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Surface molecularly imprinted nanowire for protein specific recognition

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Experimental

Materials. The anodic alumina oxide (AAO) membranes that had nominally 200-nm-diameter pores with a thickness of 60 μm were purchased from Whatman (UK). γ-Glycidoxypropylthimethoxysilane (GMPS), dopamine (DA), ammonium persulfate (APS), human and bovine hemoglobin (Hb) were purchased from Sigma (St. Louis, MO, USA) and used as received. Bovine serum albumin (BSA), ribonuclease A, horseradish peroxidase (HRP), human IgG and streptavidin were purchased from Promega (USA) and used as received. Phosphate buffer solutions (PBS) with different pHs were prepared by mixing 50 mM aqueous solution of NaH_2PO_4 and 50 mM aqueous solution of Na_2HPO_4, and PBS (pH 7.35) with different concentrations were prepared by mixing diluted NaH_2PO_4 and Na_2HPO_4 solutions. Doubly distilled water was used for preparation of all aqueous solutions.

Apparatus. The morphology of nanowires was examined with a LEO-1550 field-emission scanning electron microscope, a JEM 2100 high-resolution transmission electron microscope and an Agilent 5500 atomic force microscopy. XPS measurements were performed with an ESCALab MK2 spectrometer. A monochromatic Al Kα X-ray source was operated in CAE (constant analyzer energy) mode (CAE = 100

eV for survey spectrum and 20 eV for O1s spectrum). The static water contact angle was measured at 25^{o}C by a contact angle meter (Rame-Hart-100) employing drops of pure deionized water.

Epoxysilane modification of alumina pore walls. Before modification, alumina membrane was cleaned in an ultrasonic bath using doubly distilled water and ethanol, and then annealed (at 150 ^{o}C for 1 h) to expose the Al-O bonds on the pore wall surface.[S1] Epoxysilane modification was carried out by immersing the alumina membrane into 1% GMPS solution (ethanol/H_2O 3:1).[S2] The silanization reaction was terminated after 10 min by rinsing the membrane with ethanol thoroughly to remove the physically adsorbed GMPS. Finally, the membrane was dried under a nitrogen atmosphere at 93 ^{o}C for 2 h to produce silica nanotubes on the pore walls of AAO. The terminal epoxy groups on the inner walls of the silica nanotubes could spontaneously react with the amino groups of template protein molecules. A brief mechanical polish was necessary to remove the thin silica coating on the surface of the membrane.

Protein immobilization on pore wall of alumina membrane. The epoxysilane-coupled alumina membrane was placed into a flask under vacuum for 30 min, and 50 mM PBS containing 6.0 mg mL^{-1} template protein was then added under vacuum. After 24 h at 4 ^{o}C, the protein-immobilized alumina membrane was obtained by the reaction between amino groups of template protein molecules and terminal epoxy groups, which was then mounted into a flow device and thoroughly rinsed with pH 7.35 PBS to remove the residual protein solution. The resulting membrane was then dried at room temperature with N_2 airflow for 12 h.

Preparation of protein-imprinted polydopamine (PDA) nanowires. Several pieces of protein-immobilized alumina membranes were individually immersed into a cooled pH 7.35 PBS containing 0.3 M DA for 2 h. An equal volume of cooled 0.15 M APS solution was then added. The reaction system was sealed under vacuum for 1 h to ensure a full filling of the polymerisable DA solution into the protein-immobilized pores. After a reaction period of 2 h at 4 ^{o}C the cool reaction solution was elevated up to room temperature, at which it was maintained for 4 h, and then kept in an oil bath at 45 ^{o}C for 6 h. Afterwards, the membranes were taken out, rinsed with doubly distilled water to terminate the polymerization reaction, and dried for 5 h at 45 ^{o}C. The resulting AAO membranes were mechanically

polished to remove the undesired PDA film formed on the surface of the membrane. Finally, the alumina membrane and silica nanotubes were sequentially dissolved with 5% H_3PO_4 and 1% HF solution at 4 ^oC under oscillation, and the resulting mixture was centrifuged to obtain the protein-imprinted PDA nanowires (IPWs). During this process the protein molecules immobilized on the inner walls of silica nanotubes were also removed from the cavities of IPWs.

