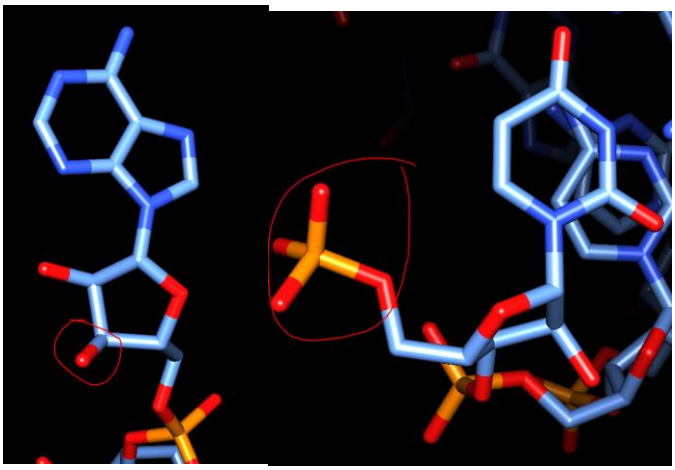
Problem Set 11 (**Due Nov 25th**)

1. Investigate the structure of the tRNA Synthase in complex with a tRNA molecule. (pdb ID 1ASY).

   a. Why don't tRNA molecules contain a 5' triphosphate like other RNA molecules do? They are not transcribed alone – instead, they are part of a long RNA molecule that contains 3 ribosomal RNAs and several tRNAs. This long polymer is processed by endonucleases to produce mature rRNA and tRNA. As such, the 5' triphosphate that is observed for all mRNA is not present on tRNA.

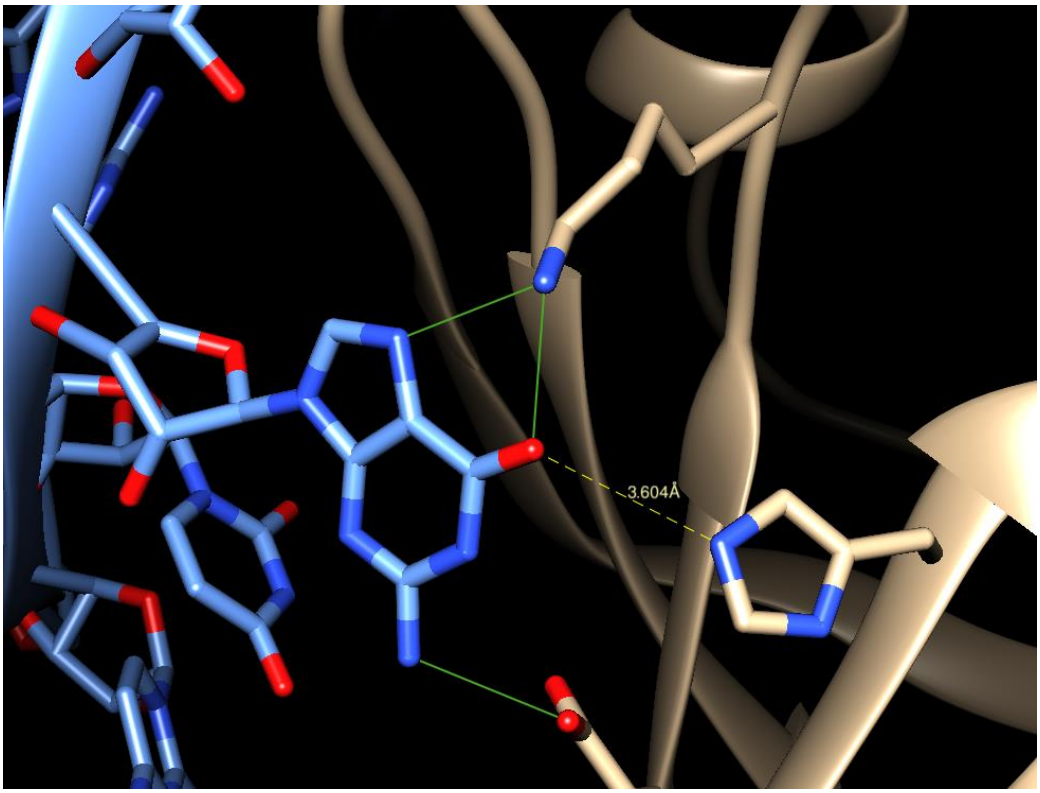
   b. Verify that the tRNA contains a 3' hydroxyl and a 5' Phosphate, as all tRNAs should. Yep – see image.



   c. What regions of the tRNA are recognized by the aaRS? Anticodon loop and acceptor stem

