Electronic Supplementary Information (ESI) for

An IMP-Reset gate-based reusable and self-powered "smart" logic aptasensor on a microfluidic biofuel cell

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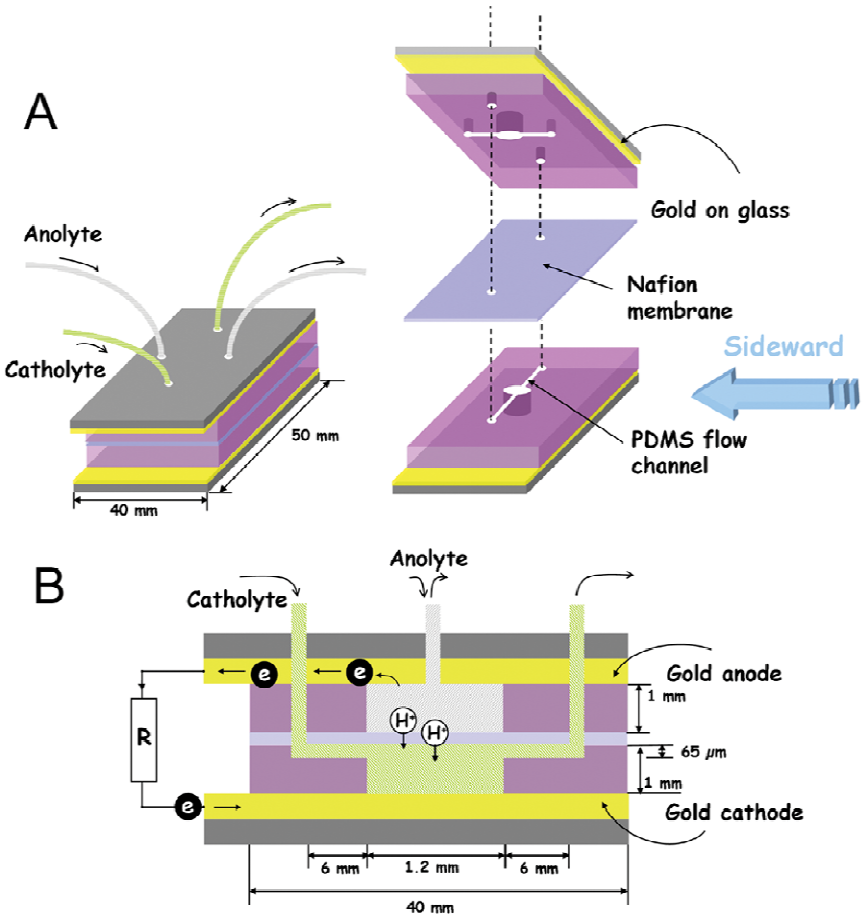
1. Fabrication of the micro-BFC

As shown in **Scheme S1**, our micro-BFC device is constructed by stacking an Au film on glass, a polydimethylsiloxane (PDMS) containing cathode chamber and flow channel, and a proton exchange membrane (PEM) in sequence onto a PDMS chip prefabricated with an Au film and a PDMS containing anode chamber and flow channel. Independent supplies of glucose (anolyte) and $K_3[Fe(CN)_6]$ electron acceptor solutions (catholyte) were delivered through the top of the device via two sets of microfluidic pathways. The details have been shown below (**Step 1 – Step 5**).



Scheme S1. (A) Schematic representation of the components and (B) sideward profile for the micro-BFC-based **IMP-Reset** logic aptasensor.

Step 1: Fabrication of Au film

Au layer (~100 nm) was vaporized onto the glass slide using a metal vacuum evaporator with Ti metal as the adherent layer.

Step 2: Manufacture of PDMS

The PDMS stamp (25 mm × 25 mm × 1 mm) containing anode (or cathode) chamber and fluidic channel was prepared in an ITO glass using standard soft-lithography techniques.[1] A polished ITO glass was first coating with AZ 50XT positive photoresist and patterned by a designed photomask, followed by an UV exposal and AZ 400K development to create the mold. A mixture of Sylgard 184 Silicone Elastomer and curing agent with a ratio of 10:1 (w/w) was then poured onto the ITO-AZ

50XT mold, shaped at 80 °C for approximately 2 h, and peeled off.

Step 3: Definition of anode (or cathode) and its chamber

The anode (or cathode) chamber was defined by PDMS stamp on Au film, and the Au surface in the channel functions was used as anode (or cathode). The cylindrical anode (or cathode) chamber of the assembled micro-BFC device was 1.2 mm diameter and 1.0 mm high.

