Supporting Information

High-fidelity determination of security threats via a Boolean biocatalytic cascade

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Preparation of chemicals and reagents

Nitroreductase from *Escherichia coli* (NRd; E.C. 1.6.99.7), peroxidase from *Horseradish* (HRP; E.C. 1.11.1.7), acetylcholinesterase from *Electrophorus electricus* (AChE; E.C. 3.1.1.7), choline oxidase from *Alcaligenes sp.* (ChOx; E.C. 1.1.3.17), bovine serum albumin (BSA), β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH), acetylcholine chloride (ACh), hydrogen peroxide (H_2O_2), diethyl 4-nitrophenyl phosphate (paraoxon; PAX), 2,4-dinitrotoluene (DNT), *o*,*o*-dimethyl-*o*-4-nitro-phenylthiophosphate (methyl parathion; MPT), potassium phosphate monobasic (KH_2PO_4), potassium phosphate dibasic (K_2HPO_4), potassium ferricyanide ($K_3[Fe(CN)_6]$), iron (III) chloride ($FeCl_3$), potassium chloride (KCl) and hydrochloric acid (HCl) were obtained from Sigma-Aldrich Corp. (St. Louis, MO) and were used as supplied. 2,4,6-trinitrotoluene (TNT; dissolved in acetonitrile) was procured from Cerilliant Corp. (Round Rock, TX). Acetonitrile was purchased from Fisher Scientific (Pittsburgh, PA). Ultrapure water (18.2 MΩ·cm) from a Barnstead NANOpure Diamond source (Waltham, MA) was used in all of the experiments. Phosphate buffer (50 mM) was prepared by mixing KH_2PO_4 and K_2HPO_4 in order to achieve a pH of 7.2. All compounds were dissolved in this buffer except for PAX, MPT, TNT, and DNT, which were prepared in acetonitrile. In order to stabilize AChE, 0.1% BSA was added to the stock solution. Likewise to avoid ACh hydrolysis, 12 mM KCl was added to the solution.

Instrumentation

A CH Instruments model 1232A Electrochemical Analyzer (Austin, TX) was used for all electrochemical measurements. An MPM model TF 100 semi-automatic screen-printer

(Franklin, MA) was employed to print the electrodes through a patterned stencil. A Salvislab ThermoCenter temperature-controlled convection oven (Schweiz, Switzerland) was employed for curing the patterned electrode.

Electrode design and fabrication

A screen printed three-electrode strip, custom-designed using AutoCAD®, consisted of a circular carbon working electrode (geometrical area: 3 mm^2) inscribed in hemispherical counter (area: 10 mm^2) and reference electrodes (area: 2 mm^2). The fabrication of the screen-printed electrode system is detailed: An Ag/AgCl-based ink from Ercon (E2414) was employed to define the conductive underlayer as well as the reference electrode. A carbon-based ink (Ercon E3449) was then overlaid on the conductor to define the working and counter electrode geometry. Finally, an insulator ink (Ercon E6165) was overlaid on the Ag/AgCl and carbon layers to insulate all except the contact pads and the active segment of the electrodes. A semi-automatic screen-printer was used to print the pattern onto an alumina substrate. Subsequent to the printing process, the patterned substrate was cured in a temperature-controlled convection oven at 120°C for 20 min. The substrate was finally cleaved to create test strips possessing overall dimensions of 10 mm × 34 mm.

Preparation of the Prussian blue (PB)-modified screen printed electrode (SPE)

The PB-modified SPE were prepared in accordance with the procedure detailed by Karyakin et al.[1] Briefly, a 0.1 M KCl / 0.1 M HCl solution was prepared and the appropriate quantity of both $K_3[Fe(CN)_6]$ and $FeCl_3$ were dissolved in this solution in order to achieve 4 mM final concentration of each of these compounds. This solution was subsequently dispensed on the SPE and E = +0.4 V (vs. Ag/AgCl) was applied for 120 sec to the working electrode. After this step, the electrode was cured at 100°C for 1 hr in a convection oven. The PB-modified SPE was then electrochemically activated by performing 40 cyclic voltammetric scans from -0.2 V to +0.5 V in the 0.1 M KCl / 0.1 M HCl solution.

Electrocatalytic activity of PB-modified SPE towards H_2O_2

Fig. S1A shows the cyclic voltammogram recorded using the PB-modified SPE in 50 mM phosphate buffer at a scan rate of 50 mV s^{-1}. A pair of redox peaks is observed at E_p^c: -0.140 V and E_p^a: -0.056 V, which is characteristic of PB electrochemistry.

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[1] A. A. Karyakin, E. A. Puganova, I. A. Budashov, I. N. Kurochkin, E. E. Karyakina, V. A. Levchenko, V. N. Matveyenko and S. D. Varfolomeyev, *Anal. Chem.,* 2004, **76**, 474-478.

