

1. (4) An E. coli cell contains many things - 70% is water plus many kinds of proteins, lipids, carbohydrates, RNA, and a one-mm-long DNA. Yet if all of these ingredients are mixed in a test tube, the result won't be living. Why not? (i.e., what is missing?)

2. (4) Give approximate numbers for the following:
 - Age of life on earth _____
 - Angstroms in one nanometer _____
 - Angle of a tetrahedral bond _____
 - Torsion angle, ω , of the trans-peptide bond _____

3. (4) Write the central dogma of information flow in molecular biology, so that you can then label: "replication", "transcription", and "translation".

4. (4) Protein X is studied in a test tube sol'n. Raising the temperature above 51°C (324 K) causes its denaturation, but X returns to its native conformation when the temperature is dropped below 51°C. Other measurements suggest that the entropy change associated with the denaturation is + 50 J/K.
 - a. Write down Gibbs expression for the free energy change ΔG as a function of ΔH and ΔS .

 - b. At $T = 37^\circ\text{C}$, is renaturation exergonic or endergonic?

 - c. When the temperature is exactly 51°C, what is the ΔG of the reaction?

 - d. Use "c" to calculate ΔH .

Multiple Choice questions: (2 pts each)

CIRCLE the letter of the one best answer.

5. An unknown polynucleotide sequence is studied by Sanger's "di-deoxy chain termination method." Why is the method called this?
 - a. because the in vitro synthesis is carried out in both the $5' \rightarrow 3'$ and $3' \rightarrow 5'$ directions
 - b. because the resulting DNA is double-stranded, from end to end
 - c. because it can sequence DNA strands that are up to 200 nucleotides long
 - d. because a small fraction of the nucleotide precursors in each tube are missing the -OH on C'-3 (as well as on C'-2) that is necessary for continued synthesis
 - e. because it can be used to sequence DNA strands but RNA strands are terminated

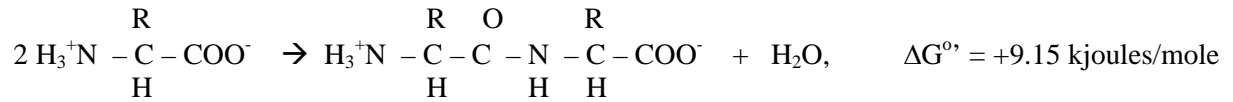
6. What is the absorbance of a 0.01 M solution of chlorophyll in a 1-cm cuvette at 410 nm? (Take the molar extinction coefficient of chlorophyll at this wavelength to be 38 L/mole-cm.)
 - a. .0038
 - b. 0.38
 - c. .026
 - d. 3800

7. Consider the following picture. This is a
- | | | |
|---|--|--|
| <p>a. nonpolar L-AA
 b. nonpolar D-AA
 c. polar uncharged L-AA
 d. polar uncharged D-AA</p> | $\begin{array}{c} \text{CH}_2\text{OH} \\ \text{CH}_2 \\ \text{NH}_3^+ \text{ === } \text{C} \text{ --- } \text{COO}^- \\ \\ \text{H} \end{array}$ | <p>(where the '='s indicate which end of the bond is closer to the viewer)</p> |
|---|--|--|
8. As we discussed in class, NH_4^+ dissociates its proton (going to NH_3) with a pK of about 9.25. Nevertheless, the pK of the amino group of alanine (which has a methyl R-group) is given as 9.87. This elevation of the pK can be explained as:
- experimental error; the history of biochemistry is rife with experimental values which have had to be adjusted now that we have recombinant DNA technologies available.
 - the “local effect” of the negatively-charged carboxyl group close-by at the other end of the alanine.
 - the tendency for a proton to migrate from the methyl group to replace the dissociating proton on the amino group.
 - The hydrophobic effect, the methyl group tends to draw extra water molecules to the vicinity of the amino acid.
9. Aspartic acid has pK's of 1.99, 3.9 and 9.9. Therefore at a pH of 3.9, its net charge would be:
- a. -1.5 b. -1.0 c. -0.5 d. 0.0 e. +0.5
10. Using the info in the previous question, the pI of Aspartic acid is:
- a. <1.99 b. 2.95 c. 5.27 d. 6.90 e. >9.9
11. “Salting out” is a protein purification process based on:
- the addition of large amounts of $(\text{NH}_4)_2\text{SO}_4$ which compete for water molecules, precipitating the protein.
 - Adding salt to a cell culture so that any salts on the outside of the cells will be transported inside.
 - Adding salt to a column chromatography apparatus, releasing bound protein.
12. In Gel Filtration (Size Exclusion) Chromatography, the first molecules to be eluted off the column are:
- the ones with the lowest pI
 - the ones with the highest pI
 - the ones with the smallest size
 - the ones with the largest size
13. During successful protein purification, the total protein tends to ___ and the specific activity tends to ___.
- increase ... increase
 - increase ... decrease
 - decrease ... increase
 - decrease ... decrease
14. An amino acid at pH 7 is called a zwitterion, because:
- both the amino and carboxyl groups are ionized
 - the amino acid is often complexed with iron at that pH
 - it belongs to the second category of amino acids
 - it flits back and forth between being protonated (positively charged) and being deprotonated (negatively charged)
15. The aromatic amino acids are important because:
- They are ionized by light of wavelength 280 nm
 - They are the amino acids most likely to be equivocal about their protonation at pH 7
 - They are the source of disulfide bonds within the exported proteins
 - They are actually imino acids that cannot rotate through the angle phi.
 - They give proteins their absorbancy at 280 nm.

