Supporting Information for

2,4-Diamino-6,7-dimethylpteridine as a Fluorescent Ligand for Binding and Sensing an Orhan Cytosine in RNA Duplexes

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Experimental

Reagents: All of the RNAs were custom-synthesized and HPLC-purified by Sigma Genosys (Hokkaido, Japan). The concentrations of RNAs were determined from the molar extinction coefficient at 260 nm (*Method Enzymol.* **1989**, *180*, 304-325.). Water was deionized (≥18.0 MΩ cm specific resistance) by an Elix 5 UV Water Purification System and a Milli-Q Synthesis A10 system (Millipore Corp., Bedford, MA), followed by filtration through a BioPak filter (Millipore Corp., Bedford, MA) in order to remove RNase. $4NH_2$-PT was synthesized according to the literature (ref. 8 in the main text). Other reagents were commercially available analytical grade and were used without further purification.

Unless otherwise stated, all measurements were performed in 10 mM sodium cacodylate buffer solutions (pH 7.0) containing 100 mM NaCl and 1.0 mM EDTA. Before measurements, the sample solutions were annealed as follows: heated at 75°C for 10 min, and gradually cooled to 5°C (3°C/min), after which the solution temperature was raised again to 20°C (1°C/min).

UV-visible spectra and fluorescence spectra measurements: Absorption and fluorescence spectra were measured at 20°C with a JASCO model V-570 UV–vis spectrophotometer and FP-6500 spectrofluorophotometer (Japan Spectroscopic Co. Ltd., Tokyo, Japan), respectively. Both instruments were equipped with thermoelectrically temperature-controlled cell holders. Measurements of absorption and fluorescence spectra were done using a 10 × 10 mm quartz cell (optical path length: 10 mm) and a 3 × 3 mm quartz cell, respectively. In the fluorescence measurements, excitation wavelengths for the ligands were set at the maximum absorption wavelength.

Salt dependence of the binding affinity: The effect of different NaCl concentrations on the 1:1 binding constants (K_{11}) of the ligands to the target nucleobase in the AP-RNAs was examined at 20 °C (pH 7.0) by fluorescence titration experiments (cf. Fig. 2 in the main text), and analyzed according to the polyelectrolyte theory proposed by Record *et al*. (ref. 12 in the main text). The observed salt dependence of the binding constants was explained by eq. (3).

$$\delta \log K_{11} / \delta \log [Na^+] = SK \qquad (3)$$

The slope (*SK*) of the plot was associated with the number of counterions released from the nucleic acids upon

ligand binding and in addition, was used to evaluate the polyelectrostatic contribution (ΔG_{pe}) to the observed binding free energy ($\Delta G_{obs} = -RT \ln K_{11}$) using the following equation: $\Delta G_{pe} = -(SK) \ln[Na^+]$.

Molecular modeling simulation: All calculations were carried out with MacroModel ver. 9.0 with an Amber* force field and the GB/SA water model. Energy-minimization was employed with the Polak-Ribiere Conjugate Gradient (PRCG, 5000 steps, convergence threshold 0.05 kJ mol^{-1} $Å^{-1}$). The initial structures of the complexes were constructed by manually inserting $4NH_2$-PT into the AP site of AP-RNA.

pK_a determination: pK_a values of the ligands were determined from pH-dependent UV-vis spectra of the ligands measured in 100 mM KCl solutions at 25°C. UV-vis spectra of the ligands were measured with increasing pH values of the solution by adding NaOH or 0.1 M NaOH solution. The obtained data of absorbance versus pH were fitted to Henderson-Hasselbach equation for the determination of pK_a values of the ligands.

