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PAPER

Simultaneous fluorescence light-up and selective multicolor nucleobase recognition based on sequence-dependent strong binding of berberine to DNA abasic site†

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Label-free DNA nucleobase recognition by fluorescent small molecules has received much attention due to its simplicity in mutation identification and drug screening. However, sequence-dependent fluorescence light-up nucleobase recognition and multicolor emission with individual emission energy for individual nucleobases have been seldom realized. Herein, an abasic site (AP site) in a DNA duplex was employed as a binding field for berberine, one of isoquinoline alkaloids. Unlike weak binding of berberine to the fully matched DNAs without the AP site, strong binding of berberine to the AP site occurs and the berberine's fluorescence light-up behaviors are highly dependent on the target nucleobases opposite the AP site in which the targets thymine and cytosine produce dual emission bands, while the targets guanine and adenine only give a single emission band. Furthermore, more intense emissions are observed for the target pyrimidines than purines. The flanking bases of the AP site also produce some modifications of the berberine's emission behavior. The binding selectivity of berberine at the AP site is also confirmed by measurements of fluorescence resonance energy transfer, excited-state lifetime, DNA melting and fluorescence quenching by ferrocyanide and sodium chloride. It is expected that the target pyrimidines cause berberine to be stacked well within DNA base pairs near the AP site, which results in a strong resonance coupling of the electronic transitions to the particular vibration mode to produce the dual emissions. The fluorescent signal-on and emission energy-modulated sensing for nucleobases based on this fluorophore is substantially advantageous over the previously used fluorophores. We expect that this approach will be developed as a practical device for differentiating pyrimidines from purines by positioning an AP site toward a target that is available for readout by this alkaloid probe.

1. Introduction

DNA nucleobase recognition by small molecules has received much attention for functional sensor design and anticancer drug screening, and because of their susceptibility to cause various common diseases.[1,2] Specifically targeting a DNA abasic site (AP site)[3] has come to light as one of the various DNA binding models in addition to intercalation and groove binding, because of the frequent occurrence of AP sites in a living cell.[4] The AP site is produced by eliminating one damaged nucleobase from a DNA duplex by specific enzymes. The AP sites' recognition by small molecules, for example, binder/insertor heterodimers,[3–6] metalloinsertors,[7,8] naphthalene derivative,[9] nitroxide spin label,[10] and DNA base analogs[11,12] has been employed to identify this type of DNA damage. Recently, covalently incorporated fluorescent non-nucleosidic surrogates at the AP site[13–16] have been successfully developed for nucleobase recognition strategies on the basis of their emission dependencies on the target bases opposite the AP site. Additionally, organic probes possessing hydrogen bond moieties that are complementary to the target bases, such as naphthyridine,[17,18] pyrazine,[19] lumazine,[20] pteridine,[21,22] and flavin[23] derivatives, can recognize DNA nucleobases upon entering into the AP site and pairing with the target bases. These label-free and cost-effective fluorescence strategies are particularly attractive for DNA nucleobase recognition[17–23] and other promising applications such as aptamer sensor designs.[2,24–27] Unfortunately, these pairwise interactions with the nucleobases usually induce fluorescence quenching.[17–23] Consequently, AP site binding-induced fluorescence light-up recognition for nucleobases still remains a challenge.[28,29] Especially, multicolor emission with individual emission energy for individual nucleobase is very promising and has been seldom attained.