Non-multiple-choice questions

16. (4) Do the 1st and 2nd laws of thermodynamics apply to Biosphere 2 (The Glass House in the Desert)? Explain.

17. (4) Amino acids will not spontaneously form a peptide bond. In fact we can quantify this condition by writing:



Calculate Keq for this reaction. (RT = 2.5 kJoules/mole)

18. (2) In the previous question draw a loop that encloses all of the atoms, but only those atoms, that lie in the peptide plane.

19. (2) Draw a picture of cysteine at pH 7 (whose R-group is $-\text{CH}_2\text{-SH}$). Then draw one water molecule hydrogen-bonding to that R group.

20. (6) A certain oligopeptide has the following Amino Acid sequence:

Ser.Gly.Cys.Lys.His.Trp Ala.Arg.Leu.Phe.Asp.Met.

a. Estimate its MW.

b. Will its pI be acidic or basic?

c. Which atomic elements will be contained in this dodecapeptide?

21. (4) Draw a titration curve of acetic acid, whose pKa is 4.76.

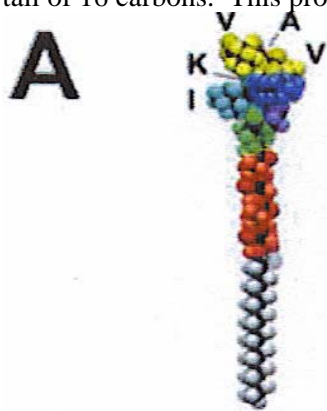
22. (4) Calculate the ratio of the concentrations of acetate and acetic acid required in a buffer system of pH 5.30.

(Background for #23 -30) In last week's **Science** magazine, Northwestern University's Samuel Stupp reported on his lab's development of nanostructures. Their nanostructure is a microscopic self-assembling device (a nanofiber) that may be a key to regeneration, a step toward finding a way to regrow nerves, cartilage, and specialized tissue. It may well be the smallest scaffolding in the world, and the easiest to set up and may help repair damaged spinal cords. Researchers have tried to build bridges across nerve gaps, but most of these are made out of a solid material such as collagen. Stupp has found a way to build a bridge out of liquid instead. When the (liquid) solution is injected into a damaged rodent spinal cord, it turns into a gel-like solid. It then serves as a scaffold for directing neuronal growth. The scaffold is designed to disintegrate after four to six weeks, hopefully leaving healthy spinal cord behind.

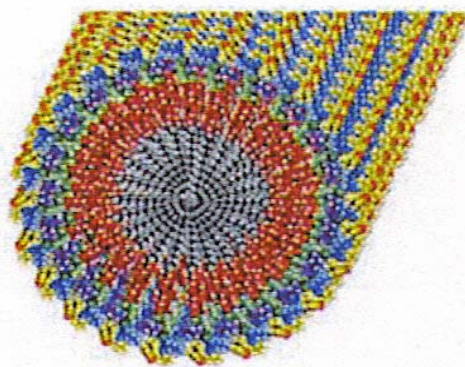
The liquid is made up of negatively charged molecules. Normally, they repel one another and keep the substance in liquid form. But when the fluid encounters positively charged molecules - such as the calcium or sodium ions found in living tissue - they clump together. "The effect happens almost instantly," says Stupp. The molecules are designed to aggregate in a particular way, forming a mass of tiny, hollow tubes. Each tube is about 5 nanometres wide - 10,000 times smaller than the width of a human hair - and several hundreds of nanometres long. The structure is porous, allowing nerve cells to grow through and around it. The team also laced each molecule in the liquid with a tiny protein fragment, an epitope, that nerve cells can recognize and latch on to. This may aid the development and growth of nerve cells.

