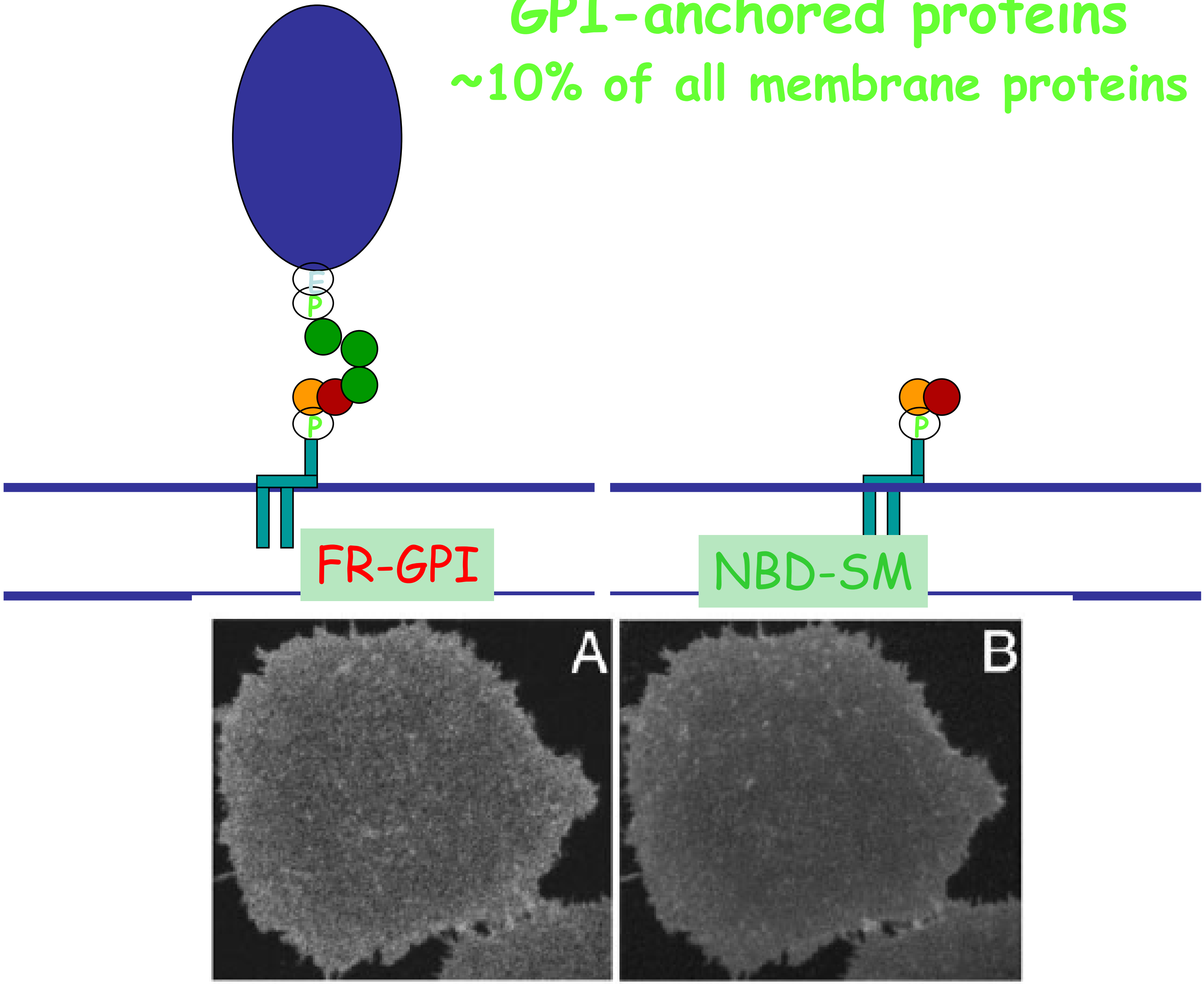
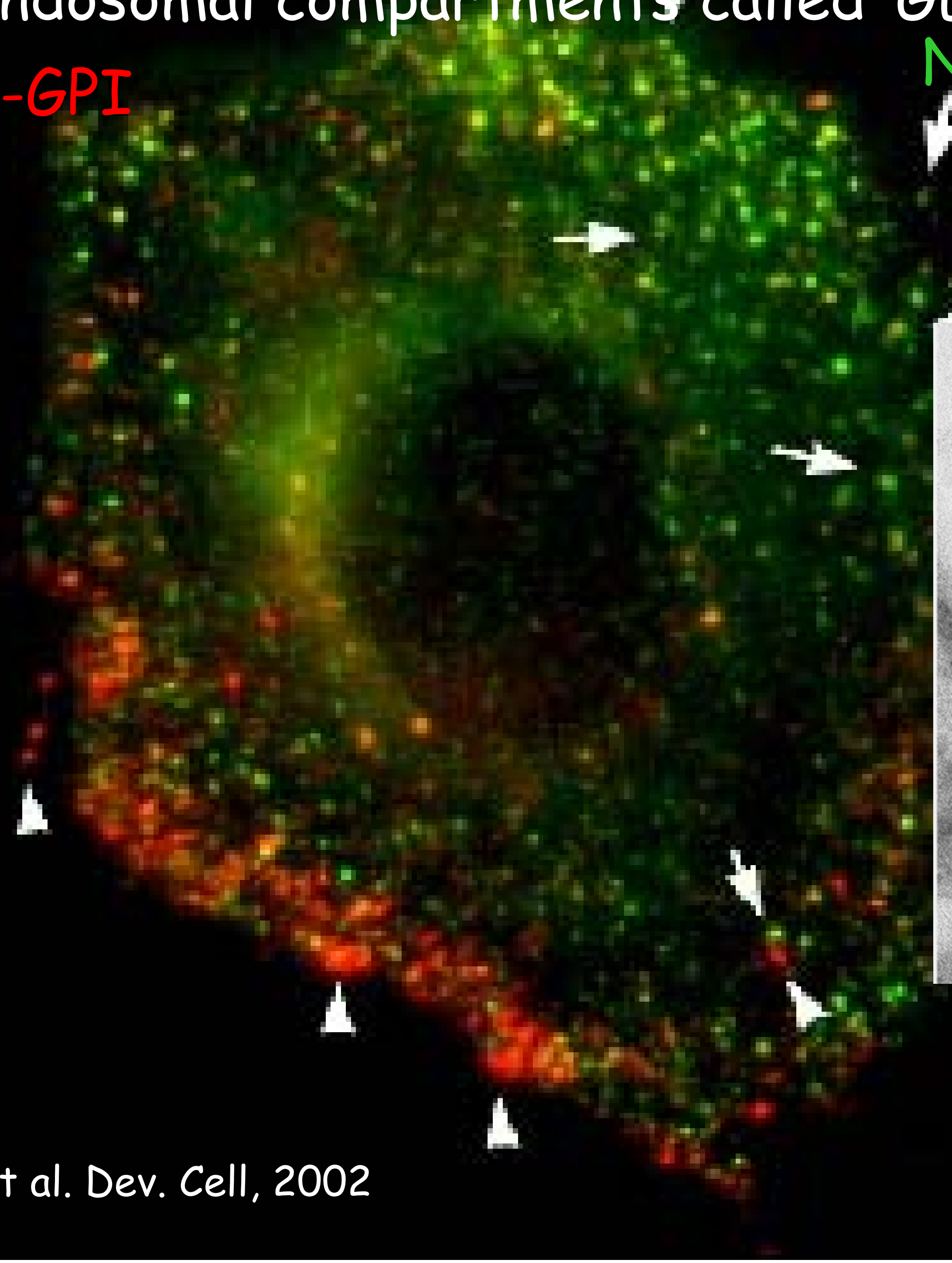
GPI-anchored proteins

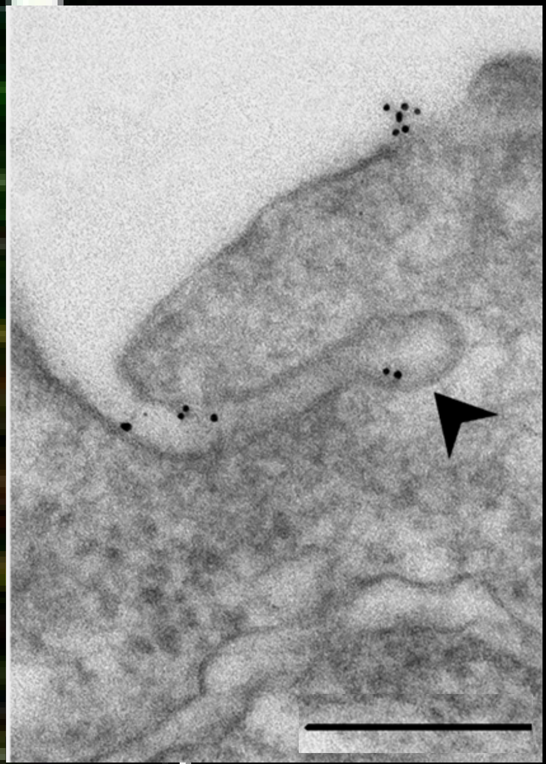
~10% of all membrane proteins



Satyajit Mayor, National Centre for Biological Sciences KITP Evo Cell, Feb 4, 2010

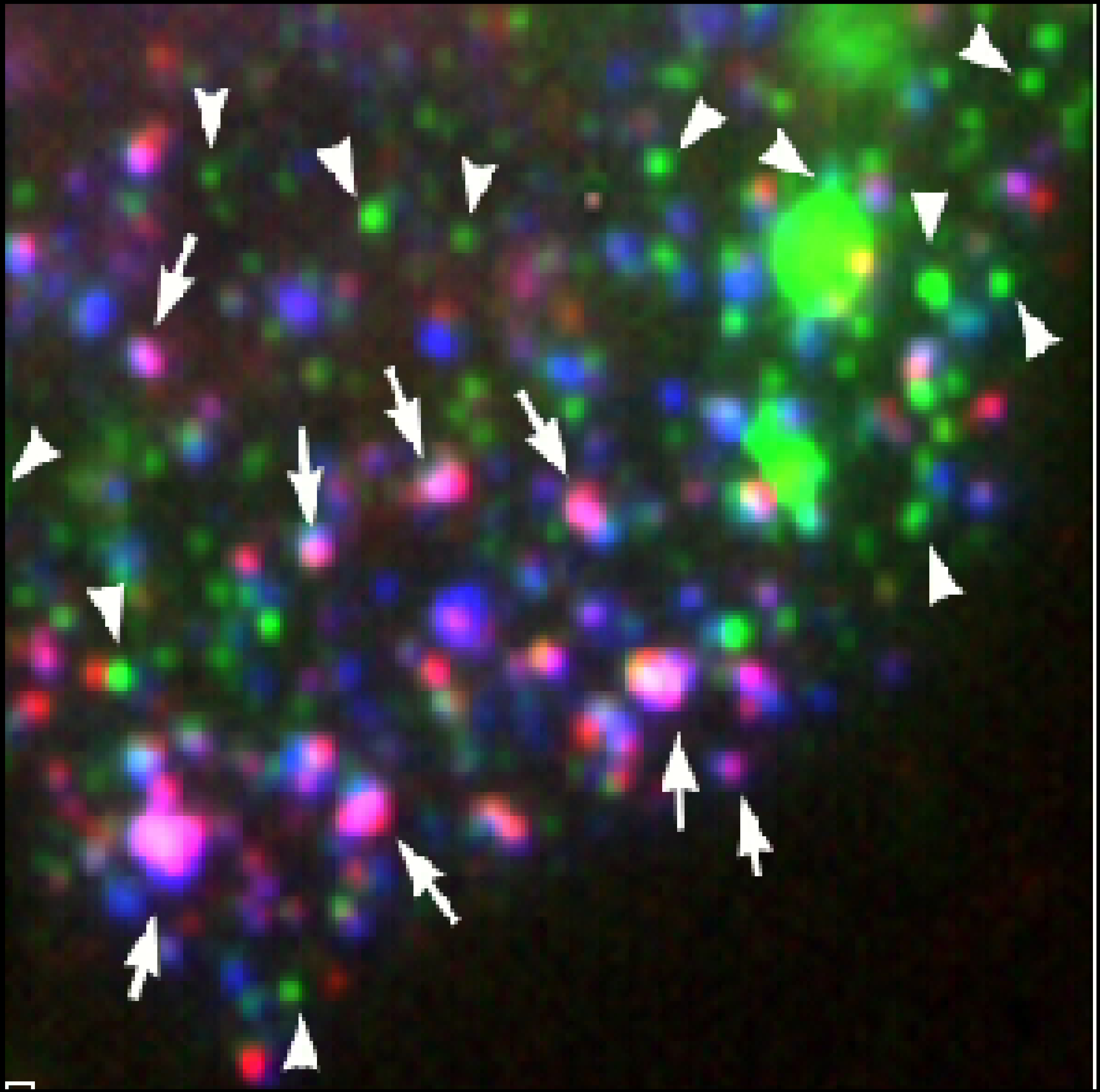
GPI-anchored proteins are selectively endocytosed into distinct endosomal compartments called 'GEECs'

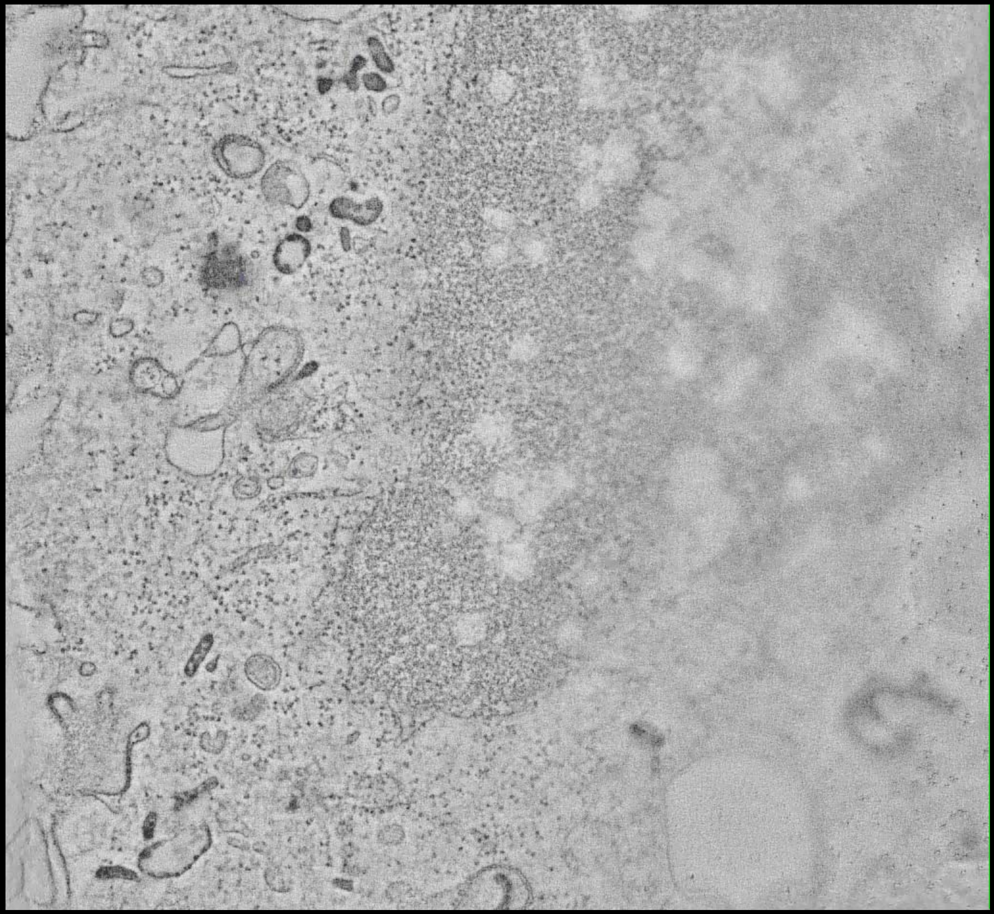




Rob Parton- Univ. Queensland

Sabharanjak et al. Dev. Cell, 2002



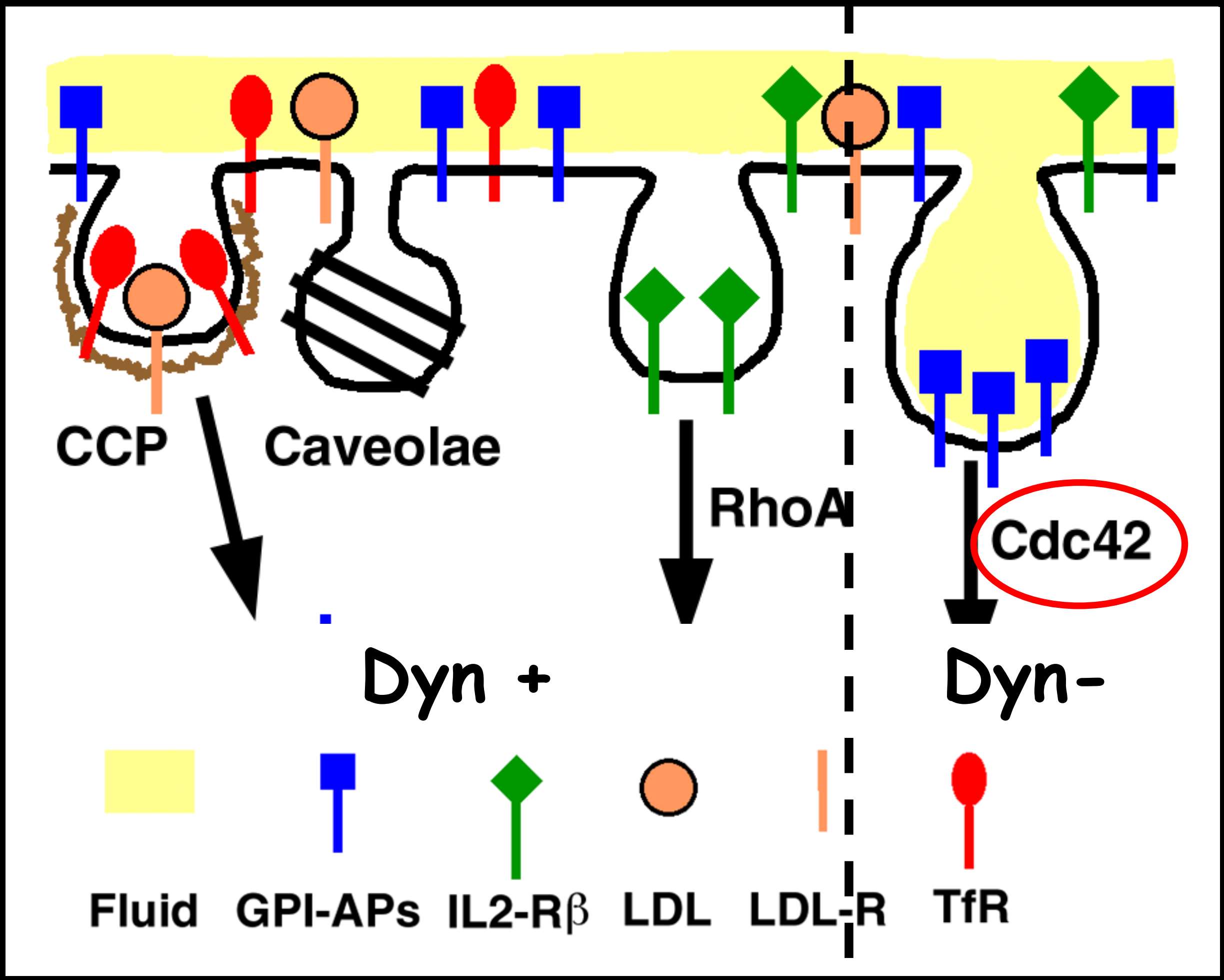


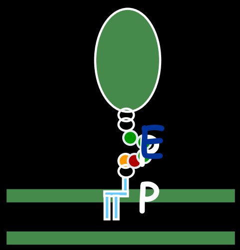
Tomogram of a CLIC/GEEC endosome:

Rob Parton

CTBHRP - 15s internalisation, Cav1-null fibroblasts, DAB-ascorbic acid method

GPI-anchored proteins are constitutively endocytosed via a specialized pathway ' the CLIC/GEEC pathway'



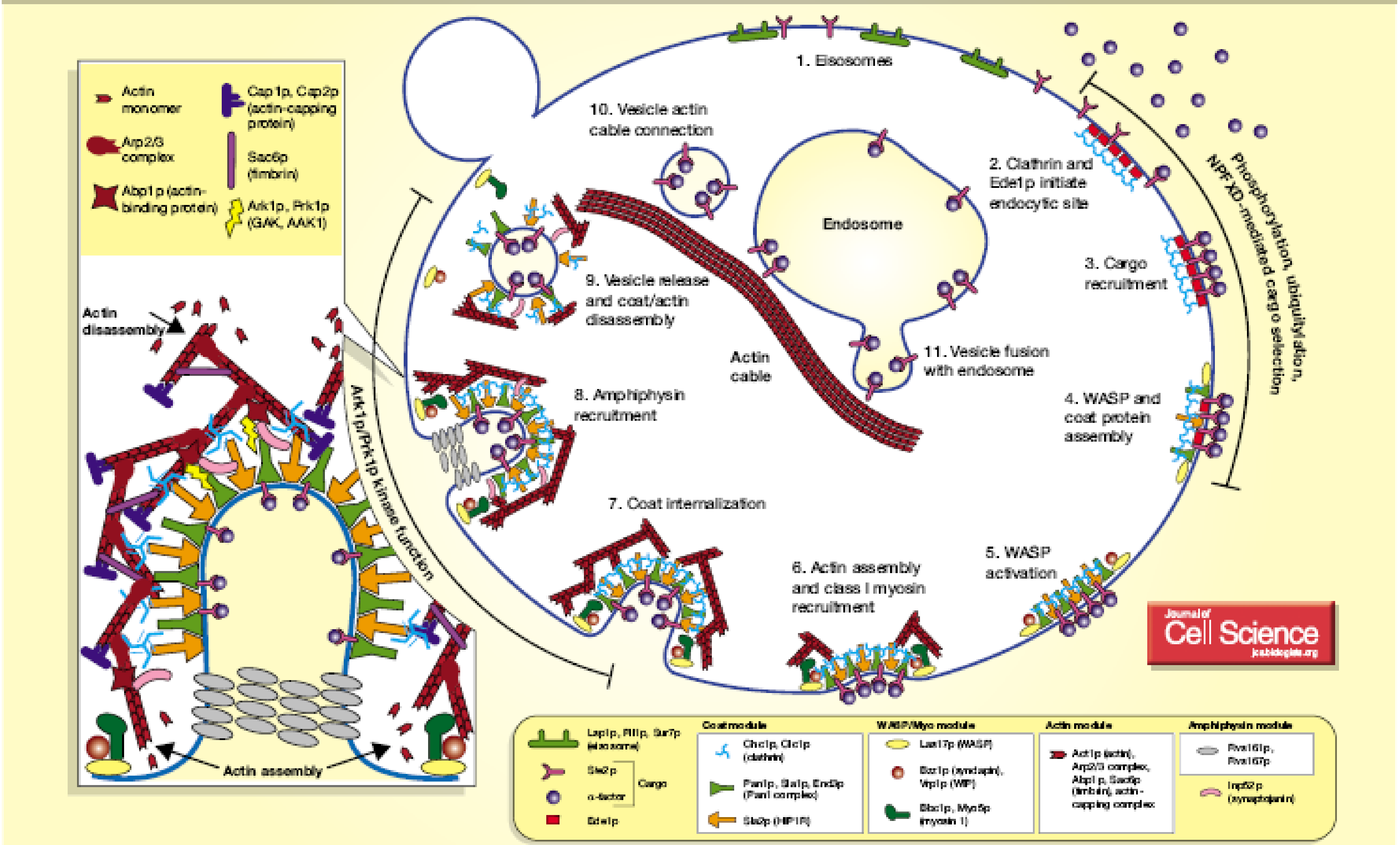


- Sabharanjak, Sharma et al. *Dev. Cell* (2002)
- Kirkham et al. *J. Cell Biol.* (2005)
- Kalia et al. *Mol. Biol. Cell* (2006)
- Chadda et al. *Traffic* (2007)
- Kumari etal. *Nature Cell Biol.* (2007)

Mayor and Riezman, *Nature Reviews MCB*, 2004
Mayor and Pagano, *Nature Reviews* MCB, 2007

The Budding Yeast Endocytic Pathway

Christopher P. Toret and David G. Drubin



Insights from Yeast

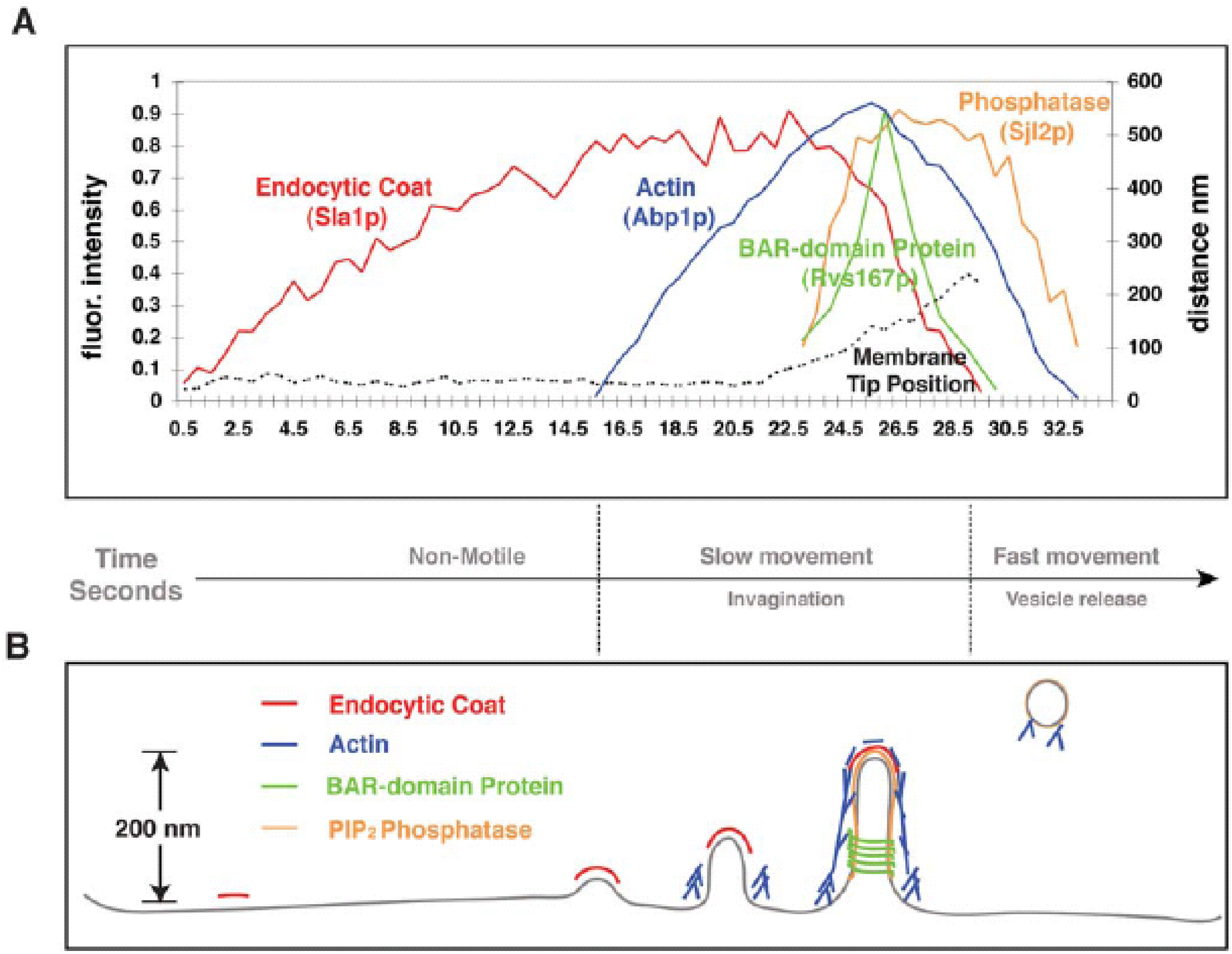
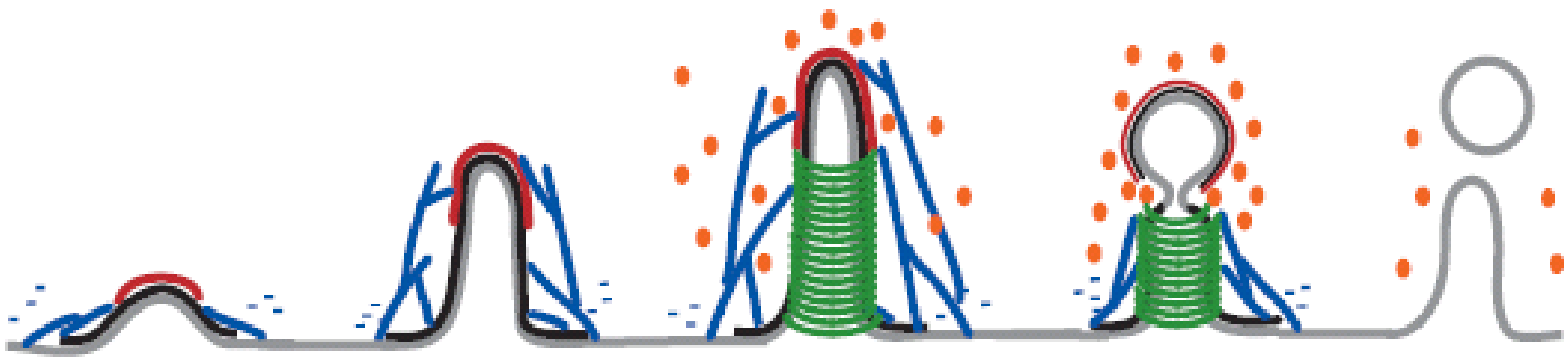


Figure 1. Endocytic dynamics in budding yeast. (A) Timelines for endocytic protein recruitment as determined by multicolor fluorescence microscopy analysis. Sla1p, which is an endocytic adaptor protein, represents the endocytic coat. Abp1p is an actin-binding protein and faithfully reports on actin dynamics. Sjl2p is the yeast synaptojanin that hydrolyzes PIP_2. PIP_2 represents the lipid module and is believed to be the recruitment signal for many endocytic proteins. Rvs167p, yeast Amphiphysin, contains a BAR domain capable of sensing/binding curved membranes and deforming membranes. (Sla1 and Abp1 data are from [8], Sjl2 data are from [11], Rvs167 data are determined in this work from six individual patches in cells expressing Rvs167-GFP and aligned to the relative timing of Sjl2 appearance.) (B) Spatial profiles of endocytic membrane and the key endocytic proteins as revealed by EM [15].
doi:10.1371/journal.pbio.1000204.g001

nd co-workers , PLoS Biol 2009

Insights from Yeast

Drubin, Oster and co-workers , PLoS Biol 2009



Stage 1

Coat module recruited to the bud: deforms the membrane & nucleates F-actin.

