

Supplementary Information

Profiling of core fucosylated N-glycans using a novel bacterial lectin that specifically recognizes α 1,6 fucosylated chitobiose

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This document provides information supplemental to the main text, in the following sections:

A - Supporting Experimental Section

B - Supporting Tables and Figures

A - Supporting Experimental Section

Gel Filtration

Gel filtration for estimation of the molecular weight was performed using a HiPrep 16/60 Sephadryl S-200 HR column (GE Healthcare Life Sciences) equilibrated with 20 mM sodium phosphate, pH 7.4 containing 150 mM NaCl. Elution was carried out with the same buffer at a flow rate of 0.5 ml/min. The calibration proteins were used as follows: conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa), ribonuclease A (13.7 kDa), and aprotinin (6.5 kDa).

Liquid chromatography mass spectrometry analysis (LC-MS)

The lectin sample was diluted in 0.1% formic acid/water and analyzed by reverse phase liquid chromatography (LC) and electrospray ionization time-of-flight mass spectrometry (ESI-TOF MS). A custom reverse phase chip (Agilent Technologies), containing an integrated trapping column (40 nl capacity), separation column and nano-ESI emitter (75 mm x 150 mm both packed with PLRP-S, 5 mm particles, 1000 Å pore size) was used for the separation of proteins. The chip trapping column was loaded at 4 ml/min and the separation column developed at a flow rate of 600 nl/min using an Agilent 1200 series nano-LC coupled to an Agilent 6210 series ESI-TOF mass spectrometer. The column was equilibrated with 0.1% formic acid water containing 5% acetonitrile. 140 ng of protein was injected onto the column and it was developed after two minutes with a twenty-five minute linear gradient from 5% to 95% acetonitrile. Protein was found to elute at approximately twelve minutes post-injection. The mass spectra were acquired from 100-3200 m/z, one cycle/sec and 10,000 transients per scan using an ionization energy of 1800 V, fragmentor of 215 V, and drying gas of 275 C at 4.0 l/min. The acquired spectra were extracted and the protein spectra deconvoluted with Agilent MassHunter Qualitative Analysis Software (with Bioconfirm) B 2.0.2 software using a mass range of 10k to 100k Daltons.

UPLC-HILIC-FLR and UPLC-HILIC-FLR-MS

The 2-AB-labeled N-glycans were separated by UPLC using a Waters ACQUITY BEH Glycan Amide column (2.1 x 150 mm, 1.7 µm) that was installed on a Waters H-Class ACQUITY instrument equipped with a quaternary solvent manager and a fluorescence detector. Solvent A was 50 mM ammonium formate buffer pH 4.4 and solvent B was acetonitrile. The gradient used was 0-1.47 min, 30% solvent A; 1.47-24.81 min, 30-47% solvent A; 25.5-26.25 min, 70% solvent A; 26.55-32 min, 30% solvent A. The flow

rate was 0.561 mL/min. The injection volume was 10 μ L and the sample was prepared in 70% (v/v) acetonitrile. Samples were kept at 5°C prior to injection and the separation temperature was 40°C. The fluorescence detection wavelengths were $\lambda_{\text{ex}} = 330$ nm and $\lambda_{\text{em}} = 420$ nm with a data collection rate of 20 Hz. A dextran hydrolysate ladder was used to convert retention times into glucose unit (GU) values. All data was processed using Waters Empower 3 chromatography workstation software.

HPLC-HILIC-FLR-MS was performed using a Waters XBridge™ BEH Amide column (3 \times 150 mm, 2.5 μ m) on a Dionex UltiMate® LC, in line with a LTQ™ Velos™ Pro Mass Spectrometer equipped with a heated electrospray standard source (HESI-II probe). The flow rate was 0.300 mL/min and the column temperature was maintained at 30°C; solvent A was 50 mM ammonium formate buffer pH 4.4 and solvent B was acetonitrile. A linear gradient of 30% to 47% A over 50 min was used. The injection volume was 98 μ L and the sample was prepared in 80% (v/v) acetonitrile. MS data was acquired in positive ion mode with a source voltage of 3 kV and capillary temperature of 275°C. Sheath gas flow, Aux gas and sweep gas flow rates were 11, 5 and 0, respectively. Full scan data for rabbit and human N-glycans were acquired over a m/z range of 400 to 2000. All data was processed using Thermo™ Xcalibur Roadmap.

BKF treatment

Recombinant α (1-2/4/6)-fucosidase (New England Biolabs) was used for the structural characterization of the glycans. 2AB-labeled N-glycans were incubated with 32 mU of α (1-2/4/6)-fucosidase in 20 μ L of 50 mM sodium acetate buffer (pH 5.5) for 24-48 h at 37°C. After digestion, the enzymes were removed from the sample by filtration through a 10 kDa MWCO ultrafiltration plate (Pall LifeSciences). The filtrate was dried in a SpeedVac and re-suspended in H₂O for analysis by UPLC-HILIC-FLR.

B – Supporting Tables and Figures

Table S1. Representation of structures of AMC-labeled oligosaccharides used for microtiter plate glycan binding screening.

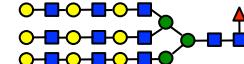
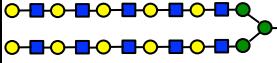
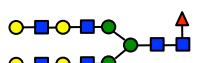
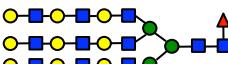
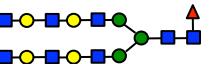
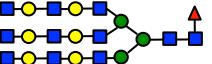
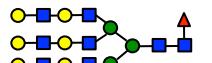
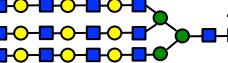
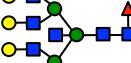
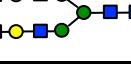
Glycan	Structure*
1	GlcNAc β 1-2Man α 1-6(GlcNAc β 1-4)(GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
2	Neu5Ac α 2-6GalNAc
3	Gal β 1-4GlcNAc
4	GlcNAc β 1-4GlcNAc β 1-4GlcNAc β 1-4GlcNAc
5	GalNAc α 1-3(Fuc α 1-2)Gal
6	Fuc α 1-2Gal β 1-4Glc
7	Gal β 1-3(Neu5Ac α 2-6)GlcNAc β 1-3Gal β 1-4Glc
8	Fuc α 1-2Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4Glc
9	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
10	Gal β 1-3GalNAc β 1-4(Neu5Ac α 2-3)Gal β 1-4Glc
11	Neu5Ac α 2-8Neu5Ac α 2-8Neu5Ac α 2-8Neu5Ac
12	Glc α 1-4Glc α 1-4Glc
13	Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc
14	Gal β 1-4(Fuc α 1-3)GlcNAc β 1-6(Fuc α 1-2Gal β 1-3(Fuc α 1-3)GlcNAc β 1-3)Gal β 1-4Glc
15	Gal α 1-3Gal β 1-4Gal α 1-3Gal
16	Gal α 1-3Gal
17	Man α 1-6(Man α 1-3)Man α 1-6(Man α 1-3)Man
18	GlcNAc β 1-2Man α 1-6 (GlcNAc β 1-2Man α 1-3)Man
19	Neu5Ac α 2-3Gal β 1-3GlcNac β 1-3Gal β 1-4Glc
20	Xyl
21	GlcA β 1-3GlcNAc β 1-4GlcA β 1-3GlcNAc β 1-4GlcA β 1-3GlcNAc β 1-4GlcA β 1-3GlaNAc
22	Xyl β 1-4Xyl β 1-4Xyl
23	Man β 1-4Man β 1-4Man
24	Xyl β 1-4Xyl β 1-4Xyl β 1-4Xyl
25	Ara α 1-5Ara α 1-5Ara α 1-5Ara
26	Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3Glc
27	Gal α 1-6Gal α 1-6Glc α 1-2Fru
28	Man α 1-3Man β 1-4GlcNAc
29	Neu5Ac α 2-3,6 Gal β 1-4GlcNAc β 1-2 Man α 1-3 (Neu5Ac α 2-3,6Gal β 1-4GlcNAc β 1-2 Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
30	Man β 1-4Man
31	GalNAc
32	GalA
33	GlcA
34	Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4(Fuc α 1-6)GlcNAc
35	GlcNAc β 1-4(Fuc α 1-6)GlcNAc
36	Gal β 1-4GlcNAc β 1-2Man α 1-6(GlcNAc β 1-Man α 1-3)Man β 1-4GlcNAc β 1-4(Fuc α 1-6)GlcNAc
37	Gal α 1,4Gal β 1-4Glc
38	Man α 1-3Man β 1-4GlcNAc
39	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2(Neu5Ac α 2-3Gal β 1-3 (Neu5Ac α 2-6)GlcNAc β 1-4)Man α 1-3(Neu5Ac α 2-3,6Gal β 1-4GlcNAc β 1-2 Man α 1-6)Man β 1-4GlcNAc β 1-4GlcNAc
40	Glc β 1-4Glc β 1-4Glc β 1-4Glc
41	Neu5Ac α 2-3,6 Gal β 1-3GalNAc

