Supplementary Information for

Facile preparation of the novel chitosan-derived porous graphitized carbon for highly efficient capture of N-glycans

Jiaxi Wang,[a] Lingxiao Weng,[a] Weizhuo Liu,[c] Hongbin Zhang,[b,*] Mingxia Gao,[a] Xiangmin Zhang,[a,*] and Li-Hao Huang,[a,*]

[a] Shanghai Key Laboratory of Metabolic Remodeling and Health, Department of Chemistry and Institute of Metabolism and Integrative Biology, Fudan University, Shanghai 200032, China.

[b] Institute for Preservation of Chinese Ancient Books, Fudan University Library, Fudan University, Shanghai 200433, China.

[c] Department of Critical Care Medicine, Shanghai Chest Hospital, Shanghai Jiaotong University, 241 West Huaihai Road, Shanghai 200030, China.

EXPERIMENTAL SECTION

Measurements and characterization. X-ray photoelectron spectroscopy (XPS) for the valences analysis of elements was recorded on an AXIS ULTRA DLD XPS System with MONO Al source (Shimadzu Corp). The temperature-programmed desorption was performed by using the AutoChem 2920 analyzer (Micromeritics), and the evolved gases (CO, CO_2 and H_2O) were identified by a quadrupole mass spectrometer (Pfeiffer, Omnistar320). The sample was heated to 110 ℃ under 50 ml/min helium flow for drying and degassing, then the sample was continuously heated to 1050 ℃ at a rate of 10 ℃/min under 50 ml/min helium flow and was maintained at 1050 ℃ for 1h. The static contact angles were measured using an OCA15+ contact-angle system (Dataphysics, Germany) at ambient temperature.

Preparation of the human serum digest. The human serum samples (obesity, impaired glucose tolerance, diabetes patients and healthy control) were centrifuged with the speed of 12000×g at 4 °C for 10 min. After being measured the concentration by bicinchoninic acid (BCA) protein assay kit, the obtained proteins were diluted with 25 mM NH_4HCO_3 and boiled for 5 min. Afterwards, the proteins were reduced with 10 mM DTT for 30 min at 55 °C, and subsequently alkylated by 20 mM IAA for 60 min at 37 °C in the dark. The mixture was diluted and transferred to an ultrafiltration tube (3 kDa). After centrifugation, the denatured protein solution was collected. The PNGase F (1 μL) was added into the solution at 37 °C overnight. The released N-glycans were collected and preserved at -20 °C for further use.

Fig. S1. (a, b) SEM images, (c, d) TEM images (inset of Fig. 1d: SAED pattern), (e) HAADF-STEM image and related elemental mapping images of the CS-900.

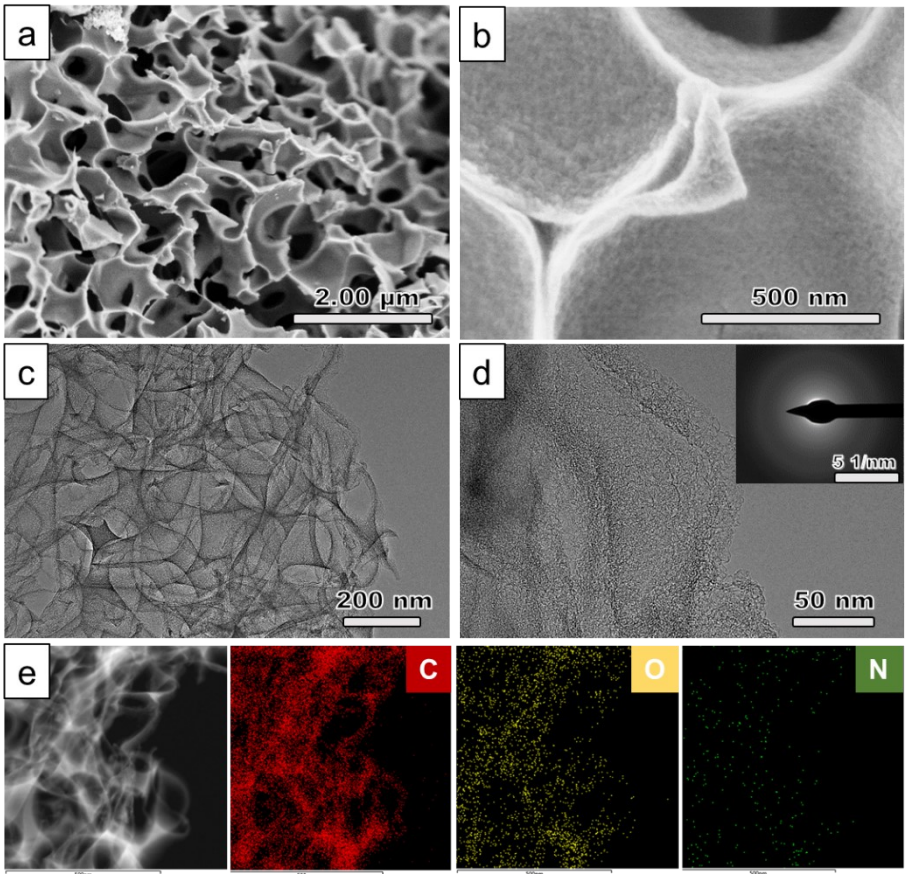


Fig. S2. (a) CS-900 and (b) CS-900-1C materials dispersed in loading buffer solution.

