Validation Plots for Mucin Runs

128.192.9.183/eln/lachele/2014/12/30/validation-plots-for-mucin-runs

December 30, 2014

To ensure that the simulation produced structures that were consistent with the NMR-software-derived structures, they are compared to each other in a series of plots.

Contents [hide]

Consistent Dihedral Angles

Here, we compare the following dihedral angles:

1. Phi and Psi values for the peptide backbone associated with each threonine and in each of the three glycosylation variants.
2. Phi and Psi values for the glycosidic linkages to the threonines in the two glycosylated molecules.

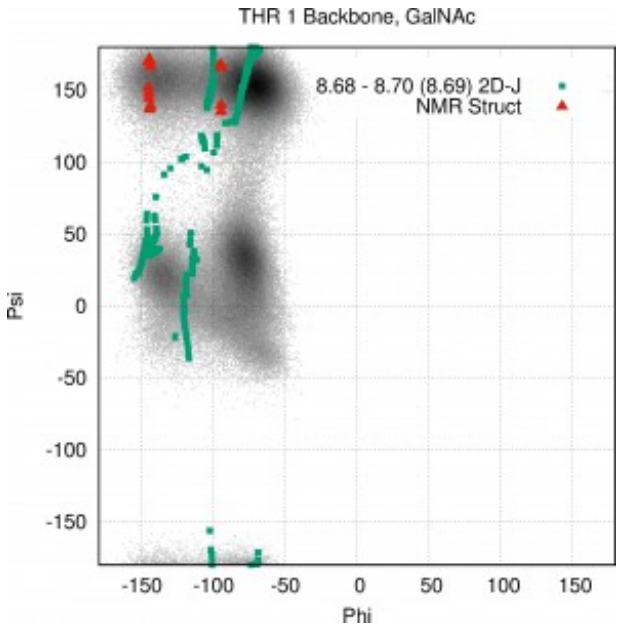
In all Phi-Psi plots:

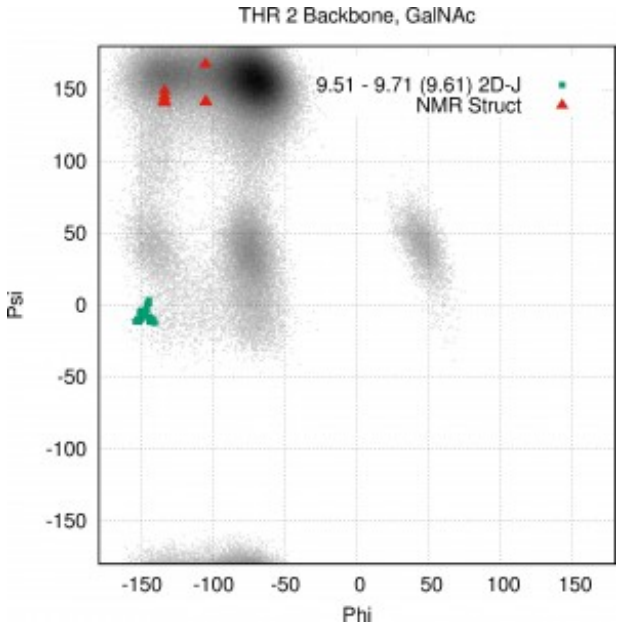
The intensities in the heat map were gamma-corrected (value of 3.2) so that they appear consistent with a 360×360-gridded 3D bar plot of the same data. The red points are values of Phi and Psi from the NMR-software-derived structures that were used as initial structures for all the simulations.

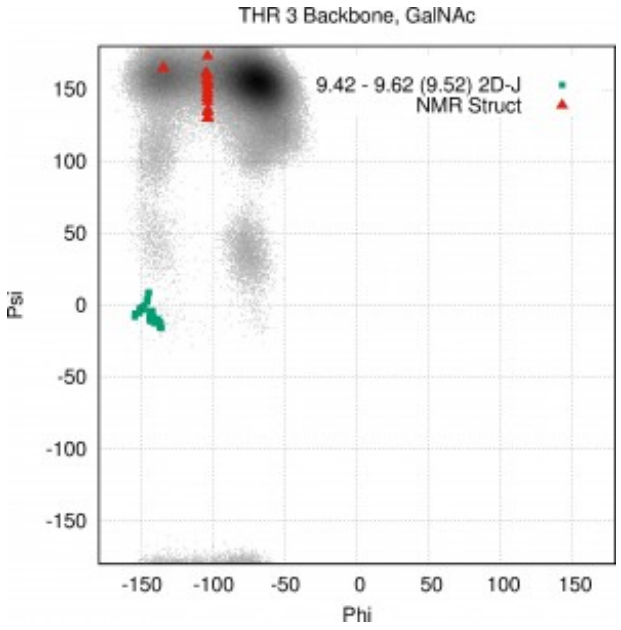
In the peptide backbone plots:

Since the 2D J-coupling calculation method is relatively new, it is appropriate to illustrate the relationship between the Phi/Psi pairs and their corresponding J-coupling values. However, the method does not use a function that can be plotted in the same way as a standard Karplus equation. To provide an illustration, green points are added to the plots that indicate the Phi/Psi values for simulation frames whose calculated 2D J-coupling values were close, as defined in each plot's key, to the observed NMR J-coupling values. In the plot key, the ranges are "Min-Max (Exp)", where Min and Max are the minimum and maximum 2D J-coupling values for which points are plotted, and Exp is the observed experimental value. The ranges were chosen for visual clarity in the graphs and are well within experimental error.

GalNAc




THR 2 Backbone, GalNAc
9.51 - 9.71 (9.61) 2D-J
NMR Struct
150
100
50
0
-50
-100
-150
Psi
-150
-100
-50
0
50
100
150
Phi


THR 3 Backbone, GalNAc
9.42 - 9.62 (9.52) 2D-J
NMR Struct
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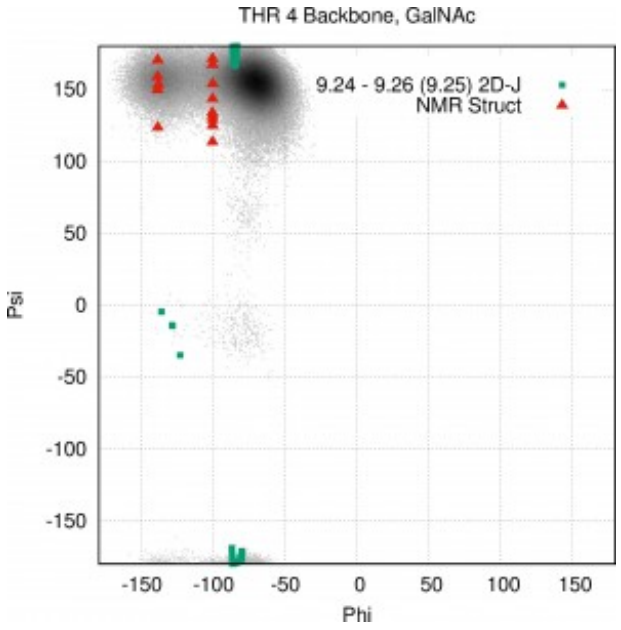
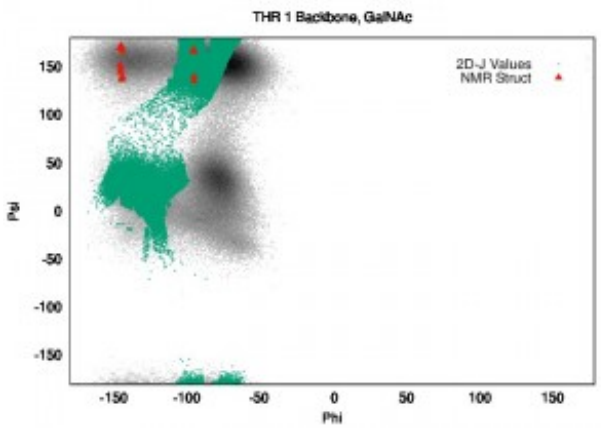
100
50
0
-50
-100
-150
Psi
-150
-100
-50
0
50
100
150
Phi



