

## 20.320 — Problem Set # 4

October 15<sup>th</sup>, 2010

*Due on October 22<sup>nd</sup>, 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. 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.

<b>90 points total.</b>
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# 1 Targeted EGFR downregulation

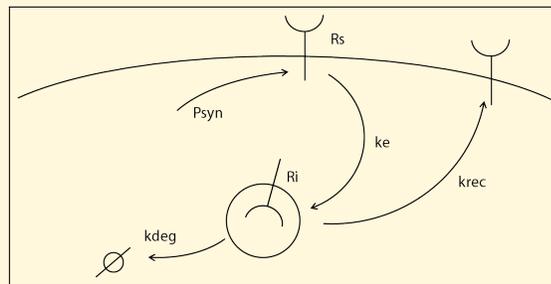
*This problem is inspired from J. Spangler et al., PNAS 2010, all the information needed to solve the problem has been given here, you do not need to read the paper.*

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 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.

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  $P_{syn}$ . Also you can assume that the ligand (nor the antibody for part b) dissociate while in the endosome and only the receptor is recycled back to the surface in its free form.

**Solution:**



$$\begin{aligned} \dot{R}_s &= P_{syn} + k_{rec}R_i - k_eR_s \\ \dot{R}_i &= k_eR_s - k_{rec}R_i - k_{deg}R_i \end{aligned}$$

*2 points*

- ii) What is the steady-state concentration of Receptor at the cell surface? (show full development)

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**Solution:**

By setting the differential equations to zero, we obtain:

$$\begin{aligned}R_{i,ss} &= \frac{k_e}{k_{\text{rec}} + k_{\text{deg}}} R_{s,ss} \\0 &= P_{\text{syn}} + k_{\text{rec}} R_{i,ss} - k_e R_{s,ss}\end{aligned}$$

Substituting the first into the latter and isolating  $R_s$  yields:

$$R_{s,ss} = \frac{P_{\text{syn}}}{k_e} \left( 1 + \frac{k_{\text{rec}}}{k_{\text{deg}}} \right)$$

*2 points*

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- iii) You are now given the kinetic rate constants listed in the table below. What value should  $k_{\text{deg}}$  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?

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

---

**Solution:**

Rearranging the equation given above to solve for  $k_{\text{deg}}$ :

$$\left( R_{s,ss} \frac{k_e}{P_{\text{syn}}} - 1 \right) k_{\text{rec}} = k_{\text{deg}} = 6 \cdot 10^{-2} \text{ min}^{-1}$$

Thus the characteristic time for receptor degradation is on the order of 15 minutes, which is in the right order of magnitude.

*Total 2 points: 1 point for correct expression, 1 point for comment.*

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- 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.

---

**Solution:**

Using two competitive antibodies, the highest order structure that can be created are dimers. However, using non-competitive antibodies, multimeric complexes can be formed.

*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 recycled 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  $K_{D,2}(\#/cell) = 3.5 \cdot 10^9 \cdot K_D(M)$ . 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?

**Solution:**

The binding event to the second receptor occurs much faster than endocytosis of the 1:1 complex.

*3 points*

- ii) Assuming that  $C_{2,S}$  are internalized ( $C_{2,i}$ ) with  $k_{e,2}$ , recycled and degraded with  $k_{rec,2}$  and  $k_{deg,2}$ , give the new system of differential equations (becareful with your units!).

**Solution:**

$$\begin{aligned} \dot{[A]} &= (-k_{on}[A]R_s + k_{off}C_{1,s}) \cdot \frac{C_e}{N_{AV}} \\ \dot{R}_s &= P_{syn} - 2k_{on}[A]R_s + k_{off}C_{1,s} - k_e R_s + k_{rec}R_i + 2k_{rec,2}C_{2,i} - k_{on,2}C_{1,s}R_s + 2k_{off,2}C_{2,s} \\ \dot{C}_{1,s} &= 2k_{on}[A]R_s - k_{off}C_{1,s} - k_{on,2}C_{1,s}R_s + 2k_{off,2}C_{2,s} \\ \dot{C}_{2,s} &= k_{on,2}C_{1,s}R_s - 2k_{off,2}C_{2,s} - k_{e,2}C_{2,s} \\ \dot{R}_i &= k_e R_s - k_{rec}R_i - k_{deg}R_i \\ \dot{C}_{2,i} &= k_{e,2}C_{2,s} - k_{rec,2}C_{2,i} - k_{deg,2}C_{2,i} \end{aligned}$$

Where  $N_{AV}$  is the avogadro number and  $C_e$  is the cell concentration.

