Supporting Information for

Triplex molecular beacon for sensitive recognition of melamine based on abasic-site-containing DNA and fluorescent silver nanoclusters

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Experimental

Materials. All of the DNA samples (sequences are listed in Table I) 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). $AgNO_3$, $NaBH_4$ were from Sinopharm Group Chemical Reagent 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.

Table 1. **The DNA Sequences Used in This Study**

Name	Sequences (5′-3′)
mDNA	CCCTT AATCC CCTTT TTTTT TTTCA GGCAT
	TTTTT TTTTT GGGTG GGGTG GGGTG GGG
mDNA-s	TTT TTTTT TTTCA GGCAT TTTTT TTTTT
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)**	

Instruments and Measurements. 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). The thermal denaturation experiment was done on a Shimadzu model UV-2450 UV–vis spectrophotometer equipped with a thermoelectrically temperature-controlled cell holder. Thermal denaturation curves were obtained by measuring absorbance of DNAs at 260 nm as a function of temperature with a heating rate of 2 °C/min. CD spectra were obtained by J-815 CD spectrometer (JASCO, Japan).

Isothermal titration calorimetry (ITC) measurements were carried out at 293K on a ITC200 microcalorimeter interfaced to a Gateway PC for data acquisition (MicroCal, LLC, Northampton, MA). Origin (version 7.0) software was used for data analysis. DNA solution (200 μL) in the sample cell was titrated using 2 μL volumes (first injection: 0.4 μL for 10 s; then 19 injections of 20 s duration at 150 s time intervals) of melamine. As a control experiment, the buffer solution without the DNA complexes in the sample cell was titrated to obtain the dilution heat. The area of each injection peak was automatically determined. A binding isotherm was obtained when the total heat per injection (kcal mol^{-1} of injectant) was plotted against the molar ratio of melamine to the tMB.

Preparation of DNA/Ag NCs. DNA/Ag NCs were prepared using mDNA as a template according to the reported method with slight modification.[1] 15μM mDNA and 90 μM $AgNO_3$ were sequentially added and mixed in 20 mM pH 6.8 sodium phosphate buffer. The mixture was incubated in the dark for 20 min after which 90 μM $NaBH_4$ was added followed by vigorous shaking for 1 min. Then the reaction was kept in the dark at room temperature for 18 hours before use.

Detection of Melamine in Milk Samples. Aliquots of the 10 mM melamine stock solution were added to milk to obtain concentrations of 5, 10 and 20 μM. Melamine-free milk was treated as the spiked milk and used to prepare blank samples. 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 detection. With respect to fluorescence detection, the melamine-free blank sample was first used as matrices to make a linear relationship between fluorescence response and concentration of melamine, and the treated spiked samples were then detected in the same way successively.

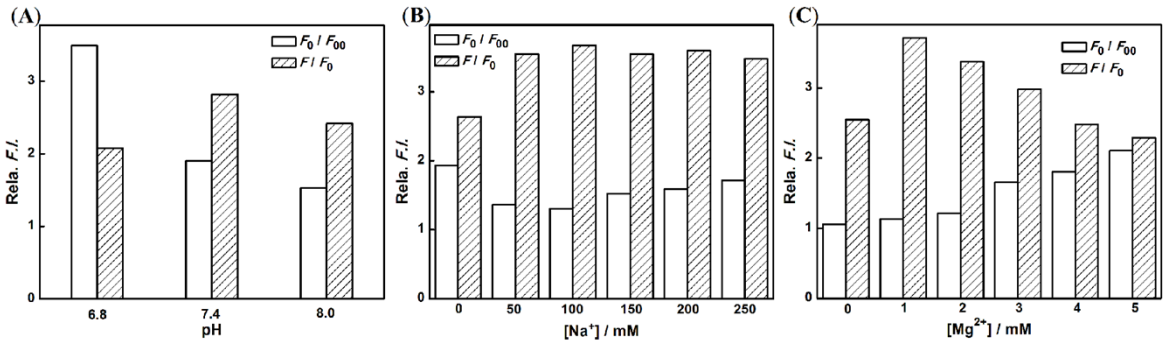

Fig. S1. Optimization of pH (A) and concentrations of Na^+ (B) and Mg^{2+} (C). F_{00}, F_0 and F represent for the fluorescence intensities of mDNA-Ag, mDNA-Ag/8A in the absence and presence of 50 μM melamine, respectively.

