Electronic Supplementary Material (ESI) for Analyst.

Dual enzyme mimics exhibited by ITO nanocubes and its application in spectrophotometric and electrochemical sensing

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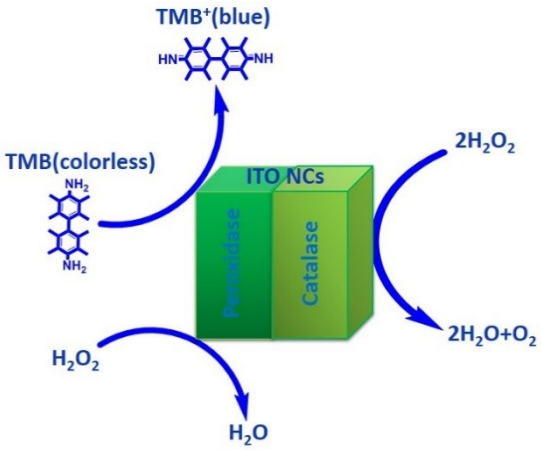

Schematic illustration of the peroxidase/ catalase-like activities of ITO nano cubes

Experimental section

Chemicals and reagents

Glucose oxidase(EC1.1.3.4, 120U/mg, from Aspergillus niger), 3,3',5,5'-tetramethylbenzidine (TMB), $SnCl_2$, $InCl_3.4H_2O$, terephthalic acid, acetic acid (glacial) and sodium acetate were purchased from Sigma-Aldrich. H_2O_2 (30%) and ethanol (99.8%) were purchased from Merck. Tin (IV) oxide was obtained from Alfa aesar. Enzymes were stored in a deep freezer at -18 °C. All other chemicals used in this experiment were of analytical grade and used without further purification. Millie Q water with a resistivity not lower than 18.2 Ω was used throughout the experiment.

Synthesis of ITO NCs

In short, $SnCl_2$ and $InCl_3.4H_2O$ were dissolved in milli-Q water separately. The two solutions were then mixed together by maintaining a mole ratio of In/ (In + Sn) as 0.95 at room temperature. The mixed solution was then added drop wise (1 ml/ min) to 5 M ammonium hydroxide solution with stirring. The reaction mixture was allowed to stand for 4 hours at 80 °C with stirring. The light yellow color precipitate was collected and washed with distilled water, air-dried and hydrothermally treated at 200°C for 24 hours using an autoclave. The product was then filtered, washed with distilled water and dried at 50 °C under vacuum.

The powder was then calcined at 700 °C for 4 h in air to obtain the ITO NCs. The various reactions involved in the co-precipitation (equation 1) and hydrothermal process (equations 2 and 3) can be summarized as follows.

$$In^{3+} + Sn^{2+} \rightarrow In(OH)_3 + Sn(OH)_2 \quad (1)$$

$$Sn^{2+} \rightarrow Sn^{4+} + 2e^- \quad (2)$$

$$4In(OH)_3 + Sn(OH)_4 \rightarrow (In_{(1-x)}Sn_x)OOH + H_2O \quad (3)$$

Apparatus and characterization

Electrochemical experiments were performed on an AUTOLAB PGSTAT 302N work station. A three electrode system, comprising of ITO NCs modified GCE (ITO/ GCE), ITO plate or bare GCE as working electrodes, saturated calomel electrode as reference electrode and a spiral platinum wire as auxiliary electrode were used for all electrochemical studies. The experimental solutions were deaerated and maintained under nitrogen atmosphere during electrochemical experiments. The surface morphology of the ITO NCs was taken using Field Emission Scanning Electron Microscope (FESEM -Zeiss Supra 55VP, CARL ZEISS). Tecnai 20 G2 (FEI make) instrument were used for obtaining the Transmission electron microscopy (TEM) images. Tecan Infinite M200 pro fluorescence spectrophotometer was used for kinetic studies, fluorescent experiments and uv-vis spectrum. XRD patterns were obtained from a Bruker D8 Advance X-ray diffraction using Ni-filtered Cu Kα radiation (l = 1.5406 Aº). The XPS analysis was performed on MULTILAB 2000 Base system with twin Anode Mg/Al (300/400W) X-Ray Source.

