Supporting Information

The influence of loops on the binding of the $[Ru(phen)_2dppz]^{2+}$ Light-Switch Compound to i-motif DNA Structures revealed by Time-resolved Spectroscopy

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Experimental

Materials

The 5'-d(CCCT$_n$)$_3$CCC-3' (n = 5 to 8, **C$_3$T$_5$**, **C$_3$T$_6$**, **C$_3$T$_7$** and **C$_3$T$_8$**) and 5'-d(CCCTAA)$_3$CCC-3' oligonucleotides were synthesised by Eurogentec (Liege, Belgium) and obtained in HPLC purified form. Oligonucleotides concentrations were determined spectrophotometrically and made up in D_2O in presence of 50 mM potassium phosphate buffer, pH 5 for the i-motifs and pH 8 for the disordered single-strand structures.

Synthesis of 1

Dichloro(1,5-cyclooctadiene)ruthenium(II), polymer [Ru(η$_4$-COD)Cl$_2$]*n* (1 equiv.) and 2 equiv. of 1,10-phenanthroline were suspended in DMF (20 mL) and degassed by bubbling with argon for 20 mins before being heated at 140 ºC for 45 mins using microwave irradiation. The reaction mixture was cooled to room temperature before being poured into acetone (200 mL) and left for 24 hrs at -10 °C. A dark purple powder was collected by filtration and washed with cold acetone to yield the Ru(phen)$_2$Cl$_2$. Dipyrido[3,2-a:2',3'-c]phenazine (1.1 equiv.) was ground into a fine powder and suspended with Ru(phen)$_2$Cl$_2$ (1 equiv.) in a H_2O/EtOH mixture (1:1 v/v, 7 mL). The suspension was degassed by bubbling with argon for 20 min before being heated at 140 ºC for 40 using microwave irradiation. The resulting orange solution was filtered before dropwise addition of conc. aq. NH_4PF_6 to precipitate the PF_6^- complex. The orange precipitate was isolated by centrifugation and the supernatant was decanted off. The precipitate was resuspended in water (5 mL) and isolated by a further centrifugation and decanting of the supernatant. The crude compound was purified by silica gel flash column chromatography, eluting with CH_3CN/H_2O/aq. $NaNO_3$(sat.) (40:4:1 v/v). To remove excess $NaNO_3$ the solvent was removed under reduced pressure and resulting solid was stirred vigorously in the CH_3CN for 2 hrs. The resulting suspension was filtered through a double thickness of filter paper, the supernatant isolated and the solvent removed under reduced pressure. The nitrate salt of the complex was then dissolved in water and the PF_6^- complex was reformed and precipitated from water by dropwise addition of conc. aq. NH_4PF_6. The precipitate was washed with H_2O (5 mL x 2) *via* centrifugation as above and was dried under vacuum. The chloride form of the complex was reformed by swirling a solution of the PF_6^- complex in MeOH (15 mL) in Amberlite ion exchange resin (chloride form) for 1 hr, filtered and dried under vacuum. ^{1}H NMR (400 MHz, Acetonitrile-d_3) δ 9.65 (2H, dd, *J* = 8.2, 1.4 Hz), 8.64 (4H, ddd, *J* = 8.3, 4.6, 1.3 Hz), 8.54 – 8.42 (2H, m), 8.29 (4H, s), 8.24 (2H, dd, *J* = 5.3, 1.3 Hz), 8.19 – 8.08 (4H m), 8.04 (2H, dd, *J* = 5.3, 1.3 Hz), 7.79 (2H, dd, *J* = 8.2, 5.4 Hz), 7.67 (4H, ddd, *J* = 12.5, 8.3, 5.3 Hz).

Infrared Spectroscopy: Infrared spectra were recorded in a Nicolet Avatar spectrometer. Samples were loaded into a demountable Harrick cell (Harrick Scientific Products Inc., New York) assembled with 25 mm diameter CaF_2 plates (Crystran, Ltd, UK), separated by a 50 μm Teflon spacer in D_2O and recorded in a N_2 flushed Transmission Accessory. Each spectrum is the average of 32 scans.

Time Resolved Spectroscopy:

rac-$[Ru(phen)_2(dppz)]^{2+}$ was synthesized according to published methods.[1] The samples for transient spectroscopy measurements were prepared by placing approximately 30 μL of sample in D_2O into a demountable liquid cell (Harrick Scientific Products, Inc., New York) assembled with 25 mm CaF_2 plates (Crystran, Ltd, UK) separated by a 50 μm Teflon spacer. ns-TrA/TRIR experiments were recorded using 400 nm excitation at 1 μJ pulse energy and a pulse width of 150 fs. The TRIR measurements were recorded using the LIFEtime apparatus and the ns-TA measurements were performed using the ULTRA laser system at the Central Laser Facility (STFC Rutherford Appleton Laboratories, Harwell).[2-4] Samples were checked before and after the experiment by UV/vis spectroscopy. Kinetic analysis was carried out at single wavenumbers by fitting to multi-exponential growth/decay functions from 1 ns to 2000 ns.

