

Supplementary Material for Chemical Communications  
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## Supplementary data

# Stimuli-Responsive Peroxidase Mimicking at a Smart Graphene Interface

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## Experimental Details

### Materials

Graphite powder ( $\leq 300$  mesh) was obtained from Beijing Chemical Reagents Company. 3,3',5,5'-tetramethylbenzidine (TMB) and *o*-phenylenediamine (OPD) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) was purchased from J&K Scientific Ltd. Horseradish peroxidase (HRP) ( $\geq 250$  U/mg) was purchased from Amresco (USA). Other reagents such as  $\text{KMnO}_4$ , NaOH, concentrated  $\text{H}_2\text{SO}_4$ , concentrated HCl,  $\text{H}_2\text{O}_2$  (30%),  $\text{HAuCl}_4$ , were all analytical-grade and purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. DNA oligomers (5'-Cy3-GCAGACACATCCAGCGATAGCCAGGACAA-3'; ssDNA-1: 5'-GCAGACACATCCAGCGATAGCCAGGACAA-3' and ssDNA-2: 5'-TTGTCTGGCTATC GCTGGATGTGTCTGC-3') were purchased from Takara Biotechnology Co. (Dalian, China). All DNA oligomers were purified by high-performance liquid chromatography (HPLC). Ultrapure water obtained from a Millipore water purification system (resistivity  $> 18.0 \text{ M}\Omega \text{ cm}^{-1}$ , Laikie Instrument Co., Ltd, Shanghai, China) was used throughout the experiments. All glassware was first cleaned with a mixture of HCl and  $\text{HNO}_3$  (ratio of HCl/ $\text{HNO}_3 = 3:1$  in volume) and thoroughly rinsed with ultrapure water. Phosphate buffer solution (PBS, 20 mM) with various pH values was prepared by mixing the stock solution of  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$ .

### Instruments

FL measurements were performed using a Hitachi F-4500 spectrofluorimeter with a scan rate at 1200 nm/min. The photomultiplier tube (PMT) voltage was 700 V. The slits for excitation and

emission were set at 5 nm/10 nm. UV-visible absorption spectra were recorded on a Jasco V-550 spectrometer. X-ray photoelectron spectroscopy (XPS) analysis was carried out on VG ESCALAB MK2 X-ray photoelectron spectrometer using a nonmonochromatized Al K $\alpha$  X-ray source (1486.6 eV). The Raman spectra were recorded on a Renishaw Micro-Raman system 2000 spectrometer with He-Ne laser excitation (wavelength 623.8 nm).

### **Preparation of Au-NPs/graphene Hybrids, Free Au-NPs and graphene**

Graphene Oxide (GO) was prepared according to a modified Hummers method reported by us previously.<sup>[1]</sup> An environment-friendly hydrothermal route was used to prepare Au-NPs/graphene hybrid, free Au-NPs and graphene. For the preparation of Au-NPs/graphene hybrid, 8 mL GO aqueous solution (0.5 mg/mL), 0.3 mL HAuCl<sub>4</sub>·3H<sub>2</sub>O (10 mM) and 4 mL NaOH solution (0.1 M) were first mixed and diluted to 100 mL. The final concentration of GO in the precursor was 40  $\mu$ g/mL. Subsequently, the mixture was sonicated for 5 h at room temperature. Then, the resulting solution was transferred to a Teflon-lined autoclave for hydrothermal reaction at 180 °C for 12 h. The product was then cooled naturally to room temperature. Synthesis of Au-NPs/graphene hybrids with different sizes of Au-NPs was similar to the above procedures, only use various concentrations of Au precursor in the initial solution. Free Au-NPs or graphene was prepared through the same steps as the synthesis of Au-NPs/graphene without the addition of GO or Au precursor in the reaction solution.

### **Sample Preparation for TEM and XPS**

TEM samples were prepared by dropping the samples onto copper grids and dried naturally. XPS samples were prepared by depositing the samples on Al<sub>2</sub>O<sub>3</sub> substrate.

### **Preparation of dsDNA**

For the preparation of dsDNA, 3.4  $\mu$ M unmodified ssDNA-1 and its perfectly complementary target ssDNA-2 were mixed in 20 mM PBS buffer (pH 7.4, containing 100 mM NaCl and 5 mM KCl). Then the mixture was heated to 95 °C for 5 min in a water bath and subsequently cooled naturally to room temperature (25 °C). The obtained dsDNA were stored at 4 °C.

