Supporting information

A Room-Temperature Adenosine-Based Molecular Beacon for Highly Senstivie Detection of Nucleic Acids

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Experimental

Chemicals. Coralyne chloride hydrate trisodium citrate, HNO_3, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), $AgNO_3$, $HgCl_2$, NaCl, Na_2CO_3, $NaNO_3$, H_3PO_4, NaH_2PO_4, Na_2HPO_4 and 3-morpholinopropanesulfonic acid (MOPS), glutathione (GSH), and cysteine were obtained from Sigma-Aldrich (St. Louis, MO, USA). All DNA samples were synthesized from Neogene Biomedicals Corporation (Taiwan). Deoxyribonuclease I was purchased from Novagen (Germany). Single-stranded DNA-binding protein (SSB) was ordered from Promega (Wisconsin, USA). Milli-Q ultrapure water (Hamburg, Germany) was used in all of the experiments.

Sample preparation. Table S1 displays four MBs consisting a donor of FAM at the 5′-end and a quencher of DABCYL at the 3′-end. The A_{16}-MB-A_{16} (20 nM, 200 μL) probe containing 200 mM HEPES (pH 7.0) and 400 mM NaCl was mixed with 200 μL of 0–20 μM coralyne. The T_{16}-MB-T_{16} probe (20 nM, 200 μL) containing 10 mM phosphate (pH 7.0) and 40 mM NaCl was added to 200 μL of 0–20 μM Hg^{2+}. The C_{16}-MB-C_{16} probe (20 nM, 200 μL) containing 20 mM MOPS, 200 mM $NaNO_3$, and 5 mM $Mg(NO_3)_2$ was incubated with 200 μL of 0–20 μM Ag^+. The as-prepared solutions were all equilibrated at ambient temperature for 0–60 min. Aliquots of tested DNA, including DNA_{pm} (1–400 nM, 100 μL) and DNA_{mm1} (30–400 nM, 100

μL), were added to each hairpin-shaped MB (400 μL). After 0–60 min, the fluorescence spectra of these solutions were recorded using a Hitachi F-4500 fluorometer (Hitachi, Tokyo, Japan) at an excitation wavelength of 480 nm. The melting point of each probe was measured by varying the temperature from 25 to 100 °C. To study the impact of the stem length, A_{16}-MB-A_{16} was replaced by A_8-MB-A_8, A_{24}-MB-A_{24}, or A_{32}-MB-A_{32}, once at a time. The base-pairing MB was prepared in a solution containing 10 mM HEPES (pH 7.6) and 300 mM NaCl.[1] The base-pairing MB (20 nM, 200 μL) was added to a solution (200 μL) containing tested DNA, including 200 nM DNA_{pm} and DNA_{mm1}. The mixture was incubated at room temperature. After 1 h, we measured the fluorescence spectra of the mixture.

To evaluate the effect of endonuclease DNase I, SSB, and aminothiols on the specificity of MB, DNase I (0.1 units/μL, 50 μL), SSB (500 nM, 50 μL), and aminothiols (4 mM, 50 μL) were separately added to each hairpin-shaped MB (400 μL). We incubated the mixtures at ambient temperature for 0–30 min and recorded their fluorescence spectra. Following the addition of DNA_{pm} (800 nM, 50 μL), the fluorescence spectra of the resulting solutions were recorded by operating the fluorescence spectrophotometer at an excitation wavelength of 480 nm.

Analysis of DNA_{pm} and DNA_{mm1} in serum. Blood samples were collected from a healthy adult female with the age of 26 years. To obtain serum samples, the collected

whole blood samples were immediately centrifuged at 3000 rpm for 10 min at 4 °C C. Serum samples were diluted to 50-fold with the solution that was used for the A_{16}-MB-A_{16}, T_{16}-MB-T_{16}, and C_{16}-MB-C_{16} probes. The obtained solutions were spiked with 80 nM DNA_{pm} and 80 nM DNA_{mm1}. After that, we incubated each hairpin-shaped MB with the spiked sample for 30 min and recorded the fluorescence spectra.

Reference

1. N. Dave and J. Liu. *J Phys. Chem. B* 2011, **114**, 15694-15699.

Table 1. DNA sequences of MBs and tested DNA

Name	Sequence (5′-3′)
A_{16}-MB-A_{16}	FAM-A_{16} **CCA GAT ACT CAC CGG** A_{16} -DABCYL
T_{16}-MB-T_{16}	FAM-T_{16} **CCA GAT ACT CAC CGG** T_{16} -DABCYL
C_{16}-MB-C_{16}	FAM-C_{16} **CCA GAT ACT CAC CGG** C_{16} -DABCYL
Base-pairing MB	FAM-ACTTAGTT**CCA GAT ACT CAC CGG** AAC TAAGT-DABCYL
DNA_{pm}	5′-CCG GTG AGT ATC TGG-3′
DNA_{mm1}	5′-CCG GTG AAT ATC TGG -3′
DNA_{mm2}	5′-CCG GTG ATT ATC TGG -3′
DNA_{mm3}	5′-CCG GTG ACT ATC TGG -3′
DNA_{mm4}	5′-CCG GAG AGT TTC TGG-3′
DNA_{mm5}	5′-CCG CTG AAT ATG TGG-3′
Non-target DNA	5′-ACA CTG GAC TAT GAT-3′

Table 2. Comparison of different types of MBs for detecting nucleic acids

	BP-MB[a]	T_7-MB -T_7	C_6-MB -C_6	T_{16}-MB -T_{16}	C_{16}-MB -C_{16}	A_{16}-MB -A_{16}
Time for forming a hairpin-shaped MB	-	2 h	NR[a]	>1 h	20 min	3 min
Time for detecting DNA_{pm}	NR[b]	2 h	10 min	8 min	3 min	3 min
Linear range (nM)	NR[b]	2–30	1–40	0.8–80	0.4–40	0.2–80
LOD (nM)	1.5	0.5	NR[b]	0.01	0.1	0.04
Resistance to SSB	No	Yes	Yes	Yes	Yes	Yes
Resistance to nuclease	No	Yes	NR[b]	Yes	Yes	Yes
Resistance to aminothiol	NR[b]	NR[b]	NR[b]	No	No	Yes
Reference	21	21	23	This study	This study	This study

[a] BP, base pairing. [b] NR, not reported

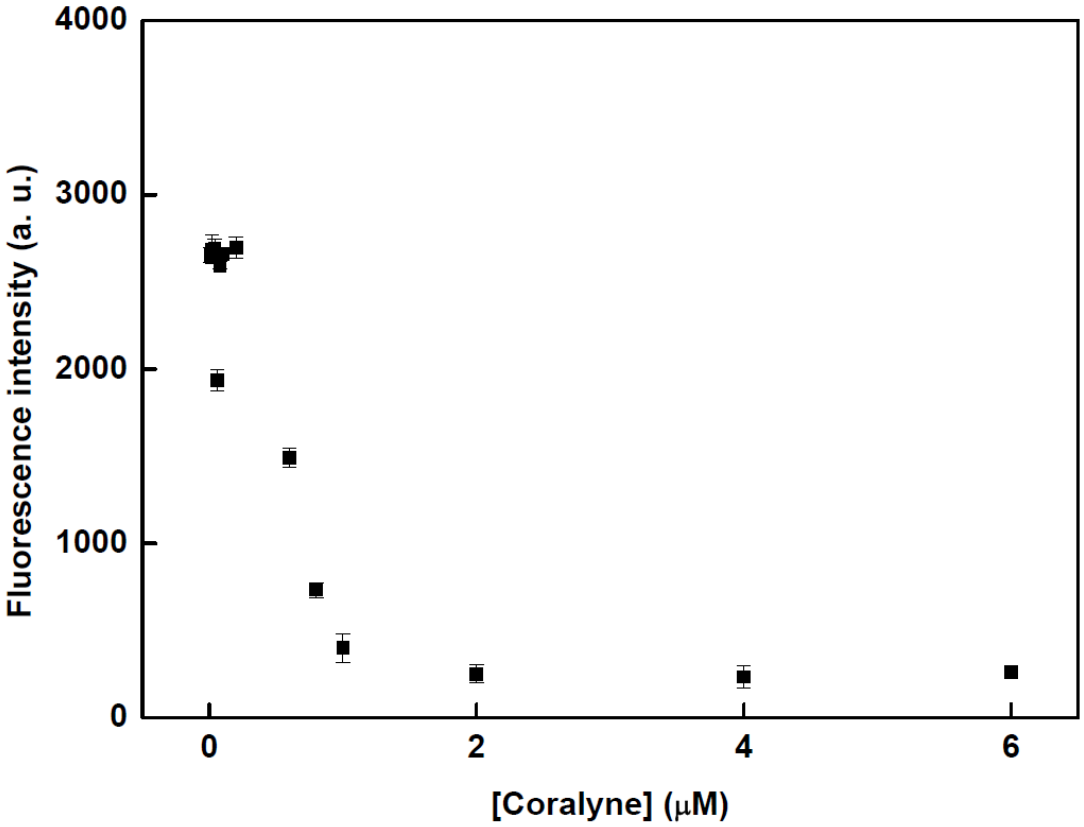

Fig. S1. Effect of coralyne concentration on the fluorescence intensity (518 nm) of 10 nM A_{16}-MB-A_{16}. A mixture of A_{16}-MB-A_{16} and coralyne was incubated in a solution containing 100 mM HEPES (pH 7.0) and 200 mM NaCl for 3 min. The error bars represent standard deviations based on three independent measurements.

