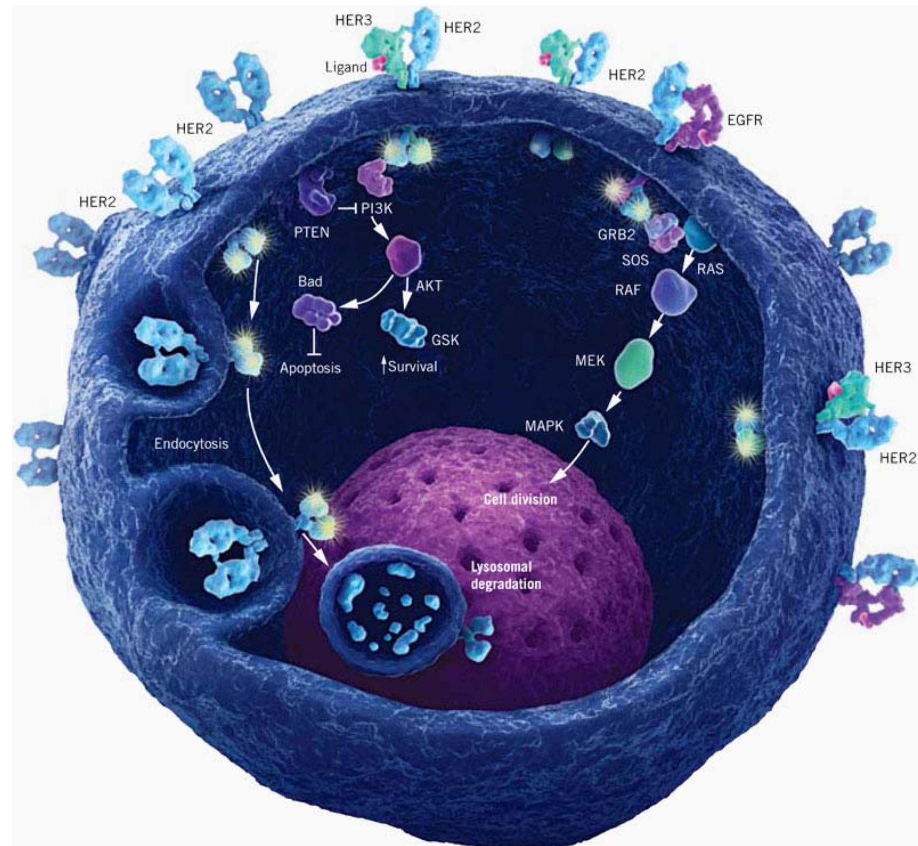
# Wilson MSM project focuses on the control of Signaling through Spatio-Temporal Organization

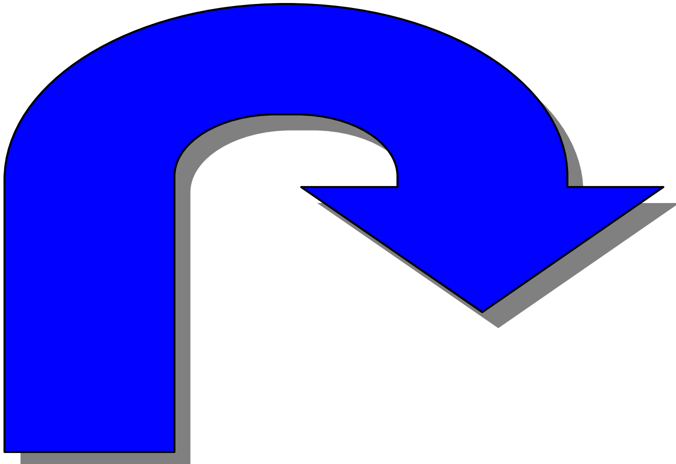
- Signals are initiated & propagated at membrane
- Membranes can be organized into subdomains
- Membrane reorganization during signaling is dynamic
- Data acquisition depends on variety of biochemical, biophysical and microscopic techniques
- Math and physics are challenging
- Powerful computing needs for imaging, analysis, visualization, simulation




one artist's view of ErbB receptors, randomly distributed in the membrane. Evidence suggests this view is not accurate.

http://www.biooncology.com/bioonc/images/her-main-lg.jpg

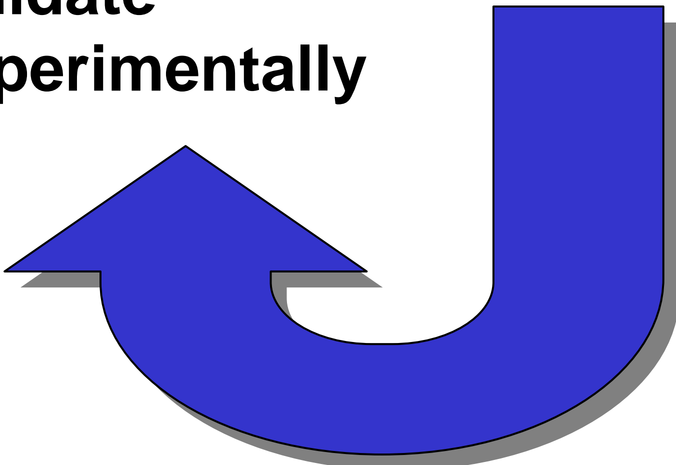
**Philosophy of the UNM Multi-Scale Modeling Team**



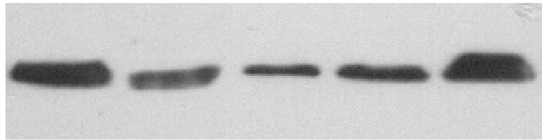
Model Data

Acquire Data

Validate Experimentally

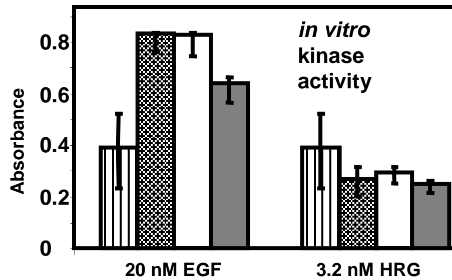


We use a variety of QUANTITATION methods to acquire data for modeling.
This presentation focuses on imaging and image analysis, as well as simulation results



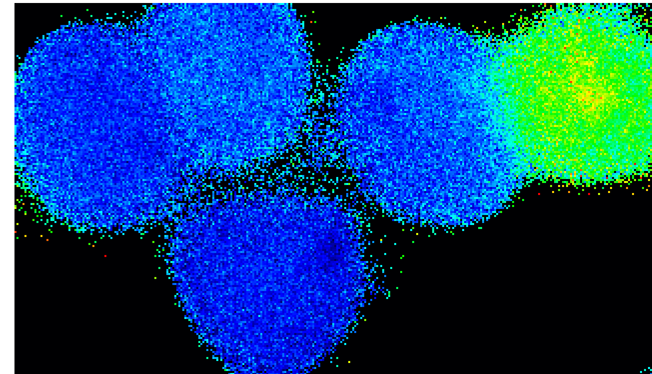
40 20 ng standard
0.25 0.5 1.0 lysate (cells x $10^6$)

western blotting



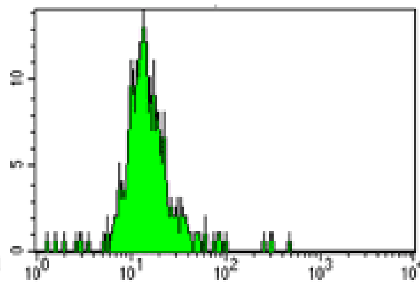

biochemical assays

live cell imaging

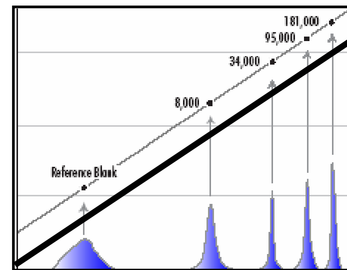


intracellular $Ca^{2+}$

flow cytometry

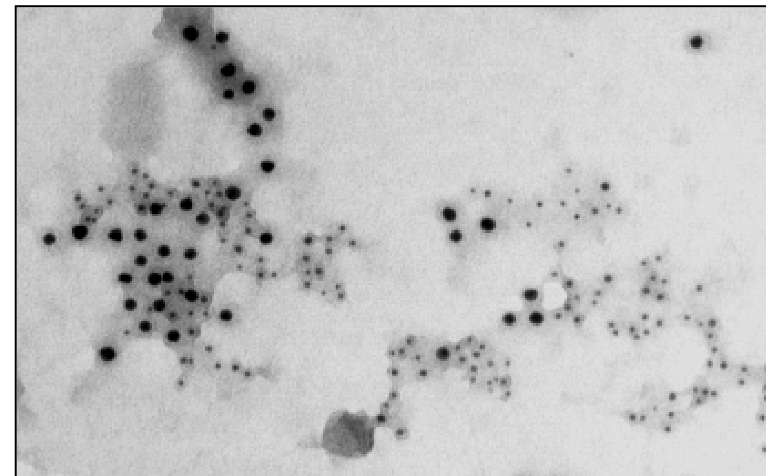


fluorescence (mAb binding to cell surface or intracellular target protein)

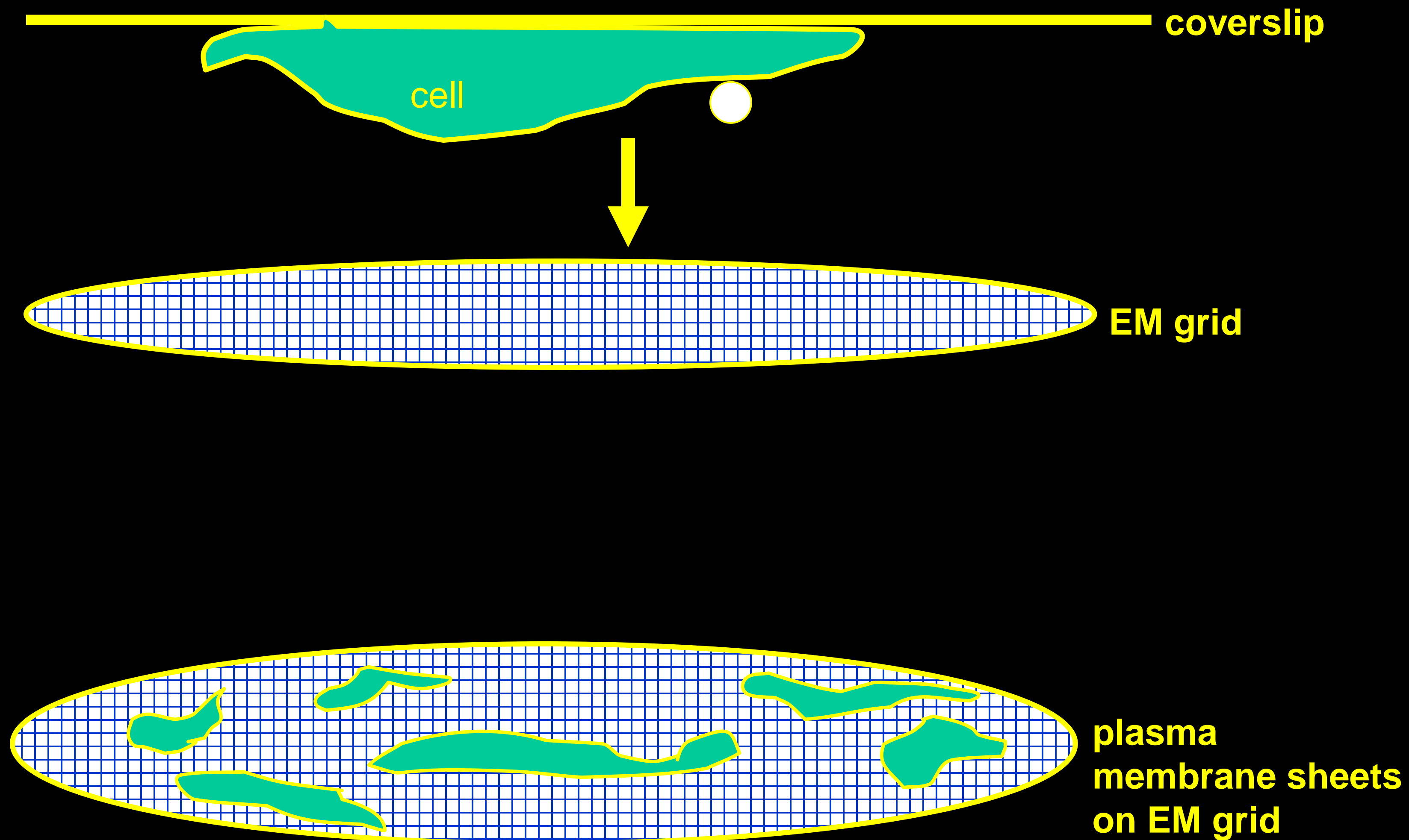



fluorescence (mAb binding to bead standards)

electron microscopy

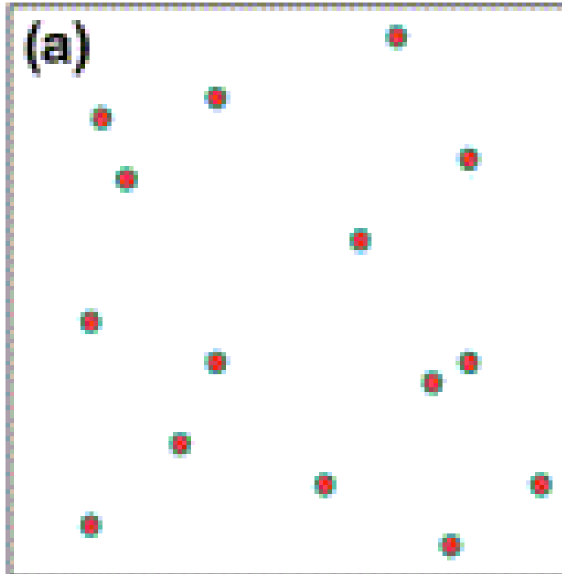


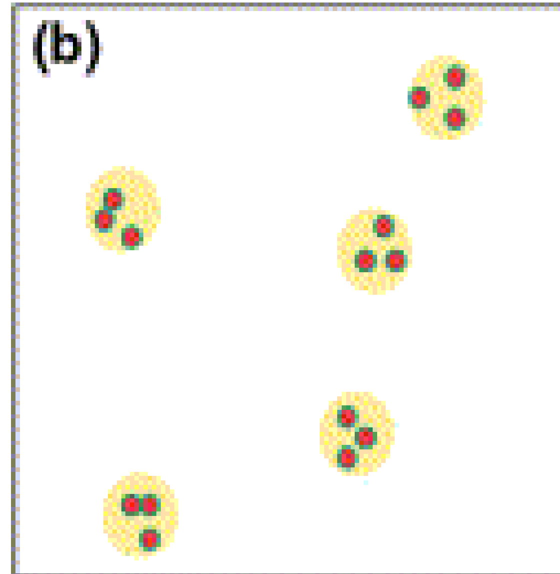
# Electron Microscopy Methods to Explore Microdomain Organization of Membranes

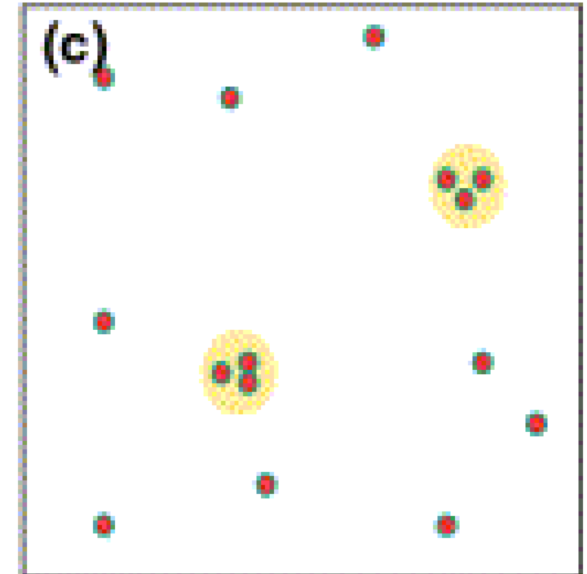



Samples are fixed with PFA before labeling & postfixed, stained, etc before TEM

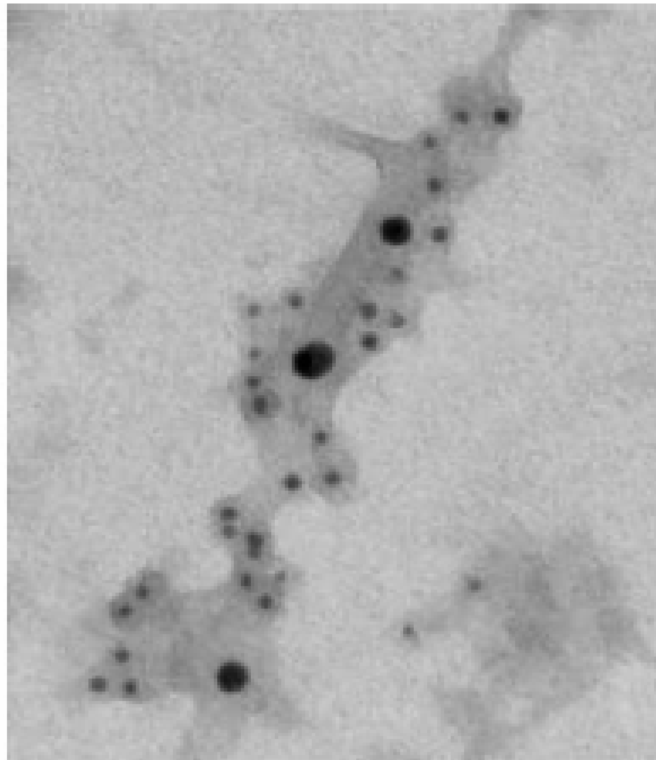
# Possible distributions of membrane constituents



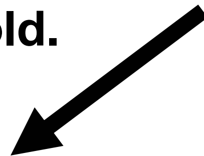



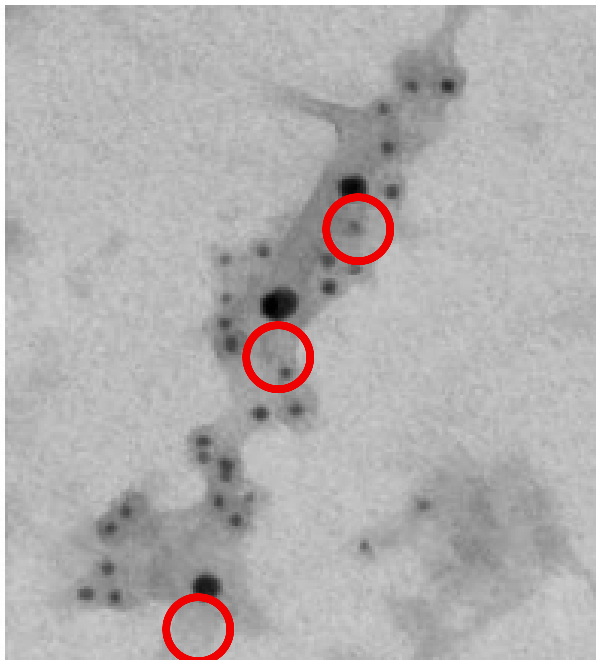



# We use spatial statistics methods to evaluate patterns in EM images.

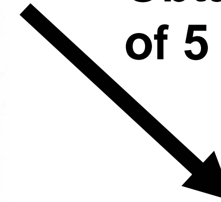


Obtain coordinates of 10 nm Gold.





Obtain coordinates of 5 nm Gold.



