20.320, notes for 11/29

Thursday, November 29, 2012
9:47 AM

Mad Cows

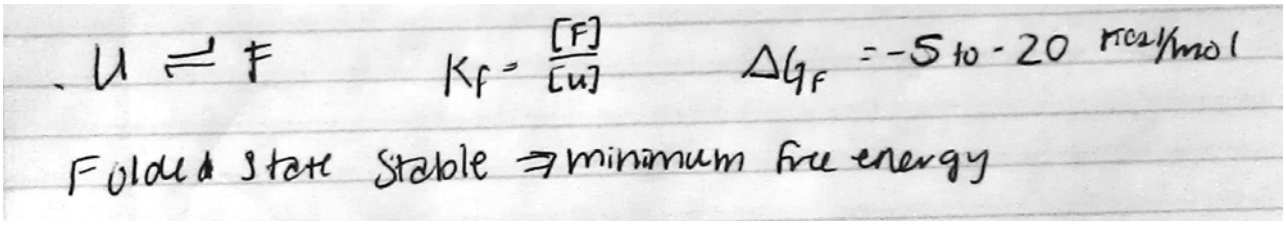
We saw a video of the symptoms of BSE, Bovine Spongiform Encephalitis. It has been linked to vCJD, variant Creutzfeldt-Jakob Disease. We also mentioned a related disease, Kuru, that affected some humans that ate the brains of other diseased humans. These are all strange diseases in that there's no living infectious agent responsible for them (no bacterium, no virus). They are all called by misformed proteins called prions, who themselves are infectious. Today, we'll talk about Protein Folding

1. Protein Folding
    a. Why do proteins fold?
        i. Atomics
        ii. Kinetics
        iii. Thermodynamics
2. Predicting folding
3. Networks of prot-prot interactions

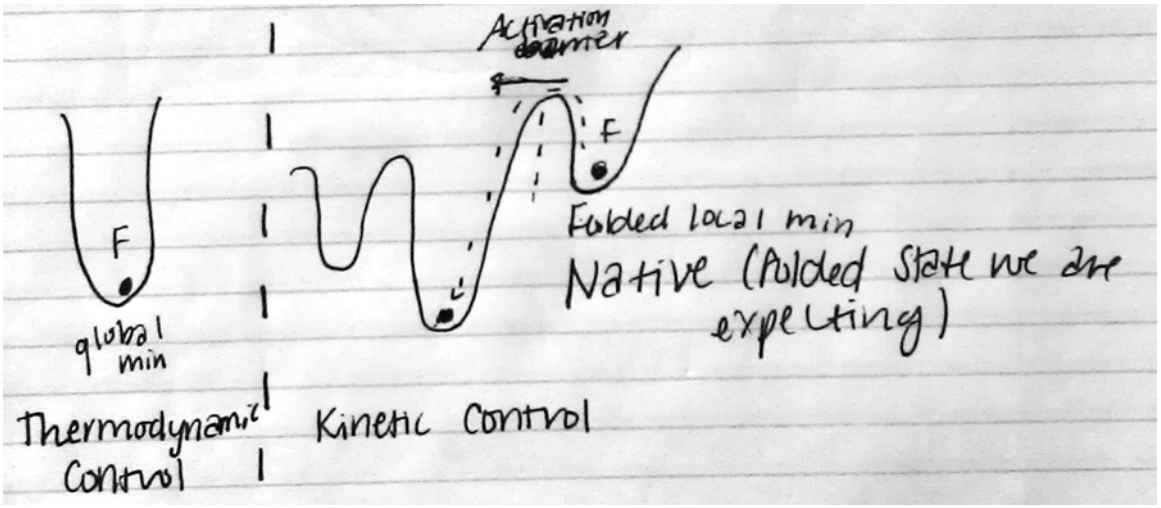
Other diseases caused by protein misfolding are Huntington's, Cystic fibrosis, etc. Let's start with Thermo.

Thermodynamics

As a starting point, let's look at some equations.

