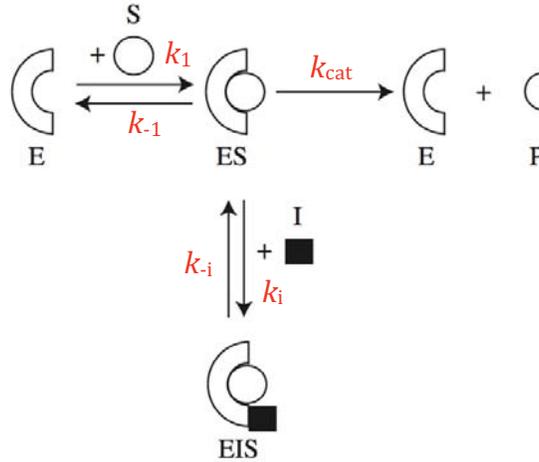


20.320 Problem Set 6
Question 1

Uncompetitive enzyme inhibitors bind to a site distant from the active site of the enzyme-substrate complex and allosterically inhibit catalysis. A schematic of this process is shown below (Figure 6.19 from Witttrup and Tidor).



- A) Write a system of Ordinary Differential Equations to describe the dynamics of uncompetitive inhibition. Label the above schematic with the rate constants you use in your equations. You should have one differential equation for each species in the system.

$$(1) \quad \frac{d[P]}{dt} = k_{cat}[ES]$$

$$(2) \quad \frac{d[EIS]}{dt} = k_i[ES][I] - k_{-i}[EIS]$$

$$(3) \quad \frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_{cat}[ES] - k_i[ES][I] + k_{-i}[EIS]$$

$$(4) \quad \frac{d[E]}{dt} = -k_1[E][S] + k_{-1}[ES] + k_{cat}[ES]$$

$$(5) \quad \frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES]$$

$$(6) \quad \frac{d[I]}{dt} = -k_i[ES][I] + k_{-i}[EIS]$$

20.320 Problem Set 6
Question 1

- B) Derive the Michaelis-Menten equation for reaction velocity in terms of [S], [I], [E₀], and the relevant rate and equilibrium constants. **Clearly state the assumptions you make in your derivation.**

Assuming quasi-steady state for enzyme-substrate complex binding the inhibitor means that we can set Equation 2 equal to zero. Noting that the same terms from Equation 2 appear in Equation 3, we can now simplify Equation 3. Applying the quasi-steady-state assumption to Equation 3 means we can set our new expression equal to zero, as well.

$$(7) \quad 0 = k_1[E][S] - (k_{-1} + k_{\text{cat}})[ES]$$

Apply conservation of enzyme to eliminate [E]:

$$(8) \quad [E_0] = [E] + [ES] + [EIS], \text{ therefore } [E] = [E_0] - [ES] - [EIS]$$

Substituting Equation 8 into Equation 7:

$$(9) \quad 0 = k_1([E_0] - [ES] - [EIS])[S] - (k_{-1} + k_{\text{cat}})[ES]$$

Using the definition of K_M allows us to replace the rate constants in Equation 9:

$$(10) \quad K_M = \frac{k_{-1} + k_{\text{cat}}}{k_1}$$

Plugging (10) into (9):

$$(11) \quad 0 = ([E_0] - [ES] - [EIS])[S] - K_m[ES]$$

Assuming rapid equilibrium binding of inhibitor allows us to relate the equilibrium inhibition constant K_I to [EIS] as follows:

$$(12) \quad K_I = \frac{[ES][I]}{[EIS]}$$

Plugging (12) into (11):

$$(13) \quad 0 = \left([E_0] - [ES] - \frac{[ES][I]}{K_I} \right) [S] - K_m[ES]$$

Following some algebra, Equation 13 can then be rearranged to solve for [ES].

$$(14) \quad [ES] = \frac{[E_0][S]}{K_m + \left(1 + \frac{[I]}{K_I} \right) [S]}$$

Plugging Equation 14 into Equation 1:

