Electronic Supplementary Information

An efficient strategy with synergistic effect of hydrophilic and electrostatic interactions for simultaneous enrichment of N- and O-glycopeptides

Zhonghan Hu[a,b], Wenqing Gao[a,b,*], Rong Liu[a,b], Jiaqian Yang[a,b], Renlu Han[a,b], Junhui Li[a,b], Jiancheng Yu[a,b,c], Danhua Ma[d*], Keqi Tang [a,b,*]

[a] *Institute of Mass Spectrometry, Zhejiang Engineering Research Center of Advanced Mass spectrometry and Clinical Application, School of Material Science and Chemical Engineering, Ningbo University, Ningbo, 315211, PR China*
[b] *Zhenhai Institute of Mass Spectrometry, Ningbo, 315211, PR China*
[c] *Faculty of Electrical Engineering and Computer Science, Ningbo University, Ningbo, 315211, PR China*
[d] *Department of Stomatology, Ningbo No.2 Hospital, Ningbo, 315010, PR China*

**Corresponding author(s)*
E-mail(s): gaowenqing@nbu.edu.cn; mdh7639@163.com; tangkeqi@nbu.edu.cn

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S1 Characterization of PDA-ADE@KCP.

The particle properties of PDA-ADE@KCP were assessed through various techniques. The morphology was inspected with scanning electron microscopy (Zeiss, Germany), while the elemental composition was determined using EDX X-Max (Oxford Instrument, UK). The surface functional groups were identified using Nicolet Fourier Transformed Infrared (FTIR) spectroscopy (Thermo Scientific, USA), and the absorbance was scanned from 450 to 4000 cm−1. Zeta potential (ζ) measurements were taken with Malvern Zetasizer Nano ZS90 (Worcestershire, UK), particles were dispersed in water, and the data was analyzed with Zetasizer Software 7.01. A K-Alpha X-ray photoelectron spectroscopy (XPS) spectrometer (Thermo Scientific, USA) was used to detect the chemical composition of samples at 2×10^{-7} mbar pressure. Additionally, thermogravimetric (TG) analysis was performed using a TG 209 F3 Tarsus thermogravimetric analyzer (Netzsch, Germany) under a nitrogen atmosphere, and water contact angles were measured with a DCAT21 Contact Angle Analyzer (Jin Mao, China).

S2 Examination of PDA-ADE@KCP glycopeptide enrichment selectivity and sensitivity.

The selectivity of PDA-ADE@KCP was assessed by enriching IgG/BSA mixtures containing 10 μg IgG and BSA ratios of 1:10, 1:100, 1:1000 or 1:5000 with 500 μg of PDA-ADE@KCP in 90% ACN and 1% TFA loading buffer. The mixture was incubated with shaking for 30 minutes at 37°C, then centrifuged. The resulting pellets were washed thrice with loading buffer before being eluted with 20 μL elution buffer (20%, 0.1% TFA) with shaking for 30 minutes at 37°C. The glycopeptide eluates were then analyzed using MALDI-TOF MS. To determine the sensitivity of PDA-ADE@KCP, IgG digest concentrations of 100 f mol/μL, 10 f mol/μL, 1 f mol/μL, and 0.1 f mol/μL were enriched using 500 μg of PDA-ADE@KCP and analyzed via MALDI-TOF MS._The selectivity and sensitivity of PDA-ADE@KCP were assessed by comparing the number and relative intensity of glycopeptide peaks.

