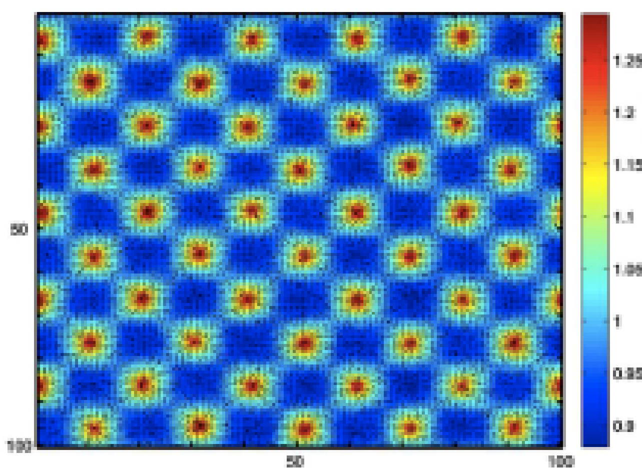
Duty ratio

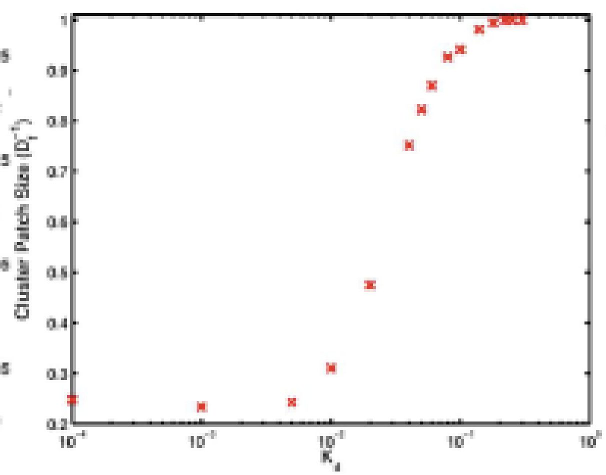
$$K \equiv \langle \Phi(t) \rangle = \frac{k^{on}}{k^{on} + k^{off}}$$

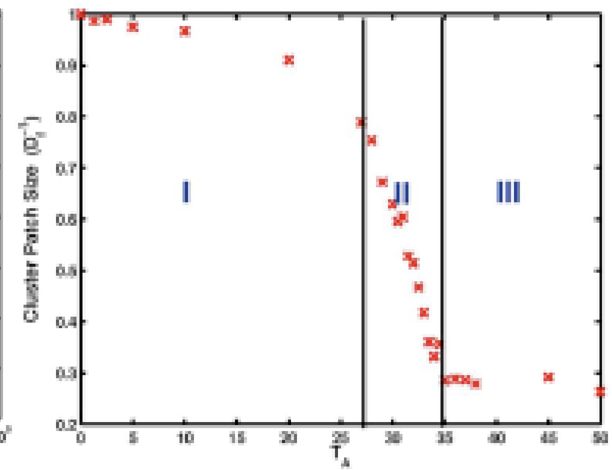
Nanoclusters and monomers







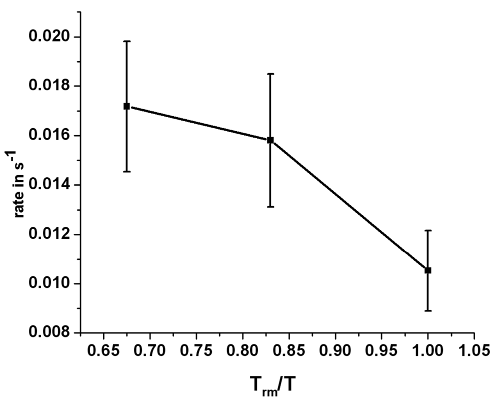




Size set by duty ratio and active temperature

Comparison with FRET experiments

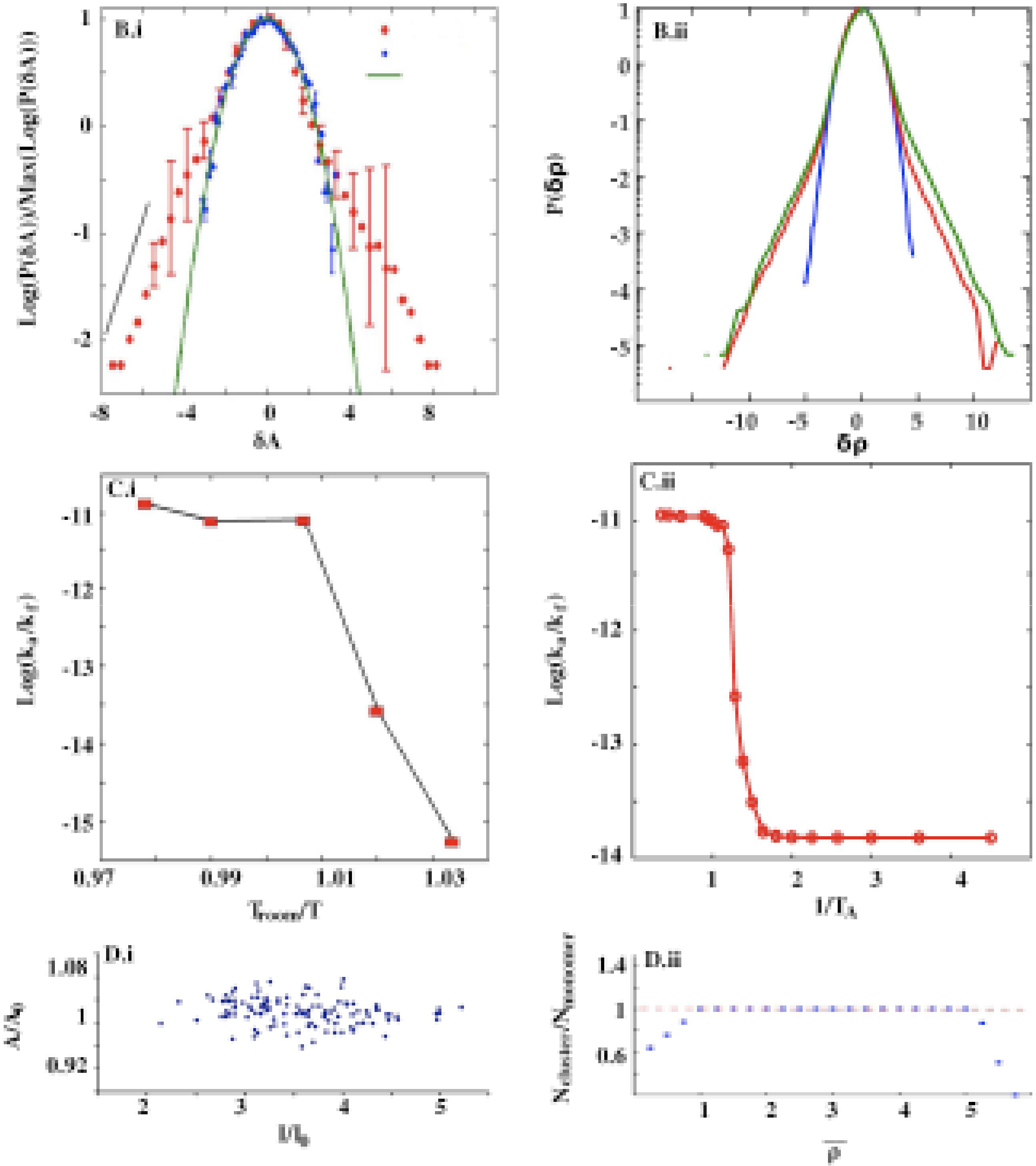
Sharma et al, Cell 2004
Goswami et al, Cell 2009



