Electronic Supplementary Information (ESI) for Green Chemistry

Enrichment of glycopeptides using environmentally friendly wood materials

Yuye Zhou, ‡[a] Xia Sheng, ‡[b] Jonas Garemark, [b] Leila Josefsson, [a] Licheng Sun, [c], [d] Yuanyuan Li, *[b] Åsa Emmer *[a]

[a] KTH Royal Institute of Technology, School of Engineering Sciences in Chemistry, Biotechnology and Health, Department of Chemistry, Division of Applied Physical Chemistry, Analytical Chemistry, Stockholm SE−100 44, Sweden

[b] KTH Royal Institute of Technology, School of Engineering Sciences in Chemistry, Biotechnology and Health, Department of Fiber and Polymer Technology, Wallenberg Wood Science Center, Stockholm SE−100 44, Sweden

[c] KTH Royal Institute of Technology, School of Engineering Sciences in Chemistry, Biotechnology and Health, Department of Chemistry, Division of Organic Chemistry, Stockholm SE−100 44, Sweden

[d] Westlake University, School of Science, Center of Artificial Photosynthesis for Solar Fuels, Hangzhou CN−310024, China

Corresponding authors: * E-mail: aae@kth.se; * E-mail: yua@kth.se

‡ These authors contributed equally to this work.

S1. Experimental Section.

Recovery from wood tips: Before estimation of the recovery, IgG glycopeptide aliquots were collected and mixed after enrichment using N-wood tips loaded with 10 μg IgG digests. The IgG glycopeptide aliquot was mixed with IgG1 internal standard (IS, EEQYN(GlcNAc)STYR, *m/z* 1392 Da, 50ng/mL, Guoping Pharmaceutical Co., LTD, Anhui, China) with the following volume ratios ($V_{glycopeptide}/V_{IS}$) sequence: 0.5/1, 1/1, 1.5/1, 2/1, 2.5/1. The IgG1 IS used here was purchased from Guoping Pharmaceutical Co., LTD, (Anhui, China). The sample containing IgG enriched glycopeptides and IgG1 IS was then mixed with HCCA (saturated in TA30) with the volume ratio of 1/1. 1 μL of the mixture was then applied on a MALDI ground steel plate, for each mixture, 12 spots were prepared and analyzed. The area ratios between enriched IgG1 main glycopeptides (*m/z* 2634, 2796, 2958) and IgG1 IS ($A_{glycopeptide}$ / A_{IS}) were calculated and plotted for each mixing ratio of $V_{glycopeptide}/V_{IS}$. This way, the correlation equation for the calibration curve was obtained as a base for the recovery calculation. A separate batch of enriched IgG glycopeptide aliquots was prepared for the recovery evaluation. A 5 μL aliquot of enriched IgG glycopeptides was enriched one more time using N-wood tips or D-wood tips. The elution fraction obtained after re-enrichment was collected and concentrated to 5 μL, and marked as recovered IgG glycopeptides. 5 μL of enriched or recovered IgG glycopeptides was then mixed with 5 μL IgG1 IS and 10 μL HCCA. 1 μL of this mixture was applied on a MALDI plate, and analyzed (totally 12 spots from 3 replicates were analyzed), the area ratios between enriched or recovered IgG1 glycopeptides (*m/z* 2634, 2796, 2958), and IgG1 IS were calculated as $A_{enriched}$ / A_{IS} or $A_{recovered}$ / A_{IS}). According to the correlation equation obtained, the actual volume ratio of $V_{enriched}/V_{IS}$ or $V_{recovered}/V_{IS}$ could be calculated. Finally, the recovery could be calculated: Recovery = [$V_{recovered}/V_{IS}$] / [$V_{enriched}/V_{IS}$] *100%. For all analyses performed for the recovery evaluation, the laser was set to 70% of total intensity, and

each mass spectrum was a sum of 10000 shots, random walk laser movement (complete sample) with 100 laser shots per raster spot was applied.

S2. Results and discussion

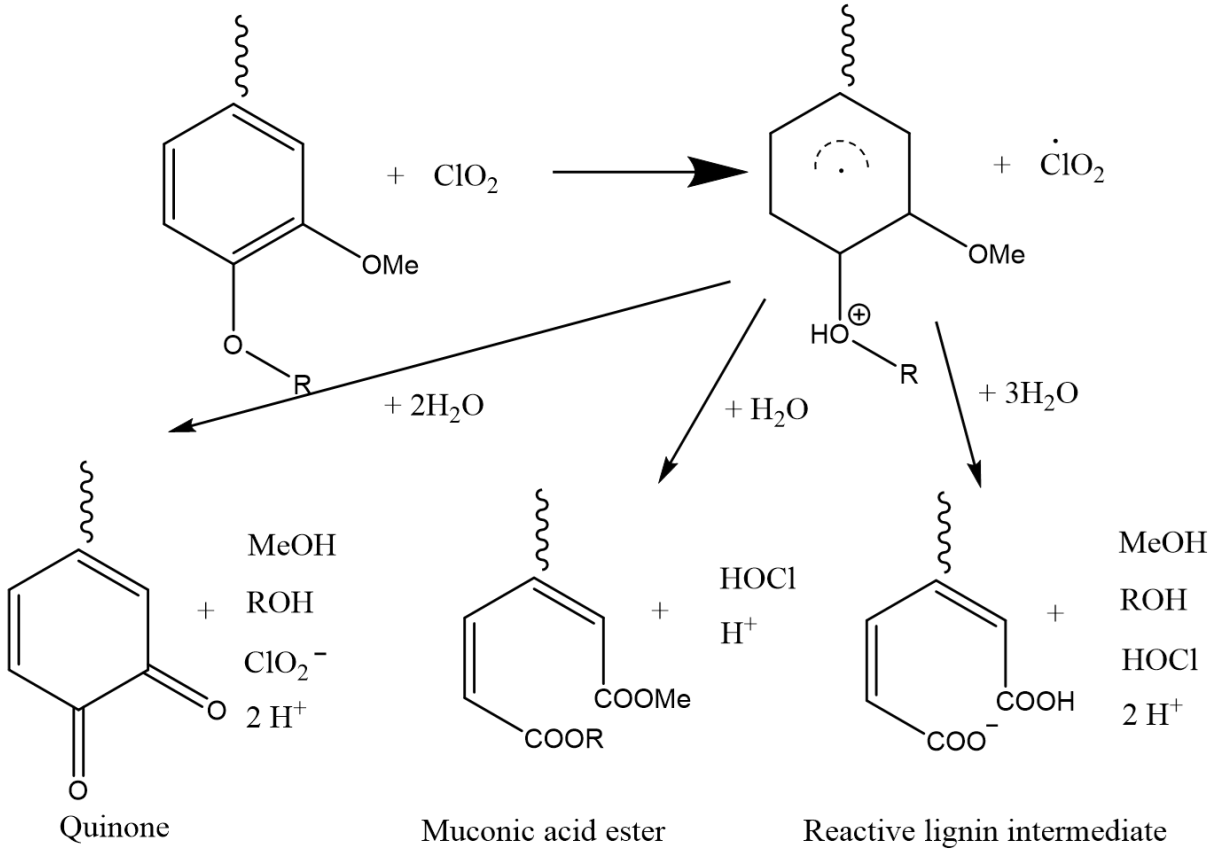


Fig. S1. Possible reactions of delignification with chlorine dioxide.[1]

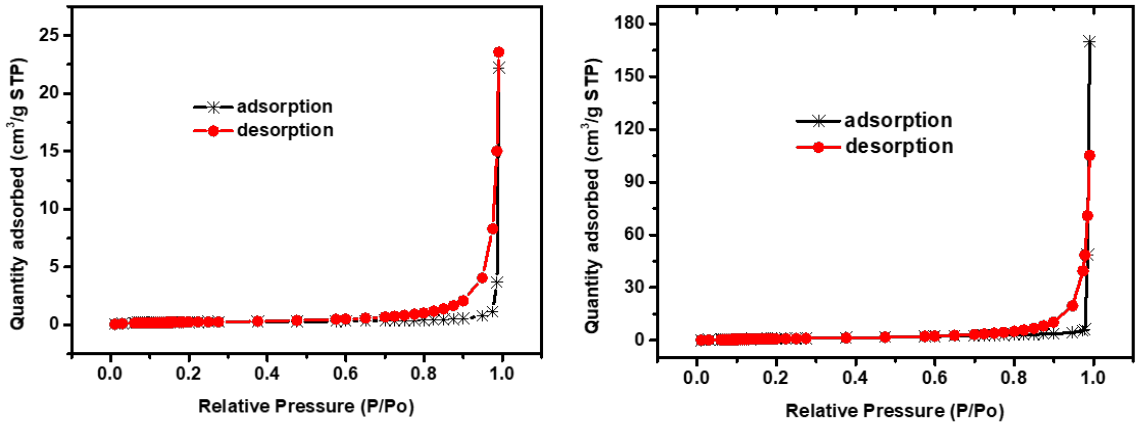


Fig. S2. N_2 adsorption/desorption isotherms of N-wood (left) and D-wood (right).

Table S1. Loading and elution solutions applied using different tips.

	Loading solution (v/v/v)	Elution solution
N-wood and D-wood tips	83% ACN / 0.1% TFA / 16.9% H_2O	H_2O
	83% ACN / 1% TFA / 16% H_2O	H_2O
	83% ACN / 3% TFA / 14% H_2O	H_2O
	86% ACN / 0.1% TFA / 13.9% H_2O	H_2O
	86% ACN / 1% TFA / 13% H_2O	H_2O
	86% ACN / 1% TFA / 13% H_2O	1% TFA
	86% ACN / 3% TFA / 11% H_2O	H_2O
	89% ACN / 0.1% TFA / 10.9% H_2O	H_2O
	89% ACN / 1% TFA / 10% H_2O	H_2O
HILIC tips	86% ACN / 1% TFA / 13% H_2O	H_2O

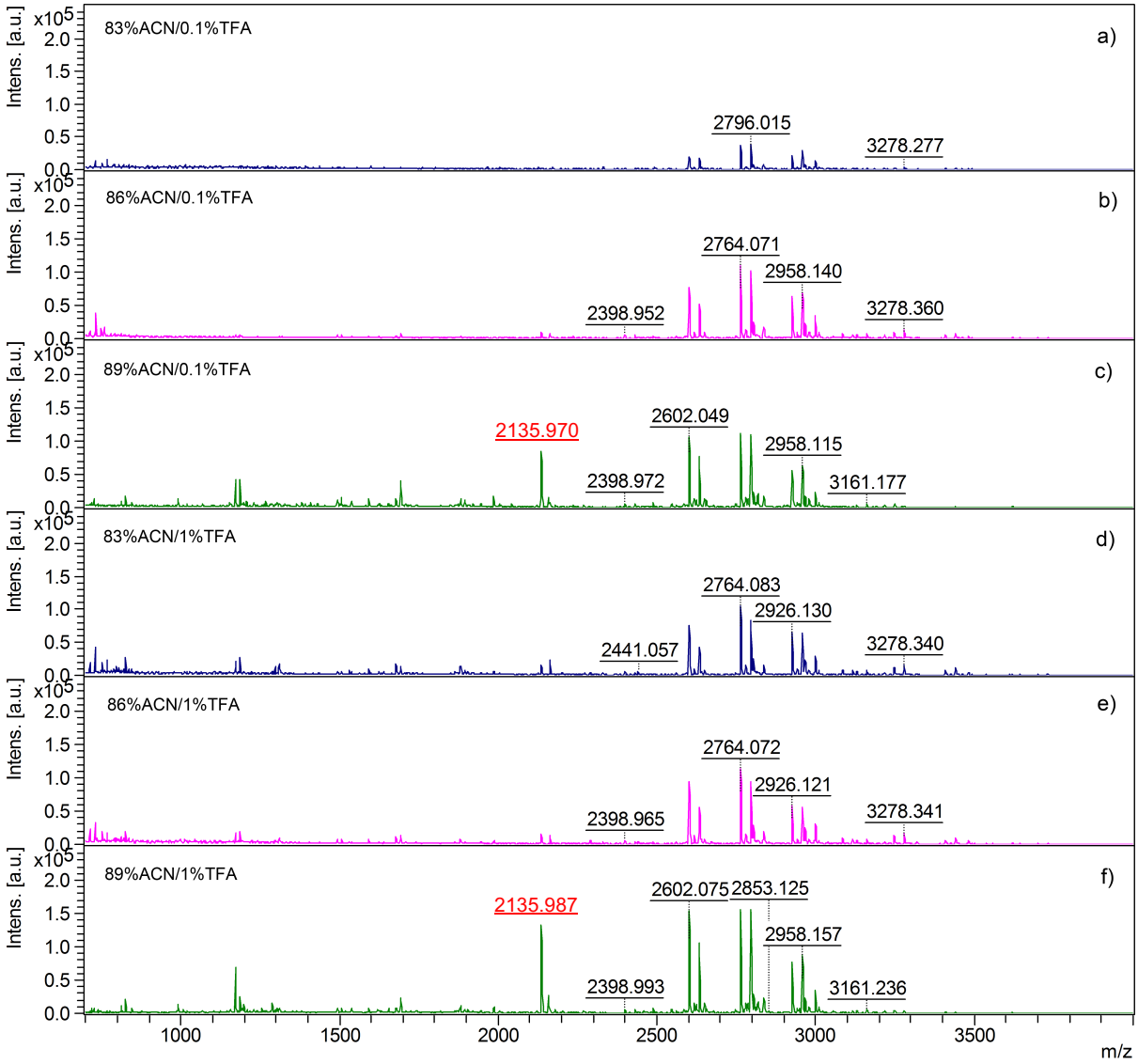


Fig. S3. MALDI-TOF-MS spectra of elution fractions obtained using N-wood tips with different loading solutions (10 µg IgG digest was loaded). MALDI matrix: DHB. Glycopeptides were detected in the range *m/z* 2200 - 3500 Da.

