

Supporting Information

**Facile and Cheap Synthesis of CS@PGMA@IDA Nanomaterial for Highly Specific  
Enrichment of Glycopeptides**

Xiajuan Zou,<sup>a</sup> \* Jianzheng Jie,<sup>b</sup> and Bin Yang<sup>a</sup>

**Experimental Procedures**

**Chemicals and Materials**

Glycidyl methacrylate (GMA, for GC, ≥97.0%, Fluke, Buchs, Switzerland), 2,5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (CHCA), tris(2-carboxyethyl)phosphine (TCEP), iodoacetamide, trifluoroacetic acid (TFA), acetonitrile (ACN) (HPLC grade), acetic anhydride, *d*<sub>6</sub>-acetic anhydride, horseradish peroxidase (HRP, 98%) and bovine serum albumin (BSA, 98%) were obtained from Sigma Aldrich (St. Louis, MO, USA). Intravenous immunoglobulin G (IgG) was from Wuhan institute of biological products Co. Ltd, PNGase F was from New England Biolabs (Ipswich, MA). Iminodiacetic acid (IDA) was obtained from Beijing chemical factory (Beijing, China). Chitosan (Mw=500,000g/mol) was obtained from Qingdao Hecreat Bio-tech Company (Qingdao, China), other chemical reagents were analytical grade.

**Synthesis of CS@PGMA@IDA nanomaterial**

CS@PGMA was synthesized according to previous report with some modification <sup>1</sup>. 0.5 g chitosan was dissolved in diluted acetic acid with strong agitation. 1.0 mL GMA was added and the reaction mixture was heated for 2 h at 70 °C with 0.035g ammonium persulfate and 0.035g sodium thiosulfate as redox catalysts. The

polymerization and grafting reaction was finalized by raising the temperature to 80 °C for an additional 2 h. The suspension was then centrifuged and washed with a large volume of deionized water in order to extract the matrix product chitosan@PGMA (**2**).

The CS@PGMA matrix (**2**) was dispersed in 0.5 g IDA solution, prepared by dissolving IDA in 20 mL of a 2 M sodium carbonate solution for 6 h at 65 °C with stirring. The final CS@PGMA@IDA (**3**) product was centrifuged and washed with deionized water until pH neutral, dried at room temperature and ground into final powder with a mortar. The total of the product is 1.50 mg.

#### **Reduction, alkylation, protein digestion**

HRP and IgG was dissolved in ammonium bicarbonate (50 mM, pH 8.3) to a final concentration of 1 mg mL/L, and denatured at 95 °C for 15 min. Sequencing grade trypsin (V5111, Promega) was added to the protein solution and the digestion performed overnight at 37 °C and was stopped with 2 % TFA.

BSA was dissolved in a solution containing ammonium bicarbonate (50 mM, pH 8.3) and TCEP (10 mM) and then incubated at 67 °C for about 15 min. The reduced protein was then alkylated with iodoacetamide (50 mM) at RT in the dark for 45 min. Sequencing grade trypsin (V5111, Promega) was added to the protein solution and the digestion performed overnight at 37 °C and was stopped with 2 % TFA.

#### **Tube-gel**

For the preparation of human serum digestion, 2 µL human serum, 14.25 µL water, 45 µL of Tris-HCl buffer (pH 8.8), 35 µL of 30% acrylamide solution, 1 µL 10% SDS and

2.5  $\mu$ L of 10% ammonium persulfate were added to a 1.5 mL Eppendorf tube. The mixture was vortexed for 0.5 min. Then 0.25  $\mu$ L TEMED was added.

The gel was fixed with 50% methanol, 12% acetic acid for 30 mins at room temperature and cut into small pieces. The gel pieces were dehydrated with ACN and reduced with 10 mM TCEP for 10 min at 67 °C, then alkylated with 50 mM iodoacetamide at RT in the dark for 45 min. Gel pieces were washed with 50% ACN / 50mM NH<sub>4</sub>HCO<sub>3</sub> buffer and dehydrated with ACN. The gel was rehydrated with 100  $\mu$ L of 10 ng/ $\mu$ L trypsin in 25 mM NH<sub>4</sub>HCO<sub>3</sub> buffer at 4 °C for 2 hrs and then incubated at 37 °C overnight. After enzyme digestion, the peptides mixture was extracted with ACN / 5% FA (2:1) twice and lyophilized to dryness.

### **Glycopeptides enrichment**

CS@PGMA@IDA nanomaterial (0.5 mg) was placed in a 0.5 mL eppendorf tube, and then added to 200  $\mu$ L of peptide mixture from tryptic digestion in binding buffer, and the entire suspension mixture was incubated on a platform shaker at 900 rpm at 30 °C for 20 min. After centrifugation at 13000 rpm for 2 min, the supernatant was discarded, followed by rinsed with of the loading buffer (3  $\times$  200  $\mu$ L). Then, 5  $\mu$ L of 50% ACN aqueous solution or more elution buffer (30% (v/v) ACN, 0.1% TFA) was added to release the glycopeptides from the CS@PGMA@IDA nanomaterial over a period of 20 min.

For glycopeptides enrichment from complex sample, digests extracted from 2  $\mu$ L serum were redissolved in 400  $\mu$ L of loading buffer ACN/H<sub>2</sub>O/TFA 89: 6: 5, v/v/v) and the entire suspension mixture was incubated on a platform shaker at 900 rpm at 30

°C for 20 min. After centrifugation at 13000 rpm for 2 min, the supernatant was discarded. Unbound peptides were eliminated by washing with 200 µL of 89% ACN / 3% TFA, 89% ACN / 2% TFA loading buffer respectively, followed by washing with 200 µL of 85% ACN / 0.5% H<sub>3</sub>PO<sub>4</sub> one time, and then eluted with the eluting buffer (30% (v/v) ACN, 0.1% TFA v/v, 30 µL) twice. Finally, the eluted glycopeptides were lyophilized and deglycosylated for LC-MS/MS analysis.

#### **Deglycosylation of N-linked glycopeptides by PNGase F**

The captured glycopeptides were redissolved in NH<sub>4</sub>HCO<sub>3</sub> (25 mM), 50 units of PNGase F was added to the solution and incubated at 37 °C for overnight. The reaction solutions were directly spotted on the target plate for MALDI-TOF-MS analysis or analysis by LC-MS/MS.

For glycopeptides enrichment from serum, 500 units of PNGase F was added to the solution and incubated at 37 °C for overnight. The reaction solutions were lyophilized, redissolved in 20 µL of 2% ACN/ 0.1% FA solution for LC-MS/MS analysis.

#### **MALDI-TOF MS process**

0.5 µL of peptides-loaded CS@PGMA@IDA nanomaterial mixture was loaded onto a stainless steel target, and 0.6 µL of a mixture of 20 mg/ mL DHB in 50% (v/v) ACN and 1% H<sub>3</sub>PO<sub>4</sub> was added as matrix. Aspirate and dispense the above mixture at least 20 cycles with tip. MALDI-TOF mass spectra were acquired on an AXIMA-CFP plus (KRATOS Analytical, Shimadzu Group Company, Japan) mass spectrometer equipped with a nitrogen laser (337.1 nm). Mass spectra were obtained in positive ion and linear mode with an acceleration voltage of 20kV and an average of over 200 laser

shots. Mass spectrometric data analysis was performed using Launchpad V 2.4 Kompact MALDI software. Data analysis was carried out using Kompact MALDI software with default parameters. Each spectrum was externally calibrated with insulin (5734.62). Each spectrum was internally calibrated with HRP digestion fragment ions at *m/z* 1842.0, 3353.5 and 4984.2.

#### **Reversed phase nano-liquid chromatography tandem mass spectrometry (nano-LC-MS/MS)**

The deglycosylated peptides (10 $\mu$ L) were loaded on a C18 pre-column (Thermo Scientific) and separated by nano-LC-MS/MS using an Easy-LC nano-HPLC (Thermo Scientific). For a gradient separation, H<sub>2</sub>O/FA (99.9:0.1) was used as the mobile phase A while ACN/FA (99.9:0.1) was mobile phase B. At first, from 5% to 30% for 90 min, from 30% to 50% for 10 min, then from 50% to 100% for 10 min, and held at 100% for 10 min. The flow rate was 300 nL/min. Mass spectrometric analysis was performed using an LTQ Orbitrap Velos pro (Thermo Scientific, Bremen, Germany). The spray voltage was operated at 2.2 kV with the ion transfer capillary at 250 °C. The MS/MS spectra were obtained in a data-dependent collision induced dissociation (CID) mode, and the full MS was acquired from *m/z* 350 to 2000 with resolution 60,000. The top 15 most intense ions were selected to for MS/MS. Parameters for acquiring CID were as follows: activation time = 10 ms, normalized energy = 35, Q-activation = 0.25. The dynamic exclusion was set as follows: repeat count 1, duration 30 s, exclusion list size 500 and an exclusion duration 30 s.

#### **Database search and data analysis**

Raw files were analyzed using Proteome Discoverer v1.4.1.14 (Thermo Scientific). MS/MS spectra were searched against UniProt Human database (Sep 2014, 146,661 entries). The search parameters were set as follows: fixed modification of cysteine residues (+57 Da), variable modification of methionine oxidation (+16 Da), asparagine and glutamine deamidation (+0.9858) and full trypsin cleavage, at most one missed tryptic cleavage sites, 10 ppm error tolerance in MS and 0.6 Da error tolerance in MS/MS. False discovery rates were obtained using Percolator<sup>2</sup> selecting identification with a q-value equal or less than 0.01.

#### **Recovery estimation of glycopeptides enrichment:**

Two of the same amounts of human IgG (3 µg) digest were firstly labeled with light and heavy isotopes by using a stable isotope acetylate labeling approaches according to a previously reported procedure with some modification<sup>3</sup>. 1 µL of 0.1 M acetic anhydride, and *d*<sub>6</sub>-acetic anhydride were added to the tubes containing the IgG digest, respectively. 10µL of 50 mM NH<sub>4</sub>HCO<sub>3</sub> was added to each tube, sonicated for 20 min. Then, 5µL of 0.1 M glycine was added to react with the excessive acetic anhydride and *d*<sub>6</sub>-acetic anhydride. The reaction solutions were lyophilized. The heavy-tagged human IgG digest was enriched with CS@PGMA@IDA according to above-mentioned procedure and the resulting eluted fraction was spiked into light-tagged human IgG digest. The combined mixture was re-enriched with CS@PGMA@IDA, and the eluant was deglycosylated, followed by MALDI-TOF MS analysis. The recovery was calculated by the peak intensity ration of heavy isotope-labeled glycopeptides to the light isotope-labeled glycopeptides.

