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Delivery of a bioactive photosensitizer to natural DNA using γ-cyclodextrin as carrier

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A challenging goal of targeted drug delivery is to discover novel administrating route for the delivery of bioactive molecules/drugs in the field of pharmaceutical, medicinal and biophysical research. Delivery of a cationic photosensitizer, namely, phenosafranin (PSF), to the most relevant biomolecular target DNA using nanocarrier, γ-cyclodextrin (γ-CD), has been explored *via* various steady state and time resolved fluorometric as well as other spectroscopic techniques. The detailed fluorometric studies including DNA helix melting experiments divulge that the binding affinity of probe with the target (DNA) is order of magnitude larger than with the carrier (γ-CD). This leads to the release of the probe from the carrier cyclodextrin to bind with the DNA. Endogenous activation, in terms of competitive binding affinity, has been exploited for this purpose. This work qualitatively illustrates that the γ-cyclodextrin can be used as a safe and potential drug delivery system (DDS) for drug targeting towards DNA.

Keywords: Phenosafranin, γ-cyclodextrin, calf thymus DNA, competitive binding, drug delivery system, drug targeting, endogenous activation

In past two decades, various drug delivery systems (DDSs) are engineered in medicinal, clinical and biophysical fields for enhanced therapeutic effects of the bioactive molecules and reduced drug-induced adverse side effects by improving their pharmacokinetics and bio-distribution profiles[1-4]. DDS protects the drug molecules and release the molecules at the target region without affecting the surrounding tissues/cells. The studied DDS models are mainly micelles, liposomes, polymers, dendrimers, nano particles, carbon nanotube, cyclodextrins, fullerenes, *etc*.[4-17] Among them, cyclodextrins (cyclic oligosaccharides) have been found as potential candidates for secure, convenient and effective administration of drugs. Cyclodextrins (CDs) contain at least 6 D-(+)-gluocopyranose units linked through α-(1,4) glucosidic bonds[18,19]. Orientation and conformation of the hydroxyl groups of CDs construct the hydrophilic outer surface and lipophilic inner cavity and hence are able to form non-covalent inclusion complexes with large variety of organic molecules. The parent α-, β-, γ-cyclodextrins consist of six, seven and eight glucose residues and differ largely in size and solubility[20]. Complexation of drug molecules with cyclodextrins changes the physiological properties like solubility, stability, *etc*. of the drug molecules. Moreover, due to the non-toxic nature and drug encapsulating ability of cyclodextrins, they can serve as successful drug delivery systems[21].

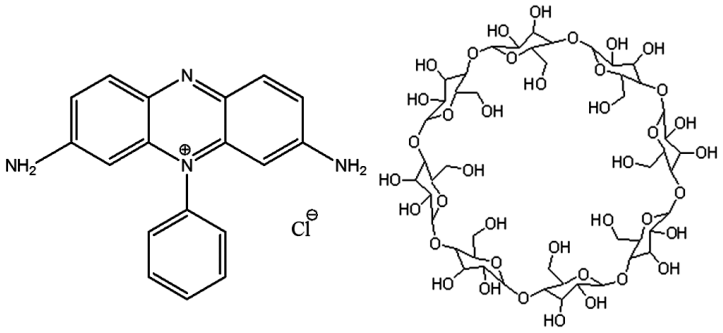
Deoxyribonucleic acid (DNA) is the most pertinent biomolecular target as it controls the inheritance of life by its base sequence and it carries the genetic instruction used in the growth, development, functioning and reproduction of all the living organisms. DNA is also involved in many important biological processes like gene transcription, gene expression, mutagenesis, *etc*.[22-25] There are mostly three modes of binding between the small drug molecules and the DNA, these are (i) electrostatic binding with the negatively charged DNA phosphate backbone, (ii) deep major or shallow minor groove binding involving H-bonding or van der Waals interaction and (iii) intercalative binding within the base pairs of the nucleic acid[26,27]. Among these, intercalative binding of drugs with DNA is the most effective for the therapeutic and/or clinical purpose as the drug molecules directly binds to the DNA basepairs[27]. Since differential binding of drug molecules to a carrier and the DNA can modulate the porting of the drugs to the target, endogenous delivery of small molecules from a nanocarrier to the natural DNA is tricky in pharmaceutical applications[28].