Preconditioning: N-wood tips were preconditioned 5 times with 100 µL loading solution

Loading: a) 83%ACN/0.1%TFA b) 86%ACN/0.1%TFA c) 89%ACN/0.1%TFA

d) 83%ACN/1%TFA e) 86%ACN/1%TFA f) 89%ACN/1%TFA

Washing: 3 times with 100 µL loading solution

Elution: H_2O

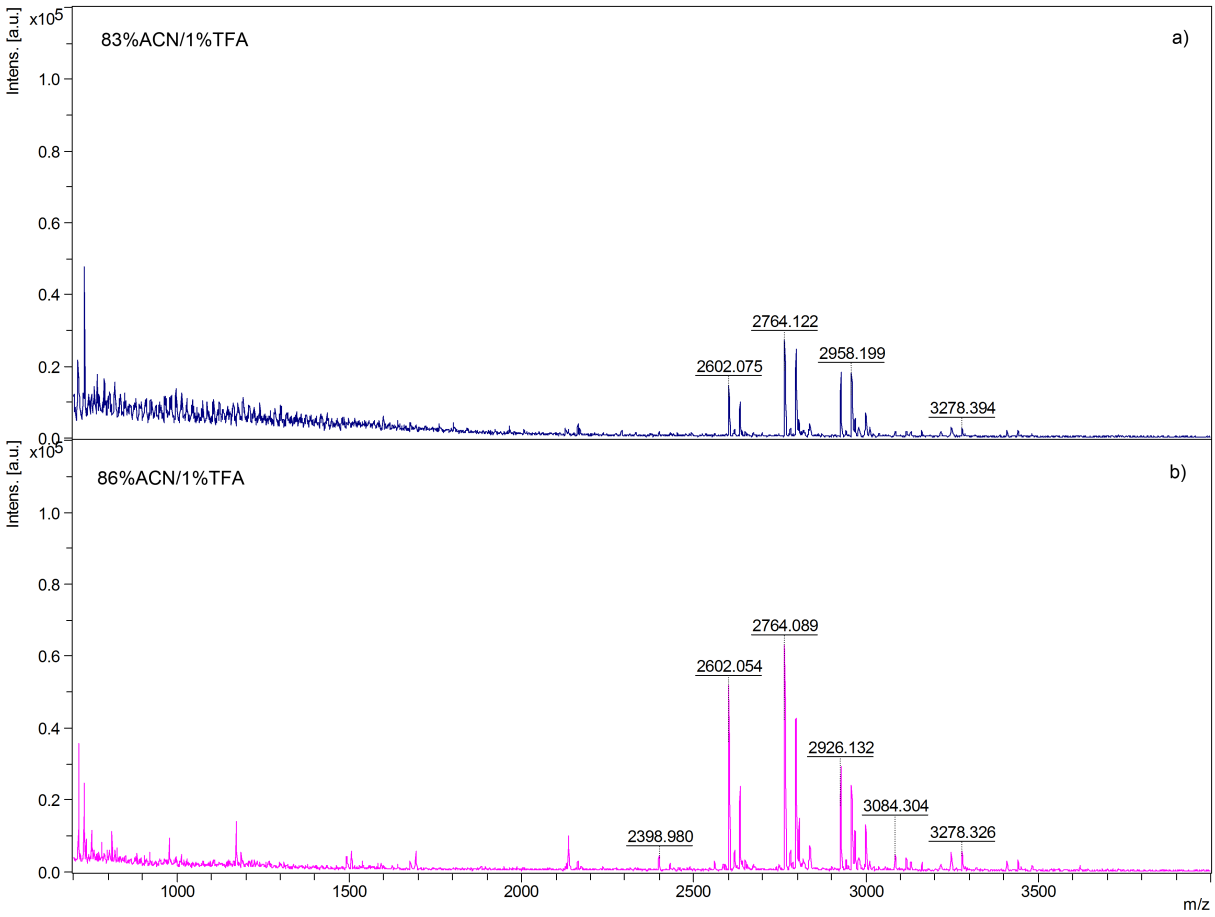


Fig. S4. MALDI-TOF-MS spectra of elution fractions obtained using N-wood tips with different loading solutions (5 µg IgG digest was loaded). MALDI matrix: DHB. Glycopeptides were detected in the range *m/z* 2200 - 3500 Da.

Preconditioning: N-wood tips were preconditioned 5 times with 100 µL loading solution

Loading: a) 83%ACN/1%TFA b) 86%ACN/1%TFA

Washing: 3 times with 100 µL loading solution

Elution: H_2O

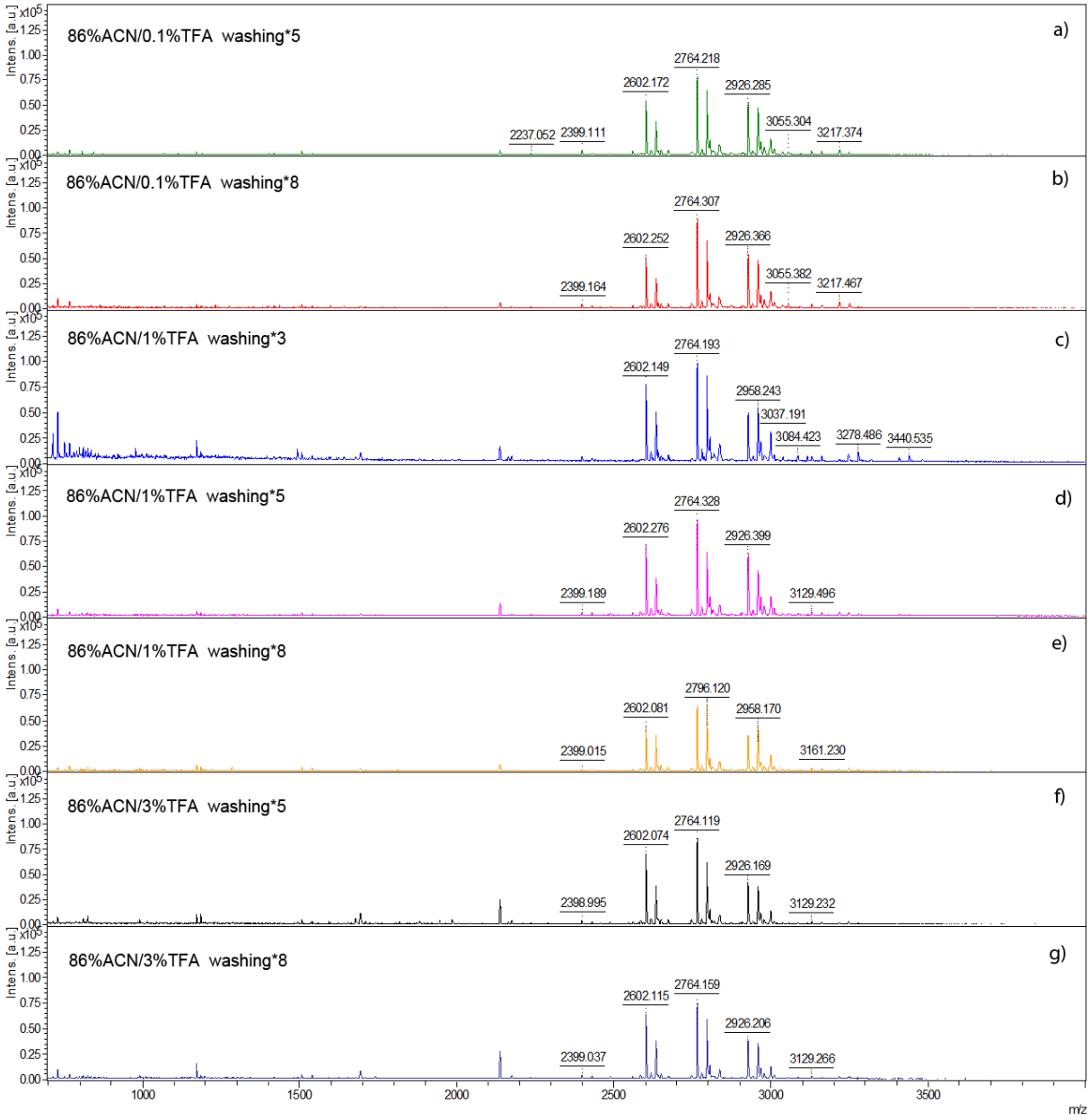


Fig. S5. MALDI-TOF-MS spectra of elution fractions obtained using N-wood tips with different loading solutions, and number of washing cycles (7.5 µg IgG digest was loaded). MALDI matrix: DHB. Glycopeptides were detected in the range *m/z* 2200 - 3500 Da.

Preconditioning: N-wood tips were preconditioned 5 times with 100 µL loading solution

Loading: a and b) 86%ACN/0.1%TFA

c, d and e) 86%ACN/1%TFA

f and g) 86%ACN/3%TFA

Washing: c) washed 3 times, a), d) and f) washed 5 times, b), e) and g) washed 8 times with 100 µL loading solution

Elution: H_2O

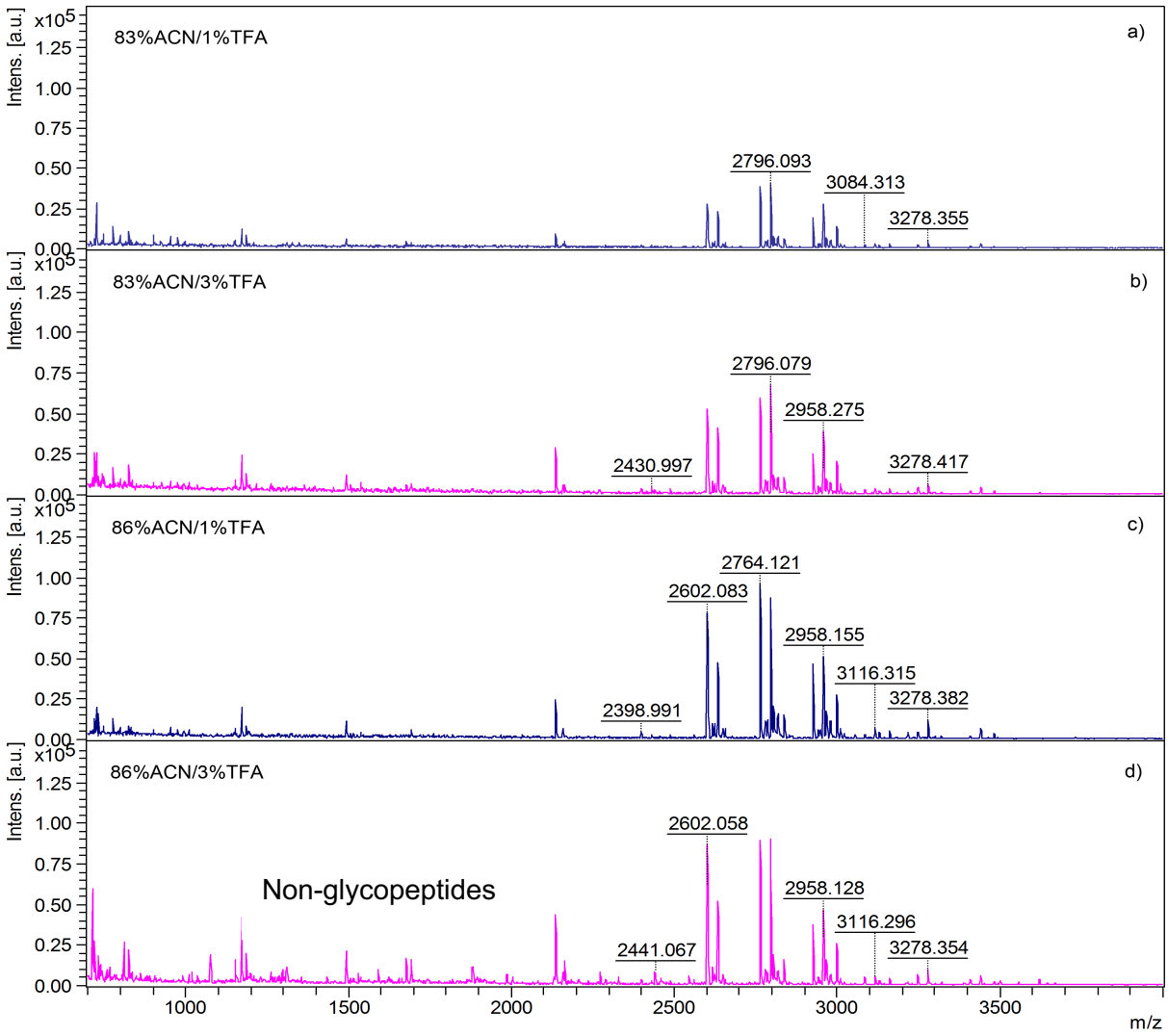


Fig. S6. MALDI-TOF-MS spectra of elution fractions obtained using D-wood tips with different loading solutions (7.5 µg IgG digest was loaded). MALDI matrix: DHB. Glycopeptides were detected in the range *m/z* 2200 - 3500 Da.