Similarly, the imprinted PDA nanowires (NIPWs) were synthesized as control according to the same procedure without the presence of template protein. The PDA nanowires were also prepared for examining the surface morphology and component of nanowires by directly using the AAO as nanomold without any modification.

Rebinding of proteins to protein-IPWs. Different amounts of the obtained protein-IPWs or NIPWs were mixed with 1.0 mL pH 7.35 PBS containing 120 μg mL^{-1} bovine or human Hb or other proteins and 0.01% Tween-20 and oscillated at 25 ^oC for 3 h. After the removal of nanowires by centrifugation, the concentrations of bovine or human Hb in the supernatants were determined using a UV-3600 spectrophotometer. The bound amounts of template proteins on nanowires were determined by the difference between total template amount and residual amount in solution, respectively.[S3]

Effect of molar ratio of DA to APS on the morphology of PDA nanowires

When the molar ratio was lower than 1:1 no obvious polymer could be formed in the pore (Fig. S1A). The AAO could even be partly or fully dissolved at high APS concentration. When the molar ratio was 1.5:1, the imprinted polymer in the pores showed wall-conglutinated nanotubes (Fig. S1B) with uncertain wall-thickness (Fig. S1C). When the molar ratio was higher than 2:1, the imprinted polymer nanowires showed breakable structure (Fig. S1D).

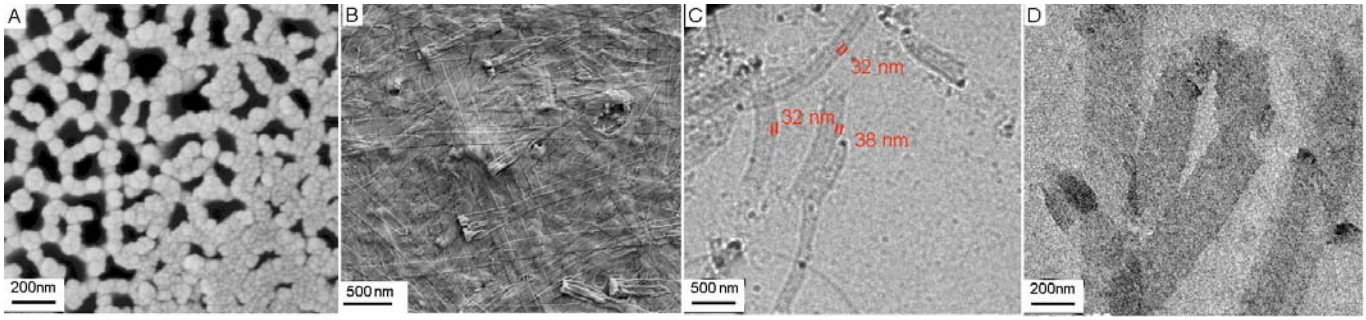

Fig. S1 SEM (A,B) and TEM (C,D) images of PDA formed in the pore of AAO at DA/APS molar ratios of 1:1 (A), 1.5:1 (B and C) and 3:1 (D).

Hydrophilicity of PDA nanowires

The hydrophilicity of MIPs plays an important role in the efficient recognition of biomolecules. So it is necessary to characterize the hydrophilicity of PDA nanowires with contact angle. The PDA nanowrie-coated glass slide (Fig. S2B) showed a much lower contact angle than the bare glass slide without any treatment (Fig. S2A), indicating better compatibility for specific binding of biomolecules to the receptors.

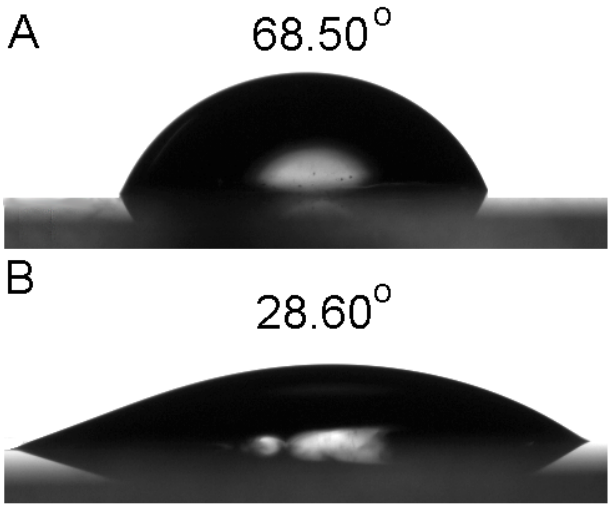

Fig. S2 Contact angles of glass slide (A) and PDA-coated glass slide (B).

