Electronic Supplementary Information

Direct growth of ordered Co doped PdCu nanoparticles on graphene oxide based on one-step hydrothermal method for ultrasensitive sensing of H_2O_2 in living cells

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Experimental section

Materials and reagents

Graphite powder (99.998%, 325mesh, Alfa Aesar), Palladium (II) acetylacetonate ($Pd(acac)_2$, 99%), copper (II) acetylacetonate ($Cu(acac)_2$, 97%), cobalt (II) acetylacetonate ($Co(acac)_2$, 97%), oleylamine(OA, 80-90%), ascorbic acid (AA, AR), uric acid (UA)and iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$,AR, 99%)were all purchased from Aldrich. H_2O_2 (30%, v/v aqueous solution)was gotfrom Tianjin Tianli Chemistry Reagent Co., Ltd (Tianjin, China),Phosphate buffer solution (PBS, 0.1 M, pH 7.2, NaH_2PO_4 and Na_2HPO_4) was used as the supporting electrolyte.

Apparatus

Field emission electron microscopic (FESEM, SU8020HITACHI Japan), field emission transmission electron microscopic (FETEM, Tecnai G^2 F20 S-TWIN, FEI, USA), X-ray diffraction (XRD, D/MAX-3C, Rigaku, Japan), fourier transform infrared spectroscopy (FTIR, TENSIR 27, Bruker, German).

Electrochemical measurements were carried out in a conventional three-electrode electroanalysis system controlled by CHI 660 electrochemical workstation (Shanghai CH Instrument Co. Ltd., China). All electrochemical experiments were conducted at room temperature (25±2°C). Cell detection was conducted at 37°C.

Synthesis of PdCu/GO and Co-PdCu/GO nanomaterials

GO was prepared from graphite powder based on a typical method.[1]Exfoliation of GO was achieved by ultrasonication of the dispersion in an ultrasonic bath.

PdCu/GO nanomaterials were synthesiszed by one-step hydrothermal method.

Typically, 15.2 mg $Pd(acac)_2$, 26.6 mg $Cu(acac)_2$, 10.8 mg $FeCl_3 \cdot 6H_2O$, 70 mg AA and 20mL OA were mixed, then ultrasonicated for 1 h. 30mg GO were dispersed in the mixture, and further ultrasonicated for 0.5 h. At last, the solution was transferred into a 50 mL Teflon-lined stainless steel autoclave and the reaction was performed under 220°C for12 h, and then cooled down to room temperature.The resulting colloidal products were collected by centrifugation and washed five times with an acetone/ethanol/cyclohexane mixture. Synthesis of Co-PdCu/GO was similar to that of PdCu/GO except for addition of 6.4 mg $Co(acac)_2$.

Comparison experiment forPdCu/GO synthesis

The comparison experiment was also performed by hydrothermal method. 15.2 mg $Pd(acac)_2$, 26.6 mg $Cu(acac)_2$ and 20mL OA were mixed, followed by ultrasonication (1 h). 30mg GO were dispersed in the mixture, and further ultrasonicated for 0.5 h.10.8 mg $FeCl_3 \cdot 6H_2O$, 70 mg AA were solved in 10 mL water, and mixed with above GO suspension solution. At last, the mixture was transferred into a 50 mL Teflon-lined stainless steel autoclave and the reaction was performed under 220°C for12 h, and then cooled down to room temperature. The resulting colloidal products were collected by centrifugation and washed five times with an acetone/ethanol/cyclohexane mixture.

Fabrication of modified electrode

The preparation method has been used in our previous work.[2] In brief, the modified glassy carbon electrode (GCE) was prepared by a e surface coating method. Prior to use, GCE was processed first: GCE was polished by 0.3 μm alumina powder and rinsed with ultrapure water. After these, the GCE was immersed into absolute ethanol and ultrapure water under ultrasound respectively. Then, the GCE was dried in a stream of nitrogen. 1 mg nanomaterials were dispersed in 0.5 mL ultrapure water and 50μL suspension was mixed with 50μL nafion (0.05%) solution under ultrasound. At last, 10μL mixture was dropped on surface of GCE and dried in fridge under 4°C. The modified electrode can be expressed as PdCu/GO/GCE and Co-PdCu/GO/GCE.

