Electronic Supplementary Information (ESI)

Thermo-sensitive imprinted polymer coating CdTe quantum dots for target protein specific recognition

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Reagents and Materials. All chemicals were of analytical grade reagents. Bovine serum albumin (BSA, MW (molecular weight) = 67 kDa), bovine hemoglobin (BHb, MW = 66 kDa), lysozyme (Lyz, MW = 14.4 kDa), ovalbumin (OB, MW = 12.4 kDa) and methylated bovine serum albumin (mBSA, MW = 68 kDa) were obtained from Sigma-Aldrich Co. (St. Louis, MO). 3-Mercaptopropionic acid (MPA, Alfa Aesar Co.) was used as the capping agent. N-isopropylacrylamide (NIPAAm) was supplied from Acros Organics USA (Morris Plains, NJ). Tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTES) were Wuhan University Silicone New Materials Co., Ltd. (Wuhan, China). L-histidine (L-His), L-glutamic acid (L-Glu), L-lysine (L-Lys), L-threonine (L-Thr), L-aspartic (L-Asp), glycine (Gly), CdCl₂•2.5H₂O, sodium dodecyl sulfate (SDS), NaBH₄, Tellurium powder and
N,N-methylenebisacrylamide (MBA) were obtained from Institute of Tianjin GuangFu Fine Chemicals (Tianjin, China).

**Synthesis and surface modification of CdTe QDs.** The water-soluble CdTe QDs were synthesized based on previous publication.\textsuperscript{S1,S2} Tellurium powder was used to prepare the NaHTe aqueous solution. In brief, it was reduced by excessive NaBH4 in water under stirring and N\textsubscript{2} bubbling. After the Te was completely reduced, a certain volume of the NaHTe solution was injected into CdCl\textsubscript{2}-MPA solution, which was deaerated by N\textsubscript{2} for 20 min. The molar ratio of Cd\textsuperscript{2+}/HTe'/MPA was set as 1: 0.5: 2.4. And then, it was heated until boiling. Under refluxing, fluorescence of the solution appeared and could be tuned in color by prolonging the refluxing time. To acquire silica coating on the surface of the QDs, a sol-gel process was adopted based on the hydrolysis of TEOS and APTES in ethanol solution in the presence of the QDs as seeds. Briefly, the QDs were added in a mixture of 10 mL ethanol, 10 mL deionized water. Subsequently, 100 \textmu L TEOS and 60 \textmu L APTES were added to the above mixture under stirring and the reaction was allowed to proceed for 2 h at room temperature. The resultant silica-coated nanoparticles were centrifuged and washed with ethanol and deionized water.

**Preparation of MIP-coated CdTe QDs.** The above composite nanoparticles were dispersed in 50 mL deionized water by ultrasonication. Then, 10 mg BHb, 100 mg NIPAAm and 40 mg MBA were added to this solution, which was incubated 2 h under stirring for pre-polymerization and the solution was deaerated by N\textsubscript{2} until the reaction finished. The polymerization was initiated by addition of 10 mg of potassium chloride.
persulfate. Polymerization was continued for 2 h and the obtained MIP-coated QDs was washed successively with SDS, ethanol, and deionized water. The non-imprinted polymer (NIP)-coated QDs was prepared using the same procedure but without addition of template molecule.

**Characterization.** Fluorescence measurements were performed on an F-4500 spectrofluorometer (Hitachi, Japan) equipped with a quartz cell (1 cm × 1 cm), the slit widths of the excitation and emission were both 5 nm, and the excitation wavelength was set at 400 nm with a recording emission range of 430-700 nm. The photomultiplier tube voltage was set at 950 V. UV-Vis spectra (200-800 nm) were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan). Transmission electron microscopy (TEM) was obtained by a Tecnai G² 20S-TWIN microscope (Philips, Holand). Fourier transform infrared (FT-IR) spectra (4000-400 cm⁻¹) in KBr were recorded using the AVATRA 360 FT-IR spectrophotometer (Nicolet, USA). X-ray photoelectron spectroscopy (XPS) measurements were performed with an ESCALAB 250 spectrometer (Thermo-VG Scientific, USA) with ultra-high vacuum generators.

**Protein adsorption experiments.** A mass of 20 mg of the particles was dispersed in certain volume of protein solution and the mixtures were agitated in a shaken bed. At different time intervals, the mixtures were centrifuged and the concentration of protein in the supernatant was measured by an UV-vis spectrophotometer at a wavelength of 405 or 280 nm. The amount of adsorbed protein can be determined by the difference in concentration before and after the adsorption.

