From Simulation to Therapy:

A Systems biology Approach to Oncogene Detection

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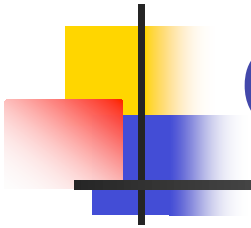
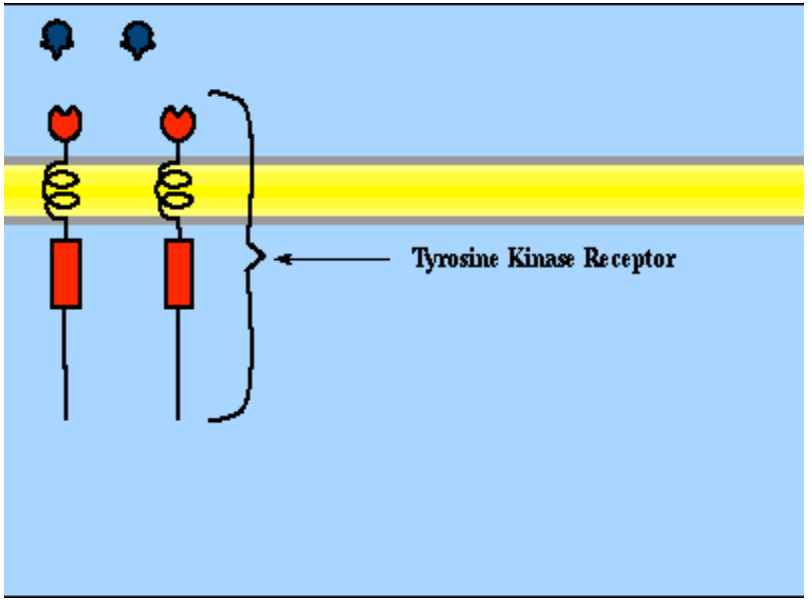


Outline

- Signal Transduction
- Mutations, Oncogenes, Proto-Oncogenes
- Mathematical Models
- Predicting Putative Oncogenes using Systems Biology
- Drug Targeting
- Overview

Signal Transduction

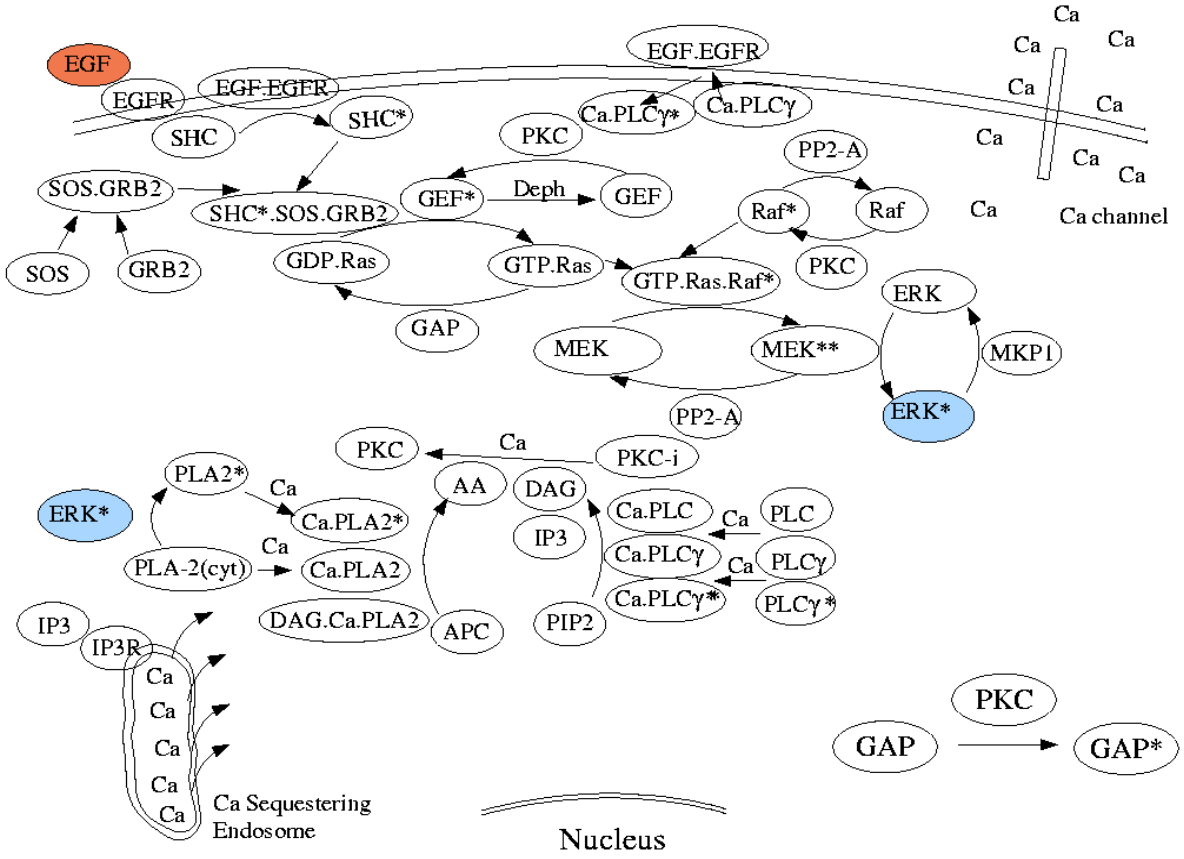
- Controls behavior of cells
  - Ion Channels
  - Metabolic Pathways
  - Cytoskeletal Proteins
  - Gene Regulatory Proteins
    - Cell cycle
    - Cytoskeletal rearrangement



D. Slish Mechanism of Tyrosine Kinase Receptors

U.Platt.