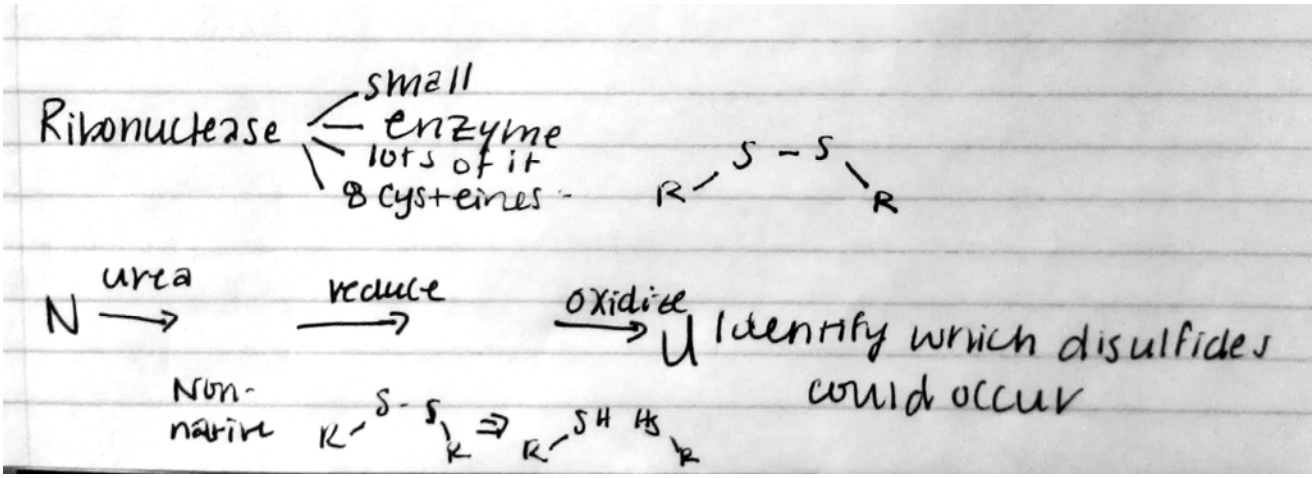


One point we haven't emphasized properly is how the global energy minimum of the protein may not be the one we actually want. The **native** state of the protein is the one we want. If everything is under thermodynamic control, then the protein will always settle to the global energy minimum. If things are under kinetic control, on the other hand, they are under kinetic control and could well land at a local minimum (which might be what you want). It's not usually clear which of these different views is the correct one.

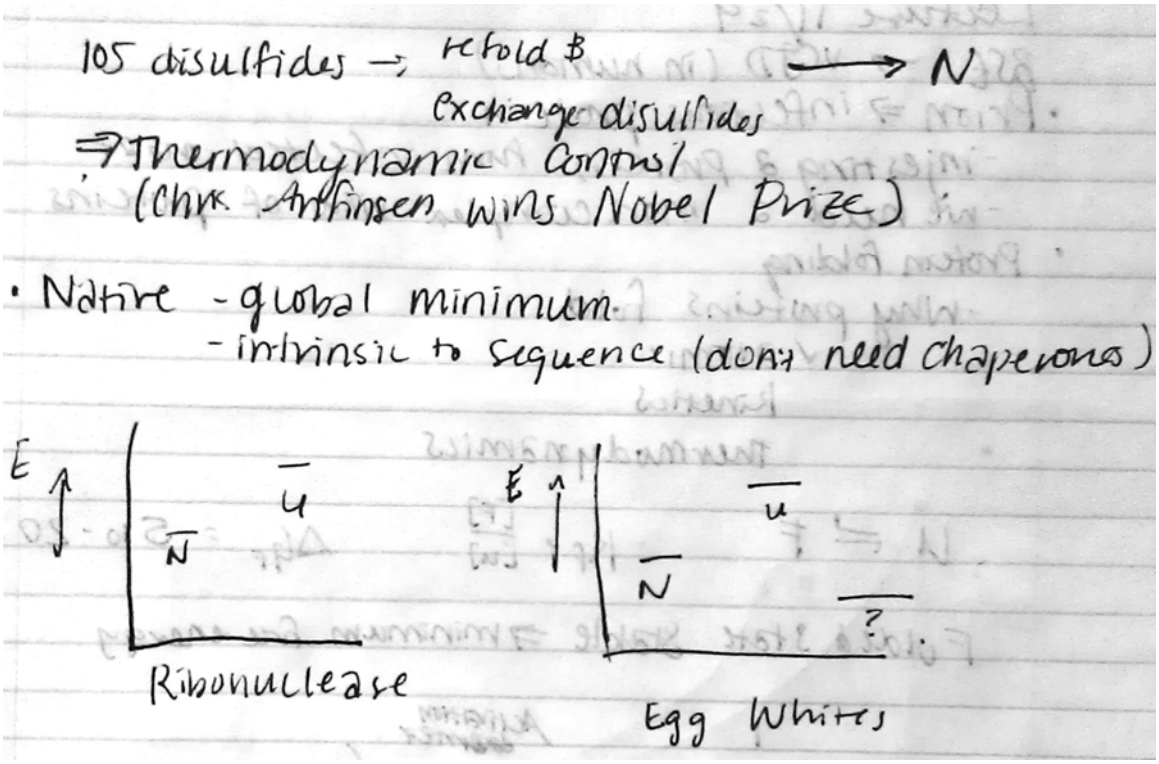


One of the simplest experiments to solve this mystery is to see whether proteins fold to the same conformation in vivo and in vitro. In vivo experiments have all sorts of kinetic control mechanisms (where the protein starts and ends its translation and modification, which chaperones help it along the way, etc.). One of the most analyzed proteins was ribonuclease, which was widely available back then. It is an enzyme with lots of cysteins.