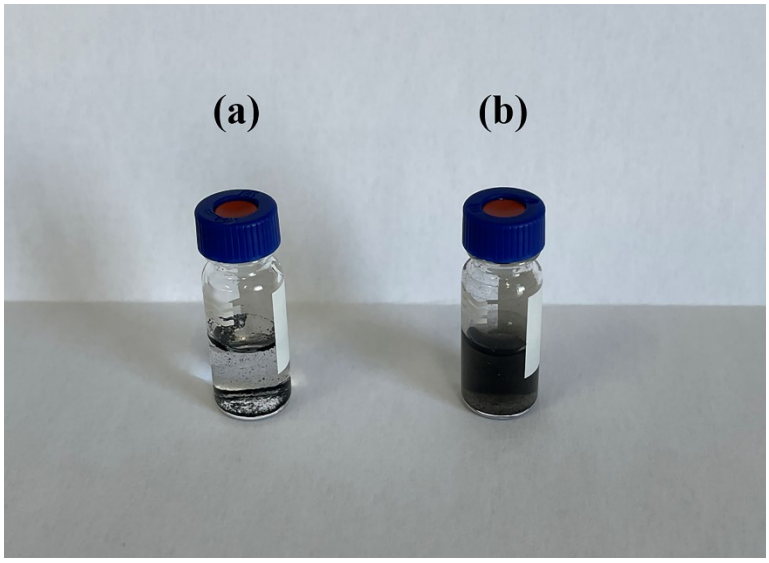


Fig. S3. The effect of different incubation time (a) 5 min; (b) 15 min; (c) 30 min and (d) 45 min in N-glycans enriched from OVA through three parallel tests.

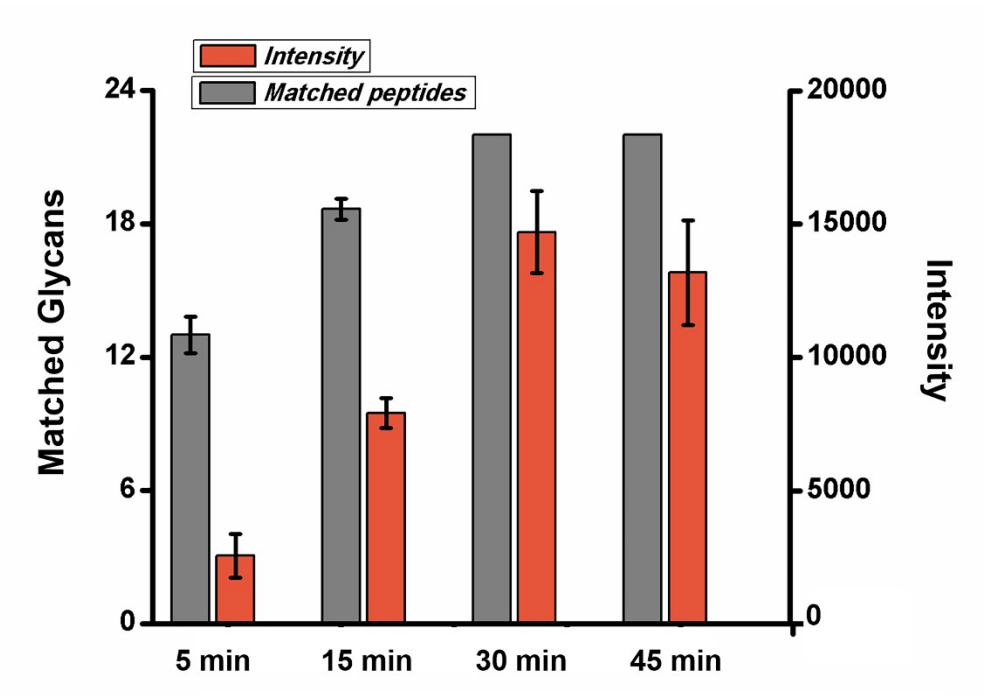


Fig. S4. MALDI-TOF-MS analysis of N-glycans derived from OVA enriched by CS-900-1C with different eluent (a) 10% ACN; (b) 30% ACN; (c) 50% ACN and (d) 80% ACN.

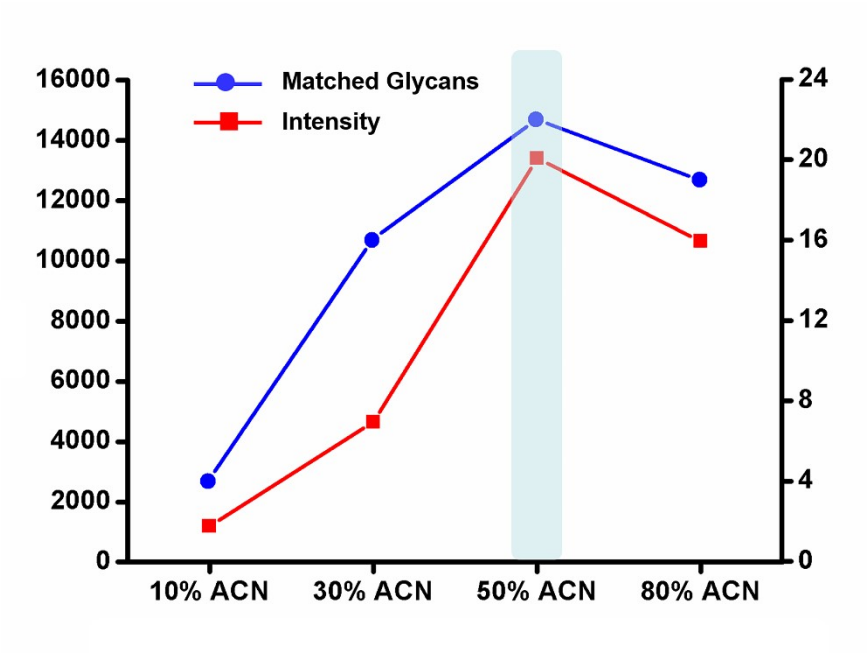


Fig. S5. MALDI-TOF mass spectra of N-glycans released from OVA digests after enrichment by (a) CS-900-1C, (b) CS-800-1C, (c) CS-700-1C and (d) CS-600-1C. The peaks of N-glycans are marked with "*".