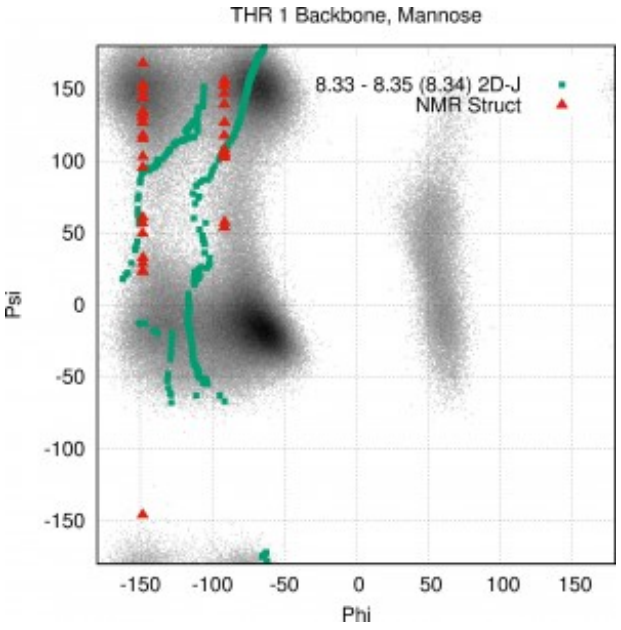
Notes

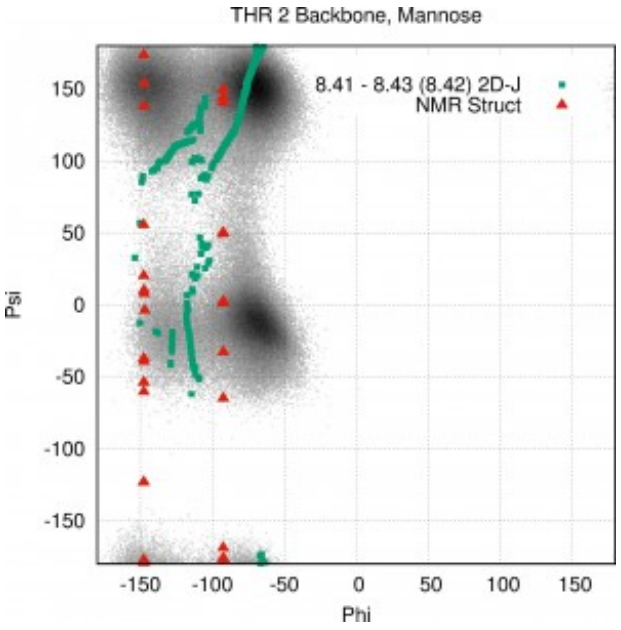
Note that there are very few "2D-J" points plotted for sites 2 and 3 in the GalNAc, even given the much larger range of values, and that the points are far from the main populations. The reason for this is that the 2D function relevant to this situation does not reach values that are high enough in the region near Psi=180 and between about -100 to -50 in Phi. The maximum value in that region is about 9.3. Although the method provides good qualitative and semi-quantitative agreement with experiment as-is, it would benefit from further optimization.

Visual clarity was significantly reduced if all points within experimental error were plotted. There were simply too many points. This graph shows the result for the first plot when all the points +/- 0.5 are added. Note that the point size used here is also much smaller than that in the graph shown above.



Mannose


THR 1 Backbone, Mannose
8.33 - 8.35 (8.34) 2D-J
NMR Struct
Psi
Phi
150
100
50
0
-50
-100
-150
-150
-100
-50
0
50
100
150


