Biology 5357

Chemistry & Physics of Biomolecules

Examination #3

Membranes, Membrane Proteins
& Glycobiology Module

December 7, 2018

Answer Key

Question 1. (8 points) Name the eight major types of lipid structures in biological membranes. Give an example of each.

(1) Fatty acyls – eicosanoides derived from arachidonic acid, prostaglandins, leukotrienes
(2) Glycerolipids – triglycerides
(3) Glycerophospholipids – phospholipids
(4) Sphingolipids – sphingomyelins, gangliosides
(5) Sterol lipids – cholesterol, steroids
(6) Prenol lipids – carotenoids, retinol
(7) Saccharolipids – lipid A
(8) Polyketides – secondary metabolites and antibiotics

1/2 point for name of each lipid class, 1/2 point for each example, round up the total for the question. For other examples not given here, look it up to confirm if correct.

Question 2. (3 points) Describe three ways that archaeal lipids can be chemically different from lipids in eubacterial membranes.

(1) Ether linkages ***(1 point)***
(2) Isoprenoid chains ***(1 point)***
(3) G1P vs. G3P glycerol backbone (also chirality) ***(1 point)***

Question 3. (10 points; A-E, 2 points each) Consider the CMC for detergent amphiphiles:

(A) What is the major driving force for micelle formation?

Hydrophobic effect

(B) What values of the critical packing parameter lead to the formation of spherical micelles?

Cpp < 1/3, i.e., the detergent monomer is cone shaped

(C) What happens to the CMC when the hydrophobic chain length of the amphiphile is increased?

CMC decreases (micelle formation free energy more favorable)

(D) What happens to CMC of an anionic detergent when the salt concentration is increased?

CMC decreases due to reduction in head group repulsion

(E) Consider the detergent CHAPS with a CMC of 10 mM. At 35 mM, what is the concentration of CHAPS monomers in solution?

Above the CMC, the concentration of free detergent is equal to CMC, therefore the concentration is 10 mM

Question 4. (8 points; A-D, 2 points each) Consider the main bilayer phase transition:

(A) What are the molecular changes that occur during the main bilayer phase transition?

(1) Cooperative rotameric disordering of hydrocarbon chains, and
(2) Gel (L_β) to liquid crystalline phase (L_α), may be via a rippled bilayer phase (P_β)

(B) Draw a graph that describes the trend of T_m as a function of acyl chain length.

The graph should show a smooth increase in T_m as the chain length increases.

(C) Draw a graph that describes the trend of T_m as a function of number of double bonds in the acyl chains.

The graph should show a smooth decrease in T_m with increasing number of double bonds. A plateau may be shown for 2-3 double bonds.

(D) Which T_m is higher? PC with 16:0, 18:1 cis-9 chains or PC with 16:0, 18:1 trans-9 chains?

T_m trans > T_m cis (trans lipids are more ordered)

Question 5. (4 points) List four different types of solvation methods (*i.e.*, structures) used for the biochemical study of purified membrane proteins.

(1) Detergent micelles
(2) Bicelles

(3) Nanodiscs or scaffolded membranes
(4) Bilayers – vesicles, liposomes
(5) Supported bilayers
(6) Black lipid membranes – suspended bilayers

Any four from the above list, or any other reasonable answer is acceptable.

Question 6. (6 points) Describe the three different types of passive transport across biological membranes. Give a specific example for each.

(1) Diffusion – e.g. non-polar substances, general anesthetics
(2) Ion channels – e.g. K+ channels, porins, CLC channels
(3) Passive transporters – e.g. uniporters like the sugar transporters

One point for each type of transport, and one point for each example.

Question 7. (4 points) Describe the two different types of active transport that are used in biological membranes. Give a specific example for each.

(1) Facilitated transport – e.g. GltpH, CLC Cl-/H+ transporter
(2) ATP driven pumps – Na+/K+ ATPase

One point for each type of transport, and one point for each example.

Question 8. (4 points; A & B, 2 points each) Consider a cell that has the following intracellular and extracellular ion concentrations. Note, there are other ions in the system that are not included here and can be ignored:

$[Na^+]_i = 20$ mM	$[Na^+]_o = 150$ mM
$[K^+]_i = 140$ mM	$[K^+]_o = 10$ mM
$[Cl^-]_i = 40$ mM	$[Cl^-]_o = 40$ mM

(A) Write down the Nernst equation for calculating the equilibrium potential across the membrane.

$$E_{rev} = -(RT/zF)\ ln([X^z]_i/[[X^z]_o) = (RT/zF)\ ln([X^z]_o/[[X^z]_i)$$

(B) In this cell, there is a single type of ion channel that is open at rest and it is selectively permeable for chloride. What is the resting membrane potential?

