Supporting Information

One-step preparation of phosphate-rich carbonaceous spheres based on hydrothermal process for phosphopeptides analysis

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Adsorption Experiment

Pyridoxal 5'-phosphate was firstly dissolved in 30% ACN (vt%) to form solutions. Then 5 mg of Ti^{4+}-IMAC carbonaceous spheres were applied in 1 mL of pyridoxal 5'-phosphate solution at room temperature with oscillation for a certain time. After centrifugation, the supernatant was determined by UV-is adsorption spectroscopy at the wavelength of 291 nm. The adsorption capacity was calculated by the following equation: $Q_e = V(C_0-C_e)/m$, where Q_e is the equilibrium adsorption capacity, C_0 and C_e are the initial and equilibrium concentrations, respectively, V is the volume of pyridoxal 5'-phosphate solution, and m is the weight of the added Ti^{4+}-IMAC carbonaceous spheres

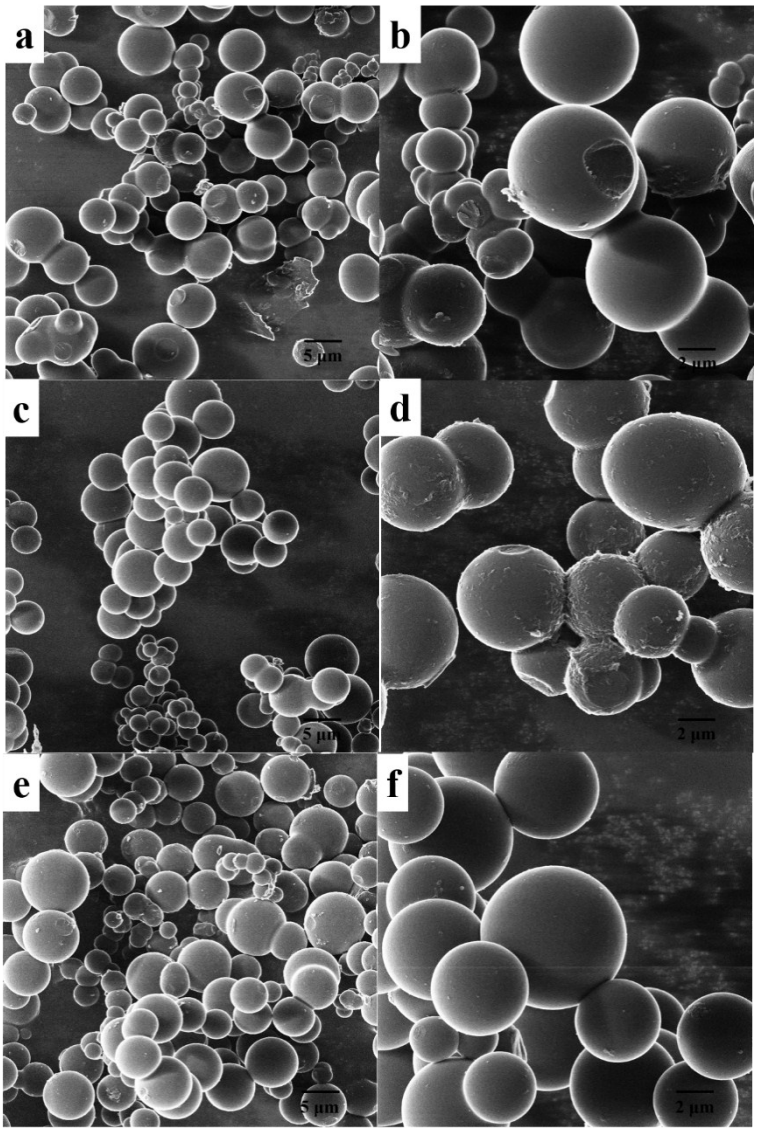

Figure S1. The SEM images of carbonaceous spheres prepared with different contents of glucose. (a, b) $G_5P_3N_0$, (c, d) $G_{15}P_3N_0$ and (e, f) $G_{20}P_3N_0$.

