DNA Binding of a Molecular-Scale Receptor in the Presence of Zinc(II) Ions

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The properties of a tritopic artificial biological probe are described. This probe consists of a luminescent pyrene–thiophene unit connected by an ethynylene group to a 2,2′:6′,2′′-terpyridine (terpy) cation binding site. The pyrene unit, as evidenced by fluorescence spectroscopy under illumination at 400 nm, is capable of intercalating into double-stranded calf-thymus DNA in H_2O (buffered, pH = 7.0) at 25 °C. The binding constant K was calculated to be $6.0 \times 10^5 M^{-1}$. Titration of zinc(II) ions in an aqueous (pH = 7.0) solution containing the intercalated probe results in fluorescence quenching which again is a consequence of the zinc(II) ions binding to the terpy site. The DNA-bound probe has also been shown to undergo singlet energy transfer to intercalated ethidium bromide with a rate constant of 9.4×10^9 s^{-1}.

Introduction

Metal ions are essential to the proper function of living cells. Their importance in biology has stimulated the development of metal-based therapeutics and the rational design of new ligands with the ability to interact with both free cations[1–3] and protein-bound ions.[4–10]Several well-known antibiotic, anticonvulsive, antitumor, and anti-inflammatory drugs[11,12] exert their pharmacological effects by interacting with the metal-based active sites of their target proteins.[13,14] For example, in Alzheimer's disease chelating ligands for zinc and copper are being investigated for potential use as a treatment.[15,16] The brain is a specialized organ that concentrates metal ions (e.g., Zn^{2+}, Cu^{2+}, Fe^{3+}) in the neocortex. During neurotransmission, an extracellular concentration of released Zn^{2+} in the vicinity of the synapse rises transiently to ca. 300 μM.[17] Aggregation of β-amyloid (Aβ) in the brain, which happens in the presence of some metallic ions such as zinc, has been suggested as the main cause of Alzheimer's disease. Whereas Aβ is neurotoxic at micromolar concentrations in vitro[18] it is neurotrophic in cell culture.[19] The presence of Zn^{2+} in the accumulated Aβ can be visualized by histological fluorescence techniques carried out within the human brain.[20] Furthermore, someother crucial roles have been recognised for zinc in gene transcription,[21] metallo-enzymes[22] and synaptic neurotransmission.[23]

Understanding the role of zinc in biology has been limited by the apparent scarcity of suitable analytical sensors. Various criteria have been suggested for the design of appropriate fluorescence-based zinc probes, and several compounds have been developed recently. Some of these new probes are zinc-chelating peptides[24,25] and proteins,[26] while others are synthetic dyes based on zinc-chelating macrocyclic receptors.[27,28] Despite the progressive improvement in this area, none of the probes currently available for the in vivo or in vitro investigation of the intracellular chemistry of labile zinc ions are perfect. For example, protein-based probes are rarely cell-permeable, so they cannot be used under physiological conditions. Also, quinoline-based chelators like TSQ, zinquin, or TFLZn, have to be illuminated with UV light,[29] which is absorbed by many other components in the biological system.

Herein we present a putative DNA intercalator that is sensitive to the coordination of zinc, and certain other cations. The investigated ligand comprises discrete pyrene, thiophene, and terpyridine units and is given the generic abbreviation of **PTT**. The structural formula of this sensor is shown in Figure 1 and it is important to note the high degree of conjugation running along the molecular backbone. The photophysical properties of this compound, and its binding affinity towards selected cations in organic solvents, have been reported.[30] It has been shown that **PTT** displays intense fluorescence that is quenched upon metal ion binding at the terpyridine site. In searching for an extension of these properties into the biological arena we note that the hydrophobic polycycle has the propensity to intercalate into double-stranded DNA. This would expose the terpyridine-based terminal to the aqueous solution where it might continue to bind cations.

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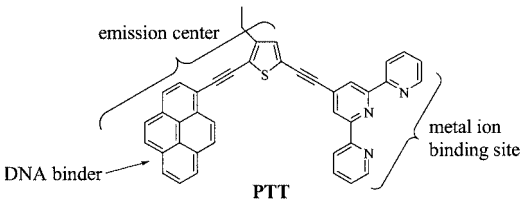

Figure 1.Structural formula and relevant features of the molecular probe **PTT**.

