Supplementary Material

Preferred Conformations of N-glycan Core Pentasaccharide in Solution and in Glycoproteins

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Running Title: Pentasaccharide conformations in solution and glycoprotein

Key words: glycan, molecular dynamics, simulation, crystal structure, information theory

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Table S1. Calculated inter-proton inter-residue distances from the simulation. The distances are calculated by using the formula, $\left\langle r^{-6} \right\rangle = \left\langle \frac{1}{N} \sum_{t} r(t)^{-6} \right\rangle$. The NMR experimental inter-proton distances are taken from literatures.

Linkage	Proton Pair	Distance (Å)	NMR Experiments
β(1→4)			
2-1	H_1/H_2'	4.7	
	H_1/H_3'	3.5	
	H_1/H_4'	2.3	1.8-2.7 [1]
	H_1/H_5'	4.0	
	H_1/H_6'	3.1	
	H_1/H_6'	3.0	
3-2	H_1/H_2'	4.7	
	H_1/H_3'	3.7	
	H_1/H_4'	2.3	1.8-2.7 [1]
	H_1/H_5'	4.1	
	H_1/H_6'	3.2	3.3-5.0 [1]
	H_1/H_6'	3.0	3.3-5.0 [1]
α(1→3)			
A-3	H_1/H_1'	4.6	3.3 [2]
	H_1/H_2'	3.5	3.1 [2]; 3.08 [3]
	H_1/H_3'	2.2	2.1-2.3 [4]; 2.36 [5]
	H_1/H_4'	3.9	3.0 [2]; 2.97 [5]
	H_1/H_5'	4.4	
α(1→6)			
A'-3	H_1/H_4'	4.2	
	H_1/H_5'	3.6	3.3 [2]; 3.3-5.0 [1]
	H_1/H_6'	2.3	2.4 [2]; 2.54 [5]; 1.8-2.7 [1]
	H_1/H_6'	2.9	2.8 [2]

Table S2. Conformational states of the N-glycan core pentasaccharide in glycoprotein from MD simulations. The initial conformational states are highlighted as bold.

	PDB:1E04		PDB:1CXP		PDB:1L6X		PDB:1RRB	
#1	**AAAAG**	56.3%	**AAAAG**	53.9%	**AAAAG**	67.6%	**AAAAG**	67.2%
#2	AAAAg	25.7%	AAAAg	31.5%	AAAAg	12.9%	AAAAg	14.3%
#3	AAABG	6.3%	AAABg	3.6%	AAABG	11.9%	BAAAG	5.8%
#4	BAAAG	3.7%	AAABG	3.5%	AAABg	6.8%	AAABG	4.7%
#5	ABAAG	3.0%	ABAAG	3.4%	BAAAG	0.5%	BAAAg	4.1%
#6	AAABg	2.1%	ABAAg	1.1%	AABAG	0.1%	ABAAg	1.7%
#7	ABAAg	0.9%	ABABG	0.8%	ABAAg	0.0%	AAABg	1.0%
#8	BAAAg	0.8%	BAAAG	0.6%	AABBG	0.0%	BAABg	0.5%
#9	BAABG	0.4%	AAAAt	0.5%	ABAAG	0.0%	BAABG	0.4%
#10	ABABG	0.2%	BAAAg	0.3%	BAABG	0.0%	AAAAt	0.1%
Sum		99.1%		99.3%		92.8%		94%

FIGURE LEGENDS

Figure S1. Efficient random walk across temperature space in the T-REXMD simulation. (A) Time series of temperature exchanges of two arbitrarily chosen replicas: replica #1 (red) and replica #48 (green). (B) Time series of different replicas visiting at temperature 300 K.

