

Supporting information

Mass Spectrometry-Based Analysis of Serum N-Glycosylation Changes in Patients with Parkinson's Disease

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Supplementary Methods

Glycan Structure Confirmation by LC-MS/MS

Human N-glycans were first labelled by an isobaric tag (QUANTITY).¹ The labelling procedure can introduce a permanently positive-charged quaternary amine to glycans, thus enhancing the ionization efficiency in MS. The dried N-glycans were re-suspended in 100 μ L reaction solution mixture consisting of anhydrous dimethyl sulfoxide (DMSO) and acetic acid (AA) (7:3, vol) in the presence of 1 M NaCNBH₃. QUANTITY 178 (100 mM) dissolved in 200 μ L mixture of DMSO and AA (7:3) were added into each sample respectively and incubated at 65 °C for 4 h. The reaction was quenched by addition of 2 mL water and 2 μ L concentrated formic acid. QUANTITY-labelled N-glycans were cleaned up by C18 purification. The purified samples were dissolved in 40 μ L of 0.2% formic acid.

Then the labelled N-glycans were separated and analyzed on an Ultimate 3000 UHPLC coupled to a Thermo Orbitrap EliteTM mass spectrometer. N-glycans were separated in an AcclaimTM PepMapTM 100 column (75 μ m \times 15 cm) packed with C18 (3 μ m, 100Å) at a flow rate of 250 nL/min. Mobile phase A (0.1 % formic acid) and mobile phase B (0.1 % formic acid in 80 % ACN) were used to establish a 120 min gradient comprised of 0.1 min of 6 % B, 54 min of 6-22 % B, 51 min of 22-50 % B, 5 min of 50-60 % B, 1 min of 60-90 % B and 9 min of 90 % B. The other parameters were the same as those in the glycopeptide analysis (please refer to Materials and Methods in the main text).

The acquired MS/MS spectra were matched with the theoretical glycan fragments created by the GlycoWorkbench software.

Supplementary Tables and Figures

Table S1. Demographic information of healthy controls and PD patients

	Control	PD	Between group differences	
			Statistic	<i>p</i> value
Number of participants	30	40		
Age mean (SD)	58.87 (4.862)	62.60 (11.08)	t=1.722	0.0896
Gender male/female	15/15	21/19	$\chi^2=0.04289$	0.8359

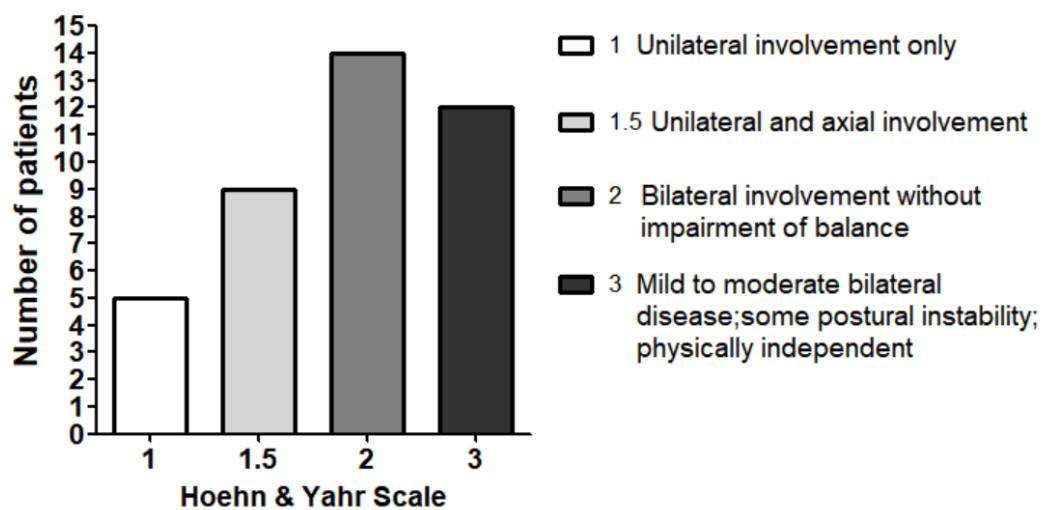


Figure S1. The Hoehn and Yahr Scales of all 40 PD patients in our study. Descriptions of symptoms at different stages are also provided.