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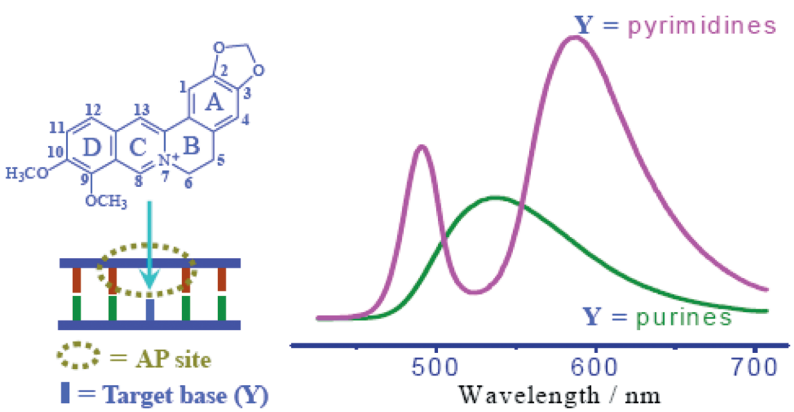
We have focused on the utilization of new fluorophores to achieve this goal, such as fluorescent silver nanoclusters[30] and flavonoids.[28] Herein, berberine, one of the clinically important natural isoquinoline alkaloids, was employed as an AP site-specific binder. Berberine is abundant in Chinese medicinal plant species such as *Coptis chinensis* possessing heat-clearing and detoxifying properties. Its powerful biological and therapeutic activities, for instance, antimicrobial, anti-inflammatory, antioxidant, and anticancer, have also been widely accepted.[31–33] Nucleic acids have been thought to be the biological targets of berberine in manifesting its anticancer activity. It is widely believed that berberine can bind with double-stranded DNA (ds-DNA),[34–41] triplex DNA,[42,43] G-quadruplex DNA,[44,45] and RNA.[46,47] Berberine can even link two DNA molecules together in an end-to-end fashion.[48] Because berberine has a polycondensate system with partial unsaturation in ring B (Scheme 1), which renders its polycyclic system slightly buckled, the binding mode of berberine to DNA could be partially intercalative,[34,35] completely intercalative,[36] groove-associative,[37] or electrostatic[41] by a sequence-dependent A/T[34,37,39] or G/C[38] selectivity, or no remarkable sequence selectivity.[40] Herein, we find that berberine binding to the AP site is dependent on the bases opposite the AP site and can be used to differentiate pyrimidines from purines with a fluorescence light-up response much higher than that for the fully matched DNAs without the AP site. More importantly, the emission of berberine is split into two new bands for the cases with pyrimidines opposite the AP site. That is, multicolor recognition for nucleobases can be achieved with berberine as readout. This light-up response with simultaneous multicolor emissions for nucleobase recognition was not previously achieved with such a simple fluorophore without complicated labeling procedures.[49,50]

2. Experimental section

DNA species (Scheme 1) were synthesized by TaKaRa Biotechnology Co., Ltd (Dalian, China) and purified by HPLC (with purity higher than 95%). The DNA concentrations were measured by UV absorbance at 260 nm using extinction coefficients calculated by the nearest neighbor analysis. Tetrahydrofuran residual was used as the chemically stable abasic site (AP site) for replacing the naturally-occurring unstable deoxyribose structure. To prepare DNA duplex solutions, the probe and target strands were mixed in equimolar amounts and annealed in a thermocycler (first at 92 °C, then cooled down to room temperature slowly) in 0.1 M phosphate buffer (pH 7.0) containing 1 mM EDTA.

A



B

DNA1-Y: 5'-ATGGTGT**X**TGCAGCG-3' Probe strand
3'-TACCACA**Y**ACGTCGC-5' Target strand
X = AP site, Target base Y = A, C, G or T

DNA2-Y: 5'-CACACT**PX**GTCGAAA-3'
3'-GTGTGAT**Y**CAGCTTT-5'
P = 2-aminopurine, Y = C or G

DNA3-Y: 5'-ATGGTGA**X**AGCAGCG-3' Probe strand
3'-TACCACT**Y**TCGTCGC-5' Target strand
X = AP site, Target base Y = A, C, G or T

Scheme 1 (A) Schematic representations of the AP site-directed binding of berberine for light-up and multicolor nucleobase recognition. (B) The sequences used in this work. X = AP site (dSpacer, tetrahydrofuran residual) for AP site-containing DNAs. Fully matched DNAs (FM-DNA) with X/Y = G/C and A/T are used as controls.

Berberine (Sigma Chemical Co., St Louis, USA), was added to the duplex DNA solution to an appropriate molar ratio at 0.1 M phosphate buffer (pH 7.0) containing 1 mM EDTA. After mixing, the solution was incubated for 15 minutes with gentle stirring. The resulting solutions were examined at room temperature within 2 h. Nanopure water (18.2 mΩ; Millipore Co., USA) was used in all experiments. Fluorescence spectra were acquired with a FLSP920 spectrofluorometer (Edinburgh Instruments Ltd, UK) at 18 ± 1 °C for the slit widths at 6 nm, equipped with a temperature-controlled circulator (Julabo, Germany). Time-resolved fluorescence decays were recorded on a time-correlated single photon counting FLSP920 system, with excitation at 360 nm. A ludox solution was used as scatter for the instrument response. The data were fitted with a multiexponential decay and χ^2 was less than 1.15. UV/Vis absorption spectra and melting temperatures (T_m) were determined with a UV2550 spectrophotometer (Shimadzu Corp., Japan), equipped with an accessory of TMSPC-8 T_m analysis system which can simultaneously control the chamber temperature and detect up to 8 samples by a micro multi-cell.

