Supplementary Information

Chemiluminescence thrombin aptasensor using high-activity DNAzyme as catalytic label

Tao Li, Erkang Wang and Shaojun Dong*

State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin, 130022, China, and Graduate School of the Chinese Academy of Sciences, Beijing, 100039, China.

Experimental Details

Materials. Three DNA sequences (**1**, 5' $HS(CH_2)_6$ TTT TTT TTT TTT TTT TTT TTG GTT GGT GTG GTT GG 3'; **2**, 5' GTG GGT AGG GCG GGT TGG TTT TTT TTT TTT GGT TGG TGT GGT TGG 3'; **3**, 5' AGT CCG TGG GTA GGG CGG GTT GGG GGT GAC TGT CCG TGG TAG GGC AGG TTG GGG TGA C 3'), and hemin were purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). Luminol were purchased from Sigma-Aldrich (St. Louis, MO, USA). 30% H_2O_2 was purchased from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). The stock solution of hemin (5 mM) was prepared in DMSO, stored in the dark at –20 ℃, and diluted to the required concentration with the HEPES buffer (25 mM HEPES, pH 7.4, 20 mM KCl, 200 mM NaCl, 0.05% (w/v) Triton X-100, 1% (v/v) DMSO). The stock solutions of luminol (50 mM) and H_2O_2 (300 mM) were prepared in 0.1 M NaOH and pure water, respectively. 18 MΩ water purified by a Milli-Q system (Millipore, Bedford, MA, USA) was used throughout.

Instrumentation. A Model MCDR-A Chemiluminescence Analyzer System (Xi'An Remax Analytical Instrument Co. Ltd., Xi'An, China) was used to record chemiluminescence (CL) emission. The CV experiments were performed with a Model 800 Electrochemical Analyzer (CHI, Shanghai, China).

Preparation of G-quadruplex-based DNAzymes. The DNAzymes were prepared as described in our previous work (*Chem. Commun.* 2007, 4209-4211) with a little modification. Briefly, 5 OD of DNAs were prepared in the TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4), and heated at 88 ℃ for 10 min to dissociate any intermolecular interaction, then gradually cooled to room temperature. An equal volume of the hybridization buffer (50 mM HEPES, pH 7.4, 40 mM KCl, 400 mM NaCl, 0.1% Triton X-100, 2% DMSO) was added to the DNA solutions, and allowed DNA sequences to fold for 40 min at room temperature. Then, an equal volume of hemin solution of same molar concentration was added, and incubated at room temperature for over 2 h to form the corresponding G-quadruplex-based DNAzymes.

Fabrication of sandwich thrombin aptasensors. (I) Before use, 1.2 mm-diameter gold disk electrodes were electrochemically cleaned in 0.1 M H_2SO_4, and then characterized by cyclic voltammetry in 10 mM $K_3Fe(CN)_6$. A solution of 1 μM thiolated aptamer **1** was prepared in the binding buffer (20 mM Tris-HCl, pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$). The cleaned substrates were immersed in this DNA solution for 22 h at room temperature to yield a self-assembled monolayer, and rinsed with the buffer, then reacted with 1 mM mercaptoethanol in the binding buffer for 1 h to fill the pinholes in the DNA monolayer. Then, the aptamer-modified substrates were incubated with different concentrations of thrombin in the binding buffer at room temperature for 4 h, and rinsed with the buffer. As described above, the DNA sequence **2** was incubated with hemin to form the G-quadruplex-based DNAzyme. This hybrid system was then introduced onto the **1**-treated electrode surface through the specific interaction between thrombin and aptamers. The whole process of DNA modification and protein loading was monitored by cyclic voltammetry in 10 mM $K_3Fe(CN)_6$. The DNAzyme-modified electrode was quickly rinsed with the HEPES buffer, and dried immediately under a slow N_2 flow, then characterized by using the luminol-H_2O_2 CL method at once.

(II) The DNA sequence **3** was pretreated with hemin as described above to form the corresponding G-quadruplex-based DNAzyme, and then diluted to 1 μM with the HEPES buffer. To this DNA solution was added an equal volume of the binding

buffer. Finally, the aptamer-thrombin-modified substrates were immersed in the DNAzyme solution (0.5 μM) for 4 h, and a thrombin-binding aptamer tethered to the DNAzyme was combined with the second binding site of thrombin. Thus, a monolayer of DNAzyme was introduced onto the substrate surface. The DNAzyme-modified substrates were quickly rinsed with the HEPES buffer, and dried immediately under a slow N_2 flow, then characterized by using the luminol-H_2O_2 CL method at once.

Chemiluminescence analysis. 50 mM luminol was diluted to 0.5 mM with an aqueous buffer consisting of 25 mM HEPES, pH 8.0, 20 mM KCl, and 200 mM NaCl. To 400 μL of 0.5 mM luminol solution was added 40 μL of 300 mM H_2O_2. The sandwich systems were quickly immersed into this mixture. The CL emission within 300 s was recorded by the MCDR-A System.

