Evidence for helical structure in a tetramer of α2-8 sialic acid: unveiling a structural antigen

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Materials and Methods

^{15}N, ^{13}C α2-8 sialic acid oligomers production and purification

Fully labeled α2-8 PSA was obtained as previously described.[1] 100 mg batches of labeled PSA were dissolved in 200 μL 0.1 M acetic acid and placed in an eppendorf tube with a hole in the lid. The tube containing the sample was partially submerged in a 50 mL plastic conical tube containing 4 mL of water, which were deposited in a beaker containing 200 mL of water. The sample was microwaved at 1.48 kW for 1 ½ minutes intervals for a total of 7 ½ minutes of microwave irradiation. The 200 mL of water were replaced for fresh water after each irradiation interval. The samples were then taken to pH = 7.0 with 1 M NaOH and left to equilibrate overnight.

Oligomer purification was carried out via ion exchange column (MonoQ HR 16/10, Pharmacia), using a salt gradient as previously reported.[2-5] Oligomers were desalted with a G10 column and lyophilized.

The degree of polymerization for α2-8-linked sialic acids on each fraction was determined by means of NMR[5] and MALDI-TOF mass spectrometry.[6] Fully labeled α2-8 sialic acid tetramer (^{15}N,^{13}C α2-8 $(SiA)_4$) was selected for this study.

NMR experiments

All NMR data was processed in NMRpipe.[7] Peaks intensities and volumes were extracted using fitting protocols provided with NMRPipe software. CCPNMR Analysis software package[8] was utilized for data display and assignments.

NMR experiments were performed on a Bruker Avance III 500 spectrometer with a conventional triple resonance probe, unless otherwise specified.

Natural abundance α2-8 $(SiA)_4$ was purchased from EY Laboratories Inc (San Mateo, CA). 20 mg of α2-8 sialic acid tetramer were dissolved in 200 μL (78.7 mM) of 20 mM phosphate buffer pH 6.5, containing 10 % ^2H_2O, 0.05 % NaN_3 and 0.1 % DSS for internal reference and placed in a Shigemi tube (Allison, PA).

All acquisition parameters for double and triple resonance experiments are summarized in Table S3 and S4, respectively.

Assignments

^1H and ^{13}C resonances were assigned via HSQC and HMBC experiments. The data were collected at 283 K, using the ***hsqcetgpsi*** and ***hmbcgplpndqf*** pulse sequences from Bruker library, respectively.

For HSQC and HMBC experiments on unlabeled $(SiA)_4$, 32 and 64 scans were accumulated for each t_1 point, respectively. The HMBC experiment was optimized for the observation of coupling constants of 6 to 8 Hz. The HMBC experiments enabled the assignment not only of the protons and carbons within a sialic acid residue but the position of residues in the tetramer through the H8(i)-C2(i+1) connectivity.

"W" coupling measurement

Intraresidue $^4J_{^1H_7-^{13}C_2}$ couplings were measured via HSQMBC[9] experiment. Acquisition parameters for the HSQMBC experiment are summarized in Table S3. The experiment was performed at 263K.

SOLEXSY

Desalted ^{15}N,^{13}C α2-8 sialic acid (SiA) oligomers were dissolved in H_2O:D_2O 1:1 and adjusted to pH 6.5 (uncorrected for isotope effect) at 298K. Data were collected at 263 K.

Parameters were optimized for H/D exchange measurement of labeled SiA oligomers.

Carbonyl and alpha carbon (methyl) offsets were set to 174 ppm and 22 ppm, respectively. The delays for the various INEPT transfer steps were set as follows: τ_a = 1.9 ms (Hα-Cα, methyl group in sialic acid), τ_c = 6 ms (Cα-C'), τ_d =17 ms (C'-N) and τ_f = 2.3ms (N-H); were the values in parentheses representing coherence transfer from the first nucleus to the second.

The data for SiA and $(SiA)_4$ were acquired using the parameters in Table S4.

As there was no spectral overlap between HN and DN resonances; no filtering scheme[10] was applied, reducing by two-fold the data collection time. Data fitting for the extraction of exchange parameters was carried out with MATLAB2007bR as previously reported.[10]

Long range CBCANH experiment

Desalted ^{15}N,^{13}C α2-8 SiA oligomers that were used for SOLEXY experiments were lyophilized and dissolved in water with 10 % D_2O and the pH was adjusted to 6.5 (uncorrected for isotope effect) at 298 K. Data were collected at 263 K.

