Cell-Signaling Dynamics in Time and Space

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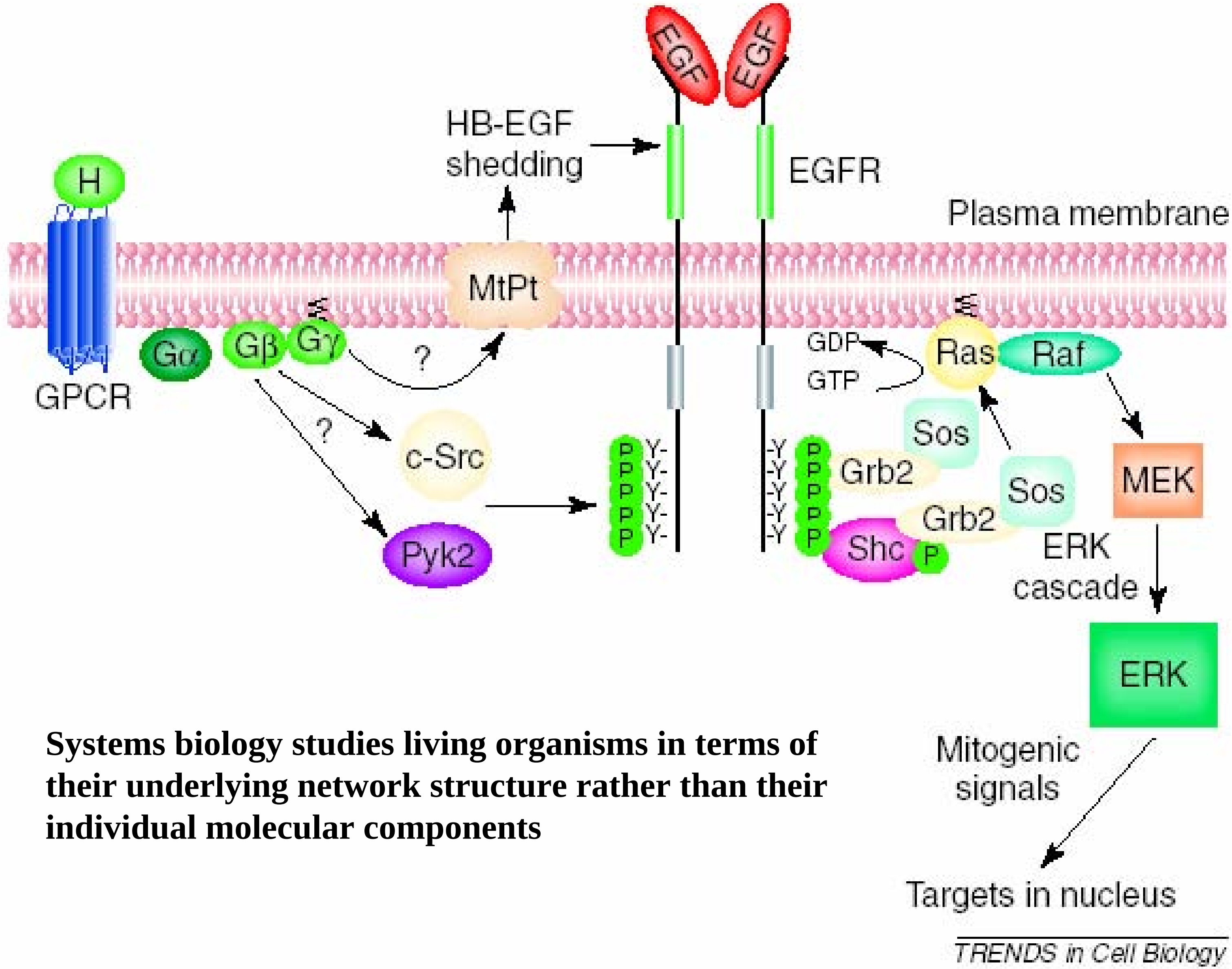
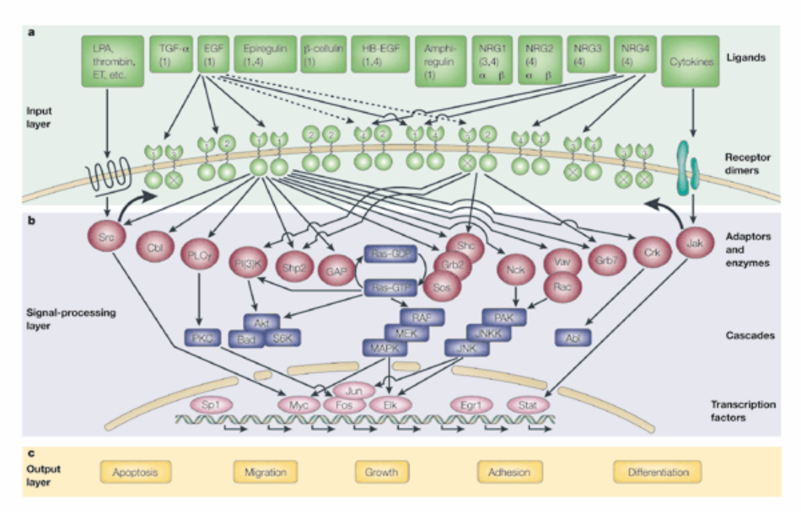
Systems Biology of Signaling Networks



TRENDS in Cell Biology

Systems biology studies living organisms in terms of their underlying network structure rather than their individual molecular components

Combinatorial Complexity Presents Problems for Models



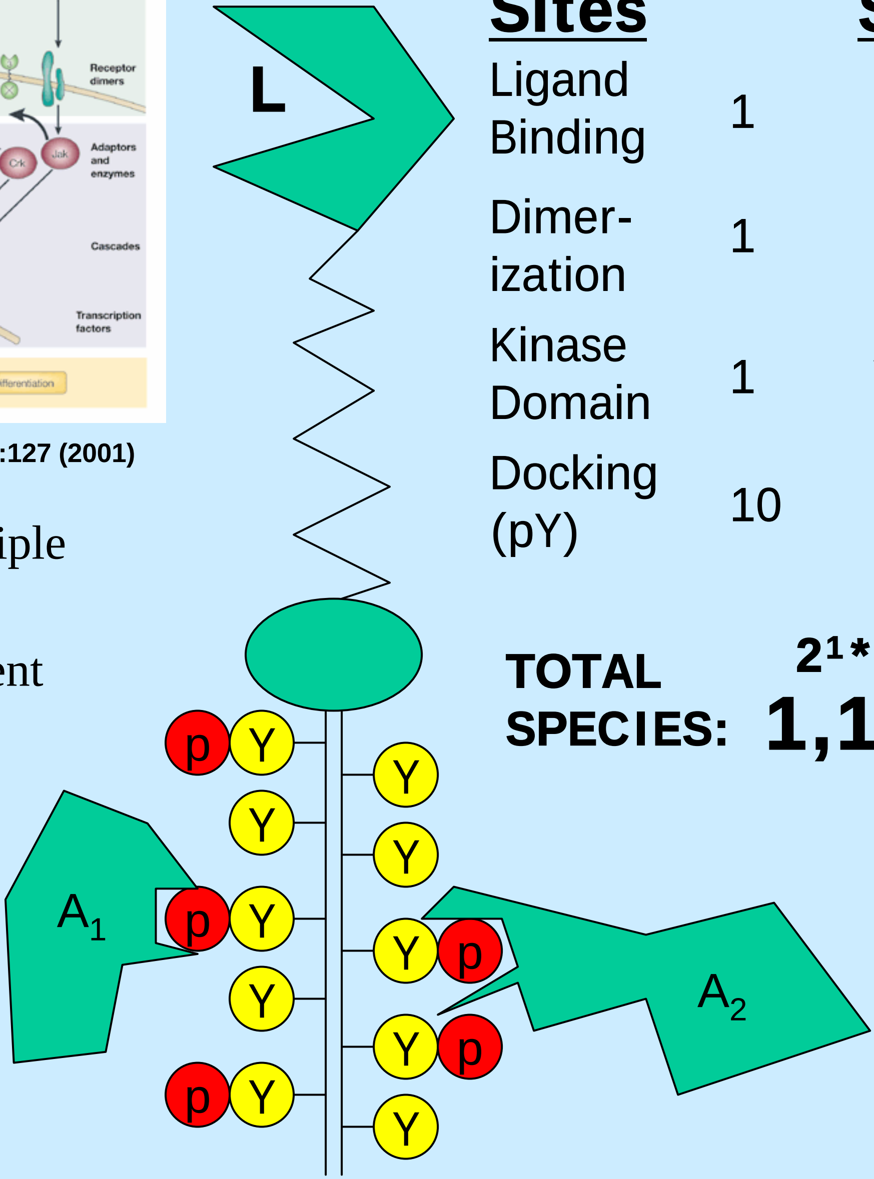
Yarden and Sliwkowski, *Nat. Rev. Mol. Cell Biol.* 2:127 (2001)

- Signaling proteins have multiple “sites” and many “states”
- Each combination is a different species that requires its own differential equation