To the naked eye, the scaffold, which consists of a combination of amino acids and fatty acids, is a blob. "It looks like a piece of transparent Jell-O from the refrigerator," Stupp said. Under a microscope, it resembles a loosely woven fabric made of cylindrical nanofibers. (See figure B.) Scientists deliver the device by injecting a pure water solution containing the disassembled scaffold molecules. (See Figure A-Left side.) Before injection, these molecules float freely. When they come into contact with fluid from a living organism, they arrange themselves into the cylindrical nanofibers that form the scaffold. The transformation is triggered by calcium and sodium ions. "Any physiological fluid of any living thing will elicit this response," said Stupp.

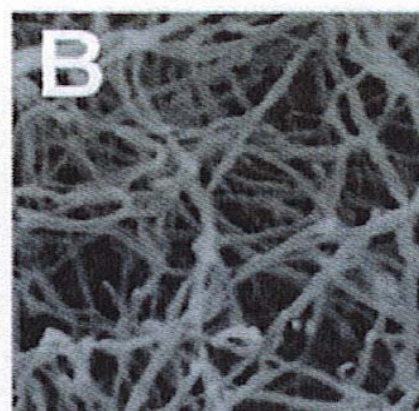
The molecular design of the scaffold incorporated the pentapeptide isoleucine-lysine-valine-alanine-valine (IKVAV) that acts as an epitope to direct neurite growth. Fig. A-Left shows the pentapeptide tip at the top of the unit. Around the tip are also some Glu residues that give the unit its negative charge at pH 7.4. When cell fluid is added, cations in the cell culture medium can screen electrostatic repulsion among them and promote self-assembly. The upper-middle of the unit contains Ala and Glycine residues and the bottom half is an alkyl tail of 16 carbons. This provides an increasingly hydrophobic sequence away from the epitope.



One peptide amphiphile molecule containing the bioactive peptide atop a stalk



one nanofiber from aggregated amphiphiles



scanning EM of a nanofiber network

23. (3) Some science reporter (see paragraph 2) has calculated that a 5nm nanofiber is 10,000 times smaller than the width of a human hair. Show that that's about the right size ratio.

24. (3) Why is a water-solution of these amphiphiles a liquid? That is, why don't the units aggregate?

25. (3) Why does it turn into a gel when mixed with cation-containing media?

26. (3) Why do Glu residues give the amphiphilic unit a negative charge at pH 7.4?

27. (3) Draw the pentapeptide Ile.Lys.Val.Ala.Val. at pH 7.4

	<u>pK_R</u>
Rile = $-\text{CH}(\text{CH}_3)-\text{CH}_2-\text{CH}_3$	-
Rval = $-\text{CH}-(\text{CH}_3)_2$	-
Rlys = $-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}_2$	10.54
Rala = $-\text{CH}_3$	-

28. (3) Estimate the pI of this pentapeptide (Assume $\text{pK}_N = 9.5$ and $\text{pK}_C = 2.5$)

29. (2) How many pentapeptides are theoretically possible, using just the standard 20 kinds of amino acids?

30. (2) If this section of peptide is encoded in DNA, how many base pairs would be needed?

.....**End of nanotubes questions**.....

31. (5) One wants to separate a solution of the following four proteins.

	<u>MW</u>	<u>pI</u>
Myoglobin	16,890	7.0
Cytochrome c	13,000	10.7
Lysozyme	13,930	11.0
Hexokinase	102,000	8.3

- a. Which protein will elute first off of a gel-filtration column? _____
- b. Which protein will elute first off of a cation-exchange column? _____
- c. Which protein might be expected to centrifuge with the largest S-value? _____
- d. Which protein(s) would migrate toward the anode during electrophoresis at pH 8.0? _____
- e. Which protein would migrate furthest during SDS-PAGE? _____

32. (4) Here four experimental techniques or terms that might be used during protein fractionation. Before each technique insert the letter of the corresponding basis of the technique or term.

- | | |
|------------------------------------|---|
| ___ SDS-PAGE | a. differential solubility in salts |
| ___ Ammonium sulfate precipitation | b. differential affinity for solid/mobile phases |
| ___ Svedberg value | c. differential response to externally applied electric field |
| ___ Anion exchange chromatography | d. differential response to centrifugal field |

33. (5) An enzyme preparation is obtained by treating a cell lysate with slow centrifugation, dialysis, isoelectric focusing, ion-exchange chromatography, and affinity column chromatography. The total amount of protein and the total activity after each step are shown in the table below.

	<u>Total Protein</u> (mg)	<u>Total Activity</u> (Enz.Units)	<u>Specific Activity</u>
Crude lysate	9400	134,000	
Isoelectric focusing	1300	111,000	
Ion-exchange chromatography	275	104,000	
Affinity column chromatography	8.3	85,200	

Calculate the specific activity after each step, and then calculate the degree of purification achieved.