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Electronic Supplementary Information

Molecular Beacon-Based NAND Logic Gate for Sensing Triplex DNA Binders

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Experimental Section

Chemicals

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium chloride (NaCl), coralyne sulfoacetate, berberine chloride, palmatine chloride, sanguinarine chloride, 4',6-diamidino-2-phenylindole, ethidium bromide, anthraquinone-2-carboxylic acid, 9-aminoacridine, $HgCl_2$, H_3PO_4, NaH_2PO_4, Na_2HPO_4, and Na_3PO_4 were obtained from Sigma-Aldrich (St. Louis, MO). All DNA samples were synthesized from Neogene Biomedicals Corporation (Taipei, Taiwan). Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, Milford, MA, USA).

Instrumentation.

The absorption and fluorescence spectra of DNA samples were collected using a double-beam UV-vis spectrophotometer (V670; JASCO, Tokyo, Japan) and a fluorometer (F-7000; Hitachi, Tokyo, Japan), respectively.

Screening of Triplex DNA Binders

Table S1 displays the DNA sequences used in our experiments. For detecting triplex DNA binders, all samples were prepared in a solution containing 10 mM HEPES (pH 6–10) and 0–400 mM NaCl. The MB (100 nM, 50 μL) probes were incubated with a mixture of polyadenosine (A_6–A_{11}; 0.01–10 μM, 50 μL) and triplex DNA binder (10 μM, 50 μL) at ambient temperature for 10 min. The volume of the resulting solutions was adjusted to 500 μL with 10 mM HEPES and 300 mM NaCl. We transferred the diluted solutions into a 1 mL quartz cuvette and recorded their fluorescence spectra by

operating the fluorescence spectrophotometer at an excitation wavelength of 480 nm. In the presence of 1 μM coralyne, the melting point of the MB-A_7-coralyne complex was measured by varying the temperature from 25 to 70 °C.

