**Supporting Information for**

# Highly selective binding of naphthyridine with a trifluoromethyl group to cytosine opposite an abasic site in DNA duplexes

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## *Experimental*

**Reagents:** All of the DNAs were custom-synthesized and HPLC-purified by Nihon Gene Research Laboratories Inc. (Sendai, Japan). The concentrations of DNAs were determined from the molar extinction coefficient at 260 nm according to the literature.[1] 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). 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).

**Synthesis of $CF_3$-AMND** (ref. 10 in the main text)**:** 2,6-Diaminopyridine (1.1 g, 10.5 mmol) in phosphoric acid (50 mL) were added to 1,1,1-trifluoro-2,4-pentanedione (1.6 g, 10.2 mmol) and the reaction mixture was stirred at 90°C for 26 h. After neutralization with NaOH, the mixture was filtered. The brown residue was extracted three times with 200 mL of $CHCl_3$ each time and the organic phase was dried over $Na_2SO_4$. The solvent was evaporated *in vacuo* to give the mixture of $CF_3$ substituent-modified AMND molecules at the 5 position and 7 position. The mixture was dissolved in 10 mL of chloroform, and filtered. Filtrate was evaporated, dissolved in 20 mL of ethyl acetate, and 2 mL of HCl (0.1 M) was added in drops to the solution. Filtration of the solution gave crystals of $CF_3$-AMND as pale yellow needles (25 mg, 0.11 mmol, 1.1 %). $^1$H NMR (DMSO-$d_6$, 600 MHz): $\delta$ = 8.17 (d, 1H, $J$ = 11 Hz), 7.65 (s, 1H), 7.08 (d, 1H, $J$ = 11 Hz), 2.68 (s, 3H). HRMS (ESI) for $C_{10}H_8N_3F_3$: calcd: ($[M+H]^+$), 228.0743; found, 228.0742.

**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 2 × 10 mm quartz cell (optical path length: 10 mm) and a 3 × 3 mm quartz cell, respectively. In the

fluorescence measurements, excitation wavelengths for CF3-AMND and AMND were set at 346 nm and 350 nm, respectively.

**Binding constants obtained from fluorescence titration:** The changes in fluorescence intensity of the ligand were monitored as a function of the concentration of DNA duplex. The resulting titration curve was analyzed by nonlinear least-squares regression based on a 1:1 binding isotherm:[2]

$$F / F_0 = \{1 + kK_{11}[D]\}/\{ 1 + K_{11}[D]\} \qquad (1)$$

where $F$ and $F_0$ are the observed fluorescence intensities of amiloride in the presence and absence of duplexes, respectively, and $k$ (= $k_{11}/k_L$) represents the ratio of proportionality constants connecting the fluorescence intensities and concentrations of the molecules (1:1 complex: $k_{11}$, free ligand: $k_L$). The concentration of the free duplex, [D], can be related to known total concentrations of the duplex ($D_0$) and ligand ($L_0$), by the following equation:

$$D_0 = [D] + \{ L_0 K_{11}[D]\}/\{1 + K_{11}[D]\} \qquad (2)$$

Together, eqs. (1) and (2) describe the system.

**Salt dependence of the binding constants.** The effect of different NaCl concentrations on the 1:1 binding constants ($K_{11}$) of $CF_3$-AMND to C and T was examined at 20 °C (pH 7.0) by fluorescence titration experiments (cf. Fig. 3A in the main text), and analyzed according to the polyelectrolyte theory proposed by Record *et al*. (ref. 11 in the main text). The observed salt dependence of the binding constants was explained by eq. (3).

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

where $Z$ is the apparent charge on the ligand and $\psi$ (0.88 for B-type DNA) is the proportion of counterions associated with each DNA phosphate group. The slope ($SK$) of the plot, which is equivalent to the number of counterions released from the duplex upon ligand binding, was obtained from the regression lines of best linear least squares fit, by which the values of $Z$ were estimated.

