

## 20.320 — Problem Set # 3

October 1<sup>st</sup>, 2010

*Due on October 8<sup>th</sup>, 2010 at 11:59am. No extensions, no electronic submissions.*

### General Instructions:

1. You are expected to state all your assumptions and provide step-by-step solutions to the numerical problems. Unless indicated otherwise, the computational problems may be solved using Python/MATLAB or hand-solved showing all calculations. Both the results of any calculations and the corresponding code must be printed and attached to the solutions. For ease of grading (and in order to receive partial credit), your code must be well organized and thoroughly commented, with meaningful variable names.
2. You will need to submit the solutions to each problem to a separate mail box, so please prepare your answers appropriately. Staple the pages for each question separately and make sure your name appears on each set of pages. (The problems will be sent to different graders, which should allow us to get the graded problem set back to you more quickly.)
3. Submit your completed problem set to the marked box mounted on the wall of the fourth floor hallway between buildings 8 and 16.
4. The problem sets are due at noon on Friday the week after they were issued. There will be no extensions of deadlines for any problem sets in 20.320. Late submissions will not be accepted.
5. Please review the information about acceptable forms of collaboration, which was provided on the first day of class and follow the guidelines carefully.

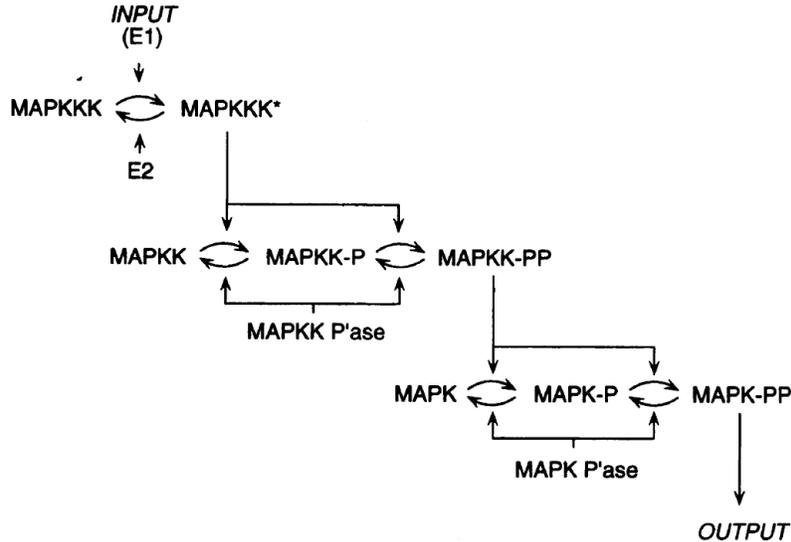
# 1 Enzyme Inhibition

A scientist is interested in developing an inhibitor for a liver enzyme whose product can be harmful. To do so he considers three strategies: i) competitive inhibition, ii) non-competitive inhibition and iii) uncompetitive inhibition. In all cases assume the following kinetic constants:  $k_1 = 10^7 \text{ M}^{-1}\text{s}^{-1}$ ,  $k_{-1} = 5 \cdot 10^{-4}\text{s}^{-1}$ ,  $k_2 = 0.2\text{s}^{-1}$ ,  $k_i = 10^5 \text{ M}^{-1}\text{s}^{-1}$ ,  $k_{-i} = 10^{-3} \text{ s}^{-1}$ ,  $[\text{E}]_0 = 10 \text{ nM}$ ,  $[\text{S}]_0 = 100 \text{ nM}$ .

- a) In uncompetitive inhibition, the inhibitor binds only to the enzyme-substrate complex in a reversible manner and impedes it from catalyzing the reaction. Draw the system including all relevant species.
- b) Give the governing differential equations that model this system.
- c) Give the product turnover rate formula for each of these inhibition mechanisms using QSSA. (The derivation to obtain product turnover rate for uncompetitive binding has been done in recitation, you do not need to repeat it).
- d) Explain what effects each of these different categories of inhibitor have on the product rate formation. Give a qualitative explanation for these effects.
- e) Using  $\text{plot}$  plot the product concentration as a function of time without inhibitors. On the same plot do the same for all three inhibition mechanisms with  $[\text{I}] = 500\text{nM}$ .
- f) For each type of inhibitor, give the concentration at which there will be 50% less product after 100, 1,000, 10,000, and 100,000 seconds. Describe qualitatively the trends you observe.

## 2 The Huang-Ferrell Model of the MAPK Cascade

The Huang-Ferrell model of the mitogen-activated protein kinase cascade captures key emergent features of its function. You have been provided with a `KinaseCascade.m` implementation of the model. In this problem, you will critically reflect on its assumptions, test its response to perturbations, and extend it to account for the effects of a drug candidate.



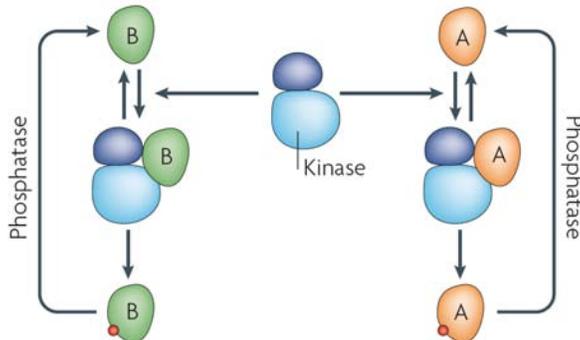
`KinaseCascade.m` contains the differential equation model of the cascade pictured above. `PS3_Huang_Ferrell.m` performs numerical integration using different sets of inputs. In Figure 1, it will plot the fractional activation of RAF, Erk, and MEK in response to an input.

