Integrating multiple signals into cellular decisions by networks of protein modification cycles

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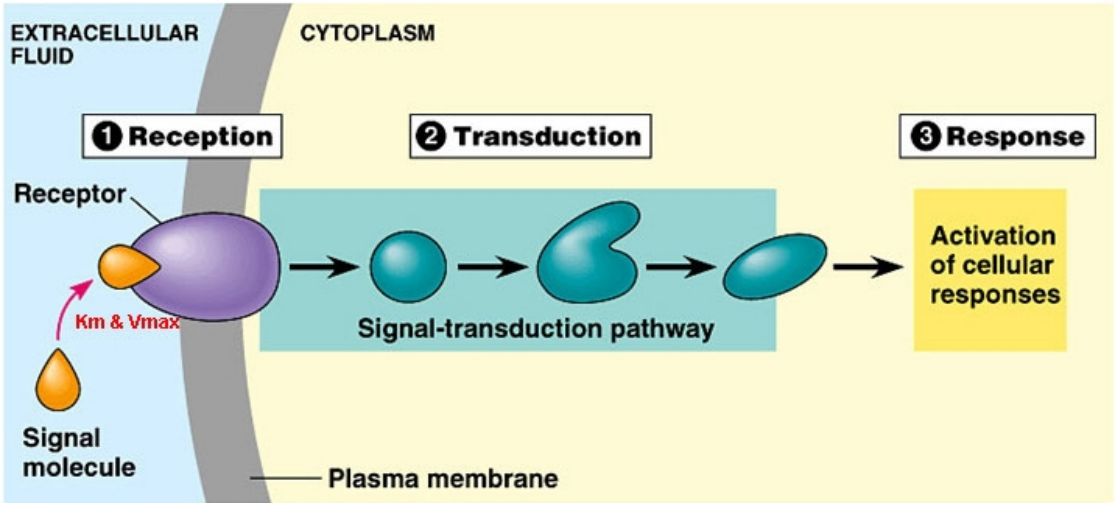
Outline

- Reversible protein modification reactions
- Phosphorylation
- Goldbeter-Koshland cycle
- Goldbeter-Koshland cycle extensions
- Multiple protein modifications
  - Two competing protein modification cycles
  - Saturated regime for two cycles
  - Saturated remine for multiple protein modifications
  - General interesting properties
- Conclusions

