

Sequence Dependent Ultrafast Electron Transfer of Nile Blue in Oligonucleotides

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Abstract In this contribution we report studies on the nature of binding of Nile blue (NB), a well known DNA intercalating drug, with three synthetic DNA oligonucleotides, (CGCAAATTTGCG)₂, (GCGCGCGCGCGC)₂ and (ATATATATATAT)₂. The nature of fluorescence quenching of the ligand upon complexation with the DNAs has been studied using steady state and picosecond-resolved optical spectroscopic techniques. The geometrical restriction on the probe in the DNA microenvironment is measured using picosecond-resolved rotational anisotropy measurements. Our experiments identify both non-specific electrostatic and intercalative modes of interaction of the probe with the DNAs at lower and higher DNA concentrations, respectively. This dual nature of binding is also confirmed through gel electrophoresis experiments. The nature of electron transfer (ET) reaction of GC base pairs with intercalated NB has also been explored. Competitive binding study reveals that binding affinity of the probe is higher with SDS micelles than with the DNAs within its structural integrity in presence of the micelles, as evidenced from circular dichroism (CD) measurements. The complex rigidity of NB with various DNAs and its fluorescence quenching with DNAs elucidate a strong recognition mechanism between NB and DNA.

Keywords Nile blue · Oligomers · Electron transfer · Picosecond dynamics

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Introduction

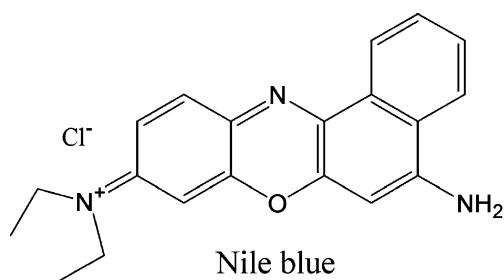
Nile blue (NB) is a nontoxic fluorescent dye that can potentially intercalate in the major groove of DNA [1, 2]. It is a member of the benzophenoxazine class of dyes, which has been found to be localized selectively in animal tumors [3] and can retard tumor growth [4, 5]. Compared to the conventional intercalators, NB has the merit of having low toxicity and high sensitivity for DNA quantification [2]. This unique feature of this red-end dye makes its interaction with DNA a potential field of study. In a recent study [6], by using a microelectrode modified with NB-DNA duplexes in cyclic voltammetry and square wave voltammetry, it has been demonstrated that the electrochemical detection of nanomolar TATA binding to DNA in presence of other proteins with a rapid and sensitive electrochemical assay is feasible. However, only a limited number of reports regarding its interaction with DNA are available in the present literature [2, 7–9]. The studies of Chen et al. [2] on the interaction of NB with calf thymus (CT) DNA concluded that NB serves as an intercalator to the stack base pairs of nucleic acids. They reported quenching of NB fluorescence at low DNA concentrations and a partial recovery of the quenching at higher DNA concentrations. However, the detailed mechanism of the quenching process and a subsequent release of it was lacking in their study. Spectrophotometric studies by Huang et al. [7] reported aggregate formation between NB-sulfate and CT DNA. Yang et al. [8] suggested that the intercalation of NB in DNA can be utilized to make NB a visible dye for DNA detection. Electrochemical studies by Ju et al. [9] concluded that the binding of NB with DNA in solution contains both electrostatic and intercalative modes of interactions. However, photophysical properties of the NB-DNA complex on the ultrafast time scale have not yet been examined. Very

recently we have reported the interaction of NB with biomimicking self organized assemblies (SDS micelles and AOT reverse micelles) and a genomic DNA (extracted from salmon sperm) (SS DNA) [1]. Our study identified two different binding modes (electrostatic and intercalative) of NB with the genomic DNA. The former is responsible for electron transfer between the probe and DNA, which eventually quenches the emission intensity of NB fluorescence in presence of low concentration of DNA. The subsequent release of quenching has been attributed to the intercalation of the dye in DNA base pairs. However, a detailed mechanism of this interaction was a question to be solved.

As a continuation of our previous study, here we report a detailed steady state and time-resolved spectroscopic studies of NB in three synthetic DNA oligonucleotides of known sequences, namely Oligo1, (CGCAAATTTGCG)₂, Oligo2, (GCGCGCGCGCGC)₂ and Oligo3, (ATATATATATAT)₂ in order to understand the electron transfer mechanism in detail. The polarization-analyzed anisotropy for the DNA–NB complexes elucidates the binding rigidity of the probe. The fluorescence quenching mechanism of NB involving electron transfer (ET) of the probe upon complexation with oligomers has been explored. The nature of DNA-binding of NB in presence of SDS micelles has also been explored in the present study. The structural integrity of the DNAs in presence of SDS micelles has been confirmed by circular dichroism (CD) spectroscopic studies. Such a comprehensive study of the photophysics of NB in DNAs of known sequences is demanded to understand the mechanism of fluorescence quenching upon interaction with DNA and to exploit this nontoxic fluorescent dye as a tool for DNA-recognition in biologically relevant macro-molecular microenvironment.

Experimental details

The fluorescent probe Nile blue (NB; Scheme 1) chloride was obtained from Sigma. Sodium dodecyl sulfate (SDS) was a product of Fluka. The purified (reverse phase HPLC) synthetic DNA oligonucleotides of 12 bases (dodecamer)



Scheme 1 Molecular structure of NB

with sequences CGCAAATTTGCG (Oligo1) and GCGCGCGCGCGC (Oligo2) were obtained from Gene Link. Oligo3 having the sequence of ATATATATATAT was a product of Sigma Aldrich. To reassociate the single strand DNA into self-complementary double-strand DNAs thermal annealing was performed as per the methodology prescribed by the vendors. The oligonucleotide solutions were dialyzed exhaustively against 10 mM phosphate buffer prior to further use. The CD spectrum of all the dialyzed oligomers was checked and all of them were found to possess the physiologically relevant B-form confirming the double strand formation in all the oligomers. The nucleotide concentrations of the DNA samples were measured by absorption spectroscopy using the average excitation coefficient of nucleotide (6,600 M⁻¹ cm⁻¹ at 260 nm) and considered to be the concentrations of the DNAs. It could be noted that Oligo3 has a low melting point (~20 °C as reported by the vendor), and we performed all the experiments with Oligo3 at 10 °C. We have also verified that there occurred marginal differences when the experiments with the other two oligomers were repeated at this temperature. SDS micelles were prepared by adding calculated amounts of SDS in known volume of buffer solution and stirring for about 45 min. The concentration of the dye NB in the sample solutions was maintained at ~2.0 μM to avoid its self-aggregation.

