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# Dual hairpin-like molecular beacon based on coralyne-adenosine interaction for sensing melamine in dairy products

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ABSTRACT

This study presents a novel dual hairpin-like molecular beacon (MB) for the selective and sensitive detection of melamine (MA) based on the conjugation of MA and thymine. In this protocol, the coordination between coralyne and adenosine (A) leaded a dual hairpin-like MB and the fluorophore–quencher pair is close proximity resulting in the fluorescence quenching. With the addition of MA, it conjugated with thymine in the loop part of dual hairpin-like MB by triple H-bonds, triggering the dissociation of the dual hairpin-like MB. The resulting spatial separation of the fluorophore from quencher induced the enhancement in fluorescence emission. Under the optimized conditions, the sensor exhibited a wide linear range of $8\times10^{-9}$–$1.6\times10^{-5}$ M ($R^2$=0.9969) towards MA, with a low detection limit of 5 nM, approximately 4000 times lower than the Drug Administration and the US Food estimated MA safety limit. The real milk samples were also investigated with a satisfying result.



## 1. Introduction

Since Tyagi and Kramer first described the molecular beacons (MBs) which can produce fluorescence without having to separate probe-target hybrids from excess probes in hybridization assays [1], it has received remarkable attention for wide applications in areas such as genetic screening [2–4], the monitoring of living systems [5–7], the investigation of enzymatic processes [8–10], the development of biosensors [11,12], the study of protein–DNA interactions [13,14], and the construction of biochips [15,16]. Generally, typical MBs are single-stranded oligonucleotides that contained a stem-loop structure with a fluorophore–quencher pair at 5′- and 3′-ends. The stem can self-hybridize that brings the fluorophore–quencher pair into close proximity, whereby fluorescence is quenched effectively. Hybridization to a complementary target disrupts the self-hybridization of the stem resulting spatial separation of the fluorophore from quencher and inducing the enhancement in fluorescence emission [1,11,16]. Although MB-based detection system is one of the most successful separation-free probes [17,18], typical MBs do not perform well at room temperature without significant and empirical optimization of their thermodynamics [19]. To solve these problems, T (thymine)–$Hg^{2+}$–T and C (cytosine)–$Ag^{+}$–C based MBs have been studied due to that the stable T–$Hg^{2+}$–T or C–$Ag^{+}$–C structures make the sensors possess relatively high stability, selectivity and short analysis time [20,21]. Nevertheless, as we know, either $Hg^{2+}$ or $Ag^{+}$ is harmful to aquatic ecosystems and human health [22–24]. Hence, it is required to explore other lowly toxic molecules for MBs.

Coralyne (5,6,7,8,13,13a-hexadehydro-8-methyl-2,3,-10,11-tetramethoxy berbinium chloride) (see Scheme 1A), a small crescent-shaped planar heterocyclic molecule, possesses pronounced antitumor activity among the protoberberine alkaloids [25,26]. It is found that its fused planar cationic aromatic ring system provides the capability of interaction with polyadenosine [27]. In the neutral pH solution coralyne promotes an excellently stable antiparallel duplex of polyadenosine, with an association constant of $1.8\times10^{6}$ $M^{-1}$ and a stoichiometry of one coralyne to four adenine bases ($A_2$-coralyne-$A_2$) [27,28–30]. What attracts the researchers most is the activity coupled with relatively low toxicity and high stability of $A_2$-coralyne-$A_2$ cooperation [31]. Thus, it is hopeful to build a MB based on the interaction between coralyne and polyadenosine.

Melamine (MA, 2,4,6-triamino-1,3,5-triazine), a chemical compound broadly used in the synthesis of melamine resins for manufacturing laminates, coatings, adhesives, dishware, plastics and so on [32,33]. Nevertheless, because of its high nitrogen level (about 66% by mass) and low cost, it has been illegally added to the protein-rich food such as infant formula and pet food abused to increase the apparent protein level and cannot be detected by

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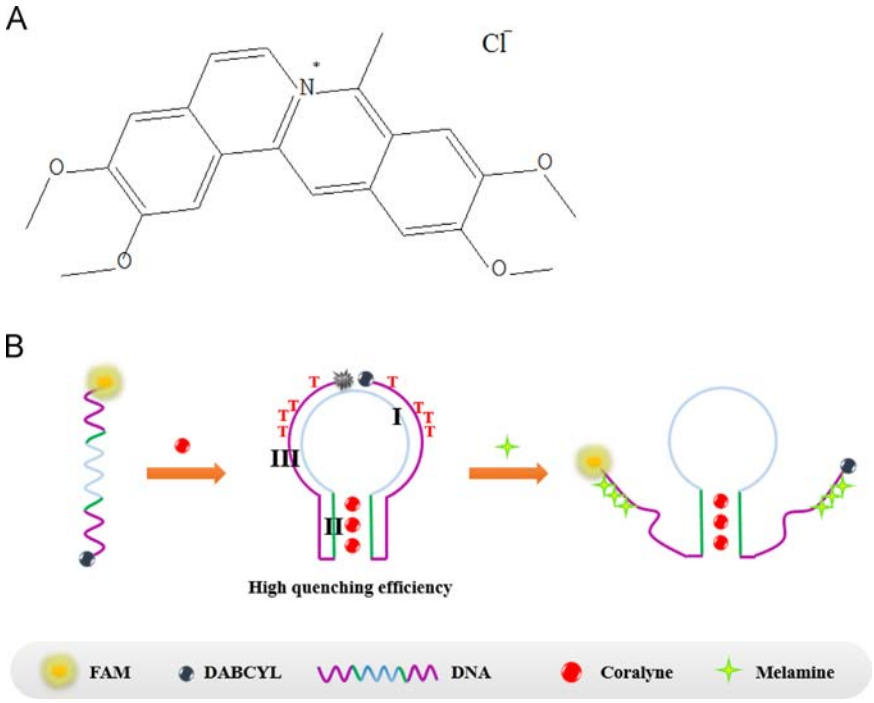



