Electronic Supplementary Material (ESI) for Chemical Communications

DNAzyme logic controlled biofuel cell for self-powered biosensors

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1. Experimental

1.1 Chemicals and Reagents

Glucose dehydrogenase (GDH, E.C. 1.1.1.47, 256 units mg^{-1} from *Pseudomonas*), L(+)-glucose, β-nicotinamide adenine dinucleotide dipotassium salt (NAD^+), Meldola's blue (MDB), glutaraldehyde solution (1:8% aqueous), bovine serum albumin (BSA), hemin (from pig), adenosine monophosphate (AMP, from yeast), hydrogen peroxide (H_2O_2, 30 wt. % in H_2O), hydroxyethyl piperazineethanesulfonic acid (HEPES), and Nafion perfluorinated membrane (Nafion® 117, thickness 0.007 in.) were obtained from Sigma-Aldrich (St. Louis, MO). Adenosine deaminase (ADA, E.C. 3.5.4.4, 20.2 U mg^{-1} from bovine spleen) was purchased from Worthington Biochemical Corporation (Lakewood, NJ). The thiolated DNA, 5'-SH-$(CH_2)_6$-AAGGGTAGGGCGGGTTGGGAACCTTCCTGGGGGAGTATTGCGGAAGGTTCCC-3' was purchased from Integrated DNA Technologies, Inc. (San Diego, CA). Multi-walled carbon nanotubes (CNT, purity ~95%, 30±5 nm diameter) were purchased from NanoLab Inc. (Waltham, MA) and purified with 3 M HNO_3 at 120 ºC for 24 h. The purified CNT was subsequently treated with a 1:3 (v:v) mixture of HNO_3 (65%) and H_2SO_4 (98%) at room temperature for 8 h with continuous ultrasonication. A 0.1 M pH 7.2 phosphate buffer solution (PBS) comprised of Na_2HPO_4, NaH_2PO_4 and H_3PO_4. All other chemicals were of analytical reagent grade and were used as received. All reagents were prepared with ultra-pure deionized water (18.2 MΩ·cm).

1.2 Instrumentation

Linear sweep voltammetry and cyclic voltammetry were performed on a μAutolab potentiostat-galvanostat (Eco Chemie, the Netherlands). Other electrochemical experiments were performed with a CHI 621A electrochemical analyzer (CH Instruments, Austin, TX). A glassy carbon (GC, 3 mm diameter) or Au (2 mm diameter) working electrode, an Ag/AgCl (saturated KCl) reference electrode, and a platinum wire counter electrode were used to establish a three-electrode electrochemical system. Without special mention, all the measurements were performed at ambient temperature (23±2 °C).

1.3 Anode Fabrication

A CNT-DMF suspension was prepared by mixing 1 mL DMF and 10 mg CNT and ultrasonicating the mixture for 0.5 h. Subsequently, 1 μL of the CNT-DMF suspension was cast on a clean GC electrode. Following evaporation of the solvent at 35 ºC for 15 min, the CNT/GC electrode was obtained. The CNT/GC electrode was immersed into a 0.5 mg mL^{-1} MDB solution for 30 min. Subsequently, the obtained CNT-MDB/GC electrode was rinsed with deionized water and dried with a nitrogen stream. A BSA solution (1% (wt.%) in 0.1 M pH 7.2 PBS) was mixed with a GDH solution (6 U μL^{-1} in 0.1 M pH 7.2 PBS) with a volume ratio of 1:2 to yield a GDH-BSA mixture. After coating 1.5 μL of the resulting mixture onto a CNT-MDB/GC electrode, a 2 μL of glutaraldehyde aqueous solution (40 mM) was further coated onto the surface in order to cross-link the GDH onto the CNT-MDB/GC electrode. After being kept at 4 ºC in the refrigerator for 12 hours, the as-prepared electrode was rinsed with distilled water, dried with a nitrogen stream, and used as an anode (i.e., GDH/CNT-MDB/GC electrode).

1.4 Cathode Preparation

The preparation method for the cathode has been described previously in the literature.[1] Briefly, 6 μL of a 1 μM thiolated DNA solution (prepared in 10 mM pH 7.2 HEPES buffer) was cast on the Au electrode for 2 h to produce a thiolated hairpin DNA self-assembly layer on the Au electrode surface. After rinsing with the 10 mM pH 7.2 HEPES buffer solution and drying with a nitrogen stream, the as-prepared electrode was employed as the cathode. As shown in **Fig. S1**, the hairpin DNA on the cathode

contained a horseradish peroxidase (HRP)-mimicking DNAzyme sequence and an AMP-binding aptamer sequence, which are both blocked in the stem region of the hairpin.

