A SYSTEMS APPROACH TO CELLULAR SIGNAL TRANSDUCTION

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A DISSERTATION

in

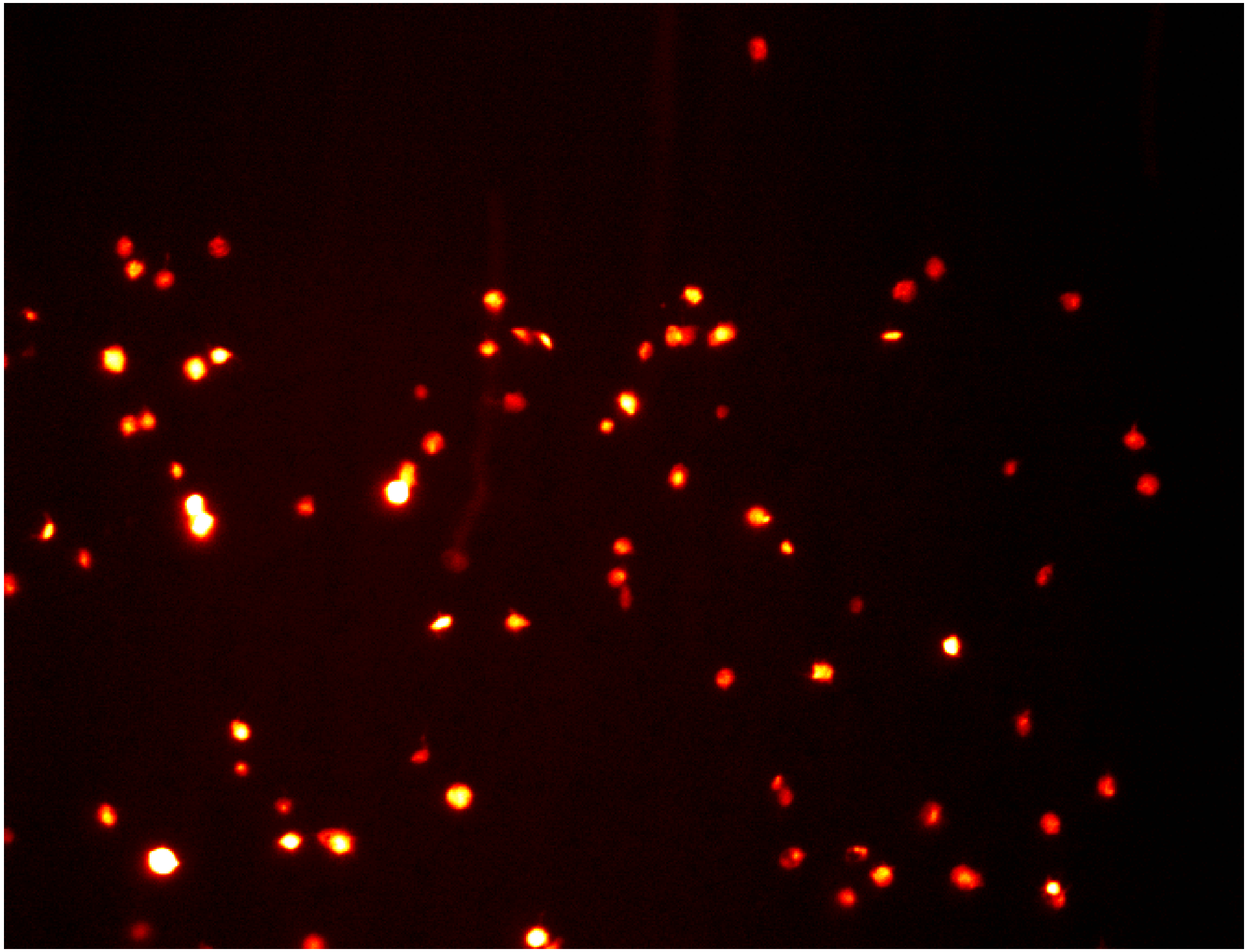
Genomics and Computational Biology

Presented to the Faculties of the University of Pennsylvania in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

2009

Cell signaling involves coordinated molecular interactions

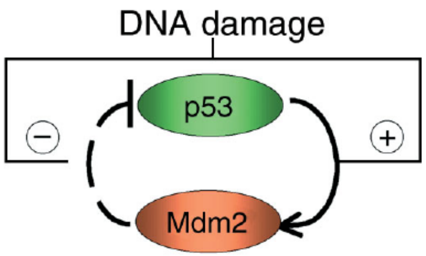
Platelets adhering to collagen, triggering intracellular calcium release

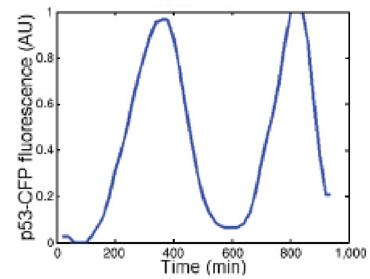


Sean Maloney | *unpublished data*

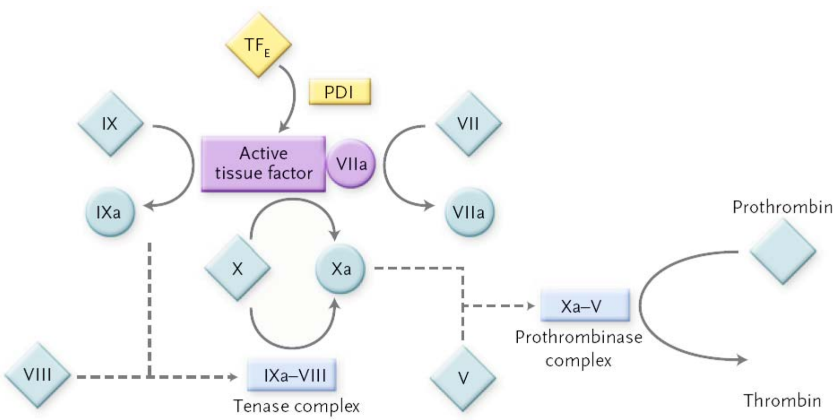
Signals are emergent behaviors with molecular causes

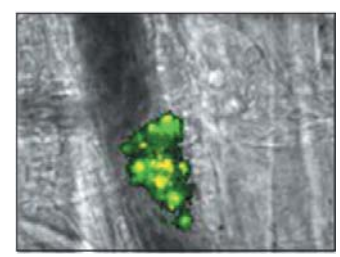
Reactions governing the DNA damage response give rise to pulses of activated p53





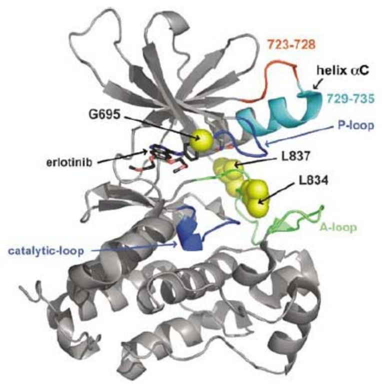
Reactions governing coagulation give rise to waves of calcium in a developing thrombus

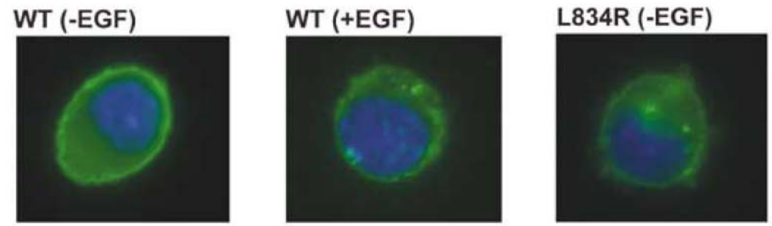




Lahav et al., 2004. Furie and Furie, 2008.

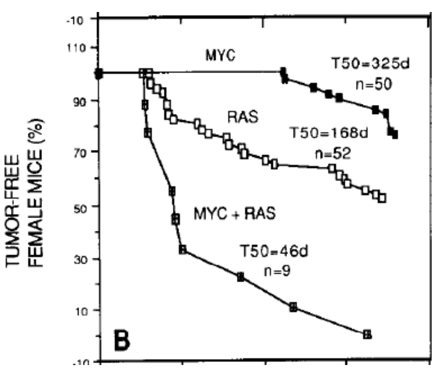
A single AA substitution in the epidermal growth factor receptor leads to constitutive growth signals





Expression of c-*myc* and *ras*^{D} in transgenic mice dramatically accelerates tumor growth

c-myc **AND** *ras*^{D}

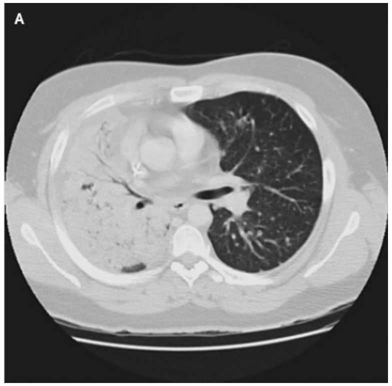


Choi et al., 2007. Sin et al., 1987.

Computational approaches elucidate two clinically-relevant signaling responses

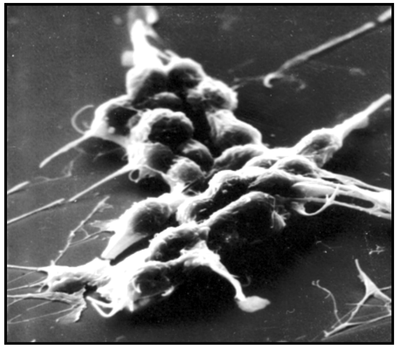
- Oncogenic signaling through the epidermal growth factor receptor

  Ravi Radhakrisnan (PI)
  Yingting Liu
  Andrew Shih
  Neeraj Agrawal



- Calcium and phosphoinositide signaling in human platelets

  Scott Diamond (PI)
  Skip Brass (PI)
  Manash Chatterjee

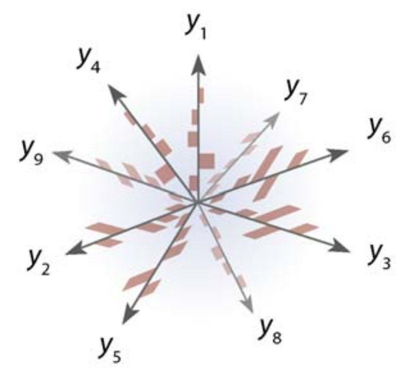


Lynch et al., 2004. White, 2007.

New modeling methods afford specific design goals

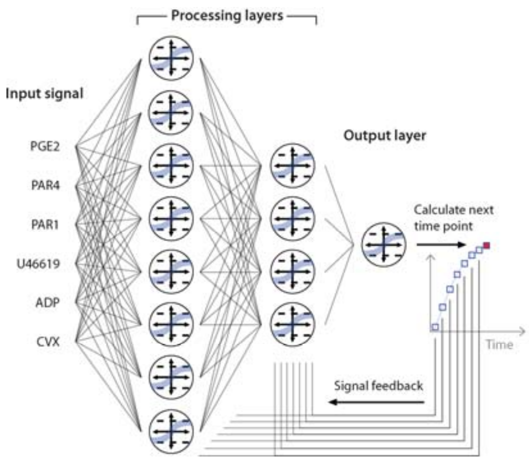
- Steady-state kinetic modeling constrains cellular resting states

  Ravi Radhakrisnan (PI)
  Scott Diamond (PI)

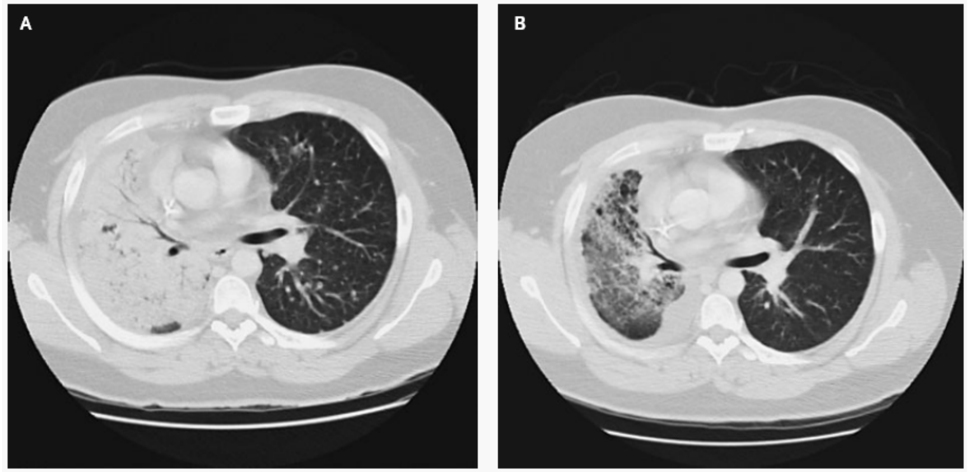


- Dynamic neural network modeling predicts responses to multi-input signals

  Scott Diamond (PI)
  Manash Chatterjee



Purvis et al., 2009. Chatterjee et al., 2009.



I. Phosphotyrosine Signaling in the Epidermal Growth Factor Receptor

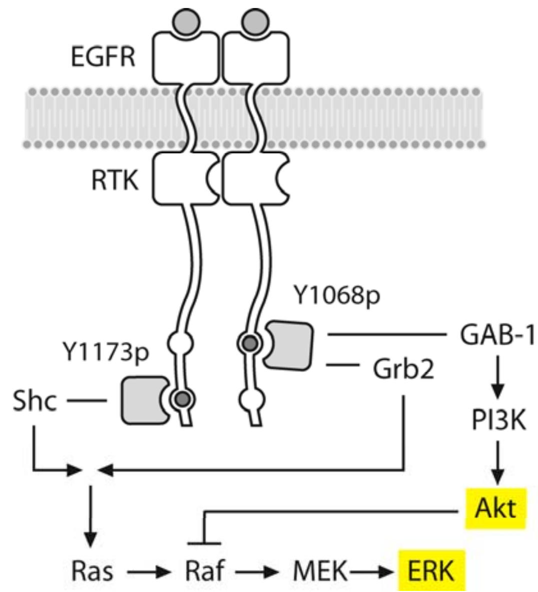
Liu et al. (2007). *Ann Biomed Eng* 35:1012-25
Purvis et al. (2008). *Biotechnol Prog* 24:540-53
Shih et al. (2008). *Mol Biosyst* 4:1151-9.

