Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information for

Effect of Substituents of Alloxazine Derivatives on the Selectivity and Affinity for Adenine in AP-site-containing DNA Duplexes†

Burki Rajendar,[a,b] Arivazhagan Rajendran,[a,c] Zhiqiang Ye,[d] Eriko Kanai,[a] Yusuke Sato,[a] Seiichi Nishizawa,[a,e] Marek Sikorski,[f] and Norio Teramae[a,e]*

[a]Department of Chemistry, Graduate School of Science, Tohoku University, Aoba-ku, Sendai 980-8578, Japan. Fax: +81 22 7956552; Tel: +81 22 7956549; E-mail: teramae@mail.tains.tohoku.ac.jp.

[b]Present address: Department of Chemistry, University of Alabama at Birmingham, 901 14th Street South, Birmingham, AL 35294, USA.

[c]Present address: Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa-Oiwakecho, Sakyo-Ku, Kyoto 606-8502, Japan.

[d]Present address: State Key Laboratory of Fine Chemicals, Department of Chemistry, Dalian University of Technology, Dalian 116012,China.

[e]CREST, Japan Science and Technology Agency (JST), Aoba-ku, Sendai 980-8578, Japan.

[f]Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland

The following items have been included as supplementary material:

Fluorescence titrations curves and fitting equations

Figure S1. Fluorescence titration curves of lumazine (Lz) for target bases (G, C, A, and T) opposite the AP site in a DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCN CGT TG-5', where X = AP site (Spacer-C3); N = G, C, A, or T).

Figure S2. Fluorescence titration curves of 6,7-dimethyllumazine (6,7-DMLz) for target bases (G, C, A, and T) opposite the AP site in a DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCN CGT TG-5', where X = AP site (Spacer-C3); N = G, C, A, or T).

Figure S3. Fluorescence titration curves of alloxazine (All) for target bases (G, C, A, and T) opposite the AP site in a DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCN CGT TG-5', where X = AP site (Spacer-C3); N = G, C, A, T).

Figure S4. Fluorescence titration curves of lumichrome for target bases (G, C, A, and T) opposite the AP site in a DNA duplex (5'-TCC AGX GCA AC-3'/3'-AGG TCN CGT TG-5', where X = AP site (Spacer-C3); N = G, C, A, or T).

Figure S5. Energy minimized complexes of alloxazine or lumichrome with adenine and their hydrogen

bonding patterns

Figure S6. Energy minimized complexes of alloxazine or lumichrome with thymine and their hydrogen bonding patterns

Figure S7. Salt dependence of binding constants for alloxazine/T, lumichrome/T interactions and alloxazine/A, lumichrome/A interactions.

Table S1. Binding constants of alloxazine and lumichrome with target A and T bases at various salt concentrations..

The titration curves were analyzed by nonlinear least-squares regression based on a 1:1 binding isotherm

