Electronic Supporting Information (ESI)

Biotemplated Synthesis of Au Nanoparticles-TiO_2 Nanotube Junctions for Enhanced Direct Electrochemistry of Heme Proteins

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Experimental Details

Materials and Reagents. Titanium foil (0.1 mm thickness, 99.6% purity) was obtained from Advent Research. Hemoglobin (from bovine blood), Bovine serum albumin (BSA) were purchased from Sigma-Aldrich Chemicals Co. Hydrochloroauric acid ($HAuCl_4$), ammonium fluoride (NH_4F) and other chemicals were of analytical grade and used without further purification. Phosphate-buffered solutions (PBS) (0.1 mol L^{-1}, pH 4.2 to 9.2) was prepared by varying the ratio of KH_2PO_4 to Na_2HPO_4. All aqueous solutions were prepared with deionized water（＞18 MΩ).

Apparatus. The morphologies of the fabricated inorganic layers were characterized using a field-emission scanning electron microscope (Hitachi FE-SEM S4800, Japan). The UV-vis absorption spectra were measured on a UV-3900

spectrophotometer (Hitachi, Japan). X-ray photoelectron spectra (XPS) were recorded on a Perkin–Elmer Physical Electronics 5600 spectrometer using Al Ka radiation at 13 kV as excitation source. The takeoff angle of the emitted photoelectrons was 45^{o}, with a resolution of 0.1 eV, using the binding energy of Ti 2p signal (458.0 eV) as the reference. Electrochemical measurements were carried out using a CHI660D electrochemical workstation (CH Instrument Co. Shanghai). The TiNT arrays acted as the working electrodes (8 mm in electrode diameter). A Pt foil and a saturated calomel electrode (SCE) were used as the counter and reference electrodes, respectively. The electrochemical impedance spectra were recorded at an open circuit potential with a signal amplitude of 5 mV over a frequency range of 0.1 – 100 000 Hz in 0.1 M KCl containing 2.0 mM Fe $(CN)_6^{3-/4-}$.

Synthesis of TiO_2 nanotube (TiNT) arrays. TiO_2 nanotube layers were formed by anodization of Ti. For this, Ti sheets of 0.1 mm thickness were degreased by sonication in acetone and ethanol, followed by rinsing with DI water and drying in a nitrogen stream. The sheets were anodized in an electrolyte of glycerol/water (50:50) with 0.27 M NH_4F at 30 V for 2 h where the Ti foils were the working electrode, and a platinum gauze served as the counter electrode. The asformed samples were annealed in ambient air at 450 ^{o}C for 1 h.

Preparation of AuNPs-TiNT junctions. All TiNT samples used for gold nanoparticles (AuNPs) decorating are anatase crystalline. TiNT samples were first incubated in a PBS solution (pH 7.0) containing 1% BSA for 12 h at 4 ^{o}C. After incubation, the samples were rinsed with DI water and then soaked in a 10 mg mL^{-1}

$HAuCl_4$ solution for 4 h and followed by a careful rinse with DI water. The samples were then irradiated for 45 min using a UVA lamp (300 W, medium pressure Hg lamp with the emission maximum centre near 360 nm, the distance between the lamps and samples was fixed at 12 cm).