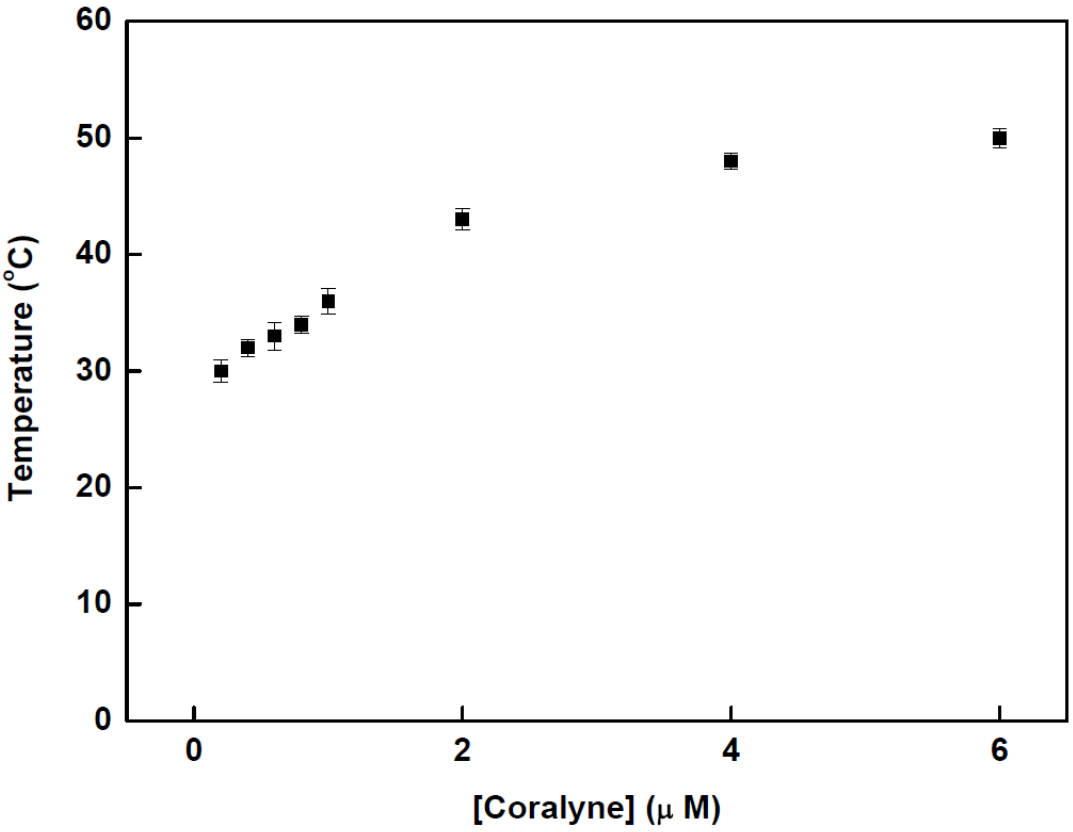

Fig. S2. Effect of coralyne concentration on the melting point of 10 nM A_{16}-MB-A_{16}. A mixture of A_{16}-MB-A_{16} and coralyne was incubated in a solution containing 100 mM HEPES (pH 7.0) and 200 mM NaCl for 3 min. The error bars represent standard deviations based on three independent measurements.

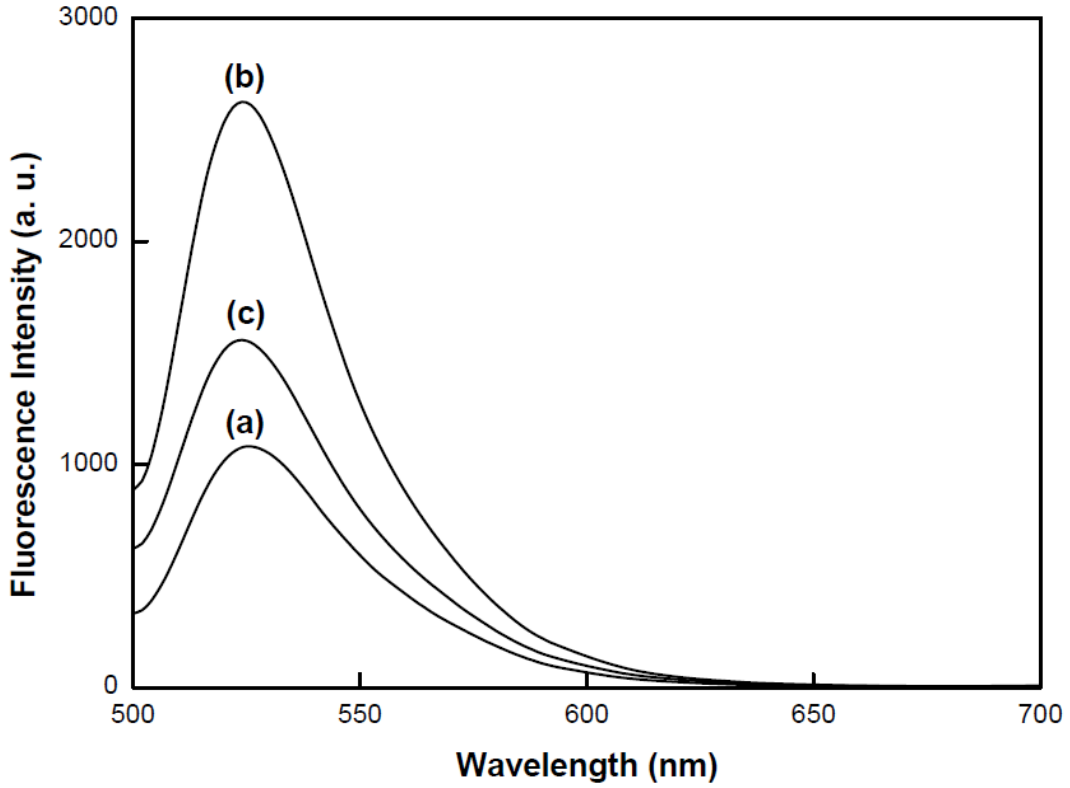

Fig. S3. Fluorescence spectra of a solution containing 10 nM A_{16}-MB-A_{16} and 1 μM coralyne in the (a) absence and (b, c) presence of (b) 80 nM DNA_{pm} and (c) 80 nM DNA_{mm1}. A mixture of A_{16}-MB-A_{16} and coralyne was incubated in a solution containing 100 mM HEPES (pH 7.0) and 200 mM NaCl for 3 min. The incubation time between the coralyne–A_{16}-MB-A_{16} complex and tested DNA was 3 min.

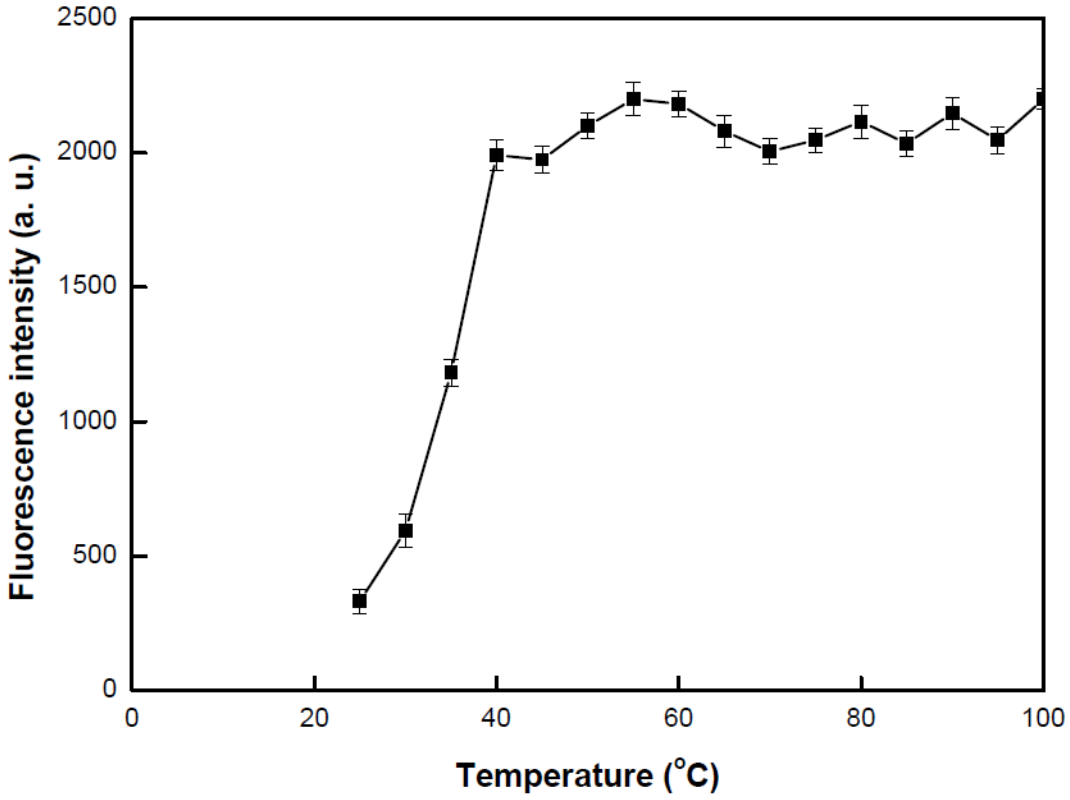

Fig. S4. Fluorescence intensity (518 nm) of a solution containing 10 nM A_{16}-MB-A_{16} and 1 μM coralyne as a functional of temperature. A mixture of A_{16}-MB-A_{16} and coralyne was incubated in a solution containing 100 mM HEPES (pH 7.0) and 200 mM NaCl for 3 min. The error bars represent standard deviations based on three independent measurements.