Bruker library pulse sequence CBCANHgpwg[11] was modified for the detection of coupled nuclei through H-bond by increasing the ^{13}C-^{15}N INEPT transfer time from 25 ms (~ 20 Hz J_{N-C}) to 133 ms (~ 3.7 Hz J_{N-C}), similar to what has been done for the detection of $^{3h}J_{NC}$ using an HNCO pulse sequence.[12]

Long-range HNC2 (HNCOgp3dHb, non TROSY based)[12]

The Bruker pulse sequence was utilized without modifications. However, the carrier in ^{13}C was set to excite C2 (100 ppm), and the pulses were tuned such not to excite other carbon frequencies (15 ppm bandwidth). The experiment was run as a 2D, without ^{15}N evolution.

Acquisition parameters are summarized in Table S4.

CNH-3D NOESY[13]

Acquisition parameters are summarized in Table S4.

NOESY-HSQC[14]

Acquisition parameters are summarized in Table S4.

α2-8 SiA tetramer structure modeling

The structure was generated and modeled with Hyperchem software package. "W" couplings and H-bond restraints were added manually by adjusting the angles according to the collected NMR data.

Table S1: Intra-residue NOEs

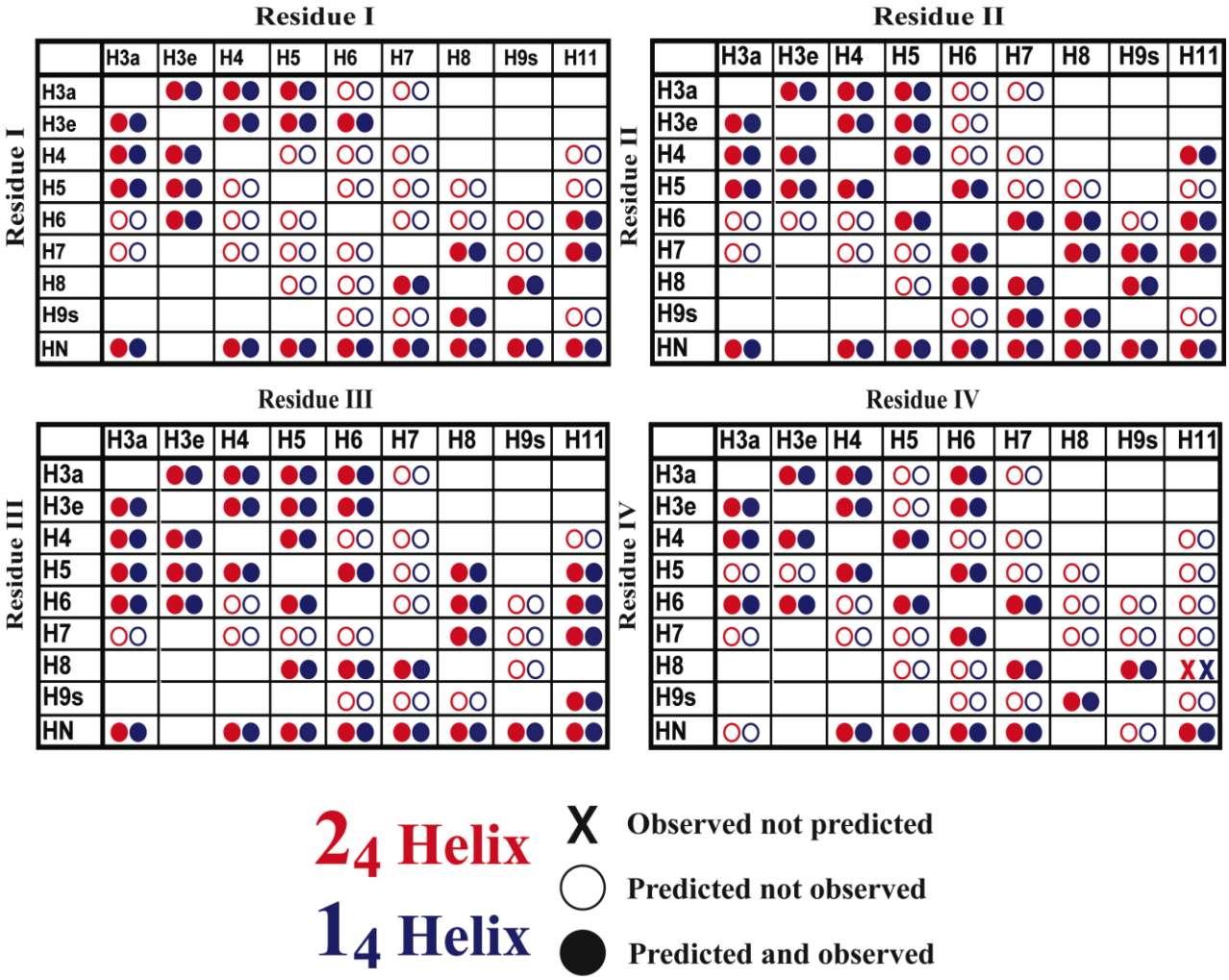

Table S2: Inter-residue NOEs

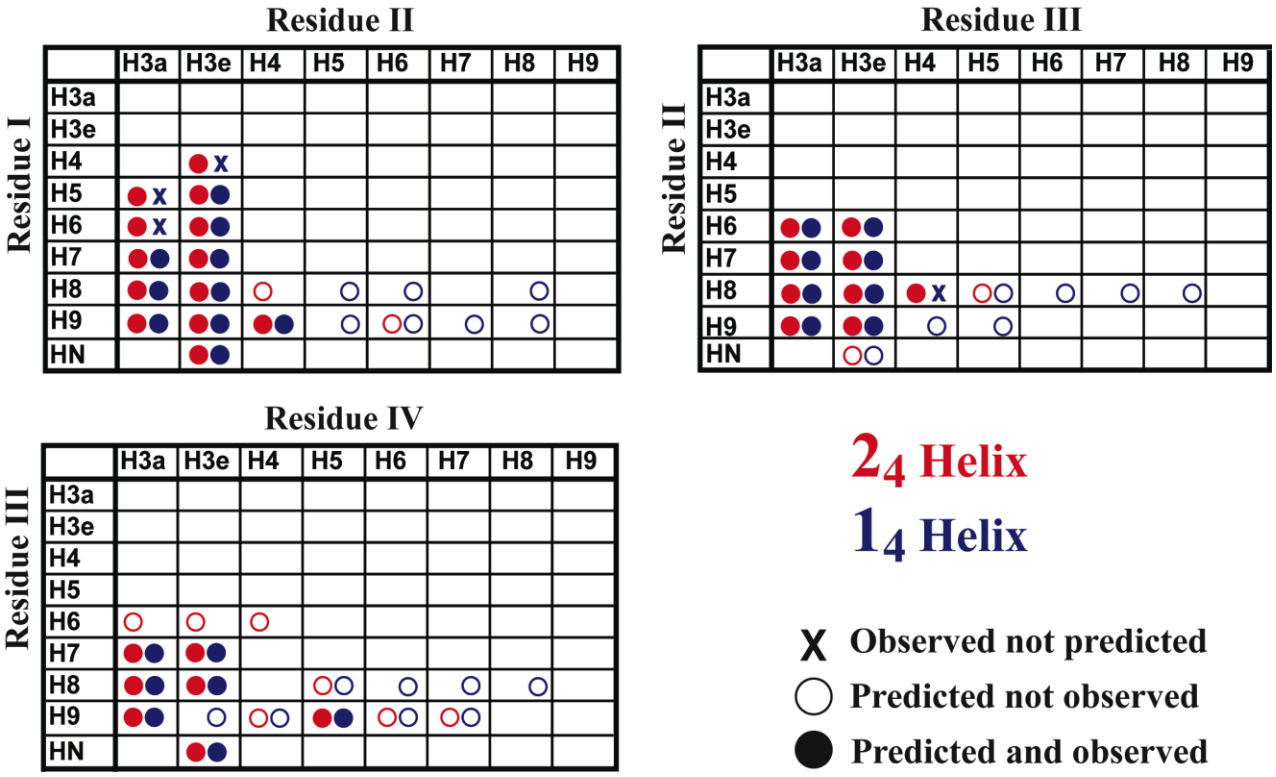

Table S3									
Double Resonance Experiments									
	HSQC			HMBC			HSQMBC		
	^{1}H	^{13}C	^{15}N	^{1}H	^{13}C	^{15}N	^{1}H	^{13}C	^{15}N
SW (ppm)	3	30	--	3	30	--	3	30	--
Complex points	4,096	128	--	4,096	128	--	1,536	256	--
Carrier (ppm)	2.75	65	--	2.75	65	--	2.75	65	--
NS	32				32		128		

