Supporting Information

TiO_2 Nanotube Array: An Intrinsic Peroxidase Mimetics

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Experimental Section

Reagents and instruments: Horseradish peroxidase (EC1.11.1.17, 300 U mg^{-1}) was purchased from Roche. Glucose oxidase (GOD) (E.C.1.1.3.4, 100 U mg^{-1}, from *Aspergillus niger*), Titanium foil (purity > 99.7%), 3, 3', 5, 5'-tetramethylbenzidine (TMB), o-phenylenediamine (OPDA) and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were obtained from Sigma-Aldrich. Hydrofluoric acid, nitric acid, acetic acid, sodium acetate, acetone, ethanol, ascorbic acid (AA), dopamine (DA), uric acid (UA), 30% hydrogen peroxide (H_2O_2), fructose, lactose, sucrose, maltose, and D-(+)-glucose were purchased from Beijing Chemical Reagent Company (Beijing, China). A fresh solution of H_2O_2 was prepared daily and glucose stock solution was left at room temperature for 24 h to mutarotate before use. Britton−Robinson buffer solutions ([CH_3COOH] = 0.1 M, [H_3PO_4] = 0.1 M, [H_3BO_4] = 0.1 M) were used in the pH range 2 < pH < 12, the pH of a solution was changed by titration using aqueous KOH solutions. 0.1 M acetate buffer (pH 3.5) was used in the colorimetric assays and 0.1 M pH 5.5 phosphate buffer solution (PBS) was used in the electrochemical assays. Serum samples were obtained from local hospital and diluted with PBS before use. All the chemicals were of analytical grade and used without further purification. Deionized water was used throughout. Scanning electron microscope (SEM) images were obtained with A XL30 ESEM field-emission scanning electron microscope at an accelerating voltage of 15 kV. Transmission electron microscopy (TEM) images were obtained with a Hitachi H-8100 EM

transmission electron microscope. X-ray diffraction (XRD) spectra was performed using a D8 ADVANCE (Germany) using Cu K (λ=1.5406 Å) radiation. UV/Vis absorption measurements were performed on a Cary 500 UV-Vis-NIR spectrometer (Varian). Electrochemical measurements were carried out on an electrochemical workstation CHI 832B (Shanghai Chenhua, China). Ag/AgCl electrode (saturated KCl) and Platinum foil were used as reference electrode and counter electrode, respectively. All of the experiments were carried out at room temperature.

Preparation of TiO_2 NTA: TiO_2 NTA was synthesized according to our previous method.[1a] In brief, high purity Ti foil with a size of 0.3 cm × 2 cm were degreased and immersed in a mixed acid solution (HF: HNO_3:H_2O (v/v) =1:4:5) to be chemically etched for 40 s. Immediately the Ti foils were rinsed with deionized water and sonicated in acetone, ethanol and water for 10 min, respectively. With the surface-clean Ti foils as the anode and a platinum foil as the cathode, a two-electrode electrochemical system was performed in the electrolyte of 0.5 wt% HF aqueous solution for 20 min at 20 V with magnetic agitation. After anodization, the Ti foils were calcined in air atmosphere at 500 ˚C for 3 h with the heating and cooling rate of 1 ˚C min^{-1}. It can be clearly seen from **Figure S1A** that high density, well-ordered, and uniform TiO_2 NTA are obtained, and the tops of the tubes were open, the diameters of these nanotubes ranging from 60 to 80 nm with wall thickness of about 16 nm. The crystal structure of the as-synthesized TiO_2 NTA was examined by means of X-ray diffraction (XRD). As shown in **Figure S1B**, the diffraction peaks at about 2θ=25.4º and 48.1º can be indexed to the (101) and (200) crystal faces of anatase TiO_2 (JCPDS file: 89-4921). In addition, there are two additional peaks (2θ=34.9º and 76.3º) corresponding to the crystal faces of rutile TiO_2. [1b] The other diffraction peaks at about 2θ=40.3º, 53.1º, 63.0º and 70.7º can be indexed to the titanium substrate (JCPDS file: 44-1294). On the basis of the results above, it can be concluded that anatase and rutile phases appeared after the anodized Ti foil being calcined at 500 °C for 3 h. Also, TiO_2 nanoporous spheres (TiO_2 NPSs) synthesized as control were synthesized by a documented two-step process according to the previous reports, titanium glycolate spheres preparation and hydrolyzation.[1c]