Colorimetric H_2O_2 detection using the ITO NCs

A typical colorimetric analysis of $H_2O_2$was realized by mixing a solution of 100 µL of 10 mM TMB, 100 µL of 0.5 mg mL^{-1} ITO NCs and 100 µL of H_2O_2 with different concentrations in 1 mL of 0.1M acetate buffer (pH 3.0) and measuring the absorbance at 652 nm using absorption spectroscopy measurement with the time drive method.

Glucose detection using Glucose oxidase (GOx) and ITO NCs

Since Glucose oxidase could be denatured at pH 3.0 acetate buffer solution, glucose detection was realized by the following procedure: 200 µL of glucose with different concentrations in 0.01M acetate buffer solution (pH 5.5) were prepared with 50 µL of 10 mg mL^{-1} GOx and incubated at 37 °C for 30 min. This solution was then added to a mixture of 50 µL of 10 mM TMB, 100 µL of 5 mg mL^{-1} ITO NCs and 200 µL of 0.1 M acetate buffer (pH 5.0). The mixed solution was incubated at room temperature for 30 min, and

used for absorbance measurement at 652nm using absorption spectroscopy measurement. Control experiments were performed using 5 mM fructose, 5 mM lactose, 5 mM maltose and 5 mM Sucrose.

Detection of Hydroxyl Radical.

Fluorescence measurements

The oxyl radical formation during the peroxidase mimics of ITO NCs was investigated by fluorescence measurements using terephthalic acid as probe. The typical procedure was as follows. A dispersion of 1, 2, 3, 4 and 5mg of the ITO NCs was prepared in 1ml of sodium acetate buffer of pH 3 containing 0.5 mM TPA and 10 mM H_2O_2. The suspension was irradiated with UV light at 365 nm for 30 min, centrifuged and the solution is subjected to fluorescence measurement. Fluorescence spectra were recorded at 315nm on a Tecan Infinite M200 pro fluorescence spectrophotometer.

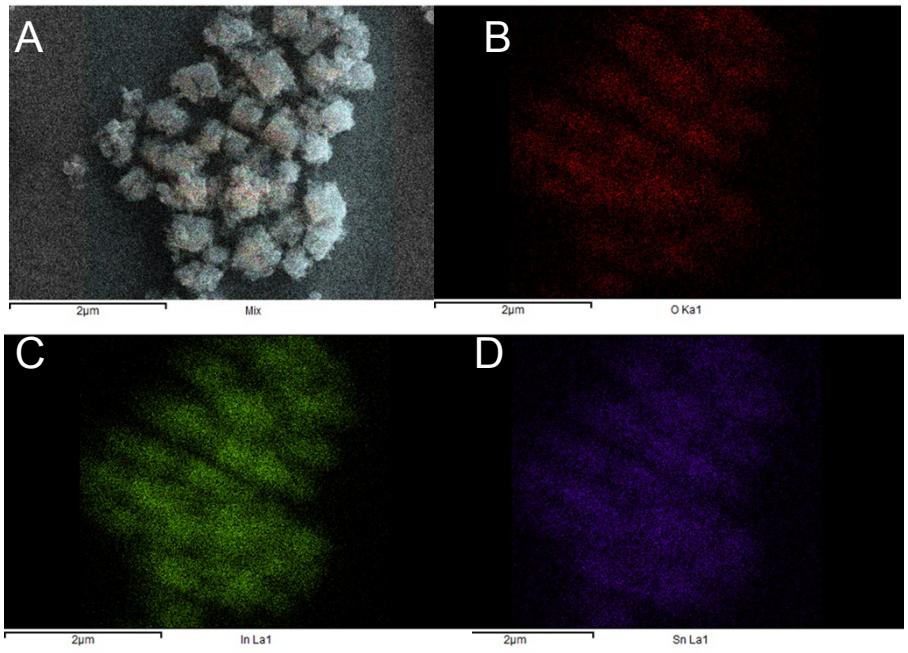

Fig. S1 EDX elemental mapping characterization of ITO nanocubes. B, C and D are the corresponding elemental mapping of the O, In and Sn elements, respectively. Scale bar, 2µm

