Photochemical & Photobiological Sciences

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/pps

Journal Name

ARTICLE

Ground and excited state interactions of metalloporphyrin PtTMPyP4 with polynucleotides $[poly(dG\text{-}dC)]_2$ and $[poly(dA\text{-}dT)]_2$

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Páraic M. Keane*[a†] and John M. Kelly*[a]

Dedicated to the memory of Dr Wil van der Putten

The ground- and excited-state interactions of Pt(II) *meso*-tetrakis(4-*N*-methylpyridyl)porphyrin (PtTMPyP4) with polynucleotides $[poly(dG\text{-}dC)]_2$ and $[poly(dA\text{-}dT)]_2$ have been investigated using UV/visible, circular dichroism, and steady-state and time-resolved emission spectroscopy. PtTMPyP4 intercalates into $[poly(dG\text{-}dC)]_2$ with $K \sim 10^6$ M^{-1}. When bound to $[poly(dG\text{-}dC)]_2$ in aerated solution there is a six-fold emission enhancement with 18 nm red-shift in emission maximum. Emission lifetimes are biexponential. In the presence of $[poly(dA\text{-}dT)]_2$ at least two distinct groove-binding modes are observed, depending on the binding ratio. In $[poly(dA\text{-}dT)]_2$ the emission intensity increases by a maximum factor of 17 with no shift in the emission spectrum. Three exponentials were required for lifetime fitting. The lower extent of emission enhancement in the presence of $[poly(dG\text{-}dC)]_2$ suggests that a slow electron transfer may take place to guanine, which is significantly less efficient than that previously observed for PtTMPyP4 in the presence of guanosine 5′-monophosphate (GMP). The results are compared to those previously recorded with free base H_2TMPyP4.

Introduction

Even though studies go back almost forty years, there is still very much interest in cationic porphyrins derived from *meso*-tetrakis *N*-methylpyridyl porphyrin (H_2TMPyP4, Fig. 1). This is due to a variety of properties of potential use for biomedical applications, including the ability to (a) bind strongly with nucleic acids,[1] (b) photo-oxidise DNA and other biomolecules[2] and (c) inhibit the telomerase enzyme by stabilising the quadruplex form of guanine-rich DNA.[3]

Substitution of metals into the macrocycle allows the structure and photophysics of the porphyrin to be tuned. PtTMPyP4 (PtP) is formally square planar, similar to the free base parent H_2TMPyP4 (H2P), and has been shown to intercalate into double-stranded DNA[4-7] and single-stranded poly(dA).[8] The excited-state properties are quite different, however, and by contrast with the short-lived fluorescence of H2P, PtP exhibits long-lived luminescence at room temperature ($\Phi \sim 2\%$[9], $\tau = 6.5$ μs,[10] in deaerated solution), due to emission from the triplet state, where the lifetime is influenced by the heavy atom effect of the Pt atom.[9] As a result, the emission is sensitive to its environment, especially the presence of collisional quenchers (O_2, Cl^-).[10,11] We have also shown that the triplet state is strongly perturbed by stacking interactions with nucleic acid constituents, resulting in emission enhancement in the presence of adenosine 5′-monophosphate (AMP) and quenching with guanosine 5′-monophosphate (GMP).[10] The former is primarily due to protection from dissolved oxygen quencher, while the latter we attributed to photoinduced electron transfer from guanine to the PtP triplet state.

Here we extend our study to examine how these properties translate to the case of polymeric DNA, and in particular whether the electron transfer to guanine can also occur with double-stranded systems. Furthermore PtP is an ideal comparison to the well studied H2P, and allows the influence of the Pt(II) metal on the ground and excited state properties to be evaluated. We have therefore studied the interactions of PtP with the alternating copolymers $[poly(dG\text{-}dC)]_2$ and $[poly(dA\text{-}dT)]_2$ and compared them to those reported for free base H2P.

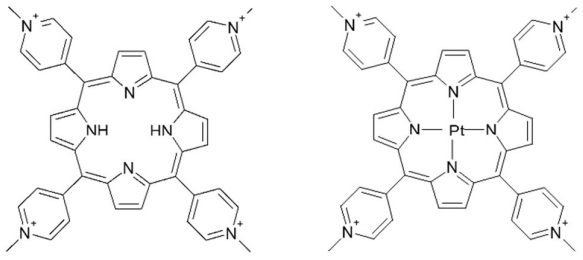

Fig. 1 Structures of H_2TMPyP4 (left) and PtTMPyP4 (right)

[a] School of Chemistry, Trinity College Dublin, The University of Dublin, Dublin 2, Ireland. Email: keanepa@tcd.ie, jmkelly@tcd.ie
† Current address: School of Chemistry, University of Reading, RG6 6AD, UK

Electronic Supplementary Information (ESI) available: [exponential fitting data, additional UV/vis, CD and emission data]. See DOI: 10.1039/x0xx00000x

Experimental

Pt(II)TMPyP4 tetrachloride was purchased from Frontier Scientific and used as received. Polynucleotides $[poly(dG\text{-}dC)]_2$ and $[poly(dA\text{-}dT)]_2$, salmon testes (st) DNA and poly(rA) were obtained from Sigma-Aldrich and used without further purification. Polynucleotide concentrations are expressed in terms of nucleotides unless otherwise stated, and were determined using extinction coefficients of (dm^3 mol^{-1} cm^{-1}) 8400, 6600, 6600 and 9800 for $[poly(dG\text{-}dC)]_2$, $[poly(dA\text{-}dT)]_2$, st-DNA and poly(rA), respectively. Concentration ratios in binding experiments are expressed as [Nucl]/[Por] (DNA nucleotide conc./porphyrin conc.) All experiments were performed in 50 mM phosphate buffer (25 mM Na_2HPO_4, 25 mM NaH_2PO_4, pH 6.8). NaCl was not used due to the ability of Cl^- to quench the triplet state of PtP.[10] Concentrations of PtP were determined using the published extinction coefficient (ε_{402} = 1.72 x 10^5 M^{-1} cm^{-1}).[8]

Absorption spectra were recorded on a Cary 50 UV/vis spectrophotometer. Spectra have been corrected for dilution (max 10%). CD spectra were recorded using 0.5 cm pathlength cuvettes on a JASCO 810 spectropolarimeter with 8 spectral accumulations. Steady-state emission spectra were recorded on a Perkin-Elmer LS55 spectrofluorimeter operating in phosphorescence mode. Where necessary, correction for photomultiplier response in the red/near-IR region was made using a solution of 4′-dimethylamino 4-nitrostilbene in orthodichlorobenzene. For steady-state emission titrations, excitation was into isosbestic points in the Q-band region (517-523 nm) under conditions of optical dilution (A<0.1 in 1 cm cell). Spectra were scaled for slight variations in absorbance at the excitation wavelength and therefore are representative of relative emission quantum yields. Relative quantum yields were calculated by integrating the emission spectra. Emission 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 via a 600 nm long pass filter. Data were fit to bi- or tri-exponential decay functions as appropriate.

