How Does Nanoconfinement within a Reverse Micelle Influence the Interaction of Phenazinium-Based Photosensitizers with DNA?

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Supporting Information

ABSTRACT: The major focus of the present work lies in exploring the influence of nanoconfinement within aerosol-OT (AOT) reverse micelles on the binding interaction of two phenazinium-based photosensitizers, namely, phenosafranin (PSF) and safranin-O (SO), with the DNA duplex. Circular dichroism and dynamic light-scattering studies reveal the condensation of DNA within the reverse micellar interior (transformation of the B-form of native DNA to ψ-form). Our results unveil a remarkable effect of the degree of hydration of the reverse micellar core on the stability of the stacking interaction (intercalation) of the drugs (PSF and SO) into DNA; increasing size of the water nanopool (that is, \( w_0 \)) accompanies decreasing curvature of the DNA duplex structure with the consequent effect of increasing stabilization of the drug:DNA intercalation. The marked differences in the dynamical aspects of the interaction scenario following encapsulation within the reverse micellar core and the subsequent dependence on the size of the water nanopool are also meticulously explored. The differential degrees of steric interactions offered by the drug molecules (presence of methyl substitutions on the planar phenazinium ring in SO) are also found to affect the extent of intercalation of the drugs to DNA. In this context, it is imperative to state that the water pool of the reverse micellar core is often argued to approach bulk-like properties of water with increasing micellar size (typically \( w_0 \geq 10 \)), so that deviation from the bulk water properties is likely to be minimized in large reverse micelles (\( w_0 \geq 10 \)). On the contrary, our results (particularly quantitative elucidation of micropolarity and dynamical aspects of the interaction) explicitly demonstrate that the bulk-like behavior of the nanoconfined water is not truly achieved even in large reverse micelles.

1. INTRODUCTION

The stable storage of crucial genetic information by deoxyribonucleic acid (DNA) is central to the development, sustaining, and functioning of various life forms and many viruses.1−4 Naturally, DNA has navigated the nucleus of a myriad of research activities for years, in particular, the remarkable upsurge of contemporary research on the application of DNA in the recognition of a myriad of life forms and many viruses.5−11 The molecular recognition of DNA in solution is comparatively sporadically addressed in the literature.10,12−15 It is usually argued that the spatial constraints within the reverse micellar nanoconfinements and low dielectric constant of the central water pool play the key roles for condensation of DNA.10,13 The degree of hydration of the nanopool of water entrapped within the reverse micelle is also reported to be crucial in the condensation phenomenon as well as in governing the degree of compactness of DNA15−20

The present study demonstrates the application of AOT reverse micelle as a condensing media to exploit the effect of nanoconfinement on the binding interaction of two phenazine-based DNA intercalator drugs, namely, phenosafranin (PSF, 3,7-diamino-5-phenyl phenazinium chloride) and safranin-O (SO, 3,7-diamino-2,8-dimethyl-5-phenyl phenazinium chloride) (Scheme 1). The photophysics of PSF and SO in various organized media is extensively studied.21−30 The cationic drug molecules PSF and SO are reported to bind to DNA principally by an intercalative mode of binding.21,22 The major focus of the present investigation aims at exploring how the binding interaction of the drugs (PSF and SO) is influenced within the nanoconfinements of reverse micelles. The variation of the binding constant values and polarity surrounding the drug microenvironment as a function of \( w_0 \) within the reverse micelle unveils the modification of the curvature of DNA within the nanocage of the reverse micelles with increasing hydration (that is, \( w_0 \)) conveying the stabilization of the intercalative binding interaction of the drugs with DNA.31 The broad
water. Properties of the water in the nanopool from those of bulk are usually equivalent to those of bulk water.48

Drug molecules within the present experimental window remain bound to well corroborate to the absorption spectral changes upon addition:15,31,41

range of compositions simply as a function of the water

The unique property of reverse micellar systems incorporating a small volume of water inside their nanoscopic core paves the way for multitudes of applications including the study of complex biological and chemical reactions.37–39 The conjugate effects of confinement and interactions with surfactant interface/headgroups lead to remarkable deviations of the properties of the water in the nanopool from those of bulk water.15,31,37–46 Furthermore, the degree of hydration within the reverse micellar interior can be effectively tuned over a wide range of compositions simply as a function of the water added:15,31,41–43,45

\[
\omega_0 = \frac{[H_2O]}{[AOT]}
\]  

This provides an additional edge toward probing the complicated effects of hydration in a given biological process for studies inside the reverse micellar core while keeping from the functional and compositional complexities associated with them. The properties of the nanoconfined water are usually reported to be enormously different from those of bulk water, particularly in small reverse micelles \((\omega_0 < 10)\).15,47,48 On the contrary, the properties of the intramicellar water are argued to resemble those of bulk water with increasing hydration level (that is, \(\omega_0\)), and especially in large reverse micelles \((\omega_0 \geq 10)\) the properties of water in the nanopool become essentially equivalent to those of bulk water.48–50 Interestingly, our results explicitly show that the microenvironments surrounding the drug molecules within the present experimental window remain significantly different from those of bulk water even in large reverse micelles. Particularly, the polarity of the drug microenvironments and the dynamical aspects of the global interaction scenario even in large reverse micelles \((\omega_0 > 10)\) have been demonstrated to be substantially different from those in bulk water.52

2. RESULTS AND DISCUSSIONS

2.1. Probing the Drug:DNA Interaction. 2.1.1. Steady-State Absorption and Fluorescence Spectroscopic Studies. The photophysical properties of the dyes PSF and SO in bulk homogeneous solvents have been extensively characterized in the literature.21–26,28–30,51–57 In bulk aqueous medium PSF and SO show the lowest energy absorption band at \(\sim 520 \text{ nm}\) and a broad unstructured fluorescence band at \(\sim 585 \text{ nm}\) arising from charge transfer emission. The characteristic absorption and fluorescence profiles of the dyes are known to be sensitive to solvent polarity, the presence of external additives, and specific interactions with microheterogeneous assemblies.21–30,51–57 As a result, these dyes have been extensively used as extrinsic molecular probes for a variety of organized media and microheterogeneous assemblies.21–30,51–57

Before moving onto probing the modulation of the drug:DNA interaction within the nanoconfinements of AOT reverse micelles, it is pertinent to unveil the pattern of interaction of the individual drug molecules (PSF and SO) with DNA alone. To this end, the drug:DNA interaction has been first investigated by spectroscopic techniques.