0 mV

Question 9. (6 points) The equation for the whole-cell current through a single type of ion channel is:

$$I = N \cdot P_O \cdot \gamma \cdot (V_m - E_{rev})$$

Describe the meaning of the following terms: N, P_O, γ, V_m, E_{rev} and $(V_m - E_{rev})$.

N – number of channels in the membrane
P_O – probability of opening
γ – single channel conductance
V_m – membrane potential
E_{rev} – equilibrium potential for permeating ion
$(V_m - E_{rev})$ – driving force for current

One point for each definition.

Question 10. (3 points; A-C, 1 pt each) In the "Cl^- dump" assay, proteoliposomes containing the functional CLC Cl^-/H^+ antiporter are prepared with 300 mM Cl^- on the inside, and the outside solution is exchanged into 1 mM Cl^-.

(A) Upon exchange into the low chloride solution there is a large concentration gradient, but no net movement of chloride out of the vesicles. Why?

Since the Cl^-/H^+ antiporter is electrogenic (two Cl^- move in the opposite direction of one H^+), a single turnover cycle produces a charge separation leading to a membrane potential that opposes further downhill chloride movement.

(B) Valinomycin is often used to initiate transport. What does valinomycin do?

Valinomycin is a K^+ specific ionophore, binds K^+ in solution and transports K^+ across the membrane.

(C) What should the counter cation be in order to observe transport with valinomycin? What should the intra- and extra-liposomal counter cation concentrations be set to?

The counter cation in the Cl^- dump assay should be K^+.

The intra- and extra-liposomal concentrations should be set to 300 mM so that the membrane potential goes to 0 mV upon addition of valinomycin.

Question 11. (4 points) Compare and contrast the two different types of membrane proteins synthesis/folding.

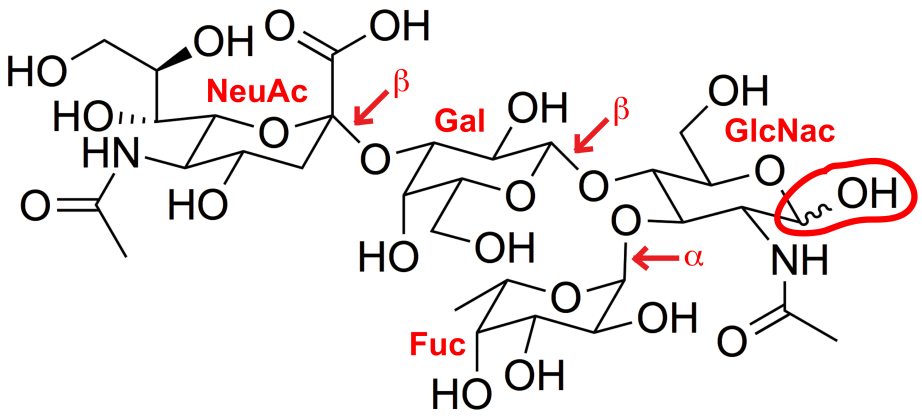
(1) Beta barrel membrane proteins (1 point each for any two of the following)

1. *are only present in bacteria and archaea*
2. *are synthesized in the cytosol/inner membrane by the ribosome coupled to the membrane translocon channel, which provides a passage for the unfolded protein to enter the periplasmic space*
3. *it is possible for beta barrel proteins to fold spontaneously into membranes in vitro from a water solvated unfolded state*
4. *in vivo, beta barrel insertion and folding into the outer membrane is assisted by chaperones such as the BAM complex*

(2) Alpha-helical membrane proteins (1 point each for any two of the following)

1. *are found in archaea, bacteria and eukaryotic cells*
2. *synthesized in the cytosol/ER membrane in eukaryotes, or cytosol/inner membrane in bacteria/archaea via the membrane embedded translocon channel*
3. *can fold spontaneously into membranes from detergent solubilized state*
4. *in vivo, alpha helical proteins follow a two-stage model for assembly – insertion of alpha helices, then equilibrium association of the helices within the membrane*

Question 12. (20 points; A-E, 4 points each) Shown below is the "sialyl Lewis x" tetrasaccharide, which is often at the terminus of *O*-linked glycans at the cell surface.



(A) What monosaccharides are present in sialyl Lewis x? Label them on the figure.

The four residues are N-Acetylglucosamine (GlcNac), Fucose (Fuc), Galatose (Gal) and Neuraminic Acid (NeuAc) as labelled on the structure above. One point for each name, either the full name or the abbreviation.

(B) Indicate on the structure the location of the reducing end of sialyl Lewis x.

The free anomeric carbon and hydroxyl group circled in red are at the Reducing end of the structure. (Note this is the only anomeric carbon not involved in a glycosidic linkage.)

(C) Mark the glycosidic linkages on the structure, and label each linkage as α or β.

The three glycosidic linkages are indicated by red arrows, and labelled as either α or β. One point for marking all three linkages and one point each for correct α/β labels.