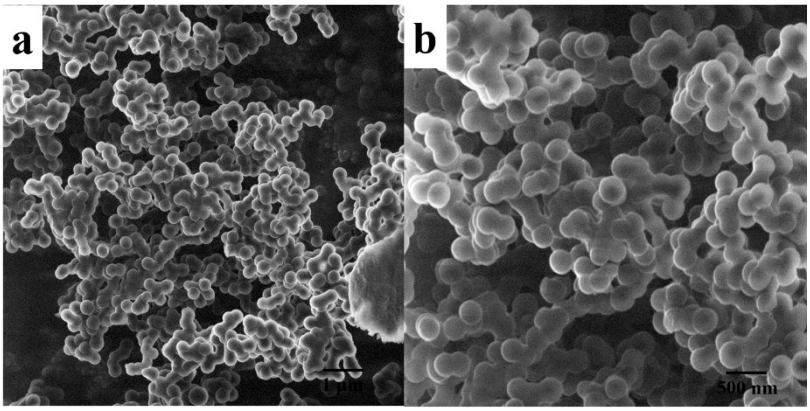

Figure S2. The SEM images of carbonaceous spheres $G_{10}P_0N_0$.

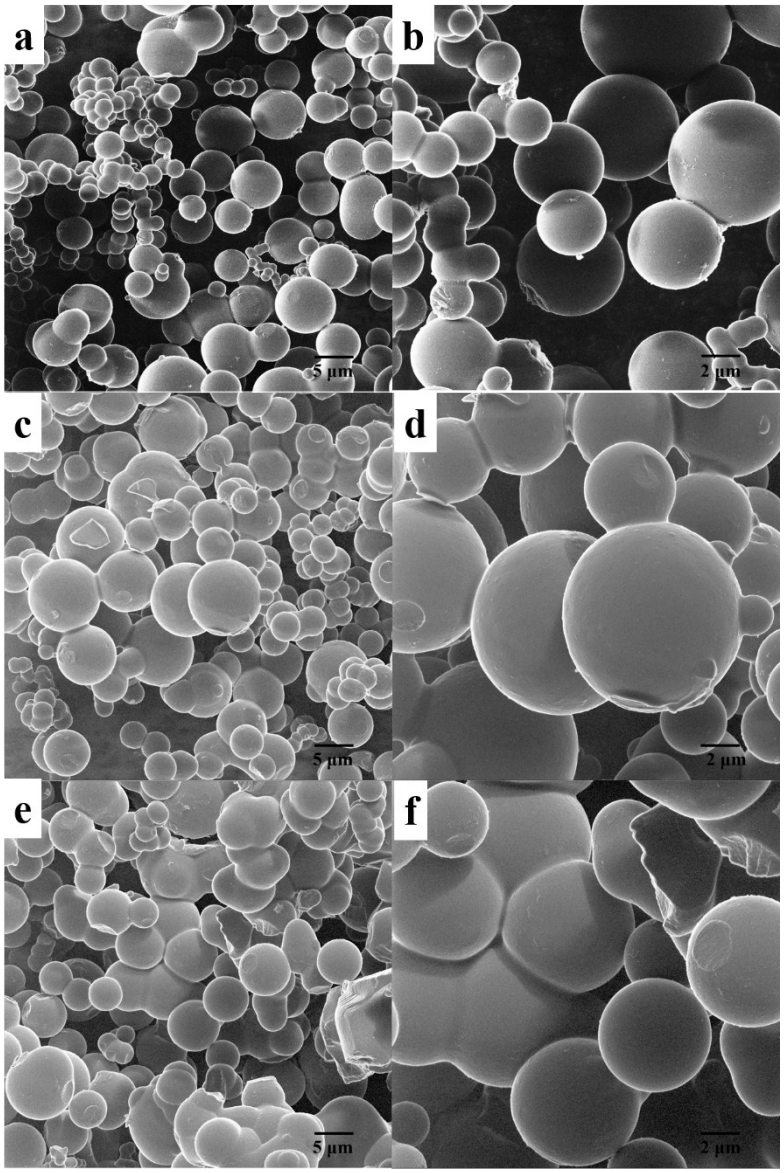

Figure S3. The SEM images of carbonaceous spheres prepared with different weight ratios of VPA to glucose. (a, b) $G_{10}P_1N_0$, (c, d) $G_{10}P_5N_0$ and (e, f) $G_{10}P_{10}N_0$.

