Supporting information

Efficient enrichment of glycopeptides using phenylboronic acid polymer brush modified silica microspheres

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1. Synthesis and characterization of monolayer PBA modified SiO_2

The azide modified silica gel was prepared according to the literature.[1,2] monolayer PBA modified SiO_2 （PBA@SiO_2） was prepared over iron salt catalyzed Click Chemistry. Azide-modified silica gel (5 g) was suspended in DMF/MeOH (v/v, 9/1, 50 mL) solution of 4-cyanophenylboronic acid (4.5 g, 30.6 mmol). Then, ferrous acetate (0.15 g, 0.85 mmol) and sodium ascorbate (1.0 g, 5 mmol) were added in the solution. The solution was stirred under 80℃ over 60 h, the obtained material was filtrated and washed with 0.1 mol/L EDTA aqueous solution (500 mL), water (1000 mL), and methanol (300 mL) respectively. The prepared materials were dried to constant weight and characterized with the elemental analysis results. The carbon content of PBA@SiO_2 was 4.5%.

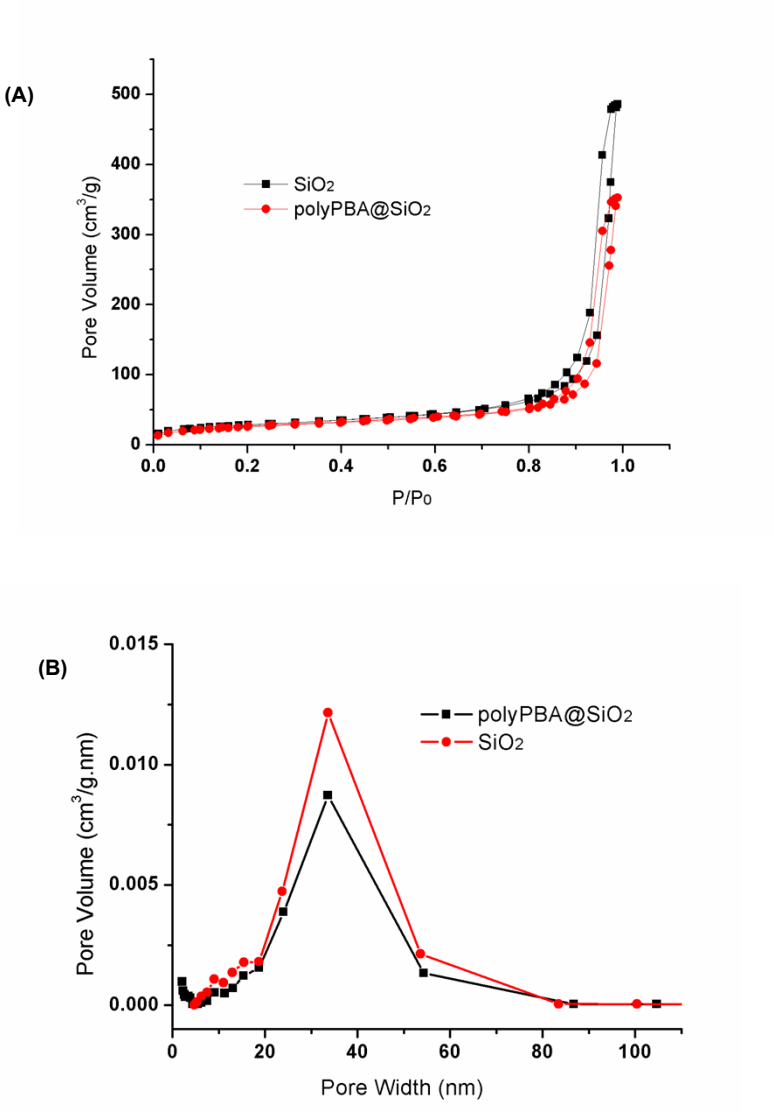


Fig. S1 The N_2 adsorption curve (A) and pore size distribution curves (B) of polyPBA@SiO_2 and SiO_2 .