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

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

$$D_0 = [D] + \{ L_0K_{11}[D]\}/\{1 + K_{11}[D]\} \qquad (2)$$

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

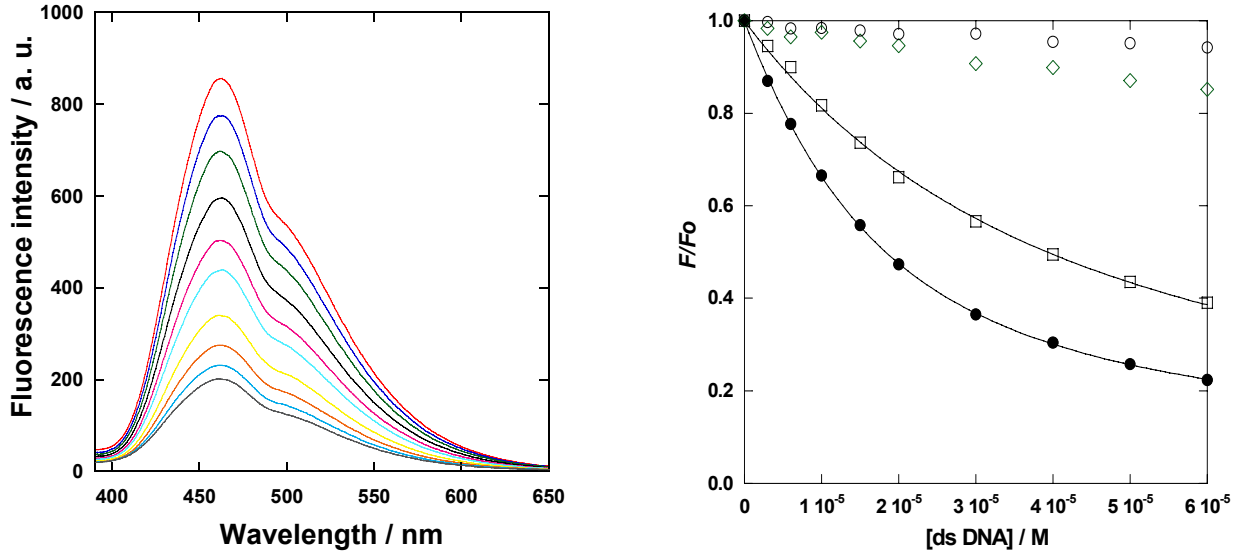


Figure S1. (Left) Fluorescence response of lumazine (10.0 μM) upon adding an AP site-containing DNA duplex (5'-TCC AGX GCA AC-3'/5'-GTT GCA CTG GA-3', X = AP site; A = target adenine) in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. (Right) Fluorescence titration curves for the binding of lumazine to 11-mer AP site-containing DNA duplexes (5'-TCCAGXGCAAC-3'/3'-AGGTCNCGTTG-5'; X = AP site (Spacer-C3), N = G (○), C (◊), A (●) or T (□)) obtained in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. The changes in the fluorescence intensity ratio at 463 nm were analyzed based on a 1:1 binding isotherm model. F and F_0 denote the fluorescence intensities of lumazine in the presence and absence of DNA duplexes, respectively. [lumazine] = 10.0 μM, λ_{ex} = 335 nm, λ_{em}= 463 nm, 5 ^{o}C.

