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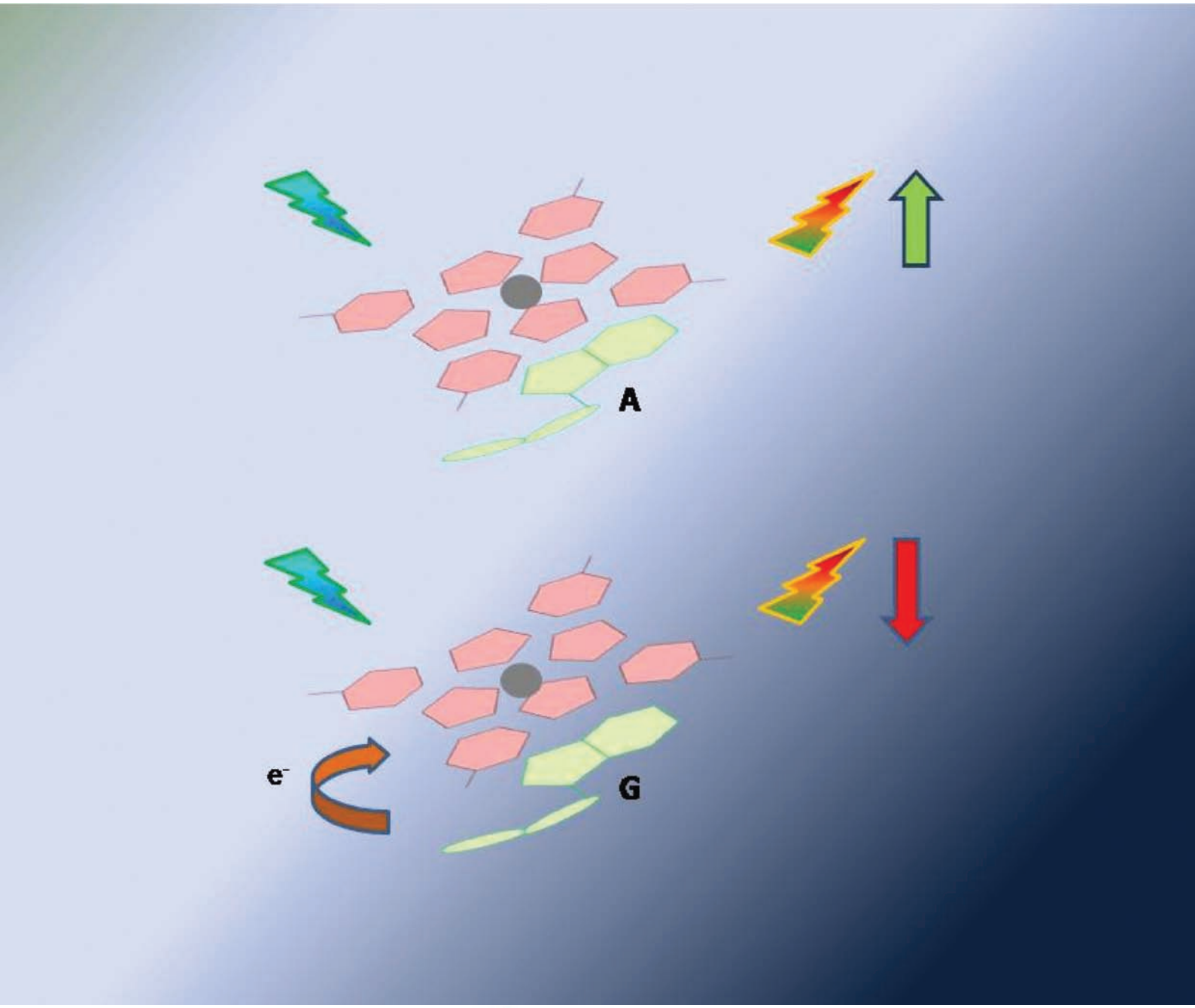

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PAPER

Triplet-state dynamics of a metalloporphyrin photosensitiser (PtTMPyP4) in the presence of halides and purine mononucleotides†

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The photophysical properties of Pt(II) *meso*-tetrakis(4-*N*-methylpyridyl)porphyrin (PtTMPyP4) have been investigated in the presence of purine mononucleotides using emission and transient UV/visible/near-IR spectroscopy. While both adenosine 5′-monophosphate (AMP) and guanosine 5′-monophosphate (GMP) form 1 : 1 and 1 : 2 complexes with PtTMPyP4, the effect on the triplet lifetime is different. With AMP, complexation gives rise to an enhancement of lifetime and quantum yield due to shielding from dissolved oxygen and a slight decrease in the non-radiative decay rate. When complexed with GMP, quenching is observed consistent with photoinduced electron transfer from guanine to triplet-excited PtTMPyP4, due to both dynamic quenching of the porphyrin and to short-lived emission from 1 : 1 (67 ns) and 1 : 2 (400 ns) complexes. No charge-separated photoproducts are observed by transient UV/vis/near-IR absorption spectroscopy on the nanosecond timescale, suggesting that rapid reverse electron transfer may prevent type 1 DNA damage.

Introduction

Many porphyrins can mediate photodynamic action in biological systems. An example is the targeted photo-oxidation of DNA, which may proceed by type 1 (electron transfer) or type 2 (singlet-oxygen mediated) mechanisms, although the latter is more common with porphyrins due to their high yields of triplet-state formation. It has also been known for many years that cationic porphyrins, such as *meso*-tetrakis(4-*N*-methylpyridyl)porphyrin (H_2TMPyP4), bind strongly to a variety of nucleic acid structures.[1–3] H_2TMPyP4, in particular, has attracted attention in recent years due to its ability to inhibit the telomerase enzyme by stabilising the quadruplex form of telomeric DNA.[4]

Numerous metalloporphyrin derivatives have been studied allowing for tuning of structure and excited-state properties. The Pt(II) derivative, PtTMPyP4, is formally square-planar and therefore structurally similar to H_2TMPyP4. It intercalates into double-stranded natural DNA and binds to single-stranded poly(dA).[5,6] It also inhibits telomerase when bound to human telomeric DNA[7] and has been tested for a number of biomedical applications.[8–10]