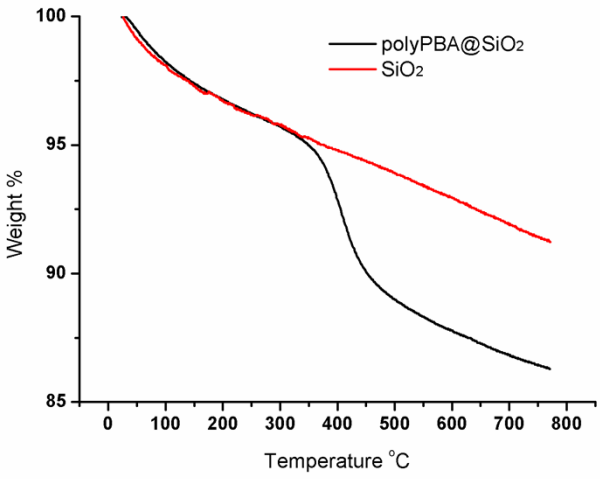


Fig. S2 Thermo-gravimetric analysis (TGA) curves of polyPBA@SiO_2 and SiO_2.

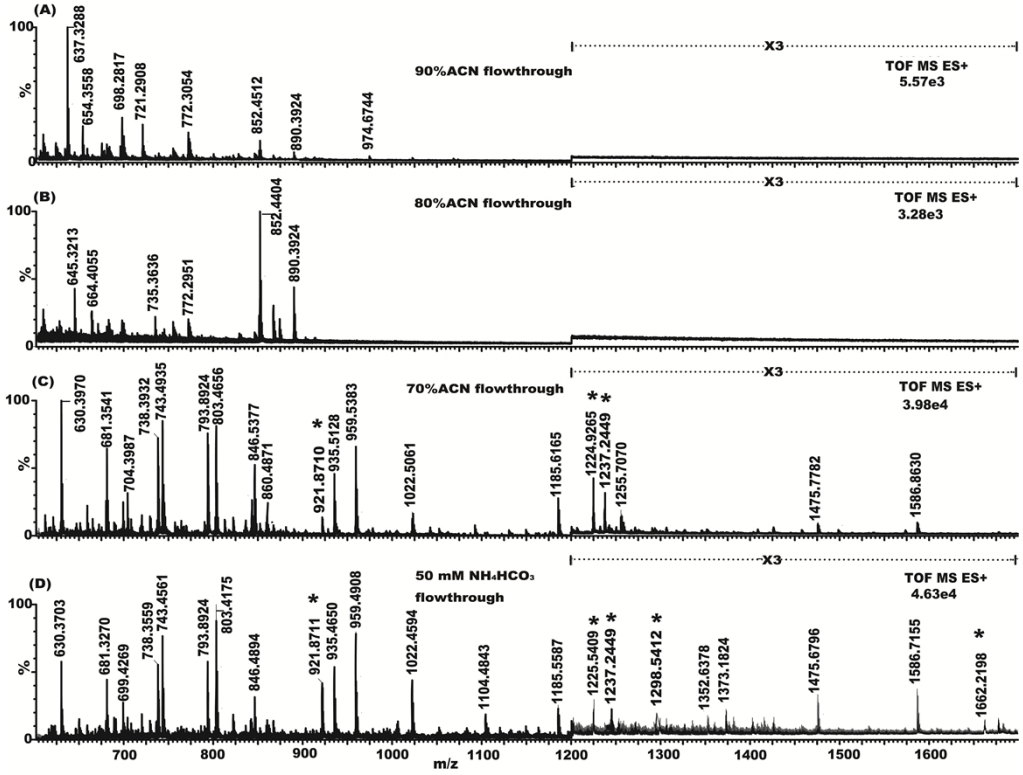


Fig. S3 Nano-ESI-Q-TOF mass spectra of flowthrough from polyPBA@SiO_2 microcolumns after loading 10□ μL tryptic HRP digests with (A) 90% ACN/50 mM NH_4HCO_3, (B) 80% ACN/50 mM NH_4HCO_3, (C) 70% ACN/50 mM NH_4HCO_3 and (D) 50 mM NH_4HCO_3. Glycopeptides are labelled with *. The non-glycopeptides were labelled with their m/z values. Glycopeptides were strongly retained by 90% CAN/50 mM NH_4HCO_3 and 80% ACN/50 mM NH_4HCO_3 and were eluted with 70% ACN/50 mM NH_4HCO_3.

