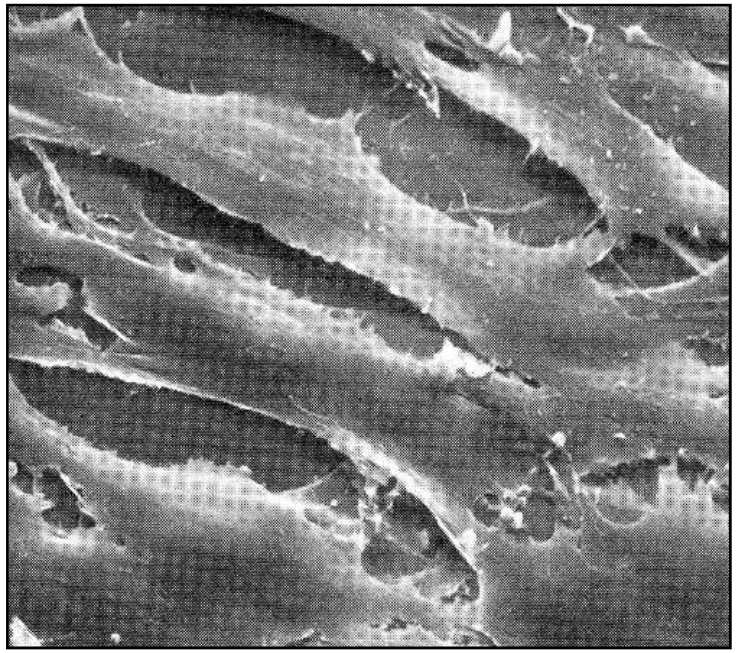
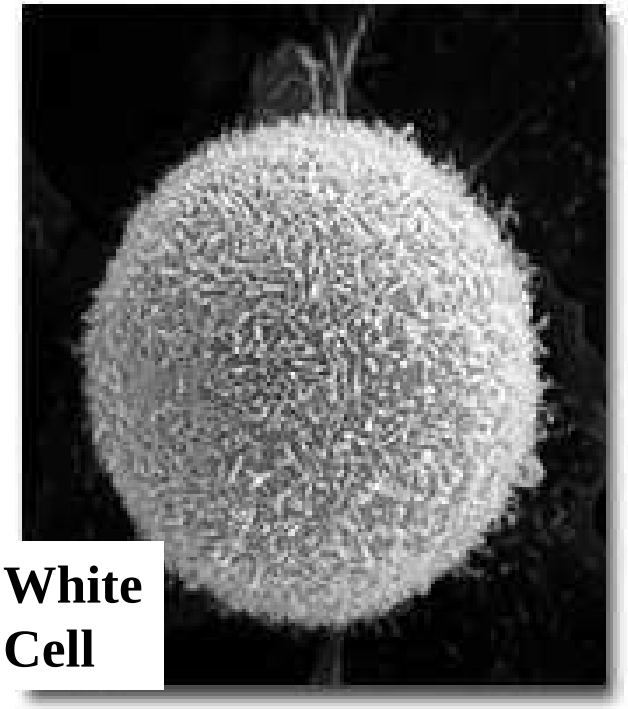
Spatiotemporal aspects of signal transduction: models and experiments

Bridget Wilson
Univ. of New Mexico
bwilson@salud.unm.edu

Our Focus: Signal Transduction in Cells
These events take place in context of Cell Geometry.



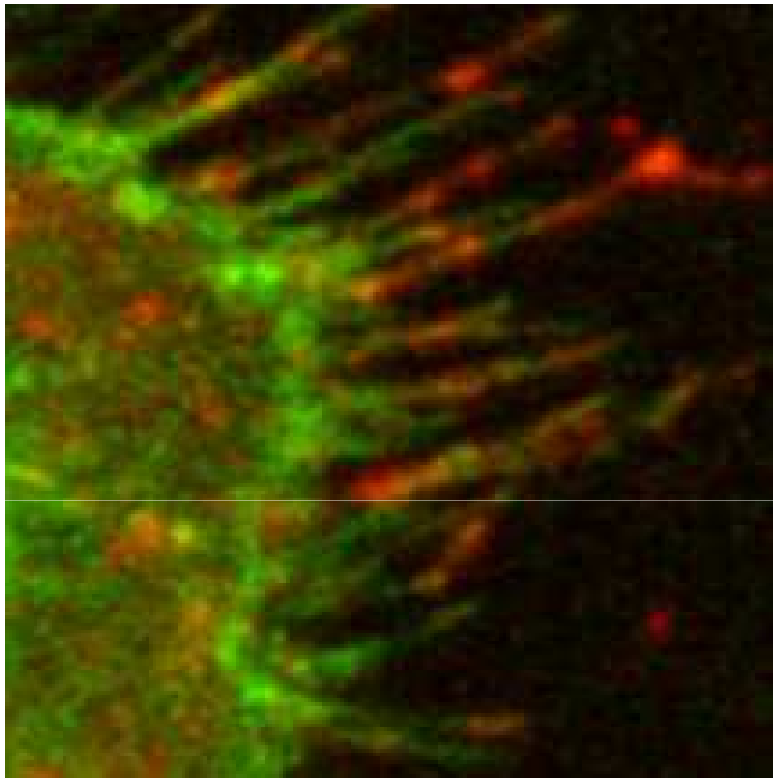
Fibroblasts in culture



White Cell

Electron Microscopy: high spatial resolution, poor temporal resolution

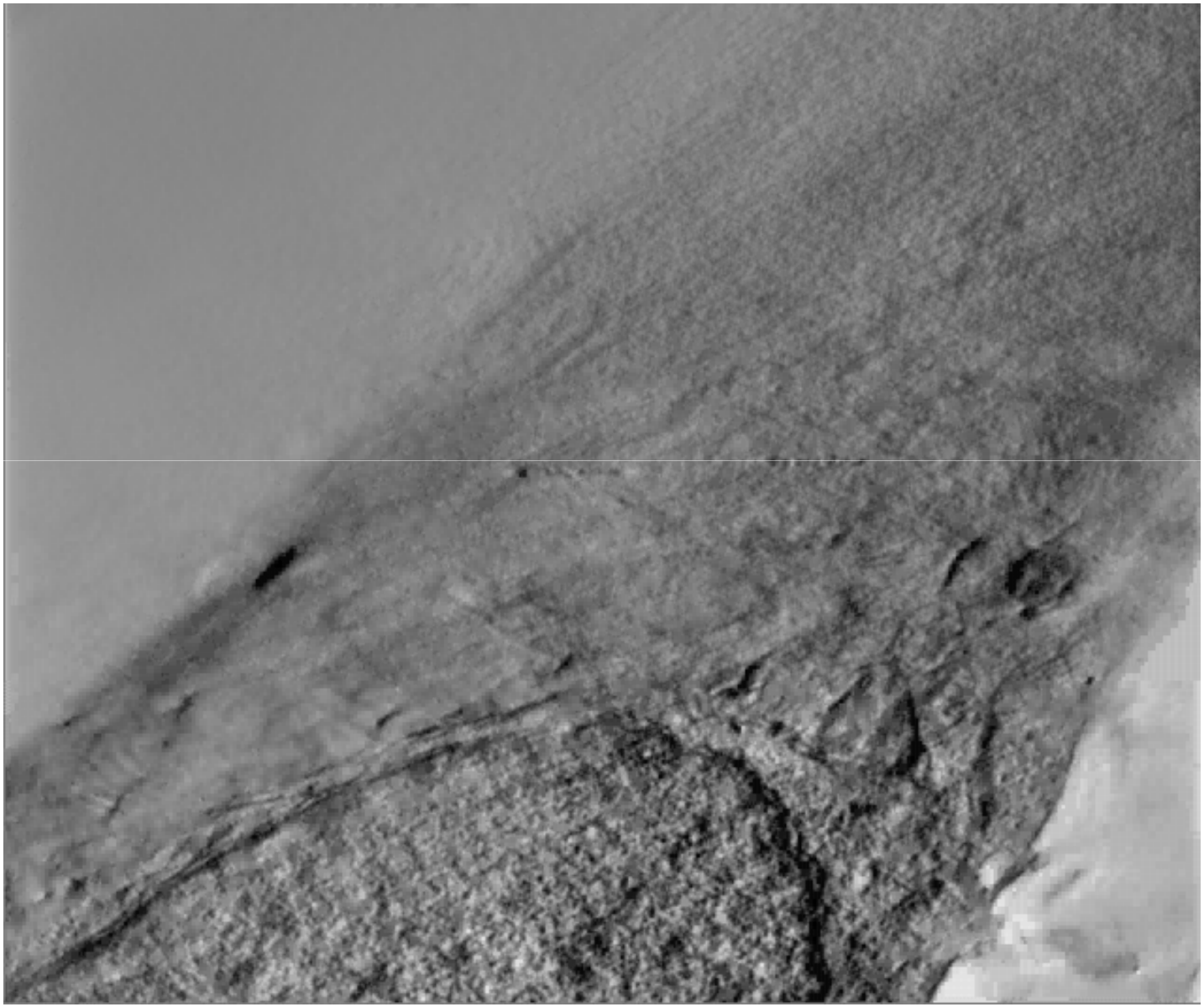
Light microscopy
- often based on fluorescence techniques
- Can offer high temporal resolution, with less spatial resolution.



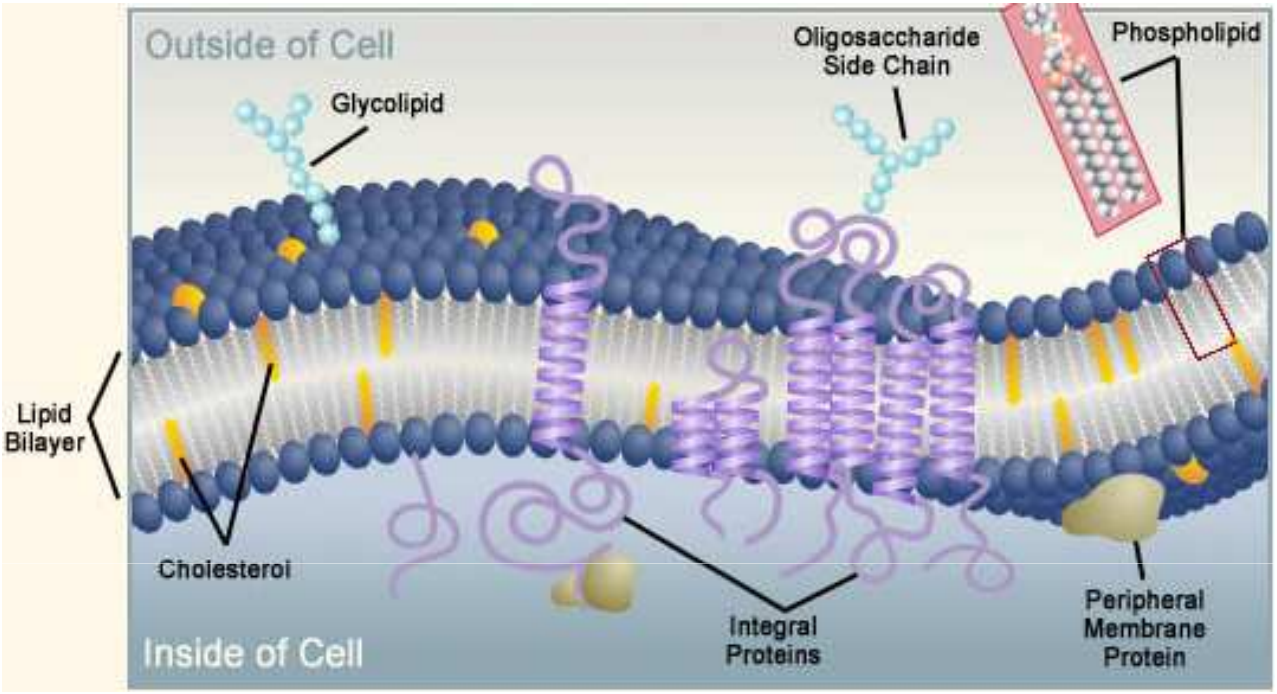
A view inside

Important Cell Compartments

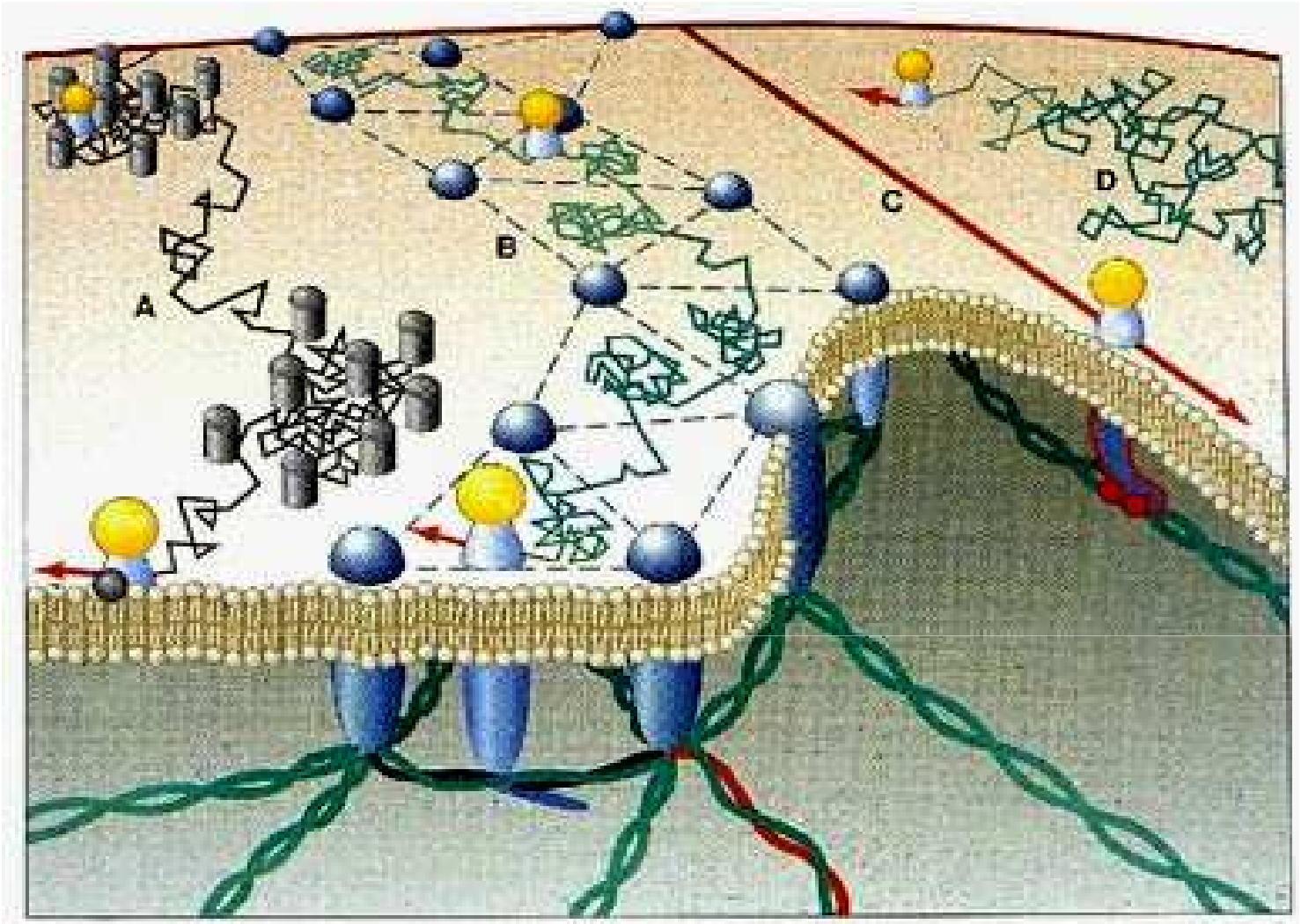
- **plasma membrane**
- **cytosol**
- **nucleus**
- **endoplasmic reticulum**
- **mitochondria**



"Textbook" View of the membrane



- membranes are asymmetrical, renewable
- Protein/Lipid composition
- Permeability barrier, with transporters & channels to move things across
- rotational and lateral mobility
- membrane receptors receive ligand input, pass on to intracellular signaling partners
- Important interactions take place in 2D membrane and 3D cytosol – and their interface
- other cellular compartments also have same 2D, 3D issues, unique composition & transport issues (ie nucleus, etc).

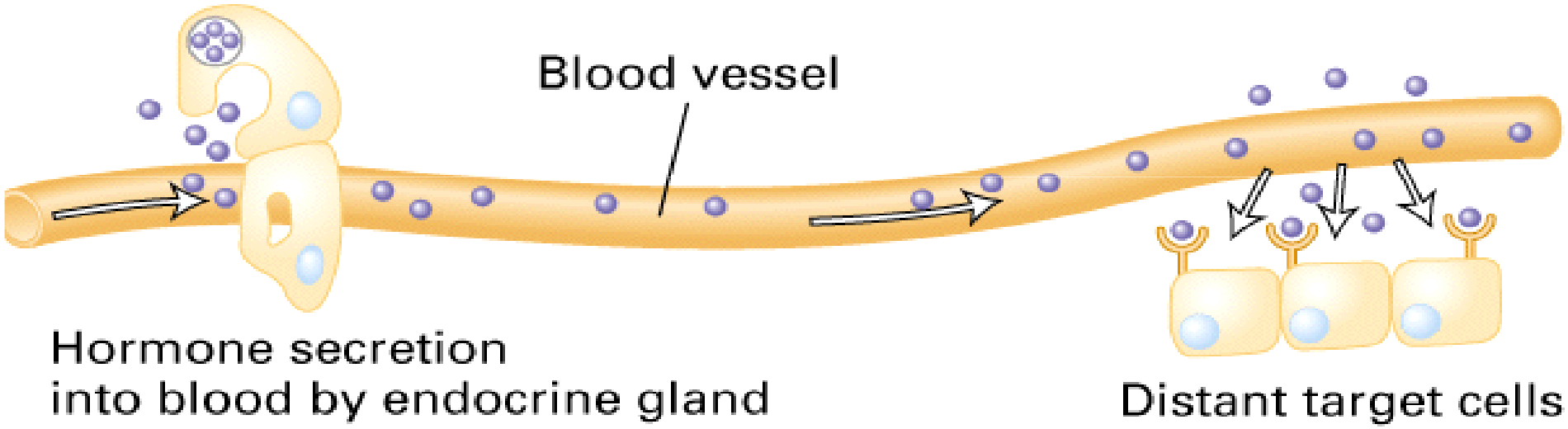


Lateral transport modes on the cell surface. (**A**) Transient confinement by obstacle clusters (**B**) or by the cytoskeleton, (**C**) directed motion, and (**D**) free random diffusion.

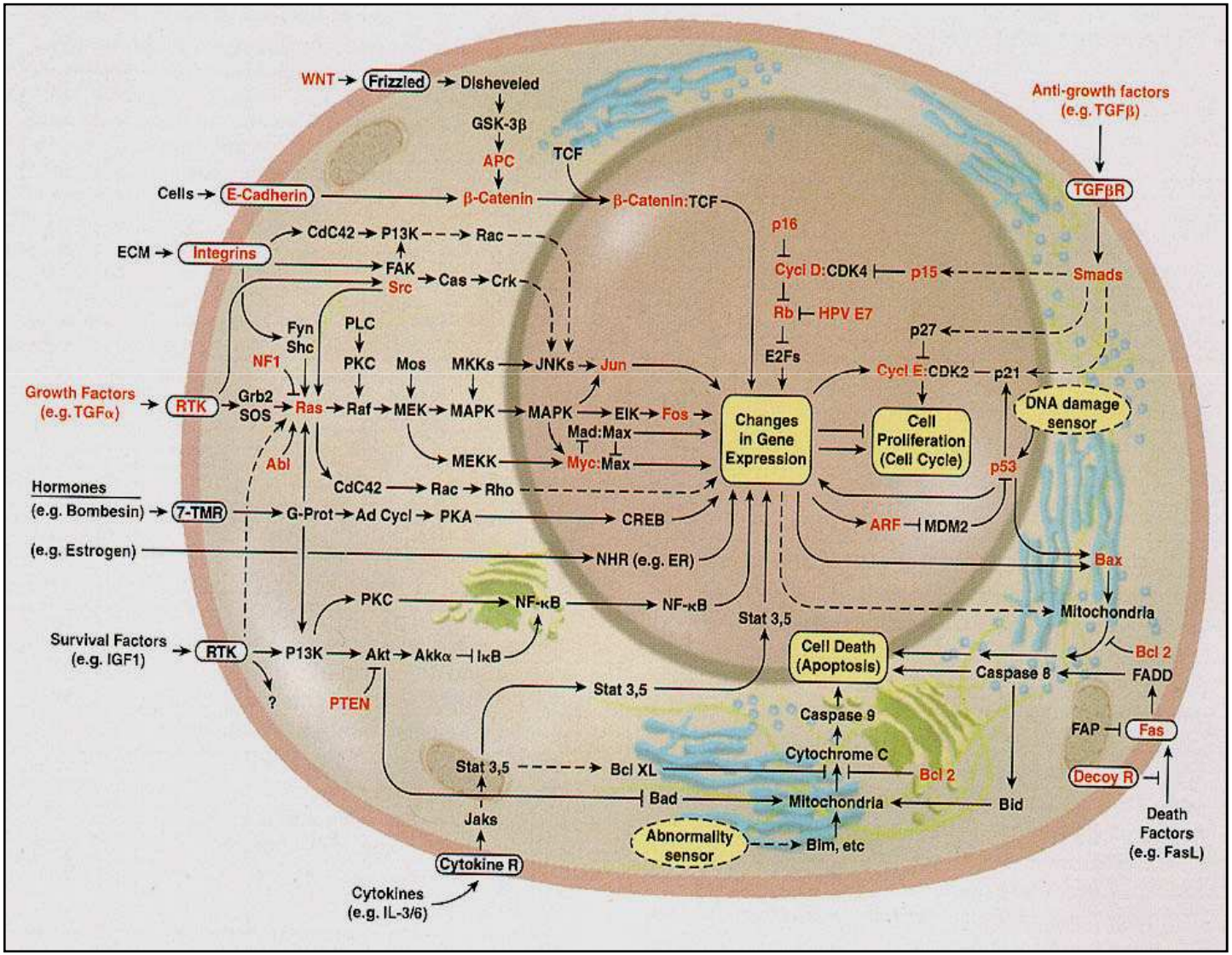
Diffusion in membranes is rarely Brownian. What mechanisms explain anomalous diffusion? How is membrane organized and how does this influence signaling?

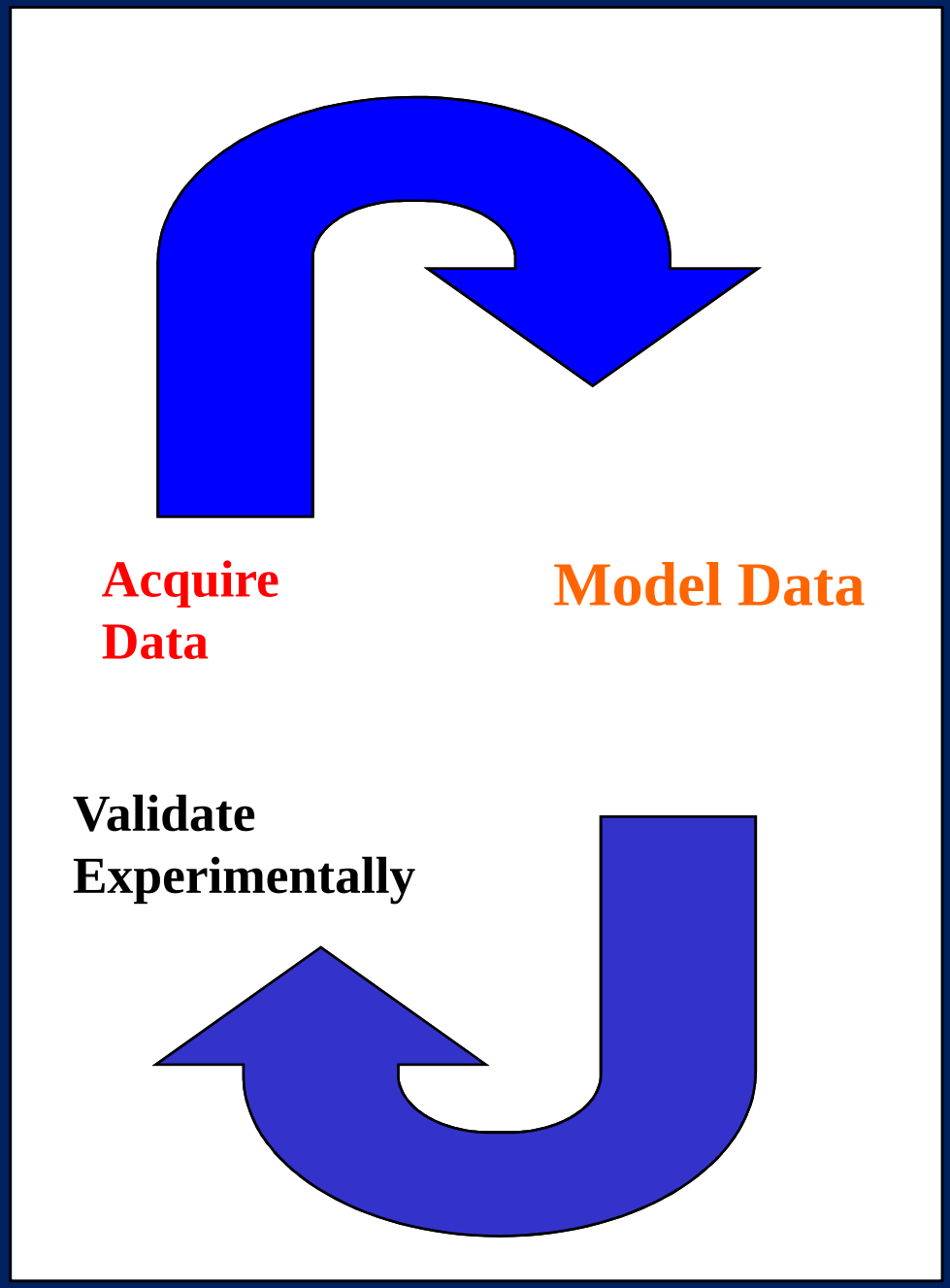
(figure from Kusumi)

One Important Function of the Plasma Membrane is to RECEIVE & PROPAGATE SIGNALS



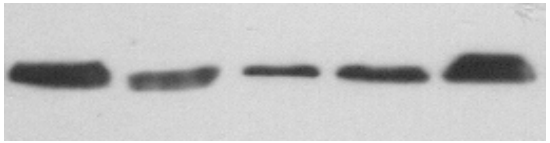
Once the ligand binds its surface Receptor, internal circuits propagate the signal. This complex, networking "circuitry" is composed of proteins, lipids and associated enzymatic reactions.





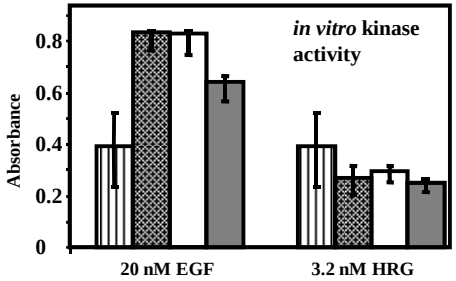
- Is biology realistic?
- Is the problem important?
- Is it feasible to get the parameters and measurements you need?
- Is the problem multi-scale?
- Which modeling approach will work best? Is the system "well mixed"? Do you need to consider spatial aspects? If so, how complex is the geometry? Will simple compartmental models do?

QUANTITATION



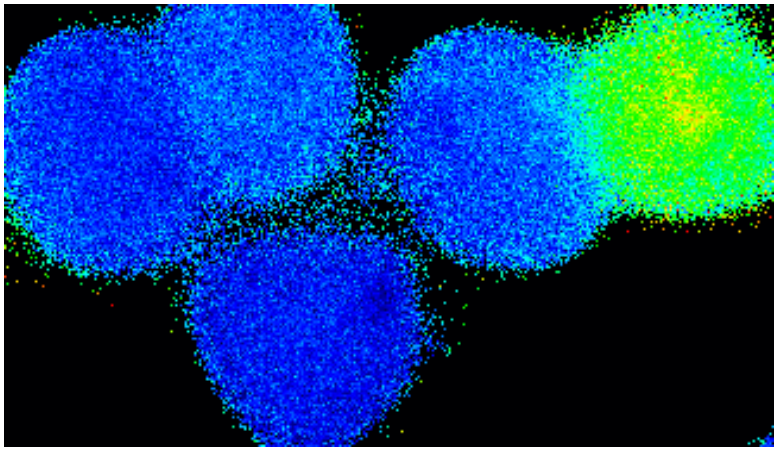
40 20 0.25 0.5 1.0
ng standard lysate (cells x 10^6)

western blotting



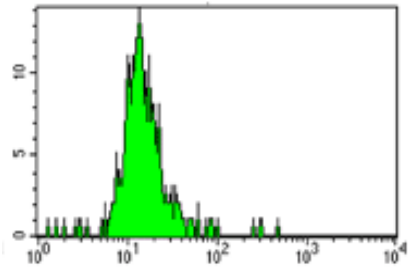
biochemical assays

live cell imaging

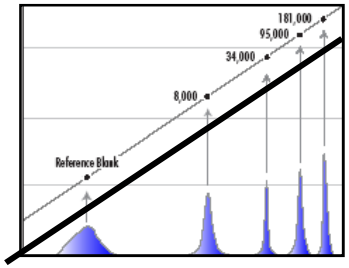


intracellular Ca^{2+}

flow cytometry

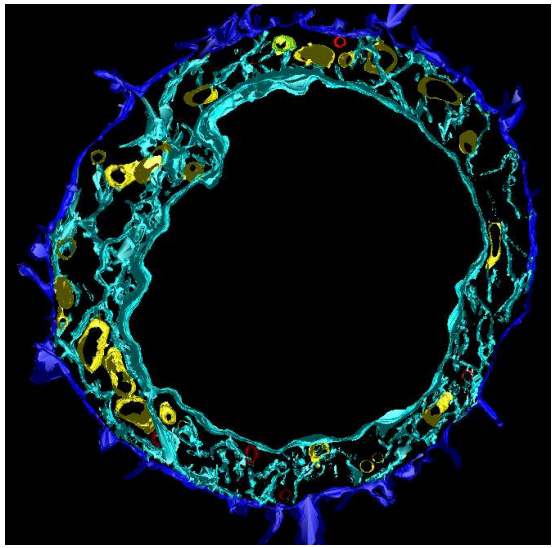


fluorescence (mAb binding to cell surface or intracellular target protein)

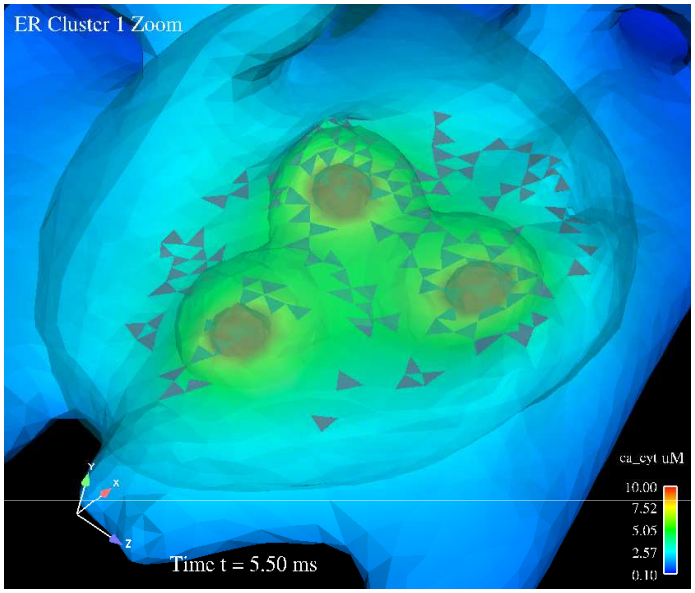


fluorescence (mAb binding to bead standards)

electron microscopy for cell & organelle reconstruction

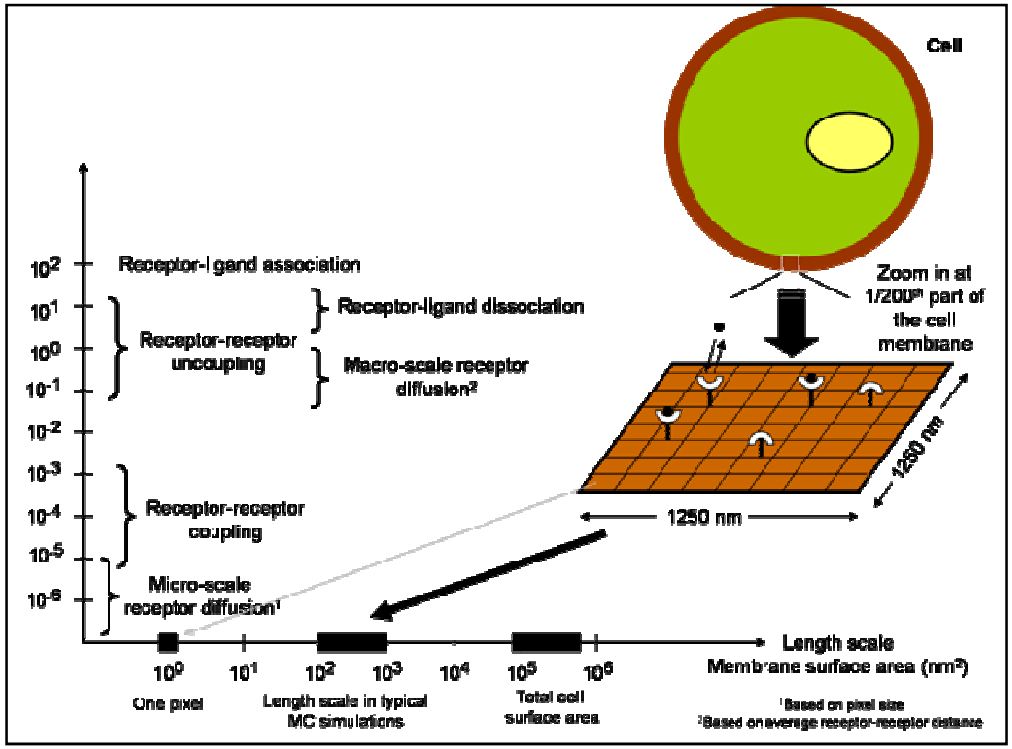


Modeling Approaches



Means et al., Biophysical J. 2006

Hybrid deterministic-stochastic reaction diffusion model for calcium transport



Niehaus et al, Biophysical J. 2007; Hsieh et al, IET Systems Biology *in press*

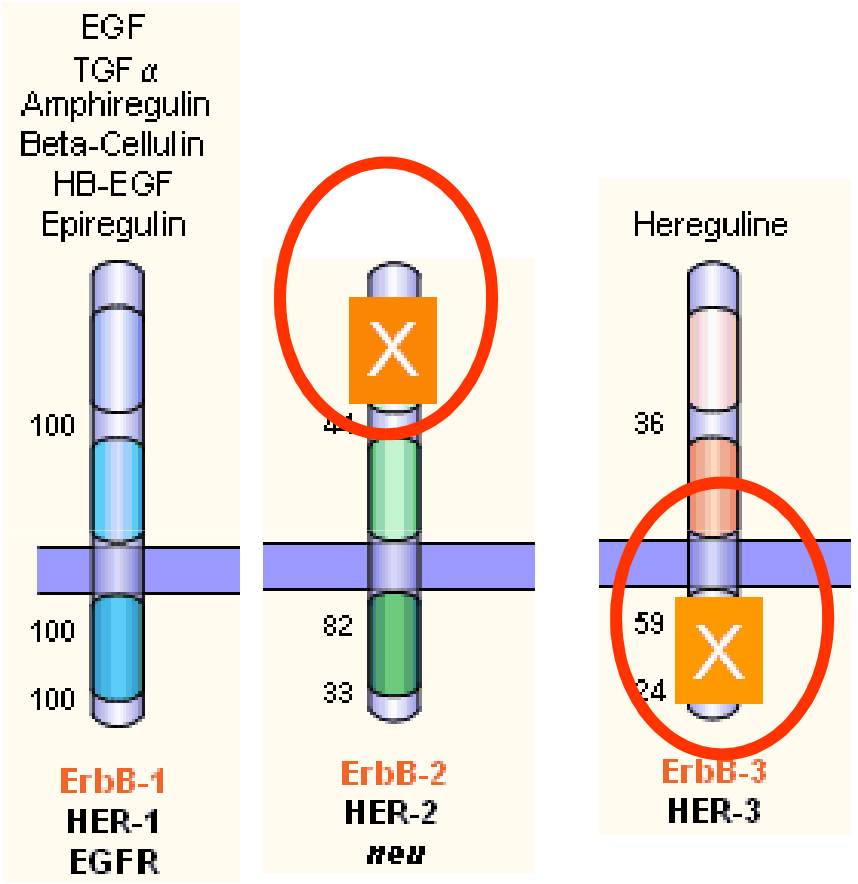
Lattice & Agent-based stochastic models for Receptor Signaling

