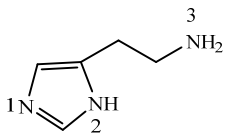
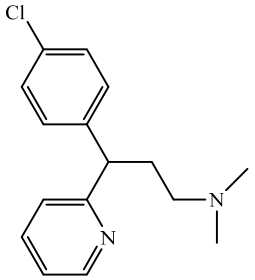
1. (15 pts) (a) Determine the protein encoded by the DNA sequence provided below. Clearly show each step of your reasoning, including transcription and translation.
(b) Cytosine can undergo deamination, in which its amino group (NH_2) is replaced by an oxygen atom to form uracil. If this mutation occurs, it may alter the corresponding mRNA sequence. Identify which cytosine in the DNA sequence, if mutated to uracil in the resulting mRNA, would have the greatest potential impact on the organism. Support your answer with a clear explanation.

G-A-A-T-G-T-C-G-C-T-A-A-T-C-T-A-G-C

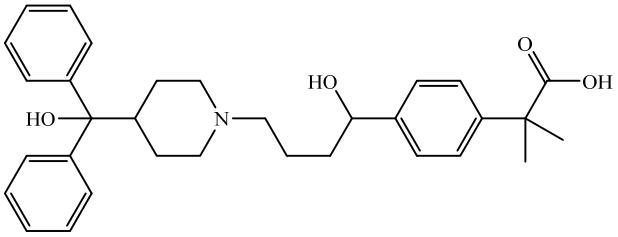
2. (20 pts) Histidine can undergo enzyme-catalyzed decarboxylation to form histamine. When histamine is produced in excess, its singly protonated (positively charged) form binds to histamine receptors, triggering the symptoms associated with the common cold and allergic reactions. Chlortrimetron is an antihistamine that binds to histamine receptors but does not activate them in the same way as histamine. However, it is able to cross the blood–brain barrier and interact with receptors in the central nervous system, leading to drowsiness—a well-known side effect of many antihistamines. To minimize this effect, nonsedating antihistamines such as Allegra (fexofenadine) have been developed.



histamine



chlorpheniramine (Chlortrimetron)

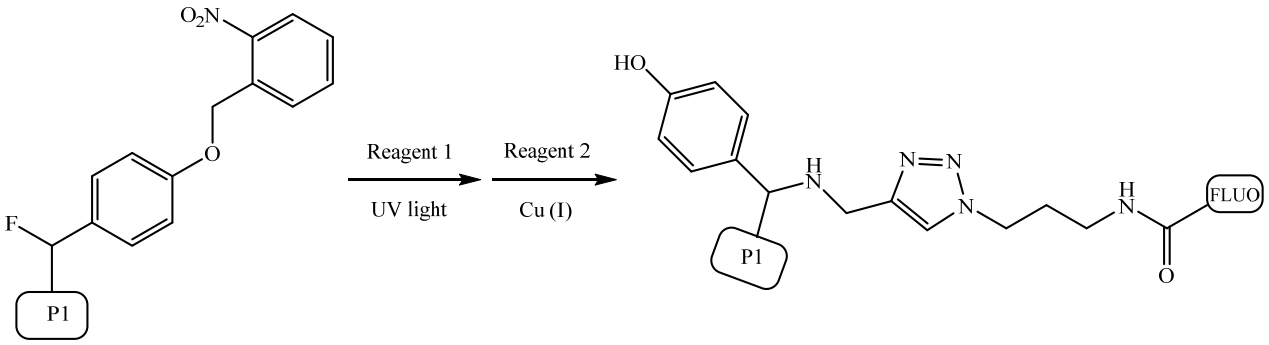


fexofenadine (Allegra)

(a) Rank the basicity of the three nitrogen atoms (N1, N2, and N3) in histamine and justify your ranking.
(b) Draw the predominant (active) form of histamine that binds to the histamine receptor.
(c) Draw the form of chlortrimetron that is most likely to bind to the histamine receptor.
(d) Propose the types of intermolecular interactions involved in ligand binding to the histamine receptor.
(e) Identify and explain the structural feature(s) that enable chlortrimetron—but not histamine—to cross the blood–brain barrier.
(f) Draw the predominant form of fexofenadine at physiological pH.
(g) Explain why Allegra (fexofenadine) functions as a nonsedating antihistamine.

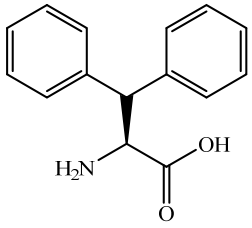
3. (16 pts) The scheme below outlines a method for site-specific labeling of protein P1 at a photocaged tyrosine analog using fluorescein (FLUO). Upon UV irradiation, P1 generates a highly reactive and short-lived intermediate from the photocaged tyrosine. This intermediate rapidly undergoes a Michael addition with reagent 1 in situ, with the reaction proceeding to completion after 10 minutes of UV exposure. In a subsequent step, the modified P1 is coupled with reagent 2 to yield the fluorescein-labeled protein.

(a) Propose the structures of reagent 1 and reagent 2.
(b) Draw the structure of the reactive intermediate that undergoes the Michael addition with reagent 1.
(c) Explain why the fluorine substituent which is not present in the reactive intermediate is essential for formation of this intermediate. Include structures if helpful.
(d) Provide a curved-arrow mechanism for the Michael addition described in part (b).