### **Fluorescent Assay for DNA Binding**

In a typical experiment, 5  $\mu$ g/mL Au-NPs/graphene and 500 nM DNA (Cy3-labeled ssDNA or dsDNA) were added to 20 mM PBS buffer (pH 7.4) for time-dependent fluorescence measurement at  $\lambda_{\text{ex}}/\lambda_{\text{em}} = 540/568$  nm. The final volume of the solution was fixed at 500  $\mu$ L. The control

experiment was carried out under the same condition without the addition of Au-NPs/graphene. All experiments were performed at 25 °C.

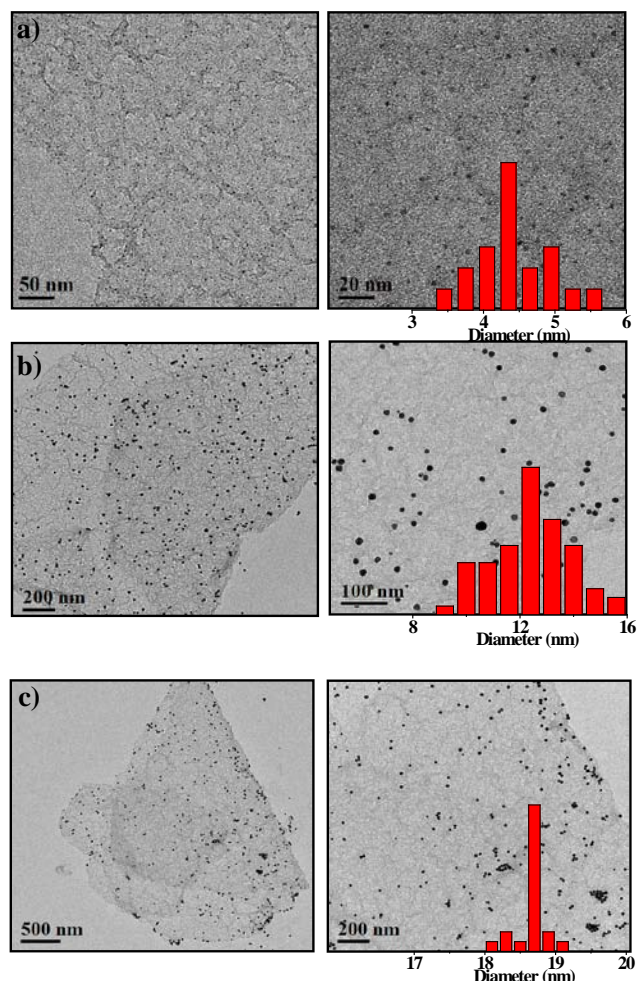
### **Catalytic Activity Inhibition Assay**

In a typical experiment, 10 µg/mL Au-NPs/graphene was firstly mixed with different concentrations of ssDNA-1 or dsDNA in 10 mM PBS buffer (pH 7.4). After 30 min reaction, the solution was transferred to a quartz cell containing 20 mM PBS buffer (pH 4.0) at 25 °C. Subsequently, 30 mM H<sub>2</sub>O<sub>2</sub> and 0.75 mM TMB were added to initiate the reaction for 15 min. UV-vis spectra were then recorded.

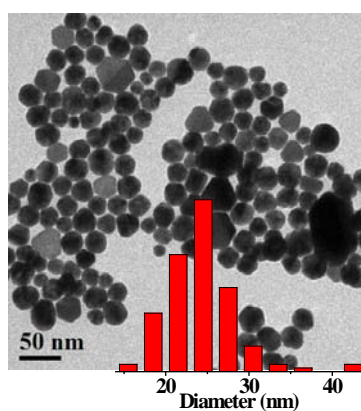
### **Catalytic Activity Recoverability Assay**

In a typical experiment, 10 µg/mL Au-NPs/graphene was firstly mixed with ssDNA-1 in 10 mM PBS buffer (pH 7.4) for 30 min. Then different concentrations of ssDNA-2 were added and heated to 95 °C for 5 min. After cooled to room temperature (25 °C), the solution was transferred to a quartz cell containing 20 mM PBS buffer (pH 4.0) at 25 °C. Subsequently, 30 mM H<sub>2</sub>O<sub>2</sub> and 0.75 mM TMB were added to initiate the reaction for 15 min. UV-vis spectra were then recorded.