20.320 Problem Set 6
Question 1

$$(15) \quad v = \frac{d[P]}{dt} = \frac{k_{\text{cat}}[E_0][S]}{K_m + \left(1 + \frac{[I]}{K_I}\right)[S]}$$

Recalling that $v_{\text{max}} = k_{\text{cat}}[E_0][S]$ and applying some algebra yields the solution in Michaelis-Menten form. Assuming that substrate is in excess allows us to replace $[S]$ with $[S_0]$.

$$(16) \quad v = \frac{\frac{v_{\text{max}}}{\left(1 + \frac{[I]}{K_I}\right)}[S_0]}{\frac{K_m}{\left(1 + \frac{[I]}{K_I}\right)} + [S_0]}$$

- C) Based on your answer to Part B), describe the effect of an uncompetitive inhibitor on the v_{max} and overall K_M of the reaction. What scaling factor(s) are applied to these terms?

Uncompetitive inhibition decreases both the v_{max} and K_M of a reaction. In this case, both terms are divided by the term $1 + \frac{[I]}{K_I}$.

- D) Given $K_I = 75 \text{ nM}$, $K_M = 25 \text{ }\mu\text{M}$ and $[S_0] = 5 \text{ mM}$, what concentration of inhibitor is needed to achieve IC_{50} ?

$$\frac{0.5v}{v} = \frac{1}{2} = \frac{\frac{v_{\text{max}}}{\left(1 + \frac{\text{IC}_{50}}{K_I}\right)}[S_0]}{\frac{K_m}{\left(1 + \frac{\text{IC}_{50}}{K_I}\right)} + [S_0]} = \left(\frac{\frac{v_{\text{max}}}{\left(1 + \frac{\text{IC}_{50}}{K_I}\right)}[S_0]}{\frac{K_m}{\left(1 + \frac{\text{IC}_{50}}{K_I}\right)} + [S_0]} \right) \left(\frac{K_m + [S_0]}{v_{\text{max}}[S_0]} \right) = \frac{\frac{K_m + [S_0]}{\left(1 + \frac{\text{IC}_{50}}{K_I}\right)}}{\frac{K_m}{\left(1 + \frac{\text{IC}_{50}}{K_I}\right)} + [S_0]}$$

$$\frac{1}{2} = \frac{K_m + [S_0]}{K_m + [S_0] \left(1 + \frac{\text{IC}_{50}}{K_I}\right)}$$

$$\frac{1}{2} \left[K_m + [S_0] + [S_0] \left(\frac{\text{IC}_{50}}{K_I} \right) \right] = K_m + [S_0]$$

$$[S_0] \left(\frac{\text{IC}_{50}}{K_I} \right) = K_m + [S_0]$$

$$\text{IC}_{50} = K_I \left(\frac{K_m}{[S_0]} + 1 \right) = (75 \times 10^{-9} \text{ M}) \left(\frac{25 \times 10^{-6} \text{ M}}{5 \times 10^{-3} \text{ M}} + 1 \right) = 75.4 \text{ nM}$$

20.320 Problem Set 6
Question 2

In order to estimate the kinetics for a given enzyme-substrate reaction, an *in vitro* reaction is typically set up with a reporter for product formation. For instance, *in vitro* kinase reactions typically use ^{32}P , a radioactive isotope of phosphate in the γ -position of ATP, and then measure the amount of radioactivity incorporated in the substrate. Although most of these reactions are performed with high substrate:enzyme ratio, often it is difficult to obtain large amounts (or large concentration) of substrate.