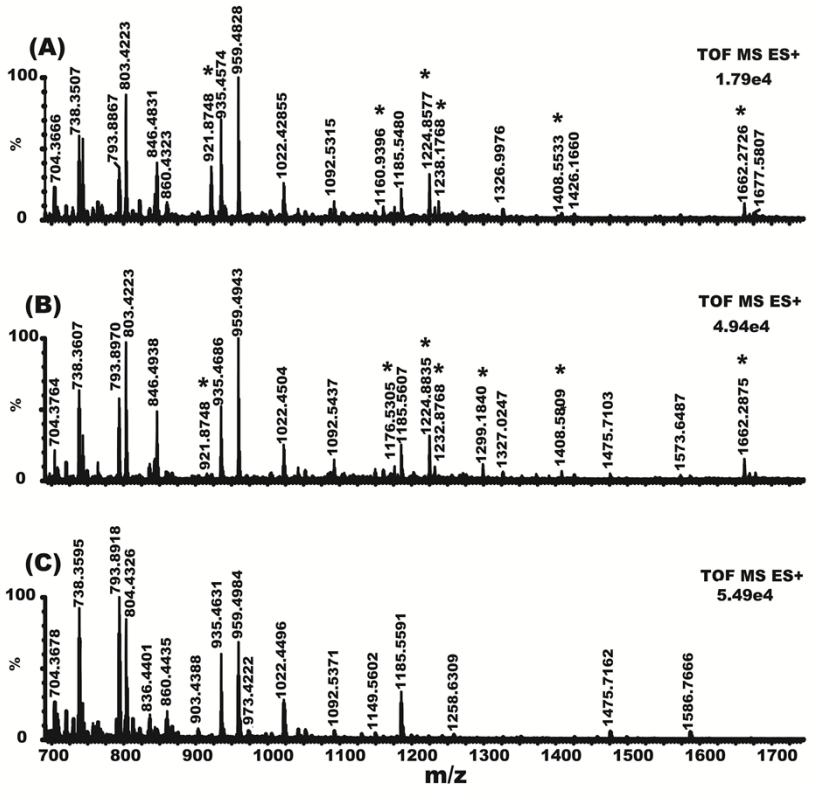
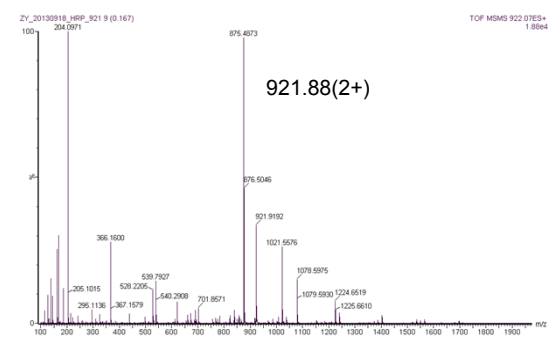
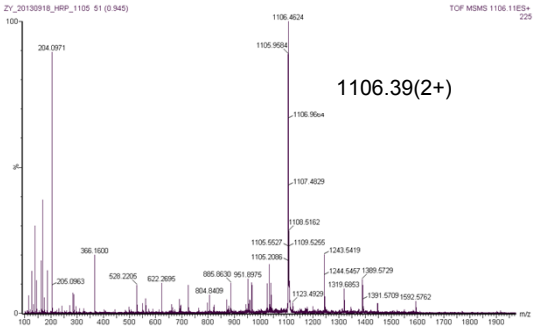


Fig. S4 Nano-ESI-Q-TOF mass spectra of HRP peptides not retained by polyPBA@SiO_2 when using different pH loading solutions. (A) 75% ACN/0.1% FA, (B) 75% ACN and (C) 75% ACN/50 mM NH_4HCO_3. Glycopeptides are labeled with *. The non-glycopeptides were labeled with their m/z values. Glycopeptides could be found when using 75% ACN in both neutral and acidic solution. On the contrary, the glycopeptides were well captured with 75% ACN in basic solution.