Results and Discussion

Binding of Zn^{2+} to PTT in DNA Media

Studies indicate that **PTT** coordinates to zinc(II) cations across an oil/water interface with a reasonable association constant (see Supporting information). Complexation is restricted to 1:1 binding and is manifest by a substantial reduction in the fluorescence yield and lifetime. There is also a slight shift in both absorption and fluorescence spectral maxima, confirming that the final fluorescence is from the complex and not from free **PTT**. Complexation of the cation to **PTT** in the presence of excess DNA is less straightforward. This is because the incoming cation can bind to the DNA, via the phosphate chains or through attachment to the nucleic acid bases, whilst the **PTT** receptor might be distributed among several disparate sites. In order to obtain a clearer description of the binding behavior in this system, several possibilities have been considered.

The absorption spectrum recorded for **PTT** shows some important changes (see Supporting information), especially in the range of 350–450 nm, after mixing with a solution of calf-thymus deoxyribonucleic acid (CT-DNA). In particular, there is a marked decrease in absorbance across the entire transition and broadening of the absorption peak. Similar changes have been seen for pyrene-based intercalators,[31] and so these observations indicate that **PTT** forms a complex with the nucleic acid. Complexation was confirmed by electrophoretic gel mobility shift assays which showed that incubation with **PTT** led to a reduction in the mobility of the duplex on agarose gel. Subsequent imaging studies indicated that the **PTT**/DNA complex was fluorescent. In order to determine the equilibrium binding constant for association of **PTT** with CT-DNA, we have carried out a series of fluorescence spectral titrations. Fluorescence spectra recorded for titration of 0.075 μM **PTT** with CT-DNA in 5 mM phosphate buffer (pH = 7.0) and 5 mM sodium sulfate are presented in Figure 2. The fluorescence yield, which is due entirely to the dye, decreases upon consecutive addition of DNA up to a phosphate/dye (p/D) ratio of ca. 10:1. The derived experimental data show obvious departure from Stern–Volmer behavior, thereby ruling out a significant dynamic process, and give the strong impression of a static quenching effect. It is interesting to note that addition of DNA causes a modest decrease in fluorescence whereas there are many compounds for which interaction with DNA leads to an increase in fluorescence.[32]

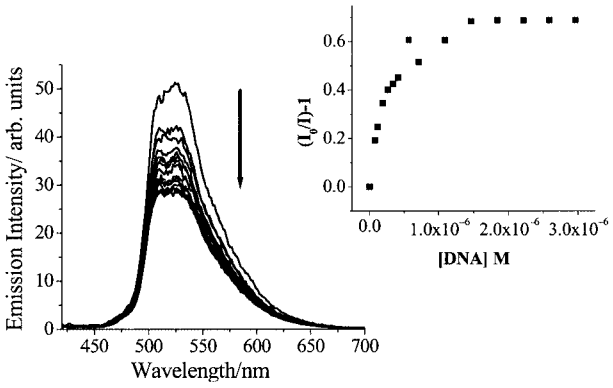

Figure 2. Change of fluorescence yield recorded for **PTT** (0.075 μM) with consecutive addition of DNA and following excitation at 400 nm. The right-hand panel depicts the Stern–Volmer plot for this titration.

A common method for examining how a dye binds to DNA involves fitting the experimental results to the Scatchard model.[33] However, **PTT** is a large molecule and might span more than one base pair. Under such conditions, the Scatchard model is inappropriate and it is generally regarded that the approach proposed by McGhee-von Hippel is more valid.[34] Furthermore, this equation considers the effect of ligand binding being cooperative, which means that a second ligand might be more or less likely to bind close to the first binding site. This can be written as in Equation (1). Here, $r = L_b/C_M$, L_f is the concentration of free ligand, L_b is the concentration of bound ligand, C_M is the DNA concentration (in terms of phosphate groups), and n is the number of bases involved in a single binding site.[34]

$$\frac{r}{L_f} = K(1-\frac{r}{n})\left[\frac{n-r}{n-(1-n)r}\right]^{\frac{1}{n}-1} \quad (1)$$

