Does microsecond sugar ring flexing encode 3D-shape and bioactivity in the heparanome?

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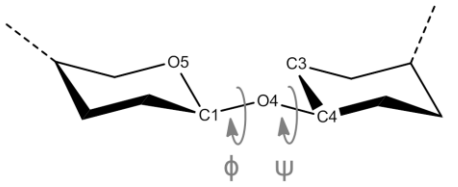
Supporting Information

Figure S1. Carbohydrate structural definitions: glycosidic torsions and pyranose ring pucker

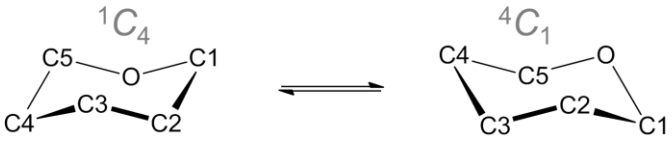
A: Glycosidic linkage definitions

Atoms defining torsions are labelled



ϕ: O5-C1-O4-C4 ; ψ: C1-O4-C4-C3

B: Pyranose ring pucker definitions: chairs



C: Pucker definitions (IdoA2S): chairs and intermediate skew-boat ^2S_O

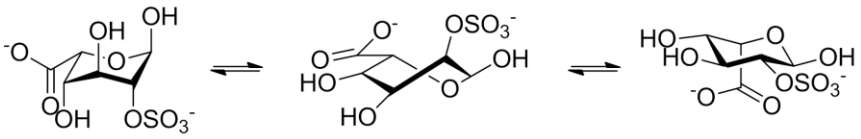


Figure S2. Convergence of puckering in the 5 μs all-atom pentasaccharide simulation

See Figures 1C and 3 for 2D- and 3D-structures of the AT-III binding pentasaccharide

