# C105A/CM205A Midterm Exam
Tuesday May 8, 2:00pm-3:50pm

Student ID #: ________________________________

Graduate / Undergraduate (Circle One)

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TOTAL: ________/300
1. (10 points) On the illustration representing a portion of a bacterium below, label five different parts of the bacterium.

![Illustration of a bacterium]

2. (9 points) We have looked at many methods to modify proteins. Our focus has been on the method to link the protein to a species we represent with a star. List three examples of what the star could be.

3. (8 points) What are two reasons why one would append a His₆ tag to a protein?
4. (24 points) Using the bank of reagents and resins below, fill in the steps necessary to synthesize the tetrapeptide: L-M-R-Q
You may use a reagent as many times as you need to.
Note that peptides are always written starting with the N-terminal residue.
<table>
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<th>Step 1:</th>
<th>Swell resin _____ in DMF</th>
<th>Step 10:</th>
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| Step 2: | _____                     | Step 11: | Wash  
| Step 3: | Wash                     | Step 12: | _____  
| Step 4: | _____                     | Step 13: | Wash  
| Step 5: | Wash                     | Step 14: | _____  
| Step 6: | _____                     | Step 15: | Wash  
| Step 7: | Wash                     | Step 16: | _____  
| Step 8: | _____                     | Step 17: | Wash  
| Step 9: | Wash                     | Step 18: | _____  

5. (35 points) The Green Fluorescent Protein (GFP) has revolutionized the ability to study proteins in living systems.

   a) Name one scientist who won the Nobel Prize for this work in 2008.

   b) List one advantage of fluorescent proteins

   c) List one disadvantage of fluorescent proteins

One of the 2008 Nobel Laureates won for elucidating the mechanism of chromophore formation and the development of colored variants.

The original GFP undergoes the following transformation:

   d) Clearly label the amino acids using three letter codes and map all carbon atoms of the amino acids onto the GFP chromophore above.
The blue fluorescent protein (BFP) was produced by performing site-directed mutagenesis to install a histidine.

e) Draw the resulting chromophore structure in BFP.

A chimera of BFP connected to GFP through a protease-sensitive linker was demonstrated to be a protease sensor through FRET.

Fill in the plots below indicating the absorbance and emission of the BFP and GFP. Clearly label BFP and GFP. Use a solid line to represent absorption and a dotted line to represent emission.

f)

![Abs/Em intensity vs Wavelength (nm)](image)

g) On your plot above, draw a squiggled arrow representing the species that is excited during this experiment.

h) Before the protease is added, what is the main emission observed during the experiment?
6. (35 points) In the scientist presentation about Carolyn Bertozzi, we saw how a HER2 antibody could be attached to a sialidase enzyme while both retained activity. Using any methods we have discussed, devise an experiment where an antibody can be irreversibly linked to a cysteine protease in vitro. Be sure your strategy allows both components to retain their activity when the two are attached.

a) Draw the final structure of the antibody-enzyme conjugate including the chemical structures of the key linkages. Use appropriate cartoons to depict the antibody and enzyme.

b) Using bullet points or a scheme, outline the experimental procedure that would be necessary to obtain the final antibody-enzyme conjugate you drew above.
7. (36 points) In 1994, Kent and coworkers reported the synthesis of Interleukin 8 by the following procedure:

a) Draw the mechanism for the transformation in arrow 1.

b) Describe this transformation in three words.
In arrow 2, Interleukin 8 is exposed to oxygen to result in the final folded protein.

c) On the structure below, label two distinct secondary structures found in Interleukin 8.

![Interleukin 8 structure](image)

d) Why was oxygen necessary for protein folding?

e) Give 2 reasons why Interleukin 8 was used for this study.

f) The synthesis of a protein was the key contribution of a number of scientists' seminal works that we have discussed in the course. Give another example of a scientist who focused on protein synthesis and a brief (less than 10 word) explanation of their seminal work.
8. (35 points) Ting and coworkers have developed a number of enzyme-mediated methods to label a peptide sequence appended to a protein of interest in a mammalian cell.

In their initial work, Ting and coworkers used the enzyme BirA to biotinylate a lysine residue on an acceptor peptide as shown in the figure below.

![Diagram of BirA acceptor peptide and BirA-mediated biotin attachment](image)

a) How were the labeled proteins detected in this experiment?

b) List two limitations of this method

In order to overcome the limitations listed above, the Ting group synthesized a keto-biotin, which the natural BirA enzyme accepted as shown below.

![Diagram of BirA acceptor peptide and keto-biotin attachment](image)

c) How were the labeled proteins detected in this experiment? Clearly draw the reagent and product.

d) List one limitation of this method
In order to overcome the above limitations, the Ting group looked to incorporate an azide-labeled biotin “azido-desthiobiotin” onto the tagged protein. A mutant version of BirA was developed that installed the azido-biotin onto the BirA acceptor peptide as shown below.

e) How could the labeled proteins be detected in this experiment?

f) Draw the mechanism for the reaction you answered above.

g) What is one limitation of this reaction?
9. (24 points) In the seminal report of the tetrazine ligation, a very simple protein (Trx) labeling experiment was performed with a mass spec readout.

a) Fill in the structures in the following scheme.

b) What are two advantages of the tetrazine ligation?

c) What are two disadvantages of the tetrazine ligation?
10. (34 points) We learned two different ways to incorporate unnatural amino acids into proteins in live cells (and *C. elegans*).

a) What are these two methods and who are the scientists associated with each of them?

b) The tRNA synthetase is a critical component for unnatural amino acid incorporation. Compare and contrast the tRNA synthetases in these two methods.

c) What is a hypothesis/problem we have seen addressed using the first method you listed? What analysis method was used in the experiment?

d) What is a hypothesis/problem we have seen addressed using the second method you listed? What analysis method was used in the experiment?
11. (38 points) We discussed activity-based protein profiling as it relates to proteases but it can be employed for a wide-range of enzymes, including kinases.

   a) Using abbreviated structures, draw the reaction that a kinase catalyzes. Be sure to indicate all starting materials and products.

An early target for an activity-based protein probe for kinases was the covalent inhibitor FSBA.

b) What do you need to add to FSBA to make it an activity-based protein probe?

c) Where on FSBA would you add the functionality you listed above?

You use the probe you designed above in an activity-based protein profiling experiment comparing healthy tissue to cancerous tissue and obtain the following results:
d) Based on the probe you designed in (b/c), what type of analysis is the figure above?

e) You are interested in the protein at 37 kDa in the diseased lane. Use bullet points or a scheme, to outline the steps necessary to identify this protein.

f) The experiment you describe in (e) shows that you have discovered a new kinase. Use bullet points or a scheme to outline an experiment that would allow you to identify the substrates for this kinase.
12. (12 points) The company Codexis uses a combination of rational design and directed evolution to generate enhanced enzymes.

   a) What is directed evolution?

   b) What are two distinct applications that Codexis has evolved enzymes for?

Bonus. (12 points) Name three current UCLA faculty and the chemical biologist we have discussed in class that they were trained by.