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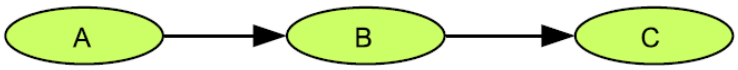
Synthetic Biology
Practical 1 Write Up

Part III: Building the first model

The simple system below was designed in CellDesigner modelling the conversion of a molecule A to B and subsequently to C. C is the degradation product of B and the rates of changes of both A and B are dependent upon their concentrations, as reflected in the differential equations below.



Below are the equations derived from the mass action law to model the conversion of A to B and from B to C:

$$\frac{d[A]}{dt} = -k_1 * [A]$$
$$\frac{d[B]}{dt} = k_1 * [A] - k_2 * [B]$$
$$\frac{d[C]}{dt} = k_2 * [B]$$

The parameters were defined per the values below:

$k_1 = 1.0$

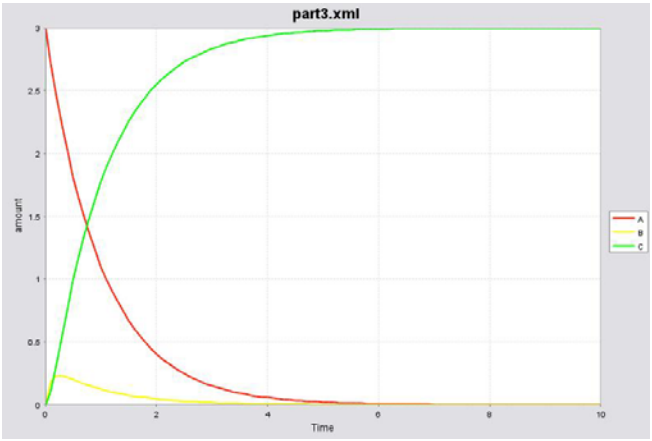
$k_2 = 10.0$

$[A]_0 = 3.0$

$[B]_0 = 0$

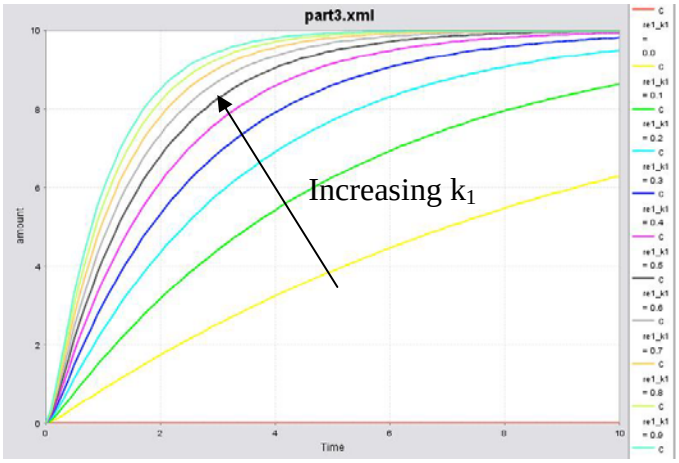
$[C]_0 = 0$

The initial results from the simulation are shown in the graph below:

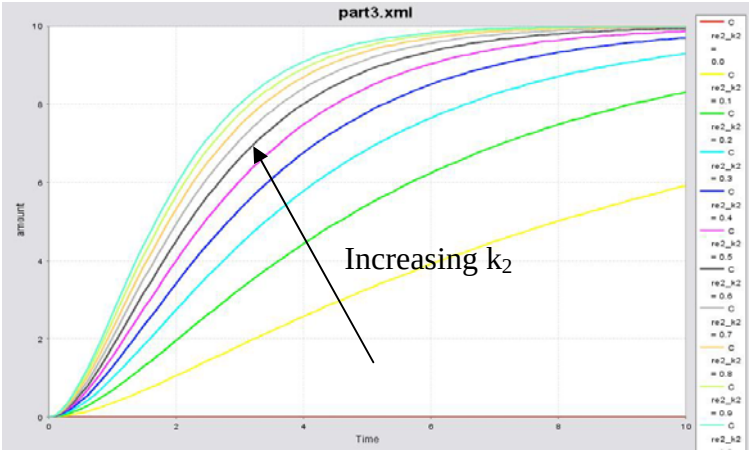


From the graph, we can see a steady exponential decrease in the amount of A, an increase in the amount of B for a tenth of a second following by a decrease and subsequent increase in the amount of C. This behaviour is expected from our model as B is a "transition" state between A and C and we are not assuming the presence of any reversible reactions. After 5 seconds (for our parameter values and initial conditions), the amount of A and C come to a steady state value where there is no A or B, and only C is present.

