Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supplementary Information

Triazine-structured covalent organic framework nanosheets with inherent hydrophilicity for highly efficient and selective enrichment of glycosylated peptides

Shi-Shu Yang,*[a] Yu-Heng Jiang,[a] Xuan Zhang,[a] Li-Hong Liu,[a] Si Liu,[b] and Hua Zhang[a]

*To whom correspondence should be addressed. E-mail:

yangshishu@htu.edu.cn

Materials and characterization.

Sodium cyanoborohydride ($NaBH_3CN$) and α-cyano-4-hydroxycinnamic acid (CHCA) were obtained from TCI (Shanghai, China). Melamine ($C_3H_6N_6$), dimethyl sulfoxide (DMSO), acetonitrile (ACN), cyanuric chloride ($C_3Cl_3N_3$), ammonium bicarbonate (NH_4HCO_3), sodium acetate (CH_3COONa), formaldehyde solution (CH_2O), deuterium formaldehyde (CD_2O) and commercial TiO_2 nanoparticles were purchased from Aladdin Chemistry Co., Ltd. Trifluoroacetic (TFA), trypsin (TPCK-treated), bovine serum albumin (BSA, from bovine milk) and horseradish peroxidase (HRP) were obtained form Sigma-Aldrich (St. Louis, MO). All chemicals used were of analytical grade.

Powder X-ray diffraction (XRD) were conducted at a scan rate of 1^o min^{-1} over a range of 2-10^o (2θ) on a Rigaku/SmartLab SE with Cu-Kα source (λ=1.5418 Å). Scanning electron microscopy (SEM) measurement was performed on a FEI Nova NaoSEM 450. Transmission electron microscopy (TEM) images and the highangle annular dark-field scanning TEM (HAADF-STEM) were obtained by the Thermo Scienific Talos F200i with an operating voltage of 200 kV. Atomic force microscopy (AFM) measurement was performed on a Bruker Dimension Icon. A MicrotracBEL BELSORP MINI X was used to analysis the N_2 adsorption/desorption isotherms at 77 K. The contact angles were measured by a contact angle analyzer (Dataphysics-OCA20). Thermogravimetric analysis (TGA) was carried out on a TA SDT Q600. The zeta potential was measured by Malvern-Zetasizer Nano S90. The X-ray photoelectron spectra (XPS) was analyzed by a Thermo Scienific ESCALAB 250Xi.

Sample preparation.

Proteins (HRP or BSA) were dissolved in NH_4HCO_3 solution (50 mM, pH ~ 10) with a final concentration of 1 μg μL^{-1} for HRP before the solution was heated at 100 ^oC and 10 μg μL^{-1} for BSA. Then the mixed solution were followed by digestion with trypsin with a concentration ratio of 50:1 (protein versus trypsin) respectively. Thereafter, they were incubated in a 37 ^oC water bath for 20 h. Finally, all the samples were lyophilized and stored at −20 ^oC. Human serum that used in the complex samples analysis were obained from patients with gastric cancer from Tongji Medical College

of Huazhong University of Science and Technology before pretreatment. Also, we were obtained the approval of patients and the ethical constent from the local committee.

In situ isotope dimethyl labeling of glycopeptides[S1].

In situ isotope labeling was performed after glycopeptides capturing and washing steps and before the elution step. At that time, the NENP-1 nanosheets with adsorbed glycopeptides were dispersed into a 200 μL CH_3COONa buffer solution (100 mM, pH ~ 5.8) and added with 8 μL of CD_2O or CH_2O and 8 μL of 600 mM $NaBH_3CN$. After being vortexed for 40 min, 10 μL of 88% formic acid (FA) was added to terminate the labeling reaction. Rinsing with 200 μL of washing buffer (95% ACN/4.9% H_2O/0.1% TFA, v/v/v) was performed to remove the excess labeling reagents. Finally, 10 μL elution buffer (30% ACN/69.9% H_2O/0.1% TFA, v/v/v) was added to the NENP-1 nanosheets and vibrated rapidly for 10 min to elute the adsorbed hydrophilic peptides. The eluent was analyzed by MALDI-TOF MS.

Glycopeptides enrichment by NENP-1 nanosheets from human serum samples.

First, 1 mg NENP-1 nanosheets were placed in a centrifuge tube and 100 μL of enrichment solution (95% ACN/4.9% H_2O/0.1% TFA, v/v/v) was added into the centrifuge tube. Then, add 3 μL of the human serum samples (serum samples were obtained from patients with gastric cancer) to the centrifuge tube and vortex the mixture at room temperature for 30 minutes. After vortex, The NENP-1 nanosheets were then separated by centrifugation and washed three times with loading buffer. Ten microliters elution buffer (30% ACN/69.9% H_2O/0.1% TFA, v/v/v) was added to the NENP-1 nanosheets and vibrated rapidly for 10 min to elute the adsorbed hydrophilic peptides. The eluent was analyzed by RPLC-ESI-MS/MS.

MS detection.

Mass spectrometry analysis was performed using Bruker's ion mobility-quadrupole-time-of-flight mass spectrometer, the timsTOF Pro. Samples were injected and separated using an UltiMate 3000 RSLCnano liquid chromatograph coupled to a mass spectrometer online. Peptide samples were aspirated by an autosampler, bound to a Trap column (75 μm × 20 mm, 2 μm particle size, 100 Å pore size, thermo), and then eluted to an analytical column (75 μm × 250 mm, 1.6 μm particle size, 100 Å pore size,

ionopticks) for separation. Two mobile phases (mobile phase A: 0.1% formic acid in H_2O and mobile phase B: 0.1% formic acid in ACN) were used to establish the analytical gradient. The flow rate of the liquid phase was set at 300 nL/min. The peptides were entered into the mass spectrometry by the CaptiveSpray nanoliter ion source for DDA scanning, with the TIMS function turned on, and the PASEF scanning mode was used. Each scan cycle time is 1.1 seconds and consists of one MS1 scan and 10 PASEF MS/MS scans, each containing 12 MS/MS spectra.

Database searching.