Reversible modifications of proteins

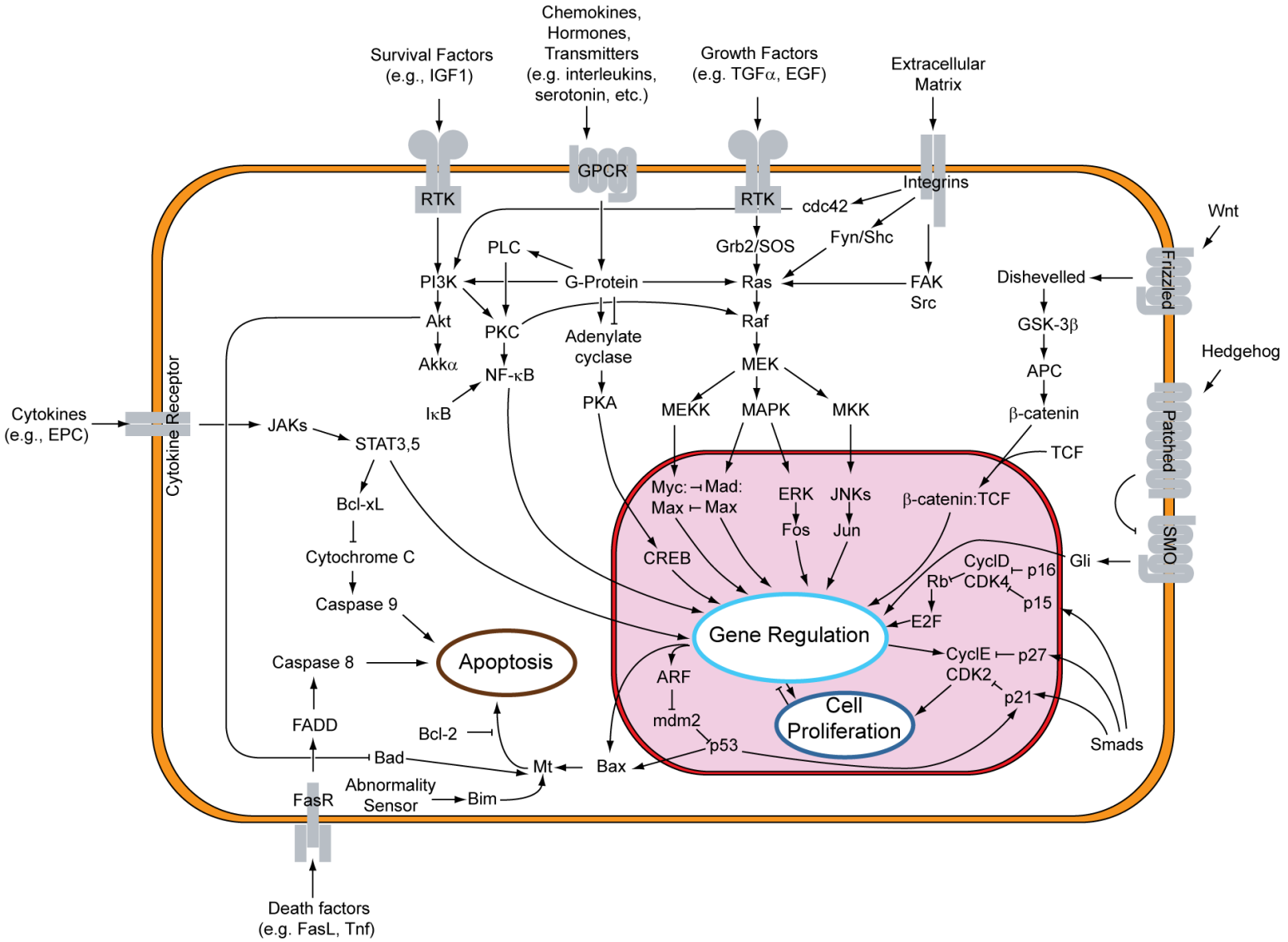
- Cell responses to internal and external stimuli are governed by protein interactions



- The activity and biological functions of proteins are modulated by (post-translational) enzymatic reversible modifications

- These modifications extend the range of functions of a protein by attaching to it other biochemical functional groups such as acetate (acetylation), various lipids (lipoyilation), phosphate (phosphorylation)...