The Normal Functioning MAPK Signal Cascade



$$\frac{d\vec{C}}{dt} = \vec{R}(\vec{C}, \vec{k}, t)$$

- **1:** Science. 1999 Jan 15;283(5400):381-7.

  Emergent properties of networks of biological signaling pathways.
  Bhalla US, Iyengar R. ICSB-2006

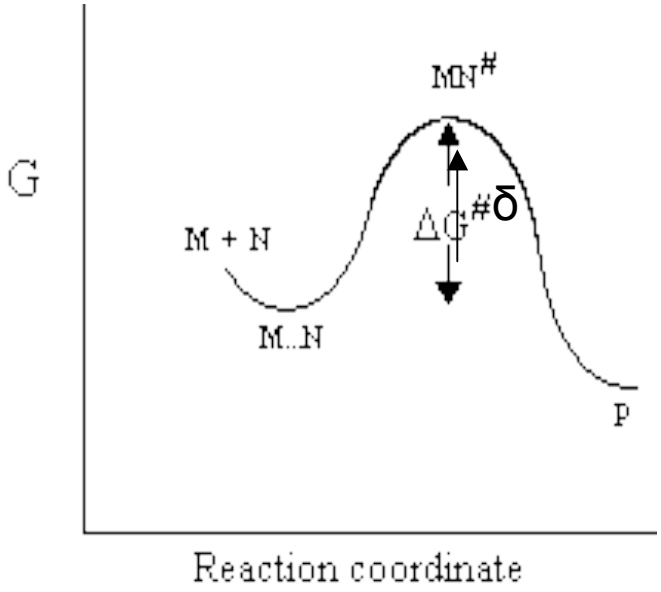
Oncogenes and Tumor Suppressor Genes

- **Oncogene** - A gene (protein) that when mutated or inappropriately expressed contributes to cancer formation
  - Mutations in signal transduction genes
  - Mutations in Tumor Suppressor genes
  - **INHERITED**
- **Activation**
  - Point Mutation
  - Chromosomal rearrangement
  - Gene amplification
  - Viral Insertion
- **Direct or Indirect**

Known Oncogenes

- EGFR - Codes for Receptor
  - Over expressed in Melanomas, Carcinomas
- FOS – Transcription Factor
  - Codes for transcription factor of DNA
  - Involved in Lung cancer
- Jun – Transcription Factor
  - Involved in Lung Cancer
- Ras – G Protein
  - Colon, Lung, Kidney, Bladder, Breast Cancer

How may a mutation affect kinetics?



$$k_{f_1} = Ae^{-\Delta G_1 / k_b T}$$

$$k_{f_2} = Ae^{-\Delta G_2 / k_b T}$$

$$\frac{k'_{f1}}{k_{f1}} = \frac{k'_{f2}}{k_{f2}} = e^{-\delta / k_b T} \quad \delta = -k_b T \log\left(\frac{k'_{f_1}}{k_{f_1}}\right)$$

Effects on signaling

$$A + B \underset{k_b}{\overset{k_f}{\rightleftharpoons}} A \cdot B$$

A mutation between two unique species

- Affects Reactant State

$$k_f' = e^{-\delta / kT} k_f$$

$$k_b' = e^{-\delta / kT} k_b$$

- Affects Product State

$$k_f' = e^{-\delta / kT} k_f$$

$$k_b' = e^{-\delta / kT} k_b$$

How may a mutation affect the initial concentration of species?

$$\text{bath} \underset{k_b}{\overset{k_f}{\longleftrightarrow}} A$$

$$k_{eq} = \frac{k_f}{k_b} = \frac{[A]}{[bath]}$$

$$\frac{[A']}{[A]} = \frac{k'_f}{k_f} = e^{-\delta / k_b T}$$

Cancer as a cumulative process

- Multiple Mutations at different sites contribute to tumor transformation
- Tumor may exist as “families” of differentially mutated cells
  - Overrides resistance of tumor to treatment