**Analysis of PCR products.** Asymmetric PCR was performed as described in our previous study (ref. 5c in the main text). After PCR amplification, the solution (200 μL) was concentrated to ca. 10 μL using a centrifugal filter (Microcon Ultracel YM-10, Millipore) and the obtained solution was freeze-dried (Freeze Dryer DC800, Yamato, Tokyo, Japan). To the freeze-dried sample, 50 μL of a buffer solution (pH 5.5, 100 mM sodium cacodylate) containing CF3-AMND (0.1 μM) and 20-mer AP site-containing probe DNA (2.0 μM; 5'-CCT ACG CCA **X**CA GCT CCA AC -3', X = AP site; dSpacer (a tetrahydrofuranyl residue)) was added.
Fluorescence spectra of the resulting solutions were then measured at 5°C, which would enable the effective analysis since the binding affinity of the AP site-binding ligand was higher at 5°C than at 20°C (ref. 5d in the main text).

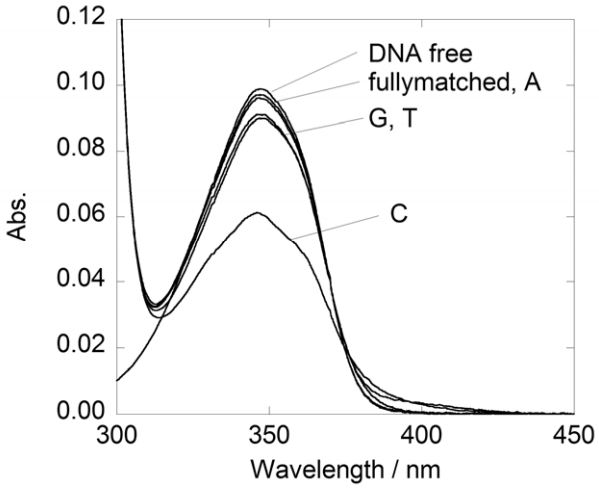


**Fig. S1** UV-visible absorption spectra of $CF_3$-AMND (10 μM) in the absence and presence of 21-meric AP site-containing DNA duplex (10 μM), measured in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. Temperature, 20°C.

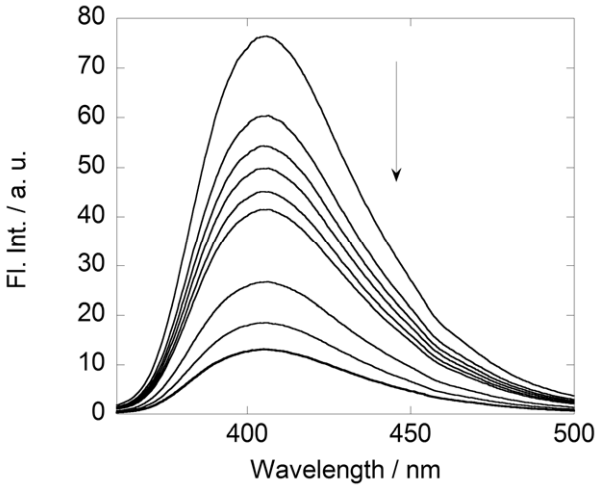


**Fig. S2** Fluorescence response of $CF_3$-AMND (1.0 μM) to C in the 21-mer AP site-containing DNA duplexes. Other solution conditions were the same as those given in Fig. 2 in the main text. Excitation, 346 nm. Temperature, 20°C.

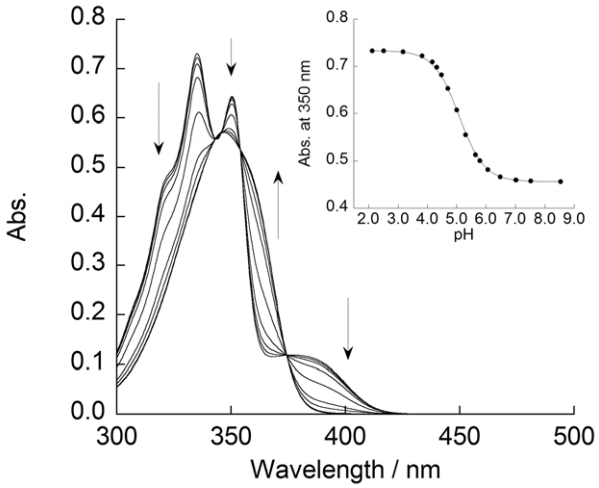


**Fig. S3** UV-visible absorption spectra of $CF_3$-AMND (45 μM) at various pH conditions from pH 2.1 to pH 8.5, measured in solutions containing 100 mM NaCl. Inset: Absorbance at 335 nm at various pH values. Temperature, 20°C.

