Lab 11. Computational Modeling and Analysis of Biological network

1. Objective

- To learn how to construct and simulate computational models of biological networks in order to have a system-level understanding of the underlying mechanisms.
- To learning about mathematical modeling of biological interactions.
- To solving ordinary differential equation in MATLAB.
- To model and analyze dynamics of cell signaling pathways through simulations.

2. Background

2.1) Systematic study of biological systems

Cellular behavior is determined not by a single molecule but by many molecules that interact strongly with one another and form a complex network. Thus, phenotype of a cellular system is an emergent outcome of complicated interactions of molecules that work together in a highly organized manner. To understand and predict biological properties in a systematic way, we need to construct, simulate and analyze a mathematical model of the interactions.

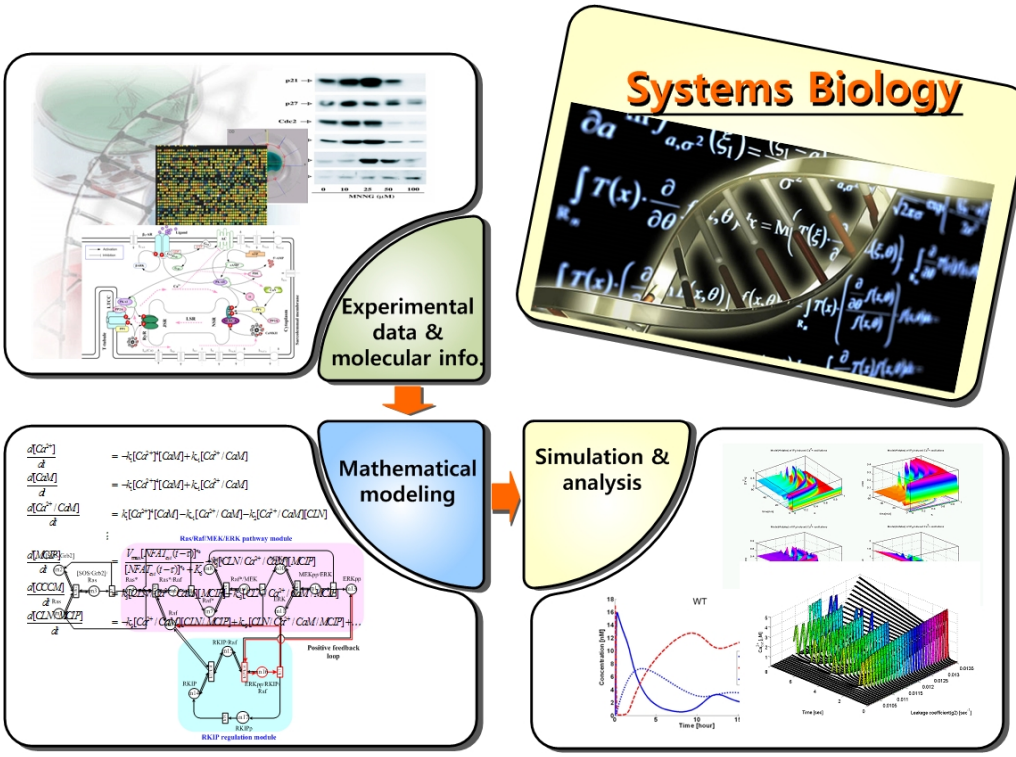


Figure 1. Workflow in Systems biology

2.2) Mathematical modeling of biological interactions

2.2.1) Dynamics of simple regulation

Let's begin by understanding the dynamics of a basic interaction, a single arrow in the biological network, which is referred to here as 'simple regulation':

This relationship may represent any activation regulation including phosphorylation(add phosphoryl group to X) or transcription between molecule X and Y. If X is produced by Y at rate β and degraded by itself at rate α, the change in the concentration of X is described by a dynamic equation:

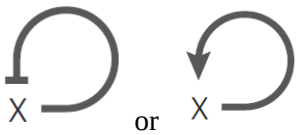
$$\frac{dX}{dt} = \beta - \alpha X$$

At steady state, the concentration of X converges to a constant concentration X_{st}, which can be found by solving for dX/dt = 0. This shows that the steady-state concentration is the ratio of the production and degradation rate:

$$X_{st} = \beta/\alpha$$

2.2.2) Positive and negative autoregulation

Biological networks typically involve positive or negative feedback loops. The simplest feedback loops are negative or positive autoregulation whereby a transcription factor represses or enhance its own transcription, respectively:



Negative autoregulation is described by $\frac{dX}{dt} = f(X) - \alpha X$ where its production rate $f(X)$ is a decresing Hill function of X:

$$f(X) = \frac{\beta}{1 + (X/K)^n}$$

Positive autoregulation is described by $\frac{dX}{dt} = \beta' + f(X) - \alpha X$ where its production rate $f(X)$ is a increasing Hill function of X:

$$f(X) = \frac{\beta}{1 + (K/X)^n}$$

Different regulation circuits give different dynamics though the steady state concentrations are the same (Fig. 2.).