Steady state absorption and emission were measured with Shimadzu UV-2450 spectrophotometer and Jobin Yvon Fluoromax-3 fluorimeter respectively. Circular dichroism (CD) spectra were taken in a Jasco-815 spectrometer using a quartz cell of path length of 1 cm. All fluorescence decays were taken by using picosecond-resolved time correlated single photon counting (TCSPC) technique. The commercially available setup is a picosecond diode laser pumped time resolved fluorescence spectrophotometer from Edinburgh Instrument, UK. It has an instrument response function (IRF) of 80 ps. The experimental and analytical details of time-resolved measurements can be found elsewhere [1, 10]. All the decay profiles are fitted using exponential decay functions with χ^2 values were close to 1. For anisotropy measurements, emission polarization was adjusted to be parallel or perpendicular to that of the excitation and anisotropy is defined as, $r(t) = \frac{I_{\parallel} - GI_{\perp}}{I_{\parallel} + 2GI_{\perp}}$. The magnitude of G, the grating factor of the TCSPC system, was determined by using a NB solution in buffer following the longtime tail matching technique [11]. According to this technique, G is a number, when multiplied with the I_⊥ values produces values comparable to the I_∥ values, specially in the long time scale (beyond 10 ns). $r(t)$ has been fitted biexponentially as, $r(t) = r_0 [\alpha_1 e^{-t/\tau_{r1}} + \alpha_2 e^{-t/\tau_{r2}}]$ following the earlier DNA-ligand studies [12–14], where τ_{r1} and τ_{r2} are the rotational time constants, and α_1 and α_2 are their amplitudes. Gel electrophoresis was carried out in an APELEX-Minigel

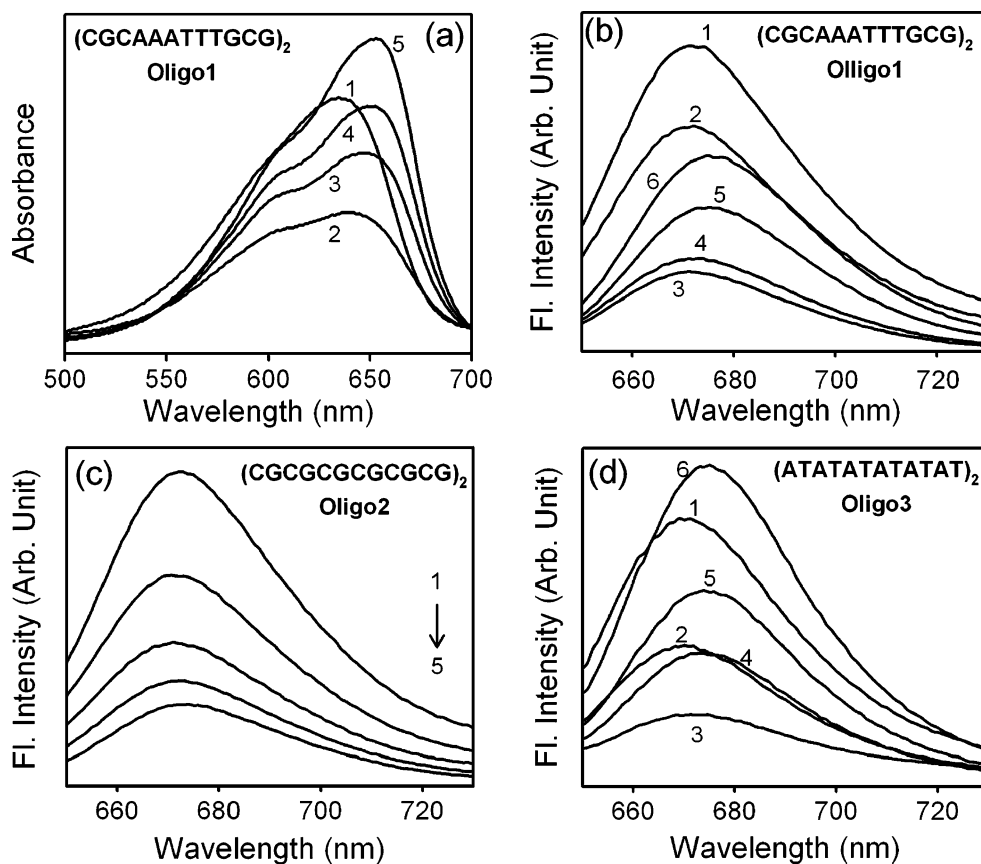
horizontal gel electrophoresis apparatus with 2% agarose gel using the standard protocol.

