SUPPLEMENTARY INFORMATION

Study of picosecond processes of an intercalated dipyridophenazine Cr(III) complex bound to defined sequence DNAs using transient absorption and time-resolved infrared methods

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Experimental

Materials

Oligonucleotides d(GGGGGCCCCC), d(GCGCGCGCGC) and d(TCGGCGCCGA) were synthesised by ATDBio Ltd (Southampton, UK). The sequences were desalted and purified by gel filtration. $[Poly(dG\text{-}dC)]_2$ was purchased from Sigma. rac-$[Cr(phen)_2dppz]^{3+}$ was prepared using previously reported methods.[1]

DNA titrations

Absorption spectra were obtained on a Varian Cary 50 spectrophotometer. Steady-state emission data were collected on a Varian Cary Eclipse fluorimeter in aerated solutions at room temperature. The oligo and Cr complex solutions for all titrations were prepared in (50 mM) sodium phosphate buffer (pH=7). The spectroscopic data of the DNA titrations were collected by increased volumetric addition of the oligo (12 mM) to 2.5 ml of a Cr complex solution in a 1 cm quartz cuvette up to a [Nucleic Acid]: [Cr complex] ratio (nucl/complex) of 20-35.

Ultrafast measurements

The samples for picosecond UV/vis transient absorption (psTA) and time-resolved infrared (psTRIR) measurements were prepared in D_2O as follows; a known volume of solution (25 – 35 μL) was dropped between two CaF_2 (25 mm diameter) windows (Crystran Ltd, UK), separated by a Teflon spacer of 50 μm pathlength, in a demountable solution IR cell (Harrick Scientific Products Inc., New York).

Ps-TA and ps-TRIR measurements were performed on the ULTRA apparatus at the Lasers for Science Facility (Harwell, UK). The time-resolved IR (TRIR) spectrometer comprises of a 10 kHz repetition rate titanium sapphire dual output amplifier (Thales), producing 0.8 mJ output with 40 fs pulse duration, at 800 nm. Optical parametric amplifiers (Light Conversion, TOPAS) and second harmonic generation of the 800 nm created the mid infrared radiation and 400 nm femtosecond pump pulses used in these experiments. The polarisation of the pump pulses at the sample were at the magic angle relative to the probe, with an energy of 1 μJ. The IR probe beam was split to form reference and probe beams which were passed through spectrographs onto MCT array detectors (IR Associates). The 5 kHz 400 nm pump pulses (~100 fs) were focussed (~ 100 μm spot sizes) and overlapped with the probe beam (~50 μm spot size) in the sample cell. High speed data acquisition systems (Quantum Detectors) allowed 10 kHz acquisition and processing of the probe and reference pulses to generate a pump-on pump-off infrared absorption difference signal. The TRIR spectra were calibrated using the characteristic polystyrene absorption lines. For the ps-TA measurements, part of the Ti:Sapphire laser output beam was used to generate a white light continuum (WLC) in a CaF_2 crystal. The crystal plate was continuously rastered to avoid colour centre formation and to improve pulse-to-pulse stability in the probe. The WLC was dispersed through the grating monochromator and detected using a linear silicon array (Quantum Detectors). In front of the monochromator, a 400 nm notch filter was placed in order to remove scatter from the excitation beam. The polarisation of the pump pulses at the sample were at magic angle relative to the probe, with energy of 1 μJ. The spectra were calibrated using five band-pass filters. Samples for both ps-TRIR and ps-TA were raster scanned in the x and y directions to minimise photodamage and re-excitation effects. Samples were checked before and after the experiment by UV/vis spectroscopy. In the case of some TRIR data, Figures 3 (manuscript) and S8 in the supporting information, 2 point adjacent averaging was applied to the raw data.

Figures

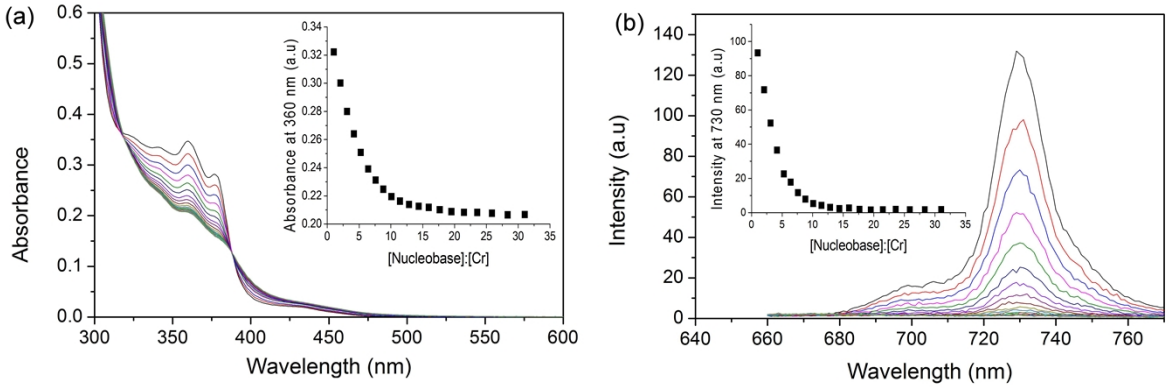


Fig. S1 (a) UV-visible absorption spectra of *rac*-$[Cr(phen)_2dppz]^{3+}$ (24 μM in 50 mM phosphate buffer solution, pH=7.4) in the presence of increasing concentrations of d(GCGCGCGCGC) (b) corresponding phosphorescence spectra (λ_{ex}= 400 nm).

