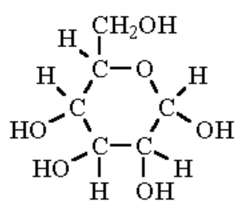
BMB170, Fall 2017
Problem Set 5: Carbohydrates

Due: 12/01/2017 by 5pm, as PDF.
OH: 11/30, 3:00-5:00PM, Broad Café
Please email questions and PDF files to Jingzhou Wang (jingzhou@caltech.edu)

Problem 1: Basic Chemistry of Carbohydrates (50 points)

a. Structure and Representation of Monosaccharides. Please draw the Fisher and Haworth projections for the following monosaccharides. Indicate whether it's an aldose or ketose. (10 points)
   I. Beta-D-Galactose
   II. Alpha-L-Ribose
   III. Beta-D-Mannose
   IV. Beta-L-Fructose
   V. Alpha-D-Erythrulose

b. Glycosidic Bond Formation. Maltose is formed by linking two glucose molecules. Below is the diagram of a glucose molecule. Assume C1 of it participates in glycosidic bond formation. Please draw a second glucose molecule next to it, and circle the atoms that are lost upon maltose formation. Label 1) "alpha" or "beta" for individual monosaccharides, and 2) reducing and non-reducing ends. How are monosaccharides modified to favor glycosidic bond formation? (10 points)



c. The Biochemistry behind glycan elongation. Glycosylttansferases are a diverse group of enzymes that catalyze the formation of varieties of glycosidic linkages. Elucidating the mechanism, structure, and roles in cell biology of glycosyltransferases is crucial for understanding glycobiology. (20 points)
   I. Subcellular Localization of Glycosyltransferases. Glycosylation of newly synthesized proteins is initiated in the ER and the carbohydrate chains are elaborated in the Golgi apparatus by sequentially acting glycosyltransferases that are localized to successive compartments of the Golgi. Schmitz et al. discovered a machinery, Vps74p, which is crucial for the localization of Golgi glycosyltransferases through direct binding interactions. Please read the paper (doi.org/10.1016/j.devcel.2008.02.016) and answer the following questions.
      i. How was Vps74p originally identified? (2.5 points)
      ii. Describe the rationales behind using mobility on SDS-PAGE to differentiate levels of glycosylation. (2.5 points)
      iii. How did the authors show that the tetrameric form of Vps74p is physiologically relevant? According to the authors, how is Vps74p crucial in glycosylation? (5 points)

iv. Based on the evidences in this paper, do you think that the author's conclusion is justified or not? Why? (1 points)

II. <u>Mechanisms of Glycosyltransferases.</u> Inverting and retaining mechanisms are two major mechanisms employed by glycosyltransferases. Please read the review article from Lairson et al. (doi.org/10.1146/annurev.biochem.76.061005.092322) and answer the following questions.

i. Inverting glycosyltransferases:

1. Describe the general mechanistic strategies employed by inverting glycosyltransferases. (2 points)
2. Describe two major differences between inverting GT-A and GT-B glycosyltransferases. (2 points)

ii. Retaining glycosyltransferases:

1. Describe the double-displacement mechanism. Based on what evidences was this mechanism proposed for glycosyltransferases? (2.5 points)
2. What are the challenges behind studying the mechanisms of retaining glycosyltransferases? (2.5 points)

d. <u>Diversity of Carbohydrate Structures.</u> The assembled architecture of carbohydrates can lead to a much larger and more diverse pool of structures when compared to other classes of biopolymers, such as protein and DNA. On the other hand, understanding structure-function relationships of glycans can be more difficult than those of other macromolecules. (10 points)

I. Briefly describe structural features of carbohydrates that result in such diverse architecture. Explain why it is hard to relate glycan structures to their functions. (8 points)

II. Describe two major differences between N-linked and O-linked glycosylation. (2 points)

Problem 2: Physiological Relevance of Glycan (50 points)

a. <u>Essential Components of Cell Wall</u>

I. Bacteria, primarily Gram-negatives, produce and secrete cellulose via a protein complex consisting of at least three subunits (BcsA, BcsB and BcsC). Morgan, et al. (doi: 10.1038/nature11744) presented a crystal structure of a catalytically active complex of *Rhodobacter sphaeroides* BcsA and BcsB1 translocating a glucan. Please answer the following questions about this work.

i. Which step in cellulose synthesis and translocation does this structure represent? How did they know this? (Include what they observed during model building.) What is missing from the catalytic site in this structure and where is the predicted position for the missing component? (2.5 points)

II. Below is a schematic representation of Gram-negative (Fig. 1) and Gram-positive (Fig. 2) bacterial cell walls. Briefly describe what are key differences in carbohydrates components between them. What do you think is the best component that can be targeted for differentiating Gram-positive and Gram-negative bacteria? Describe the existing method to differentiate Gram-negative and Gram-positive bacteria. (Figures: Varki, A., et al. (2009). Essentials of Glycobiology. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press). (5 points)