Electrochemical Detection of H_2O_2 Released from Cells

PC-12 rat adrenal medulla pheochromocytoma and normal adrenal medulla cells

were provided by Xi'an Medical University (Xi'an, Shaanxi, PR China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM).The medium was supplemented with 100 units $mL^{-1}$of penicillin, 100 units $mL^{-1}$of streptomycin and 10% bovine serum. The culture was incubated under 37°C.To utilize the cells for electrochemical measurements, the cells were separated from the culture by centrifugation at 1300 rpm and washed three times with sterile buffer to remove any remaining culture medium or serum and suspended in PBS (0.1 M).Cell number was about 4.5×10^7 which was estimated by a cell counter and the cell solution was 2 mL.

In order to detect H_2O_2 released from living cells, 1 μM AA was injected to the cells suspension every times to motivate cells generate H_2O_2. It may be due to the following reasons which have been reported before.[3-4] In general, the concentration of H_2O_2 in cells keeps a value favored for cellular proliferation. However, the relative H_2O_2 will diffuse from high concentration areas to low concentration areas when intracellular redox homeostasis was interrupted by artificial stimulation. Therefore, the extracellular and intracellular concentrations of H_2O_2 will be retained at the same level. Thus, quantitative detection of flux of H_2O_2release from living cells will be realized. The catalase was 300U/mL. The electrochemical experiments were conducted in water bath under 37°C.

References

1. G. Q. Luo, X. J. Jiang, M. Li, ACS Appl. Mater. Interfaces, 2013, **5**, 2161.

2.W. S.Bai, F.Nie, J. B.Zheng, Q. L.Sheng, *ACS Appl. Mater. Interfaces,*2014, **6**, 5439-5449.

3. Y.Zhang, X. Bai, X.Wang, *Anal. Chem.,*2014, **86**, 9459-9465.

4. G. P.Bienert, J. K.Schjoerring, T. P.Jahn, *Biochim. Biophys. Acta,*2006, **1758**, 994-1003.

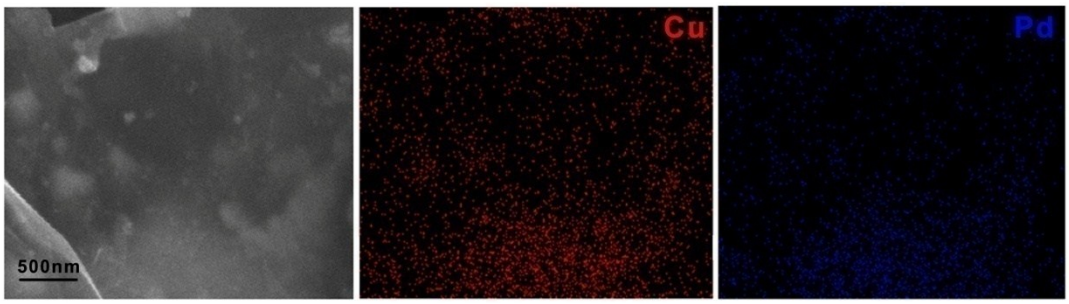

Fig. S1 SEM elemental mapping of PdCu/GO.

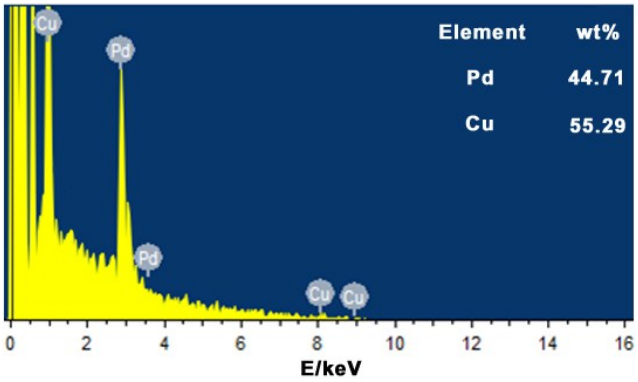

Fig. S2 SEM-EDS of PdCu/GO.