Bleb retraction rates as a function of temperature

(actomyosin contractility)

$$D_{cluster} = 0$$

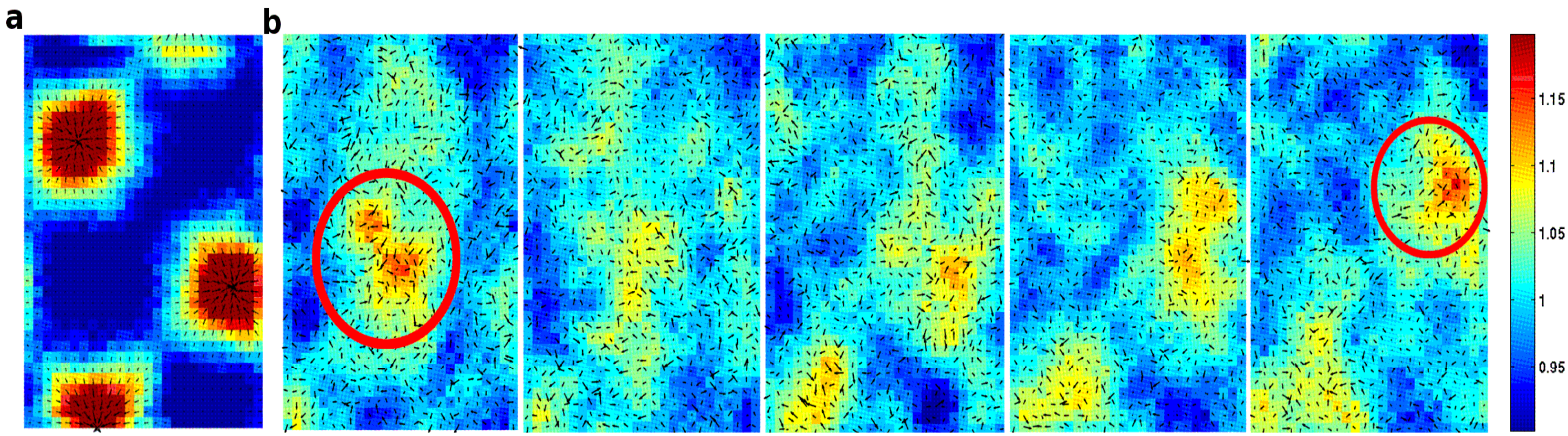


Experiment

Theory

Kripa, G., S. Ghosh et al (under review)

Active Fluctuations on cell surface



Density fluctuations of passive molecules can report on activity of underlying substrate

$$\mathbf{J} \propto \mathbf{n}$$

Current proportional to orientation

Root Mean Square Fluctuations of Number

$$\Delta N = \sqrt{S(q \to 0) L^2}$$

$$where\ S(q,t) \equiv \langle \rho(q,t)\rho(-q,t) \rangle$$

$$S(q) = q^{-2\zeta} H(\theta_{\vec{q}})$$

$$\zeta(d=2) = 3/5$$

(Toner and Tu, 1997)

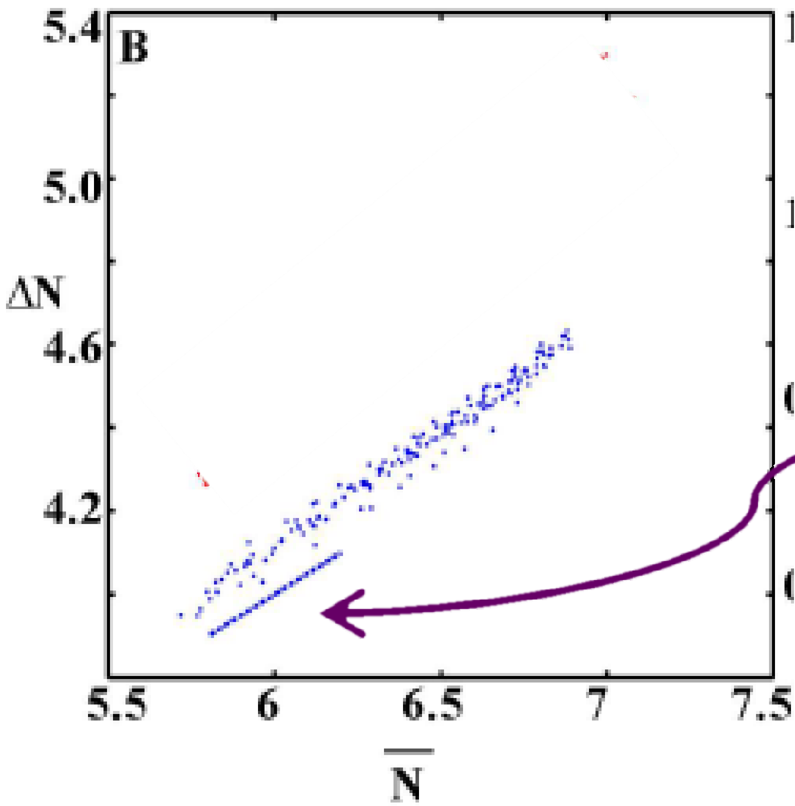
$$\text{since,} \quad \overline{N} \propto L^2$$

$$\sqrt{\Delta N^2} \propto N^{4/5}$$

Standard Deviation versus Mean

- Take `windows’ of different sizes on cell surface and measure time series of fluorescence intensity in each window
- Compute mean fluorescence intensity in each window
- Compute Standard Deviation of intensity in each window
- Plot Standard Deviation versus Mean

Number Fluctuations for an Inert Particle



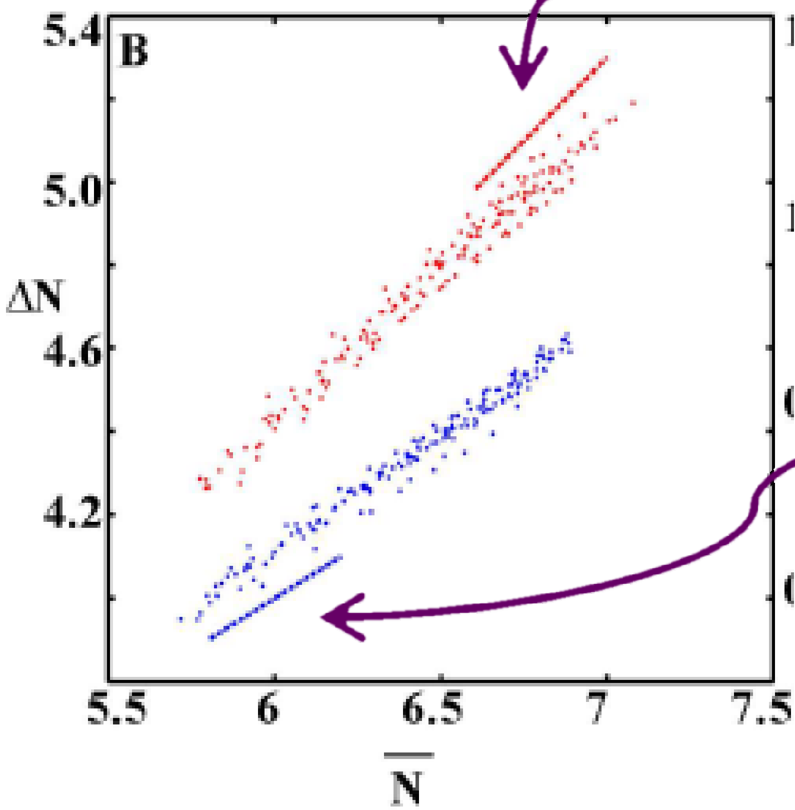
$$\sqrt{\Delta N^2} \propto \sqrt{N}$$

(Brownian Particle)

