J Fluoresc (2015) 25:1637–1643
DOI 10.1007/s10895-015-1650-x

ORIGINAL ARTICLE

A Novel Styryldehydropyridocolinium Homodimer: Synthesis and Fluorescence Properties Upon Interaction with DNA

Huirong Yao[1] · Lifang Chang[1] · Chang Liu[1] · Xiaojie Jiao[1] · Song He[1] · Haijun Liu[2] · Xianshun Zeng[1]

Received: 3 May 2015 / Accepted: 9 September 2015 / Published online: 17 September 2015

Abstract A novel homodimer of the styryldehydropyridocolinium dye (**TPTP**) has been synthesized and characterized. Free **TPTP** exhibited low fluorescence quantum yield and large Stokes shift (over 160 nm) in water. However, it showed a significant fluorescence turn-on effect upon intercalation into DNA base pairs. Meanwhile, the fluorescence intensity of the intercalated structures formed by **TPTP** and DNA decreased quickly upon addition of deoxyribonuclease I, indicating that the dye can be used to monitor deoxyribonuclease I activity and DNA hydrolysis. Electrophoresis analysis revealed that the dye had intercalative binding to DNA and can potentially be used for DNA staining in electrophoresis. Thus, the innate nature of large Stokes shift and excellent fluorescence turn on effect upon interaction with DNA endue the dye with a wide range of applications.

Keywords Cyanine · Styryldehydropyridocolinium dye · Fluorescence spectroscopy · DNA · Sensor

Electronic supplementary material The online version of this article (doi:10.1007/s10895-015-1650-x) contains supplementary material, which is available to authorized users.

✉ Xianshun Zeng
xshzeng@tjut.edu.cn

[1] Tianjin Key Laboratory for Photoelectric Materials and Devices, School of Material Science & Engineering, Tianjin University of Technology, Tianjin 300384, China

[2] Tianjin Institute of Animal Science and Veterinary Medicine, Tianjin 300112, China

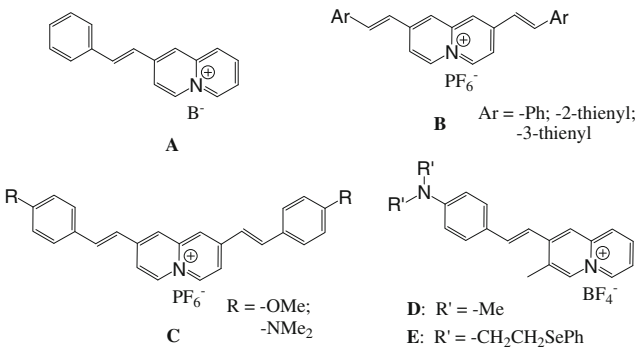
Introduction

For the past two decades, cationic planar, polycyclic cyanine dyes such as thiazole orange (TO), oxazole yellow (YO), and their homodimers TOTO and YOYO have been successfully used for fluorescence detection of DNA, RNA or other biomolecules [1–4]. As signaling elements in sensors, their nucleic acid oligomers or peptide conjugates have also found numerous applications in bioanalytical, clinical, and forensic analyses [5–8]. Recognition events/behaviors are normally characterized by a significantly enhanced fluorescence signal upon binding of the probe to its target. Compared with the fluorescence of ion-selective probes which is changed through intramolecular electron, charge, or energy transfer mechanism upon the interactions with target analytes, that of DNA-selective asymmetric cyanines is usually enhanced due to the fact that the twisting of excited-state around the central methine bridge separating the two heterocycles is restricted upon dye intercalation in the pocket defined by DNA base pairs, namely via an intramolecular charge transfer (ICT) or twisted intramolecular charge transfer (TICT) fluorescent mechanism [1–4]. Based on this intercalative mechanism, many cationic planar, polycyclic dyes derived from quinolizinium, benzooxazolinium, benzothiazolinium, and acridizinium have been developed as powerful optical probes for DNA detection [9, 10]. These DNA-selective probes demonstrate a variety of potential applications in pharmaceutical studies and molecular biology, and can be used as probes for electron and/or energy transfer and gene modulators [11–21].

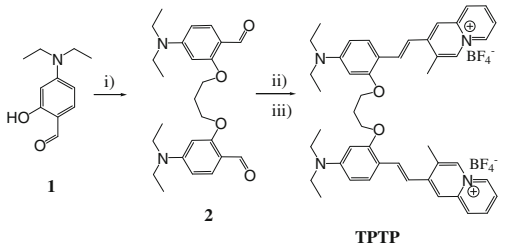
Although the synthesis of styryldehydropyridocolinium dye **A** (Scheme 1), a member of the cyanine family, was reported a few decades ago [22], the physical and photochemical properties of this type of dyes remain underdeveloped. The V-shaped derivatives of styryldehydropyridocolinium dye **B** have been reported to undergo an intramolecular cyclic