- Addresses the question of why certain cancer cell lines are responsive to tyrosine kinase inhibitor (TKI) therapy
- Seeks to resolve how structural alterations in the receptor are communicated as downstream growth or survival signals

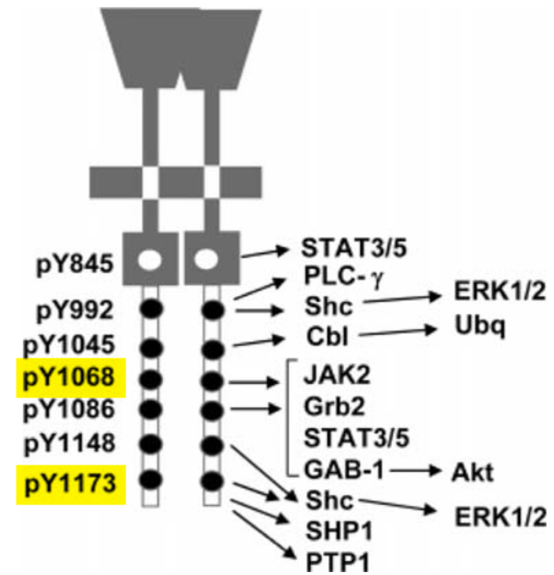
Lynch et al. (2004)

Differences in receptor signaling determine cell fate

- Growth factor signaling influences cell decisions through receptor activation and trafficking, effector processing, and transcription



- Patterns of tyrosine phosphorylation encode varying signals mediated through adaptor proteins

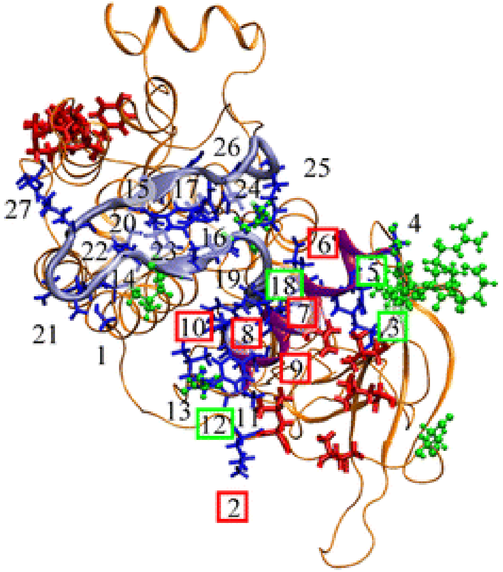


Purvis, Ilango, and Radhakrishnan (2008)

Mutant behavior resolved through molecular dynamics and molecular docking

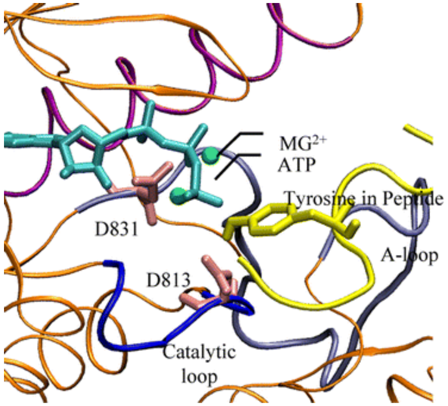
- Molecular dynamics simulations showed that the drug sensitizing mutation (L858R) of EGFR stabilizes the active conformation

  Andrew Shih



- Docking simulations showed that L858R had an increased affinity for the drug and a preference for certain phosphorylated tyrosine residues

  Yingting Liu



Liu, Purvis, Shih, Weinstein, Agrawal, and Radhakrishnan (2007)

Increased flux through Y1068 causes a relative gain in Akt activation

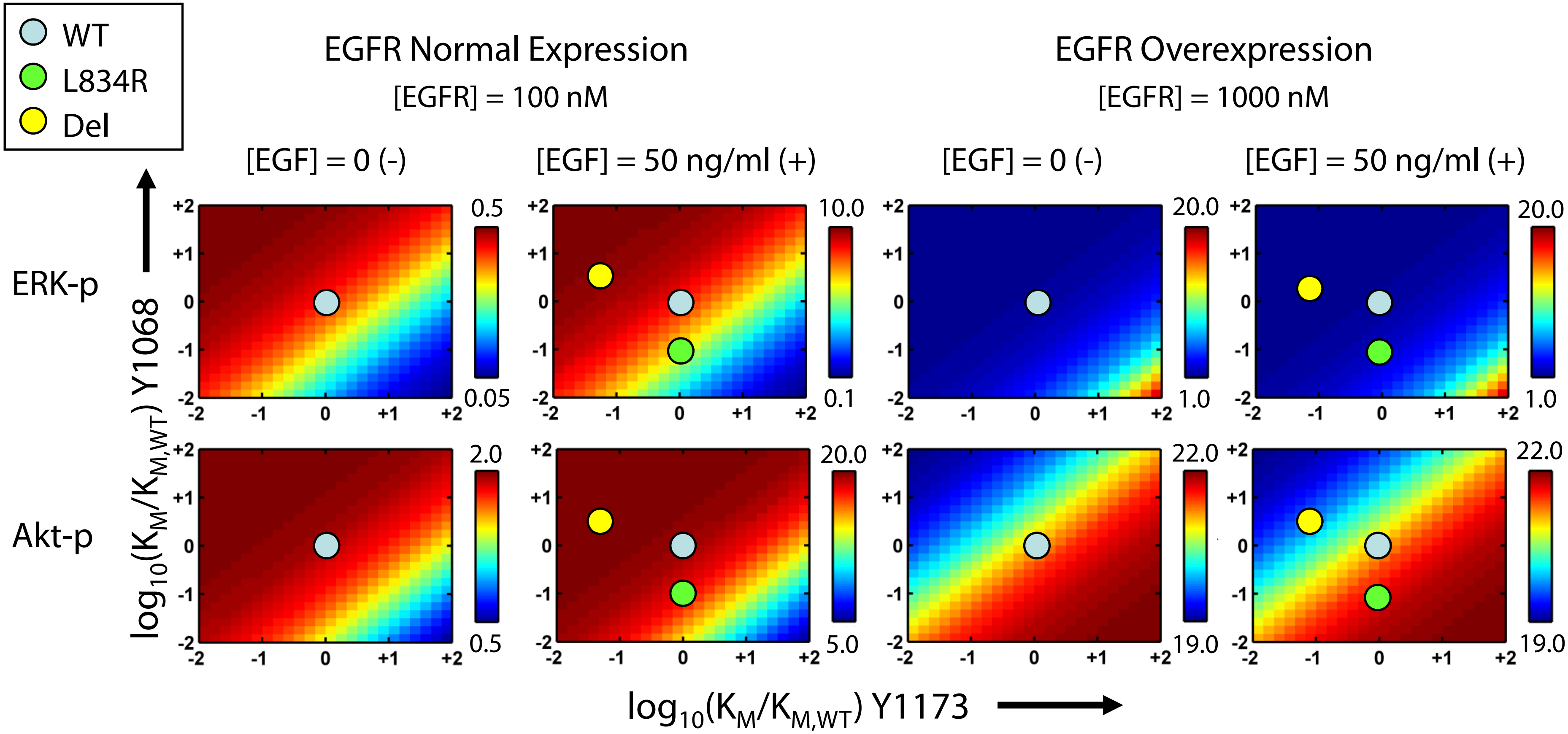
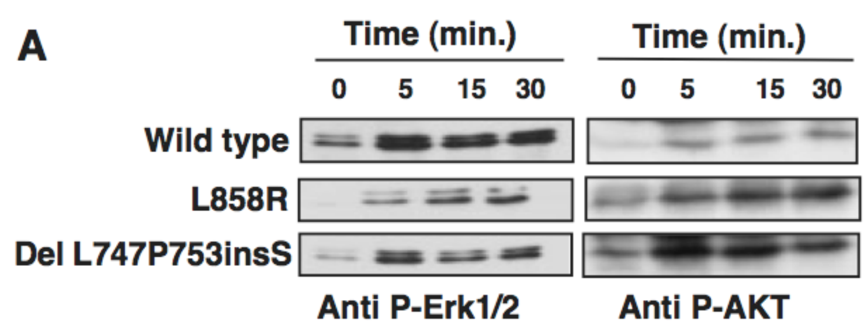


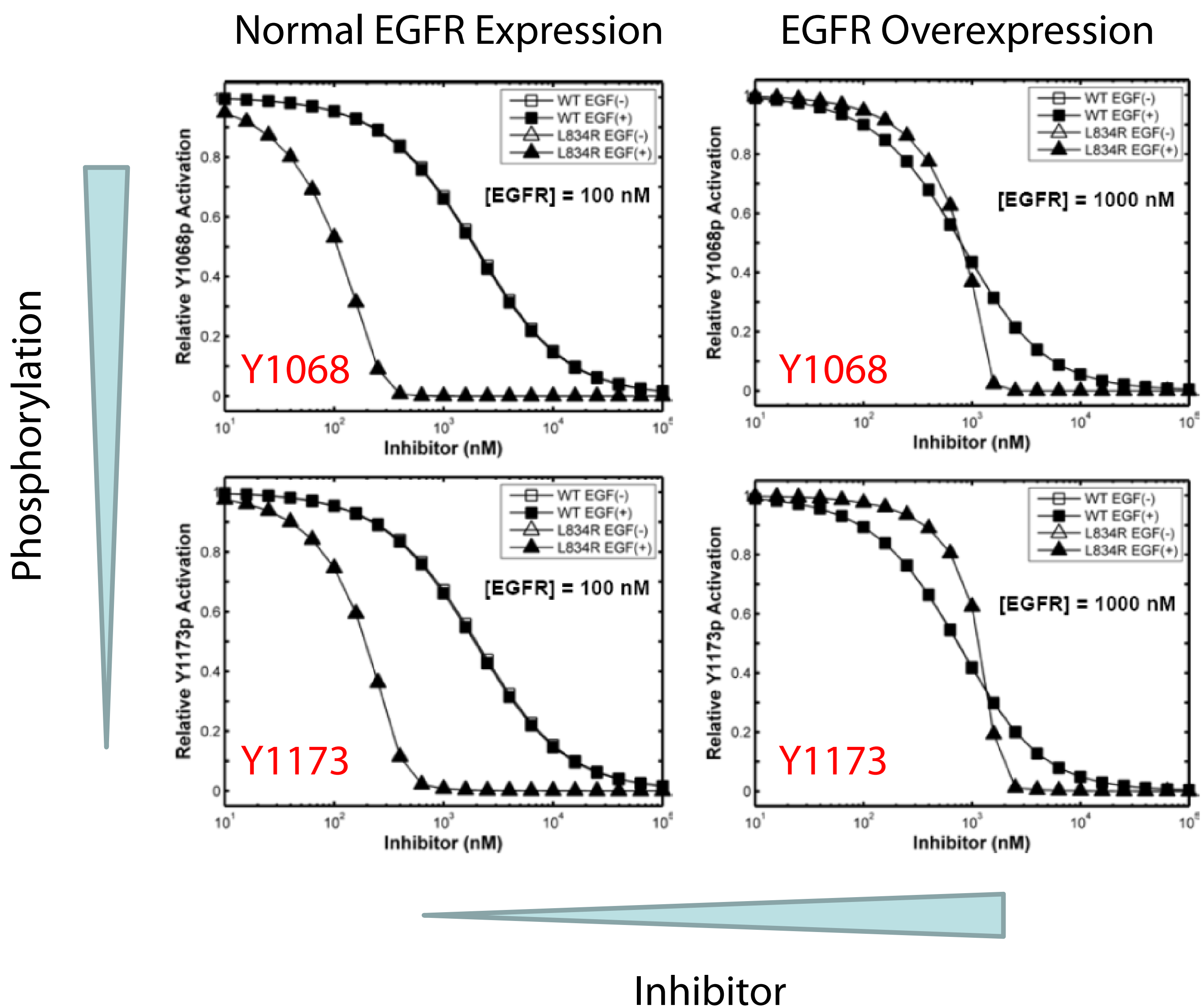
Table 1. Parametric Differences between WT and Mutant EGFR Systems Inferred from Experiments

parameter	WT	L834R	del	reference
K_M^{ATP}	5.0 μM	10.9 μM	129 μM	3
$K_I^{erlotinib}$	17.5 nM	6.25 nM	3.3 μM	3
K_M^{Y1068}	265 μM	13.3 μM	130 μM	14, 57, 58
K_M^{Y1173}	236 μM	200 μM	300 μM	14, 57, 58
k_{cat}^{Y1068}	0.29 s^{-1}	0.24 s^{-1}	0.2 s^{-1}	14, 54, 57, 58, 59
k_{cat}^{Y1173}	0.25 s^{-1}	0.21 s^{-1}	0.22 s^{-1}	14, 54, 57, 58, 59

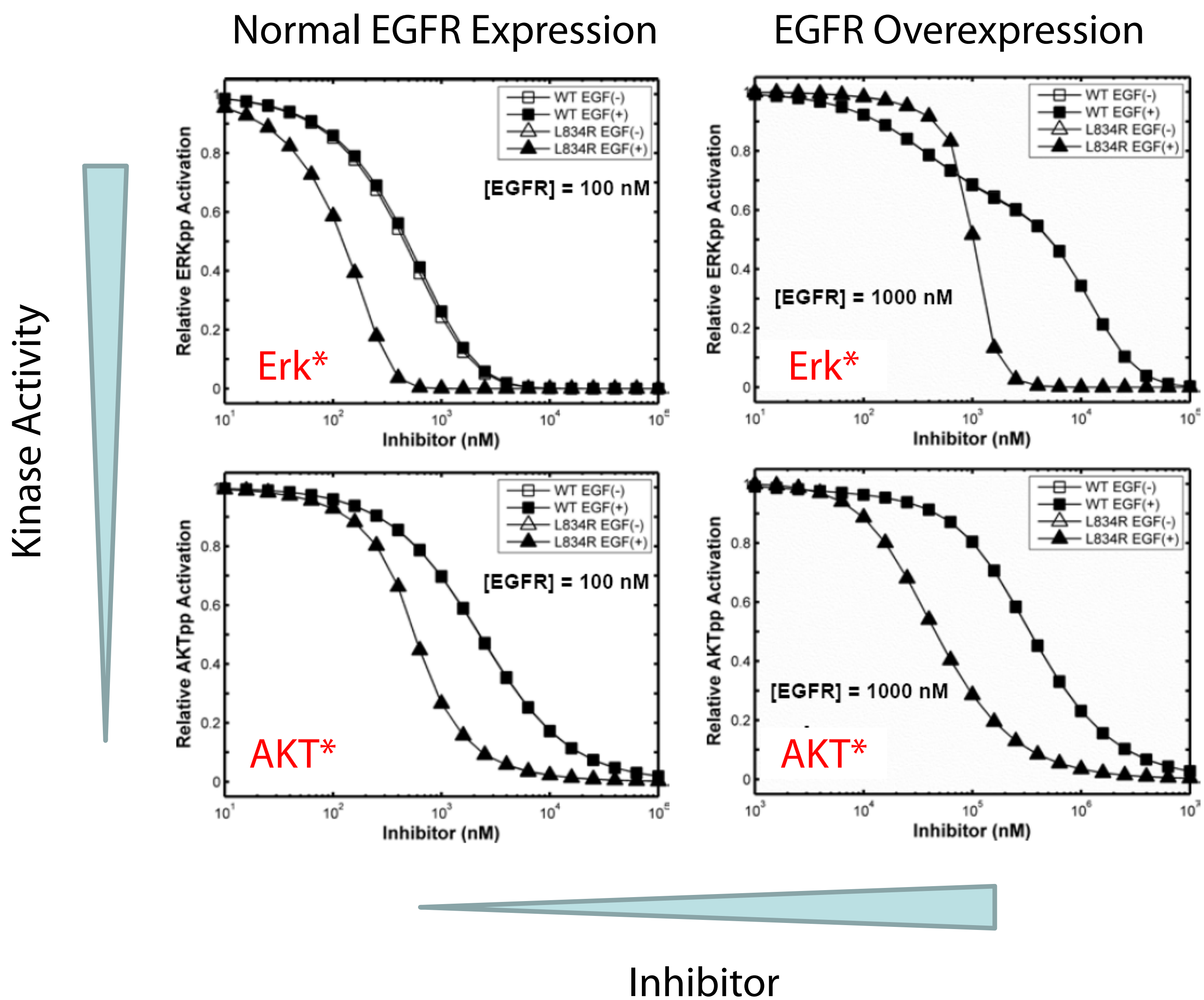


Purvis, Ilango, and Radhakrishnan (2008). Sordella et al. (2004)