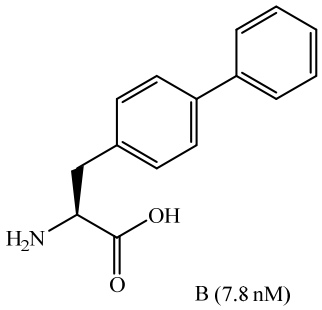


4. (12 pts) A peptide ligand exhibits strong binding affinity (5.7 nM) for its target receptor, with the C-terminal phenylalanine (Phe) playing a critical role in this interaction. To probe its importance, Phe was systematically replaced with several unnatural amino acids, generating four peptide variants. The binding affinities of these mutants are listed alongside their corresponding amino acids; lower values indicate stronger binding.

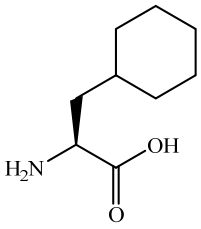
(a) Based on the binding affinity data, what can be inferred about the shape of the receptor's binding pocket?
(b) What are the dominant types of nocovalent interactions responsible for ligand binding? Support your reasoning.
(c) One mutant (containing amino acid D) shows stronger binding than the wild-type peptide. Provide a rationale for this observation.



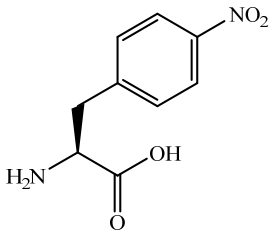
A (88 nM)



B (7.8 nM)



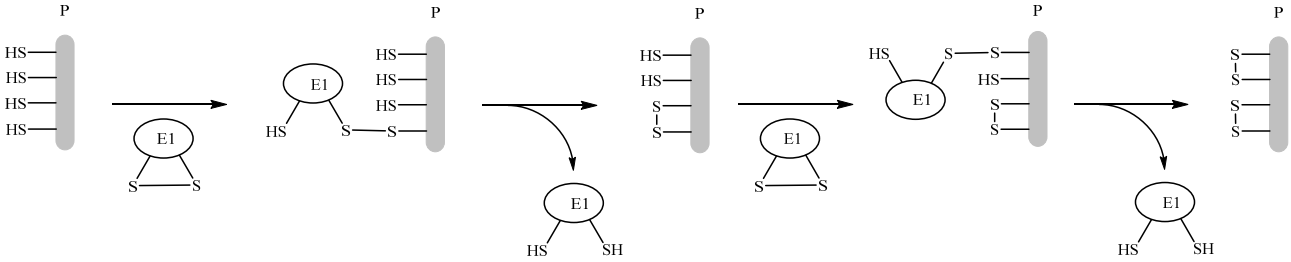
C (2.3 nM)



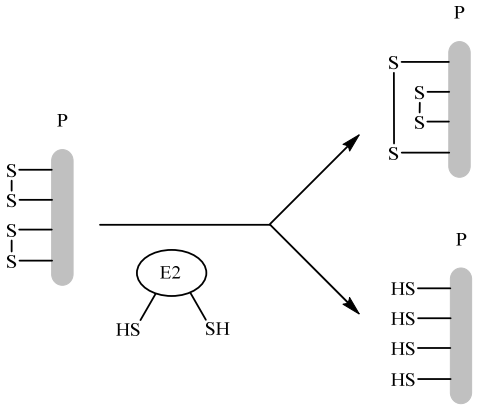
D (2.6 nM)

5. (14 pts) Scheme 1 illustrates how enzyme E1 catalyzes the formation of two intramolecular disulfide bonds in protein P via a series of thiol–disulfide exchange reactions. Enzyme E2 is proposed to operate through a similar mechanism to rearrange these disulfide bonds.
Using Scheme 1 as a guide, propose detailed stepwise reaction pathways for these rearrangement processes that account for the formation of each of the two isomeric forms of protein P shown in Scheme 2. Your answer should clearly identify all key intermediates and products at each step. Curved-arrow notation is not required.

Scheme 1



Scheme 2



6. (23 pts) The scheme below illustrates the design of a molecular beacon (MB) for target DNA detection. UV absorption spectra of DNA1 and fluorescence emission spectra (excited at 490 nm) of DNA1 and DNA5 are provided. Note that the emission spectra of DNA5 are not relevant to the questions below and may be disregarded.

DNA1 has the sequence: 3′-GTCA[TR]TTGACCGTACGTCAGTTGACTGGTCA[TO]TTGAC-5′, where TR and TO (highlighted in boxes) are fluorophores positioned within identical base environments in the stem region of the hairpin structure.

Using this information, explain the operating principle of the molecular beacon by answering the following:

(a) Assign the color codes corresponding to the UV spectra of the hairpin and duplex forms of DNA1, and justify your choices.

(b) Determine the excitation and emission maxima (λmax) for both TR and TO.

(c) Provide **spectral evidence** supporting the occurrence of Förster resonance energy transfer (FRET) in the hairpin form.

(d) Calculate the FRET energy transfer efficiency for the fluorophore pair in the hairpin. Show all work.

(e) Estimate the distances between TR and TO in both the hairpin and duplex forms. Assume the neighboring base-pair spacing of 0.34 nm.

(f) Using your result from (e), explain why FRET is minimal in the duplex form.

(g) Determine the sequence of the target DNA (5′ → 3′) shown in the scheme.

(h) Explain how binding of the target DNA induces the transition from the hairpin to the duplex form.