BE.320 Problem set #5

1. EGFR MW = 180,000 $\frac{g}{mol}$

1 unit = $\frac{1\ pmol}{min}$ @ 37°C

V_{max} = 15,000 $\frac{units}{mg}$

a. Assume Michaelis-Menten kinetics

Assume $[S] >> K_m$

$$v = \frac{V_{max}[S]}{K_m + [S]} = \frac{V_{max}[S]}{[S]} = V_{max}$$

$$15{,}000\ \frac{units}{mg} \cdot \frac{pmol/min}{units} = 15{,}000\ \frac{pmol}{mg \cdot min}$$

$$15{,}000\ \frac{pmol}{mg \cdot min} \cdot \frac{1\ min}{60\ sec} = 250\ \frac{pmol}{mg \cdot sec}$$

$\therefore 250 \times 10^{-12}\ \frac{mol}{mg \cdot sec}$ phosphorylated

b. Reaction 1

1 μM SHC, 100 mM ATP, 10 mM $MgCl_2$

Reaction 2

10 μM c-Cbl, 100 mM ATP, 10 mM $MgCl_2$

$K_{m_{EGFR\text{-}SHC}} = 1\ \mu M$

$K_{m_{EGFR\text{-}c\text{-}Cbl}} = 2\ \mu M$

$[EGFR] = 1\ \mu M$

$$[E \cdot S] = \frac{[E]_0[S]}{K_m + [S]}$$

$\therefore$ Reaction 1

$$[E \cdot S] = \frac{(1 \times 10^{-6})(1 \times 10^{-6})}{(1 \times 10^{-6}) + (1 \times 10^{-6})}$$

$$= 0.5\ \mu M$$

Reaction 2

$$[E \cdot S] = \frac{(1 \times 10^{-6})(10 \times 10^{-6})}{(2 \times 10^{-6}) + (10 \times 10^{-6})}$$

$$= 0.833\ \mu M$$

Assumptions/Approximations

1) $\frac{d[ES]}{dt} = 0$

2) $[S] = [S]_0$

3) $[S] >> [E]$ even though in this case this is not a good assumption (it's usually not in real life either)

Reaction 1

$\frac{[E \cdot S]}{[E]_0} = \frac{0.5 \times 10^{-6}}{1 \times 10^{-6}} = 50\%$ enzyme bound

Reaction 2

$\frac{[E \cdot S]}{[E]_0} = \frac{0.833 \times 10^{-6}}{1 \times 10^{-6}} = 83\%$ enzyme bound

the more enzyme bound, the faster the reaction because $v = k_2[E \cdot S]$

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%Problem 2, pset 5

%constants
Km = 17e-6;
N = 100;

%ranges
Ki = linspace(10e-9, 10e-6, N);
S = linspace(0.1e-3, 5e-3, N);

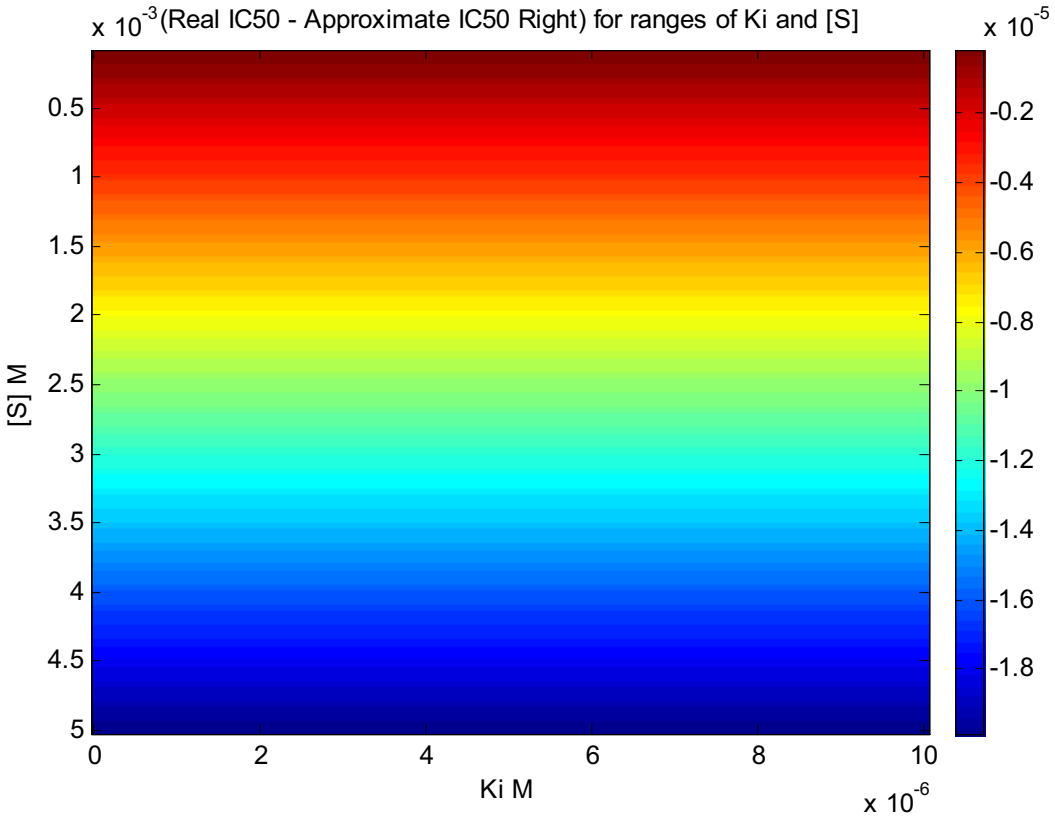
%solve for [I] where V = 1/2*Vmax
% V = (Vmax*[S]) / (Km * (1 + ([I] / Ki)) + [S])
% (1/2)Vmax = (Vmax*[S]) / (Km * (1 + ([I] / Ki)) + [S])
% 1 = (2*[S]) / (Km * (1 + ([I] / Ki)) + [S])
% Km * (1 + ([I] / Ki)) + [S] = 2*[S]
% Km * (1 + ([I] / Ki)) = [S]
% 1 + ([I] / Ki) = [S] / Km
% [I] / Ki = ([S] / Km) - 1
% [I] = Ki * (([S] / Km) - 1)

