Modeling receptor protein regulation and enzyme cascades

By

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BENG221

Background

Receptor proteins enable the cell to receive extracellular signals that stimulate certain behaviors, such as proliferate or move. The extracellular portions of receptor proteins are binding sites for specific chemicals ligands. Once a ligand is bound to a receptor protein, conformational changes occur in the intracellular region which initiate specific cellular functions[1]. Many of these cellular functions are controlled through a series of multiple enzymatic reactions. These "enzyme cascades" allow the cell to control the magnitude and timing of the resulting reactions.

The two systems modeled in this problem are the epithelial growth factor receptor (EGFR) and the Erk (MAPK) enzyme cascade. Normally, EGFR is created through transcription and translation of genetic code into receptor protein. Newly synthesized EGFR is then transported to the cell membrane via a transport vesicle. To regulate the number of EGFR on the cell surface, a fraction of the surface EGFR is continuously reabsorbed by the cell through endocytosis. Once inside the cell, EGFR will either be recycled back up into the cell membrane or undergo lysosomal degradation (Figure 1). [2]

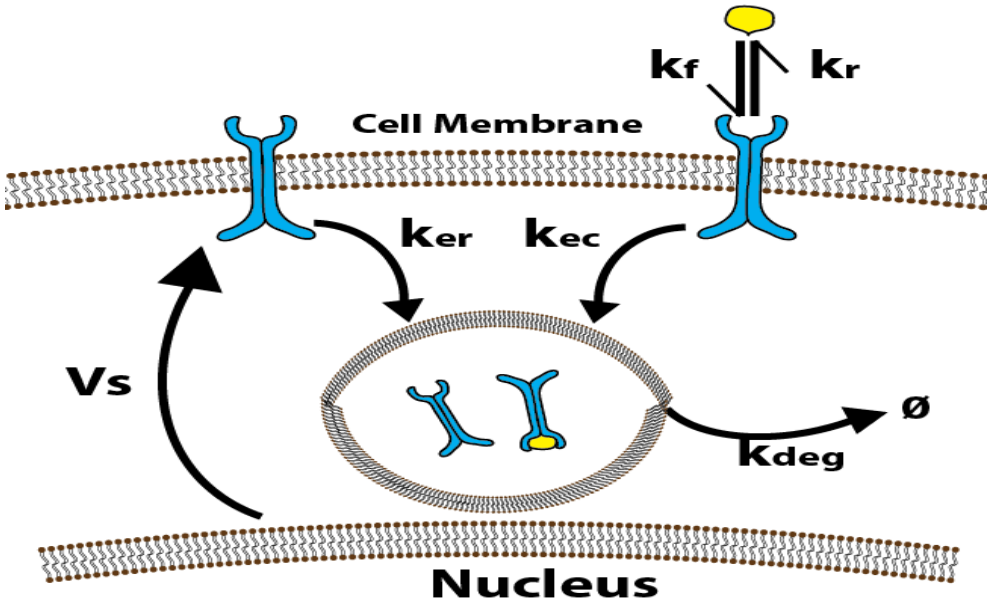

Figure 1. Diagram of the regulation of EGFR

EGFR is the receptor site for many different types of ligands. In this model, the ligand epithelial growth factor (EGF) will be used as the initiator for the activation of EGFR and the Erk enzyme cascade. When EGF and EGFR form a complex, bound receptor dimerize and the enzyme cascade is initiated by the activation of the Ras kinase by the activated EGFR complex[1]. This pathway is highly regulated; the end product, active Erk, limits its own synthesis by inhibiting Ras kinase from initiating the cascade. However the molecules of active Erk that are created go on to initiate the expression of genes responsible for cell division.[5]

