



3 points

### QUESTION 5

1. Disulfide bonds in peptides and proteins are readily broken to the respective thiol (-SH) groups by reduction with

\_\_\_\_\_

- beta-mercapto ethanol
- urea
- sodium dodecylsulfate
- acrylamide

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### QUESTION 6

1. Which of the following amino acids has a negative charge at pH 7?

- aspartate
- proline
- glutamine
- lysine

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### QUESTION 7

1. Proteins that are not catalysts are often assayed using

- a. antibody binding assays such as ELISA or western blotting
- b. amino acid analysis.
- c. catalytic activity.
- d. temperature changes.

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### QUESTION 8

1. SDS polyacrylamide gel electrophoresis (SDS-PAGE) could be used to do which of the following?

- Determine the amino acid sequence of a protein.
- Determine the apparent molecular weights of subunits of an oligomeric protein.
- Determine the apparent molecular weight of an oligomeric (multisubunit) protein.
- Determine the isoelectric point of a protein (pI = the pH at which its charge is neutral).

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### QUESTION 9

1. The mobility of proteins in SDS-PAGE is inversely correlated to its \_\_\_\_\_.

- a. mass.
- b. charge
- c. mass/charge ratio
- d. shape

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### QUESTION 10

1. Match the correct answer from the list below. Not all of the answers will be used.

The first step  
in protein  
purification  
from a  
homogenate is  
usually

Answer

- A. Isoelectric focusing
- B. Affinity chromatography
- C. Size exclusion chromatography
- D. SDS-PAGE
- E. Centrifugation
- F. Ion exchange chromatography

[Read Answer Items  
for Question 10](#)

\_\_\_\_\_ can be used to purify a protein based on its attraction for a particular chemical group.

[Read Answer Items for Question 10](#)

\_\_\_\_\_ can be used to separate proteins based on their pI.

[Read Answer Items for Question 10](#)

\_\_\_\_\_ can be used to purify a protein based on its surface charge.

[Read Answer Items for Question 10](#)

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## QUESTION 11

1. The following is a figure describing the purification of recombinant human acetylcholinesterase from transgenic plants

Legend to Figure: Characterization of plant-produced acetylcholinesterase (AChE). A) Fractions from successive purification steps were subjected to SDS-PAGE and visualized by staining (lanes 1-5) as follows: Crude protein extract from wild type plant (lane 1), crude extract from transgenic plant expressing AChE (lane 2), affinity chromatography flowthrough (lane 3), affinity chromatography eluate (lane 4), final product following anion-exchange chromatography (lane 5). B) The above fractions were subjected to immunoblot analysis (lanes 1-5) with AChE-specific antibodies. Identify the recombinant protein (AChE) by its presence in lanes 2-5 (proteins from transgenic plants that express it) and absence in lane 1 (proteins from untransformed wild type plants).

What is the apparent molecular weight of recombinant human AChE?

- 67 kDa
- 55 kDa
- 25 kDa
- 2 kDa

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## QUESTION 12

1. Two-dimensional electrophoresis is a combination of what two techniques?
- a. isoelectric focusing and affinity chromatography
  - b. isoelectric focusing and SDS-PAGE
  - c. affinity chromatography and SDS-PAGE
  - d. ion-exchange chromatography and SDS-PAGE
  - e. isoelectric focusing and ion-exchange chromatography

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### QUESTION 13

1. Which of the following is NOT a step in the process of translation:
- rRNA assembly with ribosomal proteins
  - elongation
  - termination
  - initiation

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### QUESTION 14

1. Degradation of proteins in the cytosolic 26S proteasome:
- requires tagging of target proteins by ligation of multiple subunits of ubiquitin.
  - requires tagging of proteasome component proteins with ubiquitin.
  - requires tagging of signal recognition particle with ubiquitin.
  - requires the participation of a large ribonucleoprotein complex.

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### QUESTION 15

1. During translation, the site in the ribosome that holds the amino-acyl tRNA that is linked to the growing polypeptide chain is:
- the P site
  - the A site
  - the E site
  - the polypeptide channel

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### QUESTION 16

1. mRNA found in polysomes is circularized via specific binding interactions between:

- eIF4 complex and poly(A) binding protein
- mRNA 5 phosphate and 3 hydroxyl groups
- mRNA 5 cap and mRNA poly(A) tail
- initiation complex and termination complex
- small and large ribosome subunits

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#### QUESTION 17

1. Is there an advantage to having nonstandard base-pairing at the wobble position?
- Yes; it allows the incorporation of nonstandard amino acids.
  - Yes; it allows faster protein synthesis by reducing energy requirements.
  - Yes; it reduces the number of tRNAs that are required to decode all possible codons.
  - No; the wobble phenomenon is only a fluke of evolution.

