# Ultrasensitive Self-Powered Cytosensors Based on Exogenous Redox-Free Enzyme Biofuel Cell as Point-of-Care Tools for Early Cancer Diagnosis

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## Experimental Section

### Materials and chemicals.

Pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH; E.C. 1.1.5.2, from microorganism–not specified by the company) was purchased from Toyobo Co., Japan. Bilirubin oxidase (BOD, E.C. 1.3.3.5, from *Myrothecium verrucaria*), poly (diallyldimethylammonium chloride) (PDDA, 20%, w/w in water, MW=200 000-350 000), 1-ethyl-3-(3-dimethyl-aminopropyl) carbodimiide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), 3-(N-morpholino) propanesulfonic acid (MOPS-buffer), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), calcein-AM, 6-mercapto-1-hexanol (MCH) and Dulbecco's phosphate-buffer saline (D-PBS) with $CaCl_2$ and $MgCl_2$ were purchased from Sigma-Aldrich. Both of the enzymes were used as received without further purification. Thiolated-aptamers and amino-modified part complementary strand of aptamers (cs DNA) were synthesized and purified by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequence of the thiolated-Sgc8c aptamer (SH-Sgc8c) was 5'-HS-$(CH_2)_6$-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-3'. The sequence of the part complementary strand of SH-Sgc8c was 5'-$NH_2$-$(CH_2)_6$-TTT

TTT TTT TCT AAC CGT AC-3'.The sequence of the SYL3C aptamer (SH-SYL3C) was 5'-HS-CAC TAC AGA GGT TGC GTC TGT CCC ACG TTG TCA TGG GGG GTT GGC CTG-3'. The sequence of the part complementary strand of SH-SYL3C was 5'-$NH_2$-$(CH_2)_6$-TTT TTT TTT CAG GCC AAC CCC CC-3'. Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). 0.1 M pH=7.4 phosphate buffer solutions (PBS) consisting of $Na_2HPO_4$ and $NaH_2PO_4$ were employed as the supporting electrolyte. Au NPs were prepared according to the literature by adding a sodium citrate solution to a boiling $HAuCl_4$ solution.[1] All the other reagents were of analytical grade and used without further purification. Ultrapure fresh water was obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used throughout the whole experiment.

**Apparatus.**

Field emission scanning electron microscopy (FESEM) images and transmission electron microscopy (TEM) images were measured by a HITACHI S4800 SEM and a JEOL 2010 TEM, respectively. Electrochemical impedance spectroscopy (EIS) was performed with an Autolab electrochemical analyzer (Eco Chemie, The Netherlands) in 2.5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ aqueous solution with 0.5 M $KNO_3$ as the supporting electrolyte, within the frequency range of 0.1 Hz to 100 kHz. Electrochemical measurements were performed on a CHI 660D workstation (Shanghai Chenhua Apparatus Corporation, China) with a conventional three-electrode system, which was composed of a platinum wire as the auxiliary electrode, a saturated calomel electrode (SCE) as the reference electrode, and a bioanode or

biocathode as the working electrode, respectively. The open circuit voltage of EBFC was tested by connecting the bioanode and biocathode placed in the electrolytic cell.

**Cell lines and cell culture.**

CCRF-CEM cells and K562 cells were obtained from Nanjing Key Gen Biotech Co., Ltd. and cultured in a flask in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg $mL^{-1}$) and streptomycin (100 μg $mL^{-1}$) in an incubator (5% $CO_2$, 37°C). MCF-7 cells, HL-60 cells, HeLa cells, and 293T cells were obtained from Nanjing Key Gen Biotech Co., Ltd and seeded in DMEM medium (Gibco, Grand Island, NY) supplemented with 10% fetal calf serum (Gibco, Grand Island, NY), penicillin (100 μg/mL), and streptomycin (100 μg/mL) in 5% $CO_2$, 37°C incubator. At the logarithmic growth phase, the cells were collected and separated from the medium by centrifugation at 1000 rpm for 2 min and then suspended in the binding buffer (both 4.5 g $L^{-1}$ glucose and 5 mM $MgCl_2$ dissolved in D-PBS with $CaCl_2$ and $MgCl_2$) to obtain a homogeneous cell suspension. The binding buffer was used to ensure the effective binding affinity between cells and aptamers.[2]