Residues (Res.) are numbered from the reducing end

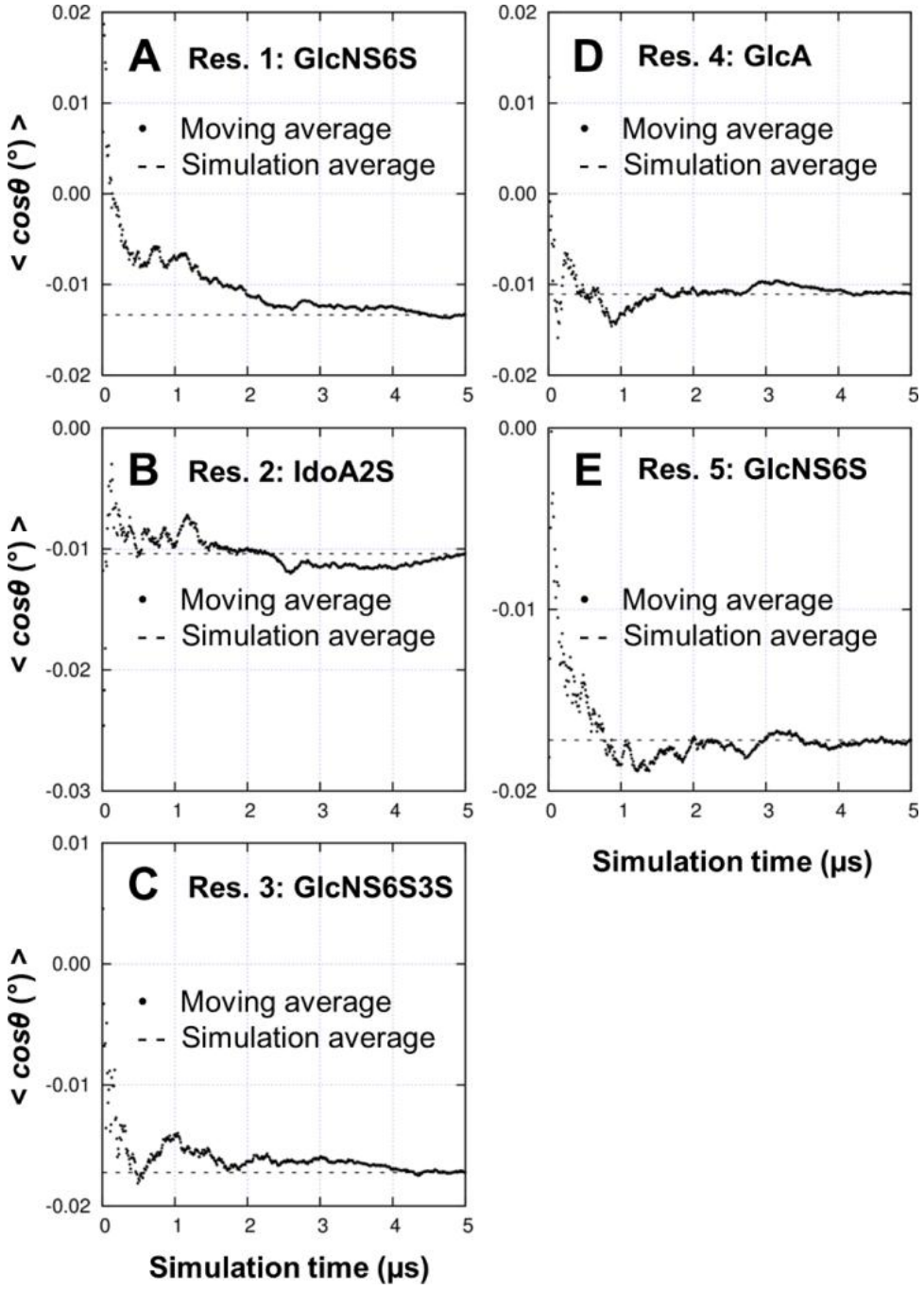


Figure S3. Time series of glycosidic torsion flexing in the 5 μs all-atom pentasaccharide simulation

See Figures 1C and 3 for 2D- and 3D-structures of the AT-III binding pentasaccharide

Linkages are numbered from the reducing end (see Figure S1 for angle definitions)

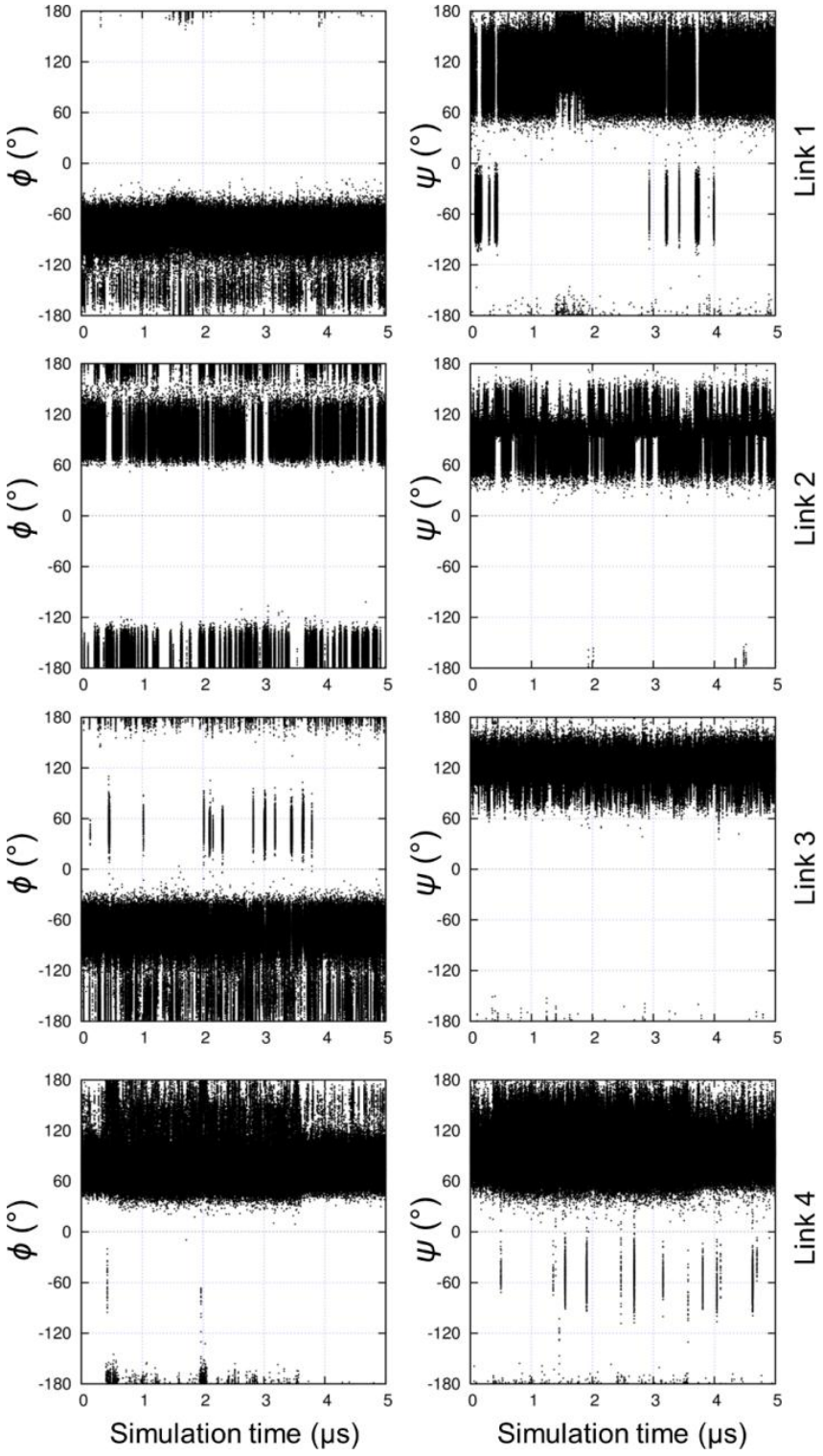


Table S4: Calculated NMR vicinal couplings: penta-, hexa-, octa- and decasaccharides

