Supporting Information

Dual enzyme-like activity of Iridium nanoparticles and their applications for the detection of glucose and glutathione

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Experimental Section

Reagents and instruments:

Sodium hexachloroiridate (III) hydrate ($Na_3IrCl_6 \cdot xH_2O$), L-ascorbic acid (AA), Glucose oxidase (GOD, from Aspergillus niger, 10 KU), and 3, 3', 5, 5'-tetramethylbenzidine (TMB) were obtained from Sigma-Aldrich. L-Glutathione (GSH), sodium borohydride ($NaBH_4$), glucose, fructose, maltose, lactose and sucrose were purchased from Beijing Chemical Reagent Company (Beijing, China). All the chemicals were of analytical grade and used without further purification. Redistilled water was used throughout the study.

UV/Vis absorption measurements were performed on a U-4100 spectrometer (Hitachi, Japan). Transmission electron microscopy (TEM) measurements were made on a Tecnai G2 F20 microscope operating at 200 kV (FEI, USA). X-ray photoelectron spectroscopy (XPS) measurement was performed on the Thermo Fisher Scientific ESCALAB 250Xi spectrometer with Al Kα X-ray radiation as the X-ray source for excitation. Inductively coupled plasma-optical emission spectroscopy (ICP-OES, iCAP 7400, Thermo Scientific, USA) was conducted to confirm the exact concentration of Ir NPs.

Preparation of Ir NPs:

Ir NPs were synthesized via a simple chemical reduction process according to literature[1] with a little modification. Briefly, 50 mL water was added into a round-bottom flask and heated up to 95 °C before 50 mg AA and 10 mg $Na_3IrCl_6 \cdot xH_2O$ were added. Subsequently, 1 mL freshly prepared $NaBH_4$ solution (25 mg mL^{-1}) was

added dropwisely into the mixed solution under vigorous stirring for 15 min. Upon cooling down to room temperature, the Ir NPs were condensed and purified by using an Amicon Ultra-4 centrifugal filter device with a molecular weight cut-off (MWCO) of 30 kDa (Millipore, Merck KGaA, Germany) for further use.

Steady-State kinetic analysis of Ir NPs as peroxidase mimetics:

Kinetic experiments were carried out in a reaction volume of 1.0 mL HAc-NaAc buffer solution (0.1 M, pH 4.0) containing 0.12 μg mL^{-1} Ir NPs, 2 mM H_2O_2 and 0.25 mM TMB as substrate, unless otherwise stated. The mixture solutions were incubated at room temperature for 5 min and then used for absorbance measurement at wavelength 652 nm. The Michaelis-Menten constant was calculated using a Lineweaver-Burk plot: $1/V = K_m/V_m (1/[S]+ 1/K_m)$, where V is the initial velocity, V_m is the maximal reaction velocity, [S] corresponds to the substrate concentration, and K_m is the Michaelis-Menten constant.

Determination of •OH radical:

50 mM H_2O_2, 0.625 mM terephthalic acid and different concentrations of the Ir NPs were first incubated in 0.1 M HAc-NaAc buffer (pH 6.0) for 1 h, and then the solutions were used for the measurement of OH radical carried out on Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc., France).

Colorimetric detection of GSH based on Ir NPs peroxidase mimic:

2 mM H_2O_2, 0.25 mM TMB, 0.25 μg mL^{-1} Ir NPs and different concentrations of GSH were incubated in 0.1 M HAc-NaAc buffer (pH 4.0) for 5 min, then the solutions were used for absorbance measurement.

Colorimetric detection of glucose based on Ir NPs peroxidase mimic:

Glucose detection was performed as follows: (1) 50 μL GOD (5 mg mL^{-1}) and 150 μL of different concentrations of glucose in 10 mM Na_2HPO_4 buffer (pH 7.4) were incubated at 37 °C for 30 min; (2) 5 μL of TMB (50 mM), 20 μL of Ir NPs (25 μg mL^{-1}) and 775 μL of 0.1 M HAc-NaAc buffer (pH 4.0) were added into the above 200 μL glucose reaction; (3) the mixed solution was incubated at room temperature for 5 min and then used for absorbance measurement.

