Spatiotemporal aspects of signal transduction: models and experiments

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

Fibroblasts in culture

Electron Microscopy: high spatial resolution, poor temporal resolution

White Cell
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).
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

- western blotting
- biochemical assays
- live cell imaging
- electron microscopy for cell & organelle reconstruction

**Absorbance in vitro kinase activity**

<table>
<thead>
<tr>
<th>Absorbance</th>
<th>20 nM EGF</th>
<th>3.2 nM HRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>0.4</td>
<td>0.6</td>
<td>0.8</td>
</tr>
</tbody>
</table>

- Absorbance bar graph
- Fluorescence (mAb binding to bead standards)
- Fluorescence (mAb binding to cell surface or intracellular target protein)
- Intracellular Ca^{2+}

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

Hybrid deterministic-stochastic reaction diffusion model for calcium transport

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

Possible distributions of membrane constituents

- Random
- Clustered
- Partially clustered

"Islands" or "Rafts"?

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 expresses 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.

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

<table>
<thead>
<tr>
<th></th>
<th>Cluster size</th>
<th>% coclustering</th>
</tr>
</thead>
<tbody>
<tr>
<td>exp</td>
<td>8.53</td>
<td>12.83%</td>
</tr>
<tr>
<td>sim</td>
<td>8.59</td>
<td>13.17%</td>
</tr>
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</table>

Experimental Data | Simulation
Agent-based simulations predict significant differences in homo- and hetero-dimerization patterns when comparing spatial stochastic results with well mixed deterministic approach.
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**

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<th>Inhibitor</th>
<th>resting</th>
<th>EGF</th>
<th>Heregulin</th>
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**Shc on membranes**

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</table>

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

Shc

# particles vs. time (min)

IP EGFR

EGF 0 30’ 1’ 2’ 5’

Blot Shc

IP EGFR

EGF 0 30’ 1’ 2’ 5’

Blot PLCγ1

SHC

# intensity vs. EGF stimulation (min)

PLCγ1

# intensity vs. LUS stimulation (min)
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 µm² 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.

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

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
acquisition 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

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.

![Graph showing relative change in D over time with varying concentrations of DNP$_{24}$-BSA.](image)

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 [Ca\textsuperscript{2+}]? ...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)
Typical Resting & Stimulated Levels of Cytoplasmic Calcium

Stimulus

Generation of Ca\(^{2+}\)-mobilizing signals

ON mechanisms

Resting Ca\(^{2+}\) 100 nM

Activated Ca\(^{2+}\) 500–1,000 nM

Ca\(^{2+}\)-sensitive processes

OFF mechanisms

Nature Reviews | Molecular Cell Biology
Also some newer GFP-based probes
In modeling, must consider flux both directions thru plasma membrane & ER membrane, as well as **buffering proteins**.
Most non-compartmental models treat mitochondria as an "immobile buffer" and represent with a single ODE.
Recommended reading: Chapters in....

dedicated
to Joel E. Keizer
1942-1999

Computational Cell Biology
Christopher P. Fall  Eric S. Marland  John M. Wagner  John J. Tyson
Editors
Calcium is important for signaling to the nucleus (such as PKC & Calmodulin/Calcineurin Pathways)
Outcomes of Elevated Calcium are Cell-type Specific

Example 1: Skeletal Muscle
Example 2: NEURON

Exocytosis

N/PQ type

Ca²⁺

ΔV

RYRI

Nuclear effects

ACI/III → cAMP
PYK2 → MAPK
CAMKII
CAM–CN

Gene transcription

Learning and memory

Synaptic effects

NMDA

Glutamate

L-type (α₁c)

Ca²⁺

InsP₃R

Ins(1,4,5)P₃

CAMKII
CAM–CN

LTP/LTD

Nature Reviews | Molecular Cell Biology
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$_3$)

Phosphatidylinositol (4,5)bisphosphate (PIP2)

diacylglycerol (DAG)
Example: Activation of phospholipase C by $G_q$- and $G_i$-coupled receptors

$G_q$-coupled-$\alpha_q$-mediated activation of PLC (M1, M3, M5, mGluR1)

($\alpha_2$AR, mGluR7, A1, $\kappa$- and $\delta$-opioid)
Starting premise: our observation that IP$_3$ receptors cluster in ER after a rise in [Ca$^{2+}$]$_i$

3D ER reconstruction
Tetrahedral mesh generation using CUBIT (Sandia)
ER & Cytoplasm MultiDomains

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


2,342,426 Tetrahedrons, Rough Ave Size 5e-6 um^3
488,491 Nodes

Vol ER Lumen (meshed) 0.452338 um^3
Vol ER Membrane (meshed) 0.054220 um^3
Surface Area ER Memb (meshed) 14.412626 um^2
Vol Cytosol (meshed) 21.032510 um^3

Means. Shepard
Decomposition for 64 Processors. Communications domain colored in grey.
For quicker simulations, we applied simpler geometries (discs & tubes).

**Disc ER Representation**

- ER Volume (Lumen & Membrane): 0.3 um³
- ER Surface Area: 8.536 um²
- Cytosolic Fraction Volume: 13.2 um³

**Cytosolic Box**

- Constructed such that Surface:Volume same as in ER reconstruction
- Surface:Volume ratio of whole ER and ER membrane: 28.46

**Total Tetrahedrons: 37,445**
**IP$_3$R regulation by calcium & IP$_3$ concentration**

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

- Paolini et al., 2004
- Patterson, 2004
- Foskett et al., 2007
Flux through IP₃ receptors in diffuse states (disc geometry)

Disc ER Geometry: 382 IP₃r Channels (100% of Total)

Diffuse Dist’n

ER Slice Plane (xy plane)

Diffuse Dist’n

Time t = 0.90 ms

Ca_er Dist’n

Cytosolic Slice Plane (xz plane)

Ca_cyt Dist’n
Flux through IP$_3$ receptors in clustered states (disc geometry)
ER Geometry: Deterministic IP$_3$R 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

381 IP$_3$R Channels (100% of Total)
2.0 uM IP$_3$O

Clustered Channel Dist’n

Time $t = 899.69$ ms

Exit for 2 movies..
THE ER EMPTY SLOWER & CYTOSOLIC CALCIUM LEVELS ARE LOWER IN THE CLUSTERED IP3R STATE

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

Difference in mean sq displacement vs free in solution

simulations in Matlab
Developing a new stochastic model simulations in Matlab
Simulating IP3 Synthesis

A

100 %

10 %

number of IP$_3$ s bound

time (ms)

0 10 20 30 40 50

100 %

10 % PLC$_{\gamma}$ active

number of IP$_3$s with 4IP$_3$s bound

time (ms)

0 10 20 30 40 50

10 % PLC$_{\gamma}$ active

B

IP$_3$ diffusion

C

IP$_3$R calcium flux

25 ms

50 ms

75 ms

100 ms
Matching simulations to data

IgE 10 ng/ml

extracellular calcium 2 mM

[Ca$^{2+}$]$_i$ (µM)

0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8

0 100 200 300 400 500 600 700

time (s)

[Ca$^{2+}$]$_e$ (µM)

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7

0 100 200 300 400 500 600 700

time (s)
One can use simulations to evaluate potential effects of changing IP$_3$R levels

- **High** (28,000 tetramers)
- **Medium** (14,000 tetramers)
- **Low** (3,500 tetramers)
Simulating Local Activation of Mitochondrial Transport
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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