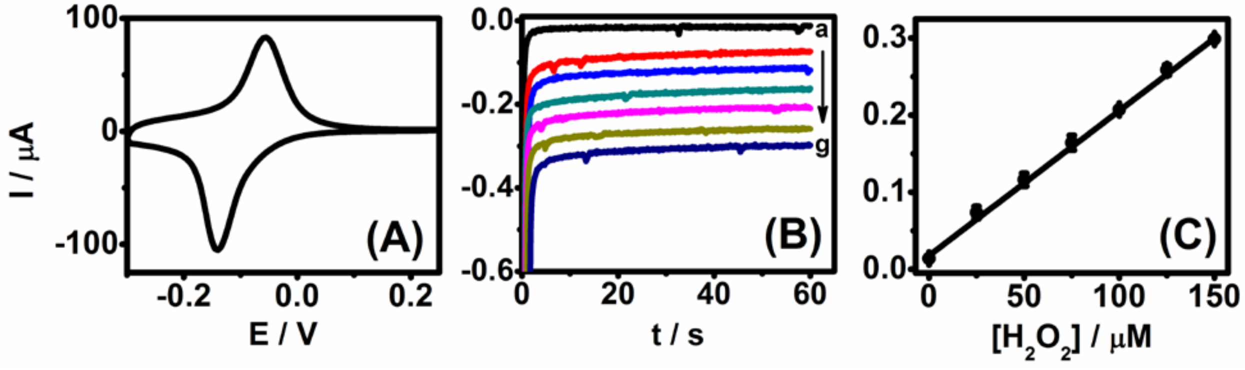

Fig. S1 (A) Cyclic voltammogram recoded at the PB-modified SPE in 50 mM phosphate buffer; scan rate: 50 mV s^{-1}. (B) Chronoamperograms recorded for the detection of H_2O_2 at SPE (a→g: 0 to 150 μM with a 25 μM interval) in 50 mM phosphate buffer; E: -0.2 V vs. Ag/AgCl. (C) Calibration curve generated from (B).

Further, the electrocatalytic activity of the PB-modified SPE was evaluated towards the reduction of H_2O_2. Fig. S1B displays the chronoamperograms recorded for increasing concentrations of H_2O_2 (0 to 150 μM, in 25 μM steps). The corresponding calibration plot is shown in Fig. S1C. The current was sampled at t = 60 sec, and a linear regression shows a high degree of correlation among increasing levels of H_2O_2 with a correlation coefficient of 0.9980 (n = 3).

Composition of the NOR logic gate and protocol

50 mM phosphate buffer containing 5 kU/L NRd, 300 kU/L HRP, 150 μM NADH, 100 U/L AChE, and 5 kU/L ChOx was mixed with the desired concentrations of TNT and PAX to obtain the corresponding (0,0), (0,1), (1,0), and (1,1) logic levels. Following a 2 min inhibition period, 1 mM ACh was added and then incubated further for an additional 5 min to allow the reaction to ensue. The solution was then dispensed onto the electrode surface. Chronoamperometric detection of the H_2O_2 product was performed at the PB-modified SPE at a step potential of E = -0.2 V (vs. Ag/AgCl) and the current value was extracted at the 30th sec. Experiments were performed at least three times at each concentration to ensure the integrity of the results.

Detection of other explosives and toxins using the *NOR logic gate*

The same protocol as mentioned above was followed for the detection of another nitro explosive, DNT, as well as for another organophosphate nerve agent, MPT.

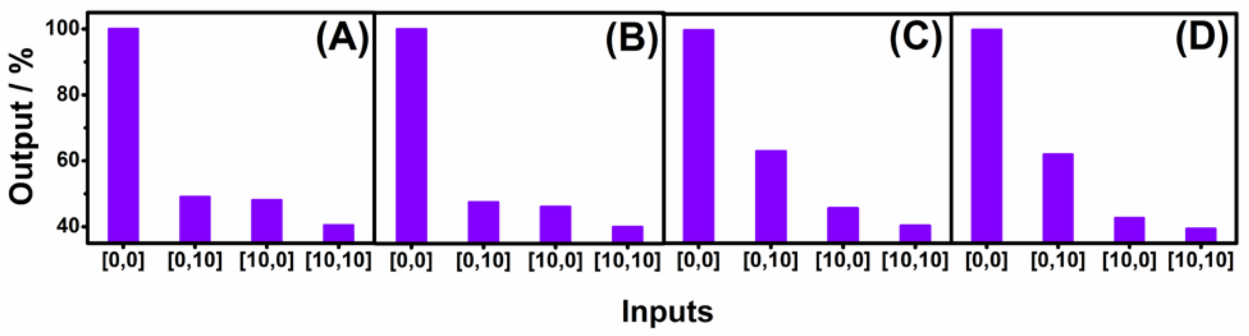

Fig. S2 Bar charts representing the magnitude of the response for four different combinations of the inputs [Input 1 (μg mL^{-1}), Input 2 (μM)] = (A) [TNT, PAX]; (B) [DNT, PAX]; (C) [TNT, MPT] and (D) [DNT, MPT]. The output current has been normalized to facilitate comparison.