For the last problem set, let’s use some of the electronic resources available to predict native disorder and inherent structure in proteins and RNA. We’ll use IUPred for native protein disorder and the RNAfold web server to predict RNA secondary structure.

1.) Generate a random DNA sequences of 60 nucleotides using the site: <http://www.faculty.ucr.edu/~mmaduro/random.htm>

Next, replace all the ‘T’s with ‘U’s to make it into RNA. Paste your RNA sequence into the RNA prediction algorithm at the RNAfold web server:

<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>

For your answer, present the MFE (minimum free energy) structure and label all of the following sites for each structure: hairpins, bulge loops, interior loops, exterior loops, multi loops, and dangling nucleotides. You must re-draw your structure, not simply cut-and-paste the answer the software gives you. Be careful here, because sometimes the bases are hard to read in the output (some get squashed together). Hint—you can download the eps or pdf file if you want the electronic output to construct your answer.

Next, randomly add two bases, NOT adjacent to each other, somewhere in the sequence. A good choice might be 1/3 and 2/3 –ish of the way through. [Hindsight—if the bases you added falls in a loop or bulge, move them! Try to disrupt the structure somehow to see what happens]. Repeat the process from above—draw the lowest energy structure (MFE) and label the structures now. Label the two new bases in BOLD (draw them in darker when you draw it, or point arrows toward them so it’s clear where the new bases lie). For each structure, write the predicted free energy of folding next to the structure. For no credit beyond your own personal reward, think about the relationship between length and stability (and chance to find more or better interaction surfaces, and almost certainly new stacking surfaces). Without knowing, I predict that your longer sequence will be more stable.

In words, describe what the major differences are in the sequences. Your answer might be that little happened—the new bases went into loops that didn’t affect the overall structure. On the other hand, it might have totally re-configured your structure! Or maybe they just ended up as a small new bulge loop. Say what you see.

2.) Create your own riboswitch! Build a riboswitch around a ribosome-binding site (RBS) in an mRNA. Here we’ll use the RBS = AGGAGGU in RNA, close to an AUG start codon. You want to create a riboswitch that, depending on conditions, either: a) makes the RBS sequence available; or b) sequesters the RBS sequence so the ribosome can’t initiate translation. In this case, a small molecule regulator binds within a bulge loop with the sequence: 5’CACAAUG (early in strand) 5’CAGUG (later in strand). The secondary structure has two extra helical A’s and looks like:



Create a riboswitch within an mRNA strand you design that accommodates all the sequence and regulatory information provided. In other words, make up an RNA sequence that can toggle between two stable secondary structures. One structure will have the small molecule (SM) bound in the bulge loop above and show the RBS sequestered. The second structure will show an alternative that makes the RBS open and available.

Three qualifiers: You must submit your own sequence and therefore structure! Turning in the same answer as someone else will be viewed as academic misconduct. Don’t worry--there are a huge number of possible answers. Provide both your one-dimensional answer (e.g. AGGAGGUCCAGC…) and your 2D riboswitch depiction. Also, send your sequence through the RNA structure prediction site (question 1) and re-draw the output next to your sequence for comparison. It’s OK to use an electronically drawn answer if you want, but you may not use the picture directly from the algorithm.