The method

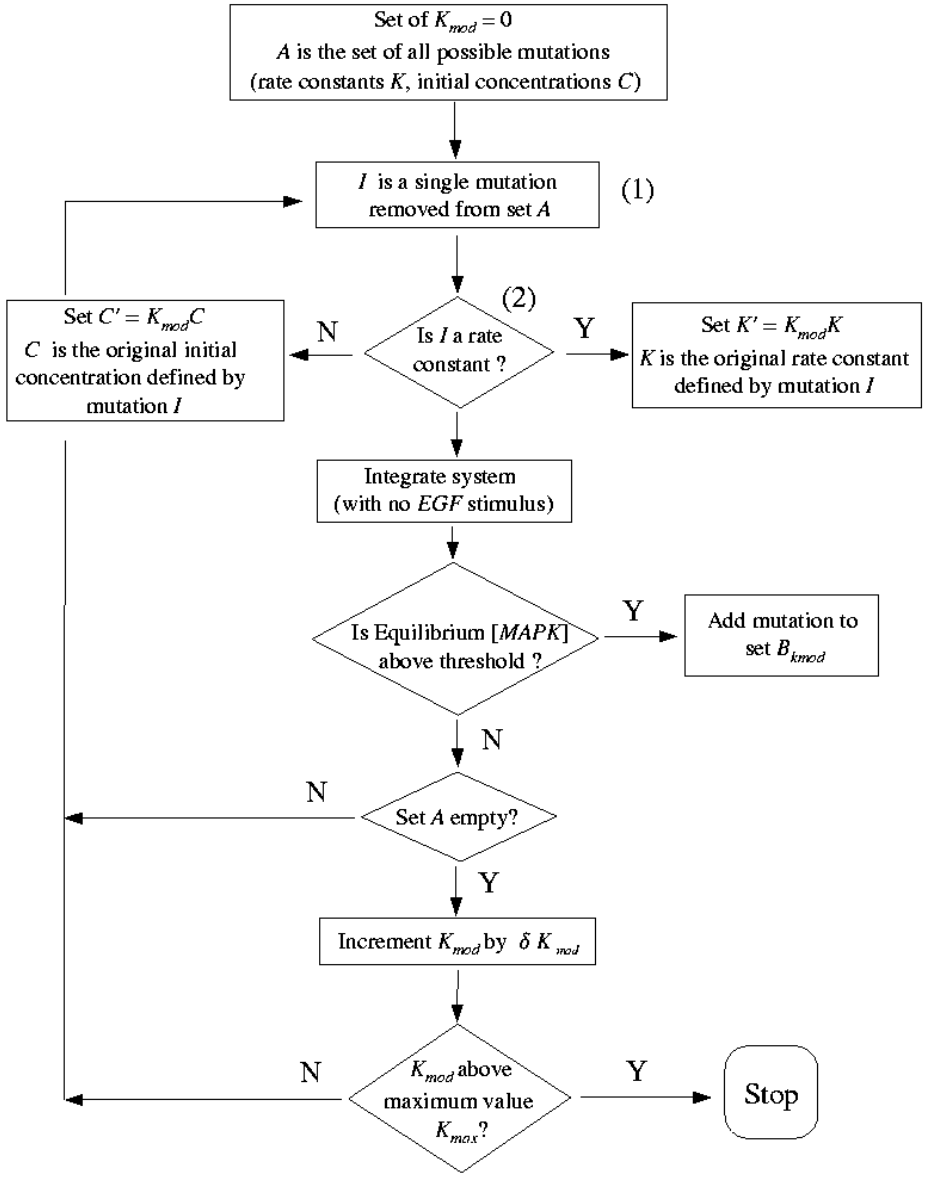
- Perturb interactions:
  k_{ij}, k_{ilm},$C_i(0)$
- Integrate (no EGF stimulation)