## Characterization

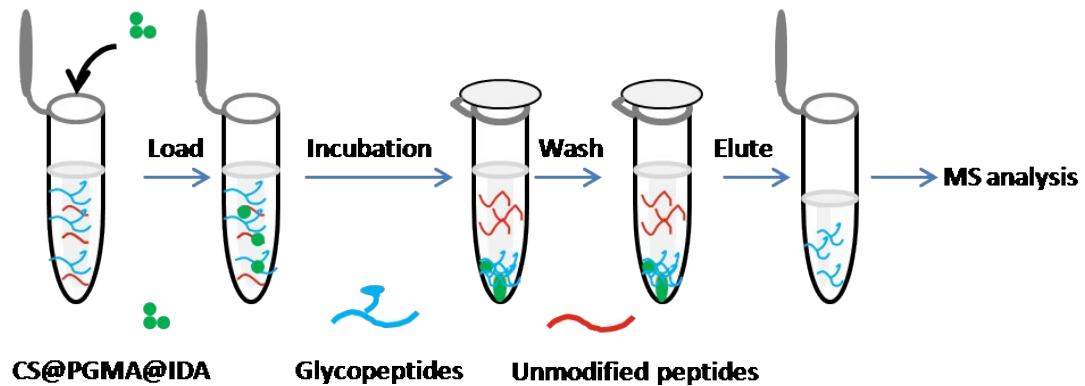
SEM (scanning electron microscope) image was performed with a JSM-5600LV (JEOL Company, Japan) instrument. Powder X-ray diffraction was analyzed using MiniFlex 300/600 (Rigaku Co., Tokyo, Japan) equipped with X-radiation source at a 40 kV voltage and 15 mA current. The samples were scanned from 3 to 100 ( $2\theta$ ) with a 5°/min scan speed. Fourier transformed infrared spectroscopy (FT-IR) characterization was measured on NEXUS-470 (nicolet, USA). Zeta potential analyses were performed using a Malvern Zetasizer Nano ZS (Malvern, U.K.) at 25 °C. Thermogravimetric analyses (TGA) were detected with SDT Q600 instrument under a nitrogen atmosphere (100 mL min<sup>-1</sup>) at a heating rate of 10 °C min<sup>-1</sup> from 20 to 600 °C(Thermal Analysis, USA). The contact angles were measured by OCA20 (Krüss, Hamburg, German). The nitrogen adsorption and desorption isotherms were measured by ASAP2020 QuadrasorbSI (Quadraorb, Wisconsin, USA). The Brunauer-Emmett-Teller (BET) method was used to calculate the specific surface areas with adsorption data in a relative pressure range from 0.0799 to 0.1998. The BJH pore-size distribution curve was obtained by nitrogen adsorption results. Elemental analyses were performed on Vario EL III (Elementar, Hanau, Germany). And the amount of IDA functionalized on the surface of nanosphere were calculated based the change of nitrogen content between before and after reaction according to a reported method <sup>4</sup>.

$$\chi_{IDA} \left( nmol.mg^{-1} \right) = \frac{\%X * 10^6}{(AM)n100 \left( 1 - \frac{X(MW)}{(AM)n100} \right)} \quad Eq.S1$$

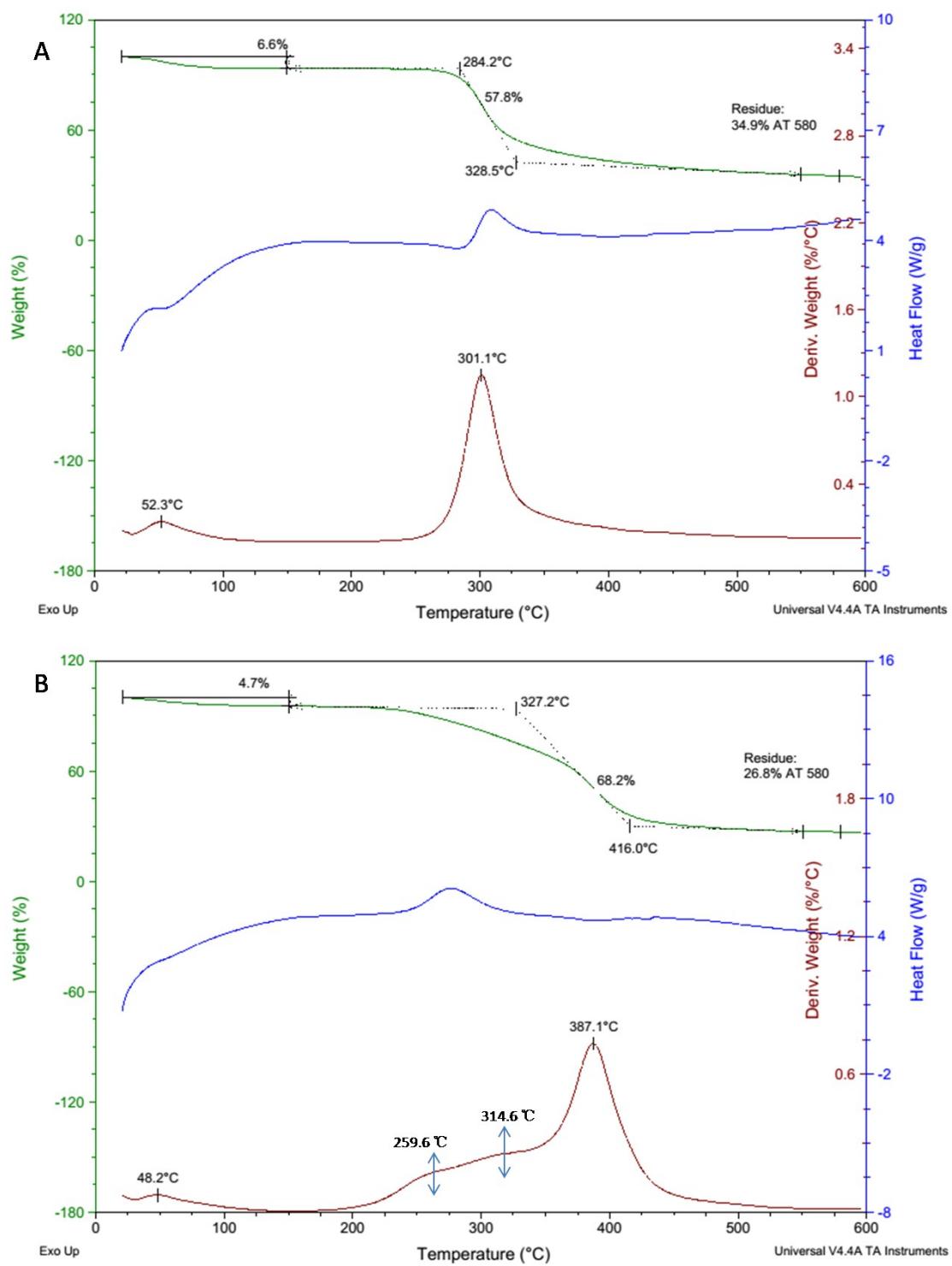
where  $\%X$  is the percent of nitrogen increase in the bonded support as determined by elemental analysis,  $AM$  is the atomic mass of nitrogen,  $MW$  is the molecular weight of the species bonded to the surface of support,  $n$  is the number of nitrogen atoms present in the bonded species.

#### Reference

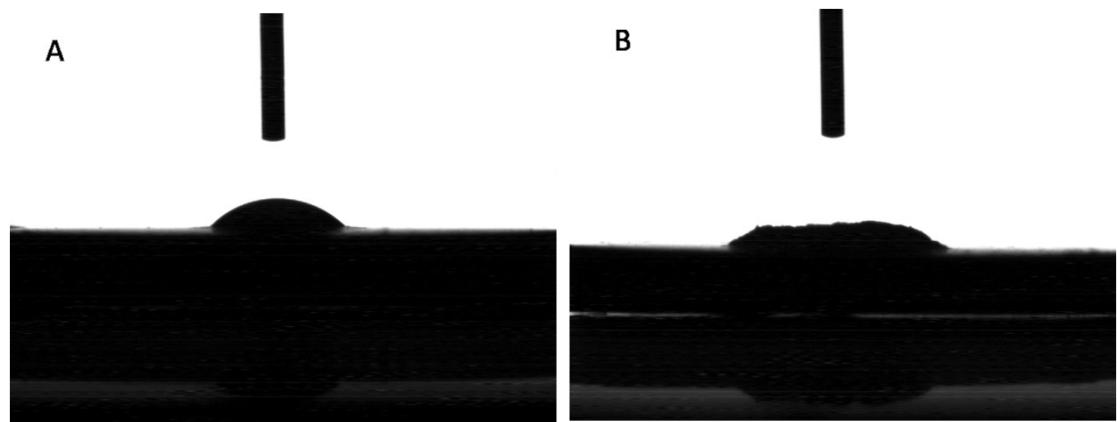
1. X. Zou, D. Liu, L. Zhong, B. Yang, Y. Lou, B. Hu and Y. Yin, *Anal. Bioanal. Chem.*, 2011, **401**, 1251-1261.
2. L. Kall, J. D. Canterbury, J. Weston, W. S. Noble and M. J. MacCoss, *Nat Methods*, 2007, **4**, 923-925.
3. Y. Yu, J. Cui, X. Wang, Y. Liu and P. Yang, *Proteomics*, 2004, **4**, 3112–3120.
4. C. E. Kibbey and M. E. Meyerhofer, *Anal. Chem.*, 1993, **65**, 2189-2196.



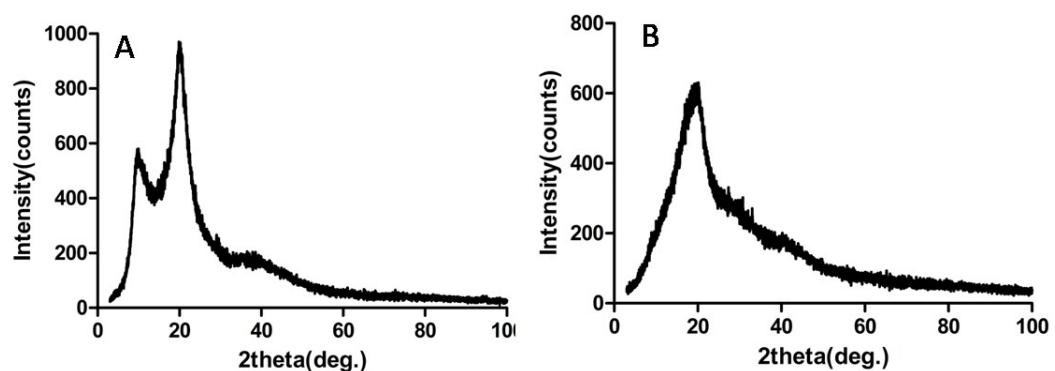
**Scheme S1** Procedure for glycopeptides enrichment



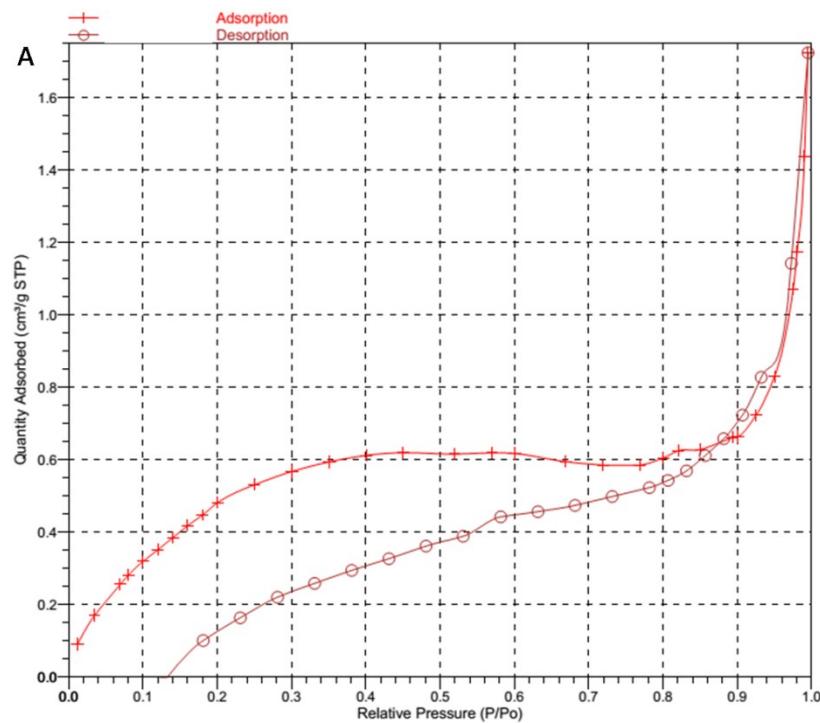
**Fig. S1** DSC-TGA curves for A) CS and B) CS@PGMA@IDA .