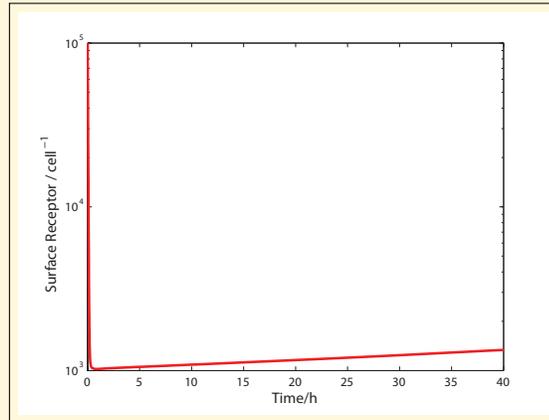
*Total 12 points*

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

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

**Solution:**



5 points

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

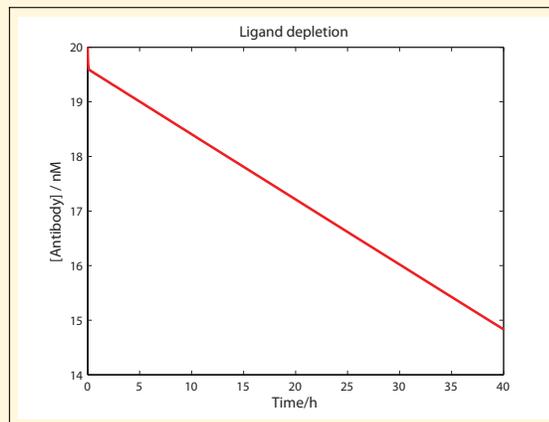
**Solution:**

First we observe receptor downregulation provoked by the antibody treatment. However, the receptor density is allowed to rise back up as the antibody is being depleted.

3 points

iii) Confirm your hypothesis on a plot.

**Solution:**



2 points

iv) In an *in vitro* experiment, how can you minimize this effect?

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**Solution:**

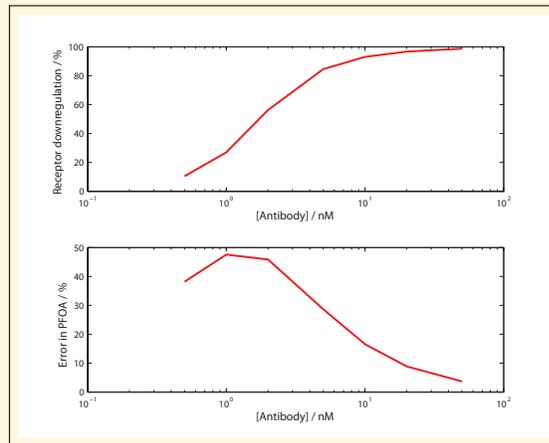
By increasing the volume, one can maintain the same antibody concentration, but obtain a much larger number of molecules.

*1 point*

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- 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 `antibody` and compare that to the value obtained using again the ODE solver but with the PFOA.*

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**Solution:**

*5 points*

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- vi) Comment on your results

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**Solution:**

At high concentration, ligand depletion is negligible. At low concentration, receptor downregulation is negligible. Therefore, only for intermediate concentration does the PFOA assumption not applicable, with error as high as almost 50%!

*2 points*

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- e) Answer the following conceptual questions:

- i) A young inexperienced 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  $k_{\text{off}}$  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 ?

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**Solution:**

□

The characteristic time for ligand dissociation is already much larger than for that of internalization. Therefore, in this example, further increase in binding affinity is useless.

*2 points*

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- ii) The phenomenon 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.

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**Solution:**

Strong binding can result in non-target cells depleting the antibody by endocytosis before the antibody has been able to reach the target cells.

*2 points*

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**44 points overall for problem 1.**