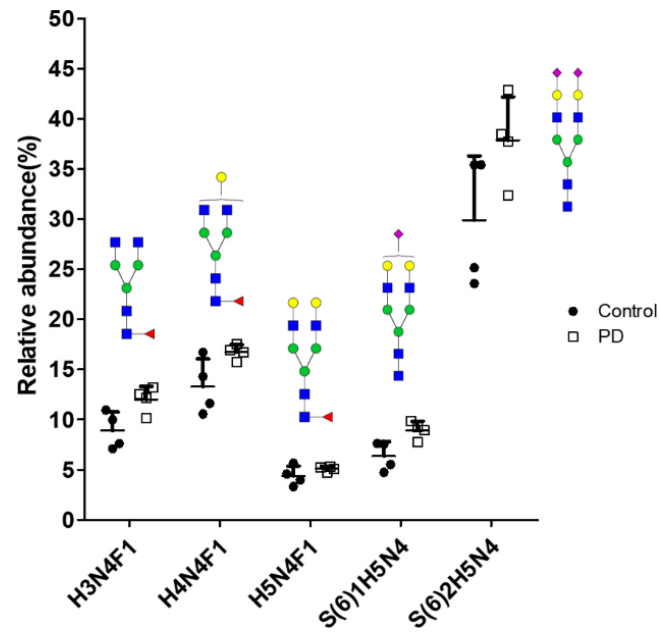


Figure S2. The scatter plot of ion abundances for 5 highly abundant N-glycans in control and PD serum samples. The black dots represent the control group while the white squares represent the PD group. Data were acquired from four independent experiments.