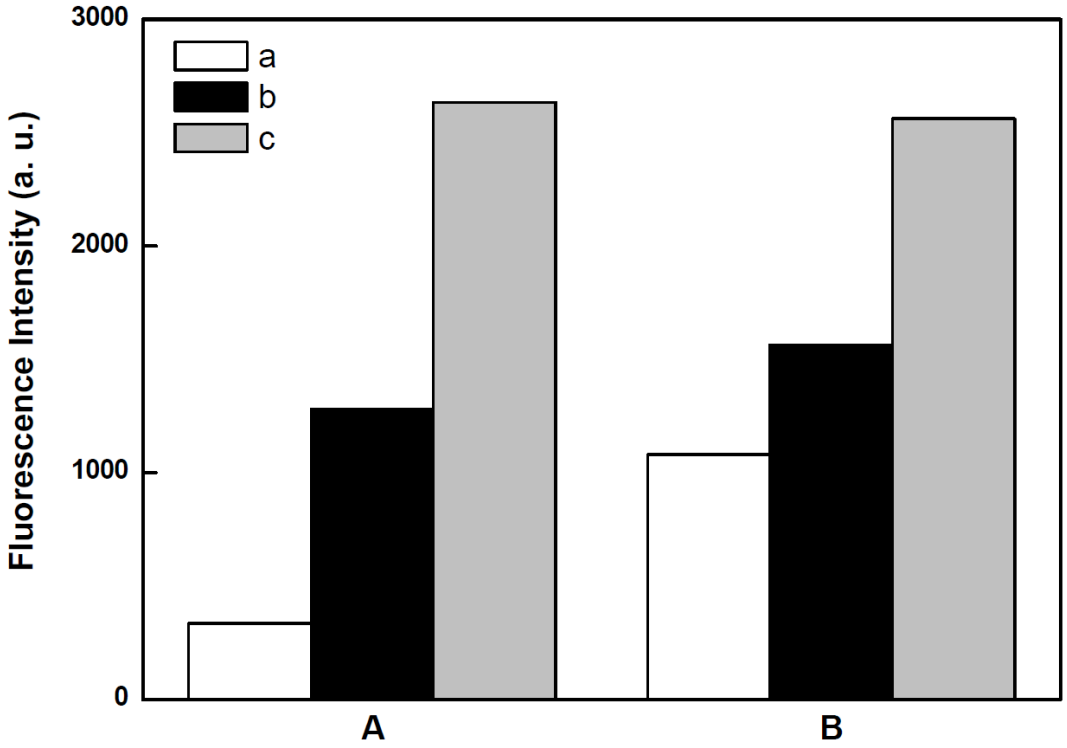

Fig. S5. Fluorescence spectra of a solution containing (A) 10 nM A_{16}-MB-A_{16} and 4 μM coralyne and (B) 10 nM A_{16}-MB-A_{16} and 1 μM coralyne in the (a) absence and (b, c) presence of (b) 80 nM DNA_{mm1} and (c) 80 nM DNA_{pm}. A mixture of A_{16}-MB-A_{16} and coralyne was incubated in a solution containing 100 mM HEPES (pH 7.0) and 200 mM NaCl for 3 min. The incubation time between the coralyne–A_{16}-MB-A_{16} complex and tested DNA was 3 min.

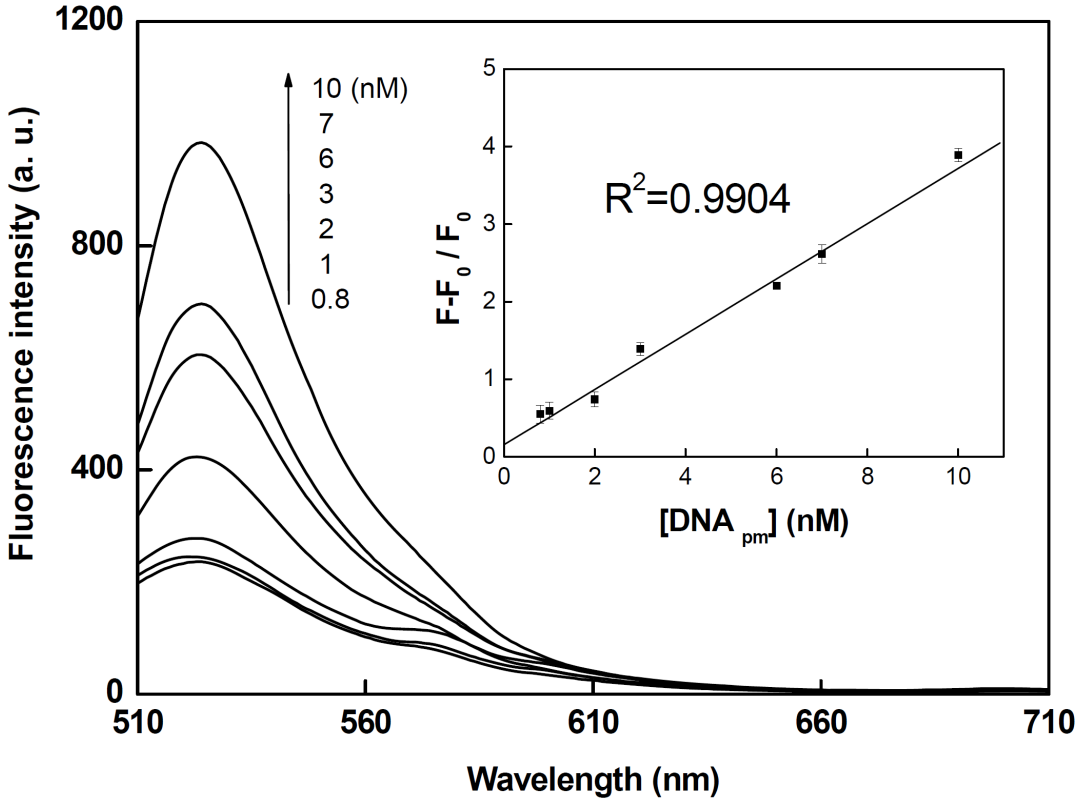

Fig. S6. Fluorescence response of a solution containing 10 nM A_{16}-MB-A_{16} and 4 μM coralyne after the addition of 0.8–10 nM DNA_{pm}. Inset: plot the value of $(F - F_0)/F_0$ against the concentration of DNA_{pm}. F_0 and F correspond to the fluorescence intensity (518 nm) of a solution containing 10 nM A_{16}-MB-A_{16} and 4 μM coralyne in the absence and presence of DNA_{pm}. A mixture of A_{16}-MB-A_{16} and coralyne was incubated in a solution containing 100 mM HEPES (pH 7.0) and 200 mM NaCl for 3 min. The incubation time between the coralyne–A_{16}-MB-A_{16} complex and tested DNA was 3 min. (A, B) The error bars represent standard deviations based on three independent measurements.