Preconditioning: D-wood tips were preconditioned 5 times with 100 µL loading solution

Loading: a) 83%ACN/1%TFA b) 83%ACN/3%TFA

c) 86%ACN/1%TFA d) 86%ACN/3%TFA

Washing: 3 times with 100 µL loading solution

Elution: H_2O

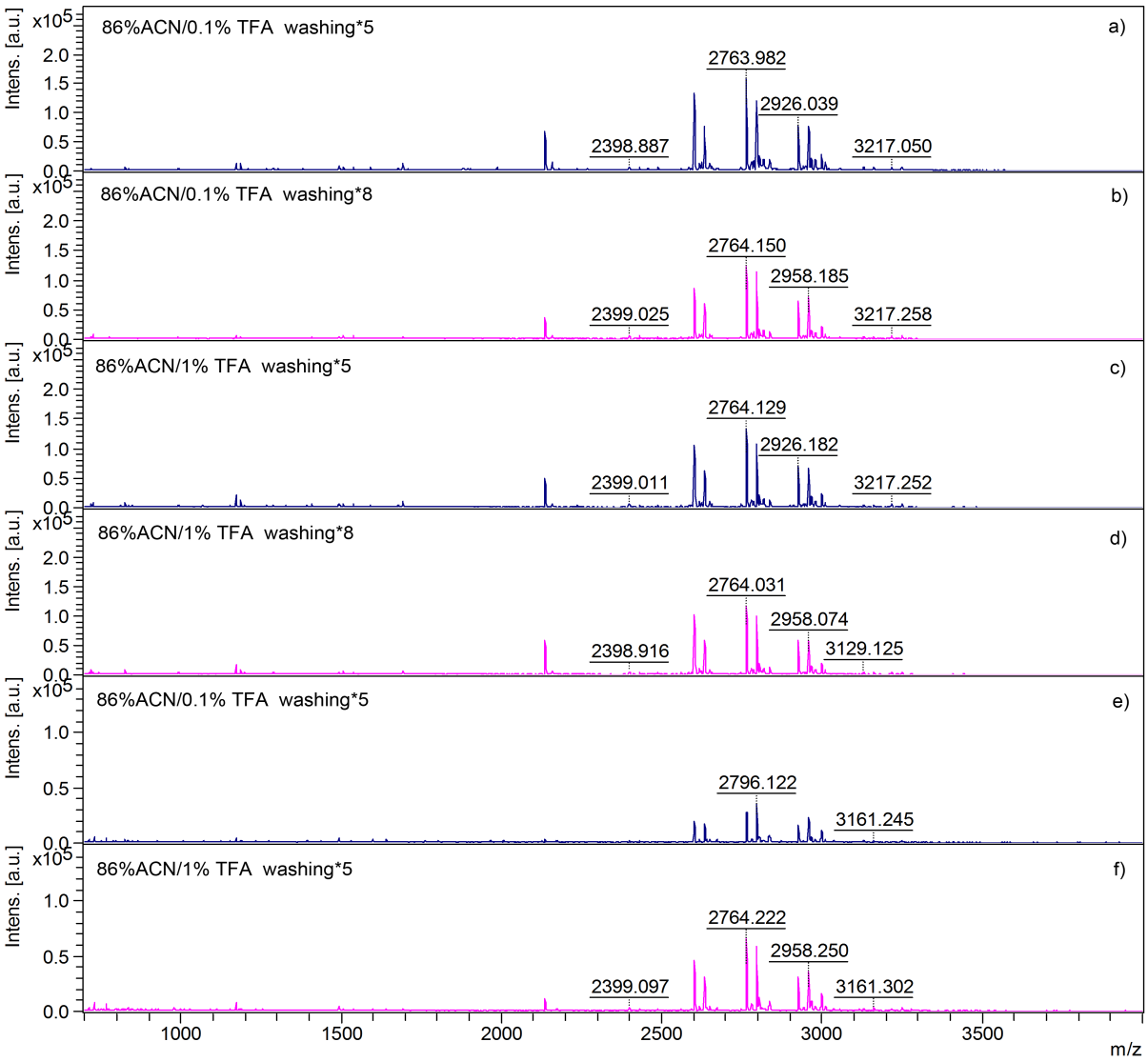


Fig. S7. MALDI-TOF-MS spectra of elution fractions obtained using D-wood tips with different loading solutions, number of washing cycles and loading amount of IgG digests. a), b), c) and d) **7.5 µg IgG digest**, e) and f) **5 µg IgG digest**. MALDI matrix: DHB. Glycopeptides were detected in the range *m/z* 2200 - 3500 Da.

Preconditioning: D-wood tips were preconditioned 5 times with 100 µL loading solution

Loading: a), b) and e) 86%ACN/0.1% TFA, c), d) and f) 86%ACN/1% TFA

Washing: a), c), e) and f) washed 5 times, b) and d) washed 8 times with 100 µL loading solution

Elution: H_2O

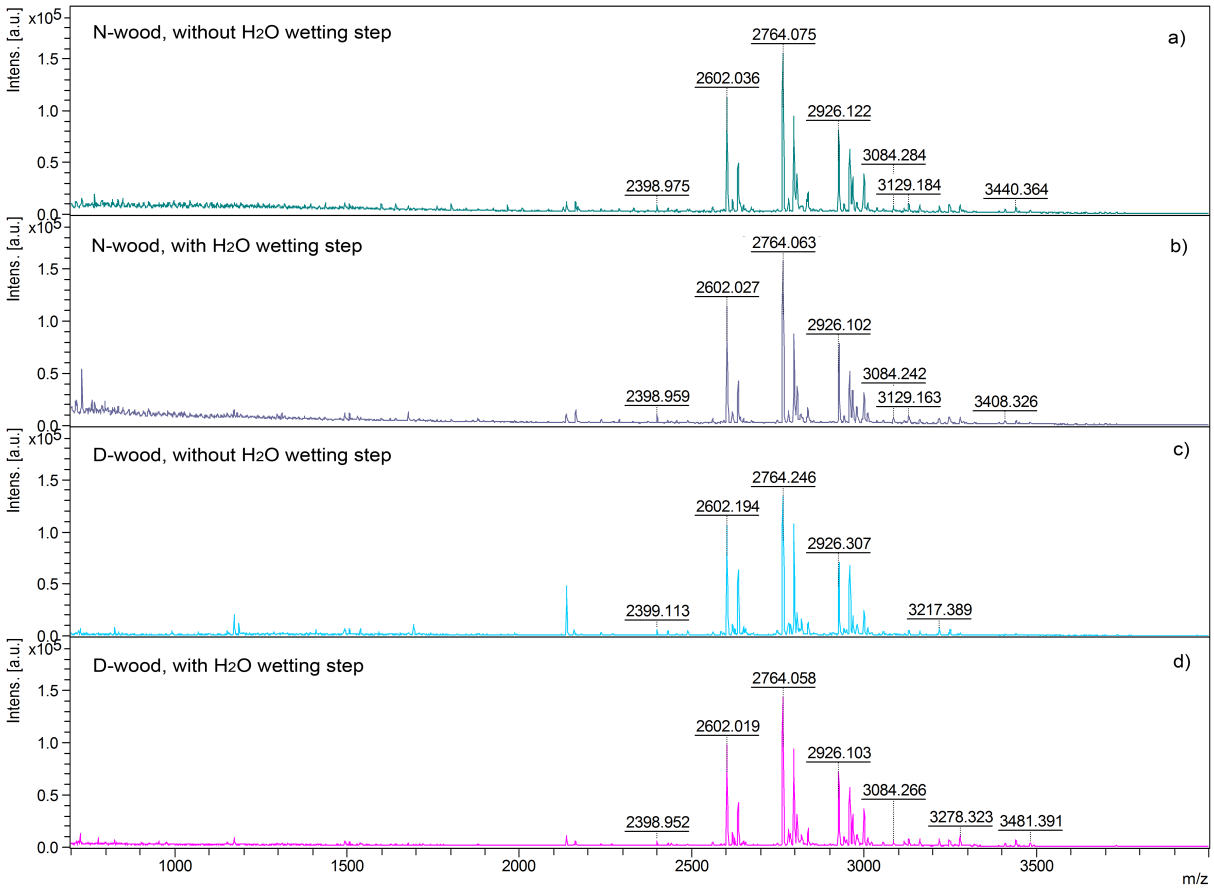


Fig. S8. MALDI-TOF-MS spectra of elution fractions obtained using a) and b) N-wood tips, c) and d) D-wood tips, with different preconditioning steps (7.5 µg IgG digest was loaded). MALDI matrix: DHB. Glycopeptides were detected in the range *m/z* 2200 - 3500 Da.

Preconditioning: a) and c) were preconditioned 5 times with 100 µL loading solution