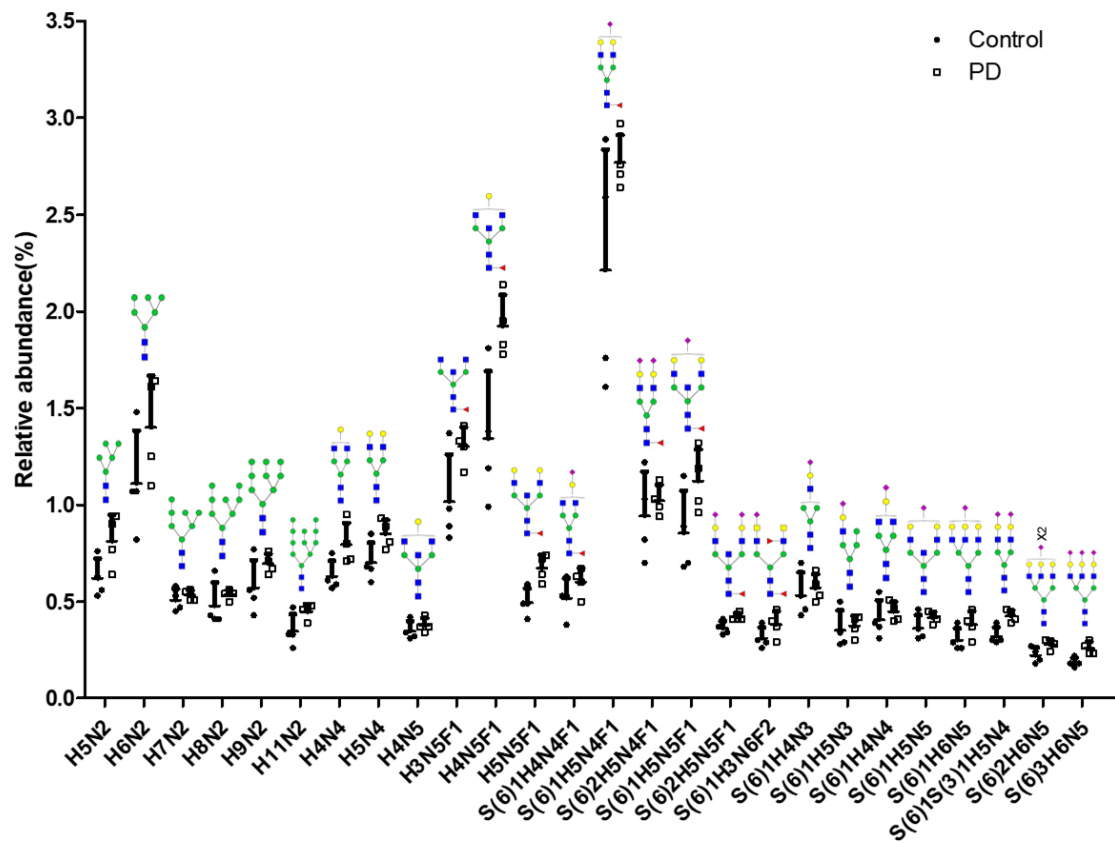


Figure S3. The scatter plot of ion abundances for 27 low abundant N-glycans in control and PD serum samples. The black dots represent the control group while the white squares represent the PD group. Data were acquired from four independent experiments.