Kripa, G., S. Ghosh et al (under review)

Anomalous Number Fluctuations from phase fluctuations of n

$$\sqrt{\Delta N^2} \propto N^{4/5} = N^{0.8} \gg N^{0.5}$$



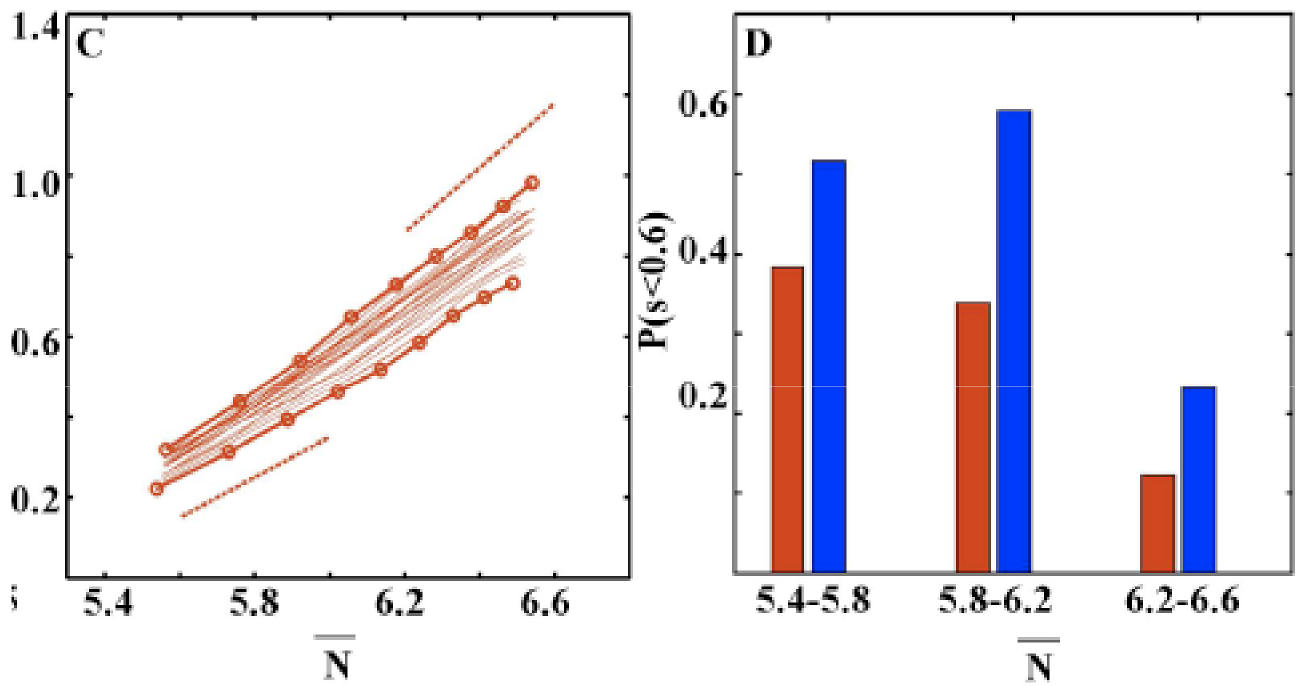
$$\sqrt{\Delta N^2} \propto \sqrt{N}$$

(Brownian Particle)

Kripa, G., S. Ghosh et al (under review)

Crossover from brownian to active (anomalous) Number Fluctuations !

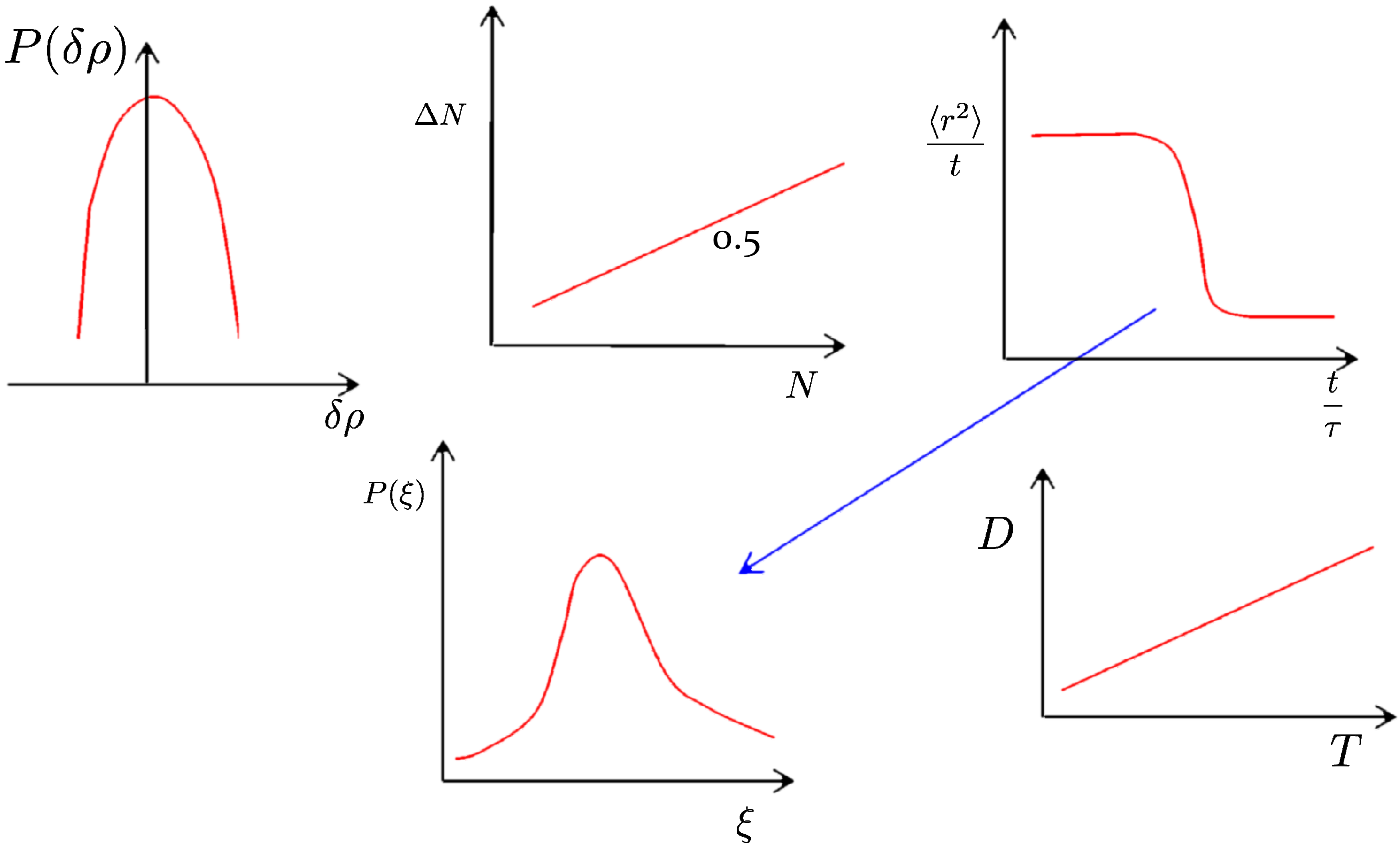
$$\sqrt{\Delta N^2} \propto N^{4/5} = N^{0.8} \gg N^{0.5}$$