Example 1:
Mapping & Modeling EGFR/ErbB Topography & Signaling

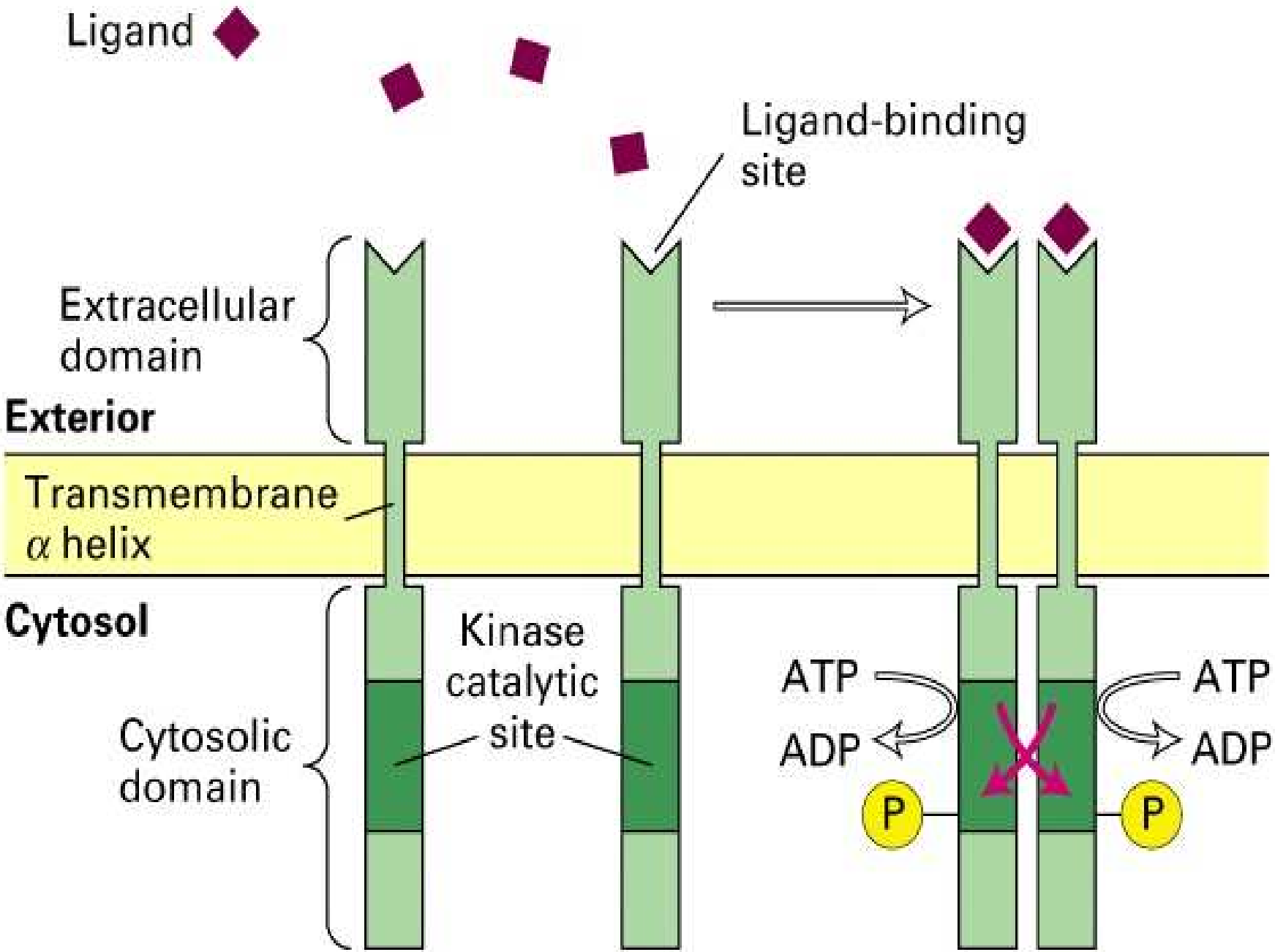
Extracellular Domain

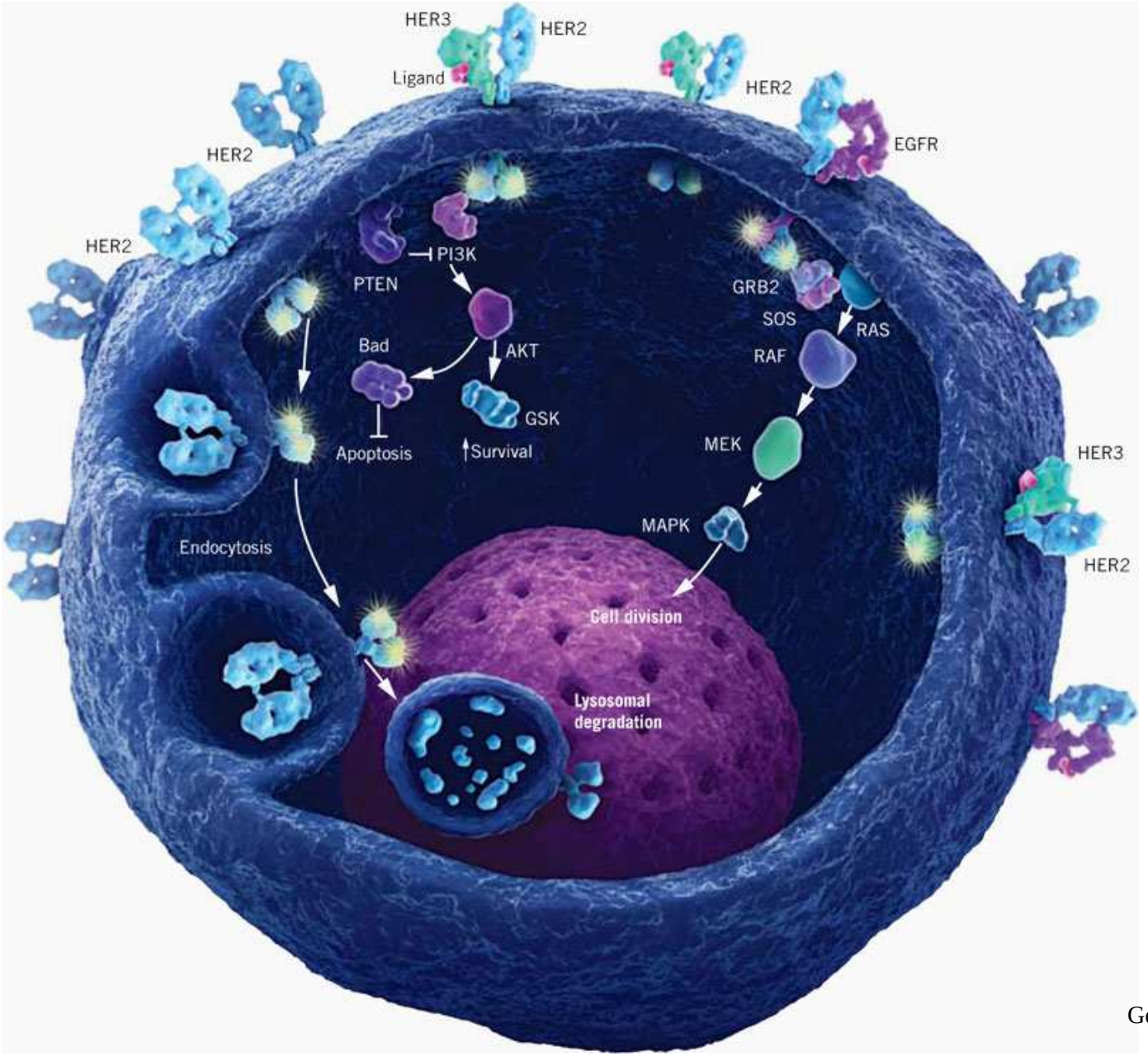
Transmembrane domain

Kinase domain + many sites for autophosphorylation



HOMODIMERS
+
HETERODIMERS

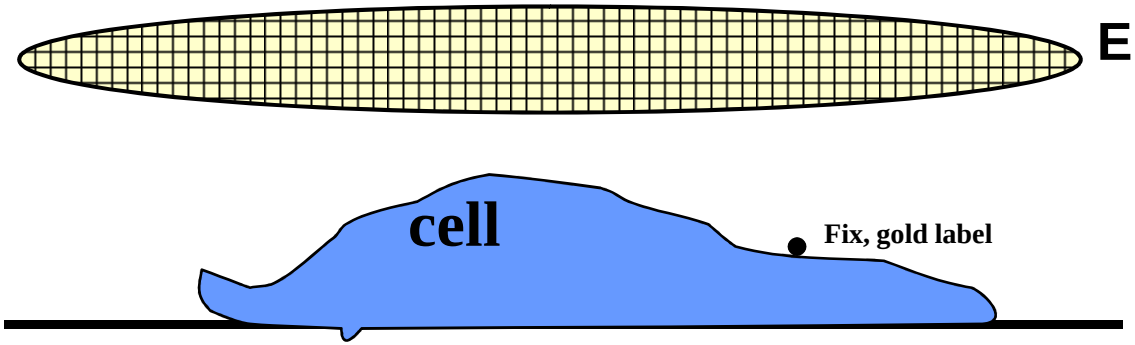

Ligand
Ligand-binding site
Extracellular domain
Exterior
Transmembrane α helix
Cytosol
Kinase catalytic site
Cytosolic domain
ATP
ADP
P
ATP
ADP
P

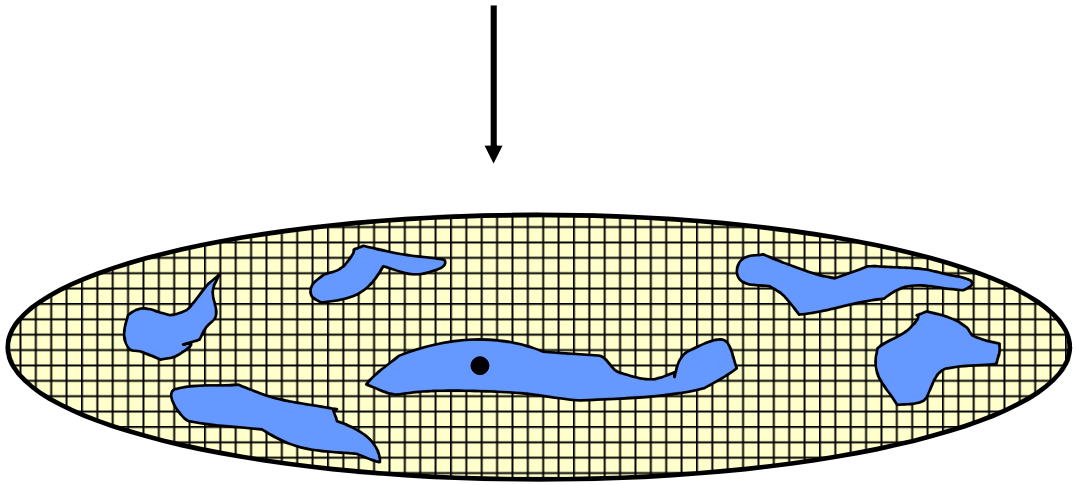


Genentech BioOncology

www.biooncology.com/bioonc/images/her-main.jpg

“Rip-Flips” offer a unique view of ErbB Signaling Domains

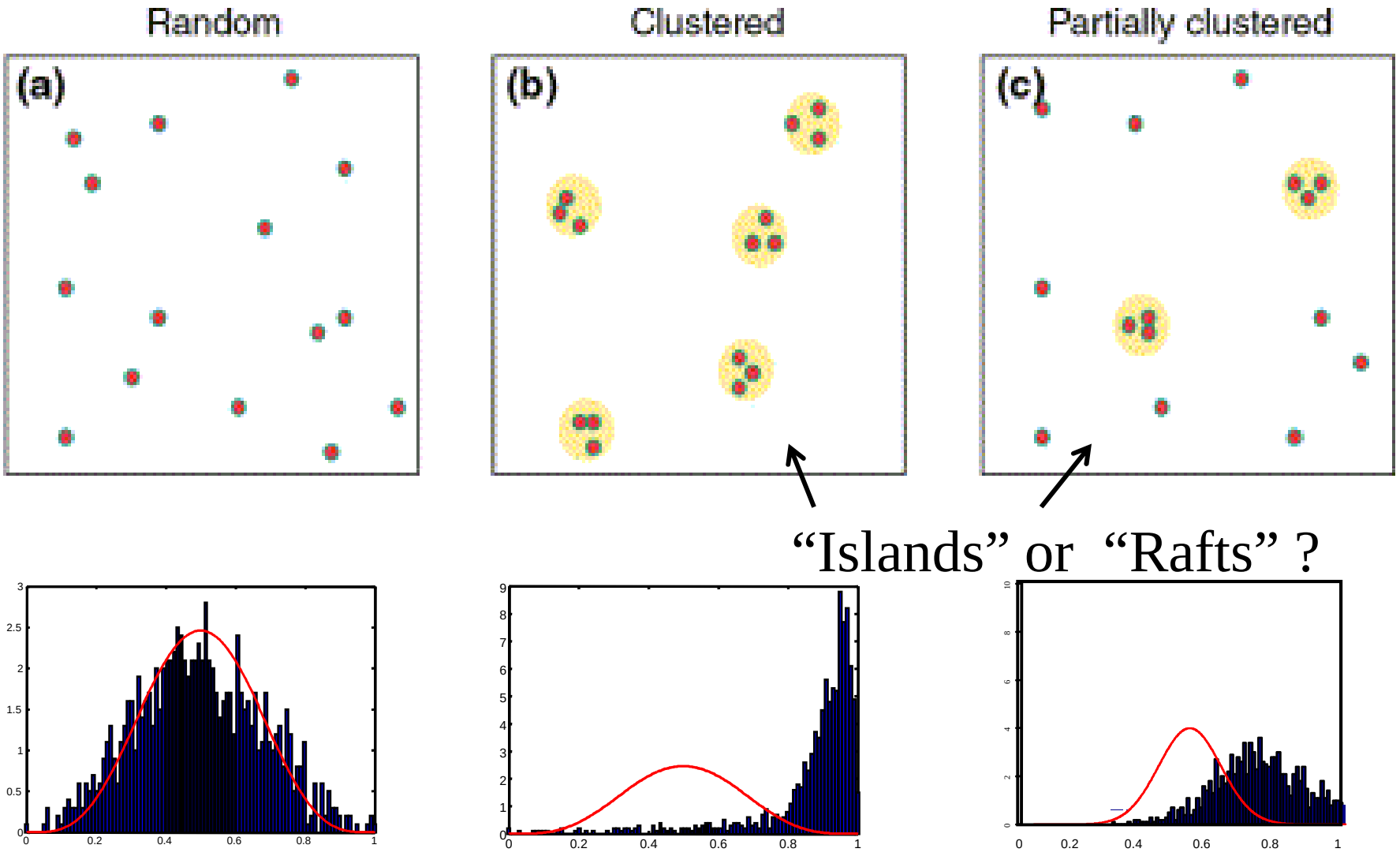




Rip leaves plasma membrane sheets on EM grid.
Fix, gold label.

Yang et al, J. Cell Science 2007

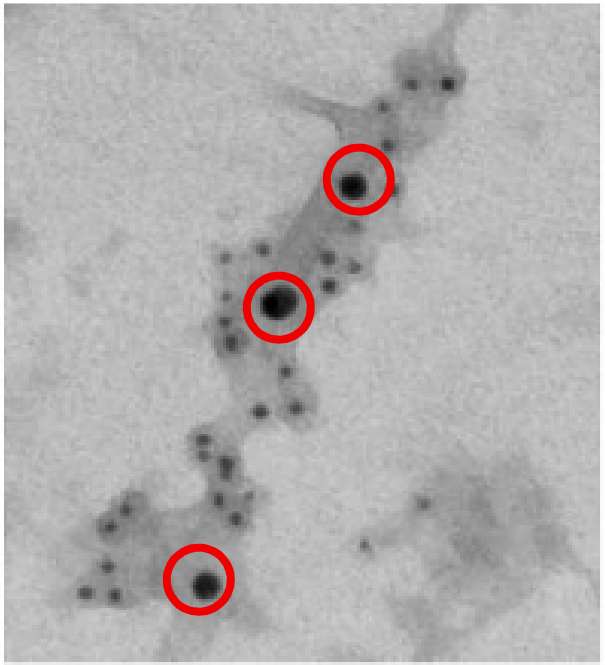
Possible distributions of membrane constituents

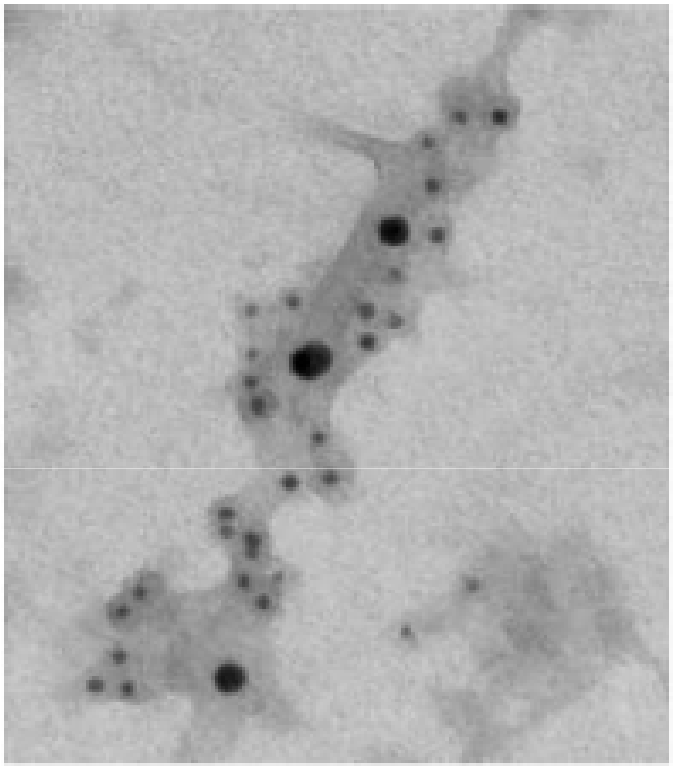


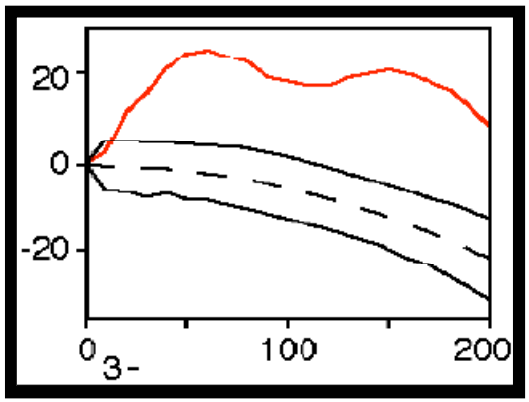
typical results, Hopkins spatial statistics test

We also use spatial statistics to evaluate co-clustering in EM images.

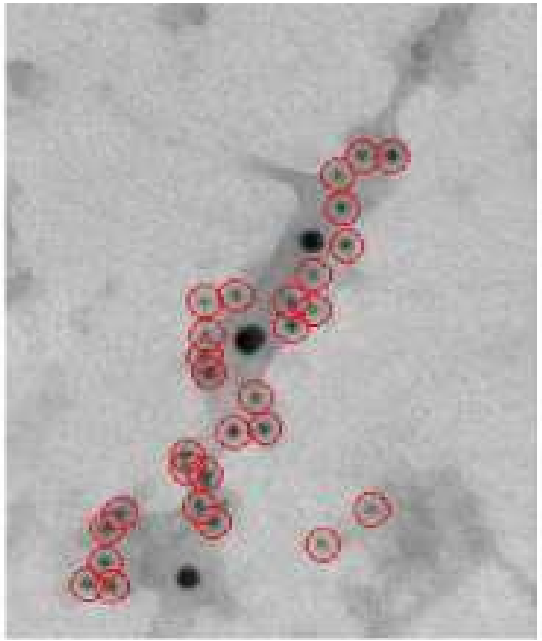
Obtain coordinates of 10 nm Gold.





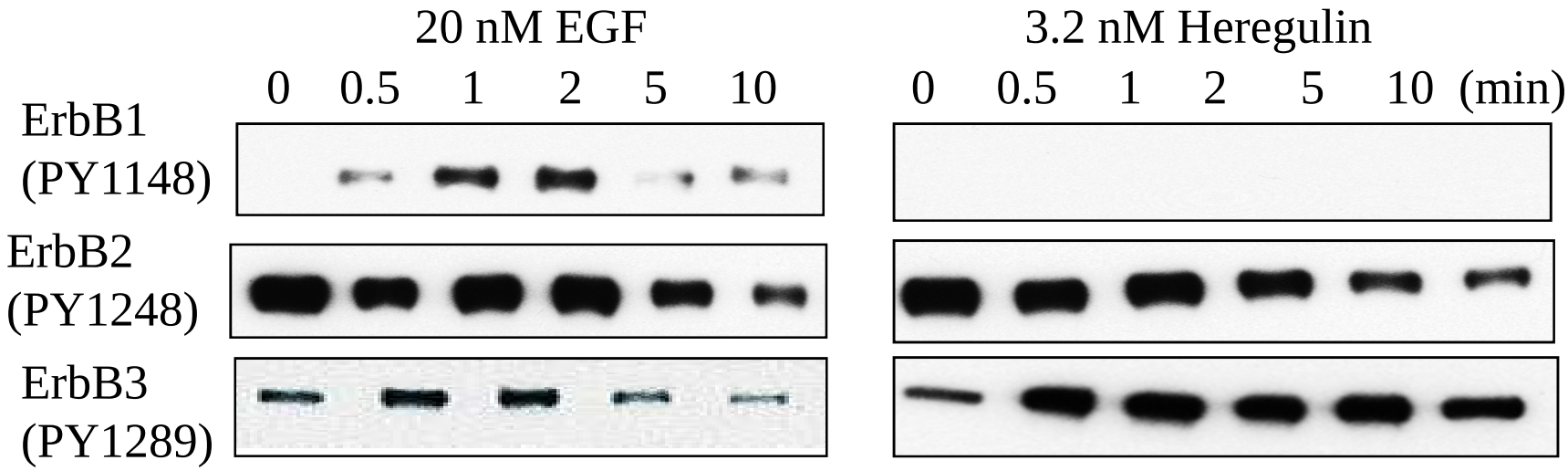


Obtain coordinates of 5 nm Gold.



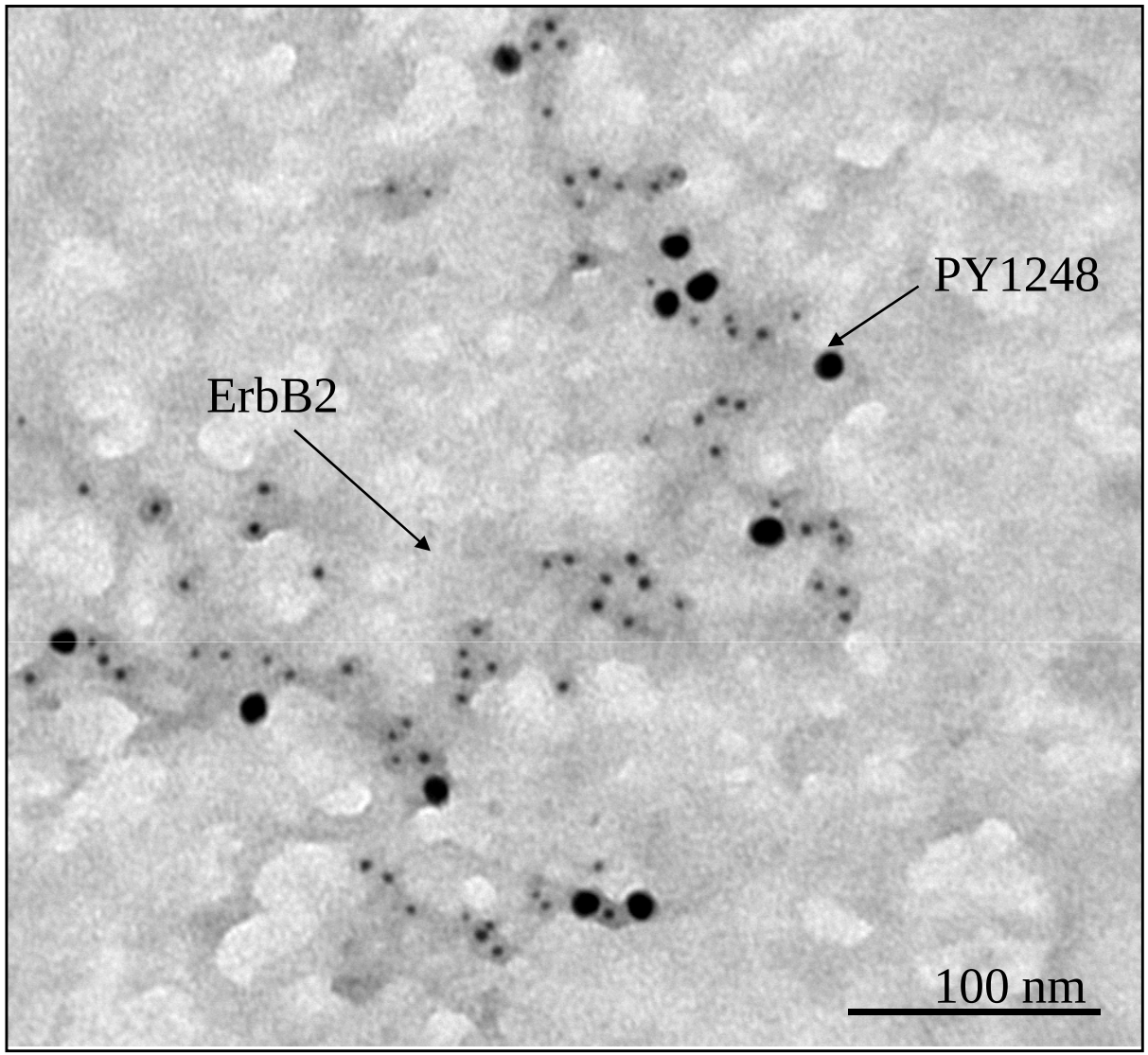
See Jun Zhang poster

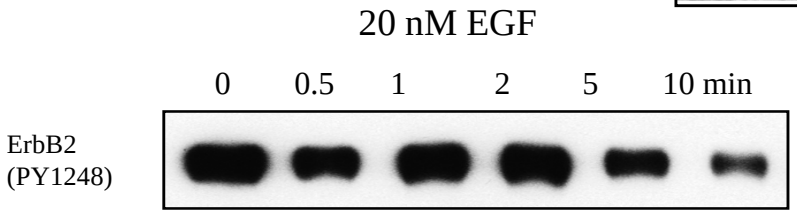
We started these studies in a cell line that express ErbB2 >> EGFR>Erb3 (measured)



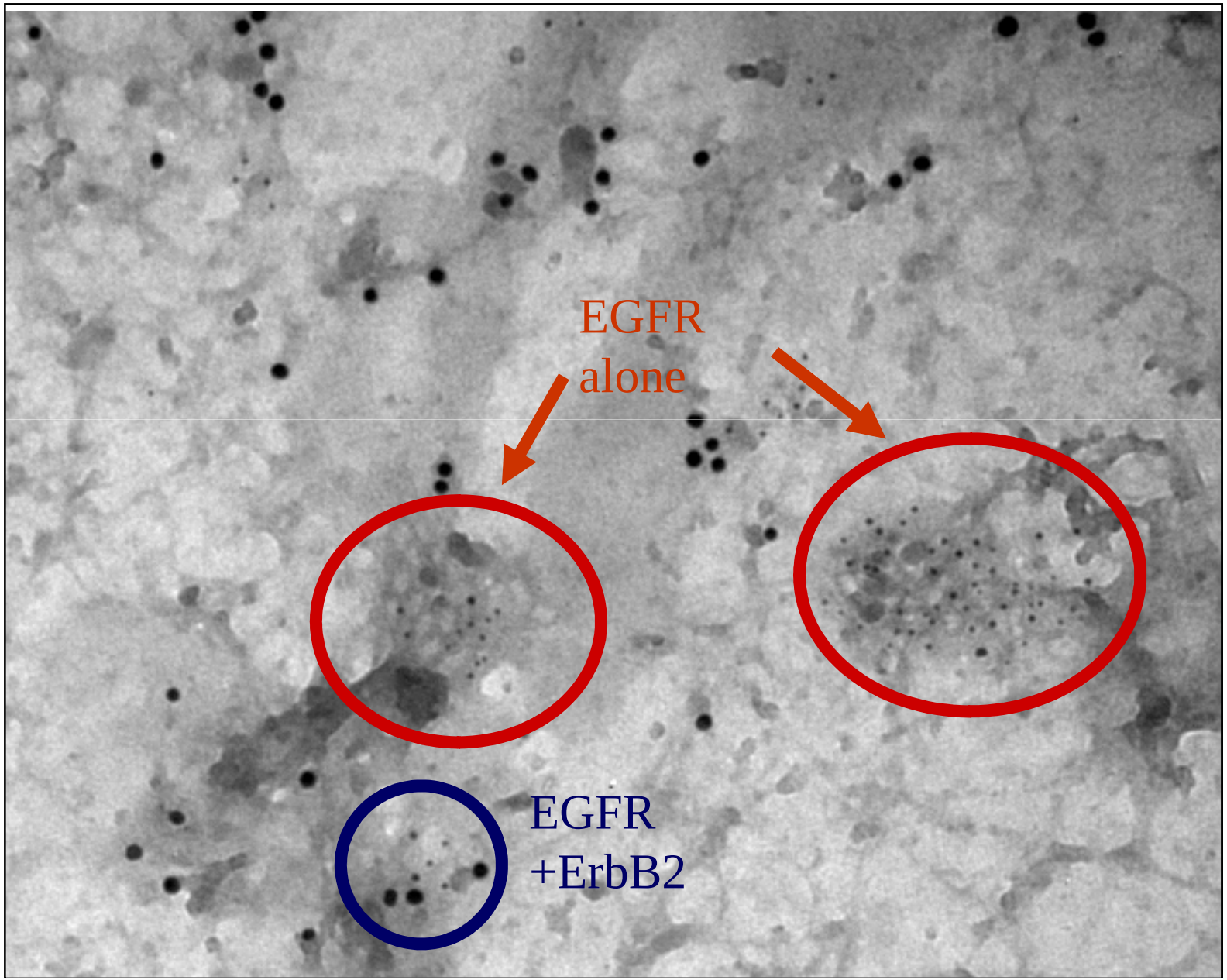
This is consistent with overexpression alone causing ErbB2 activation – a bad outcome for breast & other epithelial cancers.