O1s XPS spectrum of PDA nanowires

The well fitted O1s spectrum (Fig. S3) shows the oxygen-containing groups at 533.64 eV.

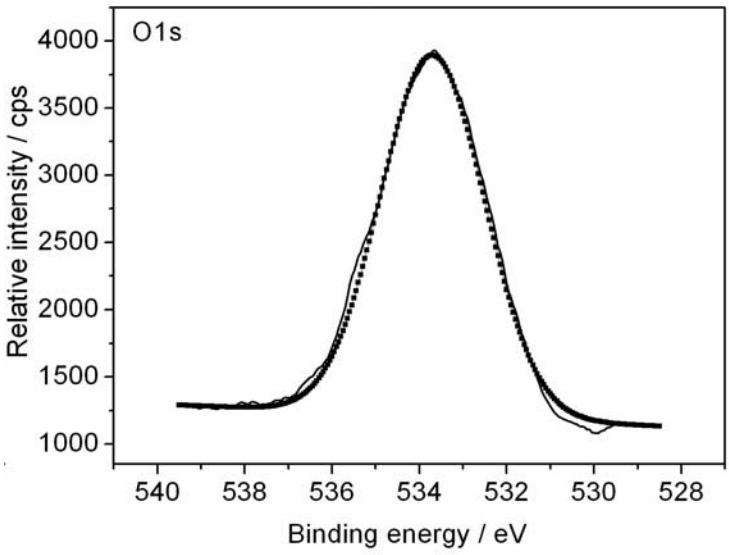

Fig. S3 O1s XPS fitted spectrum of PDA nanowires.

XPS characterization of AAO at different modifying stages

Silica nanotubes with a wall thickness of several nanometers were firstly deposited on the pore walls of AAO with an epoxysilane-related sol-gel process. The terminal epoxy groups on the inner walls of silica nanotubes then reacted spontaneously with free amino sites of protein to attach template protein molecules to the inner walls of AAO.[S4] This method simplified greatly the immobilization procedure of protein for preparation of MIPs, as compared with that using glutaraldehyde as a linker.[S3] In comparison with the XPS survey spectrum of non-modified AAO (Fig. S4A), the presence of Si2p peak at 103.05 eV in epoxysilane modified AAO (Fig. S4B) indicated that epoxy groups were successfully introduced to the pore wall of AAO. Similarly, the efficient coupling of template protein on the inner wall of silica nanotubes was clearly confirmed by the appearance of N1s peak at 401.40 eV in Fig. S4C.

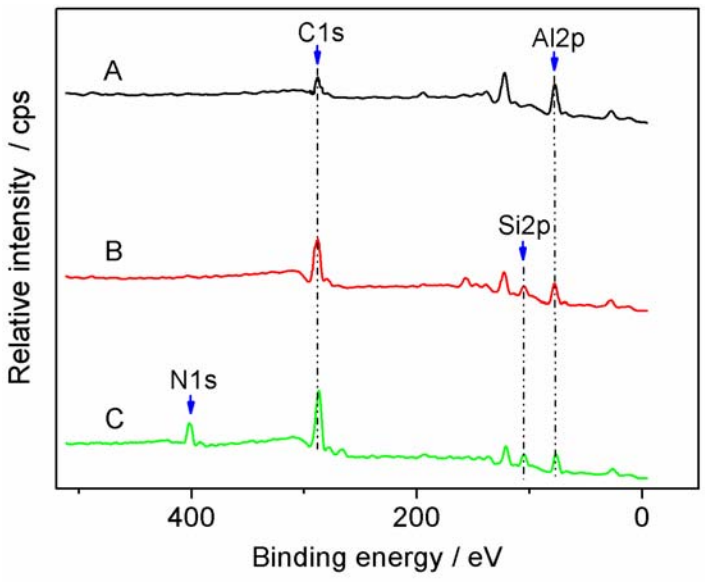

Fig. S4 XPS survey spectra for AAO (A), epoxysilane-modified AAO (B) and protein-epoxysilane-modified AAO (C).

