# 20.320 — Problem Set # 4

October 15th, 2010

*Due on October 22nd, 2010 at 11:59am. No extensions will be granted.*

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. Staples 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 Targeted EGFR downregulation

*This problem is inspired from J. Spangler et al., PNAS 2010, all the information needed to solve the problem has been given here, you do not need to read the paper.*

Spangler *et al.* demonstrate in this paper the ability to downregulate EGFR surface expression by using a combination of two non-competitive antibodies targeting the extracellular domain 3 of EGFR. By using two non-competitive antibodies, the authors hypothesize the formation of oligomeric structures that have different transport kinetics. Here we will explore a simplified version of the experiment, we will consider only a single antibody that form a 1:2 complex with EGFR. We will first explore the system without perturbations and then how the steady state surface receptor concentration is affected by the antibody treatment and its binding kinetics. For this problem, we will not explore diffusional limitations and we will never include ligands to EGFR.

a) First let us consider the system without any ligands nor antibodies:

   i) Give a schematic and the differential equation system for this model. Use the following notation: receptor at the surface $R_S$ and internalized receptor $R_i$. The receptors are internalized with rate constant $k_e$, recycled back to the surface with $k_{rec}$ and degraded with $k_{deg}$. Newly synthesized receptors are brought to the surface with zero-th order constant $P_{syn}$. *2 points.*

   ii) What is the steady-state concentration of receptor at the cell surface? (show full development) *2 points.*

   iii) You are now given the kinetic rate constants listed in the table below. What value should $k_{deg}$ take for the surface receptor density remain constant at $10^5$ receptor per cell? Does that seem reasonable, *i.e.* too fast or too slow? *2 points.*

| Parameter | Value and units |
|---|---|
| $P_{syn}$ | $5 \cdot 10^2$ min$^{-1}$ |
| $k_e$ | $2 \cdot 10^{-2}$ min$^{-1}$ |
| $k_{rec}$ | $2 \cdot 10^{-2}$ min$^{-1}$ |

b) Now consider the system with addition of antibody. It has been shown that treatment with 225 alone does not enhance surface receptor downregulation, instead Spangler *et al.* use a combination of two non-competitive antibodies. Explain how the treatment with two non-competitive antibody differs from that of two competitive ones. *1 point.*

c) To simplify the problem, we will observe the consequences of increased receptor downregulation by treating with only one antibody. Eventhough this has been shown to be non effective, if we were to consider the full model, there would be too many species and this would get extremely complicated. You will now expand your initial model to take into account the antibody treatment. When antibody binds to the receptor it first forms a 1:1 complex ($C_{1,S}$) and then binds a second receptor to form a 1:2 complex ($C_{2,S}$). Also you can assume that the antibody does not dissociate while in the endosome and that only the receptor is recylced back to the surface. The $K_D$ for the single chain fragment has been reported to be of 50pM. You may assume an appropriate $k_{on}$ given the nature of the interaction. For the second binding event, you may assume the same dissociation rate constant. We have not covered bivalent binding in class, therefore for the second binding equilibrium assume $K_{D,2}(\#/\text{cell})$ $= 3.5 \cdot 10^9 \cdot K_D(M)$. For this problem use [A] = 20nM.

i) What simplifying assumption can you make so that you do not need to consider trafficking of the $C_{1,S}$ species? *3 points.*

ii) Assuming that $C_{2,S}$ are internalized ($C_{2,i}$) with $k_{e,2}$, recycled (as free receptor) and degraded with $k_{rec,2}$ and $k_{deg,2}$, give the new system of differential equations (becareful with your units!). *Hint: We recommend you to draw a schematic of the system to help you write the system of differential equations, this is however optional. 9 points.*

d) Experiments conducted by the authors have allowed them to determine the effect of the antibody treatment to be affecting the recycling rate, which they assume to be zero. Using the values given in the table below and considering an experiment where 1 million cells are incubated in a total volume of $100\mu L$, answer the following questions:

| Parameter | Value min$^{-1}$ |
|---|---|
| $k_{e,2}$ | $2 \cdot 10^{-2}$ |
| $k_{rec,2}$ | 0 |
| $k_{deg,2}$ | $k_{deg}$ |

i) Plot the surface concentration of receptor over a period of 40 hours post antibody stimulation. *8 points.*

ii) Describe the trends you observe. Why is the surface receptor density rising back up? *3 points.*

iii) Confirm your hypothesis on a plot. *2 points.*

iv) In an *in vitro* experiment, how can you minize this effect? *1 points.*

v) On a graph, plot the surface receptor concentration at 12h under antibody stimulation with 0.5, 1, 2, 5, 10, 20 and 50nM with and without the PFOA. On a separate graph plot the % error in the PFOA approximation with antibody concentration varying from 0.5 to 50nM. *Hint: you need to extract the surface receptor density at 12h post-stimulation for varying concentration using the full model and the ode solver in          and compare that to the value obtained using again the ODE solver but with the PFOA. 5 points.*

vi) Comment on your results. *2 points.*

e) Answer the following conceptual questions:

i) A young inexperimented scientist, who has not taken 20.320 at MIT, decides to develop an higher affinity binder to enhance the downregulation of EGFR receptor on the surface. After three months in the lab he obtains a binder with a $k_{off}$ 20 times smaller. He repeats the experiment conducted by the authors above and see no differential effect of receptor downregulation after 12h. Why should he have taken 20.320 ? *2 points.*

ii) The phenomemenon you have observed represents an important limitation. Now in the context of diffusion through a tumor spheroid, explain why increased binding affinity may not be advantageous. *2 points.*

