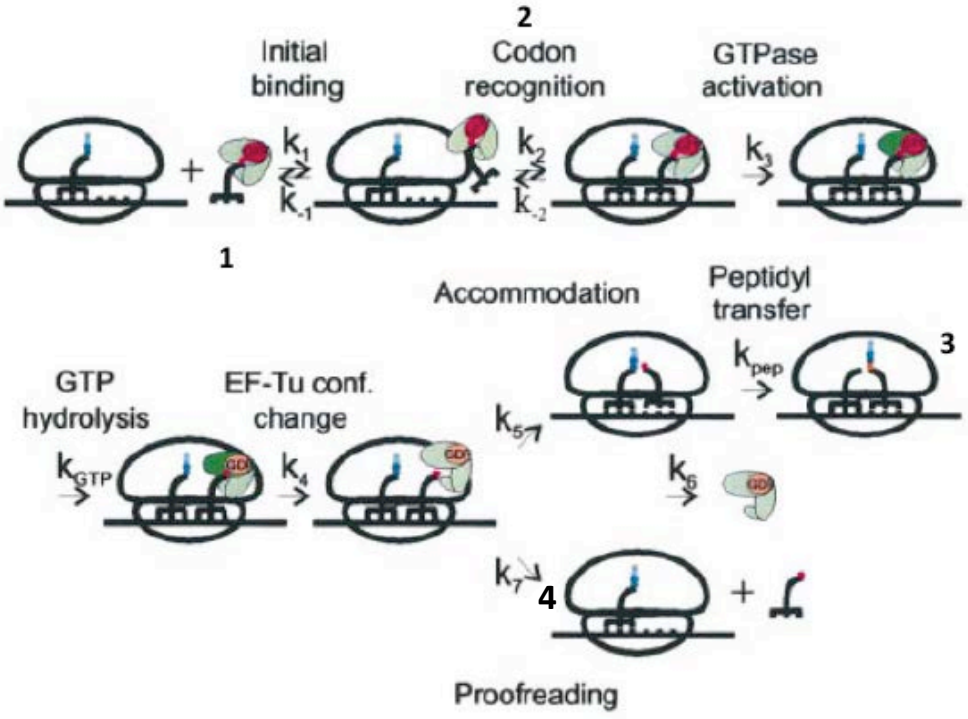
5.08 Biological Chemistry II (Spring 2016)
Problem Set #2

This problem set contains two questions and four pages.

Question 1:

The following cartoon depicts the model for EF-Tu-dependent aa-tRNA^{aa} binding to the ribosome. The E site is not shown in this depiction. This model is based on numerous experiments. You will examine a number of these experiments during recitation 3. The information that will be presented in recitation 3 is not required for answering this question.



Answer the following questions:

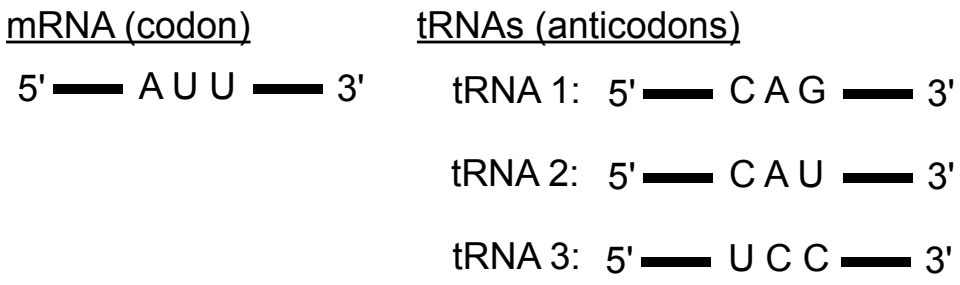
A) In the cartoon above, what is depicted by the molecule labeled as "**1**"? What is the process defined by k_1 and k_{-1}?

B) Describe the process labeled "**2**." Provide an explanation for the forward and reverse reactions (k_2 and k_{-2}).

C) What is depicted in the ribosome cartoon shown as "**3**"?

D) In the cartoon above, what steps are important for fidelity? Why?

E) Consider the following tRNAs and mRNA:



i) Draw the codon / anticodon interactions for each mRNA / tRNA pair and indicate whether the pair is a cognate, near-cognate or non-cognate match.

ii) What is the consequence of each mRNA/tRNA pair on the process labeled "**2**"?

iii) What is the consequence of each mRNA/tRNA pair on the process labeled "**4**"?

F) What occurs immediately after peptidyl transfer? Draw a cartoon of the ribosome in this state. Include and label (i) the mRNA (labeling 5' and 3' end), (ii) the three sites of the PTC, and (iii) nascent polypeptide emerging from the exit tunnel in your cartoon.