$$\frac{dC_i}{dt} = \sum_j k_{ij} C_j + \sum_{l>m} k_{ilm} C_l C_m$$

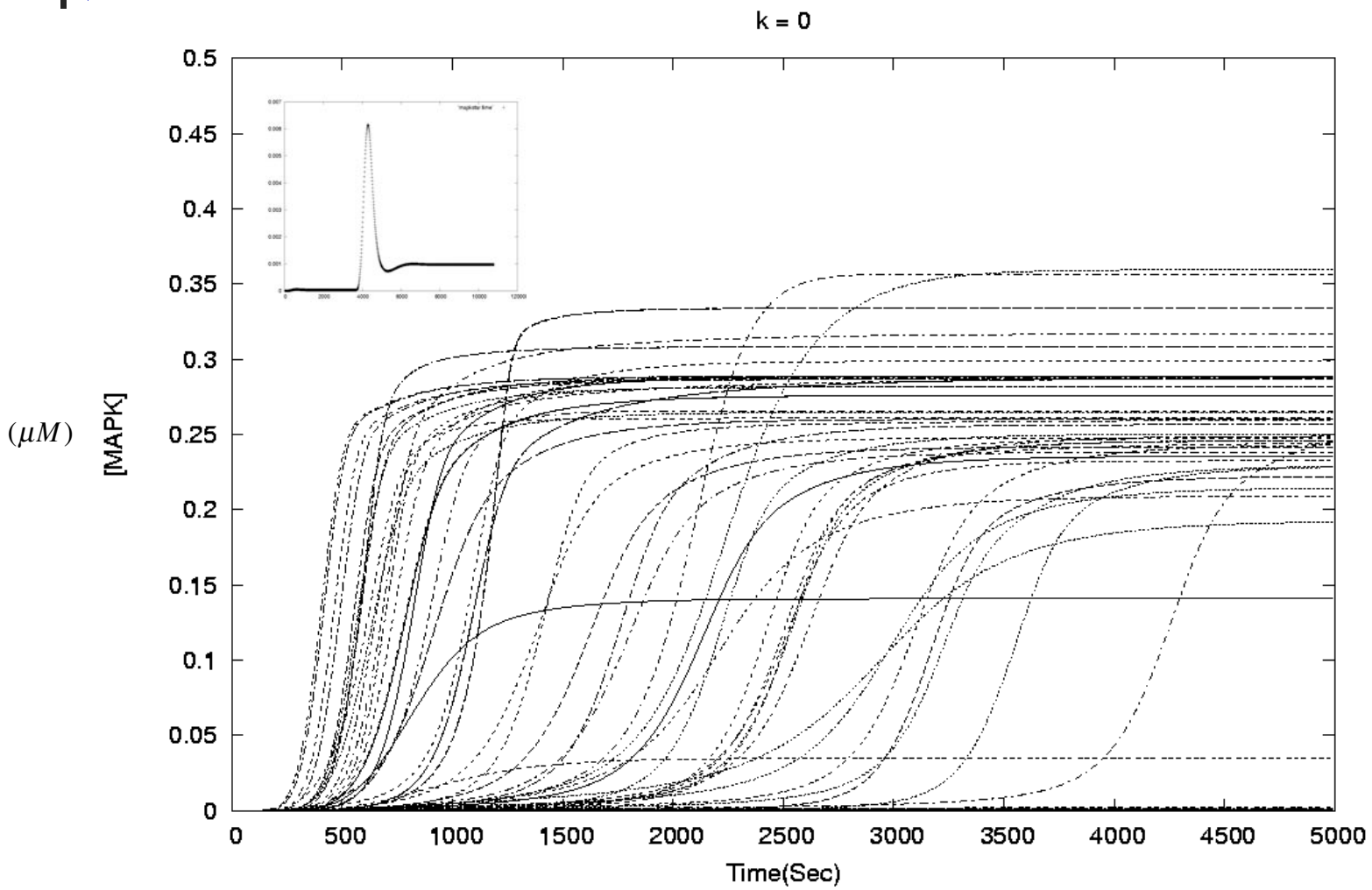
- Tag ERK* activations and

Rank according to: $e^{-\delta / kT}$

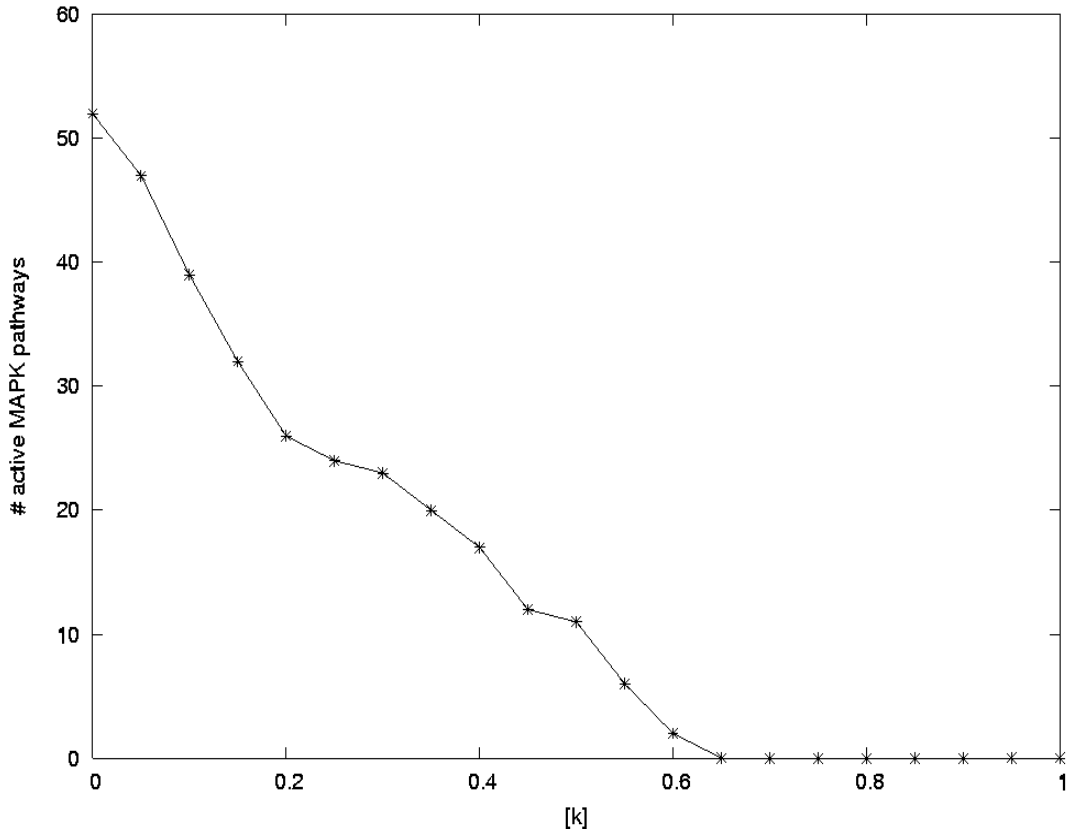
Extend to multiple mutations



“In Silico” Interaction Knockout experiments



Single Site Transformations (kf'/kf)



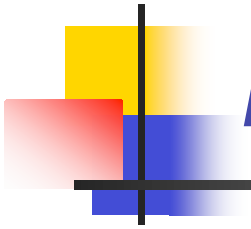
Top Ranked Mutations

X* + pp2a → X*.pp2a	0.75
X*.pp2a → X + pp2a	0.75
raf* + gtp-ras ← raf*.gtp.ras	0.60
Gtp-ras.gap →gap + gdp-ras	0.55
raf* + pp2a →raf*.pp2a	0.55
gtp-ras + gap → gtp.ras_gap	0.55
AA → apc	0.55
Ca.CaPump → capump + ca-ext	0.55
X + Raf.GTP.Ras ← X.Raf.GTP.Ras	0.50
PKC.DAG + AA ← PKC.DAG.AA*	0.50

Top Ranked expression level mutations

$\frac{[A]'}{[A]}$	Species
0.4	MKP-1
0.5	GAP
0.7	PP2-A
1.4	PKC-I
1.6	Raf
1.7	Ras

$\frac{[A]'}{[A]}$	Species
1.9	GEF
2.8	MEK
3.0	PLC
3.4	PLC-g
3.6	ERK



In Silico Cancer Cells

- We have developed a family of “in silico” cancer cells as models of pathways

What is this good for?

