Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Probing serum N-glycan patterns for rapid and precise detection of Crohn's disease

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Supplementary experimental procedures

Chemicals

Cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) and ammonium bicarbonate (NH_4HCO_3) were purchased from Sinopharm Chemical Reagent Co., Ltd. 2-methylimidazole was purchased from Adamas-beta. Polyvinylpyrrolidone (PVP, K30), chicken egg ovalbumin (OVA), bovine serum albumin (BSA), 2,5-dihydroxybenzoic acid (DHB) and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich. Peptide N-glycosidase F (PNGase F, 15,000 units, 30 μL) was purchased from Genetimes Technology (Shanghai, China). Bicinchoninic acid (BCA) protein assay kit was purchased from Beijing Solarbio Science & Technology Co., Ltd. Millipore Express® PES membrane (Filter Unit: 0.22 μm), ultrafiltration tube (100 KDa) and acetonitrile (ACN, HPLC-grade) were purchased from Merck KGaA (Darmstadt, Germany). Serum samples of healthy people and patients with Crohn disease were provided by Zhongshan Hospital (Fudan University, Shanghai, China). All aqueous solutions were prepared using ultrapure water from Milli-Q system (Merck KGaA).

Synthesis of ZIF-67 and its derived carbon matrix

ZIF-67 and the corresponding carbon material were prepared according to the previous report.[1]

Characterization of ZIF-67 and its derived carbon matrix

Scanning electron microscopy (SEM) test was conducted on a Phenom Prox scanning electron microscope (Netherlands) at 10 kV. Fourier transform infrared (FT-IR) spectrum test was operated on a Nicolet iS10 Fourier spectrophotometer (U.S.A.).

Raman spectrum test was carried out on a Hobria Jobin Yvon XploRA at 785 nm (3 mW) excitation. Nitrogen adsorption/desorption isotherm test was measured on a Micromeritcs Tristar II 3020 analyzer (U.S.A.) at 77 K. Power X-ray diffraction (XRD) pattern was obtained on Bruker D4 X-ray diffractometer with Ni-filtered Cu Kα radiation (40 kV, 40 mA).

Preparation of OVA digest

1 mg OVA protein was dissolved in 1mL NH_4HCO_3 solution (25 mM, pH=8.3) and boiled for 5 min. After cooling to room temperature, the Eppendorf tube was centrifuged to make the liquid attached onto the tube cap drop down. Then 1μL PNGase F was added into the solution and the mixture was incubated at 37 ℃ for 16 h to release N-glycans.

Preparation of serum digest

Freezing serum sample was thawed at 4 °C and centrifuged with the speed of 12000×g at 4 °C for 10 min. The protein concentration of supernatant was measured by bicinchoninic acid (BCA) protein assay kit. A certain amount of serum containing 500 μg protein was diluted to 50 μL by 25 mM NH_4HCO_3 and boiled for 5 min. Then the serum diluent was reduced at 55 °C by adding 1 μL 500 mM DTT and alkylated at 37 °C in the dark by adding 1 μL 1 M IAA. Afterwards, the mixture was diluted by adding 350 μL 25 mM NH_4HCO_3 and transferred to an ultrafiltration tube (3 kDa) for centrifugation. The centrifugation process was repeated for three times and the trapped solution onto the membrane was collected and diluted followed by adding 1 μL PNGase

F to proceed the digestion at 37 °C for 24 h. The digest was centrifuged and the supernatant was collected and preserved at -20 °C.

Extraction of N-glycans from standard sample

500 μg carbon material was dispersed in 100 μL aqueous solution containing a variable quantity of standard protein OVA digest or the mixture of OVA digest, OVA and BSA with different mass ratios. The above mixture was adequately vibrated at 37 °C for 45 min. After the washing with pure water several times, N-glycans adsorbed onto carbon material were released by 10 μL eluent (ACN/H_2O=50/50, v/v) in 30 min. The eluent containing N-glycans was immediately analyzed by MALDI-TOF MS.

Extraction of N-glycans from serum digest

500 μg carbon material was dispersed in 100 μL aqueous solution containing a fixed volume of serum digest. The mixture was adequately vibrated at 37 °C for 60 min. After washing with pure water several times, N-glycans adsorbed onto carbon material were released by 10 μL eluent (ACN/H_2O=50/50, v/v) in 45 min. The eluent containing N-glycans was immediately analyzed by MALDI-TOF MS.

MALDI-TOF MS analysis

1 μL eluent was deposited on the MALDI plate and let it dry. Then, 1 μL 10 mg mL^{-1} DHB (ACN/H_2O/TFA=20/79.9/0.1, v/v/v) was deposited onto the dried analyte to let the mixture naturally crystalized. The MALDI-TOF measurement was performed in reflector positive, linear positive and lift mode (CID on) on an UltrafleXtreme MALDI-TOF/TOF MS (Bruker Daltonic, Germany) equipped with a smartbeam Nd:YAG laser (355 nm) with the frequency of 2000 Hz. All MALDI MS data were collected using

Flexcontrol 3.4 and processed in Flexanalysis 3.4.

Data analysis

For each serum sample, three MALDI-TOF MS spectra were collected. Centroid was selected for peak detection algorithm and glycan peaks (SNR>4) detected in all three spectra were extracted. The relative content of each glycan was calculated respectively via dividing its intensity by the total intensity of all glycan peaks. The relative standard deviation (RSD) of each peak's intensity percentage should be less than 20% (n=3). Under this circumstance, few glycan peaks were discarded. Subsequently, all the glycan data of the two group were summarized and imported to SIMCA 14.1. for statistical analysis including the establishment of OPLS-DA model and permutation test. ROC curve and box plot analysis were executed based on R packages. Heatmap was depicted by OriginPro 2021b. Nonparametric test was conducted by IBM SPSS Statistics 23.

The raw MS data used in this study are available from the corresponding authors upon reasonable request.

Supplementary figures

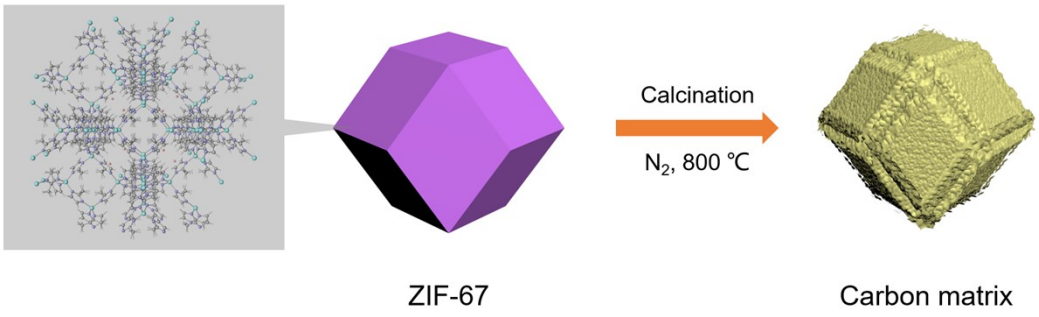


Fig. S1 Synthetic route of carbon matrix.

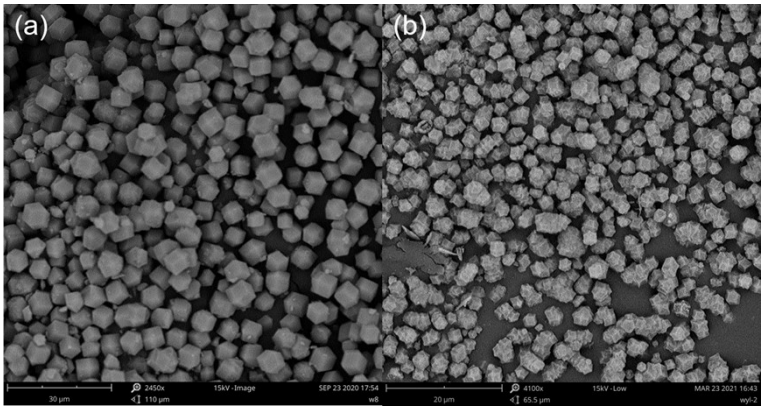


Fig. S2 SEM images of (a) ZIF-67 and (b) carbon matrix.