Signal transduction pathway

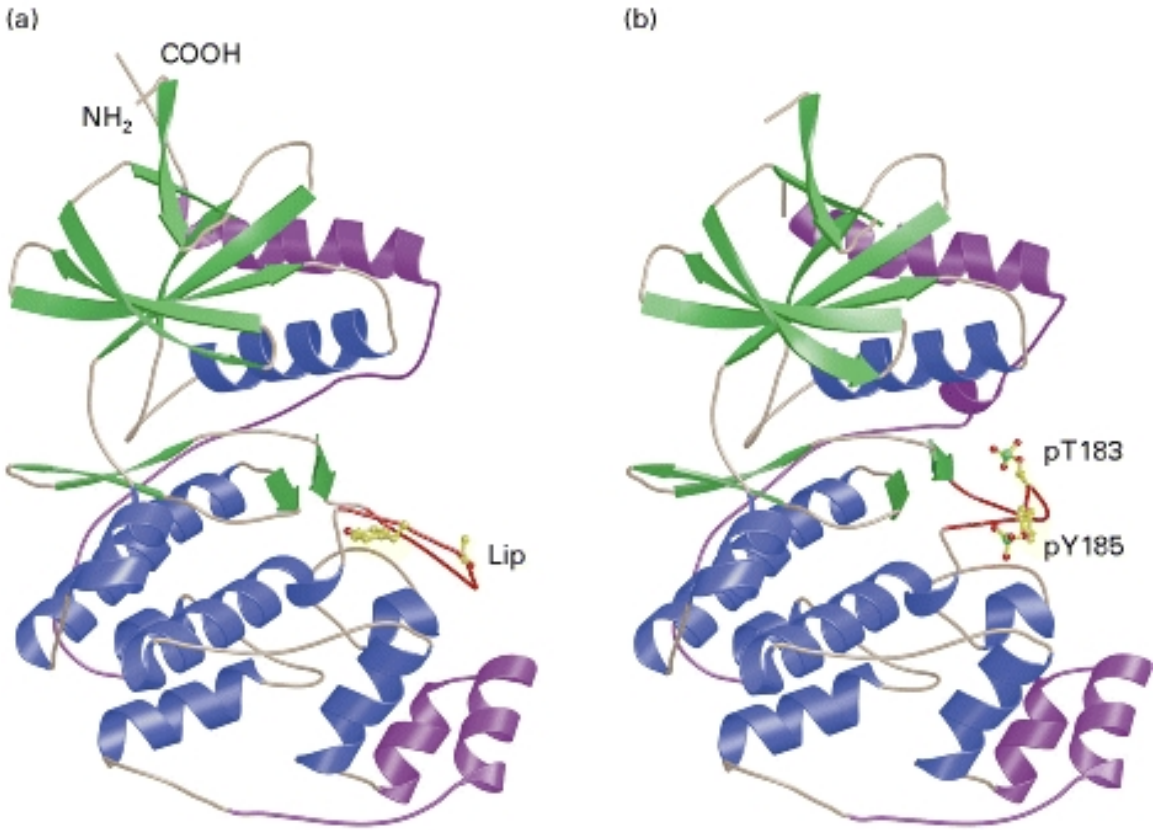


Signal transduction pathways involved in apoptosis

Phosphorylation

- Reversible phosphorylation of proteins is an important regulatory mechanism that occurs in both prokaryotic and eukaryotic organisms
- Up to 30% of all human proteins may be modified by phosphorylation, regulating the majority of cellular pathways, especially those involved in signal transduction
- Consists in the addition (catalyzed by a kinase enzime) of a phosphate (PO_4) group to a protein resulting in a conformational change in its structure
- Phosphorylation activates or deactivates many protein, by changing their enzyme activity, cellular location, or association with other proteins, causing or preventing several mechanisms
- For example, the phosphorylation of certain an amino acid residue can turn a hydrophobic portion of a protein into a polar and extremely hydrophilic molecule

Phosphorylation

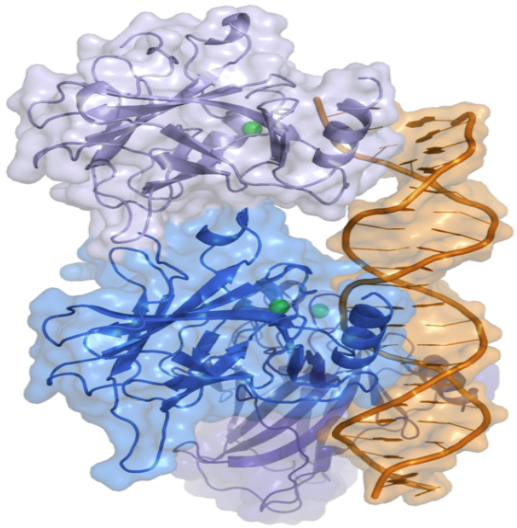


Structures of MAP kinase in its inactive, unphosphorylated form (a) and active, phosphorylated form (b)

Phosphorylation of MAP kinase by MEK at tyrosine 185 and threonine 183 residues leads to a marked conformational change in the phosphorylation lip (red). This change promotes dimerization of MAP kinase and binding of its substrates and certain proteins. [From Canagarajah et al. (1997), Cell 90:859]

Phosphorylation

- Phosphorylation often occurs on multiple distinct sites on a given protein
- For example, the p53 protein is heavily regulated and contains more than 18 different phosphorylation sites. Activation of p53 can lead to cell cycle arrest or apoptotic cell death



Tumor suppressor protein p53

Goldbeter-Koshland cycle

- A basic model of reversible protein modification is the Goldbeter-Koshland cycle [Goldbeter and Koshland (1981), PNAS 78:6840]

Proc. Natl. Acad. Sci. USA
Vol. 78, No. 11, pp. 6840–6844, November 1981
Biochemistry

An amplified sensitivity arising from covalent modification in biological systems

(protein modification/metabolic regulation/switch mechanism/enzyme cascades)

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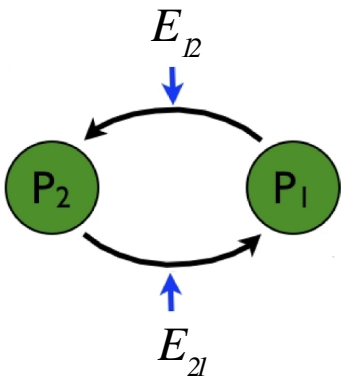
Contributed by Daniel E. Koshland, Jr., August 11, 1981