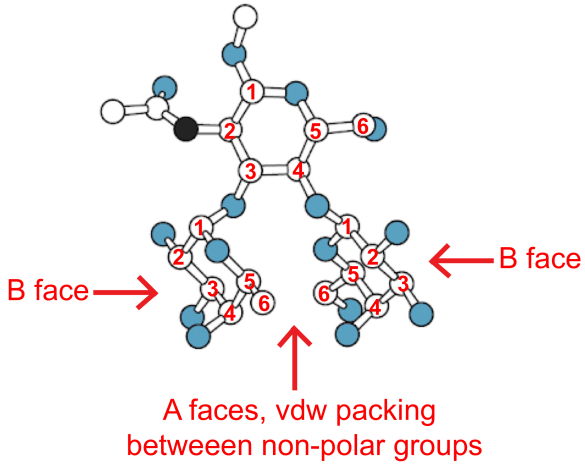
(D) Write the full name of this tetrasaccharide. Use standard abbreviations for the monosaccharides such as Gal for galactose, *etc.*, and numbers, α/β and bonds to indicate linkages and connect the structure. (Example: lactose is Galβ1-4Glc)

The full name is: Neu5Acα2-3Galβ1-4[Fucα1-3]GlcNAc. *(Partial credit for parts of the name that are correct.)*

(E) Sialyl Lewis x is frequently overexpressed on both *N*- and *O*-linked glycans of cancer cells. What effect might this have on the disease process?

High levels of expression on sialyl Lewis x on cancer cells has a positive correlation with the ability of the cells to metastasize. This is likely due to selectins on the endothelial surface of normal tissues binding to sialyl Lewis x, and migration of cancer cells into the tissue to establish a secondary tumor.

Question 13. (4 points) Below is structure of a trisaccharide conformation from the crystal structure of glycan bound to a selectin-like mutant of mannose binding protein A (PDB: 2KMB). For the lower two rings, label each face as either A or B, and explain the nature of the sugar-sugar packing interaction between these rings.



The numbering of the carbons in each saccharide ring is shown above. The "A" face is the face of the ring where the carbon numbers are clockwise as you look from above. The "B" face is the one where the numbers run counterclockwise. In the structure shown, the two A faces pack against each other. This packing is favored by van der Waals interactions between the non-polar parts of the rings.

Question 14. (5 points) Briefly summarize the similarities and differences in the attachment and processing of *N*-linked and *O*-linked glycans.

Similarities:

(1) Both O- and N-linked glycans are attached via reactions mediated by a series of glycosyltransferases.
(2) In both cases, terminal elaboration of attached glycans and final processing is done in the Golgi apparatus.
(3) In both cases the final glycoprotein is generally secreted or delivered to the plasma membrane.

Differences:

(1) For O-linked, attachment is at Ser or Thr, while Asn is the N-linked site.
(2) For O-linked, GalNAc is usually added as the first residue, for N-linked the initial sugar is most often GlcNAc.

(3) The linkage is to protein is usually via the α anomer for O-linked, and the via the β anomer for N-linked.
(4) O-linked sugars are added one at a time, while N-linked sugars first add a pre-formed "high mannose" core in the endoplasmic reticulum.

One point each for items from the above lists (others are possible as well).

Question 15. (6 points; A & B, 3 points each) Briefly explain the utility of each of the following protocols in the analysis of glycan structure.

(A) Permethylation, followed by hydrolysis, and subsequent acetylation.

Linkage analysis. Permethylation of a polysaccharide converts free -OH's to -OCH_3 groups. Hydrolysis to monosaccharides will then result in sugar rings where the remaining free -OH's are those that were involved in glycosidic linkages.

(B) Cleavage with hydrazine or a protein *N*-glycosidase, followed by labelling with 2-aminobenzamide.

Fluorescence labelling of oligosaccharides. Treatment with hydrazine or glycosidase cleaves glycans from asparagine residues. 2-Aminobenzamide reacts at the exposed reducing end of the freed oligosaccharide. Following reduction, this procedure yields a fluorescent end-labelled chain. Sequential enzyme digestion followed by chromatography is then used to identify the original glycan structure.

Question 16. (5 points) Discuss the thermodynamics of the formation and cleavage of glycosidic bonds. Why are GDP-sugars and UDP-sugars used biosynthetically during creation of glycosyl linkages?

Formation of a glycosidic bond is energetically unfavorable, and is driven the hydrolysis of two ATP phosphate linkages as the energy source. ***(2 points)*** *The first step is formation of a GDP-sugar or UDP-sugar, requiring both equivalents of ATP.* ***(1 point)*** *Then the nucleotide sugar couples with a free sugar chain in a glycosyltransferase mediated reaction to form the new glycosyl linkage.* ***(1 point)***

Cleavage of a glycosidic bond as performed by glycosidases is downhill in energy, and does not require ATP or other energy input. ***(1 point)***