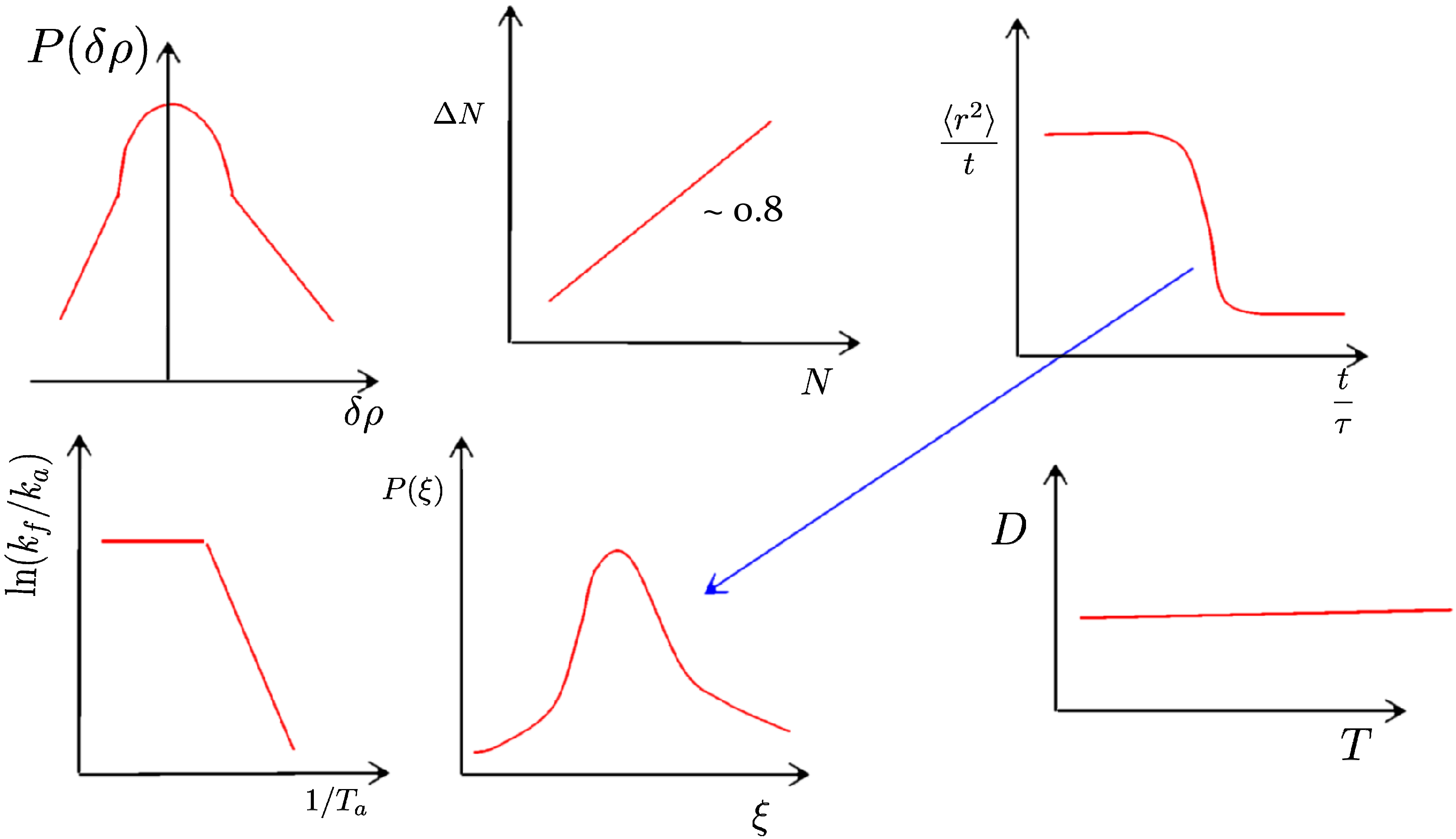
$$\Delta N = A(T) N^{1/2} F(t\, N^{3/5})$$

$$\text{where} \quad t = \frac{T_a}{T}$$

Inert Particles : NBD-SM, DOPE, ...

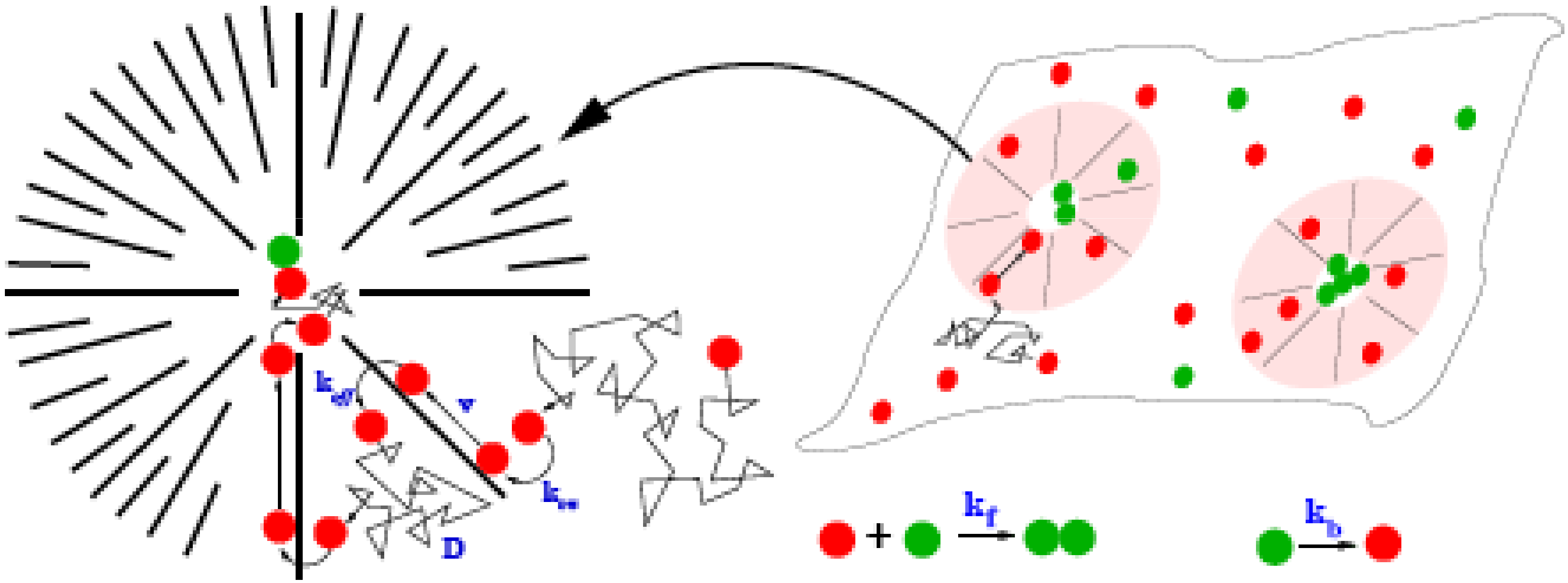


Passive Particles : GPI-Proteins, Ras, Glycolipids, ...



