Supplementary Material to Accompany: Tetrakis-acridinyl peptide: A novel fluorometric reagent for nucleic acid analysis based on the fluorescence dequenching upon DNA bind

*Hirouki Ueyama, Makoto Takagi, and Shigeori Takenaka**

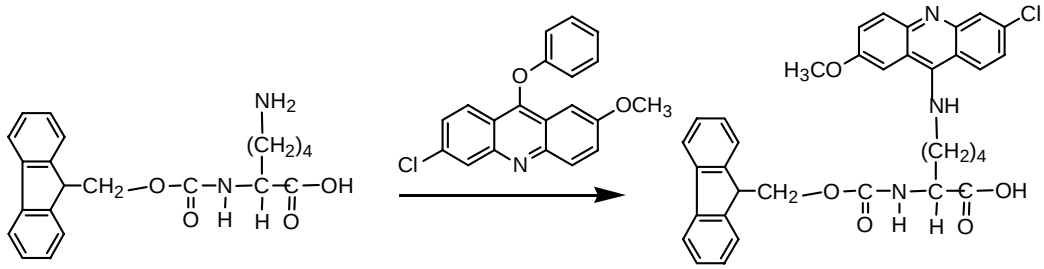
Department of Applied Chemistry, Faculty of Engineering, Kyushu University, Fukuoka 812-8581, Japan

Synthesis

6-Chloro-2-methoxy-9-phenoxyacridine

6-Chloro-2-methoxy-9-phenoxyacridine was prepared from 6,9-dichloro-2-methoxy-acridine by the procedure described previously (Takenaka, S.; Sato, H.; Ihara, T.; Takagi, M. *J. Heterocyclic Chem.* **1997**, *34*, 123-127).

Fmoc-Lys(Acr)-OH



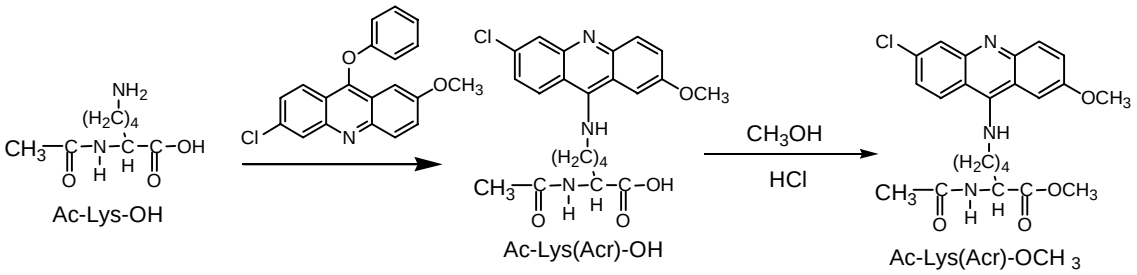
Fmoc-Lys-OH Fmoc-Lys(Acr)-OH

6-Chloro-2-methoxy-9-phenoxyacridine (4.54 g, 13.5 mmol) was dissolved at 80 °C in 30 g of phenol in a 50 ml beaker. While stirring with a magnetic bar at 55-65 °C, Fmoc-Lys-OH (4.42 g, 12.0 mmol) was added and the mixture was stirred for 2 h at 55-65 °C. After cooling to room temperature, 300 ml of ethyl ether was added while stirring vigorously. The yellow precipitate formed was filtered and washed with ethyl ether. The product was dried under vacuum to give 7.57 g (92% yield) of Fmoc-Lys(Acr)-OH as a yellow solid, mp. 142-143.5 °C. Time of flight mass spectroscopy gave 610.8 of $[C_{21}H_{33}N_3O_5Cl]^+$ for Fmoc-Lys(Acr)-OH (theory 610.1).