Strong spin–orbit coupling in PtTMPyP4 causes efficient intersystem-crossing to the triplet ππ* state and room temperature phosphorescence in aqueous solution (τ approx. 1 μs).[9,11] The long-lived emission of PtTMPyP4 is a sensitive probe, and has hence been exploited for oxygen sensing.[12,13]

However, far less attention has been paid to the photophysics of PtTMPyP4 in the presence of nucleic acids than there has been on free base H_2TMPyP4. This is despite the fact that the triplet state (the excited state involved in type 2 oxidation) can be readily monitored in PtTMPyP4. The long lifetime also means that different processes such as diffusional quenching may be observed with PtTMPyP4 than with the fluorescent H_2TMPyP4 (τ approx. 5 ns). We have therefore undertaken a detailed study of PtTMPyP4 in a variety of nucleic-acid systems, in order to understand the fundamental photophysics of PtTMPyP4-nucleic acid interactions.

Here we report on the ground and excited-state interactions of PtTMPyP4 with mononucleotides adenosine 5′-monophosphate (AMP) and guanosine 5′-monophosphate (GMP). AMP and GMP are known to form π-stacked supramolecular complexes with H_2TMPyP4 and some metal derivatives.[14–18] The fluorescence of H_2TMPyP4 is enhanced in the presence of AMP, while GMP causes efficient quenching due to possible photo-induced electron transfer (PET) from guanine to the porphyrin ^{1}ππ* state,[16,17,19] although the photoproducts of this reaction have not been observed by transient spectroscopy.

PET from guanine has, to our knowledge, not been reported with any of the metal derivatives of TMPyP, and it has been proposed that the presence of a metal lowers the oxidation potential too much for PET to occur.[19] It is therefore intriguing to examine whether electron transfer can occur between GMP and photo-excited PtTMPyP4, and to see whether the Pt(II) atom has an effect on the rates of forward and reverse electron transfer.

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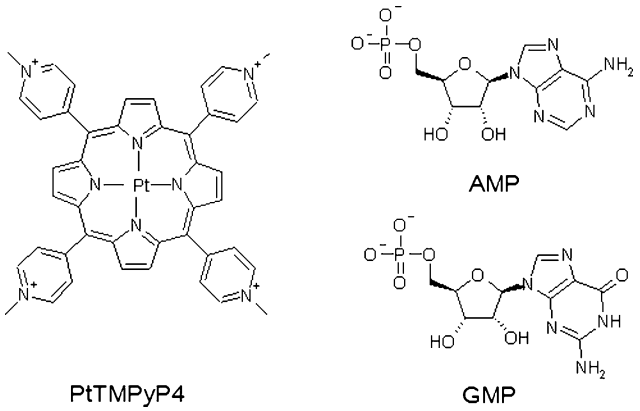
† Electronic supplementary information (ESI) available: Halide quenching plots, transient absorption decay fits, Stern–Volmer plots for quenching of triplet lifetime by GMP. See DOI: 10.1039/c1pp05125c

Materials and methods

Pt(II)TMPyP4 tetrachloride was purchased from Frontier Scientific and used as received. Sodium salts of mononucleotides AMP and GMP (Sigma) were used without further purification. All experiments were performed in 50 mM phosphate buffer (25 mM Na_2HPO_4, 25 mM NaH_2PO_4, pH 6.8). Concentrations of PtTMPyP4 were determined using the published extinction coefficient ($\varepsilon = 1.72 \times 10^5$ M^{-1} cm^{-1} at 402 nm).[5]



Absorption spectra were recorded on a Cary 50 or Shimadzu UV2401PC UV/vis spectrophotometer. Steady-state emission spectra were recorded on a Perkin-Elmer LS55 spectrofluorimeter operating in phosphorescence mode. Correction for photomultiplier response in the red region was made using 4-dimethylamino-4′-nitrostilbene in orthodichlorobenzene as a standard. Deoxygenated samples were purged with N_2 immediately prior to measurements. Transient absorption spectra and phosphorescence lifetimes were recorded on an Edinburgh Instruments FP920 kinetic absorption spectrometer using 355 nm excitation from a frequency-trebled Nd:YAG laser (Spectron, 10 ns pulse width) onto a Hamamatsu R955 PMT. Transient spectra in the near-IR range (700 nm-1100 nm) were recorded with a Hamamatsu R5108 red-sensitive PMT. A 600 nm long-pass filter was used to eliminate second-order triplet–triplet absorption. Shorter lifetimes (<1 μs) were recorded on an Edinburgh Instruments FL920 single photon counting fluorimeter using 371 nm excitation from a pulsed diode laser, or on a HORIBA Jobin Yvon FluoroLog TCSPC using 370 nm excitation from a NanoLED™ source. Emission decays were fitted to monoexponential or biexponential functions (eqn (1)).

$$I(t) = a_1 e^{-t/\tau 1} + a_2 e^{-t/\tau 2} \quad (1)$$

Fitting was performed with OriginPro 8.0 or software supplied with the apparatus (EI, HJY), using the Marquardt-Levenberg algorithm.

Results

Halide quenching

Chloride is ubiquitous in physiological solutions and is a common buffer component in DNA studies. As the luminescence of PtTMPyP4 has been reported to be quenched by NaCl,[6,11] we initially examined the effect of halide salts (NaCl and NaBr) on PtTMPyP4.

The presence of 50 mM phosphate buffer alone has no effect on the absorption or emission properties of PtTMPyP4. The addition of NaCl or NaBr to an air-equilibrated solution of PtTMPyP4 causes quenching of its steady-state emission and shortens the emission lifetime (τ_0 = 1.0 μs), but does not result in any changes in the shape of the absorption or emission spectra.

