Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information 1

1. The LC-MS conditions for *N*-glycan analysis

The mobile phase for N-glycan analysis were A: 50 mM NH_4FA (pH 4.4) and B: acetonitrile. The gradient started from 72% B and decreased to 54%B in 50 min. Then, equilibration the column at 72% B for 10 mi. The column was BEH Glycan (2.1x 150 mm, 1.7 μm) with a flow rate of 0.4 mL/min. The column temperature was set at 30 ^{o}C. Full MS scans were acquired in the Orbitrap mass analyzer with the mass range set to m/z 200–3000, a resolution setting of 120,000 at m/z 200 with an automatic gain control (AGC) target value of 3e6 and a maximum injection time of 100 ms. The polarity was positive, and the number of microscans was 1. The sheath gas and aux gas flow rate were 30 and 15 arbitrary units, respectively. The aux gas capillary and aux gas temperature were 320 °C and 350 °C, respectively. The spray voltage was 3.8 kV, and the S-lens RF level was 70.

2. The LC-MS conditions for *O*-glycopeptide and *N*-glycopeptide analysis

The mobile phase for *O*-glycopeptide and *N*-glycopeptide analysis were A: 0.1%FA aqueous and B: 0.1%FA in acetonitrile. The gradient was as follow: 0-15 min 3% B - 8%B, 15-85 min 8%-32%B, 85-90 min 32%-90%B, 90-90.1 min 90%B-3%B, 90.1-100 min 3%B. The column was BEH peptide (2.1x 150 mm, 1.7 μm) with a flow rate of 0.2 mL/min. The column temperature was set at 60 ^{o}C. DDA scans were acquired in the Orbitrap mass analyzer with the mass range for MS1 set to m/z 200–2000, a resolution setting of 60,000 at m/z 200 with an automatic gain control (AGC) target value of 3e6 and a maximum injection time of 100 ms. The MS2 was acquired by a resolution seting of 15000 at m/z 200 with first mass at 110 m/z, an automatic gain control (AGC) target value of 1e6 and a maximum injection time of 50 ms. The polarity was positive, and the number of microscans was 1. The sheath gas and aux gas flow rate were 30 and 15 arbitrary units, respectively. The aux gas capillary and aux gas temperature were 320 °C and 350 °C, respectively. The spray voltage was 3.8 kV, and the S-lens RF level was 70.

