Instructions for modeling glycosylations on protein structures **LSO Fall 2019**

0. The protein structure files of erythropoietin ("EPO") and human epidermal growth factor receptor 2 ("HER2") have already been downloaded on your computer from RCSB.org:
- EPO = 1buy.pdb
- HER2 = 2a91.pdb
  - HER2 is a protein commonly mutated in breast cancer, and is the target of the drug Herceptin

1. Now that we have our protein structures downloaded, now we can make glycosylated models of these proteins. To do so, go to glycam.org.

2. Select "**Glycoprotein Builder**" from among the "Currently available tools."
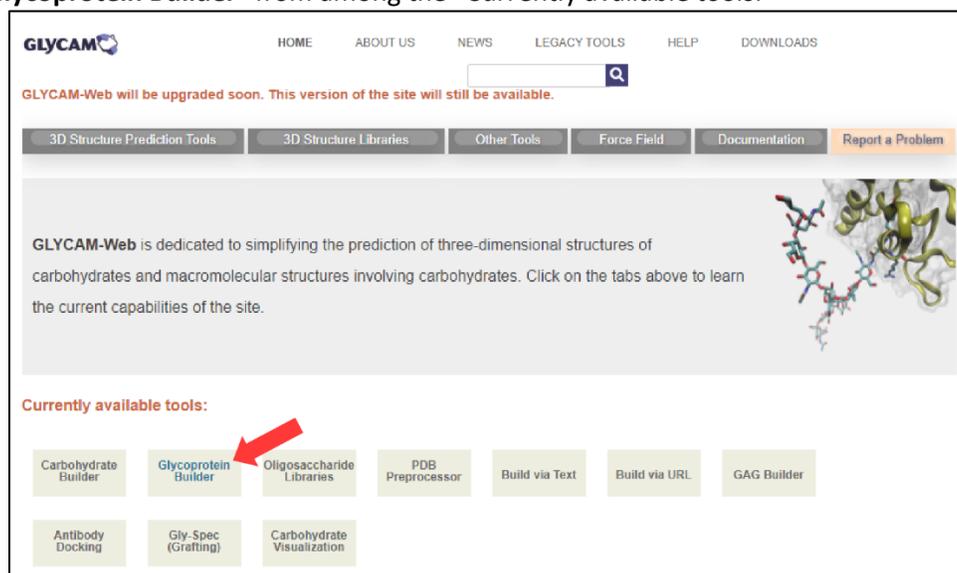
3. At the next screen, click on "**Choose File**," and choose either "1buy.pdb" or "2a91.pdb," depending on whether you want to make a glycosylated model of EPO or HER2, respectively. Then hit **continue**.
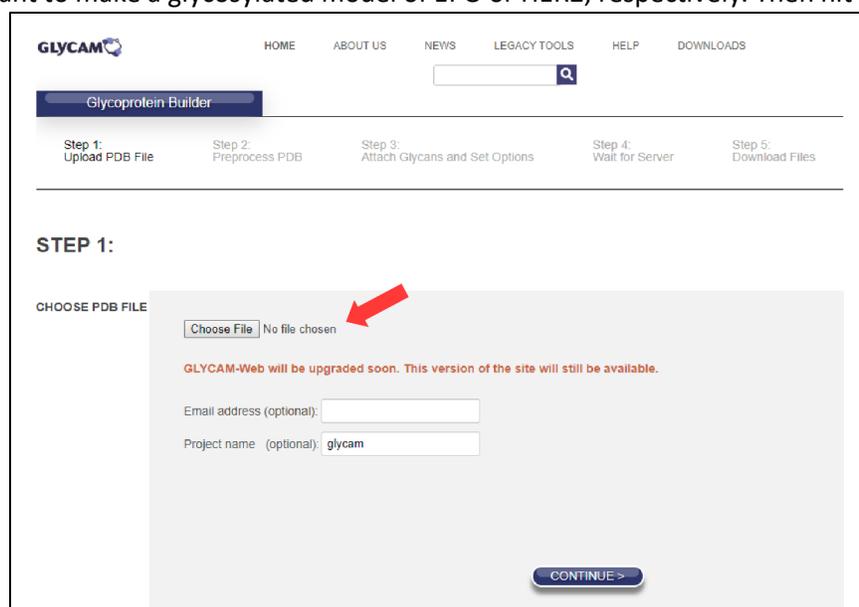
4. On the next page, which should be called "Step 2: PDB File Preprocessor," hit **continue** again.