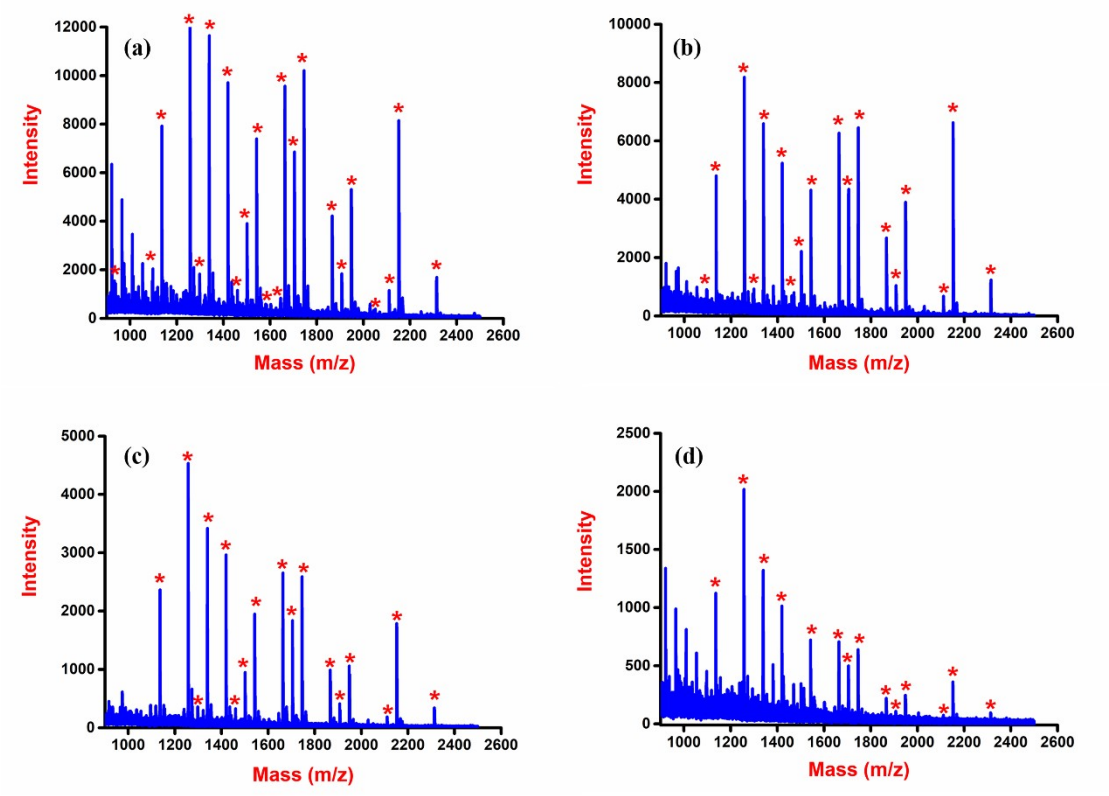


Fig. S6. The cycling performance of CS-900-1C for N-glycans capture (a). MALDI-TOF-MS for the N-glycans derived from OVA digest (50 ng/μL): (b) after treatment with CS-900-1C used for the first time, (c) after enrichment with CS-900-1C recycled 8 times.

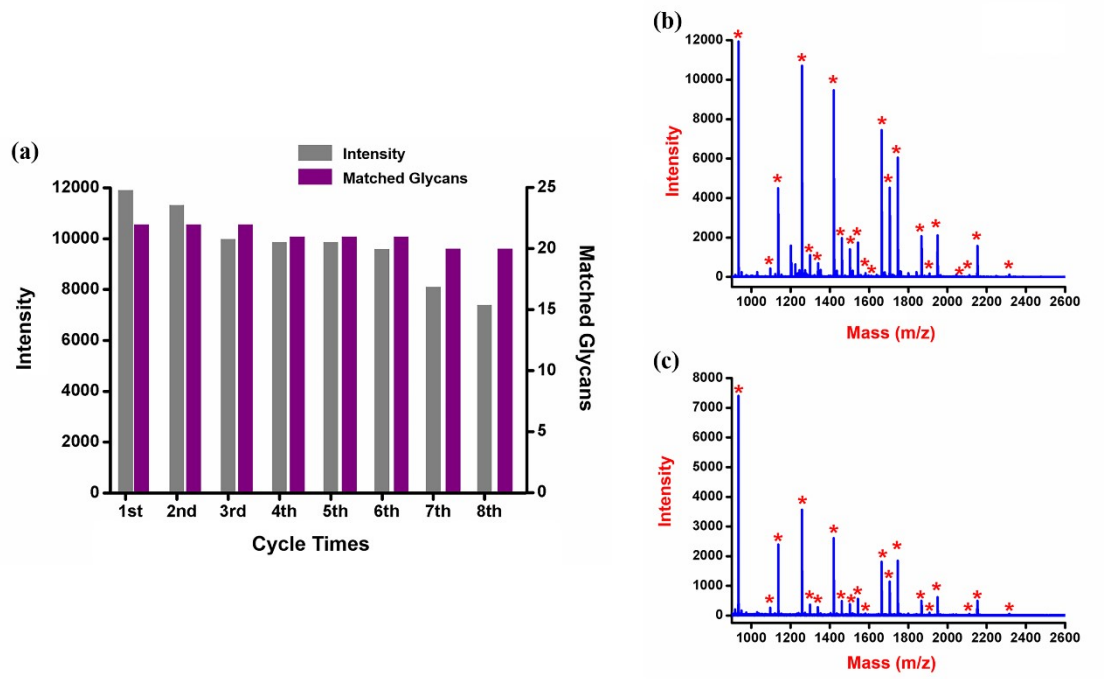


Fig. S7. MALDI-TOF-MS for the N-glycans derived from OVA digest: (a) after treatment with the CS-900-1C biomaterials for the first time and (b) after enrichment with the CS-900-1C biomaterials which had been placed for six months.