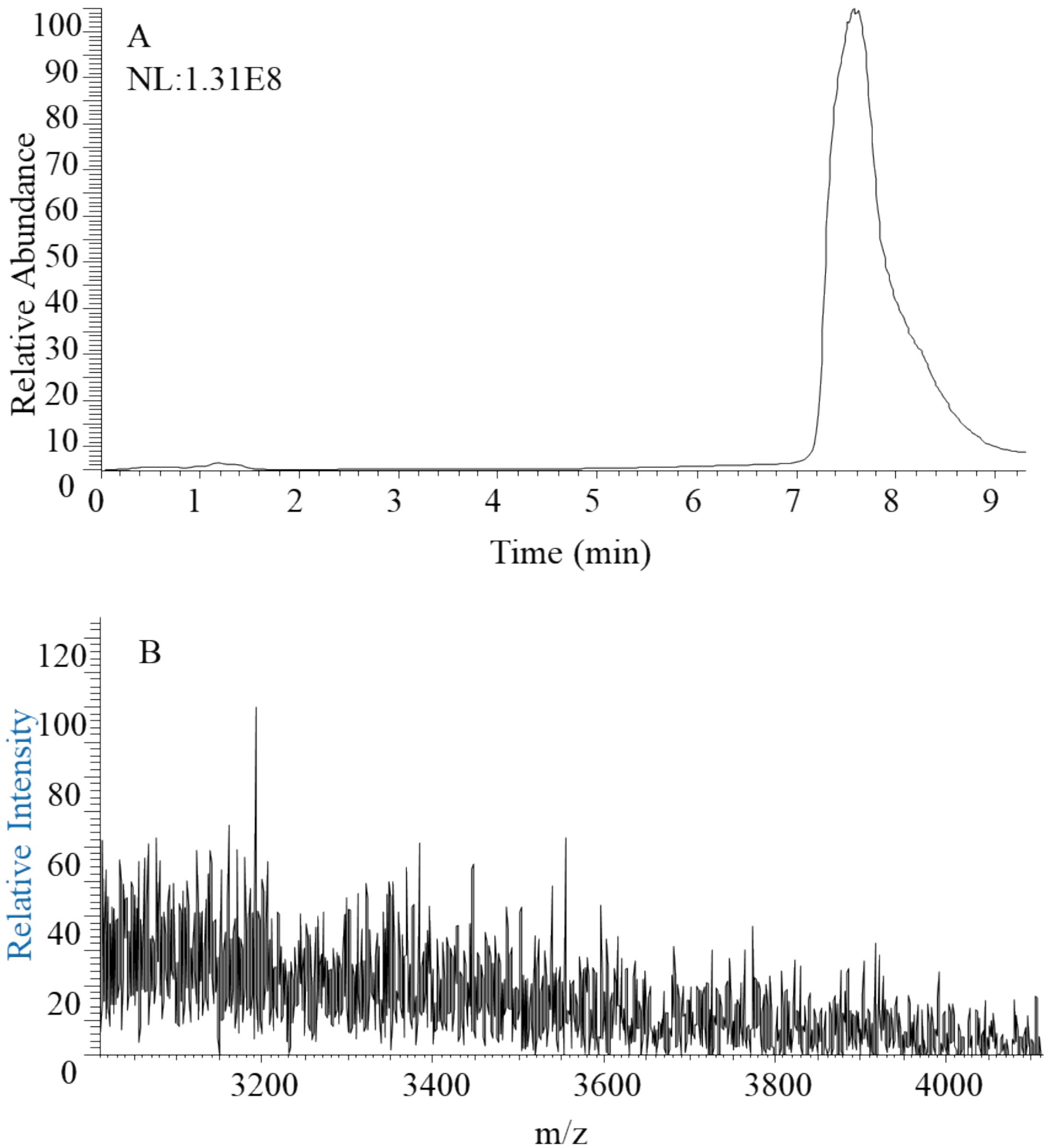
Supporting Information 2

The RPLC-MS experiment was carried on Vanquish Flex UHPLC and OE240 MS instrument. The column was MabPacRP (2.1 x 50 mm, 4 μm). The flow rate was 0.3 mL/min. The column oven temperature was set at 60 ^{o}C. The mobile phase A and B were 0.1% formic acid in water (1:1000, v/v) and 0.1% formic acid in acetonitrile (1:1000, v/v). The gradient was start from 20% and shift to 35% in 25 minutes. Full MS scans were acquired in the Orbitrap mass analyzer with the mass range set to m/z 500–4500, a resolution setting of 15,000 at m/z 200 with an automatic gain control (AGC) target value of 3e6 and a maximum injection time of 150 ms. The polarity was positive, and the number of microscans was 10. The sheath gas and aux gas flow rate were 30 and 10 arbitrary units, respectively. The aux gas capillary and aux gas temperature were 300 °C and 300 °C, respectively. The spray voltage was 3.8 kV, and the S-lens RF level was 150.

The SEC-MS experiment was also carried on Vanquish Flex UHPLC and OE240 MS instrument. The column was MabPac SEC (4.0 x 150 mm, 4 μm). The flow rate was 0.2 mL/min. The mobile phase was 50 mM ammonium acetate. The column oven temperature was set at 30 ^{o}C. Full MS scans were acquired in the Orbitrap mass analyzer with the mass range set to m/z 500–4500, a resolution setting of 15,000 at m/z 200 with an automatic gain control (AGC) target value of 3e6 and a maximum injection time of 150 ms. The polarity was positive, and the number of microscans was 10. The sheath gas and aux gas flow rate were 30 and 10 arbitrary units, respectively. The aux gas capillary and aux gas temperature were 250 °C and 250 °C, respectively. The spray voltage was 3.8 kV, and the S-lens RF level was 150.

