Electronic Supplementary Information

Graphene Encapsuled Ru Nanocrystal with Highly-efficient Peroxidase-like Activity for Glutathione Detection at Near-physiological pH†

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1. Experimental Section

1.1 Reagents

Ruthenium (III) chloride trihydrate and fumed silica were obtained from Shanghai Macklin Biochemical Co., Ltd. 3,3′,5,5′-tetramethylbenzydine (TMB), 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), dopamine hydrochloride (DA), 3,3'-diaminobenzidine tetrahydrochloride (DAB) and rhodamine B (RhB) were purchased from Alfa Aesar (Tianjin) Chemical Co., Ltd. Double-distilled water was obtained from Milli-Q Integral System (18.2 $M\Omega.cm^{-1}$). All other analytical reagents were purchased from Changsha Chemical Reagents Company. The human serum samples were obtained from Xiangya Hospital, Central South University.

1.2 Instrumentations

Hydrodynamic diameters and zeta potential measurements were carried out by the Malvern Zeta sizer Nano ZS90. High resolution transmission electron microscopic (HR-TEM) images were collected by the Tecnai G2 F20 S-TWIN TMP. UV-vis absorption spectra were obtained by Shimadzu spectrophotometer (UV-2450). Raman measurements were conducted by the Renishaw's InVia Raman system under the conditions of the 532 nm laser excitation with 0.17 mW laser power.

1.3 Synthesis of Ru@G

Graphene encapsuled Ru nanocrystal (Ru@G) was synthesized by the chemical vapor deposition (CVD) method.[1-3] Briefly, fumed silica (1.00 g) was dispersed thoroughly in methanol (200 mL) with ultrasound for 2 h, and then the ruthenium(III) chloride trihydrate (0.02 g) was added to the solution and continuing with ultrasound for 1.5 h. After elimination of the methanol and dried, the mixture powder was brought to a tube furnace for CVD growth with the CH_4 flow of 150 cm^3min^{-1} for 6 minutes. Subsequently, the product was treated with hydrofluoric acid (HF) to remove the fumed silica, and then modified with polyoxyethylenestearyl ether (C_{18}-PEG, MW = 4670) through hydrophobic interactions by using ultrasound to increase the water solubility. Finally, the product was collected by centrifugation.

1.4 Peroxidase-like activity and kinetic study of Ru@G

The study on the peroxidase (POD)-like activity of Ru@G was performed by using different POD colorimetric substrates including 3,3′,5,5′-tetramethylbenzydine (TMB), 2,2'-azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 3,3'-diaminobenzidine tetrahydrochloride (DAB)

and dopamine hydrochloride (DA). The experiments were conducted in PBS (25 mM, pH 4.0) containing Ru@G (0.33 mg L^{-1}), H_2O_2 (5 mM) and colorimetric substrate (1 mM). The absorption spectra of the reaction solution were measured within 5 min. The concentration dependence of Ru@G through TMB, ABTS, DAB and DA was conducted similar with above condition, except the concentration of Ru@G was varied (0, 0.10, 0.21 and 0.33 mg L^{-1}) and then the reaction solutions were measured using UV–vis spectrophotometer as a function of time for 3 min. The pH dependence of Ru@G on the POD-like activity was carried out at room temperature at different pH values from 1.0 to 10.0, and the temperature dependence was further conducted in 1 mL PBS (25 mM, pH 4.0), containing Ru@G (0.33 mg L^{-1}), H_2O_2 (5 mM) and TMB (1 mM) at different temperatures from 10-70 °C.

The steady-state kinetic assay was carried out at room temperature in PBS (25 mM, pH 4.0) as a reaction solution. The TMB and H_2O_2 substrates were added in the solution system, containing Ru@G (5.0×10^{-14} M) by changing the concentration of either TMB or H_2O_2 and preserving the other concentration constant (Steady-state kinetic assay toward TMB (0.01993-0.45 M), in which H_2O_2 concentration was fixed at 0.6 M and steady-state kinetic assay toward H_2O_2 (0.009-0.68 M), in which TMB concentration was fixed at 0.5 mM and then the absorbance of the reaction solution at 652 nm was measured using UV–vis spectrophotometer as a function of time for 3 min. The plots of ν against substrate concentrations were fitted using nonlinear regression of the Michaelis-Menten equation. The kinetic parameters were calculated by using Michaelis-Menten equation $\nu = V_{\max} \times [S]/[S] + K_m$, where ν is the initial velocity, [S] is the substrate concentration and Km is the Michaelis constant. K_m and V_{max} were obtained from Lineweavere-Burk double reciprocal plots.