THR 2 Backbone, Mannose
8.41 - 8.43 (8.42) 2D-J
NMR Struct
Psi
Phi
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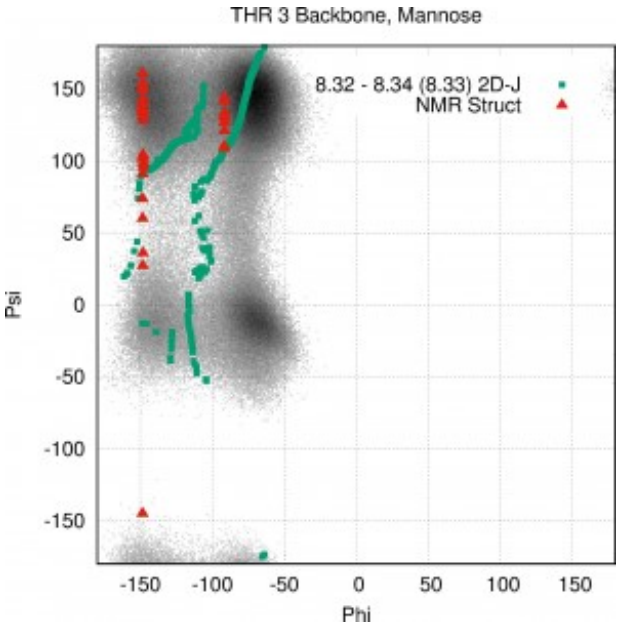
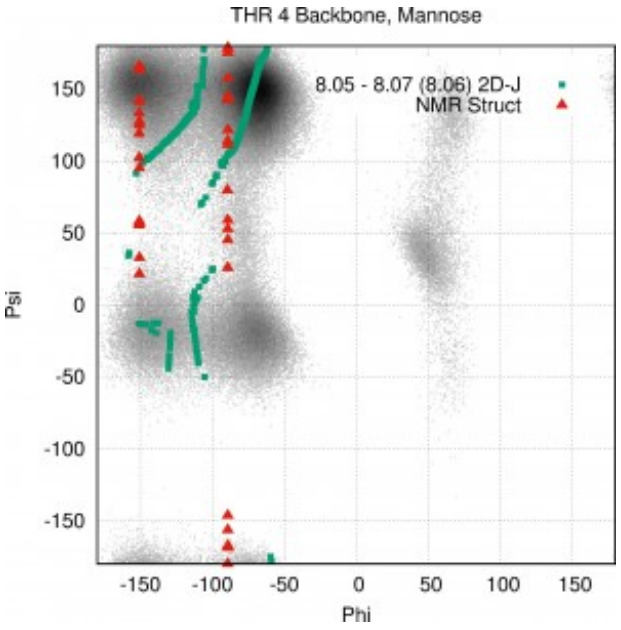
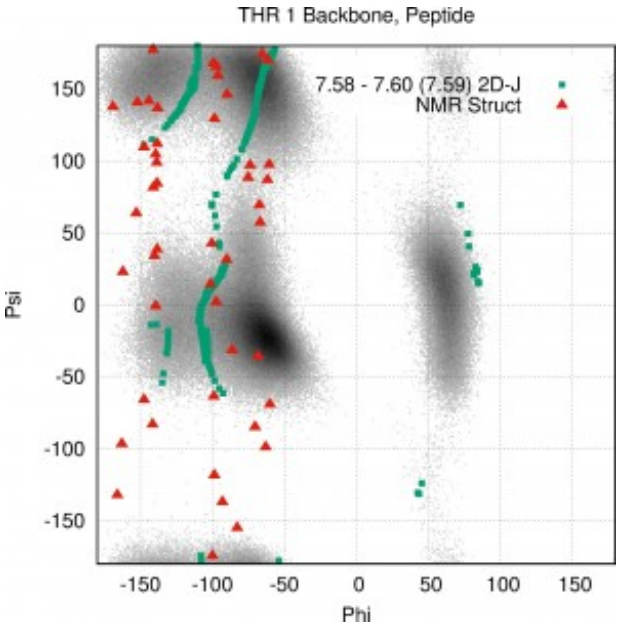
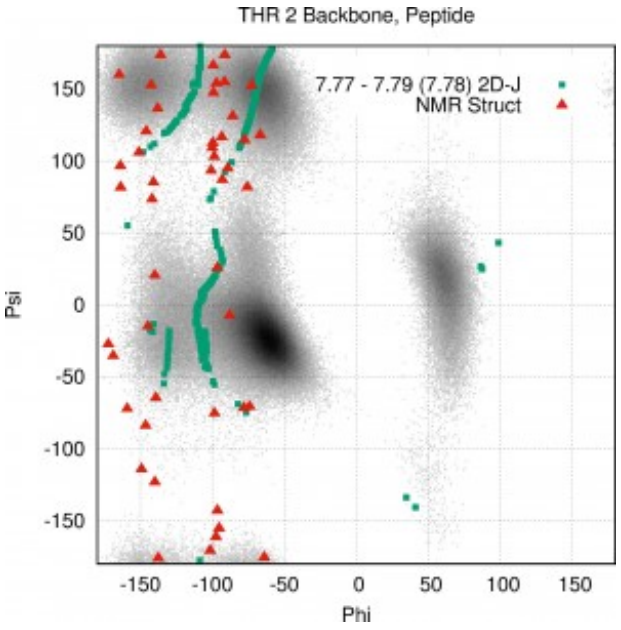
50
0
-50
-100
-150
-150
-100
-50
0
50
100
150





Unglycosylated Peptide


THR 1 Backbone, Peptide
7.58 - 7.60 (7.59) 2D-J
NMR Struct
Psi
Phi
150
100
50
0
-50
-100
-150
-150
-100
-50
0
50
100
150