ABSTRACT **The transient and steady-state behavior of a reversible covalent modification system is examined. When the modifying enzymes operate outside the region of first-order kinetics, small percentage changes in the concentration of the effector controlling either of the modifying enzymes can give much larger percentage changes in the amount of modified protein. This amplification of the response to a stimulus can provide additional sensitivity in biological control, equivalent to that of allosteric proteins with high Hill coefficients.**

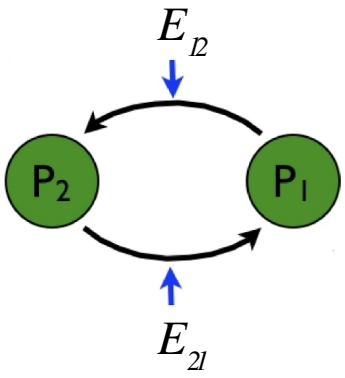
ture of covalent regulation was possible, if the differential equations could be solved analytically outside the first-order region.

This analysis has been achieved, and the results reveal that there is an added sensitivity inherent in covalent modification schemes when one or more of the converter enzymes operate in the "zero-order" region—i.e., region of saturation with respect to protein substrate. Thus there is a property of covalent systems that, in the absence of allosteric cooperativity and multiple inputs, can generate sensitivity equivalent to cooperative

- It describes reversible conversion of a protein between two forms (e.g. active or inactive) catalyzed by a pair of enzymes (E_{12} and E_{21})



Goldbeter-Koshland cycle

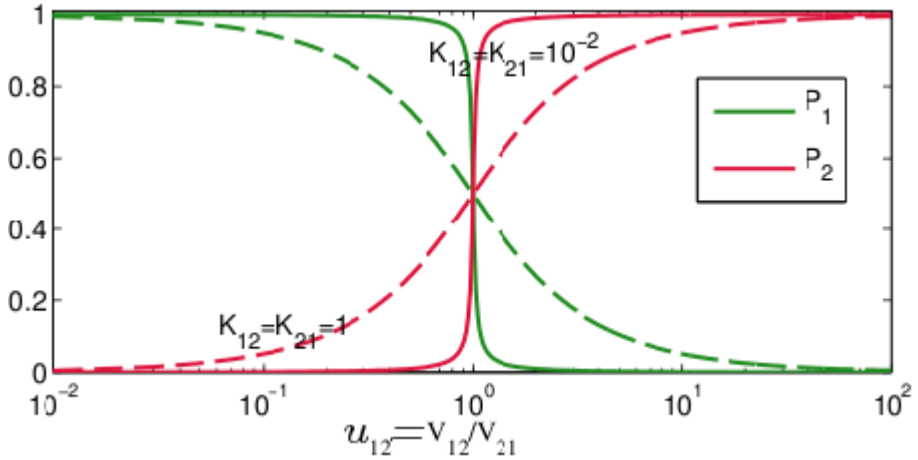


- Uniform concentration and total protein conserved (no production and degradation) $P_1+P_2=P_{tot}$

- Michaelis-Menten kinetics

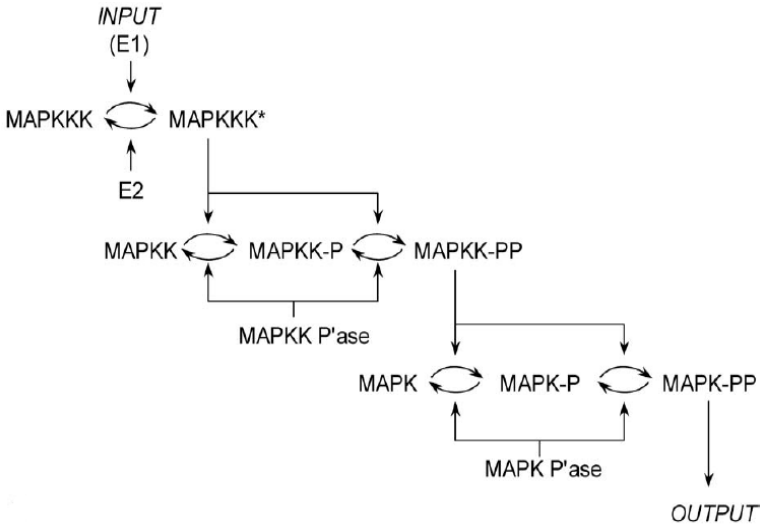
$$\frac{dP_1}{dt} = V_{21}(E_{21})\frac{P_2}{P_2 + K_{21}} - V_{12}(E_{12})\frac{P_1}{P_1 + K_{12}}$$