*Teterakis-acridinyl peptide (**1**)*

Teterakis-acridinyl peptide **1** was assembled on a peptide synthesizer (PE Applied Biosystems, model 431A) by using Fmoc chemistry. Starting with 200 mg of Fmoc-NH SAL resin, 500 mg of a **1**-immobilized resin were obtained. This resin (500 mg) was suspended in a mixture of m-cresol (0.1 ml), thioanisole (0.6 ml), and trifluoroacetic acid (TFA, 4.3 ml) and stirred at room temperature for 1 h. After removing TFA under reduced pressure, ethyl ether (20 ml) was added on ice, the mixture was sonicated and incuvated for five minutes. The supernatant was removed and ethyl acetate (20 ml) was added to this precipitate, the mixture was sonicated and incuvated for five minutes. The yellow solid collected by filtration was washed with ethyl ether and then dried under vacuum to give 318 mg of the product. Peptide **1** was purified by reversed phased HPLC (Figure S1). The eluent, consisting of 0.1% TFA (A) and 0.1% TFA in 70% acetonitrile (B), was run in a linear gradient of 0-100% of (B) over 40 min at a flow rate of 1.0 ml/min. Time of flight mass spectroscopy gave 2309.03 of $[C_{118}H_{157}N_{25}O_{15}C_{l4}]^+$ for **1**+H^+ (theory 2307.51)(Figure. S2).

*Ac-Lys(Acr)-OMe (**2**)*



6-Chloro-2-methoxy-9-phenoxyacridine (757 mg, 2.25 mmol) was dissolved at 80 °C in 5.0 g of phenol in a 50 ml beaker. While stirring with a magnetic bar at 60 °C, Ac-Lys-OH (377 mg, 2.0 mmol) was dissolved over 40 min and the mixture was stirred for 1 h at 55-60 °C. After cooling to room temperature, 50 ml of ethyl ether was added while stirring vigorously. The yellow precipitate formed was filtered and washed with ethyl ether. The solid was dried under vacuum to give 876 mg of Ac-Lys(Acr)-OH. To a suspension of crude Ac-Lys(Acr)-OH (876 mg) in dry methanol was introduced dry hydrogen chloride and the mixture was stirred for 4 h. Upon removal of the solvent under vacuum, a solid was obtained. The product was purified by recrystallization from dry methanol and dry ethyl ether to yield 633 mg (74 %) of **2** as a yellow solid, mp. 135-138 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 1.4 (q, 2H), 1.7 (quin, 2H), 1.8 (s, 3H), 1.9 (br, 2H), 3.6 (s, 3H), 4.0 (s, 3H), 4.1 (q, 2H), 4.2 (q, 1H), 7.5 (d, 8.3 Hz, 1H), 7.7 (d, 9.4 Hz, 1H), 7.9 (d, 9.4 Hz, 1H), 8.0 (s, 2H), 8.3 (d, 8.3 Hz, 1H), 8.5 (d, 8.6 Hz, 1H), 9.9 ppm (br, 1H) (Figure S3).

