Supporting information

Site-Selective Chemoenzymatic Modification on the Core Fucose of an Antibody Enhances Its Fcγ Receptor Affinity and ADCC Activity

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Table S1. Average number of contacts between the Fc chain A and FcR glycans investigated by molecular dynamics simulations.

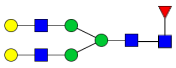
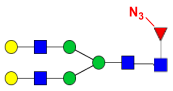
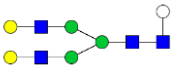
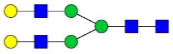
IgG glycoform	Structure	number of contacts
G2F (**1**)		0.22 ± 0.06
G2(Az)F (**2**)		0.76 ± 0.06
G2Gal (**5**)		0.70 ± 0.07
G2 (**11**)		2.48 ± 0.14

Table S2. Scaling parameters for each replica in the HREST-BP MD simulations.

Replica Index, n	T_n (K)	λ_n
0	298	0.00
1	316	0.10
2	335	0.23
3	356	0.32
4	377	0.43
5	400	0.50

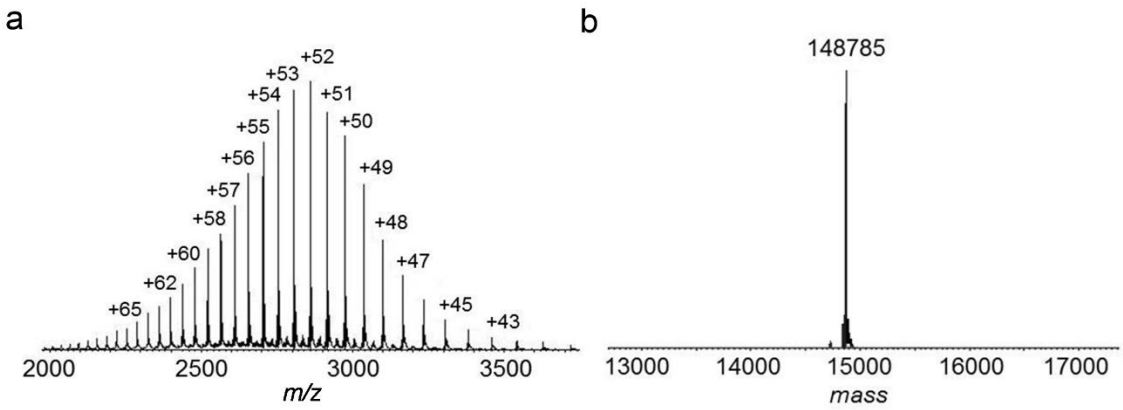


Figure S1. ESI-MS profile of the synthetic azide-core fucose antibody glycoform (G2(Az)F-Herceptin). (**a**) Original ESI-MS spectrum of G2(Az)F-Herceptin. (**b**) The deconvoluted ESI-MS spectrum of G2(Az)F-Herceptin.