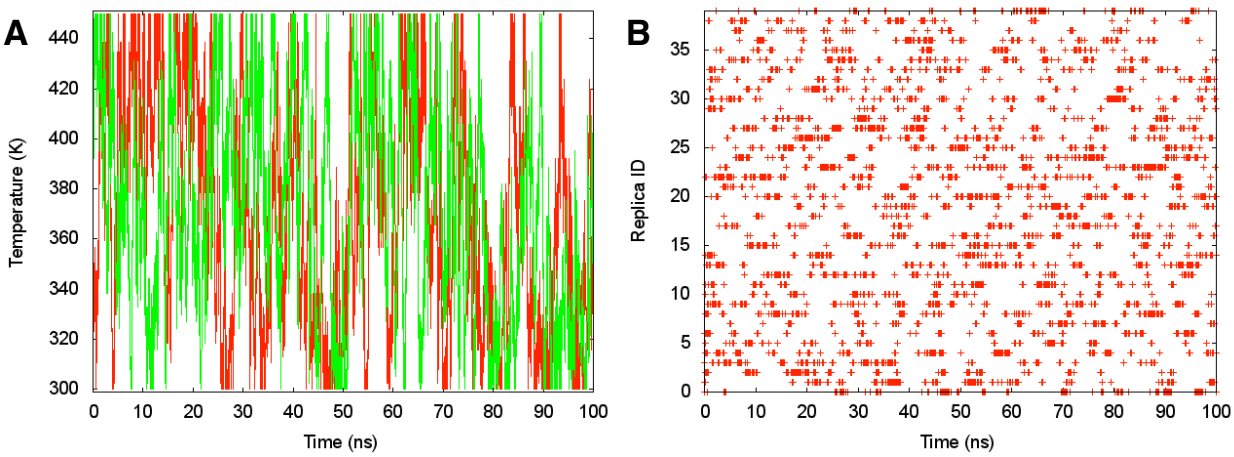
Figure S2. Torsion angle distribution for the first three glycosidic linkages. (A) GlcNAc $\beta(1\rightarrow4)$ GlcNAc, (B) Man $\beta(1\rightarrow4)$ GlcNAc, and (C) Man $\alpha(1\rightarrow3)$ Man.

Figure S3. Torsion angle distributions for the Man $\alpha(1\rightarrow6)$ Man glycosidic linkage.

Figure S4. Cumulative average of conformational state population for the top five conformational states in Table 2. Each colored line represents the population of a particular conformational state.

Figure S5. Cumulative average populations of the top five conformational states. The aggregated MD simulation trajectory was used. Each colored line represents the population of a particular conformational state.

Figure S6. Conformational variability of the pentasaccharide in solution. (A) The pair-wise RMSD distribution from the standard MD simulations. (B) Conformational variability within each of the major conformational states. Each colored line represents a pair-wise RMSD distribution from 1,000 conformers belonging to the same conformational state.



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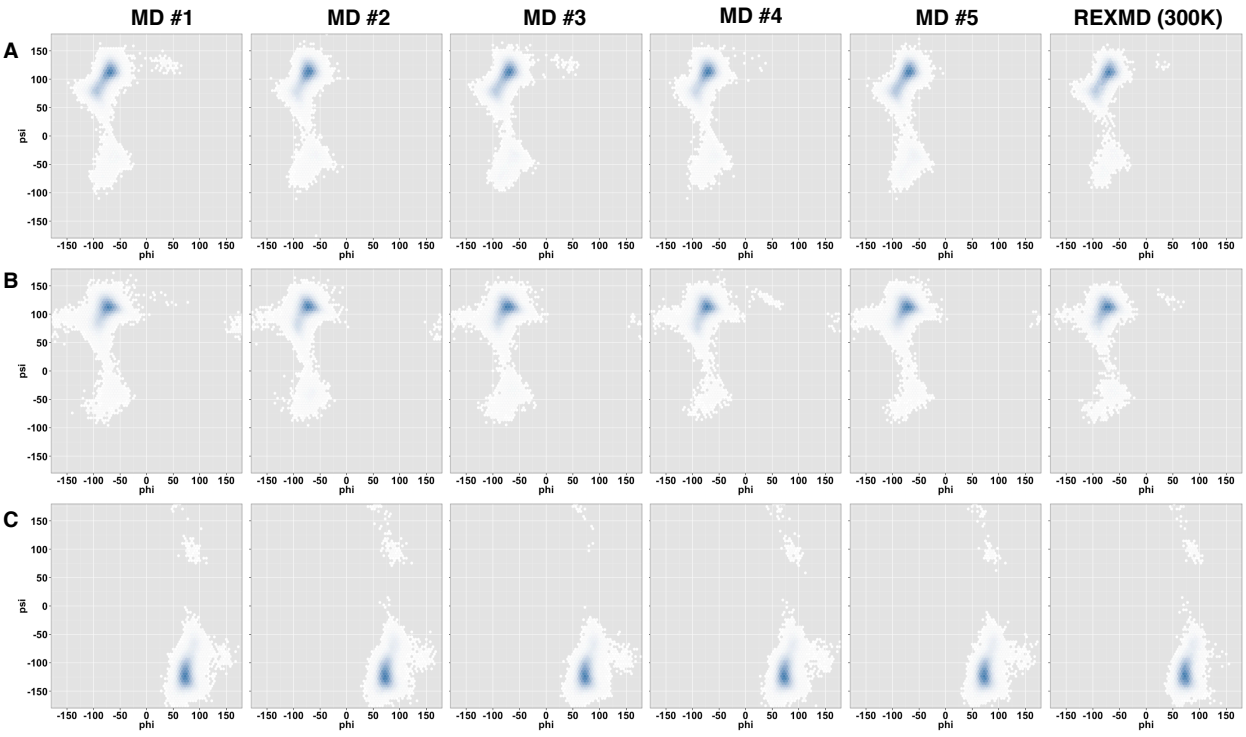