Mass spectrometry data were retrieved using MaxQuant (2.0.1) software using Andromeda database search algorithm. The database used for the search was the proteomic reference database of Human in Uniprot. The main search parameters were as follows: non-standard quantitative process was used for analysis, Oxidation (M), Deamidation (N), Acetyl (Protein N-term) were selected by variable modification, Carbamidomethyl (C) was selected by fixed modification, and Trypsin/P was selected by enzyme digestion. Results were screened at protein and peptide levels, 1% FDR, and used for follow-up analysis. In the process of functional annotation and enrichment analysis, based on the principle that the functions of proteins with the same or similar sequences are similar, the annotation information of GO, KEGG, COG and other databases of the submitted proteins is obtained through the Diamond program of Egg-NOG software, and the annotation information of all proteins identified by mass spectrometry is obtained, the relevant information of the differential proteins is extracted, the categories and numbers are counted, and the significant functional categories related to the experiment can be screened out by hypergeometric testing for functional enrichment analysis.

References

(S1) Y. Zhao, L. Zhang, Z. Chu, Z. Xiong, W. Zhang, *Anal. Methods*, 2017, **9**, 443.

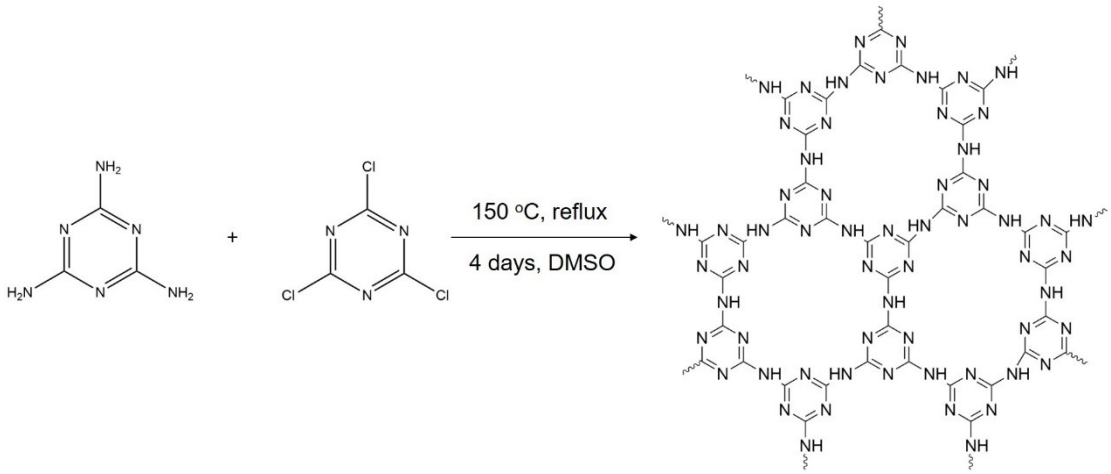
(S2) N. Sun, J. Wang, J. Yao, C.-H. Deng, *Anal. Chem.*, 2017, **89**, 1764.

(S3) J. Wang, J. Li, M. Gao, X. Zhang, *Nanoscale*, 2017, **9**, 10750.

(S4) Y. Wu, N. Sun, C.-H. Deng, *ACS Appl. Mater. Interfaces*, 2020, **12**, 9814.

(S5) Y. Ji, Y. He, R. Chen, C. Zhong, H. Li, Y. Wu, Z. Lin, *J. Mater. Chem. B*, 2022, **10**, 6507.

(S6) C. Zhang, X.-D. Jin, L.-P. Wang, C.-Z. Jin, X.-Q. Han, W.-G. Ma, X.-G. Li, G.-X. Teng, *ACS Appl. Mater. Interfaces*, 2021, **13**, 9714.



Scheme S1. Synthsis route and chemical structure of the NENP-1 nanosheets.

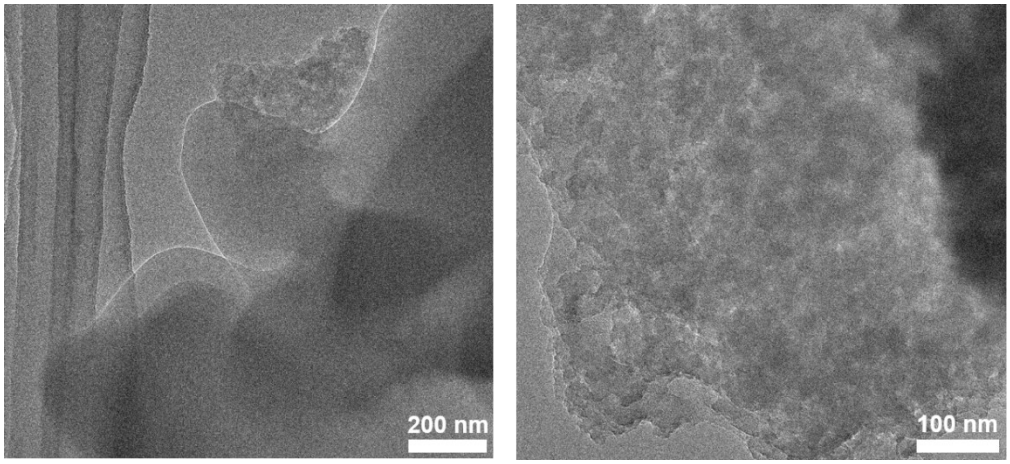


Figure S1. TEM images of NENP-1 nanosheets.