reaction to form 8a-azonia[6]helicene or 7a-azonia-3,12-dithia[6]helicene under 450 W high-pressure mercury lamp irradiation using iodine as sensitizer [23, 24]. In 2011, Maçôas et al. demonstrated firstly that V-shaped **C** derivatives can be used as two-photon fluorescent biomarkers for selective staining of vesicular organelles in the cytoplasm. Meanwhile, the cationic V-shaped **C** also shows prominent fluorescence enhancement upon interaction with DNA [25]. More recently, we found that the styryldehydropyridocolinium derivative **D** is a fluorescence turn-on probe for DNA. The fluorescence intensity of **D** increases by a factor of 60-fold upon intercalative binding with DNA [26]. On the other hand, the intercalates **DNA@SPC** formed by the intercalation of the cationic styryldehydropyridocolinium dye **E** in DNA base pairs turned out to be high Ag^+-selective fluorescence turn on chemosensors [27]. Therefore, similarly to other cationic styryl dyes, styryldehydropyridocolinium derivatives also interact with DNA with significant fluorescence enhancement due to the positively charged, planar, polycyclic nature of the dyes. Upon intercalations into DNA base pairs, the TICT is favored by reduction in mobility of the dye [1–4]. We hypothesized that the excellent fluorescence emission properties of this type of dyes would be very useful for the molecular design of probes for anionic biomolecules such as DNA and proteins.

As a continuous project for the development of DNA probes [26–29], we report herein, the molecular design of a novel positively charged homodimer of styryldehydropyridocolinium dye (**TPTP**) and its optical properties upon interaction with DNA (Scheme 2). The choice of styryldehydropyridocolinium derivative as fluorophore was based on the following considerations: a) the positively charged, planar, polycyclic nature of the dye is not only favorable for binding to nucleic acids, but also beneficial for the intercalation process as demonstrated with other DNA probes [30–35]; b) large Stokes shift (>100 nm) is an innate nature of asymmetric cyanine dyes [36–47]; c) high mobility of the electron-donating and electron-accepting moieties of the dye around the etheno bridge usually



Scheme 1 Structures of some previously reported styryldehydropyridocolinium dyes



Scheme 2 Synthesis of the probe **TPTP**. Reagents and conditions: i) 2-hydroxyl-4-diethylaminobenzaldehyde **1**, 1,3-dibromopropane and K_2CO_3 in DMF, 80 °C, 72 h; ii) 2,3-dimethyldehydroquinolizinium chloride, **2** and piperidine in anhydrous EtOH, 85 °C, 72 h; iii) KBF_4, room temperature, 2 h

leads to strong ICT or TICT optical changes before and after interaction with analytes [33–37]. Thus, it is reasonable to propose that **TPTP** could show a prominent optical signal response before and after binding with anionic biomolecules. As predicted, **TPTP** exhibited very weak fluorescence in sodium phosphate buffer solution, and showed strong turn on effect upon binding with calf thymus DNA (ct-DNA).

Experimental

All solvents and reagents (analytical grade and spectroscopic grade) were used as received unless otherwise mentioned. 2-Hydroxyl-4-diethylaminobenzaldehyde **1** was purchased from Tokyo Chemical Industry (TCI). Bovine pancreatic deoxyribonuclease I (DNase I, 2000 units/mg, Sigma D3392) and calf thymus DNA (ct-DNA, Sigma D1501) were purchased from Dingguo Biotech Co. Ltd (Beijing, China). DNA concentrations were measured by spectrophotometry (260 nm, ε=6600 M^{-1} cm^{-1}). The deoxyribonuclease I concentration was determined by Lowry method. Sodium phosphate buffer solution (pH=7.4) was used for all experiments. NMR spectra were recorded on a Bruker spectrometer at 400 (^{1}H NMR) and 100 (^{13}C NMR) MHz, with chemical shifts reported in ppm. High-resolution mass spectra (HRMS) were acquired on an Agilent 6510 Q-TOF LC/MS instrument equipped with an electrospray ionization (ESI) source. Elemental analyses were performed on a Vanio-EL elemental analyzer. Absorption spectra were obtained on a Shimadzu UV-3600 spectrophotometer (Japan). Fluorescence spectra were acquired using a Hitachi F-4600 fluorescence spectrophotometer (Japan). Melting points were recorded on a RY-2 Melting Point Analyzer (Analytical Instrument Factory, Tianjin) and are uncorrected. Electrophoresis was carried out on a DYCP-31DN electrophoresis cell, and gels were imaged using a gel rapid documentation apparatus (Bio-Rad, USA).

All anions and the chemosensor **TPTP** were dissolved in sodium phosphate buffer (2 mM, pH=7.4) to obtain 10 mM

stock solutions. Before spectroscopic measurements, the fresh solutions of bovine pancreatic deoxyribonuclease I (DNase I), calf thymus DNA (ct-DNA), ADP, AMP, and UMP were prepared. Other buffered solutions were freshly prepared by diluting the stock solutions to the required concentrations. All experiments were performed at room temperature.

