Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Ultrafine and monodispersed Iridium nanoparticles supported on nitrogen-functional carbon: an efficient oxidase mimic for glutathione colorimetric detection

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Experimental section

Chemical and Materials

Hexachloroiridium acid hydrate ($H_2IrCl_6 \cdot H_2O$), ethylene glycol, urea, ascorbic acid, and ethanol were purchased from Aladdin Ltd. (Shanghai, China). 3,3′,5,5′-tetramethylbenzidine (TMB) were obtained from Sinopharm Chemical Reagent Co., Ltd. $CaCl_2$, $MgCl_2 \cdot 6H_2O$, KCl were provided by Chengdu Kelong Chemical Reagent Factory. NaCl were provided by Shanghai Chemical Reagent General Factory. L-Serine, L-Lysine, L-Leucine, L-Cysteine, L-Valine, L-Isoleucine, L-Proline, L-Tyrosine, L-Glutamic acid, L-Histidine, D(-)-Fructose were purchased from Beijing Innochem Science&Technology Co., Ltd. L-Aspartic Acid, Glycine, L-Phenylalanine, L-(-)-Threonine were provided by Tokyo Chemical Industry Co.,Ltd. L-Arginine, Sucrose, Glutathlone, D-Alanine, D-Methionine, L-Asparagine Monohydrate, D-(+)-Maltose monohydrate were provided by Aladdin reagent Co., Ltd. (Shanghai, China). D-(+)-glucose were purchased from J&k Chemical Ltd. (China). N-Boc-D-tryptophan were provided by Accela ChemBio Co., Ltd. Boc-D-Gln-OH were purchased from Ark Pharm Chemical Technology Co., Ltd.

All chemicals were used as received without further purification. The water used throughout all experiments was purified through a Milli-Q system.

Synthesis of Ir/NC

Ir/NC was prepared following previous method. Perior to synthesis, NC was prepared via a solid-state synthesis method. Typically, 1.0 g of carbon and 1.5 g of urea were thoroughly mixed and then transferred to a ceramic crucible and calcined at

300 °C for 2 h. After being rinsed with ethanol three times, this pyrolysis product was dried in an electric oven at 60 °C overnight. Then, another 2.0 g of urea was added, and the mixture was calcinated again at a low temperature of 175 °C for 4 h. After washing and drying with the same steps, NC was obtained and stored for further use.

To prepare Ir/NC, 75 mg of NC, 150 mg of ascorbic acid, 20 mL of ethylene glycol and 16 mg of hexachloroiridium acid hydrate were added into a 50 mL vial with a cap. After being ultrasonicated for 30 min, a homogeneous mixture was achieved. Then, the mixture was transferred into a Teflon-lined stainless steel autoclave and heat treated at 180 °C for 5 h in an electric oven. When it was cooled down, the colloidal product was taken out and cleaned by ethanol three times before being fully dried at 60 ° C for 24 h in a vavuum drying oven.

As a control sample, Ir/C was synthesized with the same procedure except that carbon black was used instead of the NC matrix.

Characterizations

Powder X-ray diffraction (XRD) data were acquired on a RigakuD/MAX 2550 diffractometer with Cu Kα radiation (λ=1.5418 Å). Transmission electron microscopy (TEM) measurements were performed on a HITACHI H-8100 electron microscopy (Hitachi, Tokyo, Japan). The size distributions of Ir NPs were analyzed based on over 300 particles by measuring their diameters. XPS measurements were performed on an ESCALABMK II X-ray photoelectron spectrometer using Mg as the exciting source. Absorption spectra were recorded with a UV2550 UV-Vis Spectrophotometer (Shimadzu, Japan).

Kinetic analysis

Kinetic measurements were carried out by monitoring the absorbance change at 652 nm. Experiments were conducted using 10 μg/mL Ir/NC (or Ir/C, NC, and C) in 0.2 M HAc-NaAc buffer (pH 4) containing 80 μM TMB.

The Michaelis-Menten behavior of the Ir/NC was investigated by monitoring the absorbance of TMB at 652 nm with UV-vis spectrophotometer. The experiments were carried out at room temperature with different concentrations of TMB in NaAc-HAc buffer (0.2 M, pH 4) in presence of 10 μg/mL Ir/NC. Lineweaver-Burk plots, $1/v = (K_m/V_{max})(1/[S]+1/V_{max})$, was used to calculate the Michaelis-Menten constant, where v represents the initial velocity, V_{max} stands for the maximal reaction velocity, [S] is the concentration of substrate and K_m is the Michaelis constant.