**Scheme 1.** (A) The structure of coralyne; (B) Schematic illustration of dual hairpin-like MBs based on $A_2$-coralyne-$A_2$ complexes and the formation of triple H-bonds for MA Assay .

kjeldah method [34]. However, MA cannot be absorbed by animals metabolically, leading to subsequent tissue injury and even death above the safety regulation level. Therefore, the detection of MA are of particular significance in people's daily living and production. Recently, various methods for the detection of melamine are developed including chromatography (GC) [35–38], capillary electrophoresis [39,40], surface-enhanced Raman spectroscopy (SERS) [41–47], electrochemiluminescence (ECL) [48], and fluorescence [49,50], et al. However, some of them are not readily adaptable to routine analysis due to their time-consuming and complicated processes. Thus, developing rapid, simple and sensitive methods to detect MA becomes especially urgent.

In this work, we designed a coralyne-adenosine based dual hairpin-like MB probe for the detection of MA. We demonstrated that the presence of coralyne can efficiently form a dual hairpin-like MB. Because there is a stable triple H-bond between thymine and MA (Fig. 1) [51], the conjugation of thymine with MA triggered the separation of fluorophore–quencher pair, resulting in the restoration of fluorescence. This made the dual hairpin-like MB can detect MA at neutral pH and room temperature with high sensitivity and selectivity, even for the real sample detection.

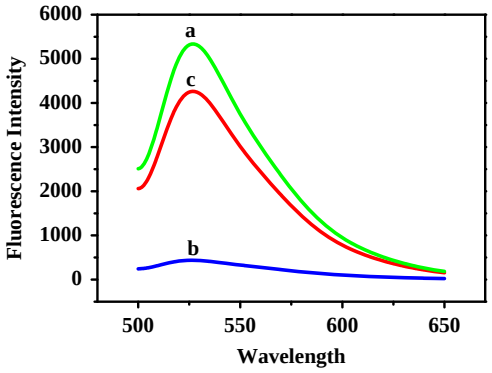



**Fig. 1.** Fluorescence spectra of solutions containing (a) 20 nM S1; (b) 20 nM S1 after 40 min of self-assembly; (c) 20 nM S1 and 4 μM coralyne; (d) 20 nM S1 and 4 μM coralyne in the presence of 2000 μg/L MA.

## 2. Experimental

### 2.1. Reagents and instrument

Oligonucleotide (S1: 5′-FAM-TCC TTT GGC GCG C6 A6 GGA GCC CCC GGA AGG CCC CCG AGG A6 C6 GCG CGG TTT CCT-DABCYL-3′) was synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and purified by HPLC. It was dissolved in Tris (hydroxymethyl) aminomethane (Tris)–HCl buffer (pH 7.4) as stock solution and the concentration was identified according to UV absorption at 260 nm. FAM: 5-carboxyfluorescein (at the 5′-end); DABCYL: 4-([4-(dimethylamino) phenoyl] azo)-benzoic acid (at the 3′-end), coralyne chloride hydrate and Tris were purchased from Sigma-Aldrich (St. Louis, MO, USA) used without further purification. MA was purchased from Sinopharm Chemical Reagent Co., Ltd. All the stocks and buffer solutions were prepared by using ultrapure water (PSDK2-10-C, Beijing, China). Fluorescence spectra were recorded using F-4600 fluorescence spectrophotometer (Hitachi, Japan).

### 2.2. Preparation of dual hairpin-like MB

DNA probe was prepared in a solution containing 20 mM Tris–HCl buffer (pH 7.4). The mixture was heated at 88 °C for 10 min, and gradually cooled to the room temperature. After heat treatment, DNA probe solution (20 nM) was mixed with coralyne solution at 1:1 volume ratio and incubated for 50 min at room temperature to form dual hairpin-like MB. The fluorescence emission spectrum was used to characterize the formation of MB at an excitation wavelength of 490 nm.