Results and discussion

Figure 1a depicts the absorption spectra of NB in 10 mM phosphate buffer in absence and presence of Oligo1 of varying concentrations. It is found that NB in buffer produces a peak at $\lambda_{max} \sim 635$ nm. When Oligo1 is added into it ([DNA]=10 μ M), a hump at 605 nm develops with a considerable decrease in the absorbance. On further increase in the Oligo1 concentration to 25 μ M, absorbance is increased with a red shift of the peak to 640 nm. When the concentration of DNA is increased to 100 μ M, the peak is further red-shifted to 652 nm with higher absorbance than that in the buffer solution. The appearance of a hump in the blue region of the spectrum signifies the possibility of formation of a probe-DNA complex in the ground state. Previously Huang et al. [7] reported the formation of an aggregate between NB-sulfate and DNA with a binding constant of 3×10^3 L mol⁻¹. Figure 1b depicts the emission spectra of the NB-Oligo1 systems. Fluorescence intensity of NB is reduced considerably at [DNA]=2 μ M with no change in the peak position with respect to the buffer solution ($\lambda_{max} \sim 672$ nm). At 10 μ M DNA, fluorescence is

significantly quenched and the peak has a little red shift to 675 nm. However, at 20 μ M DNA, the intensity of NB fluorescence increases compared to that of 10 μ M DNA. With further increase in DNA concentration, intensity gradually increases with a red shift in the peak position. The absorption spectra of NB in presence of Oligo2 and Oligo3 resemble that of Oligo1 with the appearance of a hump in the blue region confirming the formation of a ground state complex (figures not shown). The fluorescence spectra of NB in presence of Oligo2 (Fig. 1c), however, differ significantly from that of Oligo1, wherein the fluorescence of NB is quenched by Oligo2 throughout the studied concentration range and quenching is not released to any extent up to 100 μ M of Oligo2. The fluorescence spectra of NB in presence of Oligo3 are depicted in Fig. 1d. As can be observed from the figure, when Oligo3 is added into NB at low concentration (2 μ M) the fluorescence is quenched with no change in the peak position, similar to that obtained in case of Oligo1 and Oligo2. Upon gradual increase of Oligo3 concentration up to 7.5 μ M, the fluorescence intensity decreases. As the concentration is increased to 10 μ M, the intensity increases with a little red shift of the fluorescence peak. When the Oligo3 concentration is increased further, fluorescence intensity increases and at a high Oligo3 concentration, the fluorescence intensity is found to be higher than that in the buffer

Fig. 1 **a** Absorption spectra of NB in presence of Oligo1 of different concentrations, (1) 0, (2) 10, (3) 25, (4) 50 and (5) 100 μ M. **b** Fluorescence spectra of NB in presence of Oligo1 of different concentrations, (1) 0, (2) 2, (3) 10, (4) 20, (5) 50 and (6) 100 μ M. **c** Fluorescence spectra of NB in presence of Oligo2 of different concentrations, (1) 0, (2) 10, (3) 25, (4) 50 and (5) 100 μ M. **d** Fluorescence spectra of NB in presence of Oligo3 of different concentrations, (1) 0, (2) 2, (3) 5, (4) 10, (5) 30 and (6) 100 μ M



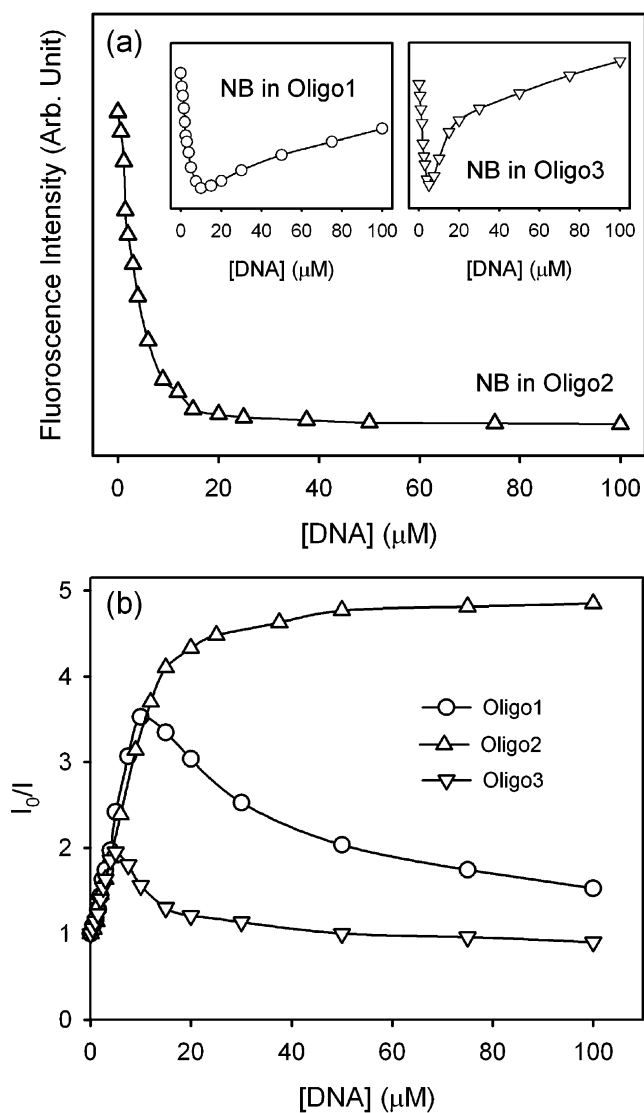


Fig. 2 **a** Fluorescence intensity of NB (at the peak emission) vs. Oligo2 concentration profile. Similar profiles for the Oligo1 and Oligo3 are presented in the inset. The solid lines are guide to eyes. **b** Stern Volmer plot of NB in Oligo1, Oligo2 and Oligo3. The solid lines are guide to eyes

solution. It could be noted that the quenching of NB, which has never been released in Oligo2 and only partially been released in case of Oligo1, is completely released in Oligo3.