Morphology of protein-IPWs

With a polymerization process by immersing the protein-coupled alumina membranes in the solution containing DA and APS at a molar ratio of 2:1, the protein-IPWs could be obtained after the alumina membranes and template protein were removed. The resulting IPWs were characterized by SEM, TEM and AFM (Fig. S5), which also showed good monodispersibility and uniform surface morphology.

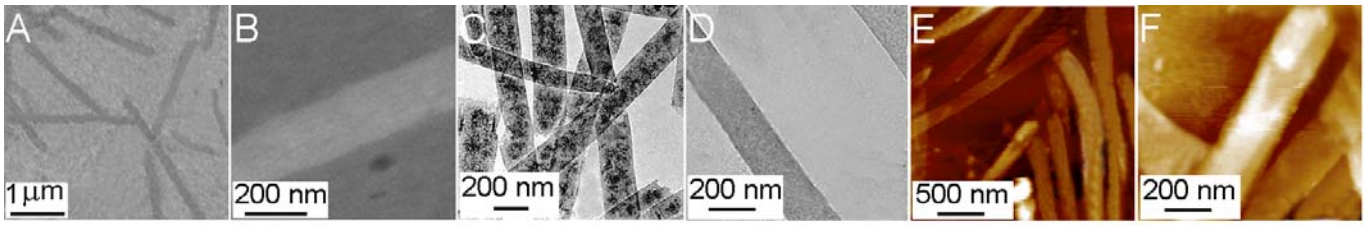

Fig. S5 SEM (A and B), TEM (C and D) and AFM (E and F) images of bovine Hb-IPWs at two amplification scales.

Effects of ionic strength and pH on binding amount of target protein

Fig. S6 shows the effects of ionic strength and pH on the binding amount of bovine Hb. The binding amount of bovine Hb at pH 7.35 slightly decreases with the increasing ionic strength due to the relatively competing of bovine Hb with PBS species, while the amount shows a maximium value at pH 7.35. To maintain the solution pH 50 mM PBS at pH 7.35 was used as the medium for the target protein binding.

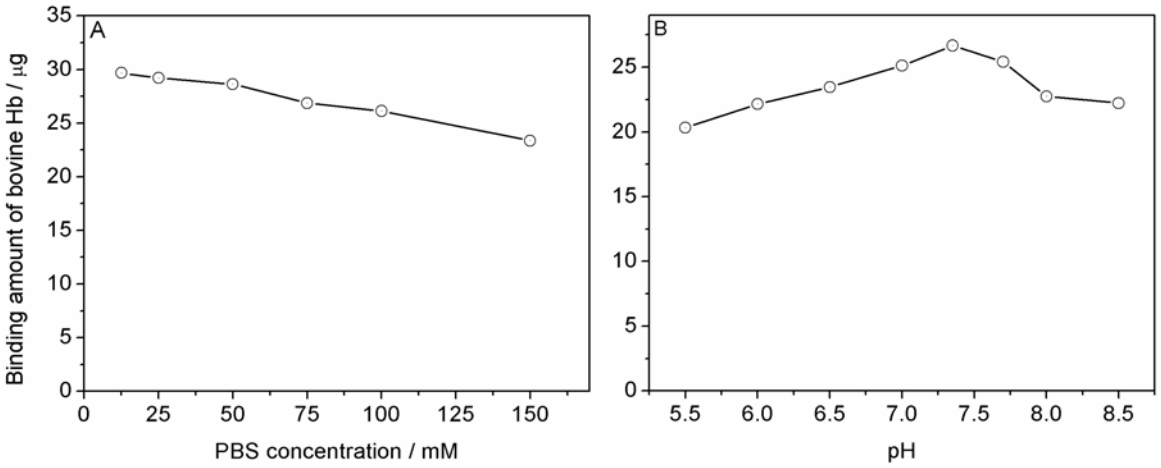

Fig. S6 Effects of ionic strength and pH on binding amount of 1.0 mL 1.0 mg mL^{-1} bovine Hb-IPWs bovine Hb.