L: Ligand Y: Tyrosine

A: Adapter pY: Phospho-Y

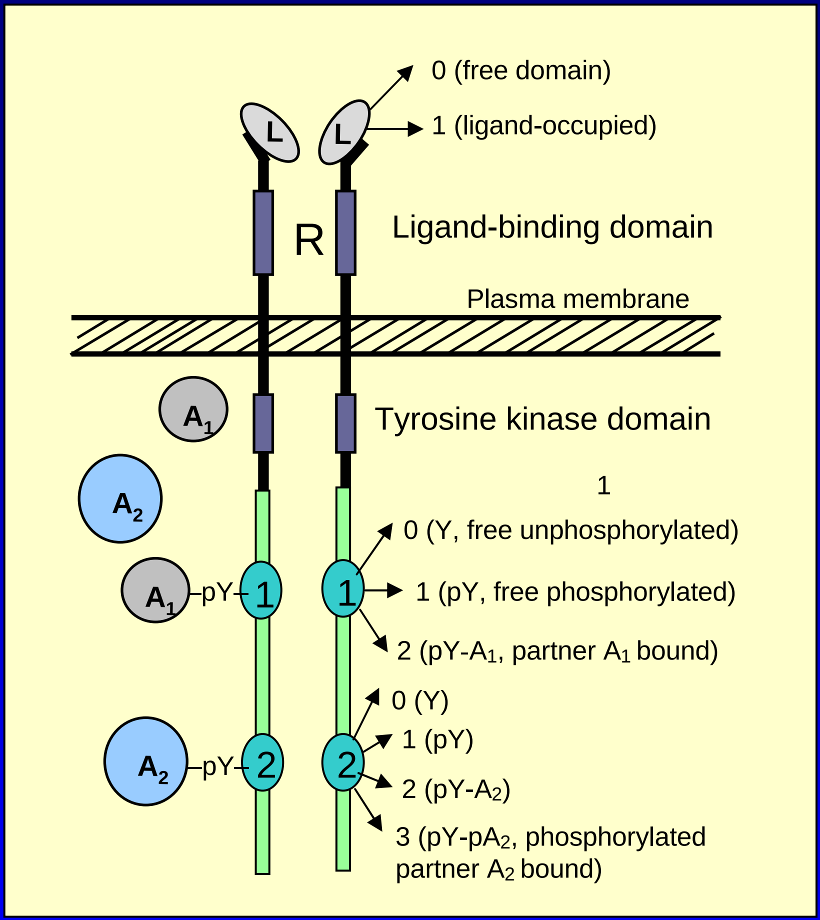
Example: The ErbB family



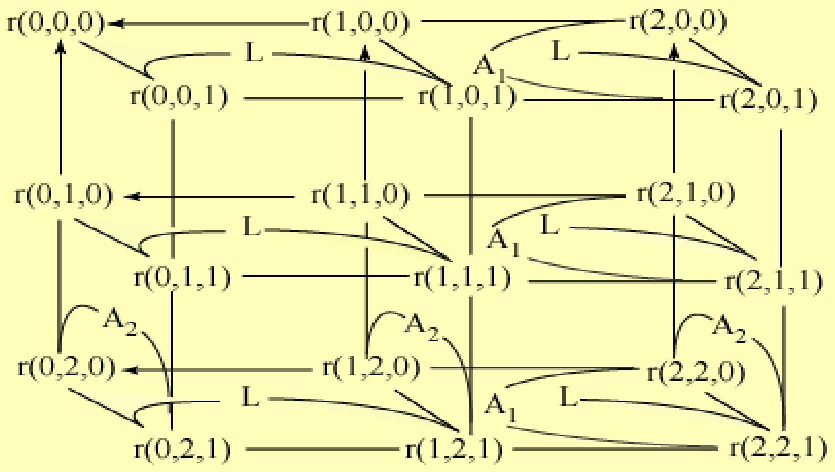
Sites		**States**	
Ligand Binding	1	Bound or Not	2
Dimer-ization	1	None or 1,2,3,4	5
Kinase Domain	1	Active or Inactive	2
Docking (pY)	10	Y, pY, Bound	3

TOTAL SPECIES: $2^1*5^1*2^1*3^{10}$ = **1,180,980!!**

Reducing Combinatorial Complexity of Multi-Domain Proteins

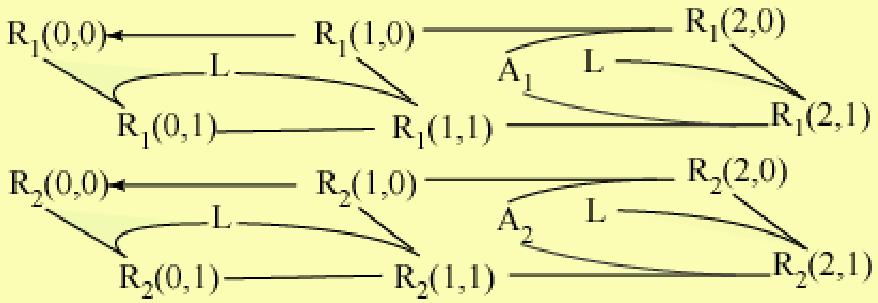


Micro-state transition graph



18 states and 33 transitions

Macro-state transition graph

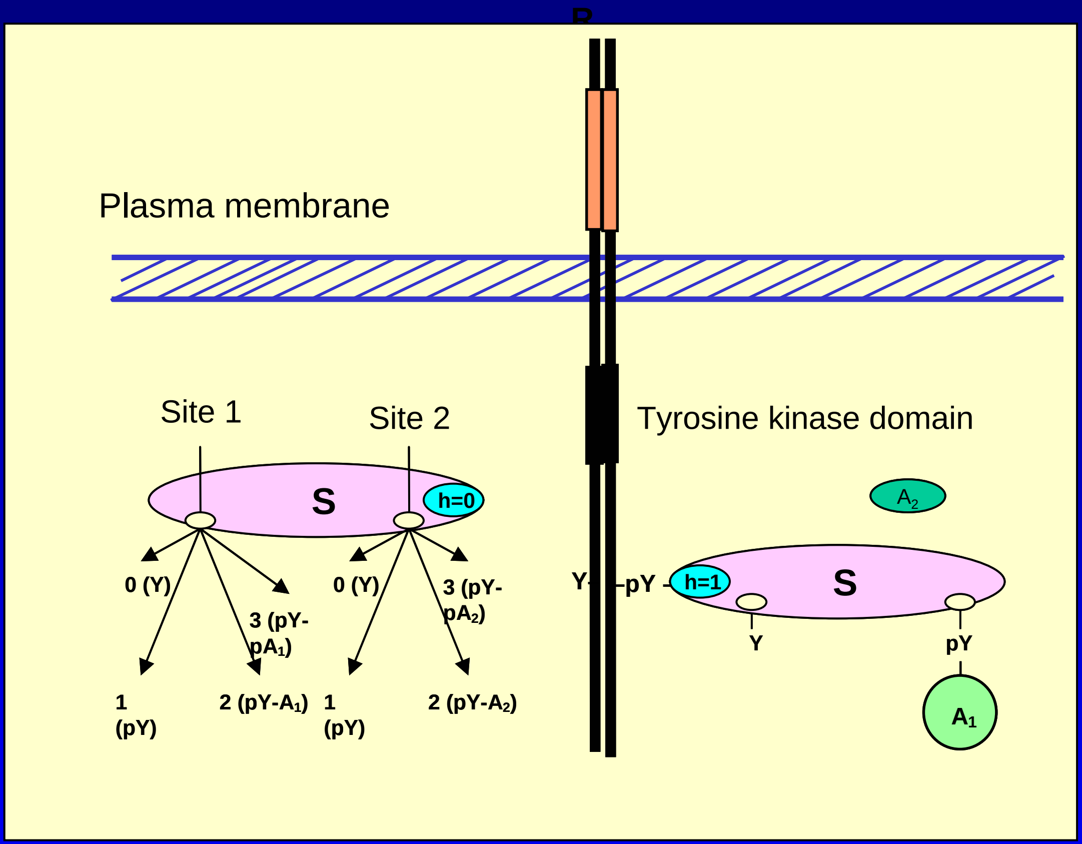


12 states and 14 transitions

Macro-state is a sum of micro-states:

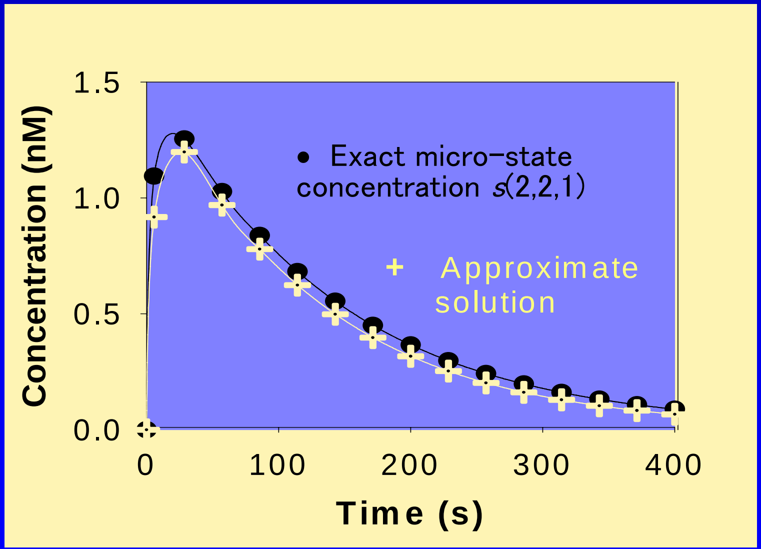
$$R_1(a_1,h)=\sum_{a_2=0}^{m_2} r(a_1,a_2,h),\quad R_2(a_2,h)=\sum_{a_1=0}^{m_1} r(a_1,a_2,h)$$

Micro- and Macro-Models of Scaffold Proteins



$$S_i(a_i,h)=\sum_{a_1=0}^{m_1}\cdots\sum_{a_{i-1}=0}^{m_{i-1}}\sum_{a_{i+1}=0}^{m_{i+1}}\cdots\sum_{a_n=0}^{m_n}s(a_1,\ldots,a_n,h)$$

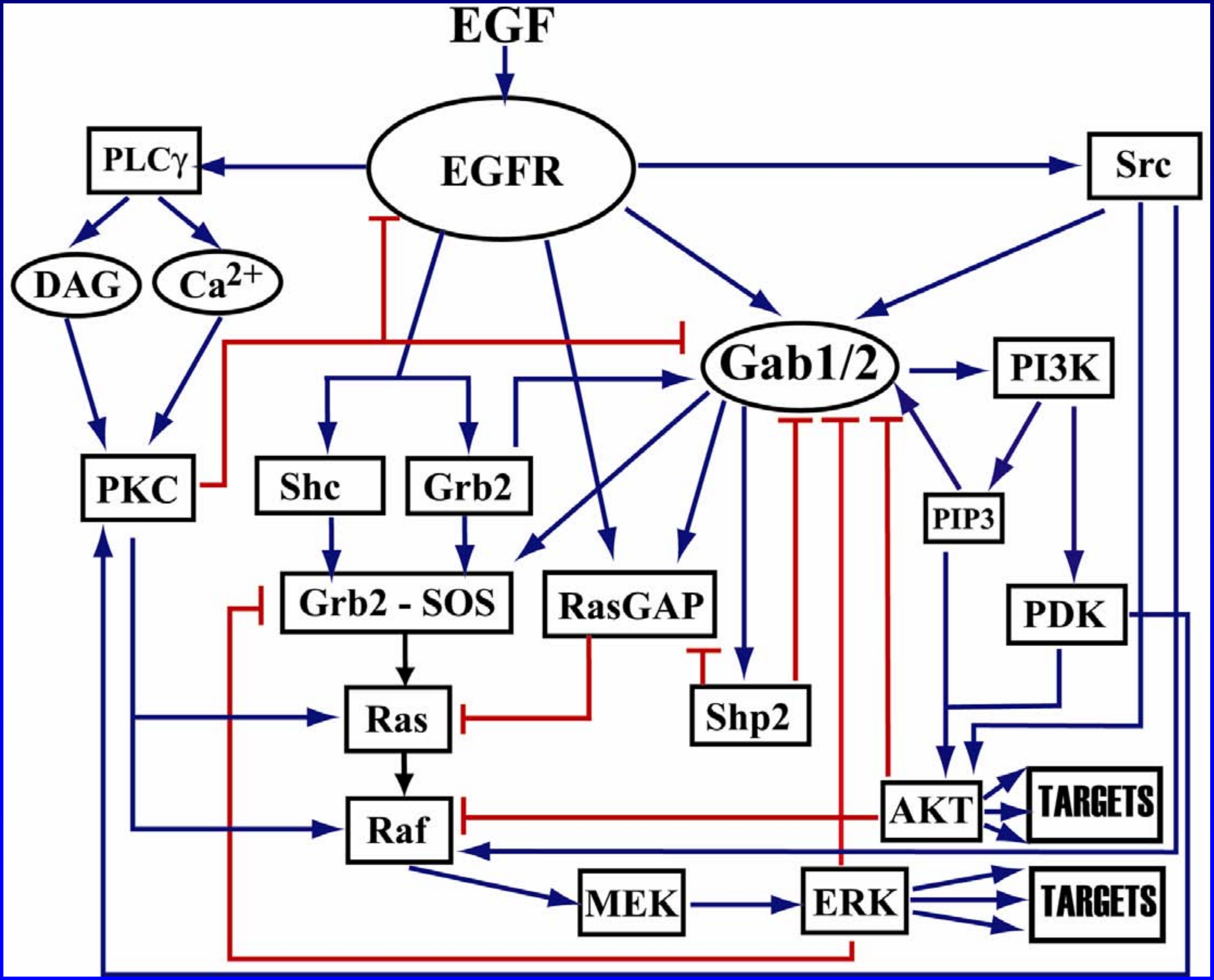
$$s(a_1,\ldots,a_n;h)\approx\frac{\prod_{i=1}^{n}S_i(a_i,h)}{S_{tot}^{n-1}(h)}$$



For the EGFR signaling network (GAB scaffold), the number of equations reduces from hundreds of thousands to ≈ 350.