921.88(2+)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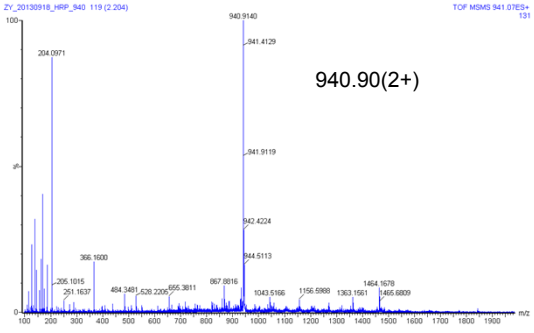
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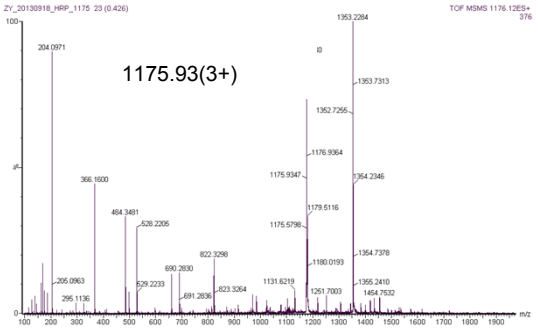
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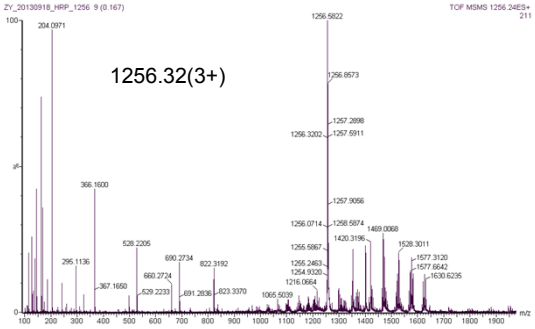
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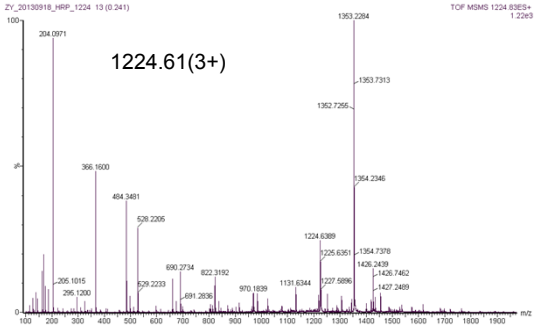
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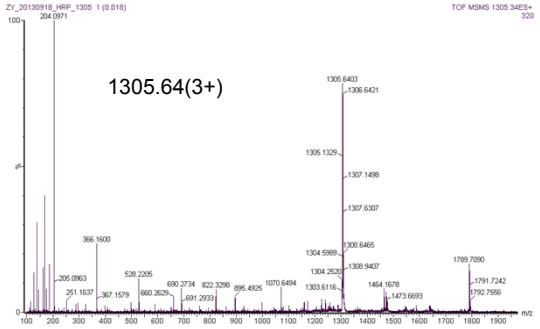