Synthesis of bis-2-(4-p-diethylaminobenzaldehyde)-1,3-propyldiether 2 To a 100 mL flask, 2-hydroxyl-4-diethylaminobenzaldehyde **1** (2.9 g, 15 mmol) was mixed with a solution of 1,3-dibromopropane (1.2 g, 6 mmol) and potassium carbonate (4.2 g, 30 mol) in DMF (30 mL). The reaction mixture was stirred for 72 h at 80 °C followed by addition of water (300 mL). Then, the organic phase was separated and the aqueous phase extracted with dichloromethane (10 mL×5). The combined organic phase was dried with anhydrous Na_2SO_4, and the solvent removed under reduced pressure. The residue was purified by column chromatography (SiO_2, petroleum ether/ethyl acetate, 3:2, v/v). The product was obtained as light green crystals with a 49 % yield (1.26 g). mp: 138-139 °C. HRMS: m/z $[M + H]^+$= 427.2596; Calcd: 427.2597; ^{1}H NMR (400 MHz, $CDCl_3$, ppm): 10.14 (s, 2H), 7.67 (d, *J*=8.8 Hz, 2H), 6.25 (dd, *J*= 8.8 Hz, 2.0 Hz, 2H), 6.02 (d, *J*= 2.0 Hz, 2H), 4.24 (t, *J*= 7.8 Hz, 4H), 3.38 (m, *J*=7.2 Hz, 8H), 2.35 (m, 2H), 1.18 (t, *J*=7.0 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): 186.8, 163.7, 154.1, 130.7, 114.4, 104.7, 93.5, 64.5, 45.0, 29.5, 12.8. Anal. Calcd for $C_{25}H_{34}N_2O_4$: C 70.39; H 8.03; N 6.57; Found: C 70.21 %, H 7.49 %, N 6.65 %.

Synthesis of the Probe TPTP In a 50 mL flask, 2,3-dimethyldehydroquinolizinium chloride (0.156 g, 0.4 mmol), bis-2-(4-p-diethylamino-benzaldehyde)-1,3-propyldiether **2** (0.171 g, 0.4 mmol), anhydrous ethanol (5 mL), and piperidine (20μL) were added. Then, the reaction mixture was stirred for 72 h in an oil bath at 85 °C. Afterwards, KBF_4 (300 mg) was added in one portion and stirred for another 2 h at room temperature. The resulting mixture was filtered and the filtrate evaporated to dryness. The residue was recrystallized from methanol. The product **TPTP** was obtained as purple red powder with a 64 % yield (0.200 g); mp: 258-260 °C. HRMS: m/z $[M\text{-}2BF_4^-]^{2+}$=353.2128; Calcd: 353.2124; ^{1}H NMR (300 MHz, DMSO-d_6, ppm): 9.09 (s, 2H), 8.98-8.96 (d, *J*=6 Hz, 2H), 8.56 (s, 2H), 8.19-8.17 (d, *J*=6 Hz, 2H), 8.07-8.03 (t, *J*= 12 Hz, 2H), 7.87-7.83 (d, *J*= 12 Hz, 2H), 7.78-7.75 (t, *J*=9 Hz, 2H), 7.48-7.46 (d, *J*=6 Hz, 2H), 7.23-7.19 (d, *J*=12 Hz, 2H), 6.28-6.26 (4H), 4.42 (4H), 3.33 (8H), 2.50-2.45 (2H, 6H), 1.06 (12H); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 160.0, 151.0, 147.9, 141.4, 136.5, 135.2, 135.0, 134.9, 132.2, 131.8, 126.4, 121.8, 117.6, 115.0, 112.7, 105.2, 95.7, 65.8, 44.4, 17.2, 13.0; Anal. Calcd for $C_{47}H_{54}B_2F_8N_4O_2\cdot 2MeOH$: C 62.30; H 6.62; N 5.93; Found: C 62.28 %, H 6.39 %, N 6.17 %.

TPTP's Fluorescence Quantum Yield The fluorescence quantum yield of the probe **TPTP** (Φ_1) was measured and calculated according to the following equation:

$$\Phi_1 = \Phi_B \frac{Abs_B \times F_1 \times \lambda_{exB} \times \eta_1^2}{Abs_1 \times F_B \times \lambda_{ex1} \times \eta_B^2}$$

Where Φ_1 and Φ_B are quantum yields of the probe **TPTP** and standard, respectively; Abs_1 and Abs_B represent the absorbance values at excitation wavelengths; F_1 and F_B are integration areas; λ_{ex1} and λ_{exB} are excitation wavelengths; η_1 and η_B are refractive indexes.

Here, rhodamine B was used as the standard, and the wavelength of 500 nm at which both absorption curves of rhodamine B and **TPTP** intersect chosen as the excitation wavelength, so the equation is:

$$\Phi_1 = \Phi_B \times \frac{F_1 \times \eta_1^2}{F_B \times \eta_B^2}$$

Results and Discussion

Synthesis of TPTP Scheme 2 illustrates the synthesis of the dye **TPTP**. The bisaldehyde **2** was prepared in 49 % yield through the nucleophilic reaction of 4-(diethylamino)-2-hydroxybenzaldehyde **1** with 1,3-dibromopropane in the presence of K_2CO_3. Subsequent reaction of **2** with 2,3-dimethyldehydroquinolizinium chloride [48] in ethanol in the presence of piperidine as a catalyst yielded the bisstyryldehydropyridocolinium derivative. The anions were exchanged by stirring the reaction mixture with excess KBF_4 in ethanol solution. The dye **TPTP** obtained was isolated as purple red powder in 64 % yield by crystallization from methanol. The structure of **TPTP** was characterized by ^{1}H and ^{13}C NMR spectroscopy, HRMS, and elemental analyses.

