

Cell-Signaling Dynamics in Time and Space

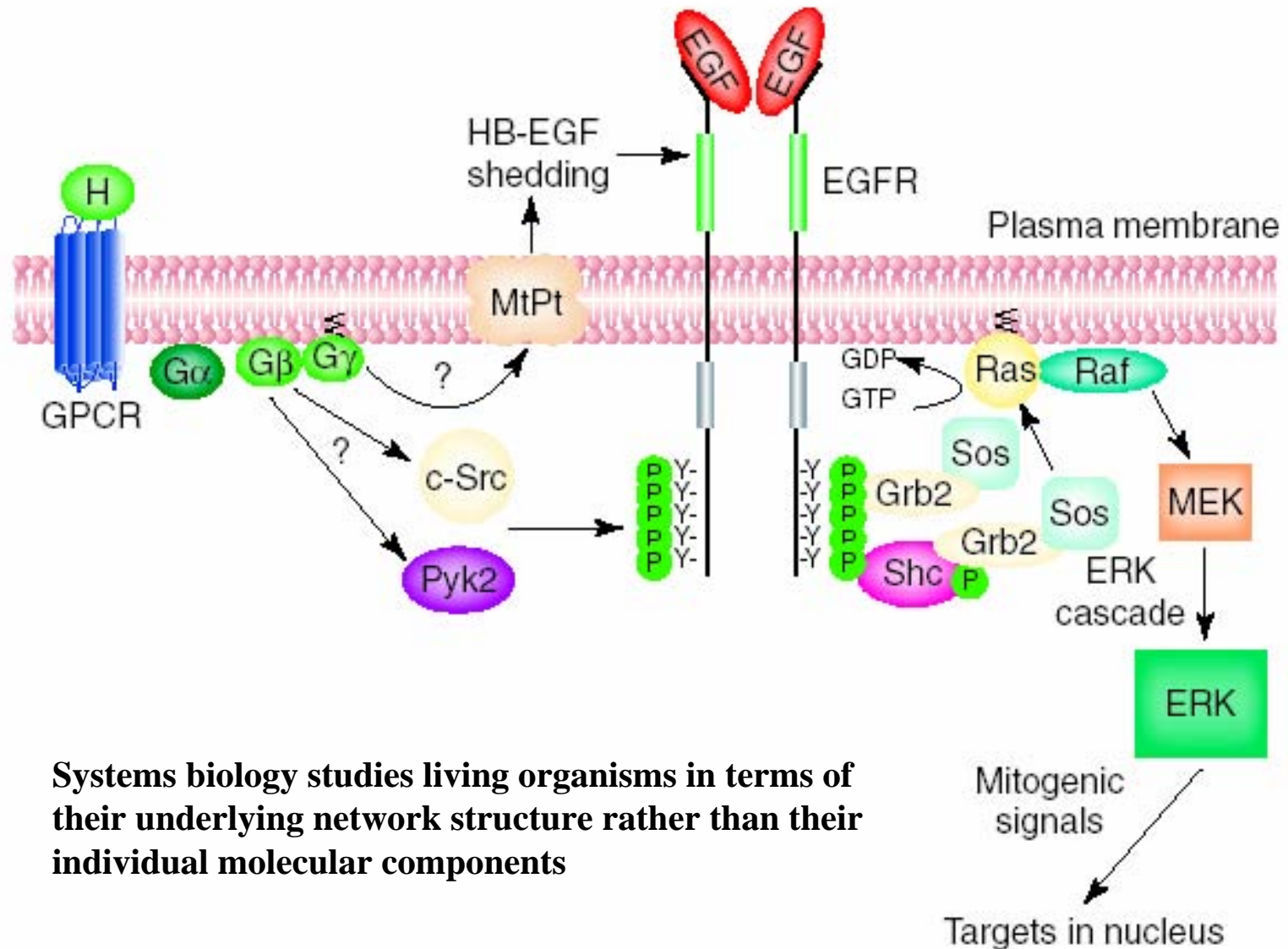
Boris N. Kholodenko

Department of Pathology, Anatomy and Cell Biology

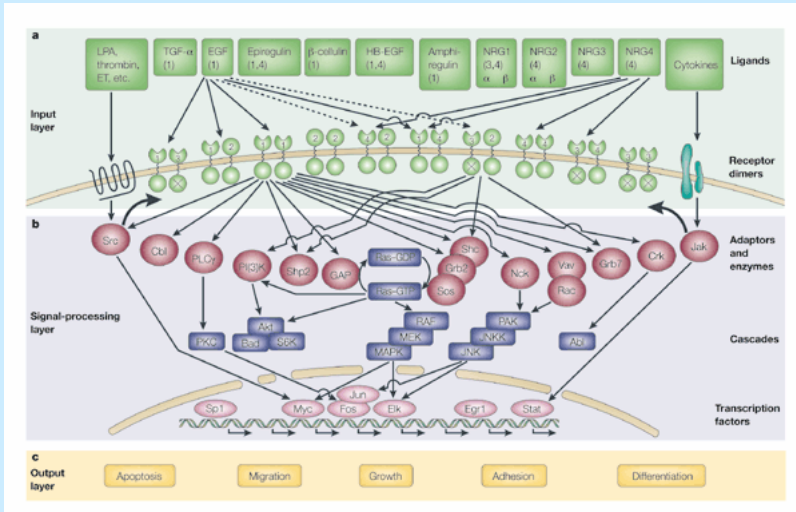
Thomas Jefferson University

Philadelphia, PA

Systems Biology of Signaling Networks



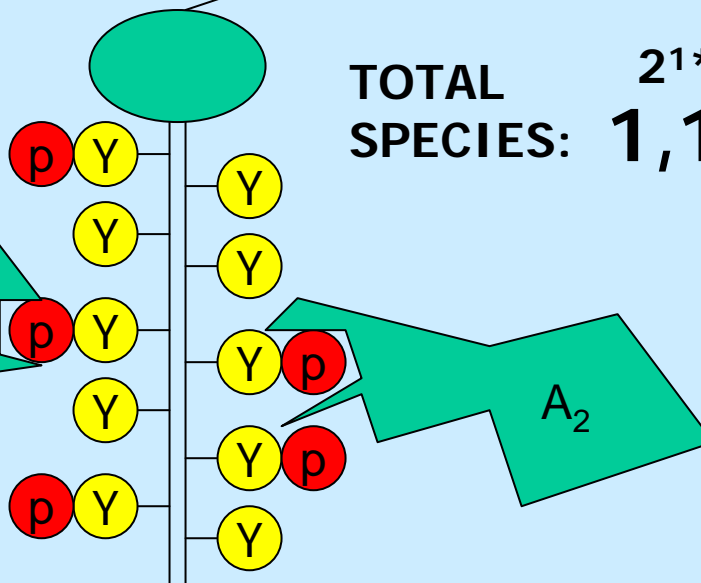
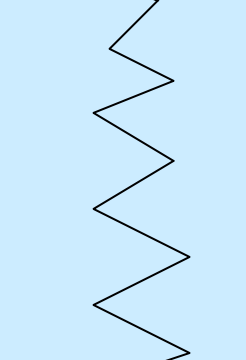
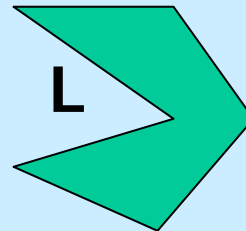
Combinatorial Complexity Presents Problems for Models



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

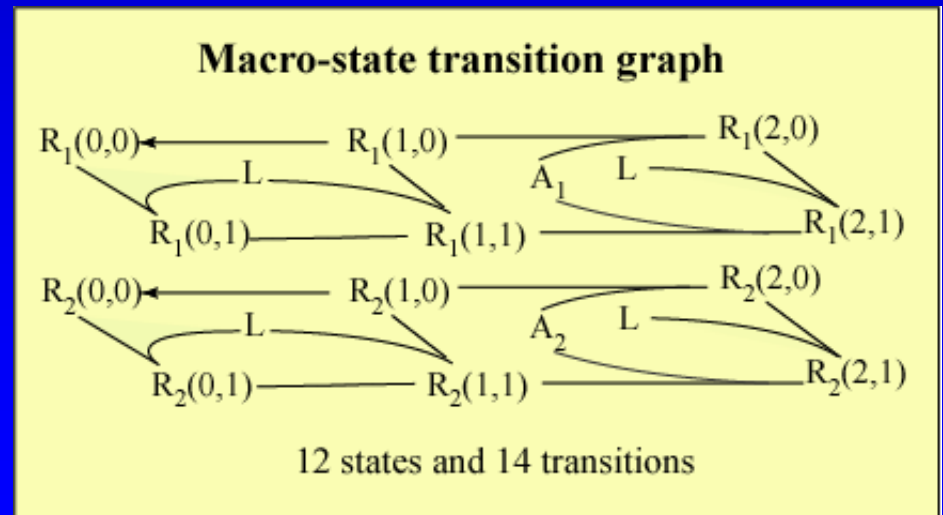
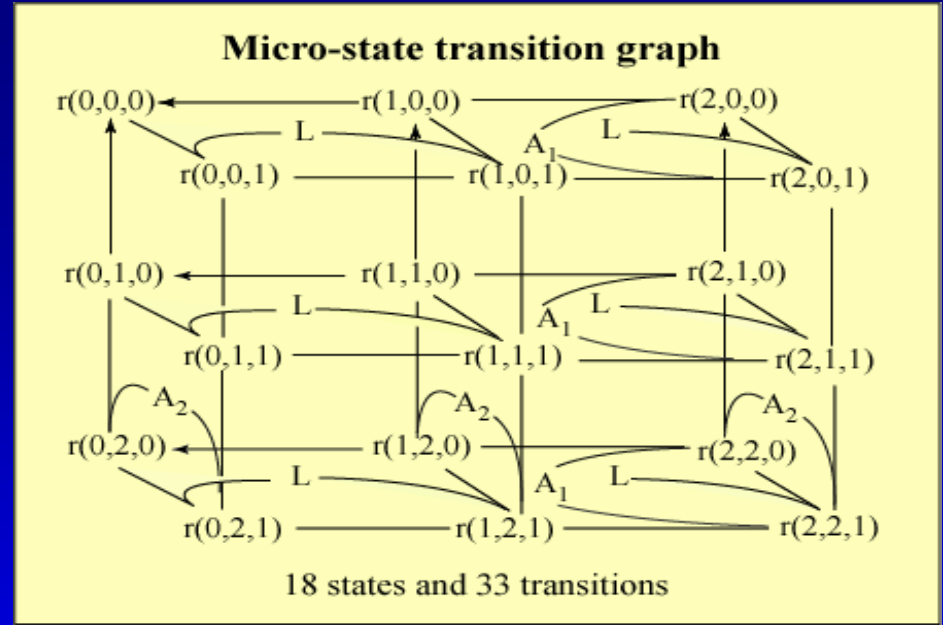
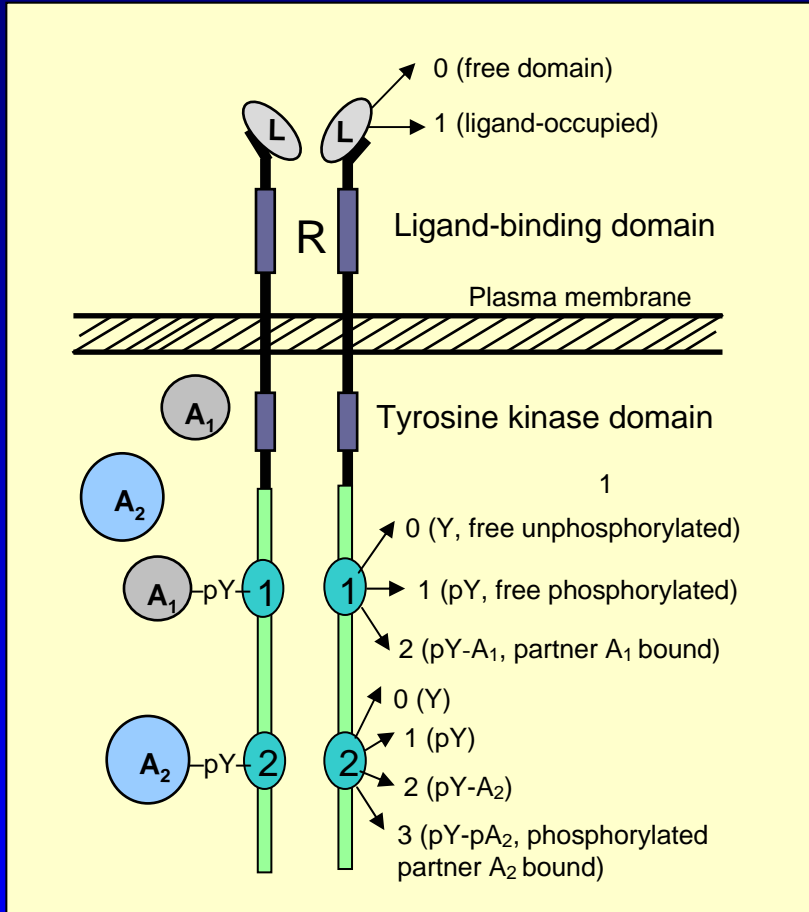
Ligand Binding	1	Bound or Not	2
Dimerization	1	None or 1,2,3,4	5
Kinase Domain	1	Active or Inactive	2
Docking (pY)	10	Y, pY, Bound	3