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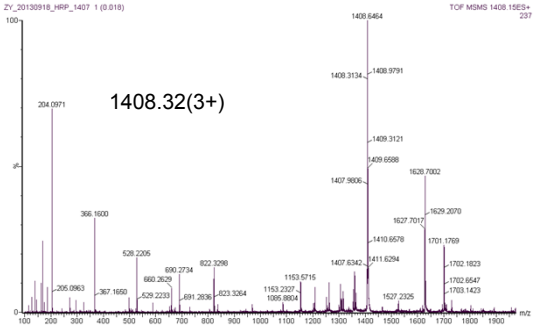
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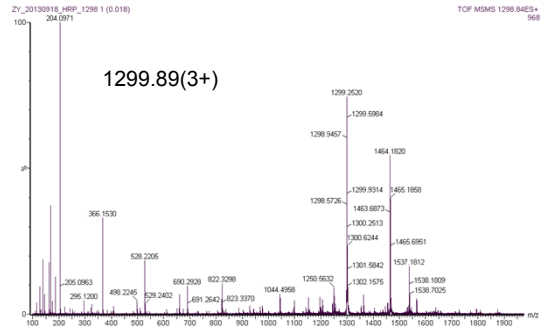
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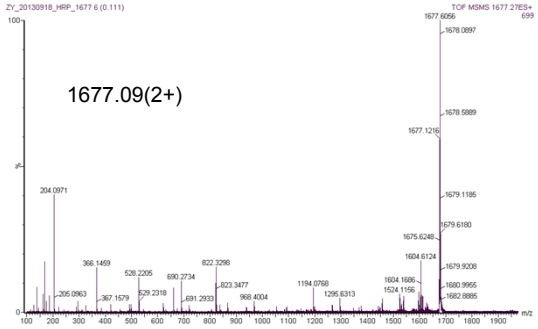
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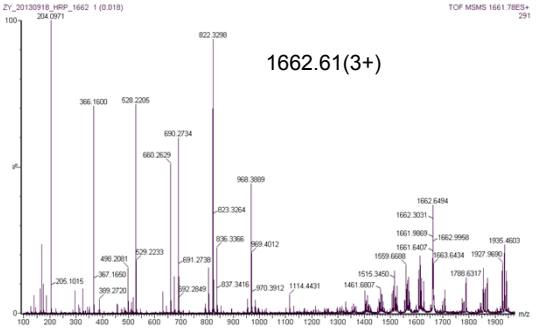
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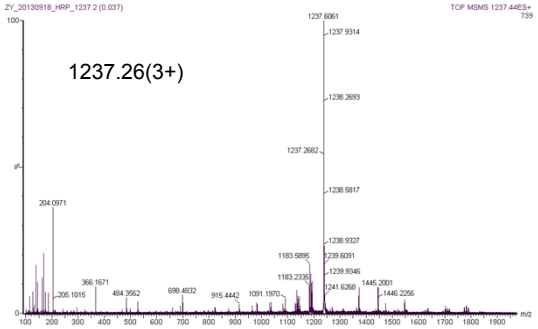
