

## Supporting information

**Direct electrochemistry and bioelectrocatalysis of horseradish peroxidase  
entrapped in a self-supporting nanoporous gold electrode:  
a new strategy to improve the orientation of immobilized enzyme**

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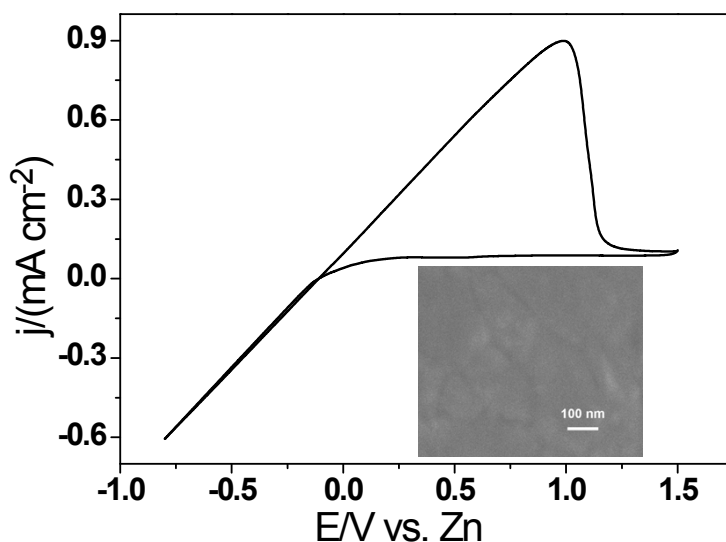
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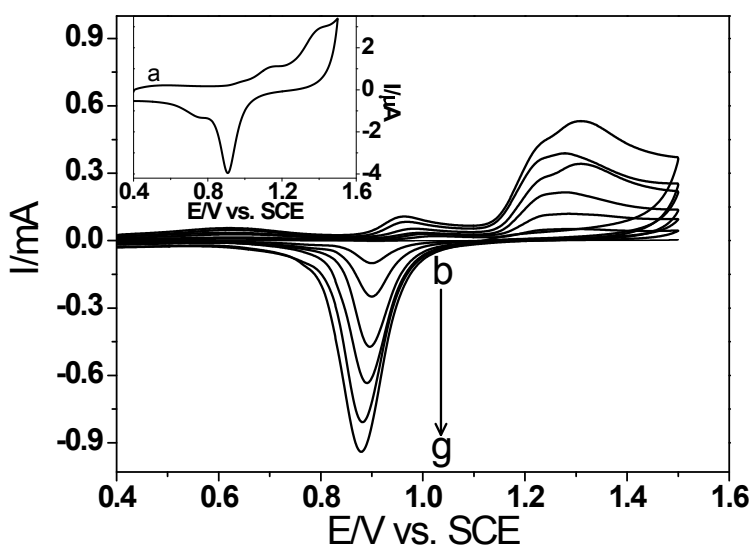
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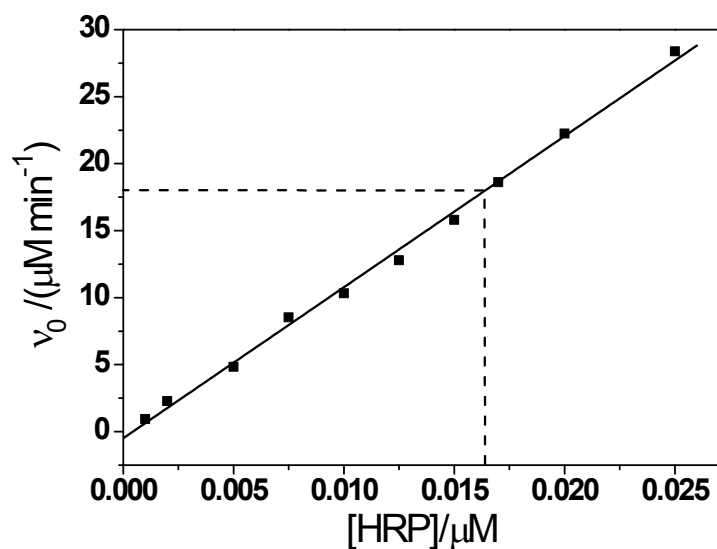
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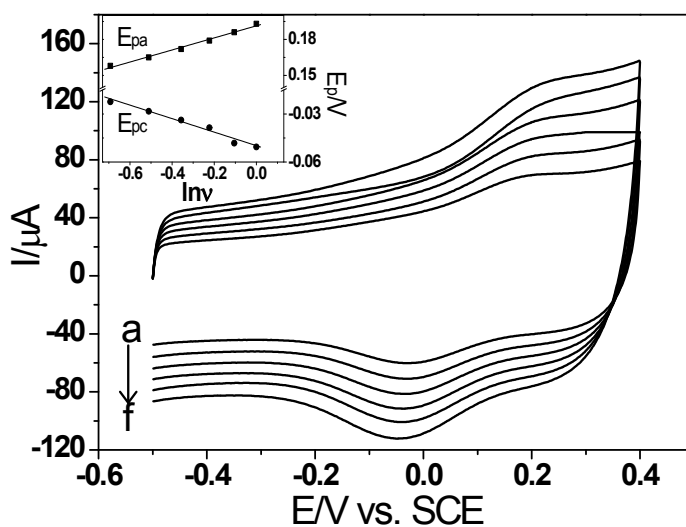
**Fig. S1.** The CV gram of a polished gold wire in [Choline]Cl·2ZnCl<sub>2</sub> at 40 °C. Scan rate: 0.01 V s<sup>-1</sup>. Inset: the SEM image of the gold wire surface after the electrochemical treatment.