The absorption profiles of PSF and SO show quite similar broad unstructured bands having maxima at \(\sim 520 \text{ nm}\) in bulk aqueous buffer. These results are found to be in good agreement with a large volume of literature reports.23–26,28–30,51–57 The occurrence of drug:DNA interaction is indicated by the decrease in absorbance and significant red-shift of the absorption profile following interaction with DNA (Figure 1).21,22 In this context, it is intriguing to note the varying extents of red-shift for the drugs at the respective saturation levels of interaction, \(\Delta \lambda \sim 17 \text{ nm}\) for PSF and \(\Delta \lambda \sim 13 \text{ nm}\), for SO which readily suggests that the microenvironment in the vicinity of the drug molecules within the DNA scaffolds is not precisely the same (Figure 1).21,22 A lesser extent of spectral modulation with SO compared to that with PSF may be comprehended from the possibility of greater degree of steric interaction with SO (because of the presence of the methyl substitutions on the planar phenazinium nucleus, Scheme 1) which may lead to a lesser degree of intercalation of SO compared to PSF within the DNA duplex. In analogy with reported literature,21,22 the red-shift of the absorption wavelength of the drugs (PSF and SO) within the DNA-bound state can be rationalized based on the modulation (decrease) of the HOMO–LUMO energy gap of the drugs.

The fluorescence profiles of both the drugs (PSF and SO) were displayed by broad, structureless bands having maxima at \(\lambda_{\text{max}} \sim 585 \text{ nm}\) (Figure 1). The occurrence of the drug:DNA interaction is found to be characterized by prominent quenching of fluorescence intensity coupled with a marginal blue-shift \((\Delta \lambda \sim 6 \text{ nm})\) of the fluorescence wavelength (Figure 1). These results corroborate well to the literature reports on the study of interaction of phenazinium drugs with DNA.21,22 The fluorescence excitation spectra of the drugs are also found to well corroborate to the absorption spectral changes upon interaction with DNA (figure not shown) implying that the DNA-induced modulations in the fluorescence profiles of

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Scheme 1. (A) Chemical Structures of PSF and SO and (B) Optimized Structures of PSF and SO (B3LYP/6-31+ +G(d,p)) Showing the Relative Orientation of the Flanking Benzene Rings with Respect to the Phenazinium Nuclei

\(\text{PSF} \quad \text{SO}\)

Front view Side view

Color scheme: hydrogen atom: white, carbon atom: gray, nitrogen atom: blue.
drugs originate from photoexcitation of the respective ground-state species. 21,22,59

In this context, it is important to establish the mode of binding of the drugs with the DNA duplex. For this purpose, the circular dichroism spectroscopic technique has been exploited here. The intrinsic far-UV circular dichroic profile of DNA shows a positive peak at ~276 nm and a negative peak at ~247 nm, typically signifying the hallmark of the native right-handed B-form of DNA 21,22,59–61 (Figure S1 of Supporting Information). The characteristic CD signal of B-DNA is attributed to the asymmetric environments surrounding the DNA bases owing to their stacking interactions and the helical suprastructure of the polynucleotide backbone. Thus, perturbation of the intrinsic CD signal of B-DNA categorically reflects an intercalative mode of binding emanating from alteration of the relative orientation of the DNA bases required to accommodate the intercalated drug(s). On the contrary, an electrostatic or groove binding is manifested by no (or only nominal) perturbation of the intrinsic CD spectrum of DNA as this induces no (or only insignificant) change on the relative orientation of the DNA bases. 21,22,59–61 Consequently, the modulation of the intrinsic CD profile of DNA following interaction with the drugs (PSF and SO) (Figure S1) implies perturbation of the stacking contacts of the DNA bases and hence establishes an intercalative mode of binding. 21,22,59–61

2.1.2. Time-Resolved Fluorescence Decay Studies. Despite the spectroscopic characterization of the drug-DNA interaction with PSF and SO, the dynamical aspects of the interaction have not been previously addressed in the literature. 60–64 Herein, the modulations in fluorescence decay behavior of the drugs (PSF and SO) following interaction with DNA are meticulously explored. In aqueous buffer, both the drugs PSF and SO are found to exhibit single exponential decay (λex = 450 nm and λem = 585 nm) having characteristic lifetimes of τ1 ~ 868 ps and τ2 ~ 1020 ps, respectively. 13,59,60,65 The variations of the fluorescence transients of the drugs with added DNA are displayed in Figure 2 with the deconvoluted data being presented in Table 1. A glimpse on the tabulated data reflects a complicated decay pattern of the drugs within the DNA scaffolds which in turn requires a triexponential decay function for an adequate description of the data (Table 1). Apart from the characteristic lifetime of the free (or unbound) drugs (τ1 in Table 1), the interaction of the drugs with DNA results in the genesis of another slow (τ3) and an ultrafast (τ2) decay component (Table 1). The pattern of modulation of the fluorescence decay behavior of the drug molecules (PSF and SO) with added DNA is found to be consistent with the decay behavior of the drugs in several other microheterogeneous environments as studied in our lab (unpublished results). In both the cases (PSF and SO), the amplitudes (α1) corresponding to the lifetime of the free drug (τ1) gradually decreases, whereas the amplitudes (α2 and α3) of the newly generated ultrafast (τ2) and relatively slow (τ3) decay components increase with added DNA (Table 1). Thus, the occurrence of the drug-DNA interaction can be argued on the basis of a steady increase (decrease) of the amplitudes corresponding to the characteristic lifetimes of the bound (unbound) drugs with increasing DNA concentration. It is

Table 1. Time-Resolved Fluorescence Decay Parameters of PSF and SO Following Interaction with DNA

| [DNA] (μM) | τ1 (ps) | τ2 (ps) | τ3 (ns) | α1 | α2 | α3 | χ2 |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Interaction of PSF with DNA | | | | | | | |
| 0 | 868 | - | 1.00 | - | - | 1.05 |
| 6.1 | 903 | 469 | 1.12 | 0.88 | 0.01 | 0.11 | 1.07 |
| 15.2 | 865 | 146 | 1.26 | 0.81 | 0.02 | 0.17 | 1.08 |
| 30.4 | 814 | 185 | 2.05 | 0.73 | 0.03 | 0.19 | 1.04 |
| 60.8 | 850 | 177 | 2.40 | 0.67 | 0.03 | 0.29 | 1.08 |
| 91.2 | 852 | 178 | 2.41 | 0.62 | 0.06 | 0.32 | 1.07 |
| 152 | 921 | 209 | 2.56 | 0.54 | 0.07 | 0.39 | 1.06 |
| Interaction of SO with DNA | | | | | | | |
| 0 | 1020 | - | 1.00 | - | - | 1.05 |
| 6.1 | 899 | 116 | 1.49 | 0.85 | 0.03 | 0.12 | 1.08 |
| 15.2 | 984 | 118 | 1.74 | 0.74 | 0.11 | 0.15 | 1.06 |
| 30.4 | 1090 | 127 | 2.19 | 0.69 | 0.12 | 0.19 | 1.06 |
| 60.8 | 1040 | 150 | 2.21 | 0.65 | 0.11 | 0.24 | 1.05 |
| 91.2 | 1150 | 161 | 2.50 | 0.61 | 0.13 | 0.26 | 1.07 |
| 152 | 1240 | 175 | 2.52 | 0.60 | 0.12 | 0.28 | 1.08 |

