

Electronic Supplementary Information

**Instrument-free visual detection of tetracycline on an autocatalytic DNA machine
using caged G-quadruplex as the signal reporter**

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Experimental Section

Chemicals and materials

Tetracycline, TMB, hemin, dimethyl sulfoxide (DMSO), hydrogen peroxide (H_2O_2 , 30wt.%), Triton X-100, and tris-(hydroxymethyl)aminomethane (Tris) were purchased from Sigma-Aldrich (St. Louis, Mo). A hemin stock solution (5 mM) was prepared in DMSO and stored in the dark at -20°C. Other reagents and chemicals were of analytical grade and used without purification. All solution was prepared with ultrapure water (18.2 MΩ/cm) from a Millipore Milli-Q water purification system (Billerica, MA).

All DNA oligonucleotides were HPLC-purified and purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences were listed as follows:

Tetracycline aptamer:

5'-CGTACGGAATTCGCTAGCCCCCGGCAGGCCACGGCTTGGGTTGGTCCACTGCGCGTGGATCCGAGCTCCACGTG-3'

a I b

DNA1: 5'-TGGAGCTCGGATGTTACACCCATGTTCGTCA-3'

c II a*

DNA2: 5'-TCTTGCAGCGATTAACATCCGAGCTCCA-3'

d

H1: 5'-CCTACCCGACTGACGATrAGGCAAGATATTTTTTTTATGGTAGGGCGGGTTGGG-3'

I b

H2: 5'-ATCCGAGCTCGTCAGTCGGTAGGTGGAGCTGGATGTTACACCCATGTTCGTCA-3'

Tetracycline assay procedure

All DNA solution was incubated at 95°C for 10 min and then gradually cooled to 25°C at a constant rate of 1°C/min. The partial DNA duplex (tetracycline aptamer/DNA1) was first prepared by mixing tetracycline aptamer and DNA1 at a final concentration of 0.2 μM in 20 mM Tris-HCl buffer (pH 7.4, 150 mM NaCl, 20 mM MgCl₂, 15 mM KCl). Subsequently, different concentrations of tetracycline was added into the partial DNA duplex solution and incubated at room temperature for 40 min. Finally, DNA2 (0.4 μM), H1 (0.6 μM), and H2 (0.2 μM) were added and the mixture was incubated for 120 min at room temperature to trigger the cleavage of the substrate and release the caged G-rich sequence.

40 μL of the above solution was mixed with 10 μL of hemin solution in 20 mM Tris-HCl buffer (pH 7.4, 2.5 μM hemin, 150 mM NaCl, 20 mM MgCl₂, 15 mM KCl, 0.01% Triton X-100, and 0.5% DMSO) and incubated at room temperature for 40 min. Then, 950 μL TMB-H₂O₂ substrate solution, which was constituted of 10 μL 0.5% (w/v) TMB, 20 μL 30% (w/v) H₂O₂, and 920 μL substrate buffer (containing 26.2 mM citric acid, 51.4 mM disodium hydrogen phosphate, and 25 mM KCl, pH 5.0) was added. After incubation at room temperature for 20 min, the colorimetric responses were observed by the naked eye and the absorption spectrum of the reaction mixture was recorded using a TU-1902 UV-vis spectrophotometer (Persee, China).

Selectivity and real sample analysis

To investigate the selectivity of the assay, other antibiotics including clindamycin, amoxicillin, ciprofloxacin, chloramphenicol, kanamycin, ribostamycin, streptomycin, and penicillin at 100 nM were also analyzed according to the procedures described above.

For real sample analysis, milk samples purchased from local market were filtered through a 0.2 μm membrane to remove the insoluble particles. Aliquots of the milk samples were spiked with different concentrations of tetracycline and diluted 10 times with the reaction buffer for recovery studies. Other procedures were the same as described above.

Optimization of experimental conditions

In this assay, the concentration of H1 substrate plays an important role in the performance of the colorimetric sensor. As shown in Fig. S1, the H1 concentration of 0.6 μ M could achieve the best signal-to-noise (S/N) ratio. The higher concentration of H1 could cause a relatively high background signal, which restricted the colorimetric response for target tetracycline detection. While the lower concentration could result in a weak colorimetric signal due to the deficient amount of G-rich sequence for signal readout. Therefore, the optimized concentration of H1 is 0.6 μ M.