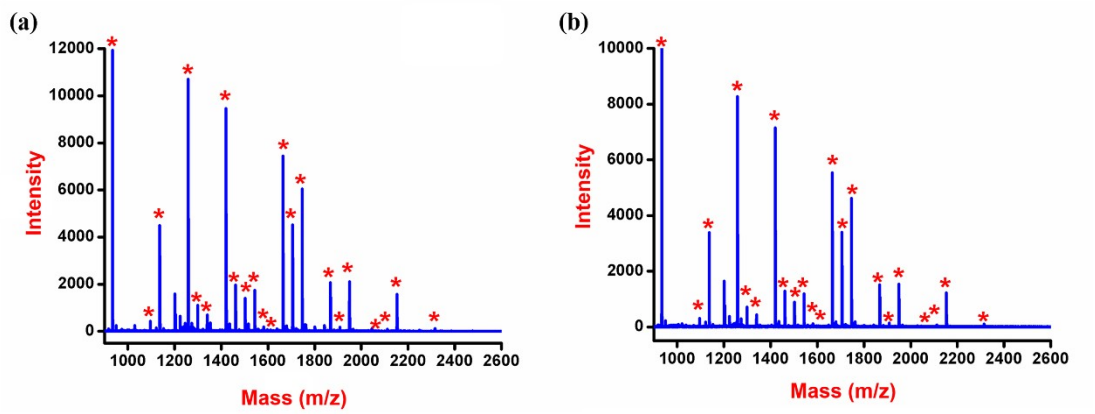


Fig. S8. MALDI-TOF-MS analysis of the N-glycans derived from the mixture of OVA digests, glycosylated proteins (OVA), and nonglycosylated proteins (BSA) (at mass ratio of 1:800:800). Supernate before enrichment (a) in positive mode and (b) in linear mode and eluate after enrichment by CS-900-1C biomaterials (c) in positive mode and (d) in linear mode. The peaks marked with red asterisks represent the identified N-glycans. The peaks marked with green asterisks represent the interferential proteins.

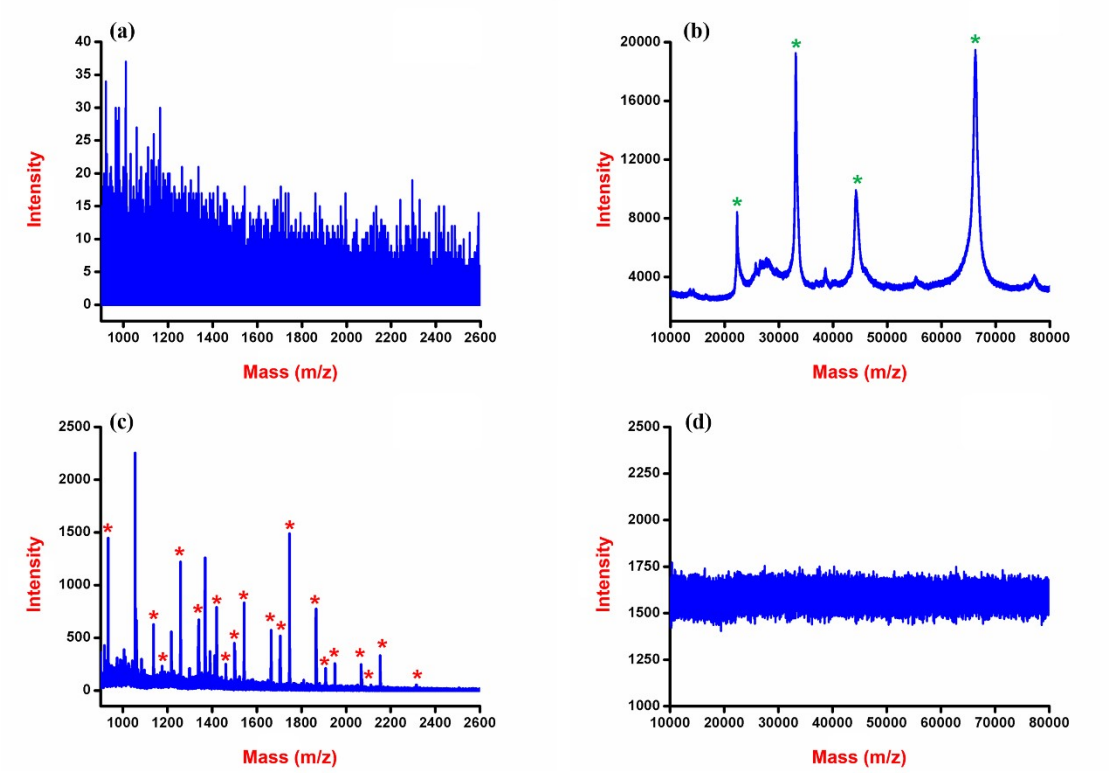


Fig. S9. MALDI-TOF-MS analysis of N-glycans identified from normal human serum (a) without enrichment and (b) after enrichment with CS-900-1C biomaterials.