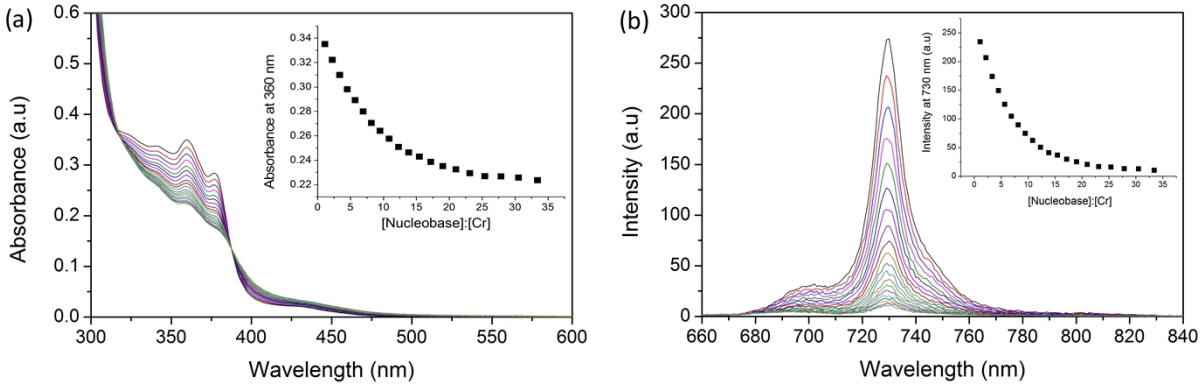


Fig. S2 (a) UV-visible absorption spectra of *rac*-$[Cr(phen)_2dppz]^{3+}$ (24 μM in 50 mM phosphate buffer solution, pH=7.4) in the presence of increasing concentrations of d(GGGGGGCCCCCC) (b) corresponding phosphorescence spectra (λ_{ex}= 308 nm)

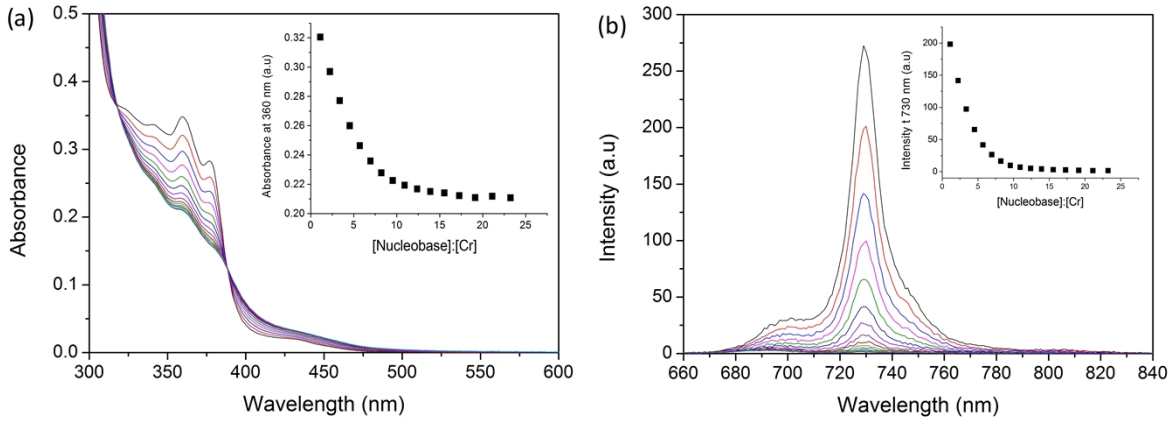


Fig. S3 (a) UV-visible absorption spectra of *rac*-$[Cr(phen)_2dppz]^{3+}$ (24 μM in 50 mM phosphate buffer solution, pH=7.4) in the presence of increasing concentrations of d(TCGGCGCCGA) (b) corresponding phosphorescence spectra (λ_{ex}= 308 nm)