- Depending on the degree of saturation the system steady states may show ultrasensitivity (switch behaviour)



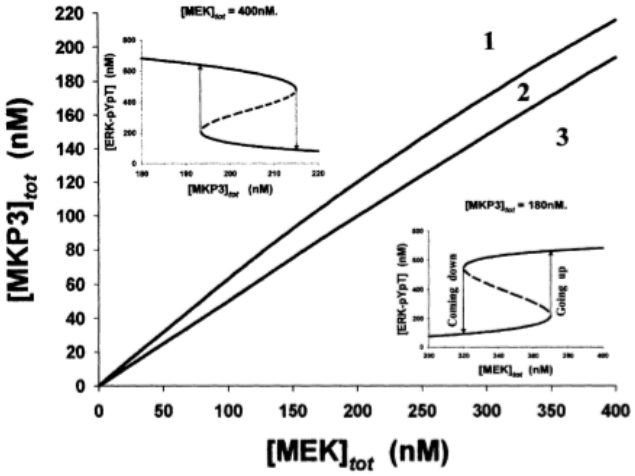
- The GK cycle is a general versatile component of pathway modules and has been found in multiple pathways involved in cycle progression, neuronal differentiation, ...

Goldbeter-Koshland cycle extensions



- Cascades of protein modification cycles (e.g.MAPK) can lead to ultrasensitivity [Huang and Ferrell (1996), PNAS 19:10078]

- Multisite phosphorylation (by the same enzyme) can lead to bistability [Markevich, Hoek, and Kholodenko (2004), J. Cell Biol. 164:353]



- General multisite phosphorylation system: reactions compete for the same enzyme pair → “unlimited” multi-stability, multi-bit memory [Thomson and Gunawardena (2009), Nature 460:274]

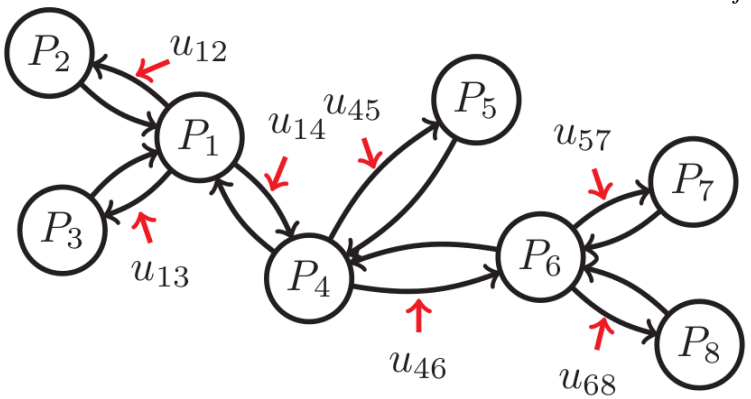
Multiple protein modifications

- Usually a protein can be modified in separate residues or by different modifications controlled by different enzimes (multiple input signals)
- Assumptions:
  - Each reaction converting P_i into P_j is reversible and catalyzed by a different enzyme E_{ij}
  - The total concentration is conserved
  - Forward and backward reaction are governed by Michaelis-Menten kinetics

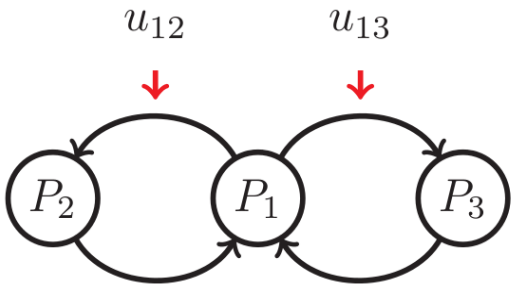
$$V_{ij}(E_{ij})\frac{P_i}{P_i+K_{ij}},\ V_{ji}(E_{ji})\frac{P_j}{P_j+K_{ji}}$$

  - The modification from a protein form to one another follows a ordered sequence, i.e. the network of protein modifications is a tree