Consider a single-substrate enzymatic reaction with no inhibition and the following parameters:

- Reaction volume: 100 μL
- Initial substrate concentration: 5 μM
- Enzyme concentration: 0.5 μM
- $k_1 = 3 \times 10^5 \text{ L mol}^{-1} \text{ sec}^{-1}$
- $k_{-1} = 5 \text{ sec}^{-1}$
- $k_{\text{cat}} = 3 \text{ sec}^{-1}$

- A) Under the above conditions, calculate the characteristic time for this system to reach quasi-steady state.

$$K_M = \frac{k_{-1} + k_{\text{cat}}}{k_1} = \frac{5 + 3 \text{ s}^{-1}}{3 \times 10^5 \text{ L mol}^{-1} \text{ s}^{-1}} = 2.7 \times 10^{-5} \text{ M}$$

$$t_{\text{QSSA}} = \frac{1}{k_1(K_M + [S]_0)} = \frac{1}{(3 \times 10^5 \text{ L mol}^{-1} \text{ s}^{-1})(2.7 \times 10^{-5} + 5 \times 10^{-6} \text{ M})} = 0.1 \text{ s}$$

- B) What is the characteristic time to deplete substrate under these conditions?

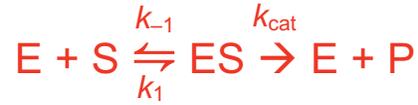
$$t_{[S]} = \frac{K_M + [S]_0}{k_{\text{cat}}[E]_0} = \frac{2.7 \times 10^{-5} + 5 \times 10^{-6} \text{ M}}{(3 \text{ s}^{-1})(0.5 \times 10^{-6} \text{ M})} = 21 \text{ s}$$

- C) Use MATLAB to compare the kinetics of product formation in this system with and without applying the Michaelis-Menten approximation.

- For simulating the reaction with no approximations, use `ode23s` to solve the representative system of differential equations with the appropriate initial conditions. Simulate the system under Michaelis-Menten conditions by simplifying your equations with the appropriate assumptions. Plot product formation over time for the first minute of the reaction on the same axes for both simulations.

20.320 Problem Set 6
Question 2

For an enzymatic reaction without inhibition, the reaction scheme is as follows:



The differential equations governing this system are:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_{\text{cat}}[ES]$$

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES]$$

$$\frac{d[P]}{dt} = k_{\text{cat}}[ES]$$

Since $[E]_0 = [E] + [ES]$, we can substitute $[E]_0 - [ES] = [E]$ in our equations, yielding the following system:

$$\frac{d[ES]}{dt} = k_1([E]_0 - [ES])[S] - k_{-1}[ES] - k_{\text{cat}}[ES]$$

$$\frac{d[S]}{dt} = -k_1([E]_0 - [ES])[S] + k_{-1}[ES]$$

$$\frac{d[P]}{dt} = k_{\text{cat}}[ES]$$

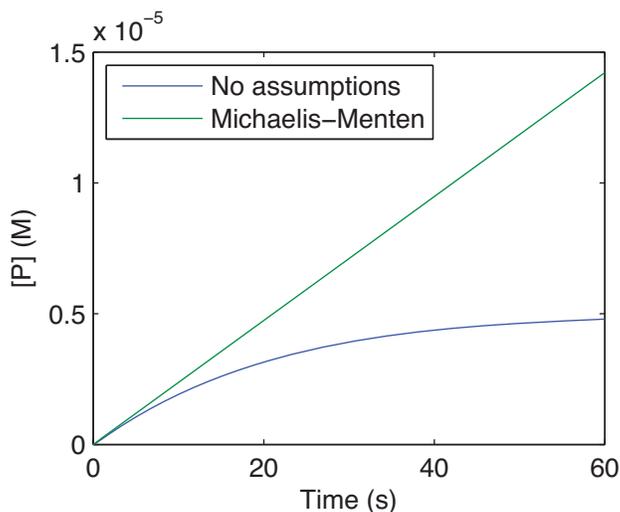
For the Michaelis-Menten approximation, the system simplifies as follows. Substrate is in excess, so $[S]$ is replaced by $[S]_0$, and $[ES]$ is calculated based on quasi-steady state conditions. Therefore:

$$[ES]_{\text{QSSA}} = \frac{[E]_0[S]_0}{K_M + [S]_0}, \text{ where } K_M = \frac{k_{-1} + k_{\text{cat}}}{k_1}$$

$$\frac{d[P]}{dt} = k_{\text{cat}}[ES]$$

Coding this system into MATLAB produces the following results:

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Question 3



- ii. Based on your plot and on the criteria discussed in class, evaluate the validity of the Michaelis-Menten approximation under these conditions. Discuss which assumptions hold and which do not. Why are your curves different?