Step 4: Pretreatment of PEM

Commercial Nafion 115 membrane was used as proton exchange membrane in this work, which was pretreated by sequentially boiling in H_2O_2 (30% v/v) and water, followed by soaking in 0.5 M sulfuric acid solution and then water, each for 1 h. The activated membranes were cut into 4×4 cm^2 and stored in water prior to use.

Step 5: Assembly of micro-BFC

To assemble the dual-chamber micro-BFC, a sandwiched structure was carefully designed. Au film (on glass), PDMS stamp (containing cathode chamber and fluidic channel), Nafion 115 membrane, PDMS stamp (containing anode chamber and fluidic channel) and Au film (on glass) were manually stacked in sequence while carefully aligning the tubing holes for the flow channels. The device was mounted on a custom chip carrier, and sandwiched between two acrylic plates. Two electrical wires were used to extend the anode and cathode, and an external load connected to the pins completed the circuit. For liquid transport, four 1.2-mm-diameter polytetrafluoroethylene (PTFE) tubes were plugged into the holes in the PDMS stamp to form two independent routes for liquid. Each device was tested by flowing sterile water through the chambers to ensure there was no cross-channel leakage.

2. Evaluation of response time

As shown in **Fig. S1**, the maximum power output for input (**0,1**), (**1,0**) or (**1,1**) changed significantly when the logic aptasensor was applied input (**0,1**), (**1,0**) or (**1,1**), and they all reached a plateau at ~60 min in this system. Therefore, 60 min was chosen as the response time.

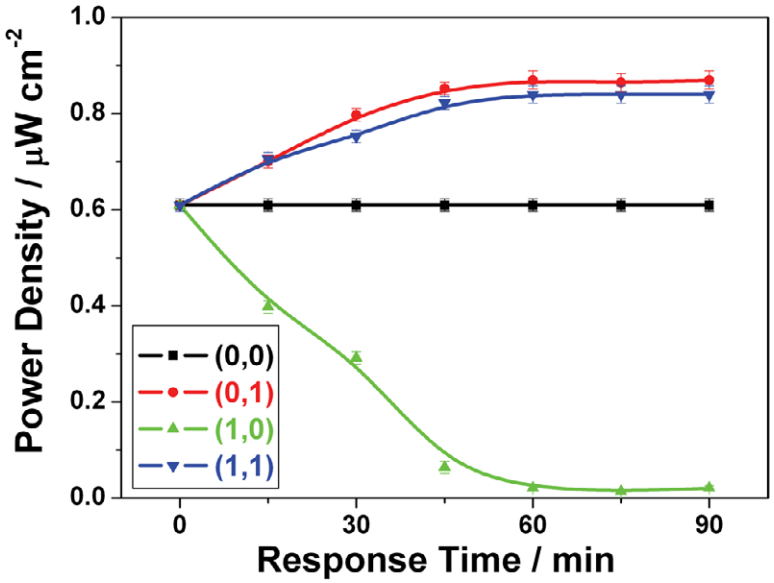


Fig. S1. Response time-dependent maximum power output for different combinations of input signals.

3. AC impedance measurements

As shown in **Fig. S2A**, the fabrication process of cathode (i.e., DNA duplex modified Au film) was monitored by AC impedance technique. Bare Au film showed a very small semicircle domain (which represented the charge-transfer resistance, i.e., R_{ct}) [**Fig. S2A**(a)]. However, for Au/5'-thiolated partly complementary strand-mixed aptamer film [**Fig. S2A**(b)], the R_{ct} signal increased obviously, due to the remarkably decreasing electron-transfer efficiency. After MCE assembled, the impedance response was reduced [**Fig. S2A**(c)].

The effect of **IMP-Reset** function on the R_{ct} value of cathode has been also charactered by AC impedance technique (**Fig. S2B**). After adding ATP [input (**0,1**)], ATP would interact with the extended ATP-binding aptamer embedded in the mixed aptamer and draw it away from the electrode surface, which made the R_{ct} signal decreased [input (**0,1**) in **Fig. S2B**]. When only thrombin was present [input (**1,0**)], the thrombin-binding aptamer in mixed aptamer on Au electrode would catch the thrombin, resulting in a blocked electrode interface. Accordingly, the R_{ct} signal increased obviously [input (**1,0**) in **Fig. S2B**]. However, further ATP addition can interact with the extended ATP-binding aptamer embedded in the mixed aptamer and draw the mixed aptamer away from the electrode surface [input (**1,1**)], making the R_{ct} signal greatly decreased [input (**1,1**) in **Fig. S2B**]. After logic operation, to re-activate the electrode, ATP and mixed aptamer were consecutively added, resulting in the regeneration of the electrode interface, thus re-activating the cathode [**Reset** in **Fig. S2B**].

