ANSWER KEY

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:

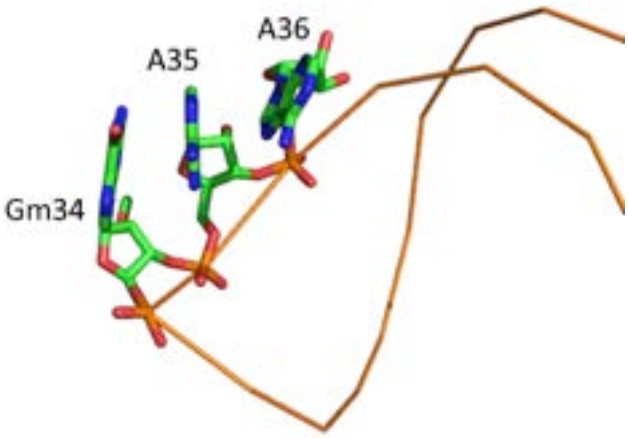
PDB files:
1EFT: EF-Tu with GDPNP bound (GDPNP is a non-hydrolyzable GTP analog).
1TTT: Ternary complex of Phe-tRNA^{Phe} •EF-Tu •GDPNP.
1TUI: EF-Tu with GDP bound.

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:

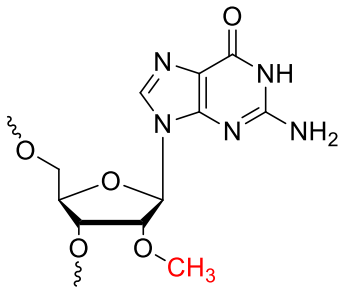
A) Look at the Phe-tRNA^{Phe} •EF-Tu •GDPNP ternary complex (**1TTT**). What is the anticodon sequence of the Phe-tRNA^{Phe}? Indicate the 5' and 3' ends, the identities of the nucleobases, and the positions of the nucleobases in your answer.

The anticodon sequence is 5'-Gm-A-A-3' (positions 34-35-36).



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

Yes, there is a little surprise. Position 34 contains a modified nucleobase (2-O-methylguanosine or Gm)! Its structure is given below. Recall from lecture (tRNA overview) that ~25% of all tRNA bases can be modified.



2-O-methylguanosine

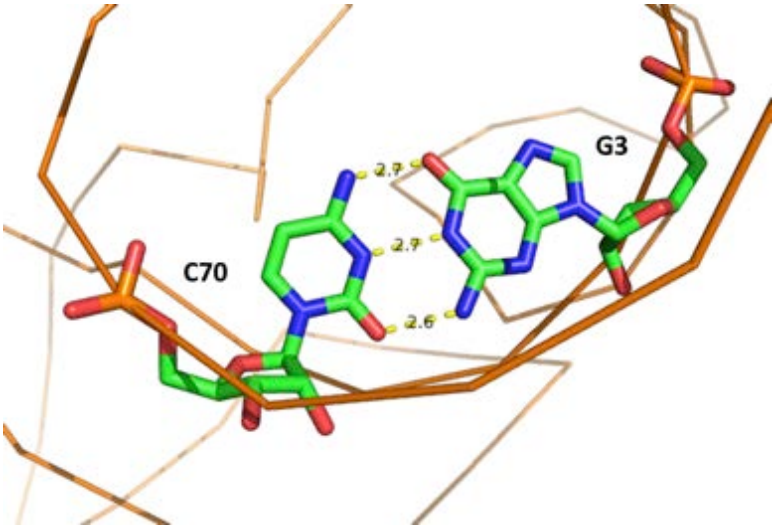
C) Look at the Phe-tRNA^{Phe}•EF-Tu•GDPNP ternary complex (**1TTT**). Is the anticodon solvent accessible?

Yes.

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.

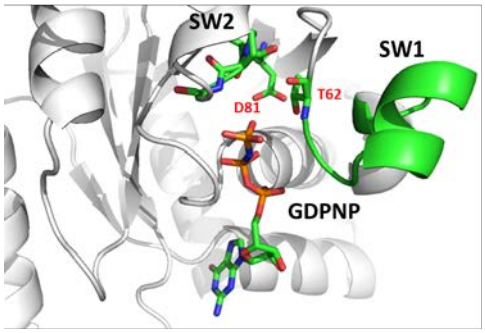
The H-bond lengths are 2.7, 2.7, and 2.6 Angstroms.



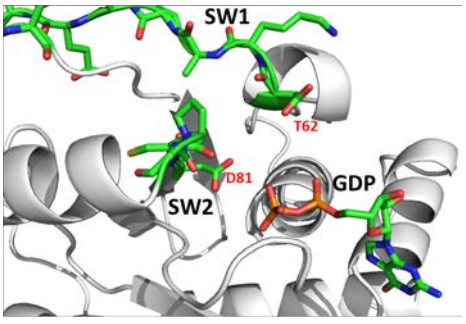
E) Compare the structures given by **1EFT** (GDPNP-bound EF-Tu) and **1TUI** (GDP-bound EF-Tu) and focus on the GTPase center of each. What conformational changes do you see (hint: focus on switch 1 and switch 2 described above)? In other words, how does the conformation of EF-Tu change following GTP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

Switch 1 and switch 2 participate in major conformational changes associated with GTP hydrolysis. In the GDPNP-bound form, the GDPNP is bound inside of EF-Tu. In the GDP-bound form, we see that the GDP is solvent exposed. A major conformational change that occurs after GTP hydrolysis is that switch 1 goes from an alpha-helix (1EFT, GDPNP-bound form) to a random coil (1TUI, GDP-bound form). Switch 2 also undergoes conformational change. Note the movement in Thr62 (switch 1) and Asp81 (switch 2). These conformational changes result in opening of a "gate" that is necessary for GTP hydrolysis.

