Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting information for

Red-emissive triplex-forming PNA probes carrying cyanine base surrogates for fluorescence sensing of double-stranded RNA

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Experimental

Reagents: All of RNAs were custom synthesized and HPLC purified by GeneDesign, Inc. (Osaka, Japan). The concentration of the RNAs was determined from the absorbance at 260 nm at 85°C using the molar extinction coefficient provided by the manufacturer. Other reagents were commercially available analytical grade and were used without further purification. Water was deinonized (> 18.2 MΩcm specific resistance) with an Elix 5 UV water purification system and a Milli-Q Synthesis A10 system (Millipore Co., Bedford, MA), followed by filtration through a BioPak filter (Millipore Co.) in order to remove RNase.

Unless otherwise mentioned, all measurements were performed in 10 mM sodium acetate (pH 5.5) or sodium phosphate buffer solution (pH 7.0) containing 100 mM NaCl and 1.0 mM EDTA. Before measurements, RNA-containing samples were annealed as follows: heating at 95°C for 5 min, and cooling at 5°C for 5 min. Then the solution temperature was gradually raised to 25°C (0.75°C/min). Typically, the probe was added to the annealed RNA-containing solution, and measurements were performed after a 15 min incubation.

UV melting experiments: UV melting experiments were conducted using a UV-Vis spectrophotometer model UV-2450 (Shimazdu Co. Ltd., Kyoto, Japan) equipped with a thermoelectrically temperature-controlled micro-multicell holder (8 cells, optical path length: 10 mm). Before each experiment, the sample solutions containing RNA and the probe were put into the cells and covered with paraffin oil and silicon caps. Then, the cells were heated at 70°C for 15 min, slowly cooled to 10°C (1°C/min), and incubated for 3 h at 10°C. We monitored absorbance change at 300 nm where the triplex-duplex transition can be selectively detected,[S1] by increasing the temperature from 10 to 110°C (0.5°C/min). The obtained melting profile was corrected by subtracting the absorbance at 400 nm. The resulting absorbance *versus* temperature was analyzed by a differential method to determine T_m values.

Fluorescence spectroscopic measurements: Fluorescence spectra were recorded at 25°C on a JASCO model FP-6500 spectrofluorophotometer (Japan Spectroscopic Co. Ltd., Tokyo, Japan) equipped with thermoelectrically temperature-controlled cell holder. Measurements of fluorescence spectra were done using a 3 × 3 mm quartz cell. Fluorescence quantum yield (ϕ_{fl}) was determined relative to Rhodamin 6G in EtOH.[S2]

CD measurements: CD spectra were measured at 25°C with a JASCO model J-800 spectropolarimeter equipped with a thermoelectrically temperature-controlled cell holder (Japan Spectroscopic Co. Ltd.) using a micro sampling disk (optical path length: 1 mm).

Probe synthesis: We synthesized carboxylated QB (Ref. 12b in the main text) and TR derivatives[S3] (Fig. S1) for the coupling with PNA backbone according to the literatures (Ref 7 in the main body). We utilized QB derivative without purification since it rapidly decomposed upon light exposure (Ref. 12b in the main text). PNA-QB and PNA-TR were manually synthesized in the solid phase synthesis using Fmoc/Bhoc PNA monomers, Fmoc-aeg(alloc)-OH, and Fmoc-Lys(Boc)-OH. Fmoc-aeg(alloc)-OH was used for introducing the base surrogate. Briefly, all monomers were assembled on the resin according to the previous report (Ref 7 in the main body). After alloc group was selectively deprotected by dimethylamine borate and tetrakis(triphenylphosphine) palladium (0), carboxylated QB or TR derivative was coupled with the resulting free secondary amine by using PyBOP as a coupling agent. We deprotected Boc/ Bhoc groups and simultaneously cleavage the product from the resin by treating with trifluoroacetic acid (TFA). The crude product was purified by a reverse-phase HPLC system (pump, PU-2086 Plus ×2; mixer, MX 2080-32; column oven, CO-1565; detector, UV-2070 plus and UV-1570M (Japan Spectroscopic Co. Ltd., Tokyo, Japan)) equipped with a C18 column (Inertsil ODS3; GL Sciences Inc., Tokyo, Japan) using a gradient of water/acetonitrile containing 0.1% TFA (Fig. S2). All probes were verified by MADLI-TOF-MS (4800 Plus MALDI TOF/ TOF analyzer: AB Sciex, Tokyo, Japan), as shown in Fig. S3.

