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PAPER

Excited state dependent electron transfer of a rhenium-dipyridophenazine complex intercalated between the base pairs of DNA: a time-resolved UV-visible and IR absorption investigation into the photophysics of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ bound to either $[poly(dA\text{-}dT)]_2$ or $[poly(dG\text{-}dC)]_2$†

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The transient species formed following excitation of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ (F_2dppz = 11,12-difluorodipyrido[3,2-*a*:2′,3′-*c*]phenazine) bound to double-stranded polynucleotides $[poly(dA\text{-}dT)]_2$ or $[poly(dG\text{-}dC)]_2$ have been studied by transient visible and infra-red spectroscopy in both the picosecond and nanosecond time domains. The latter technique has been used to monitor both the metal complex and the DNA by monitoring the regions 1900–2100 and 1500–1750 cm^{-1} respectively. These data provide direct evidence for electron transfer from guanine to the excited state of the metal complex, which proceeds both on a sub-picosecond time scale and with a lifetime of 35 ps, possibly due to the involvement of two excited states. No electron transfer is found for the $[poly(dA\text{-}dT)]_2$ complex, although characteristic changes are seen in the DNA-region TRIR consistent with changes in the binding of the bases in the intercalation site upon excitation of the dppz-complex.

Introduction

Metal complexes which intercalate between the base pairs of DNA have attracted considerable attention in recent years showing potential as light switching biosensors[1,2] and as photoreagents.[3] The molecular light switch effect, observed for complexes such as $[Ru(bpy)_2(dppz)]^{2+}$ and $[Ru(dppz)(phen)_2]^{2+}$ (bpy = 2,2′-bipyridine, phen = 1,10-phenanthroline, dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine), has been instrumental in the development of complexes which act as DNA probes.[4] In essence, this effect is typified by the absence of luminescence for these complexes in aqueous solution and the presence of appreciable luminescence in organic solvents or in aqueous solution in the presence of DNA. The effect arises due to an interplay between two close-lying ^{3}MLCT(phen) (bright) and ^{3}MLCT(phz) (dark) excited states which are distinguished by the distribution of charge on the dppz ligand framework.[5] The presence of two closely lying LUMOs can lead to even more complex photophysics for substituted Ru- and Re-dppz complexes with the excited manifold being composed of MLCT and IL ππ* (phen) and ππ*(phz) states whose population and lifetime is both solvent and environment dependent. Time-resolved vibrational spectroscopy has proved to be a useful tool with both Raman[6] and infrared[7] providing valuable insight into the photophysics of these systems.

Ruthenium polypyridyl complexes are also well known to cause photodamage to DNA and in most cases these reactions involve reactive oxygen species which are produced by interaction of the complex's excited state with molecular oxygen. However, the excited states of complexes which contain two or three ligands such as 1,4,5,8-tetraazaphenanthrene (TAP), 1,4,5,8,9,12-hexaazatriphenylene (HAT) or 2,2′-bipyrazine (bpz) are much more oxidising than those of complexes such as $[Ru(bpy)_3]^{2+}$ and can directly oxidise the guanine in both mononucleotides and polynucleotides including double-stranded DNA.[8] This photo-oxidation process can directly lead to strand breaks, alkali-sensitive sites and to photo-adduct formation. Excitation (400 nm)

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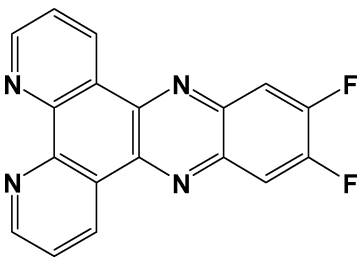
† Electronic supplementary information (ESI) available: UV/vis absorption spectra and emission ($[poly(dA\text{-}dT)]_2$ only) of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ recorded in buffered D_2O solution in the presence of increasing concentrations of $[poly(dA\text{-}dT)]_2$ or $[poly(dG\text{-}dC)]_2$. See DOI: 10.1039/c1pp05050h

of $[Ru(dppz)(TAP)_2]^{2+}$ in either H_2O or D_2O when intercalated between the base pairs of a double-stranded synthetic polynucleotide, $[poly(dG\text{-}dC)]_2$, produced the reduced complex $[Ru(dppz)(TAP)_2]^{+}$ with forward and backward rates consistent with proton-coupled electron transfer processes.[9] Picosecond transient IR measurements in D_2O solution confirm that the reduction of the metal complex was accompanied by bleaching of IR ground-state bands of guanine and cytosine and the production of the characteristic IR signature[10] of the oxidised guanine at *ca.* 1700 cm^{-1} formed by short wavelength UV irradiation.[10c] A similar signature has also been observed following the electrochemical oxidation of 2′,3′-*O*-isopropylidene-5′-*O*-(tertbutyldimethylsilyl)guanosine.[11] These results show the potential for using IR spectroscopy for interrogation of photophysical/photochemical processes of metal-dppz complexes intercalated between the base pairs of DNA. However, the IR fingerprint region where key processes in the DNA can be monitored is very congested and complex photophysical processes can be difficult to untangle. Metal carbonyls on the other hand are potentially ideal reporters of DNA intercalators since the frequencies of ν(CO) IR bands are sensitive to electronic structure, which allows these ligands to act as probes of electron distribution in the excited state and directly monitor the nature and reactivity of the excited state.[12]



F_2dppz

Complexes of the type *fac*-$[Re(CO)_3(dppz)(L)]^{+}$ (L = ligand) have been shown to bear several similarities with the Ru(II)-dppz '*light switches*'. This makes them natural candidates as DNA probes although they have been much less studied than their ruthenium counterparts. Yam *et al.*[13] and Schanze *et al.*[14] independently published work investigating the photophysics of *fac*-$[Re(CO)_3(dppz)(L)]^{+}$ complexes between the base pairs of DNA. We previously used transient Raman and IR methods to show that in CH_3CN *fac*-$[Re(CO)_3(dppz)(py)]^{+}$ has close-lying $^3\pi,\pi^*$(dppz) IL and $d\pi(Re)\rightarrow\pi^*$(phz) ^{3}MLCT excited states which dominate its photophysical behaviour and rationalize the light switch effect in the presence of DNA.[15] Addition of water to acetonitrile solutions of *fac*-$[Re(CO)_3(dppz\text{-}X_2)(py)]^{+}$ was subsequently shown to cause strikingly different behaviour.[7d] Thus while the dimethyl derivative is strongly luminescent in water, the emission of *fac*-$[Re(CO)_3(F_2dppz)(py)]^{+}$ is strongly quenched. This was attributed to the MLCT state being lower in energy than the ^{3}IL $\pi\pi^*$ state in aqueous solution. To investigate further the behaviour of the difluoro-complex as a DNA-probe, we here examine its photophysics in aqueous solutions in the presence of either double-stranded $[poly(dA\text{-}dT)]_2$ or $[poly(dG\text{-}dC)]_2$ using both time-resolved infrared (TRIR) and transient absorption (TA) on the picosecond and nanosecond timescales.

