

## Supporting Information

### **Rolling Circle Amplification, Enzyme-catalyzed Polymerization, Breast Cancer Cell, Polyaniline**

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#### Experimental

*Reagents and Materials:* Dithiothreitol (DTT), 6-mercapto-1-hexanol (MCH), Phi29 DNA polymerase and dNTP mixture were obtained from Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). Horseredish peroxide (HRP) (EC 1.11.1.7, type VI), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimidehydrochloride (EDC•HCl), N-hydroxysuccinimide (NHS) and aniline (99.5%) were purchased from Sigma (Aldrich). Oligonucleotides used in this experiment were synthesized and purified by Takara Biotechnology Co., Ltd. (Dalian, China). Immobilized capture probe, signaling probe and other oligonucleotides were designed with software OLIGO-6. Streptavidin and biotin were purchased from Sigma (USA). Human breast cancer MCF-7 cell was obtained from Cell Bank of Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). All other chemicals used in this work were of analytical grade. All solutions were prepared with ultrapure water ( $>18\text{M}\Omega\cdot\text{cm}$ ) obtained from a Millipore Milli-Q water purification system.

All oligonucleotides were dissolved with 10.0 mM TE buffer (10.0 mM Tris, 1.0 mM EDTA, pH 7.4) into stock solutions and stored at  $-20\text{ }^{\circ}\text{C}$ . They were diluted with 10.0 mM TE buffer solution to suitable concentrations prior to use. Thiol-modified capture probes were

treated with 0.05 M DTT at room temperature overnight in the dark to reduce the S–S bonds prior to use. The washing buffer was 10.0 mM PB buffer containing 0.1 M NaCl (pH 7.4).

*Apparatus.* A Model CHI660D Electrochemistry Workstation (Shanghai Chenhua Instruments Co. Ltd., China) was employed for all electrochemical experiments. A conventional three-electrode system was used. A saturated calomel electrode (SCE) and a platinum wire electrode were used as the reference electrode and the auxiliary electrode, respectively. A gold electrode (2mm in diameter) was used as a working electrode. The atomic force microscope (AFM) was a digital Instruments Multimode SPM-9500J3 (Shimadzu Corporation, Japan), operating in ex-situ Tapping Model.

*Cell Culture and Cell Treatments.* MCF-7 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/mL of penicillin, and 100 µg/mL streptomycin at 5% CO<sub>2</sub> and in humidified 95% air. The density of cells was calculated using a Petroff-Hausser cell counter. After the concentration of cells reached  $1 \times 10^6$  cells/mL, the cells were collected using centrifugation at 1000 g for 5 min.

The confocal fluorescence microscopic of MCF-7 cells were conducted by suspending cells in 0.7% low melting agarose and were applied to slides precoated with 0.6% normal melting agarose. After the agarose gel solidified, 20 mL ethidium bromide (30 mg/L) was dispensed directly onto slides and covered with a cover slip. Slides were viewed using a fluorescence microscope (Nikon 027; Nikon, Tokyo, Japan).

*Biomodification of the Au electrode.* A schematic illustration of the sensing procedure was depicted in Scheme 1. Prior to use, the bare Au electrode was polished with 0.3 and 0.05 m alumina slurries and rinsed with double distilled water. Then, the bare Au electrode was immersed in piranha solution (70% concentrated H<sub>2</sub>SO<sub>4</sub> and 30% H<sub>2</sub>O<sub>2</sub>; *Caution: piranha solution is strongly oxidizing and should be handled with care!*) for 5 min to eliminate other substances, and then ultrasonically treated in absolute ethanol and ultrapure water,

successively. Subsequently, the Au electrode was rinsed with liquid nitrogen. In order to obtain well-aligned monolayer of hairpin-aptamer capture probe on Au surface, the Au electrode was first immersed in 1.0  $\mu\text{M}$  thiol-modified capture probe P1 solution diluted with 0.1 M PBS and incubated for 1 h at 16  $^{\circ}\text{C}$  in the dark to avoid oxidation of SH-group. After that, the Au electrode was further treated with 0.1 M MCH for 2 h to block the uncovered gold electrode surface and finally rinsed with 0.01 M Tris-buffer.

