Supporting Information

Simple Bacterial Detection and High-Throughput Drug Screening Based on Graphene-Enzyme Complex

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1. Synthesis of Negatively Charged and Positively Charged Graphene Oxide (GO)

Graphene oxide (GO) was synthesized from natural graphite powder by a modified Hummer's method.[1] Briefly, graphite powder (2 grams) was ground with NaCl to reduce the particle size. After removing the salt, the graphite was added to the concentrated H_2SO_4 (80 mL) and left stirring for 2 hours. Afterwards, $KMnO_4$ (10 grams) was added gradually under stirring and the temperature of the mixture was kept to less than 20 ºC. Successively, the mixture was stirred at 35 °C for 2 h. Keeping the temperature less than 50 ºC, distilled water (180 mL) was added and then the mixture was stirred at room temperature for 3 hours. The reaction was ended by a final addition of distilled water (450 mL) and H_2O_2 (30% v/v aqueous solution, 20 mL). At the end, the mixture was repeated washed with diluted HCl (3.7 wt %) aqueous solution, and then distilled water. Exfoliation was carried out by sonicating graphene oxide (2 mg mL^{-1}) dispersion under ambient condition for 4 hours. Finally, the product was further purified by dialysis for 1 week to remove the remaining metal species. The GO aqueous suspension (about 2 mg mL^{-1}) sonicated for 1 h to give a clear solution. NaOH (1.2 g) and chloroacetic acid ($ClCH_2COOH$) (1.0 g) were added to the GO suspension and bath sonicated for 2 h to convert the -OH groups to -COOH groups. The resulting GO-COOH solution was neutralized, and purified by repeated rinsing and filtrations. The resulting GO is negatively charged since the GO sheet has chemical functional groups such as carboxylic acids.

Positively charged GO (GO-EDA) was synthesized by using EDC and EDA.[2] A negatively charged GO suspension was combined with EDC (30 mg) and ethylenediamine (2 mL) and stirred for 4 h, and afterwards the mixture solution was dialyzed to remove EDC and EDA. A positively charged GO-EDA suspension of a dark brown color was obtained.

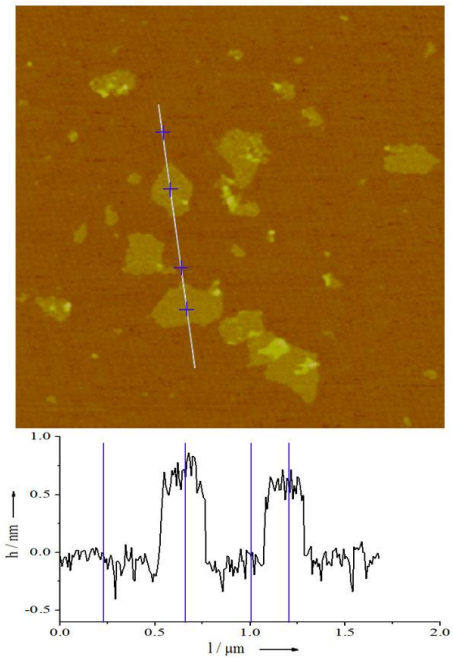

Figure S1. AFM height image of GO deposited on mica substrates.

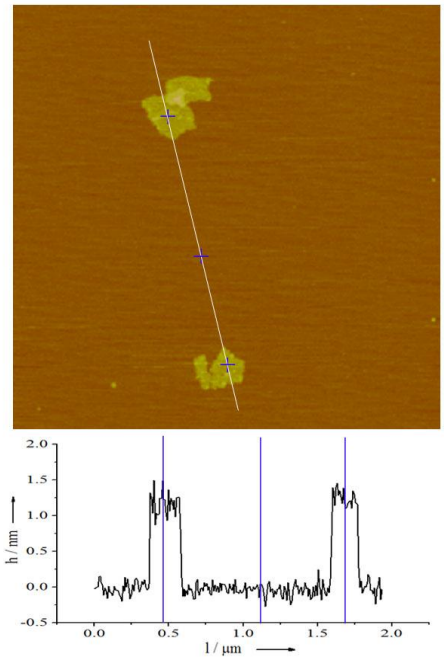

Figure S2. AFM height image of GO-EDA deposited on mica substrates.

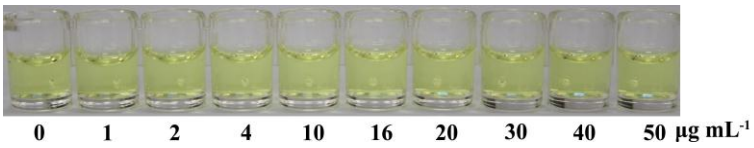

Figure S3. Image of hydrolysis of SPNA in 96 well microplates at varying concentrations of GO-EDA.

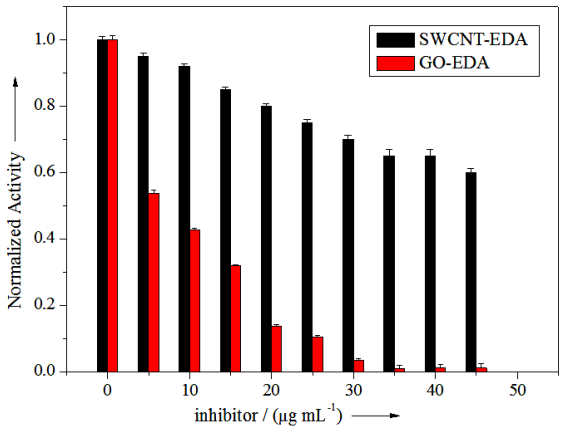

Figure S4. Activity of β-Gal plotted as a function of GO-EDA or SWCNT-EDA concentrations in 5 mM sodium phosphate buffer (pH 7.4). The data shown here represent the means and standard deviations of three independent experiments.