3. Results and discussion

3.1 Nucleobase-selective binding of berberine to the AP site of DNA

As shown in Scheme 1, the AP site (X) designed in the probe strand is placed opposite the target base (Y) of the target strand, so as to make the hydrogen bonding moieties of the target base unpaired. As shown in Fig. 1, the emission of 5 μM berberine alone is rather weak; while the presence of the fully matched DNA (FM) slightly increases the fluorescence response with emissions peaked at 538 nm by exciting at either 360 or 465 nm, which is in agreement with previous reports on the interactions of berberine with DNA.[34–45] Furthermore, berberine's emission is independent on the FM sequence used here (Fig. S1, ESI†). However, upon addition of DNA1-Y with guanine (DNA1-G) or adenine (DNA1-A) opposite the AP site to the berberine solution, berberine's emission is blue shifted to 534 nm, which is accompanied by an increase in intensity of 5 (DNA1-A) or 6 (DNA1-G) times higher than that for FM under excitation at either 360 or 465 nm, respectively (Fig. 1B and C). Interestingly, the enhancement becomes more pronounced for cytosine (DNA1-C) or thymine (DNA1-T) opposite the AP site with the intensities increased up to about 26 times when excited at 465 nm (Fig. 1C). Importantly, when the excitation wavelength is then set to 360 nm (Fig. 1B), dual emission bands appear at 492 and 584 nm for DNA1-C or DNA1-T with their intensities being 9 and 14 times higher than the FM's emission at 538 nm, respectively. Accordingly, when detected at 534 nm, the excitation bands for DNA1-C and DNA1-T between 300 and 400 nm are clearly split into two new ones that are positioned at 330 and 370 nm, respectively (Fig. 1A). Nevertheless, the excitation spectra monitored at the dual emission bands of 492 and 584 nm are quite different from those monitored at 534 nm, in which even saturation excitations are observed between 345 and 360 nm (Fig. S2, ESI†). In addition, these dual emission bands for DNA1-C and DNA1-T appear only by exciting between 330 and 370 nm (Fig. S3, ESI†). This wavelength region (between 330 and 370 nm) is just one of berberine's absorbance bands (Fig. 1D). From the absorbance spectra, the presence of DNA1-C and DNA1-T also result in more pronounced bathochromic and hypochromic changes than those for DNA1-G and DNA1-A (Fig. 1D). The nucleobase-selective dual emissions that are simultaneously dependent on the excitation wavelengths can be employed to identify DNA nucleobases. Distinguishing pyrimidines from purines can be achieved even by the naked eye under UV illumination with emissions in bright green and light blue colors, respectively (Fig. 1E). Therefore, selective multicolor nucleobase recognition could be easily attained by this isoquinoline alkaloid probe. Berberine's dual emissions were not previously observed, although Bashmakova *et al.* previously mentioned that the fluorescence spectrum of berberine consisted of two adjacent, practically unresolved bands.[51]

Note that the sequence differences among DNA1-Ys are only the target bases. Therefore, the target base-dependent emissions of berberine strongly support that the AP site should be responsible for berberine's binding. To further verify berberine's selective binding at the AP site, random sequence DNA2s were also employed, which are totally different from DNA1s in sequences and modified by a fluorescent adenine analogue of 2-aminopurine (P) just near the AP site (Scheme 1). P has been used to probe the local DNA structure and dynamics[52] and sense small molecules.[27] Here utilization of P is based on the consideration that P's emission spectrum is strongly overlapped with berberine's excitation spectrum. Therefore, as a reliable tool to probe the proximity of an energy donor to an energy acceptor, fluorescence resonance energy transfer (FRET) should occur from the adjacently labeled P to the AP site-bound berberine. As shown in Fig. 2, the addition of berberine to the solution of DNA2-T with thymine opposite the AP site leads to a dramatic decrease in P's emission between 350 and 450 nm and an increase in berberine's emission between 500 and 600 nm. In contrast, the presence of DNA2-G with guanine opposite the AP site induces smaller variations in P's and berberine's emissions than those for DNA2-T, which is in agreement with the results obtained in Fig. 1, although the excitation wavelength-dependent dual emissions are not observed in this case. Note that berberine is weakly fluorescent in the presence of FM at the same excitation wavelength of 320 nm that is also employed for DNA2s. The fact that FRET emission is highly sensitive to the base opposite the AP site again supports the conclusion that berberine's binding indeed occurs at the AP site.

As shown in Fig. 3A, relative to the case for FM, berberine's emission is dramatically and linearly increased by increasing AP-DNA concentration, indicating the dominant role of the AP-site binding on berberine's emission. From these linear dependencies, about 25 and 100 times higher responses are obtained respectively for AP-DNAs with purines and pyrimidines opposite the AP site, relative to those for FM. Interestingly, the coexistence of AP-DNAs with purines opposite the AP site does not affect the berberine binding to AP-DNAs with pyrimidines opposite the AP site. For example, further addition of DNA1-T to the solution of DNA1-G that was beforehand mixed with berberine resulted in the total transition of the characteristic emission for DNA1-G to that for DNA1-T (Fig. 3B), indicating a high selectivity of berberine binding preference to the AP site that is opposed by pyrimidines. Furthermore, berberine's

