

From Simulation to Therapy:

A Systems biology Approach to Oncogene Detection

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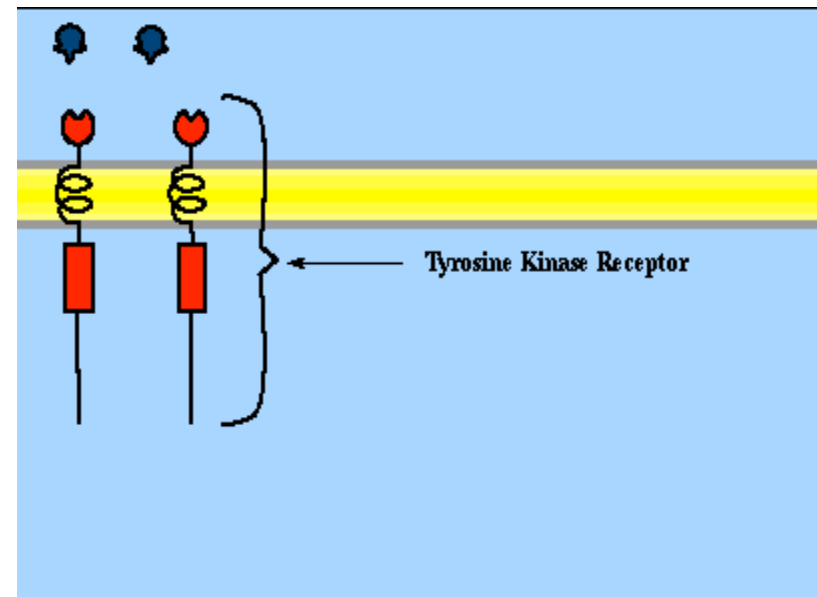


Outline

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

Signal Transduction

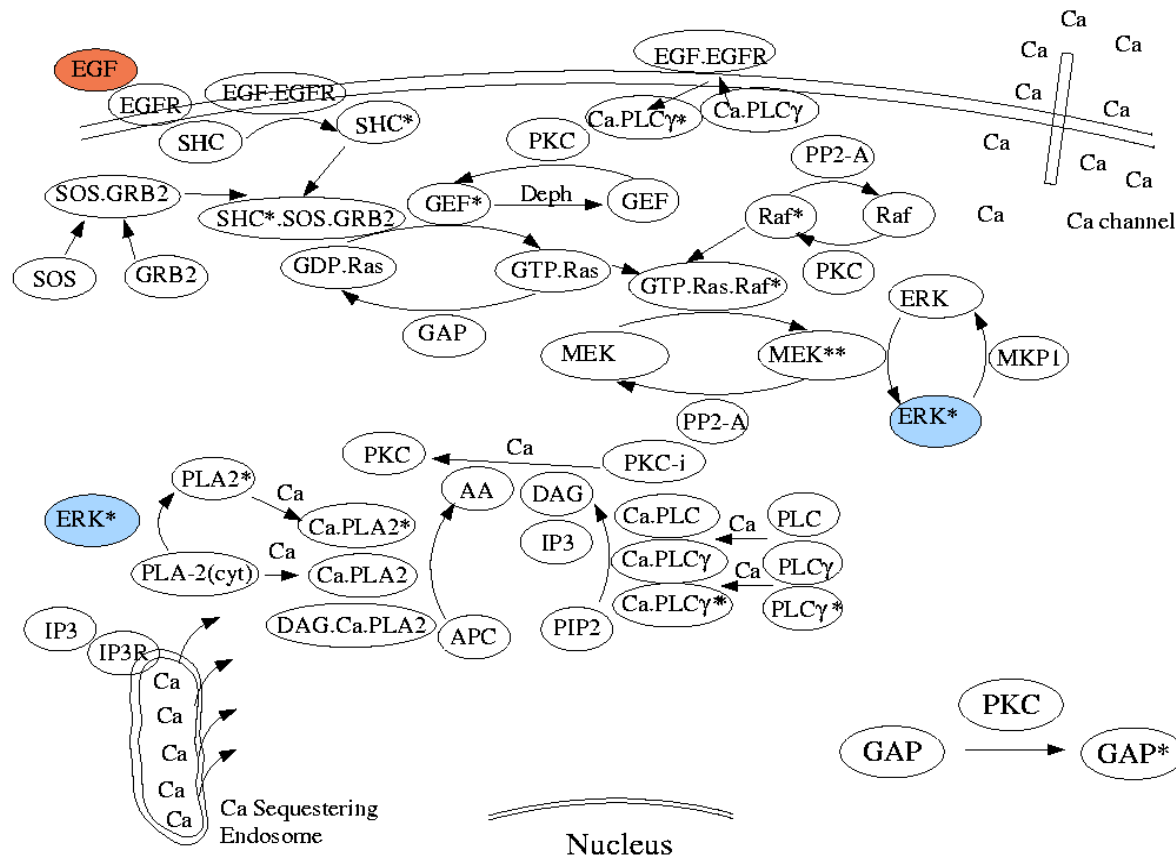
- Controls behavior of cells
 - Ion Channels
 - Metabolic Pathways
 - Cytoskeletal Proteins
 - Gene Regulatory Proteins
 - Cell cycle
 - Cytoskeletal rearrangement



