5.08 Biological Chemistry II (Spring 2016)
Problem Set #1

This problem set contains one question and two pages.

Question 1:

In the translation module, we will discuss the role of EF-Tu in aatRNA^{aa} delivery to the A-site of the ribosome. Recall from the translation overview lecture that EF-Tu is a GTPase. If the codon-anticodon interaction between the mRNA and aatRNA is cognate, then GTP hydrolysis occurs to give GDP-bound EF-Tu and free P_i. Note that GTP hydrolysis by EF-Tu is slow in solution and in the absence of cognate codon/anticodon pairing.

Background on GTPases:
GTPases have several conserved structural elements surrounding the GTP-binding site. These conserved structural elements include the P-loop, switch 1, and switch 2.

P-loop: ^{18}GXXXXGK(T/S) (X = an amino acid)
The P-loop is involved in binding the phosphate groups of GTP.
Switch 1: Residues 51-62
Note that Thr62 binds Mg(II).
Switch 2: ^{81}DXXG^{84} (X = an amino acid)
Note that Asp81 is involved in H-bonding interactions.

In this question, you will examine the structures of EF-Tu in the GTP and GDP-bound forms. To do so, first obtain the listed PDB files and use them to answer the following questions:

PDB files:
1EFT: EF-Tu with GDPNP bound (GDPNP is a non-hydrolyzable GTP analog).
1TTT: Ternary complex of Phe-tRNA^{Phe} •EF-Tu •GDPNP.
1TUI: EF-Tu with GDP bound.

You are welcome to include your PyMOL images in your problem set. If you do so, be certain to label each one appropriately in terms of the question being answered (e.g. A, B, C…) and such that your TA or professor can look at the figure and understand the point(s) you wish to make.

Use PyMOL to analyze structures and answer the following questions:

A) Look at the Phe-tRNA^{Phe} •EF-Tu •GDPNP ternary complex (**1TTT**). What is the anticodon sequence of the Phe-tRNA^{Phe}? Indicate the 5' and 3' ends, the identities of the nucleobases, and the positions of the nucleobases in your answer.

B) Is there anything surprising about this anticodon sequence? If so, what is surprising?

C) Look at the Phe-tRNA^{Phe}•EF-Tu•GDPNP ternary complex (**1TTT**). Is the anticodon solvent accessible?

D) Look at the Phe-tRNA^{Phe} •EF-Tu •GDPNP ternary complex (**1TTT**). Identify C70 and G3 in the tRNA. Draw the H-bonding interactions and label each one with the distance. You will need to find and use PyMOL functions/tools for computing H-bonds and bond distances to do so. Make sure that the image or hand drawing you provide as an answer is clear to read.

E) Compare the structures given by **1EFT** (GDPNP-bound EF-Tu) and **1TUI** (GDP-bound EF-Tu) and focus on the GTPase center of each. What conformational changes do you see (hint: focus on switch 1 and switch 2 described above)? In other words, how does the conformation of EF-Tu change following GTP hydrolysis?

F) In class, we will learn that a His residue (His84) of EF-Tu is important for catalysis. Kinetic studies performed by Rodnina and co-workers revealed that mutation of His84 to another amino acid reduced the rate of GTP hydrolysis by EF-Tu on the ribosome by five orders of magnitude! Identify the relevant His residue in both **1EFT** and **1TUI** and compare the positioning and environments of these His residues. What do you see? Note: the His of interest is His85 in the PDB structures.

G) Propose a role for this His residue in catalysis.

H) Draw the chemical structure of GDPNP. Why was GDPNP utilized to obtain the crystal structures of the GTP-bound forms?

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