- The steady state concentrations only depend on the activity ratios $u_{ij}=V_{ij}/V_{ji}$, $i<j$, between the enzyme activities

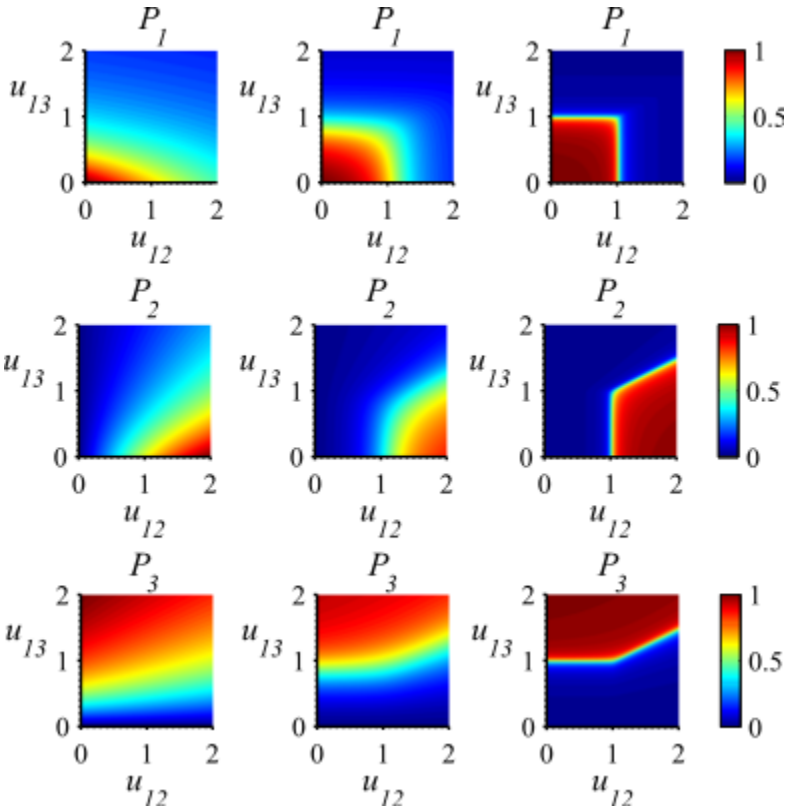


Two competing protein modification cycles



- Steady states are obtained by solving

$$\frac{u_{12}P_1}{P_1+K_{12}}=\frac{P_2}{P_2+K_{21}},\qquad \frac{u_{13}P_1}{P_1+K_{13}}=\frac{P_3}{P_3+K_{31}}$$



Linear regime

Saturated regime

– *Linear regime* (small amount of total protein concentration, $K_{ij} >> P_{tot}$):

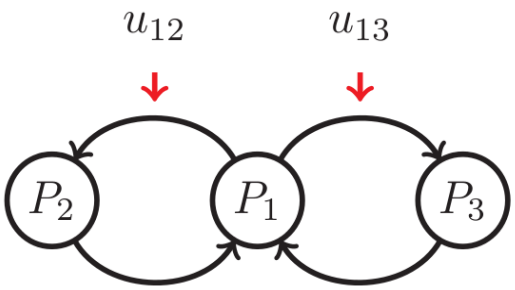
- the protein is distributed between the various forms proportionally to

$$(P_2/P_1, P_3/P_1) = (u_{12}K_{12}/K_{21},\ u_{13}K_{13}/K_{13})$$

– *Saturated regime* (large amount of total protein concentration, $K_{ij} << P_{tot}$):

- the space of activity ratios, u_{ij}, is split into *3* different regions. At each region all the protein is in only one form
- sharp transitions between protein forms when varying the activity ratio