The slope of the plot (Figure 3), considered in terms of Equation (1), allows calculation of the equilibrium binding constant (K). From the derived results, the average value for K is $8.8\times10^5\,M^{-1}$. Unfortunately, it was not possible to calculate a reliable estimate for the number of base pairs involved in the binding site, since there are too few data points around saturation. A second set of titration experiments was made in order to determine n. Here, the concentration of **PTT** was maintained at 5 μM and various concentrations of DNA were added. The data in this case were analyzed using Equation (2), where α is a constant that varies with wavelength.

$$\frac{C_M^k - C_M^j}{\rho^k - \rho^j} = \frac{L_{tot}}{\alpha}\left[\frac{\frac{C_M^k}{\rho^k} - \frac{C_M^j}{\rho^j}}{\rho^k - \rho^j}\right] + n\alpha \quad (2)$$

Here, ρ is the change in emission yield whilst the superscripts j and k refer to successive additions of DNA at concentrations C_M^j and C_M^k. The total ligand concentration is

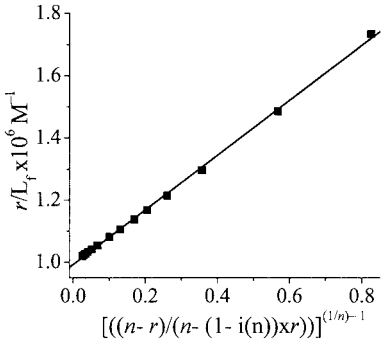

Figure 3. McGhee-von Hippel plot using the data from Figure 2 and with $n = 10$. The slope of the line gives the equilibrium binding constant between **PTT** and CT-DNA.

referred to as L_{tot}. The advantage of this method is that data at low and high loadings are not required. From an appropriate plot (see Supporting information) the slope L_{tot}/a and intercept na were evaluated. The parameters calculated by this method, after appropriate averaging, are $n = 9.2$ and $a = -2.4 \times 10^{-8}$. Using the value of a from this calculation and parameters calculated from the McGhee–von Hippel relationship,[34] the equilibrium binding constant for **PTT** with DNA was calculated to be $3.0 \times 10^5 M^{-1}$. This value is similar to that derived by the more direct titration.

Overall, it appears that **PTT** consumes 9 bases (or more likely 5 base pairs) in one binding site, with the equilibrium binding constant being equal to ca. $6 \times 10^5\ M^{-1}$ (average of the values obtained from three separate titrations). The binding affinity of **PTT** for CT-DNA is large and comparable with that of known intercalators like ethidium bromide (EB; $K = 2.6 \times 10^6\ M^{-1}$).[35] A series of experiments was carried out in order to determine whether binding involves intercalation or association with the major groove. It is recognised that to accommodate an intercalator the DNA duplex must partially unwind. This situation causes lengthening of the duplex and, in turn, this is reflected by an increase in viscosity. Furthermore, the intercalator stabilises the double helix and raises the temperature at which it melts. It was found that the melting point of DNA increased by 11.2 °C in the presence of **PTT** (p/D = 10:1). Hydrodynamic studies made with rod-like DNA indicated that the viscosity, calculated as $(\eta/\eta_0)^{1/3}$ where η and η_0 refer to the viscosity of the DNA solution in the presence and absence of dye respectively, increased in the presence of **PTT** (p/D = 10:1). The average increase, again measured in terms of $(\eta/\eta_0)^{1/3}$, was 1.15. Identical measurements made with EB, which is well known to intercalate between base pairs, gave a viscosity increase of 1.13. In contrast, the minor groove binding dye H 33258 gave an average viscosity decrease of 0.96.

All the experimental results are consistent with **PTT** intercalating between the base pairs, despite the absence of a positive charge. Such a positioning would force the terpyridine group to lie outside the duplex, where it is accessible tothe exterior aqueous phase. This notion is supported by detailed docking calculations made using the program GRAMM, which show the **PTT** intercalating between two base-pairs of the major groove in DNA. This docking geometry leaves the terpyridine unit exposed to the outer environment (Figure 4). This suggests that it might be possible to bind cations to the vacant terpyridine site. It should be noted that intercalation of **PTT** into DNA causes a loss of fluorescence from the dye. The steady-state experiments indicate that the intercalated dye is about 55% as fluorescent as the non-bound dye but it should be recalled that solubility restrictions limit the amount of dye that can be attached to the DNA duplex.