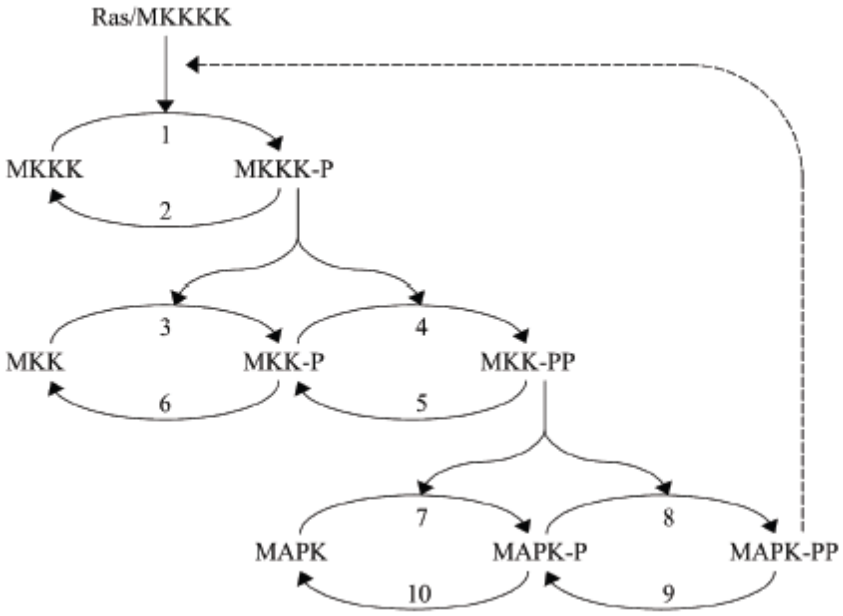

Figure 2. The resulting enzyme cascade from the activation of EGFR by EGF[3].

The clinical significance of the EGFR/Erk cascade makes it a worthwhile target for modeling. It has been shown that knockouts of EFGR are not able to form epithelial organs and therefore are not viable[3]. It has also been shown that over production of EGFR is a phenotypically correlated to many types of cancers[3]. Further, the over production and under regulation of Erk has been shown in cancer cell lines[5]. It has been thought that decreasing the activity of EGFR or increasing the negative feedback imposed by Erk may serve as a treatment for certain cancers by reducing the proliferation rate of tumors[1][5]. Therefore developing an *in silico* understanding of the EGFR/Erk system may help direct the development of *in* vivo therapies.

Problem Statement

Create and analytically solve a model of the creation of EGFR and the receptor-ligand complex. Then use this model to determine how the number of receptor complexes influences the behavior of the resulting Erk cascade.

Assumptions

The following assumptions were made to make the problem solvable (Figure 1.):

1. There is excess ligand in the extracellular space. This implies that the number of free ligands greatly outnumbers the number of free receptors. Therefore, the change in free ligand concentration is negligible, which allows L (ligand concentration) to be treated as a constant.

2. The production and deposition of receptors onto the cell surface is constant. This assumption is made to facilitate modeling by ignoring the effects of transcription, translation, modification in the golgi, etc that effect receptor synthesis.
3. As mentioned previously, when receptors and ligands are brought into the cell via endocytosis, they can either be recycled or degraded. For this model, the degradation rate is assumed to be significantly faster than the recycling rate. Thus when receptors (or complex) are taken in by the cell, it has negligible chance of returning to the cell surface. By making this assumption, the population of receptor and complex inside the endosome is disregarded.
4. Although dimerization occurs in a real system, dimerization will be ignored in order to facilitate modeling.

System of Differential Equations

The system of differential equations will consist of two equations that describe the dependent behavior of population of receptors and complexes on the cell surface. The following illustrates the factors involved along with approximate biological values:

Constants

	Definition	Value
R	EGFR receptor population	Varying
C	EGFR complex population	Varying
L	Ligand concentration	2 [mg/mL] [6]
$\mathbf{V_S}$	Receptor synthesis rate	6 x 10^2 [#/min] [6]
$\mathbf{k_f}$	Rate of forward binding between ligand and receptor	2 x 10^6 [1/M/min] [8]
$\mathbf{k_r}$	Rate of reverse unbinding of a complex	6 x 10^{-2} [min^{-1}] [8]
$\mathbf{k_{er}}$	Rate of receptor endocytosis	0.02 [#/min] [6]
$\mathbf{k_{ec}}$	Rate of complex endocytosis	0.2 [#/min] [7]

Reactions

$$R + L \overset{k_f}{\rightarrow} C$$
$$C \overset{k_r}{\rightarrow} R + L$$
$$R \overset{k_{er}}{\longrightarrow} EGFR\ receptor\ taken\ into\ the\ cell$$
$$C \overset{k_{ec}}{\longrightarrow} EGFR\ complex\ taken\ into\ the\ cell$$
$$R \overset{V_S}{\rightarrow} EGFR\ receptor\ syntehsized\ and\ brought\ onto\ cell\ surface$$

These reactions give the following differential equations:

$$\frac{dR}{dt} = -k_f LR + k_r C - k_{er} R + V_s \qquad (1)$$

$$\frac{dC}{dt} = k_f LR - k_r C - k_{ec} C \tag{2}$$

These two first order differential equations represent how receptor (1) and complex (2) populations on the cell surface change with respect to time. In order to simply the system, the coefficients of R and C are combined.