b) and d) were wetted 5 times with 100 µL H_2O

and preconditioned 5 times with 100 µL loading solution

Loading: 86%ACN/1% TFA

Washing: washed 5 times with 100 µL loading solution

Elution: H_2O

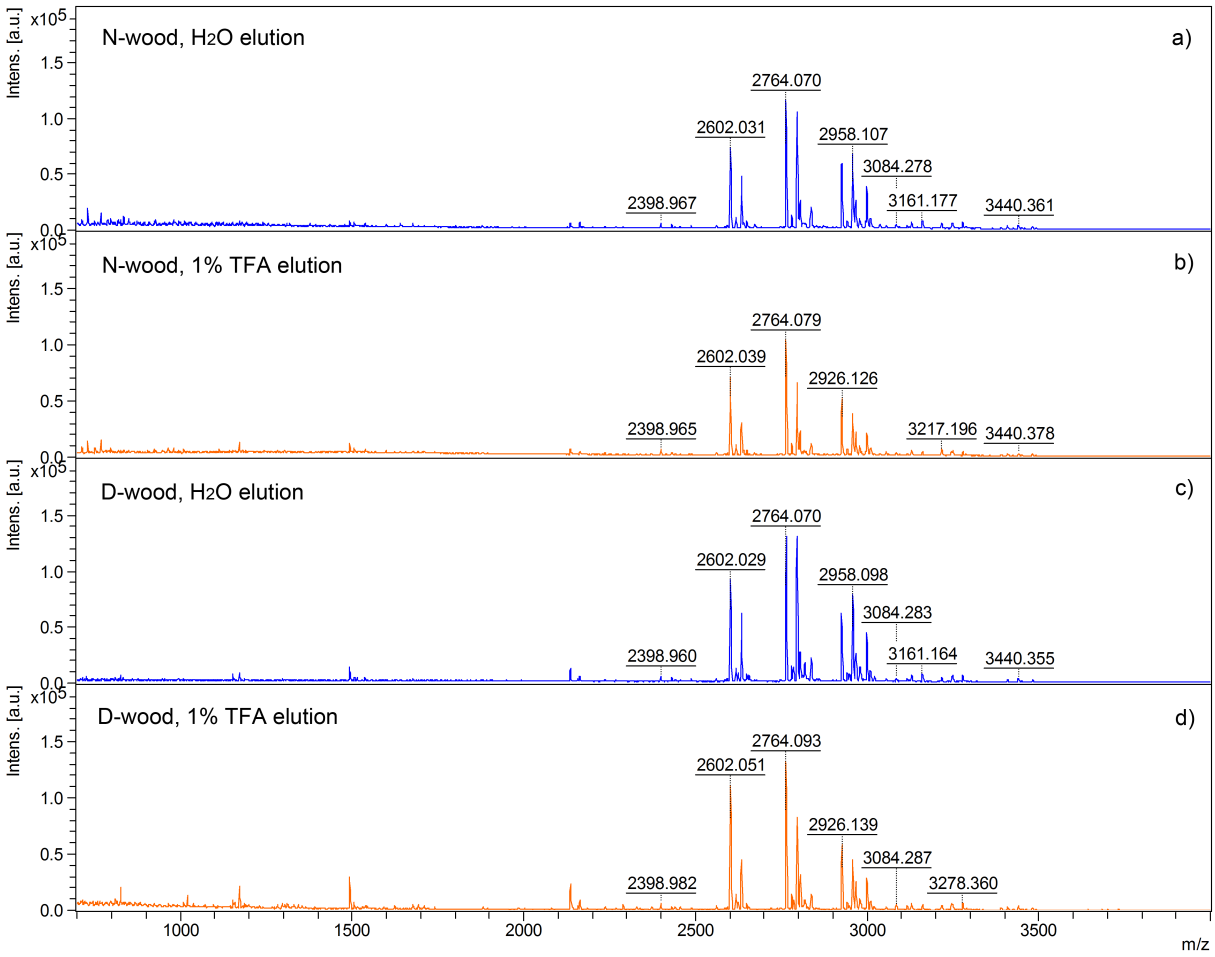


Fig. S9. MALDI-TOF-MS spectra of elution fractions obtained using a) and b) N-wood tips, c) and d) D-wood tips, with different elution solutions (7.5 µg IgG digest was loaded). MALDI matrix: DHB. Glycopeptides were detected in the range *m/z* 2200 - 3500 Da.

Preconditioning: wetted 5 times with 100 µL H_2O, and preconditioned 5 times with 100 µL loading solution 86%ACN/1% TFA

Loading: 86%ACN/1% TFA

Washing: washed 5 times with 100 µL loading solution

Elution: a) and c) H_2O, b) and d) 1% TFA

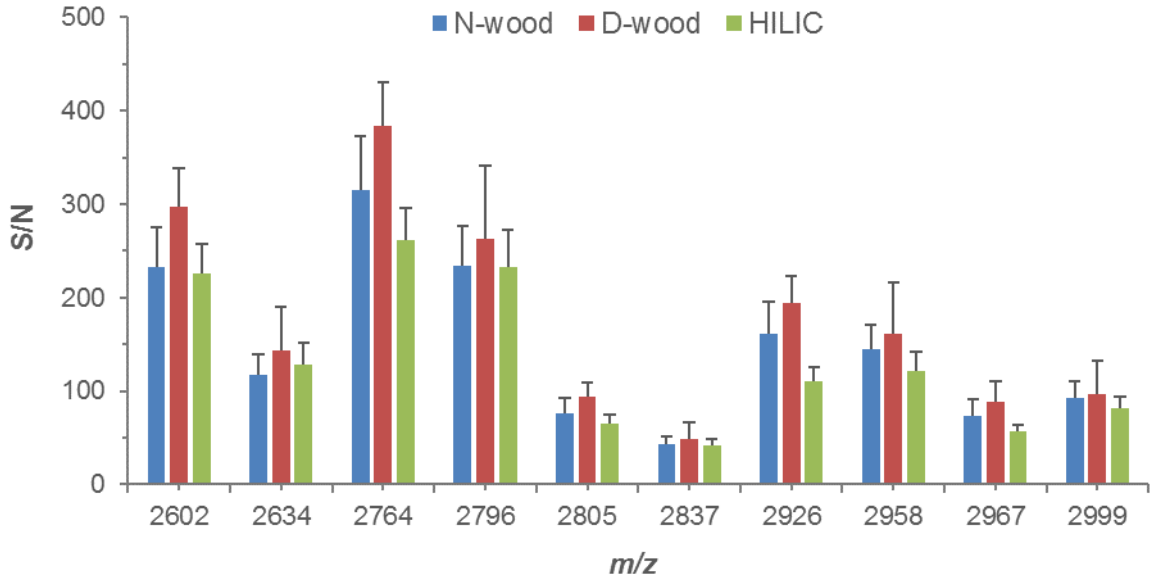
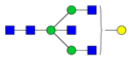
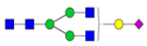
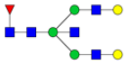


Fig. S10. Average S/N values of enriched and detected glycopeptides using N-wood, D-wood and HILIC tips. 12 spots from 3 replicates were analyzed.

Table S2. Enriched IgG glycopeptides detected using different tips. Blue squares: N-Acetylglucosamine (NAc). Red triangles: fucose (Fuc). Green circles: mannose (Hex). Yellow circles: galactose (Hex). Purple diamonds: sialic acid (Sia).

Number	Theoretical glycopeptide *m/z*	Enriched glycopeptides			Glycan composition	Amino acid sequence
		N-Wood tip	D-wood tip	HILIC tip		
1	2236.924	Yes	Yes	Yes	(Hex)2 (NAc)3 (Fuc)1 	EEQFNSTFR
2	2268.914	/	Yes	Yes	(Hex)2 (NAc)3 (Fuc)1	EEQYNSTYR
3	2398.977	Yes	Yes	Yes	(Hex)3 (NAc)3 (Fuc)1 	EEQFNSTFR
4	2430.967	Yes	Yes	Yes	(Hex)3 (NAc)3 (Fuc)1	EEQYNSTYR
5	2455.998	Yes	Yes	/	(Hex)3 (NAc)4 	EEQFNSTFR
6	2487.988	Yes	Yes	Yes	(Hex)3 (NAc)4	EEQYNSTYR
7	2561.029	Yes	Yes	Yes	(Hex)4 (NAc)3 (Fuc)1 	EEQFNSTFR
8	2593.019	Yes	Yes	Yes	(Hex)4 (NAc)3 (Fuc)1	EEQYNSTYR
9	2602.056	Yes	Yes	Yes	(Hex)3 (NAc)4 (Fuc)1 	EEQFNSTFR
10	2618.051	Yes	Yes	Yes	(Hex)4 (NAc)4 	EEQFNSTFR
11	2634.046	Yes	Yes	Yes	(Hex)3 (NAc)4 (Fuc)1	EEQYNSTYR
12	2650.041	Yes	Yes	Yes	(Hex)4 (NAc)4	EEQYNSTYR
13	2764.109	Yes	Yes	Yes	(Hex)4 (NAc)4 (Fuc)1 	EEQFNSTFR
14	2780.104	Yes	Yes	Yes	(Hex)5 (NAc)4 	EEQFNSTFR
15	2796.099	Yes	Yes	Yes	(Hex)4 (NAc)4 (Fuc)1	EEQYNSTYR
16	2805.135	Yes	Yes	Yes	(Hex)3 (NAc)5 (Fuc)1 	EEQFNSTFR
17	2812.094	Yes	Yes	Yes	(Hex)5 (NAc)4	EEQYNSTYR
18	2821.13	Yes	Yes	Yes	(Hex)4 (NAc)5 	EEQFNSTFR
19	2837.125	Yes	Yes	Yes	(Hex)3 (NAc)5 (Fuc)1	EEQYNSTYR
20	2853.120	Yes	Yes	Yes	(Hex)4 (NAc)5	EEQYNSTYR
21	2909.146	Yes	Yes	/	(Hex)4 (NAc)4 (Sia)1 	EEQFNSTFR

22	2926.162	Yes	Yes	Yes	(Hex)5 (NAc)4 (Fuc)1 	EEQFNSTFR
23	2958.152	Yes	Yes	Yes	(Hex)5 (NAc)4 (Fuc)1	EEQYNSTYR
24	2967.188	Yes	Yes	Yes	(Hex)4 (NAc)5 (Fuc)1	EEQFNSTFR
25	2999.178	Yes	Yes	Yes	(Hex)4 (NAc)5 (Fuc)1 	EEQYNSTYR
26	3055.204	Yes	Yes	Yes	(Hex)4 (NAc)4 (Fuc)1 (Sia)1 	EEQFNSTFR
27	3084.353	Yes	Yes	Yes	(Hex)3 (NAc)4 (Fuc)1	TKPREEQFNSTFR
28	3116.343	Yes	Yes	Yes	(Hex)3 (NAc)4 (Fuc)1	TKPREEQYNSTYR
29	3129.241	Yes	Yes	Yes	(Hex)5 (NAc)5 (Fuc)1 	EEQFNSTFR
30	3161.231	Yes	Yes	Yes	(Hex)5 (NAc)5 (Fuc)1	EEQYNSTYR
31	3217.257	Yes	Yes	Yes	(Hex)5 (NAc)4 (Fuc)1 (Sia)1 	EEQFNSTFR
32	3246.406	Yes	Yes	Yes	(Hex)4 (NAc)4 (Fuc)1	TKPREEQFNSTFR
33	3249.247	/	Yes	Yes	(Hex)5 (NAc)4 (Fuc)1 (Sia)1	EEQYNSTYR
34	3278.396	Yes	Yes	Yes	(Hex)4 (NAc)4 (Fuc)1	TKPREEQYNSTYR
35	3408.459	Yes	Yes	Yes	(Hex)5 (NAc)4 (Fuc)1	TKPREEQFNSTFR
36	3440.449	Yes	Yes	Yes	(Hex)5 (NAc)4 (Fuc)1	TKPREEQYNSTYR
37	3449.484	Yes	Yes	/	(Hex)4 (NAc)5 (Fuc)1	TKPREEQFNSTFR
38	3481.474	Yes	Yes	Yes	(Hex)4 (NAc)5 (Fuc)1	TKPREEQYNSTYR

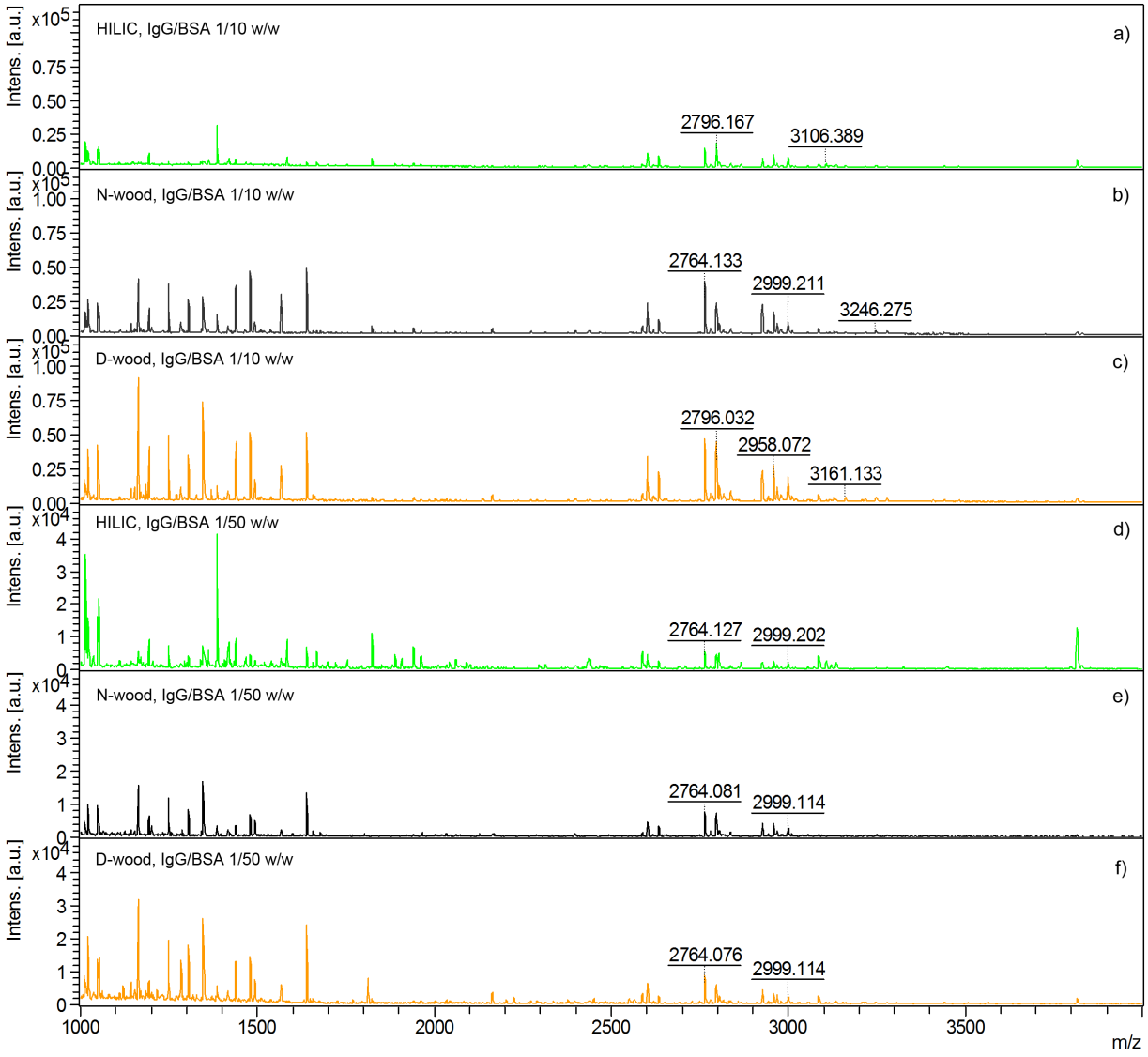


Fig. S11. MALDI-TOF-MS spectra of elution fractions obtained using a and d) HILIC tips, b) and e) N-wood tips, and c) and f) D-wood tips with sample mixtures containing IgG digest and BSA digest. a), b) and c) IgG/BSA 1/10 w/w, 5 µg/50 µg, IgG amount on spot was 1650 fmol. d), e) and f) IgG/BSA 1/50 w/w, 0.5 µg/25 µg, IgG amount on spot was 165 fmol. MALDI matrix: DHB. Glycopeptides were detected in the range *m/z* 2200 - 3500 Da.