### 2.3. Fluorescence detection of MA

A stock solution of MA (10 mg/L) was prepared in water. By serial dilution of the stock solution, MA solutions with various concentrations (1, 25, 125, 250, 500, 750, 1000, 1500, 2000, 3000, 4000 μg/L) were obtained for the sensitivity measurement. For the detection of MA, dual hairpin-like MB solution (20 nM, 1 mL) was mixed with 100 μL MA aqueous solutions with different concentrations. After 10 min, the fluorescence intensities were recorded ($\lambda^{ex}$=490 nm). For the selectivity measurement, various coexisting substances of 1000 μg/L were supplied with their salts. The fluorescence emission spectra were monitored after the introduction of coexisting substances into the test assay.

### 2.4. Preparation of milk samples

To prepare in-house reference materials, milk powder, egg powder and soy protein were obtained from local supermarket.

Sample 1: The milk powder was purchased from local supermarkets. Milk samples were prepared according to the previous report [52]. 1 mL of acetonitrile, 1 mL of $CCl_3COOH$, and 7 mL of water were added into 0.5 g of milk powder. Then the mixture in a centrifuge tube was ultrasonically extracted for 20 min and further centrifuged at 5000 rpm for 20 min. The obtained supernatant was filtered twice. The filter liquor was diluted as a 1:10 ratio with ultrapure water and the diluents were used for the MA detection.

Sample 2: For egg powder, a well-homogenized test portion was weighed (1.5 g) into a 1000 mL Erlenmeyer flask and suspended in water (3.4 g) for egg powder.

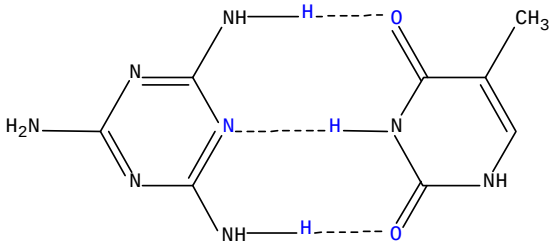
Sample 3: For soy protein, a well-homogenized test portion was weighed (1.5 g) into a 1000 mL Erlenmeyer flask and suspended in water (7.5 g) for soy protein.

The resulting slurries of sample 2 and 3 were fortified with 2.0 and 10.0 g of the 100 μg/mL unlabeled working standard solution of MA, respectively. The samples were mixed and freeze-dried overnight. The resulting powder was homogenized for 2 h in a turbula mixer with either egg powder or soy protein previously checked to be free from MA. Concentrations of both analytes in the mixtures of egg powder and soy protein were 0.1 and 0.5 mg/kg, respectively. Portions of the test materials (around 0.5 g) were packed into foil pouches and stored at room temperature.

## 3. Results and discussion

### 3.1. Sensing principle

The fabrication of the coralyne-adenosine based dual hairpin-like MBs for the detection of MA was shown in Scheme 1B. The functional oligonucleotide probe (S1) was designed containing three units: 24-mer A-rich sequences (region I, blue part), 6-mer A base pairs (region II, green part) and T-rich sequences (region III, purple part). In the absence of coralyne, the S1 probe was in a free state, and the spatial separation of the fluorophore–quencher pair resulted in a good fluorescence intensity. Whereas, a stable duplex of region II formed through the $A_2$-coralyne-$A_2$ interaction in the presence of coralyne, which forced the whole region I to form the inner loop structure. Because the inner loop part contained enough adenine bases which could hybridize with the thymine bases of the region III, thus, a dual hairpin-like MB structure formed. At this time, FAM and DABCYL were close proximity, resulting in the quenching of the fluorescence. As MA was added, because there is a strong triple-H bonds interaction between thymine and MA (Scheme 2), the conjugation of MA and thymine triggered the dissociation of region III from the inner loop part.




**Scheme 2.** The interaction between MA and thymine .

The increasing distance of FAM and DABCYL made the restoration of fluorescence. In this way, a conformational change of the dual hairpin-like MB occurred owing to the participation of MA. This strategy may provide a new insight into the detection of MA by an efficient fluorescence method.

### 3.2. Feasibility of the proposed signaling principle

We carried out a series of experiments to verify the feasibility of the proposed design. Fig. 1 showed the fluorescence emission spectra of S1 (curve *a*), S1/coralyne (curve *b*), and S1/coralyne/MA (curve *c*). As demonstrated in Fig. 1, the free S1 showed strong fluorescence emission in the absence of coralyne at 520 nm in pH 7.4 Tris–HCl buffer (curve *a*). It may be because the distance between the quencher and fluorophore was far. When the coralyne was introduced to the free S1 solution, as expected, more than 97% fluorescence was greatly quenched (curve *b*, Fig. 1), which may result from the conjugation of adenine and coralyne and further the close proximity of the fluorophore and quencher pair. Subsequently, with the addition of MA to the S1/coralyne system, the significant fluorescence recovery (curve *c*, Fig. 1) was observed. Due to the addition of MA, the hydrogen bond of thymine-adenine was destroyed by forming triple H-bonds between MA and thymine, leading to recovery of the fluorescence of this system. This fact made it possible to detect MA by the present system.