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

Binding of the porphyrin to DNA was modelled using the method of McGhee and von-Hippel with neighbour exclusion.[12]

$$\frac{r}{C_F} = K(1-nr)\left[\frac{1-nr}{1-nr+r}\right]^{n-1} \qquad (2)$$

In eqn (2), K is the apparent binding constant, C_f is the concentration of free porphyrin, r is the ratio of bound porphyrin to total base pairs and n is the number of base-pair steps per bound porphyrin.

Results

PtTMPyP4 and $[poly(dG\text{-}dC)]_2$ – Ground State

Addition of $[poly(dG\text{-}dC)]_2$ to PtP up to a [Nucl]/[Por] of 3 results in a substantial red-shift (14 nm) and maximum hypochromism of 53 % (ε_{417} = 7.77 x 10^4 M^{-1} cm^{-1}) of the Soret and Q bands in the UV/vis spectra (Fig. 2). An isosbestic point was also observed at 417 nm. As the [Nucl]/[Por] ratio is increased, there is a further 7 nm red-shift of the absorption spectrum, and a slight hyperchromism (Fig. 2 inset). The binding of PtP to $[poly(dG\text{-}dC)]_2$ was measured from the absorption spectra with a Scatchard plot according to the method of McGhee and von Hippel (eqn 2), yielding K_{app} = 3.6 x 10^6 M^{-1} and n = 2.9, implying that each binding event precludes binding at two neighbouring sites (ESI Fig. S1). By comparison, values of K = 7.7 x 10^5 M^{-1} and n = 1.8 were recorded for H2P-$[poly(dG\text{-}dC)]_2$, albeit at a higher ionic strength (μ = 0.2).[1b]

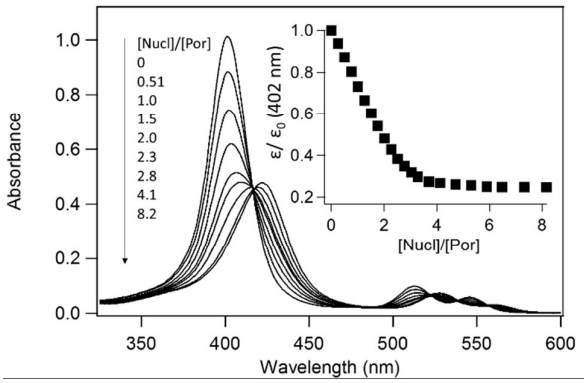

Fig. 2 UV/vis spectra of 6 μM PtP in the presence of increasing concentrations of $[poly(dG\text{-}dC)]_2$. Inset top: change in absorbance at 401 nm; bottom; change in Soret band absorbance at higher [Nucl]/[Por]. Spectral data corrected for dilution.

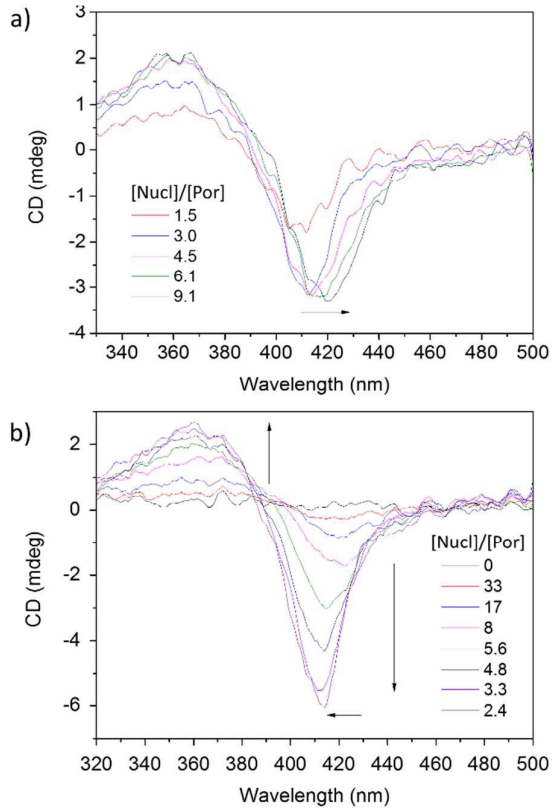

Fig. 3 Induced CD spectra of (a) 6 μM PtP in the presence of increasing concentrations of $[poly(dG\text{-}dC)]_2$ (b) 60 μM $[poly(dG\text{-}dC)]_2$ in presence of increasing concentrations of PtP. In 50 mM Na-phosphate buffer pH 6.8

As PtP is achiral, it does not produce a CD signal. However it is expected to display an induced CD signal when bound to the chiral environment of DNA. CD spectra were recorded under two conditions: addition of increasing amounts of $[poly(dG\text{-}dC)]_2$ into a fixed concentration of porphyrin (Fig. 3a), and addition of increasing amounts of porphyrin into a fixed concentration of $[poly(dG\text{-}dC)]_2$ (Fig. 3b). Binding to $[poly(dG\text{-}dC)]_2$ results in a negative induced CD signal in the Soret region of PtP, with a broad positive signal at lower wavelength. The minimum of the negative ICD was found to be dependent on the [Nucl]/[Por] ratio. At low [Nucl]/[Por], when there is a high concentation of porphyrin relative to polynucleotide, the minimum is located at 407 nm. At high [Nucl]/[Por], as more $[poly(dG\text{-}dC)]_2$ is added, this is shifted to 422 nm. There is also a notable change in the CD spectrum in the region (< 300 nm) where DNA absorbs (ESI Figs S2 & S3).