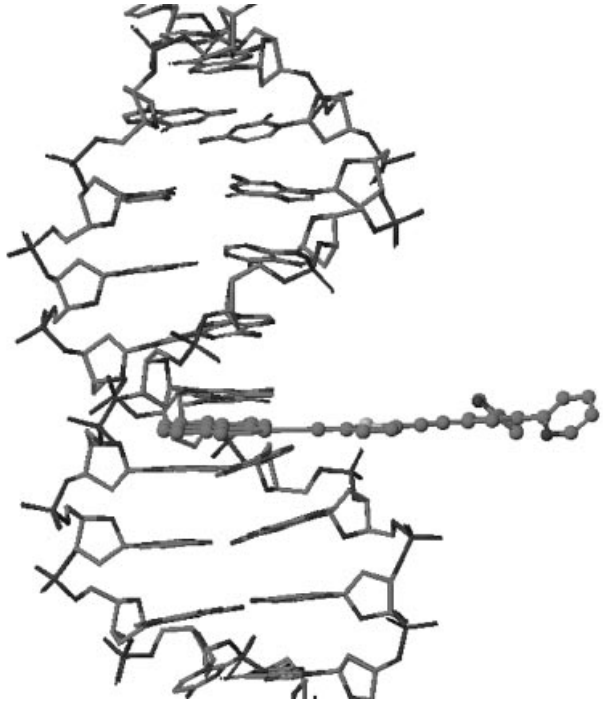

Figure 4. Representation of the docking of **PTT** into the major groove of DNA. The structure of **PTT** was energy minimized using the semi-empirical AM1 model before performing the docking calculation.

It is most likely that the pyrene subunit inserts between the base pairs and it is known from the work of Netzel et al.[36] that fluorescence from intercalated pyrene is quenched due to electron transfer to cytosine. As such, the modest reduction in fluorescence yield observed for **PTT** upon intercalation can be attributed to intrastrand electron transfer. This effect is not unduly significant and does not prevent the use of **PTT** as a fluorescent sensor. In fact, comparison of the fluorescence lifetimes recorded in micelle ($\tau_S = 1.0$ ns) and DNA media ($\tau_S = 0.58$ ns) allows estimation of the rate constant for intrastrand charge transfer as being ca. $7 \times 10^8\ s^{-1}$.

The corresponding changes in the fluorescence spectrum recorded for **PTT** in the presence of CT-DNA as a function of temperature are shown in Figure 5. It can be seen that the emission intensity decreases progressively with increasing temperature up to about 55 °C. Further increases in temperature have less effect on the fluorescence yield. There is no obvious change in the spectral profile. Similar behav-

ior is not seen in homogeneous solution such that it can be attributed to some special property of the duplex. The effect of temperature on the measured fluorescence yield is believed to refer to a change in binding affinity. If so, the fluorescence intensity at each temperature can be used to estimate the binding constant (K) at that temperature. This variation in K can now be expressed in terms of the van't Hoff isochore [Equation (3)]. Here, ΔH^0 is the change in reaction enthalpy. The experimental data were fit to Equation (3) (Figure 5), allowing calculation of ΔH^0 as $-54\ kJ{\cdot}mol^{-1}$. Consequently, increasing temperature favors the reactants and the overall binding behavior is reasonably exothermic.

$$\frac{d\ln K}{dT} = \frac{\Delta H^0}{RT^2} \quad (3)$$

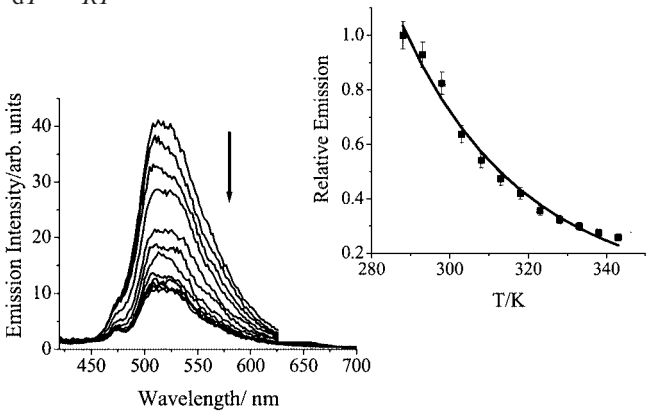

Figure 5. Effect of increasing temperature on the fluorescence spectrum of CT-DNA+**PTT**. The right-hand panel depicts the fit of the emission yield against temperature with the 1:1 binding model.