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

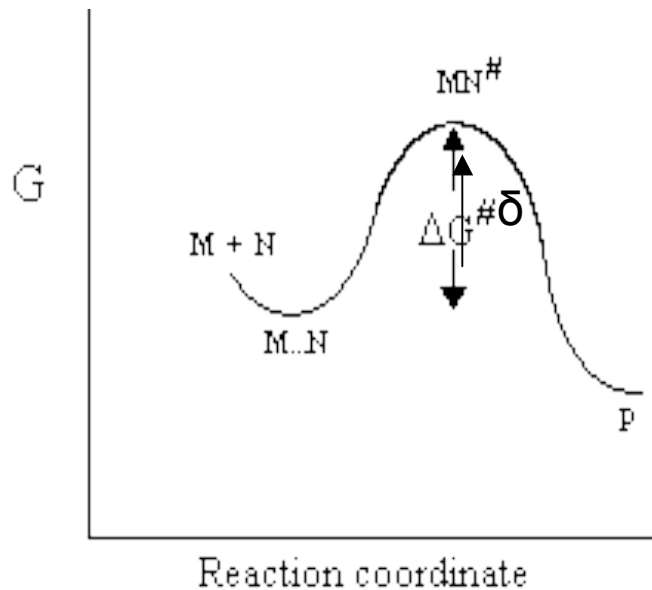
- **Oncogene** - A gene (protein) that when mutated or inappropriately expressed contributes to cancer formation
 - Mutations in signal transduction genes
 - Mutations in Tumor Suppressor genes
 - **INHERITED**
- **Activation**
 - Point Mutation
 - Chromosomal rearrangement
 - Gene amplification
 - Viral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



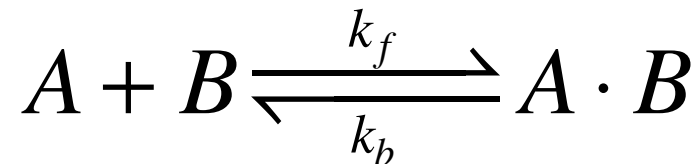
$$k_{f_1} = Ae^{-\Delta G_1 / k_b T}$$

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

$$\frac{k'_{f_1}}{k_{f_1}} = \frac{k'_{f_2}}{k_{f_2}} = e^{-\delta / k_b T} \quad \delta = -k_b T \log\left(\frac{k'_{f_1}}{k_{f_1}}\right)$$



Effects on signaling



A mutation between two unique species

Affects Reactant State

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

Affects Product State

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

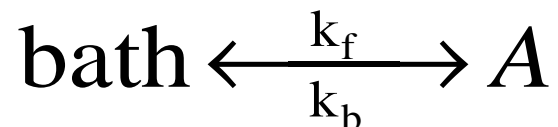
Affects Transition State

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

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

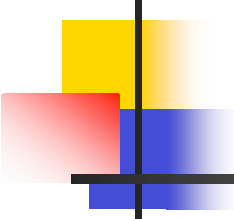


How may a mutation affect the initial concentration of species?