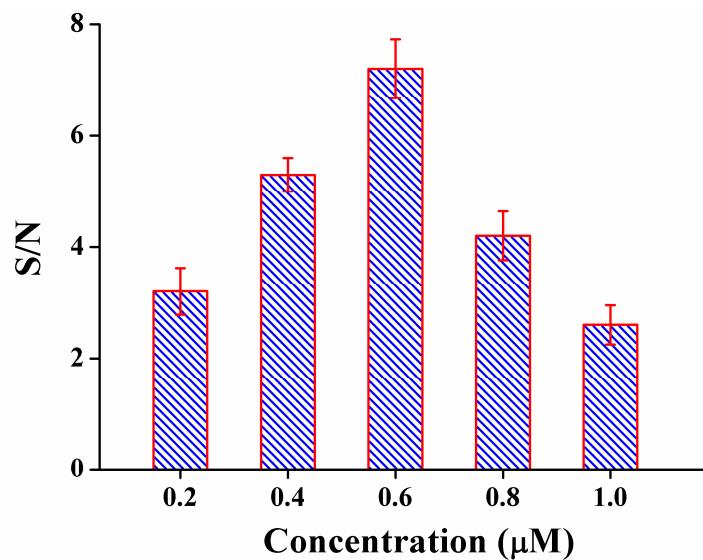


Fig. S1 Effect of the concentration of H1 substrate on the performance of the sensing system. The concentration of tetracycline aptamer, DNA1, and H2 is 0.2 μ M. The concentration of DNA2 is 0.4 μ M. The used target tetracycline concentration is 10 nM. The experiments were performed at room temperature (~ 25 $^{\circ}$ C). The error bars represent the standard deviation of three independent measurements.

The temperature at which the autocatalytic DNA machine is activated was also optimized and the performance of the sensing system was examined at different temperatures (4 °C, 25 °C, 37 °C, and 45 °C). As shown in Fig. S2, the maximum S/N ratio (blue line) was achieved at 25 °C. At low temperature (4 °C), the subunits (DNA1 and DNA1) may bind to the substrate H1 to form an active DNAzyme structure even in the absence of the target tetracycline (black histogram), giving rise to a relatively high background signal. In the presence of 10 nM tetracycline (red histogram), the active DNAzyme nearly lost all its catalytic cleavage activity at high temperature (37 °C) as the synergistically-stabilized supramolecular DNAzyme structure through DNA hybridization was unstable at this temperature. In order to obtain the best S/N ratio, a moderate temperature of 25 °C was chosen as the optimal reaction temperature in the following experiments.

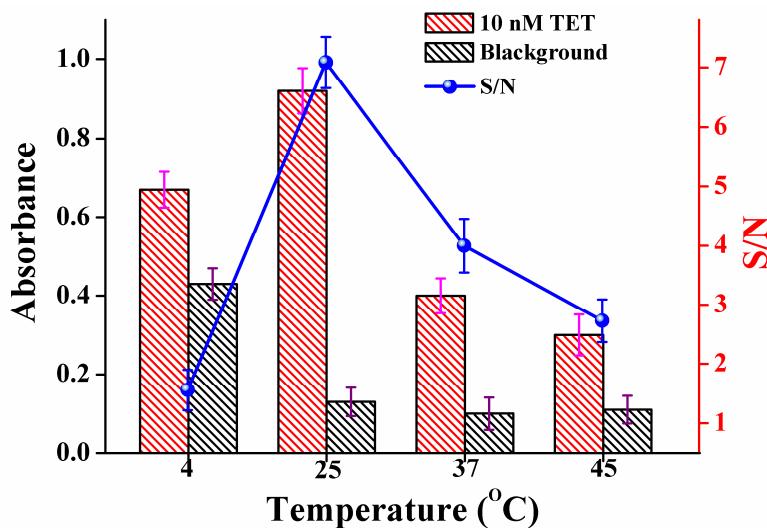


Fig. S2 Effect of the reaction temperature on the performance of the sensing system. The histograms represent the absorbance intensity of the solution with 10 nM tetracycline (red) and without tetracycline (black). The blue line represents the S/N ratio. The error bars represent the standard deviation of three independent measurements.

The reaction time of DNAzyme is another important parameter affecting the signal amplification process of the fabricated autocatalytic sensing system. As shown in Fig. S3, the colorimetric signal increased continuously with the augment of the reaction time in the presence of 10 nM tetracycline, and kept almost a constant level after 120 min (black line), which indicated that the caged G-rich sequence was almost released from H1 substrate for the colorimetric response. However, the background signal (in the absence of tetracycline) maintained its increase along with increasing the incubation time (red line). The reaction is a kinetically controlled process. Prolonged incubation times could cause more leaks and increased background signals. To achieve the best signal-to-background level and the maximal signal amplification efficiency, 120 min was chosen as the optimal reaction time to suppress small leaky signals to be "off".