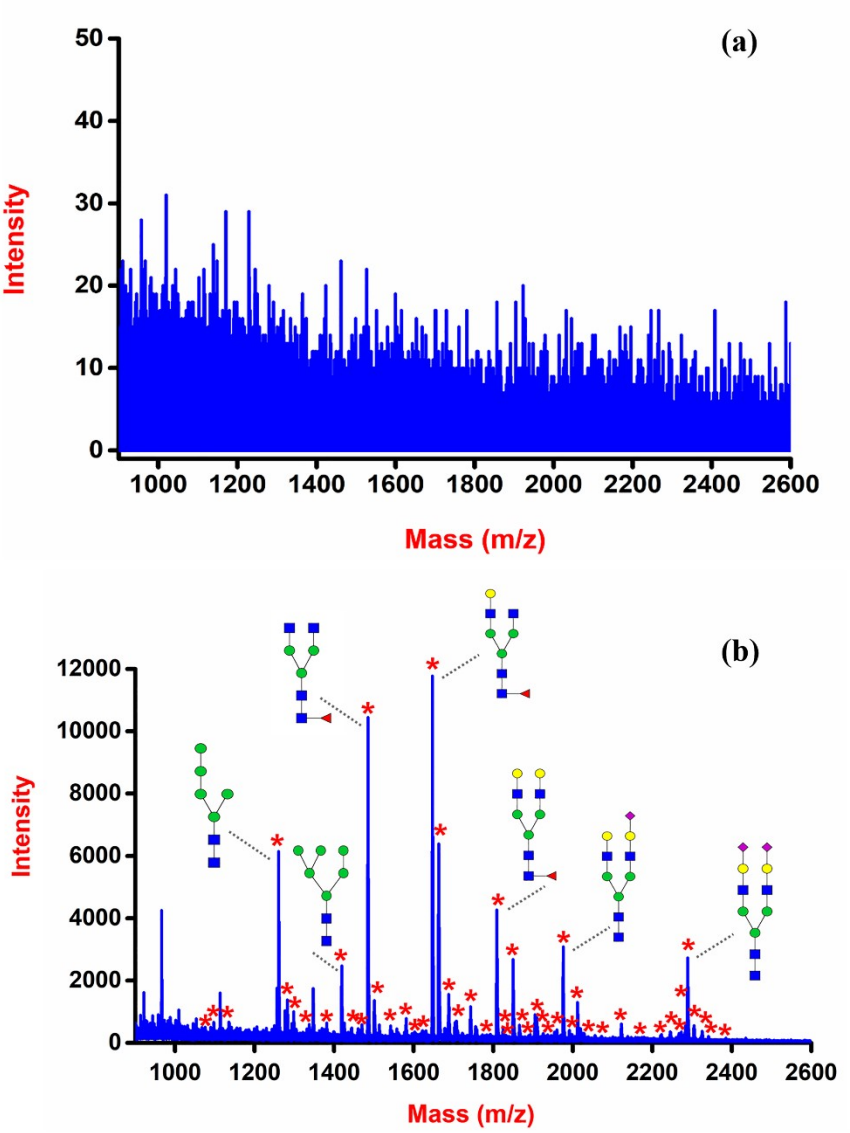


Fig. S10. Heatmap analysis based on 49 serum N-glycans in healthy controls (N), obesity (OB), impaired glucose tolerance (IGT) and diabetes (DM) groups.

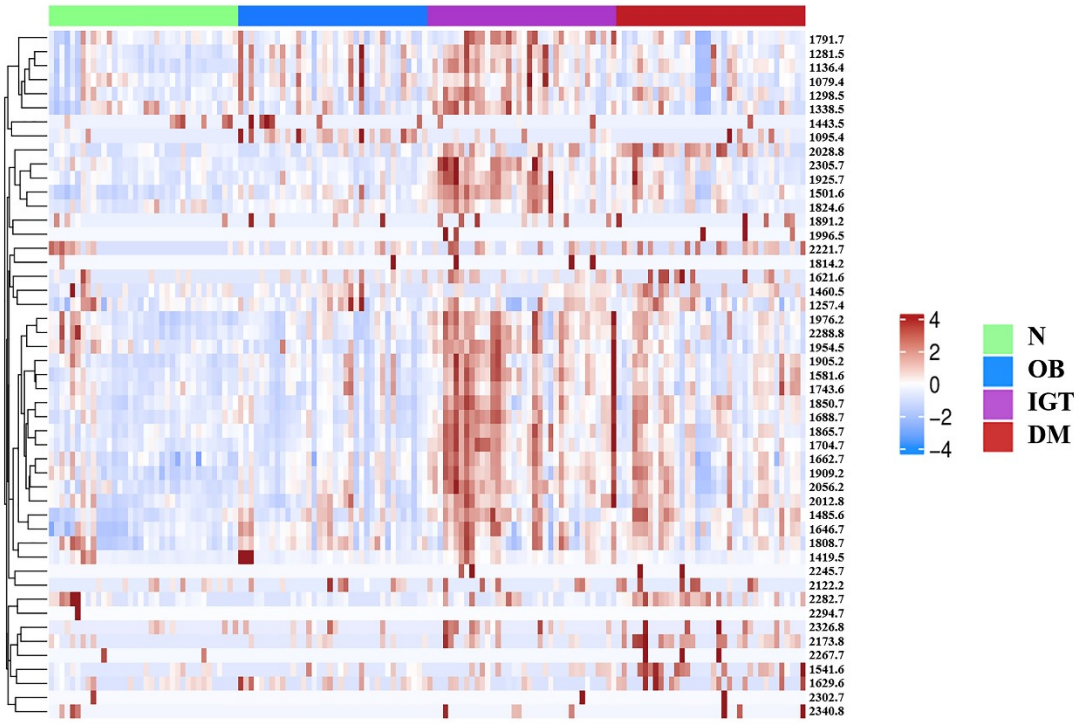


Fig. S11. Heatmap analysis based on selected 11 serum N-glycans with P value < 0.05 and VIP > 1 (a). The receiver operating characteristic (ROC) curve of sparse learning to differentiate OB patients from IGT patients afforded diagnostic performance (b-e).

