Electronic Supplementary Information

Molecular imprinting of protein in Pickering emulsion

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1. Materials

Silica nanoparticles (diameter 10 nm), acrylamide (AAm, ≥98%), *N*-hydroxymethyl acrylamide solution (HMAAm, 48%), *N,N,N',N'*-tetramethylethylenediamine (TEMED, 99%) and ammonium persulfate (APS, 98%) were purchased from Sigma-Aldrich (Gillingham, UK). *N,N*-methylenebisacrylamide (BIS) was obtained from ICN Biomedicals Inc. Fluorescein isothiocyanate (FITC, ≥ 90%) was supplied by Fluka (Dorset, UK). Bovine serum albumin (BSA; MW 69 kDa, pI 4.9), hemoglobin (Hb; MW 65 kDa, pI 6.8-7.0), myoglobin (Mb; MW 17.5 kDa, pI 6.8-7.2) and ovalbumin (OVA, albumin from egg; MW 43.0 kDa, pI 4.5) were obtained from Sigma-Aldrich. Sodium dodecyl sulphate (SDS, 98%) and dichlorodimethylsilane (DCDMS, 98%) were obtained from Merck (Darmstadt, Germany). Other solvents and inorganic salts were of analytical reagent grade and were used without further purification.

2. Fluorescent labeling with FITC

To analyze the concentration of OVA and BSA easily, OVA and BSA were labeled with FITC. OVA and BSA (50 mg) were added to 30 mL aqueous solution of FITC (20 mg mL^{-1}), and the mixture was incubated at room temperature in dark for 12 h. The proteins were separated by centrifugation with a Vivaspin 20 centrifugal concentrator (Membrane: 30,000 WMCO PES). To remove the excessive FITC, the proteins were washed with water until no fluorescence could be observed in the flow through. The FITC-labeled proteins were then lyophilized in a freeze dryer.

3. Synthesis of DCDMS modified silica nanoparticles

Silica nanoparticles with a diameter of 220 nm were prepared using a procedure described in our previous work.[1] In brief, 3.6 g of silica particles (10 nm in diameter, used as seeds) were dispersed into a mixture of methanol (160 mL), water (32 mL) and ammonia (25%, 8 mL) at room temperature. After a sonication of 3 min, 10 mL of TEOS was added to the mixture under stirring, and the mixture was again stirred at room temperature for 12 h. The silica nanoparticles were separated by centrifugation, washed with methanol and water, and dried in a vacuum chamber. Hydrophobic modification of SiO_2 particles was achieved by silanization of the particles with dichlorodimethylsilane (DCDMS) using a procedure similar to that described by Horozov *et al.*[2] Typically, the silica particles (4 g) were dried at 150 °C for 60 min. After cooling, the silica particles were dispersed in 100 mL of *n*-hexanen containing 8 mL of DCDMS. The mixture was agitated for 12 h using a magnetic stirrer. The DCDMS modified silica nanoparticles were settled by centrifugation, washed with *n*-hexanen and methanol, and then dried at 150 °C.

4. Preparation of MIP hydrogels by Pickering emulsion polymerization

A water phase was first prepared as the following: 80 mg of Hb was dissolved in 8 mL of PBS buffer (150 mM, pH=7.4). After addition of AAm (250 mg), NHMAAm solution (2000 μL) and BIS (100 mg), the mixture was shaken by hand for 5 min. To break the foams, the mixture was centrifuged at a low speed (4000 rpm) for 5 min. An oil phase was prepared as the following: 300 mg of DCDMS-SiO_2 particles, 15

mL of toluene and 15 mL of *n*-hexane were mixed and sonicated for 30 min. Both the water phase and the oil phase were kept on ice for 15 min before the two phases were mixed. A Pickering emulsion was obtained by shaking vigorously the mixture. For polymerization, 100 mg of APS and 80 μL of TEMED were added into the Pickering emulsion. The Pickering emulsion was again shaken for 10 seconds, and then kept at room temperature for 12 h. After the polymerization, the solid beads were collected by decantation, and washed with a mixture of acetonitrile and water (3:1) for 3 times. For removal of the silica nanoparticles, the composite beads were transferred into a plastic tube and stirred in a mixture of acetonitrile (6 mL), water (2 mL) and HF (30%, 2 mL) at room temperature for 12 h. The polymer gels were washed with water containing 10% acetic acid and 1% SDS for 6 times, and then washed with water until no Hb could be detected from the washing solvent by using UV spectrometric measurement. The non-imprinted polymer (NIP) hydrogels were prepared using the same procedure except for the omission of the template in the pre-polymerization mixture.

5. Characterization

To illustrate the wetting properties of the SiO_2 nanoparticles, the particles were deposited on a flat surface and pressed into a thin layer using a watch glass. The wetting properties of the polymers were estimated by comparing the spreading of water droplet (100 μL) deposited on the particle layer. In Fig. S1, complete wetting was observed for the un-modified hydrophilic SiO_2 particles (Fig. S1a). In contrast, the water droplet deposited on the hydrophobic DCDMS-SiO_2 layer did not spread, and maintained a high contact angle of approximately 160^o (Fig. S1b).