If we take a look at the production of C and what influences it, it would seem that by increasing both k_1 and k_2, this will not have any effect on the final steady state value of C, but will have an effect on the rate at which it reaches that steady state. If we take a look at one parameter at a time, we see this effect occurring quite vividly. As seen in the figure directly below, by increasing the value of k_1, the eventual steady state value of C is unchanged (except when $k_1 = 0$), but the rate at which it reaches that steady state does increase.



Looking at the parameter sweep for k_2, we see a similar effect in the figure directly below.



If we want to maximize the concentration of B, we have to look at the rates at which B is being "produced" and "degraded". To maximize this, we must have a fast rate of production and have the degradation rate be as slow as possible. This will make the "degradation" reaction the rate determining step and will limit the conversion of B to

C. With a maximum limit of 10% away from our initial values, the values that will maximise the production of B are given below.

$$k_1 = 1.1$$
$$k_2 = 9.0$$

Part IV: Synthesis-Degradation Reaction

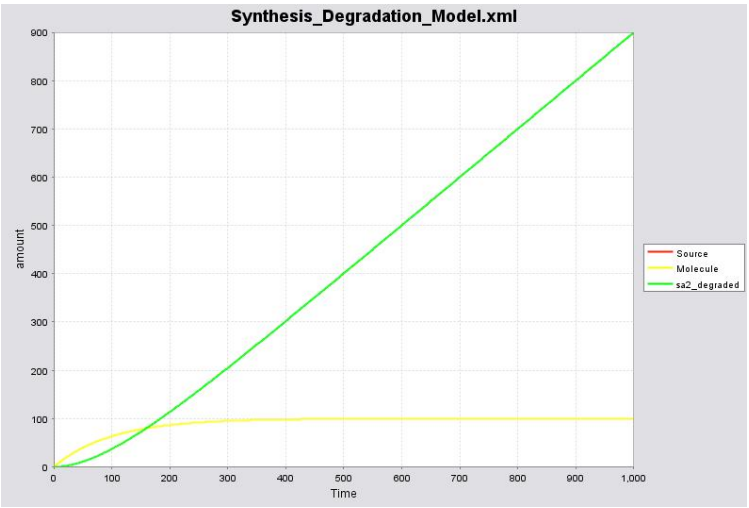
The system below was designed in CellDesigner:

Below are the equations derived from the mass action law to describe the kinetics of the above model, where A is the molecule in question.

$$\frac{d[A]}{dt} = k_1 - k_2 * [A]$$

Here, the production of A does not depend on the concentration of the source, but is constitutively expressed. The degradation of A is proportional to the amount of A present. Below is the simulation output with the following parameters and initial conditions.

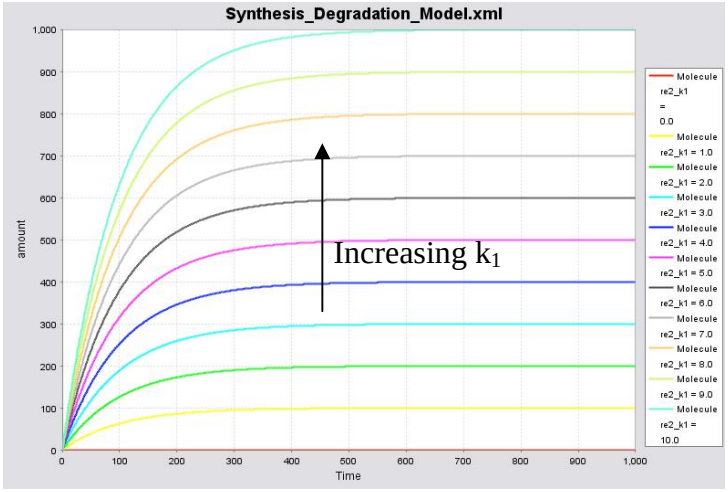
$$k_1 = 1.0$$
$$k_2 = 0.01$$
$$[A]_0 = 0$$