The effect of Drugs on Signaling in mutated cells

Competitive Binding

$$E + S \rightleftharpoons ES \longrightarrow E + P$$

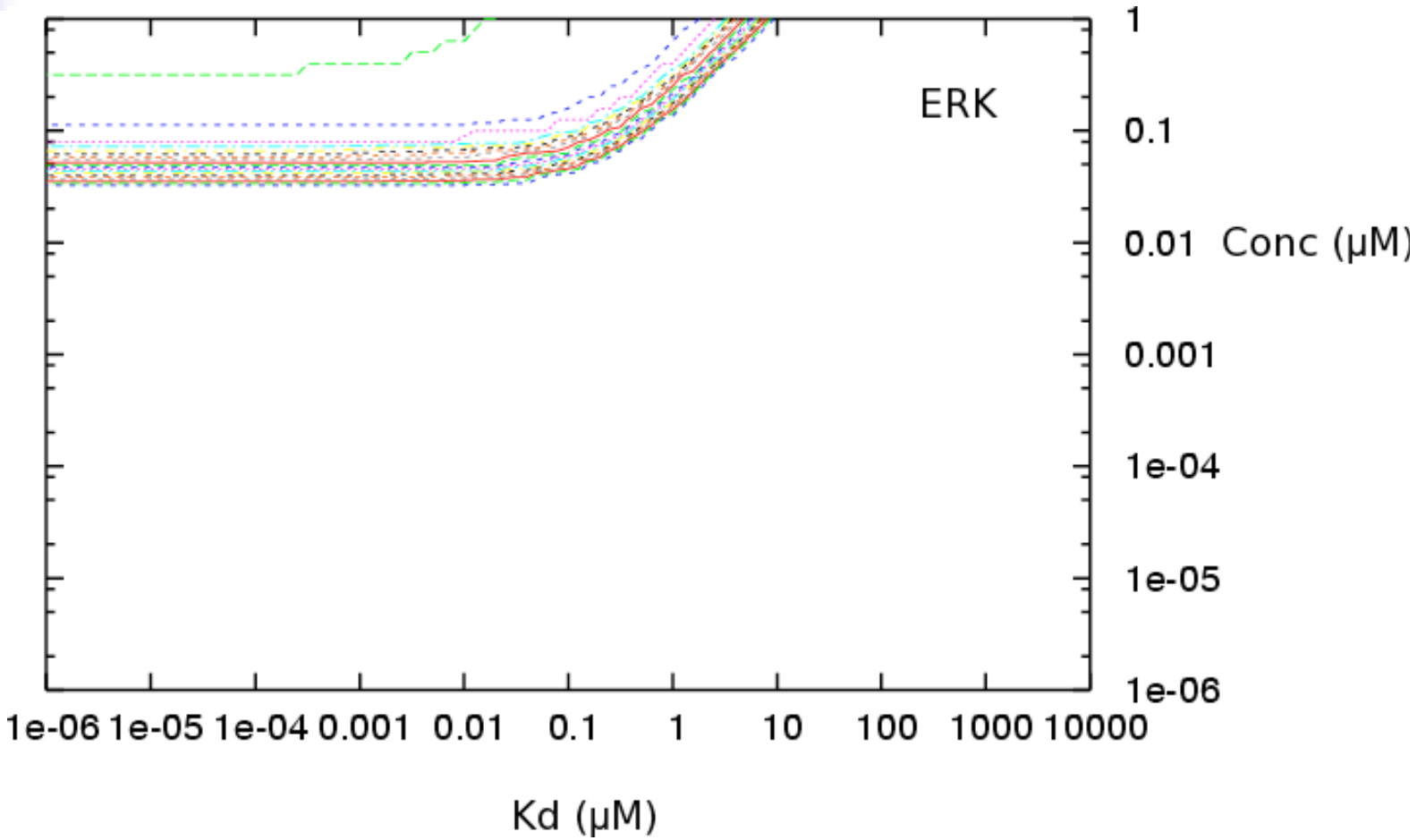
$$E + I \rightleftharpoons EI$$

Noncompetitive binding

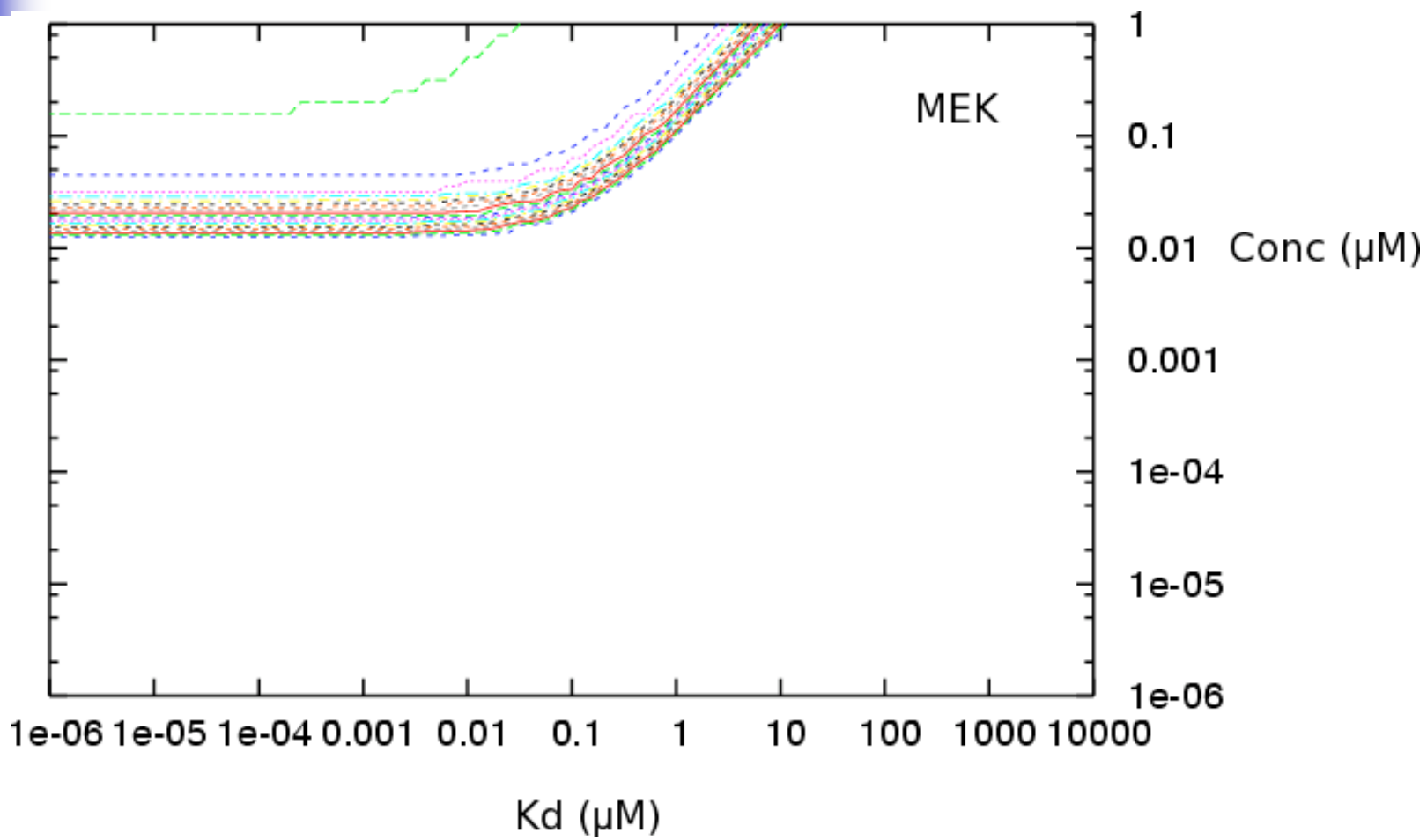
$$E + S \rightleftharpoons E.S \longrightarrow E + P$$

$$E + I \rightleftharpoons EI + S \rightleftharpoons EIS \rightleftharpoons ES + I$$