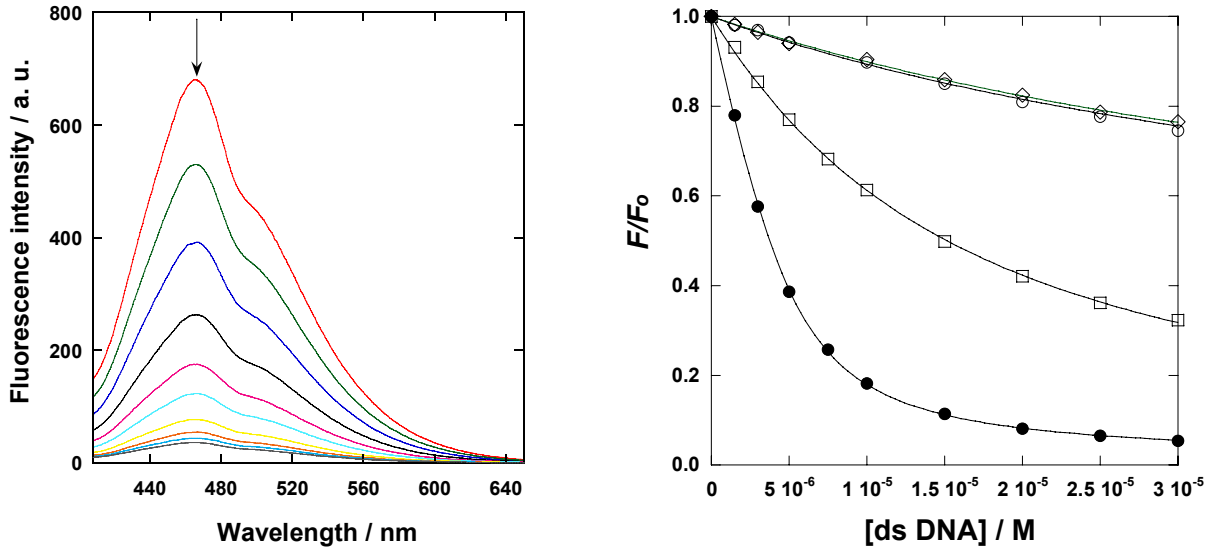


Figure S2. (Left) Fluorescence response of 6,7-dimethyllumazine (5.0 μM) upon adding an AP site-containing DNA duplex (5'-TCC AGX GCA AC-3'/5'-GTT GCA CTG GA-3', X = AP site; A = target adenine) in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. (Right) Fluorescence titration curves for the binding of 6,7-dimethyllumazine to 11-mer AP site-containing DNA duplexes (5'-TCCAGXGCAAC-3'/3'-AGGTCNCGTTG-5'; X = AP site (Spacer-C3), N = G (○), C (◊), A (●) or T (□)) obtained in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. The changes in the fluorescence intensity ratio at 466 nm were analyzed based on a 1:1 binding isotherm model. *F* and F_0 denote the fluorescence intensities of 6,7-dimethyllumazine in the presence and absence of DNA duplexes, respectively. [6,7-dimethyllumazine] = 5.0 μM, λ_{ex} = 350.5 nm, λ_{em}= 466 nm, 5 °C.