Two cycles, saturated regime

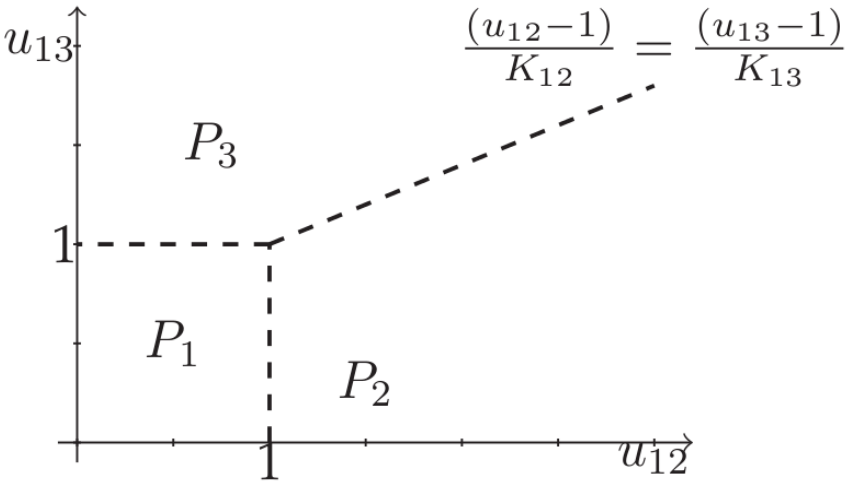


– P_1 dominant form:

$P_2 = K_{12} u_{12} / (1 - u_{12})$

$P_3 = K_{31} u_{13} / (1 - u_{13})$

$P_1 = P_{tot} - P_2 - P_3$



– P_2 dominant form:

$P_1 = K_{12} u_{12} / (1 - u_{12})$,

$P_3 = K_{31} K_{12} u_{13} / [K_{13}(u_{12} - 1) + K_{12}(1 - u_{13})]$

$P_1 = P_{tot} - P_1 - P_3$

– P_3 dominant form:

symmetric solution to P_2

- The positivity condition for these solutions partition the space parameter

Saturated multiple protein modifications

- Solutions can be found using a recursive procedure

1. We assume that P_i is saturated

$P_j=K_{ji}\,u_{ij}/(1-u_{ij})$ for each P_j (first neighbours) linked to P_i

2. This can be used recursively to evaluate the other nodes

For example, for the second neighbours, P_l, we have $P_l=K_{lj}P_j\,u_{jl}/[K_{jl}+P_j(1-u_{jl})]$

3. The positivity conditions give us a set of $N-1$ inequalities that determine the region where P_i is dominant

Repeating this procedure for each node of the network we obatin N inequalites that divide the space of activity ratios, u_{ij}, into N exclusive different regions. At each region all the protein is in only one form.

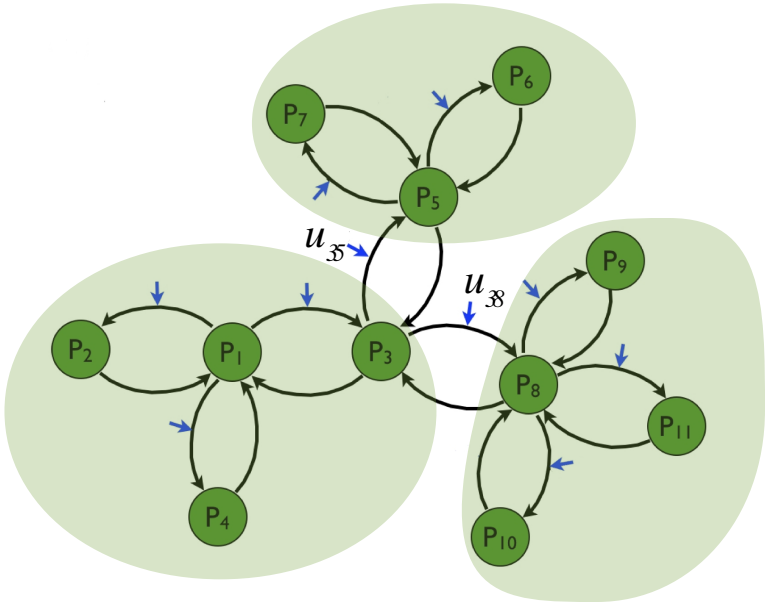
- The structure of the phase diagram can be determined from the topology of the reaction network, without specifying the values of the reaction parameters