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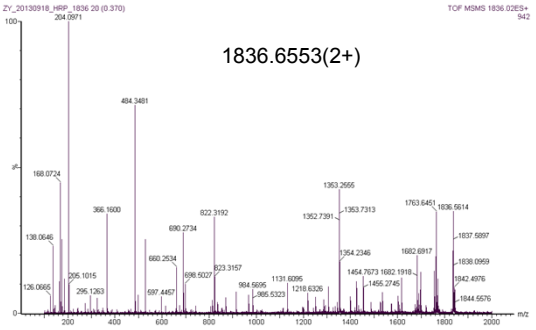
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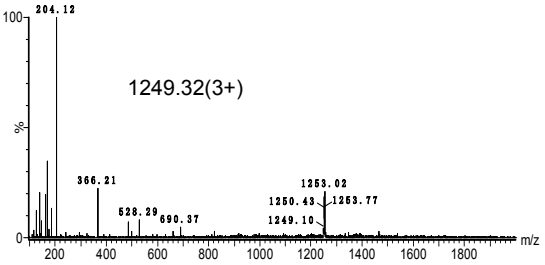
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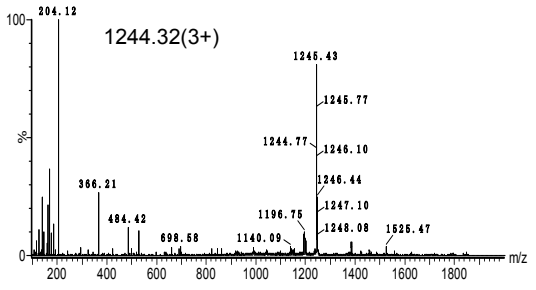
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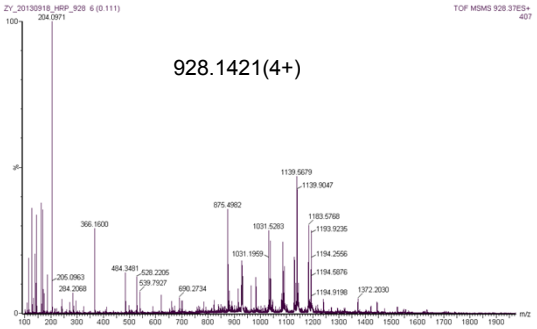
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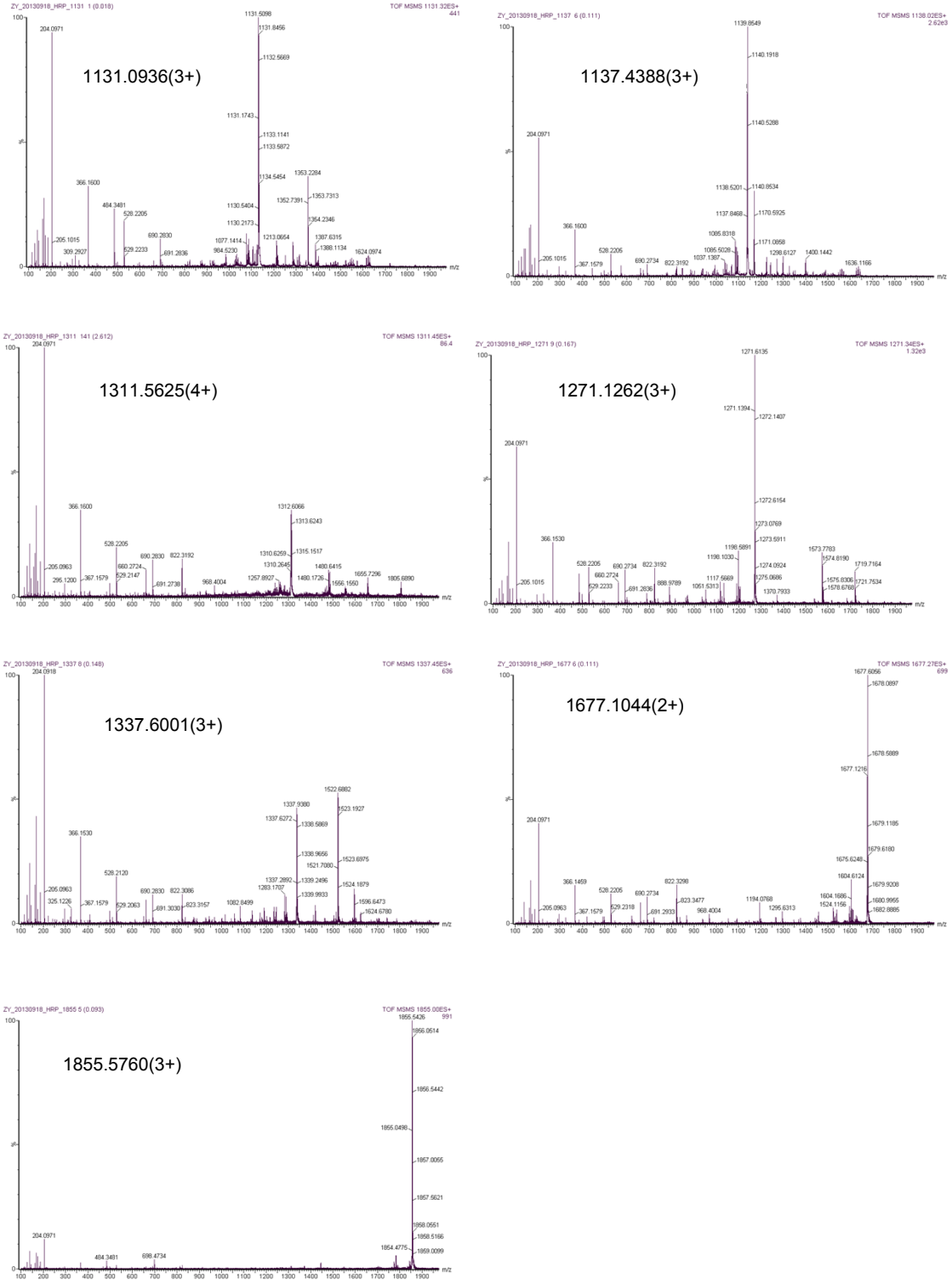