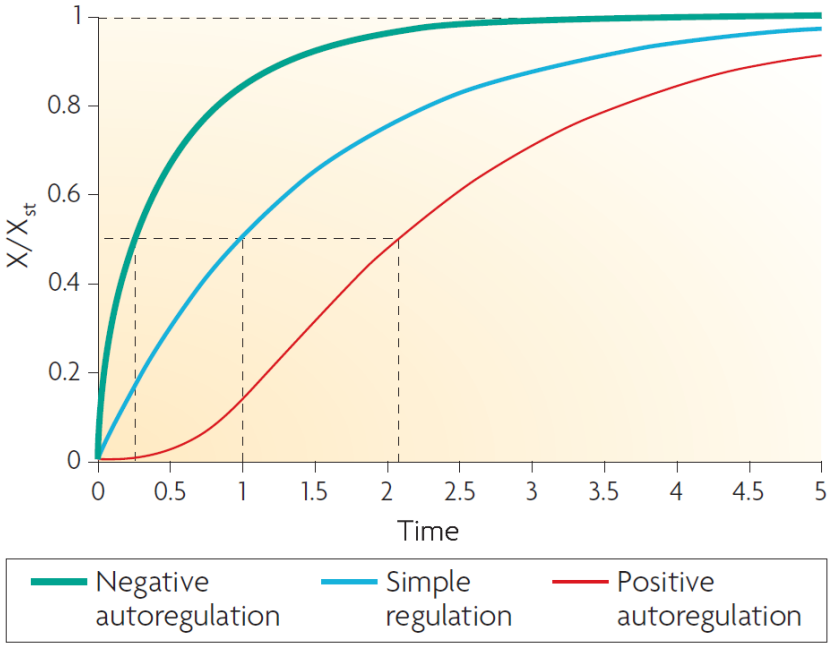


Figure 2. Simple regulation and autoregulation.

2.2.3) Solving ODE(ordinary differential equation) in MATLAB

The dynamic equations can be solved in MATLAB. MATLAB provides built-in ODE solvers such as ode15s or ode45. These ODE solvers execute a function file containing ODE equations, like as 'odefunc.m' in Fig. 3. Simulating the function file by some parameters and initial conditions for equations, we can get solved result values within a simulation time, like as 'main.m' in Fig. 3. Refer to help documentations about ODE solvers in MATLAB.

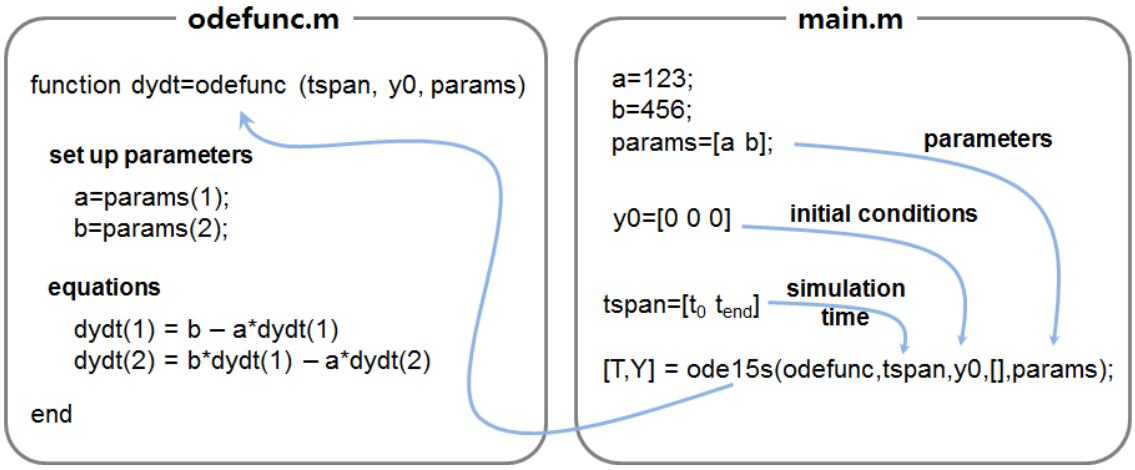


Figure 3. Using ODE solver in MATLAB.

