1) (1) If Cedric wanted to purify a native protein that was fusing to a 6xHis tag, how would he purify the protein

a) gel-filtration chromatography
b) reversed-phase HPLC
c) affinity chromatography
d) anion-exchange chromatography
e) cation-exchange chromatography

2) (1) In comparing the behavior of a 10,000 and 30,000 molecular weight protein using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and column chromatography using gel filtration, which of the following statements is most likely to be true. Assume both proteins are single polypeptide chains with no disulfide bonds. Circle the correct answer.

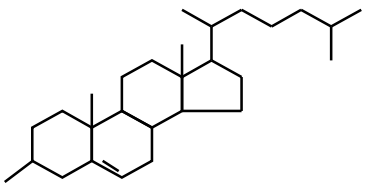
a) The smaller protein will move faster relative to the largest protein in both SDS PAGE and gel filtration.
b) The smaller protein will move slower relative to the larger protein in both SDS PAGE and gel filtration
c) The smaller protein will move faster relative to the larger protein in SDS PAGE but slower in gel filtration.
d) The smaller protein will move slower relative to the larger protein in SDS PAGE but faster in gel filtration.
e) Gel filtration operates by binding protein via electrostatic interactions so it cannot be compared with SDS PAGE.

3) (1) To obtain an accurate molecular weight of a protein we would use _mass spec technique.

4) (1) An important assumption in steady-state kinetics is

a) the concentration of the ES complex fluctuates up and down
b) the concentration of the ES complex remains constant at all substrate concentrations
c) the concentration of the free enzyme is greater than the enzyme bound in the ES complex
d) the rate of product formation is fast compared to forming the ES complex
e) the rate of formation of ES is equal to the rate of ES breakdown

5) (1) The compound shown to the right is a
A steroid
B glycerophospholipid
C phosphatidylcholine
D fatty acid
E triacylglycerol



6) The K_m for the substrate of a particular enzyme is 2.0×10^{-5} M. If the initial velocity, v_o, is 0.16 mmol/min for [S] = 0.15 M, what will be the initial velocity when [S] = 5.0×10^{-3} M.

a) (2) Work out the V_{max}.

$$v_o = \frac{V_{max}[S]}{K_m + [S]}$$

$$0.16 \text{ mmol/min} = \frac{V_{max}0.15M}{(2\times10^{-5}+0.15)M} \qquad V_{max} = 0.16 \text{ mmol/min} = 1.6 \times 10^{-4}\text{M/min}$$

b) (1) Work out the initial velocity when [S] = 5.0×10^{-3} M.

$$v_o = \frac{0.16 \text{ mmol/min}(5\times10^{-3})}{(2\times10^{-5}+5\times10^{-3})M} = 0.159 \text{ mmol/min}$$

c) (2) If the enzyme concentration is 0.1μM, what is the efficiency of the enzyme.

First, calculate k_{cat} $V_{max}=k_{cat}[E_t]$.

$k_{cat} = 160\mu\text{molmin}^{-1}/0.1\mu\text{M} = 1600 \text{ min}^{-1}$

Enzyme efficiency = k_{cat}/K_m

Enzyme efficiency = $1600/2\times10^{-5} = 8 \times 10^7$

d) (1) Comparing the V_{max}, and the initial velocity at [S] = 0.15M, at [S] = 0.15M what does indicate about the saturation of the enzyme with substrate?

The enzyme is completely (highly, almost, fully) saturated when [S] = 0.15M.

7) (4) A polypeptide chain has a _primary or AA__ sequence, α-helices and β-sheets are examples of ___secondary (structure)_ elements, folded myoglobin has a _tertiary or monomeric_ structure, whereas the subunits of hemoglobin have quaternary or tetrameric a structure.

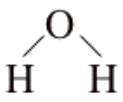
8) (3) Name three types non-covalent interactions (Any combination of any of these)

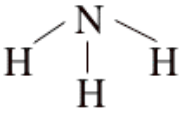
a) hydrophobic
b) H-bonding
c) Van der Waals or ionic or charge-charge or dipole-dipole or electrostatic.

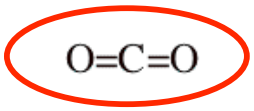
NOT disulfide bond it's covalent

9) (1) Sodium dodecyl sulfate (SDS) and fatty acids are both examples of an _amphipathic_____ molecules.

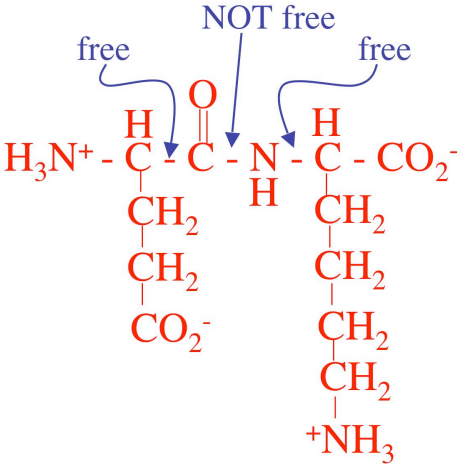
10) (1) Which of these molecules DO NOT have a fixed dipole (circle it).







