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Supporting Information

A novel dual targets simultaneous chemiluminescence signal amplification strategy for enhancing the sensitivity of multiple biomolecules detection

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Experimental section

Materials and reagents

DNA oligonucleotides used in this work were synthesized and purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of these DNA probes (Tb-HP, CEA-HP, Tb-HG4, CEA-HG4) were listed in Table S1. The oligonucleotides were used as provided and diluted in 20 mM Tris-HCl buffer solution (pH 7.4, containing 100 mM NaCl, 20 mM KCl, and 2 mM $MgCl_2$) to give a stock solutions of 10 μM. Each oligonucleotide solution was heated to 95°C for 10 min, and slowly cooled down to room temperature before use. CEA, Tb, streptavidin (SA), 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol), hemin, ethidium bromide (EB), Triton X-100, sodium 4-(2-hydroxyethyl) piperazine-1-ethanesulfonate (HEPES), tris (hydroxymethy-l)amino-methane (Tris) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2), sodium dodecylsulfate (SDS) dimethyl sulfoxide (DMSO) and sodium hydrogen carbonate ($NaHCO_3$) were obtained from Taopu Chemicals (Shanghai, China). The nicking enzyme Nb.BbvCI and 10×NEBuffer 2 (pH 7.9, containing 500 mM NaCl, 100 mM Tris-HCl, 100 mM $MgCl_2$ and 10 mM dithiothreitol) were purchased from the New England Biolabs, Inc. (Ipswich, MA, USA). Agarose G-10 was obtained from Biowest Co. (Spain). All other reagents were of analytical grade, and were used without further purification. The water used through the experiment was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA).

HEPES buffer was 250 mM HEPES-NH_4OH solution (pH 7.4, containing 0.05% (w/v) Triton X-100, 1% (v/v) DMSO, 0.2 M KCl and 2 M NaCl). The electrophoretic buffer was 15 mM borate solution (pH 9.3, containing 5 mM SDS and 1.2 mM luminol).

The oxidizer solution was 35 mM $NaHCO_3$ buffer solution (pH 9.5, containing 110 mM H_2O_2). All solutions were filtered through a 0.45 μm membrane filter before use.

Human blood samples were kindly provided by Guilin No. 5 People's Hospital. Human blood samples were centrifuged at 2000 rpm for 15 min to obtain serum. These samples were stored at −20 °C until analysis.

Apparatus

The MCE–CL detection was performed using a laboratory built system described previously.[1] A home-made glass/PDMS microchip was used in this work, and its schematic layout is illustrated in Figure S1. The channel between reservoir S and SW was used for sampling, the channel between B and BW was used for the separation, and the channel between R and BW was used for the oxidizer introduction. The width of all microchannels is 70 μm (except the width of oxidizer introduction channel is 250 μm). The depth of all microchannels is 25 μm, and the distance between two T-intersections is 60 μm.

Procedure for amplification reaction

First, 5 μL of 0.2 μM CEA-HP, 5 μL of 0.2 μM Tb-HP, 5 μL of 1 μM CEA-HG4, 5 μL of 1 μM Tb-HG4, 5 μL of varying concentrations of CEA, and 5 μL of varying concentrations of Tb solution were mixed, and incubated at 37 °C for 1 h. After that, 4 μL of 10×NEBuffer 2 and 6 μL of 2.5 U/μL Nb.BbvCI were added into above resulting mixture solution, and mixed and incubated at 37 °C for another 2 h. Then, 1 μL of 5 μM SA was added, and incubated at 37 °C for 30 min. After cooling down to room

temperature, the solution was diluted with 48 μL of water and 10 μL of 10×HEPES buffer solution. Subsequently, 1 μL of 10 μM hemin was added to obtain the mixture solution with final volume of 100 μL, and incubated for 1 h at room temperature to form the hemin/G-quadruplex structures. The resulting solution was used for MCE–CL assay.

MCE–CL assay

The procedure for MCE-CL assay was similar to that described previously.[2] Briefly, prior to the MCE separation, the microchannels were rinsed sequentially with 0.1 M NaOH, water, and electrophoretic buffer for 5 min each. Then, the reservoirs B, S, SW and BW in microchip were filled with the electrophoretic buffer, the reservoir R was filled with the oxidizer solution, and vacuum was applied to the reservoir BW in order to fill the separation channel with the electrophoretic buffer. Finally, the electrophoretic buffer in reservoir S was replaced by sample solution. For loading the sample solution, a set of electrical potentials was applied to five reservoirs: reservoir S at 700 V, reservoir B at 250 V, reservoir BW at 350 V, reservoir SW at grounded, and reservoir R floating. The sample solution was transported from reservoir S to SW in a pinched mode. After 20 s, potentials were switched to reservoir B, S, SW, and R at 2800, 1550, 1550, and 900 V, respectively, while reservoir BW was grounded for separation and detection.