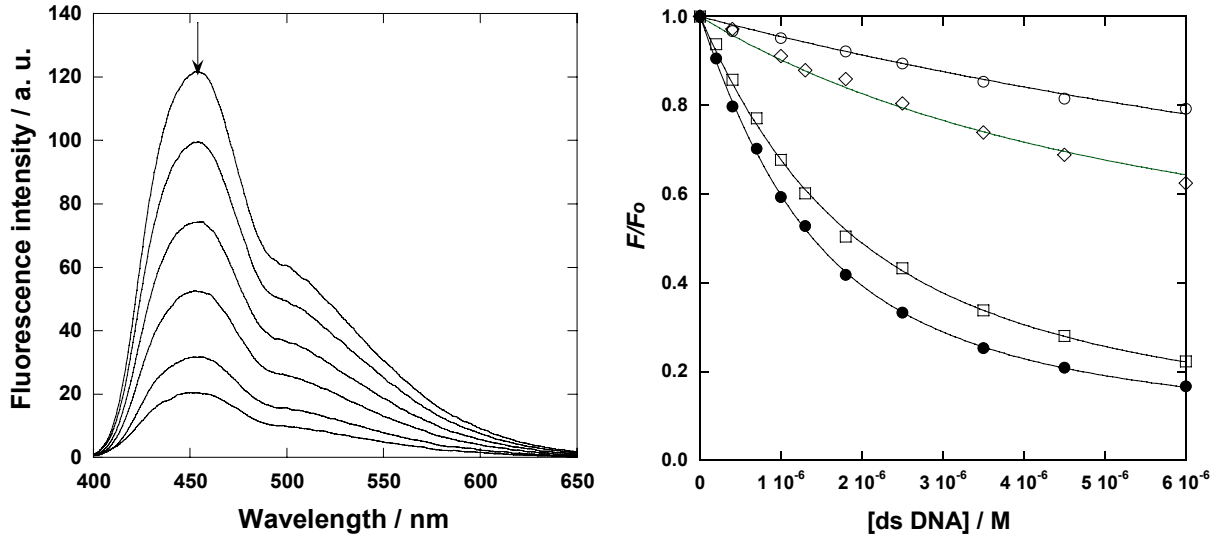


Figure S3. (Left) Fluorescence response of alloxazine (1.0 μM) upon adding an AP site-containing DNA duplex (5'-TCC AGX GCA AC-3'/5'-GTT GCA CTG GA-3', X = AP site; A = target adenine) in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. (Right) Fluorescence titration curves for the binding of alloxazine to 11-mer AP site-containing DNA duplexes (5'-TCCAGXGCAAC-3'/3'-AGGTCNCGTTG-5'; X = AP site (Spacer-C3), N = G (○), C (◊), A (●) or T (□)) obtained in solutions buffered to pH 7.0 (10 mM sodium cacodylate containing 100 mM NaCl and 1.0 mM EDTA). The changes in the fluorescence intensity ratio at 453 nm were analyzed based on a 1:1 binding isotherm model. F and F_0 denote the fluorescence intensities of alloxazine in the presence and absence of DNA duplexes, respectively. [alloxazine] = 1.0 μM, λ_{ex} = 385 nm, λ_{em}= 453 nm, 5°C.