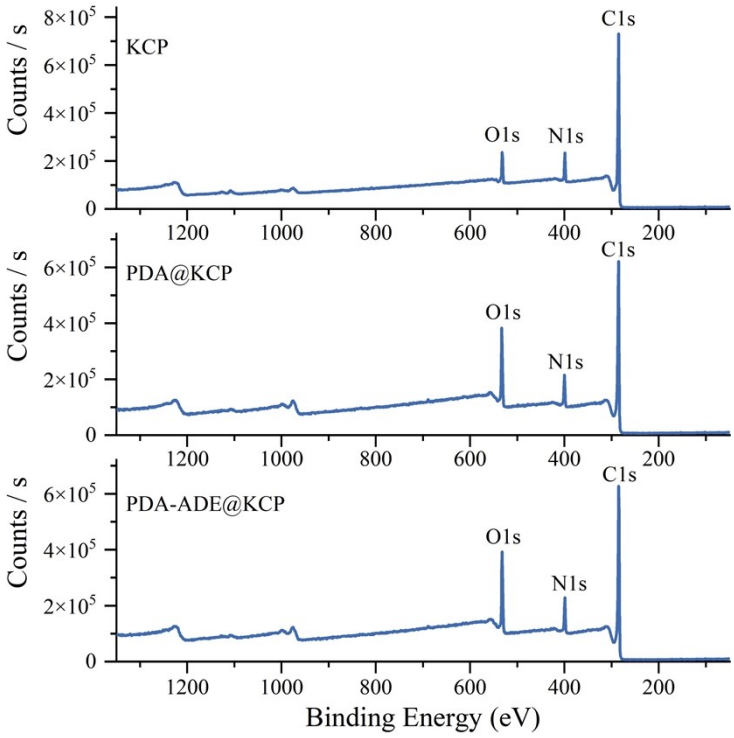

Fig. S1. XPS wide-scan spectra of KCP, PDA@KCP and PDA-ADE@KCP.

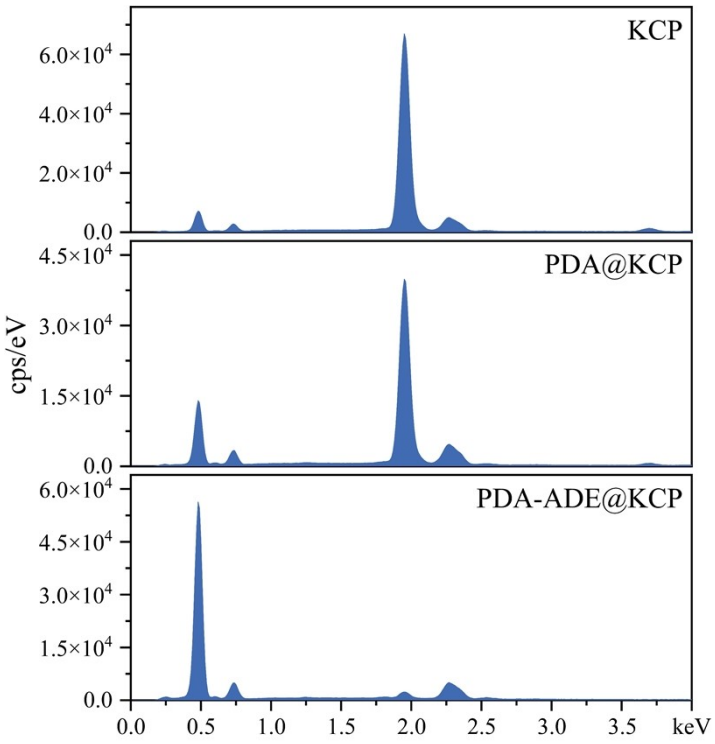

Fig. S2. EDS spectra of KCP, PDA@KCP and PDA-ADE@KCP.