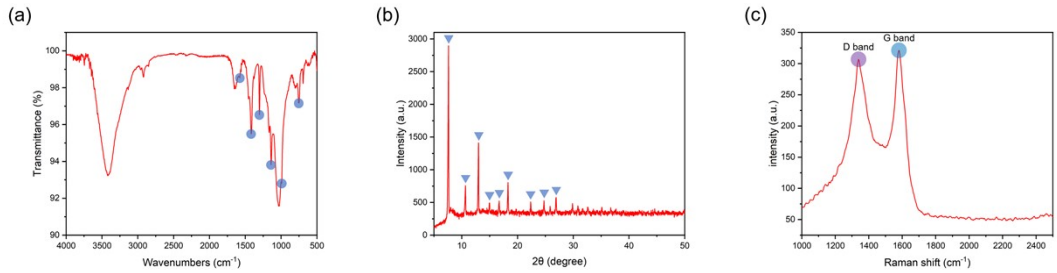


Fig. S3 (a) FT-IR spectrum and (b) XRD pattern of ZIF-67. The circles and triangles in the figures represent characteristic absorption peaks and specific diffraction peaks of ZIF-67, respectively. (c) Raman spectrum of carbon matrix.

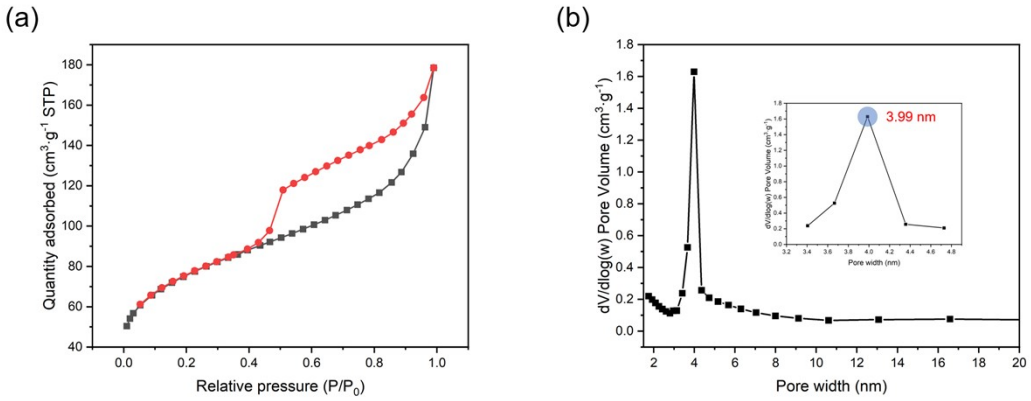


Fig. S4 (a) Nitrogen adsorption/desorption isotherm and (d) pore size distribution curve of carbon matrix.

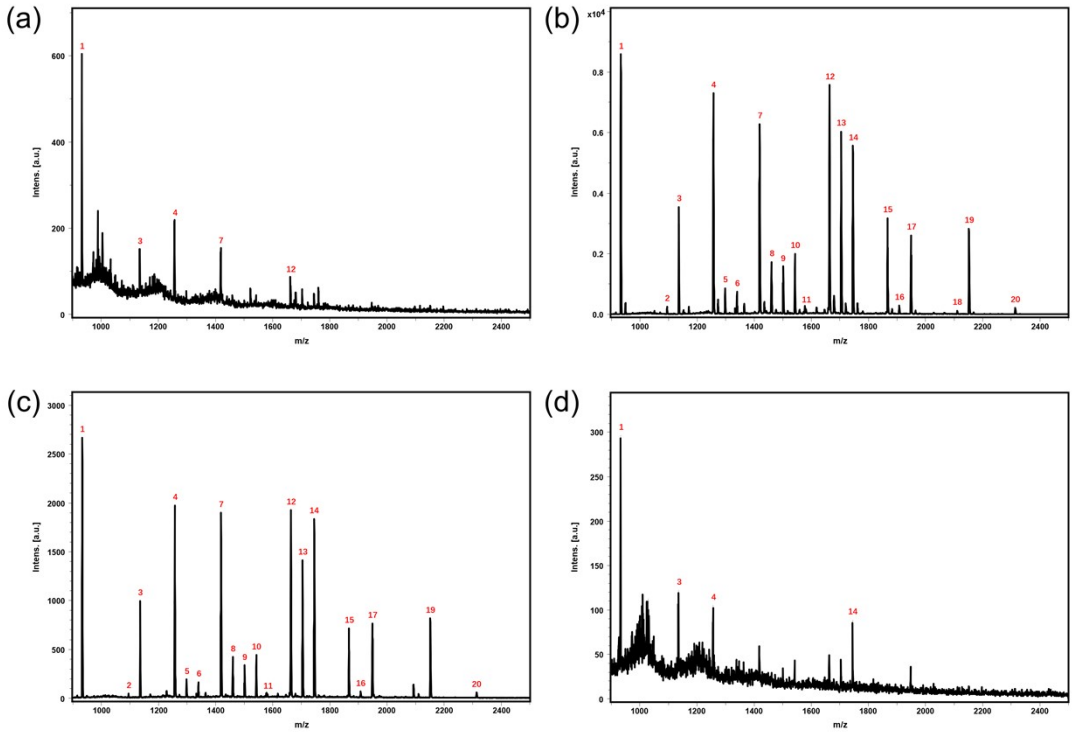


Fig. S5 MALDI-TOF MS spectra of N-glycans (a) from 50 ng μL^{-1} OVA digest before the extraction, (b-d) from 50 ng μL^{-1}, 10 ng μL^{-1} and 1 ng μL^{-1} OVA digest after the extraction by carbon matrix. (a-d) were obtained in reflector positive mode. The peaks annotated in number refers to N-glycan peaks and the detailed information of N-glycans is listed in Table S1.

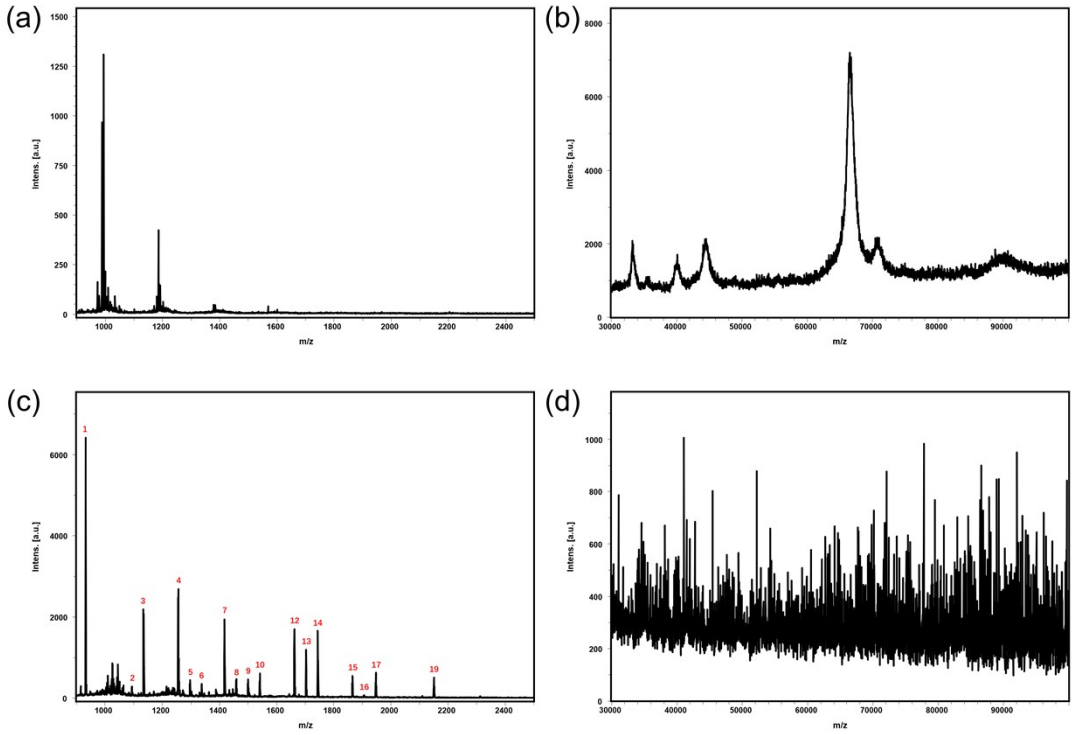


Fig. S6 MALDI-TOF MS spectra of N-glycans in the mixture of OVA digest, OVA and BSA with a mass ratio of 1:100:100: (a-b) before the extraction, (c-d) after the extraction by carbon matrix. (a) and (c) were obtained in reflector positive mode while (b) and (d) were acquired in linear positive mode. The peaks annotated in number refers to N-glycan peaks and the detailed information of N-glycans is listed in Table S1.

