20.320, notes for 9/20

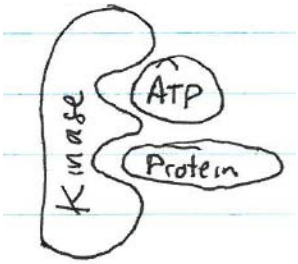
Thursday, September 20, 2012
9:38 AM

Intro

Last time we talked about EGFR and the importance of that signaling pathway. It leads to cell proliferation, so all manner of pharmaceutical companies are trying to inhibit it to treat cancer. We'll try to understand that today.

Kinase Engineering

EGFR is a kinase. As such, it has two substrates: the protein to be phosphorylated and ATP. Thus, we can draw things this way:



And we have these equations for the two substrates:

Let's define S_1 as protein, S_2 as ATP, and P as phosphorylated protein:

$$E + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES_1 \qquad E + S_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_2$$

$$ES_1 + S_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ES_1S_2 \qquad ES_2 + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES_1S_2$$

$$ES_1S_2 \xrightarrow{k_3} E + P$$

Note that we've assumed no cooperativity. Affinity for substrate doesn't change when ATP is bound. In reality, though, this is not usually how we model kinases. The concentration of ATP is so high that we ignore it by assuming its concentration stays constant throughout the reaction. Today, however, we'll not ignore it.

$$[ATP]_{in\ the\ cell} = 3\ to\ 5mM$$

So if we're trying to inhibit this kinase, which of the two substrates would we go after? Which interaction do we inhibit, ATP or the protein?

Option 1: Attack the **protein substrate binding pocket**. Inhibit interaction with the substrate. About 30% of the proteome binds ATP. Competing with ATP (rather than changing the pocket) is very difficult given that the [ATP] is so high. Also, there's one more problem: the substrate binding pocket is more of an amorphous groove than a defined lock.

Option 2: Attack the **ATP binding pocket**. There is a well-defined pocket and structure of interaction between kinase and ATP. Easy to mess it up. This is what every pharma company does to try to treat cancer.

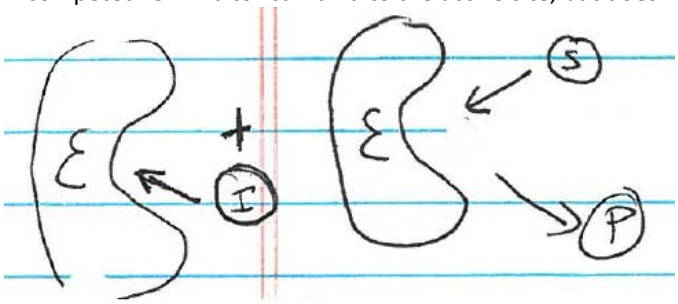
This was stated explicitly by Lipinski's Rules of 5. This guy looked at the design criteria of every successful cancer drug on the market. One of the things he discovered is that all the useful drugs were small molecules (<500 Da, which rules out all peptides above about 4 or 5 amino acids). This is a big element of the modern cancer treatment status quo.

Also, it's worth noting that any competitive ATP inhibitor is likely to interfere with other proteins that bind ATP. It depends on the specificity of the molecule. It's also worth noting that these inhibitors are frequently found to interact a lot with non-kinases. A lot of their effectiveness (and toxicity) may usually come from these molecules that are not usually tested.

So let's mess with ATP. One way to do it is with a competitive inhibitor.

Competitive Inhibition

A competetive inhibitor can bind to the active site, but doesn't lead to product.



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI$$

① $[E]_o = [E] + [ES] + [EI]$

② $\frac{dP}{dt} = v = k_2[ES]$ → product formation still dependant on catalytic rate of enzyme & ES complex

Enter Michaelis Menten, as before:

w/ M-M approximation ($[S]_0, [I]_0 >> [\mathcal{E}]_0$)

not bad for ATP, and I.