References

1 S. Zhao, X. Li and Y. M. Liu, *Anal. Chem*., 2009, **81**, 3873–3878.

2 S. Zhao, Y. Huang, M. Shi and Y. M. Liu, *J. Chromatogr. A,* 2009, **1216**, 5155–5159.

Table S1. Sequences of DNA probe used in this work

Name	Sequences (5'to 3') description
Tb-HP	AGTCCGTGGTAGGGCAGGTTGGGGTGACTGCTGATTTTT TGAGCCTCAGCAGTCACC
Tb-HG4	CCGCCCTTTTAGACTGCTGAGGCTTGGGGTAGGGCGGGT TGGGTTTTTT—Biotin
CEA-HP	CGCATACCAGCTTATTCAATTAAAAA AAAAAAACCCTCA GCTGGTATGCG
CEA-HG4	CCGCCCTTTTATACCAGCTGAGGGGT GGGGTAGGGCGGG TTGGG

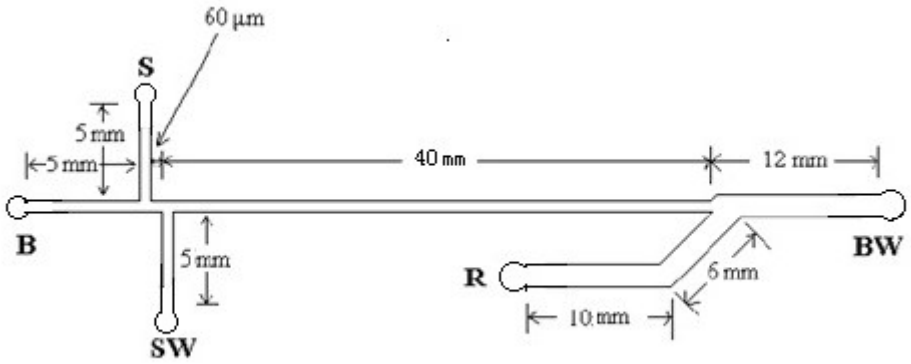

Figure S1. Dimensions and layout of the glass/PDMS microchip. S: sample reservoir; B: buffer reservoir; SW: sample waste reservoir; BW: buffer waste reservoir; R: the oxidizer reagent reservoir.

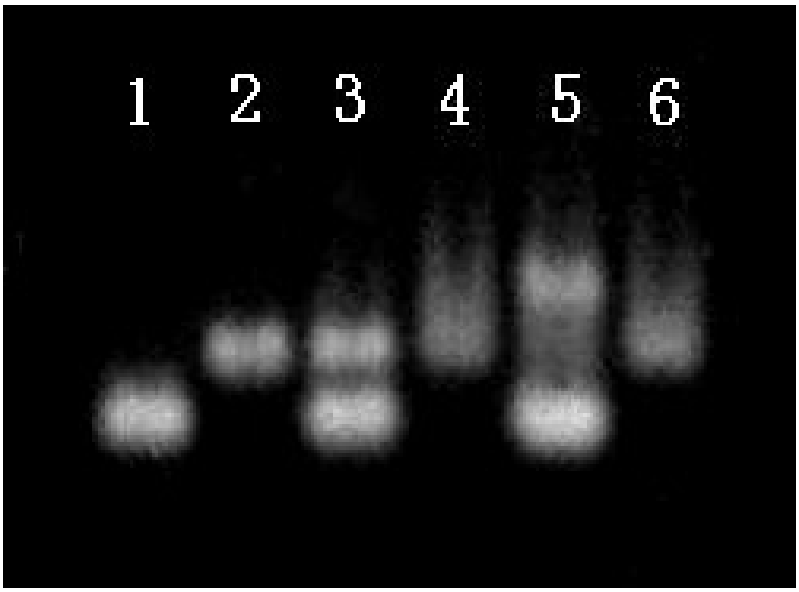

Figure S2. Agarose gel electrophoresis tests: 1. HG4s; 2. HPs; 3. HG4s+HPs+ Nb.BbvCI; 4. Tb+CEA+HPs; 5. Tb+CEA+HPs+HG4s; 6. Tb+CEA+HPs+HG4s+Nb.BbvCI.

