Supporting information for

Electrochemical Analysis of Two Analytes Based on a Dual-Functional Aptamer DNA Sequence

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Chemicals. All oligonucletides designed according to the literature in the present study were purchased from SBS Genetech Co., Ltd. (Beijing, China), and their sequences were as follows. Dual-aptamer sequence (S_1): 5'-ACC TGG GGG AGT ATT GCG GAG GAA GGT GGT TGG TGT GGT TGG-$(CH_2)_6$-SH-3'; strand complementary to adenosine aptamer (S_2): 5'-ACT CCC CCA GGT-$(CH_2)_6$-SH-3'; secondary thrombin aptamer sequence (S_3): 5'-AGT CCG TGG TAG GGC AGG TTG GGG TGA CT-$(CH_2)_6$-SH-3'; thiolated signal probe loaded on AuNP (S_4): 5'-SH-$(CH_2)_6$-TTT TTT TT-NH_2-3'.

Adenosine, cytidine, uridine, thrombin, bovine serum albumin (BSA) and lysozyme were from Sigma (St. Louis, MO), and used without further purification. 6-Mercapto-1-hexanol (MCH), Tri(2-carboxyethyl)phosphine hydrochloride (TCEP, 98%) was purchased from Alfa Aesar (MA, USA). Other chemicals employed were all of analytical grade and triple distilled water was used throughout. The human plasma samples were provided by the Medical School Hospital of Qingdao University (Qingdao, China).

Instrumentation. All electrochemical measurements were conducted in a standard electrochemical cell using an electrochemical analyzer (CHI660C, CH Instruments, USA). The three-electrode system used consisted of the working electrode of interest, a Pt-wire electrode as the counter electrode, and an Ag/AgCl reference electrode. Anodic stripping voltammetry was conducted with an *in situ* formed mercury film electrode (on a glassy carbon surface) generated as working electrode, by accumulation for 200 sec at -1.4 V. After standed for 15 sec, the stripping voltammetry was carried out between -1.0 V and -0.1 V using anodic stripping voltammetry. Faradaic impedance spectroscopy (FIS) was carried out in a degassed PBS buffer (0.1 M , pH 7.4) containing 0.1 M NaCl and 2.5 mM $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$.

Preparaton of AuNPs. Gold nanoparticles were prepared by citrate reduction of $HAuCl_4$ according to the literature.[1] Briefly, 10 mL of 38.8 mM sodium citrate was immediately added to 100 mL of 1.0 mM $HAuCl_4$ refluxing solution under stirring, and the mixture was kept boiling for another 15 min. The solution color turned to a wine red, and was cooled to room temperature with continuous stirring.

Preparation of Metal Sulfide Nanoparticles. Nanoparticles of lead sulfide and cadmium sulfide were prepared according to the reference.[2] A 2 μL of mercaptoacetic acid was added to 100 mL of 1 mM of each of the metal chloride solution under vigorous stirring, and the pH was

adjusted to 11 with 0.5 M NaOH. The solution was bubbled with nitrogen for 30 min, and 50 mL of 1.34 mM Na_2S was added dropwise to the solution. The reaction was kept for 24 h under bubbling nitrogen.

Functionalization of AuNPs with signal and reporter DNA probes. The mixture of 6.3×10^{-10} mol of S_4 and 3.1×10^{-11} mol S_2 or S_3 was activated with acetate buffer (pH 5.2) and 5 μL of 10 mM TCEP for 1 h, and then added to 2 mL of freshly prepared AuNPs and shaken gently overnight. After reaction of 16 h, the DNA-AuNP conjugates were aged in salts (0.01 M NaCl, 5.0 mM tris-acetate buffer) for another 40 h. Excess reagents were removed by centrifuging at 15,000 rpm for 30 min. The red precipitate was washed, recentrifuged, and dispersed in 1 mL of buffer containing 300 mM NaCl, 25 mM tris(hydroxymethyl)aminomethane (Tris) acetate, pH 8.2.