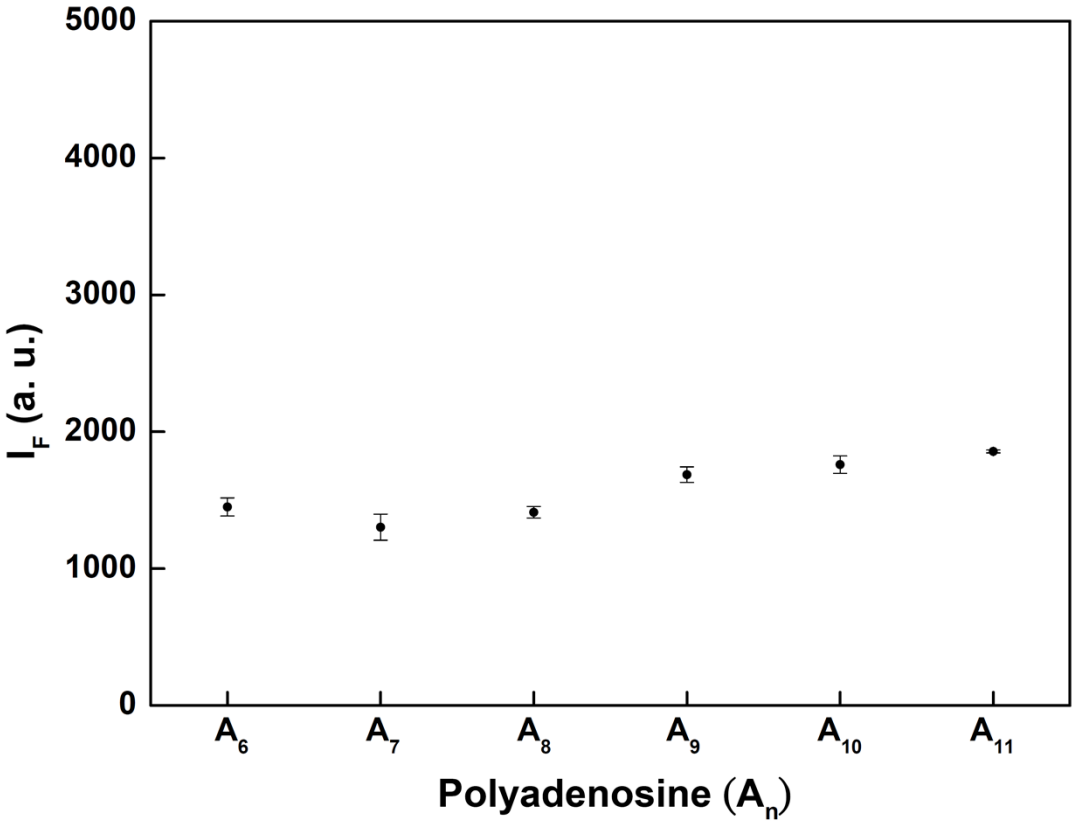

Fig. S1. Effect of the polyadenosine length on the fluorescence intensity at 520 nm of the T_8-MB-T_8·A_n–coralyne complexes. A mixture of 10 nM T_8-MB-T_8 and 100 nM A_n was incubated with 1 μM coralyne in a solution containing 10 mM HEPES (pH 7.0) and 300 mM NaCl at room temperature for 10 min. The error bars represent standard deviations based on three independent measurements.

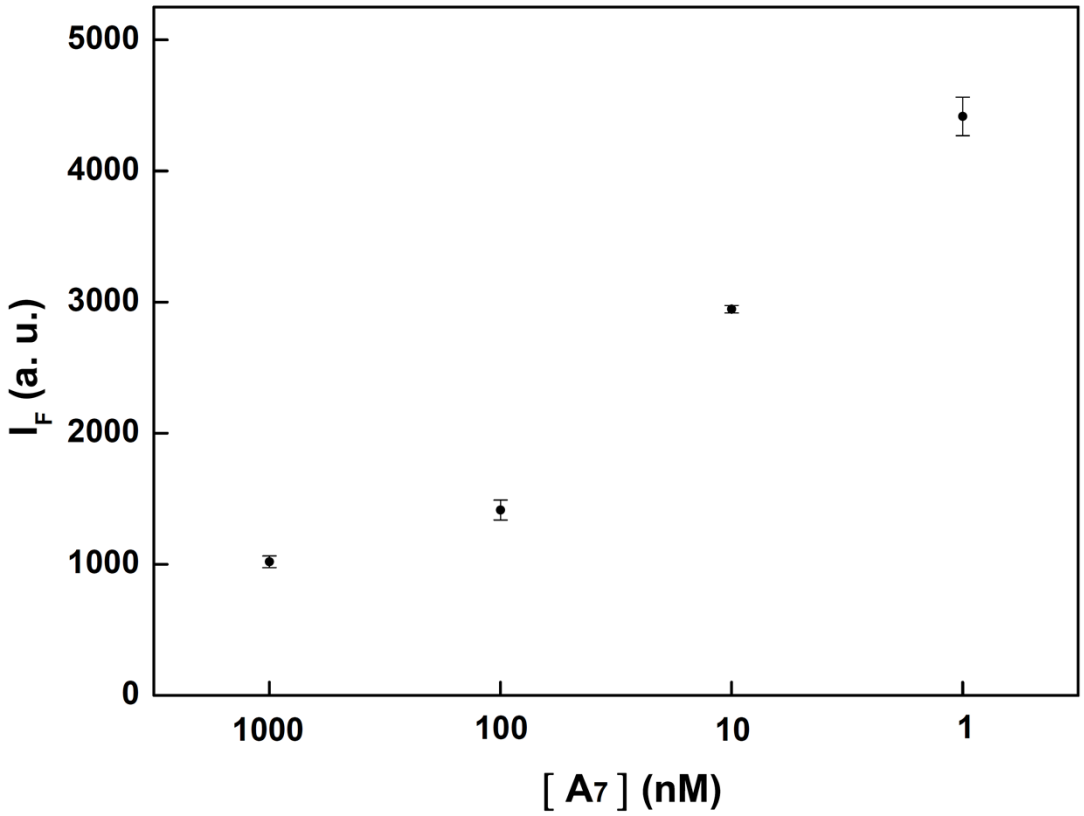

Fig. S2. Effect of the concentration of A_7 on the fluorescence intensity at 520 nm of the T_8-MB-T_8·A_n–coralyne complexes. A mixture of 10 nM T_8-MB-T_8 and 1–1000 nM A7 was incubated with 1 μM coralyne in a solution containing 10 mM HEPES (pH 7.0) and 300 mM NaCl at room temperature for 10 min. The error bars represent standard deviations based on three independent measurements.