Question 2:

Nascent polypeptide chains must fold into native conformations, and we will address protein folding in module 2. In many labs, protein folding is performed and studied in aqueous solution, hence in the "test tube." These studies provide useful information, but the folding environment provided by simple aqueous buffer is very different from what the polypeptide encounters in the crowded environment of the cell. Imagine forgetting the test tube for folding experiments and venturing into the cell. Professor Gary Pielak's lab

at University of North Carolina Chapel Hill is pioneering studies of protein folding inside living cells – wow! One approach that the Pielak Lab uses is to incorporate ^{19}F labels into the protein of interest and employ ^{19}F NMR spectroscopy to study protein folding in live *E coli* cells (see *J. Am. Chem. Soc.* **2010**, *132*, 321-327 if you are curious; you need not read the paper to answer this question). In these experiments, the *E. coli* cells produce the ^{19}F-labeled protein in the cytosol, and these *E. coli* are placed in a NMR tube and the ^{19}F signal(s) recorded.

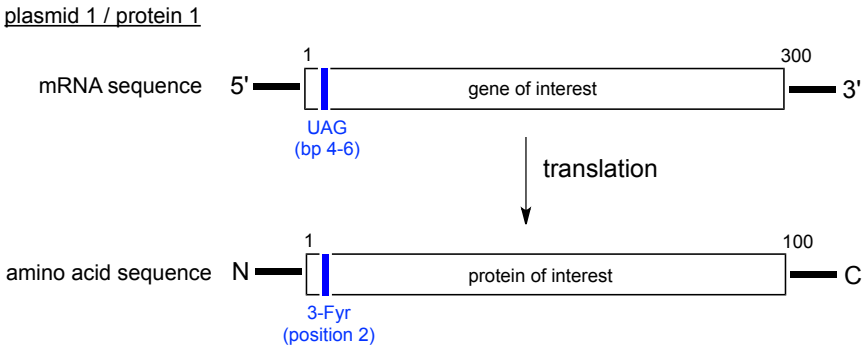
Why the choice of the ^{19}F label? In brief, ^{19}F NMR is a useful technique because ^{19}F is a spin ½ nucleus and has 100% natural abundance. The ^{19}F nucleus gives a wide range of chemical shifts (400 ppm versus 13 ppm for ^{1}H) and the chemical shifts are very sensitive to the local environment (consider that a fluorine nucleus is surrounded by 9 electrons compared to 1 electron for a proton). Moreover, taking a ^{19}F NMR spectrum is simple, very much like recording a ^{1}H NMR. Various fluorinated amino acids, including 3-fluorotyrosine, have been employed in ^{19}F NMR and in the cell-based experiments reported by the Pielak Lab. (Note: ^{19}F is not a radioisotope.)

Imagine you wish to study the folding landscape of your protein of interest in living *E. coli* cells by ^{19}F NMR. To do so, you will have *E. coli* site-specifically incorporate the unnatural amino acid (UAA) 3-fluorotyrosine (3-FTyr) into your protein using its endogenous ribosome. Your protein is 100-aa in length.

A) Draw the chemical structure of 3-fluorotyrosine.

B) You will need an evolved tRNA/aaRS pair in order to incorporate 3-fluorotyrosine into your protein. Based on class discussion, describe the essential features of this tRNA/aaRS pair.

C) You perform site-directed mutagenesis to place the Amber STOP codon at various positions in your plasmid that harbors the gene for your 100-aa protein. In plasmid 1 (encoding protein 1), the Amber STOP codon is at position 2 (the bp triplet encoding residue 2). In plasmid 2 (encoding protein 2), the STOP codon is at position 56. In plasmid 3 (encoding protein 3), the STOP codon is at position 100 (C-terminal residue). Using the diagram for the plasmid/protein 1 mutant as a guide, sketch the corresponding mRNAs that arise from transcription of the gene of interest and the full-length polypeptides that arise from translation for the position 56 and 100 variants.



D) In class, we learned about release factor 1 (RF1). This release factor is a problem for UAA incorporation by the endogenous ribosome because it recognizes the Amber STOP codon and hydrolyzes the peptidyl-tRNA, causing formation of truncated proteins. In designing your experiments further, you consider the potential effect of RF1 on the synthesis of proteins 1-3 described above. Describe the expected outcomes for translation of the mRNAs encoding proteins 1-3 taking RF1 action into account.

E) Based on your answers to C and D, what mutant protein will you select to employ in your first in-cell ^{19}F NMR experiments? Briefly explain your reasoning. Hint: to answer this question, ask what are the possible consequences of your answer in D on your planned ^{19}F NMR experiment.

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