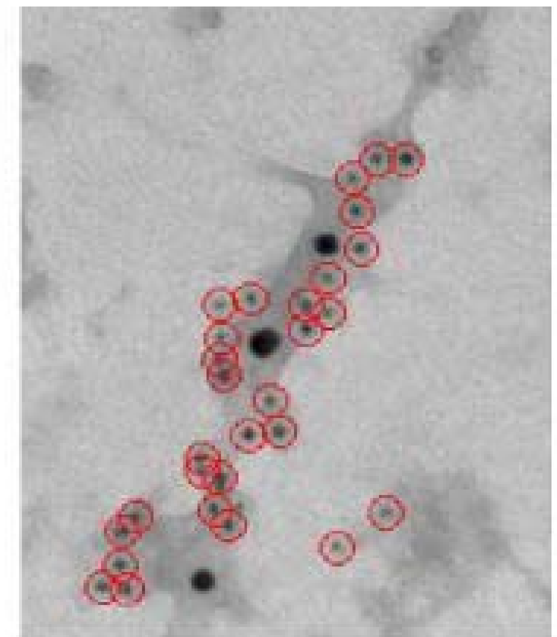
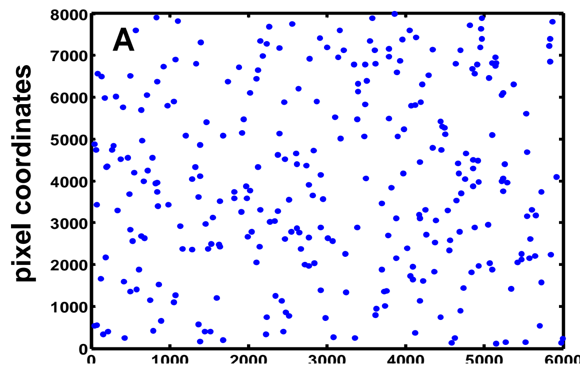


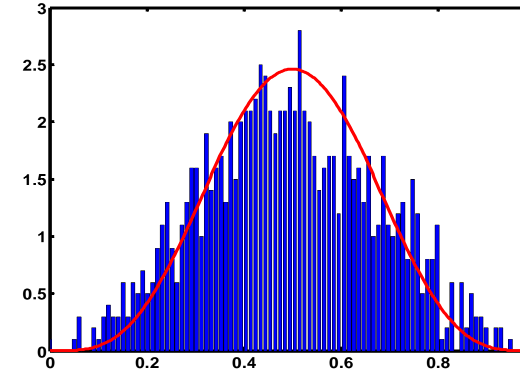
Image processing of a double-labeling experiment

Jun Zhang

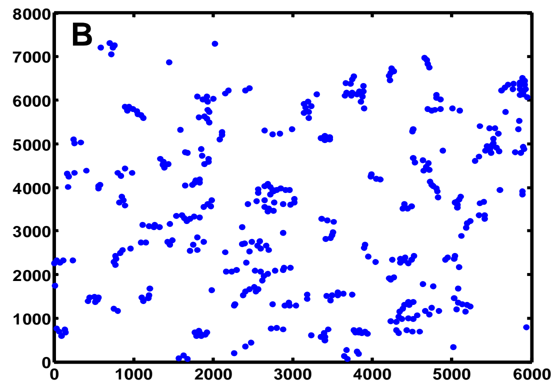
# Image processing & statistical analyses

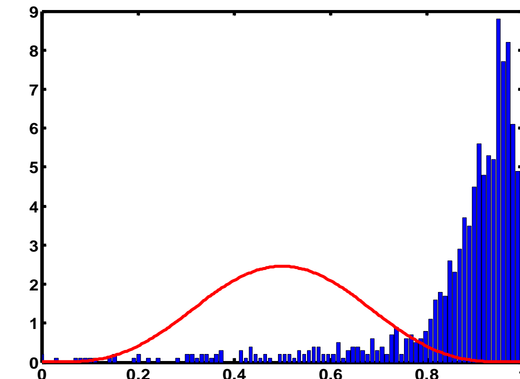
**Random (Computer Generated)**



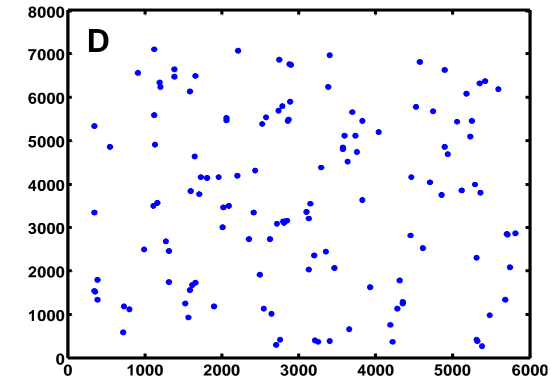

**Experimental Data 1 (Thy-1)**

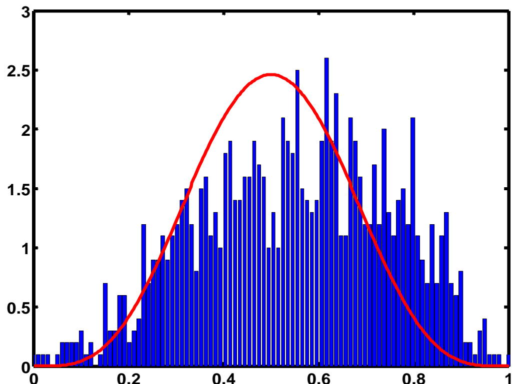



**<u>Most</u> membrane components studied thus far are non-random.**

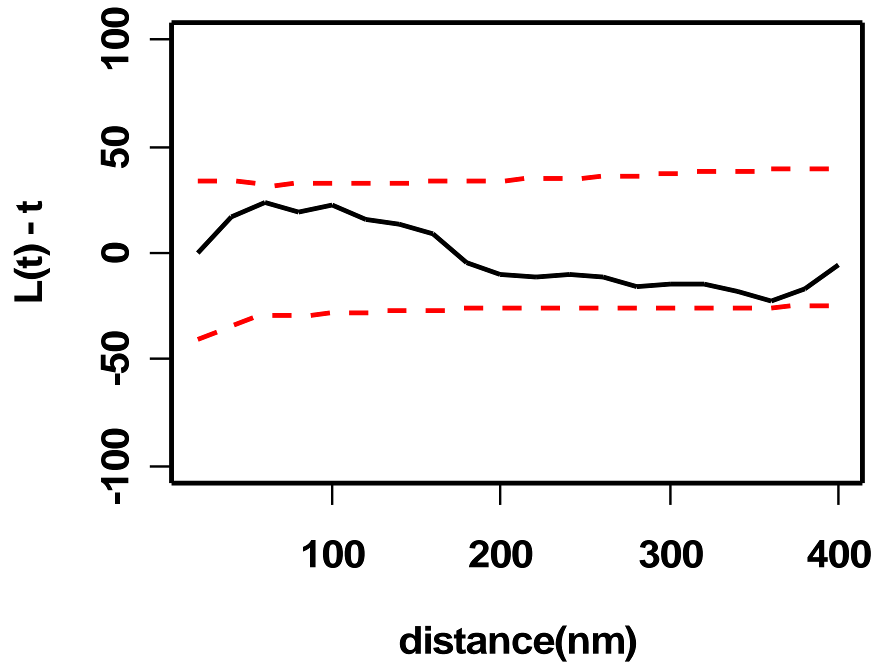
**Experimental Data 2 (GM1, Prefixed)**

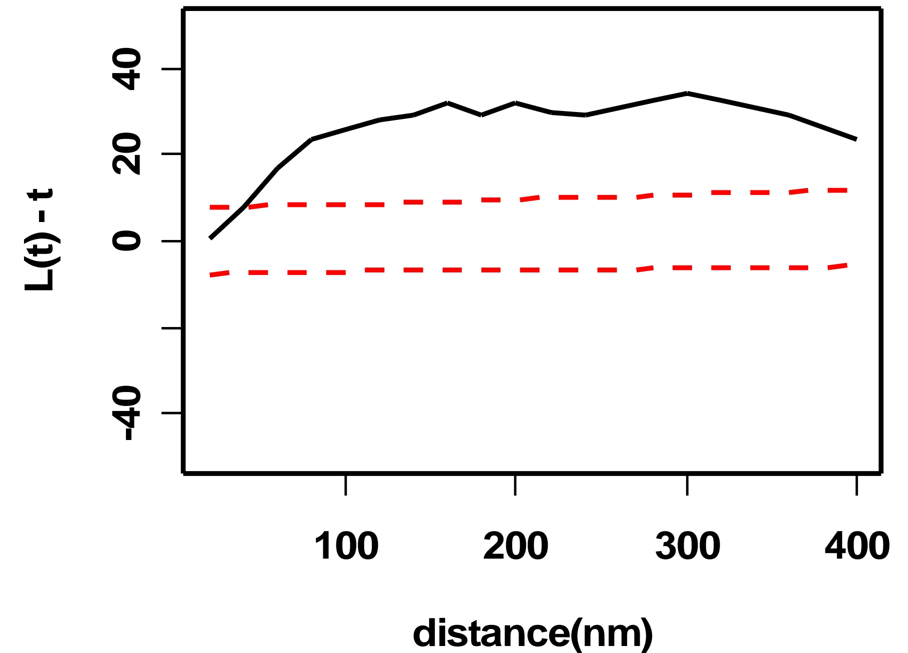


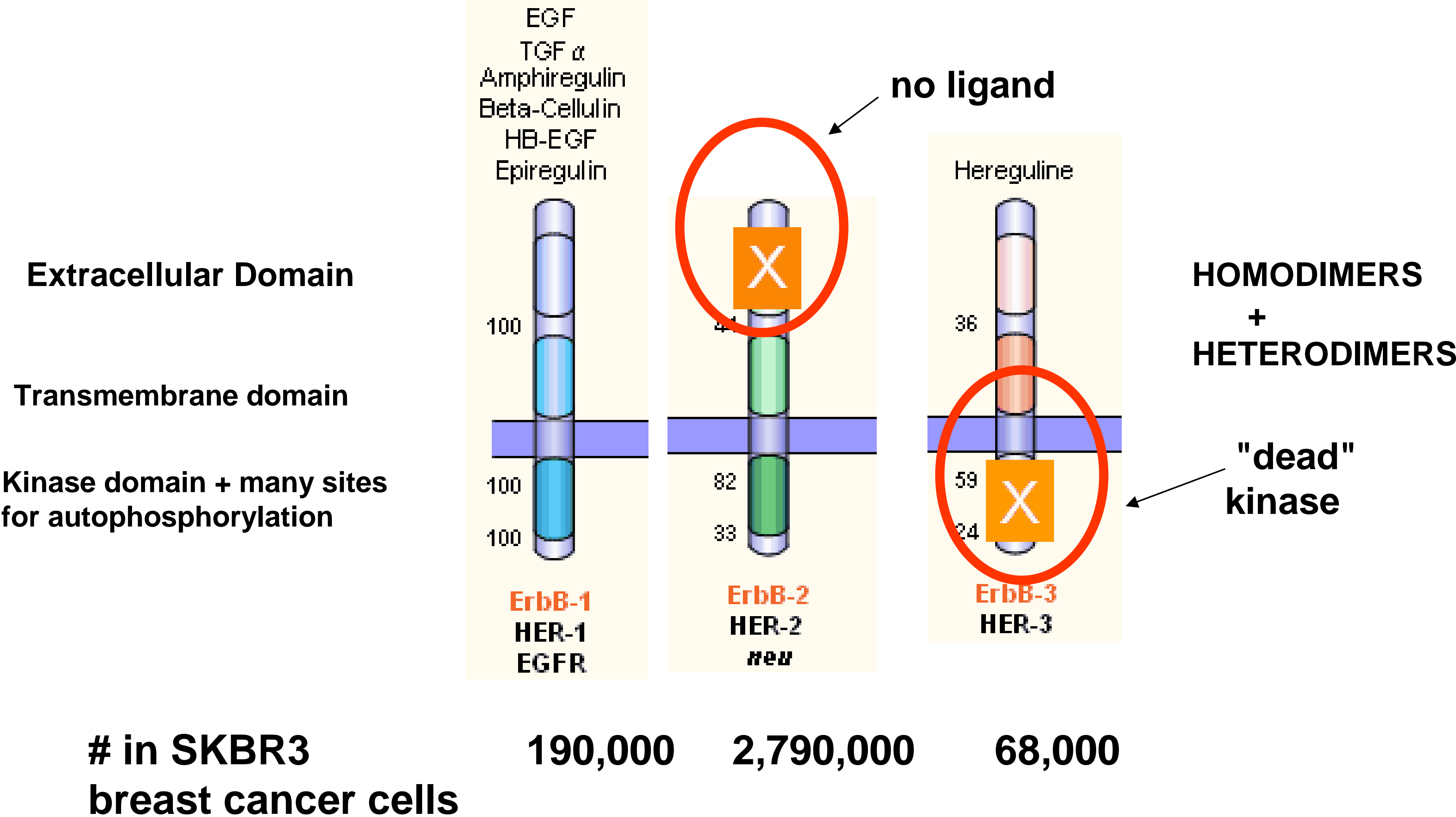

**We also apply Ripley's K statistic and co-clustering algorithms.**

# The Ripley's bivariant test is used to determine if co-clustering of two species is significant

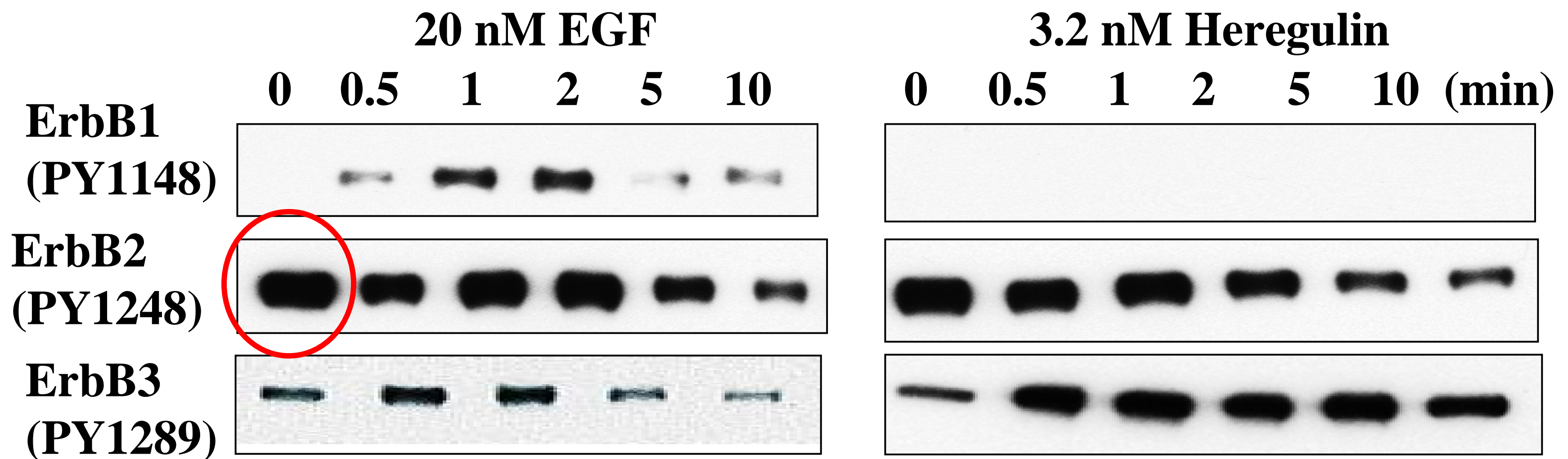


# EGFR/ErbB Family of Receptors

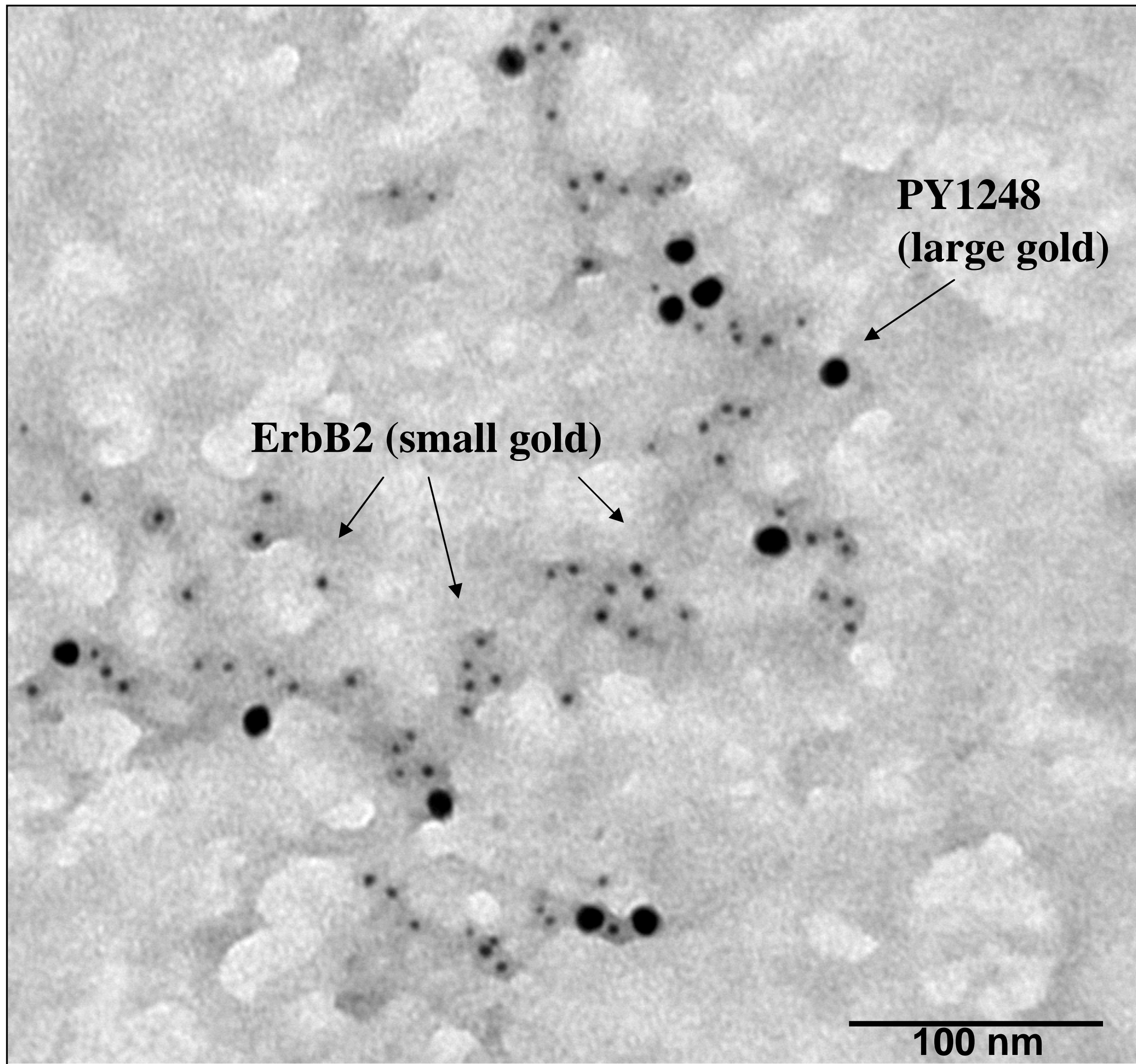


# The traditional way to track phosphorylation is using Western blotting.

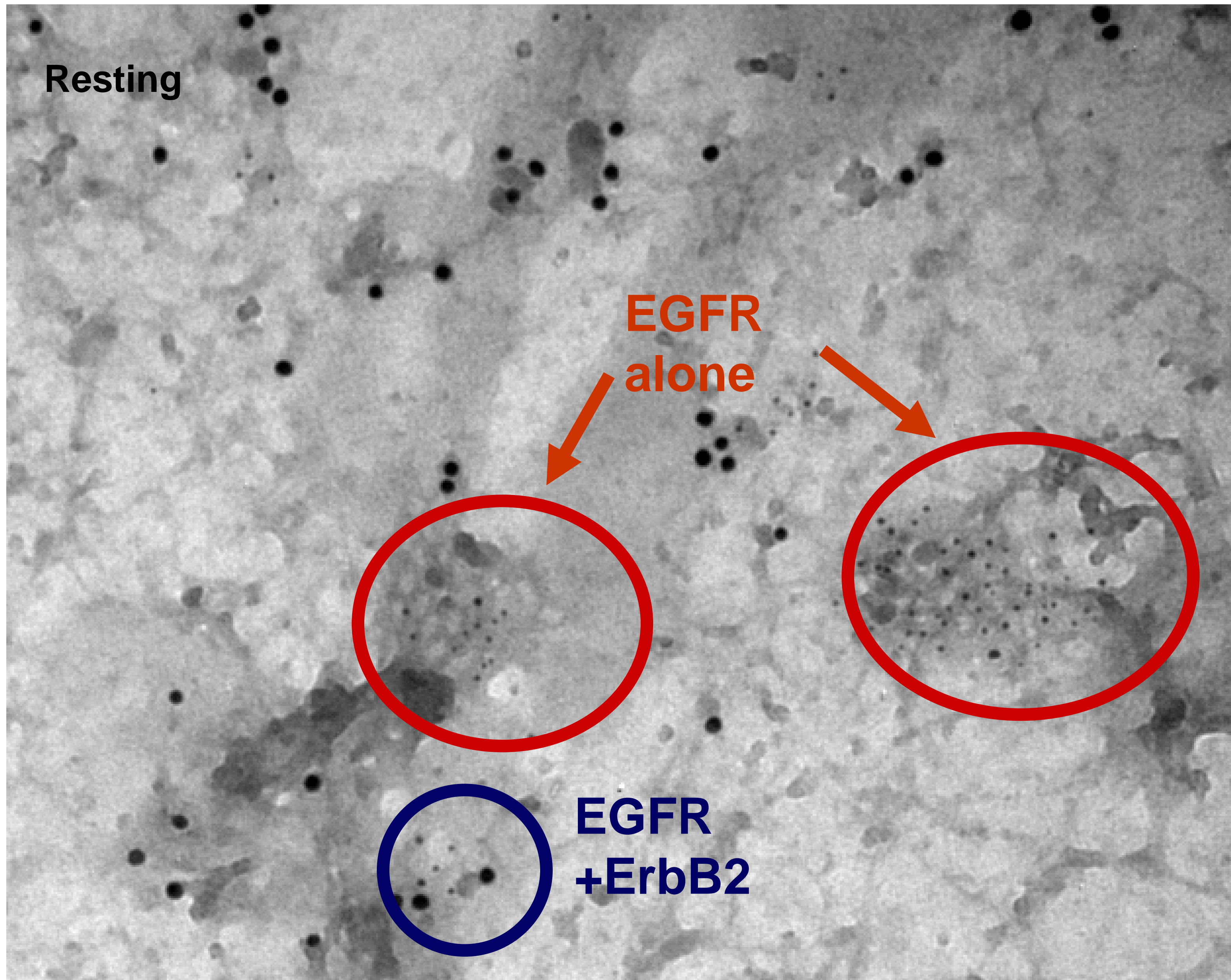



These results are from SKBR3 cells. Notice that overexpressed ErbB2 is already ACTIVE before ligands are added for its presumed dimerizing partners.