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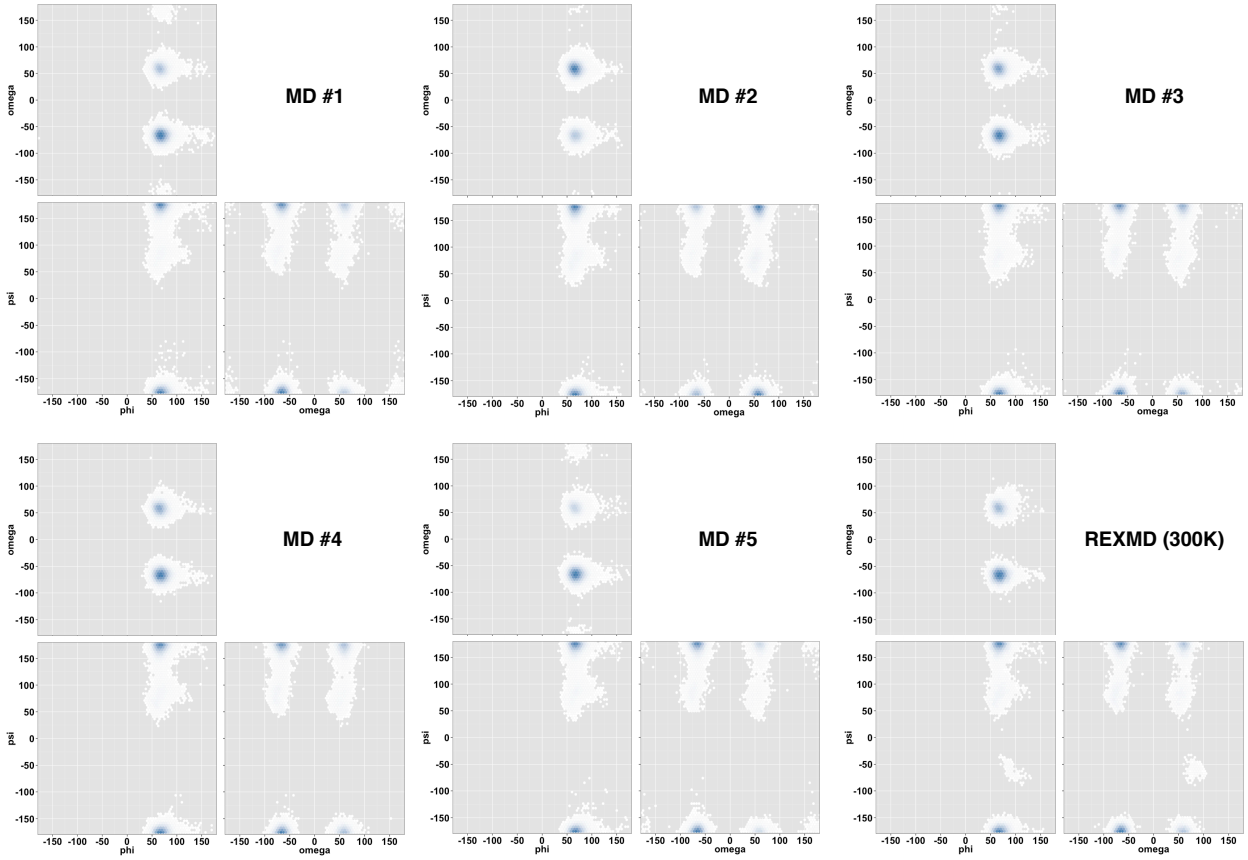