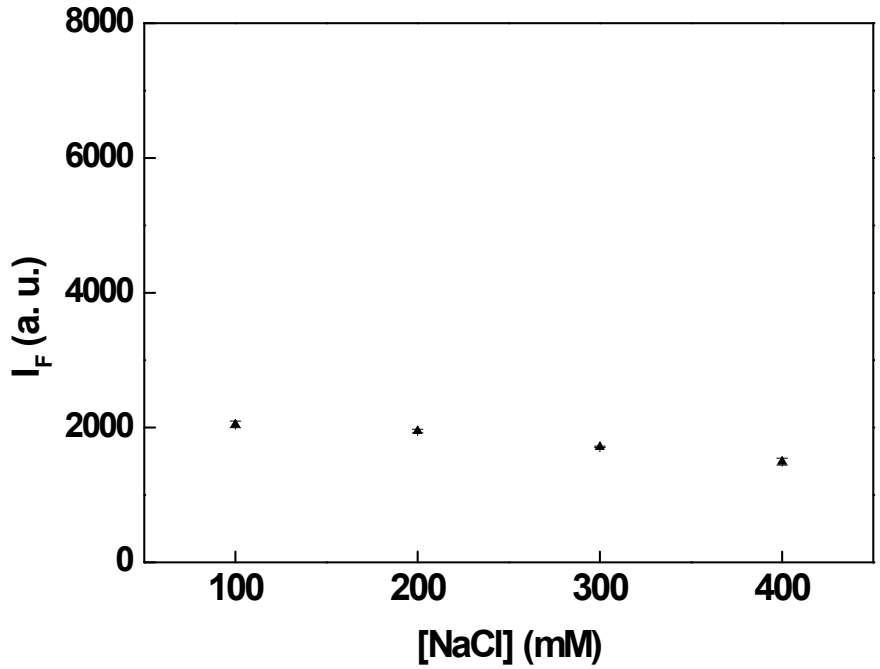

Fig. S3. Effect of the salt concentration on the fluorescence intensity at 520 nm of the T_8-MB-T_8·A_n–coralyne complexes. A mixture of 10 nM T_8-MB-T_8 and 100 nM A_7 was incubated with 1 μM coralyne in a solution containing 10 mM HEPES at pH 7.0 and 100–400 mM NaCl at room temperature for 10 min. The error bars represent standard deviations based on three independent measurements.