TOTAL

$$2^1 * 5^1 * 2^1 * 3^{10} =$$

SPECIES: **1,180,980!!**

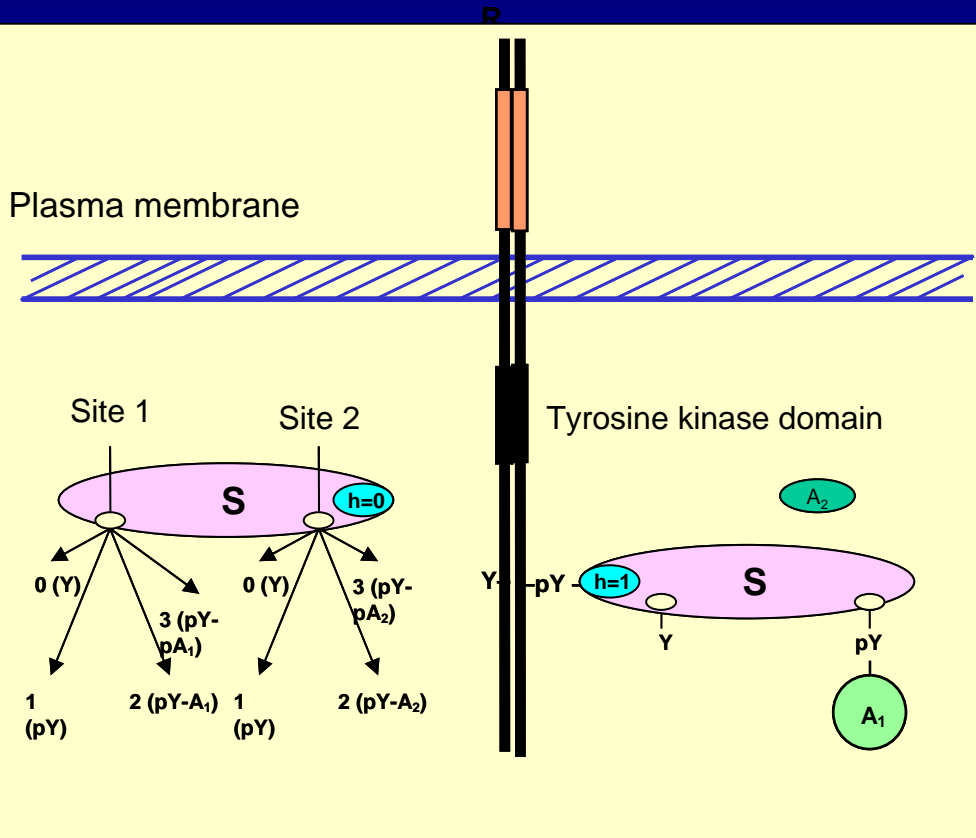
Reducing Combinatorial Complexity of Multi-Domain Proteins



Macro-state is a sum of micro-states:

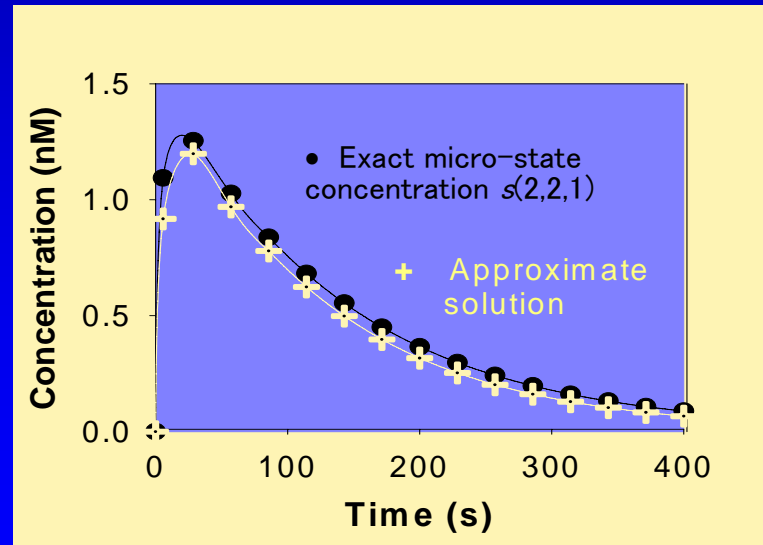
$$R_1(a_1, h) = \sum_{a_2=0}^{m_2} r(a_1, a_2, h), \quad R_2(a_2, h) = \sum_{a_1=0}^{m_1} r(a_1, a_2, h)$$

Micro- and Macro-Models of Scaffold Proteins



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

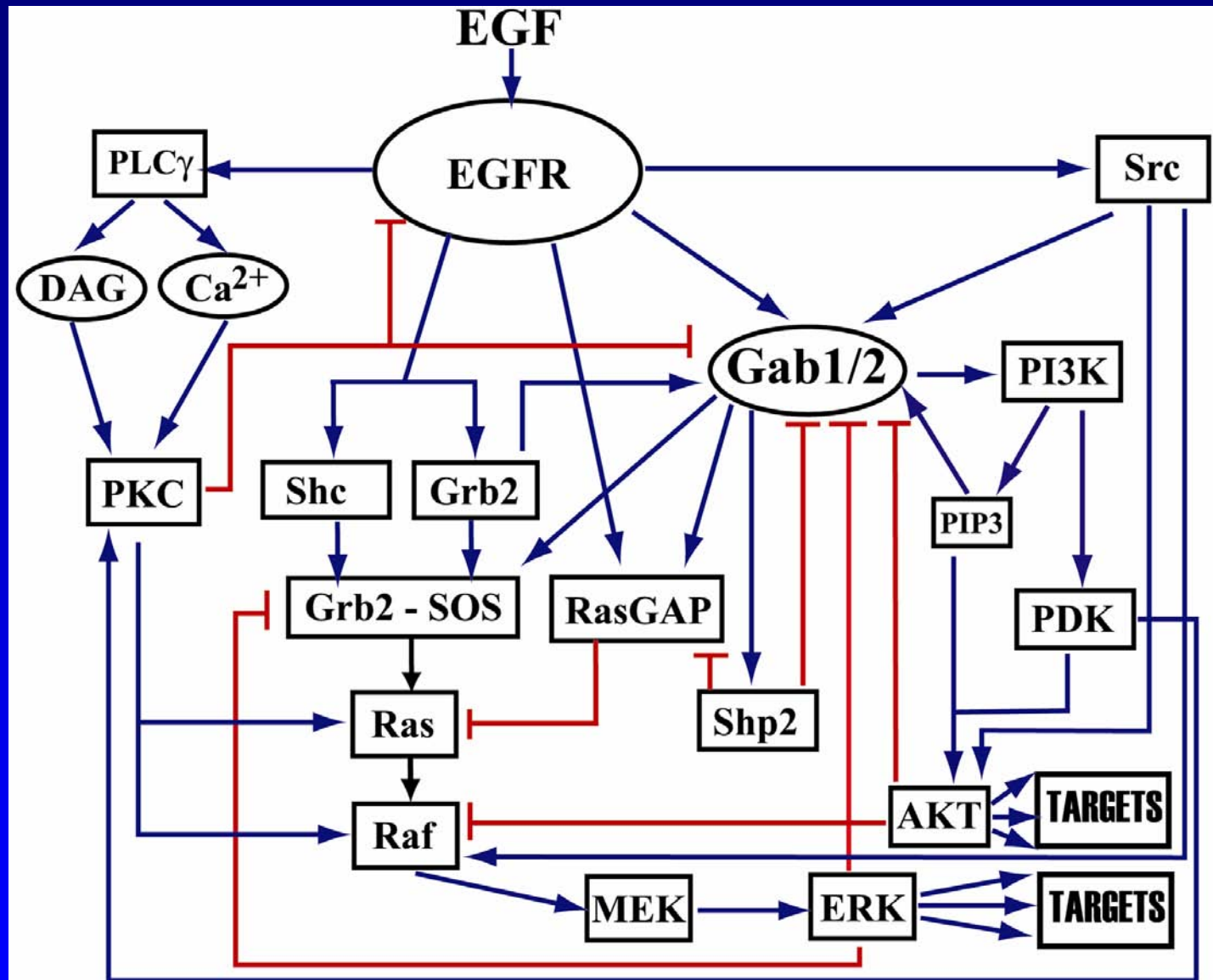
$$s(a_1, \dots, a_n; h) \approx \frac{\prod_{i=1}^n S_i(a_i, h)}{S_{tot}^{n-1}(h)}$$