**Synthesis of the G/CNTs nanocomposite.**

G/CNTs nanocomposite were prepared according to a previously published procedure with some modifications:[3] The GO colloidal suspension was prepared by oxidizing graphite powder via a modified Hummers Method, and then the solution was freeze-dried and dissolved with water to a concentration of 3 mg $mL^{-1}$. Then 60 mg CNTs were dispersed into 10 mL, 3 mg $mL^{-1}$ GO suspensions to obtain the mixed

suspensions, followed by ice bath sonication for 1h. After obtaining stable mixed suspensions, the vials with suspensions were put into a Teflon (polytetrafluoroethylene) vessel (50 mL). The vessel was then sealed in a stainless steel autoclave and heated to 180 °C for 3 h. The sonication and hydrothermal process resulted in the assembly of GO and CNTs as well as the reduction of GO into conductive G. Finally, G/CNTs nanocomposite was obtained by freeze-drying. The control material G was prepared in a similar process except that no CNTs were added.

**Preparation of G/CNTs/Au NPs hybrid, CNTs/Au NPs and G/Au NPs.**

Briefly, 5.0 mg of the G/CNTs nanocomposite was dispersed in 1% PDDA salt solution (0.02 M NaCl, 5.0 mL) and then was sonicated for 30 min to form a homogeneous suspension of positively charged G/CNTs/PDDA. Residual PDDA polymer was removed by centrifugation (15000 rpm, 10 min), and the obtained precipitate was washed with water at least three times. Subsequently, the purified G/CNTs/PDDA was dispersed in 50 mL of the prepared negatively charged Au NPs solution, and stirred at room temperature for 2 h. After that excessive Au NPs were removed by centrifugation (8000 rpm, 10 min) and the G/CNTs/Au NPs hybrid precipitate was recovered and dissolved with water to a concentration of 1 mg $mL^{-1}$. The preparation of CNTs/Au NPs or G/Au NPs was similar to the G/CNTs/Au NPs hybrid, in which CNTs or G was instead of G/CNTs nanocomposite.

**Preparation of bioanode of EBFC.**

50 μL of the prepared G/CNTs/Au NPs hybrid suspension was coated on the surface of the carbon cloth electrode. Then, the substrate electrode was dried at 37°C for 2 h

and immersed in a solution containing 1 mg $mL^{-1}$ EDC and NHS for 30 min to activate the carboxyl group of Au NPs. After rinsing with ultrapure water to eliminate excess EDC and NHS, the activated electrode was coated by 50 μL of PQQ-GDH solution (2 mg $mL^{-1}$, dissolved in MOPS-buffer (50 mM, pH 7.0) containing $Na_2SO_4$ (100 mM) and $CaCl_2$ (1 mM)) for 12 h at 4°C to obtain the bioanode of the EBFC. Before the fabrication of the EBFC, the prepared bioanode was purged with ultrapure water to wipe off unbound enzyme. The electrode was stored at 4°C when not in use.

**Preparation of biocathode of EBFC.**

Firstly, 50 μL of the prepared G/CNTs/Au NPs hybrid suspension was coated on the surface of the carbon cloth electrode. Then, the substrate electrode was dried at 37°C for 2 h. Subsquently, 50 μL 1.0 μM of aptamer SH-Sgc8c or SH-SYL3C was applied to the substrate electrode and incubated at 4°C overnight. The aptamer was assembled onto the substrate electrode surface through Au-S bond. After the aptamer-functionalized substrate electrode was rinsed with ultrapure water, 20 μL of 1 mM MCH was dropped on the surface of the prepared substrate electrode and incubated for 1 h at room temperature to block the nonspecific binding sites, and then washed with ultrapure water thoroughly to obtain the aptamer-functionalized substrate electrode.

