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Label-Free and Ultrasensitive Fluorescent Detection of Cocaine Based on a DNA-Templated Silver Nanoclusters and Nicking Endonucleases-Assisted Signaling Amplification Strategy

Kai Zhang, Ke Wang, Xue Zhu, Jue Zhang, Lan Xu, Biao Huang, Minhao Xie**

Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Key Laboratory of Molecular Nuclear Medicine, Jiangsu Institute of Nuclear Medicine. Wuxi, Jiangsu 214063, China.

* To whom correspondence should be addressed. E-mail:zhangkai@jsinm.org (K. Zhang), xieminhao@jsinm.org (M. Xie).

Chemicals: Cocaine hydrochloride, ecgonine and benzoyl ecgonine were obtained from Sigma-Aldrich (St. Louis, MO, USA). Oligounucleotides were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Nb.BbvCI and CutSmart Buffer were obtained from BioLabs (New England Biolabs). Other chemicals were all of analytical grade. All solutions were prepared with double-distilled water, which was purified with a Milli-Q purification system (Branstead) to a specific resistance of > 18 MΩ cm.

Procedure for cocaine Assay: Five strands of oligonucleotides were adopted in this experiments. The sequences were shown in Figure 1. The detailed procedure for cocaine assay was as follows. First, DNA2 (1 μL, 100 μM) and DNA3 (1 μL, 100 μM) were mixed and incubated at 80 °C for 2 min, followed by cooling down to room temperature in about 2 h. Second, 2 μL of 20 mM Tris-HCl solution that contained 50 μM of the DNA1 and 5 μL varying concentrations of cocaine or other small molecules was added into the above mixture and incubated at 37 °C for another 1 h. Then, Nb.BbvCI (1 μL, 10 U μL^{-1}) and 10 × CutSmart Buffer (10 μL) were added and allowed to incubate at 37 °C for 2 h.

The resultant products of NEASA reaction was mixed with 1 μL 1.2 mM $AgNO_3$ ($AgNO_3$:DNA2 = 12:1, molar ratio) in the 78 μL phosphate buffer (10 mM, pH 7.0) that included 200 mM $NaNO_3$ and centrifuged at 12000 rpm for 5 min at room temperature to collect the supernatant. The supernatant was incubated at room temperature in the dark for 15 min. Then, 1 μM freshly prepared $NaBH_4$ (1.2 mM, $NaBH_4$:$AgNO_3$ = 1:1, molar ratio) was added and the reaction mixture was incubated at room temperature in the dark for 15 min. Then the Ag^+ was reduced for another 14 h in the dark at 4 °C. Following reduction of Ag^+ ions by $NaBH_4$, the as-prepared fluorescent DNA-AgNCs were produced with fluorescence emission at 616 nm upon

the excitation at 530 nm.

The control experiments of two AgNCs-template-segments: The control experiments of two AgNCs-template-segments without NEASA reaction were prepared using the aforementioned methods with minor modification, that is, DNA4 (1 μL, 100 μM) and DNA5 (1 μL, 100 μM) were added in 2 μL of 20 mM Tris-HCl solution, 10 μL of 10 × CutSmart Buffer, and 6 μL of ddH_2O, and incubated at 37 °C for 1 h. The solution were sequentially added and mixed with 1 μL 1.2 mM $AgNO_3$ in the 78 μL phosphate buffer (10 mM, pH 7.0) that included 200 mM $NaNO_3$ and centrifuged at 12000 rpm for 5 min at room temperature to collect the supernatant. The supernatant was incubated at room temperature in the dark for 15 min. Then, 1 μM freshly prepared $NaBH_4$ (1.2 mM) was added and the reaction mixture was incubated at room temperature in the dark for 15 min. Then the Ag^+ was reduced for another 14 h in the dark at 4 °C. Following reduction of Ag^+ ions by $NaBH_4$, the as-prepared fluorescent DNA-AgNCs were produced with fluorescence emission at 616 nm upon the excitation at 530 nm.

Amplification and cleavage reaction time: We next optimized the amplification and cleavage reaction time. A long cleavage reaction time was expected to yield enhanced signal amplification. The experimental results indicated the fluorescence intensity increased with the increase of cleavage reaction time. By weighing the total assay time, the cleavage reaction time of 2 h was selected for the experiments.