5a. On the next page, select "Attach Glycans Using" **the oligosaccharide library**. When you select "the oligosaccharide library," another list will appear just below it. A new list with various oligosaccharide libraries will appear below:

5b. Select any library you'd like, either "**High Mannose Oligosaccharides**," or "**High Mannose Complex Hybrid N-Glycan**," or "**Sialylated or Fucosylated Complex**," or "**CFG Glycan Array Ver5.0**," or "**Complex type**," or "**Glycosaminoglycans**."

6. When you select a library, a new window will open up showing all the possible glycans you can add to your protein. **Select one** (whichever strikes your fancy!).

An example of the window that pops up when you click on the **High Mannose Oligosaccharides** library is shown on the next page, in which you can select one of the 21 possible glycans:
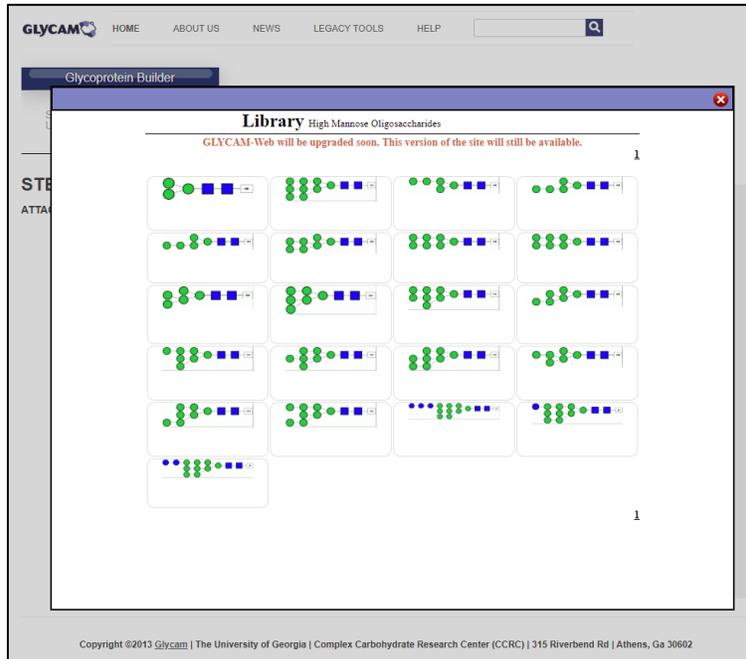
7. The next screen will indicate the glycan you selected. Hit "**Add glycan to glycosylation sites**."