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

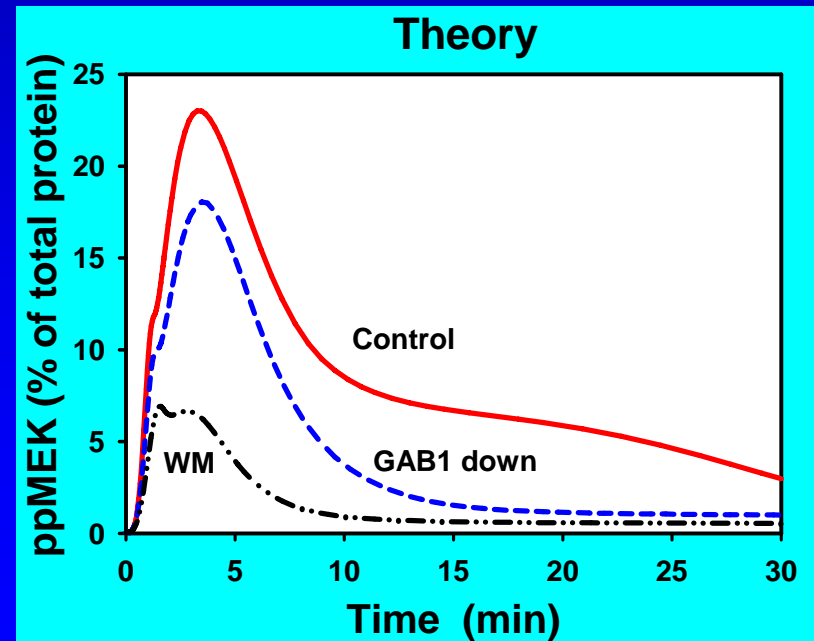
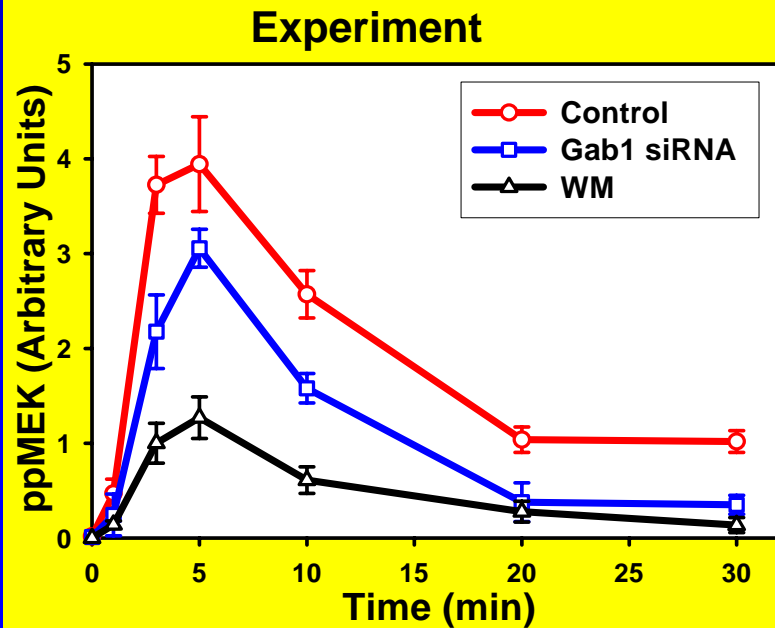
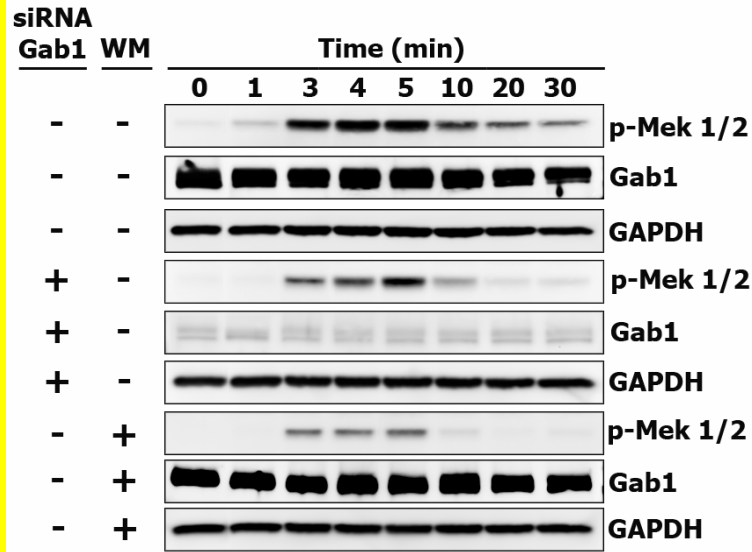
**Borisov *et al* (2005) Biophys. J. 89:951. Conzelmann *et al* (2006) BMC Bioinformatics, 7:34
Kiyatkin *et al*. J Biol Chem, (2006) 281:19925. Borisov *et al* (2006) Biosystems. 83:152**

Interactions between the Ras/ERK and PI3K/Akt pathways

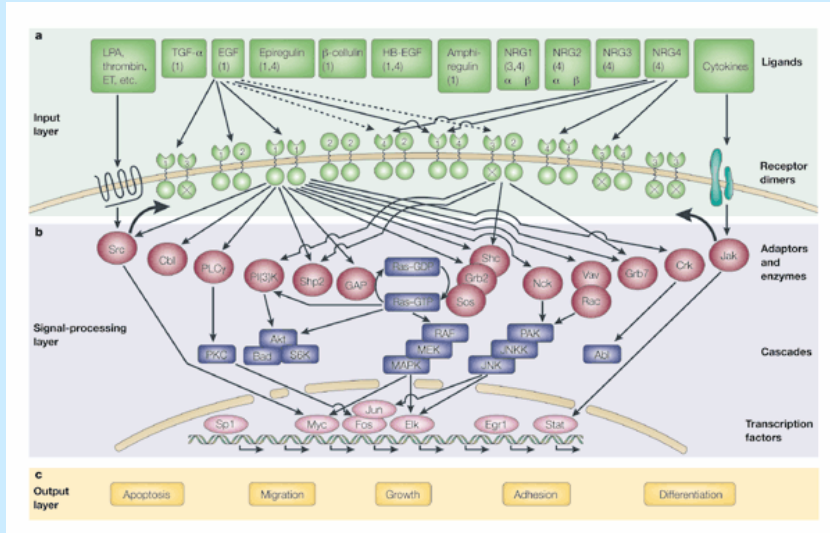


Distinct Operational Ranges of Different Feedback Loops Shape Signaling Outcome

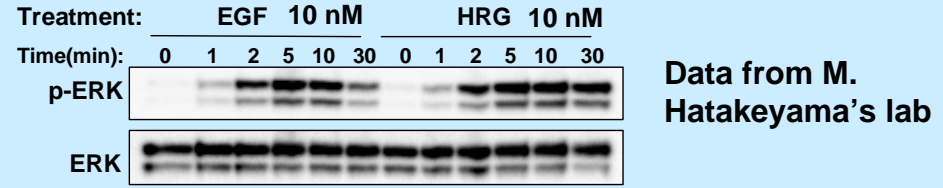
Disruption of GAB1-PI3K-GAB1 Positive Feedback: Experiment and Theory



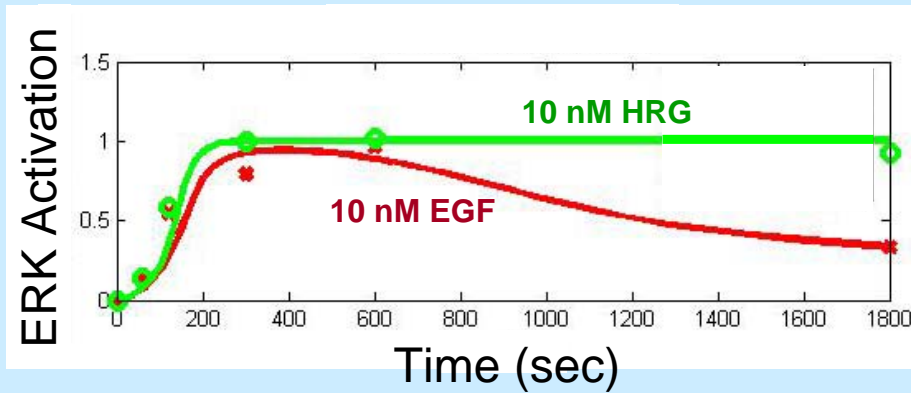
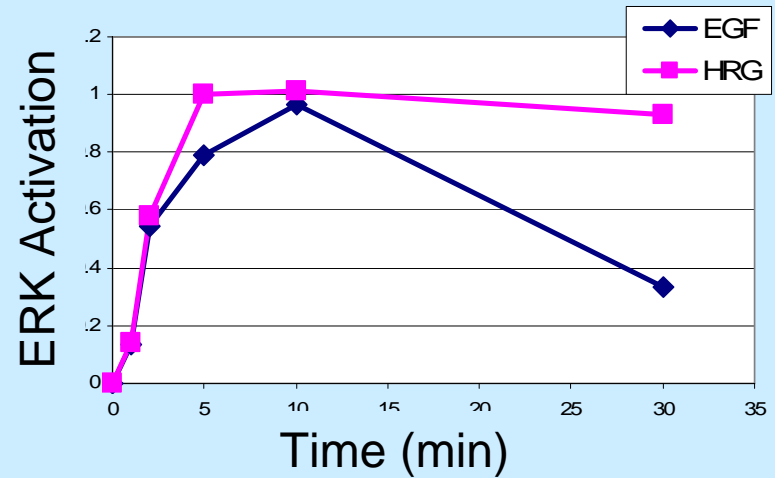
Context-Dependent Responses to EGF and HRG