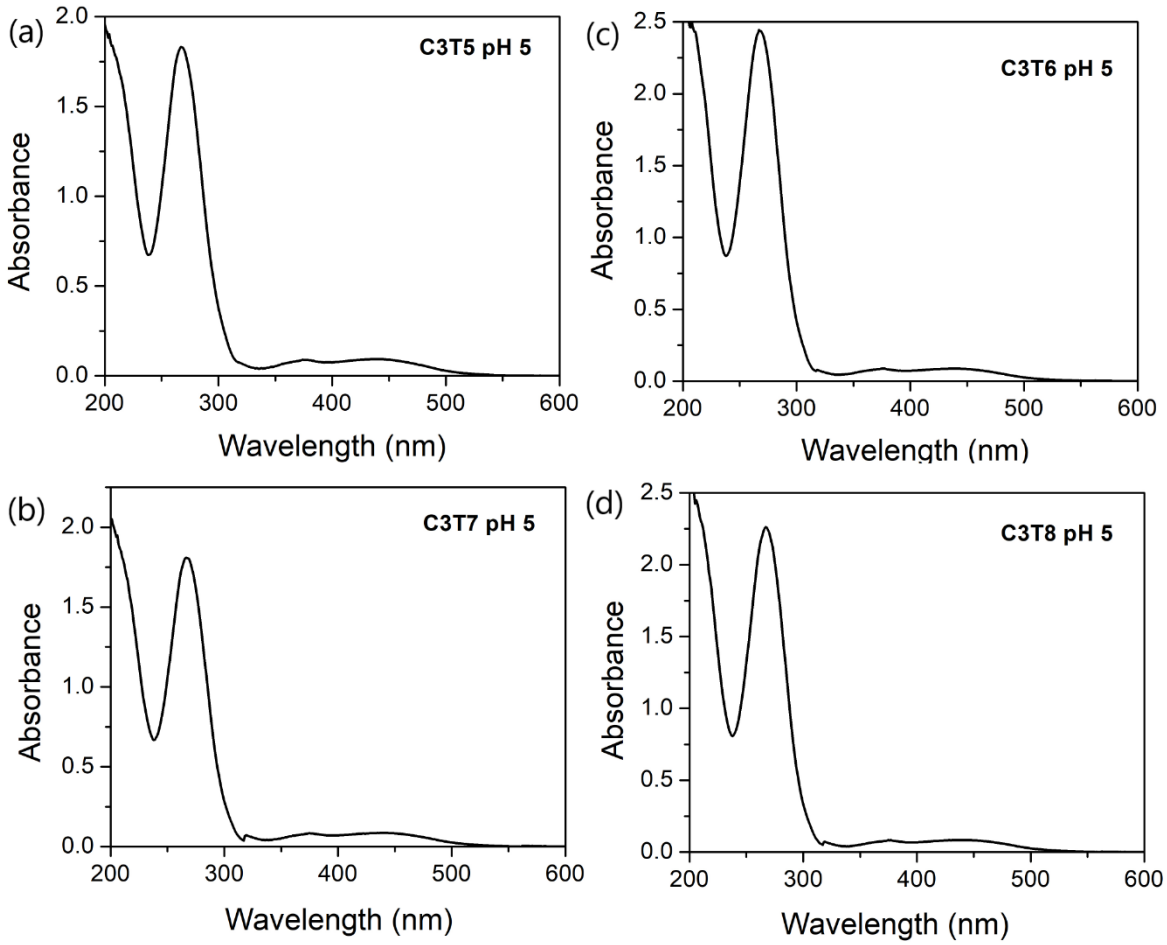

Figure S1: UV visible spectra 0.5 mM of ***rac*-[Ru(phen)$_2$dppz]$^{2+}$** in presence of 0.625 mM of (a) **C_3T_5** (b) **C_3T_6** (c) **C_3T_7** and (d) **C_3T_8** in 50 mM potassium phosphate buffer, pH 5, in D_2O.