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#### QUESTION 18

1. In eukaryotes, steps in the initiation of translation DO NOT include:
- peptide bond formation
  - scanning of the preinitiation complex along the mRNA to locate the initiation codon
  - eIF4 complex assembly at the 5 end of the mRNA
  - hydrolysis of ATP and GTP

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#### QUESTION 19

1. In prokaryotes, initiation of translation involves:
- positioning of the preinitiation complex near the initiation codon via base-pairing between mRNA and the 16S rRNA.
  - preinitiation complex formation at the mRNA 5 cap.
  - scanning of the preinitiation complex along the mRNA to locate the initiation codon.
  - positioning of the preinitiation complex near the initiation codon via binding of IF4 to IF1.
  - assembly of the preinitiation complex with the 50S ribosome subunit bound to mRNA, followed by binding of 30S ribosome subunit.

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#### QUESTION 20

1. Integral membrane proteins are classified as Type I or II based on:
- orientation of the N-terminus in cytosol or exoplasmic space.
  - presence or absence of a cytosolic C-terminal targeting domain.
  - alpha-helical or beta-sheet conformation of transmembrane domains.
  - the number of transmembrane alpha-helices.

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#### QUESTION 21

1. Energy for the transport of proteins into the mitochondrial matrix comes from:
- ATP hydrolysis by the cytosolic and matrix Hsc70 proteins and the proton motive force across the inner mitochondrial membrane.
  - ATP hydrolysis by SecA and the proton motive force across the outer mitochondrial membrane
  - GTP hydrolysis by the SRP.
  - the sequential degradation of the N-terminal matrix-targeting sequence.

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#### QUESTION 22

1. Importation of proteins from the cytosol to the mitochondrial matrix depends on:
- the N-terminal matrix-targeting sequence binding directly to an import receptor.
  - the N-terminal matrix-targeting sequence binding to SRP and SRP to the SRP receptor.
  - the N-terminal matrix-targeting sequence binding to SecA, which hydrolyses ATP to push the protein through the translocon.
  - the N-terminal matrix-targeting sequence being sequentially degraded to release the necessary energy for its translocation.

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#### QUESTION 23

1. Quality control of secretory glycoproteins in the endoplasmic reticulum (ER), which ensures correct folding before exit to the next endomembrane compartment:
- involves successive removal of terminal glucose residues from Asn-linked oligosaccharides.
  - is a co-translational process that requires hydrolysis of GTP.
  - requires the participation of signal recognition particle.
  - requires tagging of misfolded proteins.

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#### QUESTION 24

1. The figure below shows a hydropathy plot for a particular protein (positive values are hydrophobic; negative values are hydrophilic). Two hydrophobic domains are identified. This protein is most likely:

- an integral membrane protein
- a secreted soluble protein
- a lipid-anchored protein
- a peripheral membrane protein

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### QUESTION 25

1. Translocation of secretory proteins across the endoplasmic reticulum (ER) membrane involves all EXCEPT:

- transport of the nascent peptide through the ER membrane via the translocon channel.
- cleavage of the signal peptide from the nascent peptide by the translocon.
- binding of the amino-terminal region of the nascent peptide by SRP receptor alpha subunit.
- hydrolysis of GTP by both signal recognition particle (SRP) and the SRP receptor.

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### QUESTION 26

1. You are conducting experiments to evaluate the early steps in translocation and processing of secretory protein X. You prepare synthetic mRNA molecules of varying length that encode the N-terminal region of protein X, but contain no stop codons. These mRNAs are translated in a cell-free system using radioactive amino acids to label the nascent polypeptides. In this experiment, you perform the translation reactions in the absence (-) or presence (+) of microsomes, then electrophorese the samples on a SDS-polyacrylamide gel. The figure below shows an autoradiogram of the gel. Assuming that microsomes contain signal recognition particle (SRP) bound weakly to the membranes, translocation into the microsome lumen can occur.