THR 2 Backbone, Peptide
7.77 - 7.79 (7.78) 2D-J
NMR Struct
Psi
Phi
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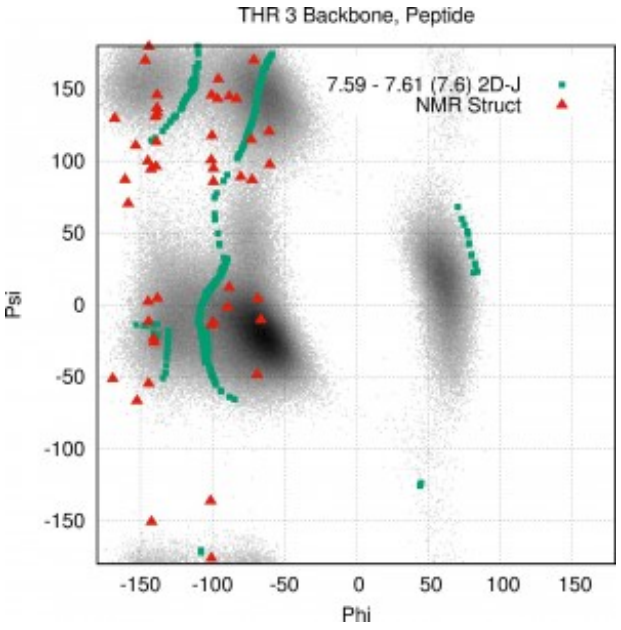
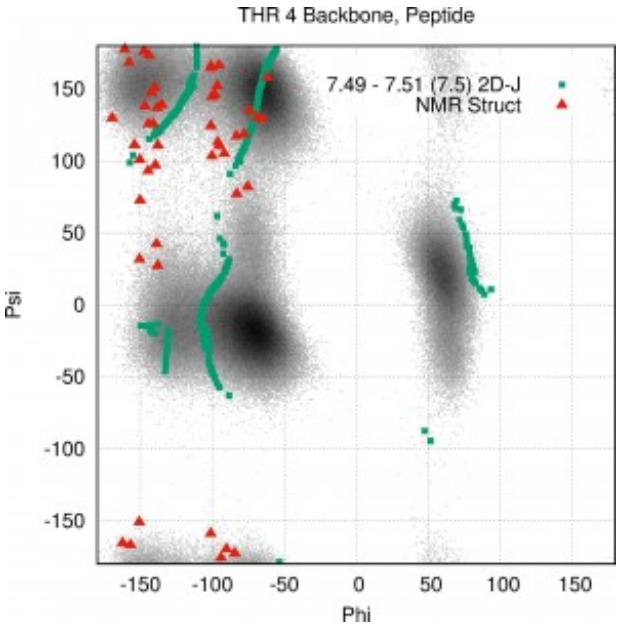
100
50
0
-50
-100
-150
-150
-100
-50
0
50
100
150