Binding of Zn^{2+} to DNA

The interaction of zinc(II) cations with DNA is well documented.[37] The so-called M-DNA is formed at pH > 8, and is associated with metal complex formation with the imino group of thymine and guanine in the DNA duplex.[38] In comparison, Wettig et al.[39]concluded that there is no formation of M-DNA with zinc(II) cations at pH = 7.5. In view of these findings, the formation of M-DNA is unlikely to take place under the pH conditions used in our experiments. Instead, the most accessible sites for complex formation are the oxygen-rich phosphate groups of the DNA backbone. The binding of zinc(II) to CT-DNA was studied by recording the absorption spectrum of the DNA solution during titration with a 500 μM solution of $ZnSO_4$. The general change of the absorption spectrum and the noted decrease in absorbance at 260 nm, where CT-DNA absorbs most strongly, were measured for various DNA (base pair)/metal molar ratios. The only significant effect of the added cation on the absorption spectral records in the absence of **PTT** was the decreased absorbance (see Supporting information).

Using the Scatchard method[33] to calculate the binding constant (K) and/or number of bases covered (n) in a binding site is inappropriate in such cases (see Supporting information). The intrinsic method using two different total substrate (i.e., Zn^{2+}) concentrations but the same DNA concentration is more valid for analyzing the data from this titration. The data analysis reveals at least two disparate binding modes for attachment of Zn^{2+} to the DNA duplex. This finding is in agreement with an association mechanism involving electrostatic attraction between Zn^{2+} cations and the phosphate groups of the backbone. The second binding mode presumably involves only a single DNA base. Metal binding to the phosphate group of DNA has been proven using different techniques, including Raman[40] and NMR[41] spectroscopy and differential pulse polarography.[42] It was shown that the phosphate groups are the preferred binding sites for divalent metallic ions at low p/D ratios.[43] Raman data have indicated a direct type of interaction between Zn^{2+} and one particular oxygen atom of the phosphate group.[44] Formation of tripartite complexes involving cation, phosphate and purine N^7 atom has also been implicated.[45]

From our results we can conclude that at low p/D electrostatic interactions are highly favorable and cause attachment of the cation to the phosphate layer. This effect, however, saturates quite quickly and is complete at a loading of about one cation per 5 base pairs. The association constant for this electrostatic binding is ca. 17500 M^{-1} such that the DNA duplex has the effect of concentrating Zn^{2+} from the solution along the phosphate chain. The facile saturation implies that the bound cation is highly mobile and can easily migrate between adjacent phosphates in a random-walk fashion. Since formation of M-DNA is unlikely[39] the weak association of Zn^{2+} (K = 2240 M^{-1}) at higher loading is presumably due to binding at the N^7 of a purine and a phosphate oxygen atom to create a chelated complex.

Attachment of Zinc(II) Cations to Intercalated PTT

Armed with the above information, it is possible to return to the full system whereby zinc(II) cations are added to a solution of intercalated **PTT**. Titration results were obtained from fluorescence quenching of solutions of **PTT** in DNA at different p/D ratios upon successive addition of $ZnSO_4$ (see Supporting information). Stern–Volmer plots constructed from the data (Figure 6) are non-linear and show clear evidence for saturation. This indicates that fluorescence quenching involves a static process. In all cases, the experimental data could be fit to a 1:1 binding model. Interestingly, the value of the apparent binding constant varies progressively over the range of 12400–22000 M^{-1} for p/D values ranging from 4:1 to 22:1. The analysis suggests that K reaches a maximum of about 22000 M^{-1} at a p/D of around 12:1. At first sight, this observation could be taken as an indication for negative cooperativity towards **PTT**; that is to say, high loadings of **PTT** prevent association with added zinc(II) cations. This could take the form of clustering of **PTT** in regions along the duplex, although this effect was not apparent from titrations made in the absence of

added cation. It is also clear that there is competition between **PTT** molecules and DNA (via the phosphate groups or to a lesser extent the bases) for interaction with Zn^{2+}.

