20.320, notes for 9/18

Tuesday, September 18, 2012
9:37 AM

Last time

We covered the graph of RU vs. time during an SPR experiment. We see a different graph from the association and dissociation parts of the experiment, and from them we can calculate k_{off} (which is independent) and k_{obs} (which contains k_{on} and k_{off}).
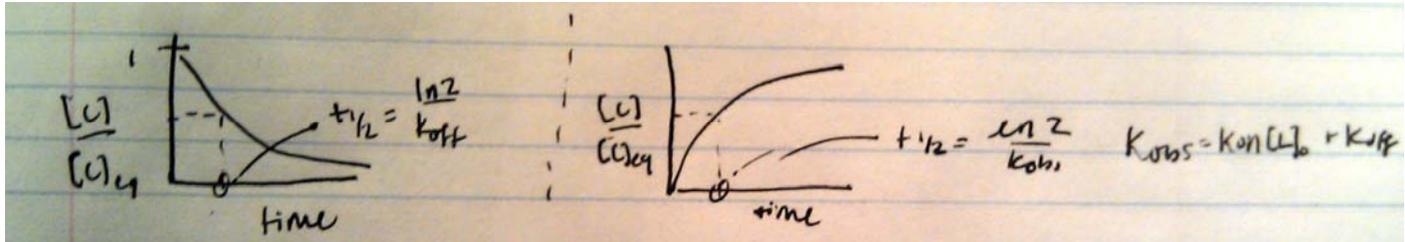
Note that the behavior of k_{off} is not necessarily what we'd expect. As k_{off} increases, the graph of RU vs. time goes down because the value of K_d (k_{off}/k_{on}) decreases with it and that sets the upper limit that the graph will asymptotically approach.
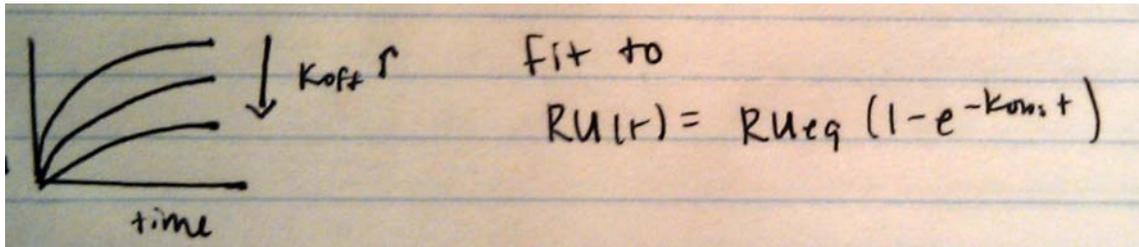
Benefits of SPR
- Redundancy.
  - We get K_d from multiple $[L]_o$
  - We get k_{off} from dissociation, which gives us k_{on} because we know K_d.
  - We get K_{on} and k_{off} from the association phase.

Limitations of SPR
- Binding of protein/ligand to the surface creates many artifacts. A protein stuck onto a surface will probably not behave like the free protein in the cell.
- K_d range between 10^{-4} and 10^{-12} only.
- Non-specific binding leads to spurious results

Overall, this is a very powerful approach. It gives you accurate kinetic parameters. The benefit of something else, like ITC, is that you can do your experiment in solution, in any solution.

Enzyme kinetics

Let's talk about a system that is representative and well-understood, the Epidermal Growth Factor Receptor (EGFR).
- Protein overexpressed or mutated in many cancers.
- It's a membrane receptor.

- Binds Epidermal Growth Factor (EGF), transforming growth factor α (TGF-α), and amphiregulin (AREG).

EGFR is a Receptor Tyrosine Kinase (RTK). It has a receptor domain outside the cell, and a kinase domain inside the cell that targets many tyrosines on a neighboring receptor of the same kind. When phosphorylated, these receptors recruit other proteins (SH2 and PTB) that have affinity for the phosphorylated tyrosines . This is the start of a signaling pathway that promotes cell proliferation. If overactive, this signal can ultimately lead to cancer. Why do we care? I just told you; cancer. EGFR is one of the most active areas of research in the pharmaceutical industry. People are screening like crazy to find small molecules that interact with these receptors. We are hoping to learn how to predict which sorts of small molecules would best interact with it.

What are enzymes?