42	GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc
43	Man α 1-6(Man α 1-3)Man α 1-6(Man α 1-3)Man β 1-4GlcNAc
44	Gal β 1-4Glc
45	Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc
46	Gal α 1-3(Fuc α 1-2)Gal β 1-4(Fuc α 1-2)Glc
47	Gal β 1-4GlcNAc β 1-6(Gal β 1-4GlcNAc β 1-2)Man α 1-6Gal β 1-4GlcNAc β 1-4(Gal β 1-4GlcNAc β 1-2)Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
48	GlcNAc β 1-4GlcNAc β 1-4GlcNAc
49	Gal β 1,4(Fuc α 1-3)GlcNAc β 1-6(Gal β 1-3(Fuc α 1-4)GlcNAc b1,3)Gal β 1-4Glc
50	Gal β 1-4(Fuc α 1-3)GlcNAc β 1-6(Fuc α 1-2Gal β 1-3(Fuc α 1-4)GlcNAc β 1-3)Gal β 1-4Glc
51	Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4-Glc
52	GalNAc α 1-3(Fuc α 1-2)Gal β 1-4Glc
53	Man α 1-6Man α 1-6Man
54	Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
55	Man α 1-6(Man α 1-3)Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
56	Fuc α 1-6GlcNAc

* GlcNAc, N-Acetyl-D-glucosamine; Man, D-Mannose; Neu5Ac, N-Acetylneuraminic acid; GalNAc, N-Acetyl-D-galactosamine; Gal, D-Galactose; Fuc, D-Fucose; Glc, D-Glucose; Xyl, D-Xylose; Ara, D-Arabinose; GalA, D-Galactouronic acid; GlcA, D-Glucuronic acid

Table S2. Mammalian glycan array (CFG) data for SL2-1. Only results of the core-fucosylated N-glycans are presented in the table. The data is arranged by RFU (high to low) to provide a list of the glycans bound with highest intensity. The rank number corresponds to the overall rank # in the complete list of 609 glycans. RFU values for available analogous of non-core-fucosylated glycans are also provided.

Rank	Glycan Structure (text)*	Glycan Structure (symbol)**	RFU	St Dev	% CV	Non-CF analog (RFU)
1	Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-4GlcNAcb1-2Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		17143	215	1	n/a
2	GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		15311	352	2	431
3	GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		14766	777	5	n/a
4	Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		14336	1075	8	622
5	Galb1-4GlcNAcb1-6(Galb1-4GlcNAcb1-2)Mana1-6(Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		13565	844	6	399
6	Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		13209	987	7	n/a
7	GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp19		12875	1156	9	399
8	Fuca1-2Galb1-4(Fuca1-3)GlcNAcb1-2Mana1-6(Fuca1-2Galb1-4(Fuca1-3)GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		11738	1064	9	187
9	Neu5Aca2-6Galb1-4GlcNAcb1-2Mana1-6(Neu5Aca2-6Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		10358	779	8	51

10	Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2)Mana1-6(Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		10241	166	2	n/a
11	GlcNAcb1-6(GlcNAcb1-2)Mana1-6(GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		9832	538	2	52
12	Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp19		9711	1226	13	630
13	Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		8211	1573	19	288
14	Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2)Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		7999	1503	19	n/a
15	GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		7646	527	7	145
16	GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2)Mana1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		7045	797	11	n/a
17	Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2)Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		6845	430	6	779
18	GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2)Mana1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2)Mana1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		6100	815	13	n/a
19	Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		5959	970	16	21
20	Galb1-4GlcNAcb1-6(Galb1-4GlcNAcb1-2)Mana1-6(GlcNAcb1-4)Galb1-4GlcNAcb1-4(Gal b1-4GlcNAcb1-2)Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAc-Sp21		5929	1156	19	18
21	GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		5659	352	6	417

22	GlcNAcb1-2 Mana1-6(GlcNAcb1-4)(GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAc-Sp21		5610	377	7	14
23	Galb1-4GlcNAcb1-2 Mana1-6(Galb1-4GlcNAcb1-4)(Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAc-Sp21		5093	636	12	n/a
24	Neu5Aca2-3Galb1-4GlcNAcb1-2Mana1-6(Neu5Aca2-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		4566	74	2	20
25	Fuca1-2Galb1-4GlcNAcb1-2Mana1-6(Fuca1-2Galb1-4GlcNAcb1-2Mana1-3)Manb1/9-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		4339	533	12	570
26	GlcNAcb1-6(GlcNAcb1-2)Mana1-6(GlcNAcb1-4)(GlcNAcb1-4(GlcNAcb1-2)Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAc-Sp21		4091	236	6	14
27	Gala1-3(Fuca1-2)Galb1-4GlcNAcb1-2Mana1-6(Gala1-3(Fuca1-2)Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		3649	529	15	564
28	Mana1-6(Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp19		3630	244	7	25
29	Fuca1-2Galb1-3(Fuca1-4)GlcNAcb1-2Mana1-6(Fuca1-2Galb1-3(Fuca1-4)GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp19		3222	144	4	120
30	Galb1-4GlcNAcb1-2Mana1-6(Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		3222	524	16	19
31	Galb1-4GlcNAcb1-2 Mana1-6(GlcNAcb1-4)(Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAc-Sp21		3216	143	4	29
32	GalNAca1-3(Fuca1-2)Galb1-3GlcNAcb1-2Mana1-6(GalNAca1-3(Fuca1-2)Galb1-3GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		2820	454	16	418
33	Fuca1-2Galb1-3GlcNAcb1-2Mana1-6(Fuca1-2Galb1-3GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		2614	126	5	421
34	Galb1-3GlcNAcb1-2Mana1-6(Galb1-3GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		2390	426	18	130
35	Gala1-3(Fuca1-2)Galb1-3GlcNAcb1-2Mana1-6(Gala1-3(Fuca1-2)Galb1-3GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		2381	293	12	n/a
36	GlcNAcb1-2Mana1-6(GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		1708	194	11	26
37	GalNAca1-3(Fuca1-2)Galb1-4GlcNAcb1-2Mana1-6(GalNAca1-3(Fuca1-2)Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		1558	87	6	511