Figure 2a shows the fluorescence quenching of NB in presence of the oligomers wherein we plot fluorescence intensity (at the peak) against concentration of the DNAs. It is evident from Fig. 2a (inset) that Oligo1 produces an inverted bell-shaped profile in which fluorescence is quenched at low DNA concentration and the quenching is released beyond 12.5 μM DNA concentration. A similar profile was reported by us for SS (salmon sperm) DNA [1] and Chen et al. [2] for CT (calf thymus) DNA. On the other hand, Oligo2 exhibits a different phenomenon in which fluorescence intensity decreases sharply up to 15 μM and then moderately to reach a plateau. The profile for Oligo3 resembles that of Oligo1, but for the former oligomer fluorescence intensity starts increasing at a lower DNA concentration and also the rise in intensity is steeper than that observed in case of Oligo1. The plots of I_0/I against the DNA concentration for all the oligomers in the entire concentration region are shown in Fig. 2b. It can be followed that for Oligo1, I_0/I increases linearly up to 10 μM DNA, beyond which it decreases indicating the release of quenching, a phenomenon similar to that obtained in SS DNA [1] and CT-DNA [2]. A Stern-Volmer equation, $\frac{I_0}{I} = 1 + K_{SV}[Q]$, (where, I_0 and I are the fluorescence intensities in absence and presence of the quencher (Q) and K_{SV} is the formation constant of the quenching complex) is fitted in the quenching regime. A good linear fit is obtained with a corresponding quenching constant (K_{SV}) of $2.6 \times 10^5 \text{ L mol}^{-1}$ for Oligo1 (Table 1). The obtained K_{SV} value is an order smaller than that reported for SS DNA ($1.6 \times 10^6 \text{ L mol}^{-1}$) [1] and CT-DNA ($3.2 \times 10^6 \text{ L mol}^{-1}$) [2]. For the other two oligomers also, I_0/I follows a linear dependency with DNA concentration with corresponding K_{SV} values of 2.1×10^5 and $2.0 \times 10^5 \text{ L mol}^{-1}$ for Oligo2 and Oligo3, respectively.

Figure 3 depicts a representative fluorescence transient and rotational anisotropy of NB in presence of the oligomers at 100 μM DNA concentration. NB in buffer

Table 1 Numerical fitting parameters of the time resolved fluorescence lifetime and rotational anisotropy studies of NB in various environments^{a,b}

System	$K_{SV} (\text{L mol}^{-1})$	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	r_0	τ_{r1} (ns)	τ_{r2} (ns)
Buffer	–	0.34(100%)	–	–	0.38	0.16(100%)	–
Oligo 1	2.6×10^5	0.10(63%)	0.40(29%)	1.3(8%)	0.37	0.06(36%)	2.3 (42%)
Oligo 2	2.1×10^5	0.07(49%)	0.35(48%)	1.2(4%)	0.33	0.05(50%)	0.25(28%)
Oligo 3	2.0×10^5	–	0.42(46%)	1.5(54%)	0.37	0.08(20%)	2.5(57%)
SS DNA ^c	1.6×10^6	0.08(54%)	0.35(25%)	1.2(21%)	0.37	0.07(31%)	2.0(13%)

a. The figures in the parenthesis represent relative contribution of the component

b. Concentration of Oligo1, Oligo 2, Oligo 3 and SS DNA is 100 μM

c. A genomic DNA extracted from salmon sperm

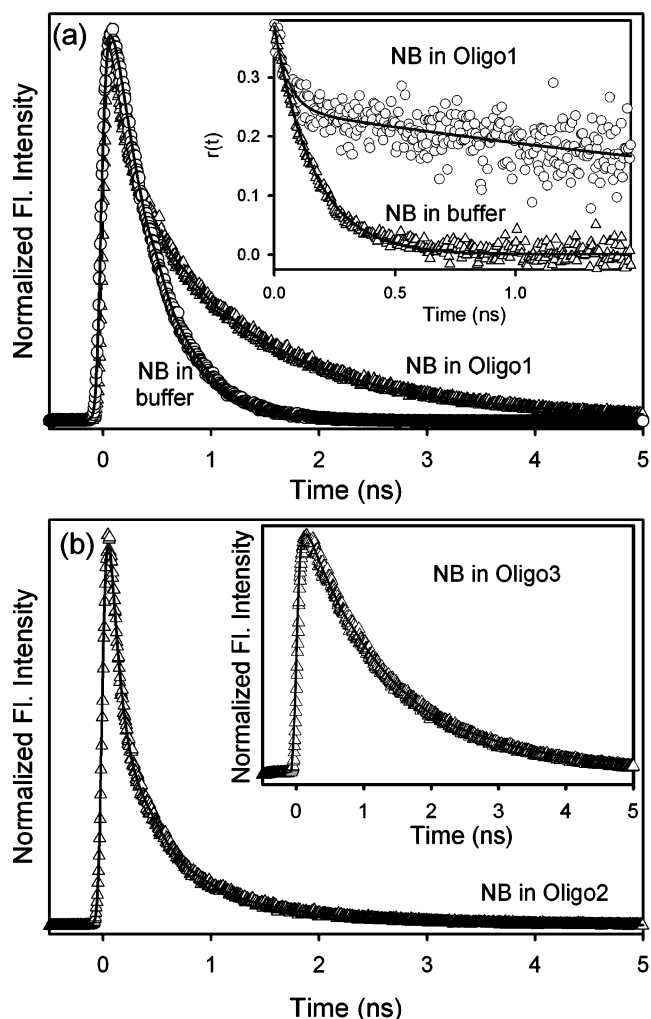


Fig. 3 Picosecond resolved fluorescence transients of NB in presence of 100 μM Oligo1 (a), Oligo2 (b) and Oligo3 (inset of b). The time resolved anisotropy, $r(t)$ profile for Oligo1 is given in the inset of Fig. 3a

produces a single-exponential decay with time constant of 0.34 ns (Fig. 3a, Table 1). At 3 μM Oligo1, a bi-exponential decay is observed with time constants of 0.3 ns (97%) and 1.2 ns (3%). The decrease in the faster lifetime indicates quenching as has also been evidenced in the steady-state experiments. With 10 μM DNA, the time constants obtained are 0.27 ns (85%) and 0.8 ns (15%). The τ_0/τ value (where, τ_0 is the excited-state lifetime of the probe in absence of the quencher and τ is that in presence of the quencher) for the former case is 1.13, whereas it is 1.25 for the latter and both of them are considerably smaller than the I_0/I values (1.74 and 3.53 respectively) in the quenching regime confirming the static nature of the quenching [15]. However, with increase in Oligo1 concentration beyond the quenching regime, the transients get slower. The fluorescence decay with 100 μM oligo1 (Fig. 3a) is well fitted tri-exponentially with time constants