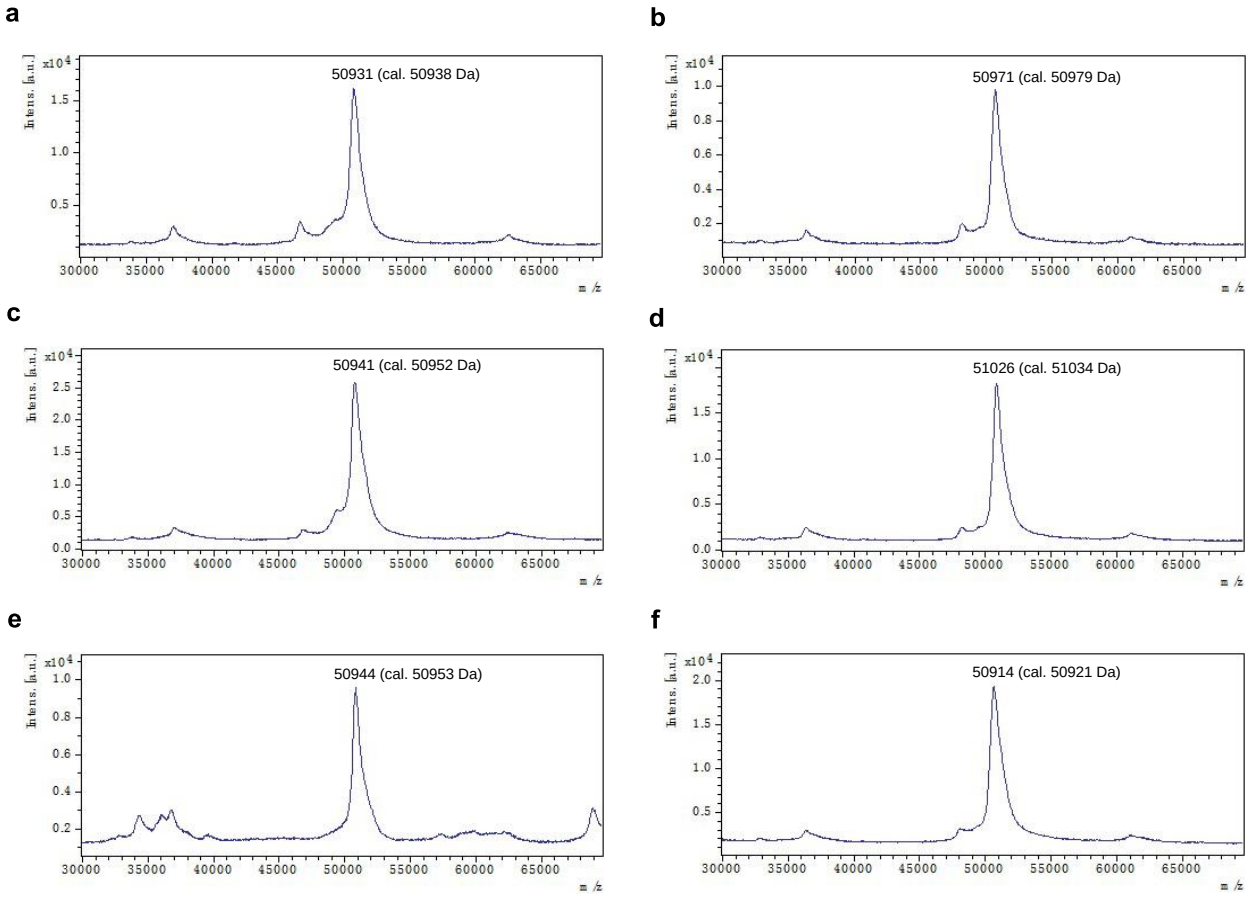


Figure S2. MALDI-TOF MS analysis of the heavy chain from the glycoforms of the engineered Herceptin after DTT treatment. (**a**) G2F-Hercpetin (**1**). (**b**) G2AzF-Herceptin (**2**). (**c**) G2NH_2F-Herceptin (**3**). (**d**) G2TriazoleF-Herceptin (**4**). (**e**) G2Gal-Herceptin (**5**). (**f**) G2Ara-Herceptin (**6**).

