Chem 570 Name_____

**Midterm 1 KEY**
**September 29, 2016**

1. Short Answer (20 points)

   a. What is "SDS" in SDS-PAGE and what is its function? (2 points)

      Sodium dodecyl sulfate (1 point) – denatures proteins and adds negative charges in proportion to mass (1 point)

   b. What is a telomere and how is telomerase involved in cancer? (3 points)

      Tandem repeats of short sequences of DNA, "measuring sticks" for cells to count divisions and protect against uncontrolled growth. (1 point)
      Telomerase extends telomeres, prevents senescence, can create immortal cancer cells. (2 points)

   c. What is the three letter code for the amber stop codon? (1 point)

      UAG

   d. Provide one example of a limitation of recombinant protein expression in bacteria. (2 points)

      Only 20 standard amino acids can be incorporated, post translational modifications can not be performed in bacterial host.

      2 points for one the above

   e. What technique would you use to detect proteins on a gel if you wanted to visualize all of the proteins present as opposed to just one protein of interest? (1 point)

      Stain such as coomassie blue, etc.

   f. Name two model organisms commonly used in biological experiments and give an advantage and disadvantage of each. (4 points)

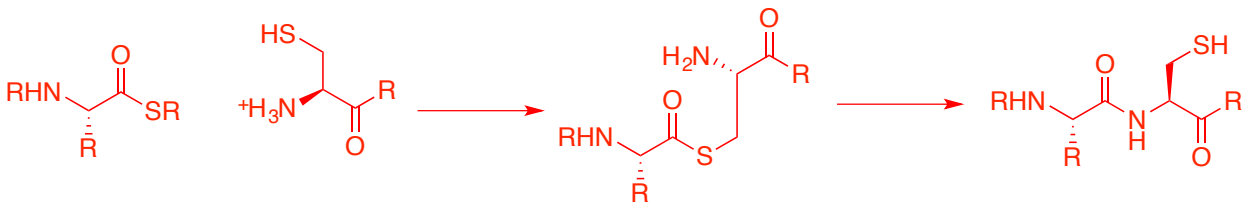
      Various answers, 1 point each for naming model organism, 1 point each for advantages and disadvantages

   g. Provide a general description of forward chemical genetics. (3 points)

1 point for phenotype to genotype
2 points for description (needed to mention small molecules because question asks about chemical genetics)

Example:
Add a variety of different compounds to plated cells, select compounds that provide desired phenotype, identify the protein target

h. Provide the starting reagents and mechanism for native chemical ligation. (4 points)




1 point each for correct starting matieral
1 point for each mechanistic step (not fully drawn here, see slides for full arrow pushing mechanism)

2. Cerulean is a CFP (cyan fluorescent protein) variant with an absorption maximum near 400 nm and an emission ranging from 450 to 550 nm. Citrine is a YFP (yellow fluorescent protein) variant with an absorption maximum near 500 nm and an emission ranging from 500 to 600 nm. Both proteins contain two surface residues (histidine and cysteine) that can coordinate to $Zn^{2+}$. (*ACS Chem. Biol.* **2016**. *In press.* DOI: 10.1021/acschembio.6b00358)

a. Describe how you could use this information to design a genetically encoded $Zn^{2+}$ sensor. What type of signal or readout would you expect from your sensor in the presence and absence of zinc? Hint: $Zn^{2+}$ is a 4-coordinate ion. (6 points)

FRET based sensor – fuse proteins together using flexible linker.
Zinc coordination will bring the two proteins together and will allow for a FRET response to occur. Excite cerulean at 400 nm. In the presence of zinc, will see emission from 500-600 (and probable some emission from 450-500). No zinc, will see emission of cerulean from 450-550.

3 points for description of design (fused proteins, zinc coordination, FRET from CFP to YFP)

3 points for expected readouts. Needed to give wavelengths of expected fluorescence in each state for full credit, partial credit was given for simply stating "FRET response" versus "no FRET response"

b. In order to properly characterize your sensor, you first need to express and purify it. In a brief paragraph, describe the general process of recombinant expression in E. coli. (6 points)

Isolate plasmid from bacterium
Insert gene into plasmid
Insert plasmid into bacterial cell
Cell multiplies and produces copies of protein of interest
Use antibiotic resistance marker and antibiotic treatment to ensure only cells that have taken up plasmid will grow
Lyse cells, purify protein

1 point for each item listed above

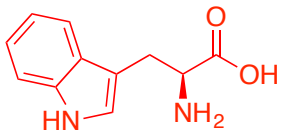
c. Give one example of a strategy you could employ to purify your sensor after recombinant expression (be specific). (4 points)

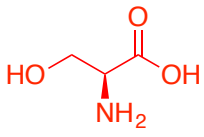
Some examples:
Maltose binding protein tag with amylose affinity resin
Glutathione S-transferase tag with Glutathione resin
Poly-His tag with Ni $2^{+}$