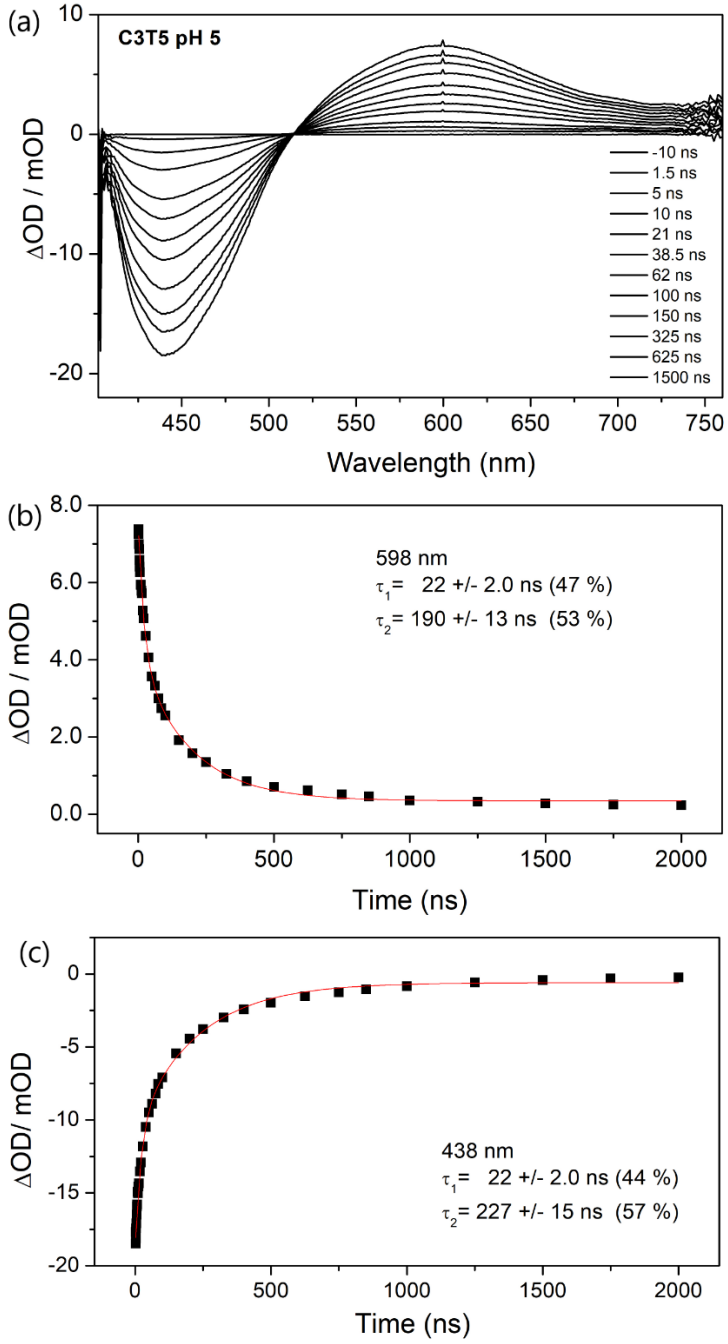

Figure S2. (a) ns-TA spectrum of 0.5 mM of ***rac*-[Ru(phen)$_2$dppz]$^{2+}$** in presence of 0.625 mM of **C_3T_5** and 50 mM potassium phosphate buffer, pH 5, in aerated D_2O. Excitation at 400 nm. Kinetic analysis for (b) transient decay and (c) bleach recovery fitted to bi-exponential profile.

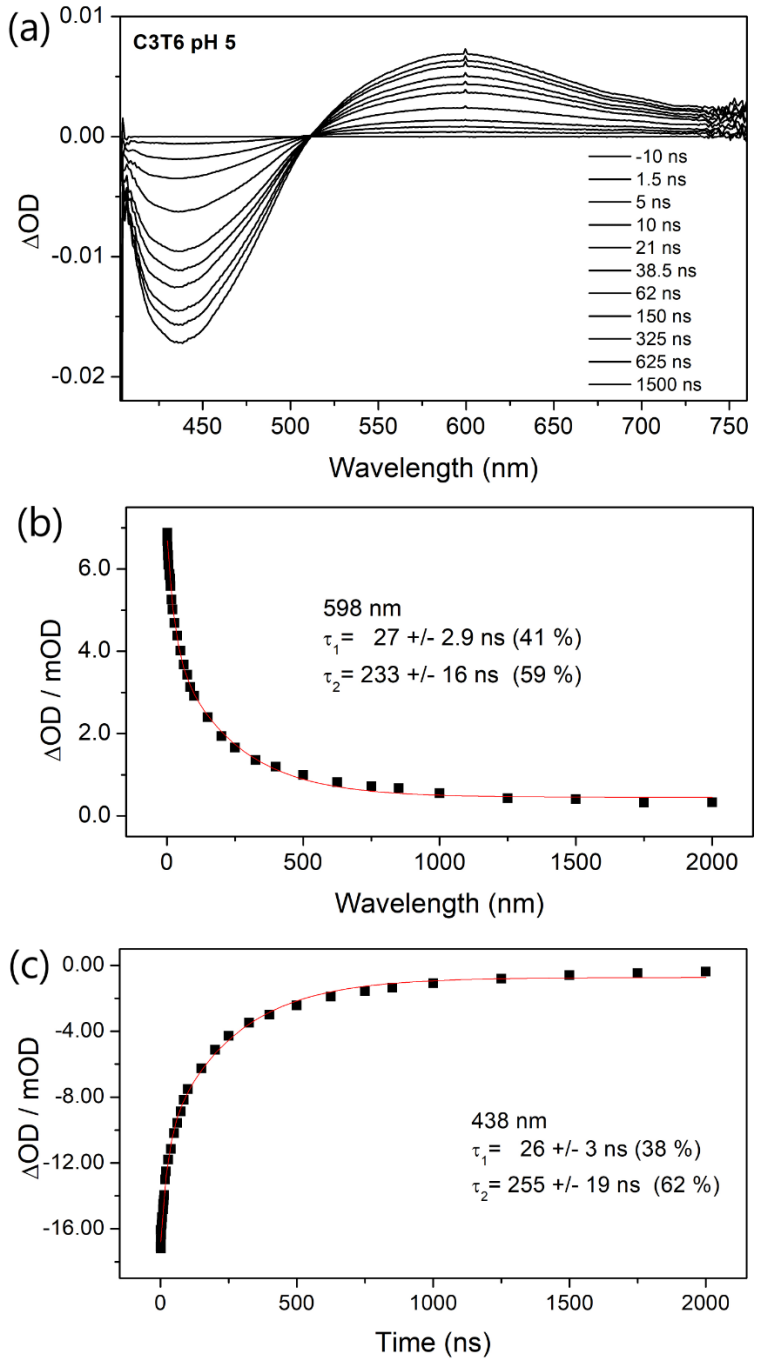

Figure S3. (a) ns-TA spectrum of 0.5 mM of ***rac*-[Ru(phen)$_2$dppz]$^{2+}$** in presence of 0.625 mM of **C_3T_6** and 50 mM potassium phosphate buffer, pH 5, in aerated D_2O. Excitation at 400 nm. Kinetic analysis for (b) transient decay and (c) bleach recovery fitted to bi-exponential profile.

