Supplementary Material (ESI) for Chemical Communications
This journal is (c) The Royal Society of Chemistry 2011

Supporting Information for

Fluorescent trimethyl-substituted naphthyridine as a ligand for C-C mismatch detection in CCG trinucleotide repeats

Yusuke Sato, Atsuko Honjo, Daisuke Ishikawa, Seiichi Nishizawa, and Norio Teramae*

Department of Chemistry, Graduate School of Science, Tohoku University, Aoba-ku, Sendai, 980-8578, Japan.

Experimental

Reagents: All of the DNAs were custom synthesized and HPLC purified by Nihon Gene Research Laboratories Inc. (Sendai, Japan). 2-Amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND) was purchased from Enamine (Kiev, Ukraine). The concentrations of DNAs were determined from the molar extinction coefficient at 260 nm:[1] 100740 cm^{-1} M^{-1} for 5'-CCG CCG CCG CCG-3'; 122180 cm^{-1} M^{-1} for 5'-CAG CAG CAG CAG-3'; 99940 cm^{-1} M^{-1} for 5'-CTG CTG CTG CTG-3'; 113780 cm^{-1} M^{-1} for 5'-CGG CGG CGG CGG-3'; 249780 cm^{-1} M^{-1} for 5'-CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG-3'; 373980 cm^{-1} M^{-1} for 5'-CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG CCG -3'. 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 105 mM NaCl and 0.1 mM EDTA. Following the procedure of reference 11 in the main text, before measurements, the sample solutions were annealed as follows: heated at 90°C for 10 min, cooled to 4°C, and kept at 4°C for 12 h.

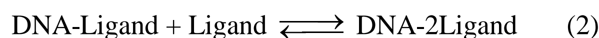
1. J. D. Puglisi, I. Tinoco, Jr., *Method Enzymol.*, **1989**, 180, 304-325.

Fluorescence measurements: Fluorescence spectra were measured at 4°C with a JASCO model FP-6500 spectrofluorophotometer equipped with a thermoelectrically temperature-controlled cell holder (Japan Spectroscopic Co. Ltd., Tokyo, Japan) using a 3 × 3 mm quartz cell. Excitation wavelength for ATMND was set at 350 nm.

Binding constants obtained from fluorescence titration: The changes in fluorescence intensity at 403 nm for ATMND were monitored as a function of the concentration of DNAs. From the results obtained by the examination of the binding stoichiometry shown in Figure S2, the following equilibriums were considered to describe the present system.

$$\text{DNA} + \text{Ligand} \rightleftharpoons \text{DNA-Ligand} \qquad (1)$$

This journal is (c) The Royal Society of Chemistry 2011



$$\text{DNA-Ligand} + \text{Ligand} \rightleftharpoons \text{DNA-2Ligand} \quad (2)$$

$$K_{11} = [\text{DNA-Ligand}]/[\text{DNA}][\text{Ligand}] \quad (3)$$

$$K_{12} = [\text{DNA-2Ligand}]/[\text{DNA-Ligand}][\text{Ligand}] \quad (4)$$

Total concentrations of DNA (C_d) and Ligand (C_l) can be expressed by the following equations.

$$C_d = [\text{DNA}] + [\text{DNA-Ligand}] + [\text{DNA-2Ligand}] \quad (5)$$

$$C_l = [\text{Ligand}] + [\text{DNA-Ligand}] + 2[\text{DNA-2Ligand}] \quad (6)$$

By combining equations (3)-(6), C_t can be expressed as follows:

$$C_d = \{(C_l - [\text{Ligand}])(1 + K_{11}[\text{Ligand}] + K_{11}K_{12}[\text{Ligand}]^2)\}/\{(K_{11}[\text{Ligand}](1 + 2K_{12}[\text{Ligand}])\} \quad (7)$$

On the other hand, the fluorescence intensity can be interpreted as the sum of each contribution as follows:

$$F = k_d[\text{DNA}] + k_l[\text{Ligand}] + k_{11}[\text{DNA-Ligand}] + k_{12}[\text{DNA-2Ligand}] \quad (8)$$

where k represent proportionality constants connecting the fluorescence intensities and concentrations of the species (k_d: free DNA, k_l: free ligand, k_{11}: 1:1 DNA-ligand complex, k_{12}: 1:2 DNA-ligand complex). In this equation, free DNA is not a fluorescence component. In addition, both 1:1 DNA-ligand complex and 1:2 DNA-ligand complex are assumed not to be contributors based on the fact that these complexes show almost no fluorescence when almost all ATMND forms the complex with DNA molecules at the high excess DNA concentration (Figure S3) and then, equation (8) can be expressed in the following simple form:

$$F = k_l[\text{Ligand}] \quad (9)$$

The following equation can be derived by using the relationship of the fluorescence intensity of ligand in the absence of DNA and target ($F_0 = k_l\, C_l$).

$$F/F_0 = [\text{Ligand}]/C_l \quad (10)$$

Together, equations (7) and (10) describe the present system.