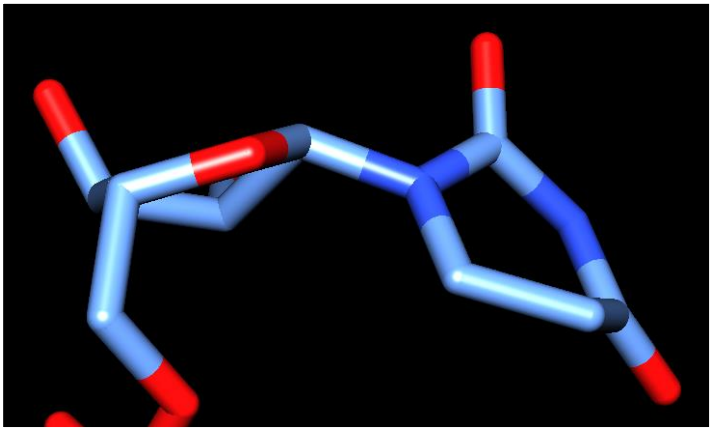
   d. Find the anticodon loop.
      i. Describe how the anticodons are positioned relative to the rest of the RNA molecule (i.e. part of a helix, base paired in non-Watson/Crick conformation, flipped out of loop, etc.) They are flipped out and not base-paired with any other bases.
      ii. What is the sequence of this anticodon? What codon would it pair with? What amino acid does it code for? 5'-GUC – 3' This would base pair with GAC codon = Asp

   e. This aaRS will recognize a tRNA molecule with an A or a G at the 3rd anticodon position. Please investigate the structure and propose a way that an A at the 3rd position will be recognized by the aaRS. AS can be seen in the image below, A major contact is found in the Hoogsteen Face of the purine; this interaction would not be possible in pyrimidines. Additionally, the Glu (shown) and the His are in close enough proximity to interact with the Watson-Crick face of purines but are not close enough to interact with a pyrimidine. Why would this aaRS recognize two different codons? Both codons code for Aspartic Acid. Having one aaRS that can recognize both tRNAs makes tRNA charging a more efficient process.

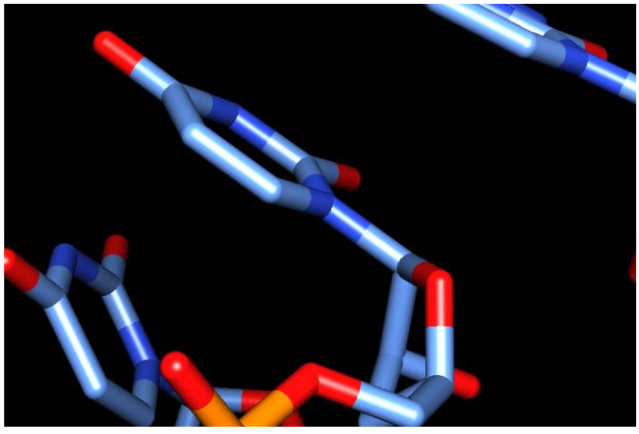


f. In addition to the 3' OH of the tRNA, what needs to be present in the active site for this aaRS to be active? Does this structure show an active conformation of the enzyme? Not completely active because it needs ATP and Asp in the active site

g. Find a Dihydrouridine in this tRNA. How does the modification change the three dimensional shape of this base?