A. Pentasaccharide

		AT-III binding pentasaccharide (see Figure 1C)				
		GlcNS6S	**GlcA**	**GlcNS6S3S**	**IdoA2S**	**GlcNS6S**
CAL_H	$J_{1,2}$	2.8	10.0	2.7	8.4	2.6
CAL_H	$J_{2,3}$	10.1	9.7	10.2	8.7	8.6
CAL_H	$J_{3,4}$	9.7	9.6	9.9	9.2	8.4
CAL_H	$J_{4,5}$	9.7	10.0	10.0	4.3	8.4
CAL_A	$J_{1,2}$	2.7	6.8	2.7	5.9	2.5
CAL_A	$J_{2,3}$	8.7	7.4	8.9	6.6	7.7
CAL_A	$J_{3,4}$	7.5	7.2	7.7	7.1	6.8
CAL_A	$J_{4,5}$	8.0	8.2	8.2	4.8	7.1

B. Hexasaccharides

		Undecorated (NA-domain, see Figure 1A)					
		GlcA	**GlcNAc**	**GlcA**	**GlcNAc**	**GlcA**	**GlcNAc**
CAL_H	$J_{1,2}$	9.9	3.2	9.7	3.2	9.5	3.5
CAL_H	$J_{2,3}$	9.8	10.1	9.6	10.1	9.5	6.7
CAL_H	$J_{3,4}$	9.9	10.0	9.6	10.0	9.6	7.8
CAL_H	$J_{4,5}$	10.1	10.0	10.0	10.0	10.0	7.8
CAL_A	$J_{1,2}$	6.7	2.9	6.6	3.0	6.5	2.8
CAL_A	$J_{2,3}$	7.5	8.7	7.3	8.7	7.2	6.0
CAL_A	$J_{3,4}$	7.5	7.8	7.3	7.8	7.3	6.4
CAL_A	$J_{4,5}$	8.3	8.3	8.2	8.3	8.2	6.6

		Sulfate-decorated (S-domain, see Figure 1B)					
		IdoA2S	**GlcNS6S**	**IdoA2S**	**GlcNS6S**	**IdoA2S**	**GlcNS6S**
CAL_H	$J_{1,2}$	2.0	2.5	8.3	2.5	7.1	2.7
CAL_H	$J_{2,3}$	2.2	10.2	8.4	10.2	7.5	10.1
CAL_H	$J_{3,4}$	2.4	9.9	8.4	10.0	7.3	9.9
CAL_H	$J_{4,5}$	3.5	10.0	4.0	10.1	4.0	10.0
CAL_A	$J_{1,2}$	2.3	2.6	5.7	2.6	5.0	2.7
CAL_A	$J_{2,3}$	3.0	8.9	6.5	8.9	6.0	8.8
CAL_A	$J_{3,4}$	3.2	7.6	6.6	7.7	5.9	7.7
CAL_A	$J_{4,5}$	2.7	8.3	4.3	8.3	4.1	8.2

Table S4 continued

C. Octasaccharides

		Undecorated (NA-domain, see Figure 1A)							
		GlcA	**GlcNAc**	**GlcA**	**GlcNAc**	**GlcA**	**GlcNAc**	**GlcA**	**GlcNAc**
CAL_H	$J_{1,2}$	9.9	3.2	9.7	3.2	9.7	3.2	9.6	3.5
CAL_H	$J_{2,3}$	9.8	10.1	9.6	10.1	9.6	10.1	9.5	6.9
CAL_H	$J_{3,4}$	9.9	10.0	9.7	10.0	9.6	10.0	9.6	8.1
CAL_H	$J_{4,5}$	10.1	10.0	10.0	10.0	10.0	10.0	10.0	8.0
CAL_A	$J_{1,2}$	6.7	2.9	6.6	2.9	6.6	3.0	6.5	2.8
CAL_A	$J_{2,3}$	7.5	8.7	7.3	8.7	7.3	8.7	7.3	6.2
CAL_A	$J_{3,4}$	7.5	7.8	7.3	7.8	7.3	7.8	7.3	6.6
CAL_A	$J_{4,5}$	8.3	8.3	8.2	8.2	8.2	8.2	8.2	6.8