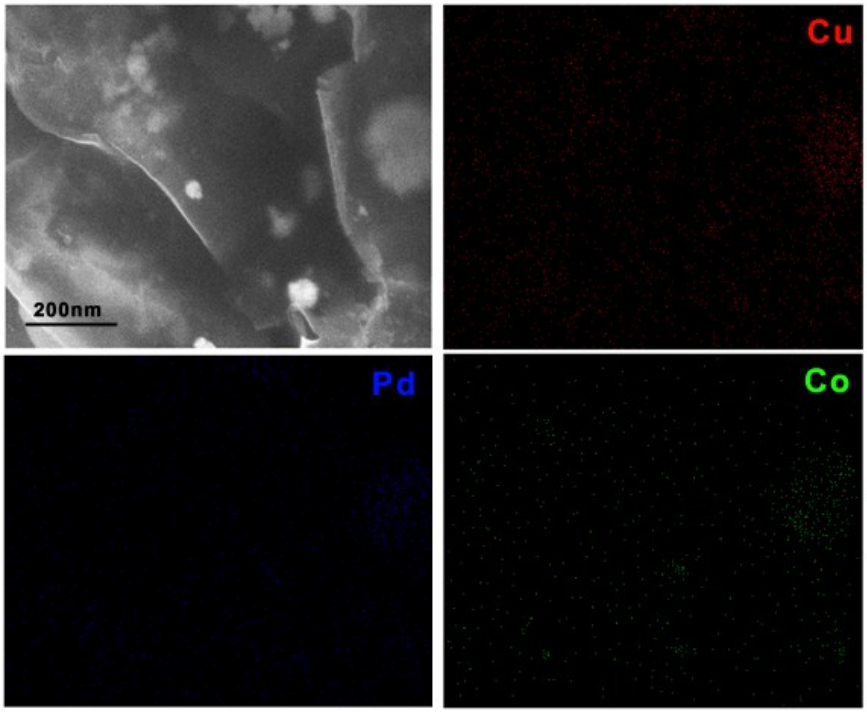

Fig. S3 SEM elemental mapping of Co-PdCu/GO.

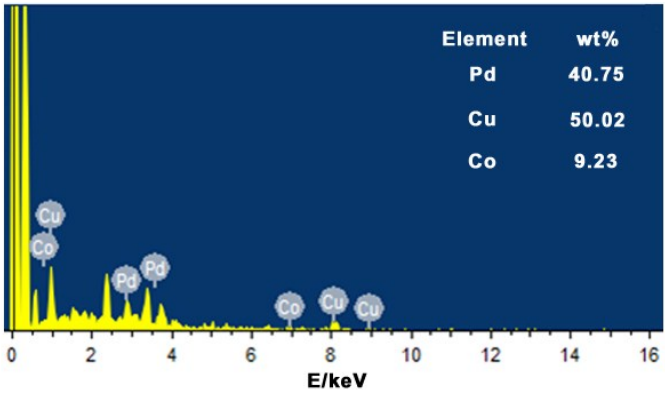

Fig. S4 SEM-EDS of Co-PdCu/GO.

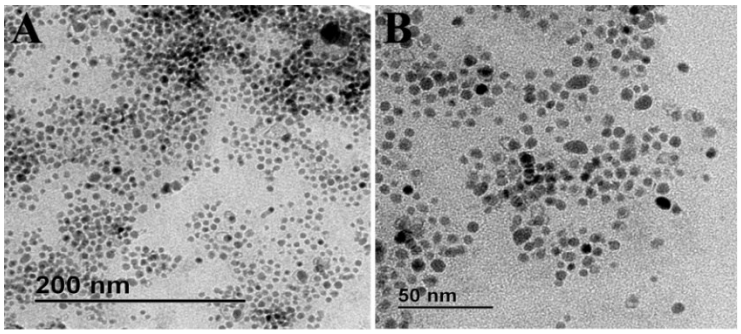

Fig. S5TEM of PdCu/GO prepared based on comparison experiment.