In the lanes marked "130", the apparent size of the labeled polypeptide in "+ microsomes" sample is smaller than that in the "- microsomes" sample, which likely results from:

- removal of Asn-linked glycans by endoglycosidase D present in the microsomes
- premature termination of translation due to contaminants in the microsomes

- proteolytic cleavage of the polypeptide by signal peptidase present in the microsomes
- proteolytic cleavage of the polypeptide by a nonspecific protease present in the microsomes

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### QUESTION 27

1. Referring to the question above: You repeat the experiment exactly as above EXCEPT that the microsomal membranes are added AFTER translation is complete. In this case, none of the samples shows a difference in mobility in the presence or absence of microsomes. What is your interpretation of (conclusion from) these results?

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### QUESTION 28

1. Recognition of the correct target membrane by a transport vesicle occurs by:
  - binding of Rab-GTP on the surface of the vesicle membrane by a Rab effector protein in the target membrane.
  - binding of Sar1 on the surface of the vesicle membrane to COPI coat proteins.
  - binding of soluble cargo receptor to vesicle coat proteins
  - binding of clathrin coat proteins to AP adapter proteins on the vesicle membrane.

3 points

### QUESTION 29

1. Clathrin-coated vesicles are destined to be delivered to which endomembrane compartment?
  - late endosome
  - cis-Golgi
  - rough ER
  - trans-Golgi network

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### QUESTION 30

1. I-cell disease is an example of an inherited human defect in protein targeting that affects an entire class of proteins, soluble enzymes of the lysosome. What is the molecular defect in this disease?
- Cells lack the lysosomal sorting signal receptor.
  - Cells lack a functional N-acetylglucosamine (GlcNAc)phosphotransferase, an enzyme that catalyzes transfer of a phosphorylated GlcNAc molecule to a terminal mannose of an N-linked glycan chain on the lysosomal enzyme.
  - Cells overproduce a ubiquitin transferase enzyme that targets lysosomal enzymes for degradation in the 26S proteasome.
  - Cells lack functional clathrin, which mediates vesicle traffic to the late endosome, and from there to the lysosome.

3 points

### QUESTION 31

1. Referring to the question above regarding I-cell disease: What other types of defect might cause a similar phenotype in which all soluble lysosomal enzymes are misdirected?
- Cells lack a functional mannose-6-phosphate receptor.
  - Cells lack the a protein of the COPI vesicle coat protein complex.
  - Cells lack the LDL receptor.
  - Cells lack a protein of the AP1 vesicle coat protein complex.

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### QUESTION 32

1. The endocytic pathway delivers extracellular molecules to the late endosome, and involves receptor-mediated uptake of:
- digestive enzymes produced by pancreatic acinar cells
  - lysosomal enzymes that contain appropriate targeting signals
  - low-density lipoprotein particles
  - iron complexed with transferrin

3 points

### QUESTION 33

1. Resident ER soluble proteins like BiP, calnexin, and PDI may be mis-sorted into vesicles that travel to and fuse with the cis-Golgi network. They are retrieved and returned to the ER because they have a C-terminal sorting signal "KDEL" (Lys-Asp-Glu-Leu). What is the KDEL receptor and where is it found?

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### QUESTION 34

1. Glycoprotein processing can be studied by assessment of the sensitivity of the glycoprotein to specific hydrolytic enzymes such as endoglycosidase D. The figure below studies the time course of processing of a temperature-sensitive mutant VSV G protein after shifting the temperature of cells from 40° to 32°C, which permits correct folding of G protein. Why did the experiment use a a temperature-sensitive mutant VSV G protein?

- Endoglycosidase D can cleave glycans only from correctly folded proteins.
- Only correctly folded proteins can move from the ER to the Golgi.
- Only correctly folded proteins can be glycosylated.
- Only correctly folded proteins can be transported into the ER.

3 points

### QUESTION 35

1. Referring to the question and figure above, the enzyme endoglycosidase D catalyzes:
- Removal of the glycan chain from the glycoprotein.
  - Addition of mannose residues to the N-linked glycan.
  - Sequential removal of glucose residues from the N-linked glycan.
  - Synthesis of protein endoglycosides.

3 points

### QUESTION 36

1. Clathrin-coated vesicles are destined to be delivered to which endomembrane compartment?
- rough ER
  - trans-Golgi network
  - late endosome
  - cis-Golgi

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