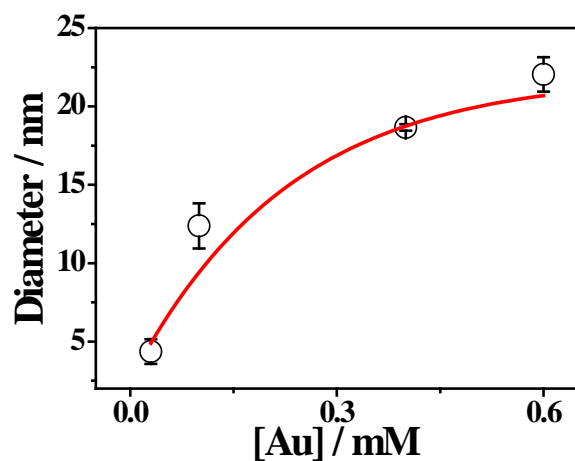
## Supplementary Data



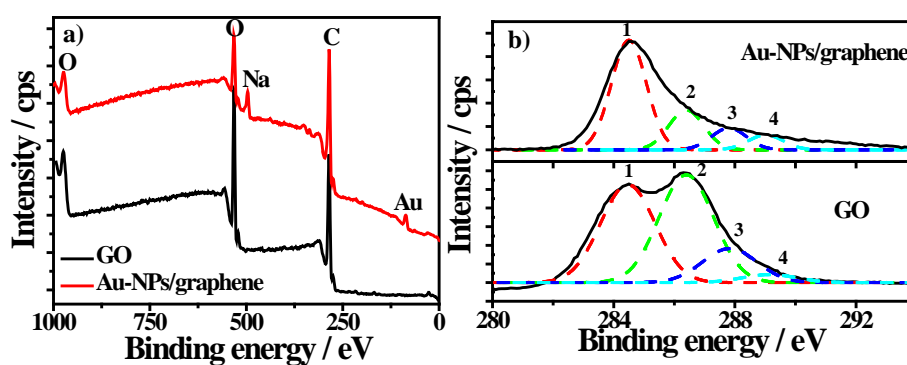
**Fig. S1.** TEM images of Au-NPs in situ grown on graphene sheets under different magnifications. Particle size distribution was estimated by measuring the size of Au-NPs on graphene sheet. The initial concentration of  $\text{HAuCl}_4$  in the sample a), b) and c) was 0.03 mM, 0.1 mM and 0.4 mM, respectively.



**Fig. S2.** TEM image and size distribution of as-prepared Au-NPs in the absence of GO. The initial concentration of  $\text{HAuCl}_4$  in the sample was 0.6 mM.



**Fig. S3.** Dependence of the diameter of Au-NPs *in situ* grown on graphene sheets on the initial concentration of Au precursor.



**Fig. S4.** a) XPS spectra and b) high-resolution C1s spectra of GO and as-prepared Au-NPs/graphene hybrid. The peaks correspond to (1) C=C/C-C in aromatic rings, (2) C-O, (3) C=O and (4) COOH groups, respectively.

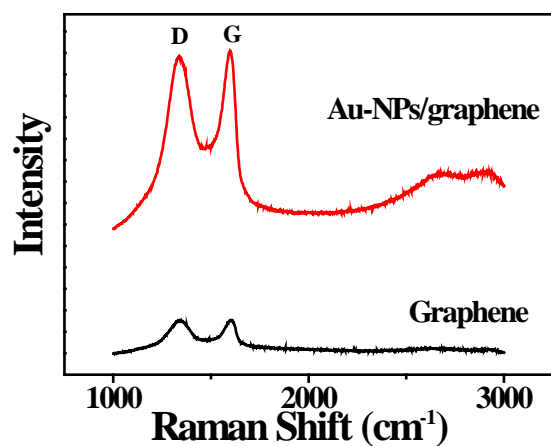


Fig. S5. Raman spectra of graphene and Au-NPs/graphene hybrids.

