

Electronic Supplementary Information

Sensitive electrochemical immunosensor using a bienzymatic system consisting of β -galactosidase and glucose dehydrogenase

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The calculation of k_{app} values for (a) FAD-GDH, (b) GOx, and (c) DI.

(a) Calculation of rate constant for FAD-GDH

$$\begin{aligned}
 & \text{From CA, at 100 s,} \\
 & I_{(\text{glucose} + \text{AN} + \text{FAD-GDH})} = 6.521 \times 10^{-6} \text{ A} \\
 & I_{lim} = 2FAC_{AN}\sqrt{D_{AN}k_{app}C_{FAD-GDH}} \\
 & k_{app} = \frac{I_{lim}^2}{D_{AN}C_{FAD-GDH}(2FAC_{AN})^2} \quad (M^{-1}s^{-1}) \\
 & = \frac{(6.521 \times 10^{-6} \text{ A})^2}{(6.3 \times 10^{-6} \text{ cm}^2/\text{s}) \times (0.231 \times 10^{-6} \text{ M}) \times (2 \times 96,485 \text{ C/mol} \times 0.28 \text{ cm}^2 \times 0.05 \text{ mM})^2} \\
 & = \frac{42.52 \times 10^{-12}}{1.455 \times (2.7)^2 \times 10^{-18} \text{ M} \cdot \text{s}} = 4.01 \times 10^6 \text{ M}^{-1}\text{s}^{-1}
 \end{aligned}$$

(b) Calculation of rate constant for GOx

$$\begin{aligned}
 & \text{From CA, at 100 s,} \\
 & I_{(\text{glucose} + \text{AN} + \text{GOx})} = 0.215 \times 10^{-6} \text{ A} \\
 & I_{lim} = 2FAC_{AN}\sqrt{D_{AN}k_{app}C_{GO_x}} \\
 & k_{app} = \frac{I_{lim}^2}{D_{AN}C_{GO_x}(2FAC_{AN})^2} \quad (M^{-1}s^{-1}) \\
 & = \frac{(0.215 \times 10^{-6} \text{ A})^2}{(6.3 \times 10^{-6} \text{ cm}^2/\text{s}) \times (0.188 \times 10^{-6} \text{ M}) \times (2 \times 96,485 \text{ C/mol} \times 0.28 \text{ cm}^2 \times 0.05 \text{ mM})^2} \\
 & = \frac{0.0462 \times 10^{-12}}{1.184 \times (2.7)^2 \times 10^{-18} \text{ M} \cdot \text{s}} = 5.35 \times 10^3 \text{ M}^{-1}\text{s}^{-1}
 \end{aligned}$$

(c) Calculation of rate constant for DI

$$\begin{aligned}
 & \text{From CA, at 100 s,} \\
 & I_{(\text{NADH} + \text{AN} + \text{DI})} = 2.29 \times 10^{-6} \text{ A} \\
 & I_{lim} = 2FAC_{AN}\sqrt{D_{AN}k_{app}C_{DI}} \\
 & k_{app} = \frac{I_{lim}^2}{D_{AN}C_{DI}(2FAC_{AN})^2} \quad (M^{-1}s^{-1}) \\
 & = \frac{(2.29 \times 10^{-6} \text{ A})^2}{(6.3 \times 10^{-6} \text{ cm}^2/\text{s}) \times (0.208 \times 10^{-6} \text{ M}) \times (2 \times 96,485 \text{ C/mol} \times 0.28 \text{ cm}^2 \times 0.05 \text{ mM})^2} \\
 & = \frac{5.24 \times 10^{-12}}{1.3104 \times (2.7)^2 \times 10^{-18} \text{ M} \cdot \text{s}} = 5.48 \times 10^5 \text{ M}^{-1}\text{s}^{-1}
 \end{aligned}$$