$$\frac{dR}{dt} = -\left(k_f L + k_{er}\right)R + k_r C + V_s$$
$$\frac{dC}{dt} = k_f LR - (k_r + k_{ec})C$$

Notice that the coefficients of R in the first equation and C in the second equation are simply sums of constants. These are grouped together and referred to as k_{rloss} and k_{closs} respectively:

$$Let\ k_{rloss} = k_f L + k_{er}\ , k_{closs} = k_r + k_{ec}$$

Then the system of equations becomes the following:

$$\frac{dR}{dt} = -k_{rloss} R + k_r C + V_s$$
$$\frac{dC}{dt} = k_f LR - k_{closs} C$$

Analytic Solution

This system can be solved by finding its eigenvalues and eigenvectors:

$$\frac{d}{dt}\begin{bmatrix} R \\ C \end{bmatrix} = \begin{bmatrix} -k_{rloss} & k_r \\ k_f L & -k_{closs} \end{bmatrix}\begin{bmatrix} R \\ C \end{bmatrix} + \begin{bmatrix} V_s \\ 0 \end{bmatrix}$$
$$A = \begin{bmatrix} -k_{rloss} & k_r \\ k_f L & -k_{closs} \end{bmatrix} \tag{3}$$

Notice that (3) is not a homogeneous equation. However, the particular solution alone will not describe the time dependent dynamics of the systems. A solution to the system must instead consist of a linear combination of its particular and homogenous solutions.

First, the homogenous solution:

$$\lambda I - A = \begin{bmatrix} \lambda & 0 \\ 0 & \lambda \end{bmatrix} - \begin{bmatrix} -k_{rloss} & k_r \\ k_f L & -k_{closs} \end{bmatrix} = \begin{bmatrix} \lambda + k_{rloss} & -k_r \\ -k_f L & \lambda + k_{closs} \end{bmatrix}$$
$$\det(\lambda I - A) = (\lambda + k_{rloss})(\lambda + k_{closs}) - k_r k_f L = \lambda^2 + (k_{rloss} + k_{closs})\lambda + k_{rloss} k_{closs} - k_r k_f L$$

Using the quadratic formula,

$$a = 1$$
$$b = k_{rloss} + k_{closs}$$
$$c = k_{rloss}k_{closs} - k_r k_f L$$
$$\lambda = \frac{-b \mp \sqrt{b^2 - 4c}}{2}$$

There are three possible homogenous solutions depending on the value of eigenvalues (λ).

Case I: $b^2 - 4c = 0$

Eigenvalues will be repeated: $\lambda_{1,2} = -\frac{b}{2}$

The solution will be in the form $\begin{bmatrix} R \\ C \end{bmatrix} = c_1 \boldsymbol{v_1} e^{-\frac{b}{2}t} + c_2 t \boldsymbol{v_2} e^{-\frac{b}{2}t}$

*Note: $\mathbf{v_1}$ and $\mathbf{v_2}$ are eigenvectors

Case II: $b^2 - 4c > 0$

Eigenvalues will be real and distinct, and the solutions will be in the following form:

$$\begin{bmatrix} R \\ C \end{bmatrix} = c_1 \boldsymbol{v_1} e^{\lambda_1 t} + c_2 \boldsymbol{v_2} e^{\lambda_2 t}$$

Case III: $b^2 - 4c < 0$

Eigenvalues will be complex. Let us call the real part λ and the imaginary part μ. Then, the solution will be in the following form:

$$\begin{bmatrix} R \\ C \end{bmatrix} = c_1 \boldsymbol{v_1} e^{\lambda t} \cos(\mu t) + c_2 \boldsymbol{v_2} e^{\lambda t} \sin(\mu t)$$

Entering biologically relevant values for these constants can determine which of these cases applies to the given system:

$$k_{rloss} = k_f L + k_{er} = (2 \times 10^6)(2) + 0.02 \approx 4 \times 10^6$$
$$k_{closs} = k_r + k_{ec} = 6 \times 10^{-2} + 0.2 = 0.26$$
$$k_f k_r L = (2 \times 10^6)(6 \times 10^{-2})(2) = 2.4 \times 10^5$$