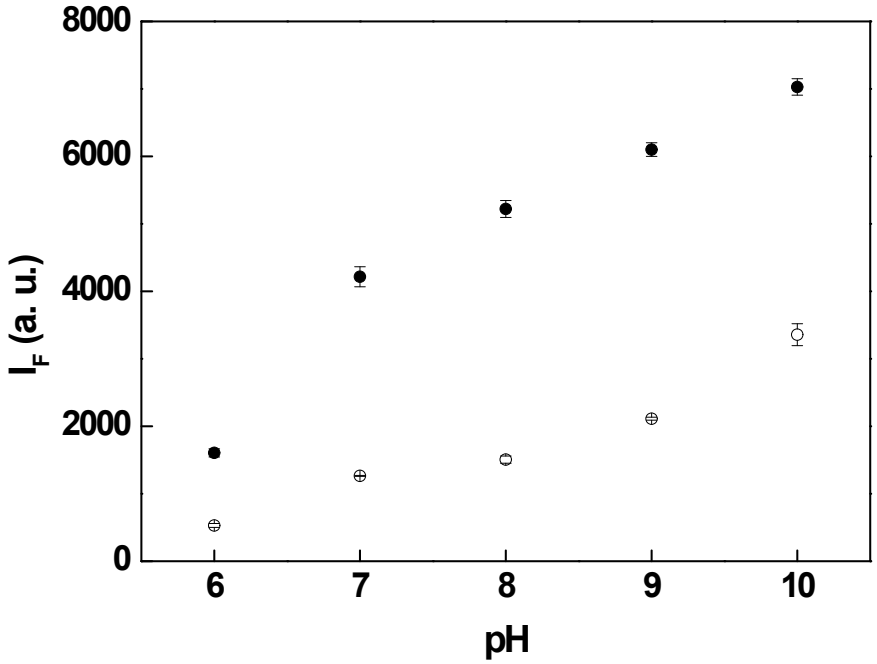

Fig. S4. Effect of the solution pH on the fluorescence intensity at 520 nm of the T_8-MB-$T_8 \cdot A_n$–coralyne complexes. Open circle: the T_8-MB-$T_8 \cdot A_7$–coralyne complexes; close circle: the T_8-MB-$T_8 \cdot A_7$ probe. A mixture of 10 nM T_8-MB-T_8 and 100 nM A_7 was incubated with 1 μM coralyne in a solution containing 10 mM HEPES at pH 6.0–7.0 and 300 mM NaCl at room temperature for 10 min. The error bars represent standard deviations based on three independent measurements.

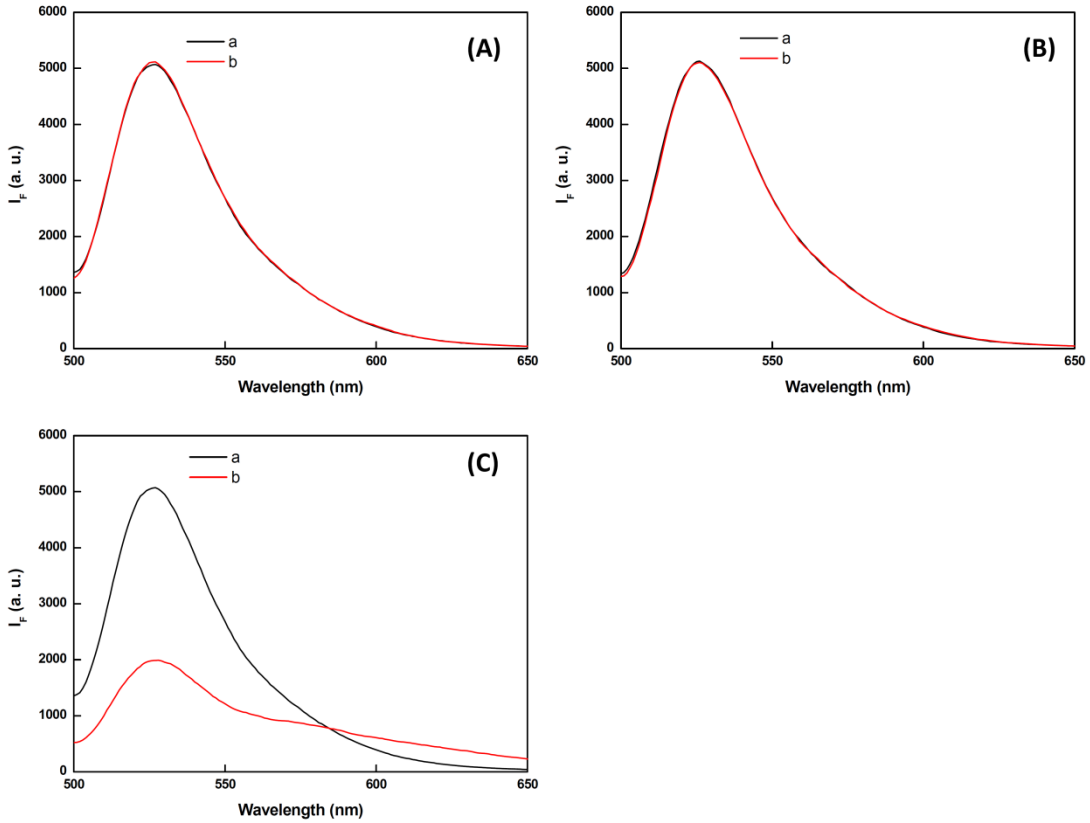

Fig. S5. Fluorescence spectra of solutions of the T_8-MB-T_8·A_7 probe obtained (a) before and (b) after the addition of (A) berberine, (B) palmatine, and (C) sanguinarine. A mixture of 10 nM T_8-MB-T_8 and 100 nM A_7 was incubated with 1 μM triplex DNA binder in a solution containing 10 mM HEPES (pH 7.0) and 300 mM NaCl at room temperature for 10 min.

