20.320, notes for 10/23

Tuesday, October 23, 2012
9:45 AM

Wow, class attendance is lower than expected. Are these class notes just *that* good that you don't benefit from hearing it from the prof directly? Studies show you're probably wrong, just saying…

Predictive models of biology

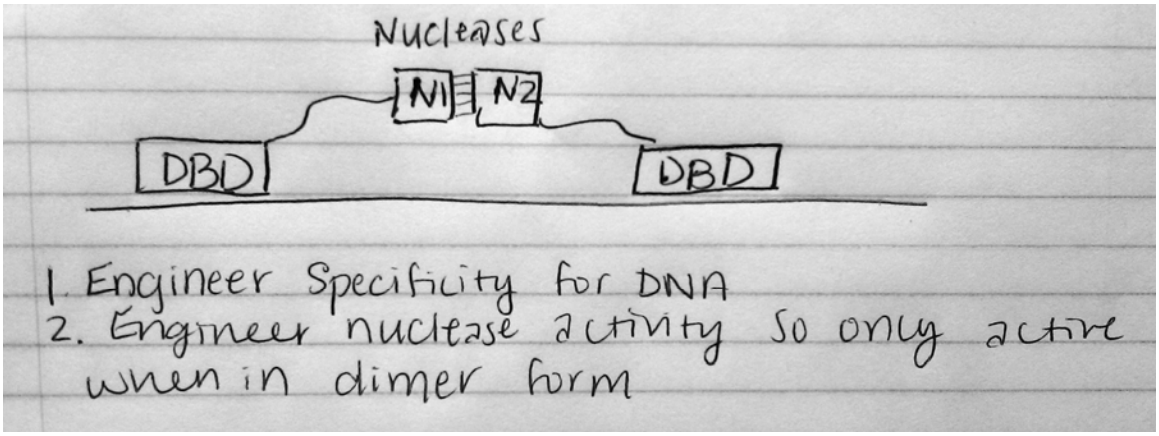
We are working on cycles of analysis and design. We have been working at the level of protein networks, but now we will go down to the details of the biomolecules themselves. How function emerges from structure.

Genome editing

The last century in biology was marked by two things. The first was the emergence of molecular biology. The second was the genomic revolution, the idea that we can