2.2.4) The law of mass action

Let's consider interactions with biochemical molecules. The law of mass action is a mathematical model that explains and predicts behaviors of solutions in dynamic equilibrium. The reaction rate of biochemical molecules is proportional to the probability of a collision of the reactants. This probability is in turn proportional to the concentration of reactants to the power of the molecularity.

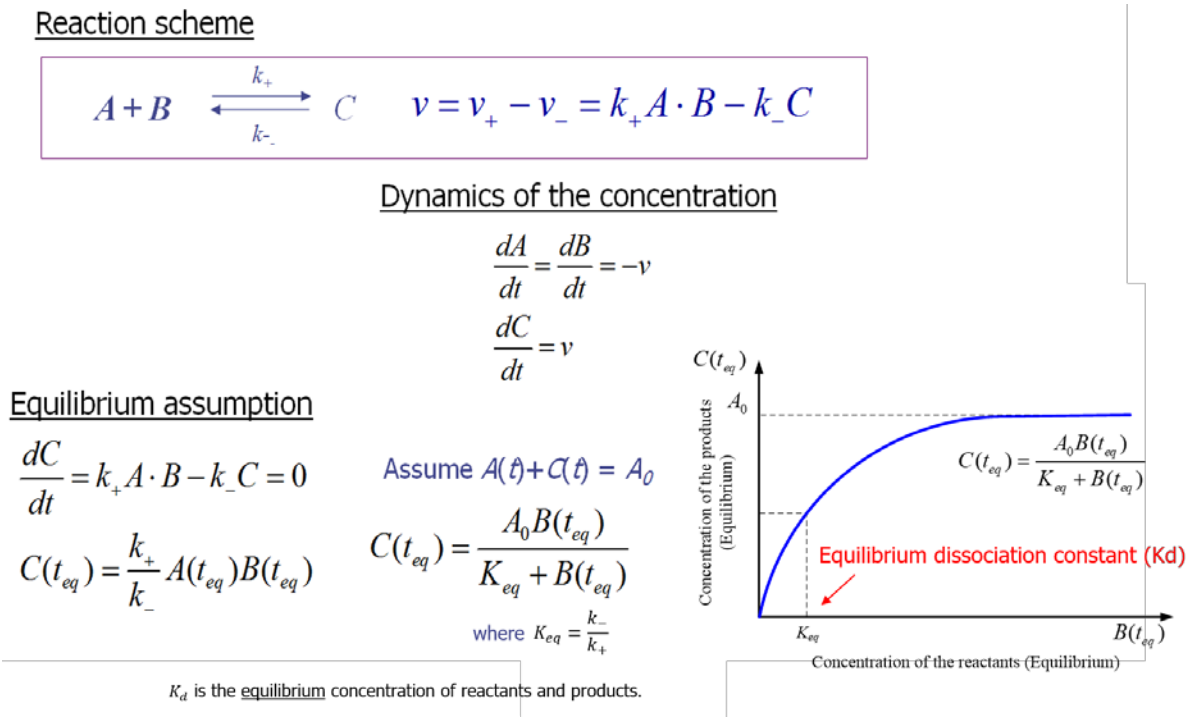
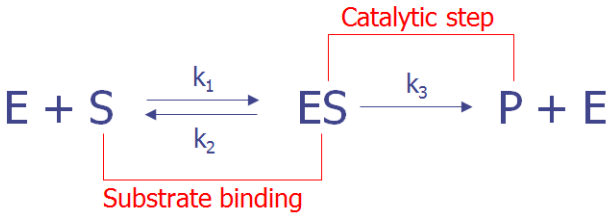


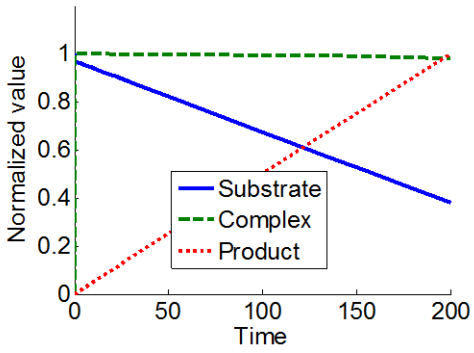
Figure 4. The law of mass action.

