Anouncements:

- remember seminar today at VanAndel
- Exam Friday over all the Kinetics stuff 8-11am and 1-4 pm. (note seminar at 3:00)

Outline:

### Enzymes

#### Michaelis-Menten

### Electrostatics

Review

**Enzymes**

Should have pointed out last time that enzymes are normally proteins. So, when I was making the point about the ribosome... RNA as an enzyme is very surprising.

Remember our 3 important experimental observations about enzyme kinetics:

1. Rate of reaction usually linear with enzyme concentration  
   *see plot at right*

2. At fixed [E], rate is linear in [S] for low [S].
   *prev. plot – low [S]*

3. But plateaus/saturates at high [S]
   *prev. plot – high [S] & saturation*

Michaelis and Menten put these together (in 1913) and came up with a mechanism that involves a binding event preceding the reaction. This is why there is saturation – if all the [E] is already bound to [S], then more [S] won’t speed up the reaction.

\[
\begin{align*}
[E] + [S] & \xrightarrow{k_1} [ES] \\
\text{low [S]} \quad \text{prev. plot} & \quad \text{high [S]} \quad \text{prev. plot} \\
[ES] & \xrightarrow{k_2} [E] + [P] \\
\text{low [S]} \quad \text{prev. plot} & \quad \text{high [S]} \quad \text{prev. plot} \\
\[E] + [S] & \xleftarrow{k_{-1}} [ES] \\
\text{low [S]} \quad \text{prev. plot} & \quad \text{high [S]} \quad \text{prev. plot} \\
[ES] & \xleftarrow{k_{-2}} [E] + [P] \\
\text{low [S]} \quad \text{prev. plot} & \quad \text{high [S]} \quad \text{prev. plot}
\end{align*}
\]

This mechanism leads to what rates for [E], [P], and [ES]?
\[
- \frac{d[S]}{dt} = k_1[E][S] - k_{-1}[ES]
\]
\[
- \frac{d[ES]}{dt} = (k_2 + k_{-1})[ES] - k_1[E][S] - k_{-2}[E][P]
\]
\[
\frac{d[P]}{dt} = k_2[ES] - k_{-2}[E][P]
\]

Note that since no enzyme is ever used up: \([E]_0 = [E] + [ES]\)

We can rewrite the second equation using this \([E]_0\) and also by using the steady-state approximation on \([ES]\). This is reasonable if we have excess \([S]\) because then we expect a basically fixed amount of the \([E]\) will be bound.

So, setting \(d[ES]/dt = 0\) and plugging in that \([E]_0\) relation to get rid of \([E]\):

\[
[ES] = \frac{k_1[S] + k_{-2}[P]}{k_1[S] + k_{-2}[P] + k_{-1} + k_2}[E]_0
\]

Plugging this back into the \(d[S]/dt\) equation gives us something that doesn’t look too bad. We do a bunch of algebra and end up with something that looks pretty nasty, but contains \(d[ES]/dt\) inside of it. So, we can set this to zero and things clean up a lot. We can then make a second approximation by saying that we will only care about the early stages of the reaction. So, the rxn velocity is \(v_0\) and \([S] \approx [S]_0\) and \([P] \approx 0\).

\[
v_0 = -\frac{d[S]_0}{dt} = \frac{k_2[S]_0[E]_0}{K_m + [S]_0}
\]

**Key equation!**

where \(K_m = \frac{k_{-1} + k_2}{k_1}\) is the Michaelis constant. It represents the apparent dissociation constant of E and S (for the reaction written \(ES \rightarrow E + S\)). **When is \(K_m\) the actual dissociation constant?** When \(k_{-1} \gg k_2\) then \(K_m\) approaches \(k_{-1}/k_1\) – the dissociation constant. In other words, when equilibrium is reached much faster than the subsequent reaction.

Notice that \(k_{-2}\) is gone from the \(v_0\) equation. **Why?** \(P=0\)
Remember, this important equation applies to the initial velocity when there is excess substrate.

Now, let’s break into groups and look at a couple questions:

1) What happens when \([S]\) is small?
2) when \([S]\) is big?

Relate these to our three observations

Notice that when \([S]\) is small... \(K_m >> [S]_0\), and we get?

\[
v_0 = \frac{k_2}{K_m} [S]_0 [E]_0
\]

and we get something linear in \([S]\) just like we should (for fixed \([E]\)).