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

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



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



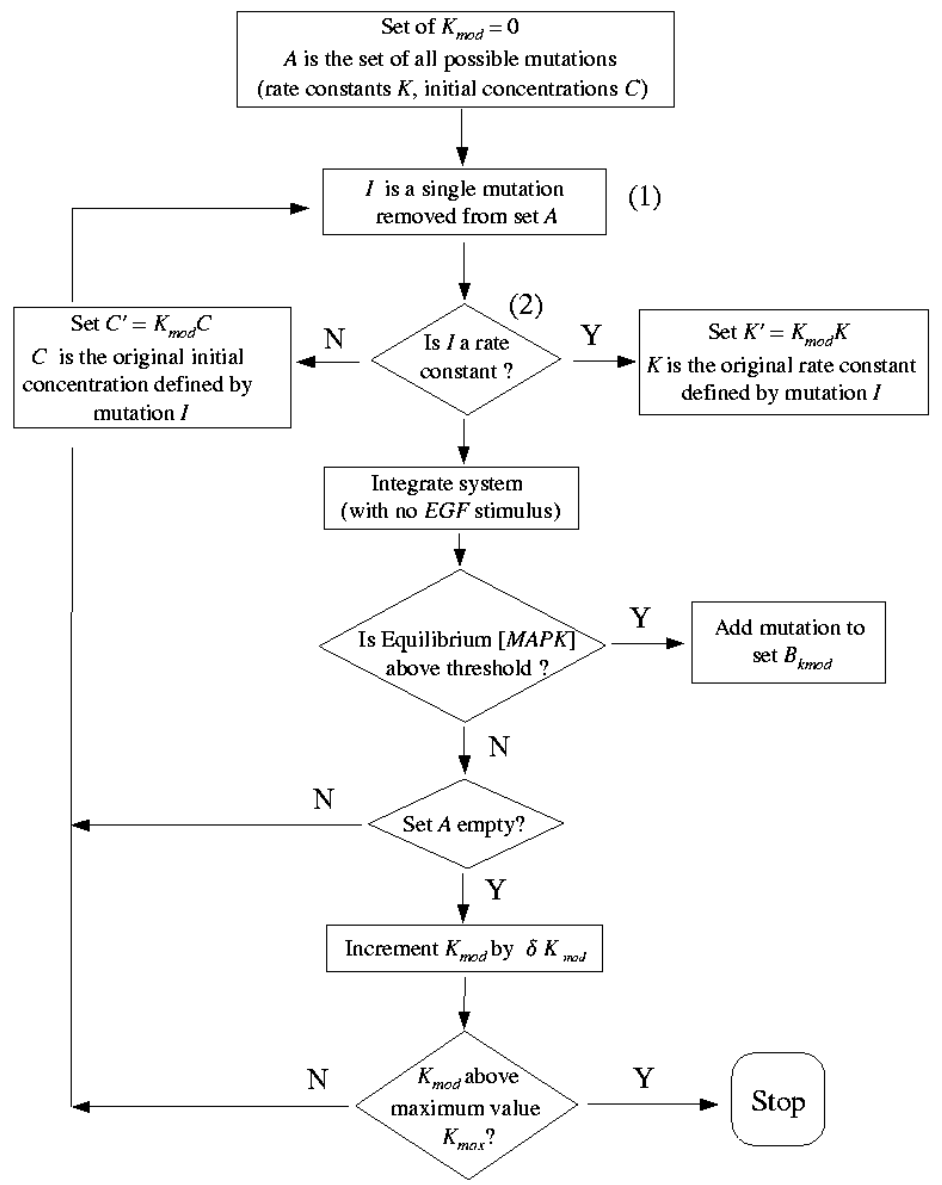
The method

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

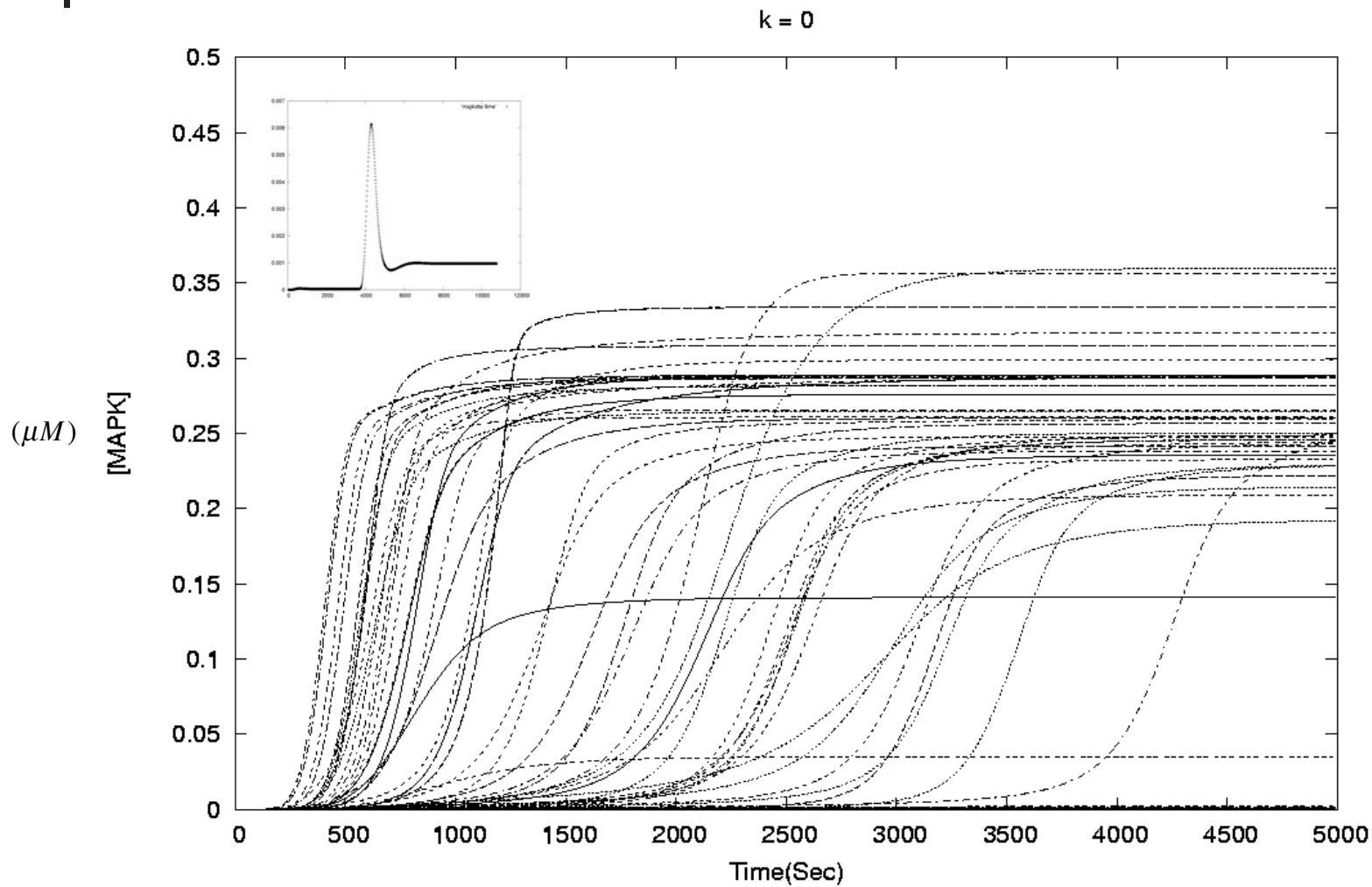
$$\frac{dC_i}{dt} = \sum_j k_{ij} C_j + \sum_{l>m} k_{ilm} C_l C_m$$