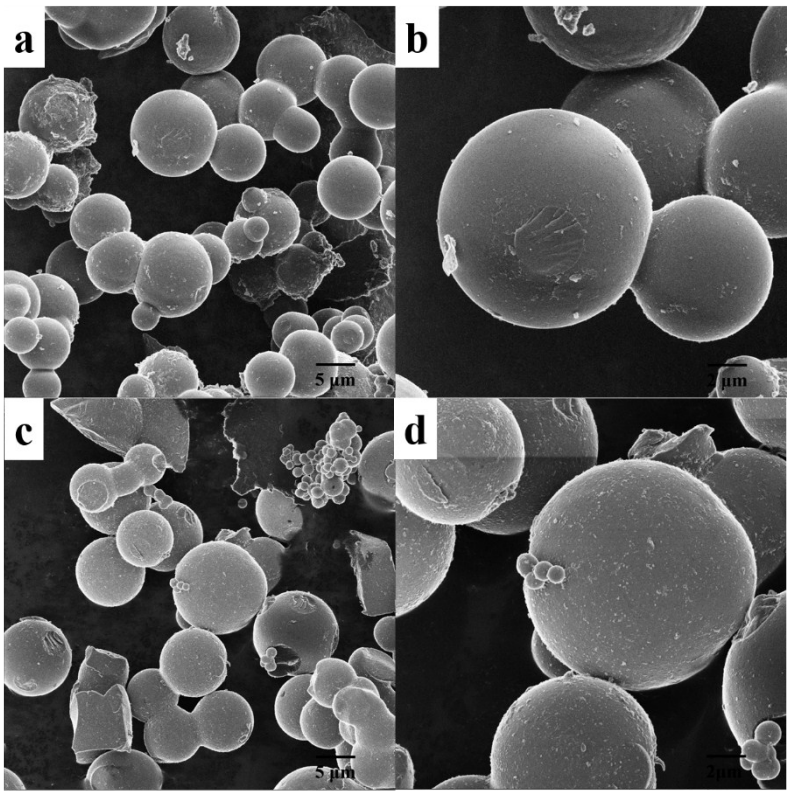

Figure S4. The SEM images of carbonaceous spheres prepared with different weight ratios of VPA to glucose. (a, b) $G_{10}P_1N_1$ and (c, d) $G_{10}P_5N_1$.

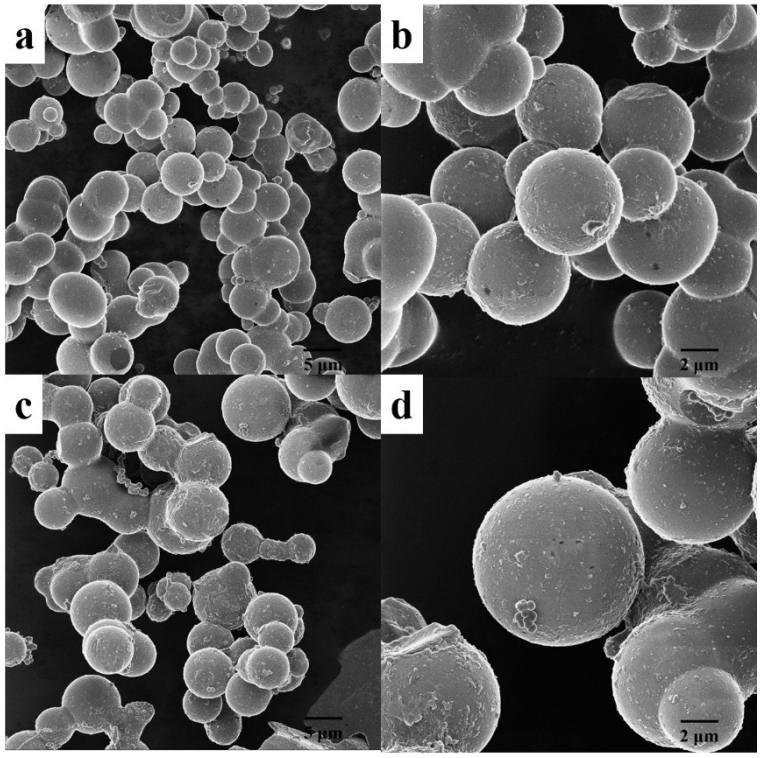

Figure S5. The SEM images of carbonaceous spheres prepared with different contents of initiator. (a, b) $G_{10}P_3N_5$ and (c, d) $G_{10}P_3N_{10}$.

