## **Electronic Supplementary Information**

## Difunctional DNA-AuNP dendrimer coupling DNAzyme with intercalator for femtomolar detection of nucleic acid

Mingdi Xu, Junyang Zhuang, Xian Chen,\* Guonan Chen and Dianping Tang\*

Ministry of Education Key Laboratory of Analysis and Detection for Food Safety, Fujian Provincial Key Laboratory of Analysis and Detection for Food Safety, Department of Chemistry, Fuzhou University, Fuzhou 350108, PR China

E-mail: dianping.tang@fzu.edu.cn (D. Tang)

Fax: +86 591 2286 6135; Tel.: +86 591 2286 6125.

## **EXPERIMENTAL SECTION**

**Materials and Reagents.** Tris (2-carboxyethyl) phosphine (TCEP) and 6-mecatohexanol was purchased from Tokyo chemical industry Co., Ltd (Japan). Gold colloids (AuNP) with 16 nm in diameter were prepared and characterized as described in our previous paper [R. Yuan, D. Tang, Y. Chai, X. Zhong, Y. Liu and J. Dai, *Langmuir*, 2004, **20**, 7240]. Phosphate buffer solution (0.1 M, pH 7.4) was prepared by mixing the stock solutions of 0.1 M NaH<sub>2</sub>PO<sub>4</sub> and 0.1 M Na<sub>2</sub>HPO<sub>4</sub>, and 0.1 M KCl was used as the supporting electrolyte. All the other chemicals were of analytical grade, and used without further purification. Ultrapure water obtained from a Millipore water purification system ( $\geq$  18 MΩ, Milli-Q, Millipore) was used in all runs. The synthesized oligonucleotides, containing the capture probe (CP), target DNA (TD), mismatch target DNA (1mDNA, 2mDNA, and 3mDNA), S1, S2, hemin-based G-quadruplex aptamer were obtained from Sangon Biotech. Co., Ltd. (Shanghai, China). The sequences of oligonucleotides are listed as follows:

Capture probe (CP): 5'-TACTCCCCAGGTGCTTTT-SH-3'

(The sequence is completely complementary with the italic letters of target DNA) S2: 5'-SH-TTTTTACTAAAAGGGTCTGAGGG-3'

G-quadruplex aptamer: 5'-SH-TTTGGGTAGGGCGGGTTGGG-3'

**Preparation of DNA-Functionalized AuNP.** Initially, 0.1 nmol S1 and 0.1 nmol G-quadruplex aptamer were directly injected into 500  $\mu$ L of the above-prepared gold colloids in a 1.5-mL centrifuge tube (*Note*: Before conjugation, S1 and G-quadruplex aptamer should be pretreated by TCEP to split the formed disulfide between the thiolated DNA probes). After gently shaking for 5 min, the mixture was transferred to the refrigerator at 4 °C for further reaction (overnight). The resulting solution in 300  $\mu$ L PBS (pH 7.4, 0.5 NaCl) was incubated

for 2 days. Following that, the mixture was centrifuged (13,000 g) for 25 min at 4 °C. The pellet (designed as DNA1-AuNP) was re-suspended in 200  $\mu$ L PBS (pH 7.4, 0.1 NaCl), and stored at 4 °C until use. By the same token, S2 and G-quadruplex-functionalized AuNP were also prepared by using the mentioned-above method, and designed as DNA2-AuNP.

**Fabrication of DNA Biosensor.** A gold electrode (2 mm in diameter) was polished repeatedly with 1.0 and 0.3  $\mu$ m alumina slurry, followed by successive sonication in distilled water and ethanol for 5 min and dried in air. Prior to modification, the gold electrode was cleaned with hot piranha solution (a 3:1 mixture of H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub>. *Cautions*!) for 10 min, and then continuously scanned within the potential range of -0.3 to 1.5 V in freshly prepared deoxygenated 0.5 M H<sub>2</sub>SO<sub>4</sub> until a voltammogram characteristic of the clean gold electrode was established. After the cleaned electrode was thoroughly rinsed with water and absolute ethanol, the cleaned electrode was immersed in 1.0  $\mu$ M capture DNA probe (*Note*: Before incubation, the capture probe was pretreated by TCEP to split the formed disulfide between the thiolated DNA probes), and incubated for 2 h at RT. During this process, the capture DNA probe was conjugated onto the gold electrode was incubated with 1.0 mM 6-mercaptohexanol in 10 mM Tris-HCl buffer, pH 7.4, for 60 min. Finally, the as-prepared DNA biosensor was suspended over pH 7.4 PBS at 4 °C when not in use.

**Electrochemical Measurement.** All electrochemical measurements were carried out on a CHI 630D Electrochemical Analyzer (CH Instruments Inc., Shanghai, China) with a conventional three-electrode system comprising of a modified gold working electrode, a platinum wire auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode. The assay was performed as follows: (i) Hybridization with target DNA by incubating the DNA biosensor with target DNA with various concentrations for 60 min at 37 °C; (ii) Formation of DNA-AuNP dendrimer by dipping the resulting sensor in a hybridization solution containing 150 µL above-prepared DNA1-AuNP and 150 µL DNA2-AuNP for 100 min at 37 °C; (iii) Formation of DNAzyme by suspending the modified sensor into 0.2 mM hemin solution for 30 min at RT; (iv) Intercalation of methylene blue by dipping the resultant

sensor in 0.5 mM methylene blue aqueous solution for 50 min at RT; and (v) Electrochemical measurement in a phosphate buffer solution (0.1 M, pH 7.4) containing 2.0 mM  $H_2O_2$  by using square wave voltammetry (SWV, potential range: 0 – -0.5 V *vs*. SCE; Amplitude: 25 mV, Frequency: 15 Hz; Increase *E*: 4 mV). After each step, the DNA biosensor was washed by using pH 7.4 PBS. All measurements were conducted at room temperature. Analyses are always made in triplicate.

**Optimization of Hybridization Time for DNA-AuNP Dendrimer.** In this work, the electrochemical signal mainly derived from the formed DNAzyme toward the catalytic reduction of  $H_2O_2$  with the aid of methylene blue. The conjugated amount of DNAzyme and methylene blue directly depended on the formed DNA-AuNP dendrimer. To achieve an optimal electrochemical signal, we investigated the effect of hybridization time between DNA1-AuNP/DNA2-AuNP and target DNA probe on the electrochemical signal of the developed DNA biosensor by using 10 pM target DNA as an example. As shown from Fig. S2, the electrochemical signal increased with the increasing hybridization time, and tended to level off after 100 min. Hence, a hybridization time of 100 min was selected for the formation of DNA-AuNP dendrimer at acceptable throughout.



**Fig. S1** SWV curves of the as-prepared DNA biosensor after incubation with 10 pM target DNA and methylene blue in sequence in pH 7.4 PBS in the (a) absence and (b) presence of 2.0 mM  $H_2O_2$ . In this case, DNA1-AuNP and DNA2-AuNP were not used for further hybridization.



**Fig. S2** The effect of hybridization time between DNA1-AuNP/DNA2-AuNP and target DNA on the electrochemical signal of the developed DNA biosensor (10 pM target DNA used in this case).



Fig. S3 Calibration curves of the electrochemical DNA biosensor toward target DNA various concentrations by using DNA1-AuNP as signal amplificaton in pH 7.4 PBS + 2 mM  $H_2O_2$  (*Inset*: the corresponding SWV curves).



**Fig. S4** The electrochemical responses of seven DNA biosensors with (a) various batches and (b) the same batch toward 10 pM target DNA.