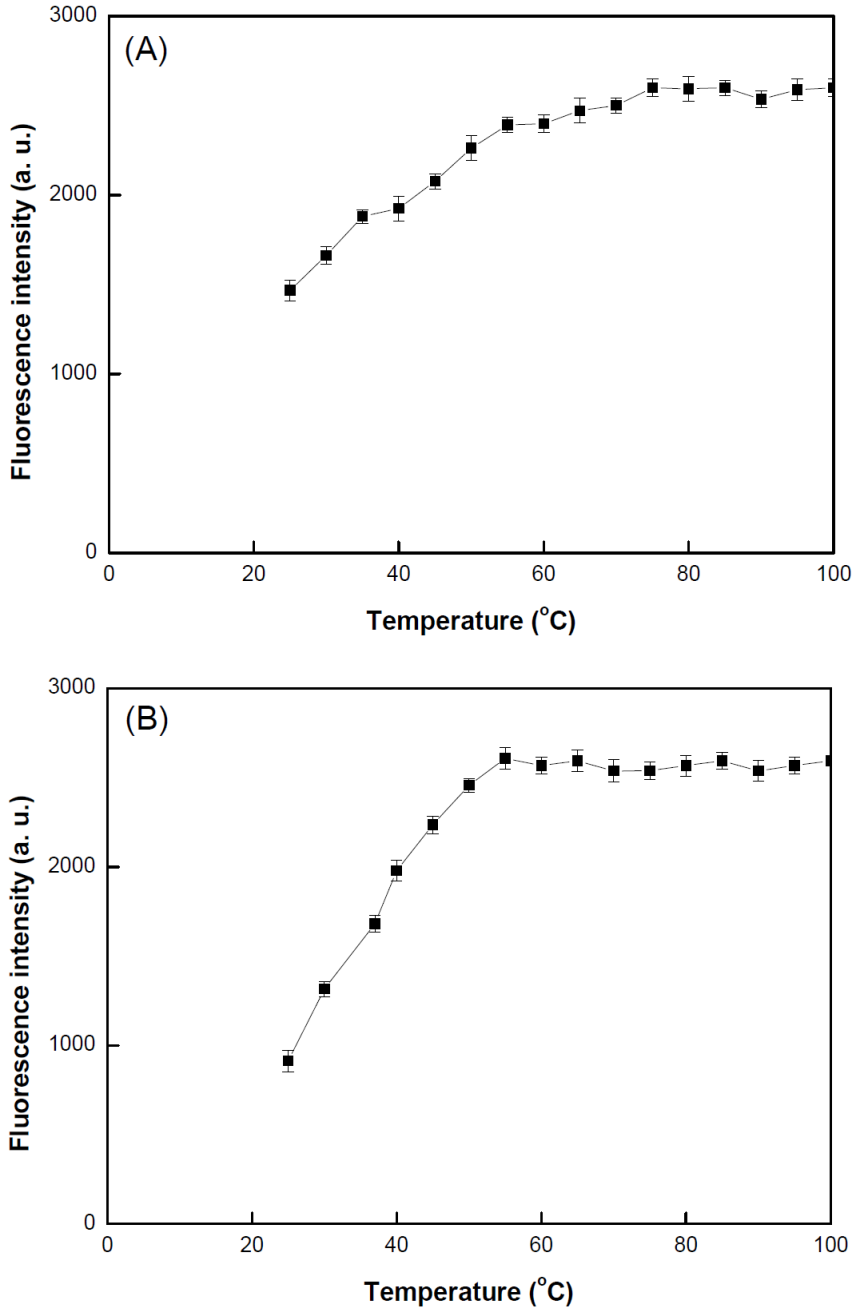

Fig. S7. Fluorescence intensity (518 nm) of a solution containing 10 nM A_{16}-MB-A_{16} and 1 μM coralyne in the presence of (A) 10 nM DNA_{pm} and (B) 10 nM DNA_{mm1} as a functional of temperature. A mixture of A_{16}-MB-A_{16} and coralyne was incubated in a solution containing 100 mM HEPES (pH 7.0) and 200 mM NaCl for 3 min. The incubation time between the coralyne–A_{16}-MB-A_{16} complex and tested DNA was 3 min. The error bars represent standard deviations based on three independent measurements.

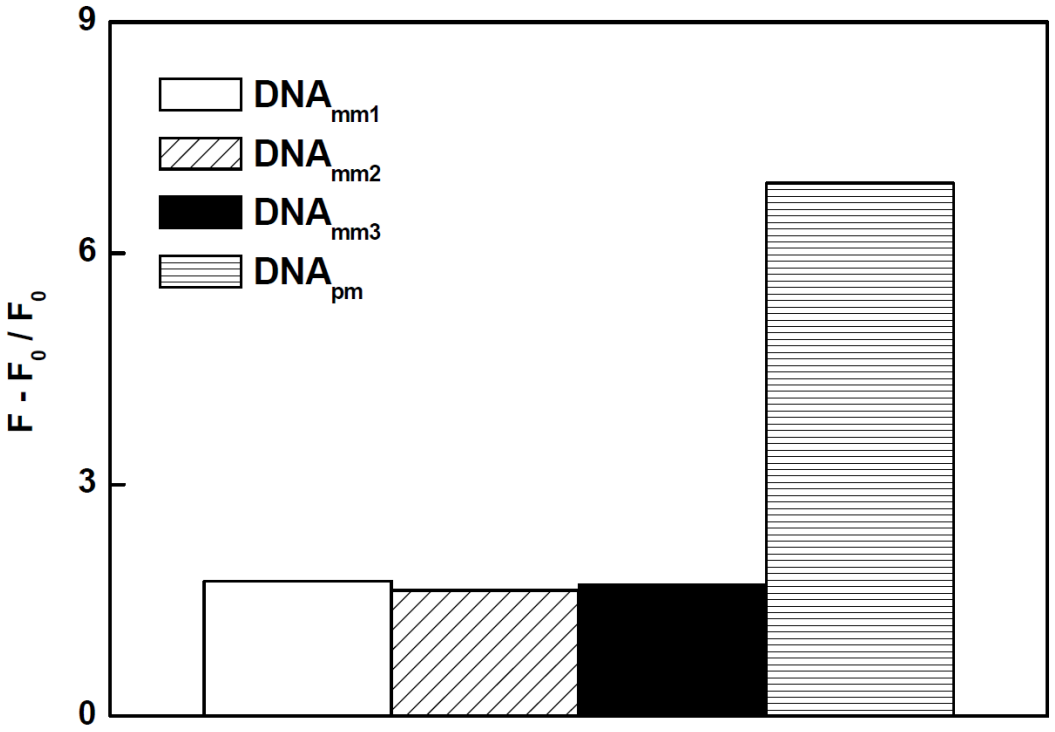

Fig. S8. Fluorescence spectra of a solution containing 10 nM A_{16}-MB-A_{16} and 4 μM coralyne in the presence of 80 nM DNA_{pm}, DNA_{mm1}, DNA_{mm2}, and DNA_{mm3}. A mixture of A_{16}-MB-A_{16} and coralyne was incubated in a solution containing 100 mM HEPES (pH 7.0) and 200 mM NaCl for 3 min. The incubation time between the coralyne–A_{16}-MB-A_{16} complex and tested DNA was 3 min.

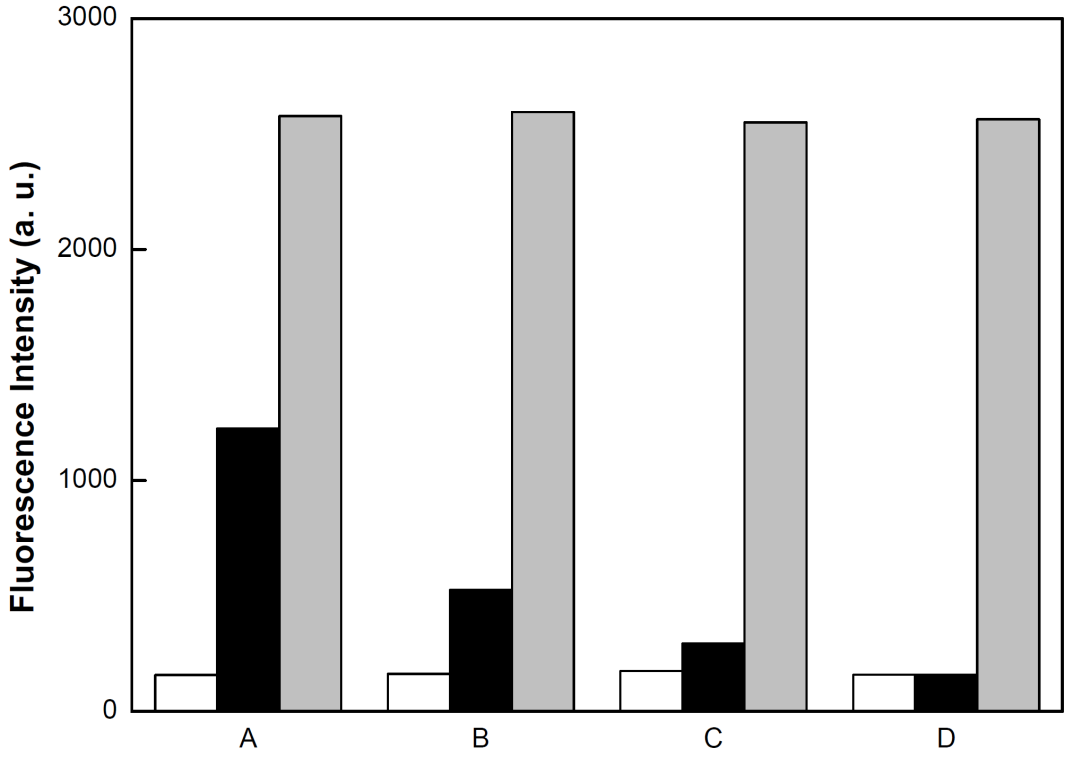

Fig. S9. Fluorescence spectra of a solution containing 10 nM A_{16}-MB-A_{16} and 4 μM coralyne in the absence (white bar) and presence of (A) 80 nM DNA_{mm1} (black bar) and a mixture of 80 nM DNA_{pm} and 80 nM DNA_{mm1} (gray bar), (B) 80 nM DNA_{mm4} (black bar) and a mixture of 80 nM DNA_{pm} and 80 nM DNA_{mm4} (gray bar), (C) 80 nM DNA_{mm5} (black bar) and a mixture of 80 nM DNA_{pm} and 80 nM DNA_{mm5} (gray bar), and (D) 800 nM non-target DNA (black bar) and a mixture of 80 nM DNA_{pm} and 800 nM non-target DNA (gray bar). The DNA sequence of DNA_{mm4}, DNA_{mm5}, and non-target DNA was shown in Table S1. A mixture of A_{16}-MB-A_{16} and coralyne was incubated in a solution containing 100 mM HEPES (pH 7.0) and 200 mM NaCl for 3 min. The incubation time between the coralyne–A_{16}-MB-A_{16} complex and tested DNA was 3 min.