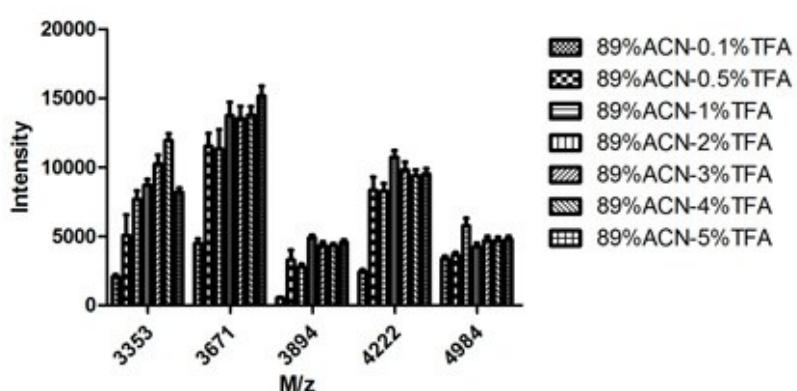
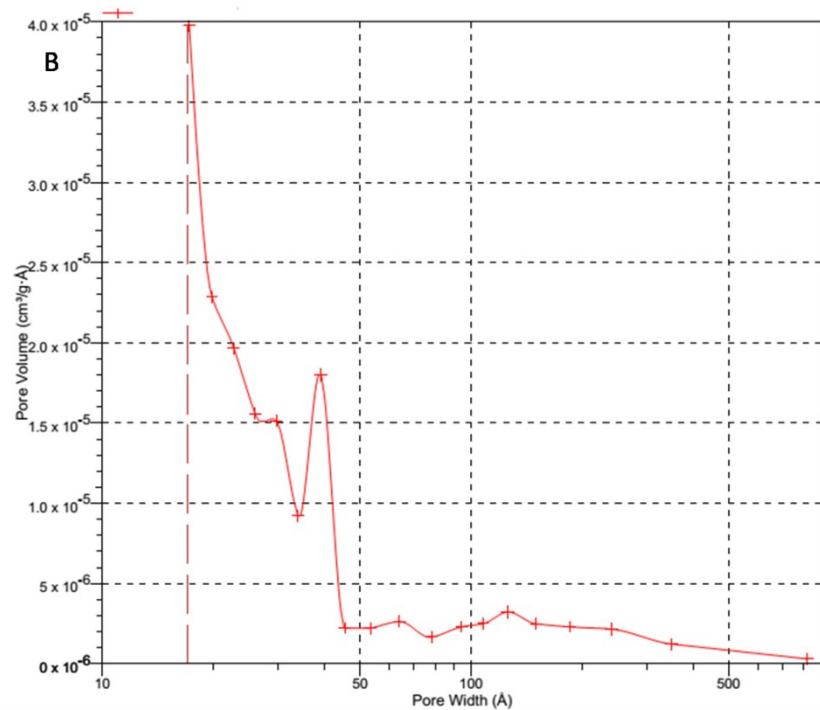


**Fig. S2** Contact angles of CS@PGMA@IDA, A) just contacted and B) 2 s later.

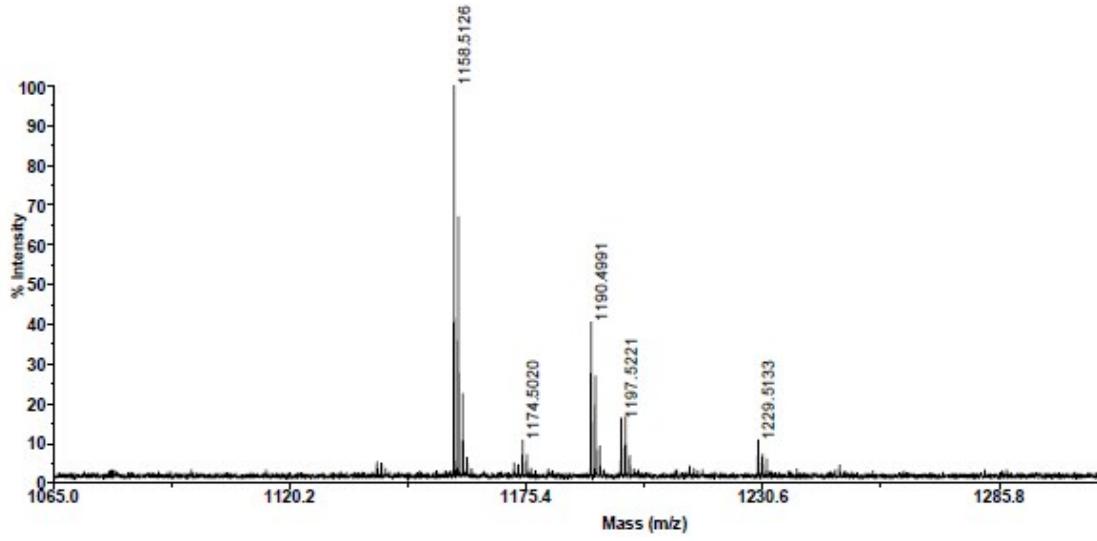


**Fig. S3** XRD profiles of A) CS and B) CS@PGMA@IDA.

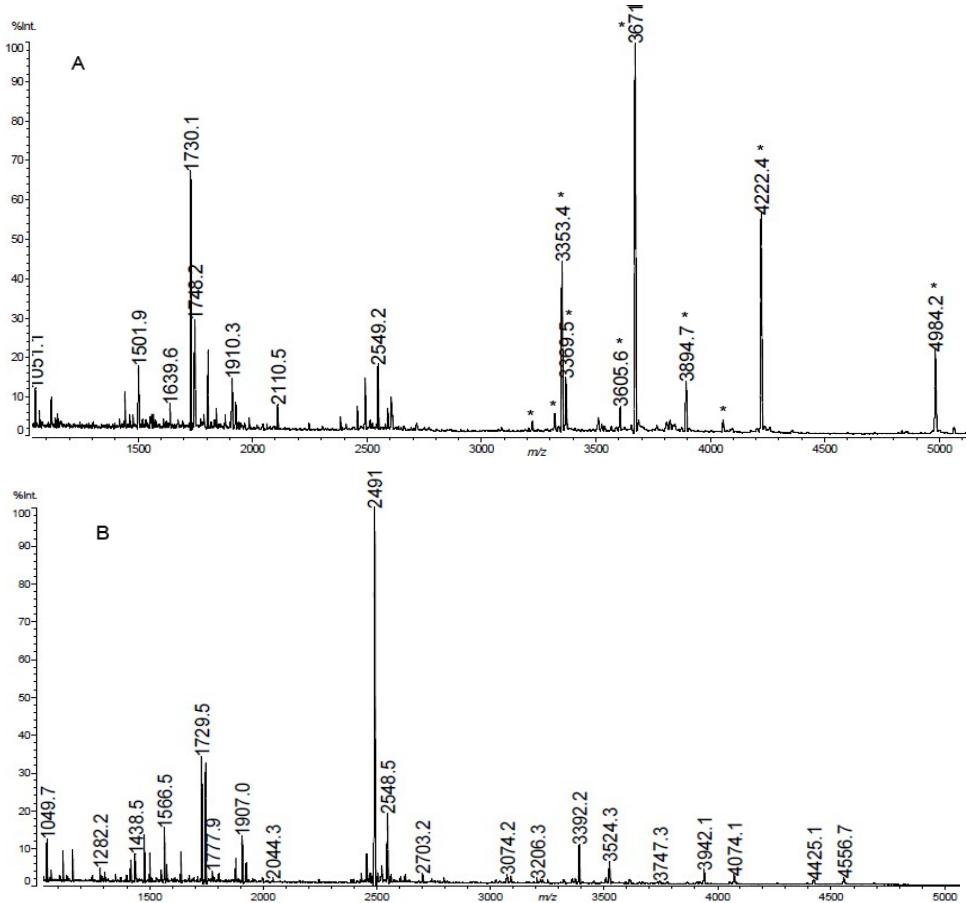


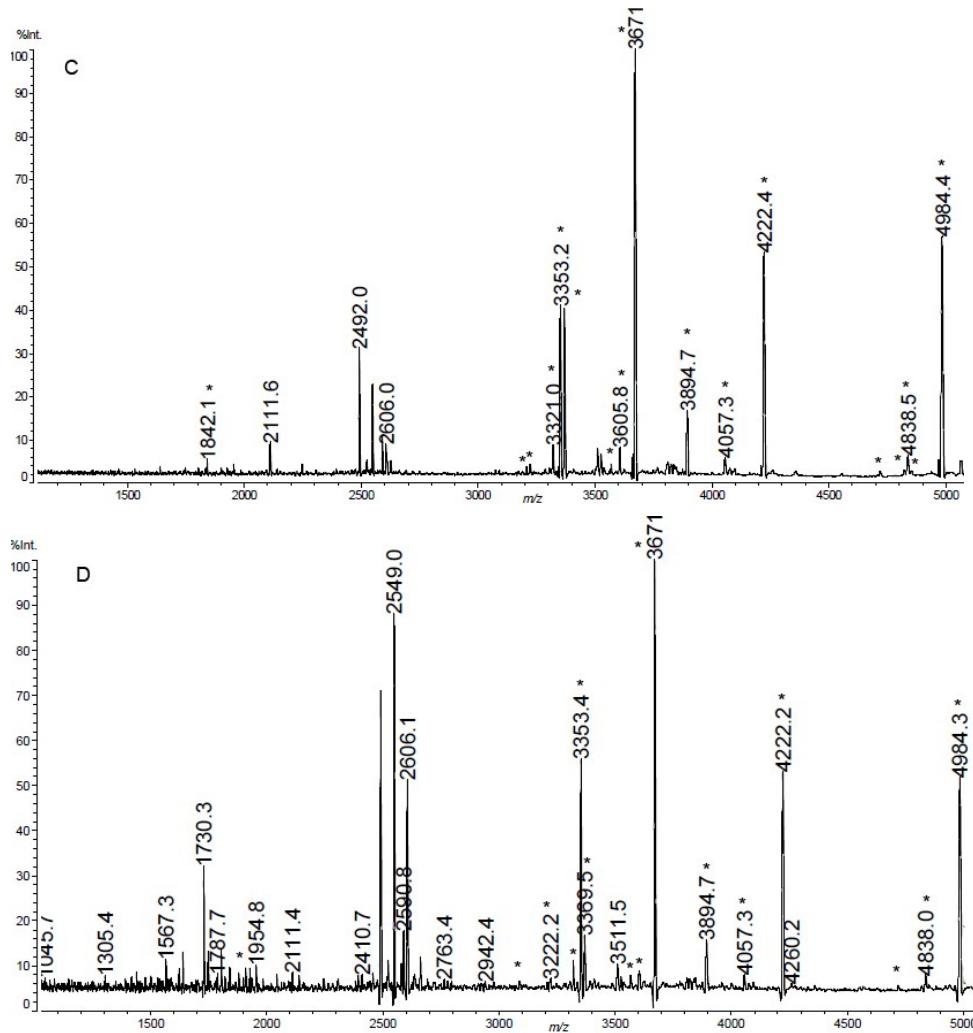


**Fig. S5** The influence of TFA IP on peak intensity of five chosen glycopeptides enriched by CS@PGMA@IDA nanospheres with four replicates.

