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Cyclodextrin induced controlled delivery of a biological photosensitizer from a nanocarrier to DNA

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Pronab Kundu, Saptarshi Ghosh*, Sinjan Das, and Nitin Chattopadhyay*

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In this article, we have addressed to a demanding physicochemical aspect of therapeutic and drug research. We have reported a simple yet prospective technique that can be exploited for the controlled delivery of drugs and/or bioactive small molecules to the most relevant biomolecular target DNA. Exploiting various steady state and time resolved spectroscopic techniques together with DNA helix melting study, we have shown that a biologically significant photosensitizer, namely, phenosafranin (PSF), can be quantitatively transferred to the DNA from the micellar nanocarrier made up of sodium tetradecyl sulfate (STS) using the external stimulant β-cyclodextrin (β-CD). The complexation property of β-CD with the nanocarrier (STS) has been utilized for the controlled release of the probe from the micelle to the DNA. Non-toxicity of the stimulant and noninvasive nature of the carrier towards the target are expected to add to the suitability of this approach from clinical perspective.

Introduction

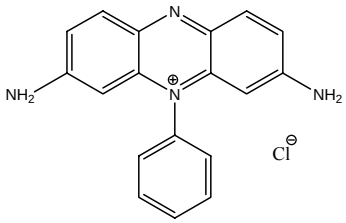
Controlled and quantitative delivery of drugs and/or biologically relevant small molecules to the site of interest is one of the major challenges of the modern day clinical and therapeutic research as it minimizes the drug induced adverse side-effects by increasing the drug efficacy.[1-3] For the purpose, a wide variety of drug delivery systems (DDS) consisting of nanoscale materials like micelles, liposomes, dendrimers, carbon nanotubes etc. have been developed in the recent past.[4-7] DDS enhances the therapeutic efficacy of the drugs by regulating the drug release in the target region.[8] Although several instances have shown successful drug loading and cellular uptake of drug, the quantitative release of the drug in a controlled manner devoid of any lethal effect still persists as a challenging task to the pharmaceutical researchers.[9] Among all the DDS, micelles are the mostly used supramolecular assembly formed by the surfactant monomers. The capability of the micelles to enhance the bioavailability of the drug molecules by accommodating them within its hydrophobic core makes micelles an efficient drug carrier.[3,10-12] Micelles can also protect the drug molecules from biodegradation and improve its transportation to the desired site.[13,14]

Deoxyribonucleic acid (DNA) is the most relevant biomolecular target due to its importance in controlling the heredity of life through its base sequence and its involvement in various key biological processes including gene transcription, mutagenesis, gene expression, etc.[15-18] Unveiling the binding mechanism of small molecules with DNA has become an emerging topic of interest of the day. Small molecules bind to the DNA double helix principally by three dominant modes referred to as (i) intercalative binding where the molecule intercalates within the base pairs of nucleic acid, (ii) groove binding involving hydrogen bonding or van der Waals interaction in the deep major groove or the shallow minor groove of the DNA helix and (iii) electrostatic binding between the negatively charged DNA phosphate backbone and cationic or positive end of the molecules.[19,20] Among these three, the intercalative mode is known to be the most effective one for the medicinal efficacy of drugs targeted to DNA.[20] Binding of small molecules to the DNA can modulate its biological activity, hence, most of the DNA associated process occurring in the living systems are likely to be modified by the introduction of the molecule having high DNA binding affinity.[21] Thus, the quantitative release of small molecules from a carrier to the DNA would be noteworthy, specially from the perspective of chemotherapeutics.

In the present work, we have demonstrated a simple but useful technique where controlled and complete release of a bioactive photosensitizer (phenosafranin, PSF) from a micellar nanocarrier (STS) to the calf thymus DNA (ctDNA) has been achieved using an external non-toxic stimulating agent (β-CD). PSF (Scheme 1), a cationic phenazinium dye, has been extensively used as sensitizer in energy and electron transfer reactions.[22] Antimicrobial photodynamic inactivation (APDI)

Department of Chemistry, Jadavpur University, Kolkata-700032, India.
Email: ghosh.saptarshi89@gmail.com (S.G.), nitin.chattopadhyay@yahoo.com (N.C.)
Fax: 91-33-2414-6584.

†Electronic Supplementary Information (ESI) available: Absorption spectra of PSF in STS and DNA, plot for the determination of the binding constant of PSF with STS, plot for determination of the aggregation number of STS, plot for the determination of the binding constant of PSF with DNA, emission spectra of micelle bound PSF with increasing BSA concentration, emission spectra of micelle bound PSF with increasing β-CD concentration, normalized emission spectra of PSF in various media and variation of the average fluorescence lifetime of micelle bound PSF with increasing β-CD concentration in the presence of ctDNA.. See DOI: 10.1039/x0xx00000x



Scheme 1. Schematic structure of phenosafranin

using the photosensitizers is emerging out, in recent times, as a promising mode of photodynamic therapy (PDT) against cancer.[23] Interestingly, among various photosensitizers, the cationic ones are found to exhibit surprising efficacy against pathogenic drug-resistant bacteria such as methicillin-resistant staphylococcus aureus, vancomycin-resistant enterococcus faecium etc.[24] In a recent work, Corio *et al*. have suggested that single-wall carbon nanotubes (SWNTs) functionalized with PSF can be applied in PDT.[25] Phenazinium dyes are also known to possess antimalarial potency.[26] PSF has already been reported to bind with ctDNA principally through intercalative mode.[20] Presence of the electrostatic interaction on the PSF-ctDNA binding has also been established by studying the effect of ionic strength of the medium on the binding process.[20] In this article, complexation of the external stimulant with the carrier has been exploited for the controlled delivery of the aforesaid bio-potent photosensitizer from micelle to the natural DNA. Differential fluorometric responses of PSF in the carrier and the target environments relative to the aqueous medium have been monitored to probe its location in the complex media. Non-toxicity of the stimulant (β-CD) and non-invasive nature of the carrier (STS) towards the target are expected to make this approach applicable for clinical purpose.