- The cascade exhibits an ultrasensitive, cooperative response to stimulus. In what ways is this cooperativity analogous to that seen in haemoglobin binding to oxygen? In what ways is it different? State and *briefly* explain two similarities and two differences.
- Both in Huang and Ferrell's paper and in this implementation, Michaelis-Menten kinetics were assumed. What is their reason for this? Do you agree with it?
- Now consider the model in detail. What would happen if the cascade did not have any phosphatases in it? State and justify a hypothesis. Then, test it computationally. Did you refute your hypothesis or is it consistent with the results? Comment.
- To interfere with pathologically upregulated cell proliferation, you consider developing an inhibitor of the MAPK cascade. You wonder if an inhibitor which binds the inactive, unphosphorylated form of MEK and prevents its phosphorylation by Raf with an  $IC_{50}$  of  $2 \mu\text{M}$  would be an effective way of downregulating the cascade. Create a new model which extends the code you have been given to incorporate such inhibition. Evaluate its impact with the same large input stimulus used in figure 2 of the given code, and plot the steady-state (maximum) output of activated ERK for a range of inhibitor concentrations. How much inhibitor must you add for the level of activated ERK to be reduced by 90%?

**HINT:** You may approximate rate constants in the same way as has been done here and in the paper.

### 3 Kinase Specificity and Competition

To parametrize physico-chemical models of cellular pathways, we often characterize their binding and catalytic properties *in vitro*. This, however, does not faithfully reflect the cellular context. The presence of a large number of competing potential substrates is particularly difficult to account for; conservative estimates put the number of amino acid sites which can potentially be phosphorylated by a protein kinase in the tens of thousands in an average human cell. Here, you will computationally investigate the ability of a protein kinase to discriminate against one particular non-cognate substrate.



The cognate substrate A (orange) and the non-cognate substrate B (green) are both bound by the kinase with equal rates and phosphorylated with equal rate. However, B dissociates much more readily from the kinase than does A. Both phosphorylated forms pA and pB are dephosphorylated by a constitutive phosphatase.

- Write out chemical equations for all reactions.
- Provide ordinary differential equations for the time-evolution of the concentrations of all chemical species.
- Formulate all necessary conservation laws (mass balance equations).
- Substitute the conservation relations into the rate equations.
- In `kinase.py`, implement a function which encodes this modified system of differential equations. Parametrize it with the following values<sup>1</sup> for rate constants and initial conditions:

Parameter	Description	Value and units
E	Initial kinase concentration	$10^{-1}$ – $10^1 \mu\text{M}$
A	Initial concentration of A	$100 \mu\text{M}$ or $0 \mu\text{M}$
B	Initial concentration of B	$100 \mu\text{M}$ or $0 \mu\text{M}$
$k_1$	Association rate constant for A and E	$1 \cdot 10^6 \text{ s}^{-1}\text{M}^{-1}$
$k_{-1}$	Dissociation rate constant for E:A	$1 \cdot 10^6 \text{ s}^{-1}$
$k_2$	Catalytic rate constant for phosphorylation of A	$3 \text{ s}^{-1}$
$k_3$	Association rate constant for B and E	$1 \cdot 10^6 \text{ s}^{-1}\text{M}^{-1}$
$k_{-3}$	Dissociation rate constant for E:B	$30 \cdot 10^6 \text{ s}^{-1}$
$k_4$	Catalytic rate constant for phosphorylation of B	$3 \text{ s}^{-1}$
$k_p$	Dephosphorylation rate constant	$0.1 \text{ s}^{-1}$

<sup>1</sup>The parameter values are mostly physiologically reasonable; however, some of the rate constants have been chosen to better illustrate a particular point.

For a range of kinase concentrations from  $10^{-1}$ – $10^1$   $\mu\text{M}$ , plot (on the same graph) the phosphorylated *steady-state* fractions of substrate A and of substrate B as a function of kinase concentration with no competition (only one substrate present at a time). Do you think the kinase will be able to discriminate between these two substrates *in vivo*?

**HINT:** As units, use  $\mu\text{M}$  for concentrations and  $10^6 \text{ M}^{-1}$  for inverse concentrations. The ODE solvers in \_\_\_\_\_ will encounter difficulties if you stay in standard SI units because the quantities they see will differ by too many orders of magnitude.

- f) Now repeat your analysis with competition (both substrates present at once). How does the result differ — do you see surprising features?
- g) The interaction between the substrates here is conceptually analogous to that between a substrate and an inhibitor. What type of inhibition does it most resemble?
- h) While numerical integration is a powerful tool to predict the time-evolution of a system, it often does not immediately clarify the parameter dependence of the system's behavior. Based on the above analogy and your knowledge of Michaelis-Menten-type rate laws for enzyme inhibition, how do you think the presence of substrate A alters the kinetics of phosphorylation of substrate B? In your answer, clearly state which parameters remain unchanged and which increase or decrease as the concentration of A is increased (assuming a *constant* concentration of the kinase).

MIT OpenCourseWare  
<http://ocw.mit.edu>

20.320 Analysis of Biomolecular and Cellular Systems  
Fall 2012

For information about citing these materials or our Terms of Use, visit: <http://ocw.mit.edu/terms>.