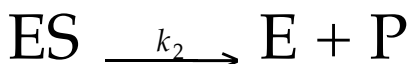
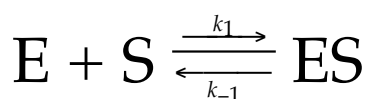


Exercise 8: Kinetics and equilibrium*Michaelis-Menten kinetics*

The study of enzyme kinetics is key to understanding the mechanism of how an enzyme performs its function. In 1913, Leonor Michaelis (German physician) and Maud Menten (Canadian physician) researched and developed a relatively simple model of enzyme kinetics.

It starts with the idea that an **enzyme** molecule (E) must meet with its **substrate** molecule (S). The substrate is the molecule on which the enzyme will perform its action. Together, they form a temporary, and until recently, unmeasurable complex called the enzyme-substrate complex (ES). That complex has two choices: fall apart back into a separate enzyme and substrate, or else let the enzyme do its work and form a **product** molecule (P), releasing the enzyme unchanged. The former of the paths is described by the backwards arrow in the first equation below, and the latter path is described by the second equation.



Since all of these reactions take place in water, it is sensible to measure the **concentrations** of various chemical species, not their mass or mole quantities. Thus, the notation [chemical species] will be used to denote their concentrations. For instance, [ES] is the concentration of enzyme-substrate complex in the solution.

The subscripted “k” letters above and below represent **rate constants** for the various reactions. A rate constant works as follows: suppose I want to know the **rate** at which the product molecule will form; I’ll call this “v”. Evidently, the rate at which the product will form is dependent on how much enzyme-substrate complex is made; after all, more ES means more P ultimately.

In other words, $v \propto [ES]$, which is translated as “the rate at which the product forms is proportional to the concentration of enzyme-substrate complex in the solution”.

The rate constant exists to turn that proportionality into an equation:

$$v = k_2 [ES]$$

The equation above is true for any time during the reaction, but the rate will vary as the reaction goes on and so is tough to measure. A fairly easy measurement is the initial rate at which the product appears; for instance, if the solution is colorless originally and the product is colored, it is an easy thing to measure how fast the product forms at first by simply measuring the amount of color the solution attains as the reaction proceeds.

This **initial rate** of product formation I'll call " v_0 " which is the rate at time zero (the start of the reaction").

$$v_0 = k_2 [ES]$$

Unfortunately for us, we cannot build a theoretical model of how enzymes work because neither of the quantities on the right side of the equation is measurable!

So we'll try a sneaky indirect method to get quantities that are measurable on the right side of the equation, so we will be able to predict what v_0 should be for a given enzyme and compare that to the actual v_0 of the enzyme. That is how science is supposed to work.

I'll make a distinction between enzymes that are "free" in solution and enzymes that are "bound" to a substrate molecule. Note that at any given time, any enzyme molecule in this solution must either be free or bound. We can then define:

$$[E_{total}] = [E] + [ES]$$

Now, recast the equation so that $[E]$ is alone on the left side.

Question 1:

$$[E] =$$

Put that aside for a minute. I will now define the rate at which the enzyme-substrate complex will form:

$$\textit{rate at which } [ES] \textit{ forms} = k_1 [E] [S]$$

How did I get that? Look at the two equations on the first page; note that the rate at which ES will form is dependent on how much E you have and how much S you have. Thus, lots of E but little S doesn't get you much ES, and lots of S but little E doesn't get you much ES, but lots of E and lots of S gets you lots of ES.

k_1 is simply the rate constant that holds the equation together along that pathway.

Now, replace the [E] in the equation above with Question 1's recasting of [E]:

Question 2:

$$\text{rate at which } [ES] \text{ forms} = k_1 (\quad) [S]$$

Next, I'll define a harder quantity: the rate at which the enzyme-substrate complex will be used up. First of all, when something is used up, the rate at which it is used up is only dependent on how much of the something there is in the first place. In other words, if you have a lot of something, you can use it up at a rate much faster than if you only had a little of something.

What I'm trying to say is that [ES] will be the only quantity involved.

Secondly, note that there are two pathways to use up ES: the "backwards" reaction in the first reaction on the first page and the second reaction on the first page. This means that there will be two rates that will need to be added together.

Question 3 (fill in the blanks before the [ES]'s on the right side):

$$\text{rate at which } [ES] \text{ is used up} = \quad [ES] + \quad [ES]$$

Now collect like terms on the right side.

Question 4:

$$\text{rate at which } [ES] \text{ is used up} = (\quad + \quad) [ES]$$

Next, we must make a crucial assumption, called the steady-state approximation. This means that, at any given instant, the amount of ES in the solution stays constant. This seems crazy, what with all the formation and using up going on, but note that if those two processes happen at the same rate, then the net change in the amount of ES is zero.

$$\text{rate at which } [ES] \text{ is used up} = \text{rate at which } [ES] \text{ forms}$$

Note that you have calculated both sides of the equation above in terms of other quantities; by the rules of algebra if $A = B$ and $B = C$ and $C = D$, then $A = D$. Write the equality above using the right sides of the equations you wrote in Questions 2 and 4.