This guy called Anfinsen did an experiment where he denatured the proteins with urea, then reduced them (breaking S-S bonds), then oxidized them. The result was that the proteins randomly created any of 105 possible S-S bonds and wound up in a whole bunch of non-native conformations. The nature of the experiment is that, if things were under Thermodynamic control, all the proteins would return to the native state. That state would correspond to the global minimum, and falling there would be pre-destined by the amino acid structure of the protein. If Ribonuclease structure were kinetically determined (that is, by the particular path it took when navigating the energy landscape), then we'd expect our batch to fall at the global minimum, somewhere other than wherever the native state had been.



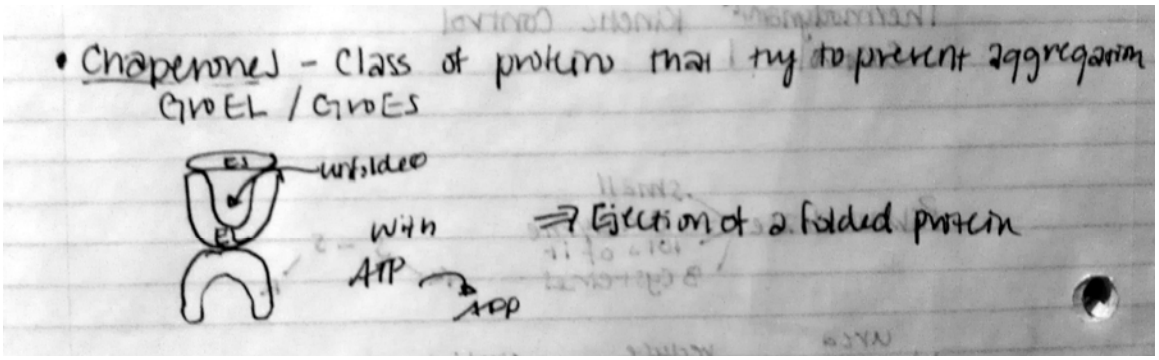
When he allowed the proteins to re-fold and exchange disulfides, he showed unambigiously that they all went back to the original, native state. Thus, Ribonuclease is thermodynamically determined. The native state **is** the global minimum. This is not necessarily the case with all proteins, as mixing egg whites can show. If you mix them into meringue, they do not come back to their original state because there is a global (thermodynamic) minimum different from the native state.



Proteins, turns out, can exist in a variety of states:

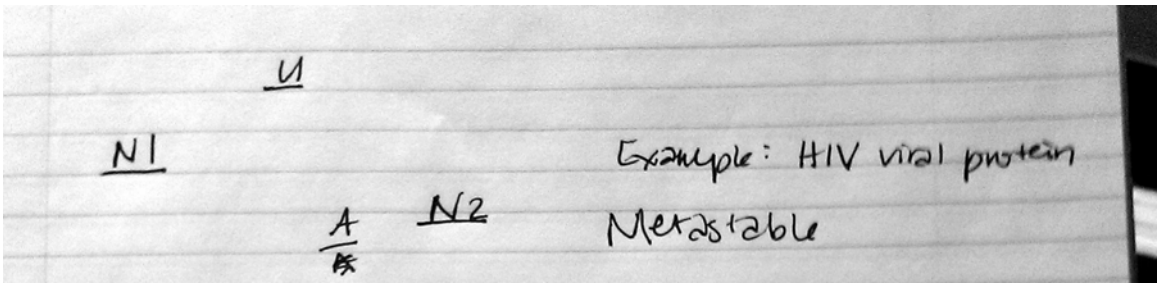
1. Native
2. Unfolded (no fixed structure)
3. Aggregates (>= 2 proteins). There's many kinds.
    a. Soluble/insoluble
    b. Covalent/non-covalent
    c. Reversible/irreversible
    d. Native/denatured

Aggregates are a big problem in any environment with lots of proteins at high concentrations, such as… well, all the cells in the body. Unwanted aggregation is prevented by many ways, and one of them is through chaperones. One class of chaperones that is well understood is called GroEL/GroES. It's shaped rather like a large, lidded basket that expands and contracts, changing the hydrophobicity of its inside as it does so (through the rotation of alpha-helices).