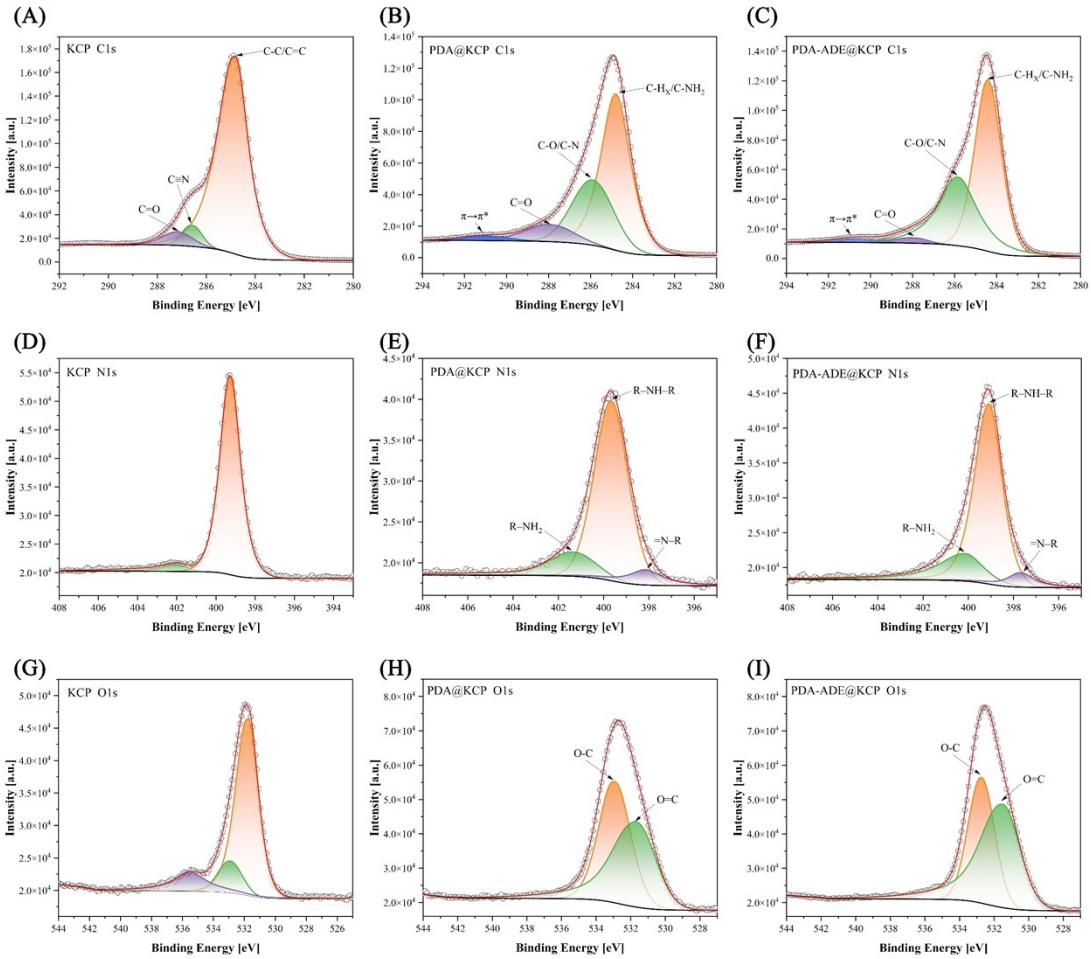

Fig. S3. The narrow-scan XPS spectra of KCP, PDA@KCP and PDA-ADE@KCP.

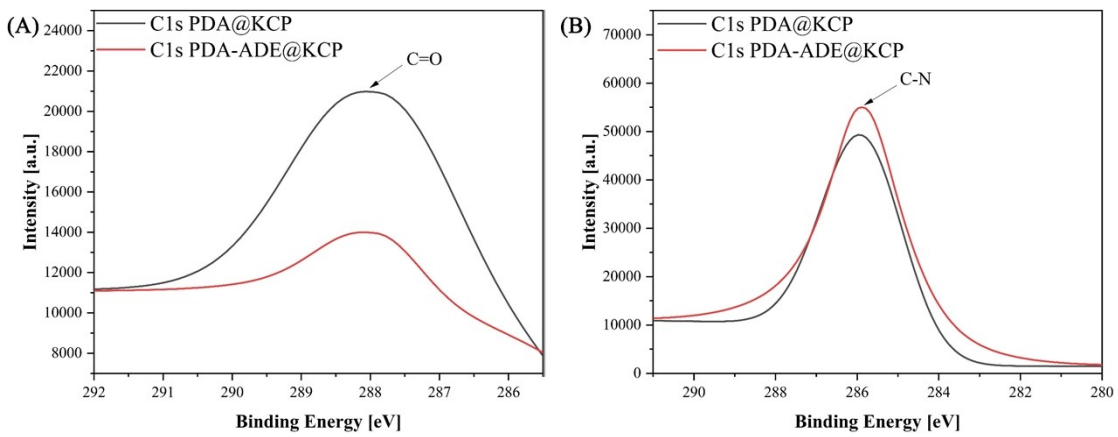

Fig. S4. Comparison of the peak intensities associated with (A) C=O and (B) C-N in the C1s spectra.

