Electronic Supplementary Material (ESI) for ChemComm.
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Electronic Supplementary Information

An Efficient Approach to Prepare Boronate Core-shell Polymer Nanoparticles for Glycoprotein Recognition via Combined Distillation Precipitation Polymerization and RAFT Media Precipitation Polymerization

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Experimental Section

1 Reagents and materials

Horseradish peroxidase (HRP), methacrylic acid (MAA, 98%), N,N-methylenebisacrylamide (MBAAm, 98%), ovalbumin (OVA, chicken egg white), conalbumin (COA, chicken egg white), N,N-dimethylformamide (DMF), hexylamine, 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (DMP, 98%, HPLC), alizarin red S (ARS) were ordered from Sigma-Aldrich (St. Louis, MO). 3-Acrylamidophenylboronic acid (APBA) was ordered from Frontier Scientific Inc (Logan, UT). Bovine serum albumin (BSA, bovine serum) was obtained from Sino-American Biotec (Luoyang, China). 2,2'-Azobisisobutyronitrile (AIBN) was purchased from Fourth Shanghai Reagent Plant (Shanghai, China), and recrystallized before usage. Acetonitrile (ACN, HPLC grade) was ordered from Merck (Darmstadt, Germany). Water was purified by a Milli-Q system (Millipore, Milford, MA). Egg white sample was commercially available.

2 Synthesis of poly(MBA-co-VPBA)@(MBA-co-APBA) nanoparticles

The synthesis procedure is schematically illustrated in Scheme 1. Hydrophilic poly(MBAAm-co-MAA) core particles were synthesized using DPP technique. A typical procedure for the DPP: 1510 mg of MBAAm, 210 μl of MAA, and 34 mg of AIBN were dissolved with 160 mL of acetonitrile in 250 ml round-bottom flask equipped with a magnetic stir bar. The solution was purged with nitrogen for 10 min before the flask was connected to the Dean-Stark receiver. Then the flask was immersed in an oil bath, and heated to 75℃ for 10 min. Subsequently, the oil bath temperature was gradually increased to 100℃ and the solvent began to be distilled from the reaction system. When half of the reaction solvent was removed within 2.5 h through the Dean-Stark receiver, the reaction was stopped. The resultant nanoparticles were washed repeatedly with ACN to remove the residual monomer and oligomer in for three times, After dried under vacuum at 40℃ for 12 h, the prepared poly(MBAAm-co-MAA) was collected.

The functional boronate shells were coated on poly(MBAAm-co-MAA) particles by the second-step RAFT media precipitation polymerization, using APBA as the functional monomer and MBAAm as the cross-linker. Briefly, the polymerization suspension in 25 ml round-bottom flask, consisting of 246 mg of MBAAm, 76 mg of APBA, 100 mg poly(MBAAm-co-MAA) particle, 10 mg of AIBN, 30 mg of DMP and 10 mL of DMF/water (95/5, v/v) mixture, was degassed in an ultrasonic bath for 3 min, then purged with nitrogen for 10 min. Subsequently, the reaction mixture was stirred at 70℃ for 24 h. After cooled to room temperature, 147 μl of hexylamine were introduced in the round bottom flask. The reaction mixture was stirred at room temperature for 12 h. During this period, the originally light-yellow solution turned colorless. The products were collected by centrifugation and washed three times with DMF/water (95/5, v/v) mixture and once with ethanol/water (50/50, V/V) to obtain purified core-shell poly(MBA-co-VPBA)@(MBA-co-APBA) nanoparticles (designated as RAFT-PP NPs). Such particles were then dried under vacuum at room temperature. As a control, polymer production was prepared by precipitation polymerization (designated as PP NPs) under identical conditions but omitting the CTAs (DMP) in the reaction system.

3 Characterization

Scanning electron microscopy (SEM) analysis was performed on JSM-6360 LV (JEOL, Tokyo, Japan). Transmission electron microscopy (TEM) analysis was performed on JEM-2000 EX (JEOL, Tokyo, Japan). Dynamic light scattering (DLS) and zeta potential measurements were carried out on Malvern Nano Z Zetasizer (Worcestershire, UK). The particles were dispersed in water during the measurement. The boron element on the surface of nanoparticles was characterized by X-ray photoelectron spectroscopy (XPS) using VG ESCALAB MKⅡ(Crawley, UK). The contents of B element in the particles were determined by inductively cou-pled plasma atomic emission spectrometry (ICP-AES) using Hitachi P-4010 instrument operated at 40.68 MHz.