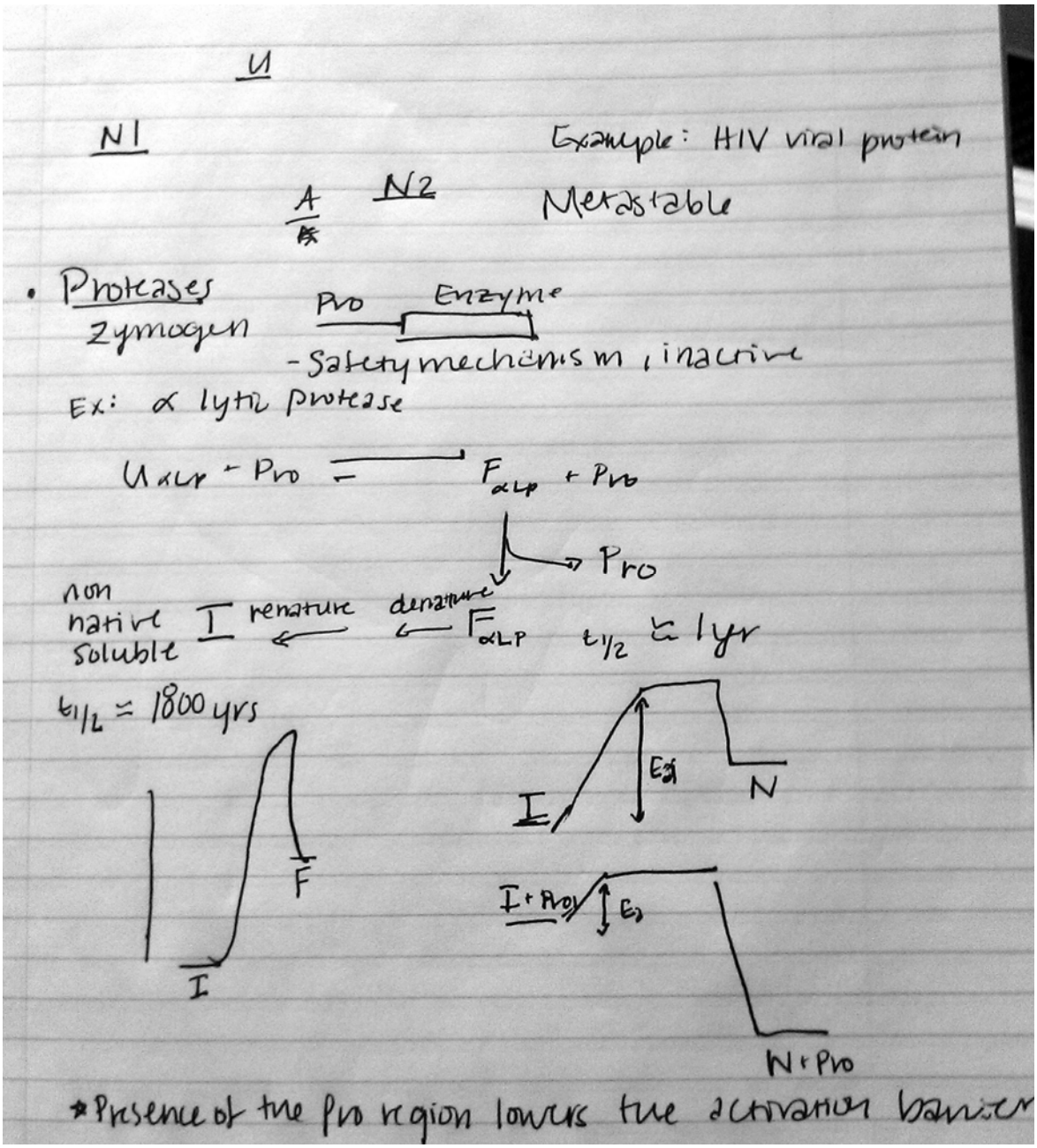


It starts with a hydrophobic inside, which is welcoming to the hydrophobic unfolded proteins. As it contracts, it becomes hydrophillic and encourages the protein to fold into its final state rather than form a hydrophobic aggregate outside somewhere.

We should mention that we usually talk about native states as if there were always a single one. In fact, there are many proteins designed to have two metastable states and transition between them.



Perhaps an even better example of the importance of energy states and protein folding is the case of protease zymogens (zymogen = enzyme precursor).



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