Isothermal titration calorimetry: ITC experiments were carried out using a Microcal VP-ITC calorimeter (Microcal Inc., Northampton, MA). The Origin software (Microcal) was used for data acquisition and analysis. All solutions were degassed by stirring under vacuum before use. Typically, the reference cell contained deionized water, and a titration was done at 4 ^{o}C so that 10 μL of ATMND solution were added by a syringe (a total of 25 injections) to 1.43 mL of solution containing 5.0 μM $(CCG)_4$ in the sample cell. The injection time was 30 s, and the interval between injections was 300 s. In order to remove any air bubbles in the tip of the syringe, the initial injection was set as 5 μL, and the resulting peak was neglected in the analysis. The peaks produced during titration were converted into heat output per injection by integration and correction for the cell volume and sample concentration. The heats of dilution for the addition of ATMND into buffer solution were determined independently, and the net enthalpy for ATMND-$(CCG)_4$ interactions was determined by subtraction of the heats of dilution. The data thus obtained were best fitted to a two-site, sequential binding model, giving the binding affinity (K), the binding enthalpies (ΔH) and the binding entropies (ΔS)

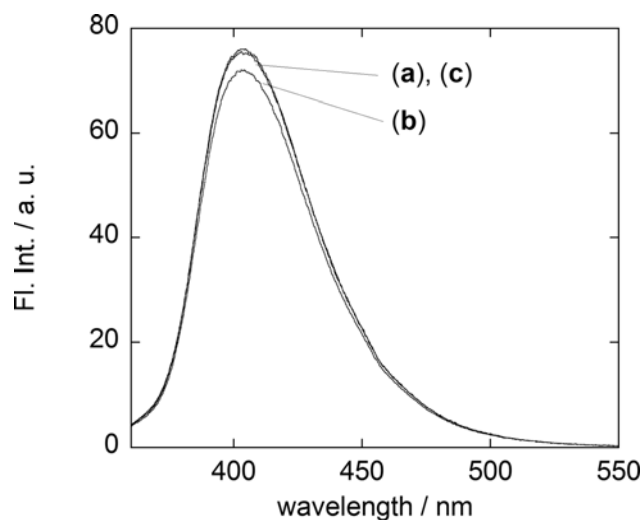


Fig. S1 Fluorescence spectra of ATMND (1.0 μM) in the (a) absence and presence of (b) $(CCG)_4/(CGG)_4$ DNA duplex (1.0 μM: 5'-CCG CCG CCG CCG-3'/3'-GCC GCC GCC GCC-5') and (c) hairpin DNA possessing an A-A mismatch in its stem moiety and a CGCC loop (1.0 μM: 5'-CAG CCG CCG CAG-3'), measured in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 105 mM NaCl and 0.1 mM EDTA. Excitation, 350 nm. Temperature, 4°C.

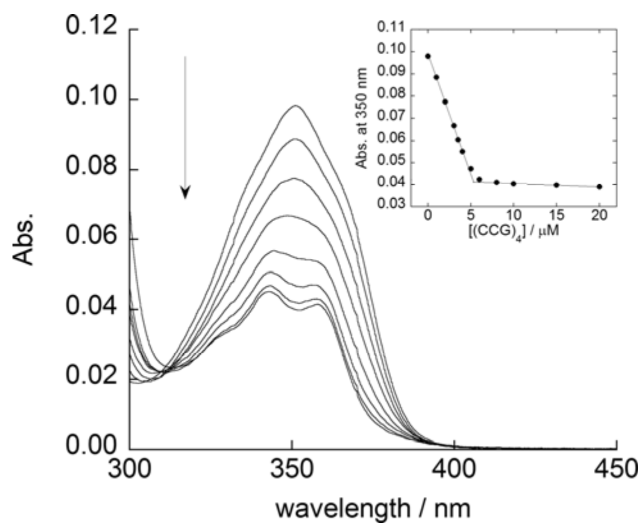


Fig. S2 Absorption spectra of ATMND (10 μM) in the presence of $(CCG)_4$ (0-20 μM). Inset: Absorbance change at 350 nm for ATMND upon binding to $(CCG)_4$. Other solution conditions are the same as those given in Fig. 2 in the main text. Temperature, 4°C.