4 boronate affinity Investigation by ARS based fluorimetricassay

The prepared RAFT-PP NPs (0.1 mg) were mixed with 0.05 mM ARS solution (100 mM phosphate buffer, pH 9.0, 1 mL). After shaking for 1 h at room temperature, the mixture was centrifugated at 10000 rpm for 15 min. After the supernatant was decanted, the deposit was rinsed repeatedly with the phosphate buffer (100 mM, pH 9.0), to remove the nonspecifically adsorbed ARS. Subsequently, the resulting particles were resuspended in the same phosphate buffer and visualized with Fluo-3/AM by laser scanning confocal microscope (LSCM, Leica TCS SP2, Germany). For comparison, the poly(MBAAm-co-VPBA) nanoparticles were also applied for ARS-responsive characterizationfollowing the same protocol.

5 Glycoprotein recognition

RAFT-PP NPs (1 mg) were suspended in 200 μL of standard glycoprotein solution (50 mM NH_4HCO_3, pH 9.0), incubated with shaking for 2 h at room temperature, and then centrifugated at 10000 rpm for 15 min. After the supernatant was decanted, the deposit was rinsed repeatedly with the same NH_4HCO_3 buffer, to remove the nonspecifically adsorbed proteins. Finally, 20 μL of ACN: H_2O: TFA (50: 49: 1, v/v/v) was added to release the glycoproteins at room temperature for 1 h, and the supernatant was deposited on MALDI plate directly after being centrifuged at 10000 rpm for 15 min. For comparison, the poly(MBAAm-co-VPBA) cores and PP NPs were also applied for glycoprotein enrichment following the same protocol.

In contrast to that from standard proteins, glycoprotein purification from egg white followed a modified protocol. Briefly, 2 μL egg white was diluted to 200 μL with 30% ACN (v/v) in 100 mM phosphate buffer (pH 9.0) and incubated with prepared boronate core-shell nanoparticles (1.5 mg). After removing the unabsorbed proteins, 100 mM acetate buffer (pH 2.7) containing 50% ACN (v/v) was added to elute the enriched glycoproteins. Finally, half of the supernatant was dried with Speed vac Concentrator for further sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

2.6 MS analysis

MALDI-TOF MS was performed on Ultraflex Ⅲ TOF/TOF (Bruker Daltonics, Bremen, Germany). Sinapic acid (SA) matrix solution (20 mg/mL) was prepared in ACN: H_2O: TFA (60: 40: 1, v/v/v). Equivalent amounts (0.5 μL) of the sample and SA were sequentially dropped onto the MALDI plate for MS analysis. Spectra was obtained in positive ionization mode using linear detection.

2.7 Protein adsorption kinetics

With HRP as the sample, the binding capacity of RAFT-PP NPs toward glycoproteins was investigated. 1 mg RAFT-PP NPs were firstly incubated with 900 μL HRP solution (1 mg/mL, 100 Mm phosphate buffer, pH 9.0) at room temperature. Then, the suspension was centrifuged and the protein concentration of supernatant was measured by HPLC at regular intervals. The adsorption capacity (Q mg/g) was calculated according to the equation below:

$$Q = \frac{(C_0 - C_t)\mathrm{V}}{10^{-3} m}(mg/g)$$

Where C_0 (mg/mL) is the initial protein concentration, C_t (mg/mL) is the supernatant protein concentration, V (mL) is the volume of protein solution and m (mg) is the weight of the RAFT-PP NPs.

2.8 SDS-PAGE analysis

The proteins were boiled in 2× reducing sample buffer at 100 ℃ for 3 min. Gel electrophoresis for the separation of proteins was carried out by regular SDS-PAGE system with 12% resolving gel and 5% stacking gel according to the operating manual (Bio-Rad, Hercules, CA, USA). Gel contents were visualized with silver staining.