Binding profiles of human Hb-IPWs and NIPWs

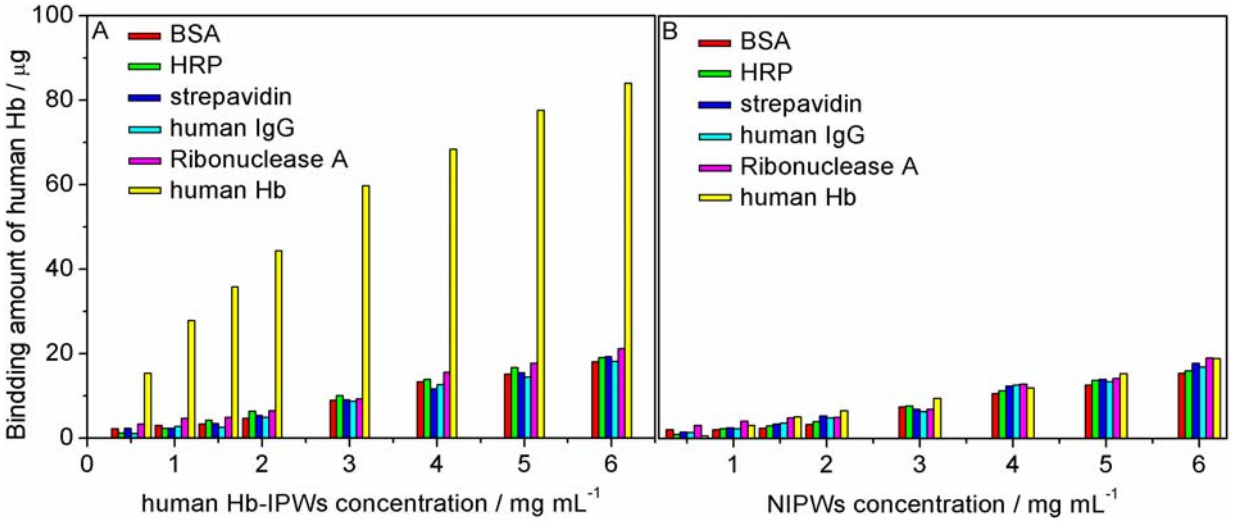

Fig. S7 Binding profiles of human Hb-IPWs (A) and NIPWs (B) to human Hb and other reference proteins.

Binding amounts of target proteins to IPWs

The binding amounts of bovine and human Hb to the individual IPWs were investigated at the different concentrations of target proteins (Fig. S8). At the nanowire concentration of 1.0 mg ml^{-1}, the binding amounts of both target proteins initially increased with the increasing target protein concentration, and then reached saturated binding. Two maximum binding amounts were 25.33±0.51 and 27.21±0.60 mg g^{-1}, respectively, indicating the high binding capacity of IPWs towards target proteins.