The adsorption capacity (Q, expressed in units of mg/g) of the protein or analogue
bound to the MIP-coated QDs is calculated by

\[ Q = \frac{(C_0 - C_t) V}{W} \]

where \( C_0 \) and \( C_t \) (mg/ml) are the initial concentration and the residual concentration of the protein or analogue, respectively, \( V \) (ml) is the volume of the initial solution, and \( W \) (g) is the weight of the MIP-coated QDs.

Stern-Volmer equation analysis for the MIP- and NIP-coated QDs with protein is by

\[ \frac{F_0}{F} = 1 + K_{SV} [Q] \]

where \( F_0 \) and \( F \) were the fluorescence intensity of QDs in the absence and presence of template, respectively, \( K_{SV} \) was the Stern-Volmer constant, and \([Q]\) was the quencher concentration.

**Sample collection and pretreatment.** The human urine samples were collected from healthy volunteers. And all the samples were diluted to a 100-fold solution and no other pretreatments were necessary.

**Analytical performance of the MIP-coated QDs.** To evaluate the analytical performance of the thermo-sensitive MIP-based QDs, the detection limit and linear range were investigated. The MIP-based QDs have linear range of \( 2.76 \times 10^{-7} - 4.04 \times 10^{-6} \) M with a correlation coefficient of 0.995 for BHb. The detection limit, which was calculated as the concentration of BHb that quenched three times the standard deviation of the blank signal, was 0.16 \( \mu \)M. And the precision for three replicate detections of 2.53 \( \mu \)M BHb was 4.2% (RSD).

**References**


*Fig. S1* TEM images of the synthesized (a) CdTe QDs, (b) silica-coated QDs, (c) MIP-coated QDs and (d) NIP-coated QDs. Scale bar: a-b 50 nm; c-d 200 nm.
Fig. S2 FT-IR spectra of QDs (a), silica-coated QDs (b), NIP-coated QDs (c) and MIP-coated QDs (d).

Fig. S3 XPS survey spectra for silica-coated QDs (a), MIP-coated QDs (b) and NIP-coated QDs.
**Fig. S4** The photographs of the (a) QDs (2.0 × 10^{-4} M), (b) silica-coated QDs (60 \mu g/mL), (c) MIP-coated QDs (60 \mu g/mL) and (d) NIP-coated QDs (60 \mu g/mL) under the ultraviolet radiation.

**Fig. S5** The normalized UV-vis absorption spectra and normalized fluorescence spectra of the BHb (C_{BHb}= 10 \mu g/mL), MIP-coated QDs (C_{MIP}= 30 \mu g/mL) and MIP-coated QDs (C_{MIP}= 30 \mu g/mL) with BHb (C_{BHb}= 10 \mu g/mL).
**Fig. S6** The pH-dependent fluorescence intensity changes of MIP- and NIP-coated CdTe QDs by template BHb. F and F₀ are the fluorescence intensities of QDs in the presence and absence of BHb, respectively. The concentration of the MIP-coated QDs was 60 μg/mL, C_{BHb} = 25 μg/mL. The error bars were from three parallel experiments.
Fig. S7 Binding behaviors of proteins and amino acid on the BHb imprinted silica-coated QDs and control NIP-coated QDs. Experimental conditions: $C_{\text{MIP}} = 0.06$ mg/mL, $C_{\text{BHb}} = C_{\text{BSA}} = C_{\text{Lyz}} = C_{\text{MBSA}} = C_{\text{OB}} = C_{\text{L-His}} = C_{\text{L-Glu}} = C_{\text{L-Lys}} = C_{\text{L-Thr}} = C_{\text{L-Asp}} = C_{\text{Gly}} = 25 \mu$g/mL. $F$ and $F_0$ are the fluorescence intensities of QDs in the presence and absence of analytes, respectively.
Fig. S8 Effect of the competitive protein BSA on the binding of template protein BHb on the MIP-coated QDs. Binding was done by fixing the concentration of BHb and increasing the concentration of BSA. $C_{\text{MIP}} = 30 \, \mu\text{g/mL}$, $C_{\text{BHb}} = 25 \, \mu\text{g/mL}$. The error bars were from three parallel experiments.