of 0.1 ns (63%), 0.4 ns (29%) and 1.3 ns (8%) (Table 1). The 0.1 ns component represents the highly quenched NB bound to the DNA whereas, the slow component of 1.3 ns represent NB molecules intercalated into the DNA. It could be noted that the 0.4 ns component is slower than that of NB in buffer but faster than that intercalated in DNA and might represent NB in monomeric or weakly DNA-bound form. To compare this result with that of a genomic DNA we measure the fluorescence decay of NB in 100 μM genomic DNA (extracted from salmon sperm) and the results are presented in Table 1. It can be found that for the genomic DNA also, similar time constants are obtained. The rotational anisotropy of NB in 100 μM Oligo1 is strikingly different from that of NB in buffer wherein it produces a single component of 0.16 ns (Fig. 3a). In Oligo1, the rotational anisotropy is associated with time constants of 0.06 ns (36%) and 2.3 ns (42%). The presence of a fast component in rotational anisotropy indicates the existence of a weakly bound NB at the DNA surface and/or wobbling dynamics of the probe NB at the site of intercalation [16]. The time constant of 2.3 ns is comparable to the tumbling motion of the oligomer [17]. The slow rotational time constant is indicative of the intercalative binding of NB with the oligomer. Similar rotational time constants are also obtained for the genomic DNA (Table 1). The large offset value (66%) obtained for the genomic DNA represents the rotational motion of the whole DNA molecule which is not complete within the experimental time window.

The decay pattern of NB in 100 μM Oligo2 is presented in Fig. 3b. It is observed that the decay is faster than the corresponding aqueous system with time components of 0.07 ns (49%), 0.35 ns (48%) and 1.2 ns (2%). It could be noted that the faster component is decreased further compared to that in Oligo1 along with a subsequent decrease in the contribution of the slower component. The fluorescence decay of Oligo3 is depicted in the inset of Fig. 3b. As can be observed from the figure, the decay is considerably slower than the other two oligomers and can only be fitted biexponentially with time constants of 0.42 ns (46%) and 1.5 ns (54%) (Table 1). The absence of a fast component in this decay transient confirms the complete removal of the NB fluorescence quenching upon intercalation with Oligo3 as has also been evidenced in the steady state measurements (Fig. 2).

Fluorescence quenching of NB upon complexation with the synthetic DNAs clearly indicates that up to 7.5–15 μM DNA concentrations the nature of binding is different from that at higher DNA concentrations. The identical K_{SV} values ($\sim 2 \times 10^5 \text{ L mol}^{-1}$) obtained for all the oligomers in this concentration range identifies the quenching process to be similar in nature for all the oligomers. For other cationic intercalators it has been shown earlier that at low DNA

concentration, the probe molecules undergo electrostatic binding (non-specific) and at higher DNA concentration the probes intercalate into the DNA [18–20]. For the intercalator ethidium bromide (EB), it is found that the probe changes its mode of interaction with DNA from electrostatic to intercalative at $[EB]:[DNA]=1:6$ [20]. It can be argued that quenching of NB fluorescence in the low concentration region (up to 10–15 μM) is due to the formation of ligand-DNA complex in the ground state (as is also evidenced from the absorption spectra) which has its origin in the electrostatic interaction between the positively charged probe and negatively charged DNA surface, an effect resembling the quenching of fluorescence by heavy anions through electrostatic interaction [21–23]. Time-resolved study confirms the static nature of the quenching, which corroborates well with the present argument. The formation of such a complex is associated with a charge-transfer mechanism, which contributes to the quenching process. The intermediate complex might also facilitate the inter system crossing (ISC) to the triplet state of the acceptor (DNA) [24]. It can be noted that the K_{SV} value obtained with oligomer-NB pair is smaller than that of the SS DNA-NB (Table 1) [1] and CT DNA-NB [2] system. The formation of a ligand-DNA complex is a consequence of close approach and fruitful collision of the reacting species. It has been reported earlier that collision between a heavy and a small particle is more probable than those between equal sized particles [25], which signifies the lower K_{SV} value in oligomers compared to that in the genomic DNAs.

The release of quenching at a higher Oligo1 and Oligo3 concentration can be correlated with the intercalation of the ligand within the base pairs of DNA. It should be noted that intercalation of NB in the close vicinity of GC base pairs might lead to electron transfer (ET) reaction [26, 27] from guanine base as evidenced by earlier studies [28]. The quenching of NB fluorescence due to ET reaction is associated with various rate constants, which are the consequences of direct and hopping mechanism of ET, revealing significant deviation from typical Stern-Volmer plot as observed in case of Oligo2 (Fig. 2b). It has been reported that guanine mediated electron transfer is associated with two time scales of ~ 5 ps and ~ 75 ps [28]. In the time resolved lifetime measurements we perhaps miss a fast component (of the order of a few picoseconds) due to the limitation of instrumental resolution (IRF ~ 80 ps), however, the 0.07 ns component (which is well within the instrumental resolution) as obtained in case of Oligo2 is consistent with the slower time scale of ET reaction as reported earlier [28]. On the other hand, in the case of Oligo1 (mixture of AT & GC) the intercalation of NB between two AT base pairs protect the NB from the ET process [26, 27] and the quenching of the probe due to

proton transfer in the buffer [29, 30] which eventually releases the quenching partially in case of Oligo1 and genomic DNA [2]. In case of Oligo3, the probe is intercalated in a purely AT environment wherein the ET possibility is very low [28] and subsequently the quenching is fully released in comparison to Oligo1, wherein quenching is only partially released due to the presence of certain extent of GC. This points out to the fact that the GC base pair is responsible for the observed quenching of NB fluorescence.