# MATLAB code for Problem 1

spanglersolution.m:

```
1 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
2 %                               Problem set #4 - Problem 1
3 %                               Targeted Receptor Downregulation
4 %
5 %                               Seymour de Picciotto
6 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
7 function spanglersolution()
8 clc;
9 clear all;
10
11 k = initk;
12 x0 = initx;
13 tspan = [0 60*60*40];
14 [T,Y] = ode15s(@(t,y)eqn_sys(t,y,k), tspan, x0);
15 %[T2,Y2] = ode15s(@(t,y)eqn_sys_constantA(t,y,k), tspan, [0.1 0 0 0]);
16
17 figure(1); % Question d ii)
18 nice_semilogy(T/3600, Y(:,2), 'Time/h', 'Surface Receptor / cell^{-1}', '', [1 0 0]);
19 figure(2); % Question d iii)
20 nice_plot(T/3600, Y(:,1), 'Time/h', '[Antibody] / nM', 'Ligand depletion', [1 0 0]);
21
22 % Receptor downregulation after 12h as a function of [Ab]
23 k = initk;
24 x0 = initx;
25 tspan = [0 60*60*12];
26
27 % Calculation: surface receptor density without Ab.
28 x0(1) = 0;
29 [T,Y] = ode15s(@(t,y)eqn_sys(t,y,k), tspan, x0);
30 Rs_12h_noA = Y(end,2);
31 % Calculation: surface receptor density with Ab.
32 A = [0.5 1 2 5 10 20 50];
33 Rs_12h = zeros(1,length(A));
34
35 for i = 1:length(A)
36     x0(1) = A(i);
37     [T,Y] = ode15s(@(t,y)eqn_sys(t,y,k), tspan, x0);
38     Rs_12h(i) = Y(end, 2);
39 end
40
41 % Calculation the surface receptor density with Ab + PFOA ([Ab] = constant).
42 Rs_12h_PFOA = zeros(1,length(A));
43 for i = 1:length(A)
44     x0(1) = A(i);
45     [T,Y] = ode15s(@(t,y)eqn_sys_PFOA(t,y,k), tspan, x0);
46     Rs_12h_PFOA(i) = Y(end, 2);
47 end
48
49 figure(3);
50 subplot(2,1,1);
51 nice_semilogx(A, 100*(Rs_12h_noA-Rs_12h)/Rs_12h_noA, '[Antibody] / nM',...
52 'Receptor downregulation / %', '', [1 0 0]);
53 subplot(2,1,2);
54 nice_semilogx(A, 100*(Rs_12h - Rs_12h_PFOA)./Rs_12h, '[Antibody] / nM',...
```

```

55     'Error in PFOA / %', '', [1 0 0]);
56
57 %-----%
58 %                               Functions
59 %-----%
60
61 function k = initk()
62 Psyn = 500/60; % s^-1
63 ke = 2e-2/60; % s^-1
64 krec = 2e-2/60; %s^-1
65 kdeg = 6e-2/60; % s^-1
66 ke2 = 2e-2/60; % s^-1
67 krec2 = 0; %s^-1
68 kdeg2 = 6e-2/60; %s^-1
69 kon = 1e5*1e-9; % nM^-1 s^-1
70 koff = 5e-6; % s^-1
71 kon2 = koff/(3.5e9*50e-12); % cell^-1
72 koff2 = koff; %s^-1
73 Ce = 1e10; % Cells*L^-1
74
75 k = [Psyn, ke, krec, kdeg, ke2, krec2, kdeg2, kon, koff, Ce, kon2, koff2];
76 end
77
78 function x = initx()
79     % x = [ A  Rs  C1s  C2s  Ri  C2i];
80     % ----x(1)-x(2)-x(3)-x(4)-x(5)--x(6)
81
82     % Starting Antigen concentration = 40nM
83     % Starting Number of Receptor on the surface = 100,000.
84     x = [20 100000 0 0 0 0];
85 end
86
87 function xdot = eqn.sys(t,x,k)
88 % x = [ A  Rs  C1s  C2s  Ri  C2i];
89 % ----x(1)-x(2)-x(3)-x(4)-x(5)--x(6)
90
91 % k = [Psyn, ke, krec, kdeg, ke2, krec2, kdeg2, kon, koff, Ce, kon2, koff2]
92 % ----k(1)--k(2)-k(3)-k(4)--k(5)--k(6)---k(7)--k(8)--k(9)-k(10)-k(11)- k(12)
93 Nav = 6.02e23*1e-9; % nmol^-1
94
95 xdot = [(-k(8)*x(1)*x(2) + k(9)*x(3))*k(10)/Nav; % dA/dt
96         k(1) + -2*k(8)*x(1)*x(2) + k(9)*x(3) - k(2)*x(2) + k(3)*x(5)...
97         + 2*k(6)*x(6) - k(11)*x(3)*x(2) + k(12)*x(4); % dRs/dt
98         2*k(8)*x(1)*x(2) - k(9)*x(3) - k(11)*x(3)*x(2) + 2*k(12)*x(4); % dC1s/dt
99         k(11)*x(3)*x(2) - 2*k(12)*x(4) - k(5)*x(4); % dC2s/dt
100        k(2)*x(2) - x(5)*(k(3) + k(4)); % dRi/dt
101        k(5)*x(4) - x(6)*(k(6) + k(7))]; % dC2i/dt
102 end
103
104 function xdot = eqn.sys_PFOA(t,x,k)
105 % x = [ A  Rs  C1s  C2s  Ri  C2i];
106 % ----x(1)-x(2)-x(3)-x(4)-x(5)--x(6)
107
108 % k = [Psyn, ke, krec, kdeg, ke2, krec2, kdeg2, kon, koff, Ce]
109 % ----k(1)--k(2)-k(3)-k(4)--k(5)--k(6)---k(7)--k(8)--k(9)-k(10)
110 Nav = 6.02e23*1e-9; % nmol^-1
111
112 xdot = [0;
113         k(1) + -2*k(8)*x(1)*x(2) + k(9)*x(3) - k(2)*x(2) + k(3)*x(5)...