41	Galb1-4(Fuca1-3)GlcNAcb1-2Man1-6(Galb1-4(Fuca1-3)GlcNAcb1-2Man1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		1069	24	2	287
46	Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-6(Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-2)Man1-6(Galb1-3GlcNAcb1-3Galb1-4GlcNAcb1-2Man1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp24		743	77	10	n/a
120	Fuca1-4(Galb1-3)GlcNAcb1-2Man1-6(Fuca1-4(Galb1-3)GlcNAcb1-2Man1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22		94	7	7	n/a

* GlcNAc, N-Acetyl-D-glucosamine; Man, D-Mannose; Neu5Ac, N-Acetylneuraminic acid; GalNAc, N-Acetyl-D-galactosamine; Gal, D-Galactose; Fuc, D-Fucose

** GlcNAc: blue square; Man, green circle; Neu5Ac: purple diamond; GalNAc: yellow square; Gal: yellow circle; Fuc: red triangle

Table S3. rIgG core-fucosylated N-glycans: enrichment efficiency. UPLC-HILIC-FLR profiles of unbound (FT) and lectin-bound (EL) fractions (including profiles of BKF-treated fractions) were integrated using Empower 3 software, and the enrichment efficiency was calculated using the obtained relative areas of each core-fucosylated glycan peak in FT (+ lectin), EL (+ lectin) and FT (no lectin) fractions.

Retention time (min)	GU	N-glycan composition	Capture efficiency (% of total)
4.64	5.82	HexNAc(4)Hex(3)dHex(1)	91
5.26	6.19	HexNAc(5)Hex(3)dHex(1)	93.1
6.03	6.6	HexNAc(4)Hex(4)dHex(1)	91.7
6.25	6.73	HexNAc(4)Hex(4)dHex(1)	92.5
6.51	6.87	HexNAc(5)Hex(4)dHex(1)	93.4
6.78	6.99	HexNAc(5)Hex(4)dHex(1)	94
7.76	7.51	HexNAc(4)Hex(5)dHex(1)	97.9
8.07	7.68	HexNAc(5)Hex(5)dHex(1)	96.6
9.42	8.38	HexNAc(4)Hex(4)Neu5Gc(1)dHex(1) HexNAc(5)Hex(4)Neu5Gc(1)dHex(1)	91
10.9	9.17	HexNAc(4)Hex(5)Neu5Gc(1)dHex(1)	91.2
11.42	9.42	HexNAc(5)Hex(5)dHex(1)Neu5Gc(1)	94.6
14.04	10.8	HexNAc(4)Hex(5)dHex(1)Neu5Gc(2)	97.8
14.29	10.97	HexNAc(5)Hex(5)dHex(1)Neu5Gc(2)	91.5

Table S4. hIgG core-fucosylated N-glycans: enrichment efficiency. UPLC-HILIC-FLR profiles of unbound (FT) and lectin-bound (EL) fractions (including profiles of BKF-treated fractions) were integrated using Empower 3 software, and the enrichment efficiency was calculated using the obtained relative areas of each core-fucosylated glycan peak in FT (+ lectin), EL (+ lectin) and FT (no lectin) fractions.

Retention time (min)	GU	N-glycan composition	Capture efficiency (% of total)
4.64	5.85	HexNAc(4)Hex(3)dHex(1)	87
5.26	6.21	HexNAc(5)Hex(3)dHex(1)	96.8
6.03	6.64	HexNAc(4)Hex(4)dHex(1)	89.3
6.25	6.76	HexNAc(4)Hex(4)dHex(1)	88.5
6.51	6.89	HexNAc(5)Hex(4)dHex(1)	87.2
6.78	7.01	HexNAc(5)Hex(4)dHex(1)	87
7.76	7.54	HexNAc(4)Hex(5)dHex(1) HexNAc(3)Hex(4)Neu5Ac(1)dHex(1)	91.1
8.07	7.71	HexNAc(5)Hex(5)dHex(1)	87
8.56	7.96	HexNAc(4)Hex(4)Neu5Ac(1)dHex(1) HexNAc(5)Hex(4)Neu5Ac(1)dHex(1)	89.4
9.22	8.32	HexNAc(5)Hex(4)Neu5Ac(1)dHex(1)	96.7
10.07	8.75	HexNAc(4)Hex(5)dHex(1)Neu5Ac(1)	92.7
10.55	9.01	HexNAc(5)Hex(5)dHex(1)Neu5Ac(1)	93.8
12.37	9.93	HexNAc(4)Hex(5)dHex(1)Neu5Ac(2)	96.9
12.65	10.07	HexNAc(5)Hex(5)dHex(1)Neu5Ac(2)	94.5