Procedure for the detection of GSH

A typical colorimetric analysis for GSH was realized as followings. Firstly, 20 μL Ir/NC (500 μg/mL) and 16 μL TMB (5 mM) were added into 954 μL 0.2 M NaAc-HAc buffer (pH 4) and mixed thoroughly. After that, the mixture was incubated at 40 °C for 20 min. Then, cool to room temperature, 10 μL GSH with different concentration was added and mixed. The mixed solution was incubated for 2 min at room temperature again. Finally, the solution was used for adsorption spectroscopy measurement.

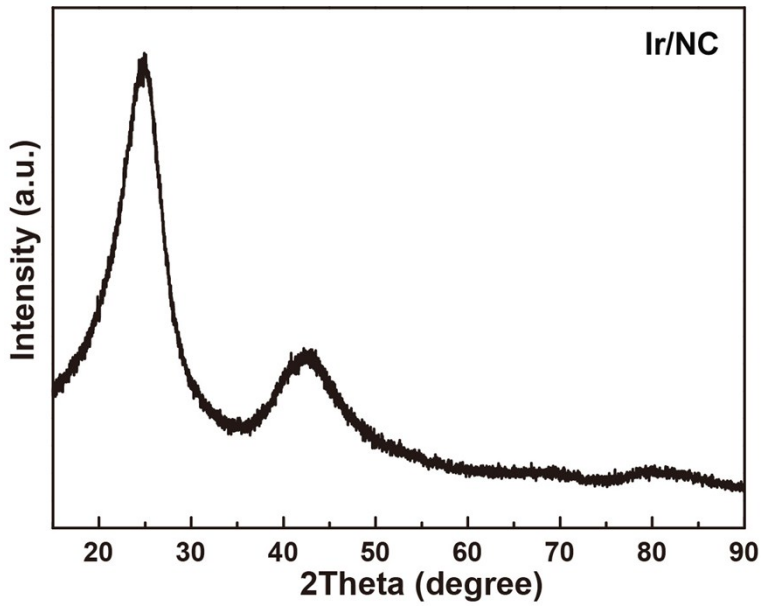

Fig. S1. XRD patterns of Ir/NC.

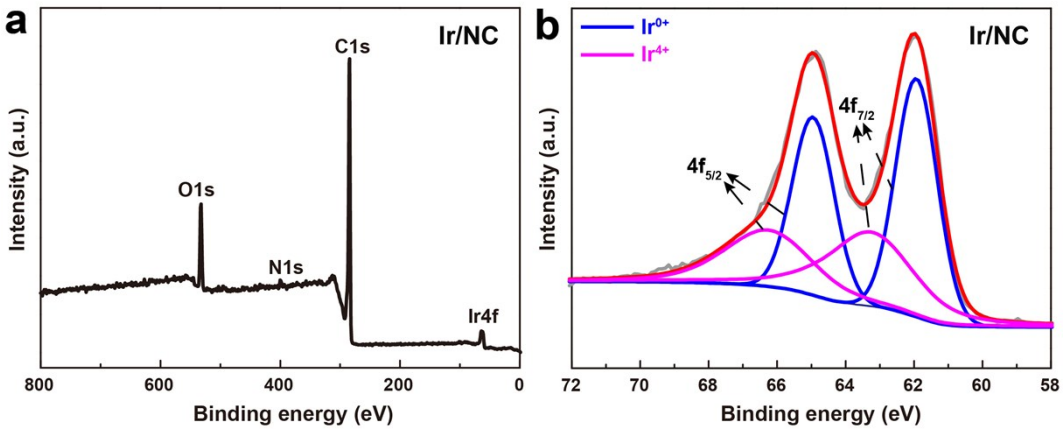

Fig. S2. (a) XPS full spectra and (b) Ir 4f spectra of Ir/NC.

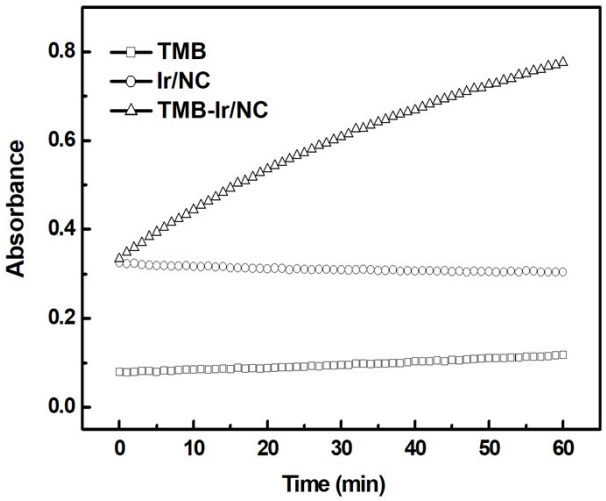

Fig. S3. Time-dependent absorbance of 80 μM TMB, 10 μg/mL Ir/NC, and according mixture solution at 652 nm under room temperature.

