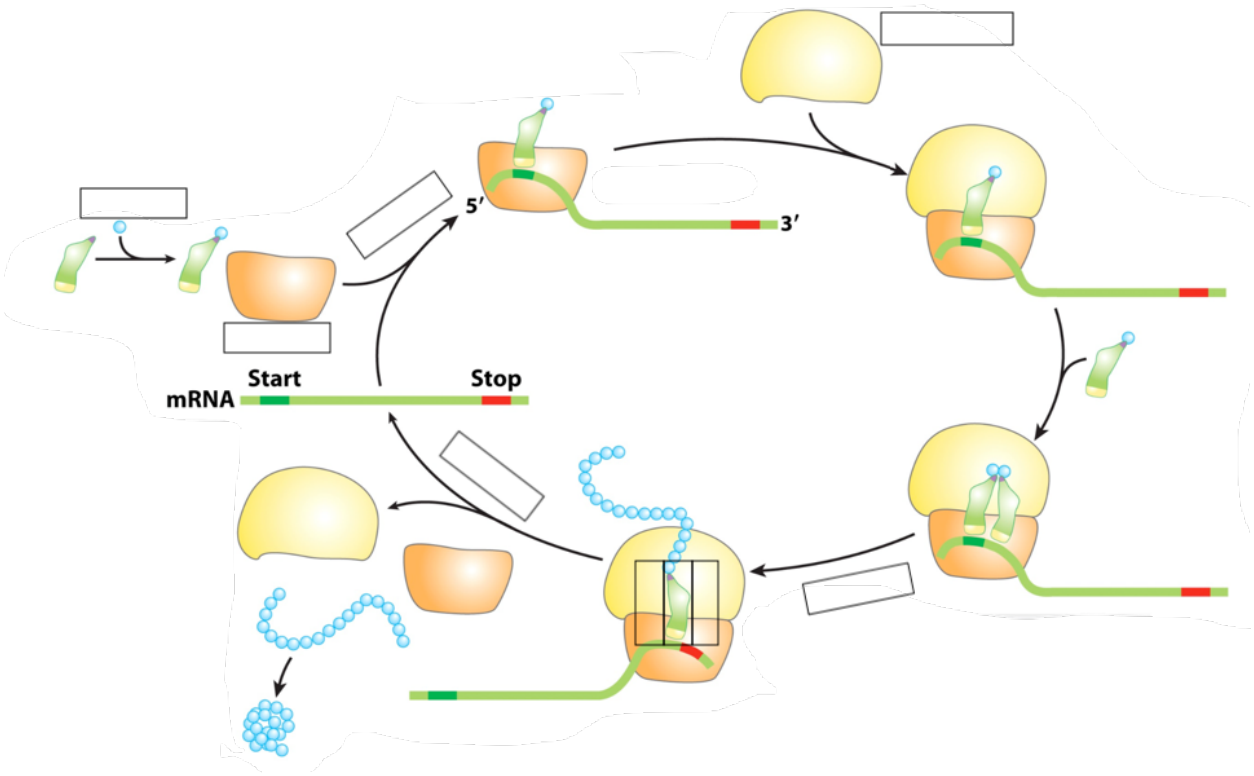
BMB170, Fall 2017
Problem Set 4: Protein Translation and Biological Membranes

Due: 11/21/2017 by noon, as PDF (preferred) or hard copy.
OH: 11/20, 3:00-5:00PM, Broad Café
Please email questions and PDF files to Jingzhou Wang (jingzhou@caltech.edu)

Problem 1: Ribosomes and Protein Translation (35 points)

Below is a simplified diagram of the ribosome assembly and protein translation cycle. The names of some specific steps, molecules, and structural elements are omitted.



a. Protein Synthesis Overview. Assume eukaryotic ribosomes are depicted in this diagram. Please fill in the blanks with one of the following words: amino-acylation, initiation step, elongation step, termination step, 60S subunit, 40S subunit, E site, P site, and A site. Please circle the binding site for EF-G on the structure that is most appropriate for EF-G to bind. What does EF-G do? (10 points)

b. Regulations of Eukaryotic Translation Initiation. The initiation step for eukaryotic translation is coordinated by multiple macromolecules, and is also tightly regulated. Please read the review article from Jackson et al. about eukaryotic translation initiation process (doi:10.1038/nrm2838). Briefly describe how is initiation regulated. What are the major two types of machineries that are targeted for regulation? For each type of regulation, which biochemical mechanism(s) is(are) used to regulate the initiation process? Please also describe one un-answered question in this field. (10 points)

