Supplementary Material for Organic & Biomolecular Chemistry

Supporting Information for

Alloxazine as a ligand for selective binding to adenine opposite AP sites in DNA duplexes and analysis of single-nucleotide polymorphisms

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The following items have been included as supplementary material:

Figure S1. 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 and T).

Figure S2. Fluorescence response and 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 and T).

Table S1. Binding constants of alloxazine to an adenine base opposite an AP site in a 11-mer DNA duplex (5'-TCCAGXGCAAC-3'/3'-AGGTCACGTTG-5', X = AP site; Spacer-C3) under different salt concentrations.

Figure S3. Salt-dependence of binding constants for the alloxazine/adenine interaction.

Figure S4. Calorimetric isothermal titration for the binding of alloxazine to an abasic site-containing DNA duplex (5'-TCCAGXGCAAC-3'/3'-AGGTCACGTTG-5', X = AP site; Spacer-C3, A = target).

Table S2. Effect of flanking nucleotides on fluorescence responses (quenching efficiency / %) and dissociation constants (K_d /μM) of alloxazine to adenine bases in 23-mer DNA duplexes (5'-TCTGC GTCCA **wXz** CAACG CACAC-3'/3'-AGACG CAGGT **w'Az'** GTTGC GTGTG-5', X = AP site; Spacer-C3).

Experimental: Preparation and Analysis of PCR products.

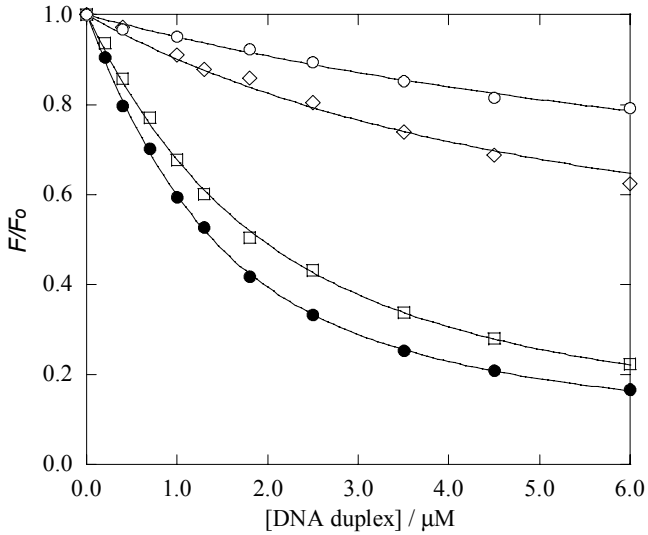

Figure S1 Fluorescence titration curves for the binding of alloxazine to 11-mer AP site-containing DNA duplexes (5'-TCCAGXGCAAC-3'/3'-AGGTCNCGTTG-5', X = AP site; Spacer-C3, N = G (○), C (◊), A (●) and T (□)) obtained in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 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, Excitation 385 nm. Temperature 5 ^{o}C.

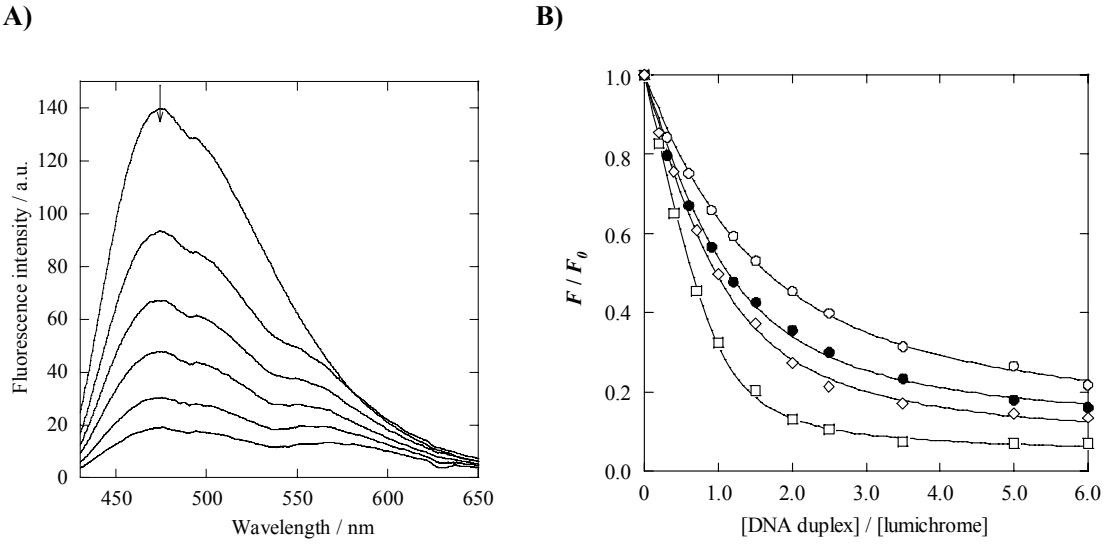

Figure S2 (A) Fluorescence responses of lumichrome (1.0 μM) to an AP site-containing DNA duplex (0, 0.6, 1.2, 2.0, 4.0, 6.0 μM; 5'-TCC AGX GCA AC-3'/3'-AGG TCA CGT TG-5', X = AP site; A = target adenine) in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 100 mM NaCl and 1.0 mM EDTA. Excitation wavelength: 420 nm. Temperature 5 ºC. (B) 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 and T) obtained in solutions buffered to pH 7.0 (10 mM sodium cacodylate) containing 400 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 (◊) titration. Excitation 420 nm, Temperature 5 ºC.

