Problem 1

Ultimately we would like to calculate the rate of product formation, which from the law of mass action is given by:

$$\frac{d[P]}{dt} = k_2[C_2] - k_{-2}[P][E]$$

Assume that $[C_2]$ is at steady state:

$$0 = \frac{d[C_2]}{dt} = k_r[C_1] + k_{-2}[P][E] - k_2[C_2] = k_r[C_1] - \frac{d[P]}{dt} \implies \frac{d[P]}{dt} = k_r[C_1]$$

Assume that $[C_1]$ is also at steady state:

$$0 = \frac{d[C_1]}{dt} = k_1[S][E] - (k_{-1} + k_r)[C_1] \implies [C_1] = \frac{k_1[S][E]}{k_{-1} + k_r}$$

Given this expression and the above result:

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

The desired rate law expression is equivalent to this statement, but does not contain the variable $[E]$, suggesting we must substitute an equivalent expression for $[E]$. Noting that the enzyme moiety is conserved, we can see that:

$$\begin{aligned}
[E_{\text{tot}}] &= [E] + [C_1] + [C_2] \\
[E] &= [E_{\text{tot}}] - [C_1] - [C_2] \\
&= [E_{\text{tot}}] - \frac{k_1}{k_{-1} + k_r}[S][E] - [C_2]
\end{aligned}$$

To simplify further, we must find an expression for $[C_2]$. The statement that the product readily rebinds free enzyme suggests that it may be appropriate to assume a rapid equilibrium assumption for the right-most reversible reaction:

$$\begin{aligned}
k_2[C_2] &= k_{-2}[P][E] \\
[C_2] &= \frac{k_{-2}}{k_2}[P][E]
\end{aligned}$$

where we have again defined K_p for convenience. Plugging this expression for $[C_2]$ into our expression for $[E]$ derived from moiety conservation and rearranging, we get:

$$
\begin{aligned}
[E] &= [E_{\text{tot}}] - \frac{k_1}{k_{-1}+k_r}[S][E] - K_p[P][E] \\
\left(1 + \frac{k_1[S]}{k_{-1}+k_r} + \frac{k_{-2}}{k_2}[P]\right)[E] &= [E_{\text{tot}}] \\
\left(\frac{k_{-1}+k_r}{k_1} + [S] + \frac{k_{-2}(k_{-1}+k_r)}{k_1 k_2}[P]\right)[E] &= \frac{k_{-1}+k_r}{k_1}[E_{\text{tot}}] \\
\left(K_m + [S] + \frac{K_m}{K_p}[P]\right)[E] &= K_m[E_{\text{tot}}] \\
[E] &= \frac{K_m[E_{\text{tot}}]}{[S] + K_m\left(1 + \frac{[P]}{K_p}\right)}
\end{aligned}
$$

where we have defined K_m and K_p for convenience. This expression for $[E]$ can be plugged into equation 1 to get:

$$
\frac{d[P]}{dt} = \frac{k_r[S]}{K_m}\left(\frac{K_m[E_{\text{tot}}]}{[S] + K_m\left(1 + \frac{[P]}{K_p}\right)}\right) = \frac{V_{\max}[S]}{[S] + K_m\left(1 + \frac{[P]}{K_p}\right)}
$$

Problem 2

We illustrate two approaches: the first uses rapid equilibrium assumptions for binding, and the second uses quasi-steady-state assumptions as the problem statement hints.

Rapid equilibrium assumptions

Enzyme moiety conservation gives the expression:

$$
[E_{\text{tot}}] = [E] + [ES] + [EI] + [ESI]
$$

To simplify this, we will assume that all substrate and inhibitor binding events are in rapid equilibrium:

$$
\begin{aligned}
k_1[E][S] = k_{-1}[ES] &\implies [E] = \frac{k_{-1}[ES]}{k_1[S]} \\
k_3[E][I] = k_{-3}[EI] &\implies [EI] = \frac{k_3[I]}{k_{-3}}\left(\frac{k_{-1}[ES]}{k_1[S]}\right) = \frac{k_{-1}k_3[I][ES]}{k_{-3}k_1[S]} \\
k_3[ES][I] = k_{-3}[ESI] &\implies [ESI] = \frac{k_3[ES][I]}{k_{-3}}
\end{aligned}
$$

Plugging these equations into the moiety conservation statement allows us to find an expression for [ES]:

$$
\begin{aligned}
[E_{\text{tot}}] &= \left(\frac{k_{-1}}{k_1[S]} + 1 + \frac{k_{-1}k_3[I]}{k_1k_{-3}[S]} + \frac{k_3[I]}{k_{-3}}\right)[ES] \\
&= \left(1 + \frac{k_3[I]}{k_{-3}}\right)\left(1 + \frac{k_{-1}}{k_1[S]}\right)[ES] \\
[ES] &= \frac{[E_{\text{tot}}]}{\left(1 + \frac{k_3[I]}{k_{-3}}\right)\left(1 + \frac{k_{-1}}{k_1[S]}\right)} = \frac{[E_{\text{tot}}][S]}{\left(1 + \frac{[I]}{K_i}\right)([S] + K_m)}
\end{aligned}
$$