8a. On this page, you can choose which amino acids of your protein you'd like to add your glycosylations to. Recall that "N-linked" glycosylations are sugars attached to the amino acid asparagine (Asn, N), and "O-linked glycosylations" are sugars attached either to the amino acid serine (Ser, S) or the amino acid threonine (Thr, T). Select "**Show All**" for both options, to see the amino acids available for glycosylation.
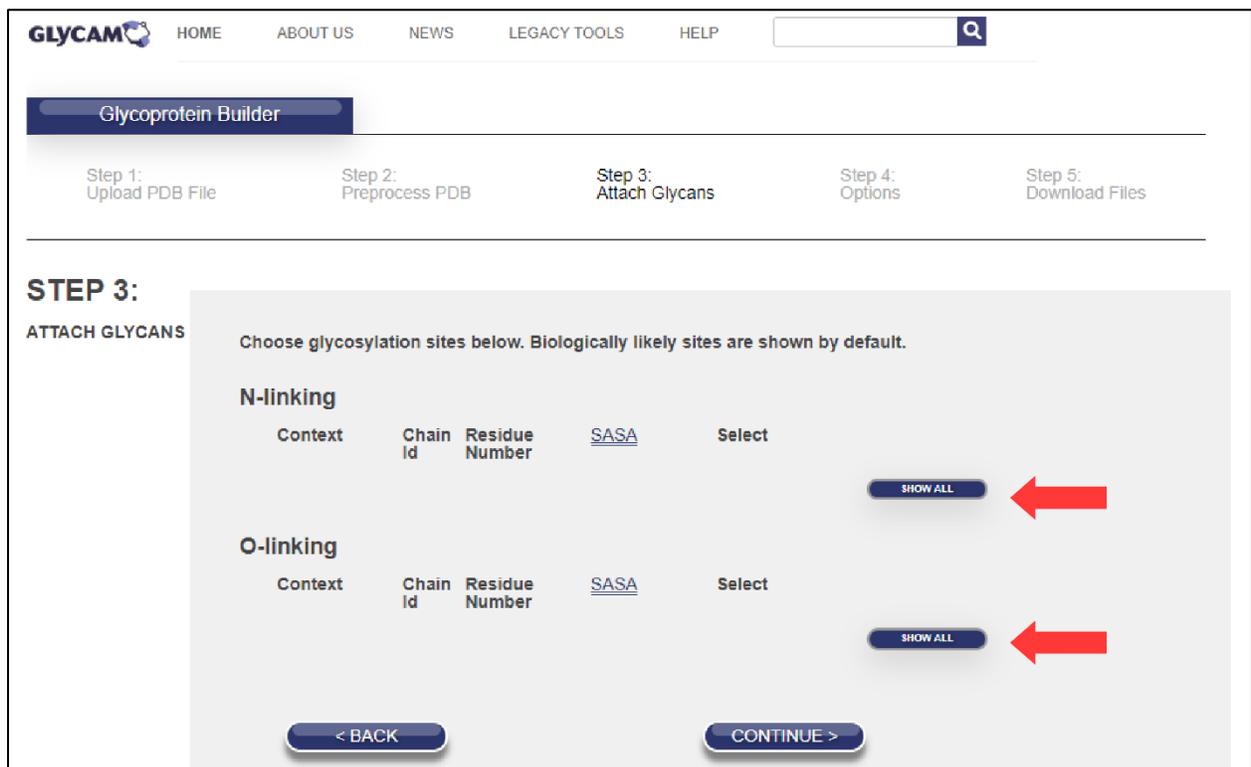
8b. Select **one** or **two** amino acid to glycosylate (whichever you prefer!), and hit **continue**. An example of selecting two amino acids for EPO is shown below:

9. If you'd like to add another type of glycosylation to your protein, you can repeat the above process at the next page. Once again, hit "**the oligosaccharide library**," choose a library, then **select** an oligosaccharide. This time, try choosing an oligosaccharide from different library. Once you select a new glycan, you will be asked which amino acid you'd like to add it to. Choose **one** amino acid to glycosylate, and hit continue.

10. Then hit "**Download current structure**." This may take 4-5 minutes for the server to build your glycosylated protein.

11. Once your glycoprotein is built, you will have a screen that looks like it does below. Click on "**Amber_style w/o H**" to download your glycoprotein file **to the desktop**:

12. We can now open your glycoprotein model using PyMOL. The file you want to open will be called "**structure_AMBER_noh.pdb**" and it will be on your desktop.