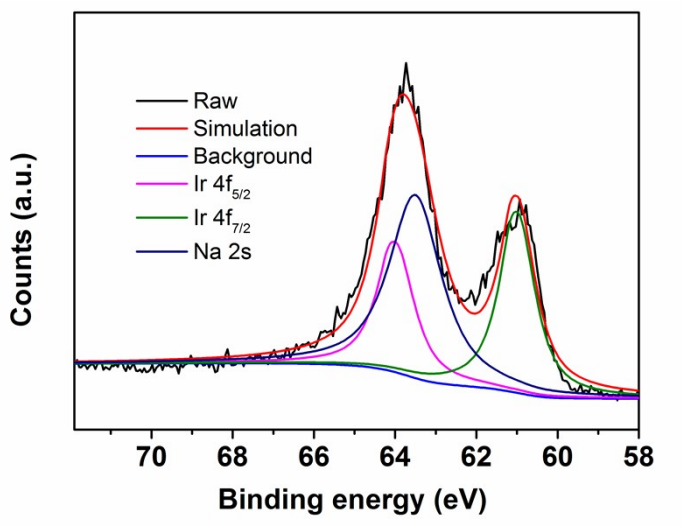

Fig. S1. High-resolution Ir 4f XPS spectra of Ir NPs.

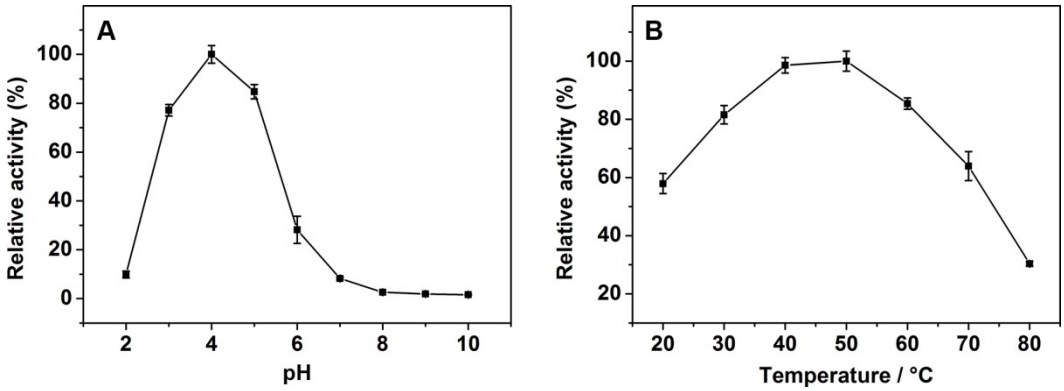

Fig. S2. Dependency of the peroxidase-like activity on (A) pH and (B) temperature. Reaction conditions: 0.25 mM TMB, 2 mM H_2O_2 and 0.12 μg mL^{-1} Ir NPs were incubated in 0.1 M HAc-NaAc buffer for 5 min for the absorbance spectroscopy measurement. The maximum point in each curve was set as 100 %.

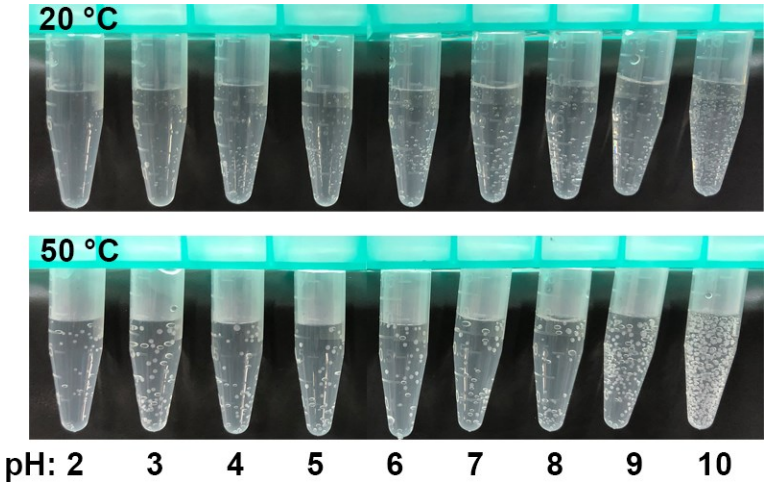

Fig. S3. Catalase-like activity of Ir NPs depended on pH and temperature. Reaction conditions: 2 μg mL^{-1} Ir NPs and 20 mM H_2O_2 were incubated in 0.1 M HAc-NaAc buffer for 10 min.

