Selective Recognition of Conformation-Specific Arylamine-DNA adduct in Frameshift Model by $[Ru(phen)_2(dppz)]^{2+}$

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Supplementary information

Experimental Section.

The preparation, purification and separation of isomers of $[Ru(phen)_2(dppz)]^{2+}$ was carried out by adopting the literature reported previously. [1] The modification and purification of oligonucleotides with AAF was carried out by following the similar methodology reported earlier. [1] To confirm the site of modification, enzymatic digestion using bovine spleen phosphodiesterase II, which cleaves from 5′-3′ end was performed with control and modified oligonucleotide using the similar MALDI-TOF protocol reported earlier.[1] Briefly, control and AAF-modified oligonucleotides were treated with phosphodiesterase-II and collected samples at different time intervals. The enzymatic reaction was quenched by adding 3-hydroxypicolinic acid matrix mixed with dihydrogen ammonium citrate (1:1).

The luminescence and quenching studies were performed by monitoring the luminescence intensity of Ru(II) complex (500 nM) upon addition of increasing amounts of oligonucleotides (0-2 μM). The excitation wavelength used here was 450 nm and luminescence intensity were measured at 610 nm in Hitachi F-7000 spectrofluorimeter.

The lifetime measurements were performed for Ru(II) (500 nM) alone and with oligonucleotides (2 μM). The room temperature excited state lifetimes were measured using Time Correlated Single Photon Counting Fluorescence Spectrophotometer (TCSPC-FS) (Edinburgh model-FLS980), UK. The lifetimes of the complexes were calculated using the software provided along with the instrument.

Microscale thermophoresis experiments were performed by using the literature reported earlier. [1,2] Briefly, sixteen different concentrations of oligonucleotides (0-45 μM) was mixed with Ru(II) complex (2 μM) and incubated for 30 min before loading. The change in fluorescence based on thermophoresis was measured using Nanotemper Monolith

instrument. The change in distribution of luminescence of Ru(II) is used to determine the ratio of free Ru(II) to bound Ru(II) to oligonucleotide. The luminescence parameters, F_{cold} and F_{hot} are collected before and after heating using laser, respectively. The ratio of F_{hot}/F_{cold} gives the normalized luminescence, F_{norm}. By plotting F_{norm} vs. log[oligo] gives sigmoidal curves from which binding parameters are calculated using Hill's plot.