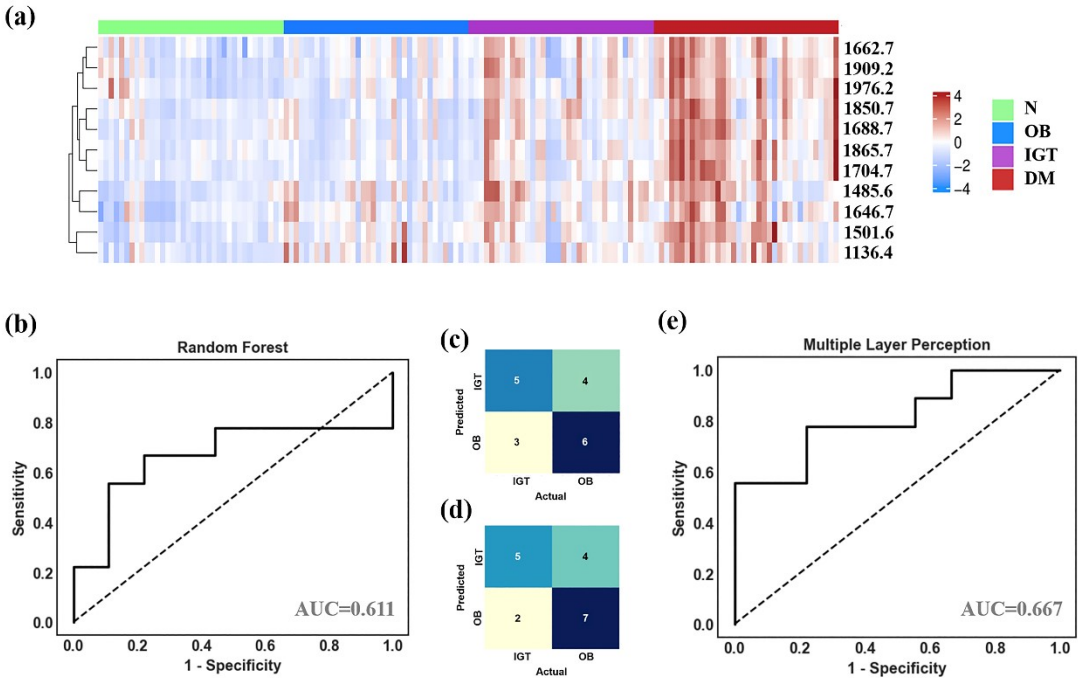
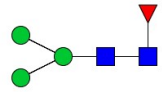
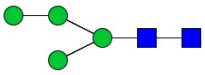
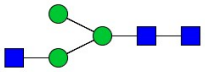
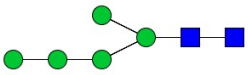
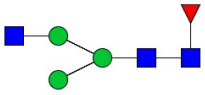
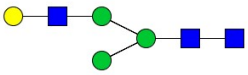
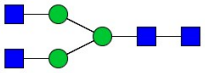
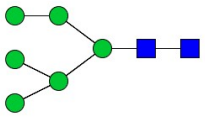
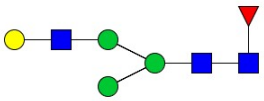
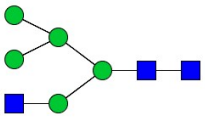
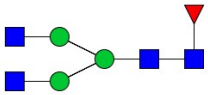
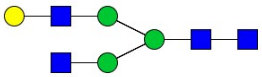
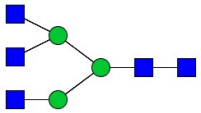
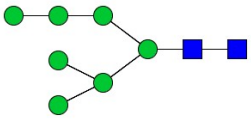
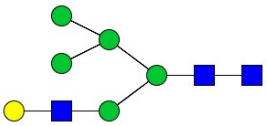
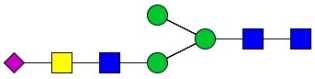
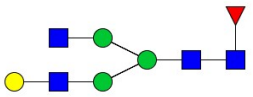
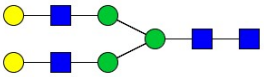
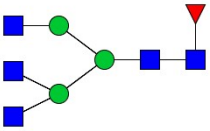
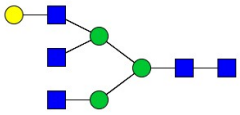
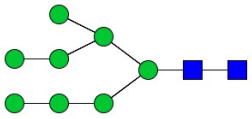
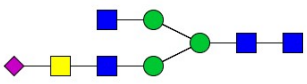


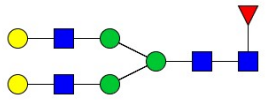
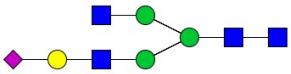
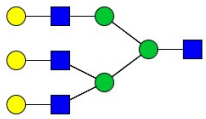
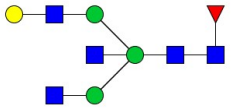
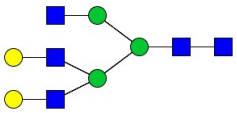
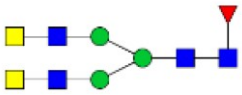
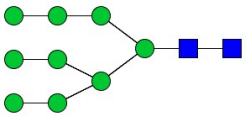
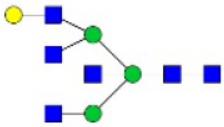
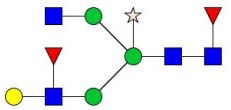
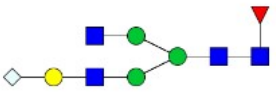
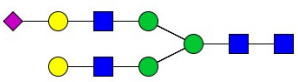
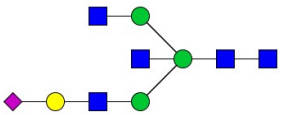
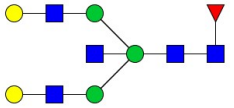
Table S1. List of identified 22 N-glycans released from OVA digests enriched by CS-900-1C porous carbon.

