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)

Electric current moves proteins and DNA through matrix made of polyacrylamide or agarose. (2 points) The porosity of the gel determines the protein size that will migrate through it, determined by polyacrylamide concentration (1 point). SDS-PAGE and Native gels, SDS-PAGE involves denaturing the proteins with sodium dodecyl sulfate, applies negative charge in proportion to mass (2 points). SDS-PAGE: proteins separated based on molecular weight. Native gel: separated based on charge and size/shape. (2 points)

* 1. Name three stains that can be used to visualize proteins in gels. Include their relative order of sensitivity. (4 points)

Coomassie Brilliant Blue (Least sensitive) silver stain (more sensitive) SYPRO ruby red (most sensitive) (1 point for each stain, 1 point for relative order)

* 1. 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)

Two dimensional gel (1 point) – separates by MW in one direction and isoelectric point in the other direction (1 point)

1. What is a localization signal and how can they be used to develop new tools for chemical biology? Give two examples. (3 points)

A localization signal is an amino acid sequence that tags a protein for import or delivery to a particular subcellular compartment. (1 point) Localization signals can be used in chemical biology for specific delivery of agents such as imaging probes, therapeutics, proteins, etc. (examples may vary, 2 points)

1. 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

Proteases (1 point). Can activate enzymes (1 point)

* 1. Acetylation/acylation

Acetylase, acylase (2 points). Can change protein charge, alter binding (1 point)

* 1. Phosphorylation/dephosphorylation

Kinases, phosphatases (2 points) Can change protein charge, regulate biochemical pathways, alter binding. (1 point)

* 1. Lipid attachment

Lipid transferases (1 point). Can anchor proteins to membrane, regulate biochemical pathways (1 point)

* 1. Glycosylation

Glycosyl transferases (1 point). Can influence cell signaling and recognition (1 point)

1. 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)

Isolate plasmid from bacterium (1 point)

Isolate DNA from from cell containing gene of interest (1 point)

Insert gene into plasmid (1 point)

Insert plasmid into bacterial cell (1 point)

Cell multiplies and produces copies of protein of interest (1 point)

Purify protein (1 point)

* 1. When performing recombinant expression in a bacterial system, how can you select for bacteria that are producing your protein of interest? (2 points)

Use plasmid containing gene for antibiotic resistance (1 point), plate bacteria on medium containing antibiotic, only bacteria containing the recombinant DNA will grow (1 point)

* 1. Describe the process of cloning into a plasmid. Include the types of enzymes necessary for this process in your description. (3 points)

Restriction enzymes cut the plasmid DNA into fragments, as well as the foreign DNA containing gene of interest. (1 point)

Foreign DNA fragment sticks to plasmid DNA by base pairing (sticky ends help this). (1 point)

DNA ligase “pastes” the strands (catalyzes formation of phosphodiester bond) to provide the recombinant DNA molecule. (1 point)

* 1. How are recombinant proteins purified? Give an example of a specific method that could be used for protein purification. (2 points)

Proteins are often expressed as a fusion with an affinity tag, this tag is used for purification on an affinity resin. (1 point) Example: Maltose Binding protein tag with amylose resin (1 point, several possible answers)

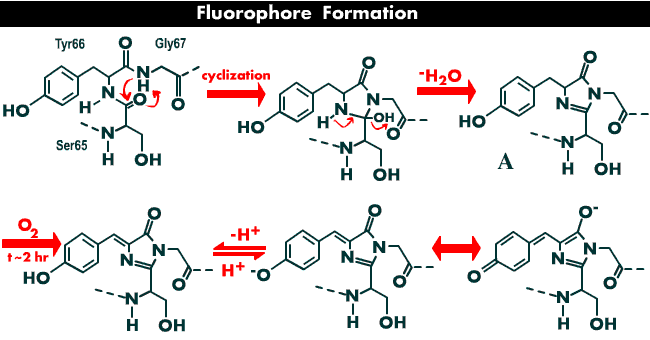
1. Describe in detail the process of Western Blotting. (4 points)

Proteins are separated using SDS PAGE. (1 point) They are then transferred to a nitrocellulose membrane throught the use of an electric current. (1 point). Proteins are labeled with a primary antibody (1 point) which is then labeled with a secondary antibody containing some type of reporter that can be used for visualization. (1 point)

1. 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)

Gram positive have a thick peptidoglycan wall and gram negative bacteria have a thin cell wall. (1 point) Gram staining – stain with crystal violet. (1 point) Gram positive bacteria will be stained dark purple. (1 point) Gram negative bacteria will be stained pink by counter stain. (1 point)