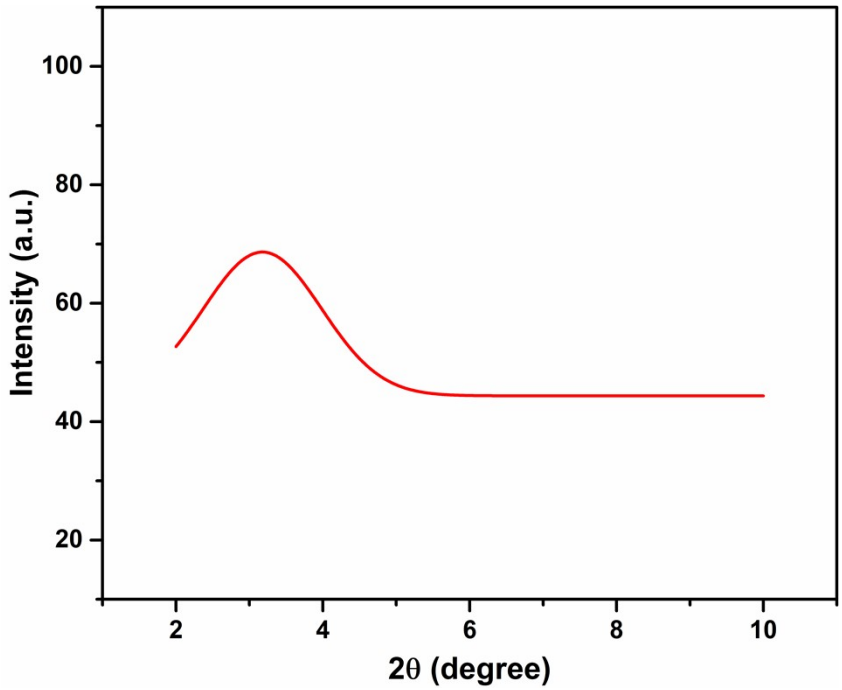


Figure S2. Gaussian curve fitting diffraction peak of NENP-1 nanosheets.

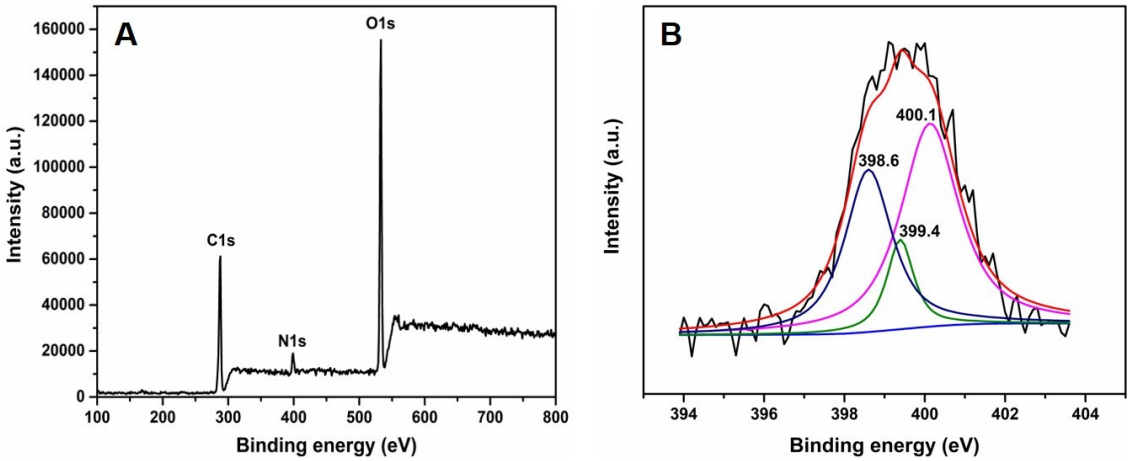


Figure S3. (A) XPS analysis, and (B) High-resolution N 1s XPS spectrum.

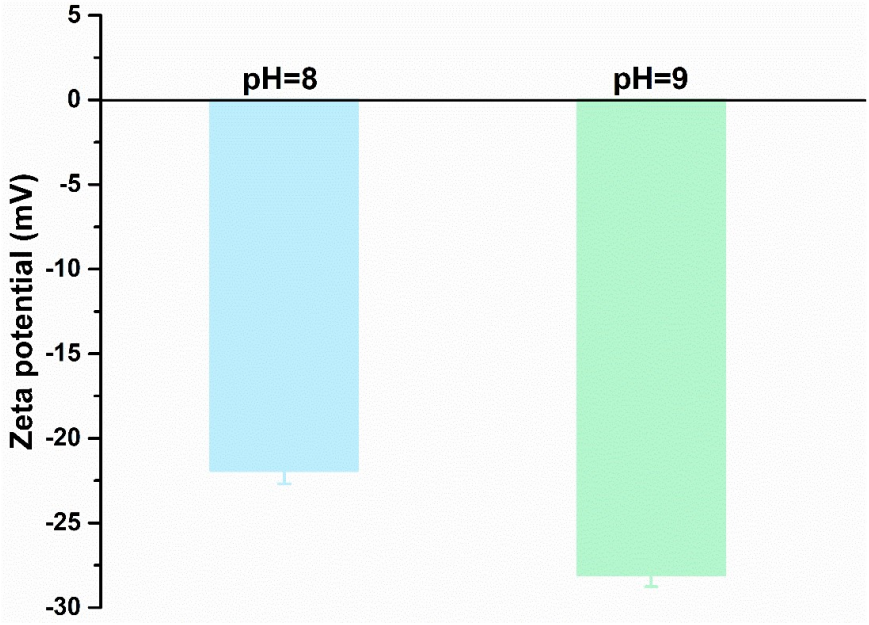


Figure S4. Zeta potential of NENP-1 nanosheets in pH=8 and pH=9.

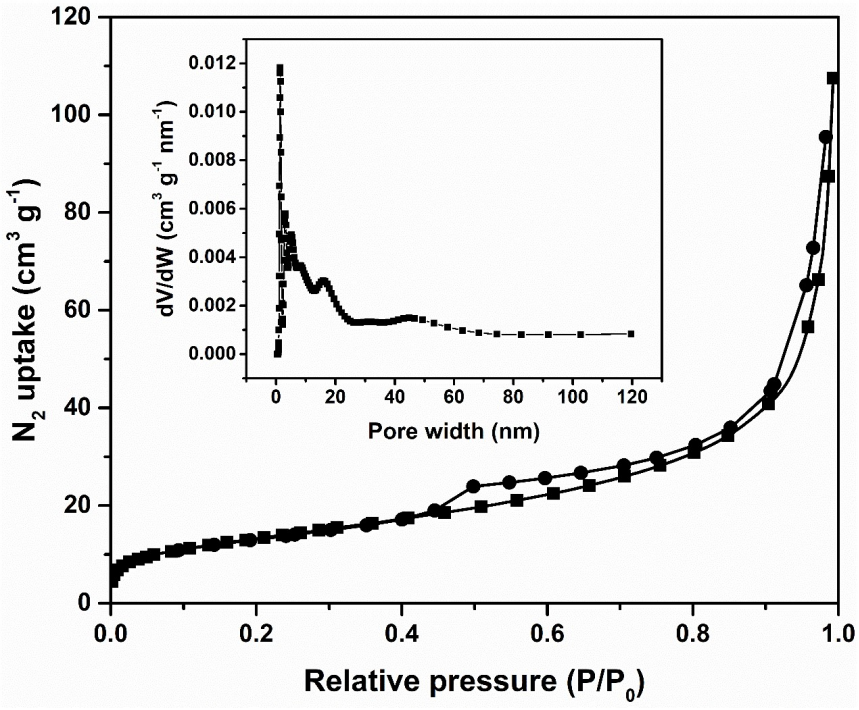


Figure S5. Nitrogen adsorption/ desorption isotherm, (insert) pore-size distribution profile of NENP-1 nanosheets.