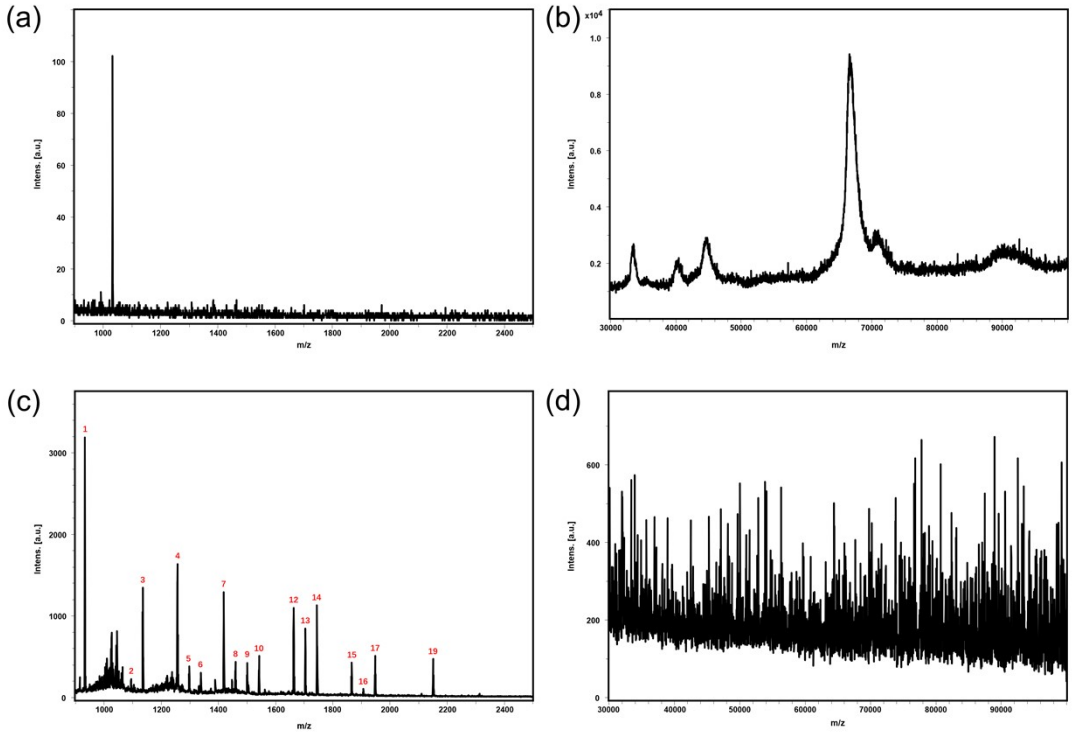


Fig. S7 MALDI-TOF MS spectra of N-glycans in the mixture of OVA digest, OVA and BSA with a mass ratio of 1:200:200: (a-b) before the extraction, (c-d) after the extraction by carbon matrix. (a) and (c) were obtained in reflector positive mode while (b) and (d) were acquired in linear positive mode. The peaks annotated in number refers to N-glycan peaks and the detailed information of N-glycans is listed in Table S1.

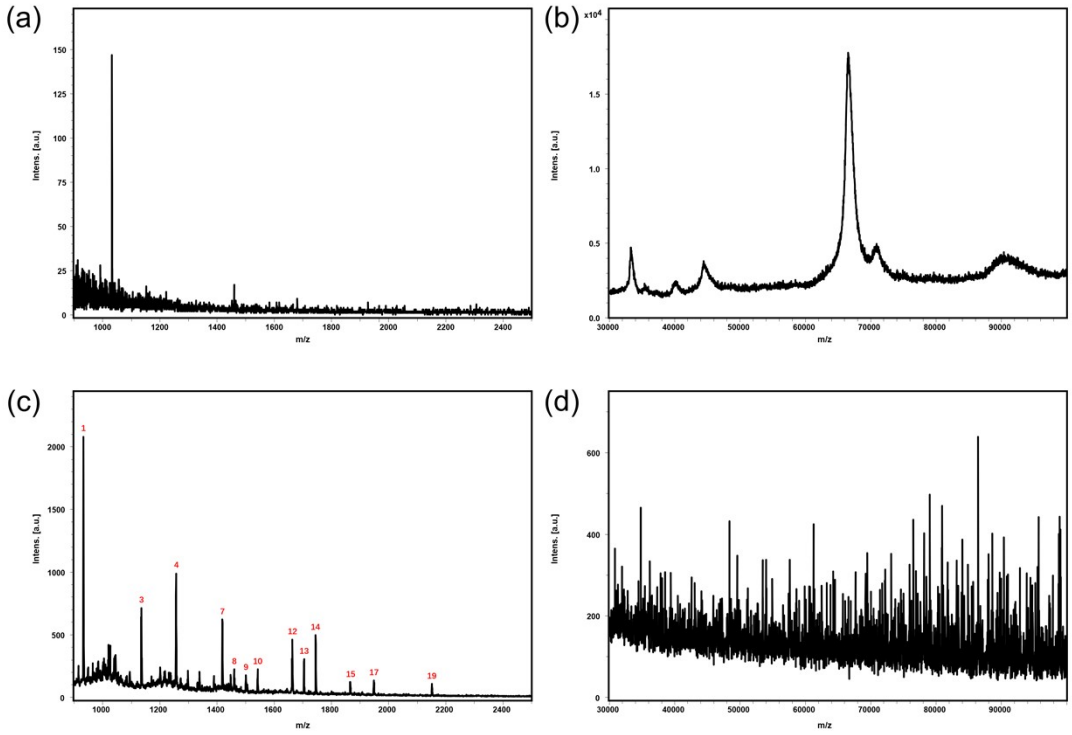


Fig. S8 MALDI-TOF MS spectra of N-glycans in the mixture of OVA digest, OVA and BSA with a mass ratio of 1:300:300: (a-b) before the extraction, (c-d) after the extraction by carbon matrix. (a) and (c) were obtained in reflector positive mode while (b) and (d) were acquired in linear positive mode. The peaks annotated in number refers to N-glycan peaks and the detailed information of N-glycans is listed in Table S1.

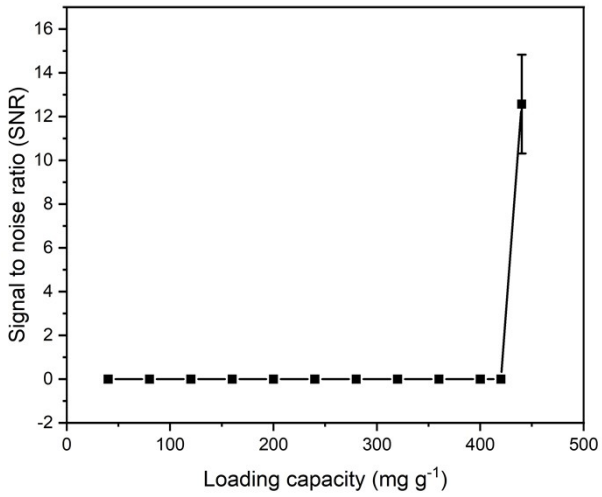


Fig. S9 SNR of the characteristic N-glycan peak (m/z=933.3) in the supernatant after the extraction by carbon matrix.