Experimental

Materials and sample handling

fac-$Re[(CO)_3(F_2dppz)(py)]^{+}$ was prepared according to a previously published procedure,[7d] both as the PF_6^- salt and as the water soluble Cl^- salt. Spectrophotometric grade D_2O was purchased (Sigma Aldrich) with a purity of 99.9 atom% D. Anhydrous acetonitrile was generated by distillation of HPLC grade solvent over calcium hydride under an argon atmosphere. The sodium salts of the polynucleotides $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$ were purchased as lyophilised solids (Sigma Aldrich) in quantities of 10 A_{260} units and were used as received. All samples were studied in 50 mM sodium phosphate (pH = 6.9).

UV-visible absorption and emission measurements

UV-visible absorption spectra were recorded on a Perkin Elmer Lambda 25 spectrophotometer. Emission measurements were performed on an Edinburgh Instruments FLS920 luminescence spectrometer.

Time-resolved infrared and transient absorption experiments

The ps- and ns-TA experiments were carried out in Nottingham on purpose-built equipment based on a pump–probe approach. The pump beam is obtained from a commercial Ti:sapphire oscillator (MaiTai)/regenerative amplifier system (Spitfire Pro, Spectra Physics) and a TP-1 harmonic generator (TimePlate Tripler, Minioptic Technology, Inc) to generate UV pulses (400 nm) or a TOPAS-C OPA (Light Conversion, Lithuania) to produce tunable UV-Vis pulses (300 nm to NIR). The probe beam is a pulsed white light continuum, generated by focusing a small amount of an 800 nm (20 nJ) laser beam onto a 4 mm thick Sapphire disk or a 3 mm thick CaF_2 disk. The white light is split into two beams. One beam passes through the sample and is spatially overlapped with the pump beam, the other beam serves as a reference. The polarization of the pump pulse is set at the magic angle (54.7°) relative to the probe pulse to recover the isotropic absorption spectrum. The time difference (up to 3 ns) between the pump and probe pulses is generated by an optical delay line. For longer timescales, a Q-switched Nd:YVO laser (ACE-25QSPXHP/MOPA, Advanced Optical Technology) is employed as a pump source which is synchronised to the Spitfire Pro amplifier. The delay between pump and probe pulses can be controlled with a pulse generator (DG535, Stanford Research Systems) from 0.5 ns to hundreds of μs. The two parts of the probe beam are monitored by a dual array detector (512 pixels) (Cronin Camera, Spectronic Device Ltd). The detector is mounted in the focal plane of a 303 mm Acton spectrograph (Acton) with a 300 g mm^{-1} and a 150 g mm^{-1} grating. The array detector outputs to a 16-bit analogue-to-digital digitizer. The pump-induced change in the absorbance, ΔA, is determined by chopping the pump pulse at half the repetition frequency of the laser and calculating the ratio between the pump-on and pump-off transmittance. The reference signal serves to normalise the shot to shot fluctuations.

A Harrick solution cell with 2 mm thick CaF_2 windows (path length 0.01–1 mm) is mounted on a motorized cell mount, which rapidly moves the cell in x and y dimensions. This ensures the laser pulses illuminate a fresh volume of the sample each time

and laser induced overheating and degrading of the sample can be minimised.

The equipment used for ps-TRIR measurements has been described in detail previously.[16] The sample solution was excited at 400 nm for the ps-measurements, using frequency-doubled pulses from a 1 kHz Ti:sapphire laser (~150 fs). The delay between pump and probe pulses in these experiments was achieved using an optical delay line. On the nanosecond timescale pumping was achieved using a Nd:YVO laser with a repetition rate of 1 kHz and an output at 355 nm. The time delay between the pump and probe pulses was achieved using a electronic delay generator.

Electrochemistry

Cyclic voltammetry was carried out in anhydrous CH_3CN using an Autolab PGSTAT20 potentiostat. Standard cyclic voltammetry was carried out under an atmosphere of argon using a three-electrode arrangement in a single compartment cell. A glassy carbon working electrode, a Pt wire secondary electrode and a saturated calomel reference electrode, chemically isolated from the test solution *via* a bridge tube containing electrolyte solution and fitted with a porous vycor frit, were used in the cell. The solution was 10^{-3} M in Re complex and 0.2 M in $[NBu_4][BF_4]$ as supporting electrolyte. Redox potentials are reported *vs.* NHE, using the conversion 0 V *vs.* SCE ≡ +0.24 V *vs.* NHE. Under these conditions $E_{\frac{1}{2}}$ Fc^+/Fc, used as the internal standard, was +0.67 V *vs.* NHE).

Density functional theory calculations

DFT geometry optimisations on *fac*-$[Re(CO)_3(py)(F_2dppz)]^+$ and *fac*-$[Re(CO)_3(py)(F_2dppz)]^+\cdot 2H_2O$ were performed using the ADF2009.01 suite of programs.[17] The calculations for the latter were performed under C_1 symmetry and the former under both C_1 and C_s symmetry using a coordinate frame where the xy plane contains the Re and N(pyridine) atoms, and bisects the N–Re–N angle that the F_2dppz ligand makes at the Re centre. The density functional calculations employed Slater type orbitals (STO) triple-ζ-plus polarisation basis sets (from TZP database of the ADF suite), the frozen core approximation (up to and including 4f for Re, 2p for S, and 1s for C, N and O), and the local density approximation (LDA) with the correlation potential due to Vosko *et al.*[18a] Gradient corrections were performed using the functional of Becke[18b] and Perdew.[18c] A scalar relativistic zero order regular approximation (ZORA) was used. The conductor-like screening model (COSMO) was used to estimate the effect of a solvent with a relative permittivity of 37.5 or 78.4 on the electronic structure. The program MOLEKEL4.2[19] was used to prepare three-dimensional plots of the electron density.

