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Viral Capsid-like Titania for Selective Enrichment of Phosphorylated Peptides

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Experimental

Materials. Poly(styrene-*b*-acrylic acid) (PS_{144}-*b*-PAA_{22}) was supplied by Polymer Source. Toluene (purity≥99.5%), hydrochloric acid (HCl, 37%), acetonitrile (ACN) (purity ≥ 99.0%) and trifluoroacetic acid (TFA) (purity≥99.0%) were obtained from Shanghai Ling Feng. Methanol (purity≥99.9%), titanium butoxide ($Ti(OC_4H_9)_4$) (purity≥99.0%) and ammonium bicarbonate (NH_4HCO_3) (putity≥99.995%) were purchased from Aladdin. Trypsin from bovine, β-casein from bovine milk and 2,5-dihydroxybenzoicacid (DHB) were obtained from Sigma-Aldrich (St Louis, MO, USA). 5% ammonia (AR). Water used in experiments was purified using a Milli-Qsystem.

Synthesis of Self-assembled PS_{144}-*b*-PAA_{22} Microsparticles and VCL-TiO_2. To synthesize the PS_{144}-*b*-PAA_{22} microparticles, 20 mg polymer was dissolved in a mixture of 0.6 ml toluene and 1.8 ml methanol. The mixture was magnetically stirred (*ca.* 800 r.p.m) at room temperature for 10 h. Then, 5 times volume of methanol was added to quench the structure. The as-prepared PS_{144}-*b*-PAA_{22} microparticles were redispersed in methanol (10 ml), followed by the slow addition of 400 µl tetrabutyl titanate with stirring. After one hour, 200 µl of 37% HCl was injected and aged for 2 days. The resulting product was collected by centrifugation, and washed with methanol. The final micro-sized VCL-TiO_2 were obtained after calcinating the white powder at 500°C.

Characterization of VCL-TiO_2. Scanning electron microscope (SEM, QUANTA FEG 250) was used to observe morphology of the VCL-TiO_2 with the accelerating voltage of 10 kV. Transmission electron microscopy (TEM) images were obtained by using a TALOS L120C transmission electron microscope (TALOS, Czech Republic). TEM operated at 120 kV was used to investigate the interior structure of the VCL-TiO_2. High-resolution transmission electron microscopy (HRTEM, JEM-2100F) was used to observe the lattice structure of VCL-TiO_2. Field emission scanning electron microscopy (FESEM) images were obtained by using S-4800 field emission scanning electron microscope (Hitachi, Japan). Powder X-ray diffraction (PXRD) patterns were collected on aBruker D8 Advance X-ray diffractometer using CuKα radiation under 0.154 nmk value, and operated at 40 kV and 40 mA. Nitrogen adsorption–desorption isotherms were obtained on an Autosorb-iQ apparatus (Micromeritics, USA). The specific surface area, pore size distribution and porevolume were determined by Brunauer–Emmett–Teller (BET) method and Barrett–Joyner–Halenda (BJH) model. Fourier transform infrared (FTIR) spectra were recorded on a Thermo Fisher Scientific Nicolet iS10 Spectrometer using spectroscopic grade KBr.

Selective Enrichment of Phosphorylated Peptides using VCL-TiO_2. 5 mg β-casein in 5 ml NH_4HCO_3 (50 mM) and 5 mg trypsin in 5 ml of HCl (1 mM) solution were mixed at a ratio of 50:1 (vol : vol) and digested at 37°C for

12 hours. The obtained peptide solution was placed at -20°C before use. The VCL-TiO_2 was suspended in the resulting peptide solution for the enrichment. The phosphopeptide-enriched VCL-TiO_2 was separated from the mixed solution by centrifugation, and then washed three times with 1 ml of loading buffer (50% ACN containing 1% TFA) to remove non-specifically adsorbed peptides. The captured phosphopeptide was eluted with 1 ml of a 5% aqueous ammonia solution for 15 min. The supernatant was collected and stored at -20°C. MALDI-TOF-MS analysis was performed at Autoflex speed. The eluted supernatant (1.0 µl) was dropped on a MALDI target, air-dried at room temperature, and then dropped into a 1.0 µl of DHB matrix solution (50% methanol, 50% acetonitrile). A positive ion reflector mode was performed, and the spectrum of each spot was obtained by accumulating 2000 laser irradiations.

