

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

1. Regarding bacterial protein secretion:
  - Sec-dependent secretion systems transport unfolded substrates
  - a co-factor requiring protein is likely to use the sec-dependent pathway
  - type I transport involves a periplasmic intermediate
  - type I secretion mediated by TolC is dependent on an ABC transporter
2. Regarding membranes and their components:
  - a synthetic bilayer can be readily formed using cholesterol
  - plasma membranes from different organisms have a similar lipid composition
  - membranes from different eukaryotic organelles have a similar bilayer thickness
  - unsaturated fatty acids enhance the packing density of phospholipids
3. With respect to FRET:
  - it is an abbreviation for fluorescence radiation energy transfer
  - it requires close proximity of the fluorophores
  - it can be applied for monitoring protein-lipid interactions
  - donor emission must overlap with acceptor excitation
4. With respect to integral membrane proteins:
  - they can be lipid linked
  - they must penetrate completely through the bilayer
  - they constitute 10% of the proteins in a typical genome
5. With respect to glycerophospholipids, they can be:
  - phosphorylated by kinases
  - used as a source of second messengers
  - required for sphingomyelin biosynthesis
6. A key feature used by algorithms to identify a potential transmembrane segment is:
  - an amplitude of 1.7 or greater (Kyte and Doolittle scale) for a hydrophilic peak
  - a hydrophobic stretch of amino acids in the range of 17-21 amino acids

**Multiple Choice (2 points each; 20 points total; one correct answer per question)**

7. Which of the following is NOT typically used in conjunction with FRET studies?
  - A) chemical fluorophores
  - B) GFP variants
  - C) enzymatic activities
  - D) fluorogenic antibodies
8. According to your reading of Bowie *et al*, the portion of a polypeptide exposed to the interfacial region of a membrane bilayer is best populated by:
  - A) apolar/nonpolar amino acids
  - B) moderately polar amino acids
  - C) strongly polar amino acids
  - D) cationic amino acids

9. Which of the following statements is false? “Epsin binding to membranes...”
- A) can be modulated by a lipid kinase
  - B) stimulates hydrolysis of PIP<sub>2</sub> into IP<sub>3</sub> and DAG
  - C) induces a structural rearrangement of the Epsin molecule
  - D) forces lipid head groups to separate
10. Regarding the hemi-fusion model for vesicle fusion, which of the following is false?
- A) the inner leaflets of the donor and target membrane intermix preceding cargo transfer
  - C) the function of SNAREs precedes the hemi-fusion state
  - B) the outer leaflets of the donor and target can intermix without intermixing of inner leaflet bilayers
  - D) failed progression past the hemi-fused intermediate (i.e. collapse of the intermediate) yields no lipid intermixing between donor and target membranes
11. The signal recognition particle has all of the following features EXCEPT for:
- A) the ability to directly bind the translocation channel
  - B) an RNA component that electrostatically interacts with the signal sequence
  - C) a methionine rich domain that interacts with the signal sequence
  - D) a flexible hinge region
12. Which of the following is NOT true about the SecY and ER translocation channels?
- A) they both transport unfolded substrates
  - B) their subunits have structural and sequence homology
  - C) they both require cytosolic chaperones to assist in the translocation process
  - D) they do not allow leakage of ions while actively transporting cargo
13. Fatty acid synthesis requires all of the following EXCEPT for?
- A) ATP
  - B) NADPH
  - C) a single enzyme activity
  - D) biotin as a cofactor
14. Which of the following is NOT known to be a phosphorylatable residue?
- A) aspartate
  - B) histidine
  - C) tyrosine
  - D) glutamate
15. Which of the following is NOT true about protein phosphorylation?
- A) it can enhance the membrane association of a target
  - B) it can occur at multiple sites on a target
  - C) it can be used to regulate the steady-state level of a target
  - D) it can be non-enzymatic
16. Which of the following statements NOT true about GPCR systems?
- A) the GPCR itself has a single transmembrane span
  - B) GPCR systems are used for sensing chemical compounds in an organism’s environment
  - C) the activity of the coupled trimeric G-protein (G $\alpha$ ) can be short-circuited by bacterial toxins
  - D) G $\alpha$  subunits can either activate or inactivate adenylate cyclase

