Electronic Supplementary Information

Uniformly distributed ruthenium nanocrystals as highly efficient peroxidase for hydrogen peroxide colorimetric detection and nitroreductase for 4-nitroaniline reduction

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Experimental section

Chemical and Materials

Potassium citrate, $NaBH_4$, 4-nitroaniline and ethanol were purchased from Aladdin Ltd. (Shanghai, China). $RuCl_3 \cdot nH_2O$ (Ru content: 37.5 wt%) were purchased from Kunming Borui Co., Ltd. 3,3′,5,5′-tetramethylbenzidine (TMB), H_2O_2 solution were purchased from Sinopharm Chemical. Metal salts were purchased from Chengdu Kelong Chemical Reagent Factory. L-Lysine, Glycine, and L-Glutamic acid were purchased from Beijing Innochem Science&Technology Co., Ltd. Glucose, Sucrose, D-(+)-Maltose monohydrate, and Fructose were provided by Aladdin reagent Co., Ltd. (Shanghai, China). All reagents were of analytical reagent grade and used as received without any purification. The water used throughout all experiments was purified through a Milli-Q system.

Synthesis of Ru/NC

Synthesis of porous carbon (PC): To prepare PC, 5 g of potassium citrate was directly carbonized at 800 ^oC for 1 h in pure argon flow. The black calcined product was pestled to powder, and immersed into 1 M H_2SO_4 solution for several hours to remove impurities, and washed with deionized water until neutral. The sample was dried at room temperature for overnight, resulting in the final PC.

Synthesis of Ru/PC: Ru/PC was synthesized via a typical impregnation method. 40 mg of PC was slowly introduced into 25 mL of deionized water by ultrasonication for 30 min to form homogenous solution (solution A). Then, 0.2 mM of $RuCl_3 \cdot nH_2O$ was dispresed in 25 mL of deionized water as solution B. Subsequently, the solution B

was added by dropwise manner into the solution A with continuous stiring. Then, the whole mixture was further stirred at a rate of 700 rpm for 12 h at room temperature. The resultant material was washed and naturally dried at room temperature and labeled as the Ru/PC. As for get a series of Ru/PC-T (T represents the temperature), different stirring temperatures of 50, 70 and 90 °C were selected for the synthesis of Ru/PC-50, Ru/PC-70 and Ru/PC-90, respectively.

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 Ru 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).

The peroxidase-like activity of Ru/PC

The peroxidase-like activity of Ru/PC is explored as followings: Firstly, 40 μL Ru/PC aqueous solution (500 μg/mL) was added to the HAc-NaAc buffer (920 μL, 0.2 M, pH 6) in the presence of 20 μL TMB (5 mM) and 20 μL H_2O_2 (100 mM). After 5 min, the absorbance at 652 nm was measured by a UV-vis spectrophotometer.

Kinetic analysis

Kinetic measurements were carried out by monitoring the absorbance change at

652 nm. Reaction conditions: 0.1 mM TMB, 2 mM H_2O_2, 20 μg/mL Ru/PC (or Ru/PC-T, PC), 0.2 M HAc-NaAc buffer (pH 6).

The Michaelis-Menten behavior of the Ru/PC was investigated by altering either the concentration of H_2O_2 or TMB and then monitoring the absorbance of oxTMB at 652 nm with UV-vis spectrophotometer. The experiments were performed at room temperature with different concentrations of TMB or H_2O_2 in NaAc-HAc buffer (0.2 M, pH 6) in presence of 20 μg/mL Ru/PC. 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 H_2O_2

A typical colorimetric analysis for H_2O_2 was realized as followings. Firstly, 40 μL Ru/PC (500 μg/mL) and 20 μL TMB (5 mM) were added into 920 μL 0.2 M NaAc-HAc buffer (pH 6) and mixed thoroughly. After that, the mixture was further added with different volume of H_2O_2 (100 mM), mixed and incubated for 5 min at room temperature. Finally, the solution was used for measurement by a UV-vis spectrophotometer.

Catalytic reduction of 4-NA

The 4-NA (0.01 M) and ice-cold $NaBH_4$ (0.05 M) aqueous solutions were freshly prepared before test. The catalytic reduction of 4-NA was performed in a quartz cuvette. Firstly, 40 μL 4-NA and 160 μL freshly prepared ice-cold $NaBH_4$ were mixed with 740 μL water. Then, 60 μL Ru/PC (500 μg/mL) was added and mixed thoroughly.

After that, the solution was used for measurement by a UV-vis spectrophotometer.

All catalytic reactions were carried out at room temperature.