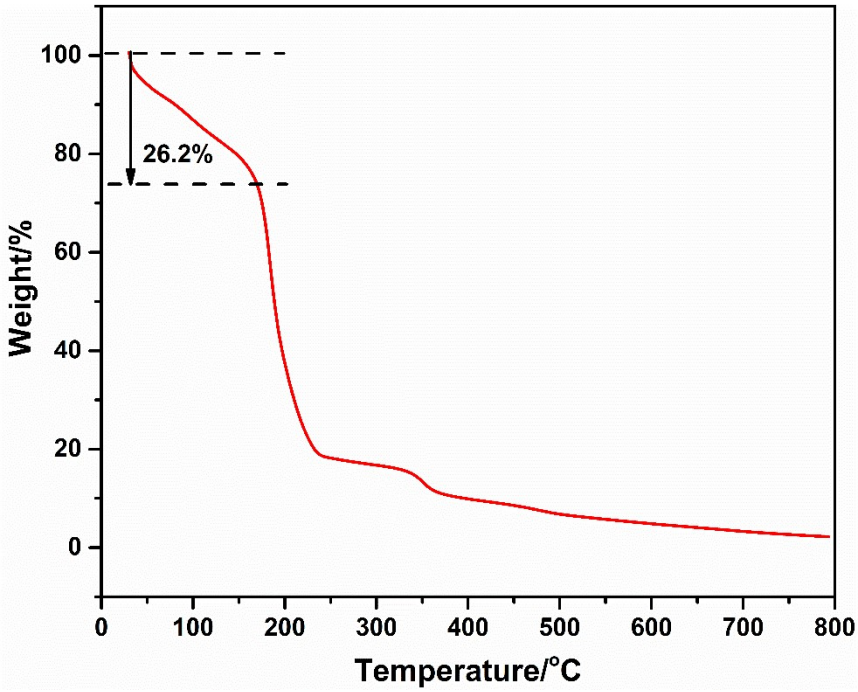


Figure S6. Thermogravimetric analysis of NENP-1 nanosheets.

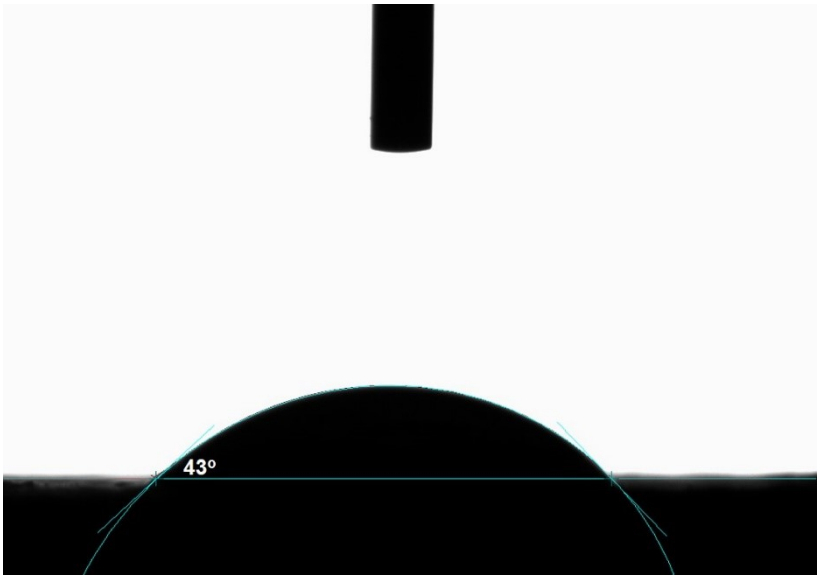


Figure S7. The wetting behavior of a water droplet on NENP-1 nanosheets.

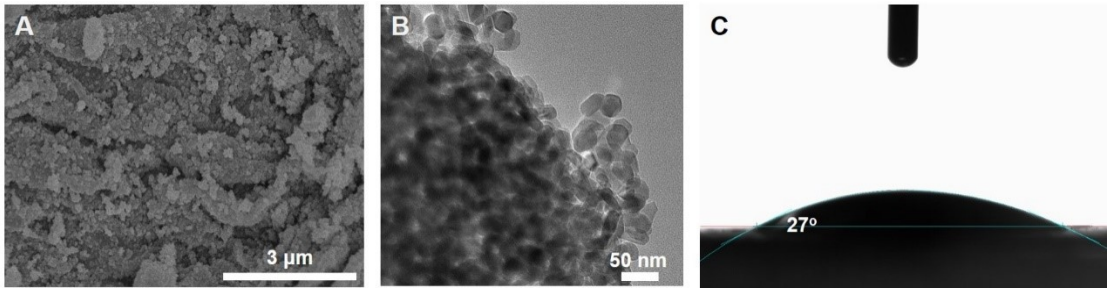


Figure S8. (A) SEM image, (B) TEM image, (C) the wetting behavior of a water droplet of the commercial TiO_2 nanoparticles.