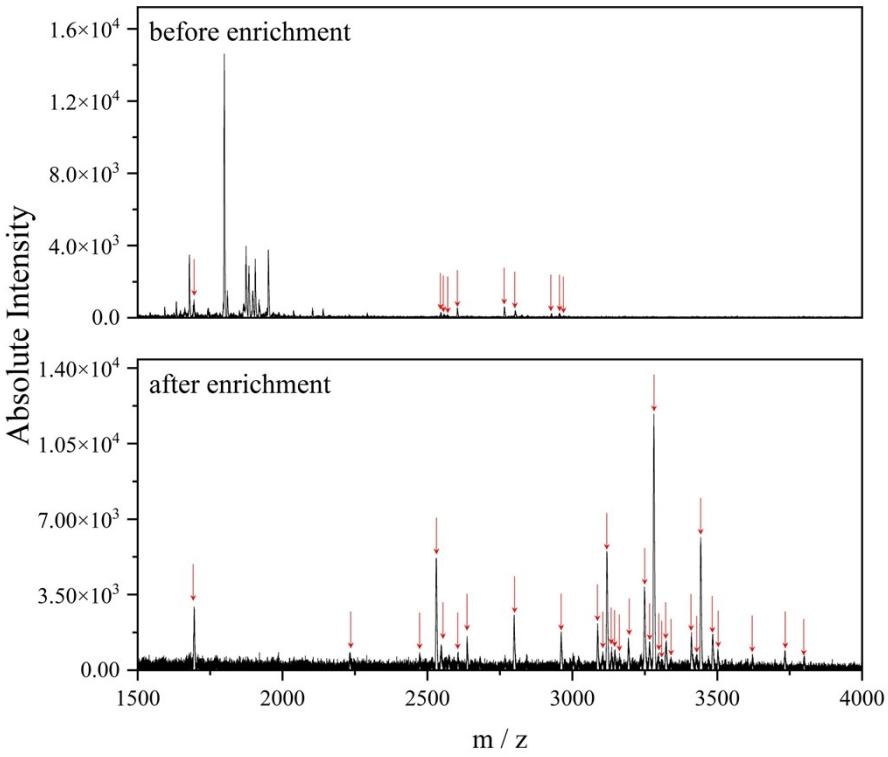

Fig. S5. PDA-ADE@KCP glycopeptide enrichment performance with standard glycoproteins. MALDI-TOF MS spectra of IgG tryptic digests. (A) before enrichment, (B) after enrichment.