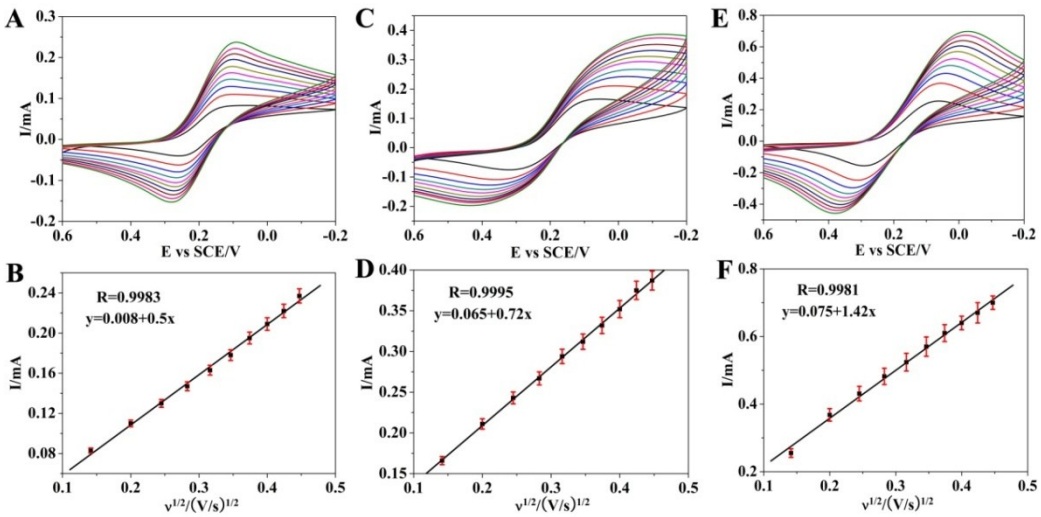

Fig.S6CVs of bare GCE(A), GO/GCE (C) and Co-PdCu/GCE(E) in 1M KCl solution containing 5mM$MK_3[Fe(CN)_6]$ (from a to j: 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200mV/s).The corresponding linear fitting programs of bare GCE(B), GO/GCE (D) and Co-PdCu/GCE (F) between peak current and square root of scan rate.

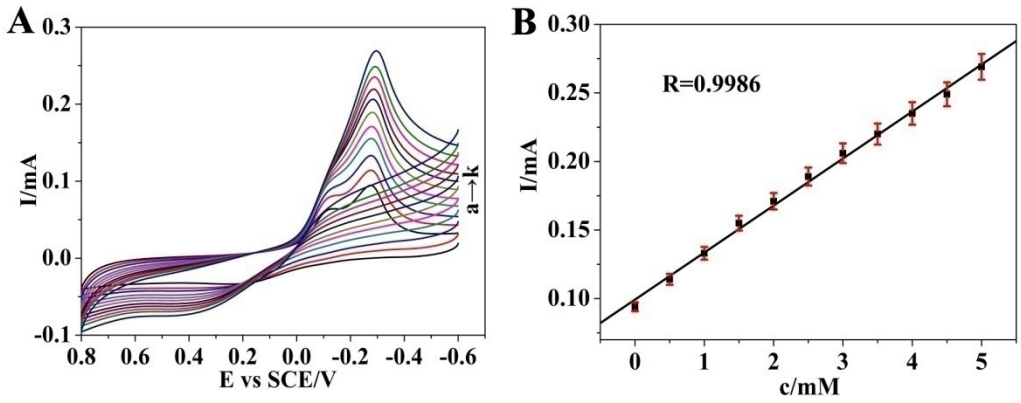

Fig. S7 (A)CVs of Co-PdCu/GCE in 0.1M PBS (pH 7.2) in absence and presence of H_2O_2 with different concentrations (from a to k: 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5,4, 4.5 and 5mM) at a scan rate of 50mV/s. (B) The corresponding linear fitting programs of Co-PdCu/GCE between peak current and H_2O_2concentration.

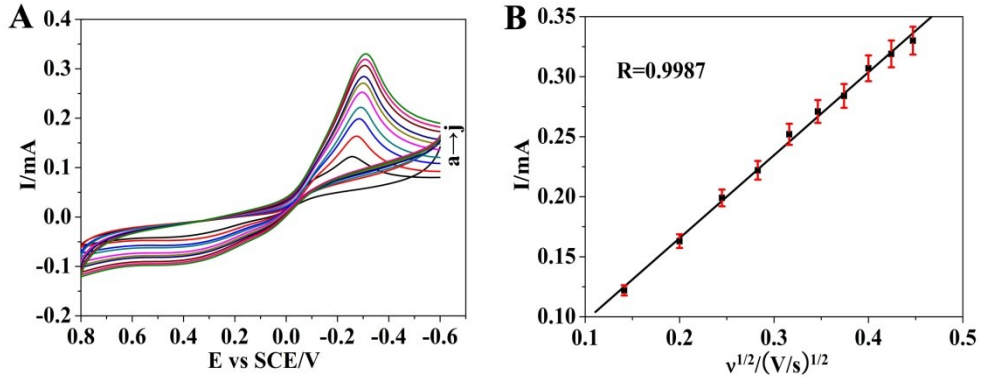

Fig. S8 (A)CVs of Co-PdCu/GCE in 0.1M PBS (pH 7.2) containing 5mM H_2O_2 at different scan rates (from a to j: 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200mV/s). (B) The linear fitting program of the reduction peak currents with the square root of scan rate.