±4%.
noteworthy at this juncture that the increase of population of the bound drugs (as estimated from the magnitudes of the corresponding amplitudes, Table 1) with added DNA is relatively greater in the case of PSF than SO which essentially reveals a greater extent of intercalation of PSF than SO. In conformity with the steady-state results stated earlier, a greater extent of intercalative interaction of PSF than SO can be intertwined with the greater degree of steric repulsion present in SO because of the methyl substitutions on the planar phenazinium ring (Scheme 1).

2.2. Characterization of DNA Conformation within Reverse Micellar Nanoconfinement. 2.2.1. Circular Dichroism Spectroscopy. The circular dichroism (CD) spectral features of the native B-form of DNA. In contrast, the CD profiles of DNA within the reverse micelles (at \( w_0 = 4 \) and 16) exhibit an increase in molar ellipticity values at the negative as well as positive wavelengths coupled with significant absorption at the longer wavelength tail (Figure 3), thus conforming to the transition of B-DNA in aqueous buffer to the \( \psi' \)-form of DNA within the nanoscopic environment of the reverse micelles.\(^{11,14,22,61,66}\)

2.2.2. Dynamic Light Scattering. Figure 4 shows a representative example of the DLS profiles of AOT reverse micelle with \( w_0 = 20 \) in the absence and presence of added DNA. The DLS profile of the reverse micelle (\( w_0 = 20 \)) without DNA exhibits a fairly monomodal distribution having hydrodynamic diameter \( d_h = 9 \pm 1 \) nm (polydispersity index = 11%) bearing reasonable resemblance with reported literature.\(^{10,12,15,31}\) The scattered intensity distribution profile of the reverse micelle containing DNA conspicuously reflects the presence of large-sized containing DNA molecules (DNA containing reverse micelles having \( d_h = 145 \pm 17 \) nm (polydispersity index = 12%)) in addition to the smaller ones (that is, empty reverse micelles).\(^{10,12,15,31}\) The presence of large reverse micelles containing encapsulated DNA (\( d_h \sim 145 \) nm) has also been confirmed with AOT reverse micelles (\([\text{AOT}] = 0.1 \) M) at various degrees of hydration (that is, \( w_0 = 10, 12, 16 \)) (results not shown). It is imperative to note that though the size of the large reverse micelles containing DNA is in good harmony with reported literature\(^{15,12,15,31}\) the DLS study may not be adequate in determining the actual morphology of these reverse micelles because the reverse micellar particles containing DNA may not be truly spherical.\(^{12,15}\) The central water pool of the AOT reverse micelle should be rich in negative charge density due to the anionic headgroup of the surfactant, which in turn is likely to govern the distribution of DNA having highly negative polyphosphate backbone within the intra (reverse) micellar nanopool of water rather than the headgroup or interfacial micellar region and thus accompanying the transformation of the B-form of DNA into its condensed \( \psi' \)-form (as evidenced from the CD spectral results discussed above).

2.3. Drug:DNA Complex within the AOT Reverse Micelle. 2.3.1. Steady-State Spectroscopic Studies. Extensive broadening of the absorption profile of drug:DNA complexes within AOT reverse micelles of varying \( w_0 \) prohibited the extraction of meaningful results with required degree of precision. However, the excitation spectra produce relatively sharper profiles, thus allowing a precise perusal of the modulation of the spectral properties. Nevertheless, it is imperative to note in this context that the study of the interaction of the drug:DNA complex within the nanocompartmentalization of AOT reverse micelles remains susceptible to significant experimental artifacts emanating from the possibility of partitioning of the drug molecules in various microheterogeneous environments.\(^{15}\) Thus, intensive care has been taken for rationalization of the spectral properties in this concern. To this end, our first attempt involves the use of a relatively high concentration of DNA in comparison to the drug concentration \(([\text{drug}]:[\text{DNA}] = 1:28, \text{molar ratio})\) in order to ensure complete complexation.

The excitation spectra of the drug:DNA complex in AOT reverse micelle with increasing \( w_0 \) display a gradual decrease in intensity with a small blue shift (\( \Delta \lambda \sim 4 \) nm) (Figure 5A). It is worth mentioning that these results apparently contrast the modulation of absorption spectral behavior of the drug molecules alone within AOT reverse micelles (which shows a significant red-shift of \( \sim 10 \) nm following encapsulation within the reverse micellar nanocage, which is rather insensitive to the variation of the water pool size, that is, variation of \( w_0 \)).\(^{65}\) Thus, an effective entrapment of the drug:DNA complexes within the reverse micellar nanopool of water can be logically assumed.

The characteristic broad, unstructured fluorescence profiles of the phenazinium dyes (PSF and SO) in bulk aqueous buffer...
having maxima at ~585 nm undergo prominent fluorescence quenching coupled with a slight blue-shift upon interaction with DNA ($\lambda_{em} \sim 581$ nm) (Figure 1). However, encapsulation of the drug:DNA complex within the AOT reverse micelle ($w_0 = 1$) is characterized by a marked blue-shift ($\lambda_{em} \sim 562$ nm) as depicted in Figure 5B. With increasing size of the nanopool of compartmentalized water within the AOT reverse micelle the fluorescence profile of the drug:DNA complex is found to undergo moderate reduction of fluorescence intensity together with a small red-shift of the fluorescence wavelength ($\lambda_{em} \sim 570$ nm at $w_0 = 20$) (Figure 5B). This can be rationalized based on the notion of increasing polarity surrounding the micropolarity of the drugs with increasing degree of hydration, that is, increasing $w_0$. Furthermore, it is known that the interfacial fluidity of the interacting reverse micelles increases with increasing nanopool size (that is, $w_0$).41–44 This can be argued to reflect an increasing degree of stabilization of the stacking (intercalation) interaction of the drugs (PSF and SO) into the DNA duplex with decreasing DNA curvature accompanying increasing hydration of the reverse micelle (that is, increasing $w_0$).15,31 However, the significant deviation of the fluorescence spectral characteristics of the drug:DNA complex within the reverse micelles from that in bulk aqueous buffer or within the DNA scaffolds reflects that the microenvironment surrounding the DNA-bound drugs is remarkably different within the reverse micelles even at high $w_0$ (that is, large reverse micelles).

