NAME: For GradeScope, please write your FIRST and LAST name in CAPITAL letters WITHIN the box:

Exam II
October 10, 2023 (Tuesday)
Biochemistry I
BI/CH 421/621

I. _____/36
II. _____/30
III. _____/24
IV. _____/10

TOTAL _____/100

I. **MULTIPLE CHOICE**. (42 points; 3 points each)
Choose the BEST answer to the question and write your answer in the box provided.

1. Which of the following is *not* considered one of the four major levels of protein structure?

   A. quaternary structure
   B. primary amino acid sequence
   C. domain structure
   D. three-dimensional conformation
   E. secondary structure

2. A D-amino acid would interrupt a β-strand made of L-amino acids. Another naturally occurring constraint on the formation of a β-strand is the presence of:

   A. a negatively charged Arg residue.
   B. a positively charged Lys residue.
   C. two Glu residues side by side.
   D. a nonpolar residue near the carboxyl terminus.
   E. a Pro residue.

3. Which statement about 3° structure determination is false?
   A. In X-ray crystallography, a structure determined at 2 Å resolution has more detail than one determined at 5 Å resolution.
   B. In NMR, COSY is able to determine bond angles.
   C. Some of the structure could be conferred by crystal packing.
   D. NMR structures are generally at higher resolution than X-ray structures, yet they reveal details of conformational flexibility.
   E. NMR can distinguish between Thr and Asp, whereas X-ray crystallography cannot.

4. A sequence of amino acids in a certain protein is found to be -Ser-Gly-Pro-Gly-Phe. The sequence is most probably part of a(n):
   A. β-turn.
   B. parallel β-sheet.
   C. α-helix.
   D. α-sheet.
   E. antiparallel β-sheet.

5. During the analysis of Amino-Acid Composition, and after heating the separated fractions in the presence of ninhydrin, the group(s) on an amino acid that react to form the final purple product is (are) the:
   A. the α-carbon and its bound hydrogen.
   B. amino group nitrogens.
   C. carboxylic acid carbon and the amino group.
   D. R group carbons.
   E. carboxylic acid oxygens.

6. Which of the following types of bonds or interactions are LEAST likely to be involved in stabilizing the tertiary structure of most proteins?

A. hydrogen bonds
B. electrostatic bonds
C. hydrophobic interactions
D. disulfide bonds
E. ester bonds

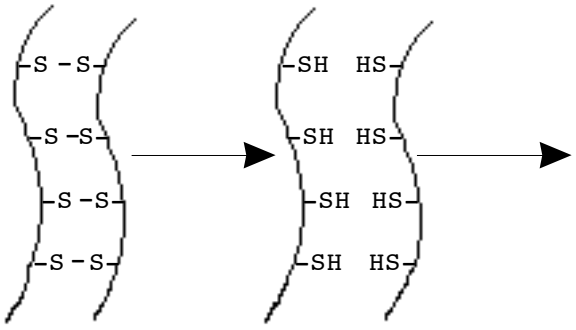
7. One method used to prevent disulfide bond interference with protein sequencing procedures is:

A. cleaving proteins with proteases that specifically recognize disulfide bonds.
B. protecting the disulfide bridge against spontaneous reduction to sulfhydryl groups.
C. removing cystines from protein sequences by proteolytic cleavage.
D. reducing disulfide bridges and preventing their re-formation by further modifying (e.g., alkylation) the -SH groups.
E. sequencing proteins that do not contain cysteinyl residues.

8. Pauling and Corey's studies of the peptide bond showed that:

A. for a protein in solution at pH 7, any one of a large number of conformations is equally probable.
B. the primary structure of all proteins is very similar, although the secondary and tertiary structure may differ greatly.
C. the peptide bonds in proteins are very unusual, bearing almost no resemblance to peptide bonds in small model compounds.
D. the peptide bond is essentially planar, with no rotation about the C-N axis of the amide bond.
E. the structure of a peptide bond is so complex that even Pauling could not understand it.

9. The □-keratin chains, indicated by the diagram below, have undergone one chemical step. To alter the shape of the □-keratin chains--as in hair waving--what subsequent steps are required?



A. chemical reduction and then chemical oxidation
B. chemical oxidation and then shape remodeling
C. shape remodeling and then chemical reduction
D. shape remodeling and then chemical oxidation
E. chemical reduction and then shape remodeling

10. You have an important tetrapeptide (MW 532) for which you only know the amino-acid composition, which is 2Ala, Lys, Trp. You don't have very much material (<10 pmol), so you perform tandem MS to determine the sequence. The molecular weights of these amino acids are 206 (W), 146 (K), and 90 (A). The MS instrument is able to capture the fragments from the C-term, the c-peptides, and the following m/z were observed: 532, 326, 236, 146. What is the sequence?