The quenching data was fitted to the Stern–Volmer equation (eqn (2), Fig. 1 and ESI Fig. S1–S3†).

$$\frac{\tau_0}{\tau} = \frac{\Phi_0}{\Phi} = 1 + k_q \tau_0 [Q] \quad (2)$$

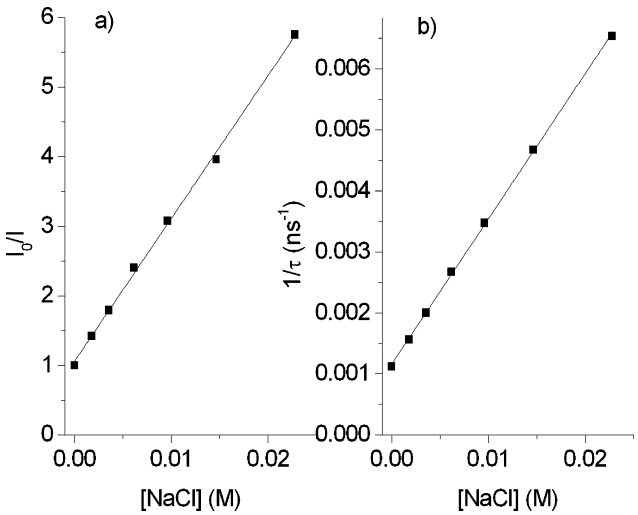


Fig. 1 Stern–Volmer plots for phosphorescence of PtTMPyP4 in the presence of NaCl in 50 mM phosphate buffer for (a) steady-state (λ_{exc} = 513 nm, λ_{em} = 665 nm) and (b) time-resolved data (λ_{exc} = 371 nm, λ_{em} = 665 nm).

Stern–Volmer plots yield k_q values of 2.4×10^8 dm^3 mol^{-1} s^{-1} (Cl^-) and 1.8×10^9 dm^3 mol^{-1} s^{-1} (Br^-), from either steady-state or time-resolved plots. These results show that in the range of halide concentrations used in this study, the quenching is by a diffusional quenching mechanism. It should be noted that the concentration of Cl^- present as counterion (~2×10^{-5} M) is too low to have a significant effect on the phosphorescence.

Quenching mechanism

The observation that Cl^- can quench the emission of PtTMPyP4 may be related to its relatively long-lived emissive state. Thus Cl^- also quenches the phosphorescence of PdTMPyP4,[11] but not the short-lived fluorescence of H_2TMPyP4 or ZnTMPyP4. Furthermore, quenching of the PtTMPyP4 singlet state would not be expected, as the S1 states of Pt(II) porphyrins typically have lifetimes in the picosecond range.[20]

Previously, it has been suggested that the quenching of PtTMPyP4 by chloride is due to aggregate formation.[6,11] However the lack of a change in the absorption spectra in our studies shows that this is not the cause of the quenching, and it appears that PtTMPyP4 shows a resistance to aggregation similar to H_2TMPyP4.[21]

Halide quenching of organic dyes has often been discussed in terms of processes such as electron transfer, exchange interactions, and spin–orbit coupling[22] and the possibility of these processes

was considered here. The probability of reductive photoinduced electron transfer can be assessed by estimating the Gibbs energy for the photoreduction from a form of the Rehm-Weller equation (eqn (3)).[23]

$$\Delta G^0 = F\left[E^0\left(X^-/X\right) - E^0\left({}^3PtP^*/PtP^{\bullet-}\right)\right] \quad (3)$$

The potential for the reduction of ^{3}PtTMPyP4* to the radical (3^+) is 1.47 V,[24] while values for the oxidation of the halides (X^-/X) are 2.6 V[25] (Cl/Cl^-) and 1.91 V[26] (Br/Br^-), all *versus* NHE. The calculated positive values of ΔG^0 mean that direct photoinduced electron transfer can be ruled out.

Spin–orbit coupling is often invoked in the quenching of singlet states, though it can only be important here if it can increase the rate of T_1–S_0 intersystem crossing. The quenching may involve a contribution from a number of processes, including charge transfer and spin orbit coupling. This is proposed in the Watkins mechanism, which has been applied to model the S_1 quenching of a Zn(II) porphyrin.[27,28] However the general mechanism for luminescence quenching by halides is clearly non-trivial, and arguably not fully understood. The important conclusion from our studies is that care needs to be taken in the use of saline solution in photophysical measurements.

Ground state interactions with nucleotides

Addition of AMP or GMP to solutions of PtTMPyP4 (Fig. 2) results in a red shift and hypochromism of the absorption bands, characteristic of a π-stacked complex.[14–18] The absorption spectra of PtTMPyP4 with AMP has an isosbestic point at 410 nm for nucleotide concentrations up to approximately 0.5 mM, corresponding to a [AMP]/[PtTMPyP4] ratio of approx. 100 in this case. The isosbestic point is lost with further addition of AMP. Similar behaviour to the above is seen with GMP (ESI, Fig. S4†). The absorption data at the Soret was fitted to Benesi–Hildebrand model (eqn (4)) in order to estimate the strength of the association.[29]

$$\frac{1}{\Delta Abs} = \frac{1}{[N]K_g\Delta Abs_i} + \frac{1}{\Delta Abs_i} \quad (4)$$

(Here ΔAbs denotes the change in absorbance at 402 nm, [N] is the concentration of nucleotide, K_g is the association constant and ΔAbs_i is the absorbance difference between the porphyrin and the complex). Apparent equilibrium binding constants (K_g) of 6200 ± 600 M^{-1} and 5300 ± 500 M^{-1} were calculated for the 1 : 1 complexes with AMP (Fig. 3) and GMP (ESI, S5†), respectively, when the data was plotted in the range of low nucleotide concentration (<0.5 mM). The loss of isosbestic points, and deviation in binding plots at higher concentration suggest that an additional 1 : 2 complex is formed under these conditions.