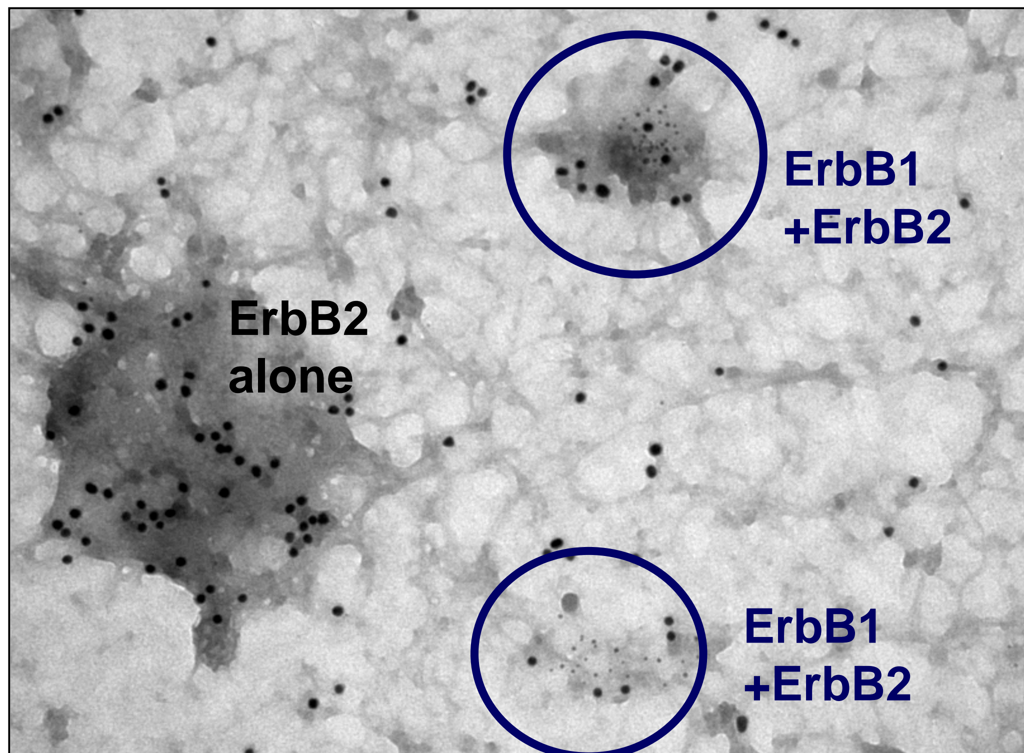
**Using EM methods, you can evaluate <u>both</u> phosphorylation state and topographical distribution of receptors. This image is from our recent *J. Cell Science* paper. It shows that ErbB2 is ACTIVE and PRECLUSTERED in serum-starved SKBR3 cells. Clusters do not break up when cells are treated with ErbB2 kinase inhibitors.**



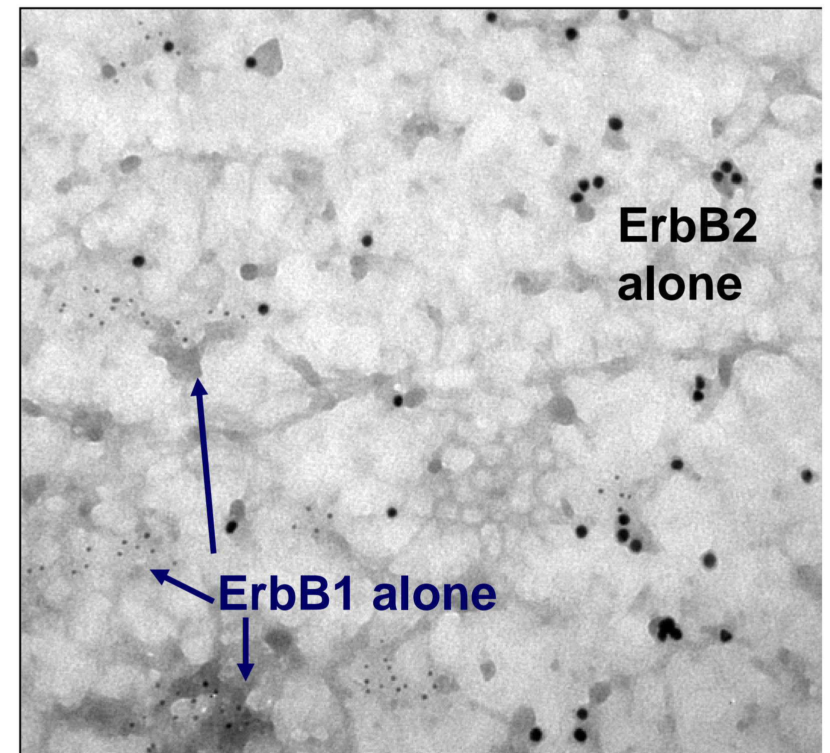
**In addition, a key result of this paper..... is that there is relatively little mixing of EGFR & ErbB2 clusters.**



## After 2' EGF, there is a bit more co-localization of EGFR & ErbB2 but only 30% of images pass statistic test



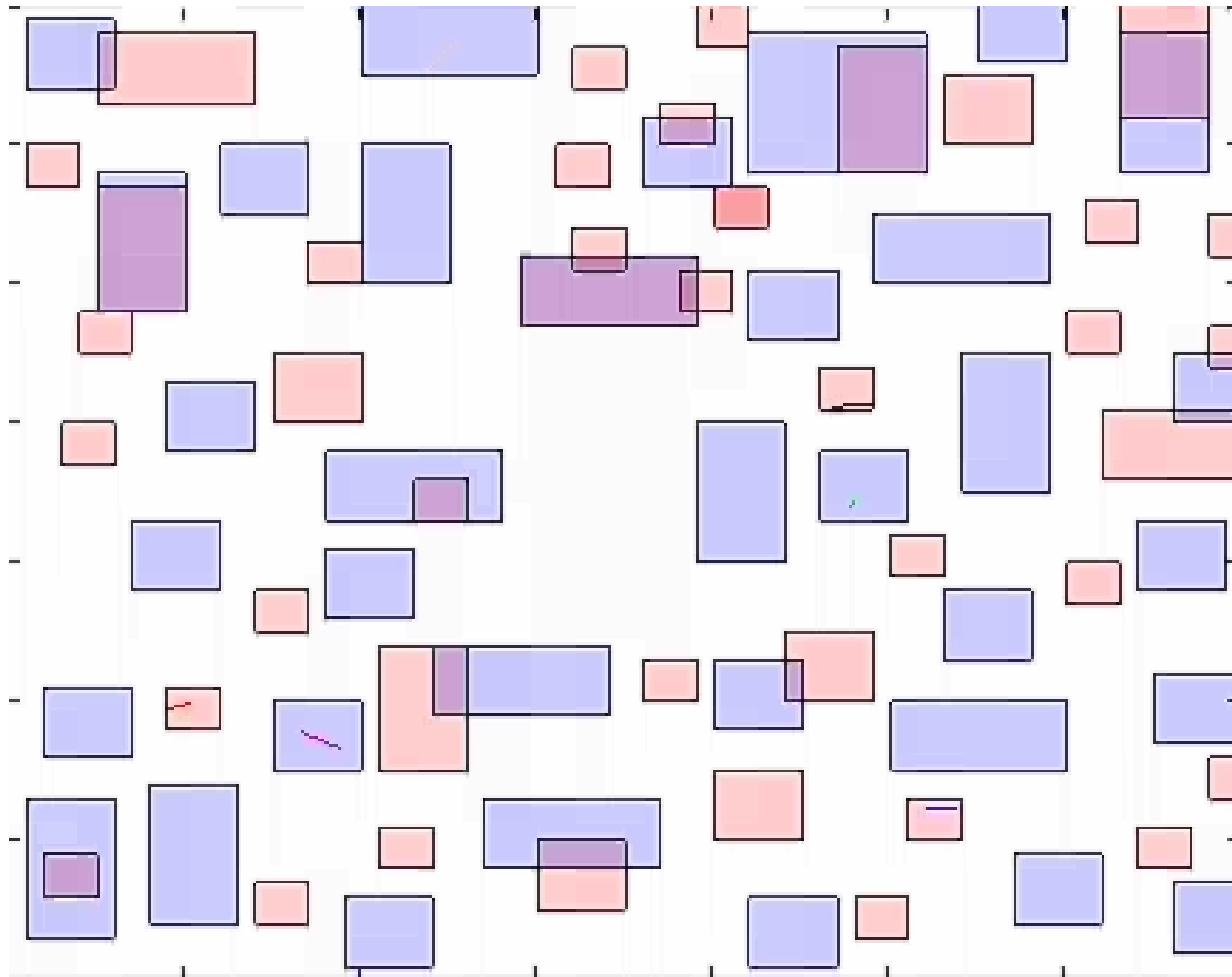

PASS



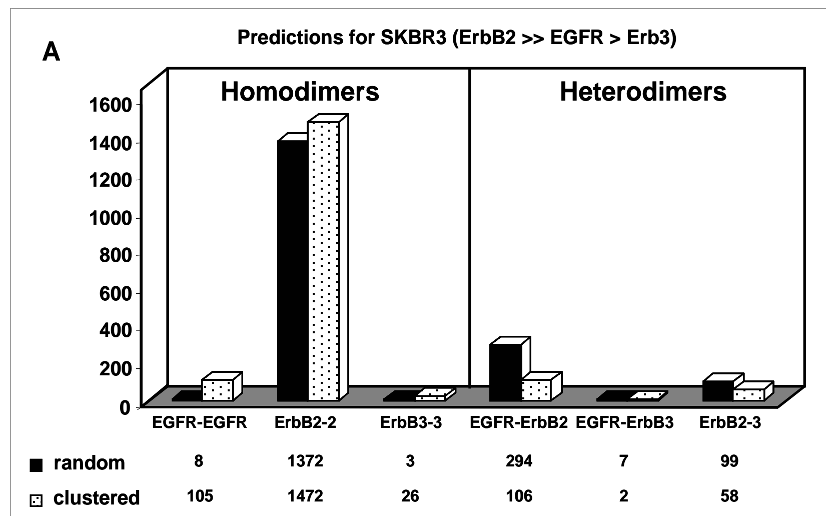

FAIL

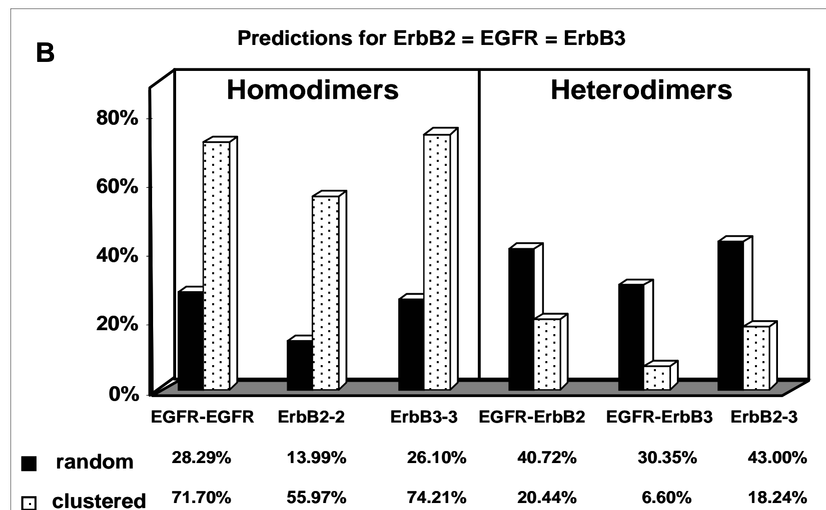
**These data suggest that prior models OVERESTIMATE HETERODIMERIZATION**

**EM approach does not directly measure dimerization. But we can use our agent-based, spatial stochastic model to simulate spatial segregation of receptor clusters. We do this by setting up "domains". Then we simulate receptors diffusing the membrane from one domain to another.**

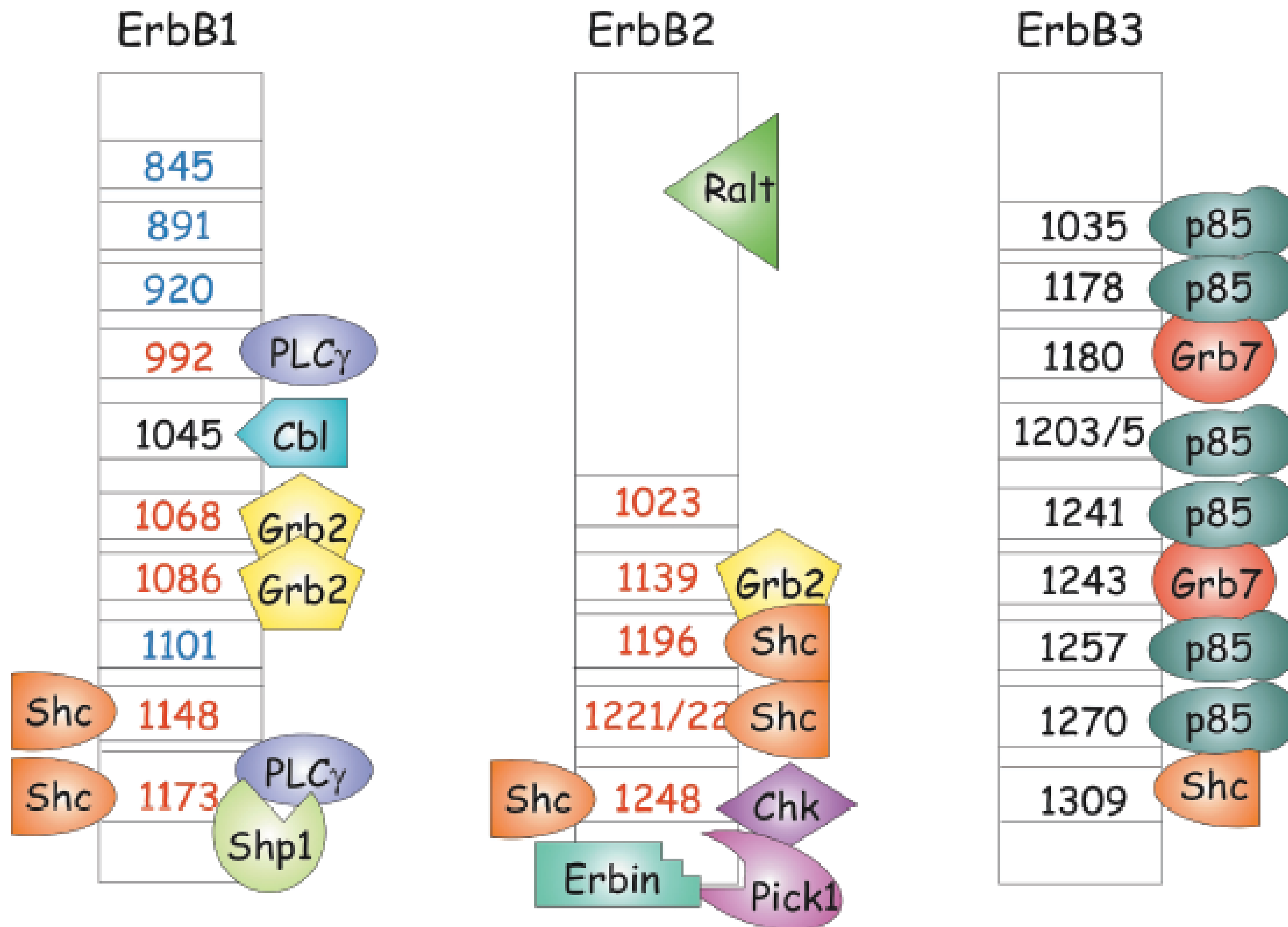


# The spatial stochastic model predicts significant differences from previously published well-mixed (deterministic*) models.



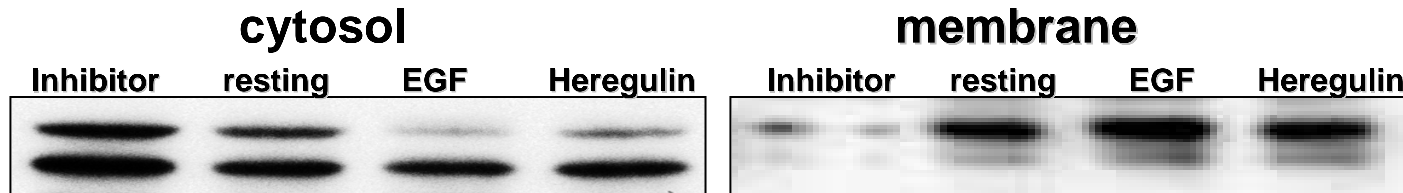



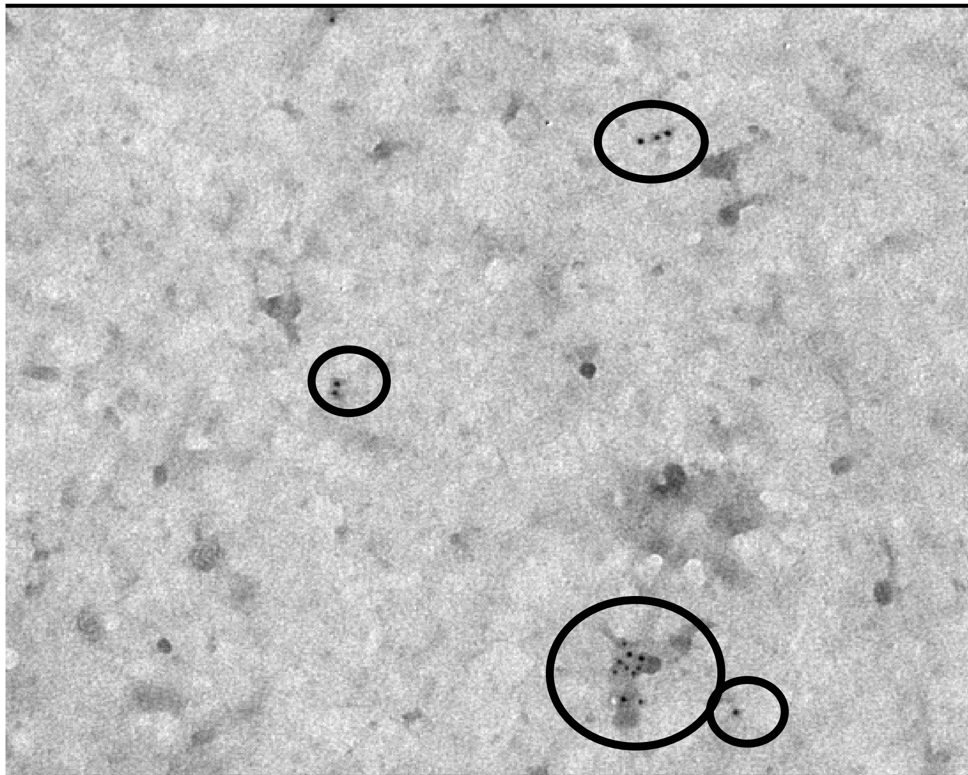
# Why is this important? Because the signals from homodimers and heterodimers are different.