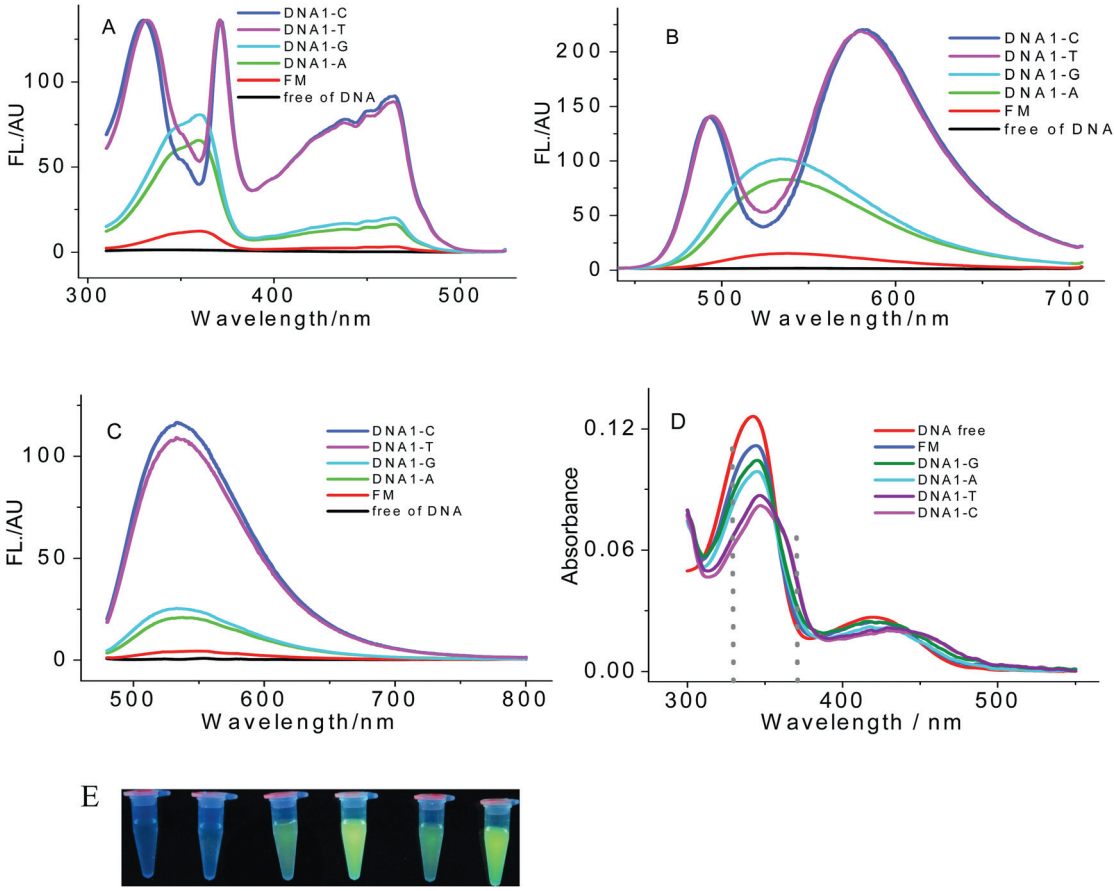


Fig. 1 Excitation (A, measured at 534 nm), emission (B and C, respectively excited at 360 and 465 nm) and absorbance (D) spectra of berberine (5 μM) in the absence and presence of DNA1-Ys (5 μM). The corresponding fully matched DNAs (FM) were used as controls. Also shown are the photographs (E) of berberine in the absence and presence of 5 μM FM, DNA1-A, DNA1-C, DNA1-G and DNA2-T (from left to right) under UV illumination.

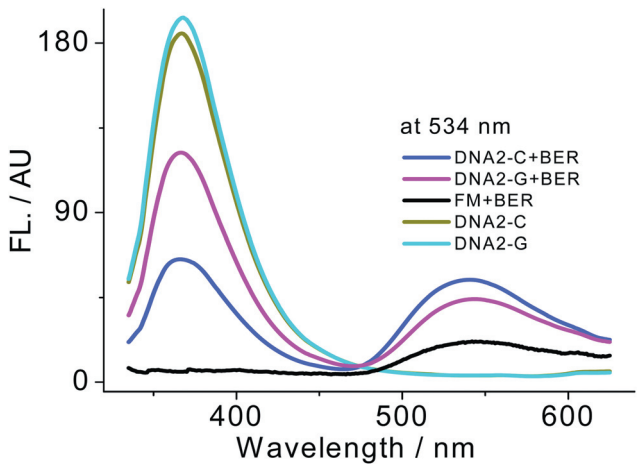


Fig. 2 Emission spectra of berberine (5 μM) in the presence of 2-aminopurine-containing DNA2s (5 μM). Excitation: 320 nm.

emissions are very weak in the presence of nucleotides or single-stranded DNAs and not dependent on their sequences (Fig. S4, ESI†). These observations suggest that this method could be very practical for nucleobase identifications.

