Electronic Supplementary Information

An omega-like DNA nanostructure upon small molecule introduction to stimulate formation of DNAzyme-aptamer conjugate

Bingqian Liu, Bing Zhang, Guonan Chen, and Dianping Tang*

MOE Key Laboratory of Analysis and Detection for Food Safety (MOE & Fujian Province), College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350108, P.R. China

E-mail: dianping.tang@fzu.edu.cn (D. Tang)
Fax: +86 591 2286 6135; Tel: +86 591 2286 6125.
EXPERIMENTAL SECTION

Material and Reagent. Adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP) and uridine triphosphate (UTP) were purchased from Dingguo Biotechnol. Co., Ltd. (Beijing, China). Hemin was purchased from Tokyo chemical industry Co., Ltd (Japan). All oligonucleotides used in this work were synthesized by Dingguo Biotechnol. Co., Ltd. (Beijing, China), which were purified by HPLC and confirmed by mass spectrometry. DNA stock solution was obtained by dissolving oligonucleotides in tris-HCl buffer solution (pH 7.4). Human IgG, human serum albumin (HAS) and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (Shanghai, China). Each oligonucleotide was heated to 90 °C for 5 min, and slowly cooled down to room temperature before usage. The sequences of oligonucleotides are listed as follows:

Strand A (SA): 5’-ACCTG GGGGA GTATT GCGGA GGAAG GT TTCTT TGG GTAGG GCGGG TTGGG -3’

Strand B (SB): 5’-TACCC AAAG TTTTTT TCC TCCGC -3’

The bold portion for strand A is the ATP-based aptamer, while the italic portion is the hemin-based aptamer. o-Phenylenediamine (OPD) was achieved from Sinopharm Chem. Re. Co. (Shanghai, China). The Britton-Robinson (BR) buffer solutions with various pH values were prepared by adding 0.04 M acetic acid and 0.04 M boric acid into 0.04 M orthophosphoric acid, with the appropriate amount of 0.2 M sodium hydroxide. All other reagents were of analytical grade and used as received without further purification. Ultrapure water obtained from a Millipore water purification system (≥18 MΩ, Milli-Q, Millipore) was used in all runs.

Preparation of Omega (Ω)-Like DNA Nanostructures. Prior to preparation, strand A and strand B were heated to 90 °C for 5 min, respectively, and then slowly cooled down to room
temperature. Following that, 100 μL of 1.0 μM strand A was diluted with 400-μL BR buffer. After gentle shaking, 100 μL of 1.0 μM strand B was added into the resulting solution. The mixture was incubated overnight at room temperature in order to adequately form the omega (Ω)-like DNA nanostructures. Finally, the resulting DNA nanostructures were used for the detection of target ATP.

**Electrochemical Measurement.** All electrochemical measurements were performed with an AutoLab μAUTIII.FRA2.v electrochemical workstation (Eco Chemie, The Netherlands). A conventional three-electrode system used in the measurements consists of a gold working electrode (2 mm in diameter), Pt wire as the counter electrode, and an Ag/AgCl reference electrode. Before measurement, a home-made electrochemical detection cell was prepared by coupling with a gold disk electrode and a micropipette (Scheme 1) *(Note: The gold electrode was strongly inserted into the micropipette, and then the top of the micropipette was cut off).* The assay was carried out as follows: (i) 50 μL of the above-prepared Ω DNA nanostructure suspension was injected into the micro-volume detection cell; (ii) 100 μL of pH 6.8 BR buffer containing 10 μM hemin, 0.02 M OPD and 0.02% (v/v) H₂O₂ was added into the resulting solution; (iii) 50 μL of target ATP standards with various concentrations were added into the detection cell and incubated for 10 min at room temperature; and (iv) the electrochemical characteristic of the gold electrode was monitored by square wave voltammetry (SWV) from 0 mV to -600 mV *(vs. Ag/AgCl)* (Amplitude: 25 mV; Frequency: 15 Hz; Increase E: 4 mV), which was registered as the signal. All incubations and measurements were performed at room temperature. Analyses were always made in triplicate.