PtTMPyP4 and $[poly(dG\text{-}dC)]_2$ – Excited State

Addition of $[poly(dG\text{-}dC)]_2$ to an air-equilibrated solution of PtP results in a six-fold enhancement in emission quantum yield, in addition to an 18 nm red shift in the emission λ_{max} (Fig. 4). Most of this spectral shift occurs with the early additions of polynucleotide ([Nucl]/[Por] 0 to 1). The phosphorescence decays can be fitted to biexponential kinetics. The short component fits to ca. 1 μs, similar to that of unbound PtP (Fig. 4b), while the second component fits as 8.0 ± 0.5 μs (Fig 4b, ESI Table S1). The relative contributions of each species plateaus at [Nucl]/[Por] = 3.5, similar to where changes in behaviour were observed in the steady-state spectra. Similar lifetimes for the long component were obtained when the first component was fixed at 1 μs (the lifetime of unbound PtP in air-equilibrated solution)[10] in the fit (ESI Table S2). An average lifetime was also calculated ($\langle\tau\rangle = \Sigma a_i\tau_i/\Sigma a_i$), and the increase in average lifetime ($\langle\tau\rangle/\tau_0$ = 6) agrees with the increase in quantum yield from the steady-state data. At high [Nucl]/[Por] (7.8) the lifetime was compared for aerated and N_2-flushed solutions. The second component lengthens in lifetime from 9 μs to 11.8 μs, yielding an O_2 k_q value (~9 x 10^7 dm^3 mol^{-1} s^{-1}) similar to that reported for H2P-$[poly(dG\text{-}dC)]_2$ (1.2 x 10^8 dm^3 mol^{-1} s^{-1})[13] and for PdTMPyP4 bound to a 5′-CG-3′ step in a double stranded oligonucleotide (1.1 x 10^8 dm^3 mol^{-1} s^{-1}).[14]

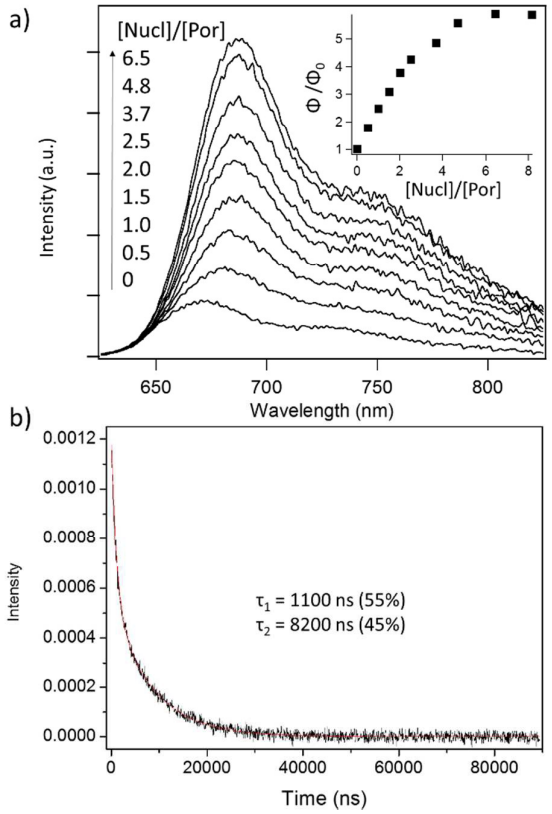

Fig. 4 (a) Emission spectra of 6 μM PtP in the presence of increasing concentrations of $[poly(dG\text{-}dC)]_2$ (λ_{exc} = 523 nm). Inset: Change in relative emission quantum yield with increasing [Nucl]/[Por]. Spectral data corrected for variations in absorbance at 523 nm. (b) Kinetic plot and biexponential fit of 8 μM PtP in presence of $[poly(dG\text{-}dC)]_2$ ([Nucl]/[Por] = 2.8, λ_{exc} = 355 nm, λ_{em} = 670 nm) % contribution = $100a_i/\Sigma a_i$. In aerated 50 mM Na-phosphate buffer pH 6.8

PtTMPyP4 and $[poly(dA\text{-}dT)]_2$ - Ground state

In PtP-$[poly(dA\text{-}dT)]_2$ the change in the absorption spectrum is less pronounced than in PtP-$[poly(dG\text{-}dC)]_2$, with only a 10 nm red-shift and a maximum of hypochromism of 40% (ε_{411} = 1.05 x 10^5 M^{-1} cm^{-1} at [Nucl]/[Por] = 3.3, see Fig. 5). The absorbance of the Soret band increases again at higher

[Nucl]/[Por] ratios. Due to the lack of a defined end-point for the titration, the binding data could not be fit using the McGhee vonHippel method.

As with $[poly(dG\text{-}dC)]_2$, induced CD titrations were performed in both directions, i.e. addition of polynucleotide to a fixed concentration of porphyrin (Fig. 6a), or addition of porphyrin to a fixed concentration of polynucleotide (Fig. 6b). The spectra in both cases differ substantially from those observed with $[poly(dG\text{-}dC)]_2$. At high [Nucl]/[Por] the spectrum is dominated by a positive signal at 409 nm. At lower [Nucl]/[Por] (i.e. with smaller $[poly(dA\text{-}dT)]_2$ concentration), the signal becomes bisignate (407 nm-/424 nm+). The presence of two distinct spectral features is characteristic of at least two binding modes, which are populated to varying degrees depending on the relative concentrations of porphyrin and polynucleotide.

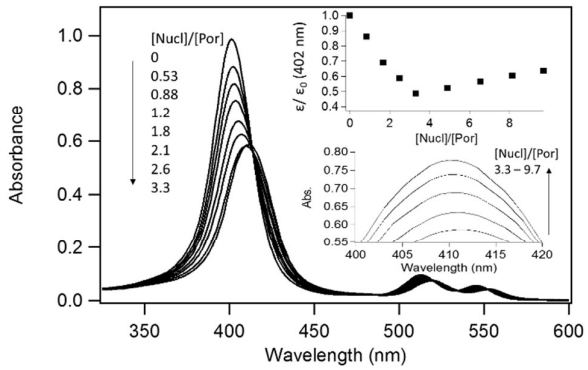

Fig. 5 UV/vis spectra of 6 μM PtP in the presence of increasing $[poly(dA\text{-}dT)]_2$ concentrations. Inset: (top) Change in absorbance at 401 nm with increasing [Nucl]/[Por] (bottom) increase in absorbance at higher [Nucl]/[Por]. In 50 mM Na-phosphate buffer pH 6.8. Spectral data corrected for dilution