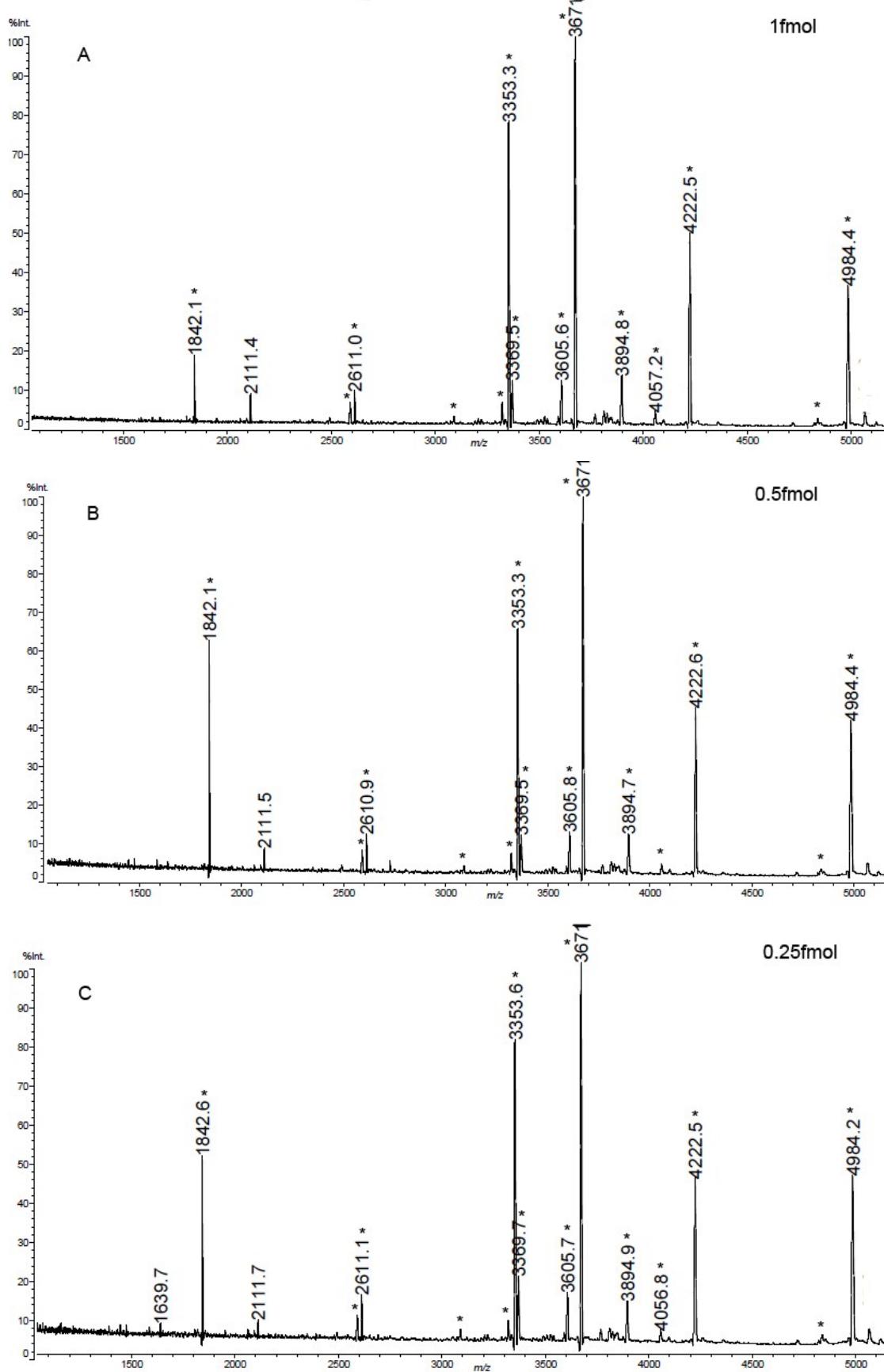


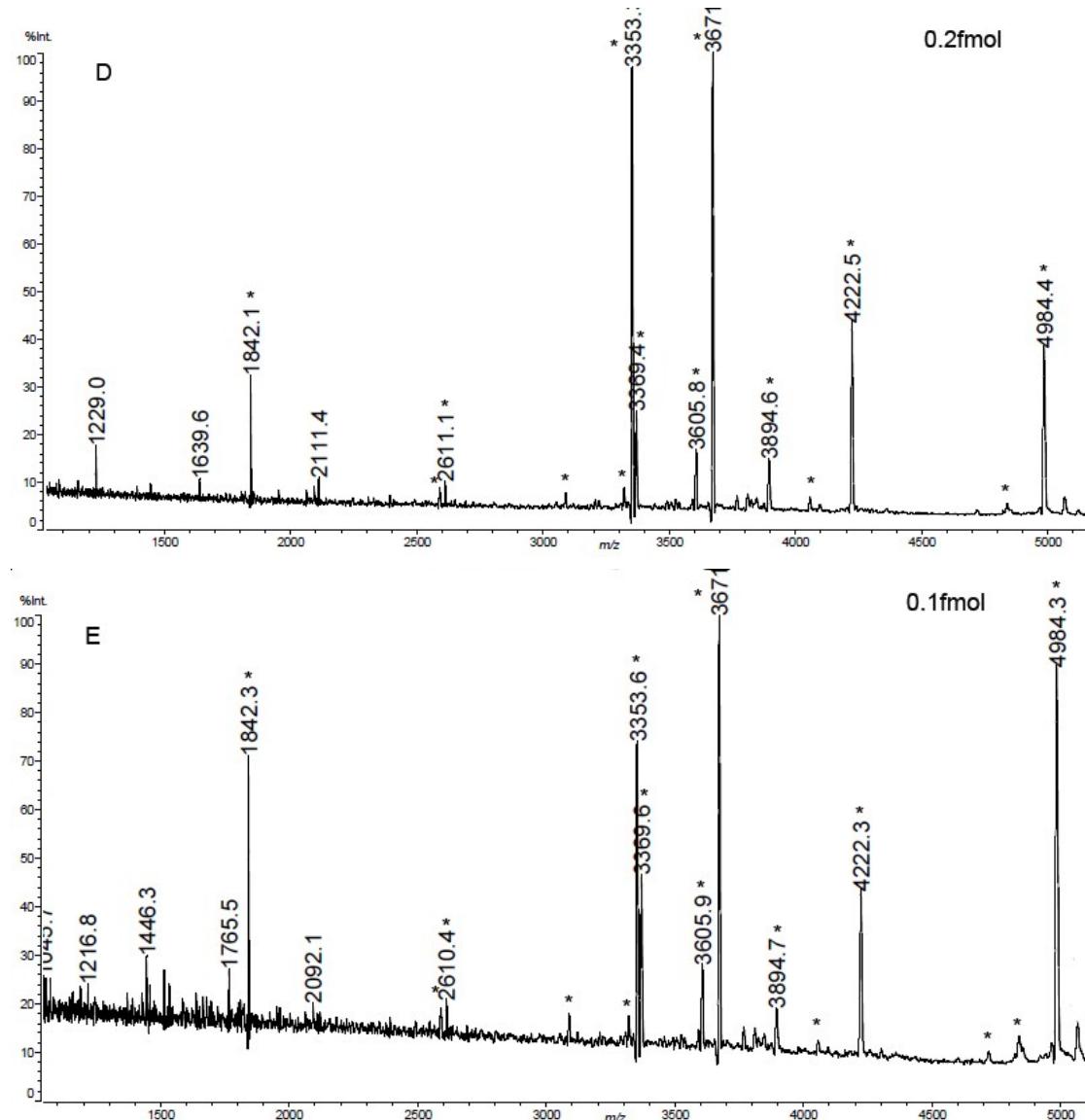
**Fig. S6** Human IgG digest after CS@PGMA@IDA enrichment and deglycosylation by PNGase F.



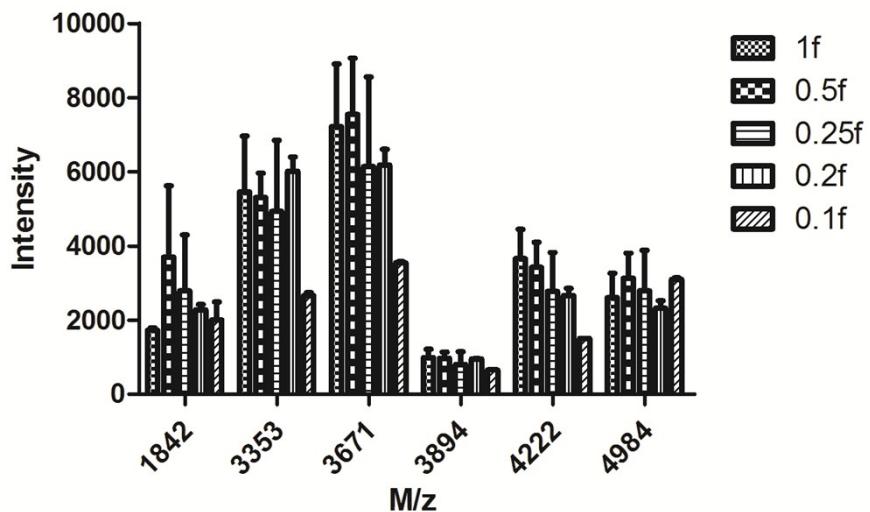


**Fig. S7** Optimization of the procedure for enrichment of glycopeptides using CS@PGMA@IDA nanospheres. MALDI mass spectra obtained from peptide mixture with the ratios of 1:100 enriched by CS@PGMA@IDA after washing three times with loading buffer (A), the eluate from washing with 85% ACN / 0.5 % H<sub>3</sub>PO<sub>4</sub> (B), and enriched by CS@PGMA@IDA after washing three times with loading buffer and 85% ACN / 0.5 % H<sub>3</sub>PO<sub>4</sub> one time (C), and enriched from peptide mixture with the ratios of 1:300 by CS@PGMA@IDA using the optimized procedure (D). The glycosylated peptides are marked with asterisks.

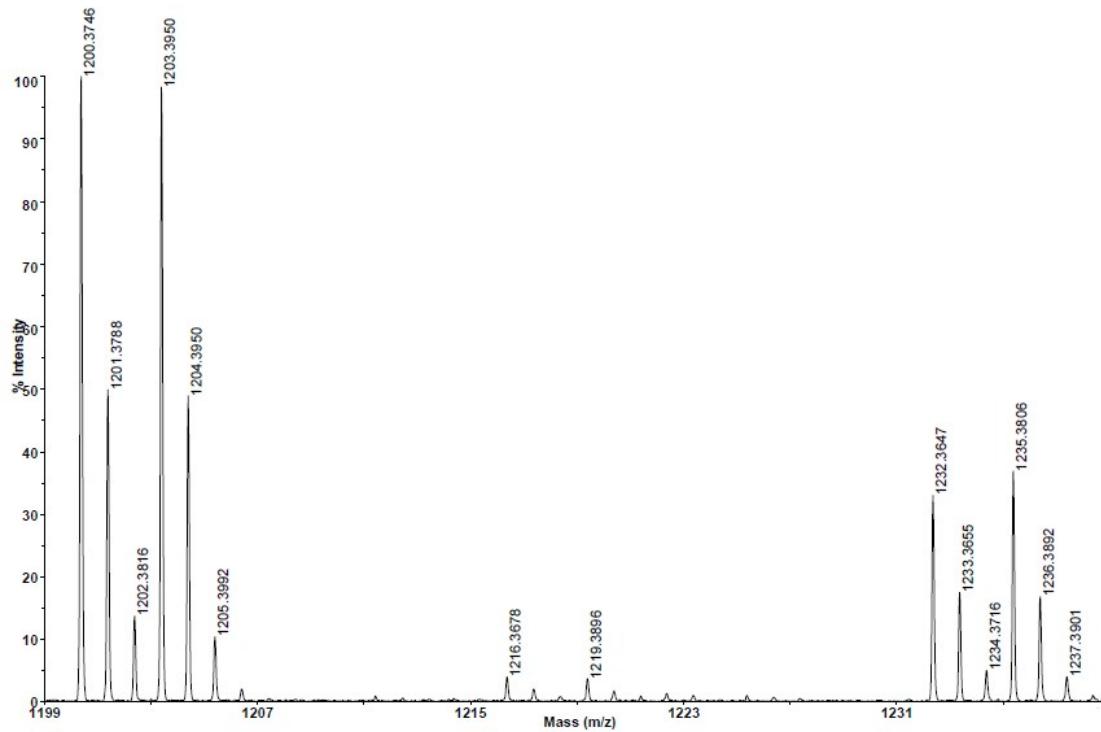




**Fig. S8** MALDI mass spectra of the enriched glycopeptides from HRP digest with the concentration of 1 fmol, 0.5 fmol, 0.25 fmol, 0.2 fmol and 0.1 fmol at different volume of 100  $\mu$ L, 200 $\mu$ L, 400 $\mu$ L, 500 $\mu$ L and 1000 $\mu$ L are shown in A, B, C, D, and E, respectively. The glycopeptides are marked with asterisks.