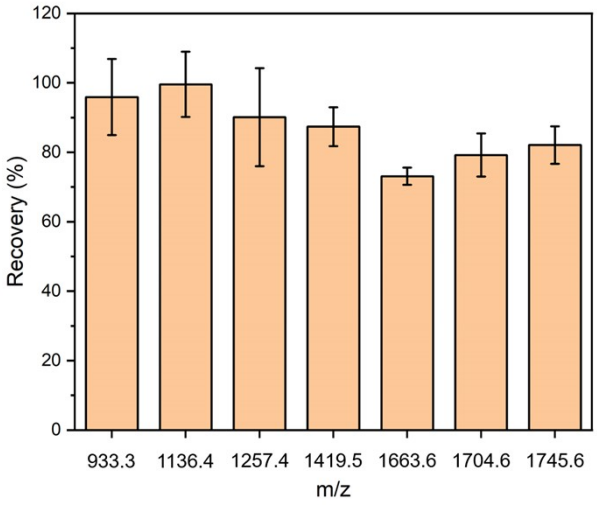


Fig. S10 Recoveries of the carbon matrix for seven N-glycans with the most signal to noise ratio (SNR) in OVA digest.

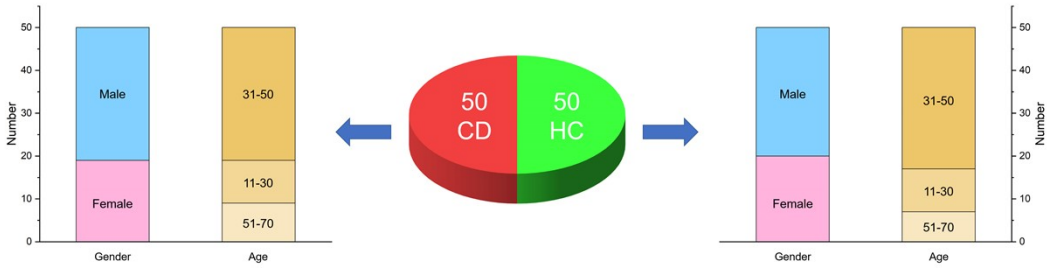


Fig. S11 Detailed information including gender and age distribution of the 50 pairs of serum samples.

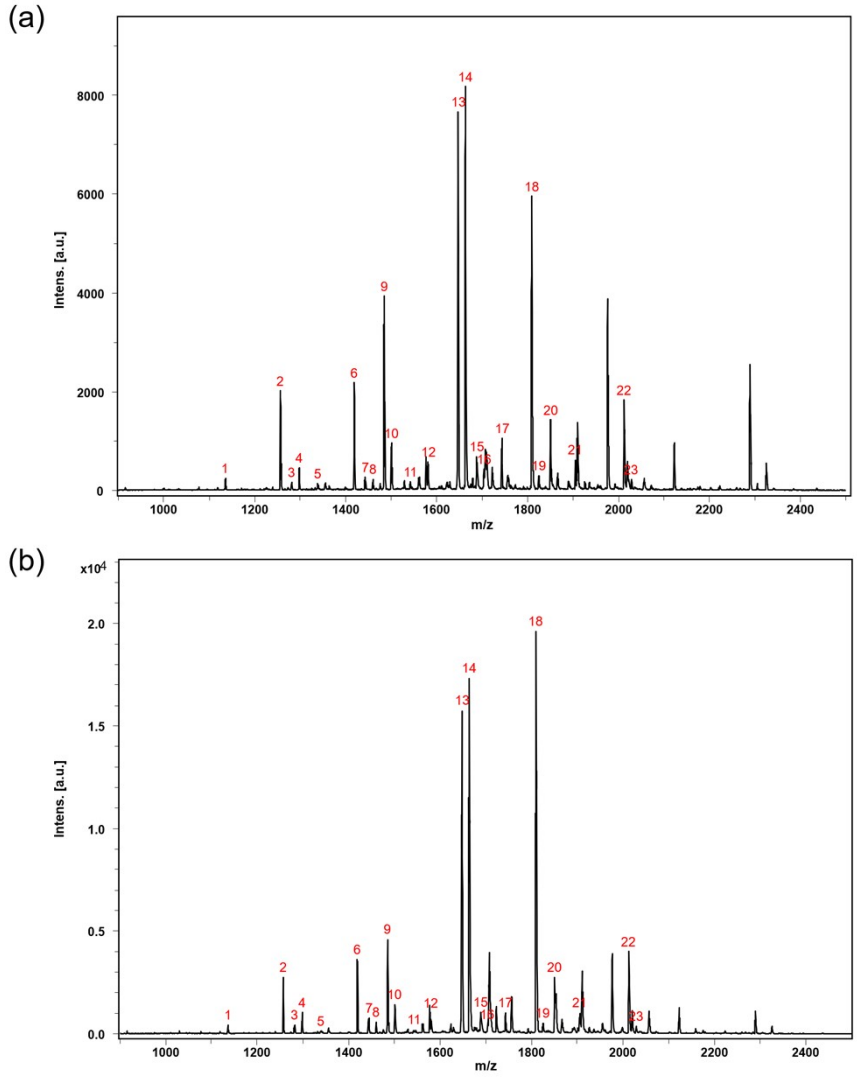


Fig. S12 MALDI-TOF MS spectra of serum N-glycans from (a) a CD patient and (b) a healthy control after the extraction by carbon matrix. (a) and (b) were obtained in reflector positive mode.The peaks annotated in number refers to N-glycan peaks and the detailed information of N-glycans is listed in Table S2.

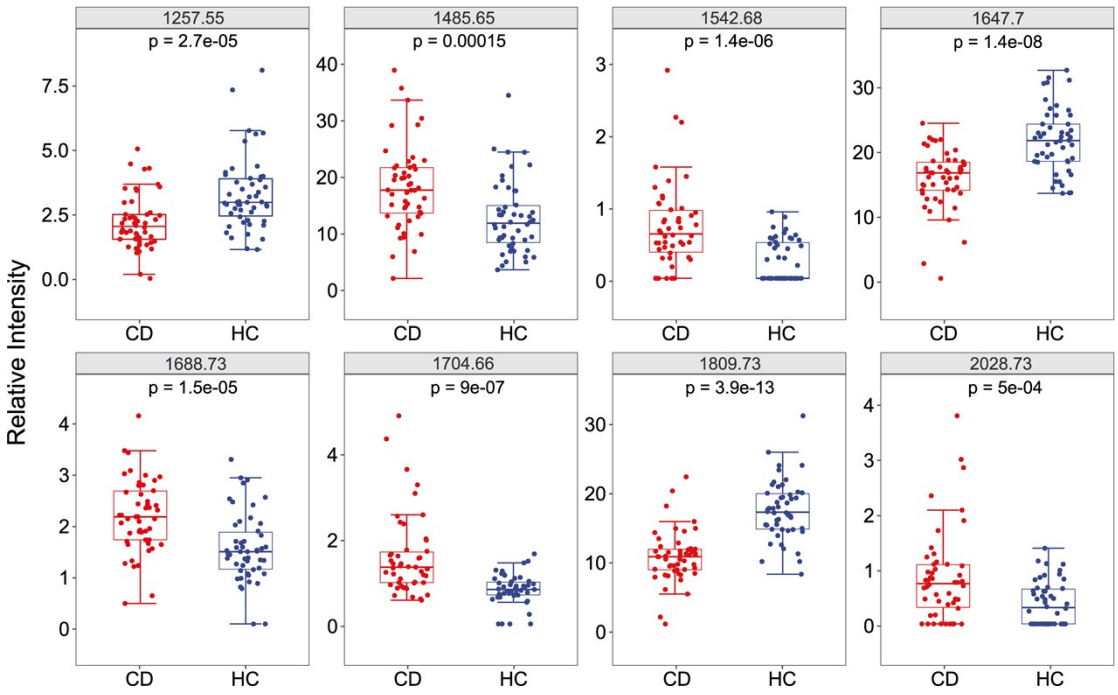


Fig. S13 Box plot analysis of eight N-glycans with great performance for CD diagnosis.