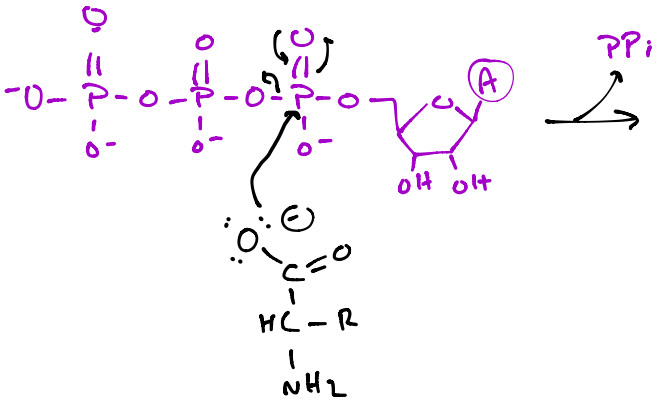


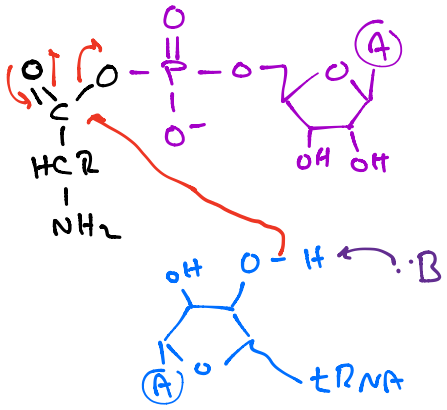
Dihydrouridine = not planar

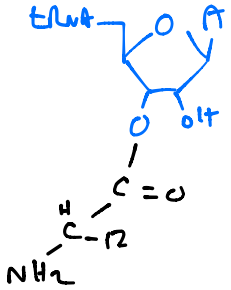


Uridine = planar

2. Show a mechanism for aaRS “charging”. Briefly discuss why this is an energy dependent process.

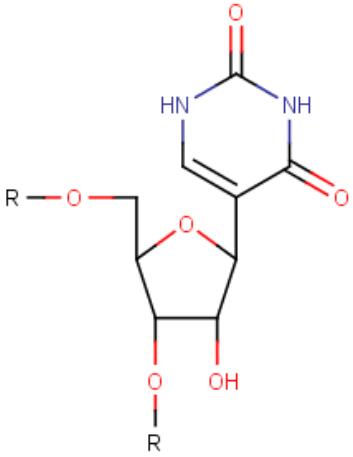


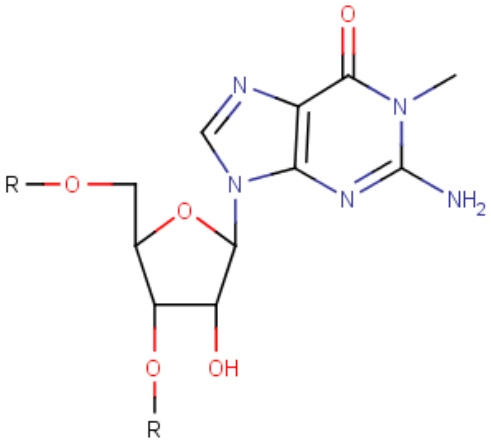




ATP = chemical energy is consumed during the reaction

3. Be familiar with the common base modifications in tRNA structures and how they can influence base pairing. Draw m^1G and Ψ.





4. Describe the steps that produce the 5' cap and 3'polyA tail in eukaryotic mRNA. Polyadenylation occurs via two steps:

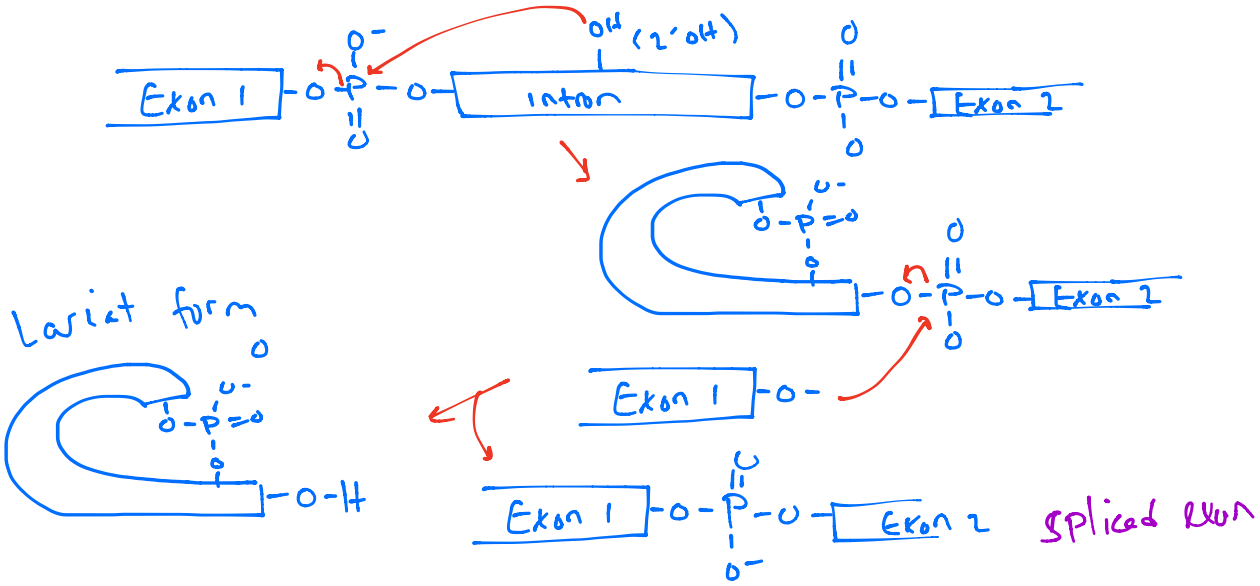
    1. Cleavage factors recognize AAUAAA – once bound, they cleave the mRNA molecule ~20 nt downstream
    2. PolyA Polymerase then binds to the cleaved sequence and catalyzes the polymerization of the poly A tail (~250 Adenosines).

    5' capping occurs via 3 steps:

    1. RNA triphosphatase – hydrolyzes the 5' triphosphate to a monophosphate
    2. Capping Enzyme – adds GDP to the 5' end – this results in a 5'-P-P-P-5- linkages of the riboses.
    3. Guanine-7-methyltransferase – uses SAM to methylate Guanine on the Hoogsteen face.