Figure S3. Torsion angle distributions for the Man α(1→6) Man glycosidic linkage.

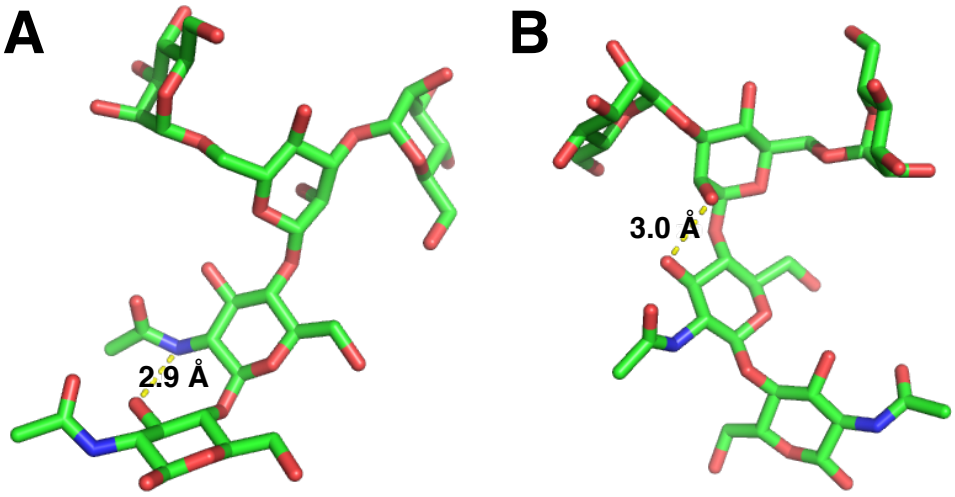

Figure S4. Two examples of less favorable exo-anomeric populations; (A) CAAAG conformational state and (B) ACAAG conformational state. The hydrogen bond formed that stabilizes the conformation is highlighted as yellow lines.

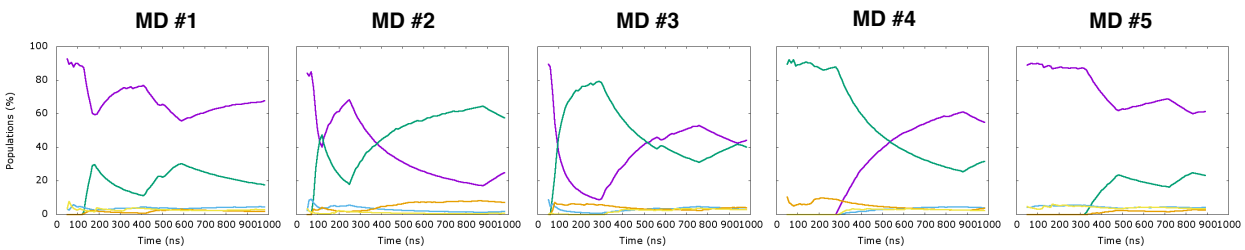

Figure S5. Cumulative average populations of the top five conformational states in Table 2. Each colored line represents the population of a particular conformational state.