Colorimetric assays based on TiO_2 NTA peroxidase mimetics: Unless otherwise stated, colorimetric assays were carried out in 0.1 M acetate buffer (pH 3.5) containing 3 mM H_2O_2 and 0.25 mM TMB incubated at 40 ˚C for 15 min. The absorbance values at 652 nm were measured. In OPDA and ABTS experiments, 0.25 mM OPDA and 0.25 mM ABTS were used instead of TMB, respectively, with the other same conditions. Kinetic measurements were carried out in 0.1 M acetate buffer (pH 3.5) with 0.3 cm × 0.3 cm TiO_2 NTA or 1 ng mL^{-1} horseradish peroxidase (HRP) as the catalysts.The kinetic data were obtained by varying one substrate concentration and fixing that of the other substrate. A series of initial reaction rates were calculated and applied to the double reciprocal of the Michaelis-Menten equation, $1/v = (K_m/V_{max}) \cdot (1/[S]) + 1/V_{max}$, where v is the initial velocity, [S] the concentration of the substrate, K_m the Michaelies-Menten constant and V_{max} the maximal reaction velocity.

Electrochemical assays based on TiO_2 NTA electrode for H_2O_2 and glucose detection: Cyclic voltammetry (CV) and chronoamperometry were used to investigate the electrocatalytic activity of TiO_2 NTA electrode towards H_2O_2. 2 mM AA, 2 mM DA, and 2 mM UA were used as the possible interferences for the selectivity assays. The GOD modified TiO_2 NTA electrode were prepared by immersing the TiO_2 NTA electrode in 1 mg mL^{-1} GOD (in 10 mM pH 5.5 PBS) overnight and then rinsing with water to remove the weak adsorbed proteins. The resulted electrode was noted as GOD/TiO_2 NTA electrode. In control experiments, 2 mM fructose, 2 mM lactose, 2 mM sucrose, and 2 mM maltose were added to the electrochemical system instead of 1 mM glucose, respectively. For real sample assays, the serum samples of a patient with diabetes from local hospital were diluted 4 times with 0.1 M PBS (pH 5.5) and the amperometric response signals were recorded, respectively.

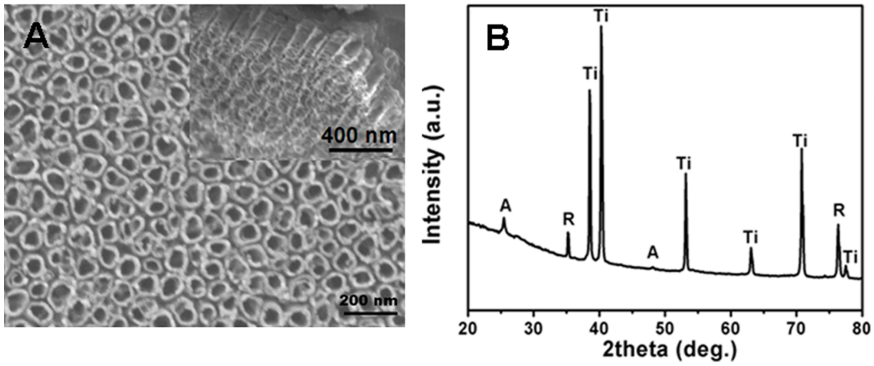

Figure S1 (A) SEM top-view image of TiO_2 NTA with the cross section in the inset; (B) The XRD pattern of TiO_2 NTA.