1.5 Catalytic mechanism of Ru@G

The catalytic mechanism of Ru@G was first conducted by investigating the effect of isopropanol on the peroxidase-like activity. In typical experiment, isopropanol (5 mM) was added in 1 mL PBS (25 mM, pH 4.0), containing TMB (1 mM), Ru@G (0.33 mg L^{-1}) and H_2O_2 (5 mM) for 5 min at room temperature and then the absorbances were measured. The RhB degradation experiment was conducted in 1 mL PBS (25 mM, pH 4.0) containing RhB (1 mM), Ru@G (0.33 mg L^{-1}) and H_2O_2 (0.5 M) for 6 h at room temperature and then the absorbances at 553 nm were measured by UV-vis spectrophotometer. The experiment on ascorbic acid (AA) oxidation was

conducted in 1 mL PBS (25 mM, pH 4.0) containing AA (1 mM) and Ru@G (0.33 mg L^{-1}) for 10 min at room temperature and then the absorbances at 240 nm were measured.

1.6 Stability study of Ru@G

The catalytic activity stability was carried out by incubating Ru@G (0.33mg L^{-1}) in DPBS, DMEM and different NaCl concentration (0, 1, 10, 50, 150 mM) for 6 h. and then the solutions were measured by using UV-vis spectrophotometer. The stability of Ru@G at different pH and temperature was also conducted. Typically, Ru@G (0.33mg L^{-1}) was incubated in different pH solution and heated with different temperature for 2 h, respectively. After treatment at different conditions as mentioned above, H_2O_2 (5 mM) and TMB (1 mM) were added into the reaction system and then the absorbances at 652 nm were measured after 5 min of reaction time. The relative catalytic activities were calculated under standard conditions, in which the activities at pH 4.0 and temperature 25 °C were set as 100 %.

1.7 Detection of GSH

A typical colorimetric assay for GSH detection was carried out by adding the various concentrations of GSH into the reaction systems. The entire experiment conditions were conducted at room temperature (~25 °C) in 1 mL PBS (25 mM, pH 7.0), containing TMB (1 mM), Ru@G (0.52 mg L^{-1}) and H_2O_2 (5 mM), then the absorbance at 652 nm of the solution was measured after 5 min. In order to test the specificity of the current assay, Ru@G were further applied to detect different interfering substrates, such as glucose, fructose, sucrose, dopamine (DA) and L-cysteine under the same condition for GSH detection. However, the optimum GSH detection was carried out in 60 μM GSH concentration, which was 40% lower than the concentration of other interferences. The selectivity of GSH was calculated after knowing the absorbance changes of GSH and other interferences. All experimental conditions were conducted in 1mL PBS (25 mM, pH 7.0), containing TMB (1 mM), Ru@G (0.52 mg L^{-1}) and H_2O_2 (5 mM) at 25 °C, the reaction time was fixed for 5 min.

The applicability of the assay was applied to detect GSH in real human serum samples by using the standard addition method. The 50-fold diluted serum samples were added into PBS (25 mM, pH 7.0) containing TMB (1 mM), Ru@G (0.52 mg L^{-1}), H_2O_2 (5 mM) and different concentrations (10, 20 and 30 μM) of GSH. The concentration of GSH was obtained from calibration plot after knowing the absorbance of the solution samples.