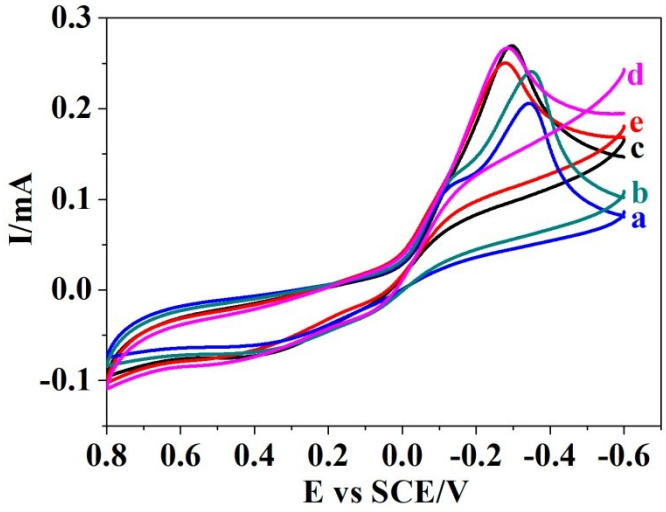

Fig. S9 CVs of Co-PdCu/GCE with different content of Co in 0.1M PBS (pH 7.2) containing 5mM H_2O_2 (from a to d: Co-PdCu nanomaterials were synthesized based on 1.6, 3.2, 6.4, 9.6, 12.8mg $Co(acac)_2$).

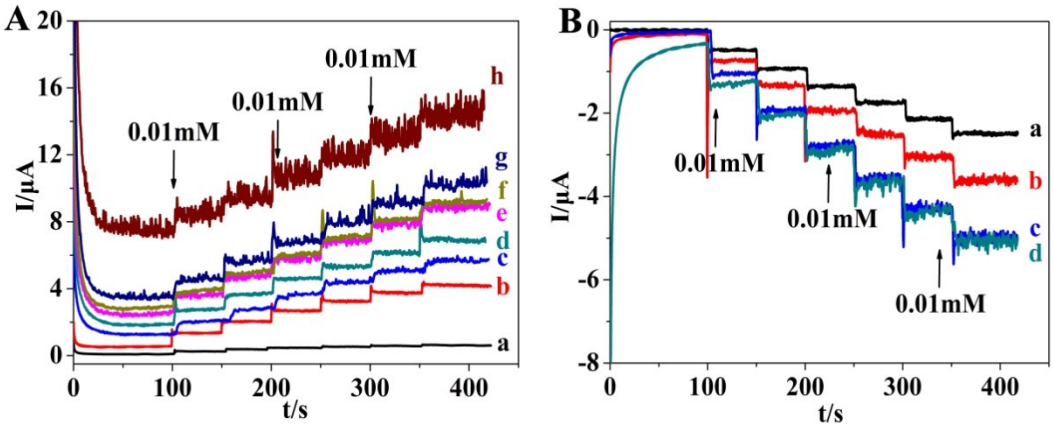

Fig. S10 Typical amperometric responses of Co-PdCu/GCE with successive injection of 0.01 mM H_2O_2 in PBS (0.1 M, pH 7.2) under different potentials: A (reduction potential, from a to h: +0.1V, 0V, -0.05V, -0.1V, -0.15V, -0.2V,-0.3V and -0.4V) and B (oxidation potential, from a to d: +0.2V, +0.3V, +0.35V and +0.4V)

Table S1. Typical amperometric responses of Co-PdCu/GCE with injection of 10μM H_2O_2 in 0.1M PBS (pH 7.2) (parallel determination for 7 times)

Time	1	2	3	4	5	6	7	RSD
Response current (μA)	0.13	0.12	0.09	0.12	0.10	0.09	0.09	1.72%