Phenosafranin (PSF, 3,7-diamino-5-phenyl phenazinium chloride) (Scheme I), a planar cationic phenazinium dye, has extensively used in semiconductor, as sensitizer in energy and electron transfer reactions[29]. Phenazinium dyes act as bacterial growth inhibitor and also possess antimalarial potency[28]. PSF, functionalized with single-wall carbon nanotubes, has been applied in photodynamic therapy (PDT) as well[30]. Apart from its biological significance, PSF has been utilized as a probe for studying various microheterogeneous assemblies like micelles, reverse micelles, lipids, proteins, *etc*.[27,29,31-34] In the present article, we have demonstrated a simple but useful technique that can be utilized for the delivery of PSF from supramolecular nanocarrier, γ-CD (Scheme I), to the natural calf thymus DNA (ctDNA). Multispectroscopic techniques including DNA helix melting suggest that the probe molecule binds with both the carrier and the target independently. PSF binds with the ctDNA through intercalative binding mode, as already reported in literature[17,27]. Binding affinity of PSF with ctDNA is found to be order of magnitude higher than that with γ-CD. Differential binding affinity of PSF towards cyclodextrin and DNA has been responsible for the quantitative transfer of PSF from the cyclodextrin nanocavity to the DNA base pairs. Retention of DNA structure in the presence of cyclodextrin, water solubility and non-toxic nature of the carrier (γ-CD) make this approach promising for clinical use.

Results and Discussion

Absorption Studies

The absorption spectra of PSF in γ-cyclodextrin (carrier) and DNA (target) environments are recorded and shown in Figure 1. The absorption spectrum of



Scheme I — Schematic structures of phenosafranin and γ-cyclodextrin

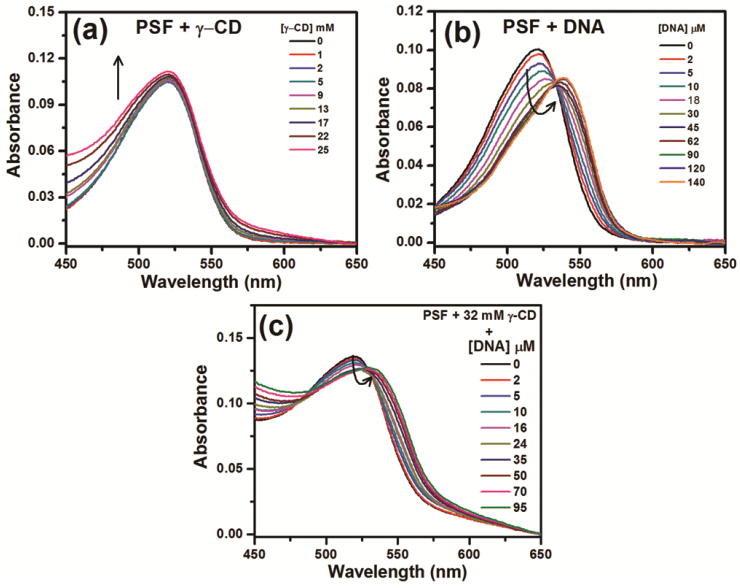


Figure 1 — Absorption spectra of PSF upon gradual addition of (a) γ-CD and (b) ctDNA. Figure (c) represents the absorption spectra of CD-bound PSF with successive addition of DNA. Concentrations of γ-CD and DNA are depicted in the legends. [PSF] = 5 μM.

PSF in aqueous buffer medium shows a broad and unstructured lowest energy absorption band with maximum at 520 nm, attributed to the charge transfer transition of the chromophore system[27,29,35]. Addition of γ-CD to the aqueous solution of PSF leads to a slight enhancement of the absorption intensity along with a nominal red shift (1-2 nm) of the band maximum, suggesting weak binding interaction between these two partners (Figure 1a). Observation of the red shift of the absorption maximum signifies that the polarity at the immediate vicinity of PSF in cyclodextrin environment is lower compared to that in the buffer medium[36]. With the successive addition of calf thymus DNA (ctDNA), on the other hand, the absorbance of PSF gradually decreases along with a significant bathochromic shift of the absorption maximum by ~ 17 nm (from 520 nm in aqueous buffer to 537 nm in DNA medium) at the saturation level of interaction (Figure 1b)[27,36]. Such type of large red shift of the absorption maximum for the probe-DNA interaction implies an intercalative binding of PSF with the DNA[27]. Absence of any precise isosbestic point in the dye-DNA absorption spectral profile accounts for the partial involvement of electrostatic binding between the cationic probe and negatively charged phosphate backbone of DNA in addition to the intercalative binding between PSF and DNA[17,27,36].

Upon addition of DNA to the CD-bound probe, the absorption spectral profile of PSF changes radically, *i.e.*, the absorbance of PSF decreases along with a large bathochromic shift of the emission maximum, as depicted in Figure 1c. The spectral modification of PSF in the composite medium (PSF + CD + DNA) grossly mimics its behavior in pure DNA environment, indicating identical microenvironment around the probe in both cases. Therefore, the absorption spectral study implies that in the presence of both CD and DNA, PSF preferentially resides in the DNA environment. The higher binding affinity of PSF towards DNA compared to CD is ascribed to this observation (*vide infra*).