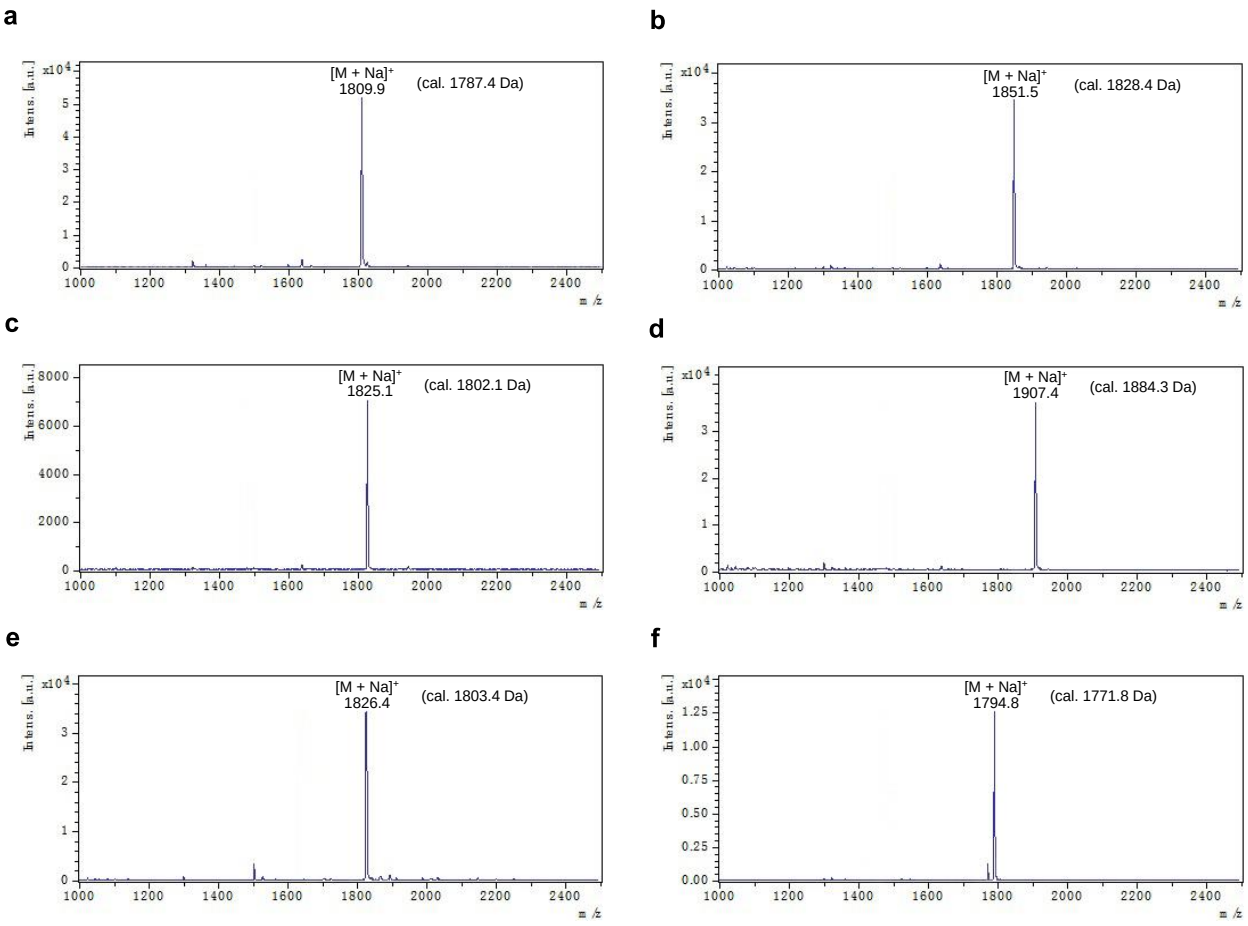


Figure S3. MALDI-TOF MS analysis of the Fc N-glycans released by PNGase F treatment of the engineered Herceptin glycoforms. (**a**) G2F-Hercpetin (**1**). (**b**) G2AzF-Herceptin (**2**). (**c**) G2NH_2F-Herceptin (**3**). (**d**) G2TriazoleF-Herceptin (**4**). (**e**) G2Gal-Herceptin (**5**). (**f**) G2Ara-Herceptin (**6**).

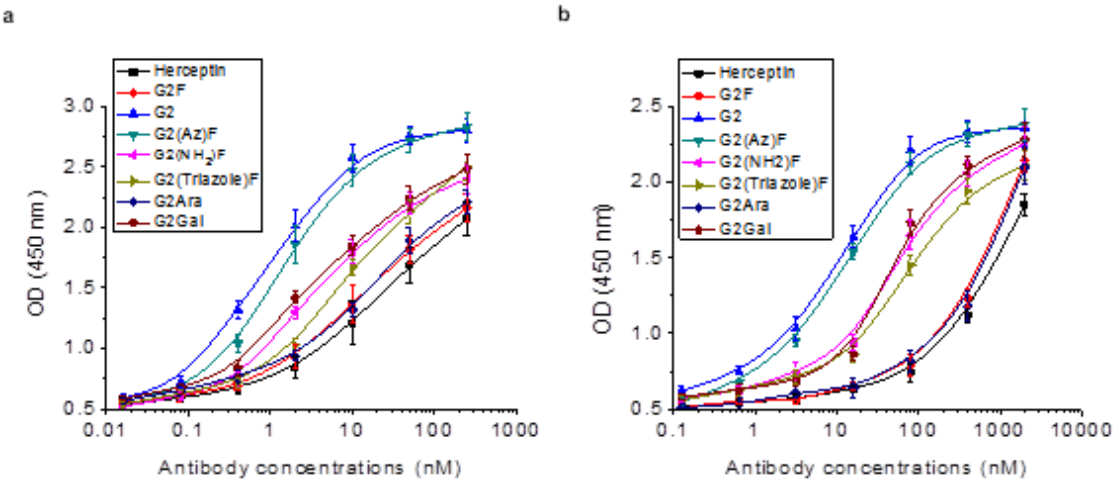


Figure S4. ELISA analysis of the binding between human FcγIIIa receptor and various synthetic glycoforms of Herceptin. (**a**) Binding with FcγRIIIa V158 allele. (**b**) Binding with FcγRIIIa F158 allele.