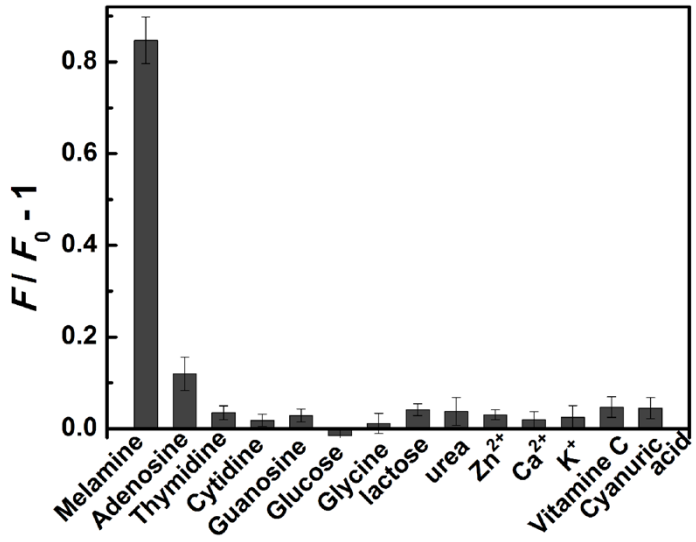

Fig. S2. The selectivity of the proposed system towards melamine detection. The concentrations of melamine and other substances are 4 μM. The error bars illustrate the standard deviations of three independent measurements. F: the fluorescence intensities of mDNA-Ag/8A in the presence of melamine or other substances, F_0: the fluorescence intensity of mDNA-Ag/8A.

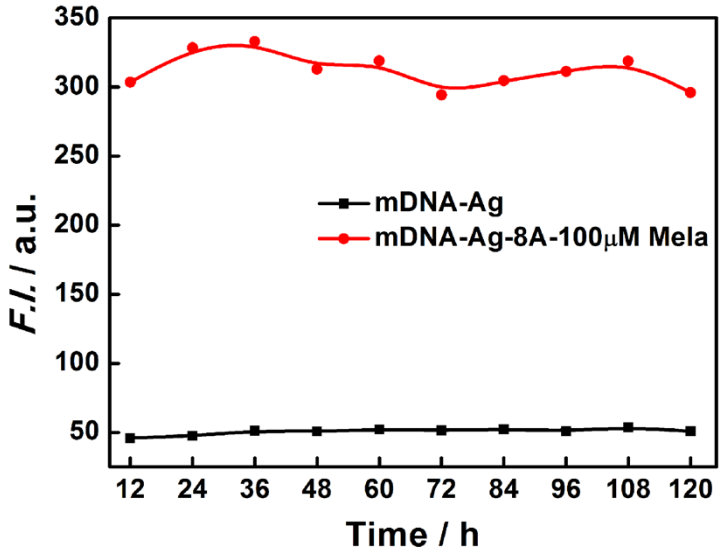

Fig. S3. The stability of the fluorescence of mDNA-Ag itself (black, square) and its responses to melamine (red, round). The fluorescence spectra were measured when the solutions had been stored at 4 °C for corresponding time (from 12 to 120 hours).

Table 2 Recovery of Melamine from Spiked Milk Samples

Samples	Amount added (μM)	Amount found [a] (μM)	Recovery (%)	RSD (%)
1	0.5	0.50 ± 0.04	100.2	6.5
2	1.0	1.01 ± 0.11	101.2	10.5
3	2.0	2.05 ± 0.08	102.5	3.8

[a] Average of three determinations.

References:

1. H. C. Yeh, J. Sharma, J. J. Han, J. S. Martinez and J. H. Werner, *Nano Lett.*, 2010, 10, 3106