The fluorescence intensity decay measurements (Table 1) show the presence of a short-lived and a long-lived excited state for DNA1-G and DNA1-A as well as for FM, most possibly resulting from the different DNA-bound berberine states, which is in agreement with berberine's two unresolved emission bands (Fig. 1).[41,51] However, only the long-lived excited states are observed for both the 492 nm and 584 nm emission bands of DNA1-C and DNA1-T, indicating that only one species is responsible for the corresponding emission bands.

The optical properties of small molecules bound to the AP site are also affected by the flanking nucleobases as well as the opposite nucleobases,[17–23,28,29] since these neighboring nucleobases would be stacked directly above and below with the bound small molecules. In this work, we checked the effect of changing the flanking bases from pyrimidines to purines on berberine's emission behavior. As shown in Fig. 4, when the flanking thymines of the AP site in DNA1-Ys are changed to adenines in DNA3-Ys, the dual emission bands still appear for DNA3-T and DNA3-C with thymine and cytosine opposite the AP site under excitation at 360 nm (Fig. 4A), whereas 465 nm excitation only produces a single band (Fig. 4B). However, the single band is always observed for DNA3-A and DNA3-G with adenine and guanine opposite the AP site under all the excitation conditions. As occurred for DNA1-Ys, the excitation spectra for DNA3-Ys

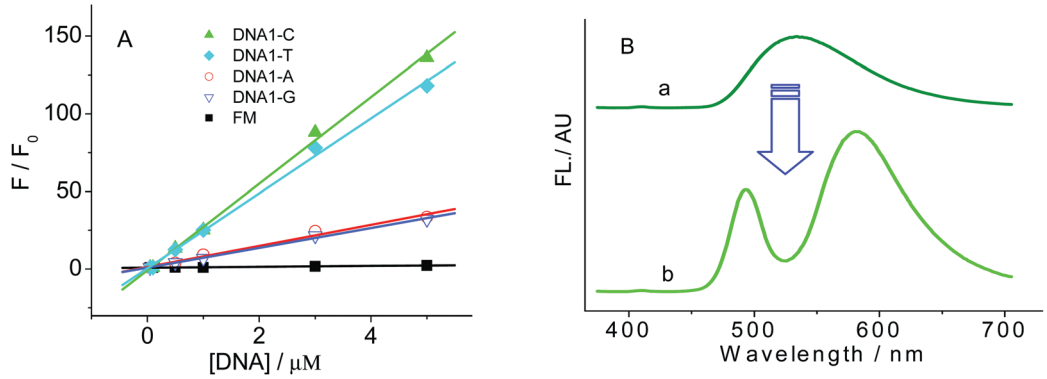


Fig. 3 (A) Fluorescence responses of 5 μM berberine to the DNA concentrations. F_0 and F represent the fluorescence responses of berberine in the absence and presence of the DNAs, respectively. (B) Fluorescence spectra of 5 μM berberine firstly in the presence of 5 μM DNA1-G (a) and then with further addition of 5 μM DNA1-T (b).

Table 1 Fluorescence decay fitting parameters (τ_1 and τ_2) of 5 μM berberine in the absence and presence of 5 μM DNAs

	τ_1/ns	τ_2/ns	χ^2
DNA free	0.33		1.095
DNA1-A	3.02 (17.94%)	12.21 (82.06%)	1.115
DNA1-C	12.14 (584 nm)		1.084
	12.71 (492 nm)		1.088
DNA1-G	2.23 (10.97%)	13.21 (89.03%)	1.003
DNA1-T	12.77 (584 nm)		1.008
	12.97 (492 nm)		1.001
FM	2.47 (37.37%)	12.66 (62.63%)	1.094

(inset of Fig. 4B) are also observed to be dependent on the nucleobases opposite the AP site. In spite of the similarities in the experimental results for these DNAs, the dual emission bands for DNA3-pyrimidines are much less separated than those for DNA1-pyrimidines. These minor differences in the emission profiles should result from the effect of the flanking bases on berberine binding at the AP site.