2.3.2. Polarity of the Drug Microenvironment. A quantitative estimation of the polarity surrounding the drug microenvironment under various experimental conditions has been elucidated on the basis of polarity-sensitive fluorescence properties of the drugs (PSF and SO). The characteristic fluorescence maxima of the drugs (PSF and SO) are found to display a significant blue-shift with decreasing medium polarity, e.g., $\lambda_{em} \sim 585$ nm in bulk aqueous medium and $\lambda_{em} \sim 564$ nm in 1,4-dioxane (Figure S2). Drawing on this, a calibration curve is constructed by observing the variation of the characteristic fluorescence wavelength of the drugs (PSF and SO) in a reference solvent mixture of water/1,4-dioxane of varying compositions having known polarity (on $E_T(30)$ scale).37 The polarity surrounding the drug microenvironment under the as-employed experimental conditions is then evaluated by the method of interpolation on the calibration curve (a detailed description of the results and experimental protocol is provided in the Supporting Information).

Figure 6 displays the variation of micropolarity surrounding the drug as a function of $w_0$ when the drug:DNA complex is encapsulated within the reverse micellar core. A steady increase of polarity (on $E_T(30)$ scale) of the drug microenvironment with increasing $w_0$ ($E_T(30)$ (kcal mol$^{-1}$) = 47.1 at $w_0 = 1$ and 53.06 at $w_0 = 20$ for DNA-bound PSF; $E_T(30)$ (kcal mol$^{-1}$) = 49.4 at $w_0 = 1$ and 53.8 at $w_0 = 20$ for DNA-bound SO) is found to be consistent with the notion of increasing polarity with increasing water content (increasing $w_0$) of the reverse micellar water pool and thus accounts for the gradual red-shift of the fluorescence wavelength of the drug:DNA complex within the reverse micelle with increasing $w_0$. It is usually argued that the water molecules near the interface can be engaged in hydration of the anionic AOT headgroups, in sodium counterions, or in interaction with other water molecules.41–44 The water molecules at the interface perturbed by interactions with surfactant headgroups or sodium counterions continuously equilibrate with bulk-like water molecules present in the reverse micellar interior.12 Thus, it is not surprising that a polarity-sensitive probe molecule (here PSF and SO) will sense a lower polarity of the central water pool in small reverse micelles (that is, small $w_0$). However, in large reverse micelles, typically $w_0 \geq 10$, with increasing water content of the central pool the intramicellar water is often argued to resemble bulk-like properties of water.13,45,47,49,68 It is crucial to note that the micropolarity of the DNA-bound drugs within the reverse micelle-encapsulated state even at the highest $w_0$ studied, that is, $w_0 = 20$, still remains notably lower (more hydrophobic environment; $E_T(30)$ (kcal mol$^{-1}$) = 53.06 at $w_0 = 20$ for DNA-bound PSF; $E_T(30)$ (kcal mol$^{-1}$) = 53.8 at $w_0 = 20$ for DNA-bound SO) compared to that in the drug:DNA complex in the bulk aqueous buffer phase ($E_T(30)$ (kcal mol$^{-1}$) = 61.23 for PSF and SO; Figure S2). Our results thus comprehensively suggest that even in large reverse micelles ($w_0 \geq 10$) the properties of the nanopool of water do not typically approach the bulk-like properties. This is consistent with a recent simulation study which shows a marked reduction of the dielectric constant of water upon confinement.69

Furthermore, the data presented in Figure 6 reveal that the polarity surrounding microworlds of the DNA-bound drugs at any given level of hydration of the reverse micelle (that is, a given $w_0$) is relatively lower (more hydrophobic) for PSF compared to that for SO. In corroboration to our earlier results,
a lesser degree of intercalation of SO into the DNA arising from the greater degree of steric hindrance due to the presence of methyl substitutions on the planar phenezinium ring (Scheme 1) can be argued to provide a reasonable rationale underlying the aforesaid variation of polarity.

2.3.3. Time-Resolved Fluorescence Decay: Determination of Drug:DNA Binding Constant. The modulation of fluorescence decay transients of the drug:DNA complex entrapped in the AOT reverse micellar systems as a function of \( w_0 \) is presented in Figure 7, and the relevant data are assembled in Table 2. At this stage, it is imperative to note that the fluorescence decay behavior of the drugs alone (PSF and SO) in AOT reverse micelles remarkably contrasts the present results; the excited-state fluorescence lifetime of the AOT reverse micelle-encapsulated drugs (PSF and SO) exhibits a monoexponential decay with a moderate decrease of lifetime with increasing \( w_0 \). On the contrary, the pattern of modulation of the fluorescence decay parameters of the drug:DNA complex with increasing \( w_0 \) within the reverse micelle-encapsulated state is found to bear reasonable harmony with the results obtained in the course of binding interaction of the drugs with DNA. The data summarized in Table 2 reveal that a triexponential decay function is required to aptly describe the data. In analogy to the data for binding interaction of the drugs (PSF and SO) with DNA as stated earlier (section 3.1.2, Table 1), the decay constant \( \tau_3 \) can be ascribed to the free drug, while the occurrence of the drug:DNA binding interaction is associated with the appearance of a relatively slow (\( \tau_1 \) and an ultrafast (\( \tau_2 \)) decay component (Table 2). Thus, a careful perusal of the decay parameters (Table 2) enabled the determination of a legitimate estimate of the variation of the fraction of DNA-bound drug within AOT reverse micelles with increasing \( w_0 \). A progressive increase of the population of the bound drugs (as estimated from the gradual decrease of the relative amplitude, \( \alpha_i \), corresponding to the free drug, Table 2) with increasing \( w_0 \) is found to be in good agreement with our earlier results and thus reflects an enhanced stabilization of the intercalative interaction of the drugs with DNA with increasing nanopool size, that is, \( w_0 \). In general, a steady increase of the fluorescence lifetime values (Table 2) with incremental addition of DNA suggests the impartation of increasing degree of motional restrictions on the drug molecules. The data compiled in Table 2 further show that the relative population of the DNA-bound drug at a given \( w_0 \) within the reverse micelles is greater for PSF in comparison to SO (as estimated from the relative magnitudes of the amplitude, \( \alpha_i \), corresponding to the free drug (Table 2)). This is consistent with the notion that stabilization of the intercalative interaction of the drugs occurs to a greater extent with PSF compared to SO, which in turn can be rationalized on the basis of the greater degree of steric interaction available with SO because of the presence of methyl substitutions on the planar phenezinium nucleus (Scheme 1; such steric interaction may impede the stacking interaction of the cationic dyes within the DNA base pairs).