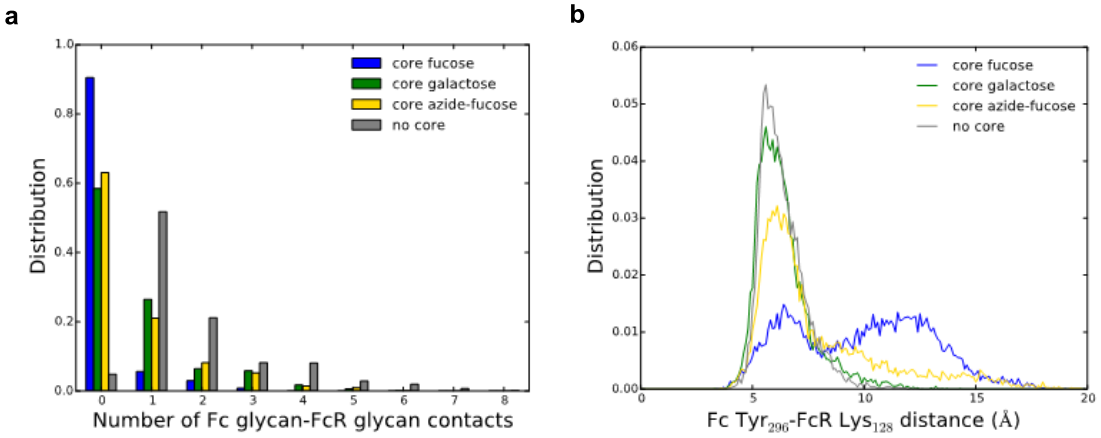


Figure S5. Interactions between core glycoengineered Fc domain and FcR from the MD simulations. **(a)** Distribution of conformations in which FcR glycan makes 0, 1, 2, 3, *etc*. contacts (distance between glycan centers of mass < 7 Å) with Fc glycans with core fucose, galactose, azide-fucose, or no core. Contacts with the core sugar in the Fc glycan are excluded. FcR glycans have greater likelihood of making multivalent contacts with Fc glycans with modified or no core. (**b**) Distribution of distances between Fc Tyr296 and FcR Lys128 from Fc-FcR systems with varying core sugars in the Fc glycan.