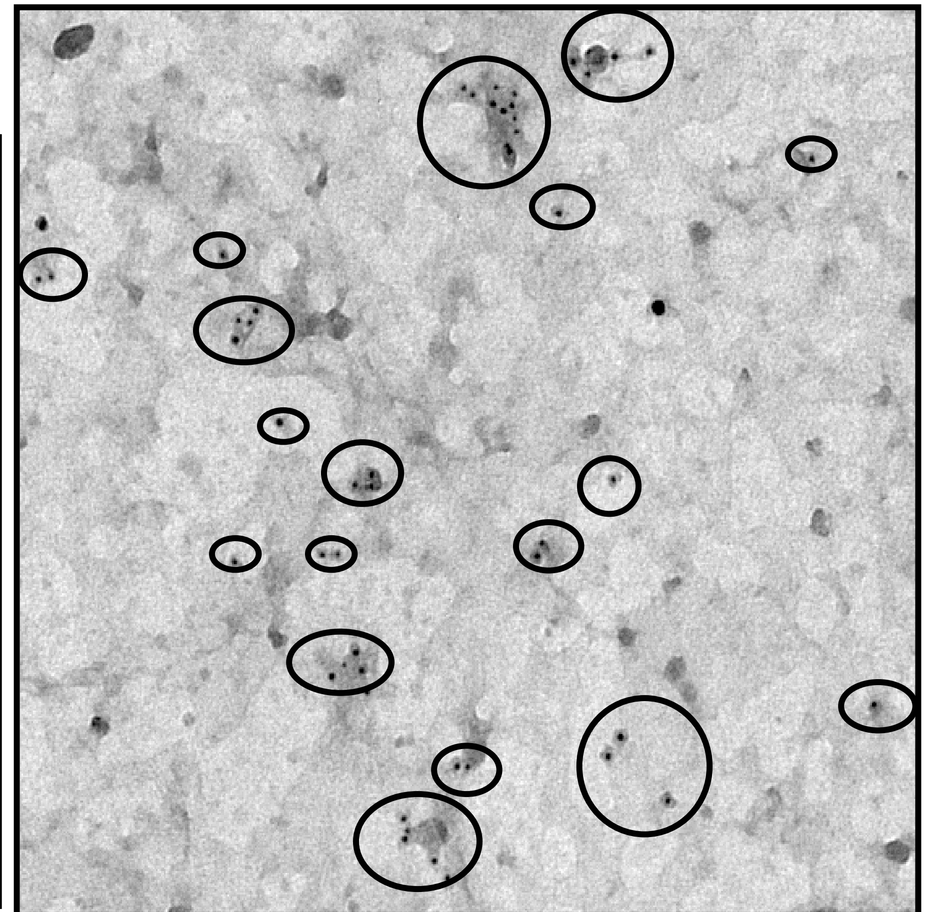



*Breast Cancer Res* 2001, 3:385-389

**We can also use the EM method to spatially map and quantify recruitment of adaptor molecules. The example shown here is SHC.**



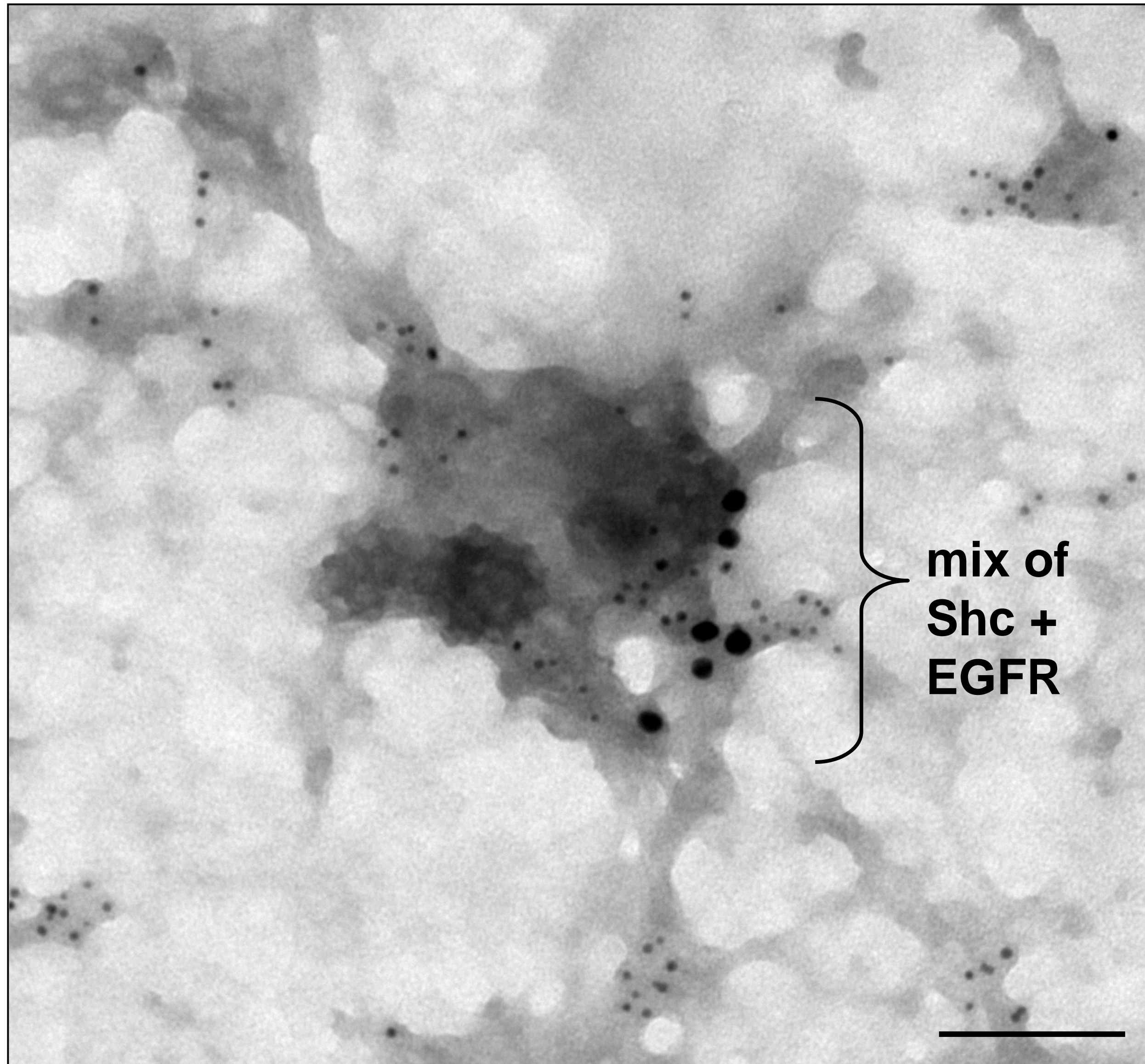





**Resting: 17 particles/sq micron**

**EGF 2': 71 particles/sq micron**

# As expected, Shc is found with activated EGFR

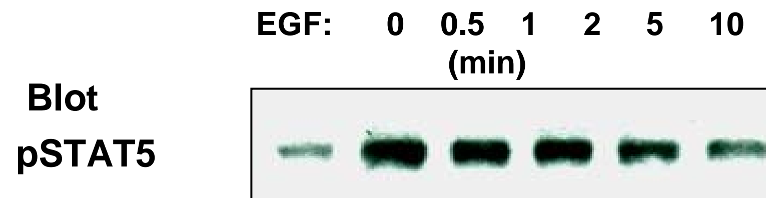



This kind of spatial data is critical for SPATIAL MODELING

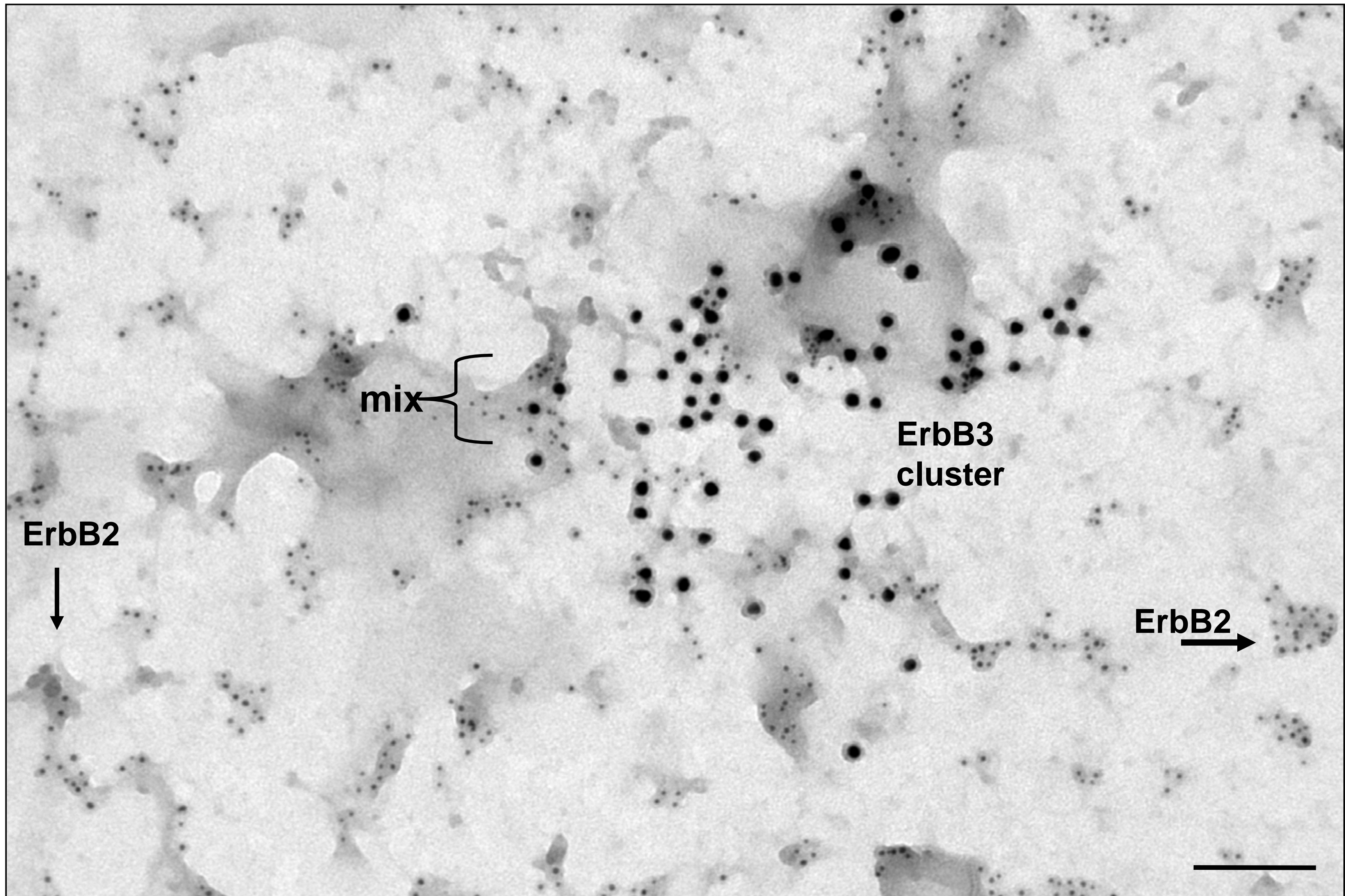
# EM also shows dramatic increase in phospho-STAT5 at the membrane after EGF

resting

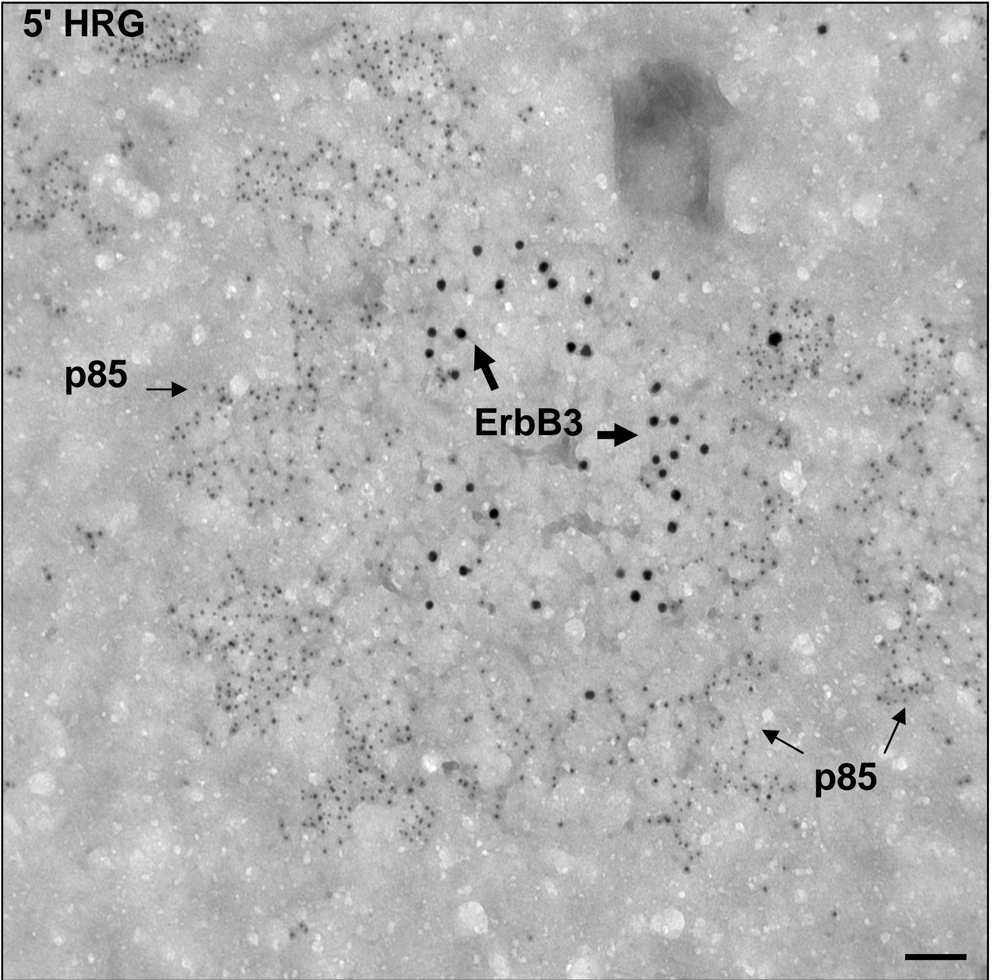
2' EGF

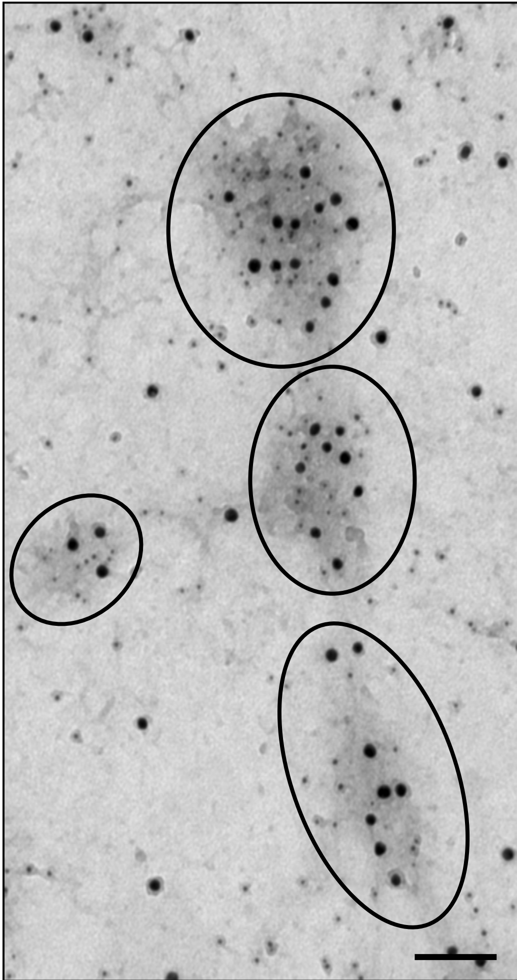


# Dramatic result: ErbB3 clustering in response to Heregulin



# PI 3-kinase is strongly recruited to ErbB3 clusters



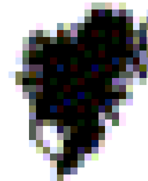



# Membrane components are in constant motion (rotational and lateral movements)

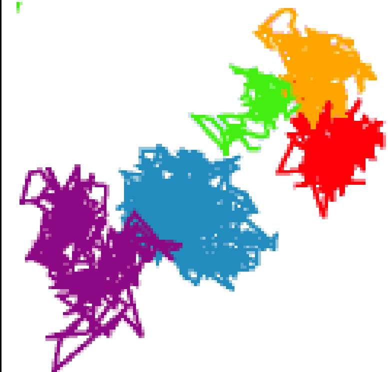
**Membrane constituent ungoing rapid diffusion**