know the sequence of the whole genome. This got people thinking: 'can we correct genomic diseases?' Gene therapy was all the rage in the 90s, and we'll now go through a quick history of it.

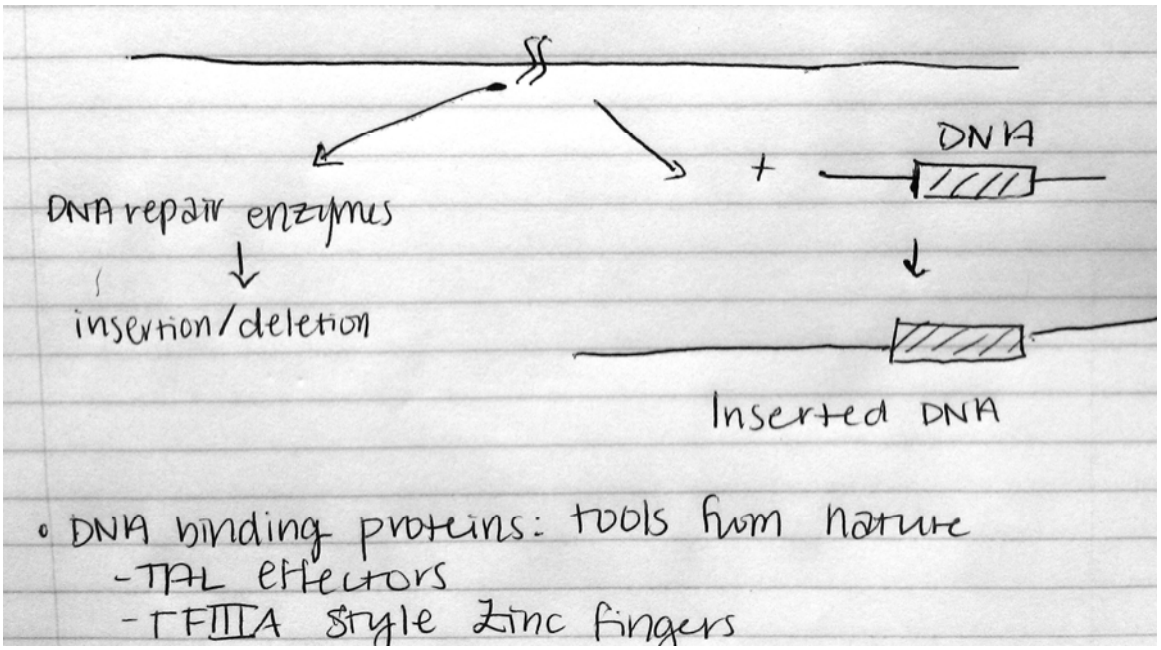
1989	1st clinical trial of a gene therapy
2000	SCID-X1 seems successfully treated with gene therapy. Lots and lots of excitement
2002	2 out of 10 patients treated with the above developed leukemia, because the viral vector had inserted itself upstream of LM02. A third case of cancer came up later.
2012	Glybera is the only gene therapy waiting for approval in Europe. Likely to happen, but it's the only one in the entire European pipeline. There are no drugs in the American pipeline.