Results and discussion

Density functional theoretical studies

In order to provide a framework to interpret the fast time-resolved measurements that follow,[15] we have calculated the ordering and numbering of the frontier molecular orbitals of $[Re(CO)_3(py)(F_2dppz)]^+$ in its ground state both in the gas phase and in media of relative permittivity of 37.5 and 78.4 (representing CH_3CN and H_2O respectively). Furthermore we have investigated the effect of explicit water molecules coordinated to the hydrogen bonding sites on the central phenazine nitrogen atoms of the dppz ligand in the ground state. Fig. 1 shows the partial energy level diagram and the three dimensional isosurface plots of the electron density for the LUMO (103a) and LUMO+1 (104a) of the $[Re(CO)_3(py)(F_2dppz)]^+\cdot 2H_2O$ in a relative permittivity medium of 78.4. The frontier molecular orbital manifold of the previously reported[15] *fac*-$[Re(CO)_3(py)(dppz)]^+$ and *fac*-$[Re(CO)_3(py)(F_2dppz)]^+$ can be compared. This comparison shows qualitatively that the HOMO is d-orbital based and the LUMO and LUMO+1 are phenazine and phenanthroline based for both complexes.

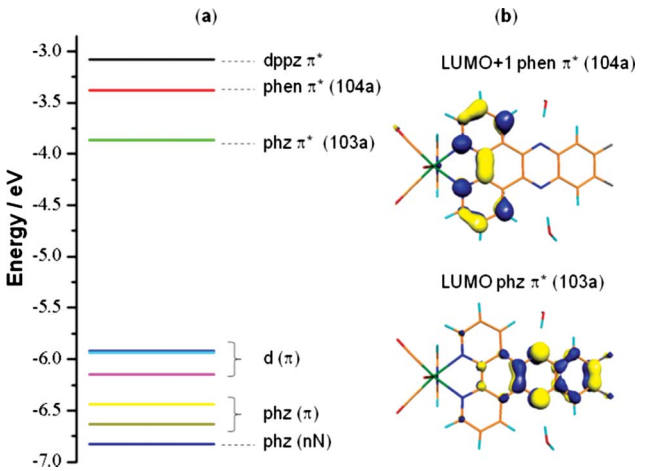


Fig. 1 (a) Energy level diagram for the frontier molecular orbitals of *fac*-$[Re(CO)_3(py)(F_2dppz)]^+\cdot 2H_2O$ in its ground state derived from DFT calculations in a relative permittivity medium of 78.4. (b) Isosurface plots of the frontier orbitals of *fac*-$[Re(CO)_3(py)(F_2dppz)]^+\cdot 2H_2O$ at the 0.05 eÅ^{-3} isosurface level.

Spectroscopic properties of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in the presence of $[poly(dA\text{-}dT)]_2$ or $[poly(dG\text{-}dC)]_2$

The UV/visible absorption spectra of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in phosphate buffered aqueous solution were recorded in the presence of increasing concentrations of $[poly(dG\text{-}dC)]_2$ or $[poly(dA\text{-}dT)]_2$. In water, *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ exhibits a strong maximum at 274 nm and characteristically structured π→π*(dppz) IL transitions between 360 and 400 nm, with maxima at 363 and 382 nm. Addition of either $[poly(dA\text{-}dT)]_2$ or $[poly(dG\text{-}dC)]_2$ induces hypochromism and a red-shift of the absorptions between 360 and 400 nm as the [nucleotide] : [Re] ratio is increased from 0 to *ca.* 12. (see Fig. S1 & S2, ESI†). This is consistent with *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ binding to either $[poly(dA\text{-}dT)]_2$ or $[poly(dG\text{-}dC)]_2$ *via* intercalation in an analogous way to that reported for *fac*-$[Re(CO)_3(dppz)(py)]^+$ and other dppz complexes.[13] The intrinsic binding constants (K_b) were estimated to be $2.2\ (\pm 0.2)\times 10^4$ and $1.2\ (\pm 0.1)\times 10^4$ dm^3 mol^{-1} for the complex with $[poly(dA\text{-}dT)]_2$ and $[poly(dG\text{-}dC)]_2$ respectively (see ESI†).

The steady state emission and excitation spectra were also recorded for *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in D_2O solutions in the presence of increasing concentrations of the polynucleotides. Addition of $[poly(dA\text{-}dT)]_2$ to the complex up to a ratio of [nucleotide] : [Re] of 12 : 1 switches on luminescence having a band

maximum at 590 nm, similar to the spectrum found for the emitting ^{3}IL ππ*(dppz) state in organic solvents (see Fig. S3†). These results are consistent with the relatively hydrophobic environment of the intercalation pocket of [poly(dA-dT)]$_2$ raising the energy of the MLCT state of the complex so that the ^{3}IL ππ*(dppz) state is now the lowest-lying (and emissive) state.

By contrast, in the presence of a high concentration of [poly(dG-dC)]$_2$ there is no emission from [Re(CO)$_3$(F$_2$dppz)(py)]$^+$, even though the dielectric properties of the intercalation site should be broadly similar to that in [poly(dA-dT)]$_2$. The lack of emission is consistent with the excited state being quenched by electron transfer in the presence of [poly(dG-dC)]$_2$. In order to determine whether this is thermodynamically possible we have performed cyclic voltammetry and transient UV/visible and time-resolved IR absorption spectroscopy of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ bound to the polynucleotides.