500 nm

**Membrane constituent with restricted diffusion**

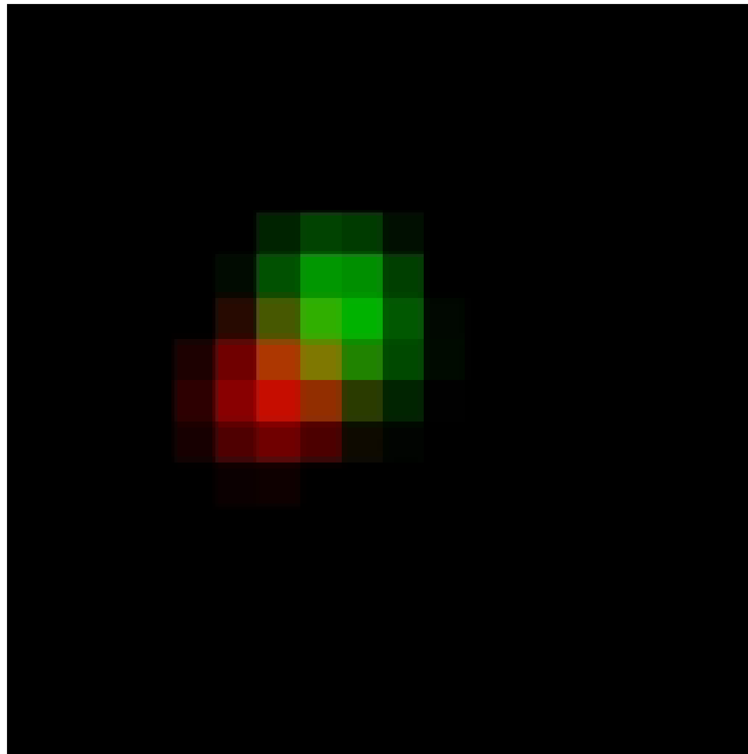


**Restricted movement – then "Hop Diffusion"**



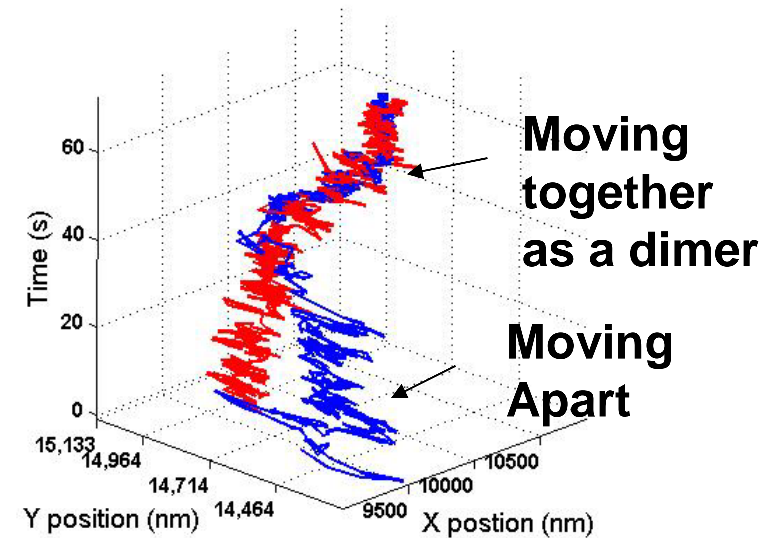
Kusumi laboratory, Nagoya Univ
http://www.supra.bio.nagoya-u.ac.jp/

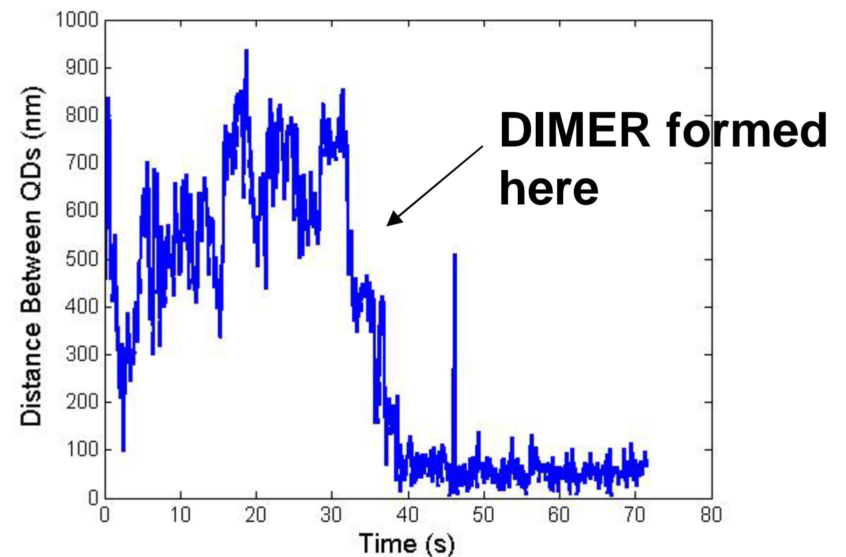
**Dr. Diane Lidke is new member of our MSM team. A biophysicist, she uses novel quantum dot approaches for single particle tracking. With this method, can measure receptor dimer formation in real time.**



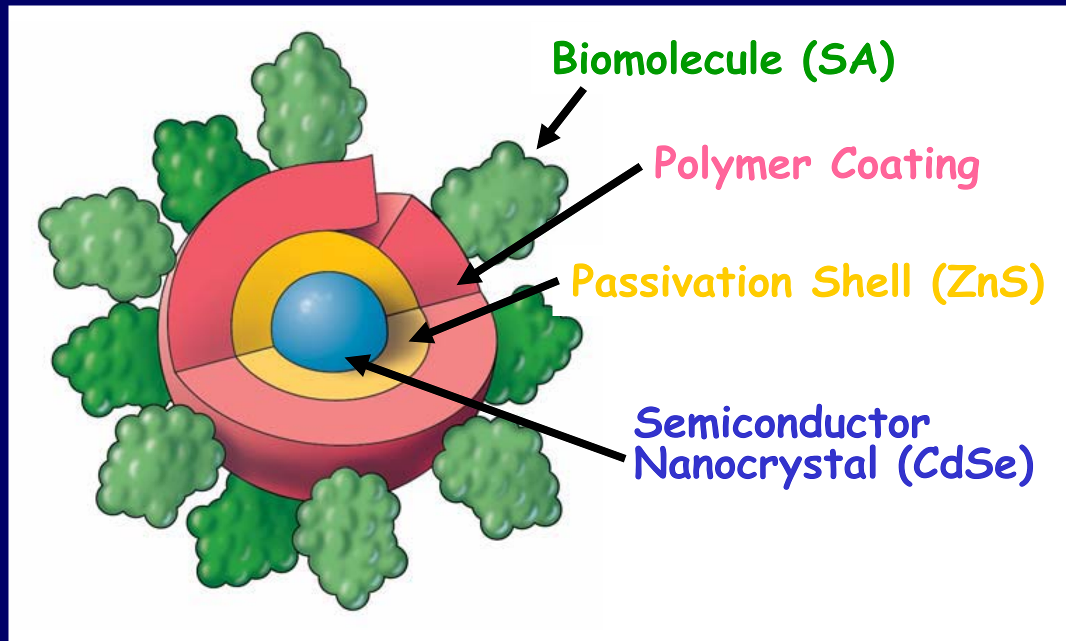
EGF-QD585 EGF-QD655

Interaction dynamics on the cell surface

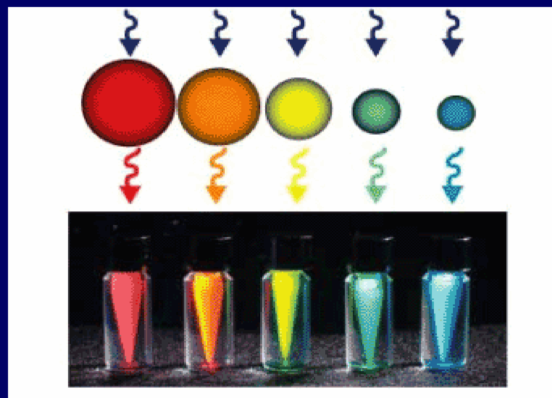


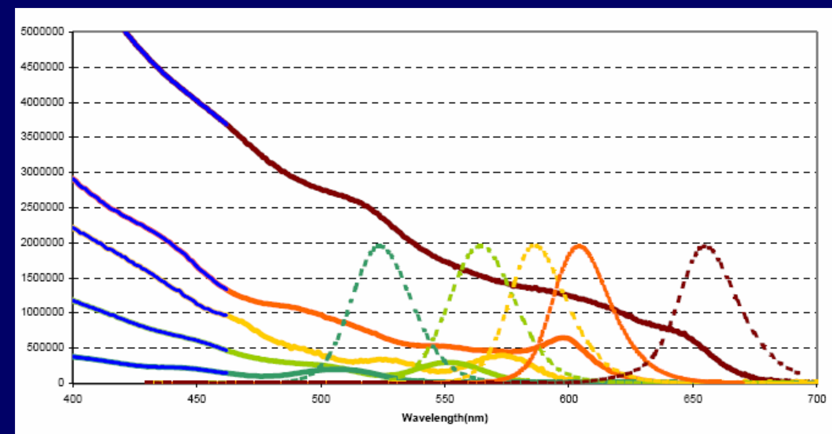



# Properties of Quantum Dots



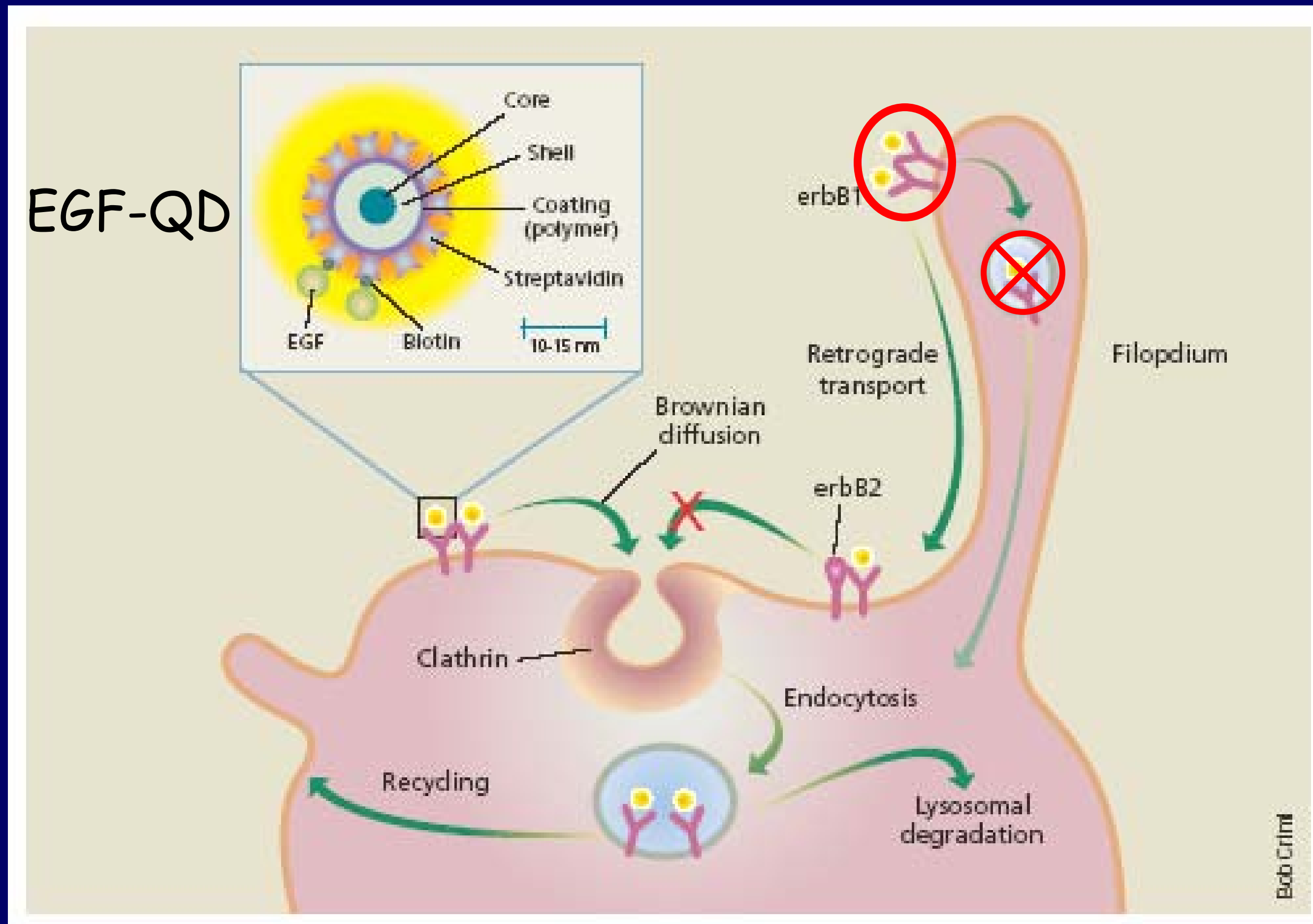

- Broad excitation spectrum
- Narrow emission band
- Brightness
- Photostability
- Single molecule sensitivity
- Bioconjugates (Streptavidin, Protein A, IgG...)
- Non-toxic
- Donors for FRET






*Commercial sources*: Quantum Dot Inc., Evident Technologies

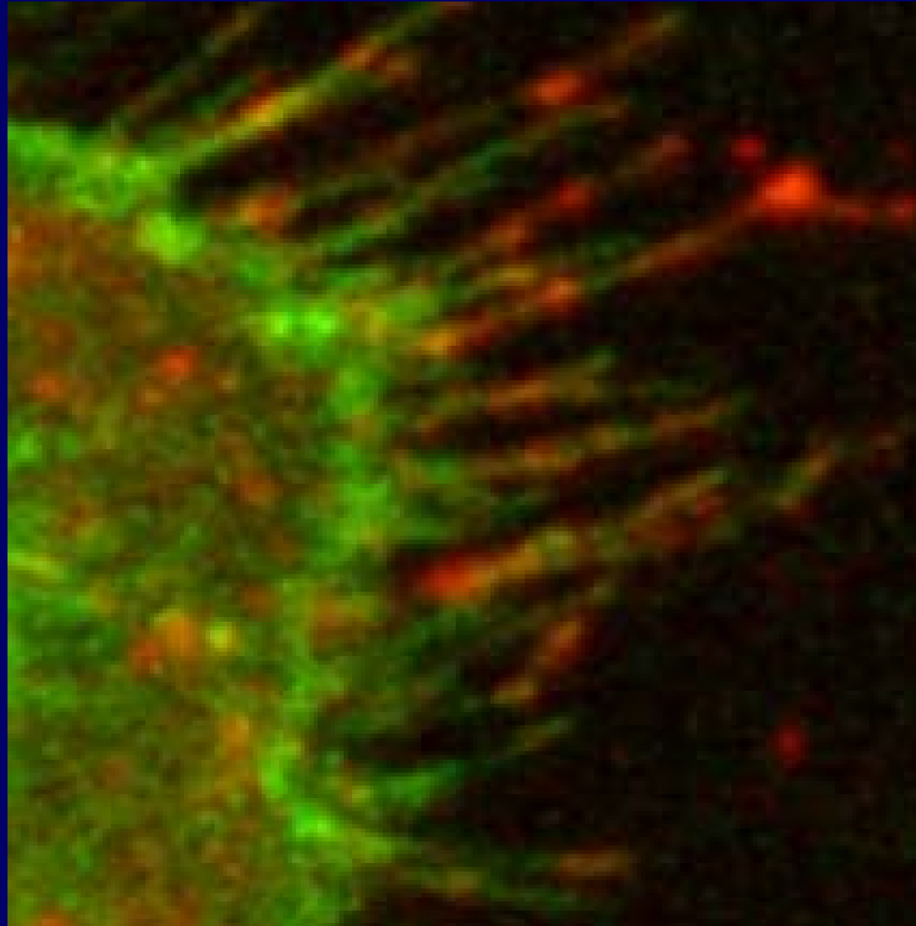
# QDs make it possible to multiple events in live cells…




Gur and Yarden
*Nature Biotechnology*
**22**:169 (2004)

Biotinylated-EGF + Streptavidin QDs = EGF-QD

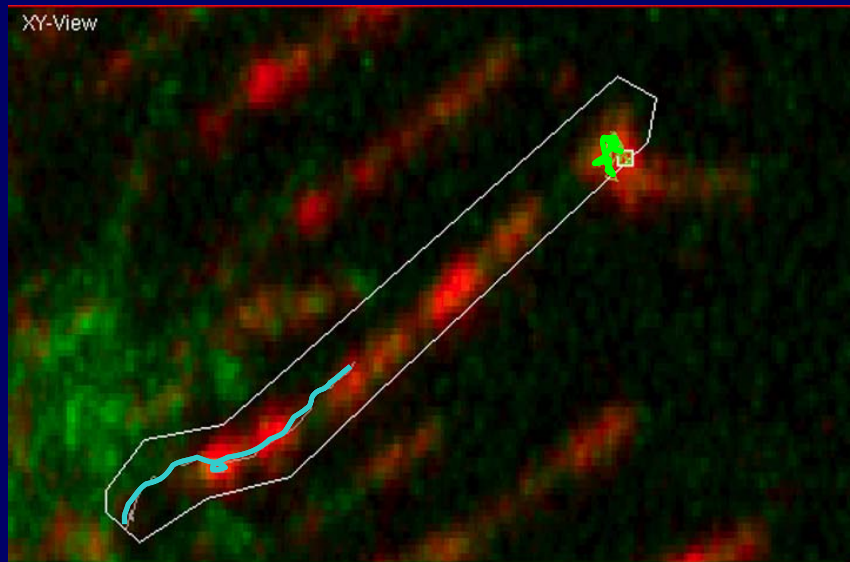
# Retrograde Transport



A431 cell expressing erbB1-GFP (green)
after addition of EGF-QD (red)

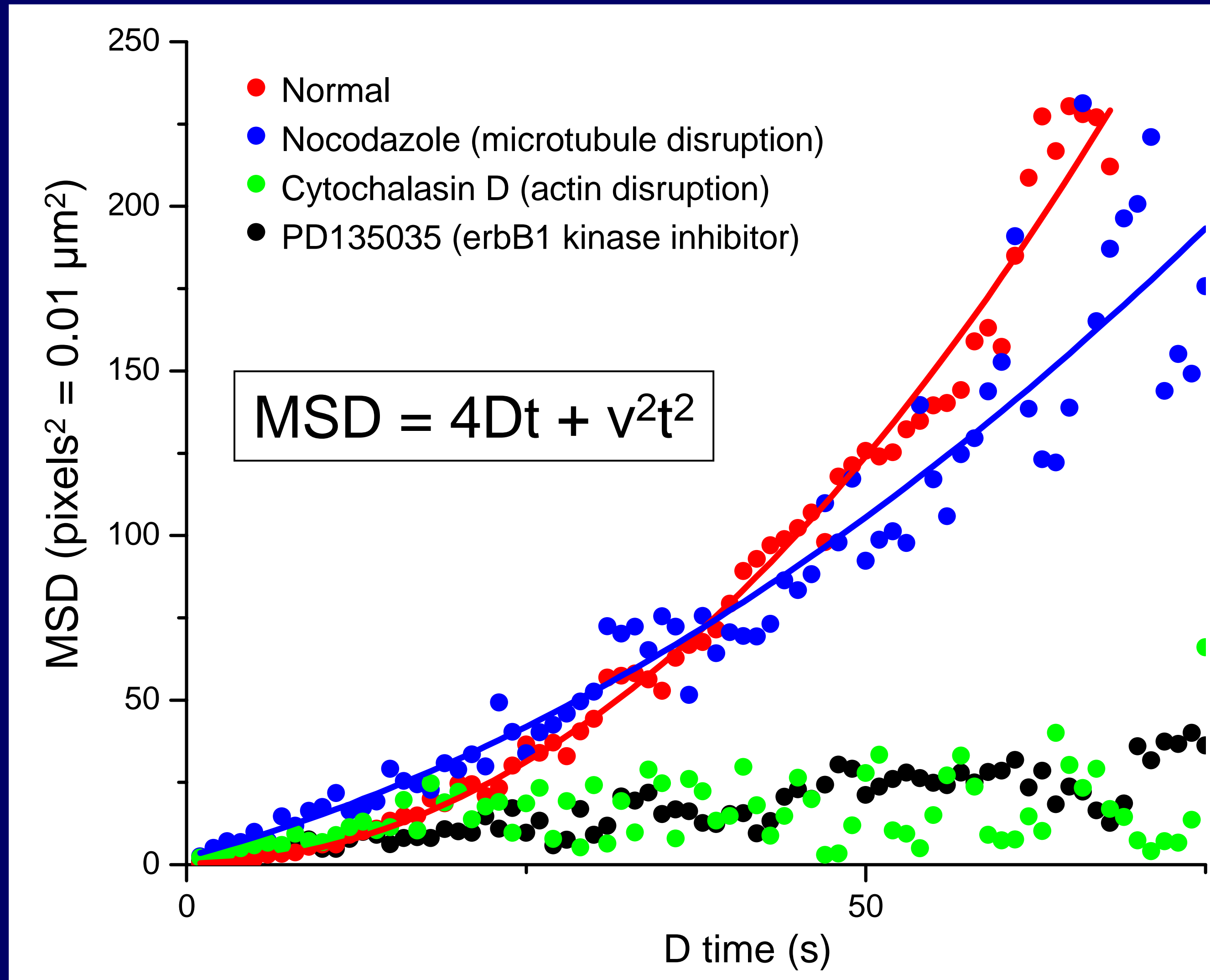
Lidke et al., Nature Biotech 2004

# Tracking Retrograde Transport



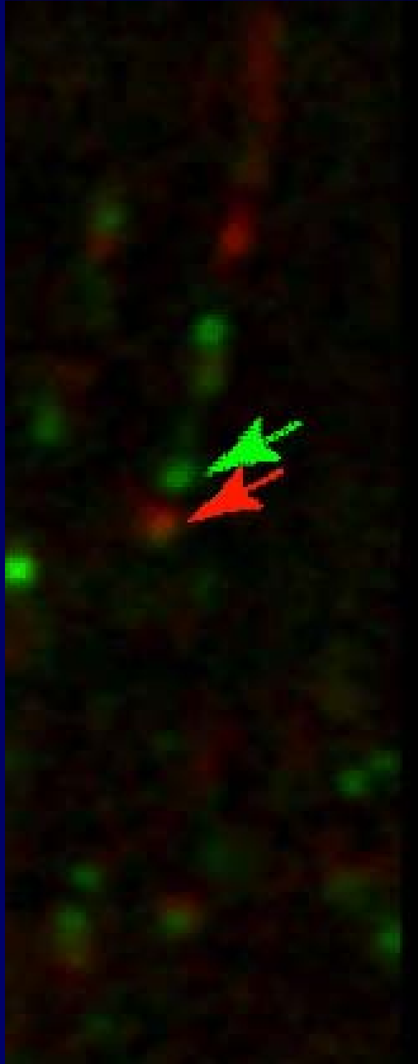

Track loci over time using the “5D Viewer” (Image J plug-in developed by Dr. Rainer Heintzmann) or Matlab/DIPimage routine, which calculates the center of intensity in a region around the maximum in each time step

# Typical MSD plots of QD-EGF-ErbB1 retrograde transport on A431 cells under different conditions




These plots can be fit to determine diffusion coefficients and velocities...

