IDENTIFICATION OF THE DESIGN PRINCIPLES FOR METABOLIC ENGINEERING

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Background and Scope

Signal transduction pathways relay extracellular stimuli from the plasma membrane to targets in the cytoplasm and nucleus, initiating diverse responses involving cell growth, mitogenesis, differentiation and stress responses in mammalian cells. The mitogen-activated protein kinase (MAPK) cascades are ubiquitous in eukaryotic signal transduction, and these pathways are conserved in cells from yeast to mammals. Metabolic engineering of mammalian cells requires the redesign of the steady-sate and the dynamic responses of signal transduction pathways. Therefore, understanding the design principles of these pathways is a key to success of metabolic engineering for cell culture development and drug target discovery.

In recent years, much effort has been devoted in the development of detailed kinetic models of MAPK network as tools for the rational design of metabolic engineering strategies. These models are mostly large systems of differential-algebraic equations (DAEs) and they link molecular (protein-protein, protein-DNA, and protein-RNA) interactions, gene expression and chemical reactions to cellular behavior. In this DAE description of signaling pathways, the differential equations express the mass-action kinetics, whereas the algebraic equations enforce conservation relations among the constituents. Moreover, these models typically involve a relatively large number of parameters, such as the rate constants and strength of protein-protein interactions, the values of which are not directly accessible in vivo and are subject to large uncertainty.

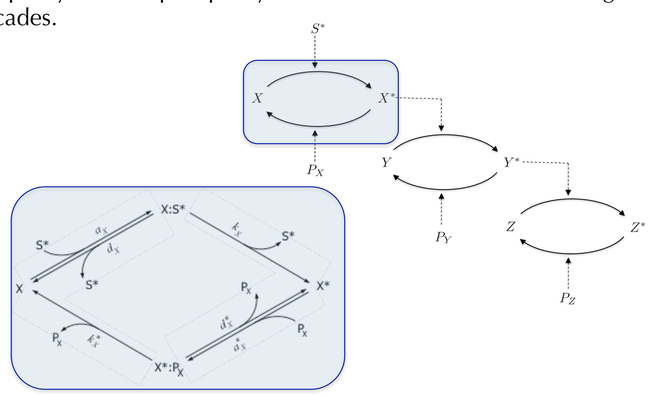
In this work, we investigate the application of dynamic optimization techniques to study the relationships between the biophysical and biochemical parameters and the functions of the MAPK cascades.

Modeling

Signaling Cascades

Simple biochemical networks consisting of the enzyme catalyzed chemical modification of a protein molecule and the reverse reaction catalyzed by a different enzyme (monocyclic cascades) are studied.

This basic unit is found repeatedly in multiple configurations, throughout a wide variety of biological pathways, including phosphorylation-dephosphorylation of MAP kinase signaling cascades.



Steady-state Model

Using mass-conservation principles and mass-action kinetic models, the steady-state concentrations of the foregoing species are described by differential-algebraic equations (DAEs):

$$0 = 1 - x - x^* - \rho^{S/X}\{x : s^*\} - \rho^{P_X/X}\{x^* : p_X\}$$

$$0 = 1 - s - s^* - \{x : s^*\}$$

$$0 = 1 - p_X - \{x^* : p_X\}$$

$$0 = \{x : s^*\} - \frac{x}{x + \tilde{K}_X}$$

$$0 = \{x^* : p_X\} - \frac{x^*}{x^* + \tilde{K}_X^*}$$

$$0 = \alpha_X\{x : s^*\} - \{x^* : p_X\}$$

- $\rho^{S/X}$ and $\rho^{P_X/X}$ denote the ratios of input signaling enzyme and of phosphatase to substrate concentration, respectively;
- $\tilde{K}_X$ and $\tilde{K}_X^*$ are dimensionless Michaelis-Menten constants:

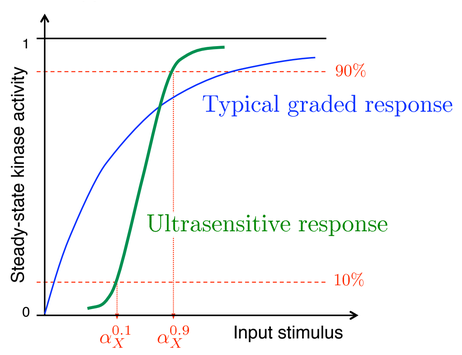
$$\tilde{K}_X^* := \frac{\tilde{d}_X^* + 1}{\tilde{a}_X^*} \qquad \tilde{K}_X := \frac{\tilde{d}_X + \tilde{k}_X}{\tilde{a}_X},$$