*Recognition of MCF-7 cells and RCA reaction.* The recognition of MCF-7 cells was conducted by immersing the hairpin-aptamer P1 modified Au electrode into a 50.0  $\mu\text{L}$  0.01M Tris-buffer solution containing certain concentration of target DNA and incubated for 20 min. After rinsed with 0.1 M pH 7.4 Tris-HCl buffer containing 0.05% Tween 20, the electrode was transferred into the incubation solution containing variable concentrations of target MCF-7 cells and incubated for 1 h at 37  $^{\circ}\text{C}$  to form the cell-aptamer complex, followed by rinsing thoroughly with pH 7.4 phosphate buffer saline (PBS, 137 mM NaCl, 2.7 mM KCl, 8.72 mM  $\text{Na}_2\text{HPO}_4$  and 1.41 mM  $\text{KH}_2\text{PO}_4$ ) containing 0.05% Tween 20.

RCA reaction on electrode surface was performed by immersing the electrode into a 20  $\mu\text{L}$  reaction mixture (0.2 units of phi29 DNA polymerase, 100 nM biotinylated primer, 1 mM dNTP, 10 mM magnesium acetate, 33 mM potassium acetate, 1 mM dithiothreitol in 10 mM pH 7.4 Tris-HCl buffer) and incubated at 37  $^{\circ}\text{C}$  for 1 h.

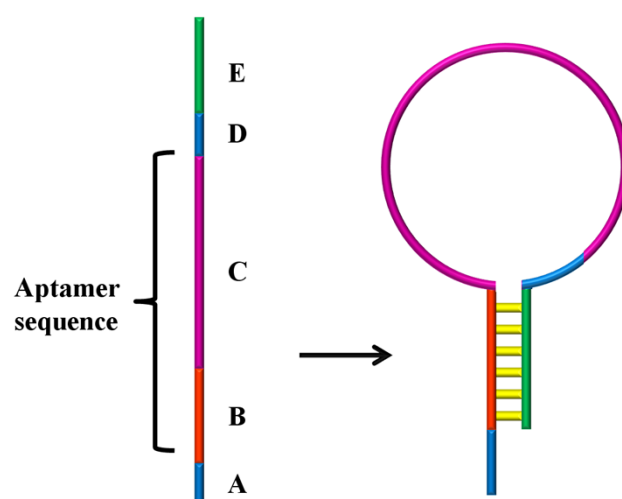
*Ligation of streptavidin and biotin-labeled HRP.* After washing with PBS containing 0.05% Tween 20, the hybridized electrode was immersed into 500  $\mu\text{L}$  of 0.5 M streptavidin solution diluted with 0.1 M PBS for 30 min. Streptavidin could specifically ligate to biotins labeled at the end of the signaling probe. After that, the electrode was thoroughly rinsed with PBS to remove unligated streptavidin and was incubated in biotin-labeled HRP solution for 30 min. Because of strong biotin-streptavidin binding interaction, the biotin-labeled HRP binds to the

streptavidin-conjugated of the detection probe on the electrode surface. This incubation step was followed by thoroughly rinsing of the surface with 0.1M PBS to eliminate detergents.