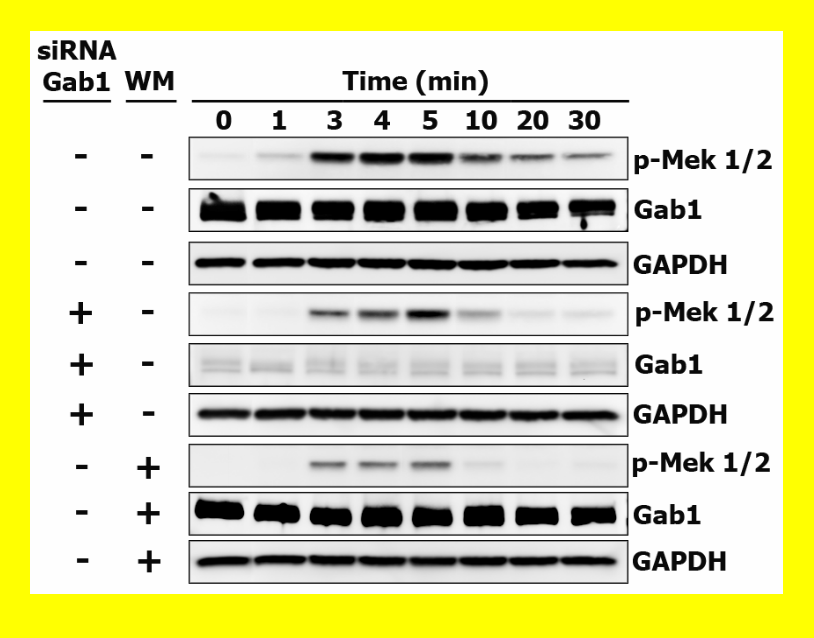
Borisov *et al* (2005) Biophys. J. 89:951. Conzelmann *et al* (2006) BMC Bioinformatics, 7:34
Kiyatkin et al. J Biol Chem, (2006) 281:19925. Borisov *et al* (2006) Biosystems. 83:152

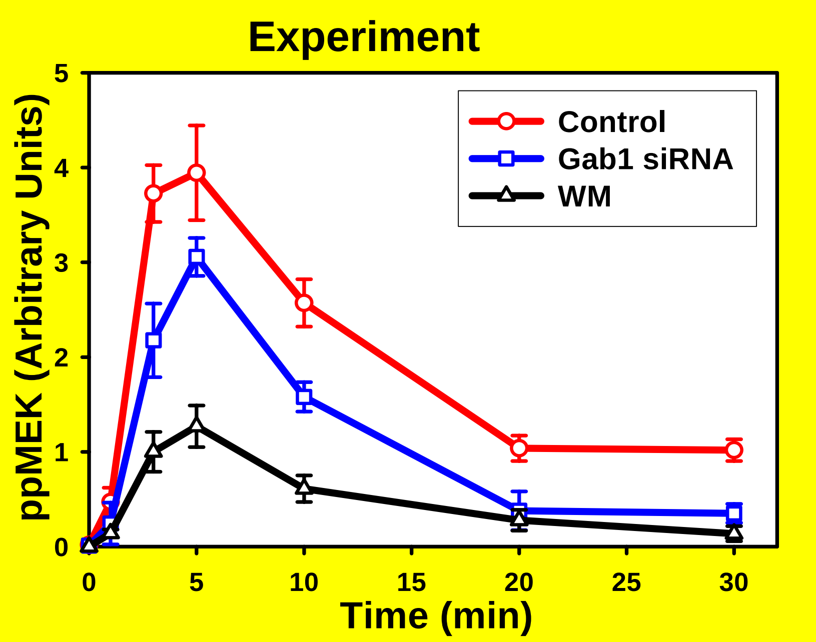
Interactions between the Ras/ERK and PI3K/Akt pathways

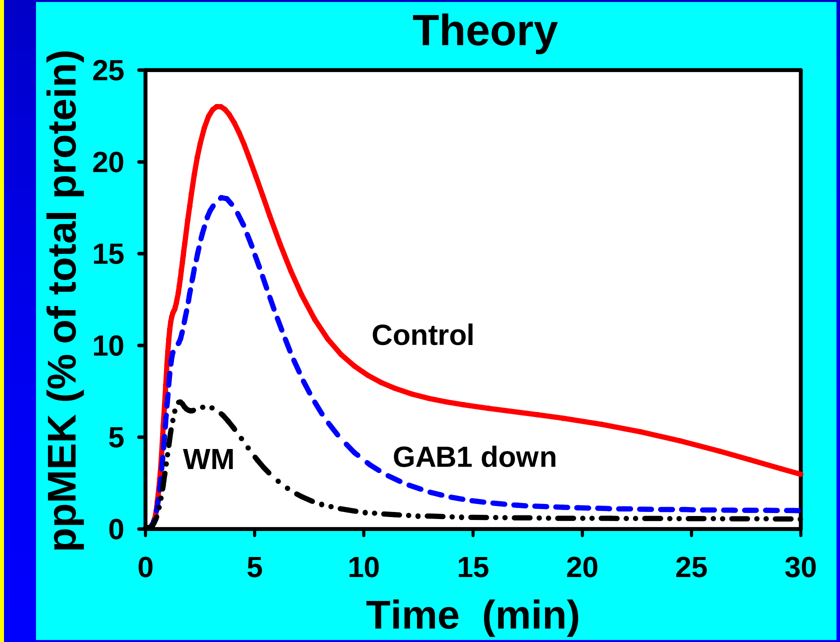


Distinct Operational Ranges of Different Feedback Loops Shape Signaling Outcome

Disruption of GAB1-PI3K-GAB1 Positive Feedback: Experiment and Theory

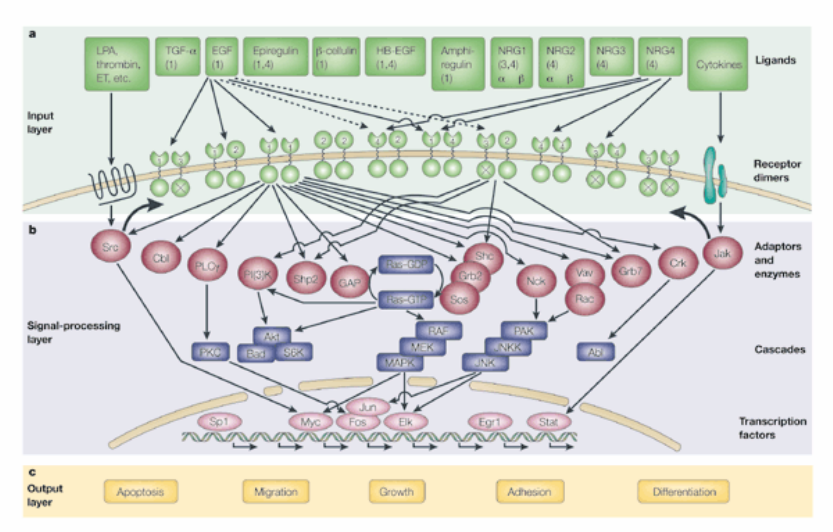



Experiment

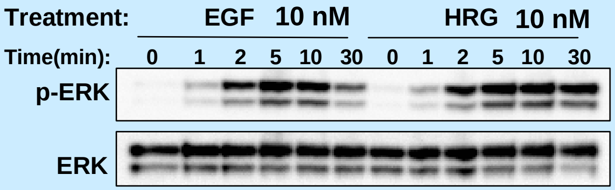

Theory

Kiyatkin et al. J Biol Chem, (2006) 281:19925

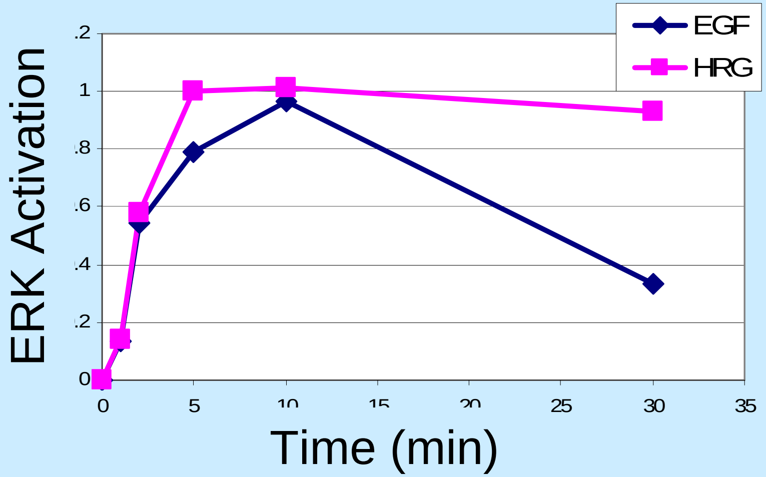
Context-Dependent Responses to EGF and HRG

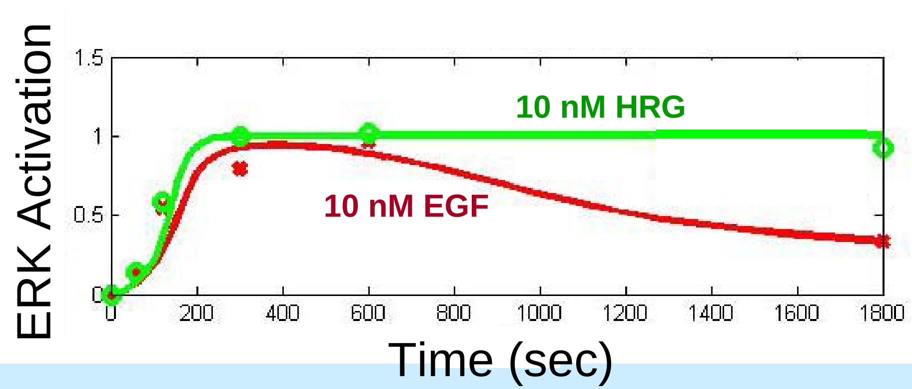


Yarden and Sliwkowski, *Nat. Rev. Mol. Cell Biol.* 2:127 (2001)