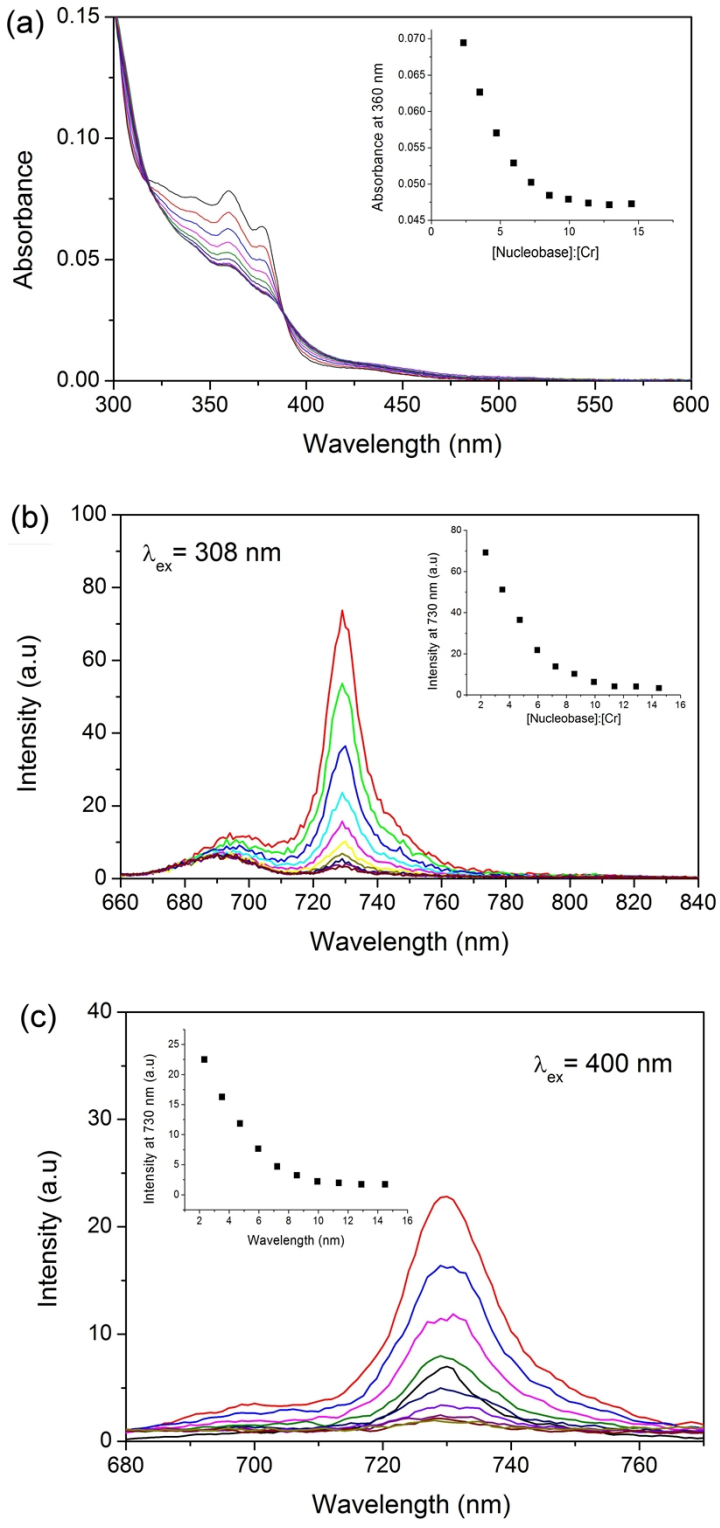


Fig. S4 (a) UV-visible absorption spectra of *rac*-$[Cr(phen)_2dppz]^{3+}$ (5.6 µM in 50 mM phosphate buffer solution, pH=7.4) in the presence of increasing concentrations of $[poly(dG\text{-}dC)]_2$ and corresponding phosphorescence spectra at (b) λ_{ex}= 308 nm and λ_{ex}= 400 nm.