2 points for affinity tag, 2 points for corresponding resin

(1 point was given if affinity chromatography was mentioned but no specific examples provided)

d. Upon determining the binding affinity of $Zn^{2+}$ to your sensor, you determine that the $K_d$ is not in the desired range, and you hypothesize that this is due to a hydrogen bond interaction taking place between Trp-66 and Ser-205 that is causing a disfavorable conformation in the $Zn^{2+}$ binding domain. Draw the structures of these two amino acids and describe a possible experiment that would allow you to verify your hypothesis. (4 points)

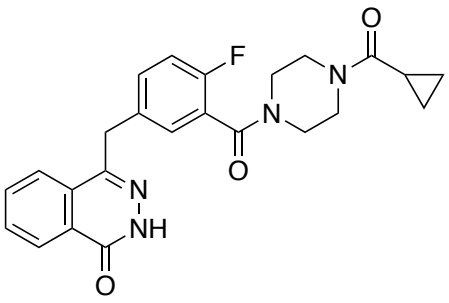


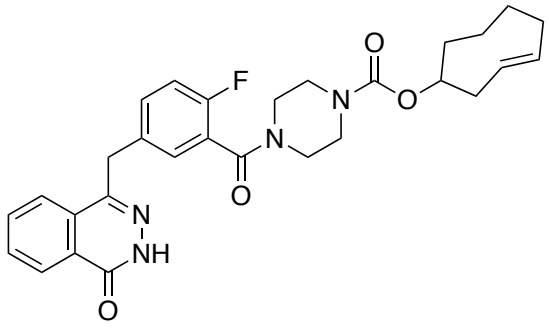
Design a mutant variant of the sensor in which one of these residues is mutated to an alternate residue that does not permit this H-bond interaction, see if the binding affinity changes. Also could repeat this experiment to mutate both residues.

1 point each for AA structures (including stereochem)
2 points for experiment proposal

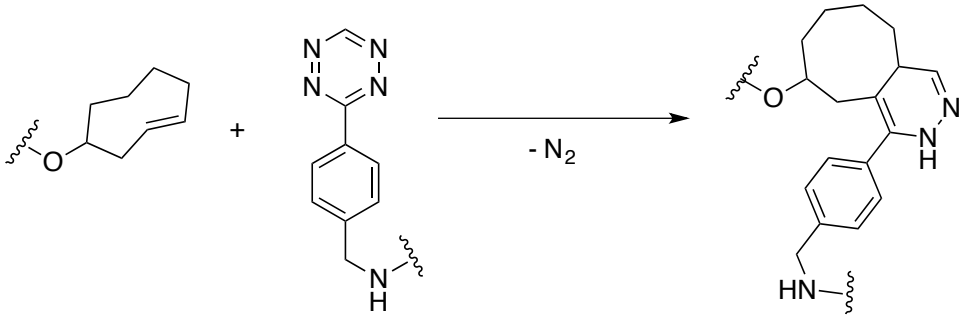
3. Olaparib is a compound that has been recently approved for the treatment of advanced ovarian cancer. It has been shown that functionalization of Olaparib with the trans-cyclooctene shown below does not greatly affect its activity, and also enables a "click" type reaction in which the cyclooctene undergoes a Diels-Alder cycloaddition with a tetrazine moiety (shown below). (*ACS Chem. Biol.* **2016**. 11, 2541-2550)



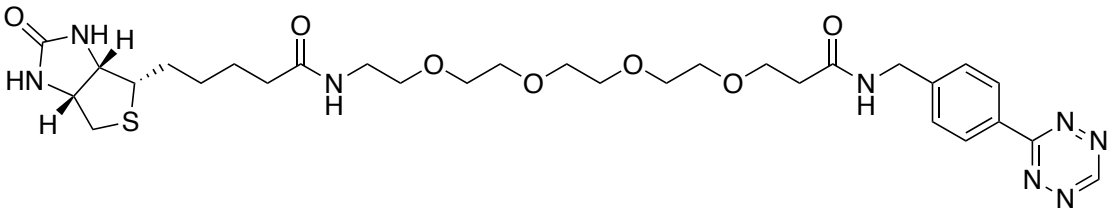

**Olaparib**




**Cyclooctene derivative**




a. Describe how you could use the cyclooctene derivative of Olaparib to isolate its biological target from cancer cells and determine its identity. Hint: the following tetrazine-biotin conjugate is commercially available. (6 points)



Incubate cell or cell extracts with cyclooctene compound.
Add tetrazine-biotin conjugate, will react to biotinylate bound ligands
Use streptavidin resin to purify protein
Use western blot, immunostaining, mass spec etc. to identify protein

3 points for using streptavidin-biotin affinity for purification
3 points for identification

b. You determined that the biological target of Olaparib is PARP-1, an enzyme that is involved in DNA damage repair and is overexpressed in a variety of different cancer types. Describe how you could use the cyclooctene derivative of Olaparib to determine the subcellular localization of PARP-1 after binding to Olaparib. (4 points)

Incubate cells with cyclooctene derivative, then incubate with fluorescent dye with appended tetrazine functionality. Important to add dye AFTER cyclooctene derivative has bound because we know that cylooctene will not affect binding. Wash out excess dye. Perform organelle specific staining with dyes such as lyso tracker, mito tracker, etc. Overlay FL signals to determine subcellular localization of compound.