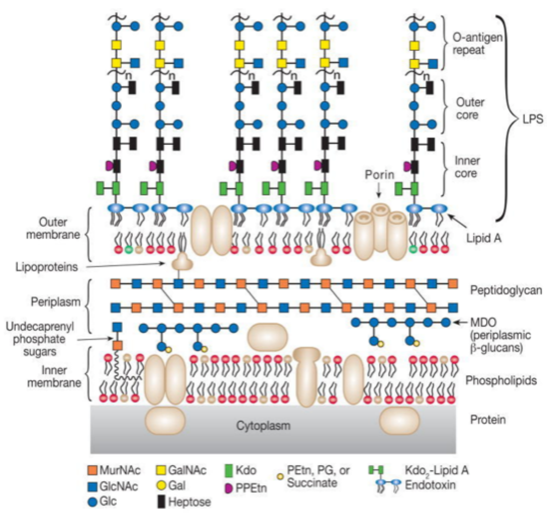


Figure 1

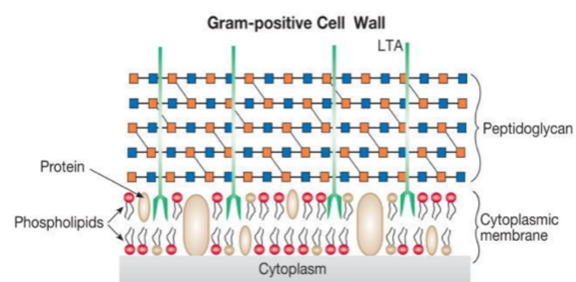


Figure 2

III. Beta-lactam antibiotics inhibit enzymes catalyzing transpeptidation between glycan chains of cell wall, and therefore suppress cell wall biosynthesis. However, methicillin-resistant *S. aureus* (MRSA) evades beta-lactam inhibition through PBP2a, which catalyzes transpeptidation without inhibited by nearly all beta-lactam drugs. In 2001, Lim et al. provided the first structural insight into the antibiotic resistance of PBP2a (doi:10.1038/nsb858).

i. From the kinetic perspective, which strategy does PBP2a use to achieve broad spectrum resistance? What structural changes happen in PBP2a to help implement this strategy? (5 points)

ii. From the structure of PBP2a and the suggested antibiotic-resistant mechanisms, what are potentially effective drug design methods that can inhibit PBP2a? (2.5 points)

iii. Acebron et al. discussed the allosteric perspectives of PBP2a (Curr Med Chem. 2015; 22(14): 1678–1686). Which initial evidences suggest allosteric changes of PBP2a? How does ceftaroline successfully inhibit PBP2a? (5 points)

b. Glycosylated HIV Env. HIV Env proteins are highly glycosylated viral spikes. The glycans on HIV Env impose challenges to therapeutic designs and structural studies, but are also proven to be potential therapeutic targets.

   I. Due to the heterogeneity of glycans, powerful technologies that can profile glycosylation patterns are crucial. Please answer the following questions regarding a proteomic-based analytical strategy of HIV Env glycoprotein (doi:10.1038/ncomms14954).

      i. Briefly describe the workflow of the method present in this study. (2.5 points)

      ii. Which strategy did the author use to maximize sequence coverage for analysis? How did the authors validate that their method can correctly identify the types of glycan? (5 points)

      iii. Based on your knowledge and experience, what are potentially the biggest challenges of analyzing glycolysis profile through proteomics? (2.5 points)

   II. Gristick et al. solved the crystal structure of two broadly neutralizing antibodies in complex with natively glycosylated HIV-1 Env (doi:10.1038/nsmb.3291).

      i. Why gp120 glycosylation reduces immunogenicity of HIV virus? (2.5)

      ii. Why gp120 glycosylation makes crystallographic study more difficult? What strategies did the authors use to overcome this limitation? Can you think of other strategies? (5 points)

      iii. Open structure 5T3X. Identify 10-1074 Fab and Env with appropriate labeling. Show N332 glycan as stick with appropriate labeling. Identify a glycosylation site with disordered glycan structure. Why this glycan chain is disordered while some other glycans have visible electron density? (5 points)

c. Biomarker. Glycoproteins are clinically utilized as serological markers for various cancers in diagnostic and prognostic purposes. Give two criteria that you think are important in selecting a suitable cancer biomarker. Melo et al. (doi: 10.1038/nature14581) reported GPC1+ circulating exosomes (crExos) as a potential diagnostic and screening target for early stages of pancreatic cancer. Explain how the early detection of pancreatic cancer can be done more effectively with the cancer-cell- derived exosomes than with other glyco-biomarkers previously identified. (7.5 points)