Inserting these values into b and c:

$$b = k_{rloss} + k_{closs} \approx 4 \times 10^6$$
$$c = k_{rloss}k_{closs} - k_f k_r L = 8 \times 10^5$$

It is clear that $b^2 - 4c >> 0$ and that the system falls under Case II. Therefore, the homogeneous solution is of the following form:

$$\begin{bmatrix} R \\ C \end{bmatrix} = c_1 \boldsymbol{v_1} e^{\lambda_1 t} + c_2 \boldsymbol{v_2} e^{\lambda_2 t}$$

Where:

$$\lambda_1 = \frac{-b + \sqrt{b^2 - 4c}}{2} = \frac{-4 \times 10^6 + \sqrt{(4 \times 10^6)^2 - 4(8 \times 10^5)}}{2} = -0.2\lambda_2 = \frac{-b - \sqrt{b^2 - 4c}}{2}$$
$$= \frac{-4 \times 10^6 - \sqrt{(4 \times 10^6)^2 - 4(8 \times 10^5)}}{2} \approx -4 \times 10^6$$

Both eigenvalues are negative therefore:

1. The system is stable.
2. As t → ∞, the receptor and complex populations converge to values given by the particular solution.

Numerical Analysis

Due to the size and complexity of the combined receptor-Erk cascade system solutions were determined numerically. The coupling of these two systems required the consideration of two essential system components. The first is the input signal into the Erk cascade. Biologically, the input signal is given by Ras which, by phosphorylating RAF kinase, initiates the cascade. However, Ras is activated via phosphorylation by the kinase activity of EGF bound EGFR dimers. The model simplifies this interaction by using the number of active EGFR complexes on the surface of the cell as a proxy for the input signal into the cascade.

The second component is the negative feedback imposed by active Erk. Here feedback is assumed to follow non-competitive inhibition of the input signal. Although this model has not been verified from the literature, it is not an unreasonable assumption to make. It is a common mode of inhibition in other systems and it avoids out-competing the effect of feedback by increasing levels of RAF. Together these two components act as opposing forces on the dynamics of active Erk. It is also important to note the lack of symmetry in the system. Although the input signal provided by the surface EGFR complexes stimulates Erk activation and Erk feedback, the increased Erk feedback does not affect the number of surface EGFR complexes able to signal into the cascade. This sets the number of surface EGFR complexes apart as a fundamentally independent variable, from the viewpoint of the cascade, while feedback, by scaling with the number of active Erk molecules, is a function of the number of surface complexes

Both of these system components can be altered to give different time varying behaviors to the levels of active Erk. Considering feedback exclusively, three distinct modes of behavior are seen (Figure 3). The first mode (A) is the product of a feedback-free system. Without feedback, the levels of active Erk exhibit a sharp initial increase, in accordance with the sharp initial increase in the number of surface EGFR complexes, followed by a leveling off to a sustained steady-state value. The second mode (B) illustrates the result of extreme feedback. Levels of Erk exhibit the same initial rise as in the first mode but then rapidly fall off to a near-zero value and experiences a sustained suppression. Here the presence of a small amount of active Erk provides sufficient negative feedback to inhibit further activation regardless of the magnitude of the input signal. The third mode (C) represents an intermediate, and

biologically relevant, level of feedback. Under this case, the levels of Erk oscillate in time. The levels rise when Erk concentration is low in response to the stimulus provided by surface EGFR complexes and fall when Erk concentration high due to the heightened feedback imposed by the sufficiently large number of active Erk molecules.