Optical Properties of TPTP To understand the optical properties of **TPTP**, absorption and fluorescence emission spectra of freshly prepared **TPTP** solutions in solvents with different polarity were analyzed (Fig. 1). According to spectrophotometric measurements, strong solvatochromic effects were observed for the dye. **TPTP** in water exhibited a maximum absorption at 464 nm (ε=4.8×10^4 M^{-1} cm^{-1}) which can be assigned to the π-π^* transitions [36–40]. Compared with the absorption in water, ca 30 nm red shift with increased molar extinction coefficients (ε=6.2 - 7.2×10^4 M^{-1} cm^{-1}) in DMF, EtOH, CH_2Cl_2, acetone, DMSO, and acetonitrile were observed. However, the maximum absorption bands blue-shifted to ca. 410 nm (ε=~ 2.0×10^4 M^{-1} cm^{-1}) in THF, ethyl acetate, toluene, and 1,4-dioxane. These findings are indicative of intramolecular charge transfer (ICT) [49]. Upon excitation at 500 nm, **TPTP** emission in toluene peaked at ~570 nm, and shifted bathochromically to ~630 nm with

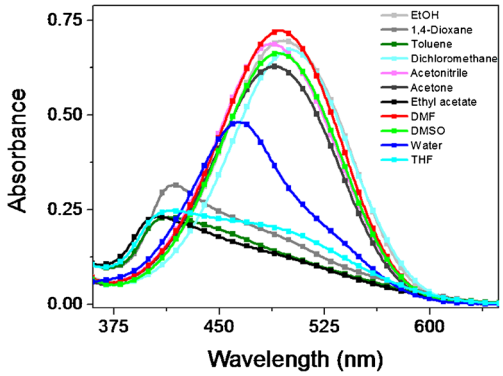


Fig. 1 UV–Vis absorption spectra of **TPTP** (10 μM) in various freshly prepared solvents: ethanol, 1,4-dioxane, toluene, CH_2Cl_2, acetonitrile, acetone, ethyl acetate, DMF, DMSO, H_2O, and THF

increased solvent polarity (Fig. 2). Of note, **TPTP** in water exhibited maximum emission at 625 nm along with very weak emission intensity. The innate weak emission nature and large Stokes shift of over 100 nm of the dye is a pivotal factor of a signaling fluorophore for the molecular design of "light-up probes". [50].

Fluorescence Evaluation of DNA Binding Interaction

Fluorescence emission spectra of **TPTP** with incremental amounts of ct-DNA were recorded in sodium phosphate buffer (pH=7.4). As shown in Fig. 3, the free probe **TPTP** showed a low fluorescence intensity at 625 nm (Φ_{FL}=0.0035). Upon addition of ct-DNA, the fluorometric titration reaction curves displayed a steady and smooth increase at 616 nm. An enhanced fluorescence (Φ_{FL}=0.0504) with a turn-on ratio of ca. 14.4-fold at 616 nm was triggered upon binding to ct-DNA (50.0 equivalents) via a TICT mechanism. Job's plot using fluorescence changes showed a ct-DNA-to-**TPTP** ratio of 2:1 (Figure S1) [51]. The binding association constant derived from fluorometric titrations was estimated at 2.92×

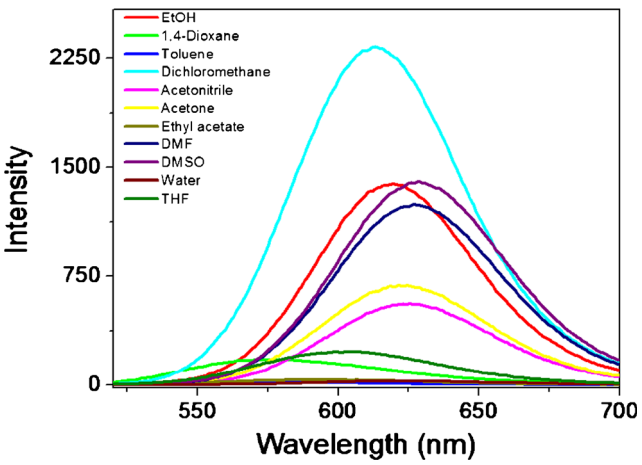


Fig. 2 Fluorescence emission spectra of **TPTP** (10 μM) in various freshly prepared solvents: ethanol, 1,4-dioxane, toluene, CH_2Cl_2, acetonitrile, acetone, ethyl acetate, DMF, DMSO, H_2O, and THF. λ_{ex}=500 nm, slit: 5.0, 5.0

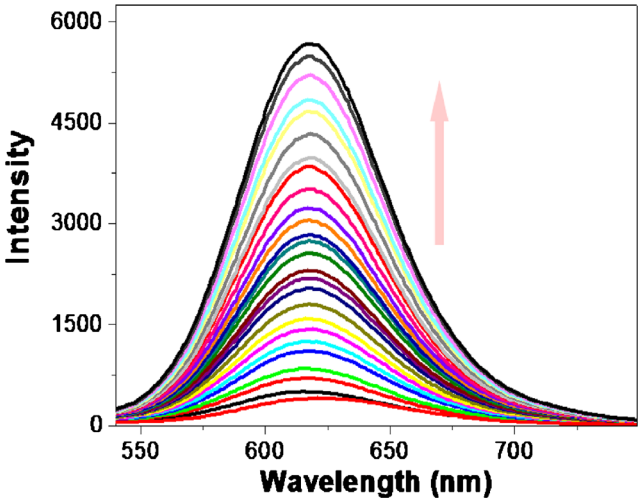


Fig. 3 Spectrofluorimetric titration of ct-DNA (0-50.0 equivalents) to **TPTP** (10 μM) in 2 mM sodium phosphate buffer (pH=7.4). Excitation wavelength λ_{ex}=500 nm, slit 10.0 nm, 10.0 nm

10^7 M^{-2} (R>0.99) by using nonlinear least-square analysis with a 2:1 ct-DNA-to-**TPTP** ratio (Figure S2). These results indicated a strong association of the dye with DNA, providing a straightforward way to quantify DNA by fluorescence.