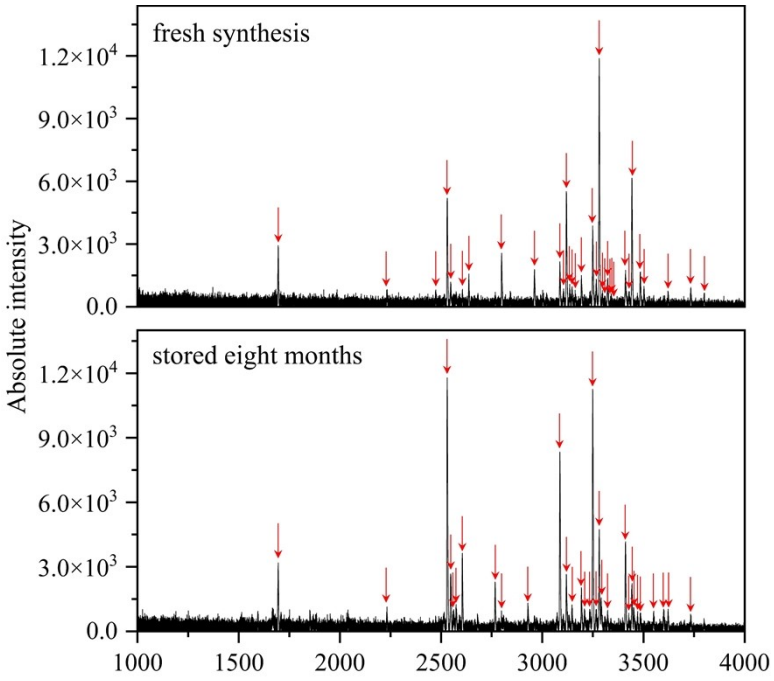

Fig. S6. PDA-ADE@KCP storage stability. MALDI-TOF MS spectra of IgG tryptic digests. (A) fresh synthesis, (B) stored eight months.

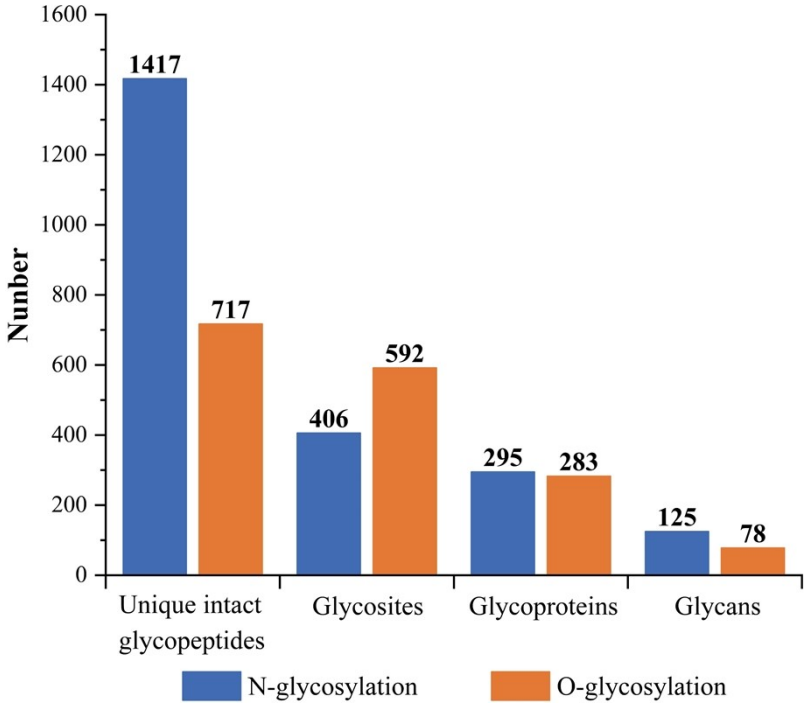

Fig. S7. The number of identified N- and O-glycoproteins, glycosites, intact glycopeptides and glycans using commercial ZIC-HILIC.