**Fig. S9** The influences of HRP digest concentration on peak intensity of six chosen glycopeptides enriched by CS@PGMA@IDA.



**Fig. S10** MALDI mass spectra of the recovery of the light- and heavy- acetylute labeling deglycosylated peptides of human IgG.

**Table S1. Elemental analysis of the title product**

C%	H%	N%	$X_{\text{IDA unit}}^{\text{a}}$ [mmol mg <sup>-1</sup> ]

CS@PGMA	50.38	6.734	2.020	
CS@PGMA@IDA	49.27	6.955	2.188	122

<sup>a</sup> The amount of IDA was obtained via a calculation based on the nitrogen content from the elemental analysis.

**Table S2. Zeta potential of CS@PGMA@IDA nanospheres in different binding buffer**

Time	89%ACN-0.1%TFA	89%ACN-1%TFA	89%ACN-3%TFA	89%ACN-5%TFA
1	0.089	0.09	-0.193	-0.024
2	0.0102	0.0918	0.169	0.0176
3	-0.136	0.00526	0.127	-0.216
Average	-0.0123±0.11417	0.06235±0.04945	0.03433±0.19799	-0.0741±0.12461

**Table S3: Detailed information of the glycopeptides enriched by CS@PGMA@IDA**

**from HRP digest, N# denotes the N-linked glycosylation site.**

No.	Observed m/z	Glycan composition	Amino acid sequence
H1	1842.3	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	NVGLN#R
H2	2532.8	FucGlcNAc	SFAN#STQTFFNAFVEAMDR
H3	2590.8	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	PTLN#TTYLQTLR
H4	2611.0	XylMan <sub>3</sub> GlcNAc <sub>2</sub>	MGN#ITPLTGTQQQIR
H5	3074.5	FucGlcNAc	LHFHDCFVNGCDASILLDN#TTSFR
H6	3088.1	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	GLCPLNGN#LSALVDFDLR
H7	3191.1	XylMan <sub>2</sub> FucGlcNAc <sub>2</sub>	SFAN#STQTFFNAFVEAMDR
H8	3207.1	XylMan <sub>3</sub> GlcNAc <sub>2</sub>	SFAN#STQTFFNAFVEAMDR
H9	3222.0	Man <sub>3</sub> FucGlcNAc <sub>2</sub>	SFAN#STQTFFNAFVEAMDR
H10	3320.9	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	QLTPTFYDNSCP#VSNIVR
H11	3353.0	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	SFAN#STQTFFNAFVEAMDR
H12	3369.6	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	SFAN#STQTFFNAFVEAM*DR
H13	3508.7	XylMan <sub>2</sub> FucGlcNAc <sub>2</sub>	GLIQSDQELFSSPN#ATDTIPLVR
H14	3525.6	XylMan <sub>3</sub> GlcNAc <sub>2</sub>	GLIQSDQELFSSPN#ATDTIPLVR
H15	3539.6	Man <sub>3</sub> FucGlcNAc <sub>2</sub>	GLIQSDQELFSSPN#ATDTIPLVR
H16	3605.5	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	NQCRLCPLNGN#LSALVDFDLR
H17	3671.6	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	GLIQSDQELFSSPN#ATDTIPLVR

H18	3894.6	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	LHFHDCFVNGCDASILLDN#TTSFR
H19	4056.9	XylMan <sub>3</sub> GlcNAc <sub>2</sub>	QLTPTFYDNSC(AAVESACPR)PN#VSNIKR-H <sub>2</sub> O
H20	4222.5	XylMan3FucGlcNAc2	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR
H21	4720.1	Man <sub>3</sub> FucGlcNAc <sub>2</sub> , Man <sub>3</sub> FucGlcNAc <sub>2</sub>	LYN#FSNTGLPDPTLN#TTYLQTLR
H22	4821.7	XylMan <sub>2</sub> FucGlcNAc <sub>2</sub> , XylMan <sub>2</sub> GlcNAc <sub>2</sub>	LYN#FSNTGLPDPTLN#TTYLQTLR
H23	4838.2	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub> , XylMan <sub>3</sub> GlcNAc <sub>2</sub>	LYN#FSNTGLPDPTLN#TTYLQTLR
H24	4852.7	Man <sub>3</sub> FucGlcNAc <sub>2</sub> , XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	LYN#FSNTGLPDPTLN#TTYLQTLR
H25	4984.3	XylMan <sub>3</sub> FucGlcNAc <sub>2</sub> , XylMan <sub>3</sub> FucGlcNAc <sub>2</sub>	LYN#FSNTGLPDPTLN#TTYLQTLR

**Table S4. Detailed information of the glycopeptides enriched by CS@PGMA@IDA from human IgG digest. N# denotes the N-linked glycosylation site.**

No.	Observed m/z	Glycan composition	Amino acid sequence
I1	2237.4	[Hex]3[HexNAc]2[Fuc]1	EEQFN#STFR
I2	2268.8	[Hex]3[HexNAc]2[Fuc]1	EEQYN#STYR
I3	2398.8	[Hex]3[HexNAc]3[Fuc]1	EEQFN#STFR
I4	2414.6	[Hex]3[HexNAc]3[Fuc]1	EEQFN#STYR
I5	2431.5	[Hex]3[HexNAc]3[Fuc]1	EEQYN#STYR
I6	2456.4	[Hex]3[HexNAc]4	EEQFN#STFR
I7	2472.0	[Hex]3[HexNAc]4	EEQFN#STYR
I8	2488.4	[Hex]3[HexNAc]4	EEQYN#STYR
I9	2561.8	[Hex]4[HexNAc]3[Fuc]1	EEQFN#STFR
I10	2593.2	[Hex]4[HexNAc]3[Fuc]1	EEQYN#STYR
I11	2602.5	[Hex]4[HexNAc]4[Fuc]1	EEQFN#STFR
I12	2618.7	[Hex]4[HexNAc]4[Fuc]1, or [Hex]4[HexNAc]4	EEQFN#STYR, or EEQFN#STFR

I13	2634.8	[Hex]4[HexNAc]4[Fuc]1, or [Hex]4[HexNAc]4	EEQYN#STYR, or EEQFN#STYR,
I14	2650.9	[Hex]4[HexNAc]4	EEQYN#STYR
I15	2660.1	[Hex]3[HexNAc]5	EEQFN#STFR
I16	2674.8	[Hex]3[HexNAc]5	EEQFN#STYR
I17	2691.9	[Hex]3[HexNAc]5	EEQYN#STYR
I18	2765.0	[Hex]4[HexNAc]4[Fuc]1	EEQFN#STFR
I19	2781.2	[Hex]4[HexNAc]4[Fuc]1, or [Hex]5[HexNAc]4	EEQFN#STYR, or EEQFN#STFR
I20	2797.2	[Hex]4[HexNAc]4[Fuc]1, or [Hex]5[HexNAc]4	EEQYN#STYR, or EEQFN#STYR
I21	2806.8	[Hex]3[HexNAc]5[Fuc]1	EEQFN#STFR
I22	2822.7	[Hex]3[HexNAc]5[Fuc]1, or [Hex]4[HexNAc]5	EEQFN#STYR, or EEQFN#STFR
I23	2838.3	[Hex]3[HexNAc]5[Fuc]1, or [Hex]4[HexNAc]5	EEQYN#STYR or EEQFN#STYR
I24	2854.6	[Hex]4[HexNAc]5	EEQYN#STYR
I25	2927.5	[Hex]5[HexNAc]4[Fuc]1	EEQFN#STFR
I26	2943.6	[Hex]5[HexNAc]4[Fuc]1	EEQFN#STYR
I27	2959.6	[Hex]5[HexNAc]4[Fuc]1	EEQYN#STYR
I28	2969.0	[Hex]4[HexNAc]5[Fuc]1	EEQFN#STFR
I29	2985.1	[Hex]4[HexNAc]5[Fuc]1, or [Hex]5[HexNAc]5	EEQFN#STYR, or EEQFN#STFR
I30	3002.5	[Hex]4[HexNAc]5[Fuc]1, or [Hex]5[HexNAc]5	EEQYN#STYR, or EEQFN#STYR
I31	3017.5	[Hex]5[HexNAc]5	[Hex]5[HexNAc]5
I32	3057.4	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQFN#STFR
I33	3089.4	[Hex]4[HexNAc]4[Fuc]1[NeuAc]1	EEQYN#STYR
I34	3105.7	[Hex]5[HexNAc]4[NeuAc]1	EEQYN#STYR
I35	3131.2	[Hex]5[HexNAc]5[Fuc]1	EEQFN#STFR
I36	3162.9	[Hex]5[HexNAc]5[Fuc]1	EEQYN#STYR
I37	3219.5	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQFN#STFR
I38	3235.9	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQFN#STYR
I39	3251.7	[Hex]5[HexNAc]4[Fuc]1[NeuAc]1	EEQYN#STYR

**Table S5. Recovery of three deglycosylated peptides from human IgG digest by using CS@PGMA@IDA**

Ratio(%)	EEQFN#STYR	EEQFN#STFR	EEQYN#STYR
D/H	99.5	106.7	99.1
D/H	93.3	99.3	105.7
D/H	95.2	92.6	103.7

Average recovery	96±3.16	102.8±7.06	99.5±3.43
± S. D			

**Table S6. Identified N-glycoptides containing deamidated Asn within N-X-S/T from 1 1µL human serum digest. N# denotes the N-linked lycosylation site. The total of LC-MS/MS run time is 120mins. (c denotes Carbamidomethyl; n and q denote Deamidated; m denotes Oxidation).**