Next, the fluorescence responses of **TPTP** to ct-DNA and other anionic species such as ATP, Br^-, Cl^-, ct-DNA, F^-, HCO_3^-, HSO_4^-, $P_2O_7^{4-}$, ADP, AMP, and UMP in sodium phosphate buffer (2 mM, pH=7.4) were compared (Fig. 4). Among the anionic species examined, **TPTP** only showed overtly enhanced fluorescence (over 9-fold) upon addition of 20.0 equivalents ct-DNA. However, no marked effects on fluorescence emission were observed with other anions (30.0 equivalents). In addition, the detection limit was calculated to be 1.13×10^{-7} M from the ct-DNA-fluorescence dependence

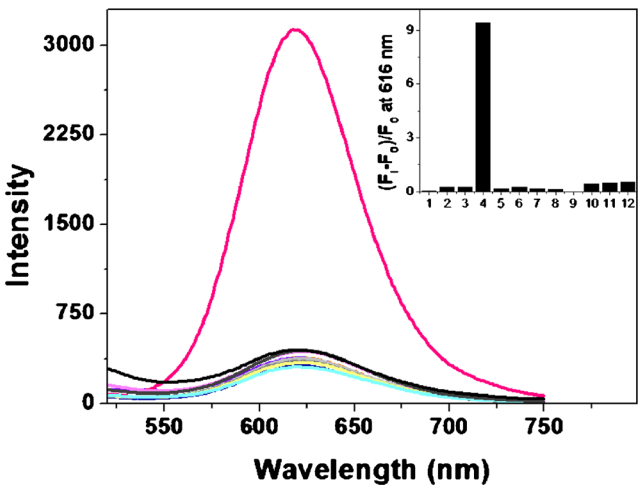


Fig. 4 Fluorescence profile of **TPTP** (10 μM) in the presence of various anions (20.0 equivalents for ct-DNA and 30.0 equivalents for other anions). Inset: histogram representing fluorescence enhancement at 616 nm. From 1 to 12: ATP, Br^-, Cl^-, ct-DNA, F^-, HCO_3^-, HSO_4^-, $P_2O_7^{4-}$, **TPTP** alone, ADP, AMP, and UMP in sodium phosphate buffer. λ_{ex}=500 nm, slit 10.0 nm, 10.0 nm

curve of the probe (Figure S3) [52]. On the other hand, **TPTP** also showed a strong fluorescence turn on effect upon interaction with fish sperm DNA (fs-DNA) (Figure S4). These results indicated that the dye could be used as a selective probe for DNA.

To further elucidate the binding modes and strengths between **TPTP** and ct-DNA, melting experiments were carried out in sodium phosphate buffer (2 mM, pH=7.4). Herein, helix melting temperatures, i.e., temperatures at which the double helix structure disassembles to form single stranded DNA, respectively in the presence and absence of the dye were recorded by monitoring the absorbance of ct-DNA at 260 nm as a function of temperature. The melting temperature of ct-DNA (63.4 °C) increased by 12.5 °C at 1/7.6 ratio of C_{TPTP}/C_{DNA} (Figure S5). These results suggested that the intercalation of the dye to duplex DNA base pairs largely stabilizes the DNA's double helix.

To compare the properties of **TPTP** with that of ethidium bromide (**EB**) which has been widely used as powerful fluorescent probes for DNA, the competitive experiments of **TPTP** and **EB** with DNA were carried out under the same conditions. The addition of DNA (10 equivalents) to the sodium phosphate buffer solutions of **EB** led to a significant increase (13.1-fold) of the florescence at 600 nm. At the same time, the florescent increase at 618 nm for **TPTP** was found to be 33.1-fold (Figure S6). The results indicated that the fluorescence turn-on property of **TPTP** is better than that of **EB** under the same conditions. However, addition 1 equivalent of **EB** to the equilibrated **TPTP** + DNA solution or addition of **TPTP** to the equilibrated **EB** + DNA solution, they showed an emission peak at 600 nm as those of equilibrated **EB** + DNA solution. The results indicated that both **TPTP** and **EB** can intercalative binding to duplex DNA [8].

Electrophoresis Assay To examine the potential use of **TPTP** as a fluorescent DNA indicator, electrophoresis was conducted, and the intercalation properties between the probe and DNA evaluated. As shown in Fig. 5, the mobility of ct-DNA was not affected by the intercalation of **TPTP**, which showed the same mobility as that obtained with Goldenview™ staining. However, higher dye concentrations led to a fluorescence self-quenching effect. These findings indicated that the probe has intercalative binding to ct-DNA and can potentially be used for DNA staining in electrophoretic analyses.