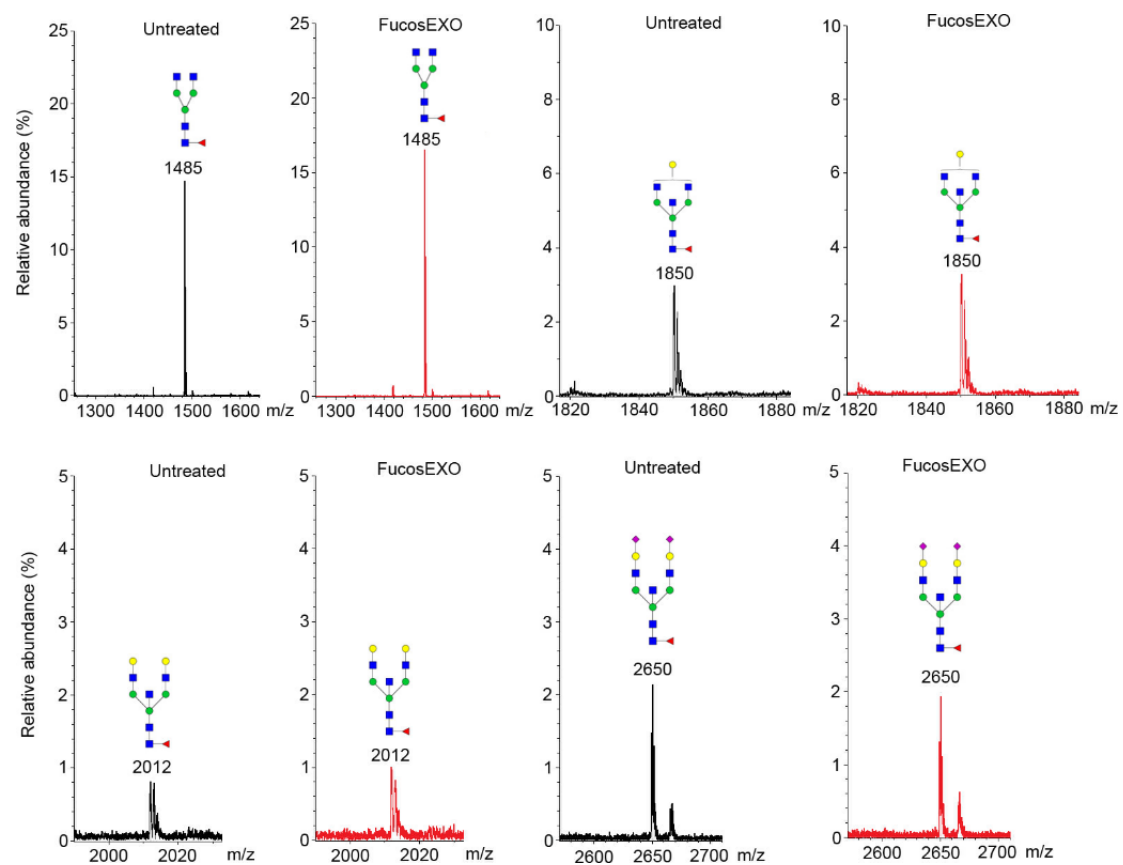


Figure S4. MALDI-MS spectra of fucose-attached glycans (H3N4F1, H4N5F1, H5N5F1 and S(6)2H5N5F1) treated without and with α 1-2,3,4 fucosidase (FucosEXO).