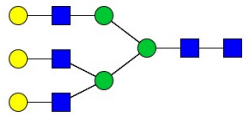
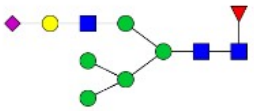
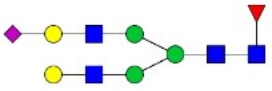
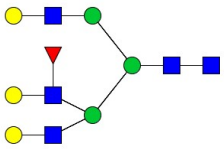
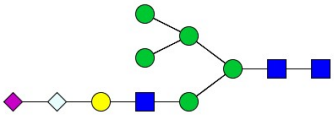
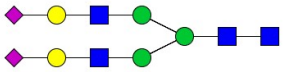
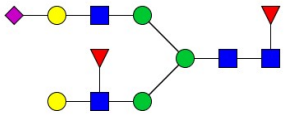
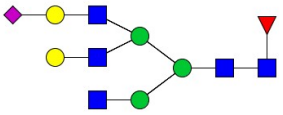
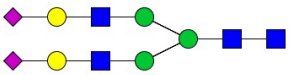
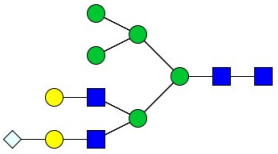
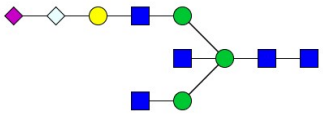
No.	m/z [M+ Na]$^+$	Composition
1	933.4	$Hex_3HexNAc_2$
2	1095.4	$Hex_4HexNAc_2$
3	1136.5	$Hex_3HexNAc_3$
4	1257.5	$Hex_5HexNAc_2$
5	1298.6	$Hex_4HexNAc_3$
6	1339.6	$Hex_3HexNAc_4$
7	1419.6	$Hex_6HexNAc_2$
8	1460.7	$Hex_5HexNAc_3$
9	1501.7	$Hex_4HexNAc_4$
10	1542.7	$Hex_3HexNAc_5$
11	1581.7	$Hex_7HexNAc_2$
12	1622.7	$Hex_6HexNAc_3$
13	1663.8	$Hex_5HexNAc_4$
14	1704.8	$Hex_4HexNAc_5$
15	1745.8	$Hex_3HexNAc_6$
16	1866.9	$Hex_5HexNAc_5$
17	1907.0	$Hex_4HexNAc_6$
18	1948.0	$Hex_3HexNAc_7$
19	2028.0	$Hex_6HexNAc_5$
20	2110.1	$Hex_4HexNAc_7$
21	2151.1	$Hex_8HexNAc_4$
22	2313.2	$Hex_3HexNAc_8$

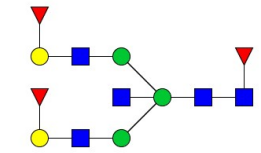
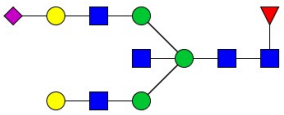
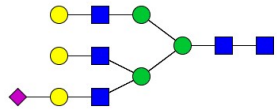
Table S2. Detailed information of the observed N-glycans from normal human serum by enriching with CS-900-1C biomaterials (N-linked glycans were released by PNGase F digestion, and glycan strcutures were searched from Glycoworkbench through the Mol. wt of oligosaccharide. Part of the strcutures has been demonstrated.

No	M/Z ($[M+nNa]^+$)	Composition	Structure (M)
1	1079.4	H3N2F1	
2	1095.4	H4N2	
3	1136.4	H3N3	
4	1257.4	H5N2	
5	1281.5	H3N3F1	
6	1298.5	H4N3	
7	1338.5	H3N4	
8	1419.5	H6N2	
9	1443.5	H4N3F1	
10	1460.5	H5N3	

11	**1485.6**	**H3N4F1**	
12	**1501.6**	**H4N4**	
13	**1541.6**	**H3N5**	
14	**1581.6**	**H7N2**	
15	**1621.6**	**H6N3**	
16	**1629.6**	**H3N4A1**	
17	**1646.7**	**H4N4F1**	
18	**1662.7**	**H5N4**	
19	**1688.7**	**H3N5F1**	
20	**1704.7**	**H4N5**	
21	**1743.6**	**H8N2**	
22	**1791.7**	**H3N5A1**	

23	1808.7	H5N4F1	
24	1814.2	H4N4A1	
25	1824.6	H6N4	
26	1850.7	H4N5F1	
27	1865.7	H5N5	
28	1891.2	H3N6F1	
29	1905.2	H9N2	
30	1909.2	H4N6	
31	1925.7	H4N4F2X1	
32	1954.5	H4N4F1A1	
33	1976.2	H5N4A1	
34	1996.5	H4N5A1	
35	2012.8	H5N5F1	

36	**2028.8**	**H6N5**	
37	**2056.2**	**H6N3F1A1**	
38	**2122.2**	**H5N4F1A1**	
39	**2173.8**	**H6N5F1**	
40	**2221.7**	**H6N3A2**	
41	**2245.7**	**H5N4A2**	
42	**2267.7**	**H5N4F2A1**	
43	**2282.7**	**H5N5F1A1**	
44	**2288.8**	**H5N4A2**	
45	**2294.7**	**H7N4A1**	
46	**2302.7**	**H4N5A2**	

47	**2305.7**	**H5N5F3**	
48	**2326.8**	**H5N5F1A1**	
49	**2340.8**	**H6N5A1**	

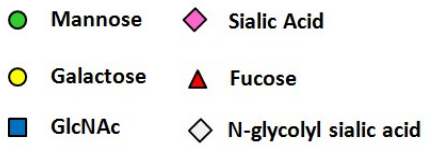


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Table S3. Clinical characteristics of healthy controls (N) and patients with obesity (OB), impaired glucose tolerance (IGT), and diabetes patients (DM).