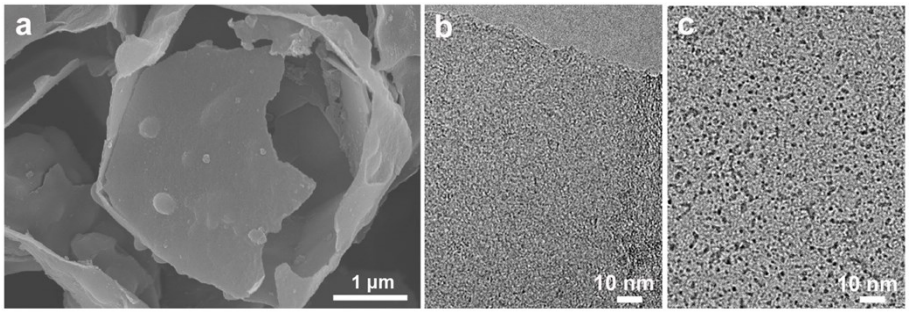

Fig. S1. (a) SEM and (b) TEM images of PC support. (c) TEM image of Ru/PC.

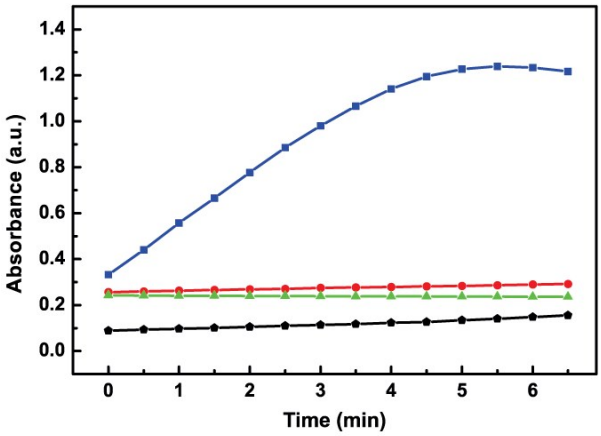

Fig. S2. Time-dependent absorbance change of oxTMB at 652 nm for those reaction systems. System 1 (black line): 0.1 mM TMB + 2 mM H_2O_2; System 2 (green line): 2 mM H_2O_2 + 20 μg/mL Ru/PC; System 3 (red line): 0.1 mM TMB + 20 μg/mL Ru/PC; System 4 (blue line): 0.1 mM TMB + 2 mM H_2O_2 + 20 μg/mL Ru/PC.

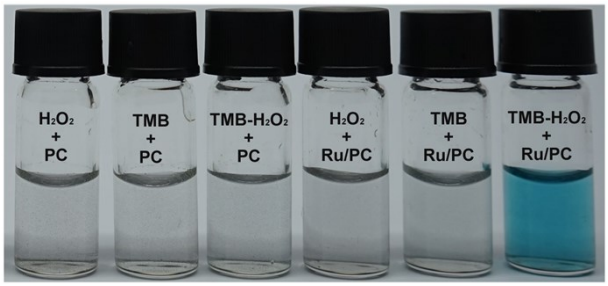

Fig. S3. Photographs of different reaction systems. Reaction conditions: 5 mM TMB, 2 mM H_2O_2, 20 μg/mL PC or Ru/PC, 0.2 M HAc-NaAc buffer (pH 6).

