Electronic Supplementary Information (ESI)

for

Proximity-Dependent Surface Hybridization Strategy for Constructing Efficient Signal-On Electrochemical DNAzyme Sensing System

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1. Experimental Details

Reagents and Apparatus. All oligonucleotides were purchased from Takara Biotechnology Co., Ltd. (Dalian, China), and their sequences are listed in Table S1. 1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC), N-Hydroxysulfosuccinimide (Sulfo-NHS), L-histidine and ferrocenecarboxylic acid (Fc) were purchased from Sigma Aldrich Chemical Co. All chemicals were of analytical grade unless otherwise specified. All solutions were prepared in Milli-Q water (resistance >18 MΩ·cm) from a Millipore system. Electrochemical experiments were carried at CHI 760B electrochemical workstation (Shanghai, China). A normal three-electrode configuration was involved, which was comprised of a saturated calomel electrode (SCE), a working electrode, and a platinum counter electrode. The differential pulse voltammogram (DPV) measurements were performed in 5 mL of 0.1M $KClO_4$ solution and the DPV curves scanned from 0 to 0.5 V. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter.

Table S1. Sequences of oligonucleotides used in this work.[a]

Oligonucleotide	Sequences (from 5′ to 3′)
uDNAzyme	AGATCATGTGACGGAGGACATrAGGAAGAGATGTTTTTTTTTT CATCTCTTAACGGGGCTGTGCGGCTAGGAAGTAATGTCCTCC
Capture DNA(DNA3)	ATGTCCTCCTAGTGAC-$(CH_2)_6$-SH
Reporter DNA(DNA2)	NH_2-$(CH_2)_6$-GTCACTA AAGTCACATGATCT
DNA1	AGATCATGTGACGGAGGACATrA

The underlined sequences in DNAzyme sequence represent the linker between the enzyme and substrate strand, and rA denotes adenosine ribonucleotide at that position while all others are deoxyribonucleotides.

Preparation of Ferrocene-Labeled Oligonucleotides. The Fc-conjugated Oligonucleotides was prepared according to the literature method with a minor modification.[1] Briefly, 2 mg of Fc monocarboxylic acid was added to 1 mL of 10 mM PBS (pH=7.4) buffer containing EDC/sulfo-NHS

(0.1M each) solution under stirring. Afterward, 100 mL of 10 μM reporter DNA (dissolved in water) was injected, the resulting solution was stirred at room temperature overnight. Subsequently, the mixture (Fc-conjugated Oligonucleotides solution) was stored in refrigerator at 4°C until use.

Fabrication of sensing interface. The gold electrode (~2 mm diameter) was polished with a 0.05-μm alumina powder, followed by sonication in ethanol and water for 5 min, respectively. Then, the gold electrode was incubated with piranha solution (H_2SO_4/H_2O_2, 7:3 by volume) for 20 min. Finally, the electrode was washed with distilled water and dried in a nitrogen stream.

The fabrication of electrochemical sensing interface was accomplished as follows: Firstly, 10 μL of 1 μM capture probe was dropped on the pretreated gold electrode for 2 hours at room temperature. Then, the modified electrode was treated with 1 mM mercaptohexanol (MCH) for 30min to cover the nonspecific sites and to optimize the orientation of the capture probes to make hybridization easier.

Electrochemical Detection. A 3.5 μL droplet of uDNAzyme solution (1 μM) was mixed with 3.5 μL of L-histidine at different concentration, and the incubation was kept for 10 min at 30 °C. Then, 3 μL of Fc-conjugated oligonucleotides was injected, followed by incubation for another 20 min. The resulting solution was dropped onto the capture probe modified gold electrode for 1 h at 30°C. Subsequently, the treated electrode surface was washed with buffered solution prior to electrochemical measurements. The peak current recorded in ~0.213 V was then performed to estimate the analytical performances of the proposed sensing system.

Kinetic Fluorescence Measurement. To investigate the reaction kinetics of the DNAzyme, 200 nM of uDNAzyme was first incubated with 1×SYBR I at room temperature for 30 min in a buffer solution containing 25 mM HEPES (pH 7.4) and 500 mM KCl. A 95 μL aliquot of the sample was transferred into a quartz fluorescence cell, which was then placed in the fluorometer. The detection was carried out in kinetics mode. The excitation and emission were set at 494 nm and 525 nm respectively. After the initial reading, the quartz cell was taken out, and a 5 μL of L-histidine stock solution was added to induce the DNAzyme catalyzed cleavage reaction. The final concentration of L-histidine was 5 mM. After vortexing to mix components thoroughly, the cell was quickly put back into the fluorometer to

continue the kinetics measurement. Fluorescence spectra were measured using a Hitachi F-4500 fluorescence spectrometer (Hitachi, Japan) with both excitation and emission slit set at 5.0 nm.