<u>AGP58216</u>	1	-----MLTGTFLGLGALALAATAVAPAANA-----APVPVTSLNCDGDTYKTAGLRFGRWVAQWNVNVFHQSPA--	67
WP_037947329	1	MS-aRRMLTGTFLGLGALALAATAVAPAANA-----APVPVTGLNCDCDTYKCTANLRFGRWVAQWNVNVFHQSAA--	72
WP_059148527	1	MS-aRRMLTGTFLGLGALALAATAVAPAANA-----APVPVTLNCDCDTYKCTANLAFGRWVAQWNVNVFHQSAA--	72
WP_030825832	1	MS-aRRMLTGTFLGLGALALAATAVAPAANA-----APVPVTLTCDCDTYKCTANLAFGRWVAQWNVNVFHQSAA--	72
WP_062012936	1	MS-aRRMLTGTFLGLGALALAATAVAPAANA-----APVPVTLTCDCDTYKCTANLAFGRWVAQWNVNVFHQSAA--	72
WP_055550715	1	MS-aRRMLTGTFLGLGALLMVSTAVAPAATA-----APVPVTLNCDCDTYKCTAGLRFGRWFADWNNANVFHQSAArs	74
WP_018088730	1	M---RRTIVGTCAALGALAIAGLAG---PATA----APVPVTLNCDCDTYQCTANLRFGRDNNWKA\$GANIFHQTPR--	67
KJA18763	1	MQ----LTNSFLSLLAAAQAIAVPFGSSHvtntLAPVPVTKLVCDFGSFKCTASLD\$FGDGNWVAQW\$TAVFHTGLFg-	74
WP_042158013	1	M---RRTIVGTCAALGALAIAGLAG---PATA----APVPVTLNCDCDTYQCTANLRFGRDNNWKA\$GANIFHQTPR--	67
WP_030083396	1	M---RRTIVGTCAFAALGIGLAG---PAVA----APVPVTLNCDCDTYKCTANLRFGRDNNWAGWGANIFHQAR--	67
WP_030983681	1	M---RRTIVGTCAFAVGIGLAG---PAVA----APVPVTLNCDCDTYKCTANLRFGRDNNWRAGWGANIFHQVTR--	67
WP_033267611	1	M---RRTIAGICVALVMASAPVFGASAVERPRD---APVPVTLNCDCDTYKCTASLRFGDGNWKA\$SANVYHQAVR--	67
WP_051772026	1	MT-fKKALVALVMMASAPVFGASAVERPRD---PVPVTLWCDCDTYKCTAAL\$FGDGGWVADWSADVYH-EDRa-	72
BAL02930	1	MN---FTASFLALLAAAQSIAAFPGTTGaktaLAPVPVTKLTCDFGSFMCTANLDFADGNWVAQWTSPVFRGNFg-	74
WP_006601771	1	M---RRTIVGTCAALAVLGIGSAA---PAVA----APVPVTLNCDCDTYKCTANLRFGRDNNWKA\$DASIFHQANR--	67
WP_054290187	1	-----MSCDCDTYRCTAAL\$FGDGGWVADWSANVYH-A-Rv-	34
WP_015801300	1	MNitTKRIAATSLAAASLLAAVV---PASAd----PVDASGLTCDGQTYECVANLDFGDGNWTA\$WGAGVMHVDDLa-	71
<u>AGP58216</u>	68	sqgraarsadapRGAAPVPVTTLCDCDTYKCTAGLQFGDGRWVAQWNANVFHQAMANAVPTasaktvkaprEAAAPVP	147
WP_037947329	73	shqrvttrsadapRGAAPVPVTTLCDCDTYKCTAGLQFGDGRWVAQWNANVFHQAVASAPatsakvkaprEAAAPVP	152
WP_059148527	73	shqratrsadalsRAAAPPVPVTTLCDCDTYKCTAGLQFGDGRWVAQWNANVFHQAVTNAVFaasaktvkaprEAAAPVP	152
WP_030825832	73	shqratgsadalsRAAAPPVPVTTLCDCDTYKCTAGLQFGDGRWVAQWNANVFHQAMAHESAasaktvkaprEAAAPVP	152
WP_062012936	73	srqratgsadalsRAAAPPVPVTTLCDCDTYKCTAGLQFGDGRWVAQWNANVFHQAMAHAVAasasaktvkaprEAAAPVP	152
WP_055550715	75	asaqsaesaqsaESVRAPVPVTTLCDCDTYKCTAGLQFGDGRWVAQWDARVFHQ-----gagamkaprGEAAPVP	146
WP_018088730	68	-----SAYRAPVPVTLNCDCDTGKCIANLQFGDGYWRA\$WSANVHFQAFRSADGA-----RLAAPVP	125
KJA18763	75	-----apdhkefmhEMTLAPVPVTKLVCDFDTFQCTANLDFGDGRWVAQW\$TSVFNHSLRSETTH-----L-APVP	139
WP_042158013	68	-----SASRAPVPVTLNCDCDTGKCIANLQFGDGYWRA\$WSATIFHQGLRSGDGT-----RLAAPVP	125
WP_030083396	68	-----SAYRAPVPVGLSCDGDGTGKCVANLQFGDGYWRA\$WPATIFHQALRSADGA-----RLAAPVP	125
WP_030983681	68	-----SAYRAPVPATSLSCDGDGTGKCVANLQFGDGYWRA\$WPATIFHQALRSADGA-----RLAAPVP	125
WP_033267611	68	-----SAYRAPVPATSLSCDGDGTGQCVAQGLQFGDGRWRA\$WPANVHFSGFH\$PGRK-----RLAAPVP	125
WP_051772026	73	-----QRDRMVPVTRLRCDDGDTYCTCtanLRFGDGRWVA\$WSDVYRTG--RAVQL-----DPVE	124
BAL02930	75	-----apdhkdiieQMAMAPVPVTELTCDGSSFLCTANLDFGDGNWVAQWPANVHFSGFH\$PGRK-----LFAPVP	140
WP_006601771	68	-----SASRAPVPATSLNCDDGDNCTCVA\$ALMFGDGRWRA\$WPATVFP\$ALRSAEG-----RLVAPVP	124
WP_054290187	35	-----EPRAMPVTRLRCDDGDTYKCTANLKF\$FGDGRWVA\$WDSVRVHHTN--RAEPR-----SPVR	86
WP_015801300	72	-----QQRSPVGVTKVTCDDGTWTCTANLDFGDGGWTAEWGAWIAHEDKD\$EDARE-----APVD	125
<u>AGP58216</u>	148	VTA\$LNCDGDTYKCTAGLQFGDGRWVAQWDASVFHQ\$-----	183
WP_037947329	153	VTA\$LNCDGDTYKCTANLQFGDGRWVAQWDASVFHQ\$-----	188
WP_059148527	153	VTA\$LNCDGDTYKCTANLQFGDGRWVAQWDASVFHQ\$-----	188
WP_030825832	153	VTA\$LNCDGDTYKCTANLQFGDGRWVAQWDASVFHQ\$-----	188
WP_062012936	153	VTA\$LNCDGDTYKCTANLQFGDGRWVAQWDASVFHQ\$-----	188
WP_055550715	147	VTA\$LNCDGDTGLCTANLQFGDGRWVAQW\$GARIFHQ-----	181
WP_018088730	126	ANS\$LNCDGNIGRC\$SVNLQFGDGNWKA\$NPATIYHL-----	160
KJA18763	140	VTKLVCDDGSFKCTGSLDFCGDKWVAQW\$TSVPHQ\$Safflegk	182
WP_042158013	126	ANS\$LNCDGNIGRC\$SVNLQFGDGNWKA\$NPATIYHL-----	160
WP_030083396	126	ANS\$LNCDGDTGRCSVNLQFGDGNWKA\$NPATIYHQ-----	160
WP_030983681	126	ANS\$LNCDGDTGRCSVNLQFGDGNWKA\$NPATIYHQ-----	160
WP_033267611	126	ANS\$LNCDGDTGRCSVNLQFGDGNWKA\$NPATIYHQ-----	161
WP_051772026	125	VTGLWCDGDTYKCKAAL\$FGDGRWVA\$WDSADIFHT-----	159
BAL02930	141	VTDLNCDGETFMCTGNLDFADGNWKAQWNTNVP\$HNnfffkqn-	182
WP_006601771	125	ANS\$LNCDGDTGRCSVNLQFGDGGWKA\$WAATIYHQ-----	160
WP_054290187	87	VTGLWCDGDTYKCKAAL\$FGDGRWVA\$WNVK\$VYHS-----	121
WP_015801300	126	VYELTCDDGDEGKCVAKLDFGDGRWAA\$WSARIKHS-----	160