③ $\frac{d[\mathcal{E}S]}{dt} = k_1[\mathcal{E}][S] - k_{-1}[\mathcal{E}S] - k_2[\mathcal{E}S] = 0$

④ $\frac{d[\mathcal{E}I]}{dt} = k_3[\mathcal{E}][I] - k_{-3}[\mathcal{E}I] = 0$

define $K_I = \frac{k_{-3}}{k_3} = \frac{[\mathcal{E}][I]}{[\mathcal{E}I]}$

※ And after the usual math, we get a M-M equation with competitive inhibition (!)

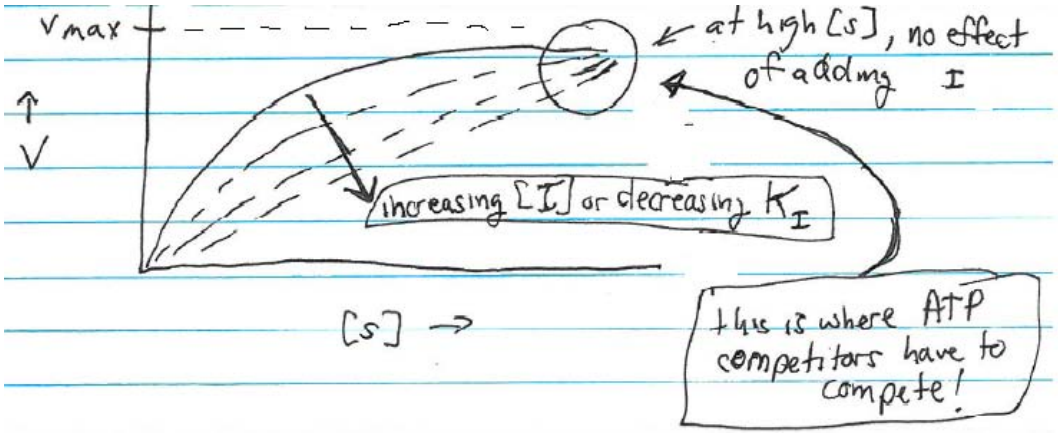
$$v = \frac{V_{max}[S]}{K_m\left(1 + \frac{[I]}{K_I}\right) + [S]}$$

$V_{max} = k_2[\mathcal{E}]_0$

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

$K_I = k_{-3}/k_3$

As you can see, a greater concentration of inhibitor (or a higher inhibitor affinity for the substrate, which shows up as a lower K_I.) both result in a lower K_M. When graphed out, the effect looks like this:



Note that, at high substrate concentrations, you just need a **whole** lot of inhibitor to have any effect. [ATP] is not an easy concentration to compete with. Just how much inhibitor would we have to have to compete?

$$V_i = \frac{V_{max}[S]}{K_m\left(1 + \frac{[I]}{K_I}\right) + [S]} \qquad V = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{V_i}{V} = 0.1 = 90\% \text{ inhibition} = \frac{K_m + [S]}{K_m\left(1 + \frac{[I]}{K_I}\right) + [S]}$$

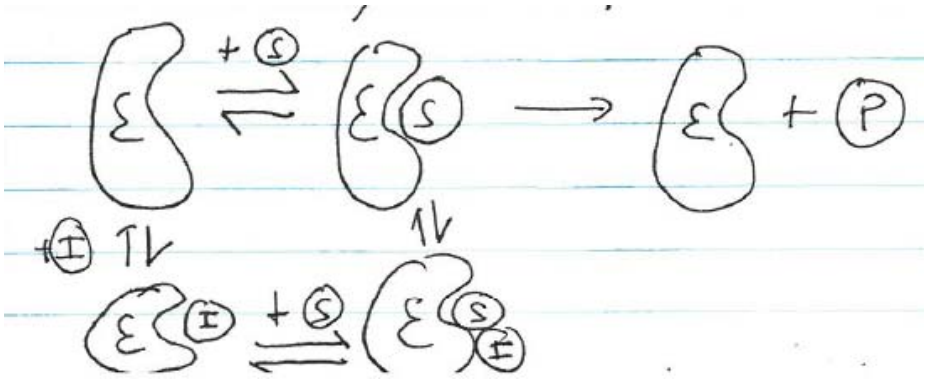
Plug in: $[S] = 3nM$ $K_m = 50\mu M$ $K_I = 10nM$

Result: $[I] = 5.5\mu M$ for 90% inhibition.