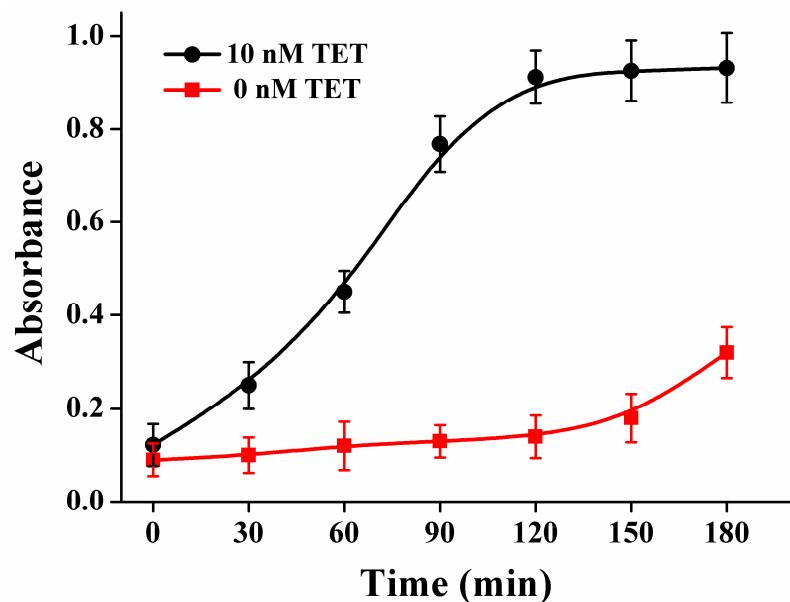


Fig. S3 Effect of the reaction time of DNAzyme on the performance of the sensing system in the presence of 10 nM tetracycline (red line) or without tetracycline (black line). The experiments were performed at room temperature (~ 25 $^{\circ}\text{C}$). The error bars represent the standard deviation of three independent measurements.

The number of complementary bases between a of DNA1 and a* of DNA2 was also optimized. As shown in Fig. S4, the S/N ratio increased with increasing complementary bases from 6 bp to 12 bp, further increasing the number of complementary bases caused a decrease of the S/N ratio. The reason can be attributed to the fact that with shorter complementary bases between segments a and a*, the complementary domains are insufficient to stabilize the formation of the active DNAzyme, and the partially complementary duplex between DNA1 and the aptamer is unstable and will cause a high background signal. In contrast, longer complementary bases is not conducive to the displacement reaction between tetracycline and aptamer because of the complementarity between segment a of DNA1 and aptamer, thus resulting in a weak signal. Thus, to maximize the S/N ratio, the number of complementary bases between a and a* was chosen as 12 bp for subsequent experiments. The hybrid duplexes of DNA1 and DNA2 (active DNAzyme) is stable and can undergo many cycles to catalyze the cleavage of numerous H1 substrates. after cleavage reaction, the released trigger DNA (T) coupled with excess DNA2 and H2 to initiate another signal amplification process.

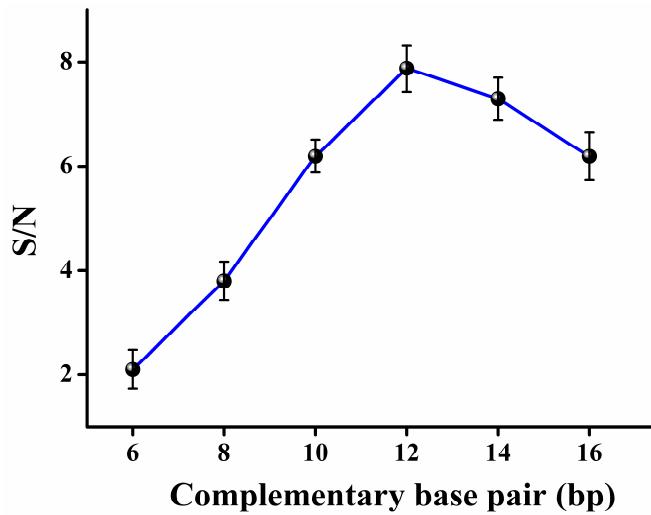


Fig. S4 Effect of the complementary bases between a of DNA1 and a* of DNA2 on the performance of the sensing system. The concentration of tetracycline aptamer, DNA1, and H2 is 0.2 μ M. The concentration of DNA2 is 0.4 μ M. The concentration of H2 is 0.6 μ M. The used target tetracycline concentration is 10 nM. The experiments were performed at room temperature (~25 $^{\circ}$ C). The error bars represent the standard deviation of three independent measurements.

Table S1 Recovery experiments of tetracycline determination in milk samples.

Sample	Added (nM)	Found (mean ^a \pm SD ^b) (nM)	Recovery (%)	LC-MS/MS ^c (nM)	Relative error (Re) ^d (%)
Milk sample 1	0.1	0.089 \pm 0.04	89	0.093	4.5
Milk sample 2	1	0.96 \pm 0.24	96	0.98	2.1
Milk sample 3	10	10.8 \pm 0.72	108	11.2	3.7
Milk sample 4	25	21 \pm 2.56	84	22.6	7.6
Milk sample 5	50	51.5 \pm 4.28	103	49.7	-3.5

^aMean of three determinations. ^bSD, standard deviation. ^cThe concentration of tetracycline in milk samples was certified using LC-MS/MS. ^dOur constructed autocatalytic biosensor vs LC-MS/MS method.