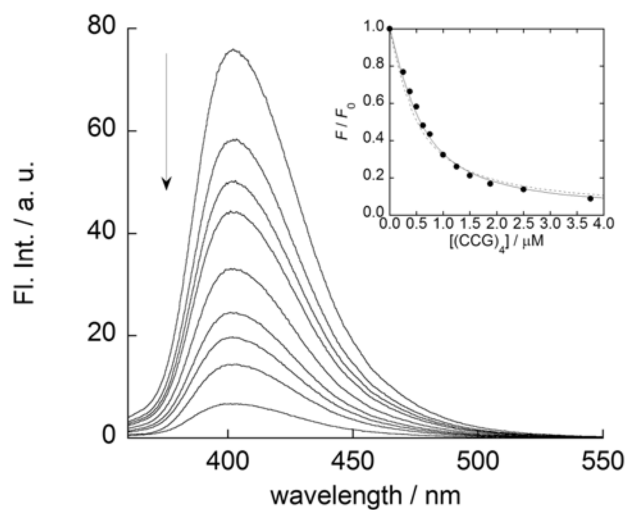


Fig. S3 Fluorescence response of ATMND (1.0 μM) to $(CCG)_4$. Inset: titration curve for ATMND binding to (CCG)4 based on the $K_{11} \neq K_{12}$ model (solid line); the dotted curve represents the titration curve based on the $K_{11} = K_{12}$ model. Other solution conditions are the same as those given in Fig. 2 in the main text. Excitation, 350 nm. Analysis, 403 nm. Temperature, 4°C.

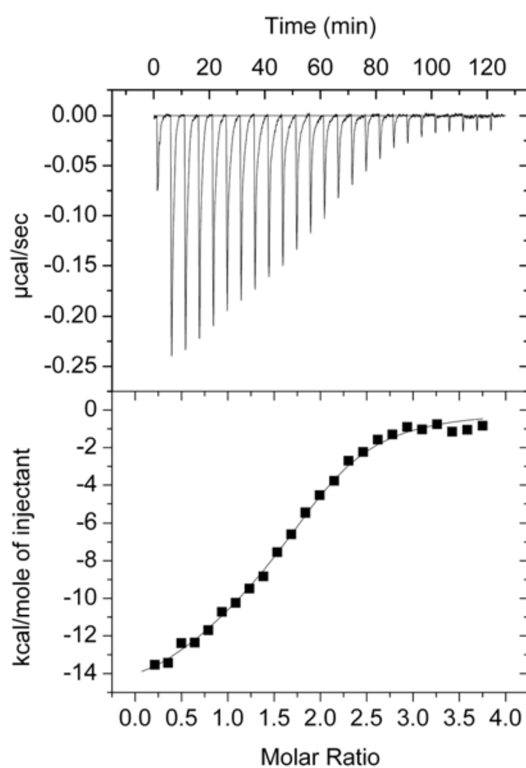


Fig. S4 ITC data for the addition of ATMND aliquots (each 15 μl of 100 μM) into the solution containing $(CCG)_4$ (1.43 ml of 5.0 μM, 5'-CCG CCG CCG CCG -3'). Sample solutions were buffered to pH 7.0 with 10 mM sodium cacodylate, containing 105 mM NaCl and 0.1 mM EDTA. Temperature, 4°C. The data were best fitted to a two-site sequential binding model.

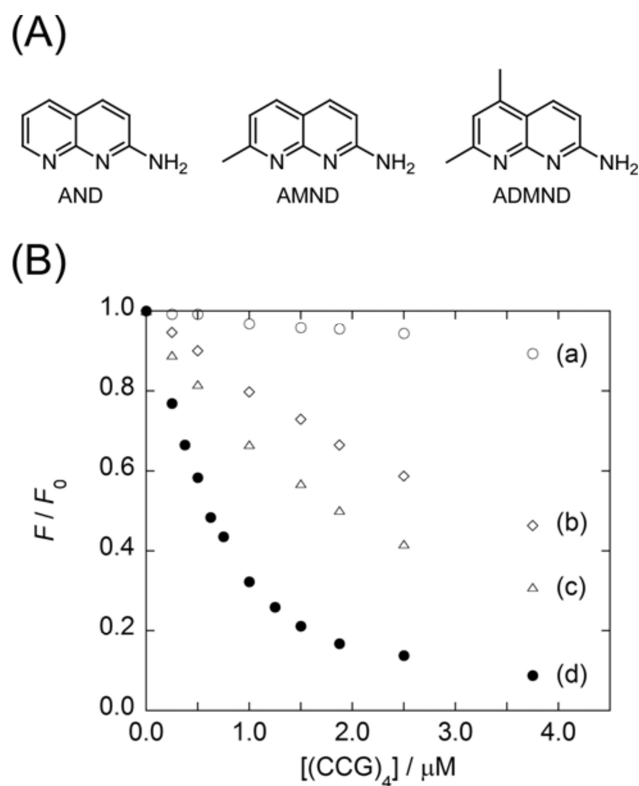


Fig. S5 (A) Chemical structures of a series of 2-amino-1,8-naphthyridines used for comparison with ATMND for the binding to $(CCG)_4$. (B) Fluorescence response of 2-amino-1,8-naphthyridines (1.0 μM): (**a**) AND, (**b**) AMND, (**c**) ADMND, and (**d**) ATMND upon binding to $(CCG)_4$. Other sample conditions are the same as those given in Fig. 2 in the main text. Excitation, 350 nm. Analysis, 392 nm for AND, 400 nm for AMND and ADMND, 403 nm for ATMND. Temperature, 4°C.