Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Glycoproteins Recognition by Water-Compatible Core-shell Polymeric Submicron Particles

Electronic Supplementary Information

Experimental Section

1. Chemicals

Horseradish peroxidase (HRP), ribonuclease B (RNase B, bovine pancreas), myoglobin (MYO, equine heart), ovalbumin (OVA, chicken egg white), conalbumin (COA, chicken egg white), urea (99.5%), alizarin red S (ARS), N,N-methylenebisacrylamide (MBAAm, 98%), and 4-vinylphenylboronic acid (VPBA) were obtained from Sigma-Aldrich (St. Louis, MO). Bovine serum albumin (BSA, bovine serum) was obtained from Sino-American Biotec (Luoyang, China). 2,2'-Azobisisobutyronitrile (AIBN) was purchased from the Fourth Shanghai Reagent Plant (Shanghai, China) and recrystallized before use. Anhydrous ethanol (analytical reagent) was obtained from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Water was purified by a Milli-Q system (Millipore, Milford, MA). A commercially available egg white sample was used.

2. Synthesis of poly(MBAAm-co-VPBA)@VPBA nanoparticles

In the first stage, the poly(MBAAm-co-VPBA) core was prepared with VPBA as the functional monomer and MBAAm as the crosslinker by precipitation polymerization. MBAAm (1.2 mmol), VPBA (0.2 mmol), and free-radical initiator AIBN (2 wt% relative to the total monomer) were dissolved in 10 mL of water/ethanol (4/1, v/v) in a 50 mL round-bottomed flask. The polymerization solution was then purged with nitrogen for 10 min. Subsequently, the flask was submerged in an oil bath, heated to 70 °C, and then maintained for 18 h with magnetic stirring. For the second reaction period, an additional 2 mL of ethanol solution containing VPBA (0.2 mmol) and AIBN (2 wt% relative to the total monomer) was added into the same reaction flask,

and polymerization was allowed to occur for an additional 6 h at 70 °C with magnetic stirring. After cooling to room temperature, the products were collected by centrifugation and washed repeatedly with water/ethanol (4/1, v/v) to obtain purified poly(MBAAm-co-VPBA)@VPBA core-shell nanoparticles. Finally, the collected particles were dried under vacuum at room temperature.

3. Characterization

Scanning electron microscopy (SEM) analysis was performed on a JSM-6360 LV (JEOL, Tokyo, Japan) instrument. Dynamic light scattering (DLS) and zeta potential measurements were performed on a Malvern Nano Z Zetasizer (Worcestershire, UK). The nanoparticles were dispersed in water during the measurement. Infrared measurements were acquired with a PerkinElmer Spectrum GX spectrometer (San Jose, CA) by mixing nanoparticles with KBr to prepare samples. Dried nanoparticles were analyzed by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) using a Netzsch STA 449 F3 Jupiter® simultaneous thermal analyzer (Burlington, MA) in argon with a heating rate of 2.5 °C min^{-1}. ^{1}H NMR spectra in deuterated DMSO were recorded on a Bruker Advance 400 spectrometer (Bruker AXS, Inc., Madison, WI, USA). The monomer and collected oligomer after 1^{st} step was immersed in deuterated DMSO for approximately 1 h, respectively. Then the solution was recovered and concentrated for the NMR analysis.

4. ARS-based colorimetric assay

Briefly, 10 μL of a VPBA stock solution in ethanol (0, 3.5, 7, 35 or 140 mM) and prepared poly(MBAAm-co-VPBA) nanoparticles (0.5 mg) were mixed with 0.1 mM ARS (100 mM phosphate buffer, pH 9.0, 200 μL). After incubation for 1 h at room temperature, the UV-Vis absorption of the VPBA solutions (after 5-fold dilution) was measured in an Agilent Technologies Cary 60 UV-Vis spectrophotometer (Santa Clara, CA). The suspension of polymer nanoparticles was centrifuged at 7155 g for 10 min. After the supernatant was decanted, the pellet was rinsed repeatedly with phosphate buffer (100 mM phosphate buffer, pH 9.0) to remove nonspecifically

adsorbed ARS. The color changes of the nanoparticles before and after the binding of ARS were observed.

5. Glycoprotein recognition

Poly(MBAAm-co-VPBA)@VPBA core-shell nanoparticles (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 centrifuged at 7155 g for 15 min. After the supernatant was decanted, the deposit was rinsed repeatedly with the same NH_4HCO_3 buffer to remove nonspecifically adsorbed proteins. Finally, acidic aqueous solution (20 μL) containing 50% ACN and 1% TFA was added at room temperature for 1 h to release glycoproteins. After centrifugation at 7155 g for 15 min, the supernatant was directly deposited on a MALDI plate. For comparison, poly(MBAAm-co-VPBA) nanoparticles were also used for glycoprotein enrichment following the same protocol.