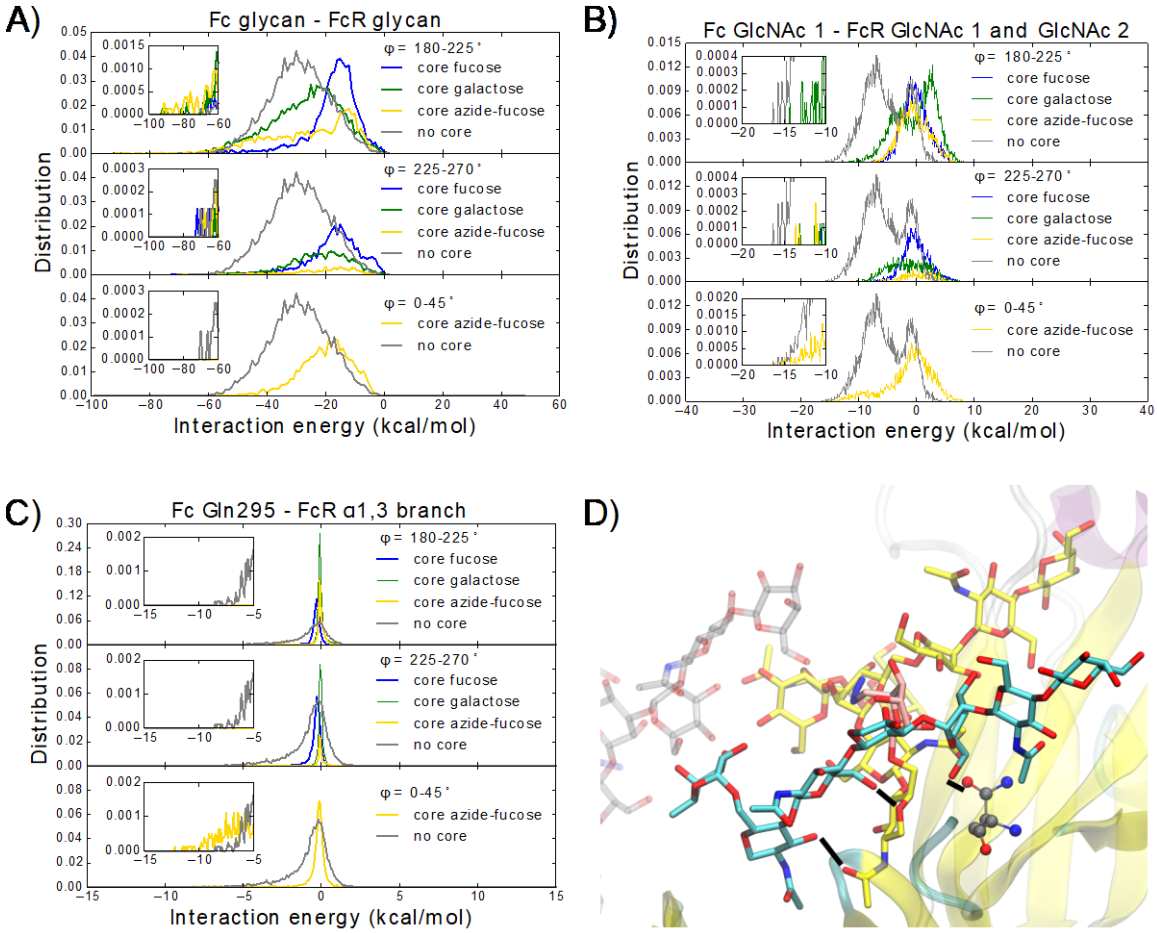


Figure S6. Interaction energy distributions between Fc chain A and selected FcR components by population of core sugar conformations in the Fc chain A glycan ((**a-c**). Inserts in each panel present expanded views of most favorable regions of the interaction energy distributions. Interaction energy distributions for the afucosylated Fc (no core) are included in gray in all panels for reference. Snapshot of the interaction orientation between the FcR glycan and Fc chain A glycan with core azide-fucose at ϕ=0-45° orientation (**d**). FcR glycan carbons are in cyan, Fc glycan carbons are in yellow, core azide-fucose carbons are in pink, and Fc Gln295 carbons in gray beads. Oxygens and nitrogens in red and blue, respectively. Hydrogen bonds between Fc GlcNAc 1 and FcR GlcNAc 1 and 2 and between Man 3 of the FcR α1,3 branch and Fc Gln295 are indicated by black dashes. The Fc chain A and FcR proteins and Fc chain B glycan in gray are made transparent, and the FcR α1,6 branch is removed for clarity.