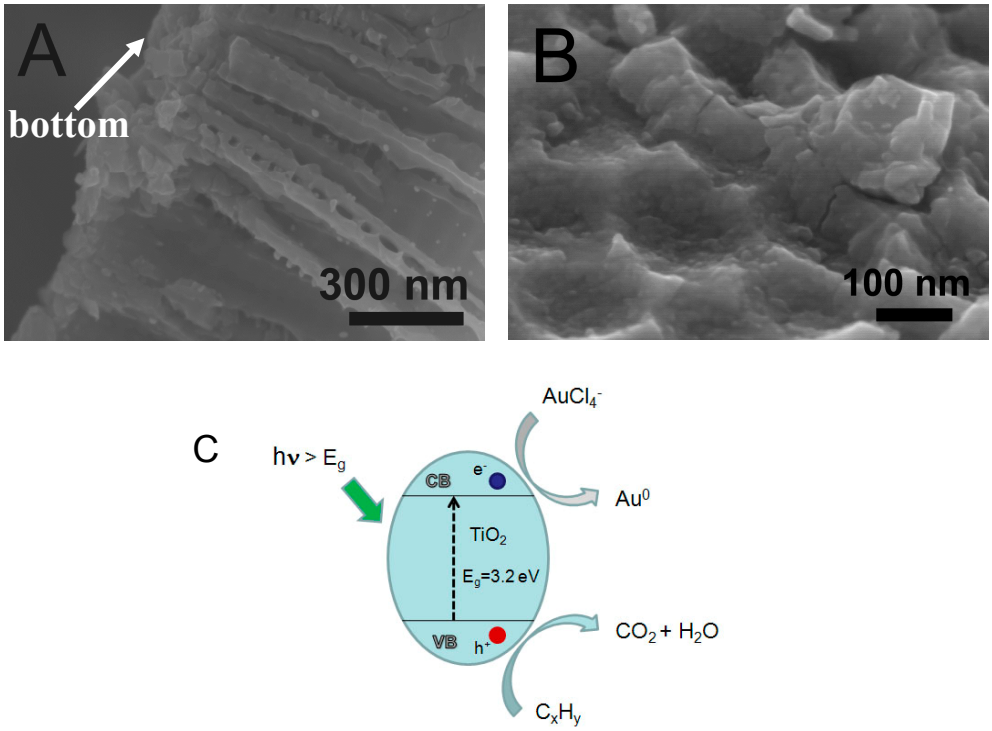

Fig. S1. SEM images of A) cross-sectional view of the TiO_2 nanotubes after Au nanoparticles decorated and B) the corresponding Ti substrate after nanotube removed. C) A schematic of preparing AuNPs-TiNT junctions by UV illumination.

Au nanoparticles are found in the channels and on Ti substrate. Herein, electrons are supposed to transfer from tube wall to Ti substrate directly through these nanoparticles, and avoid the block from barrier layer.

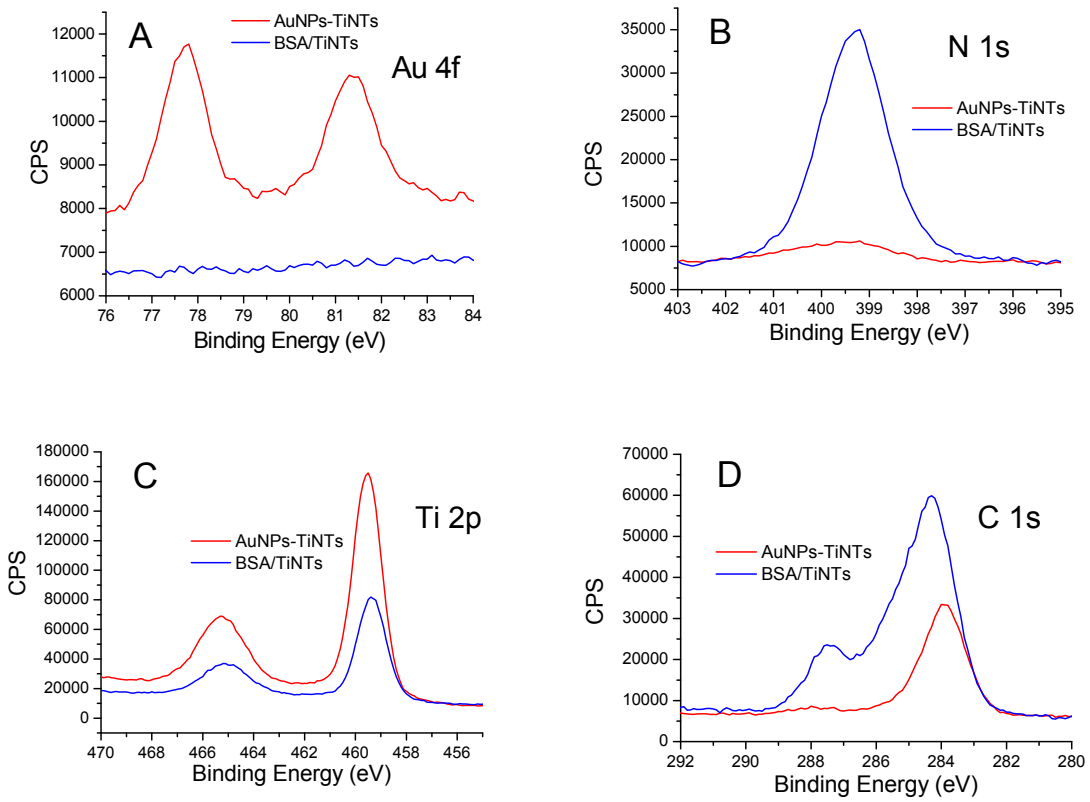

Fig. S2 XPS characterization: A) Au 4f, B) N 1s, C) Ti 2p and D) C 1s peaks for BSA/TiNTs and AuNPs-TiNT surface. CPS = counts per second.