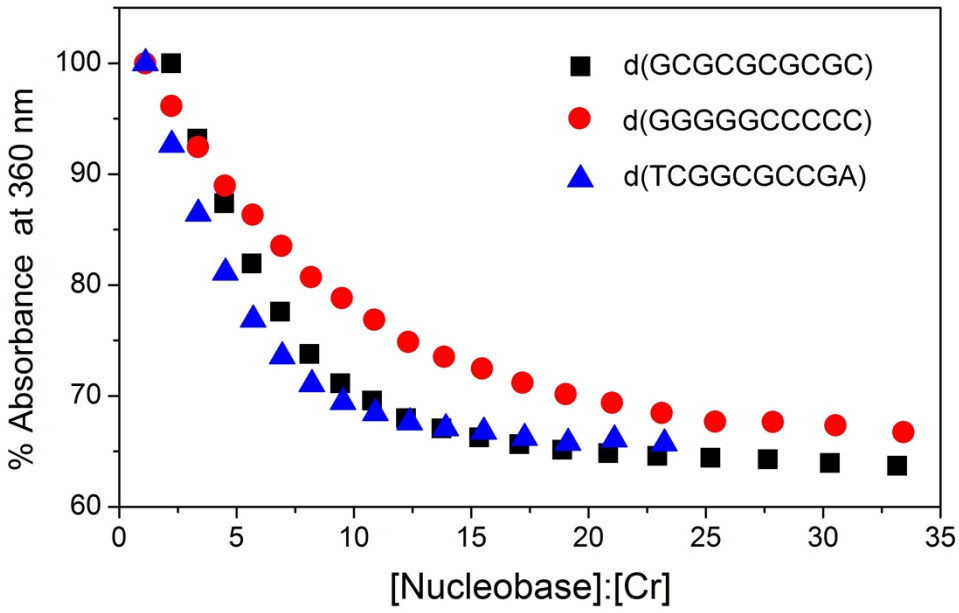
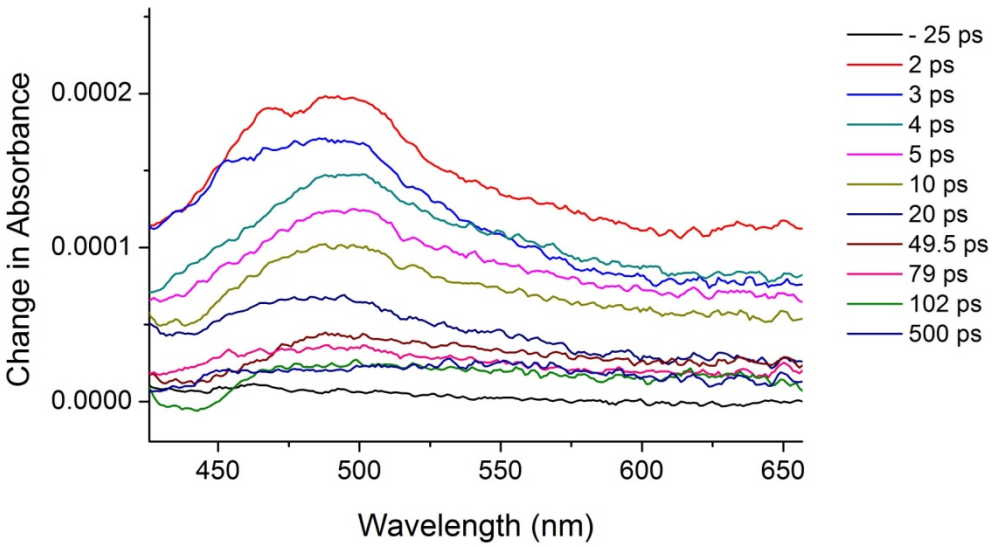
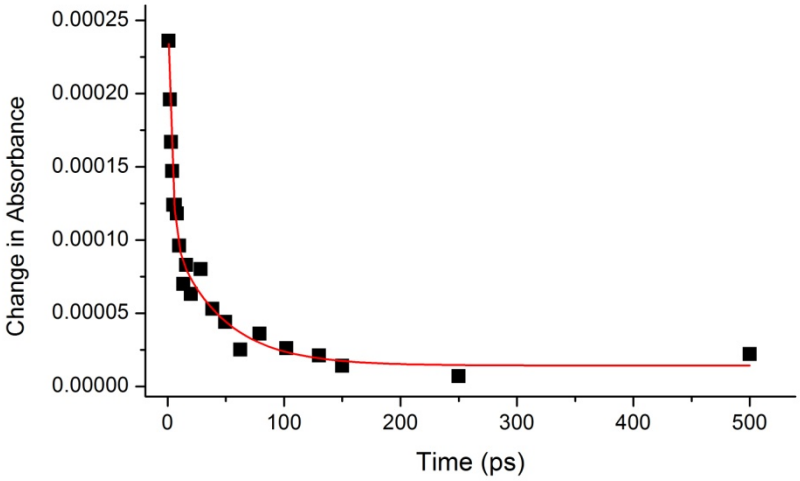