When \([S]\) is big... \([S]_0 >> K_m\), and we get?

\[
v_{Max} = k_2 [E]_0
\]

we get something linear in \([E]_0\) and independent of \([S]\) like we should.

Because this corresponds to the saturated region, we call it \(v_{max}\). \(k_2\) then is the turnover or \(k_{cat}\) we talked about before. Note again, \([E]_0\) is the concentration of active sites not just of enzymes.

Also in your groups, what about when \(K_m = [S]_0\)?

Finally, when \(K_m = [S]_0\), we get:

\[
v_0 = \frac{k_2 [S]_0 [E]_0}{2[S]_0} = \frac{1}{2} v_{Max}
\]

So, the Michaelis constant is the substrate concentration that gives half \(v_{max}\).
Note that we can rearrange the important equation above:
(substitute \( v_{\text{max}} = k_2[E]_0 \) then invert the rest)

\[
    v_0 = \frac{v_{\text{Max}}}{1 + \frac{K_M}{[S]}}
\]

and then invert to give

\[
    \frac{1}{v_0} = \frac{1}{v_{\text{Max}}} + \frac{K_M}{v_{\text{Max}}[S]}
\]

So, plotting \( 1/v_0 \) vs. \( 1/[S] \) gives \( K_M \) and \( v_{\text{Max}} \). This is called a Lineweaver-Burke plot. Several other plotting techniques exist.
Finally, note that $k_{cat}/K_M$ is the specificity constant. If two substrates are competing for one enzyme...

$$\frac{v_A}{v_B} = \left(\frac{k_{cat}/K_M}{k_{cat}/K_M}\right)_A \frac{[A]}{[B]}$$

So the specificity constant tells you the selectivity of an enzyme for a particular substrate. If $A$ has a large $k_{cat}/K_m$ then its velocity is really fast and $A$ outcompetes $B$ for enzyme.

Brief intro to Electrostatics:

**Intermolecular Interactions**

Okay, for this week we are changing our focus to talk about various kinds of intermolecular interactions. I’m a little irritated with the book that this central topic comes so late but we’ll do our best.

We begin with the strongest interactions between molecules – electrostatics. (nuclear forces are stronger, but that’s about it.)

Electrostatics includes charge-charge interactions, charge-dipole, dipole-dipole, dipole-quadruple, etc. (often described as monopole/multipole interactions)
Coulomb’s law

Let’s start with Coulomb’s law which everyone remembers from physics

\[ u(r) = \frac{1}{4\pi\varepsilon_0} \frac{q_A q_B}{r} \]

\( u \) is the interaction energy (units J = V\cdot C) (note W = J/s = V\cdot C/s = V\cdot A)

\( \varepsilon_0 \) is the permittivity of vacuum (units F/m = C/V\cdot m = C^2/J\cdot m)

So, we can calculate the electrostatic interaction between two charged particles. In practice this is a difficult calculation. Why? Because the interaction falls off very slowly with distance \( \sim r^{-1} \)

Text does an example of NaCl crystal. If you just look at one line of charges that surround a central Na+: 

we get something that converges VERY slowly. In this case there is a regular arrangements of charges, so we can find a series approximation that helps us out [defines the Madelung constant]. e.g.

This is great for crystalline solids, but for more general charge distributions (like dissolved salts, amorphous materials, or proteins you have to try to just add them all up.

Fortunately, in most media charges are shielded by the surroundings. This is especially important in water. We can write Coulombs law the way it is really defined

\[ \varepsilon = \varepsilon_0 D \]

where \( D \) is the dielectric constant

\( D \equiv 1 \) for vacuum
\( D = 1.00059 \approx 1 \) for air
\( D \approx 2 \) for hydrocarbons
\( D \approx 1.4 \) often used for proteins
D = 33 MeOH
D = 78.54 for water

Book mentions Bjerrum length, which is a handy tool. Remember our golden tool for connecting microscopic properties to macroscopic behavior? Partition function.

Energy always appears as $E/kT$ or $E/RT$ depending on units.

The Bjerrum length is just the distance two charged bodies are apart that corresponds to $U = RT$.

Book points out that Bjerrum length is 80 times shorter ($i.e.$ a factor of $D$, 560 Å versus 7.13 Å) for NaCl dissolved in water than for two bare Na$^+$ Cl$^-$ particles. Their interaction is severely weakened by the presence of the water.

If we had many charges, we would just add up the $u$ for each of them, until we had accounted for everybody. However, there are easier ways, which we will work up to now.