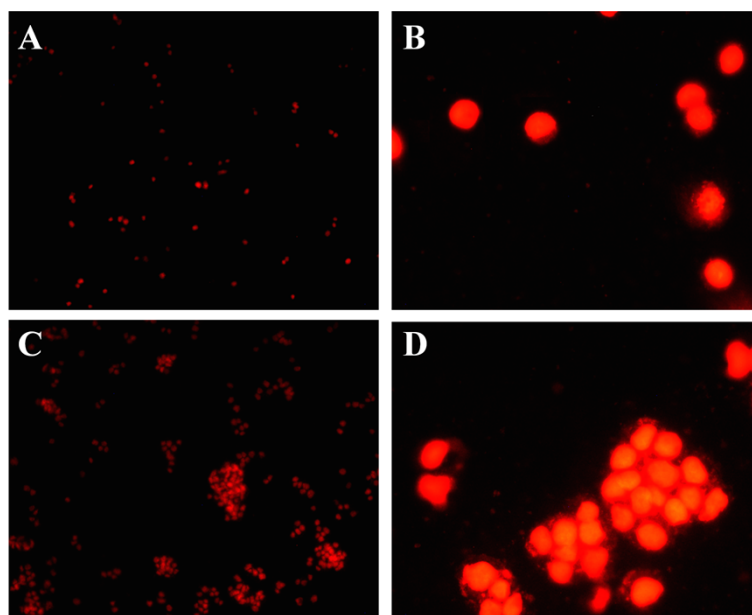
*Enzymatic deposition of PANI and electrochemical measurements.* To perform the deposition of PANI under enzymatic condition and the voltammetric detection, the resulting modified electrode was immersed into a 0.1 M HAc–NaAc solution (pH 4.3) containing 1.0 mM aniline and 5.0 mM H<sub>2</sub>O<sub>2</sub> for a desirable accumulation time (about 15 min). After that, the electrode was removed from the above solution and transferred into a 0.1 M HAc–NaAc solution (pH 4.3), after which cyclic voltammetry and SWV were conducted. EIS was performed in 5.0 mM K<sub>3</sub>Fe(CN)<sub>6</sub>/K<sub>4</sub>Fe(CN)<sub>6</sub> (1:1) containing 0.2 M KCl. The impedance measurements were recorded at a bias potential of +195 mV (vs. SCE) within the frequency range of 10<sup>5</sup> to 10<sup>-2</sup> Hz.

**Table S1:** Oligonucleotides used in this paper.

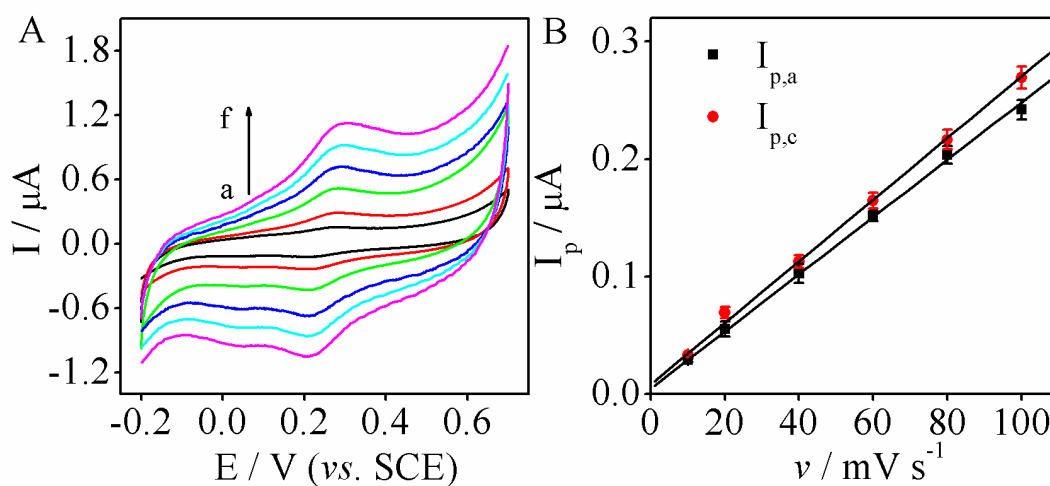
Name	Sequence
P1	5'-HS-C6-TTTTTCACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTTGGC -CTGTTTTTCTCTGTAGTG-3'
P2	5'-biotin-CACTACAGAG-3'
P3	5'-HS-C6-GGGGGGTAAAAGAACTGATCAGCACGGGATGGGATAGGGAGGGGAGT -GTTGAAAAGGGGGTGATCAGTTCTT-3'
P4	5'-biotin-AAGAACTGAT-3'
P5	5'-HS-C6-TTTTTGCAGCTCAAGTCACCCTCCCCCTCAACACAACACAACCCTCCTCT -CCTTTTTTCTTGAGCTGC-3'