- Tag ERK* activations and Rank according to:
 $e^{-\delta/kT}$

Extend to multiple mutations

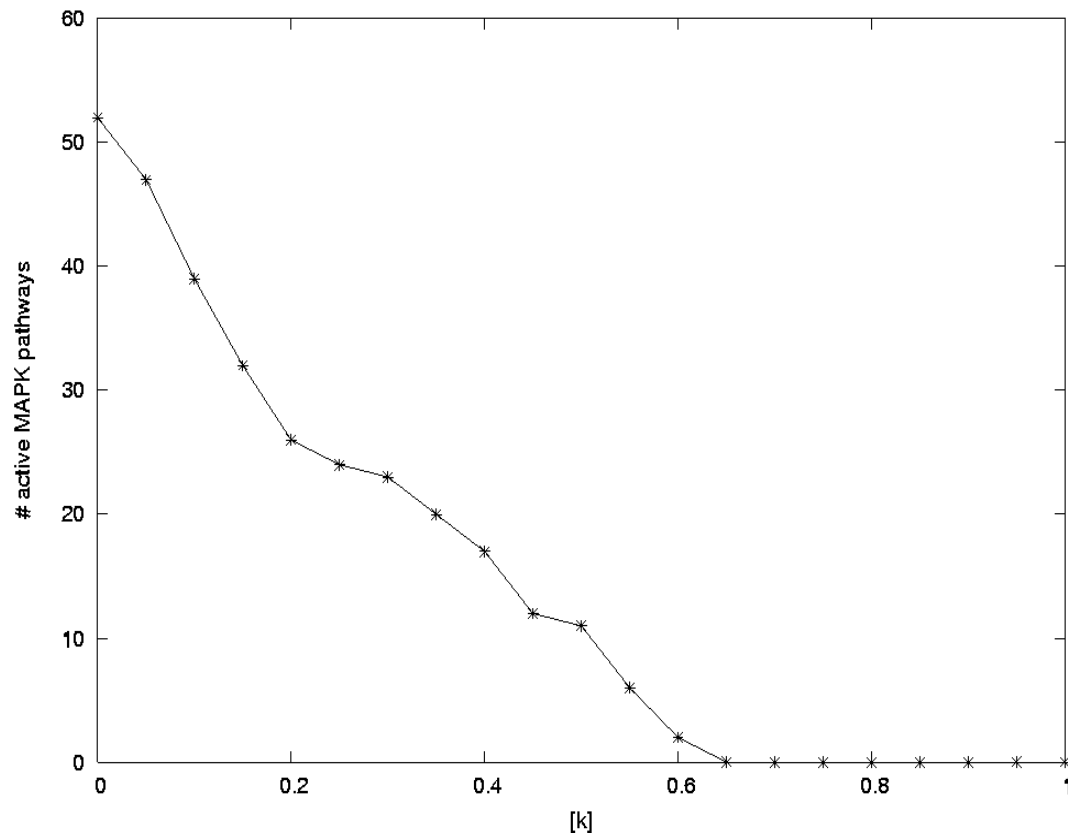


“In Silico” Interaction Knockout experiments



Single Site Transformations

(kf'/kf)

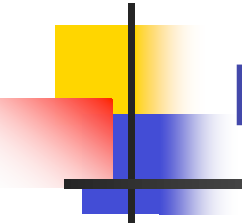




Top Ranked Mutations

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

Top Ranked expression level mutations



$\frac{[A]'}{[A]}$	Species	$\frac{[A]'}{[A]}$	Species
0.4	MKP-1	1.9	GEF
0.5	GAP	2.8	MEK
0.7	PP2-A	3.0	PLC
1.4	PKC-I	3.4	PLC-g
1.6	Raf	3.6	ERK
1.7	Ras		



In Silico Cancer Cells

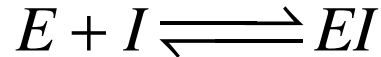
- We have developed a family of “in silico” cancer cells as models of pathways

What is this good for?