# Minimum requirement for transport is a liganded dimer



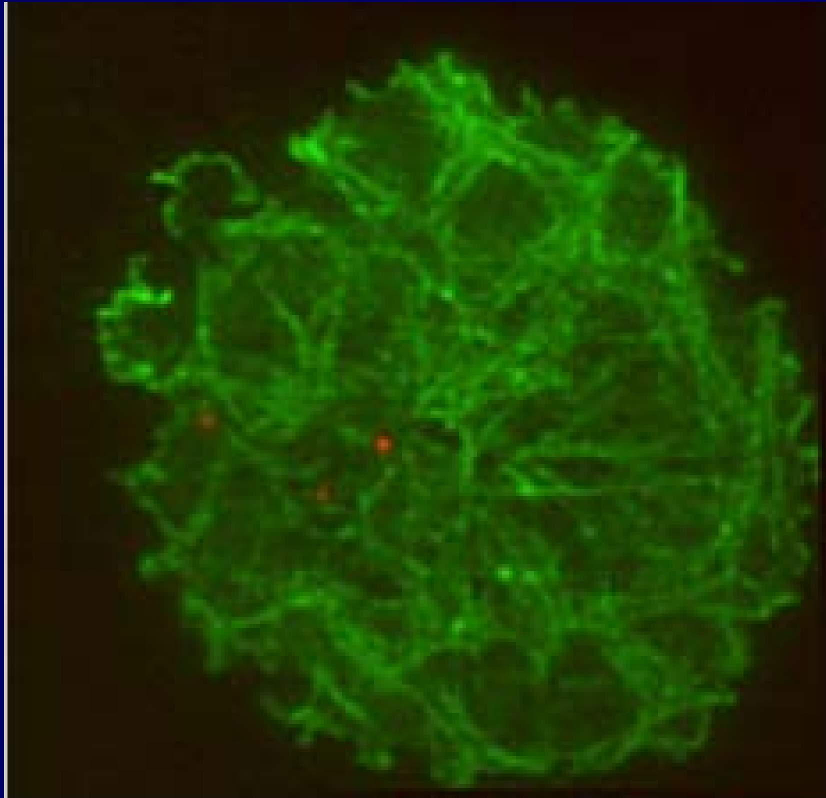
EGF-QD525 (green) and EGF-QD605 (red) are added simultaneously to A431 cells at room temperature.

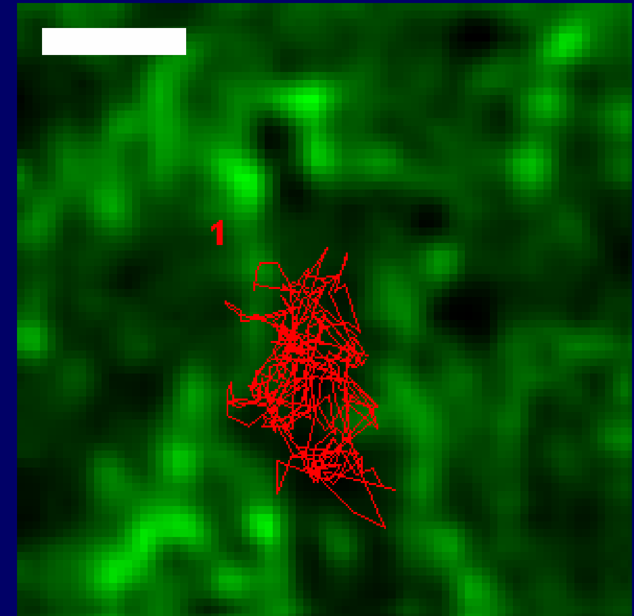
Single molecule sensitivity
When imaged with a CCD camera.

One green QD and one red QD are seen to merge and then transport together.

Lidke et al., JCB 2005

New work at UNM has provided proof for the "Corral" hypothesis.






Example trajectory tracing

Andrews et al., submitted

- **Signal propagation occurs in multiple membrane domains. For fine spatial details, we use EM. For fine kinetics details, we use SPT.**

- **Membrane properties are altered by cell activation**
  - **cluster size**
  - **composition**
  - **distribution**

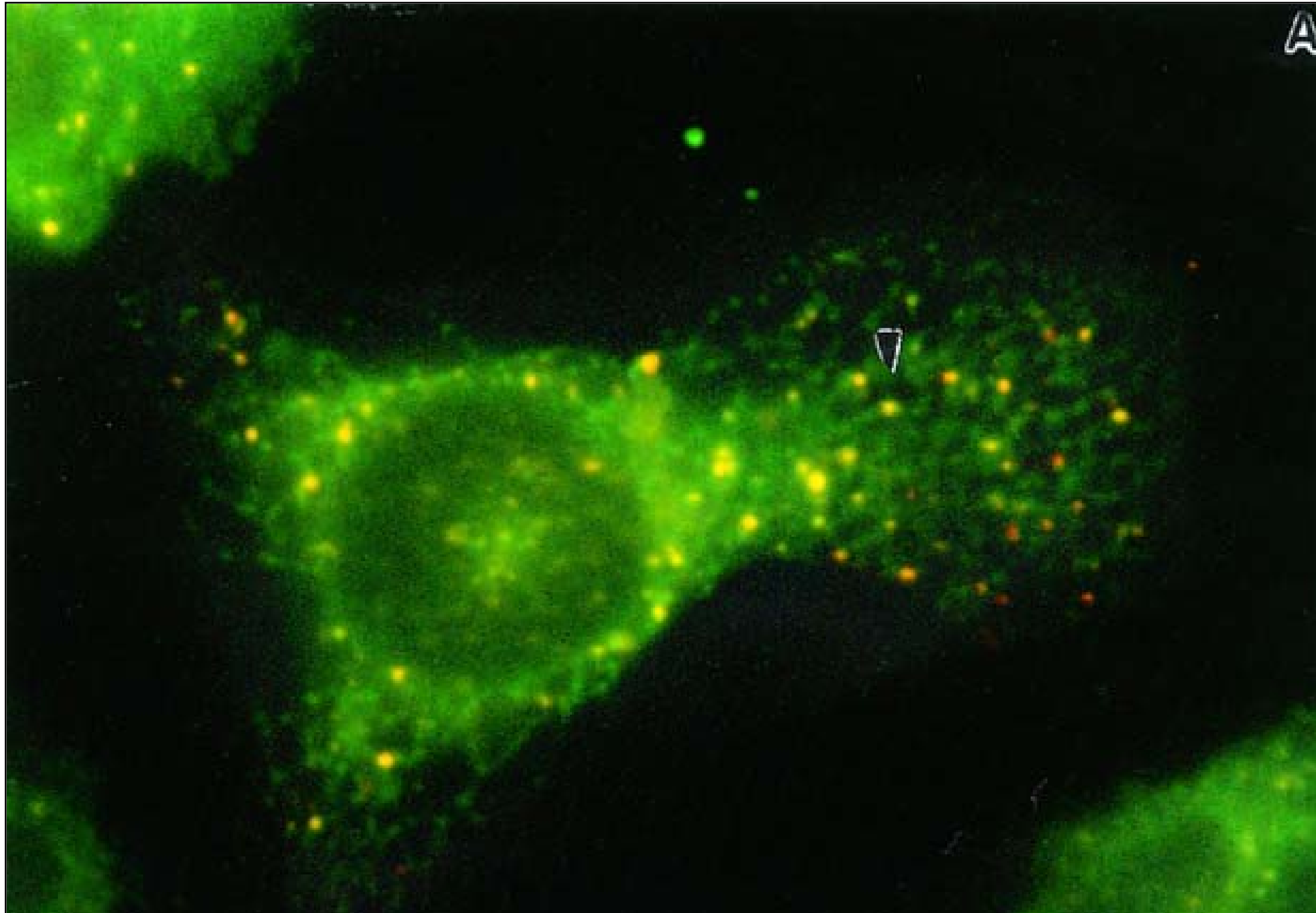
- **Activated receptors form dimers & removed from the membrane by endocytosis.**

- **What is the significance and mechanism underlying the clustering of membrane proteins?**

- **How do spatial relationships of molecules, corrals, etc affect the kinetics of signal initiation, propagation, and down regulation?**

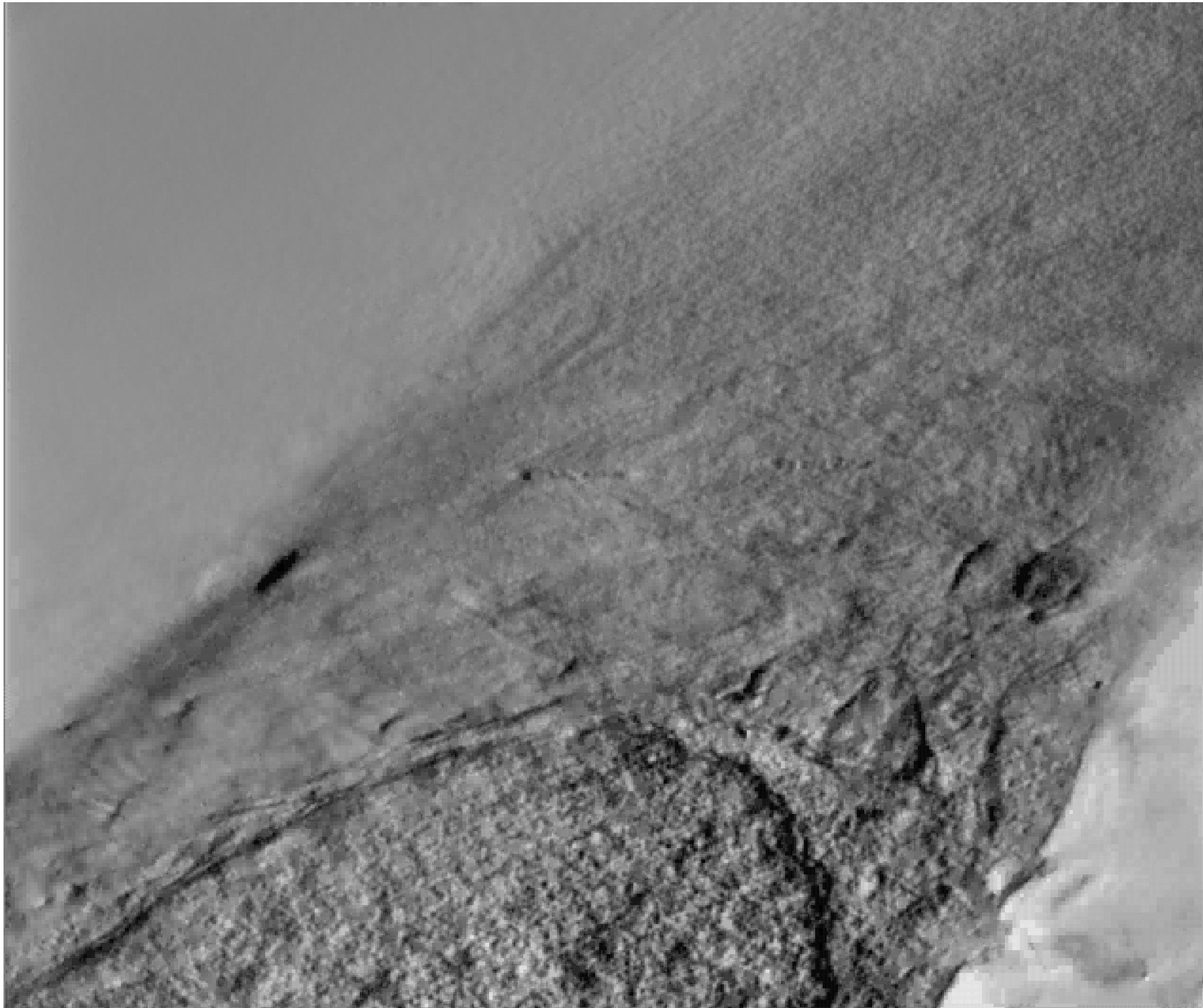
- **Mathematical modeling will continue to help us both test and develop hypotheses.**

# Clustering is not restricted to the PM… for example, $IP_3$ receptors cluster in the ER after rises in $[Ca^{2+}]_i$




Wilson et al, 1998

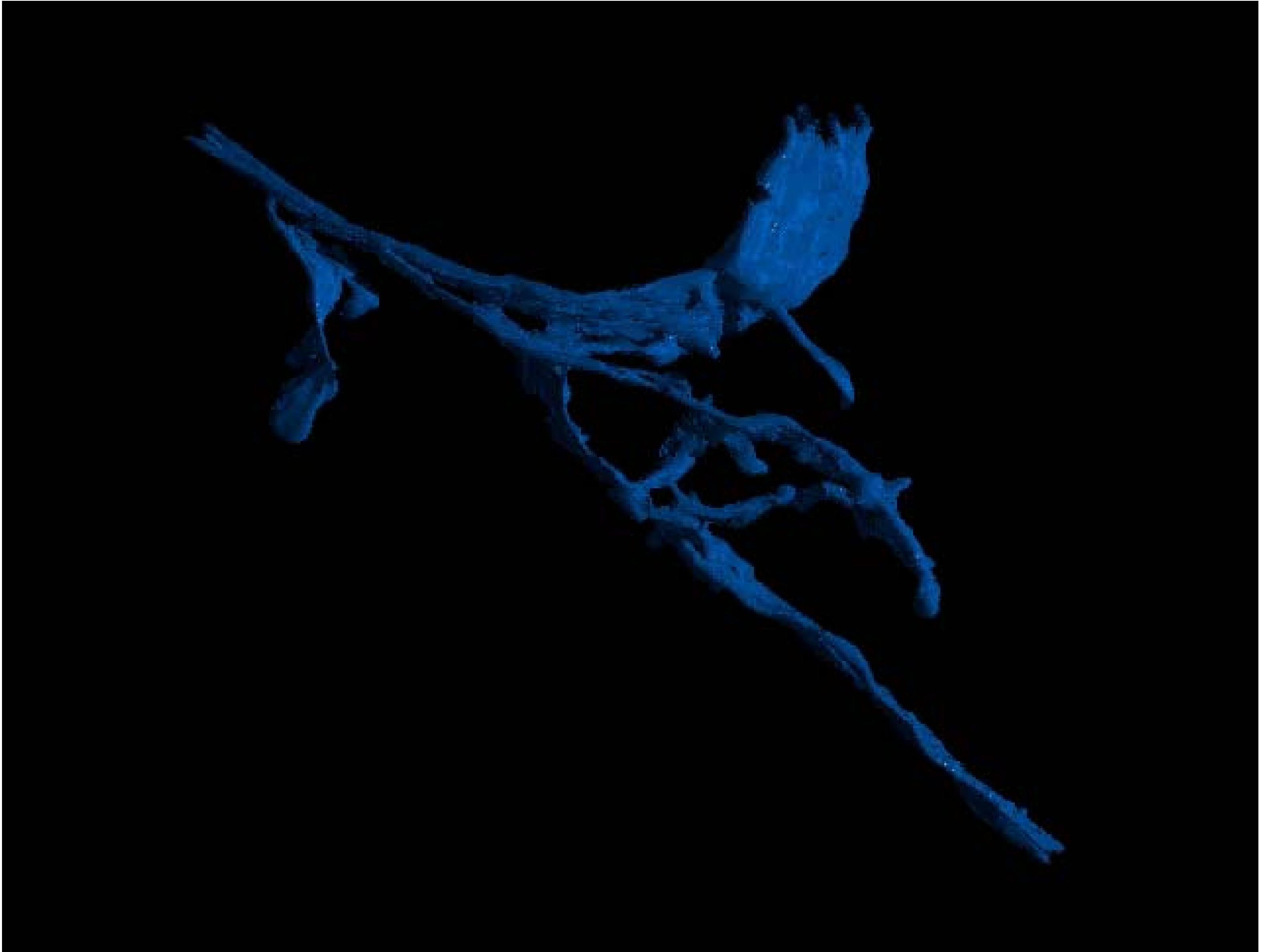
# We also model cell calcium, specifically in context of cell geometry based upon tomography (and serial TEM sections)