		Sulfate-decorated (S-domain, see Figure 1B)							
		IdoA2S	**GlcNS6S**	**IdoA2S**	**GlcNS6S**	**IdoA2S**	**GlcNS6S**	**IdoA2S**	**GlcNS6S**
CAL_H	$J_{1,2}$	1.6	2.5	8.0	2.5	6.6	2.5	7.0	2.7
CAL_H	$J_{2,3}$	1.8	10.2	8.2	10.2	7.0	10.2	7.3	10.2
CAL_H	$J_{3,4}$	2.0	9.9	8.2	10.0	6.8	10.0	7.2	10.0
CAL_H	$J_{4,5}$	3.4	10.0	4.0	10.1	4.0	10.1	3.9	10.0
CAL_A	$J_{1,2}$	2.1	2.6	5.5	2.5	4.7	2.6	5.0	2.7
CAL_A	$J_{2,3}$	2.8	8.9	6.4	8.9	5.8	8.9	5.9	8.8
CAL_A	$J_{3,4}$	3.0	7.6	6.5	7.7	5.5	7.7	5.9	7.7
CAL_A	$J_{4,5}$	2.6	8.3	4.2	8.3	3.9	8.3	4.0	8.3

Table S4 continued

D. Decasaccharides

		Undecorated (NA-domain, see Figure 1A)									
		GlcA	**GlcNAc**	**GlcA**	**GlcNAc**	**GlcA**	**GlcNAc**	**GlcA**	**GlcNAc**	**GlcA**	**GlcNAc**
CAL_H	$J_{1,2}$	9.9	3.2	9.7	3.2	9.7	3.2	9.7	3.2	9.6	3.5
CAL_H	$J_{2,3}$	9.8	10.1	9.7	10.1	9.6	10.1	9.6	10.1	9.6	6.8
CAL_H	$J_{3,4}$	9.9	10.0	9.7	10.0	9.6	10.0	9.6	10.0	9.6	7.9
CAL_H	$J_{4,5}$	10.1	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	7.9
CAL_A	$J_{1,2}$	6.7	2.9	6.6	3.0	6.6	2.9	6.6	3.0	6.6	2.8
CAL_A	$J_{2,3}$	7.5	8.7	7.4	8.7	7.3	8.7	7.3	8.7	7.3	6.1
CAL_A	$J_{3,4}$	7.5	7.8	7.3	7.8	7.3	7.8	7.3	7.8	7.3	6.5
CAL_A	$J_{4,5}$	8.3	8.3	8.2	8.3	8.2	8.3	8.2	8.3	8.2	6.7

		Sulfate-decorated (S-domain, see Figure 1B)									
		IdoA2S	**GlcNS6S**	**IdoA2S**	**GlcNS6S**	**IdoA2S**	**GlcNS6S**	**IdoA2S**	**GlcNS6S**	**IdoA2S**	**GlcNS6S**
CAL_H	$J_{1,2}$	3.1	2.5	7.1	2.5	8.7	2.5	8.4	2.6	8.0	2.7
CAL_H	$J_{2,3}$	3.2	10.2	7.5	10.2	8.8	10.2	8.6	10.2	8.1	10.2
CAL_H	$J_{3,4}$	3.4	9.9	7.3	10.0	8.9	10.0	8.5	10.0	8.1	10.0
CAL_H	$J_{4,5}$	3.5	10.0	4.0	10.1	4.1	10.1	4.1	10.1	4.0	10.1
CAL_A	$J_{1,2}$	2.9	2.6	5.0	2.6	5.9	2.6	5.7	2.6	5.6	2.7
CAL_A	$J_{2,3}$	3.6	8.9	6.0	8.9	6.8	8.9	6.6	8.9	6.4	8.8
CAL_A	$J_{3,4}$	3.8	7.6	5.9	7.7	6.9	7.7	6.6	7.7	6.5	7.8
CAL_A	$J_{4,5}$	3.0	8.3	4.0	8.3	4.5	8.3	4.4	8.3	4.3	8.3

$^3J_{H,H}$ values are reported in Hz. Calculated (CAL) values are averages from 5 µs simulations computed using the Karplus equations of Altona and Haasnoot[1] (CAL_A) and Hricovini et. *al*.[2] (CAL_H).

(1) Altona, C.; Haasnoot, C. A. G. *Org. Magn. Reson.* **1980**, *13*, 417.
(2) Hricovini, M.; Bizik, F. *Carbohydr. Res.* **2007**, *342*, 779.