Biology is much more complicated than we expected. These leukemias happened because the gene insertions happened in uncontrollable locations. In the traditional view of gene therapy, a viral vector carries the desired change and inserts randomly in the genome. Ideally, though, what we want is a system that recognizes a DNA sequence that occurs only once, at the target location. We want to engineer specificity. Imagine, say, a protein with a couple of nucleases bound to DNA Binding Domains (DBDs).



We don't want the enzymes to cut anywhere they diffuse to, just at the target, so we make the nucleases active only as a dimer. In that case, they are most likely to only work at the desired location where their DBDs have brought them together. DNA repair enzymes can then come and work on

insertion/deletion.



So how do we design DNA binding domains that have specificity for particular regions of DNA and nowhere else? That's what we'll do in this class, use the software tools used in the real world to design specificity for a particular sequence of DNA or protein. Some examples out there are TAL effectors and TFIIIA-style Zn fingers. One interesting example is CCR5, the cell receptors recognized by HIV. There's a clinical trial going on to knock it out and cure HIV. Results should be available soon.

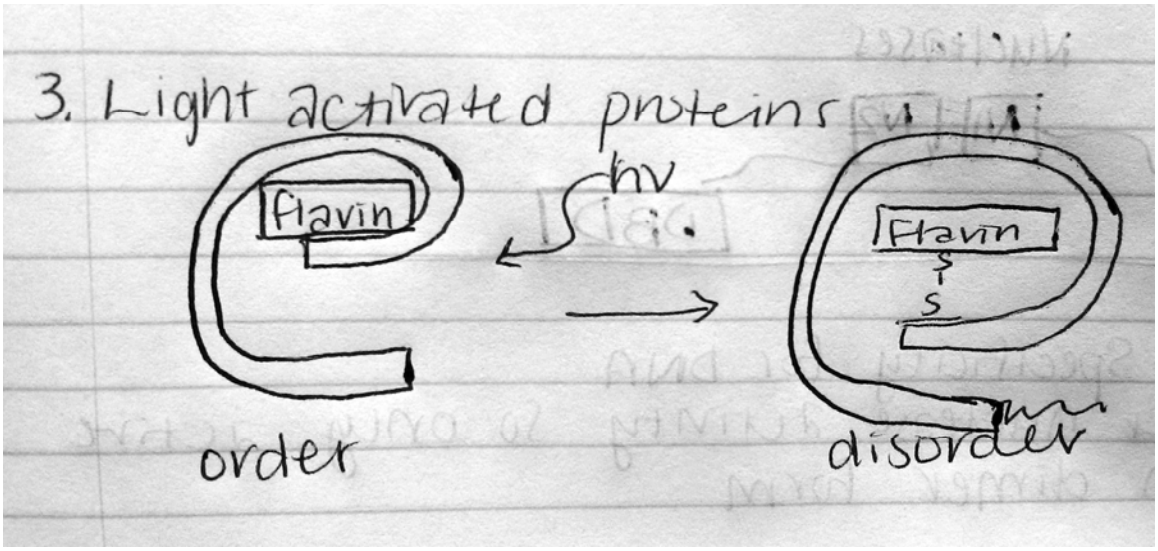
Sensors

The second example we want to talk about are sensors. MRI is really popular out there, but we usually don't get too much information because we look at glucose uptake or blood flow and try to use that as a proxy for brain activity. There's also such a thing as high-resolution fMRI, where people like Alan Jasanoff (MIT) are trying to make MRI markers for neurotransmitter release. What they've engineered is to create a sensor that responds to, say, dopamine binding to produce an MRI signal. How do we do this?

One way is to create a protein that specifically traps or releases water when bound to a ligand. MRI is really sensitive to water, and can detect changes based on the interactions of water. We don't want just affinity for dopamine, though. We want *specific* affinity for dopamine, so that it doesn't bind to any other molecules. We'll go in and actually modify the amino acids that interact with dopamine, changing them to change the specificity of the entire molecule.

Light-activated proteins

Ever wondered how plants manage to follow the sun across the sky? They use proteins with something called a LOV domain. LOV domains have a flavin at their core, which creates an S-S bond in response to light and disorders the nice and ordered structure of the protein, unfolding it and resulting in very measurable motion. The upswing of this is that we have light-activated proteins.



New enzymes

If we could design protein specificity, we could do a whole lot of things. One particularly important one is to design new enzymes. One way to do it is to modify an existing protein to bind a new substrate rather than an old one. We have to worry about creating specificity (so it binds only to the new target) and retaining activity (which is easy to mess up).

One interesting example is David Baker, who started with a model of the desired transition state (of substrate to product) and performed quantum mechanical modeling to design a completely new protein with predicted activity. Amazingly enough, it worked.

This module

A roadmap.

1. Protein energetics
    a. Starting today!
2. Protein structure
    a. Focused on some genomic tools
3. Specificity
    a. Protein-protein
    b. Protein-DNA
4. Protein engineering
5. Drug design

Electrostatics

Back to elementary physics. The potential energy between two particles interacting at a distance r is related to their charges.