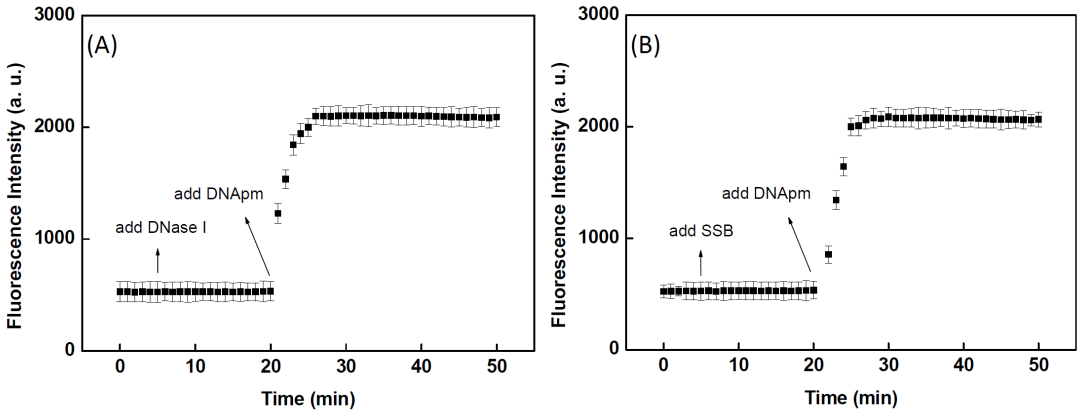

Fig. S10. Fluorescence response of a solution containing 10 nM C_{16}-MB-C_{16} and 6 μM Ag^+ after the addition of (A) 0.01 units/μL DNase I (5th min) and 80 nM DNA_{pm} (20th min) and (B) 50 nM SSB (5th min) and 80 nM DNA_{pm} (20th min). A mixture of C_{16}-MB-C_{16} and Ag^+ was incubated in a solution containing 10 mM MOPS (pH 7.0), 100 mM $NaNO_3$, and 2.5 mM $Mg(NO_3)_2$ for 20 min. The incubation time between the Ag^+–C_{16}-MB-C_{16} complex and tested DNA was 3 min. The error bars represent standard deviations based on three independent measurements.

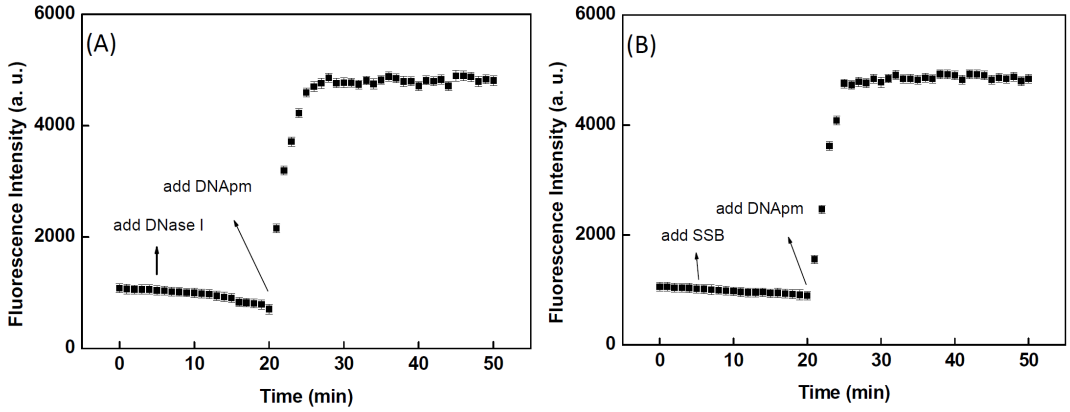

Fig. S11 Fluorescence response of a solution containing 10 nM T_{16}-MB-T_{16} and 1 μM Hg^{2+} after the addition of (A) 0.01 units/μL DNase I (5th min) and 80 nM DNA_{pm} (20th min) and (B) 50 nM SSB (5th min) and 80 nM DNA_{pm} (20th min). A mixture of T_{16}-MB-T_{16} and Hg^{2+} was incubated in a solution containing 5 mM phosphate (pH 7.0) and 20 mM NaCl for 60 min. The incubation time between the Hg^{2+}–T_{16}-MB-T_{16} complex and tested DNA was 8 min. The error bars represent standard deviations based on three independent measurements.

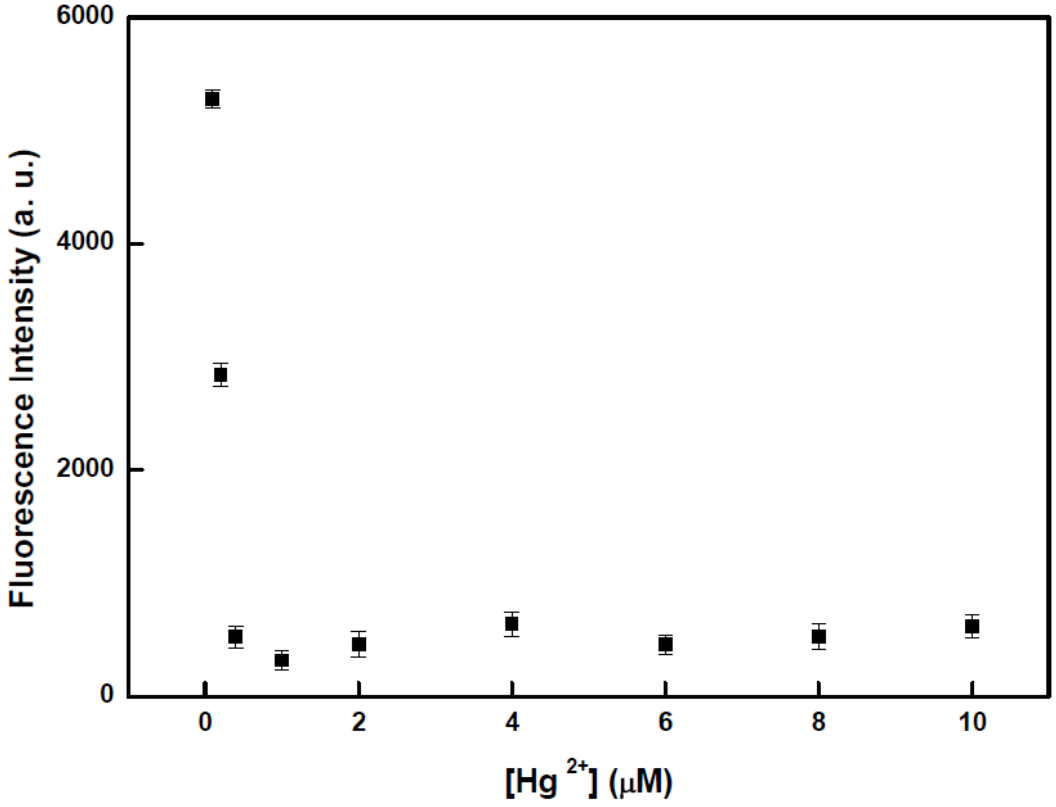

Fig. S12. Effect of Hg^{2+} concentration on the fluorescence intensity (518 nm) of 10 nM T_{16}-MB-T_{16}. A mixture of T_{16}-MB-T_{16} and Hg^{2+} was incubated in a solution containing 5 mM phosphate (pH 7.0) and 20 mM NaCl for 60 min. The incubation time between the Hg^{2+}–T_{16}-MB-T_{16} complex and tested DNA was 8 min. The error bars represent standard deviations based on three independent measurements.