Enzyme Activity Monitoring The probe **TPTP** was then utilized as a fluorescent sensor for monitoring the activity of bovine pancreatic deoxyribonuclease I (DNase I). Digestion by DNase I of a mixture containing the probe (10 μM) and DNA (10.0 equivalents) in sodium phosphate buffer solution was assessed (Fig. 6). The

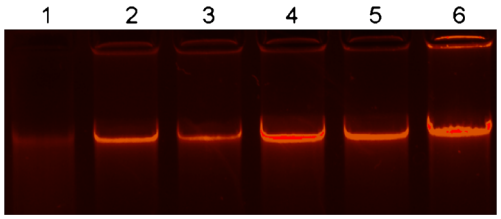


Fig. 5 Electrophoresis gel showing the interactions between **TPTP** and ct-DNA (500 ng) at increasing concentrations. The ct-DNA/**TPTP** ratio is 1, 5, 10, 20, and 30 for lane 1 to lane 5, respectively. Lane 6 is the reference Goldenview with ct-DNA

fluorescence intensity of the probe and DNA mixture at 616 nm decreased rapidly upon addition of DNase I, indicating the release of intercalated probes from the space confined by DNA base pairs, upon the enzyme-catalyzed hydrolysis of the DNA double helix. These results demonstrated that the probe can be used to monitor enzyme activity and hydrolysis [53, 54].

Conclusion

In summary, a novel DNA-responsive, positively charged fluorescent homodimer of styryldehydropyridocolinium dye (**TPTP**) has been synthesized and characterized. It exhibits a typical maximum π-π^* transition absorption at 464 nm, and very weak emission at 625 nm in water. The positively charged homodimer showed a marked selective fluorescence turn-on effect with calf thymus DNA among various anions examined in sodium phosphate buffer. It can also be used as a potential indicator to monitor deoxyribonuclease I activity and DNA hydrolysis process. Meanwhile, DNA electrophoresis data indicated that TPTP can be used as a probe for DNA staining in electrophoresis as well.

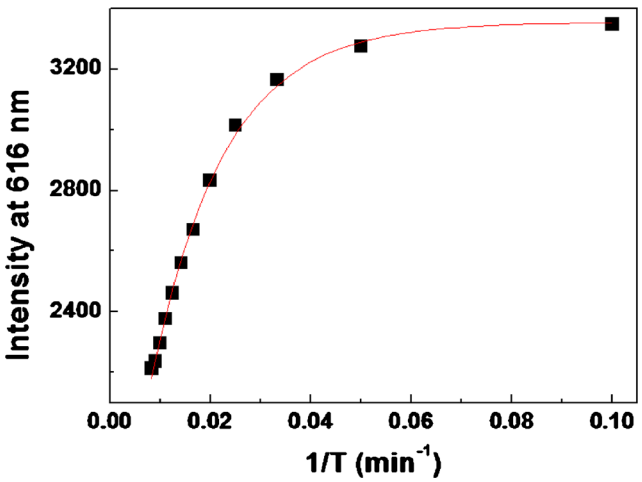


Fig. 6 Fluorescence intensity (at 616 nm) against 1/T in **TPTP** (10 μM) solution in the presence of 10.0 equivalents ct-DNA and 1.0 equivalent DNase I

Acknowledgments We gratefully acknowledge the Natural Science Foundation of China (NNSFC 21272172), and the Natural Science Foundation of Tianjin (12JCZDJC21000).