Figure S1. Multiple sequence alignment of the hypothetical proteins identified through similarity search. Aligned protein sequences are from *Streptomyces rapamycinicus* NRRL 5491 (AGP58216); *Streptomyces* sp. PRh5 (WP_037947329); *Streptomyces violaceusniger* (WP_059148527); *Streptomyces hygroscopicus* (WP_030825832); *Streptomyces sporocinereus* (WP_062012936); *Streptomyces* sp. NBRC 110028 (WP_055550715); *Streptomyces* sp. FxanaC1 (WP_018088730); *Hypoloma sublateritium* FD-334 SS-4 (KJA18763); *Streptomyces* sp. NBRC 110027 (WP_042158013); *Streptomyces decoyicus* (WP_030083396); *Streptomyces* sp. NRRL S-1813 (WP_030983681); *Streptomyces lydicus* (WP_033267611); *Saccharothrix* sp. NRRL B-16314 (WP_051772026); *Pholiota nameko* (BAL02930); *Streptomyces auratus* (WP_006601771); *Kibdelosporangium phytohabitans* (WP_054290187) and *Actinosynnema mirum* (WP_015801300).

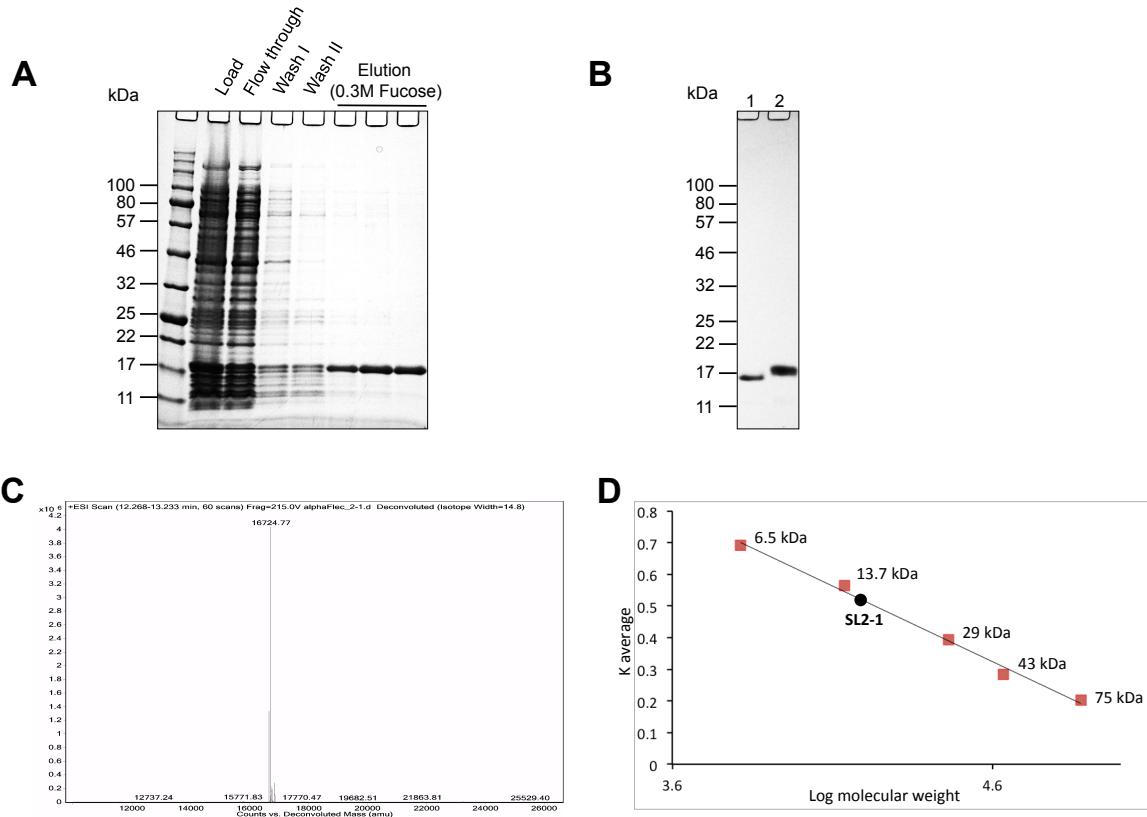


Figure S2. Expression, purification and characterization of recombinant SL2-1. (A) Recombinant protein was expressed in *E. coli* and purified by affinity chromatography using α -L-fucose agarose resin. (B) Non-reduced (lane 1) and reduced (lane 2) purified protein detected as single band of \sim 17 kDa on the denaturing polyacrylamide gel. (C) Electrospray ionization mass spectrometry of recombinant SL2-1. (D) The apparent molecular mass of the native SL2-1 was estimated by gel filtration on a HiPrep 16/60 Sephadex S-200 HR column.

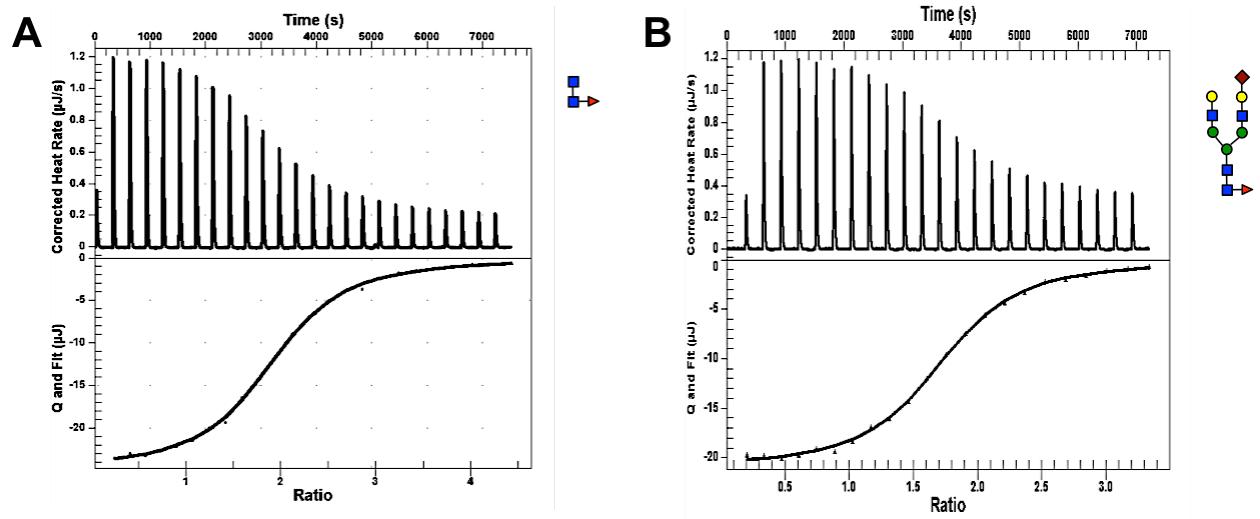


Figure S3. Typical ITC titration curves obtained for α 1-6 fucosylated chitobiose (N2F) and a monosialo-fucosylated biantennary N-glycan (A1F).