### 3.3. Optimization of the variables of the measuring system

In order to obtain the highest quenching efficiency and the best binding ability of MA with thymine, optimization for the experimental conditions were necessary. Because the sensing capability of dual hairpin-like MB was highly dependent on the interaction between coralyne and adenines, we investigated the effect of the concentration of coralyne. The dual hairpin-like MB (20 nM, 500 μL) probe was mixed with series concentrations of coralyne from 0 to 10 μM. Fig. 2A showed that the fluorescence intensity of FAM reduced sharply as the concentration of coralyne increasing from 0 to 4 μM ($\lambda^{ex}$=490 nm). After that, the fluorescence quenching reached a saturation level. So we selected the optimal concentration of coralyne as 4 μM.

To investigate whether buffer pH value could affect the dual hairpin-like MB system, we carried out the reaction in buffers at different pH values (pH 6–9), respectively. Fig. 2B showed the effects of the pH value on the performance of the sensor. The fluorescence intensity was not significantly affected by pH value, suggesting the buffer pH may not affect the fluorescence properties of the dyes obviously. Because the dual hairpin-like MB and $A_2$-coralyne-$A_2$ coordination was stable at neutral pH, dual hairpin-like MB was prepared with a neutral pH to conjugate with coralyne.

The effect of the incubation time for $A_2$-coralyne-$A_2$ complex on the performance of the sensor was also investigated. Fig. 2C showed the time-dependent fluorescence intensity response of

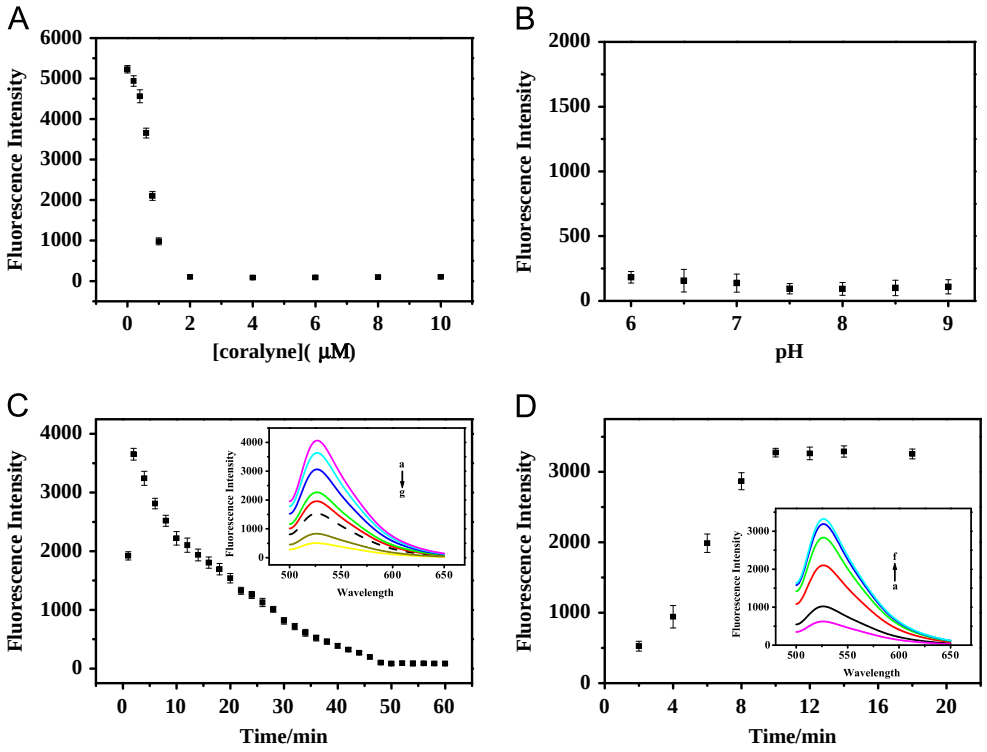



**Fig. 2.** Effect of different (A) concentrations of coralyne, (B) solution pH, (C) time course of coralyne reaction and (D) time course of MA reaction on the fluorescence intensity of the dual hairpin-like MB; ($\lambda^{ex}$=490 nm); (C) and (D) inset: Fluorescence spectra of the intensity change of dual hairpin-like MB ($\lambda^{ex}$=490 nm). The arrow from *a* to *g* or *f* indicates the increasing time. The error bars represent standard deviations based on three independent measurements.

FAM in the presence of coralyne. As shown, the coralyne-induced fluorescence quenching was nearly complete after approximately 50 min, suggesting the interaction of adenosine with coralyne reached equilibrium within 50 min. Therefore, 50 min was chosen as the optimal reaction time for conjugation with coralyne. We also investigated the incubation time of MA with the system. We can observe that the fluorescence response increased with the increasing of the incubation time from 0 to 10 min, and reached a plateau after 10 min (Fig. 2D), so we chose 10 min as the optimal reaction time between thymine and MA.