References

1. Glazer AN, Rye HS (1992) Stable dye-DNA intercalation complexes as reagents for high-sensitivity fluorescence detection. Nature 359:859–861
2. Lee LG, Chen C, Liu LA (1986) Thiazole orange: a new dye for reticulocyte analysis. Cytometry 7:508–517
3. Rye HS, Yue S, Wemmer DE, Quesada MA, Haugland RP, Mathies RA, Glazer AN (1992) Stable fluorescent complexes of double-stranded DNA with bisintercalating asymmetric cyanine: properties and applications. Nucleic Acids Res 20:2803–2812
4. Nygren J, Svanvik N, Kubista M (1998) The interactions between the fluorescent dye thiazole orange and DNA. Biopolymers 46:39–51
5. Ishiguro R, Saitoh J, Yawata H, Otsuka M, Inoue T, Sugiura Y (1996) Fluorescence detection of specific sequence of nucleic acids by oxazole yellow-linked oligonucleotides. Homogeneous quantitative monitoring of in vitro transcription. Nucleic Acids Res 24: 4992–4997
6. Seitz O, Bergmann F, Heindl D (1999) A convergent strategy for the modification of peptide nucleic acids: novel mismatch-specific PNA-hybridization probes. Angew Chem Int Ed 38:2203–2206
7. Svanvik N, Westman G, Wang D, Kubista M (2000) A high-performance liquid chromatographic method to measure sphingosine 1-phosphate and related compounds from sphingosine kinase assays and other biological samples. Anal Biochem 281:26–34
8. Carreon JR, Mahon KP Jr, Kelley SO (2004) Thiazole orange-peptide conjugates: sensitivity of DNA binding to chemical structure. Org Lett 6:517–519
9. Fürstenberg A, Julliard MD, Deligeorgiev TG, Gadjev NI, Vasilev AA, Vauthey E (2006) Ultrafast excited-state dynamics of DNA fluorescent intercalators: newinsight into the fluorescence enhancement mechanism. J Am Chem Soc 128:7661–7669
10. Mohanty J, Barooah N, Dhamodharan V, Harikrishna S, Pradeepkumar PI, Bhasikuttan AC (2013) Thioflavin T as an efficient inducer and selective fluorescent sen-sor for the human telomeric G-quadruplex DNA. J Am Chem Soc 135:367–376
11. Friedman AE, Chambron JC, Sauvage JP, Turro NJ, Barton JK (1990) A molecular light switch for DNA: $Ru(bpy)_2(dppz)^{2+}$. J Am Chem Soc 112:4960–4962
12. Jung Y, Lippard SJ (2007) Direct cellular responses to platinum-induced DNA damage. Chem Rev 107:1387–1407
13. Wu J, Zou Y, Li C, Sicking W, Piantanida I, Yi T, Schmuck C (2012) A molecular peptide beacon for the ratiometric sensing of nucleic acids. J Am Chem Soc 134:1958–1961
14. Hernandez-Folgado L, Baretić D, Piantanida I, Marjanović M, Kralj M, Rehm T, Schmuck C (2010) Guanidiniocarbonylpyrrole-aryl derivatives: structure tuning for spectrophotometric recognition of specific DNA and RNA sequences and for antiproliferative activity. Chem Eur J 16:3036–3056
15. Tanada M, Tsujita S, Kataoka T, Sasaki S (2006) Cu^{2+}-mediated assembly of the minor groove binders on the DNA template with sequence selectivity. Org Lett 8:2475–2478
16. Neto BAD, Lapis AAM, Mancilha FS, Vasconcelos IB, Thum C, Basso LA, Santos DS, Dupont J (2007) New sensitive fluorophores for selective DNA detection. Org Lett 9:4001–4004
17. Law GL, Man C, Parker D, Walton JW (2010) Observation of the selective staining of chromosomal DNA in dividing cells using a luminescentterbium(III) complex. Chem Commun 46:2391–2393
18. Davis SK, Bardeen CJ (2003) Cross-linking of histone proteins to DNA by UV illumination of chromatin stained with hoechst. Photochem Photobiol 77:675–679
19. Pfeifer GP, You YH, Besaratinia A (2005) Mutations induced by ultraviolet light. Mutation Res 571:19–31
20. Smith PJ, Blunt N, Wiltshire M, Hoy T, Teesdale-Spittle P, Craven MR, Watson JV, Amos WB, Errington RJ, Patterson LH (2000) Characteristics of a novel deep red/infrared fluorescent cell-permeant DNA probe, DRAQ5, in intact human cells analyzed by flow cytometry, confocal and multiphoton microscopy. Cytometry 40:280–291
21. Wojcik K, Dobrucki JW (2008) Interaction of a DNA intercalator DRAQ5, and a minor groove binder SYTO17, with chromatin in live cells-influence on chromatin organization and histone-DNA interactions. Cytometry Part A 73:555–562
22. Richards A, Stevens TS (1958) Synthesis and properties of behydropyridocolinium salts. J Chem Soc 25:3067–3073
23. Sato K, Okazaki S, Yamagishi T, Arai S (2004) The synthesis of azoniadithia[6]helicenes. J Heterocyclic Chem 41:443–447
24. Sato K, Arai S, Yamagishi T, Tanase T (2003) An azonia derivative of hexahelicene. Acta Crystallographica Section C C59:o162
25. Maçôas E, Marcelo G, Pinto S, Cañeque T, Cuadro AM, Vaquero JJ, Martinho JMG (2011) V-shape cationic dye for nonlinear bioimaging. Chem Commun 47:7374–7376
26. Chang L, Liu C, He S, Lu Y, Zhang S, Zhao L, Zeng X (2014) Novel styryldehydropyridocolinium derivative as turn-on fluorescent probe for DNA based on an intercalative binding mechanism. Sen Actuators B 202:483–488
27. Liu Q, Liu C, Chang L, He S, Lu Y, Zeng X (2014) Tunable PET process by the intercalation of cationic styryl dye in DNA base pairs and its application as turn-on fluorescent sensor for Ag^{+}. RSC Adv 4:14361–14364
28. Lu Y, Sun J, Wang L, Cheng D, Sun Y, Zeng X (2013) Fluorescence turn-on detection of DNA based on the aggregation-induced emission of conjugated poly(pyridinium salt)s. Polym Chem 4:4045–4051
29. Han F, Lu Y, Zhang Q, Sun J, Zeng X, Li C (2012) Homogeneous and sensitive DNA detection based on polyelectrolyte complexes of cationic conjugated poly(pyridinium salt)s and DNA. J Mater Chem 22:4106–4112
30. Prunkl C, Pichlmaier M, Winter R, Kharlanov V, Rettig W, Wagenknecht HA (2010) Optical, redox, and DNA-binding properties of phenanthridinium chromophores: elucidating the role of the phenyl substituent for fluorescence enhancement of ethidium in the presence of DNA. Chem Eur J 16:3392–3402
31. Gill MR, Garcia-Lara J, Foster SJ, Smythe C, Battaglia G, Thomas JA (2009) A ruthenium(II) polypyridyl complex for direct imaging of DNA structure in living cells. Nat Chem 1:662–667
32. Kuruvilla E, Nandajan PC, Schuster GB, Ramaiah D (2008) Acridine-viologen dyads: selective recognition of single-strand DNA through fluorescence enhancement. Org Lett 10:4295–4298
33. Feng XJ, Wu PL, Bolze F, Leung HWC, Li KF, Mak NK, Kwong DWJ, Nicoud JF, Cheah KW, Wong MS (2010) Cyanines as new fluorescent probes for DNA detection and two-photon excited bioimaging. Org Lett 12:2194–2197
34. Peng X, Wu T, Fan J, Wang J, Zhang S, Song F, Sun S (2011) An effective minor groove binder as a red fluorescent marker for live-cell DNA imaging and quantification. Angew Chem Int Ed 50: 4180–4183
35. Shank NI, Zanotti KJ, Lanni F, Berget PB, Armitage BA (2009) Enhanced photostability of genetically encodable fluoromodules based on fluorogenic cyanine dyes and a promiscuous protein partner. J Am Chem Soc 131:12960–12969
36. Özhalici-Ünal H, Pow CL, Marks SA, Jesper LD, Silva GL, Shank NI, Jones EW, Burnette JM III, Berget PB, Armitage BA (2008) A rainbow of fluoromodules: a promiscuous scFv protein binds to and

