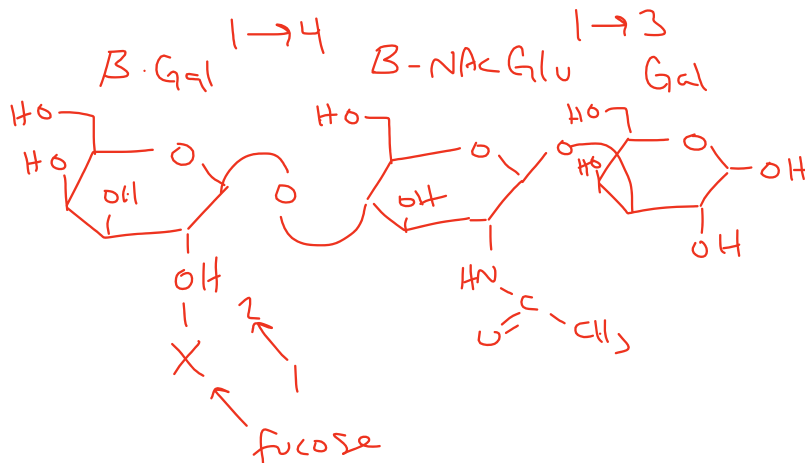
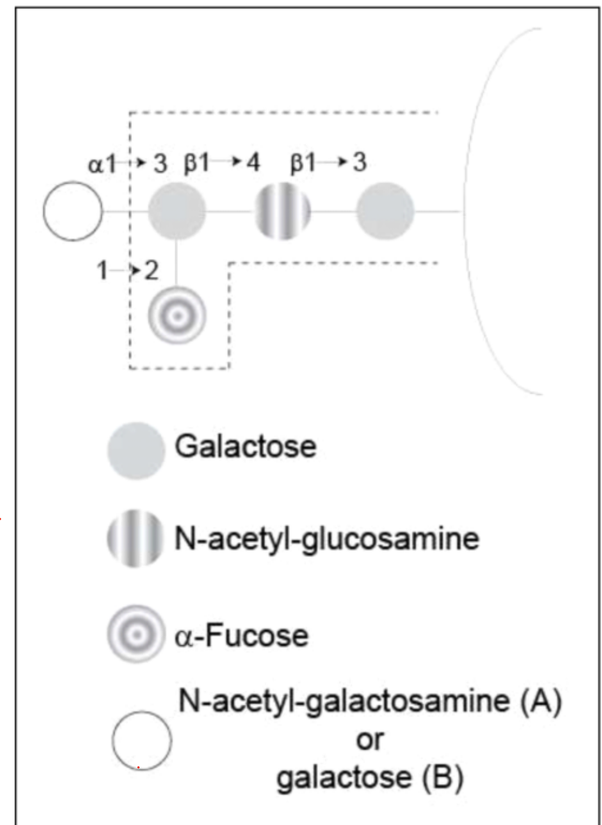
Structure of Biomolecules

There are 35 human blood group systems. The two most important are the ABO and the Rh systems. These are incredibly important contributors to your immune response to blood transfusions. In the ABO system, antigens, made of small chains of carbohydrates, decorate the surface of red blood cells (as well as most other cells in your body) and are recognized by antibodies. The Rh blood group system is defined by the presence or absence of 50 different blood group antigens (this gets complex really quickly) with the most important being the D antigen. The presence of the D antigen is Rh positive and the absence is Rh negative. Let's explore these systems in a little bit of detail and use it as a way to reinforce some of the most important concepts that we've learned about biomolecule structure.

The ABO Antigens

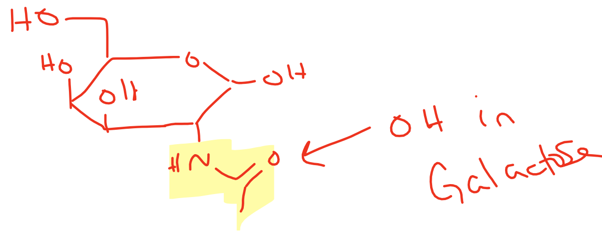
1. The image to the right shows the H-antigen (dashed box) linked to the surface of a cell. Recalling that galactose is the C4 epimer of glucose and that glucosamine has an amine substitution at C2, draw the first three sugars of the H-antigen as they would look on the surface of the cell. Put an "X" where the fucose would be attached.