```

```

114         + 2*k(6)*x(6) - k(11)*x(3)*x(2) + k(12)*x(4); % dRs/dt
115         2*k(8)*x(1)*x(2) - k(9)*x(3) - k(11)*x(3)*x(2) + 2*k(12)*x(4); % dC1s/dt
116         k(11)*x(3)*x(2) - 2*k(12)*x(4) - k(5)*x(4); % dC2s/dt
117         k(2)*x(2) - x(5)*(k(3) + k(4)); % dRi/dt
118         k(5)*x(4) - x(6)*(k(6) + k(7))]; % dC2i/dt
119     end
120
121     function nice_plot(x,y, Xlab, Ylab, Title, ColorCode)
122         plot(x,y, 'Color', ColorCode, 'LineWidth', 2);
123         xlabel(Xlab, 'FontSize', 12);
124         ylabel(Ylab, 'FontSize', 12);
125         title(Title, 'FontSize', 12);
126     end
127
128     function nice_semiplotx(x,y, Xlab, Ylab, Title, ColorCode)
129         semilogx(x,y, 'Color', ColorCode, 'LineWidth', 2);
130         xlabel(Xlab, 'FontSize', 12);
131         ylabel(Ylab, 'FontSize', 12);
132         title(Title, 'FontSize', 12);
133     end
134
135     function nice_semiploty(x,y, Xlab, Ylab, Title, ColorCode)
136         semilogy(x,y, 'Color', ColorCode, 'LineWidth', 2);
137         xlabel(Xlab, 'FontSize', 12);
138         ylabel(Ylab, 'FontSize', 12);
139         title(Title, 'FontSize', 12);
140     end
141
142     end

```

## 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.

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**Solution:**

- No ligand depletion: can be guaranteed by sufficient incubation volume, and media changes.
- Ordered binding: site 1 binds first to a free receptor, then site 2.
- Dissociation of ligand bound to receptor by site 2 occurs extremely fast: characteristic time for the dissociation time via site 2 is:

$$\tau = k_{\text{off},2}^{-1} = 62.5\text{min}$$

The dissociation is thus not *extremely fast* and it is ambiguous as to why the author included this assumption here.

4 points

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

---

**Solution:**

This is the simplest mathematical form for a saturable process such as proliferation. Indeed, nutrient limitations for example limit the exponential growth of cells. Here, this form assumes that the proliferative signal is linear for low amount of dimer formation and then becomes saturable. This hyperbolic response lumps the activation of the kinases, recruitment of STATs and etc. into one term.

2 points

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

---

**Solution:**

As the ligand concentration increases, the likelihood of forming single complex is higher than dimers.

2 points

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

---

**Solution:**

Figure 2a from the paper shows the proliferation dose responses for the wild-type hGH and two mutants, one with high and one with low site 1 affinity. We can see that the variants can differ by orders of magnitude in terms of affinity and still obtain similar on rates. Dissociation constant  $k_{r1}$  was assumed to be 30-fold lower and 700-fold higher for the high affinity and low affinity mutants respectively. The EC50 is correctly estimated by the high affinity mutant but not the IC50. The low affinity mutant was unable to predict correctly neither the EC50 or IC50.

In figure 3, the affinity of site 2 was modified by changing the parameter  $K_X$ . Three different values of  $K_X$  were assessed: 41/R0, 410/R0, 4.1/R0. The impact on the EC50 is minimal whereas the larger  $K_X$  the larger the IC50. Therefore, the broadness of the bell-shaped dose-response seems to depend on the site 2 affinity. This makes sense since the stronger the affinity for site 2, the easier it is to compete with free receptor to have them dimerize instead of forming 1:1 complex with some other ligand through site 1.

*4 points: 2 points for figure 2a explanation, 2 points for figure 3 explanation.*

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- 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?

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**Solution:**

1 minute post stimulation, there is a large difference across ligand concentrations while all ligand concentration signal somewhat equally at steady state, with a very weak signal. This allows the cell to respond differentially to the input at early times, but then desensitize itself for prolonged stimulations.

*2 points*

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- f) As it turns out, the biological model on which Haugh's mathematical model is based is incorrect<sup>1</sup>. What does this tell you on the utility and validity of this model?

---

**Solution:**

All models are wrong, some are useful. In this case, this model was useful because it allowed scientists to understand the importance of the binding difference between site 1 and 2.

*2 points*

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**16 points overall for problem 2.**

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<sup>1</sup>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).

*This problem is inspired by a computational study [1] by Kholodenko in 2000 and the experimental verification of its predictions [2] by Shankaran and colleagues in 2009.*