They are catalysts. As such, they facilitate a reaction that neither creates nor consumes them. They reduce the activation energy barrier that prevents the reaction from happening, by stabilizing the intermediate compound that reagents have to transition through on their way to being products. The canonical equation for enzyme kinetics is:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$$
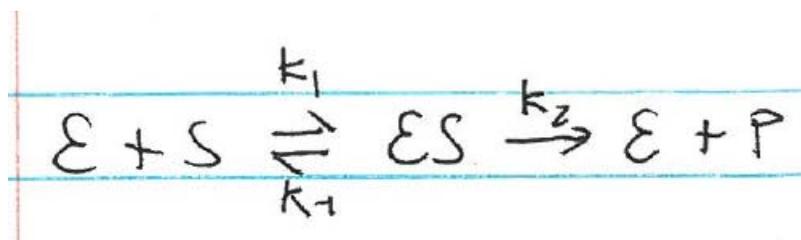
Assumptions:
- ES -> E + P is irreversible.
- E does not bind P.

$$\frac{d[E]}{dt} = -k_1[E][S] + k_{-1}[ES] + k_2[ES]$$

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

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

$$\frac{d[P]}{dt} = k_2[ES]$$

$$\frac{d[E]}{dt} + \frac{d[ES]}{dt} = 0 \qquad \leftarrow \text{Since enzyme is not created or destroyed.}$$

Assumptions Commonly used

(Michaelis Menten assumptions)

1. Substrate is in excess

2. $\dfrac{d[ES]}{dt} = 0 \qquad \leftarrow$ Quasi-Steady-State Assumption, QSSA.

If we turn put everything in terms of the original amount of enzyme, which is what we do know, we get

$$[E]_0 = [E] + [ES]$$

$$[ES] = \frac{[E]_0[S]}{\underbrace{\left(\frac{k_{-1}+k_2}{k_1}\right)}_{K_M \; [=]\frac{M}{L}} \underset{s^{-1}}{\underbrace{}} + [S]}$$

$\overset{\cdot}{s} \longrightarrow$ s^{-1} $\frac{L}{mol \cdot s}$

$$v = \text{rate} = \text{product formation} = \frac{d[P]}{dt} = k_2[E] \cdot [S]. \qquad k_2 = k_{cat}$$

What's the max. rate of the rxn?

$$V_{Max} = k_2[E]_o.$$

$$v = \frac{V_{max}[S]}{K_M + [S]} \qquad \frac{v}{V_{max}} = \frac{[S]}{K_M + [S]} \qquad \text{fractional saturation}$$

Lineweaver-Burke

$$\frac{1}{v} = \frac{K_M}{V_{Max}} \frac{1}{[S]} + \frac{1}{VM_{2x}}$$

slope = $\frac{K_M}{V_{max}}$

intercept $\frac{1}{V_{max}}$
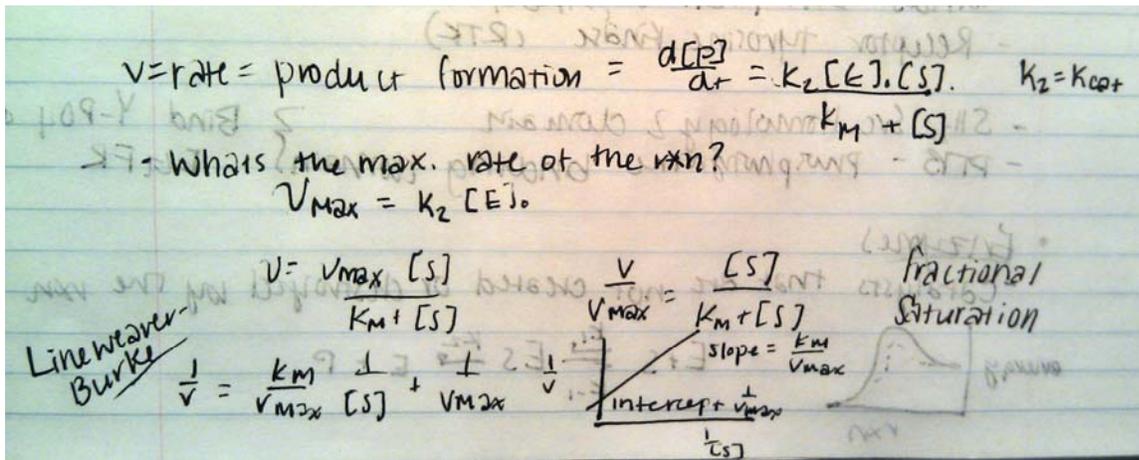
And this is the famous Michaelis-Menten Equation. Note that the ratio of V to V_{max} has a very familiar form. It is essentially a form of fractional saturation. And again, we can take advantage of excess substrate to make the all-familiar assumption that $[S] = [S]_o$.