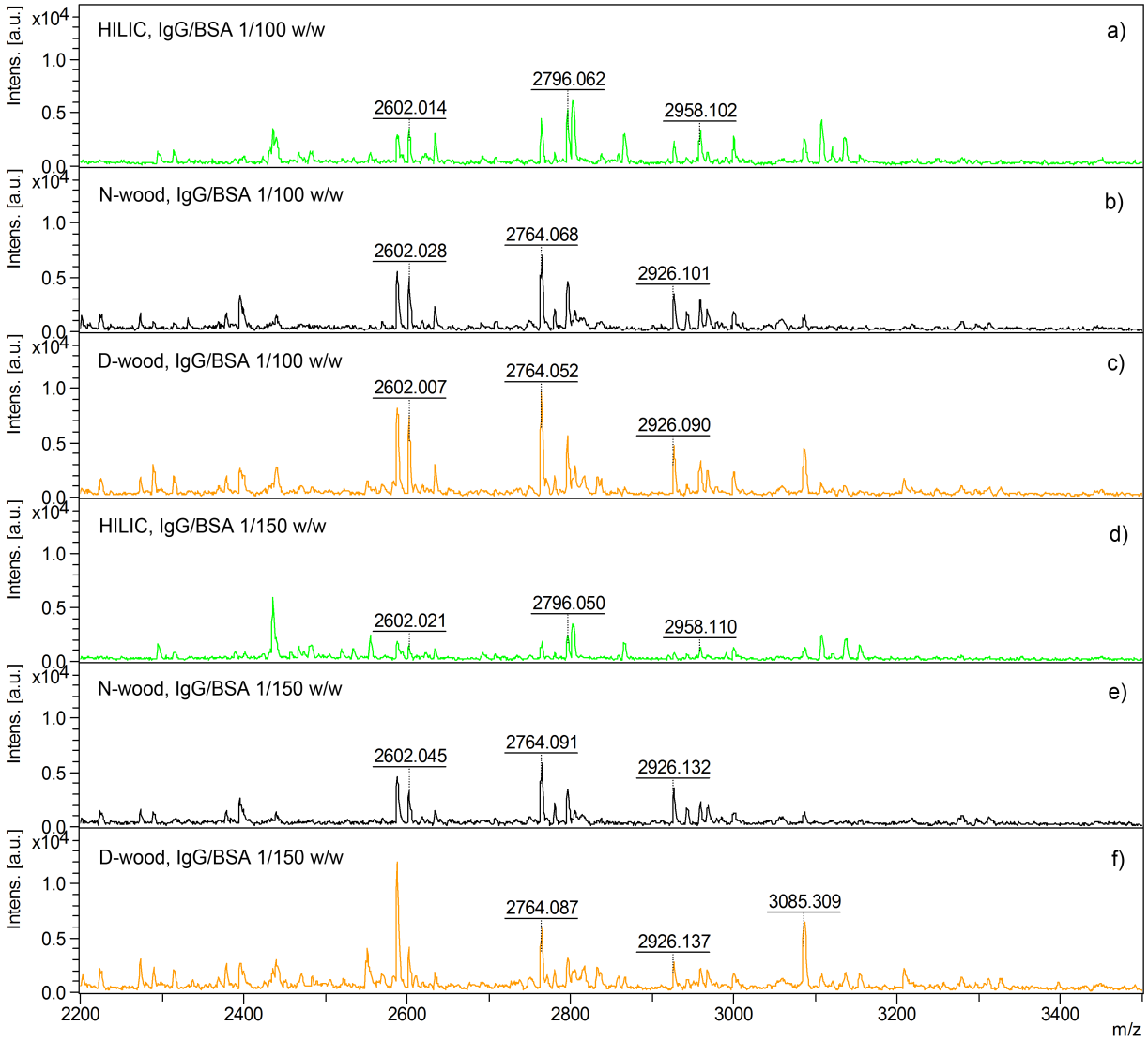


Fig. S12. MALDI-TOF-MS spectra of elution fractions obtained using a) and d) HILIC tips, b) and e) N-wood tips, c) and f) D-wood tips with sample mixtures containing IgG digest and BSA digest. a), b) and c) IgG/BSA 1/100 w/w, 0.5 µg/50 µg, d), e) and f) IgG/BSA 1/150 w/w, 0.5 µg/75 µg. IgG amount on each spot was 165 fmol. MALDI matrix: DHB. Glycopeptides were detected in the range *m/z* 2200 - 3500 Da.

Table S3: S/N values of enriched glycopeptides detected using different tips when loading the same amount of IgG digest (0.5 µg, 165 fmol on each spot), and different amounts of BSA digest (25 µg, 50 µg, 75 µg), corresponding to weight ratios of 1/50, 1/100 and 1/150. Totally 12 spots from 3 replicates were analyzed.

	IgG/BSA 1/50 w/w			**IgG/BSA 1/100 w/w**			**IgG/BSA 1/150 w/w**		
m/z	N-wood tips	D-wood tips	HILIC tips	N-wood tips	D-wood tips	HILIC tips	N-wood tips	D-wood tips	HILIC tips
2602	17.9 ± 5.1	18.7 ± 6.1	11.3 ± 2.9	14.5 ± 3.7	17.3 ± 4.3	9.4 ± 2.6	8.2 ± 1.5	7.8 ± 1.3	4.1 ± 1.9
2634	13.4 ± 6.4	6.3 ± 1.7	6.8 ± 1.8	7.5 ± 1.7	6.4 ± 2.0	6.8 ± 2.9	3.8 ± 3.4	0.0	2.3 ± 2.1
2764	29.7 ±7.3	26.5 ± 8.8	13.0 ± 3.4	23.5 ± 6.5	23.6 ± 5.6	12.3 ± 4.0	14.2 ± 2.6	11.4 ± 1.7	5.6 ± 2.4
2796	31.7 ± 14.7	13.8 ± 3.4	14.1 ± 3.6	17.1 ± 3.9	14.4 ± 4.0	15.3 ± 5.1	10.5 ± 4.9	5.9 ± 2.4	7.0 ± 2.8
2805	5.7 ± 2.6	6.8 ± 2.0	0.0	4.6 ± 2.2	4.2 ± 2.8	0.0	0	0	0
2837	4.5 ± 2.7	0.8 ± 1.5	0.0	0	0	0	0	0	0
2926	15.4 ± 3.3	13.2 ± 4.4	5.7 ± 1.2	12.1 ± 3.2	11.4 ± 2.8	5.9 ± 2.4	8.1 ± 1.9	5.7 ± 0.9	0.0
2958	19.0 ± 8.0	8.3 ± 2.1	7.8 ± 1.9	10.0 ± 1.8	8.8 ± 2.5	9.1 ± 2.6	6.3 ± 4.0	3.3 ± 1.7	3.8 ± 2.0
2967	7.1 ± 2.1	7.9 ± 2.5	0.0	4.8 ± 3.2	4.9 ± 1.9	0.0	3.3 ± 2.2	0.0	0.0
2999	11.5 ± 5.5	5.5 ± 1.4	6.3 ± 1.4	6.6 ± 1.7	5.9 ± 1.7	6.8 ± 1.9	3.5 ± 2.5	0.0	2.3 ± 1.8

Table S4. Average MALDI-MS S/N values of abundant enriched glycopeptides in elution fractions loading different amounts of IgG digests using N-wood tips. 12 spots from 3 replicates were analyzed.

m/z	4 μg	5 μg	6 μg	7 μg	8 μg	9 μg	10 μg
2602	125.9 ± 26.6	163.0 ± 26.2	187.1 ± 30.0	211.7 ± 29.5	217.4 ± 49.2	223.0 ± 35.4	232.6 ± 43.0
2634	54.7 ± 12.9	94.7 ± 34.1	90.7 ± 20.0	90.8 ± 13.4	94.0 ± 26.0	98.1 ± 33.2	116.8 ± 21.7
2764	187.0 ± 29.2	225.3 ± 33.9	273.4 ± 43.1	303.1 ± 40.1	321.0 ± 75.5	319.3 ± 48.1	314.7 ± 57.6
2796	121.4 ± 27.7	192.7 ± 61.9	198.1 ± 39.9	192.7 ± 29.7	200.0 ± 52.4	210.8 ± 67.2	233.7 ± 42.8
2805	42.8 ± 8.0	53.3 ± 8.6	64.9 ± 10.6	70.5 ± 9.4	75.7 ± 19.4	75.7 ± 12.1	76.0 ± 16.6
2837	20.8 ± 5.8	35.6 ± 12.5	34.8 ± 7.7	34.8 ± 4.8	35.8 ± 10.8	36.8 ± 12.5	43.6 ± 7.5
2926	94.8 ± 13.9	111.1 ± 17.1	140.3 ± 25.4	159.3 ± 22.4	173.7 ± 45.9	162.9 ± 25.2	160.8 ± 34.8
2958	73.0 ± 19.5	119.2 ± 40.9	123.3 ± 27.3	123.3 ± 19.3	129.5 ± 36.9	129.3 ± 43.4	144.3 ± 26.3
2967	43.6 ± 6.5	51.3 ± 7.9	64.0 ± 11.9	70.1 ± 9.8	77.2 ± 21.7	74.9 ± 11.7	73.8 ± 16.6
2999	47.1 ± 11.9	75.0 ± 26.1	78.4 ± 17.5	76.8 ± 10.7	79.4 ± 24.3	82.2 ± 25.5	92.3 ± 18.0

Table S5. Average MALDI-MS S/N values of abundant enriched glycopeptides in elution fractions loading different amounts of IgG digests using D-wood tips. 12 spots from 3 replicates were analyzed.

m/z	4 μg	5 μg	6 μg	7 μg	8 μg
2602	188.0 ± 37.2	192.5 ± 16.8	217.7 ± 34.4	212.5 ± 28.0	233.0 ± 26.0
2634	83.9 ± 21.5	80.4 ± 21.0	92.2 ± 21.4	82.7 ± 17.7	101.5 ± 25.4
2764	242.9 ± 47.4	261.1 ± 21.6	290.9 ± 42.9	330.8 ± 46.9	351.1 ± 34.7
2796	169.4 ± 42.2	164.2 ± 36.9	184.3 ± 42.5	191.4 ± 37.3	224.7 ± 52.1
2805	62.8 ± 12.0	64.0 ± 6.7	72.2 ± 11.0	74.6 ± 11.6	80.5 ± 8.3
2837	32.1 ± 9.2	29.3 ± 9.6	34.6 ± 9.4	32.8 ± 7.1	40.3 ± 9.7
2926	117.4 ± 25.3	127.3 ± 13.9	138.3 ± 22.2	180.8 ± 28.9	186.8 ± 18.7
2958	102.6 ± 28.1	98.3 ± 23.4	108.8 ± 26.2	126.3 ± 25.7	145.2 ± 34.7
2967	57.6 ± 12.1	60.2 ± 5.8	68.2 ± 11.1	77.4 ± 13.2	80.5 ± 8.2
2999	67.2 ± 19.5	61.4 ± 15.5	73.7 ± 19.0	73.2 ± 16.2	84.5 ± 21.7

m/z	9 μg	10 μg	11 μg	12 μg
2602	275.6 ± 41.0	302.8 ± 40.6	315.4 ± 51.7	318.1 ± 61.5
2634	119.3 ± 27.8	126.5 ± 46.6	138.5 ± 35.3	135.7 ± 24.5
2764	371.3 ± 53.7	380.3 ± 46.1	384.8 ± 62.3	403.5 ± 74.4
2796	242.4 ± 51.1	238.8 ± 77.9	259.2 ± 63.4	252.5 ± 51.3
2805	92.2 ± 14.3	95.3 ± 14.4	100.7 ± 18.6	104.0 ± 20.9
2837	45.4 ± 9.8	43.3 ± 17.9	48.4 ± 14.2	46.0 ± 11.5
2926	180.5 ± 28.0	185.1 ± 29.0	190.8 ± 35.1	199.8 ± 42.1
2958	141.0 ± 30.8	142.1 ± 55.6	154.3 ± 49.0	152.8 ± 30.4
2967	86.4 ± 13.0	83.4 ± 22.0	91.3 ± 19.1	96.6 ± 20.6
2999	93.2 ± 20.7	92.2 ± 35.4	100.7 ± 26.8	94.8 ± 20.2