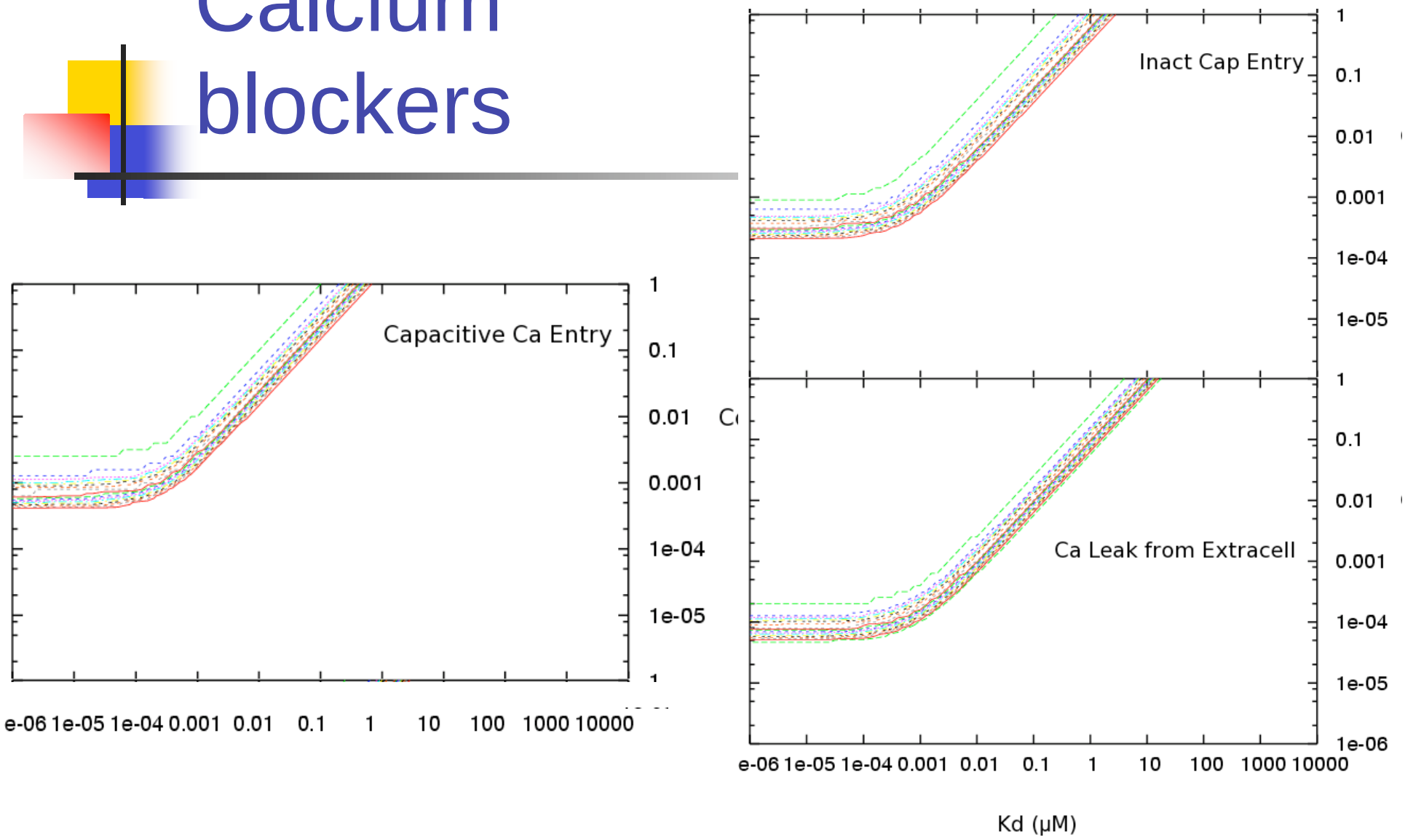
ERK Target


ERK
1
0.1
0.01
0.001
1e-04
1e-05
1e-06
Conc (μM)
1e-06 1e-05 1e-04 0.001 0.01 0.1 1 10 100 1000 10000
Kd (μM)

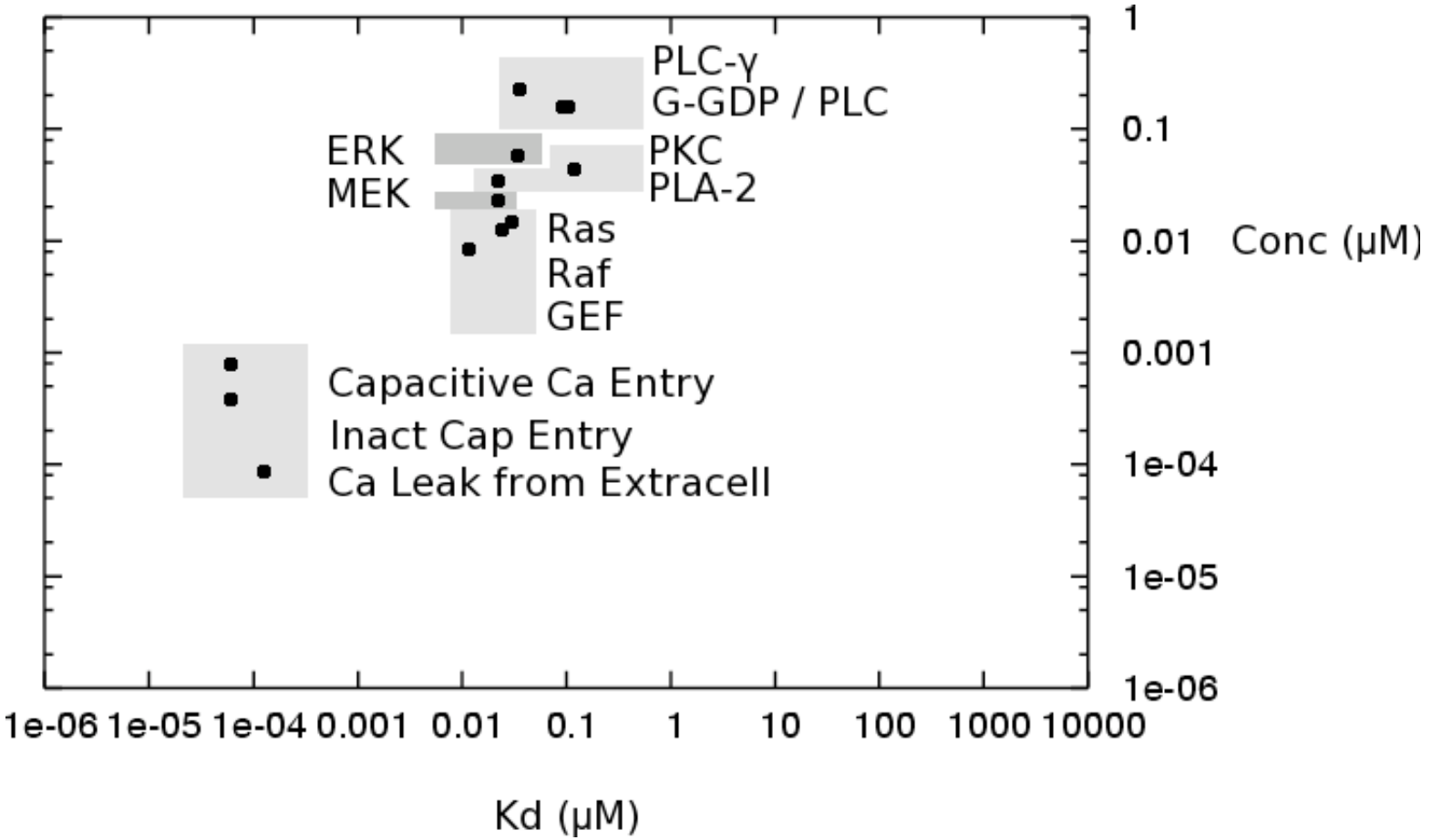
MEK Target


MEK
1
0.1
0.01
0.001
1e-04
1e-05
1e-06
Conc (μM)
1e-06 1e-05 1e-04 0.001 0.01 0.1 1 10 100 1000 10000
Kd (μM)

Calcium blockers

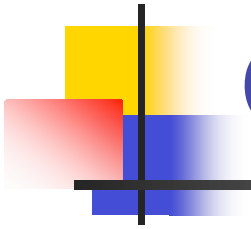


Threshold “Ranking”



Experimental Assays

- PLC-γ - peptide based inhibitors
- PLC - U73122
- PKC - Tamoxifen , Go6976, SPC-100221
- PLA-2 - Quinacrine (anti-inflammatory)
- ERK - PD98059
- MEK - AZD6244/ARRY-142886, CI-1040 , PD98059
- Ras - R115577, BMS-214462 SCH-66336
- Raf - Bay43-9006 ISIS-5132
- GEF -
- Calcium Blockers Verapamil , Nicardipine



Overview

- Ras/Gap mutations predicted
- PP2A mutations predicted
- Ras/Raf interaction “importance” predicted
- Much of the overexpression/underexpression mutations predicted
- Drug targets are for the most part have experimental counter parts.