Glycoprotein purification from egg white followed a modified protocol. Briefly, 2 μL of 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 unabsorbed proteins were removed, 100 mM acetate buffer (pH 2.7) containing 50% ACN (v/v) was added to release the enriched glycoproteins. Finally, half of the supernatant was dried with a Speed Vac Concentrator prior to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

6. Binding capacity and recovery measurements

The binding capacity and recovery of glycoprotein by poly(MBAAm-co-VPBA)@VPBA nanoparticles were investigated using HRP. For binding capacity evaluation, 1 mg poly(MBAAm-co-VPBA)@VPBA was incubated with 1 mL HRP solution (0.2 mg/mL, 50 mM NH_4HCO_3, pH 9.0) at room temperature for 1 h. The supernatant was collected, and the residual protein concentration was measured by HPLC. The recovery of glycoprotein after enrichment with poly(MBAAm-co-VPBA)@VPBA nanoparticles was evaluated by the ratio of the peak area in the

HPLC chromatograms specifically eluted from poly(MBAAm-co-VPBA)@VPBA nanoparticles to the peak area before enrichment. The same experiment was repeated three times. For comparison, poly(MBAAm-co-VPBA) nanoparticles were also applied for the measurement of binding capacity following the same protocol.

7. SDS-PAGE analysis

Proteins were boiled in 2X reducing sample buffer at 100 °C for 3 min and then separated by SDS-PAGE (12% resolving gel and 5% stacking gel) according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). Gel contents were visualized by silver staining.

8. MS analysis

MALDI-TOF MS was performed on an Ultraflex Ⅲ TOF/TOF (Bruker Daltonics, Bremen, Germany) instrument. 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 sample and SA were sequentially dropped onto the MALDI plate for MS analysis. Spectra were obtained in positive ionization mode using linear detection.

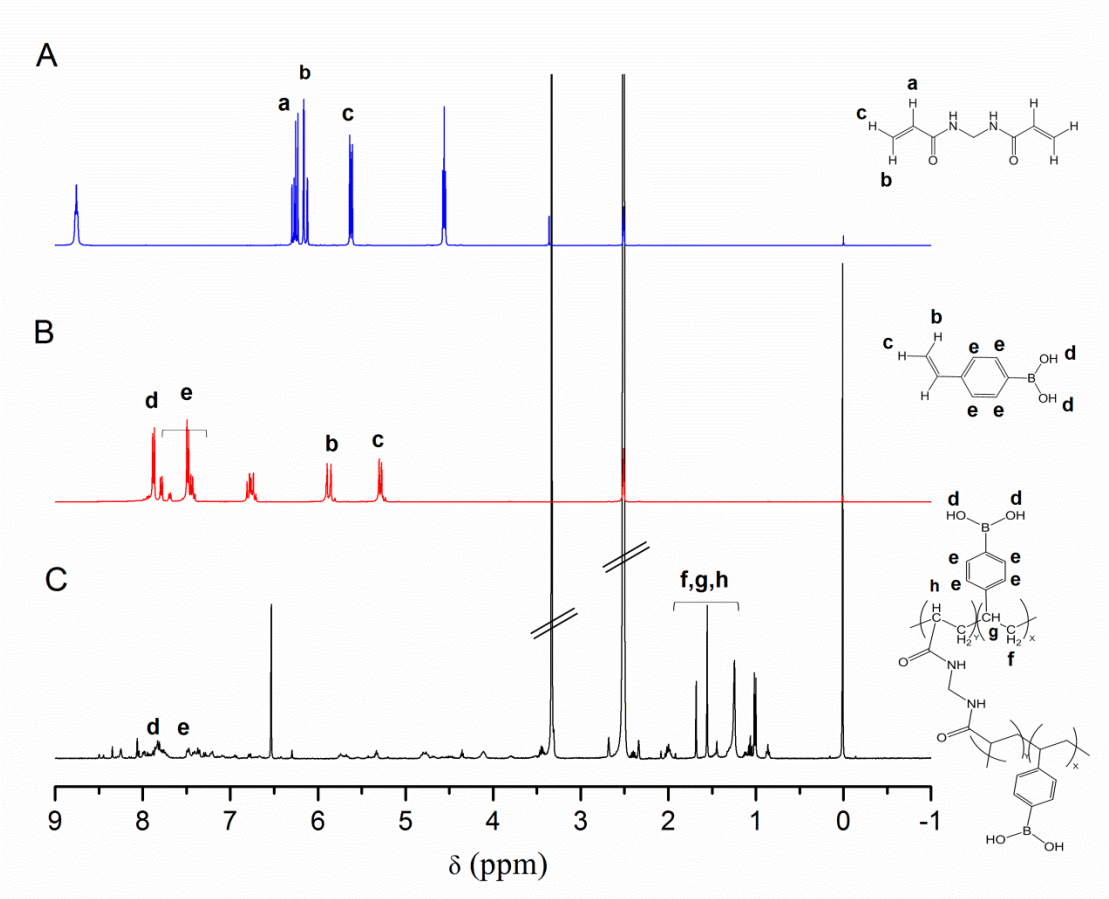

Fig. S1 ^{1}H NMR spectrum of (A) MBAAm, (B) VPBA monomer and (C) residual soluble oligomer after 1st step polymerization in deuterated DMSO.