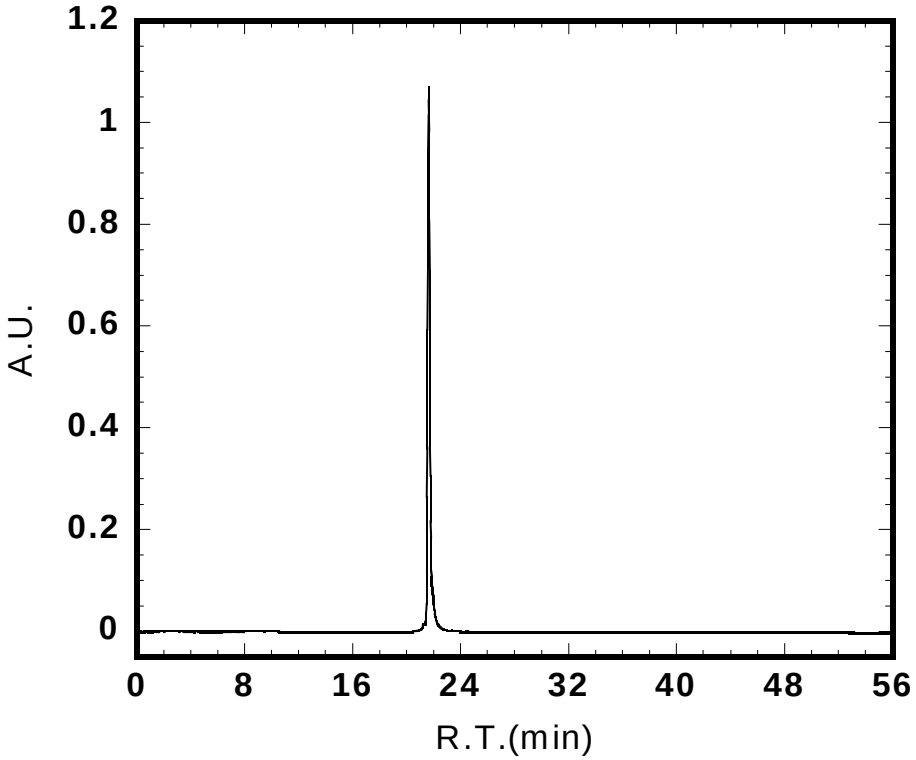


Figure 1S. Reversed phase HPLC of **1**. The eluent, consisting of 0.1% TFA (A) and 0.1% TFA in 70% acetonitrile (B), was run in a linear gradient of 0-100% of (B) over 40 min at a flow rate of 1.0 ml/min.