In Fig. 4. v is the net rate, v_+ the rate of the forward reaction, v_- the rate of the backward reaction, and k_+ and k_- are the respective proportionality factors (kinetic or rate constant).

2.2.5) Michaelis-Menten kinetics

Michaelis-Menten kinetics is a model of enzyme kinetics. In particular, the Michaelis-Menten equation describes the rates of irreversible enzymatic reactions by relating reaction rate to the concentration of the substrate.





Differential equations for the enzyme kinetics

$$\frac{ds}{dt} = -k_1 \cdot s \cdot e + k_2 \cdot c$$

$$\frac{de}{dt} = (k_2 + k_3) \cdot c - k_1 \cdot s \cdot e$$

$$\frac{dc}{dt} = k_1 \cdot s \cdot e - (k_2 + k_3) \cdot c$$

$$\frac{dp}{dt} = k_3 \cdot c$$

Conserved quantities

$$s + c + p = s_0$$

$$e + c = e_0$$

The equilibrium approximation

$$\frac{dc}{dt} = k_1 \cdot s \cdot e - (k_2 + k_3) \cdot c = 0 \quad \Rightarrow \quad k_1 \cdot s \cdot e = (k_2 + k_3) \cdot c$$

$$K_m = \frac{s \cdot e}{c} = \frac{s \cdot (e_0 - c)}{c} \leftarrow e+c=e_0$$

$$c = \frac{s \cdot e_0}{K_m + s} \quad \text{where} \quad K_m = (k_2 + k_3)/k_1 \quad \text{, which denotes the } \textit{Michaelsi-Menten} \text{ constant.}$$

$$\frac{dp}{dt} = k_3 \cdot c$$

Michaelis-Menten equation

$$\frac{dp}{dt} = \frac{k_3 \cdot e_0 \cdot s}{K_m + s} = \frac{V_{\max} \cdot s}{K_m + s}$$

Figure 5. Michaelis-Menten kinetics.

The enzyme *E* converts the substrate *S* into the product P through a two-step process. First *E* combines with *S* to form a complex *C (substrate binding step)* which then breaks down into the product *P* by releasing *E (catalytic step)*. In Fig. 5, s=[S], c=[C], e=[E], and p=[P], s_0 describes the total concentration of substrate, and e_0 the total concentration of enzyme. When enzymes are saturated with substrates (i.e., [S]>>[E]) we can assume that the complex is at an instantaneous equilibrium. $V_{max}=k_3 \cdot e_0$ is the maximum reaction velocity, attained when all the enzyme is complexed with the substrate. Above enzymatic reaction is simply represented by

$$S \xrightarrow{E} P.$$

2.3) Background of MAPK signaling pathway

The mitogen-activated protein kinase (MAPK) cascades are widely involved in eukaryotic signal transduction, and these pathways are conserved in cells from yeast to mammals. The MAPK cascades relay extracellular stimuli from the plasma membrane to targets in the cytoplasm and nucleus, initiating diverse responses involving cell growth, mitogenesis, differentiation and stress response in mammalian cells.

The MAPK pathway consists of three levels and described by the network as:

In this pathway, the activated kinase at each level phosphorylates the kinase at the next level down the cascade. Phosphorylation can occur on serine, threonine and tyrosine side chains(often called ‘residues’). Detailed interactions are illustrated in Figure 6. The MAPKs(bottom of Figure 6) are activated by the MKK (MAPK kinase) that phosphorylate MAPKs at two sites, conserved threonine and tyrosine residues(reaction 7, 8 in Figure. 6). Dephosphorylation of either residue is thought to inactivate MAPKs, and mutant kinases lacking either residue are almost inactive. At one level upstream, MKKs are themselves phosphorylated at serine and threonine residues by the MKKK (MAPK kinase kinase). The kinases of the first level, MKKKs are activated by several mechanisms involving phosphorylation at a tyrosine residue. At each cascade level, the protein phosphatases inactivate the corresponding kinases.