Notice that the definition of K_m here is a bit unexpected: this is due to our use of rapid equilibrium rather than steady-state assumptions. Plugging this into the equation for rate of product formation, we get:

$$
\frac{d[P]}{dt} = k_2[ES] = \left(\frac{k_2[E_{\text{tot}}]}{1 + \frac{[I]}{K_i}}\right)\frac{[S]}{[S] + K_m} = \left(\frac{V_{\max}}{1 + \frac{[I]}{K_i}}\right)\frac{[S]}{[S] + K_m}
$$

Quasi-steady-state assumptions

The quasi-steady-state assumptions give three expressions:

$$
\begin{aligned}
\frac{d[EI]}{dt} &= k_3[E][I] + k_{-1}[ESI] - (k_1[S] + k_{-3})[EI] = 0 \\
\frac{d[ES]}{dt} &= k_1[E][S] + k_{-3}[ESI] - (k_3[I] + k_{-1} + k_2)[ES] = 0 \\
\frac{d[ESI]}{dt} &= k_1[EI][S] + k_3[ES][I] - (k_{-3} + k_{-1})[ESI] = 0
\end{aligned}
$$

We can eliminate [E] from these expressions using moiety conservation, i.e.

$$
[E] = [E_{\text{tot}}] - [ES] - [EI] - [ESI]
$$

Plugging in and rearranging, we get:

$$
\begin{aligned}
\frac{d[EI]}{dt} &= k_3[E_{\text{tot}}][I] - k_3[I][ES] + (k_{-1} - k_3[I])[ESI] - (k_1[S] + k_{-3} + k_3[I])[EI] = 0 \\
\frac{d[ES]}{dt} &= k_1[E_{\text{tot}}][S] - k_1[S][EI] + (k_{-3} - k_1[S])[ESI] - (k_3[I] + k_{-1} + k_2 + k_1[S])[ES] = 0 \\
\frac{d[ESI]}{dt} &= k_1[EI][S] + k_3[ES][I] - (k_{-3} + k_{-1})[ESI] = 0
\end{aligned}
$$

The third quasi-steady-state assumption gives us that:

$$
[ESI] = \frac{k_1}{k_{-3} + k_{-1}}[EI][S] + \frac{k_3}{k_{-3} + k_{-1}}[ES][I]
$$

$$\frac{d[EI]}{dt} = k_3[E_{\text{tot}}][I] - k_3[I]\left(\frac{k_{-3}+k_3[I]}{k_{-3}+k_{-1}}\right)[ES] - \left(k_1[S]\left(\frac{k_{-3}+k_3[I]}{k_{-3}+k_{-1}}\right) + k_{-3} + k_3[I]\right)[EI] = 0$$

$$\frac{d[ES]}{dt} = k_1[E_{\text{tot}}][S] - k_1[S]\left(\frac{k_{-1}+k_1[S]}{k_{-3}+k_{-1}}\right)[EI] - \left(k_3[I]\left(\frac{k_{-1}+k_1[S]}{k_{-3}+k_{-1}}\right) + k_{-1} + k_2 + k_1[S]\right)[ES] = 0$$

```
function []  = mutualrepression()
    % Pick some parameter values for plotting
    global k n
    k = 0.5; n=3;

    [x, y] = meshgrid(0:0.05:1.5, 0:0.05:1.5);
    dx = k ./(k+y.^n) - x;
    dy = k ./(k+x.^n) - y;
    r = (dx.^2 + dy.^2).^0.5;
    dx = dx ./ r;
    dy = dy ./ r;

    quiver(x,y,dx,dy); hold on;
    xlabel('[X]')
    ylabel('[Y]')
    axis([0,1.5,0,1.5])
    [t, c] = ode45(@updater, [0 50], [0.7, 0.8]);
    plot(c(:,1),c(:,2),'-r', 'LineWidth', 3)
    plot(0.7, 0.8, 'or');
    [t, c] = ode45(@updater, [0 50], [0.6,0.5]);
    plot(c(:,1),c(:,2),'-g', 'LineWidth', 3);
    plot(0.6, 0.5, 'og');
    [t, c] = ode45(@updater, [0 50], [1.4,0.2]);
    plot(c(:,1),c(:,2),'-b', 'LineWidth', 3);
    plot(1.4, 0.2, 'ob');
    [t, c] = ode45(@updater, [0 50], [0.3,1.2]);
    plot(c(:,1),c(:,2),'-k', 'LineWidth', 3);
    plot(0.3, 1.2, 'ok');
    [t, c] = ode45(@updater, [0 50], [0.3,0.3]);
    plot(c(:,1),c(:,2),'-m', 'LineWidth', 3);
    plot(0.3, 0.3, 'om');

end

function dc = updater(t, c)
    x = c(1);
    y = c(2);
    global k n
    dx = k/(k+y^n) - x;
    dy = k/(k+x^n) - y;
    dc = [dx; dy];
end
```