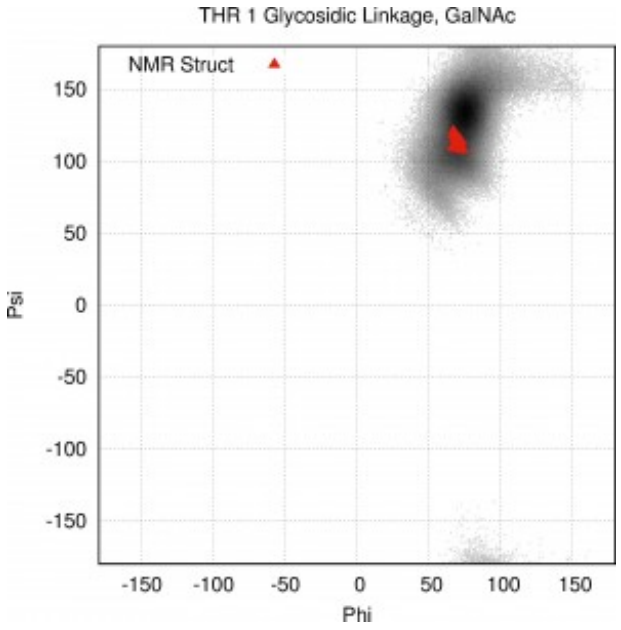


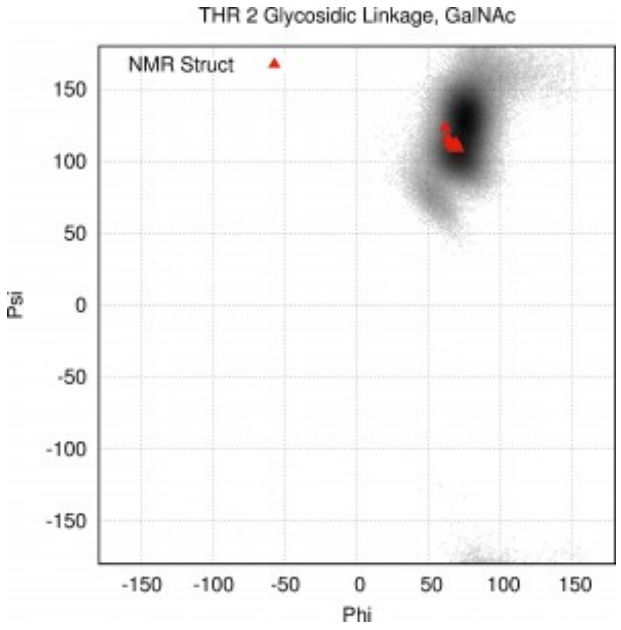


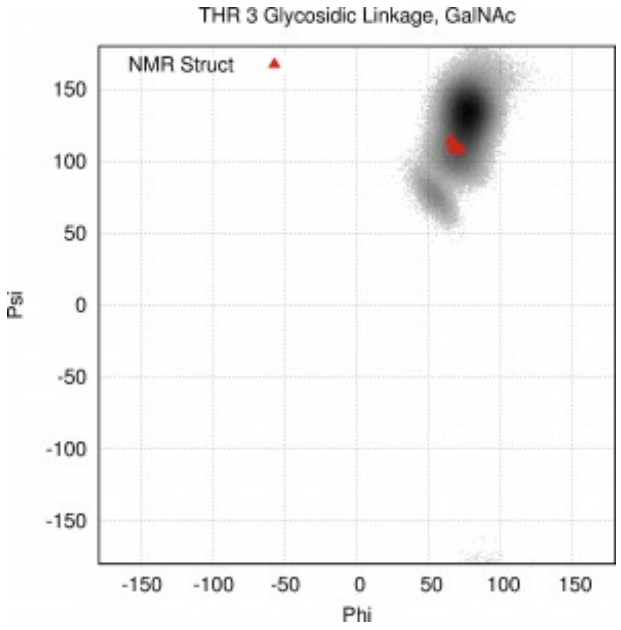
In the glycosidic linkage plots:

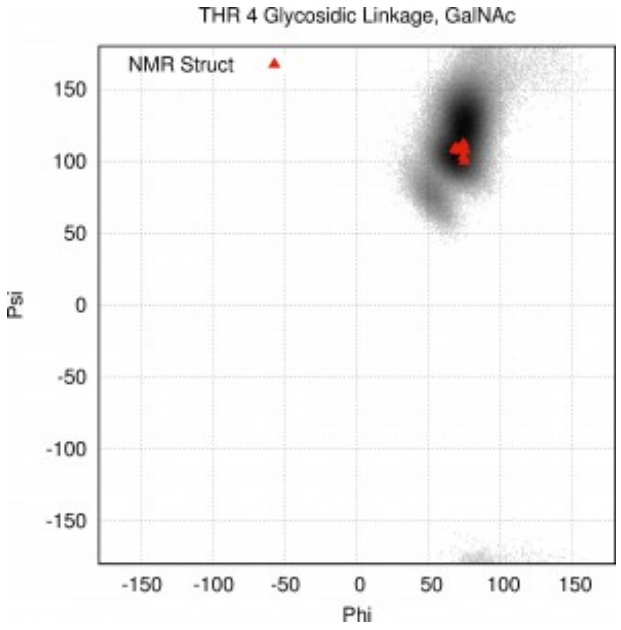
These plots are essentially the same as the peptide backbone plots except that there is no information regarding 2D J-coupling because the method applies only to peptide linkages.

GalNAc


THR 1 Glycosidic Linkage, GalNAc
NMR Struct
150
100
50
0
-50
-100
-150
Psi
-150
-100
-50
0
50
100
150
Phi


THR 2 Glycosidic Linkage, GalNAc
NMR Struct
150
100
50
0
-50
-100
-150
Psi
-150
-100
-50
0
50
100
150
Phi


THR 3 Glycosidic Linkage, GalNAc
NMR Struct
150
100
50
0
-50
-100
-150
Psi
-150 -100 -50 0 50 100 150
Phi


THR 4 Glycosidic Linkage, GalNAc
NMR Struct
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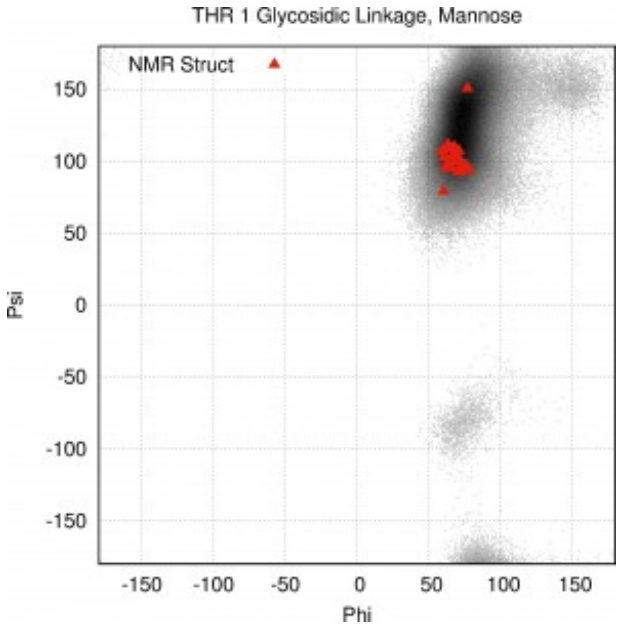
100
50
0
-50
-100
-150
Psi
-150 -100 -50 0 50 100 150
Phi