Stage 2

Membrane invagination is intiated by F-actin & myosin.

Stage 3

The high curvature RAPIDLY recruits:
(1) BDP to the tubule region via its intrinsic postivie feedback between curvature sensing and deforming;
(2) Phosphatase to all over the endocytic site.

Stage 4

BDP protection of the tubule PIP_2 creates a PIP_2 phase segregation. The resulting interfacial force squeezes the neck, creating even higher curvature, induces more PIP_2 hydrolysis, resulting in an even larger interfacial force.

Stage 5

Rapid vesicle scission and disassembly of endocytic apparatus.

Membrane — PIP_2 — Coat — Actin — BDP — Phosphatase

The Mechanochemistry of Endocytosis

Jian Liu[☯¤], Yidi Sun[☯], David G. Drubin*, George F. Oster*

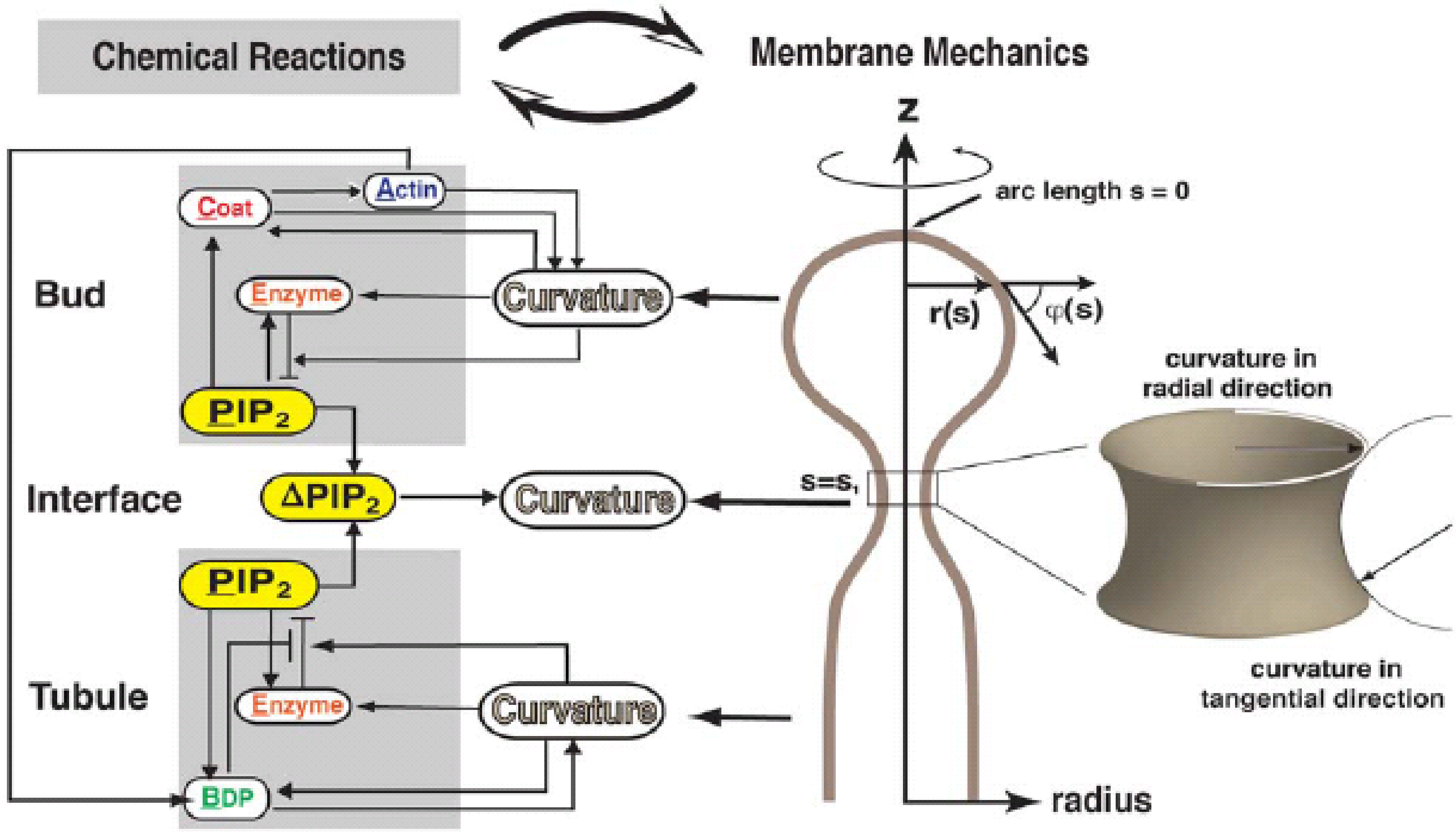
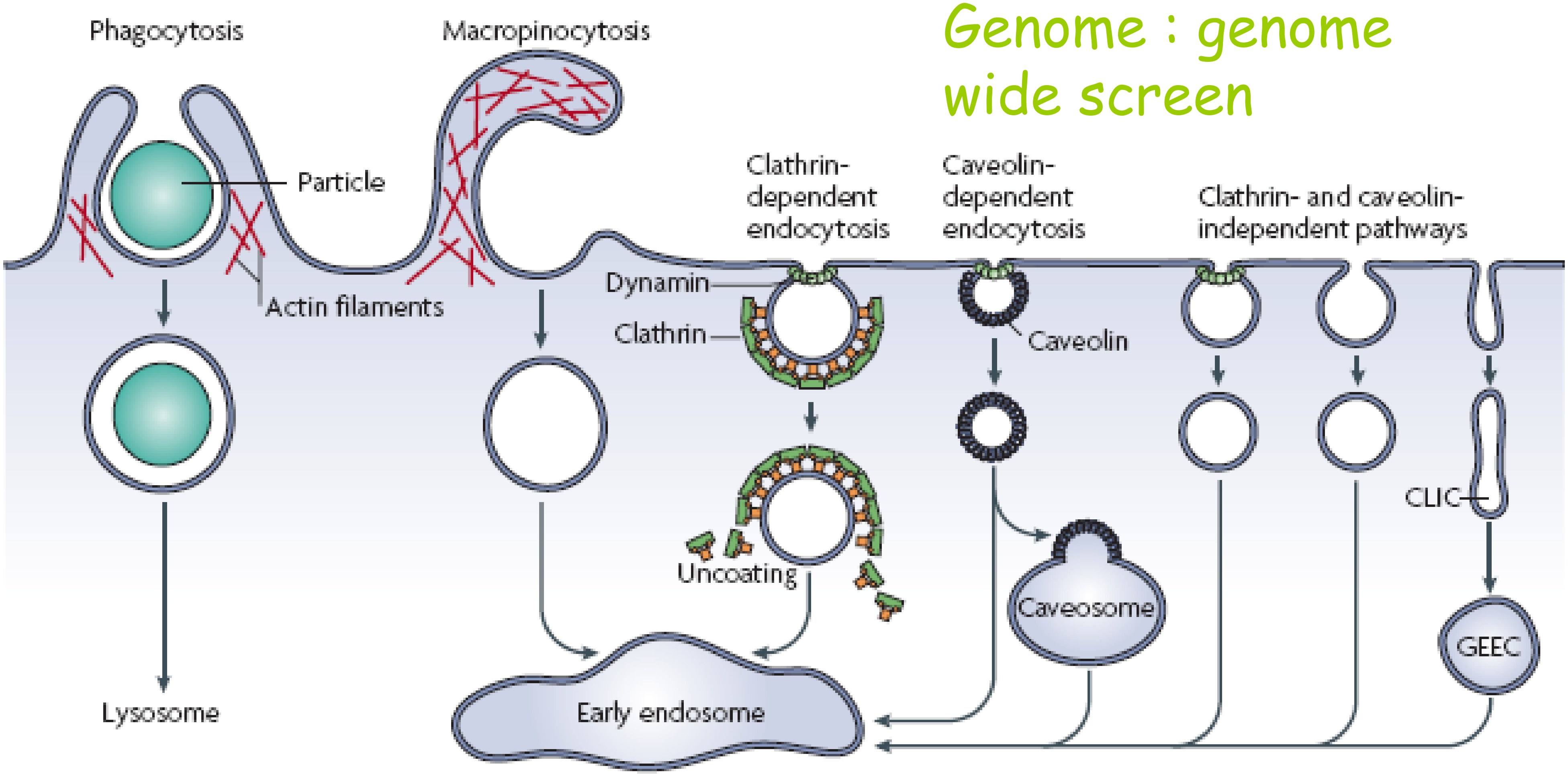


Figure 2. Mechanochemical feedback mechanism for endocytosis in budding yeast. The thin arrows represent activation effects, and the bar ends represent inhibition effects. The local spatial coordinate along the membrane surface is the arc length s with unit length 1 nm. The bud region is defined by the arc length $0 \le s \le s_1$, the lipid phase boundary is at $s = s_1$, and the tubule region starts from $s = s_1 + 1$, where s_1 is chosen to be 100. We assume that membrane shape is cylindrically symmetric. $\varphi(s)$ is the membrane tangential angle and $r(s)$ is the radius of the tubule. The mean curvature, $\Omega(s)$, is the average of the curvatures in the radial and tangential directions; it measures the overall extent of the PIP_2 head group exposure.
doi:10.1371/journal.pbio.1000204.g002

Drubin, Oster and co-workers , PLoS Biol 2009

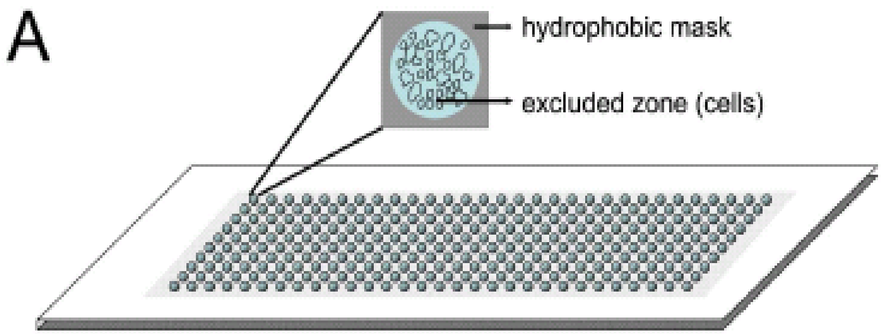
A high throughput screening strategy to identify molecular components of the pinocytic pathway in *Drosophila* SR+ cells.

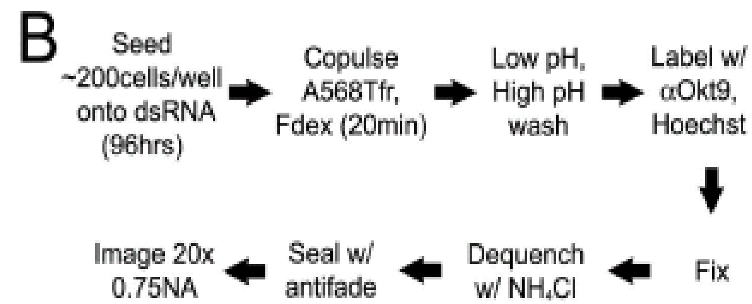
Genome : genome wide screen

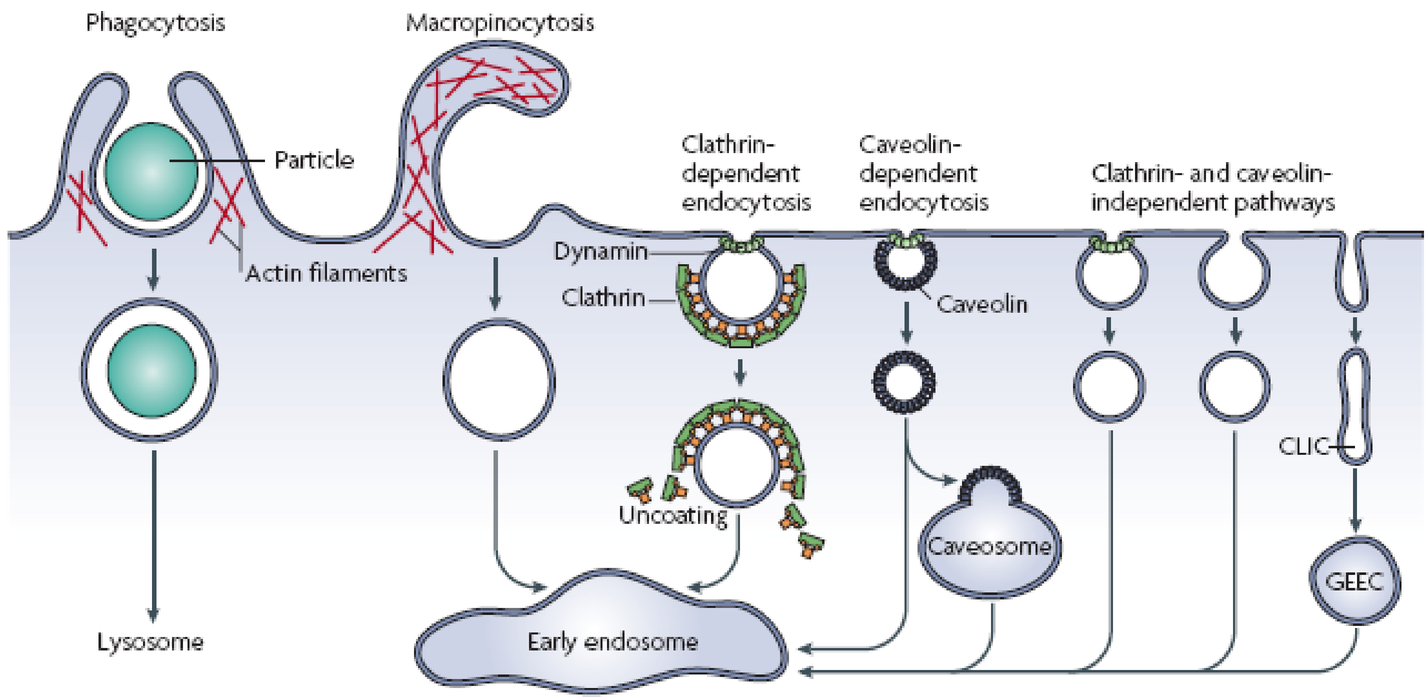


Balaji R./Swetha MG/Gagan Gupta/ Gautam Dey /
Mukund Thattai/ Shameer/R. Sowdhamini/Krishnamurthy

A high throughput screening strategy to identify molecular components of the pinocytic pathway in *Drosophila* SR+ cells.

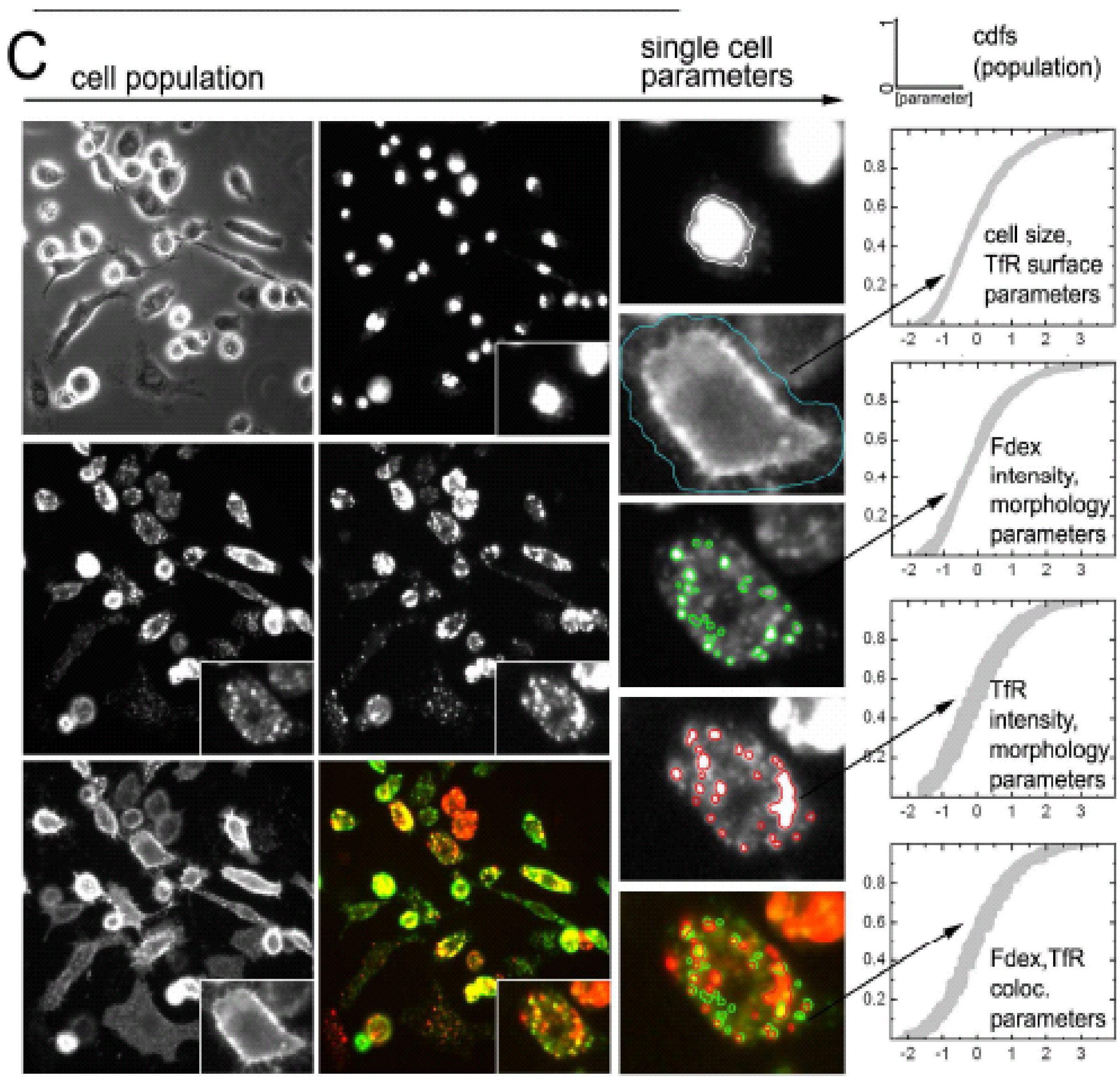






Balaji R/Swetha MG/Joseph Mathew/Gagan Gupta,/ Gautam Dey/ Mukund Thattai/Shameer K/R.Sowdhamini

A high throughput screening strategy to identify molecular components of the pinocytic pathway in *Drosophila* SR+ cells.



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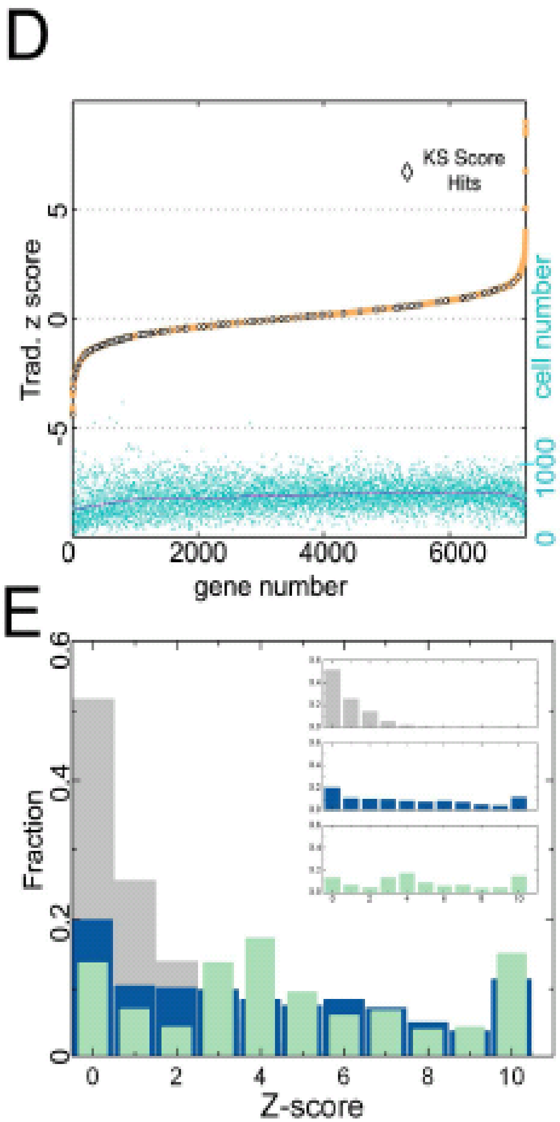
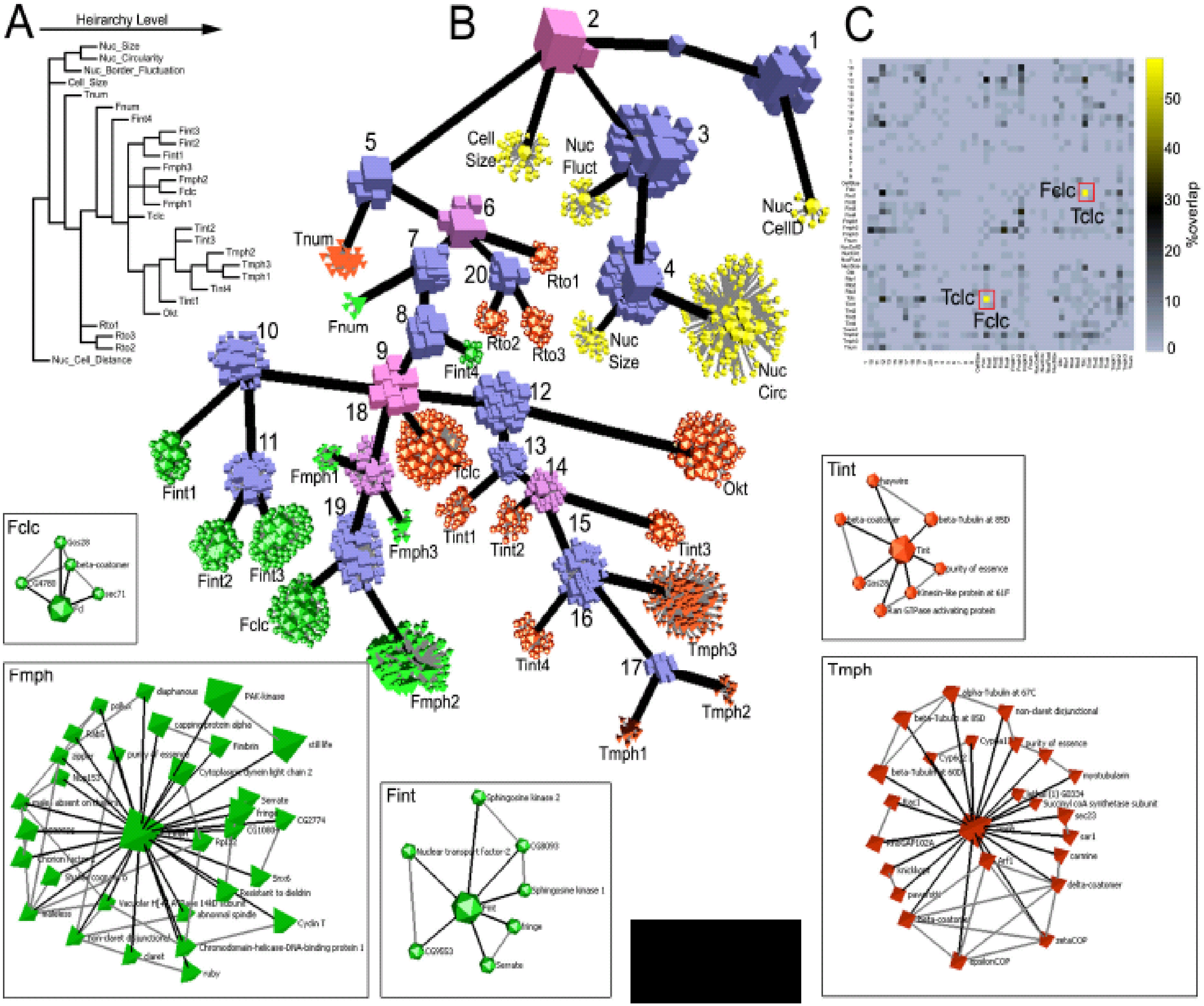
A high throughput screening strategy to identify molecular components of the pinocytic pathway in *Drosophila* SR+ cells.