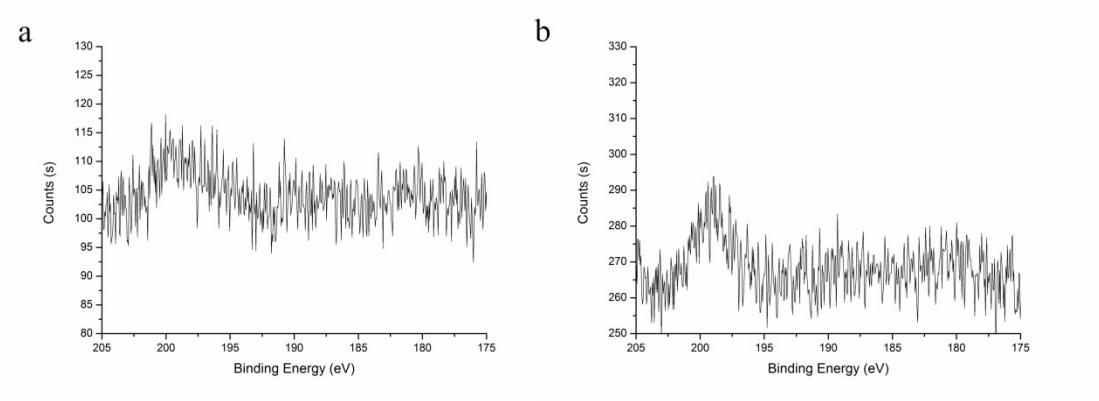

Fig S1 a) XPS spectra of elemental boron from PP NPs B) poly(MBA-co-MAA) particles.

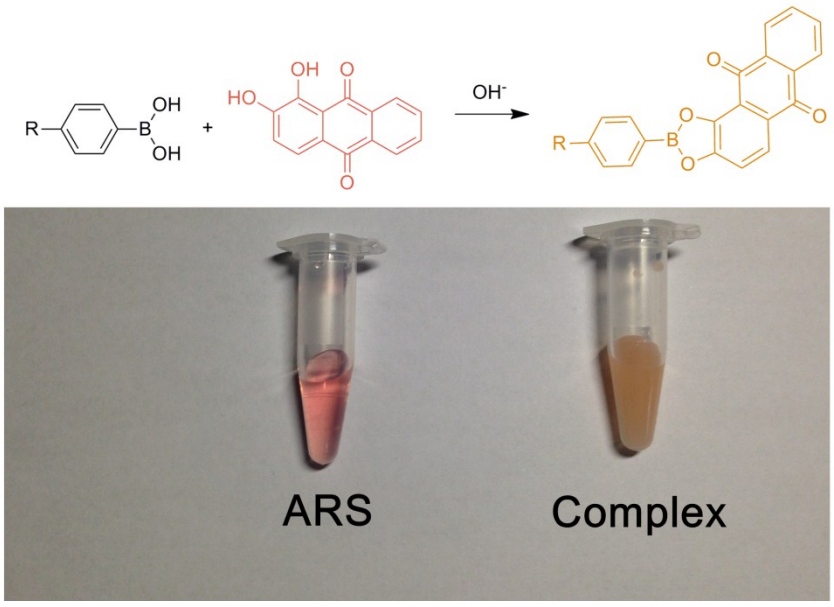

Fig. S2 The illustration and color changes of reaction between ARS and boronic aicd.

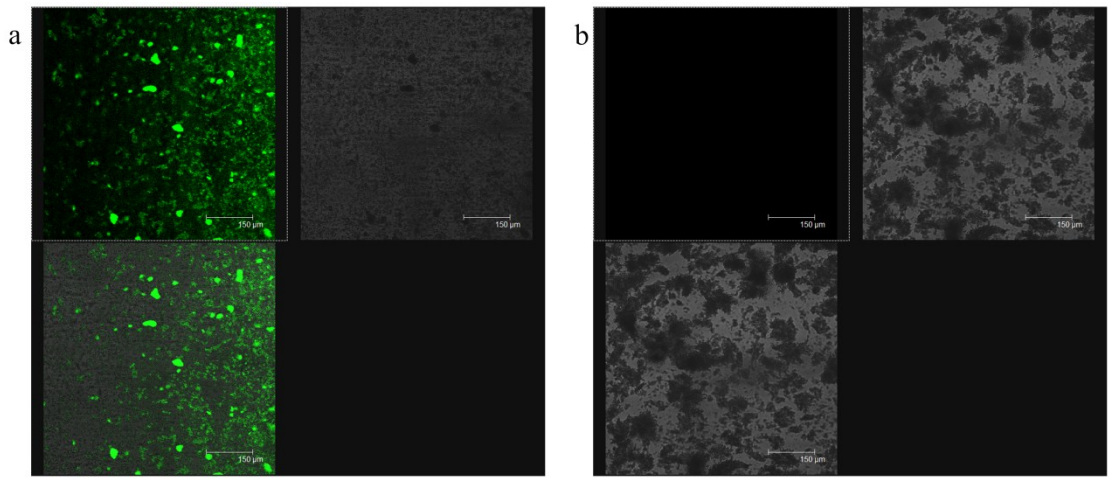

Fig S3 a) The laser scanning confocal microscope images of RAFT-PP NPs b) poly(MBA-co-MAA) particles