Instrumentation: The fluorescence spectra were recorded by a SpectraMax M5 Multi-Mode Microplate Readers (Molecular Devices, USA), using a black 384 microtiter plate (No. 781209, Greiner Bio-one, Frickenhausen, Germany).

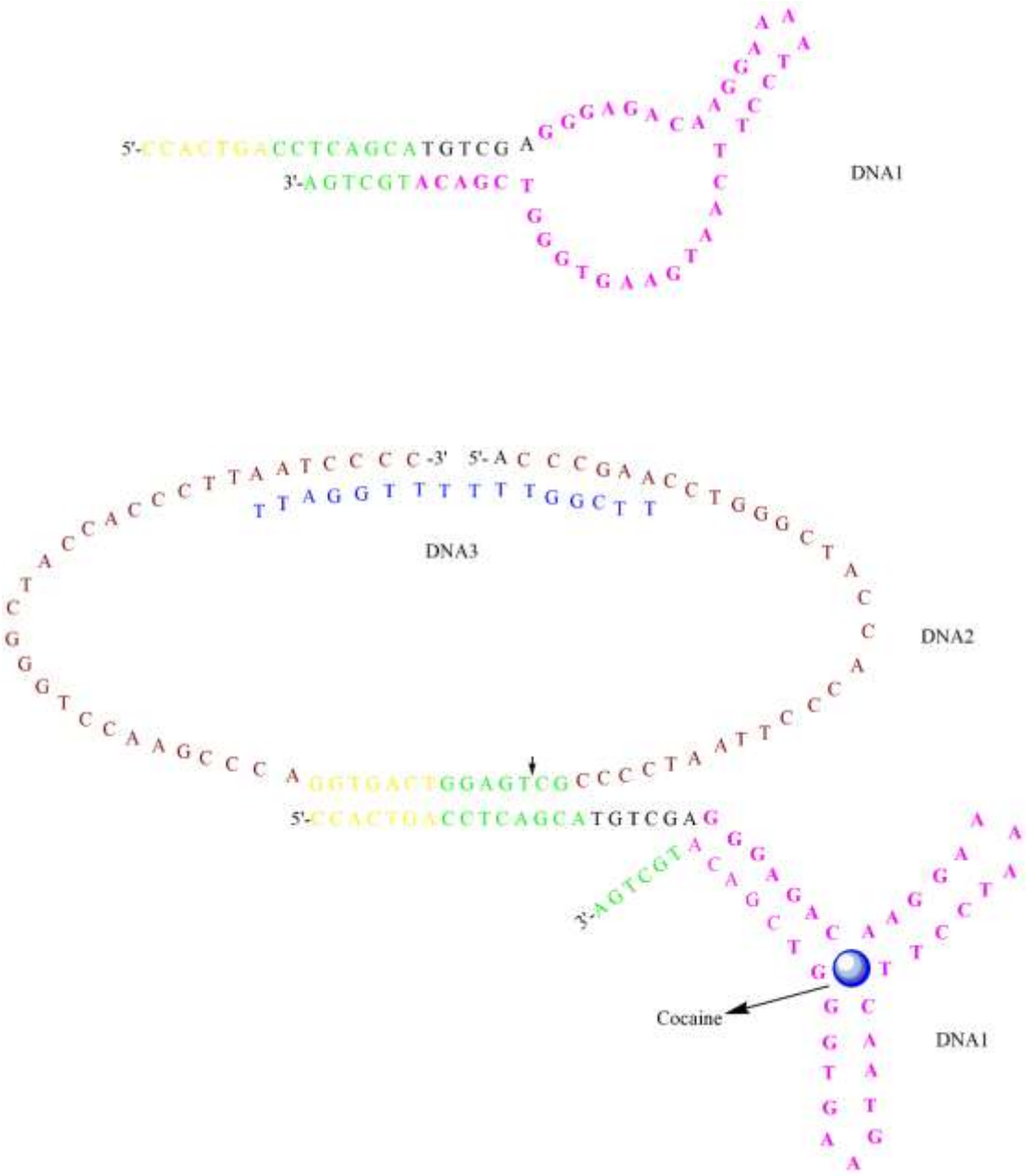

Fig. S1. The Structures of DNA1 and cocaine/DNA1/DNA2/DNA3 complex.

Samples	cocaine added (nM)	found (U L^{-1})	recovery (%)
1	10	9.8	98.0
2	100	99.2	99.2
3	1000	998.4	99.8

Table S1. Results of the recovery test of cocaine in 10% human serum.