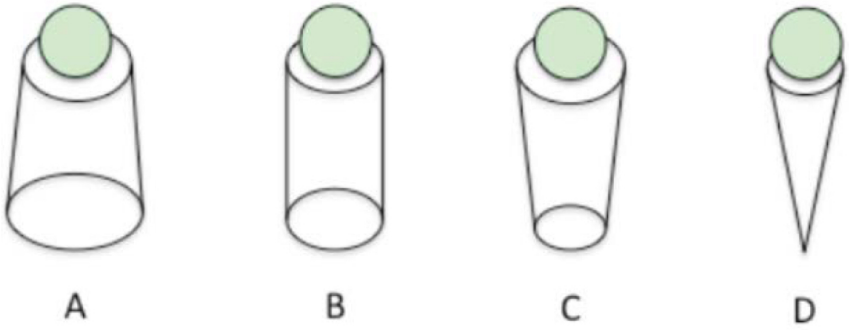
c. Ribosomes are Ribozymes. In 2000, the Tom Steitz group published the first crystal structure of the 50S ribosome subunit (doi:10.1126/science.289.5481.905). Please open the structure with PyMOL (PDB ID: 1FFK). Color RNAs in grey and proteins in yellow. Highlight the 23S rRNA in red with an appropriate label. Based on the structure, what roles do you think are respectively played

by the protein components and nucleotide components? Identify three distinct binding interfaces that you think are major stabilization contributors for the structure, and rationalize your choices. (15 points)

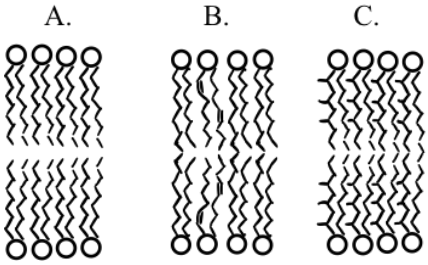
Problem 2: Lipids (30 points)

a. Hydrophobic Effect
   1) Describe the concept of hydrophobic effect. (2 points)
   2) How does the hydrophobic effect mediate the formation of lipid bilayers? (2 points)

b. Phospholipid Arrangement in Membranes
   1) Below are four types of phospholipid with different tail curvature. Estimate the packing parameter for each one of them. Based on the estimated packing parameter, how would each phospholipid assemble? Which one will assemble into the biologically relevant structure? (Hint: *P=v/al*) (5 points)



   2) Below are three different types of biological membranes. Please rank them by thermo-stability. Briefly explain your choices. Are there other factors that might change membrane thermo-stability? If yes, please elaborate. (5 points)



c. Lipid flipping: Flipping of lipids across cell membrane is mediated by flippases. In 2015, Perez et al. solved the crystal structure of a flippase, PglK, and proposed a catalytic mechanism (doi:10.1038/nature14953). Please read the paper and answer the following questions.
   1) To monitor the flipping process, the authors developed an *in vitro* essay. Briefly describe the rationales behind the designs of the essay. (5 points)
   2) When solving the structure of ADP-bound PglK, the authors started with a dataset of low resolution. What did the authors do to overcome this limit and build the model? (6 points)
   3) The authors studied external helix EH extensively in this study. What prompted them to focus on this helix? How did they design the experiments? What did they conclude? (5 points)

Problem 3: Membrane Proteins (35 points)

a. Crowded Membrane.
   1) For *E. coli*, about 1/3 of its proteome are membrane proteins. The median length for proteins in *E. coli* is ~277 amino acids. The average mass of amino acids is ~100 Da. Assume that each membrane protein is a sphere and occupy a patch of membrane corresponding to the area of its middle cross-section. Roughly estimate the average distance between every two membrane proteins for an *E. coli* bacterium. Can all membrane proteins fit in the membrane through the way described here? (3 points)
   2) What strategies, do you think, do cells employ to effectively fit all their membrane proteins in the membrane? (3 points)

b. Co-translational Translocation. Voorhees et al. published the structure of the ribosome-Sec61 complex to gain insights into the detailed mechanism of co-translational translocation (doi:10.1016/j.cell.2014.05.024). Please read the paper and answer the following questions.
   1) Which technique did they choose to visualize this structure? Why this technique is advantageous in solving structures of protein complexes compared to other structural determination methods? (3 points)
   2) How did the authors prepare their samples before collecting structural data? Which procedures helped stabilize the transmembrane domain of the translocon? How did they make sure that the ribosome in complex with the translocon was actively translating? (5 points)
   3) Based on the structural data, they generated a two-step model for Sec61 activation. Briefly describe this model. Based on what evidences did they come up with this model? (5 points)

c. Membrane Protein Topology. Describe the positive inside rule. How is this rule supported by data from biochemical essays? How is this rule supported by bioinformatics data? (4 points)

d. Crystallography of Membrane Proteins.
   1) Expression: What factors would you consider when you design a system for heterologous expression of membrane proteins? Name two factors, and explain why you believe that they are crucial for membrane protein expression. Please name an *E. coli* strain that is optimized for membrane protein expression. For the strain that you choose, what makes it more suitable for membrane protein expression? (6 points)
   2) Isolating Membrane Proteins: What methods can you use to extract membrane proteins from living cells? How do you purify them? (3 points)
   3) Crystallization: describe three crystallography methods that are beneficial for membrane protein crystallization. (3 points)