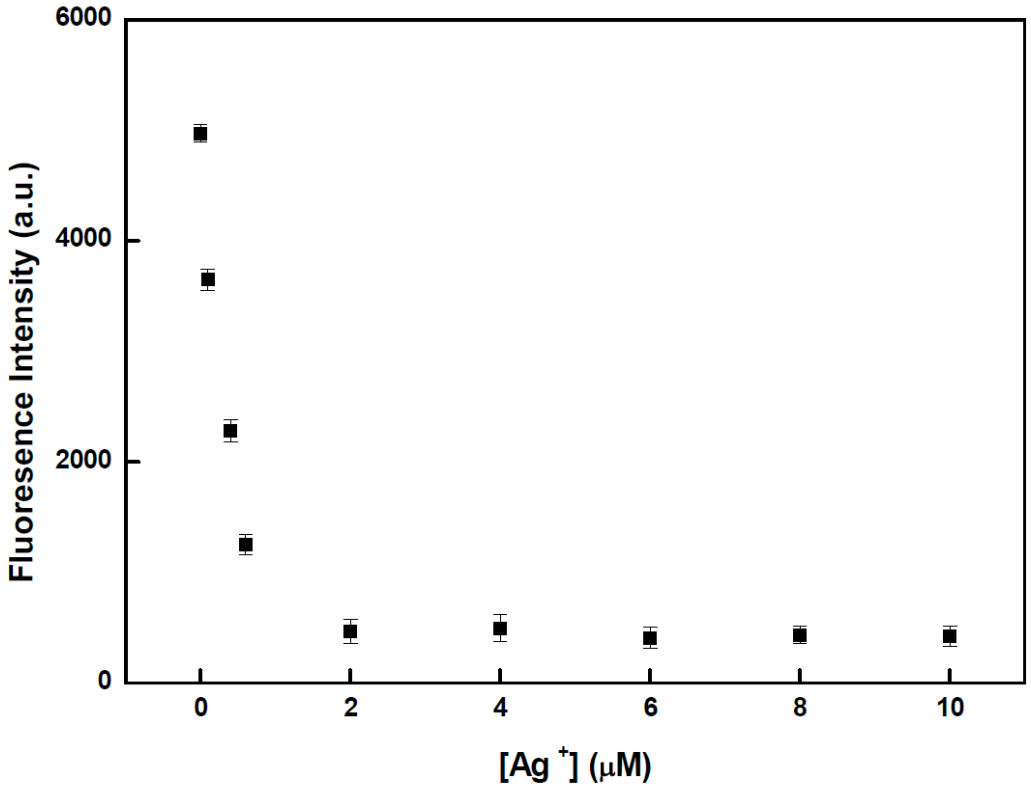

Fig. S13. Effect of Ag^+ concentration on the fluorescence intensity (518 nm) of 10 nM C_{16}-MB-C_{16}. A mixture of C_{16}-MB-C_{16} and Ag^+ was incubated in a solution containing 10 mM MOPS (pH 7.0), 100 mM $NaNO_3$, and 2.5 mM $Mg(NO_3)_2$ for 20 min. The incubation time between the Ag^+–C_{16}-MB-C_{16} complex and tested DNA was 3 min. The error bars represent standard deviations based on three independent measurements.

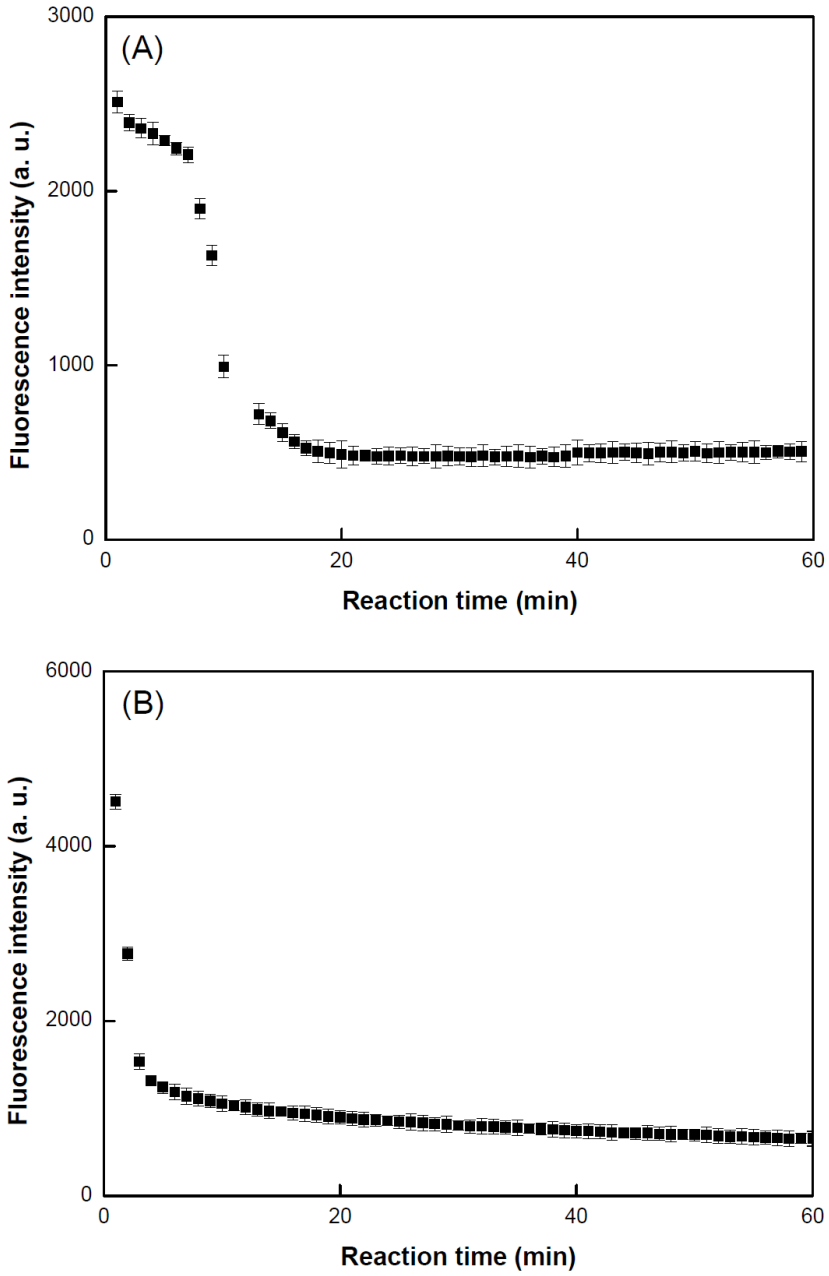

Fig. S14. Temporal change in the fluorescence intensity (518 nm) of a solution containing (A) 10 nM C_{16}-MB-C_{16} and 6 μM Ag^{+} and (B) 10 nM T_{16}-MB-T_{16} and 1 μM Hg^{2+}. The error bars represent standard deviations based on three independent measurements.

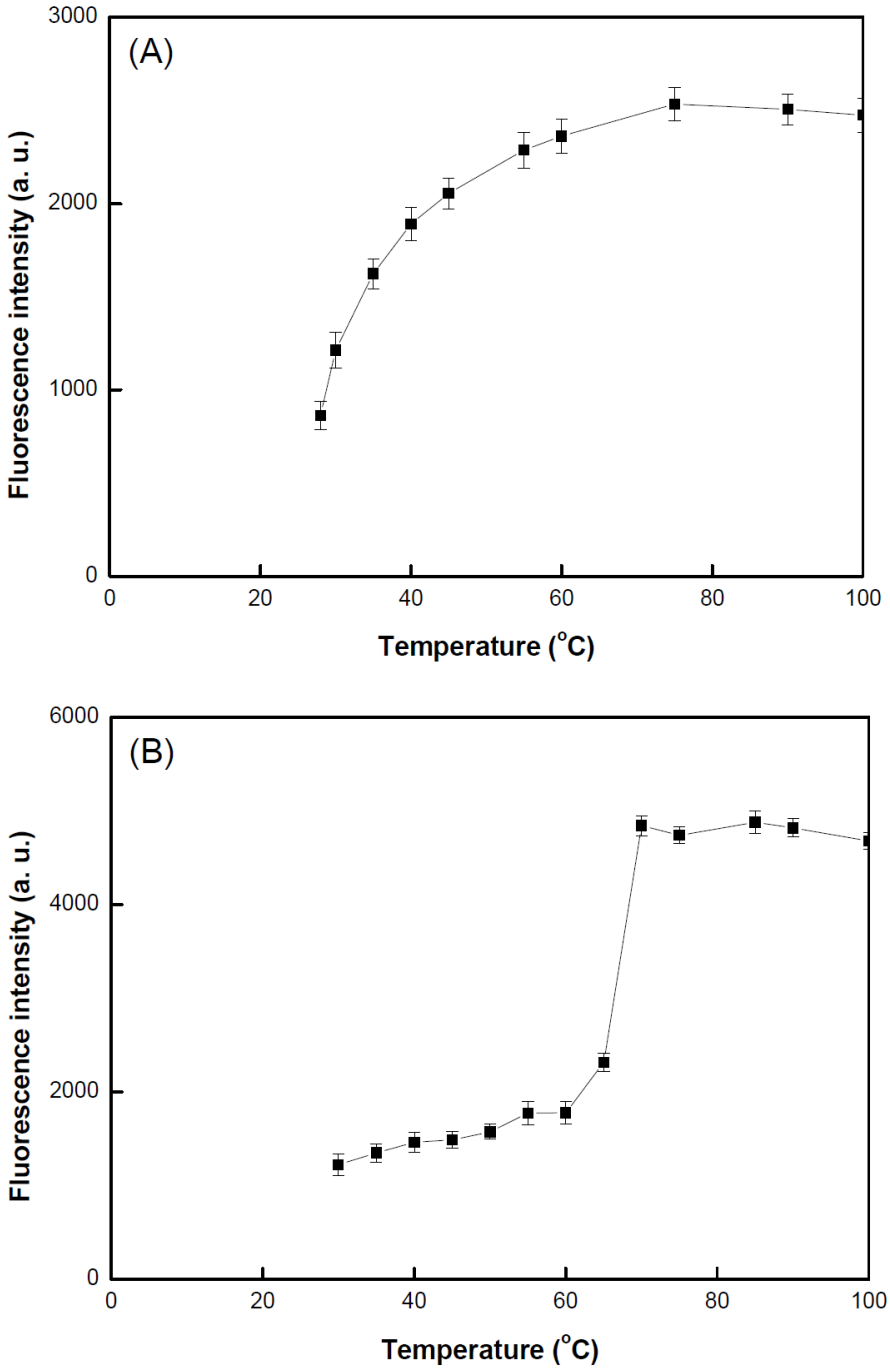

Fig. S15. Effect of (A) 6 μM Ag^{+} and (B) 1 μM Hg^{2+}concentration on the melting point of 10 nM (A) C_{16}-MB-C_{16} and (B) T_{16}-MB-T_{16}. The error bars represent standard deviations based on three independent measurements.