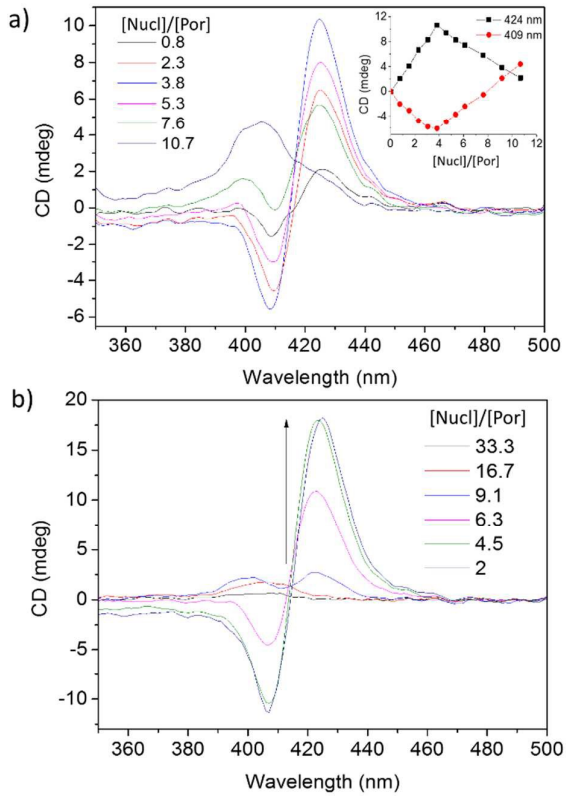

Fig. 6 Induced CD spectra of (a) 6 μM PtP in the presence of increasing concentrations of $[poly(dA\text{-}dT)]_2$ (b) 70 μM $[poly(dA\text{-}dT)]_2$ in presence of increasing concentrations of PtP. In 50 mM Na-phosphate buffer pH 6.8

PtTMPyP4 and $[poly(dA\text{-}dT)]_2$ - Excited state

Phosphorescence titrations of PtP with $[poly(dA\text{-}dT)]_2$ in aerated solution show a significant enhancement of emission up to $\Phi/\Phi_0 = 17$, but with no shift in emission λ_{max} (Fig. 7a). After [Nucl]/[Por] ~ 3.5 the emission intensity drops, and there is a slight red-shift in the emission λ_{max} ($\Delta\lambda = 10$ nm at [Nucl]/[Por] =10). Notably, this is the same [Nucl]/[Por] region where the absorption and CD spectra change profile. The phosphorescence lifetime decays were initially fit to biexponential kinetics (similar to the PtP-$[poly(dG\text{-}dC)]_2$ experiment) with the first component of ca. 1 μs and a longer component freely fitting to ca. 13 μs (ESI Fig S5 Tables S3 & S4). However, in contrast to the PtP-$[poly(dG\text{-}dC)]_2$ system, better fitting residuals could be obtained with three exponentials (see Fig 7b ESI Figs S6, Tables S5 & S6). When the first component is fixed as 1 μs in the fit, two long components (7.5 ± 1.0 μs, 22 ± 3 μs)‡ grow in with increasing [Nucl]/[Por], though the 22 μs lifetime falls away at high [Nucl]/[Por] (ESI Fig. S6, ESI Table S5). Although the assignment of three exponentials should generally be treated with caution, the resolution of two long lifetime components is consistent with the dual binding inferred from the CD, UV/vis and steady-state emission data. We suggest that the 22 μs lifetime corresponds to the binding mode responsible for the

bisignate CD signal, which predominates at lower [Nucl]/[Por]. At higher [Nucl]/[Por], the 7.5 μs species predominates, and this corresponds to the postive CD signal at 409 nm. This assignment is also consistent with the drop in emission intensity observed above [Nucl]/[Por] ~ 3.5.

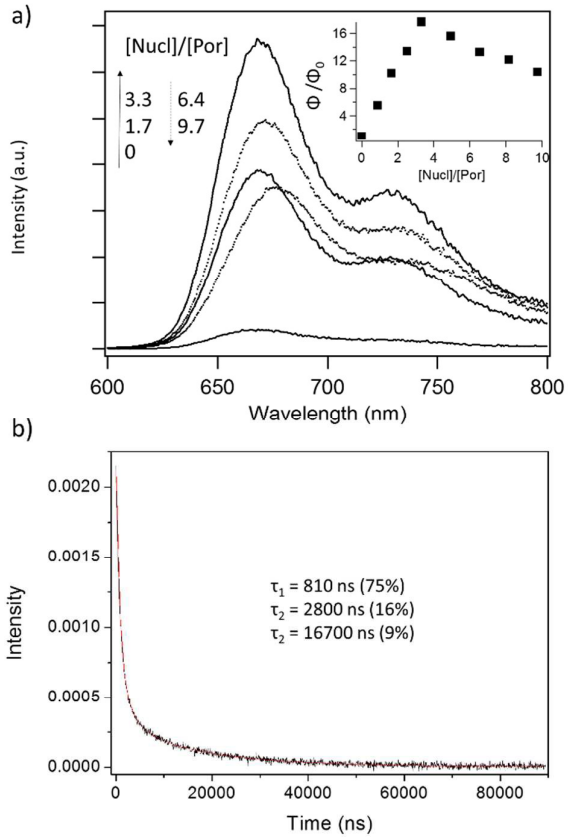

Fig. 7 (a) Emission spectra of 6 μM PtP in the presence of increasing concentrations of $[poly(dA\text{-}dT)]_2$ Inset: change in relative emission quantum yield as a function of [Nucl]/[Por] ratio. (λ_{exc} = 519 nm). Some spectra removed for clarity. Spectral data corrected for variations in absorbance at 519 nm (b) Kinetic plot and triexponential fit of 6 μM PtP in presence of $[poly(dA\text{-}dT)]_2$ ([Nucl]/[Por] = 2.5, λ_{exc} = 355 nm, λ_{em} = 670 nm). % contribution = $100a_i/\Sigma a_i$. In aerated 50 mM Na-phosphate buffer pH 6.8

Natural DNA

In order to compare our work to others in the field[4-7] we also studied PtP in the presence of mixed sequence DNA. UV/vis absorption titrations show a strong hypochromism and red-shift consistent with strong binding, as previously reported (ESI Fig S8). The emission spectra has an initial increase in intensity, followed by a spectral shift at higher [Nucl]/[Por]. The extent of emission enhancement is similar to that found by Zhang et al[6]. It is lower than that reported by Nyarko et al[4] and Sabharwal et al,[7] though it is possible that Cl^- ions in the buffer solution effectively quench the unbound species in the latter study, resulting in a larger relative enhancement when the complex is bound to DNA.