The strong increases in the Au 4f signal at 77.8 eV and 81.3 eV in the additional decreases in the N 1s signal at 399.2 eV and C 1s signal at 284.2 eV are observed. Obviously, these results suggest that biological templates are decomposed during the AuNPs formation process.

Table S1 Electrochemical parameters determined from AC impedance spectra by fitting Z_{im} with Randle'equivalent circuit at different incubation times.

Incubation time (min)	R_{ct}^{Hb} (Ω)	θ (%)	k^0 (m s^{-1})
0	1057	0	0.25
10	1450	27.1	0.18
30	1678	37.0	0.16
60	2030	47.9	0.13
90	2218	52.3	0.12
120	2346	54.9	0.11
180	2362	55.2	0.11

The increase in impedance demonstrates that the adsorbed Hb molecules prevented the electron transfer between the probe molecules and electrode surface, which indicates more Hb adsorbed on the electrode. The surface coverage can then be derived from the following Equation 1 [1, 2]:

$$\theta = 1 - R_{ct}^{network}/R_{ct}^{Hb} \quad [1]$$

where $R_{ct}^{network}$ denotes the charge transfer resistance of the AuNPs-TiNT electrode and R_{ct}^{Hb} the corresponding resistance of the passivated electrode covered by Hb for different times. θ is the surface coverage of Hb on the AuNPs-TiNT electrode.

From EIS results, the heterogeneous charge kinetics can be derived from the following Equation 2 and 3 [3]:

$$R_{ct} = RT/nFi^0$$

$$i^0 = nFAk^0C$$

where k^0 is the electron transfer rate constant between the probe molecules $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ redox couple; C is the concentration of the redox couple in the bulk solution; n is the number of electrons involved in an electrode reaction, for the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ redox couple, n = 1; A, R, T, and F denote the electrode area, gas constant, temperature, and Faraday constant, respectively. Clearly, increasing the Hb coverage results in the decrease of charge-transfer rate constant. The charge-transfer rate constant of the redox probe on a bare AuNPs-TiNT is 0.25 m s^{-1}. Up to a saturated monolayer of Hb immobilized, the charge-transfer rate constant decreases to 0.11 m s^{-1}.

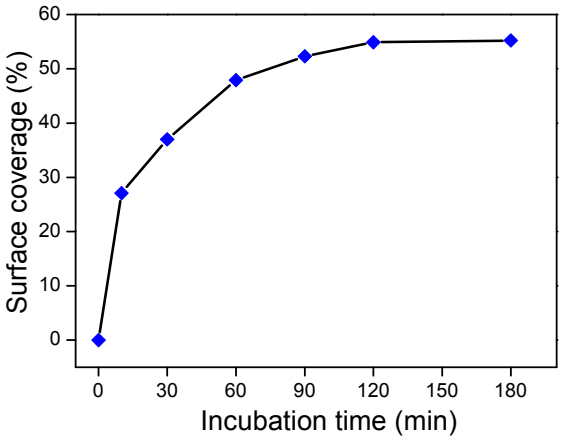

Fig. S3 Plot of the surface coverage against incubation time.

The surface coverage of Hb on the AuNPs-TiNT electrode increased exponentially within the first several minutes and then leveled off after 120 min where saturation coverage of Hb was reached.