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

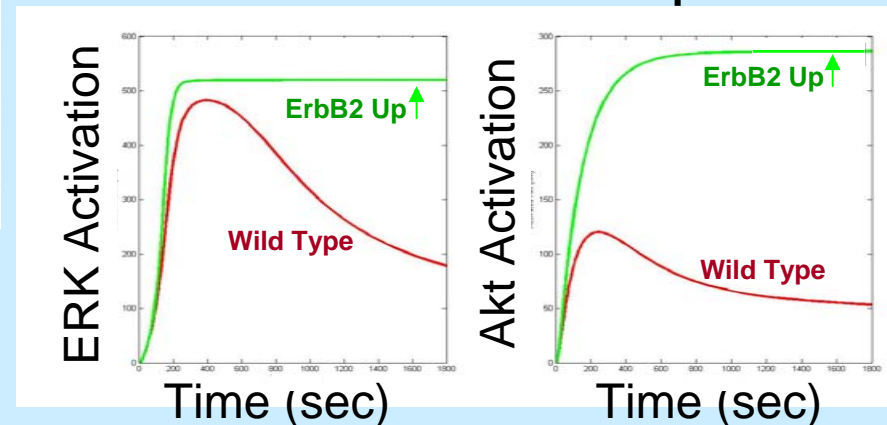


Data from M. Hatakeyama's lab



See Poster #269 by M. Birtwistle et al.

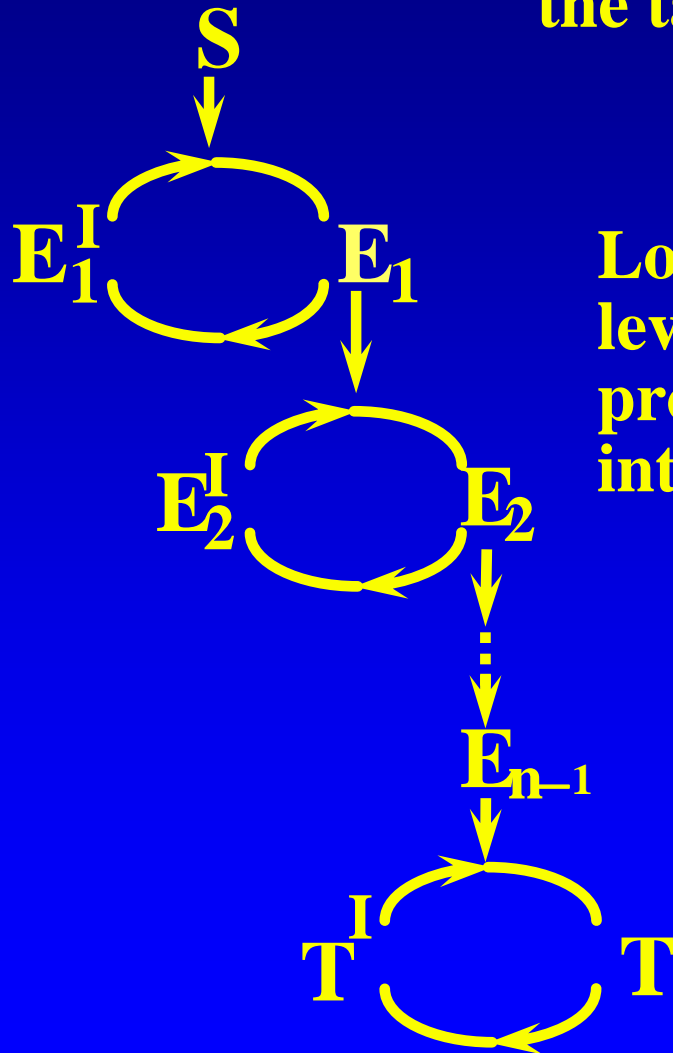
ErbB2 Overexpression Transforms a Transient into Sustained Response



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

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



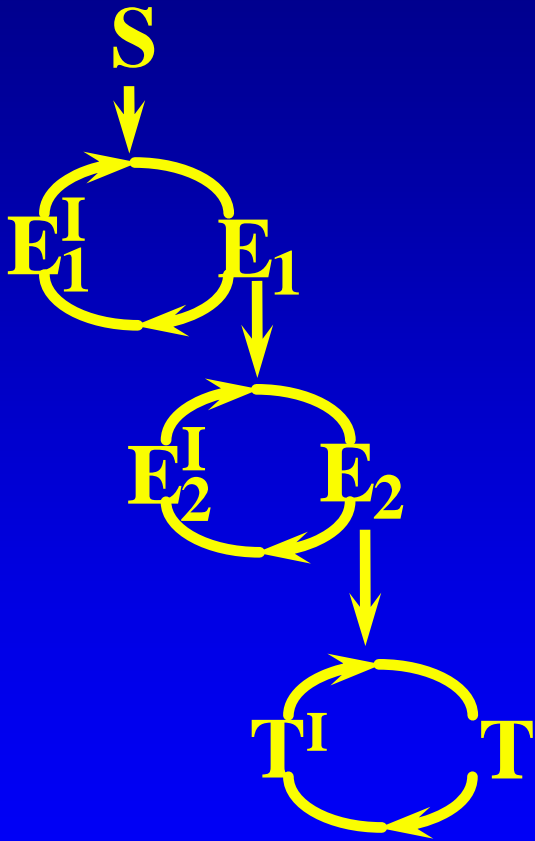
Local Response: the sensitivity (r) of the level i to the preceding level $i-1$. Active protein forms (E_i) are “communicating” intermediates:

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

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

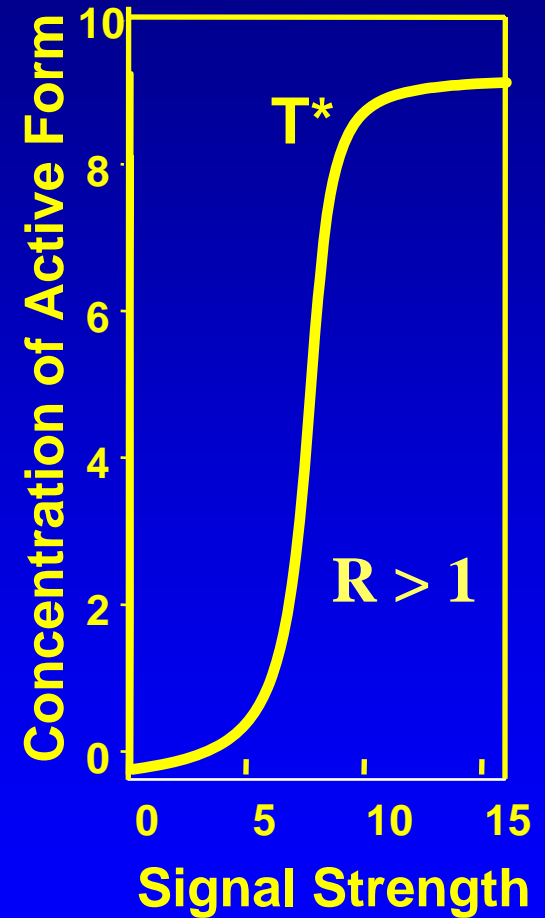
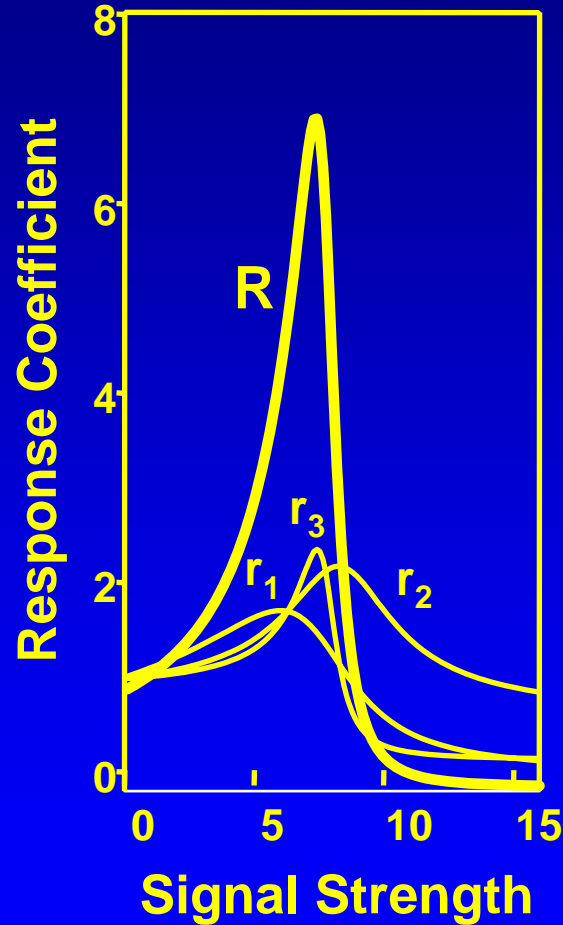
$$R_S^T = r_1 \cdot r_2 \cdot \dots \cdot r_{n-1} \cdot r_n = \prod (\text{path})$$