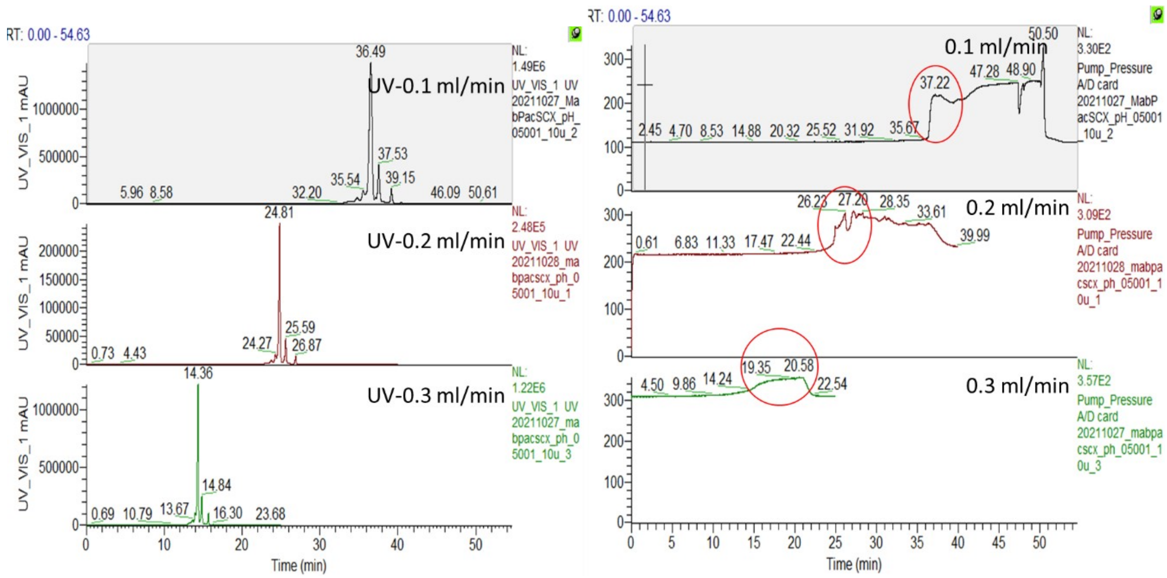
Supporting Information 3

The total ion chromatography (TIC) of etanercept (A) and mass spectra (B) obtained by SCX-MS. The gradient used for was 0-15 min 30-70% B. The flow rate was 0.3 mL/min.