Table S1 Binding constants of alloxazine to an adenine base opposite an AP site in a 11-mer DNA duplex (5'-TCCAGXGCAAC-3'/3'-AGGTCACGTTG-5', X = AP site; Spacer-C3) under different salt concentrations.[a)]

$[Na^+]$ / mM	K_{11}/ M^{-1}
110	1.21×10^6
160	1.19×10^6
210	1.17×10^6
310	1.16×10^6
410	1.15×10^6

[a)]The binding constants were obtained by fluorescence titration experiments in solutions buffered to pH 7.0 with 10 mM sodium cacodylate containing 1.0 mM EDTA (cf. Figure 2). The concentration of NaCl was ranged from 110 mM to 410 mM. For the titration: [alloxazine], 1.0 μM; [DNA duplex], 0-6.0 μM; excitation wavelength, 385 nm; analysis, 453 nm. Temperature 5 °C.

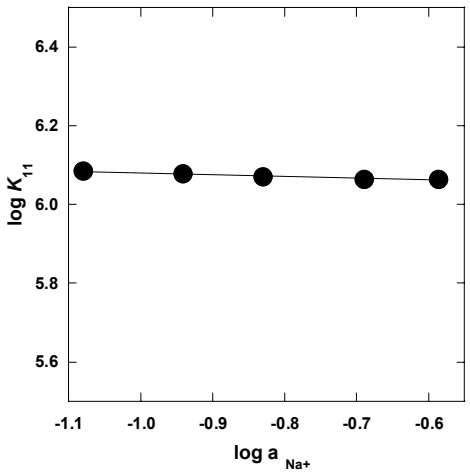

Figure S3 Salt-dependence of binding constants for the alloxazine /adenine interaction. The data are given in Table S1. The linear least squares fit to the data yielded a slope of –0.044, from this value, an apparent charge of alloxazine was obtained (Z = +0.05).

The effect of added NaCl on the binding constant for the alloxazine/adenine interaction (cf. Table S1) was analyzed according to the polyelectrolyte theory by Record et al.[1] The observed linear relationship between log K_{11} and log a_{Na+} (cf. Figure S3) is described by:

$$\delta \log K_{11} / \delta \log a_{Na+} = -Z\psi \qquad (1)$$

where ψ is the proportion of counterions associated with each DNA phosphate group (ψ = 0.88 for double-stranded *B*-type DNA) and *Z* is the apparent charge on the ligand.

The $Z\psi$ value, obtained from the slope analysis (cf. Figure S3), was used to evaluate the electrostatic or polyelectrolyte contribution (ΔG_{pe}) to the observed binding free energy (ΔG_{obs}), using the following equation:

$$\Delta G_{pe} = Z\psi RT \ln a_{Na+} \qquad (2)$$

The non-polyelectrolyte contribution (ΔG_t) is then given by:

$$\Delta G_{obs} = \Delta G_t + \Delta G_{pe} \qquad (3)$$

1. M. T. Record, C. F. Anderson, T. M. Lohman, *Q. Rev. Biophys.*, 1978, **11**, 103.

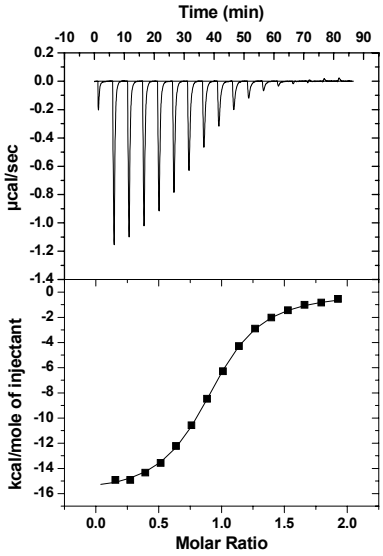

Figure S4 ITC data obtained at 5 ^{o}C for the addition of DNA aliquots (each 15 µl of 200 µM) into the solution containing alloxazine (1.42 ml of 20 µM). Sample solutions were buffered to pH 7.0 with 10 mM sodium cacodylate, containing 100 mM NaCl and 1 mM EDTA. DNA duplex: 5'-TCCAG<u>X</u>GCAAC-3'/3'-AGGTCACGTTG-5', <u>X</u> = AP site; Spacer-C3.