13. Once you open the file in PyMOL, hit "S" at the bottom right corner to show the **S**equence of the protein at the top of the window (red arrows on the next image indicate both the "S" button in the bottom right corner, and the resulting protein sequence that appears at the top of the screen):
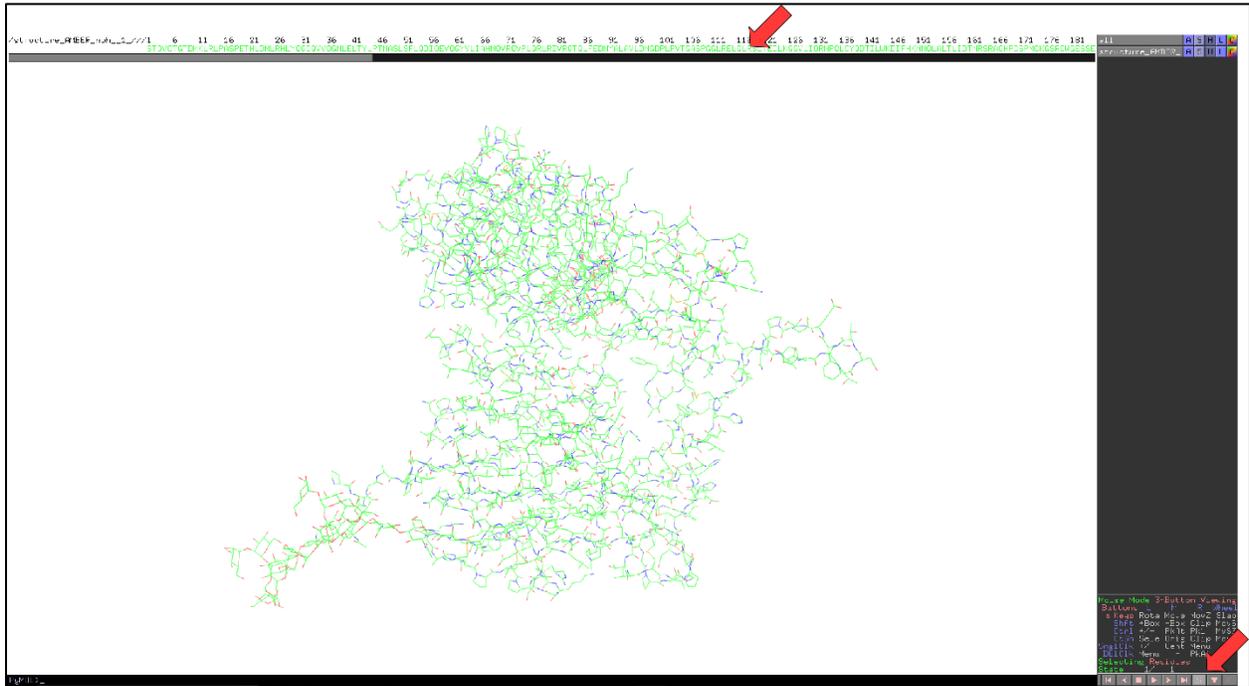
14. Click on the "S" near the top right of the screen, next to the term "structure_AMBER_noh":

15. Scroll down the resulting menu options and select "**cartoon**":
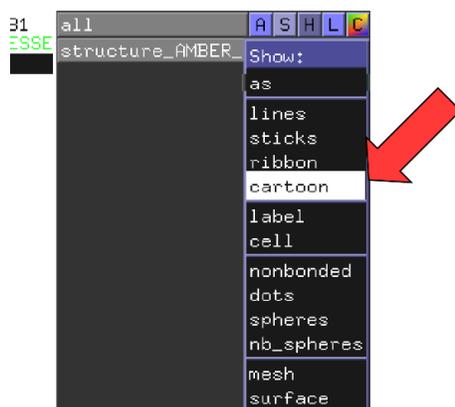
As a result, the amino acids of the protein will be represented as a cartoon.

16. The cartoon representation does not yet show the glycans we added to our protein. To make the glycans visible, click on the scroll bar at the **top** of the window, and pull it to the right to get to the end of the glycoprotein sequence.
- EPO has about 166 amino acids, so its last amino acid is #166.
- HER2 has about 506 amino acids, so its last amino acid is #506.