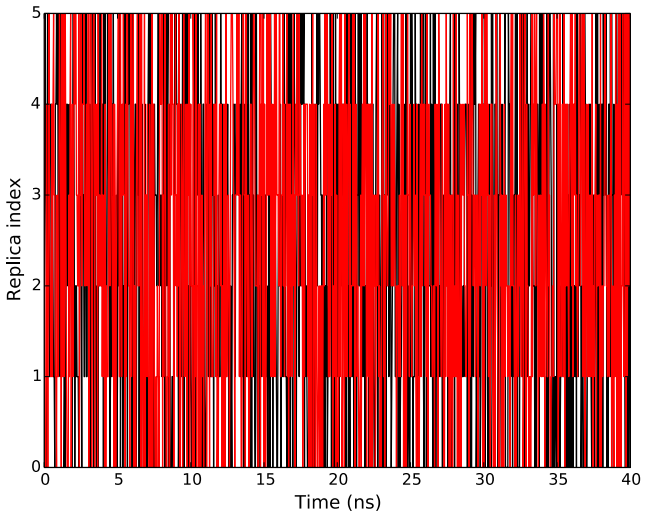
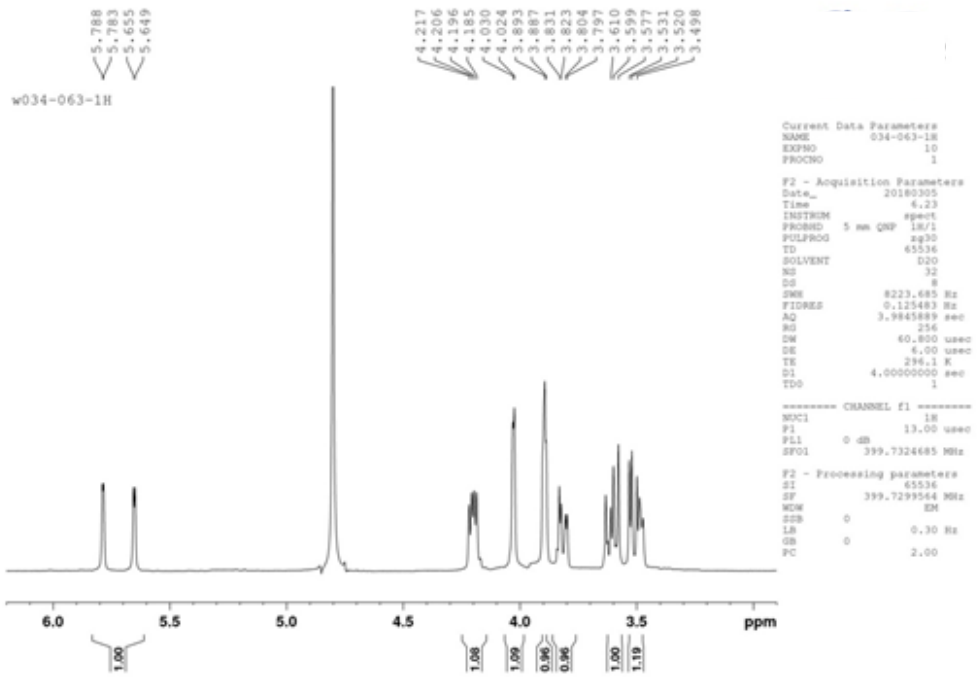
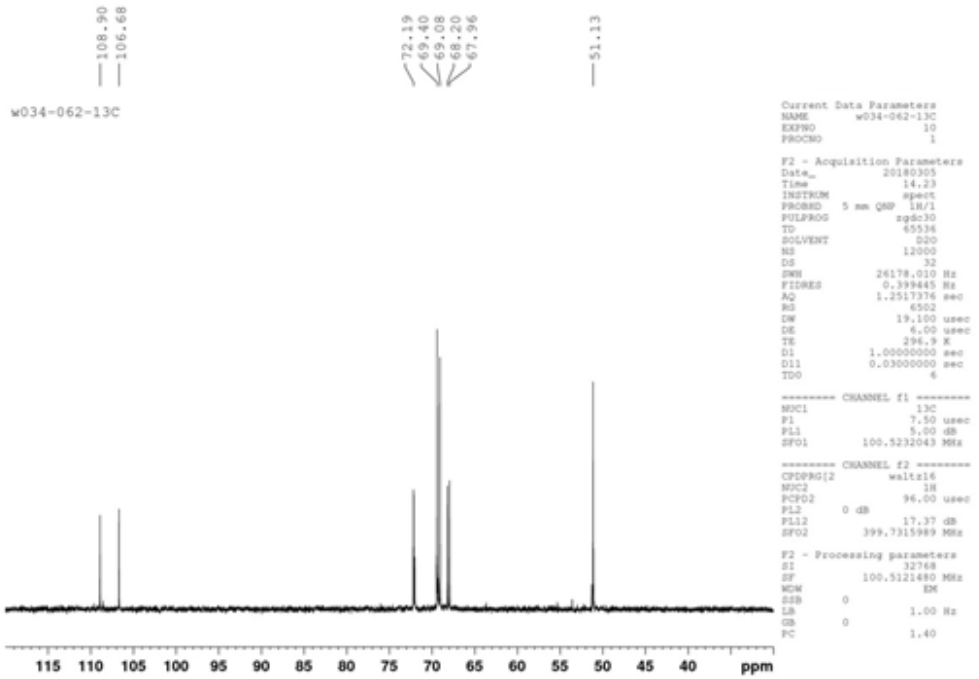


Figure S7. Replica walk of the original ground-state replica (black) and the highest replica (red) *vs.* time across 40 ns of HREST-BP simulations for the system with Fc glycan with core fucose.