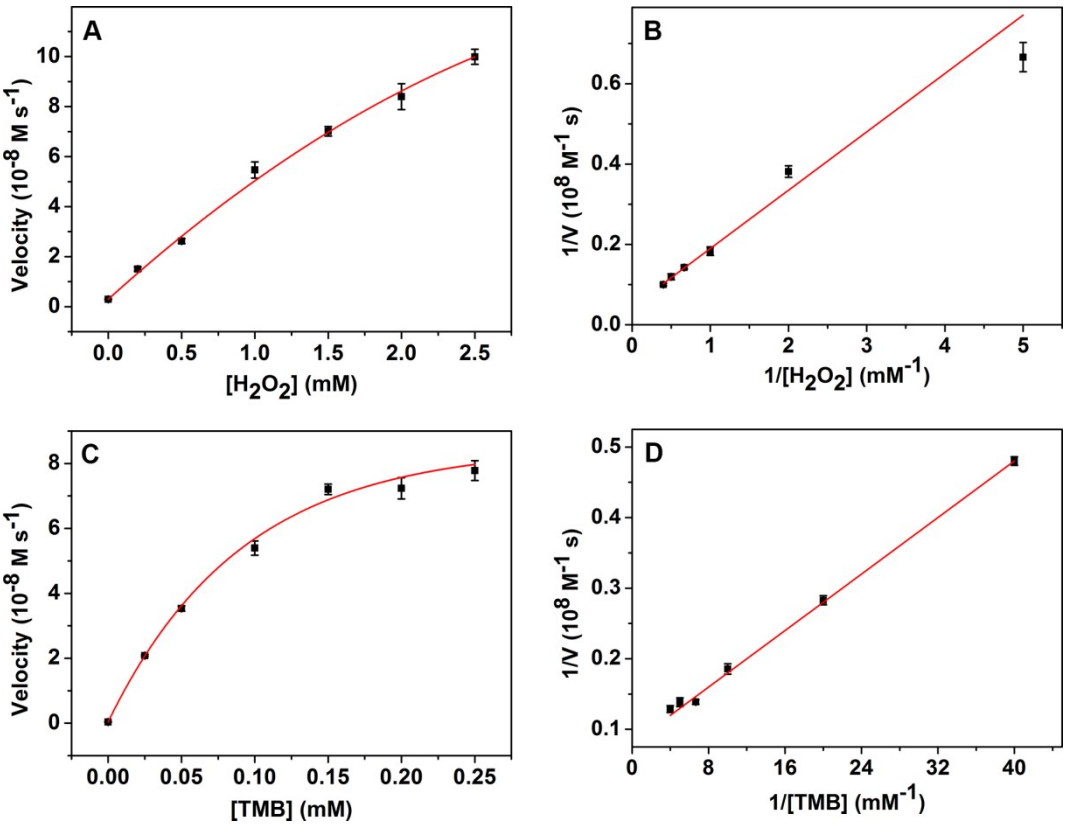

Fig. S4. Steady-state kinetic assay of Ir NPs. The concentration of TMB in A was 0.25 mM and the H_2O_2 concentration in C was 2 mM. (B) and (D) Double reciprocal plots of activity of Ir NPs. The error bars represented the standard deviation of three measurements.