Emission lifetime experiments returned a biexponential decay, with one component of 1 μs and another component of 6.5 μs, somewhat shorter than those recorded by Zhang et al (1.9 and 9.9 μs)[6] and Borsch (8 μs).[5]

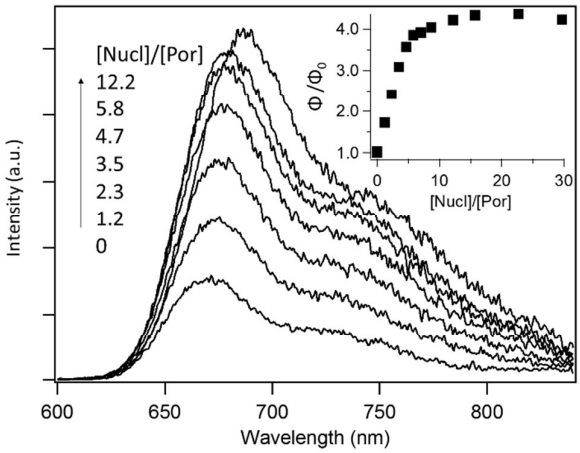

Fig. 8 Emission spectra 6.6 μM PtP in the presence of increasing concentrations of salmon testes DNA. λ_{exc} = 521 nm. In aerated 50 mM Na-phosphate buffer pH 6.8.

Table 1 Emission parameters for PtP in the presence of polynucleotides and mononucleotides. Recorded in air-equilibrated solution, 50 mM Na-phosphate buffer pH 6.8.

sequence	Δλ (nm)	I/Io	τ_{bound} (aer.)[a]
$[poly(dG\text{-}dC)]_2$	18	6	8.0 μs
$[poly(dA\text{-}dT)]_2$	0,10	17	7.5 μs, 22 μs
ds-DNA	21	4	6.5 μs
poly(rA)	5	14	13 μs
AMP[b]	7	6	2.5 μs, 6 μs
GMP[b]	7	0.3	70 ns, 400 ns

[a] errors in lifetime are ± 10% [b] from ref. 10

Discussion

Binding to polynucleotides

Large changes in UV/vis spectra (hypochromism and bathochromic shifts), and negative induced CD, suggest that PtP intercalates between the GC base-pairs in $[poly(dG\text{-}dC)]_2$, consistent with the behaviour observed for H2P and planar metal derivatives such as Ni(II)TMPyP4 and Cu(II)TMPyP4.[15] There has been much discussion on the mode of intercalation of cationic porphyrins with DNA polymers. A symmetric intercalation has been proposed for H2P in the 5′-CG-3′ step,[16,17] though a crystal structure of CuTMPyP4 in $[d(CGATCG)]_2$ suggests a hemi-intercalation with some flipping out of the neighbouring base.[18] The 5′-CG-3′ step is also believed to be the favoured site for intercalation by planar metal derivatives (NiTMPyP4/PdTMPyP4).[19]

The slight shift observed in the induced CD of PtP bears some resemblance to the behaviour of H2P in $[d(GCACGTGC)]_2$[17] and $(dG\text{-}dC)_{10}$[20] and may be due to

saturation of intercalation sites at high [porphyrin]/[DNA] ratios (low [Nucl]/[Por]), whereby some porphyrin may be forced to bind by an alternative mode such as external binding. Other possibilities include a disortion of the helix, which has been suggested to occur at high [pophyrin]/[nucleotide] ratios in a number of studies on TMPy systems[21-23] or to varying distributions of the porphyrin amongst the two possible intercalation sites (5′-CG-3′ and 5′-GC-3′).[21] Conformational changes in the $[poly(dG\text{-}dC)]_2$ helix are suggested by the changes in the CD spectra below 300 nm (ESI Figs S2 & S3).

Fundamentally different binding behaviour is observed in $[poly(dA\text{-}dT)]_2$. It is known that H2P and its metal derivatives do not intercalate at AT-rich sites (possibly due to steric interference from the T methyl group[16] or to clashes with the sugar-phosphate groups that are overcome only at GC sites through partial melting.[24]) There has been significant work done on the induced CD spectra of H2P in the presence of $[poly(dA\text{-}dT)]_2$, and it has been proposed that a positive CD signal corresponds to porphyrins bound in the minor groove, while the bi-signate signal is due to porphyrins self-stacked along the major groove.[25] The CD data for PtTMPyP4-$[poly(dA\text{-}dT)]_2$ may hence be interpreted as follows. At high [Nucl]/[Por], PtTMPyP4 binds mainly in the minor groove. At low [Nucl]/[Por] (i.e. high relative porphyrin concentration) most available sites become saturated and the porphyrins are forced to stack along the major groove. Conversely, when the $[poly(dA\text{-}dT)]_2$ is added to the PtTMPyP4 the porphyrins can initially occupy both sites, but will bind preferentially in the minor groove when free sites become available at high [Nucl]/[Por]. The shape of the excitonic induced CD signal has been described as 'extensively stacked' while a reversal of the signal implies 'moderate' stacking.[23] The latter is not observed in our spectra though it may be expected to be weak.

Triplet state dynamics

The enhancement in emission quantum yield and lifetime of PtP in the presence of $[poly(dG\text{-}dC)]_2$ contrasts with the quenching we reported previously with GMP, which we attributed to photo-induced electron transfer from guanine to the PtP $^3\pi\pi^*$ state.[10] The greater ease of electron transfer to guanine in GMP vs. $[poly(dG\text{-}dC)]_2$ is an intriguing observation, as it contrasts with what has been found for other photosensitisers such as naphthaldiimides[26] or phenothiazinium dyes[27] where base-pairing to cytosine is found to make the oxidation of guanine more favourable. This is also consistent with recent electrochemical measurements such as those of Shinde et al., which showed that the oxidation potential (E^o = 1.22 V)[28] was somewhat lower than that for isolated isolated GMP (1.29 V,[29] 1.31 V,[30]). By contrast, other authors have reported that there is an increased difficulty of oxidizing the nucleobases in single-stranded or double-stranded DNA.[31] The increased lifetime of the PtP triplet state when intercalated into the polynucleotide compared to that when complexed with GMP may be associated with a number of factors such as (i) the expected reduction in non-radiative decay of the excited state as is well known for other intercalated photosensitisers, (ii) better protection from oxygen quenching (iii) a decrease in the reduction potential of the porphyrin excited state when bound to $[poly(dG\text{-}dC)]_2$ as is shown by the red-shift in the phosphorescence (see Table 1, the reduction potential of the $^3\pi\pi^*$ state of unbound PtP has been reported as 1.47V vs NHE).[32] Another factor which will influence the rate of electron transfer is the probable different geometry of the porphyrins and guanine moieties,[33] which unfortunately is not known (a precise study of this latter factor would require carrying out the photo-induced electron transfer in crystals, as has recently been carried out with an intercalating ruthenium complex.)[34]

