

True / False (1/2 point each; 10 points total)

1. With respect to a pure lipid membrane,
___ water crosses more efficiently than glucose.
___ ions cross more efficiently than glucose.
2. The flow of water across a biological membrane is:
___ regulated by the opening and closing of water channels.
___ correlates with ion flow.
3. Phosphorylation enhances:
___ the polarity of the modified side chain.
___ ionic interactions with glutamate and aspartate side chains.
4. SCF-type E3 ligases:
___ bind phosphorylated substrates.
___ are covalently modified with ubiquitin.
5. Facilitated transport:
___ is typically highly specific with respect to cargo.
___ can involve the transport of molecules up their concentration gradients.
6. Porins:
___ have a larger pore size than water channels.
___ are found on the inner membrane of Gram-negative bacteria.
7. Type I bacterial secretion:
___ is mechanistically similar to ER translocation.
___ involves the action of an ABC transporter.
8. Regarding protein-protein interaction domains:
___ PH domains bind phosphoserine residues.
___ SH2 domains bind phosphotyrosine residues.
9. Regarding the use of radiolabeled azido-ATP to probe enzyme function:
___ kinase substrates can be labeled using $^{32}\text{P}(\gamma)\text{-ATP}$.
___ ATPases can be labeled using $^{32}\text{P}(\gamma)\text{-ATP}$.
10. Requirements for the application of FRET include:
___ fluorophores that emit at different wavelengths.
___ physical interaction between the fluorophores.

Multiple Choice (2 points each; 20 points total)

11. Based on the analogies presented in class, where proteins are represented by plants and lipids by water, a biological membrane is most like a:
- A) a dense forest with a few pools of water
 - B) a grassland with the occasional lake and river
 - C) a swamp with waterways, bogs and small forested islands
 - D) a sea dotted with the occasional forested island
12. All of the following are key characteristics of plasma membranes, except for:
- A) they are composed of lipids, proteins and carbohydrates
 - B) they have similar ratios of lipids, proteins and carbohydrates from organism to organism
 - C) the leaflets of the bilayer are asymmetric in composition
 - D) they are generally permeable to ions
13. Which of the following amino acids would you expect to find, more likely than the others, in a transmembrane segment?
- A) glutamate
 - B) leucine
 - C) lysine
 - D) serine
14. All of the following are hallmarks of eukaryotic secretory signal sequences, except for:
- A) they have a tripartite composition
 - B) they interact with RNA
 - C) they are proteolytically cleaved at two or more sites
 - D) they target proteins to an ABC-type transporter on the ER
15. P-type transporters are phosphorylated on which of the following residues during their catalytic cycle?
- A) aspartate
 - B) cysteine
 - C) serine
 - D) tyrosine
16. Which of the following proteolytic mechanisms does not require a water nucleophile during the hydrolytic cycle?
- A) aspartate
 - B) cysteine
 - C) metallo
 - D) serine

17. As a group, ABC transporters exhibit which one of the following characteristics:

- A) they can transport a variety of substrates
- B) they function only in the import of molecules
- C) they are smaller than the average 30 kDa protein
- D) they can be composed of four identical subunits

18. All of the following can occur during the transport of ions via channels, except for:

- A) desolvation
- B) transport of cargo down a concentration gradient
- C) weak interactions with carbonyl atoms of helices that line the channel
- D) generation of electrochemical gradients

19. The following are features of GPCR systems, except for:

- A) they are used for transducing extracellular signals to the inside of the cell
- B) they require a peptide ligand
- C) they require a receptor that has multiple membrane spans
- D) they are coupled to trimeric G-proteins

20. All of the following contain a fatty acid component, except for:

- A) sphingolipids
- B) prostoglandins
- C) GPI proteins
- D) isoprenylated proteins

Short Answer - These can be answered using one or two keywords, diagrams, and/or a short sentence (2-5 points each; 36 points total)

21. Draw the stick figure chemical structure of a 16:1(Δ^9) fatty acid. (4 points)

22. Draw the chemical structure of an activated isoprene. (3 points)

23. What radiolabeled precursor would you use to specifically radiolabel the following: (1 point each)

a) all lipids.

b) fatty acid-based lipids, to the exclusion of isoprene-based lipids

c) glycerophospholipids and triacylglycerol, to the exclusion of other lipids.

d) sphingolipids.

24. Historically, why have phosphohistidine residues been difficult to detect? (2 points)

25. If a protein has a PH domain, where is the likely subcellular localization of this protein. (2 points)

26. Fatty acids have relatively low solubility in aqueous solutions. What facilitates the circulation of fatty acids in the blood? (2 points)
27. Of the four proteolytic mechanisms, which involve the formation of a covalent intermediate between the protease and its substrate? (2 points)
28. List the representative groups of lipid molecules that can be found covalently attached to proteins? (5 points)
29. As discussed in class, list the four key events associated with signal transduction in whole cells. (4 points)
30. List the post-translational modifications associated with regulation of I κ B function and the resultant effect of these modifications. (4 points)
31. What is the purpose of a PDZ domain? (2 points)
32. What structural feature of chymotrypsin explains why it is inhibited by TPCK but not TLCK. (2 points)

Long Answer - answer the following questions using several sentences and/or diagrams. (4-8 points each; 34 points total)

33. Describe the general events associated with NF κ B activation? (6 points)

34. Outline or diagram the general steps associated with SREBP-mediated cholesterol homeostasis. (8 points)

35. You are comparing the transport rates of ions across a synthetic membrane bilayer in the presence of two ionophores. After measuring the transport rates at room temperature, you chilled the same samples and reassessed transport rates. At the lower temperature, you notice that ionophore A no longer functions in ion transport, while ionophore B remains functional, albeit at a lower rate. Provided that the temperature change does not alter the conformation of ionophore A or its ability to bind ions, what reason do you posit as a simple explanation for your result. Explain your reasoning. (6 points)

36. You have identified a periplasmic enzyme in *E. coli* that is comprised of two subunits (A and B) that are encoded on separate genes. You have recently determined that subunit A has a secretion signal sequence, but subunit B does not have a signal sequence of any sort. Provide a reasonable explanation for how the B subunit ends up in the periplasm. (4 points)

37. You have determined through genetics (*e.g.*, two-hybrid analysis) that Protein X and Protein Y are likely to interact. You are now attempting to confirm this interaction using biochemical approaches. When you epitope tag Protein A at its C-terminus, you can co-immunoprecipitate untagged Protein B. However, you are unable to co-immunoprecipitate untagged Protein A when using Protein B that has been epitope tagged at its C-terminus. Provide a reasonable explanation for this observation given your knowledge of protein-protein interaction domains. (5 points)
38. You have identified that a small peptide can restore oxidative phosphorylation, but not ATP synthesis in the presence of oligomycin. What type of compound do you likely have? Explain your reasoning. (5 points)

Bonus Questions – Answer any two of the following (6 points total; 2 points each)

1. Other than yourself, list at least two other members of your group. (2 points)

2. What is the role of biotin in fatty acid synthesis? (2 points)

3. Long before the first structure of a membrane protein was determined, it was predicted that only regular secondary structure elements (*e.g.*, α helices and β sheets) could occur within the lipid bilayer. Why? (2 points)