Characterization of DNA modification and protein loading

Figure S1A shows the characterization of the above self-assemble process by potentiometry. It is observed that the treatment of electrode with DNA, mercaptoethanol and then thrombin all cause obvious current decrease. This indicates the coverage of such monolayers on gold surface, which blocks the electron transfer. According to the previous studies (*Anal. Chem.* 1998, **70**, 4670-4677), the surface density of DNA immobilized on gold substrate can be quantified using chronocoulometry. The coverage rate of the thrombin/DNA/mercaptoethanol monolayer on gold surface can also be estimated using potentiometry and electrochemical impedance spectroscopy (*Anal. Chem.* 2005, **77**, 6320-6323), although the quantitative value cannot be obtained. According to this report, from Figure S1A we estimate that the coverage rate of the thrombin/DNA/mercaptoethanol monolayer on gold surface is about 53%.

The protein loading is also monitored by the CL method (Figure S1B), indicating the specific association of thrombin with TBA immobilized on gold surface.

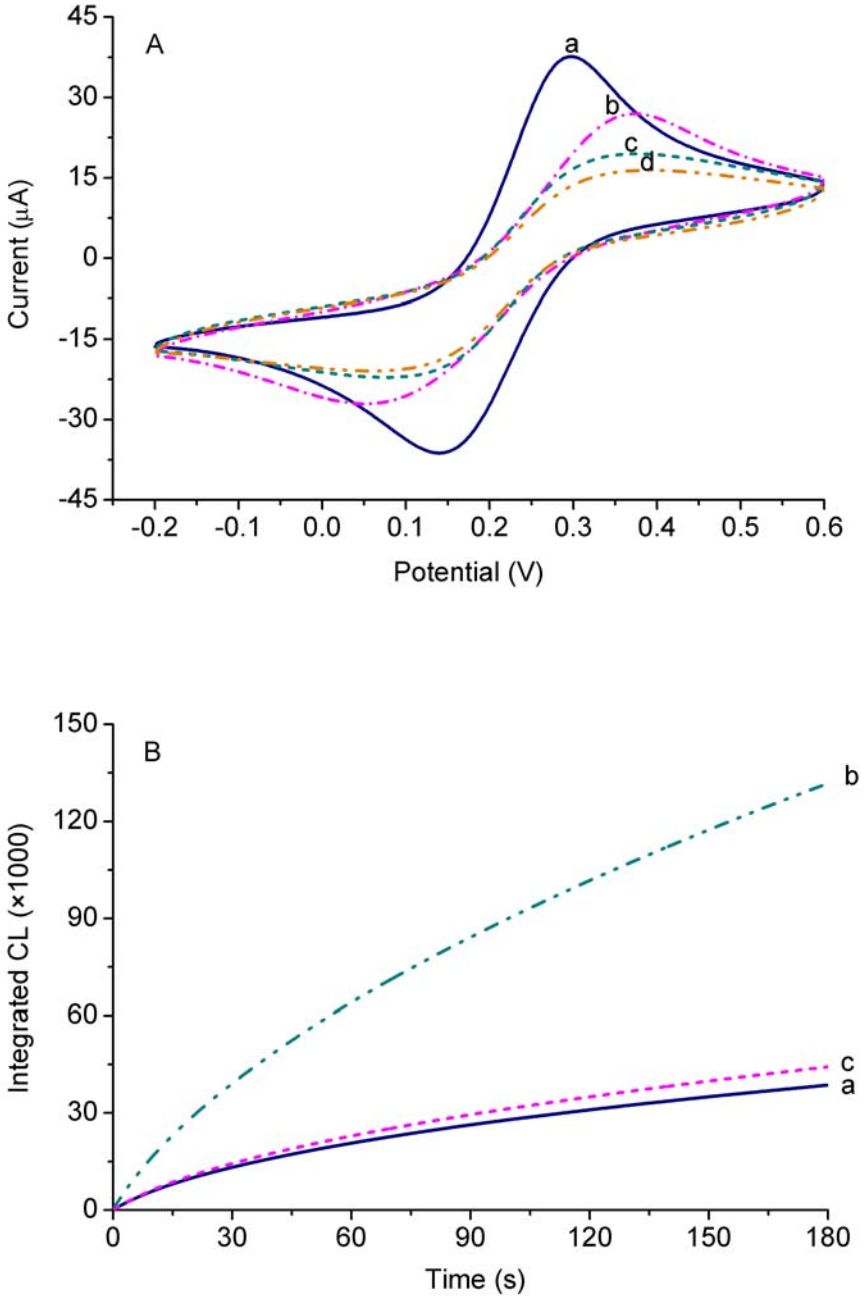

Figure S1. Characterization of DNA modification and protein loading on gold substrates by using cyclic voltammetry and the CL method. (A) Cyclic voltammograms of 10 mM $K_3Fe(CN)_6$ in the binding buffer: a) bare electrode; b) after modified with the thiolated DNA **1**; c) after treated with mercaptoethanol; d) after incubated with thrombin. (B) CL analysis of DNAzyme loading introduced by different proteins in the luminol-H_2O_2 system: a) no protein; b) 1 μM thrombin; c) 1 μM BSA.