Herein, the variation of the fluorescence decay characteristics of the drug:DNA complex encapsulated in AOT reverse micelles as a function of \( w_0 \) is further processed toward an endeavor to elucidate a relative estimate of the variation of the drug:DNA binding constant (\( K \)). Knowing the total concentrations of the drugs and DNA (as determined from their respective molar absorption coefficient values, Section 2) and the signature of the relative population of the drug:DNA complex obtained from analysis of the decay parameters, the binding constant (\( K \)) values are estimated using the following equation:

\[
K = \frac{[\text{drug}:\text{DNA}]}{([\text{drug}] - [\text{drug}:\text{DNA}]) \times ([\text{DNA}] - [\text{drug}:\text{DNA}])}
\]

where the terms within square brackets indicate the molar concentrations of the respective species. The variation of the binding constant values as a function of \( w_0 \) (Figure 8) unveils a discernible increase with increasing water loading (that is, \( w_0 \)) of the interacting reverse micelles. This is consistent with the decreasing curvature of DNA within the nanocage of the reverse micelles with increasing hydration (that is, \( w_0 \)), leading to the resultant effect of increasing degree of stabilization of the intercalative binding interaction of the drugs with DNA.

Furthermore, a comparatively higher binding constant (\( K \)) for PSF:DNA interaction than that for SO:DNA interaction at a given \( w_0 \) within the reverse micelle is in parity with the lesser extent of intercalation of SO into the DNA duplex owing to the presence of greater degree of steric hindrance available with SO.

![Figure 7. Modulation of fluorescence decay transients (\( \lambda_{ex} = 450 \text{ nm} \)) of (A) DNA-bound PSF and (B) DNA-bound SO within AOT reverse micelle with variation of \( w_0 \). Curves i \( \rightarrow \) vii represent \( w_0 = 1, 2, 5, 10, 12, 16, \) and 20. The scattered symbols represent the raw data, and the best fit lines are shown by the solid lines. The sharp black profile at the extreme left denotes the instrument response function (IRF).](image-url)

Table 2. Time-Resolved Fluorescence Decay Parameters of DNA-Bound Drugs within the AOT Reverse Micelle at Various \( w_0 \)

| \( w_0 \) | \( \tau_1^w (\text{ns}) \) | \( \tau_2^w (\text{ps}) \) | \( \tau_3^w (\text{ns}) \) | \( \alpha_1 \) | \( \alpha_2 \) | \( \alpha_3 \) | \( \chi^2 \) |
|---|---|---|---|---|---|---|---|
| DNA-Bound PSF in AOT Reverse Micelle |
| 1 | 1.19 | 219 | 3.07 | 0.48 | 0.10 | 0.42 | 1.08 |
| 2 | 1.71 | 312 | 3.11 | 0.47 | 0.08 | 0.45 | 1.09 |
| 5 | 1.78 | 412 | 3.12 | 0.39 | 0.09 | 0.52 | 1.06 |
| 10 | 1.85 | 515 | 3.15 | 0.35 | 0.10 | 0.55 | 1.08 |
| 12 | 1.87 | 621 | 3.24 | 0.31 | 0.12 | 0.57 | 1.09 |
| 16 | 1.90 | 889 | 3.31 | 0.29 | 0.12 | 0.59 | 1.05 |
| 20 | 1.95 | 995 | 3.71 | 0.26 | 0.13 | 0.61 | 1.07 |
| DNA-Bound SO in AOT Reverse Micelle |
| 1 | 1.72 | 146 | 3.65 | 0.58 | 0.13 | 0.29 | 1.09 |
| 2 | 2.12 | 264 | 3.35 | 0.55 | 0.04 | 0.41 | 1.06 |
| 5 | 2.14 | 268 | 4.29 | 0.50 | 0.03 | 0.47 | 1.08 |
| 10 | 2.20 | 477 | 3.49 | 0.41 | 0.08 | 0.51 | 1.07 |
| 12 | 2.38 | 686 | 3.9 | 0.34 | 0.13 | 0.53 | 1.09 |
| 16 | 2.53 | 796 | 3.03 | 0.32 | 0.13 | 0.55 | 1.07 |
| 20 | 2.68 | 897 | 3.85 | 0.31 | 0.12 | 0.57 | 1.04 |

\( \alpha \pm 4\% \)
because of the methyl substitutions present on the planar phenazinium nucleus (Scheme 1).

2.3.4. Rotational Relaxation Dynamics. a. Rotational Relaxation Dynamics of the Drugs within the DNA-Intercalated State. The study of rotational relaxation dynamics of the drugs within the DNA-intercalated state can provide critical insights regarding the interaction behavior of the drugs with DNA. To this end, the time-resolved fluorescence anisotropy decay transients of the drugs (PSF and SO) have been meticulously explored in bulk aqueous buffer as well as within the DNA scaffolds (Figure 9A). In bulk aqueous buffer, both the drug molecules are found to exhibit monoexponential anisotropy decay profiles having rotational correlation time of $\tau_r = 147 \pm 6$ ps for PSF and $\tau_r = 179 \pm 7$ ps for SO. A monoexponential anisotropy decay pattern is suggestive of a homogeneous environment surrounding the drug molecules in aqueous buffer.\textsuperscript{58,70–72} The lack of any significant residual anisotropy (Figure 9A) further suggests that the rotational depolarization of the drugs is essentially completed within the excited-state fluorescence lifetime, as is also evidenced from the relatively slower lifetimes of the drugs in aqueous buffer (PSF: $\tau_i = 868 \pm 35$ ps, SO: $\tau_i = 1020 \pm 41$ ps, Table 2).

