## **Supporting Material**

## A new electrochemical substrate for rapid and sensitive *in-vivo* monitoring of $\beta$ -galactosidase gene expressions

Kesavan Manibalan<sup>1</sup>, Veerappan Mani<sup>1</sup>, Chih-Hung Huang<sup>1,2</sup>, Sheng-Tung Huang<sup>1,2\*</sup>, Pu-Chieh Chang<sup>1,2</sup>

<sup>1</sup>Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, Taipei 10608, Taiwan (R.O.C.)

<sup>2</sup>Institue of Biochemical and Biomedical Engineering , National Taipei University of Technology, Taipei 10608, Taiwan (R.O.C.)

\*Corresponding author. Tel.: +886 2771-2171 2525, \*E-mail: <u>ws75624@ntut.edu.tw</u>



Fig. S1. Preparation of graphene oxide from graphite



Fig. S2 Mechanism for the electrochemical oxidation of 4-MP



**Fig. S3** *E. coli*: CVs obtained at GO/GCE in tris buffer (pH 7.3) containing different concentrations of pristine 4-MP. Inset:  $I_{pa}$  versus [4-MP];  $I_{pa}/\mu A = 0.0171$  [4-MP] ( $\mu M$ ) + 0.602.



**Fig. S4** *E. coli*: (A) Kinetic linear transformation curves at increasing concentrations of 4-MPGal (a = 0.1 mM, b = 0.2 mM, c = 0.3 mM, d = 0.4 mM, e = 0.5 mM, f = 0.6 mM, g = 0.7 mM, h = 0.8 mM and i = 0.9 mM) containing 3.8 µg of  $\beta$ -Gal. (B) Lineweaver-Burk plot of hydrolyzed 4–MP against different concentration of 4-MPGal (0.2–0.9 mM).



**Fig. S5** *A. oryzae*: (A) CVs obtained at GO/GCE in 50 mM acetate buffer (pH 4.5) (a), containing 1 mM 4-MPGal (b), 10 μg β-Gal (c) and mixture of 10 μg β-Gal and 1 mM (d). Inset: CV obtained at GO/GCE in 50 mM acetate buffer (pH 4.5) containing 0.1 mM of 4-MP. The scan rate was 0.1 Vs<sup>-1</sup>. (B) CVs obtained at bare GCE and GO/GCE in 50 mM acetate buffer (pH 4.5) containing 1 mM 4-MPGal and 10 μg β-Gal at scan rates of 0.1 Vs<sup>-1</sup>. Inset: pH optimization: [current] (μA)/pH. CVs obtained at GO/GCE in Tris buffer (different pH) containing 1 mM 4-MPGal and 0.63 μg of β-Gal. (C) CVs obtained at GO/GCE in 50 mM acetate buffer (pH 4.5) containing 1 mM 4-MPGal and 10 μg β-Gal at different scan rates from 0.01 Vs<sup>-1</sup> to 1 Vs<sup>-1</sup>. Plot of *I*<sub>pa</sub> and *I*<sub>pc</sub> versus  $v^{1/2}$ ; *I*<sub>pa</sub>/μA = 45.60  $v^{1/2}$  /(Vs<sup>-1</sup>)<sup>1/2</sup> + 1.712; *I*<sub>pa</sub> /μA = -60.76  $v^{1/2}$  /(Vs<sup>-1</sup>)<sup>1/2</sup> - 1.523. (D) CVs obtained at GO/GCE in tris buffer (pH 7.3) containing different concentrations of 4-MP. Inset: *I*<sub>pa</sub> versus [4-MP]



**Fig. S6** *A. oryzae*: (a-i) Kinetic linear transformation curves for the increasing concentration of 4-MPGal (a= 0.02 mM, b= 0.05 mM, c= 0.1 mM, d= 0.2 mM, e= 0.3 mM, f= 0.4 mM, g= 0.6 mM, h= 0.8 mM, i= 1.0 mM) upon treated with 12.5  $\mu$ g  $\beta$ -Gal (1U). (B) Michaelis–Menten plot of hydrolyzed 4-MP against different concentration of 4-MPG (0.02-1 mM).