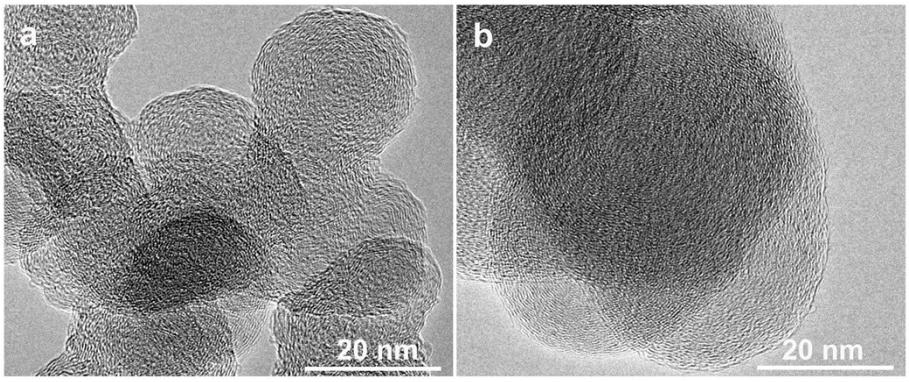

Fig. S4. TEM images of (a) C and (b) NC.

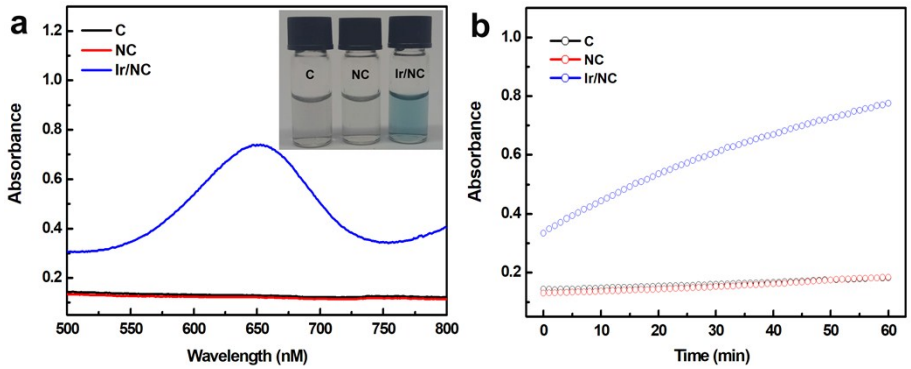

Fig. S5. (a) The absorption spectra of 80 μM TMB in presence of 10 μg/mL C, NC, and Ir/NC at 652 nm under room temperature. Inset shows the digital photos. (b) The corresponding time-dependent absorbance spectra.