In order for the Michaelis-Menten approximation to hold, the following criteria must be met:

- Must reach quasi-steady state well before substrate depletion ($t_{QSSA} \ll t_{[S]}$). From Parts A and B, we can see that this condition is met.

- $\frac{[E_0]}{K_M + [S_0]} \ll 1$

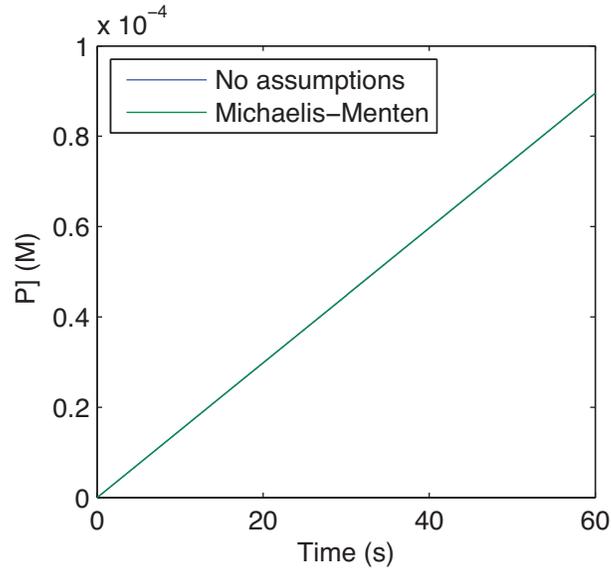
In this case: $\frac{0.5 \times 10^{-6} \text{ M}}{\frac{5+3 \text{ s}^{-1}}{3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}} + 5 \times 10^{-6} \text{ M}} = 0.016$ Therefore, this condition is met.

- Substrate must be in great excess, since we are assuming $[S] = [S_0]$. Since the other two conditions have been met, we are likely entering a substrate-limiting regime when the two curves begin diverging. Therefore, $[S_0]$ should be increased.

- iii. Change an aspect of the original system (either rate constants or initial conditions) such that the Michaelis-Menten approximation is valid for this time scale. On a new plot, overlay your two curves to show they are the same.

Increase $[S_0]$ by a factor of 1000, such that $[S_0] = 5 \text{ mM}$. This produces the following results:

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Question 3



Code:

```
% Initial conditions
E0 = 0.5e-06;
S0 = 5e-06; % Change this to 5e-03 for Part Ciii.
ES0 = 0;
P0 = 0;

% Rate constants
kf = 3e+05;
kr = 5;
kcat = 3;

% Initialize parameters vector
params = [P0 ES0 S0 E0 kf kr kcat];

% Set timespan
time = [0:0.1:60];

[t y] = ode23s(@reaction, time, params); % Solve with no assumptions

% Calculate Michaelis-Menten constant and apply QSSA for [ES]:
Km = (kr + kcat)/kf;
ES = (E0*S0)/(Km + S0);

params = [P0 ES kcat];
[t z] = ode23s(@MMrxn, time, params); % Solve with Michaelis-Menten

plot(t, y(:,1), t, z(:,1))
legend('No assumptions', 'Michaelis-Menten', 'location', 'NorthWest');
xlabel('Time (s)');
ylabel('[P] (M)');
```