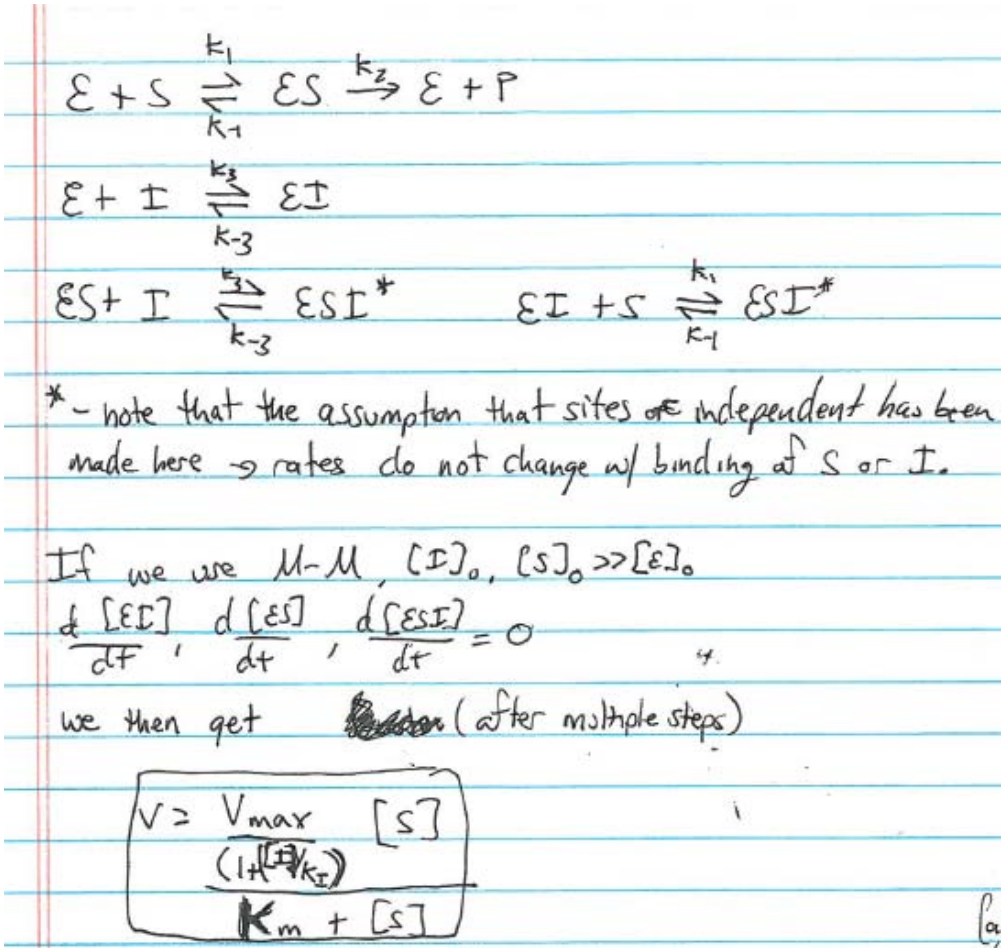
And this is a really difficult amount to deal with. 5.5µM inside the tumor means a whole lot more has to be given to the patient, and there is great potential for toxicity.

Non-competitive inhibition

How are things different now?



