BMB170, Fall 2017

Problem Set 4: Protein Translation and Biological Membranes

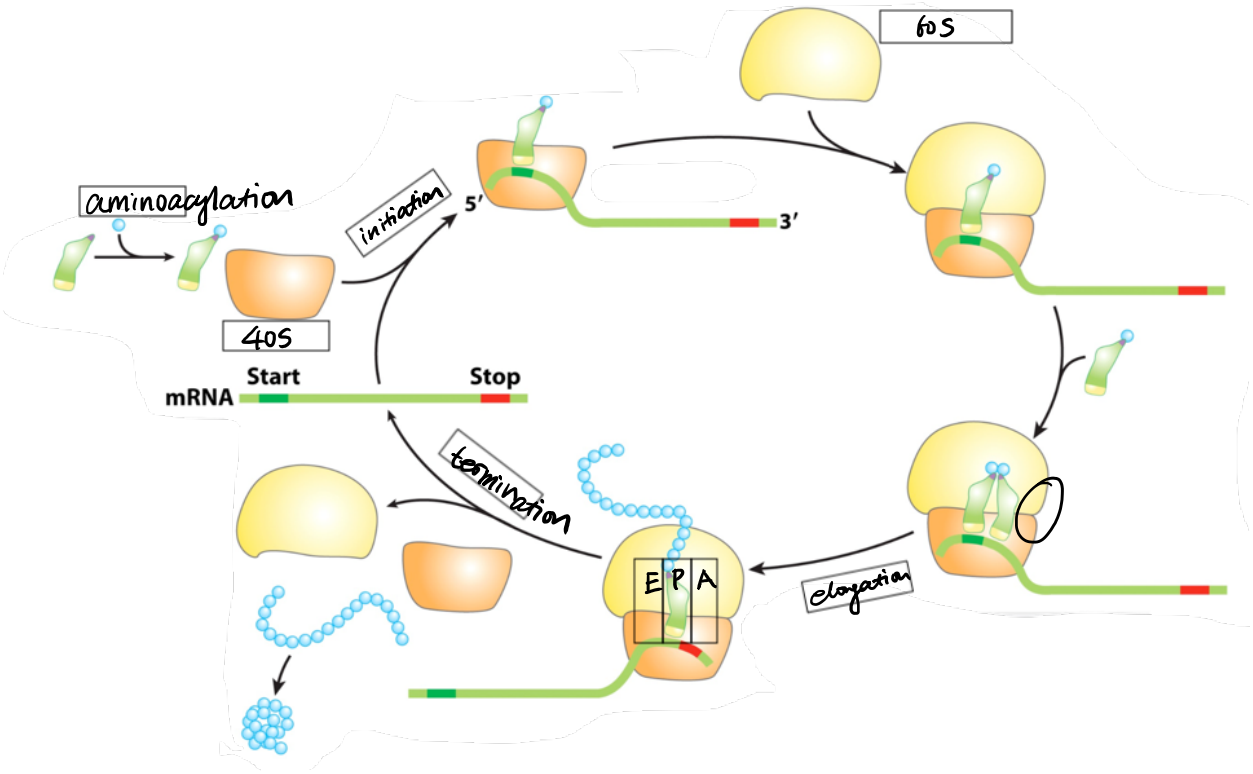
Due: 11/21/2017 by noon, as PDF (preferred) or hard copy.

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)
EF-G initiates elongation by translocating previous amino-acyl tRNAs downstream.
1 point each for filling in the correct blank;
for EPA sites, if all correct give 1 point, and for every wrong filling, deduct 1/3 point.
For circling the binding site of EF-G: 2 points if circle on the correct structure and correct location.
1 point if either on the wrong structure or the wrong location.
1 point if correct explanation for EF-G function.

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)

Two main categories: eukaryotic initiation factors (eIFs) regulation and mRNA regulation. For eIFs regulation: reversible phosphorylation and proteolysis. For mRNA regulation: protein, miRNA, or a combination of both directly bind to mRNA through sequence-specific interactions. Unanswered questions: (one of the following) elucidating IF binding sites on 40S subunits, miRNA-mediated repression mechanisms, mechanism of regulation of vertebrate TOP mRNAs.

2 points for correct main categories.

3 points for naming the detailed mechanism for eIFs regulation.

3 points for naming the detailed mechanism for mRNA regulation.

2 points for naming one unanswered question. If the student gives an answer different from the one listed above, points can be given based on the reasoning that the student gives.

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)

Labeling the structure: 1 point if a structure is shown. 2 point if yellow proteins. 2 point if the largest RNA (23S rRNA) is colored red. 1 point if the rest of other RNA is colored gray.

Function of the proteins: 3 points if the student answers that the proteins stabilize the rRNAs to allow rRNAs to properly perform catalysis.