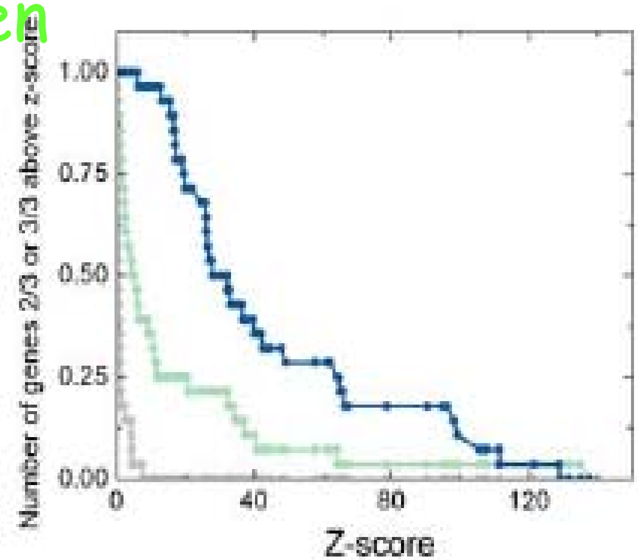


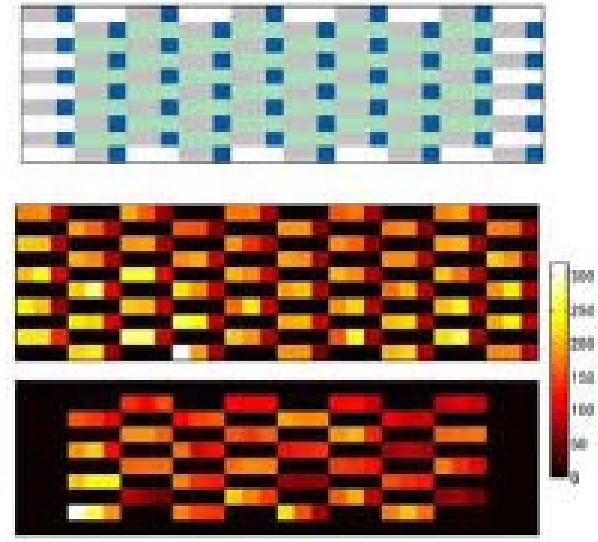
Balaji R/Swetha MG/Joseph Mathew/Gagan Gupta,/ Gautam Dey/ Mukund Thattai/Shameer K/R.Sowdhamini



Balaji R/Swetha MG/Joseph Mathew/Gagan Gupta,/ Gautam Dey/ Mukund Thattai/Shameer K/R.Sowdhamini

Classsification Screen



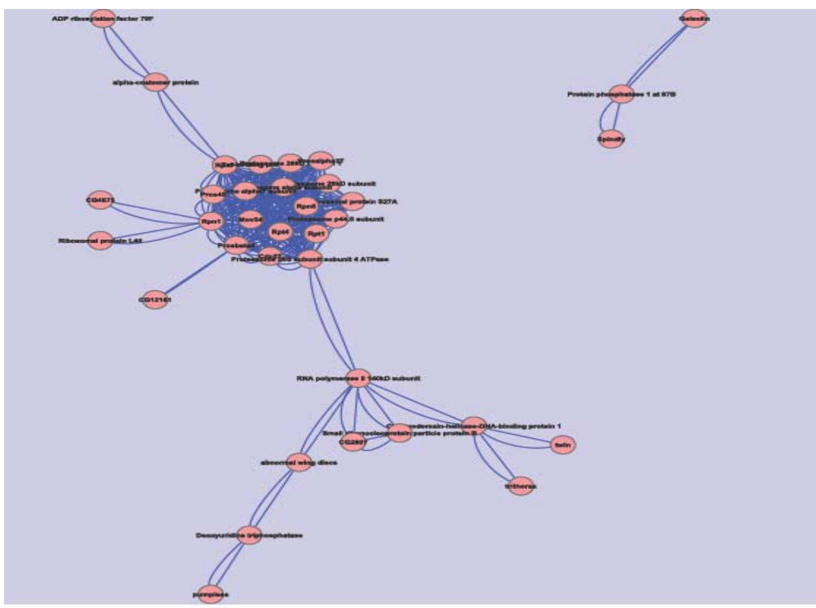


Pulse

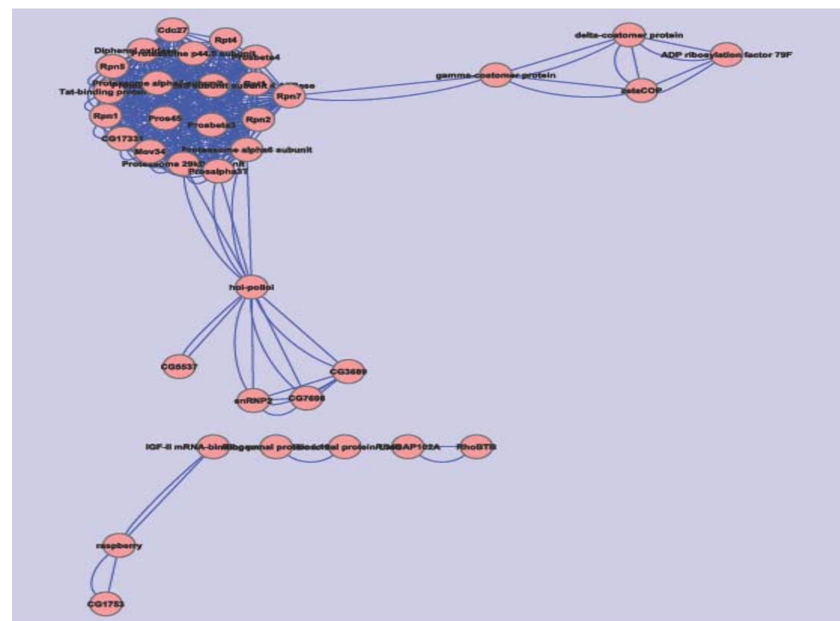
Chase

Pulse

Short Pulse Intensity down



Fint and Tint down



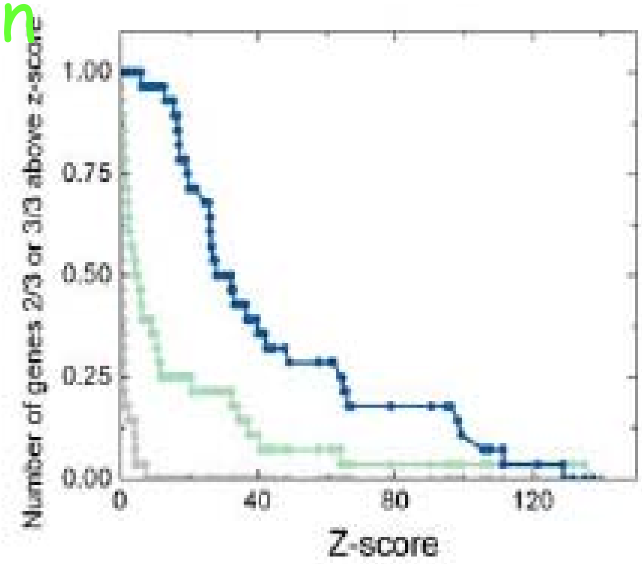
Balaji R/Swetha MG/Joseph Mathew/Gagan Gupta,/ Gautam Dey/ Mukund Thattai/Shameer K/R.Sowdhamini

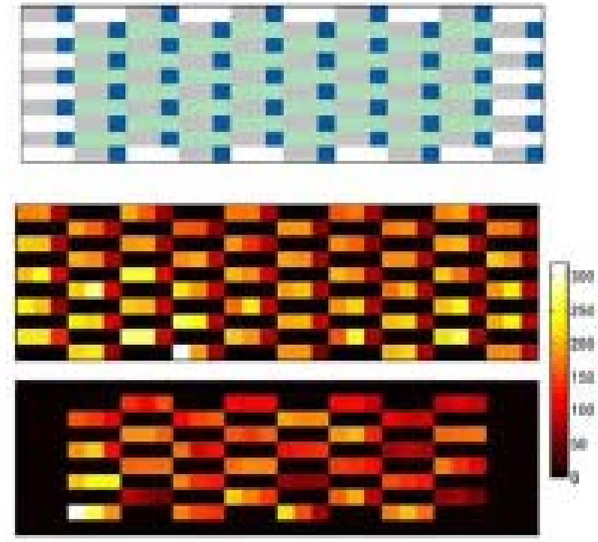
Classsification Screen

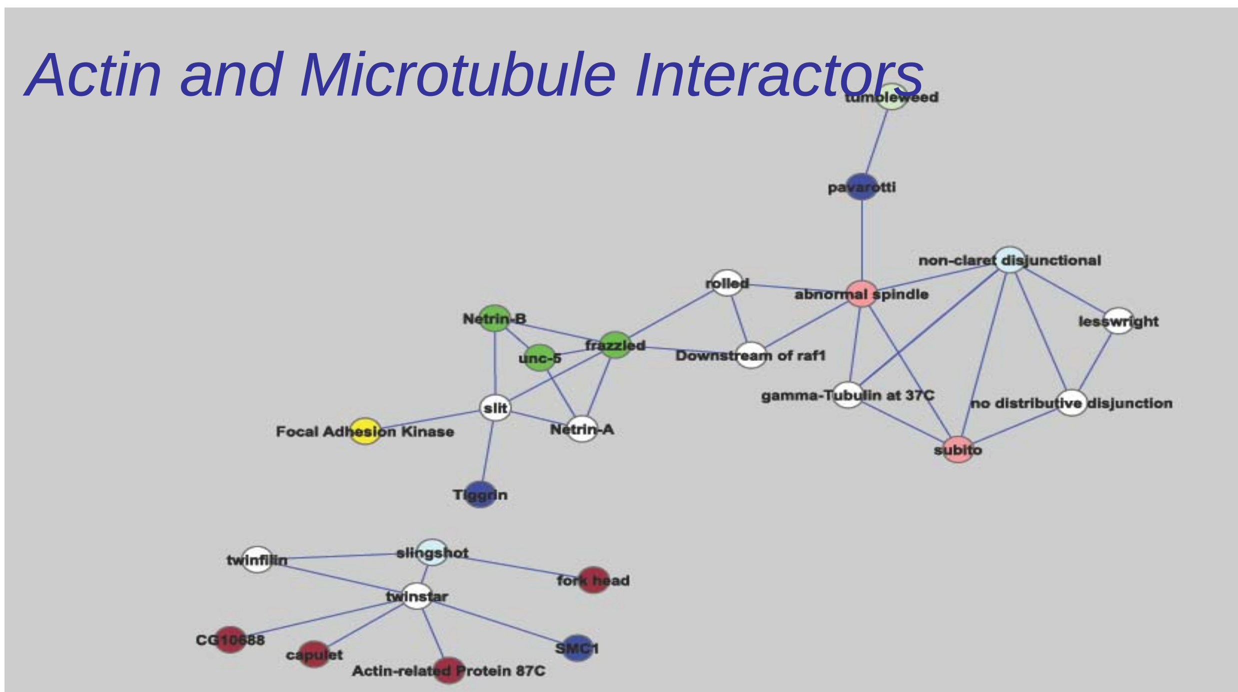
Pulse

Chase

Pulse

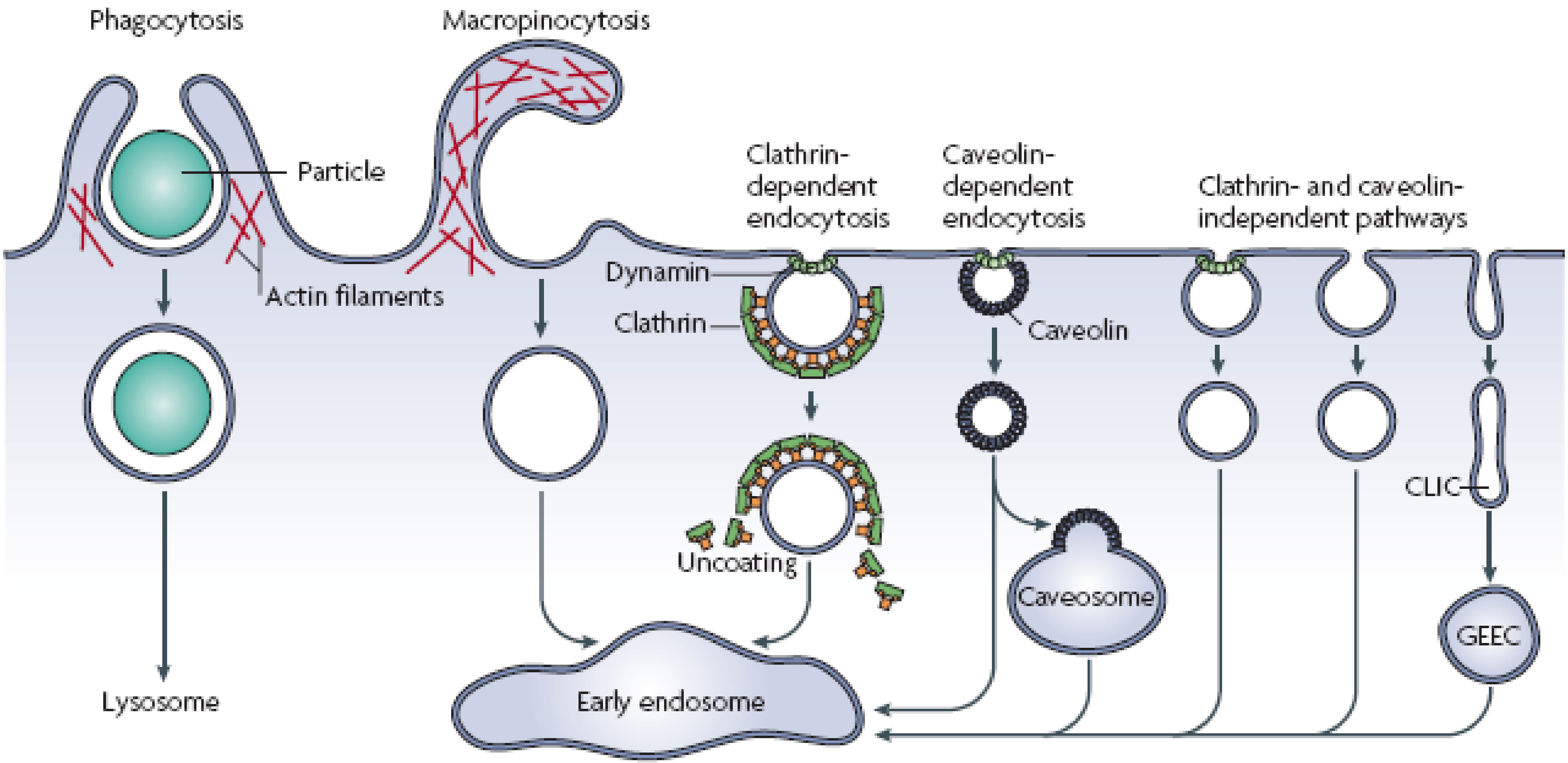






Balaji R/Swetha MG/Joseph Mathew/Gagan Gupta,/ Gautam Dey/ Mukund Thattai/Shameer K/R.Sowdhamini

Lists of Genes: What are they good for?



Manufacturing Rafts:
Active membrane organization in living cells

Satyajit Mayor
National Centre for Biological Sciences (NCBS),
Bangalore, India

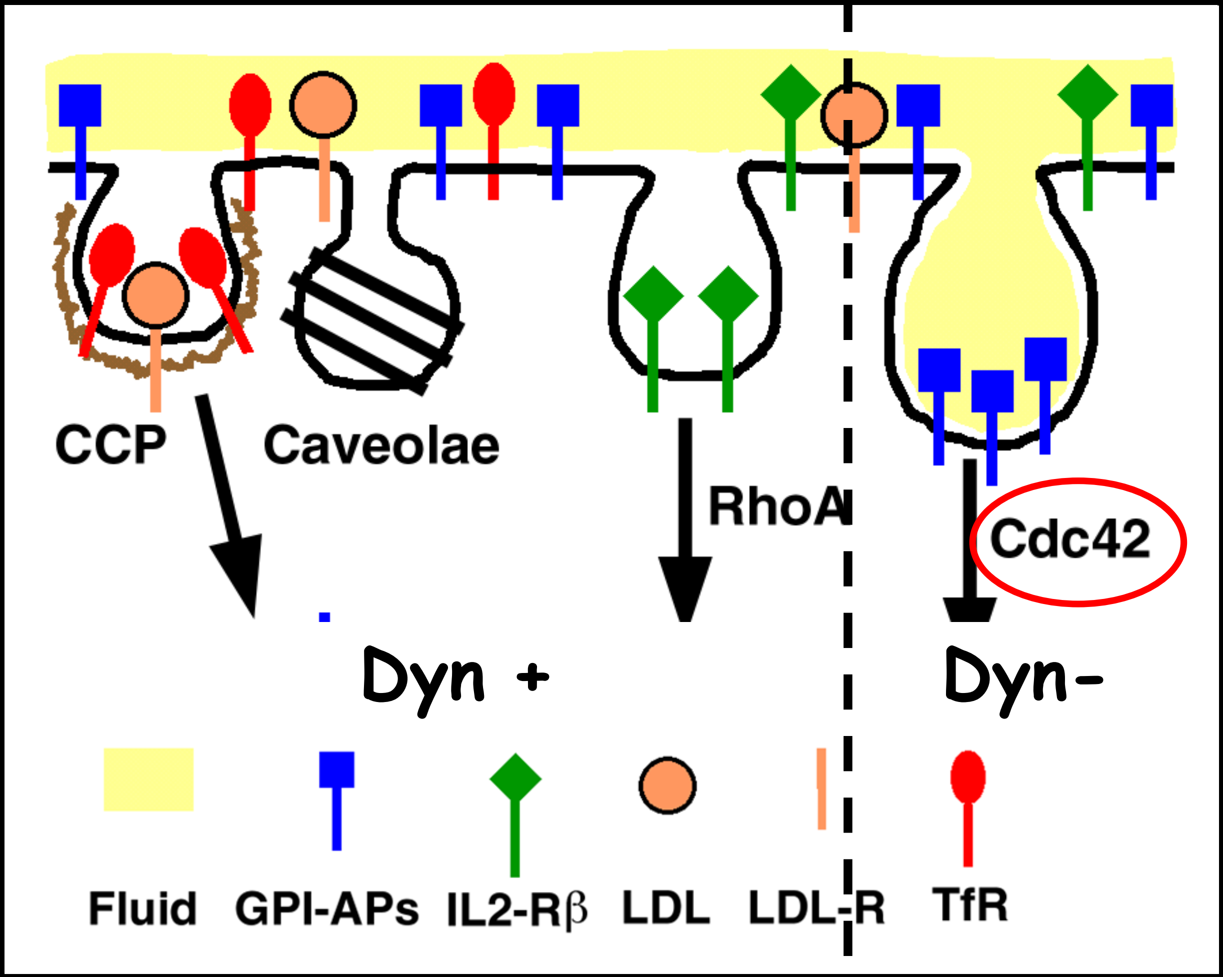
in collaboration with

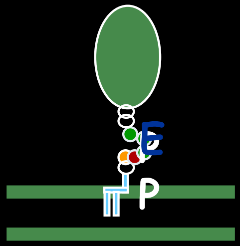
Madan Rao
NCBS/ Raman Research Institute (RRI) ,
Bangalore

ncbs

www.ncbs.res.in

GPI-anchored proteins are constitutively endocytosed via a specialized pathway ' the CLIC/GEEC pathway'





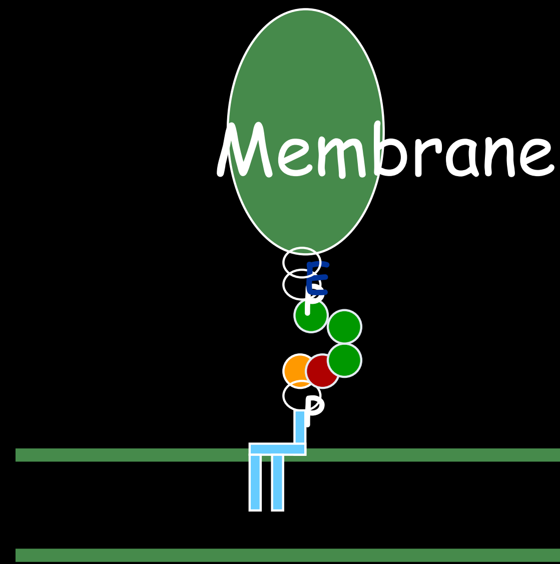
- Sabharanjak, Sharma et al. *Dev. Cell* (2002)
- Kirkham et al. *J. Cell Biol.* (2005)
- Kalia et al. *Mol. Biol. Cell* (2006)
- Chadda et al. *Traffic* (2007)
- Kumari etal. *Nature Cell Biol.* (2007)

Mayor and Riezman, *Nature Reviews MCB*, 2004
Mayor and Pagano, *Nature Reviews* MCB, 2007

GPI-anchored proteins are internalized via a pinocytic pathway that is

•Constitutive
•Lipid-selective
•Cdc42-regulated
•Cholesterol sensitive
•Actin dependent

Membrane domains ?



Does not utilize
•Dynamin
•Clathrin/ Caveolin
•Arf6
•RhoA
•Rac1

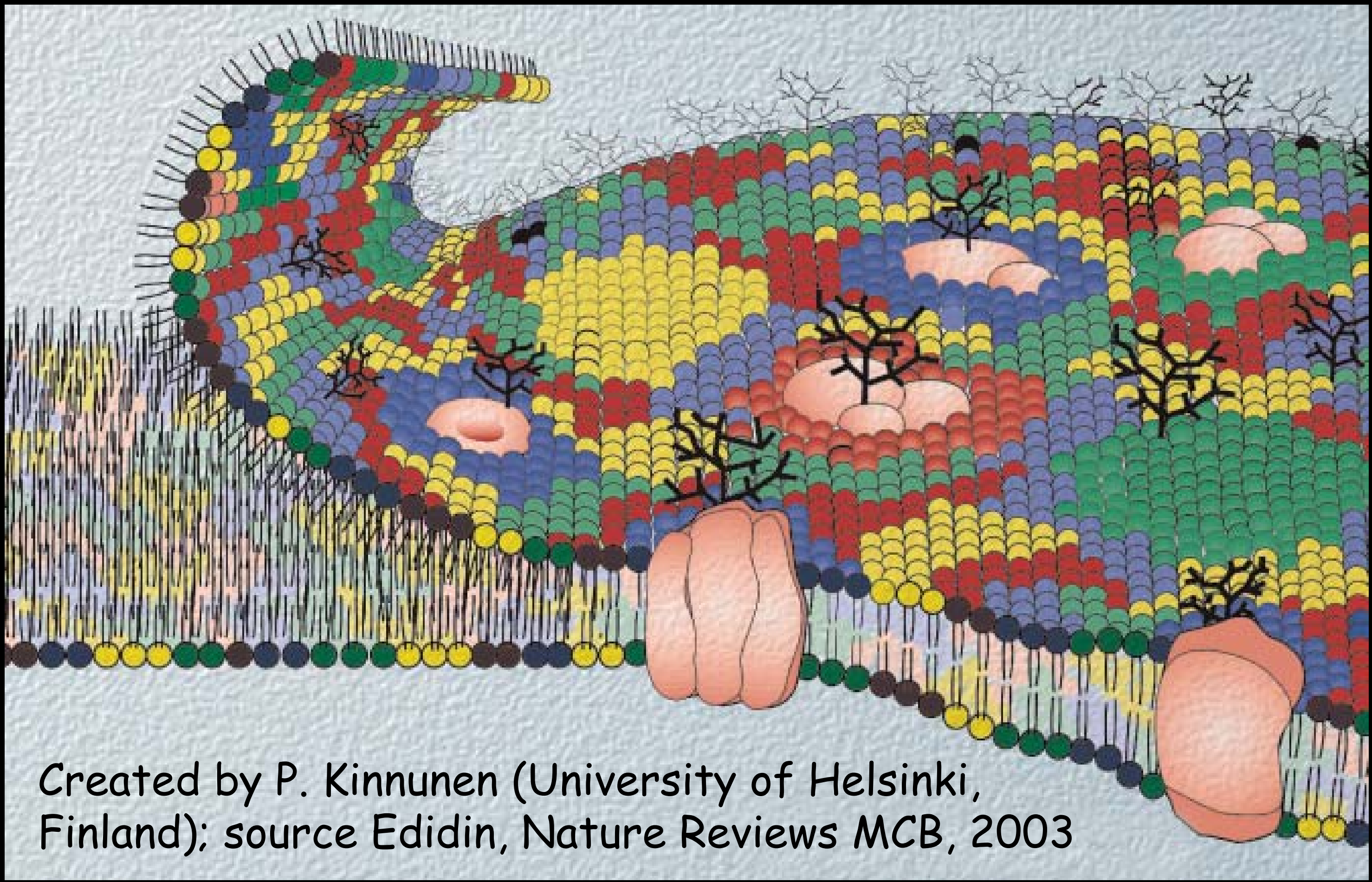
Mayor and Riezman, *Nature Reviews MCB*, 2004
Mayor and Pagano, *Nature Reviews* MCB, 2007