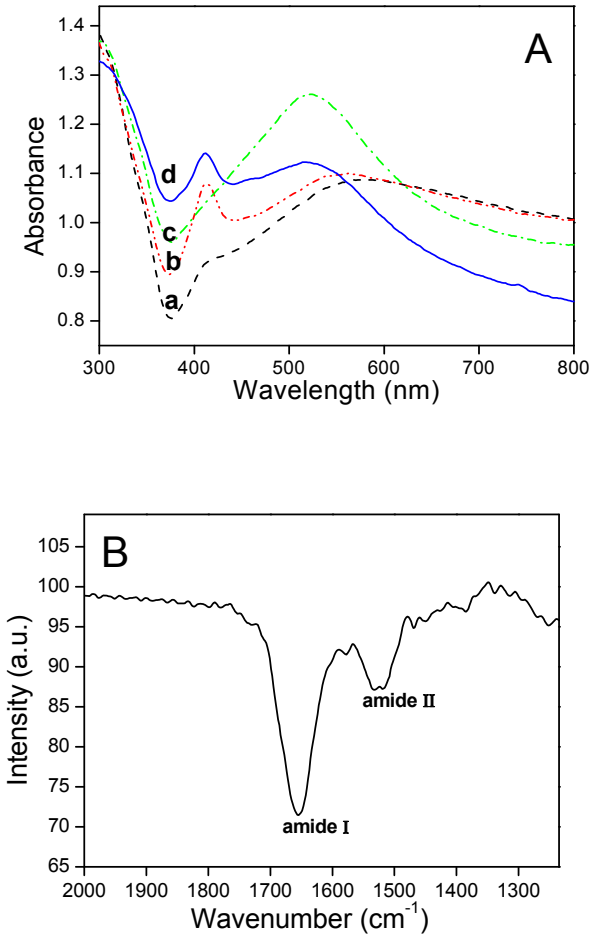

Fig. S4. A) UV-visible diffuse reflectance spectra of TiNT (a), Hb/TiNT (b), AuNPs-TiNT (c), Hb/AuNPs-TiNT (d). B) FTIR spectra (128 scans, 8 cm^{-1} resolution) of Hb/AuNPs-TiNT.

The UV-vis characterization shows an adsorption peak at 410 nm for Hb/AuNPs-TiNT (curve d). This position is coincident with the adsorption position of Hb by dropping PBS containing Hb solution onto the TiNT chips directly (curve b). Furthermore, the amide I band at 1660 cm^{-1} and amide II band at 1550 cm^{-1} also obeserved in FTIR spectrum. These results indicate Hb molecules absorbed on AuNPs-TiNT keeping the native conformation.

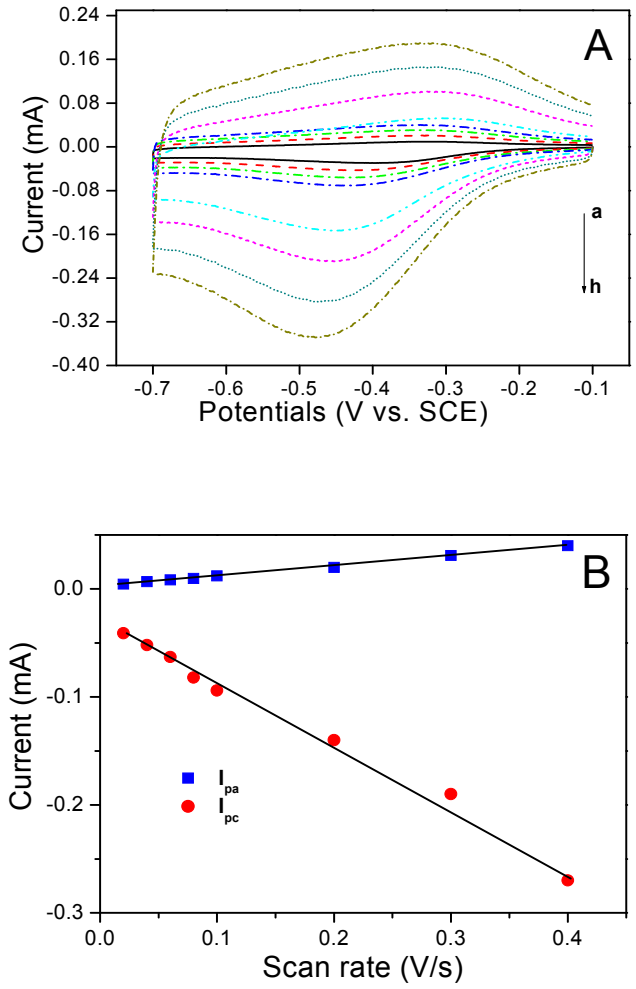

Fig. S5 A) Cyclic voltammograms of Hb/AuNPs-TiNT electrode in PBS (0.1 mol/L, pH 7.0) solution at different scan rates. From inner to outer CVs, the scan rates are 0.02, 0.04, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4 V/s. B) The plot of the anodic and cathodic peak currents versus scan rate.