### 3.4. The analytical performance of the sensor

Under optimal conditions, the analytical performance of the dual hairpin-like MB for the detection of MA was investigated. The fluorescence emission intensity of the assay was measured with various concentrations of MA (1, 25, 125, 250, 500, 750, 1000, 1500, 2000 μg/L) in the dual hairpin-like MBs system. As shown in Fig. 3A, the fluorescence intensity increased with the concentration of MA increasing from 1 to 2000 μg/L, indicating that the fluorescence intensity was highly dependent on the concentration of MA. Fig. 3B showed the relationship between the fluorescence intensity and the concentration of MA. Under the optimal conditions, a good linear correlation from 1 to 2000 μg/L is obtained (Fig. 3C). The linear regression equation was $F=550.50+1.82C$ ($R^2=0.9969$), where $F$ refers to fluorescence intensity and $C$ refers to the concentration of MA. The Food and Drug Administration (FDA), the European Community, and the other countries have established criteria for maximum residua limits of MA in a variety of foods [53]. Generally, standard limits of 1 ppm (8 μM) for MA in infant formula and 2.5 ppm (20 μM) in other milk powder milk products have been introduced by many countries. Our method here was very sensitive to MA with a detection limit measured by $3\sigma b$/slope ($\sigma b$=standard deviation of the blank samples) to be 6 nM lower than the standard limits, which is comparable to or even lower than those of some previously reported fluorescent methods (Table 1 [54–59]).

### 3.5. Selectivity and reproducibility

Selectivity is an important property of the fluorescent sensor. Under the optimal conditions, to evaluate the effect of the coexisting substances on the specificity of dual hairpin-like MB, $K^+$, $Na^+$, $NH_4^+$, $Cl^-$, $PO_4^{3-}$, $SO_4^{2-}$, $Ac^-$, glucose, alanine, arginine, tyrosine, monosodium glutamate, $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, $Zn^{2+}$, and $Cu^{2+}$ were studied with the dual hairpin-like MB. Following the addition of 1000 μg/L MA, the fluorescence spectra of the resulting solutions were recorded. Fig. 4 showed that only MA caused a remarkable increase in the fluorescence intensity, showing that this probe is highly selective to MA over other ions. These results here clearly revealed the high selectivity of the sensing method toward MB against other ions, which was primarily associated with the high binding specificity of the thymine in dual hairpin-like MB probe toward MA. And it also indicated that the approach is not only insensitive to other interferences but also selective toward MA in their presence. The reproducibility of the sensor was conducted by determining 1000 μg/L MA with six dual hairpin-like MB sensors which were fabricated following the same procedure. The relative standard deviation (RSD) of 3.2% was obtained, which indicated that the sensor had good reproducibility.

### 3.6. Detection of MA in real samples

Although the proposed sensor showed high selectivity towards MA, a much more challenging goal is to detect MA in real samples, since the milk powder is one of the most heavily regulated products in the food industry. In order to validate the reliability of the proposed method, we applied a standard addition method to detect MA in milk powder, egg powder and soy protein from local supermarket. The addition of the samples spiked with