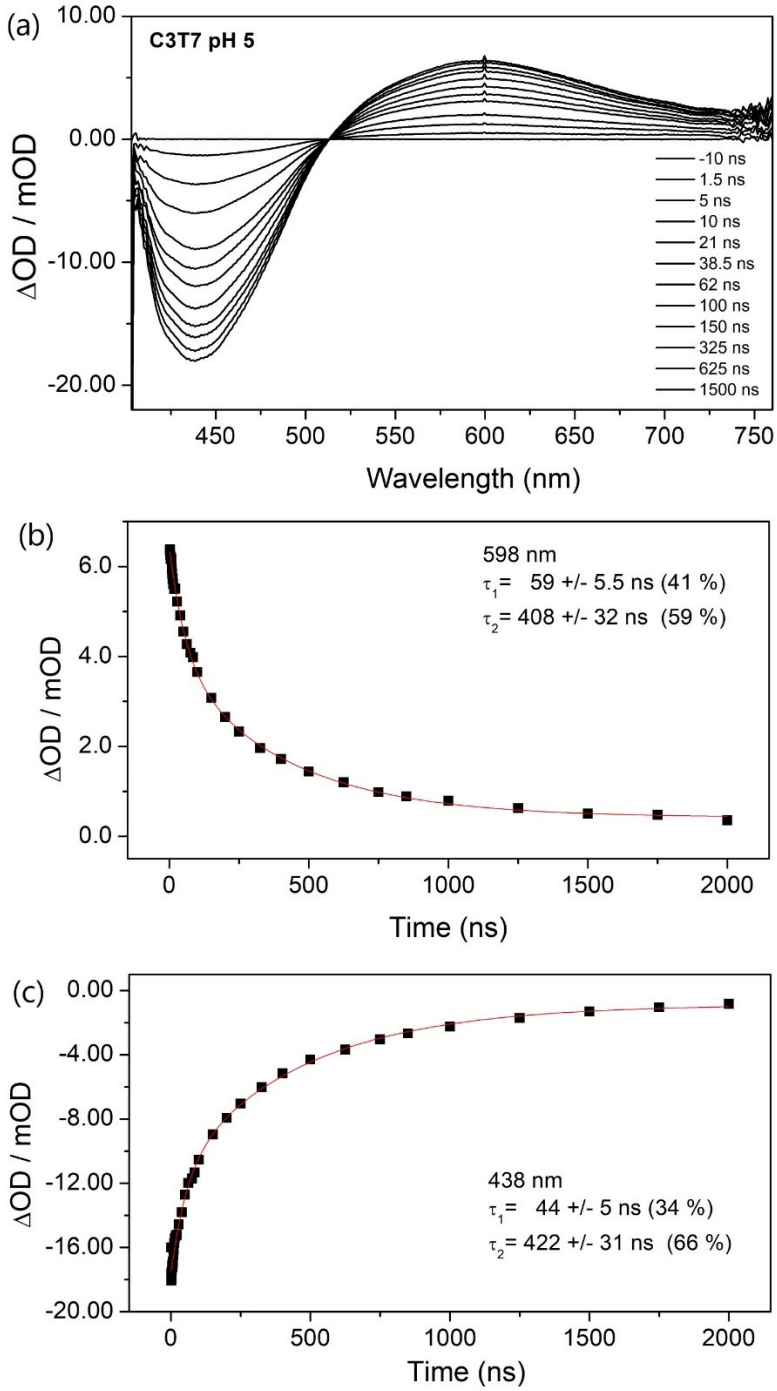

Figure S4. (a) ns-TA spectrum of 0.5 mM of ***rac*-[Ru(phen)$_2$dppz]$^{2+}$** in presence of 0.625 mM of **C_3T_7** and 50 mM potassium phosphate buffer, pH 5, in aerated D_2O. Excitation at 400 nm. Kinetic analysis for (b) transient decay and (c) bleach recovery fitted to bi-exponential profile.