Preparation of Bio-Bar-Coded AuNPs Labeled with Metal Sulfide Nanoparticles. Preparation of bio-bar-coded AuNPs containing metal sulfide nanoparticles was performed according to our previous report.[3] A 200-μL of 0.1 M imidazole solution (pH 6.8) was added to 1.0 mL of the prepared oligonucleotide-functionlized AuNP solution. After 30 min, 100 μL of 0.1 M EDC solution and 2.0 mL of PbS or CdS colloid were added to the mixture and incubated at room temperature for 12 h with gentle shaking. Excess reagents were removed by centrifugation at 10,000 rpm for 30 min. The precipitate was washed and then dispersed into water. The solution of bio-bar-coded gold nanoparticles containing PbS or CdS NPs was stored at 4 °C for the hybridizations.

Dual-Aptamer DNA immobilization and hybridization on Gold Electrodes. The gold electrode was polished with a 0.05-μm alumina powder and soaked in an ultrasonic bath successively with distilled water, absolute alcohol, and distilled water for 5 min each. The gold electrode was dipped in piranha solution (H_2SO_4/H_2O_2, 7:3 V/V) for 5 min at 90 ºC and electrochemically treated by cycling the potential between +0.1 and +1.5 V in 0.1 M H_2SO_4 until a stable gold oxide cyclic voltammogram was obtained. The pretreated electrodes were immersed into the $HAuCl_4$ (6.0 mM) solution containing 0.1 M KNO_3, where electrochemical deposition was conducted at -400 mV by single potential mode.

Then the gold electrode was immersed into an immobilization buffer (IB, 20 mM Tris-HCl + 0.1 M NaCl + 5.0 mM $MgCl_2$ at pH 7.4) containing 2.5×10^{-8} M of the dual-aptamer DNA for 12 h. The DNA-modified electrodes were further treated with 1 mM MCH for 1 h to obtain well aligned

DNA monolayers, followed by washing with triple distilled water to remove unspecific adsorbed DNA. Then, it was immersed into Tris-HCl solution containing AuNP labeled with S_2 and S_4 for a desired time at 37 °C. After hybridization, electrode was extensively rinsed with washing buffer (10 mM Tris-HCl, pH 7.0).

Simultaneous Electrochemical Detection of Adenosine and Thrombin. For the detection procedure, the sensing interface was immerged into IB buffer containing of various concentrations of adenosine and thrombin. The electrode was rinsed with 10 mM Tris-HCl buffer (pH 7.0) and then hybridized with the secondary thrombin aptamer loaded on AuNPs for 1 h at room temperature. Then, the metal sulfide nanoparticles released into the sample solution and remained on the surface were dissolved by treatment of the gold substrates with a solution of 135 μL 2.0 M HNO_3 and 200 μL 1.0 M HNO_3 for 20 min, respectively. The solution containing the dissolved quantum dots were transferred into 2 mL of acetate buffer (0.10 M, pH=5.3) containing 0.01 g/L of mercury ions and ASV was conducted.

Plasma Samples Treatment. Plasma samples were pretreated as with salt solution (2 M ammonium sulfate and 0.1 M NaCl) to avoid the formation of fibrin and the rapid sample clotting according to the previous reports.[4,5] The blood and stop solution mixture was then immediately centrifuged for 5 min at 10000 rpm at 4 ºC. The plasma was removed and frozen at -70 ºC until used. Different concentrations of standard solutions of adenosine and thrombin were spiked into the pretreated plasma. Adenosine and thrombin were measured after appropriate dilution and was calculated from the measured concentrations after correction for dilution.

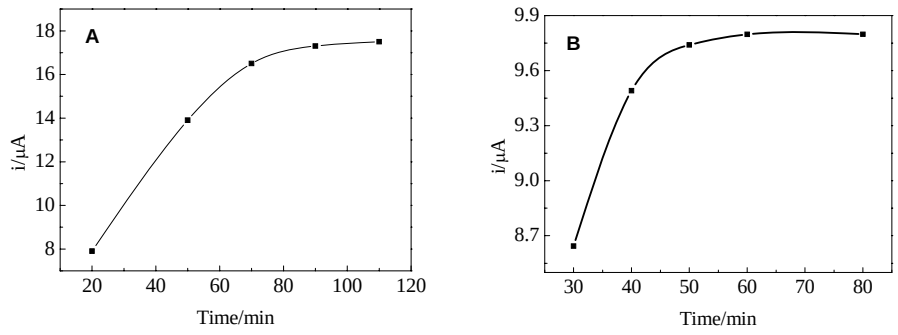

Figure S1. Time-dependent ASV signal response for adenosine (A) and thrombin (B) detection.