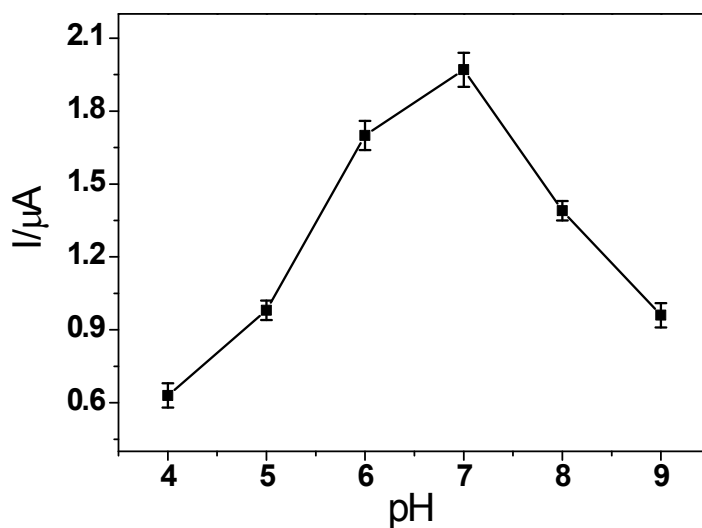
**Fig. S2.** CV grams of a polished gold electrode (a) and NPGEs (b-g) in 0.5 M H<sub>2</sub>SO<sub>4</sub> solution at a scan rate of 0.1 V s<sup>-1</sup>. Inset: the magnified curve of curve a. The NPGEs were obtained from the gold electrode after alloying/dealloying at 50 °C (b), 70 °C (c), 90 °C (d), 100 °C (e), 110 °C (f) and 120 °C (g) at a scan rate of 0.01 V s<sup>-1</sup> after 15 cycles.



**Fig. S3.** Calibration curve of the initial rate of catalytic oxidation of OPD by HRP versus the concentration of HRP in 0.1 M phosphate buffer (pH 7.0). Each datum was an average of three replicate determinations.



**Fig. S4.** CV grams of Nafion/HRP/NPGE in 0.1 M phosphate buffer (pH 7.0) at different scan rates (a-f: 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 V s<sup>-1</sup>). Inset: plot of redox peak potential versus lnv.



**Fig. S5.** The effect of the buffer pH on the electrochemical response current of 0.1 mM H<sub>2</sub>O<sub>2</sub> on Nafion/HRP/NPGE.

**Table S1.** The real area ( $A_{real}$ ) and  $R_f$  of the NPGEs fabricated under different temperatures.<sup>a, b</sup>

Temperature/°C	$A_{real}/\text{mm}^2$	$R_f$
50	11	6.2
70	27	15.3
90	58	32.8
100	76	42.9
110	104	58.8
120	156	88.1

<sup>a</sup> NPGEs fabricated after 15 scan cycles at a scan rate of 0.01 V s<sup>-1</sup>.

<sup>b</sup> Each datum was an average of three replicate determinations.

**Table S2.** Comparison of analytical performance of several HRP biosensors for H<sub>2</sub>O<sub>2</sub>.

HRP biosensors	Linear range/ $\mu\text{M}$	Sensitivity/ $\mu\text{A mM}^{-1}$	Detection limit/ $\mu\text{M}$	References
Nafion/HRP-GNS <sup>a</sup> -TiO <sub>2</sub> /GCE <sup>b</sup>	41-630	0.23	5.9	1
Ti/TiO <sub>2</sub> /Au/HRP	5-400	Not reported	2	2
MWNTs <sup>c</sup> /chitosan/GCE	16.7-740	4.9	10.3	3
HRP/HNT <sup>d</sup> /chitosan/GCE	2.6-75	12	0.7	4
SPCE <sup>e</sup> /GS <sup>f</sup> -Nafion/Fe <sub>3</sub> O <sub>4</sub> -Au-HRP	20-2500	Not reported	12	5
Nafion/HRP/NPGE	10-380	21	2.6	This work

<sup>a</sup> Gold nano-seeds.

<sup>b</sup> Glassy carbon electrode.

<sup>c</sup> Multi-wall carbon nanotubes.

<sup>d</sup> Halloysitenanotubes.

<sup>e</sup> Screen-printed carbon electrode.

<sup>f</sup> Graphene sheets.

## References

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**Table S3.** Determination of H<sub>2</sub>O<sub>2</sub> in real samples (diluted disinfectant).\*

Real sample	Value found in diluted sample/mM	Added/mM	Total found/mM	Recovery/%
1	0.168	0.050	0.214	98.2
2	0.168	0.100	0.273	101.9
3	0.168	0.150	0.328	103.1

\* Each datum was an average of three replicate determinations.