Electrochemistry

The redox behaviour of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ was studied in CH$_3$CN, with an oxidation observed at E_p = +2.11 V and a reversible reduction at $E_{\frac{1}{2}}$ = –0.56 V (both *vs.* NHE). Interestingly, the latter is ~0.08 V more positive than the corresponding reduction of the unsubstituted *fac*-[Re(CO)$_3$(dppz)(py)]$^+$ complex.‡

Visible transient absorption spectroscopy

As previously reported[7d] there is a major difference between the transient behaviour observed on excitation at 400 nm of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ in CH$_3$CN and in D$_2$O. In CH$_3$CN at 4 ps a broad absorption with a maximum at 475 nm and shoulders at *ca.* 540 and 595 nm are evident. There is only a small increase in intensity (*ca.* 5%) at the maximum observed over the first 100 ps, with minor changes occurring elsewhere. This final spectrum is similar to that reported by Schanze *et al.* for *fac*-[Re(CO)$_3$(dppz)(4-Mepy)]$^+$ and is assigned to the ^{3}IL ππ*(dppz) excited state.[14] By contrast in D$_2$O in the absence of polynucleotides, a strong transient band at 450 nm and broad weak shoulder band to lower energy (*ca.* 550 nm) were recorded {Fig. 2(a), Table 1}. These absorption bands both decay with a lifetime of 450 ± 20 ps§ {Fig. 2(a) *inset*}This transient absorption was assigned previously, by comparison with ps-TRIR studies, to an equilibrated mixture of ^{3}MLCT and ^{3}IL ππ*(dppz) excited states.

The ps-transient absorption spectrum of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ in buffered D$_2$O in the presence of [poly(dA-dT)]$_2$ ([nucleotide] : [Re] = 20 : 1) exhibits a similar band shape to that observed in CH$_3$CN, with broad maxima at 475 nm and *ca.* 575 nm (Fig. 2(b), Table 1). There is a partial decay on a timescale of 235 ± 80 ps (Fig. 2(b) *inset*) but most of the absorption persists beyond the timescale of the experiment (>2 ns). The rapid partial decay is difficult to definitively assign, occurring on the same timescale as changes observed in the carbonyl stretching region of the time-resolved infrared spectra (see below), tentatively assigned to vibrational cooling of the excited state. These results are consistent with the excited-state of the polynucleotide-bound complex being predominantly ^{3}IL ππ*(dppz) in character and as such agree with the observation of luminescence enhancement in the presence of [poly(dA-dT)]$_2$.

In contrast, the ps-transient absorption spectra of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ in buffered D$_2$O in the presence of [poly(dG-dC)]$_2$ are quite different (Fig. 2(c), Table 2). Two broad, strong transient bands are observed at early time (Δt = 2 ps) with

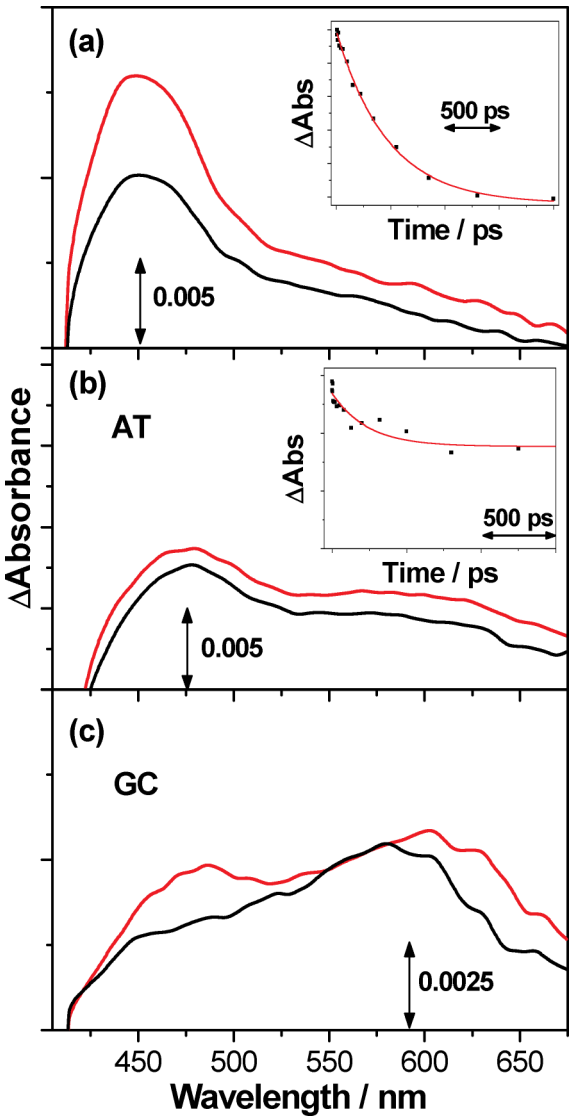


Fig. 2 ps-TA spectra at 2 ps (*red lines*) and 200 ps (*black lines*) after 400 nm excitation of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ in (a) D$_2$O (ps-decay at 450 nm inset); (b) buffered D$_2$O in the presence of [poly(dA-dT)]$_2$ ([nucleotide] : [Re] = 20 : 1) (ps-decay at 595 nm inset), and (c) buffered D$_2$O in the presence of [poly(dG-dC)]$_2$ ([nucleotide] : [Re] = 20 : 1).

‡ In principle this value can be used to estimate an approximate value for the excited state reduction potential for both the singlet and triplet excited state IL(ππ*) of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$. From the absorption spectrum of the complex in the polynucleotide we estimate the onset of absorption of the singlet IL(ππ*) to be at *ca.* 400 nm allowing us to estimate the excited-state potential as being in excess of 2.4 V. The triplet IL(ππ*) excited state energy can be crudely estimated from the onset of the phosphorescence at about 520 nm (see Figure S3) giving a value of $E°(Re^{+*}/Re)$ = 1.77 V, by assuming that the redox potentials are the same in acetonitrile and water. Although the oxidation potential of guanine is still the subject of debate (values ranging from 1.3 to 1.53 V have been reported recently) it is clear that both the singlet and triplet IL(ππ*) excited-states should be able to oxidise guanine in [poly(dG-dC)]$_2$.[22]

§ We previously described this as a biexponential decay (79 and 810 ps). The data were found to be satisfactorily fit by first-order kinetics in the present case.