Experimental section

Materials

Phenosafranin (PSF), sodium tetradecyl sulfate (STS), β-cyclodextrin (β-CD) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (USA) and used without further purification. ctDNA (Molecular wt. 8.4 MDa), Tris-HCl buffer were obtained from Sisco Research Laboratories (India) and used as received. Deionised water from Milli-Q water purification system (Millipore) was used throughout the study. All the experiments were performed using Tris-HCl buffer of 0.01 M at pH = 7.4.

Stock solution of ctDNA was prepared by dissolving solid ctDNA in Tris-HCl buffer and was stored at 4 °C. The measured ratio of the absorbance at 260 nm to that at 280 nm of the prepared ctDNA solution was in the range 1.8—1.9 indicating that the DNA is free from protein molecules.[21] The concentration of ctDNA solution was determined spectrophotometrically using ε_{DNA} = 13,600 mol^{-1} dm^3 cm^{-1} at 258 nm.[27] The concentration of PSF was kept at 5 μM throughout the study unless otherwise specified. Freshly prepared micellar solutions were used for all the measurements to avoid aging. Concentration of STS was maintained at 2 mM throughout the study.

Methods

Shimadzu UV-2450 absorption spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used for the steady state absorption studies. Steady state fluorescence and fluorescence anisotropy measurements were carried out in a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer. Fluorescence anisotropy (r) is defined as

$$r = (I_{VV} - G.\ I_{VH}) / (I_{VV} + 2G.\ I_{VH}) \quad (1)$$

where I_{VV} and I_{VH} are the emission intensities obtained with the excitation polarizer oriented vertically and emission polarizer oriented vertically and horizontally, respectively. The G factor is defined as[28]

$$G = I_{HV}/ I_{HH} \quad (2)$$

where the intensities I_{HV} and I_{HH} refer to the vertical and horizontal positions of the emission polarizer, with the excitation polarizer being horizontal.

Time resolved fluorescence decay measurements were performed by the time-correlated single photon counting (TCSPC) technique in Horiba–Jobin–Yvon FluoroCube system using NanoLED at 490 nm (IBH, UK) as the excitation source and TBX photon detection module as the detector. The decays were analyzed using IBH DAS-6 decay analysis software. The lamp profile was collected by placing a scatterer (dilute micellar solution of sodium dodecyl sulfate in water) in place of the sample. Goodness of fits was evaluated from χ^2 criterion and visual inspection of the residuals of the fitted function to the data. Mean (average) fluorescence lifetimes (τ_{avg}) for biexponential iterative fittings were calculated from the decay times (τ_1 and τ_2) and the normalized pre-exponential factors (a_1 and a_2) using the following relation

$$\tau_{avg} = a_1\tau_1 + a_2\tau_2 \quad (3)$$

Circular dichroism spectra were recorded on a JASCO J-815 spectropolarimeter (Jasco International Co., Hachioji, Japan) using a rectangular quartz cuvette of path length 1 cm. The CD profiles were obtained employing a scan speed of 50 nm/min after appropriate baseline corrections were made by aqueous buffer solution. All the experiments were performed at room temperature (298 K) with air-equilibrated solutions.

For DNA helix melting experiment, the pre-fixed temperatures were set using a high precession peltier (Wavelength Electronics, USA, Model No. LFI-3751, temperature stability within 0.002 ^{0}C) that was attached to the abovementioned spectrophotometer.

Results and discussion

Circular dichroism spectral measurement

Retention of structure of the target in the presence of the carrier is an essential criterion for a potential drug delivery system in order not to degrade the normal biological activity of the target. To verify whether STS (the choice of STS is clarified later) has any significant impact on the structure of DNA or not, we have recorded the intrinsic circular dichroism (CD) spectrum of

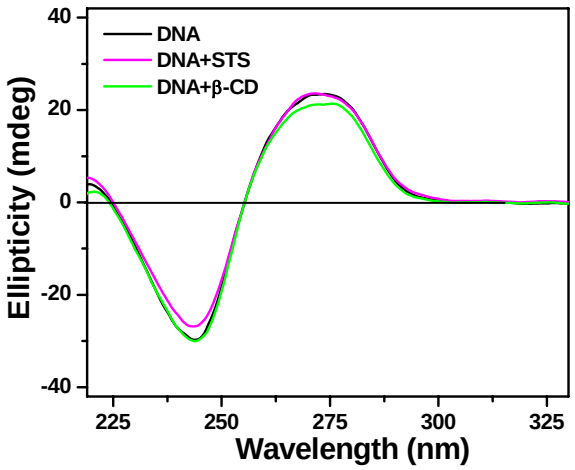


Fig. 1. Circular dichroism spectra of ctDNA in aqueous buffer (black), in the presence of 2 mM STS (red) and in the presence of 3 mM β-CD (blue). [ctDNA] = 100 μM.