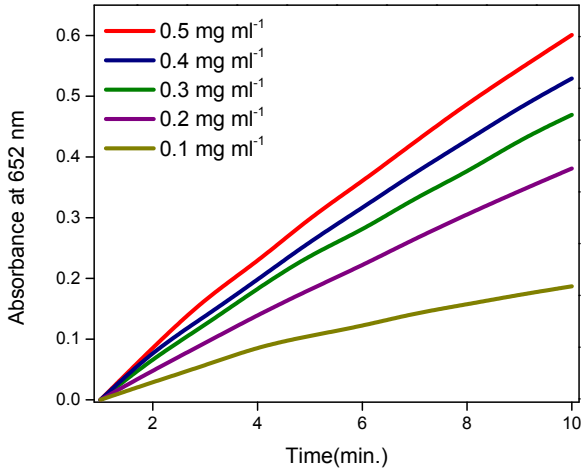

Fig. S2 Dependency of the peroxidase-like activity of ITO NCs on ITO NCs concentration.

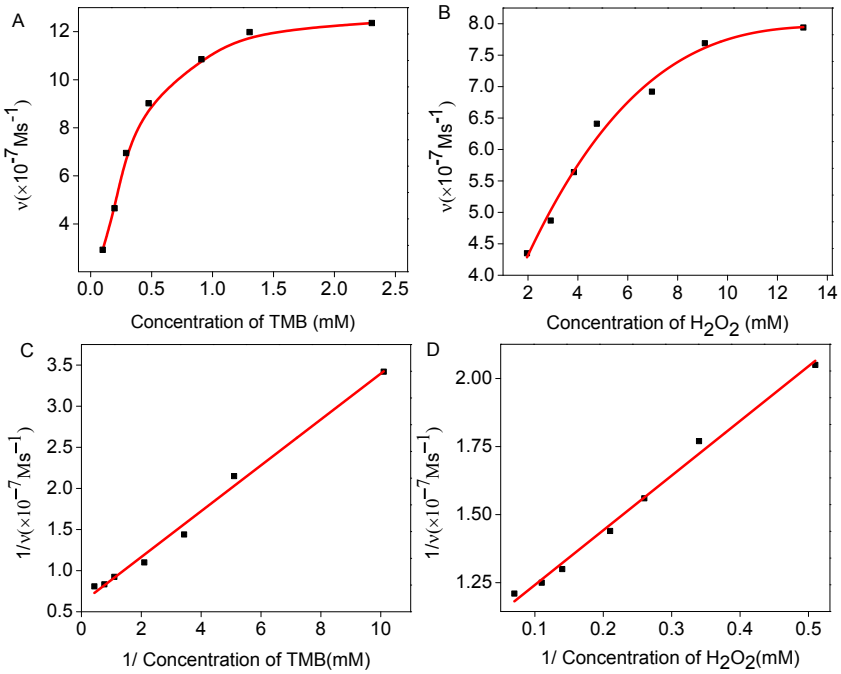

Fig. S3 Steady-state kinetic analysis of ITO NCs. The velocity of the reaction was measured using 0.5 mgmL^{-1} ITO NCs in 0.1M acetate buffer (pH 3.0) at 28 °C. (A) The concentration of H_2O_2 was fixed at 9.09 mM and the concentration of TMB was varied. (B) The concentration of TMB was fixed at 0.99 mM and the H_2O_2 concentration was varied. Lineweaver-Burk plots of the ITO NCs activity using TMB (C) and H_2O_2 (D) by fixing the concentration of one substrate and varying the concentration of the second substrate.

Table. S1 Comparison of Michaelis–Menten constants of ITO NCs for H_2O_2 and TMB substrate with other metal oxide peroxidase mimic and HRP from the literature.