Tarceva® (erlotinib) reduces Y-phosphorylation under normal EGFR expression

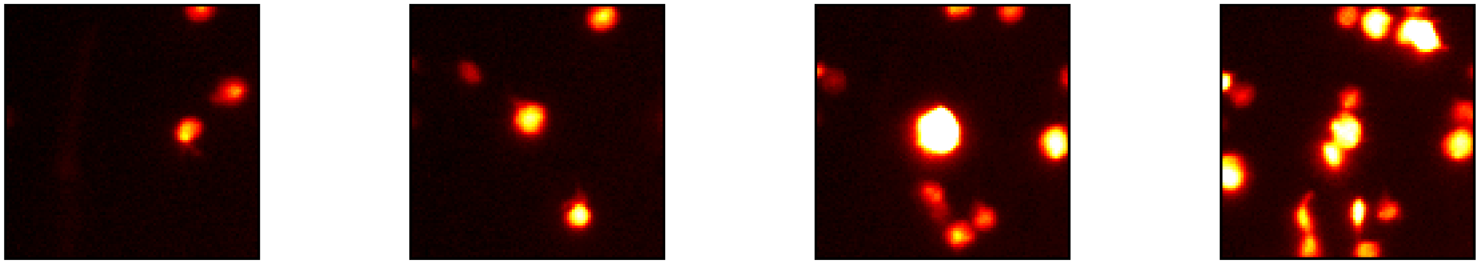


Tarceva® (erlotinib) reduces Akt activation under normal and overexpressed EGFR



Multiscale modeling offers mechanistic explanation for oncogene addiction

- Response of L858R to TKI therapy is an example of "oncogene addiction"
- Sensitivity may be used to identify probable resistance mechanisms
- Method provides a link between mutational status and kinetic properties of the system
- Akt, and not Erk, is the major mediator of cell survival in oncogenic mutants of EGFR



II. Calcium Signaling in Human Platelets

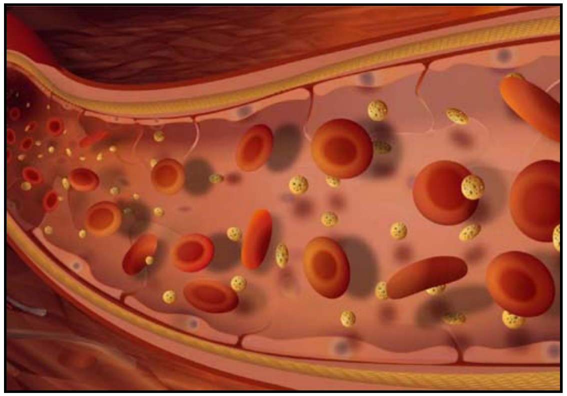
Purvis et al. (2008). *Blood* 112:4069-4079
Purvis et al. (2009). *PLoS Comput Biol*
Chatterjee et al. (2009). *submitted*

- Platelet activation is central to the 1.75 million heart attacks and strokes that occur annually in the US
- Quantitative models can make accurate predictions of patient-specific risks and biological mechanism

Sean Maloney | *unpublished data*

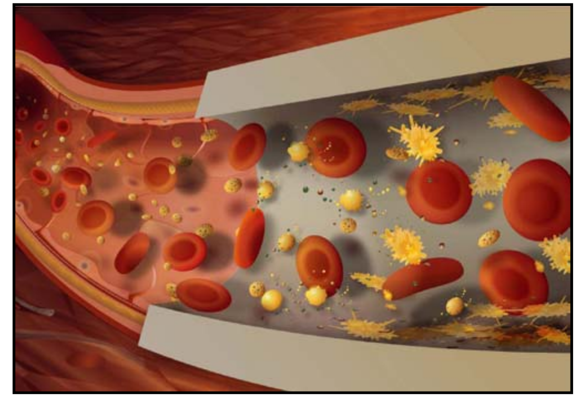
Platelet signaling regulates hemostasis and thrombosis

Normal circulation

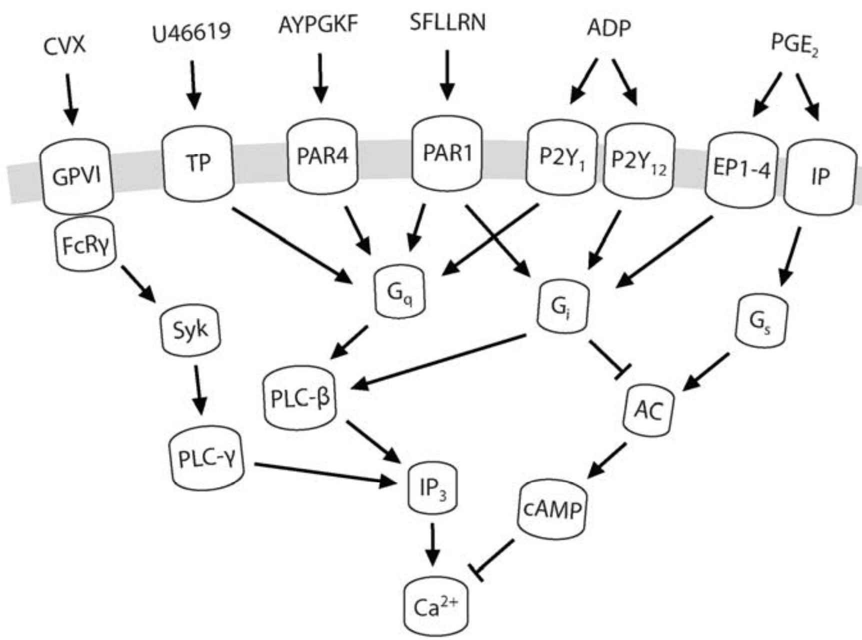


[1]

Injury



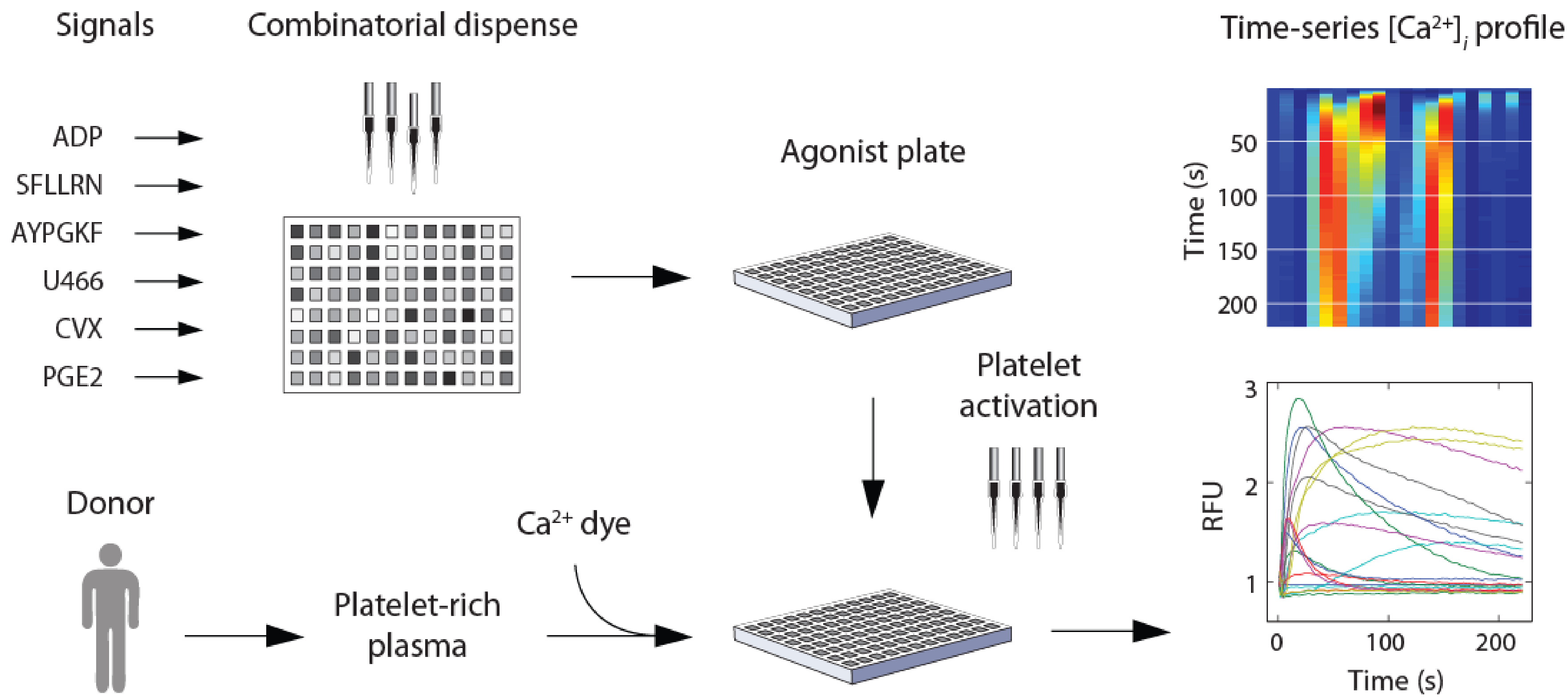
[1]



[2]

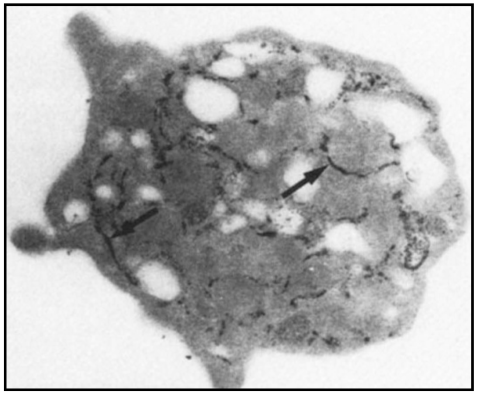
[1] Brubaker (2006). [2] Chatterjee, Purvis, and Diamond (2009)

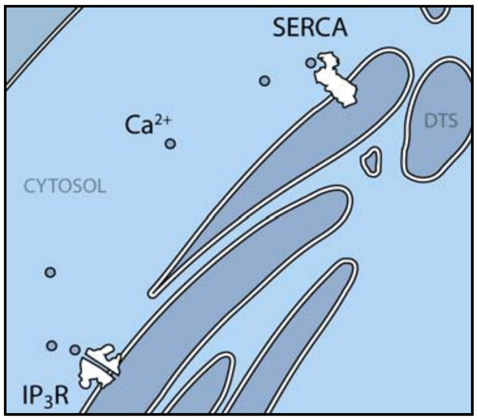
Platelet activation is monitored by dynamic intracellular calcium measurements



[1] Brubaker, 2006. [2] Chatterjee, Purvis, and Diamond, 2009

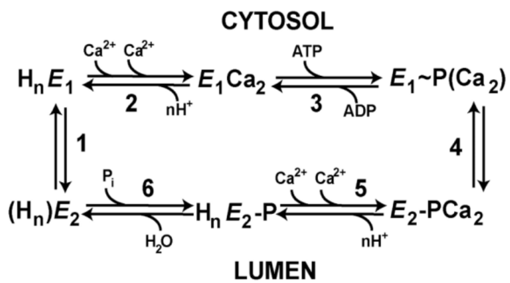
Opposing kinetic processes balance calcium in the platelet



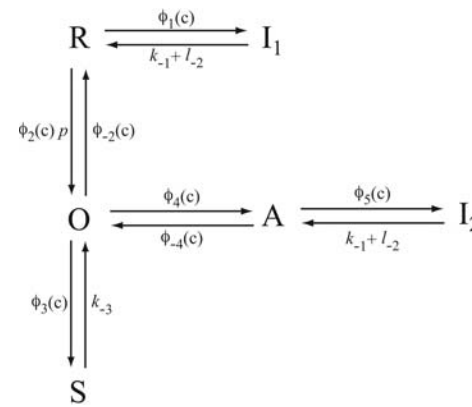


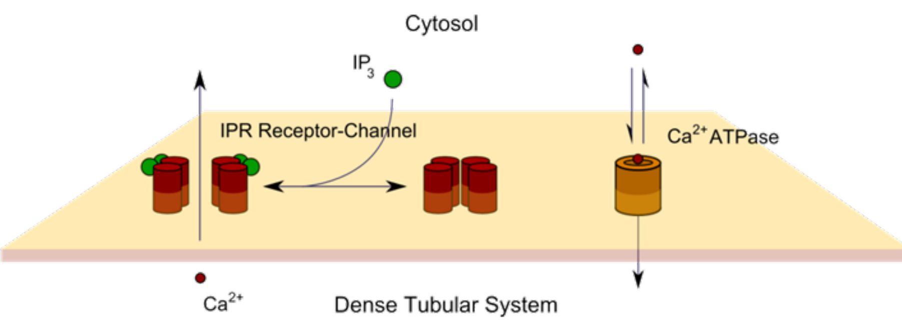
- Cytosolic calcium concentration ($[Ca^{2+}]_{cyt}$) ≈ 100 nM
- Lumenal calcium concentration ($[Ca^{2+}]_{dts}$) ≈ 200-650 μM
- Extracellular calcium concentration ($[Ca^{2+}]_{dts}$) ≈ 1 mM
- Lumenal fraction appears to occupy ~0.1 to 10% of reactive volume

SERCA3 Calcium ATPase



IP_3 Receptor Channel



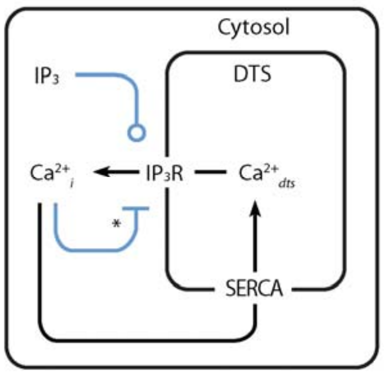


$$\frac{d}{dt}([Ca^{2+}]_i) = I = N \, \gamma \, P_o \, \frac{RT}{zF} \log\left(\frac{[Ca^{2+}]_{dts}}{[Ca^{2+}]_i}\right)$$

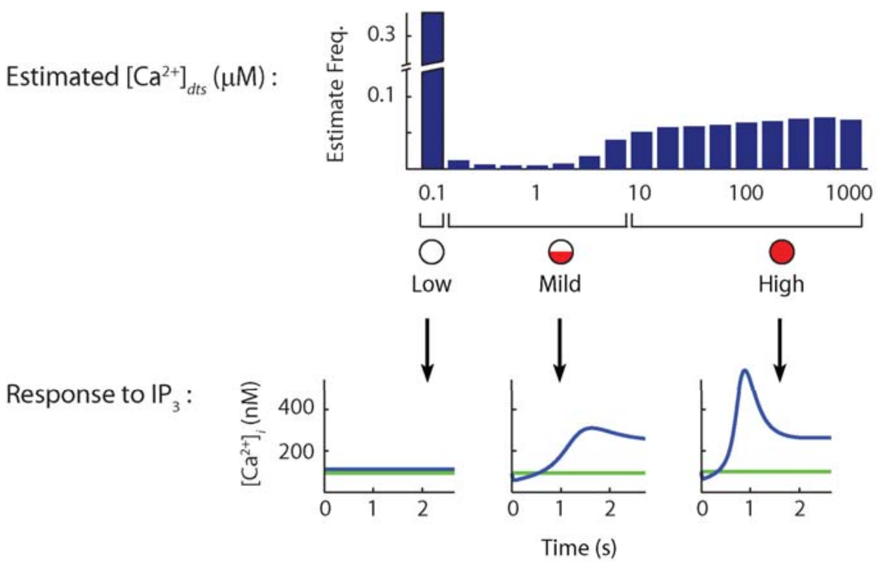
Ebbeling et al., 1992. *Blood* 80:718. Dode *et al*, 2002. Sneyd and Dufour, 2002.