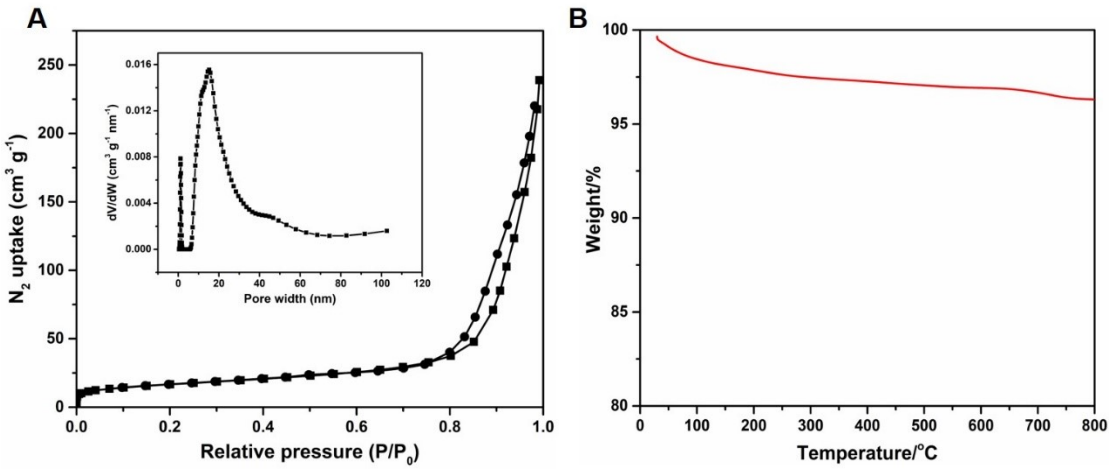


Figure S9. (A) Nitrogen adsorption/ desorption isotherm, (insert) pore-size distribution profile and (B) thermogravimetric analysis of the commercial TiO_2 nanoparticles.

Table S1. The detailed information of glycopeptides identified from HRP tryptic digest by NENP-1 nanosheets. N# denotes the N-linked glycosylation site.

NO.	Glycan composition	Peptide sequence	Observed m/z
1	XylMan3FucGlcNAc2	PnVSNIVR	2105.2
2	XylMan2FucGlcNAc2	SILLDN#TTSFR	2299.6
3	FucGlcNAc	SFAnSTQTFFNAFVEAMDR	2532
4	XylMan3FucGlcNAc2	SSPnATDTIPLVR	2551.7
5	XylMan3GlcNAc2	MGnITPLTGTQGQIR	2609
6	FucGlcNAc	GLIQSDQELFSSPnATDTIPLVR	2862.7
7	XylMan2GlcNAc2	SFAnSTQTFFNAFVEAMDR	3057.6
8	FucGlcNAc	LHFHDCFVNGCDASILLDnTTSFR	3083.3
9	XylMan3FucGlcNAc2	GLCPLNGnLSALVDFDLR	3097.4
10	XylMan3GlcNAc2	SFAnSTQTFFNAFVEAMDR	3212.1
11	Man3FucGlcNAc2	SFAnSTQTFFNAFVEAMDR	3235.3
12	XylMan3FucGlcNAc2	QLTPTFYDNSCPnVSNIVR	3326.7
13	XylMan3FucGlcNAc2	SFAnSTQTFFNAFVEAMDR	3358.6
14	XylMan3FucGlcNAc2	SFAnSTQTFFNAFVEAMDR	3374.1
15	[Hex]2[HexNAc]2[Fuc]1	GLIQSDQELFSSPN#ATDTIPLVR	3386.4
16	[Hex]2[HexNAc]2[Fuc]1[Xyl]1	GLIQSDQELFSSPN#ATDTIPLVR	3511.9
17	XylMan3GlcNAc2	GLIQSDQELFSSPnATDTIPLVR	3527.8
18	Man3FucGlcNAc2	GLIQSDQELFSSPnATDTIPLVR	3541.3
19	XylMan3FucGlcNAc2	NQCRGLCPLNGnLSALVDFDLR	3607.3
20	XylMan3FucGlcNAc2	GLIQSDQELFSSPnATDTIPLVR	3672.9
21	Man2GlcNAc2	LHFHDCFVNGCDASILLDnTTSFRTEK	3822.5
22	XylMan3FucGlcNAc2	LHFHDCFVNGCDASILLDnTTSFR	3893.6
23	[Hex]4[HexNAc]2[Fuc]1[Xyl]1	QLTPTFYDNSC(AAVESACPR)PN#VSNIVR	4055.2
24	XylMan3FucGlcNAc2	QLTPTFYDNSC(AAVESACPR)PnVSNIVR	4218.1
25	Man3FucGlcNAc2,	LYN#FSNTGLPDPTLN#TTYLQTLR	4713.2
26	XylMan2FucGlcNAc2, XylMan2GlcNAc2	LYnFSNTGLPDPTLnTTYLQTLR	4813.6
27	XylMan3FucGlcNAc2, XylMan3GlcNAc2	LYnFSNTGLPDPTLnTTYLQTLR	4829.5
28	Man3FucGlcNAc2, XylMan3FucGlcNAc2	LYnFSNTGLPDPTLnTTYLQTLR	4843.4
29	XylMan3FucGlcNAc2, XylMan3FucGlcNAc2	LYnFSNTGLPDPTLnTTYLQTLR	4975.5

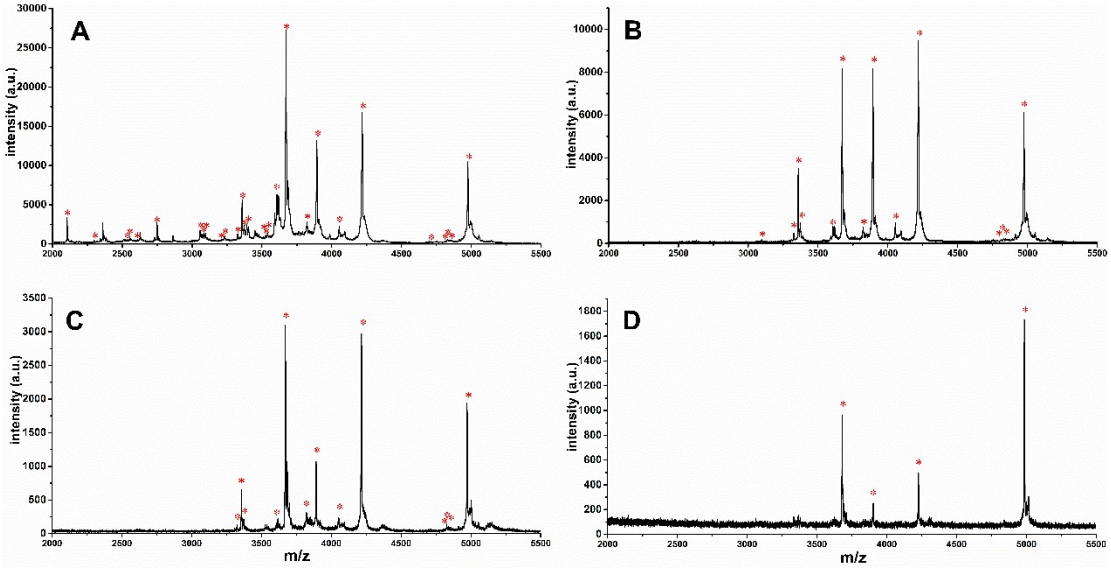


Figure S10. Optimization of the concentration of ACN in loading buffer: (A) 95% ACN, (B) 90% ACN, (C) 85% ACN, and (D) 80% ACN.