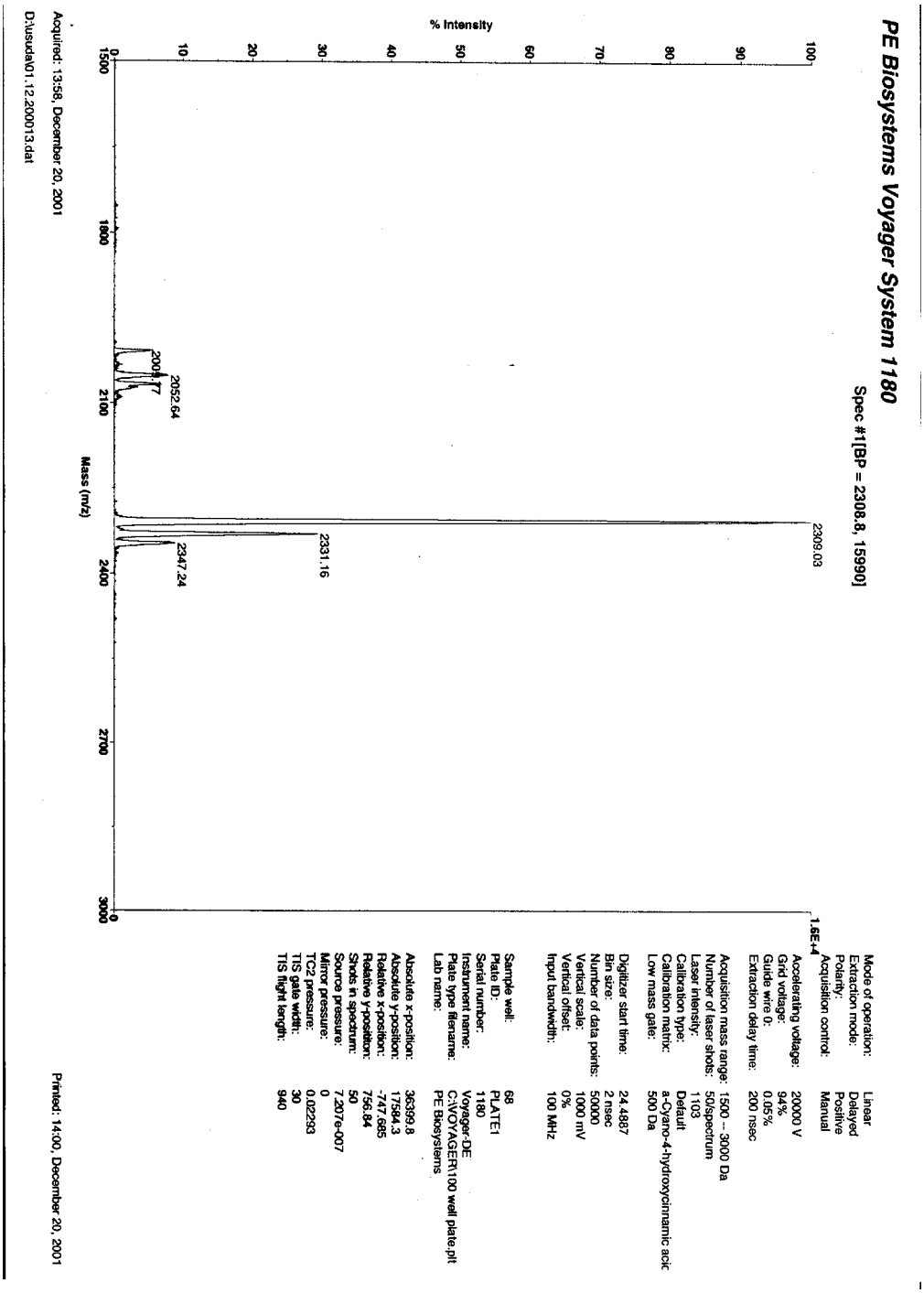


Figure S2. Time of flight mass spectrum of **1**. The m/z value of 2309.03 is consistent with that of $[C_{118}H_{157}N_{25}O_{15}Cl_4]^+$ (theory 2307.51).