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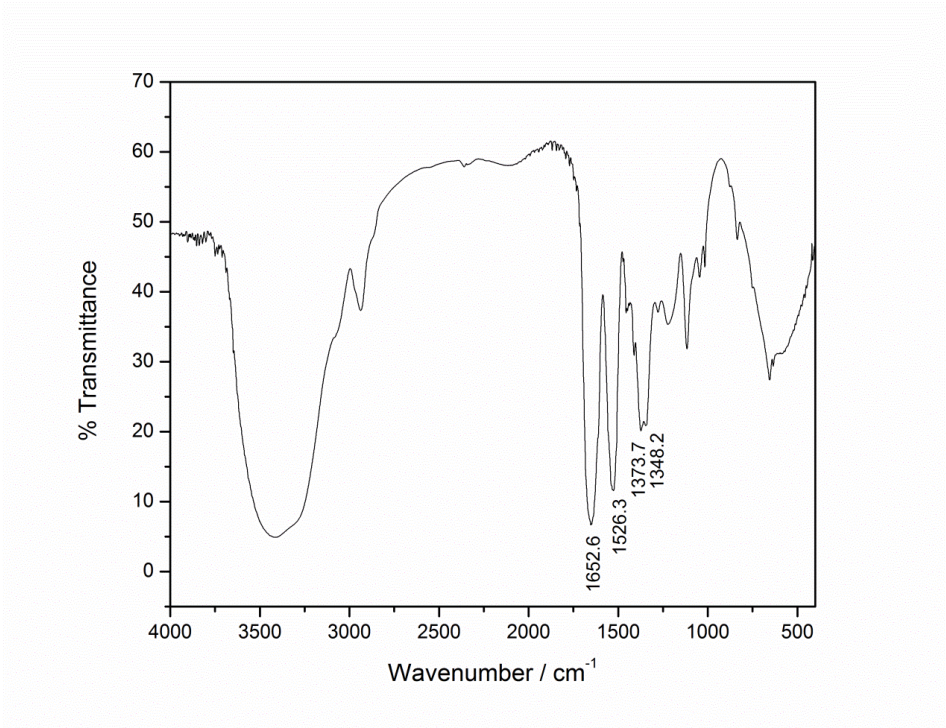

Fig. S2 FTIR spectrum of poly(MBAAm-co-VPBA)@VPBA nanoparticles.

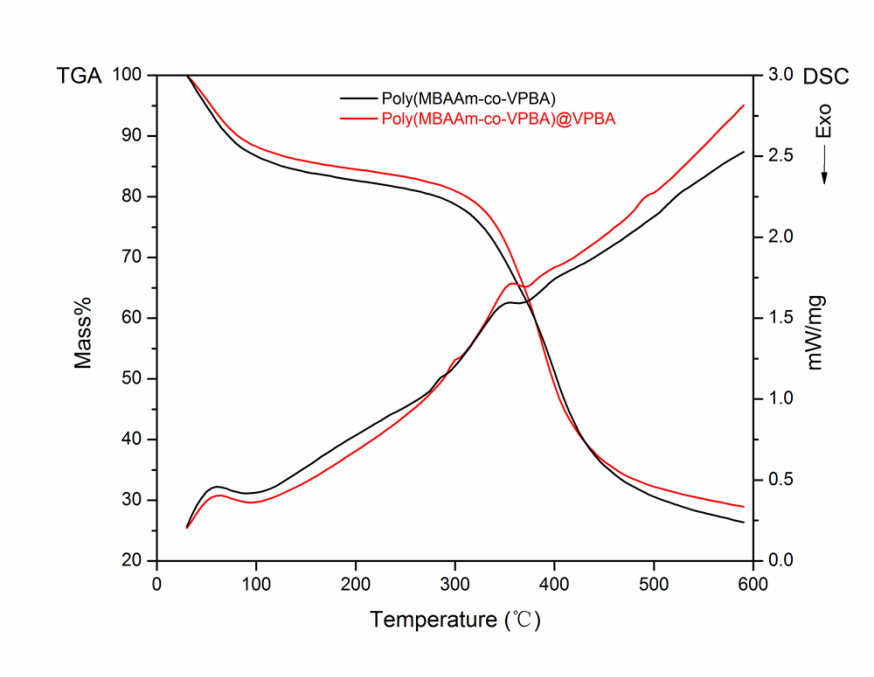

Fig. S3 TGA and DSC curves of poly(MBAAm-co-VPBA) and poly(MBAAm-co-VPBA)@VPBA nanoparticles.