activates a diverse set of fluorogenic cyanine dyes. J Am Chem Soc 130:12620–12621
37. Brown AS, Bernal LM, Micotto TL, Smith EL, Wilson JN (2011) Fluorescent neuroactive probes based on stilbazolium dyes. Org Biomol Chem 9:2142–2148
38. Li Y, He S, Lu Y, Zeng X (2011) Novel hemicyanine dye as colorimetric and fluorometric dual-modal chemosensor for mercury in water. Org Biomol Chem 9:2606–2609
39. Arunkumar E, Chithra P, Ajayaghosh A (2004) A controlled supramolecular approach toward cation-specific chemosensors: alkaline earth metal ion-driven exciton signaling in squaraine tethered poda. J Am Chem Soc 126:6590–6598
40. Li Y, He S, Lu Y, Zhao L, Zeng X (2013) Novel mercury sensor based on water soluble styrylindolium Dye. Dyes Pigm 96:424–429
41. Chang J, Lu Y, He S, Liu C, Zhao L, Zeng X (2013) Efficient fluorescent chemosensor for HSO_4^- based on a strategy of anion-induced rotation-displaced H-aggregate. Chem Commun 49: 6259–6261
42. Liu C, Lu Y, He S, Wang Q, Zhao L, Zeng X (2013) The nature of the styrylindolium dye: transformations among monomer, aggregates and water adducts and its photophysical properties. J Mater Chem C 1:4770–4778
43. McEwen JJ, Wallace KJ (2009) Squaraine dyes in molecular recognition and self-assembly. Chem Commun 6339–6351
44. Wang Q, Liu C, Lu Y, He S, Liu C, Zhao L, Zeng X (2013) Novel water soluble styrylquinolinium boronic acid as a ratiometric reagent for the rapid detection of hypochlorite. Dyes Pigm 99:733–739
45. Arunkumar E, Ajayaghosh A, Daub J (2005) Selective calcium ion sensing with a bichromophoric squaraine foldamer. J Am Chem Soc 127:3156–3164
46. Ajayaghosh A, Arunkumar E, Daub J (2002) A highly specific Ca^{2+}-ion sensor: signaling by exciton interaction in a rigid-flexible-rigid bichromophoric "H" foldamer. Angew Chem Int Ed 41:1766
47. Varghese R, George SJ, Ajayaghosh A (2005) Proton controlled intramolecular photoinduced electron transfer (PET) in podand linked squaraine-aniline dyads. Chem Commun 593–601
48. Delgado F, Linares ML, Alajarín R, Vaquero JJ, Builla JA (2003) Westphal reaction in solid-phase. Org Lett 5:4057–4060
49. Rodríguez-Romero J, Aparicio-Ixta L, Rodríguez M, Ramos-Ortíz G, Maldonado JL, Jiménez-Sánchez A, Farfán N, Santillan R (2013) Synthesis, chemical–optical characterization and solvent interaction effect of novel fluorene-chromophores with D–A–D structure. Dyes Pigm 98:31–41
50. Cosa G, Focsaneanu KS, McLean JRN, McNamee JP, Scaiano JC (2001) Photophysical properties of fluorescent DNA-dyes bound to single-and double-stranded DNA in aqueous buffered solution. Photochem Photobiol 73:585–589
51. Wang L, Qin W, Tang X, Dou W, Liu W, Teng Q, Yao X (2010) A selective, cell-permeable fluorescent probe for Al^{3+} in living cells. Org Biomol Chem 8:3751–3757
52. Shortreed M, Kopelman R, Kuhn M, Hoyland B (1996) Fluorescent fiber-optic calcium sensor for physiological measurements. Anal Chem 68:1414–1418
53. Xu XY, Han MS, Mirkin CA (2007) A gold-nanoparticle-based real-time colorimetric screening method for endonuclease activity and inhibition. Angew Chem Int Ed 46:3468–3470
54. Glazer AN, Peck K, Mathies RA (1990) A stable double-stranded DNA-ethidium homodimer complex: application to picogram fluorescence detection of DNA in agarose gels. Proc Natl Acad Sci U S A 87:3851–3855