# 2 Human growth factor receptor

*This exercise is based on the Jason M. Haugh (pronounced Hawk) paper discussed in class. You do not need to read the full paper to answer this problem, but it might help to look over the first three pages and the appendix.*
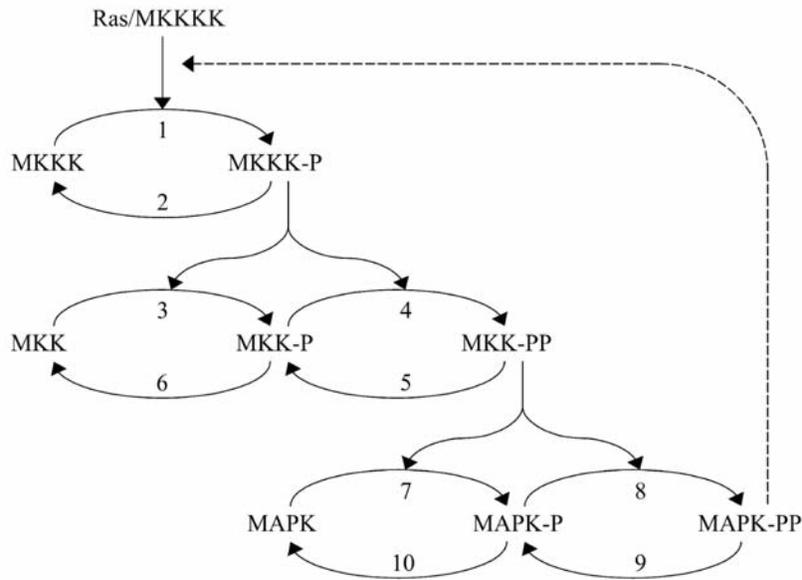
We will explore in this problem the importance of both binding sites in the hGH molecule for inducing dimerization and subsequent signaling.

a) Equations A1a-d describe fully the system. Give two assumptions used in establishing this model and discuss their validity. *4 points.*

b) Equation A5 in the paper describes the signal potency of the system. Is this a relevant mathematical form? Explain. *2 points.*

c) Why is the dose-dependent proliferation signal in the form of a bell-shaped curve? *2 points.*

d) Looking at figure 2a and 3, discuss the effects of different $k_{off}$ for site 1 or 2 on the dose-dependent proliferation signal. *4 points.*

e) Figure 6 shows the dimer fraction for various ligand concentration at different time points. What is the difference across the ligand concentration at 1 minute versus at steady-state? How can this be advantageous to the cell in terms of downstream signaling? *2 points.*

f) As it turns out, the biological model on which Haugh's mathematical model is based is incorrect[1]. What does this tell you on the utility and validity of this model? *2 points.*

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[1] Brown et al., 2005 - doi:10.1038/nsmb977

# 3 Negative feedback in the MAPK cascade: A closer look

In class, the effect of positive and negative feedback on the response of the MAPK cascade to various stimuli was discussed. Here, we will consider it in more detail for the case of negative feedback. You have been provided with a MATLAB implementation of the MAPK cascade as shown here, with negative feedback from Erk-pp. The model is less drastically simplified than the implementation you were given for problem set 3; for example, proper Michaelis-Menten terms were retained (see the code for details).



a) What functional form does the negative feedback take in the provided implementation? Is this justified? Why or why not? *7 points.*

b) Complete the provided code to ascertain that the model behaves as discussed in class (only 2 lines of code required). Plot the system response for the given initial concentrations and rate constants, setting the strength of the step stimulus `v1` to 10 nM s$^{-1}$ and the `Feedback` parameter to 0 or 100 nM$^{-1}$. *2 points.*

c) Now change the parameters. Reduce the initial concentrations of all kinases to one half their original initial vaues, then plot the cascade response against time to input `v1` at 2 nM s$^{-1}$ with `Feedback` strength of 0.15 nM$^{-1}$. What do you observe? *3 points.*

d) To explore this change in behavior, vary the input strength and plot the minimal and maximal Erk-pp levels over time.

   i) Follow the instructions in the comments in the code to let the system evolve for some time under each set of conditions, and then follow it for a second time interval. Plot the minimal and maximal Erk-pp concentrations from that second interval against the input. What do you see? *6 points.*

   ii) Find all critical input strength values and comment on what happens there. *3 points.*

   iii) In each distinct region along the x-axis, pick a typical input strength value. Plot a time course of Erk-pp concentration each such input value for two hours. How is the system responding to the stimulus? *4 points.*

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iv) In the middle section of the plot of extrema in Erk-pp concentration against input strength, what is [Erk-pp] doing *over time*? Indicate this in the extrema vs. input graph by adding trajectories and arrows by hand. *2 points.*

e) What biological role could this phenomenon play? How does it relate to the behavior discussed in class as resulting from negative feedback? *3 points.*

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