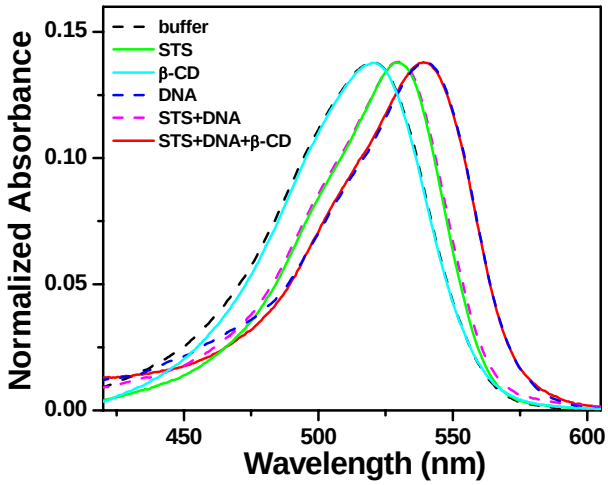


Fig. 2. Normalized absorption spectra of PSF in different environments as labeled in the legends. [PSF] = 5 μM, [STS] = 2 mM, [ctDNA] = 150 μM, [β-CD] = 3 mM.

ctDNA in the absence and in the presence of STS. Fig. 1 represents the CD spectra of ctDNA in aqueous buffer and in the presence of 2mM STS which is higher than its critical micellar concentration (CMC of STS is reported to be 1.8 mM in aqueous medium[29] although the CMC values of sodium alkyl sulfate micelles drop down in buffer media[30]). The circular dichroism spectrum of free ctDNA in buffer solution shows a positive peak at ~ 274 nm and a negative peak at ~ 244 nm, characteristic of the right handed B form.[20] Fig. 1 reveals that there is no significant change in the CD spectrum of the DNA in the presence of STS, indicating that STS does not distort the secondary structure of DNA. To ensure that the stimulant, β-CD, also has no effect on the structure of DNA, we have further monitored the CD spectrum of ctDNA in the presence of 3 mM β-CD (Fig. 1). The figure demonstrates that β-CD does not bring a considerable change in the CD spectrum implying insignificant interaction of β-CD with the DNA. Thus, both the carrier and the stimulant have negligible effect on the target, so far as the structural aspect is concerned.

Absorption study

Absorption spectra of PSF have been recorded in various media and are presented in Fig. 2. Compared to the intensity of the absorption spectrum the position of the absorption maximum of the probe is found to be more sensitive towards the local environments. Hence, we have presented the normalized absorption spectra of PSF in different media for an easy visualization of the spectral positions. In aqueous buffer solution PSF shows a broad and unstructured lowest energy absorption band with maximum at ~ 520 nm.[20] In STS micelle, the absorbance of PSF slightly increases but the absorption maximum reveals significant bathochromic shift from 520 nm to 529 nm.[31] The absorption spectrum shows remarkable modification in the DNA medium relative to that in buffer. With gradual addition of ctDNA to the aqueous buffer solution of PSF the absorbance first decreases and then slightly increases with a large bathochromic shift of the band maximum from 520 nm in aqueous buffer medium to 539 nm in DNA.[20]

The absorption spectra of PSF in both STS and ctDNA media are provided in the ESI† (Fig. S1). The feeble shoulder near 510 nm in DNA medium may be due to the very little contribution from the residual uncomplexed PSF molecules. Nonexistence of the isosbestic point in the absorption spectra of the probe in DNA accounts for the involvement of both intercalative and electrostatic binding of the probe with the ctDNA.[20] The absorption spectral modifications of PSF in STS and ctDNA media imply binding interactions of the probe with both the media. The significant bathochromic shifts in both micelle and DNA environments (Fig 2) suggest that the probe experiences less polar environments around itself in these media compared to the aqueous buffer milieu. Addition of 3 mM β-CD in the aqueous solution of the probe, however, does not reveal any visible change in the absorption spectrum. The spectral changes in these media are consistent with the existing reports on the probe in microheterogeneous media.[20,22,31]

With the addition of ctDNA to the PSF—STS assembly, the absorption maximum of PSF remains in the same position as observed in STS micelle implying that the probe resides in the STS micelle even in the presence of DNA. A relatively stronger binding of the cationic probe with the anionic STS micelle than that of the DNA is ascribed for this preferential residence of the probe (the detailed discussion is in the upcoming section). Interestingly, with the gradual addition of β-CD to the PSF—STS assembly in the presence of DNA, the absorption maximum moves to the higher wavelength and eventually, upon addition of 3 mM β-CD the position of the absorption maximum reaches the value that is observed for PSF in pure DNA medium. This observation indicates that upon addition of β-CD in the solution containing both STS and ctDNA, the probe comes out of the STS micellar medium to bind exclusively with the ctDNA.