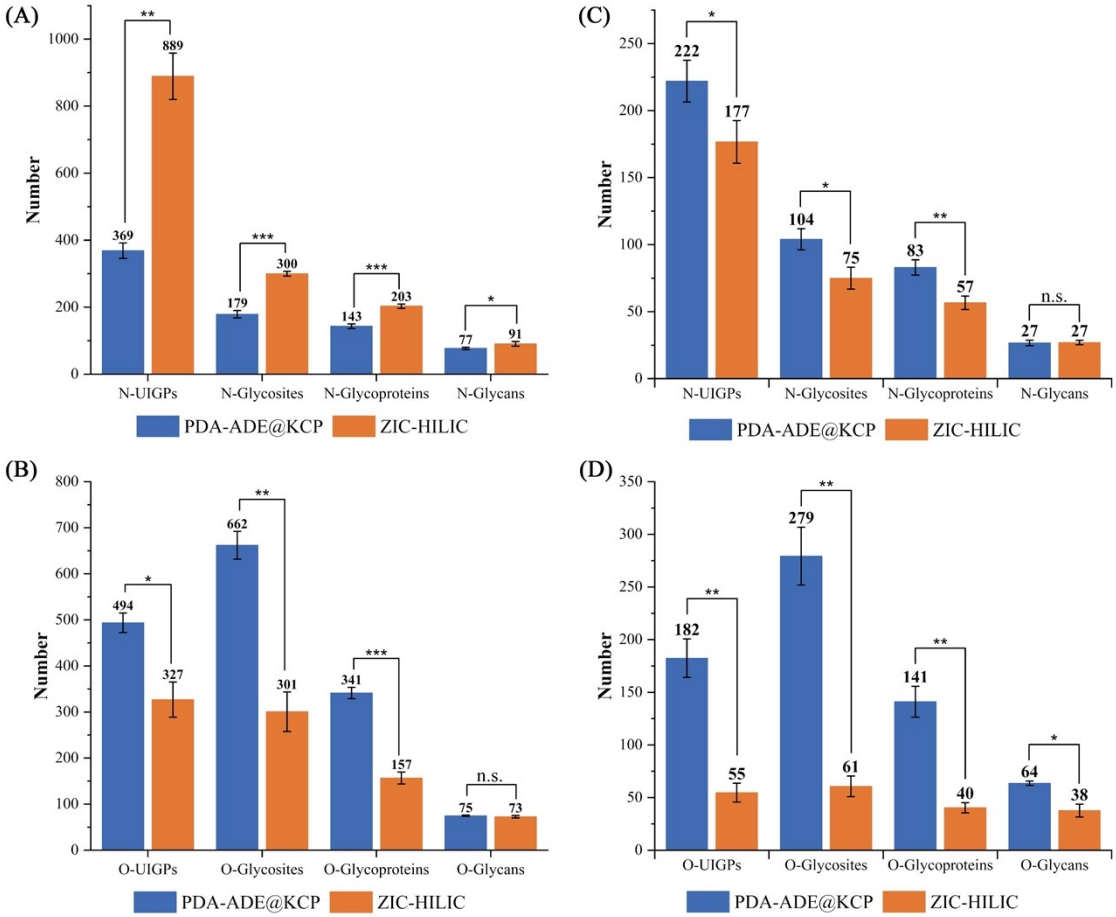

Fig. S8. The number of identified N- and O- linked intact glycopeptides, glycosites, glycoproteins compared between PDA-ADE@KCP and ZIC-HILIC from mouse liver. N-glycosylation (A) and O-glycosylation (B) modification with PEP 2D < 0.01, FDR 2D < 0.01 and |Log Prob| ≥ 2 as cut off. N-glycosylation (C) and O-glycosylation (D) modification with Byonic Score > 500 as cut off. "*" means $p < 0.05$, "**" means $p < 0.01$, "***" means $p < 0.001$ and "n.s." means not statistically significant.