Supporting Results

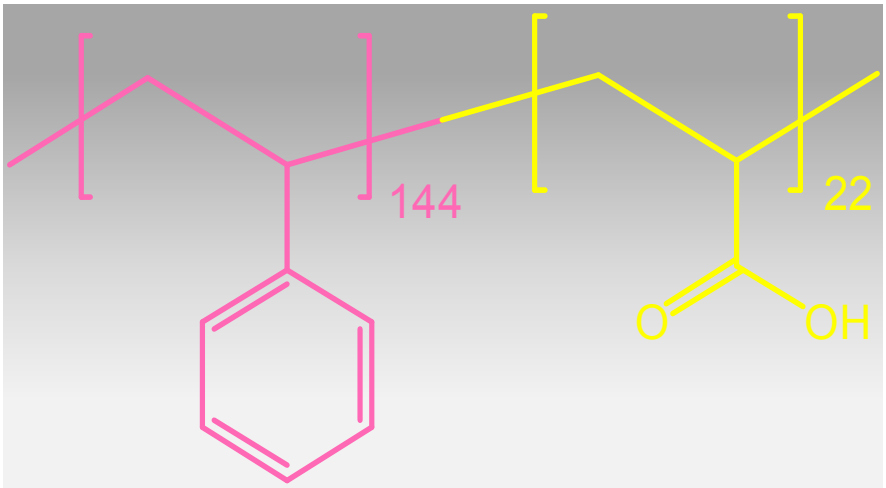

Figure S1. Molecular formula of block copolymer PS_{144}-*b*-PAA_{22}.