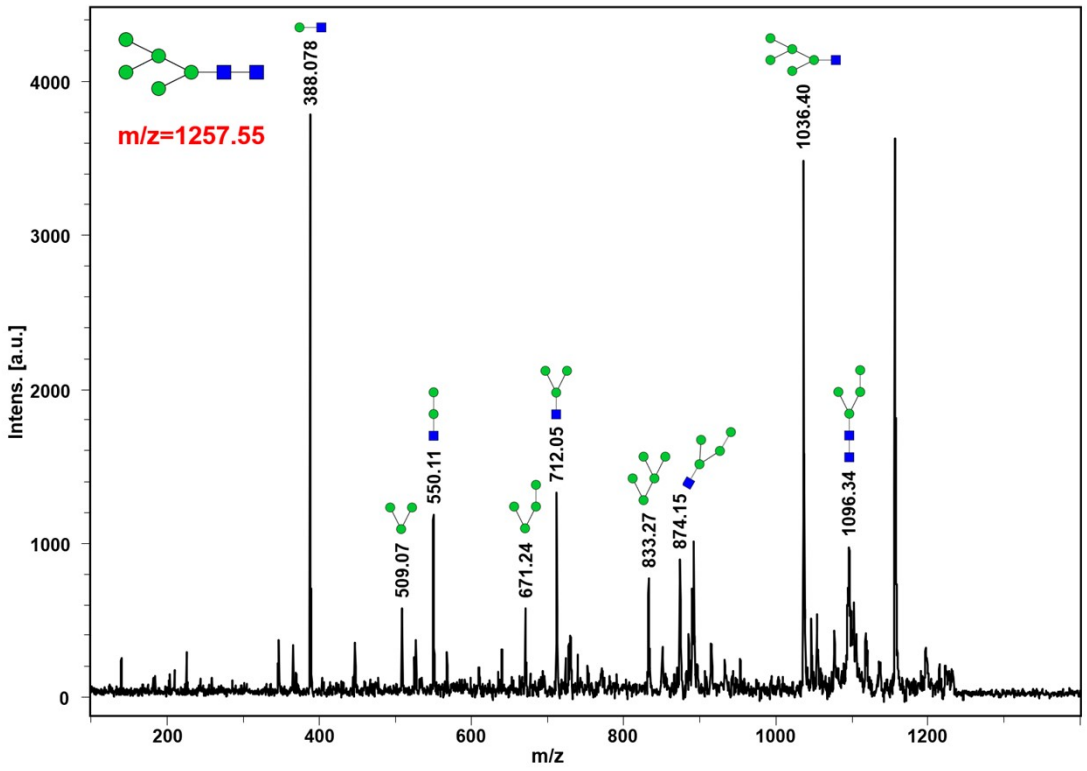


Fig. S14 MALDI-TOF MS/MS spectrum of the N-glycan with a m/z of 1257.55 obtained in lift mode. The specific structures of corresponding fragment peaks are annotated in the spectrum. The yellow circle, green circle and blue square represent galactose, mannose and N-acetylglucosamine, respectively.

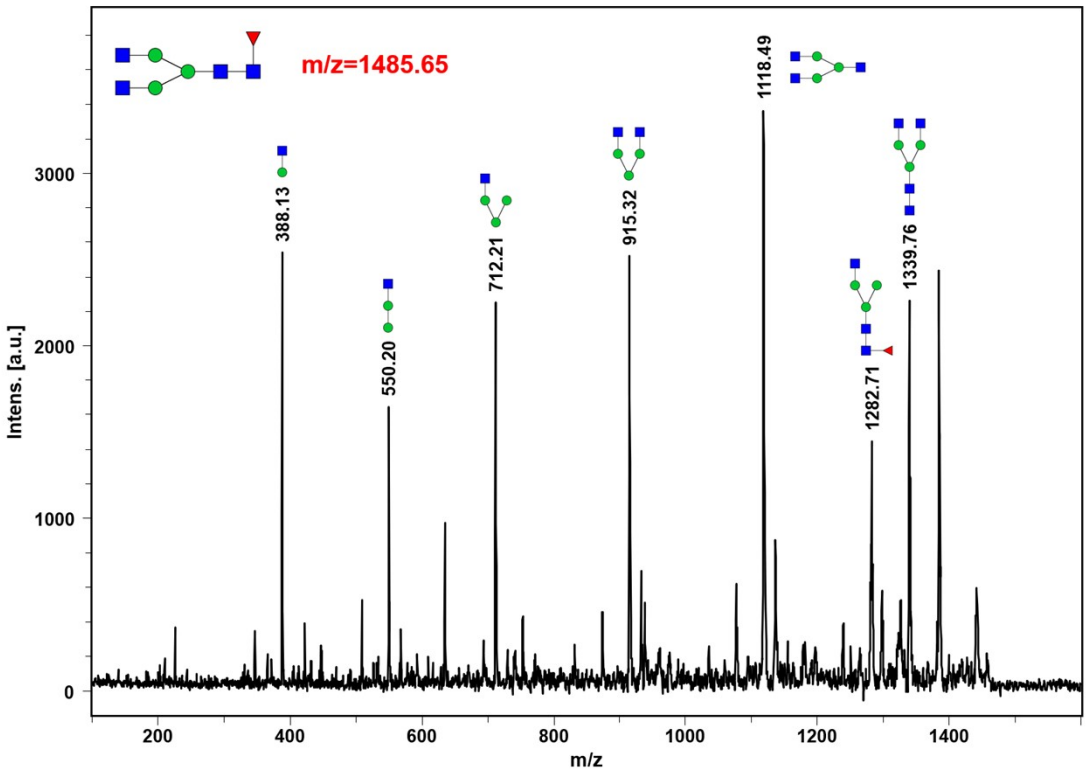


Fig. S15 MALDI-TOF MS/MS spectrum of the N-glycan with a m/z of 1485.65 obtained in lift mode. The specific structures of corresponding fragment peaks are annotated in the spectrum. The yellow circle, green circle and blue square represent galactose, mannose and N-acetylglucosamine, respectively.