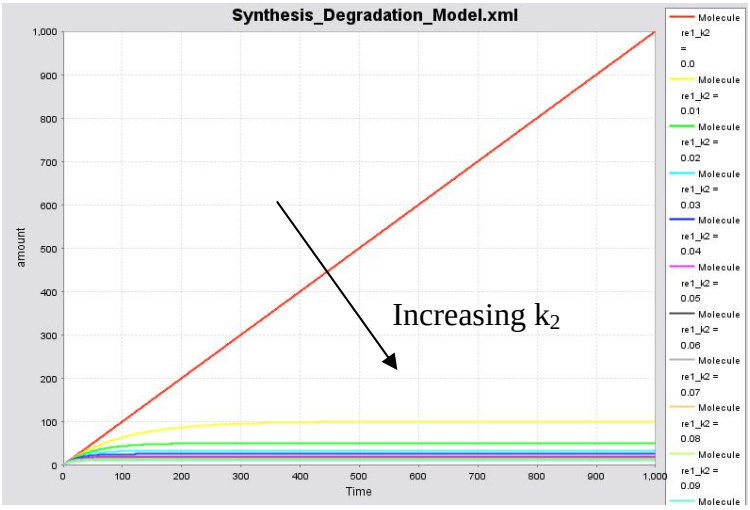
We can see from the simulation graph above that the amount of molecule will reach a steady state value, but the degradation products continue to grow as expected (due to the continuous production of A). If we take a look at the equations, we can put a quantitative value on the stead state concentration of A by setting $\frac{d[A]}{dt} = 0$. We obtain the equilibrium value of A to be: $[A_{eq}] = \frac{k_1}{k_2}$. In our case, this comes out to be

100 and is verified by the graph above. Furthermore, we can allow the source to be zero because the production of the molecule (yellow line) is not dependent upon the concentration of the source. Here, it is constitutively expressed and reaches steady state after about 300 seconds.

Using the parameter scan, we can see that as we increase k_1, the steady state value of A will increase, but increasing the value of k_2, will decrease the value of the steady state concentration of A. Below, we first show the effect of k_1 in the range 0 to 10 with interval 1.



And below we show the effect of increase k_2 in the range 0 to 0.1 with interval 0.01. If we do not allow for degradation, A will continually be produced with a linear relationship leading to the red line below. If degradation is allowed for, a dynamic equilibrium is set up between the rate of production and degradation of molecule A, and the amount of A reaches a steady state being determined by the ratio of k_1 and k_2.



The analytical solution to the differential equation was obtained from matlab and is displayed below with the initial condition $A(0) = 0$.

$$[A](t) = \frac{k_1}{k_2}\left(1 - e^{-k_2 t}\right)$$

Now if we consider $k_1 = 0$, we are left with the equation below:

$$\frac{d[A]}{dt} = k_2[A]$$

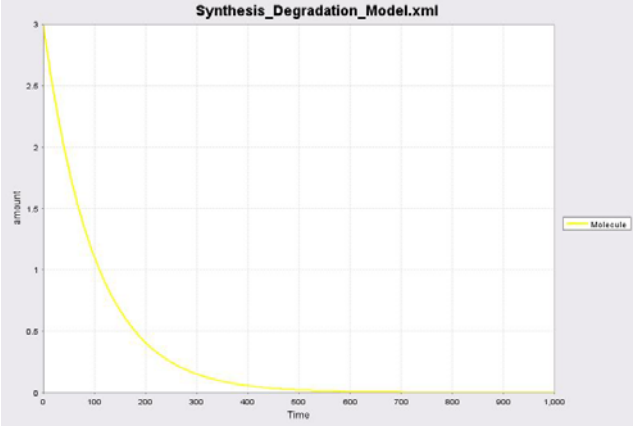
We can also solve this equation analytically to give the solution to the first order reaction whose differential equation is above.

$$[A](t) = [A]_0 e^{-k_2 t}$$

The half-life of the species A is the time it takes for the remaining concentration to decrease by half. This time is given by the equation below ([A](t) = (½)A_0).

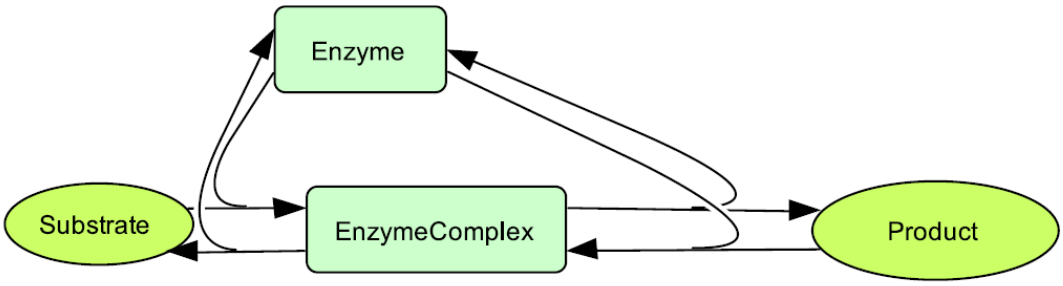
$$t = \frac{\ln 2}{k_2}$$

We can visualize this on CellDesigner by altering the parameters and the initial conditions where A_0 is not equal to 0. For our values, $k_2 = 0.01$, so the half-life is approximately 69 s, which is seen on the graph below as the time the graph takes to reach the amount of 1.5 (or also the time it takes the amount to decrease from 1.5 to 0.75).