	All (n=48)	N (n=12)	OB (n=12)	IGT (n=12)	DM (n=12)
BMI (kg/m^2)	34.6±8.8	23.1±1.9	36.9±5.4 ↑	38.4±6.5 ↑	40.0±7.7 ↑
Sex, male (%)	24 (50%)	6 (50%)	6 (50%)	6 (50%)	6 (50%)
Glucose (mmol/L)	6.2±1.9	5.0±0.4	5.1±0.4	5.6±0.5 ↑	8.9±1.9 ↑
Insulin (mU/L)	38.9±22.8 (n=36)	-	32.0±15.9 ↑	44.1±14.4 ↑	40.6±33.3 ↑
C-peptides (µg/L)	4.7±1.2 (n=35)	-	4.2±1.2	5.2±0.9 ↑	4.6±1.3 ↑ (n=11)
Glucose(2h) (mmol/L)	9.9±4.2 (n=38)	5.4±1.0 (n=6)	6.4±1.4 (n=8) ↑	9.2±1.3 ↑	15.3±2.2 ↑
Insulin (2h) (mU/L)	183.4±156.7 (n=32)	-	144.7±138.3 (n=8)	273.5±185.2	119.1±90.9
C-peptides (2h) (µg/L)	12.6±5.0 (n=32)	-	11.4±5.5 (n=8)	15.9±4.5	10.0±3.5
HbA1c (%)	6.3±1.2 (n=41)	5.4±0.3 (n=6)	5.5±0.3 (n=11)	5.9±0.3 ↑	7.8±1.1 ↑
IGF-1 (µg/L)	154.6±52.0 (n=36)	-	190.4±57.5	147.3±35.0	126.1±41.6
ALT (U/L)	61.5±61.1 (n=42)	14.3±10.0 (n=6)	58.8±45.2 ↑	61.4±36.1 ↑	88.0±92.3 ↑
AST (U/L)	35.5±35.8 (n=42)	15.0±5.3 (n=6)	30.6±24.1	35.2±19.4 ↑	51.0±57.4 ↑
Γ-GGT (U/L)	48.5±33.0 (n=42)	14.8±5.0 (n=6)	48.3±18.3 ↑	44.9±26.9	74.3±40.0 ↑
Cholesterol (mmol/L)	4.8±0.7 (n=36)	-	4.7±0.6	4.5±0.5	5.2±0.7
TG (mmol/L)	1.9±1.3 (n=35)	-	1.5±1.0	2.1±1.2 ↑	2.0±1.7 ↑ (n=11)
HDL-c (mmol/L)	1.0±0.2 (n=36)	-	1.0±0.3	0.9±0.2	1.0±0.2
LDL-c (mmol/L)	3.1±0.6 (n=33)	-	3.1±0.5 (n=10)	2.9±0.6	3.5±0.7 ↑ (n=11)
APOA-1 (g/L)	0.9±0.1 (n=33)	-	0.9±0.1 (n=10)	0.9±0.1	0.9±0.1 (n=11)
APOB (g/L)	0.7±0.1 (n=33)	-	0.6±0.1 (n=10)	0.6±0.1	0.7±0.1 (n=11)
nHDL-c (mmol)	3.8±0.7 (n=32)	-	3.7±0.7 (n=9)	3.6±0.5	4.1±0.7 (n=11)
NEFA (mmol/L)	0.6±0.2 (n=36)	-	0.6±0.3	0.7±0.3 ↑	0.6±0.1
HCY (µmol/L)	12.2±8.4 (n=28)	-	16.6±15.2 ↑	12.4±4.7	9.1±2.5

			(n=7)	(n=10)	(n=11)
TP (g/L)	70.8±4.2 (n=42)	69.2±5.0 (n=6)	70.3±3.2	71.3±2.8	71.5±5.7
ALB (g/L)	43.4±2.7 (n=42)	45.8±2.2 (n=6)	43.3±2.7	42.7±2.5	42.8±2.6
PA (mg/L)	255.1±49.5 (n=42)	262.3±50.8 (n=6)	265.3±55.3	244.7±41.0	251.6±54.7
CRE (μmol/L)	56.1±14.7 (n=39)	60.3±10.1 (n=3)	60.9±16.1	58.4±15.4	47.8±10.9
UA (mmol/L)	0.5±0.1 (n=39)	0.4±0.1 (n=3)	0.5±0.1 ↑	0.5±0.2 ↑	0.5±0.1 ↑
CysC (mg/L)	0.8±0.1 (n=31)	0.7±0.01 (n=3)	0.8±0.2 (n=7)	0.9±0.1 (n=10)	0.8±0.1 (n=11)
FE (μmol/L)	17.1±5.6 (n=35)	-	16.1±5.9 (n=11)	17.0±5.3	18.2±5.9 (n=11)
GA(%)	11.3±3.4 (n=35)	-	8.7±1.0	9.9±1.2	15.5±2.7 (n=11)
FIB (g/L)	3.2±1.0 (n=42)	2.3±0.6 (n=6)	3.4±0.9	3.4±0.7	3.4±1.2
FERR (ng/mL)	347.5±367.7 (n=35)	-	266.7±198.0 (n=11)	331.6±279.0	459.0±527.0
CER (g/L)	0.3±0.05 (n=28)	-	0.3±0.05 (n=6)	0.3±0.05 (n=10)	0.3±0.05
β2-MG (mg/L)	1.7±0.4 (n=28)	-	1.7±0.3 (n=6)	1.9±0.5 (n=10) ↑	1.6±0.3
IgG4 (g/L)	0.7±0.6 (n=28)	-	0.4±0.2	0.8±0.5 (n=10)	0.7±0.7 (n=6)
WBC (/10^9)	8.0±1.8 (n=42)	6.4±1.4 (n=6)	7.7±1.5	8.8±2.1	8.3±1.6

Data are reported as means±SD. *N*, normal; *OB*, obesity; *IGT*, impaired glucose tolerance; *DM*, diabetes mellitus; *HbA1c*, glycated hemoglobin; *IgF-1*, insulin like growth factor; *ALT*, Alamine aminotransferase; *AST*, aspartate amino transferase; *γ-GGT*, γ-glutamyltransferase; *TG*, triglyceride; *HDL-c*, high density lipoprotein cholesterol; *LDL-c*, low density lipoprotein cholesterol; *APO-A*, apolipoprotein-A; *APO-B*, apolipoprotein-B; *nHDL-c*, non high density lipoprotein cholesterol; *NEFA*, free fatty acids; *HCY*, Homocysteine; *TP*, total protein; *ALB*, albumin; *PA*, prealbumin; *CRE*, creatinine; *UA*, uric acid; *CysC*, serum cystatin; *FE*, serum Ferrium; *GA*, Glycosylated serum protein ratio; *FIB*, fibrinogen; *FERR*, Ferritin; *CER*, ceruloplasmin; *β2-MG*, β2-microglobulin; *IgG4*, immunoglobuin 4; *WBC*, White blood cell; black arrow indicated values higher than normal.