Electronic supplementary information of the paper entitled “Zeolite nanoparticles with immobilized metal ion: isolation and MALDI-TOF-MS/MS identification of phosphopeptides”.

Experimental details:

Protein digestion:

Bovine β-casein, 2 mg/mL in 50 mM ammonium bicarbonate buffer at pH 8.3, was incubated at 37 °C with trypsin (E:S = 1:50) for 18 h.

Isolation of phosphopeptide from β-casein digest by Fe$^{3+}$ ion-immobilized zeolite nanoparticles (Fe$^{3+}$-nanozeolites):

Fe$^{3+}$-nanozeolites suspension (10 µL of 3 mg/mL) was added into 0.2 mL of 5ng/µL or 0.5 ng/µL β-casein peptide digests solution. Then the solution containing peptides and Fe$^{3+}$-nanozeolites was vibrated at 37 °C for 90 min, and centrifugated at 17000 rpm for 15 min. After removing the supernate, the residual peptides/Fe$^{3+}$-nanozeolites were redispersed into 5 µL of 50% acetonitrile aqueous solution.

MALDI-TOF-MS process:

Peptides/Fe$^{3+}$-nanozeolites were deposited on the MALDI target using dried droplet method. 0.35 µL of the above slurry was spotted onto the MALDI plate and then another 0.35 µL of α-CHCA saturated solution (in 50% acetonitrile aqueous solution containing 0.1% trifluoroacetic acid) was introduced. Positive ion MALDI-TOF mass spectra were acquired on 4700 Proteomics Analyzer (Applied Biosystems, USA). Sample desorption was achieved using an Nd-YAG laser (355 nm) operated at a repetition rate of 200 Hz and acceleration voltage of 20 kV.
**Fig. S1** SEM images of zeolite-beta nanoparticles before (a) and after (b) immobilizing metal ions. The SEM images indicate that this covalent modification has no influence on the morphology of nanoparticles. 1 bar is 1µm.

**Fig. S2** FT-IR spectrum of the Fe³⁺-nanozeolites. The absorption on 1604 cm⁻¹ and 1458-1394 cm⁻¹ could be assigned to the anti-symmetric and symmetric stretching vibration of carboxylate, respectively.
Fig. S3 Energy dispersive spectrum (EDS) analyses of the Fe$^{3+}$-nanozeolites. The EDS analysis was carried out by means of a Philips XL30 scanning electron microscope. The appearance of Au element can be attributed to the sprinkling Au on the sample before SEM analysis.