The behaviour is consistent with that observed with H_2TMPyP4, which undergoes less efficient fluorescence (i.e. singlet state) quenching in H2P-$[poly(dG\text{-}dC)]_2$ ($k = 4 \times 10^8$ s^{-1}) than H2P-GMP (1.7×10^9 s^{-1}).[33] Furthermore, the triplet state of H2P is quenched by GMP but not by $[poly(dG\text{-}dC)]_2$.[33,35,36] The increase in triplet emission and lifetime observed in the current study initially suggest that PtP, like H2P, does not undergo electron transfer from the triplet state when intercalated in $[poly(dG\text{-}dC)]_2$. However it is interesting to note that the PtP-$[poly(dG\text{-}dC)]_2$ triplet lifetime (aerated solution) is shorter than that for PtP in $[poly(dA\text{-}dT)]_2$. By contrast, the triplet lifetime of H2P-$[poly(dG\text{-}dC)]_2$ (30 μs) is longer than H2P-$[poly(dA\text{-}dT)]_2$ (5.5 μs and 20.5 μs), and the rate constants for collisional O_2 quenching are larger in the latter.[37] Similarly, Bork et al reported higher O_2 k_q values for PdTMPyP4 bound to an oligonucleotide hairpin containing TA steps, which would favour outside binding (2.5×10^8 dm^3 mol^{-1} s^{-1}), than for one containing a 5′-CG-3′ intercalation site (1.1×10^8 dm^3 mol^{-1} s^{-1}),[14] while Brun and Harriman have also observed a significantly greater degree of protection from dissolved oxygen in intercalated PdTMPyP4 vs an externally bound derivative.[38] Test experiments with PtP in the presence of poly(rA) (to which TMPy metalloporphyrins have been shown to bind strongly)[39] show that binding increases the emission intensity by a factor of 13 in aerated solution and 35 when deoxygenated (ESI Figs S9 & S10). This enhancement is notably more than for PtP-$[poly(dG\text{-}dC)]_2$. Therefore it is possible that a (very) slow electron transfer does indeed take place ($k < 1 \times 10^5$ s^{-1}) for PtP-$[poly(dG\text{-}dC)]_2$.

The biexponential kinetics measured for PtP-$[poly(dG\text{-}dC)]_2$ suggest that there are two types of bound porphyrin. The first component is similar in lifetime to that of unbound porphyrin, however the experiments were conducted at [Nucl]/[Por] values where no unbound species would be expected. Furthermore, T-jump experiments by Pasternack[15] measured the rate of dissociation of an intercalated porphyrin as 1.8 s^{-1}, suggesting that it is unlikely that a photoexcited porphyrin would escape from the intercalation site within its excited-state lifetime.[§] The presence of two bound species is also consistent with the [Nucl]/[Por] – dependent shifts observed in the CD and phosphorescence spectra.

Interestingly, Chirvony et al observed two fluorescence lifetimes (2.5 ns, 7 ns) for H2P bound to $[poly(dG\text{-}dC)]_2$.[40] Notably the second component is longer than that of unbound H2P (5 ns), leading the authors to suggest that some bound porphyrin was not undergoing electron transfer. However it may also be suggested that this 7 ns species is undergoing a

weaker electron transfer, as its lifetime is still shorter than that of H2P bound to $[poly(dA\text{-}dT)]_2$, 5′-AMP or natural double-stranded DNA. A similar interpretation for the PtP system would suggest that the 8 μs component corresponds to weakly reactive porphyrin, while the ca. 1 μs component is that of a more strongly quenched porphyrin.

Based on our assignment of the 7.5 μs and 22 μs lifetimes in $[poly(dA\text{-}dT)]_2$ to PtP in the minor and stacked in the major groove, respectively, we can surmise that porphyrins in the minor groove are more susceptible to non-radiative excited-state decay and/or O_2 quenching. By comparison, H2P shows lengthening in both S_1 and T_1 lifetime in the presence of $[poly(dA\text{-}dT)]_2$, and two triplet decay constants for the bound form have also been observed. Interestingly these lifetimes (5.5 μs and 20.5 μs) are similar to those we observe with PtP. However, the authors of that study proposed that the longer-lived component was that of minor-groove bound porphyrins, where they suggested protection from dissolved oxygen would be greater.

It is noteworthy that the lifetime of the bound form of both PtP and H2P in $[poly(dA\text{-}dT)]_2$ is longer than that of the unbound porphyrin. As discussed earlier, CD data suggests some 'self-stacking' interactions in the major groove, and these may be expected to result in efficient quenching, rather than enhancement, of the excited state.[41] The observed enhancement implies that any self-stacking interactions in the major groove are not sufficient to quench the excited states.

Conclusions

PtP intercalates into GC base pairs in $[poly(dG\text{-}dC)]_2$ and binds to two sites in $[poly(dA\text{-}dT)]_2$, assumed to be the minor and major grooves. The binding is similar to that observed with H_2TMPyP4. Despite the emission enhancement in PtTMPyP4-$[poly(dG\text{-}dC)]_2$ the data is consistent with a very slow photoinduced electron transfer from the porphyrin triplet to the guanine base, which is much less efficient than that recorded in the presence of the GMP nucleotide. Large changes in the phosphorescence yields and lifetimes indicate that the luminescence of air-equilibrated PtTMPyP4 has a more sensitive response to DNA-binding than exhibited by the fluorescence of the analagous H_2TMPyP4 systems, demonstrating the usefulness of this molecule as a probe.

Acknowledgements

Science Foundation Ireland (06/RFP/CHP035 and 07/RFP/CHEF437) and Trinity College Dublin are thanked for financial support. We should like to dedicate this article to the memory of Dr. Wil van der Putten, who carried out pioneering studies on the transient spectroscopy of dyes such as methylene blue bound to DNA.

Notes and references

‡ When the first component was freely fit, the lifetimes of the longer components were shorter (3.5 ± 1.0 and 16 ± 3 μs; see ESI Fig S7 and Table S6).

§ Kubat et al have observed 20% contribution from a free porphyrin chromophore at a DNA [Nucl]/[Por] of 70, which they suggested may be due to dissociation within the excited state lifetime. In this case the lifetime of the bound species is ca. 114 μs.[41b]

1 (a) R. J. Fiel, J. C. Howard, E. H. Mark and N. Datta-Gupta, Interaction of DNA with a porphyrin ligand – evidence for intercalation, *Nucleic Acids Res.*, 1979, **6**, 3093–3118. (b) R. F. Pasternack, E. J. Gibbs and J. J. Villafranca, Interactions of porphyrins with nucleic acids, *Biochemistry*, 1983, **22**, 2406-2414; (c) J. M. Kelly, M. J. Murphy, D. J. McConnell and C. OhUigin, A comparative study of the interaction of 5,10,15,20-tetrakis(N-methylpyridinium-4-yl)porphyrin and its zinc complex with DNA using fluorescence spectroscopy and topoisomerisation, *Nucleic Acids Res.*, 1985, **13**, 167-184

2 (a) R. J. Fiel, N. Datta-Gupta, E. H. Mark and J. C. Howard, *Cancer Res.*, 1981, **41**, 3543-3545 (b) D. Praseuth, A. Gaudemer, J. Verlhac, I Kraljic, I. Sissoeff and E. Guille, *Photochem. Photobiol.* 1986, **44**, 717–724. (c) C. Kasturi and M. S. Platz, Inactivation of lambda phage with 658 nm light using a DNA-binding porphyrin sensitiser, *Photochem. Photobiol.*, 1992, **56**, 427–429.