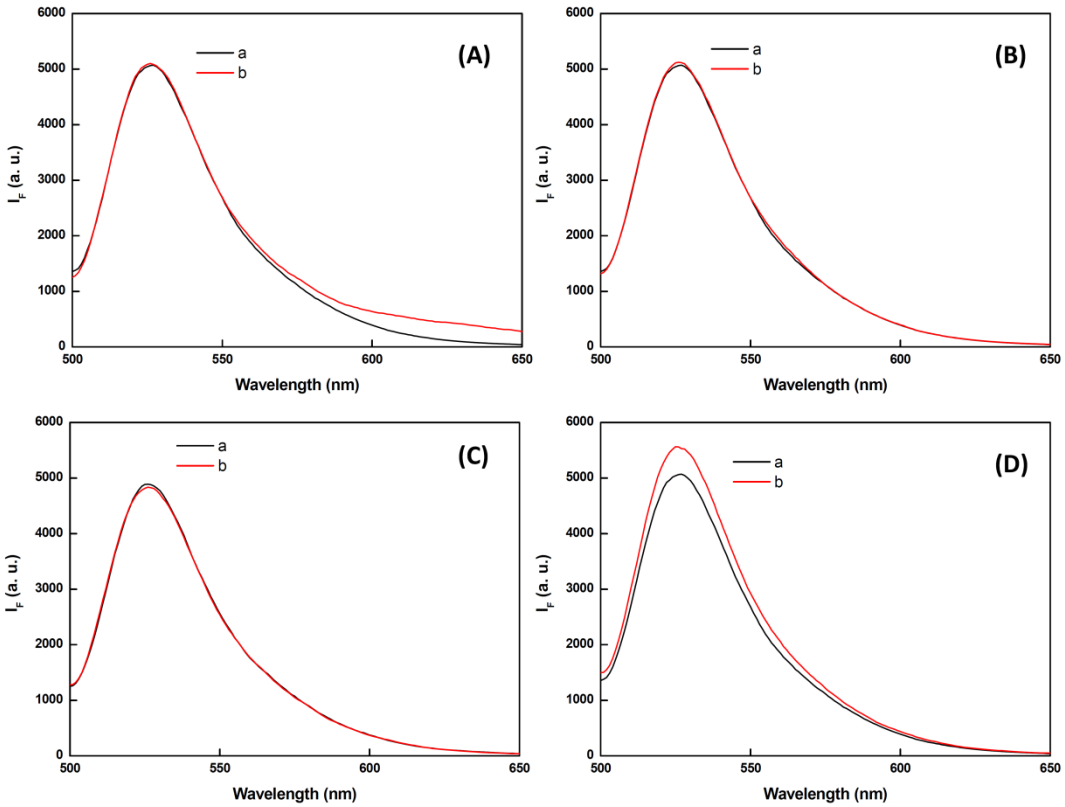

Fig. S6. Fluorescence spectra of solutions of the T_8-MB-T_8·A_7 probe obtained (a) before and (b) after the addition of (A) ethidium bromide, (B) DAPI, (C) AQ2A, and (d) 9-aminoacridine. A mixture of 10 nM T_8-MB-T_8 and 100 nM A_7 was incubated with 1 μM duplex DNA binder in a solution containing 10 mM HEPES (pH 7.0) and 300 mM NaCl at room temperature for 10 min.