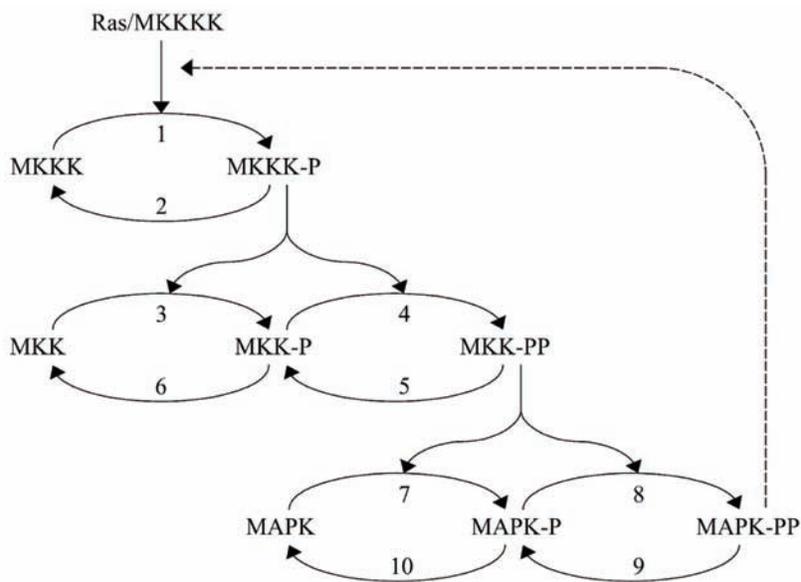


Figure reproduced from [1].

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

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**Solution:**

The negative feedback affects only reaction 1 in the above reaction network, i.e. the phosphorylation of MKKK (Raf) by active MKKKK (Ras). The relevant line in the code is `r1 = k(1)*y(1)/((1+(y(8)*Feedback)^n)*(KM(1)+y(1)));`, which encodes the following expression for the reaction rate,  $r_1$ :

$$\begin{aligned} r_1 &= \frac{\frac{v_1}{1+(\text{Feedback} \cdot [\text{Erk-pp}])^n} \cdot [\text{Raf}]}{K_{M,1} + [\text{Raf}]} \\ &= \frac{\frac{v_1}{1+\left(\frac{[\text{Erk-pp}]}{K_I}\right)^n} \cdot [\text{Raf}]}{K_{M,1} + [\text{Raf}]} \end{aligned}$$

This corresponds to nonlinear, non-competitive inhibition of Ras-mediated Raf phosphorylation by activated Erk with an inhibition constant  $K_I = \text{Feedback}^{-1}$ .

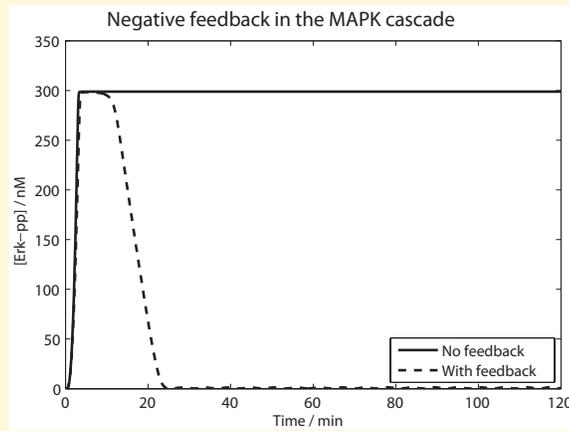
It is certainly reasonable to suppose non-competitive inhibition of a protein kinase: both it and its substrate are macromolecules, and it is conceivable that an inhibiting protein should bind only the complex of the two but neither of them individually, for example. The Hill coefficient,  $n$ , and the stronger nonlinearity which it introduces can, conceivably, arise from intermediate reactions, as Erk-pp does not directly bind to Ras to inhibit its action. A nice feature of non-competitive inhibition in this model is that its action cannot be competed out by substrate, so that it should exert a qualitatively similar effect over a wide range of parameters. For biological relevance, it would be interesting to see if the model predictions hold true if a different type of negative feedback is encoded, and to determine from the experimental literature what type of inhibition is most likely.

*7 points total: 1 for identifying the relevant term in the code, 1 for identifying the proteins and constants, 1 for noting it is in Michaelis-Menten-like form, 2 for recognizing it corresponds to noncompetitive inhibition (or 1 point partial credit for pointing out that only the maximal rate is scaled down by the feedback), 2 points for discussion.*

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- 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  $v_1$  to  $10 \text{ nM s}^{-1}$  and the **Feedback** parameter to 0 or  $100 \text{ nM}^{-1}$ .

**Solution:**

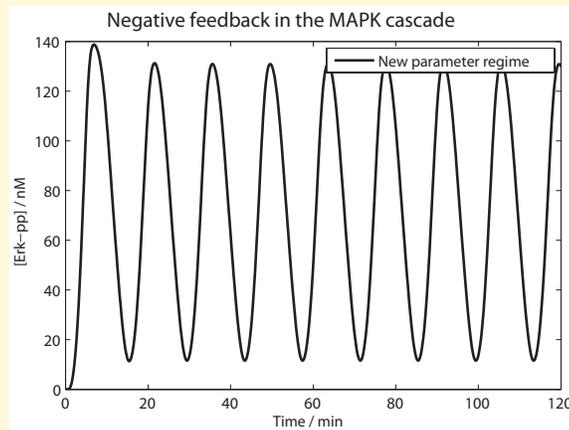


As expected from the discussion in class, negative feedback changes the network response to a step stimulus from step activation to transient activation.