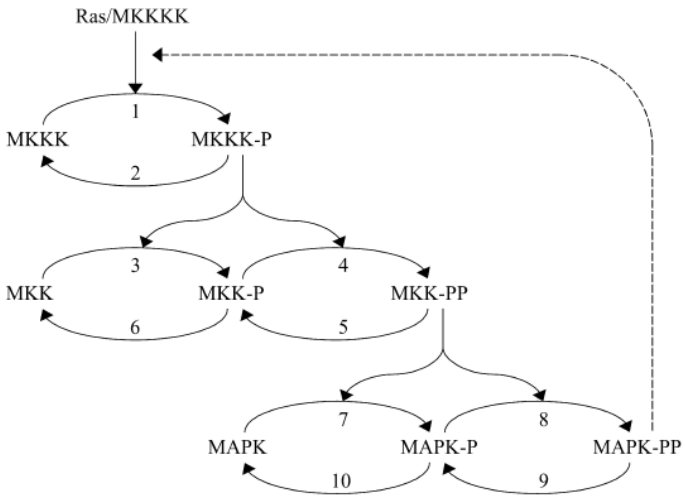


Figure 6. Schematic diagram of the MAPK cascade [1].

3. Prelab activities

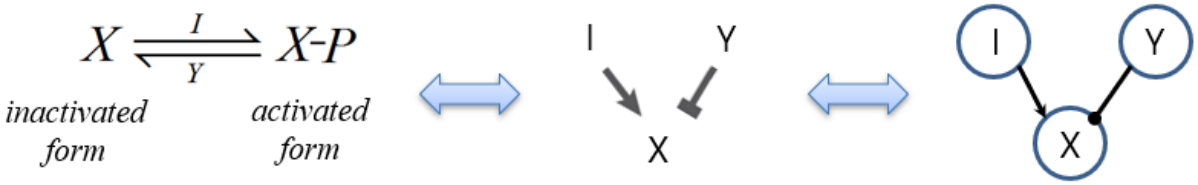
3.1) Read the main text for Lab. 11.

3.2) Read the paper.

Boris N. Kholodenko. (2000) Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *Eur. J. Biochem*. 267, 1583-1588.

3.3) Simulate phosphorylation and dephosphorylation

3.3.1) Derive the Michaelis-Menten equation of following interactions:



where I phosphorylate X and Y dephosphorylate X. Note that main text 2.2.4 explains the equation of one simple interaction but this case includes two interactions. Assume that total concentration of X and X-P is constant as 1.

3.3.2) Construct MATLAB codes for 3.3.1). Assume all the parameter values (including kinetic constant k and Michaelis Menten constant K) as 1.

3.3.3) Simulate and plot the graph of X-P using ODE solver.

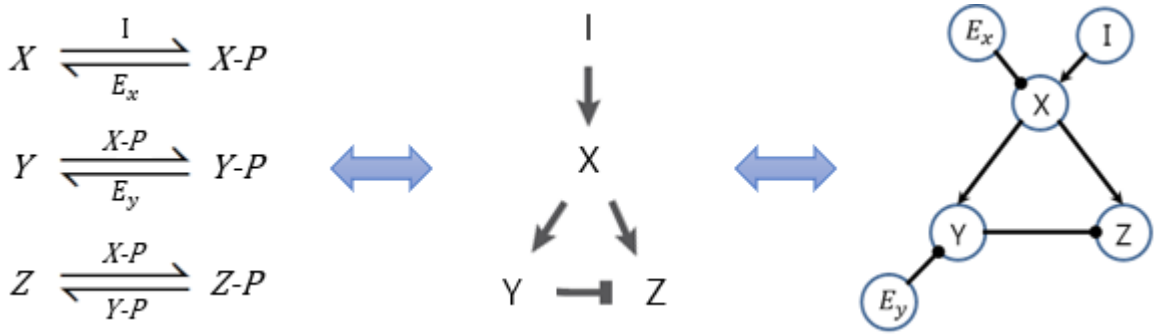
3.3.4) Change the values of I and Y and discuss about the effect to the steady-state of X-P.

4. Mainlab activities

- Construct and simulate three node feedback loop
- Unravel the functional role of cellular signaling pathways of MAPK cascades
- Construct and simulate a computational model of the MAPK cascades pathway
- Analyze the underlying dynamics and explain about your new insight obtained from this *in silico* experiment.

4.1) Modeling feedforward circuit

① Derive the Michaelis-Menten kinetics of following interactions:



where X directly activate Z but indirectly inactivate Z via Y. (I phosphorylate X and E_x dephosphorylate X-P. X-P phosphorylate Y and E_x dephosphorylate Y-P. X-P phosphorylate Z and Y-P dephosphorylate Z-P) This circuit is called by 'incoherent feedforward loop'(i-FFL).

