Supporting Information

DNA tetrahedron and star trigon nanostructures for target recycling detection of nucleic acid

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EXPERIMENTAL SECTION

Materials and chemicals

Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma (USA). All other reagents were of analytical grade and were used as received. Human serum samples were from local hospitals. Water used to prepare all solutions was purified by a Millipore system (>18 MΩ·cm resistivity). All oligonucleotides were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). Detailed sequences and modification information were listed in Table S1.

Instrumentation

Electrochemical experiments were performed on a CHI 660D electrochemical workstation (CH instruments, Shanghai, China). A traditional three electrode system was employed, which consisted of a saturated calomel reference electrode (SCE), a platinum wire counter electrode and DNA tetrahedron modified gold electrode as the working electrode. SWV experiments were carried out in the electrolyte of 20 mM Tris-HCl (pH 7.5). Potential scan range was -0.05 to -0.55 V, potential step was 4 mV, amplitude was 25 mV, and frequency was 20 Hz.

Preparation of tetrahedral DNA modified gold electrode

Tetrahedral DNA was formed through the hybridization of four ssDNAs (Tetrahedron A, B, C, D).[1] The four ssDNAs were firstly dissolved in 10 mM Tris-HCl buffer solution (10 mM TCEP, 50 mM $MgCl_2$, pH 8.0), respectively. Then, 25 μL of the four strands were mixed (4 μM) and heated to 95 °C for 2 min. After cooled to 4 °C, DNA tetrahedron was formed.

The pretreatment of substrate gold electrode following the reported protocol.[2] It was firstly incubated with piranha solution (98% H_2SO_4 : 30% H_2O_2 = 3 : 1) for 5 min (*Caution: Piranha*

solution reacts violently with organic solvents and must be handled with great care!). Next, the electrode was successively polished using P5000 sand paper and 1 μm, 0.3 μm, 0.05 μm alumina slurry. After that, it was soaked in ethanol and then in pure water during ultra-sonicating, each for 5 min.[3-4] Subsequently, the electrode was immersed in 50% HNO_3 for 30 min and further electrochemically cleaned with 0.5 M H_2SO_4 to remove any remaining impurities. After dried by nitrogen, 10 μL of DNA tetrahedron was dipped on the electrode surface for a duration of 8 h.

Target induced stem-loop opening, star trigon formation and target recycling

Standard target DNA solutions were prepared in 10 mM phosphate buffered saline (PBS, pH 7.4) containing 0.25 M NaCl, which were then used to incubate DNA tetrahedron modified electrode for 1 h. Next, the electrode was further treated with the mixture of 3 μM auxiliary probe and 3 μM signal probe for 2 h. After carefully rinsing with pure water, the electrode was electrochemically tested and SWV responses were recorded.

Electrochemical assay in human serum

To monitor whether the developed method could measure target DNA in real samples, recovery experiments were performed in human serum samples. With different standard additions, three concentrations of target DNA (10 fM, 1 pM, 100 pM) were supplemented in 1% human serum samples, which were then tested by the proposed electrochemical method.

References

1. Z. L. Ge, M. H. Lin, P. Wang, H. Pei, J. Yan, J. Y. Sho, Q. Huang, D. N. He, C. H. Fan and X. L. Zuo, *Anal. Chem.*, 2014, **86**, 2124-2130.
2. J. Zhao, Y. L. Yan, L. Zhu, X. X. Li and G. X. Li, *Biosens. Bioelectron.*, 2013, **41**, 815-819.

3. I. Akseli, M. Miraskari, H. A. Zhang, W. Q. Ding and C. Cetinkaya, *J. Adhes. Sci. Technol.*, 2011, **25**, 407-434.

4. W. Ding, H. Zhang and C. Cetinkaya, *J. Adhes.*, 2008, **84**, 996-1006.

Table S1. DNA sequences used in this study.

Name	Sequence (from 5' to 3')
Tetrahedron A	TTTTCCACATTCGCATACGTAAATGTGGAAAATCTCTAGCAGTTTCAAC ATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCA TAGTA
Tetrahedron B	SH-C_6- TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGT CCAATAC
Tetrahedron C	SH-C_6- TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCG GCTCTTC
Tetrahedron D	SH-C_6- TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCC TCGCAT
target HIV DNA	ACTGCTAGAGATTTTCCACAT
single-base mismatched	ACTGCTAGAGATTTTCCA<u>T</u>AT
double-base mismatched	AC<u>C</u>GCTAGAGATTTTCCA<u>T</u>AT
unmatched	TCACTGAATCTCAGGTCGTCG

auxiliary probe	TCGCATACGTACTGCTAGAGAACGTATGCGAATGTGGAAAA
signal probe	MB-ACTGCTAGAGATTTTCCACATTCTCTAGCAGTACGTATGCGA