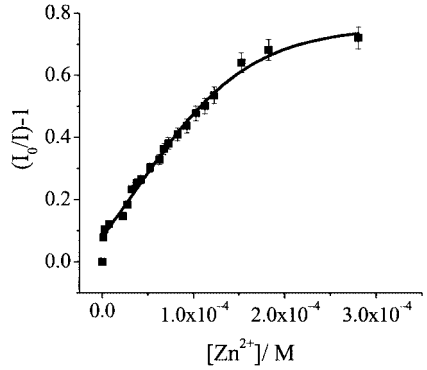

Figure 6. Stern–Volmer plot for addition of Zn^{2+} to a solution of **PTT** (5 μM) bound to DNA (50 μM·bp^{-1}).

Singlet Energy Transfer from PTT to Ethidium Bromide in DNA

The above results indicate that **PTT** intercalates into double-stranded DNA and binds zinc(II) cations at the vacant terpyridine unit. It is also likely that the **PTT**/DNA conjugate could be used to facilitate other functions, such as excitation energy transfer, that will not occur in dilute solution. With respect to excitation energy transfer, it is recognized that this can take place over a considerable distance if suitable reactants can be identified. It is also known that DNA provides a good medium for such processes because it serves to align the transition dipoles.[46] The most common mechanism for long-range energy transfer involves coulombic dipole–dipole interactions, for which the rate constant depends on the inverse sixth power of the donor–acceptor distance.[47] The concept of DNA-mediated excitation energy transfer was first described by Le Pec and Paoletti for transfer from DNA bases to intercalated ethidium bromide (EB).[48] Thereafter, it has been used as an indicator for a drug being intercalated between or in contact with DNA bases.[49–52] Considering these findings one can consider fluorescence energy transfer from **PTT** (as donor) to ethidium bromide (as acceptor) with both reagents being intercalated into the same duplex. Such experiments would confirm that **PTT** intercalates between the bases and determine the transfer efficiency. Ethidium bromide (EB) is a classic DNA intercalator and has been very well studied. The binding constant (K) is 2.6×10^6 M^{-1} and a saturation (n) of 2 base pairs was reported for intercalation of EB into CT-DNA (5 mM phosphate buffer, pH = 7, containing 5 mM Na_2SO_4).[53]

For the energy-transfer experiments, a solution of [**PTT**] = 5 μM and [DNA] = 70 μM·bp^{-1} in the usual buffer was used. A non-random distribution of **PTT** with an average separation of 7 base pairs (ca. 24 Å) is assumed to prevail under these conditions,[46] although this is obviously a gross approximation. It should be stressed that it is possible to excite **PTT** at a wavelength (λ_{max} = 400 nm) where EB shows little absorption. Upon addition of successive aliquots of EB, up to 40 μM, the fluorescence characteristic of **PTT** was quenched and a new fluorescence peak appeared around 600 nm (Figure 7). The latter peak can be assigned to fluorescence from intercalated EB by reference to control experiments. There is no fluorescence from EB free in solution under these conditions, because of quenching by water molecules,[46] and the observed emission is clearly that of the intercalated EB dye. A crude isosbestic point at 570 nm is preserved throughout much of the titration but is lost at high loading of EB onto the duplex. This latter effect arises because EB begins to displace **PTT** at high concentrations from the duplex.

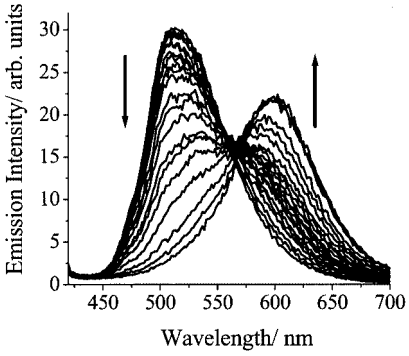

Figure 7.The effect of added EB on the fluorescence spectrum of **PTT** (5 μM) bound to DNA (70 μM·bp^{-1}).