No.	Protein Group Accessions	Description	Sequence
1	X6RLJ0	Complement C1q subcomponent subunit A (Fragment) OS=Homo sapiens GN=C1QA PE=4 SV=1 - [X6RLJ0_HUMAN]	RNPPmGGNVVIFDTVITNQEEPYQN#HSGR RNPPmGGNVVIFDTVITnQEEPYQN#HSGR
2	B1AHM6	Fibulin-1 (Fragment) OS=Homo sapiens GN=FBLN1 PE=4 SV=1 - [B1AHM6_HUMAN]	cATPHGDN#ASLEATFVK
3	Q9Y5Y7	Lymphatic vessel endothelial hyaluronic acid receptor 1 OS=Homo sapiens GN=LYVE1 PE=1 SV=2 - [LYVE1_HUMAN]	KANQQLN#FTEAK ANQQLN#FTEAK
4	Q9UK55	Protein Z-dependent protease inhibitor OS=Homo sapiens GN=SERPINA10 PE=1 SV=1 - [ZPI_HUMAN]	ETFFN#LSK
5	Q9UGM5-2	Isoform 2 of Fetuin-B OS=Homo sapiens GN=FETUB - [FETUB_HUMAN]	VLYLAAYN#cTLRPVSK
6	Q9HDC9-2	Isoform 2 of Adipocyte plasma membrane-associated protein OS=Homo sapiens GN=APMAP - [APMAP_HUMAN]	AGPN#GTLFVADAYK
7	Q96PD5	N-acetylmuramoyl-L-alanine amidase OS=Homo sapiens GN=PGLYRP2 PE=1 SV=1 - [PGRP2_HUMAN]	LEPVHLQLQcmSQEQLAQVAAN#ATK LEPVHLQLQcMSQEQLAQVAAN#ATK GFGVAIVGN#YTAALPTEAALR
8	Q96IY4-2	Isoform 2 of Carboxypeptidase B2 OS=Homo sapiens GN=CPB2 - [CBPB2_HUMAN]	KQVHFFVN#ASDVDNVK QVHFFVN#ASDVDNVK
9	Q8WUT4	Leucine-rich repeat neuronal protein	AFAcFPALqLLN#LScTALGR

		4 OS=Homo sapiens GN=LRRN4 PE=1 SV=3 - [LRRN4_HUMAN]	
10	Q7Z7M0-2	Isoform 2 of Multiple epidermal growth factor-like domains protein 8 OS=Homo sapiens GN=MEGF8 - [MEGF8_HUMAN]	LTcEDcLAN#SSqcAWcQSTHTcFLFAAYLAR
11	Q7Z2Y8	Interferon-induced very large GTPase 1 OS=Homo sapiens GN=GVINP1 PE=2 SV=2 - [GVIN1_HUMAN]	DNELVTFVIGLAN#LTLINIFGENPSEMQDIL QIVVQAFLRMK
12	Q7L4P6-2	Isoform 2 of BEN domain-containing protein 5 OS=Homo sapiens GN=BEND5 - [BEND5_HUMAN]	YlcEKImDIN#K
13	Q6UXB8	Peptidase inhibitor 16 OS=Homo sapiens GN=PI16 PE=1 SV=1 - [PI16_HUMAN]	SLPNFPN#TSATAN#ATGGR
14	Q6EMK4	Vasorin OS=Homo sapiens GN=VASN PE=1 SV=1 - [VASN_HUMAN]	LHEITN#ETFR
15	Q5VVQ7	C4b-binding protein beta chain (Fragment) OS=Homo sapiens GN=C4BPB PE=4 SV=1 - [Q5VVQ7_HUMAN]	EWDN#TTTEcR LGHcPDPVLVNGEFSSGPVN#VSDK LGHcPDPVLVnGEFSSGPVN#VSDK
16	Q5T985	Inter-alpha-trypsin inhibitor heavy chain H2 OS=Homo sapiens GN=ITIH2 PE=1 SV=1 - [Q5T985_HUMAN]	GAFISN#FSmTVDGK GAFISN#FSMTVDGK
17	Q5SSB9	Ficolin-3 OS=Homo sapiens GN=FCN3 PE=4 SV=1 - [Q5SSB9_HUMAN]	VELEDFNGN#R VELEDFnGN#R
18	Q5SQ12	Prostaglandin-H2 D-isomerase OS=Homo sapiens GN=PTGDS PE=3 SV=1 - [Q5SQ12_HUMAN]	SVVAPATDGGLN#LTSTFLR
19	Q5H9B4	Metalloproteinase inhibitor 1 (Fragment) OS=Homo sapiens GN=TIMP1 PE=1 SV=2 - [Q5H9B4_HUMAN]	FVGTPEVN#QTTLYQR
20	Q16609	Putative apolipoprotein(a)-like protein 2 OS=Homo sapiens GN=LPAL2 PE=5 SV=1 - [LPAL2_HUMAN]	WEYcN#LTR
21	Q14624-4	Isoform 4 of Inter-alpha-trypsin inhibitor heavy chain H4 OS=Homo sapiens GN=ITIH4 - [ITIH4_HUMAN]	KAFIT N#FSmIIDGmTYPGIK KAFIT N#FSMIIDGmTYPGIK AFIT N#FSmIIDGmTYPGIK NQALN#LSLAYSFVTPLTSMVVTKPDDQEQS

			QVAEKPMEGESR AFIT N#FSMIIIDGmTYPGIK
22	Q08380	Galectin-3-binding protein OS=Homo sapiens GN=LGALS3BP PE=1 SV=1 - [LG3BP_HUMAN]	GL N#LTEDTYKPR EPGS N#VTmSVDAEcVPmVR ALGFE N#ATQALGR TVIRPFYLTN#SSGVD
23	Q06033-2	Isoform 2 of Inter-alpha-trypsin inhibitor heavy chain H3 OS=Homo sapiens GN=ITIH3 - [ITIH3_HUMAN]	NAHGEEKEN#LTAR nAHGEEKEN#LTAR
24	Q04756	Hepatocyte growth factor activator OS=Homo sapiens GN=HGFAC PE=1 SV=1 - [HGFA_HUMAN]	DSVSVVLGQHFFN#R
25	Q03591	Complement factor H-related protein 1 OS=Homo sapiens GN=CFHR1 PE=1 SV=2 - [FHR1_HUMAN]	LQNNENN#IScVER
26	P80188	Neutrophil gelatinase-associated lipocalin OS=Homo sapiens GN=LCN2 PE=1 SV=2 - [NGAL_HUMAN]	SYN#VTSVLFR
27	P55058	Phospholipid transfer protein OS=Homo sapiens GN=PLTP PE=1 SV=1 - [PLTP_HUMAN]	VSN#VScQASVSR GKEGHFYYN#ISEVK N#WSLPNR EGHFYYN#ISEVK
28	P51884	Lumican OS=Homo sapiens GN=LUM PE=1 SV=2 - [LUM_HUMAN]	LHINHNN#LTEVGPLPK LHINHnnN#LTEVGPLPK LGSFEGLVN#LTFIHLQHNR AFEN#VTDLQWLILDHNLLNSK
29	P49917	DNA ligase 4 OS=Homo sapiens GN=LIG4 PE=1 SV=2 - [DNLI4_HUMAN]	APN#LTNVNK
30	P43652	Afamin OS=Homo sapiens GN=AFM PE=1 SV=1 - [AFAM_HUMAN]	HN#FSHccSK YAEDKFN#ETTEK DIENFN#STQK
31	P40197	Platelet glycoprotein V OS=Homo sapiens GN=GP5 PE=1 SV=1 - [GPV_HUMAN]	LLDLSGN#LTHLPK
32	P35858	Insulin-like growth factor-binding protein complex acid labile subunit OS=Homo sapiens GN=IGFALS PE=1 SV=1 - [ALS_HUMAN]	AGAFLGLTNVAVmN#LSGNcLR
33	P29622	Kallistatin OS=Homo sapiens GN=SERPINA4 PE=1 SV=3 -	FLN#DTmAVYEAK DFYVDEN#TTVR

		[KAIN_HUMAN]	SQILEGLGFN#LTELSESDVHR
34	P22792	Carboxypeptidase N subunit 2 OS=Homo sapiens GN=CPN2 PE=1 SV=3 - [CPN2_HUMAN]	AFGSNPN#LTK LYLGSNN#LTALHPALFQN#LSK LEDLEVTGSSFLN#LSTnIFSNLTSLGK
35	P20742	Pregnancy zone protein OS=Homo sapiens GN=PZP PE=1 SV=4 - [PZP_HUMAN]	TFSSmTcASGAN#VSEQLSLK
36	P19652	Alpha-1-acid glycoprotein 2 OS=Homo sapiens GN=ORM2 PE=1 SV=2 - [A1AG2_HUMAN]	EN#GTVSR QNQcFYN#SSYLNQVR QnQcFYN#SSYLNQVR
37	P14151	L-selectin OS=Homo sapiens GN=SELL PE=1 SV=2 - [LYAM1_HUMAN]	FcRDN#YTDLVAIQNK IGGIWTWVGTN#K
38	P12259	Coagulation factor V OS=Homo sapiens GN=F5 PE=1 SV=4 - [FA5_HUMAN]	NSVLN#SSTAEHSSPYSEDPIEDPLQPDVTGIR
39	P10909-4	Isoform 4 of Clusterin OS=Homo sapiens GN=CLU - [CLUS_HUMAN]	HN#STGcLR EDALN#ETR LAN#LTQGEDQYYLR LKELPGVcN#ETmmALWEEcKPcLK ELPGVcN#ETmmALWEEcKPcLK ELPGVcN#ETMmALWEEcKPcLK LKELPGVcN#ETMmALWEEcKPcLK LKELPGVcN#ETMMALWEEcKPcLK ELPGVcN#ETMMALWEEcKPcLK QLEEFLN#QSSPFYFWmnGDR mLN#TSSLLEQLNEQFNWVR MLN#TSSLLEQLNEQFNWVR
40	POCOL5	Complement C4-B OS=Homo sapiens GN=C4B PE=1 SV=2 - [CO4B_HUMAN]	GLN#VTLSSTGR FSDGLESN#SSTQFEVK FSDGLESN#SSTQFEVK TYNVLDmKN#TTcQDLQIEVTVK TYNVLDmKN#TTcqDLQIEVTVK
41	P08603;Q03591	Complement factor H OS=Homo sapiens GN=CFH PE=1 SV=4 - [CFAH_HUMAN]	SPYEmFGDEEVmcLnGN#WTEPPQcK ISEEN#ETTcYmGK mDGASN#VTcINSR ISEEN#ETTcYMGK IPcSQPPQIEHGTIN#SSR
42	P08185	Corticosteroid-binding globulin OS=Homo sapiens GN=SERPINA6 PE=1 SV=1 - [CBG_HUMAN]	AQLLQGLGFN#LTER AVLQLNEEGVDTAGSTGVTLN#LTSKPIILR AVLQLnEEGVDTAGSTGVTLN#LTSKPIILR VTISGVYDLGDVLEEEmGIADLFTNQAN#FSR
43	P07996	Thrombospondin-1 OS=Homo	VVN#STTGPGEHLR