Organization of GPI- tethered proteins at the cell surface- nano-scale clusters

The role of an active actin cortex- experiments and theory

Towards a new picture of membrane organization

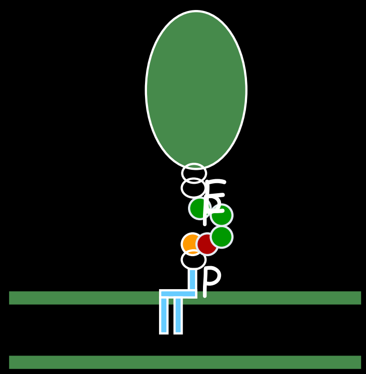
Fluid Mosaic- patchwork quilt

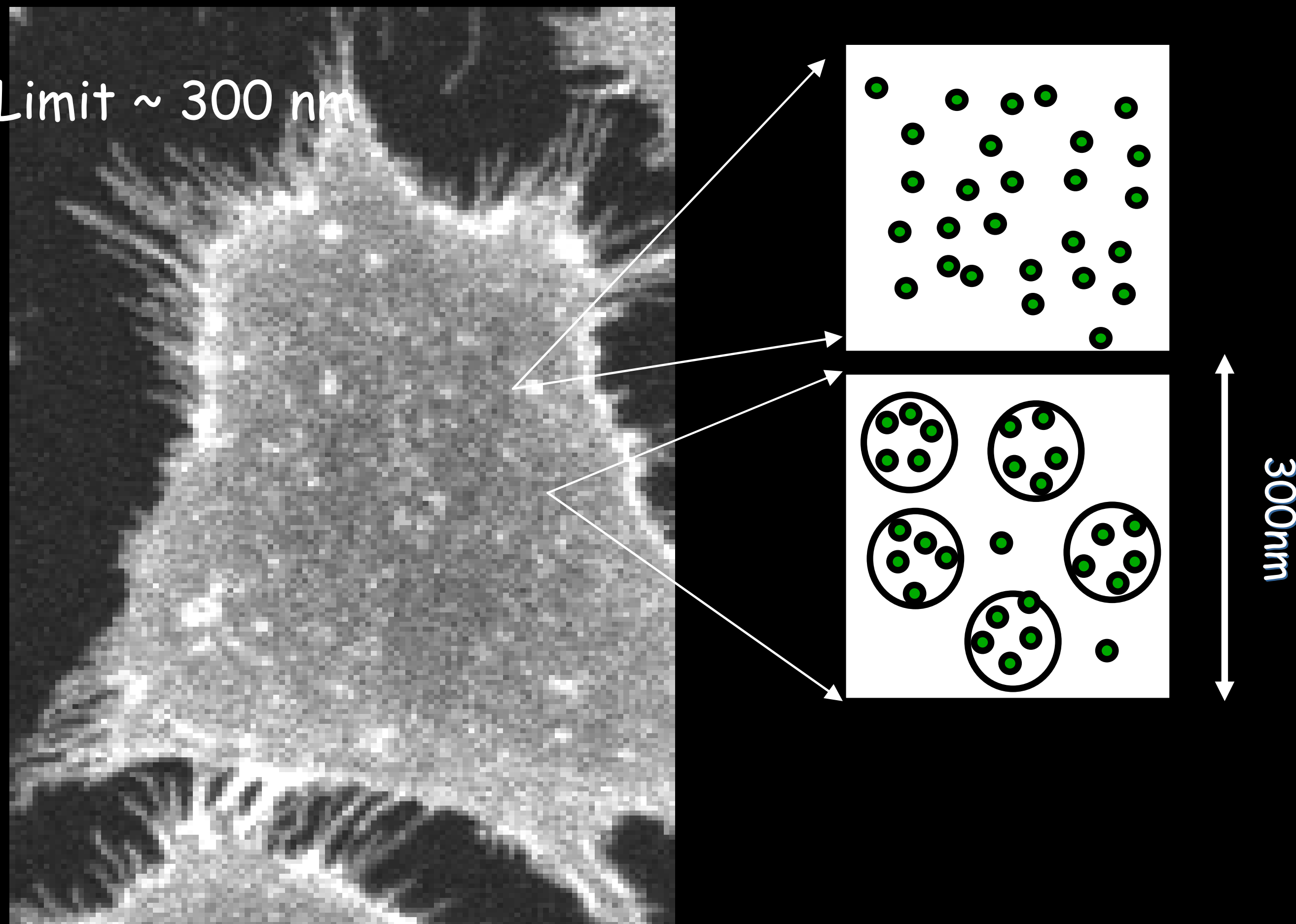


Created by P. Kinnunen (University of Helsinki, Finland); source Edidin, Nature Reviews MCB, 2003

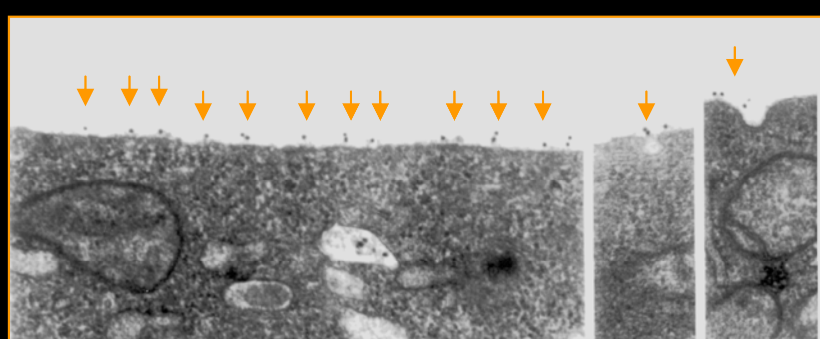
Surface distribution of GPI-APs in CHO cells

Diffraction Limit ~ 300 nm



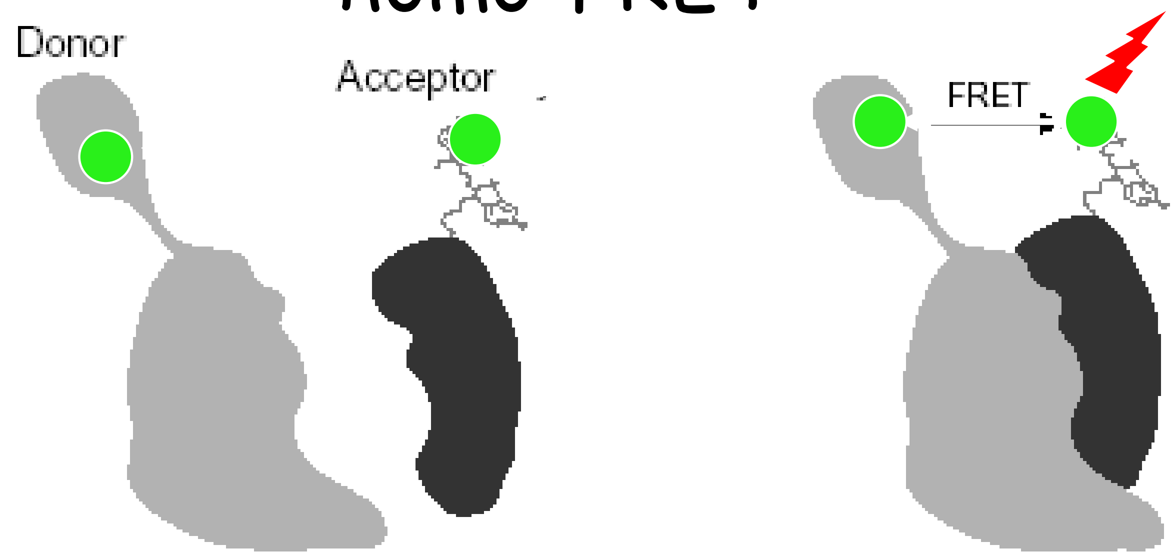


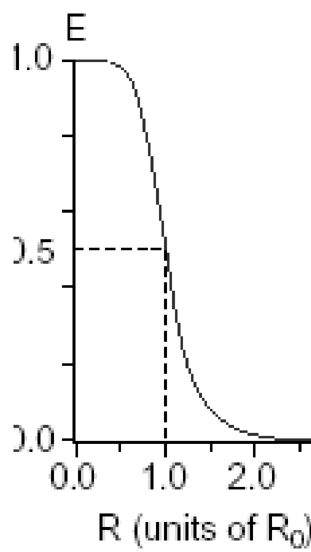
GPI-anchored protein immunoGold EM analysis



Forster's energy transfer mechanism

homo-FRET





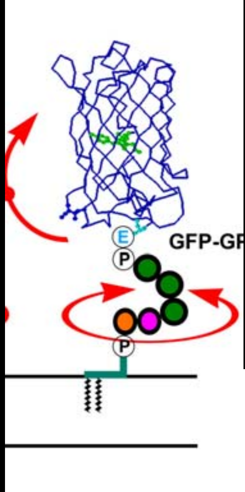
measure fluorescence anisotropy

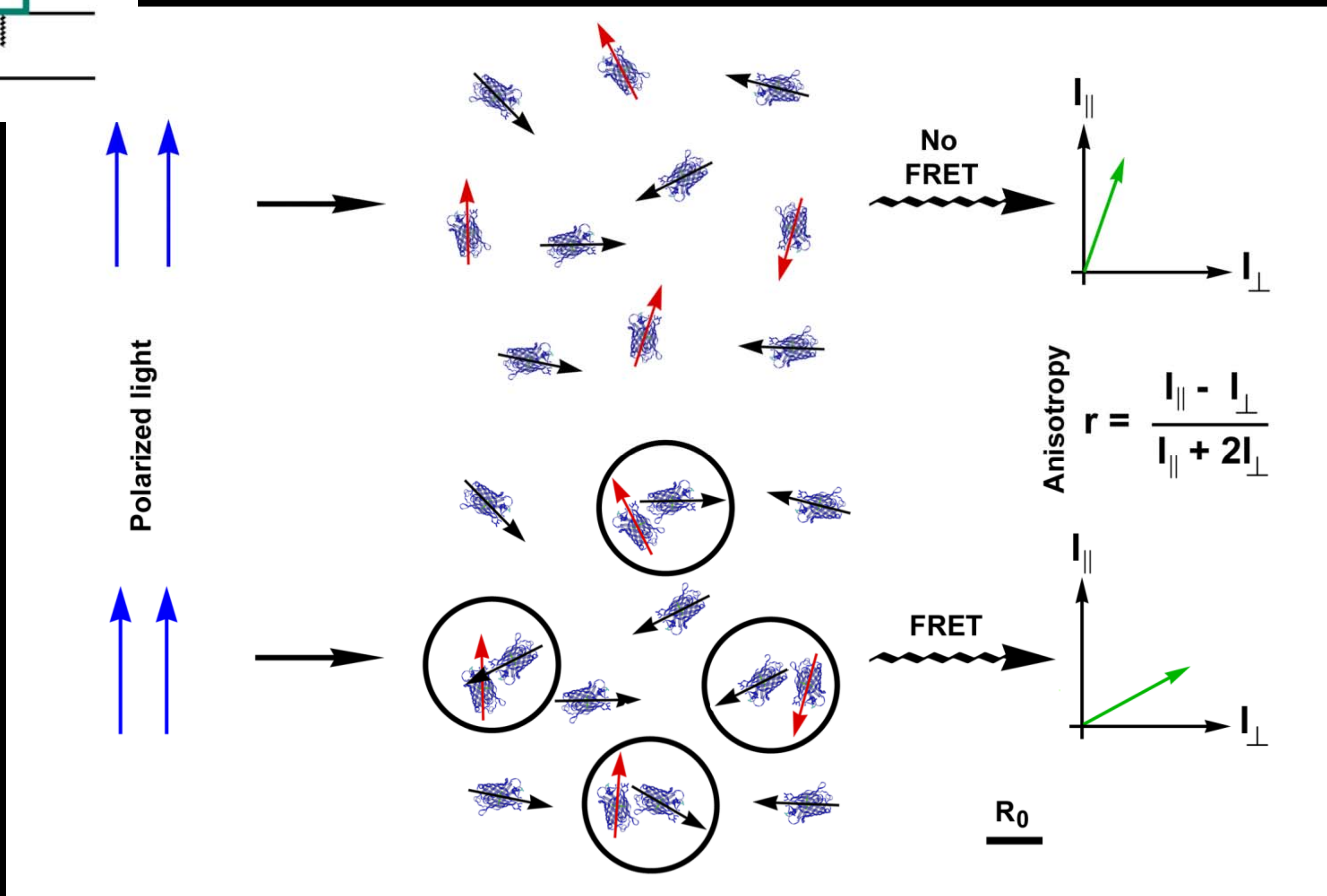
Varma and Mayor, *Nature* (1998)

Depolarization FRET

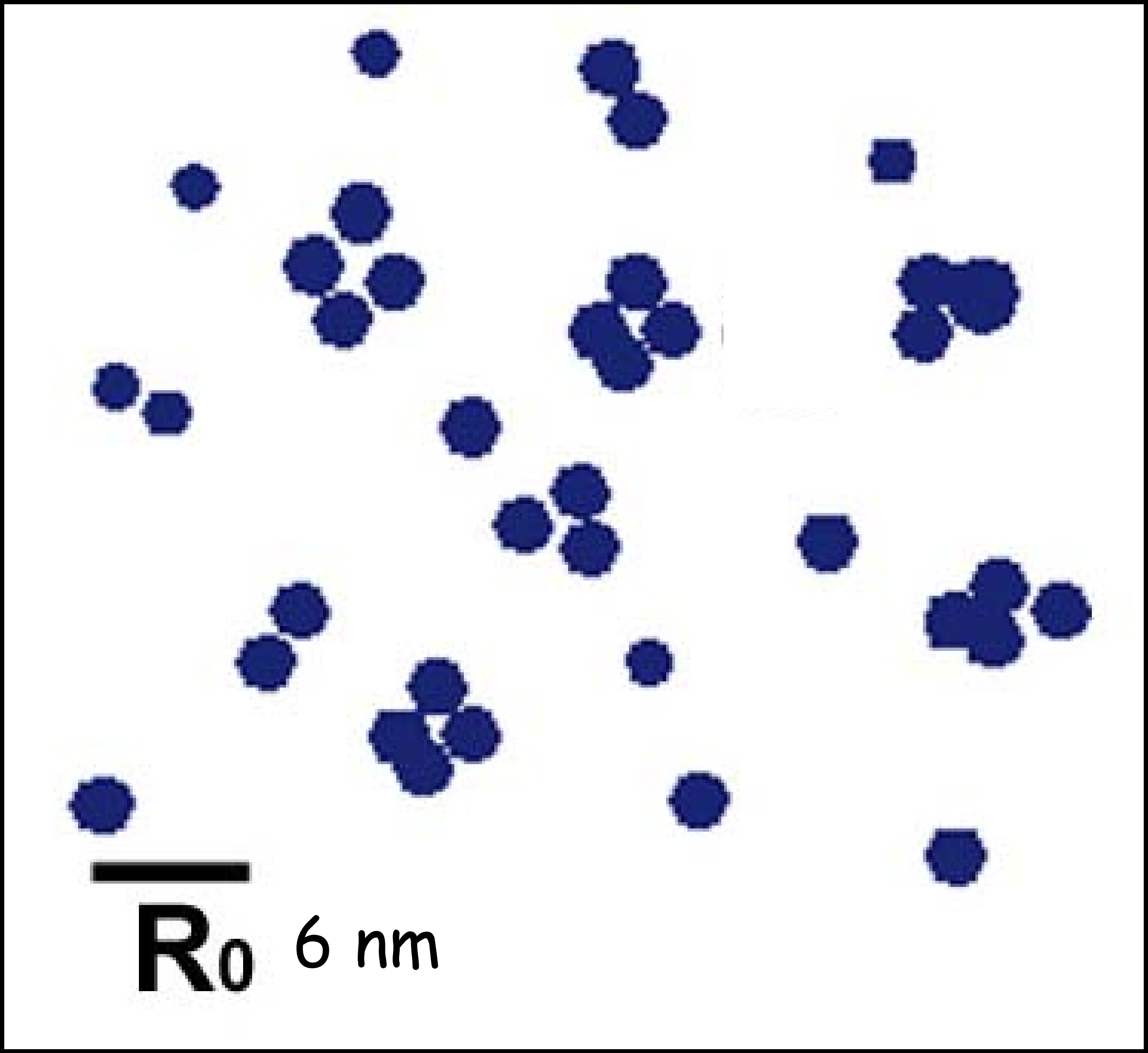
Weber G., *Trans. Farad. Soc.* 1954

homo-FRET


E
P
GFP-GP
P


Polarized light
No
FRET
FRET
Anisotropy
r = (I|| - I⊥) / (I|| + 2I⊥)
R0

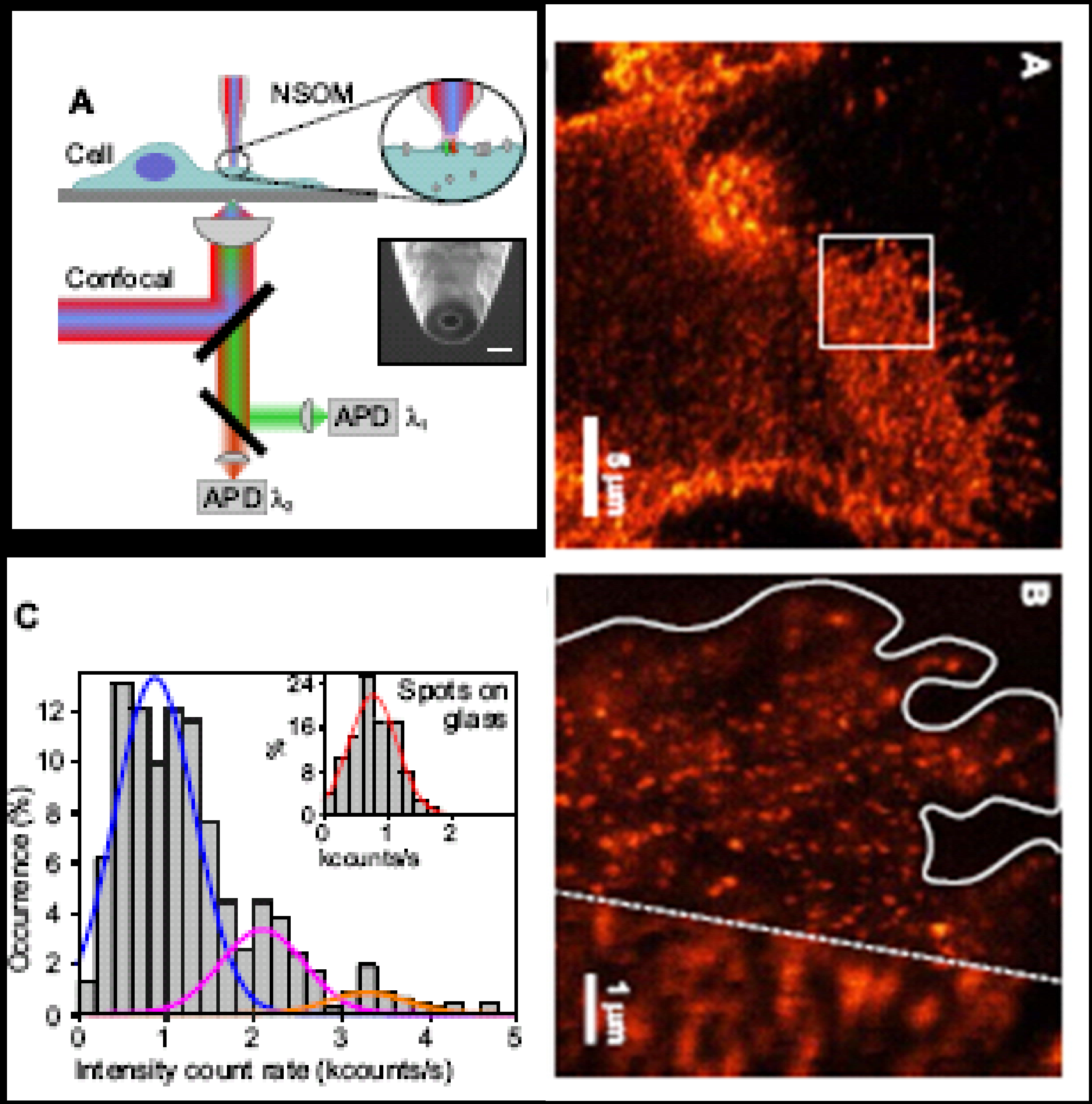
Mainly monomers and 20-40 % as nano-clusters -



Varma and Mayor, *Nature 1998*
Sharma, Varma, Sarasij et al., *Cell* 2004

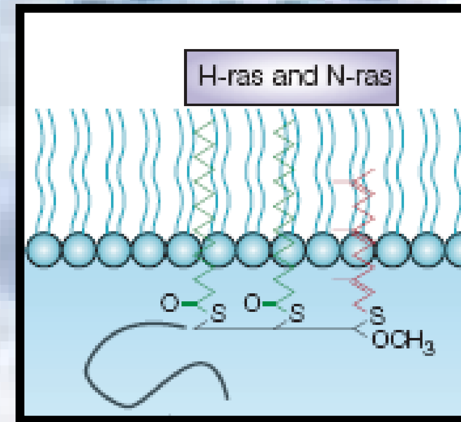
Freidrichson and Kurchalia, *Nature 1998*
Paladino et al., *JCS* 2008

NSOM Imaging of GPI-anchored proteins



van-Zanten et al *PNAS*, 2009

Nano-clustering of H-Ras and Gangliosides



< 10 nm clusters of lipid-anchored proteins, sugars

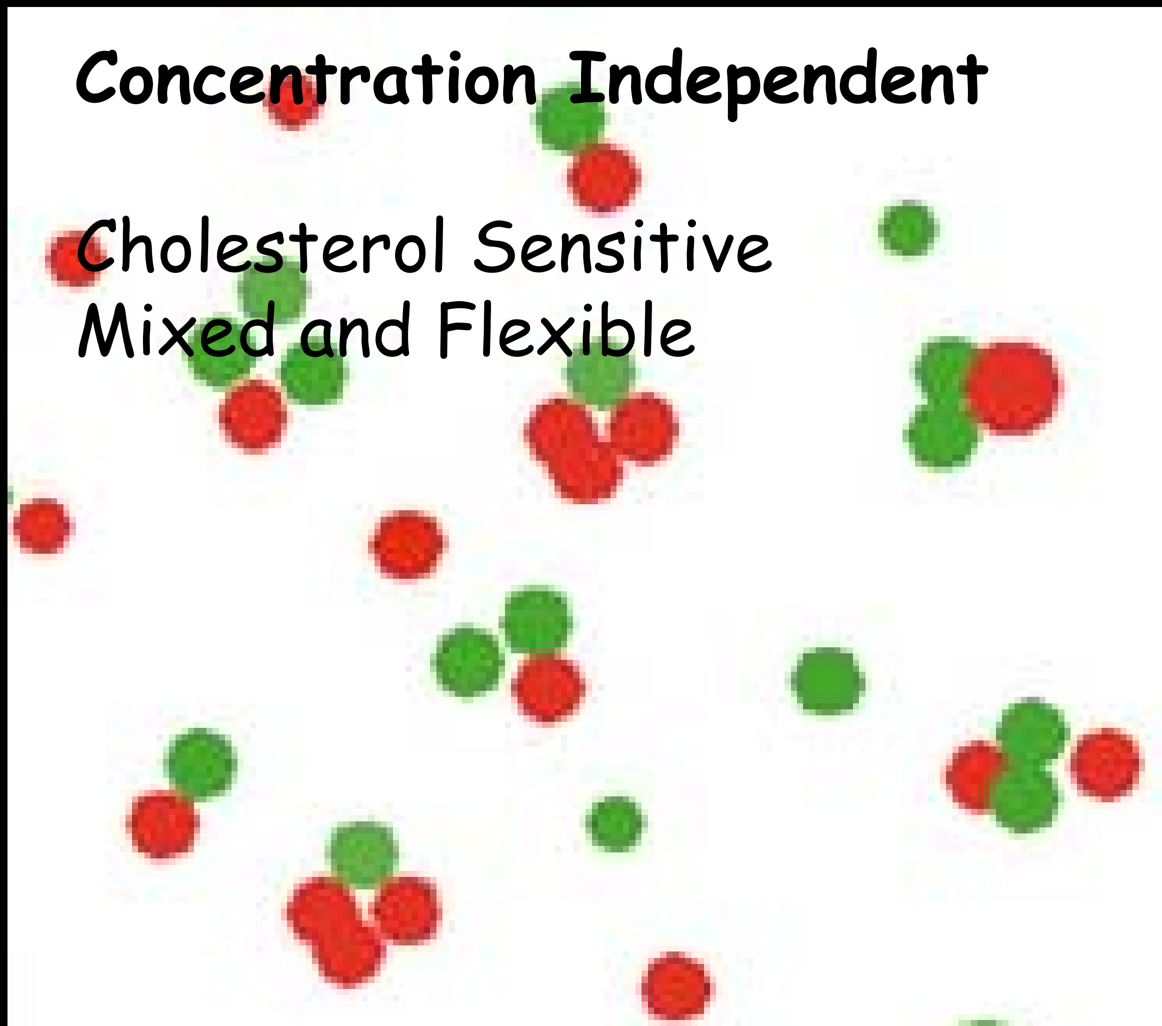
Cholesterol-dependent
Concentration independent
Actin-cytoskeleton sensitive

Oncogenic signaling/ Toxin Delivery

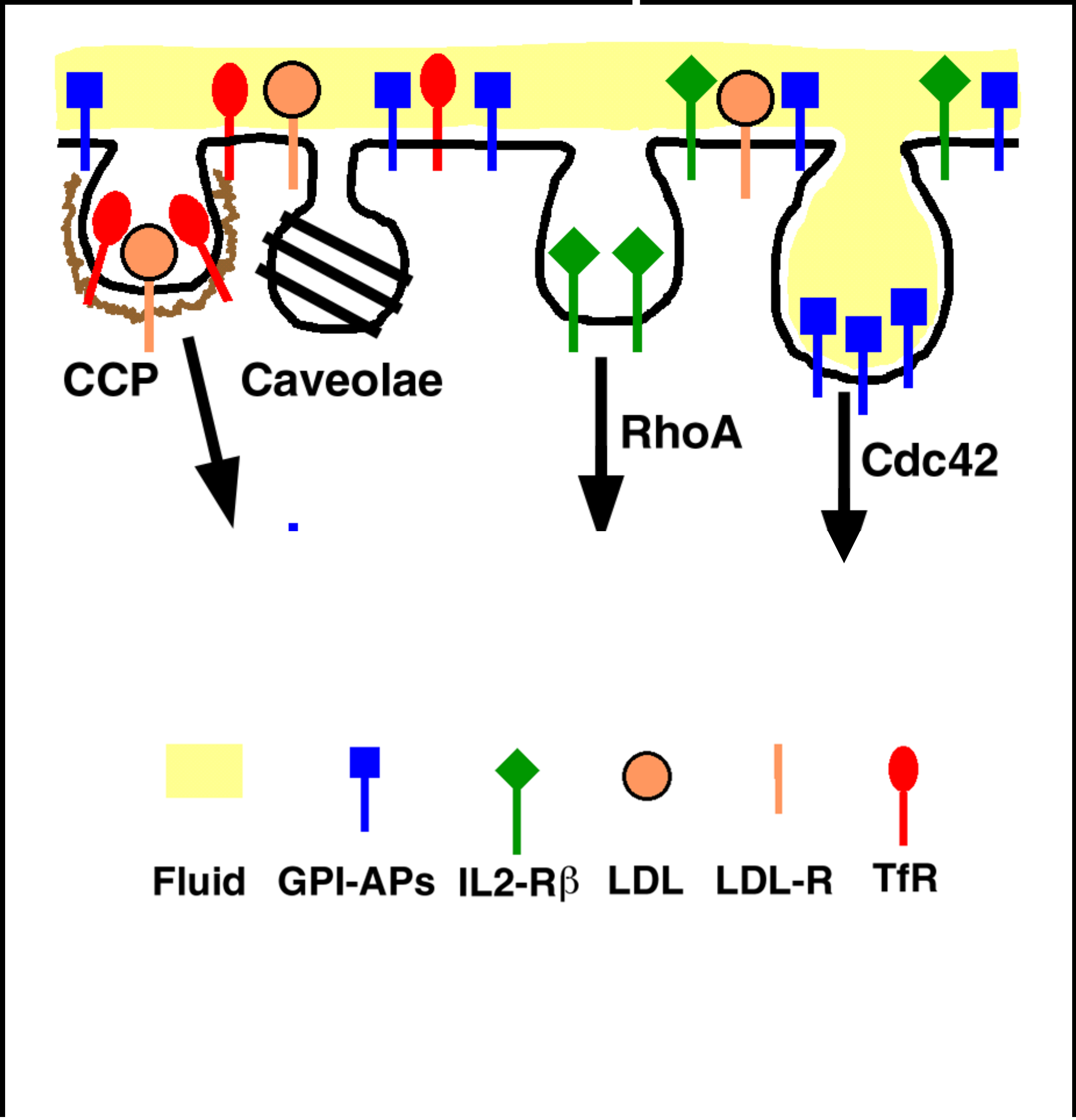
Prior et al , JCB, 2003
Plowman et al, 2005
Fujita et al, MBC 2007

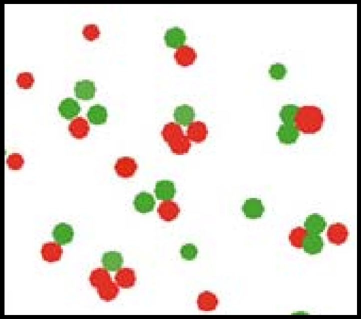
Hancock, *Nature Reviews MCB*, 2003

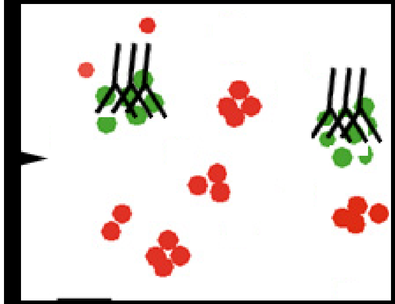
GPI-APs are flexibly organized as monomers and mixed nano-clusters



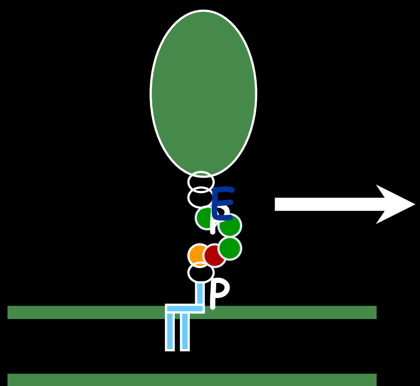
Endocytosis of GPI-anchored proteins and nanoclusters?