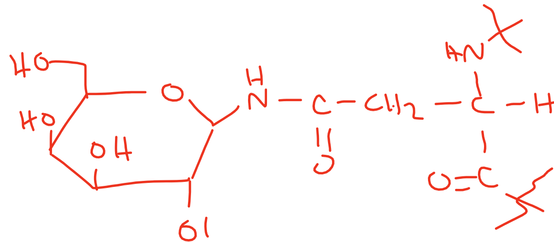


2. O blood type does not have the 2nd sugar (the white circle) attached to the H antigen. A and B types have an extra carbohydrate attached through an α 1→3 as the image suggests.
    a. Is this extra sugar locked into the cyclical form or is it free to interconvert to the linear form? Explain your answer. Yes. The anomeric carbon is part of a glycosidic bond. This removes the potential to be hydrolyzed.

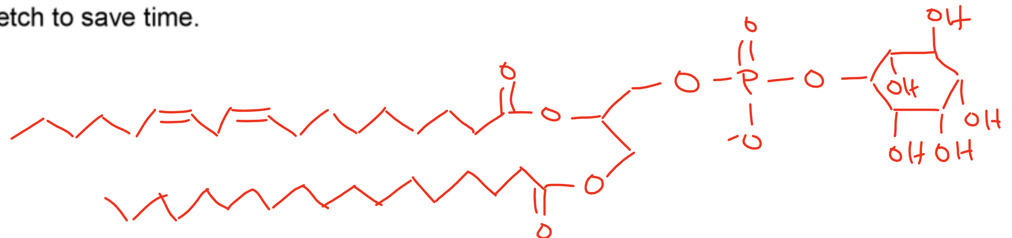
b. Draw N-acetylgalactosamine in the ring form. How does this differ from galactose? It has a N-acetyl group at C2.



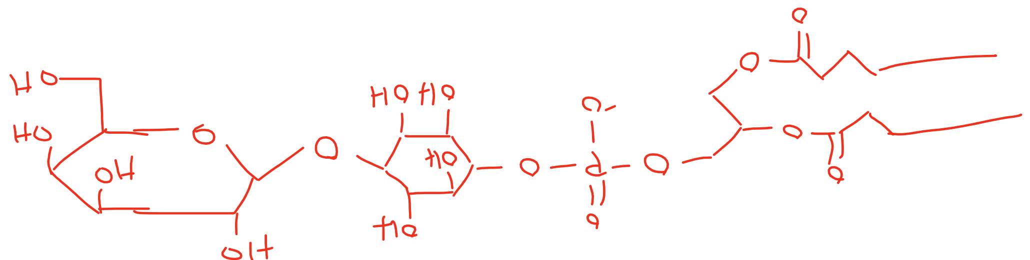
3. These antigens can attach to the surface of a cell via a linkage to a protein or a phospholipid.
   a. As you may recall from your reading, sugars can be O-linked or N-linked. For N-linked sugars, the anomeric carbon is covalently bonded to an Asn residue. Draw an N-linked β-galactose.



   b. Commonly, the antigens are linked to phosphoditylinositol.
      i. The structure of inositol is shown. Draw phosphotidylinositol with 18:0 and 18:2n-6 at the appropriate positions on the glycerol backbone. Feel free to make a shorthand sketch to save time.



      ii. Now show how a galactose could be anchored to the membrane by phosphotiylinositol.



      iii. Is this a stable linkage or is it susceptible to hydrolysis?

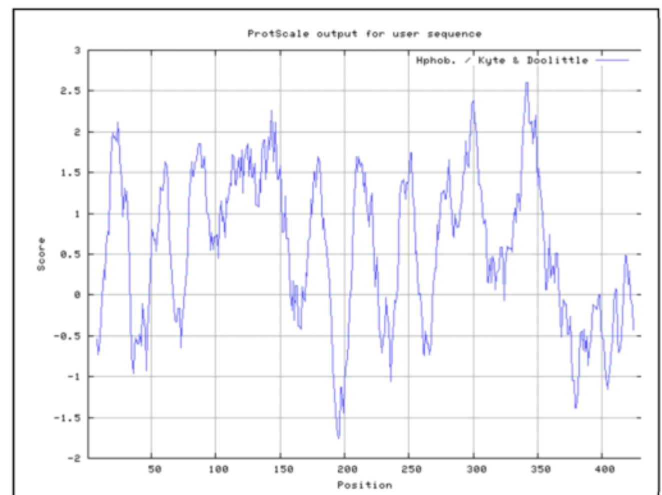
as drawn, no because the anomeric carbon is tied up in a glycosidic bond