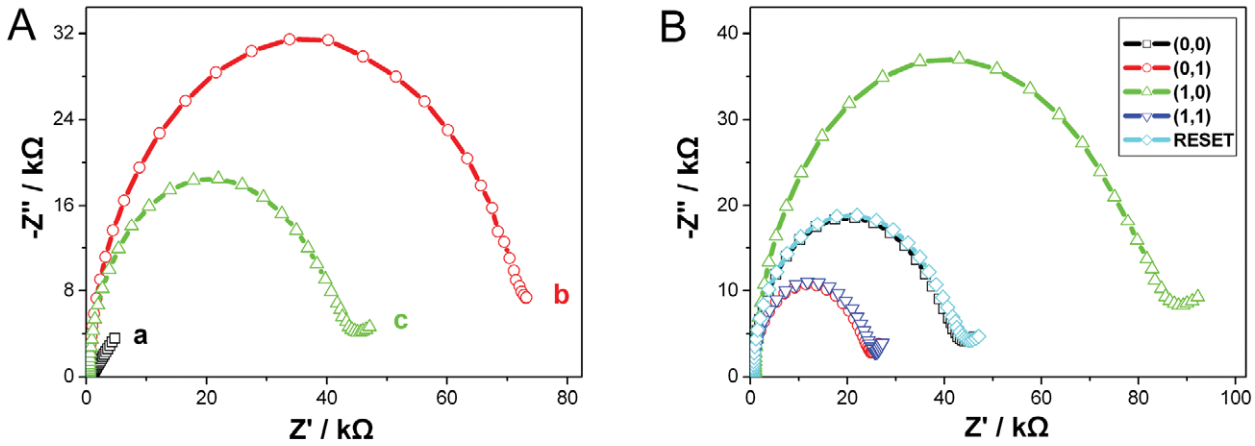


Fig. S2. (A) Formation process of the cathode (i.e., DNA duplex modified Au film) measured using AC impedance technique: Au film (a), Au/5'-thiolated partly complementary strand-mixed aptamer film (b), and Au/5'-thiolated partly complementary strand-mixed aptamer/MCE film (c). (B) Nyquist plots of the cathode after the input signals: (**0,0**); (**0,1**); (**1,0**); (**1,1**) and **Reset** function. Electrolyte: 25 mM pH = 8.2 Tris-HCl containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and 100 mM NaCl.

4. Electrooxidation of glucose at anode

As shown in **Fig. S3**, after glucose addition, the anodic peak current at anode increased obviously, indicating the fabricated anode can electrooxidize glucose in this system.

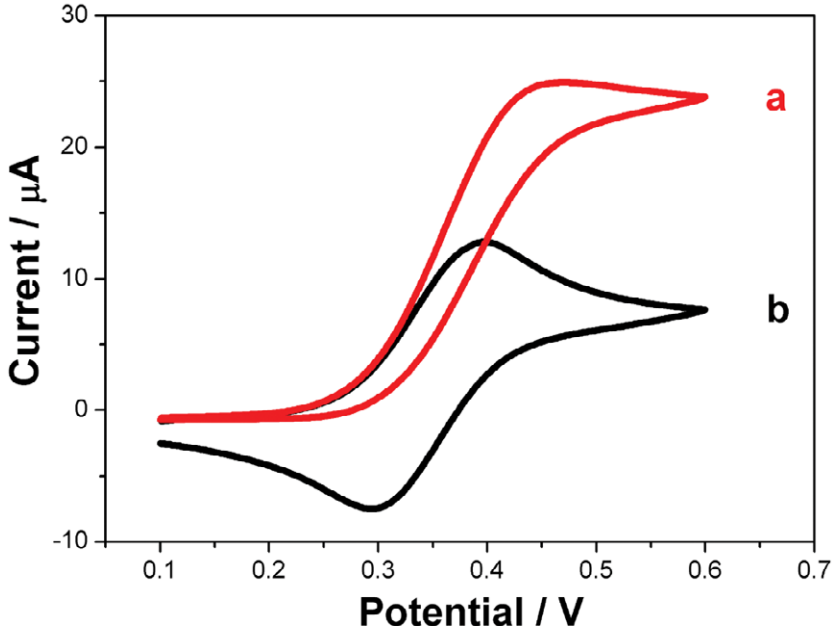


Fig. S3. Cyclic voltammograms of anode in air-saturated pH 7.4 PBS containing 200 units mL^{-1} GOD and 0.5 M FMCA with (a) and without (b) 80 mM glucose. Scan rate: 10 mV s^{-1}.

5. Reference for Electronic Supplementary Information (ESI)

1 G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang and D. E. Ingber, *Annu. Rev. Biomed. Eng.*, 2001, **3**, 335-373.