The effect of Drugs on Signaling in mutated cells

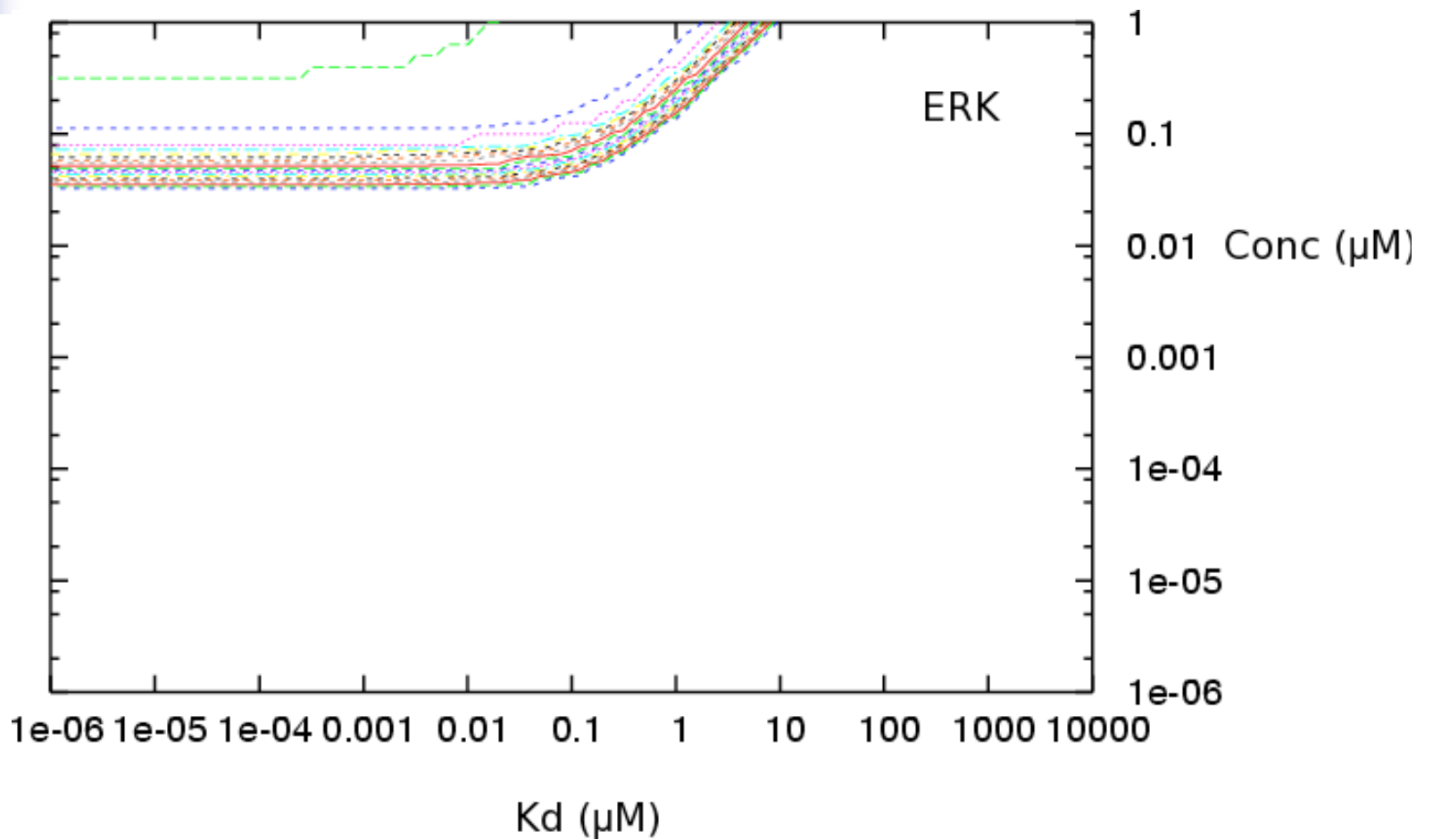