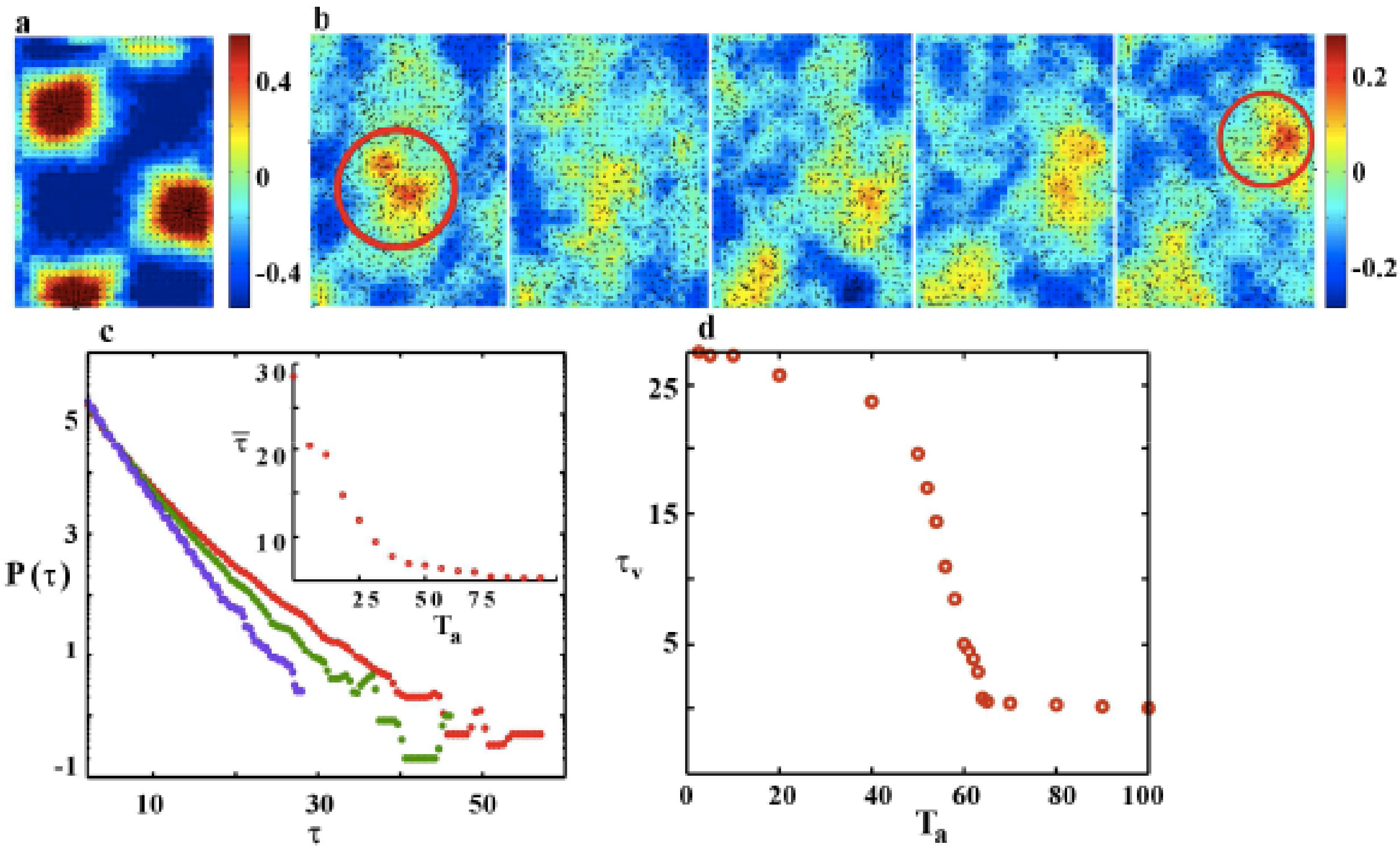
Spatiotemporal regulation of chemical reactions by active cytoskeletal remodeling

A. Chaudhuri, B. Bhattacharya et al. (2010)



enhances multi-particle encounters

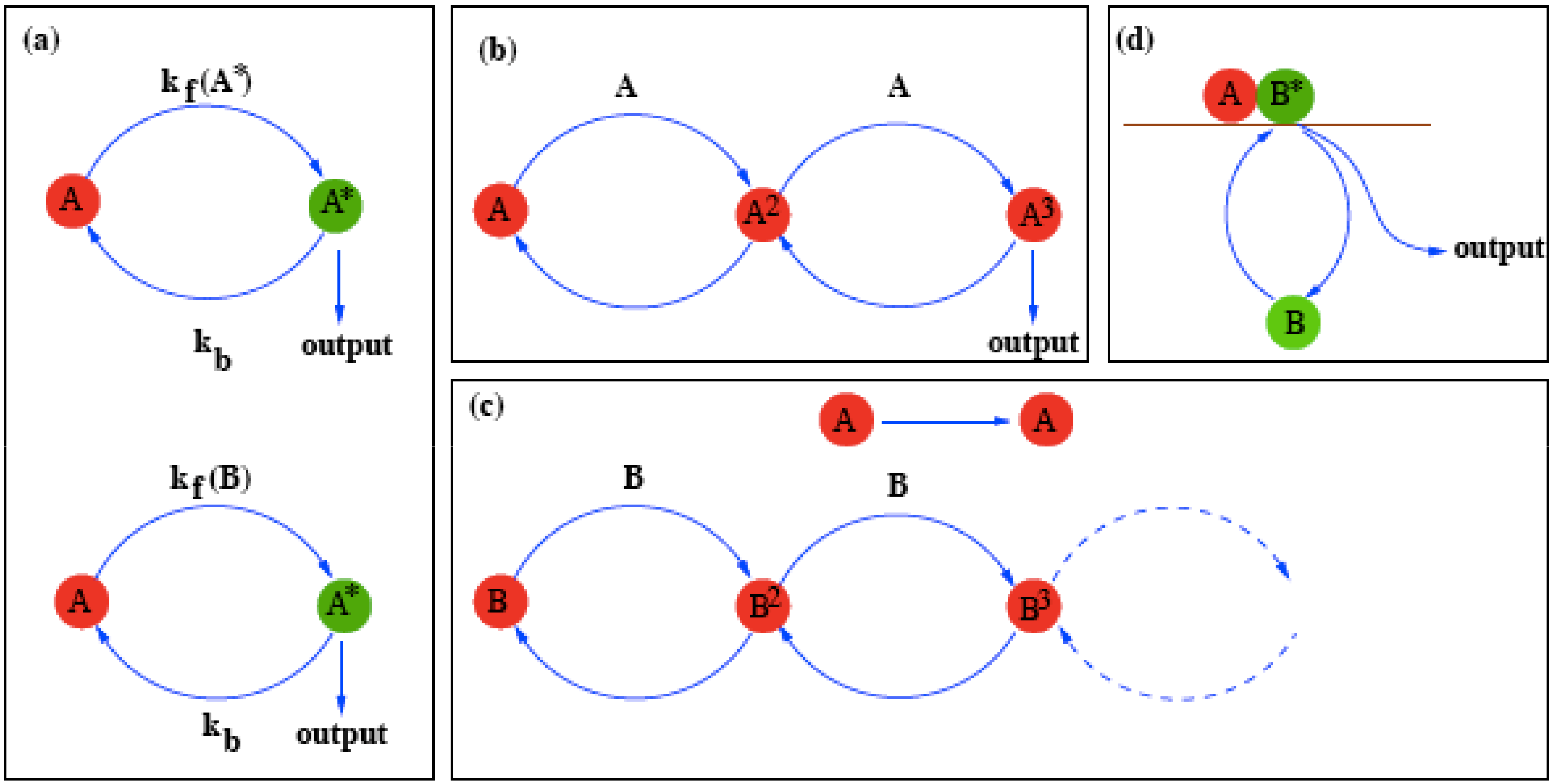
Steady State Remodeling of Active Filaments



Lifetime Distribution of Asters

Mean Advection Time (Aster Size)