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Question 3

```
function [out] = reaction(t, params)

    % Initial conditions
    P = params(1);
    ES = params(2);
    S = params(3);

    % Parameters and Rate constants
    E0 = params(4);
    kf = params(5);
    kr = params(6);
    kcat = params(7);

    % System of differential equations
    dPdt = kcat * ES;
    dESdt = kf*(E0 - ES)*S - kr*ES - kcat*ES;
    dSdt = -kf*(E0 - ES)*S + kr*ES;

    % Return changing values for P, ES, and S
    out = [dPdt; dESdt; dSdt; 0; 0; 0; 0];

return

function [out] = MMrxn(t, params)

    % Initial conditions
    P = params(1);
    ES = params(2);

    % Parameters
    kcat = params(3);
    % Differential Equation
    dPdt = kcat * ES;

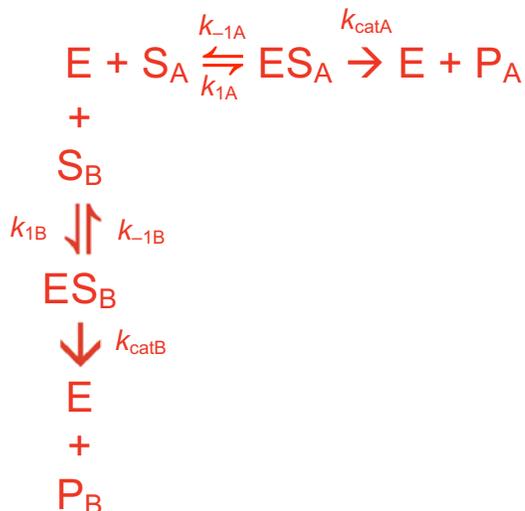
    % Return changing values for P, and S
    out = [dPdt; 0; 0];

return
```

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Question 3

Enzymes can typically catalyze reactions involving many different substrates, and can therefore be used to produce multiple products. Often these reactions have different K_M and k_{cat} values, which provides a degree of specificity. This problem will examine the effects of competition for enzyme binding on the enzyme's substrate specificity.

- A) Provide a schematic diagram and write out the differential equations with the appropriate rate constants for two substrates reacting with the same enzyme to form two different products. Assume that the enzyme has one active site that can be occupied by a single substrate molecule at a time.



$$\frac{d[P_A]}{dt} = k_{catA}[ES_A]$$

$$\frac{d[P_B]}{dt} = k_{catB}[ES_B]$$

$$\frac{d[S_A]}{dt} = -k_{1A}[E][S_A] + k_{-1A}[ES_A]$$

$$\frac{d[S_B]}{dt} = -k_{1B}[E][S_B] + k_{-1B}[ES_B]$$

$$\frac{d[ES_A]}{dt} = k_{1A}[E][S_A] - (k_{-1A} + k_{catA})[ES_A]$$

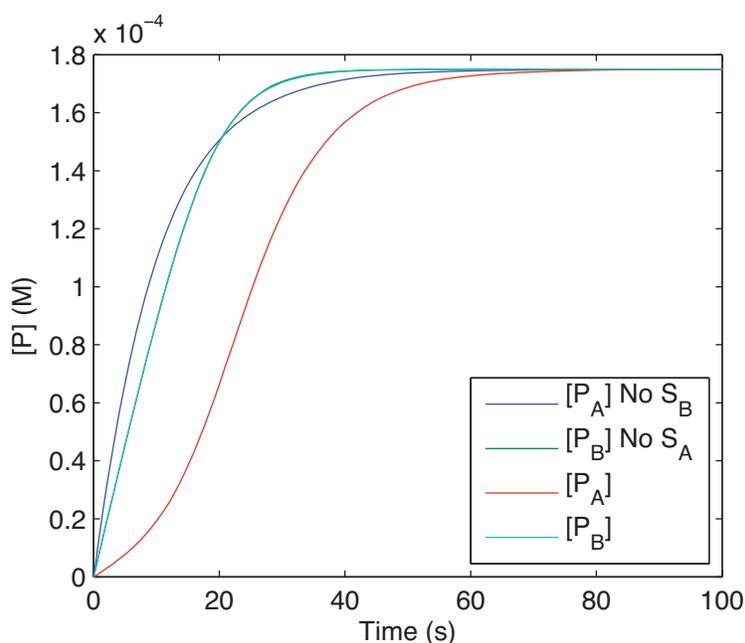
$$\frac{d[ES_B]}{dt} = k_{1B}[E][S_B] - (k_{-1B} + k_{catB})[ES_B]$$

$$\frac{d[E]}{dt} = -k_{1A}[E][S_A] + (k_{-1A} + k_{catA})[ES_A] - k_{1B}[E][S_B] + (k_{-1B} + k_{catB})[ES_B]$$