Part V: Michaelis-Menten Kinetics

The model below was designed in CellDesigner:



The following kinetic rate laws were derived from this model:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E + P$$

$$\frac{d[E]}{dt} = k_2[ES] - k_1[E][S]$$
$$\frac{d[S]}{dt} = k_2[ES] - k_1[E][S]$$
$$\frac{d[ES]}{dt} = k_1[E][S] - k_2[ES] - k_3[ES] + k_4[E][P]$$
$$\frac{d[P]}{dt} = k_3[ES] - k_4[E][P]$$

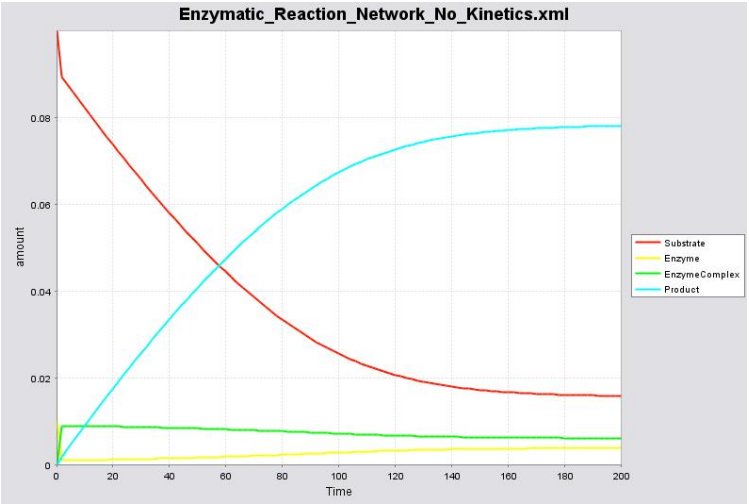
The kinetic rate laws that were used are shown below:

Substrate + Enzyme →Enzyme Complex: s*e*k1
Enzyme Complex → Substrate + Enzyme: es*k2
Enzyme Complex → Product + Enzyme: es*k3
Product + Enzyme → Enzyme Complex: p*e*k4

The following parameters and initial conditions were used in the model:

$k_1 = 100000$
$k_2 = 1000$
$k_3 = 0.1$
$k_4 = 2.0$
$[E]_0 = 0.01$
$[S]_0 = 0.1$
$[ES]_0 = 0$
$[P]_0 = 0$

The results of the simulation were shown below. As is seen, all the variables achieve a steady-state concentration after a long period of time (about 60 sec). Initially, there is a fast drop in substrate and enzyme levels as they are bound to the enzyme very quickly. This causes a spike in the enzyme-substrate complex amount. As product begins to form, the enzyme-substrate complex amount decreases and reaches a steady level, while free enzyme also increases again as it is not saturated. It can also be seen that all of the concentrations reach a steady state after about 200 seconds as expected, but in the transient state, especially at very short times, it is not possible to assume that the reaction will be at steady state.



Also, we can note that the concentrations of the substrate and product are in a dynamic equilibrium with each other since reversible reactions are allowed in the model.

Let us investigate the Michaelis-Menten approximation, where we make the following assumptions:

1) That the steady state approximation applies and the concentration of the enzyme complex remains constant: $\frac{d[ES]}{dt} = 0$. It is important to note that this approximation only holds at long enough time scales. From the above graph where we modelled the system individually with both forward and reverse rates, it was seen that steady state is not achieved until 200 seconds.
2) The reverse reaction of product and enzyme to enzyme complex does not take place: $k_4 = 0$. Again, this is a simplification that we can make to help our analysis, but is not necessarily correct for all enzymes. For some enzymes, it is almost impossible to ensure that the reaction goes to completion and the product and substrate are usually in a dynamic equilibrium. This dynamic equilibrium can be seen in the previous model where the substrate concentration does not go to zero. Taking this assumption allows for the concentration of the substrate to go to zero, which is acceptable for some enzymatic reactions, but not plausible for others.