2. Results and Discussion

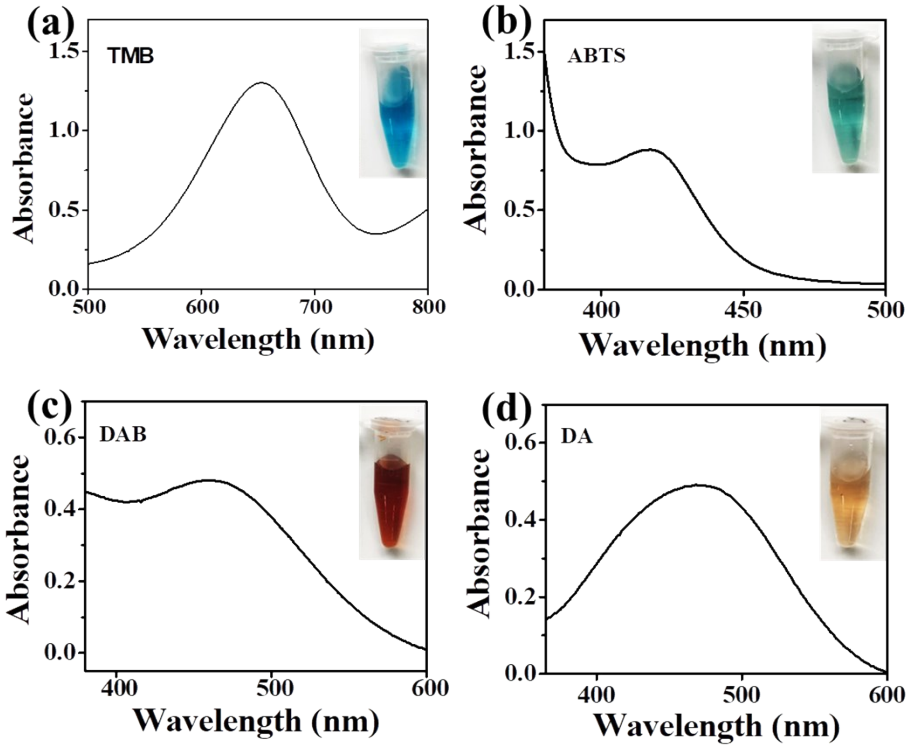

Fig. S1. UV-vis absorption spectra of (a) TMB, (b) ABTS, (c) DAB and (d) DA in the presence of POD-like Ru@G (insets: photos of the corresponding products).

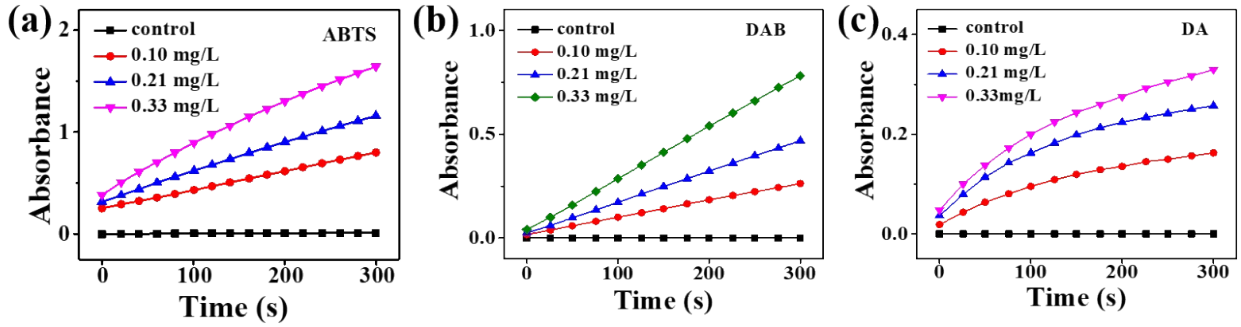

Fig. S2. Concentration dependence of the POD-like activity of Ru@G using (a) ABTS, (b) DAB and (c) DA as the colorimetric probes.

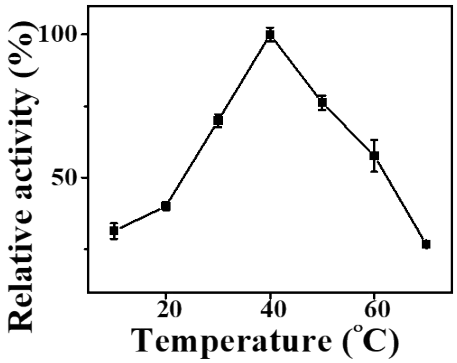

Fig. S3. Temperature dependence of the POD-like activity of Ru@G using TMB as the colorimetric probe.

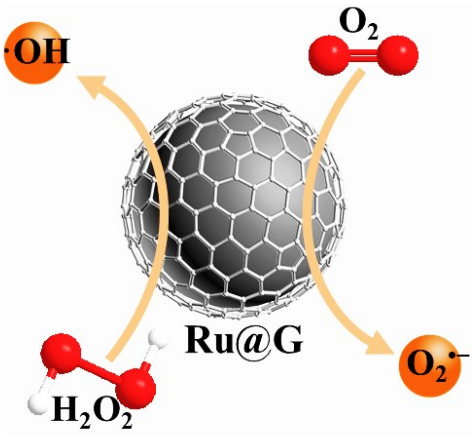

Fig. S4. Possible catalytic mechanism for TMB oxidation by Ru@G.

