Supporting Information

Activation of Mg^{2+}-dependent DNAzymes based on AP site-containing triplex for specific melamine recognition

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Experimental section

Materials. All of the DNA samples (sequences are listed in Table S1) were custom synthesized and HPLC purified (>97 %) by Sangon Biotech. Inc. (Shanghai, China). The concentrations of DNAs were determined from the molar extinction coefficient at 260 nm. Melamine was purchased from Aladdin Reagents Co., Ltd. (Shanghai, China). The other reagents were commercially available analytical grade and were used without further purification. Water was deionized (18.0 MΩ·cm specific resistance) by Hitech laboratory water purification system.

Apparatus. Absorption spectra were measured with a Cary 60 UV–vis spectrophotometer (Agilent Technologies, Palo Alto, CA, USA). Fluorescence spectra were measured with a Cary Eclipse spectrofluorophotometer equipped with a thermoelectrically temperature-controlled cell holder (Agilent Technologies, Palo Alto, CA, USA). Emission spectra were recorded upon excitation at 492 nm. All fluorescence measurements were performed at 20°C. Circular dichroism (CD) spectra were obtained by J-815 CD spectrometer (JASCO, Japan).

Table S1. **The DNA sequences used in this study**

Name	Sequences (5′-3′)
Substrate (1)	6-FAM-AGAGTATrAGGATATC-BHQ1
Substrate-D	AGAGTATAGGATATC
DNAzyme (2)	GATATCAGCGATCTTATTTTTTTTTTTCCCCTTTTTTTTTTTAGCACCC AT GTTACTCT
11A	AAAAA X AAAAA
10A	AAAAA X AAAA
9A	AAAA X AAAA
8A	AAAA X AAA
7A	AAA X AAA
***X denotes the AP site having a propyl residue (Spacer C3)**	

Fluorescence assay. The regulation system for melamine recognition was studied in a solution consisting of the substrate (**1**) (1 μM), DNAzyme (**2**) (1 μM) and 8A (1 μM) in 25 mM HEPES

buffer (100 mM NaCl, 10 mM Mg^{2+}, pH=7.2). After addition of melamine with different concentrations, the solutions were incubated at 20 °C for 6 hours. The fluorescence intensity was then measured.

Detection of melamine in milk samples. Aliquots of the 10 mM melamine stock solution were added to milk to obtain spiked milk of corresponding concentrations. The extraction procedures of milk pretreatment were carried out as follows: 5 mL of spiked milk was placed into a 10 mL centrifuge tube, then 1.5 mL of 2 M trichloroacetic acid was introduced. After 15 min sonication and 10 min shaking for precipitating the protein, the mixture was centrifuged at 10,000 rpm for 10 min to separate the deposit. The supernatant was adjusted to pH 7.0 with 1 M NaOH solution and further filtered with 0.22 μm filter to obtain the samples for subsequent detection.

Table S2. Determination results of melamine in spiked milk samples

Samples	Concentration of melamine added (μM)	Recovery (%)
1	0.5	100.6
2	5.0	95.9
3	10.0	98.0

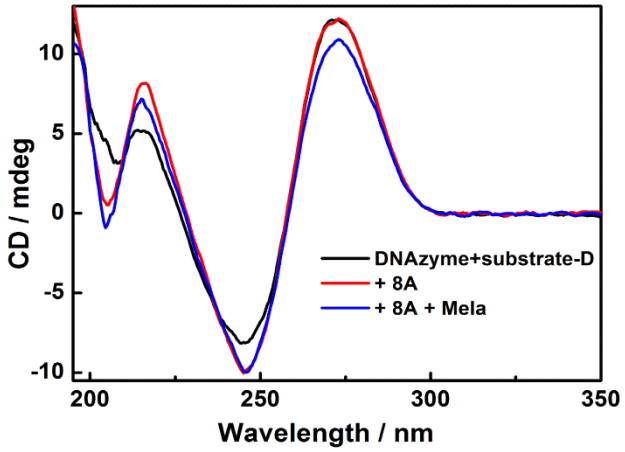

Fig. S1. CD spectra of complex of the DNAzyme and substrate-D (black line), complex of the DNAzyme, substrate-D and 8A in the absence of melamine (red line) and presence of 120 μM melamine (blue line). Concentrations of DNA were 8 μM.

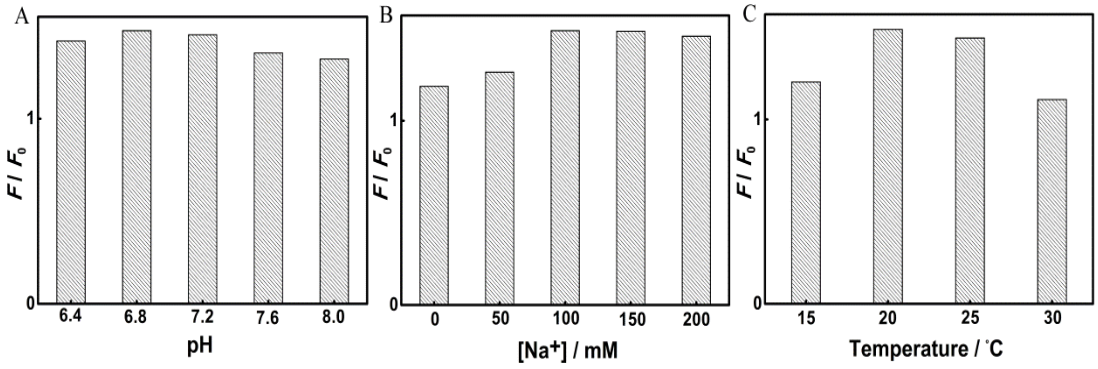

Fig. S2. Optimization of pH (A), concentrations of Na^+ (B) and temperature of reaction (C). F and F_0 represent for the fluorescence intensities of the system in the presence and absence of 10 μM melamine. DNAs (1 μM), Mg^{2+} (10 mM), time of reaction (6 h).