② Simulate X-P, Y-P, Z-P over the time interval of 10s. Assume that X-P, Y-P, Z-P are initially absent and starts at t = 0. Set all the parameter values of Michaelis-Menten equation (including kinetic constant k and Michaelis Menten constant K) and concentration of enzymes (I, E_x, E_y) as 1. Total concentration of X, X-P are constant as 1. (Same for the (Y, Y-P), (Z, Z-P) respectively)

③ Plot three figures of the temporal profile of X-P, Y-P, Z-P using three different parameter value of Y-|Z.
(x-axis: time, y-axis: concentrations)

④ Compare the dynamics of incoherent feedforward loop and simple regulation.

Q. What is the function of incoherent feedforward loop?

4.2) Modeling MAPK signaling pathway

① Construct a computational model of the MAPK cascade pathway in Figure 6 using the rate equations and parameter values described in Table 1

② Simulate the dynamics of MAPK-PP, MKK-PP, MKKK-P over the time interval of 5000s. Assume that MAPK-PP, MKK-PP, MKKK-P are initially absent and starts at t = 0.

③ Plot the temporal profile of three nodes MAPK-PP, MKK-PP, and MKKK-P.
(x-axis: time, y-axis: concentrations)

Reaction number	Rate equation	Parameter values
1	$V_1 \cdot [MKKK]/((1 + ([MAPK\text{-}PP]/K_I)^n) \cdot (K_1 + [MKKK]))$	$V_1 = 2.5$; $n = 1$; $K_I = 9$; $K_1 = 10$;
2	$V_2 \cdot [MKKK\text{-}P]/(K_2 + [MKKK\text{-}P])$	$V_2 = 0.25$; $K_2 = 8$;
3	$k_3 \cdot [MKKK\text{-}P] \cdot [MKK]/(K_3 + [MKK])$	$k_3 = 0.025$; $K_3 = 15$;
4	$k_4 \cdot [MKKK\text{-}P] \cdot [MKK\text{-}P]/(K_4 + [MKK\text{-}P])$	$k_4 = 0.025$; $K_4 = 15$;
5	$V_5 \cdot [MKK\text{-}PP]/(K_5 + [MKK\text{-}PP])$	$V_5 = 0.75$; $K_5 = 15$;
6	$V_6 \cdot [MKK\text{-}P]/(K_6 + [MKK\text{-}P])$	$V_6 = 0.75$; $K_6 = 15$;
7	$k_7 \cdot [MKK\text{-}PP] \cdot [MAPK]/(K_7 + [MAPK])$	$k_7 = 0.025$; $K_7 = 15$;
8	$k_8 \cdot [MKK\text{-}PP] \cdot [MAPK\text{-}P]/(K_8 + [MAPK\text{-}P])$	$k_8 = 0.025$; $K_8 = 15$;
9	$V_9 \cdot [MAPK\text{-}PP]/(K_9 + [MAPK\text{-}PP])$	$V_9 = 0.5$; $K_9 = 15$;
10	$V_{10} \cdot [MAPK\text{-}P]/(K_{10} + [MAPK\text{-}P])$	$V_{10} = 0.5$; $K_{10} = 15$;
Total concentrations: $[MKKK]_{total} = 100$; $[MKK]_{total} = 300$; $[MAPK]_{total} = 300$		

Table 1. Rate equations and parameter values [1].

4.3) Ultra-sensitivity of MAPK signaling pathway

① Change input parameter V_1 values (0:0.1:3) and investigate the input (MKKK-P) – output (MAPK-PP) relationship. (simulation time : 5000ms)

② Obtain steady-state (after 5000ms) maximum concentrations of MAPK-PP, MKK-PP, and MKKK-P.

③ Plot three stimulus/response curves in one figure.
(x-axis: input parameter, y-axis: concentration)

④ Plot again after normalizing three graphs by their maximum values.
(x-axis: input parameter, y-axis: normalized concentration)

⑤ Compare the responses of the output nodes.