To reconfirm the dual binding nature of NB with the oligomers, we perform gel electrophoresis experiment using NB and another well known DNA intercalator, ethidium bromide (EB) [14, 31] in both low ($[NB]:[DNA]=1:2.5$) (in the electrostatic binding regime) and high ($[NB]:[DNA]=1:25$) (in the intercalative binding regime) concentrations of DNA. The results are depicted in Fig. 4. Note that we have not used Oligo3 for the gel electrophoresis study as it has a very low melting point and might not retain its physiologically relevant conformation at room temperature. As can be observed from the figure, distinct band is obtained for NB at high DNA concentration in both the oligomers (lane F and H), specially for Oligo 2, identical to that obtained for the EB system. The distance traveled by NB-DNA is comparable to that of EB-DNA confirming the intercalative nature of binding of NB with DNA. On the other hand, for low DNA concentration systems, the bands are not prominent and the lanes are smeared for NB-DNA (lane B and D), whereas EB-DNA travel identical distances (lane A and C) as that in the high DNA concentration systems (lane E and G). It could be noted that DNA is negatively charged at neutral pH and during an electrophoresis measurement when an electric potential is applied in it, it moves to the positive pole. As NB is positively charged and electrostatically binds with DNA, it neutralizes the charge of DNA surface resulting in the slow movement of the DNA in the

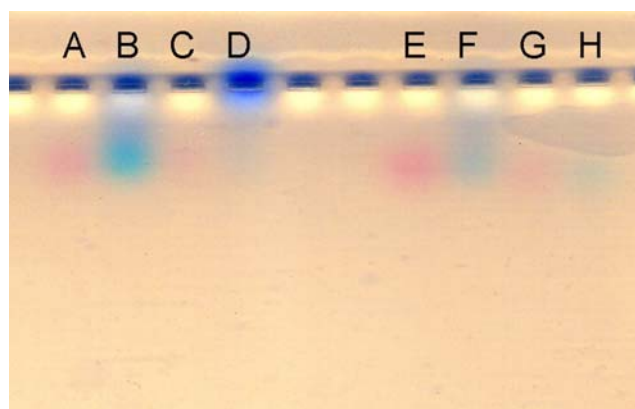


Fig. 4 Electropherogram for diluted DNA samples ($[NB]:[DNA]=1:2.5$): (a) EB-Oligo 1, (b) NB-Oligo 1, (c) EB-Oligo 2, (d) NB-Oligo 2; concentrated DNA samples ($[NB]:[DNA]=1:25$): (e) EB-Oligo 1, (f) NB-Oligo 1, (g) EB-Oligo 2, (h) NB-Oligo 2

low DNA concentration regime. Also, while moving through the lane, DNA releases weakly surface-bound (electrostatically) NB and makes the lane smeared. Thus the gel electrophoresis study confirms both electrostatic as well as intercalative binding mode of NB with DNA.

Intercalation of small ligands into the base pairs of DNA requires formation of an intercalation cavity, into which drugs/ ligands may penetrate, followed by the formation of additional molecular interactions (like hydrogen bonding, Van der Waals stacking etc.) to anchor the complex. Thermodynamic studies reveal that binding of intercalators is clearly driven by large favorable enthalpy contribution and is opposed by entropy [32]. Base pair separation upon intercalation costs energy, which is partially regained upon intercalation by the formation of base pair-drug stacking. Also anchoring interactions like hydrogen-bond formation between drug and nearby bases provide additional favorable enthalpy. Intercalation results in both lengthening and stiffening of the DNA duplex, along with a damping of motions within the helix [33]. This rigidity of the DNA ought to extract an entropic cost making intercalation an entropically forbidden process. To check whether the gain in enthalpy is sufficient to compensate the entropic cost under stressed conditions, we study the effect of SDS micelles on the photophysical properties of NB in presence of the oligomers. Formation of micelles is a strongly entropy driven process [34] and its hydrophobic core can accommodate small hydrophobic molecules like NB. The effect of SDS micelles on the DNA-binding of NB at DNA concentration of 100 μM is shown in Fig. 5. As evident from Fig. 5a, in the presence of DNA a red shift of the fluorescence maximum occurs along with a decrease in the intensity. Upon addition of 50 mM SDS, the peak suffers a blue shift with a huge increase in fluorescence intensity indicating a protected residence of the dye in micellar environment, compared to that in the DNA. To check the structural integrity of DNA in presence of SDS micelles, we perform the CD experiment on 20 μM Oligo1 in absence and presence of 50 mM SDS (Fig. 5b). As evidenced from Fig. 5b, the DNA retains its native B-form in presence of SDS micelles. The corresponding time-resolved study is presented in Fig. 5c. It can be observed that in presence of 50 mM SDS, the contribution of the fast component, originally present in Oligo1 becomes insignificant with a slow component of 1.2 ns as the major one, similar to that of an SDS micellar system. Identical conclusion can also be drawn from the anisotropy study (inset of Fig. 5c) wherein a clear indication of a migration of the dye NB from DNA to micellar environment is evidenced. Similar trends are also observed for the Oligo2 and Oligo3 systems.

Exclusion of the intercalated dye from the oligomers by SDS micelles is interesting because the DNAs and SDS micelles are supposed to be non-interacting owing to their