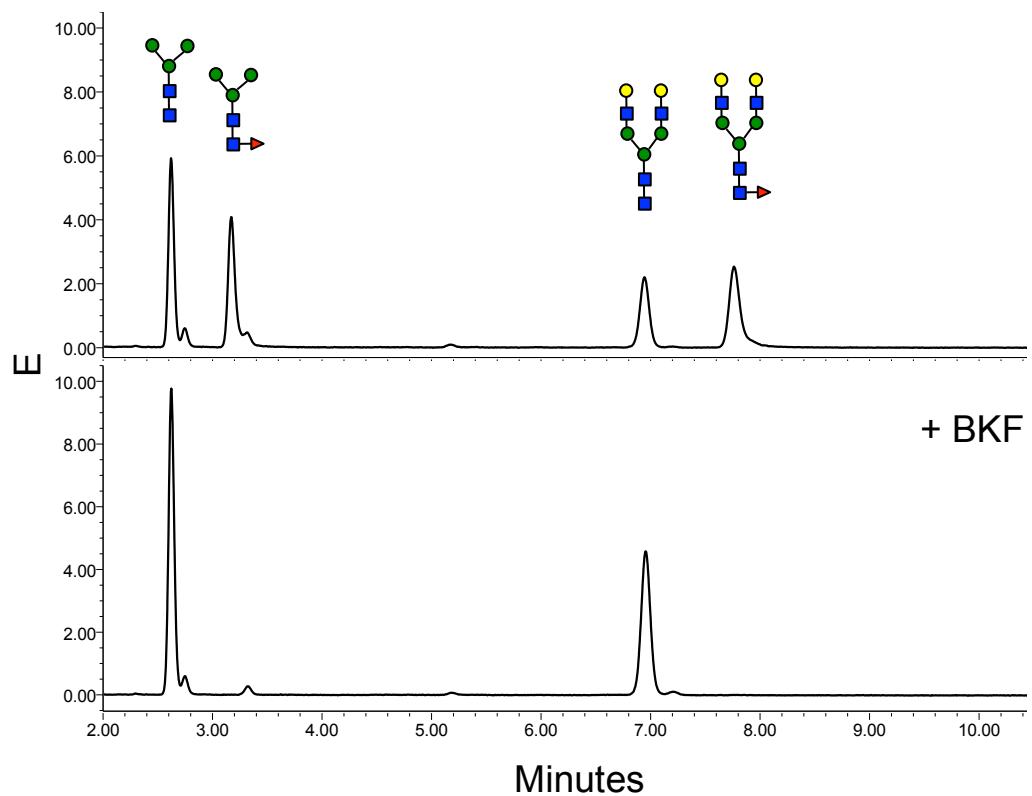


Figure S4. UPLC-HILIC-FLR profiles of undigested vs BKF-digested M3N2, M3N2F, NA2 and NA2F N-glycans.

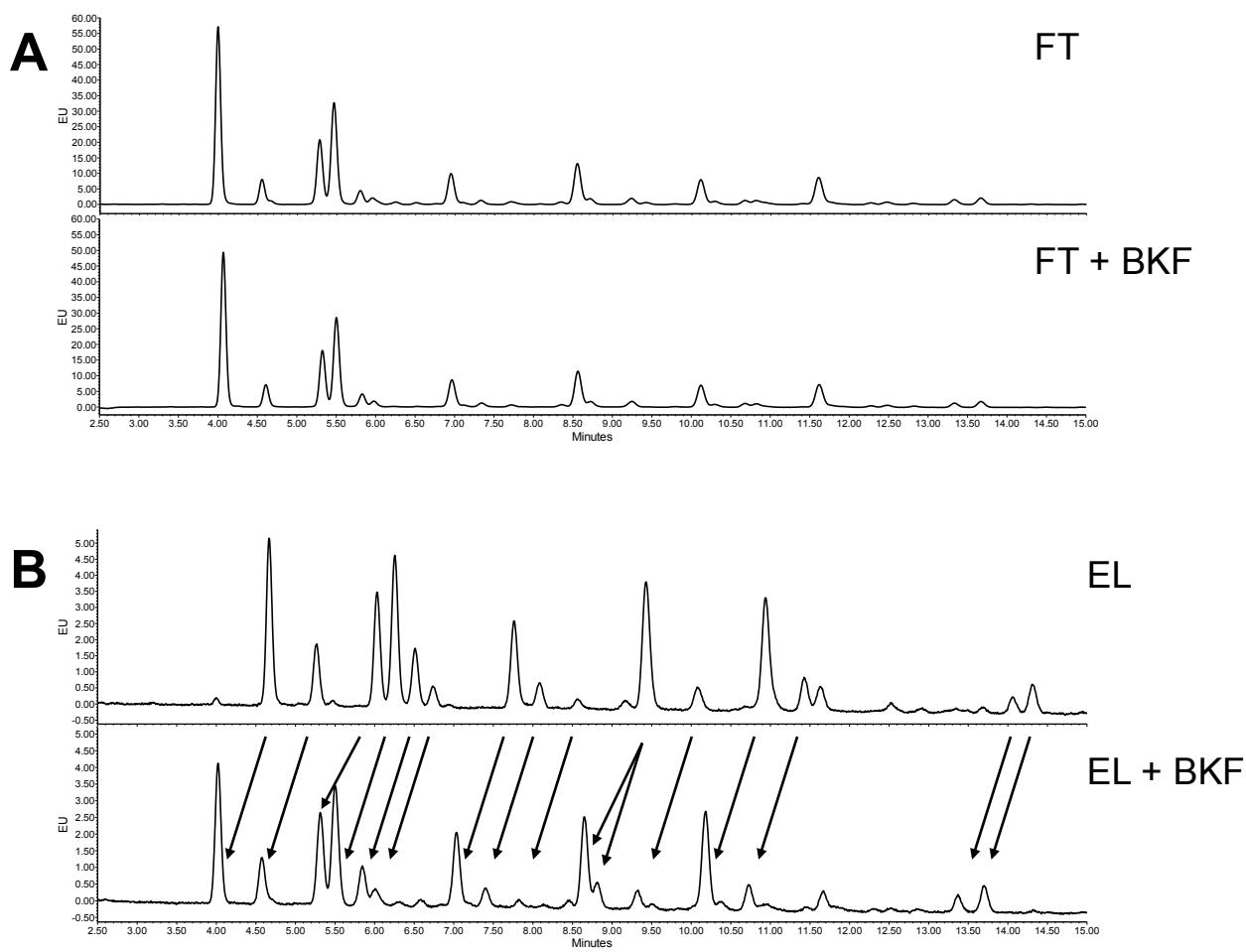
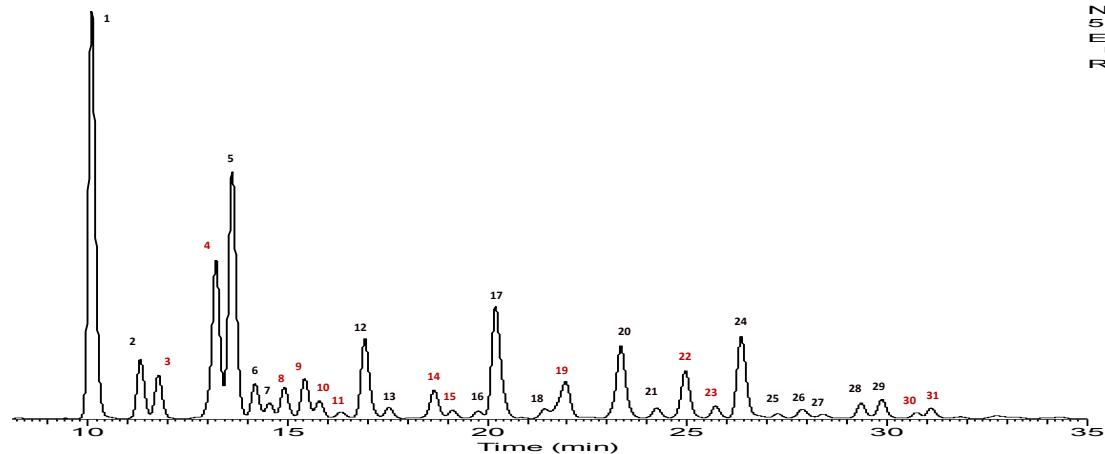