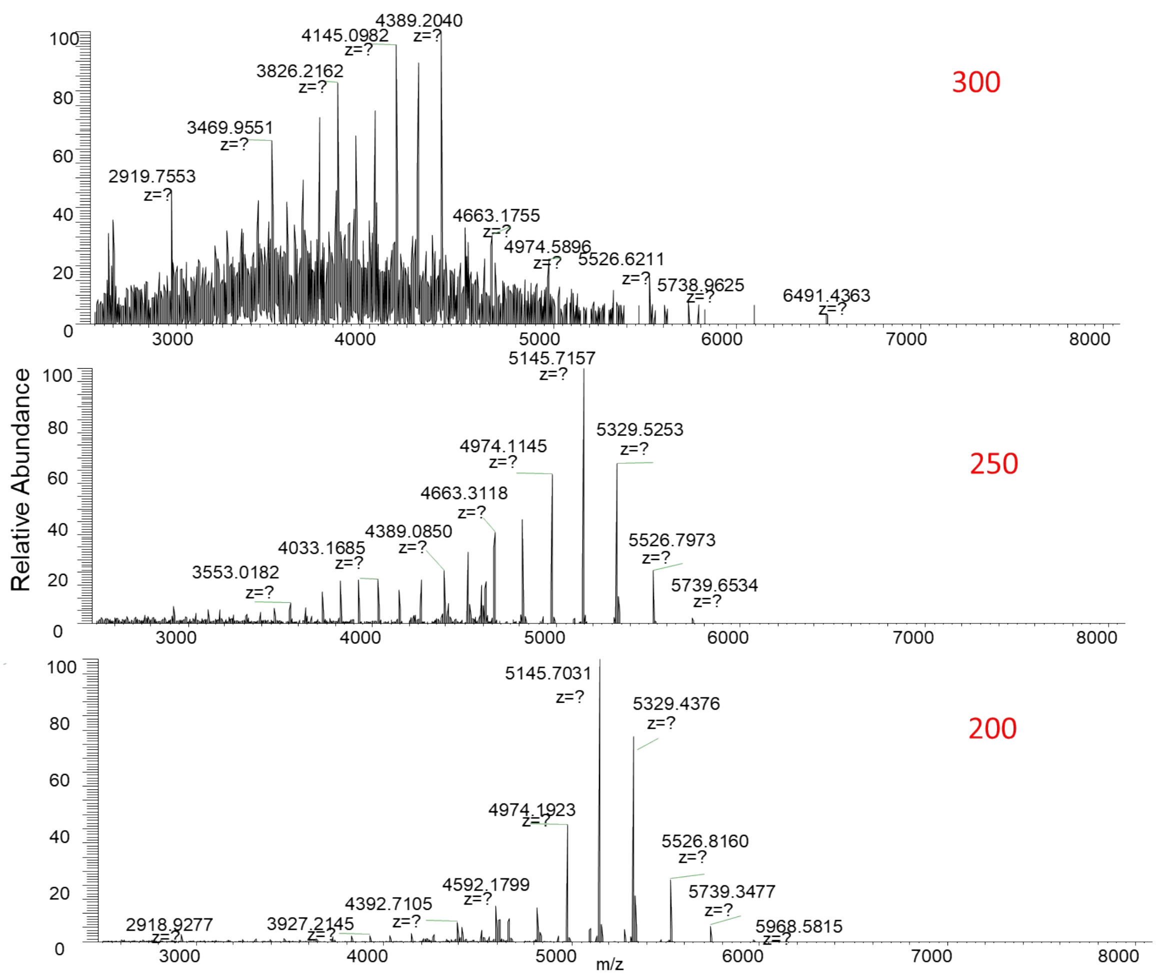
Supporting Information 4

The chromatogram (left) and related system pressure (right) of proteins obtained on SCX column with different flow rate.



Supporting Information 5

The MS spectra of IgG 1 obtained with different aux gas temperature (300, 250 and 200 ^{o}C).



Supporting Information 6

The annotation of peaks shown in Fig.5

Molecular Weight (Da)	Modification
87,005.04	4x FA2S2, 2x A2S1G0F,1x GalNAc-6GGn-3SG
87,045.57	4x FA2S2, 2x A2S1G0F,1xGalNAc-3SG, 2x GalNAc
87,083.08	4x FA2S2, 2x A2S1G0F,3xGalNAc-3SG
87,126.49	4x FA2S2, 2x A2S1G0F,2xGalNAc-3SG, 1x GalNAc-6Gn
87,166.37	4x FA2S2, 2x A2S1G0F,1xGalNAc-3SG, 2x GalNAc-6Gn
87,209.42	4x FA2S2, 2x A2S1G0F,3x GalNAc-6Gn
87,251.83	4x FA2S2, 2x A2S1G0F,1xGalNAc-3SG, 1x GalNAc-6Gn, 1x GalNAc-6S
87,323.18	3x FA2S2， 2x A2S1G0F,1x A1S1M5F, 1x GalNAc-3G
87,328.06	3x FA2S2， 2x A2S1G0F,1xA3S1G1F, 1x GalNAc-3G
87,371.09	3x FA2S2， 2x A2S1G0F,1xA4S1G0F, 1x GalNAc-3G
87,415.90	4x FA2S2， 2x A2S1G0F,1x GalNAc-3G
87,532.83	3x FA2S2， 2x A2S1G0F,1xA4S1G1F, 1x GalNAc-3G
87,576.65	5x FA2S2，1x A2S1G1， 1x GalNAc-3SG
87,619.61	5x FA2S2， 1x A2G2M5, 1x GalNAc-3SG
87,655.21	5x FA2S2，1x A2S1G1F
87,662.17	5x FA2S2, 1x A2S1G1F
87,696.69	5x FA2S2, 1x A4S1G1
87,698.39	5x FA2S2， 1x A2S1G0F,1x GalNAc
87,704.54	5x FA2S2， 1x A3S1G0F
87,738.09	5x FA2S2， 1x A3G3F
87,779.20	5x FA2S2， 1xA4G2F
87,823.99	5x FA2S2， 1xA3S1G1F
87,859.93	5x FA2S2， 1x A2S1G0F,1x GalNAc-3G
87,902.79	5x FA2S2， 1x A2S1G0F,1x GalNAc-6Gn
87,943.34	6x FA2S2
87,986.04	
87,986.63	
88,021.26	6x FA2S2， 1x GalNAc-3SG
88,065.60	6x FA2S2， 1x GalNAc-6Gn
88,109.08	5x FA2S2， 1x A2S2M4F
88,150.29	6x FA2S2， 1x GalNAc
88,191.71	5x FA2S2， 1x GalNAc, 1xA2S1G1M5F
88,226.43	5x FA2S2，1x A2S1G0F,2x GalNAc-3G
88,267.33	5x FA2S2， 1xA3Sg1G2F, 1x GalNAc
88,277.20	5x FA2S2， 1xA4S1G2, 1x GalNAc