Emission studies

The triplet state of PtTMPyP4 phosphoresces at room temperature in degassed aqueous buffer solution with a lifetime of 6.5 μs and a quantum yield of ~2%.[11] Upon aeration of the solution the lifetime is reduced to 1.0 μs, corresponding to a rate constant $k_{O_2} = 3 \times 10^9$ dm^3 mol^{-1} s^{-1} (assuming a dissolved O_2 concentration of 2.8×10^{-4} M at 20 °C.[30]) As pointed out earlier this long lifetime means that potentially other processes can be monitored that cannot

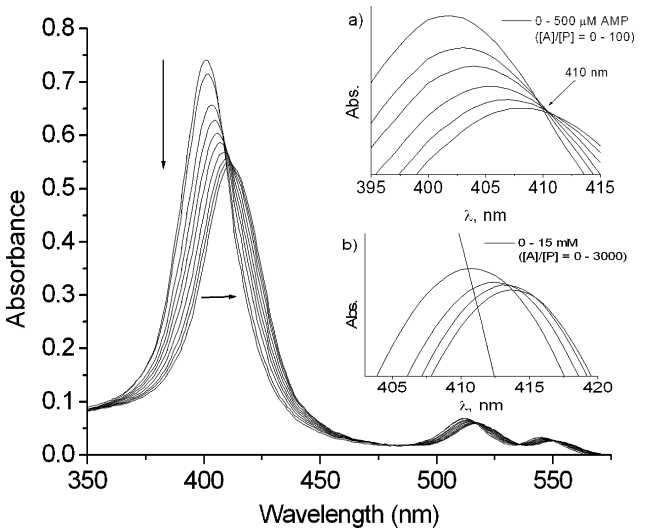


Fig. 2 UV/vis spectra of 4.5 μM PtTMPyP4 in presence of increasing AMP concentration in 50 mM phosphate buffer. Inset: (a) isosbestic points in Soret region for titration at low AMP concentrations. (b) loss of isosbestic point at higher AMP concentrations.

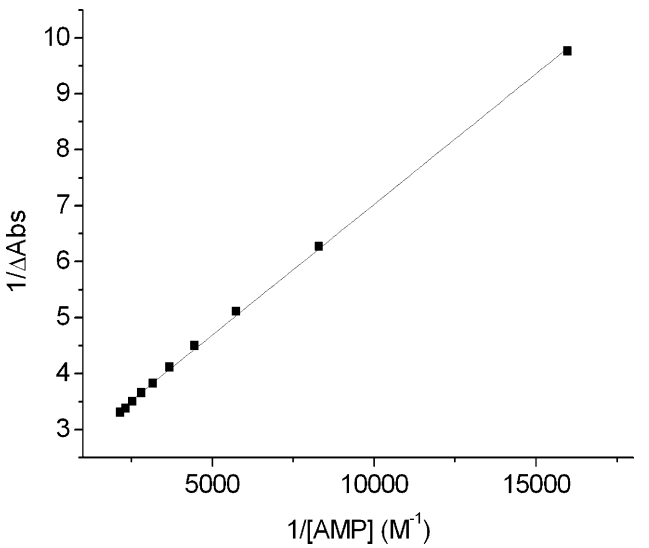


Fig. 3 Benesi–Hildebrand plot for 4.5 μM PtTMPyP4 in presence of AMP (up to 0.5 mM) in 50 mM phosphate buffer.

be readily probed by fluorescence. In particular it may be noted that the lifetime of the phosphorescence falls into approximately the same range as that of the formation and dissociation of the porphyrin-nucleotide complex. This enhanced lifetime range could mean that the kinetics may be rather complex and this has indeed proved to be the case.

PtTMPyP4 and AMP

When the solutions are deoxygenated, the addition of 10 mM AMP to PtTMPyP4 (5.0 μM) causes an enhancement of steady-state emission quantum yield, which plateaus at $I/I_0 = 1.5$ (Fig. 4a). At the same time the emission maximum also shifts from 668 nm to 675 nm. At this concentration we expect >98% of the porphyrin molecules to be complexed to the nucleotides, with a significant fraction being in the form of 1 : 2 complexes. The triplet

Table 1 Photophysical data for PtTMPyP4 in presence of nucleotides[a]

	τ_{aer} (μs)	τ_{deox} (μs)	k_{nr}[b] (s^{-1})	k_{O_2} (M^{-1} s^{-1})
P	1.0	6.5	1.4×10^5	3×10^9
PA	2.5	10	1×10^5	1×10^9
PA_2	6.0	10	1×10^5	3×10^8
PG	0.067	0.067	1.5×10^7	nd
PG_2	0.4	0.4	2.3×10^6	nd

[a] In 50 mM phosphate buffer. [b] k_r assumed to be 1300 s^{-1} in all cases (see ref. 11). nd = not determined. Errors in lifetime ± 10%.

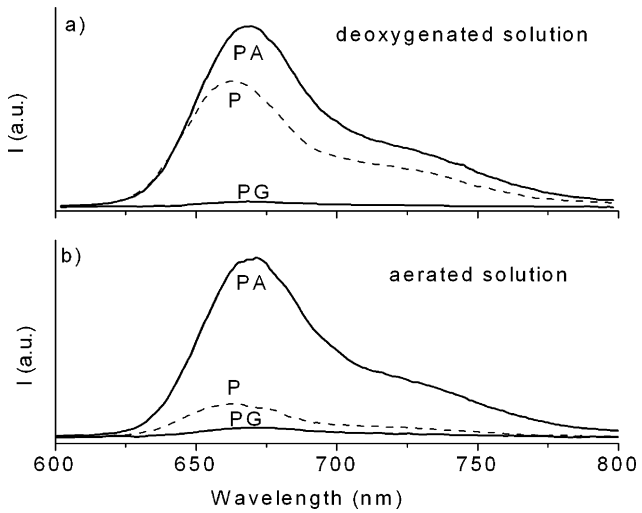


Fig. 4 Steady-state emission spectra of 5 μM PtTMPyP4 in the absence (P) and in the presence of 10 mM AMP (PA) or GMP (PG). Recorded in (a) deoxygenated solution and (b) aerated solution. λ_{exc} = 517 nm.

state lifetimes also increases from 6.5 μs to 10.0 μs. Both values were monoexponential. This increase in lifetime and quantum yield of PtTMPyP4 in the absence of dissolved oxygen may be attributed to a decrease in the non-radiative decay rate (k_{nr}), which was calculated assuming $\tau = (k_r + k_{nr})^{-1}$ (see Table 1)

When 10 mM AMP is added to an aerated 5.0 μM solution of PtTMPyP4 the emission is enhanced by a factor of 6.0 (Fig. 4b), and the lifetime also increases from 1.0 μs to 6.0 μs. Comparing the lifetime in aerated and deaerated solution gives a value of $k_{O_2} = 3 \times 10^8$ dm^3 mol^{-1} s^{-1} under these high AMP concentration conditions.