Agarose gel electrophoretic analysis. To confirm the formation of the ternary "Y" junction structure, four samples are prepared for agarose gel electrophoresis. They are composed of (1) 2μl DNA1 (10μM); (2) 2μl DNA2 (10μM); (3) 2μl DNA3 (10μM); (4)DNA1, DNA2 and DNA3 (each 2μl, 10μM). After incubation at 30ºC for 2 hours, the sample was applied to an agarose gel (3% agarose). The electrophoresis was carried out in 1×Tris-borate-EDTA (TBE) buffer (90 mM Tris, 90 mM boric acid, and 10 mM EDTA, pH 8.0) at 100 V for 40 mim. The gels were Gold View staining. The resulting gel was excited using a WD-9403F UV device and imaged with a Canon digital camera.

To investigate the L-histidine-dependent catalyzing capability for substrate-hydrolysis of DNAzyme, five samples are prepared for agarose gel electrophoresis. They are composed of (1) uDNAzyme and buffer; (2) uDNAzyme and 10 mM mixed other amino acids (cysteine/ lysine/phenylalanine/arginine); (3) uDNAzyme and 5 mM L-histidine; (4) uDNAzyme and 200 μM L-histidine; (5) uDNAzyme and 50 μM L-histidine. A 2-μL droplet of uDNAzyme solution (10 μM) was incubated with 2 μL of target L-histidine. When performing the control experiments, the corresponding buffers or mixed other amino acids (cysteine/ lysine/phenylalanine/arginine) are used instead of the reagent solutions. After incubation at 30ºC for 0.5 hours, the sample was applied to an agarose gel (3% agarose). The electrophoresis was carried out in 1×Tris-borate-EDTA (TBE) buffer (90 mM Tris, 90 mM boric acid, and 10 mM EDTA, pH 8.0) at 100 V for 1 h.

Preparation of cellular homogenate. The lung caner A-549 cells (5000 cells) were first centrifuged for 5 min at 25 °C (1000 rpm) with the supernatant being removed, and the cells precipitate was redispersed in 25mM HEPES (pH 7.4, 0.5M KCl). Then, the redispersed cells were subjected to a sonication treatment (20 min with 4 s on and 8 s off) in an ice-water bath using a probe-type sonicator (200 W). Finally, the resulting cellular homogenate was stored at 4 °C.

2. Supplementary figures

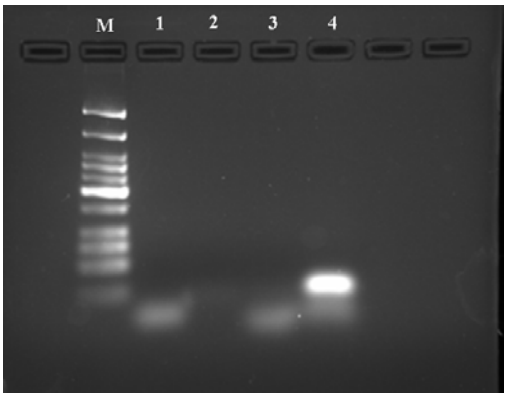

Figure S1. Agarose gel electrophoresis of the sensing system under different conditions: lane M: DNA marker; lane 1: DNA1 only; lane 2: DNA2 only; lane 3: DNA3 only; lane 4: DNA1, DNA2 and DNA3 incubated for 2 h.