The decrease in **PTT** emission and concomitant increase in EB emission are progressive and interrelated. Complete loss of **PTT** fluorescence is observed at a loading of EB of about 30 μM whilst 10 μM EB is sufficient to reduce the initial fluorescence by 50%. Higher concentrations of EB cause displacement of the intercalated **PTT** whilst 20 μM EB corresponds to full loading of all the available sites on the duplex. Under these latter conditions, the nearest EB molecule will be about 10.2 Å away from the **PTT**. The probability for energy transfer, as calculated from the respective fluorescence yields, increases with increasing concentration of EB. After correction for any direct absorption by EB molecules, it is found that the maximum transfer probability is about 82% (see supporting information).

According to Förster theory, the rate of energy transfer depends on the mutual orientation of donor and acceptor and on their spectral overlap integral, in addition to the separation distance. Such transfer can occur over large distances, e.g. 20–80 Å, with appropriate reagents.[53] The excited singlet state lifetime of **PTT** intercalated into DNA in the absence of EB is 600 ps. At full loading of EB the excited state lifetime of the donor was reduced to 90 ps. Comparison of these two lifetimes provides an estimate for the rate constant for energy transfer (k_{ET}) as being 9.4×10^9 s^{-1}. Presumably, energy transfer occurs to the closest acceptor molecule,[46] which will be 10.2 Å away. The fluorescence lifetime of the EB acceptor is 24 ns under these conditions.

Titration of a solution comprising DNA/**PTT**/EB (70:5:5, with [DNA bp] being 70 μM) with $ZnSO_4$ solution

caused a progressive decrease in the overall fluorescence intensity without affecting the lifetime of the EB excited singlet state. Under these conditions, both **PTT** and EB fluoresce and the presence of Zn^{2+} is seen to quench both species with the same efficiency (Figure 8). Separate experiments showed that the cation did not quench the fluorescence of EB but we know from earlier studies that bound Zn^{2+} readily quenches the excited singlet state of **PTT**. As such, we can conclude that Zn^{2+} binds to **PTT** and curtails energy transfer to intercalated EB. The latter effect arises from the shortened lifetime of the cation complex. As might be expected, the resultant Stern–Volmer plot is non-linear (see Supporting information). Fitting the total fluorescence yield to a 1:1 binding model indicates that the equilibrium binding constant for attachment of Zn^{2+} to **PTT** in the presence of EB is only 3,240 M^{-1}. This is much less than the corresponding value found in the absence of EB ($K = 1.3 \times 10^4$ M^{-1}). Apparently, intercalation of EB, with its single positive charge, blocks some of the phosphate groups and hinders approach of the Zn^{2+} cation.

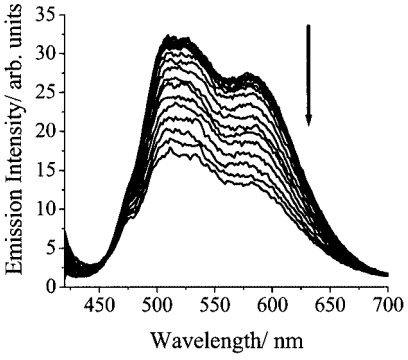

Figure 8. Fluorescence quenching of the sample involving DNA/**PTT**/EB (70:5:5 μM) upon titration with Zn^{2+}.

Conclusions

The detection of Zn^{2+} with **PTT** is possible after the dye has been intercalated into DNA. The **PTT** probe, which is insoluble in water, binds to DNA by way of intercalation between base pairs. This process is reasonably efficient, especially when taking into account the fact that the dye is neutral, and positions the terpyridine unit on the exterior of the duplex. It is interesting to consider here if the DNA/**PTT** conjugate could be used to detect zinc(II) cations in biological environments. Firstly, the complexity of the system makes for a difficult calibration of the fluorescence probe. It is clear that high loadings of intercalator inhibit coordination of the cation to the terpyridine site on the receptor. This behavior is observed at high loadings of **PTT**, where the binding constant decreases, or when high loadings of EB are used. In the latter case, the binding constant for coordination of zinc(II) cations is reduced even further because EB can pack more closely into the duplex than does **PTT**, because of its lower *n* value.