Kinetic processes enforce natural constraints on molecular makeup and structure

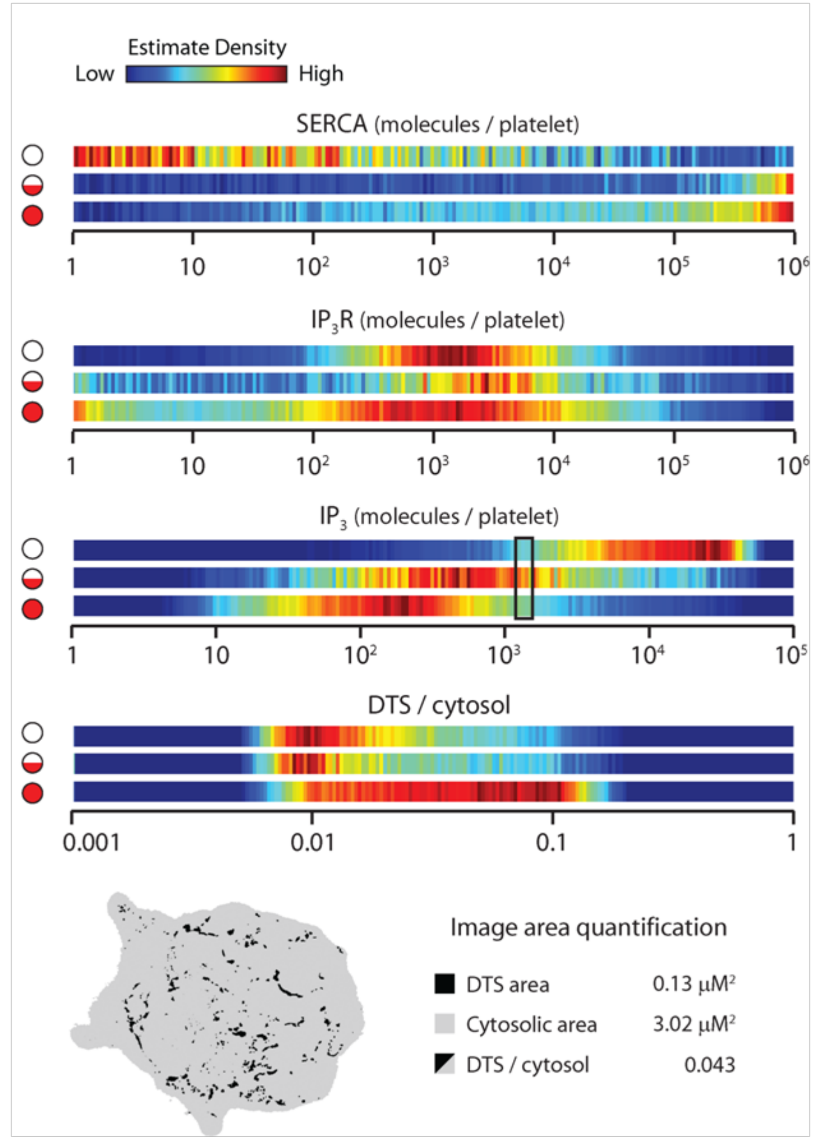
Fix topology and sample Ca^{2+} space



Parameter	Value Range
IP_3R	$1 - 10^6$ platelet^{-1}
SERCA	$1 - 10^6$ platelet^{-1}
$[IP_3]$	$1 - 10^4$ nM
$[Ca^{2+}]_{dts}$	0.01 – 10 mM
Cytosol	5 – 10 fL
DTS	0.01 – 1 fL



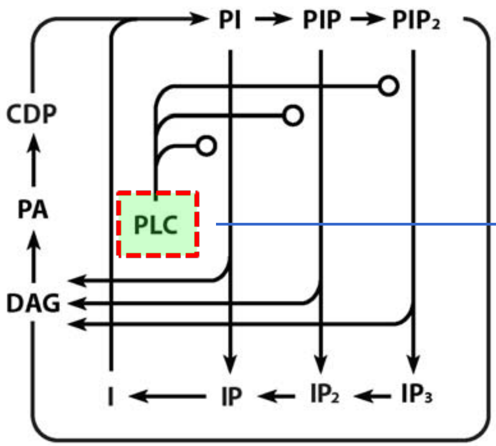
Preferred concentrations/structures



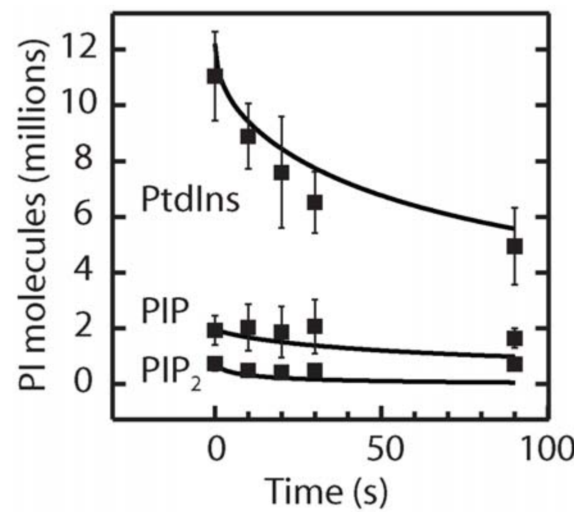
Purvis, Chatterjee, Brass, and Diamond, 2008

Simple signaling module cannot explain regeneration of PIs

PI reaction cycle[1]



PI turnover in response to thrombin/elevated PLC-β[1,2]

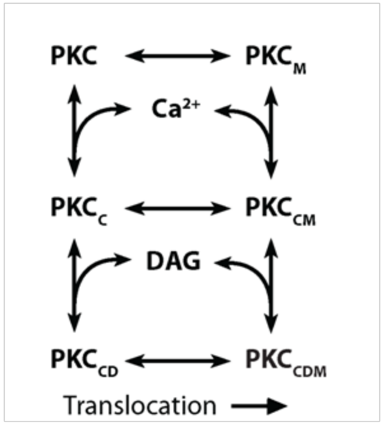


No repletion of PIP_2 in module

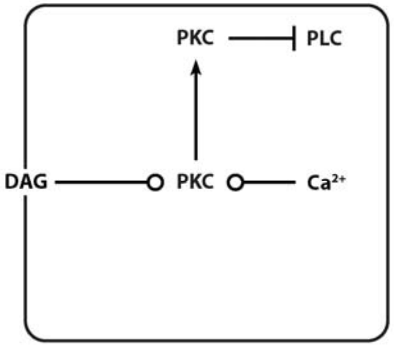
[1] Purvis, Chatterjee, Brass, and Diamond, 2008. [2] Wilson, Neufeld, and Majerus, 1985.

Negative feedback through PKC reproduces synchronized calcium pulses

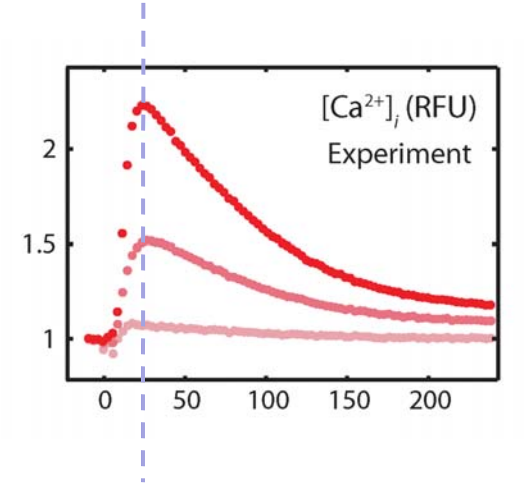
Modulation of PKC activity by second messengers, Ca^{2+} and DAG



Activated PKC translocates to PM and phosphorylates PLC-β

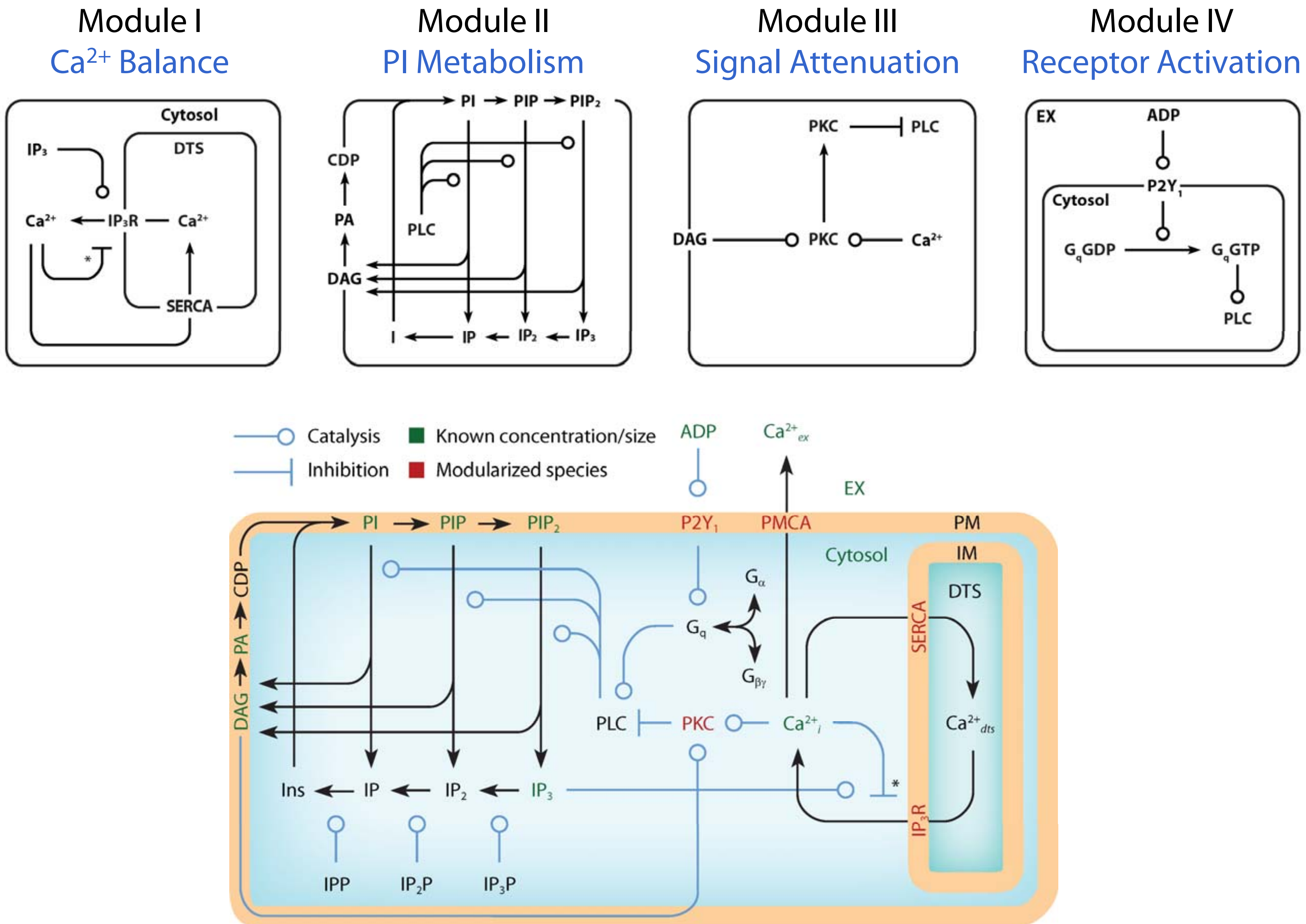


Negative feedback necessary for synchronized responses



Bhalla and Iyengar, 1999

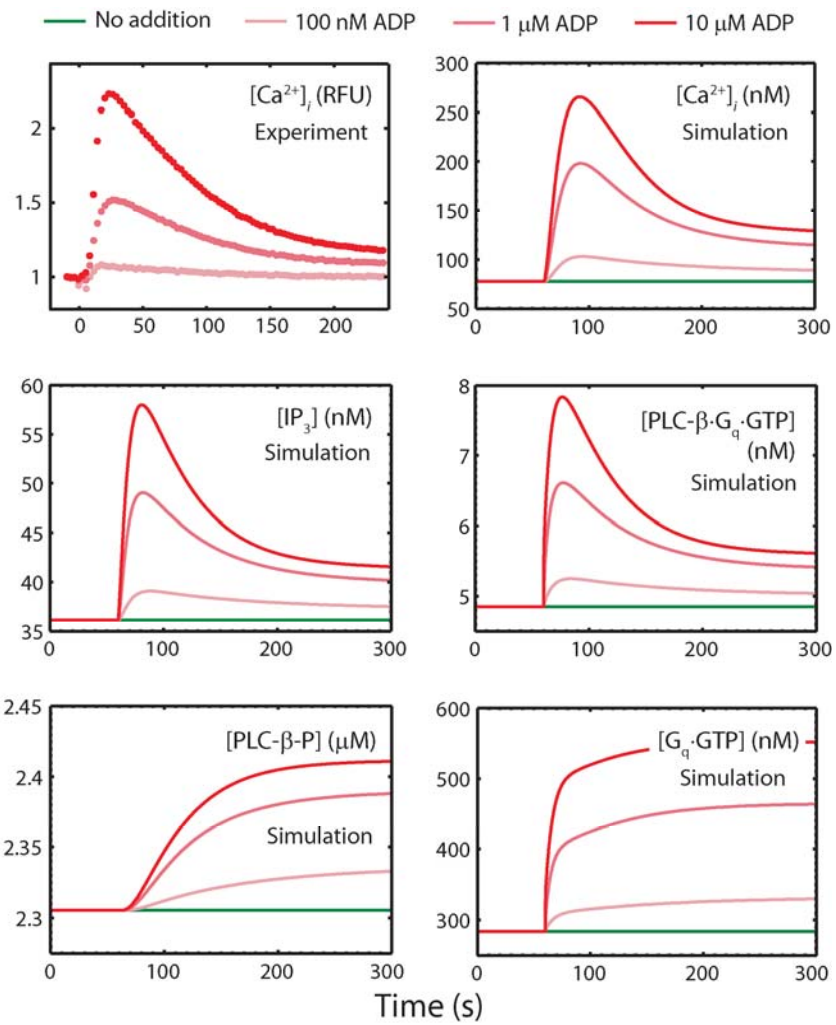
Resting modules are merged to form a full kinetic model



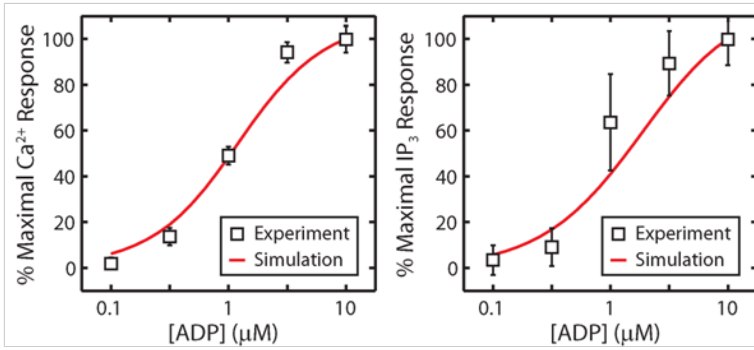
Purvis, Chatterjee, Brass, and Diamond, 2008. *Blood* 112:4069.

Model reproduces measured dynamic behavior of ADP-stimulated platelets

Measured/simulated time-course behavior



Dose response to ADP



Population Responses:

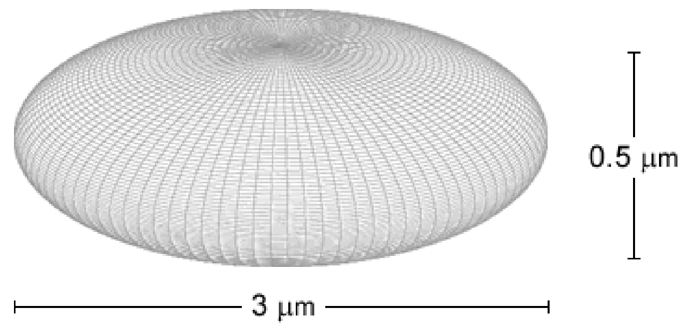
Smooth, average responses are observed when cells are pooled

Purvis, Chatterjee, Brass, and Diamond , 2008.

