Please print your name: ______________________________________

Instructions:

Please write only on these pages, in the spaces allotted and not on the back. Write your number on each page (not your name), so that we can split them up and grade them anonymously. There are a total of 6 pages including this cover page. You may not use any books or notes, and no electronic aids, including calculators.

Answer only in the space provided; short, concise answers are preferred and will be rewarded. Please be as neat as possible.
1. Of the three tight junction-associated marvel proteins (TAMPs), which family is the main determinant of the characteristics of the paracellular pathway? (1 point)

2. What is the function of the cytosolic PDZ domain of the tight junction, single span protein: Junctional Adhesion Molecule (JAM)? (1 point)

3. On a cellular level, death is characterized by the inability to replenish depleted intracellular ATP stores. Another hallmark of cell death is an increase in intracellular calcium. Please answer the following: (3 points)
   a) Why does calcium increase in cells during cell death?
   b) What is the response of gap junctions to the increased intracellular calcium?
   c) What property of gap junctions makes this response important to maintain the health of cells connected to the dead cell?

4. Please outline the experimental method used to determine that apical and basolateral directed vesicles have different protein complements. (4 points)
5. GPI anchoring of protein to lipid rafts may be an important sorting signal to send some proteins to the ________________ surface. (1 point)

6. For the following ions, please indicate whether the intracellular or extracellular concentration is typically higher in a generic mammalian cell: (3 points)
   - Na⁺
   - K⁺
   - Cl⁻

7. Glucose transporters (GLUT) are examples of proteins involved in facilitated diffusion. Briefly describe the process of facilitated diffusion. Make sure to touch on the direction of flux and binding affinity of the protein on both sides of the membrane. (2 points)

8. The Na,K-ATPase is an active transport protein that uses the energy from the hydrolysis of ATP to transport _____ (#) Na ions extracellularly and _______ (#) K ions intracellularly. (2 points)

9. The Na/Ca exchanger is a bidirectional transport protein that is expressed in cardiac myocytes (muscle cells) and functions to remove calcium from the cytoplasm by exchanging it with sodium. Cardiac myocytes also express the Na,K-ATPase. Using your knowledge of the relative concentrations of ions (intra vs. extracellularly) and the important role of the Na,K-ATPase in maintaining the electrochemical gradient, please state and provide a brief explanation of the change in the calcium ion concentration that you would expect if a Na,K-ATPase inhibitor like digoxin was used on a cardiac myocyte cell culture. (3 points)

10. True or false. If false, please correct (4 points).
A. In the direct route of apical-basolateral sorting, proteins achieve their asymmetric distribution by selective stabilization or retention at one cell surface. (1 point)

B. In addition to regulating the binding of belt desmosomes to the actin skeleton β-catenins are also transcription factors (1 point)

C. Desmoplakin is a spot desmosome plasma membrane protein that anchors microtubule filaments. (1 point)

D. Generally, the Na,K-ATPase is located exclusively on the basolateral membrane. (1 point)

**Colin Nichols Section (21 points)**

1. The plasma membrane of cells is a selectively permeable barrier. Please name one type of molecule that is freely permeable to this barrier and another type that requires facilitation by a protein carrier or channel. (2 points)

2. Using the concept of ion selectivity through size or filter, please explain why Na ions do not pass through ion channels that are selective for K ions. (2 points)

3. Please describe the Nernst potential of an ion. If the resting membrane potential is more negative than the Nernst potential for a cation, will the cation move intra or extracellularly in response to an increase in conductance for that ion? (3 points)

4. Contrast the Nernst equation from the Goldman-Hodgkin-Katz (GHK) equation. Why does the GHK equation provide a better description of membrane potential as a function of potassium concentration? (4 points)
5. Label what a, b, c, d, and e represent on the figure below: (5 points)

![Figure: Currents During an Action Potential](image)

6. Briefly explain how the K-ATP channel functions as a metabolic sensor of the cell. Be sure to mention the channels’ response to cellular substrates and what the presence of these substrates indicate in terms of cellular metabolic state. (3 points)

7. True or false. If false, please correct (2 points).

A. Biologic membranes function as capacitors with capacitance of the membrane being inversely proportional to area and directly proportional to membrane thickness (1 point)