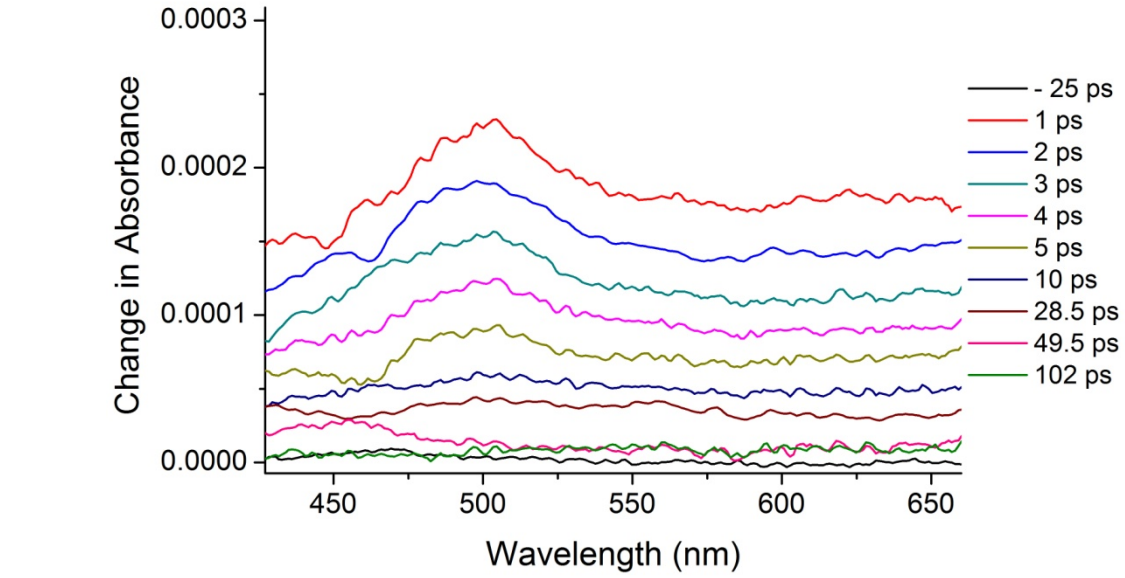
Fig. S5 Comparison of UV-visible absorption at 360 nm of *rac*-$[Cr(phen)_2dppz]^{3+}$ (24 μM in 50 mM phosphate buffer solution, pH=7.4) in the presence of increasing concentrations of the GC family of oligonucleotides.

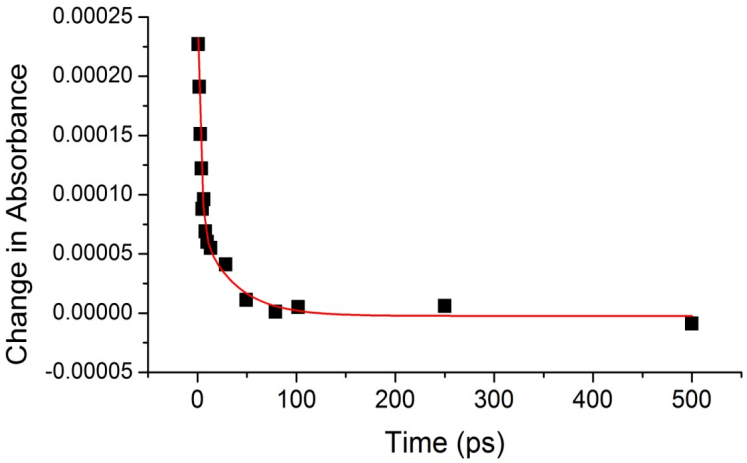




Model	ExpDec2		
Equation	y = A1*exp(-x/t1) + A2*exp(-x/t2) + y0		
Reduced Chi-Sqr	7.86217E-11		
Adj. R-Square	0.98083		
		Value	Standard Error
C250	y0	1.42966E-5	5.3425E-6
C250	A1	1.782E-4	1.62603E-5
C250	t1	3.11854	0.60742
C250	A2	9.25467E-5	1.15588E-5
C250	t2	44.25378	12.04328

Fig. S6 (a) ps-TA of 800 μM *rac*-$[Cr(phen)_2dppz]^{3+}$ in presence of 2 mM d(GGGGGCCCCC) in 50 mM phosphate buffer solution, pH = 7.4. (b) Biexponential fit for the excited state decay at 500 nm. Excitation at 400 nm, 1 μJ.





Model	ExpDec2		
Equation	y = A1*exp(-x/t1) + A2*exp(-x/t2) + y0		
Reduced Chi-Sqr	7.6073E-11		
Adj. R-Square	0.98546		
		Value	Standard Error
E	y0	-2.3334E-6	5.72233E-6
E	A1	7.84256E-5	1.56802E-5
E	t1	35.05382	13.90211
E	A2	2.26111E-4	1.81805E-5
E	t2	2.80338	0.5227

Fig. S7 (a) ps-TA of 800 μM *rac*-$[Cr(phen)_2dppz]^{3+}$ in presence of 2 mM $[poly(dG\text{-}dC)]_2$ in 50 mM phosphate buffer solution, pH=7.4. (b) Biexponential fit for the excited state decay at 500 nm. Excitation at 400 nm, 1 μJ.