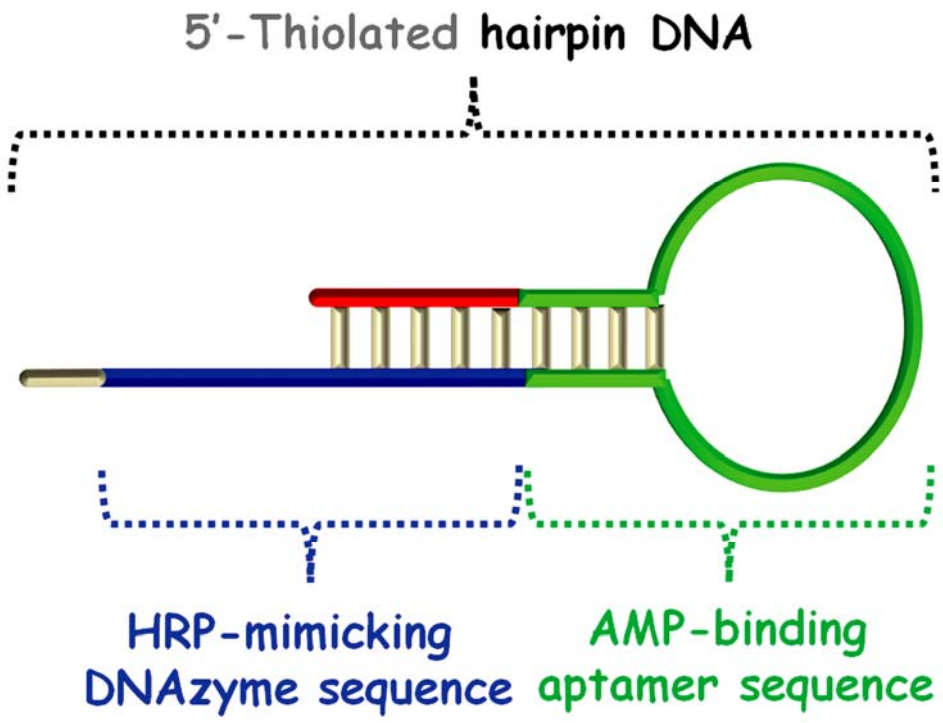

Fig. S1 *Components of the hairpin DNA on cathode.*

1.5 BFC Design

The BFC was comprised of two compartments (cathode and anode) separated by a Nafion perfluorinated membrane.

1.6 Logic Action

All possible permutations of biochemical input A (ADA, 10 units mL^{-1}) and biochemical input B (AMP, 50 mM) were added to the cathodic compartment. Following addition of the inputs to the system, the signals were extracted after 2 h transpired.

2. Cathode

2.1 Control Experiments

As shown in the CVs (**Fig. S2**) and LSVs (**Fig. S3**) at the cathode, only the addition of both hemin and AMP on cathode can lead to substantially increased cathodic currents for the H_2O_2 electroreduction compared with the absence of hemin or AMP, which is consistent with recent reports.[1] Hence, the presence of both hemin and AMP together in the system can lead to the HRP-mimicking DNAzyme formation on the electrode interface that can be applied as the cathode of a BFC for H_2O_2 electroreduction.