Steady state emission study

The room temperature emission spectra of PSF in different media are presented in Fig. 3. In the aqueous buffer solution PSF shows a single broad and unstructured emission band with

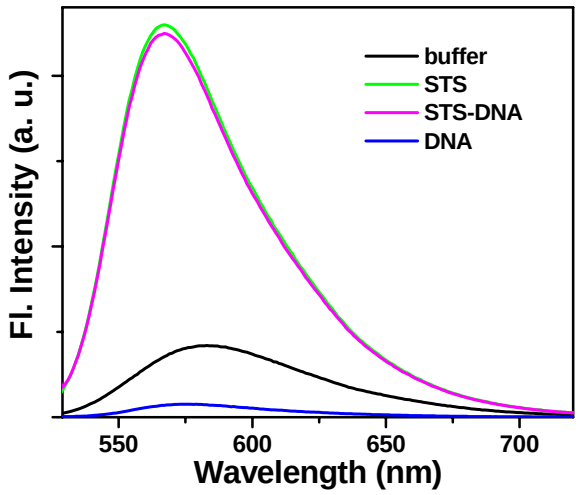


Fig. 3. Fluorescence spectra of PSF in aqueous buffer, 2 mM STS, 150 μM ctDNA and in the presence of both STS and ctDNA. Different environments are labeled in the legends. [PSF] = 5 μM, λ_{ex} = 520 nm.

a maximum at 583 nm ascribed to the charge transfer emission.[32] In 2 mM STS micelle, the emission intensity of PSF increases remarkably with substantial hypsochromic shift of 16 nm (from 583 nm in aqueous buffer to 567 nm in STS micelle). However, on intercalation within the DNA, fluorescence intensity of PSF decreases drastically with a considerable blue shift of 8 nm (from 583 nm in aqueous buffer to 575 nm in ctDNA). The present observations go parallel to the literature reports on PSF in anionic micellar media and DNA environment revealing strong binding interaction of the probe with both the STS micelle and ctDNA.[20,31] Addition of ctDNA to the micelle bound PSF does not bring any notable change in the fluorescence intensity as well as in the position of the emission maximum of the probe corroborating the outcome of the absorption study and implies that PSF prefers to reside in the micelle even in the presence of ctDNA. Being cationic in nature, PSF binds strongly with the anionic STS micelle. Binding constant of PSF with STS micelle has been estimated from the fluorescence intensity data following the method described by Almgren *et al.*[33] Corresponding plot for the probe-micelle binding is given in the ESI† (Fig. S2) and the binding constant has been determined to be 1.35×10^6 M^{-1}. Binding affinity of PSF with anionic micelles is found to be increased significantly with increasing length of the hydrophobic chain of the surfactants.[31] Higher binding affinity of the probe with STS micelle ($C_{14}S$) compared to the other anionic micelles of the series having smaller chain lengths ($C_{10}S$ and $C_{12}S$) has prompted us to use STS as the carrier of PSF so that the probe is not released through the interaction with other biosystems present on its way during transportation. By the way, the aggregation number of STS micelle under the experimental condition has been determined following the standard fluorescence quenching method[34] (corresponding plot is given in the ESI† (Fig. S3)) and the value comes out to be 75, consistent with the value reported by Gratzel *et al.*[35] Our calculation further suggests that roughly a single PSF molecule binds per micelle. The binding constant of PSF with ctDNA has been determined using the well-known Benesi-Hildebrand equation[36] (the corresponding plot for the determination of the binding constant is provided in the ESI† (Fig. S4)) and the value comes out to be 5×10^4 M^{-1}. Much higher binding affinity of PSF with the STS micelle compared to that with the DNA is ascribed to be responsible for the preferential residence of the probe in the micellar environment in the presence of both micelle and ctDNA (see above).

It is pertinent to mention here that release of the carrier encapsulated drug/small molecules during their transportation through the body system due to the interactions with other components in the blood stream like serum albumin etc. is of concern during targeted delivery. To ensure safety in this respect we have monitored the emission spectral behavior of micelle bound PSF with the addition of bovine serum albumin (BSA), one of the most abundant transport protein in the blood plasma (Fig. S5, in the ESI†). Fluorometric response of the micelle bound probe remains unaltered with the addition of BSA indicating insignificant interaction between the micelle bound PSF and BSA. As justified before, this observation is rationalized from a much lower binding affinity of the probe with the BSA (6.5×10^3 M^{-1})[37] compared to that with the STS micelle (1.35×10^6 M^{-1}). Selection of STS as carrier is thus justified.

Cyclodextrins (CDs) are made up of glucopyranose units capable of forming supramolecular host-guest inclusion complexes with a verity of small molecules involving non covalent interactions.[38] β-CD is reported to modulate the binding interactions between the dyes and DNA.[39,40] A couple of recent works with acridine dye and an anticancer drug ellipticine have shown the relocation of the probes from the CDs to the DNA on altering the solution pH.[41,42] In the present study, STS has been used as the carrier of PSF and β-CD is used to activate delivery of the probe from the carrier micelle to the target ctDNA. Cyclodextrins are known to form inclusion complexes with the common surfactant molecules and hence disrupt the self assembled micellar structures.[43-45] As a result the CMC values increase in the presence of CDs.[46,47] Jiang *et al.* have shown that CMC of surfactants increases steadily with the gradual addition of CDs.[47] In the present study also we have verified the similar effect of β-CD on STS micelle from the fluorometric studies. With gradual addition of β-CD to the micelle bound PSF we observe a concomitant decrease in the fluorescence intensity of the probe with a bathochromic shift of the emission maximum (Fig. S6, in the ESI†). Agreement of the emission spectrum of the probe in the micellar medium after the addition of 3 mM β-CD to that in the buffer medium suggests that addition of β-CD leads to the disruption of the micellar structure of STS resulting in the release of the probe in the buffer milieu. Fluorescence anisotropy measurements (to be discussed in the next section) also corroborate the disruption of the micellar structure by the addition of β-CD. This complexation property of β-CD with the hydrophobic chain of the surfactant has been exploited here for the controlled release of the micelle bound PSF to the DNA. Recently, we have shown that β-CD does not affect the structures of the serum proteins under physiological condition.[48] Hence, use of β-CD