Figure S5: Puckering in 5 μs all-atom and coarse-grained dynamics: NA-domain decasaccharide

Key:

N: hexosamine (GlcNAc), U: uronic acid (GlcA), see Figure 1A for structures

Residues are numbered from the reducing end

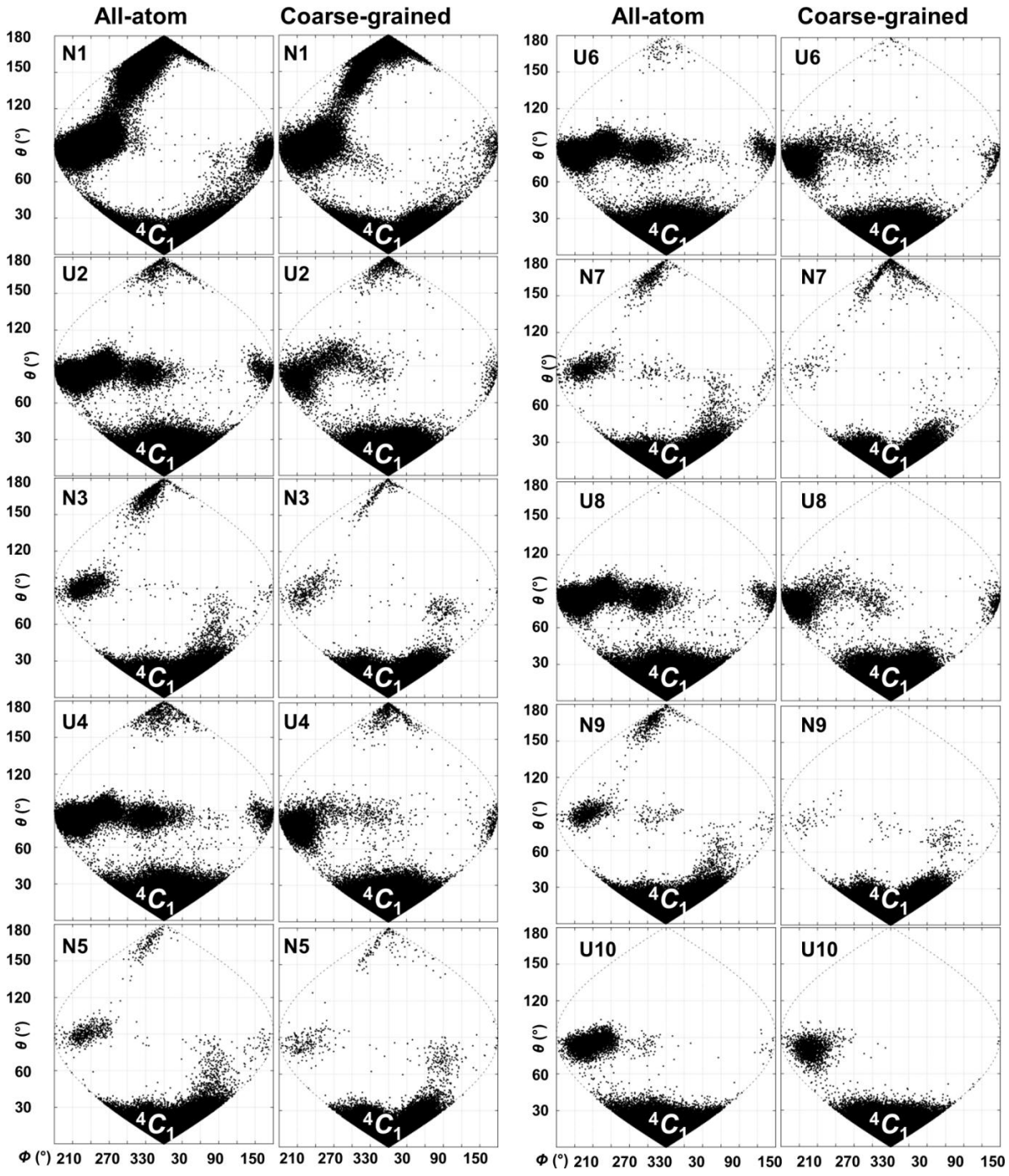


Figure S6: Puckering in 5 μs all-atom and coarse-grained dynamics: S-domain decasaccharide

Key:

N: hexosamine (GlcNS6S), U: uronic acid (IdoA2S), see Figure 1B for structures

Residues are numbered from the reducing end

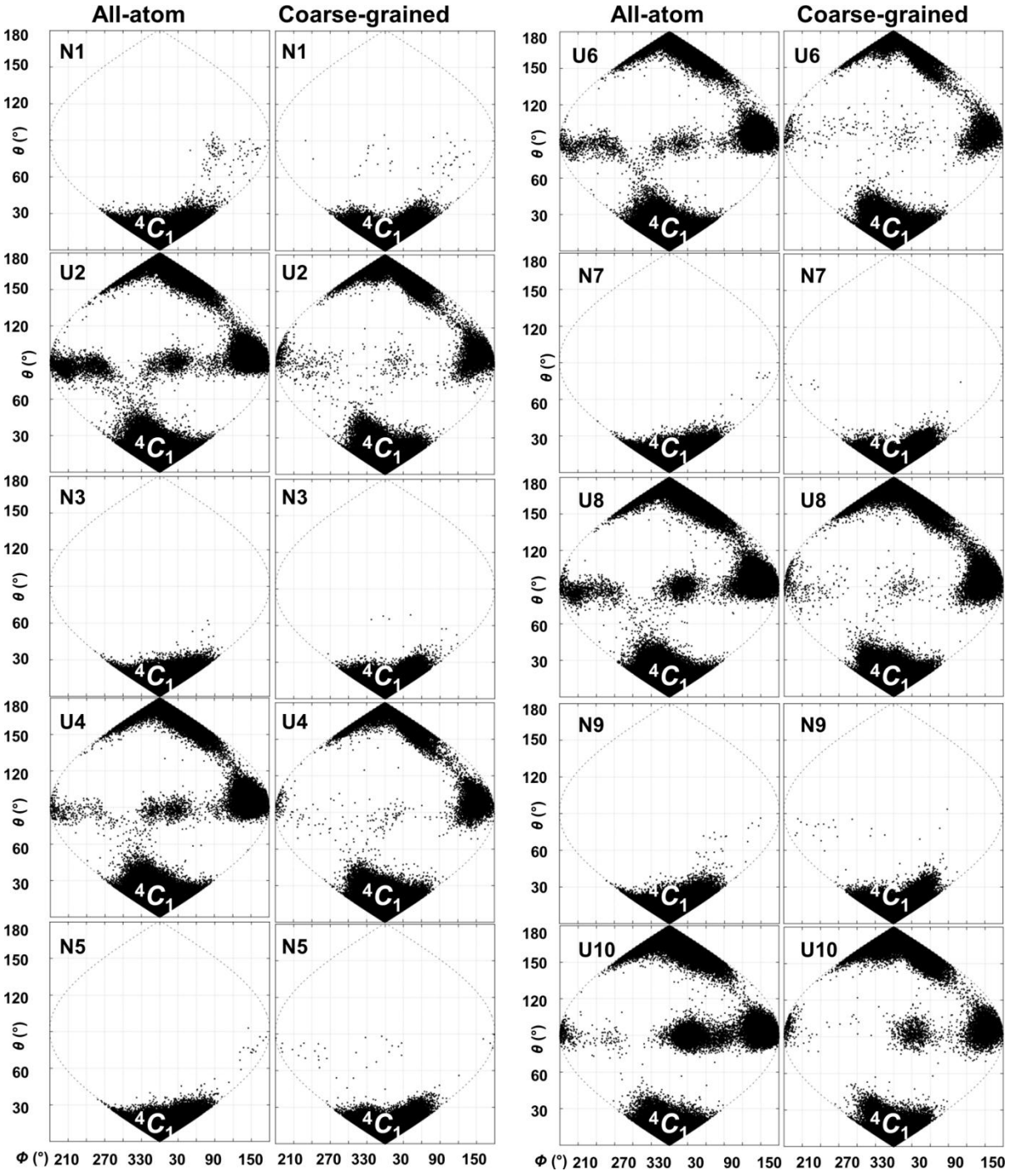


Figure S7: Computed puckering rates in 5 μs all-atom and coarse-grained dynamics: pentasaccharide

See Figures 1C and 3 for 2D- and 3D-structures of the AT-III binding pentasaccharide

Key:

N1: GlcNS6S at position 1 numbered from the reducing end (see Figures 1C and 3)

U2: IdoA2S at position 2 numbered from the reducing end (see Figures 1C and 3)