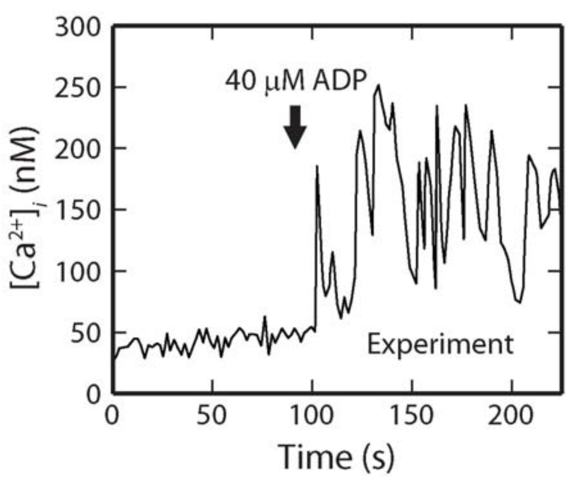
Individual platelets exhibit noisy calcium release behavior

Single Platelet EM[1]

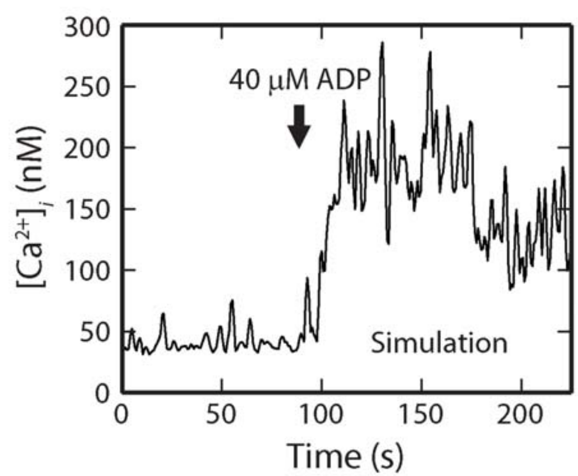
Simulated Platelet Structure



Single Platelet Ca^{2+} Release[2]



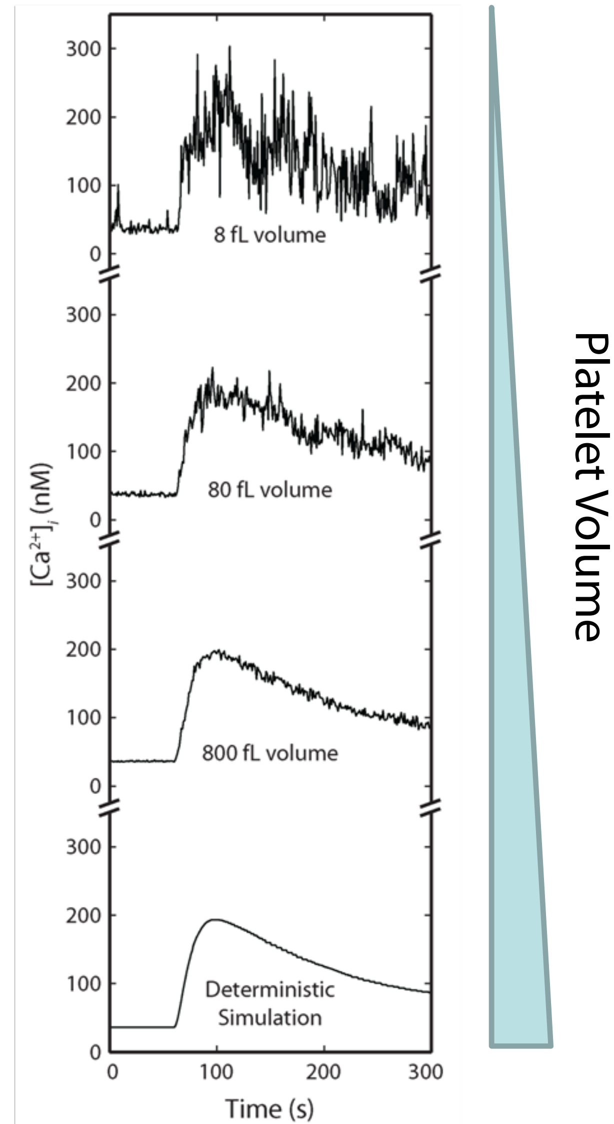
Simulated Platelet Ca^{2+} Release



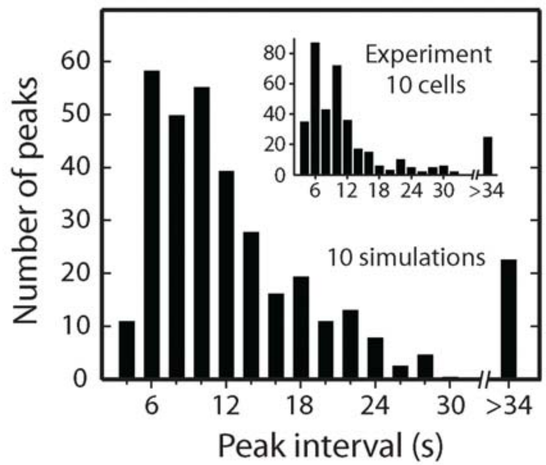
[1] White, 2007. [2] Heemskerk *et al*, 1992. [3] Purvis *et al*, 2008.

Small cell volume gives rise to stochastic response

Ca2+ response as a function of cell volume



Ca2+ peak intervals for observed and simulated platelets



Platelet calcium signaling is stochastic

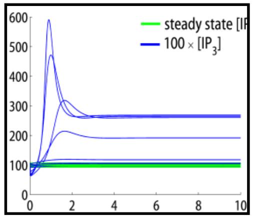
Platelet volume: ~ 8 fL

1 nM = 4 molecules per platelet

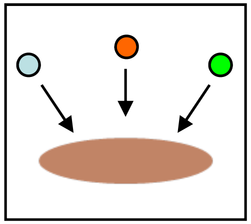
Purvis, Chatterjee, Brass, and Diamond , 2008.

Platelet model is extensible to comprehensive models of coagulation

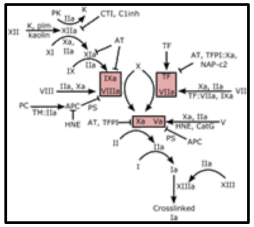
- Quantitatively understand Ca^{2+} balance/release mechanisms



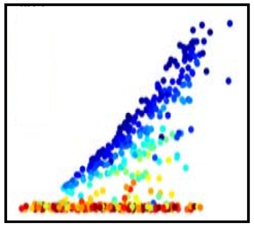
- Recapitulate platelet response to multiple agonists



- Integrate into larger scale models of coagulation



- Predict blood function and pharmacological sensitivity



Homeostasis Requirement

Resting cell:

$$d\,\mathbf{C}/dt = \mathbf{0}$$

Transient cell:

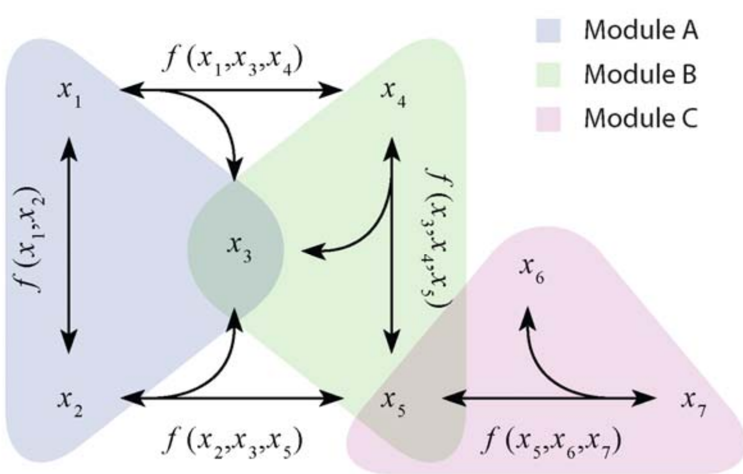
$$d\,\mathbf{C}/dt \neq \mathbf{0}$$

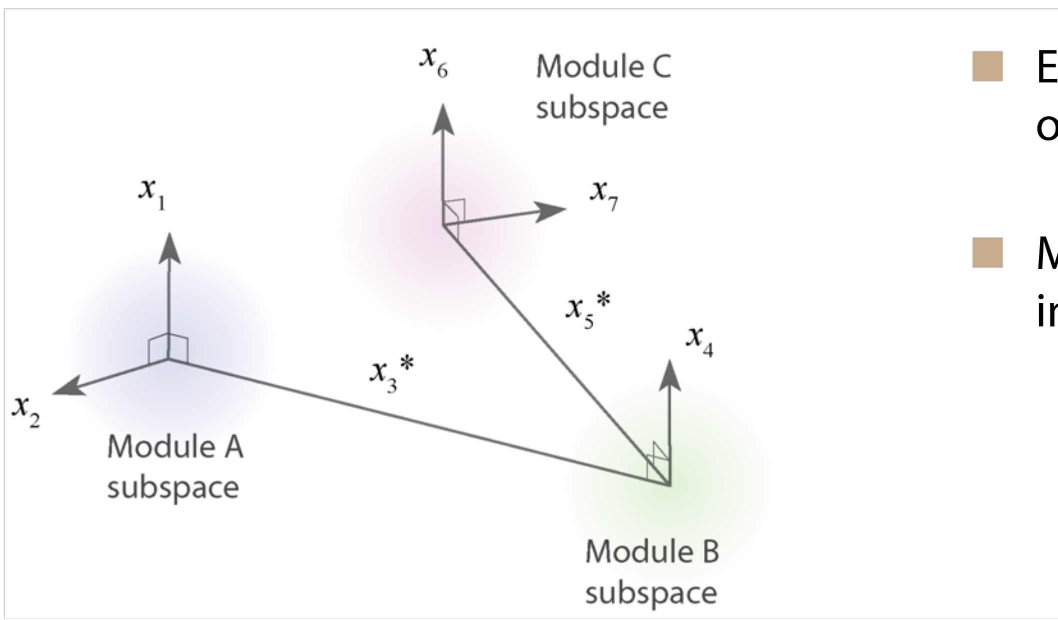
III. Steady-State Kinetic Modeling Constrains Cellular Resting States

Purvis et al. (2008). *Blood* 112:4069-4079
Purvis et al. (2009). *PLoS Comput Biol*

- Signaling systems are robust to small perturbations and have a steady state
- Large kinetic models have many unknown values that are possibly correlated

System with fixed kinetics is represented as a space of unknown concentrations

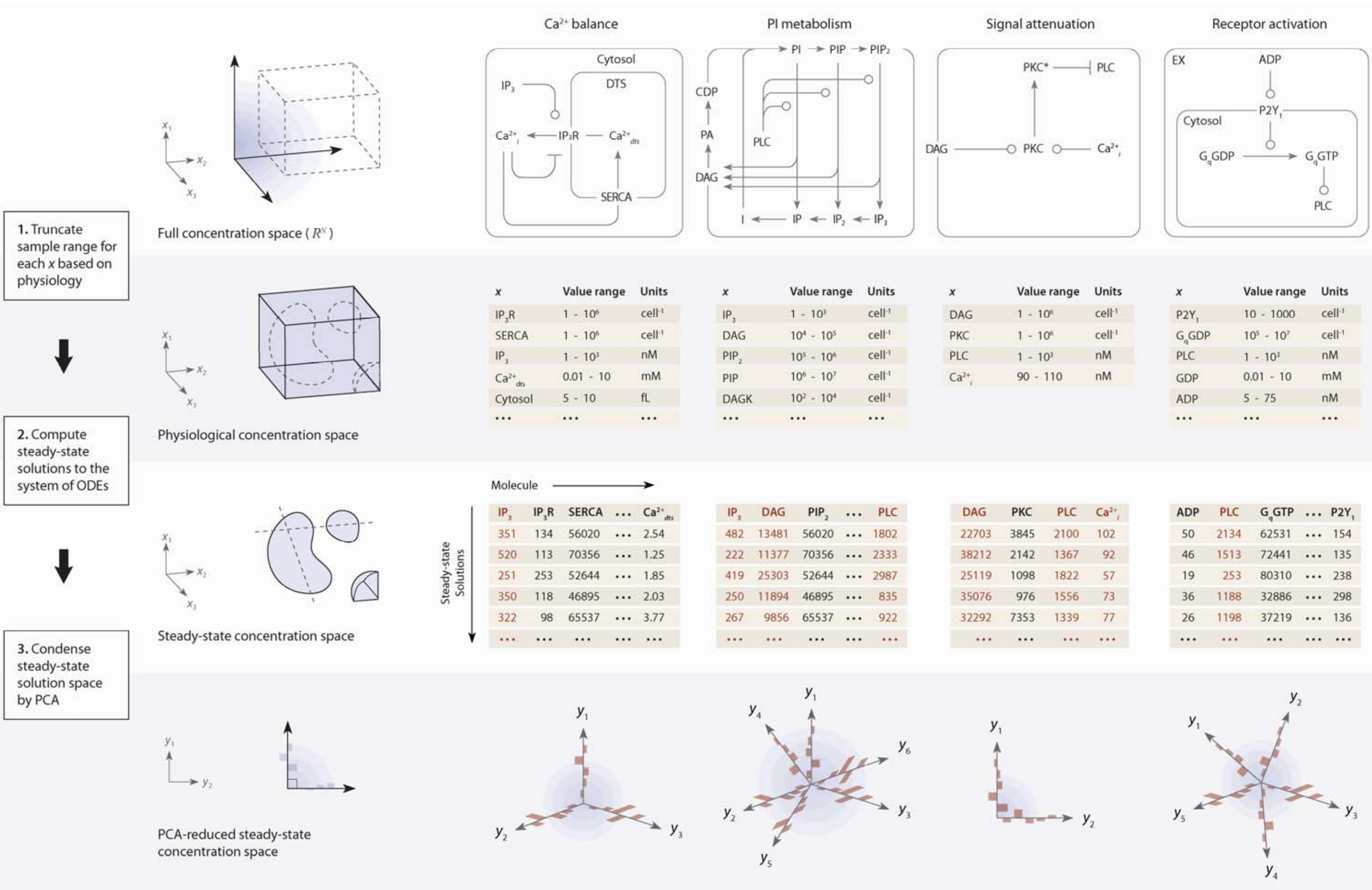




- Often, topology is know but concentrations are not
- Each molecule occupies a separate linear dimension
- Each module comprises a subspace of the full concentration space
- Modules that share a species have intersecting subspaces

Purvis, Radhakrishnan, and Diamond (2009). *PLoS Comput Biol* 5:e1000298

Homeostasis constraint and PCA reduce allowable cellular configurations



x	Value range	Units
IP_3R	$1 - 10^6$	$cell^{-1}$
SERCA	$1 - 10^6$	$cell^{-1}$
IP_3	$1 - 10^3$	nM
Ca^{2+}_{dts}	0.01 - 10	mM
Cytosol	5 - 10	fL
...	...	...

x	Value range	Units
IP_3	$1 - 10^3$	$cell^{-1}$
DAG	$10^4 - 10^5$	$cell^{-1}$
PIP_2	$10^5 - 10^6$	$cell^{-1}$
PIP	$10^6 - 10^7$	$cell^{-1}$
DAGK	$10^2 - 10^4$	$cell^{-1}$
...	...	...

x	Value range	Units
DAG	$1 - 10^6$	$cell^{-1}$
PKC	$1 - 10^6$	$cell^{-1}$
PLC	$1 - 10^3$	nM
Ca^{2+}_i	90 - 110	nM

x	Value range	Units
$P2Y_1$	10 - 1000	$cell^{-1}$
G_qGDP	$10^5 - 10^7$	$cell^{-1}$
PLC	$1 - 10^3$	nM
GDP	0.01 - 10	mM
ADP	5 - 75	nM
...	...	...