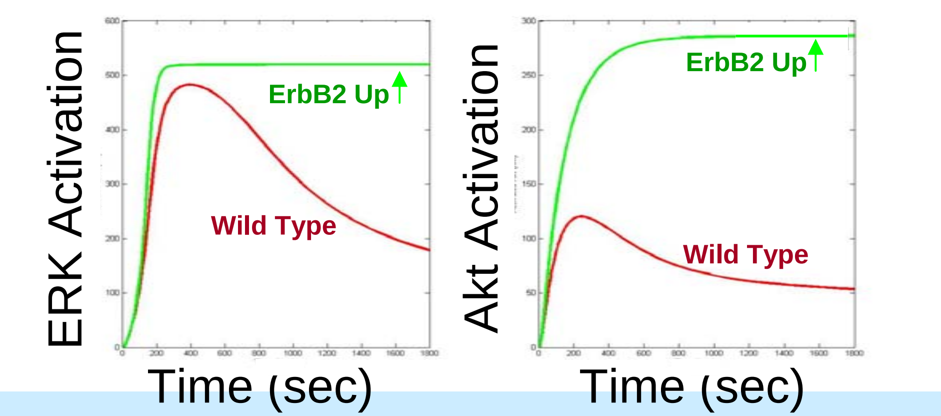
Data from M. Hatakeyama's lab



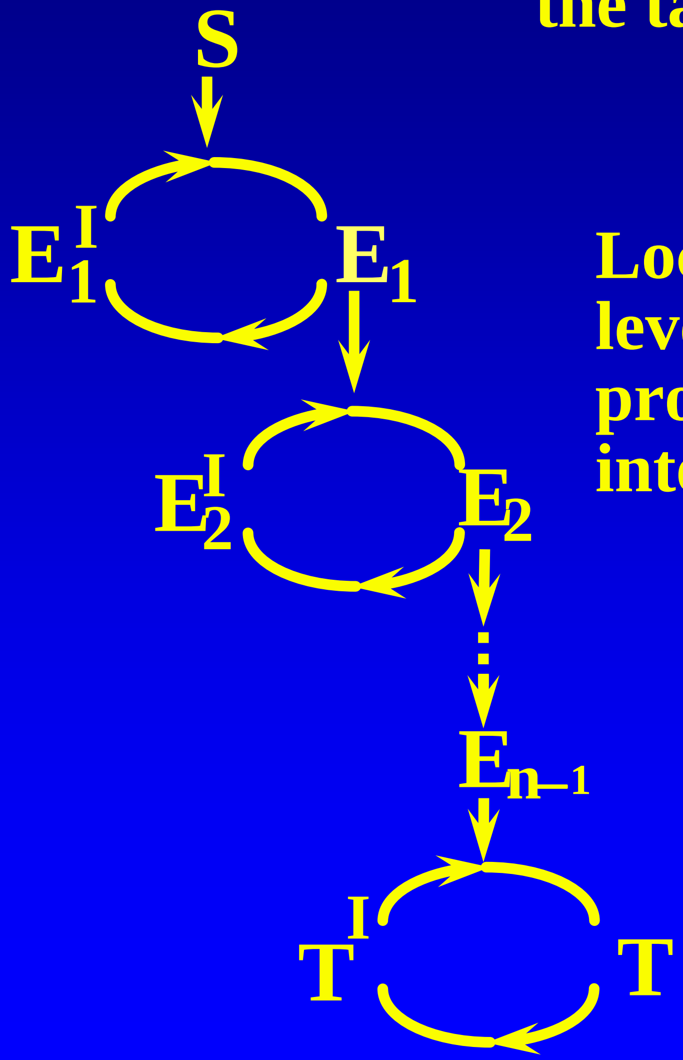


See Poster #269 by M. Birtwistle et al.

ErbB2 Overexpression Transforms a Transient into Sustained Response



How to Quantify the Control Exerted by a Signal over a Target?



System Response: the sensitivity (*R*) of the target *T* to a change in the signal *S*

$$R_S^T = d\ln T / d\ln S\big|_{steady\ state}$$

Local Response: the sensitivity (*r*) of the level *i* to the preceding level *i*-1. Active protein forms (E_i) are "communicating" intermediates:

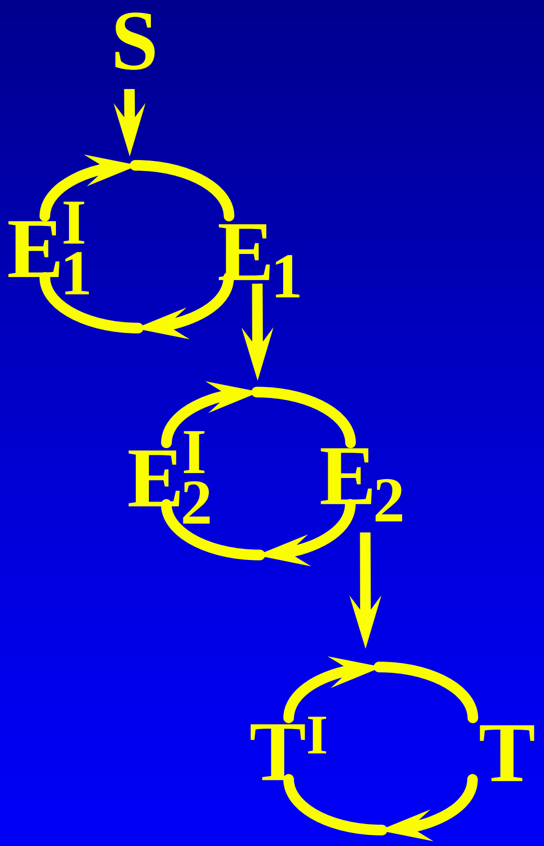
$$r_i = \partial \ln E_i / \partial \ln E_{i-1}\big|_{Level\ i\ steady\ state}$$

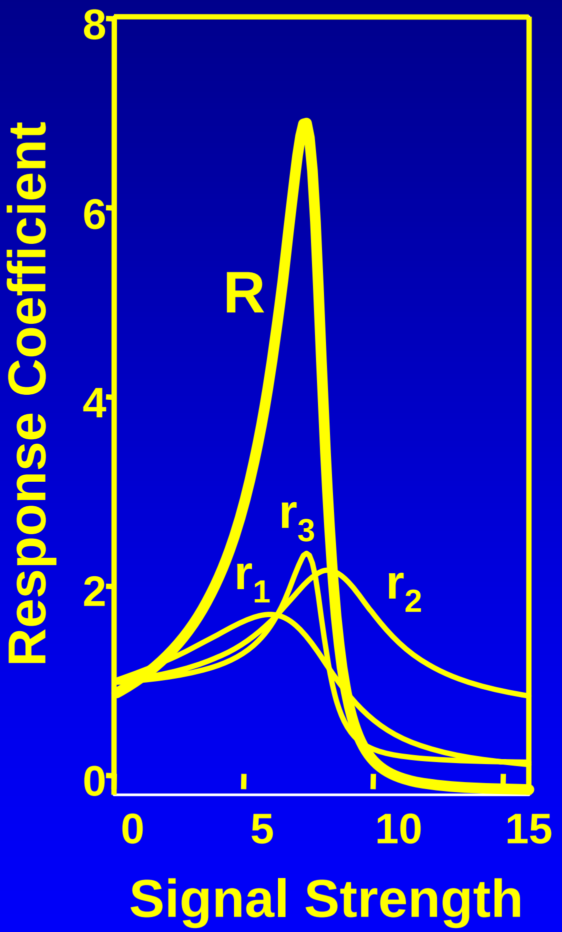
System response equals the PRODUCT of local responses for a linear cascade:

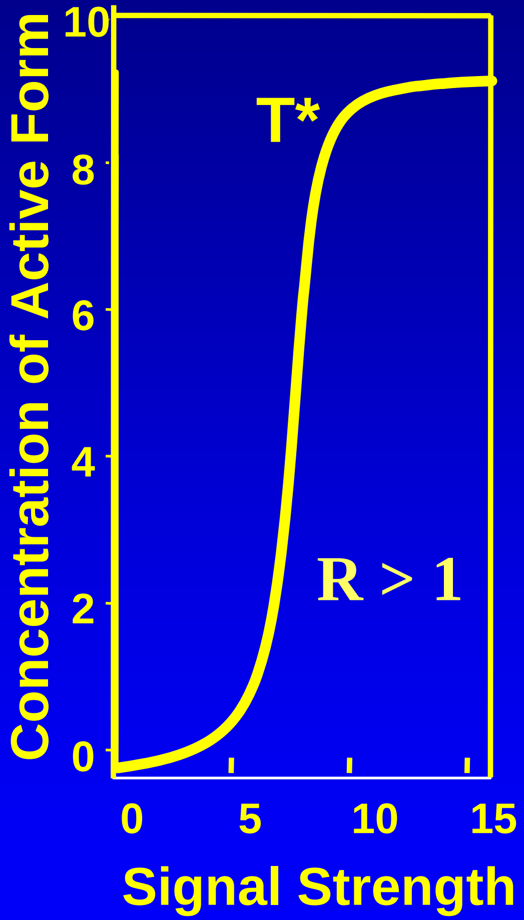
$$R_S^T = r_1 \cdot r_2 \cdot \ldots \cdot r_{n-1} \cdot r_n = \prod (path)$$

Kholodenko *et al* (1997) FEBS Lett. 414: 430

Functional Implications of the Multiplication Rule: When Does a Signaling Cascade Operate as a Switch?