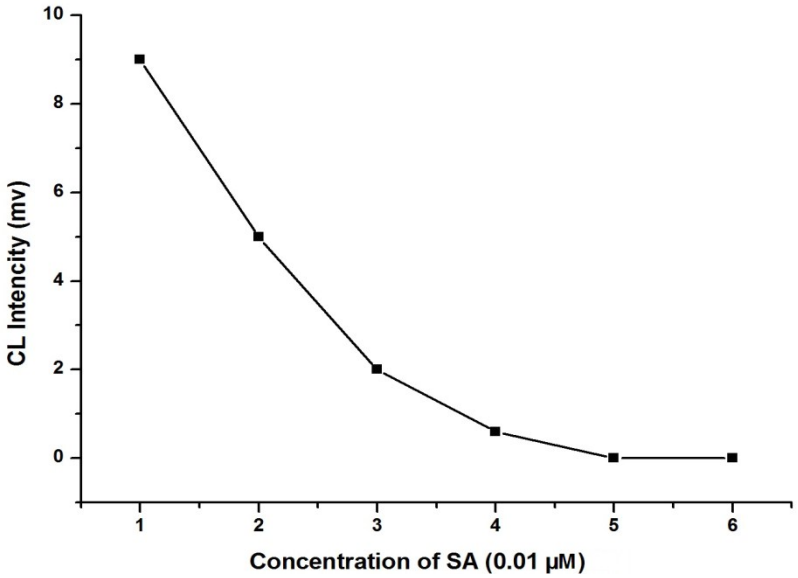

Figure S3. Influence of SA concentration on the formation of G-quadruplex DNAzyme-SA complex.

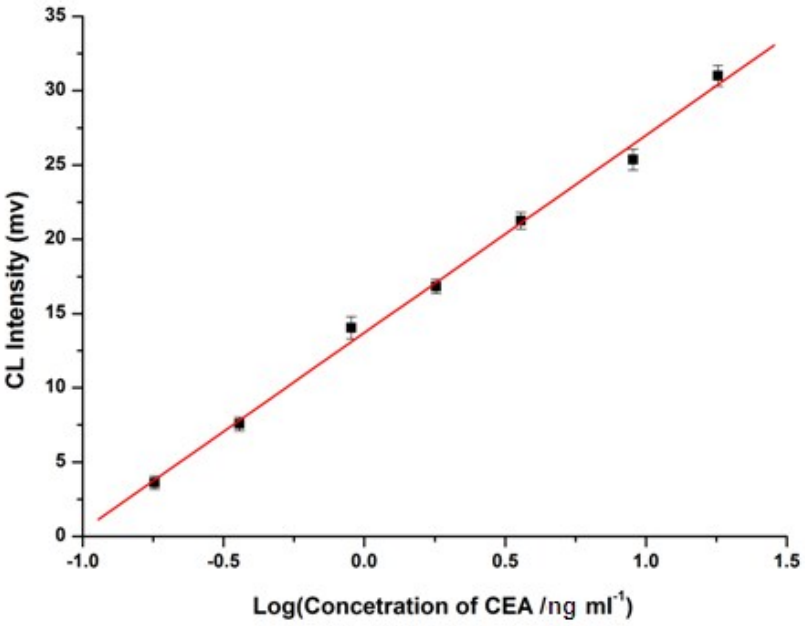

Figure S4. The plot of relative CL intensity versus the logCEA concentration.

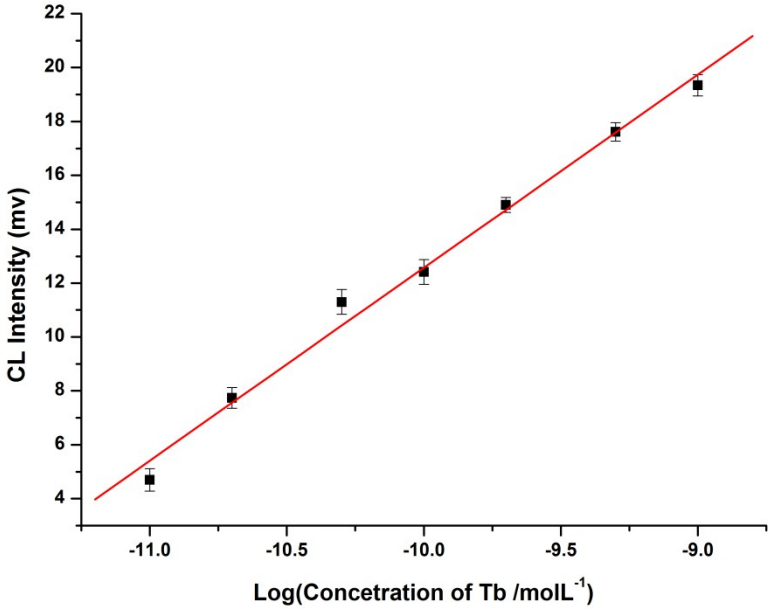

Figure S5. The plot of relative CL intensity versus the logTb concentration.