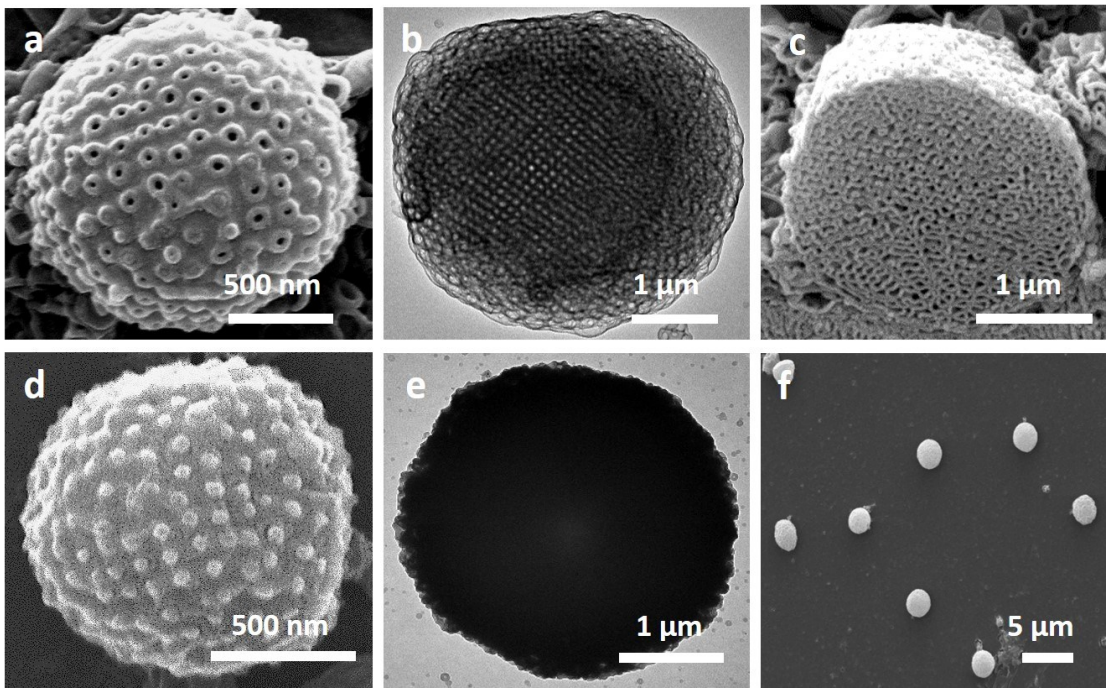

Figure S2. (a, b, c) SEM, TEM and cross-sectional images of self-assembled PS_{144}-*b*-PAA_{22} microparticles. (d, e) SEM and TEM images of PS_{144}-*b*-PAA_{22}/TiO_2 hybrid before calcination. (f) SEM image of VCL-TiO_2 at low magnification.

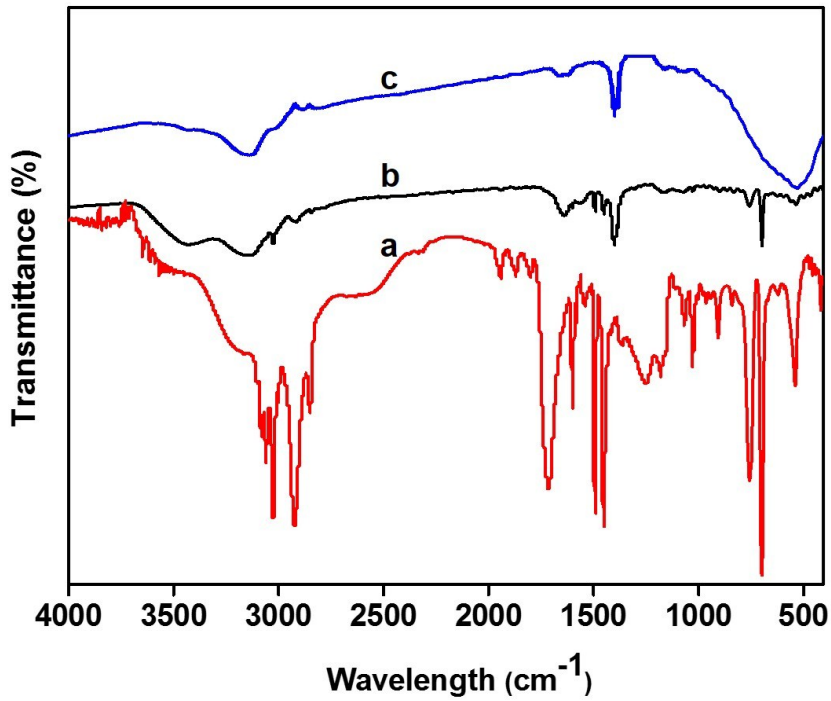

Figure S3. FT-IR spectra of (a) PS_{144}-*b*-PAA_{22} microparticles, (b) PS_{144}-*b*-PAA_{22}/TiO_2 hybrid and (c) VCL-TiO_2.

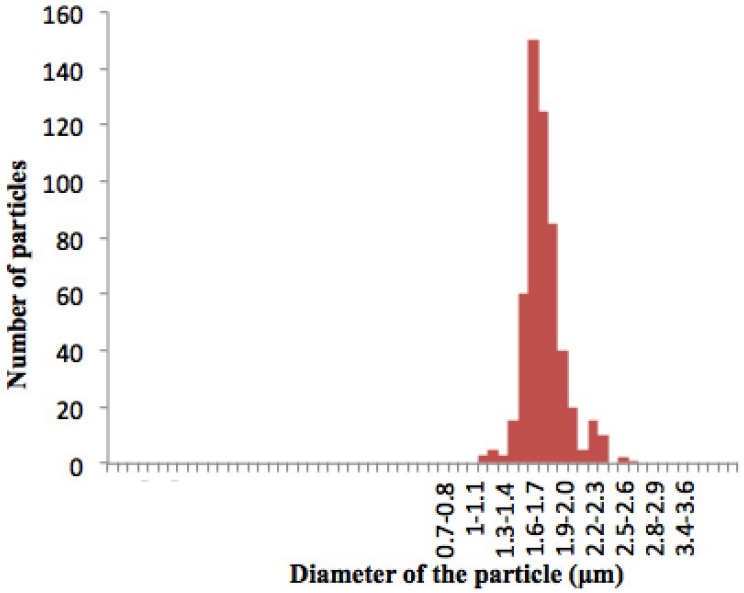

Figure S4. Histogram shows the diameter and distribution of the VCL-TiO_2.