The fluorescence depolarization profiles of the drugs (PSF and SO) are found to be remarkably modified in the presence of DNA, signifying the occurrence of the drug:DNA interaction (Figure 8A). However, it is imperative to note that the anisotropy decay transients of the DNA-bound drugs could not be aptly described by a simple monoexponential or biexponential decay function; rather the decay transients are found to produce a weak signature of dip-and-rise anisotropy decay profile, which is also designated as associated anisotropy decay (Figure 9A).\textsuperscript{58,70–72} In general, the following observations are noted regarding the modulation of anisotropy decay behavior of the drugs following interaction with DNA: (i) with incremental addition of DNA the dip-and-rise anisotropy decay pattern (although weak) becomes relatively more prominent, and (ii) at a given concentration of DNA, the dip-and-rise anisotropy decay pattern is comparatively more prominent with PSF than SO (Figure 9A).

The glimpse of a dip-and-rise anisotropy decay pattern for DNA-bound drugs reflects the spectroscopic signature for the coexistence of at least two classes of population of fluorophores having significantly different fluorescence lifetimes and rotational correlation times.\textsuperscript{58,70–72} Usually, the origin of such anisotropy decay profiles is associated with partial encapsulation of the fluorophore molecule(s) with the ensuing effect of a slow fluorescence lifetime and rotational correlation time emanating from the bound part of the fluorophore, while on the contrary, the unbound (or solvent exposed) functional moieties of the fluorophore accounts for the ultrafast motions.\textsuperscript{58,70–72} According to literature reports,\textsuperscript{58,70–72} an associated exponential model can be exploited to describe such dip-and-rise anisotropy decay, in which the fluorescence depolarization parameters are coupled with the excited-state fluorescence decay parameters through the following relationships

\begin{equation}
    r(t) = \sum_{i=1}^{n} f_i(t) \gamma_i(t)
\end{equation}

where the time-dependent weighting factor is described as

\begin{equation}
    f_i(t) = \alpha_i \exp\left(-t/\tau_i\right)
\end{equation}

and the total fluorescence intensity decay is given as

\begin{equation}
    I_T(t) = \sum_{i=1}^{n} \alpha_i \exp\left(-t/\tau_i\right)
\end{equation}

and the time-dependent anisotropy decay profile is then described as

\begin{equation}
    \gamma_i(t) = (\gamma_{0,i} - \gamma_{\infty,i}) \exp\left(-t/\tau_r\right) + \gamma_{\infty,i}
\end{equation}

The terms $\alpha_i$ and $\tau_i$ designate the corresponding parameters for the fluorescence decay of the fluorophore (amplitude and lifetime, respectively); $\tau_r$ represents the rotational relaxation time constant; and $\gamma_{0,i}$ is the pre-exponential anisotropy (or fundamental anisotropy).\textsuperscript{58,70–72} The coexistence of two remarkably different fluorescence decay time constants is argued to be crucial to underlying the origin of such dip-and-rise anisotropy decay behavior, which rightfully rationalizes the importance on the time-dependent weighting factor ($f_i(t)$) as stated above in...
eqs 3 and 4,60,70–72 Evidently, the modulation of the fluorescence decay behavior of the drugs (PSF and SO) within the DNA-intercalated state is found to conform to this essential prerequisite as shown in Table 2. A monoexponential decay behavior of the drugs in bulk aqueous buffer implies an essentially homogeneous environment surrounding the drug molecules. However, the marked modulation of the fluorescence decay behavior of the drugs within the DNA scaffolds accompanies the genesis of an ultrafast ($\tau_2$) and a slow ($\tau_1$) decay component having considerably different relative amplitudes ($\alpha_2$ and $\alpha_1$) (Table 2). The differential fluorophore populations (justifying the faster and slower decay components due to the solvent-exposed functional moieties of the fluorophore and the embedded part, respectively) leading to varying contributions to the total fluorescence decay ($I(t)$), in conjunction with their variable local mobilities, are argued to result in the dip-and-rise fluorescence depolarization profile.63

Herein, the necessity of introducing the "pseudorise" component in the fitting analysis of the anisotropy decay transients is cross-validated by a comparative scrutiny of the distribution of the weighted residuals and the "goodness of fit" parameters as obtained for various functions used for the fitting analyses (Figure S4 in the Supporting Information).71

In this context, a careful perusal of the structural features of the drug molecules (PSF and SO) appears to provide a critical insight into the rotational dynamical behavior in parity with the aforementioned discussion. The optimized geometries of the drugs (PSF and SO, Scheme 1) reveal that the drug molecules are not fully planar; the flanking benzene nucleus is found to be slightly out-of-plane with respect to the planar phenazine rings in PSF and SO. Such geometric landscape of the drug molecules provides a critical understanding in deciphering the mode of binding of the drugs with DNA in the sense that the planar phenazine ring of the cationic drugs is suitably intercalated within the DNA base pairs accounting for the embedded part of the fluorophore, while the contiguous benzene nuclei accounts for the solvent-exposed part which in turn leads to the ultrafast decay component found in the overall fluorescence decay profile of the DNA-bound drugs (Table 2). Moreover, the general observation that the dip-and-rise anisotropy decay pattern is comparatively more prominent with PSF than SO at a given concentration of DNA (Figure 9A) can also be argued to be consistent with the interpretation of a lesser extent of intercalation of SO as compared to PSF, as stated earlier on the basis of a greater degree of steric bulk available with SO because of the methyl substitutions present on the planar phenazine ring.

b. Rotational Relaxation Dynamics of the Drug:DNA Complex within the Nanocage of Reverse Micelles. Figure 9B illustrates the time-resolved fluorescence anisotropy decay transients of the drug:DNA complexes entrapped within the nanocage of the reverse micelles as a function of $w_0$. The deconvoluted decay parameters are summarized in Table S1. In this context, it is noteworthy that the fluorescence anisotropy decay transients of the drug:DNA complexes within the reverse micelle-encapsulated state are markedly different from those of the drugs in bulk aqueous buffer phase and/or DNA-intercalated state (Figure 9A). The encapsulation of the drug:DNA complexes within the nanoscope of the reverse micelles leads to a biexponential anisotropy decay pattern (Figure 9B, Table S1 in Supporting Information) comprised of a major ultrafast component ($\tau_1$) along with a minor contribution from a relatively slower decay component ($\tau_2$) (Table S1). The functional form anisotropy decay is described as

$$r(t) = r_0 \sum_i \alpha_i \exp(-t/\tau_i)$$

and the average rotational correlation time is calculated from the following relationship

$$\langle \tau \rangle = \alpha_1 \tau_1 + \alpha_2 \tau_2$$

where the $\alpha$ terms represents the relative amplitudes of the corresponding rotational correlation time constants ($\tau_i$).