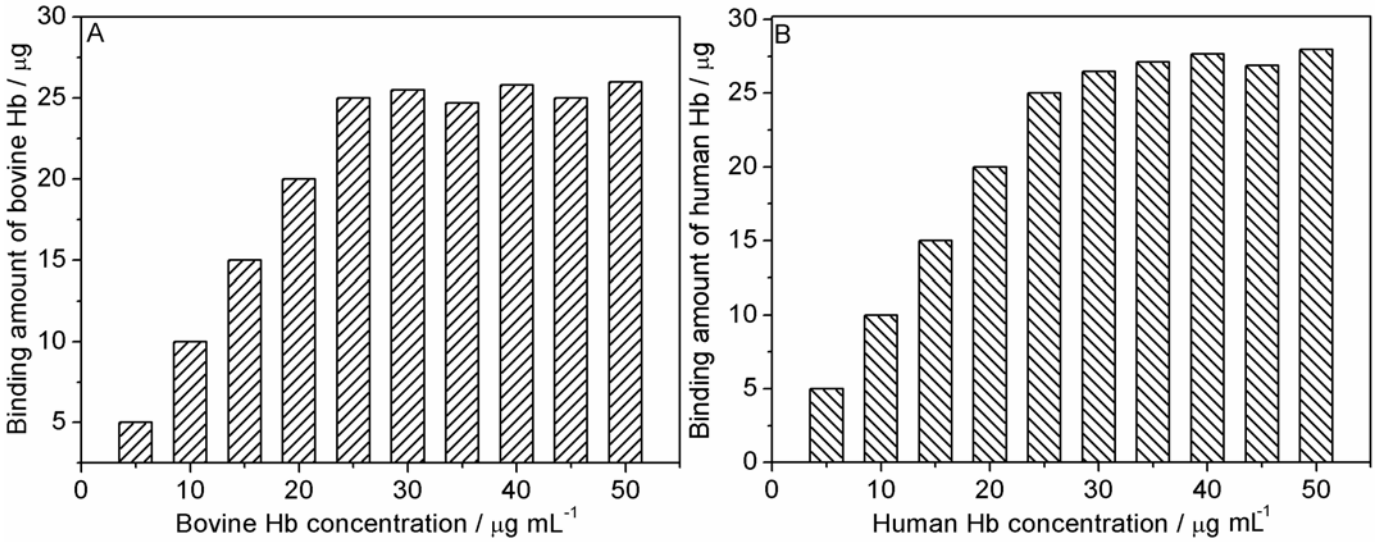

Fig. S8 Binding amount of 1.0 mL 1.0 mg mL^{-1} IPWs to bovine (A) and human (B) Hb at different concentrations.

Specificity of bovine and human Hb-IPWs

Five proteins including BSA (isoelectric point (IP) 4.7), streptavidin (IP 5.0), HRP (IP 7.2), human IgG (IP 9.0) and ribonuclease A (IP 9.6) were used as competitors in the coexistence of equivalent template protein for investigating the competitive bindings of bovine and human Hb-IPWs (Fig. S9). In the presence of the competing protein, the relative rebinding of template proteins to the IPWs still reached above 80%, much higher than 60% of polyacrylamide nanowires.[S3]

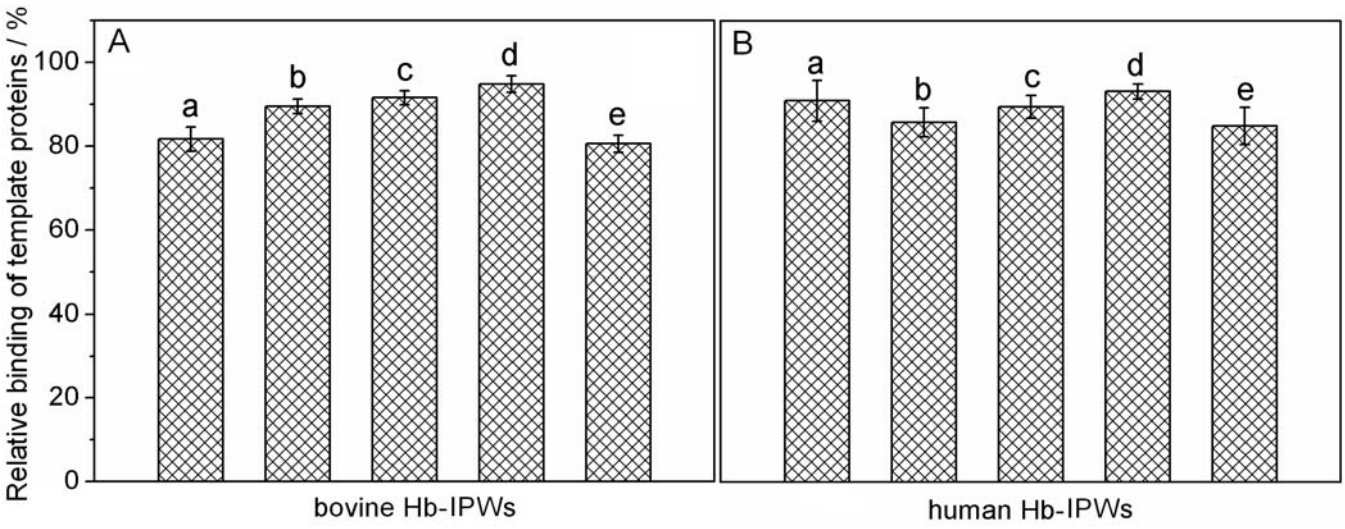

Fig. S9 Competitive binding of template bovine (A) and human (B) Hb with nontemplate BSA (a), HRP (b), streptavidin (c), human IgG (d), and ribonuclease A (e) at 120 μg mL^{-1} to IPWs at pH 7.35.

Additionally, the binding equilibrium constants of the two IPWs towards these proteins could be calculated according to the following equations:

$$K_B = C_s / C_l \qquad (1)$$

It can be seen that the IPWs showed much greater binding constants towards target proteins than five reference proteins (Table S1), displaying high selectivity of IPWs towards the template proteins.