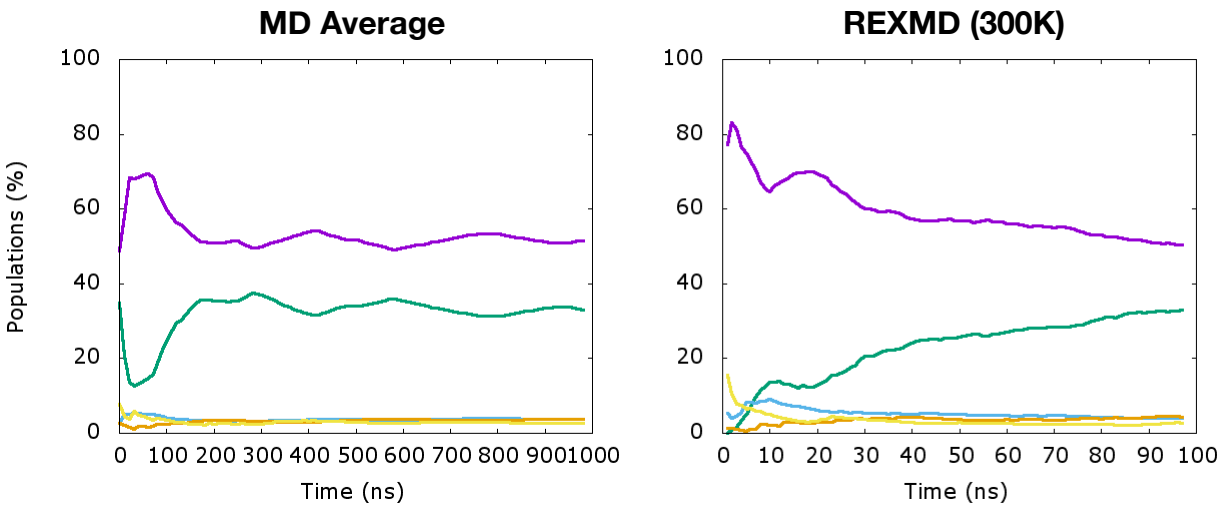

Figure S6. Cumulative average populations of the top five conformational states. The aggregated MD simulation trajectory was used. Each colored line represents the population of a particular conformational state.

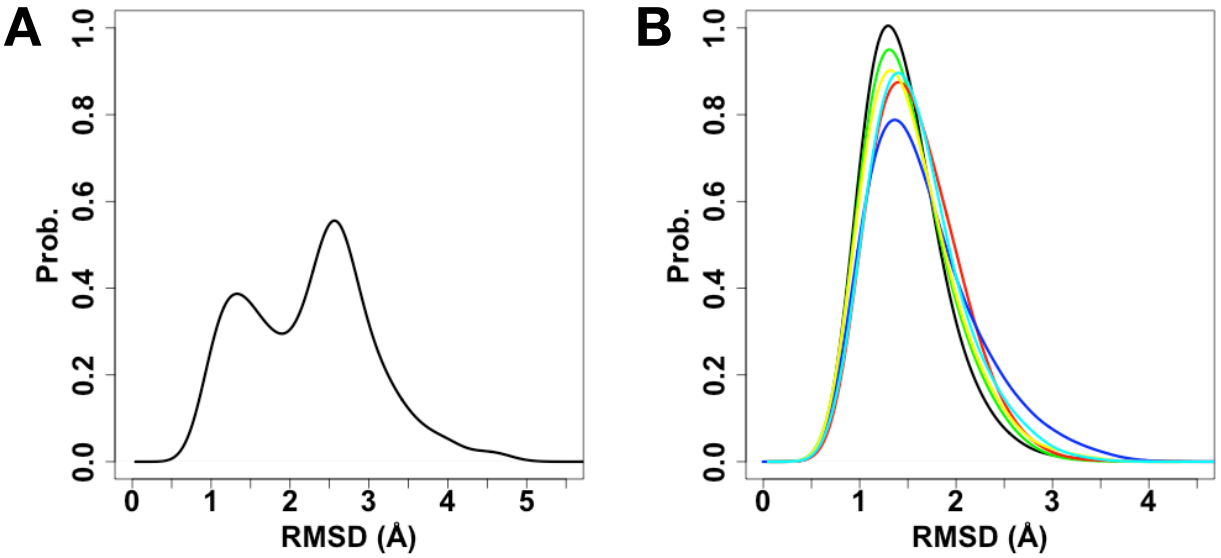

Figure S7. Conformational variability of the pentasaccharide in solution. (A) The pair-wise RMSD distribution from the standard MD simulations. (B) Conformational variability within each of the major conformational states. Each colored line represents a pair-wise RMSD distribution from 1,000 conformers belonging to the same conformational state.

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