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Question 3

B) To estimate the temporal effects as well as the specificity effects, we will compare the level of product formation for each substrate at various times up 100 s, in the presence and absence of competition. Using an initial enzyme concentration of 50 μM and an initial substrate concentration of 175 μM for each substrate, graph the formation of product 1 assuming no product 2 is formed, product 2 assuming no product 1 is formed, and product 1 and product 2 assuming that the other can be formed on the same graph in MATLAB (you should have 4 lines total on the graph) for the time period of 0 to 100 seconds. Use the following rate constants:

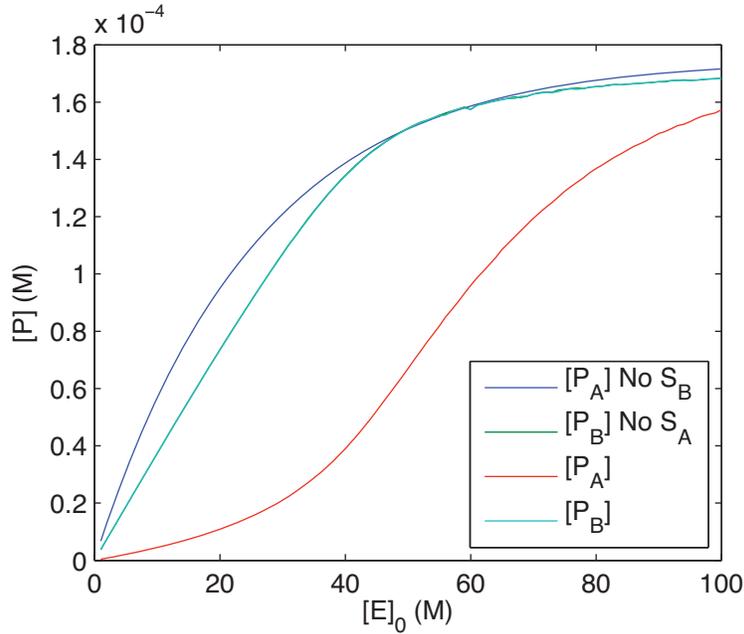
Rate of association between Enzyme and Substrate 1:	$5 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$
Rate of dissociation of the Enzyme–Substrate 1 complex:	$3 \times 10^1 \text{ s}^{-1}$
Rate of formation of Product 1 from Enzyme–Substrate 1 complex:	$2 \times 10^1 \text{ s}^{-1}$
Rate of association between Enzyme and Substrate 2:	$2 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$
Rate of dissociation of the Enzyme–Substrate 2 complex:	$2 \times 10^1 \text{ s}^{-1}$
Rate of formation of Product 2 from Enzyme–Substrate 2 complex:	$2 \times 10^{-1} \text{ s}^{-1}$



See Part C for code.

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Question 3

Compare the concentration of each product at a time of 20 seconds as the enzyme concentration increases from 1 to 100 μM , repeat for each substrate in the absence of competition, then repeat with both substrates together as in Part B).



Code:

```
% Initial Conditions
PA0 = 0;
PB0 = 0;
SA0 = 175e-06;
SB0 = 175e-06;
ESA0 = 0;
ESB0 = 0;
E0 = 50e-06;

% Rate Constants
kfA = 5e+03;
krA = 3e+01;
kcatA = 2e+01;
kfB = 2e+06;
krB = 2e+01;
kcatB = 2e-01;

% Time span for solver
time = (0:1:100);

% Establish parameters array for solver
params = [PA0 PB0 SA0 SB0 ESA0 ESB0 E0 kfA krA kcatA kfB krB kcatB];

% Solve system with Product 1 alone: i.e. [SB]_0 = 0
params(4) = 0;
[t A_only] = ode15s(@reaction, time, params);
params(4) = SB0;
```