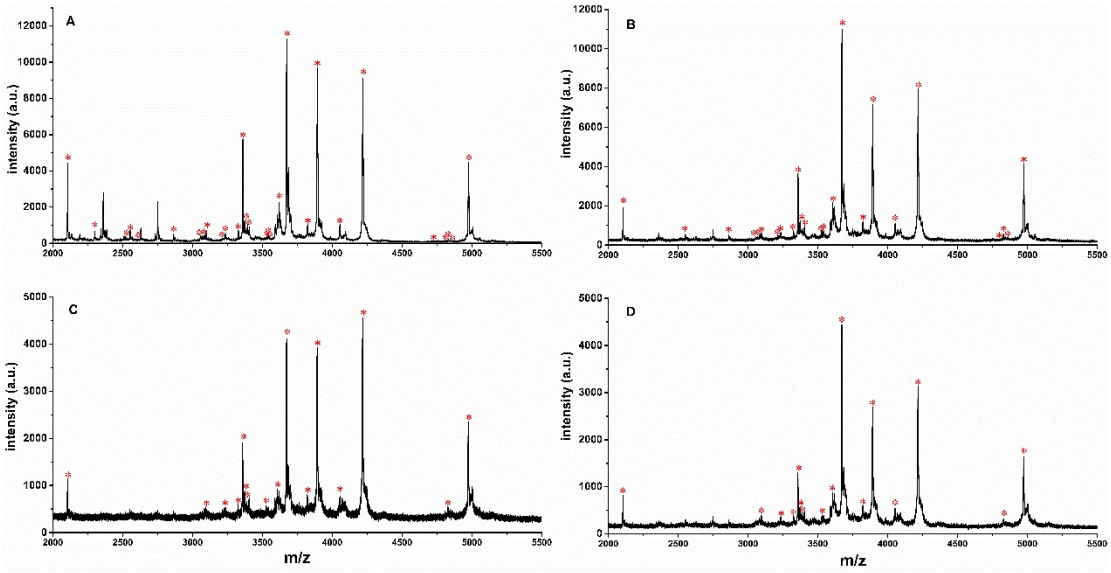


Figure S11. Optimization of the concentration TFA in elution buffer: (A) 0.1% TFA, (B) 0.5% TFA, (C) 1% TFA, and (D) 1.5% TFA.

Table S2. Comparison of the enrichment efficiency of the NENP-1 nanosheets towards glycopeptides with those of hydrophilic materials reported before.

Materials	Number of detected glycopeptides	Concentrations of HRP tryptic digest (pmol/μL)	Ref.
Fe_3O_4@mSiO_2-IDA	23	1	(S2)
MagG@COF-5	16	1	(S3)
mCTpBD	26	0.1	(S4)
HFH-COFs@Au@GSH	25	4	(S5)
$MnFe_2O_4$@C@APBA	23	0.1	(S6)
NENP-1 nanosheets	29	2.5	This work

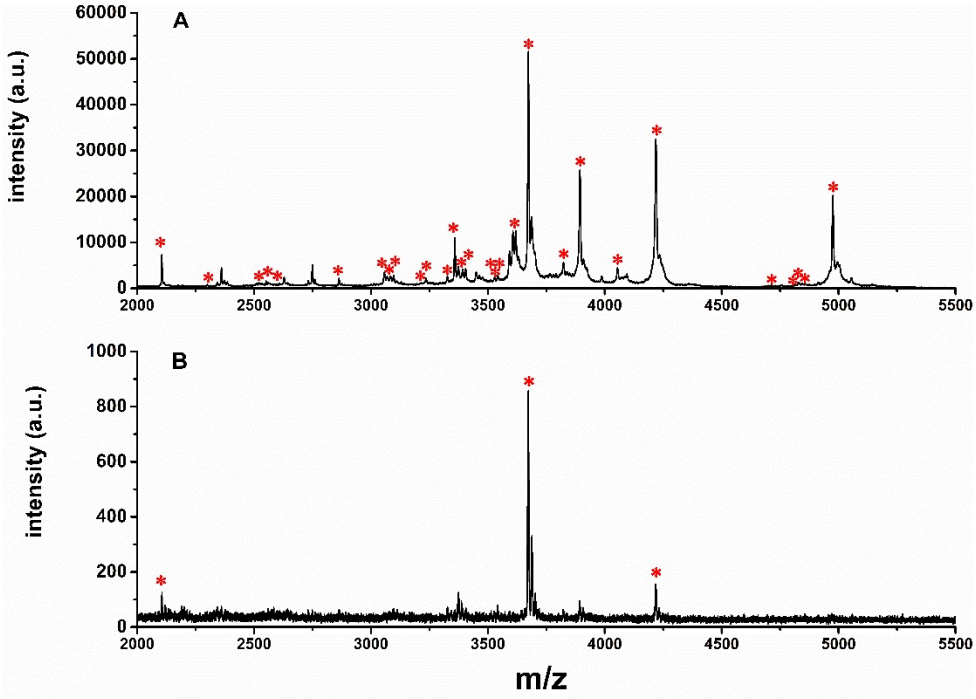


Figure S12. MALDI-TOF mass spectra of 2.5×10^{-6} M digest of HRP glycoprotein after enrichment by (A) NENP-1 nanosheets and (B) TiO_2 nanoparticles. Glycopeptide peaks are marked with "*". The detected glycopeptides with m/z value and amino acid sequences are listed in Table S1.