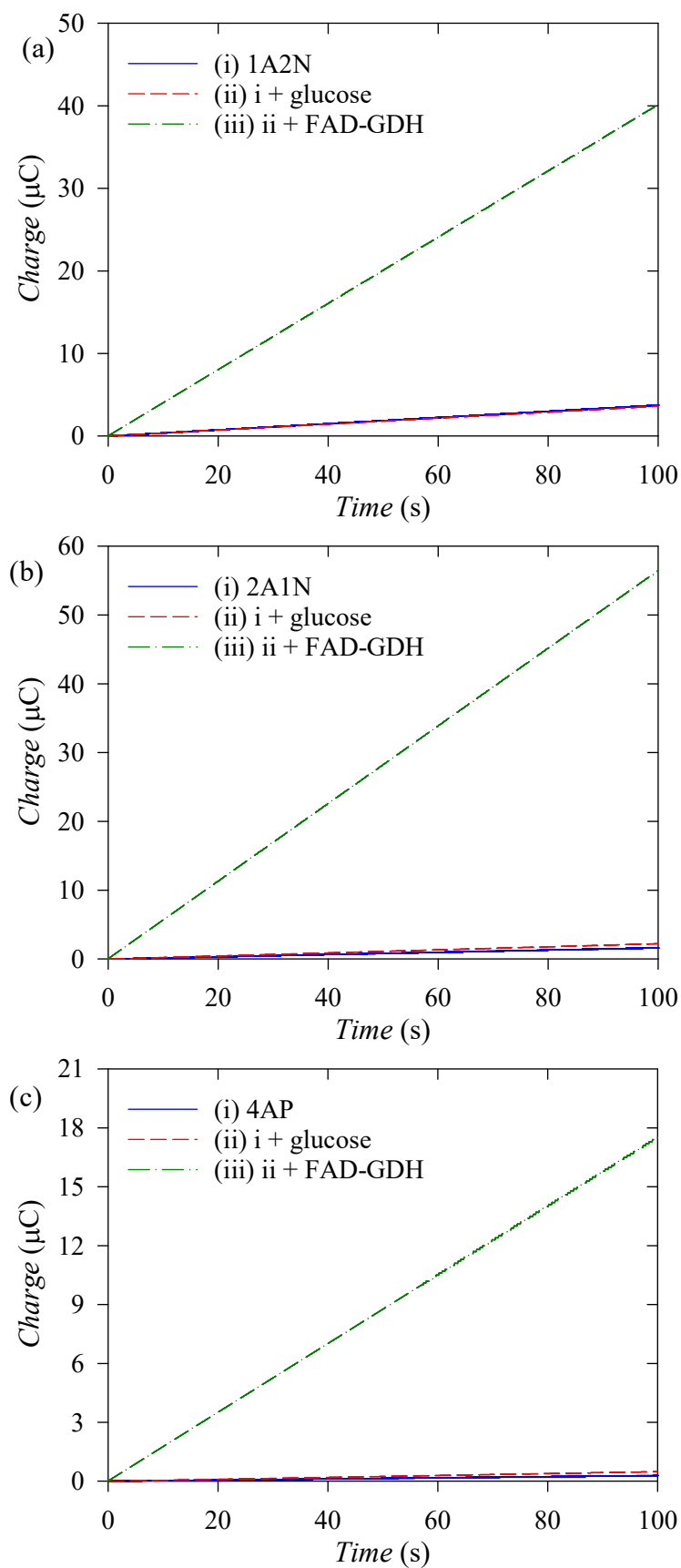


Fig. S1 Chronocoulograms obtained at 0.2 V using bare ITO electrodes after an incubation period of 10 min at 25 °C in Tris buffer (pH 7.5) containing (i) 50 μM product of Gal reaction [(a) 4A1N, (b) 1A2N, (c) 2A1N, and (d) 4AP, respectively], (ii) 50 μM product of Gal reaction and 1 mM glucose, and (iii) 50 μM product of Gal reaction, 1 mM glucose and 10 $\mu\text{g/mL}$ FAD-GDH.