2 points for FL dye with tetrazine, 2 points for experimental design

c. Give two reasons for the importance of identifying biological targets of compounds with interesting biological activity. (4 points)

Some examples:
To understand the mode of action (required for FDA approval in most cases)
To allow design of more potent variants
To learn about fundamental biology

List two of the above, 2 points each

d. In order to better understand the binding interaction between Olaparib and PARP-1 and potentially design more potent analogs, it is necessary to identify the

compound binding site. Describe two techniques that allow you to determine the protein binding site of a small molecule. (6 points)

Some examples:
X-ray crystallography
SAR by NMR
Displacement with a ligand with a known binding site
Photoaffinity labeling

Describe 2 of the above, 3 points each

4. Mcl-1 (induced myeloid leukemia cell differentiation protein) is often overexpressed in tumor cells and plays a role in increasing the apoptotic resistance of these cells. Specifically, Mcl-1 neutralizes pro-apoptotic proteins Bak and Bax to prevent the activation of apoptosis. Because of this, significant effort has been aimed at the development of Mcl-1 inhibitors as possible anti-cancer therapeutics. (*Nature Chem. Biol.* **2016**. *In press.* DOI: 10.1038/nchembio.2174)

    a. Upon developing an inhibitor of Mcl-1 and showing that this inhibitor can sufficiently increase apoptotic activity in cells, you need to show that these results are in fact dependent on on the interaction between Mcl-1 and Bak. Describe how you could use siRNA to verify this interaction, and include a brief overview of how siRNA achieves post-transcriptional gene silencing. (8 points)

    Several possible answers, best answer:
    Use siRNA to knock down gene expressing Bak, incubate with inhibitor. Should see decrease in cell death (increase in cell viability) in cells with no Bak.

    Description of how siRNA works

    dsRNA containing gene of interest is introduced
    dsRNA is cleaved by dicer into short fragments
    strands are separated, incorporated into RISC
    binding of antisense strand to complementary mRNA prevents its translation
    degraded by nuclease

    3 points for using siRNA to verify interaction
    5 points for describing of how siRNA works (1 point for each item above)

    b. What technique could you use to verify that the Mcl-1 inhibitor is not affecting the total protein levels of Mcl-1 or Bak in cells? Describe the process of how this technique works. (6 points)

Best answer:
Treat cells with increasing concentrations of inhibitor. Lyse cells, perform western blot analysis, see if protein levels are affected by increasing concentration of inhibitor.

Western blot: mixture of proteins from cell lysate is separated by gel electrophoresis (SDS-page). An electric current is applied to the gel to transfer proteins to a membrane. POI is labeled with specific antibody. Specific antibody is labeled with secondary antibody that can facilitate some sort of readout to visualize protein.

2 points for western blot, 4 points for describing how western blot works

Partial credit was given for verifying mRNA levels instead of protein levels

c. Name two techniques that you could use to determine the binding kinetics of your inhibitor with Mcl-1. Choose one of these techniques and briefly describe how it works. (6 points)

Surface plasmon resonance, Isothermal titration calorimetry, etc.

3 points for giving two techniques, 3 points for describing how one of them works, (see slides for full description on how these techniques work)

Bonus (10 points)

Purifying and quantifying mRNA levels is an important process in chemical biology. The current state of the art technology for analyzing global mRNA levels in a cell is the use of microarrays. Describe in detail how mRNA is quantified using DNA microarrays, beginning with the isolation and purification of mRNA from cells. (8 points)

mRNA is purified on a poly-T column. (1 point)
mRNA is generally poly adenylated, therefore sticks to poly-T column and can be eluted with salt buffer. (2 points)
mRNA is reverse transcribed to cDNA, labeled with a fluorophore (2 point)
Array is constructed with immobilized cDNA (full length genes or oligonucleotides) (1 point)
Apply cDNA to microarray, fluorescent response indicates presence of mRNA in cell (2 points)

Describe the technique that was used to quantify mRNA before the development of microarrays. (2 points)

Northern blot

1 point for northern blot
1 point for description of how it works, including complementary hybridization probe for detection (see slides for full description)