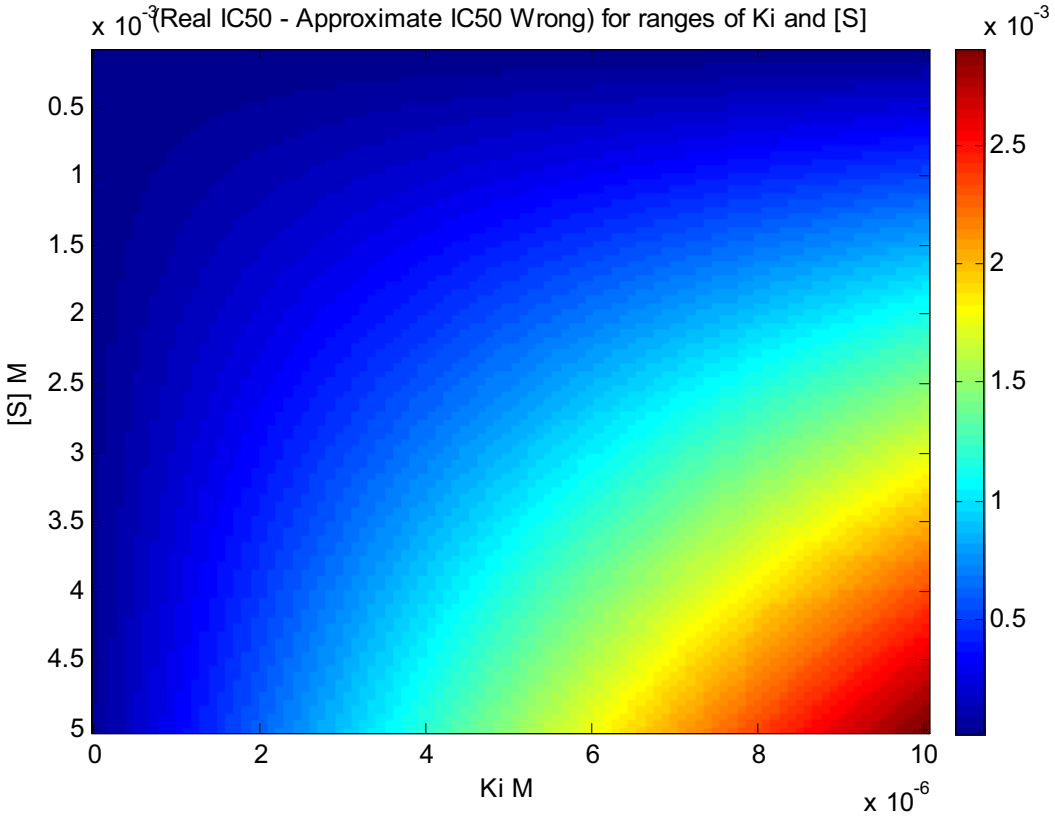
for i = 1:N
    for j = 1:N
        IC50_real = Ki(i)*((S(j)/Km)-1);
        IC50_approx_wrong = Ki(i)/(1 + (S(j)/Km));
        IC50_approx_right = Ki(i)*(1 + (S(j)/Km));
        diff_wrong = IC50_real - IC50_approx_wrong;
        diff_right = IC50_real - IC50_approx_right;
        diff_mat_wrong(i,j) = diff_wrong;
        diff_mat_right(i,j) = diff_right;
    end
end