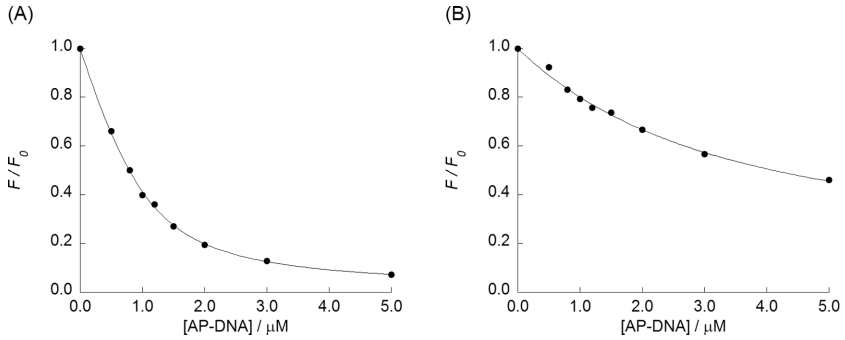

Fig. S1 Fluorescence titration curves for the binding of (A) $4NH_2$-PT and (B) 4OH-PT to the target C in AP site-containing DNA duplexes (5'-ATT TGG GTG AXA TTG CTC ACA-3'/3'-TAA ACC CAC TCT AAC GAG TGT-5', X = AP site (spacer C3)). F and F_0 denote the fluorescence intensities of the ligand in the presence and absence of DNAs, respectively. Excitation: (A) 362.5 nm; (B) 349.5 nm. Analysis: (A) 451.5 nm; (B) 432.5 nm. Solution conditions were the same as those given in Fig. 2 in the main text. Temperature, 20°C.

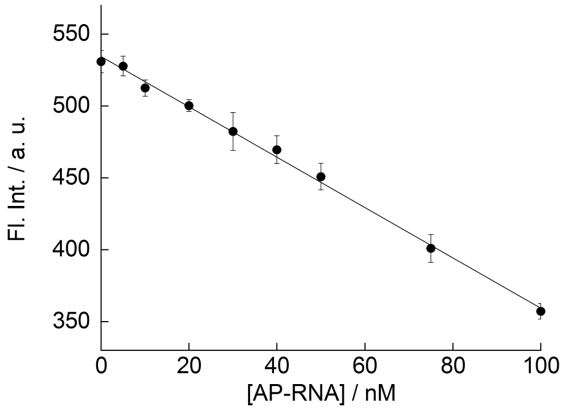

Fig. S2 Determination of the limit of detection (LOD) for AP-RNA containing target C based on the binding-induced fluorescence response of $4NH_2$-PT (100 nM).

The obtained equation by the linear fitting is y = 534.5–1.75 x (r^2 = 0.9967). From three independent experiments, the standard deviation of the fluorescence intensity of $4NH_2$-PTin the absence of AP-RNAs was determined as 7.69. LOD was then estimated according to the following equation: LOD = 3 × 7.69 / (slope obtaind from the linear fitting). Therefore, the estimated LOD was 13 nM.

Other solution conditions were the same as those given in Fig. 2 in the main text. Temperature, 20°C. Excitation: 362.5 nm. Analysis: 451.5 nm.

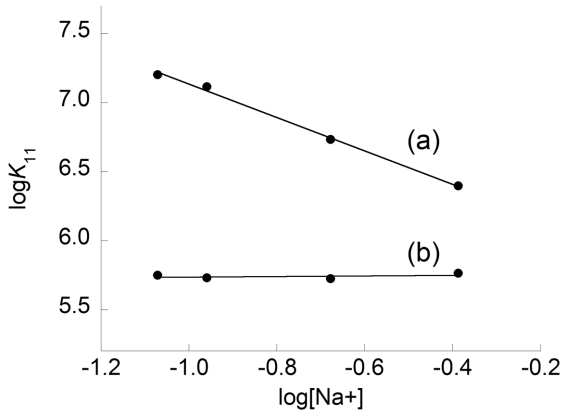

Fig. S3 Salt dependence of the binding constants (K_{11}) of (a) $4NH_2$-PT and (b) 4OH-PT for the target C in the AP-RNA. Solution conditions were the same as those given in Fig. 2 in the main text. Temperature, 20°C. K_{11} was calculated from $K_{11} = 1/K_d$.