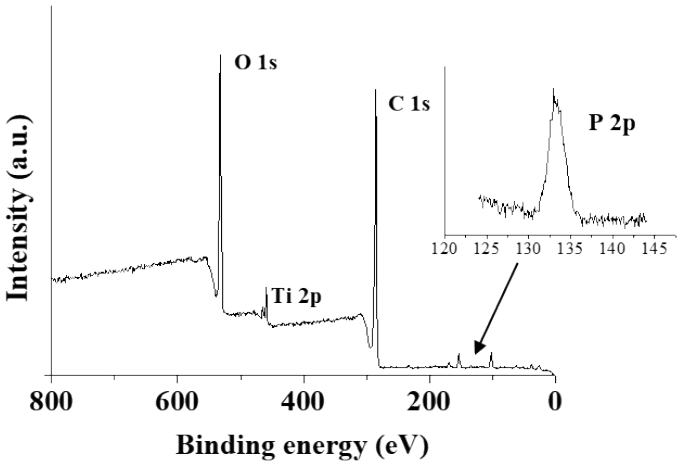

Figure S6. Full XPS spectrum of $G_{10}P_3N_0@Ti^{4+}$.

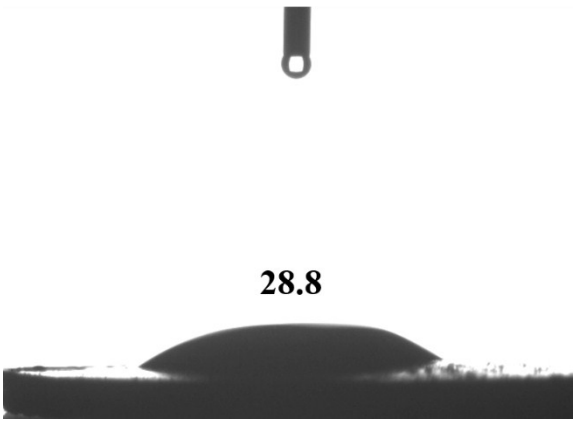

Figure S7. Water contact angle of carbonaceous sphere $G_{10}P_0N_0$.

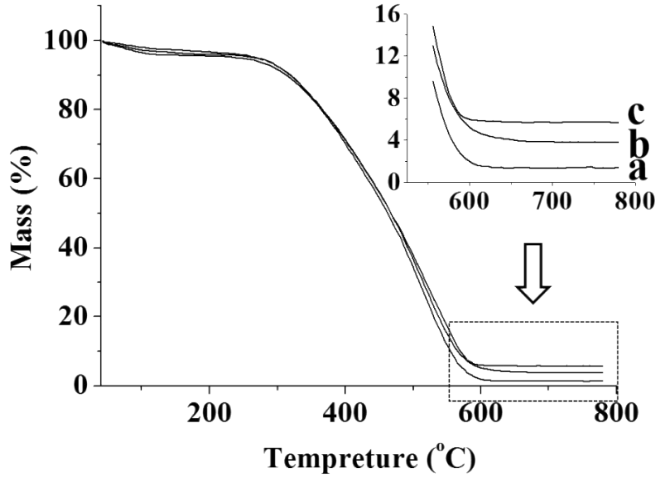

Figure S8. Thermal gravimetric analysis of carbonaceous spheres (a) $G_{10}P_1N_0$, (b) $G_{10}P_3N_0$ and (c) $G_{10}P_3N_0$@Ti^{4+}. Heating rate, 10 °C/min in air. Residues: (a) 1.40%, (b) 3.79% and (c) 5.70%.