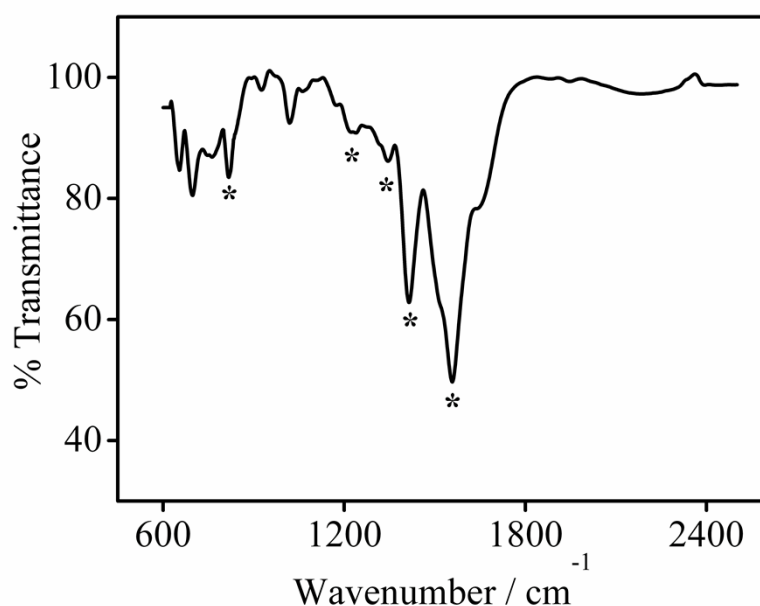
**Figure S1.** The structure of the selected hairpin-aptamer.



**Figure S2.** Confocal fluorescence microscopy images of MCF-7 cells treated without (A and B) or with P1 (C and D).



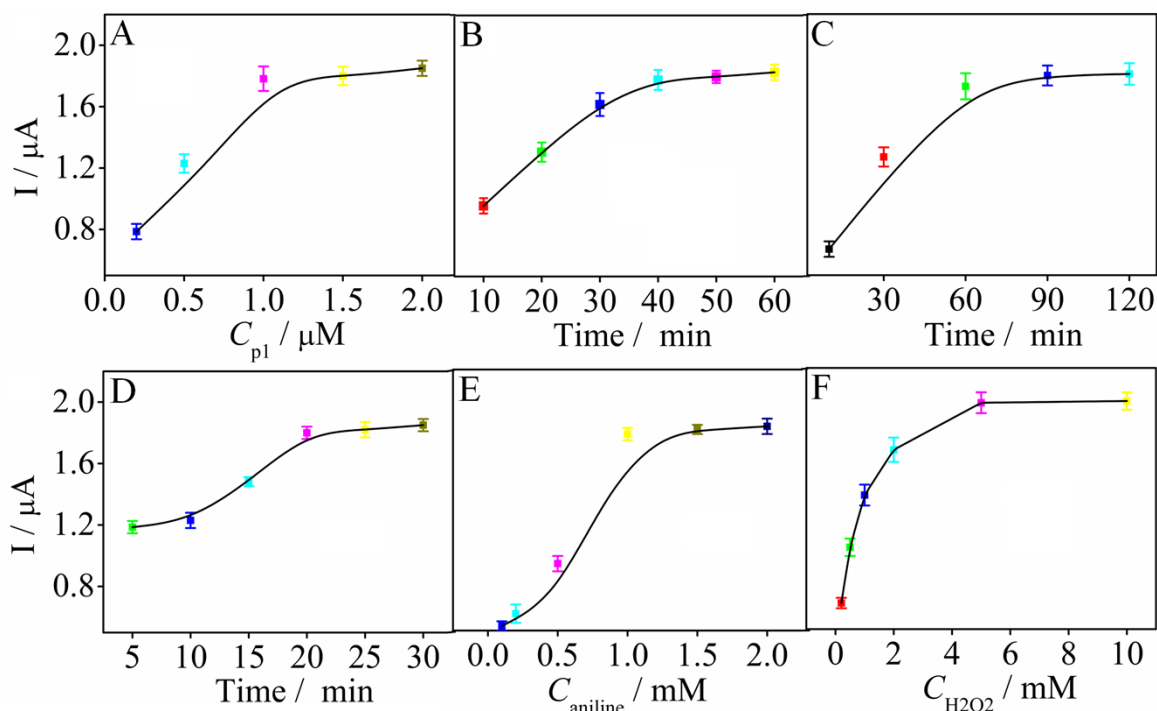
**Figure S3.** (A) Cyclic voltammograms of the composite film modified electrode in 0.1M HAc–NaAc solution (pH 4.3) solution at various scan rates (from a to f): 10, 20, 40, 60, 80, and 100  $\text{mV} \cdot \text{s}^{-1}$ . (B) Plots of peak currents versus scan rates.