**Short Answer** - These can be answered using one or two keywords, lists, diagrams, and/or a short sentence (1-4 points each; 35 points total)

17. Based on your reading of Reguenga *et al*, list two distinct methods that can be used for monitoring protein-protein interactions. (2 points)
18. Explain why the observed intensity after recovery in a FRAP experiment is typically less than expected by simple diffusion modeling. (1 point)
19. Incubation of cells with radiolabeled  $^{14}\text{C}$  radiolabeled versions of the indicated compounds would immediately yield which radiolabeled lipid(s)?
- a) serine (2 points)
  - b) phosphatidic acid (2 points)
  - c) acetate (1 point)
20. What chemical process is utilized by food chemists to create solid margarine from plant triglycerides that are normally viscous liquids at colder temperatures. (1 point)
21. Which is the only sphingolipid type that could be a target of phospholipases? (1 point)
22. Historically, why have phosphohistidine residues been difficult to detect? (2 points)

23. Using the stick figure method, draw the chemical structure of a naturally occurring 14:1( $\Delta^8$ ) fatty acid. (4 points)
24. List the representative types of lipid molecules that can be found covalently attached to proteins and whether they are found on intracellular or extracellular proteins? I am requesting a response that is more specific than the two broad categories defined in class. (4 points)
25. What chemical feature (bond) is typically incorporated into the HMG-CoA reductase inhibitors called statins in order to increase their membrane permeability? (1 point)
26. Typically, what is the attacking nucleophile in the intein splicing reaction? Do not draw the chemical reaction! (1 point)
27. Why is palmitoylation not an appropriate term for the S-acylation of proteins. (1 point)
28. What features of the aquaporin channel contribute to its dipole filter? (2 points)

29. Using your best artistic talents, sketch the calmodulin domain in its substrate-free and substrate-bound states. You can sketch each folded subdomain as a rigid body if you wish, but point out the major difference(s) between the structures. (4 points)

30. What are the steps associated with the maturation of Ras? (3 points)

31. List three characteristics that define a channel? (3 points)

**Long Answer** - answer the following questions using several sentences, descriptive diagrams, and/or descriptive lists. (4-10 points each; 35 points total). For all questions in this section, *use sufficient detail to convey your knowledge of the material (i.e. do not use one word responses or diagrams without labels)!*

32. Answer only one of the following questions on this page. Circle the question that you are answering. (8 points)

- a) In what four major ways do biological membranes NOT conform to textbook descriptions of membranes?
- b) In general terms, describe the steps associated with a FRAP experiment designed to evaluate the mobility of a protein in a particular membrane?

33. Answer only one of the following questions on this page. Circle the question that you are answering. (10 points)

- a) Describe the steps associated with the activation of the ATF6 transcription factor. Begin with a description of the unactivated state.
- b) Describe the life cycle of Notch and explain why it is considered both a receptor and a transcription factor and how one is derived from the other.

34. Answer only one of the following questions on this page. Circle the question that you are answering.  
(8 points)

- a) Explain what is meant by ion solvation and how ion transporters deal with this issue to effectively transport ions across membranes.
- b) In general terms, outline the steps associated for empirically determining whether a protein is a peripherally associated membrane protein? Begin your description with intact cells.

35. What empirical observations about oxidative phosphorylation (i.e. ATP synthesis) support the tenets of the chemiosmotic model/hypothesis? You may paraphrase but not quote the text directly! (4 points)

36. Explain how vanadate can be used to assess ATP hydrolysis by an ABC protein. A summary description of key experimental steps is required for full credit. (5 points)

**Bonus Questions – (6 points total)**

1. With respect to your group,
  - a) what is your group number? (1 point)
  - b) list three members of your group other than you; three names are required for credit. (1 point)
  
2. Describe the following features for a protein interaction domain that you identified from Dr. Pawson's website. Any domain will suffice as long as its structure was not discussed in class (i.e. not PDZ, leucine zippers, ENTH, calmodulin, and certain others).
  - a) name of the domain (1 point)
  
  - b) an example of a protein having the domain (1 point)
  
  - b) the binding mediated by the domain (1 points)
  
  - c) the general structural aspects of the domain, such as size in amino acids, unique 2° structure elements, and/or 3° structure (1 point)