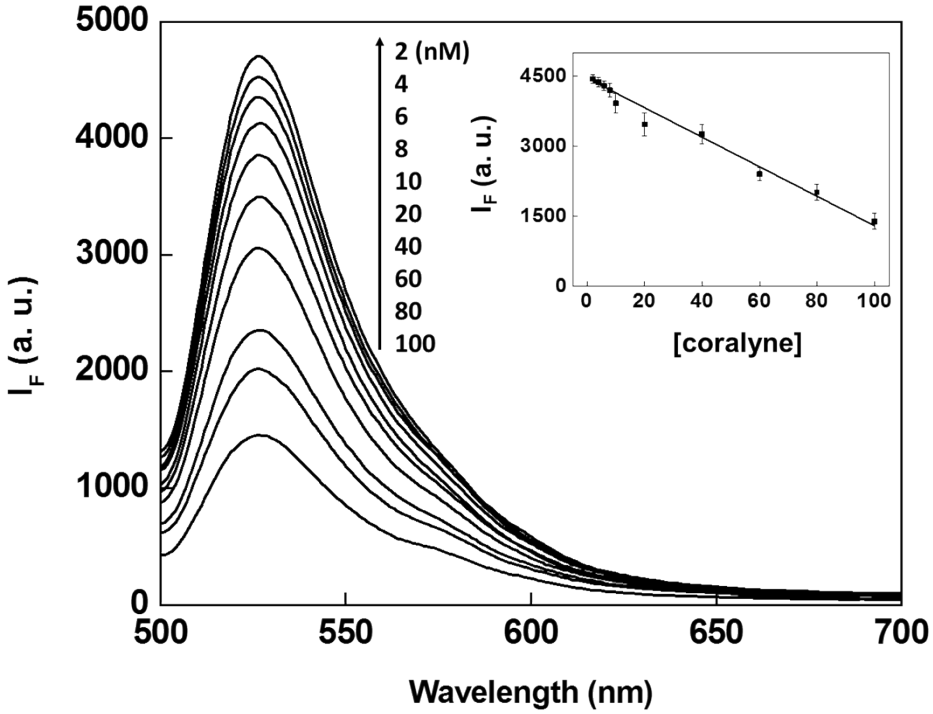

Fig. S7. Fluorescence spectra of the T_8-MB-T_8·A_7 probe in the presence of increasing concentration of coralyne. Inset: a plot of the value of the fluorescence intensity at 520 nm *versus* the concentration of coralyne. A mixture of T_8-MB-T_8 and A_7 was incubated with triplex DNA binder in a solution containing 10 mM HEPES (pH 7.0) and 300 mM NaCl at room temperature for 10 min. The error bars represent standard deviations based on three independent measurements.