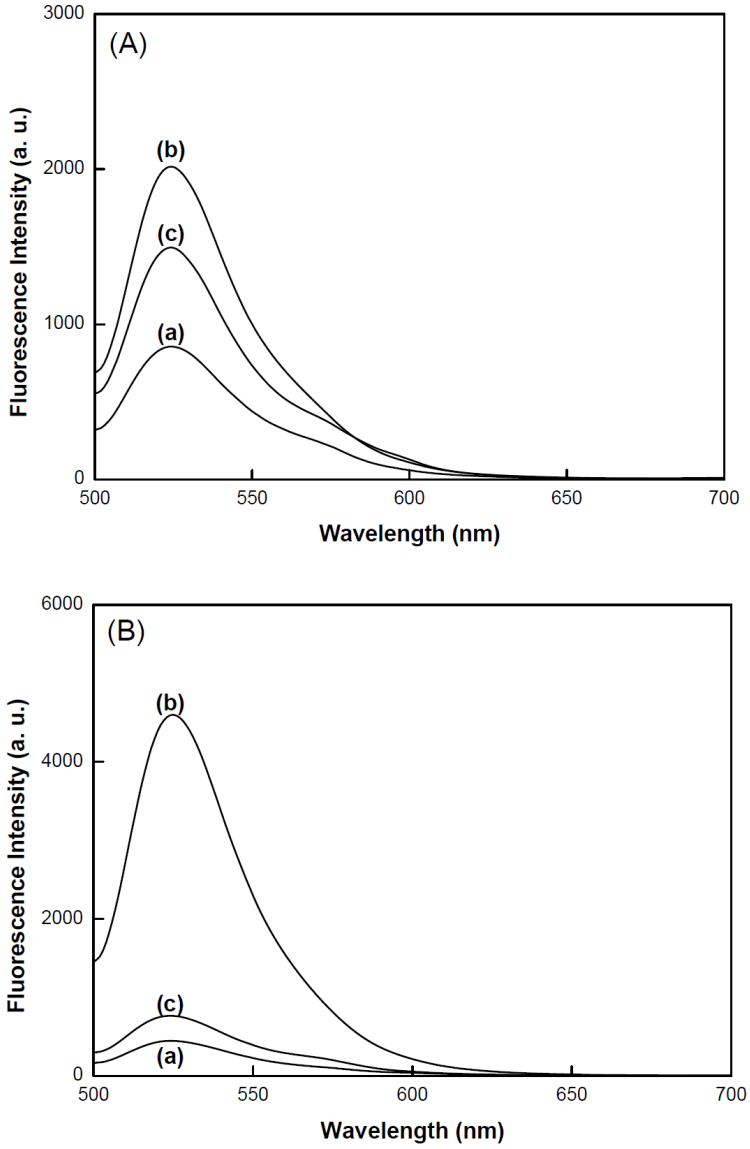

Fig. S16. Fluorescence spectra of a solution containing (A) 10 nM C_{16}-MB-C_{16} and 6 μM Ag^{+} and (B) 10 nM T_{16}-MB-T_{16} and 1 μM Hg^{2+} in the (a) absence and (b, c) presence of (b) 80 nM DNA_{pm} and (c) 80 nM DNA_{mm1}. (A) A mixture of C_{16}-MB-C_{16} and Ag^{+} was incubated in a solution containing 10 mM MOPS (pH 7.0), 100 mM $NaNO_3$, and 2.5 mM $Mg(NO_3)_2$ for 20 min. The incubation time between the Ag^{+}–C_{16}-MB-C_{16} complex and tested DNA was 3 min. (B) A mixture of T_{16}-MB-T_{16} and Hg^{2+} was incubated in a solution containing 5 mM phosphate (pH 7.0) and 20 mM NaCl for 60 min. The incubation time between the Hg^{2+}–T_{16}-MB-T_{16} complex and tested DNA was 8 min.

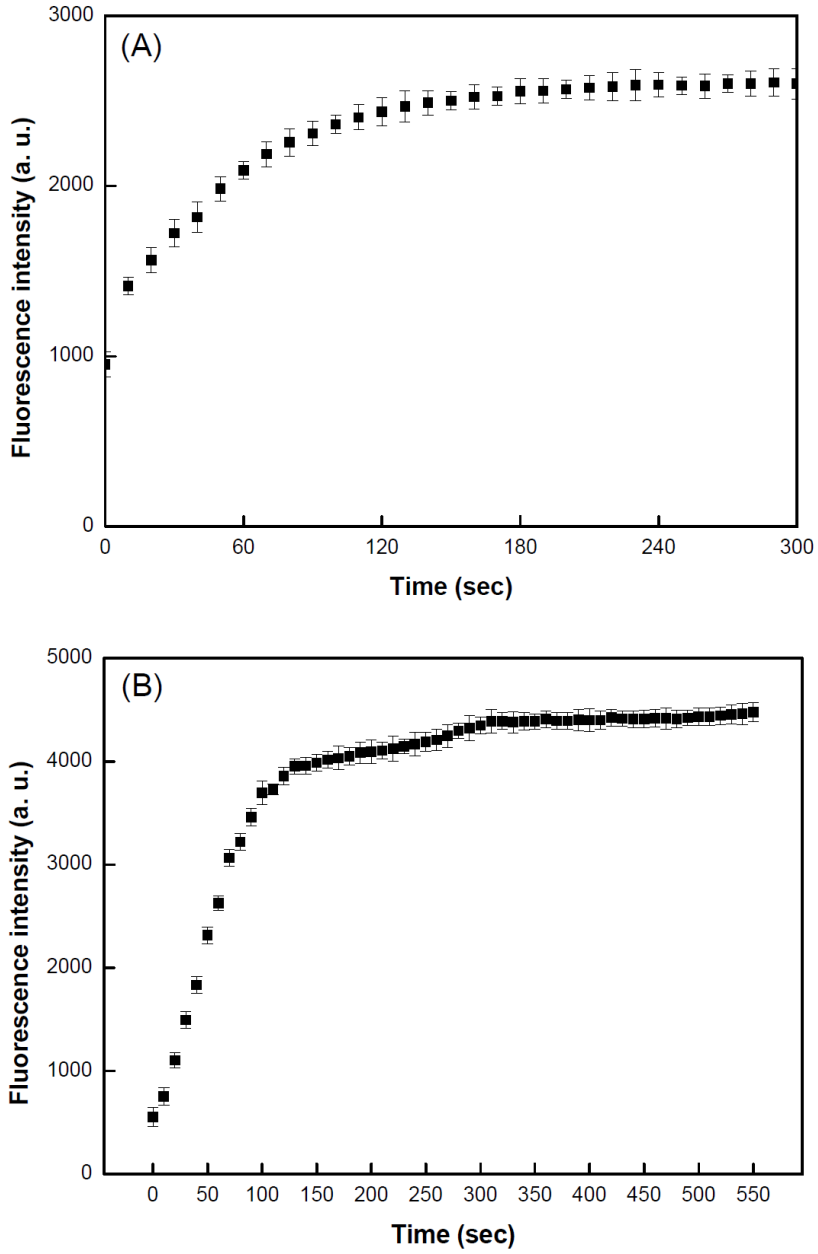

Fig. S17. Time course measurement of fluorescence intensity (518 nm) of a solution containing (A) 10 nM C_{16}-MB-C_{16} and 6 μM Ag^{+} and (B) 10 nM T_{16}-MB-T_{16} and 1 μM Hg^{2+} upon the addition of 80 nM DNA_{pm}. (A) A mixture of C_{16}-MB-C_{16} and Ag^{+} was incubated in a solution containing 10 mM MOPS (pH 7.0), 100 mM $NaNO_3$, and 2.5 mM $Mg(NO_3)_2$ for 20 min. (B) A mixture of T_{16}-MB-T_{16} and Hg^{2+} was incubated in a solution containing 5 mM phosphate (pH 7.0) and 20 mM NaCl for 60 min. The error bars represent standard deviations based on three independent measurements.