Who did the real work?

- **Dhruv Pant**

- **Aparna Kumar**
- **Ray Zou**
- **Dave Miller**

- **Uli Rodeck (TJU)**

- Yihua Wang
- Hanbing Lin
- Tom Shortell
- Travis Hoppe
- Jordan Viss

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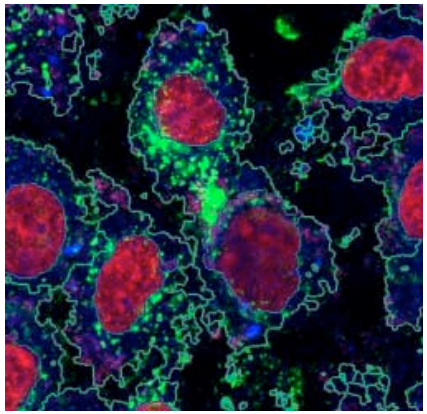
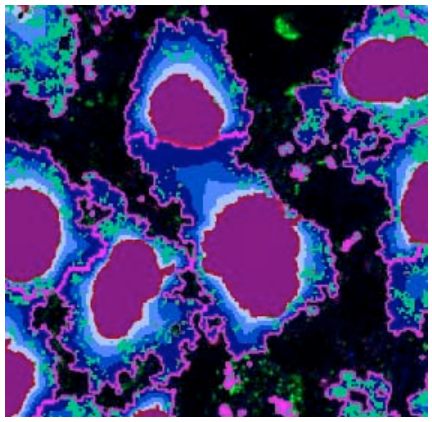
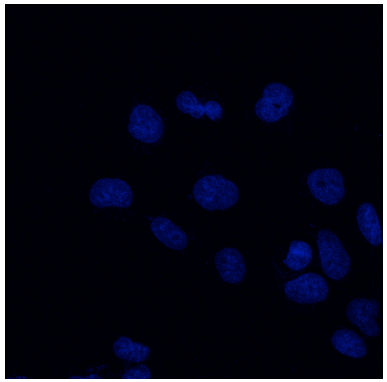
• NSF, Penn. Health Initiative, Sun, Drexel Startup Funds

Confocal Imaging and Quantum Dots





(**Elisabeth S. Papazoglou, Andres Kriete)**



QDots

- Tunable
- Photostable
- Narrow Emission Spectra
- Broad Absorption Spectrum

Confocal Imaging

- Live Cells and fixed cells
- sub micron imaging

Quantitative Signaling Analysis

Data from Confocal Imaging

- derivation of “in vivo” rate constants through global analysis

Given time sliced images and model

Derived rate constants will “fit” experimental images best (global optimization problem) with confidence intervals

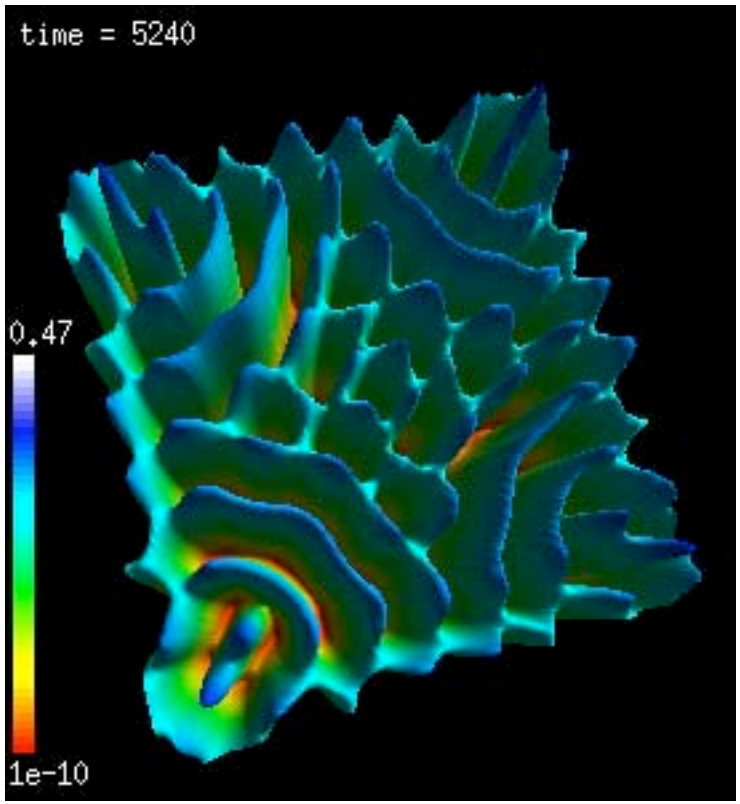
- wealth of data helps prevent over fitting C(x,y,z,t)
- “Automated” Sensitivity Analysis will be used for fitting

Cell(ular)Sim(ulator)

•Cross Platform (Linux, Windows, MacOS X, Solaris)
•Multiprocessor (MPI) (linear scaling)
•Freely Available (GPL)

•Pseudo-C Scripting Language

Adaptive Time Stepping Integrators (Reaction Diffusion Advection) with Sensitivity Analysis



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