Competitive Binding



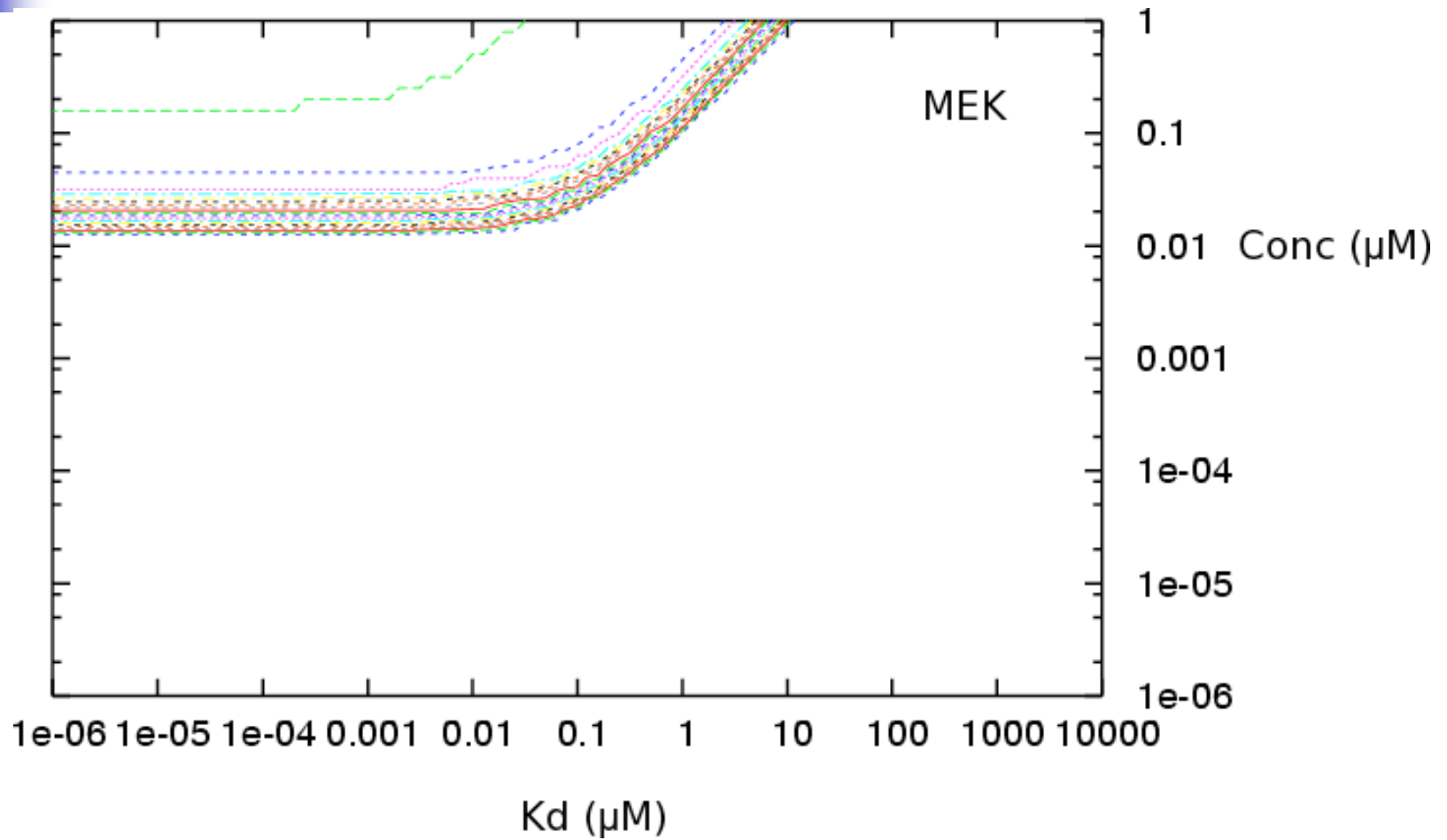
Noncompetitive binding