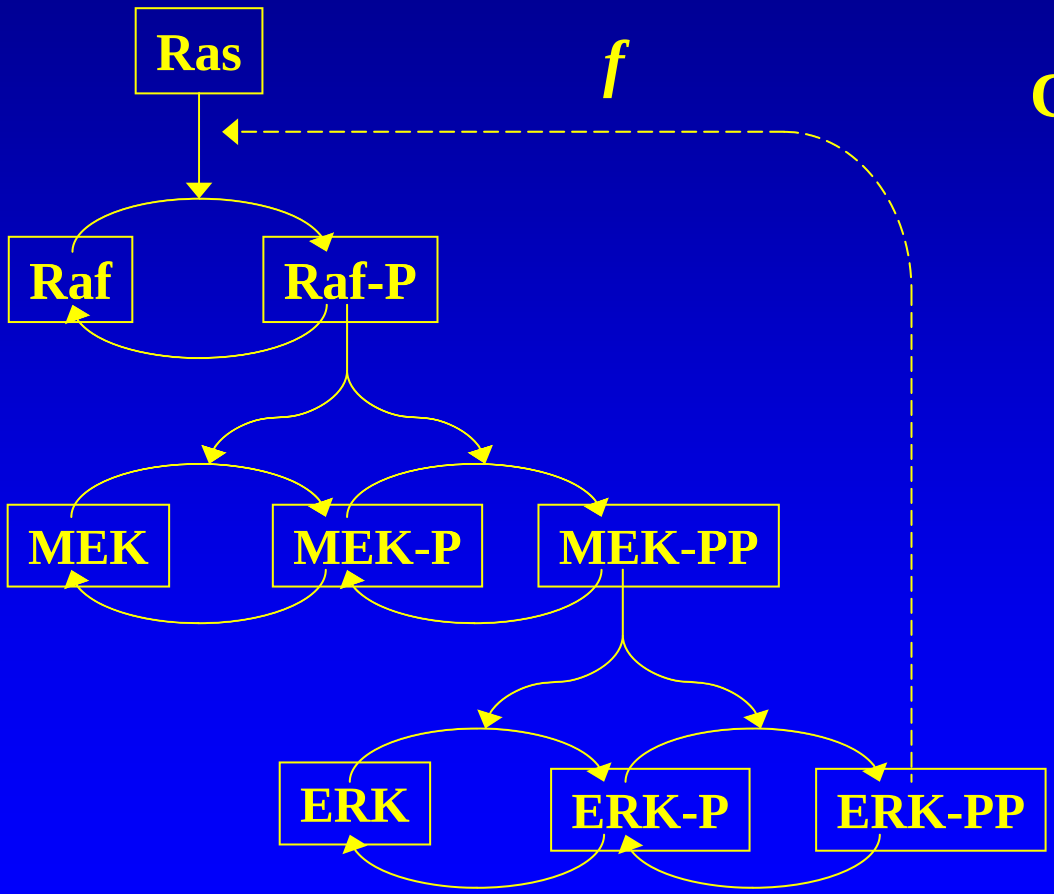


Cascade response

$$R = r_1 \cdot r_2 \cdot r_3$$

Kholodenko *et al* (1997) FEBS Lett., 414: 430

Control and Dynamic Properties of the MAPK Cascades



Cascade response

$$R = \mathrm{d}\ln[\mathrm{ERK\text{-}PP}]/\mathrm{d}\ln[\mathrm{Ras}]$$

Cascade with no feedback

$$R = r_1 \cdot r_2 \cdot r_3$$

Feedback strength

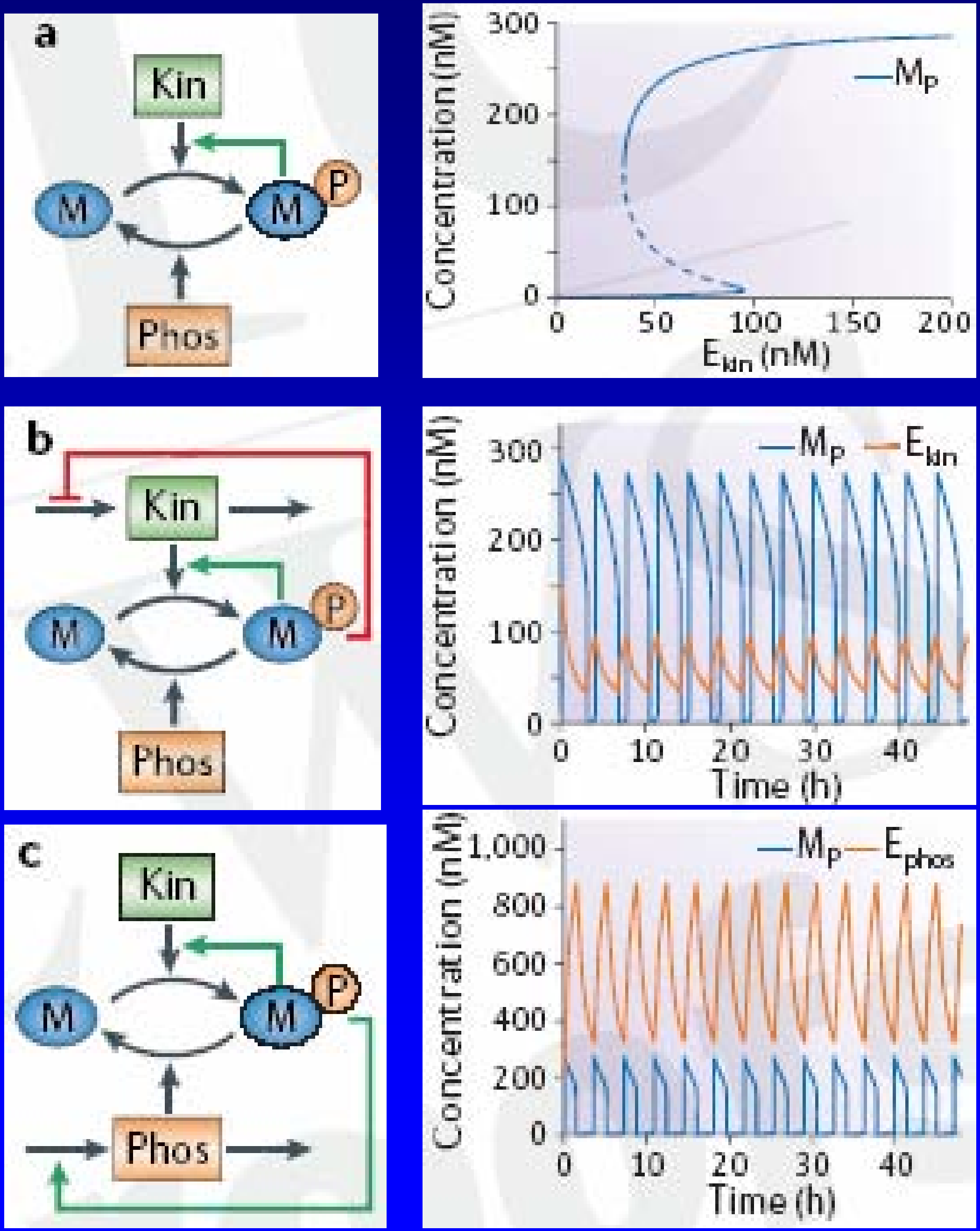
$$f = \partial \ln v/\partial \ln[\mathrm{ERK\text{-}PP}]$$

Cascade with feedback

$$R_f = R/(1 - f \cdot R)$$

Kholodenko, B.N. (2000) Europ. J. Biochem. 267: 1583.

Simple motifs displaying complex dynamics

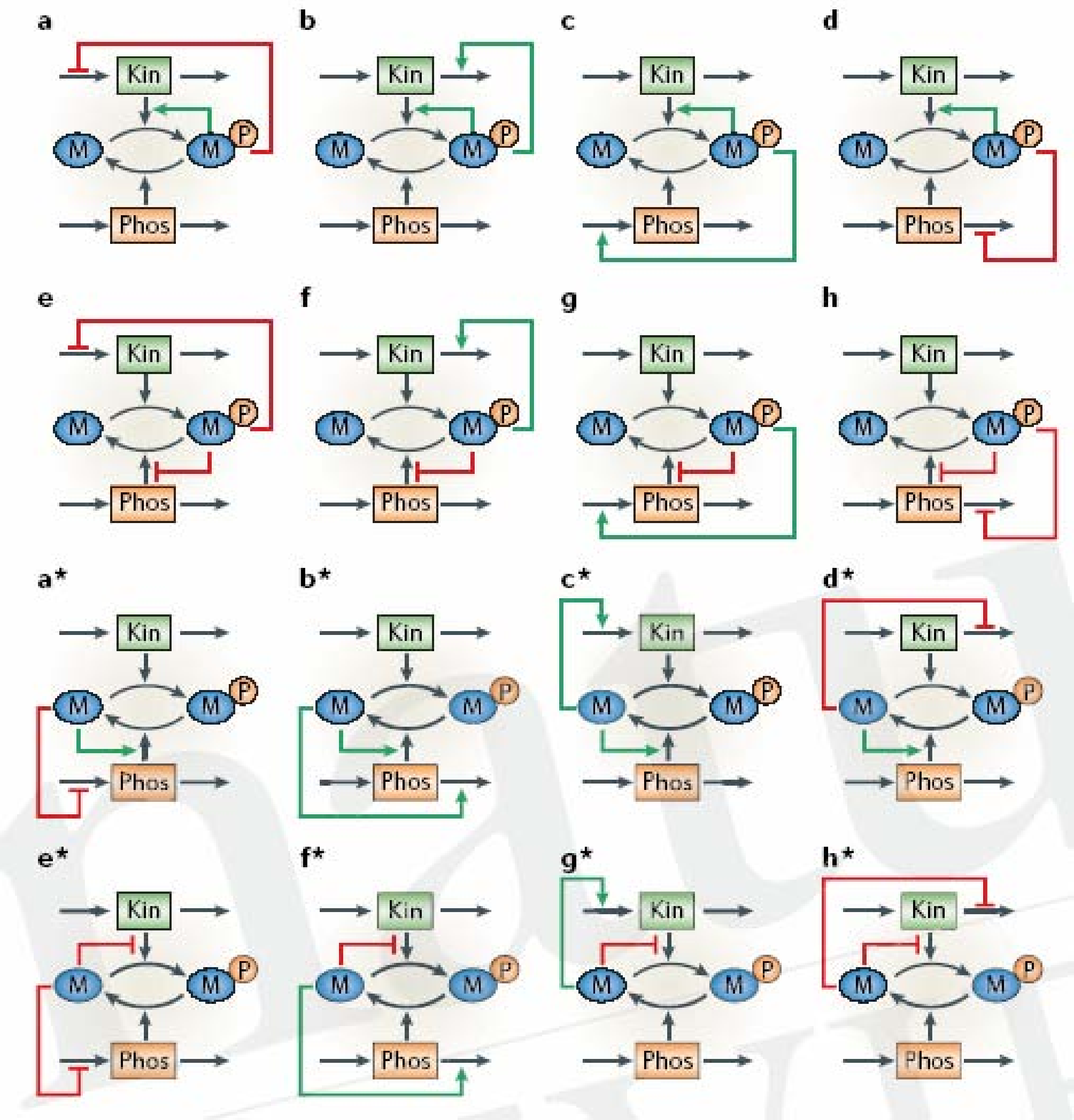


Bistability and hysteresis arise from product activation (destabilizing positive feedback)

Relaxation oscillator is brought about by positive feedback plus negative feedback

Kholodenko, B.N. (2006)
Nat. Rev. Mol. Cell Biol.

Simple motifs displaying complex dynamics



32 feedback designs that turn a universal signaling motif into a bistable switch and a relaxation oscillator.

Complex dynamics is a robust design property.

All rates and feedback loops obey simple Michaelis-Menten type kinetics

Kholodenko, B.N. (2006) Nat. Rev. Mol. Cell Biol.

Interaction Map of a Cellular Regulatory Network is Quantified by the Local Response Matrix

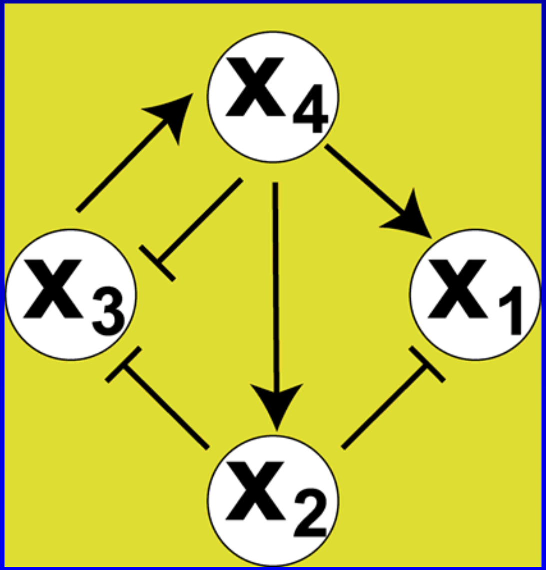
A dynamic system: $dx/dt = f(x, p).\ \ x = x_1, \dots, x_n,\ \ p = p_1, \dots, p_m$

The Jacobian matrix: $\mathbf{A} = (\partial f/\partial x).$

Signed incidence matrix

$$\begin{matrix} - & - & 0 & + \\ 0 & - & 0 & + \\ 0 & - & - & - \\ 0 & 0 & + & - \end{matrix}$$

If $\partial f_i/\partial x_j = 0$, there is no connection from variable x_j to x_i on the network graph.