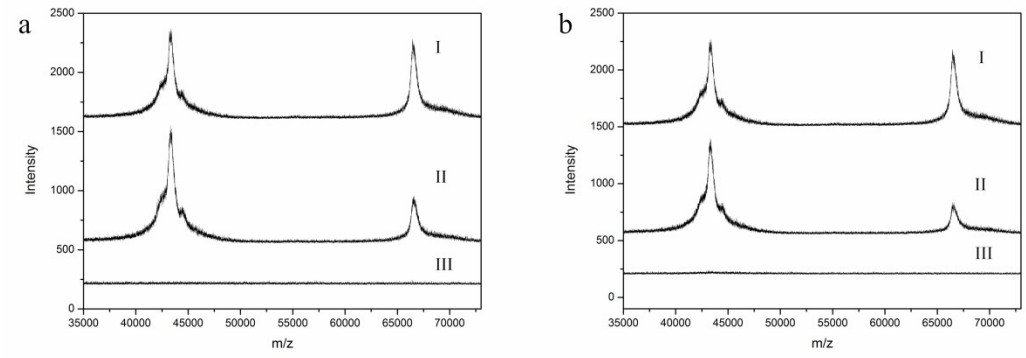

Fig S4. MALDI-TOF mass spectra of HRP and BSA mixture at a ratio of 1:1 (m/m, 50 µg/µL): (I) before enrichment; (II) supernatant; and (III) elute after enrichment with (a) PP NPs and (b) poly(MBA-co-MAA)

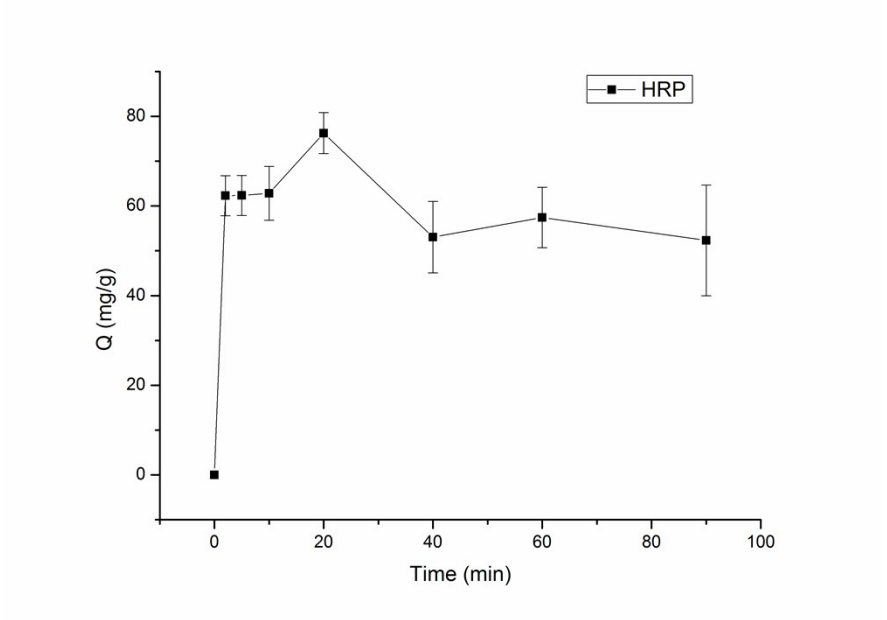

Fig S5. Adsorption kinetics for HRP and BSA on RAFT-PP. NPs: 1 mg; V: 1.0 mL; temperature: RT; incubation time: 90 min;

Table S1 The mass concentration percentage of B elements obtained by ICP-AES of three kind of particles

sample	B element wt (%)[a]
Cores	0
PP NPs	0.09
RAFT-PP NPs	0.89

[a]Without B element in the core particle poly(MBAAm-co-MAA), the data was corrected based on that B element in poly(MBAAm-co-MAA) was zero.