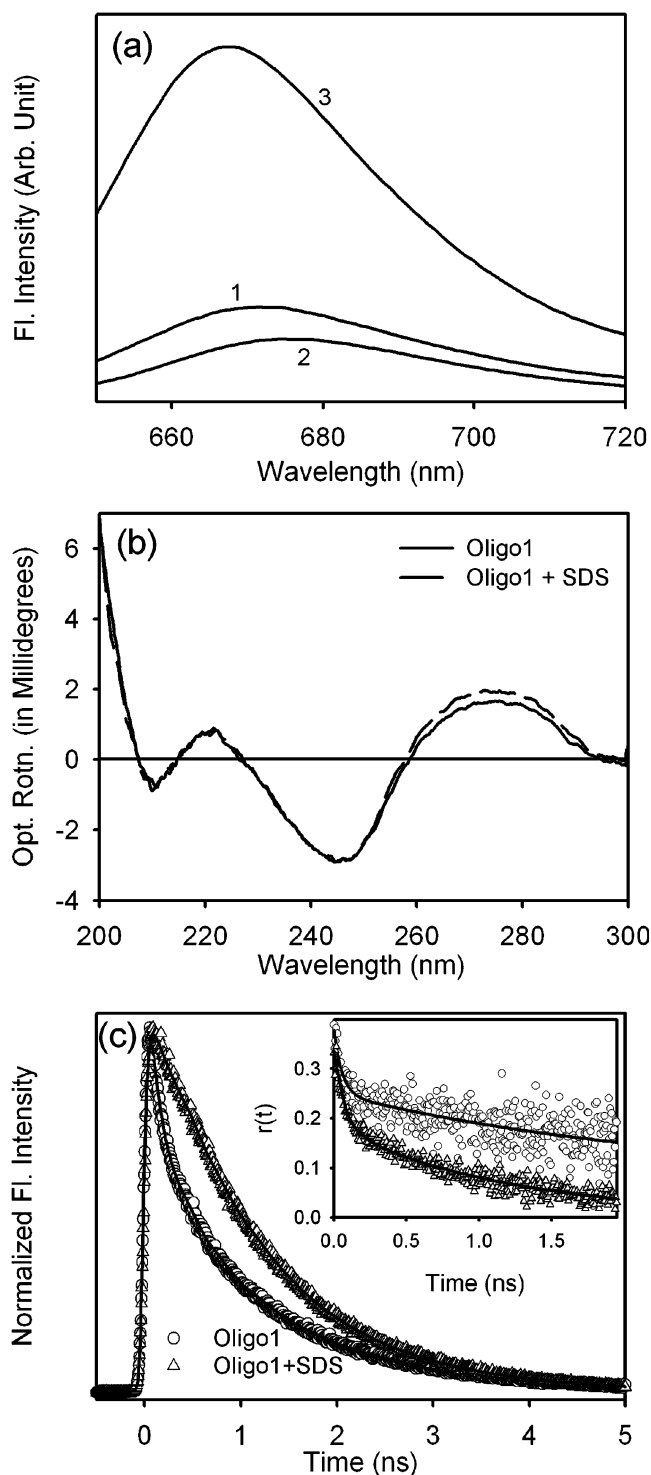


Fig. 5 a Fluorescence spectra of NB in (1) water, (2) 100 μM Oligo1, (3) 100 μM Oligo1 in presence of 50 mM SDS. b Circular dichroism (CD) spectra of 20 μM Oligo1 in absence and presence of 50 mM SDS. c Picosecond resolved fluorescence transients of NB in presence of 100 μM Oligo1 and 50 mM SDS. The corresponding time resolved anisotropy, $r(t)$ profiles are given in the insets

similar surface charge. Izumrudov et al. [35] earlier proposed that an exclusion of intercalated EB from DNA in presence of cationic micelles was prompted by hydrophobic interactions between the planar aromatic ethidium ring and the surfactant tail group. It could be noted that the intercalation process of a small ligand into the base pairs of DNA is a dynamical equilibrium process in which ligand monomers are in equilibrium with the intercalated ones [36]. The present study shows that in presence of SDS micelles, the equilibrium process is shifted toward the dissociation of intercalated NB monomers from the DNAs. This result infer that in order to affect the rate of dissociation of NB from DNA, the SDS micelles have to bind or at least have to get very close to the negatively charged DNA surface. However, the presence of aromatic ring systems in combination with positive charge of NB might produce transient electro-neutral regions on the DNA surface that attracts the negatively charged SDS micellar surface [37]. Also the hydrophobic environment of SDS micelles might favor transient openings of the otherwise quite compact DNA duplex structure, which provides a safe residence for exposed hydrophobic moieties of the intercalated ligands. A detailed thermodynamic study of the intercalated NB-DNA dissociation and free NB-SDS micelle association is of potential importance and is underway in our laboratory.

Conclusions

In the report we explore the nature of binding interaction of a fluorescent dye/drug Nile blue (NB) with synthetic oligonucleotides of known sequences. Picosecond resolved fluorescence quenching and polarization analyzed anisotropy of NB in the DNAs clearly reveal the interaction of the probe with the DNA molecules. Our studies depict two types of binding modes of the probe drug with DNAs; non-specific electrostatic at lower DNA concentration and intercalative at the higher DNA concentration in the solution. The former is responsible for quenching of NB fluorescence, whereas the latter partially releases the quenching in Oligo1 and fully in Oligo3. The nature of photo induced electron transfer (ET) reaction of NB upon intercalation into the DNA base pairs, are also evident from our studies. The nature of the binding of NB in presence of the oligomers and SDS micelles reveals more stable NB-micelle complexation compared to that with the DNA revealing entropic contribution in the process of NB-complexation with other macromolecules. The structural integrity of the DNAs in presence of SDS micelles is confirmed by CD studies. Our studies can be effectively used to understand the complexation mechanism of the non-toxic drug NB with DNA.