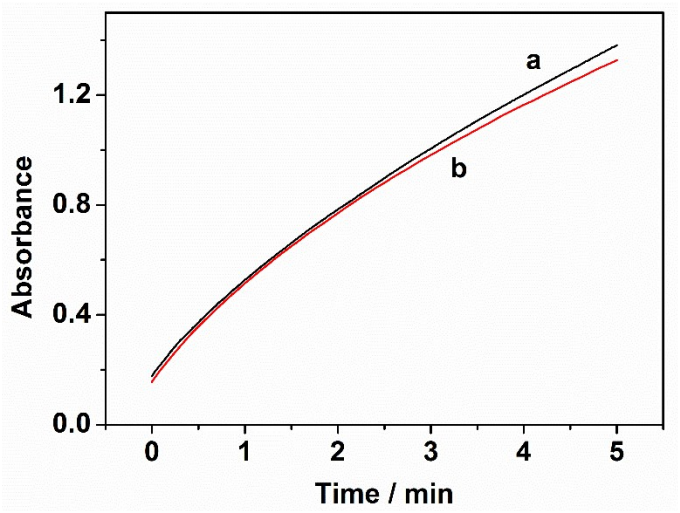

Fig. S5. Time-dependent absorbance changes at 652 nm of TMB using Ir NPs (a) and Ir NPs after five-month storage (b).

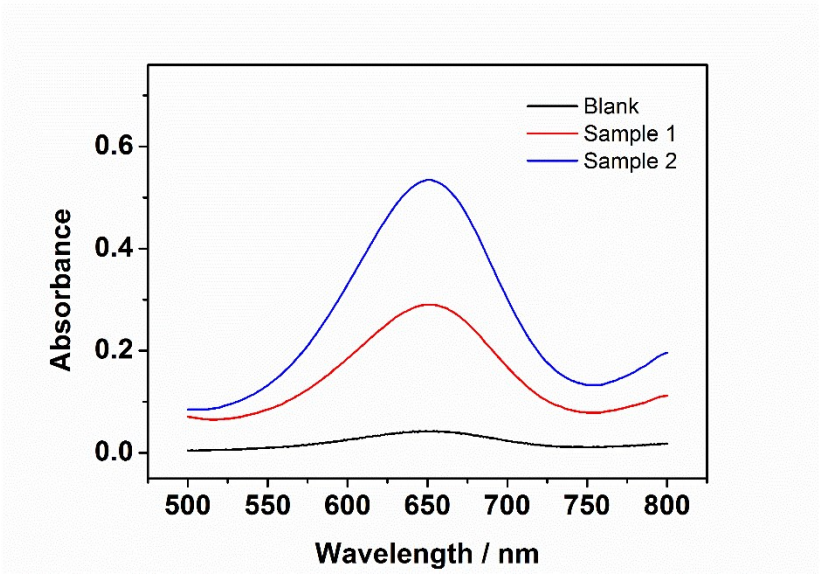

Fig. S6. UV-vis spectra of the buffer solution and 50-fold dilution human serum samples.

Table S1. Comparison of the apparent Michaelis-Menten constant (K_m) and maximum reaction rate (V_m).

Catalyst	K_m (mM)		V_m (10^{-8} M s^{-1})		Ref.
	H_2O_2	TMB	H_2O_2	TMB	
HRP	3.7	0.434	8.71	10	2
Fe_3O_4	154	0.098	9.78	3.41	2
Pd-Ir cubes	340	0.13	5.1	6.5	3
Ir NPs	3.27	0.12	22.57	12.56	This work

Table S2**.** Comparison of the detection limit (LOD) of glucose using different nanomaterials.

Materials	**Linear range (μM)**	**LOD (μM)**	**Method**	**Ref.**
Co_3O_4 NPs	20-200	5	colorimetry	4
CeO_2/NT-TiO_2	10-500	6.1	colorimetry	5
CZIS NCs	16-60	4.1	colorimetry	6
Fe_3O_4	50-1000	30	colorimetry	7
Ir NPs	10-2000	5.8	colorimetry	This work

Table S3. Determination results of glucose in the fetal bovine serum samples.

Original amount (mM)	**Added (mM)**	**Found (mM)**	**Recovery (%)**	**RSD (%)**
	0.1	0.104	104	0.2
0.058	0.2	0.206	103	1.9
	0.3	0.280	93.3	0.4

Reference

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