Q. What is the ultra-sensitivity? Explain with the graph of our simulations. What is the difference from amplification?

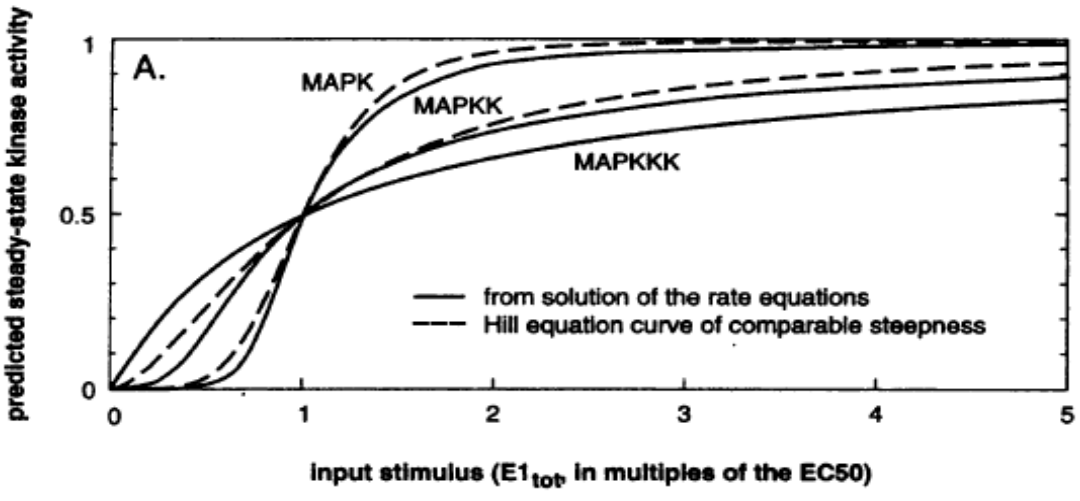


Figure 7. Stimulus/response curves for MAPK cascade components.

4.4) Sustained oscillations in the MAPK cascades

① Change feedback parameters K_I and investigate the input-output relationship.
Notice that $K_I > 0$.

② Plot two graphs about the max values and min values of steady state MAPK-PP according to the different parameter values of V_1 or K_I.
(x-axis: input parameter V_1 or K_I, y-axis: concentration)
* Plotting results in log scale with Log2X
(using parameter range like as "Log2X=?:?:?; x=2.^Log2X;")

③ Find out the range of negative feedback parameter value which results in such sustained oscillations of MAPK.

Q. How sustained oscillation behavior is occurred in MAPK signaling pathway? Pinpoint two key components.

Q. Discuss what we can learn from mathematical modeling and analysis of biological system approach compared to a conventional wet experimentation.

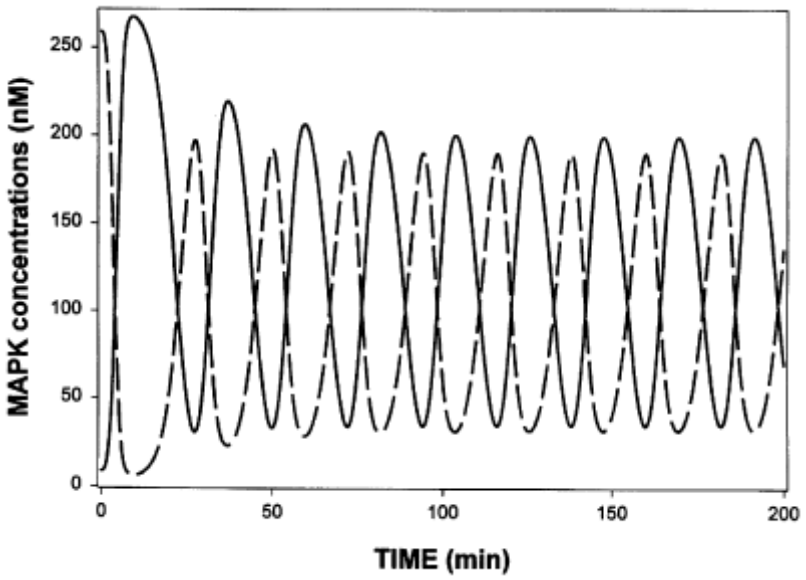


Figure 8. Oscillations in the MAPK concentrations.

5. Reference

[1] Boris N. Kholodenko. (2000) Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *Eur. J. Biochem*. 267, 1583-1588.

[2] Chi-Ying F. Huang., James E. Ferrell, JR. (1996) Ultrasensitivity in the mitogen-activated protein kinase cascade. *Proc. Natl. Acad. Sci. USA* Vol. 93, pp. 10078-10083.

[3] Alon, U. (2007). Network motifs: theory and experimental approaches. Nature Reviews Genetics, 8(6), 450-461.