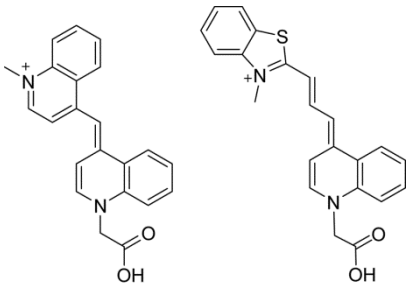

Fig. S1 Carboxylated QB (left) and TR derivative (right) used for the probe synthesis

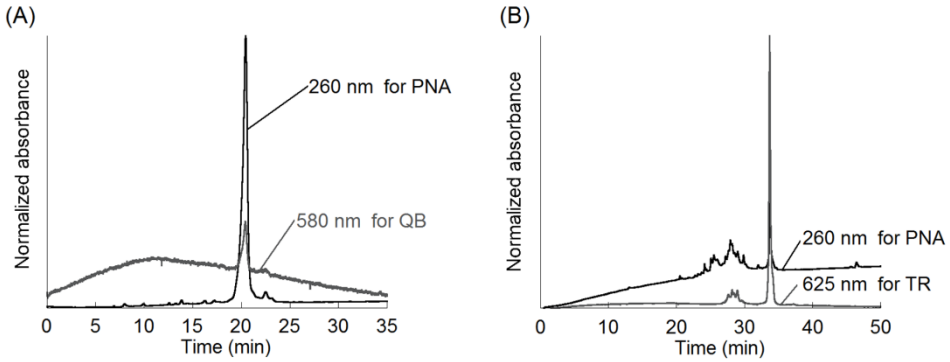

Fig. S2 HPLC profile for probe purification: (A) PNA-QB and (B) PNA-TR. Absorbance of PNA unit and cyanine base were monitored. The peak (*) was collected and identified as the purified probe by MALDI-TOF-MS.

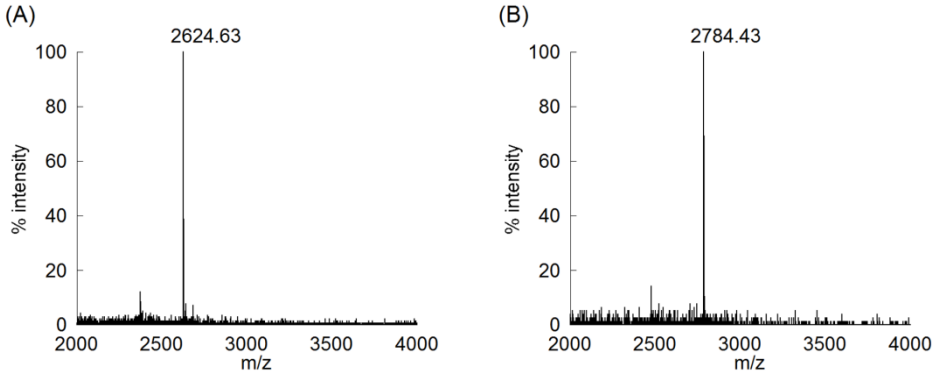

Fig. S3 MALDI-TOF-MS spectra of the purified probe: (A) PNA-QB and (B) PNA-TR.