a

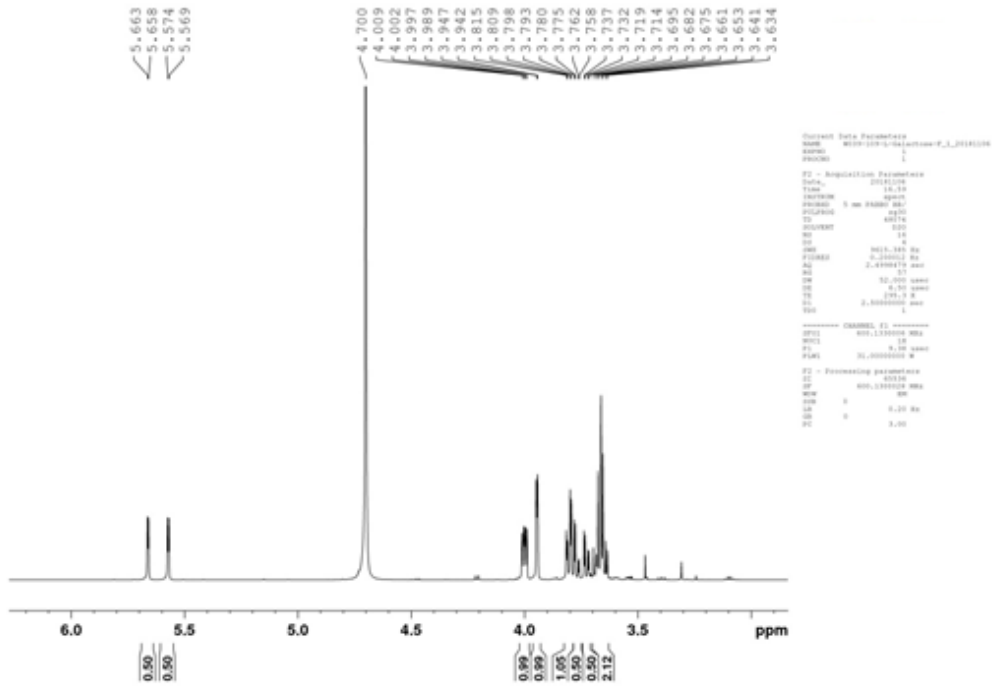


b

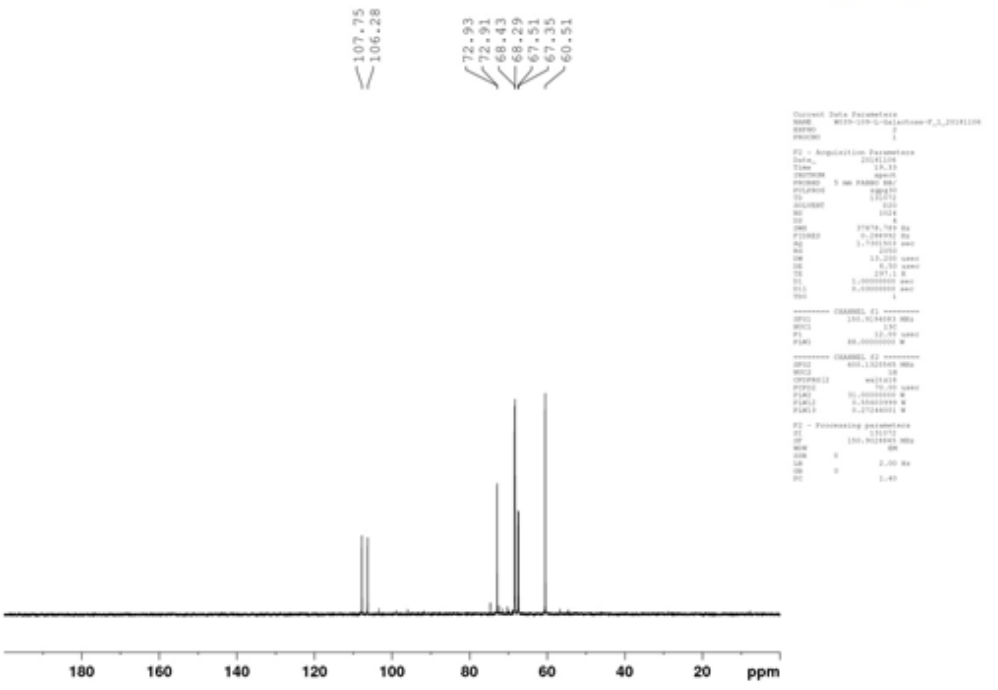


NMR spectra of 6-azido α-L-fucosyl fluoride (8). (a) ^{1}H NMR. (b) ^{13}C NMR.