*2 points.*

- c) Now change the parameters. Reduce the initial concentrations of all kinases to one-half their original initial values, then plot the cascade response against time to input  $v_1$  at  $2 \text{ nM s}^{-1}$  with Feedback strength of  $0.15 \text{ nM}^{-1}$ . What do you observe?

**Solution:**

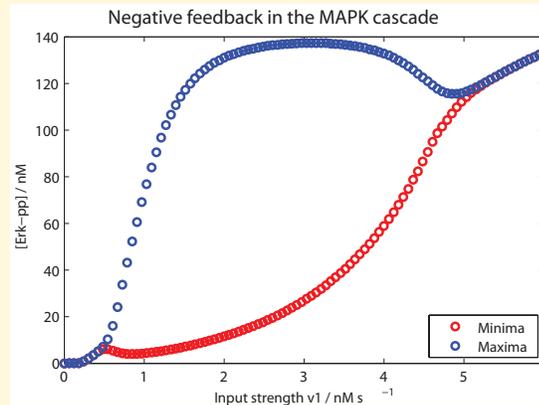


The network response as quantified by Erk-pp concentration now oscillates, settling into a sustained oscillation after an initial transient overshoot.

*3 points: 2 for plot, 1 for noting a sustained oscillation.*

- 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?

**Solution:**



Minimal and maximal values coincide for high and low values of input strength,  $v_1$ , but diverge at intermediate input strengths.

*6 points: 3 for code, 2 for plot, 1 for observation.*

- ii) Find all critical input strength values and comment on what happens there.

**Solution:**

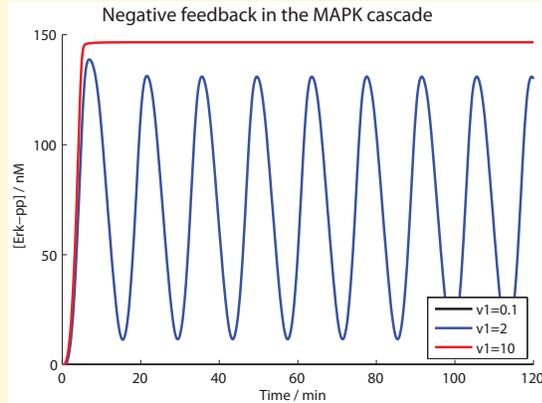
At an input strength of  $0.5 \text{ nM s}^{-1}$ , the system ceases to have a (low) stable steady-state and enter a regime where it continues to vary over time. This regime vanishes at an input strength of about  $5 \text{ nM s}^{-1}$ , after which the system again has a stable steady-state (now high).

Specifically, the intermediate regime is sustained oscillation on a limit cycle. The transitions at the critical input strengths are called Hopf bifurcations.

*3 points: 1 for the numerical values of the bifurcation points, 2 for discussion of the stable steady state / oscillation transition.*

- 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?

**Solution:**

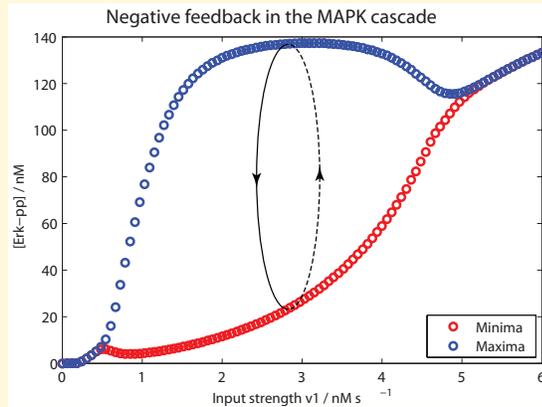


Both the low and the high steady states are reached monotonically here, but can include an initial damped oscillation if the input is close to the bifurcation points. The time-variant regime in the middle is indeed a sustained oscillation.

*4 points: 2 for plot, 2 for discussion.*

- 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.

**Solution:**



This graph indicates only the extrema. From c) and from d)iii), but not from d)i) alone, we know that the nature of the time-varying behavior on that region is a regular and sustained oscillation. This can be indicated by arrows between the extrema, or by waves propagating into the plane of the paper. The important thing to realize is that in this plot, time has to be added as a third dimension – understanding this is what this subproblem asks for.

*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?

---

**Solution:**

In relation to the behavior discussed in class — a transient response with a duration which smoothly varies with feedback strength — this is qualitatively different, and illustrates that different sets of parameter values can lead to drastic changes in the behavior of a biological network (although most often they don't).