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



Cascade response

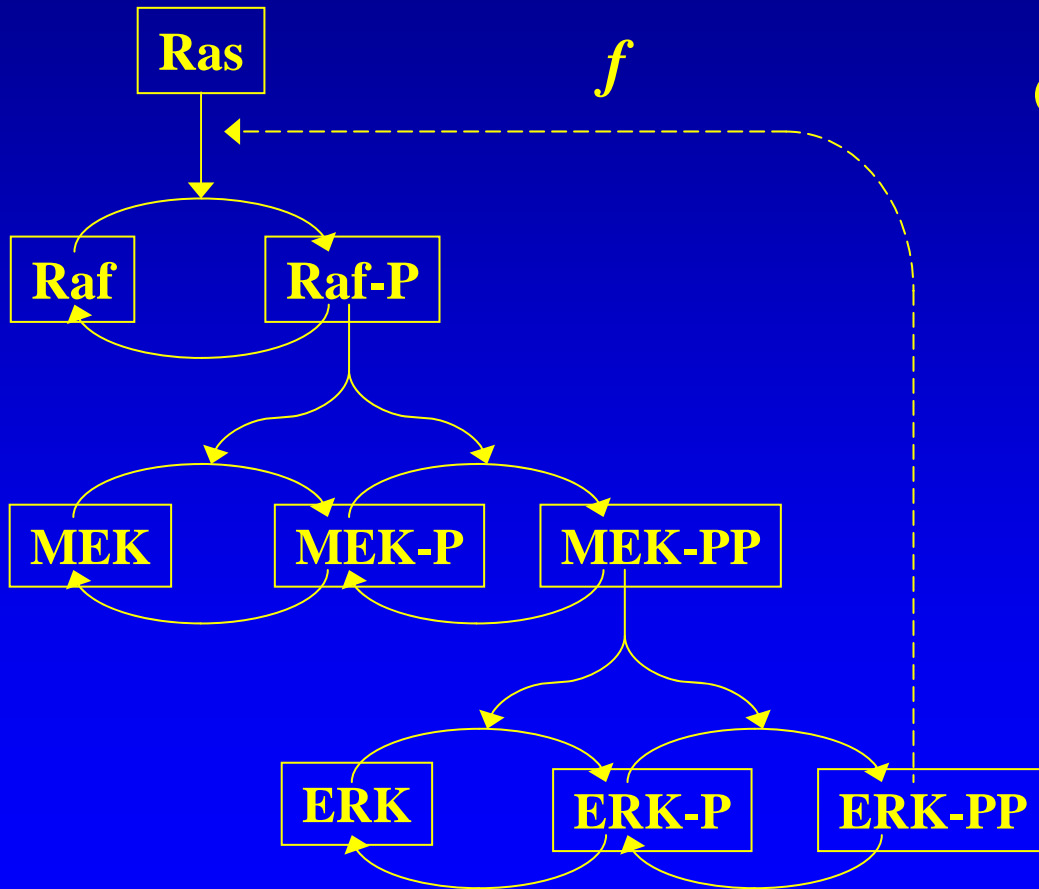
$$R = r_1 \cdot r_2 \cdot r_3$$



Control and Dynamic Properties of the MAPK Cascades

Cascade response

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



Cascade with no feedback

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

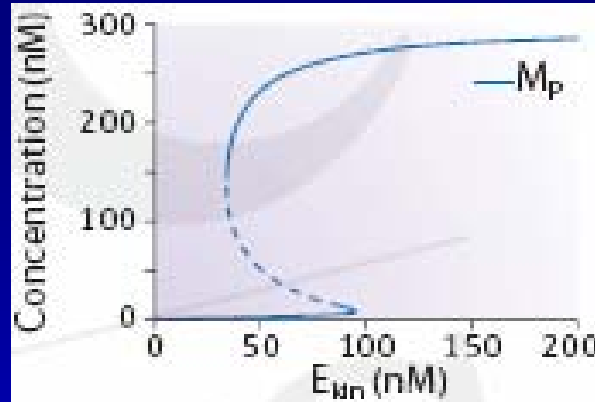
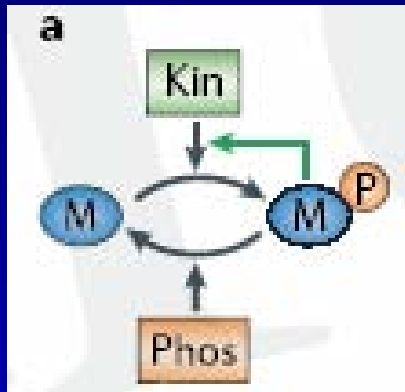
Feedback strength

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

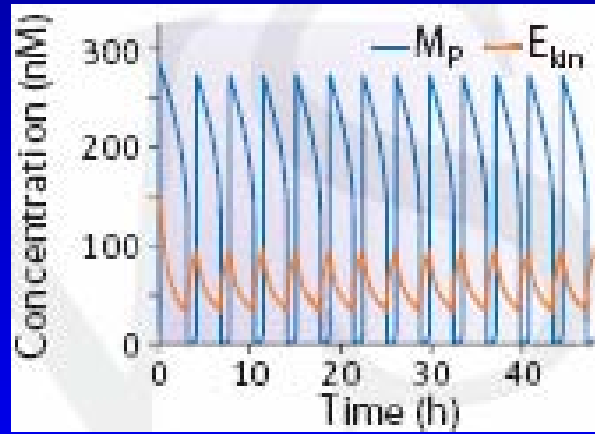
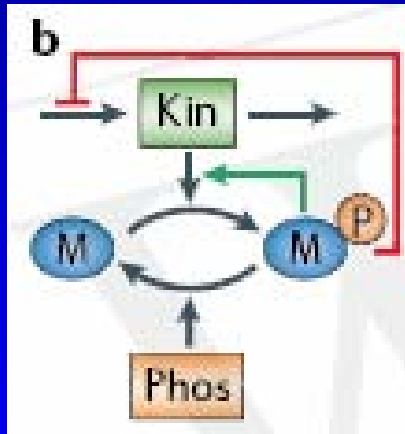
Cascade with feedback

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

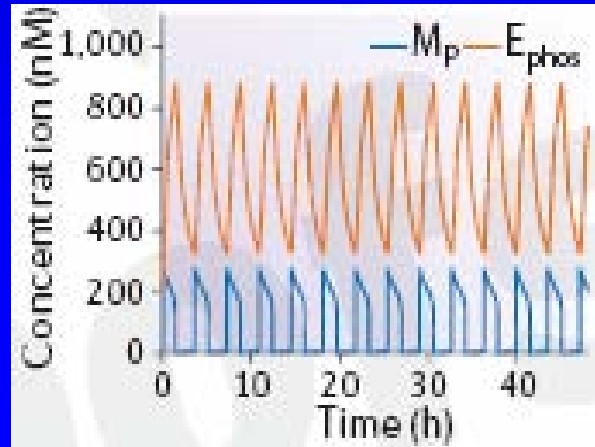
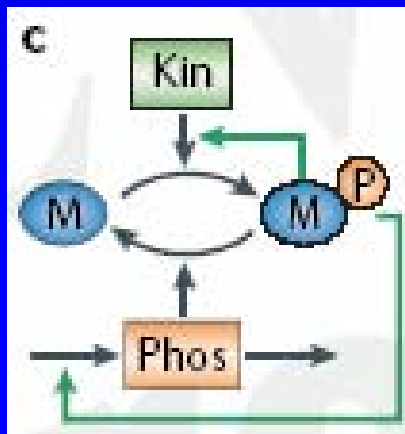
Simple motifs displaying complex dynamics



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

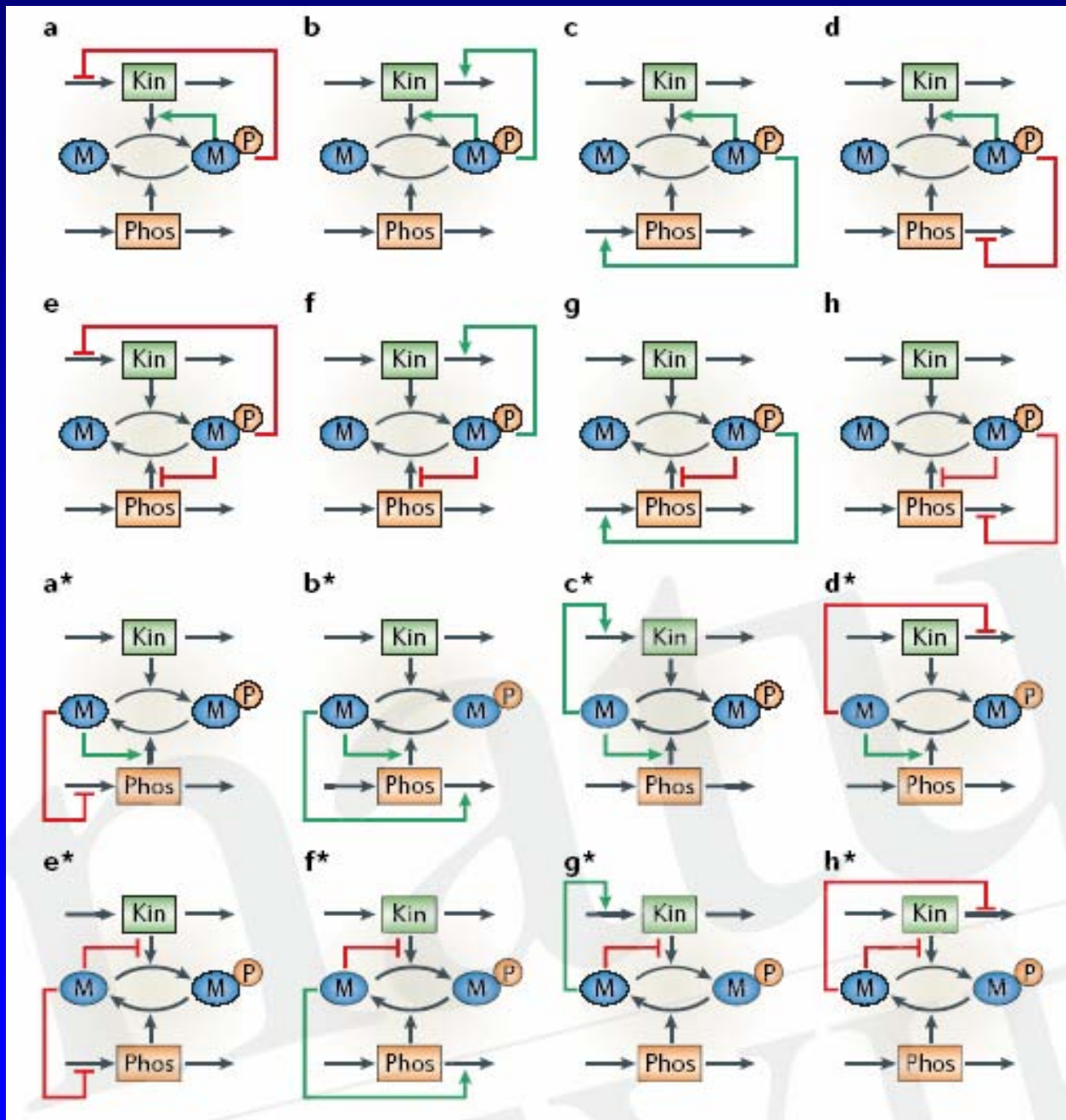


Relaxation oscillator is brought about by positive feedback plus negative feedback



