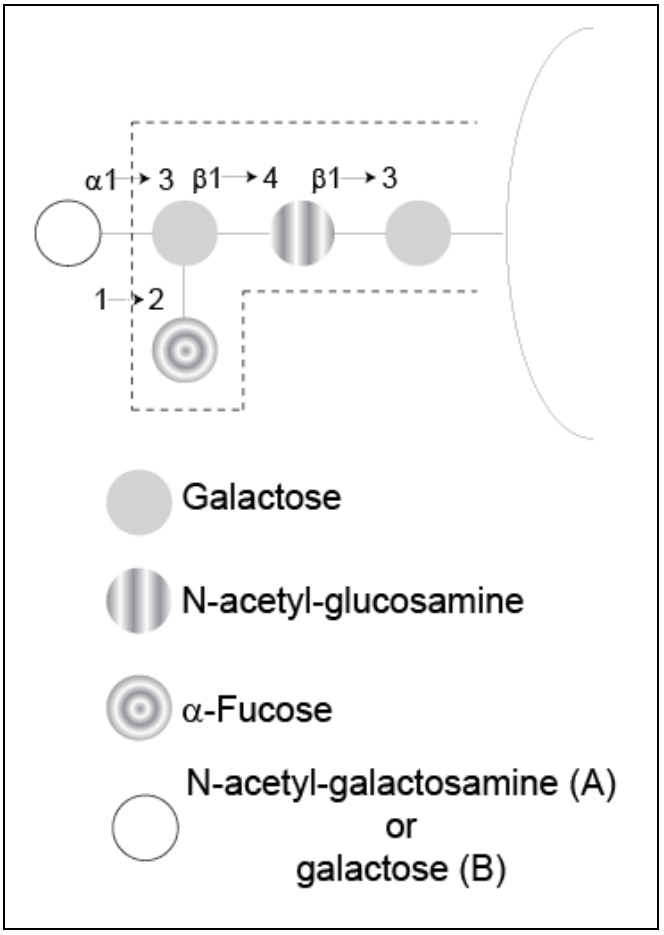
Structure of Biomolecules

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.

b. Draw N-acetylgalactosamine in the ring form. How does this differ from galactose?

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?

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?

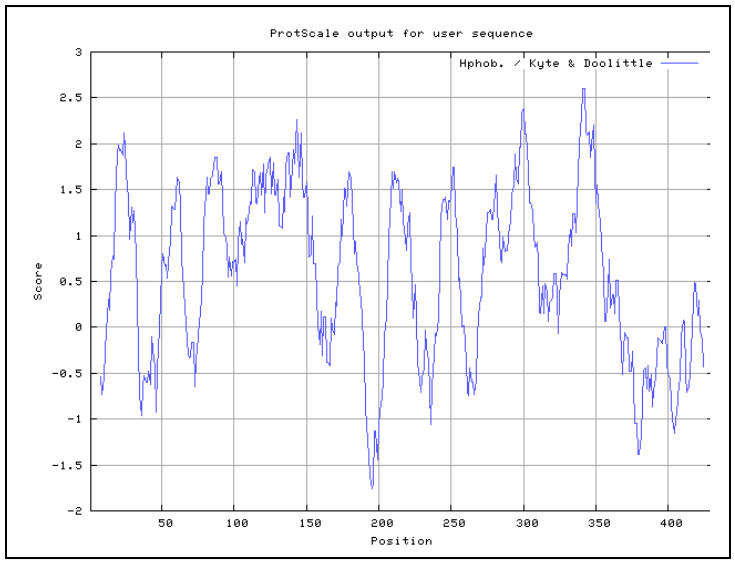
    b. Part of the process of hemagglutination is the denaturation of some secreted globular proteins.
        i. Why would the denaturation of these proteins lead to visible clumping in the blood?

        ii. What are two other ways to denature a protein?

        iii. Why can some proteins tolerate more stress before denaturing than others? Make sure to consider which forces keep a protein folded.

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?

    b. How is this image made and what do the values on the x-axis mean?



c. Proline is commonly found three amino acids before a transmembrane helix but not part of the helix. Why?

d. Is it possible for a globular, water soluble protein to have a similar hydrophobicity plot? What would be the main driving force that would allow this protein to stay folded in aqueous media?

e. Would the melting of this protein be a cooperative process?

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?

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.

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.

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

III. What happens to ammonia when it is dissolved in water (so become aqueous ammonia)?

IV. Based on your answer to III, which side of the protein do you expect aqueous ammonia to be attracted to? Justify your answer.

V. Is your answer consistent with the actual direction of transport (in → out). 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? Is it attracted to the protein? Do you think that is important?

    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.

    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?

    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?