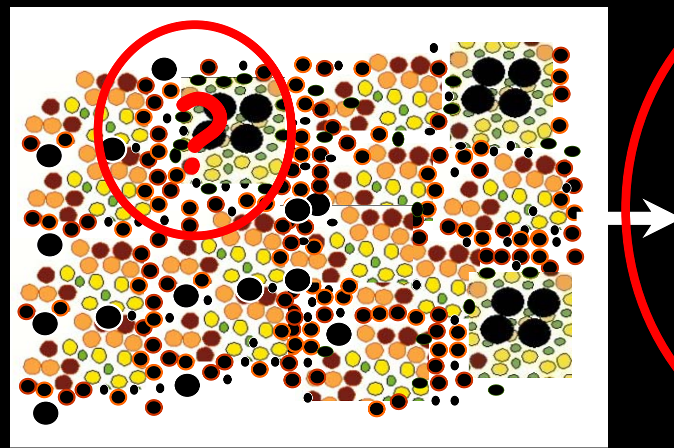




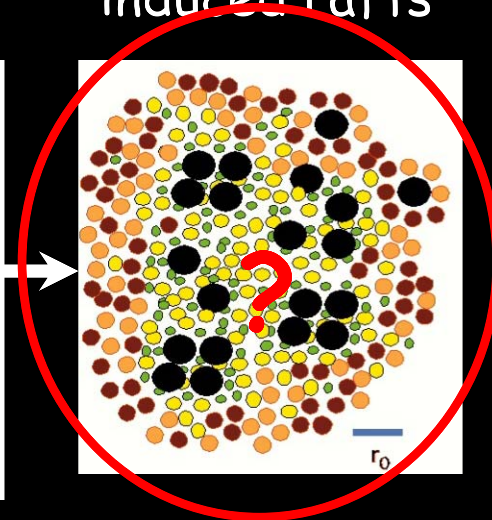
GPI-anchored protein domains



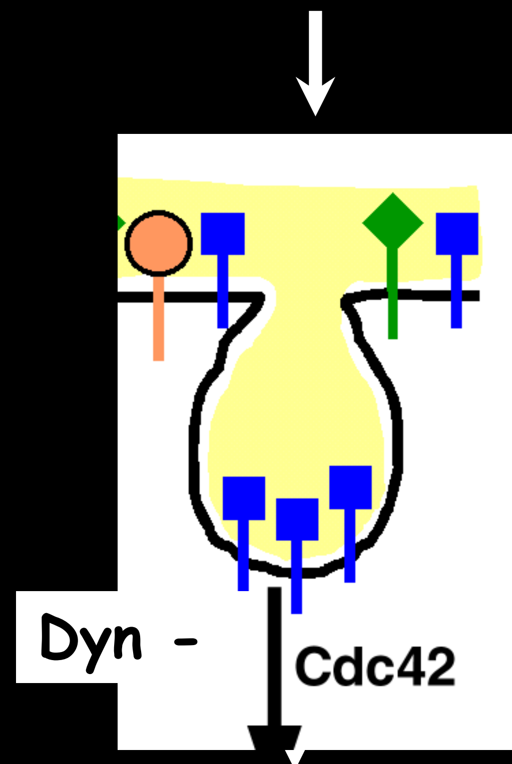
pre-existing organization



induced rafts



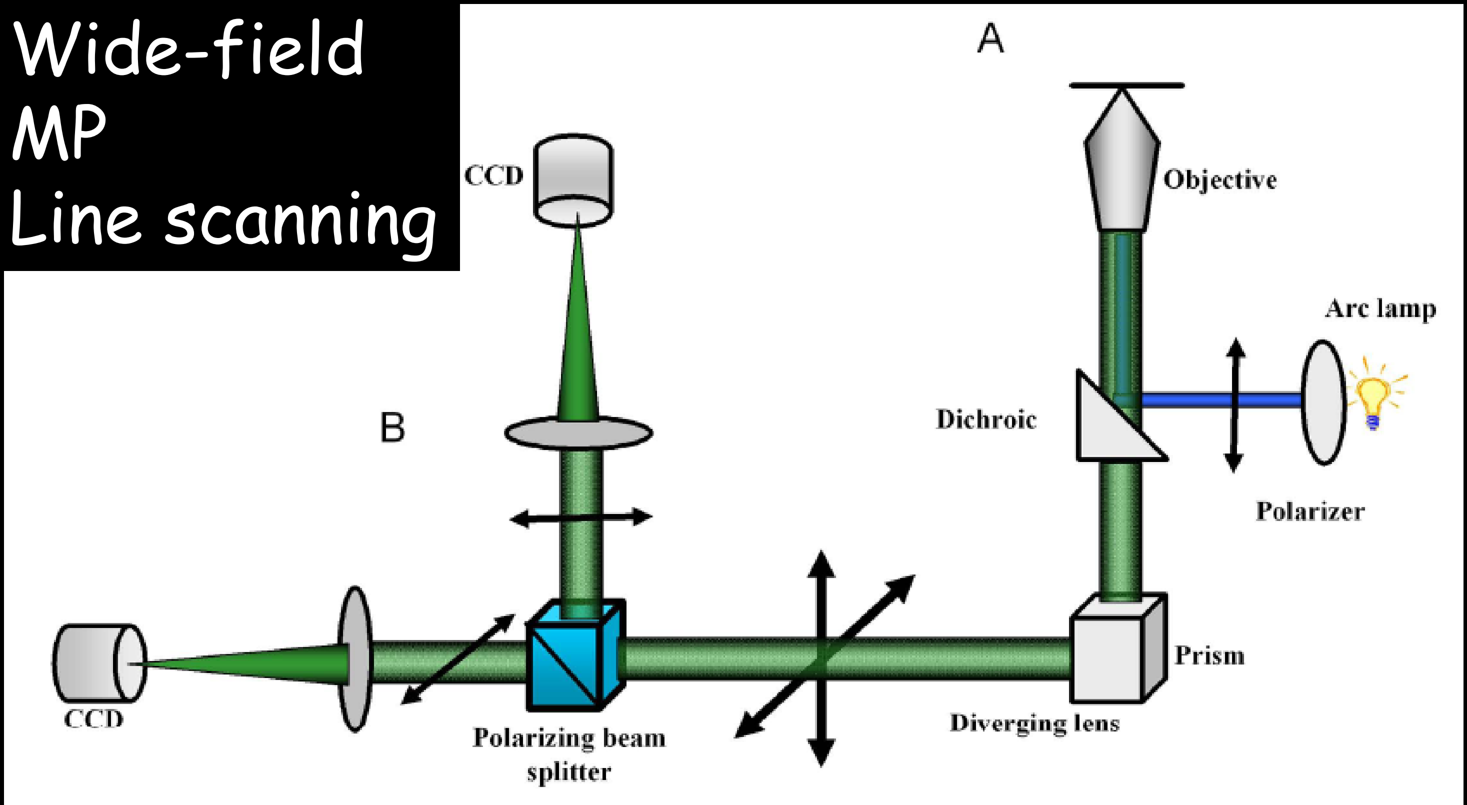
- Nanoclusters as sorting signals



Mayor and Rao, *Traffic* (2004)
Sharma et al, *Cell (2004)*

High resolution FRET imaging

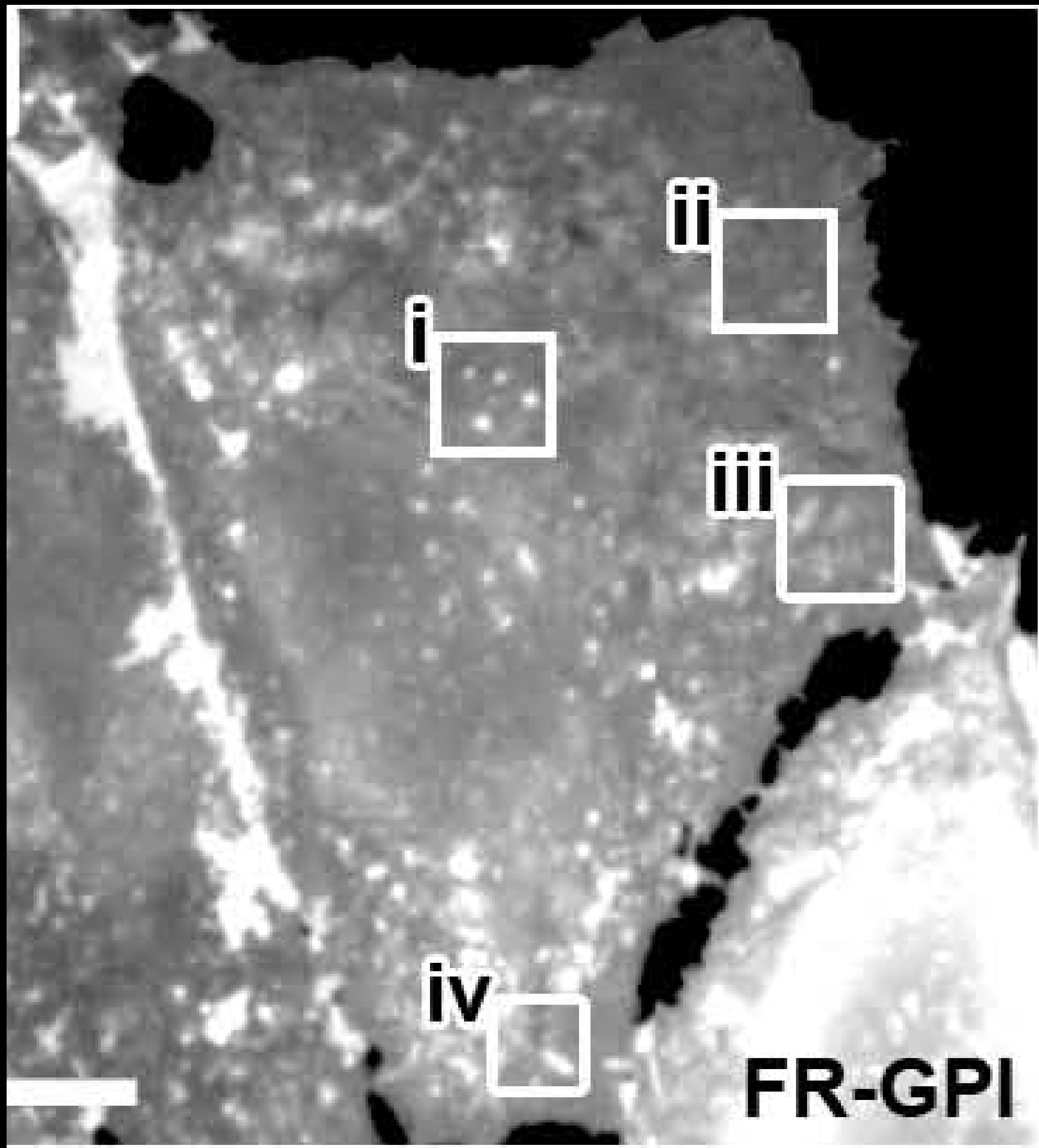
Wide-field
MP
Line scanning



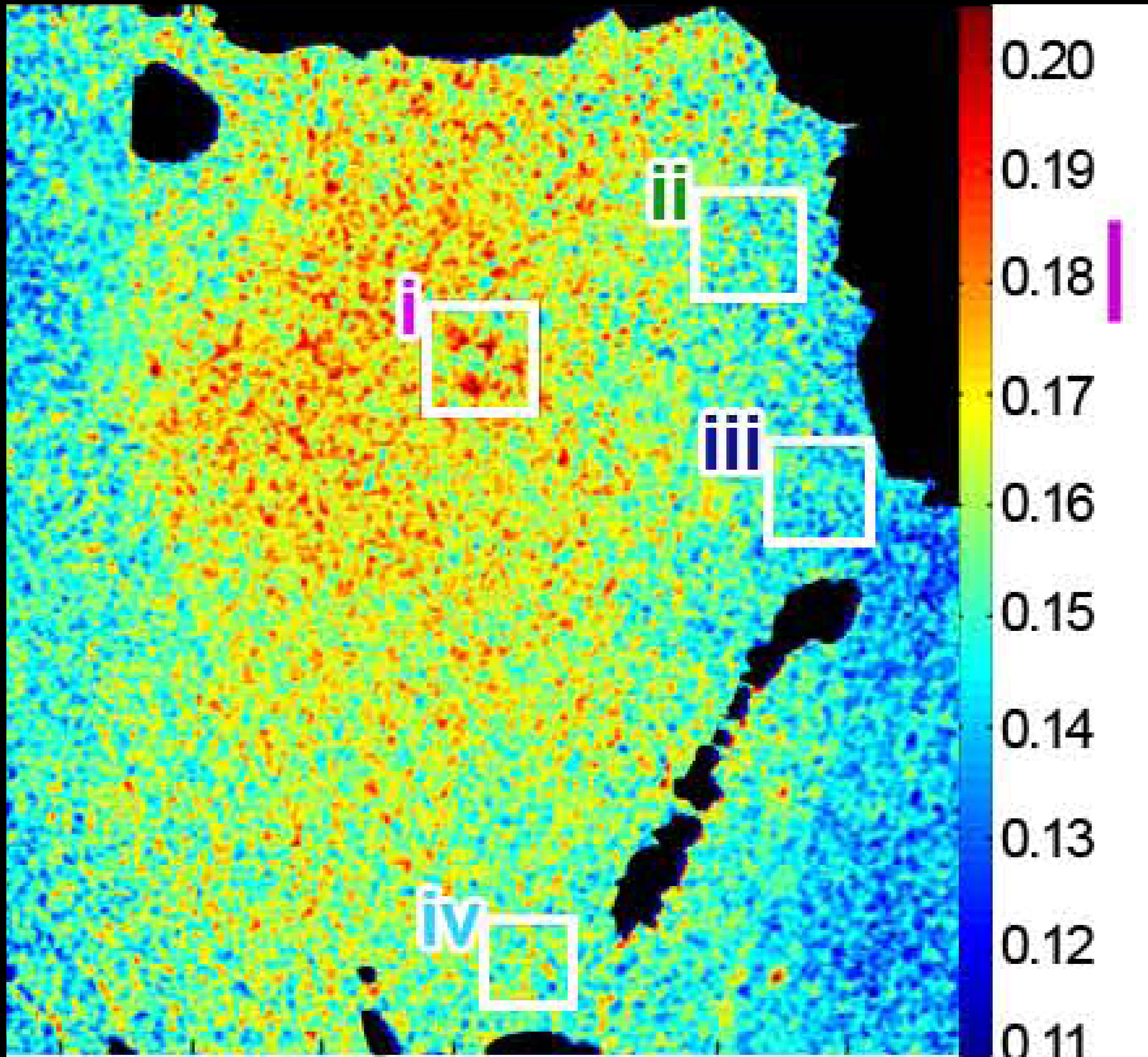
High resolution ~ FRET imaging at 300 nm spatial resolution

Goswami et al. *Cell 2008*

Spatial distribution of nanoclusters

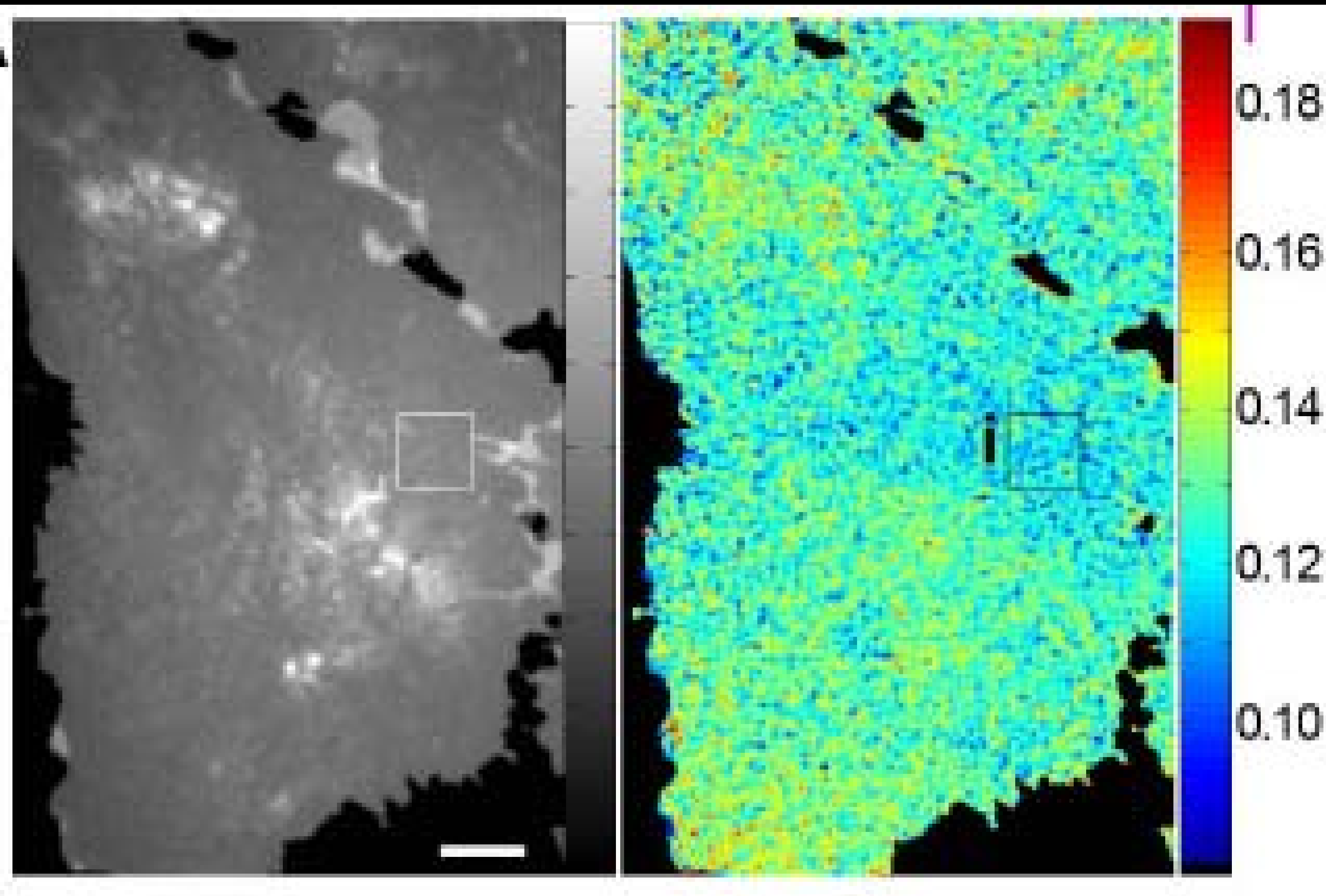


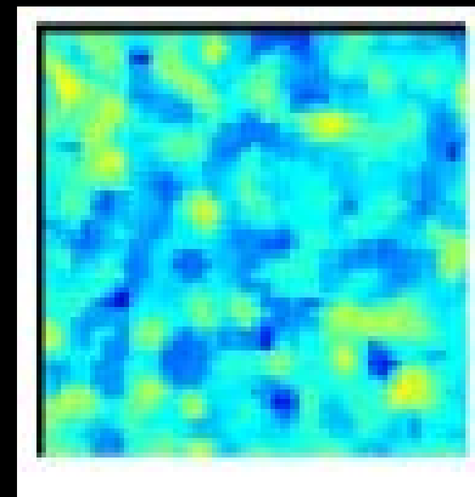
Spatial distribution of nanoclusters



Steady State Distribution:

Nanoclusters are enriched in flat-scapes of the cell membrane





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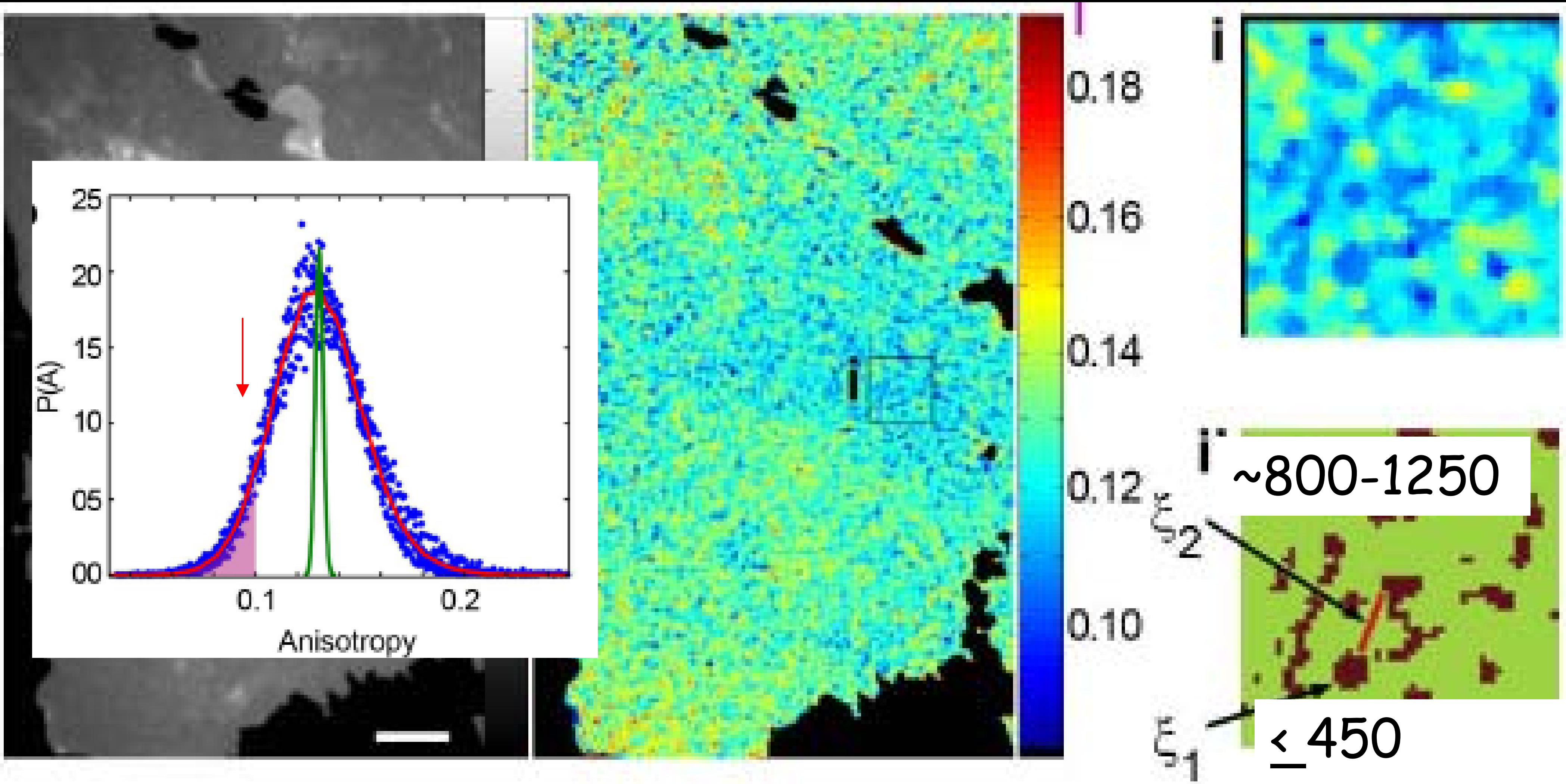
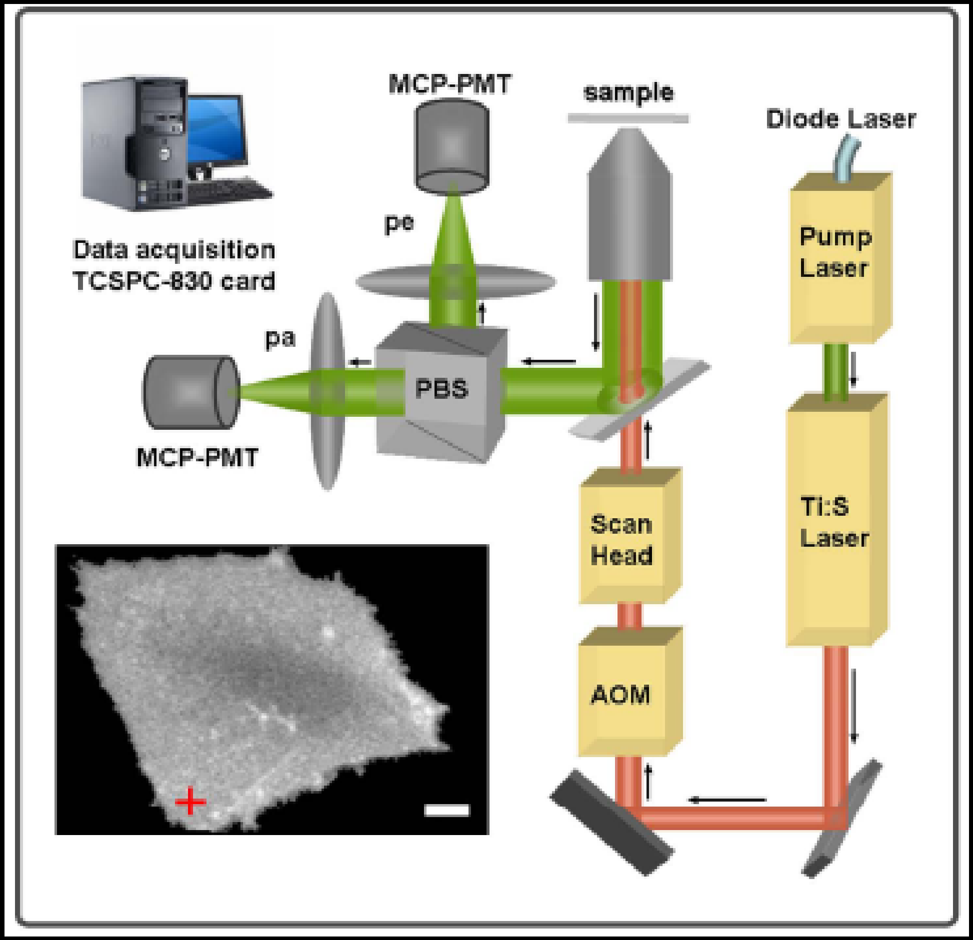
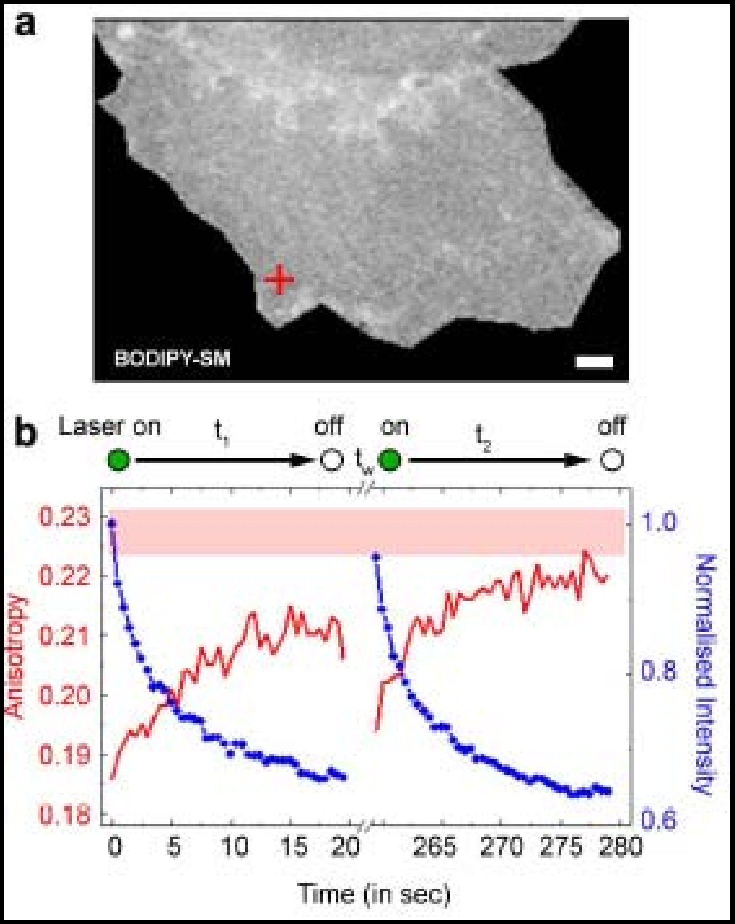


