Supporting Information

Hybridization Chain Reaction-DNAzyme Amplified Switch Microplate Assay for Magnesium Ions

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目录

Experimental section

Materials and reagent. PBS Buffer**,** the Tris(2-carboxyethyl) phosphine (TCEP), DNA Marker 1 (25-500 bp), DNA Marker 2 (50-500 bp) 4SGelblue, 10000X in water, Acryl/Bis 40% Solution (37.5:1), 50X TBE Buffer, 5X TBE Buffer, 6X DNA Loading Dye, TBE buffer, TAE buffer, Premixed Powder (1X), 1,2-bis(dimethylamino)-ethane (TMED), APS (10%) and all DNA sequences were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences were listed in **Table 1.** The chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Beijing Huawei Ruike Chemical Co., Ltd. (Beijing, China), 6-mercapto-1-hexanol (MCH) was purchased from Meryer Chemical Technology Co., Ltd. (Shanghai, China). The 96-well plate coated streptavidin was purchased from Shanghai Muchen Biotechnology Co. Ltd. (Shanghai, China). Calcium chloride ($CaCl_2 \cdot 2H_2O$), potassium ferrocyanide trihydrate ($K_4[Fe(CN)_6]$), potassium ferricyanide ($K_3[Fe(CN)_6]$), Iron(II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$) were ordered from Sinopharm Chemical Reagent Co., Ltd. (China). Sodium phosphate monobasic dihydrate ($NaH_2PO_4 \cdot 2H_2O$), manganese (II) sulfate monohydrate ($MnSO_4 \cdot H_2O$) were obtained from Xilong Chemical Industry Co., Ltd. (Shanghai, China). Zinc Chloride ($ZnCl_2$), Cupric Chloride ($CuCl_2$) were purchased from Shanghai Sinpeuo Fine Chemical Co., Ltd. (Shanghai, China). Potassium chloride (KCl) and Sulfuric acid (H_2SO_4) were ordered from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China). Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) was obtained from Aladdin Biotechnology Co., Ltd. (Shanghai, China), Drinking water, Beer water, Luzhou Laojiao, Multivitamin were bought from local shop. Yiqingtongji Lake water and Tap water were from Nanjing Tech University. ITO-coated glass (sheet resistance 5-7 Ωsp^{-1}; thickness 1.1 mm; size, 100 mm × 100 mm) was purchased from Luoyang Shangzhou technology Co., Ltd. (HeNan, China), respectively. Scotch Magic Tape was bought from Minnesota Mining and Manufacturing Material Technology Co., Ltd. (Suzhou, China). All other chemicals were of analytical grade and were used as received without purification. All water used in this work was RNase-free.

Apparatus. The polyacrylamide gel electrophoresis (PAGE) and agarose gel electrophoresis (AGE) were imaged with a BIO-RAD ChemiDoc XRs (Tanon, China). The electrode surface conductivity and electrochemical signal were determined on CHI660E (Chen-Hua, China). The content Mg^{2+} in actual samples were monitored by iCAP PRO Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (Thermo Fisher, USA).

Optimization of the concentration of H5-MB. Different concentrations of H5-MB (200 nM, 320 nM, 400 nM, 800 nM, 1.6 μM) were combined with the HCR (800 nM) structure for 2 h in a 37 ℃ water bath, respectively, and then terminated for 1 h in a 4 ℃ refrigerator. For validation, a 12 μL mixture was loaded on the gel. After separation,

the gel was stained with Sybr Green I (3 μL) for 10 min and the final gel was photographed by a MINI SPACE 1000 system (Tanon).

Optimization of the Mg^{2+} concentration. In order to explore the optimal concentration of Mg^{2+} for cleavage, the HCR-DNAzyme (H5-FAM) system was incubated with a series of Mg^{2+} concentration (200 μM, 1 mM, 2 mM,4 mM, 6 mM, 8 mM, 10 mM) for 2 h in a 30℃ water bath, respectively. After that, 10 μL reaction product with 2.5 μL 6× loading buffer was loaded onto a freshly prepared 12% native PAGE, electro-phoresis was performed at a constant voltage of 150 V in 1×TBE buffer for 90 min. After separation, the final gel was photographed by a MINI SPACE 1000 system (Tanon) before and after stained with Sybr Green I (3 μL) for 10 min.