- α_X is the activity coefficient of the signaling cascade:

$$\alpha_X := \tilde{k}_X \frac{\rho^{S/X}}{\rho^{P_X/X}}$$

Design Objective: Ultrasensitivty

The variation of activated substrate with respect to input signal, at steady state, is an important response property of signaling cycles. A signaling cycle is said to exhibit *ultrasensitivity* when the response is significantly more sensitive to changes in input signal activity than enzymes that follow hyperbolic kinetics.

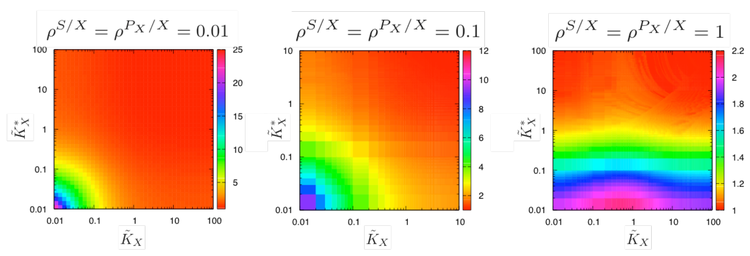


$$n_H := \frac{\ln(81)}{\ln(\alpha_X^{0.9}) - \ln(\alpha_X^{0.1})}$$

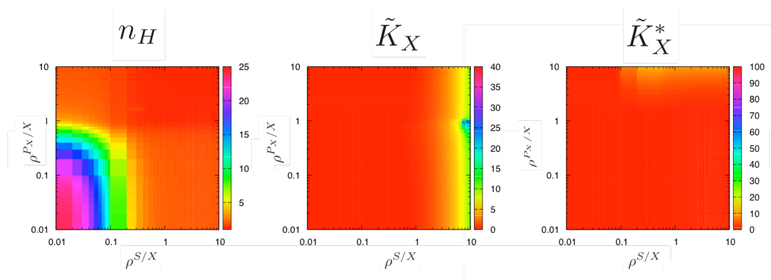
Results

Monocyclic Cascade

Optimal Hill coefficient n_H and Michaelis-Menten constants $\tilde{K}_X, \tilde{K}_X^*$, for various values of $\rho^{S/X}, \rho^{X/Y}$:



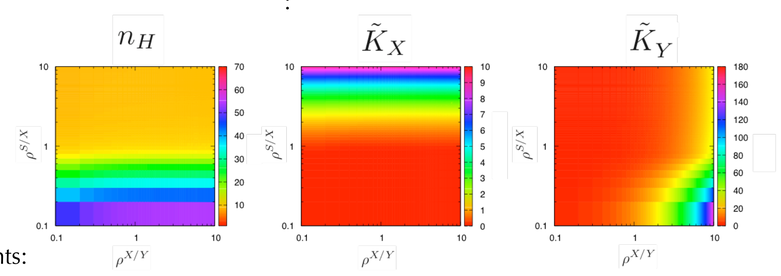
Landscape of Hill coefficient values for various Michaelis-Menten constants:



- ✓Ultrasensitivity can always be achieved for small values of concentration ratios
- ✓The maximal Hill coefficient values is always obtained for the least possible values of the Michaelis-Menten constants

Bicyclic Cascade

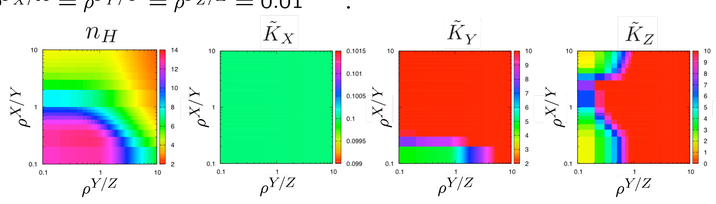
Optimal Hill coefficient and Michaelis-Menten kinase constants $\tilde{K}_X, \tilde{K}_Y$, for values $\rho^{S/X}, \rho^{X/Y} \in [0.01, 100]$; the Michaelis-Menten phosphatase constants are $\tilde{K}_X^* = \tilde{K}_Y^* = 0.01$ and $\rho^{P_X/X} = \rho^{P_Y/Y} = 0.01$:



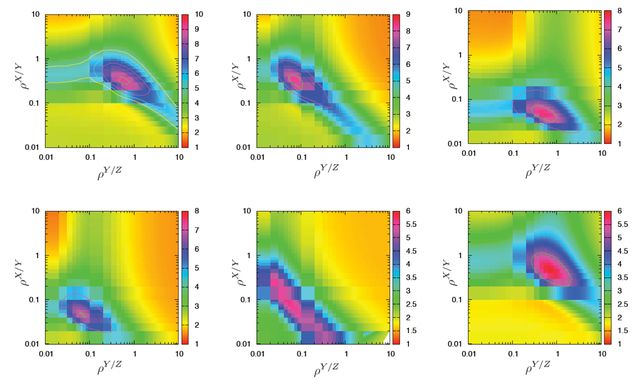
- ✓Much larger Hill coefficients are obtained than for monocyclic cascades, with the corresponding sets of parameters
- ✓The maximal Hill coefficient increases when the concentration ratio between the first and second levels is increased
- ✓The maximal Hill coefficient is attained when the first kinase is saturated, but not the second kinase
- ✓An optimal bicyclic cascade does not correspond to a series of optimal monocyclic cascades !

Tricyclic Cascade

Optimal Hill coefficient and Michaelis-Menten kinase constants $\tilde{K}_X, \tilde{K}_Y, \tilde{K}_Z$ for values of $\rho^{S/X} = 0.1$ $\rho^{X/Y}, \rho^{Y/Z} \in [0.01, 100]$; the Michaelis-Menten phosphatase constants are $\tilde{K}_X^* = \tilde{K}_Y^* = \tilde{K}_Z^* = 0.1$ and $\rho^{P_X/X} = \rho^{P_Y/Y} = \rho^{P_Z/Z} = 0.01$:



Optimal Hill coefficient for various Michaelis-Menten kinase constants:



- ✓Optimal ultrasensitivity is achieved if the first kinase is saturated by its target kinase, but not the subsequent two kinases.

Conclusions

➢ Large values of the Hill coefficient can be obtained in monocyclic cascades, when both the signaling-enzyme-to-substrate and phosphatase-to-substrate ratios are small. However, this condition alone is not sufficient to allow ultrasensitivity. It is also required that both the activation and deactivation reactions operate at enzyme saturation.

➢ The analysis of bicyclic cascades, with the corresponding conformation of parameters, shows that significantly larger values of the Hill coefficient can be obtained by adding an extra level. The Highest values are obtained when $\tilde{K}_X$ remains at its lower bound and $\tilde{K}_Y$ at its upper bound.

➢ The conditions under which ultrasensitivity is promoted in tricyclic cascades differ from the conditions promoting ultrasensitivity in monocyclic cascades. This demonstrates the importance of considering the actual network, rather than just the core module.

References

•A. Goldbeter, and D.E. KoshlandJr, "An amplified sensitivity arising from covalent modification in biological systems", Proc NatlAcadSciUSA, 78(11):6840-4, 1981.
•E. Klipp, R. Herwig, A. Kowald, C. Wierling, and H. Lehrach. "Systems Biology in Practice. Concepts, Implementations and Applications," Wiley-VCH, Weinheim (Germany), 2005.
•T.R. Reiger, and Vassily Hatzimanikatis, "Understanding the ultrasensitive responses of tricyclic cascade networks", To be submitted to: Proc NatlAcadSci USA
•C. Y. Huang, and J. E. Ferrel Jr. "Ultrasensitivity in the mitogen-activated protein kinase cascade," Proc. Natl. Acad. Sci. U.S.A., 93:10078–10083, 1996.
•R. Heinrich, B. G. Neel, and T. A. Rapoport. "Mathematical models of protein kinase signal transduction," Mol. Cell, 9:957–970, 2002.
•B. Chachuat, D. Bonvin, and V. Hatzimanikatis. "Analysis of signal transduction pathways through dynamic optimization – Application to MAP kinase cascades," AIChE Annual Meeting, Salt Lake City (UT), 2007.