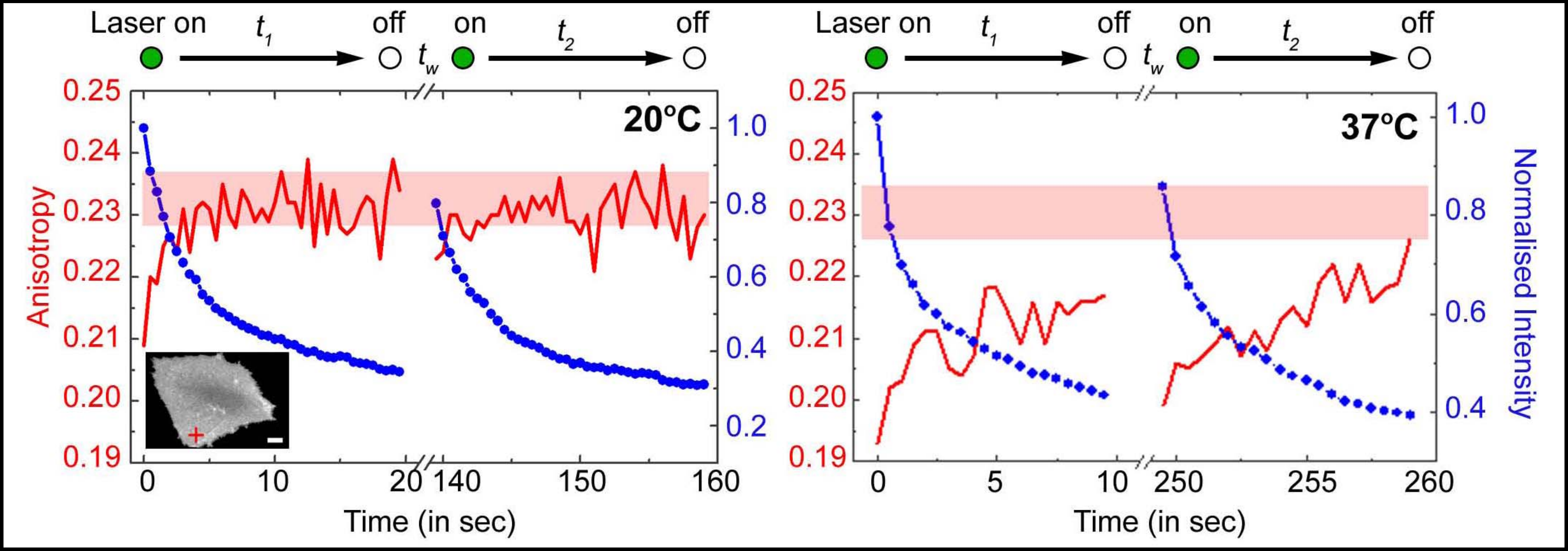
Steady State Dynamics:





Steady State Dynamics:

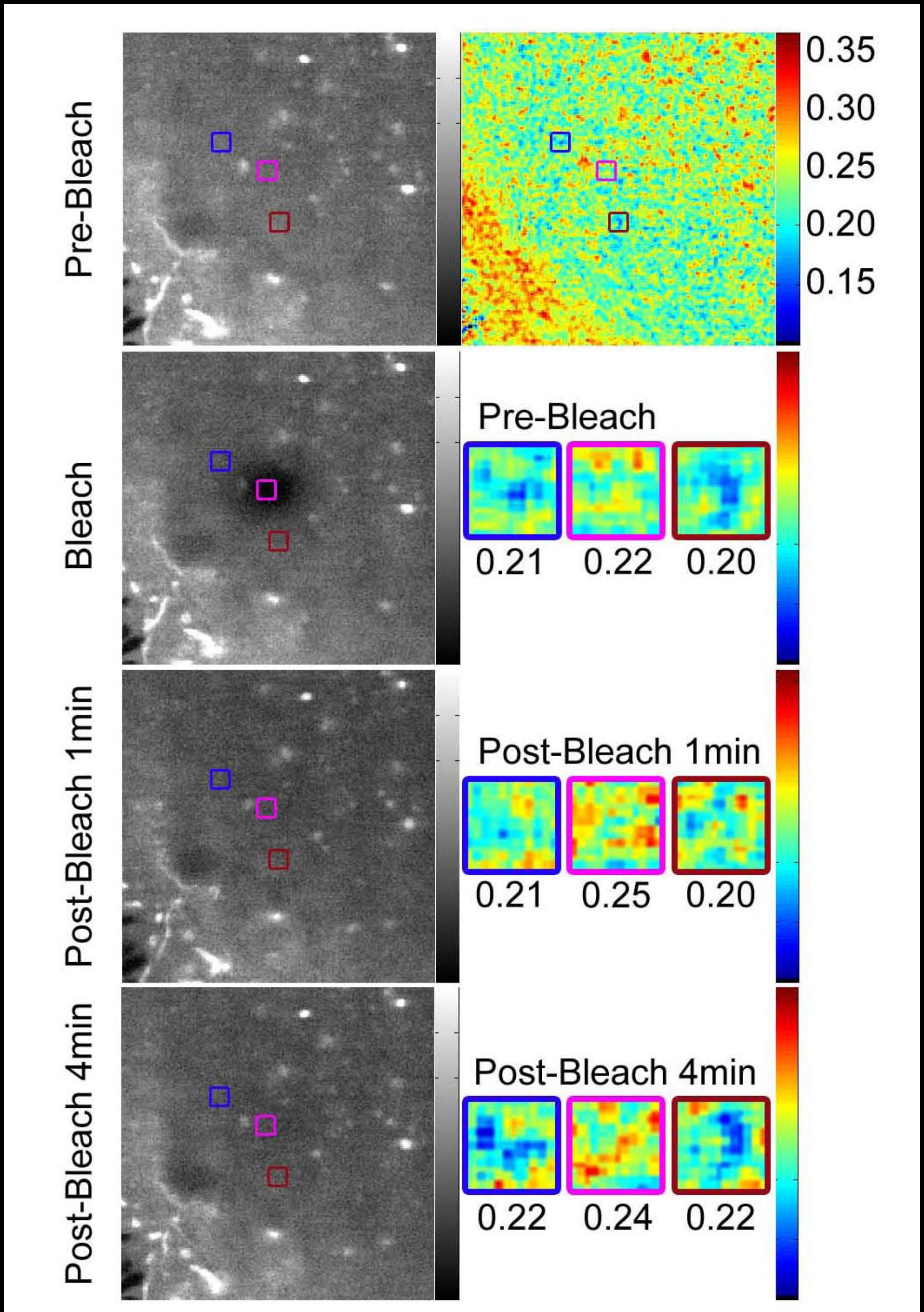
Temperature sensitive reformation

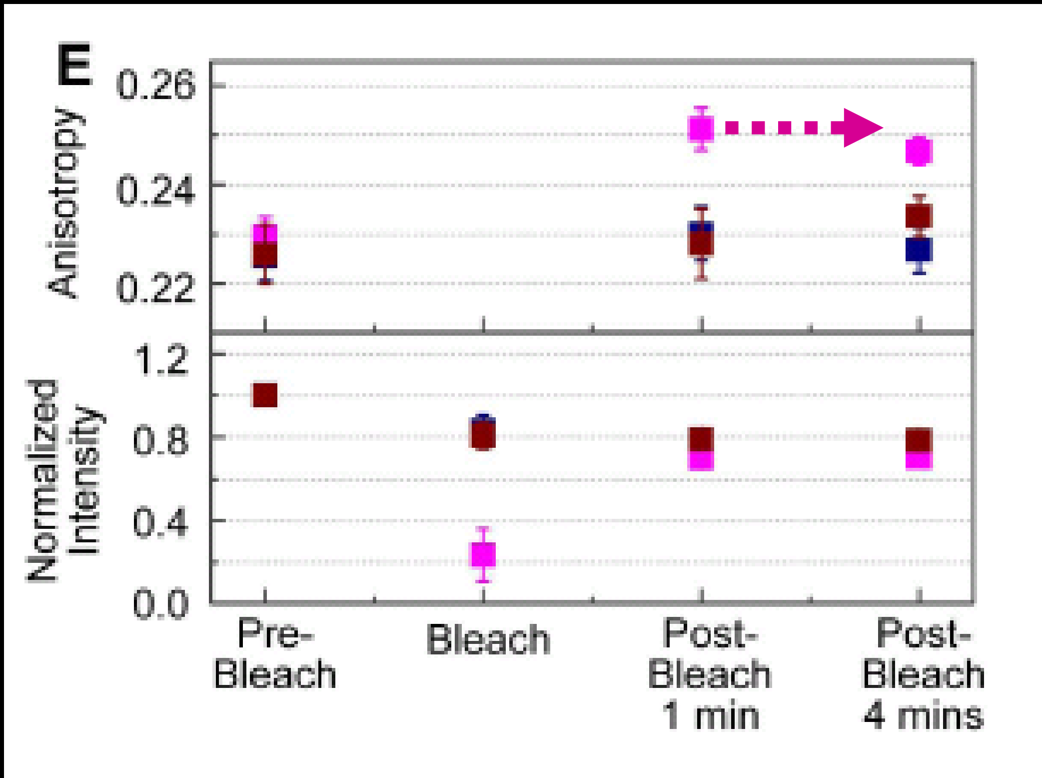


Nanoclusters do not reform at lower temperatures
Nanoclusters are immobile ?

20 °C

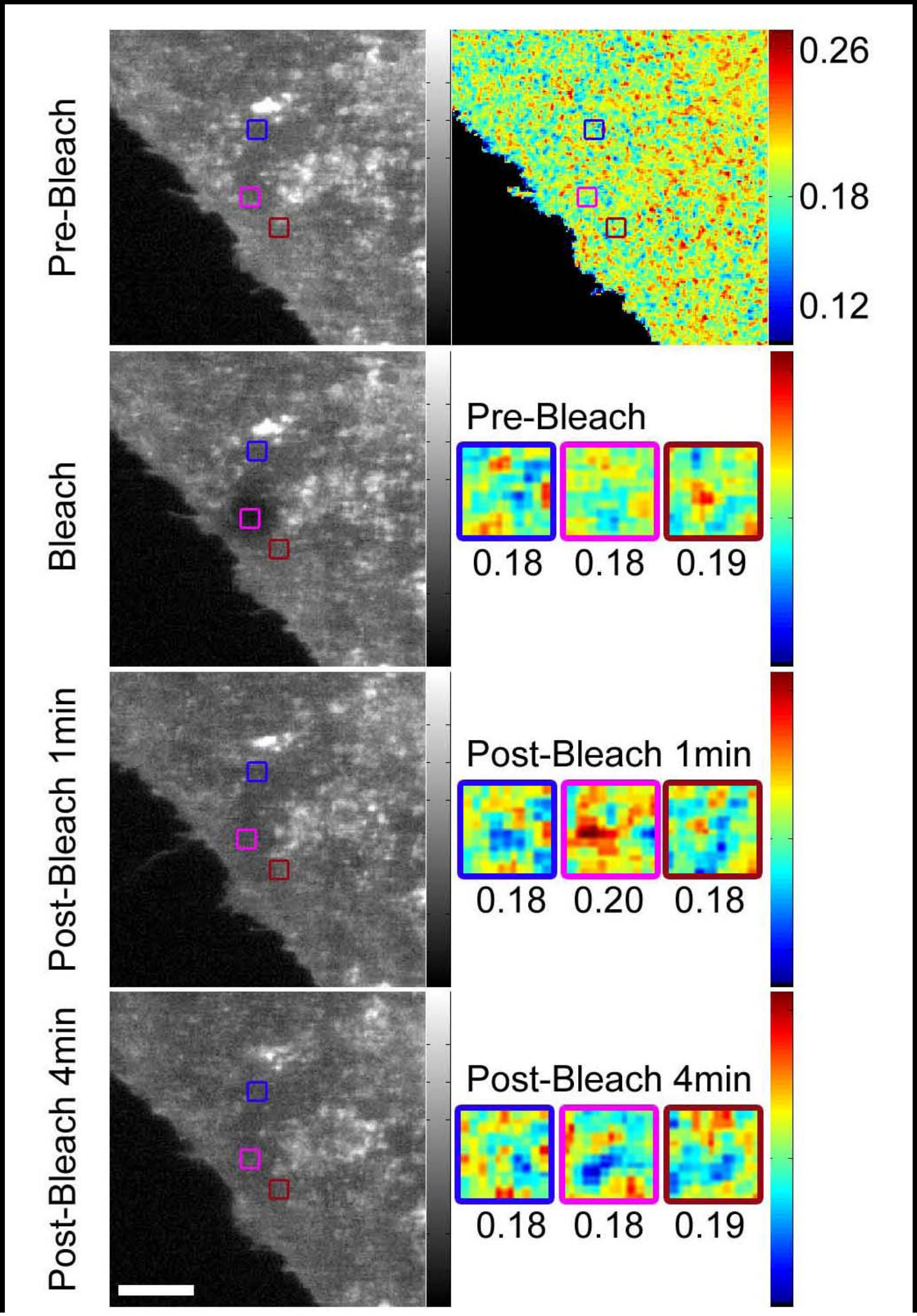
Steady State Dynamics:
Immobile Nanoclusters

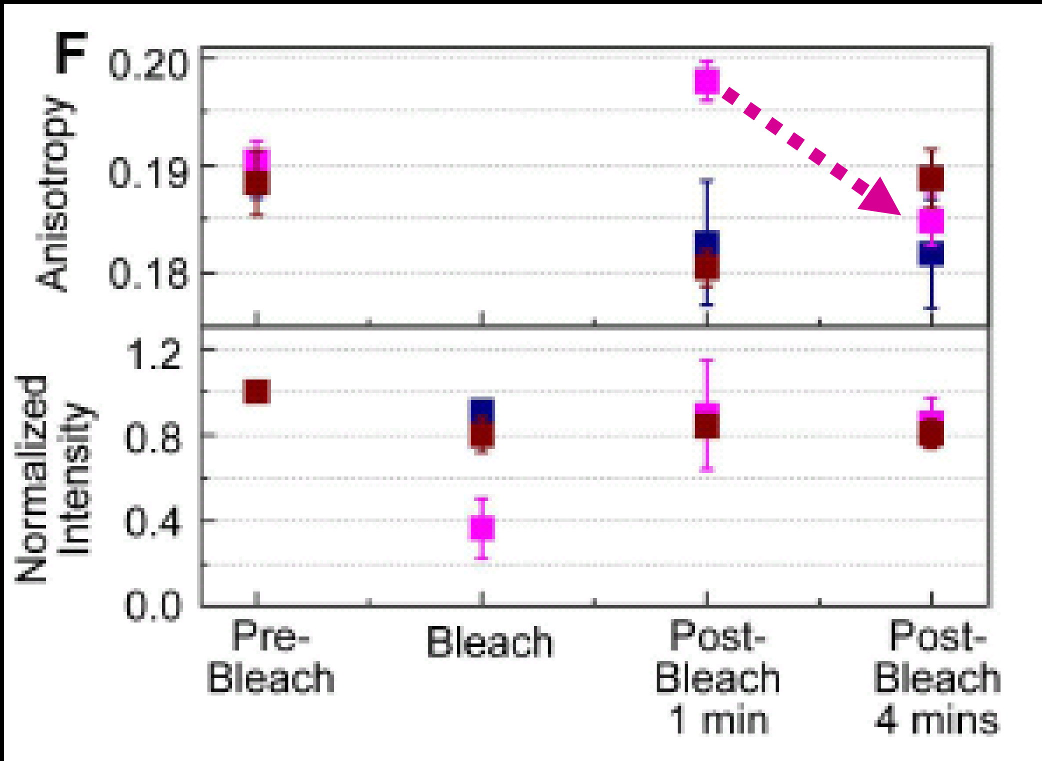




37 °C

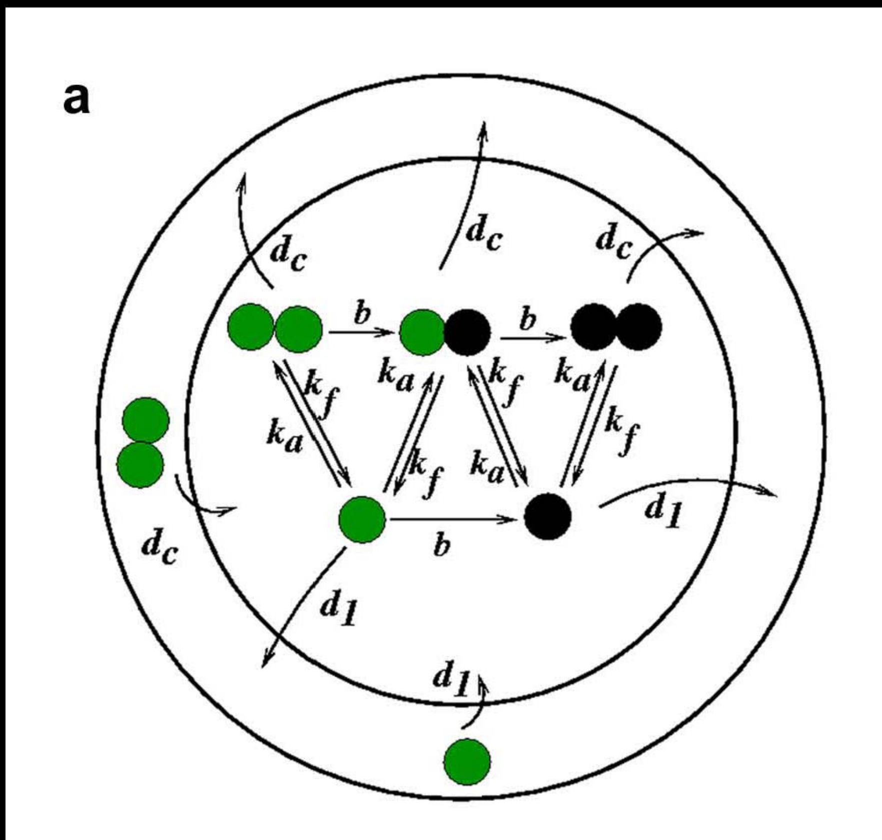
Steady State Dynamics:
Reforming Nanoclusters

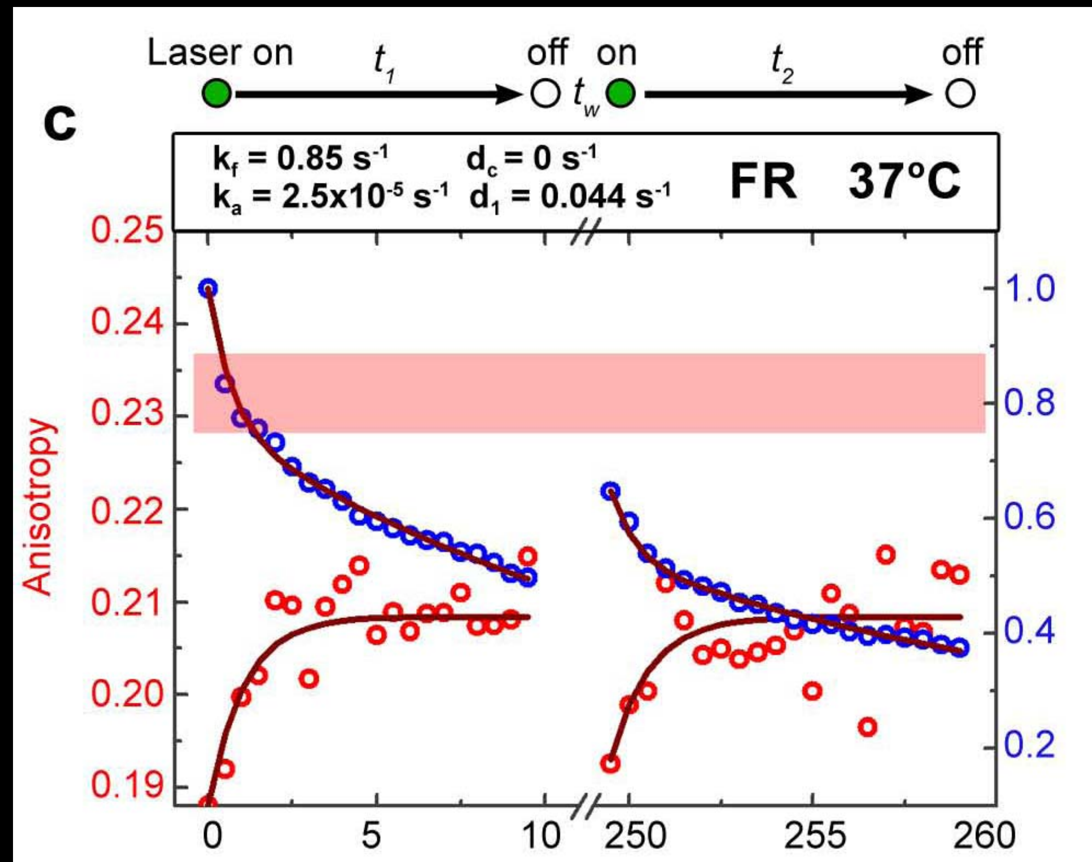




Steady State Dynamics:

Kinetics of aggregation and fragmentation

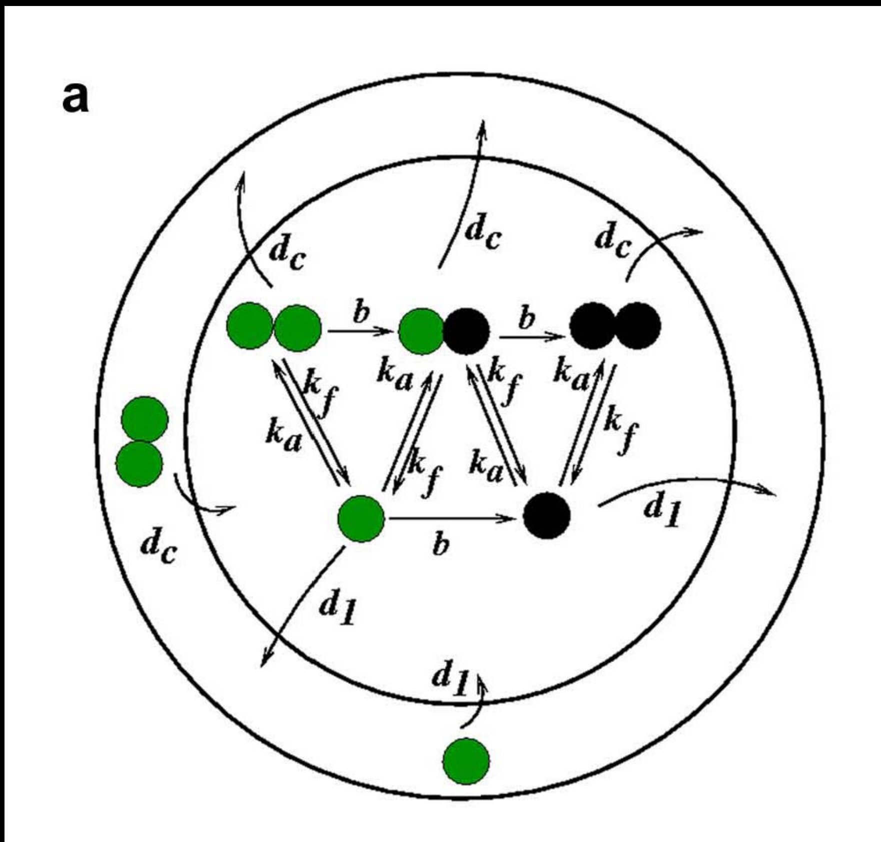


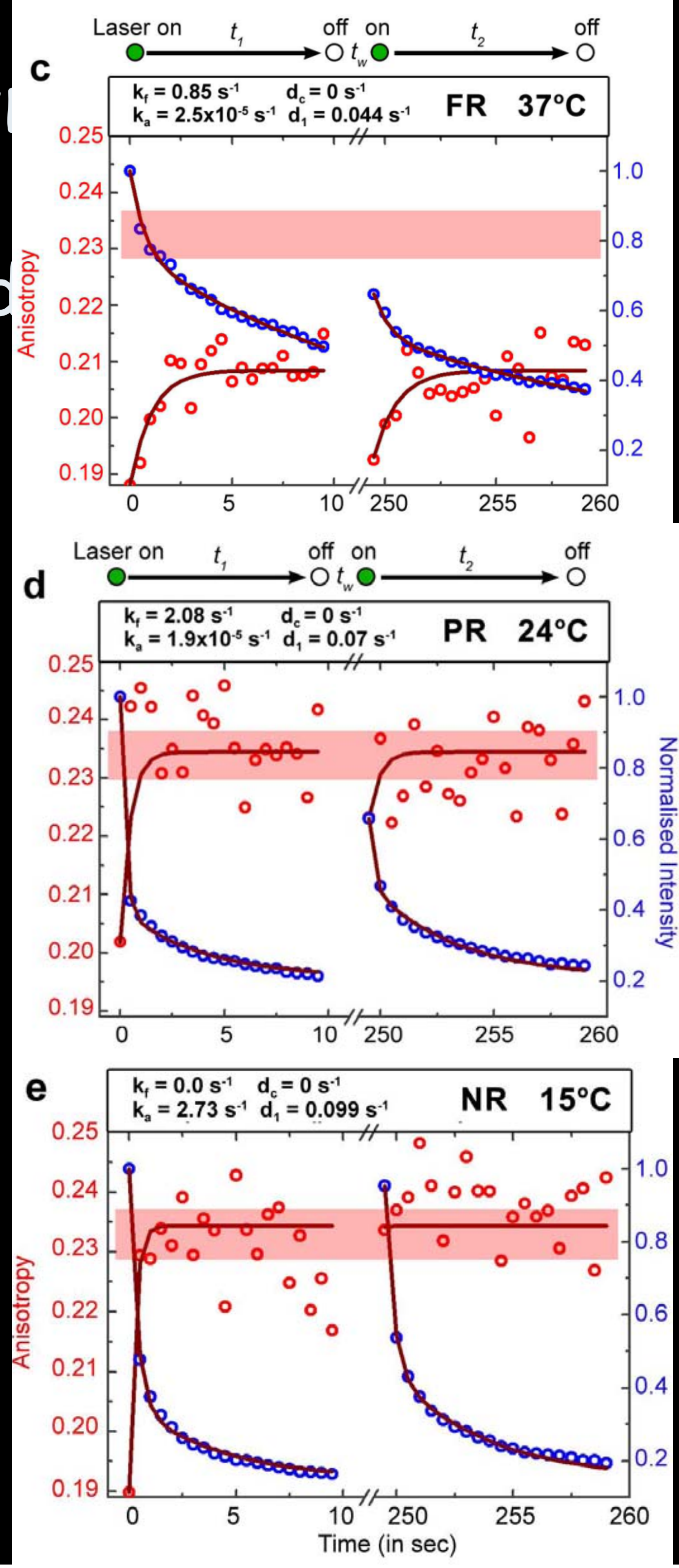


Life time~ < 200 msec - 1 sec

Steady State Dy

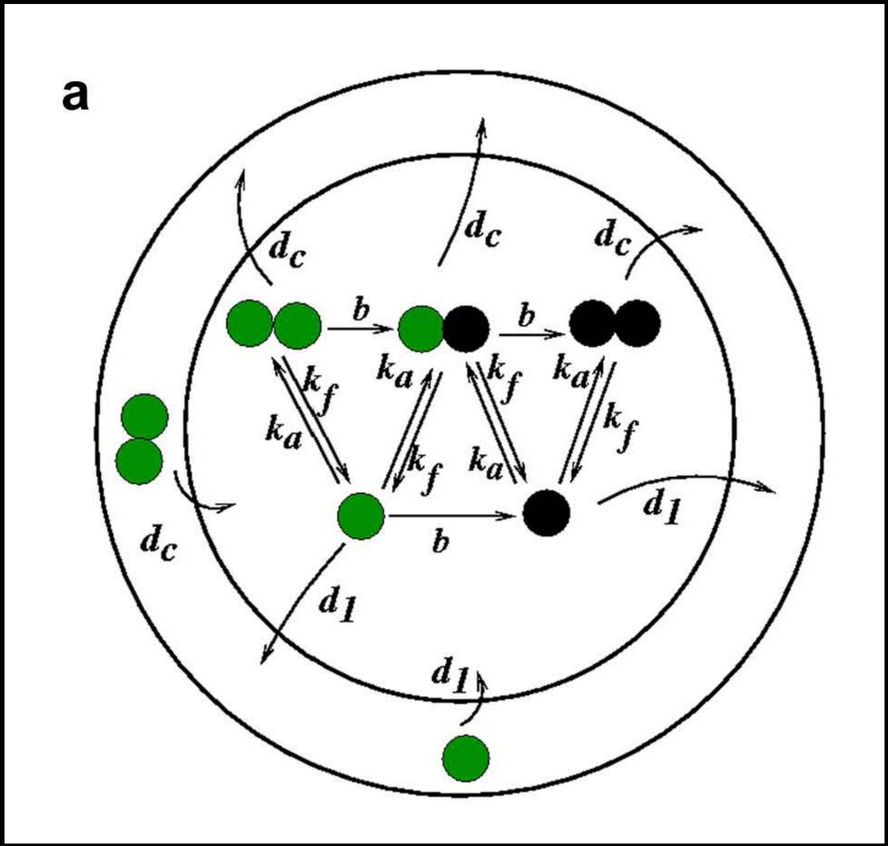
Kinetics of aggregation and

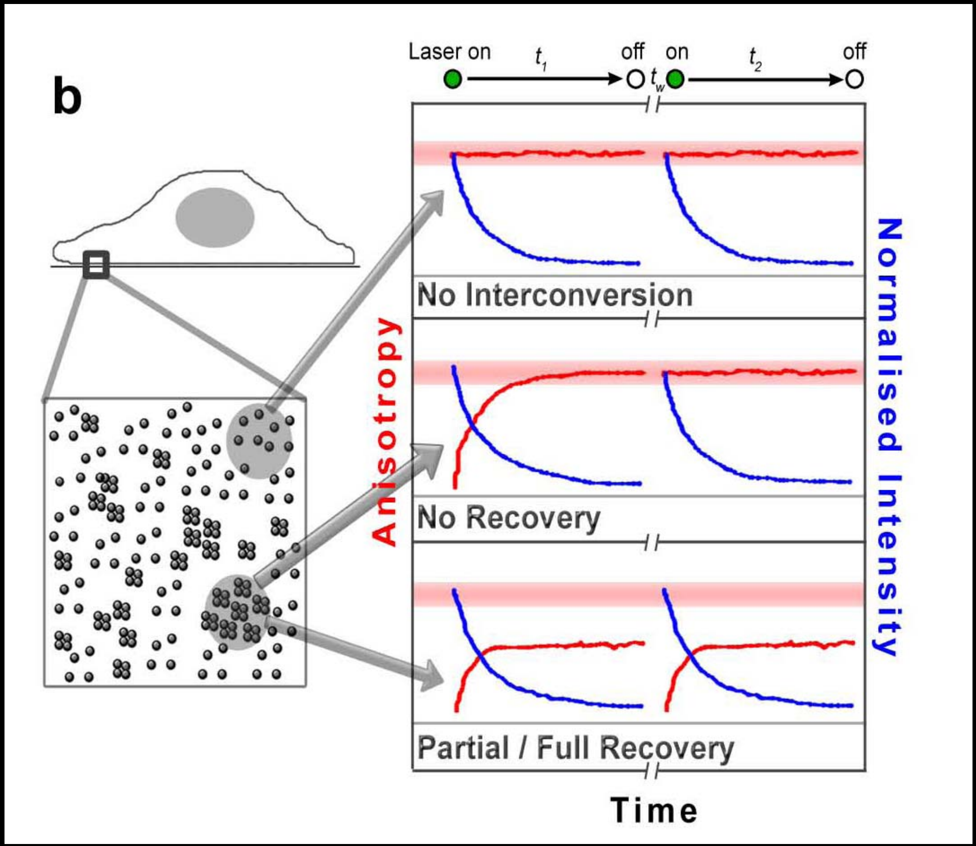




Steady State Dynamics:

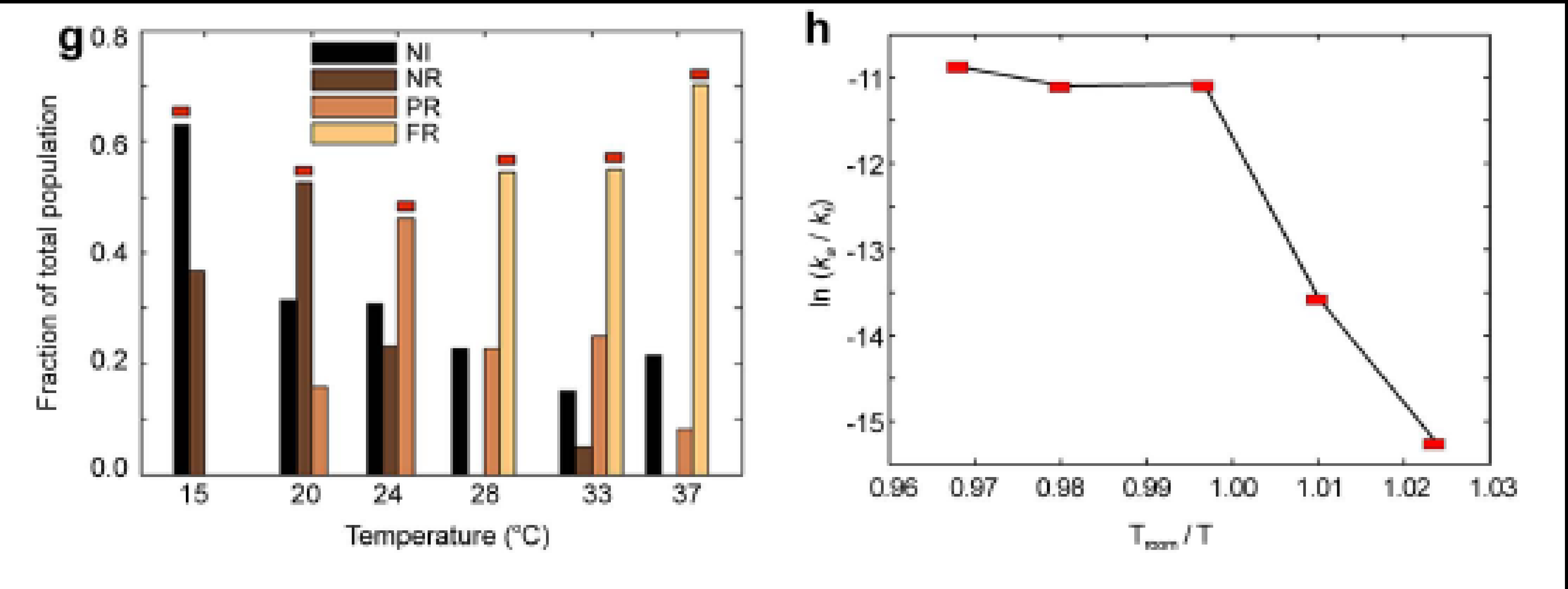
Non-homogenous





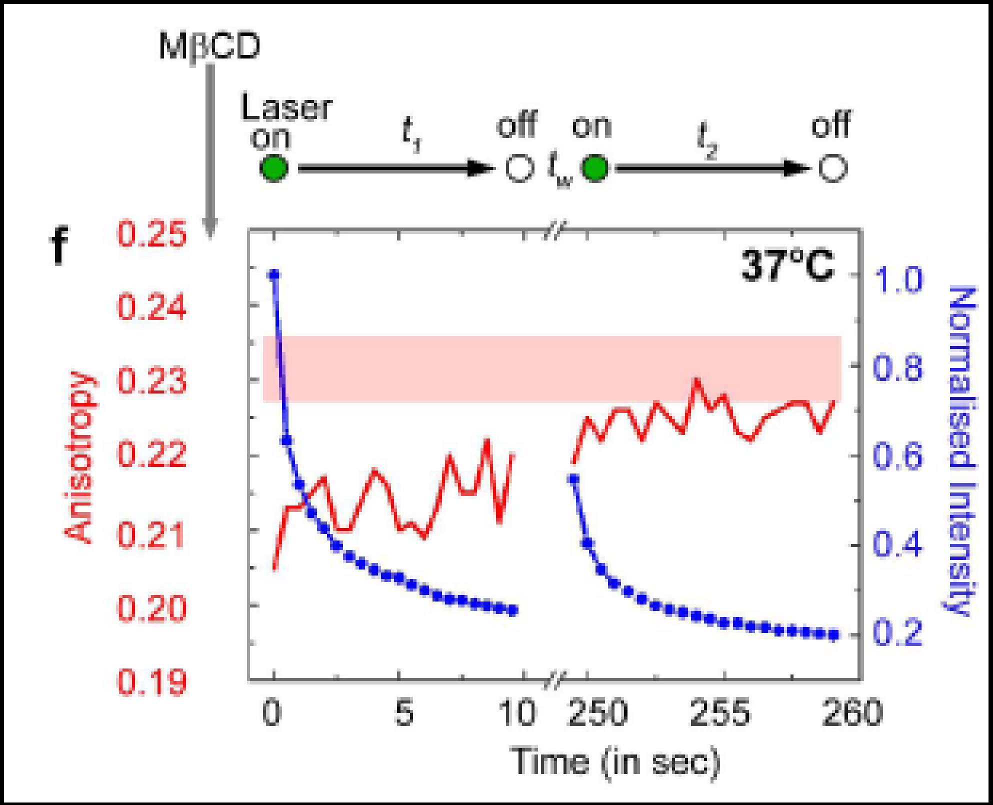
Steady State Dynamics:

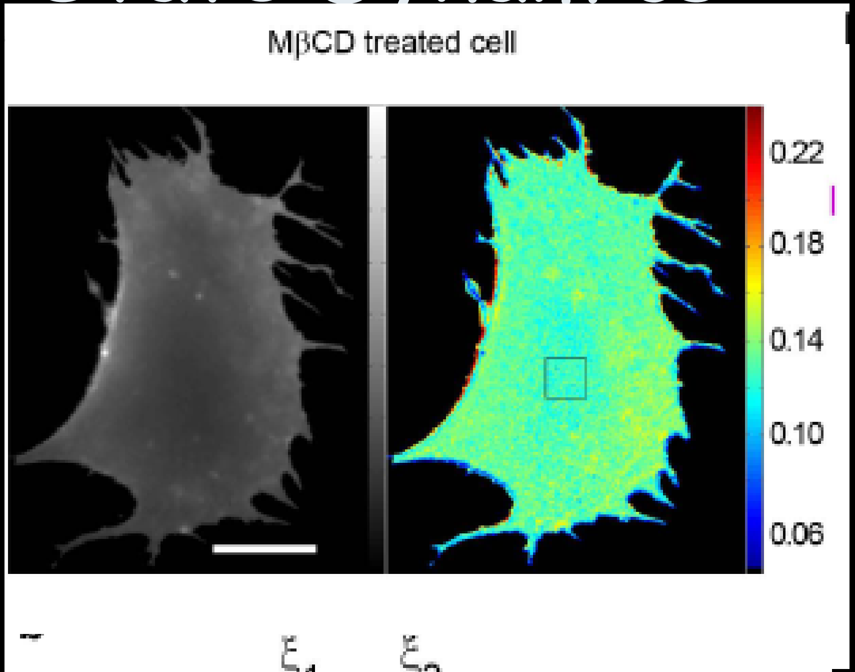
Non-Arrhenius

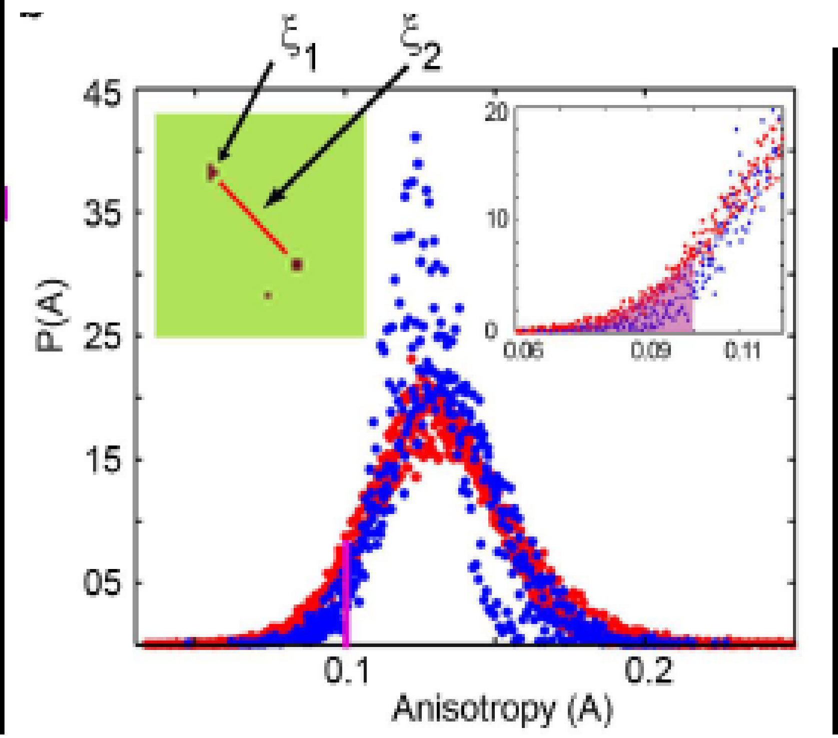


Perturbations of Steady State Dynamics

Cholesterol Depletion

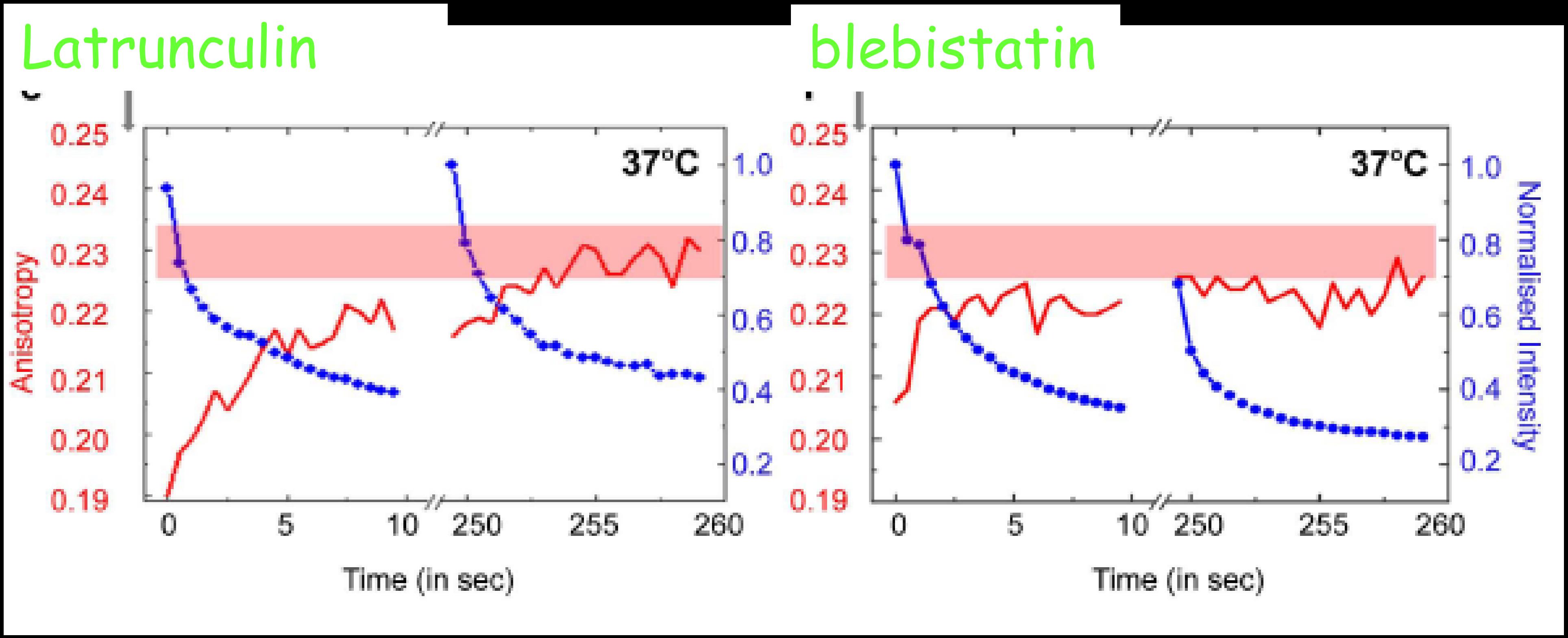




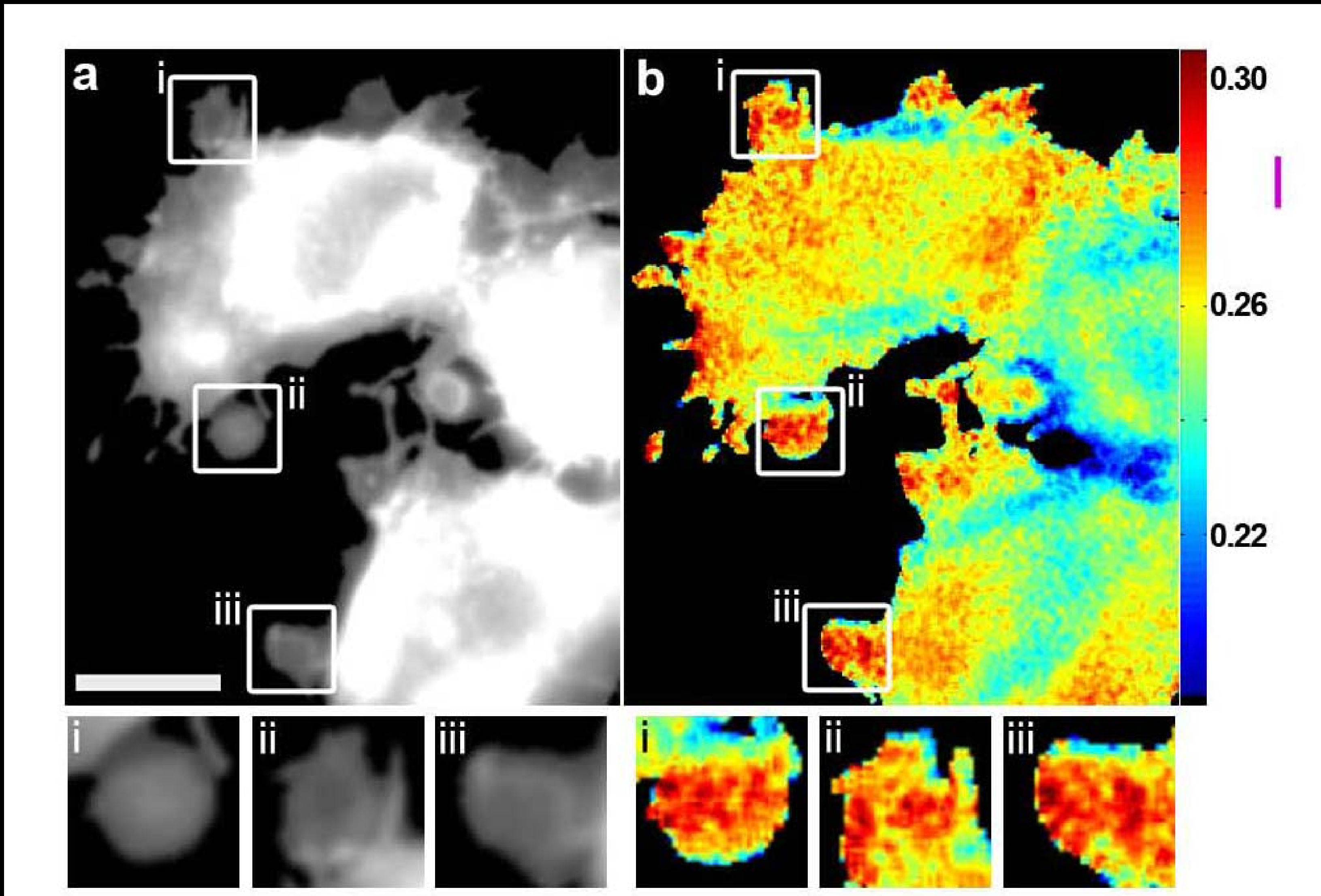


Perturbations of Steady State Dynamics

Actin and Myosin perturbation



Actin perturbation and nanocluster distribution

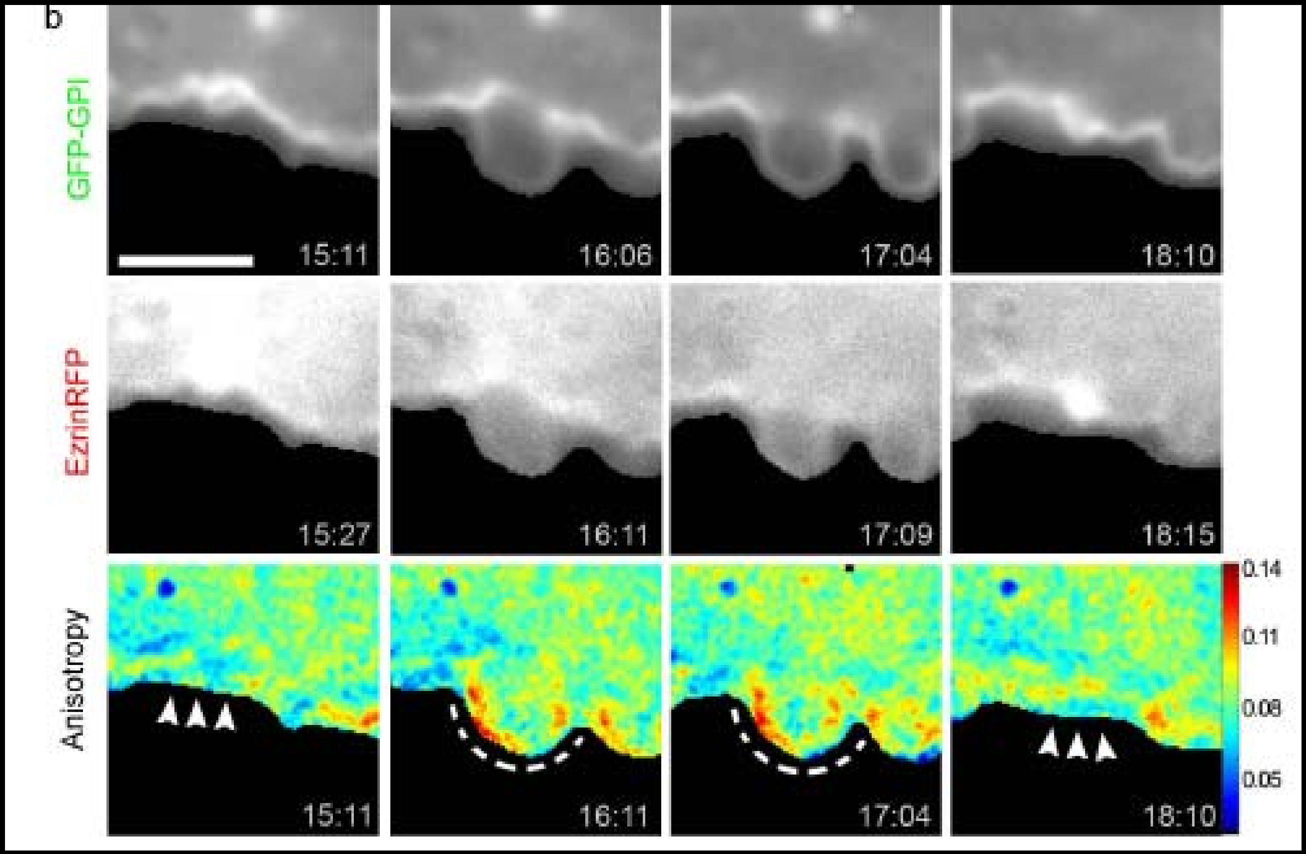


Characteristics of Nanoclusters

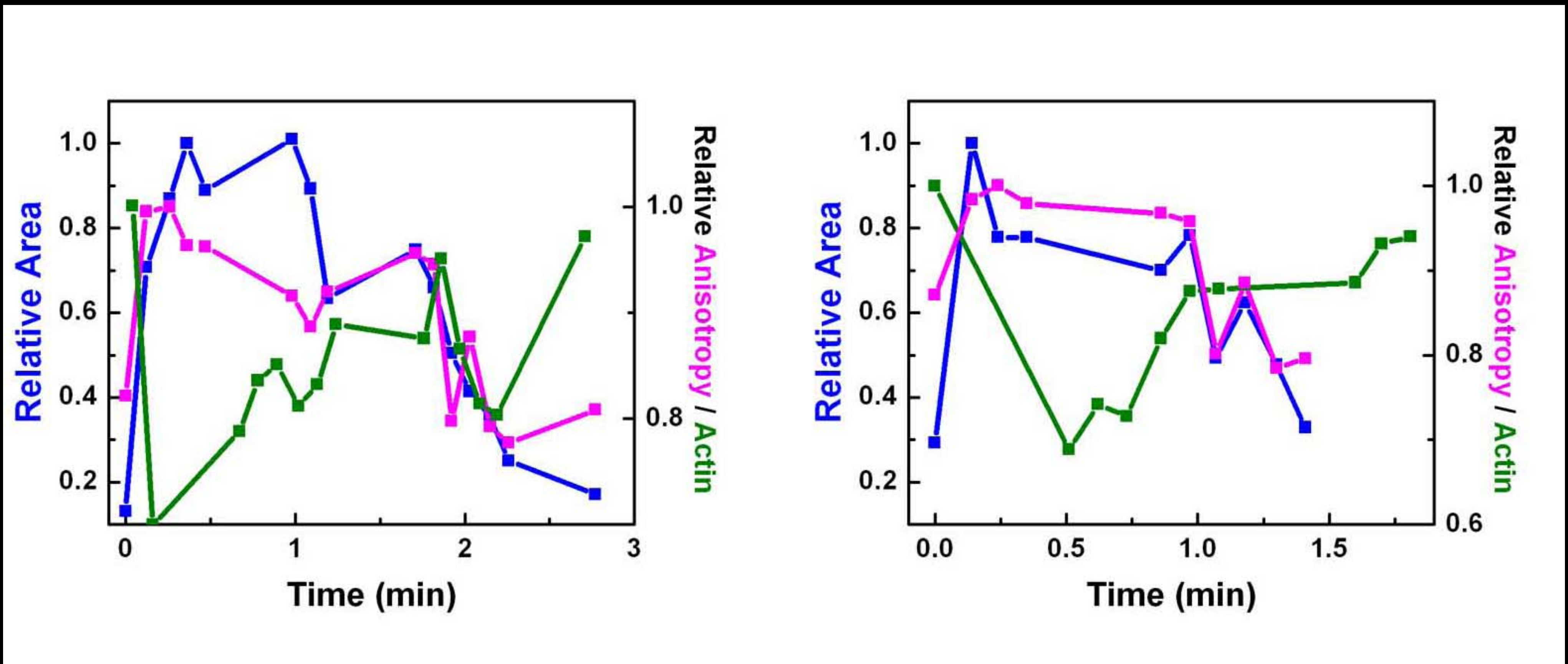
- Dynamics is Non-Arrhenius
- Sensitive to actin and myosin perturbation
- Spatially inhomogeneous
- Formed in regions that are supported by actin cortex
- Immobile