ErbB2 is preclustered & active in serum-starved cells

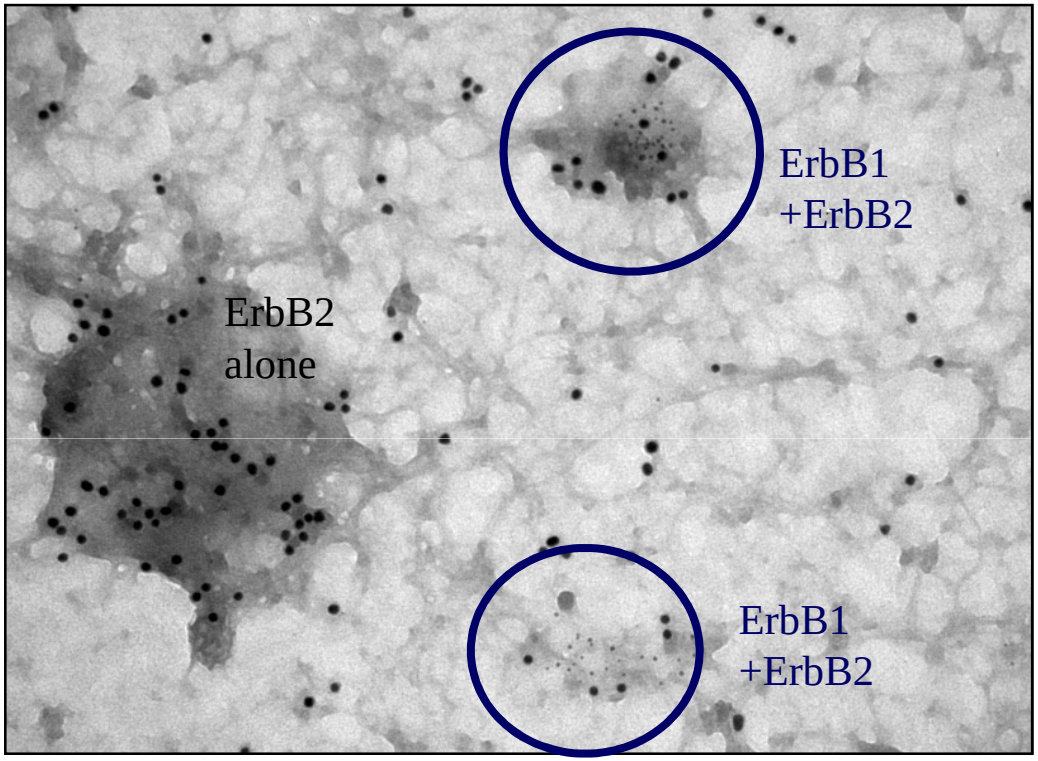




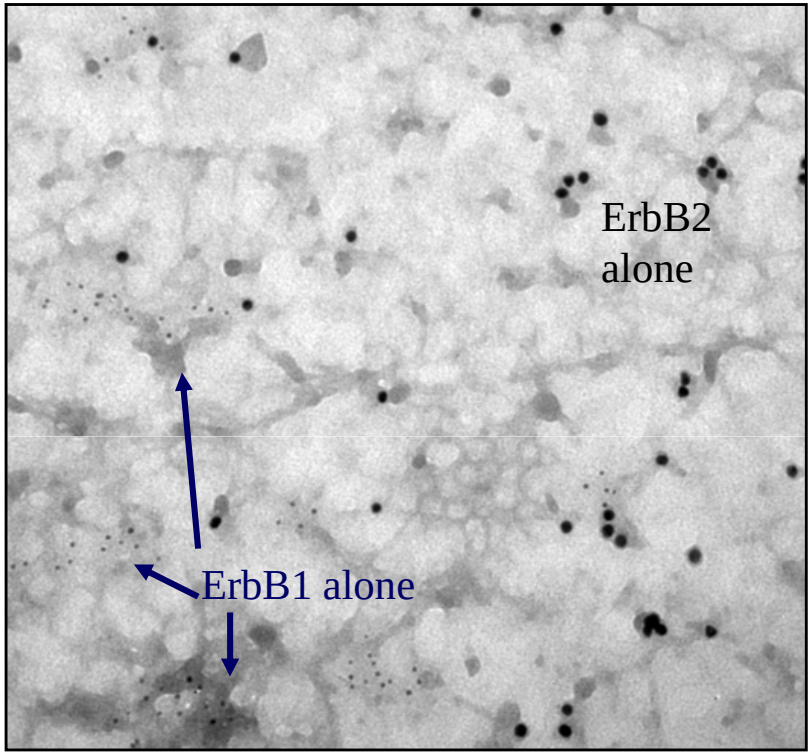
In resting membranes, there is sparse co-localization of EGFR & ErbB2
(typically fails statistics test)



After 2' EGF, there is slightly more co-localization of EGFR & ErbB2 but most images fail statistics test for co-clustering



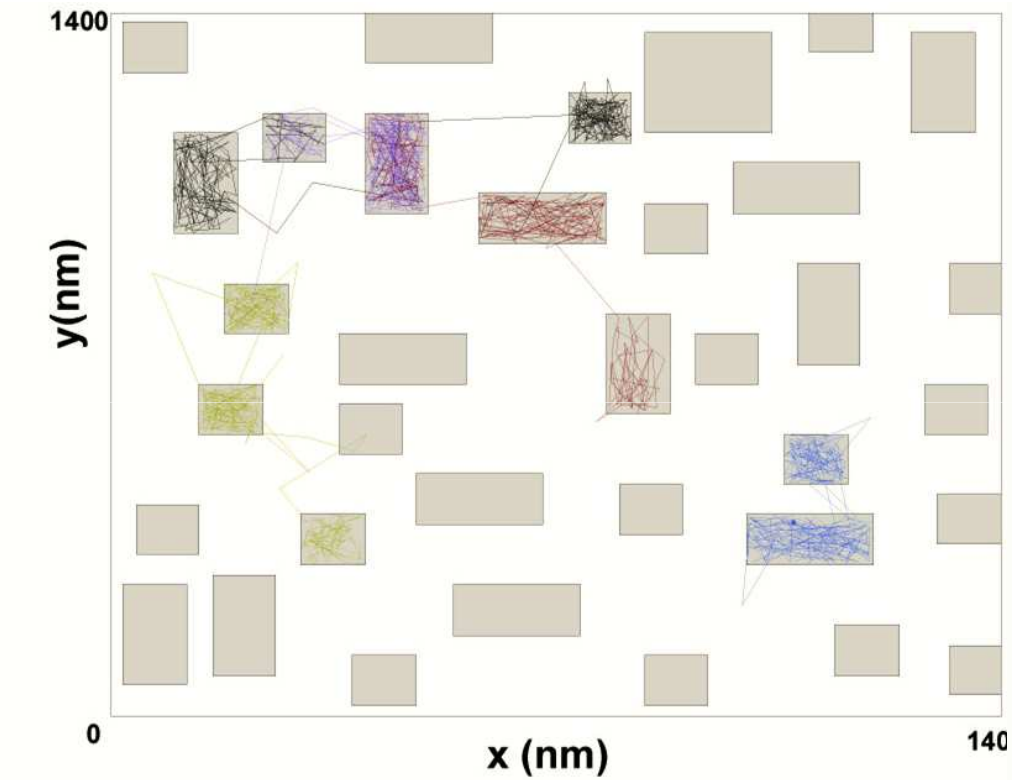
PASS (30%)

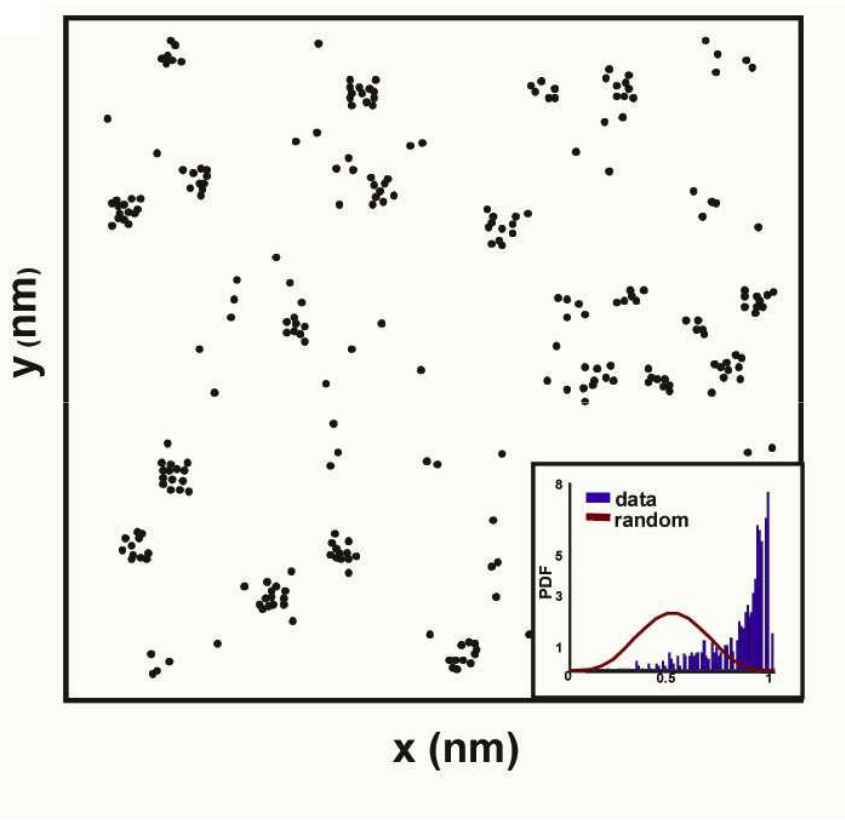


FAIL (70%)

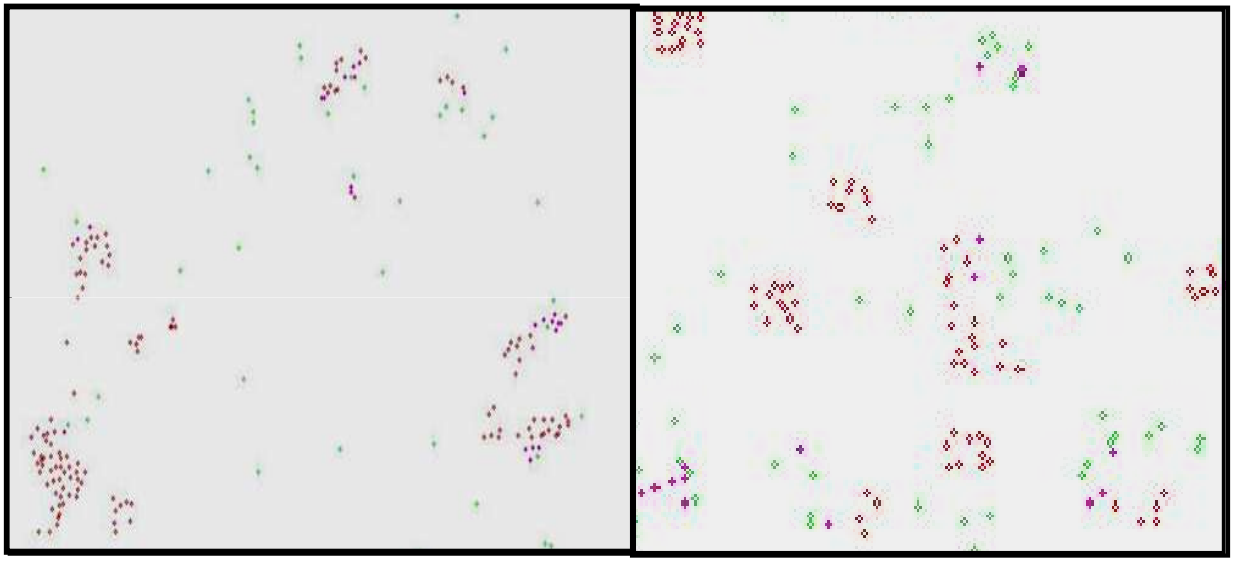
These data suggest that homodimers, not heterodimers, predominate.

To test this by simulation, we used our agent based model.





Simulating EGFR & ErB2 cluster distributions based upon EM data

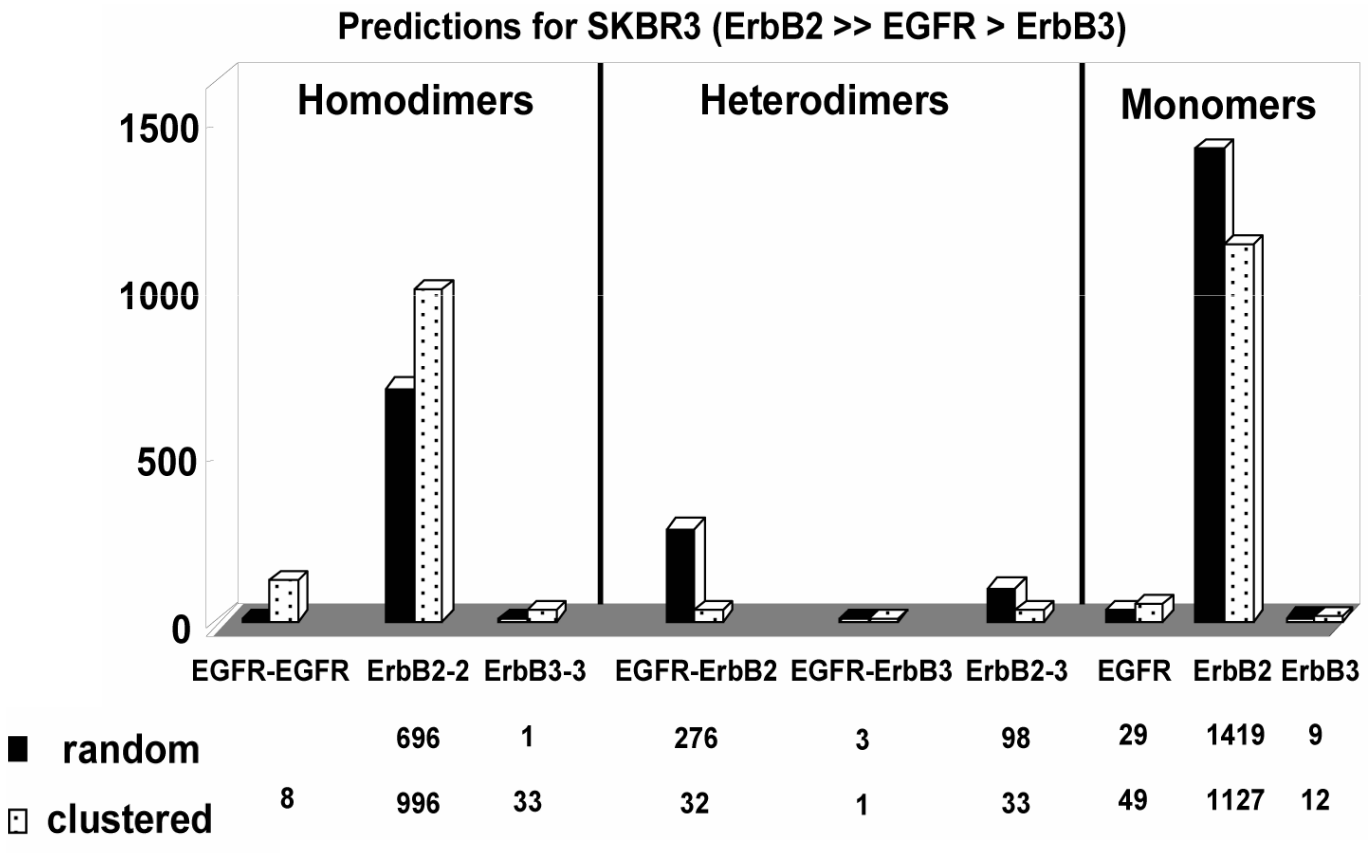


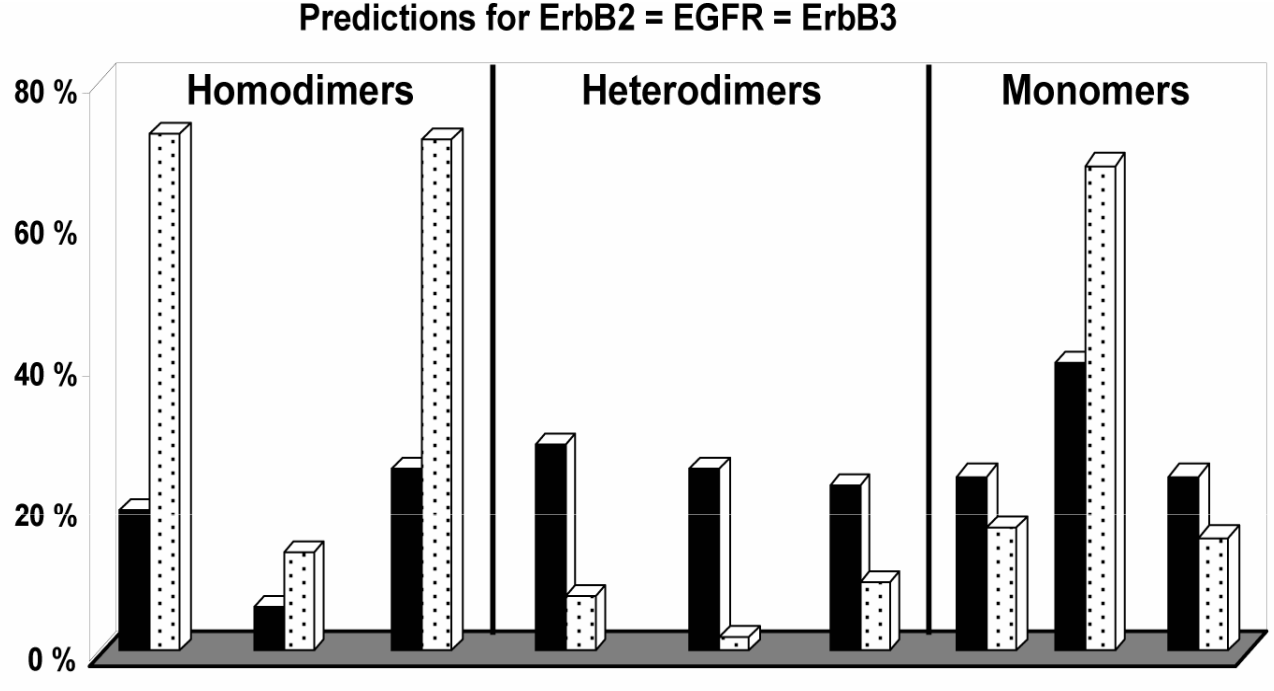
Experimental Data

Simulation

	Cluster size	% coclustering
exp	8.53	12.83%
sim	8.59	13.17%

Agent-based simulations predict significant differences in homo- and hetero-dimerization patterns when comparing spatial stochastic results with well mixed deterministic approach

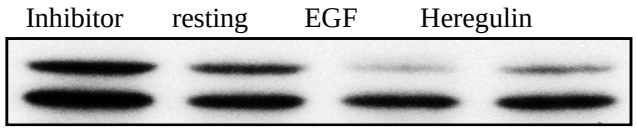




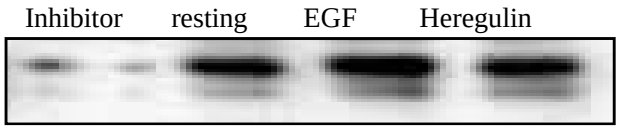
	EGFR-EGFR	ErbB2-2	ErbB3-3	EGFR-ErbB2	EGFR-ErbB3	ErbB2-3	EGFR	ErbB2	ErbB3
■ random	20.13%	6.29%	25.79%	29.25%	25.79%	23.58%	24.84%	40.88%	24.84%
▯ clustered	72.96%	13.84%	72.33%	7.86%	1.89%	9.75%	17.30%	68.55%	16.04%

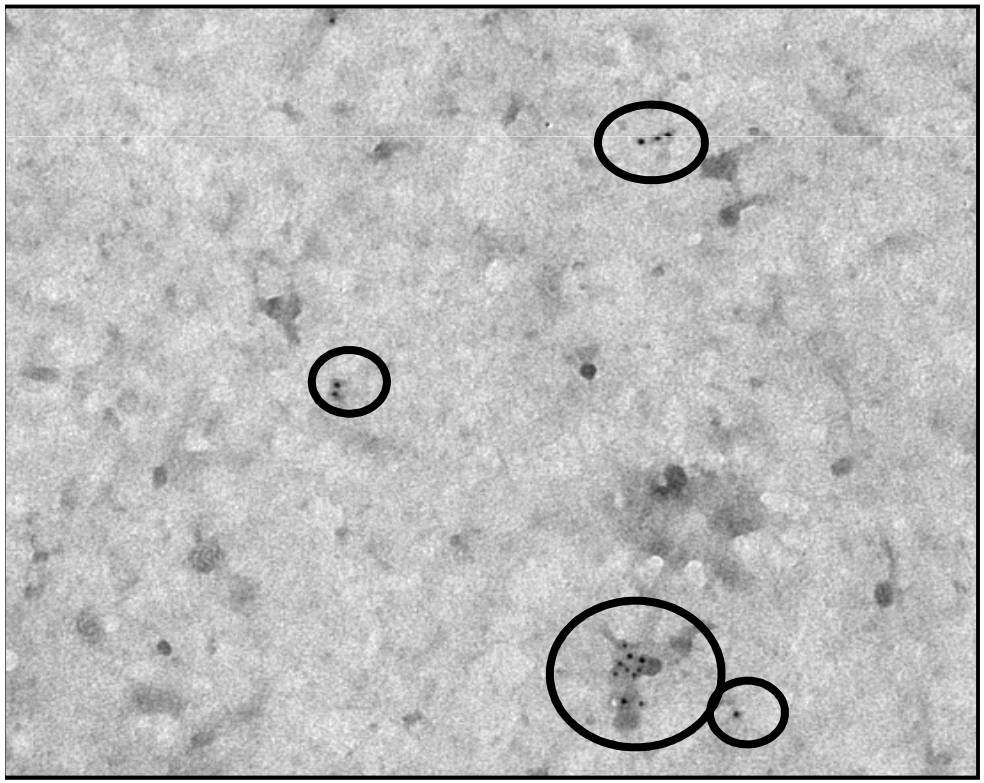
In our 3D agent based model, we explicitly consider individual diffusing receptors and their adaptors. We will make use of our unique data sets. For example, using EM, we can spatially map and quantify recruitment of adaptor molecules.

Shc in cytosol

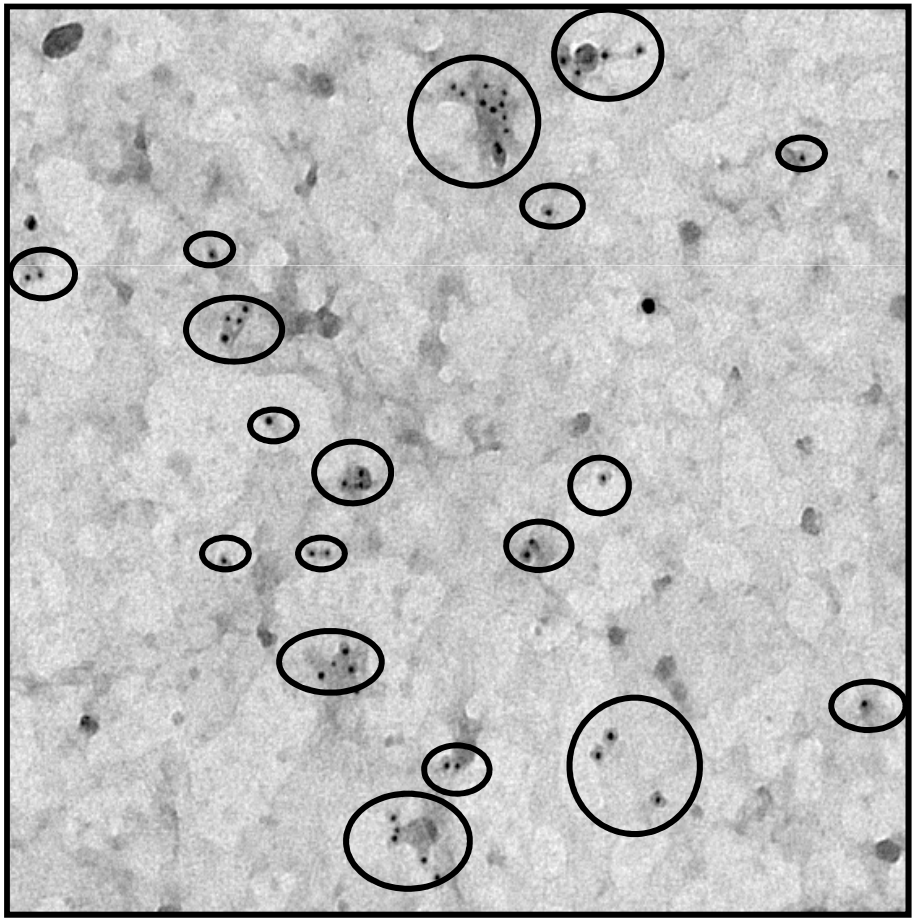


Shc on membranes



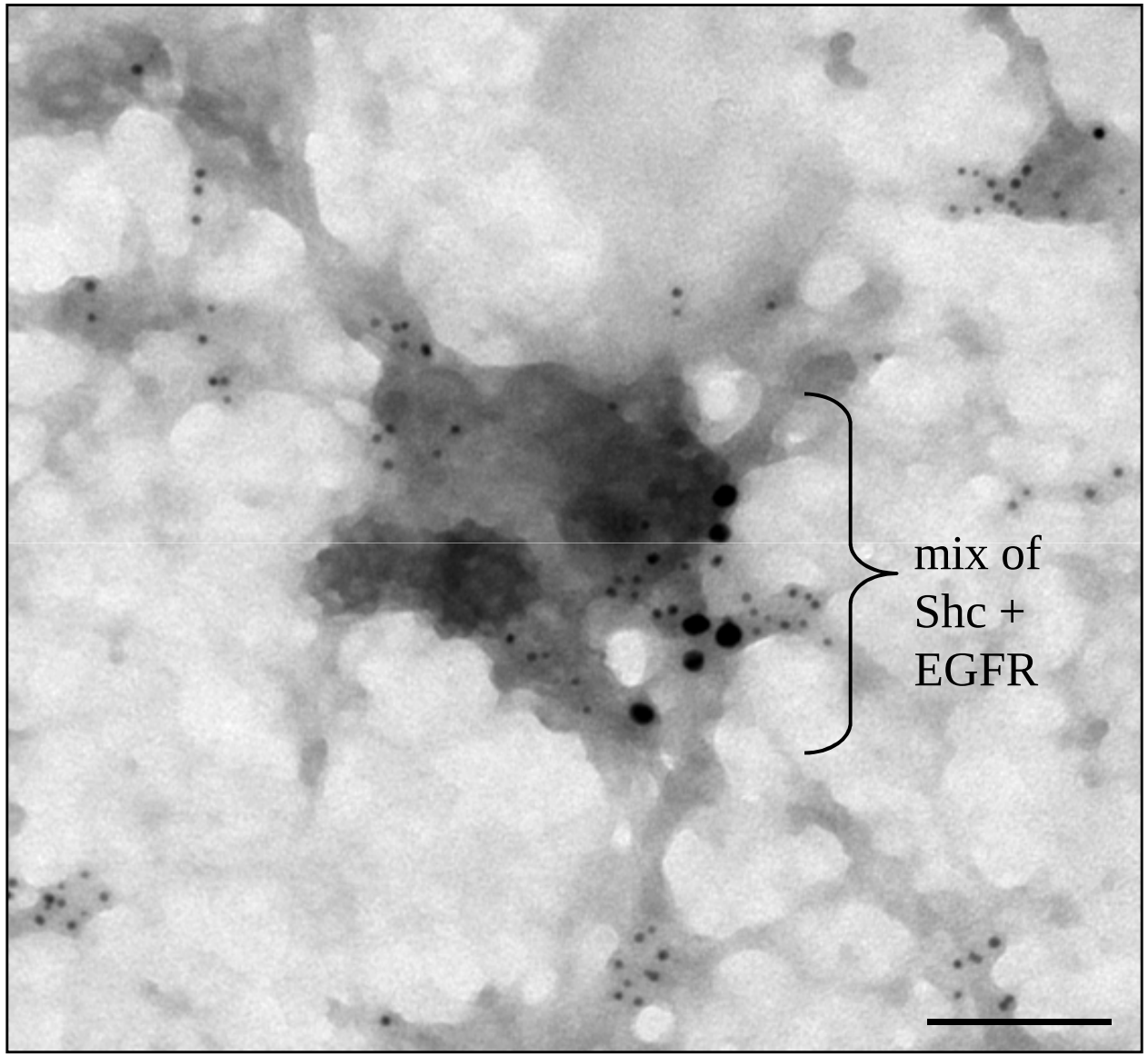


Resting: 17 particles/sq micron



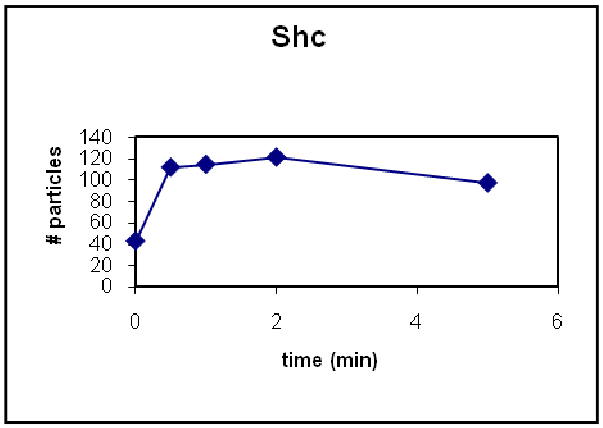
EGF 2': 71 particles/sq micron

As expected, Shc is found with activated EGFR



Examples of data from EGF activated A431 cells

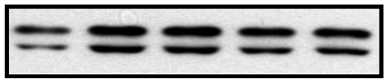
EM data

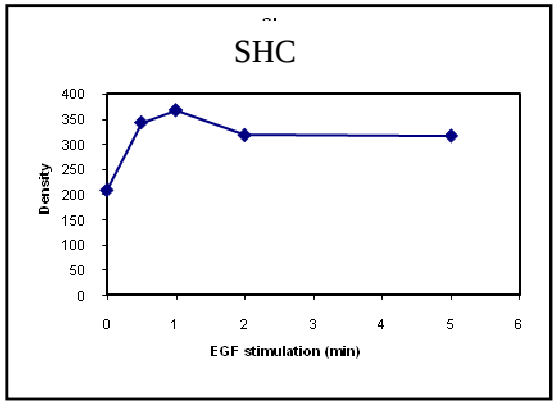


IP EGFR

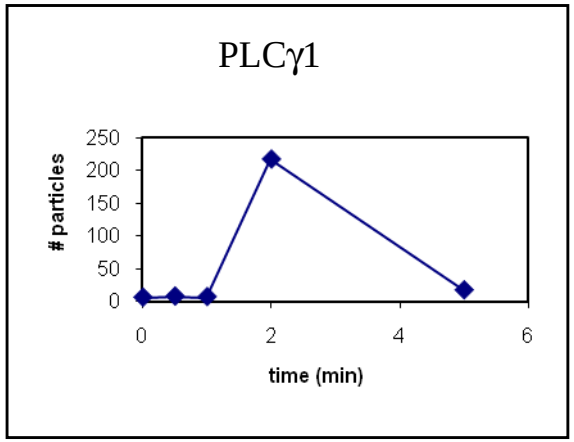
EGF 0 30” 1’ 2’ 5’

Blot Shc





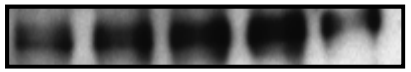
EM data

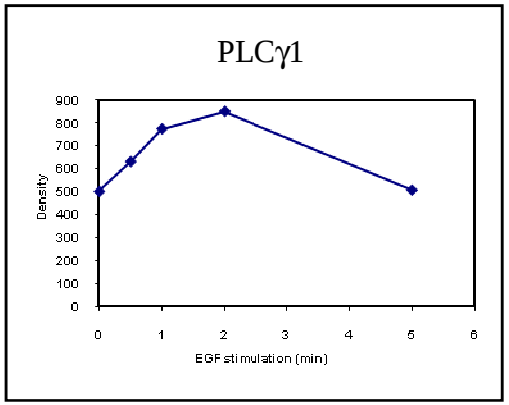


IP EGFR

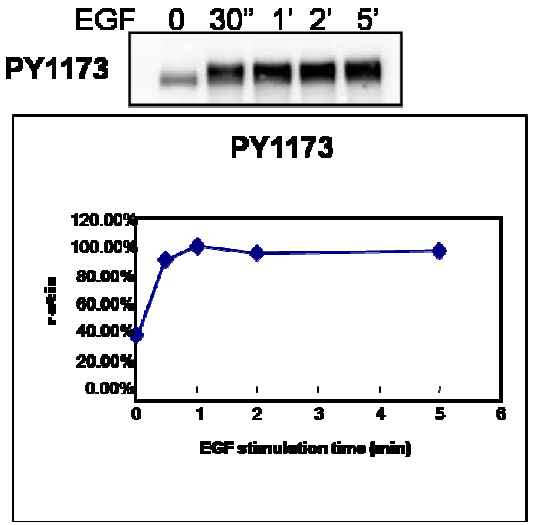
EGF 0 30” 1’ 2’ 5’

Blot PLCγ1

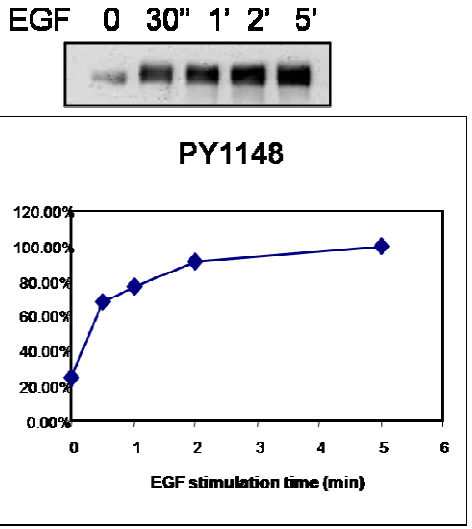




Typical for 992, 1069, 1173



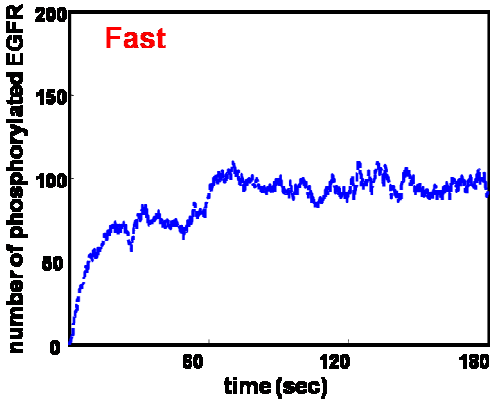
fast

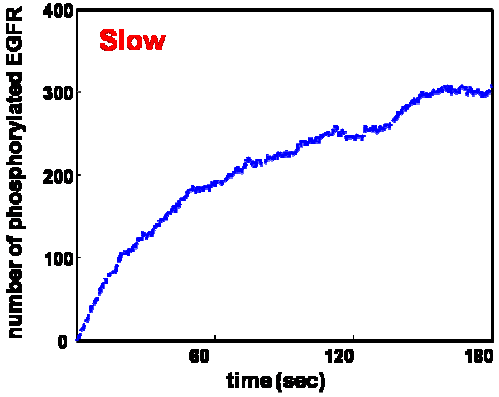


slow

One goal is to evaluate the impact of membrane topography on signal propagation in our agent based model.

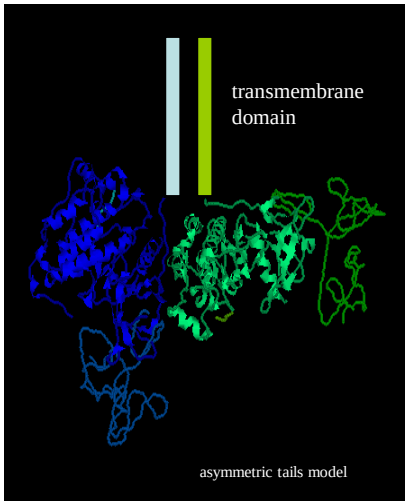
Since adaptor proteins dock on specific phosphotyrosines, we need to document and simulate kinetics of phosphorylation.