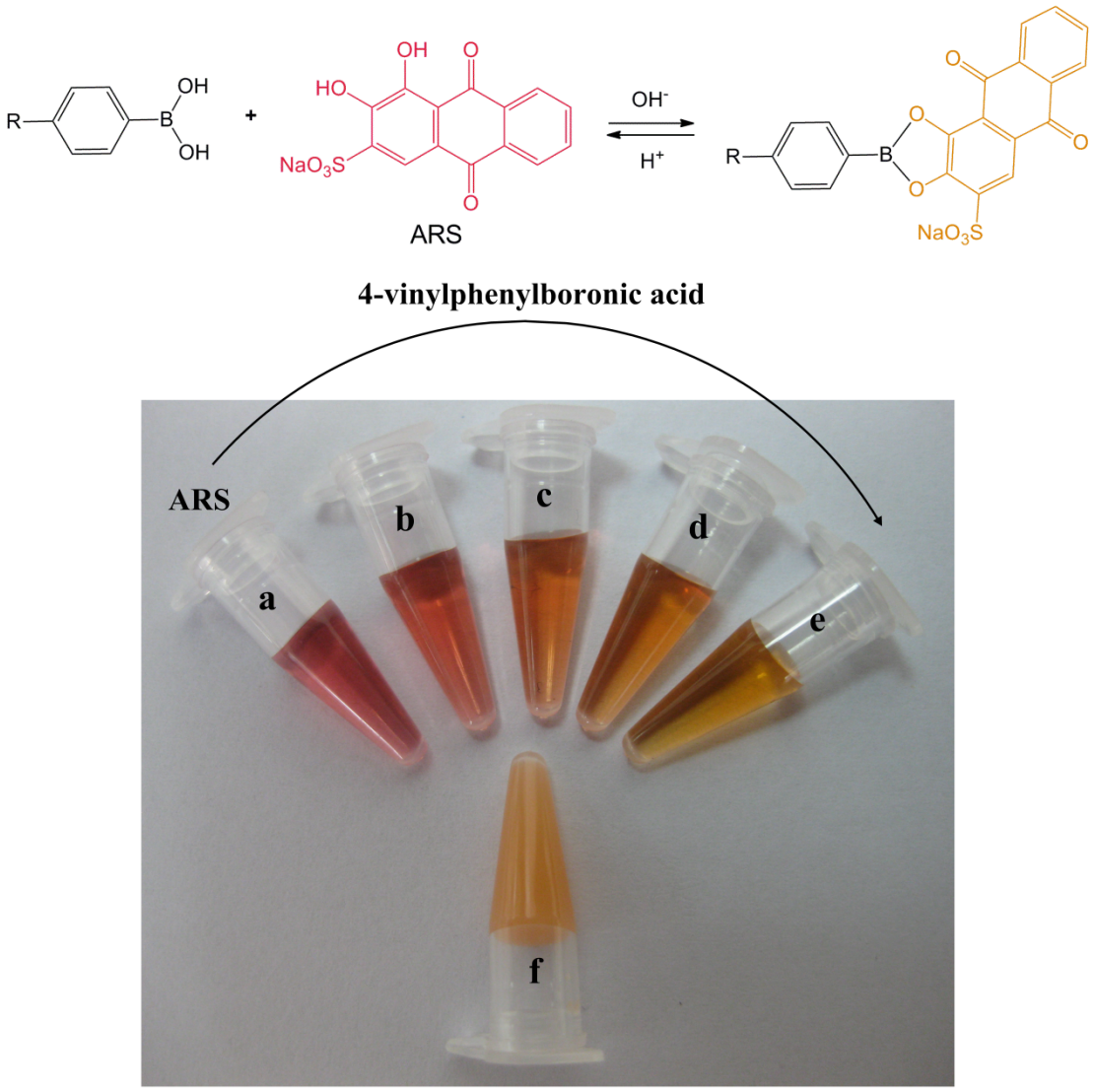

Fig. S4 Color changes of 0.1 mM ARS (100 mM phosphate buffer, pH 9.0) when mixed with 4-vinylphenylboronic acid at various concentrations. a) 0 μmol; b) 0.035 μmol; c) 0.07 μmol; d) 0.35 μmol; e) 1.4 μmol; f) poly(MBAAm-co-VPBA)@VPBA nanoparticles (0.5 mg).