Second and higher-order chemical networks in signaling and sorting



Active Gain in reaction time

Active Peclet Number

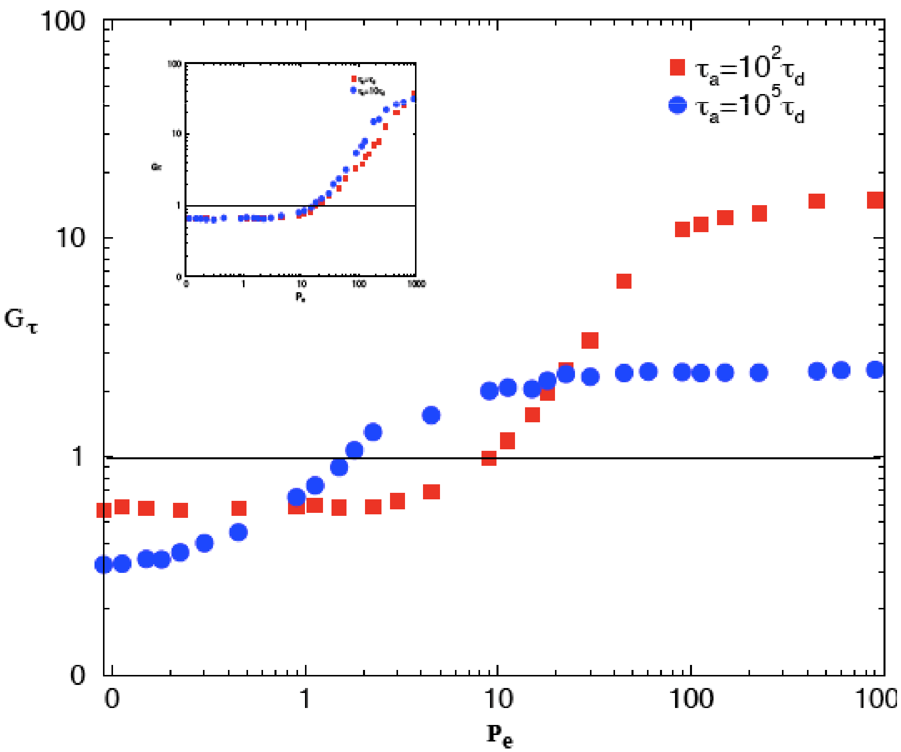
$$P_e > P_e^* \approx 10$$

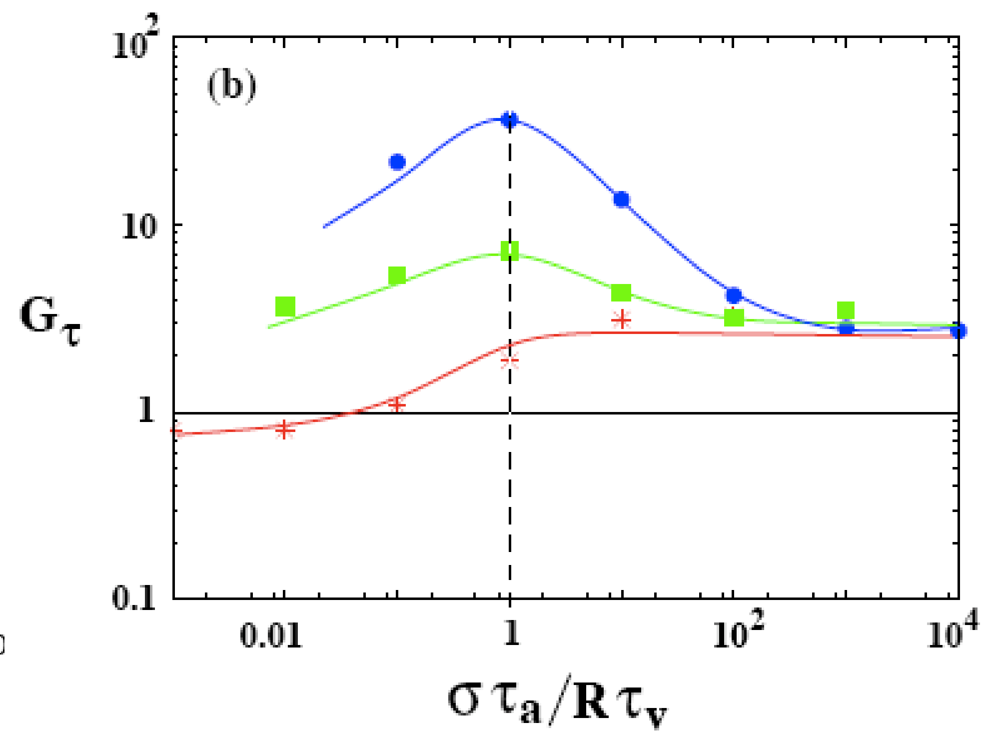
$$P_e = K \frac{R}{\sigma} \frac{\tau_d}{\tau_v} = \frac{K v R}{D}$$