1. GFP’s fluorescence comes 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)



3 points for drawing the peptide, 1 point for each mechanistic step

1. 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)

Forster Resonance Energy Transfer (1 point)

10-100 angstroms (1 point)

Diagram – (5 points) (label donor, label acceptor, show excitation, show emission, show energy transfer)

Example of application (1 point)

1. What are Lipinski’s rules? List them and describe their purpose. (5 points)

Lipinski’s rules define characteristics of drug molecules that will have enable optimal absorption and permeability. (1 point)

Less than 5 hydrogen bond donors (1 point)

Greater than 10 hydrogen bond acceptors (1 point)

Molecular weight greater than 500 (1 point)

CLogP > 5 (1 point)

1. What is the difference between a gain of signal assay and a loss of signal assay? Which is more beneficial? (2 points)

Gain of signal: read-out involves turn on response such as fluorescence or colorimetric output. Loss of signal: read-out involves loss of an existing signal. (1 point)

Gain of signal more beneficial because there is greater signal to noise. (1 point)

1. When performing high-throughput screening, what are three experiments that should be performed before a “hit” compound is accepted as real? (3 points)

Confirm dose-dependent effect (1 point)

Test hit compound in independent assays (1 point)

Rule out non-specific inhibition (1 point)

1. 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)

Large tags may alter solubility, affect binding affinity. (1 point)

Use “label-free” method – click on analytical tag AFTER binding. (2 points for diagram)

1. Cancer-causing mutations
   1. Name and define the two major gene types whose alteration can promote tumor formation. (4 points)

Oncogenes: genes that are mutated such that protein is produced in higher quantity or is more active and can initiate tumor formation (2 points)

Tumor suppressor: genes that normally inhibit tumor formation (2 points)

* 1. 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)

UV can induce [2+2] cycloaddition of adjacent pyrimidines in DNA, forms DNA dimers

1. Anti-cancer agents
   1. Name the three major types of DNA damaging agents that are commonly used in cancer therapy. (3 points)

DNA alkylating agents, DNA intercalators, topoisomerase poisons

* 1. 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)

Cytotoxin: drug that kill rapidly dividing cells better than killing other cells. “anti-proliferative” (1 point)

Disadvantage: Dose limiting toxicity; normal cells that rapidly divide are also targeted (1 point)

Alternative: targeted therapies that hit a cancer specific target (1 point)

1. Stem cells
   1. List two key properties of stem cells. (2 points)

Ability to go through many cycles of cell division while maintaining undifferentiated state (1 point)

Capacity to differentiate into “any” mature cell type (1 point)

* 1. What are the three main types of stem cells and what cell types are they capable of differentiating into? (3 points)

Totipotent – can differentiate into any cell type (1 point)

Pluripotent – can differentiate into any of the three major tissue types (1 point)

Multipotent – can differentiate into cell within major tissue type (1 point)

1. 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)

Active sites are highly conserved making specificity for one class of phosphatase very challenging. Bidentate ligands can bind to non-conserved suppocket, allowing greater specifitiy to be achieved. (2 points)

1. Imaging probes
   1. Describe two strategies for developing imaging probes for detection of analytes in a cellular system. (4 points)

Chelation based – signal change upon chelation of metal ion (2 points)

Reaction based – probe is involved in chemical reaction of analyte (2 points)

* 1. List two important characteristics that need to be considered when selecting a fluorophore for use as an imaging probe. (2 points)

Answers may vary: optical brightness, ex and em wavelengths, stability, solubility, etc.

1. 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)



Aqueous media required, marrow window of pH and temperature, low protein substrate concentration, not site selective, could modify other regions of protein

2 points each

* 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)

Any amino acid with an alkyne, fluorescent dye with azide, or vice versa. Also could use any bioorthogonal conjugation reaction.

2 points for unnatural aa, 2 points for other reactive group, 2 points for final product

* 1. Describe the process of incorporating this unnatural amino acid into the protein at the desired location. (10 points)

Directed evolution to evolve a tRNA/synthetase pair that recognizes amber stop codon and will charge tRNA with unnatural amino acid.

Inject plasmids: 1 with tRNA gene and gene for Cmr resistance, 1 with gene for mutated aminoacyl-tRNA synthetase

Treat with chloramphenicol, positive selection

Negative selection, toxic gene product produced if natural amino acid is incorporated instead of unnatural amino acid

2 points each for each of the above points