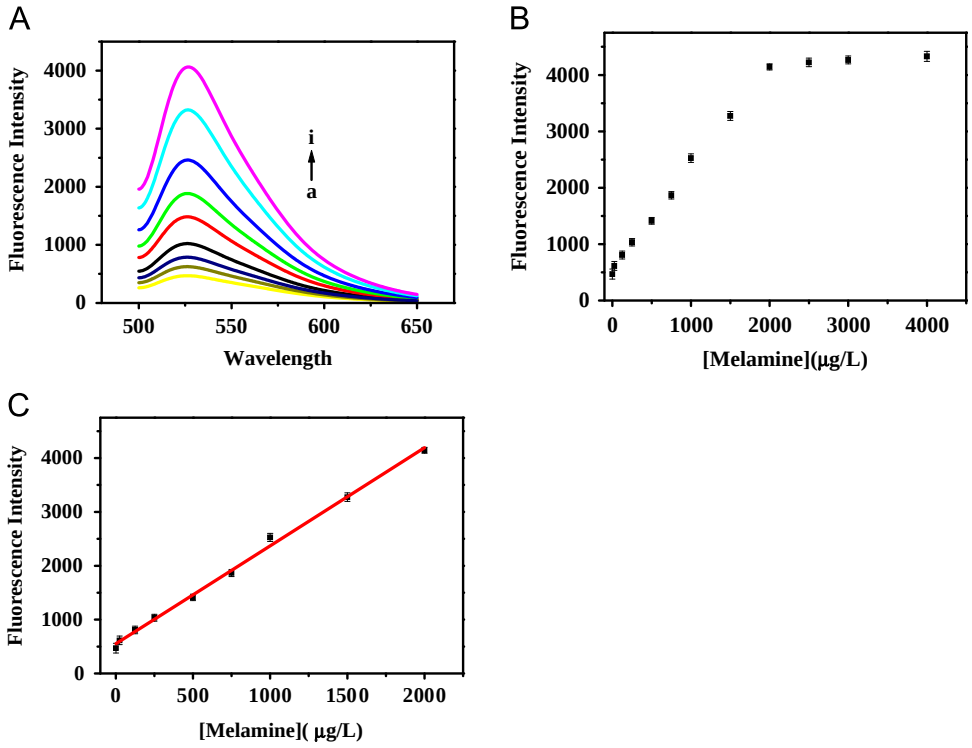



**Fig. 3.** (A) Fluorescence emission spectra at different concentrations of MA (1, 25, 125, 250, 500, 750, 1000, 1500, 2000 μg/L from *a* to *i*) ($\lambda^{ex}$=490 nm); (B) Relationship between the fluorescence intensity and the concentration of MA; (C) The linear relationship of the fluorescence intensity and the concentrations of MA. The error bars represent standard deviations based on three independent measurements.

**Table 1**
Comparison of different methods for the determination of MA.

| Detection | Principle | Linear range | Detection limit | Signal reporter | Reference |
|---|---|---|---|---|---|
| Colorimetry | Melamine-induced color change | 0.08–10.0 μM | 80 nM | Dopamine-stabilized Ag NPs | [57] |
| Colorimetric visualization | Melamine-induced color change | 4.0–170 μM | 2.32 μM | Label-free Ag NPs | [58] |
| visualization | Melamine-induced color change | 4–88 μM | 1.2 μM | Unmodified Au NPs | [54] |
| Visualization and light scattering | Hydrogen-bonding recognition-induced aggregation | 0.08–1 μM | 20 nM | Polythymine-modified Au NPs | [59] |
| SERS | Enhanced-Raman- Scattering | – | 0.1 μM | Ag-NPs coated PSA | [56] |
| Fluorescence | Fluorescence enhancement | 0.099–9.91 μM | 0.059 μM | 5-(9-Anthracenylmethylene) barbituric acid (DBA) | [55] |
| Fluorescence | Fluorescence enhancement | 0.05–7 μM | 10 nM | DNA/Ag NCs | [49] |
| Fluorescence | Fluorescence enhancement | $2\times10^{-9}$ –$5\times10^{-5}$ M | 1 nM | CdS QDs | [50] |
| Fluorescence | Hydrogen-bonding recognition-induced | $8\times10^{-9}$ –$1.6\times10^{-5}$ M | 6 nM | FAM | This work |

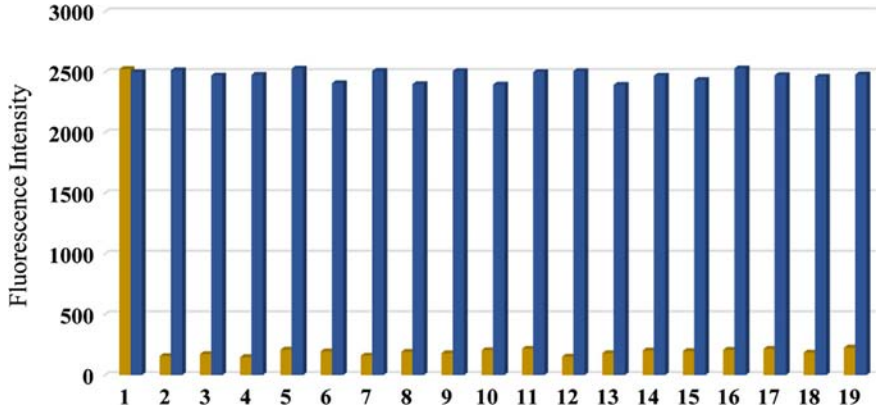



**Fig. 4.** Effect of MA and other relevant analytes on the fluorescence intensity of dual hairpin-like MB. The concentration of MA was 1000 μg/L. For others the concentration was 1500 μg/L.

**Table 2**
Results of the determination of the MA in real samples.

| Sample | MA spiked (μg/L) | MA founded (μg/L) | Recovery ($n$=6) |
|---|---|---|---|
| Sample 1 | 0 | – | – |
| | 25 | 24.82 | 99.28 |
| | 50 | 49.26 | 98.52 |
| | 125 | 123.84 | 98.72 |
| Sample 2 | 0 | – | – |
| | 25 | 24.86 | 99.44 |
| | 50 | 49.53 | 99.06 |
| | 125 | 123.12 | 98.5 |
| Sample 3 | 0 | – | – |
| | 25 | 24.77 | 99.08 |
| | 50 | 49.31 | 98.62 |
| | 125 | 123.29 | 98.63 |

different concentrations of MA led to a significant increase in the fluorescence intensity of the probe. Table 2 indicated the recovery of the MA was in the range of 98.5–99.44%, indicating that this method could be applied successfully for determination MA in various real world samples.