Local response matrix r (Network Map)

$$\begin{matrix} -1 & r_{12} & 0 & r_{14} \\ 0 & -1 & 0 & r_{24} \\ 0 & r_{32} & -1 & r_{34} \\ 0 & 0 & r_{43} & -1 \end{matrix}$$

Relative strength of connections to each x_i is given by the ratios,

$$r_{ij} = \partial x_i/\partial x_j = -(\partial f_i/\partial x_j)/(\partial f_i/\partial x_i)$$

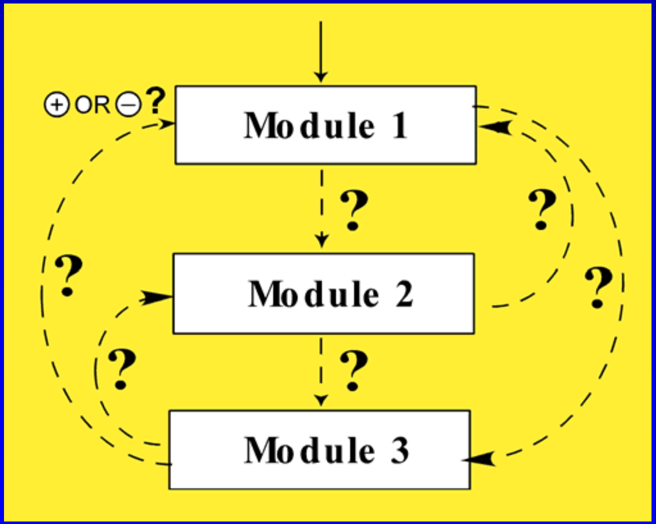
$$\mathbf{r} = -(dg\mathbf{A})^{-1} \cdot \mathbf{A}$$

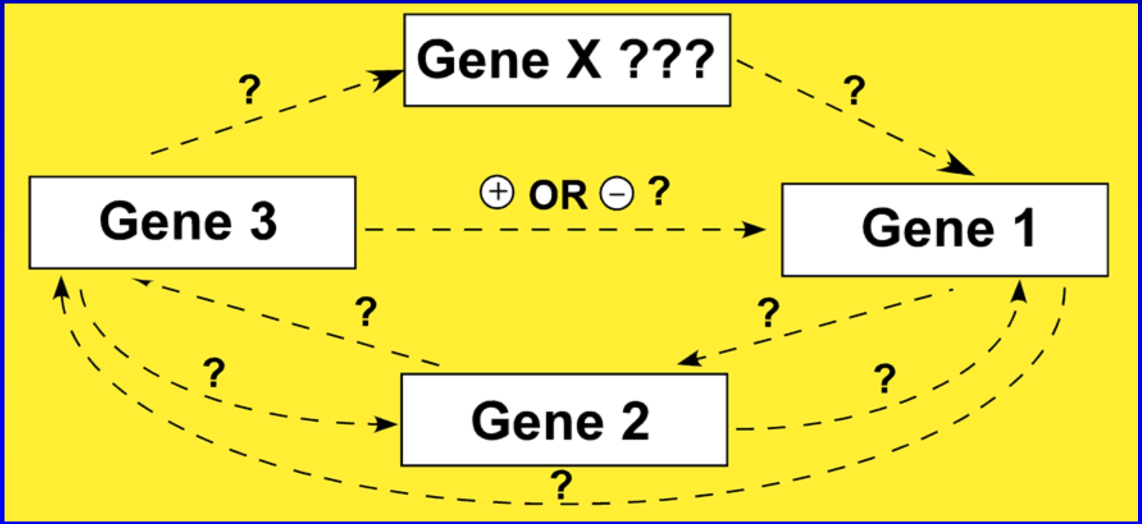
Kholodenko *et al* (2002) PNAS 99: 12841.

Untangling the Wires: Tracing Functional Interactions in Signaling and Gene Networks.

Goal: To Determine and Quantify Unknown Network Connections

Problem: **Network (system) responses (*R*) can be measured in intact cells, whereas local response matrix, r (network interaction map), cannot be captured unless entire system is reconstituted "*in vitro*".**



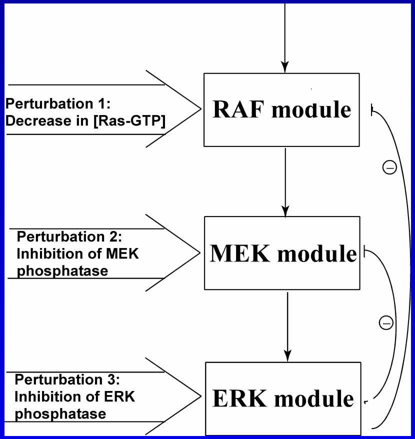


Kholodenko et al (2002) PNAS 99: 12841: Unraveling network structure (including feedback) from responses to gradual perturbations

Sachs et al. (2005) Science, 308: Bayesian network algorithm infers connections from perturbation data. No feedback loops are allowed.

Step 1: Determining System Responses to Three Independent Perturbations of the Ras/MAPK Cascade.

a). Measurement of the differences in steady-state variables following perturbations: $\Delta \ln X \approx 2(X^{(1)} - X^{(0)})/(X^{(1)} + X^{(0)})$



Perturbation 1:
Decrease in [Ras-GTP]

RAF module

Perturbation 2:
Inhibition of MEK phosphatase

MEK module

Perturbation 3:
Inhibition of ERK phosphatase

ERK module

⊖

⊖

1

$$\begin{pmatrix} \Delta_1 \ln(Raf{-}P) \\ \Delta_1 \ln(MEK{-}PP) \\ \Delta_1 \ln(ERK{-}PP) \end{pmatrix}$$

2

$$\begin{pmatrix} \Delta_2 \ln(Raf{-}P) \\ \Delta_2 \ln(MEK{-}PP) \\ \Delta_2 \ln(ERK{-}PP) \end{pmatrix}$$

3

$$\begin{pmatrix} \Delta_3 \ln(Raf{-}P) \\ \Delta_3 \ln(MEK{-}PP) \\ \Delta_3 \ln(ERK{-}PP) \end{pmatrix}$$

b) Generation of the system response matrix

$$\begin{matrix} -7.4 & 6.9 & 3.7 \\ -6.2 & -3.1 & 8.9 \\ -12.7 & -6.3 & -3.4 \end{matrix}$$

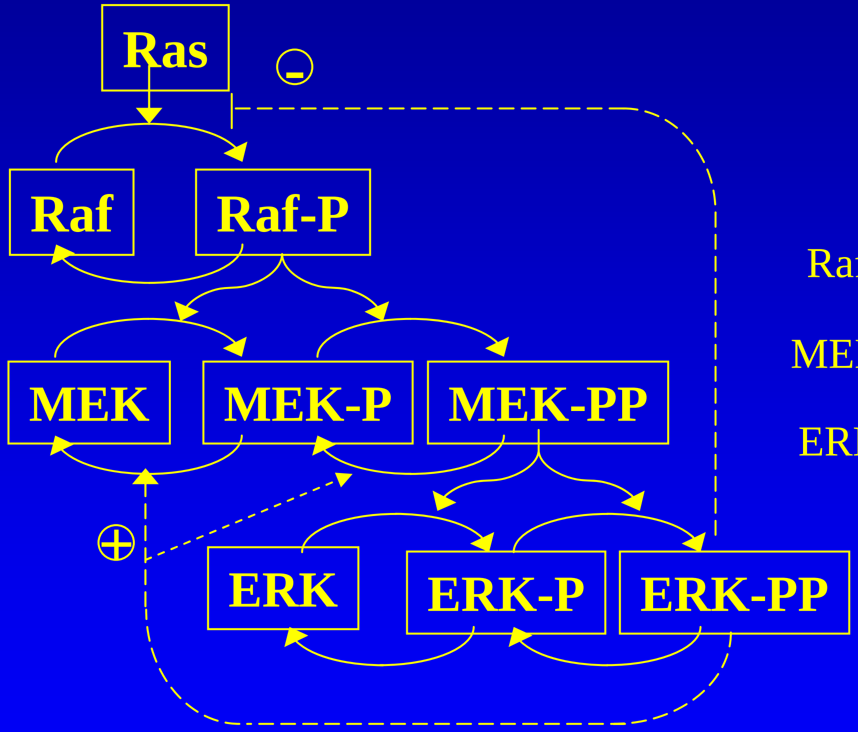
10% change in parameters

$$\begin{matrix} -55.5 & -46.3 & -25.0 \\ -44.8 & 20.3 & -56.8 \\ -85.7 & 39.4 & 21.8 \end{matrix}$$

50% change in parameters

Step 2: Calculating the Ras/MAPK Cascade Interaction Map from the System Responses

$$\mathbf{r} = -\left(dg(\mathbf{R}^{-1})\right)^{-1}\cdot\mathbf{R}^{-1}$$



Two interaction maps (local response matrices) retrieved from two different system response matrices

$$\begin{array}{c|ccc} & \text{Raf-P} & \text{MEK-PP} & \text{ERK-PP} \\ \hline \text{Raf-P} & -1 & 0.0 & -1.1 \\ \text{MEK-PP} & 1.9 & -1 & -0.6 \\ \text{ERK-PP} & 0.0 & 2.0 & -1 \end{array} \qquad \begin{array}{ccc} -1 & 0.0 & -1.2 \\ 1.8 & -1 & -0.6 \\ 0.0 & 2.0 & -1 \end{array}$$