Table 1 UV/visible ground-state and transient absorption (TA) spectra band positions and kinetics of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ under different conditions

Solvent	Abs. (nm)	Emission (nm)	TA bands (nm)	lifetimes from ps-TA
CH_3CN	361, 379	540, 583 (5 μs)	475, 540, 595	>2 ns
D_2O	363, 382	[a]	450, 550	450 ± 20 ps
D_2O+AT[b]	366, 385	590	475, 575	235 ± 80 ps[b], >2 ns
D_2O+GC[b]	366, 385	[a]	485, 600, 580	34 ± 3 ps[c], >2 ns

[a] No detectable emission. [b] See text for details. [c] Lifetime is fitted as both a decay of one species and growth of another, see text.

Table 2 IR band positions (cm^{-1}) and kinetics of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ under different conditions

Solvent	Ground state	Excited state	τ	Excited state assignment[a]
CH_3CN	2037, 1934	2032, 1927	18 ± 2 ps, >500 ps	^{3}IL(π–π*)
D_2O	2036, 1935	2029, 1924	↓430 ± 40 ns, ↓2.3 ± 0.3 μs[b]	^{3}IL(π–π*)
		2108, 2047, 2003	↑400 ± 40 ns, ↓2.5 ± 0.1 μs[b]	^{3}MLCT
D_2O+AT	2037, 1936	2029, 1916	9.8 ± 4 ns, 218 ± 31ns	^{3}IL(π–π*)
		1650, 1633, 1585	7.5 ± 5 ns, 205 ± 70 ns	^{3}IL(π–π*)
D_2O+GC	2037, 1936	2027, 1910	30 ± 10 ns, 317 ± 21 ns	[Re^I(CO)$_3$(F$_2$dppz)$^{\bullet-}$(py)][c]
		1695	↑39 ± 5 ps, >1 ns	[Re^I(CO)$_3$(F$_2$dppz)$^{\bullet-}$(py)][c]

[a] See text for discussion of species assignment. [b] Equilibrium formed between IL(π–π*) and MLCT states. [c] One-electron reduced species, see text.

maxima at *ca.* 485 nm and *ca.* 600 nm. Over the first 200 ps the higher energy band at 485 nm decays significantly. The lower energy band decays to a lesser extent and sharpens to a peak position of *ca.* 580 nm. The spectral shape then persists beyond the timescale of the experiment (>2 ns). These results clearly demonstrate the formation of another transient than observed with [poly(dA-dT)]$_2$ (Fig. 2(b)). As it is formed on a timescale shorter than 235 ps, this suggests that it could correspond to a product of rapid quenching of the excited IL state, maybe a reduced complex (with associated oxidation of the guanine) as the electron transfer is thermodynamically possible. The spectral temporal evolution can be analysed by fitting of each spectrum to two model spectra, namely the final spectrum (Δt = 200 ps) from the experiment in the presence of the [poly(dG-dC)]$_2$ and the spectrum from the experiment in the presence of [poly(dA-dT)]$_2$ recorded at the same Δt as the spectrum being fitted. The percentage of each component at every time delay is calculated and the kinetic evolution of the two species is shown in Fig. 3. Thus the excited state species is modelled by the experiment with [poly(dA-dT)] and the charge transfer species is modelled by the 200 ps spectrum in the presence of [poly(dG-dC)]$_2$. The initial spectrum at Δt = 1 ps comprises *ca.* 34% excited-state and 66% charge-transfer species. Over the next 200 ps, the excited-state component decays with a corresponding growth of the charge-transfer species (t = 34 ± 3 ps), indicative of electron transfer quenching of the excited-state occurring on two timescales, *i.e.* <1 ps and 34 ps.

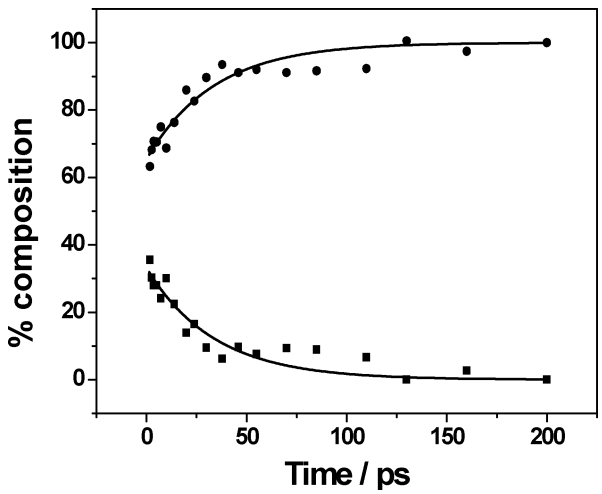


Fig. 3 Kinetic evolution of the ps-TA spectral models after 400 nm excitation of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ in buffered D_2O in the presence of [poly(dG-dC)]$_2$ ([nucleotide] : [Re] = 20 : 1) showing growth of the final spectrum (*circles*) and decay of the excited state (*squares*) (see text for details of calculation of the % composition).

Time-resolved infrared spectroscopy

To gain further insights into the photophysics of these systems we used ps-TRIR[20] to probe *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ in the presence of the polynucleotides. This allows us to monitor both the metal complex (by recording its carbonyl stretching vibrations in the range 1900–2100 cm^{-1}) and the nucleobases of DNA (by monitoring their vibrations in the range 1500–1750 cm^{-1}, Table 2).

ps-TRIR spectra of *fac*-[Re(CO)$_3$(F$_2$dppz)(py)]$^+$ in the presence of [poly(dA-dT)]$_2$ ([nucleotide] : [Re] = 20 : 1) in buffered D_2O solution obtained at 2 and 200 ps following 400 nm excitation are shown in Fig. 4(a). It is clear that the parent bands (2037 and 1936 cm^{-1}) are bleached and new transient features are produced. The early time TRIR spectra (*e.g.* Δt = 2 ps) show two new broad absorption bands centred at. 2026 and 1915 cm^{-1}. Over the first 100 ps these bands narrow and shift to higher wavenumber (2029 and 1916 cm^{-1}) consistent with relaxation from an initially formed vibrationally hot excited state. The apparent decay and recovery of these bands is attributed to this cooling process, due to the appreciable overlap of bands of the IL(π–π*) and the ground state, and occurs on the same timescale as changes observed in