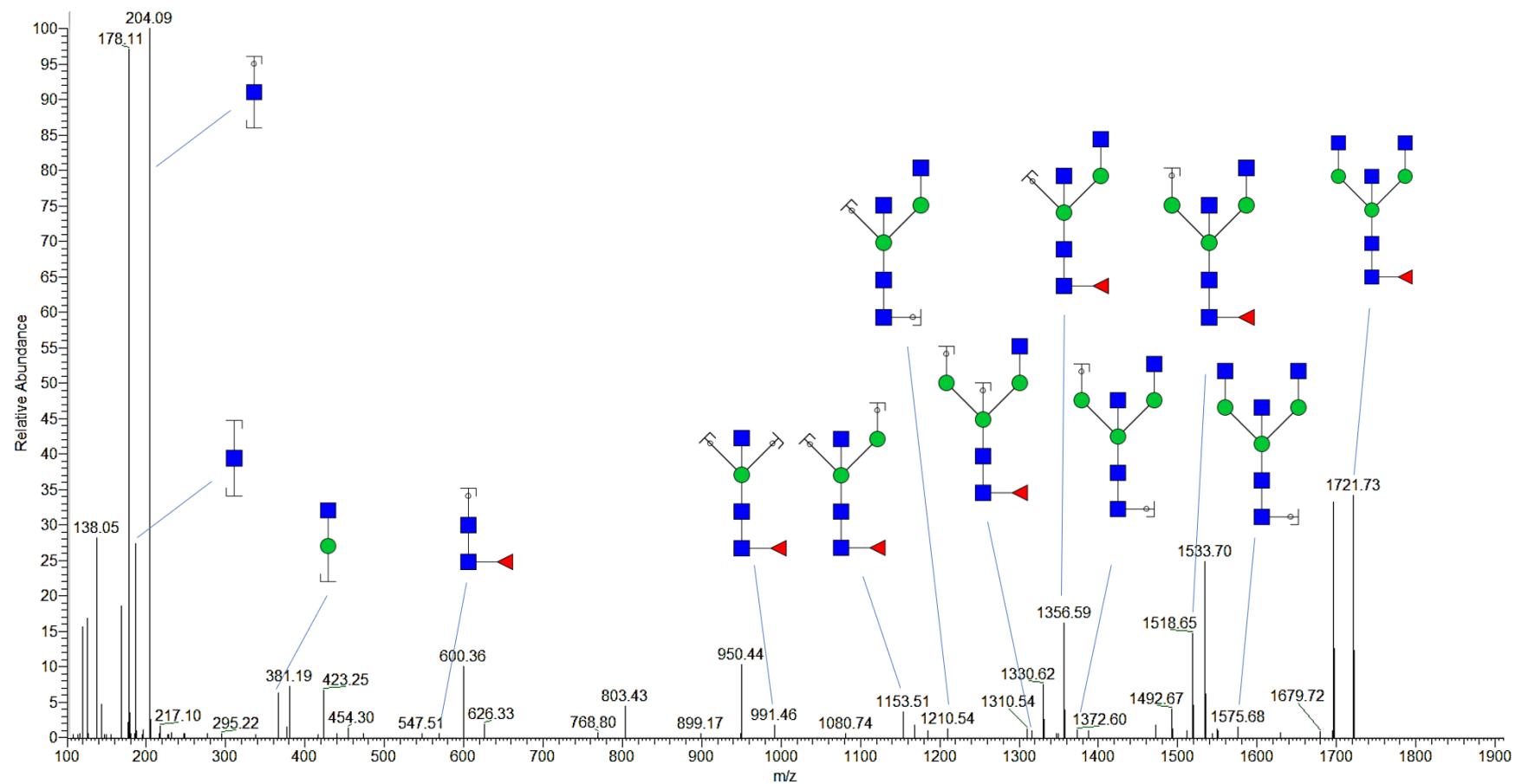


Figure S5. MS/MS fragment ion spectra of the selected bisecting structure (H3N5F1) detected in human serum. Human serum N-glycans were labeled with an isobaric tag (QUANTITY 178) for better ionization efficiency. As a result, fragment ions containing the first GlcNAc on the reducing end showed a mass increase of 55 Da. The reporter ion with a m/z of 178 was clearly observed in the spectrum, demonstrating the success of QUANTITY labelling.

Table S2. List of proteins identified by lectin affinity purification

AAL		SNA		MALII	
Accession No.	Protein name	Accession No.	Protein name	Accession No.	Protein name
A0AUZ9	KAT8 regulatory NSL complex subunit 1-like protein	P00450	Ceruloplasmin	O95497	Pantetheinase
O75443	Alpha-tectorin	P00734	Prothrombin	P00450	Ceruloplasmin
O94916	Nuclear factor of activated T-cells 5	P00738	Haptoglobin	P00734	Prothrombin
P00450	Ceruloplasmin	P00739	Haptoglobin-related protein	P00738	Haptoglobin
P00738	Haptoglobin	P01008	Antithrombin-III	P01008	Antithrombin-III
P00739	Haptoglobin-related protein	P01009	Alpha-1-antitrypsin	P01009	Alpha-1-antitrypsin
P01009	Alpha-1-antitrypsin	P01011	Alpha-1-antichymotrypsin	P01011	Alpha-1-antichymotrypsin
P01011	Alpha-1-antichymotrypsin	P01023	Alpha-2-macroglobulin	P01023	Alpha-2-macroglobulin
P01023	Alpha-2-macroglobulin	P01024	Complement C3	P01024	Complement C3
P01024	Complement C3	P01031	Complement C5	P01031	Complement C5
P01860	Immunoglobulin heavy constant gamma 3	P01042	Kininogen-1	P01042	Kininogen-1
P01861	Immunoglobulin heavy constant gamma 4	P01860	Immunoglobulin heavy constant gamma 3	P01860	Immunoglobulin heavy constant gamma 3
P01871	Immunoglobulin heavy constant mu	P01861	Immunoglobulin heavy constant gamma 4	P01861	Immunoglobulin heavy constant gamma 4
P02766	Transthyretin	P01871	Immunoglobulin heavy constant mu	P01871	Immunoglobulin heavy constant mu
P02787	Serotransferrin	P01876	Immunoglobulin heavy constant alpha 1	P01876	Immunoglobulin heavy constant alpha 1
P07333	Macrophage colony-stimulating factor 1 receptor	P01877	Immunoglobulin heavy constant alpha 2	P02671	Fibrinogen alpha chain
P08603	Complement factor H	P02671	Fibrinogen alpha chain	P02748	Complement component C9
P10909	Clusterin	P02749	Beta-2-glycoprotein 1	P02749	Beta-2-glycoprotein 1
P35125	Ubiquitin carboxyl-terminal hydrolase 6	P02751	Fibronectin	P02751	Fibronectin