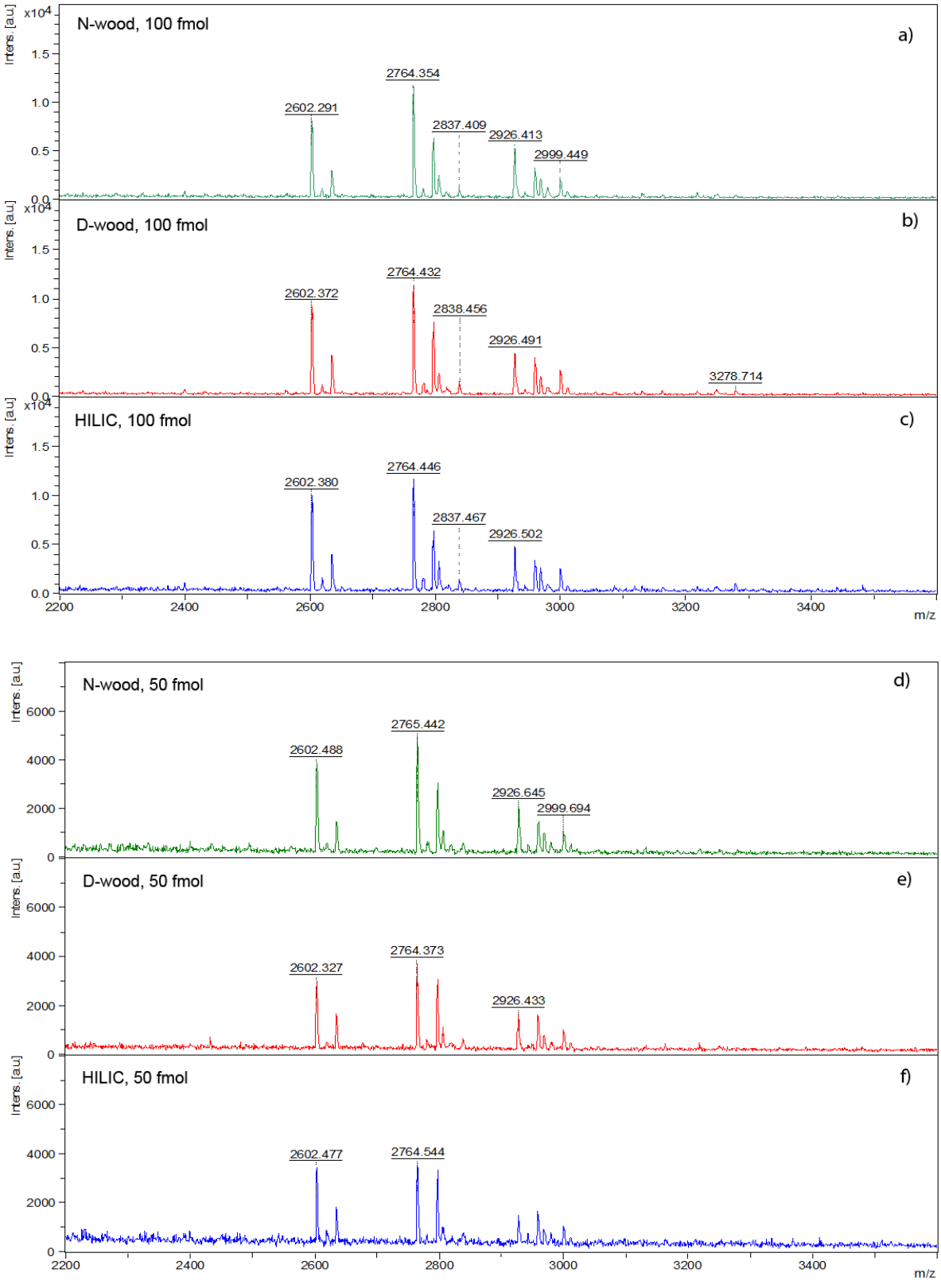


Fig. S13. MALDI-TOF-MS spectra of enriched glycopeptides obtained using a) and d) N-wood tips, b) and e) D-wood tips, c) and f) HILIC tips loaded with different amounts of IgG digest: a), b) and c) 300 ng (100 fmol on plate), d), e) and f) 150 ng (50 fmol on plate).

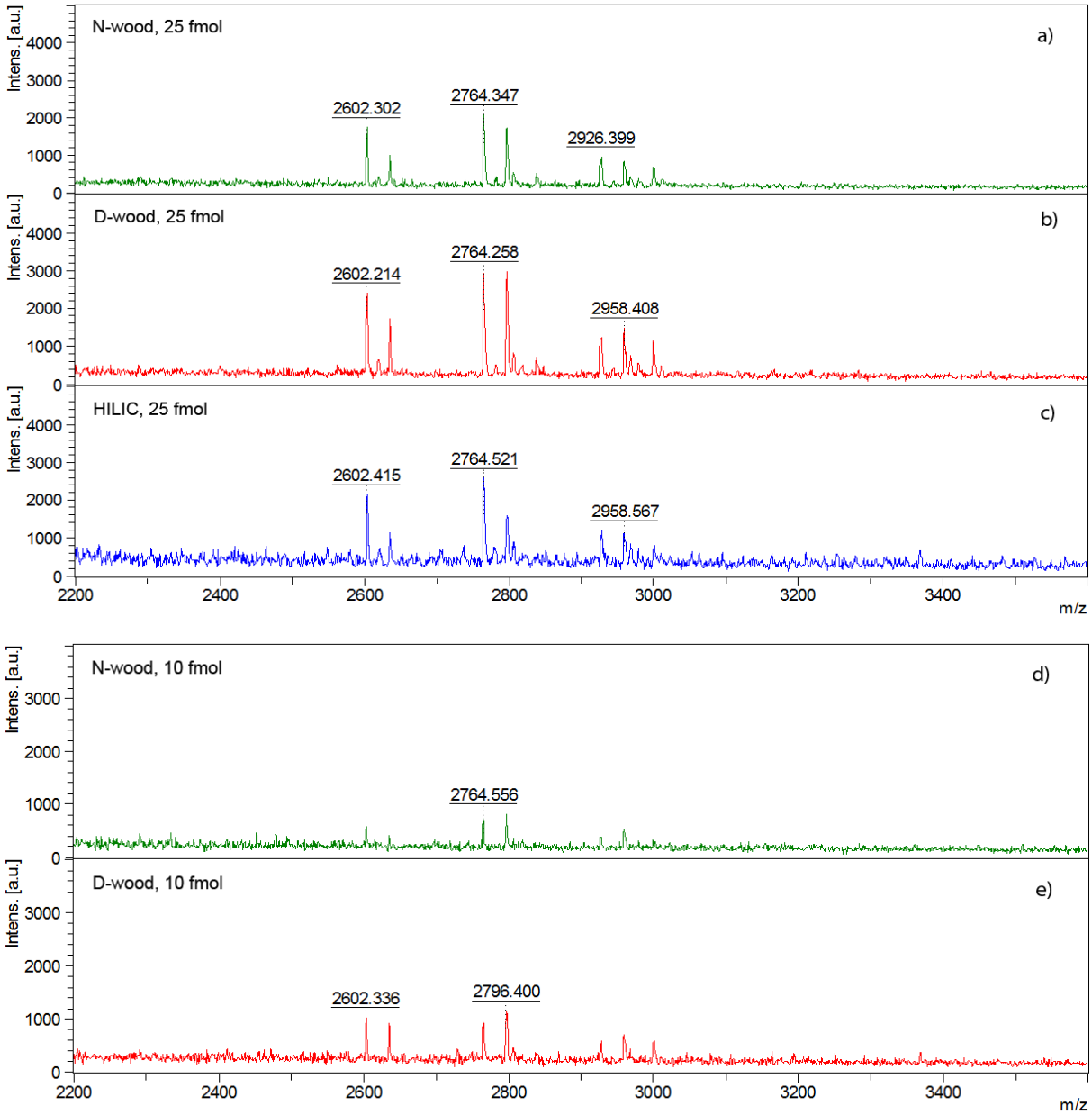


Fig. S14. MALDI-TOF-MS spectra of enriched glycopeptides obtained using a) and d) N-wood tips, b) and e) D-wood tips, c) HILIC tips loaded with different amounts of IgG digest: a), b) and c) 75 ng (25 fmol on plate), d) and e) 30 ng (10 fmol on plate).

Table S6. Average MALDI-MS S/N values of abundant enriched glycopeptides in elution fractions loading different amounts of IgG digests (300 ng, 150 ng and 75 ng) using N-wood, D-wood and HILIC tips. 12 spots from 3 replicates were analyzed.

m/z	**100 fmol** N-wood tips	D-wood tips	HILIC tips	**50 fmol** N-wood tips	D-wood tips	HILIC tips	**25 fmol** N-wood tips	D-wood tips	HILIC tips
2602	30.1 ± 9.0	30.7 ± 7.6	27.3 ± 7.1	13.0 ± 2.7	13.1 ± 3.6	12.3 ± 2.8	9.8 ± 3.6	10.3 ± 2.8	6.4 ± 1.0
2618	3.6 ± 2.2	3.7 ± 2.1	3.8 ± 1.4	0	0	0	0	0	0
2634	16.8 ± 6.0	17.0 ± 6.9	13.1 ± 3.1	8.2 ± 2.0	7.5 ± 2.3	6.4 ± 1.8	4.5 ± 1.8	6.3 ± 1.8	1.2 ± 1.7
2764	45.5 ± 14.4	41.8 ± 9.5	30.9 ± 9.2	19.2 ± 3.8	18.7 ± 4.1	14.0 ± 3.5	13.6 ± 4.7	13.1 ± 3.3	7.0 ± 1.3
2780	4.9 ± 2.4	4.2 ± 2.2	4.5 ± 4.6	0	0	0	0	0	0
2796	37.3 ± 14.3	35.8 ± 16.0	22.3 ± 7.6	18.6 ± 5.3	15.6 ± 4.9	11.6 ± 3.4	10.2 ± 2.9	12.2 ± 3.6	4.6 ± 1.7
2805	9.6 ± 2.8	9.4 ± 2.1	7.3 ± 3.4	4.6 ± 3.8	4.5 ± 1.2	2.8 ± 2.1	2.0 ± 2.2	2.7 ± 1.7	0
2837	5.8 ± 2.8	5.6 ± 4.0	3.4 ± 2.6	1.9 ± 2.1	1.2 ± 1.7	0	0	0	0
2926	21.8 ± 6.9	17.5 ± 5.6	12.6 ± 4.5	9.3 ± 2.4	8.6 ± 1.8	5.8 ± 1.8	5.8 ± 2.4	5.5 ± 1.6	1.6 ± 1.7
2958	20.7 ± 8.2	18.3 ± 10.2	11.9 ± 3.4	10.3 ± 3.0	8.3 ± 2.6	5.9 ± 2.0	5.3 ± 2.2	6.1 ± 2.1	0.9 ± 1.7
2967	9.6 ± 2.8	7.8 ± 2.9	6.6 ± 2.0	4.3 ± 0.9	3.8 ± 1.6	3.4 ± 1.2	1.3 ± 1.9	1.6 ± 2.0	0
2999	12.4 ± 4.8	11.8 ± 4.5	7.8 ± 2.1	6.1 ± 1.9	5.1 ± 1.8	2.8 ± 2.6	3.0 ± 1.9	4.4 ± 1.2	0

Table S7. Area ratios of IgG1 glycopeptides and IgG1 IS ($A_{glycopeptide}/A_{IS}$) for different mixing ratios ($V_{glycopeptide}/V_{IS}$). Glycoeptides were enriched from 10 µg IgG digests using 1 mg wood tips.