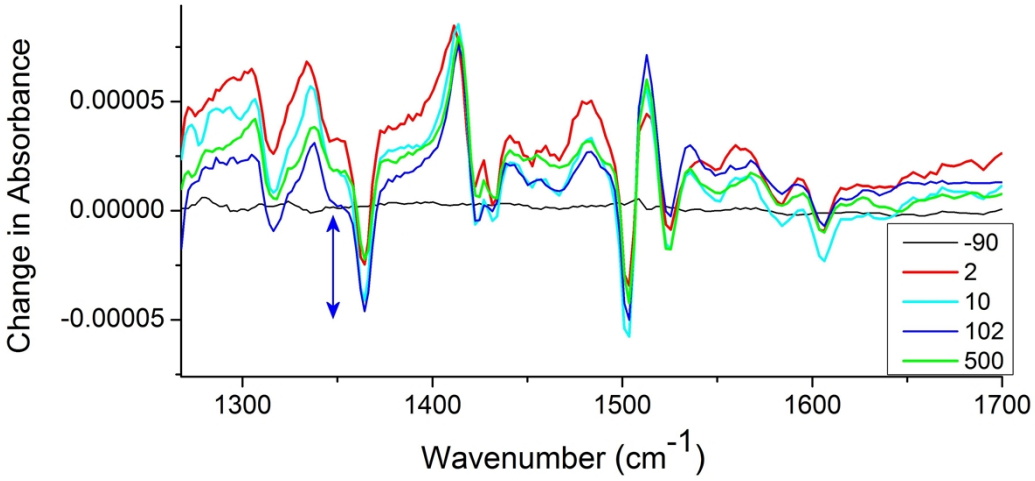


Fig. S8 (a) ps-TRIR of 400 μM *rac*-$[Cr(phen)_2dppz]^{3+}$ in 50 mM phosphate buffer solution, pH=7.4.

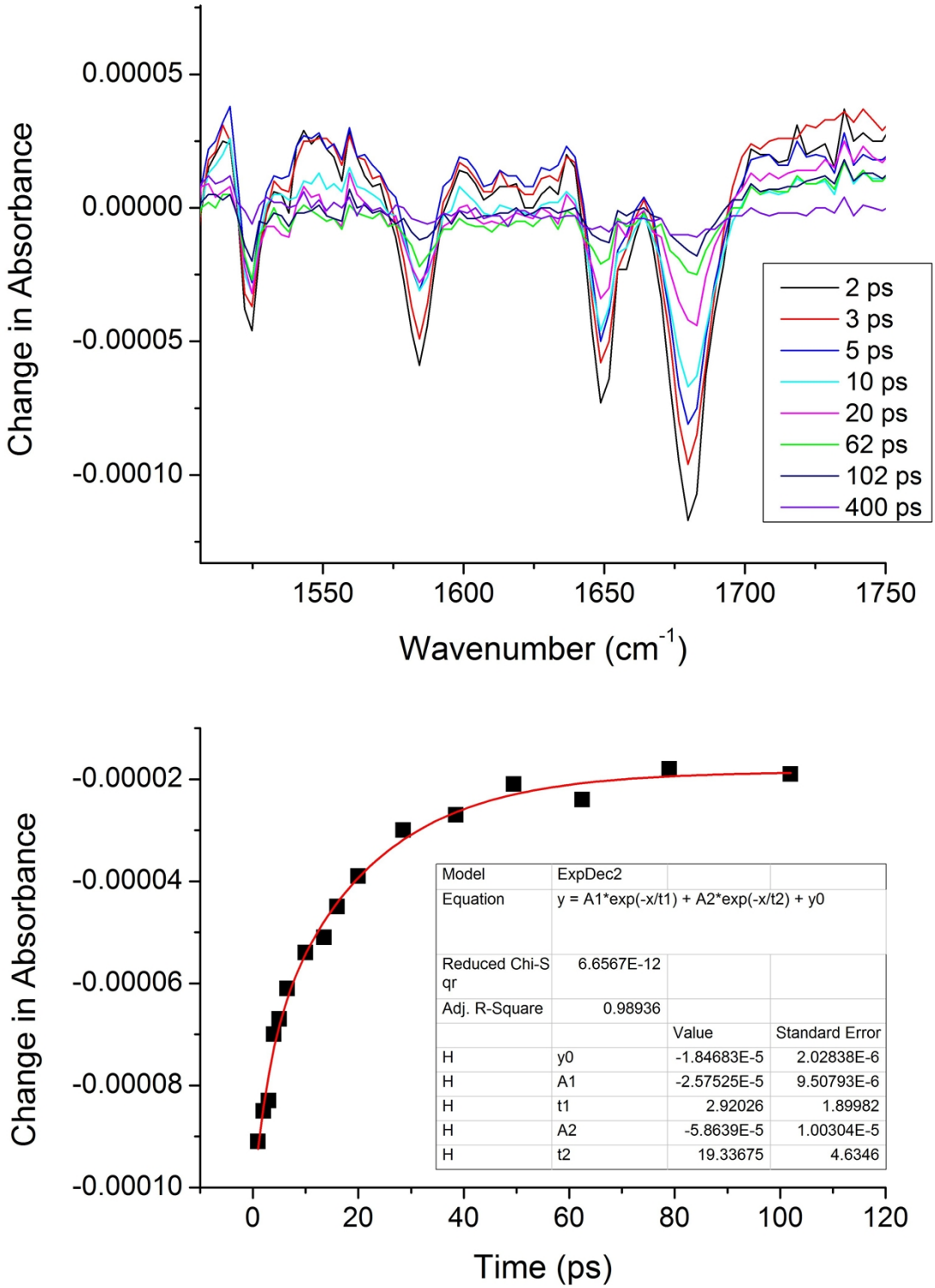


Fig. S9 (a) ps-TRIR of 400 μM *rac*-$[Cr(phen)_2dppz]^{3+}$ in 1 mM d(GGGGGCCCCC) in 50 mM phosphate buffer solution, pH=7.4. (b) Biexponential fit for the excited state recovery of guanine carbonyl bleach at 1680 cm^{-1}. Excitation at 400 nm, 1 μJ.