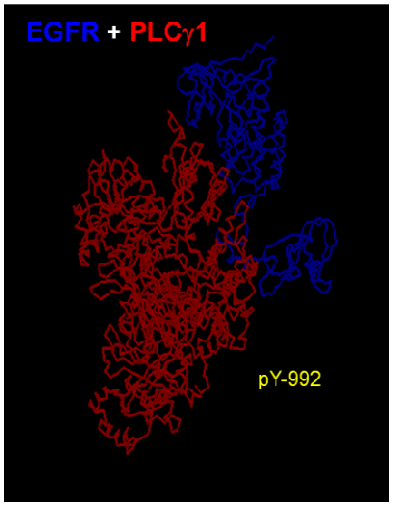


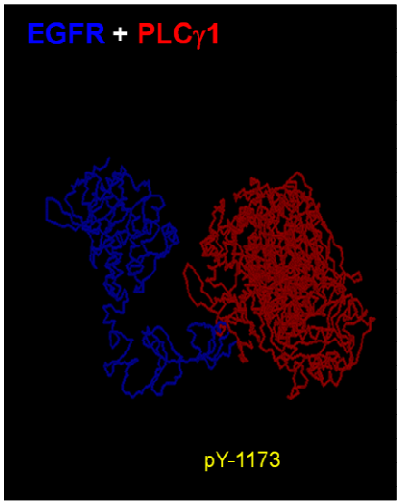


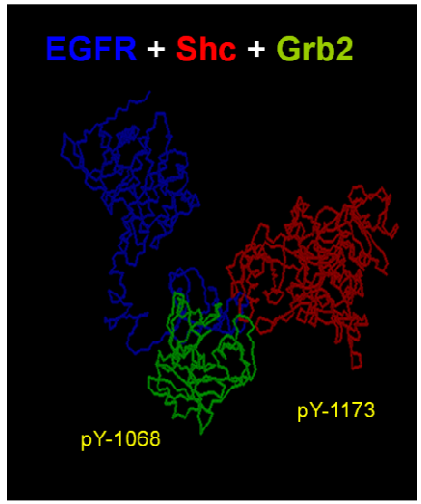
•1592 EGFR in 0.49 μm^2 simulated space (4 million per A431 cell) with 20 nM EGF
• incorporate phosphorylation and dephosphorylation rate constants
• parameter fitting using PottersWheel

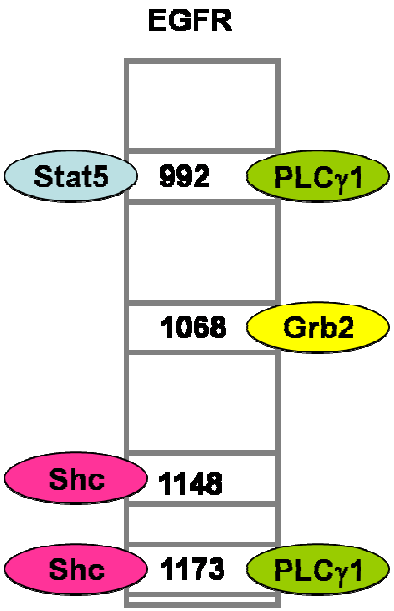
To establish docking "rules", we used coarse grain molecular docking methods







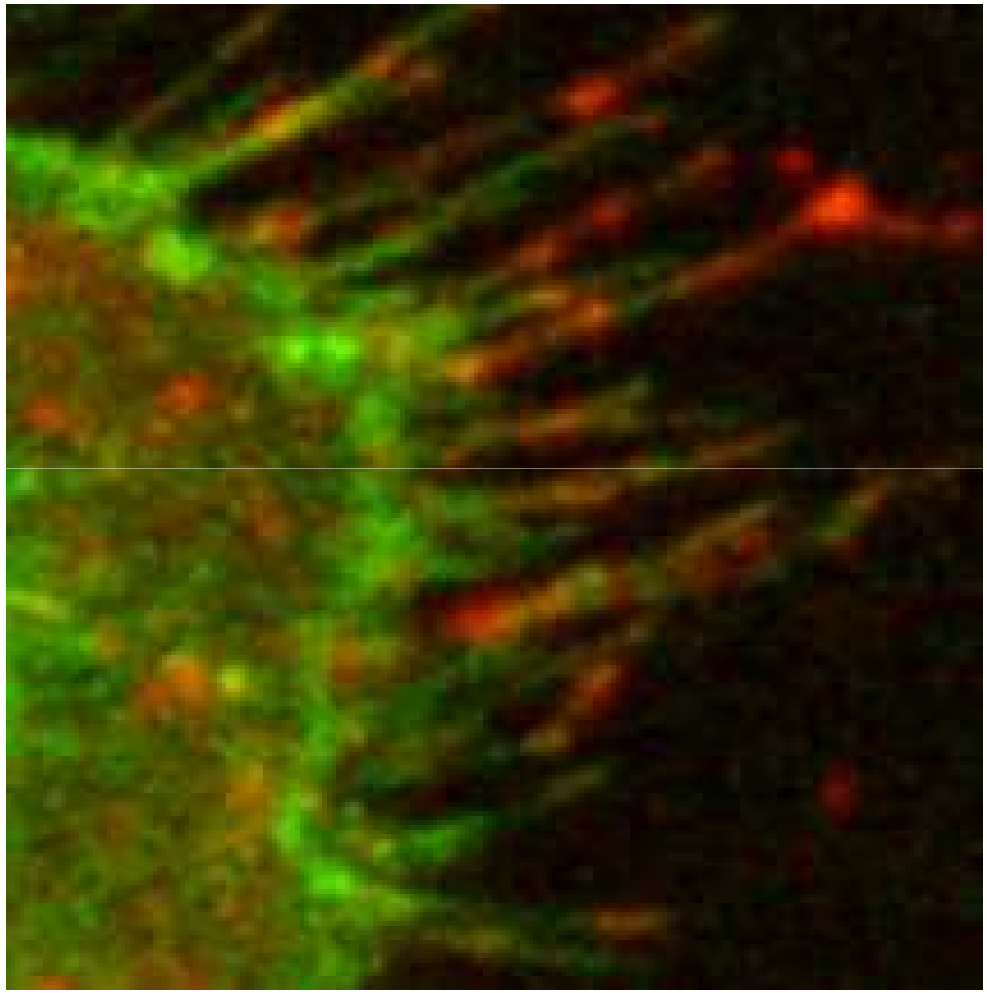




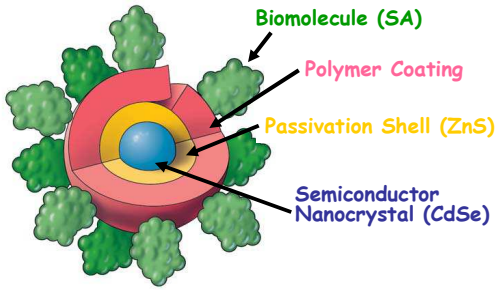
	Stat5 (PY992)	PLCγ1 (PY992)	PLCγ1 (PY1173)	Shc (PY1148)	Shc (PY1173)	Grb2 (PY1068)
Stat5 (PY992)			+	+	+	+ +
PLCγ1 (PY992)			X	+	+	+
PLCγ1 (PY1173)	+	X		X	X	+
Shc (PY1148)	+	+	X		+ +	+ +
Shc (PY1173)	+	+	X	+ +		+ +
Grb2 (PY1068)	+ +	+	+	+ +	+ +	

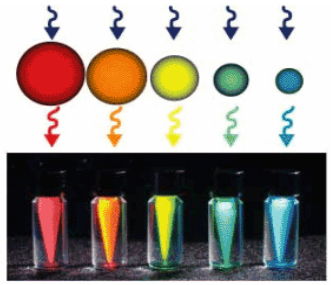
These predictions need to be tested experimentally. We are now running simulations, using values for proteins based on quantitative flow, western blotting.

Studying receptor diffusion with Quantum Dot Probes



EGF-bound receptors travel towards the cell biology down filopodia
Lidke et al., Nature Biotechnology 2005

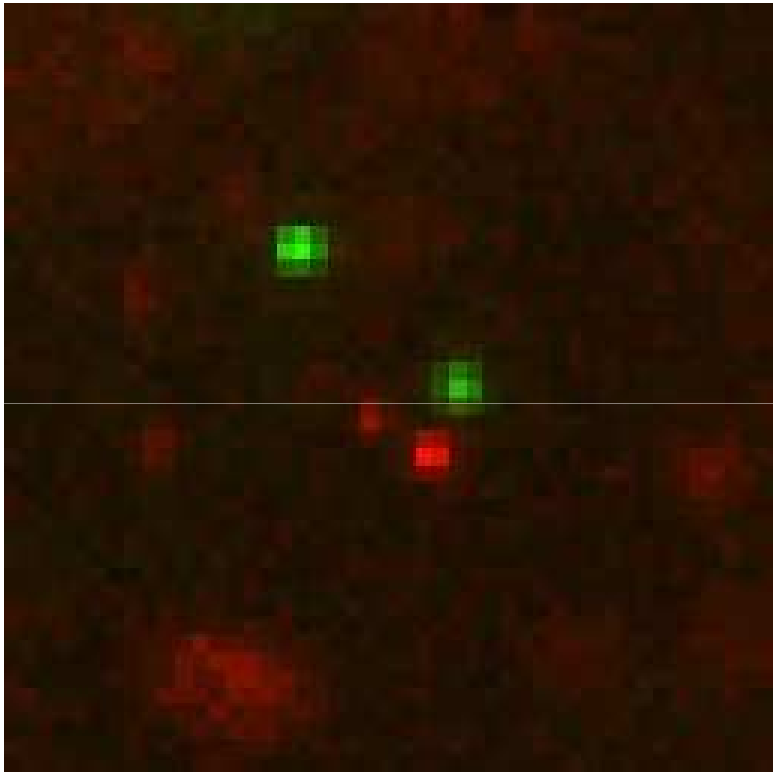




EGF-QD655

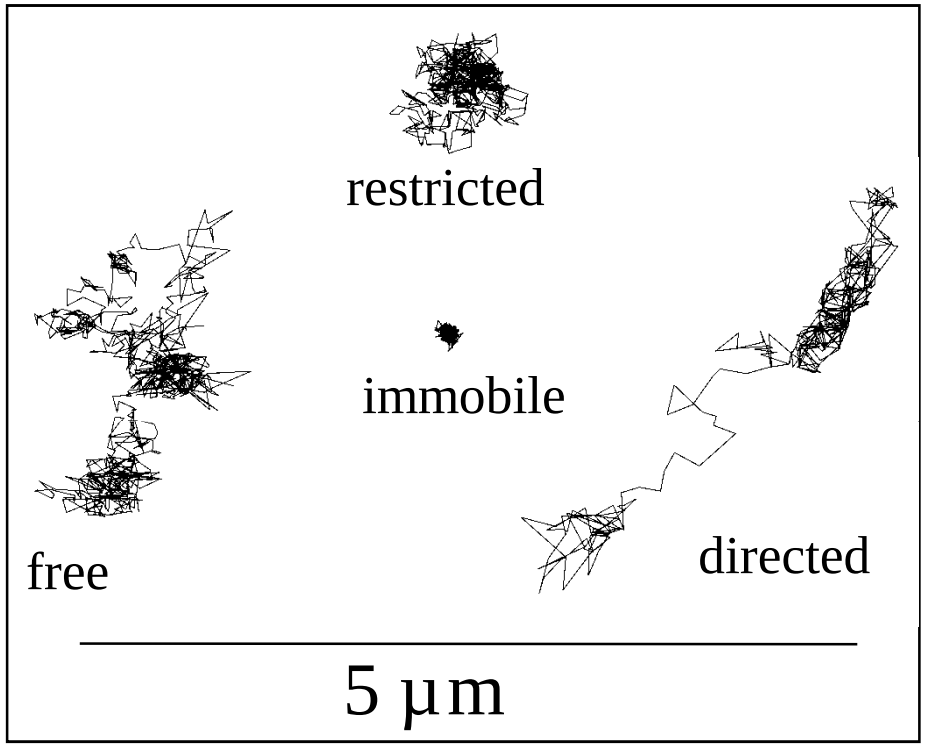
Commercial sources: Quantum Dot Inc., Evident Technologies

Single Particle Tracking shows diverse modes of motion for individual (resting) IgE receptors



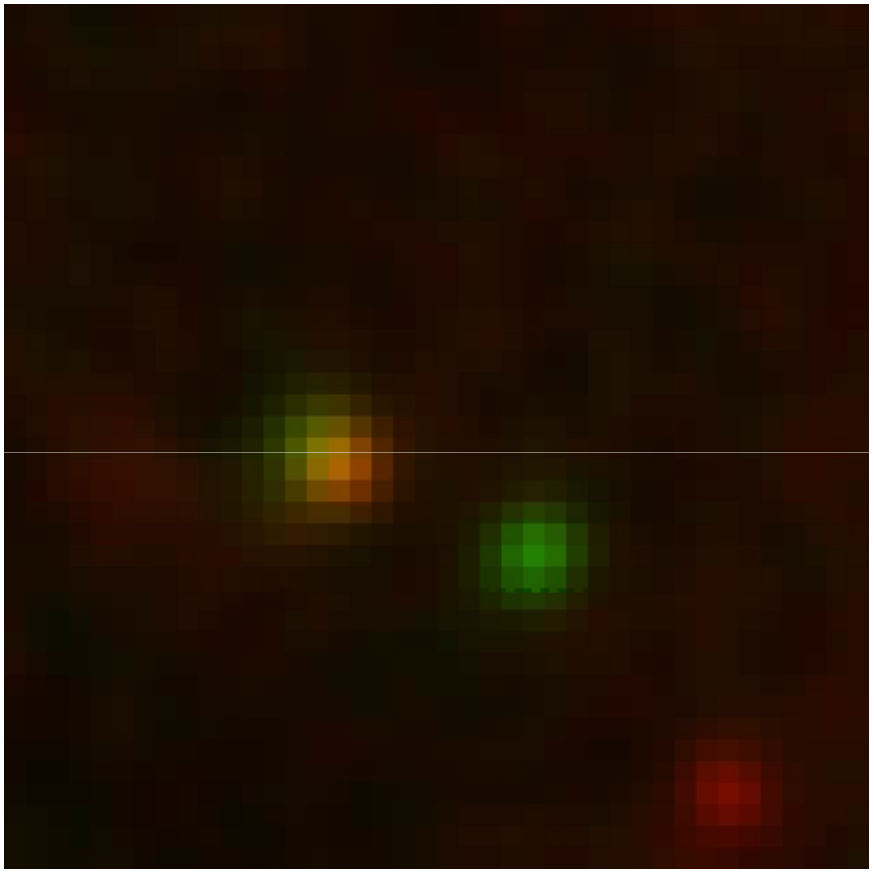
QD-IgE

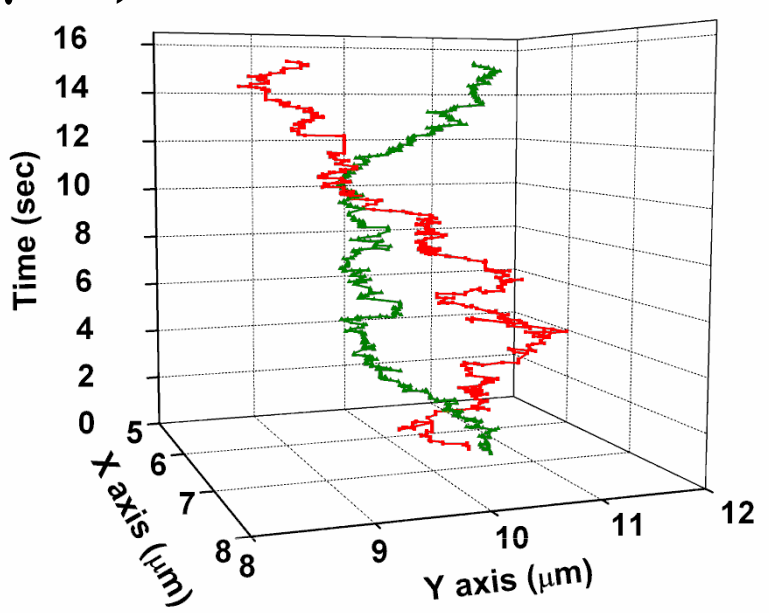
1 pixel = 0.267 µm
acquition 33 frames/sec
playback realtime

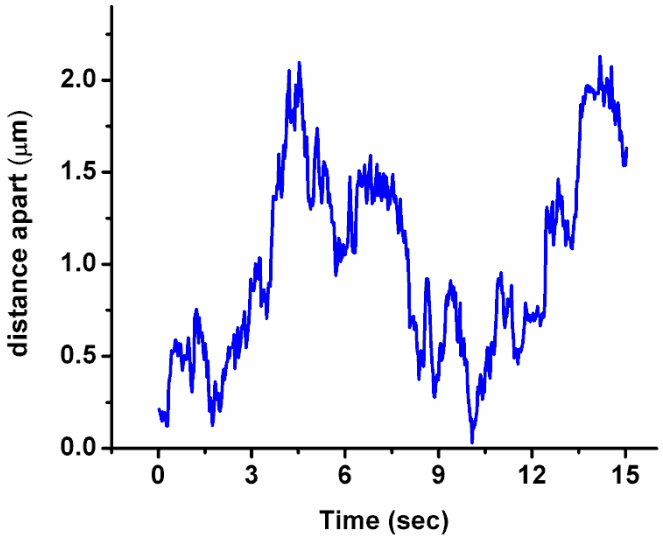


Andrews et al,
Nature Cell Biology 2008

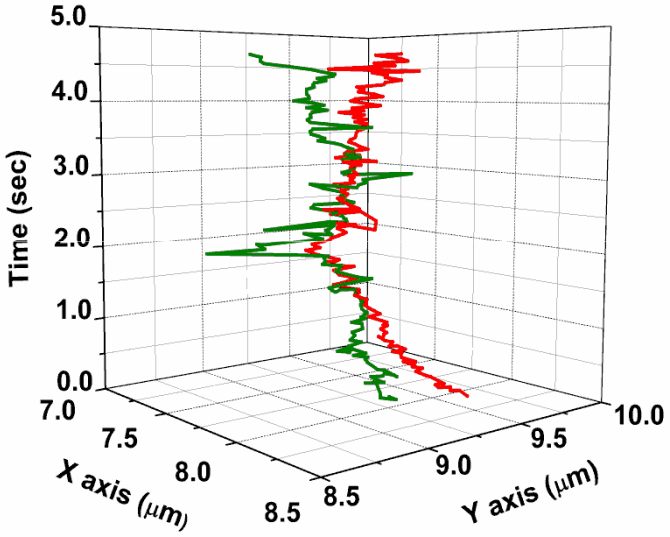
IgE receptors can repeatedly come together within the same large (1-2 μm) microdomain

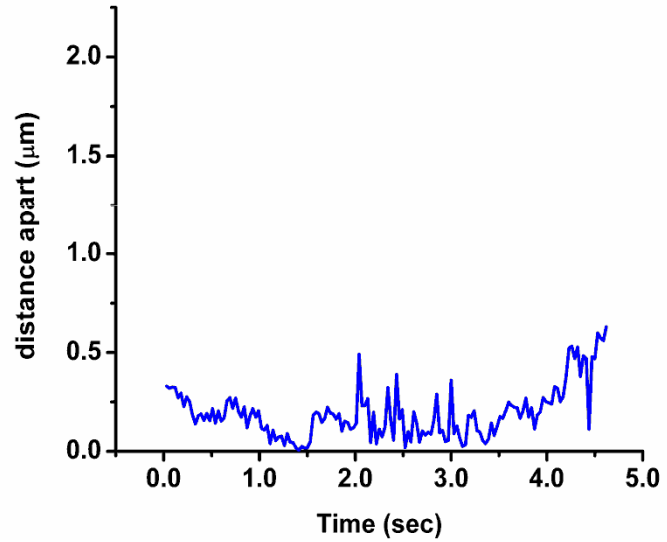




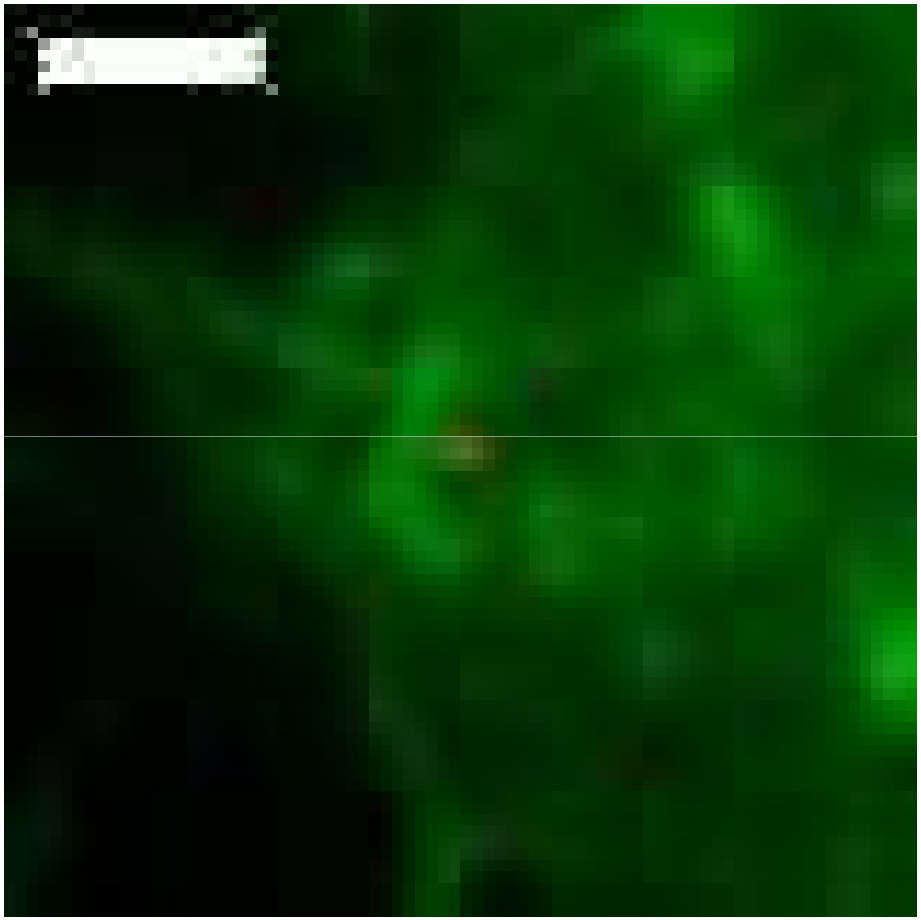


Mobile IgE receptors can also apparently occupy the same microdomain for a while





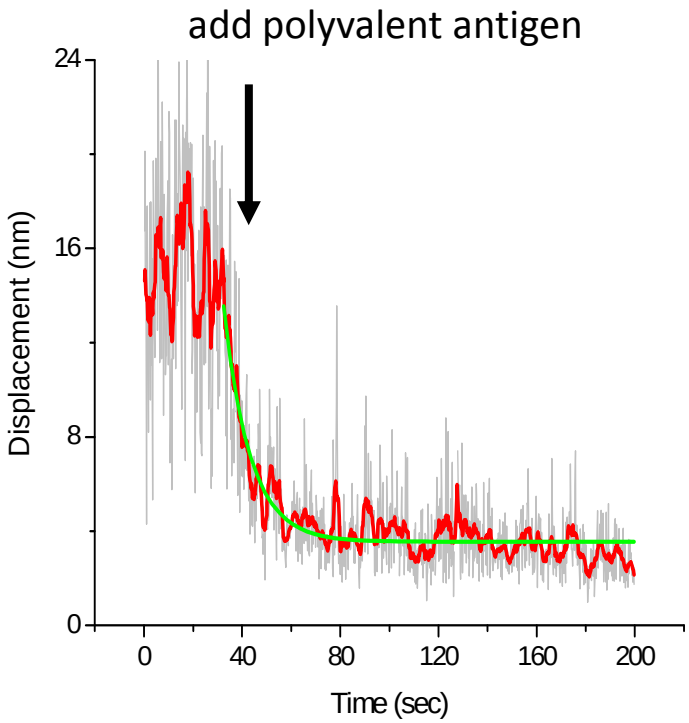
Monovalent QD probes follow diffusing resting receptors, show cytoskeletal corrals when GFP-actin is also imaged

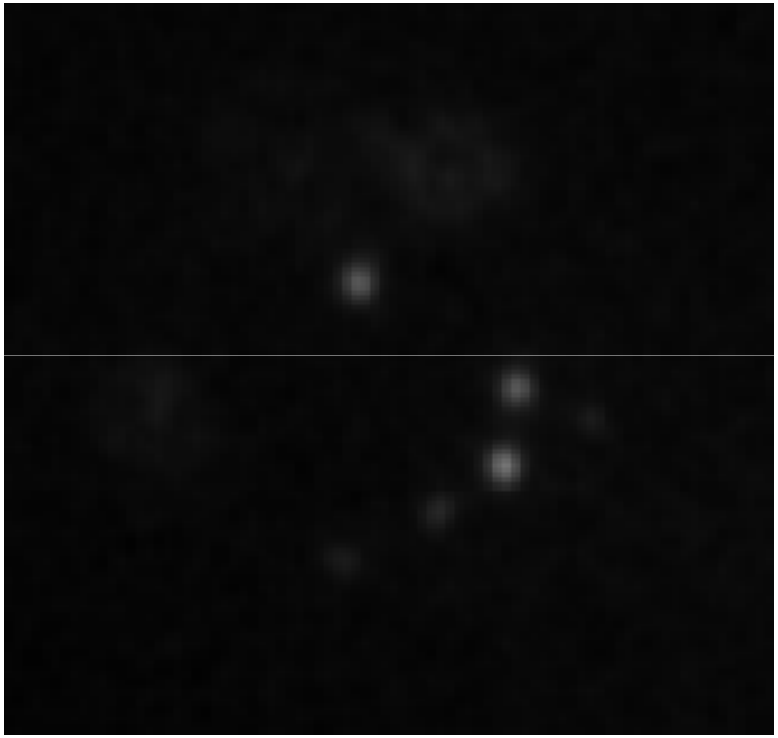


Andrews et al, *Nature Cell Biology*, 2008

35 frames/second IgE-QD/FcεRI

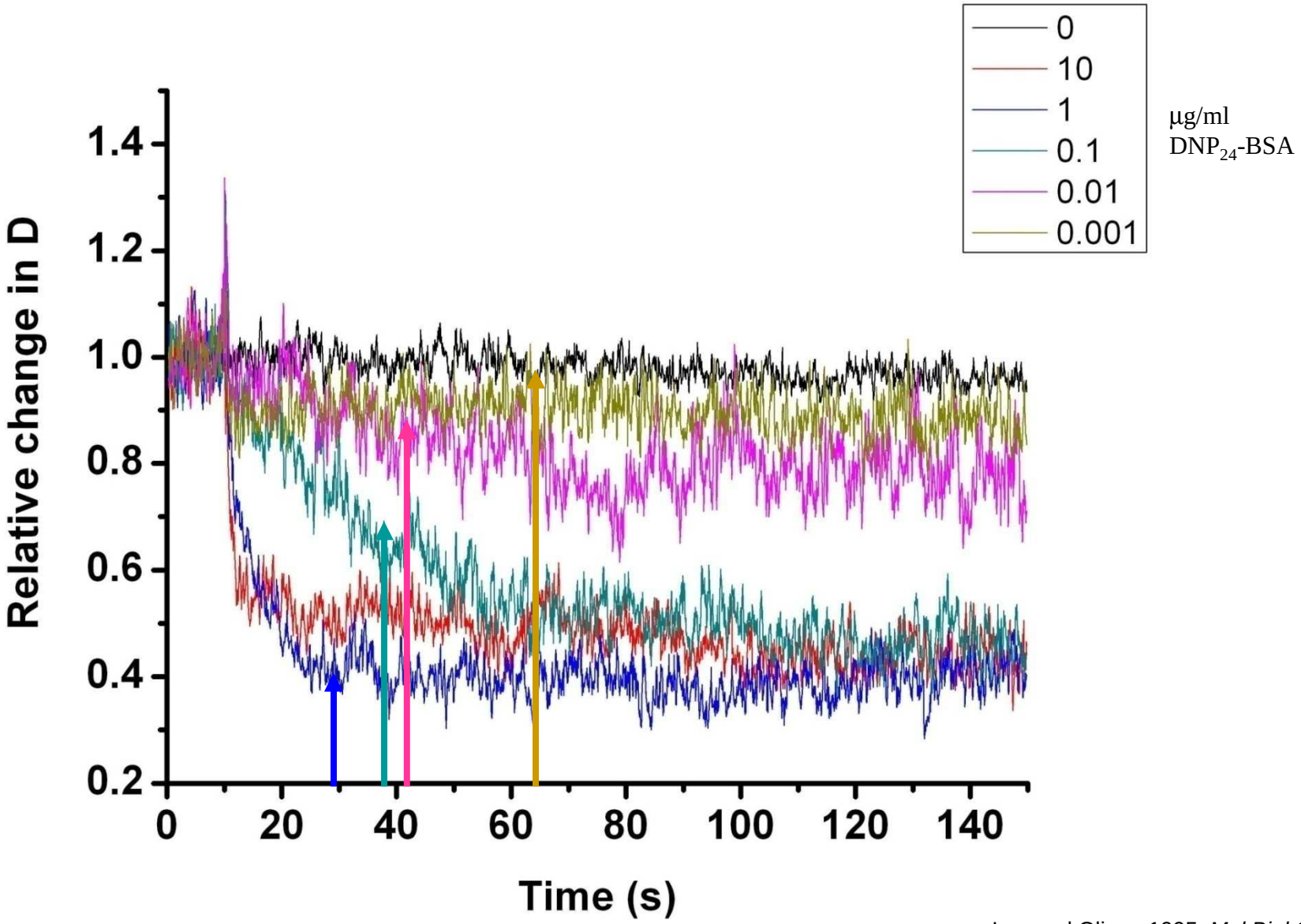
Fast immobility of FcεRI upon crosslinking





Andrews et al, Immunity in press

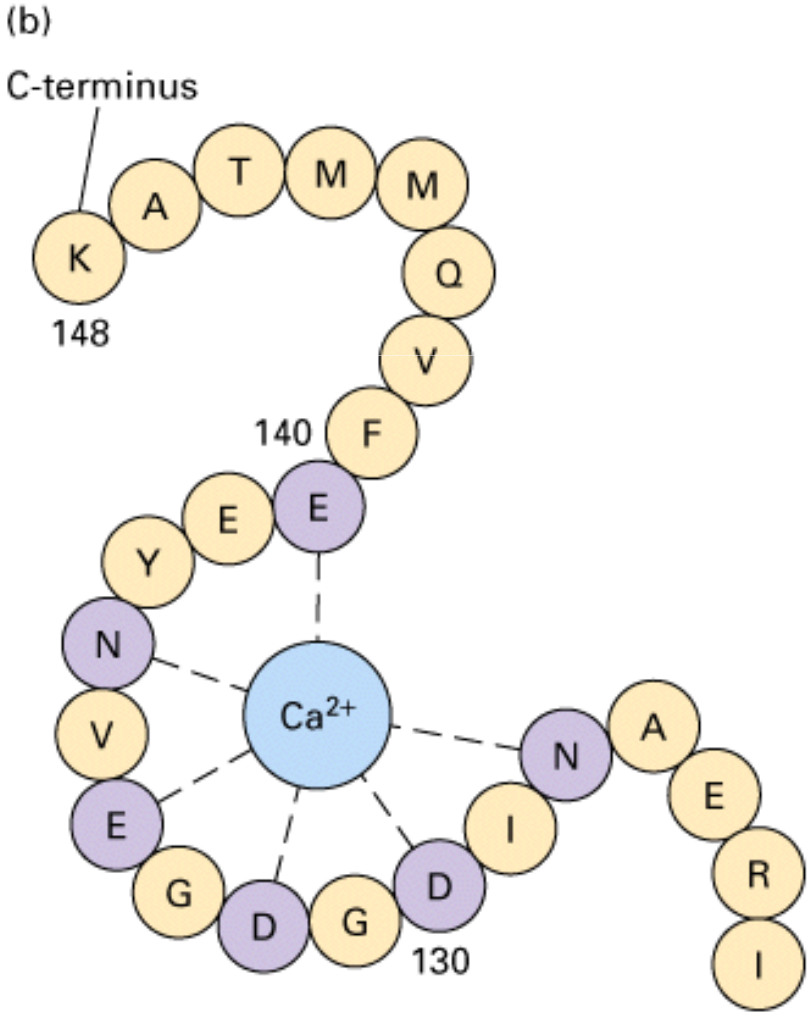
However, immobilization is not necessary for signal initiation – as indicated by calcium response

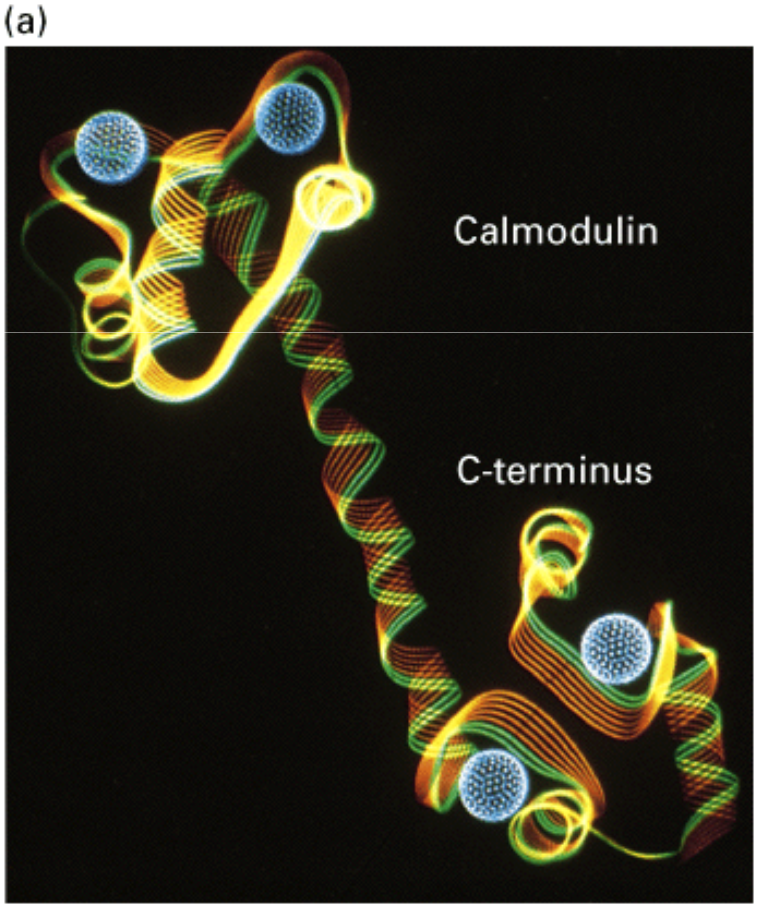


Lee and Oliver, 1995, *Mol Biol Cell*

Example 2: Modeling calcium fluxes in cells

Calcium is a 2nd Messenger & binds directly to proteins such as calmodulin.