When is Michaelis-Menten valid?
- Substrate in excess
- $\frac{d[ES]}{dt} = 0$

Note that we also have the hidden assumption that our products are forming instantly. In reality, they take a moment at the beginning of the reaction. As the reaction advances through time, there is a linear domain where QSSA applies and the substrate is in great excess. Eventually, though, the substrate gets depleted and your product formation plateaus again.
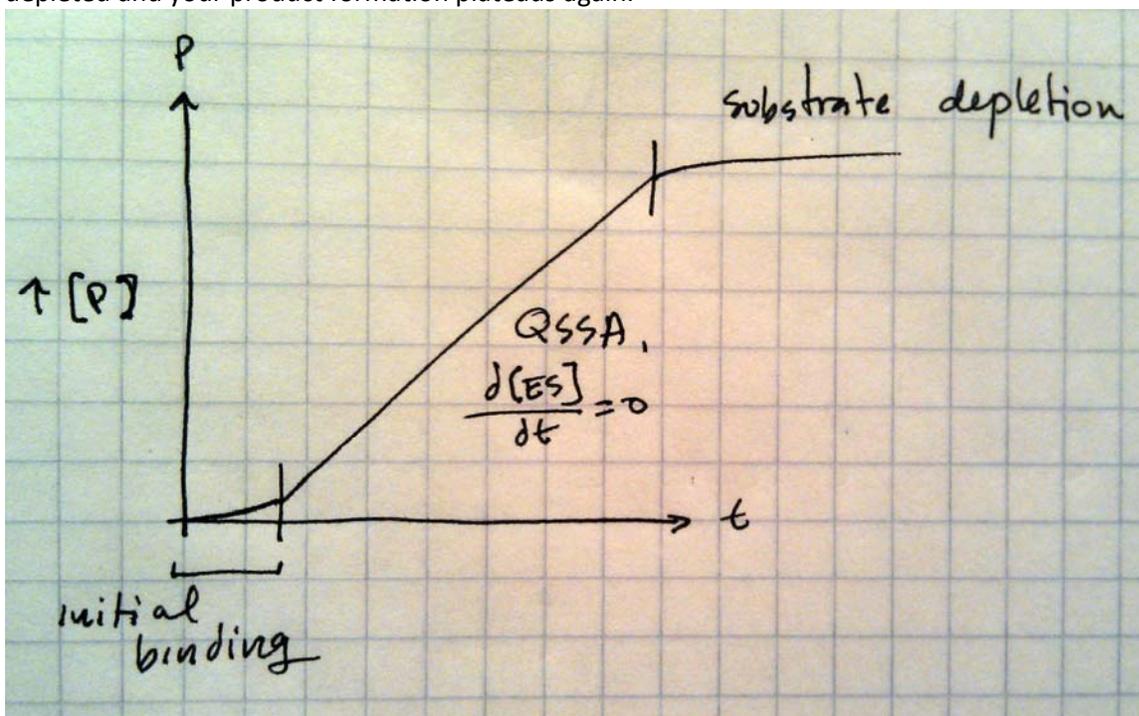
We will calculate the time it takes for a given reaction to undergo these deviations from the ideal case. We'll see when we can assume M-M as we'd like.

If our assumption is that $[S] = [S]_o$, **how long to equilibrium?**

• How long does it take to reach equilibrium?

Assumption:

$$[S] \cong [S]_0$$

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

$$[ES] = \underbrace{\frac{[E]_0[S]_0}{K_M + [S]_0}}_{[ES]_{QSSA}} (1 - e^{-\lambda t}) \qquad \lambda = k_1(K_M + [S]_0)$$

$$[ES] = [ES]_{QSSA}(1 - e^{-\lambda t})$$

$$t_{1/2} = \frac{\ln 2}{\lambda} = \frac{\ln 2}{k_1(K_M + [S]_0)} = \frac{\ln 2}{(k_{-1} + k_2) + k_1[S]_0}$$

$$t_{QSSA} = \frac{1}{\lambda} \quad \leftarrow 63\% \text{ to equilibrium}$$

Again, does this look familiar? We had very similar equations for SPR, since that was modeling protein-protein interactions. Remember that the first part of enzyme kinetics is also just protein-protein interactions.

t_{QSSA} is the time it takes to reach 63% of equilibrium.