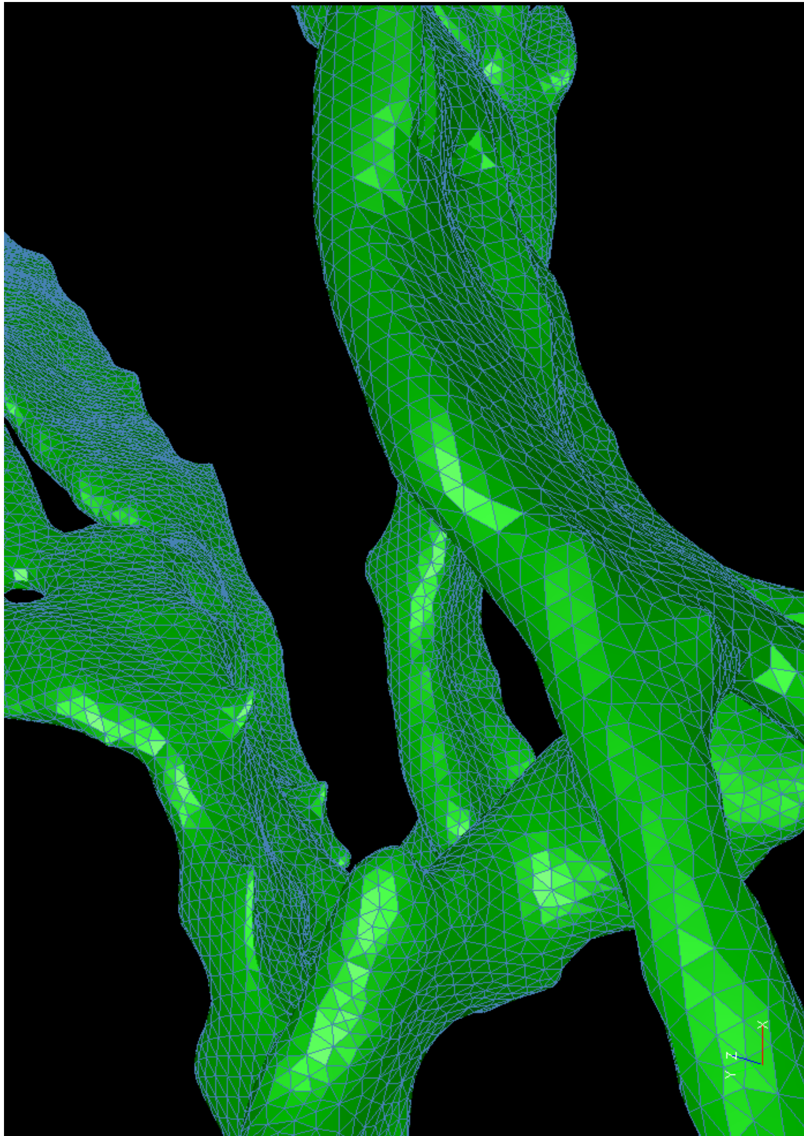
UCSD

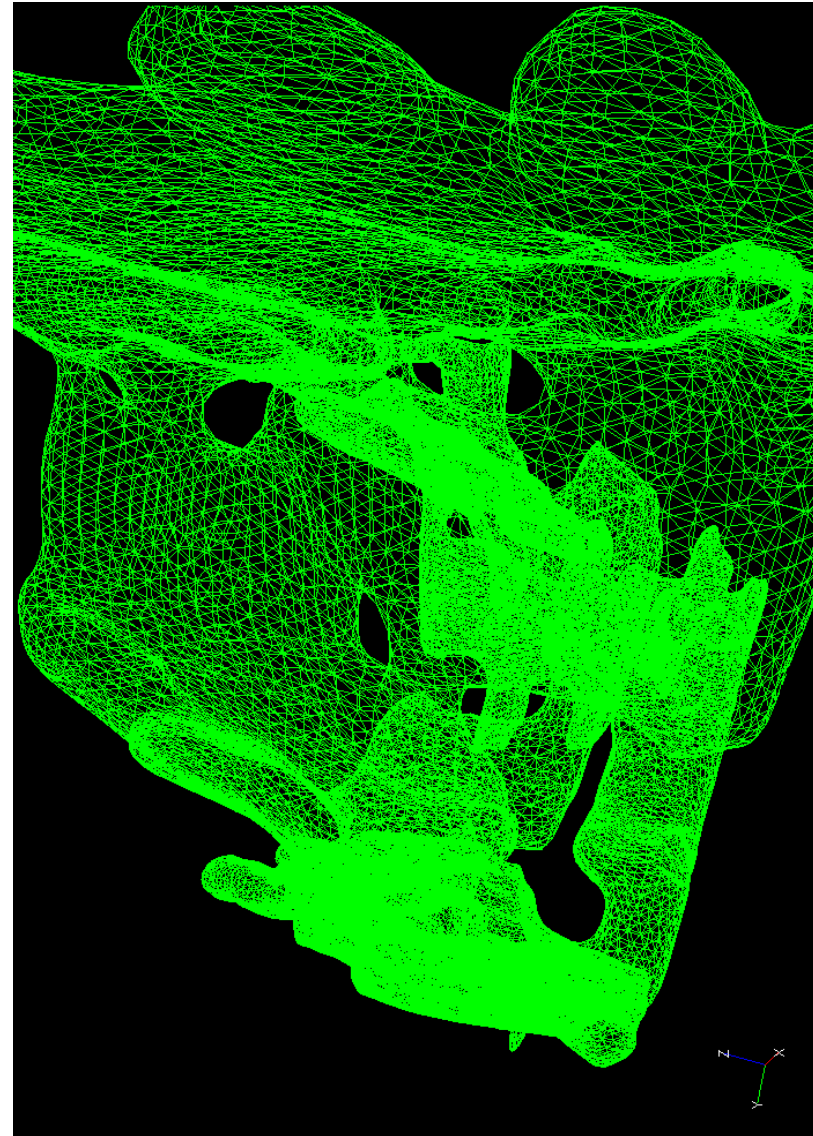
Means et al, Biophysical J. 2006

# Endoplasmic Reticulum (ER) Reconstruction



# Tetrahedral mesh generation



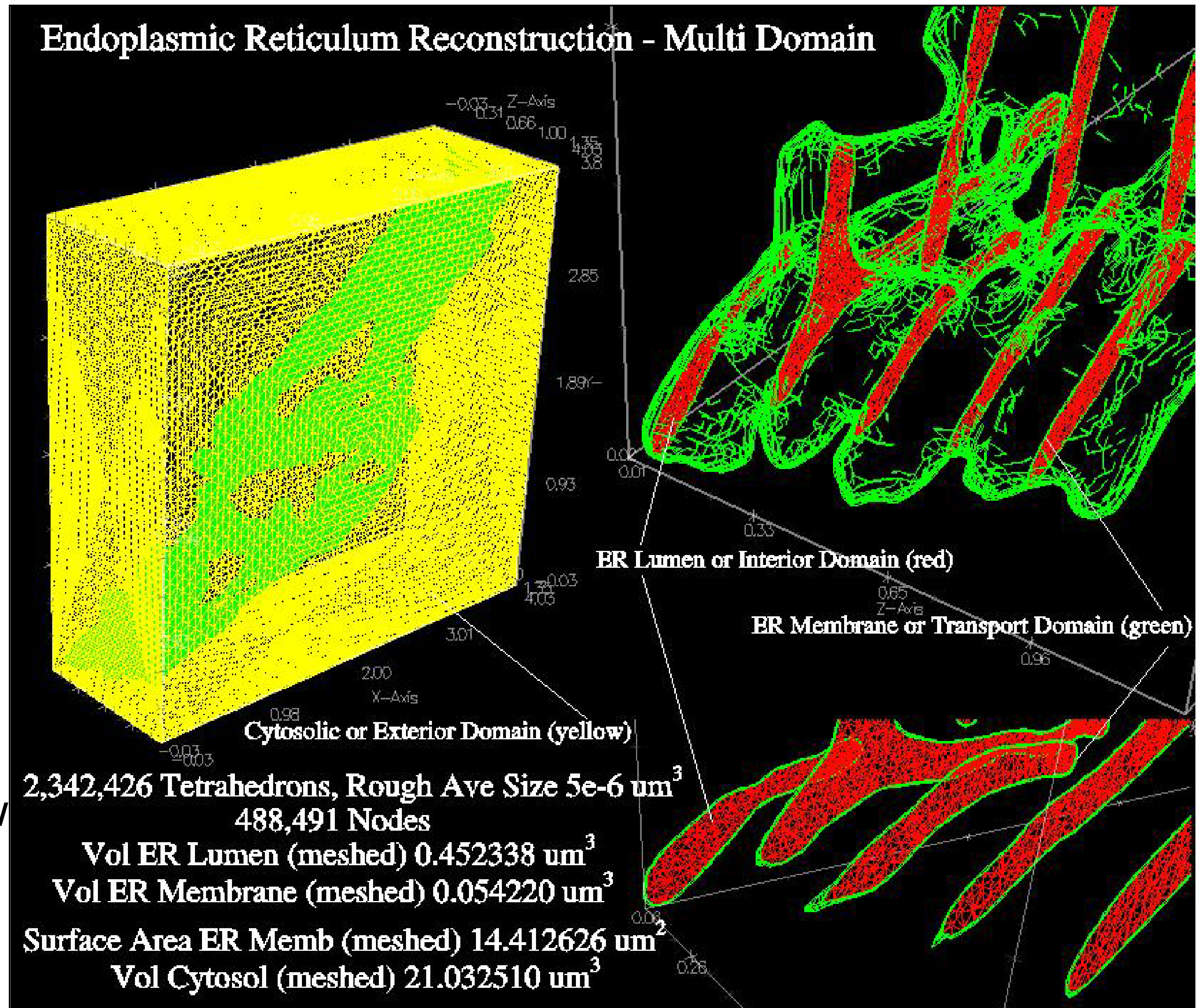


# ER & Cytoplasm MultiDomains

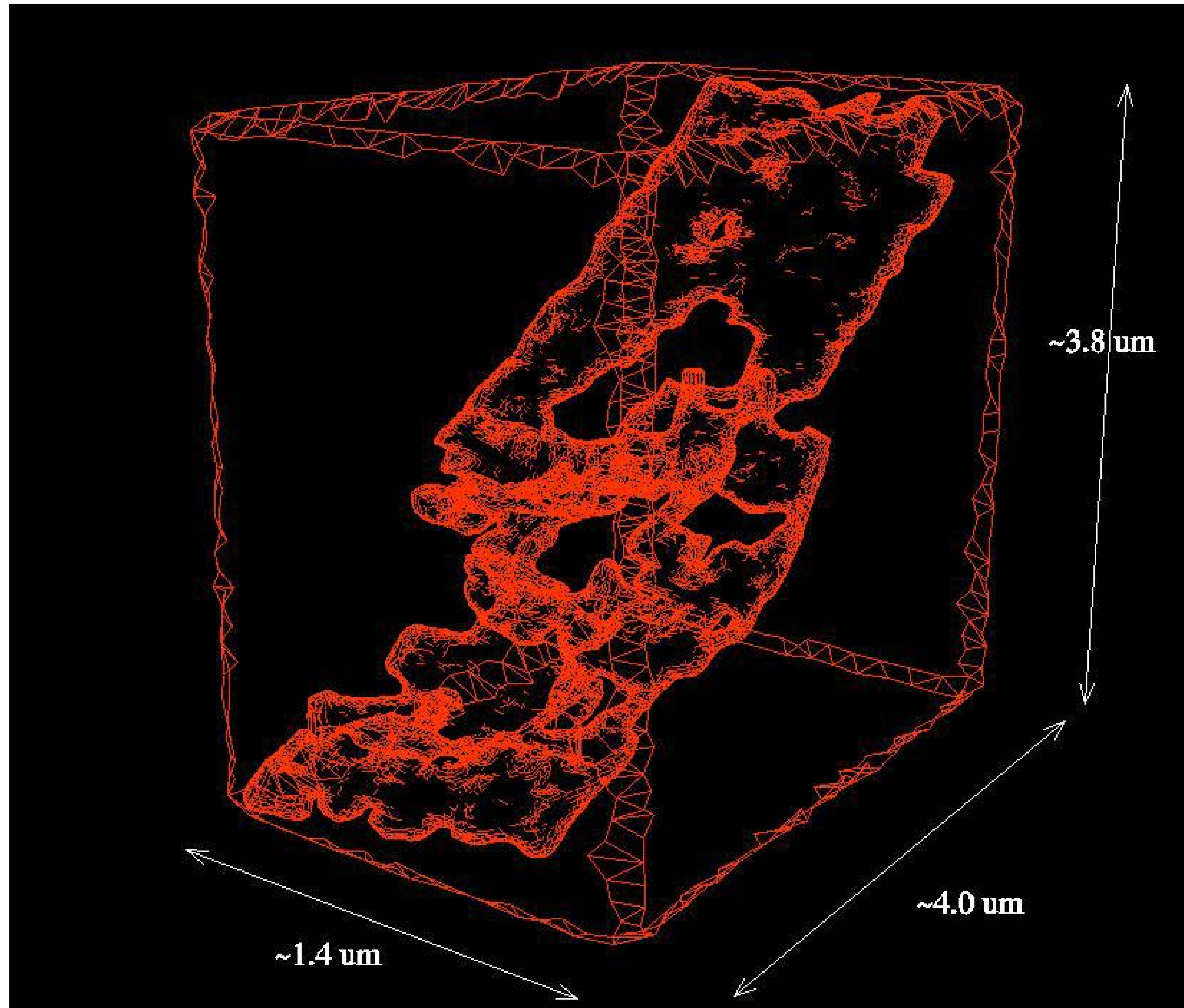
Simulations use
**MPSalsa**

**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)**

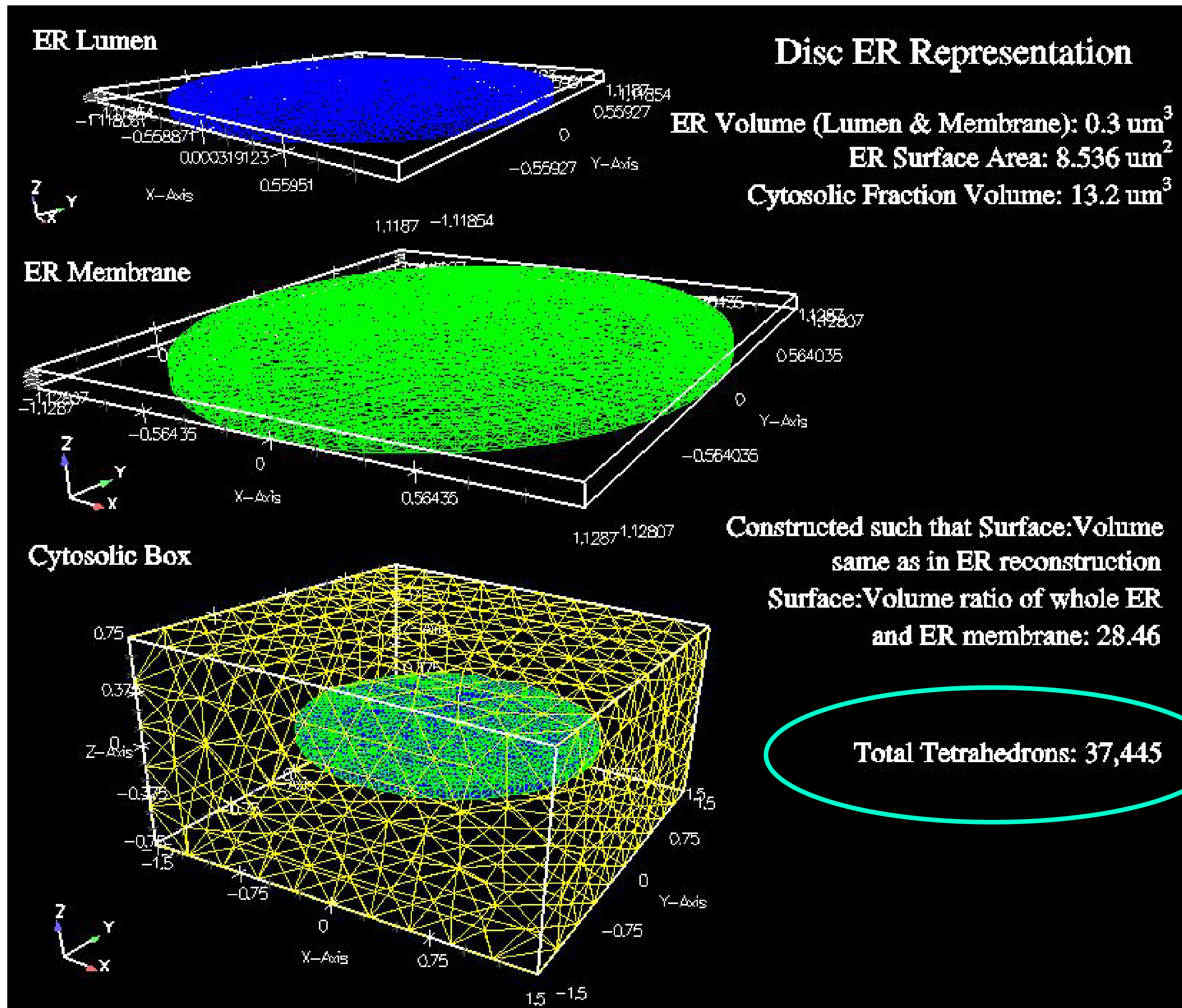
**http://www.cs.sandia.gov/CRF/MPSalsa/**



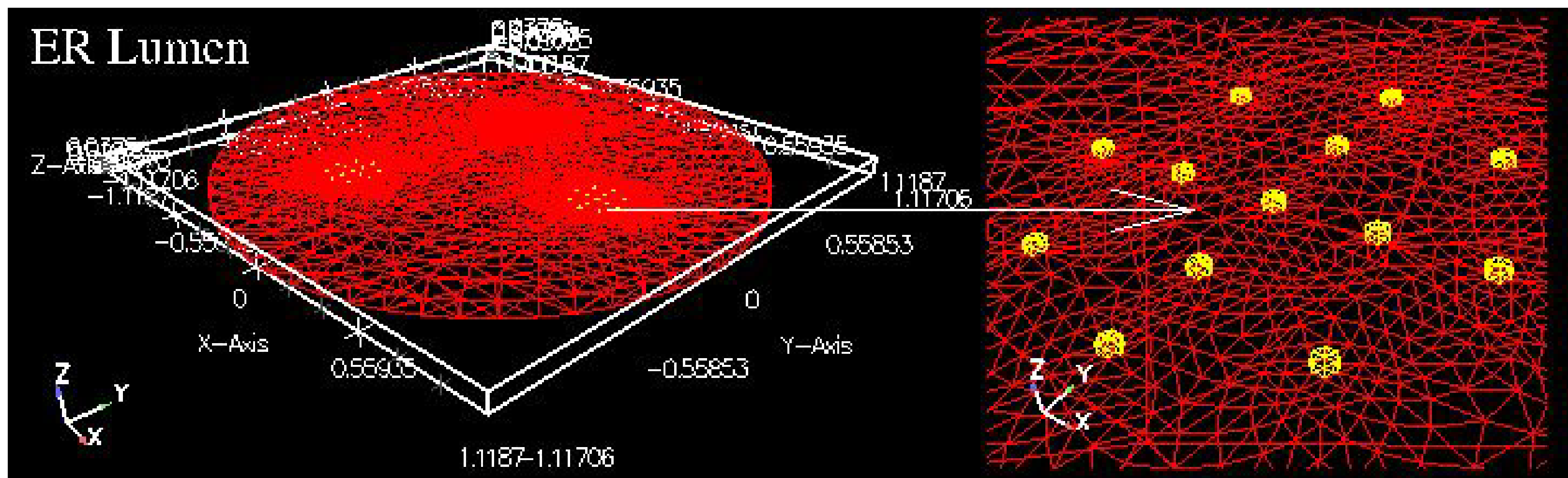
# Mesh added to represent the plasma membrane.



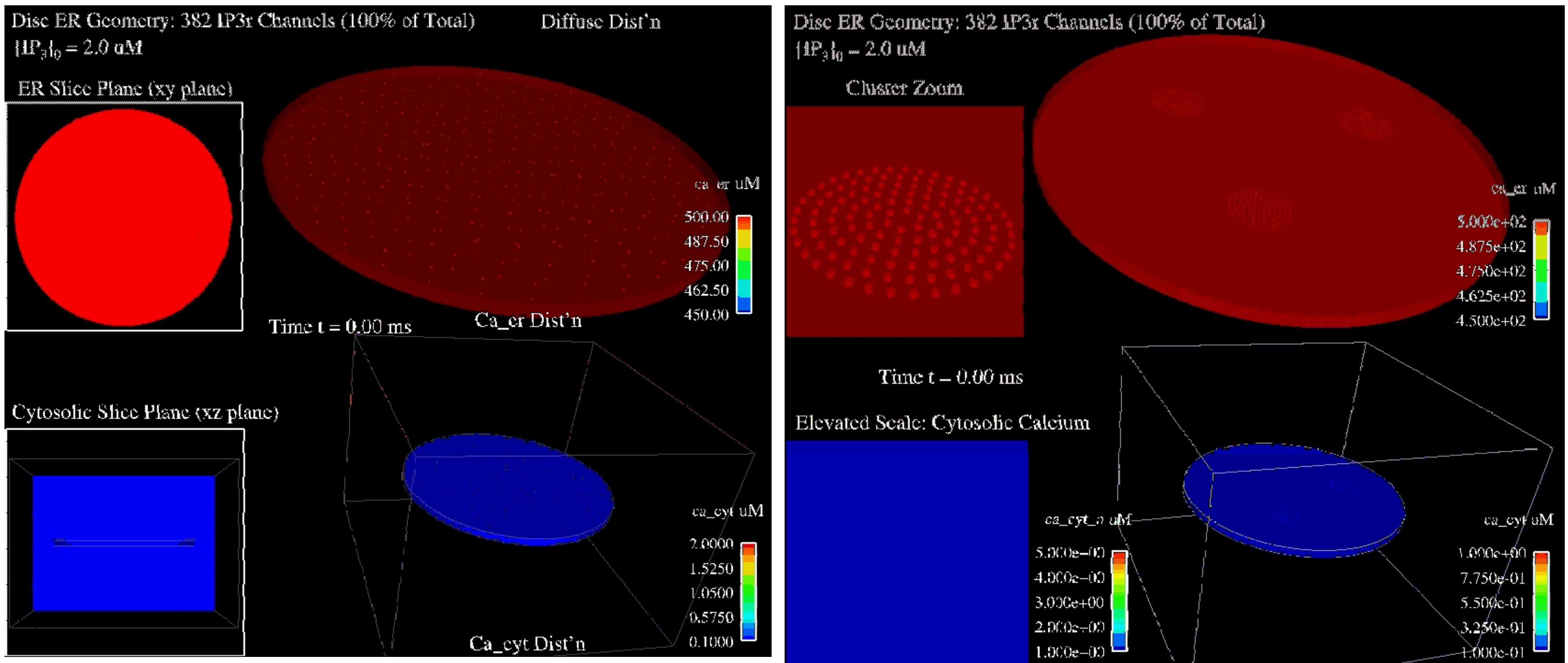
## For quick simulations, apply simpler geometries (discs & tubes).