Fig. S5. Tandem mass spectra of identified HRP peptides enriched with polyPBA@SiO_2. Fragment ions of all detected HRP glycopeptides have at least three indicator oxonium ions including m/z 163 (Hex+), m/z 204 (HexNAc+) and m/z 366.

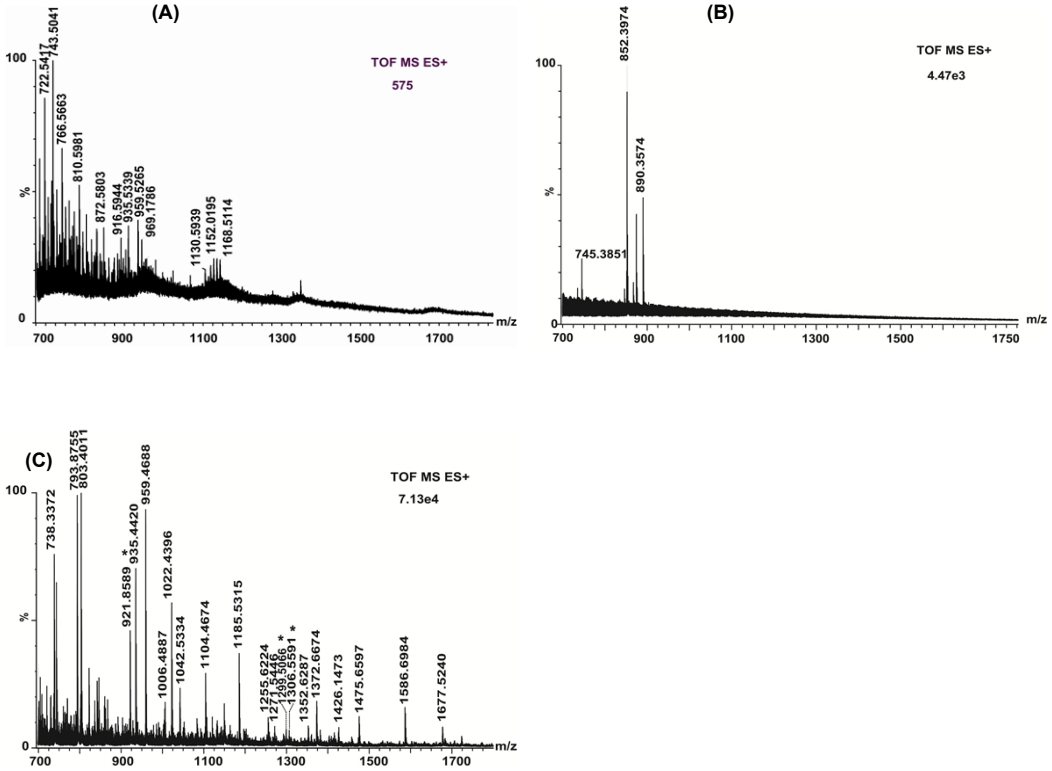

Fig. S6. Nano-ESI-Q-TOF mass spectra of HRP peptides in flowthrough fractions from PBA-agarose (A), polyPBA@SiO_2 (B), monolayer PBA@SiO_2 without incubation (C). Glycopeptides are labelled with *. The non-glycopeptides were labelled with their m/z values. PBA@SiO_2 and PBA-agarose showed strong affinity for peptides.

References

1. *A. Shen, Z. Guo, L. Yu, L. Cao and X. Liang, Chem. Commun., 2011, **47**, 4550.*
2. *L. Yu, X. Li, Z. Guo, X. Zhang and X. Liang, Chem．Eur. J, 2009, **15**, 12618.*