Concentration exploration of LDNA conjugated to the bottom of microplates. The assembly of the HCR-DNAzyme structure was carried out after different concentrations of LDNA (0, 50 nM, 100 nM, 200 nM, 500 nM, 1 μM, 2 μM) were placed on the bottom of the microtiter plate and ligated. The supernatant was taken for gel validation.

Electrochemical sensor detection. All electrochemical experiments were performed on a CHI600E electrochemical workstation (CH Instruments Inc.), including cyclic voltammetry (CV), electrochemical impedance spectra (EIS), Amperometric i-t Curve (IT) and differential pulse voltammetry (DPV). Electrochemical experiments were performed on three electrodes system with a bare gold electrode or ITO/Au/CDNA as working electrode, a platinum electrode as the counter electrode, and a saturated calomel reference electrode (SCE) as the reference electrode. The CV used for verification of Au electrode surface modification was scanned at a rate of 100 mV/s from +1 V to +1 V. The DPV used for characterization of methylene blue (MB) signal was scanned from -0.4 V to 0 V. Error bars showed the standard deviation of three individual tests.

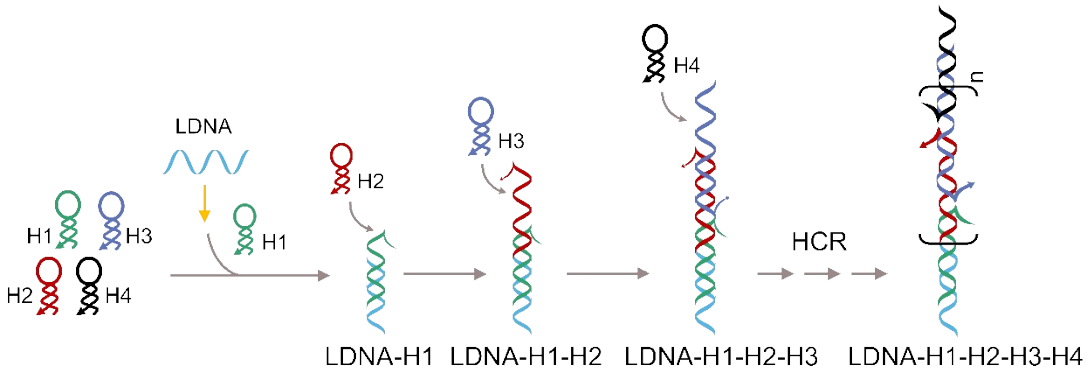
Pre-treatment of ITO electrodes. The ITO electrodes were cut into rectangles of 2 cm × 0.7 cm. They were then cleaned by sonication in acetone, ethanol, and ultrapure water for 10 min and dried under a stream of nitrogen.

Deposition and the optimization of Au film on ITO electrode. Au was deposited on the surface of ITO electrode by constant charge method (potential: 0.5 V vs. Hg/Hg_2Cl_2) in 1 wt % $HAuCl_4$ solution. CV and EIS were employed to study the electrode property when different thickness of Au film were deposited onto the surface of ITO electrode.

Optimization of the CDNA concentration hybridized with $H5_B$-MB. Prior to modification, the thiol-modified CDNA were reduced for 1 h at RT by mixing 200 μL of 1 μM of CDNA with 20 μL of 1 mM TCEP. Different concentrations of CDNA (800 nM, 1.2 μM, 1.6 μM) were hybridized with H5-MB generated after Mg^{2+} cleavage of

HCR-DNAzyme for 2h in a 37 ℃ water bath. Finally, 10 μL reaction product with 2.5 μL 6× loading buffer was loaded onto a freshly prepared 12% native PAGE, electrophoresis was performed at a constant voltage of 150 V in 1×TBE buffer for 90 min. After separation, the final gel was photographed by a MINI SPACE 1000 system (Tanon) before and after stained with Sybr Green I (3 μL) for 10 min.