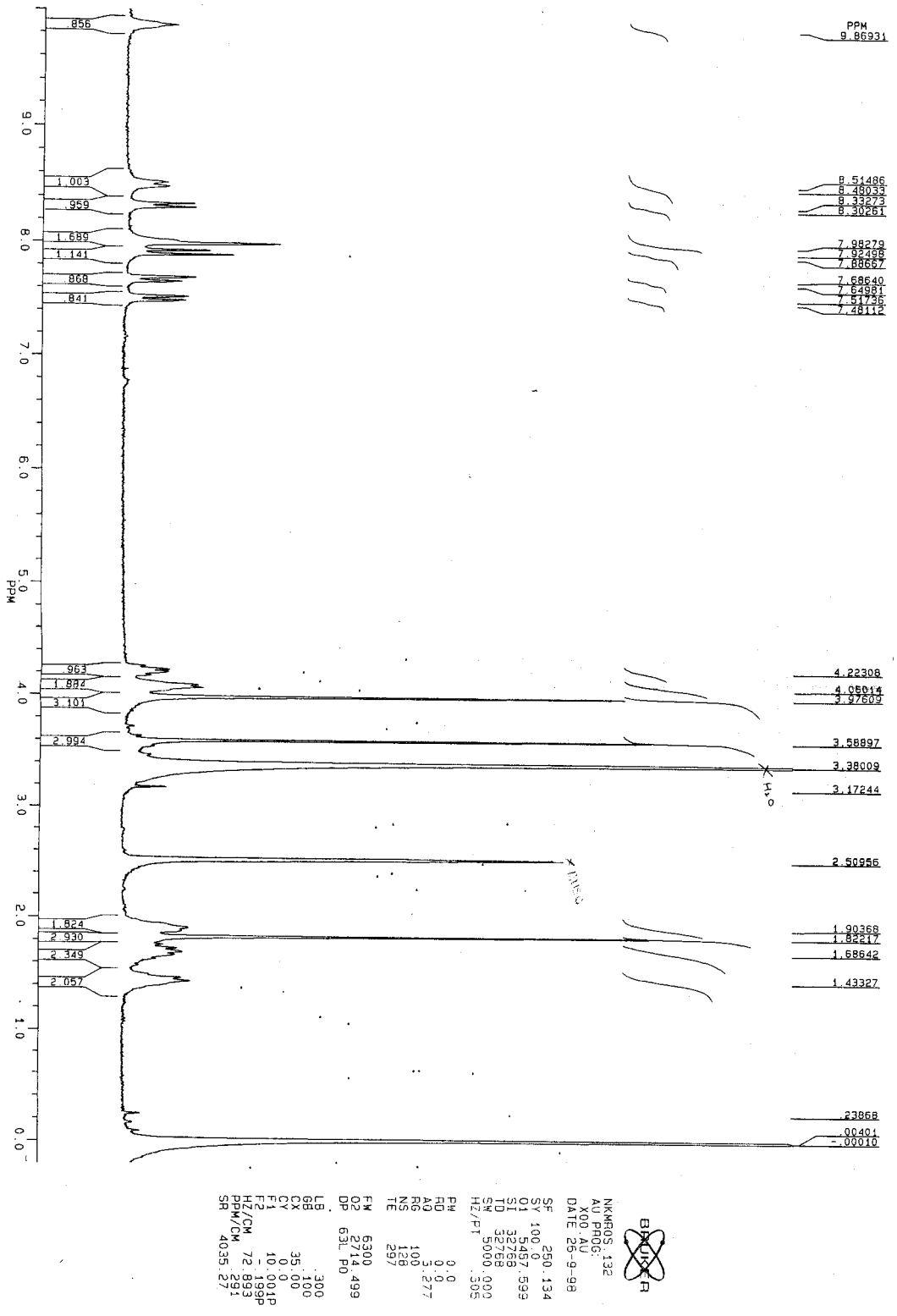


Figure S3. ^{1}H NMR (250 MHz, DMSO-d_6) of Ac-Lys(Acr)-OMe, **2**.

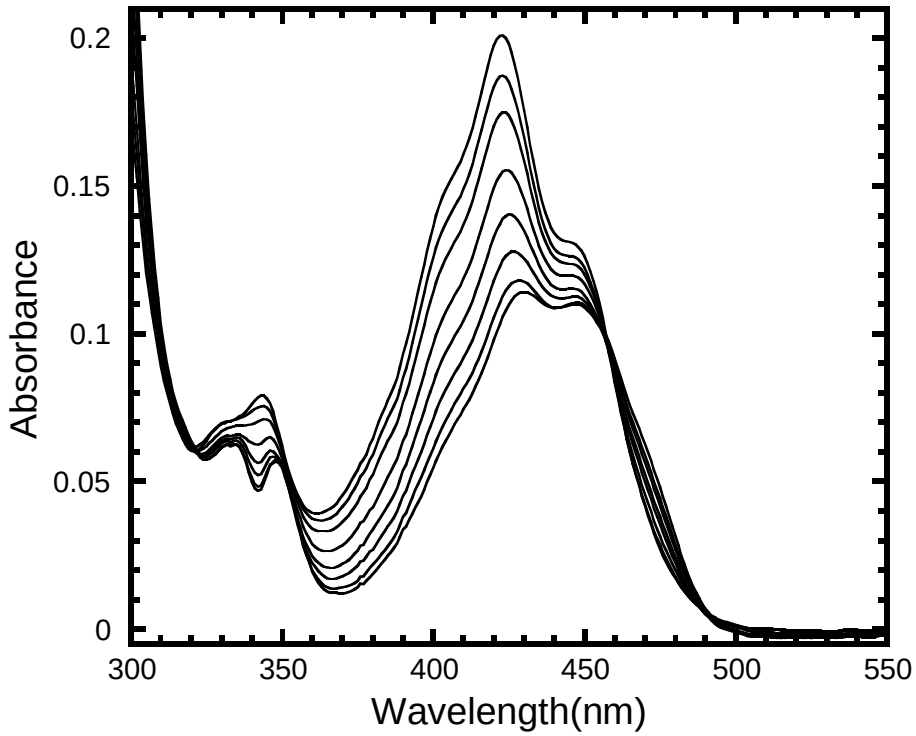


Figure S4. Spectral shifts of 0.7 μM **1** in 10 mM MES buffer and 1 mM EDTA containing 0.2 M NaCl (pH 6.25) on titration with $[poly(dA\text{-}dT)]_2$. The DNA base pair concentrations were 0, 0.30, 0.75, 1.50, 2.10, 2.70, 3.23, and 3.68 μM from top to bottom.

