A more focused to-do list

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Important issues

There are three main issues to deal with WRT these mucin runs:

1. Validation of the computational technique.
2. Demonstration that there is a marked flexibility difference between peptides that are O-GalNAc'd versus the others.
3. Discussion of findings regarding the likelihood (or lack thereof) of a bridging water being important to the O-GalNAc structure.

Things that are done, or essentially done, are in light bluish text.

Tactics to use

Point 1, Validation

The only serious problem here is that our J-coupling values are off, quite systematically, from the measured ones, by about 1 Hz. This is not surprising. We often have this issue. In other work, it is becoming plain that the relatively simple Karplus equations we've been using aren't necessarily so useful in these situations. In this work, that point is illustrated by the fact that only the 2D j-coupling software could differentiate between the three peptide types, and the other methods didn't work as well.

List of Possible Figures

- 2D J-coupling figure (done previously)
- Other J-coupling methods figures for SI (done previously)
- 1/r^6 averaging of H-H distances (really not a good way… might not bother except maybe to compare with the previous)
- RMSD's vs initial NMR structures (all to all)
  Use background highlighting to make it more sense-making
- Phi-Psi contour plots showing values for NMR structures and NMR data implied values

Point 2, Flexibility

Someone suggested rmsd values from MD, but that isn't really the best way to show flexibility. For example, an average rmsd of 2.5 means that, on average (ish), the atoms in the structure are 2.5 A away from the reference coordinates. That could mean anything. Their coords could be distributed around the reference, or displaced from it, and give the same value. So… that, by itself, isn't enough.

List of Possible Figures

- N-to-N length distributions for each type of peptide. Choose N's on either side of the THR's in the backbone. This shows the changes in backbone flexibility very well.
- A special rmsd of the sugars as follows: For each THR, one at the time, align the molecule on the THR. But, get the rmsd values for the heavy atoms of the attached sugar. Best, probably, to use only the ring, and maybe glycosidic linkage, atoms in the sugar to ensure that there are no size effects in the comparison.
- The Phi-Psi plots, for both the peptide backbone and for the glycosidic linkages, will do a nice job of illustrating the change in flexibility. Using the backbone means it is possible to include the unglycosylated variant, too.

Point 3, Does a Bridging Water Contribute?

The issue here is that a previous group reported that, for a much smaller system, they saw evidence that a water molecule forming a bridging bond between the GalNAc's NAc H and the backbone H was stabilizing the structure. So far, our evidence is inconclusive. We see bridging bonds, but it isn't at all obvious that they are doing any significant stabilizing.

Here are some points of note:

1. It is very difficult to say for certain whether or not bridging H2Os are there merely because they are one of many waters nearby and, occasionally, one might be expected to form a bridge.

2. We and the previous group both used the TIP3P water model. This model is well known to have a much lower viscosity than real water. So, it is possible that the model is causing the water molecules to behave in slightly unusual ways. Perhaps they are more able to slip into a bridging position than they might normally be. Or, conversely, perhaps the more proper geometry of a model that approximates lone pairs, e.g., TIP5P, might cause waters to form bridges far more often than we see. It seems unnecessary to do TIP5P calculations for the purpose of this paper, so we won't. But, that could happen later.
3. There is a very stable structure formed by the backbone O and H and by the glycosidic O and the NAc H. This structure is present ~90% of the time. A bridging water is present only ~15-30% of the time.
4. When bridges do form, they are not particularly long-lived. If a bridge were a strongly stabilizing influence, one might expect the bridging waters to persist for a long time. But, it is also possible that they don't need to.

To attempt to get a handle on this, I am currently running a zillion implicit solvent calculations. In these, there are no bridging waters because there are no explicit water molecules. So, if the structures are stable there, then the bridging waters seem less likely to be important. The problem, here, is that there are no implicit solvent models that perform well for carbohydrates. So, no matter what the results say, I won't know much more than I did. Anyhow, I'm running all the IS variants that I can. I'll find the model(s) that give N-to-N lengths closest to the explicit simulations and check their structures. Details of these calculations will be posted here eventually.

List of Possible Figures

- RDFs for solvent for comparison to the earlier work
- Distribution of persistence times for bridging bonds
- Figures of the 4-way,
    - Overlay of all trajectory positions with an average structure highlighted
    - A simple view of it
    - Showing a bridging bond in the strucrture
- N-N lengths and 4-way interaction probability versus IS (and explicit) method.
- Contour plots of 4-way distances with and without bridging bonds
    Also the big one from the main sim where waters weren't saved