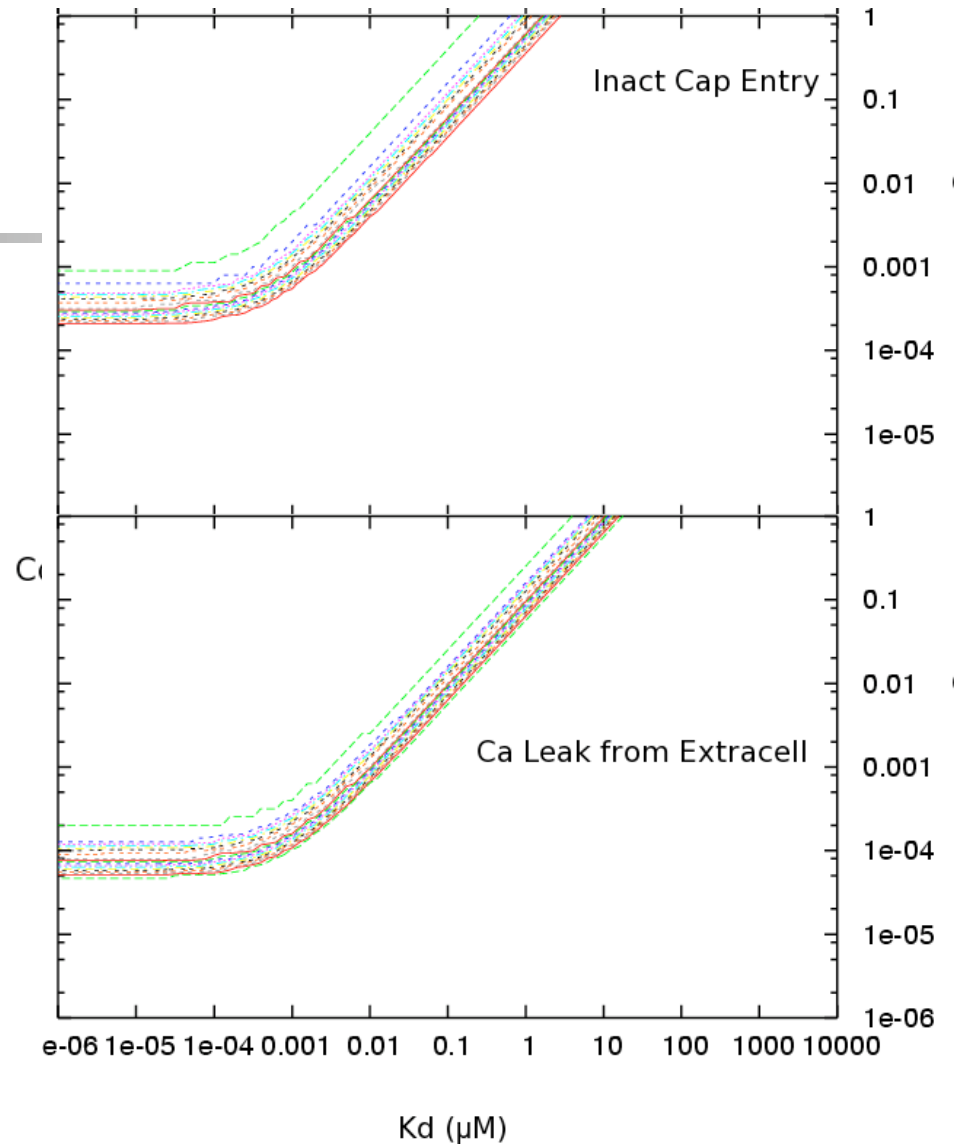
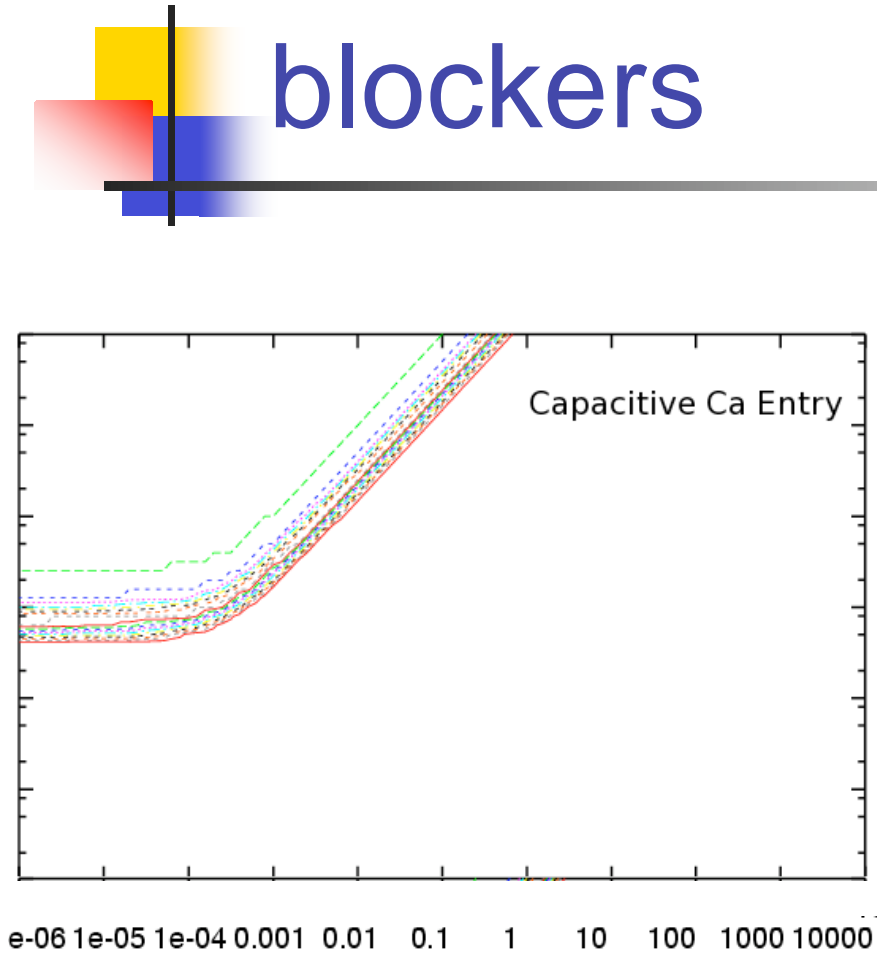
ERK Target