In addition to this, the amount of CDNA attached to the surface of the Au/ITO electrode was optimized as follows. The cleaned ITO/Au electrode was immersed in TCEP-treated CDNA solution and left in the dark at RT for 2 h. After that, the electrode was washed with ultrapure water. 20 μL of 6-mercapto-1-hexanol (MCH) was sealed on the surface of the electrode for 1 h to occupy the active site, and the electrode was washed with RNase-free water. This electrode was inserted into a Mg^{2+}-cut HCR-DNAzyme microplate and incubated at 37 ℃ for 2 h to complete the capture of H_5B-MB by CDNA. Finally, the electrochemical biosensor signal of CDNA capture of H_5B-MB was detected by DPV method.



Scheme S1. Schematic diagram of HCR structure assembly process.

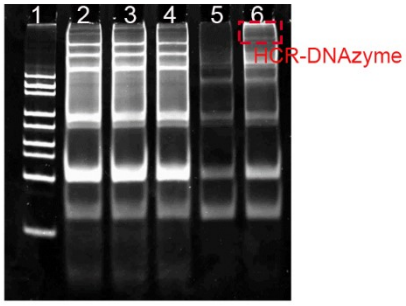


Figure S1. 12% native-PAGE characterization of the construction of HCR with H5-MB of different concentration. Lane 1: Marker 1; Lane 2: LDNA+H1+H2+H3+H4+H5-MB (LDNA, 4 μM; H1-H4, 800 nM; H5-MB, 200 nM); Lane 3: LDNA+H1+H2+H3+H4+H5-MB (LDNA, 4 μM; H1-H4, 800 nM; H5-MB, 320 nM); Lane 4: LDNA+H1+H2+H3+H4+H5 (LDNA, 4 μM; H1-H4, 800 nM; H5-MB, 400 nM); Lane 5: LDNA+H1+H2+H3+H4+H5-MB (LDNA, 4 μM; H1-H4, 800 nM; H5-MB, 800 nM); Lane 6: LDNA+H1+H2+H3+H4+H5 (LDNA, 4 μM; H1-H4, 800 nM; H5-MB, 1.6 μM).