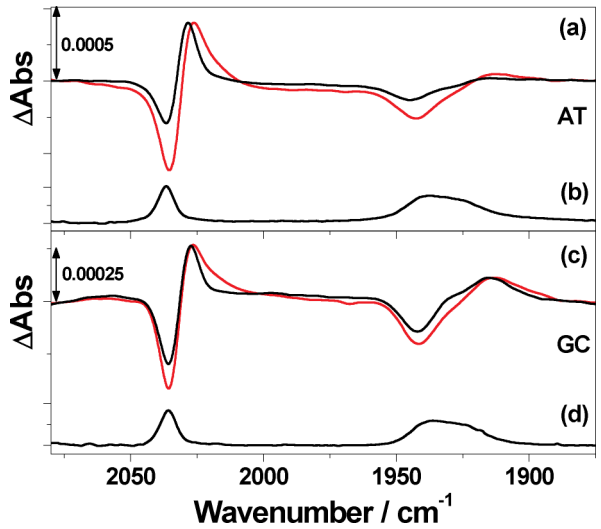


Fig. 4 Buffered D_2O solution (a) ps-TRIR difference spectra and (b) ground-state FTIR of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in the presence of $[poly(dA\text{-}dT)]_2$ ([nucleotide] : [Re] = 20 : 1); (c) ps-TRIR difference spectra and (d) ground-state FTIR of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in the presence of $[poly(dG\text{-}dC)]_2$ ([nucleotide] : [Re] = 20 : 1). Difference spectra recorded at 2 ps (*red lines*) and 200 ps (*black lines*) after 400 nm excitation.

the ps-TA experiment described above. The possibility that these spectral changes may be linked to the conversion between other IL states cannot be excluded. The small shift to lower wavenumber compared with the parent absorptions is consistent with the formation of an IL(π–π*) excited state. No further changes are observed up to 1000 ps. This behaviour is similar to the results we have reported[7d] from TRIR of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in CH_3CN and is in marked contrast to experiments in pure D_2O where the transient species decay rapidly on the picosecond timescale. These results are again consistent with the solvent environment of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ when bound to this polynucleotide being more similar to that of a polar organic solvent. On the nanosecond timescales the ν(CO) IR bands of the ^{3}IL(π–π*) decay concominant with the reformation of the parent (τ_1 = 9.8 (± 4) ns; τ_2 = 218 (± 31) ns), Fig. 5.[4]

Excitation of the metal complex, when bound to $[poly(dA\text{-}dT)]_2$ also induced large transient signals in the nucleobase region of the DNA. Thus, strong bleaching of the bands at 1690, 1662, 1638 and 1619 cm^{-1} (associated primarily with the CO stretch of the thymine and the ring vibration of adenine) is observed on the ps-timescale, along with formation of a transient band at 1585 cm^{-1}.¶ These signals persist onto the ns-timescale (Fig. 6a shows the TRIR spectrum taken after 4.5 ns). This transient band decays at the same rates as the bleaches recover (τ_1 = 7.5 ± 5 ns; τ_2 = 205 ± 70 ns). These kinetics are similar to those of the decay of the ^{3}IL(π–π*) monitored using the ν(CO) IR bands. This suggests that the $[poly(dA\text{-}dT)]_2$ structure is perturbed upon formation of the excited state of the intercalated complex, and that this changed structure relaxes back to the equilibrium position as the excited state decays.

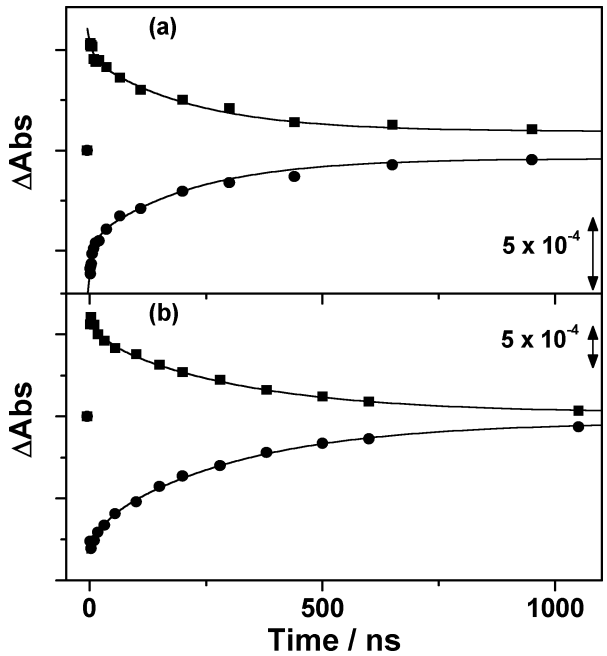


Fig. 5 Buffered D_2O solution (a) ns-TRIR difference spectra kinetics of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in the presence of $[poly(dA\text{-}dT)]_2$ ([nucleotide] : [Re] = 20 : 1) recorded at 2037 (*bleach, circles*) and 2029 (*transient, squares*) cm^{-1}; (b) ns-TRIR difference spectra kinetics of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in the presence of $[poly(dG\text{-}dC)]_2$ ([nucleotide]:[Re] = 20 : 1) recorded at 2039 (*bleach, circles*) and 2027 (*transient, squares*) cm^{-1}.

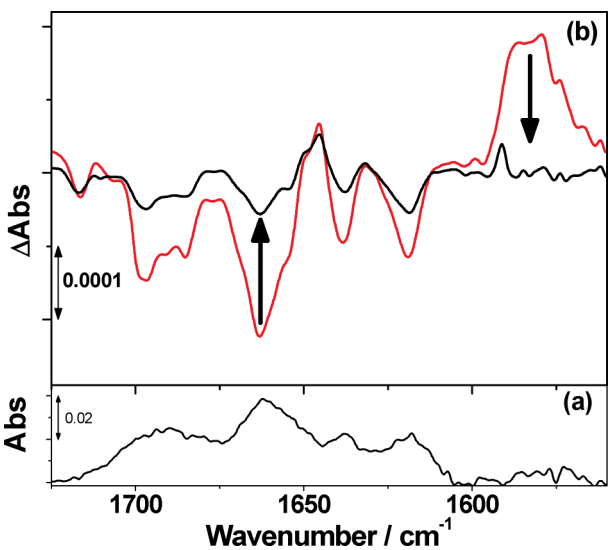


Fig. 6 (a) Ground-state FTIR and (b) ns-TRIR DNA-region difference spectra of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in buffered D_2O in the presence of $[poly(dA\text{-}dT)]_2$ ([nucleotide] : [Re] = 20 : 1) recorded at 4.5 ns (*red line*) and 320 ns (*black line*) after 355 nm excitation.