$V_{glycopeptide}/V_{IS}$ 0.5/1	**$A_{glycopeptide}/A_{IS}$**												**mean**	**std**
m/z 2634	0.43	0.44	0.41	0.51	0.49	0.46	0.50	0.49	0.54	0.51	0.41	0.53	0.48	0.04
m/z 2796	0.88	0.91	0.83	1.02	1.00	0.94	1.08	1.03	1.12	1.06	0.86	1.08	0.98	0.10
m/z 2958	0.58	0.57	0.52	0.64	0.63	0.60	0.65	0.64	0.68	0.65	0.52	0.65	0.61	0.05
$V_{glycopeptide}/V_{IS}$ 1.0/1	**$A_{glycopeptide}/A_{IS}$**												**mean**	**std**
m/z 2634	1.18	1.07	1.07	1.03	1.09	0.94	1.14	1.13	1.07	1.12	1.10	0.94	1.07	0.07
m/z 2796	2.28	2.06	2.16	1.99	2.16	1.85	2.26	2.19	2.11	2.22	2.19	1.82	2.11	0.15
m/z 2958	1.35	1.27	1.33	1.22	1.35	1.16	1.42	1.36	1.32	1.39	1.38	1.12	1.31	0.09
$V_{glycopeptide}/V_{IS}$ 1.5/1	**$A_{glycopeptide}/A_{IS}$**												**mean**	**std**
m/z 2634	1.50	1.61	1.53	1.64	1.59	1.47	1.48	1.45	1.55	1.61	1.59	1.59	1.55	0.06
m/z 2796	3.06	3.25	3.09	3.28	3.16	2.98	2.98	2.98	3.19	3.28	3.14	3.24	3.14	0.12
m/z 2958	1.93	2.02	1.90	2.02	1.97	1.84	1.85	1.84	1.90	2.01	1.87	2.00	1.93	0.07
$V_{glycopeptide}/V_{IS}$ 2.0/1	**$A_{glycopeptide}/A_{IS}$**												**mean**	**std**
m/z 2634	2.04	1.95	2.00	2.00	2.14	2.19	1.93	2.06	2.30	2.11	2.22	1.99	2.08	0.12
m/z 2796	4.13	3.93	4.09	3.98	4.24	4.19	3.88	4.01	4.35	4.12	4.12	3.95	4.08	0.14
m/z 2958	2.43	2.47	2.65	2.40	2.56	2.64	2.53	2.60	2.69	2.64	2.81	2.51	2.58	0.12
$V_{glycopeptide}/V_{IS}$ 2.5/1	**$A_{glycopeptide}/A_{IS}$**												**mean**	**std**
m/z 2634	2.32	2.25	2.25	2.36	2.33	2.32	2.36	2.46	2.40	2.39	2.42	2.34	2.35	0.06
m/z 2796	4.81	4.65	4.58	4.81	4.84	4.81	4.81	5.03	5.01	4.99	4.96	4.89	4.85	0.14
m/z 2958	2.98	2.87	2.65	2.90	2.94	2.93	2.89	3.11	3.05	3.10	2.92	3.00	2.95	0.12

IS: internal standard. EEQYN(GlcNAc)STYR, *m/z* 1392, 50ng/mL. MALDI matrix: HCCA, 10000 shots, laser movement of random walk (complete sample).

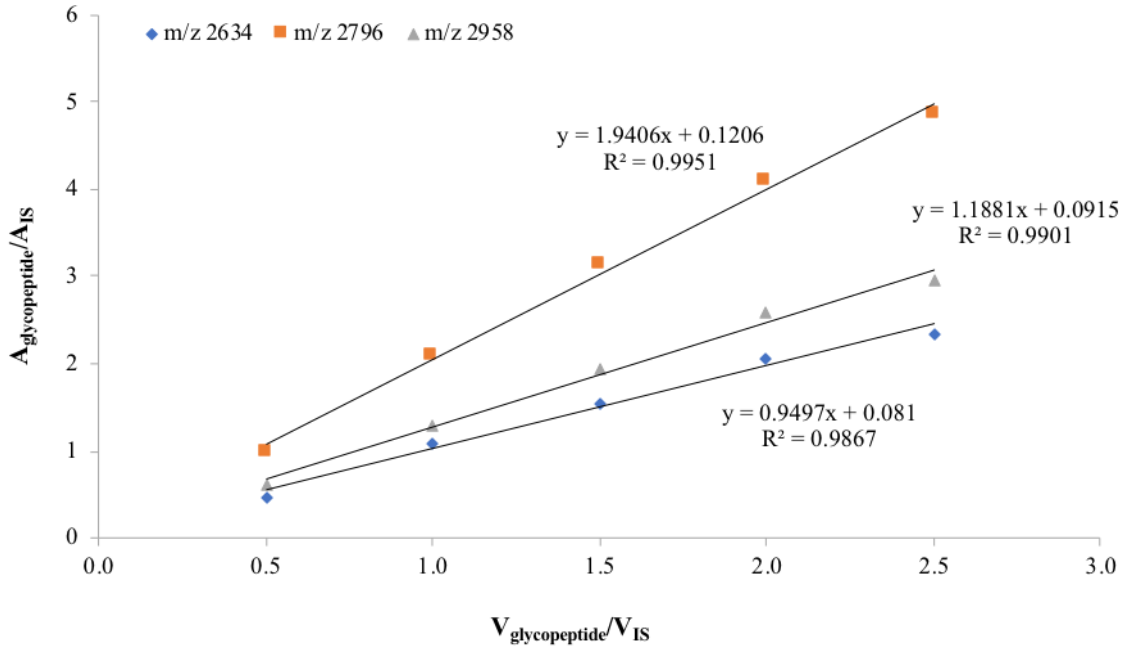


Fig. S15. Calibration curves for selected IgG1 glycopeptides (*m/z* 2634, 2796 and 2958).

Table S8. Detected area ratios of IgG1 glycopeptides to IgG1 IS for enriched ($A_{enriched}/A_{IS}$) and recovered samples ($A_{recovered}/A_{IS}$). 12 spots from 3 replicates.

m/z	**$A_{enriched}/A_{IS}$**												**mean**
2634	0.89	0.90	1.04	0.96	1.09	1.03	1.10	0.93	0.94	0.91	0.86	0.90	0.96
2796	1.78	1.79	2.04	1.99	2.15	2.05	2.16	1.81	1.86	1.87	1.92	1.85	1.94
2958	1.08	1.13	1.28	1.25	1.31	1.24	1.25	1.09	1.23	1.14	1.15	1.09	1.19
m/z	**$A_{recovered}/A_{IS}$ N-wood tips**												**mean**
2634	0.52	0.68	0.61	0.52	0.61	0.51	0.61	0.56	0.60	0.52	0.56	0.59	0.57
2796	1.24	1.69	1.57	1.29	1.52	1.27	1.53	1.41	1.54	1.30	1.48	1.41	1.44
2958	0.89	1.20	0.95	0.90	1.08	0.91	1.11	1.02	1.09	0.88	1.05	0.94	1.00
m/z	**$A_{recovered}/A_{IS}$ D-wood tips**												**mean**
2634	0.81	0.70	0.76	0.84	0.80	0.79	0.72	0.76	0.79	0.83	0.85	0.80	0.79
2796	1.93	1.62	1.75	1.93	1.84	1.79	1.63	1.54	1.55	1.62	1.66	1.69	1.71
2958	1.26	1.07	1.15	1.28	1.20	1.18	1.08	0.91	0.91	0.96	0.99	1.05	1.09

Table S9. Detected and calculated (from calibration curves) area ratios of IgG1 glycopeptides to IgG1 IS for enriched and recovered samples, using N-wood and D-wood tips.

m/z	**$A_{enriched}/A_{IS}$**	**Calculated $V_{enriched}/V_{IS}$**	
2634	0.96	0.93	
2796	1.94	0.94	
2958	1.19	0.92	
N-Wood tips			
m/z	**$A_{recovered}/A_{IS}$**	**Calculated $V_{recovered}/V_{IS}$**	**Recovery (%)**
2634	0.57	0.52	55.85
2796	1.44	0.68	72.49
2958	1.00	0.77	83.25
D-wood tips			
m/z	**$A_{recovered}/A_{IS}$**	**Calculated $V_{recovered}/V_{IS}$**	**Recovery (%)**
2634	0.79	0.74	80.12
2796	1.71	0.82	87.56
2958	1.09	0.84	90.95

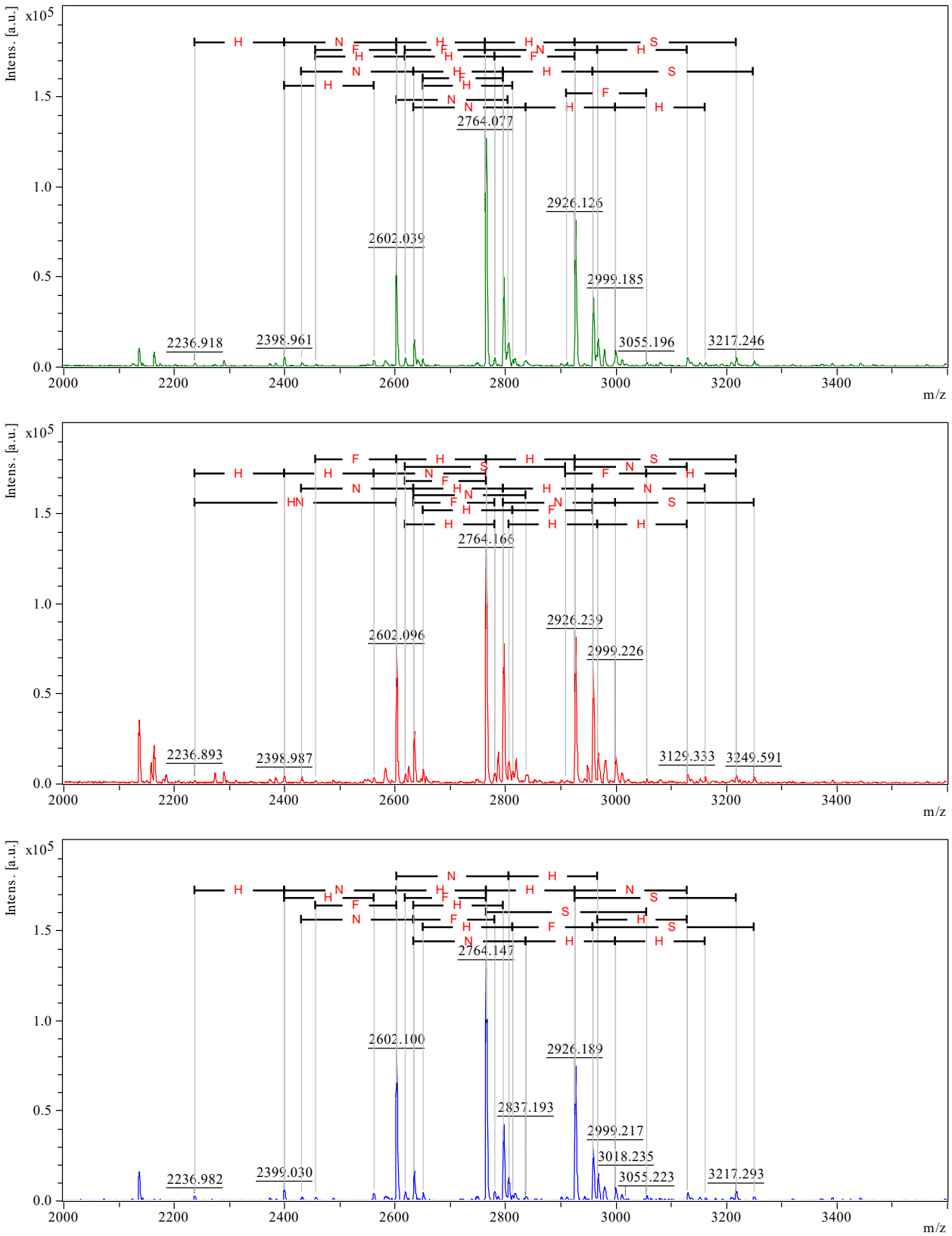


Fig. S16. MALDI-TOF-MS spectra of enriched IgG glycopeptides with glycan annotations from human plasma using top) N-wood tip, middle) D-wood tip and bottom) HILIC tip. F: Fucose. H: Hexose (mannose or galactose). N: N-Acetylglucosamine.