## 4. Conclusion

In the investigation described above, we have designed a new MA sensing strategy based on $A_2$-coralyne-$A_2$ MB and the triple H-bonds between MA and thymine. This method can detect MA at room temperature in neutral solutions. Our work showed the present system has a wide linear range and a low detection limit. The excellent selectivity toward MA was also demonstrated with multiple coexisting substances. Compared with others, the sensor is more environmentally friendly and lowly toxic because of the use of coralyne. This sensor was successfully used to detect MA in real milk sample. It is expected that the proposed approach may have a great promise to be used in other applications such as pet foods and milk-based foods.

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## References

[1] S. Tyagi, F.R. Kramer, Nat. Biotechnol. 14 (1996) 303–308.
[2] P. Zhang, T. Beck, W.H. Tan, Angew. Chem. Int. Ed. 113 (2001) 416–419.
[3] Z.Y. Xiao, X.T. Guo, L.S. Ling, Chem. Commun 49 (2013) 3573–3575.
[4] F.L. Gao, J.P. Lei, H.X. Ju, Chem. Commun 49 (2013) 4006–4008.
[5] D.P. Bratu, B.J. Cha, M.M. Mhlanga, F.R. Kramer, S. Tyagi, Proc. Nat. Acad. Sci. U. S.A. 100 (2003) 13308–13313.
[6] C.D. Medley, T.J. Drake, J.M. Tomasini, R.J. Rogers, W.H. Tan, Anal. Chem. 77 (2005) 4713–4718.
[7] X.Y. Lin, C. Zhang, Y.S. Huang, Z. Zhu, X. Chen, C.Y.J. Yang, Chem. Commun 49 (2013) 7243–7245.
[8] J.J. Li, R. Geyer, W. Tan, Nucleic Acids Res. 28 (2000) e52.
[9] Z.W. Tang, K.M. Wang, W.H. Tan, J. Li, L.F. Liu, Q.P. Guo, X.X. Meng, Nucleic Acids Res. 31 (2003) e184.
[10] Z.W. Tang, K.M. Wang, W.H. Tan, C.B. Ma, J. Li, L.F. Liu, Q.P. Guo, X.X. Meng, Nucleic Acids Res. 33 (2005) e97.
[11] X.H. Fang, X.J. Liu, S. Schuster, W.H. Tan, J. Am. Chem. Soc. 121 (1999) 2921–2922.
[12] J. Li, W. Tan, K. Wang, D. Xiao, X. Yang, X. He, Z. Tang, Anal. Sci. 17 (2001) 1149–1153.
[13] X.H. Fang, J.J. Li, W.H. Tan, Anal. Chem. 72 (2000) 3280–3285.
[14] J.W.J. Li, X.H. Fang, S.M. Schuster, W.H. Tan, Angew. Chem. Int. Ed. 112 (2000) 1091–1094.
[15] W.H. Tan, K.M. Wang, T.J. Drake, Curr. Opin. Chem. Biol. 8 (2004) 547–553.
[16] H. Wang, J. Li, H. Liu, Q. Liu, Q. Mei, Y. Wang, J. Zhu, N. He, Z. Lu, Nucleic Acids Res. 30 (2002) e61.
[17] Y. Guo, P. Xu, H. Hu, X. Zhou, J. Hu, Talanta 114 (2013) 38–142.
[18] X.H. Fang, X.J. Liu, S. Schuster, W.H. Tan, J. Am. Chem. Soc. 121 (1999) 2921–2922.
[19] Y. Xiao, K.J.I. Plakos, X.H. Lou, R.J. White, J.R. Qian, K.W. Plaxco, H.T. Soh, Angew. Chem. Int. Ed. 48 (2009) 4354–4358.
[20] R. Yang, J. Jin, L. Long, Y. Wang, H. Wang, W. Tan, Chem. Commun. (2009) 322–324.
[21] Y. Wang, J. Li, H. Wang, J. Jin, J. Liu, K. Wang, W. Tan, R. Yang, Anal. Chem. 82 (2010) 6607–6612.
[22] E.M. Nolan, S.J. Lippard, Chem. Rev. 108 (2008) 3443–3480.
[23] F. Yilmaz, N. Ozdemir, A. Demirak, A.L. Tuna., Food Chem. 100 (2007) 830–835.
[24] C. Zhao, K.G. Qu, Y.J. Song, C. Xu, X.G. Qu, Chem. Eur. J 16 (2010) 8147–8154.
[25] S. Lee Jeremy, J.P.L., J.H. Ken Laura, Biochemistry 32 (1993) 5591–5597.
[26] M.S. Keppler, S. Neidle, K.R. Fox, Nucleic Acids Res. 29 (2001) 1935–1942.