# Placement of discrete channels in ER membrane.

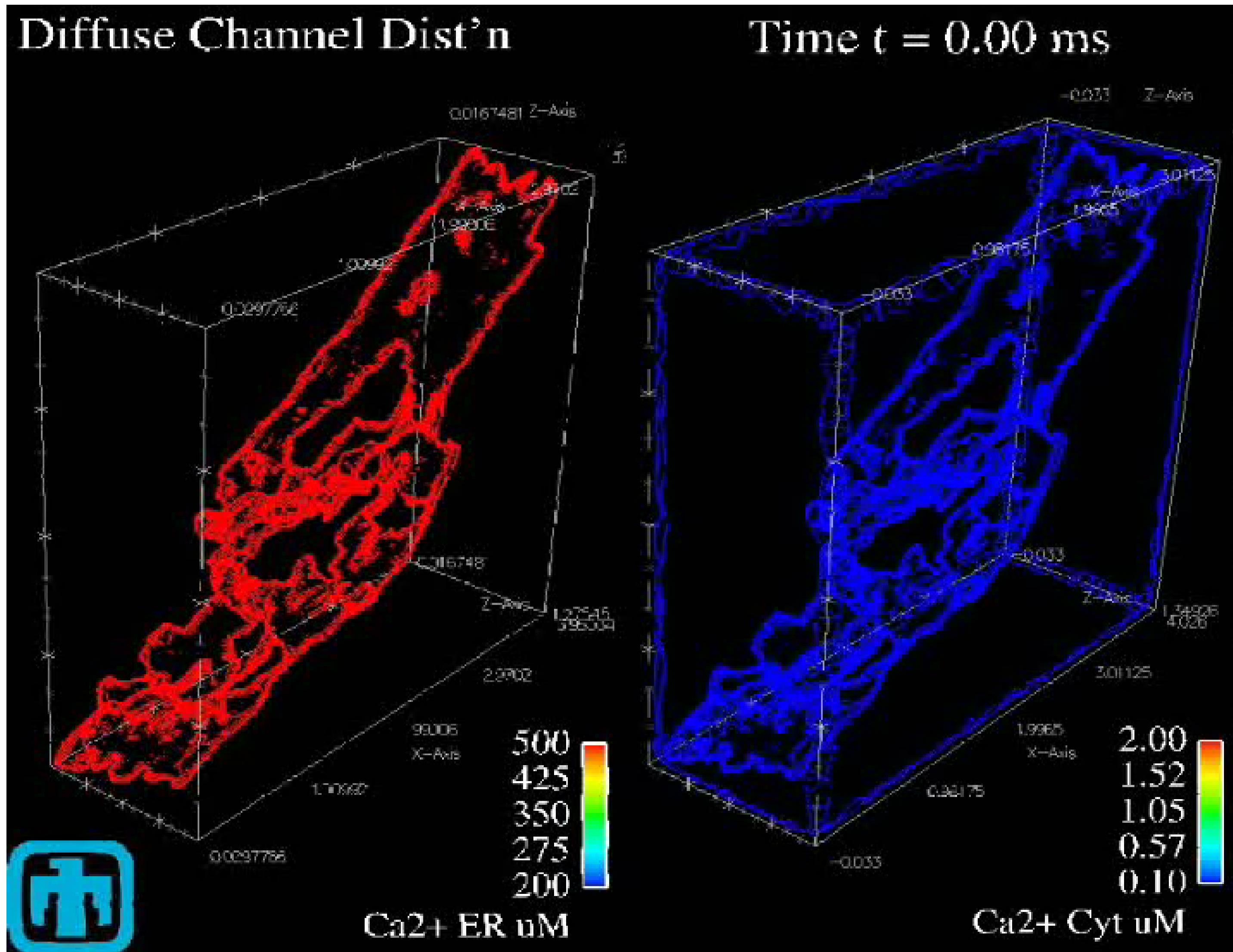


# Comparison of flux through $IP_3$ receptors in clustered & unclustered states (disc geometry)

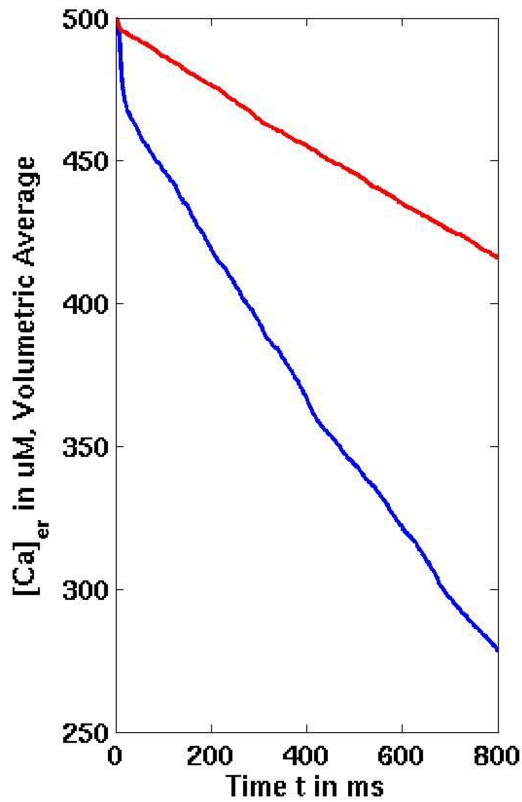


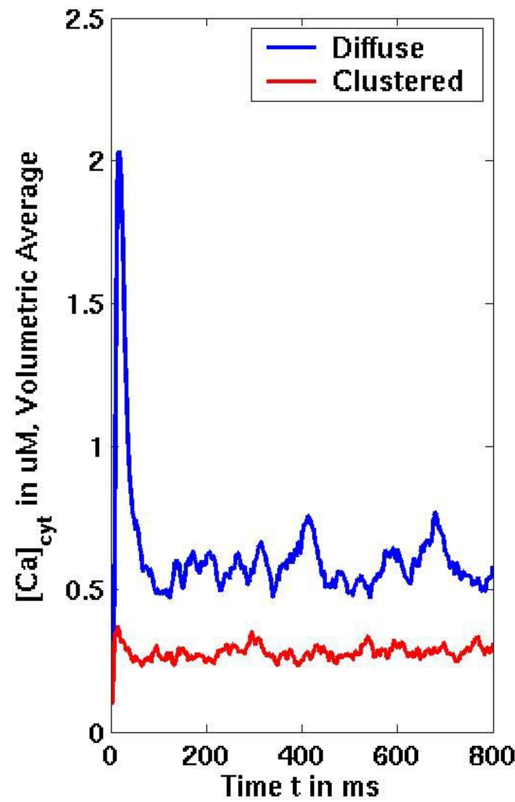

**Shawn Means, Tomas Mazel**

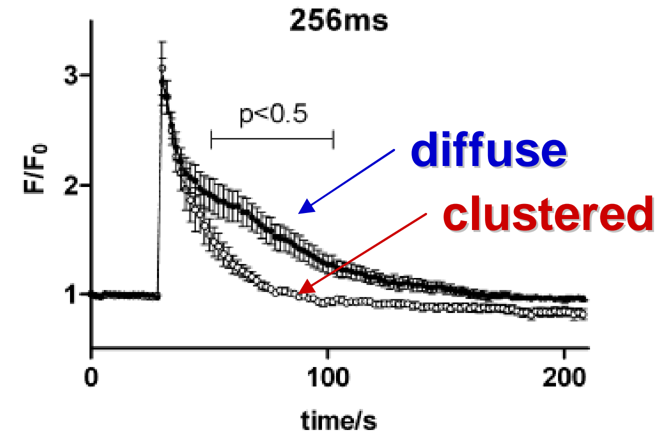
# Demonstration of simulations in FULL GEOMETRY



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



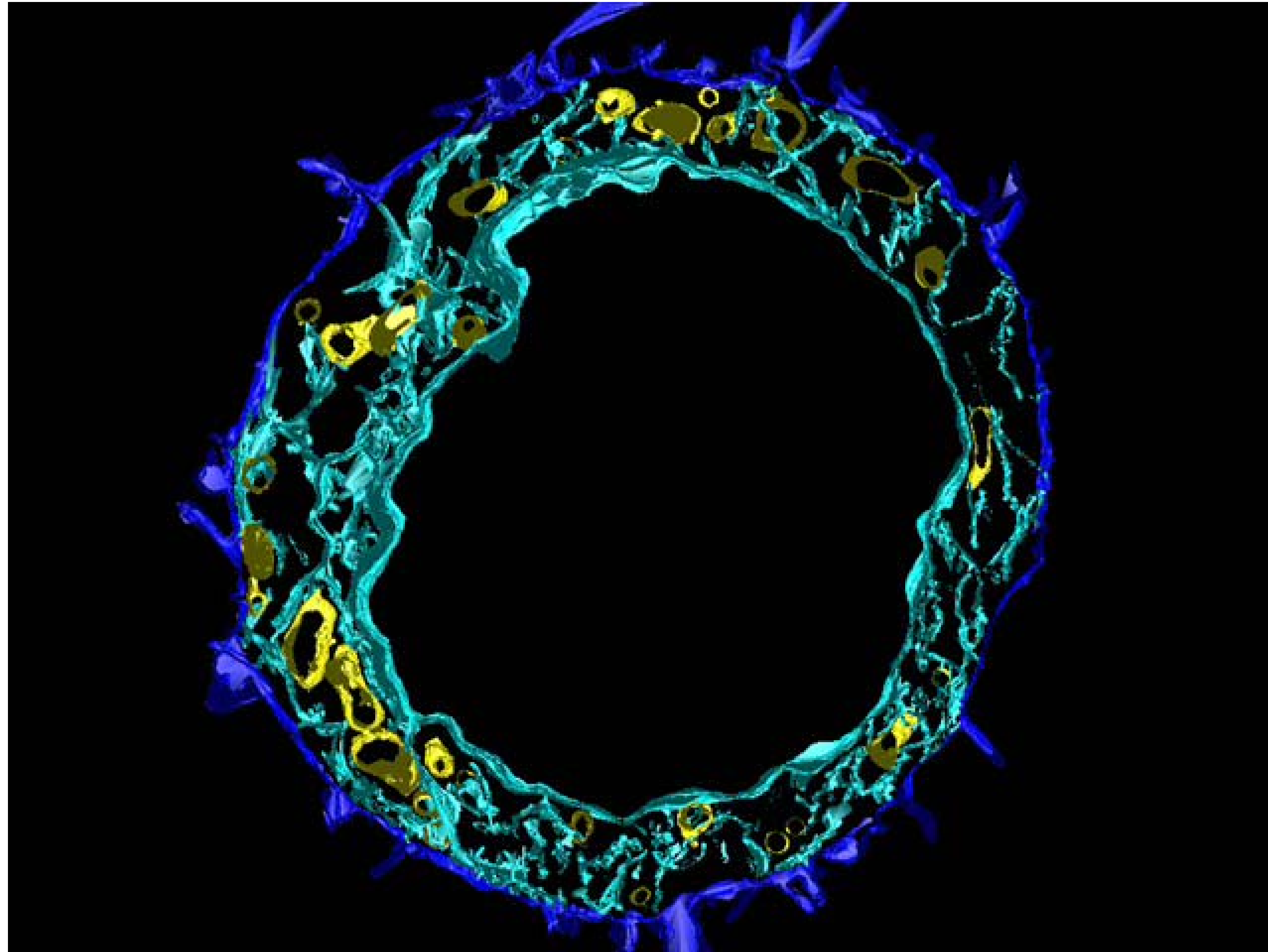




**EXPERIMENT (IP3 UNCAGING)**

**Alex Smith**

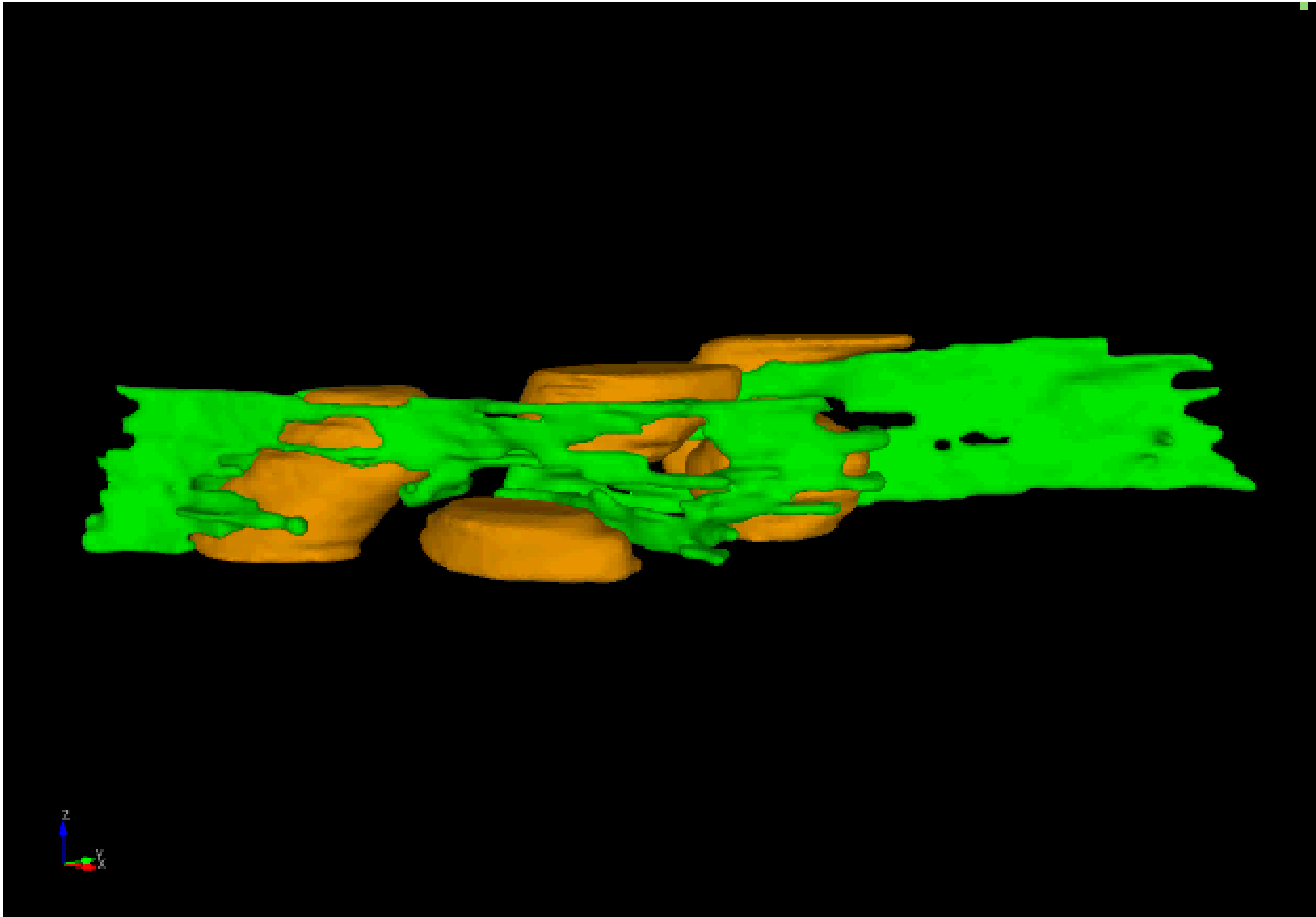
**SIMULATION**

# Ongoing work... More complete cell reconstructions

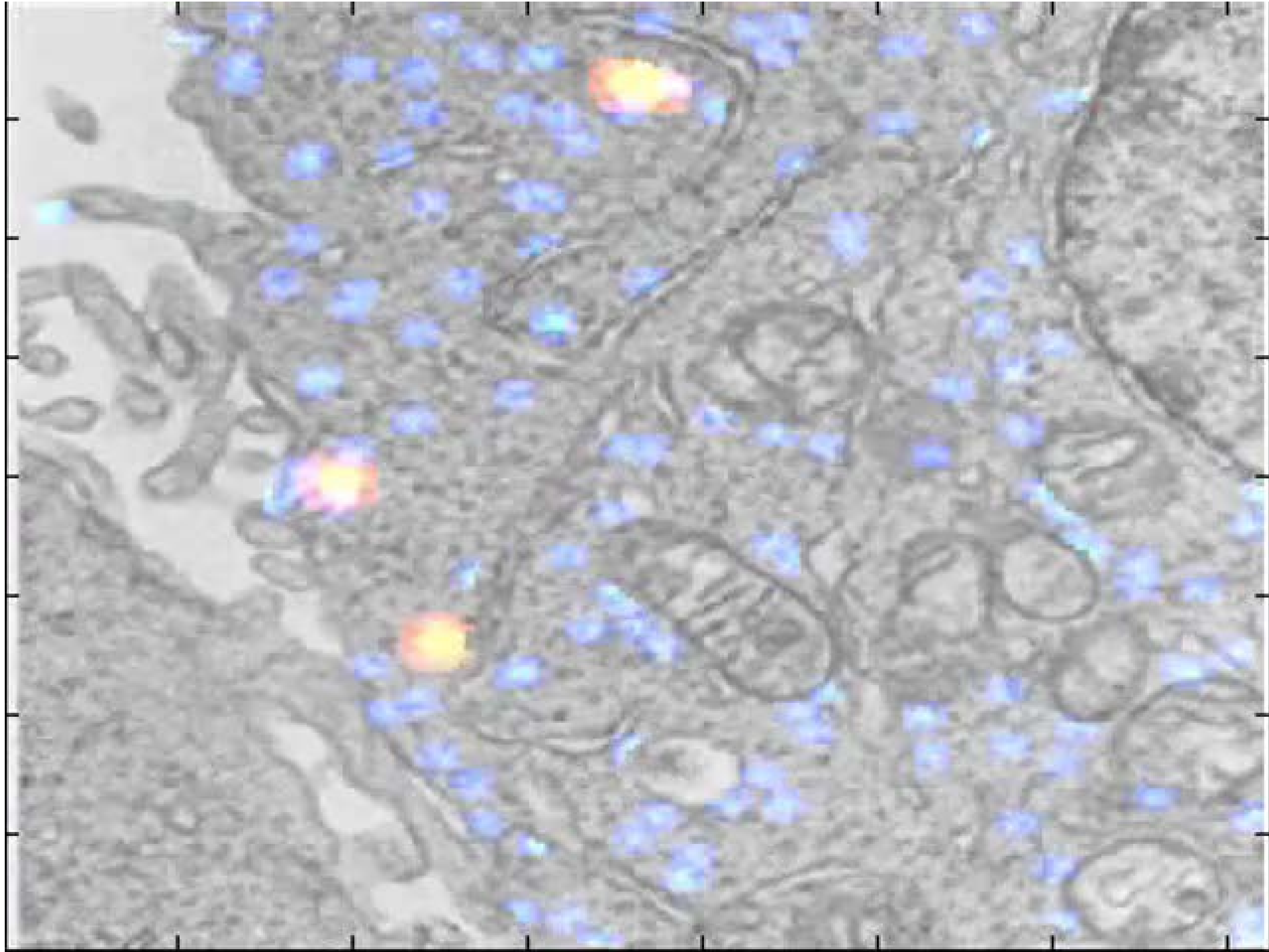


Tomas Mazel

# Note close contact between mitochondria and ER



# and a fully stochastic model for calcium



2D version of this model: blue - calmodulin    Yellow - calcium (coarse grain)