88,313.21	6x FA2S2， 1x GalNAc-3G
88,354.53	6x FA2S2， 1x GalNAc-6Gn
88,364.05	6x FA2S2， 2x GalNAc
88,382.44	5x FA2S2， 1xA3Sg1S1G0, 1x GalNAc-3G
88,391.63	5x FA2S2， 1x A3S1G2F, 1x GalNAc-3G
88,397.14	5x FA2S2， 1x A3Sg2G0, 1x GalNAc-3G
88,440.53	6x FA2S2， 1x GalNAc-6S
88,476.98	6x FA2S2， 1x GalNAc-6Gn,2x GalNAc-3SG
88,517.93	6x FA2S2， 1x GalNAc-3G, 1XGalNAc
88,558.07	6x FA2S2， 1x GalNAc-6Gn, 1XGalNAc
88,569.12	5x FA2S2， 1xA3S2G0， 1x GalNAc-3G, 1XGalNAc
88,609.18	6x FA2S2， 1x GalNAc-3SG
88,640.43	5x FA2S2， 1xA4S1G0F, 2x GalNAc-3G
88,679.51	6x FA2S2， 2x GalNAc-3G
88,723.47	6x FA2S2， 1x GalNAc-3G, 1XGalNAc-6Gn
88,731.94	5x FA2S2， 1x A3S2G0, 2x GalNAc-3G
88,754.93	5x FA2S2， 1x A3Sg1S1G0, 2x GalNAc-3G
88,759.04	5x FA2S2， 1xA3Sg2G0, 2x GalNAc-3G
88,772.07	5x FA2S2， 1xA3Sg2G0, 2x GalNAc-3G
88,803.04	6x FA2S2， 1x GalNAc-3G， 1x GalNAc-6S
88,806.96	6x FA2S2， 1x GalNAc,1x GalNAc-3SG
88,813.02	5x FA2S2， 1XA4G4, 1x GalNAc,1x GalNAc-3SG
88,841.29	6x FA2S2， 1x GalNAc-3G， 1x GalNAc-3SG
88,884.86	6x FA2S2， 2x GalNAc-3G， 1x GalNAc-3SG
88,894.79	6x FA2S2， 1x GalNAc-3G， 1x GalNAc-3SG， 1x GalNAc-6S
88,937.31	5x FA2S2， 1X A3S2G0， 2x GalNAc-3G, 1X GalNAc
88,968.10	6x FA2S2， 1x GalNAc-3G,1x GalNAc-3SG
88,973.35	6x FA2S2， 1x GalNAc-3SG, 1x GalNAc-6S-3SG
89,012.10	6x FA2S2， 2x GalNAc-3G, 1x GalNAc-6S
89,048.75	6x FA2S2， 3x GalNAc-3G
89,059.47	6x FA2S2， 1xA2S2M4， 3x GalNAc-3G
89,097.03	5x FA2S2， 1X A3S2G0， 3x GalNAc-3G
89,130.78	6x FA2S2， 1x GalNAc-3G
89,175.75	6x FA2S2， 2x GalNAc-3G,1x GalNAc-6S
89,183.06	5x FA2S2， 3x GalNAc-3G,1xA4Sg1G1F
89,212.31	6x FA2S2， 2x GalNAc-3G， 1x GalNAc-3SG
89,251.43	6x FA2S2， 2x GalNAc-3G, 1x GalNAc-6Gn-3SG
89,260.59	6x FA2S2， 2x GalNAc-3SG
89,338.30	6x FA2S2， 2x GalNAc-3G,1x GalNAc-3SG