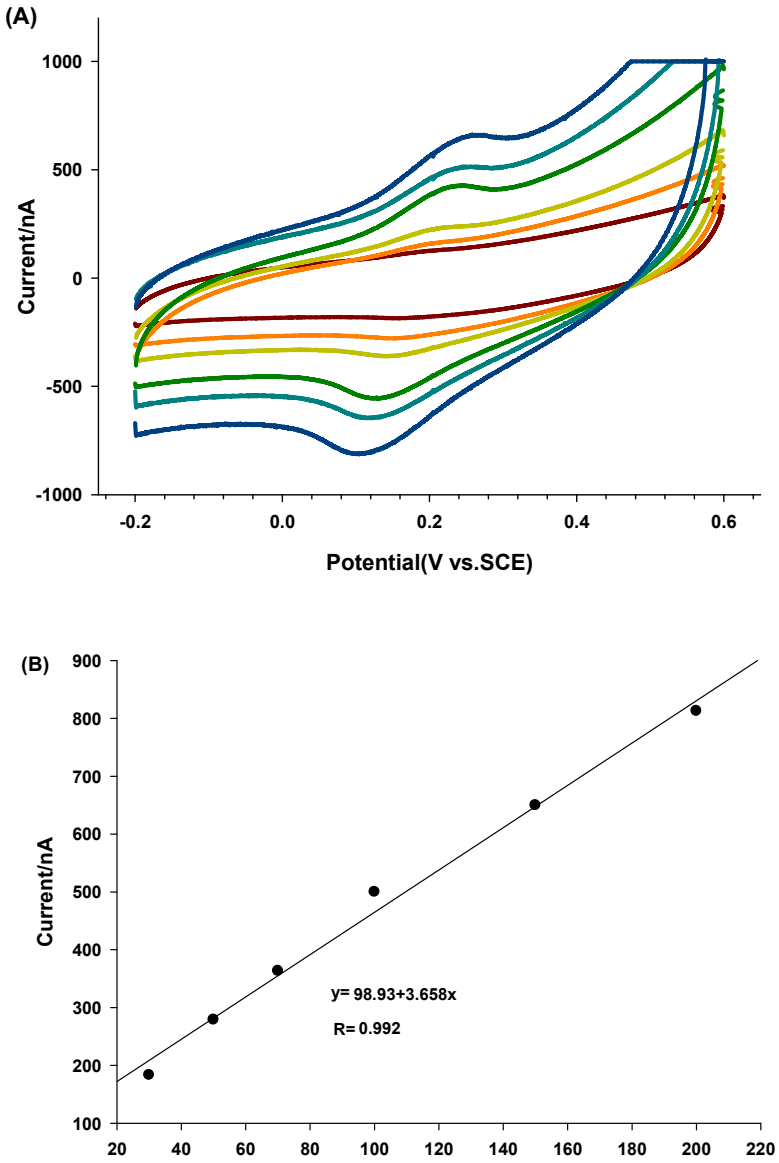

Figure S2. Cyclic voltammograms of the modified gold electrode at various scan rates, 30, 50, 70, 100, 150, 200 mV/s after addition of 5 mM L-histidine. (B) The plots of oxidation peak currents vs scan rate.

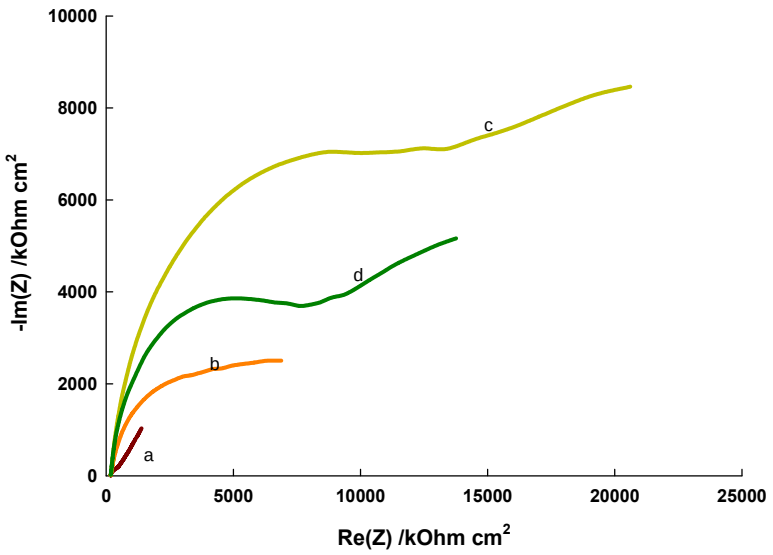

Figure S3. Nyquist diagrams of electrochemical impedance for the different modification stages: (a) bare gold electrode; (b) the thiolated DNA (capture probe) modified gold electrode; (c) capture probe/MCH modified gold electrode; (d) DNAzyme cleavage products (probe A)/ferrocene-conjugated detection probe hybridized gold electrode. Electrochemical measurements were carried out in 10 mM PBS (pH 7.4) containing 0.1M KCl and 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a redox probe.