Table S2. Effect of flanking nucleotides on fluorescence responses (quenching efficiency / %) and dissociation constants (K_d /μM) of alloxazine to adenine bases in 23-mer DNA duplexes (5'-TCTGC GTCCA **wXz** CAACG CACAC-3'/3'-AGACG CAGGT **w'Az'** GTTGC GTGTG-5', X = AP site; Spacer-C3).

5'-z'Aw'-3'/3'-zXw-5'	quenching efficiency [a] (%)	K_d (μM) [b]
5'-TAC-3'/3'-AXG-5'	57.6	0.28
5'-GAC-3'/3'-CXG-5'	61.3	0.22
5'-CAC-3'/3'-GXG-5'	46.3	0.59
5'-AAC-3'/3'-TXG-5'	42.8	0.72
5'-TAT-3'/3'-AXA-5'	41.5	0.79
5'-GAT-3'/3'-CXA-5'	51.0	0.44
5'-CAT-3'/3'-GXA-5'	33.0	1.30
5'-AAT-3'/3'-TXA-5'	26.9	1.90
5'-TAG-3'/3'-AXC-5'	15.6	4.4
5'-GAG-3'/3'-CXC-5'	20.6	3.0
5'-CAG-3'/3'-GXC-5'	14.7	4.8
5'-AAG-3'/3'-TXC-5'	14.3	5.0
5'-TAA-3'/3'-AXT-5'	10.8	7.2
5'-GAA-3'/3'-CXT-5'	14.3	5.0
5'-CAA-3'/3'-GXT-5'	8.4	9.6
5'-AAA-3'/3'-TXT-5'	9.7	8.2

[a] Sample solutions were buffered to pH 7.0 with 10 mM sodium cacodylate, containing 100 mM NaCl and 1 mM EDTA. Excitation 385 nm; detection 453 nm. Temperature 5 ºC. [Ligand] = 1.0 μM; [DNA duplex] = 1.0 μM.

[b] The dissociation constants (K_d/μM, pH 7.0, I = 0.11 M, at 5 ºC) were roughly estimated from the observed fluorescence quenching (453 nm), using a 1:1 binding isotherm model. For the estimation, the complexation-induced maximal quenching effciency was assumed to be 84 %, which was obtained for the titation using an 11-mer DNA duplex (5'-TCCAGXGCAAC-3'/3'-AGGTCACGTTG-5', X = AP site; Spacer-C3, cf. Fig. 2 and Fig. S2).

Preparation of PCR products. Asymmetric PCR[1] amplification of 107-mer sense strands of *K-ras* gene (codon 12)[2] was done with a 20-mer forward primer (5'-GACTGAATATAAACTTGTGG-3') and a 20-mer reverse primer (5'-CTATT GTTGG ATCAT ATTCG-3'). The reaction solution (100 μl) contained dNTPs (2.5 mM each), 10×PCR buffer (10 μl; TaKaRa), forward primer (300 pmol), reverse primer (20 pmol), template (0.5 ng), and Ex-Taq (2.5 U; TaKaRa). PCR conditions: 94 ˚C for 5 min, followed by 40 cycles of 94 ˚C for 30 s, 52 ˚C for 30 s, and 72 ˚C for 30 s, and then 72 ˚C for 7 min and kept at 4 ˚C. 107-mer PCR product: 5'-GACTG AATAT AAACT TGTGG TAGTT GGAGC TG**G/A**TG GCGTA GGCAA GAGTG CCTTG ACGAT ACAGC TAATT CAGAA TCATT TTGTG GACGA ATATG ATCCA ACAAT AG-3'.

Analysis. After PCR amplification, two aliquots (40 μl) from the PCR product (100μl) were buffered to pH 7.0 with 100 mM sodium cacodylate containing EDTA (1.6 mM). Then, alloxazine or diMe-pteridine (100 nM), and a 20-mer AP site-containing probe oligonucleotide (5.0 μM, 5'-CCT ACG CCA XCA GCT CCA AC-3'; X = AP site) were added. Fluorescence spectra of the resulting solutions (50 μl) were then measured at 5 ˚C with a JASCO FP-6500 spectrofluorophotometer (Japan Spectroscopic Co. Ltd., Tokyo, Japan) equipped with a thermoelectrically temperature controlled cell holder (quartz cuvette: 3 mm × 3 mm); the slits for the excitation and emission monochromators were 5 and 5 nm, respectively.

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1 (*a*) M. A. Innis, K. B. Myambo, D. H. Gelfand, and M. A. D. Brow, *Proc. Natl. Acad. Sci. U. S.A.*,1988, **85**, 9436; (*b*) M. Kiviniemia, J. Nurmi, H. Turpeinen, T. Lovgren, J. Ilonen, *Clin. Biochem*., 2003, **36**, 633.

2 J. L. Bos, *Mutat. Res*., 1988, **195**, 255.