89,414.52	6x FA2S2， 1x GalNAc-3SG， 1x GalNAc-6GGn-3G
89,426.48	6x FA2S2， 1x GalNAc-3SG， 1x GalNAc-6S
89,465.83	6x FA2S2， 1x GalNAc-3SG,， 1x GalNAc
89,543.51	5x FA2S2， 1xA3Sg2G0， 2x GalNAc-3SG,， 1x GalNAc
89,551.10	6x FA2S2， 2x GalNAc-3SG
89,626.94	6x FA2S2， 1x GalNAc-3G,2x GalNAc-3SG
89,632.03	6x FA2S2， 2x GalNAc-3SG， 1x GalNAc-3G
89,705.80	6x FA2S2， 1xA3Sg2G0， 1x GalNAc-3G,2x GalNAc-3SG
89,789.01	6x FA2S2， 1x GalNAc,2x GalNAc-3SG
89,832.28	6x FA2S2， 1x GalNAc-3GnG,2x GalNAc-3SG
89,919.05	6x FA2S2， 3x GalNAc-3SG
89,919.88	6x FA2S2， 2x GalNAc-3SG,1X GalNAc-6S-3G
89,994.09	6x FA2S2， 1xGalNAc-6GGn-3G,2x GalNAc-3SG
90,000.77	5x FA2S2， 1xA3Sg2G0， 3x GalNAc-3SG
90,072.70	5x FA2S2， 1xA4G4F， 3x GalNAc-3SG
90,080.81	5x FA2S2， 1xA2S2M4F， 3x GalNAc-3SG
90,154.45	5x FA2S2， 1xA3Sg1S1G1， 3x GalNAc-3SG
90,209.11	6x FA2S2， 2x GalNAc-3SG, 1x GalNAc-6S-3SG
90,283.69	6x FA2S2， 1x GalNAc-3G,2x GalNAc-3SG
90,363.29	5x FA2S2， 1xA3S1G2F， 1x GalNAc-3G,2x GalNAc-3SG
90,494.54	6x FA2S2， 1x GalNAc-3GnG,2x GalNAc-3SG
90,525.44	5x FA2S2， 1xA2Sg2F， 1x GalNAc-3SG,1x GalNAc-6GGn-3GnG, 1x GalNAc-6S-3SG
90,650.24	6x FA2S2， GalNAc-6GGn-3G,2x GalNAc-3SG
90,729.84	6x FA2S2， 1x GalNAc-6GGn-3G,2x GalNAc-3SG
90,747.93	6x FA2S2， 1x GalNAc-6GGn-3SG,2x GalNAc-3SG
90,940.40	5x FA2S2， 1xA3Sg2G0， 2x GalNAc-6S-3SG, 1x GalNAc-6GGn-3SG
91,018.44	4x FA2S2， 2xA3Sg2G0， 2x GalNAc-6S-3SG, 1x GalNAc-6GGn-3SG
91,164.95	6x FA2S2， 1x GalNAc-6GGn-3SG, 1x GalNAc-6Gn-3GnG,1 x GalNAc-6S-3SG