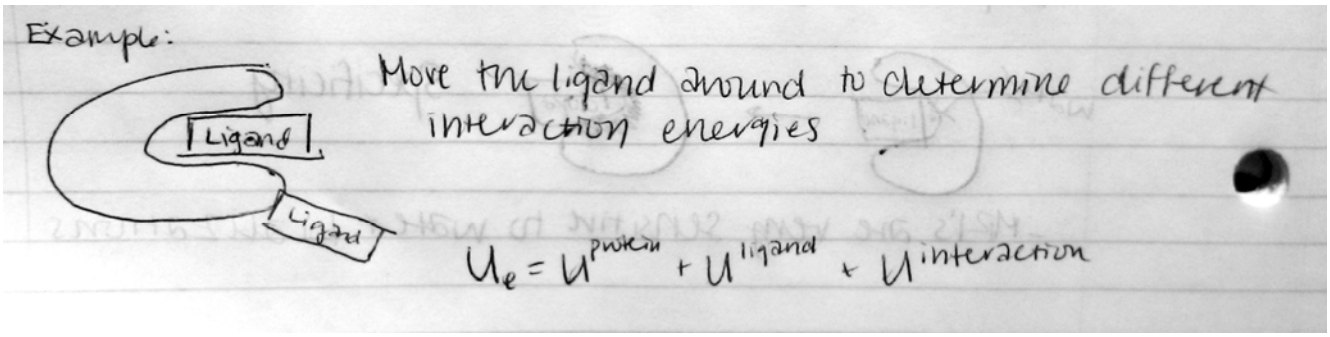
$$U \propto \frac{q_1 q_2}{r}$$

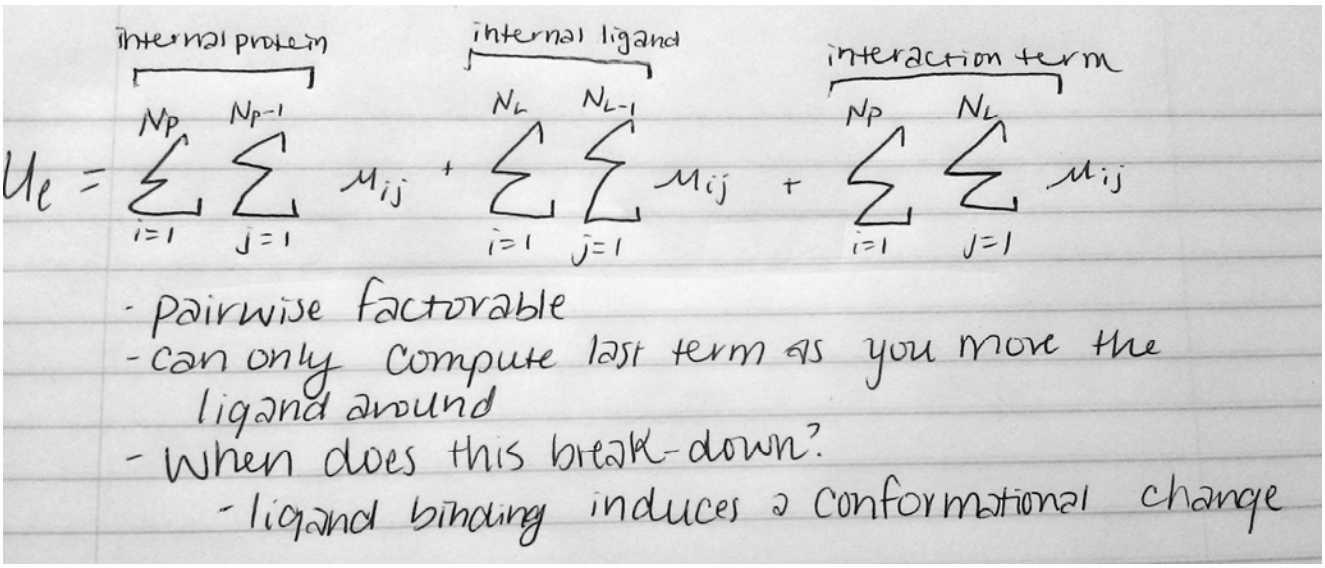
Remember that this is potential, not force. This potential will be much higher in vacuum than in water, because water shields this interaction. This is represented by the dielectric constant, ε.

$$U = \frac{q_1 q_2}{\varepsilon\, r}$$

Medium	Value of ε
Vacuum	1
Water	80
Proteins	2-4, heavy approximation

In protein things are different. It's not vacuum, because there's charged stuff in the middle. It's not water either, though, because water is inherently more fluid than the rest of the protein. Assigning a value to the dielectric constant is always a matter of gross estimation. So let's write up some equations to describe how a protein interacts with small molecules.





So when does this break down? One case is when the protein changes conformation. Suddenly we have to re-compute a whole lot of terms in this equation. Interactions are happening through protein instead of solvent, all locations have changed, etc. Lots of things to re-compute.

Coming attractions

(For next class, that is)

1. Electrostatics
2. Hydrogen Bonding
3. Van der Waals forces
4. Solvent interactions

20.320 Analysis of Biomolecular and Cellular Systems
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