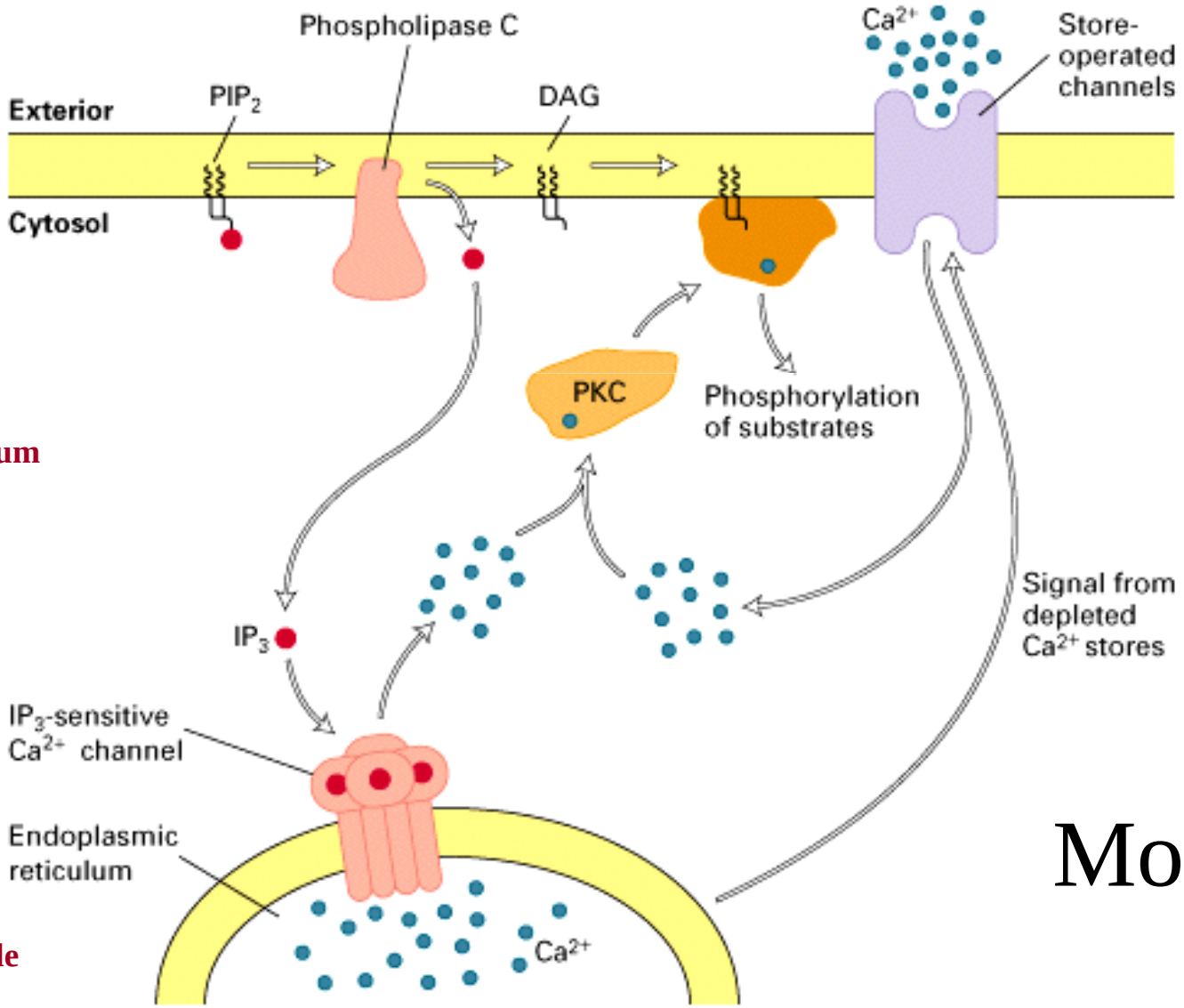




The cartoon version of calcium

("non-excitable" cells)

mM calcium outside

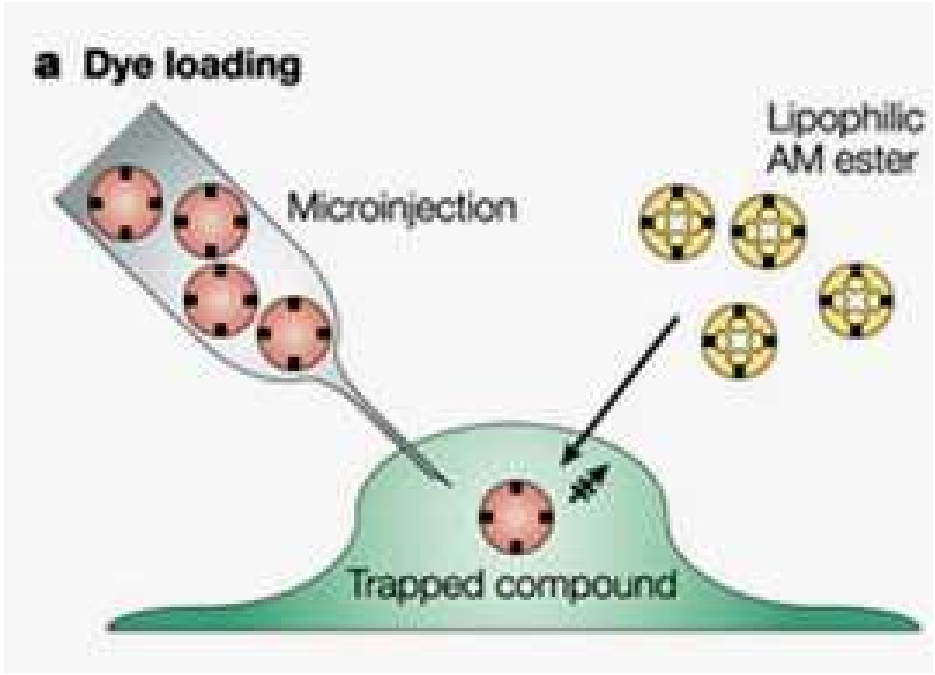


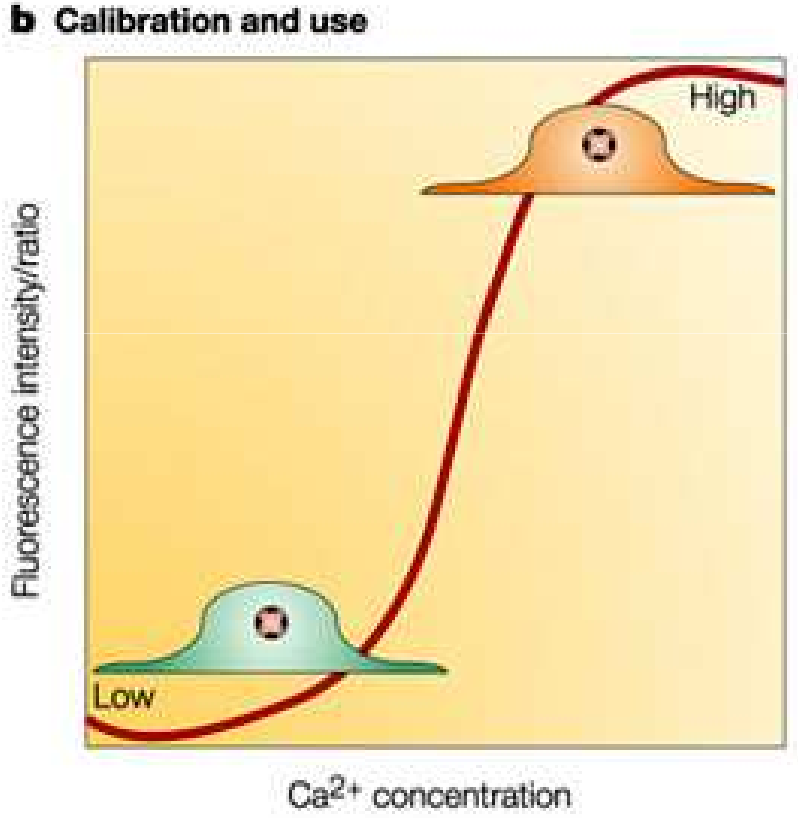
Cytosol: 100 nm calcium (resting)

ER: ~500µM calcium inside

Movie

How to measure [Ca2+]? ...typically use Fluorescent Probes

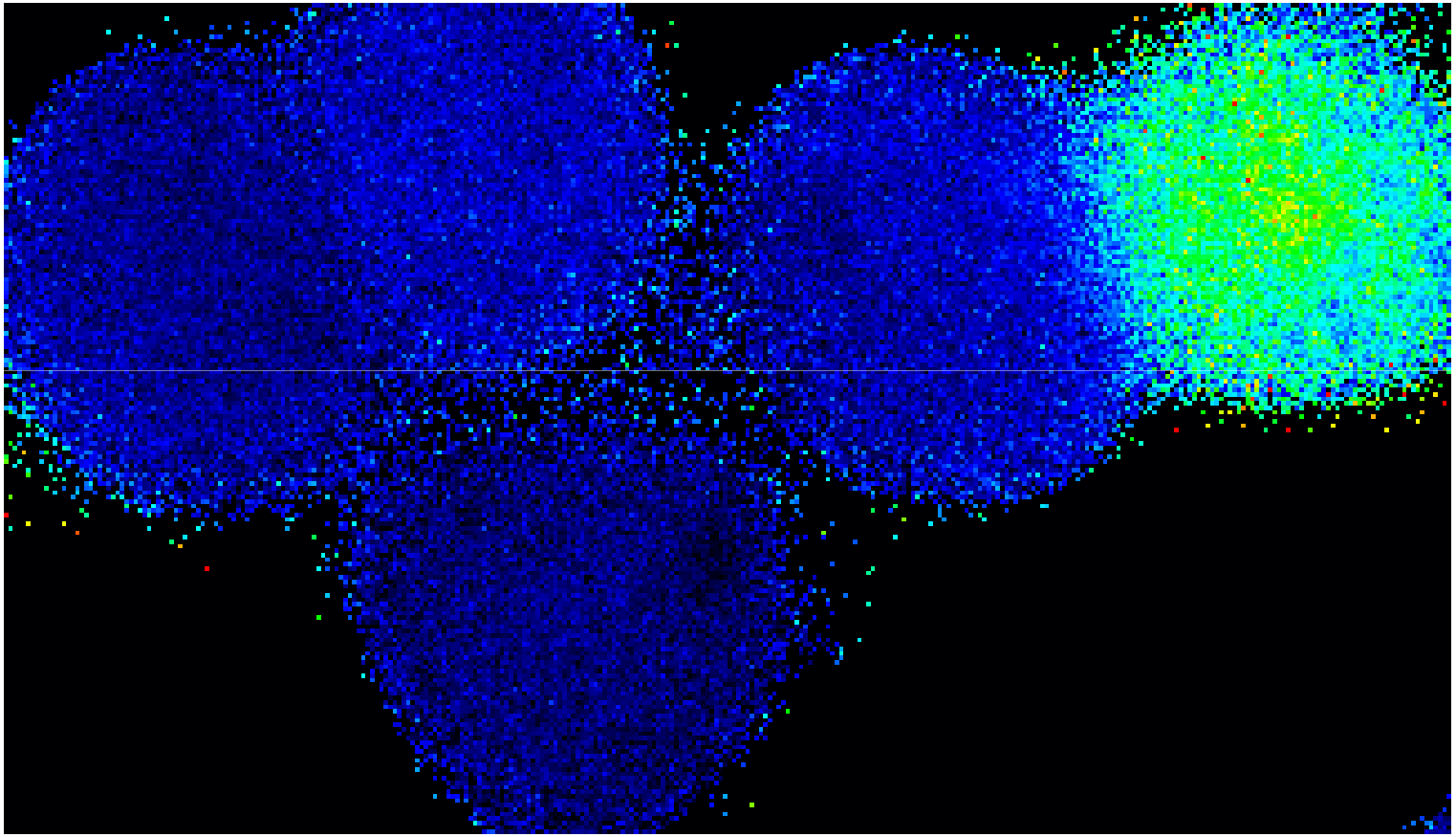




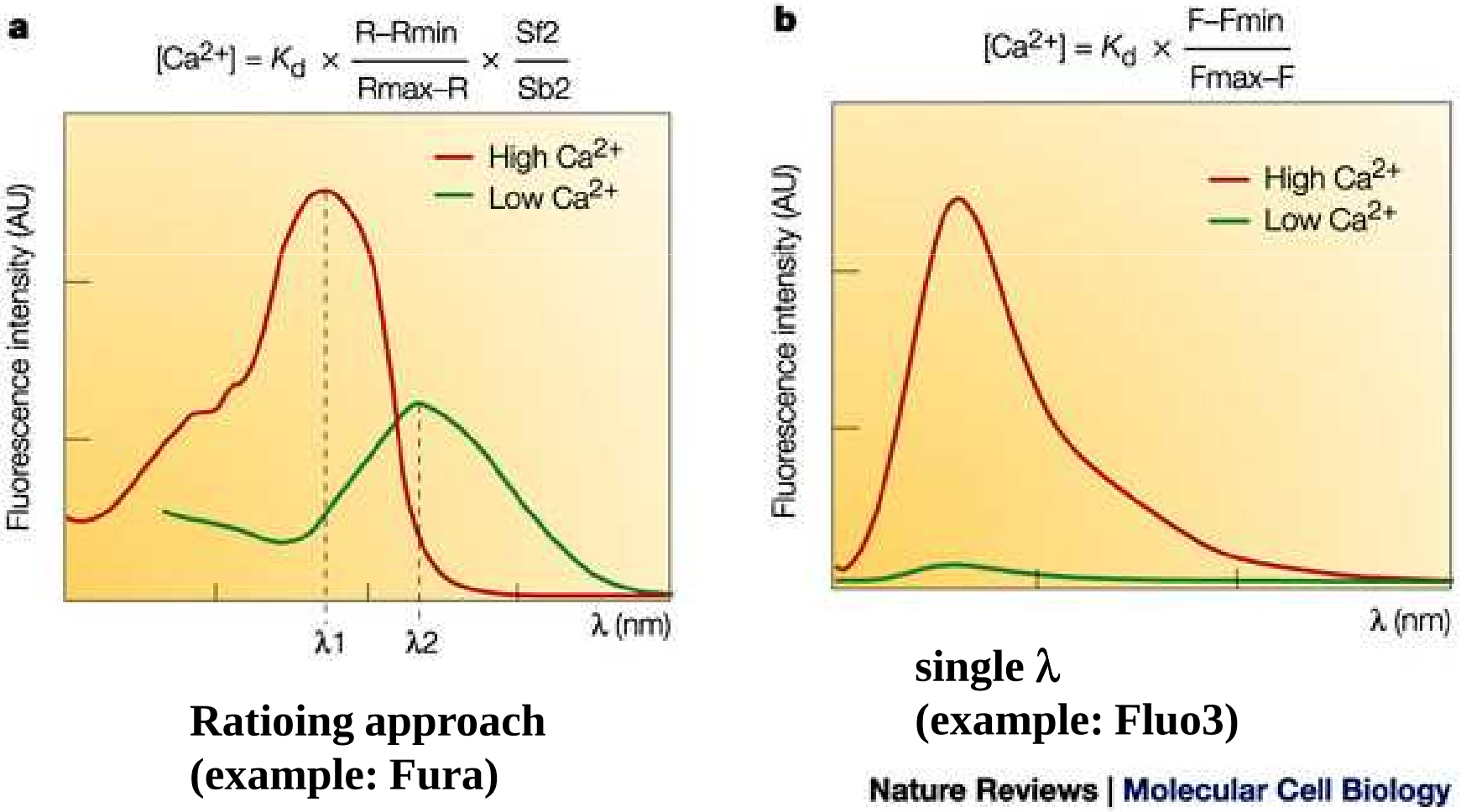
Movie

http://www.jcb.org/cgi/content/full/jcb.200206089/DC1/2

Imaging Live Cells



More on measurements...pick best dye for instrument you have access to.



Ratioing approach (example: Fura)

single λ (example: Fluo3)

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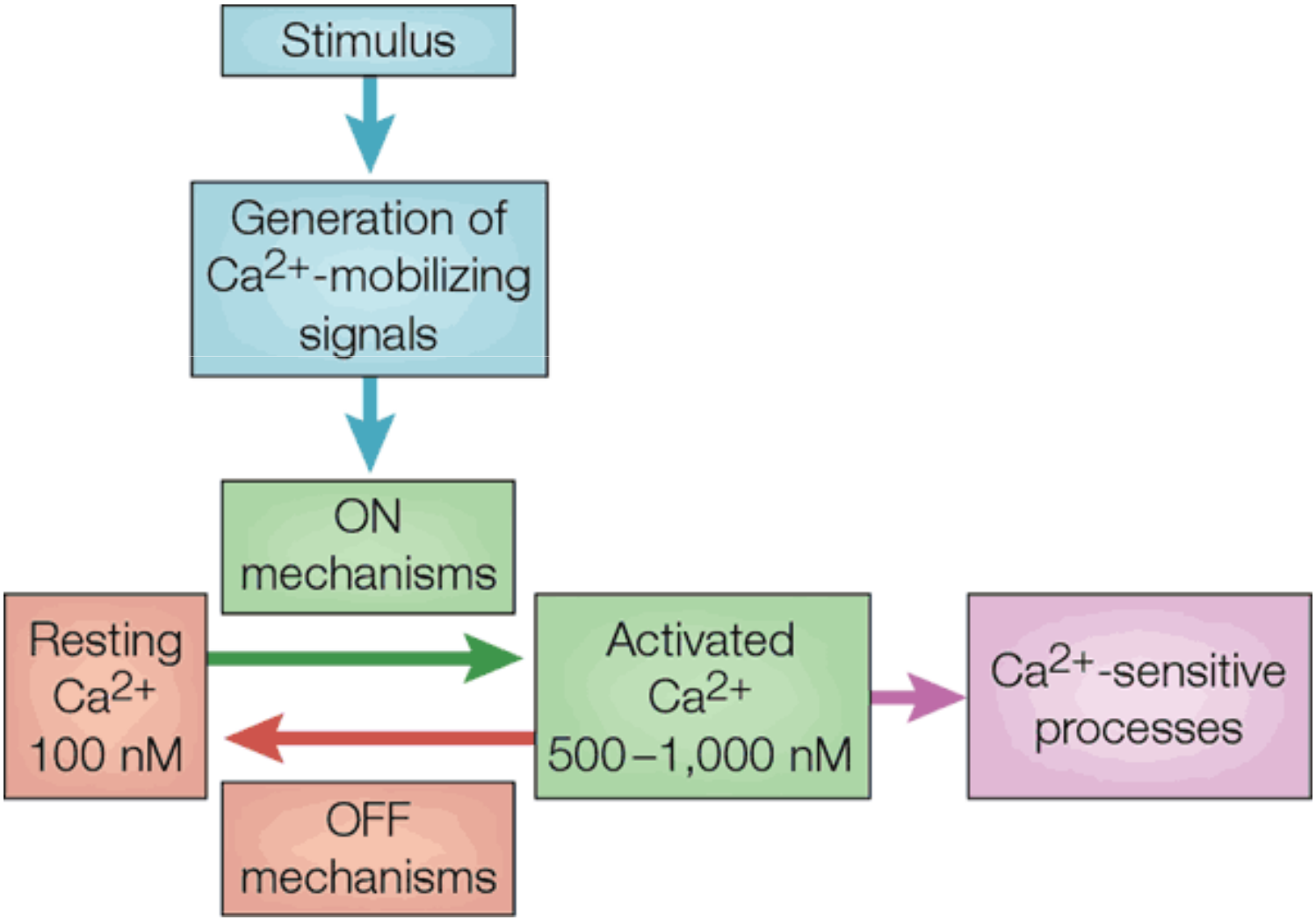
Typical Resting & Stimulated Levels of Cytoplasmic Calcium



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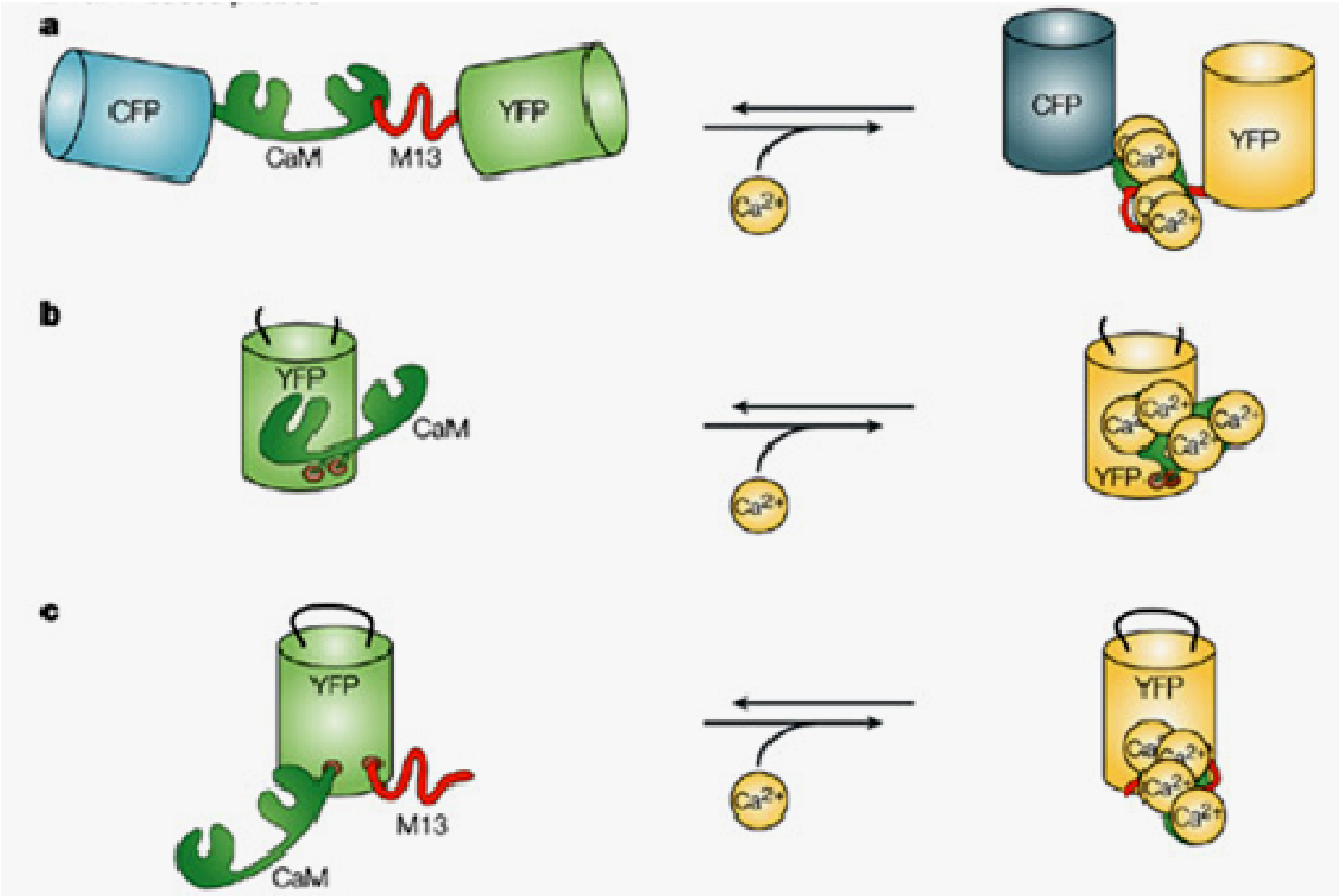
Also some newer GFP-based probes



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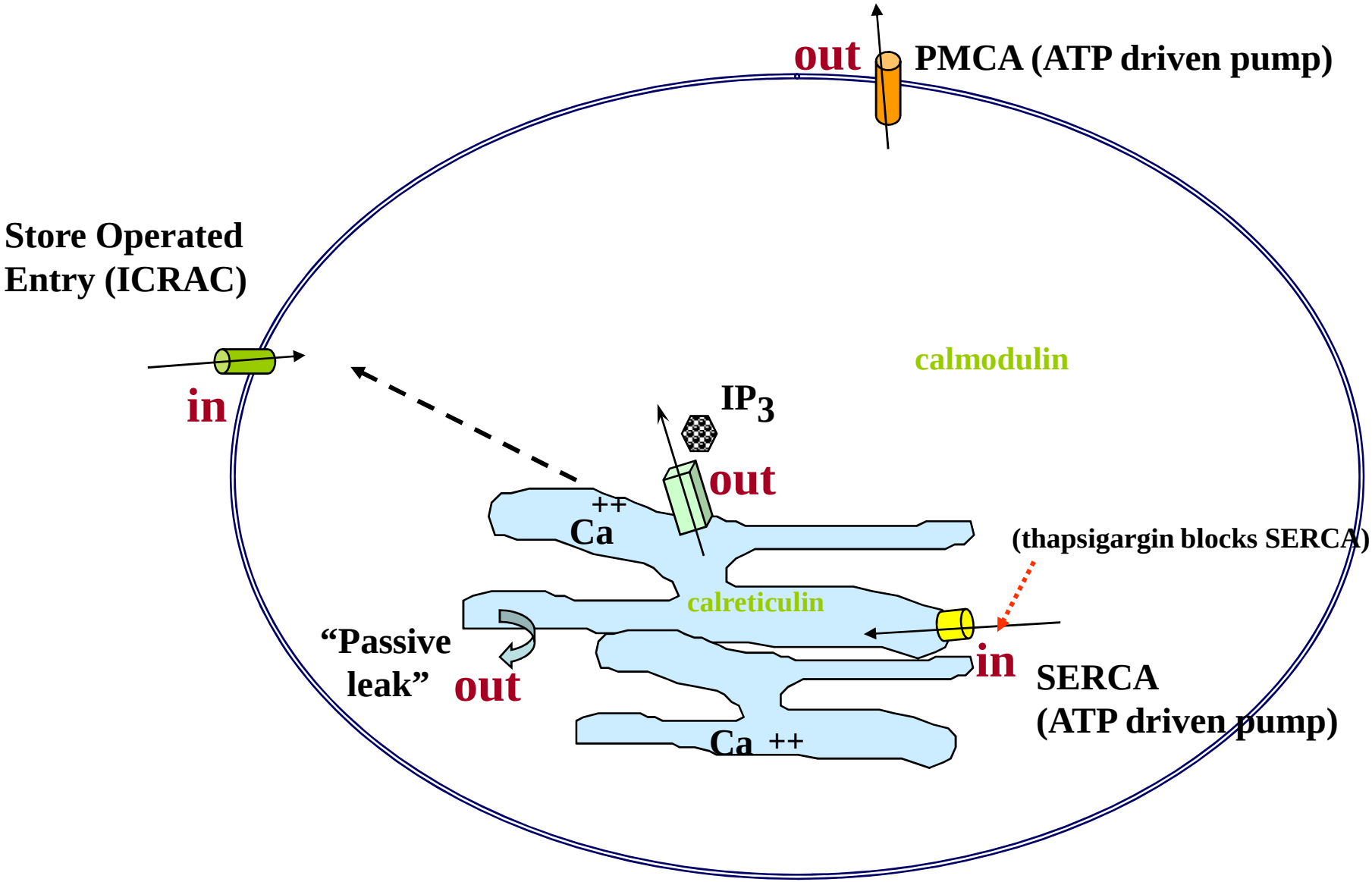
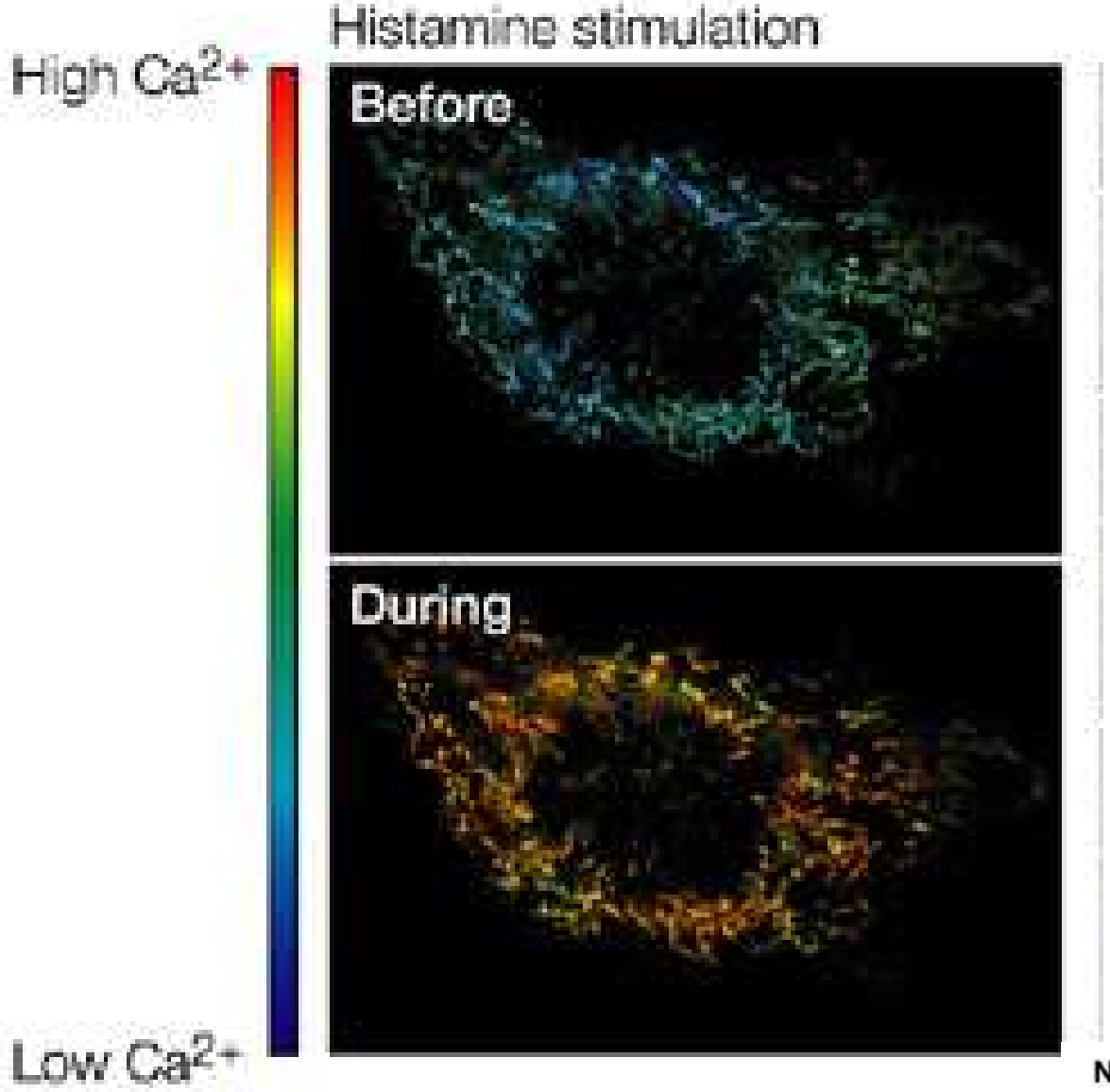
In modeling, must consider flux both directions thru plasma membrane & ER membrane, as well as buffering proteins



....and Mitochondria, too!



Most non-compartmental models treat mitochondria as an "immobile buffer" and represent with single ODE.