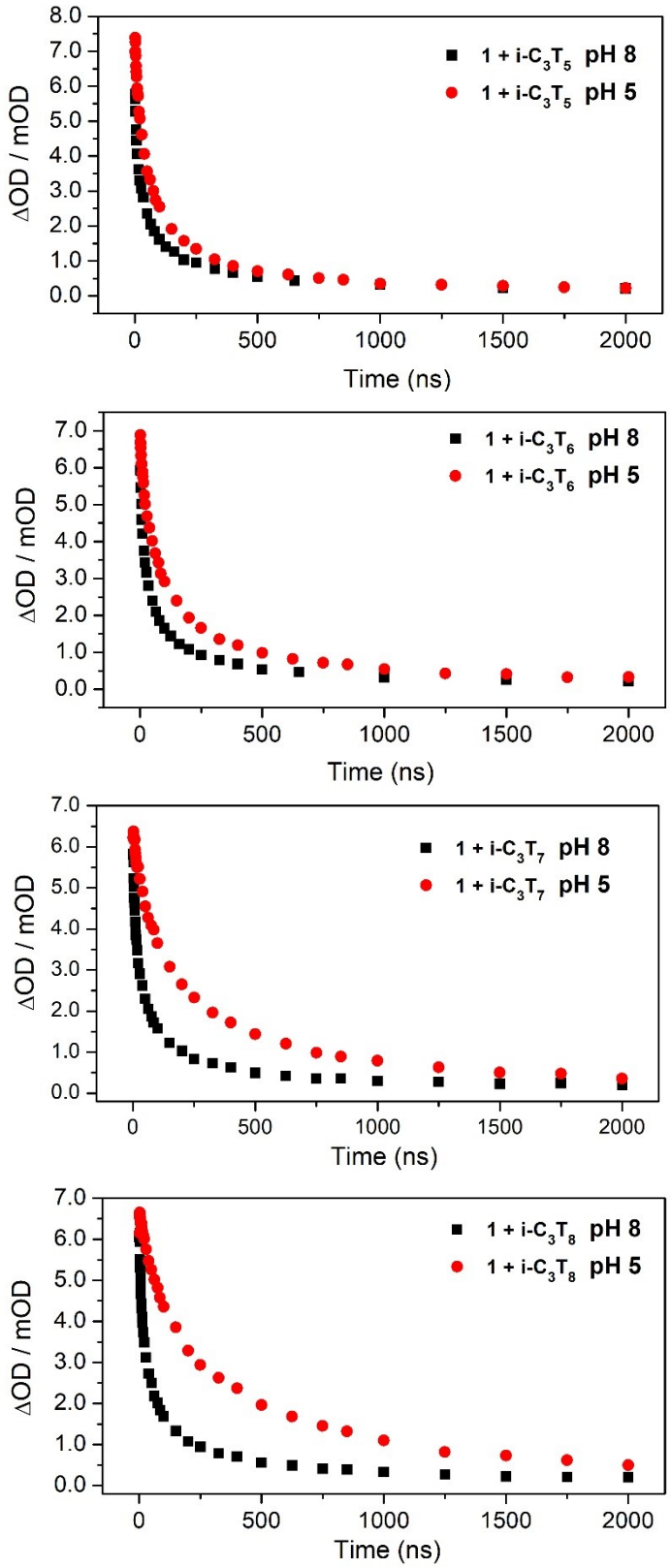

Figure S5. Comparison of ns-TA kinetics for transient decay monitored at 598 nm of 0.5 mM of **1** in presence of 0.625 mM of **single-stranded** and **i-motif structures** in aerated 50 mM potassium phosphate buffer, pH 5 and at pH 8, in D_2O. Excitation at 400 nm.