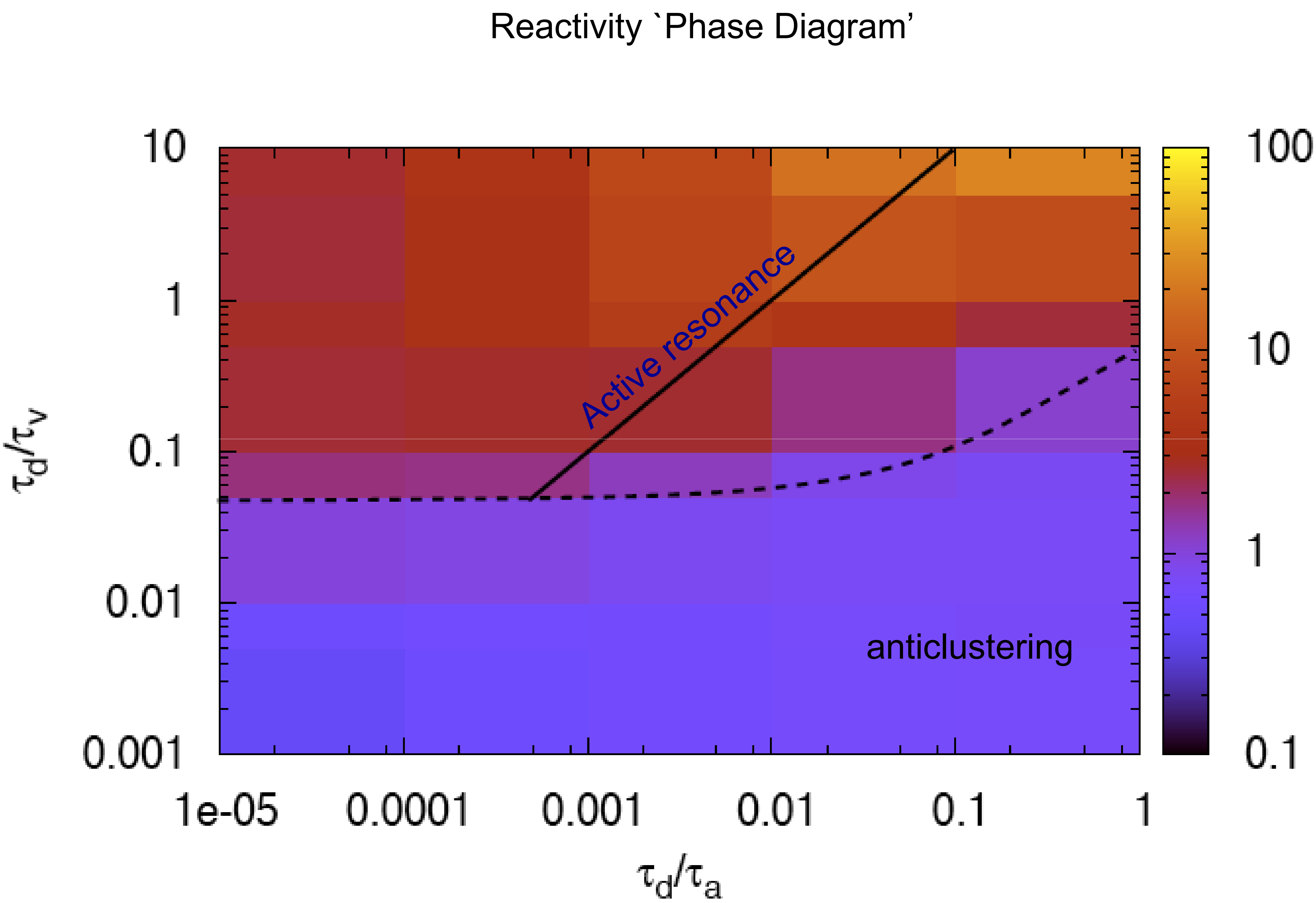
Active Resonance

Advection=Fragmentation

$$\tau_v = K \frac{\sigma \tau_a}{R}$$





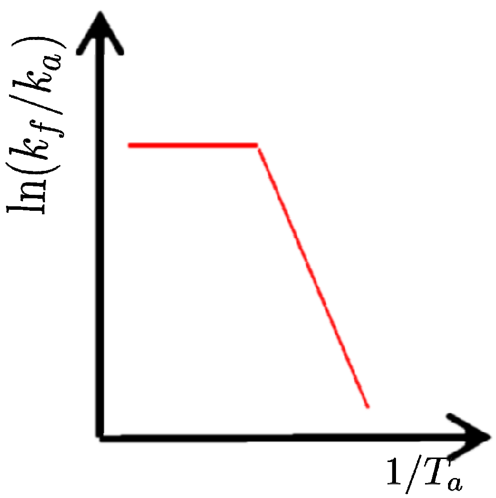


Reactivity `Phase Diagram'
Active resonance
anticlustering
10
1
0.1
0.01
0.001
1e-05
0.0001
0.001
0.01
0.1
1
100
10
1
0.1
τ_d/τ_v
τ_d/τ_a

Active Chemical Thermodynamics : Aster as an enzyme (E)

Passive Molecule, A $$E + A \leftrightharpoons EA \rightarrow A^{*} + E$$

with Michelis-Mentin kinetics



Reaction Cascade

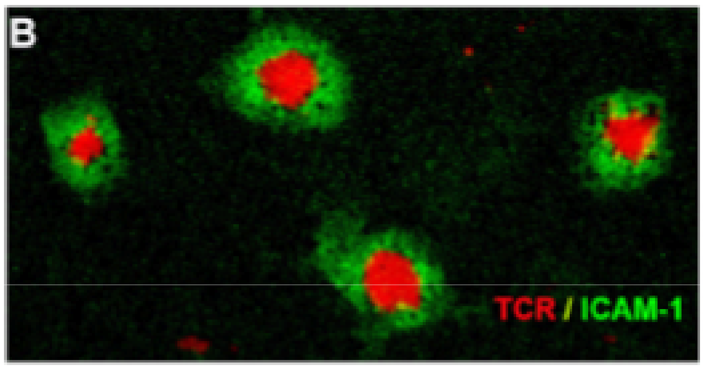
$$E + A_1 \leftrightharpoons EA_1 \leftrightharpoons EA_1A_2 \leftrightharpoons \cdots \leftrightharpoons EA_1 \ldots A_p \rightarrow A^{*} + E$$

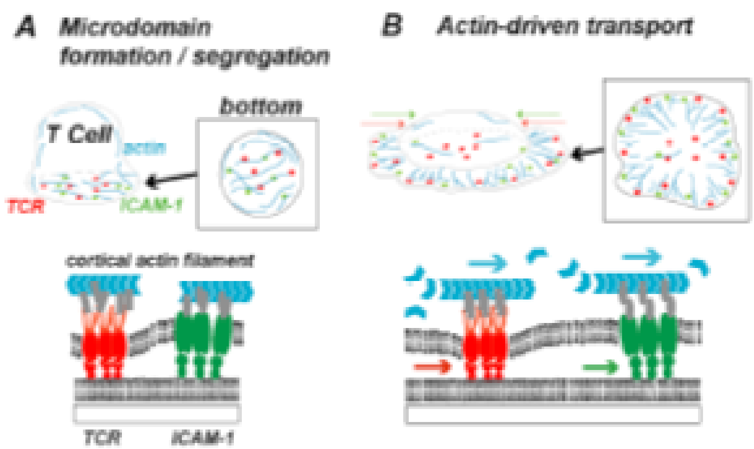
High Hill Coefficient

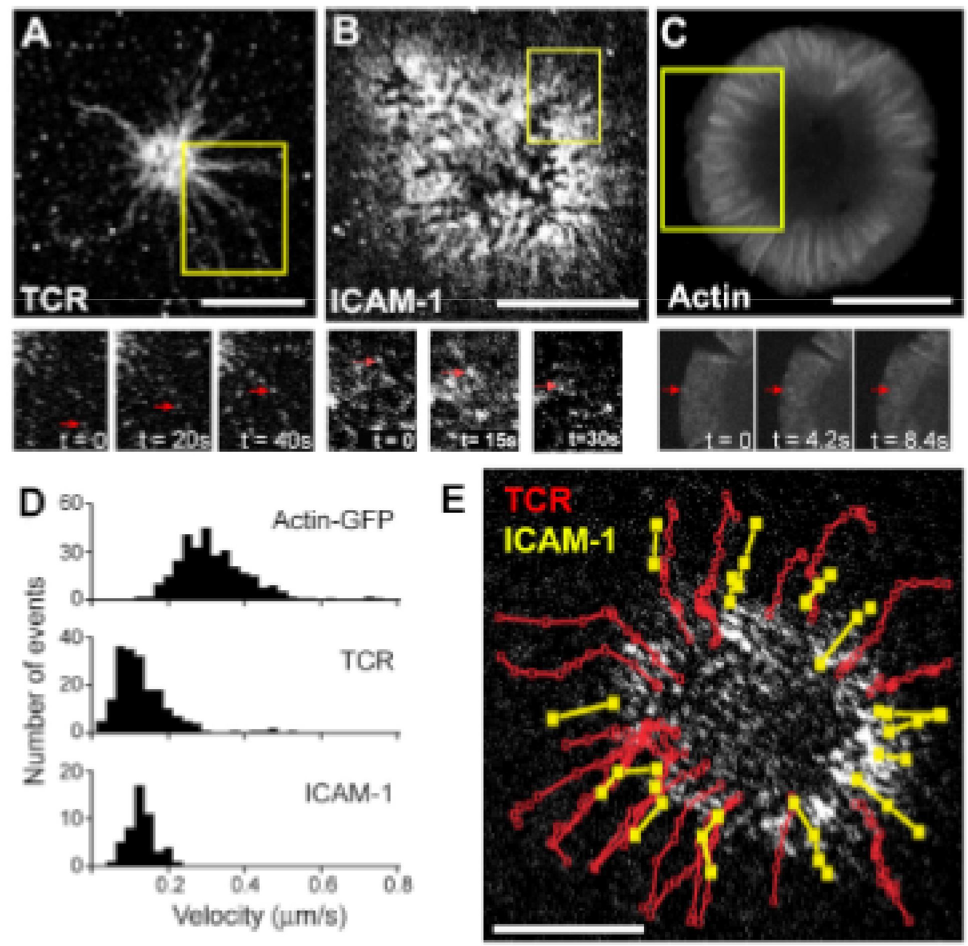
Active Segregation of Molecules and Patterning

Mechanisms for Segregating T Cell Receptor and Adhesion Molecules During Immunological Synapse Formation in Jurkat T Cells

Yoshihisa Kaizuka[1*], Adam D. Douglass[1*], Rajat Varma[2], Michael L. Dustin[2†], and Ronald D. Vale[1†]