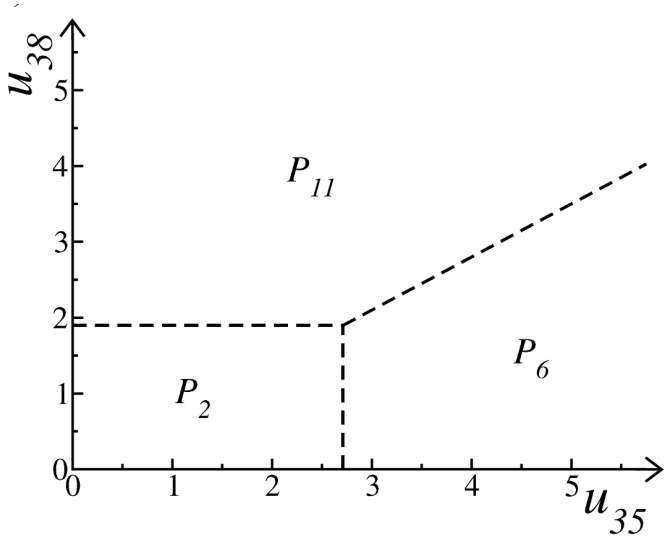
Interesting properties

- Varying only one activity ratio produces a single switch between the dominant protein forms of the two subnetworks obtained by removing the corresponding edge [thus, changing a selected activity ratio can trigger switching between two very different (far) forms]

- The dominant nodes within each subnetwork are determined by their own activity ratios

- The reduced phase diagram obtained by varying two activity ratios is qualitatively the same as for the two cycles system





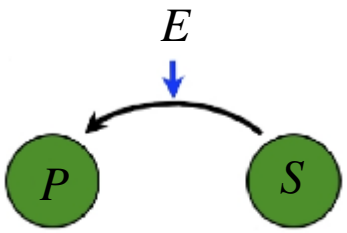
Conclusions

- We have analyzed a general model of reversible protein modifications catalyzed by a set of different ezymes that extends the GK cycle to any sequential protein modification system
- Far from saturation there is a gradual linear response an the protein concentration is distributes proportional to the relative strength of the activity ratios (input signal)
- In the saturated limit, only one form is possible depending on the activity ratios
- Thus, this systems can integrate multiple inputs into robust cellular decisions by switching between different protein forms (that can activate different cellular processes)
- We have presented a procedure to obtain the phase diagram and determine analytically the conditions for switching between different responses

Thanks for your attention!!!

This work: L. Cerone, J. Muñoz-García, and Z. Neufeld (2011), Submitted

Michaelis Menten kinetics



- The Michaelis–Menten equation describes the rates of irreversible enzymatic reactions by relating reaction rate to the concentration of the substrate

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} E + P$$

- The concentration of the substrate-bound enzyme ([ES]) changes much more slowly than those of the product ([P]) and substrate ([S])

$$\frac{d[ES]}{dt} = k_1[E][S] - [ES](k_{-1} + k_2) \stackrel{!}{=} 0$$

$$\frac{d[P]}{dt} = k_2[ES]$$

- The total enzyme concentration ([E]) does not change over time

$$[E]_0 = [E] + [ES] \stackrel{!}{=} \text{const.}$$

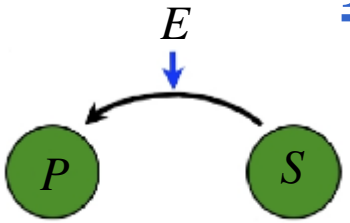
- Substituting this, we obtain an expression for [ES] which can use to find an expression for the rate of product formation

$$v_0 = \frac{v_{\max}[S]}{K_M + [S]}$$

v_{max} =maximum reaction rate = $k_2[E_0]$

K_M = inverse of enzyme affinity = $[k_{-1}+k_2]/k_1$

Michaelis Menten kinetics



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$0 = k_1[S]([E]_0 - [ES]) - [ES](k_{-1} + k_2)$$

$$k_1[S][E]_0 = k_1[S][ES] + [ES](k_{-1} + k_2)$$

$$[S][E]_0 = [S][ES] + [ES]\underbrace{\frac{(k_{-1} + k_2)}{k_1}}_{K_M}$$

$$[S][E]_0 = (K_M + [S])[ES]$$

$$[ES] = \frac{[S][E]_0}{K_M + [S]}$$

$$\frac{d[P]}{dt} = v_0 = k_2[ES] = \underbrace{k_2[E]_0}_{v_{\max}} \frac{[S]}{K_M + [S]}$$

$$v_0 = \frac{v_{\max}[S]}{K_M + [S]} \qquad (4)$$

$$\frac{1}{v_0} = \frac{K_M}{v_{\max}} \cdot \frac{1}{[S]} + \frac{1}{v_{\max}} \qquad (5)$$