Now, when have we **reached substrate depletion**?

• When do we deplete substrate?

$$t_{[S]} = \frac{\text{total substrate}}{\text{rate of depletion during QSSA}} = \frac{[S]_0}{k_{cat}[ES]_{QSSA}} = \frac{[S]_0}{k_{cat}\left(\frac{[E]_0[S]_0}{K_M + [S]_0}\right)}$$

$$t_{[S]} = \frac{K_M + [S]_0}{k_{cat}[E]_0}$$
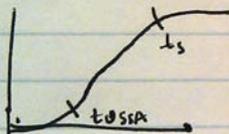
$$t_{QSSA} \ll t_{[S]}$$

In order for our system to be useful, then, what we want is $t_{QSSA} \ll t_{[S]}$. That will be achieved when:

$$\frac{1}{k_1(K_M + [S]_0)} \ll \frac{K_M + [S]_0}{k_{cat}[E]_0}$$

$$\frac{[E]_0}{K_M + [S]_0} \ll \left(1 + \frac{k_{-1}}{k_{cat}}\right)\left(1 + \frac{[S]_0}{K_M}\right)$$

Now, all of the above will be valid only when substrate is in excess. What happens when the substrate is depleted during the association phase? For that, we need to look at the ratio of change in [S] to total [S].

And this is the true condition that is necessary for QSSA—and therefore, for Michaelis-Menten. Now, how much error is introduced if these conditions are not met? If we did the math, we'd come up with a graph that looks like this.
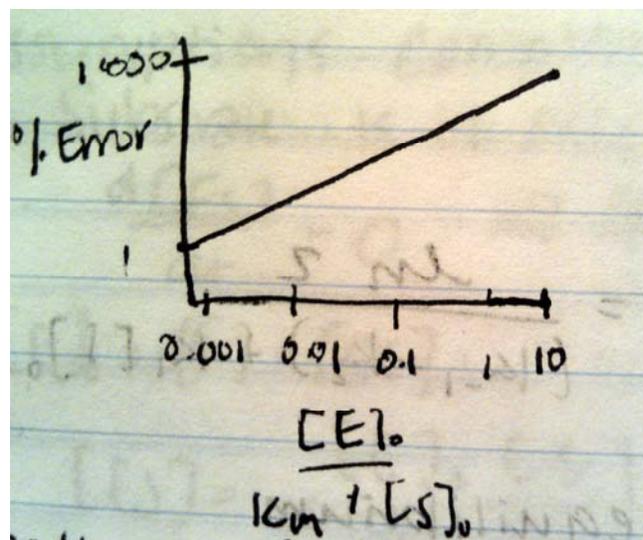
Now, how biologically relevant is this? In the case of EGFR,

E = EGFR
S = EGFR
P = EGFR + PO$_4$

In this case, the substrate and the enzyme are the same molecule. M-M does not apply. Why do we teach M-M, then, if it doesn't apply in this enormously important system? Well, because there are lots of cases where it does; around the world of biology, the equation can give us a great approximation. M-M is so easy, that it is a great way to get a first approximation in a myriad of systems where we really

don't want to go through all the math. This was a really big deal, during most of the history of biology. We are very lucky, because we have MATLAB and we can bypass these approximations. We can just go and calculate the actual rates of reaction without having to rely on dubious approximations like excess substrate.

And one note on 1000% error. How bad is that? This is a good moment to remember a central mantra of modeling.

All models are wrong… but some are useful.

During your modeling, you should vary your parameters one way and another by 1000%. See if the model cares about variation in that parameter, and thereby determine which things *you* should care about. Once you pin the important parameters, you can always go and do SPR to figure them out exactly. The point is that there will be a lot of cases where you'll easily get away with 1000% error. Learn to accept that there are different degrees of wrong, and that a little *wrong* can pay for a whole lot of *useful.* Finding the balance is our job as bioengineers.

20.320 Analysis of Biomolecular and Cellular Systems
Fall 2012