**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

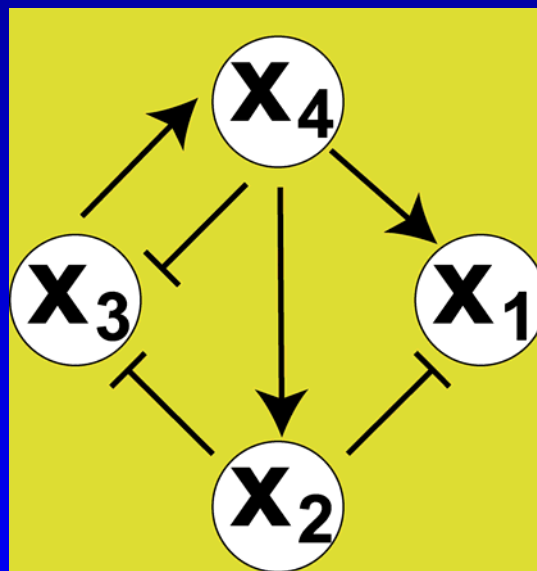
A dynamic system: $dx/dt = f(x, p)$. $x = x_1, \dots, x_n$, $p = p_1, \dots, p_m$

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

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

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

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



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

Signed incidence matrix

-	-	0	+
0	-	0	+
0	-	-	-
0	0	+	-

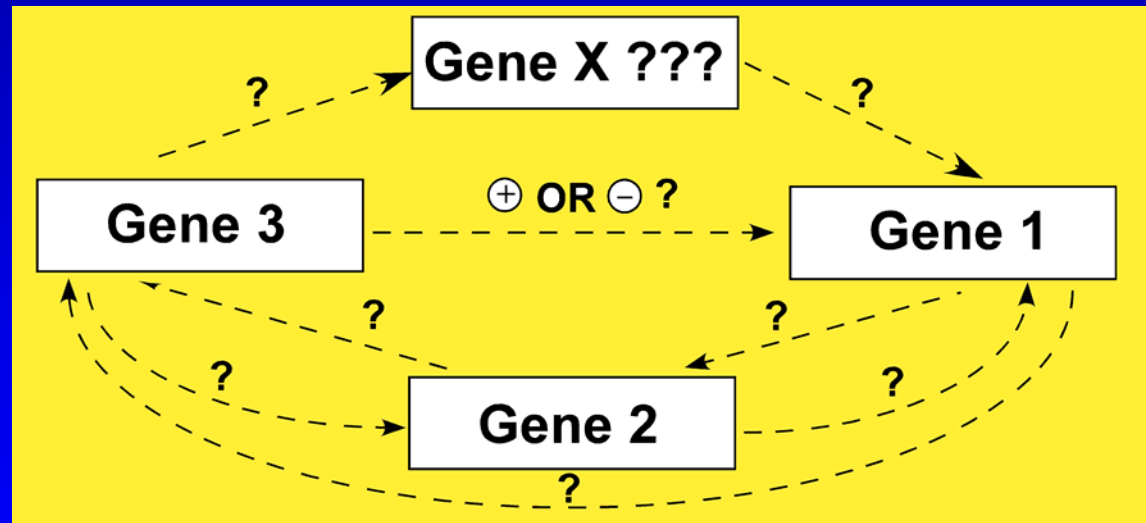
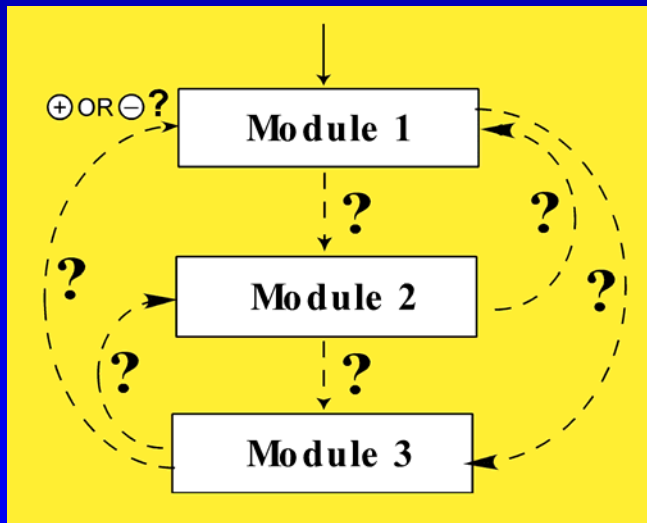
Local response matrix r (Network Map)

-1	r_{12}	0	r_{14}
0	-1	0	r_{24}
0	r_{32}	-1	r_{34}
0	0	r_{43}	-1

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

Goal: To Determine and Quantify Unknown Network Connections

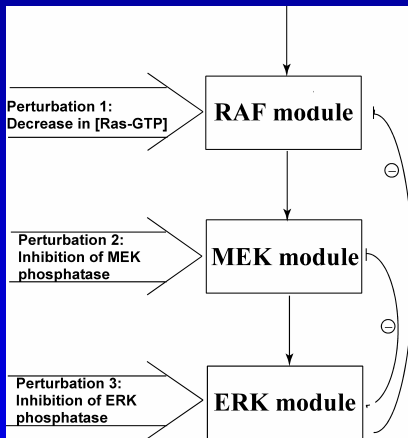
Problem: Network (system) responses (R) can be measured in intact cells, whereas local response matrix, r (network interaction map), cannot be captured unless entire system is reconstituted “*in vitro*”.



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: $\Delta \ln X \approx 2(X^{(1)} - X^{(0)}) / (X^{(1)} + X^{(0)})$



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

-7.4	6.9	3.7
-6.2	-3.1	8.9
-12.7	-6.3	-3.4

10% change in parameters

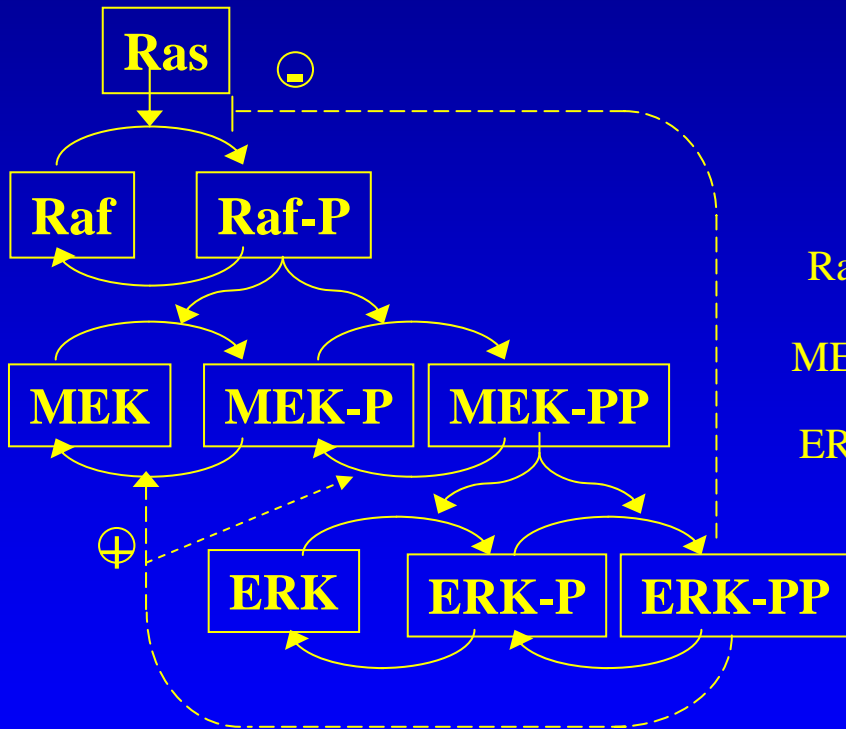
-55.5	-46.3	-25.0
-44.8	20.3	-56.8
-85.7	39.4	21.8