Catalysts	**Km[H_2O_2]/ mM**	**Km[TMB]/ mM**	**References**
Fe_3O_4	154	0.098	Perrett et al.[1]
Ceria	293	0.147	Qu et al. [2]
$AgVO_3$	14	8.03	Zhang et al.[3]
Co_3O_4	140.07	0.037	Wang et al.[4]
HRP	7.08	0.123	Zhang et al.[5]
ITO NCs	5.47	0.26	This work

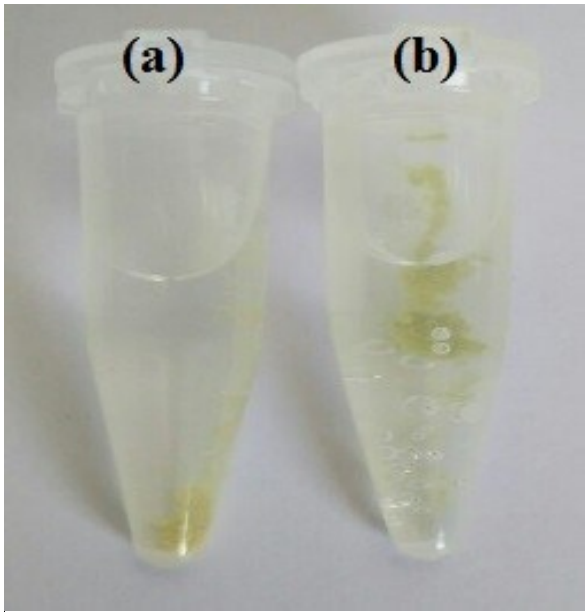

Fig. S4 Catalase-like activity of ITO NCs (a) ITO NCs (b) H_2O_2+ ITO NCs in acetate buffer of pH 3.0 (0.1M) at 45 °C for 30 minutes.

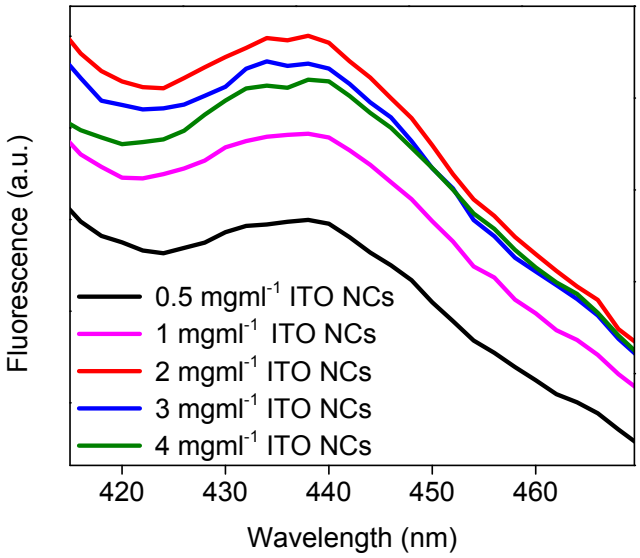

Fig. S5 The relation between ITO NCs and the formation of hydroxyl radicals with terephthalic acid (TPA) as the fluorescence probe.0.5, 1, 2, 3 and 4 mg mL^{-1} different concentrations of the ITO NCs were incubated in 0.1M acetate buffer (pH 5.0) containing 0.5 mM TPA and 10 mM H_2O_2 and exposed to UV light at 365 nm for 30 min. The fluorescence spectra recorded at 315nm excitation wavelength.

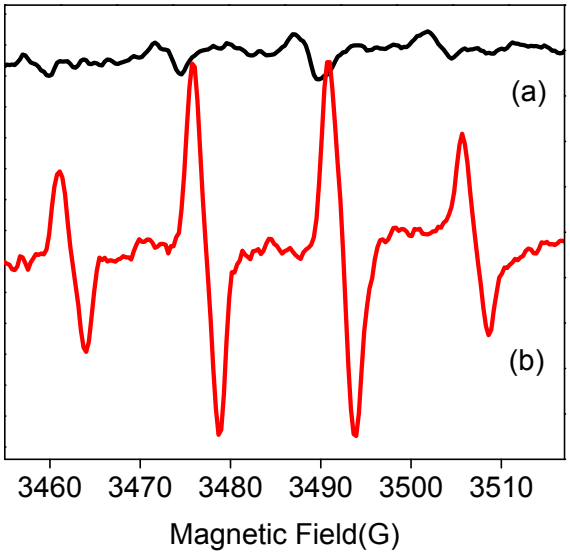

Fig. S6 EPR spectra of the DMPO–OH adduct observed from the (a) blank experiment and (b) ITO nanocube system.