Mannose


THR 1 Glycosidic Linkage, Mannose
NMR Struct
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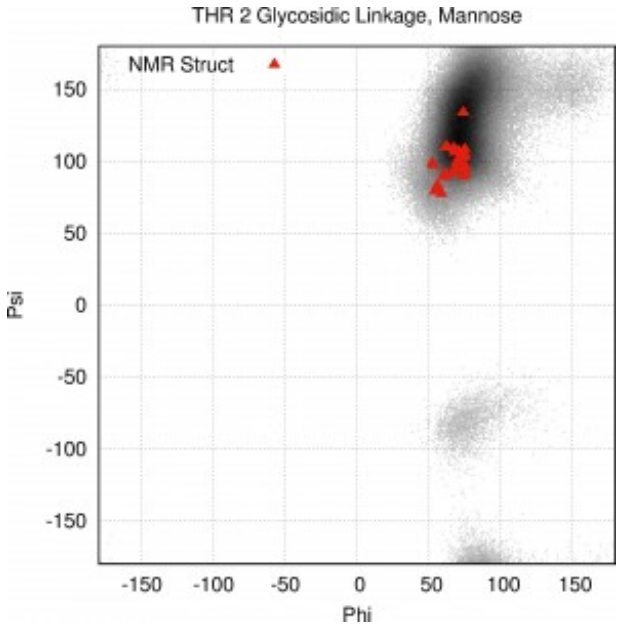
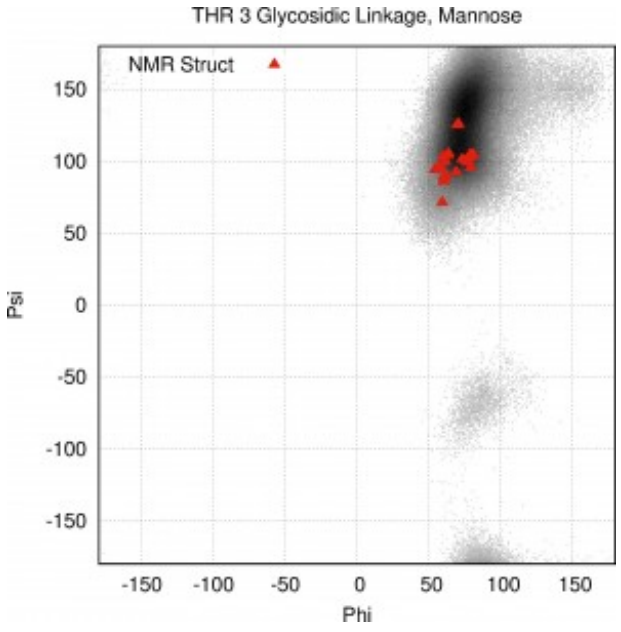
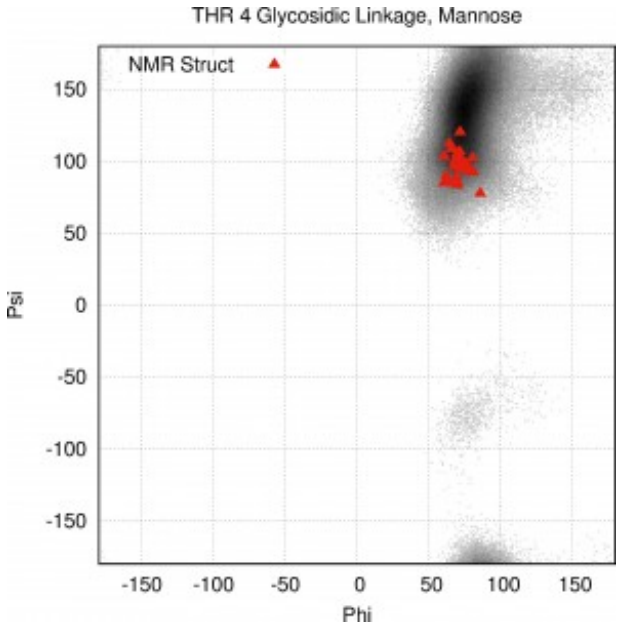
100
50
0
-50
-100
-150
Psi
-150
-100
-50
0
50
100
150
Phi


THR 2 Glycosidic Linkage, Mannose
NMR Struct
150
100
50
0
-50
-100
-150
Psi
-150
-100
-50
0
50
100
150
Phi





Overall notes on Phi-Psi plots

Although there is little change in the allowed glycosidic angles for the GalNAc across the four positions, there is a marked change in the allowed backbone dihedral angles. There is no evidence of such restriction in the backbone of the unglycosylated peptide. There is only an indication of a restriction in the Mannose. This latter indicates that the effect in the GalNAc must be related either to the increase in size due to the NAc moiety and/or to interactions between it and the backbone.