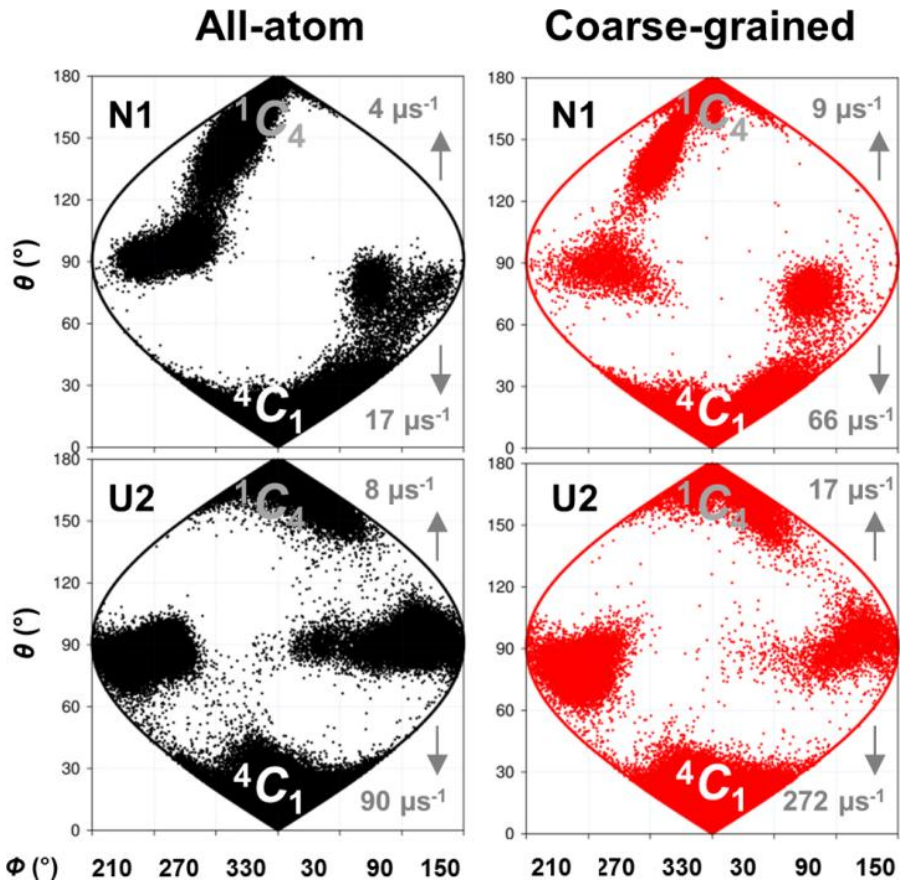


Figure S8: Effect of puckering on computed radii of gyration (*Rg*) in 5 µs coarse-grained simulations

Key:

Undecorated: NA-domain (see Figure 1A)

Sulfate-decorated: S-domain (see Figure 1B)