50% change in parameters

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

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

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



Raf-P MEK-PP ERK-PP

Raf-P	-1	0.0	-1.1	-1	0.0	-1.2
MEK-PP	1.9	-1	-0.6	1.8	-1	-0.6
ERK-PP	0.0	2.0	-1	0.0	2.0	-1

Known Interaction Map

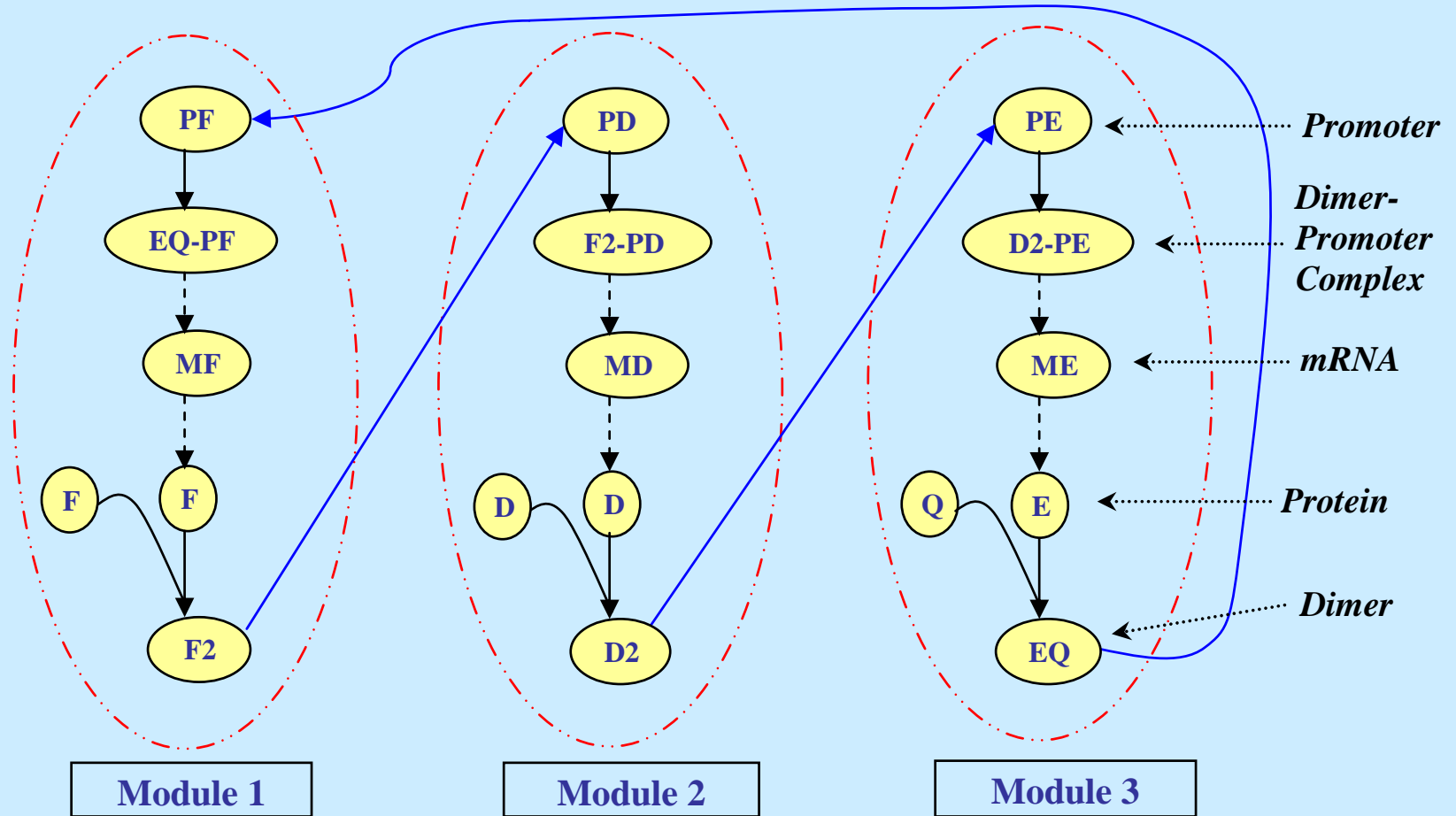
Raf-P MEK-PP ERK-PP

Raf-P	-1	0.0	-1.1
MEK-PP	1.9	-1	-0.6
ERK-PP	0.0	2.0	-1

Kholodenko et al (2002) PNAS 99: 12841

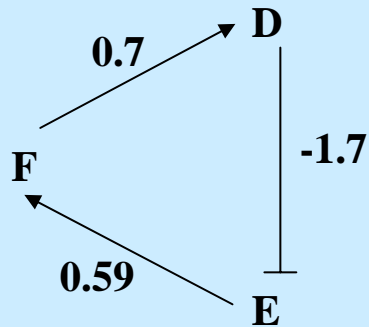
Testing in Silico: Unraveling the Wiring of a Gene Network

Modular description



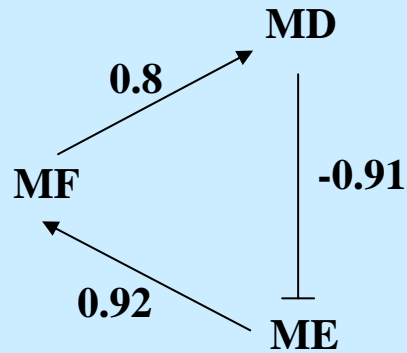
Inferring Interaction Maps

Protein interaction map



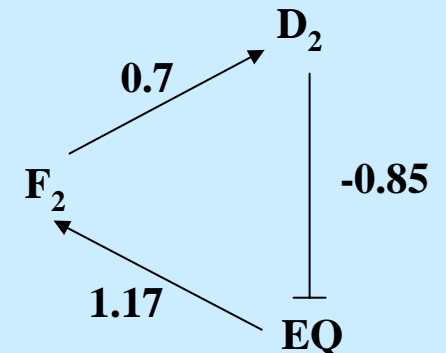
	D	E	F
D	-1.00	0.00	0.70
E	-1.70	-1.00	0.00
F	0.00	0.59	-1.00

mRNA interaction map



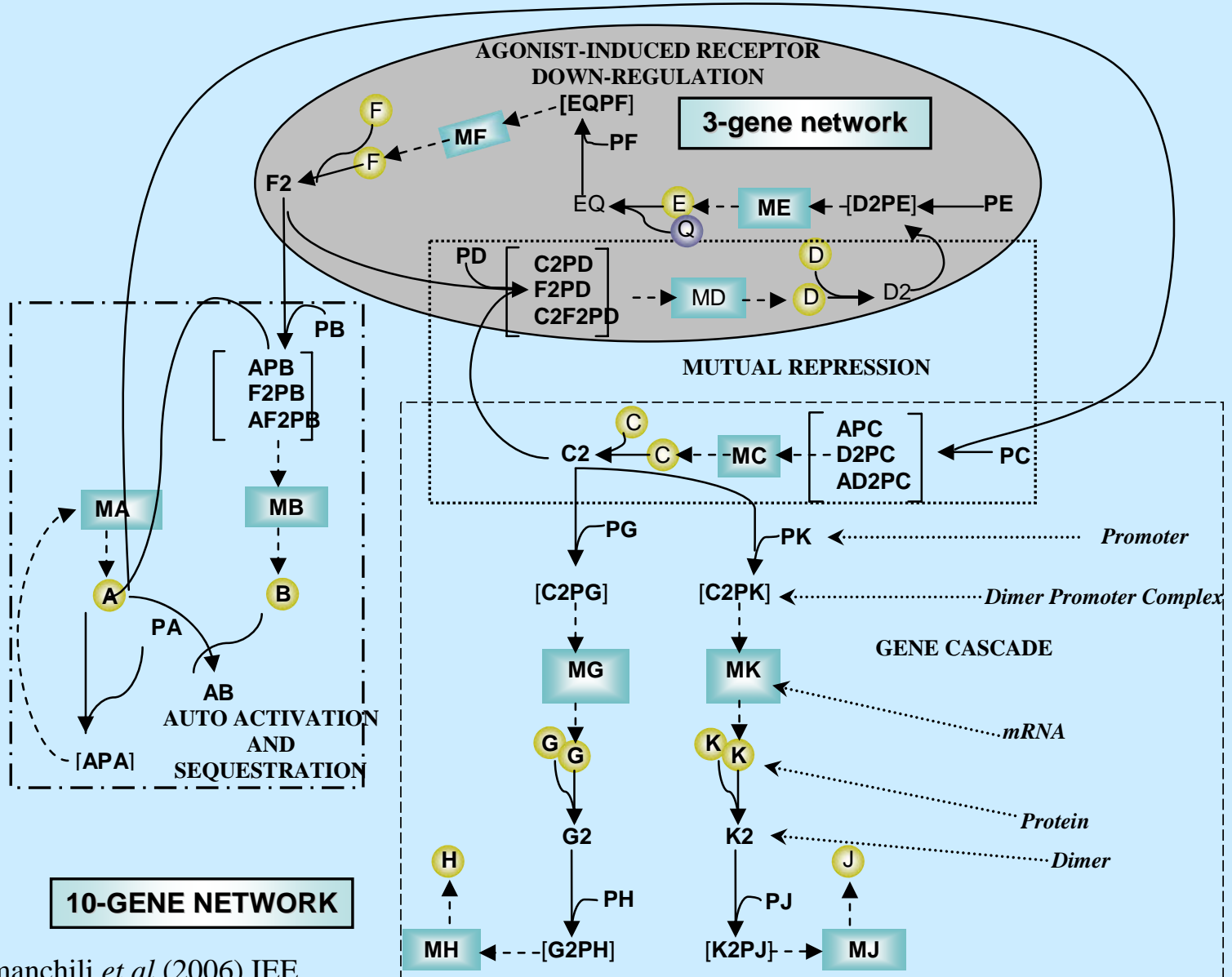
	MD	ME	MF
MD	-1.00	0.00	0.80
ME	-0.91	-1.00	0.00
MF	0.00	0.92	-1.00

Interaction Map for Dimers

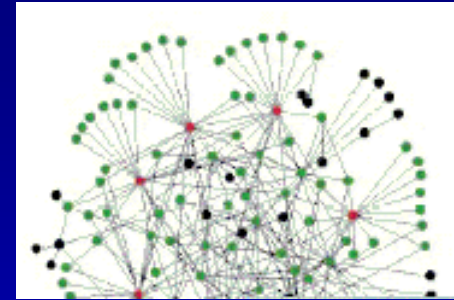
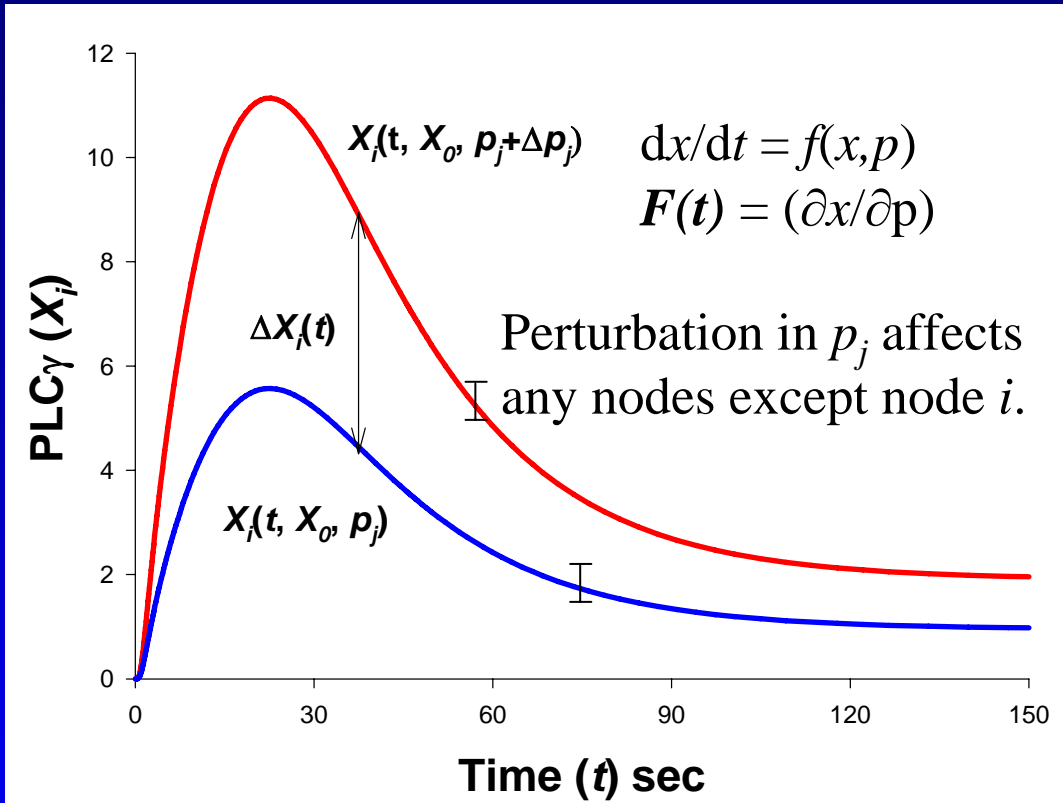


