

Supplementary Information

Florescence and electrochemical detection of pyrimidine/purine transversion by a ferrocenyl aminonaphthyridine derivative

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Synthesis of 7-methyl-1,8-naphthyridin-2-amine: AMND

2,6-diaminopyridine (6.5 g, 59.6 mmol) and 98% phosphoric acid (100 mL) were added to 1,1-dimethoxy-3-butanone (7.5 g, 56.7 mmol) and the mixture was stirred for 24 h at 90°C. After neutralization by addition of NaOH aq. (ca. 3 M, 800 mL), the mixture was filtered to remove a black gum and phosphate salt. The filtrate was extracted seven times with 700 mL of chloroform each time, and the chloroform phases were dried over Na₂SO₄, before being evaporated to dryness to give AMND as brown solid. Yield: 6.5 g (40.8 mmol, 72%). ¹H NMR (CDCl₃-d, J / Hz, δ / ppm): δ = 7.83 (d, 1H, J = 8.1 Hz), 7.82 (d, 1H, J = 8.5 Hz), 7.08 (d, 1H, J = 8.1 Hz), 6.75 (d, 1H, J = 8.5 Hz), 5.23 (br., 2H), 2.69 (s, 3H).

Synthesis of N-ferrocenylmethyl-7-methyl-1,8-naphthyridin-2-amine: FcAMND

Ferrocenecarboxaldehyde (0.68 g, 3.1 mmol) and AMND (0.50 g, 3.1 mmol) were dissolved in CH₂Cl₂ (30 ml) and stirred for 6 h at reflux. Sodium borohydride (1.2 g, 31 mmol) was added, and stirring was continued for another hour. After this period the excess borohydride was destroyed by the dropwise addition of HCl (1 M, ca. 40 ml) until effervescence had stopped and the solution was acidic (pH 2). Sodium hydroxide (1 M, ca. 25 ml) was then added until the solution was slightly basic (pH 10). The mixture was extracted into CH₂Cl₂, washed with water, and dried over Na₂SO₄. Evaporation to dryness gave the crude product as orange solid. The crude product was purified by size exclusion chromatography (CHCl₃), followed by silica gel column chromatography (MeOH-CHCl₃). Yield = ca. 0.2 mg (0.6 mmol, 20%). ¹H-NMR (CDCl₃-d, J / Hz, δ / ppm): δ = 2.69 (s, 3H, CH₃), 4.14 (t, 2H, J = 1.6, *m*-CpH), 4.19 (s, 5H, unsubst. CpH), 4.25 (t, 2H, J = 1.6, *o*-CpH), 4.49 (d, 2H, J = 4.9, NHCH₂Cp), 5.02 (br, 1H, NH), 6.56 (d, J = 8.9, 1H, PhH), 7.03 (d, J = 7.8, 1H, PhH), 7.72 (d, J = 8.9, 1H, PhH),

7.78 (*d*, $J = 7.8$, 1H, PhH). ESI-MS: calcd. for $[M+H]^+$, 358.1001; found, 358.1000. Elemental analysis: calcd. for $C_{20}H_{19}FeN_3 \cdot 0.3 H_2O$: C, 66.24; H, 5.45; N, 11.59; found: C, 66.16; H, 5.49; N, 11.80%.

General experimental procedures

All reagents were purchased from Wako Pure Chemicals (Sendai, Japan). All of the oligodeoxynucleotides used in the present study were custom synthesized by Nihon Gene Research Laboratories Inc. (Sendai, Japan). For the synthesis of abasic (AP) site-containing DNAs, a propylene residue (Spacer phosphoramidite C3, Spacer C3) was utilized. The concentration of DNA was determined from the molar extinction coefficient at 260 nm. Water was deionized ($\geq 18.0 M\Omega cm$ specific resistance) with an Elix 5 UV Water Purification System and a Milli-Q Synthesis A10 system (Millipore Corp., Bedford, MA). The other reagents were commercially available analytical grade and were used without further purification. Unless otherwise stated, measurements were performed in 10 mM sodium cacodylate buffer solutions (pH 7.0) containing 100 mM NaCl and 1 mM EDTA. Before the measurements, the sample solutions were annealed as follows: heating at 75°C for 10 min, and then gradually cooling down to 5°C (3°C/min).

UV-vis absorption measurements

Unless otherwise stated, absorption spectra were measured with a JASCO V-570 spectrophotometer (Japan Spectroscopic Co. Ltd., Tokyo, Japan) equipped with a thermoelectrically temperature controlled cell holder (quartz cuvette, 1 mm \times 10 mm).

Fluorescence measurements

Unless otherwise stated, fluorescence spectra were measured with a JASCO FP-6500 spectrofluorophotometer (Japan Spectroscopic Co. Ltd.) equipped with a thermoelectrically temperature controlled cell holder (quartz cuvette, 3 mm \times 3 mm); the slits for the excitation and emission monochromators were 3 and 3 nm, respectively. All emission spectra were corrected for the instrumental spectral response.