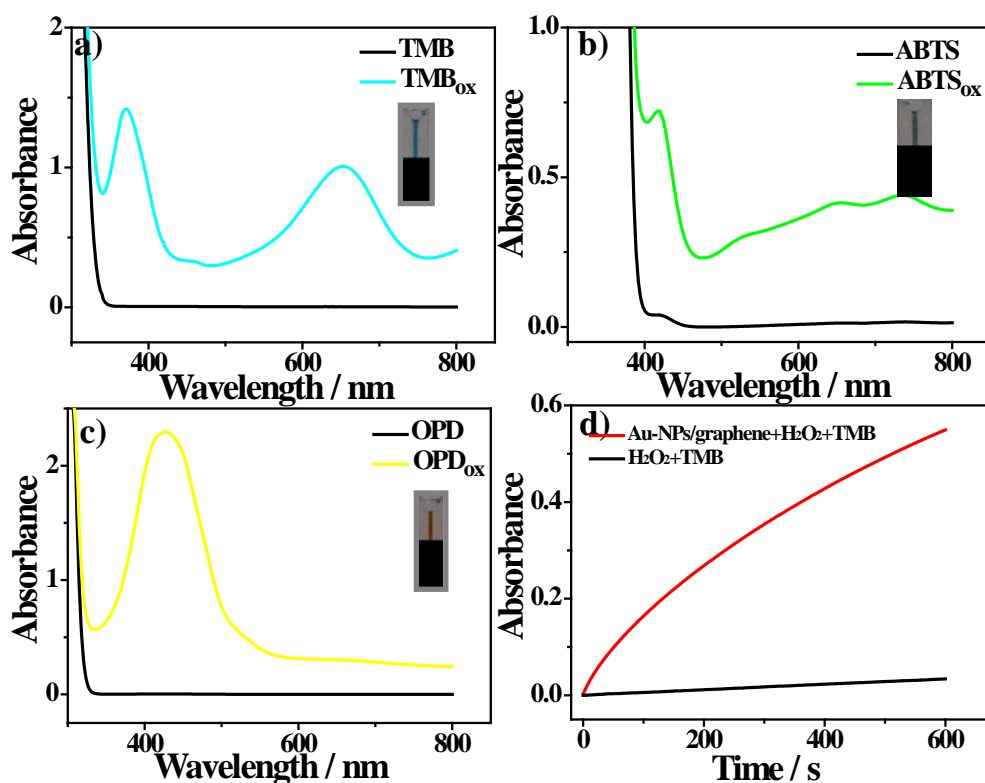
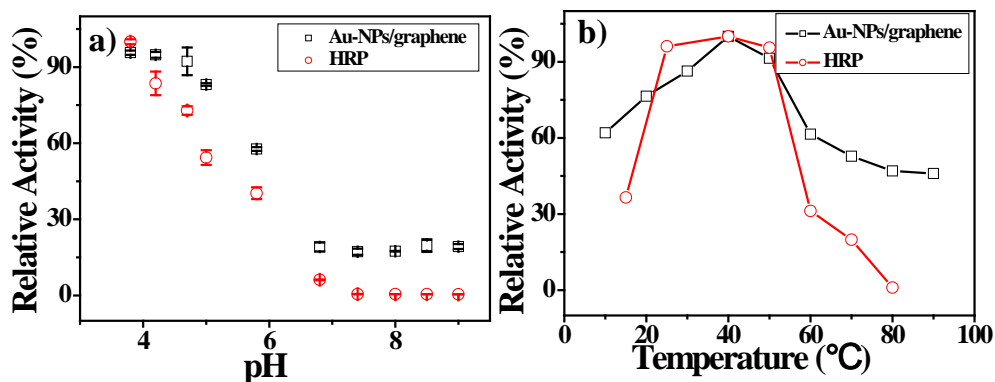
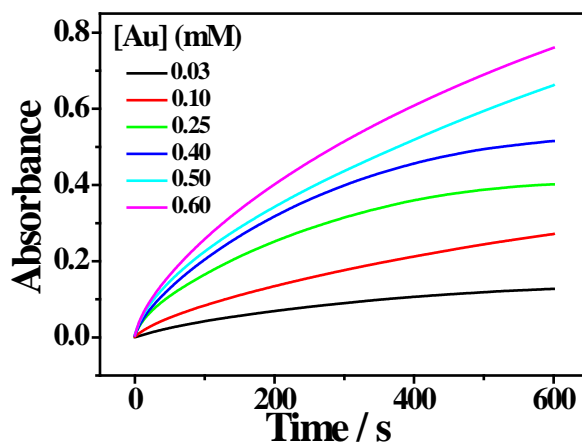