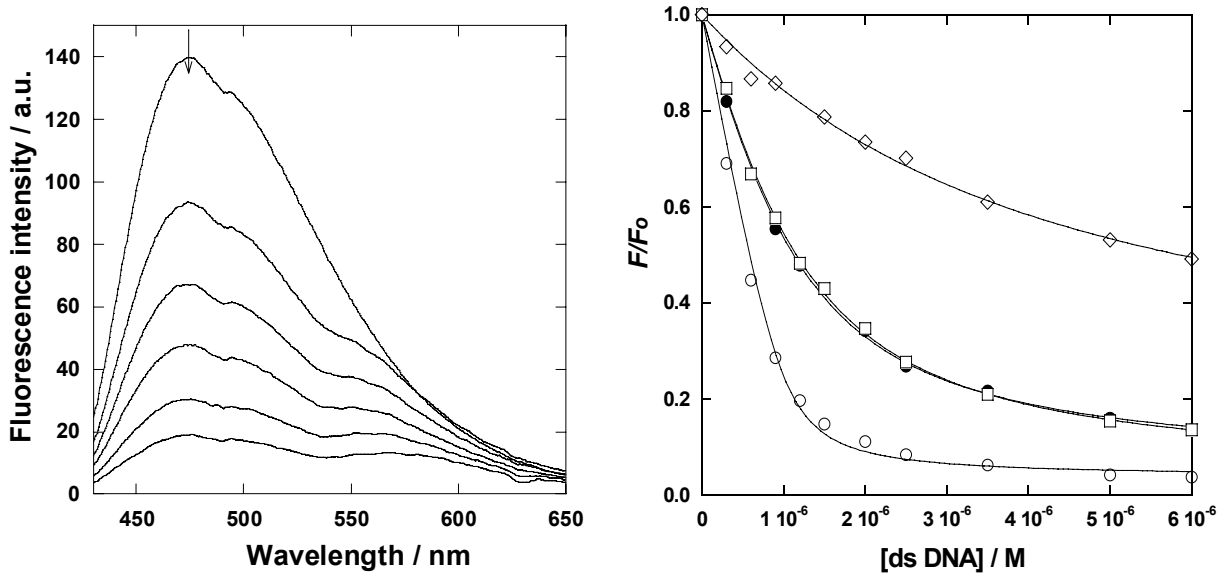
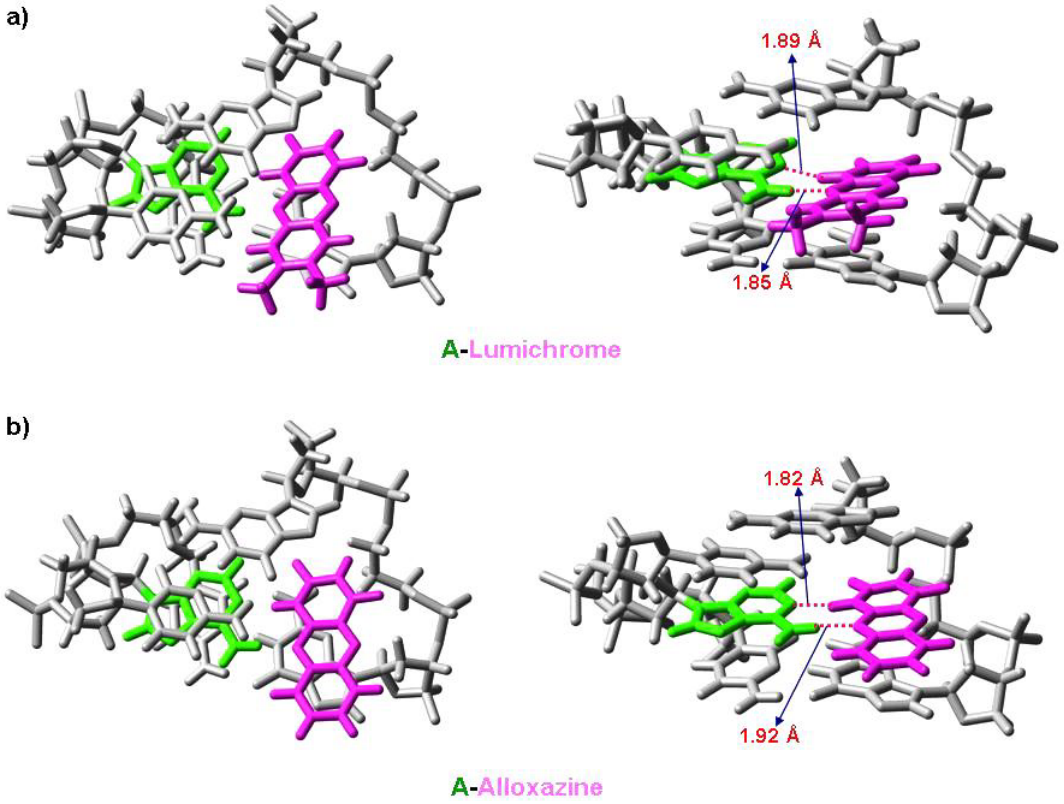
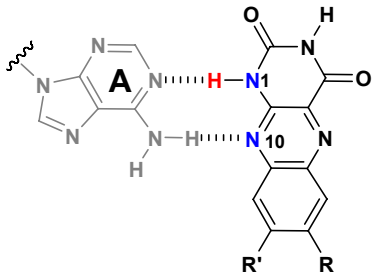