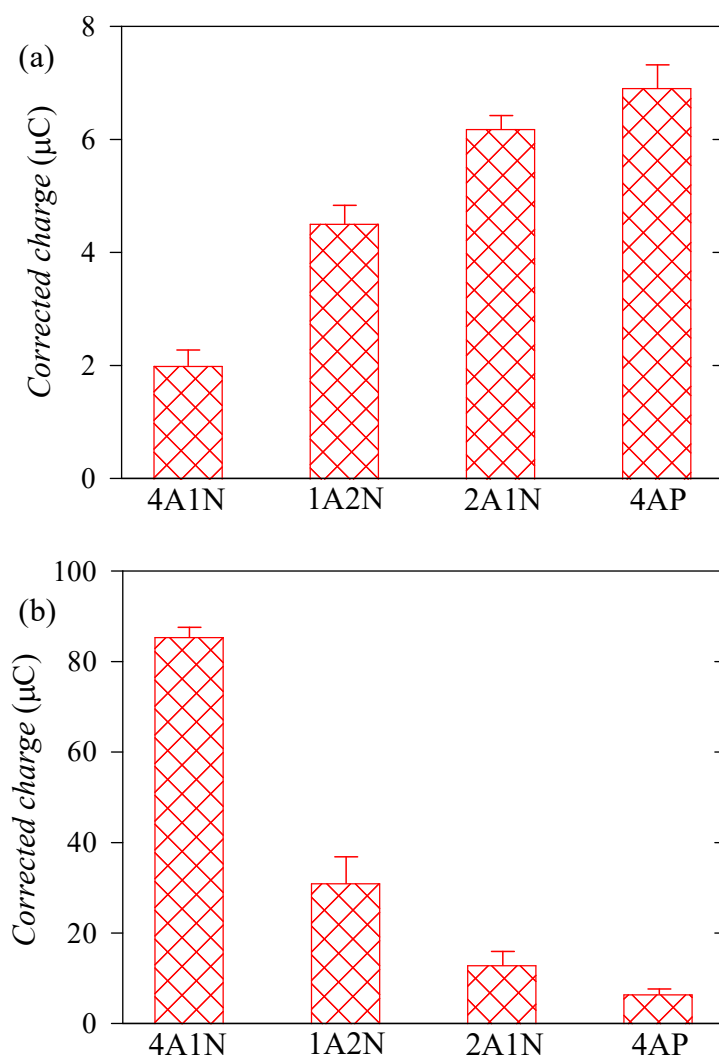


Fig. S2 Histograms of corrected charge values measured by charge difference between charge obtained at 100 s from chronocoulograms obtained at 0.2 V using bare ITO electrodes in Tris buffer (pH 7.5) containing (a) (i) 50 μM product of Gal reaction and 1.0 mM NADH and (ii) 50 μM product of Gal reaction, 1 mM NADH, and 10 $\mu\text{g/mL}$ DI and (b) (i) 50 μM product of Gal reaction and 1 mM glucose and (ii) 50 μM product of Gal reaction, 1 mM glucose, and 10 $\mu\text{g/mL}$ GOx.