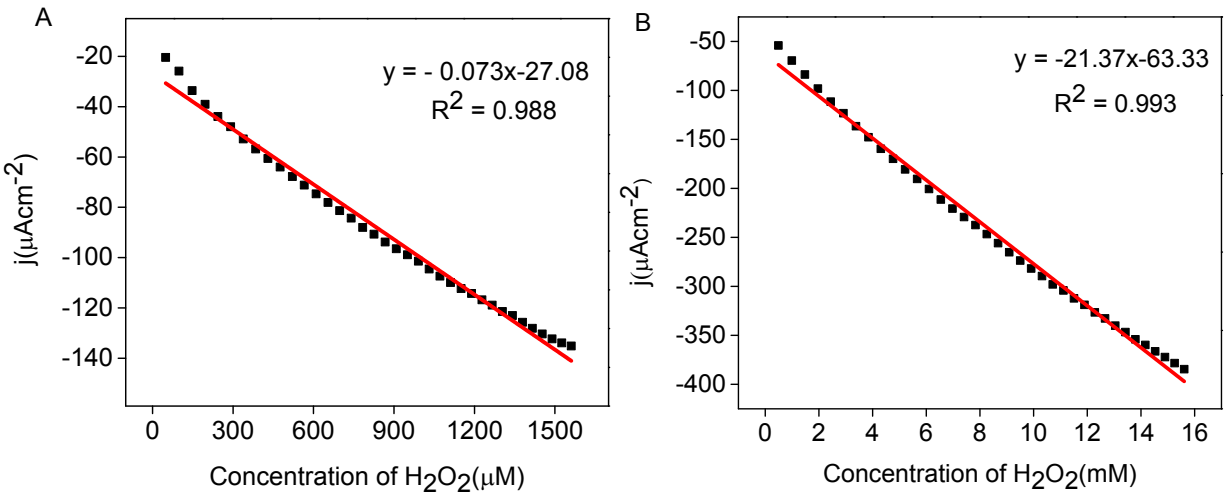

Fig. S7 (A) Amperometric response of ITO NCs modified GCE upon successive additions of 49 μM H_2O_2 (b) and upon successive additions of 0.49 mM H_2O_2 in 0.1M acetate buffer solution (pH 3.0) at applied potential of -0.4 V.