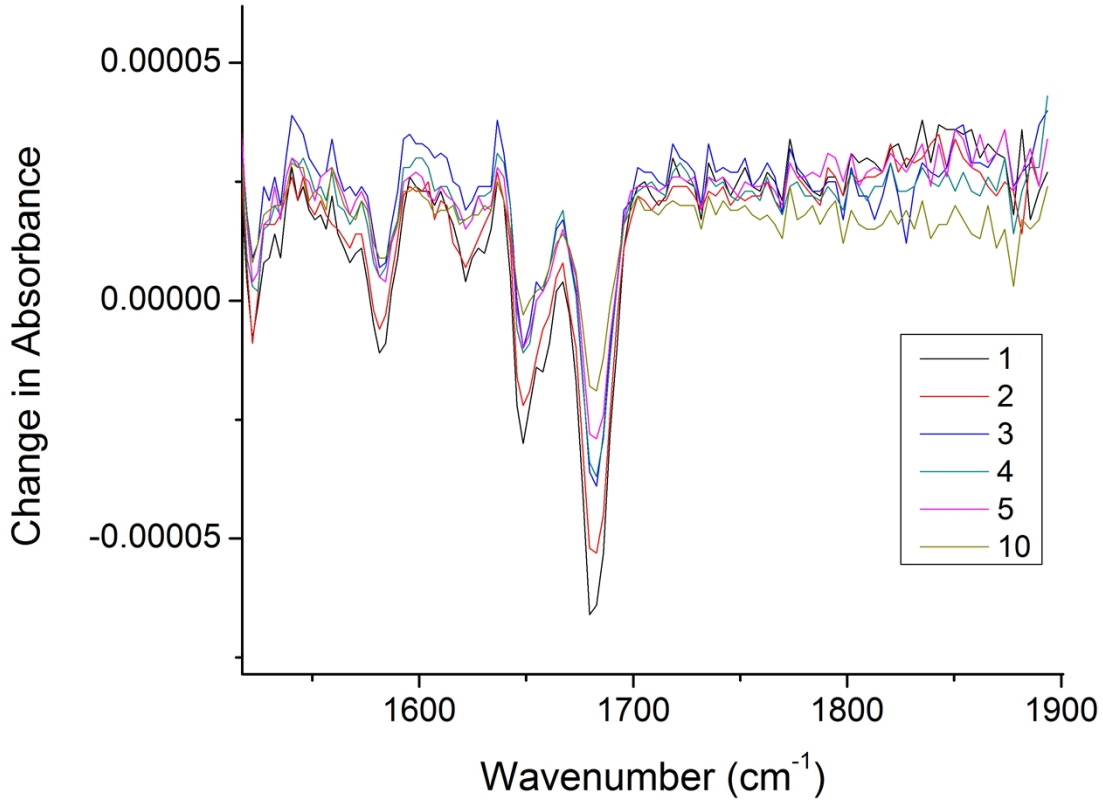


Fig. S10 ps-TRIR of 400 μM *rac*-$[Cr(phen)_2dppz]^{3+}$ in 1 mM $[poly(dG\text{-}dC)]_2$ in 50 mM phosphate buffer solution, pH=7.4.