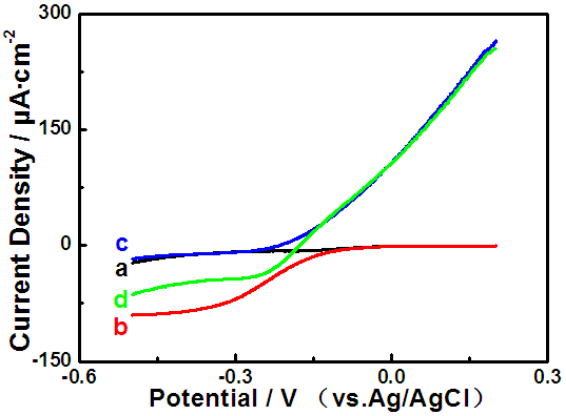

Figure S2 Polarization curves of TiO_2 NTA electrode at a scan rate of 10 mV s^{-1} in 0.1 M pH 6.5 PBS without (curves a, b) and with (curves c, d) the UV light illumination (365 nm) in the absence of H_2O_2 (curves a, c) and in the presence of 1 mM H_2O_2 (curves b, d).

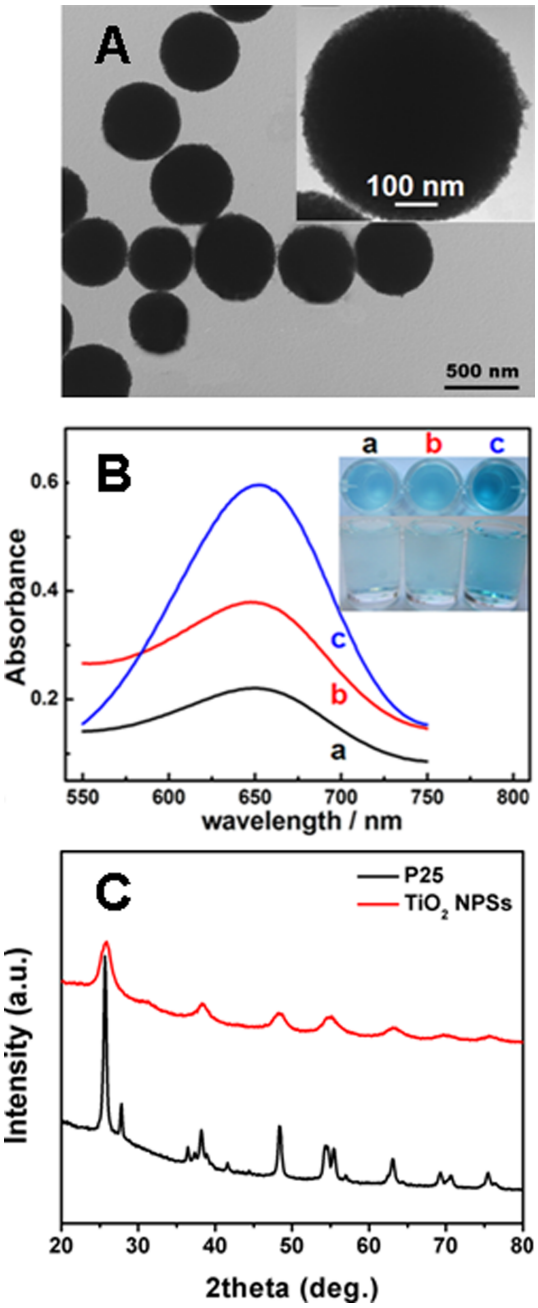

Figure S3 (A) TEM images of TiO_2 NPSs with the large magnification in the inset; (B) The absorption spectra and digital photos of colorimetric reaction of TMB and H_2O_2 in 0.1 M pH 3.5 NaAc buffer solution with P25 (a), TiO_2 NPSs (b), and TiO_2 NTA (c) as the catalysts. (C) The XRD pattern of P25 and TiO_2 NPSs.