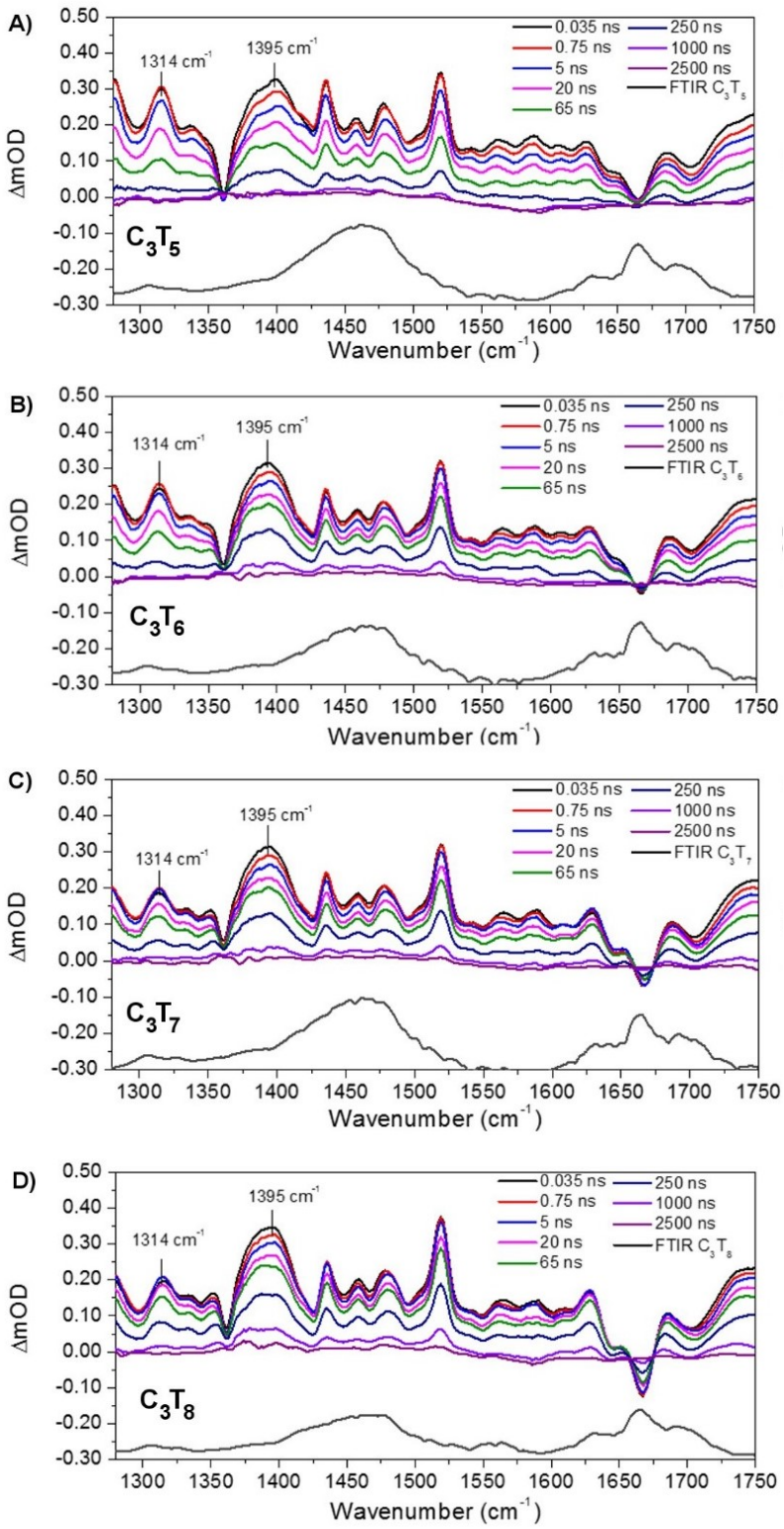

Figure S6. ns-TRIR spectrum of 0.5 mM of ***rac*-[Ru(phen)$_2$dppz]$^{2+}$** in presence of 0.625 mM of (a-d) **C$_3$T$_{5\text{-}8}$** and 50 mM potassium phosphate buffer, pH 5, in D_2O. Excitation at 400 nm.

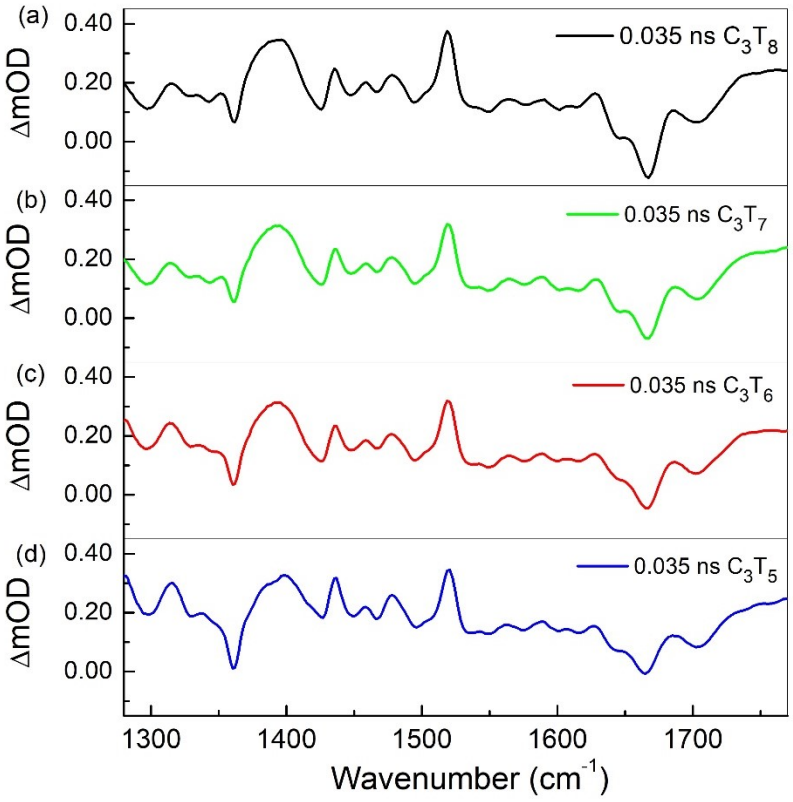

Figure S7. ns-TRIR spectrum recorded at 35 ps after excitation of 0.5 mM of ***rac*-[Ru(phen)$_2$dppz]$^{2+}$** in presence of 0.625 mM of (a) **i-C$_3$T$_8$**, (b) **i-C$_3$T$_7$**, (c) **i-C$_3$T$_6$** and (d) **i-C$_3$T$_5$** in 50 mM potassium phosphate buffer, pH 5, in D_2O.

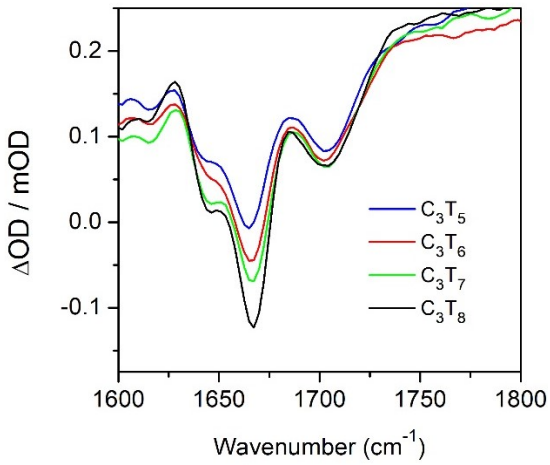

Figure S8. Comparative TRIR of the DNA carbonyl vibrations for **i-C$_3$T$_{5\text{-}8}$** spectra of 0.5 mM of **1** in presence of 0.625 mM of C_3T_8 in 50 mM potassium phosphate buffer, pH 5, in D_2O (λ_{ex}= 400 nm, 150 fs).