11) (5) Draw the structure of the dipeptide Glu-Lys with the correct ionization at pH 7.0 Indicate which bonds along the polypeptide chain have a) freedom to rotate (arrow with FREE written next to it), and b) no freedom to rotate (arrow with No FREE written next to it, not including the N- and C- termini of the peptide). Hint: There are 2 bonds that rotate, and 1 that does not.



12) (2) If a protein has a molar extinction coefficient of 16,000 $M^{-1}cm^{-1}$ and an Abs $(OD)_{280}$ = 0.1, what is the concentration of the protein. (You must assume that the pathlength of the cell is 1cm, we went over this in lecture).

Abs = εcl
c = 0.1/16000x1 = 6.25 μM, 0.00000625M, or 6.25 x 10^{-6}M

13) (1) Which amino acid has more than one chiral center?

A) Cys
B) Gly
C) Thr
D) Glu
E) Leu

14) (3) A particular peptide has the following amino acid composition (Lys, Phe, Met, Asp, Thr). The following experimental observations have been made.

a) Trypsin yields 2 peptides with composition (Lys, Met, Asp) and Thr, Phe)
b) Cyanogen bromide cleavage gives 2 peptides with composition (Met, Asp) and Lys, Thr, Phe).
c) Chymotrypsin give 2 peptides with composition (Lys, Met, Phe, Asp) and (Thr).

What is the amino acid sequence? Use the 3-letter amino acid code. (no partial credit).

Asp-Met-Lys-Phe-Thr

15) The beta _anomer__ of D-glucopyranose is slightly more stable than the alpha __ anomer _.

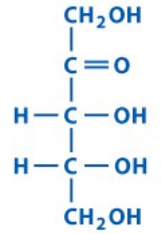
16) (5) In the first step of the chymotrypsin peptide cleavage mechanism the His57 acts as a _base__ to act upon Ser195 to generate a _nucleophile_ that attacks the carbonyl_ carbon of the peptide bond to form a covalently bound tetrahedral/acyl/unstable intermediate. What is the second nucleophile in the mechanism? _H_2O or ^-OH_

17) (2) In the allosteric regulation of hemoglobin, O_2 acts a _homotropic__ regulator and BGP acts as a _heterotropic allosteric regulator.

18) (1) How many diasteromer names does D-ribulose have? (Remember not to count D- and L-)

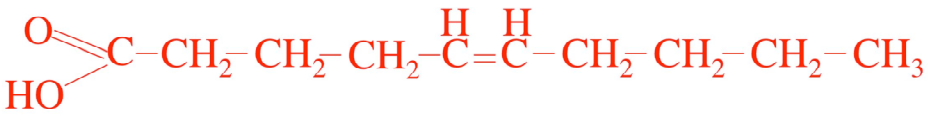
There are two chiral center.

No of diasteromer names – $2^2/2 = 2$



D-Ribulose

19) (3) Draw the structure of a 10:1cΔ5 (remember to be very clear about the stereochemistry).



20) (3) Acetic acid has a pK_a is 4.76. In an acetic acid-acetate buffer at 4.0, what is the ratio of acetate to acetic acid?

$$pH = pKa + \log \frac{[A^-]}{[HA]}$$

$$4.0 = 4.76 + \log \frac{[\text{acetate}]}{[\text{acetic acid}]}$$

$$-0.76 = \log \frac{[\text{acetate}]}{[\text{acetic acid}]}$$

$$0.174 = \frac{[\text{acetate}]}{[\text{acetic acid}]}$$

21) (3) In glycolysis, the enzyme phosphofructokinase I catalyzes the reaction

Fructose 6-phosphate + ATP ⟶ fructose 1,6-bisphosphate + ADP

Given the data below, calculate the equilibrium constant for this reaction:
$R = 8.315\ Jmol^{-1}K^{-1}$
$T = 25°C$

ATP ⟶ ADP + Pi $\Delta G^0 = -30.5\ kJmol^{-1}$

Fructose 1,6-bisphosphate ⟶ fructose 6-phosphate + Pi $\Delta G^0 = +16.0\ kJmol^{-1}$

We allowed two answers as this was confusing.

1) Overall for the reaction, $\Delta G^0 = -14.5\ kJmol^{-1}$

$Keq = e^{(-\Delta G^0/RT)}$
$Keq = e^{(14500/8.315X298)} = 348$

Or

2) Overall for the reaction, $\Delta G^0 = -46.5\ kJmol^{-1}$

$Keq = e^{(-\Delta G^0/RT)}$
$Keq = e^{(46500/8.315X298)} = 1.41 \times 10^8$

22) (4) If an enzyme is allosterically regulated in a cooperative manner, after plotting initial velocity vs [S], the curve is sigmodial or S in shape.

If there is a feedback inhibitor that binds in the regulatory site, then K_m is **increased/decreased**, as the affinity for substrate is lower or decreased. The equilibrium of the enzyme has shifted towards the T-state.

23) (1) An enzyme operates by _____

a) shifting the equilibrium of reaction from reactants toward products.
b) increasing the energy of the substrate in the enzyme-substrate complex.
c) decreasing the energy of the product in the enzyme-produced complex
d) decreasing the activation free energy.

24) (1) What is the pH of 10^{-2}M HCl is 2

25) (1) Hemoglobin has a high content of
a) α-helix
b) β-turn
c) parallel β-sheet
d) anti-parallel β-sheet