At this point, it is important to underscore that the anisotropy decay profiles of the drugs (PSF and SO) alone in reverse micelles show significantly different behavior in terms of a monoexponential decay profile with gradually decreasing rotational correlation time with increasing $w_0$.65 Herein, a progressive increase of the average rotational relaxation time ($\langle \tau \rangle$, Table S1) of the drug:DNA complex within the AOT reverse micelles with increasing hydration (that is, $w_0$) implies the impartation of increasing degree of motional constraints on the drug molecules as a function of $w_0$. This finding reasonably corroborates our earlier results in the sense that an increasing hydration (that is, $w_0$) of the reverse micelles accompanies greater degree of stabilization of the intercalative interaction of the drugs with DNA.

Initially, the observation of a biexponential anisotropy decay having two rotational relaxation time constants on markedly different time scales appears to invoke the argument of contributions from bound and unbound fractions of the total fluorophore population accounting for the slow and fast decay components, respectively. However, this would naturally entail the relative amplitudes ($\alpha_{1r}$ and $\alpha_{2r}$) of the fast ($\tau_{1r}$) and slow ($\tau_{2r}$) components to reflect the relative populations of the unbound and bound fluorophores, respectively. Within the present experimental window, the drug:DNA molar ratio is maintained at [drug]:[DNA] = 1:28 in order to ensure a complete complexation naturally and does not conform to the magnitudes of the relative amplitudes ($\alpha_{1r}$ and $\alpha_{2r}$, Table S1) and thus leads to negating this proposition.

Based on numerous literature reports,68–60,67–78 we have resorted to the application of the wobbling in cone model for a fruitful rationalization of such fluorescence anisotropy decay patterns. This model describes the global fluorescence depolarization of a fluorophore encapsulated within a macro-molecular system by interplay of various motions treated independently:68–60,67–78

(i) wobbling motion of the fluorophore having characteristic time constants, $\tau_w$

(ii) translational motion of the fluorophore along the surface of the microheterogeneous system/aggregate having characteristic time constant, $\tau_{tr}$, and

(iii) overall rotational dynamics of the macromolecular system/aggregate having characteristic time constant, $\tau_p$.

At the outset, the involvement of more than one type of rotational motions can simply be invoked to rationalize the deviation of the anisotropy decay profiles of drug:DNA complex within the reverse micellar encapsulated state from a monoexponential pattern. The overall wobbling motion ($\tau_w$) of the fluorophore is connected to the individual fast ($\tau_{1r}$) and slow ($\tau_{2r}$) anisotropy decay components as follows:68–60,67–78

$$\tau_w = \tau_{1r} + \tau_{2r}$$

$$\tau_p = \frac{\tau_{1r} \tau_w}{\tau_{1r} + \tau_{2r}}$$

$$\tau_{tr} = \frac{\tau_{1r} \tau_{2r}}{\tau_{1r} + \tau_{2r}}$$

Thus, the overall rotational relaxation time ($\tau_p$) in the presence of wobbling motion ($\tau_w$) and translational motion ($\tau_{tr}$) can be expressed as

$$\tau_p = \tau_{1r} + \tau_{2r} = \frac{\tau_{1r} \tau_{2r}}{\tau_{1r} + \tau_{2r}}$$

$$\tau_p = \frac{\tau_{1r} \tau_{2r}}{\tau_{1r} + \tau_{2r}} + \tau_{tr}$$

The above representation is based on the assumption that the rotational relaxation time ($\tau_{1r}$) and translational motion ($\tau_{tr}$) are independent of each other. However, in the case of a fluorophore embedded within a macromolecular system, these motions are coupled, and the overall rotational relaxation time ($\tau_p$) can be expressed as

$$\tau_p = \frac{\tau_{1r} \tau_{2r}}{\tau_{1r} + \tau_{2r}} + \tau_{tr}$$

$$\tau_p = \frac{\tau_{1r} \tau_{2r}}{\tau_{1r} + \tau_{2r}}$$

Thus, the overall rotational relaxation time ($\tau_p$) in the presence of wobbling motion ($\tau_w$) and translational motion ($\tau_{tr}$) can be expressed as

$$\tau_p = \frac{\tau_{1r} \tau_{2r}}{\tau_{1r} + \tau_{2r}} + \tau_{tr}$$

$$\tau_p = \frac{\tau_{1r} \tau_{2r}}{\tau_{1r} + \tau_{2r}}$$

Thus, the overall rotational relaxation time ($\tau_p$) in the presence of wobbling motion ($\tau_w$) and translational motion ($\tau_{tr}$) can be expressed as

$$\tau_p = \frac{\tau_{1r} \tau_{2r}}{\tau_{1r} + \tau_{2r}} + \tau_{tr}$$

$$\tau_p = \frac{\tau_{1r} \tau_{2r}}{\tau_{1r} + \tau_{2r}}$$

Thus, the overall rotational relaxation time ($\tau_p$) in the presence of wobbling motion ($\tau_w$) and translational motion ($\tau_{tr}$) can be expressed as

$$\tau_p = \frac{\tau_{1r} \tau_{2r}}{\tau_{1r} + \tau_{2r}} + \tau_{tr}$$
\[
\frac{1}{\tau_w} = \frac{1}{\tau_{\text{fast}}} - \frac{1}{\tau_{\text{slow}}} = \frac{1}{\tau_{\text{fl}}} - \frac{1}{\tau_{\text{st}}} \tag{9}
\]

According to the wobbling in cone model the rotational motion of the drug molecules can be characterized by a single (isotropic) diffusion coefficient \(D_w\) within a cone of semiangle \(\theta_{w}\) however, the diffusion motion is energetically prohibited to penetrate the rest of the space. This restricts the limiting potential describing the rotational motion by \(\theta_{w} = \theta_{\text{max}}\). Thus, according to this model, the faster rotational relaxation \(\tau_{fl}\) is essentially treated as the wobbling motion of a restricted rotor having transition dipoles undergoing orientational relaxation in a cone of semiangle \(\theta_{w}\). The slower rotational relaxation \(\tau_{st}\), however, accounts for the global orientational diffusion which subsequently leads to orientational randomization (that is, orientational diffusion under no angular restriction). The geometric restraint governing the rotational motion of the fluorophore within the conical volume of semiangle \(\theta_{w}\) is linked to the generalized order parameter \(S\) through the following relationship:

\[
S = \frac{1}{2} \cos \theta_{w}(1 + \cos \theta_{w}) \tag{10}
\]

The physical significance of the order parameter \(S\) can be realized in terms of revelation of the degree of spatial restriction imposed on the drug molecules within the limit:

\[
0 \leq S \leq 1 \tag{11}
\]

where a complete restriction on the rotational motion is designated by \(S = 1\) and an unrestricted motion by \(S = 0\).