Emission Studies

To understand the binding interactions of PSF in different environments, *i.e.*, cyclodextrin, DNA and in composite (CD + DNA) medium, steady state emission studies have been performed (Figure 2). In the aqueous buffer solution PSF shows a single broad and unstructured emission band with a maximum at 583 nm ascribed to the charge transfer (CT) emission[35]. Gradual addition of γ-CD to the aqueous solution of PSF results in a slight enhancement of the emission intensity associated with a small blue shift (~ 2 nm) of the emission maximum (Figure 2a). The spectral modification reveals weak binding interaction between the probe and the cyclodextrin. On the other hand, addition of DNA to the aqueous solution of PSF results in a significant decrease in emission intensity together with an appreciable blue shift (~ 7 nm) of the emission maximum (Figure 2b). The fluorescence quenching with a considerable blue shift of the band maximum reveals an intercalative binding between the probe and the DNA, consistent with the literature reports on PSF in DNA environment[17,27,36].

To acquire a qualitative idea about the strength of binding of PSF with cyclodextrin as well as with DNA, we have determined the binding constant from the fluorescence titration data using modified Benesi-Hildebrand equation[37] as given below

$$\frac{1}{\Delta F} = \frac{1}{\Delta F_{max}} + \frac{1}{K\Delta F_{max}}\frac{1}{[L]}$$

where $\Delta F_{max} = F_\infty - F_0$ and $\Delta F = F_x - F_0$ where F_0 and F_x are the fluorescence intensities of PSF in the

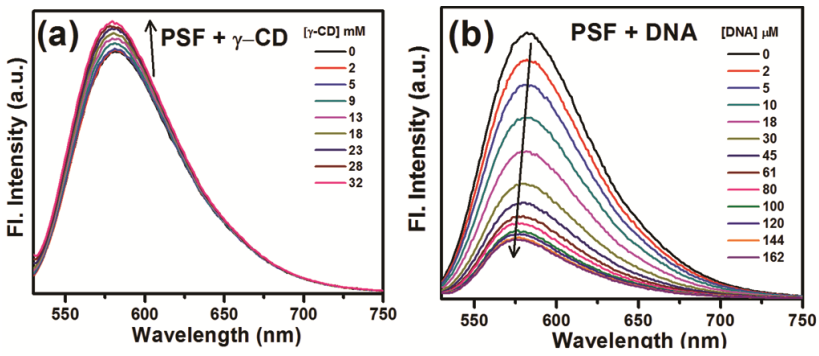


Figure 2 — Variation in the emission spectra of PSF upon gradual addition of (a) γ-CD and (b) DNA. Concentrations of the added γ-CD and DNA are depicted in the legends. [PSF] = 5 μM.

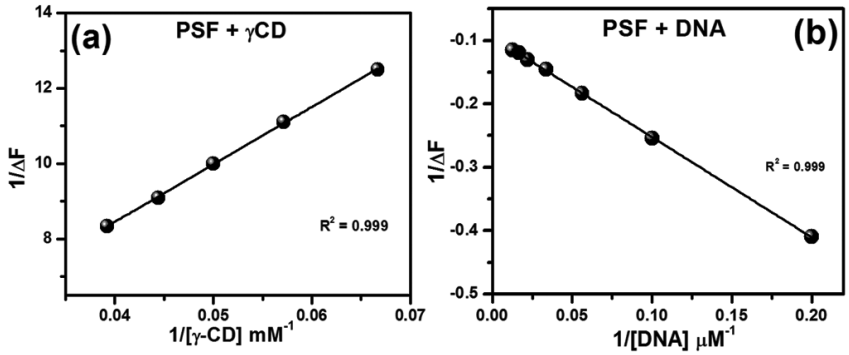


Figure 3 — Double reciprocal plots for the binding of PSF with (a) γ-CD and (b) DNA.

absence and at an intermediate concentration of CD or DNA. F_∞ is the intensity at a concentration of CD or DNA for complete interactions; K is the binding constant and [L] is the concentration of CD or DNA. Binding constants for the PSF-CD and PSF-DNA system have been determined from the double reciprocal plots of 1/ ΔF against 1/[L] and the values come out to be 15 M^{-1} and 6 × 10^4 M^{-1} respectively (Figure 3(a and b)). Linearity of both the binding plots suggests one-to-one binding of PSF with the two individual environments. A low value of the binding constant of PSF in γ-CD environment infers that the probe forms a weak inclusion complex with γ-cyclodextrin. On the contrary, the high binding constant for the PSF-DNA complexation is rationalized by considering the intercalative binding of the drug with the DNA[17,27,36]. It is apparent from these two binding constant values that the binding strength of PSF towards DNA is much higher (10^3 times) than that with γ-CD. The negative values of ΔG (−6.75 kJ mol^{-1} and −27.4 kJ mol^{-1} for PSF with CD and DNA systems, respectively, at 300 K) imply that the binding interactions are spontaneous.