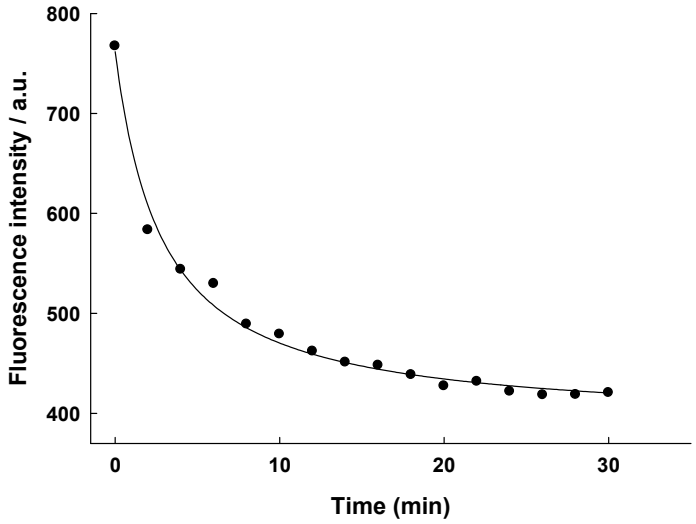

Figure S4. The kinetics of fluorescence decrease for the sensing system induced by 5 mM L-histidine.

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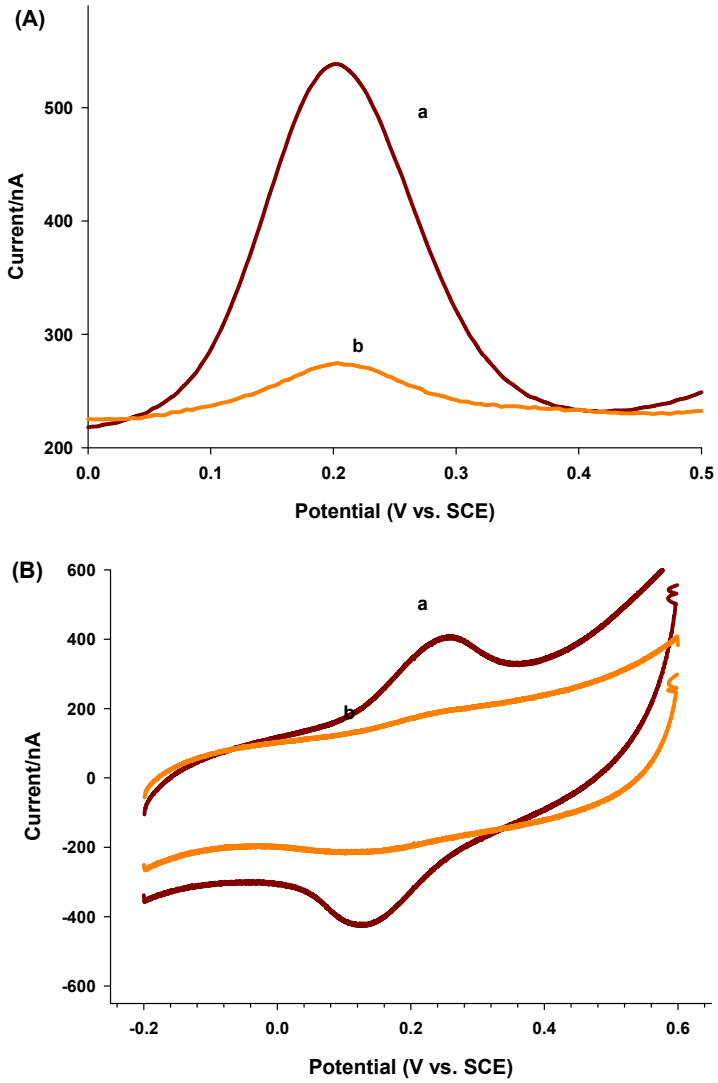

Figure S5. Electrochemical responses correspond to sensing systems recorded in 0.1 M $KClO_4$. (A) Typical DPV curves of the sensor in response to 5 mM (line a) and 0 mM (line b) L-histidine, respectively. (B) The cyclic voltammograms a and b correspond to lines a and b in (A), respectively. Potential scanning rate is 100 mV/s.