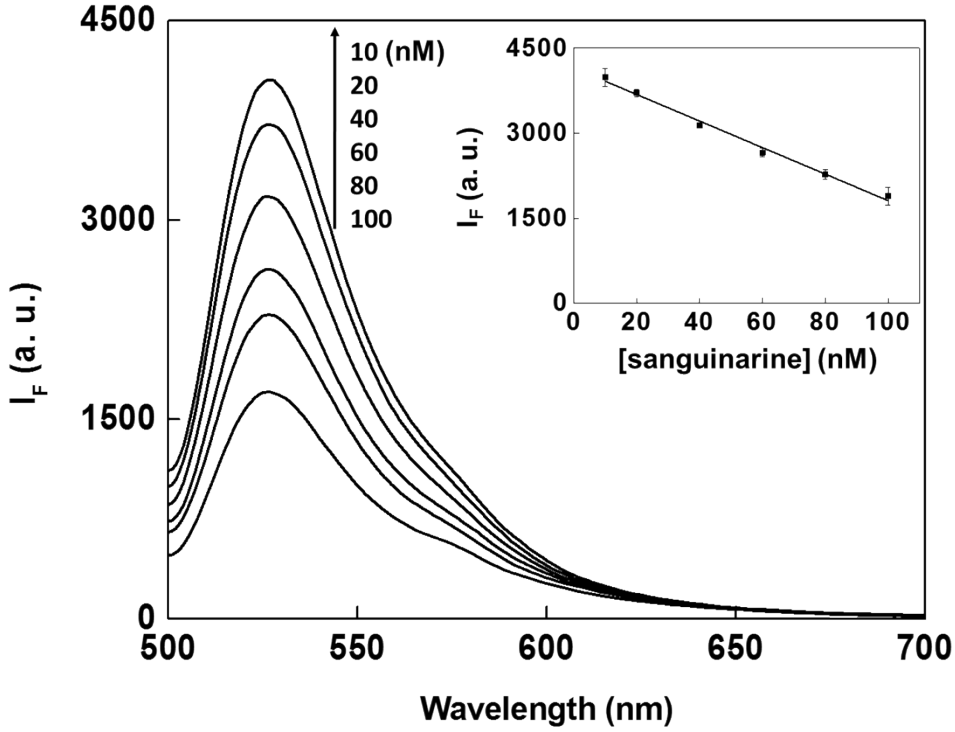

Fig. S8. Fluorescence spectra of the T_8-MB-T_8·A_7 probe in the presence of increasing concentration of sanguinarine. Inset: a plot of the value of the fluorescence intensity at 520 nm *versus* the concentration of sanguinarine. A mixture of T_8-MB-T_8 and A_7 was incubated with triplex DNA binder in a solution containing 10 mM HEPES (pH 7.0) and 300 mM NaCl at room temperature for 10 min. The error bars represent standard deviations based on three independent measurements.

Table S1. Comparison of other methods for the determination of coralyne.

Material	detection	LOD	linear range	Reference
G-quadruplex DNAzyme	absorption	19 nM	0.06 to 10 μM	Anal. Method 2013, 5, 4671-4674
graphene oxide-based aptamer sensor	fluorescence	Not given	10 to 700 nM	Talanta 2013, 112, 117-122
poly(dA)-based DNA sensor	fluorescence	65 nM	0.1 to 10 μM	Chem. Commun., 2011, 47, 11134-11136
poly(dA)-adsorbed gold nanoparticle	absorption	Not given	0 to 728 nM	Analyst, 2009, 134, 1647–1651
coralyne-induced formation of peroxidasemimicking split DNAzyme	Absorption	31 nM	50 to 5000 nM	Analyst, 2013, 138, 4728-4731
molecular beacon	fluorescence	2 nM	6 to 1000 nM	Analyst 2014 139 1436-1441
DNA-functionalized graphene	absorption	0.1 μM	0.1 to 1 μM	Biomaterial 2013 34 4810-4817
Polyadenosine	dual polarization interferometry	0.22 μM	0.5 to 12 μM	Anal. Chem. 2012 84 924-930
DNA-modified silica-Au/core-shell nanoparticles	SERS	0.1 μM	0.1 to 100 μM	Anal. Methods 2013 5 3927-3932
Molecular beacon and polyadenosine	fluorescence	0.6 nM	2 to 100 nM	This study

Table S2. Comparison of other methods for the determination of sanguinarine

Methods	detection	LOD	linear range	Reference
high performance liquid chromatography	fluorescence	0.5 ng/mL	10 to 2000 ng/mL	J. Chromatogr. B 2004, 799, 195-200
capillary electrophoresis	fluorescence	5 μg/mL	50000 to 250000 ng/mL	Talanta 2006, 70, 202-207
high performance liquid chromatography	fluorescence	3 nM	10 to 10000 nM	J. Sep. Sci. 2003, 26, 679-685
high performance liquid chromatography	ESI-MS	1.60 ng/mL	5.25 to 1050 ng/mL	Chromatographia 2004, 60, 347-351
capillary electrophoresis	absorption	3 μM	20 to 500 μM	J. Chromatogr. A 2004, 1040, 141-145
capillary electrophoresis	absorption	3 μM	20 to 500 μM	J. Chromatogr. A 2000, 866, 293-298
glutathhone-capped CdTe/CdS quantum dots	fluorescence	3.4 ng/mL	0.2 to 40 μg/mL	Luminescence 2014, 29, 176-182
molecular beacon and polyadenosine	fluorescence	3 nM (~1 ng/mL)	10 to 100 nM (~3 to 30 ng/mL)	This study

Table S3. Sequence of molecular beacons and polyadenosine

Name	Sequence (5' to 3')
T_8-MB-T_8	FAM-T_8 CCA GAT ACT CAC CGG T_8-DABCYL
T_8-MB-T_8 (no dye)	T_8 CCA GAT ACT CAC CGG T_8
Poly A_6	AAA AAA
Poly A_7	AAA AAA A
Poly A_8	AAA AAA AA
Poly A_9	AAA AAA AAA
Poly A_{10}	AAA AAA AAA A
Poly A_{11}	AAA AAA AAA AA