After investigating the binding of PSF with the carrier (γ-CD) and the target (DNA) independently, we have performed the fluorometric studies to understand the effect of DNA on the CD-bound probe. The spectral modification of the CD-bound probe upon gradual addition of DNA is shown in Figure 4. The figure reveals that upon gradual addition of DNA to the cyclodextrin-bound PSF, the emission intensity drastically decreases along with a considerable blue shift (~ 7 nm) of the emission maximum. Similar spectral trend as well as same position of the emission maximum (576 nm) of PSF in the composite medium (γ-CD + DNA) and in pure DNA environment suggests that in the presence of both CD and DNA, the probe molecule gets dislodged

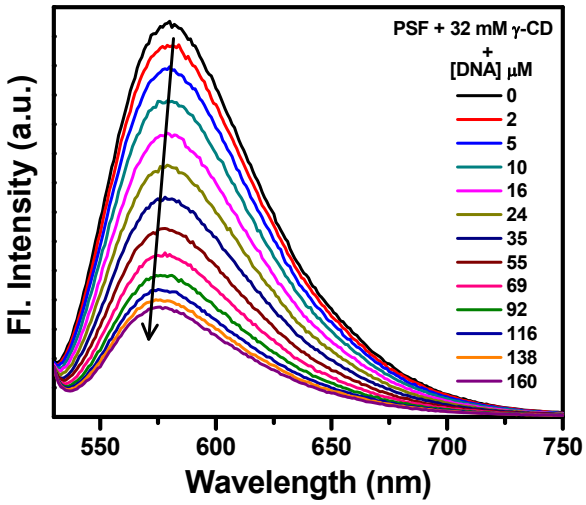


Figure 4 — Fluorescence spectra of cyclodextrin-bound PSF upon gradual addition ctDNA. Concentrations of the added DNA are depicted in the legends. [PSF] = 5 μM and [γ-CD] = 32 mM.

from the CD and is intercalated within the DNA base pairs. The transfer of probe molecule from the CD cavity to the DNA is rationalized from the higher binding affinity of PSF towards DNA than γ-CD. Thus, delivery of PSF from the cyclodextrin cavity to the biomolecular target, DNA has been achieved simply due to the competitive binding, *i.e.*, by means of endogenous activation.

Micropolarity Studies

The micropolarity studies have been performed to inspect the micropolarity and hence the location of PSF within different microenvironments. Local polarity around a probe in a microheterogeneous medium is determined in the $E_T(30)$ scale based on the transition energy for the intramolecular charge transfer absorption of the betaine dye, namely, 2,6-diphenyl-4(2,4,6-triphenyl-1-pyridono)phenolate of varying solvent polarity, as developed by Kosower and Reichardt[38,39]. To assess the possible location of

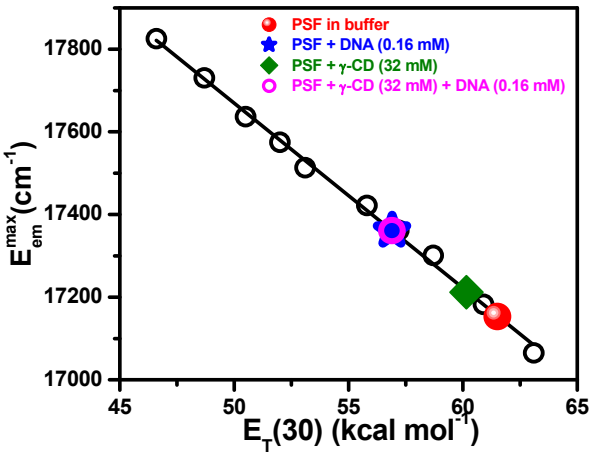


Figure 5 — Variation of the energy corresponding to the maximum of the PSF emission as a function of $E_T(30)$ in water–dioxane mixtures. Media for the interpolated values are provided in the legends. [PSF] = 5 μM.