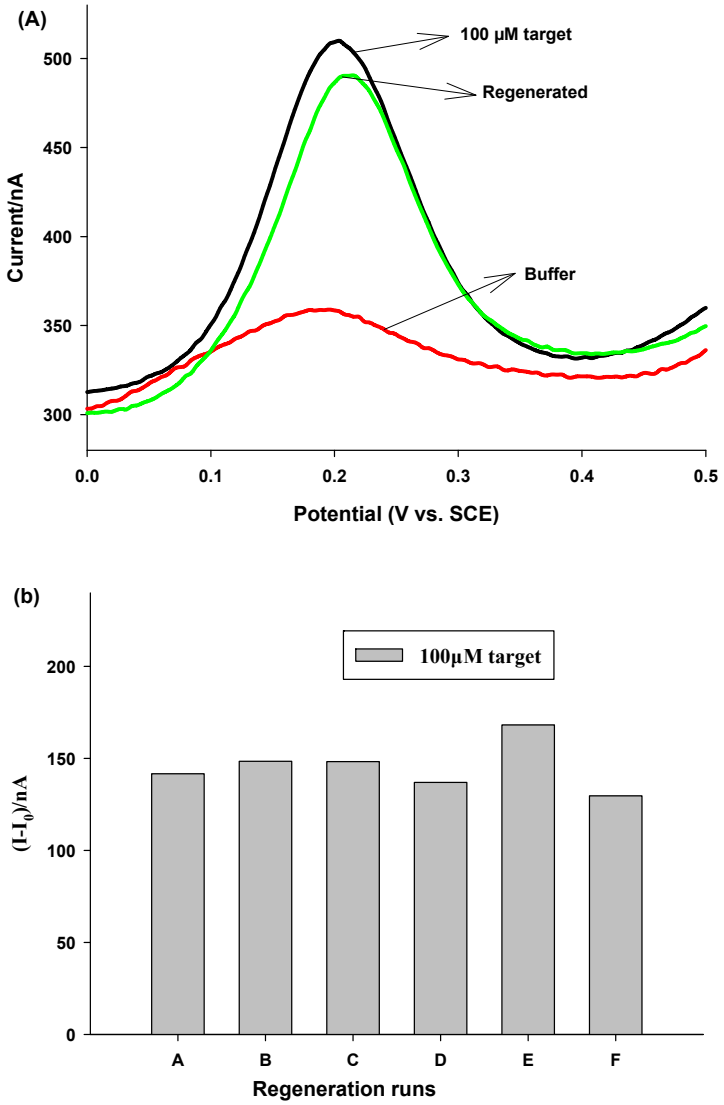

Figure S6. Typical DPV curves of the sensor in response to 100 μM and 0 μM L-histidine, and the current response after regenerated (A). The sensor shows no significant degradation after 6 cycles of use and regeneration (B). I and I_0 represented the DPV peak current in the presence of and absence of 100 μM target.

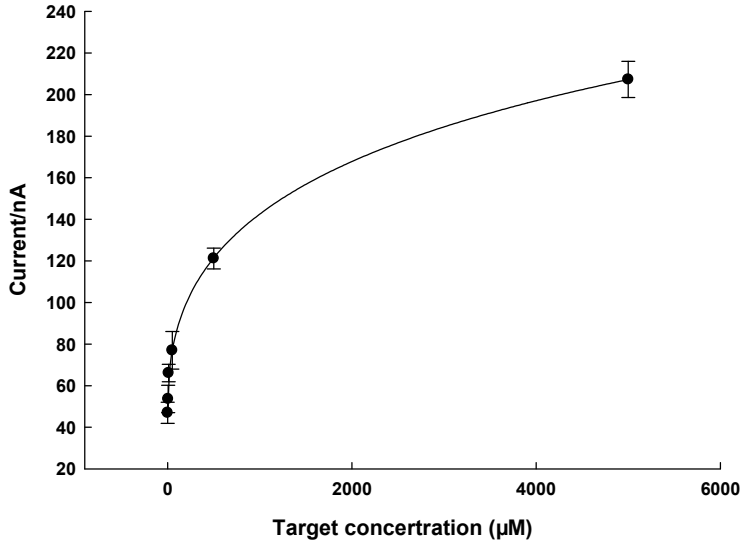

Figure S7. DPV peak currents for different L-histidine concentrations in cellular homogenate samples.

Reference:

1. Fahlman, R. P.; Sen, D. *J. Am. Chem. Soc.* **2002**, *124*, 4610–4616.