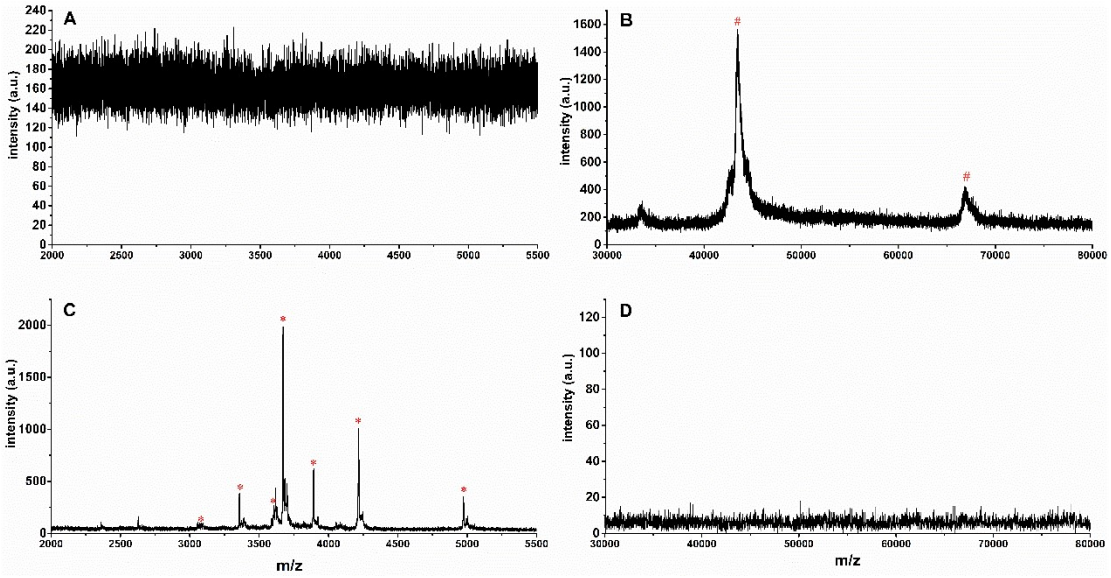


Figure S13. MALDI-TOF mass spectra of glycopeptides enriched by NENP-1 nanosheets enriched from the mixture of HRP digests and nonglycoprotein BSA at the mass ratio of 1:200. Before enrichment (A) in reflection positive mode and (B) in high linear mode; elute after enrichment by NENP-1 nanosheets (C) in reflection positive mode and (D) in high linear mode. BSA peaks are marked with "#". Glycopeptide peaks are marked with "∗". The detected glycopeptides with m/z value and amino acid sequences are listed in Table S1.

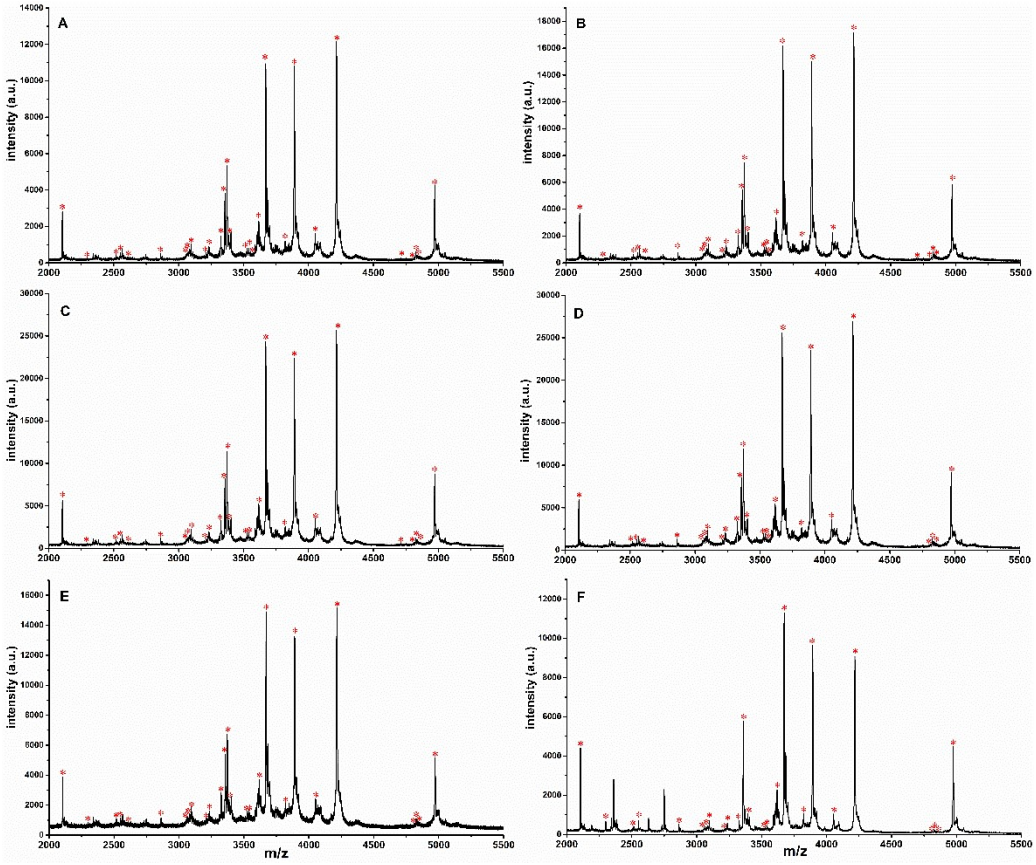


Figure S14. The cycling performance of the NENP-1 nanosheets: (A) the first time, (B) the second time, (C) the third time, (D) the fourth time, (E) the fifth time and (F) after enrichment with the NENP-1 nanosheets placed for two weeks. Glycopeptide peaks are marked with "∗". The detected glycopeptides with m/z value and amino acid sequences are listed in Table S1.