P49746	Thrombospondin-3	P02766	Transthyretin	P02760	Protein AMBP
Q02224	Centromere-associated protein E	P02787	Serotransferrin	P02763	Alpha-1-acid glycoprotein 1
Q5VV63	Attractin-like protein 1	P02790	Hemopexin	P02765	Alpha-2-HS-glycoprotein
Q8NDA2	Hemicentin-2	P04004	Vitronectin	P02766	Transthyretin
Q9BSJ5	Uncharacterized protein C17orf80	P04114	Apolipoprotein B-100	P02787	Serotransferrin
Q9NYQ6	Cadherin EGF LAG seven-pass G-type receptor 1	P05154	Plasma serine protease inhibitor	P02790	Hemopexin
		P05155	Plasma protease C1 inhibitor	P03952	Plasma kallikrein
		P05546	Heparin cofactor 2	P04003	C4b-binding protein alpha chain
		P08603	Complement factor H	P04114	Apolipoprotein B-100
		P10909	Clusterin	P05155	Plasma protease C1 inhibitor
		P19652	Alpha-1-acid glycoprotein 2	P05546	Heparin cofactor 2
		P19823	Inter-alpha-trypsin inhibitor heavy chain H2	P07357	Complement component C8 alpha chain
		P19827	Inter-alpha-trypsin inhibitor heavy chain H1	P08603	Complement factor H
		P02763	Alpha-1-acid glycoprotein 1	P09871	Complement C1s subcomponent
		P23142	Fibulin-1	P10643	Complement component C7
		P35916	Vascular endothelial growth factor receptor 3	P10909	Clusterin
		P82094	TATA element modulatory factor	P13725	Oncostatin-M
		P98164	Low-density lipoprotein receptor-related protein 2	P19652	Alpha-1-acid glycoprotein 2
		Q13029	PR domain zinc finger protein 2	P19823	Inter-alpha-trypsin inhibitor heavy chain H2
		Q15303	Receptor tyrosine-protein kinase erbB-4	P19827	Inter-alpha-trypsin inhibitor heavy chain H1
		Q494X3	Zinc finger protein 404	P22792	Carboxypeptidase N subunit 2
		Q52LW3	Rho GTPase-activating protein 29	P22891	Vitamin K-dependent protein Z
		Q5VV63	Attractin-like protein 1	P25311	Zinc-alpha-2-

					glycoprotein
		Q6N041	Uncharacterized protein DKFZp686O16217	P27169	Serum paraoxonase/arylesterase 1
		Q6N091	Uncharacterized protein DKFZp686C02220	Q03591	Complement factor H- related protein 1
		Q86TH1	ADAMTS-like protein 2	Q2KJY2	Kinesin-like protein KIF26B
		Q8N139	ATP-binding cassette sub-family A member 6	Q5T1H1	Protein eyes shut homolog
		Q8N6G6	ADAMTS-like protein 1	Q6S5L8	SHC-transforming protein 4
		Q96MP5	Zinc finger SWIM domain-containing protein 3	Q8N139	ATP-binding cassette sub-family A member 6
		Q96PD5	N-acetylmuramoyl-L- alanine amidase	Q8N6G6	ADAMTS-like protein 1
		Q96RV3	Pecanex-like protein 1	Q96PD5	N-acetylmuramoyl-L- alanine amidase
		Q9NRF2	SH2B adapter protein 1	Q9P2N2	Rho GTPase-activating protein 28
		Q9NZR2	Low-density lipoprotein receptor-related protein 1B		
		Q9UK05	Growth/differentiation factor 2		