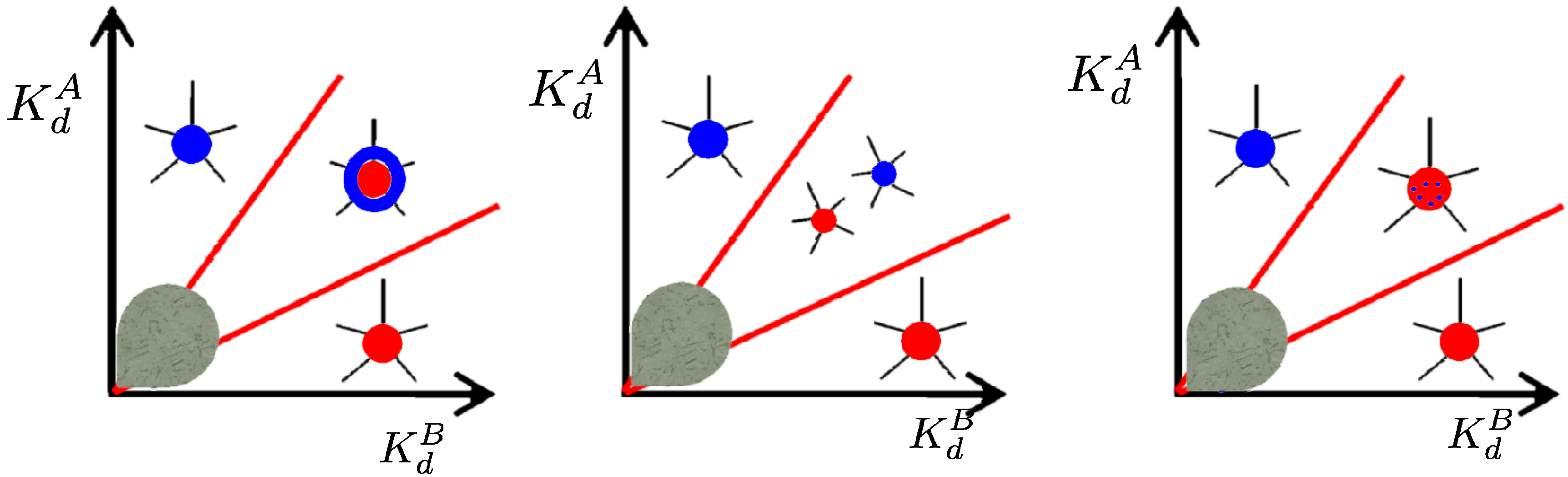


A. Chaudhuri, R Vale, S. Mayor, MR

Include Protein-Protein Interactions

Morse Potential

$$V_{\alpha\beta}(r) = V_{\alpha\beta}\left(1 - e^{-a(r-r_0)}\right)^2$$



$V_{AA} \gg V_{BB}$

V_{AB} small

(eg., TCR, ICAM-1)

$V_{AA} \sim V_{BB}$

(eg., TCR, LAT)

V_{AB} large

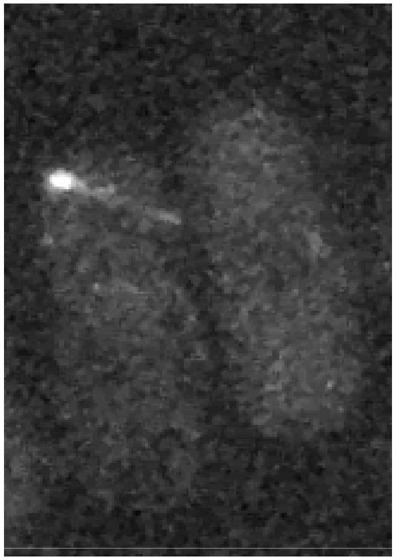
(eg., GPI-APs)

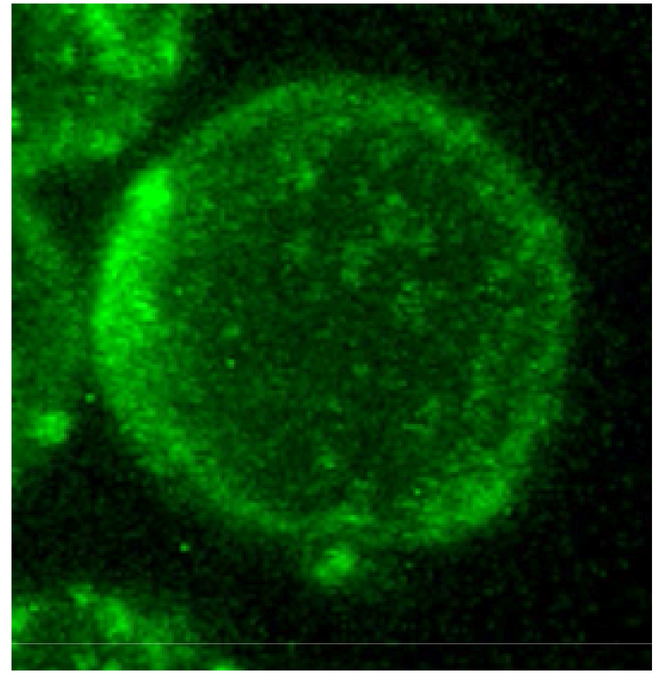
$$g(r) \text{ has a cusp as } r \to 0$$

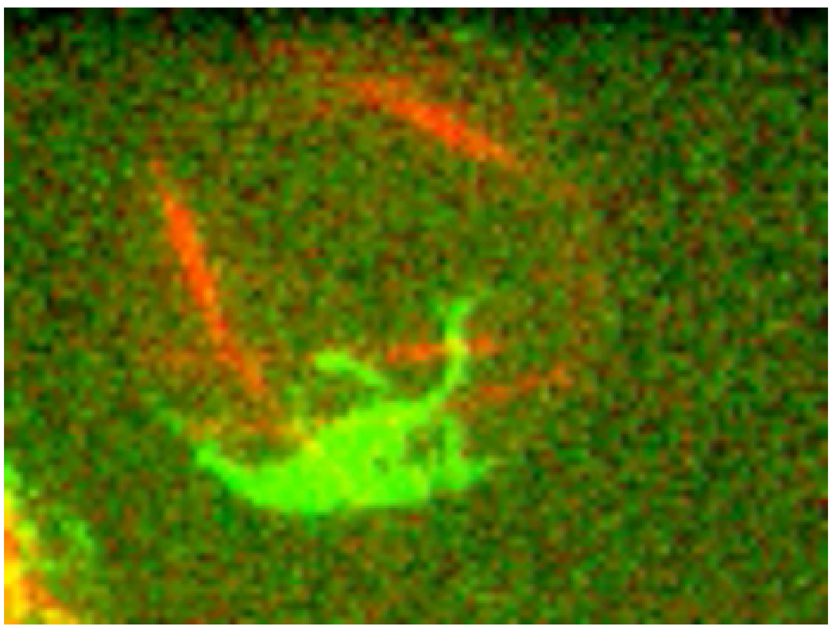
Phases of Active Cortical Actin on spherical and cylindrical cells

Pragya Srivastava, RRI

Fission Yeast : Misplaced Cortical Actin Ring







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Active Hydrodynamics Theory

Kripa Gowrishankar (RRI*)

Pragya Srivastava (RRI)

Roie Shlomovitz (Weizmann, Israel)

Fluorescence-based experiments

Debanjan Goswami (NCBS*)

Subhashri Ghosh (NCBS)

Applications : Cell Surface Signaling, Sorting, Immunological Synapse

Abhishek Chaudhuri (Postdoc, RRI*)

Bhaswati Bhattacharya (JNCASR*)

Ron Vale (UCSF)

Discussions

Sriram Ramaswamy (IISc)
John Toner (Oregon)

Contractile Slipping in Yeast

Mohan Balasubramanian (TLL)
Mithilesh Mishra (TLL)
P. Srinivasan (TLL)

Funding : CEFIPRA 3504-2, HFSP