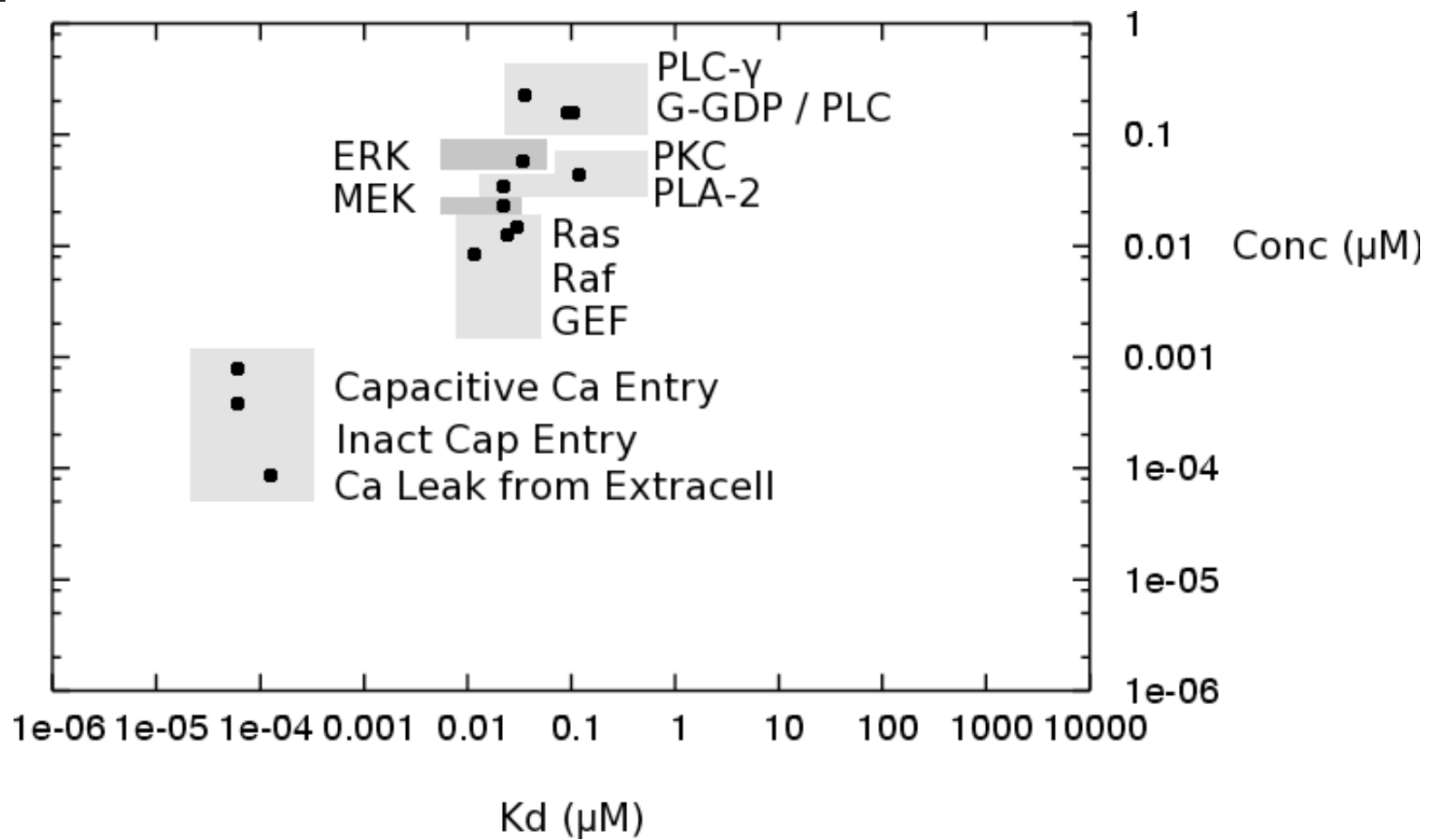
MEK Target



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 , Nifedipine



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

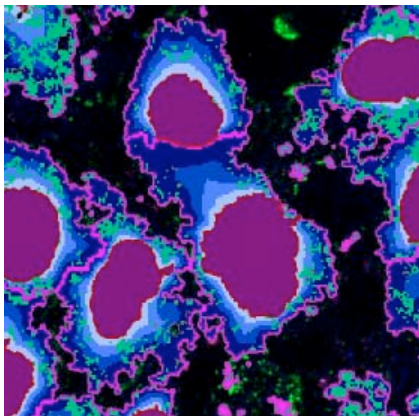
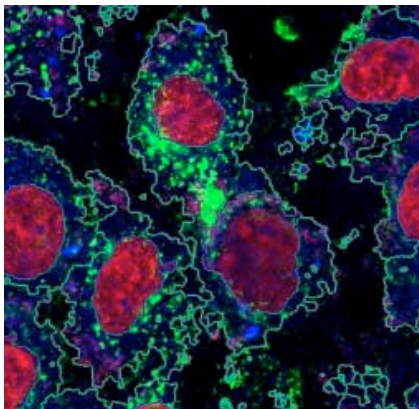


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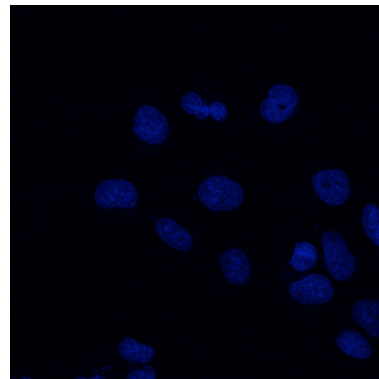
Email : avijit@physics.drexel.edu

- 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

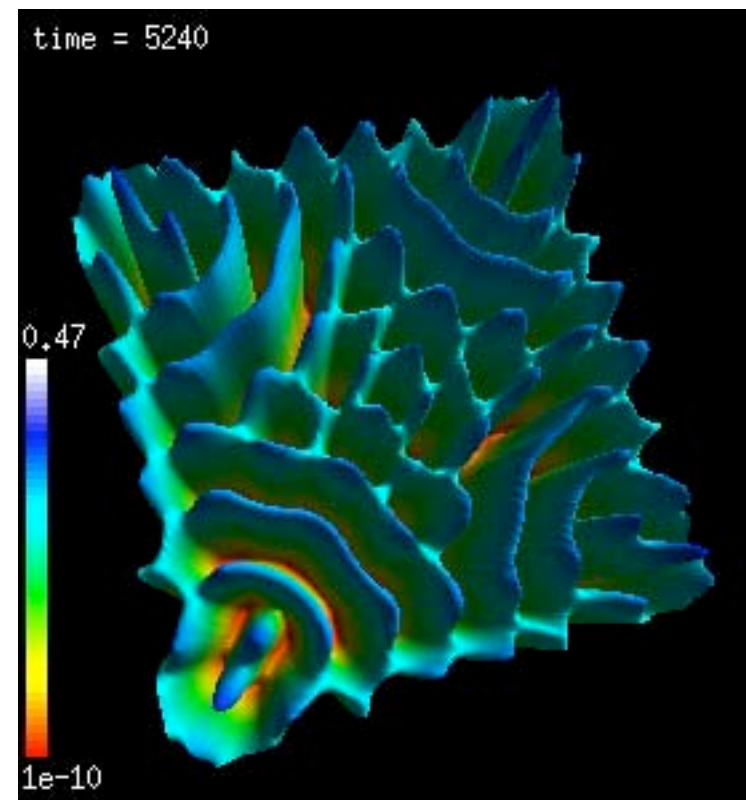
Given time sliced images and model

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

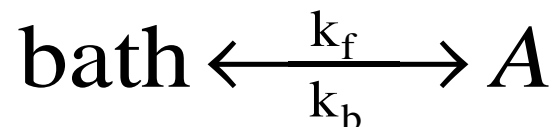
Cell(ular)Sim(ulator)

- 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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