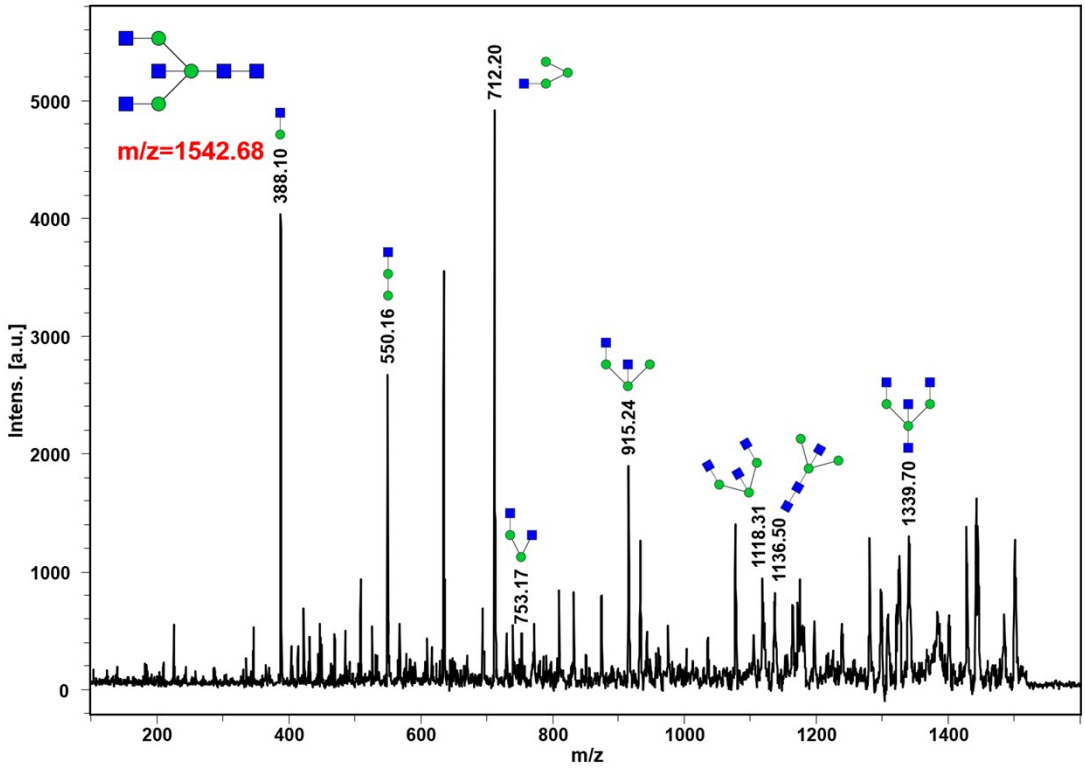


Fig. S16 MALDI-TOF MS/MS spectrum of the N-glycan with a m/z of 1542.68 obtained in lift mode. The specific structures of corresponding fragment peaks are annotated in the spectrum. The yellow circle, green circle and blue square represent galactose, mannose and N-acetylglucosamine, respectively.

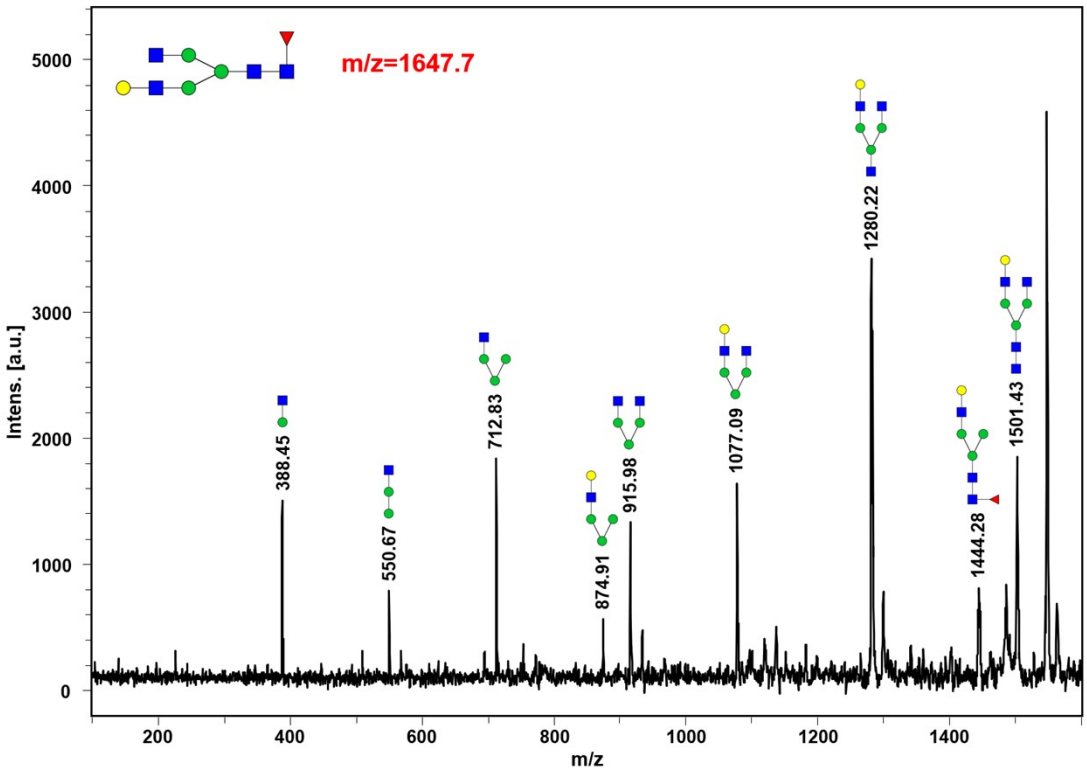


Fig. S17 MALDI-TOF MS/MS spectrum of the N-glycan with a m/z of 1647.7 obtained in lift mode. The specific structures of corresponding fragment peaks are annotated in the spectrum. The yellow circle, green circle, red triangle and blue square represent galactose, mannose, fucose and N-acetylglucosamine, respectively.

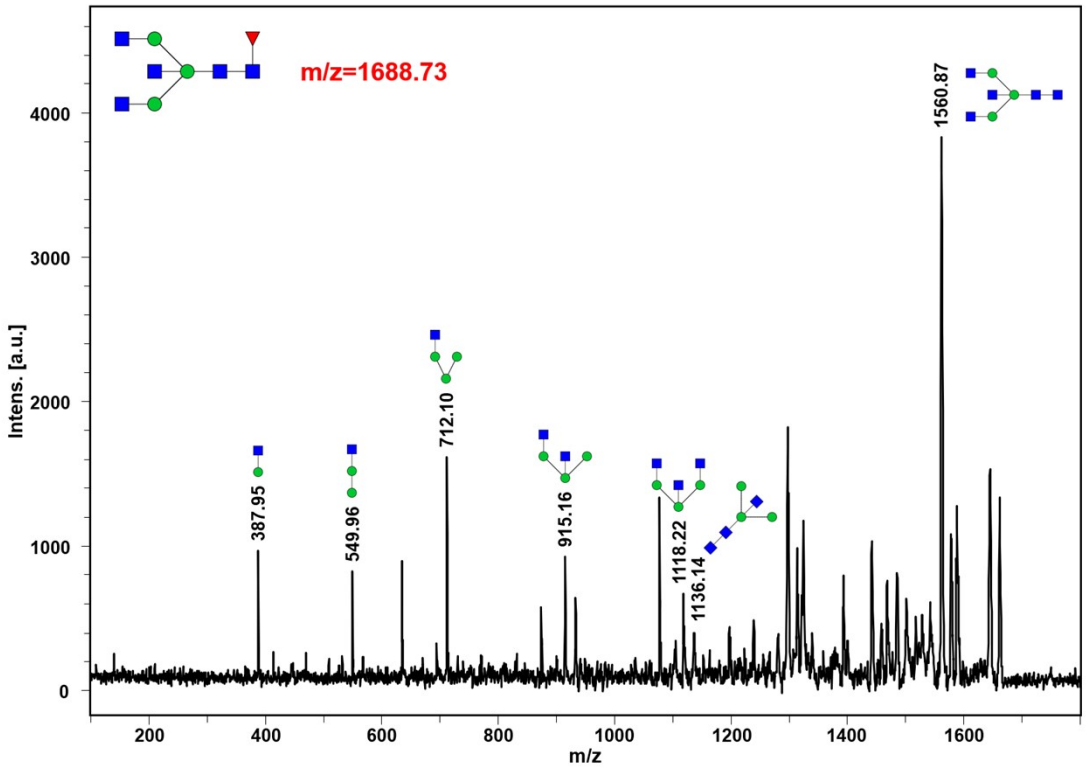


Fig. S18 MALDI-TOF MS/MS spectrum of the N-glycan with a m/z of 1688.73 obtained in lift mode. The specific structures of corresponding fragment peaks are annotated in the spectrum. The yellow circle, green circle, red triangle and blue square represent galactose, mannose, fucose and N-acetylglucosamine, respectively.