B. The shape of an action potential is dependent on the complement of ion channels expressed by the excitable cell firing it. (1 point)
1. How are proteins separated by 2D gel electrophoresis? What is the major limitation of this technique? (3 points)

2. In mass spectrometry, tryptic peptides are broken into fragment ions by collision with an inert gas. Measuring the mass of these fragment ions gives us a final spectrum that is difficult to distinguish. This spectrum contains: (4 points)
   1) 
   2) 
   3) 
   4) 

3. Metabolic labeling and quantitative proteomics can be used to map Her2 Tyrosine kinase signaling. Describe the experimental conditions that allow you to differentiate between the target proteins containing phospho-Tyrosine residues. How do these conditions affect the final spectrum? How is the amount of protein quantified based upon the mass spectrometry results? (3 points)

4. Nuclear hormone receptors dimerize and bind to hormone response elements (HREs) to regulate gene expression. What is the general difference between HREs bound by homodimers versus those bound by heterodimers? (1 point)
5. List two ways that protein kinases can be regulated. (2 points)

6. In class, several examples of how misregulated signal transduction pathways confer human disease, were discussed, including:
   - Her2/EGFR
   - β-adrenergic receptors
   - Renin-Angiotensin system
   - EPO deficiency
Choose two of these pathways.

   Name the disease associated with misregulation of each pathway (cancer, heart disease, kidney disease, hypertension, pain/pain relief). (2 points)

   What type of pathway is implicated in each disease (cytokine receptor, cAMP/second messenger, nuclear hormone receptor, RTK, GPCR)? (2 points)

   Name a drug or treatment used to target these pathways for treatment. (2 points)

7. (True/False) The 9;22 translocation results in a chromosomal rearrangement called the Philadelphia chromosome and a fusion protein called BCR-ABL in chronic myeloid leukemia (CML). The treatment of CML was revolutionized by the drug Gleevec, a monoclonal antibody that treats the disease by recognizing and binding to the fusion protein. (1 point)

Ken Blumer Section (35 points)

1. List the four modes of cellular communication. (4 points)
2. List the three basic modes by which signals can be transmitted within a cell. (3 points)

3. This graph represents the results of a ligand binding assay in order to determine the amount of insulin-specific receptors present per cell. Label the three curves on the graph, then briefly describe the calculation or experimental conditions used to generate the curve. (6 points)
4. Scatchard plots are used in order to measure receptor abundance on a cell. Label the point on the plot that represents the total number of receptors on a cell. What does the slope of the line represent? What can you deduce from this graph if the slope of the line is steeper? (3 points)

5. Receptors are responsible for generating second messengers in order to transmit signals into and throughout the cell. Name two of these second messengers that were discussed in class and one of the downstream effectors that each of these messengers activates. (4 points)

6. Which subunit of heterotrimeric G-proteins do small G-proteins resemble? (1 point)
7. The Arg and Gln residues in the GTP-binding site are responsible for stabilizing the transition state for GTP hydrolysis to occur by the G-alpha subunit of the heterotrimeric G protein. Would a mutation in this site be considered dominant-negative or dominant-positive? Briefly explain. (2 points)

8. Small G proteins are activated by guanine nucleotide exchange factors (GEFs) that have a Dbl homology (DH) domain responsible for catalyzing the nucleotide exchange of GDP for GTP. List three ways that this GEF can be activated in order to interact with its target small G protein. (3 points)

9. What are the four steps involved in activating receptor Tyrosine kinases (RTKs) and its downstream effectors? (4 points)
10. Progesterone is responsible for inducing germinal vesicle breakdown of *Xenopus* oocytes via the MAPK kinase cascade. If we add a concentration of progesterone that half-maximally activates MAPK, do we see a graded or a switch-like response of the MAPK pathway? Describe the results from this experiment that would allow you to make this conclusion, including what you would measure in order to evaluate activation of the pathway. (3 points)

11. Phosphoinositide 4-phosphate (PIP2) is phosphorylated by PI3K, resulting in Phosphoinositide 4,5-bisphosphate (PIP3). What protein can remove this phosphate group in order to reduce PIP3 signaling? (1 point)

12. (True or False) G protein coupled receptors (GPCRs) have a conserved 5 transmembrane domain architecture and are robust switches with multiple effectors. (1 point)