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Question 3

```
% Solve system with Product 2 alone: i.e. [SA]_0 = 0
params(3) = 0;
[t B_only] = odel15s(@reaction, time, params);
params(3) = SA0;

% Solve system with both products present
[t both] = odel15s(@reaction, time, params);

% Plotting for Part B
figure(1)
plot(t, A_only(:,1), t, B_only(:,2), t, both(:,1), t, both(:,2))
legend('[P_A] No S_B', '[P_B] No S_A', '[P_A]', '[P_B]', 'location',
'SouthEast');
xlabel('Time (s)')
ylabel('[P] (M)')

% Part C: Concentrations at t = 20s
figure(2)
time = [0:10:20];

for E0 = (1:100)
    % Solve for range of initial enzyme concentrations from 1 - 100  $\mu\text{M}$ 
    params(7) = E0 * 1e-06;

    % Solve system with Product 1 alone: i.e. [SB]_0 = 0
    params(4) = 0;
    [t A_only] = odel15s(@reaction, time, params);
    A_curve(E0) = A_only(size(A_only, 1), 1); % Add to array of [P1] vs. [E0]
    params(4) = SB0;

    % Solve system with Product 2 alone: i.e. [SA]_0 = 0
    params(3) = 0;
    [t B_only] = odel15s(@reaction, time, params);
    B_curve(E0) = B_only(size(B_only, 1), 2); % Add to array of [P2] vs. [E0]
    params(3) = SA0;

    % Solve system with both products present
    [t both] = odel15s(@reaction, time, params);

    % Add to array of [P1] & [P2] vs. [E0]
    both_curve(E0,:) = [both(size(both, 1),1) both(size(both, 1),2)]; end

% Plot four curves vs [E0] on same axes
E0 = (1:100);
plot(E0, A_curve, E0, B_curve, E0, both_curve(:,1), E0, both_curve(:,2))
xlabel('[E]_0 (M)')
ylabel('[P] (M)')
legend('[P_A] No S_B', '[P_B] No S_A', '[P_A]', '[P_B]', 'location',
'SouthEast');
```

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Question 3

```
function [out] = reaction(t, params)

% Initial Conditions
SA = params(3);
SB = params(4);
ESA = params(5);
ESB = params(6);
E = params(7);

% Rate Constants
kfA = params(8);
krA = params(9);
kcatA = params(10);
kfB = params(11);
krB = params(12);
kcatB = params(13);

% System of differential equations
dPA dt = kcatA * ESA;
dPB dt = kcatB * ESB;
dSA dt = -kfA * E * SA + krA * ESA;
dSB dt = -kfB * E * SB + krB * ESB;
dESA dt = kfA * E * SA - (krA + kcatA) * ESA;
dESB dt = kfB * E * SB - (krB + kcatB) * ESB;
dE dt = -kfA * E * SA + (krA + kcatA) * ESA - kfB * E * SB + (krB + kcatB) * ESB;

out = [dPA dt; dPB dt; dSA dt; dSB dt; dESA dt; dESB dt; dE dt; 0; 0; 0; 0; 0; 0];

return
```

- C) Explain the shape of the curve of product 1 formation in Parts B) and C). What type of inhibition is the early part of the curve analogous to? How does the overall curve shape from this type of inhibition differ with the curves you produced and why?

The shape of the curve for product 1 formation is due to competition between substrate 1 and substrate 2 for enzyme binding. This is analogous to competitive inhibition: substrate 2 is a stronger binder to the enzyme than substrate 1, but it is converted to product at a slower rate after binding. This makes it a pseudo-competitive inhibitor since it is essentially blocking substrate 1 from entering the site. It is different than normal competitive inhibition, however, in the sense that substrate 2 is used up with time and therefore has a diminishing effect of preventing the formation for product 1. This is the reason that we see an S-like curve shape in part b. In part d we see an increasing amount of product formed at 20 seconds because as more enzyme is available, more of substrate 1 will be able to bind as the substrate 2 concentration is more rapidly depleted.

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20.320 Analysis of Biomolecular and Cellular Systems
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