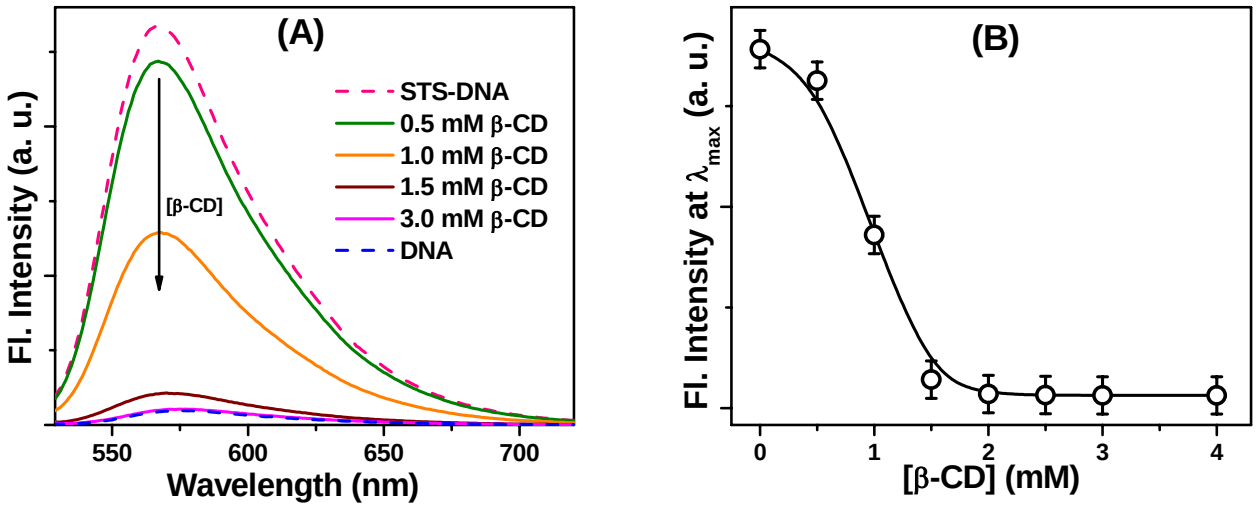


Fig. 4. (A) Fluorescence spectra of micelle bound PSF in the presence of ctDNA with the gradual addition of β-CD. Different environments and the different β-CD concentrations are labeled in the legends. λ_{ex} = 520 nm. **(B)** Variation of the fluorescence intensity of PSF in STS-DNA mixture with a variation in the concentration of β-CD. [PSF] = 5 μM, [STS] = 2 mM, [ctDNA] = 150 μM.

for the purpose of releasing the micelle entrapped PSF to the target DNA can be considered as safe for both the target (as already discussed) and serum proteins so far as their structures are concerned.

Differential fluorometric responses of the probe in the micellar and DNA environments have been utilized to find its location in the composite media. With the gradual addition of β-CD to the micelle bound PSF in the presence of DNA, fluorescence intensity of the probe decreases drastically and ultimately, upon addition of 3 mM β-CD the emission intensity becomes same as the intensity of the probe in pure ctDNA medium. Fig. 4(A) represents the emission spectra of micelle bound probe in the presence of ctDNA with the gradual addition of β-CD and the variation in the fluorescence intensity of the probe is shown in Fig. 4(B). Emission maximum of PSF in the composite medium (upon addition of 3mM β-CD to the micelle bound PSF in the presence of DNA) appears at 575 nm, coinciding with the position observed for the probe in DNA medium. The normalized emission spectra of PSF are provided in Fig. S7 (in the ESI†) for a better understanding about the positions of the fluorescence maxima in different environments.

The observations indicate that with the gradual addition of β-CD to the micelle bound probe, the probe gets released from the micelle and binds with the DNA. Agreement of the emission spectrum of PSF in STS—DNA mixture after the addition of 3 mM β-CD to that in pure ctDNA medium imply complete transfer of the probe from the STS micelle to ctDNA at this CD concentration. Formation of PSF—β-CD inclusion complex in the present case is ruled out considering the very low binding affinity of PSF towards β-CD (binding constant = 82 M^{-1}).[49] Fig. 4(B) also demonstrates that the amount of PSF released depends on the concentration of β-CD till the complete release of the probe. Thus, it is possible to tune the release of the probe from the STS carrier to the DNA easily by controlling the β-CD concentration. Therefore, β-CD induced release of PSF from micelle to the DNA is targeted, quantitative as well as controlled. As mentioned in the experimental section, we have used 2 mM STS throughout our study. This concentration of the surfactant is sufficient to accommodate all the PSF molecules in the micellar units in 1:1 fashion.