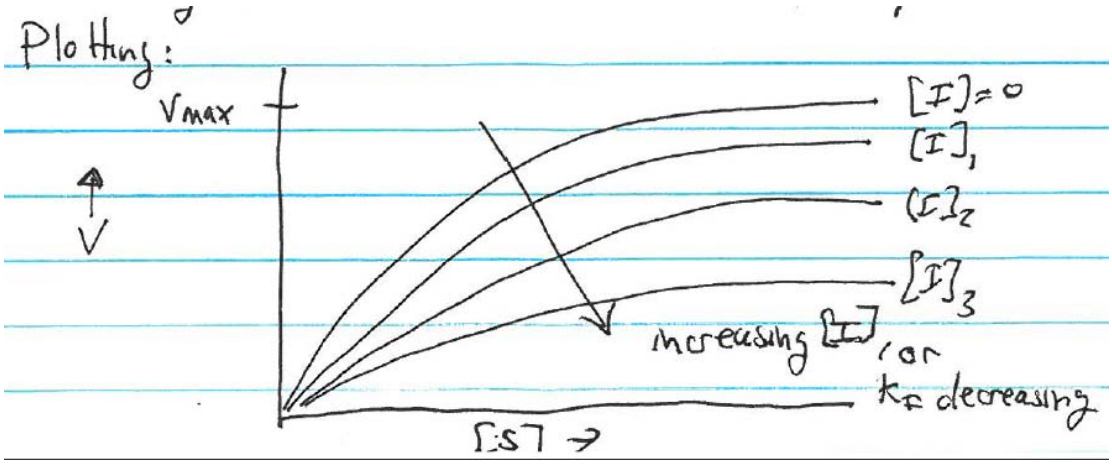
And applying the same equations,



Notice that in this case, what's changing is the apparent V_{max}. This makes sense, given that

$V_{max} = k_2 * [E]_o$

And our inhibitor is effectively reducing the available enzyme by capturing it into something useless. This is always independent of the amount of substrate.



Inhibiting EGFR in Cancer

Let's not lose track of what we're trying to do here. Back in the 90's, there was an amazing success story for pharmaceuticals targeting kinases. Gleevec, or Imatinib, targets the Abl protein in the Bcr-Abl mutation that leads to chronic myeloid leukemia. 90% of people tended to die before 5 years. Now 90%

live past 5 years.

3% of non-smokers with lung cancer have the EML4-Alk mutation. Crozotinib targets Alk, and gives people an extra 6 months to live.

Both of these drugs, however, suffer from a common problem in cancer drug development called **Oncogene Addiction**. When you add a flashy new drug, it will kill all the cancer cells that depend on your particular target for survival. The problem is that this creates a strong selection for cells that don't depend on your particular pathway, and cancer cell microevolution will probably develop another strain of cells that bypasses your roadblock.

Why drugs fail

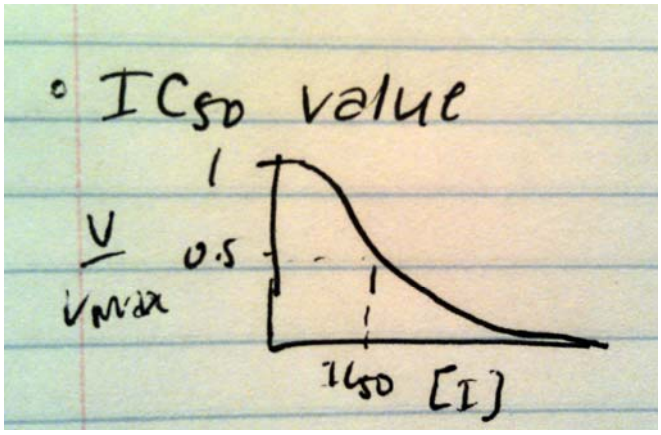
EGFR in cancer can be 10- to 100-fold overexpressed. To make sure that we eliminate the effect on downstream targets, we need to inhibit EGFR by about 90% (or up to 99%). The problem is that EGFR is not only present in cancer cells. The E is for Epidermal, and EGFR regulates the process of skin cell regeneration. In normal skin cells, EGFR is expressed to about 1/10 the levels in cancer cells. So if you inhibit EGFR enough to kill cancer, you will obliterate normal EGFR skin cells.

This is the problem called **Dose-Limited Toxicity.** Unless your target is *specific* to cancer calls, you will mess up other stuff. Going over the most overexpressed protein is not usually the best target. You need a lot more drug and you are much more likely to kill (or harm the functionality of) normal cells that use that protein. The solution is modeling, so that you may identify the most susceptible nodes in the interaction network. Find the protein that will have the greatest benefit at the lowest cost. This is not easy to do, but that's what we learn to do here.

