Problem Set 9 (**Due Nov 11th**)

1. Although the two processes are very similar on the chemical level, DNA replication is much more intricate than transcription. Why? There needs to be a lot more effort put into ensuring no errors are made in replication. Errors in the replication process result in mutations in the DNA that will be passed on to daughter cells and effect the lineage forever.

2. Name and discuss the role of all proteins that are important in DNA replication. For each of the enzymes, determine the class of reaction that they catalyze (note that you can always verify your answer by determining the EC number).

   DNA Polymerase – Pol III is the replicase. It is responsible for catalyzing the elongation of DNA on both chains at the replication fork (transferase). This enzyme also has 3'→5' exonuclease activity (hydrolase). Pol I is important for replacing the RNA primer with DNA – two activities: exonuclease (3'→5' and 5' →3' = hydrolase) and polymerase (transferase)

   Primase (DnaG) – synthesizes the RNA primer (transferase) on the lagging strand

   Helicase (DnaB) – melts apart the dsDNA at the replication fork (isomerase)

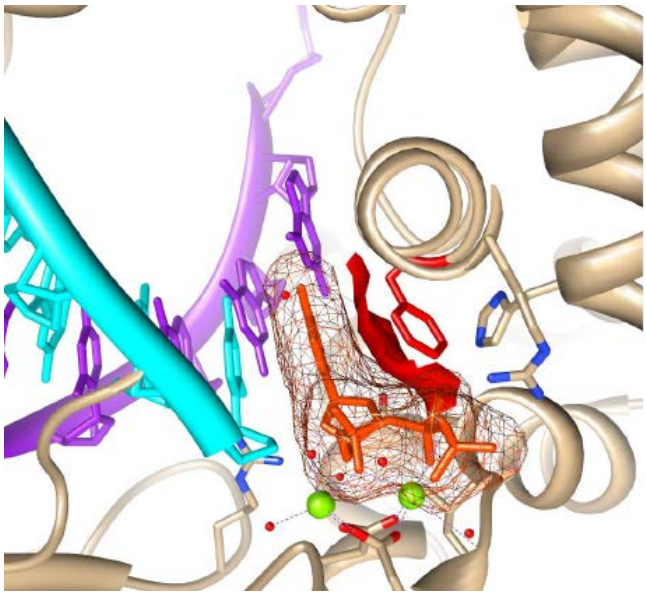
   Topoisomerase – eliminates superhelical structure upstream of the replication fork (isomerase)

   DNA Ligase – seals the breaks in the lagging strand (ligase)

   SSB – Single Stranded DNA binding Protein – prevents ssDNA from reannealing. This is not an enzyme, so no class.

3. DNA polymerase structure/function. Use *Taq* Pol I (pdb id 3KTQ) and *E. coli* Pol I (1KLN) to answer these questions.
   a. Describe how Pol I selects the correct base pair. The *Taq* structure shows the appropriate conformation of the enzyme. You don't necessarily have to show an image; just understand what is important for bases selection.