Question 5:

=

With a couple of deft manipulations, you should be able to isolate all of the k (rate constant) terms on the right side of the equation. In fact, I'll help you with that; all you have to do is fill in the left side.

Question 6:

$$= \frac{k_{-1} + k_2}{k_1}$$

The quantity on the right side of the equation above is itself a constant and so is redefined as K_M , or the Michaelis-Menten constant.

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

K_M will be a good number to compare the activity of different enzymes, as we'll see later.

Now, I will take over the algebra. With the definition of K_M and one multiplication, I can change question 6's equation into:

$$([E_{total}] - [ES]) [S] = K_M [ES]$$

Expanding the left side of the equation:

$$[E_{total}] [S] - [ES] [S] = K_M [ES]$$

Gathering all the $[ES]$ terms on one side:

$$[E_{total}] [S] = (K_M + [S]) [ES]$$

Finally, isolating [ES] on one side:

$$[ES] = \frac{[E_{total}] [S]}{(K_M + [S])}$$

Remember $v = k_2 [ES]$ from the first page? I can now replace the [ES] in the equation above with the equation at the top of the page:

$$v = k_2 \left(\frac{[E_{total}] [S]}{(K_M + [S])} \right)$$

And remember how I complained that nothing on the right side of the equation was measurable? Well, now nearly everything is...except k_2 . We can get around this, though.

We have to think of the **fastest possible reaction rate**, given a certain amount of enzyme. Call this " v_{max} ". This will occur when there is so much substrate floating around in the solution, that every single enzyme molecule is bound and trying to work on the substrate.

$$v_{max} = k_2 [ES] = k_2 [E_{total}]$$

since all of the enzyme is bound up. Notice that the right side of the equation above is hidden in the equation just above that.

Now, you get to write the Michaelis-Menten equation for enzyme kinetics by replacing v_{max} into the expression for v .

Question 7:

$$v =$$

How does this help us understand enzyme kinetics? The graph below shows **reaction rate** plotted against **substrate concentration**.

Question 8: Calculate v for the case where the substrate concentration is very large (in other words, if $[S] \gg K_M$). Hint: the answer should be quite short!

Question 9: Calculate v for the case where the substrate concentration is very small (in other words, if $[S] \ll K_M$).

Question 10: Which of the two numbers, the question 8 answer or the question 9 answer, is bigger? Where does this value correlate to, on the graph above?

Question 11: Take the case where $[S] = 0$ (in other words, there is no substrate). What will v be? Where does this value correlate to, on the graph above?

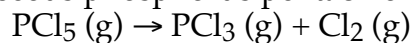
Question 12: The enzyme hexokinase catalyzes the transfer of a phosphate group from ATP to a sugar. K_M of hexokinase for a glucose substrate is 0.15; for a fructose substrate, it is 1.5. Suppose you have two beakers of **dilute** sugar solutions, one containing glucose and the other fructose. You add the same amount of hexokinase to both beakers. Which beaker will contain more

phosphorylated sugar product, shortly after the addition of the enzyme? **Show your reasoning.**

Equilibrium calculations

13. 5.0 moles of ammonia are introduced into a 5.0 liter reactor vessel in which it partially dissociates at high temperatures: $2 \text{NH}_3 (\text{g}) \rightarrow 3 \text{H}_2 (\text{g}) + \text{N}_2 (\text{g})$. At equilibrium at 700°C , 1.0 moles of ammonia remains. Calculate K_c for the reaction.

14. Gaseous phosphorus pentachloride decomposes according to:



K_c for the reaction is $1.00 \times 10^{-3} \text{ mol/L}$. Suppose 2.00 moles of phosphorus pentachloride in a 2.00 liter vessel is allowed to come to equilibrium. Calculate the equilibrium concentrations of all species.

15. For the reaction $\text{N}_2\text{O}_4 (\text{g}) \rightarrow 2 \text{NO}_2 (\text{g})$, $\Delta H_{\text{rxn}} = +58 \text{ kJ/mole}$. **Write** the equilibrium expression and, using that, predict the results (in terms of the direction the reaction will go) of the following changes to an equilibrium mixture of the reaction.

a) Addition of $\text{NO}_2 (\text{g})$

b) Addition of $\text{He} (\text{g})$ (not a catalyst, nor does it react)

c) Decreasing the container volume

d) Increasing the temperature

e) Addition of a $\text{Ni} (\text{s})$ catalyst

16. An example of thermodynamics and kinetics from biochemistry: F_{ab} is the variable part of the antibody molecule and it binds to a substrate, called a hapten. At 25°C , the dissociation constant of an F_{ab} -hapten complex is 3.0×10^{-7} .

The rate constant of the release of the hapten from the complex is 120 s^{-1} . What is the **rate constant** for the capture of the hapten molecule by F_{ab} ?