4. In your blood, you have large proteins called antibodies that will recognize these antigens. Someone that has an A antigen on the cell surface has Anti-B in their blood; this is an antibody that will specifically seek out and bind to the B-antigen. If Anti-B binds to the B antigen, hemagglutination (the clumping of red blood cells) ensues.
    a. It's pretty amazing to consider that these antibodies are so selective. Consider the differences between the A and B antigens. Based on this difference, what differences do you think may exist in the recognition pocket of the antibody? Anti-B recognizes an OH at the C2 position but excludes $NH(CO)CH_3$ while Anti-A is the opposite. There are two main differences here – the size of the group hanging off the C2 position and the potential for chemical interactions. If the Anti-B has a small binding pocket, the A antigen will not be able to fit, so it can be excluded. However, the Anti-A has a big pocket that will allow the OH or the NAc. Consequently, the Anti-A must offer some sort of favorable interaction (perhaps a small hydrophobic pocket for the CH_3) that is the driving force needed for tight binding to the antigen.

    b. Part of the process of hemagglutination is the denaturation of some secreted globular proteins.
        i. Why would the denaturation of these proteins lead to visible clumping in the blood? Hydrophobic side chains would be exposed to the polar solvent. These would have a strong tendency to "find" other hydrophobic groups to interact with and if they are not able to repackage into the core of a protein, they will aggregate with other denatured proteins and eventually precipitate.
        ii. What are two other ways to denature a protein? Lots of options here. Heat, pH, chaotropic salts, etc.

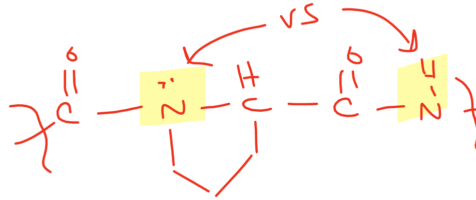
        iii. Why can some proteins tolerate more stress before denaturing than others? Make sure to consider which forces keep a protein folded. The have stronger forces holding them in the correct structure. Two really good ideas would be that a protein has a lot of disulfide bridges (like we saw with RNase) which covalently lock a protein into the best structure – also, you may hypothesize that a protein has a large number of hydrophobic residues that form a particularly stable core.

5. Now let's think about the Rh protein. This is a primarily alpha helical transmembrane protein.



    a. The hydrophobicity plot is shown. Based on this, predict how many transmembrane helices exist? 11, but other answers are ok – the point is to recognize the alternating pattern of hydrophobic/hydrophilic
    b. How is this image made and what do the values on the x-axis mean? The hydrophobicity of each amino acid is scored and plotted vs. the amino acid number (on the x-axis). This allows a scientist to get an idea about the clustering of hydrophobic and hydrophilic regions of the protein.

c. Proline is commonly found three amino acids before a transmembrane helix but not part of the helix. Why? The backbone imide does not have the potential to H-bond in the pattern needed to stabilize secondary structure.