We can model the system with the following equations:

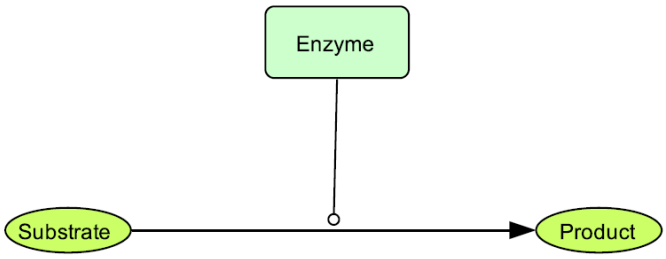
$$\frac{d[E]}{dt} = -k_1[E][S] + k_2[ES] + k_3[ES]$$
$$\frac{d[S]}{dt} = -k_1[E][S] + k_2[ES]$$
$$\frac{d[ES]}{dt} = k_1[E][S] - k_2[ES] - k_3[ES]$$
$$\frac{d[P]}{dt} = k_3[ES]$$

From the first and third equations, we can see that $\frac{d[ES]}{dt}+\frac{d[E]}{dt}=0$, and we can say that the total enzyme concentration $[E_0] = [ES] + [E]$.

Also from the third equation and our steady state assumption, we can define the Michaelis-Menten constant, k_m as follows: $k_m=\frac{k_2+k_3}{k_1}=\frac{[E][S]}{[ES]}$. Substituting $[E_0] = [ES] + [E]$, we obtain that $k_m[ES]=([E_0]+[ES])[S]$ and rearrange to give $[ES]=\frac{[E_0][S]}{k_m+[S]}$.

Substituting into the fourth equation, we obtain $\frac{d[P]}{dt}=k_3[ES]=k_3\frac{[E_0][S]}{k_m+[S]}$. We can define now $v_{max}=k_3[E_0]$ to obtain the general Michaelis-Menten equation form: $v=\frac{d[P]}{dt}=v_{max}\frac{[S]}{k_m+[S]}$.

In CellDesigner, we can model this system as per the diagram below.

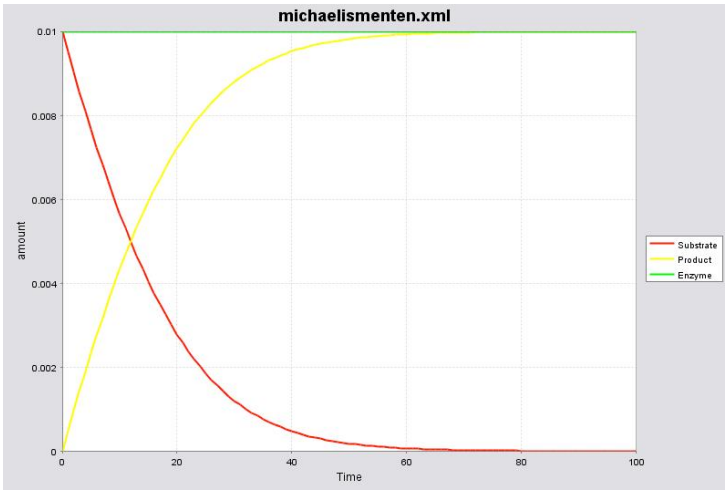


Here, we only have one kinetic law describing the production of the product using the Michaelis-Menten equation.

Kinetic Law used: $\frac{d[P]}{dt}=v_{max}\frac{[S]}{k_m+[S]}$, where v_{max} and k_m are defined per the original parameters k_1, k_2, k_3, and $[E_0]$ above.

$$v_{max}=k_3[E_0]=0.1\times 0.01=0.001$$
$$k_m=\frac{k_2+k_3}{k_1}=\frac{1000+0.1}{100000}=0.01$$

When simulated, this produces the graph below.



If we compare the above graph to the one produced when not making any assumptions, we can see that the concentration of the substrate does go to zero and there is a constant enzyme concentration (not seen in the previous model). Furthermore, the steady state concentration of product and substrate is close, but not the same as the predicted values in the previous model. This is probably because we are assuming here that all the substrate can be converted to product, but in the previous model, a dynamic equilibrium was set up between the substrate, enzyme complex, and formation of product. No equilibrium is present here and after a certain period of time, only product is present in the solution.

In the Michaelis-Menten model, we also assume that the enzyme complex concentration does not change over time. We can see in the previous model that this is not exactly the case, especially at the beginning of the reaction when free enzyme is suddenly bound to reactants. Furthermore, we can see that the enzyme complex concentration decreases over time to reach the dynamic equilibrium, an effect which is assumed not to happen in the Michaelis-Menten model.

Overall, the Michaelis-Menten model is a reasonably good model to describe enzyme kinetics, although it is not perfect. To a first approximation, it is relatively good and it is a way of reducing a four-dimensional model to a one-dimensional model that is straight-forward and easy to understand. With advances in computing, equation reduction might not be necessary any longer, but is still desired in order to simplify analysis of complicated mathematical models.