[27] O. Persil, C.T. Santai, S.S. Jain, N.V. Hud, J. Am. Chem. Soc. 126 (2004) 8644–8645.
[28] F. Xing, G. Song, J. Ren, J.B. Chaires, X. Qu, FEBS Lett. 579 (2005) 5035–5039.
[29] T. Hou, X.Z. Wang, X.J. Liu, Z.F. Du, F. Li, Analyst 138 (2013) 4728–4731.
[30] T. Hou, C.X. Li, X.Z. Wang, C. Zhao, F. Li, Anal. Methods 5 (2013) 4671–4674.
[31] B. Gatto, M.M. Sanders, C. Yu, H.Y. Wu, D. Makhey, E.J. LaVoie, L.F. Liu, Cancer Res. 56 (1996) 2795–2800.
[32] B. Roy, P. Bairi, A.K. Nandi, RSC Adv 4 (2014) 1708–1734.
[33] M.X. Tan, Y.N. Sum, J.Y. Ying, Y.G. Zhang, Energy Environ. Sci. 6 (2013) 3254–3259.
[34] Y.N. Wu, Y.F. Zhao, J.G. Li, Biomed. Environ. Sci. 22 (2009) 95–99.
[35] R.A. Yokley, L.C. Mayer, R. Rezaaiyan, M.E. Manuli, M.W. Cheung, J. Agric. Food Chem. 48 (2000) 3352–3358.
[36] X.L. Zhu, S.H. Wang, Q. Liu, Q. Xu, S.X. Xu, H.L. Chen, J. Agric. Food Chem. 57 (2009) 11075–11080.
[37] Q.R. Cai, Y.Y. Ouyang, Z.J. Qian, Y.F. Peng, Chin. J. Chromatogr. 26 (2008) 339–342.
[38] Y. Sun, W. Ma, L. Xu, Y. Zhu, L. Liu, C. Peng, L. Wang, H. Kuan, C. Xu, Trends Anal. Chem. 29 (2010) 1239–1249.
[39] J.G. Xia, N.Y. Zhou, Y.J. Liu, B. Chen, Y.N. Wu, S.Z. Yao, Food Control 21 (2010) 912–918.
[40] N. Yan, L. Zhou, Z.F. Zhu, X.G. Chen, J. Agric. Food Chem. 57 (2009) 807–811.
[41] L. He, Y. Liu, M. Lin, J. Awika, D.R. Ledoux, H. Li, A. Mustapha, Sens. Instrum. Food Qual. Saf. 2 (2008) 66–71.
[42] B. Liu, M. Lin, L. Hao, Sens. Instrum. Food Qual. Saf. 4 (2010) 13–19.
[43] M. Lin, L. He, J. Awika, L. Yang, D.R. Ledoux, H. Li, A. Mustapha, J. Food Sci. 73 (2008) 129–134.
[44] H.B. Hu, Z.H. Wang, L. Pan, S.P. Zhao, S.Y. Zhu, J. Phys. Chem. C 114 (2010) 7738–7742.
[45] Y. Cheng, Y.Y. Dong, J.H. Wu, X.R. Yang, H. Bai, H.Y. Zheng, D.M. Ren, Y.D. Zou, M. Li, J. Food Compos. Anal. 23 (2010) 199–202.
[46] Y. Cheng, Y.Y. Dong, Food Control 22 (2011) 685–689.
[47] N.N. Yazgan, I.H. Boyac, A. Topcu, U. Tamer, Anal. Bioanal. Chem. 403 (2012) 2009–2017.
[48] Z. Guo, P. Gai, T. Hao, S. Wang, D. Wei, N. Gan, Talanta 83 (2011) 1736–1741.
[49] S. Han, S.Y. Zhu, Z.Y. Liu, L.Z. Hu, S. Parveen, G.B. Xu, Biosens. Bioelectron. 36 (2012) 267–270.
[50] G.L. Wang, H.J. Jiao, X.Y. Zhu, Y.M. Dong, Z.J. Li, Talanta 93 (2012) 398–403.
[51] M. Ma, A. Paredes, D. Bong, J. Am. Chem. Soc. 130 (2008) 14456–14458.
[52] N. Ding, N. Yan, C.L. Ren, X.G. Chen, Anal. Chem. 82 (2010) 5897–5899.
[53] C.W. Kim, J.W. Yun, I.H. Bae, J.S. Lee, H.J. Kang, K.M. Joo, H.J. Jeong, J.H. Chung, Y.H. Park, K.M. Lim, Chem. Res. Toxicol. 23 (2009) 220–227.
[54] L.Q. Guo, J.H. Zhong, J.M. Wu, F.F. Fu, G.N. Chen, X.Y. Zheng, S. Lin, Talanta 82 (2010) 1654–1658.
[55] H. Huang, D.S. Xiang, L. Li, H.G. Li, G.P. Zeng, Z.K. He, Chem. J. Chin 32 (2011) 2504–2508.
[56] J.M. Li, W.F. Ma, C. Wei, L.J. You, J. Guo, J. Hu, C.C. Wang, Langmuir 27 (2011) 14539–14544.
[57] Y.R. Ma, H.Y. Niu, X.L. Zhang, Y.Q. Cai, Analyst 136 (2011) 4192–4196.
[58] H. Ping, M.W. Zhang, H.K. Li, S.G. Li, Q.S. Chen, C.Y. Sun, T.H. Zhang, Food Control 23 (2012) 191–197.
[59] W.J. Qi, D. Wu, J. Ling, C.Z. Huang, Chem. Commun. 46 (2010) 4893–4895.