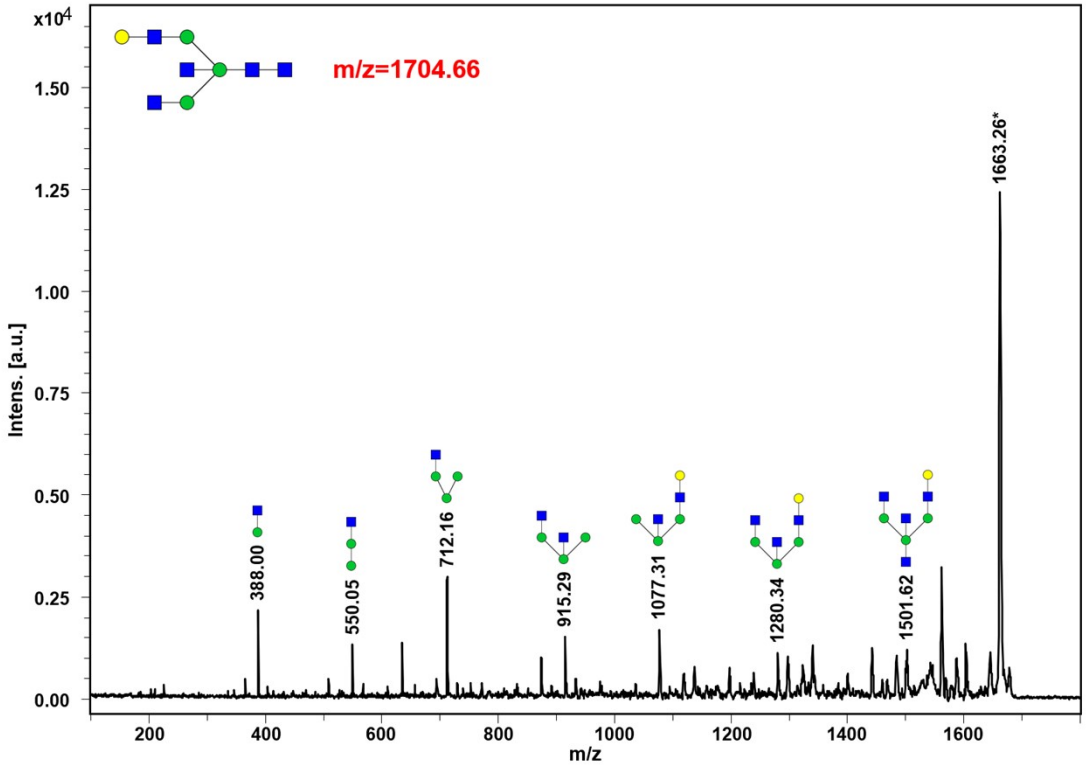
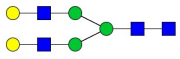


Fig. S19 MALDI-TOF MS/MS spectrum of the N-glycan with a m/z of 1704.66 obtained in lift mode. The specific structures of corresponding fragment peaks are annotated in the spectrum. The yellow circle, green circle, red triangle and blue square represent galactose, mannose, fucose and N-acetylglucosamine, respectively. * probably represents an high SNR impurity peak (m/z=1663.26) whose structure is



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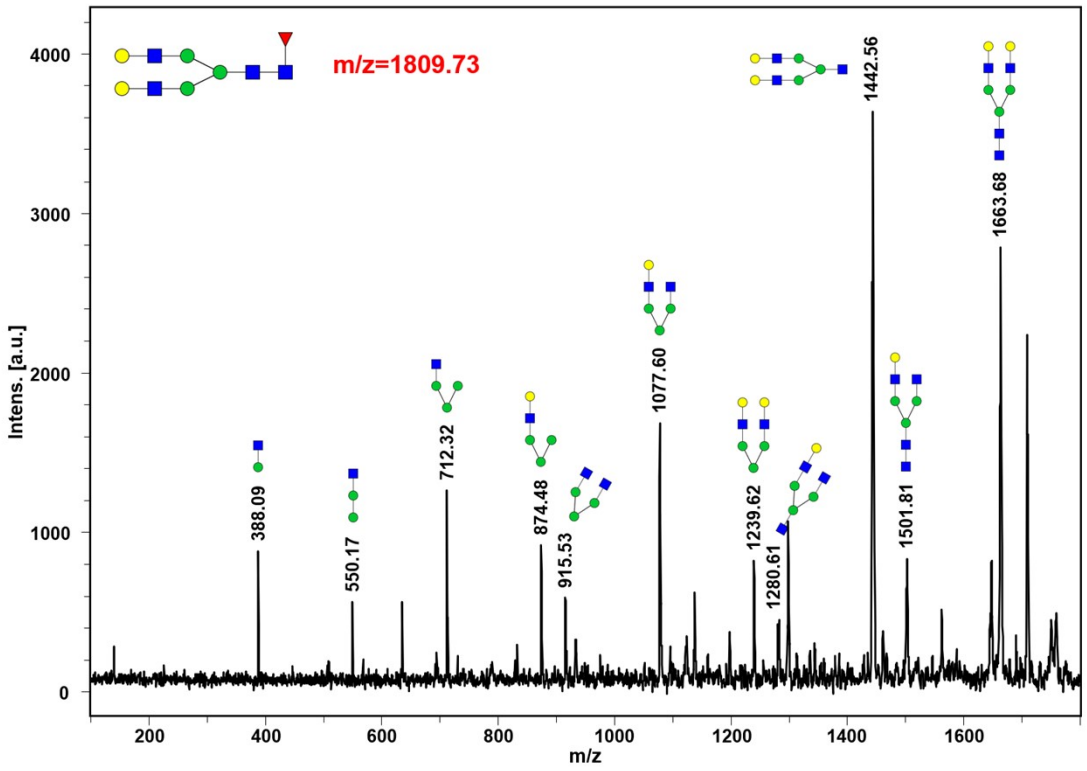


Fig. S20 MALDI-TOF MS/MS spectrum of the N-glycan with a m/z of 1809.73 obtained in lift mode. The specific structures of corresponding fragment peaks are annotated in the spectrum. The yellow circle, green circle, red triangle and blue square represent galactose, mannose, fucose and N-acetylglucosamine, respectively.

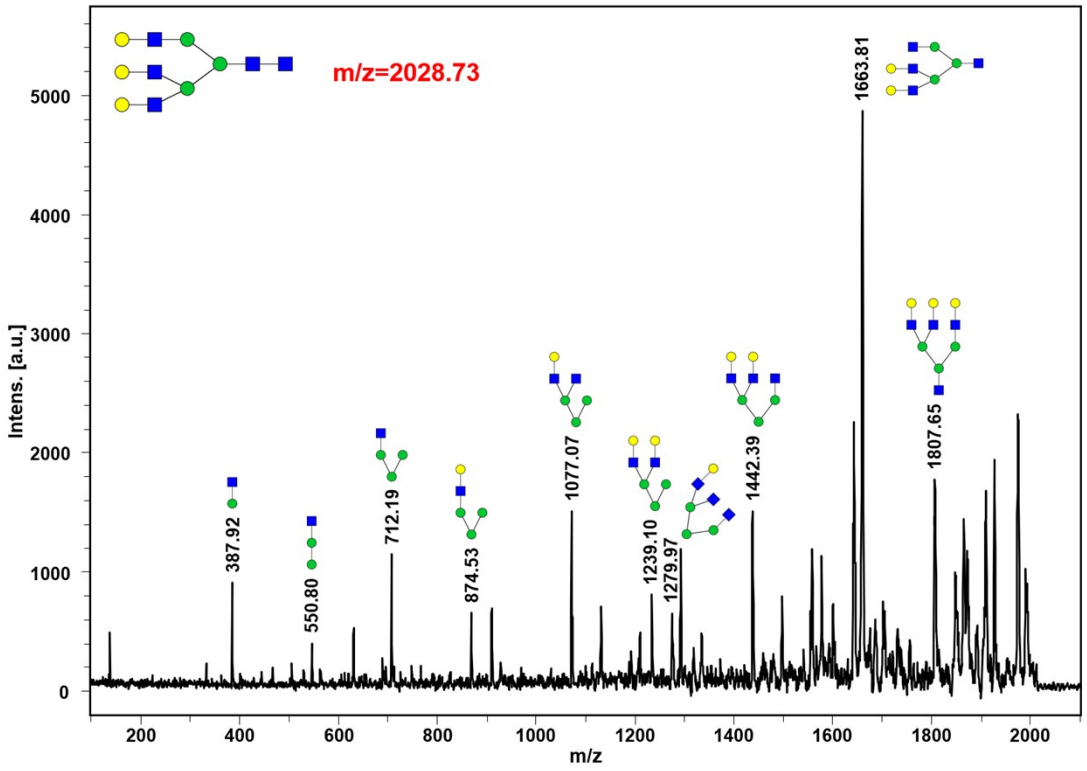
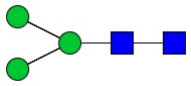
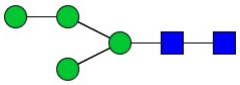
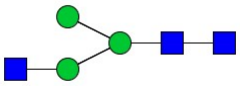
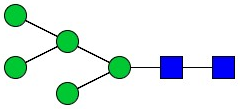
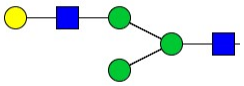
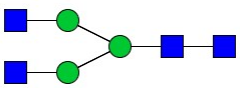
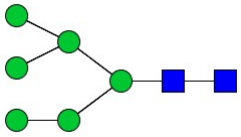
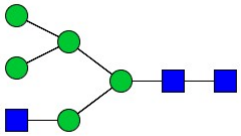
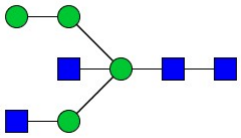
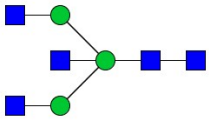
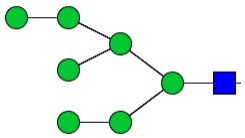
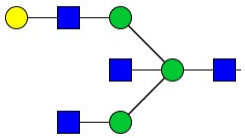
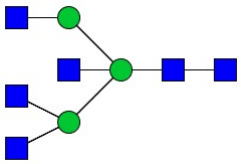
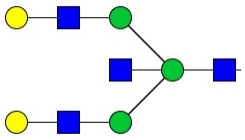