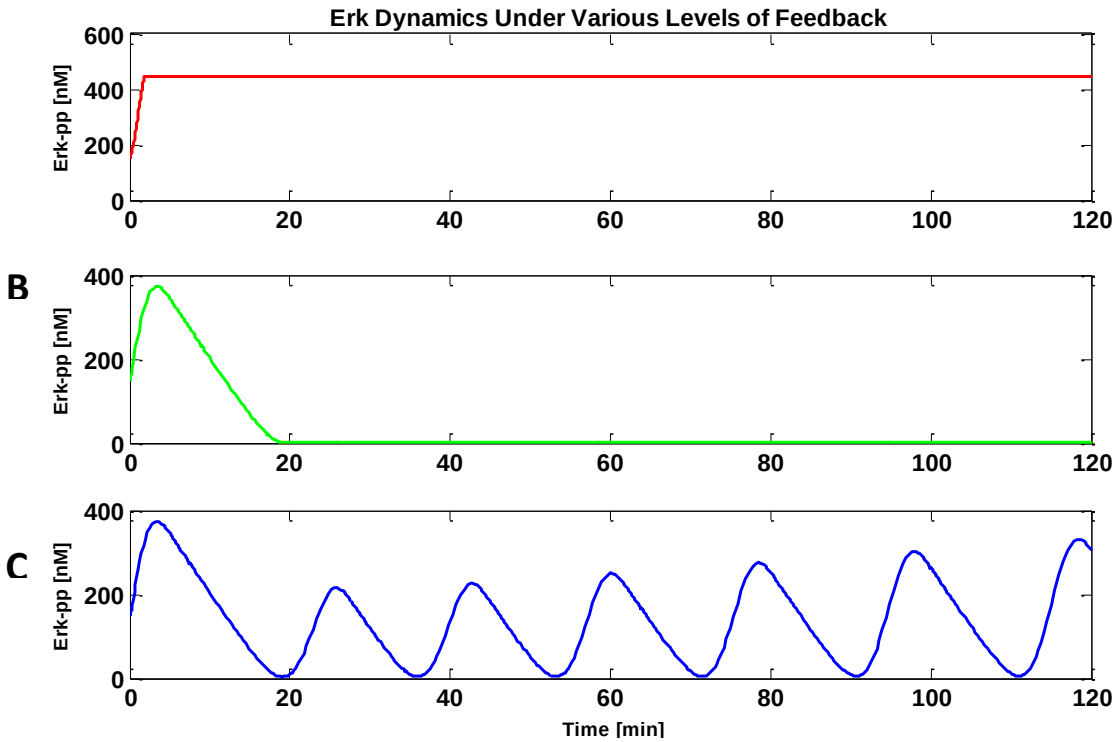

Figure 3. Erk Demonstrates Three Distinct Modes of Time Varying Behavior Under Various Levels of Feedback

In a system free of feedback levels of active Erk increase rapidly to their steady state value (A). Under extreme feedback minimal levels of active Erk are sufficient to quench further activation (B). Intermediate levels of feedback lead to oscillations in active Erk concentrations (C).

While the existence of oscillations is biologically relevant, as the period of oscillation can encode information on the timing of gene activation, the model can also reveal how oscillation amplitude varies with feedback. Active Erk has a number of downstream targets with various binding affinities; high affinity pathways are active with relatively little active Erk while low affinity substrates are only active when Erk is present in sufficient quantities to saturate higher affinity pathways. Understanding how feedback effects the maximum level of active Erk can shed light on which of these pathways are able to be activated at any given time. Sweeping through a suite of feedback values (in arbitrary units) reveals a large dynamic range in the amplitude of active Erk oscillations (Figure 4). Note that active Erk maxima follow the same trend as the input signal of surface EGFR complexes.

With this examination of the effect of feedback it is now possible to finely tune the levels of active Erk present in a cell by manipulating the reaction kinetics of active Erk/Ras binding. But the power of this model is that the concentration of any species in the system, its reaction kinetics, and

interspecies interactions can all be modified to reflect how the system can be changed to elicit a desired effect. Such flexibility in *in silico* experiments makes it possible to determine which species or interaction is the most relevant target, and how to target it, to elicit a particular response before moving on to more time consuming and laborious *in vitro* and *in vivo* experiments.