A. It cannot be determined from the data given
B. W-A-A-K
C. K-A-A-W
D. A-A-K-W
E. Because lysine has the same MW as glutamine, the sequence cannot be unambiguous.

11. You identify an important protein involved in neuronal cell communication by western blot analysis, which uses SDS-PAGE. It had a M_r of 80 kD. Yet, after you purified the protein and verified the molecular weight by analytical ultracentrifugation using sedimentation equilibrium, the data did not fit a molecule of 80 kD, and looked like it was larger. Explain.

A. sedimentation equilibrium isn't as reliable as SDS-PAGE
B. you should have performed sedimentation velocity instead
C. the protein is oligomeric
D. the protein was aggregating
E. sedimentation

12. You have just purified a protein of interest, but you don't have enough for either X-ray crystallography or NMR. How can you best determine if your protein has mostly helical or mostly beta-sheets?

A. circular dichroism spectroscopy
B. COSY spectroscopy
C. dansylation of the amino-terminus
D. UV/VIS spectroscopy
E. fluorescence spectroscopy

II. **SHORT ANSWER.** (30 points)
Give a **brief** answer or number for each problem or question below.
Write your answer in the box provided.

13. A biochemist purified a new enzyme and subjected a sample to SDS-PAGE (SDS-polyacrylamide gel electrophoresis). The results showed that there were three different proteins present with relative molecular weights of 20 kDa, 25 kDa, and 40 kDa. When sedimentation equilibrium ultracentrifugation was performed on the preparation, a molecular weight of 130 kDa was determined. What is the quaternary structure of this enzyme? Use proper nomenclature, and make the largest subunit alpha. (4 pts)

14. How does one determine the three-dimensional structure of a protein? You need only describe ONE technique and list the steps in order, for the use of that technique. Use the numbers from the list of steps given on the right. (7 pts)

Name of chosen technique

1. crystallize protein
2. purify protein
3. make assignments to protons in spectra
4. from phi and psi angles and long-distance contacts, compile a group of structures that fit the data
5. collect diffraction pattern
6. collect various 2-D spectra like COSY and NOESY
7. use Fourier transform to convert diffraction data into electron density map
8. fit a molecular model into the electron density map
9. analyze COSY to get long-distance contacts
10. analyze NOESY to get close contacts
11. concentrate protein in solution

15. Using the information below, determine the primary structure of an enkephalin isolated from brain extracts. (10 pts)

(a) Complete hydrolysis by 6 M HCl at 110 °C followed by amino acid analysis indicated the presence of G, L, F, and Y in a 2:1:1:1 ratio.
(b) Treatment of the peptide with 1-fluoro-2,4-dinitrobenzene (FDNB) followed by complete hydrolysis and amino acid analysis indicated the presence of 2,4-dinitrophenyltyrosine.
(c) Complete digestion of the peptide with chymotrypsin, followed by separation of the peptides and amino-acid analysis of each peptide yielded a tripeptide containing G and F in a 2:1 ratio, free Y, and free and L.

a. What is the sequence of the peptide?

b. How does this peptide mimic opiates such as morphine?

16. What advantages does NMR provide over X-ray crystallography in characterizing protein structure? What is the major limitation of NMR analysis for this purpose? (4 pts)

NMR advantages

NMR limitations

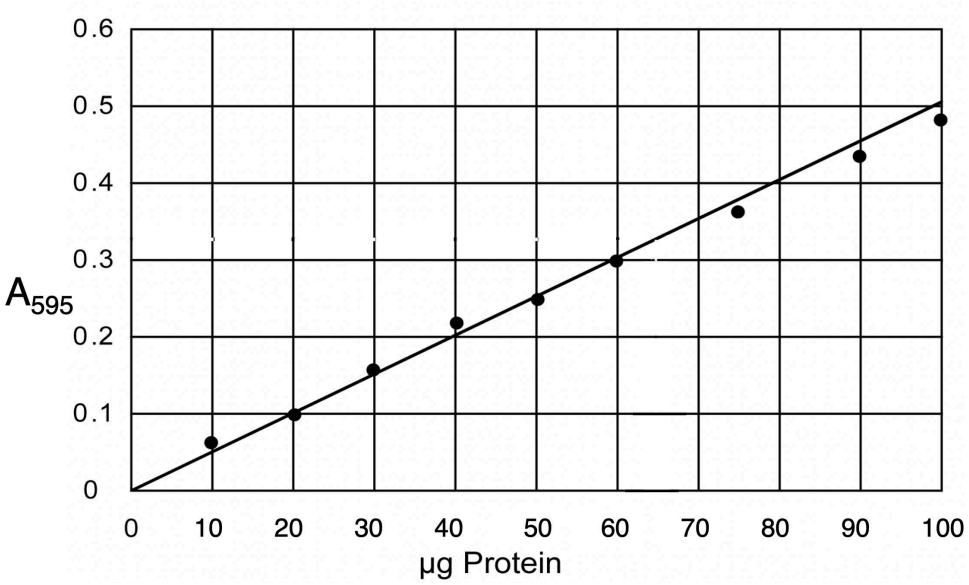
17. You are using the dye-binding assay developed by Marion Bradford. The final step in purification of your protein requires a high enough concentration for NMR spectroscopy (>1 mM). After performing this final step, you remove a 10 μL aliquot and perform three 2-fold serial dilutions. From the most diluted solution, you remove 13 μL and perform the dye-binding assay. You measure an absorbency at 595 nm of 0.325. Using the standard curve given, how much protein was in your 13 μL aliquot? Be sure to enter the units. (5 pts)