The TRIR spectra obtained following irradiation of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in buffered D_2O solution in the presence of $[poly(dG\text{-}dC)]_2$ ([nucleotide] : [Re] = 20 : 1) at 2 and 200 ps following 400 nm excitation are shown in Fig. 4(c). At early times the parent ν(CO) bands are bleached and two new broad

¶ We find that the IR spectrum obtained 10 ps after photolysis shows that bands associated with both the A and T bases are bleached and new bands are produced at *ca.* 1650, 1633 and 1585 cm^{-1}. The two small transient bands at 1633 and 1650 cm^{-1} decay rapidly over the first 50 ps but the parent bands do not appear to significantly recover and the precise assignment of these bands is unknown but occurs on a similar timescale to vibrational cooling.

absorption bands centred at *ca.* 2026 and 1908 cm^{-1} are formed which narrow and shift slightly (2027 and 1910 cm^{-1}) over the first 100 ps. At first glance these TRIR spectra at later time seem to show an effect similar to that observed for the complex in the presence of $[poly(dA\text{-}dT)]_2$. However more careful inspection shows that a different species appears to be produced which is most evident by the difference in low frequency ν(CO) band in the spectra taken 200 ps after photolysis ($[poly(dA\text{-}dT)]_2$: 1916 *vs.* $[poly(dG\text{-}dC)]_2$: 1910 cm^{-1}). This spectrum of this transient, which is stable on the time-scale of the experiment (up to 1000 ps), is consistent with that expected for the reduced rhenium complex and returns to the parent on the ns-timescale ($\tau_1 = 30\ (\pm 10)$ ns; $\tau_2 = 315\ (\pm 25)$ ns) somewhat slower than the recovery of the excited state formed in the presence of $poly[dA\text{-}dT]_2$.

The ps-TRIR spectra in the DNA region recorded following irradiation of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in buffered D_2O solution in the presence of $[poly(dG\text{-}dC)]_2$ ([nucleotide] : [Re] = 20 : 1) are shown in Fig. 7. The parent (predominantly) guanine (1685 cm^{-1}) and cytosine (1648 and 1658 cm^{-1}) bands are clearly bleached and a new transient band is observed at 1695 cm^{-1}. This band is similar in position to that observed previously for the transients formed upon 200 nm excitation of $[poly(dG\text{-}dC)]_2$ and 400 nm excitation of $[Ru(dppz)(TAP)_2]^{2+}$ intercalated between the base pairs of $[poly(dG\text{-}dC)]_2$ in D_2O and which was attributed to an oxidised guanine species (either the radical cation or its deprotonated form) (at *ca.* 1700 cm^{-1}). The transient band is overlapped with a parent depletion, but the kinetics can be fitted to a growth in intensity with a lifetime of 39 ± 5 ps. The initial intensity of the band ($\tau = 2$ ps) is *ca.* 64% of its final intensity ($\tau >$ 200 ps). These values agree well with those observed in the ps-TA experiment ($\tau = 34$ ps; initial intensity *ca.* 66%) for the spectrum also attributed to the formation of the guanine radical cation and the reduced Re complex.