	D ₂	EQ	F ₂
D ₂	-1.00	0.00	0.70
EQ	-0.85	-1.00	0.00
F ₂	0.00	1.17	-1.00

Testing in Silico: Unraveling the Wiring of a Gene Network



Unraveling the Wiring Using Time Series Data



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,
 $G_j(t) = (\partial \Delta x_i / \partial t, \Delta x_i, \dots, \Delta x_n)$

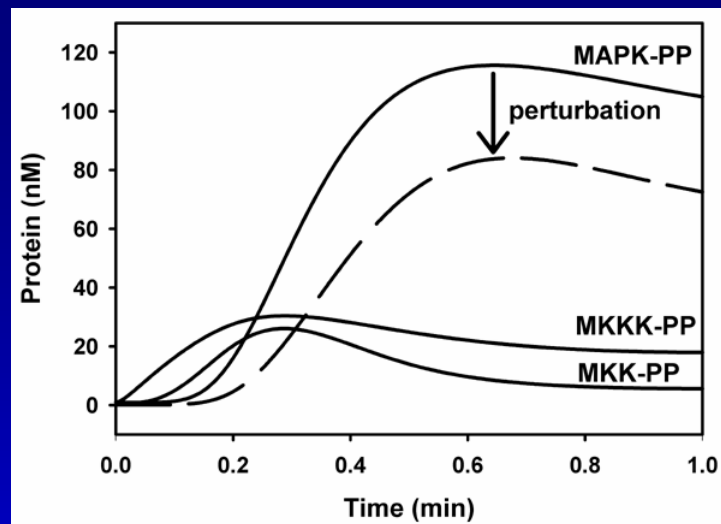
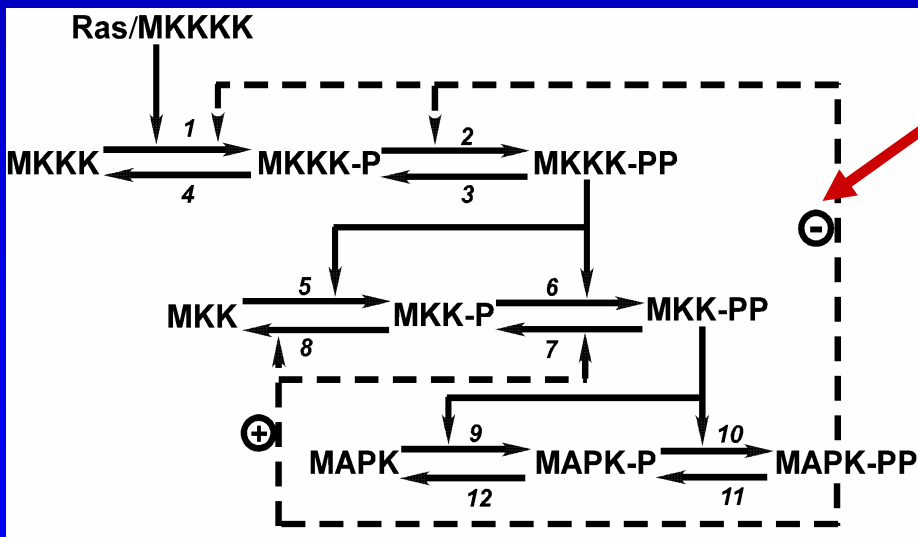
Orthogonality theorem: $(F_i(t), G_j(t)) = 0$
Sontag *et al.* (2004) *Bioinformatics*, 20, 1877.

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

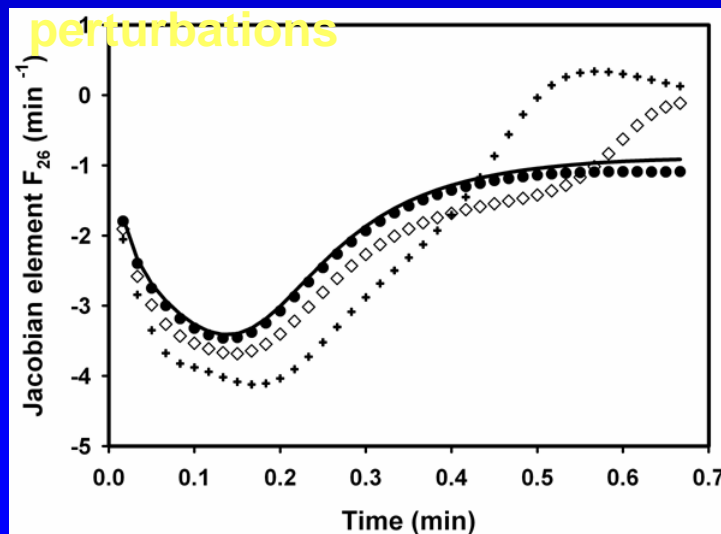
Inferring dynamic connections in MAPK pathway successfully

Transition of MAPK pathway from resting to stable activity state 

MAPK pathway kinetic diagram



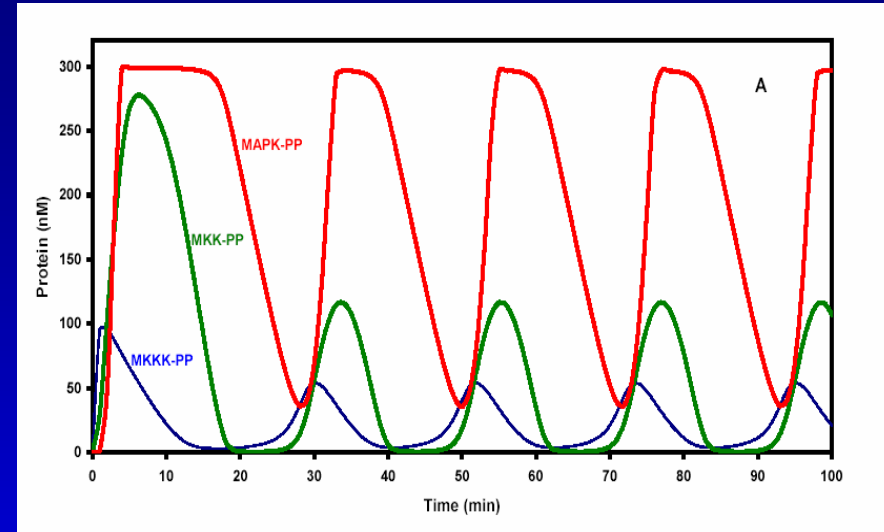
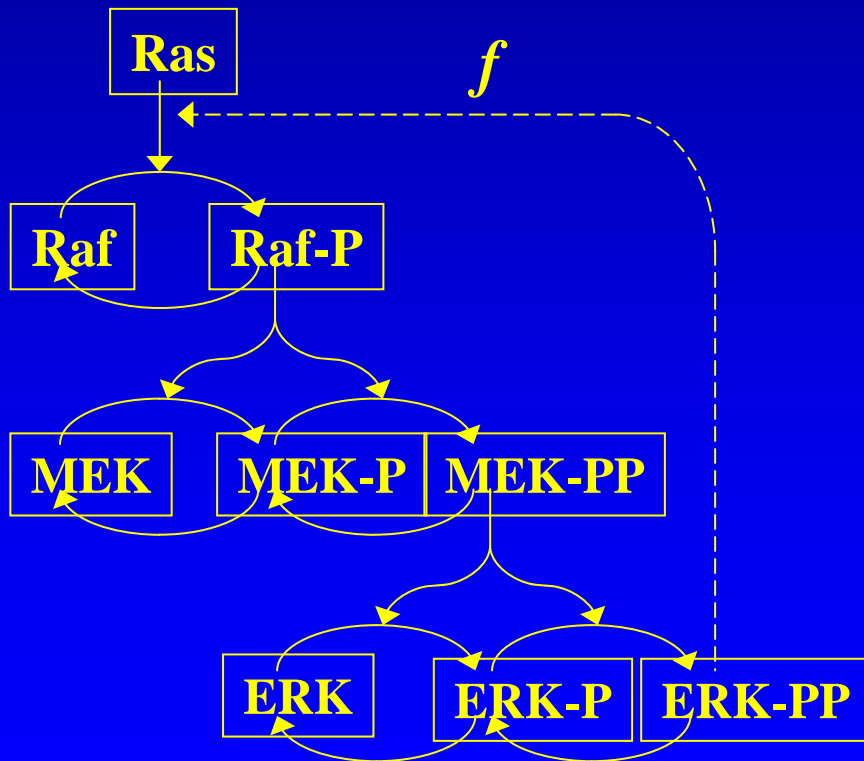
Deduced time-dependent strength of a negative feedback for 5, 25, and 50% perturbations



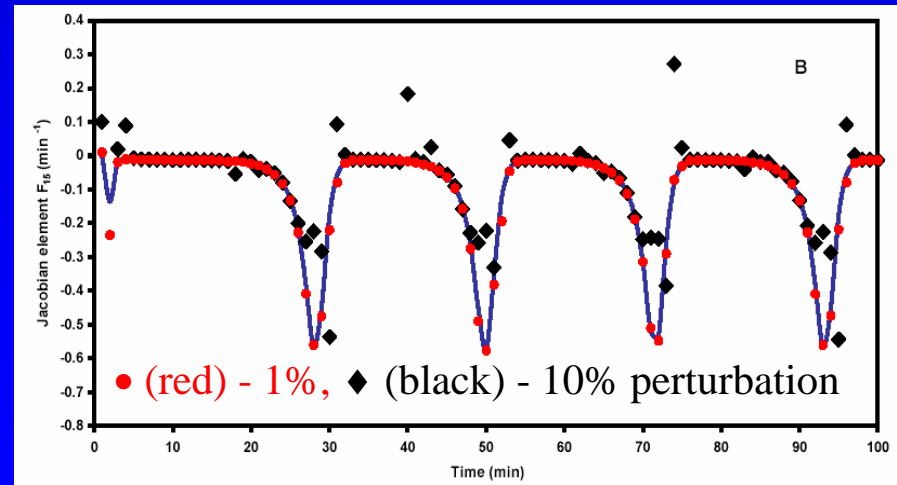
Sontag *et al.* (2004) *Bioinformatics* 20, 1877.

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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Japan)**

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