3.2 Mechanism of berberine's multicolor emission upon binding to the AP site

The excitation spectra monitored at 492, 534, and 584 nm for DNA1-G and DNA1-A are somewhat similar to their corresponding absorption spectra (Fig. 1A, D and Fig. S1†). However, the excitation spectra monitored especially at 534 nm for DNA1-C and DNA1-T are quite different from the corresponding absorption spectra in the region between 300 and 400 nm. It has been reported[53,54] that absorption and excitation spectra do not correspond to each other if the sole present species has different forms in the ground state (aggregates, complexes, *etc.*). Because berberine does not dimerize in aqueous solution at such concentrations as used here,[55] it is not possible that these differences in fluorescence spectra may be caused by different berberine's aggregation states. The specific binding model of berberine to AP-DNA should be responsible for these fluorescence behaviors. It is widely accepted that in normal DNA complexes of berberine, only the moieties of rings C and D (Scheme 1) are responsible for intercalative binding to DNA helix, while the portions from rings A and B protrude out of the helix interior into the minor groove.[56,57] However, by considering the berberine structure, we can conclude that berberine's intercalating into DNA base pairs could experience more steric hindrance because berberine is a non-planar molecule[58] with the plane of the ring A being tilted by approximately 10–16° relative to the plane formed by rings C and D.[57] In addition, the two *ortho*-positioned methoxy groups on the ring D are twisted about 0.1 Å below and 0.3 Å above the C–D plane. Therefore, only groove binding[37] was observed for certain DNAs. It is expected that the space created here by the AP site could be used to accommodate the intercalated moieties of berberine with less steric hindrance.

In order to investigate the DNA binding behavior of berberine, fluorescence quenching experiments with an anionic quencher ($Fe(CN)_6^{4-}$)[42,59] were carried out to provide further evidence for the binding dependence of berberine on the DNA sequence. It is expected that the quencher could be readily attainable to berberine that is bound on the groove of DNA, while berberine intercalated between base pairs should be shielded from direct contact with the quencher. Hence, relative to the groove binding, very little or no quenching may be observed for berberine with an intercalative binding due to the large electrostatic barrier from the negatively charged DNA backbone and the anionic quencher that hinders the quencher to penetrate into the interior of the helix. As shown in Fig. 5A, significant quenching is observed for DNA1-G and DNA1-A with the same magnitude as that for FM by titration with $Fe(CN)_6^{4-}$, while the presence of DNA1-T and DNA1-C induce the occurrence of almost unquenching with the $Fe(CN)_6^{4-}$ concentration up to 5 mM. On the other hand, it has been reported that DNA binding of berberine is accompanied by Na^+ release from DNA[40] and the interaction between berberine and DNA is partially electrostatic in nature on the basis of Na^+ dependence of DNA-bound berberine emission.[41] Interestingly, as observed in Fig. 5B, addition of Na^+, however, almost doesn't affect the emission of berberine bound to DNA1-T and DNA1-C, although FM, DNA1-A and DNA1-G show a Na^+-dependent quenching. These results indicate that the chromophore moiety of berberine can intercalate into the interior of DNA1-T and DNA1-C helix. By contrast, groove binding of berberine to DNA1-A and DNA1-G is expected as that occurs for FM, although a higher fluorescence response for the former is observed (Fig. 1).

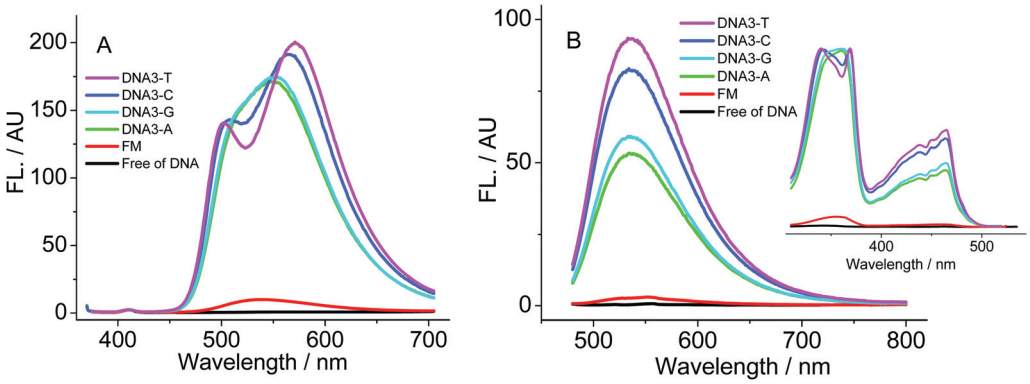


Fig. 4 Emission spectra of berberine (5 μM) in the absence and presence of DNA3-Ys (5 μM) under excitation at 360 (A) and 465 (B) nm. Inset: the corresponding excitation spectra (measured at 534 nm).