Table S4														
Triple Resonance Experiments														
	CNH-NOESY			NOESY-HSQC			CBCANH			HNCA			SOLEXSY	
	^{1}H	^{13}C	^{15}N	^{1}H	^{15}N	^{1}H	^{13}C	^{15}N	^{1}H	^{13}C	^{15}N	^{1}H	^{13}C	^{15}N
SW (ppm)	10	35	6	10	6	10	30	6	10	6.5	6	10	30	6
Complex points	2,048	128	16	2,048	10	2,048	52	1	2,048	22	1	2,660	1	60
Carrier (ppm)	4.75	65	123.8	4.75	123.8	4.73	65	123.8	4.73	99.2	123.8	4.702	22	123.8
NS	128			256		4,096			6,144			72		

Figure S1

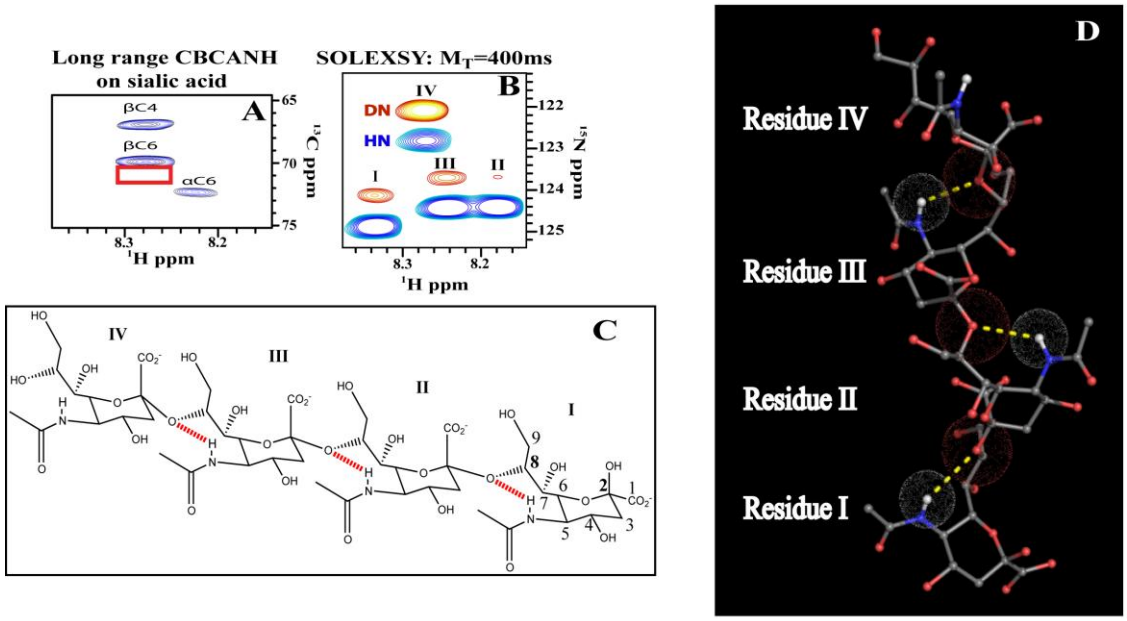

Figure S1. A) Long range CBCANH experiment on SiA. The red square indicates the absence of a C8 correlation. B) SOLEXSY spectra collected at 400 ms mixing time (M_T). Residue IV ^{1}HN (blue) and ^{2}HN (red) peaks show similar intensities (fully exchanged), whereas for residues I-III, ^{2}HN peaks are weak (partially exchanged). C) $(SiA)_4$ chemical structure depicting the H-bond pattern as evidenced by NMR. H-bonds are indicated in red between HN proton (donor) and the glycosidic oxygen atom (acceptor) at $C8_i$ and $C_{2i+1.}$ Atom numbering and residue numbering is according to IUPAC and is indicated with Arabic and Roman numerals, respectively. D) $(SiA)_4$ structure in stick representation. Atoms are color coded as follows: carbon, hydrogen, nitrogen and oxygen atoms are grey, white, blue and red respectively. The donor and acceptor hydrogen bond pair is highlighted and linked by a yellow dashed line.