Experiments were also carried out at lower concentrations of AMP (0 to 0.5 mM), where the UV/visible spectroscopic studies reported above showed that there is an equilibrium between the free porphyrin and the 1 : 1 AMP complex. In deoxygenated solution, a similar yield and lifetime is recorded in 0.5 mM as in 10 mM. However, different behaviour is observed in aerated solution. At low AMP concentrations (<0.5 mM AMP) an increase in emission intensity and a red shift in emission λ_{max} is observed. However the lifetimes are biexponential. Fixing the first component as 1.0 μs (the lifetime of unbound PtTMPyP4) the second fits as 2.5 ± 0.3 μs. As more AMP is added the contribution from this longer-lived component increases at the expense of the short component (Fig. 5 and inset). This is consistent with the oxygen quenching rate being significantly lower for the 1 : 1 complex than for the free PtTMPyP4.

At high AMP concentrations (*i.e.* from 0.5 mM to 10 mM), the lifetimes further increases with satisfactory fits to monoexponential kinetics (Fig. 5). The value plateaus at 6.0 ± 0.3 μs, when as mentioned above the 1 : 2 complex is likely dominant (see Scheme 1). The observed monoexponential decay kinetics in this concentration range may be caused by an equilibrium between the 1 : 1 and 1 : 2 complex, assuming that the latter complex dissociates within its excited state lifetime.

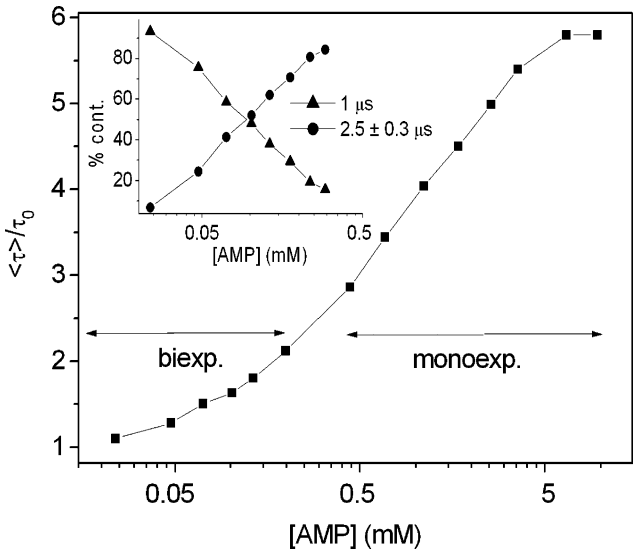
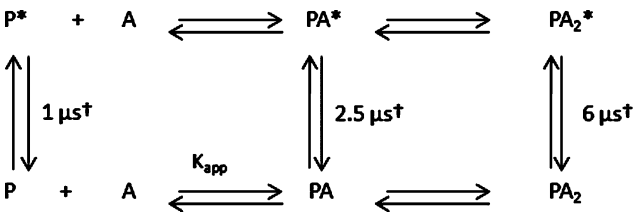


Fig. 5 Increase in lifetime with increasing AMP concentration, showing regions where lifetimes fit to either two or one exponentials (λ_{exc} = 370 nm, λ_{em} = 670 nm). Average lifetime is calculated from amplitude average $\langle\tau\rangle = \sum a_i\tau_i / \sum a_i$. Inset: variation in contribution of two decay components in biexponential region, where % contribution = $100a_i / \sum a_i$ (see eqn (1)).



Scheme 1 Suggested photophysical scheme for interactions of PtTMPyP4 with AMP in aerated solution. † Decay determined by both non-radiative pathways and O_2 quenching.

Titrations of NaCl into the PtTMPyP4-AMP systems (5 μM PtTMPyP4, 15 mM AMP) were also performed, in order to see how formation of the complex affects the ability of Cl^- ions to quench the triplet state. Downward curvature is observed in the Stern–Volmer plots (ESI, Fig. S6†). This may be related to decreases in binding efficiency of the PtTMPyP4-AMP complex, though very little change was observed in the steady-state absorption spectra. An approximate quenching constant for Cl^- in the complex was recorded as 1.5×10^6 s^{-1}. This value is notably two orders of magnitude lower than that recorded for the free PtTMPyP4, showing that complex formation effectively inhibits Cl^- quenching.

PtTMPyP4 and GMP

By contrast with what is found with AMP the emission of PtTMPyP4 in both deoxygenated and aerated solution is strongly

quenched in the presence of GMP (Fig. 4a,b). With 10 mM GMP, I/I_0 values of 0.05 and 0.3 were recorded in deoxygenated and aerated solutions, respectively. The λ_{max} shifts from 668 nm to 675 nm, consistent with this emission now originating from PtTMPyP4-GMP complexes. The emission in both deoxygenated and aerated solution fits to single exponential decay with a lifetime of 400 ± 40 ns.

As for the PtTMPyP4-AMP system, lifetime determinations were performed over the whole concentration range in aerated solution. In general at lower concentrations biexponential decay kinetics is observed. The 'long' component may be assigned to the free PtTMPyP4, as its lifetime decreases from 1000 ns to 400 ns with increasing [GMP] (Fig. 6). The decay of the long component was plotted in Stern–Volmer form ($1/\tau$ *vs.* [GMP]) in the concentration range up to 0.4 mM, yielding an apparent k_q value of 4×10^9 dm^3 mol^{-1} s^{-1} (Fig. 6, inset) assuming dynamic quenching by unbound GMP. The 'short' component has a constant lifetime of 67 ± 7 ns, and may be attributed to that of the 1 : 1 PtTMPyP4-GMP complex. Consistent with this the contribution of this component to the decay kinetics rises to about 50% at 0.5 mM (Fig. 7); at this point 50% of the porphyrin would be bound based on an association constant of 5000 M^{-1}.