		sapiens GN=THBS1 PE=1 SV=2 - [TSP1_HUMAN]	
44	P07357	Complement component C8 alpha chain OS=Homo sapiens GN=C8A PE=1 SV=2 - [CO8A_HUMAN]	GGSSGWSGGLAQN#R
45	P05546	Heparin cofactor 2 OS=Homo sapiens GN=SERPIND1 PE=1 SV=3 - [HEP2_HUMAN]	DFVN#ASSK GETHEQVHSILHFDFVN#ASSK GETHEqVHSILHFDFVN#ASSK N#LSmPLLPADFH GGETAQSAQPQWEQLNNKN#LSmPLLPADFH GGETAQSAQPQWEQLNNKN#LSmPLLPADFH
46	P05543	Thyroxine-binding globulin OS=Homo sapiens GN=SERPINA7 PE=1 SV=2 - [THBG_HUMAN]	VTAcHSSQPN#ATLYK TLYETEVFSTDFSN#ISAAK
47	P05154	Plasma serine protease inhibitor OS=Homo sapiens GN=SERPINA5 PE=1 SV=3 - [IPSP_HUMAN]	VVGVPYQGN#ATALFILPSEGK
48	P04275	von Willebrand factor OS=Homo sapiens GN=VWF PE=1 SV=4 - [VWF_HUMAN]	mEAcLN#GTVIGPGK
49	P04220	Ig mu heavy chain disease protein OS=Homo sapiens PE=1 SV=1 - [MUCB_HUMAN]	THTNISEHPN#ATFSAVGEASICEDDWDSGER THTN#ISEHPnATFSAVGEASICEDDWDSGER
50	P04217	Alpha-1B-glycoprotein OS=Homo sapiens GN=A1BG PE=1 SV=4 - [A1BG_HUMAN]	EGDHEFLEVPEAQEDVEATFPVHQPGN#YScSYR EGDHEFLEVPEAqEDVEATFPVHQPGN#YScSYR EGDHEFLEVPEAqEDVEATFPVHQPGN#YScSYR FQSPAGTEALFELHNISVADSAN#YScVYVDLK KPPFGGSAPSER FqSPAGTEALFELHN#ISVADSAnYScVYVDLK PPFGGSAPSER
51	P04196	Histidine-rich glycoprotein OS=Homo sapiens GN=HRG PE=1 SV=1 - [HRG_HUMAN]	VIDFN#cTTSSVSSALAnTK VIDFN#cTTSSVSSALANTK
52	P04180	Phosphatidylcholine-sterol acyltransferase OS=Homo sapiens GN=LCAT PE=1 SV=1 - [LCAT_HUMAN]	AELSN#HTRPVILVPGcLGNQLEAK
53	P04114	Apolipoprotein B-100 OS=Homo	FVEGSHN#STVSLTTK

		sapiens GN=APOB PE=1 SV=2 - [APOB_HUMAN]	FN#SSYLQGTnQITGR FN#SSYLQGTNQITGR YDFN#SSmLYSTAK SKPTVSSSmEFKYDFN#SSmLYSTAK VNQNLVYESGSLN#FSK VNQNLVYESGSLN#FSKLEIQSQVDSQHVGH SVLTAK QVLFLDTVYGN#cSTHFTVK qVLFLDTVYGN#cSTHFTVK FEVDSPVYN#ATWSASLK TIHDLHLFIENIDFN#K ELcTISHIFIPAmGN#ITYDFSFK YNQN#FSAGNnEnImEAHVGINGEANLDLF NIPLTIPEmR SSVITLNTNAELFN#qSDIVAHLLSSSSVIDAL QYK
54	P04004	Vitronectin OS=Homo sapiens GN=VTN PE=1 SV=1 - [VTNC_HUMAN]	NN#ATVHEQVGGPSLTSDLQAQSK nN#ATVHEQVGGPSLTSDLQAQSK N#GSLFAFR N#ISDGFDGIPDnVDAALALPAHYSGR FEDGVLDPDYPRN#ISDGFDGIPDNVDAALA LPAHYSGR
55	P04003	C4b-binding protein alpha chain OS=Homo sapiens GN=C4BPA PE=1 SV=2 - [C4BPA_HUMAN]	LSVDKDqYVEPENVTIQcDSGYGVVGPQSITc SGnR LSVDKDqYVEPEN#VTIQCDSGYGVVGPQSIT cSGnR FSLLGHASIScTVEN#ETIGVWRPSPPTcEK
56	P03951-2	Isoform 2 of Coagulation factor XI OS=Homo sapiens GN=F11 - [FA11_HUMAN]	LETTVN#YTDSQRPIcLPSK
57	P02790	Hemopexin OS=Homo sapiens GN=HPX PE=1 SV=2 - [HEMO_HUMAN]	GHGHRN#GTGHGN#STHHGPEYmR N#GTGHGN#STHHGPEYmR SWPAVGN#cSSALR ALPQPQN#VTSLLGcTH cSDGWSFDATTLDDN#GTmLFFK cSDGWSFDATTLDDN#GTmLFFK
58	P02787	Serotransferrin OS=Homo sapiens GN=TF PE=1 SV=3 - [TRFE_HUMAN]	cGLVPVLAENYN#K cGLVPVLAEnYN#K QQQHLFGSN#VTDcSGNFcLFR QQQHLFGSN#VTDcSGnFcLFR
59	P02765	Alpha-2-HS-glycoprotein OS=Homo sapiens GN=AHSG PE=1 SV=1 - [FETUA_HUMAN]	AALAAFNAQnN#GSNFQLEEISR
60	P02763;P1	Alpha-1-acid glycoprotein 1	NEEYN#K

	9652	OS=Homo sapiens GN=ORM1 PE=1 SV=1 - [A1AG1_HUMAN]	SVQEIQATFFYFTPN#K SVQEIQATFFYFTPN#KTEDTIFLR NEEYNKSVqEIQATFFYFTPN#KTEDTIFLR EN#GTISR QDQcIYN#TTYLnVQR QDQcIYN#TTYLNVQR
61	P02751-10	Isoform 10 of Fibronectin OS=Homo sapiens GN=FN1 - [FINC_HUMAN]	RHEEGHmLN#cTcFGQGR HEEGHmLN#cTcFGQGR HEEGHMLN#cTcFGQGR DQcIVDDITYNVN#DTFHKR DQcIVDDITYNVN#DTFHK LDAPTNLQFVN#ETDSTVLVR LDAPTNLqFVN#ETDSTVLVR
61	P02750	Leucine-rich alpha-2-glycoprotein OS=Homo sapiens GN=LRG1 PE=1 SV=2 - [A2GL_HUMAN]	mFSQN#DTR KLPPGLLAN#FTLLR LPPGLLAN#FTLLR SDHGSSIScQPPAEIPGYLPADTVHLAVEFFN #LTHLPANLLQGASK
62	P02749	Beta-2-glycoprotein 1 OS=Homo sapiens GN=APOH PE=1 SV=3 - [APOH_HUMAN]	VYKPSAGN#nSLYR VYKPSAGN#nSLYR LGN#WSAMPScK DTAVFEcLPQHAmFGnDTITcTTHGN#WTK
63	P02748	Complement component C9 OS=Homo sapiens GN=C9 PE=1 SV=2 - [CO9_HUMAN]	AVN#ITSENLIIDVVSLIR
64	P02743	Serum amyloid P-component OS=Homo sapiens GN=APCS PE=1 SV=2 - [SAMP_HUMAN]	ESVTDHVNLTITLEKPLQN#FTLcFR ESVTDHvnLTITLEKPLQN#FTLcFR
65	P01876;P0 1877	Ig alpha-1 chain C region OS=Homo sapiens GN=IGHA1 PE=1 SV=2 - [IGHA1_HUMAN]	LSLHRPALEDELLGSEAN#LTcTLTGLR LAGKPTHVN#VSVVmAEVDGTCY LAGKPTHVN#VSVVMAEVDGTCY
66	P01871;P0 4220	Ig mu chain C region OS=Homo sapiens GN=IGHM PE=1 SV=3 - [IGHM_HUMAN]	STGKPTLYN#VSLVmSDTAGTcY STGKPTLYN#VSLVMSDTAGTcY YKN#NSDISSTR YKN#nSDISSTR GLTFQQN#ASSmcVPDQDTAIR GLTFqQN#ASSmcVPDQDTAIR THTN#ISESHPN#ATFSAVGEASICEDDWNS GER THTN#ISESHPN#ATFSAVGEASICEDDWnSG ER THTN#ISESHPNATFSAVGEASICEDDWNSG ER

			GLTFQQN#ASSMcVPDQDTAIR
67	P01861	Ig gamma-4 chain C region OS=Homo sapiens GN=IGHG4 PE=1 SV=1 - [IGHG4_HUMAN]	EEQFN#STYR
68	P01860	Ig gamma-3 chain C region OS=Homo sapiens GN=IGHG3 PE=1 SV=2 - [IGHG3_HUMAN]	EEQYN#STFR
69	P01859	Ig gamma-2 chain C region OS=Homo sapiens GN=IGHG2 PE=1 SV=2 - [IGHG2_HUMAN]	TKPREEQFN#STFR EEQFN#STFR
70	P01857	Ig gamma-1 chain C region OS=Homo sapiens GN=IGHG1 PE=1 SV=1 - [IGHG1_HUMAN]	TKPREEQYN#STYR EEQYN#STYR
71	P01042-2	Isoform LMW of Kininogen-1 OS=Homo sapiens GN=KNG1 - [KNG1_HUMAN]	ITYSIVQTN#cSK YNSQN#QSNNQFVLYR YnSQN#QSNNQFVLYR YnSQN#QSnNQFVLYR HGIQYFNN#NTQHSSLFmLNEVKR LNAEnN#ATFYFK HGIQYFnN#NTQHSSLFmLNEVKR LNAENN#ATFYFK HGIQYFnN#nTQHSSLFmLNEVKR HGIQYFnN#NTQHSSLFmLNEVK HGIQYFNN#NTQHSSLFMLNEVK HGIQYFnN#NTQHSSLFMLNEVK
72	P01031	Complement C5 OS=Homo sapiens GN=C5 PE=1 SV=4 - [CO5_HUMAN]	AN#ISHKDmQLGR YN#FSFR
73	P01024	Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2 - [CO3_HUMAN]	TVLTPATNHmGN#VTFTIPANR TVLTPATNHMGN#VTFTIPAnR TVLTPATNHmGN#VTFTIPAnR TVLTPATNHmGN#VTFTIPAnREFK TVLTPATnHmGN#VTFTIPAnREFK TVLTPATNHMGN#VTFTIPANR
74	P01023	Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3 - [A2MG_HUMAN]	SLGNVN#FTVSAEALESqELcGTEVPSVPEHG RK SLGNVN#FTVSAEALESqELcGTEVPSVPEHG RK SLGNVN#FTVSAEALESqELcGTEVPSVPEHG R GNEANYYSN#ATTDEHGLVQFSIN#TTNVm GTSLTVR IITILEEEmN#VSVCGLTYGKPVPGHVTVSIC R

			SLGNVN#FTVSAEALESqELcGTEVPSVPEHGR GNEANYYSN#ATTDEHGLVQFSIN#TTnVmGTSLTVR IITILEEMMN#VSVCGLTYGKPVPGHVTVSICR GcVLLSYLN#ETVTVSASLESVR GNEANYYSN#ATTDEHGLVQFSIN#TTNVMGTSLTVR VSN#qTLSLFFTQLQDVPVR
75	P01019	Angiotensinogen OS=Homo sapiens GN=AGT PE=1 SV=1 - [ANGT_HUMAN]	LQAILGVPWKDKN#cTSR VYIHPFHLVIHN#ESTcEQLAK
76	P01011	Alpha-1-antichymotrypsin OS=Homo sapiens GN=SERPINA3 PE=1 SV=2 - [AACT_HUMAN]	TLN#QSSDELQLSmGnAmFVK YTGN#ASALFILPDQDK TLN#QSSDELQLSMGNAMFVK GLKFN#LTETSEAEIHQSfqHLLR FN#LTETSEAEIHQSfqHLLR YTGN#ASALFILPDQDKmEEVEAmLLPETLKR YTGN#ASALFILPDQDKmEEVEAmLLPETLK APDKNIVSPLSISTALAFSLGAHN#TTLTEILK NVIFSPLSISTALAFSLGAHN#TTLTEILK
77	P01009	Alpha-1-antitrypsin OS=Homo sapiens GN=SERPINA1 PE=1 SV=3 - [A1AT_HUMAN]	YLGN#ATAIFFLPDEGK YLGN#ATAIFFLPDEGKLQHLENELTHDIITK YLGN#ATAIFFLPDEGKLQHLENELTHDIITK ADTHDEILEGLNFN#LTEIPEAqIHEGFQELLR ADTHDEILEGLNFN#LTEIPEAqIHEGFQELLR qLAHQSN#STnIFFSPVSIATAFAmLSGTK
78	P01008	Antithrombin-III OS=Homo sapiens GN=SERPINC1 PE=1 SV=1 - [ANT3_HUMAN]	WVSN#KTEGR LGAcN#DTLQQLmEVFK LGAcN#DTLQQLmEVFKFDTISEK LGAcN#DTLQQLMEVFK LGAcN#DTLQQLMEVFKFDTISEK SLTFN#ETYQDISELVYGA SLTFN#ETYqDISELVYGA
79	P00748	Coagulation factor XII OS=Homo sapiens GN=F12 PE=1 SV=3 - [FA12_HUMAN]	RN#HScEPcQTLAVR
80	P00747	Plasminogen OS=Homo sapiens GN=PLG PE=1 SV=2 - [PLMN_HUMAN]	GNVAVTVSGHTcQHWSAQTPHTHN#R
81	P00738	Haptoglobin OS=Homo sapiens	NFLNHSEN#ATAK