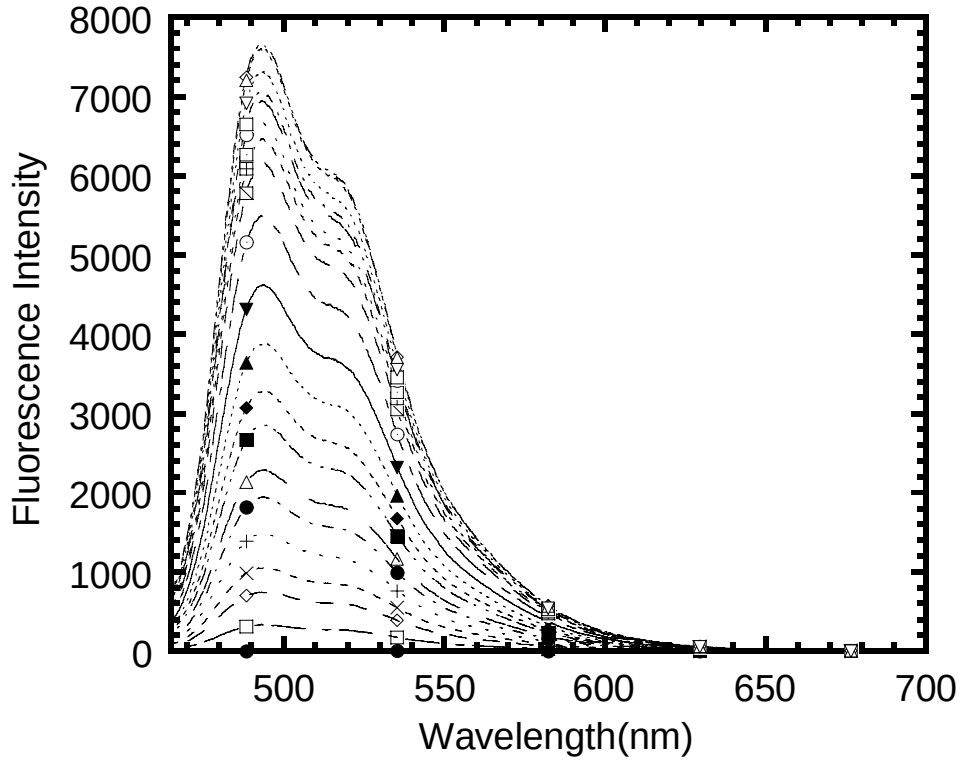


Figure S5. Fluorometric titration of 1.6 μM **1** with $[poly(dA\text{-}dT)]_2$ in 10 mM MES buffer and 1 mM EDTA (pH 6.25) containing 0.2 M NaCl at 25 °C. The DNA base pair concentrations were 0, 1.13, 2.26, 3.39, 4.52, 5.65, 6.78, 7.91, 9.04, 10.17, 11.30, 12.43, 13.56, 14.69, 15.82, 18.08, 20.34, 24.86, 33.90, and 42.94 μM from bottom to top. The excitation wavelength was 457 nm.