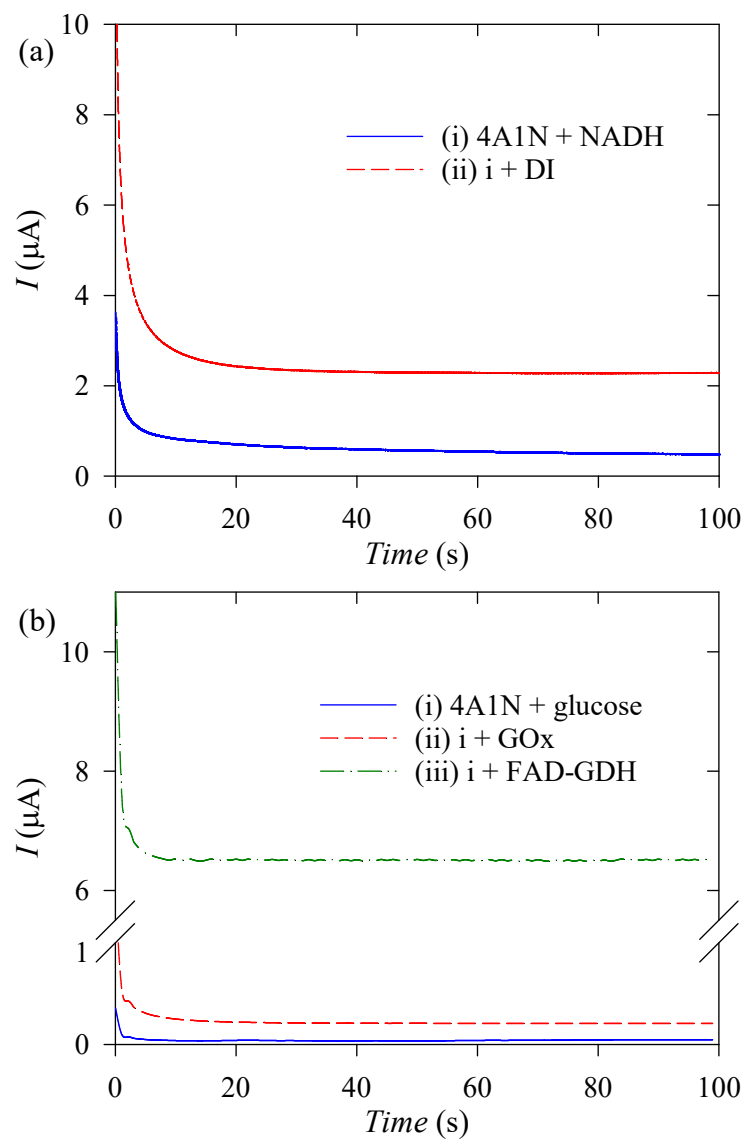


Fig. S3 Chronoamperograms obtained (at 0.35 V) using avidin and BSA modified ITO electrodes in Tris buffer (pH 7.5) containing (a) (i) 50 μM 4A1N and 5 mM NADH and (ii) 50 μM 4A1N, 5 mM NADH, and 10 $\mu\text{g/mL}$ DI and (b) (i) 50 μM 4A1N and 5 mM glucose and (ii) 50 μM 4A1N, 5 mM glucose, and 30 $\mu\text{g/mL}$ GOx, and (iii) 50 μM 4A1N, 5 mM glucose, and 30 $\mu\text{g/mL}$ FAD-GDH.

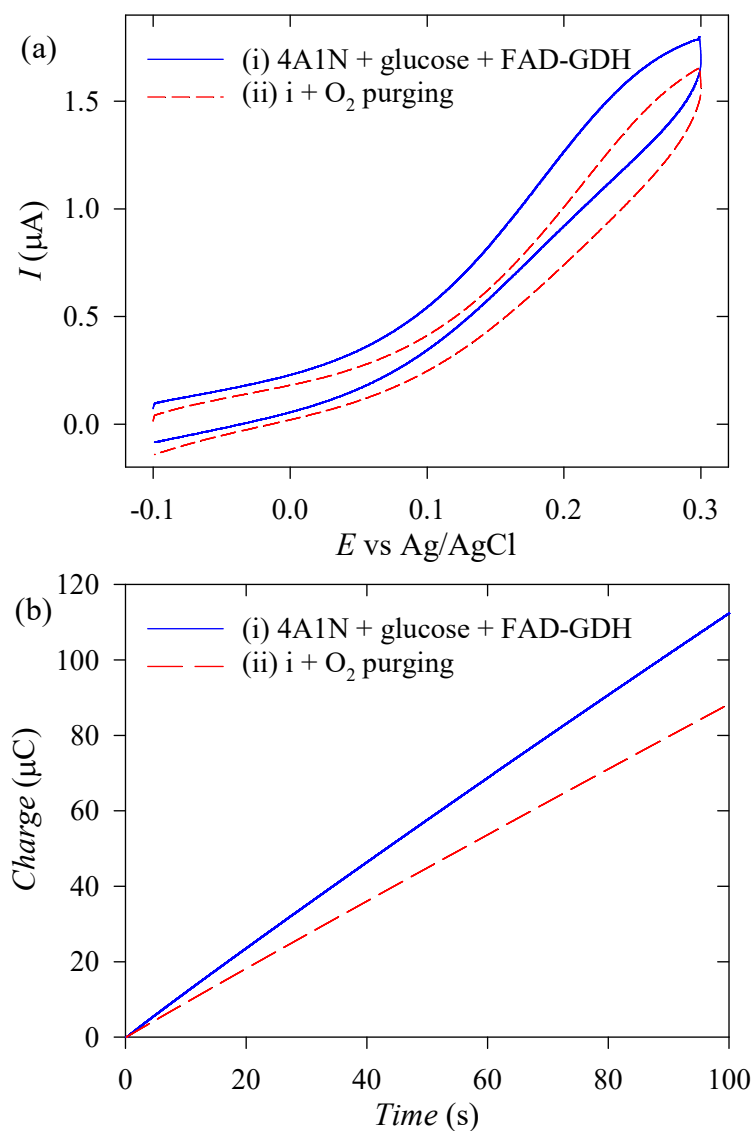


Fig. S4 (a) Cyclic voltammograms (at a scan rate of 20 mV/s) and (b) chronocoulograms (at 0.2 V) obtained at a bare ITO electrode after an incubation period of 15 min at 25 °C in Tris buffer (pH 7.5) containing (i) 50 μM 4A1N and 1 mM glucose, (ii) 50 μM 4A1N, 1 mM glucose, and 10 $\mu\text{g/mL}$ FAD-GDH, (iii) 50 μM 4A1N and 1 mM glucose treated by O_2 gas purging for the incubation period, and (iv) 50 μM 4A1N, 1 mM glucose, and 10 $\mu\text{g/mL}$ FAD-GDH treated by O_2 gas purging for the incubation period.