Figure S5. UPLC-HILIC-FLR profiles of undigested vs BKF-digested N-glycans from rabbit serum IgG. Unbound (FT) (A) and lectin-bound (EL) (B) fractions were treated with α 1-2,4,6 fucosidase (BKF) and analyzed by Glycan BEH Amide HILIC chromatographic separation.

Figure S6. Structural identification of N-glycans from rabbit serum IgG by mass spectrometry. Identified carbohydrate compositions, observed masses and their sensitivity to BKF treatment provided in the table below (core-fucosylated structures labeled in red; non-fucosylated – in black).



Peak #	Composition	m/z [+1] [‡] theor.	m/z [+1] [‡] observed	BKF-sensitive
1	HexNAc(4)Hex(3)	1437.5625	1437.65	-
2	HexNAc(5)Hex(3)	1640.6419	1640.66	-
3	HexNAc(4)Hex(3)dHex(1)	1583.6205	1583.65	+
4	HexNAc(4)Hex(4) HexNAc(5)Hex(3)dHex(1)	1599.6154 1786.6998	1599.66 1786.75	- +
5	HexNAc(4)Hex(4)	1599.6154	1599.66	-
6	HexNAc(5)Hex(4)	1802.6947	1802.73	-
7	HexNAc(5)Hex(4)	1802.6947	1802.73	-
8	HexNAc(4)Hex(4)dHex(1)	873.3403*	873.36*	+
9	HexNAc(4)Hex(4)dHex(1)	873.3403*	873.36*	+
10	HexNAc(5)Hex(4)dHex(1)	974.88*	974.95*	+
11	HexNAc(5)Hex(4)dHex(1)	974.88*	974.95*	+
12	HexNAc(4)Hex(5)	1761.6682	1761.75	-
13	HexNAc(5)Hex(5)	1964.7476	1964.84	-
14	HexNAc(4)Hex(5)dHex(1)	954.367*	954.37*	+
15	HexNAc(5)Hex(5)dHex(1)	1055.9064*	1055.96*	+
16	?	?	1055.46*	-
17	HexNAc(4)Hex(4)Neu5Gc(1)	953.8565*	953.87*	-
18	HexNAc(5)Hex(4)Neu5Gc(1)	1055.3962*	1055.46*	-
19	HexNAc(4)Hex(4)Neu5Gc(1)dHex(1) HexNAc(5)Hex(4)Neu5Gc(1)dHex(1)	1026.8854* 1128.4251*	1026.95* 1128.46*	+
20	HexNAc(4)Hex(5)Neu5Gc(1)	1034.8829*	1034.87*	-
21	HexNAc(5)Hex(5)Neu5Gc(1)	1136.4226*	1136.42*	-
22	HexNAc(4)Hex(5)Neu5Gc(1)dHex(1) HexNAc(4)Hex(5)Neu5Ac(2)	1107.9119* 1172.4332*	1107.96* 1172.46*	+
23	HexNAc(5)Hex(5)Neu5Gc(1)dHex(1)	1209.4515*	1209.47*	+
24	HexNAc(4)Hex(5)Neu5Ac(2)	1172.4332*	1172.46*	-
25	HexNAc(5)Hex(6)Neu5Gc(1)	1217.449*	1217.47*	-
26	HexNAc(4)Hex(5)Neu5Ac(1)Neu5Gc(1)	1180.4306*	1180.46*	-
27	HexNAc(5)Hex(5)Neu5Ac(1)Neu5Gc(1)	1281.9703*	1281.97*	-
28	HexNAc(4)Hex(5)Neu5Gc(2)	1188.4281*	1188.46*	-
29	HexNAc(5)Hex(5)Neu5Gc(2)	1289.9678*	1289.97*	-
30	HexNAc(4)Hex(5)Neu5Gc(2)dHex(1)	1261.457*	1261.47*	+
31	HexNAc(5)Hex(5)Neu5Gc(2)dHex(1)	1362.9967*	1362.97*	+

[‡]2AB-labeled glycans

*m/z [+2]