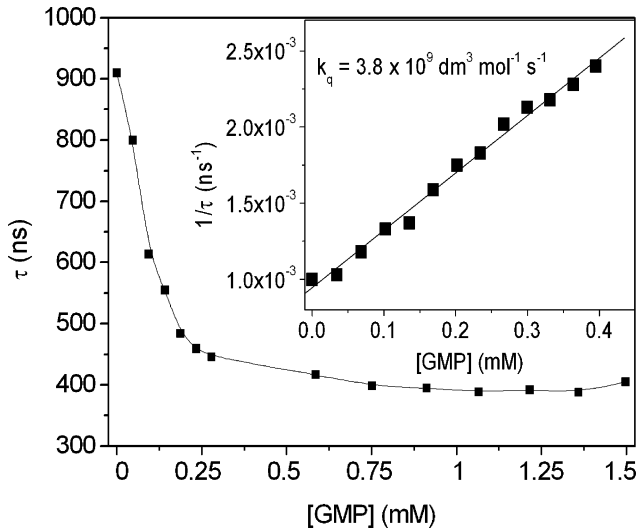


Fig. 6 Dependence of long lifetime component in 5 μM PtTMPyP4 on GMP conc. Inset: Stern–Volmer plot for lifetimes of long component at low [GMP] (< 0.5 mM). Recorded using single-photon counting (λ_{exc} = 370 nm, λ_{em} = 670 nm).

Interestingly, on increasing the GMP concentration further, the contribution from the short component decreases until a mononexponential lifetime of 400 ± 40 ns is obtained, presumably due to the dominance of the 1 : 2 PtTMPyP4:GMP complex under these conditions. Notably, there is very little change in the steady-state intensity above a GMP concentration of approx. 0.5 mM, in either aerated or deoxygenated solution (Fig. 8).

Given that guanine is more readily oxidised than adenine, the quenching of the phosphorescence yield and lifetime of PtTMPyP4 by GMP may be indicative of a photoinduced electron transfer (PET) from guanine to the excited triplet state of PtTMPyP4, and we propose that the electron transfer proceeds at different rates in the 1 : 1 and 1 : 2 complexes (see Scheme 2). Due to the oxidative robustness of Pt(II), reduction of the porphyrin

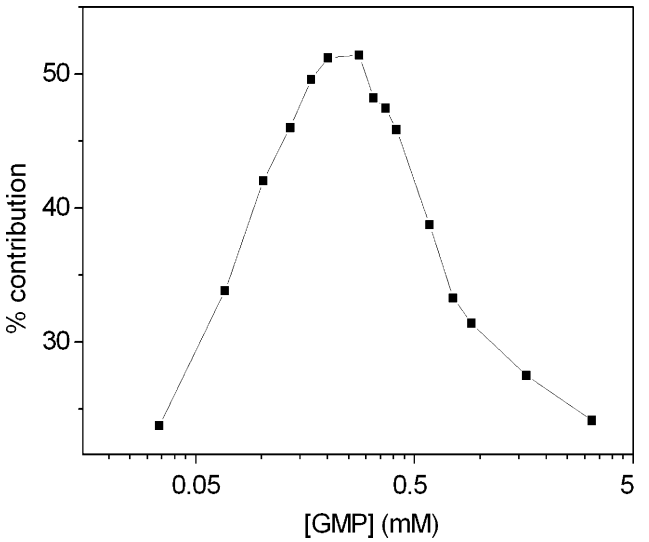


Fig. 7 Dependence of the relative amplitude of the short (67 ± 7 ns) lifetime component of the PtTMPyP4-GMP complex on [GMP]. Recorded using single-photon counting (λ_{exc} = 370 nm, λ_{em} = 670 nm).

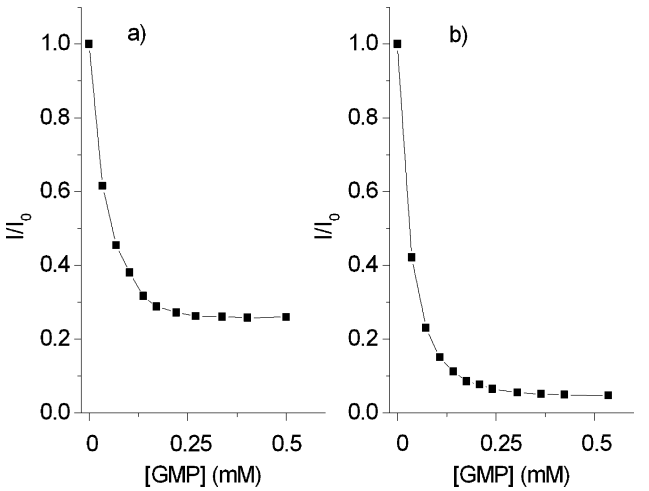
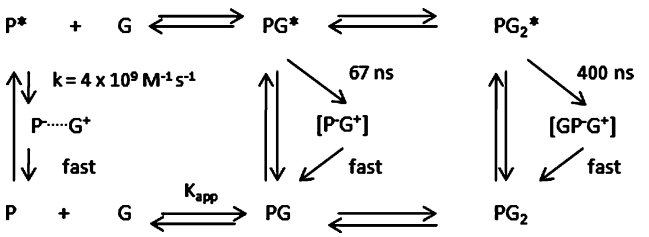


Fig. 8 Quenching of steady-state emission of 5 μM PtTMPyP4 in presence of increasing concentration of GMP in (a) aerated and (b) deoxygenated solution. λ_{exc} = 517 nm in 50 mM phosphate buffer.



Scheme 2 Suggested photophysical scheme for interactions of PtTMPyP4 with GMP.

would be expected to be localised on the TMPy ligand. Eqn (3) can be used to estimate ΔG^0 for an electron transfer to the PtTMPyP4 triplet state. A value of 1.31 V *vs.* SHE (1.07 V *vs.* SCE) has been proposed for the one-electron oxidation of GMP,[31] implying a favourable photoreduction of PtTMPyP4 (ΔG^0 = –0.16 V). However, difficulties have been encountered in measuring the

oxidation potential of guanine due to rapid deprotonation of the guanine radical cation, and hence some higher values have been reported (*e.g.* 1.47 V,[32] 1.58 V[33]). Therefore depending on the chosen potential for GMP, a positive ΔG^0 may be obtained. It may be noted that the apparent electron transfer rates in the 1 : 1 (k = 1.5×10^7 s^{-1}) and 1 : 2 complexes ($k = 2.5 \times 10^6$ s^{-1}) are relatively slow, and likely to be associated with a small driving force.