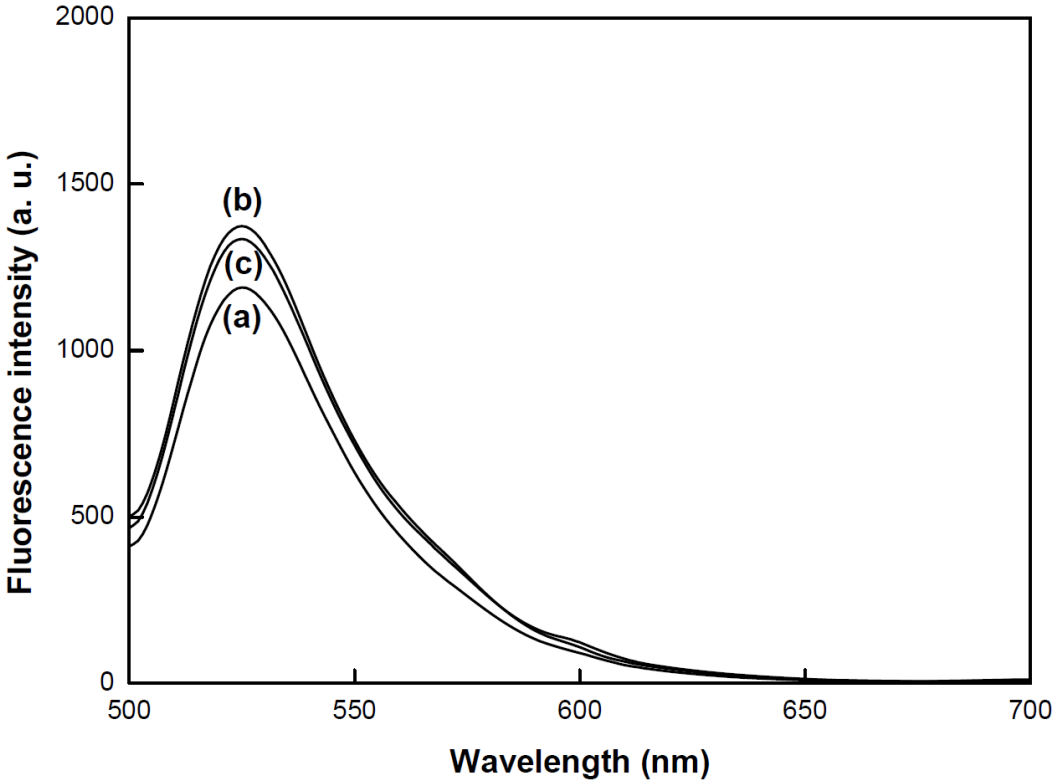

Fig. S18. Fluorescence spectra of a solution containing 10 nM base-pairing MB in the (a) absence and (b, c) presence of (b) 100 nM DNA_{pm} and (c) 100 nM DNA_{mm1}.

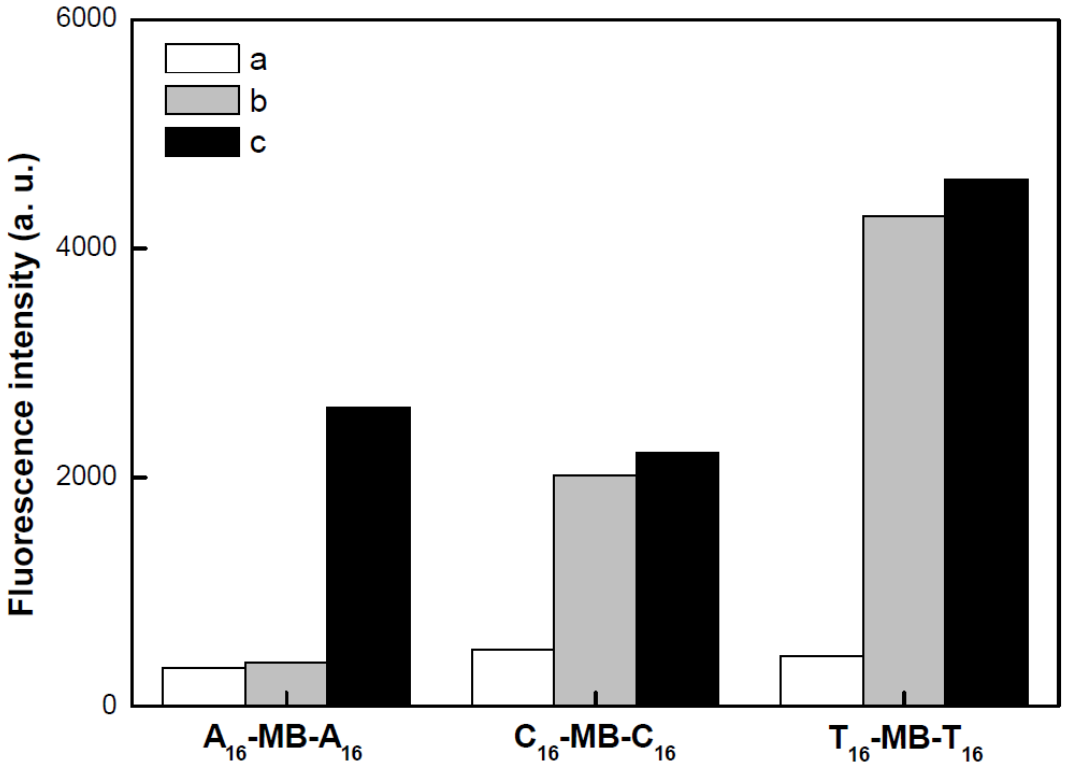

Fig. S19. Fluorescence intensity (518 nm) of three MBs in the (a) absence and (b, c) presence of (b) GSH and (c) GSH and DNA_{pm}. The incubation times between 10 nM A_{16}-MB-A_{16} and 4 μM coralyne, 10 nM C_{16}-MB-C_{16} and 6 μM Ag^{+}, and 10 nM T_{16}-MB-T_{16} and 1 μM Hg^{2+} were 3 , 20, and 60 min, respectively. Three MBs were equilibrated with 4 mM GSH at ambient temperature for 30 min. The coralyne–A_{16}-MB-A_{16}, Ag^{+}–C_{16}-MB-C_{16}, and Hg^{2+}–T_{16}-MB-T_{16} complexes were incubated with 80 nM DNA_{pm} for 3, 3, and 8 min, respectively.

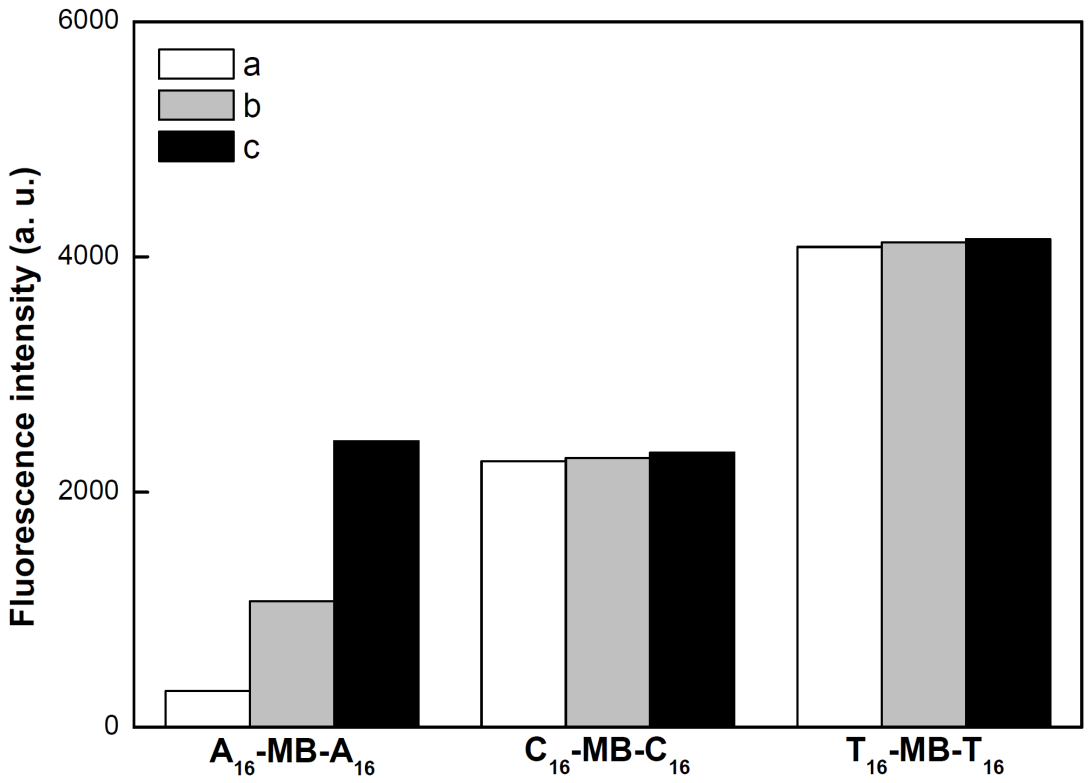

Fig. S20. Fluorescence intensity (518 nm) of three MBs obtained after the addition of (a) a diluted serum, (b) a mixture of diluted serum and 80 nM DNA_{mm1}, and (c) a mixture of diluted serum and 80 nM DNA_{pm}. Serum samples were diluted to 50-fold with the solutions, which were used in the preparation of three MBs. Diluted serum samples were spiked with DNA_{mm1} and DNA_{pm}. The resulting solutions were incubated with three MBs at ambient temperature for 30 min. The incubation times between 10 nM A_{16}-MB-A_{16} and 4 μM coralyne, 10 nM C_{16}-MB-C_{16} and 6 μM Ag^{+}, and 10 nM T_{16}-MB-T_{16} and 1 μM Hg^{2+} were 3 , 20, and 60 min, respectively.