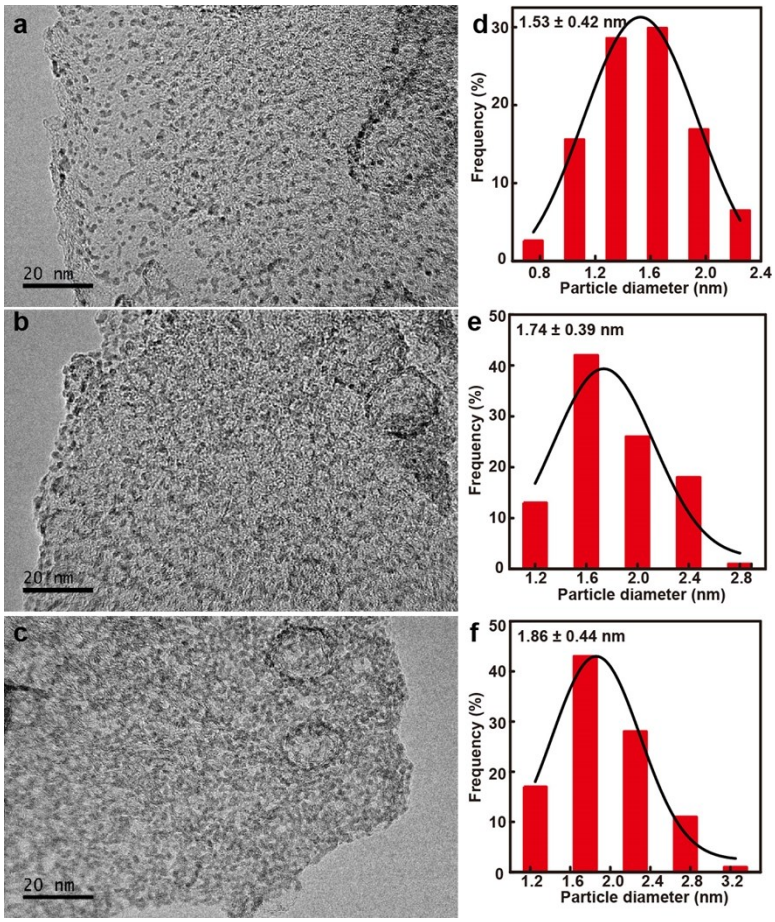

Fig. S4. TEM images of Ru/PC-50 (a), Ru/PC-70 (b), and Ru/PC-90 (c), and the corresponding NPs' size distribution histograms of Ru/PC-50 (d), Ru/PC-70(e), and Ru/PC-90 (f).

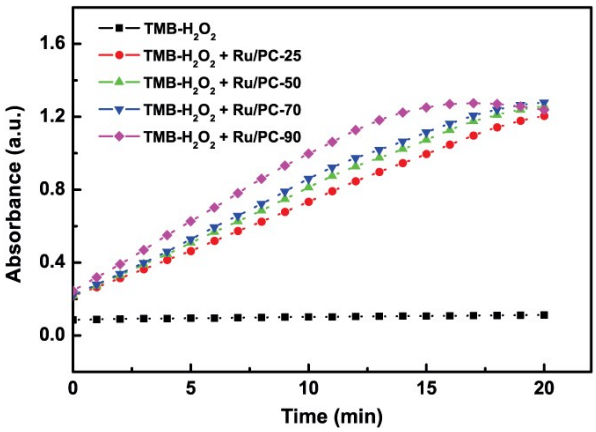

Fig. S5. Time-dependent absorbance change of oxTMB at 652 nm for TMB-H_2O_2 (black line) and TMB-H_2O_2 with the addition of Ru/PC-T. Reaction conditions: 0.1 mM TMB, 1 mM H_2O_2, 15 μg/mL Ru/PC-T, 0.2 M HAc-NaAc buffer (pH 6).

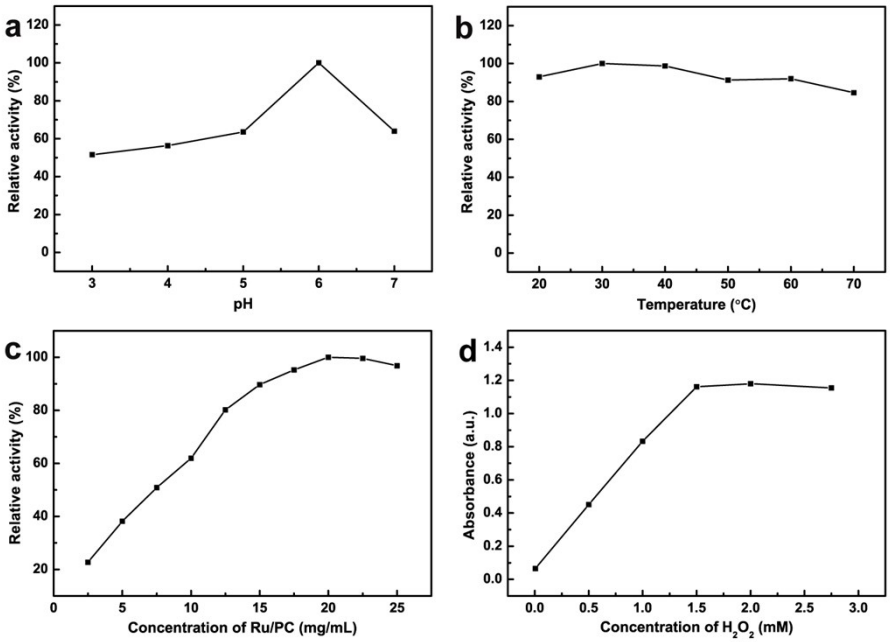

Fig. S6. Dependency of Ru/PC peroxidase-like activity on (a) pH (experiment conditions: Ru/PC 20 μg/mL, TMB 0.1 mM, H_2O_2 2mM, HAc-NaAc buffer 0.2 M with pH ranging from 3.0 to 7.0, 30 ^oC), (b) incubation temperature (experiment conditions: Ru/PC 20 μg/mL, TMB 0.1 mM, H_2O_2 2mM, HAc-NaAc buffer 0.2 M (pH 6)), (c) concentration of Ru/PC (experiment conditions: TMB 0.1 mM, H_2O_2 2mM, HAc-NaAc buffer 0.2 M (pH 6), 30 ^oC) and (d) concentration of H_2O_2 (experiment conditions: Ru/PC 20 μg/mL, TMB 0.1 mM, HAc-NaAc buffer 0.2 M (pH 6), 30 ^oC).