Reasons drugs fail:

1. **Dose-limited toxicity**
2. **Resistance pathways**
    a. Compensatory signaling through other pathways
3. **Heterogeneity in the tumor**
    a. They don't all depend on a single mutation

Imagine you're getting an inhibitor from a catalog. You're actually not likely to get the K_I value, which is rather hard to compute. Instead, what you'll likely get is the IC_{50}, or the inhibitor concentration yielding 50% activity.



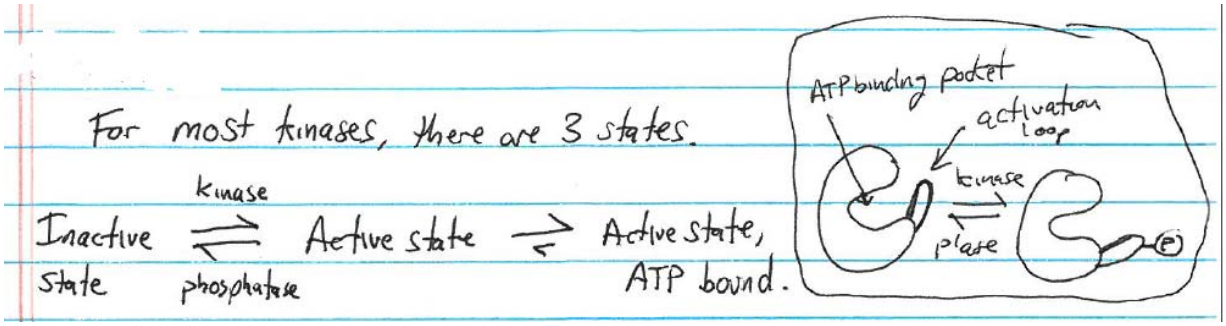
Or, alternatively, the EC_{50} or effective inhibitor concentration yielding 50% activity (in vivo, in a phenotypic assay where you measure proliferation, migration, or apoptosis).

Regulation of kinase activity

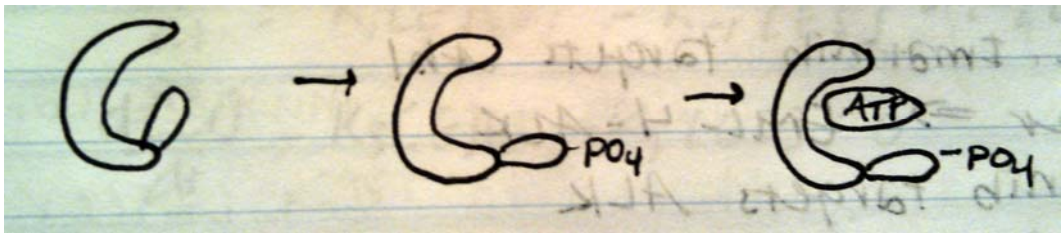
There are several options, given this equation:

1. Feedback inhibition: shut down activity entirely.
    a. This is one of the fastest things the cell can do.
2. Reduce the amount of enzyme
    a. Transcription/translation/degradation
    b. This stuff takes a relatively long time to happen
3. Localization
    a. Also slow.

What the cell often winds up using is post-translational modification. Phosphorylation is how the activity of most kinases is regulated. One relevant example is **Src**, which has a tyrosine that shuts the protein down when phosphorylated. Most kinases, turns out, have an ATP-binding pocket and what's called an activation loop, the "T-loop". These proteins have three states:



When the loop is active, it allows ATP to bind.



This is a great drug target, turns out, because if you trap it in the closed position then you don't have to compete with ATP or anything like that to inactivate it. Type II inhibitors bind the "DFG" out conformation (the un-phosphorylated state at the far left) and lock the enzyme there, inactivating it.

ATP analogue halftime = 0.1-10 seconds
Type II inhibitor halfitem = 20 hours

And that's an example of how structure-guided design can dramatically increase your drug's effectiveness. There's plenty more drug targets here. If you know which kinases and phosphatases catalyze the steps above, then you can target those as well. Next class we'll go into the gory details of complex cascades of kinases activating cascades.

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