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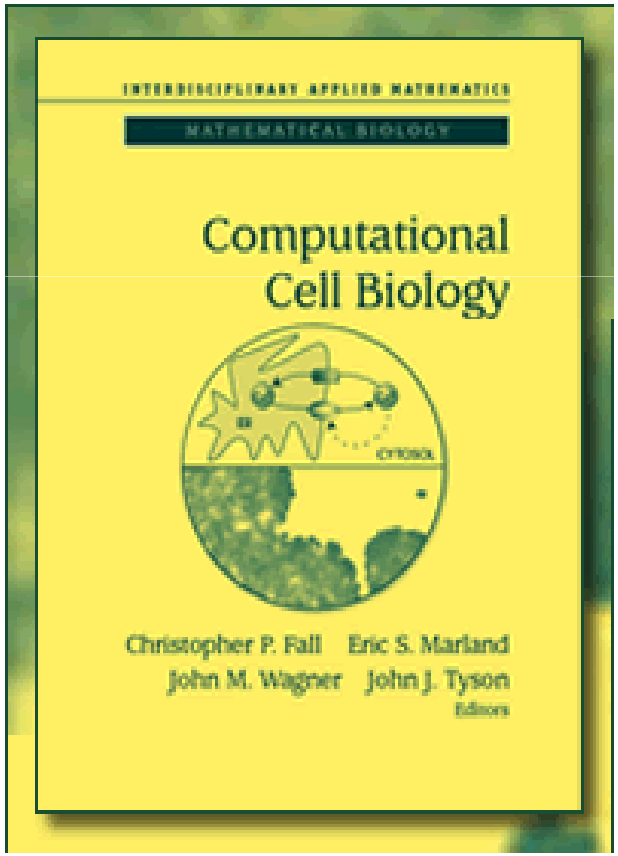
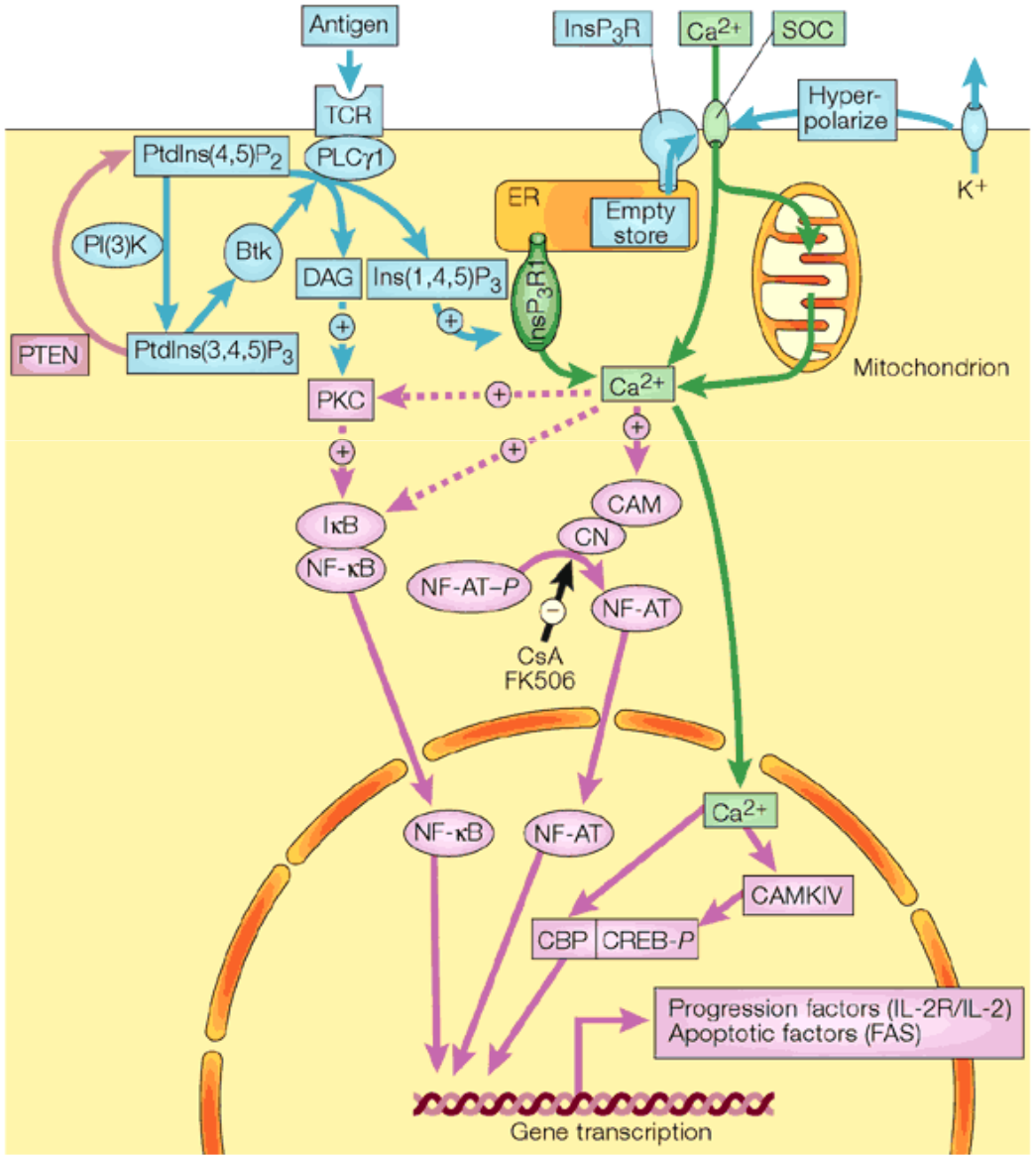
Recommended reading: Chapters in....



dedicated
to Joel E. Keizer
1942-1999

Calcium is important for signaling to the nucleus (such as PKC & Calmodulin/Calcineurin Pathways)



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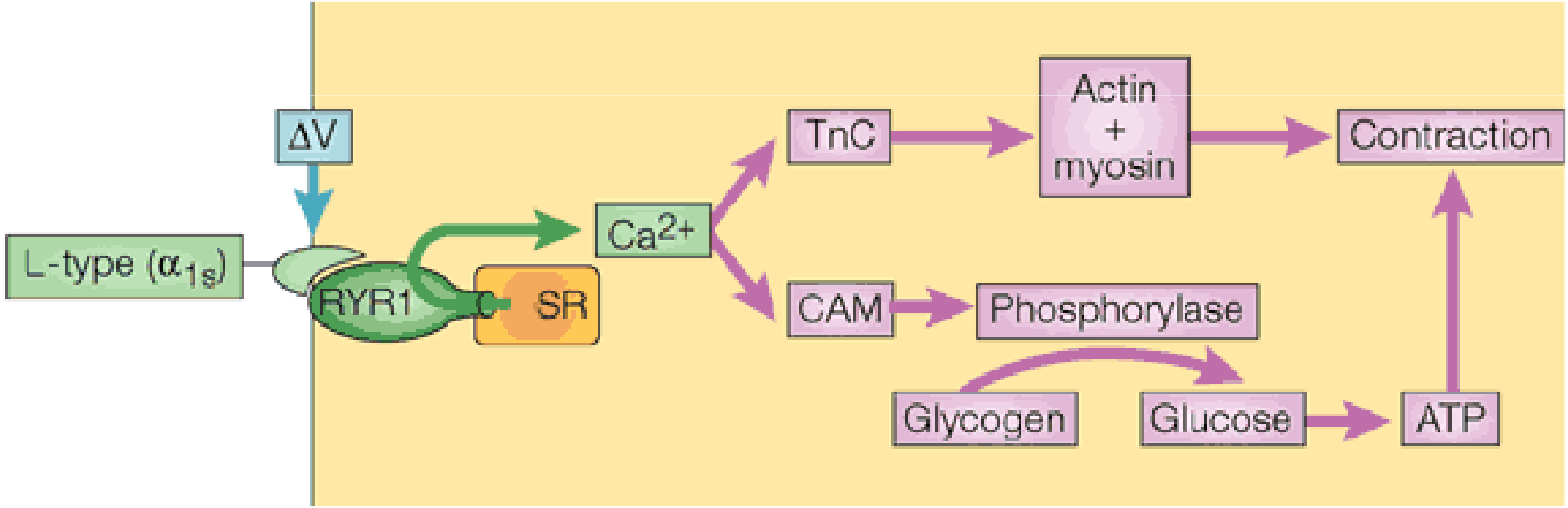
Outcomes of Elevated Calcium are <u>Cell-type Specific</u>

Example 1: Skeletal Muscle



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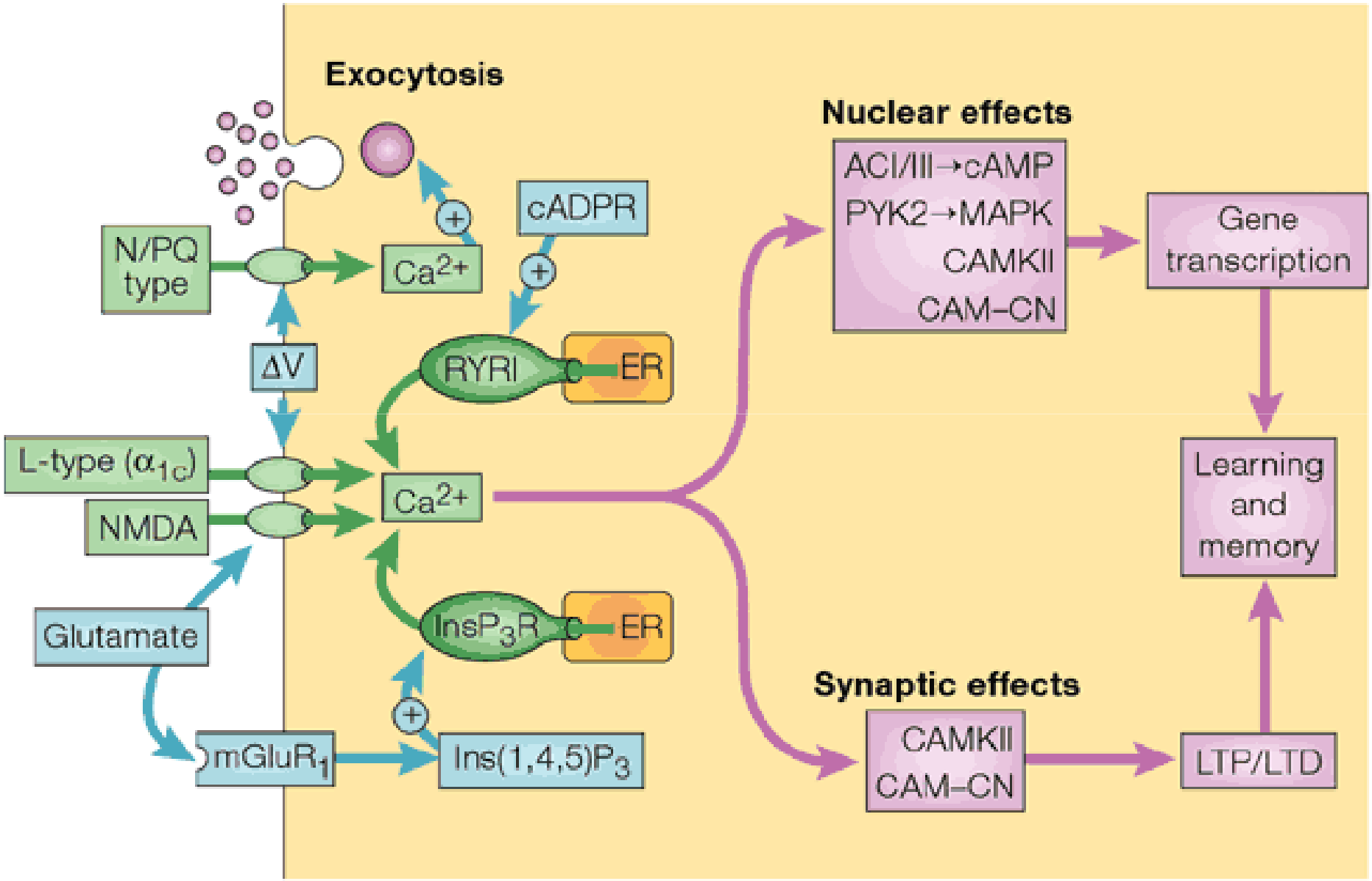
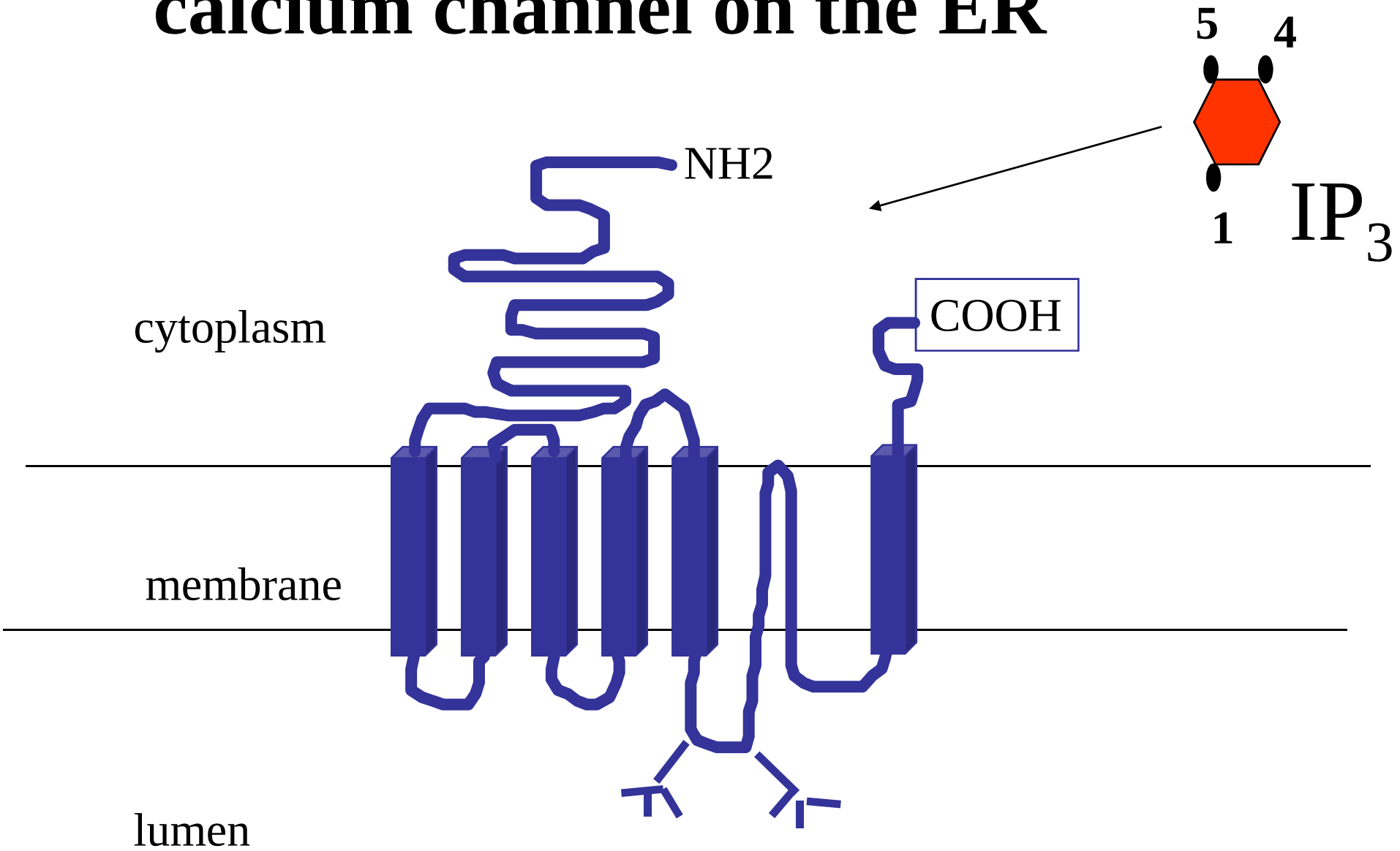
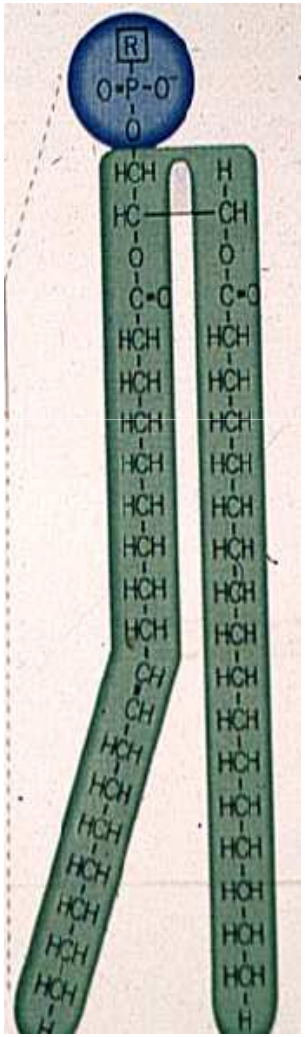
Example 2: NEURON

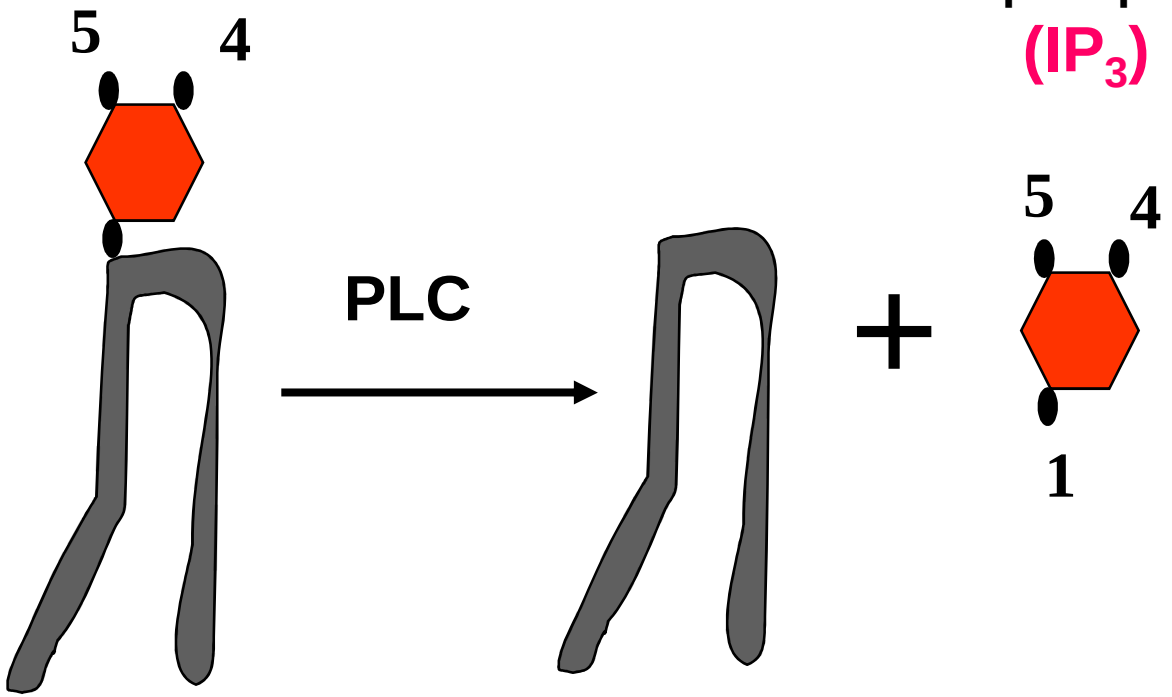


The IP_3 receptor is a ligand-gated calcium channel on the ER



The lipid PIP_2 is critical to this pathway



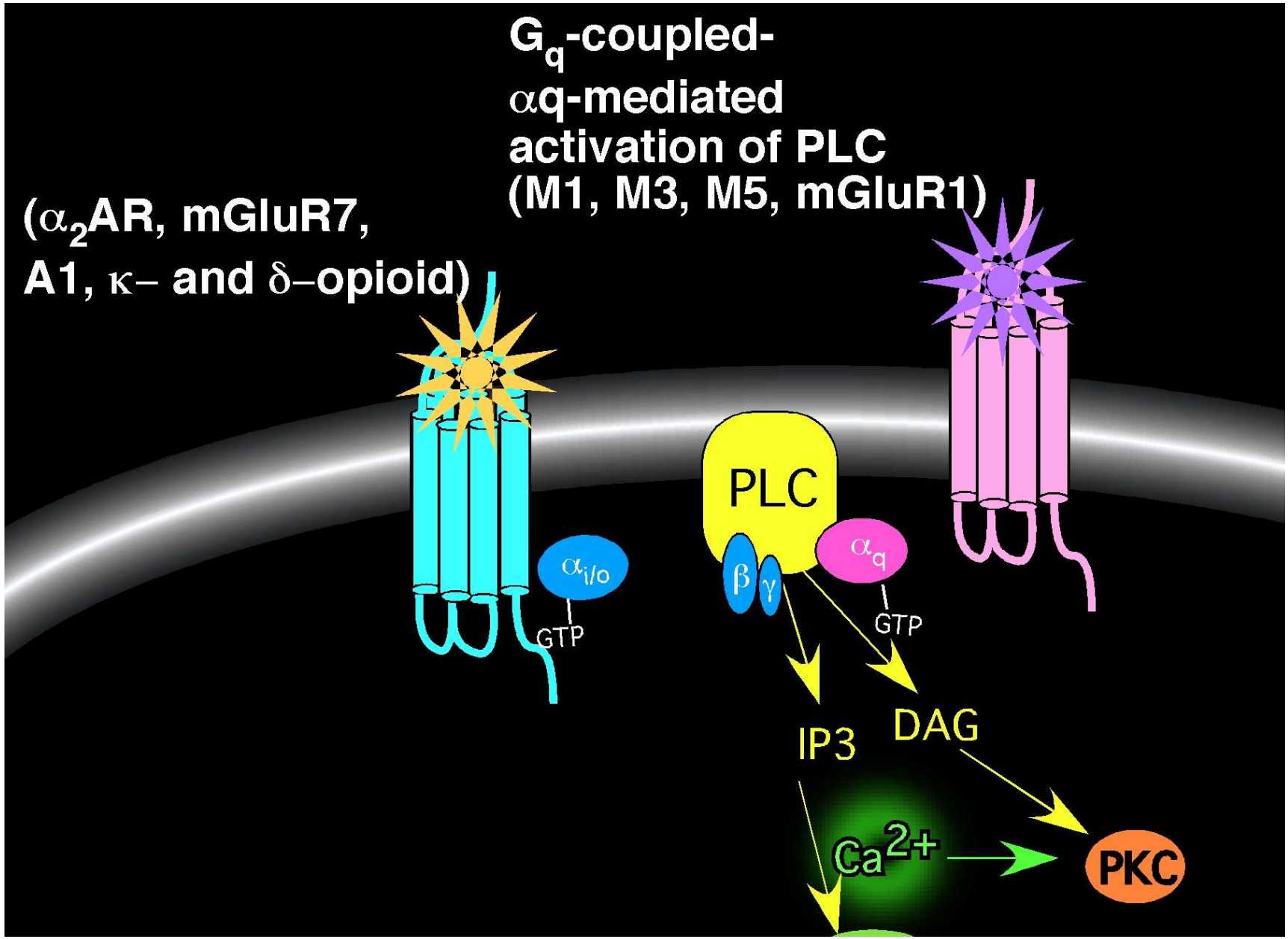


Inositol(1,4,5) trisphosphate (IP_3)

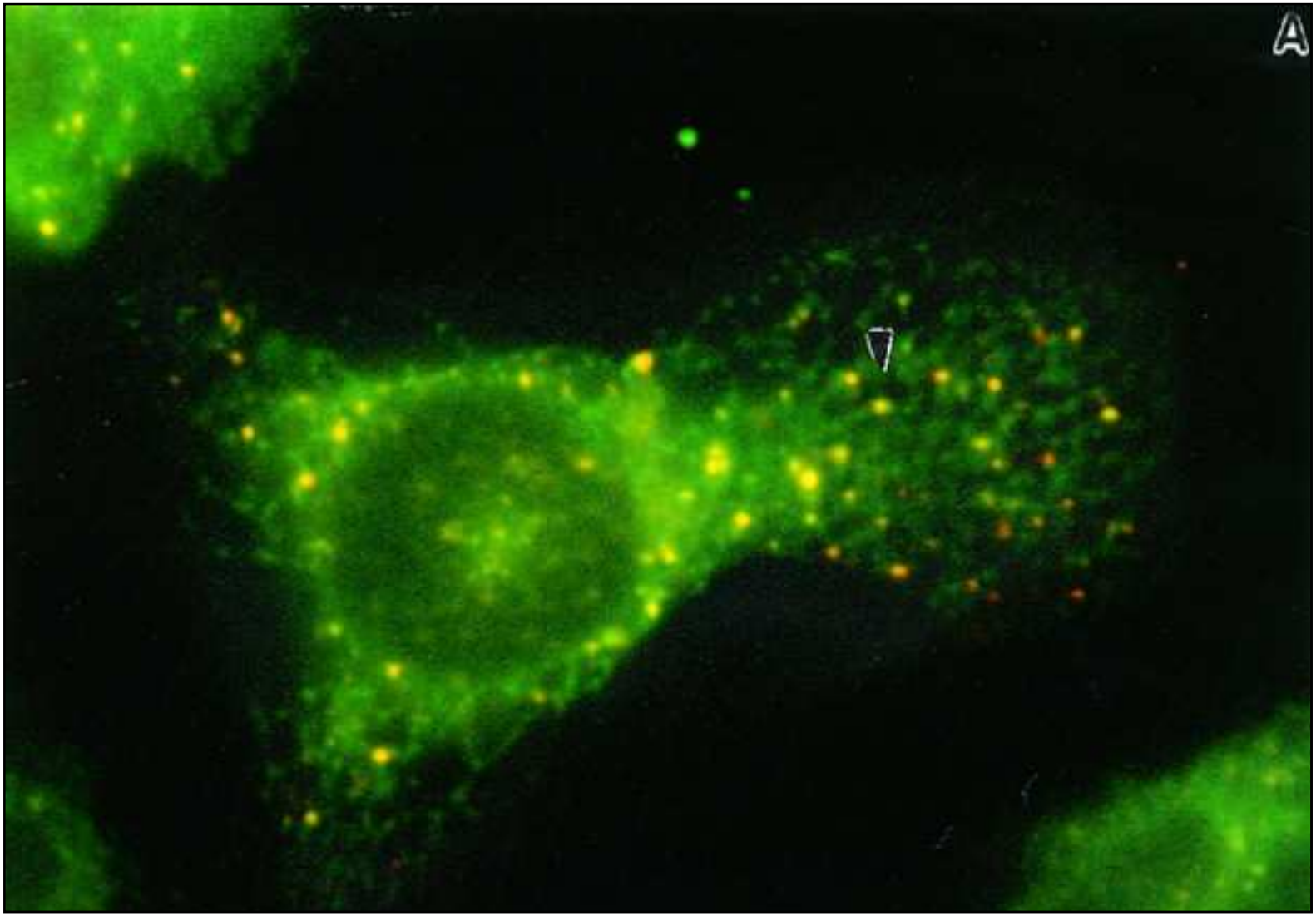
diacylglycerol (DAG)

Phosphatidylinositol (4,5)bisphosphate (PIP2)

Example: Activation of phospholipase C by G_q- and G_i-coupled receptors

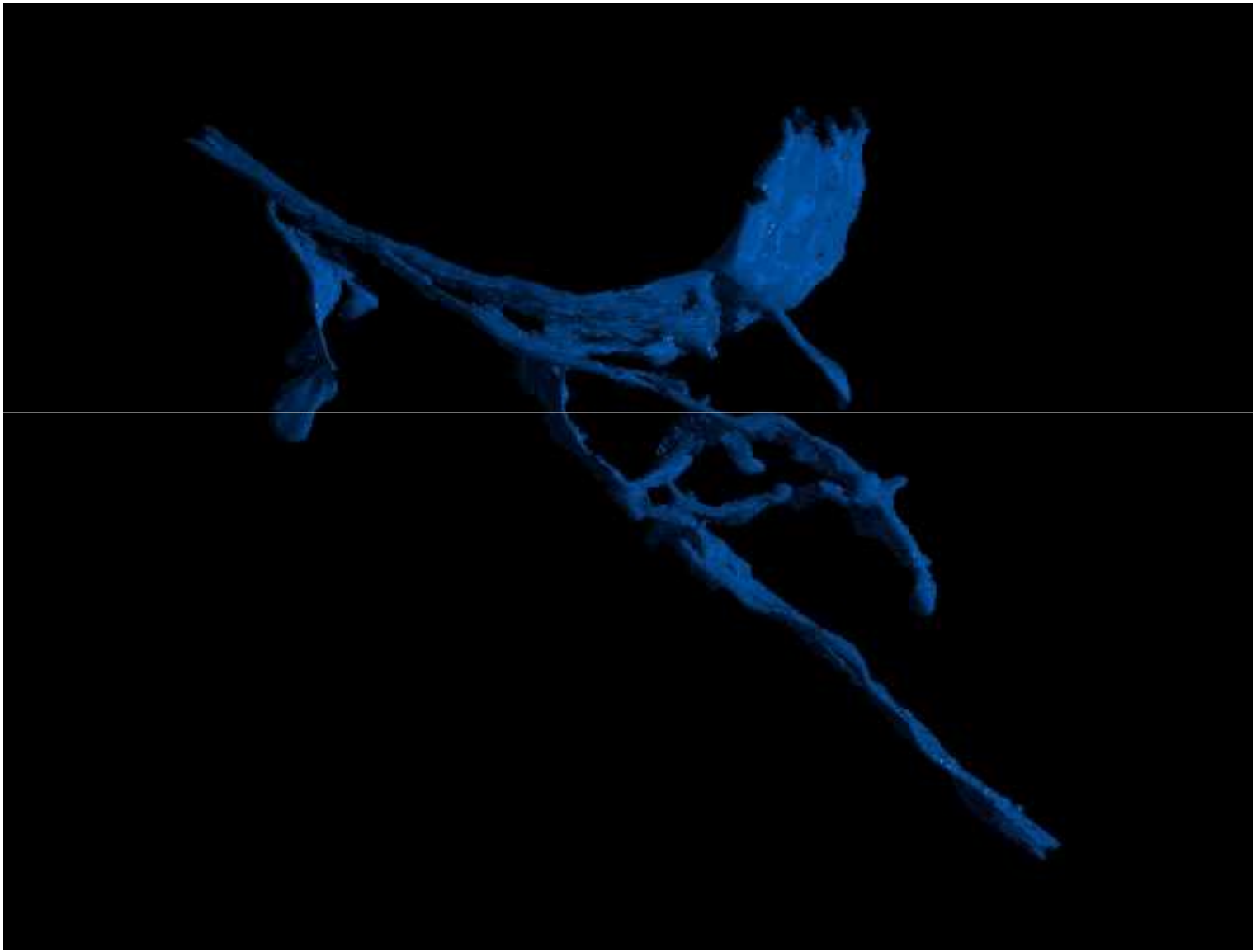


<u>Starting premise:</u> our observation that IP_3 receptors cluster in ER <u>after</u> a rise in $[Ca^{2+}]_i$

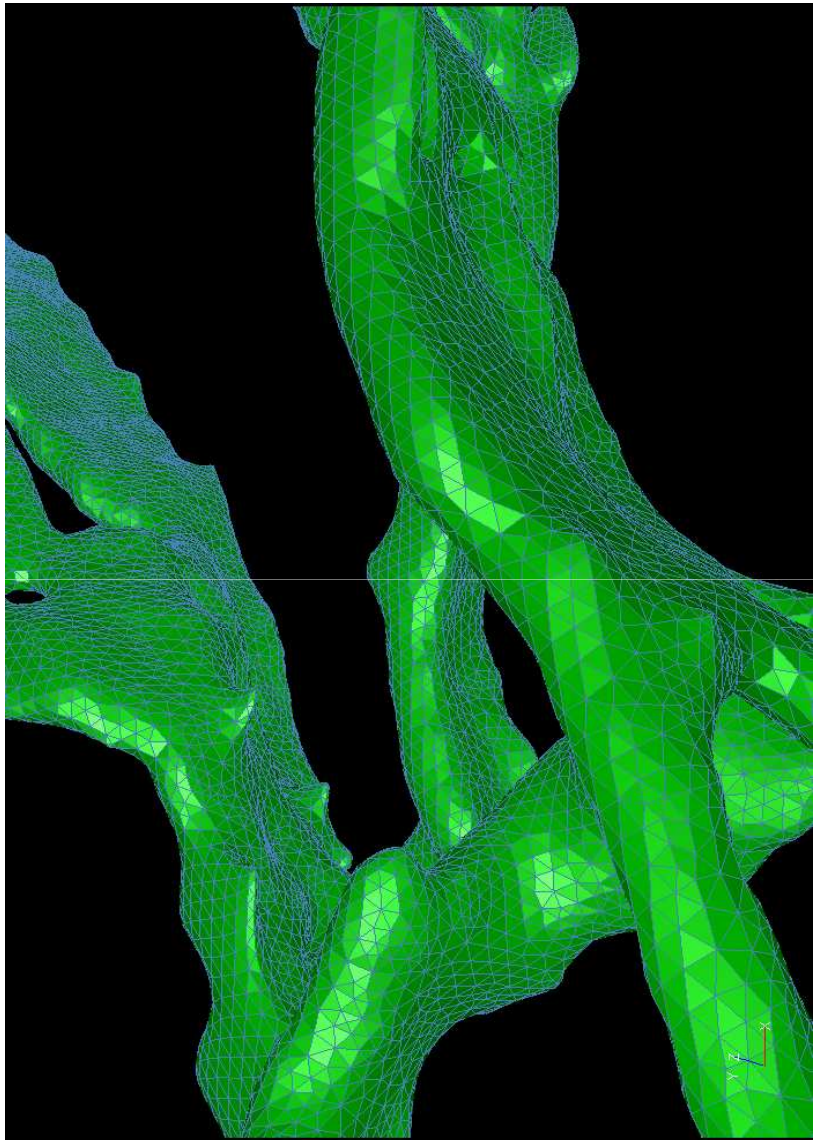


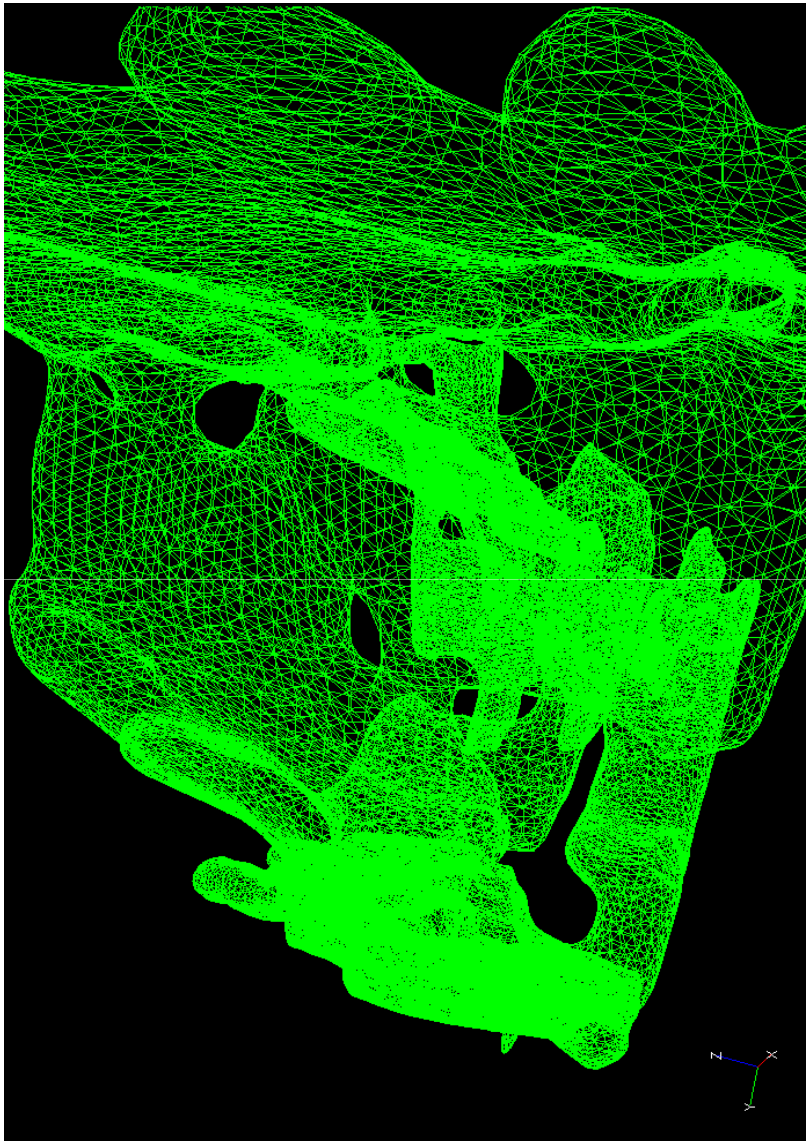
Wilson et al, Molecular Biol.Cell 1998

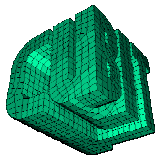
3D ER reconstruction



Tetrahedral mesh generation







using CUBIT (Sandia)