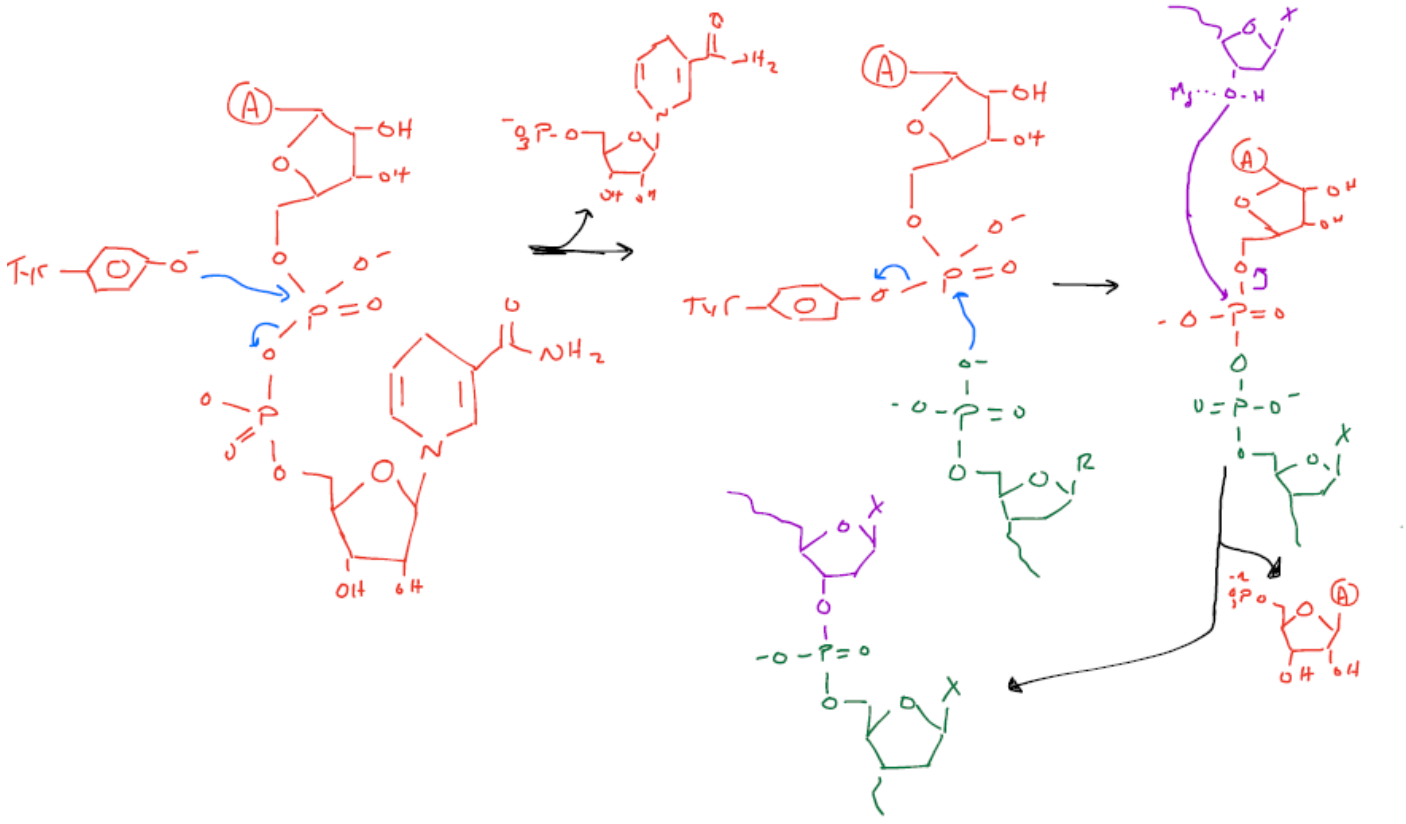
   Close Examination of 3KTQ shows that the orientation of Phe667 ensures a certain shape in the active site. This shape requirement can only be satisfied by a purine-pyrimidnie pair. In the image below, the red surface is that of Phe667 – you can see that it presents a surface that is perfectly complementary to the purine-pyrimidine pair.



b. When the wrong base pair is added to the 3' end of the growing strand, how does Pol I sense this mistake and correct it. 1KLN shows Pol I with the growing strand in the 3'→5' exonuclease active site. The 3' end of the growing DNA polymer has an affinity for both the polymerization active site as well as the exonuclease site. The most widely accepted model of proofreading is that equilibrium exists between occupancy of these two sites; any instability in the polymerization active site will trigger a shift in the equilibrium that favors the 3' end at the exonuclease site.

4. Newly synthesized dsDNA is arranged in the A-form just after it is made. Why is this important for DNA Polymerases? It is a weaker helical form. If an error is made at the 3' end, the helix is destabilized even more and the dsDNA can peel apart to get repaired at the exonuclease domain.

5. In class, we discussed how DNA Ligase can use ATP as an energy source for the ligation reaction. Please draw a mechanism showing how this enzyme could use NADH to drive the ligation reaction. Note that some NADH dependent ligases use Tyr in place of the catalytic Lys in the ATP-dependent reaction. You may show the reaction using either amino acid as the nucleophile.



6. If you wanted to incorporate ^{32}P into a DNA strand, you could use the strategy shown below.
    a. Why is Pol I a better choice than Pol III? Pol I contains a 5'→3' exonuclease domain which can remove unlabeled DNA and replace them with labelled nucleotides. Pol III does not have this domain, so you would need to start with ssDNA and have all replication machinery available.
    b. So as not to be wasteful, only one phosphorus in dNTPs needs to be isotopically enhanced. Which one and why? The α position – the other phosphates are not incorporated into the DNA backbone.