PSF in the presence of cyclodextrin, ctDNA and the composite medium (CD + ctDNA), we have determined the $E_T(30)$ values of the probe in these media independently. For the judgment, a calibration plot monitoring the energies in cm^{-1} corresponding to the emission maxima of PSF in water-dioxane mixtures of known polarities are constructed (Figure 5). $E_T(30)$ values for the different compositions of the dioxane-water mixtures are adopted from literature[38]. Since the water-dioxane mixtures cover a much broader range of polarity compared to the other homogeneous solvent mixtures like water-alcohol, we have planfully chosen water-dioxane for constructing the calibration plot[40,41]. Interpolating the energy values corresponding to the emission maxima of the probe in aqueous buffer and in different environments on the calibration line, the micropolarity ($E_T(30)$) values around PSF in different environments are determined (Figure 5). The $E_T(30)$ values extracted from the plot are collected in Table I. The values reveal that the micropolarities around PSF in all three microheterogeneous media (γ-CD, ctDNA and γ-CD + DNA) are less compared to that in the aqueous buffer medium, implying that the probe resides in rather hydrophobic regions in these assemblies. Inspection of the micropolarity values further discloses that PSF shows similar polarities in the composite medium and the ctDNA environment, reiterating that relocation of PSF takes place from the CD to the DNA in the presence of both the hosts.

Fluorescence Anisotropy Studies

Measurements of fluorescence anisotropy serve an important role to assess the location of the drug molecules within complex microheterogeous environments. Any modulation in the size, shape, or segmental flexibility of a molecule affects the fluorescence anisotropy value[42]. The rotational diffusion of a fluorophore in an environment is reflected from its anisotropy value[42]. In aqueous medium, the probe molecules rotate very quickly within its lifetime giving rise to a very low anisotropy value. However, binding with bio- or biomimetic microheterogeneous environments like lipids, proteins, micelles, reverse micelles, cyclodextrins, *etc*. enhances the effective size of the fluorophore remarkably resulting in an increased fluorescence anisotropy value[42]. Thus, fluorescence anisotropy studies have great impact on determining the probable location of the probe in various microheterogeneous environments. The variations of fluorescence anisotropy of PSF in different environments are depicted in Figure 6 and the anisotropy values at the saturation level of interactions are presented in Table I. The figure reveals that the anisotropy value of PSF increases radically in ctDNA environments (0.24) relative to that in aqueous buffer medium (0.03) (Figure 6a), suggesting that the rotational motion of the probe gets slower. A significant enhancement in the fluorescence anisotropy value in DNA is attributed to the strong binding of PSF with the DNA, *i.e*., intercalative binding[27,36]. Whereas, in CD environment, the anisotropy value of PSF is enhanced slightly compared to that in the aqueous buffer medium, suggesting formation of a feeble inclusion complex (Figure 6b (black part)). With the successive addition of ctDNA to the CD-bound PSF, the anisotropy value of the probe rises gradually and the value approaches 0.24 at high DNA concentration (160 μM) (Figure 6b (red part)). The observation of same anisotropy values of PSF in DNA medium and in the composite medium (CD + DNA) implies that the probe experiences similar microenvironment around it in both the situations. Thus, the fluorescence anisotropy studies also imply that in the presence of both CD and DNA, the probe molecule gets free from the CD cavity and is intercalated within the DNA base pairs.

Fluorescence Lifetime Studies

Fluorescence lifetime measurement serves as an excellent experimental tool to investigate the local atmosphere around the fluorophore within the microheterogeneous assemblies such as lipids, proteins, micelles, reverse micelles, cyclodextrins, *etc*. and it is extremely sensitive to the excited state