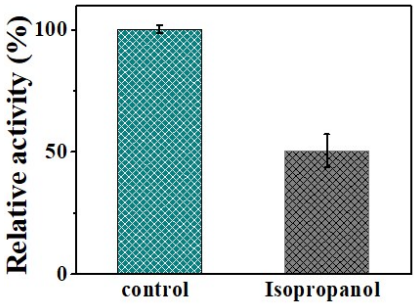

Fig. S5. Effect of isopropanol on the catalytic activity of Ru@G. In typical experiment, isopropanol (5 mM) was added in 1 mL PBS (25 mM, pH 4.0), containing TMB (1 mM), Ru@G (0.33 mg L^{-1}) and H_2O_2 (5 mM) for 5 min at room temperature.

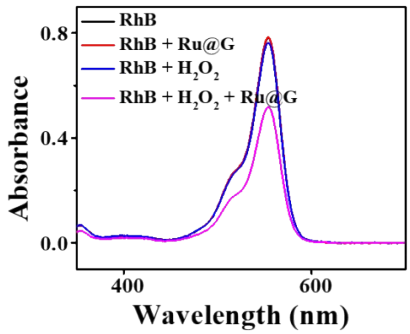

Fig. S6. UV–vis spectra of RhB in different conditions. The RhB degradation experiment was conducted in 1 mL PBS (25 mM, pH 4.0) containing RhB (1 mM), Ru@G (0.33 mg L^{-1}) and H_2O_2 (0.5 M) for 6 h at room temperature.

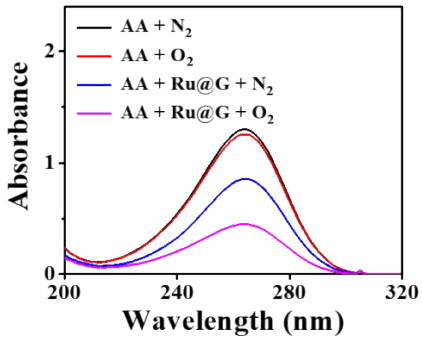

Fig. S7. UV-vis absorption spectra of AA in different conditions. The experiment on ascorbic acid (AA) oxidation was conducted in 1 mL PBS (25 mM, pH 4.0) containing AA (1 mM) and Ru@G (0.33 mg L^{-1}) for 10 min at room temperature and then the absorbances at 240 nm were measured.

Table S1 Comparison of Michaelis–Menten constant (K_m) and maximum reaction rate (V_{max}) between Ru@G* and other catalysts (nanoparticles).

Catalysts	Substrates	K_m (mM)	V_{max} (M s^{-1})	Reference
HRP	TMB	0.43	1×10^{-7}	3
	H_2O_2	3.7	8.71×10^{-8}	
Rh NSs	TMB	0.26	1.25×10^{-7}	4
	H_2O_2	4.51	6.8×10^{-7}	
Rh	TMB	0.189	$6.78\text{x}10^{-8}$	5
	H_2O_2	0.38	2.41×10^{-7}	
Pd NSs	TMB	0.1098	5.82×10^{-8}	6
	H_2O_2	4.398	6.51×10^{-8}	
Ru frames	TMB	0.06	1.34×10^{-7}	7
	H_2O_2	300	7.41×10^{-8}	
Pd-Ir cubes	TMB	0.13	6.5×10^{-8}	8
	H_2O_2	340	5.1×10^{-8}	
Fe_3O_4@C	TMB	0.072	17.99×10^{-8}	9
	H_2O_2	0.38	73.99×10^{-8}	
PtCNs	TMB	0.0375	14.9×10^{-8}	10
	H_2O_2	0.1	1.4×10^{-9}	
Pd cubes	TMB	0.054	9.7×10^{-8}	11
	H_2O_2	700	6.5×10^{-8}	
Ru@G	TMB	0.027	16.3×10^{-7}	This work
	H_2O_2	5.8	1.37×10^{-7}	

*In this experiment (kinetic assay), the concentration of Ru@G used was 5×10^{-14} M by the calculation of nanoparticles amount. The Ru nanoparticles were considered as main catalyst center.

Reference

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