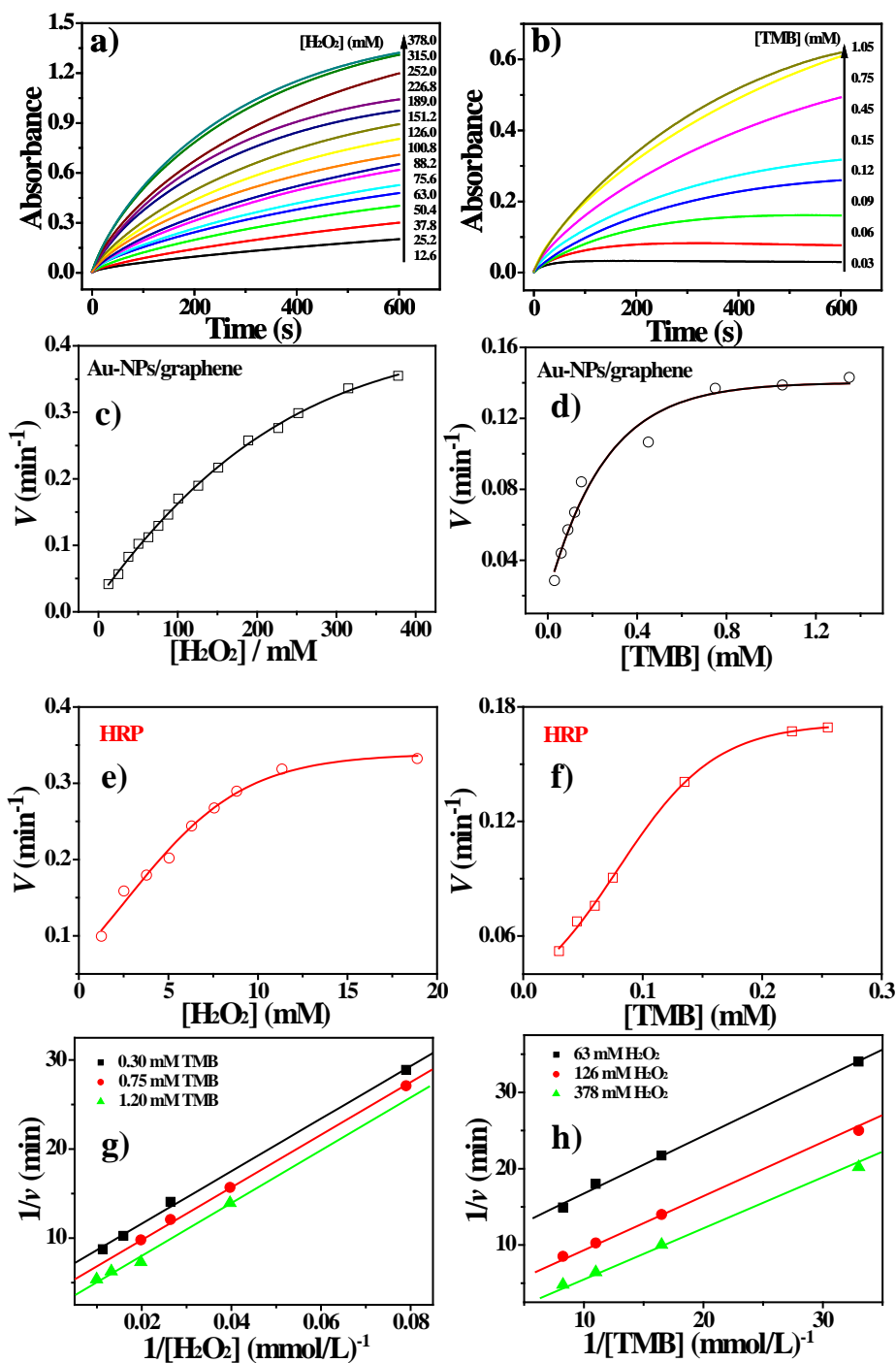
Fig. S6. Au-NPs/graphene hybrids mediate the oxidation of a) TMB, b) ABTS and c) OPD in the presence of H<sub>2</sub>O<sub>2</sub> in phosphate buffer (pH 4.0) at 25 °C. d) The time-dependent absorbance changes at 652 nm in the absence (black) or presence (red) of the peroxidase mimic in phosphate buffer (20 mM, pH 4.0) containing 63 mM H<sub>2</sub>O<sub>2</sub>, 0.75 mM TMB and 5 μg/mL catalyst.