Moreover, looking beyond the GTPase center, movement of switch 1 and switch 2 results in a 90 degree shift in domain I relative to domains II and III.



EF-Tu·GDPNP
PDB: 1EFT



EF-Tu·GDP
PDB: 1TUI

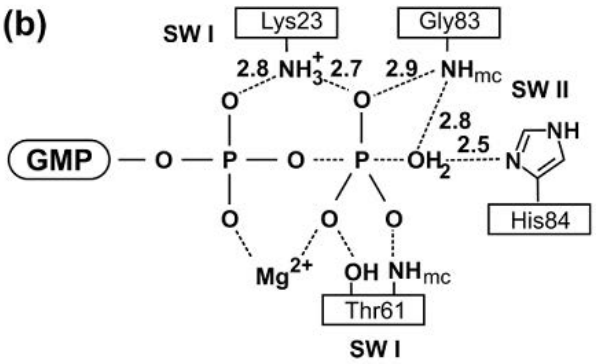
F) In class, we will learn that a His residue (His84) of EF-Tu is important for catalysis. Kinetic studies performed by Rodnina and co-workers revealed that mutation of His84 to another amino acid reduced the rate of GTP hydrolysis by EF-Tu on the ribosome by five orders of magnitude! Identify the relevant 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.

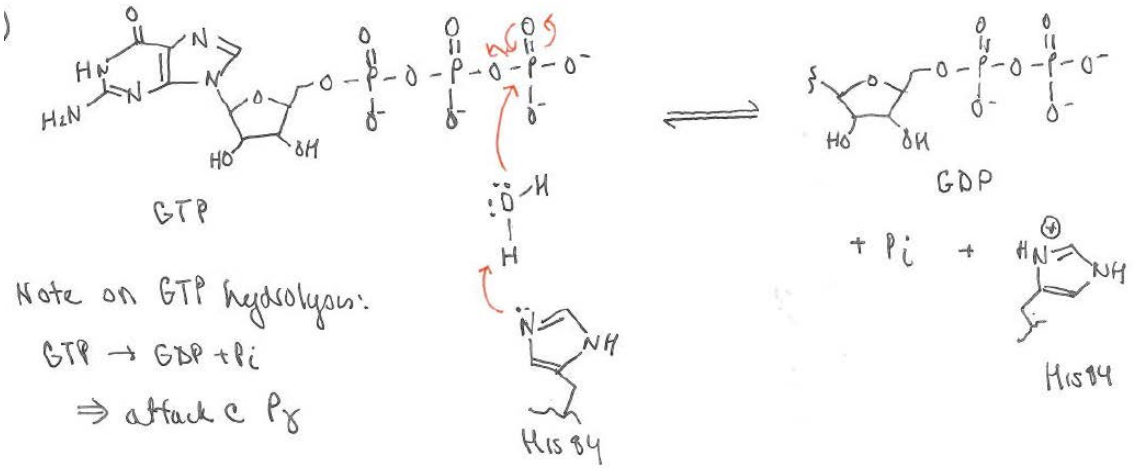
In the GDPNP-bound form (1EFT, left), His84 is pointing towards the γ-phosphate of GDPNP. The distance between N3 of His and oxygen of phosphate is about 7.0 Angstroms. A water molecule can fit into this distance to form hydrogen bonding with the His residue and the γ-phosphate of GDPNP.

In contrast, in the GDP-bound form (1TUI, right), His84 is pointing away from the β-phosphate of GDP. The distance between N3 of His and oxygen of phosphate is about 10.5 Angstroms. At this distance, we can assume that there is no interaction between the His residue and the β-phosphate.

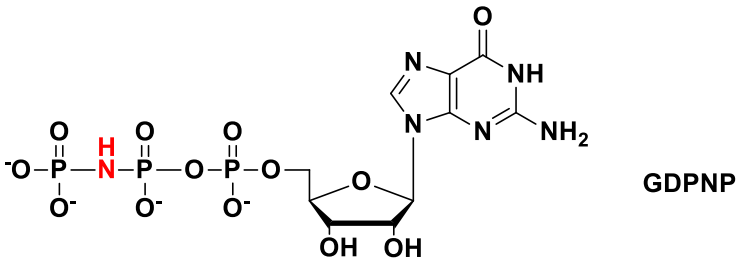
G) Propose a role for this His residue in catalysis.

One potential role for His84 in catalysis is it activates the water molecule for attack on the γ-phosphate of GTP as shown below. The following figure depicting interactions in the GTPase region is adapted from Rodnina and co-workers (*J. Mol. Biol.* **2003**, *332*, 689-699).





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



GDPNP is a non-hydrolyzable GTP analog. It forms a complex with EF-Tu and aa-tRNA^{ss}, but it cannot be hydrolyzed. Depending on the experiment and system, this feature is important because it prevents background GTP hydrolysis or enzymatic GTP hydrolysis. Non-hydrolyzable analogs are very useful for crystallography. Sometimes it takes many months for a crystal to form, and so the crystallographer does not want the GTP hydrolyzing while waiting for crystals of the GTP-bound form.

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