IP_3	IP_3R	SERCA	...	Ca^{2+}_{dts}
351	134	56020	...	2.54
520	113	70356	...	1.25
251	253	52644	...	1.85
350	118	46895	...	2.03
322	98	65537	...	3.77
...	...	...	...	...

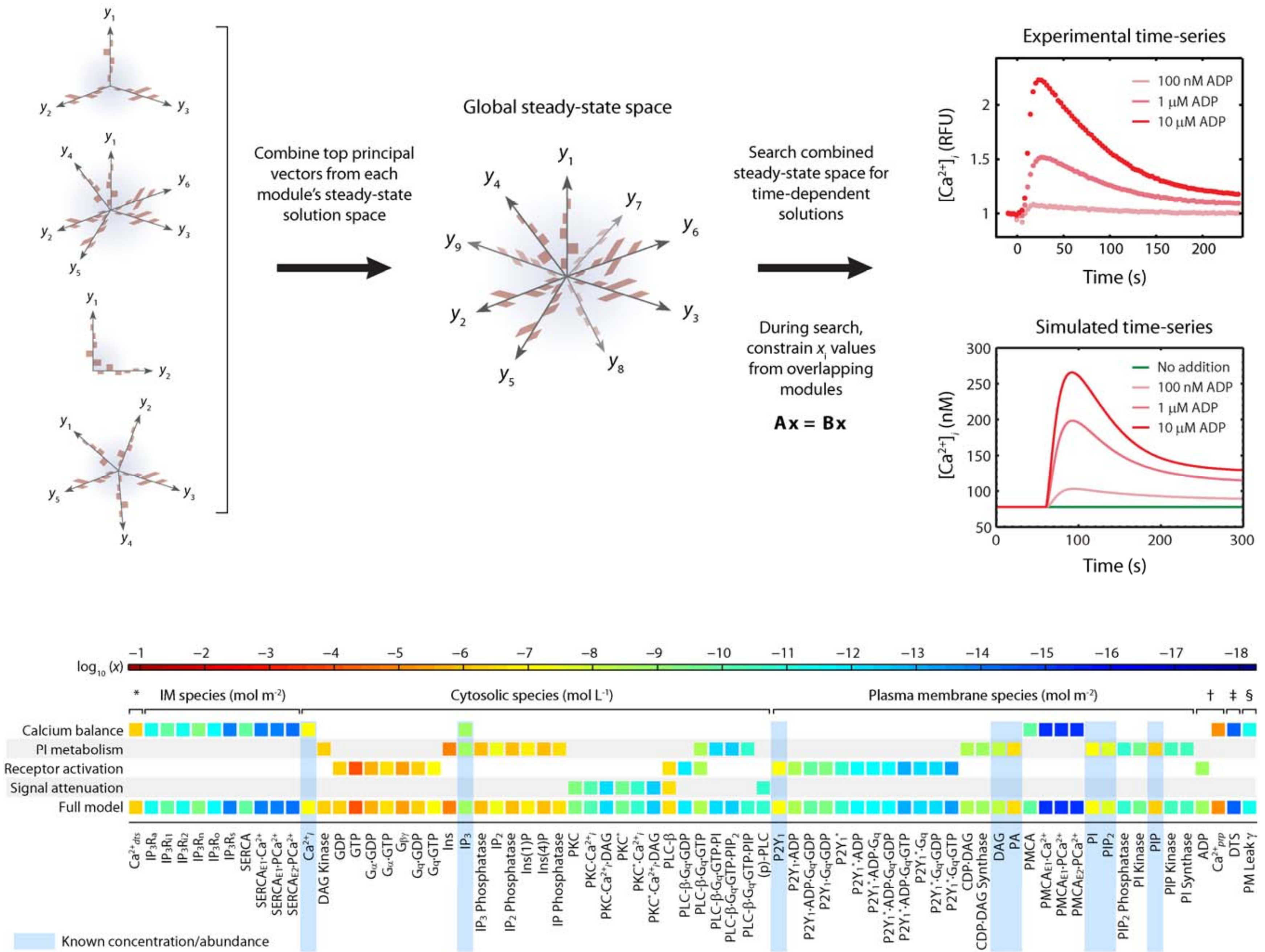
IP_3	DAG	PIP_2	...	PLC
482	13481	56020	...	1802
222	11377	70356	...	2333
419	25303	52644	...	2987
250	11894	46895	...	835
267	9856	65537	...	922
...	...	...	...	...

DAG	PKC	PLC	Ca^{2+}_i
22703	3845	2100	102
38212	2142	1367	92
25119	1098	1822	57
35076	976	1556	73
32292	7353	1339	77
...	...	...	...

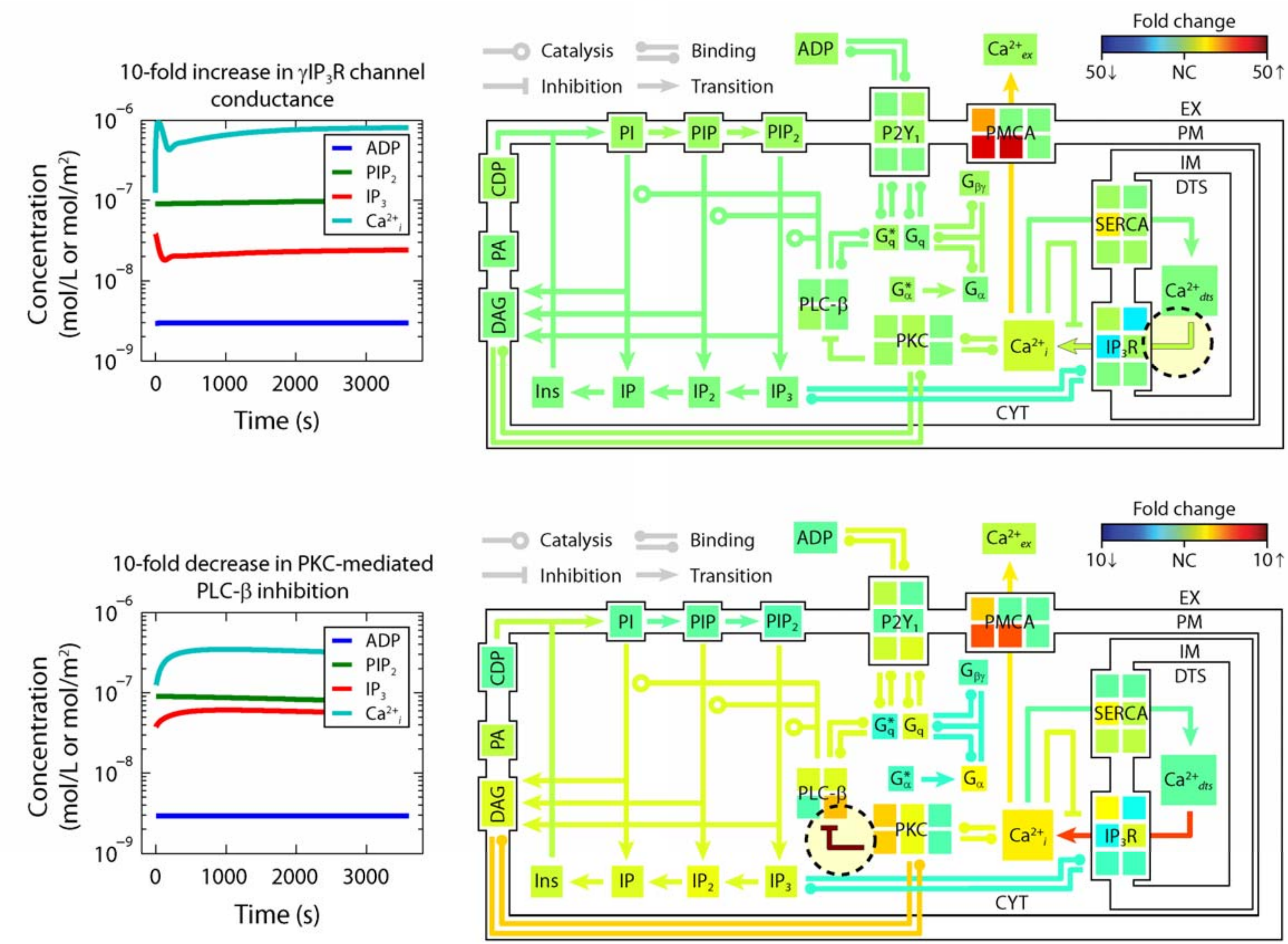
ADP	PLC	G_qGTP	...	$P2Y_1$
50	2134	62531	...	154
46	1513	72441	...	135
19	253	80310	...	238
36	1188	32886	...	298
26	1198	37219	...	136
...	...	...	...	...

Purvis, Radhakrishnan, and Diamond (2009). *PLoS Comput Biol* 5:e1000298

Resting modules are merged to find global time-dependent solution

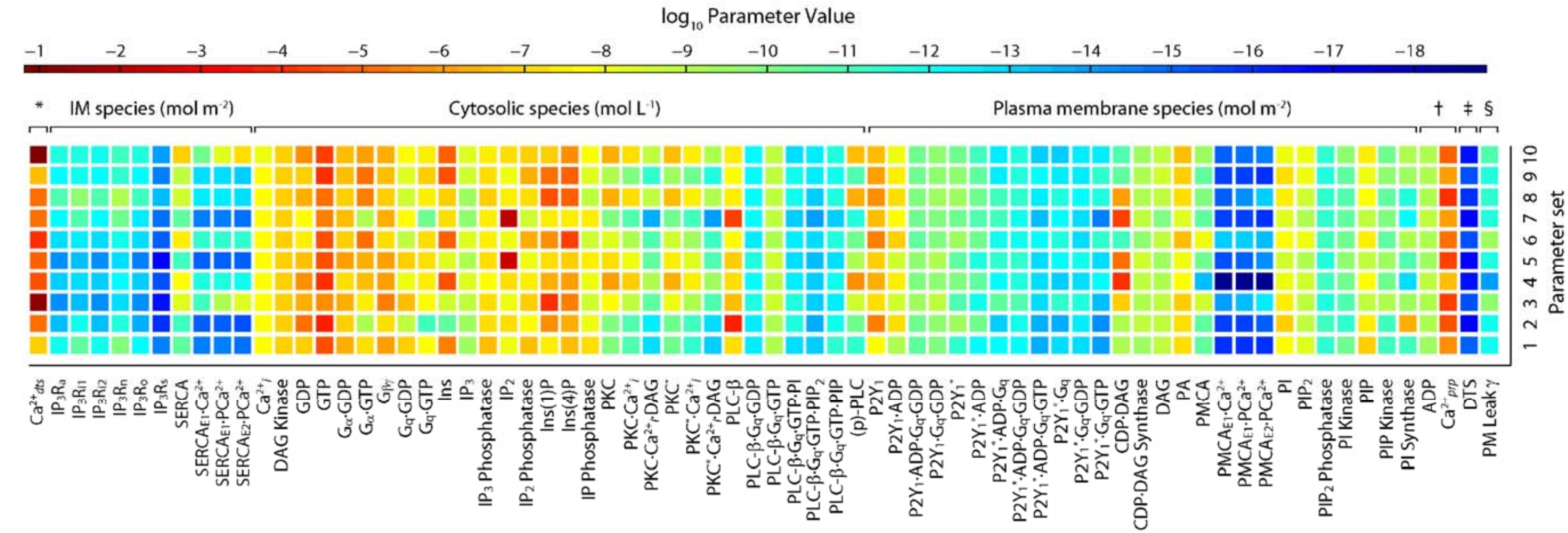


Kinetic perturbations cause cells to adopt new steady states



Purvis, Radhakrishnan, and Diamond (2009). *PLoS Comput Biol* 5:e1000298

Alternate steady state profiles reveal `rigid´ and ` flexible´ concentrations



`Rigid' nodes:

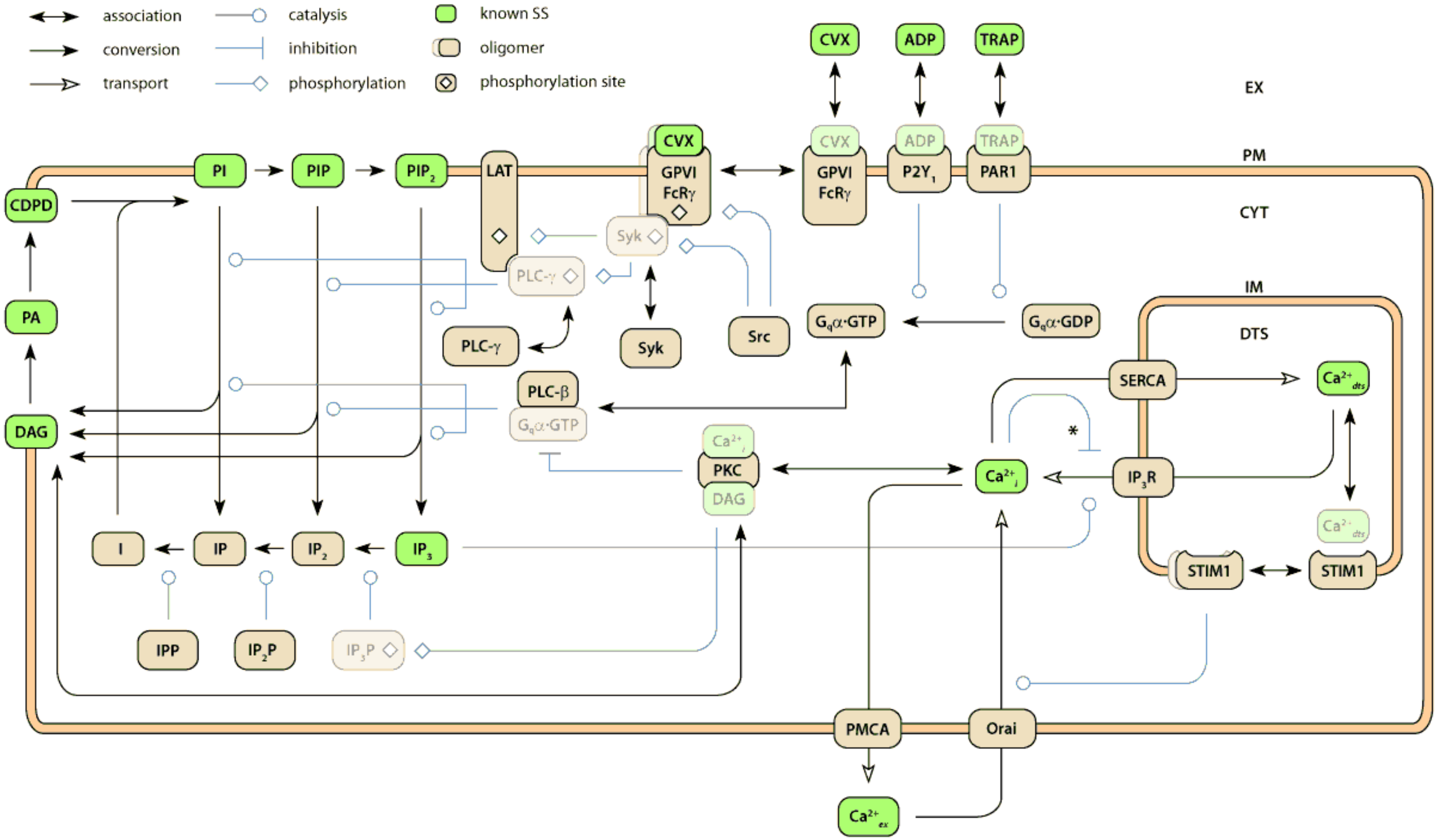
`Flexible' nodes:

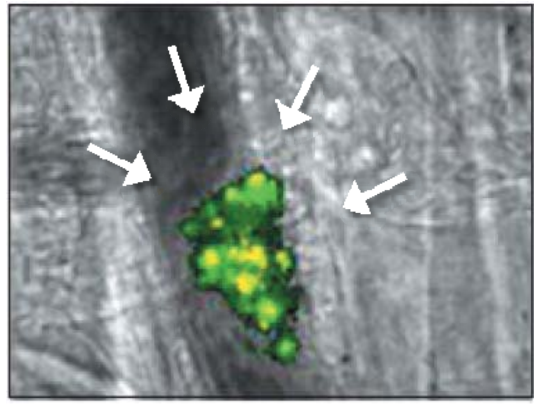
Values fixed during estimation: $[Ca^{2+}]_i$, PIP_2

`Rigid' nodes	`Flexible' nodes
$[Ca^{2+}]_i$	PKC
PIP_2	G_q-GTP
GTP	$P2Y_1$
PLC-β	SERCA

Purvis, Chatterjee, Brass, and Diamond, 2009. *Blood* 112:4069

A realistic description of signaling requires a multitude of reactions



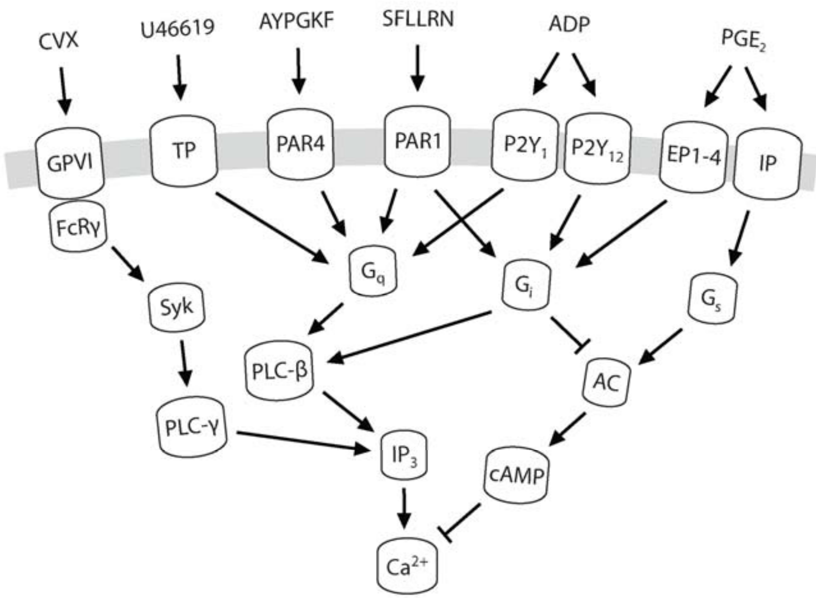


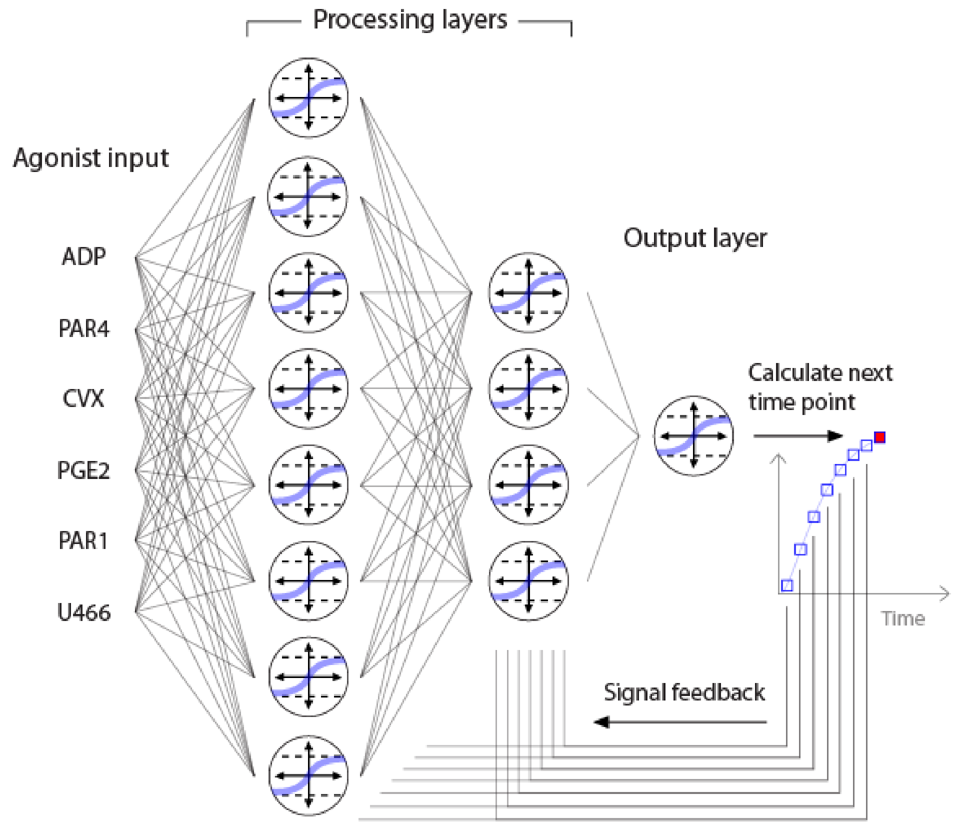
IV. Dynamic neural network modeling predicts responses to multi-input signals

Chatterjee, Purvis, and Diamond (2009) *submitted*

- Cells must respond to multiple potent signals *in vivo*
- Addresses question of how multiple simultaneous signals are integrated
- Sensitive enough to resolve differences in signaling among individuals

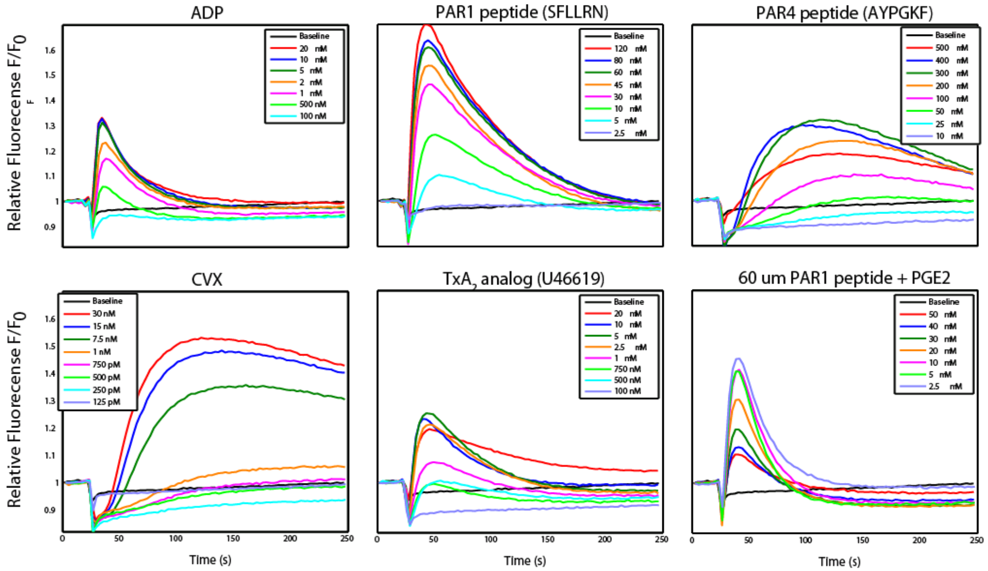
Multiple signaling pathways converge on intracellular calcium release





Chatterjee, Purvis, and Diamond, 2009 *submitted*

Signal inputs are normalized according to EC_{50}



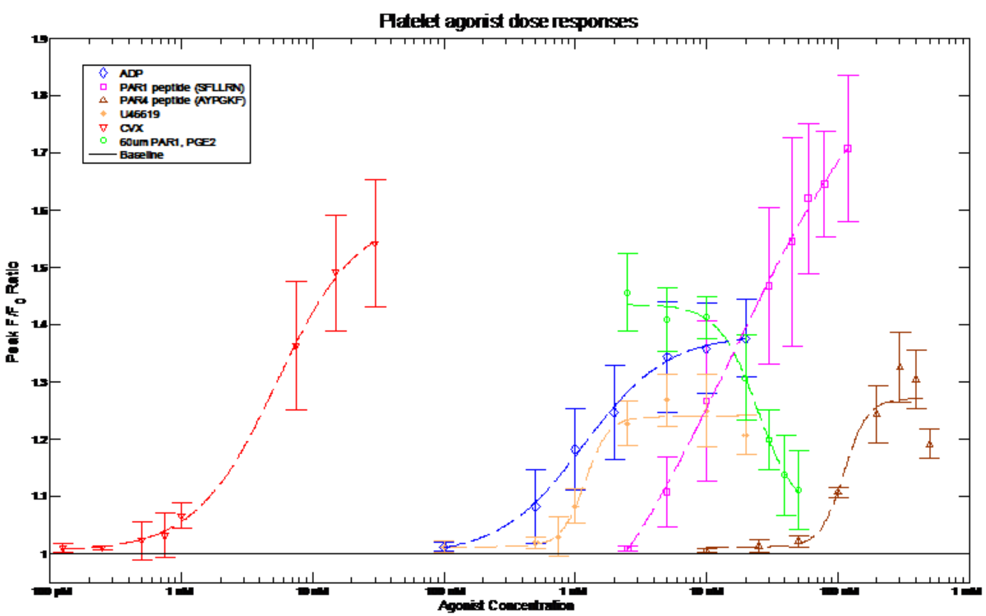
4 agonists states:

$0 \times EC_{50}$

$0.1 \times EC_{50}$

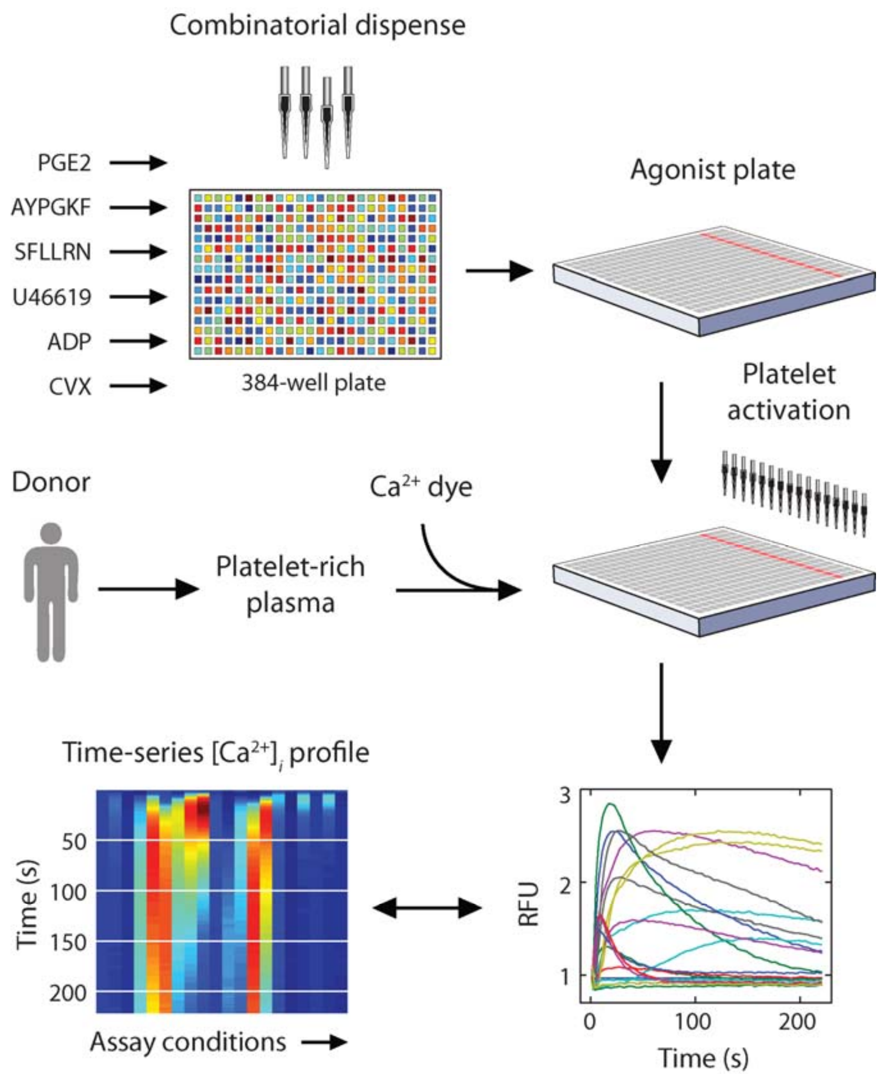
$1 \times EC_{50}$

$10 \times EC_{50}$



Selecting low, moderate, and high doses for each agonist **normalizes** the input signals and **captures the dynamic range** of each agonist

A high-throughput assay measures platelet response to combinatorial inputs

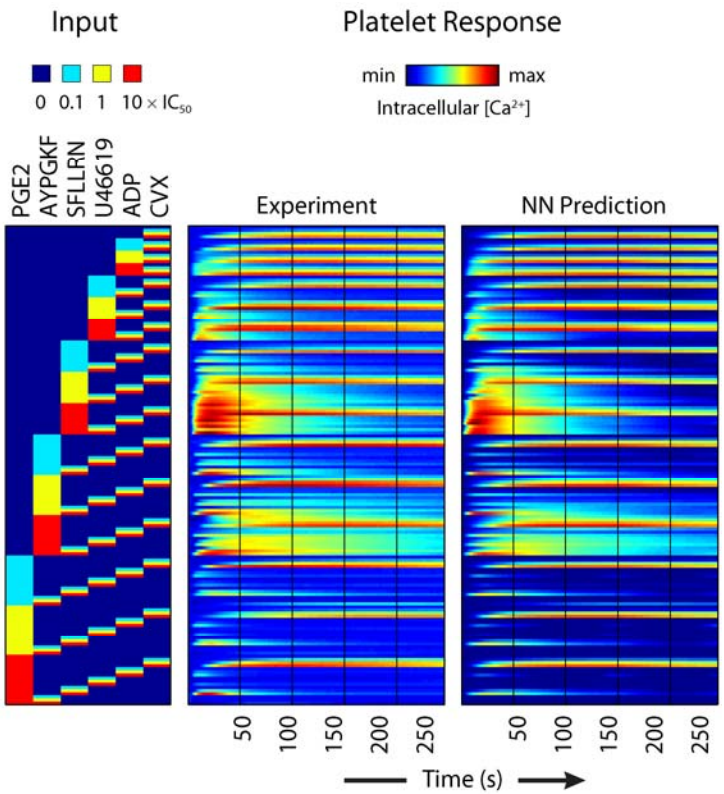


Chatterjee, Purvis, and Diamond, 2009 *submitted*

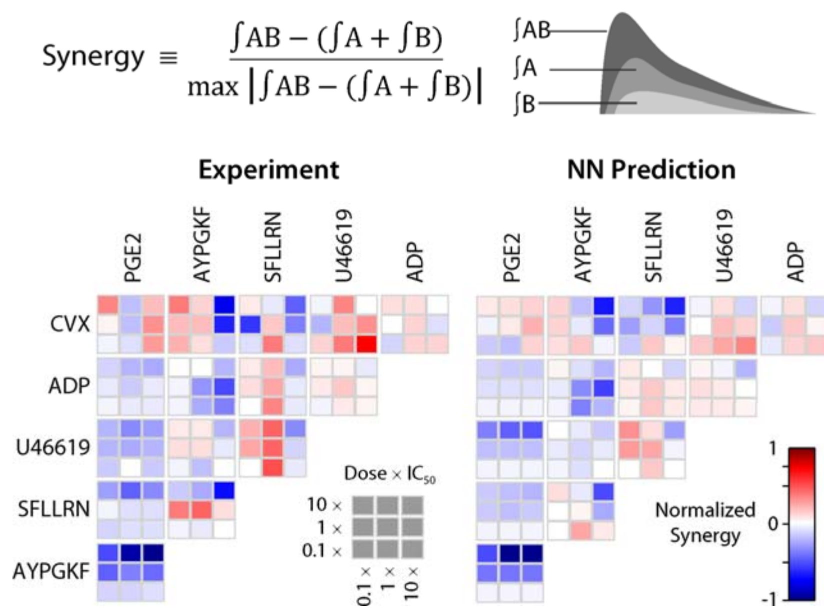
NN is trained on responses to all pairwise combinations of agonist