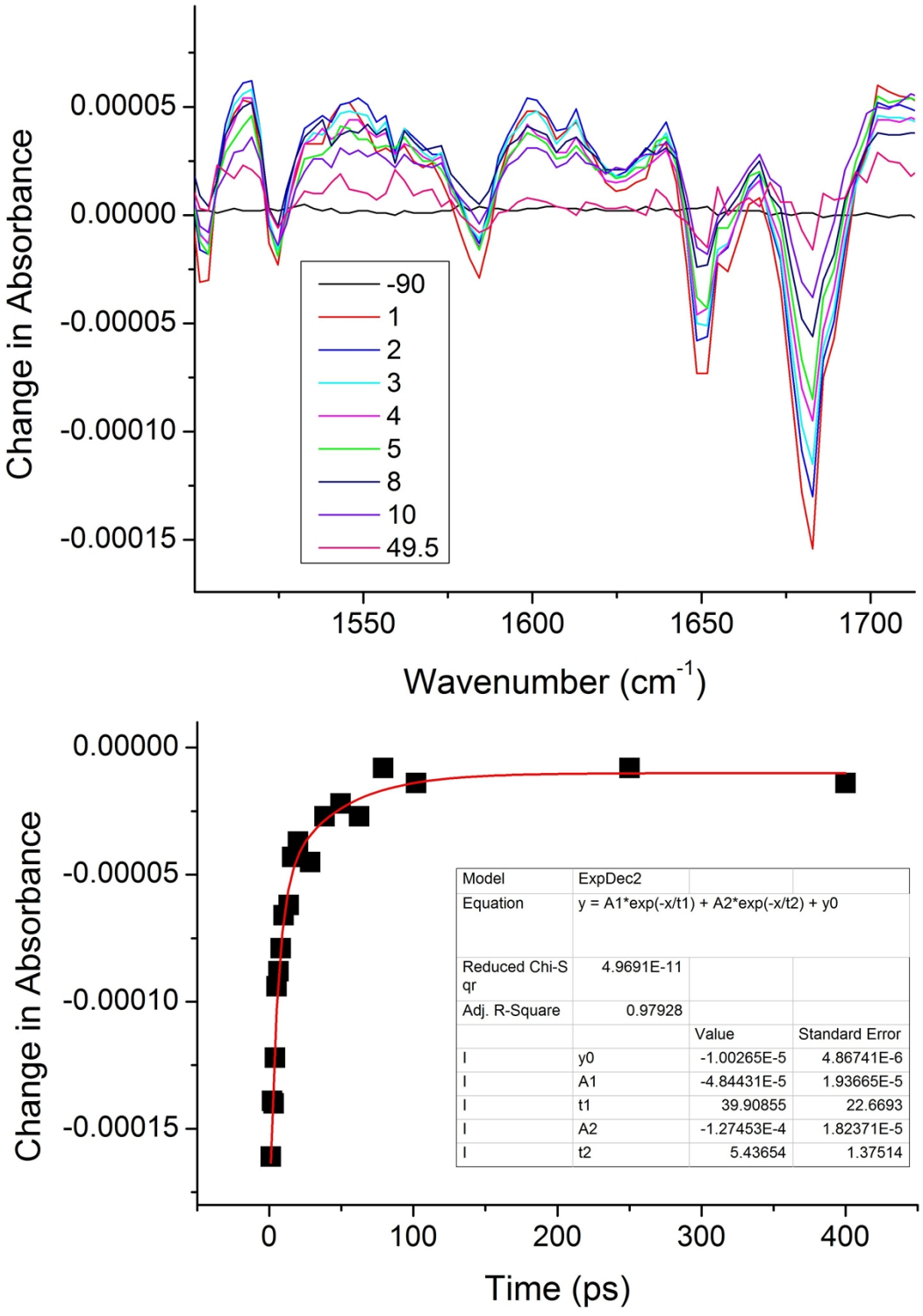


Fig. S11 (a) ps-TRIR of 400 μM *rac*-$[Cr(phen)_2dppz]^{3+}$ in 1 mM d(TCGGCGCCGA), in 50 mM phosphate buffer solution, pH=7.4. (b) Biexponential fit for the excited state recovery of guanine carbonyl bleach at 1680 cm^{-1}. Excitation at 400 nm, 1 μJ.

Tables

Table S1 Biexponential fitting of ps-TA data for the excited state decay at 500 nm obtained after 400 nm excitation of 800 μM *rac*-$[Cr(phen)_2dppz]^{3+}$ in the presence of 2 mM GC oligonucleotides.

	Transient (500 nm)
d(GCGCGCGCGC)	2.6± 0.7 ps (70 %) 34.5 ± 8.9 ps (30 %)
d(GGGGGCCCCC)	3.1± 0.6 ps (66 %) 44 ± 12 ps (34 %)
$[poly(dG\text{-}dC)]_2$	2.8 ± 0.5 ps (74 %) 35.0 ± 13.9 ps (26 %)

Table S2 Biexponential fitting of ps-TRIR data for the recovery of guanine carbonyl bleach at 1680 cm^{-1} obtained after 400 nm excitation of 400 μM *rac*-$[Cr(phen)_2dppz]^{3+}$ in the presence of 1 mM oligonucleotides.

	G bleach (1680 cm^{-1})
d(GCGCGCGCGC)	2.5 ± 0.7 ps (60%) 16.5 ± 2.3 ps (40%)
d(GGGGGCCCCC)	2.4 ± 1.8 ps (70 %) 19± 4 ps (30 %)
d(TCGGCGCCGA)	5.4 ± 1.4 ps (73 %) 39 ± 22 ps (27 %)

References

1. K.D. Barker, K.A. Barnett, S.M. Connell, J.W. Glaeser, A.J. Wallace, J. Wildsmith, B.J. Herbert, J.F. Wheeler, N.A.P. Kane-Maguire, *Inorg. Chim. Acta*., 2001, **316**, 41