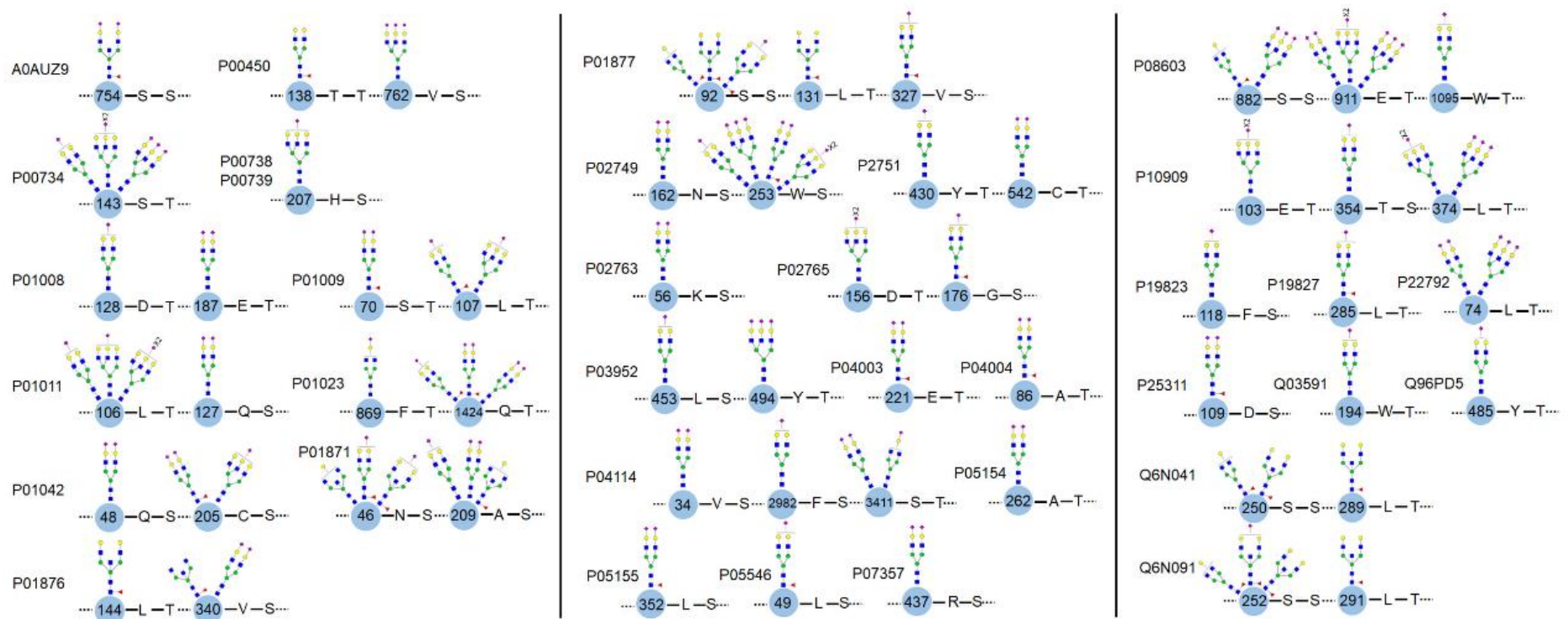


Figure S6. Uniquely identified site-specific N-glycosylation containing core fucose, sialic acids and bisecting GlcNAc in PD patients' sera. The accession numbers and N-glycosylation motifs of relevant glycoproteins are displayed. The number in the blue circles represents the position of asparagine on the protein sequence.

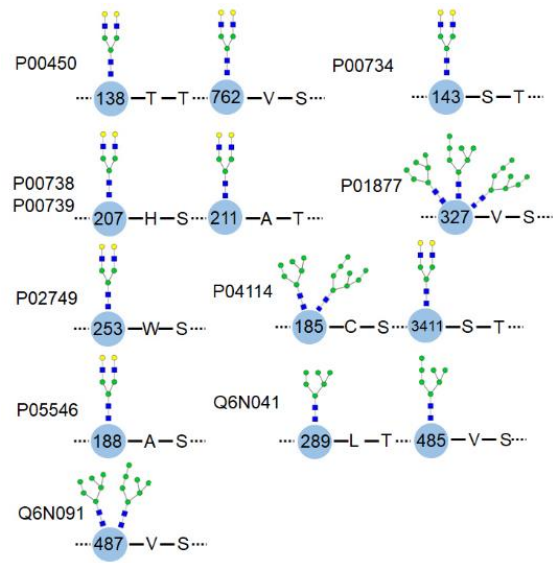


Figure S7. Uniquely identified site-specific N-glycosylation containing complex (without core fucose or sialic acid) and high-mannose N-glycans in PD patients' sera.