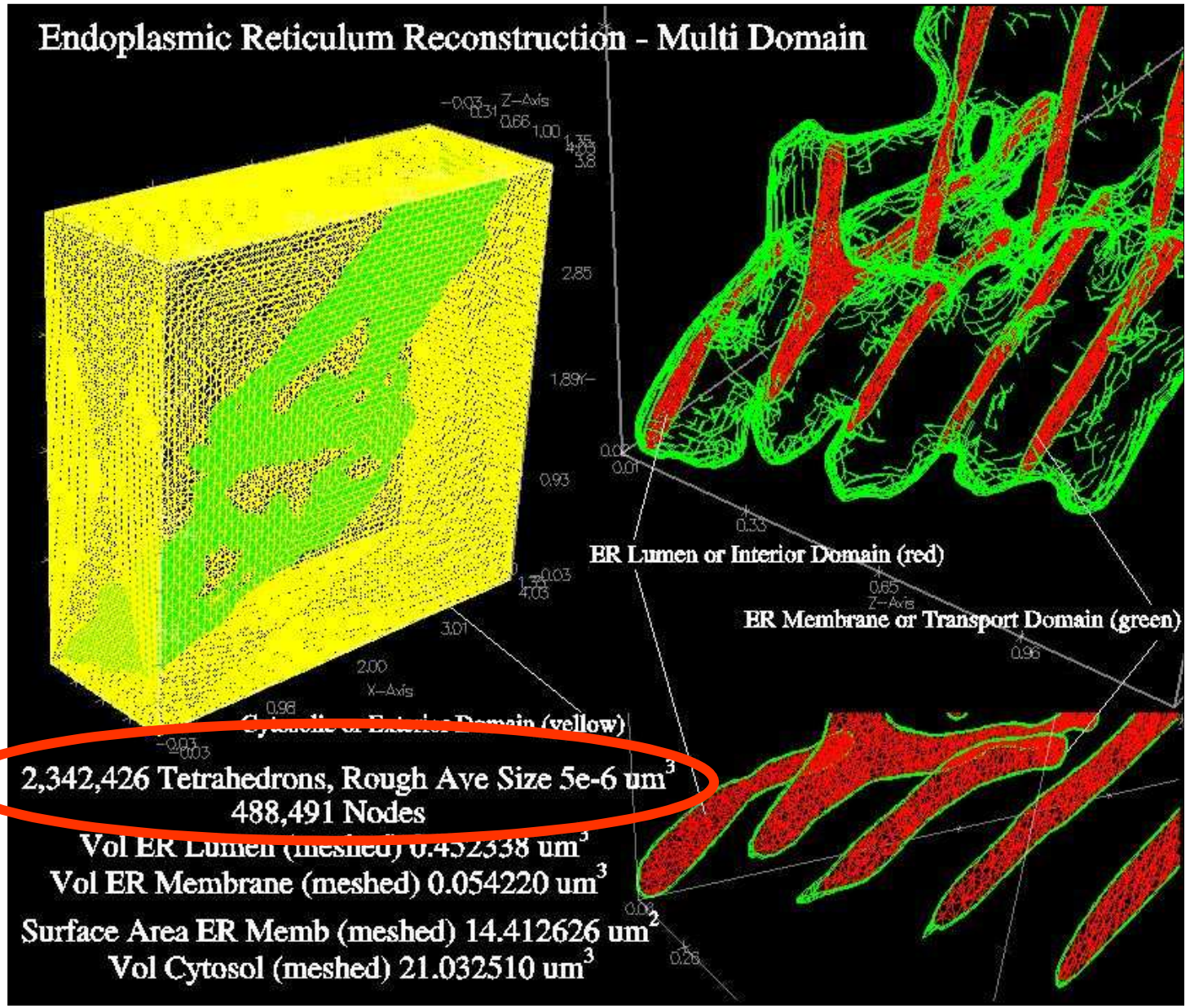
Smith, Shadid

ER & Cytoplasm MultiDomains

Simulations use

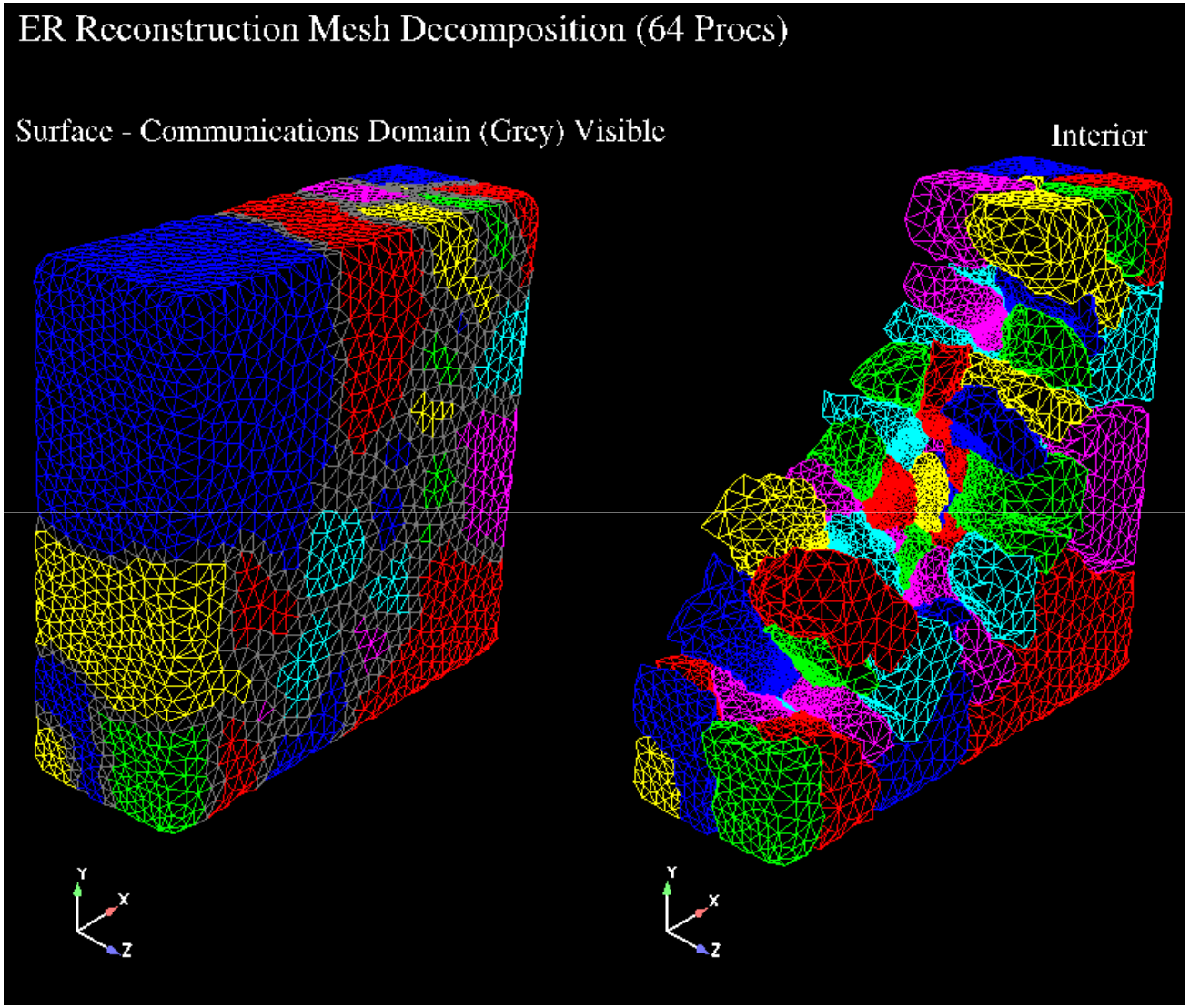
FEM Reacting Flows Solver, not originally designed for multiple domain problems.
Code modifications allow for accurate representation of surface transport (Neumann Flux) with spatially-localized reactions (source term)

http://www.cs.sandia.gov/CRF/MPSalsa/

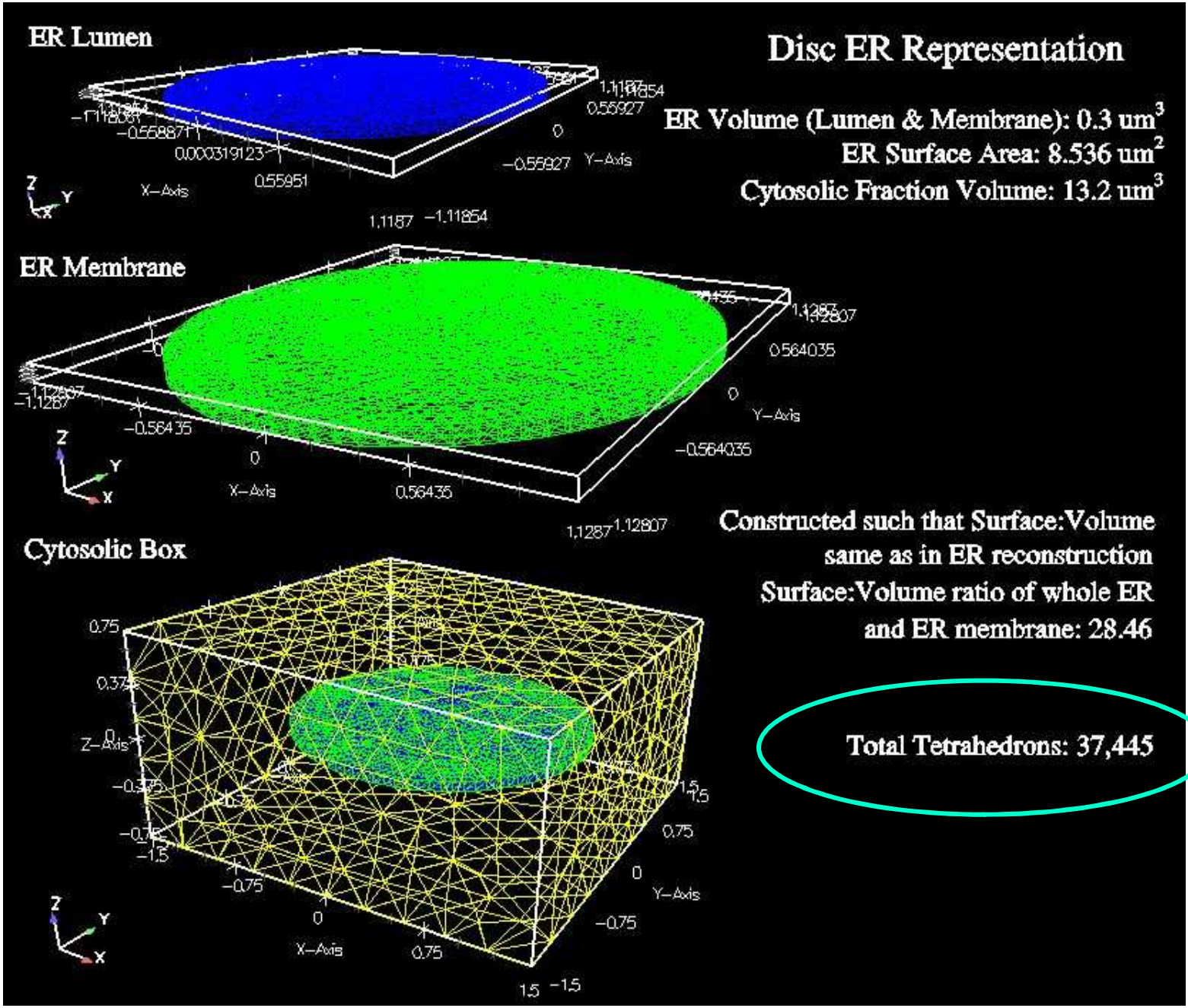


Means, Shepard

Decomposition for 64 Processors. communications domain colored in grey.

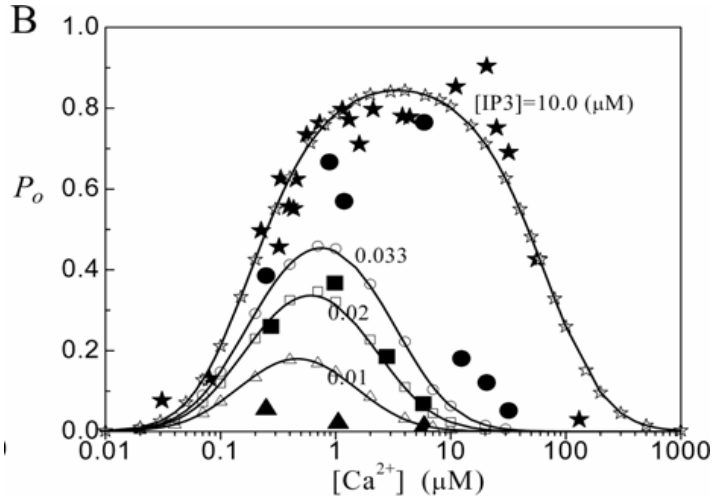


For quicker simulations, we applied simpler geometries (discs & tubes).

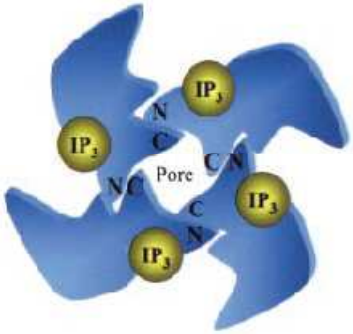


IP_3R regulation by calcium & IP_3 concentration

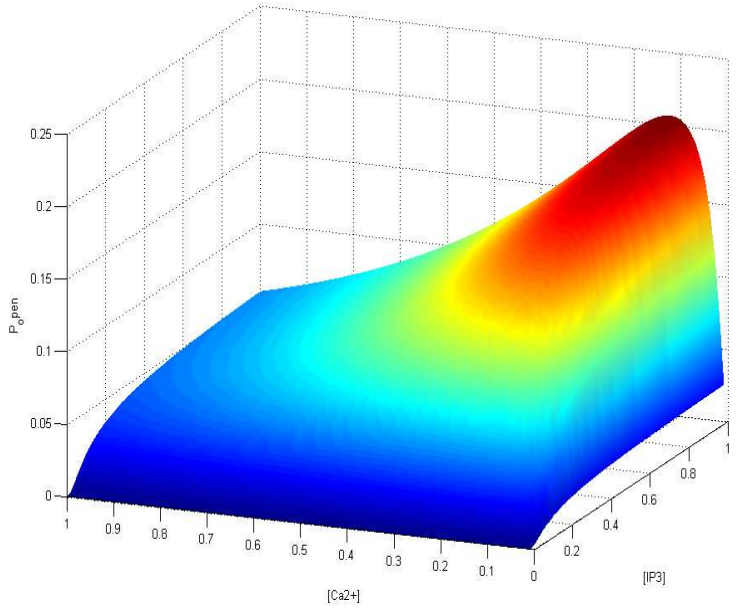
Paolini et al., 2004



Foskett et al., 2007

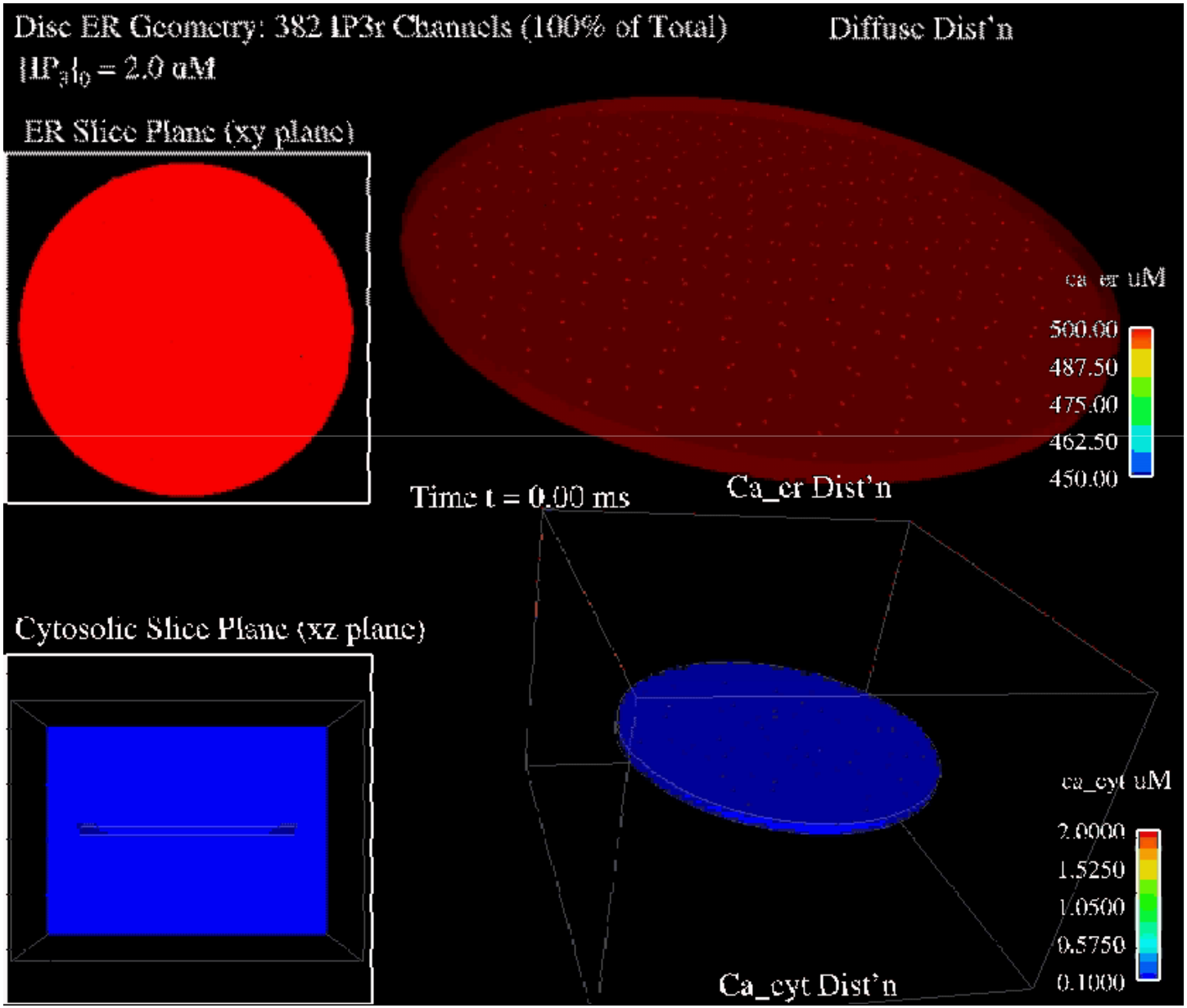


Patterson, 2004

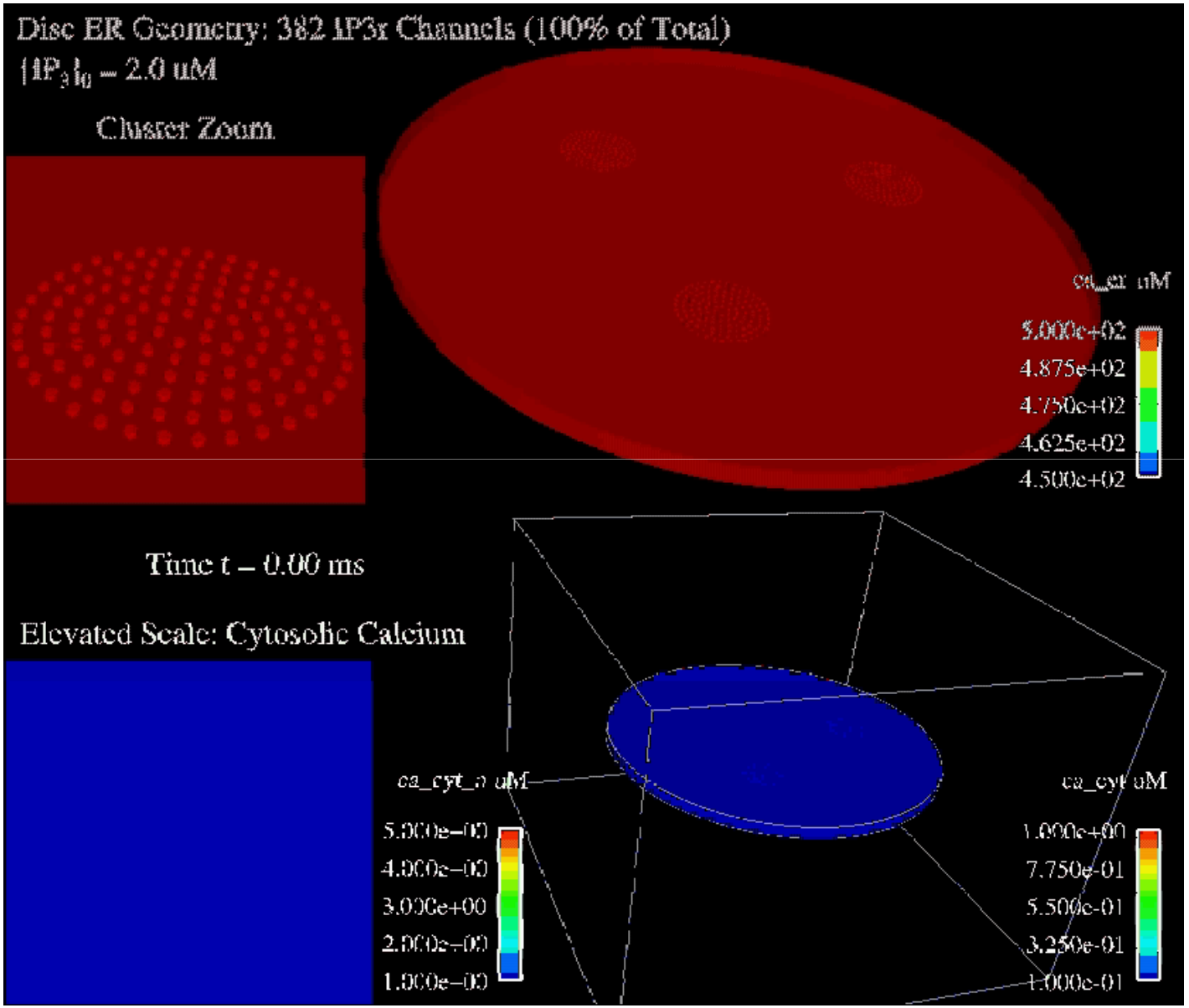


$$J_{IP3r} = V_{IP3r} \times \phi_o(IP_3, Ca_{cyt}, t) \times (Ca_{er} - Ca_{cyt})$$

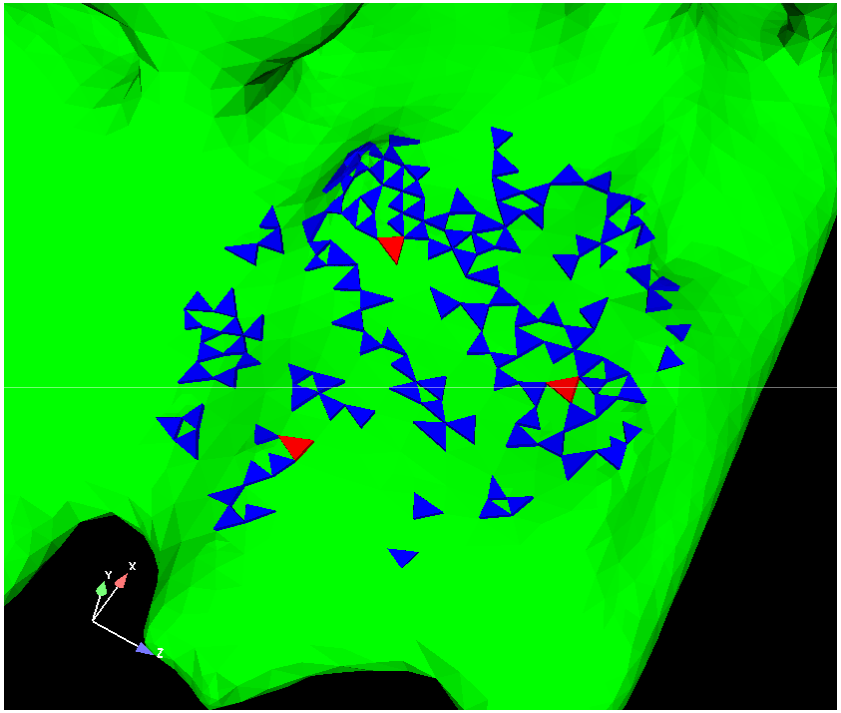
Flux through IP_3 receptors in diffuse states (disc geometry)

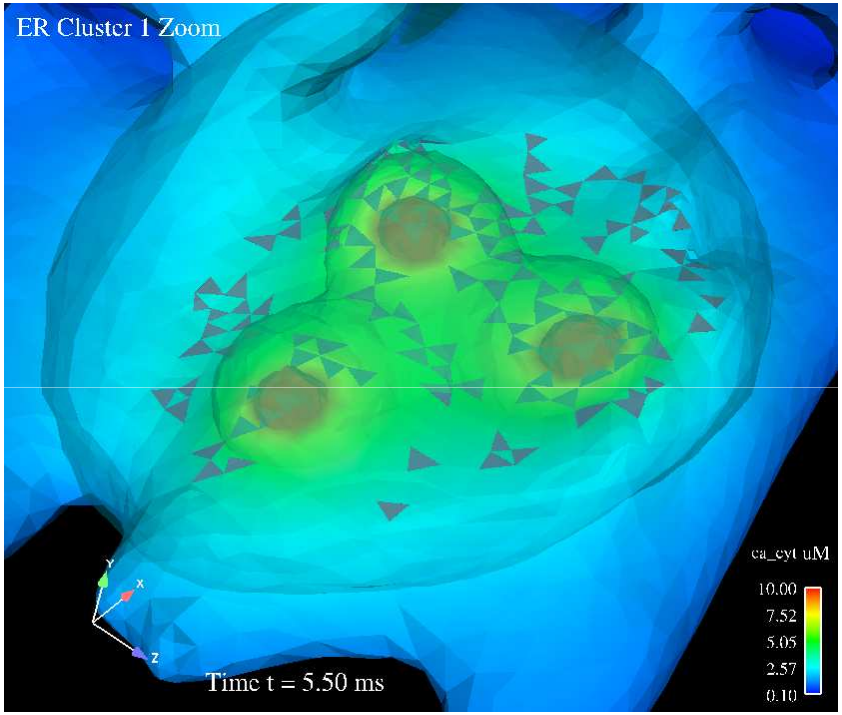


Flux through IP_3 receptors in clustered states (disc geometry)



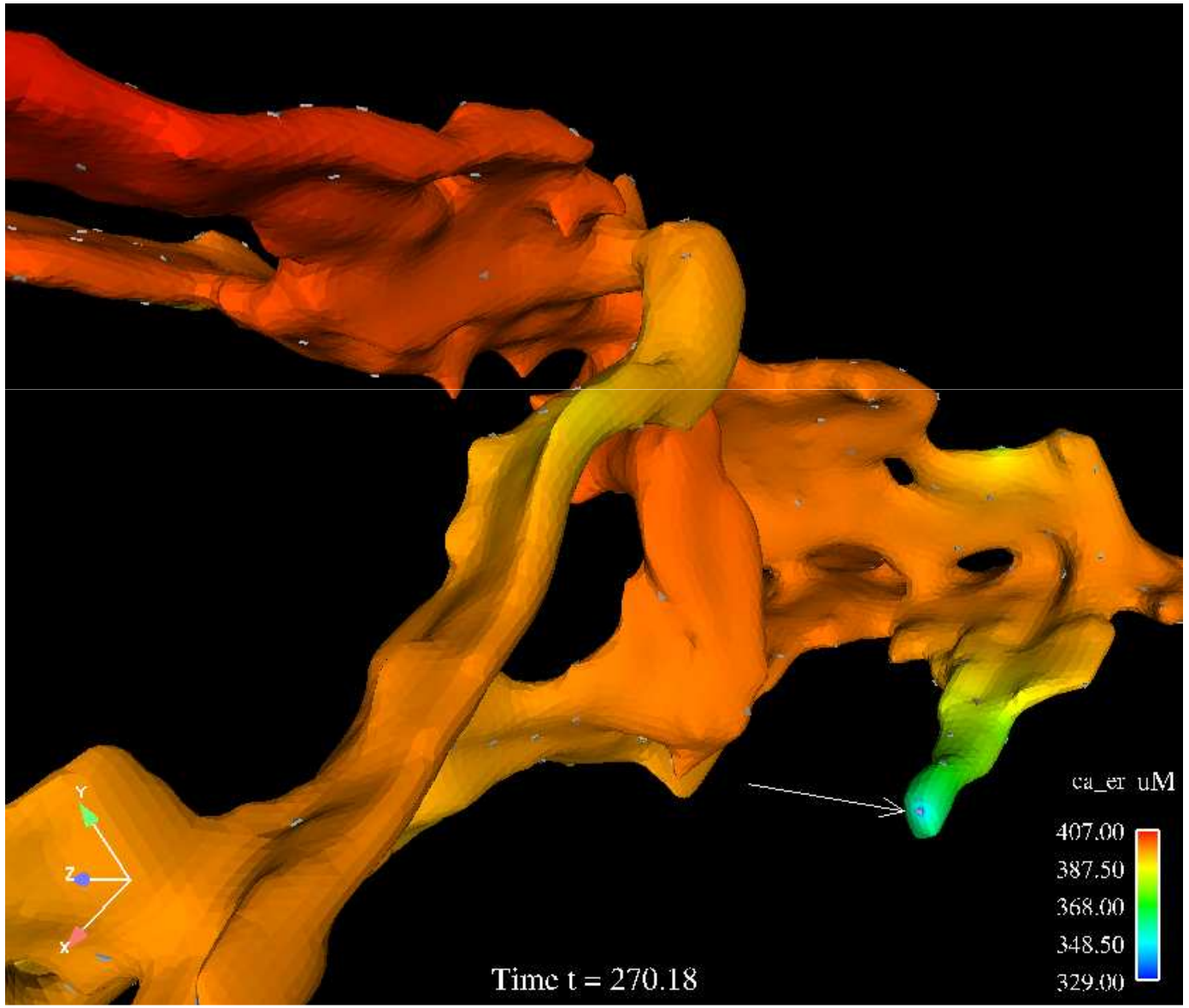
ER Geometry: Deterministic IP_3R Trial



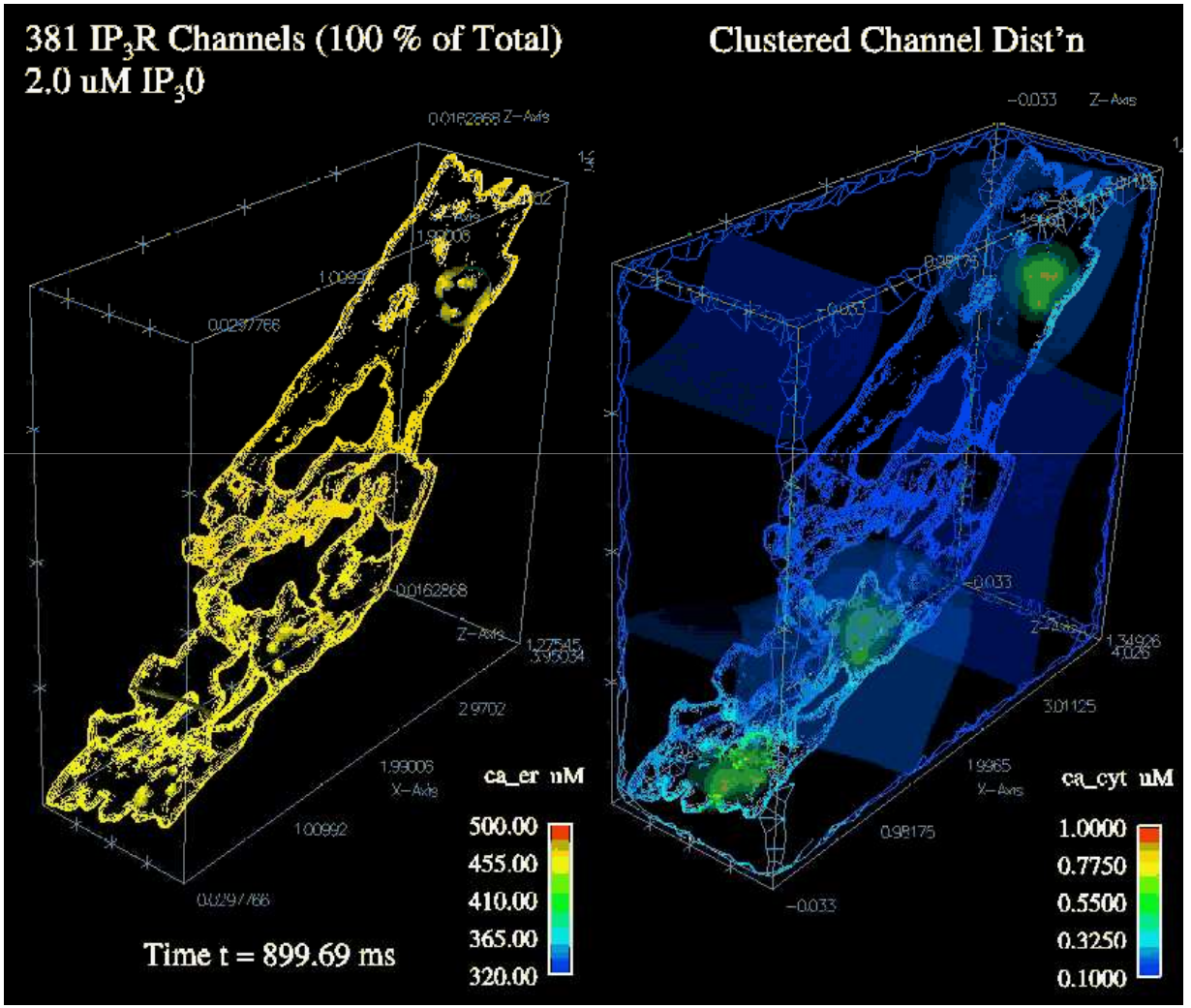


Three channels forced open and closed for 10 ms. Trio in clustered channels (left, red) and resulting calcium cloud shown.

ER Geometry: Small, Transient Concentration Gradients (Diffuse IP3R distribution)

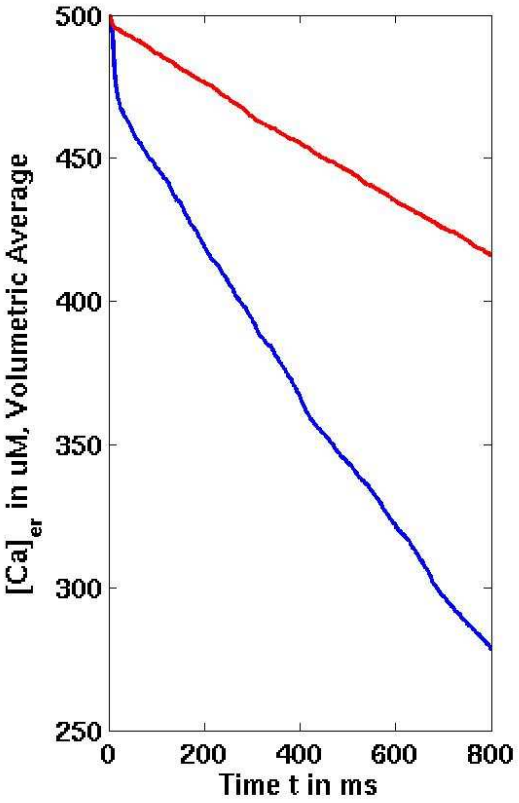


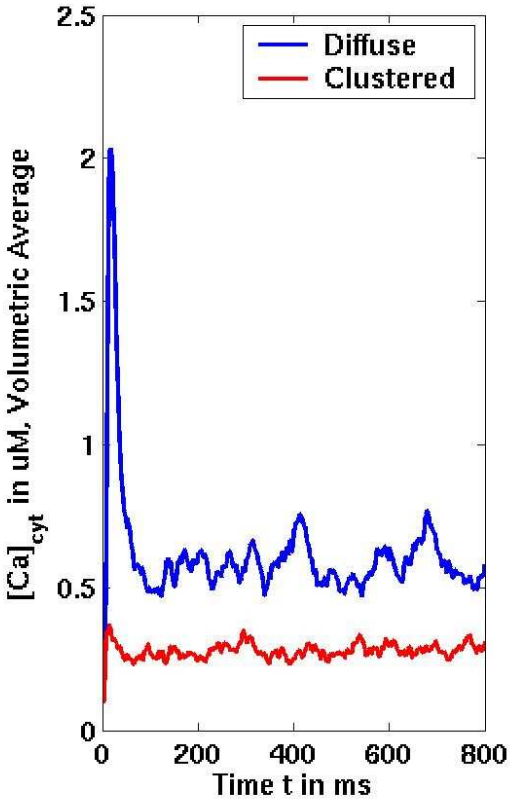
RESULTS IN THE FULL GEOMETRY



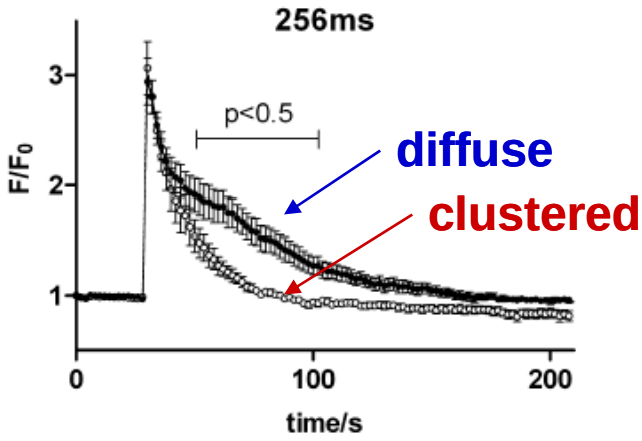
Exit for 2 movies..