Describing binding interface: 1 point for pointing out the binding interface and gives proper description. 1 point for a reasonable explanation that backs up why the described interface is important for the structure.

Problem 2: Lipids (30 points)

a. Hydrophobic Effect

1) Describe the concept of hydrophobic effect. (2 points)

Favorable aggregation of hydrophobic molecular groups in water while excluding water molecules. 1 point The favorability is explained by breaking ordered water cage formed around hydrophobic molecular entities to increase entropy. 1 point

2) How does the hydrophobic effect mediate the formation of lipid bilayers? (2 points)

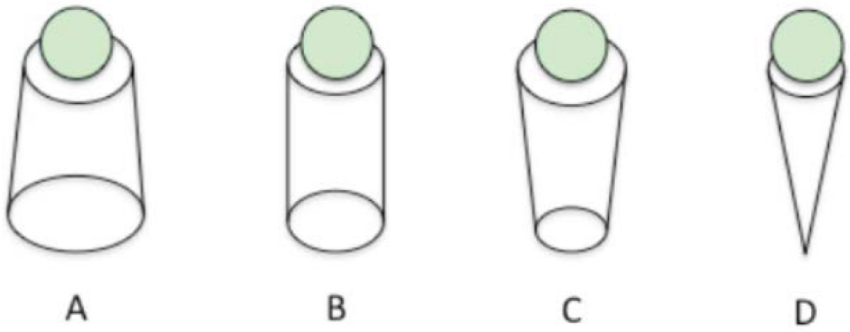
phospholipid molecules contain a hydrophilic group and a hydrophobic group. Hydrophobic effect mediates the hydrophobic group into excluded from water molecules. Therefore, the bilayer structure is formed as the hydrophobic groups are buried in the middle and only the hydrophilic heads are exposed to solvent.

1 point if pointing out that phospholipids are amphipathic.

1 point if stating the relationship between hydrophobic effect and the amphipathic characteristics of lipid bilayers.

b. Phospholipid Arrangement in Membranes

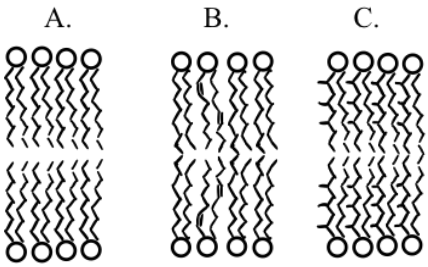
1) Below are four types of phospholipid with different tail curvature. Estimate the packing parameter for each one of them. Based on the estimated packing parameter, how would each phospholipid assemble? Which one will assemble into the biologically relevant structure? (Hint: $P=v/al$) (5 points)



A: P >1. B: P=1 C: 1<P<1 D: P<1/3. B will assemble into the correct structure.

1 point for each correct approximation of P value. 1 point for pointing out the biologically relevant choice.

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)



Ranking as highest thermostability to lowest: A>B>C or A>C>B. A is the most tightly packed because no disruptive element of molecular interactions is present.

(For B and C, it is still a question under debate in the field. The answer will be judged by reasonable thinking that backs up the choice) Answer examples: *If it is estimated to be B>C*: the introduction of branched chains can potentially be far more disruptive than double bonds due to potential steric clashes. *If it is estimated to be C>B*: the introduction of double bonds adds strains that prevent each aliphatic chain from sampling the most energetically favorable packing conformation, whereas in C the bonds are allowed to sample more rotamer conformations that may result in a tighter packing than B.

2 points for giving a reasonable ranking. 3 points for describing individual traits of A, B, and C that allow the student to give that ranking.

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)

PglK can process LLOs with shortened oligosaccharides in vitro. 1 point The in vitro translocating environment is in proteoliposomes with co-reconstituted PglK and tLLO that has two GalNAc moieties. tLLO flipping is determined by radiolabeling GalNAc residues on tLLOs that are translocated to the outside of the proteoliposomes with PglH, a specific glycosyltransferase. 4 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)

A previous finding shows that transmembrane helices of ABC exporters move in pairs during structural transitions helps them to help the model building.2 points They later confirmed that the model was correct through anomalous diffraction data from Hg-soaked crystals.2 points A selenomethionine derivative also allowed the authors to position crystaine and methionine residues to further improve the model building. 2 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)

The helix EH is on an unusual location (a loop) and has unusual surface. 2 points They generated a deletion mutant of helix EH by replace the entire helix with a flexible linker to study the general effect of the helix. They also generated a triple mutant that converts prominently located T into A, and a double mutant that disrupts stabilizing interactions involving positively charged residues. All of the three mutants showed ATPase activity irresponsive to LLO stimulation. Suggesting LLO binding on PglK is disrupted. They therefore speculated that the hydrophobic grooves near the EH might contribute to the binding of LLO to PglK. 3 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)

3 points will be given if the student gives resonable calculation that concludes that through the way describe in the question prompt all membrane proteins cannot or can barely fit on the cell membrane.