%plot all differences
figure(1)
imagesc(Ki, S, diff_mat_right);
xlabel('Ki M');
ylabel('[S] M');
title('(Real IC50 - Approximate IC50 Right) for ranges of Ki and [S]');
colorbar;

figure(2)
imagesc(Ki, S, diff_mat_wrong);
xlabel('Ki M');
ylabel('[S] M');
title('(Real IC50 - Approximate IC50 Wrong) for ranges of Ki and [S]');
colorbar;
```

BE.320 Problem Set 5 Problem 2 Figures





BE 320 Problem Set #5 (Continued)

3a. $E + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES_1$

$E + S_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_2$

$ES_1 + S_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_1S_2 \xrightarrow{k_3} E + P$

$ES_2 + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES_1S_2 \xrightarrow{k_3} E + P$

$v = k_3[ES_1S_2]$

Assume $\frac{d[ES_1S_2]}{dt} = 0$

Assume $\frac{d[ES_1]}{dt} = 0$

Assume $\frac{d[ES_2]}{dt} = 0$

$$\frac{d[ES_1S_2]}{dt} = k_2[ES_1][S_2] + k_1[ES_2][S_1] - (k_{-2} + k_{-1} + 2k_3)[ES_1S_2]$$

$$\frac{d[ES_1]}{dt} = k_1[E][S_1] - k_{-1}[ES_1] - k_2[ES_1][S_2] + k_{-2}[ES_1S_2]$$

$$\frac{d[ES_2]}{dt} = k_2[E][S_2] - k_{-2}[ES_2] - k_1[ES_2][S_1] + k_{-1}[ES_1S_2]$$

$E_0 = [E] + [ES_1] + [ES_2] + [ES_1S_2]$

$E + S_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_2$ in equilibrium

$\therefore 0 = k_2[E][S_2] - k_{-2}[ES_2]$

$\therefore \frac{d[ES_2]}{dt} = -k_1[ES_2][S_1] + k_{-1}[ES_1S_2]$

$0 = \frac{d[ES_2]}{dt} = -k_1[ES_2][S_1] + k_{-1}[ES_1S_2]$

$k_1[ES_2][S_1] = k_{-1}[ES_1S_2]$

$\therefore [ES_2] = \left(\frac{k_{-1}}{k_1}\right)\left(\frac{[ES_1S_2]}{[S_1]}\right)$

Now,

$$0 = \frac{d[ES_1S_2]}{dt} = k_2[ES_1][S_2] + k_1[ES_2][S_1] - (k_{-2} + k_{-1} + 2k_3)[ES_1S_2]$$

$$\therefore 0 = k_2[ES_1][S_2] + k_1\left(\frac{k_{-1}}{k_1}\right)\left(\frac{[ES_1S_2]}{[S_1]}\right)[S_1] - (k_{-2} + k_{-1} + 2k_3)[ES_1S_2]$$

$$\therefore 0 = k_2[ES_1][S_2] - (k_{-2} + 2k_3)[ES_1S_2]$$

Now,

$$0 = \frac{d[ES_1]}{dt} = k_1[E][S_1] - k_{-1}[ES_1] - k_2[ES_1][S_2] + k_{-2}[ES_1S_2]$$

$$0 = k_1([E]_0 - [ES_1] - [ES_2] - [ES_1S_2])[S_1] - k_{-1}[ES_1] - k_2[ES_1][S_2] + k_{-2}[ES_1S_2]$$

$$0 = k_1[E]_0[S_1] + [ES_1](-k_1[S_1] - k_{-1} - k_2[S_2]) - k_1[ES_2][S_1] + [ES_1S_2](k_{-2} - k_1[S_1])$$

$$0 = k_1[E]_0[S_1] + [ES_1](-k_1[S_1] - k_{-1} - k_2[S_2]) - k_1\left(\frac{k_{-1}}{k_1}\right)\left(\frac{[ES_1S_2]}{[S_1]}\right)[S_1] + [ES_1S_2](k_{-2} - k_1[S_1])$$

$$0 = k_1[E]_0[S_1] + [ES_1](-k_1[S_1] - k_{-1} - k_2[S_2]) + [ES_1S_2](k_{-2} - k_1[S_1] - k_{-1})$$