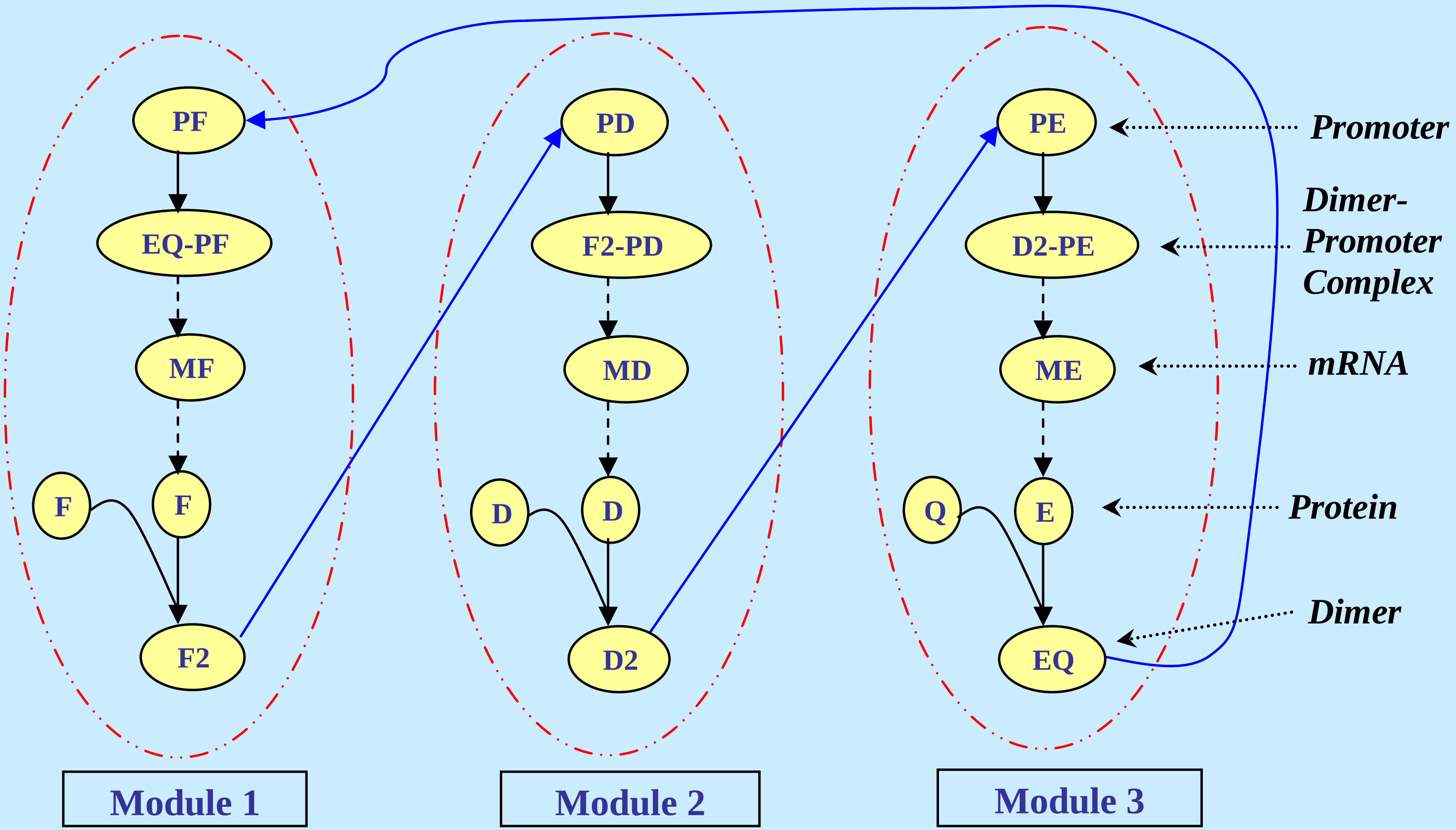
Known Interaction Map

$$\begin{array}{c|ccc} & \textbf{Raf-P} & \textbf{MEK-PP} & \textbf{ERK-PP} \\ \hline \textbf{Raf-P} & -1 & 0.0 & -1.1 \\ \textbf{MEK-PP} & 1.9 & -1 & -0.6 \\ \textbf{ERK-PP} & 0.0 & 2.0 & -1 \end{array}$$

Kholodenko et al (2002) PNAS 99: 12841

Testing in Silico: Unraveling the Wiring of a Gene Network

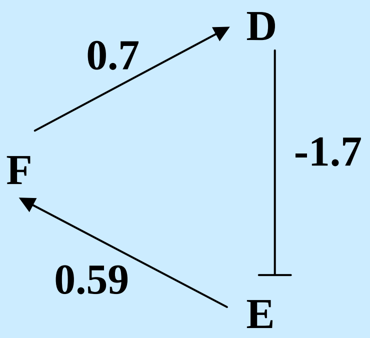
Modular description



Yalamanchili *et al* (2006) IEE Proc Systems Biol, 153, 236-246

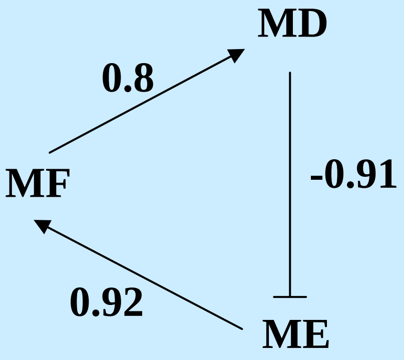
Inferring Interaction Maps

Protein interaction map



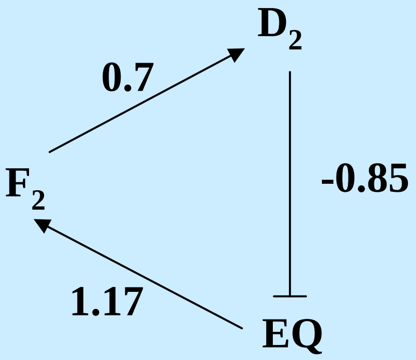
	D	E	F
D	-1.00	0.00	0.70
E	-1.70	-1.00	0.00
F	0.00	0.59	-1.00

mRNA interaction map



	MD	ME	MF
MD	-1.00	0.00	0.80
ME	-0.91	-1.00	0.00
MF	0.00	0.92	-1.00

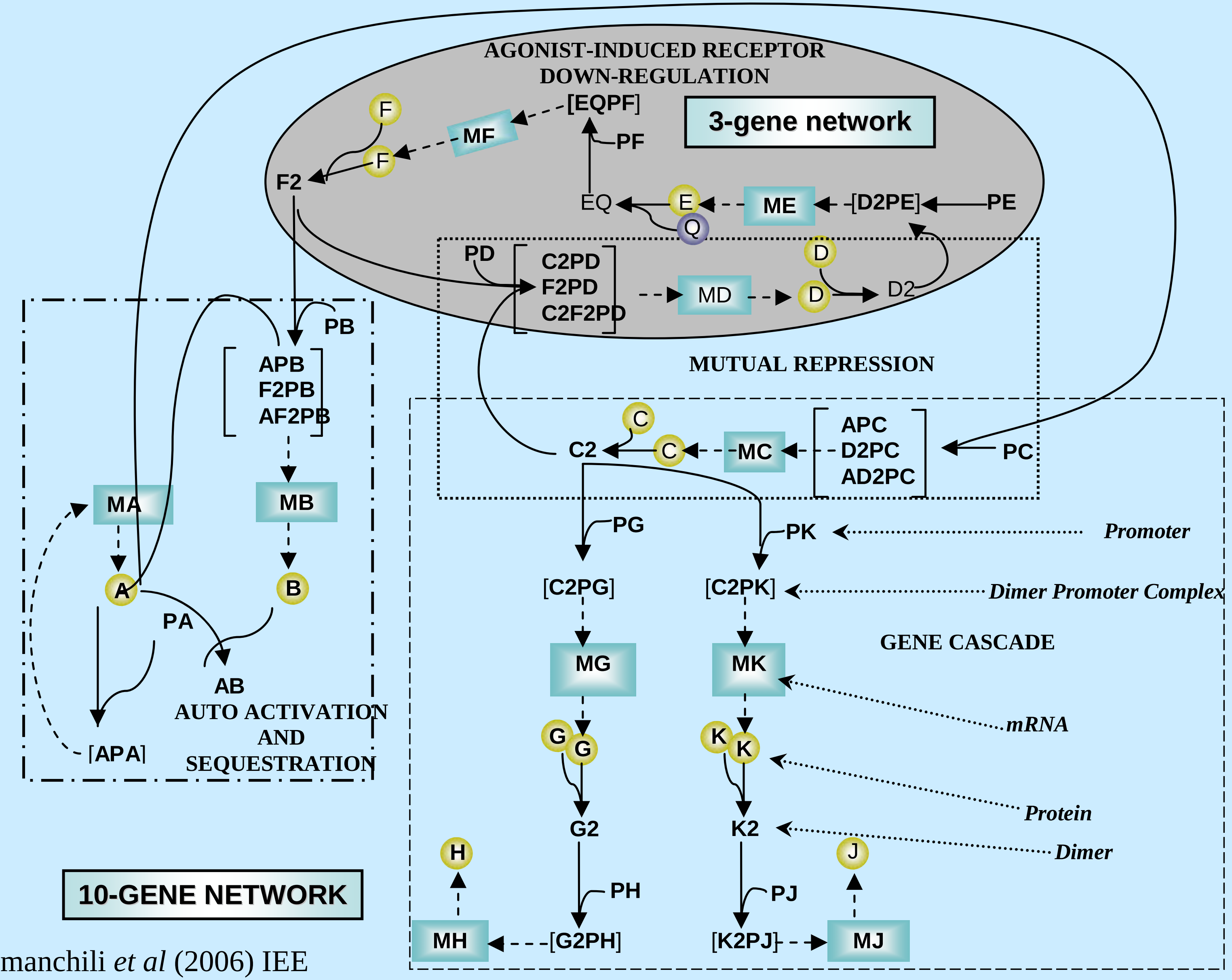
Interaction Map for Dimers



	D_2	EQ	F_2
D_2	-1.00	0.00	0.70
EQ	-0.85	-1.00	0.00
F_2	0.00	1.17	-1.00

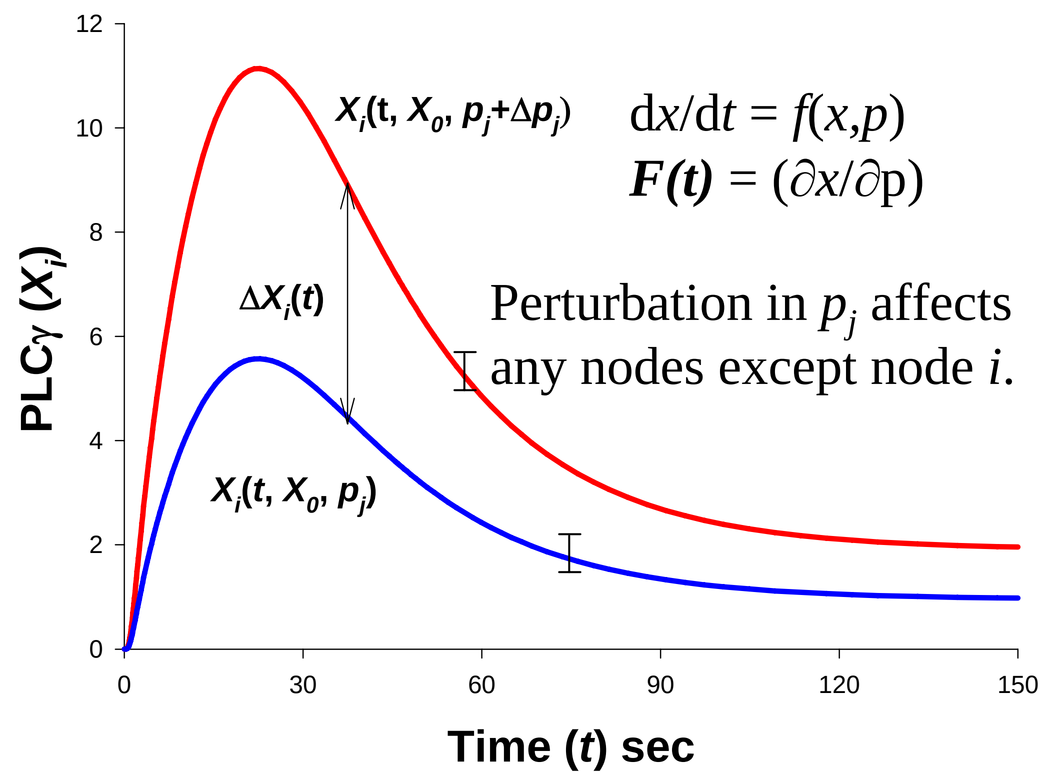
Yalamanchili *et al* (2006) IEE Proc Systems Biol, 153, 236-246

Testing in Silico: Unraveling the Wiring of a Gene Network



Yalamanchili *et al* (2006) IEE Proc Systems Biol, 153, 236-246

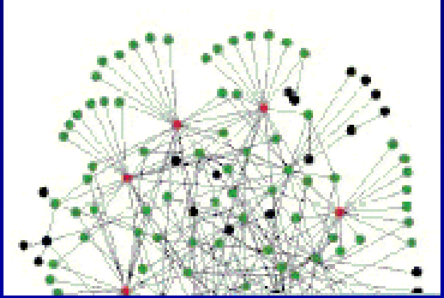
Unraveling the Wiring Using Time Series Data



$dx/dt = f(x,p)$

$\boldsymbol{F(t)} = (\partial x/\partial p)$

Perturbation in p_j affects any nodes except node i.