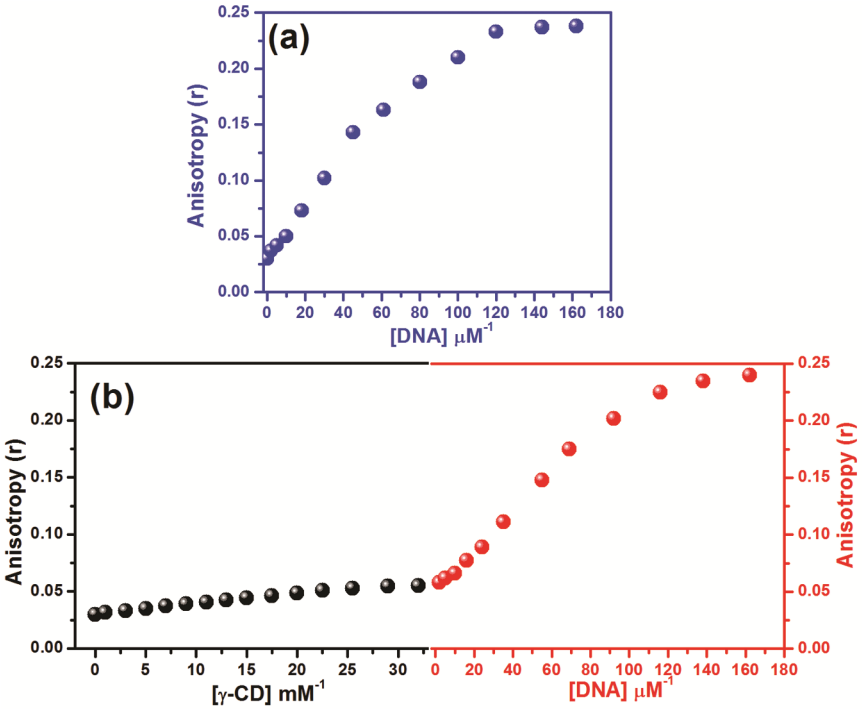


Figure 6 — Variation in the fluorescence anisotropy of PSF with increasing concentrations of (a) ctDNA and (b) γ-CD (black part) and in that of the CD-bound probe with increasing DNA concentrations (red part). Each data point is an average of 15 consistent individual measurements. λ_{ex} = 520 nm and $\lambda_{monitor} = \lambda_{em}^{max}$ in the respective media.

Table I — λ_{abs}^{max}, λ_{em}^{max}, $E_T(30)$ values and fluorescence anisotropy of PSF in different environments

Environments	Absorption maximum	Emission maximum	E_T (30)/(kcal mol^{-1})	Fluorescence anisotropy (r)
Buffer	520	583	61.5	0.03
γ-CD	521	581	60.1	0.056
DNA	537	576	56.9	0.24
γ-CD + DNA	534	576	56.9	0.24

Table II — Time resolved fluorescence decay parameters of PSF in different environments

Environments	τ_1 (ns) (± 0.1)	a_1	τ_2 (ns) (± 0.1)	a_2	τ_{avg} (ns) (± 0.1)	χ^2
Buffer	0.90				0.90	1.09
25 mM γ-CD	0.91	0.93	1.65	0.07	0.96	1.02
100 μM ctDNA	0.92	0.85	2.94	0.15	1.22	1.10
25 mM γ-CD + 100 μM ctDNA	0.90	0.85	3.01	0.15	1.21	1.05

reactions[42]. In this work, the fluorescence decay profiles of PSF have been measured in aqueous buffer, γ-CD, ctDNA and (CD + DNA) environments, respectively, as depicted in Figure 7 and the deconvoluted lifetime data are collected in Table II. In aqueous buffer, PSF gives a mono-exponential decay profile yielding a lifetime ~ 0.9 ± 0.1 ns[31]. With increasing CD and DNA concentrations, a significant modification in the decay profiles are observed. In both the media, the decay profiles of PSF become bi-exponential giving fluorescence lifetime values 0.91 ± 0.1 ns and 1.65 ± 0.1 ns in CD environment and 0.92 ± 0.1 and 2.94 ± 0.1 ns in DNA medium, respectively. The component with lifetime around 0.9 ns is ascribed to correspond to the free fluorophore. The multi-exponential nature of the decay profiles reveals binding interaction of PSF with both the carrier (CD) and the target (DNA)[17,36]. With the addition of ctDNA to the carrier bound probe, the decay profile of PSF remains bi-exponential and at high DNA concentration, the lifetime values are obtained as 0.90 ± 0.1 and 3.01 ± 0.1 ns, respectively. Both the lifetime

values as well as the pre-exponential factors (a_1 and a_2) in the composite medium (CD + DNA) become equal to those in DNA medium only, implying that the probe resides in the same environment in both the cases and hence justify the transfer of the probes from the CD nanocavity to the DNA, consistent with other spectroscopic studies.

Circular Dichroism Studies

The most important pre-requisite of targeted drug delivery towards DNA is that the carrier should not affect the secondary structure of DNA. To confirm that γ-cyclodextrin does not influence the conformation of the DNA, we have studied the intrinsic circular dichroism of DNA in the absence and in the presence of cyclodextrin (Figure 8a). The intrinsic circular dichroism spectrum of DNA consist of one positive peak at ~ 276 nm due to the base pair stacking and a negative peak at ~ 244 nm ascribed to the polyneucleotide helicity[43]. Retention of DNA secondary structure even in the presence of cyclodextrin (inset of Figure 8a) implies that γ-cyclodextrin can be used as a safe drug carrier targeted to DNA. However, addition of PSF in pure DNA results in a significant alteration of both the positive and the negative CD bands, confirming intercalative binding of PSF with DNA (Figure 8a)[27]. To confirm the delivery of the probe in the DNA medium leaving the cyclodextrin cavity in the composite medium, circular dichroism studies have also been performed. Being planar and achiral, the fluorophore (PSF) does not show any circular dichroism spectrum (Figure 8b). Inclusion of PSF in γ-cyclodextrin also does not develop any CD signal. However, addition of DNA on the cyclodextrin-bound PSF gives two signature peaks for DNA intercalated probe. It is relevant to mention here that the CD spectral profile of the DNA (90 μM) with PSF matches exactly with the CD spectrum of the composite medium (PSF + CD (24 mM) + DNA (90 μM)) as depicted in Figure 8b (inset), indicating the environments around the probe are similar in both the systems. Thus, the circular dichroism spectral studies confirm the delivery of probe molecule from the cyclodextrin to the DNA in the composite medium.