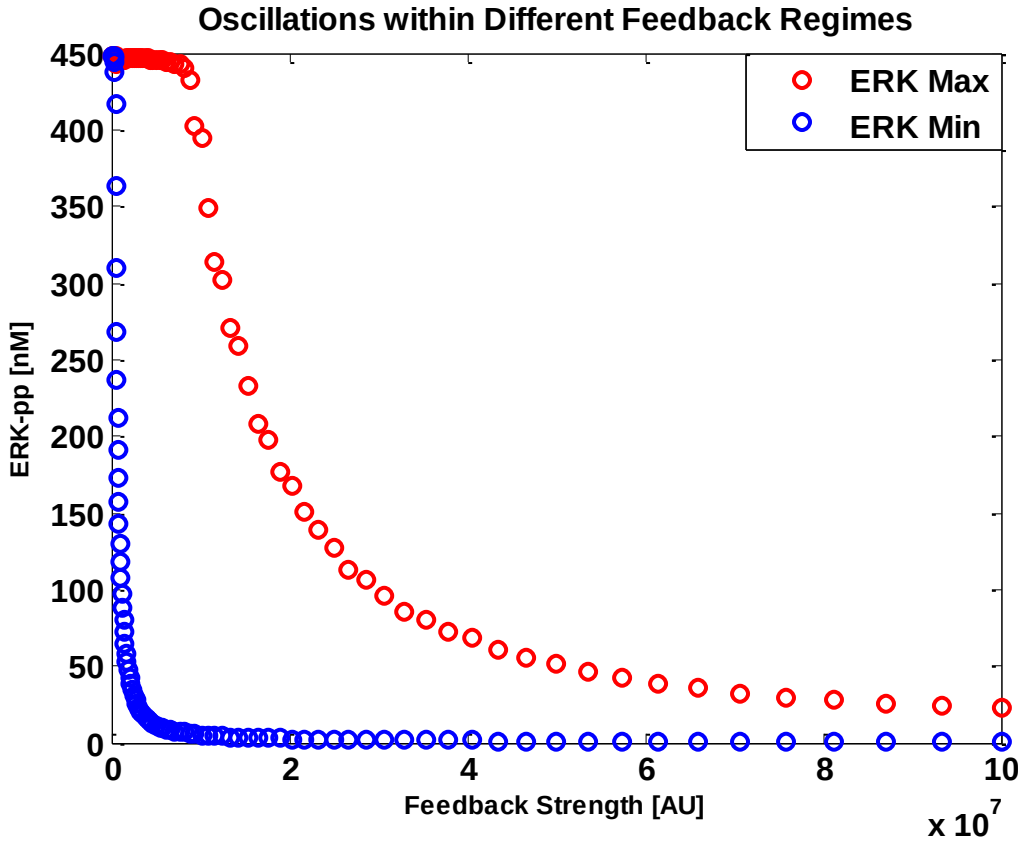

Figure 4. The Amplitude of active Erk oscillations Exhibits a Large Dynamic Range. Sweeping through a suite of feedback values (in arbitrary units) reveals a large dynamic range in the amplitude of active Erk oscillations.