**Figure S4.** FTIR spectrum of the deposited PANI. The bands at  $1558\text{ cm}^{-1}$  is characteristic absorption bands arising from the vibration mode of both ring stretching of the quinoid diimine. The bands near  $1416\text{ cm}^{-1}$  is the weak C–N stretching vibration, and the band at  $1342\text{ cm}^{-1}$  peak is assigned to C–N stretching of secondary aromatic amines. The C–H out-of-plane bending located at  $816\text{ cm}^{-1}$  in both spectra is due to a para-substitution pattern, indicating a head-to-tail coupling of aniline occurs during the polymerization

In order to obtain good assay results, some factors that may influence the performances of the electrochemical cytosensor are investigated in detail. The performances of the cytosensor fabricated under different conditions (such as probe concentration, incubation time, enzymatic reaction concentration and time) were studied under the same concentration of  $1 \times 10^5$  cells/mL MCF-7 cells. The surface density of the assembled hairpin-aptamer probe is the first issue that can be controlled by changing the probe P1 concentration during the assembling of the cytosensor sensing units. The change of SWV peak current was used to characterize the change of the above factors. It was observed that the peak current increased with the increase of surface density with the P1 concentration range from 0.2 to  $1.0\text{ }\mu\text{M}$ . After which, the peak currents level off (Figure S5(A)). Hence,  $1.0\text{ }\mu\text{M}$  hairpin-aptamer probe P1 was chosen as the optimal concentration for the hairpin-aptamer assembling. Meanwhile, 40 min of the assembling time of hairpin-aptamer probe P1 on the Au electrode surface was used in the standard procedures (Figure S5(B)). In order to evaluate the kinetic binding between aptamer and cells, the recognition time was investigated and the results was shown in Figure S5(C). It can be seen that the current elevates with increasing incubation time and starts to level off

after 1 h. Thus, 1 h was used as the optimum recognition time. In addition, the effect of PANI deposition time on the cytosensor was investigated. As expected in Figure S5(D), the longer was the deposition time, the higher was the peak current, with the maximum signal achieved after 20 min. Therefore, a deposition time of 20 min was selected. The effects of aniline concentration as well as the  $\text{H}_2\text{O}_2$  concentration for enzymatic deposition of PANI were studied by varying the aniline concentration the  $\text{H}_2\text{O}_2$  concentration. As illustrated in Figure S5(E and F), 20.0 mM of aniline and 5.0 mM of  $\text{H}_2\text{O}_2$  were therefore used as the optimum concentrations for subsequent experiments.



**Figure S5.** Influence of (A) hairpin-aptamer P1 concentration, (B) assembling time, and (C) aptamer-cell incubation time on the response of the cytosensor; Influence of D) deposition time, E) aniline concentration, and F)  $\text{H}_2\text{O}_2$  concentration on the response of the cytosensor.