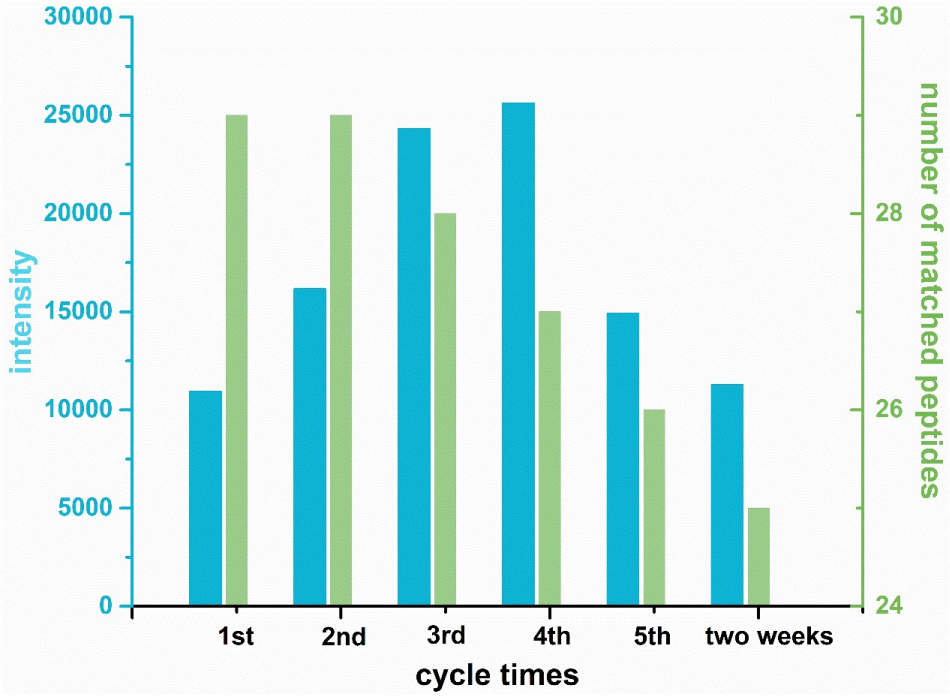


Figure S15. Cycling performance of NENP-1 nanosheets for glycopeptides enrichment.

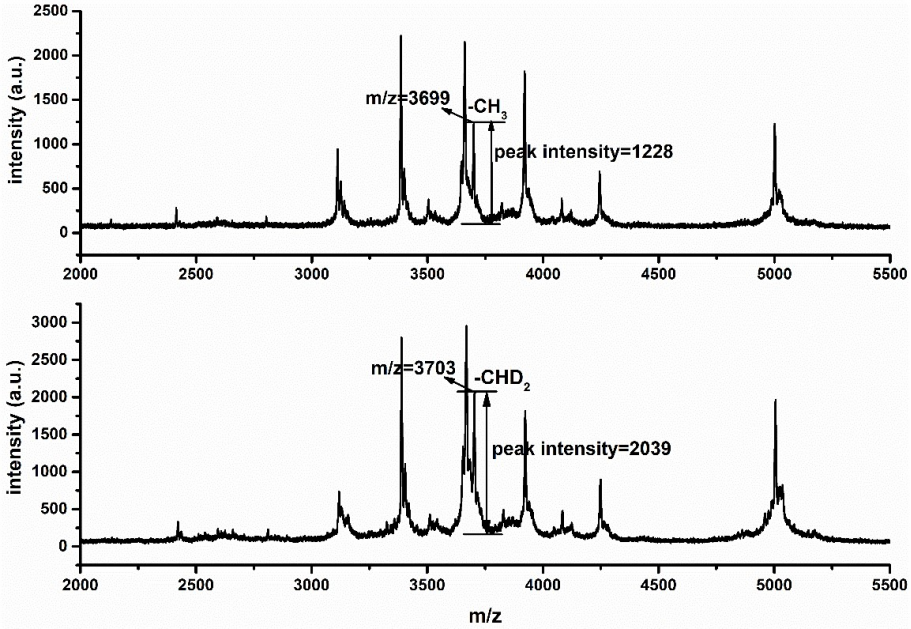
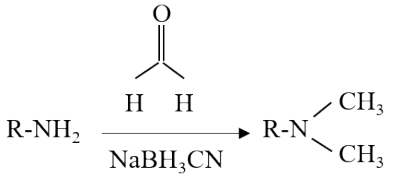
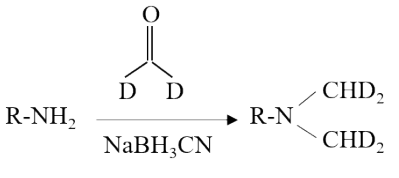


Figure S16. The experiment of the recovery of the light- and heavy dimethyl labeling deglycosylated peptides from HRP tryptic digest and the labeling reactions are as follows, recovery was calculated from the measurements of 1:1 mixture of the H-labeled and D-labeled of the m/z = 3672 peaks(S1).



+28Da, light-labeled

,



+32Da, heavy-labeled

,

Table S3. Identified N-glycopeptides containing deamidated Asn by 3 μL human serum digest. N# denotes the N-linked glycosylation site.

NO.	Peptide Sequence	Protein Group Accessions
1	AAQRIRAANSNGLPR	Q9P0V3
2	AVPPNNSNAAEDDLPTVELQGVVPR	P00488
3	AYLLPAPPAPGNASESEEDRSAGSVESPSVSSTHR	P17936
4	EEIPVNDGIELLQMVLNFDTK	Q9HAV4
5	GNTEGLQKSLAELGGHLDQQVEEFR	P06727
6	GNTEGLQKSLAELGGHLDQQVEEFRR	P06727
7	HGIQYFNNNTQHSSLFMLNEVK	P01042
8	HGIQYFNNNTQHSSLFMLNEVKR	P01042
9	HVDAHATLNDGVVVQVMGLLSNNNQALR	Q13283
10	KIVLDPSGSMNIYLVLDGSDSIGASNFTGAKK	P00751
11	LLHGDPGEEDGAELDLNMTR	P01127
12	LNPEAVR	Q63HM2
13	MKELLETVVNRTR	P55056
14	MLTHLSLAENALKDEGAK	Q86W28
15	NLQNNAEWVYQGAIR	P04114
16	NPNVQQALR	P15538
17	NVHSGSTFFK	Q14624
18	NVHSGSTFFKYYLQGAK	Q14624
19	PFLAPGRQSVDNVTNPEK	Q9UPN6
20	PTNVKENTIK	Q9BX40
21	RAVPPNNSNAAEDDLPTVELQGVVPR	P00488
22	SGVQQLIQYYQDQKDANISQPETTKEGLR	Q13790
23	VSFLSALEEYTKKLNTQ	P02647
24	YVKALAEENKNTVDVENGASMAGYADLK	Q96EE4