Transient absorption studies

To further study the decay processes of the triplet excited state and to look for transient photoproducts, nanosecond UV/visible/near-IR transient absorption experiments were carried out on PtTMPyP4 and its nucleotide complexes. Studies in the 700–1100 nm range are particularly useful as the triplets and reduction products of TMPy porphyrins absorb in this region.[34–36] An electron transfer from guanine to triplet-excited PtTMPyP4 is expected to yield transient photoproducts (guanine radical cation, porphyrin radical). The guanine radical cation has a broad, weak absorption in the visible region 500–700 nm[37] while the porphyrin π-radical anion is expected in the 700–800 nm region.[36]

The prompt transient UV/vis spectrum of PtTMPyP4 shows bleaching of the Soret band at 402 nm, and a broad T-T absorption at 450 nm. In the presence of AMP and GMP the bleaching is red-shifted and less intense, matching the changes in the ground-state absorption spectra (Fig. 9). The lifetimes of transients and bleaches for PtTMPyP4 and its AMP/GMP complex are monoexponential (in agreement with the phosphorescence lifetimes). This indicates that triplet–triplet annihilation is not a factor under our experimental conditions (see ESI, Fig. S7 & S8†). The PtTMPyP4-GMP system was also studied at low GMP concentrations. Similar biexponential decay is observed as in the SPC experiments, with a short (*ca.* 70 ns) and a longer (1000–400 ns) component. When the solutions were deoxygenated, the long component lengthened in lifetime. A Stern–Volmer plot to these lifetimes yielded a similar k_q (4.5×10^9 dm^3 mol^{-1} s^{-1}) to that recorded in aerated solution (ESI, Fig. S9†).

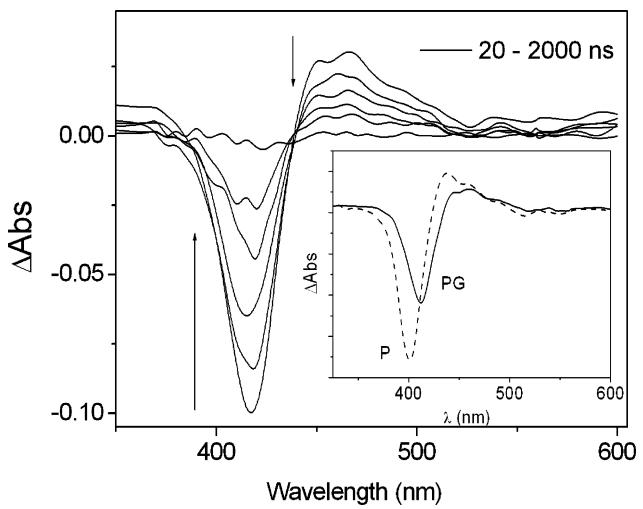


Fig. 9 Decay of T-T absorption spectrum of 5 μM PtTMPyP4 in the presence of 10 mM GMP. Inset: Comparison of spectra of free PtTMPyP4 with PtTMPyP4-GMP recorded 20 ns after laser pulse. In aerated 50 mM phosphate. λ_{exc} = 355 nm.

In the near-IR PtTMPyP4 has a broad triplet–triplet absorption around 1000 nm, similar to other TMPy derivatives.[34] The maximum is red-shifted by 30 nm (303 cm^{-1}) in the presence of either AMP or GMP, indicating a lowering in the T-T energy gap on formation of the complex (Fig. 10). The lifetimes are similar to those recorded in the UV/vis region (ESI, Fig. S10 & S11). The spectrum shape does not change as the species decays, suggesting that no other long-lived transient species are produced as the triplet relaxes to the ground state. The absence of long-lived photoproducts in the UV/vis or near-IR regions for the PtTMPy4-GMP system suggests that efficient charge recombination occurs within the time limits of the apparatus.

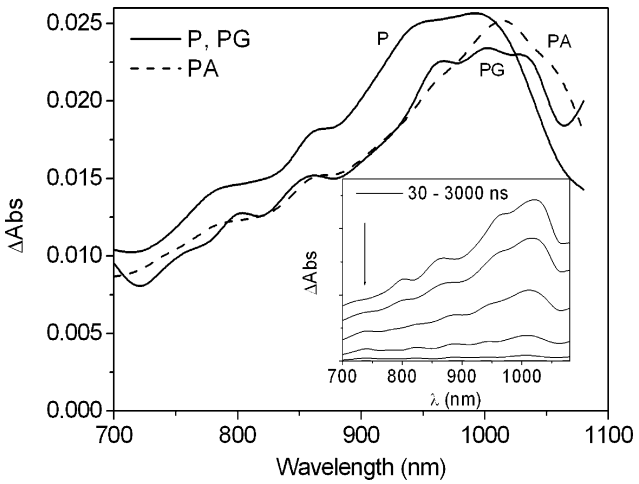


Fig. 10 Near-IR T-T absorption spectra of 5 μM PtTMPyP4 in the absence (P) and in the presence of 10 mM AMP (PA) or GMP (PG). Recorded 25 ns after laser pulse. Inset: Decay of PtTMPyP4-GMP spectrum. In aerated 50 mM Na-phosphate. λ_{exc} = 355 nm.

Discussion

The visible absorption spectroscopic measurements are consistent with strong ground-state complex formation between the PtTMPyP4 and either AMP or GMP. At lower nucleotide concentrations (<0.5 mM) a 1 : 1 complex dominates, while at higher values we anticipate that the 1 : 2 species is present. This behaviour mirrors that found by Pasternack *et al.* for CuTMPyP4 and NiTMPyP4[14] and by Mojzes *et al.* who have reported that CuTMPyP4 forms both 1 : 1 and 1 : 2 complexes with dTMP.[38] 1 : 2 association has also been recorded for other planar dyes (*e.g.* thionine, methylene blue) in the presence of nucleotides at low ionic strength.[39]

The phosphorescence lifetime of PtTMPyP4 increases slightly upon complexation with AMP in deoxygenated solution. This decrease in the rate of non-radiative decay upon complex formation may be attributed to the greater rigidity of the porphyrin in the complex, which has previously accounted for phosphorescence enhancement of PtTMPyP4 in mesoporous substrates,[13] or to the decrease in solvent mediated relaxation due to reduction of contact of the solvent with the excited state. It may be noted that for H_2TMPyP4, the increase in fluorescence lifetime in the presence of AMP has been attributed to perturbation of an intramolecular charge transfer state[40] and it is possible that a similar explanation

might apply for PtTMPyP4. However, the sensitivity to medium polarity need not necessarily be a general property for the metal derivatives of H_2TMPyP4, as for example the fluorescence of ZnTMPyP4 is only very slightly affected by the association of AMP.