Table S1 Probe characterization

Probe	Molecular Formula	Calculated ([M^+])	Found ([M^+])
PNA-QB	$C_{115}H_{147}N_{44}O_{33}$	2624.13	2624.63
PNA-TR	$C_{121}H_{159}N_{46}O_{31}S$	2784.20	2784.43

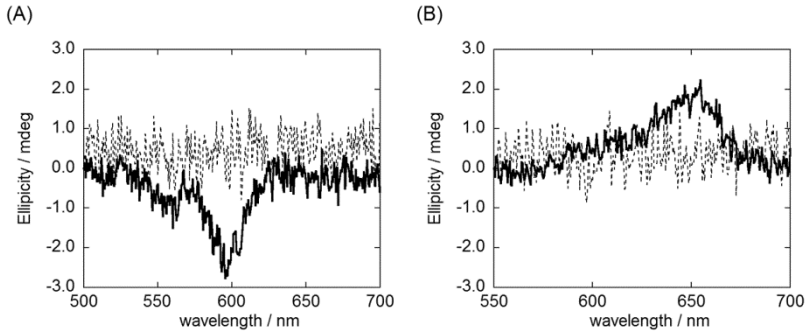

Fig. S4 CD spectra of the (A) PNA-QB (40 μM) and (B) PNA-TR (30 μM) in the presence (bold line) or absence (dashed line) of equimolar target dsRNA: (A) RNA1 and (B) RNA4. Other solution conditions are the same as those given in Table 1 in the main body. Temperature, 25°C.

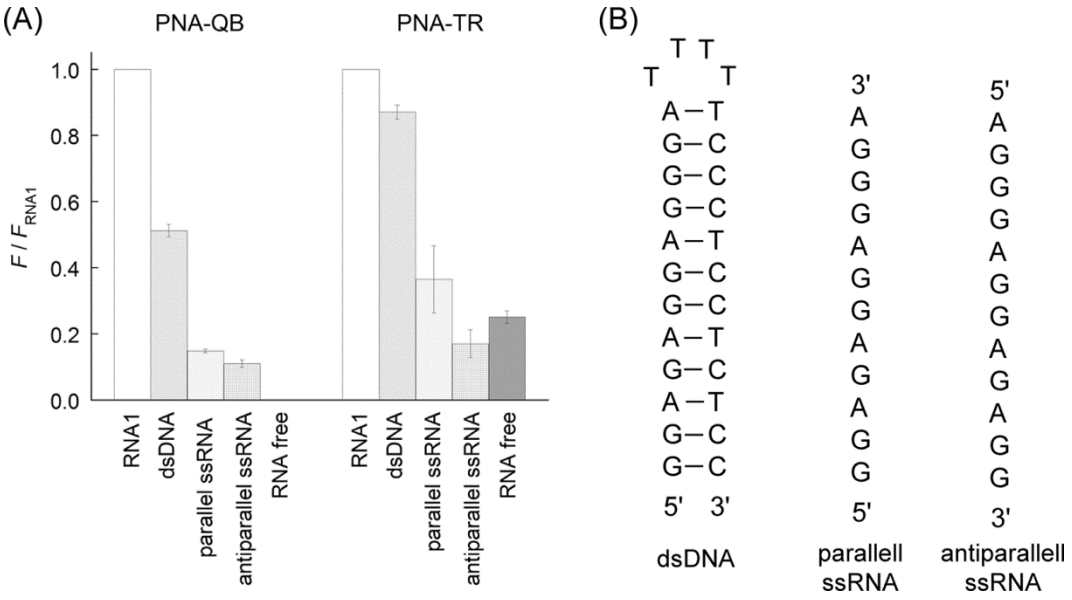

Fig. S5 (A) Normalized fluorescence intensity of the probe (1.0 μM) at 608 nm (PNA-QB) and 663 nm (PNA-TR) upon addition of various target (5.0 μM: dsDNA or single-stranded RNAs). We also showed the normalized intensity in the absence of targets. Other solution conditions are the same as those given in Table 1. Excitation: 584 nm (PNA-QB) and 639 nm (PNA-TR). Temperature, 25° C. (B) Sequences of target dsDNA and single-stranded RNAs.