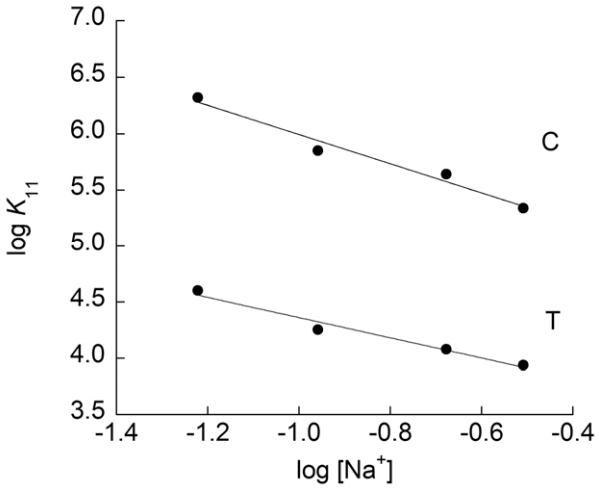


**Fig. S4** Salt dependence of the binding constants ($K_{11}$) of $CF_3$-AMND to C and T in the 21-mer AP site-containing DNA duplexes. Data are also given in Table S1. Other solution conditions were the same as those given in Fig. 3A in the main text. Temperature, 20°C. The obtained linear equation: y = 4.70 – 1.29 x (r = 0.9859) for C, y = 3.46 – 0.898 x (r = 0.9859) for T.

**Table S1.** Binding constants ($K_{11}$) of $CF_3$-AMND to C and T in an AP site-containing DNA duplex at different salt concentrations.[a)]

| | $[Na^+]$ = 60 mM[b)] | $[Na^+]$ = 110 mM[c)] | $[Na^+]$ = 210 mM[b)] | $[Na^+]$ = 310 mM[b)] |
|---|---|---|---|---|
| C | $2.1 (\pm 0.1) \times 10^6$ | $7.2 (\pm 0.2) \times 10^5$ | $3.9 (\pm 0.3) \times 10^5$ | $1.3 (\pm 0.2) \times 10^5$ |
| T | $4.0 (\pm 0.1) \times 10^4$ | $1.4 (\pm 0.2) \times 10^4$ | $1.2 (\pm 0.1) \times 10^4$ | $8.7 (\pm 0.4) \times 10^3$ |

a) The binding constants were determined by fluorescence titration experiments in solutions buffered to pH 7.0 with 10 mM sodium cacodylate, containing 1.0 mM EDTA. The concentration of NaCl was ranged from 100 mM to 300 mM.

b) Errors are the fitting errors.

c) Errors are the standard deviations obtained from three independent experiments.

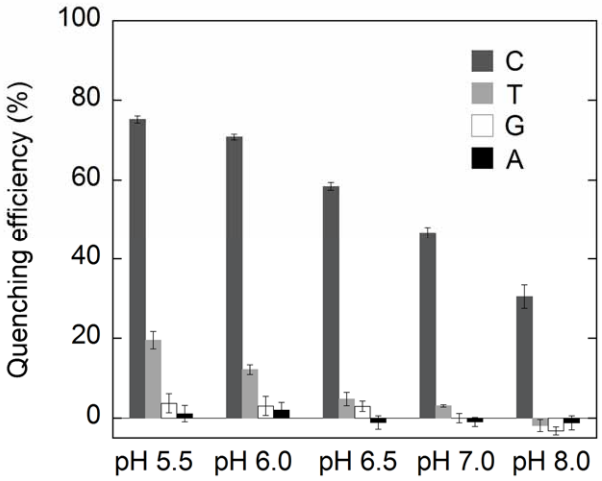


**Fig. S5** pH dependence of the quenching efficiency of $CF_3$-AMND (1.0 μM) upon binding to the target nucleobase (C, dark gray bar; T, light gray bar; G, white bar; A, black bar) in 21-mer AP site-containing DNA duplexes (1.0 μM). Other solution conditions were the same as those given in Fig. 2 in the main text. Quenching efficiency (%) was calculated by $(F_0\text{-}F)/F \times 100$, where $F$ and $F_0$ denote the fluorescence intensities of $CF_3$-AMND in the presence and absence of DNA duplexes, respectively. Excitation, 346 nm. Analysis, 403 nm. Temperature, 20°C. Error bars represent the standard deviations obtained from three independent experiments.