The cathodic and anodic peak currents increase lineally with the scan rate and their potentials don't exhibit obvious shift, suggesting a diffusionless, surface-controlled electrode process.

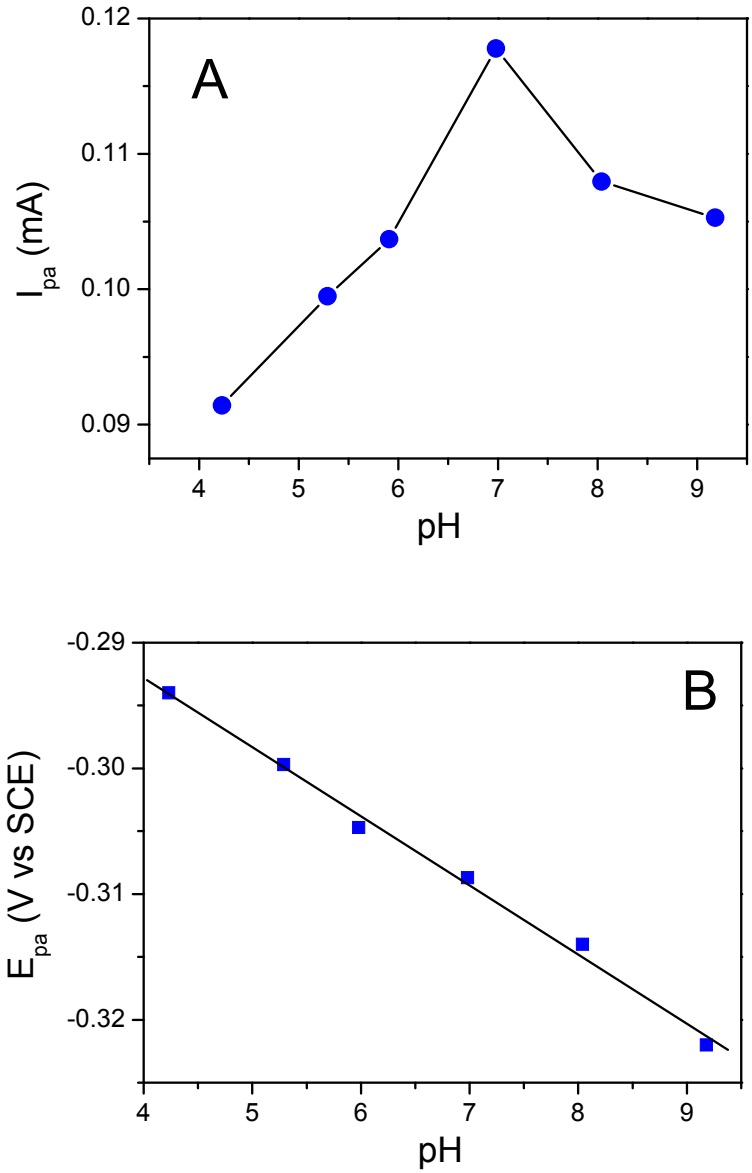

Fig. S6. Influence of solution on A) the anodic peak current and B) the anodic peak potential

In Fig. S6A, the peak current reaches a maximum at pH 7.0, corresponding to the isoelectric point of Hb (pI = 6.93). Increasing pH also causes a negative shift of the peak potentials. In Fig. S6B, the anodic peak potential shifts with a slop of -49.33 mV pH^{-1}.

[1] E. Sabatani, J. Cohen-Boulakia, M. Bruening, and I. Rubinstein, *Langmuir*, 1993, **9**, 2974.

[2] C. Henke, C. Steinem, A. Janshoff, G. Steffan, H. Luftmann, M. Sieber, H. J. Galla, *Anal. Chem.*, 1996, **68**, 3158.

[3] E. Sabatini, I. Rubinstein, *J. Phys. Chem.*, 1987, **91**, 6663.