As for biological relevance: this is not yet known, and any reasonable discussion should be awarded credit. From Shankaran et al. [2]:

“Although ERK oscillations are remarkable for their persistence and regularity, whether they contain information that can cause differential cell responses is unclear. Extracellular signal-regulated kinase is a potent activator of many nuclear transcription factors, and oscillations could be a means to selectively activate a subset of ERK-responsive genes, analogous to oscillatory calcium signaling. In the case of calcium oscillations, information about stimulus dose can be encoded both in the amplitude and frequency of oscillations, which in turn have been proposed to control the level and specificity of gene expression (Dolmetsch et al, 1998). Unlike calcium oscillations, however, ERK oscillations do not display strong frequency or amplitude modulation in response to ligand dose. However, the strong dependence of the oscillation on cell density is consistent with it being a highly regulated process that could encode contextual information. It has been reported that different primary stimuli in PC12 cells can induce either transient or sustained activation of ERK and that these induce different cellular fates (Sasagawa et al, 2005; Santos et al, 2007). Conditions giving rise to oscillations are associated with an apparent sustained activation of ERK, whereas conditions that suppress oscillations give rise to transient ERK activation (Figure 3E and F). Thus, oscillation could be a mechanism underlying different cellular responses to persistent versus transient ERK activation. Although a direct role for ERK oscillations in controlling gene expression is intriguing, the oscillation could also simply be a consequence of the feedback control and the regulatory structure of the ERK pathway without directly encoding information. Experiments are underway to explore these different possibilities.”

*3 points.*

---

<b>30 points overall for problem 3.</b>
---

## MATLAB code for Problem 3

PS4FeedbackMAPKSolution.m:

```
1 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
2 % 20.320 PS4 Q3: Negative feedback in the MAPK cascade
3 % Solution
4 % Fall 2010
5 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
6
7 function PS4FeedbackMAPK()
8 clc;
9 close all;
10 k=[0.4; % v1 / nM s^-1
11     0.25; % v2 / nM s^-1
12     0.025; % k3 / s^-1
13     0.025; % k4 / s^-1
14     0.75; % v5 / nM s^-1
15     0.75; % v6 / nM s^-1
16     0.025; % k7 / s^-1
17     0.025; % k8 / s^-1
18     00.5; % v9 / nM s^-1
19     0.5]; % v10 / nM s^-1
20 KM=[10; % all in nM
21     8;
22     15;
23     15;
24     15;
25     15;
26     15;
27     15;
28     15;
29     15];
30 Feedback=0.1; % nM^-1
31 n=1; % Hill coefficient
32 yo=[100; % y1 = MKKK; all in nM
33     0; % y2 = MKKK-p
34     300; % y3 = MKK
35     0; % y4 = MKK-p
36     0; % y5 = MKK-pp
37     300; % y6 = MAPK
38     0; % y7 = MAPK-p
39     0]; % y8 = MAPK-pp
40
41 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
42 % b) Plot system response to step stimulus with and without negative
43 % feedback
44
45 tspan=[0 7200];
46 k(1)=10; % strength of input stimulus
47 Feedback=0; % Try 0 vs. 100; plot in same graph
48 [TOUT1,YOUT1] = ode23s(@CascadeFB, tspan, yo, [],k,KM,Feedback,n);
49 activatedERK_no_FB = YOUT1(:,8);
50 Feedback=100;
51 [TOUT2,YOUT2] = ode23s(@CascadeFB, tspan, yo, [],k,KM,Feedback,n);
52 activatedERK_with_FB = YOUT2(:,8);
53 figure();
54 plot(TOUT1./60,activatedERK_no_FB, 'k-', TOUT2./60, ...
```

```

55     activatedERK_with_FB, 'k--', 'LineWidth', 2);
56     legend('No feedback','With feedback','Location','SouthEast');
57     title('Negative feedback in the MAPK cascade','FontSize', 16, ...
58           'FontWeight', 'bold');
59     xlabel ('Time / min','FontSize', 12, 'FontWeight', 'bold');
60     ylabel ('[Erk-pp] / nM', 'FontSize', 12, 'FontWeight', 'bold');
61     set(gca,'FontSize',12, 'FontWeight', 'bold');
62     axis([0 120 0 350]);
63
64     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
65     % c) Adjust parameters, repeat
66     k(1)=2; % strength of input stimulus
67     Feedback=0.15;
68     yo(1)=50; % y1 = MKKK
69     yo(3)=150; % y3 = MKK
70     yo(6)=150; % y6 = MAPK
71     [TOUT,YOUT] = ode23s(@CascadeFB, tspan, yo,[],k,KM,Feedback,n);
72     activatedERK = YOUT(:,8);
73     figure();
74     plot(TOUT./60,activatedERK, 'k-', 'LineWidth', 2);
75     legend('New parameter regime','Location','NorthEast');
76     title('Negative feedback in the MAPK cascade','FontSize', 16, ...
77           'FontWeight', 'bold');
78     xlabel ('Time / min','FontSize', 12, 'FontWeight', 'bold');
79     ylabel ('[Erk-pp] / nM', 'FontSize', 12, 'FontWeight', 'bold');
80     set(gca,'FontSize',12, 'FontWeight', 'bold');
81
82     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
83     % d) 1. Draw bifurcation diagram:
84     %     i) Vary v1
85     %     ii) For each v1, let system evolve for 10 000 s
86     %     iii) Record and plot min and max [Erk-pp] between 5 000 - 10 000 s.
87     %           Plot the data as points, not lines.
88     %
89     % HINT: Let system evolve for 5 000 s. Use endpoint concentrations as
90     %        initial conditions for another 5 000 s run. Then extract the min
91     %        and max values from this second run only.
92     vlrange=linspace(0,6,100); % reasonable range to iterate over
93
94     for j = 1:length(vlrange)
95         %iterate through input strengths
96         k(1) = vlrange(j);
97         tspan=[0 5000];
98         % Run for 10 000 s
99         [TOUT1,YOUT1] = ode23s(@CascadeFB, tspan, yo,[],k,KM,Feedback,n);
100        % Run for next 10 000 s, with results from previous run as ICs
101        [TOUT2,YOUT2] = ode23s(@CascadeFB, tspan, YOUT1(end,:), [], k, ...
102            KM,Feedback,n);
103        activatedERK = YOUT2(:,8);
104        % Save min and max
105        ymin(j) = min(activatedERK);
106        ymax(j) = max(activatedERK);
107    end
108    figure();
109    plot(vlrange,ymin, 'ro', vlrange,ymax, 'bo', 'LineWidth', 2);
110    legend('Minima','Maxima','Location','SouthEast');
111    title('Negative feedback in the MAPK cascade','FontSize', 16, ...
112          'FontWeight', 'bold');
113    xlabel ('Input strength v1 / nM s^{-1}','FontSize', 12, ...