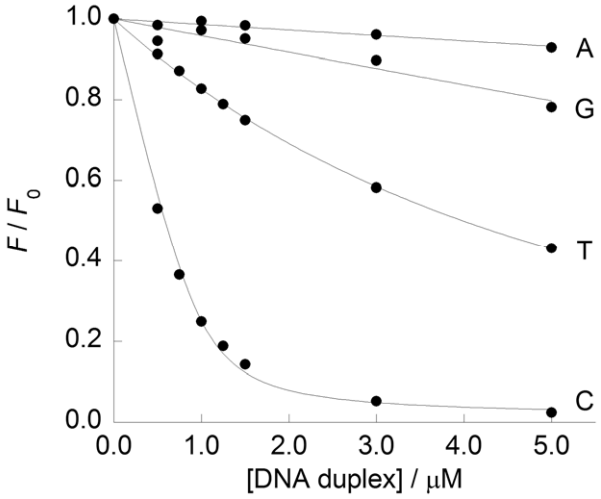


**Fig. S6** Fluorescence titration curves for the binding of $CF_3$-AMND (1.0 μM) to 21-mer AP site-containing DNA duplexes, obtained in solutions buffered to pH 5.5 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. The changes in the fluorescence intensities at 403 nm were analyzed based on a 1:1 binding isotherm model. $F$ and $F_0$ denote the fluorescence intensities of $CF_3$-AMND in the presence and absence of DNA duplexes, respectively. Excitation, 346 nm. Temperature, 20°C.

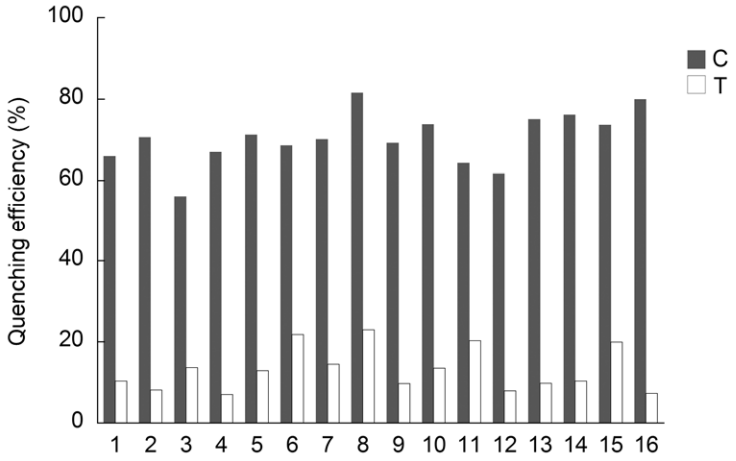


**Fig. S7** Effect of flanking nucleobases on the fluorescence quenching efficiency of $CF_3$-AMND (0.5 μM) to C (gray bars) and T (white bars) in the 21-mer AP site-containing DNA duplexes (1.0 μM; 5'-GCT CCT CTG WXW' CCC TCG ACG-3'/3'-CGA GGA GAC Z**N**Z' GGG AGC TGC-5', **X** = AP site (Spacer C3) or G, **N** = C, or T). W, W' represent the nucleobases flanking the AP site and Z, Z' represent complementary nucleobases flanking the target nucleobase, where W**X**W'/Z**N**Z' = **1**, T**X**T/A**N**A; **2**, G**X**T/CAN; **3**, C**X**T/G**N**A; **4**, A**X**T/T**N**A; **5**, T**X**G/A**N**C; **6**, C**X**G/G**N**C; **7**, A**X**G/T**N**C; **8**, G**X**G/C**N**C; **9**, T**X**C/A**N**G; **10**, G**X**C/C**N**G; **11**, C**X**C/G**N**G; **12**, A**X**C/T**N**G; **13**, T**X**A/A**N**T; **14**, G**X**A/C**N**T; **15**, C**X**A/G**N**T; **16**, A**X**A/T**N**T. Other solution conditions were the

same as those given in Fig. S6. Quenching efficiency (%) was calculated by $(F_0\text{-}F)/F \times 100$, where $F$ and $F_0$ denote the fluorescence intensities of $CF_3$-AMND in the presence and absence of DNA duplexes, respectively. Excitation, 346 nm. Analysis, 403 nm. Temperature, 20°C.

References

(1) Puglisi, J. D.; Tinoco, I. *Method Enzymol.* **1989**, *180*, 304-325.

(2) Connors, K. A. *Binding constants*, John Wiley & Sons, New York, 1987, pp. 339-343.