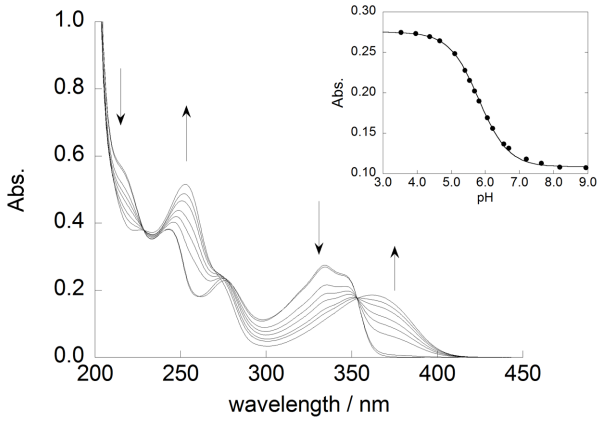

Fig. S4 Determination of pK_a value of $4NH_2$-PT from pH dependence of UV-visible absorption spectra, ranged from pH 3.9 to pH 8.9. Inset: Absorbance at 334.4 nm at various pH values. The concentration of $4NH_2$-PT was 32 μM and the spectra were measured in solution containing 100 mM KCl.

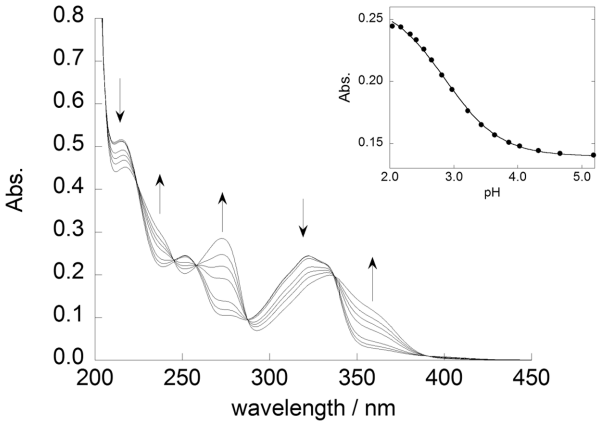

Fig. S5 Determination of pK_a value of 4OH-PT from pH dependence of UV-visible absorption spectra, ranged from pH 2.0 to pH 5.2. Inset: Absorbance at 322.2 nm at various pH values. The concentration of 4OH-PT was 33 μM and the spectra were measured in solution containing 100 mM KCl.

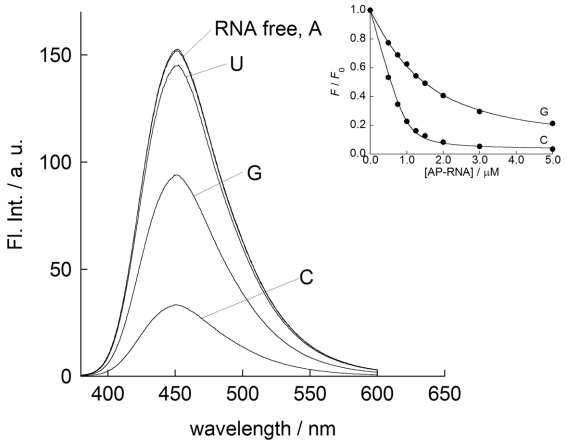

Fig. S6 Application of $4NH_2$-PT to detection of the target C in other RNA sequence. The target RNA sequence is 5'-UGA GGU AGU AGN UUG UAC AGU-3' (N = target nucleobase; C, G, A or U), where the length and sequence are identical to those of let-7g (N = U; *Cell*, 2005, **120**, 635-647.). The probe sequence is the same as shown in our previous study (ref. 5 in the main text).

$4NH_2$-PT shows the significant fluorescence quenching for the target C (K_d = 53 nM, Inset) compared to other target nucleobases. The results indicate that $4NH_2$-PT is applicable to detection of the target C in various kinds of microRNA sequences.