Figure S4 (Left) Fluorescence responses of lumichrome (1.0 μM) to an AP site-containing DNA duplex (0, 0.6, 1.2, 2.0, 4.0, 6.0 μM; 5'-TCC AGX GCA AC-3'/3'-AGG TCA CGT TG-5', X = AP site; A = target adenine) in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. Excitation wavelength: 420 nm. Temperature 5 ºC. (Right) Fluorescence titration curves for the binding of lumichrome to 11-mer AP site-containing DNA duplexes (5'-TCCAGXGCAAC-3'/3'-AGGTCNCGTTG-5'; X = AP site (Spacer-C3), N = G, C, A or T) obtained in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. The changes in the fluorescence intensity ratio at 475 nm were analyzed based on a 1:1 binding isotherm model. F and F_0 denote the fluorescence intensities of lumichrome in the presence and absence of DNA duplexes, respectively. [lumichrome] = 1.0 μM for C (□), A (●) and T (○), 10 μM for G (◊). λ_{ex} = 420 nm, 5 ^{o}C.

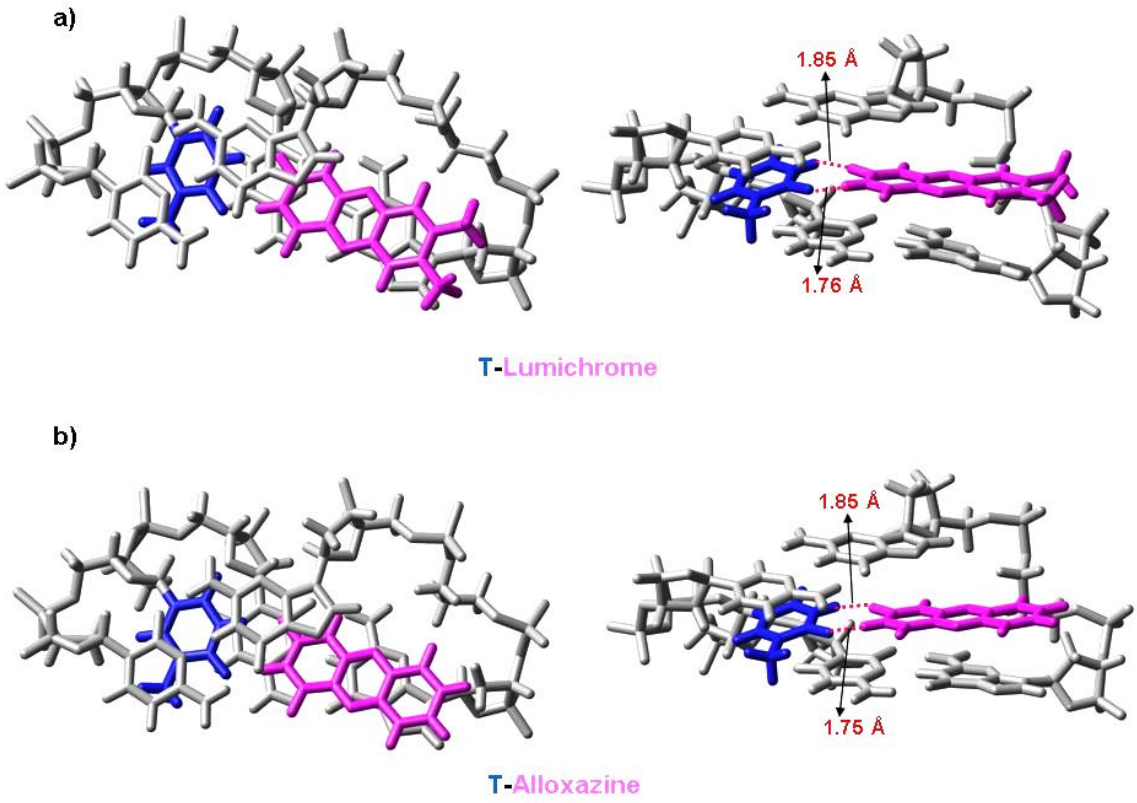


c) lumichrome or alloxazine - adenine

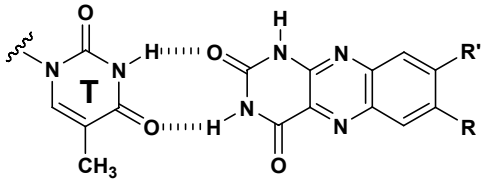


R, R' = H - Alloxazine
R, R' = CH_3 - Lumichrome

Figure S5. Amber* force filed energy minimized structures of a) lumichrome-adenine complex (left: bottom-view, right: side-view) and b) alloxazine-adenine complex (left: bottom-view, right: side-view) colored green for adenine base and pink for ligand (lumichrome or alloxazine). c) Possible hydrogen-bonding pattern of alloxazine or lumichrome with adenine.



c) lumichrome or alloxazine-thymine



R, R' = H - Alloxazine
R, R' = CH_3 - Lumichrome

Figure S6. Amber* energy minimized structures of a) lumichrome-thymine complex (left: bottom-view, right: side-view) and b) alloxazine-T complex (left: bottom-view, right: side-view) colored blue for thymine base and pink for ligand (lumichrome or alloxazine). (c) Hydrogen-bonding pattern of lumichrome or alloxazine with thymine base.