		GN=HP PE=1 SV=1 - [HPT_HUMAN]	NFLN#HSEN#ATAK nLFLN#HSEN#ATAK VVLHPN#YSQVDIGLIK mVSHHN#LTTGATLINEQWLLTAK mVSHHN#LTTGATLInEQWLLTAK MVSHHN#LTTGATLInEqWLLTAK MVSHHN#LTTGATLINEQWLLTAK VVLHPN#YSqVDIGLIK
82	O95497	Pantetheinase OS=Homo sapiens GN=VNN1 PE=1 SV=2 - [VNN1_HUMAN]	LTGVAGN#YTVcQK
83	O95445-2	Isoform 2 of Apolipoprotein M OS=Homo sapiens GN=APOM - [APOM_HUMAN]	TEGRPDmKTELSScPGGImLN#ETGQGYQR TELFSScPGGImLN#ETGQGYQR
84	O75882-3	Isoform 3 of Attractin OS=Homo sapiens GN=ATRN - [ATRN_HUMAN]	IDSTGN#VTNELR ISN#SSDTVEcEcSENWK N#HScSEGQISIFR YN#WSFIHcPAcQcnGHSK EWLPLN#R VFHIHN#ESWVLLTPK mPSQAPTGNFYPQPLLN#SSmcLEDSR
85	O75144	ICOS ligand OS=Homo sapiens GN=ICOSLG PE=1 SV=2 - [ICOSL_HUMAN]	TVVTYHIPQN#SSLENVDSR
86	K7ER74	Protein APOC4-APOC2 OS=Homo sapiens GN=APOC4-APOC2 PE=4 SV=1 - [K7ER74_HUMAN]	mKELLETVVN#R ELLETVVN#R
87	K7EL19	Zinc finger protein 235 (Fragment) OS=Homo sapiens GN=ZNF235 PE=4 SV=1 - [K7EL19_HUMAN]	IYLN#ETQnYQR
88	I3L4B9	Sex hormone-binding globulin OS=Homo sapiens GN=SHBG PE=4 SV=1 - [I3L4B9_HUMAN]	LDVDQALN#R
89	H9KV43	Dynein heavy chain 14, axonemal (Fragment) OS=Homo sapiens GN=DNAH14 PE=4 SV=1 - [H9KV43_HUMAN]	IN#mScAVFITMnPRYGGVELPDNLK
90	H3BRJ9	Cholesteryl ester transfer protein OS=Homo sapiens GN=CETP PE=4 SV=1 - [H3BRJ9_HUMAN]	GVVVN#SSVmVK N#VSEDLPLPTFSPTLLGDSR
91	G3XAM2	Complement factor I light chain OS=Homo sapiens GN=CFI PE=3 SV=1 - [G3XAM2_HUMAN]	FLNN#GTcTAEGK LSDLSIN#STEcLHVHcR SIPAcVPWSPYLQPN#DTcIVSGWGR
92	F8WEX7	Cholinesterase OS=Homo sapiens	WSDIWN#ATK

		GN=BCHE PE=4 SV=1 - [F8WEX7_HUMAN]	
93	F8WCZ6	Complement C1s subcomponent OS=Homo sapiens GN=C1S PE=3 SV=1 - [F8WCZ6_HUMAN]	YTcEEPYYYmEnGGGGEYHcAGN#GSWVNE VLGPELPK NcGVN#cSGDVFTALIGEIASPNYPKPYPENS R NcGVN#cSGDVFTALIGEIASPnYPKPYPENS R
94	F8W1Q3	Biotinidase OS=Homo sapiens GN=BTD PE=4 SV=1 - [F8W1Q3_HUMAN]	FN#DTEVLR NPVGLIGAEN#ATGETDPSHSK nPVGLIGAEN#ATGETDPSHSK DVQIIVFPEDGIHGFN#FTR
95	F5H7E1	Uncharacterized protein OS=Homo sapiens GN=ITIH1 PE=1 SV=1 - [F5H7E1_HUMAN]	IcDLLVANNHFAHFFAPQN#LTNmNK DKIcDLLVANnHFAHFFAPQN#LTNmNK IcDLLVAnnHFAHFFAPQN#LTnmNK IcDLLVANNHFAHFFAPQN#LTnmNK DKIcDLLVANnHFAHFFAPQN#LTNmNK DKIcDLLVANnHFAHFFAPQN#LTnmNK AN#LSSQALQmSLDYGFVTPLTmSIR AN#LSSqALQMSLDYGFVTPLTmSIR
96	F5H4W9	Serum paraoxonase/arylesterase 1 OS=Homo sapiens GN=PON1 PE=4 SV=1 - [F5H4W9_HUMAN]	HAN#WTLPLK VTQVYAEN#GTVLQGSTVASVYK
97	F5GY80	Complement component C8 beta chain OS=Homo sapiens GN=C8B PE=4 SV=1 - [F5GY80_HUMAN]	EYESYSDFERN#VTEK
98	E9PIT3	Thrombin light chain OS=Homo sapiens GN=F2 PE=3 SV=1 - [E9PIT3_HUMAN]	SRYPHKPEIN#STTHPGADLQEnFcR SRYPHKPEIN#STTHPGADLQENFcR YPHKPEIN#STTHPGADLqEnFcR YPHKPEIN#STTHPGADLQENFcR YPHKPEIN#STTHPGADLQEnFcR N#FTENDLLVR
99	E9PFZ2	Ceruloplasmin OS=Homo sapiens GN=CP PE=4 SV=1 - [E9PFZ2_HUMAN]	EHEGAIYPD#TTDFQR ELHHLqEQN#VSnAFLDK ELHHLQE#VSNAFLDK ELHHLQE#VSnAFLDK ELHHLQE#VSnAFLDKGEFYIGSK EN#LTAPGSDSAVFFEQGTTR AGLQAFFQVQE#N#K EN#LTAPGSDSAVFFEqGTTR
100	E9PBC5	Plasma kallikrein heavy chain OS=Homo sapiens GN=KLKB1 PE=3 SV=1 - [E9PBC5_HUMAN]	GVNFN#VSK LQAPLN#YTEFQKPIcLPSK IYSGILN#LSDITK IYPGVDFGGEELN#VTFVK

			IVGGTN#SSWGEWPWQVSLQVK
101	E7ETN3	Uncharacterized protein OS=Homo sapiens PE=3 SV=1 - [E7ETN3_HUMAN]	LTDTlcGVGN#mSAnASDQER TmFPN#LTDVR AlqAVYSmmSWPDDVPPEGWN#R SPYYN#VSDEISFHcYDGYTLR KIVLDPSGSmNIYLVLGSDSIGASN#FTGAK KIVLDPSGSmNIYLVLGSDSIGASN#FTGAK IVLDPSGSmNIYLVLGSDSIGASN#FTGAK
102	E7ESS4	Intercellular adhesion molecule 1 OS=Homo sapiens GN=ICAM1 PE=1 SV=1 - [E7ESS4_HUMAN]	LNPTVTYGN#DSFSAK
103	E7EQ48	Proteoglycan 4 OS=Homo sapiens GN=PRG4 PE=1 SV=1 - [E7EQ48_HUMAN]	N#GTLVAFR
104	E7EPG1	Multimerin-1 OS=Homo sapiens GN=MMRN1 PE=4 SV=1 - [E7EPG1_HUMAN]	VN#ESVVSIAAQKQ
105	D6RIS9	Selenoprotein P (Fragment) OS=Homo sapiens GN=SEPP1 PE=4 SV=1 - [D6RIS9_HUMAN]	EGYSN#ISYIVVNHQGISSR
106	D6RD17	Immunoglobulin J chain (Fragment) OS=Homo sapiens GN=IGJ PE=1 SV=2 - [D6RD17_HUMAN]	EN#ISDPTSPLR IIVPLNNREN#ISDPTSPLR
107	C9JX71	Apolipoprotein D (Fragment) OS=Homo sapiens GN=APOD PE=4 SV=1 - [C9JX71_HUMAN]	cIQAN#YSLmENGK cIQAN#YSLmEnGK ADGTVNqIEGEATPVN#LTEPAK ADGTVNQIEGEATPVN#LTEPAK ADGTVnQIEGEATPVN#LTEPAKLEVK ADGTVnQIEGEATPVN#LTEPAKLEVK
108	C9JMA2	Mannan-binding lectin serine protease 1 (Fragment) OS=Homo sapiens GN=MASP1 PE=4 SV=2 - [C9JMA2_HUMAN]	FGYILHTDN#R
109	C9JEV0	Zinc-alpha-2-glycoprotein OS=Homo sapiens GN=AZGP1 PE=3 SV=1 - [C9JEV0_HUMAN]	FGcEIENN#R DIVEYYNDSN#GSHVLQGR DIVEYYN#DSN#GSHVLQGR AREDIFmETLKDIIVEYYN#DSNGSHVLQGR EDIFmETLKDIIVEYYNDSN#GSHVLQGR
110	C9JCF5	Kaliocin-1 (Fragment) OS=Homo sapiens GN=LTF PE=1 SV=1 - [C9JCF5_HUMAN]	TAGWNVPIGTLRPFLN#WTGPPEPIAAVAR
111	B7Z3Y2	Prenylcysteine oxidase 1 OS=Homo sapiens GN=PCYOX1 PE=1 SV=1 - [B7Z3Y2_HUMAN]	GELN#TSIFSSRPIDK LLHALGGDDFLGmLN#R

112	B7Z3A7	Calcium/calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase 1A OS=Homo sapiens GN=PDE1A PE=2 SV=1 - [B7Z3A7_HUMAN]	LmqEEEMnILIN#LSKDDWRDLR
113	B4E1H2	Plasma protease C1 inhibitor OS=Homo sapiens GN=SERPING1 PE=2 SV=1 - [B4E1H2_HUMAN]	DTFVN#ASR VLSN#NSDANLELINTWVAK VGQLQLSHN#LSLVILVPQNLK
114	B4DWL3	Lysosome-associated membrane glycoprotein 1 OS=Homo sapiens GN=LAMP1 PE=1 SV=1 - [B4DWL3_HUMAN]	GHTLTLN#FTR
115	B3WK7	Insulin-like growth factor binding protein 3, isoform CRA_b OS=Homo sapiens GN=IGFBP3 PE=2 SV=1 - [B3WK7_HUMAN]	GLcVN#ASAVSR AYLLPAPPAPGN#ASESEEDR
116	P80108	Neutrophil gelatinase-associated lipocalin OS=Homo sapiens GN=LCN2 PE=1 SV=2 - [NGAL_HUMAN]	LGTSLSSGHVLmN#GTLK N#LTTSLTESVDR LNVEAAN#WTVR FHDVSESTHWTPFLN#ASVHYIR