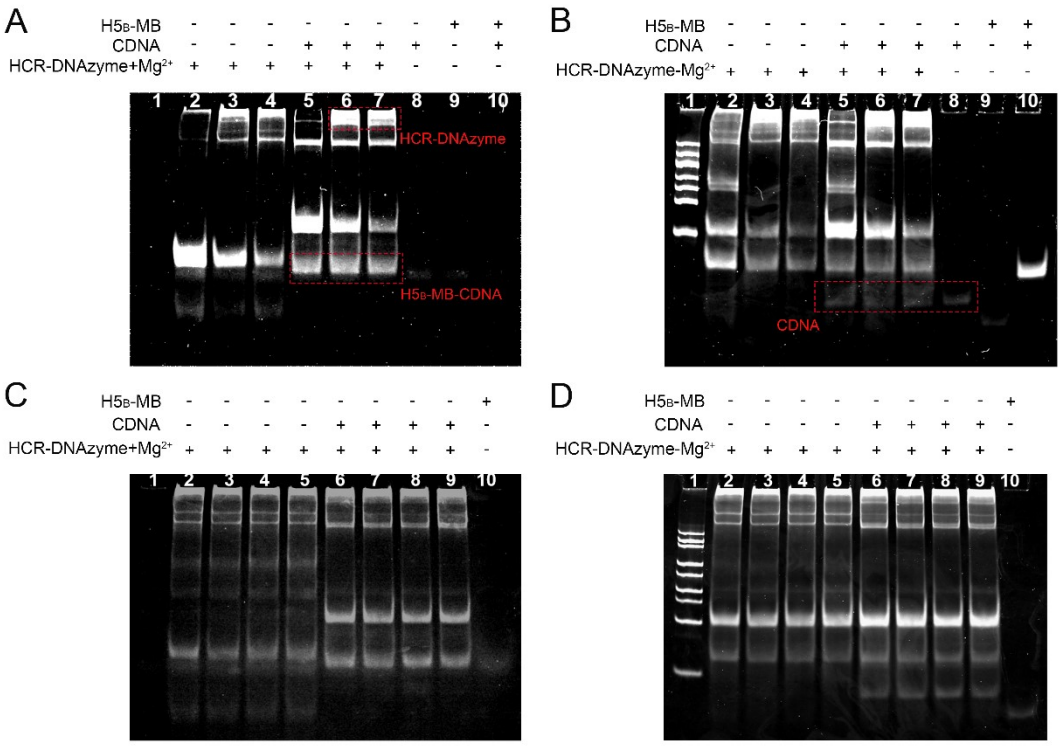


Figure S2. Verification of the binding ability of HCR-DNAzyme to Mg^{2+} with different concentration. (A)(B) Lane 1: Marker 2; Lane 2: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 200 μM); Lane 3: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 1 mM); Lane 4: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 2 mM); Lane 5: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 200 μM) /CDNA (1.6 μM); Lane 6: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 1 mM) /CDNA(1.6 μM); Lane 7: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 2 mM) /CDNA(1.6 μM); Lane 8: CDNA (200 nM); Lane 9: $H5_B$-MB(200 nM); Lane 10: $H5_B$-MB+CDNA ($H5_B$, 200 nM; CDNA, 200 nM); (C)(D) Lane 1: Marker 1; Lane 2: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 4 mM); Lane 3: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 6 mM); Lane 4: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 8 mM); Lane 5: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 10 mM); Lane 6: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 4 mM)/CDNA(1.6 μM); Lane 7: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 6 mM)/CDNA(1.6 μM); Lane 8: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 8 mM)/CDNA(1.6 μM); Lane 9: HCR-DNAzyme+Mg^{2+} (Mg^{2+}, 10 mM)/CDNA(1.6 μM); Lane 10: $H5_B$-MB(200 nM). ((A) (C) and (B) (D) were taken before and after staining with 4SGelblue nucleic acid dye, respectively, i.e. (A) and (B) are the same image, and (C) and (D) are the same image, both containing molecular mass markers (lane 1). The bands presented in the Figure S2A and C are modified FAM groups at the end of the H5 strand).

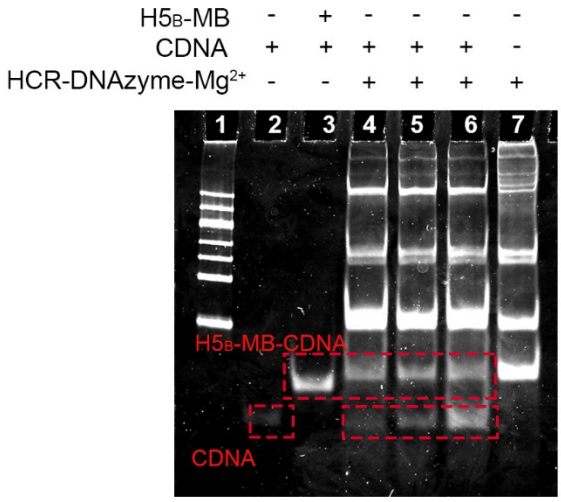


Figure S3. PAGE characterization plots used for the effect of CDNA concentration on the process of capture. Lane 1: Marker 2; Lane 2: CDNA (250 nM); Lane 3: $H5_B$-MB+CDNA($H5_B$, 200 nM; CDNA, 200 nM); Lane 4: HCR-DNAzyme+Mg^{2+}+CDNA (800 nM); Lane 5: HCR-DNAzyme+Mg^{2+}+CDNA (1.2 μM); Lane 6: HCR-DNAzyme+Mg^{2+}+CDNA (1.6 μM); Lane 7: HCR-DNAzyme+Mg^{2+} (HCR-DNAzyme, 200 nM; Mg^{2+}, 400 μM).