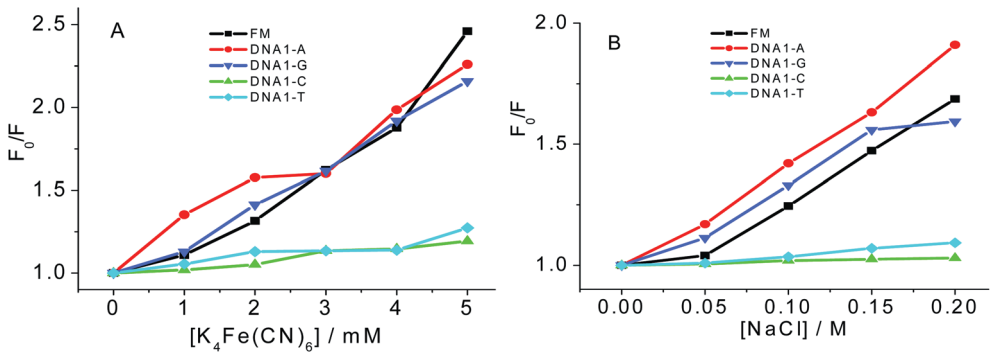


Fig. 5 Quenching of berberine fluorescence by increasing concentrations of $K_4Fe(CN)_6$ (A) and NaCl (B). F_0 and F represent the fluorescence responses of the berberine–DNA complexes in the absence and presence of the quenchers, respectively.

In order to verify the occurrence of stacking interactions of berberine with DNA base pairs near the AP site, DNA melting (T_m) experiments were conducted. As shown in Table 2, the presence of berberine stabilizes DNA1-C and DNA1-T by T_m increases of 1.6 and 1.8 °C. On the other hand, only 0.6 and 0.7 °C changes in the T_m values are observed for DNA1-A and DNA1-G. However, berberine induces a minor change in the FM's T_m (ΔT = 0.1 °C). These results confirm the intercalative binding mode of berberine at the AP site of DNA1-C and DNA1-T, while this interaction mode should become much weaker for DNA1-A and DNA1-G.

Fluorescence enhancement of berberine after binding to DNA should be ascribed to the less polar environment of DNA[41] and protection of attack from the electrolyte ions[60] or hydrogen bond donors.[61] However, there are several possibilities to explain the dual emissions. Firstly, binding at the AP site would make a better coplanarity of the berberine's rings A, B, C and D, which could produce the dual emissions because of the presence of dual emissions for sanguinarine, a rings-coplanar alkaloid but with its structure similar to berberine, and the absence of dual emission for non-coplanar dihydrosanguinarine.[51,62,63] But this reason can not explain the phenomenon of the excitation energy-dependent emissions. Secondly, the excited triplet state of berberine could be involved in the emission process because the triplet state energy is theoretically a little close to the 584 nm emission band.[64] However, the measured lifetimes (Table 1) are much less than that for the excited triplet state of berberine.[65] Thirdly, the stacking of electron-donating DNA base with berberine would reduce the energy gap between the ground state and excited singlet state of berberine,[66] which would induce the low-energy emission band.

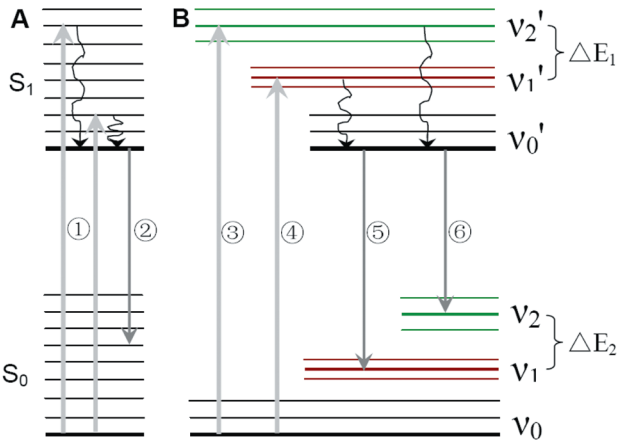
However, all these possibilities do not match the experimental results well and another process should be preferred to control the berberine's emissions. As shown in Fig. 1, the full width at half maxima (FWHM) of the 370 nm excitation bands monitored at 534 nm and the 492 nm emission bands excited at 360 nm for DNA1-C and DNA1-T are less than 12 and 30 nm, indicating a vibration-alike pattern. Such narrow fluorescence spectra are rarely observed for simple organic dyes. Interestingly, the dual emission bands can be observed only under the excitation of berberine's strong absorption band of 330–370 nm, although berberine has another absorption band of 400–500 nm. It is believed that the absorbance between 300 and 370 nm is mainly derived from the ring C of the berberine isoquinoline moiety.[67] In addition, this 330–370 nm absorption band of berberine is just located in the range of DNA fluorescence (the maximum at about 350 nm, dependent on the DNA sequences[68,69]). This means that the overlap integral of these bands can be rather large, which facilitates an opportunity for Förster dipole–dipole resonance interaction between the π-electron systems of the involved molecules. Accordingly, the fluorescence spectra of berberine in the presence of DNA1-C and DNA1-T show such a