Appendix

```
function FeedbackMAPK()

clc;
close all;

k=[0.25;  % v2 / nM s^-1
   0.025; % k3 / s^-1
   0.025; % k4 / s^-1
   0.75;  % v5 / nM s^-1
   0.75;  % v6 / nM s^-1
   0.025; % k7 / s^-1
   0.025; % k8 / s^-1
   0.5;   % v9 / nM s^-1
   0.5];  % v10 / nM s^-1

KM=[10; % all in nM
    8;
    15;
    15;
    15;
    15;
    15;
    15;
    15;
    15];

Feedback=0.0; % nM^-1

n=1; % Hill coefficient

yo=[1e5; % y1 = Initial number of Rs
    10;  % y3 = Ligand concentration in nM
    0;   % y4 = Initial Cs
    100; % y6 = MKKK; all in nM
    0;   % y7 = MKKK-p
    300; % y8 = MKK
    0;   % y9 = MKK-p
    0;   % y10 = MKK-pp
    300; % y11 = MAPK
    0;   % y12 = MAPK-p
    0;
    0];  % y13 = MAPK-pp

tspan=[0 3600];


%%
% System response to stimulus with and without negative
% feedback from MAPK

Feedback=0;
[TOUT1,YOUT1] = ode23s(@EGFRtraffickingPFOA, tspan, yo,[],k,KM,Feedback,n);
```

```
activatedERK_no_FB = YOUT1(:,11);
Feedback=1e10;

[TOUT2,YOUT2] = ode23s(@EGFRtraffickingPFOA, tspan, yo,[],k,KM,Feedback,n);
activatedERK_with_FB = YOUT2(:,11);

figure();
plot(TOUT1./60,activatedERK_no_FB, 'k-', TOUT2./60,activatedERK_with_FB, 'k--
', 'LineWidth', 2);
    legend('No feedback','With feedback','Location','NorthEast');
    title('Negative feedback in the MAPK cascade','FontSize', 16,
'FontWeight', 'bold');
    xlabel ('Time [min]','FontSize', 12, 'FontWeight', 'bold');
    ylabel ('Erk-pp [nM]', 'FontSize', 12, 'FontWeight', 'bold');
    set(gca,'FontSize',12, 'FontWeight', 'bold');
figure();
plot(TOUT1./60,YOUT1(:,1), 'r', TOUT1./60,YOUT1(:,3), 'b', 'LineWidth', 2);
    legend('Unbound EGFR','Surface Complex','Location','NorthEast');
    title('Impact of EGF on EGFR levels','FontSize', 16, 'FontWeight',
'bold');
    xlabel ('Time [min]','FontSize', 12, 'FontWeight', 'bold');
    ylabel ('Receptor Number', 'FontSize', 12, 'FontWeight', 'bold');
    set(gca,'FontSize',12, 'FontWeight', 'bold');


% %%
% % ADJUST PARAMETERS FOR OSCILLATORY BEHAVIOR IN MAPKpp
Feedback=1e7;
yo(6)=50;  % y6 = MKKK
yo(8)=150; % y8 = MKK
yo(11)=150; % y11 = MAPK

[TOUTC,YOUTC] = ode23s(@EGFRtraffickingPFOA, tspan, yo, [], k, KM,
Feedback,n);
activatedERK_with_FB = YOUTC(:,11);
figure(2)
plot(TOUTC./60, activatedERK_with_FB, 'g-', 'LineWidth',2);
legend('Active Erk','Location','NorthEast');
title('Erk-pp Levels Oscillation with Increased Feedback','FontSize', 16,
'FontWeight', 'bold');
xlabel ('Time [min]','FontSize', 12, 'FontWeight', 'bold');
ylabel ('Erk-pp [nM]', 'FontSize', 12, 'FontWeight', 'bold');
set(gca,'FontSize',12, 'FontWeight', 'bold');
% %%
%
%
%
% Demonstrate oscillatory behavior with various values of feedback in the
% cascade system. Here Feedback varies logarithmically between 1e5 to 1e10.
% System evolves for 10000 seconds and the maximum and minimum concentration
% of Erk-pp ar given, showing the feedback regime within which oscillations
% occur.

tspan = [0 5000];
tspan1 = [5000 10000];
ERK_max = [];
```

```
ERK_min = [];

FeedbackRange = logspace(2,8,200);
for rate = 1:length(FeedbackRange);
    Feedback = FeedbackRange(rate);
    [TOUTD,YOUTD] = ode23s(@EGFRtraffickingPFOA, tspan,
yo,[],k,KM,Feedback,n);
    newRs_with_FB = YOUTD(:,1);
    newL_with_FB = YOUTD(:,2);
    newCs_with_FB = YOUTD(:,3);
    newTrans_with_FB = YOUTD(:,12);

    newRAF_with_FB = YOUTD(:,4);
    newRAFP_with_FB = YOUTD(:,5);
    newMEK_with_FB = YOUTD(:,6);
    newMEKP_with_FB = YOUTD(:,7);
    newMEKPP_with_FB = YOUTD(:,8);
    newERK_with_FB = YOUTD(:,9);
    newERKP_with_FB = YOUTD(:,10);
    newERKPP_with_FB = YOUTD(:,11);
    y1 = [newRs_with_FB(length(newRs_with_FB));
     newL_with_FB(length(newL_with_FB));
     newCs_with_FB(length(newCs_with_FB));
     newRAF_with_FB(length(newRAF_with_FB));
     newRAFP_with_FB(length(newRAFP_with_FB));
     newMEK_with_FB(length(newMEK_with_FB));
     newMEKP_with_FB(length(newMEKP_with_FB));
     newMEKPP_with_FB(length(newMEKPP_with_FB));
     newERK_with_FB(length(newERK_with_FB));
     newERKP_with_FB(length(newERKP_with_FB));