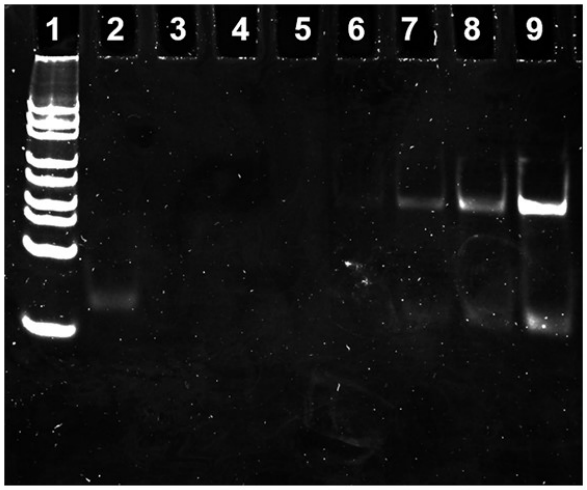


Figure S4. PAGE characterization characterized to optimize the amount of LDNA modified at the bottom of microplate. Lane 1: Marker 1; Lane 2: single-stranded LDNA; Lane 3: LDNA (0 μM); Lane 4: LDNA (50 nM); Lane 5: LDNA (100 nM); Lane 6: LDNA (200 nM); Lane 7: LDNA (500 nM); Lane 8: LDNA (1 μM); Lane 9: LDNA (2 μM). (Lane 3-Lane 9 was obtained from the supernatant after incubation in 96-well plates).

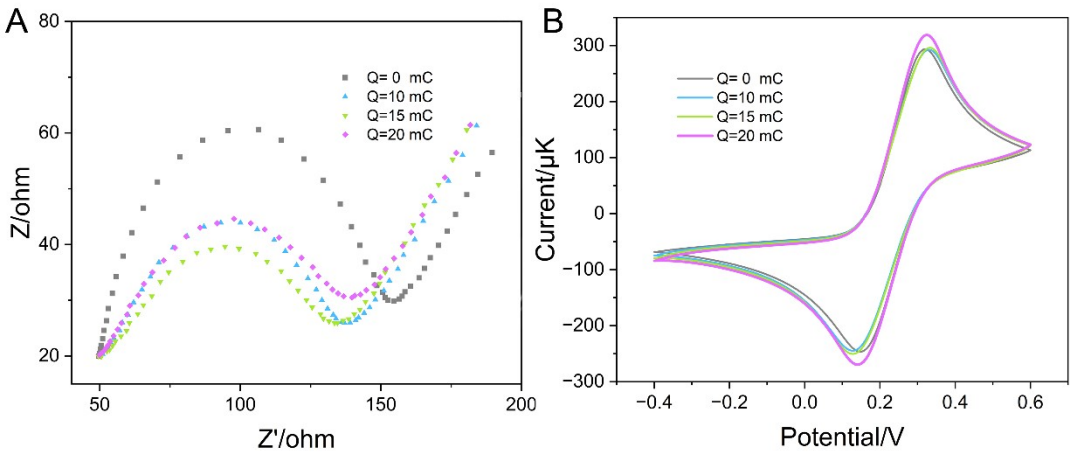


Figure S5. Optimization of the amount of Au deposited on the bare ITO electrode characterized by electrochemical impedance spectra (A) and cyclic voltammetry (B) in 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN]_6$ solution containing 0.1 M KCl.