We can recognize at least two distinct pathways for coordination of Zn^{2+} to intercalated **PTT**. First, we can consider the possibility that zinc(II) cations free in solution bind directly to the DNA/**PTT** conjugate. In competition to this step, zinc(II) cations can first bind to DNA and migrate along the duplex, using the phosphate groups as pathways, until they become localized at a vacant **PTT** site. The effect of intercalator loading is consistent with this latter situation being the major binding mode. Indirectly, this provides an interesting opportunity to detect the presence of intercalated species that do not emit or absorb strongly in the visible region. Simply measuring the binding constant for attachment of Zn^{2+} cations to intercalated **PTT** at a fixed loading would provide this latter information. Otherwise, the best experimental conditions for detecting Zn^{2+} cations involves the use oflow loadings of **PTT**. The high fluorescence yield facilitates the use of such conditions.

Experimental Section

The ligand **PTT** was prepared and purified by the method described in the literature.[54] Purity of the compound was checked by ^1H NMR spectroscopy, mass spectrometry, thin layer chromatography and fluorescence spectroscopy. All other chemicals were purchased commercially and used as received. Double-stranded calf-thymus DNA (CT-DNA) was obtained from Sigma and dialysed for 24 h at room temperature. A stock solution was prepared by carefully dissolving CT-DNA in neutral (phosphate buffer) deionised water containing 5 mM Na_2SO_4 with gentle sonication. Subsequent analysis by agarose gel electrophoresis indicated no fragmentation. The CT-DNA concentration was calculated from the absorbance at 260 nm (ε = 6600 M^{-1} cm^{-1}).[55] Samples of rod-like double-stranded DNA for hydrodynamic measurements were prepared by sonication of CT-DNA. A Cannon-Ubbelhode semi-microdilution viscometer, thermostatted at 21 °C, was used for all viscosity measurements. A sample of rod-like DNA (4×10^{-4} M) with average fragment size of 600 base pairs was prepared in the usual buffer and incubated with dye (p/D = 10:1). Solutions were filtered with an Acrodisc CR PTFE syringe filter and the flow time was measured with a digital stopwatch.

Absorption spectra were recorded with a Hitachi U3310 spectrophotometer while corrected fluorescence spectra were recorded with a Hitachi F4500 spectrophotometer. All fluorescence measurements were made using optically dilute solutions and were corrected for spectral imperfections of the instrument by reference to a standard lamp. Quantum yields were measured[56] by reference to fluorescein in 0.1 M NaOH solution (Φ_F = 0.925). Fluorescence lifetimes were measured with a Fluorolog tau-3 spectrometer after deconvolution of the instrument response function. The thermal denaturation experiments were made with **PTT**/DNA mixtures (p/D = 10:1) (8 μM) in buffer solution. Melting curves were measured at 260 nm with a Perkin–Elmer Lambda-3 spectrophotometer equipped with a PTP-1 Peltier temperature programmer. The temperature was increased at a rate of 0.5 °C/min.

The equilibrium binding constant for association of **PTT** with CT-DNA was measured using a series of fluorescence spectral titrations. The dye was first dissolved in 10% DMSO/H_2O and various aliquots of a concentrated solution of DNA were added. The solution was allowed to equilibrate for 10 min before recording the fluorescence spectrum. Both solutions were buffered to pH = 7.0 with phosphate and the ionic strength was fixed by the presence of 5 mM sodium sulfate. Studies were made with different initial

concentrations of **PTT** and all experiments were repeated at least three times. All measurements were carried out at room temperature with solutions protected against room light and incubated for 20 min before use.

Molecular modelling calculations were carried out in conjunction with Dr. Ata Amini (Imperial College, London). The basic DNA structure was selected from the PDB data bank (entry 109D) and the **PTT** structure was optimized using the semi-empirical AM1 method.[57] Molecular docking calculations were performed using the software GRAMM.[58] In a typical calculation the **PTT** location with respect to the DNA backbone was set and molecular docking was allowed to take place. Several starting trajectories of the **PTT** molecule were used and the corresponding energy of the conjugate calculated. The structure was refined until the minimum energy was attained. The derived structure is far from unique and there exists an entire family of related geometries having the pyrene unit intercalated between base pairs and the terpyridine group directed into the surrounding aqueous phase.

Supporting information includes the description of the quenching of **PTT** in Triton X-100 micelles, as well as plots and figures referred to in the main body of the text.

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