At the end of the amino acid sequence, select all the sugar groups, which will appear as "**4YB**," **VMB**," "**OMA**," "**2MA**," etc. Select them all by clicking on one and dragging across the sequence as you hold down (try to select everything after amino acid 166 for EPO, or after amino acid 506 for HER2):
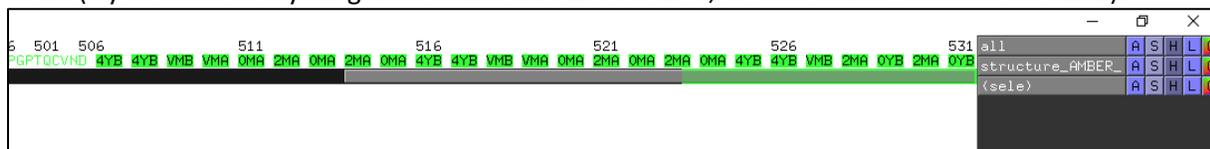

17. **Right-click** on a highlighted sugar, and a menu will appear. Select "**show**," then scroll down to "**sticks**." This will make your modeled glycans appear!
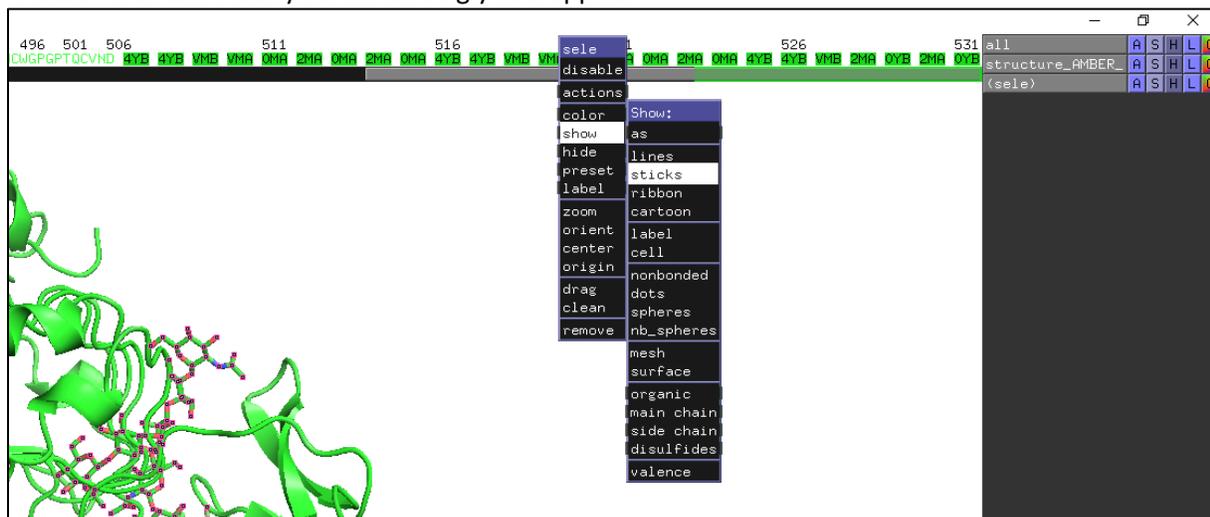