Steady state fluorescence anisotropy measurements

Fluorescence anisotropy dictates the motional restriction imposed on the fluorophore by the surrounding environment and hence, often serves as a tool for exploring the location of the fluorophore in a bio- or biomimetic microheterogeneous environments like protein, lipid, DNA, micelle, cyclodextrin etc.[45,48-52] A fluorophore in a fluid medium without appreciable interaction with the latter rotates almost freely within its fluorescence lifetime resulting in a very low fluorescence anisotropy while an increase in the rigidity or viscosity in the microenvironment around the fluorophore results in an enhancement in the anisotropy value.[28,49-54] In the present study, we have measured the fluorescence anisotropy of PSF in different environments (Fig. 5, data compiled in Table 1) to assess its precise location in these media. Fig. 5 reveals that the anisotropy values of PSF in both STS micelle and ctDNA increase from the value observed in aqueous buffer suggesting imposition of motional restriction on the probe upon binding with these environments. Much higher value of anisotropy for PSF in the DNA medium is rationalized from the intercalative mode of binding of PSF with ctDNA.[20] It is generally observed that due to the constrained environment within the DNA basepairs, intercalation of probes to the DNA results in fluorescence anisotropy value greater than or around 0.25.[27,53] Gradual addition of β-CD to the micelle bound probe causes a

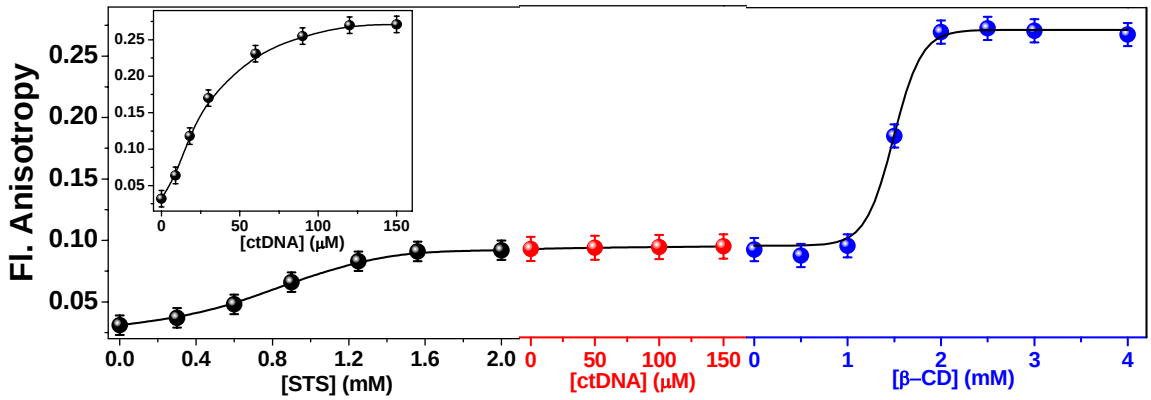


Fig. 5. Variation in the steady state fluorescence anisotropy of PSF in various environments. Inset shows the same as a function of ctDNA concentration. λ_{ex} = 520 nm and $\lambda_{monitor} = \lambda_{em}^{max}$. Each data point is an average of 15 individual measurements.

Table 1. Steady state fluorescence anisotropy of PSF in different media

Environments	Anisotropy (r)
Aqueous buffer	0.032
2 mM STS micelle	0.092
2 mM STS micelle + 3 mM β-CD	0.036
2 mM STS micelle + 150 μM ctDNA	0.094
2 mM STS micelle + 150 μM ctDNA + 3 mM β-CD	0.271
150 μM ctDNA	0.268
3 mM β-CD	0.078

lowering in the anisotropy value (figure not shown) and at 3 mM β-CD concentration the value comes out to be 0.036 which resembles the anisotropy of the free probe in the buffer medium (Table 1). The observation substantiates that addition of β-CD leads to the disruption of the STS micellar structure and therefore, the probe gets released from the micellar environment to the buffer medium. With the addition of DNA to STS micelle bound PSF, however, anisotropy of the probe remains invariant implying that PSF does not change its location even in the presence of DNA (Fig. 5). Fig. 5 further reveals that upon gradual addition of β-CD to the micelle—DNA mixed system, the anisotropy of PSF increases remarkably and at high enough β-CD concentration, the value reaches at 0.27, the same value as is observed for PSF being intercalated within ctDNA. The observation demonstrates that the probe experiences the same motional restriction by its microenvironment in free DNA and micelle—DNA—β-CD mixed system. Thus, fluorescence anisotropy measurements corroborate the proposition made from the absorption and emission studies that transfer of PSF from the micelle to the DNA is achieved by the addition of β-CD to the micelle—DNA mixed system. That PSF—β-CD complex is not formed, is confirmed since in that case the fluorescence anisotropy would have been much lower (0.078, see Table 1).