Our lifetime studies in aerated solution show that oxygen quenching reduces the lifetime of the 1 : 1 complex to 2.5 μs and that of the 1 : 2 complex to 6 μs (Scheme 1), indicating that the formation of π-stacked complexes between PtTMPyP4 and AMP offers shielding from quenching by dissolved oxygen and decreases k_{O_2}, as has been observed for other porphyrins associated with mononucleotides.[41] The large decrease in quenching efficiency of Cl^- (approx. 100-fold) compared to O_2 (10-fold) in the 1 : 2 complex may be attributed to electrostatic repulsion experienced by Cl^- from the nucleotide phosphate groups.

Our photophysical studies of PtTMPyP4 in the presence of GMP are again consistent with the formation of 1 : 1 and 1 : 2 complexes in the different concentration ranges (Scheme 2). In contrast to what is found for AMP the lifetime of the triplet state is markedly decreased by GMP. In the case of the uncomplexed porphyrin a quenching rate constant of 4×10^9 dm^3 mol^{-1} s^{-1} is observed, consistent with highly efficient deactivation. This quenching appears to lead to the formation of the excited 1 : 1 complex, which is also formed directly by excitation of the 1 : 1 ground state complex. This excited state complex has a lifetime of 67 ns. At higher concentrations of GMP where the 1 : 2 complex is dominant the lifetime of the excited state is longer (400 ns). As shown in Fig. 7, the proportion of the 67 ns species initially increases and subsequently decreases as the concentration of GMP becomes larger – entirely consistent with the proposed ground state equilibrium. A similar behaviour was reported in a time-resolved resonance Raman investigation of CuTMPyP4 in the presence of dTMP.[38]

The reduction in lifetime of the porphyrin in the presence of GMP may be attributed to electron transfer from the nucleotide to the porphyrin excited state, as has been proposed for similar systems.[16,17,40,42] The longer lifetime of the 1 : 2 complex implies that electron transfer is less efficient than in the 1 : 1 complex. This might be due to differences in the orientation of porphyrin and nucleotide, that may affect the electronic coupling required for electron transfer. (In this context it is worth noting that Sazanovich *et al.* report at least four different types of H_2TMPyP4-GMP complexes on the basis of different fluorescence lifetimes.[40]) Alternatively the thermodynamic driving force may also be affected both because the triplet state energy of the bound porphyrin is reduced upon complexation, and because it is likely that the energy of the electron transfer species is raised by the presence of the second nucleotide.

By probing in the near-IR region we had hoped that our nanosecond flash photolysis experiments might reveal the presence of the reduced porphyrin which would be formed by electron transfer. Unfortunately this was not found to be the case, even though one could argue that the initially formed caged product would be in its triplet state, so that the reverse electron transfer to form the ground state species should be spin forbidden.[43] However it is equally possible that the presence of the heavy Pt(II) atom facilitates the spin flip and hence aids recombination. The apparent efficiency of the reverse transfer may also relate to the fact that the donor and acceptor form a strong complex (at least in the ground state), thus decreasing the likelihood that the photoproducts can undergo cage escape before recombination. Based on an association constant of 5000 M^{-1} and an association rate constant (k_{on}) of 4×10^9 dm^3 mol^{-1} s^{-1} the dissociation rate of the redox pair (k_{off}) can be estimated as 8×10^5 s^{-1}. Clearly the back transfer would need to be very slow for cage escape to dominate.

This behaviour may be compared and contrasted with that of other systems where quenching of excited states by electron transfer from guanine has been proposed.[44] Thus in quenching by the singlet state of thionine by guanine, the forward and back electron transfer have been shown to proceed in less than 1 ps.[45] Similarly with the Cr(III) dipyridophenazine (dppz) complexes no evidence for the electron transfer products could be observed despite strong quenching by GMP or upon binding to double stranded $[poly(dG\text{-}dC)]_2$.[46] By contrast, with the metal complexes $[Ru(TAP)_2(dppz)]^{2+}$ (TAP = 1,4,5,8-tetraazaphenathrene)[47] or $[Re(CO)_3(dppzF_2)]^{+}$[48] spectroscopic evidence for both the reduced metal complex and the oxidised guanine have been obtained using both transient visible and mid-IR absorption spectroscopy. The reasons for this disparity are not fully clear and will require further investigation.

Conclusions

This study confirms that the long-lived luminescence of PtTMPyP4 makes it a valuable photophysical probe for probing the dynamics of biological molecules in solution, especially for these processes in the microsecond and tens-to-hundreds of nanoseconds range. At the same time, however, this long lifetime makes it vulnerable to dynamic bimolecular quenching by species which are commonly found in the solution, such as molecular oxygen or halide ions. The spectroscopic and photophysical properties measured over an extended concentration range of the nucleotide reveal the presence of 1 : 1 and probably 1 : 2 complexes with AMP and GMP. The quenching with GMP proceeds, apparently by electron transfer, approximately six times faster in the 1 : 1 complex than in the 1 : 2 complex. To the best of our knowledge PtTMPyP4 is so far the only metal derivative of H_2TMPyP4 to exhibit such electron transfer quenching by guanine. However, our transient absorption measurements reveal that the reverse electron transfer is very rapid. This means that PtTMPyP4 will not be as efficient a type 1 photo-oxidant as H_2TMPyP4, consistent with earlier reports.[8] We have recently extended our studies on the triplet state of PtTMPyP4 to polynucleotide systems, and these results will be discussed in a forthcoming publication.

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