18. If you'd like, you can also color your glycans a different color. Once again, **right-click** on the highlighted sugars at the end of the protein sequence. This time choose "**color**," and choose whichever color you'd like!
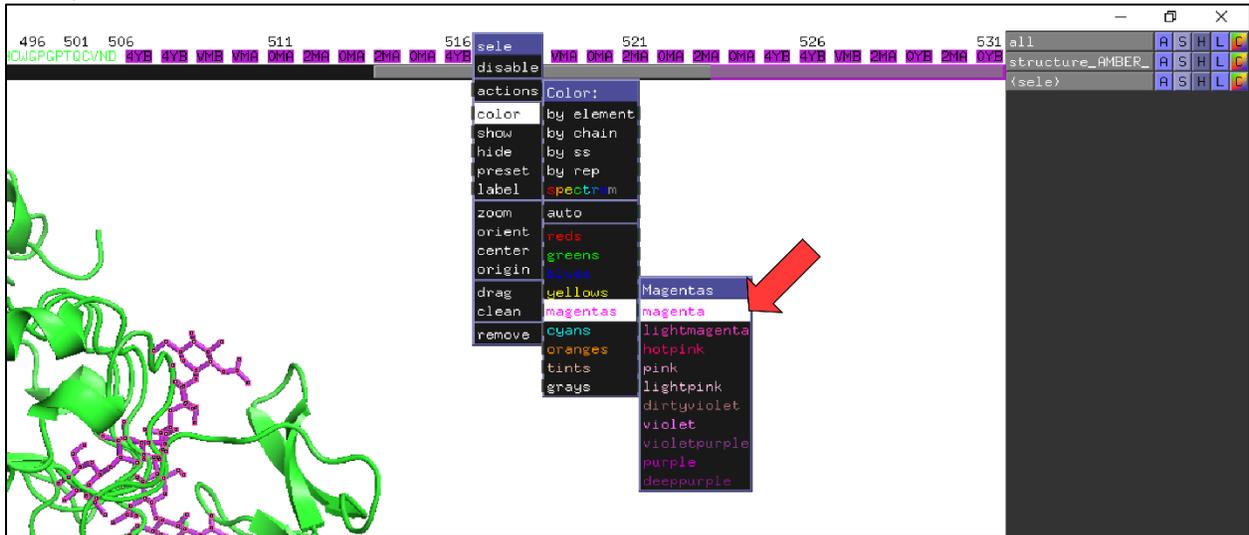
Zoomed out structure of a glycosylated-HER2 model:
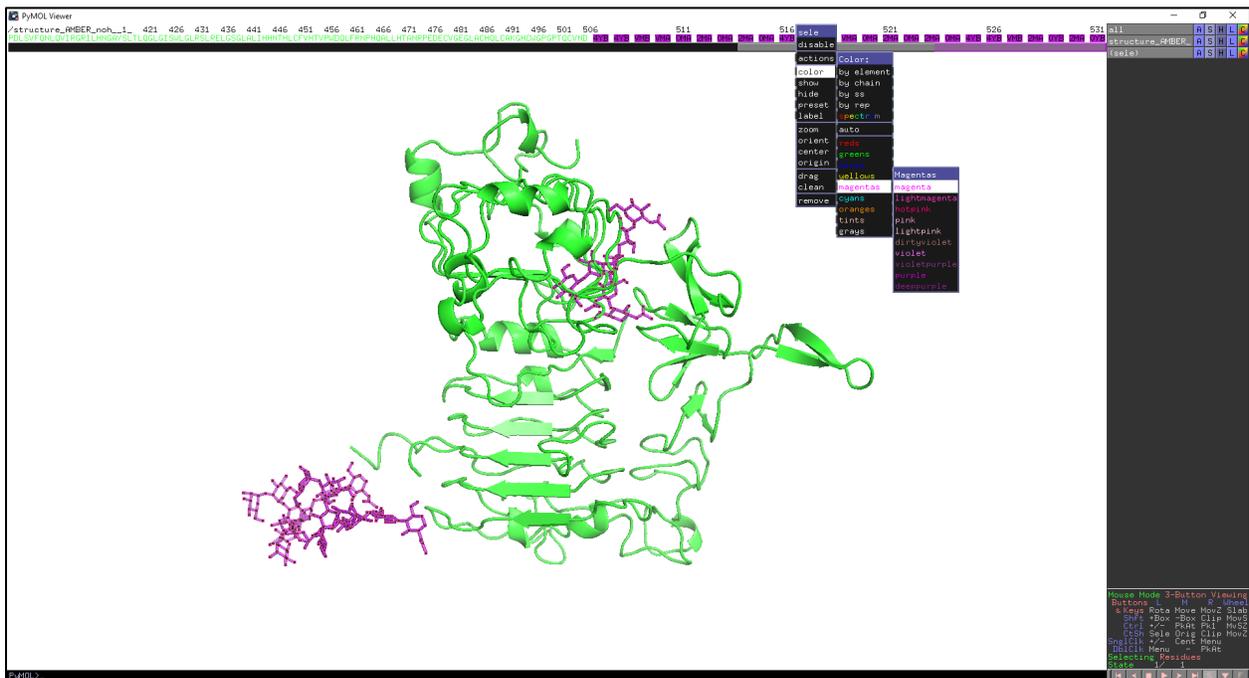
Discussion Questions

Does your glycoprotein structure look similar to those made by your table mates?
- Where are the glycans on your protein relative to theirs?
- How much of the protein's surface is covered by glycans?