Time resolved fluorescence decay measurements

Fluorescence lifetime of a fluorophore is sensitive to its immediate environment and excited state interactions and hence, it is often exploited to extract valuable information about the binding and location of the probe in complex microheterogeneous environments.[49-54] We have recorded the time resolved fluorescence decays of PSF in different environments to verify whether addition of β-CD to the micelle—DNA mixed system leads to the transfer of PSF from micelle to the DNA or not. Characteristic fluorescence decay profiles of PSF in different environments are shown in Fig. 6(A) and the deconvoluted lifetime data are collected in Table 2. In aqueous buffer, PSF shows single exponential decay with lifetime value around 1 ns.[22] In STS micelle also PSF exhibits monoexponential decay with a much higher lifetime value of 2.28 ns suggesting a strong binding of the cationic probe with the anionic micelle.[31] The fluorescence decay of PSF in pure ctDNA has been found to be biexponential. However, addition of ctDNA to the micelle bound PSF neither brings any change in the decay profile nor in the lifetime value of the probe from that in the micellar medium. Single exponential fluorescence decays of the fluorophore suggest one type of location of the probe whereas the multiexponential decay of the fluorophores in microheterogeneous environments is ascribed to originate from the location of the fluorophore in different polarity regions as frequently observed in bio- or biomimicking assemblies.[28,50-54] The biexponential fluorescence decay in ctDNA environment might originate from the two types of binding of the probe with the DNA as described in the section 'Absorption study'. However, it is often difficult to provide specific mechanistic models to explain the individual components of the decays of a probe in complex microheterogeneous environments. Hence, instead of putting much emphasis on the individual decay components, use of the average fluorescence lifetime of the probe is often preferred for

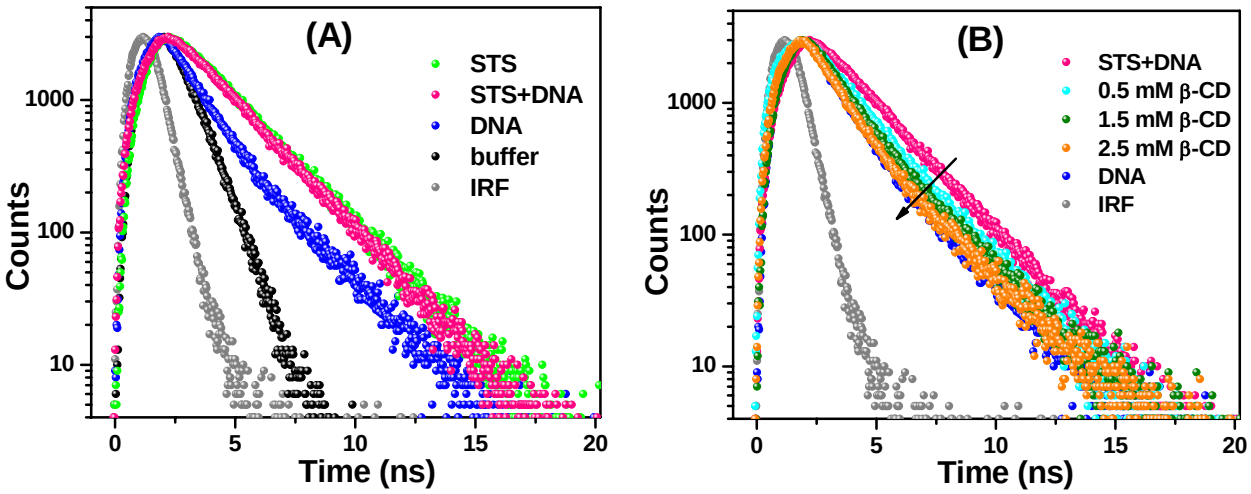


Fig. 6. (A) Time resolved fluorescence decays of PSF in various environments as labeled in the legends and **(B)** time resolved fluorescence decays of micelle bound PSF in the presence of ctDNA with the addition of β-CD. Different β-CD concentrations are labeled in the legends. λ_{ex} = 490 nm and λ_{em} = λ_{em}^{max}. [PSF] = 5 μM, [STS] = 2 mM, [ctDNA] = 150 μM.

Table 2 Time resolved fluorescence decay parameters of PSF in different environments

Solution	τ_1 (ns) (± 0.1)	a_1	τ_2 (ns) (± 0.1)	a_2	τ_{avg} (ns) (± 0.1)	χ^2
buffer	1.02				1.02	1.12
2 mM STS	2.28				2.28	1.05
150 μM ctDNA	1.25	0.82	2.94	0.18	1.52	1.10
2 mM STS + 150 μM ctDNA	2.24				2.24	1.10
[β-CD] (mM) on (2 mM STS + 150 μM ctDNA)						
0.5	1.83	0.93	3.66	0.07	1.96	1.14
1.0	1.61	0.78	2.80	0.22	1.84	1.08
1.5	1.34	0.77	2.70	0.23	1.65	1.09
2.0	1.26	0.83	2.89	0.17	1.54	1.09
2.5	1.25	0.78	2.94	0.22	1.58	1.11
3.0	1.27	0.82	2.93	0.18	1.56	1.05

understanding the behavior of the probe within the microheterogeneous environments.[52-54] In the present case also we have dealt with the average lifetime to find the location of the probe in the complex media. With the gradual addition of β-CD to the micelle bound probe in the presence of ctDNA the decays become biexponential (Fig. 6(B)). At higher β-CD concentration, the decay profile of the PSF becomes same as the decay of the probe in pure ctDNA. The decay parameters of the probe with the addition of β-CD to the micelle-DNA mixed systems are also provided in Table 2 and the variation in the average lifetime of the probe is depicted in Fig. S8 (in the ESI†). A glance on Table 2 reflects that the average fluorescence lifetime of PSF in the micelle—DNA mixed system decreases steadily with the addition of β-CD and at 3 mM β-CD concentration the value approaches the value observed in pure DNA medium implying the complete transfer of the probe from micelle to the DNA. Thus, time resolved fluorescence study strengthens the proposition made from the steady state results confirming the complete transfer of PSF from STS micelle to ctDNA upon addition of β-CD.

