Chem 570 Name

**Final Exam**

**December 16, 2016**

1. Biochemical analysis of proteins
   1. Gel electrophoresis is a common technique for biochemical analysis of proteins. Describe this process in detail, including answers to the following questions: How does gel electrophoresis work? What determines the size of proteins that will migrate through a gel? What different types of gel electrophoresis are used and how do they differ? For each type, what features of the proteins determine how they are separated? (7 points)
   2. Name three stains that can be used to visualize proteins in gels. Include their relative order of sensitivity. (4 points)
   3. Some proteins can be very difficult to resolve using gel electrophoresis if they are similar in MW. Name a type of gel electrophoresis that can be useful in separating proteins of similar MW and describe how separation is achieved using this technique. (2 points)
2. What are localization signals and how can they be used to develop new tools for chemical biology? Give two examples. (3 points)
3. Name the enzyme class(es) responsible for each of the following post-translational modifications, and give one example of a specific function of each modification. (12 points)
   1. Proteolysis
   2. Acetylation/acylation
   3. Phosphorylation/dephosphorylation
   4. Lipid attachment
   5. Glycosylation
4. Recombinant Expression
   1. Provide an overview of the process of recombinant expression in E. coli. List all major steps necessary for this process. (6 points)
   2. When performing recombinant expression in a bacterial system, how can you select for bacteria that are producing your protein of interest? (2 points)
   3. Describe the process of cloning into a plasmid. Include the types of enzymes necessary for this process in your description. (3 points)
   4. How are recombinant proteins purified? Give an example of a specific method that could be used for protein purification. (2 points)
5. Describe in detail the process of Western Blotting. (4 points)
6. What is the difference between gram-negative and gram-positive bacteria? How can you determine if bacteria in a sample are gram-positive or gram-negative? (Be specific, include necessary reagent(s) and expected results of this experiment). (3 points)
7. GFP’s fluorescence comes from the autocatalytic conversion of the tripeptide Ser65-Tyr66-Gly67 to a fluorophore. Draw this tripeptide and show the mechanism of its conversion to the active fluorophore. (7 points)
8. What is FRET? At what distance between biomolecules can FRET occur? Draw a detailed labeled diagram explaining how this process work. Give an example of an application of FRET in the development of chemical biology tools. (8 points)
9. What are Lipinski’s rules? List them and describe their purpose. (5 points)
10. What is the difference between a gain of signal assay and a loss of signal assay? Which is more beneficial? (2 points)
11. When performing high-throughput screening, what are three experiments that should be performed before a “hit” compound is accepted as real? (3 points)
12. What is a major drawback of incorporating large analytical tags into activity based protein profiling probes? Draw a diagram illustrating a possible strategy that could overcome the use of a large analytical tag. (3 points)
13. Cancer-causing mutations
    1. Name and define the two major gene types whose alteration can promote tumor formation. (4 points)
    2. A common cause of mutations is UV radiation. Describe a common mechanism of UV-induced DNA damage (accounting for 80% of all UV-induced mutations). (2 points)
14. Anti-cancer agents
    1. Name the three major types of DNA damaging agents that are commonly used in cancer therapy. (3 points)
    2. Cancer treatment often involves the use of cytotoxic drugs. Define “cytotoxin,” provide a major disadvantage to using these types of drugs for cancer therapy, and provide an alternative strategy that is aimed at overcoming this disadvantage. (3 points)
15. Stem cells
    1. List two key properties of stem cells. (2 points)
    2. What are the three main types of stem cells and what cell types are they capable of differentiating into? (3 points)
16. Bidentate ligands are commonly used in designing phosphatase inhibitors. What is the role of these bidentate ligands in designing inhibitors with high specificity? (2 points)
17. Imaging probes
    1. Describe two strategies for developing imaging probes for detection of analytes in a cellular system. (4 points)
    2. List two important characteristics that need to be considered when selecting a fluorophore for use as an imaging probe. (2 points)
18. Proper folding of complex proteins can have a critical impact on important cellular functions. One way of studying protein folding is to label proteins with fluorescent dyes and visualize the FRET response between the two dyes based on their proximity to one another during the folding process. This strategy has been demonstrated on p97, an AAA+ chaperone protein involved in many critical cell functions.
    1. One method for attaching fluorescent dyes to proteins involves the covalent modification of cysteine residues with malemide-containing fluorophores, as shown below. Provide two drawbacks of functionalizing proteins using this method. (4 points)



* 1. An alternate method for attaching fluorophores to proteins is through the use of unnatural amino acid incorporation. Show the structure of a potential unnatural amino acid that could be incorporated into the protein of interest, and show the chemical reaction that would result in conjugation of the fluorophore to this unnatural amino acid (You can abbreviate the structure of the fluorophore) Include the structure that will react with the amino acid and the structure of the final product. (6 points)
  2. Describe the process of incorporating this unnatural amino acid into the protein at the desired location. (10 points)