**Fig. S7.** a) pH- and b) temperature-dependent catalytic activity of Au-NPs/graphene hybrids or HRP. Experiments are carried out in 20 mM phosphate buffer using 5  $\mu\text{g/mL}$  Au-NPs/graphene or 1.4 ng/mL HRP at pH 4.0, 25  $^{\circ}\text{C}$  (unless otherwise stated).  $\text{H}_2\text{O}_2$  concentration was 63 mM for Au-NPs/graphene and 7.56 mM for HRP, TMB concentration was 0.75 mM for Au-NPs/graphene and 0.28 mM for HRP. The maximum point was set as 100%.



**Fig. S8.** Time-dependent absorbance changes at 652 nm in the presence of Au-NPs/graphene hybrid with various Au content. Experiments were carried out in 20 mM phosphate buffer (pH 4.0) containing 8  $\mu\text{g/mL}$  Au-NPs/graphene, 63 mM  $\text{H}_2\text{O}_2$  and 0.75 mM TMB at 25  $^{\circ}\text{C}$ .



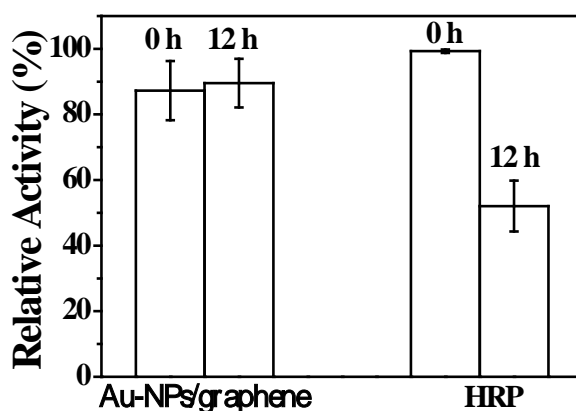
**Fig. S9.** a), b) The time-dependent absorbance changes at 652 nm in the presence of different concentrations of H<sub>2</sub>O<sub>2</sub> or TMB. c), d), e), f) Steady-state kinetic assay of Au-NPs/graphene hybrids or HRP. Experiments are carried out in 20 mM phosphate buffer (pH 4.0) using 5 μg/mL Au-NPs/graphene a)-d) or 1.4 ng/mL HRP e), f) at 25 °C. a), c), e) TMB concentration is fixed at 0.75 mM (Au-NPs/graphene) or 0.28 mM (HRP) and the H<sub>2</sub>O<sub>2</sub> concentration is varied. b), d), f) H<sub>2</sub>O<sub>2</sub> concentration is fixed at 63 mM (for Au-NPs/graphene) or 7.56 mM (for HRP) and the TMB concentration is varied. g), h) Double-reciprocal plots of the catalytic activity of Au-NPs/graphene hybrids.



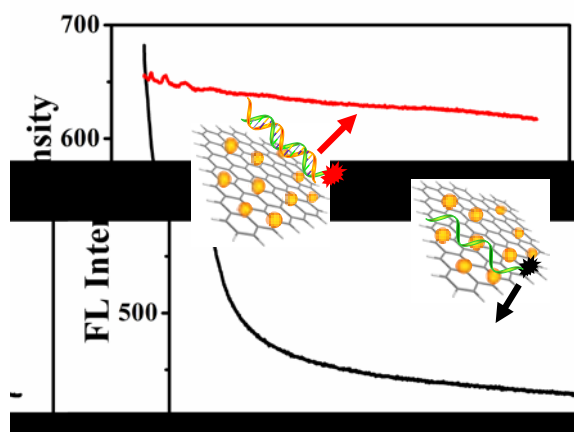
**Tab. S1** Comparison of the Kinetic Parameters between Au-NPs/graphene and HRP<sup>[a]</sup>

|                 | Substrate                     | $K_m$ (mM) | $v_{max}$ ( $10^{-8}$ M/s) |
|-----------------|-------------------------------|------------|----------------------------|
| Au-NPs/graphene | H <sub>2</sub> O <sub>2</sub> | 140.52     | 17.3±1.0                   |
| Au-NPs/graphene | TMB                           | 0.14       | 7.1±0.1                    |
| HRP             | H <sub>2</sub> O <sub>2</sub> | 3.42       | 14.1±0.5                   |
| HRP             | TMB                           | 0.069      | 5.8±0.3                    |