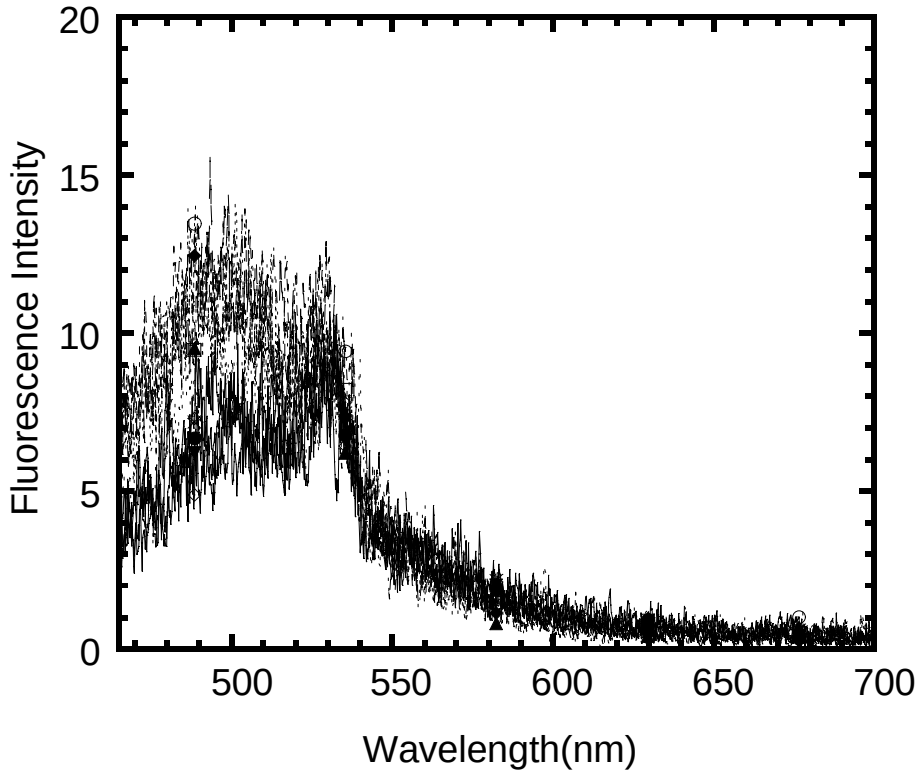


Figure S6. Fluorometric titration of 1.6 μM **1** with $[poly(dG\text{-}dC)]_2$ in 10 mM MES buffer and 1 mM EDTA (pH 6.25) containing 0.2 M NaCl at 25 °C. The DNA base pair concentrations were 0, 1.08, 2.16, 3.24, 4.32, 9.72, 15.12, 20.52, 25.92, and 31.32 μM from bottom to top. The excitation wavelength was 451 nm.

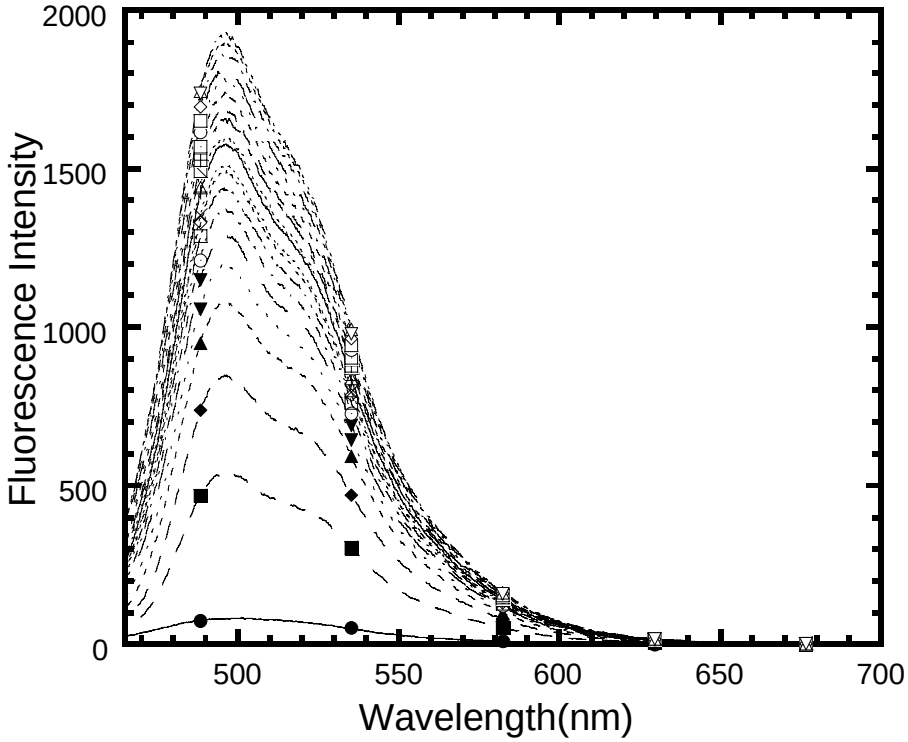


Figure S7. Fluorometric titration of 1.6 μM A666 with $[poly(dA\text{-}dT)]_2$ in 10 mM MES buffer and 1 mM EDTA (pH 6.25) containing 0.2 M NaCl at 25 °C. The DNA base pair concentrations were 0, 1.22, 2.44, 3.66, 4.88, 6.10, 7.32, 8.54, 9.76, 10.98, 12.20, 13.42, 15.86, 18.30, 23.18, 32.94, 43.92, 73.20, 106.14, and 117.12 μM from bottom to top. The excitation wavelength was 453 nm.