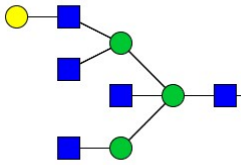
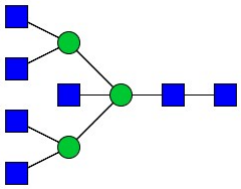
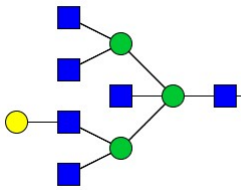
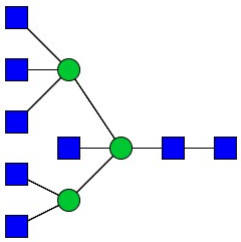
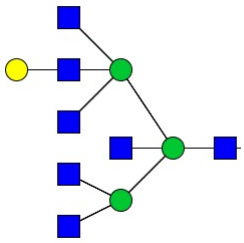
Fig. S21 MALDI-TOF MS/MS spectrum of the N-glycan with a m/z of 2028.73 obtained in lift mode. The specific structures of corresponding fragment peaks are annotated in the spectrum. The yellow circle, green circle and blue square represent galactose, mannose and N-acetylglucosamine, respectively.

Supplementary tables

Table S1 Detailed information of the observed N-glycans extracted from standard glycoprotein OVA digest. The corresponding glycan structures were searched from GlycomeDB through the Mol. wt of oligosaccharide by GlycoWorkbench. The capital "H" and "N" in "Composition" represent hexose and N-acetylglucosamine, respectively.

No.	m/z ([M+Na]$^+$)	Compositions	Structures
1	933.3	H3N2	
2	1095.4	H4N2	
3	1136.4	H3N3	
4	1257.4	H5N2	
5	1298.4	H4N3	
6	1339.5	H3N4	

7	1419.5	H6N2	
8	1460.5	H5N3	
9	1501.5	H4N4	
10	1542.6	H3N5	
11	1581.5	H7N2	
12	1663.6	H5N4	
13	1704.6	H4N5	
14	1745.6	H3N6	
15	1866.7	H5N5	

16	1907.7	H4N6	
17	1948.7	H3N7	
18	2110.8	H4N7	
19	2151.8	H3N8	
20	2313.8	H4N8	

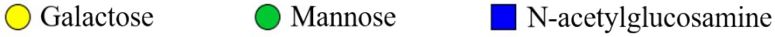
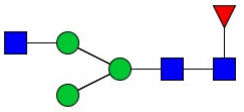
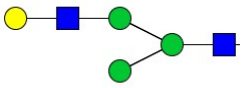
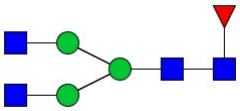
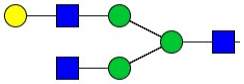
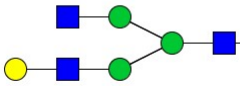
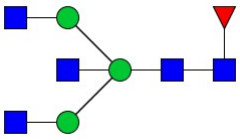


Table S2 Detailed information of the observed N-glycans extracted from human serum digest. The corresponding glycan structures were searched from GlycomeDB through the Mol. wt of oligosaccharide by GlycoWorkbench. Structures of eight N-glycans with credible performance for CD diagnosis have been demonstrated by MS/MS while the others are not. The capital "H", "N" and "F" in "Composition" represent mannose and galactose, N-acetylglucosamine and fucose, respectively.

No.	m/z ([M+Na]$^+$)	Compositions	Structures
1	1136.4	H3N3	
2	1257.4	H5N2	
3	1282.5	H3N3F1	
4	1298.4	H4N3	
5	1339.5	H3N4	
6	1419.5	H6N2	
7	1444.5	H4N3F	

8	1460.5	H5N3	
9	1485.5	H3N4F	
10	1501.5	H4N4	
11	1542.6	H3N5	
12	1581.5	H7N2	
13	1647.6	H4N4F	
14	1663.6	H5N4	
15	1688.6	H3N5F	
16	1704.6	H4N5	

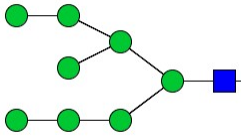
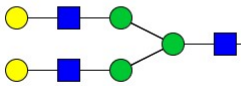
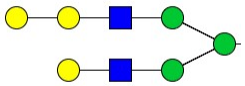
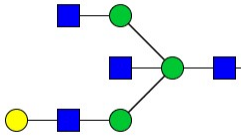
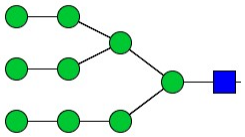
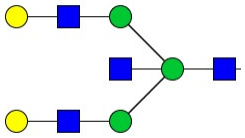
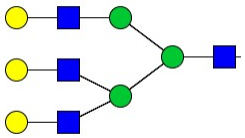
17	1743.6	H8N2	
18	1809.6	H5N4F	
19	1825.6	H6N4	
20	1850.7	H4N5F	
21	1905.6	H9N2	
22	2012.7	H5N5F	
23	2028.7	H6N5	

Table S3 P and VIP values of the eight N-glycans selected for ROC curve, heatmap and box plot analysis.

m/z ($[M+Na]^+$)	P values	VIP values
1257.55	2.55E-05	1.17667
1485.65	1.51E-04	1.10749
1542.68	8.34E-07	1.29295
1647.7	1.38E-08	1.4081
1688.73	1.54E-05	1.19075
1704.66	3.76E-07	1.31619
1809.73	3.46E-13	1.6403
2028.73	4.23E-04	1.0502

Table S4 Detailed information including structures, N-glycan types and expression changes of eight N-glycans with credible performance for CD diagnosis.

m/z ([M+Na]$^+$)	Structures	N-glycan types	Expression changes
1257.55		High mannose	Down-regulation
1485.65		Complex	Up-regulation
1542.68		Complex	Up-regulation
1647.7		Complex	Down-regulation
1688.73		Complex	Up-regulation
1704.66		Complex	Up-regulation
1809.73		Complex	Down-regulation
2028.73		Complex	Up-regulation

Reference

1 N. Sun, X. Zhang and C. Deng, *Nanoscale*, 2015, **7**, 6487–6491.