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References

- Mitra RK, Sinha SS, Pal SK (2008) Interactions of Nile blue with Micelles, reverse Micelles and a genomic DNA. *J Fluoresc* 18:423–432
- Chen Q, Li D, Yang H, Zhu Q, Xu J, Zhao Y (1999) Interaction of a novel red-region fluorescent probe, Nile Blue, with DNA and its application to nucleic acids assay. *Analyst* 124:901–907
- Staveren HJ, Speelman OC, Witjes MJH, Cincotta L, Star WM (2001) Fluorescence imaging and spectroscopy of ethyl Nile blue A in animal models of (Pre)malignancies. *Photochem Photobiol* 73:32–38
- Morgan J, Potter WR, Oseroff AR (2000) Comparison of photodynamic targets in a carcinoma cell line and its mitochondrial DNA-deficient derivative. *Photochem Photobiol* 71:747–757
- Singh G, Espiritu M, Shen XY, Hanlon JG, Rainbow AJ (2001) In vitro induction of PDT resistance in HT29, HT1376 and SK-N-MC cells by various photosensitizers. *Photochem Photobiol* 73:651–656
- Gorodetsky AA, Ebrahim A, Barton JK (2008) Electrical detection of TATA binding protein at DNA modified micro-electrodes. *J Am Chem Soc* 130:2924–2925
- Huang CZ, Li YF, Zhang DJ, Ao XP (1999) Spectrophotometric study on the supramolecular interactions of nile blue sulphate with nucleic acids. *Talanta* 49:495–503
- Yang Y, Hong HY, Lee IS, Bai DG, Yoo GS, Choi JK (2000) Detection of DNA using a visible dye, Nile Blue, in electrophoresed gels. *Anal Biochem* 280:322–324
- Ju H, Ye Y, Zhu Y (2005) Interaction between nile blue and immobilized single- or double-stranded DNA and its application in electrochemical recognition. *Electrochim Acta* 50:1361–1367
- Mitra RK, Sinha SS, Pal SK (2007) Temperature dependent hydration at micellar surface: activation energy barrier crossing model revisited. *J Phys Chem B* 111:7577–7581
- O'Connor DV, Philips D (1984) Time correlated single photon counting. Academic Press, London
- Banerjee D, Pal SK (2008) Dynamics in the DNA recognition by DAPI: exploration of the various binding modes. *J Phys Chem B* 112:1016–1021
- Shaw AK, Pal SK (2007) Fluorescence relaxation dynamics of acridine orange in nanosized micellar systems and DNA. *J Phys Chem B* 111:4189–4199
- Sarkar R, Pal SK (2007) Interaction of Hoechst 33258 and ethidium with histone1-DNA condensates. *Biomacromolecules* 8:3332–3339
- Lakowicz JR (1999) Principles of fluorescence spectroscopy. Kluwer Academic/Plenum, New York
- Millar DP, Robbins RJ, Zewail AH (1982) Torsion and bending of nucleic acids studied by subnanosecond time-resolved fluorescence depolarization of intercalated dyes. *J Chem Phys* 76:2080–2094
- Pal SK, Zhao L, Zewail AH (2003) Water at DNA surfaces: ultrafast dynamics in minor groove recognition. *Proc Natl Acad Sci USA* 100:8113–8118
- McMurray CT, Small EW, van Holde KE (1991) Binding of ethidium to the nucleosome core particle. 2. Internal and external binding modes. *Biochemistry* 30:5644–5652
- McMurray CT, van Holde KE (1991) Binding of ethidium to the nucleosome core particle. 1. Binding and dissociation reactions. *Biochemistry* 30:5631–5643

20. Sarkar R, Pal SK (2006) Ligand-DNA interaction in a nanocage of reverse micelle. *Biopolymers* 83:675–686
21. Bortolus P, Bartocci G, Mazzucato U (1975) Excited state reactivity of aza aromatics. III. Quenching of fluorescence and photoisomerization of azastilbenes by inorganic anions. *J Phys Chem* 79:21–25
22. Davidson RS, Lewis A (1973) Formation of exciplexes between aromatic hydrocarbons and halogen anions. *J Chem Soc Chem Comm* 262
23. Watkins AR (1973) Quenching of biphenyl fluorescence by inorganic ions. *J Phys Chem* 77:1207–1210
24. Atherton SJ, Beaumont PC (1987) Laser flash photolysis of DNA-intercalated ethidium bromide in the presence of methylviologen. *J Phys Chem* 91:3993–3997
25. Heller W, Rowe E, Berg R, Watson JHL (1959) Effect of partial coagulation upon the size distribution curve in heterodisperse colloidal systems. *J Phys Chem* 63:1566–1569
26. Kobayashi T, Takagi Y, Kandori H, Kemnitz K, Yoshihara K (1991) Femtosecond intermolecular electron transfer in diffusionless, weakly polar systems: Nile blue in aniline and N,N-dimethylaniline. *Chem Phys Lett* 180:416–422
27. Kandori H, Kemnitz K, Yoshihara K (1992) Subpicosecond transient absorption study of intermolecular electron transfer between solute and electron-donating solvents. *J Phys Chem* 96:8042–8048
28. Wan C, Fiebig T, Kelley SO, Treadway CR, Barton JK, Zewail AH (1999) Femtosecond dynamics of DNA-mediated electron transfer. *Proc Natl Acad Sci USA* 96:6014–6019
29. Grofcsik A, Kubinyi M, Jones WJ (1996) Intermolecular photoinduced proton transfer in Nile blue and Oxazine 720. *Chem Phys Lett* 250:261–265
30. Douhal A (1994) Photophysics of Nile blue A in proton accepting and electron donating solvents. *J Phys Chem* 98:13131–13137
31. McMurray CT, Van Holde KE (1991) Binding of ethidium to the nucleosome core particle. 1. Binding and dissociation reactions. *Biochemistry* 30:5631–5643
32. Chaires JB (2006) A thermodynamic signature for drug–DNA binding mode. *Arch Biochem Biophys* 453:26–31
33. Bloomfield VA, Crothers DM, Tinoco JI (2000) *Nucleic acids: structures, properties and functions*. University Science Books, Sausalito, California
34. Tanford C (1980) *The Hydrophobic effect: formation of micellar and biological membranes*, 2nd edn. Wiley, New York
35. Izumrudov VA, Zhiryakova MV, Goulko AA (2002) Ethidium bromide as a promising probe for studying DNA interaction with cationic amphiphiles and stability of the resulting complexes. *Langmuir* 18:10348–10356
36. Wilhelmsson LM, Westerlund F, Lincoln P, Norden B (2002) DNA-binding of semirigid binuclear ruthenium complex, [-(11,11'-bidppz)(phen)₄Ru₂]⁴⁺: extremely slow intercalation kinetics. *J Am Chem Soc* 124:12092–12093
37. Westerlund F, Wilhelmsson LM, Norden B, Lincoln P (2003) Micelle-sequestered dissociation of cationic DNA-intercalated drugs: unexpected surfactant-induced rate enhancement. *J Am Chem Soc* 125:3773–3779