What is the concentration of protein in mg/mL?

What is the concentration of your protein in your undiluted prep?

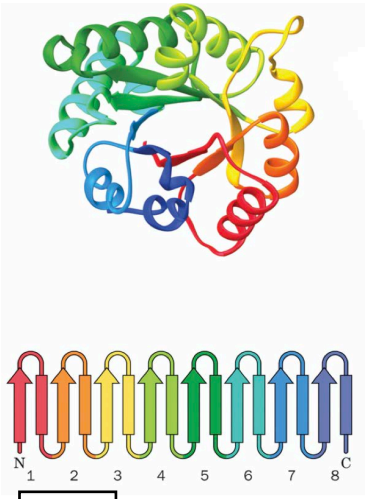
If your protein has a molecular weight of 20,000 g/mol, and NMR requires a concentration of at least 1 mM, is your protein sufficiently concentrated?

yes or no



III. MATCHING. Follow directions given for each question. (24 points)

18. Match the letters from the list below that best describes each of the pictures or descriptions of structure. Write that letter in the boxes provided. Below each box say whether the structure is a super-secondary structure (SSS), a domain (D), a tertiary structure (3°), or a quaternary structure (4°). All choices (A-I) will be used, and used only once. (18 pts)

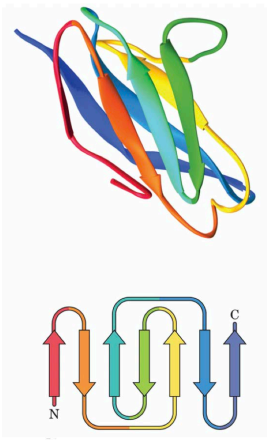


1. ☐

☐

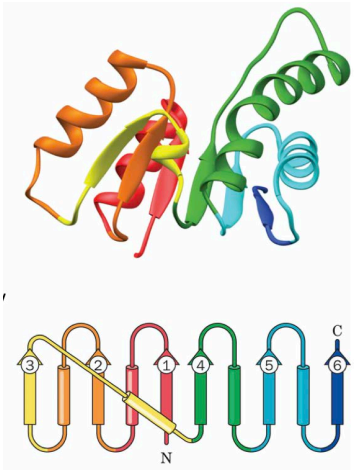
2. ☐

☐



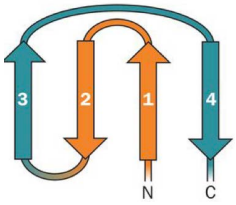
3. ☐

☐



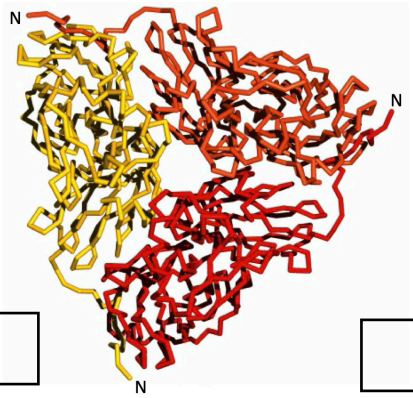
4. ☐

☐



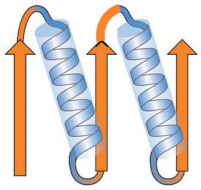
5, ☐

☐



6. ☐

☐



7. ☐

☐

($\square$)$_3$ (anti-parallel)

8. ☐

☐

(α)$_4$ (anti-parallel)

9. ☐

☐

A. Greek key

B. Rossmann fold

C. $\square$-hairpin

D. Immunoglobulin fold

E. $\square$/$\square$ barrel

F. β-barrel

G. α_3

H. α/β-saddle

I. helical bundle

19. From the types of bonds and interactions on the right, identify which is *most* responsible for the structures described on the left. (6 points)

☐	1.	a keratin coiled-coiled
☐	2.	secondary structure of proteins
☐	3.	a β-sheet
☐	4.	an α -helix
☐	5.	the compactness of the interior of myoglobin
☐	6.	the association of immunoglobulin subunits in its quaternary structure

A. ionic interactions
B. hydrophobic interactions
C. van der Waals interactions
D. covalent bonds
E. hydrogen bonds

IV. **TRUE/FALSE**. (10 points)

20. Write the appropriate letter in the box to the left of each statement.

☐ 1. In an α helix, the R groups on the amino acid residues are found on the inside of the helix spiral

☐ 2. The C_α-C and N-C_α bond-pairs within a peptide backbone show free rotation.

