HW 3: Synthetic transcriptional circuits.

(Dated: October 20, 2010)

1. SMALL BACTERIAL RNAS.

As discussed in class, small RNAs ArcZ, DsrA and RprA all positively regulate expression from the *rpoS* mRNA.

- Using the EcoCyc webpage, find the sequence of the 5'UTR of the *rpoS* gene. You will need about 200 nucleotides upstream of the start codon. Similarly, find the sequences of small RNAs ArcZ and DsrA.
- Use the NUPACK analysis tool, an online nucleic acid folding and structure predication resource, to find the minimum free energy structure (the most stable fold) of ArcZ, DsrA and the *rpoS* leader sequence at 37 $^{\circ}C$. What do you observe? What are the free energies for those structures?
- Using NUPACK, predict the structures that are formed when ArcZ and DsrA bind to their target. Assume that the sRNA and target are both at a concentration of 1 μM. Use NUPACK utilities to label the sequence with numbers. How many base pairs are formed between the two sRNAs and their target?
- Based on these results or based on other online resources, try to identify the RBS of the *rpoS* mRNA.

CELLULAR MOLECULES AND THEIR ABUNDANCE.

- How long is the *E. Coli genome*, how many genes are there, and how many of those genes encode small RNA?
- What is a plasmid and what are main features of a plasmid? Give an example of an *E. Coli* plasmid.
- What's the size and volume of E. Coli and what are the copy numbers of (i) the genome, (ii) a typical plasmid, (iii) a typical mRNA, (iv) a sRNA and (v) a protein? In the previous example we made the assumption that the concentrations of all RNAs are 1 μM. Is this in the reasonable range?

3. SMALL RNA MODELS.

- Propose a model for the positive regulation of an mRNA by a sRNA and solve for the protein concentration in steady state.
- Propose a model for negative regulation where the sRNA acts catalytically. That is, binding of the sRNA to the mRNA leads to degradation of the mRNA but not of the sRNA (which can be reused). Solve this model for steady state mRNA and protein concentration.