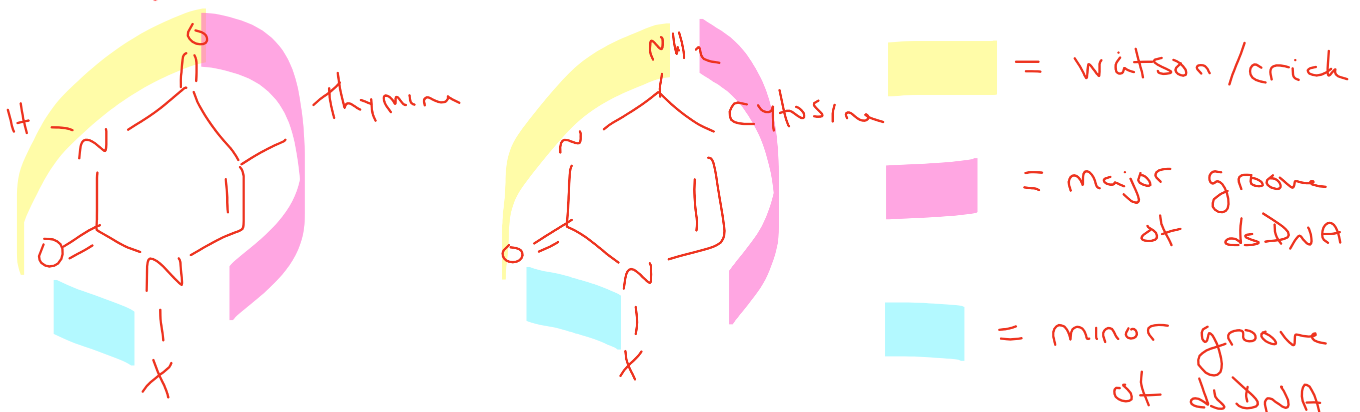


d. Is it possible for a globular, water soluble protein to have a similar hydrophobicity plot? yes What would be the main driving force that would allow this protein to stay folded in aqueous media? The hydrophobic effect – the nonpolar regions pack together into the core of the protein and the polar remain on the exterior. This is an entropy driven process (water being displaced from the non-polar groups.

e. Would the melting of this protein be a cooperative process? Yes – protein folding and unfolding is always cooperative. This means that once the protein begins to denature, it is much easier for the subsequent unfolding steps to occur.

f. The Rh D antigen differs from the RhC antigen by only a few amino acids. How can this changes be made? That is, how does a different polypeptide sequence form? There is not a good way to change the amino acid within a protein, so the changes come at the level of the DNA.

6. I couldn't 'figure out how to tie in a good DNA/RNA question, so I'm just throwing it in here. We talked about endonucleases (restriction enzymes) in the discussion about working with DNA. You may recall that endonucleases have very specific recognition sequences. Consider, for example, NdeI which recognized CATATG but not TATATG. Propose a way that NdeI will bind to the first sequence but not the second. Remember that endonucleases do not cut single stranded DNA, so the recognition must be something other than looking for the Watson-Crick base pairing pattern. Sketch the two bases (C and T) to support your answer. Proteins that bind to DNA cannot look for the H-bonding patterns in the Watson-Crick face because they are already H-bonding with the other chain. Consequently, the protein must be recognizing another structural component of the DNA. Comparing C and T, the two big differences are the presence of absence of a methyl group in the major groove (remember this is the part of the base opposite the glycosidic bond – yes, the ribose → base bond is a glycosidic bond) and the presence of an C=O or C-NH_2.



7. The rhesus family of protein (the namesake for Rh) are ammonia transporters. Their role is to excrete NH_3 and ensure renal pH balance. Consider these facts to answer the following questions.
    a. The extracellular portion of the proteins has an isoelectric point of 6.01.
    b. The intracellular portion has an isoelectric point of 4.17
    c. The pH inside the cell is 7.3.
    d. The pH outside the cell is 5.5.

I. What is the charge on the extracellular side of the protein? Positive or negative.

pH < pI, so there is a positive charge

II. What is the charge on the intracellular side of the protein? Positive or negative.

pH > pI, so it's a negative charge

III. What happens to ammonia when it is dissolved in water (so become aqueous ammonia)? It reacts with water to become the ammonium cation (NH_4^+)

IV. Based on your answer to III, which side of the protein do you expect aqueous ammonia to be attracted to? Justify your answer. The intracellular side because cations are attracted to negative charges

V. Is your answer consistent with the actual direction of transport (in → out). No. The ammonium ion is not attracted to the extracellular side of the protein. Instead, it seems logical that it would stay interacting with the anionic intracellular part of the protein. If not, think critically about what role the unexpected polarity might have. Answer the following question to help understand how this protein works

a. What is the form (and charge) of aqueous ammonia when it enter the transporter? It's a cation (NH_4^+) Is it attracted to the protein? Yep, it's attracted because opposite charges attract each other Do you think that is important? Yes – if the ammonium were not attracted to the transporter, it would have no reason to come into close proximity (which is needed for transport.

b. The core of the transport channel is very narrow and cannot accommodate water or an ion. What is the form (and charge) of ammonia as it passes through the core. If an ion cannot be accommodated, it must be the neutral form (NH_3)

c. When ammonia reaches the extracellular side of the channel, it is once again exposed to water and a pH of 5.5. What is the form (and charge) of ammonia as it exits the transporter? It reforms the cation (NH_4^+)

d. Does the attraction or repulsion of aqueous ammonia to the extracellular side of the rhesus protein play a role in the ability of the protein to function properly? Now the ammonium is exposed to the (+) charge on the transporter. This will encourage the ammonium to be repelled from the protein into the extracellular solvent and allowing another NH_3 to be pushed through.