n is one disaccharide

Rg values are averages computed from 5 µs coarse-grained simulations

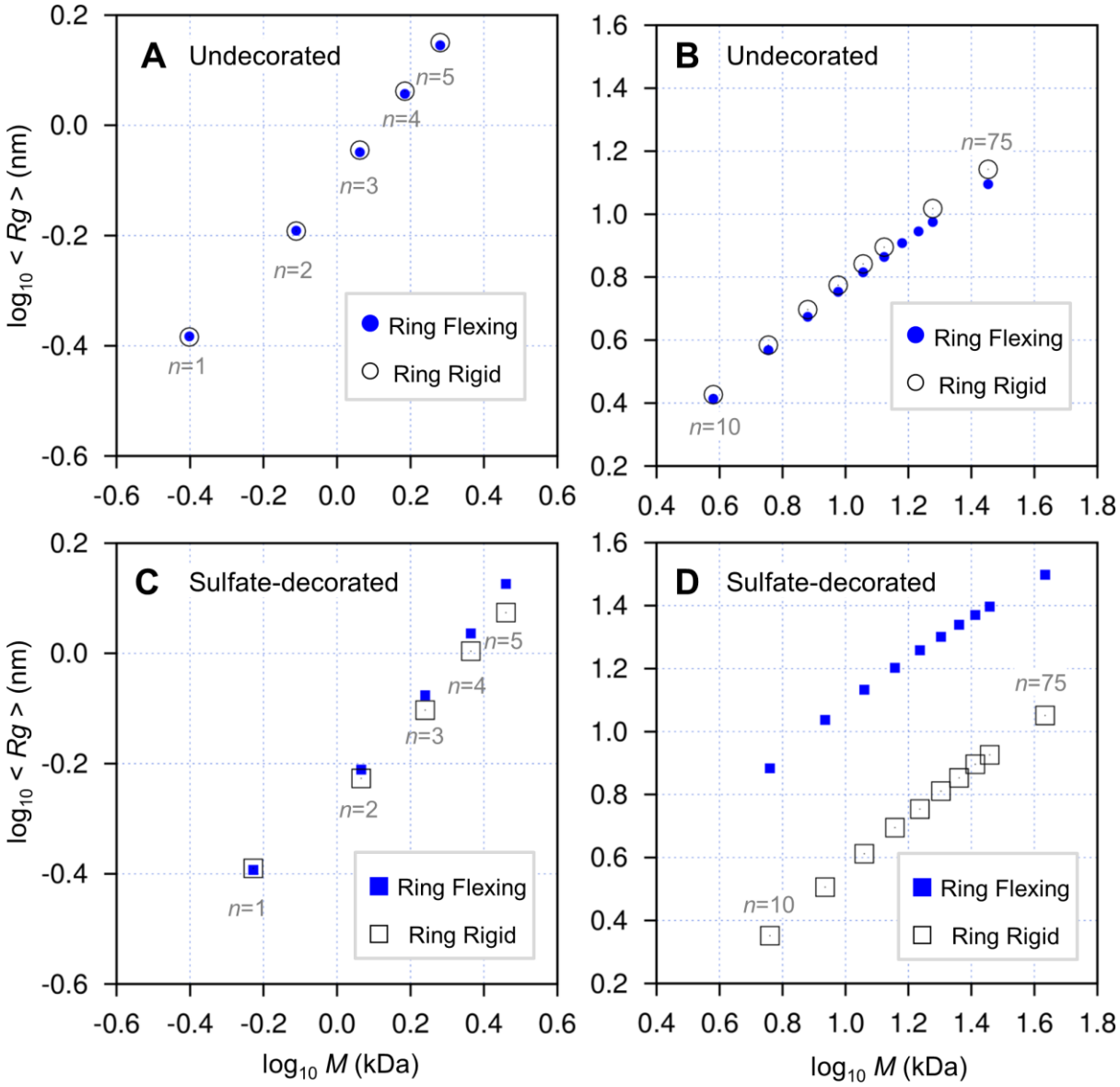


Table S9: Example coarse-grained model parameters for an undecorated disaccharide: GlcA-GlcNAc

A. Linkage parameters (*a* in equation 4)

Coefficient	Link1	Coefficient	Link1
a11	7.68416	**a51**	1.29052
a12	-1.40903	**a52**	-0.09979
a13	0.25870	**a53**	-0.11105
a14	1.24834	**a54**	-0.63034
a15	0.90768	**a55**	-0.08483
a16	0.05457	**a56**	0.15591
a17	0.05667	**a57**	-0.02683
a21	2.46828	**a61**	-0.23076
a22	0.10199	**a62**	0.20548
a23	0.53080	**a63**	-0.06387
a24	-0.66081	**a64**	-0.30119
a25	-0.47651	**a65**	-0.02941
a26	-0.09475	**a66**	-0.13548
a27	-0.98742	**a67**	0.04598
a31	-0.13343	**a71**	0.72569
a32	0.43569	**a72**	0.66938
a33	0.44937	**a73**	-0.19506
a34	0.69129	**a74**	-0.17980
a35	-0.58730	**a75**	-0.11033
a36	-0.11151	**a76**	-0.12909
a37	-0.28179	**a77**	0.17149
a41	0.13703		
a42	-0.52469		
a43	-0.01765		
a44	-0.11525		
a45	0.52223		
a46	-0.14909		
a47	-0.02410		

Table S9 Continued

B. Ring parameters (*b* and *c* in equation 5)

	Residue			Residue	
Coefficient	**GlcNAc**	**GlcA**	**Coefficient**	**GlcNAc**	**GlcA**
b11	0.24169	-0.00750	**b51**	-1.60031	-1.34011
b12	0.95609	0.68256	**b52**	0.53981	0.38765
b13	0.58833	0.40260	**b53**	-0.03052	0.36467
b14	0.01369	-0.23797	**b54**	-0.26366	-0.21763
b15	0.31891	-0.01549	**b55**	-0.37721	-0.05755
b16	-0.19525	0.06458	**b56**	-0.20607	0.02100
b17	0.15045	-0.05535	**b57**	-0.03668	-0.19842
b21	-0.18692	0.69771	**b61**	-0.64929	-0.06412
b22	-1.72237	0.37600	**b62**	-0.35763	-0.04622
b23	0.74411	0.11657	**b63**	-0.24990	0.00399
b24	0.05477	-0.20069	**b64**	0.07214	-0.19693
b25	-0.12889	0.03784	**b65**	0.13338	0.04944
b26	0.52664	0.00745	**b66**	0.21996	-0.02659
b27	0.11943	0.06835	**b67**	-0.05496	-0.01811
b31	-0.67553	-0.16194	**b71**	-0.17659	-0.22226
b32	0.51826	-0.89460	**b72**	-0.44646	-0.19701
b33	-0.47089	-0.97007	**b73**	-0.19497	0.06537
b34	0.29983	0.20345	**b74**	0.07793	-0.20244
b35	-0.09301	0.20647	**b75**	-0.32710	-0.06931
b36	-0.28609	-0.02025	**b76**	-0.09421	0.11462
b37	0.09158	-0.14991	**b77**	-0.04022	-0.05448
b41	-0.60477	-1.28403			
b42	-0.84503	-0.26587	**c1**	0.51083	0.54762
b43	0.17131	-0.64415	**c2**	6.70621	5.58854
b44	-0.04156	0.33844	**c3**	-1.86733	-0.82020
b45	0.28483	0.18385			
b46	-0.00316	-0.19445			
b47	0.13508	-0.01212			

C. Cremer-Pople *Q* values computed from 5 μs aqueous simulations: GlcA-GlcNAc disaccharide

	Q (Ave.)	**STDEV**
GlcNAc	0.55	0.05
GlcA	0.57	0.05