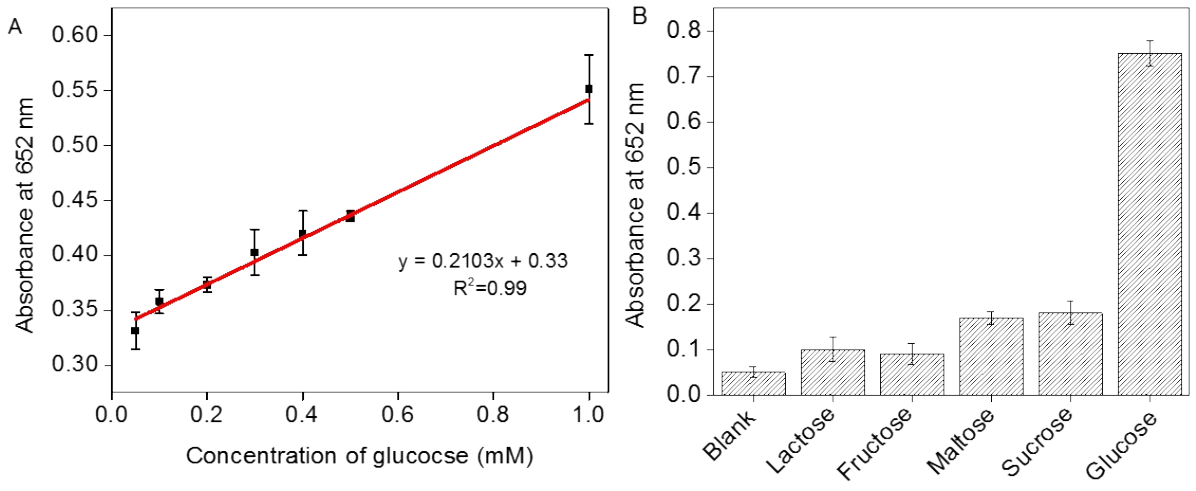

Fig. S8 (A) The calibration plot for glucose determination. (B) The selectivity studies of glucose determination with 5 mM fructose, 5 mM maltose, 5 mM lactose and 1 mM glucose. Insert: the color change of different solutions (from left to right: blank, lactose, fructose, maltose, sucrose and glucose).

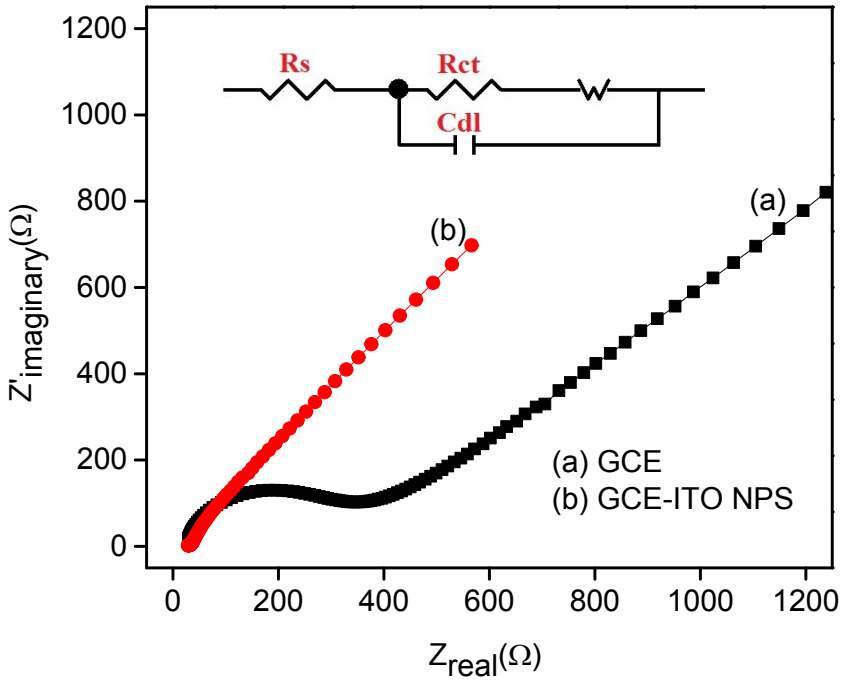

Fig. S9 Nyquist plots at the GCE (curve a) and GCE-ITO NC electrode (curve b) in 5 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl as supporting electrolyte. Inset is the equivalent circuit used for fitting the circuit.