Talbe S1 Binding constants of IPWs and NIPWs to target and reference proteins

Proteins	K_{BI} (bovine Hb-IPWs)	K_{BI} (human Hb-IPWs)	K_{AN} (NIPWs)
BSA	0.100±0.085	0.081±0.062	0.070±0.052
HRP	0.090±0.073	0.079±0.072	0.069±0.056
Streptavidin	0.091±0.073	0.084±0.066	0.075±0.059
Human IgG	0.081±0.068	0.077±0.063	0.072±0.057
Ribonuclease A	0.102±0.064	0.106±0.075	0.082±0.059
Bovine Hb	0.670±0.42	0.073±0.051	0.071±0.064
Human Hb	0.077±0.060	0.735±0.58	0.074±0.062

Homologous proteins, bovine and human Hb, were chosen as templates to further evaluate the specific recognition ability of the designed imprinted nanowires (Fig. S10). The relative binding amounts of template proteins against homologous proteins were unexpectedly up to 4.1 and 4.5 for bovine and human Hb-IPWs, respectively, in despite of their similar amino acid sequences and 3D structures. The result confirmed the high specificity and selectivity of the imprinted nanowires towards template proteins, which could be contributed to the difference of homologous proteins in polar interactions and steric complementarity with the well-defined network of the imprinted cavity.

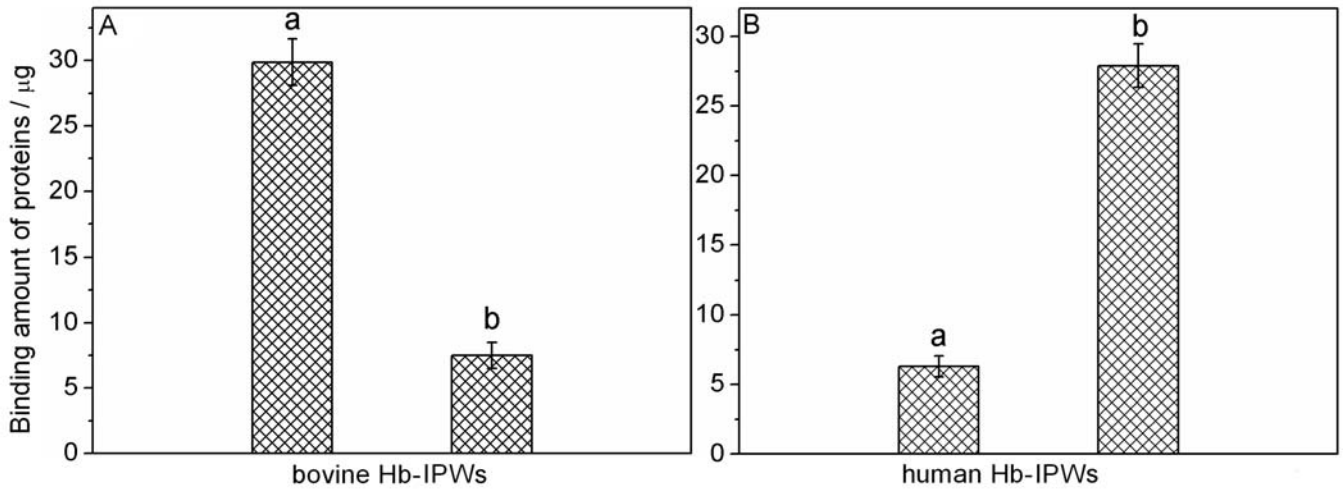

Fig. S10 Homologous protein recognitions of bovine (A) and human (B) Hb-IPWs at the nanowire concentration of 1.0 mg mL^{-1}. a: bovine Hb; b: human Hb.

References

S1 (*a*) M. Steinhart, J. H. Wendorff, A. Greiner, R. B. Wehrspohn, K. Nielsch, J. Schilling, J. Choi and U. Gösele, *Scienc*e, 2002, **296**, 1997; (*b*) J. J. Qiao, X. H. Zhang, X. M. Meng, S. M. Zhou, S. K. Wu and S. T. Lee, *Nanotechnology*, 2005, **16**, 433.

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