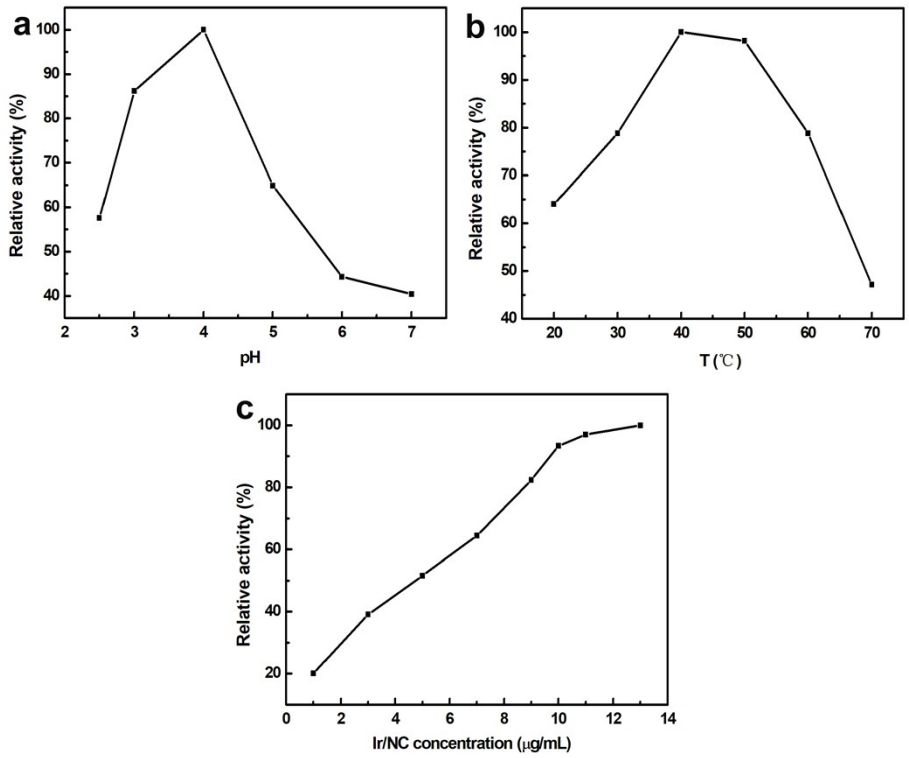

Fig. S6. Dependency of the Ir/NC oxidase-like activity on (a) pH, experiment conditions: Ir/NC 10 μg/mL, TMB 80 μM, HAc-NaAc buffer 0.2 M, 40 ^{o}C; The maximum point in each curve was set as 100%. (b) Temperature, experiment conditions: Ir/NC 10 μg/mL, TMB 80 μM, HAc-NaAc buffer 0.2 M (pH 4); (c) The concentration of Ir/NC, experiment conditions: TMB 80 μM, HAc-NaAc buffer 0.2 M (pH 4), 40 ^{o}C. All adsorption spectroscopy measurements were performed after it cool to the room temperature.

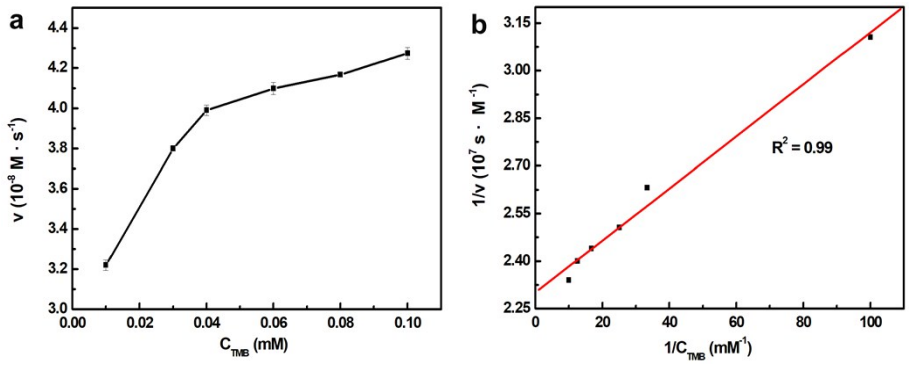

Fig. S7. (a) Steady-state kinetic assays of the 10 μg/mL Ir/NC in 0.2 M HAc-NaAc buffer (pH 4) under room temperature. (b) Double reciprocal plots of the Michiaelis-Menten equation.

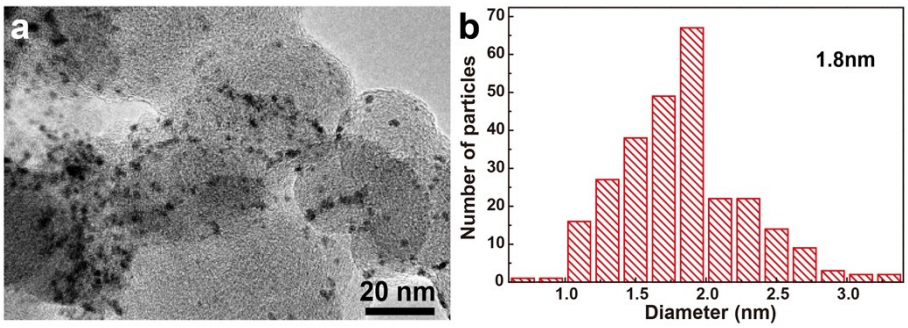

Fig. S8. (a) TEM images and (b) The corresponding Ir NPs distribution for Ir/C.