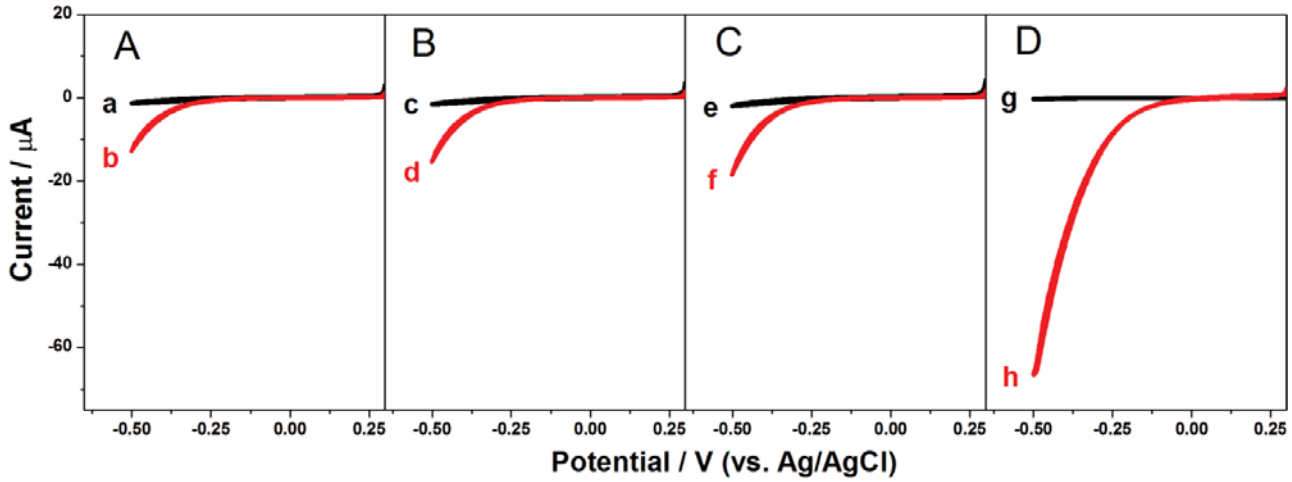

Fig. S2 *(A) CVs at the cathode for 0 (a) and 1 mM H_2O_2 (b). (B) CVs at the cathode in the presence of 1 μM hemin for 0 (c) and 1 mM H_2O_2 (d). (C) CVs at the cathode in the presence of 50 μM AMP for 0 (e) and 1 mM H_2O_2 (f). (D) CVs at the cathode in the presence of 1 μM hemin and 50 μM AMP for 0 (g) and 1 mM H_2O_2 (h). Electrolyte: N_2-saturated 10 mM pH 7.2 HEPES buffer containing 50 mM KCl, and 100 mM NaCl. Scan rate: 20 mV s^{-1}.*

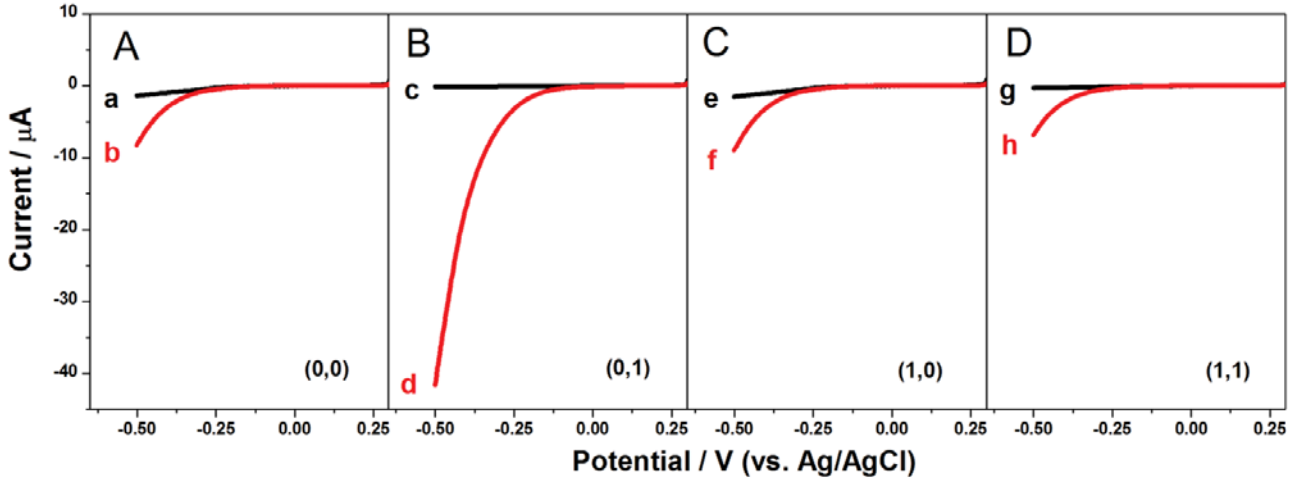

Fig. S3 *LSVs at the cathode for 0 (a, c, e and g) and 1 mM H_2O_2 (b, d, f and h) upon different input signals (A) (0,0), (B) (0,1), (C) (1,0) and (D) (1,1). Input A, 10 units mL^{-1} ADA; Input B, 50 μM AMP. Cathode electrolyte: N_2-saturated 10 mM pH 7.2 HEPES buffer containing 50 mM KCl, 100 mM NaCl, and 1 μM hemin. Scan rate: 2 mV s^{-1}.*