3 (a) R. T. Wheelhouse, D. Sun, H. Han, F. X. Han and L. H. Hurley, Cationic porphyrins as telomerase inhibitors: the interaction of tetra-(-N-methyl-4-pyridyl)porphine with quadruplex DNA, *J. Am. Chem. Soc.*, 1998, **120**, 3261-3262; (b) D. F. Shi, R. T. Wheelhouse, D. Sun and L. H. Hurley, Quadruplex-interactive agents as telomerase inhibitors: synthesis of porphyrins and structure-activity relationship for the inhibition of telomerase, *J. Med. Chem.*, 2001, **44**, 4509-4523

4 E. Nyarko, N. Hanada, A. Habib and M. Tabata, Fluorescence and phosphorescence spectra of Au(III), Pt(II) and Pd(II) porphyrins with DNA at room temperature, *Inorg. Chim. Acta*, 2004, **37**, 739-745

5 M. Borsch, Targeting cytochrome C oxidase in mitochondria with Pt(II)-porphyrins for photodynamic therapy, *Proc. SPIE-Int. Soc. Opt. Eng.* 2010, 7551, 75510G

6 H. Zhang, M. A. Bork, K. J. Reidy, D. R. McMillin and J. H. Choi, Understanding photophysical interactions of semiconducting carbon nanotubes with porphyrin chromophores, *J. Phys. Chem. C*, 2014, **118**, 11612-11619

7 N. C. Sabharwal, O. Mendoza, J. M. Nicoludis, T. Ruan, J.‐L. Mergny and L. A. Yatsunyk, Investigation of the interactions between Pt(II) and Pd(II) derivatives of 5,10,15,20‐tetrakis (N‐methyl‐4‐pyridyl) porphyrin and G‐quadruplex DNA, *J. Biol. Inorg. Chem.*, 2016, **21**, 227-239

8 R. F. Pasternack, R. A. Brigandi, M. J. Abrams, A. P. Williams and E. J. Gibbs, Interactions of porphyrins and metalloporphyrins with single-stranded poly(dA), *Inorg. Chem.*, 1990, **29**, 4483-4486.

9 V. A. Blinova and V. V. Vasil'ev, Luminescence properties of water soluble cationic platinum(II) and palladium(II) porphyrins, *Russ. J. Phys. Chem.*, 1995, **69**, 1097-1101

10 P. M. Keane and J. M. Kelly, Triplet state dynamics of a metalloporphyrin photosensitiser (PtTMPyP4) in the presence of halides and purine mononucleotides, *Photochem. Photobiol. Sci.*, 2011, **10**, 1578-1586

11 (a) H. Zhang, Y. Sun, K. Ye, P. Zhang and Y. Wang, Oxygen sensing

materials based on mesoporous silica MCM-41 and Pt(II)-porphyrin complexes, *J. Mater. Chem.*, 2005, **15**, 3181–3186. (b) V. V. Vasil'ev and S. M. Borisov, Optical oxygen sensors based on phosphorescent water-soluble platinum metals porphyrins immobilized in perfluorinated ion-exchange membrane, *Sens. Actuators, B*, 2002, **82**, 272–276.

12 J. D. McGhee and P. H. VonHippel, Theoretical aspects of DNA-protein interactions – cooperative and non-cooperative binding of large ligands to a one-dimensional homogeneous lattice, *J. Mol. Biol.*, 1974, **86**, 469-489

13 V. S. Chirvony, V. A. Galievsky, N. N. Kruk, B. M. Dzhagarov and P.-Y. Turpin, Photophysics of cationic 5,10,15,20-tetrakis-(4-*N*-methylpyridyl)porphyrin bound to DNA, $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$: on a possible charge-transfer process between guanine and porphyrin in its excited singlet state, *J. Photochem. Photobiol., B*, 1997, **40**, 154-162.

14 M. A. Bork, C. G. Gianopoulos, H. Zhang, P. E. Fanwick, J, H Choi and D. R. McMillin, Accessibility and external versus intercalative binding to DNA as assessed by oxygen-induced quenching of the palladium(II)-containing cationic porphyrins Pd(T4) and Pd(tD4), *Biochemistry*, 2014, **53**, 714-724

15 R. F. Pasternack, E. J. Gibbs and J. J. Villafranca, Interactions of porphyrins with nucleic acids, *Biochemistry*, 1983, **22**, 5409-5417

16 K. G. Ford, L. H. Pearl and S. Neidle, Molecular modelling of the interaction of tetra(4-N-methylpyridyl) porphin with TA and CG sites on DNA, *Nucleic Acids Res.*, 1987, **15**, 6553-6562

17 A. B. Guliaev and N. B. Leontis, Cationic 5,10,15,20,-tetrakis(*N*-methylpyridinium-4-yl)porphyrin fully intercalates at 5'-CG-3' steps of duplex DNA in solution, *Biochemistry*, 1999, **28**, 15425-15437

18 L. A. Lipscomb, F. X. Zhou, S. R. Presnell, R. J. Woo, M. E. Peek, R. R. Plaskon and L. D. Williams, Structure of a DNA-porphyrin complex, *Biochemistry*, 1996, **35**, 2818-2823

19 J. A. Strickland, L. G. Marzilli, W. D. Wilson and G. Zon, Metalloporphyrin DNA interactions: insights from NMR studies of oligodeoxyribonucleotides, *Inorg. Chem.*, 1989, **28**, 4191-4198

20 J. Novy, M. Urbanova and K. Volka, Electronic and vibrational circular dichroism spectroscopic study of non-covalent interactions of *meso*-5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin with $(dG\text{-}dC)_{10}$ and $(dA\text{-}dT)_{10}$, *Vib. Spectroscopy*, 2007, **43**, 71-77

21 B. Hudson, J. Sou, D. J. Berger and D. R. McMillin, Luminescence studies of the intercalation of Cu(TMPyP4) into DNA, *J. Am. Chem. Soc.*, 1992, **114**, 8997-9002