THE ER EMPTIES SLOWER & CYTOSOLIC CALCIUM LEVELS ARE LOWER IN THE CLUSTERED IP3R STATE





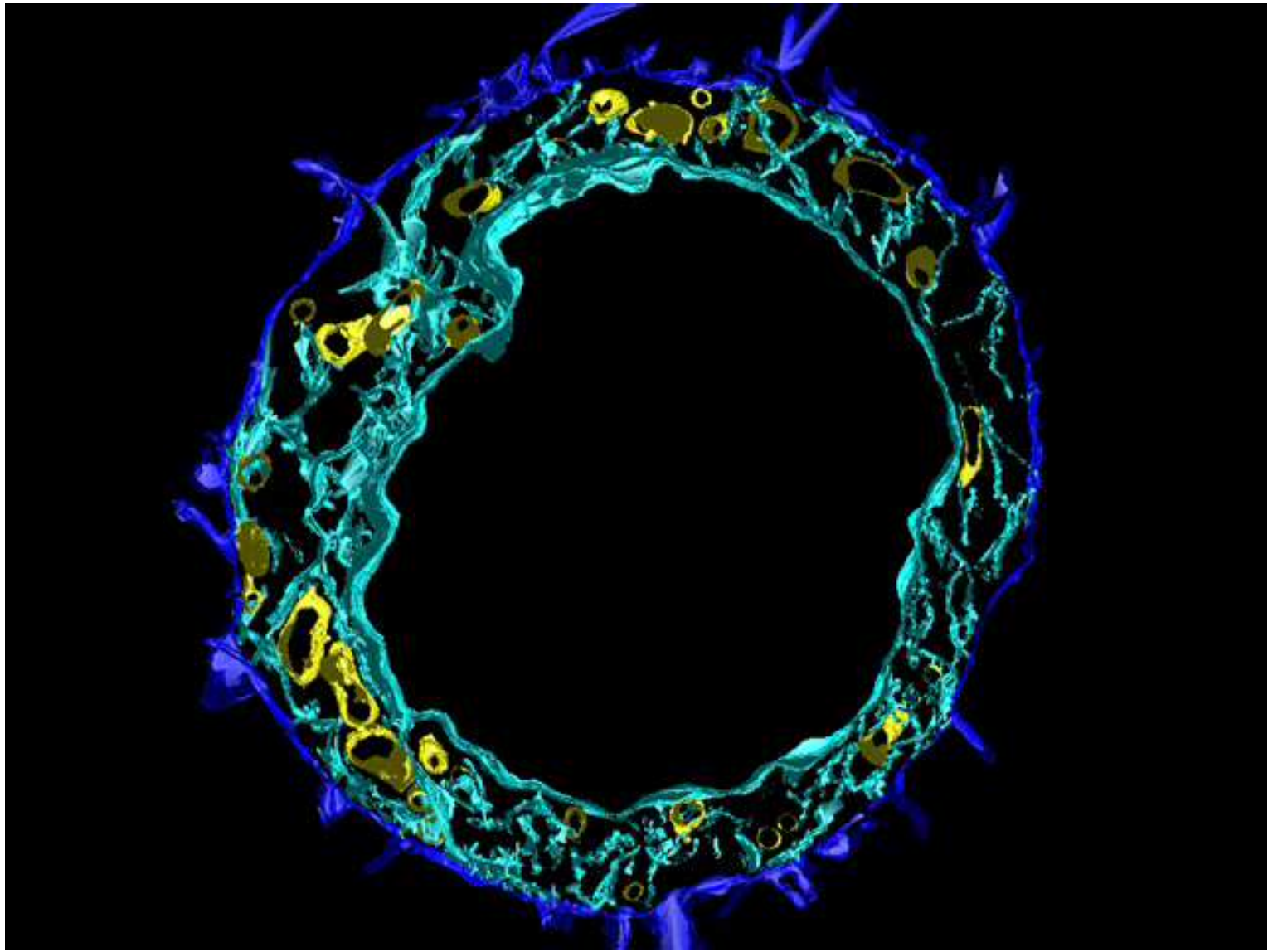
SIMULATION



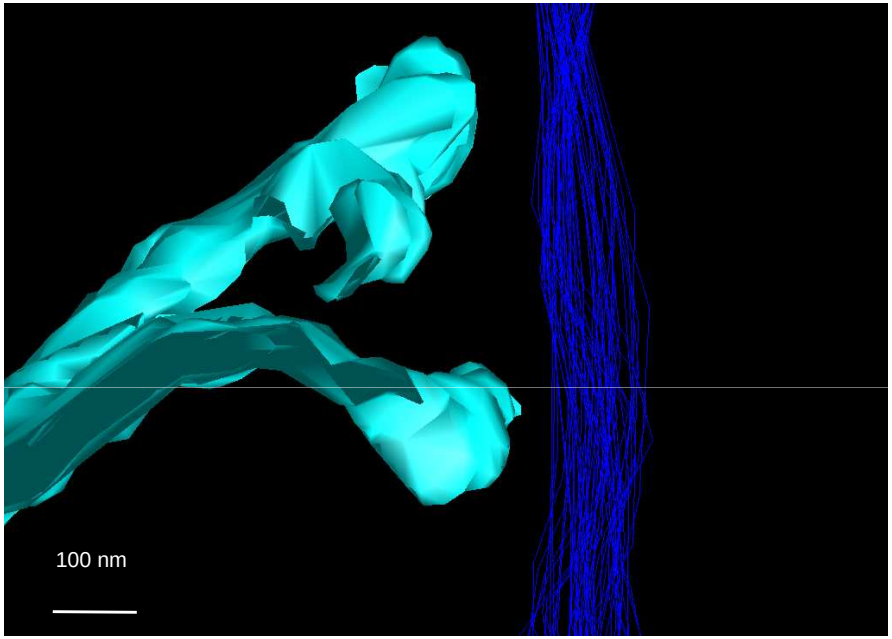
EXPERIMENT
(IP3 UNCAGING)

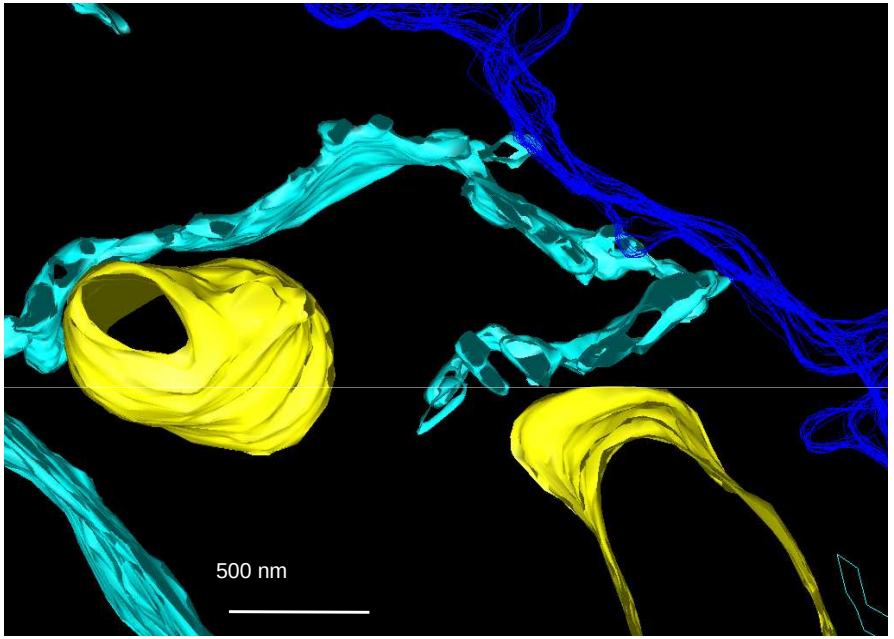
Alex Smith

In next phase, we have been focused on building more features of the cell, including explicit representation of mitochondria.

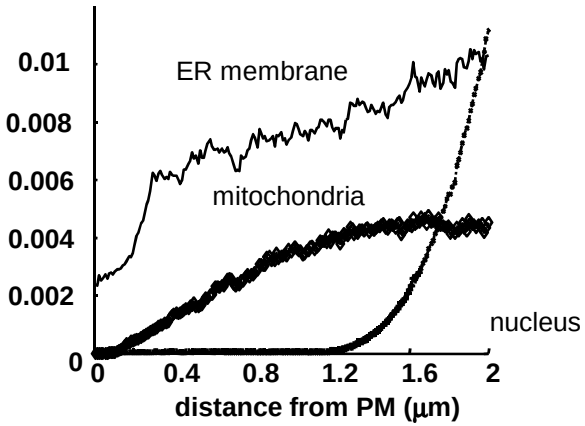


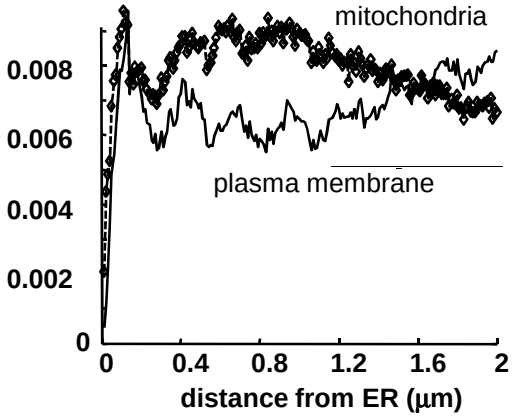
CLOSE UP VIEWS

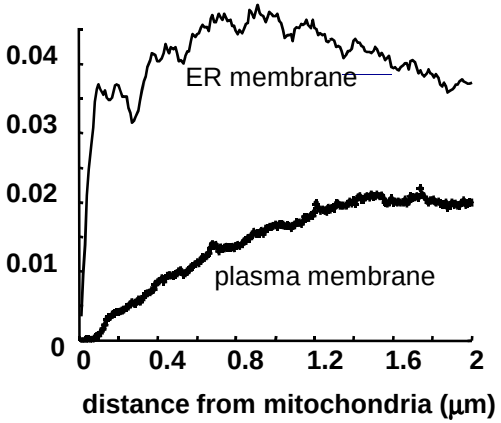




Useful parameters derived from the reconstruction

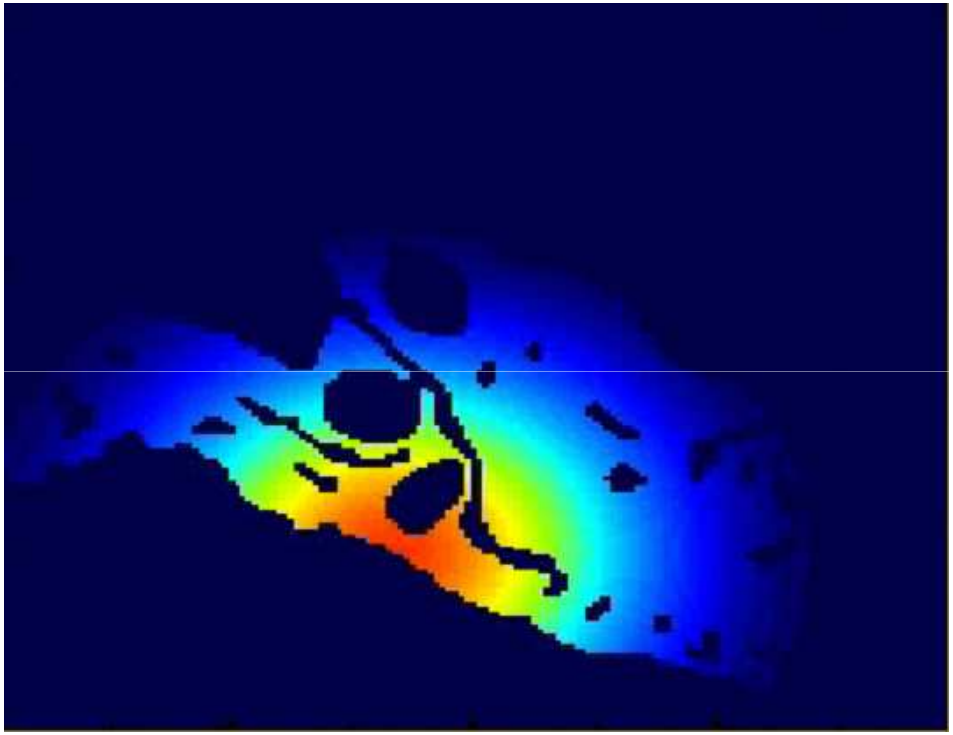






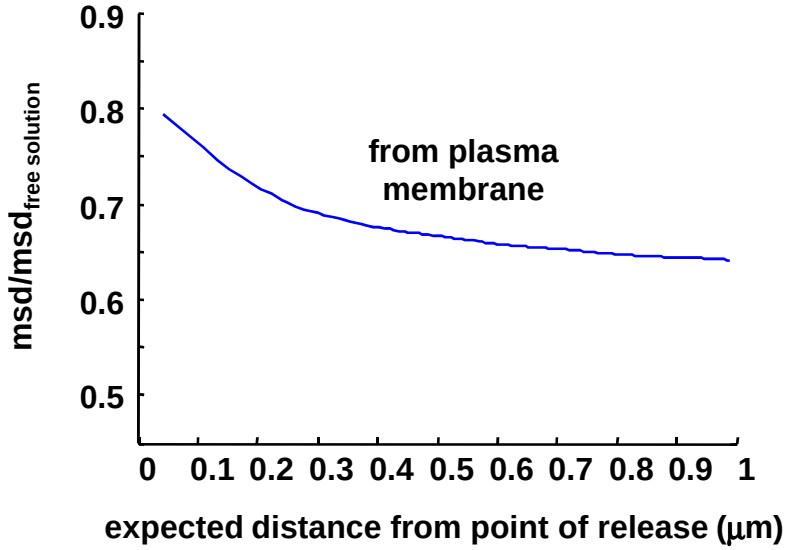
done in Matlab

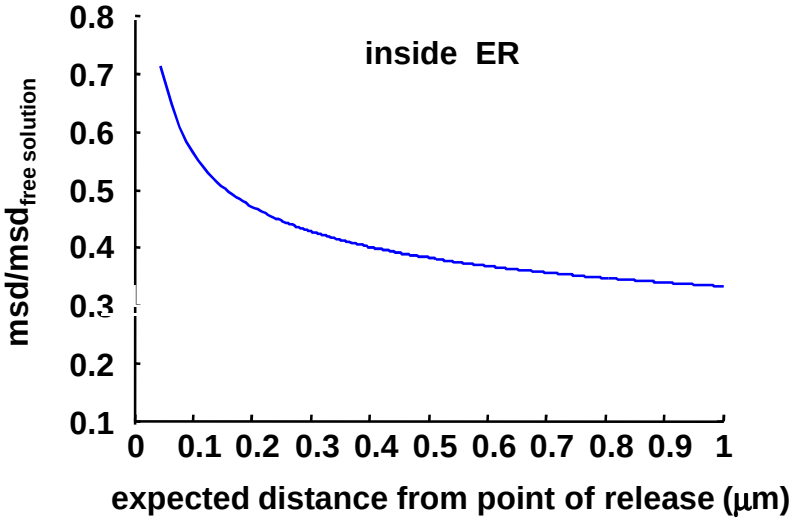
Predicting travel inside cells



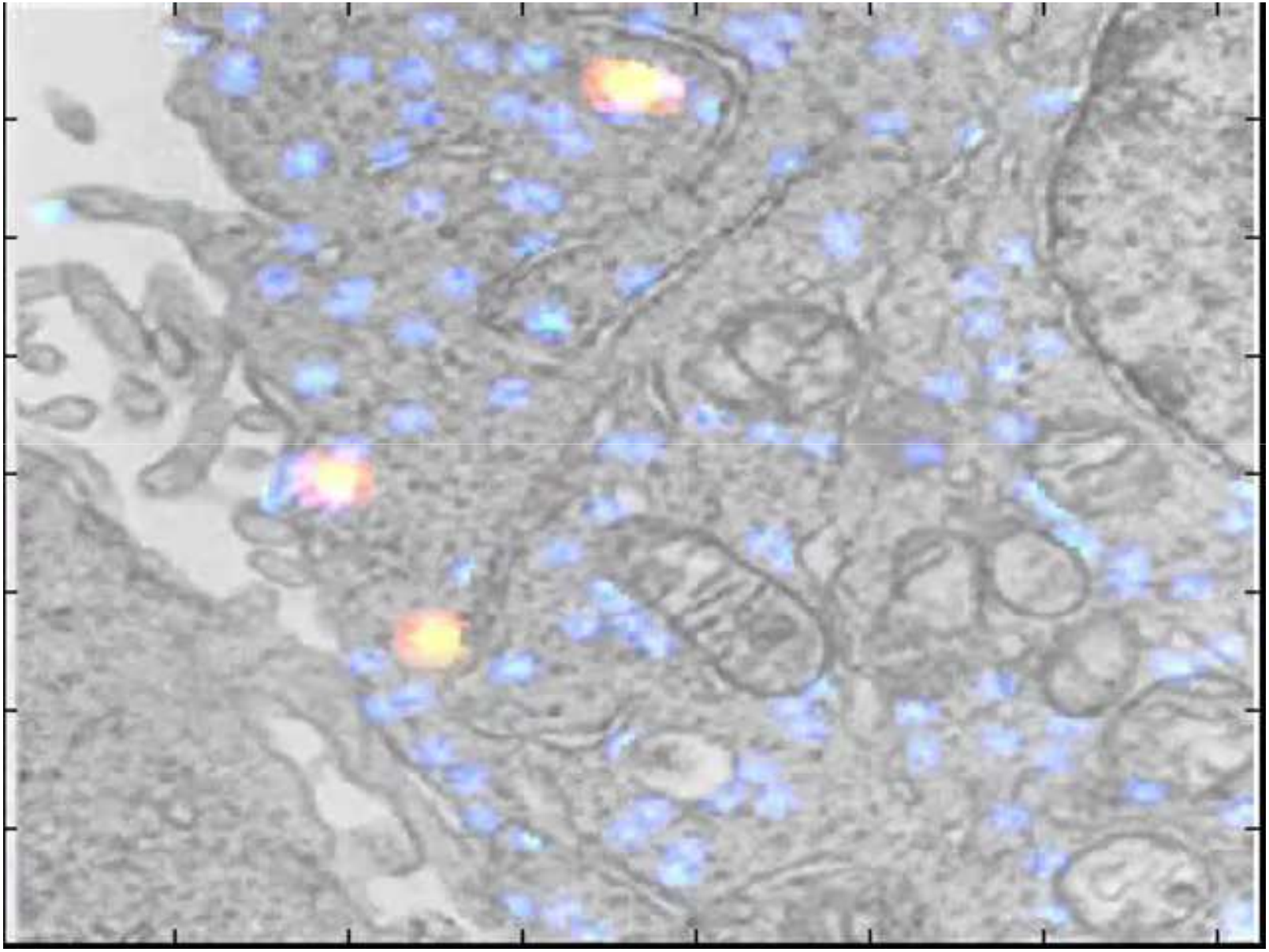
simulations in Matlab

Difference in mean sq displacement vs free in solution



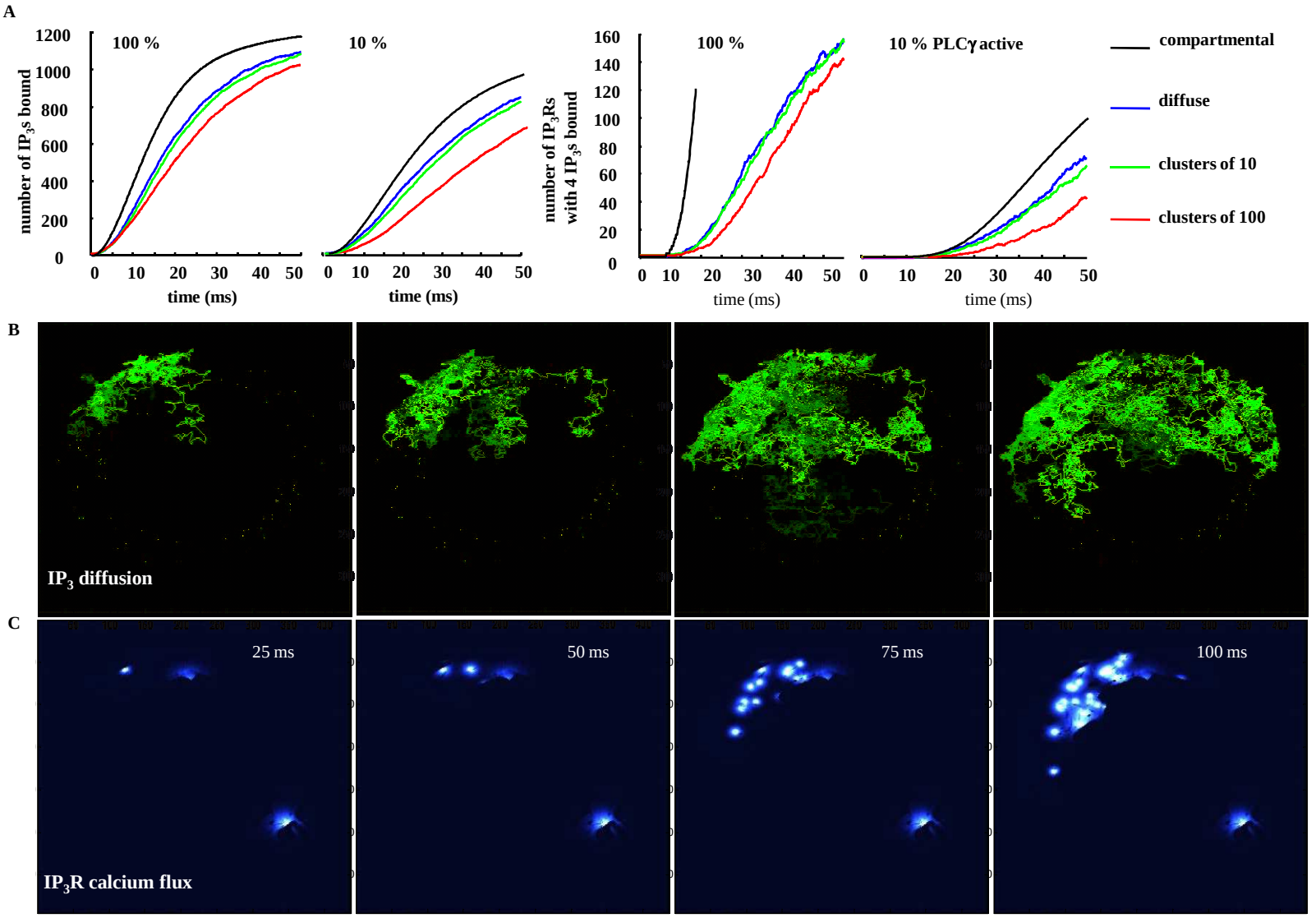


Developing a new stochastic model



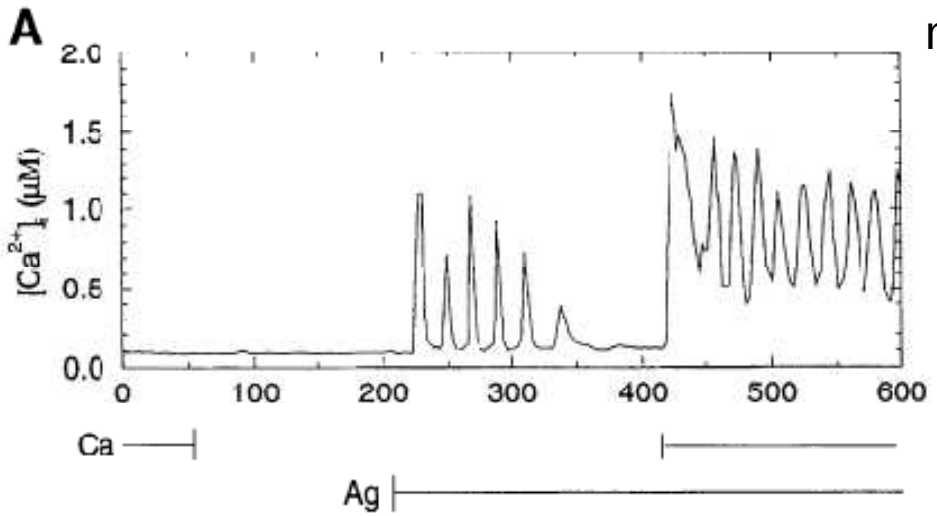
simulations in Matlab

Simulating IP3 Synthesis

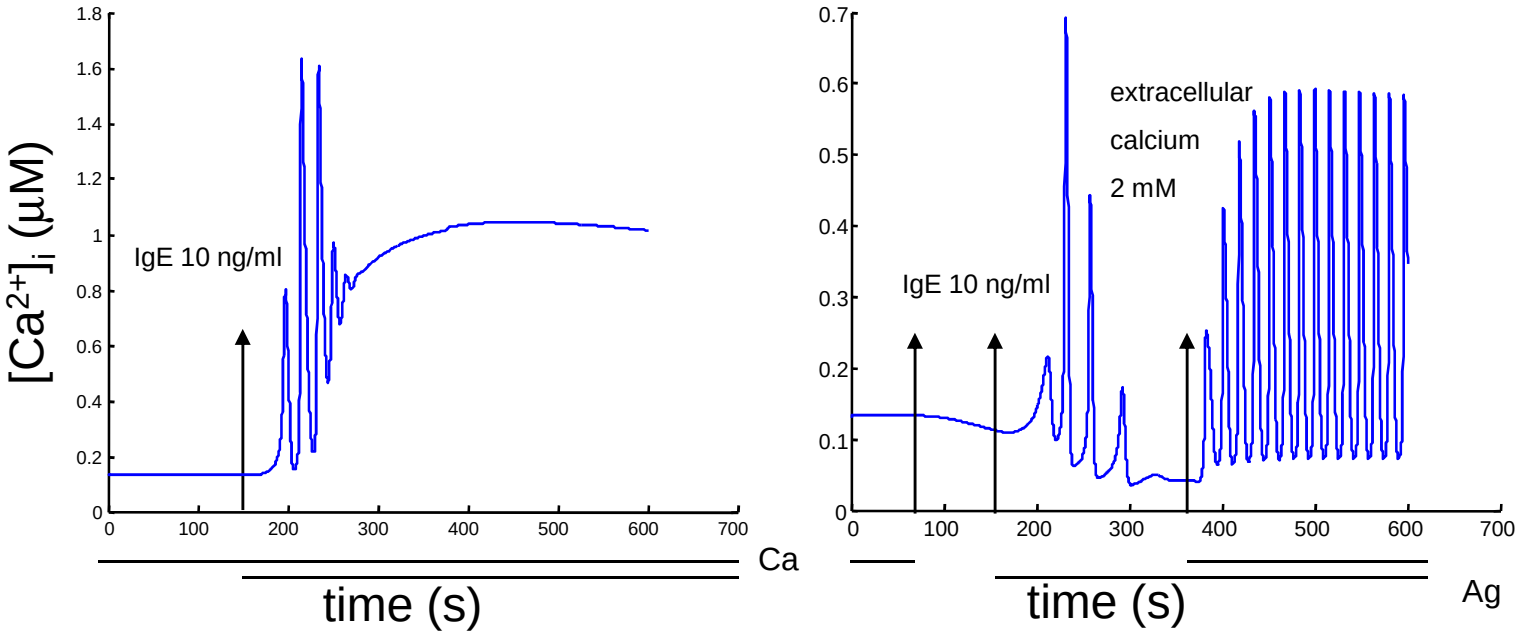


Matching simulations to data

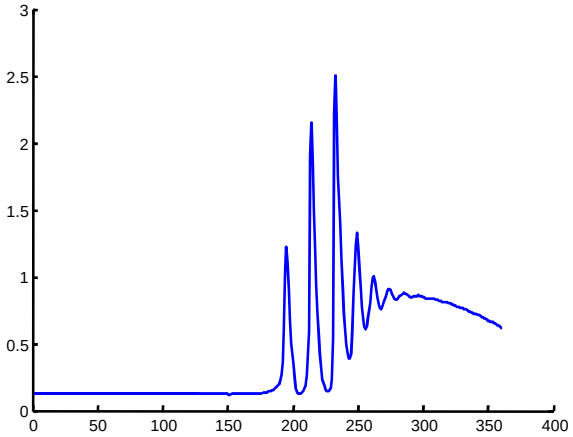
measurement



simulations

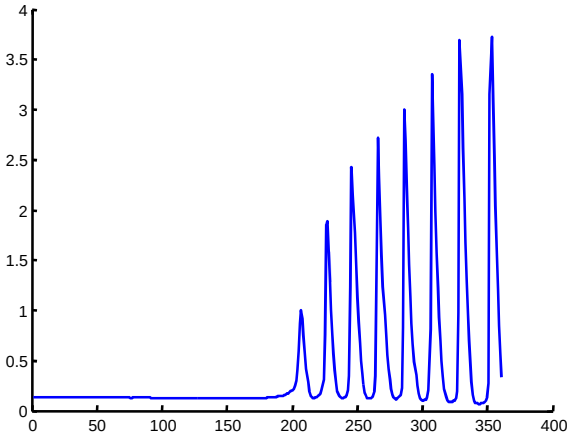


One can use simulations to evaluate potential effects of changing IP_3R levels



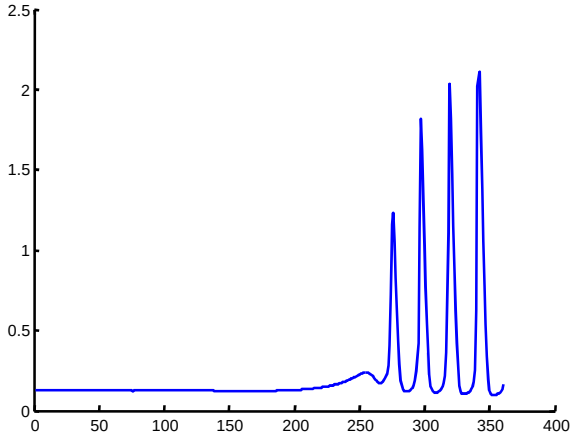
high

(28,000 tetramers)



medium

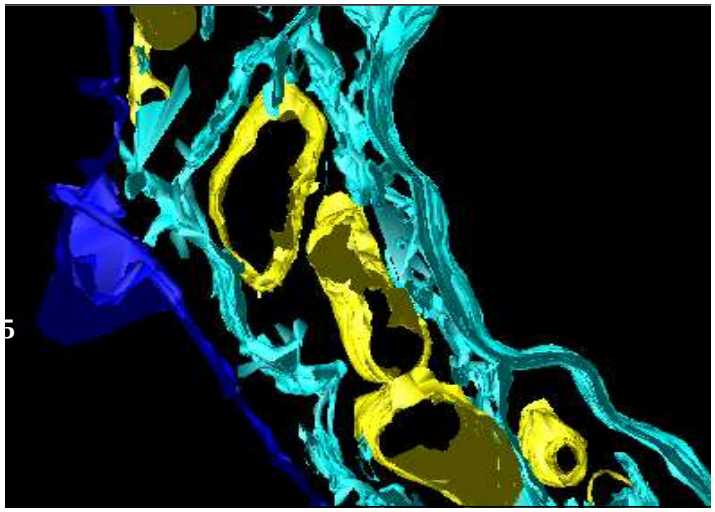
(14,000 tetramers)

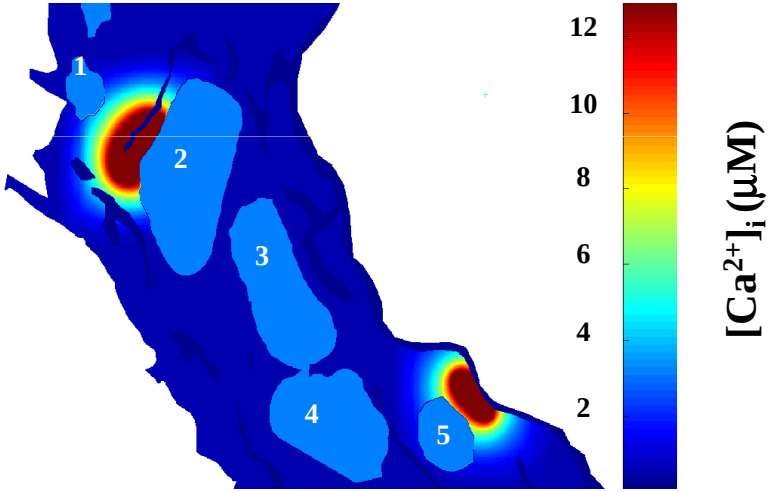


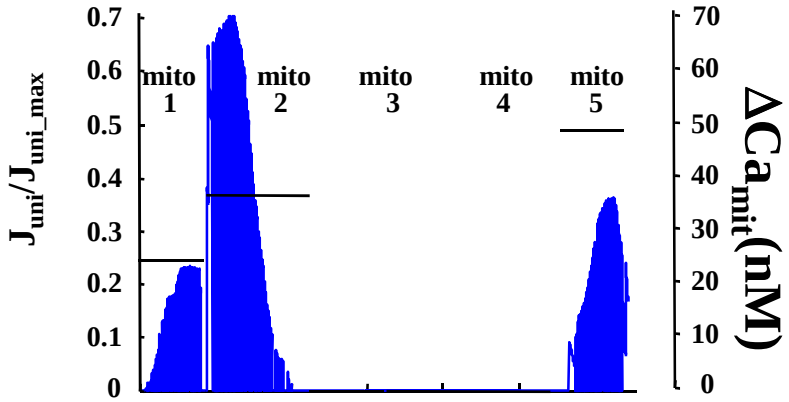
low

(3,500 tetramers)

Simulating Local Activation of Mitochondrial Transport







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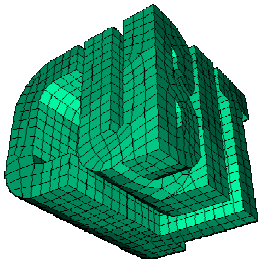
- Jason Shepherd, SNL
- John Fowler



Digital
Data
Viewer

Salsa

THE UNIVERSITY OF NEW MEXICO • HEALTH SCIENCES CENTER
SCHOOL OF MEDICINE

New Mexico Center for Spatio-Temporal Modeling

- Emphasis on cell signaling in immune function, carcinogenesis
- Signals are initiated & propagated at membrane: roles for membrane domains
- Also consider cell geometry, fine-scale spatial features
- Data acquisition depends on variety of biochemical, biophysical and microscopic techniques

UNM Experimentalists

Janet Oliver
cell biologist

Bridget Wilson
cell biologist

Diane Lidke
biophysics

Keith Lidke
optical physics, instrumentation development

UNM modelers

Jeremy Edwards
stochastic & deterministic methods

Stanly Steinberg
spatial statistics, anomalous diffusion

National Laboratory Partners

Bill Havalacek

Byron Goldstein
Rules-Based Modeling

Yi Jiang
tumor modeling

Andrew Bradbury
recombinant probes

Anup Singh
microfluidics devices