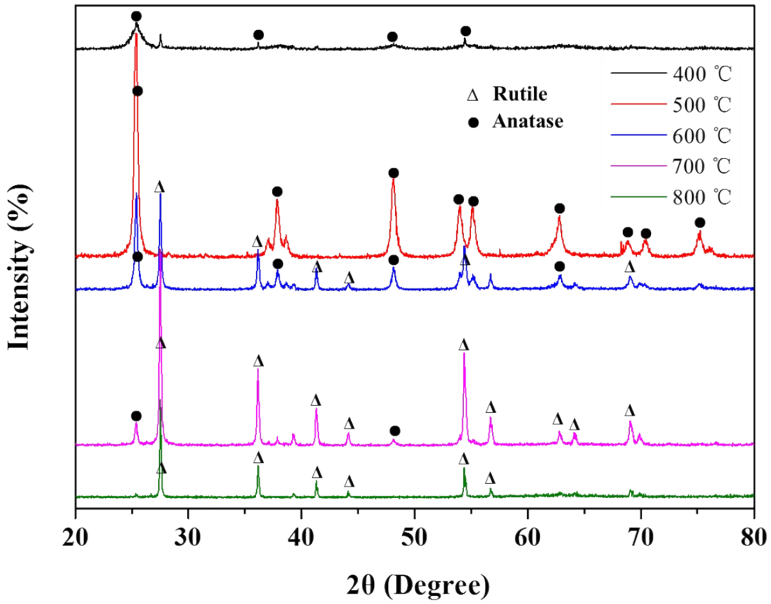

Figure S5. XRD spectra show the crystal form change of titania with temperature.