Table 2 Melting temperatures of DNAs in the absence and presence of berberine

	FM	DNA1-A	DNA1-C	DNA1-G	DNA1-T
With berberine/°C	65.1	49.6	49.0	49.7	49.0
Without berberine/°C	65.0	49.0	47.4	49.0	47.2
ΔT/°C	0.1	0.6	1.6	0.7	1.8

strong vibration pattern, which reflects a strong resonance coupling of the electronic transitions to the particular vibration mode. This point is supported by the appearance of the dual emissions with excitation at the 330–370 nm absorption band and the disappearance of them with excitation at the 400–500 nm absorption band. The vibration coupling was also observed for another alkaloid of sanguinarine when it interacted with some amino acids.[70] Although the resolution of fluorescence spectra is insufficient to unambiguously identify the precise mode of vibration coupled to the electronic transition, the narrow spectra could be used to roughly predict the binding model. It is proposed that the negative charge moieties in DNA minor groove facilitate berberine binding at this site.[51] Coincidentally, the binding selectivity of berberine at the AP site is sustained for target bases C and T over A and G, in accordance with the presence of the electronegative carbonyl moieties of C and T toward the minor groove, while against the absence of any carbonyl moiety in the minor groove for A and G. However, we can't exclude the possibility that the less bulky target bases C and T than A and G in size would leave more space at the AP site for easily accommodating berberine. In spite of these possibilities, it seems that the stacking interaction at the AP site should orient berberine in the DNA toward a preferable state for the occurrence of the vibration-coupling electron transition. The excited singlet state is produced by excitation of the isoquinoline moiety of berberine. The highest occupied molecular orbital (HOMO) of these alkaloids is localized on the dimethoxy benzene moiety,[41] while the lowest unoccupied molecular orbital (LUMO) is mainly localized at the N^+ heterocycle.[71] Therefore, it is expected that the vibration of the N^+ heterocycle would be strongly affected by the electron-rich flanking bases through the stacked π–π interaction.

On the basis of these considerations, the berberine energy diagrams in the presence of DNAs are depicted in Scheme 2. Binding of berberine with FM, DNA1-A or DNA1-G only produce 534 nm emission band (②) under different excitation wavelengths (①). Berberine binding to DNA1-C and DNA1-T under excitation between 330 and 370 nm induces resonance coupling of the electron transitions to a certain vibration level by the stacking interaction between them. The vibration energy levels of ground (S_0) and excited singlet (S_1) states are symmetrical according to the Franck–Condon principle and by the evidence that the excitation energy difference (ΔE_1 = 3276 cm^{-1}) that can produce the dual emissions under excitations between 330 (③) and 370 (④) nm is very similar to that (ΔE_2 = 3202 cm^{-1}) for the dual emissions at 492 (⑤) and 584 (⑥) nm. The dual emission bands at 492 and 584 nm for DNA1-C and DNA1-T have energy differences of 1598 and 1604 cm^{-1} relative to the single 534 nm emission bands for FM, DNA1-A and DNA1-G, which could be attributed to the



Scheme 2 Energy diagrams of berberine in the absence (A) and presence (B) of a coupling of the electronic transitions to a particular vibration mode. Only vibration energy levels responsible for excitation and emission (ν_0, ν_1, ν_2, ν_0', ν_1', ν_2') are presented here with narrower line separations representing a strong vibration coupling (ν_1, ν_1'). The possibility of firstly exciting to higher energy level (for example, S_2) and then radiationlessly transiting to S_1 during these processes was omitted for clarity.

vibrations of the ring A and the C–N bond of ring B.[72,73] Infrared and Raman spectra were not attempted here to assign the specific vibration mode of berberine due to interference from DNA in this wavenumber region. Because only one long-lived excited state is observed for 492 nm (⑤) and 584 nm (⑥) emission bands, the ratio of the emission integrals of these two bands (1 : 5.8) is proportional to the ratio of the transition probabilities of the two processes from the lowest vibration level of S_1 to the corresponding vibration levels of S_0 (namely, the Franck–Condon ground state) that could be controlled by the densities of the vibration states.

4. Conclusion

In summary, label-free DNA nucleobase recognition was achieved by berberine on the basis of the AP site design with fluorescent light-up and emission energy-modulated sensing merits. When pyrimidines are opposite the AP site, dual emission bands can be realized, whereas only a single band is witnessed for purines opposite the AP site, although fluorescence enhancement is always accompanied for these AP-DNAs relative to the fully matched DNA without the AP site. Thus multicolor recognition for nucleobases can be developed as a practical detection method for differentiating pyrimidines from purines by positioning an AP site toward a target. The dual emissions are believed to be caused by a strong resonance coupling of the electronic transitions to the particular vibration mode that is evidenced by excitation energy-dependent emission behaviors and narrow spectra profiles. These featured emission properties of berberine in the presence of AP-DNAs is very likely to be developed into practical and functional DNA-based sensors and optical devices.[2]

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