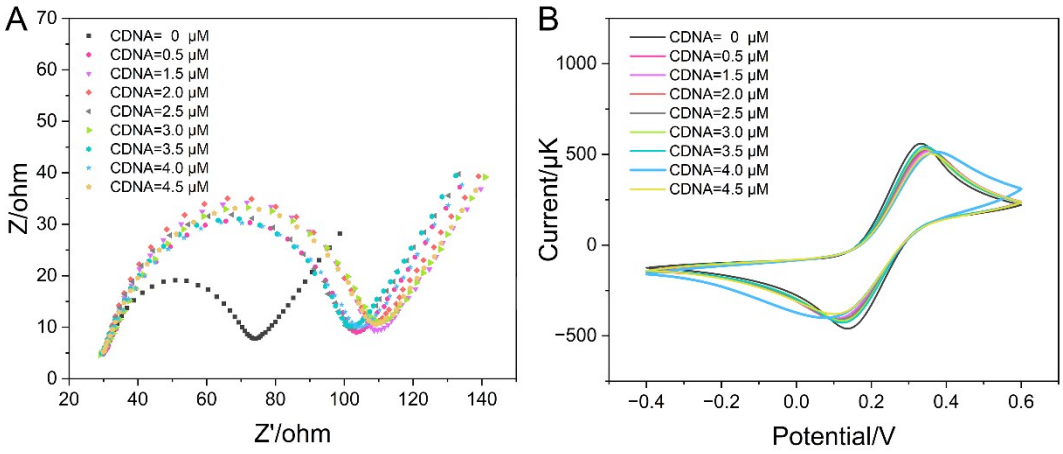


Figure S6. Optimization of the amount of CDNA connected on the ITO/Au determined by electrochemical impedance spectra (A) and cyclic voltammetry (B) in 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN]_6$ solution containing 0.1 M KCl. ITO/Au/ (black), ITO/Au/ CDNA (0.5 μM) (magenta), ITO/Au/ CDNA (1.5 μM) (purple), ITO/Au/ CDNA (2.0 μM) (red), ITO/Au/ CDNA (2.5 μM) (grey), ITO/Au/ CDNA (3.0 μM) (green), ITO/Au/ CDNA (3.5 μM) (lake green), ITO/Au/ CDNA (4.0 μM) (blue) , ITO/Au/ CDNA (4.5 μM) (yellow).

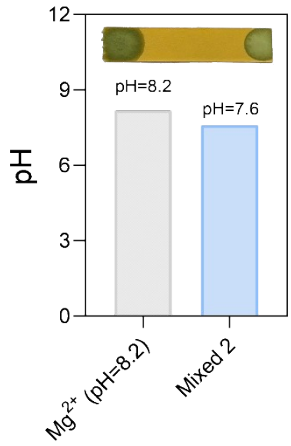


Figure S7. pH of solutions during detection of Mg^{2+} and mixed 2 with microplate strategy.

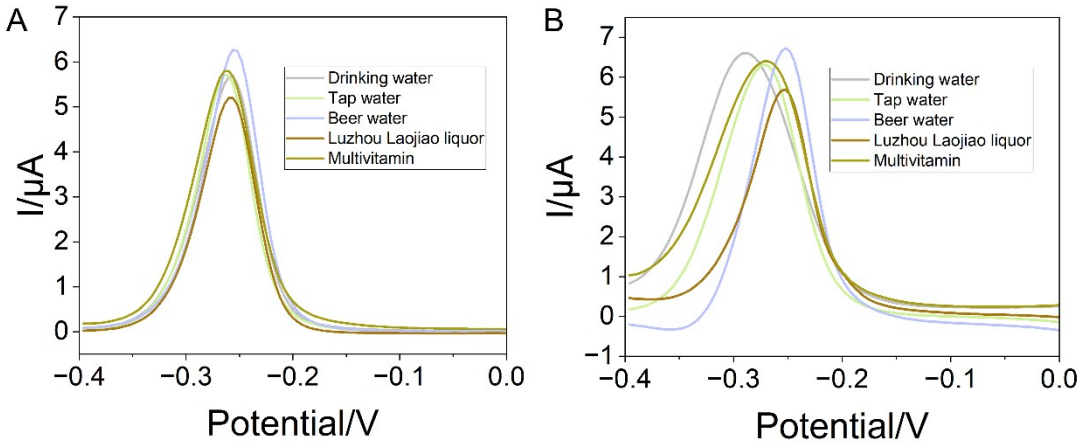


Figure S8. DPV curve for actual sample testing with (A) or without (B) spiking standard samples.