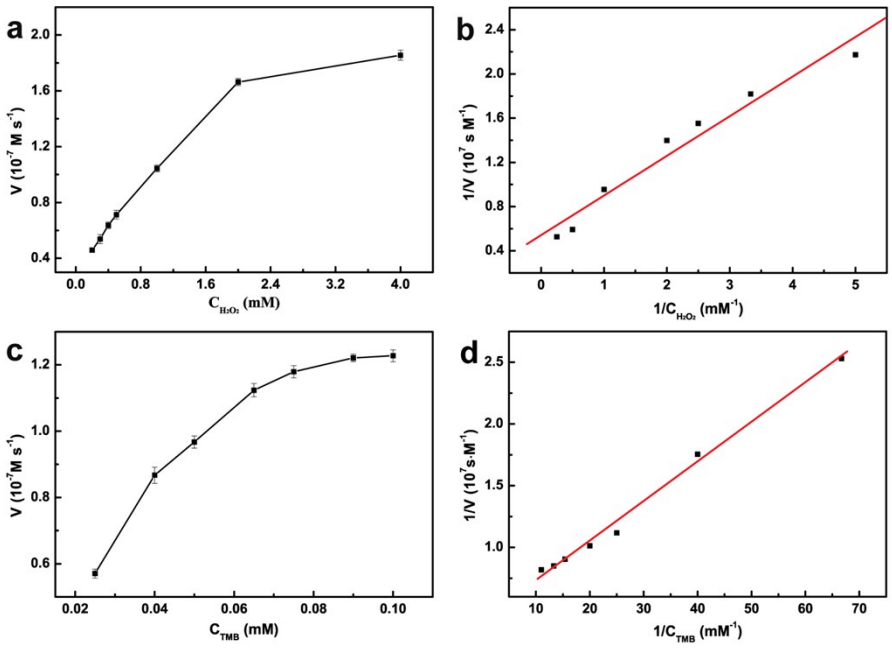

Fig. S7. (a) Steady-state kinetic assays by varying of H_2O_2 concentrations while keeping the concentration of TMB constant (0.1 mM). (b) The Lineweaver-Burk plots of the double reciprocal plots of the Michiaelis-Menten equation for (a). (c) Steady-state kinetic assays by varying of TMB concentrations while keeping the concentration of H_2O_2 constant (2 mM). (d) The Lineweaver-Burk plots of the double reciprocal plots of the Michiaelis-Menten equation for (c).

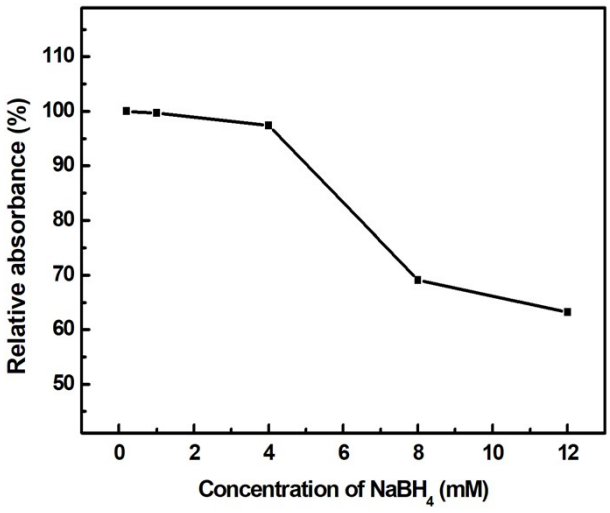

Fig. S8. Dependency of relative absorbance of 4-NA at 380 nm on $NaBH_4$ concentration. (experiment conditions: Ru/PC (30 μg/mL), 4-NA (0.4 mM) and freshly prepared ice-cold $NaBH_4$ with different concentration.)

Table S1. Comparison of the peroxidase-like activity of other Ru nanoenzymes and HRP.

Catalyst	Substrate	K_m (mM)	V_{max} (10^{-8} M s^{-1})	Ref
Ru/PC	TMB	0.071	2.285	**This work**
	H_2O_2	0.969	1.79	**This work**
Ru NPs	TMB	0.234	8.25	28
	H_2O_2	2.206	58.2	28
Ru frames	TMB	0.0603	13.4	29
	H_2O_2	318	7.41	29
HRP	TMB	0.434	10	30
	H_2O_2	3.7	8.71	30