Numbers can be found through Google:

$1.35\ g/cm^3$: protein density $6\ \mu m^2$: e.coli surface area

$0.95\ pg$: e.coli cell weight 15% : percentage of protein weight of e.coli cell

weight of one protein: $277 \times 100 / 6.02\times10^{23}\ g = 4.6\times10^{-20}\ g$

weight of total membrain protein $0.95\times10^{-12} \times 0.15 \times \frac{1}{3} = 4.75\times10^{-14}\ g$

# of total membrain protein: $4.75\times10^{-14} / 4.6\times10^{-20} = 103260$

V of one protein $= 4.6\times10^{-20} / 1.35 = 3.33\times10^{-20}\ cm^3$ | area of cross section $= \left(\frac{2.33\times10^{-22}\times3}{4\pi}\right)^{\frac{2}{3}}\pi = 1.25\times10^{-13}\ cm^2$

Total area occupied by membrane proteins: $1.25\times10^{-13} \times 103260 = 1.29\times10^{-7}\ cm^2 = 12.9\ \mu m^2$

$\therefore 12.9 > 6$

$\therefore$ This is not how membrane proteins fit on the cell membrane

2) What strategies, do you think, do cells employ to effectively fit all their membrane proteins in the membrane? (3 points)

Membrane proteins fit in lipid bilayers effectively through a rather 3-dimentional scheme of fitting, through which the intra-membrane space, membrane surface, and extra-cellular space are all effectively occupied. More specifically, membrane proteins effectively use the

intramembrane space by integral membrane proteins, and membrane surface or extracellular space through membrane-anchored proteins.

3 points will be given if the two types of membrane proteins are described.

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)

Cryo-EM. 1 point Cryo-EM can study interactions in large complexes without harsh limitations such as requirement of homogeneous crystals in X-ray crystallography, or a size limit above which resolution will be dramatically reduced in NMR. 2 points

Note: if a comparison between cryl-em and other methods is not given, points can be deducted accordingly.

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)

The ribosome-translocon specimen was generated by fractionation of detergent-solubilized rough microsomes from porcine pancreas. 2 points Detergent helped stabilize the transmembrane domain. 1 point The presence of translationally active ribosomes in the microsomes was verified by labeling of their associated nascent polypeptides with puromycin. 2 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)

Sec61 changes from quiescent state to primed state and finally to translocating conformation. Change from quiescent state to primed state is caused by binding of the ribosome. Change of primed state to translocating state is caused by binding of substrate peptide. Evidence supporting the transformation from quiescent state to primed state is the structural difference between Sec61-ribosome complex and previous structures of Sec61 itself, which shows a crack in the cytosolic side of the lateral gate in the Sec61-ribosome structure. The transformation from primed state to translocating state is supported by the dynamic plug domain observed in translocating Sec61, as the density for the plug was no longer visible.

3 points: describing the model.

1 point: structural difference between primed state and quiescent state

1 point: structural difference between translofacint state and primed state

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)

Transmembrane domains are inserted in the membrane in a way that keeps the positively charged face towards to cytoplasmic side and negatively charged face towards the outer face of cell membrane. 1 point

Bioinformatic: positively charge amino acids are more abundant in cytoplasmic loops that periplasmic loops. 2 points Biochemical: mutation of positively charged residues can be used to change the orientation of transmembrane domains and loops 2 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)
   For each factor: 1 point for naming it. 1 point for describing why it is important for membrane protein expression. An example:
   Translocation machinery, including SRP and SecY may be saturated when membrane proteins are overexpressed. Membrane proteins are more aggregation-prone, and therefore saturation of these chaperones may affect protein stability and cell viability, which can contribute to toxicity of membrane protein overexpression.
   1 point for naming the strain. 1 point for describing the beneficial trait of the strain. An example:
   Optimizing levels of membrane protein expression by using strains such as Lemo21(DE3), where the T7 promoter is tunable by varying L-rhamnose concentration in media, is one way to address such problem.
2) Isolating Membrane Proteins: What methods can you use to extract membrane proteins from living cells? How do you purify them? (3 points)
   Differential centrifugation to separate inclusion bodies together with detergent solubilization to stabilize hydrophobic domains in micelles can help extract membrane proteins. 2 points After extraction, standard column-based purification methods can be applied such as denatured-state affinity-chromatography. 1 point
3) Crystallization: describe three crystallography methods that are beneficial for membrane protein crystallization. (3 points)
   2D crystallization, antibody stabilization, and nanodisc.
   1 point for each naming. If a different choice than than the ones given in this answer, point can be given if that choice is reasonable.