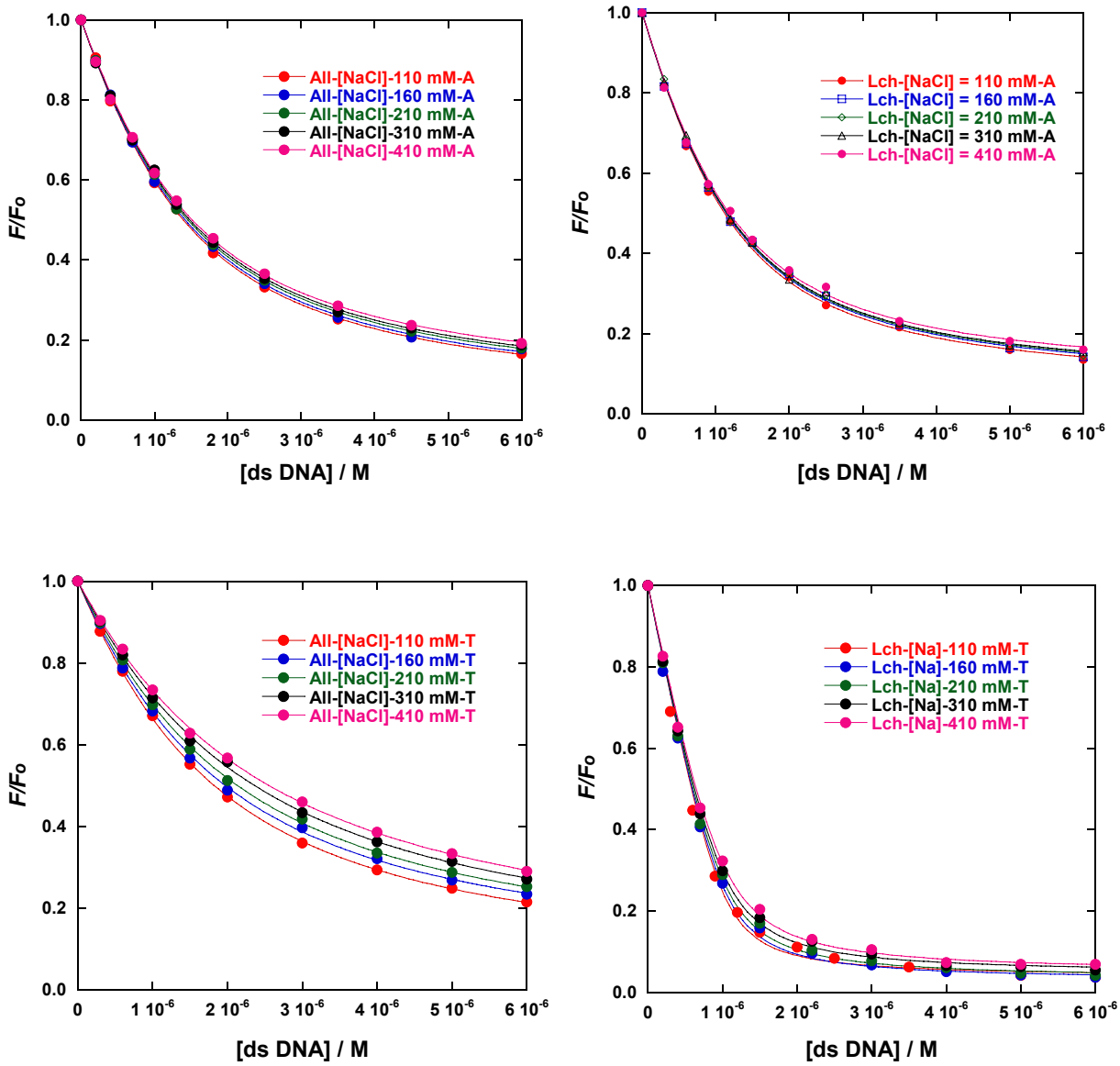


Figure S7. Fluorescence titration curves of alloxazine/A (upper, left), lumichrome/A (upper, right), alloxazine/T (lower, left), and lumichrome/T (lower, right) at various salt concentrations. (experimental conditions, see captions of Figures S3 and S4).

Table S1. Binding constants of alloxazine, lumichrome with target A base and T base in AP-site-containing DNA duplexes; binding constant values were obtained by fluorescence titration analysis. (See Figure S7).

	Binding constants (K_{11}) / 10^6 M^{-1}			
[NaCl] / mM	**alloxazine/A**	**7,8-dimethyl alloxazine/A**	**alloxazine/T**	**7,8-dimethyl alloxazine/T**
110	1.21 ± 0.04	1.87 ± 0.12	0.8 ± 0.01	16.21 ± 3.91
160	1.19 ± 0.03	1.85 ± 0.11	0.75 ± 0.03	13.06 ± 2.31
210	1.17 ± 0.02	1.84 ± 0.08	0.67 ± 0.02	11.47 ± 1.69
310	1.16 ± 0.04	1.84 ± 0.06	0.58 ± 0.02	10.49 ± 1.27
410	1.15 ± 0.04	1.83 ± 0.13	0.53 ± 0.02	8.84 ± 0.87