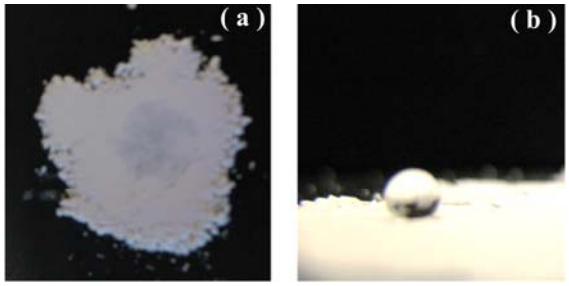

Fig. S1 Images of water drops on SiO_2 (a) and DMDCS-SiO_2 (b) thin layers.

The wettability of polymer particles was also studied in a separate experiment. The different SiO_2 nanoparticles (5 mg) were added into 1 mL of water. The mixtures were shaken for 1 min, and their photo images were taken with a digital camera (Canon A630).

The surface structures of the different SiO_2 nanoparticles were analysed using attenuated total reflection (ATR) infrared spectra on a Perkin-Elmer FTIR instrument (Perkin-Elmer Instruments). Approximately 1 mg of dry particles were placed onto the sample plate of the instrument, and the spectra in the 4000-375 cm^{-1} region were recorded with a resolution of 4 cm^{-1}, using 24 scans at room temperature.

The MIP and NIP gels were deposited on a glass slide before their optical microscope images were collected with a Nikon Eclipse E400 epifluorescence microscope equipped with a CCD camera.

6. Swelling properties of the MIP and NIP hydrogels

The swelling properties of the MIP and NIP gels were measured in PBS buffer (pH=7.4). Typically, 100 mg of MIP or NIP gels (dried by filter paper) and 2 mL of PBS buffer were placed in a 2-mL plastic tube. After a vigorous shaking for 10 min, the mixture was gently shaken on a rocking table at room temperature overnight. The excess buffer was removed by centrifugation and blotting by filter paper. The amount of PBS buffer adsorbed by the MIP or NIP gels (V_{water}) was calculated using the following equation:

$$V_{water} = \frac{m_s - m_0}{m_0 \times d}$$

where m_0 is the mass of MIP or NIP gels used (100 mg), m_s is the mass of the swollen gels, and d is the density of PBS buffer (150 mM, pH=7.4). The amounts of solvent adsorbed by the MIP and the NIP gels were 1.22 ± 0.05 mL g^{-1} and 0.62 ± 0.13 mL g^{-1}, respectively (Fig. S2).

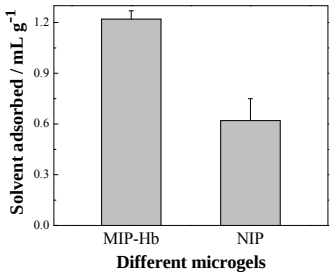

Fig. S2. Amount of water adsorbed by different hydrogels.

7. Binding experiments

Hydrogel particles (50 mg) were added into 1 mL PBS buffer (150 mM, pH 7.4) containing different amount of proteins. The mixture was gently stirred at room temperature for 12 h. After centrifugation, about 600 μL of supernatant was collected. The concentrations of the proteins were measured with a DU 800 Spectrophotometer (Beckman Coulter). The UV detection wavelengths were fixed at 408 nm for Hb and Mb, and at 492 nm for FITC-labelled OVA and BSA, respectively.

8. Synchronous fluorescence spectra

Synchronous fluorescence spectra of Hb solutions were recorded on a QuantaMaster C-60/2000 spectrofluorimeter (Photon Technology International, Lawrenceville, NJ, USA). The samples were prepared as the following: i) 250 mg of AAm, 2000 μL of HMAAm and 100 mg of BIS were dissolved in 8 mL of PBS buffer (150 mM, pH=7.4). This mixture was named as monomer phase. ii) A stock solution of Hb was prepared at a concentration of 500 mg L^{-1}. iii) A series of samples containing Hb (250 mg L^{-1}) and different percentage of the monomer phase were prepared according to Table S1. Synchronous fluorescence spectra of the samples were recorded by scanning simultaneously the excitation and emission wavelength using a fixed interval of 15 nm ($\Delta\lambda$ = 15 nm, λ_{ex} = 235-335 nm) and of 60 nm ($\Delta\lambda$ = 60 nm, λ_{ex} = 220-320 nm), respectively.

Table S1. Samples containing 250 mg L^{-1} Hb and different percentage of the monomer phase.

Samples	Amounts of the different solutions used			Components of the sample solution	
	Stock solution of Hb (μL)	Monomer phase (μL)	PBS buffer (μL)	Hb concentration (mg L^{-1})	Percentage of monomer phase (%)
1	1000	0	1000	250	0.0
2	1000	50	950	250	2.5
3	1000	200	800	250	10.0
4	1000	500	500	250	25.0
5	1000	800	200	250	40.0

References

1. Shen, X.; Ye, L. *Macromolecules* **2011**, 44, 5631-5637.
2. Horozov, T. S.; Aveyard, R.; Clint, J. H.; Binks, B. P. *Langmuir* **2003**, 19, 2822-2829.