22 M. Tabata, K. Nakajima and E. Nyarko, Metalloporphyrin mediated DNA cleavage by a low concentration of HaeIII restriction enzyme, *J. Inorg. Biochem.*, 2000, **78**, 383-389

23 S. Lee, Y.-A. Lee, H. M. Lee, J. Y. Lee, D. H. Kim and S. K. Kim, Rotation of periphery methylpyridine of *meso*-tetrakis(*n*-*N*-methylpyridiniumyl)porphyrin (n = 2,3,4) and its selective binding to native and synthetic DNAs, *Biophys. J.*, 2002, **83**, 371-382

24 D. R. McMillin, A. H. Shelton, S. A. Bejune, P. E. Fanwick and R. K. Wall, Understanding binding interactions of cationic porphyrins with B-form DNA, *Coord. Chem. Rev.*, 2005, **249**, 1451-1459

25 B. Jin, J. E. Ahn, J. H. Ko, W. Wang, S. W. Han and S. K. Kim, Effect of the position and number of positive charges on the intercalation and stacking of porphyrin to $poly[d(G\text{-}C)_2]$, $poly[d(A\text{-}T)_2]$ and native DNA, *J. Phys. Chem. B*, 2008, **112**, 15875-15882

26 K. Kawai, Y. Wata, N. Ichinose and T. Majima, Selective enhancement of the one electron oxidation of guanine by base-pairing with cytosine, *Angew. Chem. Int. Ed.,* 2000, **39,** 4327-4329.

27 (a) G. S. Beddard, J. M. Kelly and W. J. M. van der Putten, *J. Chem. Soc., Chem. Commun.,* Picosecond study of luminescence and transient absorption of methylene blue-polynucleotide Complexes. 1990, 1346-1347. (b) G. D. Reid, D. J. Whittaker, M. A. Day, D. A. Turton, V. Kayser, J. M. Kelly and G. S. Beddard, Femtosecond electron-transfer in mono and polynucleotides and in DNA, *J. Am. Chem. Soc.*, 2002, **124**, 5518– 5527.

28 S. S. Shinde, A. Maroz, M. P. Hay and R. F. Anderson, One-electron reduction potential of the neutral guanyl radical in the GC base pair of duplex DNA, *J. Am. Chem. Soc.*, 2009, **131**, 5203-5207

29 S. Steenken and S. V. Jovanovic, How easily oxidizable is DNA? One electron reduction potentials of adenosine and guanosine radicals in aqueous solution, *J. Am. Chem. Soc.*, 1997, **119**, 617–618.

30 S. Fukuzumi, H. Miyao, K. Ohkubo and T. Suenobu, Electron transfer oxidation properties of DNA bases and DNA oligomers, *J. Phys. Chem. A*, 2005, **109**, 3285–3294.

31 M. Zhou, Y. Zhai and S. Dong, Electrochemical sensing and biosensing platform based on chemically reduced graphene oxide, *Anal. Chem.,* 2009, **81,** 5603-5613

32 V. V. Vasil'ev, I. A. Blinova, I. V. Golovina and S. M. Borisov, Photophysical and photochemical properties of the water-soluble porphyrin complexes of the platinum group*, J. Appl. Spectrosc.*, 1999, **66**, 583–587

33 V. S. Chirvony, Primary photoprocesses in cationic 5,10,15,20-meso-tetrakis(4-N-methylpyridiniumyl)porphyrin and its transition metal complexes bound with nucleic acids, *J. Porphyrins Phthalocyanines*, 2003, **7**, 766-774

34 J. P. Hall, F. E. Poynton, P. M. Keane, S. P. Gurung, J. A. Brazier, D. J. Cardin, G. Winter, T. Gunnlaugsson, I. V. Sazanovich, M. Towrie, C. J. Cardin, J. M. Kelly and S. J. Quinn, Monitoring one-electron photo-oxidation of guanine in DNA crystals using ultrafast infrared spectroscopy, *Nat. Chem.*, 2015, **7**, 961-967

35 C. Belin, M.Sc. Thesis, Trinity College Dublin, 1992

36 R. Jasuja, D. M. Jameson, C. K. Nishijo and R. W. Larsen, Singlet excited state dynamics of tetrakis(4-N-methylpyridyl)porphine associated with DNA nucleotides, *J. Phys. Chem. B*, 1997, **101**, 1444–1450.

37 N. N. Kruk, B. M. Dzhagarov, V. A. Galievsky, V. S. Chirvony and P.-Y. Turpin, Photophysics of the cationic 5,10,15,20-tetrakis(4-*N*-methylpyridyl)porphyrin bound to DNA, $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$: interaction with molecular oxygen studied by porphyrin triplet-triplet absorption and singlet oxygen luminescence, *J. Photochem. Photobiol. B*, 1998, **42**, 181-190

38 A. M. Brun and A. Harriman, Energy- and electron-transfer processes involving palladium porphyrins bound to DNA, *J. Am. Chem. Soc.*, 1994, **116**, 10383

39 Z.-X. Zhou, F. Gao, X. Chen, X.-J. Tian and L.-N. Ji, Selective binding and reverse transcription inhibition of single-strand poly(A) RNA by metal TMPyP complexes, *Inorg. Chem.*, 2014, **53**, 10015−10017

40 V. S. Chirvony, V. A. Galievsky, N. N. Kruk, B. M. Dzhagarov and P.-Y. Turpin, Photophysics of cationic 5,10,15,20-tetrakis-(4-*N*-methylpyridyl)porphyrin bound to DNA, $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$: on a possible charge-transfer process between

guanine and porphyrin in its excited singlet state, *J. Photochem. Photobiol., B*, 1997, **40**, 154-162.

41 (a) K. Lang, P. Anzebacher, P. Kapusta, V. Kral, P. Kubat and D. Wagnerova, Long-range assemblies on poly(dG-dC)$_2$ and poly(dA-dT)$_2$: phosphonium cationic porphyrins and the importance of the charge, *J. Photochem. Photobiol. B*, 2000, **57**, 51-59; (b) P. Kubat, K. Lang, V. Kral and P. Anzebacher, Preprogramming of porphyrin-nucleic acid assemblies via variation of the alkyl/aryl substituents of phosphonium tetratolylporphyrins, *J. Phys. Chem. B*, 2002, **106**, 6784-6792

Binding of PtTMPyP4 to synthetic polynucleotides poly(dA-dT)$_2$ and poly(dG-dC)$_2$ results in an increase in triplet state emission quantum yield and lifetime.