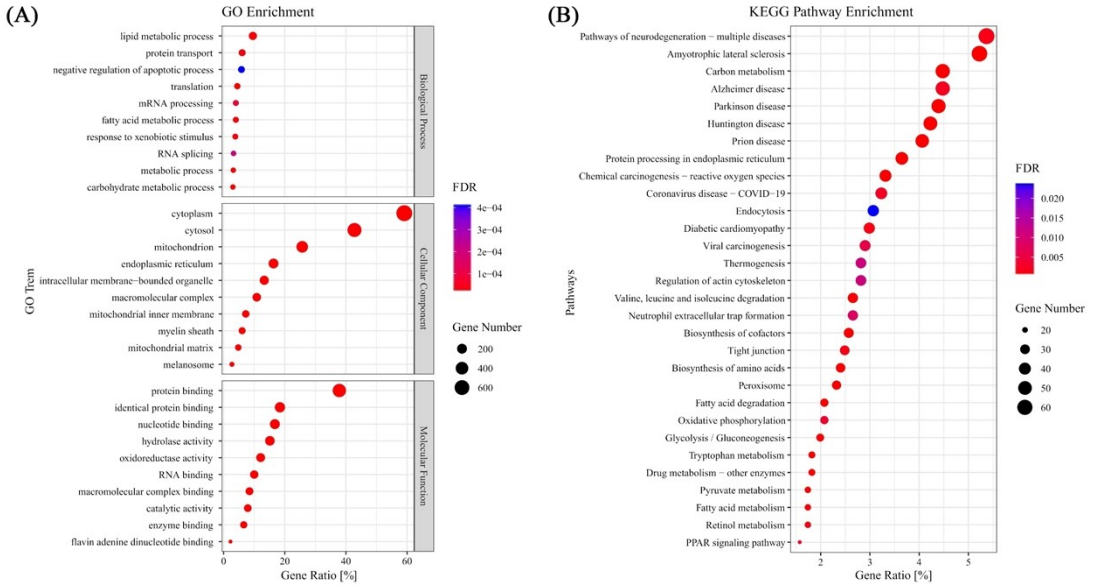

Fig. S9. Functional annotation analysis of global O-glycoproteins in mouse liver. (A) GO enrichment analysis; (B) KEGG enrichment analysis.

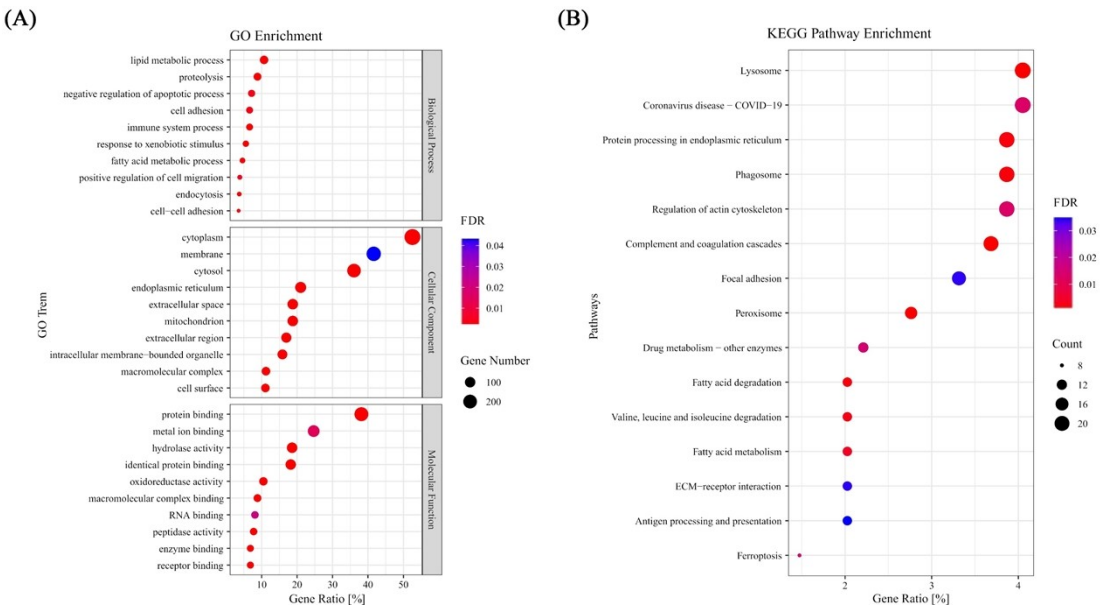

Fig. S10. Functional annotation analysis of global N-glycoproteins in mouse liver. (A) GO enrichment analysis; (B) KEGG enrichment analysis.

Table S1. Chemical compositions (by XPS) of KCP, PDA@KCP and PDA-ADE@KCP.

Samples	C (at. %)	N (at. %)	O (at. %)
KCP	70.02	11.78	18.19
PDA@KCP	58.24	9.12	32.64
PDA-ADE@KCP	58.82	10.24	30.94