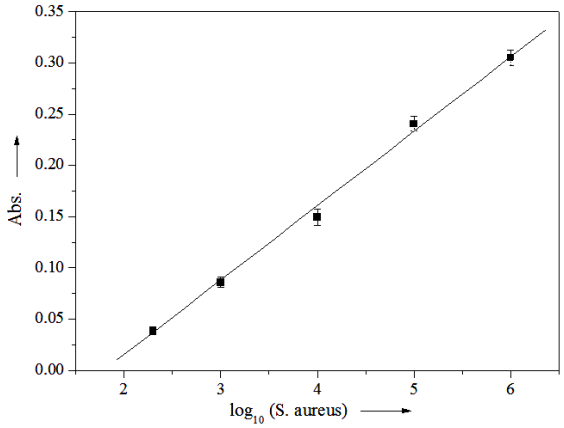

Figure S5. The absorption intensity at 410 nm was plotted as a function of the logarithm of bacteria concentrations. The data shown here represent the means and standard deviations of three independent experiments.

2. The influence of GO-EDA on bacterial survival

For investigating whether the influence of GO-EDA on bacterial survival, we used staphylococcus aureus (S. aureus) as a model analyte. Upon incubation S. aureus with the mixture of GO-EDA and β-Gal, the reaction samples were stained by propidium

iodide (PI) for fluorescent confocal microscopy (Nikon, C2 Si, Japan). As shown in Figure S6, the result demonstrated the GO-EDA had no noticeable influence on bacterial survival.

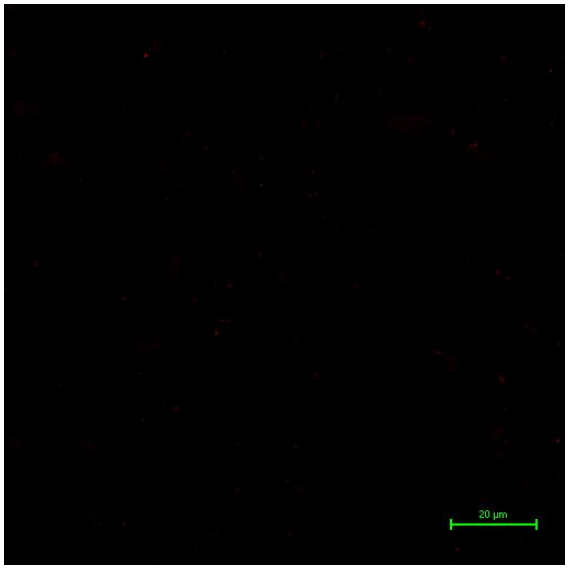

Figure S6. Fluorescent microscope micrographs of S. aureus staining with propidium iodide (PI).

3. An approximate cost of per analysis

An approximate cost of per analysis is listed as follows:

1). Graphene oxide (GO) was synthesized from natural graphite powders. The price of one kg of natural graphite powder is $ 15.8. The amount of other used reagents is very small and the cost is negligible.

Positively charged GO-EDA was synthesized by using EDC chemistry. The price of 25 g of EDC powder is $ 186 and the price of 250 mL of ethylenediamine (EDA) is $ 57.0.

The synthesis of 20 mg GO-EDA used about 30 mg graphite powders, 300 mg EDC and 2 mL EDA. So, the cost of 20 mg GO-EDA is $ 2.68. Per analysis used 6 μg GO-EDA and the cost of per analysis is 0.080 cent.

2). the price of 1KU (about 2 mg) of β-galactosidase is $ 56.7. Per analysis used 46.5 ng β-galactosidase and the cost of per analysis is 0.13 cent.

3). the price of 1 g of 2-nitrophenyl β-D-galactopyranoside (ONPG) is $ 33.7. Per analysis used 60 μg ONPG and the cost of per analysis is 0.20 cent.

In summary, the cost of per analysis is 0.41 cent (the cost of the used solvent is negligible). Therefore, low cost is one of the advantages of the system.

Table S1. A single factor analysis of variance between concentration groups in E. coli detection

A single factor analysis of variance (ANOVA) 95% confidence						
variation	SS	df	MS	F	P-value	F crit
between group variation	0.181623	6	0.030271	24.57802	1.13×10^{-6}	2.847726
within group variation	0.017243	14	0.001232			
total	0.198866	20				

Table S2. A single factor analysis of variance between concentration groups in S. aureus detection

A single factor analysis of variance (ANOVA) 95% confidence						
variation	SS	df	MS	F	P-value	F crit
between group variation	0.161405	4	0.040351	50.84823	1.3×10^{-6}	3.47805
within group variation	0.007936	10	0.000794			
total	0.16934	14				

1. W. S. Hummers, R. E. Offeman, *J. Am. Chem. Soc.* 1958, **80**, 1339.
2. J. Yang, J. W. Kim, H. S. Shin, *Adv. Mater.* 2012, **24**, 2299.