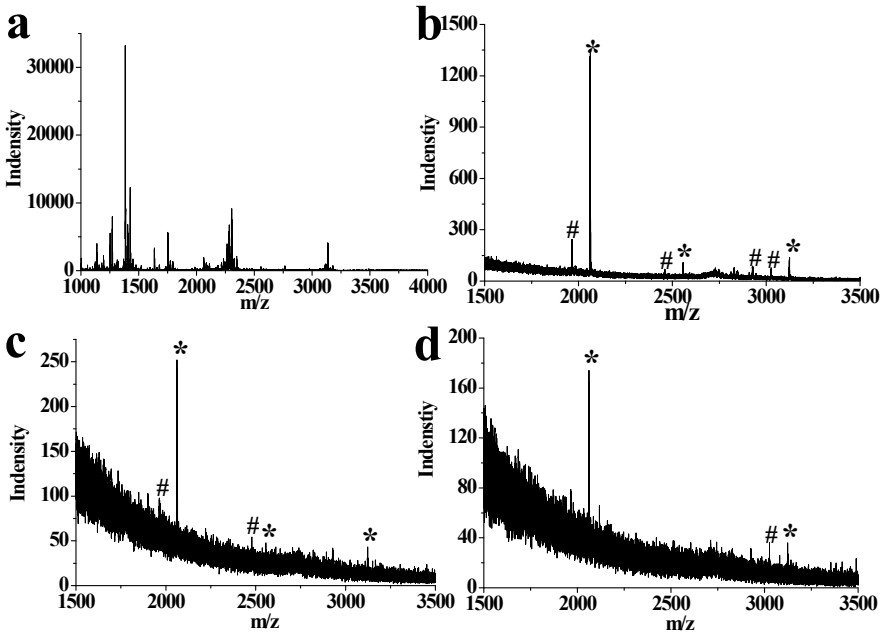

Figure S9. MADLI-TOF MS analysis of tryptic digest of *β*-casein. (a) Direct analysis without any enrichment; analysis of (b) 100 fmol, (c) 10 fmol and (d) 5 fmol tryptic digest of *β*-casein after enrichment by Ti^{4+}-IMAC carbonaceous spheres. The asterisk (*) indicates phosphopeptides, and (#) indicates dephosphorylated peptides.

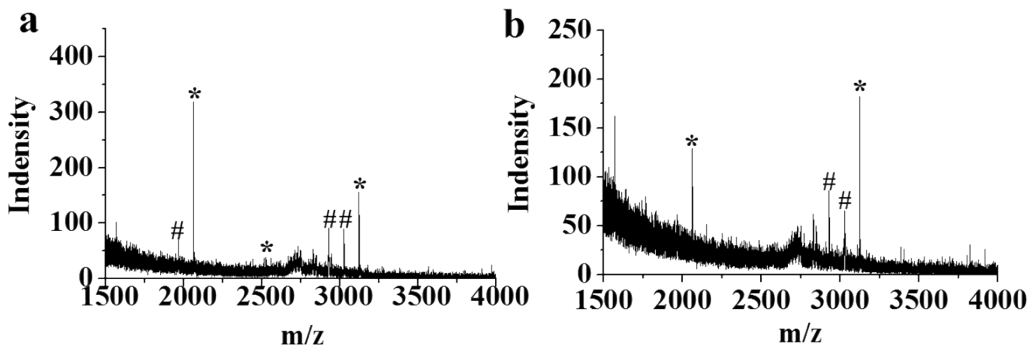

Figure S10. MADLI-TOF MS analysis of *β*-casein and BSA digests after enrichment by Ti^{4+}-IMAC carbonaceous spheres at the molar ratios of (a) 500/1 and (b) 1000/1. The asterisk (*) indicates phosphopeptides, and (#) indicates dephosphorylated peptides.

Table S1. List of phosphopeptides from diluted human serum after enriched by Ti^{4+}-IMAC carbonaceous spheres in MALDI-TOF MS analysis.

No.	Peptide sequence	Observed m/z
HS_1	D[pS]GEGDFLAEGGGV	1389
HS_2	AD[pS]GEGDFLAEGGGV	1460
HS_3	D[pS]GEGDFLAEGGGVR	1545
HS_4	AD[pS]GEGDFLAEGGGVR	1616