The assembly of oligonucleotides on electrodes and the formation of double-stranded DNA on the support can be followed by Faradaic impedance spectroscopy (FIS).[6] Compared with bare Au electrode, the charge-transfer resistance (*R*ct) of probe DNA-modified Au electrode was increased, due to the immobilization of negatively charged ODN probes on the electrode surface resulting in a negatively charged interface that electrostatically repels the negatively charged redox probe $[Fe(CN)_6]^{3-/4-}$ and inhibits interfacial charge-transfer.[7] After hybridization with S_2 loaded on the AuNPs, the value of *R*ct was further increased. After treatment of adenosine and thrombin, the interfacial electron resistance increased remarkably. Thrombin is a bulky protein with negative charges, which makes the density of negative charge on the electrode increase. Accordingly, the FIS signal was increased. Subsequently, the interaction with thrombin secondary aptamer loaded on AuNPs induced an increase, due to the negative charge of DNA.

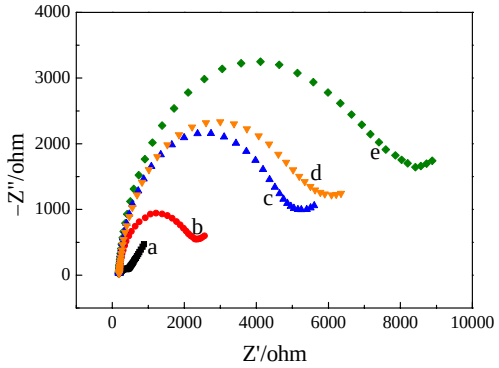

Figure S2. Faradaic impedance spectra corresponding to Au electrodes (a), after immobilization of dual-aptamer DNA (b), hybridization with S_2 and bio-bar-coded AuNPs (c), after treatment with 1.0×10^{-10} M of adenosine and 1.0×10^{-11} M of thrombin (d), introduction of secondary thrombin aptamer and bio-bar-coded AuNPs (e). The data were recorded in the presence of 2.5 mM $[Fe(CN)_6]^{3-/4-}$ as redox label, and upon application of the biasing potential 0.21 V, applying 5 mV alternative voltage in the frequency range of 50 mHz to 10 kHz.

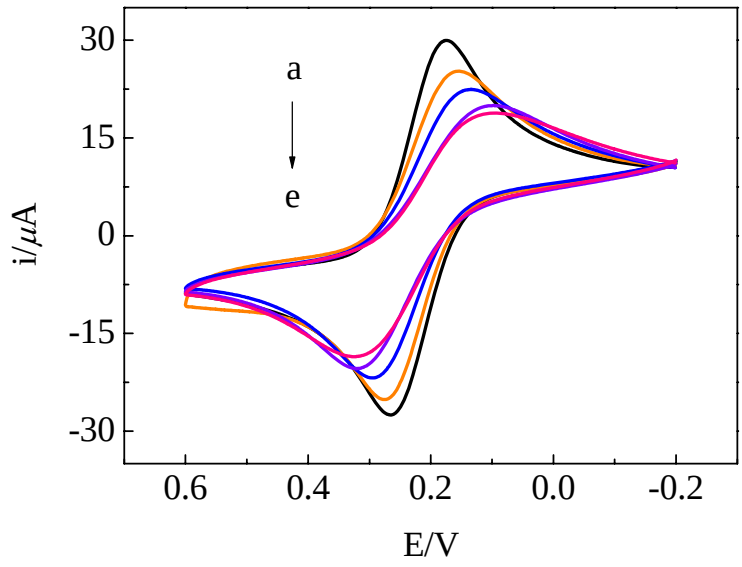

Figure S3. CVs corresponding to Au electrodes (a), after immobilization of dual-aptamer DNA (b), hybridization with S_2 and bio-bar-coded AuNPs (c), after treatment with 1.0×10^{-10} M of adenosine and 1.0×10^{-11} M of thrombin (d), introduction of secondary thrombin aptamer and bio-bar-coded AuNPs (e). The data were recorded in the presence of 2.5 mM $[Fe(CN)_6]^{3-/4-}$ as redox label, with a scan rate of 100 mV s^{-1}.

References:

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