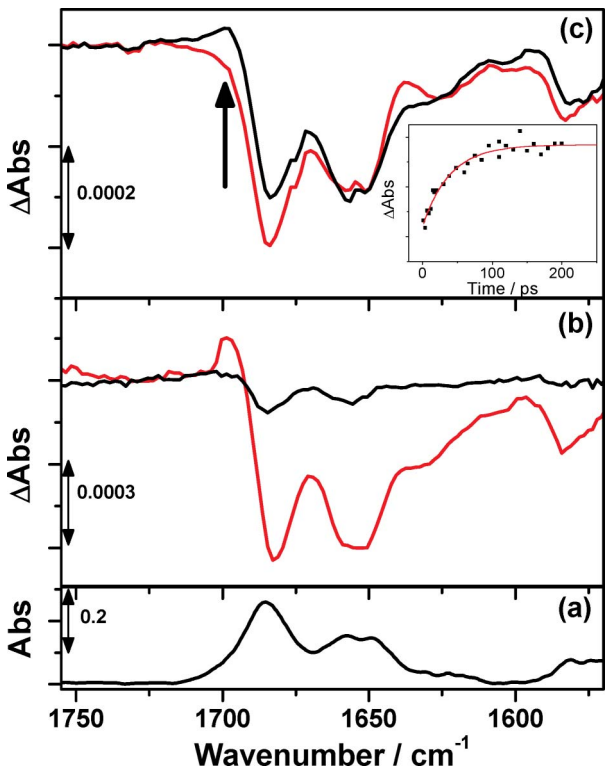


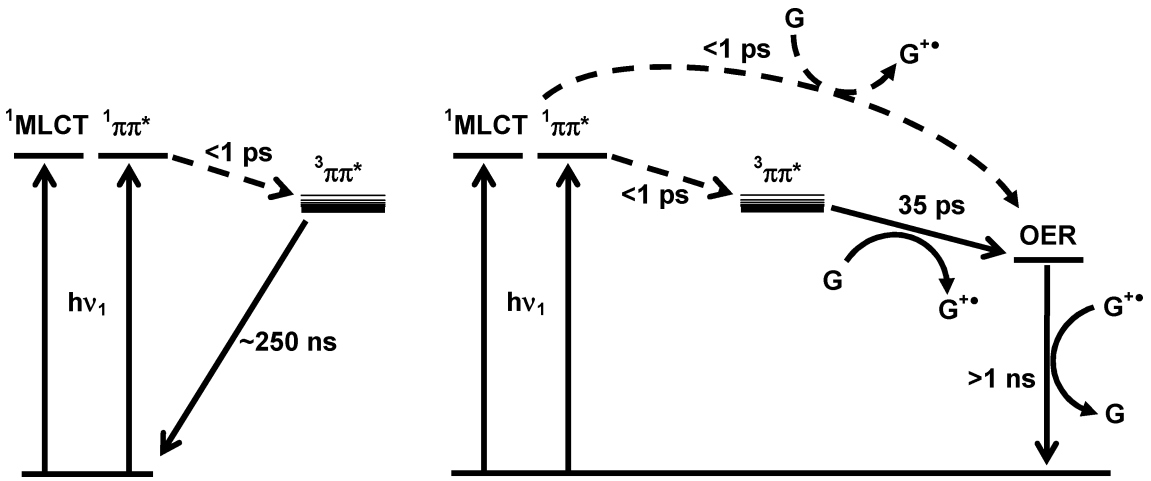
Fig. 7 (a) Ground-state FTIR, (b) ns-TRIR DNA-region difference spectra of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in buffered D_2O in the presence of $[poly(dG\text{-}dC)]_2$ ([nucleotide] : [Re] = 20 : 1) recorded at 3.2 ns (*red line*) and 1000 ns (*black line*) after 355 nm excitation and (c) ps-TRIR DNA-region difference spectrum of the same recorded 3.7 ps (*red line*) and 190 ps (*black line*) after 400 nm excitation. Kinetic plot (*inset*) recorded at 1695 cm^{-1}, red line denotes exponential fit (see text).

Conclusions

As was previously noted[7d] substituting the 11,12 positions in the dppz ring has a significant effect on the photophysical properties of *fac*-$[Re(CO)_3(dppz\text{-}X_2)(py)]^+$. In the case of the difluoro-complex it is found that the emission is quenched in water, with the lifetime which in acetonitrile is over 5 μs being reduced to 450 ps (as shown by ps-TA measurements). This was previously ascribed to the energetic proximity of the ^{3}MLCT and the emissive ^{3}IL(π–π*) excited states in the difluorocomplex. As the complex is non-luminescent in water, but luminescent in organic solvents, it would be expected that it would, like the much studied $[Ru(bpy)_2(dppz)]^{2+}$,[4] behave as a light switch in the presence of a polynucleotide. This is indeed found to be the case with the double–stranded homopolymer $[poly(dA\text{-}dT)]_2$. However with $[poly(dG\text{-}dC)]_2$ no emission is observed. This complex is therefore interesting as it behaves as a sequence-selective light switch – only becoming luminescent when it binds in guanine-free sites. The lack of emission from the complex bound to guanine-containing polynucleotide may be ascribed to the reduction potential of the excited state of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ being sufficient to allow this species to oxidise guanine. The transient absorption studies presented here unambiguously show this to be the case. Thus the visible TA studies clearly show the formation of the reduced complex, confirmed by ps-TRIR studies of the CO stretch region. Direct evidence for guanine oxidation is provided by studying the vibrations in the 1550-1750 cm^{-1} region. This excited state redox process is markedly faster than that reported for $[Ru(dppz)(TAP)_2]^{2+}$, where the process is believed to proceed by proton-coupled electron transfer.[9] Interestingly 66% of the complexes undergo electron transfer within 1 ps, while the other 34% proceeds with a lifetime of 34 ps. This may be due to reaction of two excited states (for example the fast process involving a singlet species or vibrationally hot excited state) and the slower reaction originating from the thermally equilibrated ^{3}IL(π–π*) state, Scheme 1. Alternatively the two decay processes might originate from excited states bound in different sites of the polynucleotide.‖

The subsequent decay of the products takes place on a timescale more than four orders of magnitude slower than that of the majority of the initial electron transfer reaction. In particular the diagnostic band at 1695 cm^{-1} allows the monitoring of the oxidised guanine. This has been attributed to either the guanine radical

‖ A referee suggested an alternative hypothesis that these results could be due to formation of the radical anion of *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ from the IL excited state (1ps) and afterwards its transformation into a protonated or hydrogen-bonded species (34 ps). We think this is less likely because it would require the TA and TRIR spectra of the reduced complex and its protonated form to be identical *i.e.* identical UV/visible and IR spectra produced at 1 ps and on the 34 ps timescale.



Scheme 1 Proposed major excited-state decay pathways for *fac*-$[Re(CO)_3(F_2dppz)(py)]^+$ in D_2O after 400 nm laser excitation in the presence of $[poly(dA\text{-}dT)]_2$ (*left*) and $[poly(dG\text{-}dC)]_2$ (*right*). Solid arrows denote observed processes, kinetics obtained from TA and TRIR measurements, see text for details. The dotted arrows represent unobserved processes and the rapid (<1ps) oxidation of guanine may occur from the singlet states that are shown or from some other short-lived excited state, *e.g.* ^{3}MLCT. OER = one electron reduced species.

cation or the guanine radical formed by its deprotonation, the former attribution being favoured in more recent publications.[10b,10c,21] The slow decay of this species is qualitatively consistent with the predictions of Marcus theory given the considerable exothermicity of this back electron transfer process. The relatively long lifetime of the oxidation product, compared to those generated from dyes such as those of the thionine family,[22] suggests that these rhenium-based complexes may be useful photosensitisers for guanine oxidation in natural DNA.

Our transient spectroscopic measurements confirm that the excited state is unable to oxidise the nucleobases in $[poly(dA\text{-}dT)]_2$, as the transient visible absorption spectra reveal that the species present after excitation of the complex bound to this nucleic acid is the ^{3}IL(π–π*) state not a ^{3}MLCT state. Most of this species has a lifetime of over 200 ns. However, while the evidence is therefore clear that electron transfer from the nucleobase is unimportant, the TRIR measurements in the 1550–1750 cm^{-1} region do reveal that the excited state does perturb the nucleobases substantially. This is not entirely unexpected as there will be a major change in the environment due to the formation of the dppz localised excited state. The nature of this perturbation will require further study. A possible probe for this perturbation is the band observed at 1580 cm^{-1} – which again will require further study.

In summary, the experiments reported in this paper show that this rhenium-dppz complex is a photosensitiser which facilitates the examination of time-resolved photosensitised oxidation of DNA.

Acknowledgements

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