☐ 3. The angle of rotation around the N-C_α bond is called the φ(phi) angle.

☐ 4. The double bond character of the peptide bond results in a partial positive charge on the carbonyl group.

☐ 5. An α-helix has a φ(phi) angle of -57° and a ψ(psi) angle of -47°.

☐ 6. An α-helix is stable for poly(Glu) at high pH and for poly(Lys) at low pH.

☐ 7. A naturally occurring constraint on the formation of an α-helix is the presence of proline.

☐ 8. The angle of rotation around the C_α-C=O bond is called the ψ(psi) angle.

☐ 9. In proteins, often an Arg can be found at the N-terminal end of an α-helix

☐ 10. A β-turn is often seen connecting parallel β-strands in proteins.

Answer Key for Exam II, 10/10/2023

No. on Test	Correct Answer
1	C
2	E
3	D
4	A
5	B
6	E
7	D
8	D
9	D
10	B
11	C
12	A
13	Since there must be at least one of each subunit, the MW would be ≥ 85 kDa. Sedimentation equilibium ultracentrifugation measured the native MW of 130 kDa, therefore the only combination that gives 130 would be 45 kDa more, so there must be two each of the smaller subuinnts (20x2 + 25x2 + 40 = 130). So the quaternary structure would be $\alpha\alpha_2\beta_2$, where either the 20 or 25 kDa subunits are α or α and the 40 kDa subunit is β. Make sure each subunit is defined.

14.

X-ray crystallography:

2. The protein is purified

1. crystallized

5. collect diffraction pattern

7. use Fourier transform to convert diffraction data into electron density map

8. matching electron density with the known sequence to model

NMR:

2. The protein is purified

11. concentrate protein

3. various methods used to make assignments.

6. collect 2-D COSY and 2D-NOSEY methods are used to determine the spacial relationships.

4. from phi and psi angles and long-distance contacts, compile a group of structures that fit the data

18

No. on Test	Correct Answer

15. a. Y must be at the N-term. The tripeptide must be GGF because chymotrypsin cleaves at F and this must follow the Y because chymotrypsin does not cleave after L, which must be at the C-term.

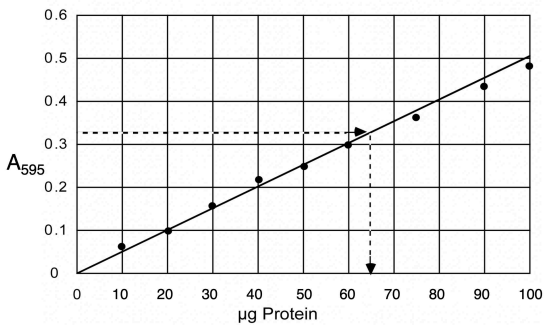
YGGFL

b. These peptides bind to specific receptors that also bind morphine and therefore must fold up into a conformation that is similar to the structure of morphine.

16. The advantages include (a) structural information from proteins that fail to crystallize and (2) information about protein folding and dynamics since protein movements can be traced over relatively long time scales.

The primary limitations of NMR is that the protein must be no larger than ~40 kDa or assignments are difficult for larger proteins.

17. From the standard curve, an absorbance of 0.325 would be about **65 μg**



65 μg from 13 μL is 65/13 = 5 μg/μL or **5 mg/mL**

You did three 2-fold dilutions, which is a dilution factor of 2x2x2 = 8. So, your stock concentration is 8 x 5 mg/mL = **40 mg/mL.**

40 mg/mL = 40 g/L ÷ 20,000 g/mol = 0.002 M or 2 mM; **Yes**

18.
1. E; tertiary or domain
2. F; tertiary or domain
3. D; tertiary or domain
4. H; tertiary or domain
5. A; super-secondary structure
6. G; quaternary structure
7. B; super-secondary structure
8. C; super-secondary structure
9. I; tertiary or domain

19.
1. B
2. E
3. E
4. E
5. B
6. D

20.
1. F
2. T
3. T
4. F
5. T
6. F
7. T
8. T
9. F
10 F