     newERKPP_with_FB(length(newERKPP_with_FB));
     newTrans_with_FB(length(newTrans_with_FB))];
    [TOUTD,YOUTD] = ode23s(@EGFRtraffickingPFOA, tspan1,
y1,[],k,KM,Feedback,n);
    activatedERK_with_FB = YOUTD(:,11);
    ERK_max(rate) = max(activatedERK_with_FB);
    ERK_min(rate) = min(activatedERK_with_FB);
end
% Crit_point(1) = FeedbackRange(find(ERK_max-ERK_min > 1,1,'first'))
% Crit_point(2) = FeedbackRange(find(ERK_max-ERK_min > 1,1,'last'))
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%
figure(3)
plot(FeedbackRange,ERK_max,'ro','LineWidth',2);
hold on
semilogx(FeedbackRange,ERK_min,'bo','LineWidth',2);
legend('ERK Max','ERK Min','Location','NorthEast')
xlabel('Feedback Strength [AU]', 'LineWidth', 14, 'FontWeight', 'bold')
ylabel('ERK-pp [nM]', 'LineWidth', 14, 'FontWeight', 'bold')
title('Oscillations within Different Feedback
Regimes','Fontweight','b','Fontsize',14)
set(gca,'FontSize',12, 'FontWeight', 'bold');
% % %
% Plots Erk-pp as a function of time in different feedback regimes

FBrange = [0,1e7,1e10];
```

```
t_2hours = [0 7200];
colorIndex = {'r'; 'b';'g'; 'm'};

for index = 1:length(FBrange)
    Feedback = FBrange(index);
    [TOUTD,YOUTD] = ode23s(@EGFRtraffickingPFOA, t_2hours, yo, [], k, KM,
Feedback,n);
    activatedERK_with_FB = YOUTD(:,11);
    figure(4)
    subplot(length(FBrange),1, index);
    plot(TOUTD./60, activatedERK_with_FB, char(colorIndex(index)),
'LineWidth',2);
    xlabel('Time [min]', 'LineWidth', 14, 'FontWeight', 'bold')
    ylabel('Erk-pp [nM]', 'LineWidth', 14, 'FontWeight', 'bold')
    title(['Feedback =
',num2str(FBrange(index))],'fontsize',14,'fontweight','b');
    set(gca,'FontSize',12, 'FontWeight', 'bold');

end


function dAll = EGFRtraffickingPFOA(t,y,k,KM,Feedback,n)
%EGFRtraffickingPFOA models the endocytosis and trafficking of EGFR in the
%presence of ligand under the Pseudo First Order Approximation of ligand
concentration
%in excess and therefore unchanging throughout the reaction. Feeds value of
%Cs into the system controlling the ERK-MAPK enzyme cascade.

%Inputs:
%t = time vector [min]
%y = paramters in the system

%Output:
%dAll = a vector containing the ODEs for each species in the model

%Constants (untis given in comments):
%Original values have time units of mintues. These were convereted to
%seconds to conform with time units of cascade component.

Psyn = (6e2)/60;        %receptors/min/cell
ker = (2e-2)/60;        %receptors/min
kec = (.2)/60;          %receptors/min
krec = (.2)/60;         %receptors/min
kdeg = (.1)/60;         %receptors/min
kon = (2e6)/60;         %1/(M*min)
koff = (6e-2)/60;       %1/min
% cells = 1e6;    %cell number
% vol = 100e-6;   %L
% Na = 6.022e23;  %Avagadro's Constant -- #/mol

%Paramters
Rs = y(1);      %Receptors at surface
% Ri = y(2);      %Internalized receptors
L = y(2);       %Antibody concentration
Cs = y(3);      %1R:1A number at surface
```

```
% Ci = y(5);      %Internalized 2R:1A
Transition = y(12);

dRs = -kon*L*Rs + koff*Cs - ker*Rs + Psyn;
% dRi = ker*Rs - krec*Ri - kdeg*Ri;
dL = 0;
dCs = kon*L*Rs - koff*Cs - kec*Cs;
% dCi = kec*Cs - krec*Ci - kdeg*Ci;
dTransition = Cs;

%%
%%%KI = 1/Feedback;

% Pre-calculate terms for rate equations
r1  = Transition*y(4)/((1+(y(11)*Feedback)^n)*(KM(1)+y(4)));
r2  = k(1)*y(5)/(KM(2)+y(5));
r3  = k(2)*y(5)*y(6)/(KM(3)+y(6));
r4  = k(3)*y(5)*y(7)/(KM(4)+y(7));
r5  = k(4)*y(8)/(KM(5)+y(8));
r6  = k(5)*y(7)/(KM(6)+y(7));
r7  = k(6)*y(8)*y(9)/(KM(7)+y(9));
r8  = k(7)*y(8)*y(10)/(KM(8)+y(10));
r9  = k(8)*y(11)/(KM(9)+y(11));
r10 = k(9)*y(10)/(KM(10)+y(10));

% Calculate derivatives
dMKKK = r2-r1;
dMKKKp = r1-r2;
dMKK = r6-r3;
dMKKp = r3+r5-r4-r6;
dMKKpp = r4-r5;
dMAPK = r10-r7;
dMAPKp = r7+r9-r8-r10;
dMAPKpp = r8-r9;
%%
dAll = [dRs;
        dL;
        dCs;
        dMKKK;
        dMKKKp;
        dMKK;
        dMKKp;
        dMKKpp;
        dMAPK;
        dMAPKp;
        dMAPKpp
        dTransition];
end
```

Citations

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