a

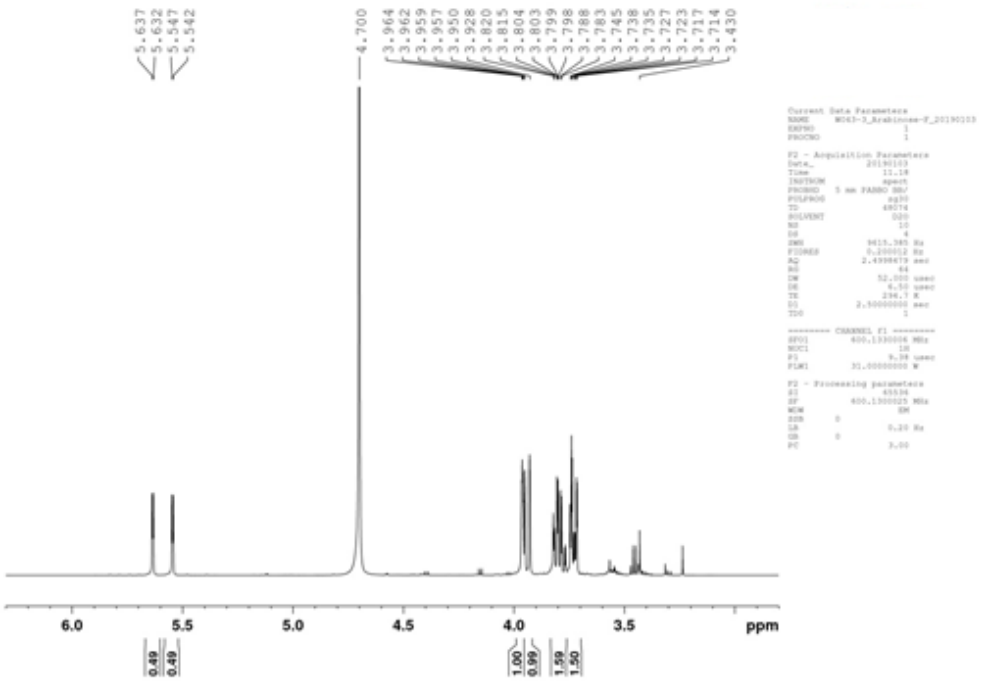


b

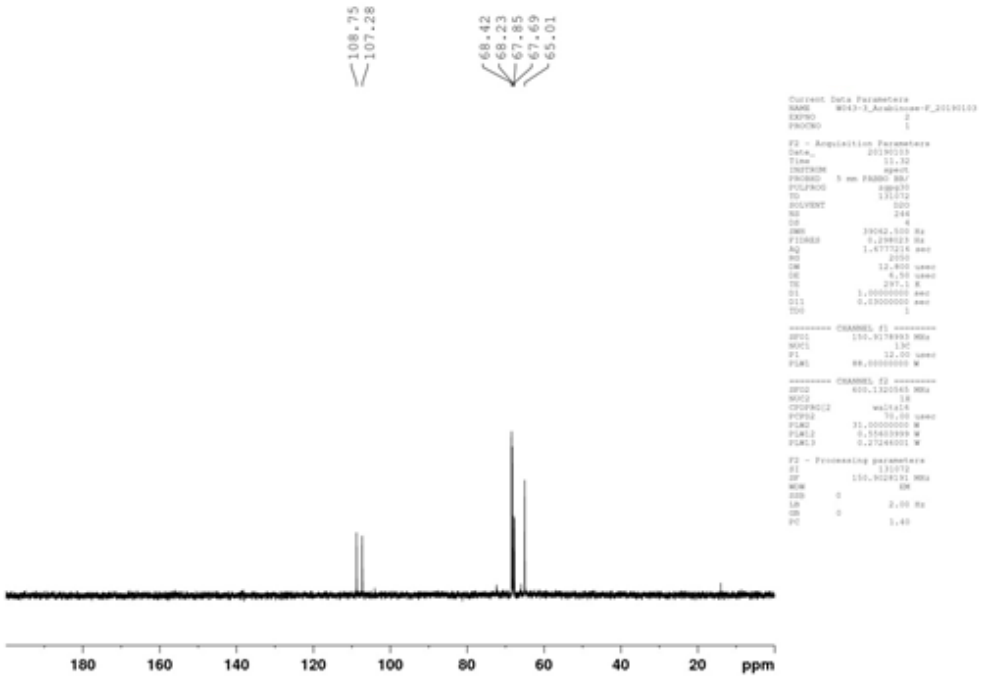


NMR spectra of α-L-galactosyl fluoride (12). (a) ^{1}H NMR. (b) ^{13}C NMR.

a



b



NMR spectra of α-D-arabinosyl fluoride (13). (a) ^{1}H NMR; (b) ^{13}C NMR.