Figure S2

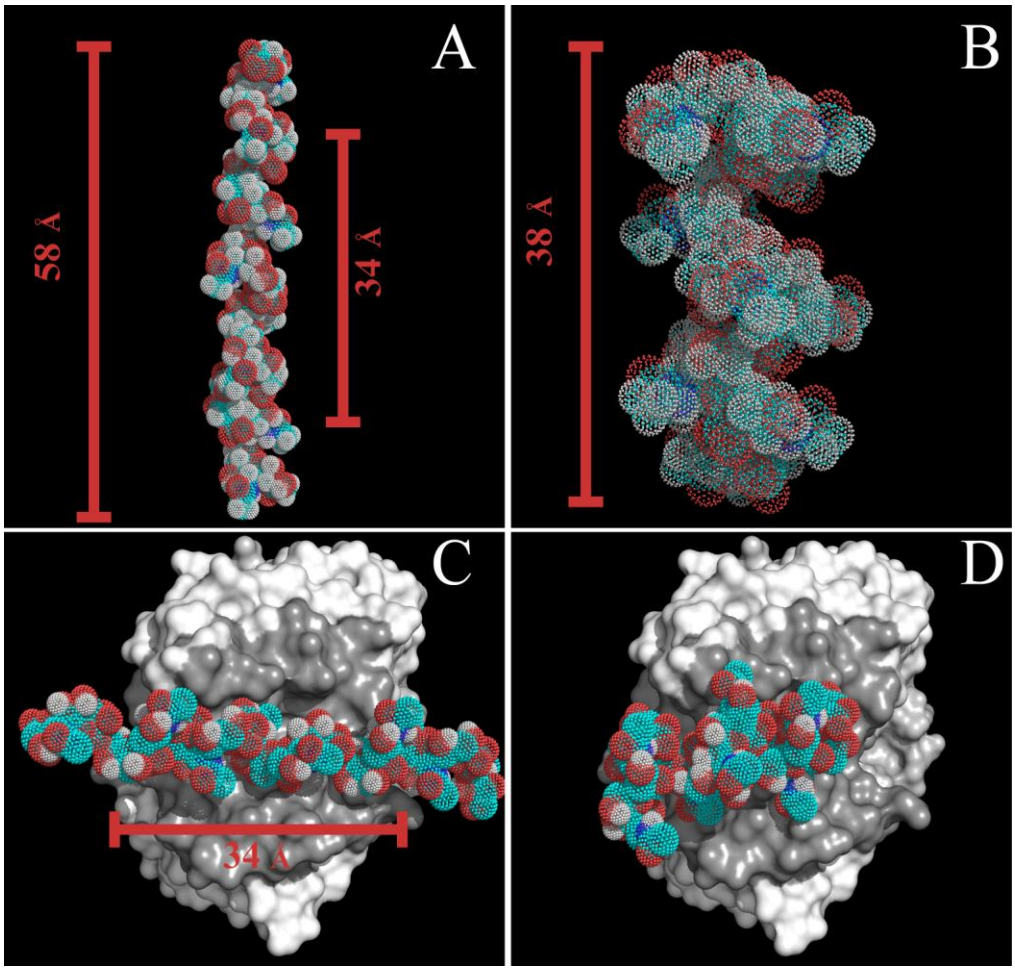

Figure S2. α2-8 $(SiA)_{10}$ helical models built propagating the 2_4 (Panel A) or 1_4 (Panel B) conformations. Panel C and D depict the decamer models (built propagating either 2_4 or 1_4 models, respectively) docked on the mAb735 crystallographic structure surface (PDB ID: 1PLG). The mAb735 FAB binding site is colored dark gray. $(SiA)_{10}$ carbon, hydrogen, oxygen and nitrogen atoms are colored cyan, white, red and blue respectively. Docking experiments were performed with Autodock4.0 software. The molecular and binding site lengths are indicated in red.

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