A mixture of 1 mg $mL^{-1}$ CNTs/Au NPs, 1 mg $mL^{-1}$ EDC and NHS was sonicated at room temperature for 30 min to activate the carboxyl group of Au NPs. Then 5 μL, 10 μM of the cs DNA and 10 μL, 5 mg $mL^{-1}$ BOD were added to 50 μL of the above mixture and shaken in a small vial overnight at room temperature. The reaction

mixture was then centrifuged at 10 000 rpm at 4 °C for 15 min, and the supernatant was discarded to remove any free BOD and cs DNA. The above wash process was repeated for three times. Finally, 50 μL of 0.01 M pH 7.4 PBS containing 0.1 M NaCl was added to the collected precipitate to form a homogeneous dispersion, denoted as BOD bioconjugate. After then, 50 μL of BOD bioconjugate solution was dropped onto the aptamer-functionalized substrate electrode surface and incubated for 1 h at 37°C to obtain the biocathode of EBFC.

**Fabrication and measurement of the EBFC cytosensor.**

A membrane-less glucose/$O_2$ EBFC could be constructed by using the prepared bioanode and biocathode in the room temperature (25$^o$C). The supporting electrolyte was oxygen-saturated 0.1 M PBS (pH=7.4) containing 5 mM of glucose.

For cytosensing, the prepared biocathode was first soaked in 50 μL of target cell suspension at a certain concentration and incubated at 4°C for 30 min to capture the cells. After the electrode was taken out and rinsed with incubation buffer to remove the non-captured cells carefully, it was put back into the electrolytic cell, and the open circuit voltage of EBFC cytosensor was measured.

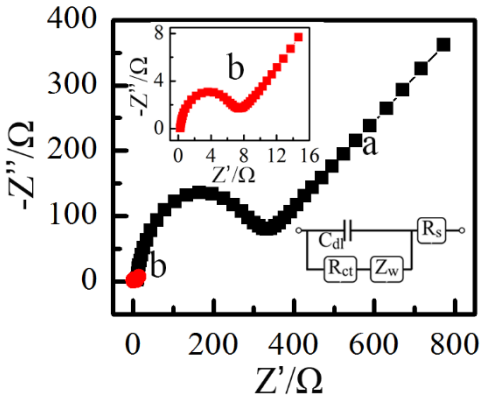


**Fig. S1** EIS of the bare carbon cloth electrode (a) and G/CNTs/Au NPs hybrid modified carbon cloth electrode (b) Insets are the enlarged view of curve b (left) and the Randles equivalent circuit used to fit the EIS data (right).