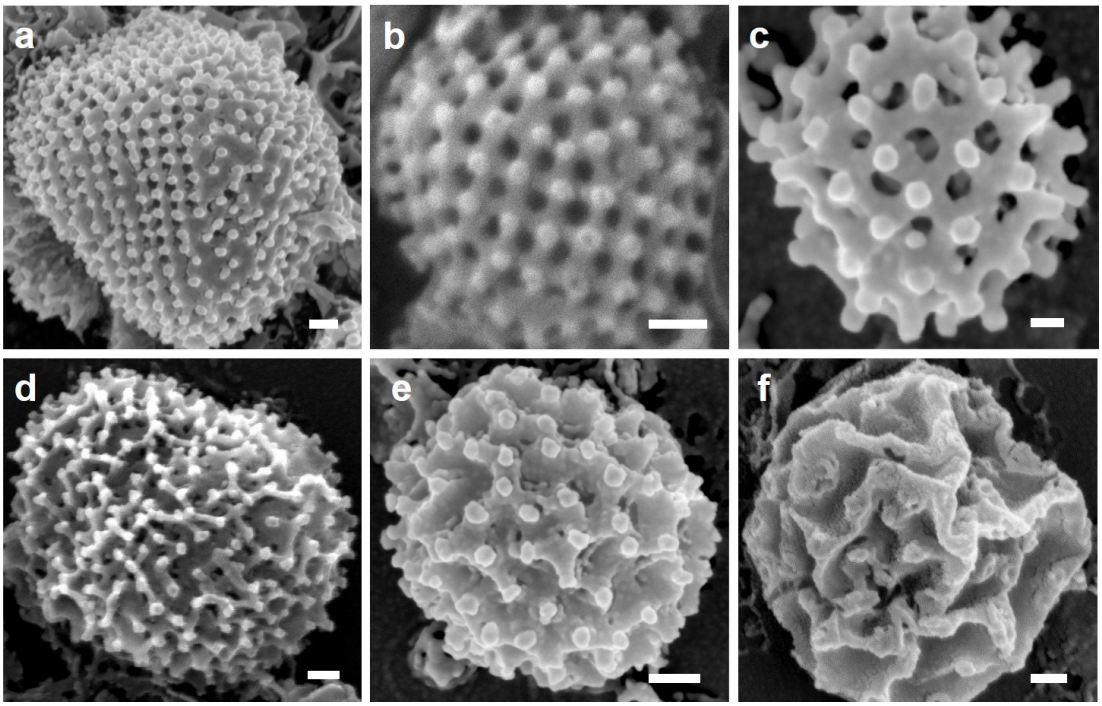

Figure S6. FESEM images of some fascinating intermediate structures of TiO_2. The scale bar represents 100 nm.

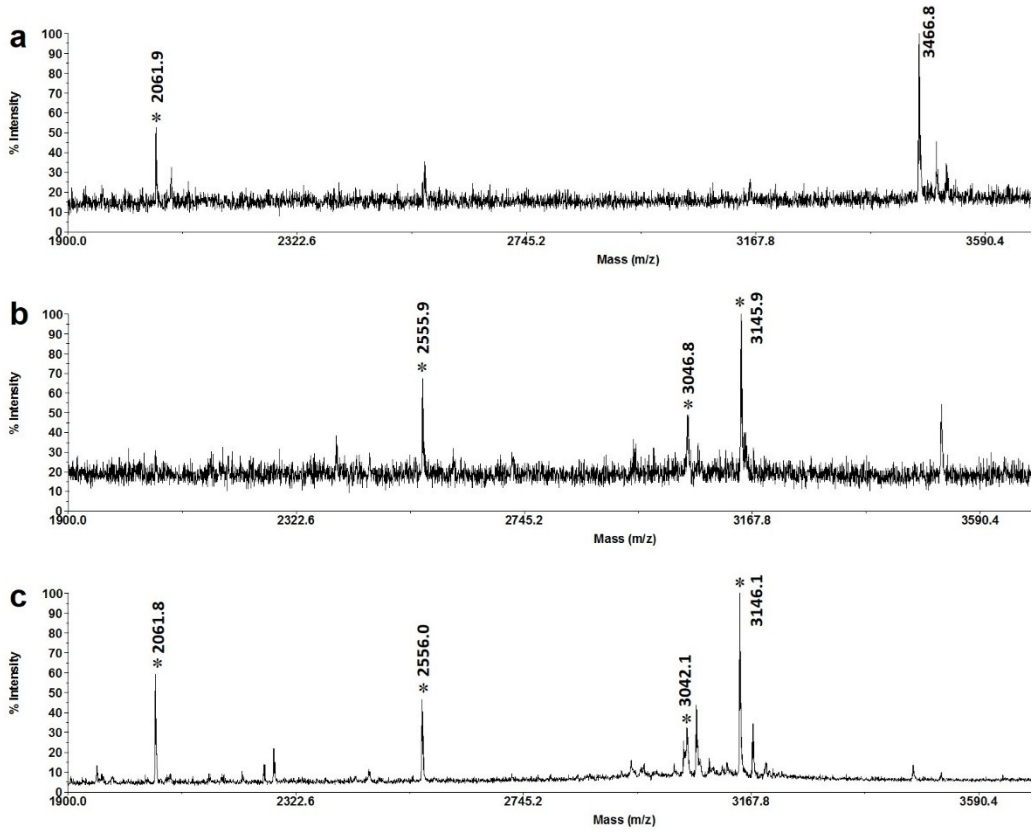

Figure S7. MALDI-TOF-MS spectra of the tryptic digest of β-casein. After enrichment by the VCL-TiO_2 particles with different enrichment time (a) 0 h, (b) 0.5 h, (c) 1 h. The peaks of phosphopeptides are marked with "*".