- Network trained on all pairwise combinations of 6 agonists at 3 concentrations

  (6 choose 2) × 3^2 + 19 = 154 combinations



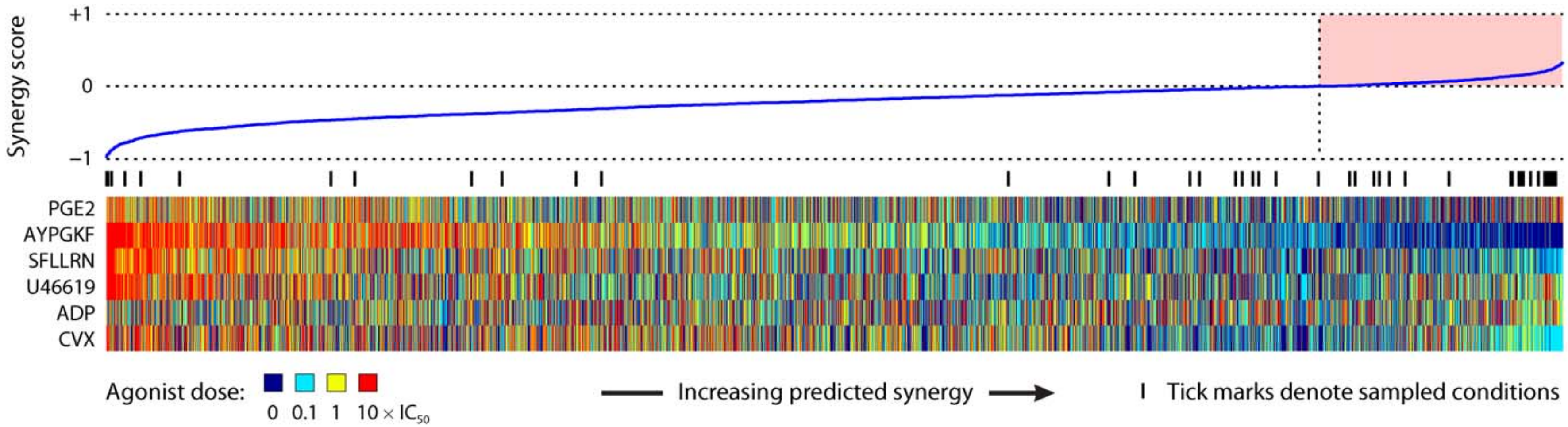
- Define synergy as the difference between the integrated calcium transient for the combined response and the integrated area for the individual responses



Chatterjee, Purvis, and Diamond, 2009 *submitted*

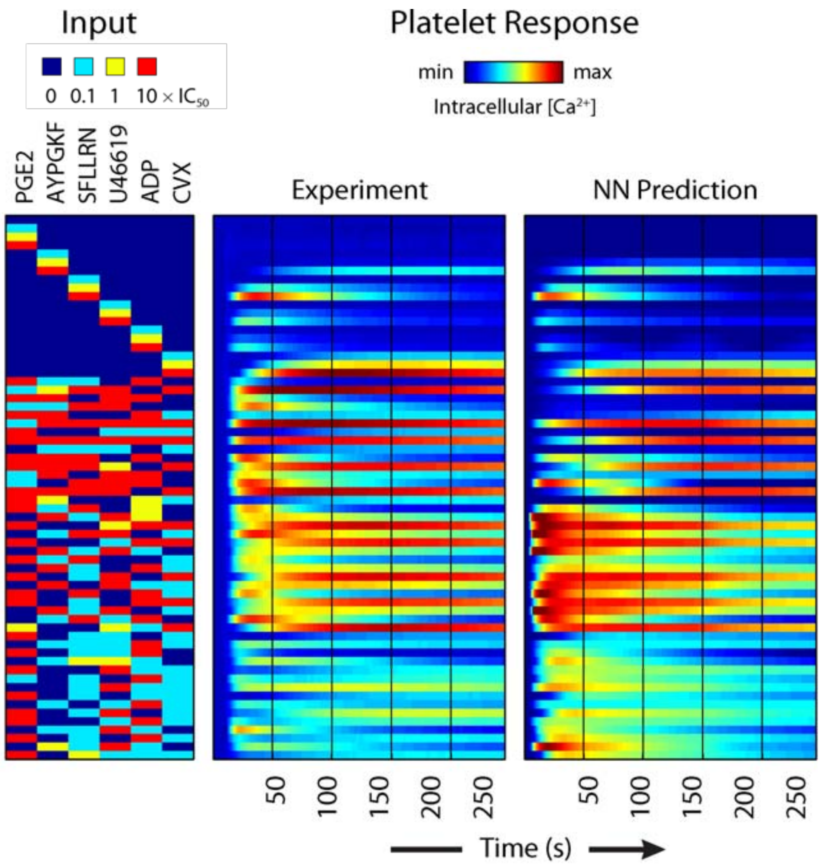
NN predicts entire 6-dimensional platelet response

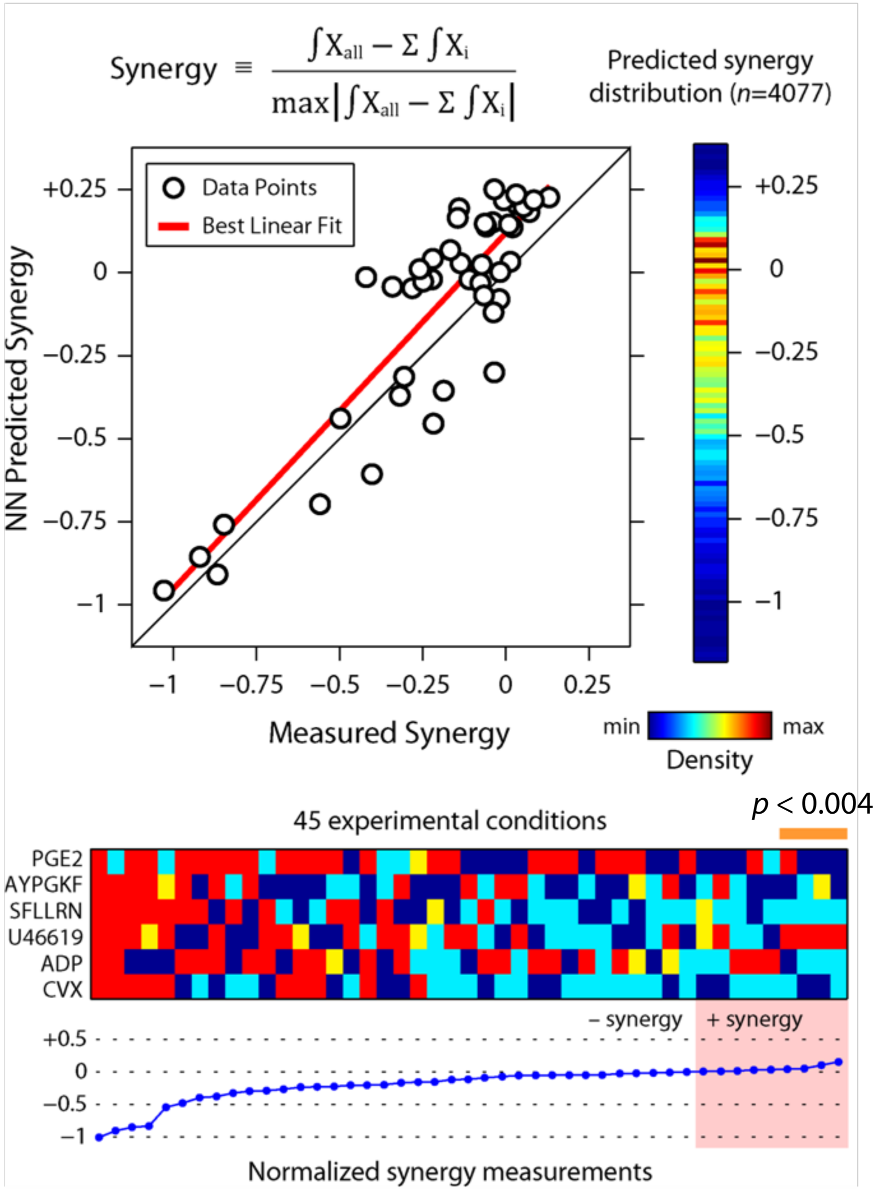
- NN made 4077 predictions of synergy among all combinations of 6 agonists at 3 doses
- 45 dissimilar conditions were chosen for experimental verification



Chatterjee, Purvis, and Diamond, 2009 *submitted*

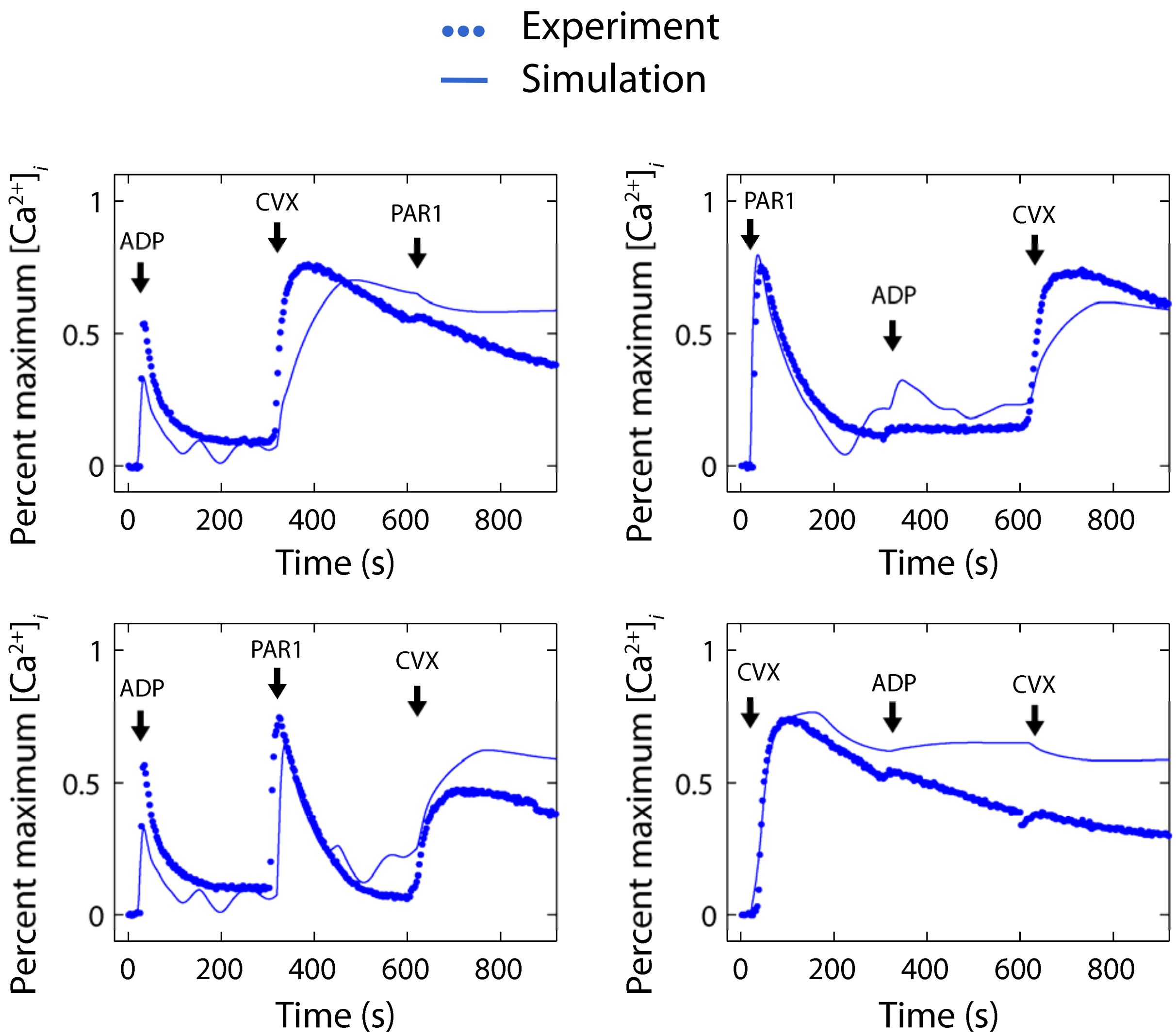
Sample ($n = 45$) of global response space identifies high-synergy conditions





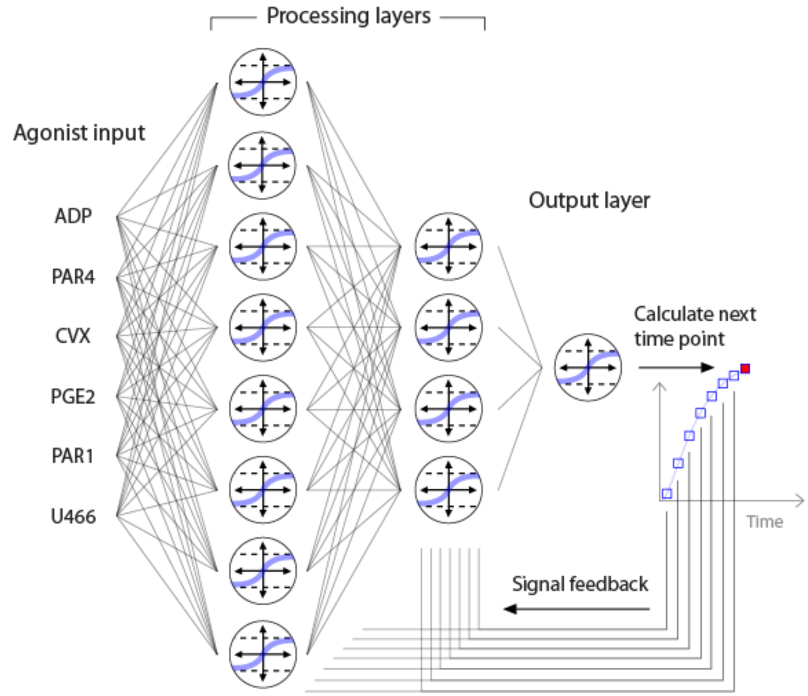
Chatterjee, Purvis, and Diamond, 2009 *submitted*

NN predicts sequential responses and downregulation

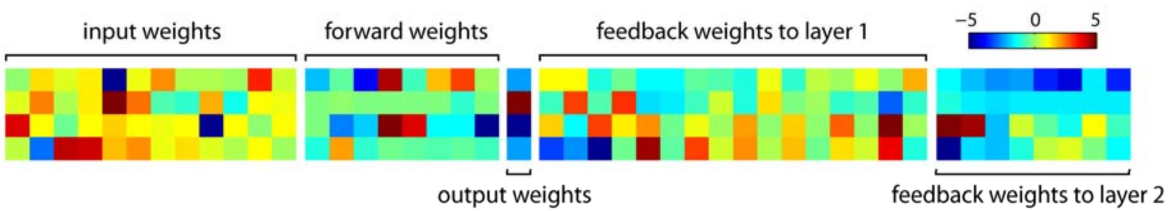


NN models provide a compact, patient-specific predictive tool

Train NN to match calcium profile for specific donor:



Weight values for donor-trained NN:



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