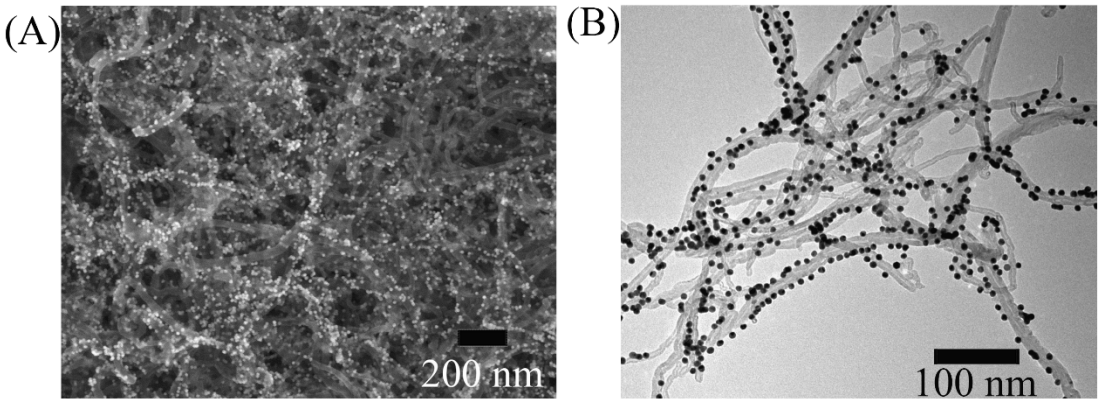


**Fig. S2** SEM (A) and TEM (B) of CNTs/Au NPs

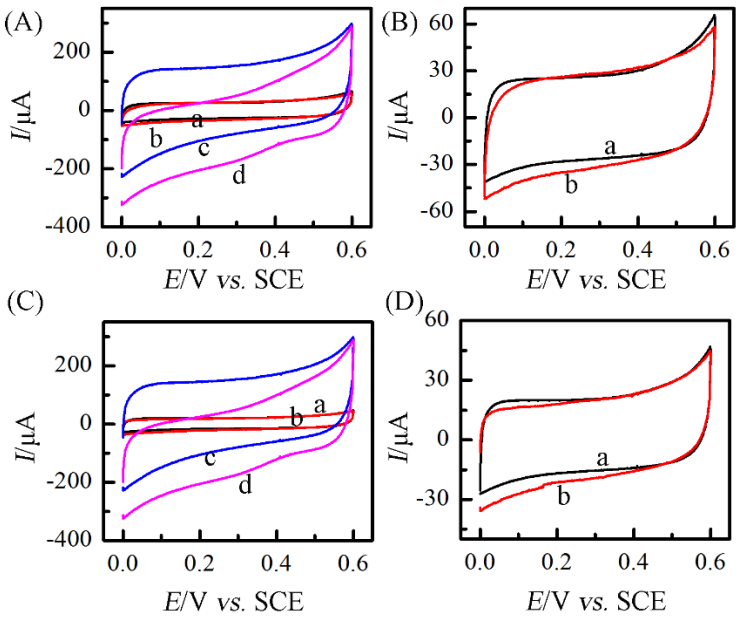


**Fig. S3** (A) CVs of BOD bioconjugate-aptamer-functionalized CNTs/Au NPs biocathode in PBS (pH=7.4) saturated with $N_2$ (a) and $O_2$ (b), and CVs of BOD bioconjugate-aptamer-functionalized G/CNTs/Au NPs biocathode in PBS (pH=7.4) saturated with $N_2$ (c) and $O_2$ (d). (B) The enlarged view of curve a and b in (A). (C) CVs of BOD bioconjugate-aptamer-functionalized G/Au NPs biocathode in PBS (pH=7.4) saturated with $N_2$ (a) and $O_2$ (b), and CVs of BOD bioconjugate-aptamer-functionalized G/CNTs/Au NPs biocathode in PBS (pH=7.4) saturated with $N_2$ (c) and $O_2$ (d). (B) The enlarged view of curve a and b in (C).

As shown in Fig. S3, at the same potential, the bioelectrocatalytic currents at the control biocathodes were both much smaller than that at BOD bioconjugate-aptamer-functionalized G/CNTs/Au NPs biocathode, suggesting that the BOD bioconjugate-aptamer-functionalized G/CNTs/Au NPs biocathode possessed higher-efficient catalytic activity for oxygen reduction compared with the BOD bioconjugate-aptamer-functionalized CNTs/Au NPs or G/Au NPs electrodes. The results also demonstrated that the highly porous 3D architectures of G/CNTs/Au NPs improved the bioelectrocatalysis of enzyme.

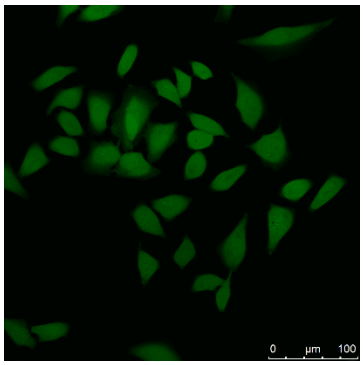


**Fig. S4** Fluorescence microscopy image of MCF-7 cells stained with calcein-AM after they were captured on the biocathode surface.

## References


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2. D. Shangguan, Y. Li, Z. Tang, Z. C. Cao, H. W. Chen, P. Mallikaratchy, K. Sefah, C. J. Yang and W. Tan, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 11838-11843.
3. Z. Niu, L. Liu, L. Zhang, Q. Shao, W. Zhou, X. Chen and S. Xie, *Adv. Mater.*, 2014, **26**, 3681-3687.