The rotational decay parameters calculated from the analysis based on the wobbling in cone model are assembled in Table S1. A gradual increase of the order parameter \(S\) with increasing \(w_0\) is consistent with the idea of impartation of increasing degree of motional restrictions on the rotational degrees of freedom of the drug molecules. This is further substantiated from the progressive decrease of the semiangle \(\theta_{w}\) with increasing \(w_0\) (Table S1). These results corroborate well to the variation (increasing) of average rotational time of the reverse micelle-encapsulated drug:DNA complex with increasing \(w_0\).

The wobbling diffusion coefficient \(D_w\) values are evaluated using the following relationship:

\[
D_w = \frac{7\theta_{w}^2}{24\tau_{w}}, \quad \text{for } \theta_{w} \leq 30^\circ \tag{12}
\]

and for \(\theta_{w} > 30^\circ\, D_w\) is given as

\[
D_w = \left\{ (1 - S^2)\tau_{w} \right\}^{-1} \left\{ \frac{x^3(1 + x^2)}{2(x - 1)} \ln \left( \frac{1 + x}{2} \right) + \left( \frac{1 - x}{2} \right) \right\}
+ \left( \frac{1 - x}{24} \right) (6 + 8x - x^2 - 12x^3 - 7x^4) \tag{13}
\]

where \(x = \cos \theta_{w}\).

As exemplified by the data presented in Table S1, the semiangle \(\theta_{w} < 30^\circ\) in the present case of study, thus eq 13 is used for calculation of \(D_w\).

Usually, a faster water relaxation dynamics within reverse micelles with increasing \(w_0\) results in lowering of fluorescence anisotropy of the reverse micelle-encapsulated drug molecules as the water pool size (that is, \(w_0\)) increases. However, the typically contrasting results obtained with DNA-bound drugs within the reverse micelle even at high \(w_0\) (large reverse micelle) imply that the microenvironments surrounding the drug molecules do not approach bulk-like aqueous environment even in large reverse micelles.

3. CONCLUSIONS

The salient findings of the present investigation can be summarized as follows:

First, the condensation of the DNA duplex structure within the reverse micellar core is found to accompany significant modification of the binding interaction (intercalation) of the phenazinium drugs (PSF and SO) to DNA as compared to that in bulk aqueous buffer phase. The gradual increase of the drug:DNA binding constant values as a function of \(w_0\) reveals a discernible increase with increasing degree of hydration of the nanopool of water (that is, \(w_0\)) of the interacting reverse micelles. This is consistent with the decreasing curvature of DNA within the nanocage of the reverse micelles with increasing \(w_0\), leading to increasing degree of stabilization of the intercalative binding interaction of the drugs with DNA.

Our results also unveil the impact of steric hindrance available with SO due to the presence of methyl substitution on the planar phenazinium ring leading to a lesser extent of intercalation of SO compared to PSF.

Second, a meticulous comparison of the spectroscopic and dynamical aspects of the interactions within the reverse micellar core with those obtained in bulk aqueous buffer conspicuously reveals that the properties of the nanoconfined water in reverse micelles do not typically approach the bulk-like properties even in large reverse micelles (\(w_0 = 10\) or more). This is in apparent contradiction with many literature reports characterizing the water in large reverse micelles (that is, \(w_0 \geq 10\)) as bulk water.

4. EXPERIMENTAL SECTION

4.1. Materials. The drugs Phenosafranin (PSF) and Safranin-O (SO) (Scheme 1), herring sperm DNA, Tris-HCl buffer, and the surfactant sodium bis(2-ethylhexyl) sulfosuccinate (Aerosol-OT, abbreviated as AOT) were used as received from Sigma-Aldrich Chemical Co., USA. UV spectroscopic grade n-heptane and 1,4-dioxane were used as procured from Spectrochem, India. The surfactant (AOT) was extensively dried in vacuum desiccator for no less than 48 h. 0.01 M Tris-HCl buffer of pH 7.4 was prepared in triply distilled deionized Millipore water. All the experiments were performed with only freshly prepared solutions maintaining a low concentration of the drugs ([drug] = 4.0 \(\mu\)M) in order to ensure minimization of inner-filter effects. The concentrations of the drugs and DNA were determined from respective molar absorption coefficient values \(\varepsilon = 33,000\, \text{M}^{-1}\text{cm}^{-1}\) for PSF and \(\varepsilon = 25,000\, \text{M}^{-1}\text{cm}^{-1}\) for SO at \(\lambda = 250\, \text{nm}\). The absorption and steady-state fluorescence spectra were acquired on Hitachi UV–vis U-3501 spectrophotometer and PerkinElmer LS55 fluorometer, respectively. The time-resolved fluorescence decay measurements (lifetime and fluorescence anisotropy measurements) were carried out by the time-correlated single photon counting (TCSPC) method on a FluoroCube-01-NL spectrometer using a laser diode (model: DD-450L-8666, typical fwhm ~170 ps) as the light source for excitation of the...
samples at $\lambda_{ex} = 450$ nm. The signals were collected at the magic angle polarization of 54.7° in order to eliminate the contribution from fluorescence depolarization. Dynamic light-scattering (DLS) measurements were performed on a Malvern Nano-ZS instrument equipped with a thermostated sample chamber (Laser source: 4 mW He–Ne laser having $\lambda = 632.8$ nm, scattering angle = 173°). Each of the fluorescence decay and fluorescence depolarization profiles reported in this work were reproduced for four individual measurements. Detailed description of the experimental protocols and experimental protocols/procedures is given in the Supporting Information.

- ASSOCIATED CONTENT

- Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01820.

Details of the experimental protocols, methods and instrumentation, CD spectra of interaction of the dyes with DNA, estimation of polarity around the drug microenvironment, comparative analysis of deconvolution of the fluorescence depolarization data, table of the time-resolved fluorescence decay parameters (PDF)

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Notes

The authors declare no competing financial interest.

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