2.2 Effects of Different Inputs on Cathode

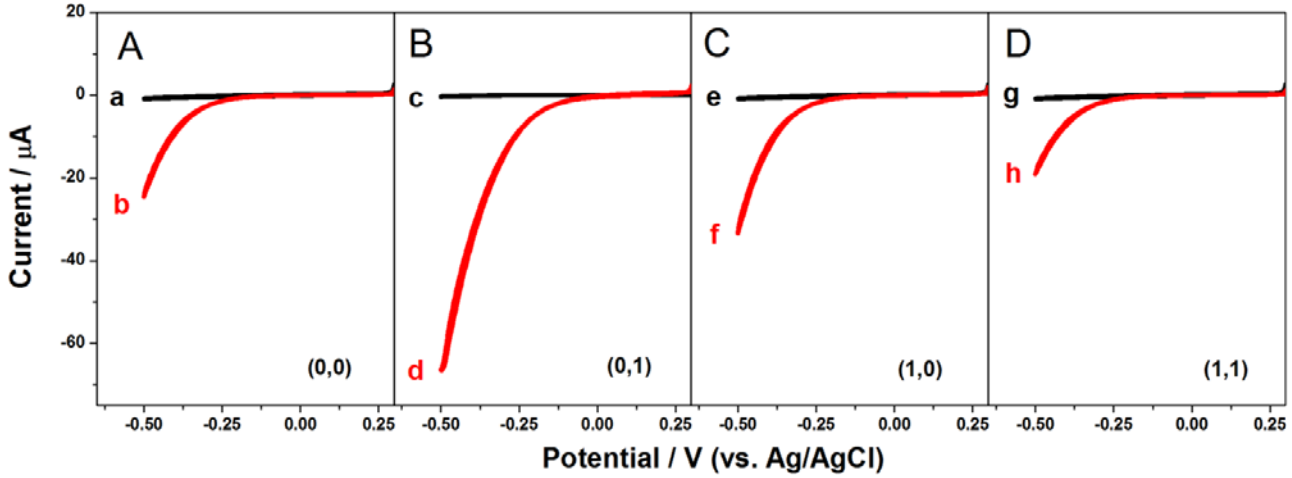

Fig. S4 *CVs at the cathode for 0 (a, c, e and g) and 1 mM H_2O_2 (b, d, f and h) upon the application of various input signals (A) (0,0), (B) (0,1), (C) (1,0) and (D) (1,1). Input A, 10 units mL^{-1} ADA; Input B, 50 μM AMP. Cathode electrolyte: N_2-saturated 10 mM pH 7.2 HEPES buffer containing 50 mM KCl, 100 mM NaCl, and1 μM hemin. Scan rate: 20 mV s^{-1}.*