Nanoclusters are depleted and then repleted in blebs

Spontaneous blebs - Whole Cell



Nanoclusters are depleted and then repleted in blebs *after actin is recruited*

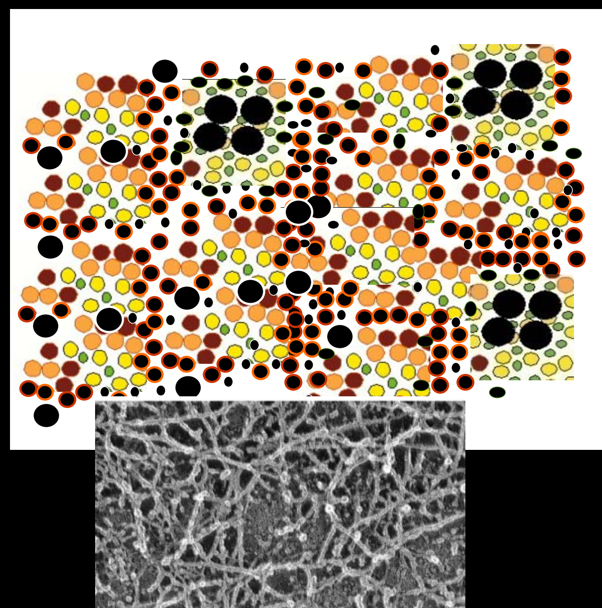


Active Membrane Complexes : a central role for actin

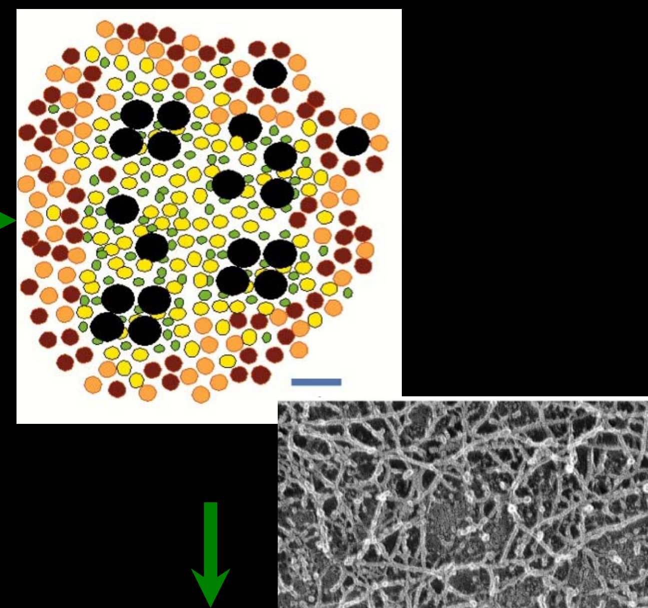
Localization of nanoclusters with specific types of actin organization

Actin polymerization and contractility perturbation dramatically modifies nanocluster distribution

pre-existing organization



induced rafts



Sorting or Signaling Function

Membrane templated by cortical actin

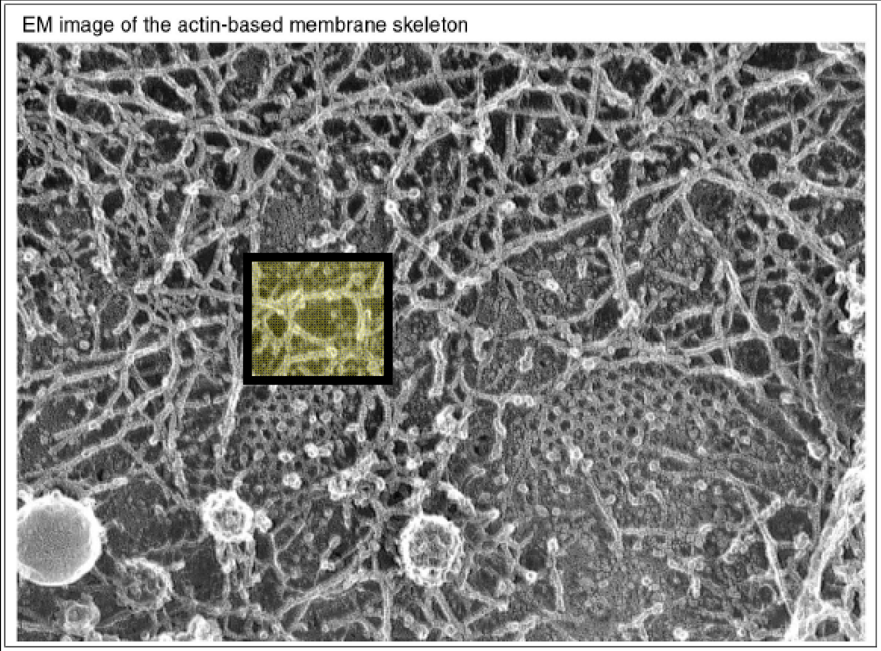
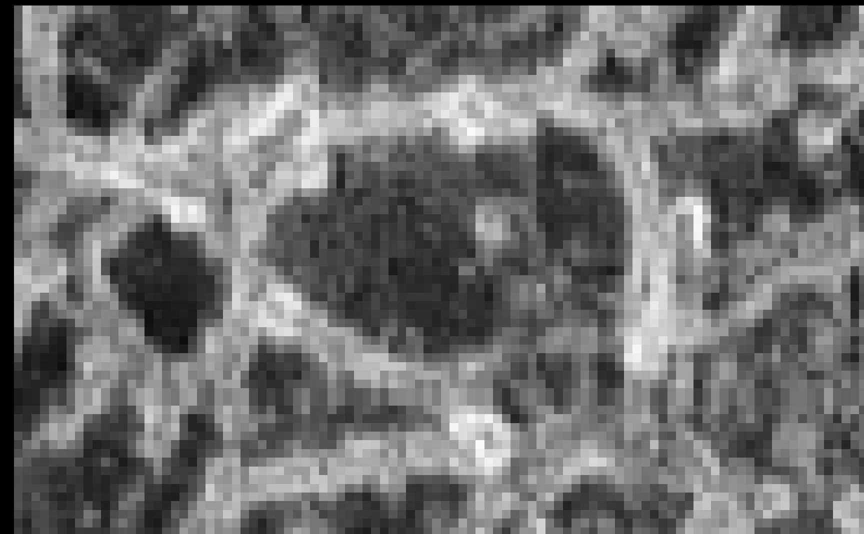


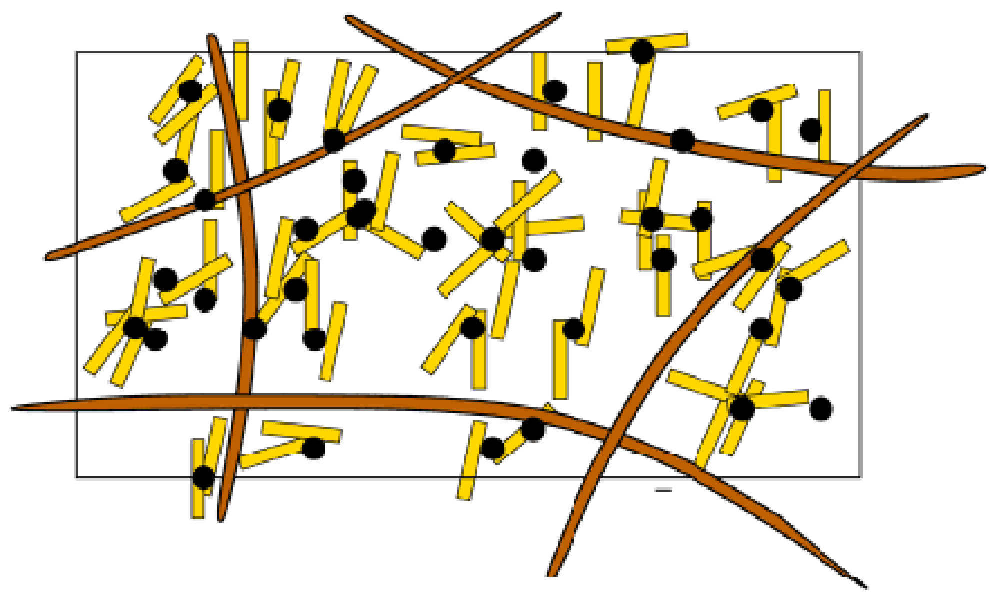
Figure 24. The membrane skeleton is largely comprised of actin. The thin white bands seen on the filaments in this high magnification image indicate that the membrane skeleton in close proximity to the lipid bilayer is comprised of actin filaments.



Long Static crosslinked actin filaments

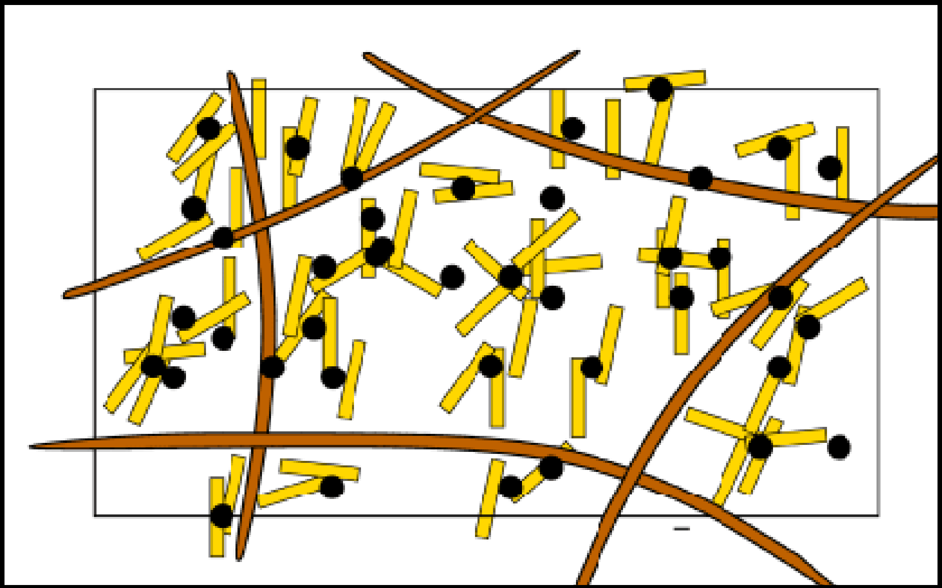
Morone et al JCB 2007

Horizontal crosslinked short dynamic actin



Membrane templated by cortical actin

Short dynamic actin
Orientation n
Concentration c

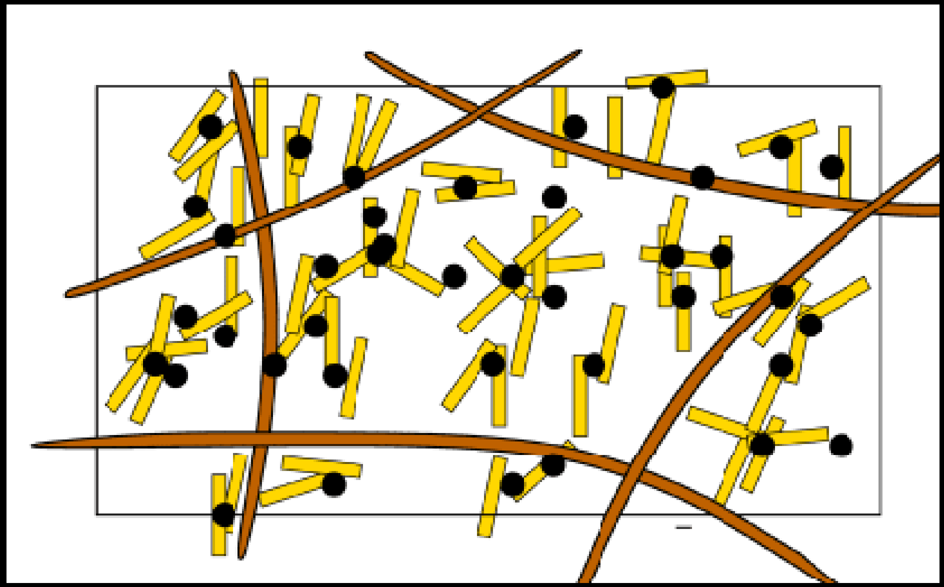


Long Static actin
Orientation N
Concentration C

Acto-Myosin Contractility

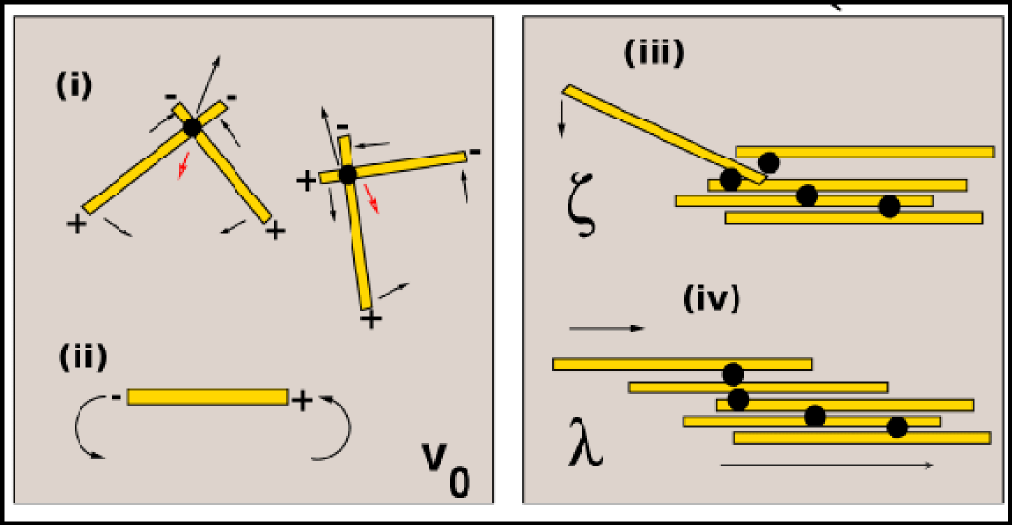
Active hydrodynamics of cortical actin filaments

Short dynamic actin
Orientation n
Concentration c



Long Static actin
Orientation N
Concentration C

Acto-Myosin Contractility



Joanny, Prost, Kruse, Julicher
Sriram, Madan

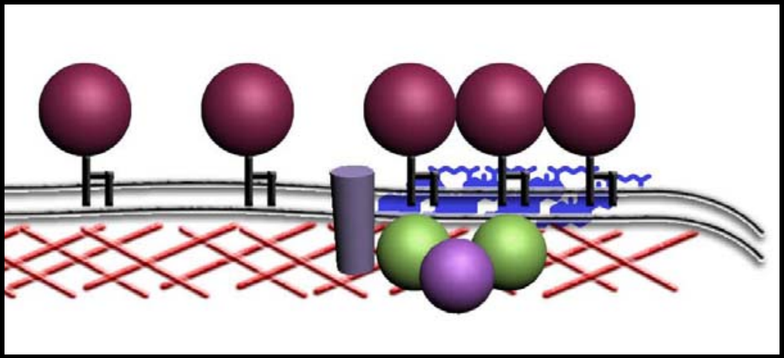
Liverpool, Marchetti

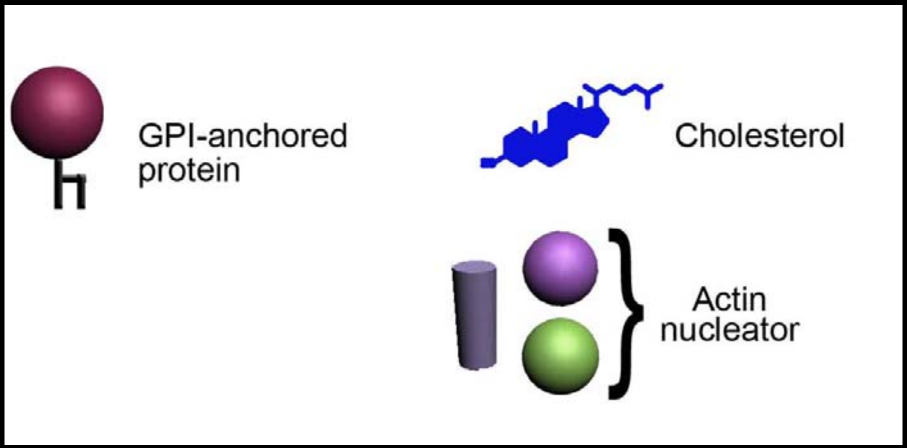
Simha+Ramaswamy, PRL (2002)
Hatwalne et al, PRL (2004)
Muhuri et al, EPL (2006)
Ramaswamy+Rao, NJP (2007)

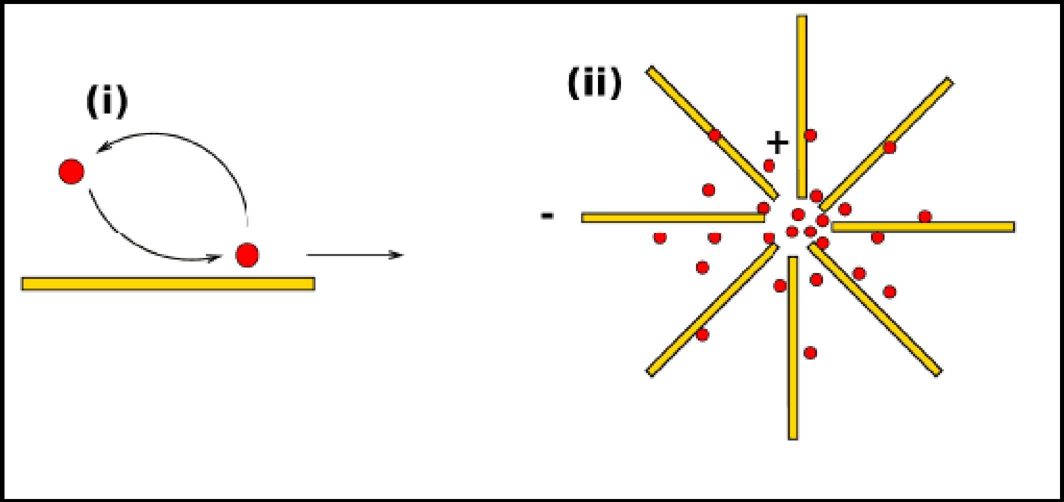
Cytoskeletal activity drives and regulates molecular complexation on cell surface

Active composite cell surface

Inert Particles
Passive Particles
Active Particles







Active actin membrane composite

Flip-flop: lipid flux: secretion: endocytosis

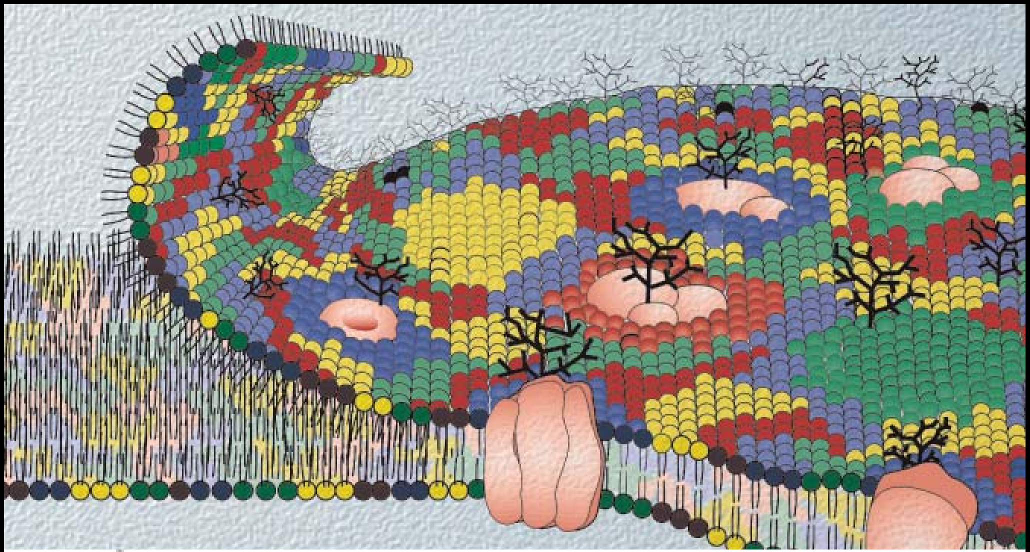
Passive:

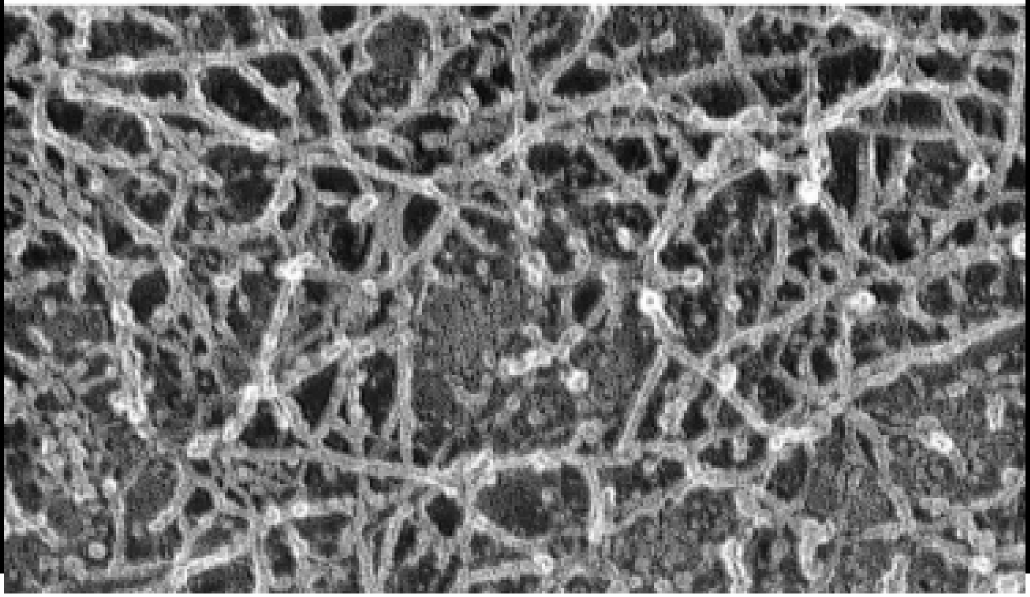
Pre-existing phase segregation

Equilibrium principles

Membrane lipid environment

Passively formed





Active:

Actively manufactured

Non-equilibrium principles

Composite of membrane and cytoskeleton

Living - regulated

People

Membrane Organization

*Rajat Varma, NYU/NIH
*Pranav Sharma, CSH

Endocytosis

*Shefali Sabharanjak, B'lore
*Samit Chatterjee, CSH, NY
*Pranav Sharma, CSH, NY
*Manjula Kalia, ICGEB, N. Delhi

Sameera Bilgrami
Debanjan Goswami
Subhashri Ghosh

Riya Raghupathy
Suvrajit Saha

H. Krishnamurthy, CIFF NCBS

Rob Parton, (Australia),
G.Krishnamoorthy, (TIFR, Mumbai)
Aki Kusumi, (Japan)
Ram Vishwakarma (NII, Delhi)

Madan Rao, (RRI/NCBS)
G. Kripa, (RRI/NCBS)
Abhishek Chaudhry (NCBS)
*Sarasij RC, (RRI/NCBS)

Rahul Chadda
Sudha Kumari

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