BE.320 Problem Set #5 (Continued)

3a.

$$[ES_1]\left(-k_1[S_1]-k_{-1}-k_2[S_2]\right) = -k_1[E]_0[S_1]-[ES_1S_2](k_{-2}-k_1[S_1]-k_{-1})$$

$$\therefore [ES_1] = \frac{k_1[E]_0[S_1]+[ES_1S_2](k_{-2}-k_1[S_1]-k_{-1})}{k_1[S_1]+k_{-1}+k_2[S_2]}$$

Now,

$$0 = k_2[ES_1][S_2]-(k_{-2}+2k_3)[ES_1S_2]$$

$$0 = \frac{k_1k_2[E_0][S_1][S_2]}{k_1[S_1]+k_{-1}+k_2[S_2]} + [ES_1S_2]\left(-k_{-2}-2k_3+\frac{k_2[S_2](k_{-2}-k_1[S_1]-k_{-1})}{k_1[S_1]+k_{-1}+k_2[S_2]}\right)$$

$$0 = \frac{k_1k_2[E]_0[S_1][S_2]}{k_1[S_1]+k_{-1}+k_2[S_2]} - [ES_1S_2]\left(\frac{k_1k_{-2}[S_1]+k_1k_2[S_1][S_2]+k_{-1}k_2[S_2]+2k_3k_1[S_1]+2k_3k_{-1}+2k_3k_2[S_2]}{k_1[S_1]+k_{-1}+k_2[S_2]}\right)$$

$$\therefore [ES_1S_2] = \frac{k_1k_2[E]_0[S_1][S_2]}{k_1k_{-2}+k_1k_{-2}[S_1]+k_{-1}k_2[S_2]+k_1k_2[S_1][S_2]+2k_3k_1[S_1]+2k_3k_{-1}+2k_3k_2[S_2]}$$

$$\therefore [ES_1S_2] = \frac{k_1k_2[E]_0[S_1][S_2]}{(k_1k_{-2}+2k_3k_{-1})+(k_1k_{-2}+2k_3k_1)[S_1]+(k_{-1}k_2+2k_3k_2)[S_2]+k_1k_2[S_1][S_2]}$$

$$\therefore [ES_1S_2] = \frac{[E]_0[S_1][S_2]}{\left(\frac{k_1k_{-2}+2k_3k_{-1}}{k_1k_2}\right)+\left(\frac{k_{-2}+2k_3}{k_2}\right)[S_1]+\left(\frac{k_{-1}+2k_3}{k_1}\right)[S_2]+[S_1][S_2]}$$

$$\therefore V = \frac{k_3[E]_0[S_1][S_2]}{\left(\frac{k_1k_{-2}+2k_3k_{-1}}{k_1k_2}\right)+\left(\frac{k_{-2}+2k_3}{k_2}\right)[S_1]+\left(\frac{k_{-1}+2k_3}{k_1}\right)[S_2]+[S_1][S_2]}$$

BE.320 Problem Set #5 (continued)

3b.

$$E + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES_1$$

$$E + S_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_2$$

$$ES_1 + S_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_1S_2 \xrightarrow{k_3} E + P$$

$$ES_2 + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES_1S_2 \xrightarrow{k_3} E + P$$

$$E + I \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} EI$$

$$ES_1 + I \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} ES_1I \qquad K_I = \frac{k_{-4}}{k_4}$$

$$EI + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES_1I$$

Equations

$$\frac{d[ES_1S_2]}{dt} = k_2[ES_1][S_2] + k_1[ES_2][S_1] - (k_{-2} + k_{-1} + 2k_3)[ES_1S_2]$$

$$\frac{d[ES_1]}{dt} = k_1[E][S_1] - k_{-1}[ES_1] - k_2[ES_1][S_2] + k_{-2}[ES_1S_2] - k_4[ES_1][I]$$

$$\frac{d[ES_2]}{dt} = k_2[E][S_2] - k_{-2}[ES_2] - k_1[ES_2][S_1] + k_{-1}[ES_1S_2]$$

$$\frac{d[EI]}{dt} = k_4[E][I] - k_{-4}[EI] - k_1[EI][S_1] + k_{-1}[ES_1I]$$

$$\frac{d[ES_1I]}{dt} = k_4[ES_1][I] - (k_{-4} + k_{-1})[ES_1I] + k_1[EI][S_1]$$

$$[E] = [E]_0 + [ES_1] + [ES_2] + [ES_1S_2] + [EI] + [ES_1I]$$