Electrochemical measurements

An Ag/AgCl (sat. KCl) electrode and a platinum wire were used as reference and counter electrodes, respectively. Au electrodes (1.6 mm ϕ) were purchased from BAS (Tokyo, Japan) and used as a working electrode. Before each experiment, Au electrodes were polished successively by 1.0 and 0.05 μm

alumina slurries (Bühler, Illinois, USA) with Milli-Q water on polishing cloths (Bühler). After polishing, the electrodes were rinsed with Milli-Q water several times and sonicated in Milli-Q water for three 20 s periods. Cyclic voltammogram (CV) and square wave voltammogram (SWV) measurements were performed using a voltammetric analyzer (ALS model 1030, BAS) equipped with a thermoelectrically temperature controller. CVs were recorded with a scan rate of 0.1 V s⁻¹ in a potential range of -0.2 to 0.4 V and the formal potential of FcAMND was determined by CV. The pH dependant CV measurements were performed in an aqueous buffer solution containing 0.1 M NaCl, 10 mM phosphoric acid, 10 mM acetic acid and 10 mM boric acid. SWVs were recorded in the same potential range as CV measurements with a modulation amplitude of 20 mV, a step potential of 5 mV, and a frequency of 10 Hz.

Molecular modeling simulations

Molecular modeling simulations were carried out by using MacroModel Ver. 9.0 (Schörringer, LLC, Portland, OR). The energy minimizations were performed with Amber* force field and GB-SA treatment water for the initial structures of the complex between FcAMND and 11-meric AP site-containing duplex (5'-d(TCC AGX GCA AC)-3'/3'-d(AGG TCT CGT TG)-5', X= Spacer C3), which were obtained by manually inserting the ligand into the AP site.

Fluorescence intensity of FcAMND

As can be seen from Fig. S1, FcAMND and AMND show absorption maxima at 363 nm and 343 nm, respectively. While both the shape and position of the emission peak of FcAMND almost coincide with those of AMND (cf. Fig. S2), the intensity at 405 nm is reduced to *ca.* 1/10 for FcAMND compared with AMND. The quantum yield of AMND ($\Phi = 0.386$) was determined by an absolute PL quantum yield measurement system, model CC9920-02 (Hamamatsu Photonics). The quantum yield of FcAMND ($\Phi \approx 0.04$) was estimated by using AMND as a reference. The decrease in the fluorescence intensity of FcAMND is explained by an electron transfer mechanism, in which Fc and AMND moieties act as an electron donor and an acceptor, respectively. The feasibility of electron transfer between AMND and Fc moieties can be assessed according to the Rehm-Weller equation:

$$\Delta G_{el} = 23.06 [E(D)_{ox} - E(A)_{red}] - w_p - \Delta G_{00}$$

where ΔG_{el} , $E(D)_{ox}$, $E(A)_{red}$, w_p , and ΔG_{00} are the free energy change of electron transfer (kcal mol⁻¹), the oxidation potential of a donor (D), the reduction potential of an acceptor (A), the ion-pairing energy, and

the excitation energy of the donor (A), respectively. The oxidation potential of Fc, $E(\text{Fc})_{\text{ox}}$, is 0.25 V vs. Ag/AgCl. The value of ω_p , is usually estimated to be $-0.5 \text{ kcal mol}^{-1}$ in water. $E(\text{AMND})_{\text{red}}$ is assumed to be the same as the reported reduction potential of naphthyridine derivatives (*ca.* -1.5 V vs. Ag/AgCl), and $\Delta G_{00}(\text{AMND})$ is 83 kcal mol^{-1} . The free energy change, ΔG_{el} , within the present system calculated from the above values is negative. Accordingly, fluorescence quenching of FcAMND can be ascribed to the photoelectron transfer where AMND and Fc moieties act as an electron acceptor and a donor, respectively.

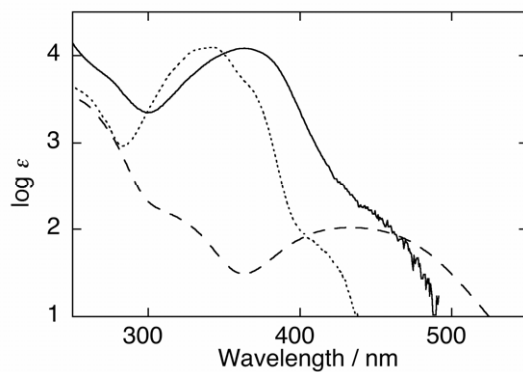


Fig. S1 Absorption spectra of FcAMND (solid line) and AMND (dotted line) Me₂NMeFc (broken line) in a buffer solution. The vertical axis plots the logarithm of the molar absorption coefficient. [FcAMND] = 30 μM, [AMND] = 50 μM, [Me₂NMeFc] = 500 μM, temperature = 20°C.

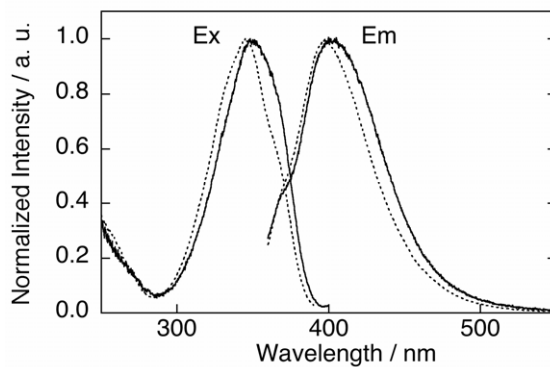


Fig. S2 Normalized excitation and emission spectra of FcAMND (solid line) and AMND (dotted line) in a buffer solution. [FcAMND] = 15 μM, [AMND] = 1 μM, temperature = 20°C. Excitation wavelength = 350 nm, emission wavelength = 405 nm, excitation band width = 3 nm, emission band width = 3 nm.