5. What is the role of the 5' cap in eukaryotic mRNA? It is important for initiating translation (it is recognized by one of the elongation Initiation Factors – eIF4E)

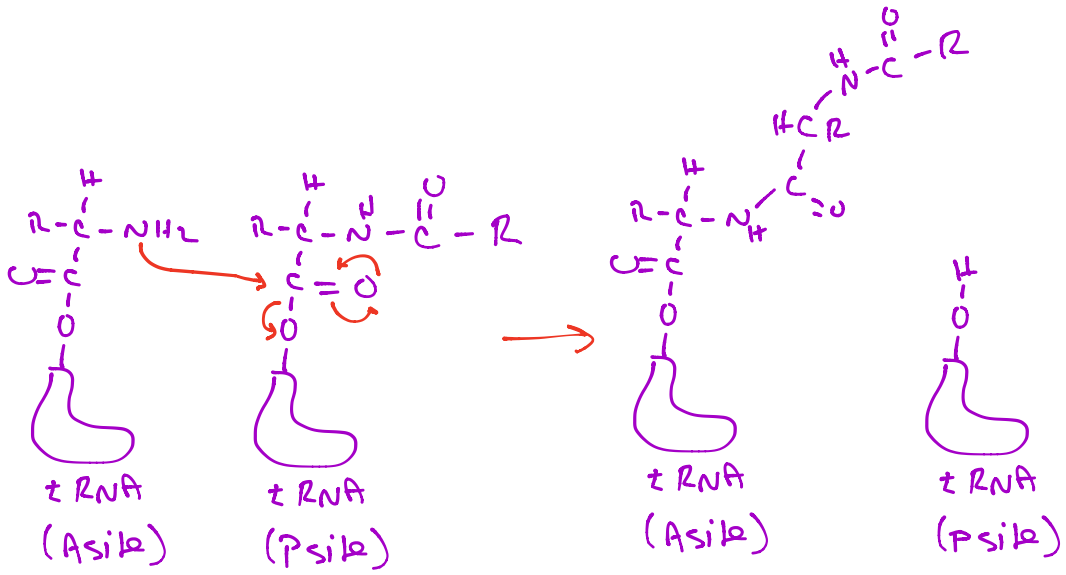
6. What is the lariat form of introns? It is a cyclical conformation that forms by the spliceosome when the exons are spliced together. Sketch how it is formed – this can be very general, but make sure your sketch shows the important steps and what happens to the RNA chain during this process.



7. What is the role of base modifications in tRNA? The modifications prevent certain base pairing interactions and allow other non-traditional RNA structures to form. For example, m^1G prevents standard GC base pairs from forming.

8. How do the mRNA and the tRNAs interact with the ribosome. Which subunits make the primary contacts with each polymer? mRNA interacts at the interface between the large and small subunits – the tRNA-mRNA interaction also occurs there. mRNA makes primary contacts with the small (30S) subunit while the tRNA makes nearly all contacts with the larget subunit. The acylated 3' end of the tRNAs are presented completely in the large subunit; for this reason, the 50S subunit is considered the catalytic subunit.

9. Draw the peptidyl transfer reaction. In your sketch, label the A site and the P site.



10. Describe the initiation process in prokaryotes – include how the initiation factors participate, the role of $tRNA_f^{Met}$, and which steps are energy dependent. IF3 binds to the small subunit and prevents the two ribosomal subunits from interacting; this allows the mRNA to bind. $tRNA_f^{Met}$ is recognized by IF2. This complex then interacts with the mRNA and small subunit of the ribosome (at the correct position). IF1 is positioned on the 30S subunit in what will become the E site and IF3 is in the A site; this leaves the P-site available for the $tRNA_f^{Met}$ and mRNA to interact. The proper placement is further ensured by an interaction between the Shine-Dalgarno sequence on the mRNA base pairing with the 3' end of the small subunit.

11. Describe the elongation process prokaryotes – include how the elongation factors participate and which steps are energy dependent.

    Decoding:
    EF-Tu (GTP-bound) interacts with an incoming aminoacyl-tRNA. Hyrdolysis of GTP puts the tRNA into the A-site of the ribosome. EF-Tu ejects GDP and is stabilized by interacting with EF-Ts. This complex is broken upon addition of GTP and another aminoacyl-tRNA

    Transpeptidation:
    The N-term of the aminoacyl-tRNA in the A site attacks the carbonyl of the aminoacyl-tRNA in the P-site. This causes the entire growing polypeptide chain to be moved over to the A-site.

    Tranlocation:
    GTP hydrolysis by EF-G powers the translocation of the mRNA/tRNAs such that the tRNA with the peptide is now present in the P-site and the A-site is empty.