```

```

114         'FontWeight', 'bold');
115     ylabel ('[Erk-pp] / nM', 'FontSize', 12, 'FontWeight', 'bold');
116     set(gca, 'FontSize', 12, 'FontWeight', 'bold');
117
118     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
119     % 2. Plot Erk-pp as a function of time in response to stimuli of different
120     % strengths
121
122     vlrange=[0.1 2 10];
123     linecols = {'k-', 'b-', 'r-'};
124     figure();
125     hold on;
126     for j = 1:length(vlrange)
127         %iterate through input strengths
128         k(1) = vlrange(j);
129         tspan=[0 7200];
130         % Run for 2 h
131         [TOUT,YOUT] = ode23s(@CascadeFB, tspan, yo, [],k,KM,Feedback,n);
132         activatedERK = YOUT(:,8);
133         plot(TOUT./60,activatedERK, linecols{j}, 'LineWidth', 2);
134     end
135     legend('v1=0.1','v1=2','v1=10','Location','SouthEast');
136     title('Negative feedback in the MAPK cascade','FontSize', 16, ...
137           'FontWeight', 'bold');
138     xlabel ('Time / min','FontSize', 12, 'FontWeight', 'bold');
139     ylabel ('[Erk-pp] / nM', 'FontSize', 12, 'FontWeight', 'bold');
140     set(gca, 'FontSize', 12, 'FontWeight', 'bold');
141     hold off;
142
143     %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
144     function dydt = CascadeFB(t,y,k,KM,Feedback,n)
145
146     % Pre-calculate terms for rate equations
147     r1 = k(1)*y(1)/((1+(y(8)*Feedback)^n)*(KM(1)+y(1))); %KI = 1/Feedback
148     r2 = k(2)*y(2)/(KM(2)+y(2));
149     r3 = k(3)*y(2)*y(3)/(KM(3)+y(3));
150     r4 = k(4)*y(2)*y(4)/(KM(4)+y(4));
151     r5 = k(5)*y(5)/(KM(5)+y(5));
152     r6 = k(6)*y(4)/(KM(6)+y(4));
153     r7 = k(7)*y(5)*y(6)/(KM(7)+y(6));
154     r8 = k(8)*y(5)*y(7)/(KM(8)+y(7));
155     r9 = k(9)*y(8)/(KM(9)+y(8));
156     r10 = k(10)*y(7)/(KM(10)+y(7));
157
158     % Calculate derivatives
159     dydt=[r2-r1;          % y1 = MKKK
160          r1-r2;          % y2 = MKKK-p
161          r6-r3;          % y3 = MKK
162          r3+r5-r4-r6;    % y4 = MKK-p
163          r4-r5;          % y5 = MKK-pp
164          r10-r7;         % y6 = MAPK
165          r7+r9-r8-r10;   % y7 = MAPK-p
166          r8-r9];         % y8 = MAPK-pp

```

## References

- [1] B. N. Kholodenko. Negative feedback and ultrasensitivity can bring about oscillations in the mitogen-activated protein kinase cascades. *European Journal of Biochemistry*, 267(6):1583–1588, March 2000.
- [2] H. Shankaran, D. L. Ippolito, W. B. Chrisler, H. Resat, N. Bollinger, L. K. Opresko, and H. S. Wiley. Rapid and sustained nuclearcytoplasmic erk oscillations induced by epidermal growth factor. *Molecular Systems Biology*, (5):332, December 2009.

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