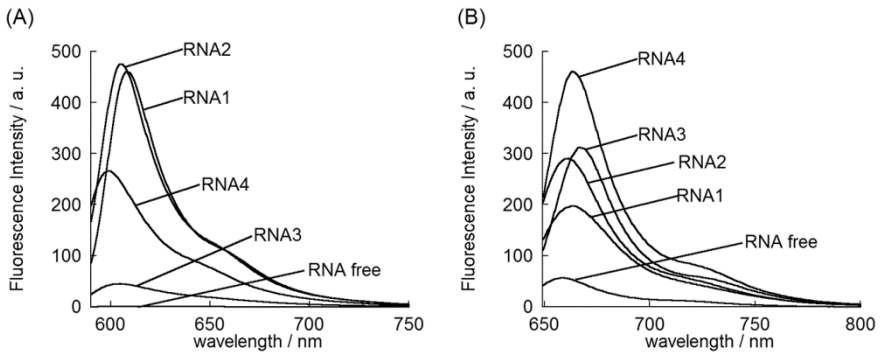

Fig. S6 Fluorescence response of the probes (1.0 μM) for RNA1-RNA4 (5.0 μM): (A) PNA-QB and (B) PNA-TR. Other solution conditions are the same as those given in Table 1. Excitation: (A) 574 nm and (B) 639nm. Temperature, 25°C.

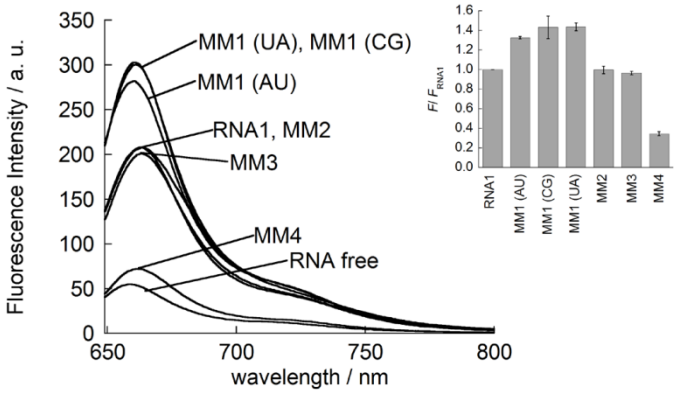

Fig. S7 Fluorescence spectra of PNA-TR (1.0 μM) in the absence and presence of various kinds of dsRNAs (5.0 μM): RNA1, single-base pair mismatch (MM1-MM3) and three-base pair mismatches-containing sequences (MM4). Inset: Sequence-selectivity of the fluorescence response of PNA-TR. Other solution conditions are the same as those given in Table 1. F and F_{RNA1} denote the fluorescence intensity of PNA-QB at 663 nm in the presence of target dsRNAs and RNA1, respectively. Error bars are the standard deviations obtained from three independent experiments. Excitation, 639 nm. Temperature, 25°C.

Table S2 Summary of fluorescence quantum yield (ϕ_{fl}) of the probes in the absence and presence of dsRNAs[a]

	PNA-QB	PNA-TR	PNA-TO
RNA free	0.0017	0.012	0.0028[b]
RNA1	0.33	0.038	0.41[b]
RNA2	0.36	0.045	0.48[b]
RNA3	0.029	0.062	0.083[b]
RNA4	0.17	0.060	0.27[b]
MM1(AU)	0.014	-----	0.10
MM1(CG)	0.013	-----	0.093
MM1 (UA)	0.020	----	0.11
MM2	0.068	-----	0.18
MM3	0.30	------	0.30

[a] [Probe] = 1.0 μM, [dsRNA] = 1.0 μM in 10 mM sodium acetate buffer solution (pH 5.5) containing 100 mM NaCl and 1.0 mM EDTA. Temperature, 25°C. Excitation: 530 nm for PNA-QB; 485 nm for PNA-TO. Rhodamine 6G was used as a reference dye to determine ϕ_{fl} values for PNA-QB and PNA-TR.[S2] As for PNA-TO, we used fluorescein as the reference. [b] Values are from the literature (Ref 7b in the main body).

References

S1. B.-W. Sun, B. R. Babu, M. D. Sorensen, K. Zakrzewska, J. Wengel, and J. S. Sun, *Biochemistry*, 2004, **43**, 4160.

S2. D. Magde, R. Wong and P. G. Seybold, *Photochem. Photobiol.*, 2002, **75**, 327.

S3. J. R. Carreon, K. M. Stewart, K. P. Mahon, S. Shin and S. O. Kelly, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 5182.