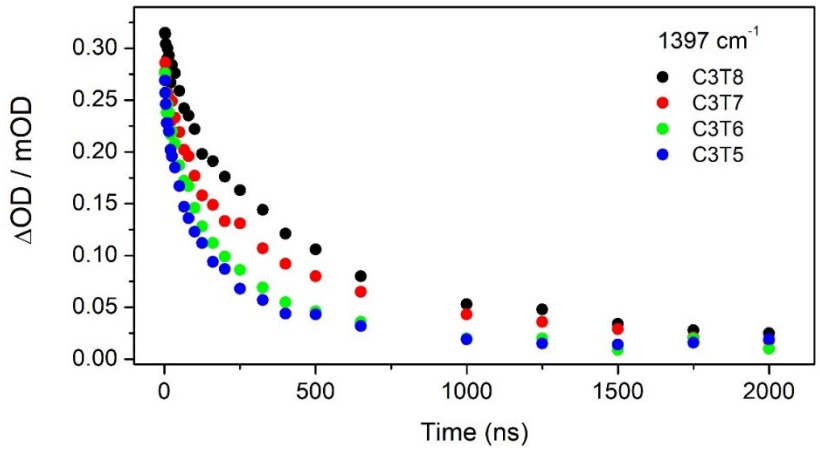

Figure S9. Decay of the transient '*bright*' state band at 1395 cm^{-1} monitored for **1** in presence of 0.625 mM of $\mathbf{C_3T_{5\text{-}8}}$ and aerated 50 mM potassium phosphate buffer, pH 5, in D_2O after excitation at 400 nm.

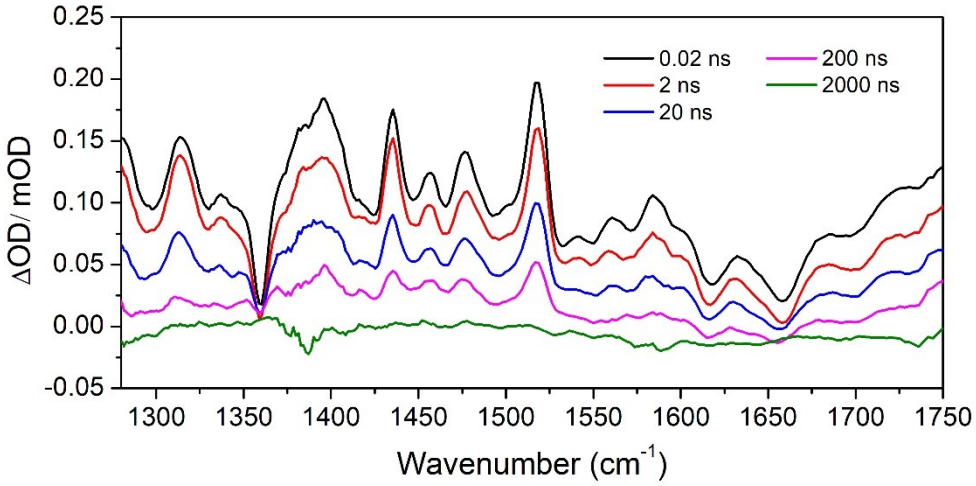

Figure S10. ns-TRIR spectrum of 0.5 mM of **1** in presence of 0.625 mM of human telomer i-motif (**CCCTAA)$_3$CCC** and 50 mM potassium phosphate buffer, pH 5, in D_2O. Excitation at 400 nm.

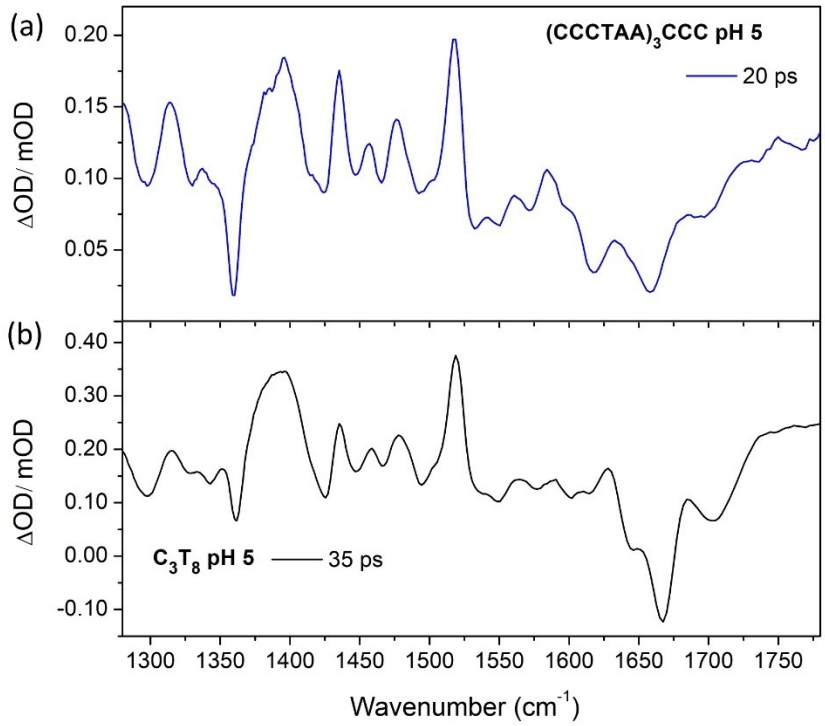

Figure S11. Comparative TRIR spectra of ns-TRIR spectra of 0.625 mM of **1** in presence of (a) (**CCCTAA)$_3$CCC** and (b) **i-C$_3$T$_8$** potassium phosphate buffer, pH 5, in D_2O.