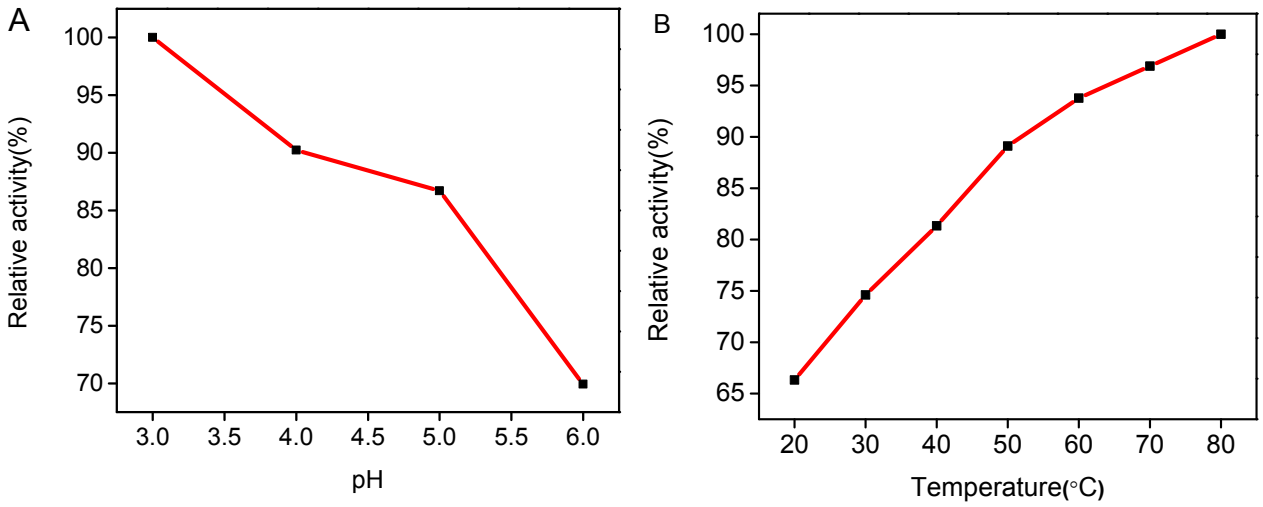

Fig. S10 Dependency of the peroxidase-like activity of ITO NCs on (A) pH and (B) temperature.

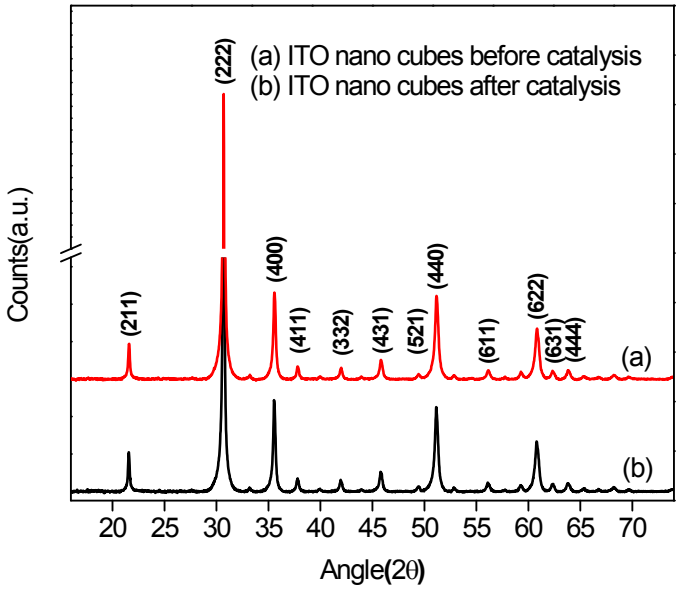

Fig. S11 XRD patterns of indium tin oxide nano cubes (a) before and (b) after the catalytic reaction.

Table. S2 Analytical results of glucose in serum

Sample	Proposed method (mM)	Standard method (mM)	RSD (%)
1	5.16	5.22	0.91
2	4.34	4.38	2.60

Table S3 The recovery of standard addition of hydrogen peroxide in serum.

Sample	Spiked(mM)	Found(mM)	Recovery (%)
1	0.55	0.545	99.09
2	0.55	0.546	99.27
3	0.55	0.565	102.72

Notes and references

1. L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang and S. Perrett, *Nat. nanotechnol.*, 2007, **2**, 577-583.

2. Z. Tian, J. Li, Z. Zhang, W. Gao, X. Zhou and Y. Qu, *Biomaterials*, 2015, **59**, 116-124.

3. Z. Xiang, Y. Wang, P. Ju and D. Zhang, *Microchimica Acta*, 2016, **183**, 457-463.

4. J. Mu, Y. Wang, M. Zhao and L. Zhang, *Chem. Commun*., 2012, **48**, 2540-2542.

5. L. Zhang, L. Han, P. Hu, L. Wang and S. Dong, *Chem. Commun.*, 2013, **49**, 10480-10482.