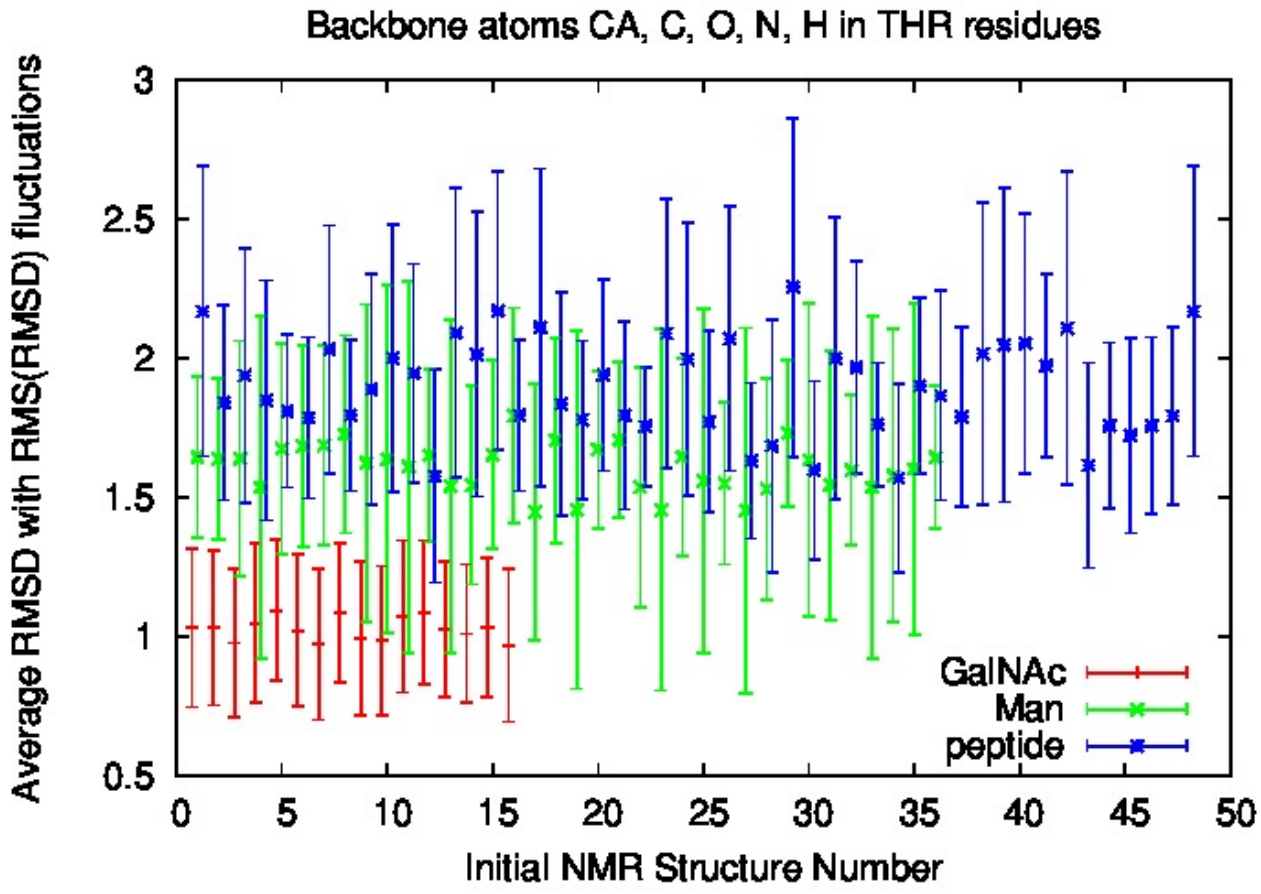
Consistent RMSDs

The following plots show RMSD values across all runs when referenced to each starting structure. The point is to show that the simulations and the initial NMR structures are consistent with each other. That is, the RMSDs are all pretty much the same no matter which structure is considered as a reference. This suggests, but does not prove, that not only are the initial structures reasonable, but also that the simulations, collectively, were sufficient to capture a significant portion of the conformation space.

Note that there are only 16 initial structures for the GalNAc runs, but that the RMSDs represent all 32 simulations (each structure was used twice). *Note: Actually, there were only 15 structures. One was used twice, so four times… There was a similar issue with the Mannose. I'll fix the figures for this eventually*. The error bars are RMS fluctuations in the RMSDs.

Considering just the backbone atoms

This plot shows the RMSDs for just the backbone atoms. A statistical test (p or similar) could give a more quantitative statement about the distributions for Man and Pep being different. But, it seems quite obvious that they are different. There is little doubt that the GalNAc variants are different from the others.



Considering the sugar atoms

Here is a similar plot, but with the RMSD being taken on the glycan heavy atoms.