**Problem Set 5:**

**1.** Antibody diversity is partially created by the joining of random DNA segments to create the exons that code for the variable region. In this problem you will discover additional mechanisms to increase diversity.

Sequence 1:

-EWGGAAFQRTYESTTY

-V--|--J---|--- C-light

Sequence 2:

-EWGGAFQRTYESTTY

-V--|--J--|--- C-light

You are sequencing the light chain gene in B-cells (i.e. after VJ joining). These two B-cells happened to use the same V and J segments when the light chain was created. You find the following protein sequences around the J-segments in two B-cells (one letter amino acid code. The gray and blue highlighted regions indicate sequences from the V-segment and the constant light exon, respectively. Suggest what might have occurred during the joining process to generate the second sequence.

**2.** You are an oncologist and one of your patients is no longer responding to chemotherapy to treat their cancer. You determine the sequence of the genome of the tumor cells and find a mutation in several nuclear proteins (including p53) and a cell surface protein. Since it is easy to purify p53 you use the altered p53 to produce antibodies. You then use the antibodies to produce a bispecific antibody to activate T-cells. Will this approach help the patient? Why or why not?

**3.** What disease is the drug Blinatumomab (also known as MT103) used to treat? Briefly describe how it works to cure the patient (*please use the web and provide the appropriate citation*).

**4.** The Jmol page associated with this problem set shows wild-type and a mutant HIV protease in complex with a number of different HIV drugs. One of these drugs is the same as the one presented in class. This drug contains a cyclohexane ring and it binds to the wild-type enzyme with high affinity. The cyclohexane ring interacts with Val82 on the wild-type (non-mutant) enzyme.

Three different drugs, with alteration in the cyclohexane ring, have been developed for the purpose of inactivating a *mutant* HIV protease. Enzyme kinetic data for the three different inhibitors are plotted. Please answer the following questions.

i) What feature of the HIV life-cycle leads to a high level of mutations in the HIV genetic material?

ii) Which residue is altered in the mutant HIV protease? That is, what has the valine at position 82 been changed to? You will need to use the structure of the mutant to determine this.

iii) Explain, with reference to the *structure* of the enzyme-inhibitor complex for the wild-type and mutant enzymes, why the affinity to the original cyclohexane drug has been decreased by this mutation.

iv) Which of the three drugs would be the *worst* inhibitor of the mutant protease? Justify your answer with reference to the kinetic data, as well as the interaction between the drug and the mutant enzyme. A simple sketch of the interaction between the drug and the inhibitor would be useful.

v) Which of the three drugs would be the *best* inhibitor of the mutant protease? Justify your answer with reference to the kinetic data, as well as the interaction between the drug and the mutant enzyme. A simple sketch of the interaction between the drug and the enzyme would be useful.

**Structures of Drugs:**

J-mol page instructions:

* The “Wild-type+CycloHex” button will load the “wild-type” or non-mutant enzyme with the original drug bound.
* The “simple view” button will show the backbone of the protein, the sidechain of key residues, and the bound drug.
* The check boxes will add surfaces to the indicated features, to orient you with respect to the molecule.
* The “Mut+cyclohexane” button will load the mutant HIV protease and the original, non-modified drug. This drug has a cyclohexane group that contacts the enzyme and is the same as the drug from lecture.
* The buttons labeled “Drug1”, “Drug2”, etc. will load the structure of the *mutant* HIV protease with a different drug bound in the active site.

**5.** Lovastatin is an competitive inhibitor of HMG-CoA reductase, an enzyme in the synthesis pathway of cholesterol. Lovastatin is used to reduce cholesterol levels to prevent heart disease. This enzyme catalyzes the reaction shown at the top of the figure. SCoA is a large organic group that is bound to the rest of the substrate via a -S- bond.

The structure of lovastatin is shown on the lower part of the figure on the right. The regions labeled “A” and “B” represent part of the active site of HMG-CoA reductase.

i) Two regions of Lovastatin that interact with the enzyme are indicated, “A” and “B”. What type of amino acid residues would you most likely find on HMG-CoA reductase that would contact these two regions? Would they be polar or non-polar? What type of interaction between the indicated region and the enzyme would stabilize the bound drug? Justify your answer.

**6.** α-Amanitin is a natural product that is a potent inhibitor of *eukaryotic* RNA polymerase.

i) What are the consequences to the cell of inhibiting its RNA polymerase?

ii) Where is α-Amanitin produced – what organism makes it (please use the web)?

iii) Both rifampicin and α-Amanitin inhibit RNA synthesis. Rifampicin can be used to treat bacterial infections, but α-Amanitin cannot. Why?

**7.** This question will help you understand the basics of genome editing with Cas9. The partial sequence of a gene is indicated below (both strands are given). The amino acid sequence for this gene is given below the DNA sequence. The three bases to use as the PAM sequence are bold and highlighted in yellow. The start and stop codons are highlighted in green and red, respectively.

ATG...AAGCGTGGACCGTACGTCGTACAA**CTA**CGACCGCGTAATT**TGG**CGACATTT...TAACCTTTA

TAC...TTCGCACCTGGCATGCAGCATGTTGATGCTGGCGCATTAAACCGCTGTAAA...ATTGGAAAT

Met...LysArgGlyProTyrValValGln**Leu**ArgProArgAsnLeuAlaThrPhe...

i) Give the sequence of the 5’ end of the guide RNA that would target this gene. Your answer should be first 20 bases.

ii) You wish to change the Leu codon (bold, underlined) to a Glycine. Give the sequence of a template DNA that would result in this change after Cas9 caused the double stranded break. Indicate the important features of the template DNA.

iii) You also wish to inactivate the gene using Cas9. You treat your cells with Cas9 and the guide RNA you designed in part i of this question, but don’t include any template DNA. You sequence the entire genome of resultant cells and find indels at the above target gene as well as at a different gene. The sequence of the other gene (before Cas9 treatment) is:

AAGCGTGGACCGTACGTCGTACTTCTACGACCGCGTAATT**TGG**CGACATTT

Suggest a reason why the second gene became inactivated.