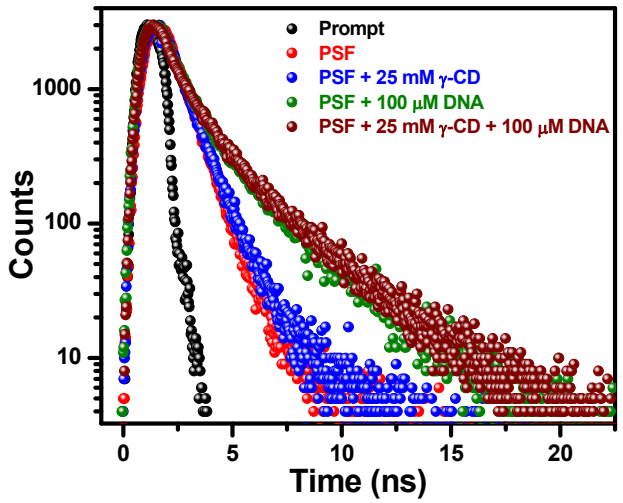


Figure 7 — Fluorescence decay profiles of PSF in buffer, 25 mM γ-CD, 100 μM ctDNA and composite medium (25 mM γ-CD + 100 μM ctDNA). The sharp profile on the extreme left represents the instrument response function. [PSF] = 5 μM, λ_{ex} = 450 nm and $\lambda_{monitor} = \lambda_{em}^{max}$.

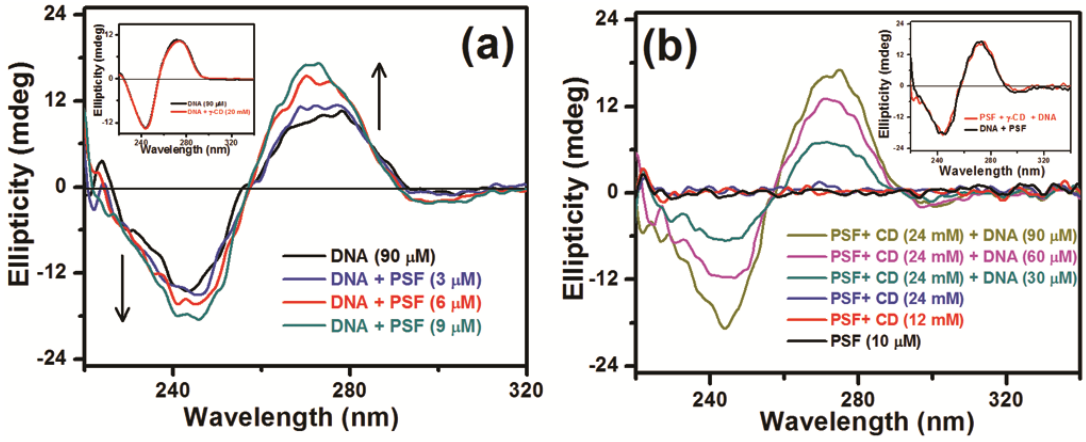


Figure 8 — Circular dichroism spectral profiles of (a) DNA with gradual addition of PSF, and (b) PSF, addition of γ-CD on PSF and addition of DNA on γ-CD-bound PSF. Inset of (a) shows the intrinsic circular dichroism spectra of DNA in the absence and in the presence of 24 mM γ-CD. Inset of (b) depicts the circular dichroism spectra of PSF-bound DNA and DNA on γ-CD-bound PSF. Concentrations of PSF, γ-CD and ctDNA are depicted in the legends.