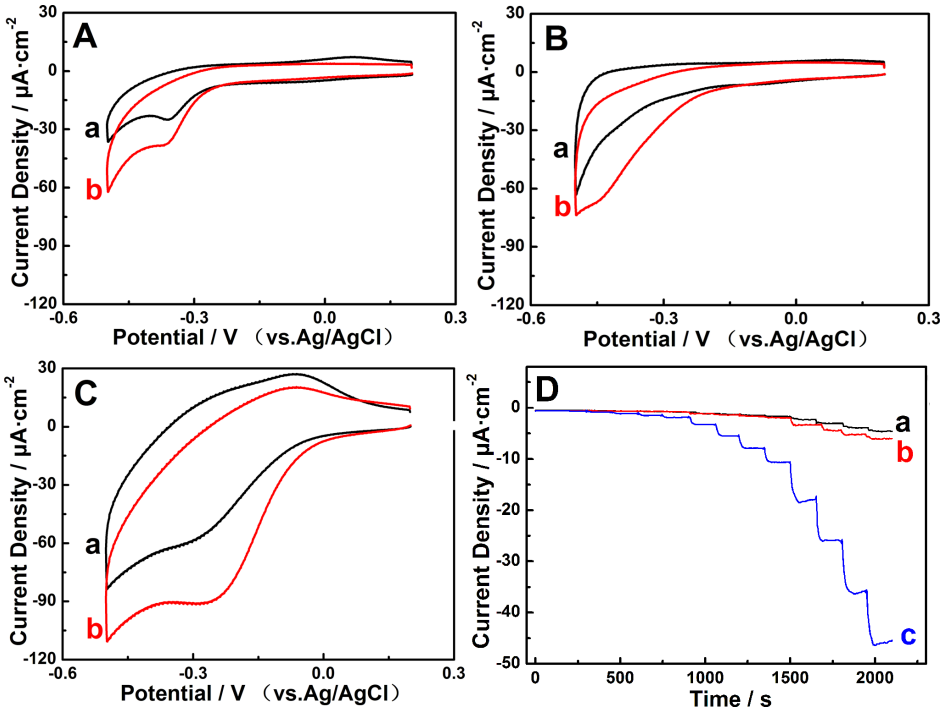

Figure S4 (A) CVs obtained at P25/ITO electrode in 0.1 M pH 5.5 PBS without (a) and with (b) 0.2 mM H_2O_2; (B) CVs obtained at TiO_2 NPSs/ITO electrode in 0.1 M pH 5.5 PBS without (a) and with (b) 0.2 mM H_2O_2; (C) CVs obtained at TiO_2 NTA electrode in 0.1 M pH 5.5 PBS without (a) and with (b) 0.2 mM H_2O_2. Scan rate is 50 mV s^{-1}. (D) Amperometric responses at P25/ITO electrode (a), TiO_2 PSs /ITO electrode (b), andTiO_2 NTA electrode (c) in 0.1 M pH 5.5 PBS to continuing injection of H_2O_2 at -0.25 V.

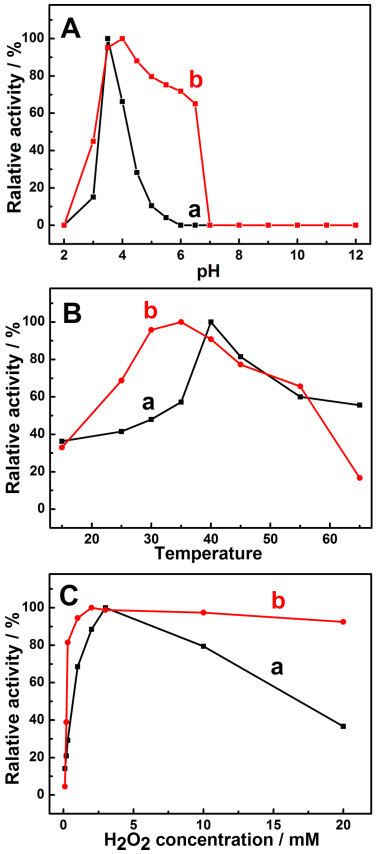

Figure S5 Dependency of the peroxidase-like activity of the TiO_2 NTA (a) and HRP (b) on (A) pH, (B) temperature, and (C) H_2O_2 concentrations. Experiments were carried out using 0.3 cm × 0.3 cm TiO_2 NTA or 1 ng mL^{-1} HRP in 0.1 M pH 3.5 NaAc solution containing 0.2 mM TMB, the absorbance value of the colorimetric reaction was used to probe the activity.