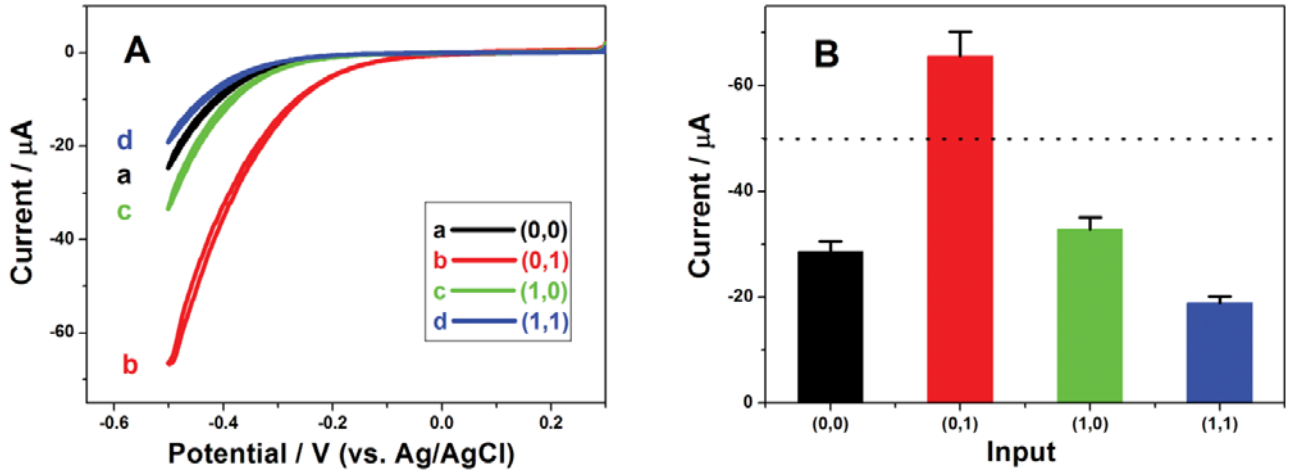

Fig. S5 *(A) CVs at the cathode for 1 mM H_2O_2 upon the input signals (0,0) (a), (0,1) (b), (1,0) (c), and (1,1) (d). Scan rate: 20 mV s^{-1}. (B) Bar diagrams, illustrating the current response at -0.5 V for the different combinations of the input signals, derived from* ***Fig. S5A****. The dotted line is established at the threshold (-50 μA). Input A, 10 units mL^{-1} ADA; Input B, 50 μM AMP. Cathode electrolyte: N_2-saturated 10 mM pH 7.2 HEPES buffer containing 50 mM KCl, 100 mM NaCl, and 1 μM hemin.*

3. Anode

With glucose addition, the anodic peak currents at the anode from the CV (**Fig. S6A**) and LSV (**Fig. S6B**) both increased (curves b and d), which are similar with those at a GDH-electrode with MDB as the mediator.[2] This suggests that the fabricated anode is able to electrooxidize glucose in this system and can be used as the bioanode constituent of a BFC.

As shown in **Fig. S6C**, upon performing a calibration, the electrocatalytic anodic current did not saturate until the glucose (biofuel) concentration exceeded 24 mM. Glucose played an important role in the power output performance of the BFC, so we chose 24 mM as the biofuel concentration for anode.