The goal is to determine $F_i(t) = (1, F_{i1}, \dots, F_{in})$ the Jacobian elements that quantify connections to x_i

Vector $G_j(t)$ contains experimentally measured network responses $\Delta x_i(t)$ to parameter p_j perturbation, $G_j(t) = (\partial \Delta x_i/\partial t, \Delta x_i, \dots, \Delta x_n)$

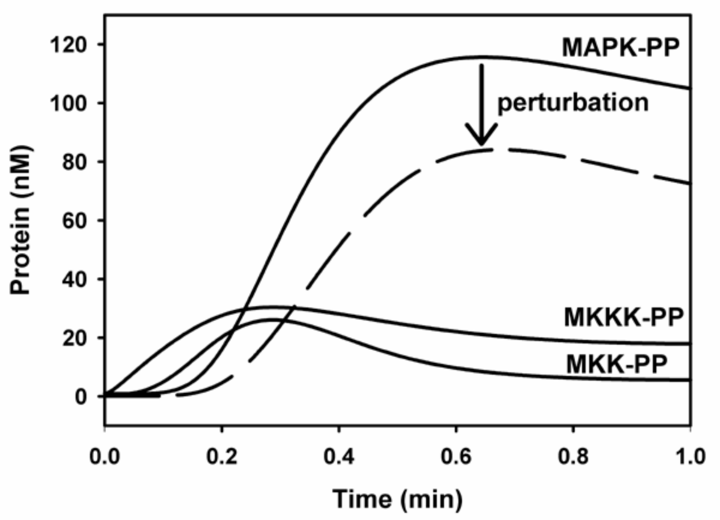
Orthogonality theorem: $(F_i(t), G_j(t)) = 0$

Sontag *et al*. (2004) Bioinformatics, 20, 1877.

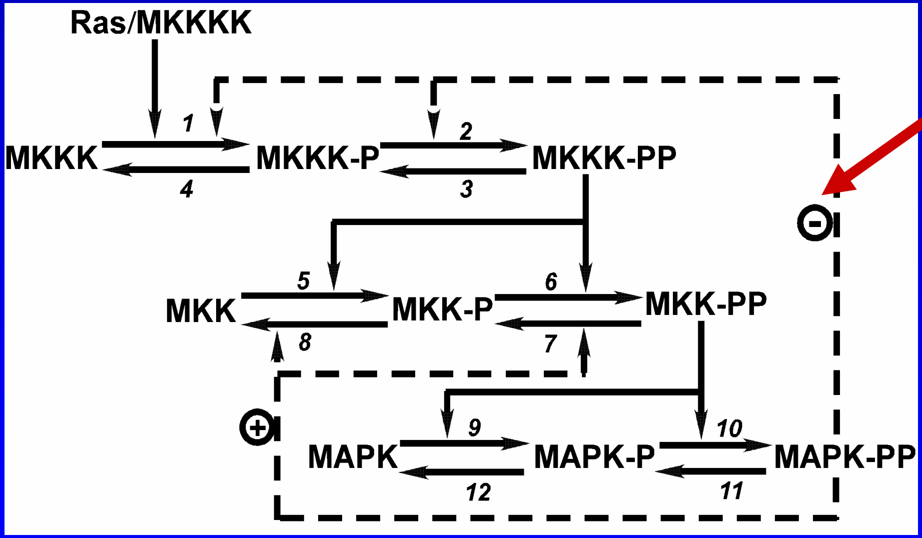
A vector $A_i(t)$ is orthogonal to the linear subspace spanned by responses to perturbations affecting either one or multiple nodes different from *i*

Inferring dynamic connections in MAPK pathway successfully

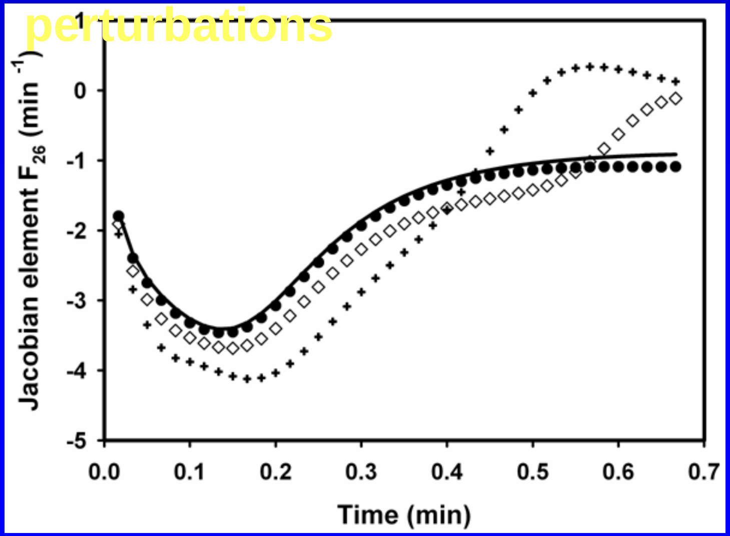
Transition of MAPK pathway from resting to stable activity state



MAPK pathway kinetic diagram



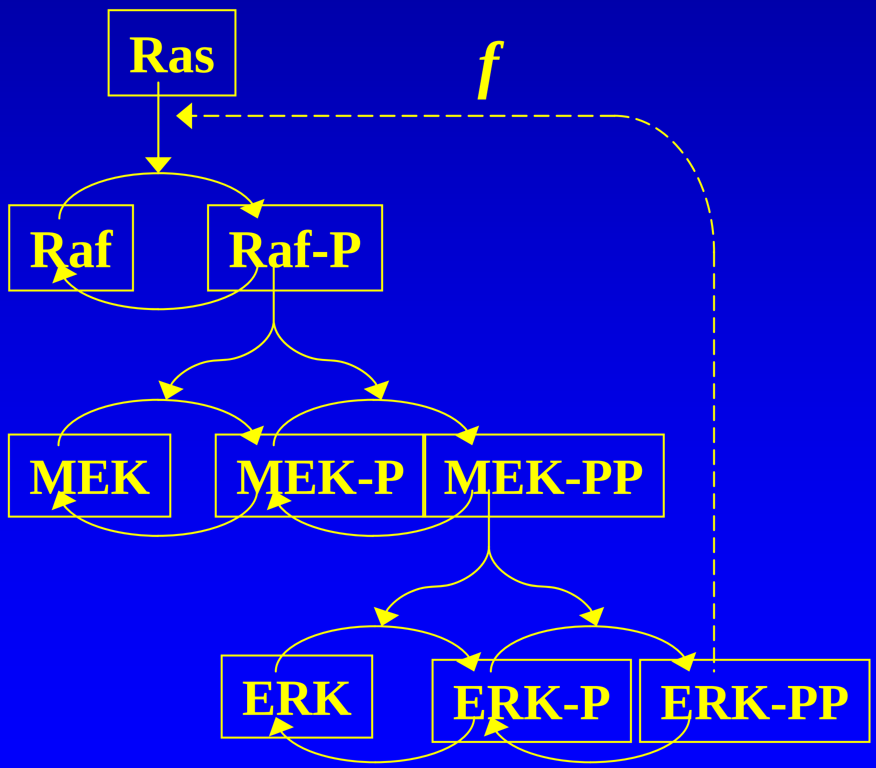
Deduced time-dependent strength of a negative feedback for 5, 25, and 50% perturbations

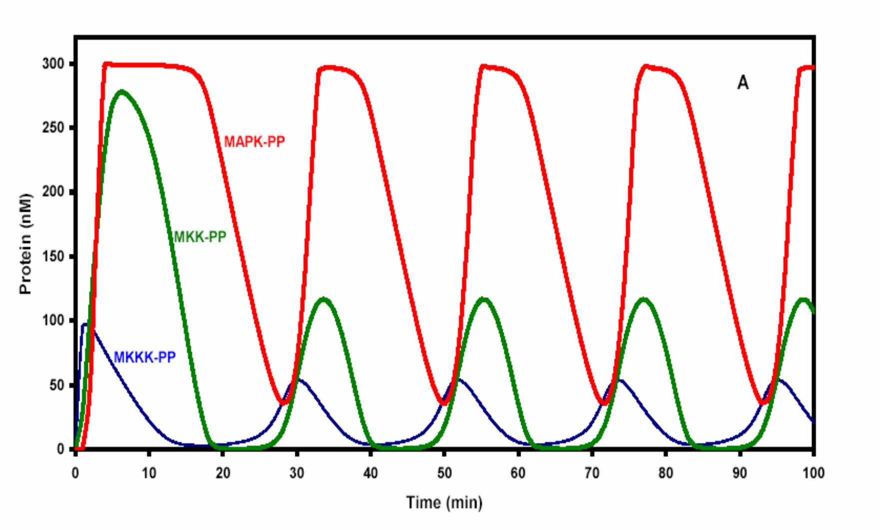


Sontag *et al*. (2004) Bioinformatics 20, 1877.

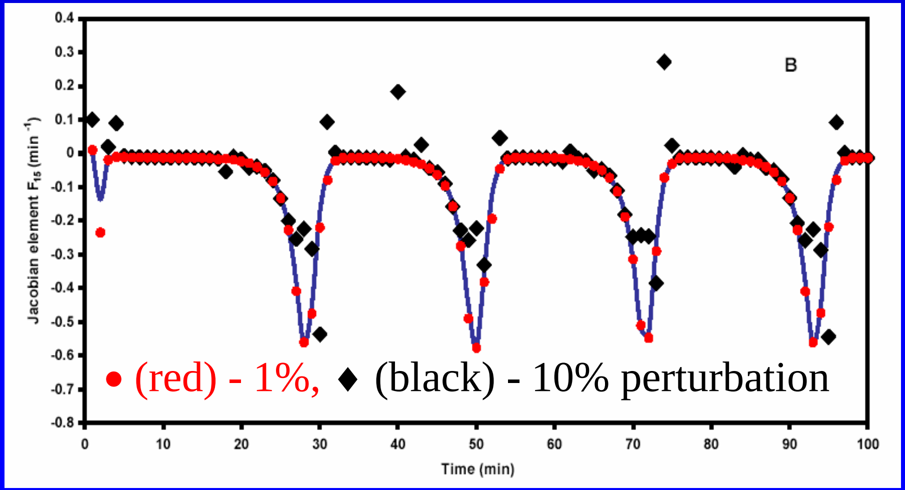
Oscillatory dynamics of the feedback connection strengths is successfully deduced

Oscillations in MAPK pathway





The Jacobian element F_{15} quantifies the negative feedback strength



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