**Fig. S7** *A-oryzae*: CVs obtained at GO/GCE in 50 mM acetate buffer (pH 4.5) containing 12.5 μg of β-Gal and different concentration of 4-MPGal (each addition of 10 μM) at scan rate from 0.1 Vs<sup>-1</sup>. Inset:  $I_{pa}$  versus [4-MPGal];  $I_{pa}/\mu A = 0.0461$  [4-MPGal] (μM) + 1.024.



**Fig. S8** *A. oryzae*: CVs obatined sat GO/GCE in 50 mM acetate buffer (pH 4.5) containing various concentration of β-Gal and 1 mM 4-MPGal (a= 0.1 ng, b= 0.5 ng, c= 1 ng, d= 2.5 ng, e= 5 ng, f= 7.5 ng, g= 10 ng, h= 20 ng, i= 30 ng, j= 40 ng, k= 50 ng, l= 60 ng, m= 70 ng, n= 80 ng, o= 90 ng, p= 200 ng, q=400 ng, r= 600 ng, s= 800 ng, t= 1000 ng). (B)  $I_{pa}$  vs. [β-Gal];  $I_{pa}/\mu$ A = 0.069 [β-Gal]/ ( $\mu$ A/ng) + 1.046. (C)  $I_{pa}$  vs. [β-Gal];  $I_{pa}/\mu$ A = 23.14 [β-Gal]/ ( $\mu$ A/µg) + 5.837.



**Fig. S9** *A. oryzae*: DPVs obtained at GO/GCE in 50 mM acetate buffer (pH 4.5) (a) containing 4-MPGal (1 mM) with increasing concentration of  $\beta$ -Gal (b= 10 pg, c= 20 pg, d= 30 pg, e= 40 pg, f= 50 pg, g= 60 pg, h= 70 pg, i= 80 pg, j= 90 pg, k=100 pg). Inset: Linear calibration plot: [*I*]/ $\mu$ A vs. [ $\beta$ -Gal]; *I*/ $\mu$ A = 12.3 [ $\beta$ -Gal]/ (nA/ $\mu$ g) + 0.034.



Fig. S10 (A) CVs obtained at GO/GCE in broth medium (a) with the presence of different concentrations of 4-MP (b-k; each additions of 100  $\mu$ M). (B) CVs obtained at GO/GCE in broth medium (a) containing 5.3  $\mu$ g  $\beta$ -Gal from *E. coli* and different concentration of 4-MPGal (each addition (1 mM (b), 2 mM (c), 3 mM (d), 4 mM (e) and 5 mM (f)).

In order to prove that our scheme works in the LB broth medium, we presents the cyclic voltammograms carried out for LB broth medium containing different concentrations of pristine 4-MP (Fig. S10 (A)) and different concentrations of 4-MPGal (Fig. S10 (B)). The linear dependence of the response currents with the concentration of pristine 4-MP and 4-MPGal is indicating the satisfactory performance of our scheme in broth medium.

Optimized	Data
Parameters	
рН	pH 7.3 for <i>E. coli</i> (Inset b, Fig. 1B)
	pH 4.5 for A. oryzae (Inset, Fig. S5 (B))
Concentration of GO	5 µl (inset a, Fig. 1B)
Scan rate	100 mV (Fig. 1C for <i>E. coli</i> ; Fig. S5 (C) for <i>A</i> .
	oryzae)
CV parameters	Potential range = $-0.3$ V to $+1.0$ V and scan rate of
	0.1 Vs <sup>-1</sup>
DPV parameters	Amplitude= 0.05 V, sampling width= 0.0167 s,
	pulse period= $0.5$ s.
K <sub>m</sub>	0.21 mM for <i>E. coli</i> (Fig. S2)
	0.27 mM for A. oryzae (Fig. S7)

Table S1 Data for the optimized parameters