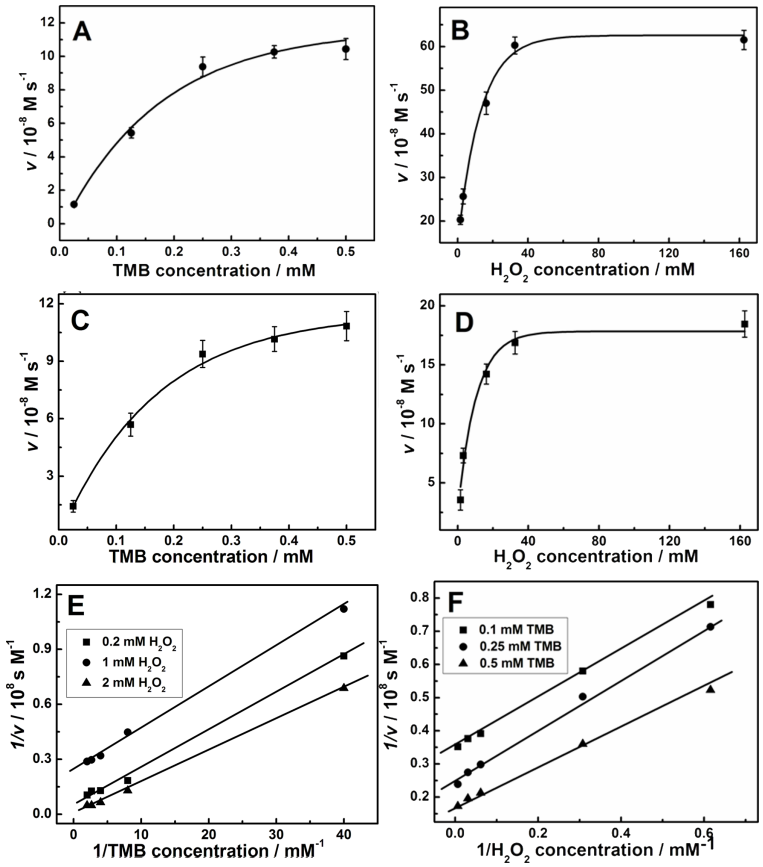

Figure S6 Steady-state kinetics of TiO_2 NTA (A, B) and HRP (C, D) relied on TMB concentration (A, C) and H_2O_2 concentration (B, D), respectively. Experiments were carried out using 0.3 cm × 0.3 cm TiO_2 NTA and 1 ng mL^{-1} HRP in 0.1 M pH 3.5 NaAc solution. The H_2O_2 concentration in A and C is 1 mM and the TMB concentration in B and D is 0.5 mM TMB; Double reciprocal plots of peroxidase-like activity of TiO_2 NTA at a fixed concentration of one substrate versus varying concentration of the second substrate for TMB and H_2O_2 (E, F).

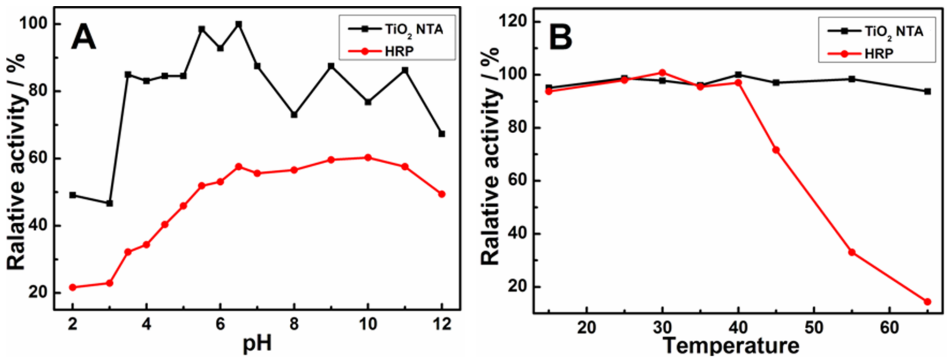

Figure S7 The tolerability comparison of the TiO_2 NTA and HRP to pH (A) and temperature (B). The activity of TiO_2 NTA and HRP was evaluated after incubation in buffer solution range from 2 to 12 for 2h and at 15-65 ˚C for 2h, respectively.

Table S1 Comparison of the Mochaelis-Menten constant (K_m) for H_2O_2 with different nanomaterials as catalysts.

Catalysts	K_m [H_2O_2] / mM
Fe_3O_4 MNPs[2]	154
Graphene oxide[3]	3.99
Carbon nanodot[4]	26.77
Co_3O_4 NPs[5]	140.07
TiO_2 NTA	5.26

Reference

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