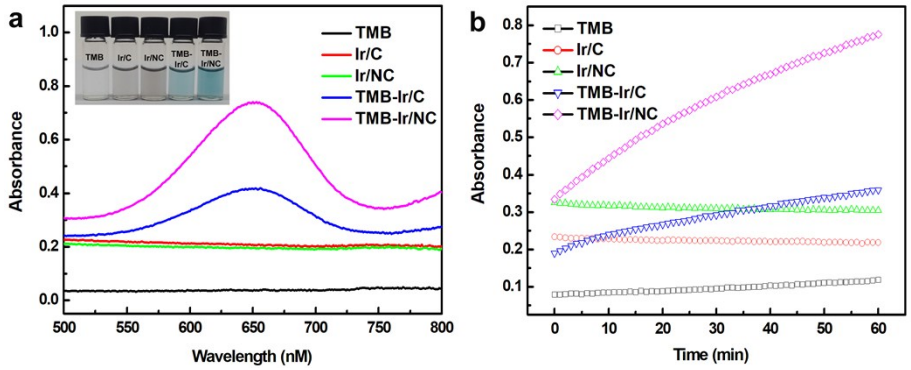

Fig. S9. (a) The absorption spectra and digital photos of 80 μM TMB, 10 μg/mL Ir/C, 10 μg/mL Ir/NC, 80 μM TMB in presence of 10 μg/mL Ir/C, and 80 μM TMB in presence of 10 μg/mL Ir/NC. (b) The corresponding kinetic curves of these solutions under room temperature.

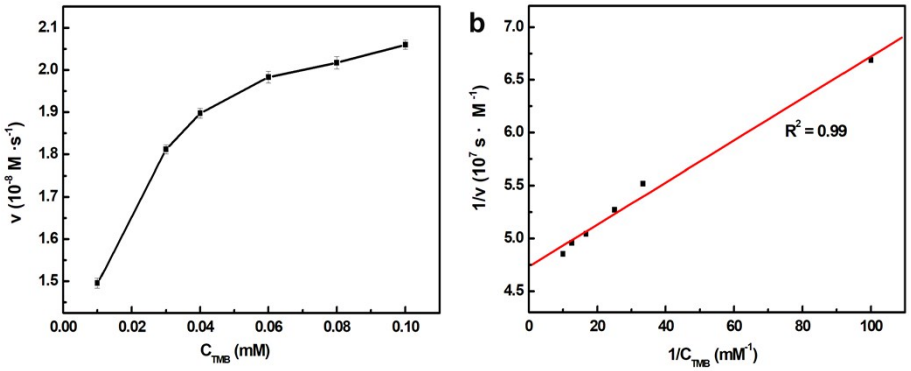

Fig. S10. (a) Steady-state kinetic assays of the 10 μg/mL Ir/C in 0.2 M HAc-NaAc buffer (pH 4) under room temperature. (b) Double reciprocal plots of the Michiaelis-Menten equation.

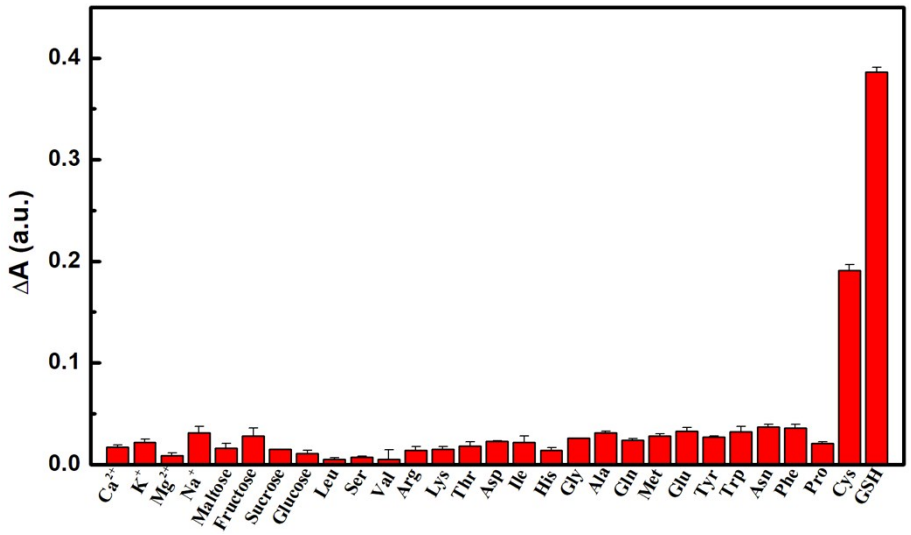

Fig. S11. Interferences test of Ir/NC-TMB system for GSH detection. Reaction conditions: 80 μM TMB, 10 μg/mL Ir/NC, 0.2 M HAc-NaAc buffer (pH 4). Concentrations of the tested substances: 150 μM except 15 μM GSH, 15 μM Cys.

Table S1. Comparison of the apparent Michaelis-Menten constant (K_m) and maximum reaction rates (V_{max}) of Ir colloids, Ir/C and Ir/NC.

Oxidase mimics	**Substrate**	K_m **(mM)**	V_{max} **(M · s^{-1})**	**Ref**
Ir colloids	TMB	0.280	1.365×10^{-7}	26
Ir/**N**C	TMB	0.0036	4.35×10^{-8}	This work
Ir/C	TMB	0.0042	2.11×10^{-8}	This work