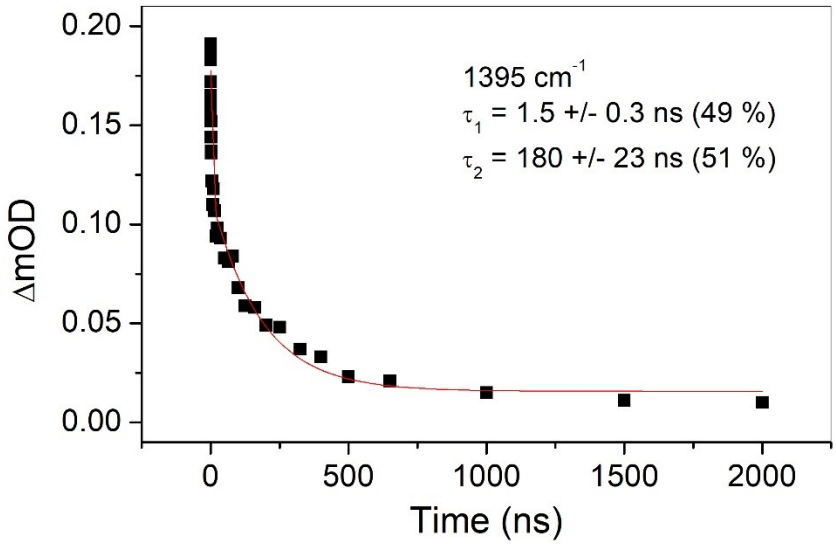

Figure S12. Decay of the transient '*bright*' state band at 1395 cm^{-1} monitored for **1** in presence of 0.625 mM of (**CCCTAA)$_3$CCC** and aerated 50 mM potassium phosphate buffer, pH 5, in D_2O after excitation at 400 nm.

Table S1. Comparison of the kinetics for the decay of the 598 nm excited state measured by ns-TA of **[Ru(phen)$_2$dppz]$^{2+}$** in the presence of single stranded and i-motif structures.

i-motif	pH	τ_1 (ns)	A_1	τ_2 (ns)	A_2
i-C$_3$T$_5$	5	22 ± 2	0.47	190 ± 13	0.53
ss-C$_3$T$_5$	8	12 ± 1	0.50	158 ± 18	0.50
i-C$_3$T$_6$	5	27 ± 2	0.41	233 ± 16	0.59
ss-C$_3$T$_6$	8	12 ± 1	0.50	151 ± 16	0.50
i-C$_3$T$_7$	5	59 ± 6	0.41	408 ± 32	0.59
ss-C$_3$T$_7$	8	10 ± 1	0.50	144 ± 10	0.50
i-C$_3$T$_8$	5	92 ± 6	0.41	603 ± 44	0.59
ss-C$_3$T$_8$	8	12 ± 8	0.49	158± 12	0.51

Table S2. Comparison of the kinetics for the decay of the 598 nm excited state measured by ns-TA and the "bright" excited-state at 1395 cm^{-1} measured by TRIR, of **[Ru(phen)$_2$dppz]$^{2+}$** in the presence of **i-C$_3$T$_5$**, **i-C$_3$T$_6$**, **i-C$_3$T$_7$** and **i-C$_3$T$_8$** i-motifs, pH 5.

i-motif	λ (nm) or $\bar{\upsilon}$ (cm^{-1})	τ_1 (ns)	A_1	τ_2 (ns)	A_2
i-C$_3$T$_5$	598 nm	22 ± 2	0.47	190 ± 13	0.53
i-C$_3$T$_5$	1395 cm^{-1}	13 ± 2	0.31	189 ± 14	0.69
i-C$_3$T$_6$	598 nm	27 ± 2	0.41	233 ± 16	0.59
i-C$_3$T$_6$	1395 cm^{-1}	37 ± 6	0.41	278 ± 16	0.59
i-C$_3$T$_7$	598 nm	59 ± 6	0.41	408 ± 32	0.59
C$_3$T$_7$	1395 cm^{-1}	51 ± 14	0.33	484 ± 83	0.67
C$_3$T$_8$	598 nm	92 ± 6	0.41	603 ± 44	0.59
C$_3$T$_8$	1395 cm^{-1}	63 ± 21	0.24	515 ± 66	0.76

References

1. C. Hiort, P. Lincoln and B. Norden, *J. Am. Chem. Soc.*, 1993, **115**, 3448-3454.

2. G. M. Greetham, P. Burgos, Q. Cao, I. P. Clark, P. S. Codd, R. C. Farrow, M. W. George, M. Kogimtzis, P. Matousek, A. W. Parker, M. R. Pollard, D. A. Robinson, Z. J. Xin, M. Towrie, *Appl. Spectrosc.* 2010, **64**, 1311.

3. G. M. Greetham, D. Sole, I. P. Clark, A. W. Parker, M. R. Pollard, M. Towrie, *Rev. Sci. Instrum.* 2012, **83**, 103107.

4. G. M. Greetham, P. M. Donaldson, C. Nation, I. V. Sazanovich, I. P. Clark, D. J. Shaw, A. W. Parker, M. Towrie, *Appl. Spectrosc.* 2016, **70**, 645– 653.