Helix melting study

Location of the probe in the composite medium is further confirmed from the DNA helix melting experiment. DNA melting is the process by which the hydrogen bonding and base stacking interactions between the strands of the DNA double helix are disrupted by heating, resulting in the separation of the double helix ultimately into two single strands.[55] The melting temperature (T_m) of DNA is defined as the temperature at which half of the double helical DNA strands are unfolded to single strands.[56] Helix melting of DNA was performed by measuring the absorbance at 260 nm as a function of temperature. The molar extinction coefficient of DNA bases at 260 nm in the double-helical form is much less than in the single stranded form.[50,54] Intercalation of probes into the DNA double helix increases the thermal stability of the helix through hydrogen bonding between the probe and the base pairs leading to a significant enhancement in the melting temperature,[53,57] while other modes of binding result in imperceptible changes in T_m.[50,54] The helix melting temperatures of ctDNA have been determined in different environments (Fig. 7) and are reported in Table 3.

In aqueous buffer, melting temperature of the native ctDNA comes out to be 67.7 °C. Upon addition of PSF to the solution of the native DNA, melting temperature of the ctDNA increases to the value 72.4 °C implying the intercalation of the probe within the DNA basepairs. In the presence of STS and 3 mM β-CD, however, melting temperature of ctDNA does not change significantly corroborating the results of circular dichroism study that STS and β-CD have imperceptible effect on the

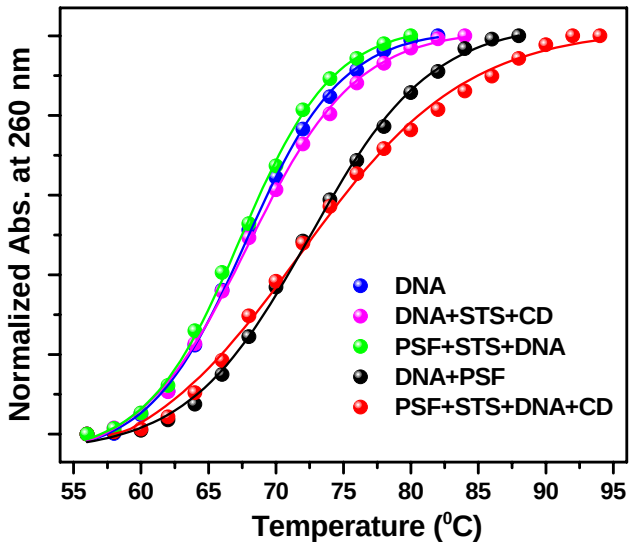


Fig. 7. Thermal melting profiles of ctDNA in the different environments as mentioned in the legends. [ctDNA] = 100 μM, [PSF] = 5 μM, [STS] = 2 mM and [β-CD] = 3 mM.

Table 3 Melting temperatures (T_m) of ctDNA in different environments

Environment	T_m (± 0.3 °C)
Free ctDNA	67.7
ctDNA + STS + 3 mM β-CD	67.9
PSF + STS + ctDNA	67.4
ctDNA+ PSF	72.4
PSF + STS + ctDNA + 3 mM β-CD	71.8

structure and stability of the native DNA. In the presence of micelle bound PSF also the melting temperature of ctDNA is found to remain unchanged indicating that the probe remains exclusively entrapped in the micelle in the presence of DNA. Upon addition of 3mM β-CD on the micelle bound PSF in the presence of DNA, however, the melting temperature of the DNA is determined to be 71.8 °C, a value close to that of the native ctDNA intercalated with PSF (Table 3). Thus, the helix melting study confirms the proposition made from the steady state and time resolved spectroscopic studies that with the addition of β-CD to the micelle bound PSF in the presence of ctDNA, the probe gets intercalated in the DNA base pairs leaving the micellar environment.

Conclusion

The present work articulates the controlled release of a bioactive photosensitizer (PSF) from the micellar carrier to the most relevant biological target DNA, by the use of an external stimulant (β-CD). Steady state absorption and fluorescence study, anisotropy measurements, time resolved fluorescence decay study and thermometric experiment relating to the helix melting of ctDNA unambiguously reveal that in the presence of both the micelle and the ctDNA, the probe remains exclusively within the micellar environment. Interestingly, upon addition of β-CD the probe is dislodged from the micelle to get intercalated within the DNA base pairs. At around 3mM β-CD concentration the transfer process is revealed to be complete. This work is expected to stimulate the development of newer chemical systems for controlled and quantitative drug delivery.

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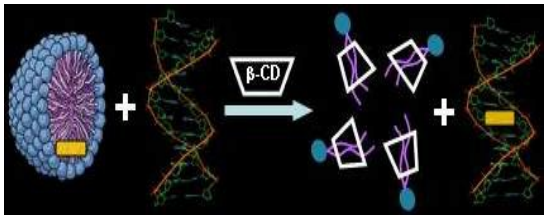

Addition of β-cyclodextrin to the micelle bound phenosafranin in the presence of ctDNA leads to quantitative transfer of the fluorophore from the micelle to the DNA.