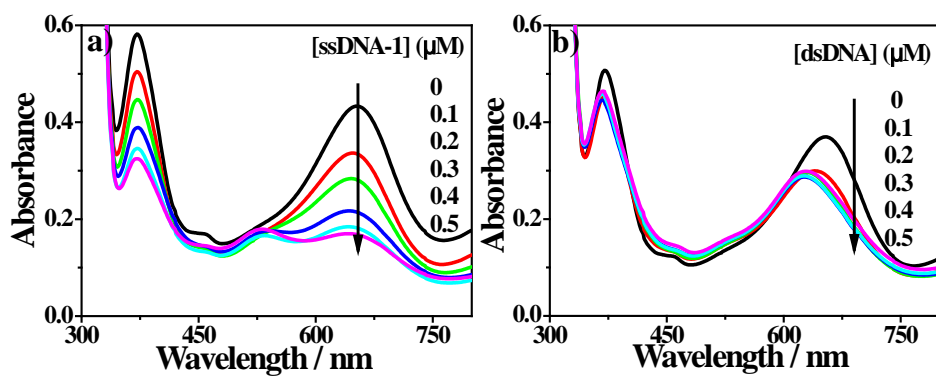
<sup>[a]</sup>The apparent kinetic parameters were calculated based on Michaelis-Menten function:  $v = v_{max} [S] / (K_m + [S])$ , where  $K_m$  is the Michaelis constant;  $v_{max}$  is the maximal reaction velocity and  $[S]$  is the substrate concentration.



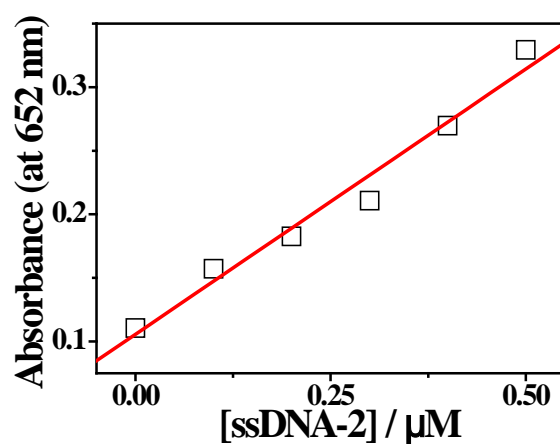
**Fig. S10.** Comparison of the catalytic activity of Au-NPs/graphene with HRP during the storage. Experiments are carried out in 20 mM phosphate buffer using 5  $\mu$ g/mL Au-NPs/graphene or 1.4 ng/mL HRP at pH 4.0, 25 °C. H<sub>2</sub>O<sub>2</sub> concentration was 63 mM for Au-NPs/graphene and 7.56 mM for HRP, TMB concentration was 0.75 mM for Au-NPs/graphene and 0.28 mM for HRP. The maximum point was set as 100%.



**Fig. S11.** Time-dependent fluorescence changes of Au-NPs/graphene/Cy3-labeled ssDNA (red line) and Au-NPs/graphene/Cy3-labeled dsDNA (black line) in PBS buffer solution (20 mM, pH 7.4). Au-NPs/graphene concentration was fixed at 5  $\mu$ g/mL,  $\lambda_{ex}/\lambda_{em} = 540$  nm/568 nm.



**Fig. S12.** Au-NPs/graphene hybrids mediate the oxidation of TMB in the presence of different concentrations of a) ssDNA-1 or b) dsDNA. Details are described in the Experimental section.



**Fig. S13.** Plots of absorbance changes as a function of ssDNA-2 concentrations.

## Reference

- [1] M. Liu, Q. Zhang, H. M. Zhao, S. Chen, H. T. Yu, Y. B. Zhang and X. Quan, *Chem. Commun.*, 2011, **47**, 4084-4.86.