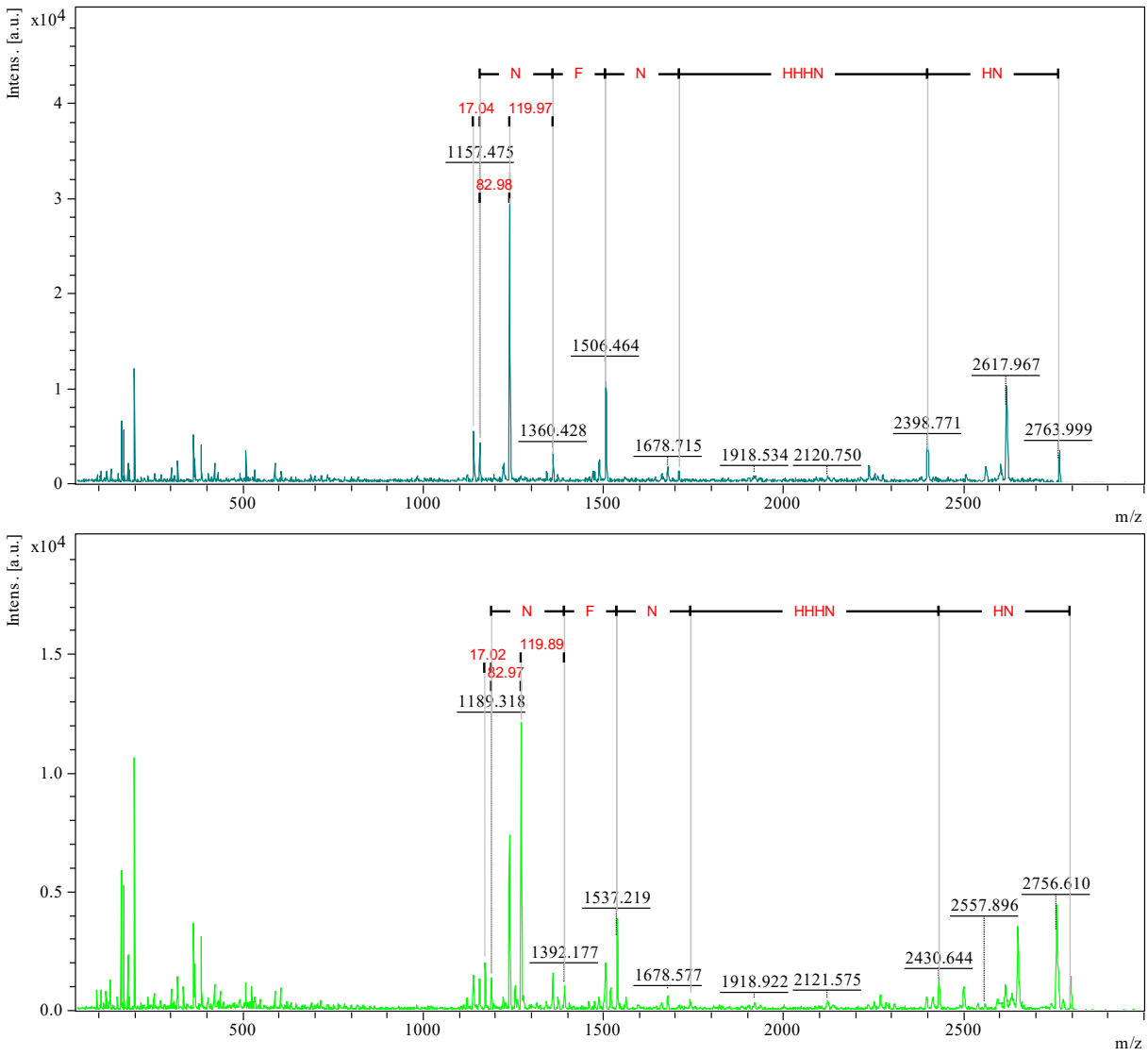
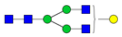
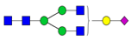
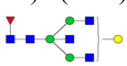


Fig. S17. MALDI-TOF-MS/MS spectra of IgG glycopeptides enriched from human plasma using N-wood tip. Top: *m/z* 2764, amino acid sequence EEQFNSTFR (*m/z* 1157). bottom: *m/z* 2796, amino acid sequence EEQYNSTYR (*m/z* 1189). F: Fucose. H: Hexose (mannose or galactose). N: N-Acetylglucosamine.

Table S10. Enriched IgG glycopeptides from human plasma samples using different tips. Blue square: N-acetylglucosamine (NAc). Red triangle: fucose (Fuc). Green circle: mannose (Hex). Yellow circle: galactose (Hex). Purple diamond: sialic acid (Sia).

Number	Theoretical glycopeptide *m/z*	Enriched glycopeptides			Glycan composition	Amino acid sequence
		N-wood tip	D-wood tip	HILIC tip		
1	2236.924	Yes	Yes	Yes	(Hex)2 (NAc)3 (Fuc)1 	EEQFNSTFR
2	2398.977	Yes	Yes	Yes	(Hex)3 (NAc)3 (Fuc)1 	EEQFNSTFR
3	2430.967	Yes	Yes	Yes	(Hex)3 (NAc)3 (Fuc)1	EEQYNSTYR
4	2455.998	Yes	Yes	Yes	(Hex)3 (NAc)4 	EEQFNSTFR
5	2561.029	Yes	Yes	Yes	(Hex)4 (NAc)3 (Fuc)1 	EEQFNSTFR
6	2602.056	Yes	Yes	Yes	(Hex)3 (NAc)4 (Fuc)1 	EEQFNSTFR
7	2618.051	Yes	Yes	Yes	(Hex)4 (NAc)4 	EEQFNSTFR
8	2634.046	Yes	Yes	Yes	(Hex)3 (NAc)4 (Fuc)1	EEQYNSTYR
9	2650.041	Yes	Yes	Yes	(Hex)4 (NAc)4	EEQYNSTYR
10	2764.109	Yes	Yes	Yes	(Hex)4 (NAc)4 (Fuc)1 	EEQFNSTFR
11	2780.104	Yes	Yes	Yes	(Hex)5 (NAc)4 	EEQFNSTFR
12	2796.099	Yes	Yes	Yes	(Hex)4 (NAc)4 (Fuc)1	EEQYNSTYR
13	2805.135	Yes	Yes	Yes	(Hex)3 (NAc)5 (Fuc)1 	EEQFNSTFR
14	2812.094	Yes	Yes	Yes	(Hex)5 (NAc)4	EEQYNSTYR
15	2837.125	Yes	Yes	Yes	(Hex)3 (NAc)5 (Fuc)1	EEQYNSTYR
16	2909.146	Yes	Yes	/	(Hex)4 (NAc)4 (Sia)1 	EEQFNSTFR
17	2926.162	Yes	Yes	Yes	(Hex)5 (NAc)4 (Fuc)1 	EEQFNSTFR
18	2958.152	Yes	Yes	Yes	(Hex)5 (NAc)4 (Fuc)1	EEQYNSTYR
19	2967.188	Yes	Yes	Yes	(Hex)4 (NAc)5 (Fuc)1 	EEQFNSTFR
20	2999.178	Yes	Yes	Yes	(Hex)4 (NAc)5 (Fuc)1	EEQYNSTYR
21	3055.204	Yes	Yes	Yes	(Hex)4 (NAc)4 (Fuc)1 (Sia)1 	EEQFNSTFR

22	3129.241	Yes	Yes	Yes	(Hex)5 (NAc)5 (Fuc)1 	EEQFNSTFR
23	3161.231	Yes	Yes	Yes	(Hex)5 (NAc)5 (Fuc)1	EEQYNSTYR
24	3217.257	Yes	Yes	Yes	(Hex)5 (NAc)4 (Fuc)1 (Sia)1 	EEQFNSTFR
25	3249.247	Yes	Yes	Yes	(Hex)5 (NAc)4 (Fuc)1 (Sia)1	EEQYNSTYR

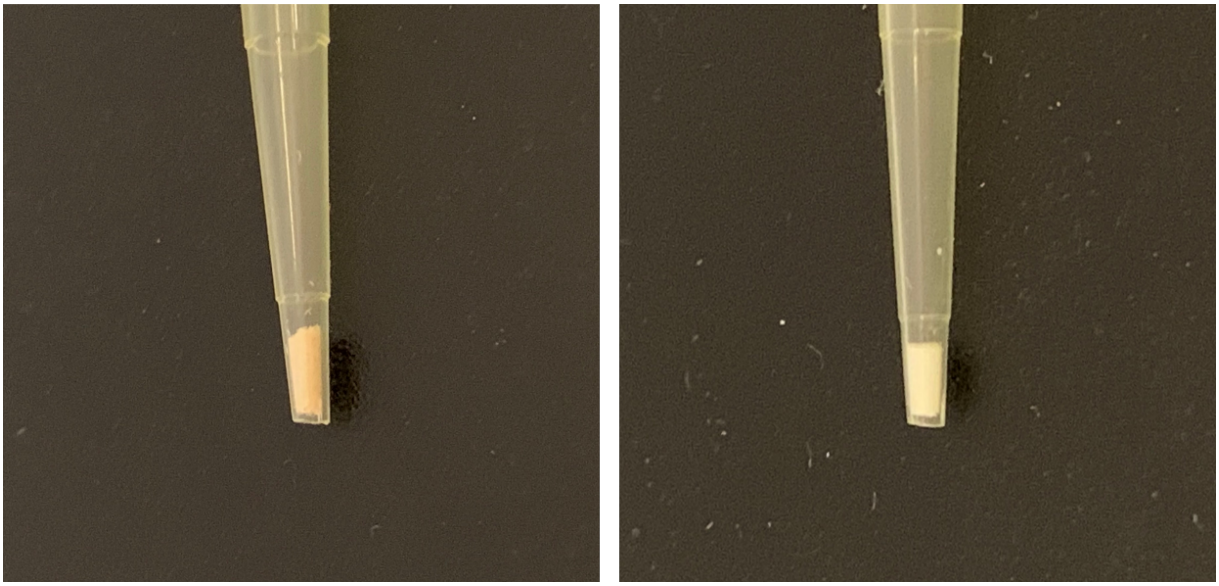


Fig. S18. N-wood tip (left) and D-wood tip (right).

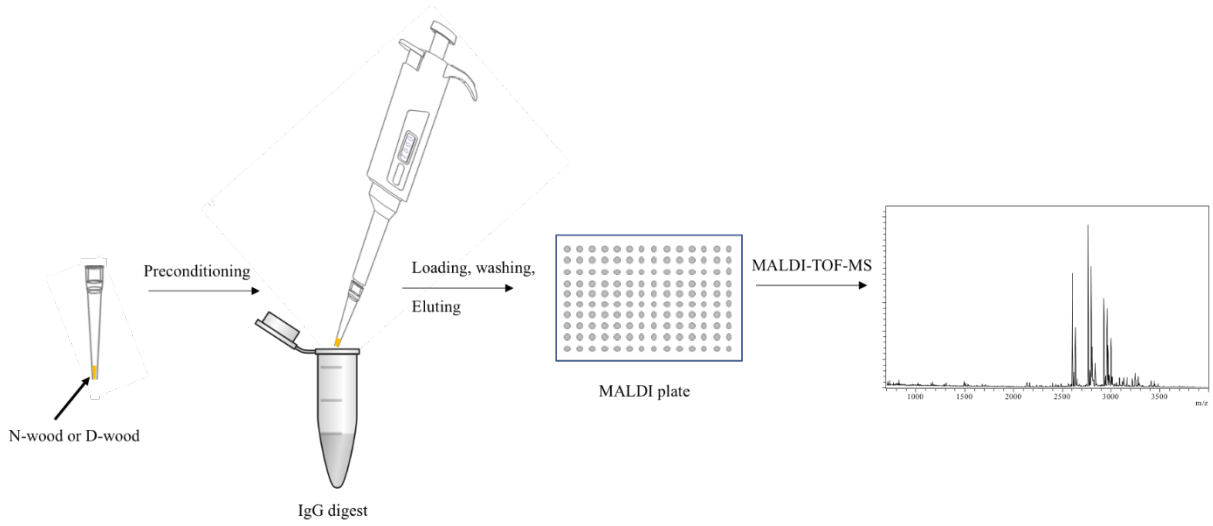


Fig. S19. Schematic illustration of the IgG glycopeptide enrichment procedure.

Reference

1. V. Tarvo, T. Lehtimaa, S. Kuitunen, V. Alopaeus, T. Vuorinen and J. Aittamaa, *J. Wood Chem. Technol.*, 2010, **30**, 230-268.