Helix Melting Study

The location of the probe molecule in the composite medium (PSF + γ-CD + ctDNA) is further corroborated by the DNA helix melting studies. The melting temperature (T_m) of DNA is referred to the temperature at which half of the double stranded DNA (dsDNA) is converted into single stranded DNA (ssDNA)[44]. The extent of strand separation, *i.e.*, DNA melting is assessed by measuring the absorbance of DNA at 260 nm as a function of temperature. The molar extinction coefficient of the single stranded DNA is much higher than that of the double stranded form[40,41]. The DNA melting profiles in different microenvironments are presented in Figure 9 and the T_m values obtained from the inflection points of the sigmoidal curves are listed in Table III. The melting temperature of native DNA in aqueous buffer solution is found to be 71.9°C, consistent with the literature reports[15,17,36]. Addition of PSF to the DNA solution leads to a substantial enhancement (~ 5 °C) in the T_m value, suggesting intercalative binding of PSF with the DNA as it increases the thermal stability of the dsDNA by stabilizing the π-π stacking[36,45]. Addition of γ-cyclodextrin to the aqueous solution of DNA does not alter the T_m value of DNA, suggesting insignificant effect of the carrier (γ-CD) on the structure of dsDNA and it goes parallel to the similar observations from the circular dichroism studies. However, the T_m value of DNA (77.4°C) of the composite medium containing ctDNA, PSF and γ-CD results in a ~ 5.5°C increment of the melting temperature, close to the T_m value of DNA intercalated with PSF molecules. In tune with other spectroscopic studies, the DNA helix melting studies unambiguously reveal that in the composite medium PSF preferentially resides within the DNA base pairs and confirms the delivery of the probe molecule to the DNA.

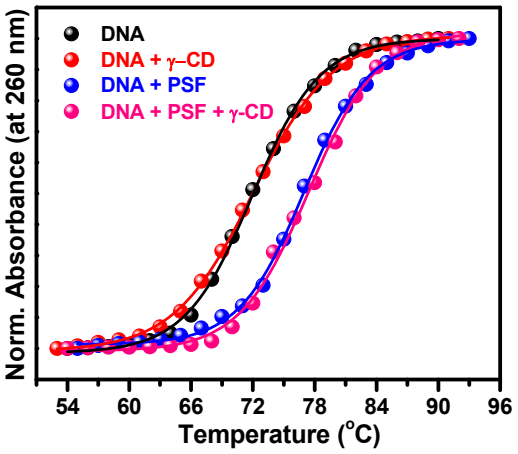


Figure 9 — Thermal melting curves of native DNA in different environments. The environments are given in the legends. [DNA] = 100 μM, [PSF] = 10 μM, γ-CD = 30 mM.

Table III — Melting temperatures of ctDNA in different environments

Environments	$T_m \pm 0.4$ (°C)
Buffer	71.9
PSF	77.0
γ-CD	72.0
PSF + γ-CD	77.4

Experimental Section

Materials

Phenosafranin (PSF), γ-cyclodextrin (γ-CD), sodium salt of calf thymus DNA (ctDNA) and HEPES buffer were procured from Sigma-Aldrich (USA) and used as received. Deionised water from a Milli-Q water purification system (Millipore) was used for the preparation of buffer solutions. The *p*H of 50 mM HEPES buffer solution was maintained at the physiological *p*H of 7.4 throughout the experiment.

The stock solution of ctDNA was prepared by dissolving solid ctDNA in HEPES buffer and was stored at 4°C. The ratio of the absorbance of ctDNA solution at 260 nm to that at 280 nm, which was in the range of 1.8−1.9, ensures its purity. 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.

Methods

The steady state absorption studies were carried out on a Shimadzu UV-2450 absorption spectrophotometer (Shimadzu Corporation, Kyoto, Japan). A Horiba Jobin Yvon Fluoromax-4 spectrofluorometer was exploited for the steady state fluorescence and fluorescence anisotropy measurements. Fluorescence anisotropy (r) is defined as[42]

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

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[42]

$G = I_{HV}/I_{HH}$

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 on a Horiba Jobin Yvon FluoroCube fluorescence lifetime system using NanoLED at 450 nm (IBH, UK) as excitation source and TBX photon detection module as detector. The decays were analyzed using IBH DAS-6 decay analysis software. 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

$$\tau_{avg} = a_1\tau_1 + a_2\tau_2$$

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 and appropriate baseline corrections were made by using aqueous HEPES buffer solution.

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°C) that was attached to the above mentioned spectrophotometer. All the other experiments were performed at room temperature (298 K) with air-equilibrated solutions.

Conclusions

The present work articulates the delivery of a bioactive fluorophore, PSF from the γ-cyclodextrin nanocavity to the natural DNA. Comprehensive steady state and time resolved emission studies along with circular dichroism and DNA helix melting studies unequivocally ascertain that upon addition of the DNA to the γ-cyclodextrin-bound probe, the probe molecule gets released from the cyclodextrin nanocavity and is intercalated within the DNA base pairs. Stronger binding affinity of PSF towards the ctDNA, a signature of intercalative binding, compared to that with γ-cyclodextrin, is responsible for the transfer of the probe from the CD to the DNA. Non-toxic nature of γ-cyclodextrin and the preservation of the secondary structure of DNA in its presence imply that γ-cyclodextrin is a suitable and safe carrier for the delivery of the drug molecule targeted to the natural DNA.

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