Table S3. Common site-specific glycosylation of selected proteins shared by control and PD sera

Protein name	Glycosite	Glycan
Ceruloplasmin	N(138)TT	S(6)1H4N3, S(6)1H5N4, S(6)2H5N4, S(6)2H6N5, S(6)1S(3)1H6N5, S(6)3H6N5, S(6)1H5N4F1, S(6)2H5N4F1
	N(358)KS	S(6)1H5N4, S(6)2H5N4, S(6)2H5N4F1
	N(397)LT	S(6)1H5N4, S(6)2H5N4, S(6)2H6N5, S(6)1S(3)1H6N5, S(6)3H6N5, S(6)1H5N4F1, S(6)2H5N4F1
	N(762)VS	S(6)1H4N3, S(6)1H5N4, S(6)2H5N4, S(6)2H6N5, S(6)1S(3)1H6N5, S(6)1H5N4F1, S(6)2H5N4F1
Haptoglobin	N(184)LT	S(6)1H4N3, S(6)1H4N4, H5N4, S(6)1H5N4, S(6)2H5N4, S(6)1H6N5, S(6)2H6N5, S(6)1S(3)1H6N5, S(6)3H6N5, S(6)1H5N4F1, S(6)2H5N4F1
	N(207)HS	S(6)1H5N4, S(6)2H5N4
	N(211)AT	S(6)1H5N5
	N(241)YS	S(6)1H4N3, S(6)1H4N4, H4N4, H5N4, S(6)1H5N4, S(6)2H5N4, S(6)1H6N5, S(6)2H6N5, S(6)1S(3)1H6N5, S(6)3H6N5, S(6)1H5N4F1, S(6)2H5N4F1
Kininogen-1	N(169)NT	S(6)1H5N4, S(6)2H5N4, S(6)2H6N5, S(6)1S(3)1H6N5
	N(205)CS	S(6)2H5N4, S(6)2H6N5, S(6)1S(3)1H6N5, S(6)3H6N5
	N(294)AT	S(6)2H5N4, S(6)2H6N5, S(6)1S(3)1H6N5, S(6)3H6N5, S(6)2H5N4F1
Complement factor H	N(217)GS	S(6)2H5N4
	N(882)SS	S(6)1H4N3, H5N4, S(6)1H5N4, S(6)2H5N4, S(6)2H6N5, S(6)1S(3)1H6N5
	N(911)ET	S(6)1H5N4, S(6)2H5N4
	N(1029)VT	S(6)1H5N4, S(6)2H5N4, S(6)1H5N4F1
Clusterin	N(86)ET	S(6)1H5N4, S(6)2H5N4, S(6)2H6N5, S(6)1S(3)1H6N5, S(6)3H6N5
	N(103)ET	S(6)2H5N4, S(6)3H6N5
	N(374)LT	S(6)1H4N3, S(6)1H5N4, S(6)2H5N4

SUPPLEMENTARY RAW MS DATA

The original LC-MS/MS data for HILIC-enrichment glycopeptides were uploaded to Baidu Netdisk. The link for downloading the raw files is: <https://pan.baidu.com/s/109oAAe5Lh2uOocNeYasvsA>. The code for file extraction is 2248.

REFERENCES

1. Yang, S.; Wang, M.; Chen, L.; Yin, B.; Song, G.; Turko, I. V.; Phinney, K. W.; Betenbaugh, M. J.; Zhang, H.; Li, S., QUANTITY: An Isobaric Tag for Quantitative Glycomics. *Sci. Rep.* **2015**, *5*, 17585.