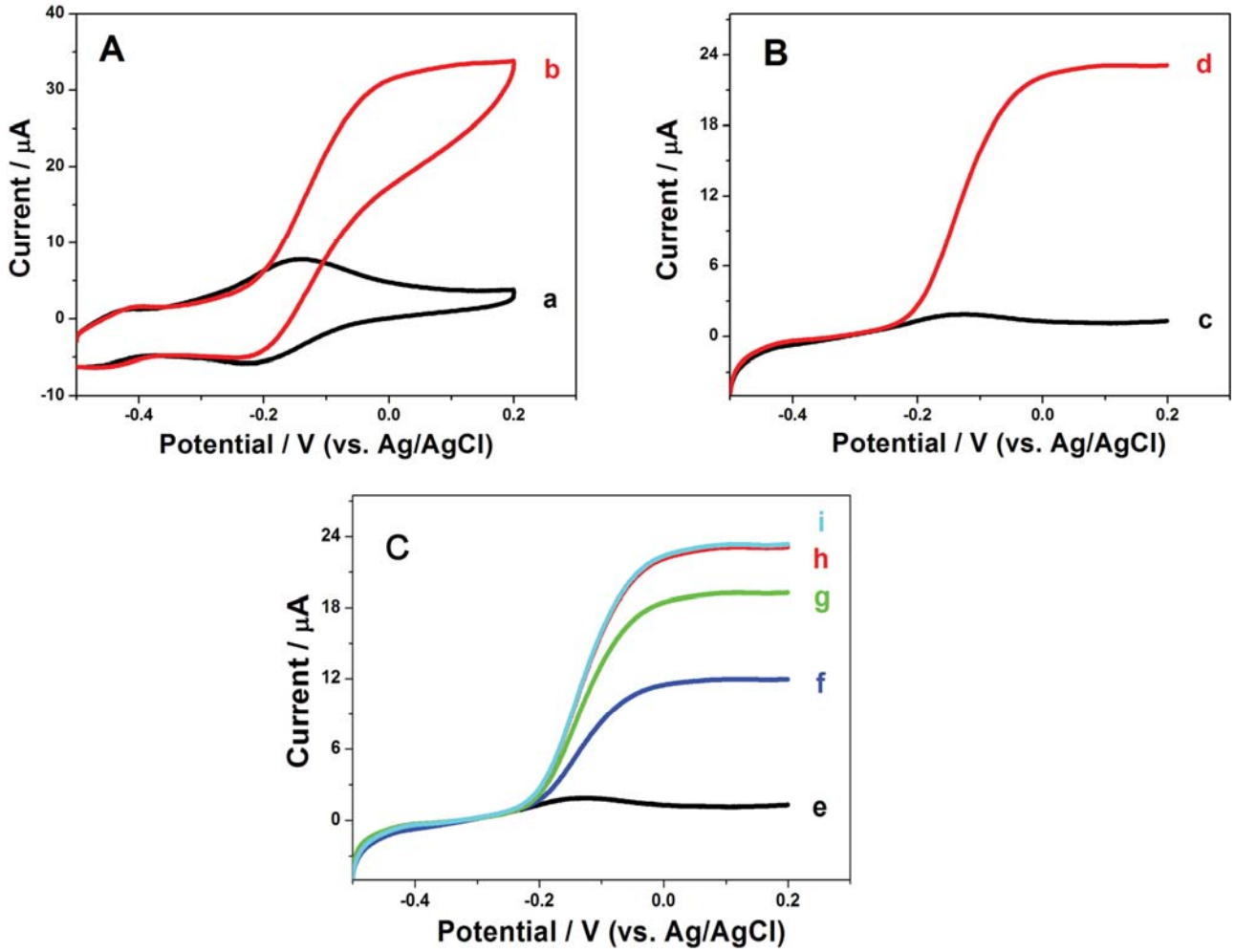

Fig. S6 *(A) Cyclic voltammograms (CVs) at the anode for 0 (a) and 24 mM glucose (b). Scan rate: 20 mV* s^{-1}*. (B) Linear sweep voltammograms (LSVs) at the anode for 0 (c) and 24 mM glucose (d). Scan rate: 2 mV* s^{-1}*. (C) LSVs at the anode for 0 (e), 8 (f), 16 (g), 24 (h) and 32 mM glucose (i). Scan rate: 2 mV* s^{-1}*. Anode electrolyte:* N_2*-saturated 0.1M pH 7.2 PBS containing 20 mM* NAD^+*.*

4. BFC

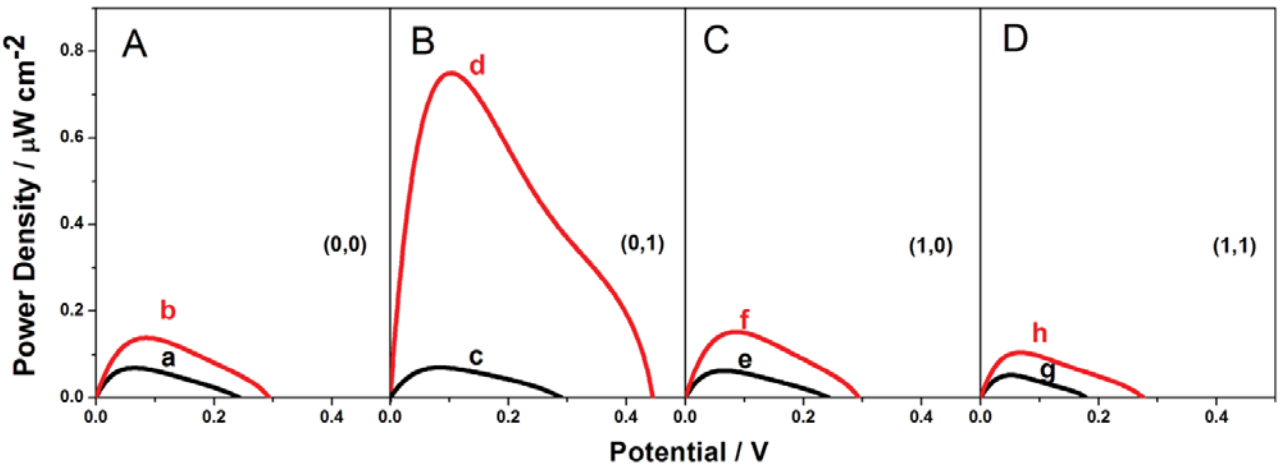

Fig. S7 *Dependence of the power density on the BFC voltage by using 24 mM glucose as the biofuel at the anode and 0 (a, c, e and g) or 1 mM H_2O_2 (b, d, f and h) as the oxidizer for the cathode upon the application of different input signals (A) (0,0), (B) (0,1), (C) (1,0) and (D) (1,1). Input A, 10 units mL^{-1} ADA; Input B, 50 mM AMP. Cathode electrolyte: N_2-saturated 10 mM pH 7.2 HEPES buffer containing 50 mM KCl, 100 mM NaCl, and 1 μM hemin. Anode electrolyte: N_2-saturated 0.1 M pH 7.2 PBS containing 20 mM NAD^+.*

References for Electronic Supplementary Information (ESI)

1 G. Pelossof, R. Tel-Vered, J. Elbaz and I. Willner, *Anal. Chem.*, 2010, **82**, 4396.
2 M. Zhou, L. Deng, D. Wen, L. Shang, L. Jin and S. Dong, *Biosens. Bioelectron.*, 2009, **24**, 2904.