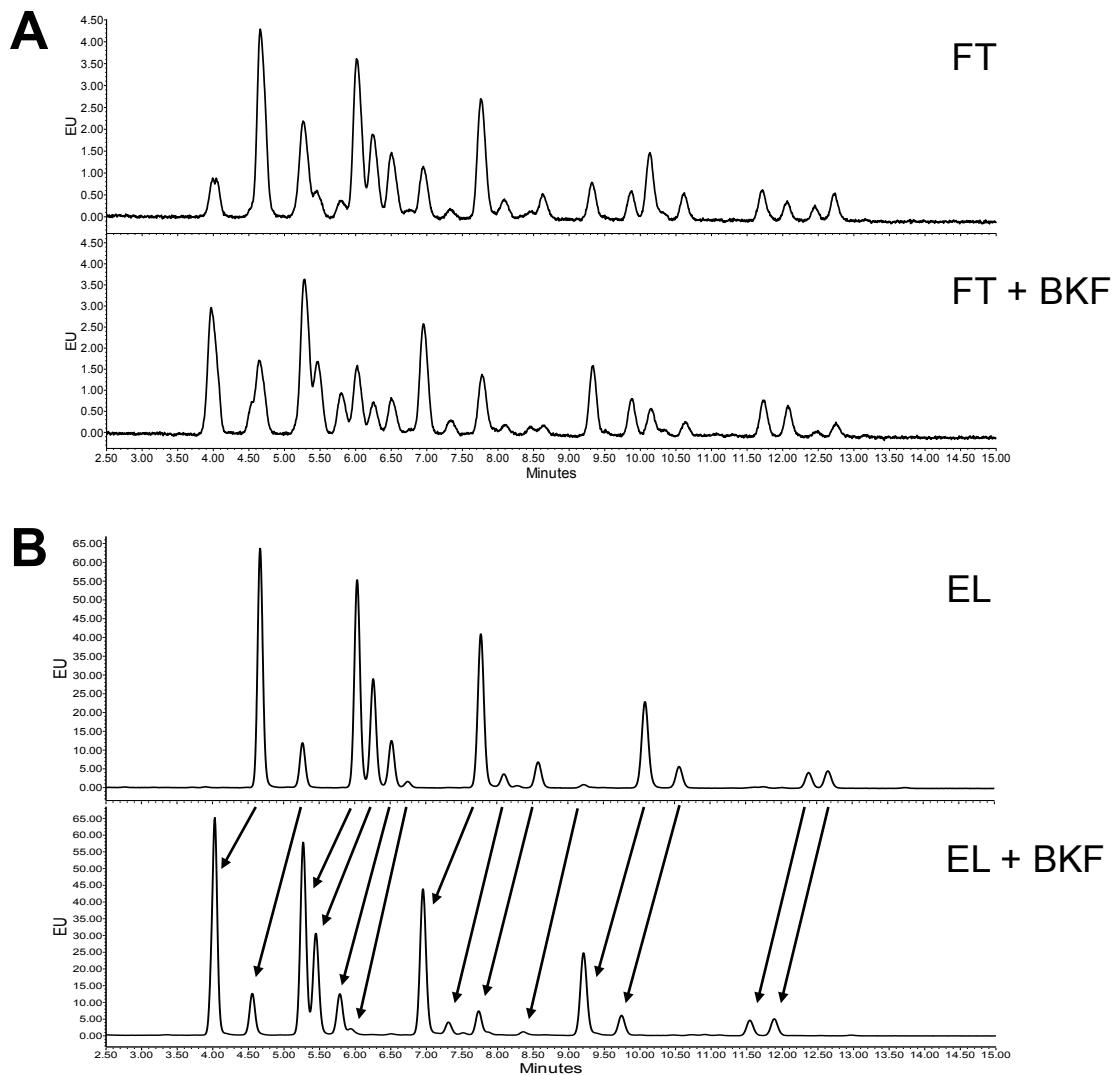
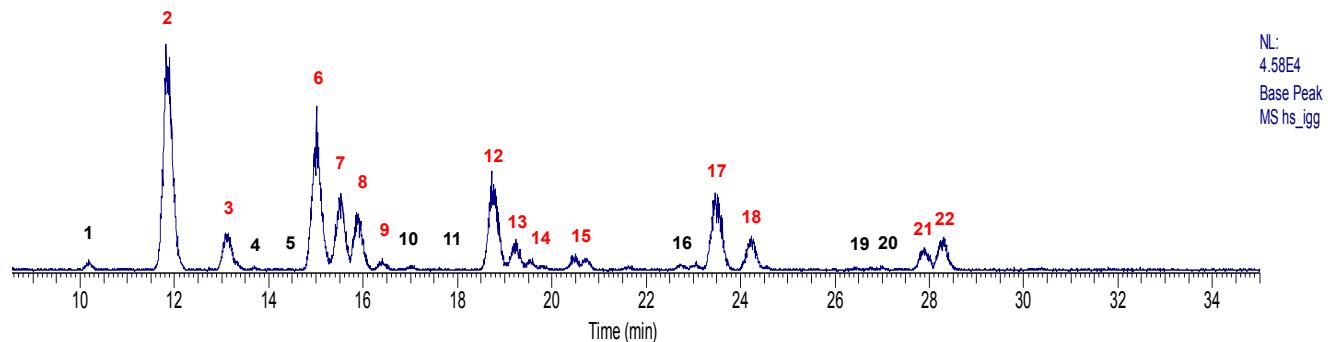


Figure S7. UPLC-HILIC-FLR profiles of BKF-digested vs undigested N-glycans from human serum IgG. (Unbound (FT) (A) and lectin-bound (EL) (B) fractions were treated with α 1-2,4,6 fucosidase (BKF) and analyzed by Glycan BEH Amide HILIC chromatographic separation.

Figure S8. Structural identification of N-glycans from human serum IgG by mass spectrometry.
 Identified carbohydrate compositions, observed masses and their sensitivity to BKF treatment provided in the table below (core-fucosylated structures labeled in red; non-fucosylated – in black).



Peak #	Composition	m/z [+1] [‡] theor.	m/z [+1] [‡] observed	BKF-sensitive
1	HexNAc(4)Hex(3)	1437.5625	1437.65	-
2	HexNAc(4)Hex(3)dHex(1) HexNAc(5)Hex(3)	1583.6205 1640.6419	1583.65 1640.66	+ -
3	HexNAc(5)Hex(3)dHex(1)	1786.6998	1786.75	+
	HexNAc(4)Hex(4)	1599.6154	1599.66	-
4	HexNAc(4)Hex(4)	1599.6154	1599.66	-
5	HexNAc(5)Hex(4)	1802.6947	1802.75	-
6	HexNAc(4)Hex(4)dHex(1) HexNAc(5)Hex(4)	1745.6733 1802.6947	1745.75 1802.75	+ -
7	HexNAc(4)Hex(4)dHex(1)	1745.6733	1745.75	+
8	HexNAc(5)Hex(4)dHex(1)	1948.7527	1948.84	+
9	HexNAc(5)Hex(4)dHex(1)	1948.7527	1948.68	+
10	HexNAc(4)Hex(5)	1761.6682	1761.75	-
11	HexNAc(5)Hex(5)	982.8774*	982.87*	-
12	HexNAc(4)Hex(5)dHex(1) HexNAc(3)Hex(4)Neu5Ac(1)dHex(1) HexNAc(5)Hex(4)Neu5Ac(1)	1907.7261 917.3483* 1047.3987*	1907.76 917.36* 1047.37*	+ + -
13	HexNAc(5)Hex(5)dHex(1) HexNAc(5)Hex(4)Neu5Ac(1)	1055.9064* 1047.3987*	1055.96* 1047.46*	+ -
14	HexNAc(4)Hex(4)Neu5Ac(1)dHex(1) HexNAc(5)Hex(4)Neu5Ac(1)dHex(1)	1018.8880* 1120.4277*	1018.87* 1120.46*	+ +
15	HexNAc(5)Hex(4)Neu5Ac(1)dHex(1) HexNAc(4)Hex(5)Neu5Ac(1)	1120.4277* 1026.8854*	1120.46* 1026.87*	+ -
16	HexNAc(5)Hex(5)Neu5Ac(1)	1128.4251*	1128.46*	-
17	HexNAc(4)Hex(5)Neu5Ac(1)dHex(1)	1099.9144*	1099.96*	+
18	HexNAc(5)Hex(5)Neu5Ac(1)dHex(1)	1201.4541*	1201.46*	+
19	HexNAc(4)Hex(5)Neu5Ac(2)	1172.4332*	1172.38*	-
20	HexNAc(5)Hex(5)Neu5Ac(2)	1273.9728*	1273.97*	-
21	HexNAc(4)Hex(5)Neu5Ac(2)dHex(1)	1245.4621*	1245.47*	+
22	HexNAc(5)Hex(5)Neu5Ac(2)dHex(1)	1347.0018*	1347.06*	+

[‡]2AB-labeled glycans ,

*m/z [+2]