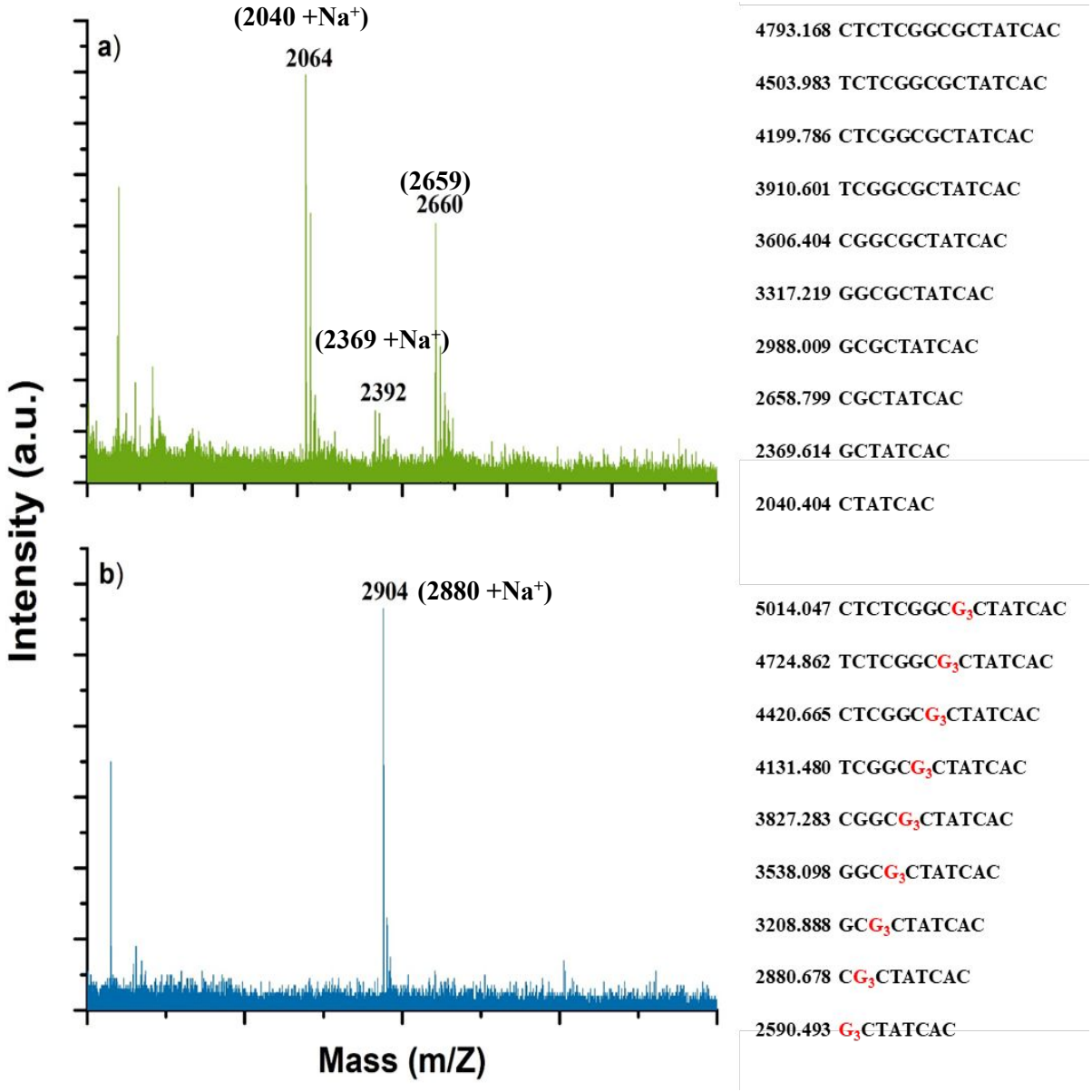

Figure S1: MALDI spectra for PDE II enzyme digested a) *Nar*I G_3CT unmodified, and b) *Nar*I $G_3^{AAF}CT$ oligonucleotide. In parenthesis, theoretical mass with or without sodium ions are provided. The theoretical mass values are provided for the digested oligonucleotide sequences.

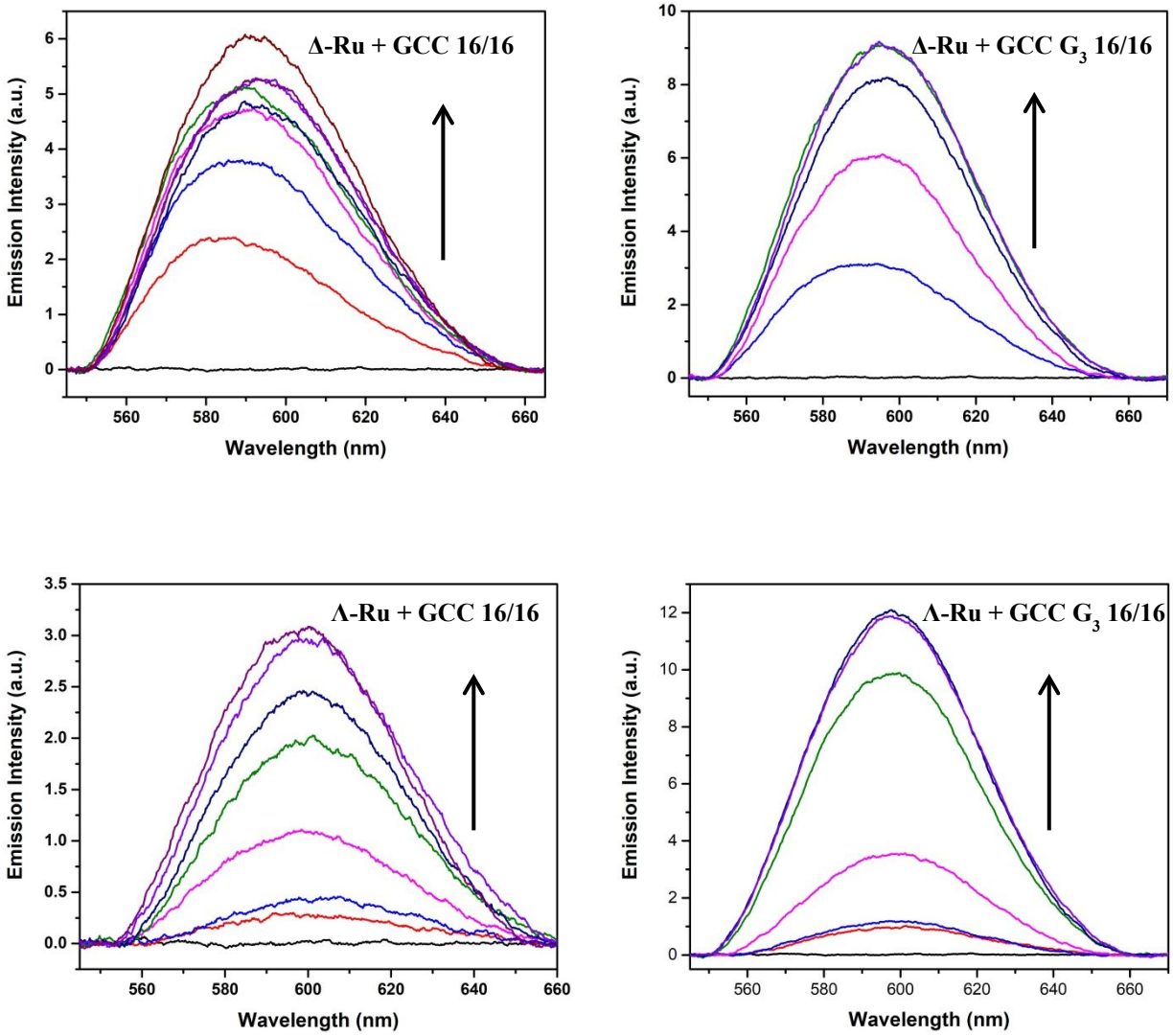

Figure S2: Luminescence spectra of Ru(II) with unmodified (left) and AAF modified oligonucleotides(right) in full-duplex model. Δ-(top) and Λ-(bottom)

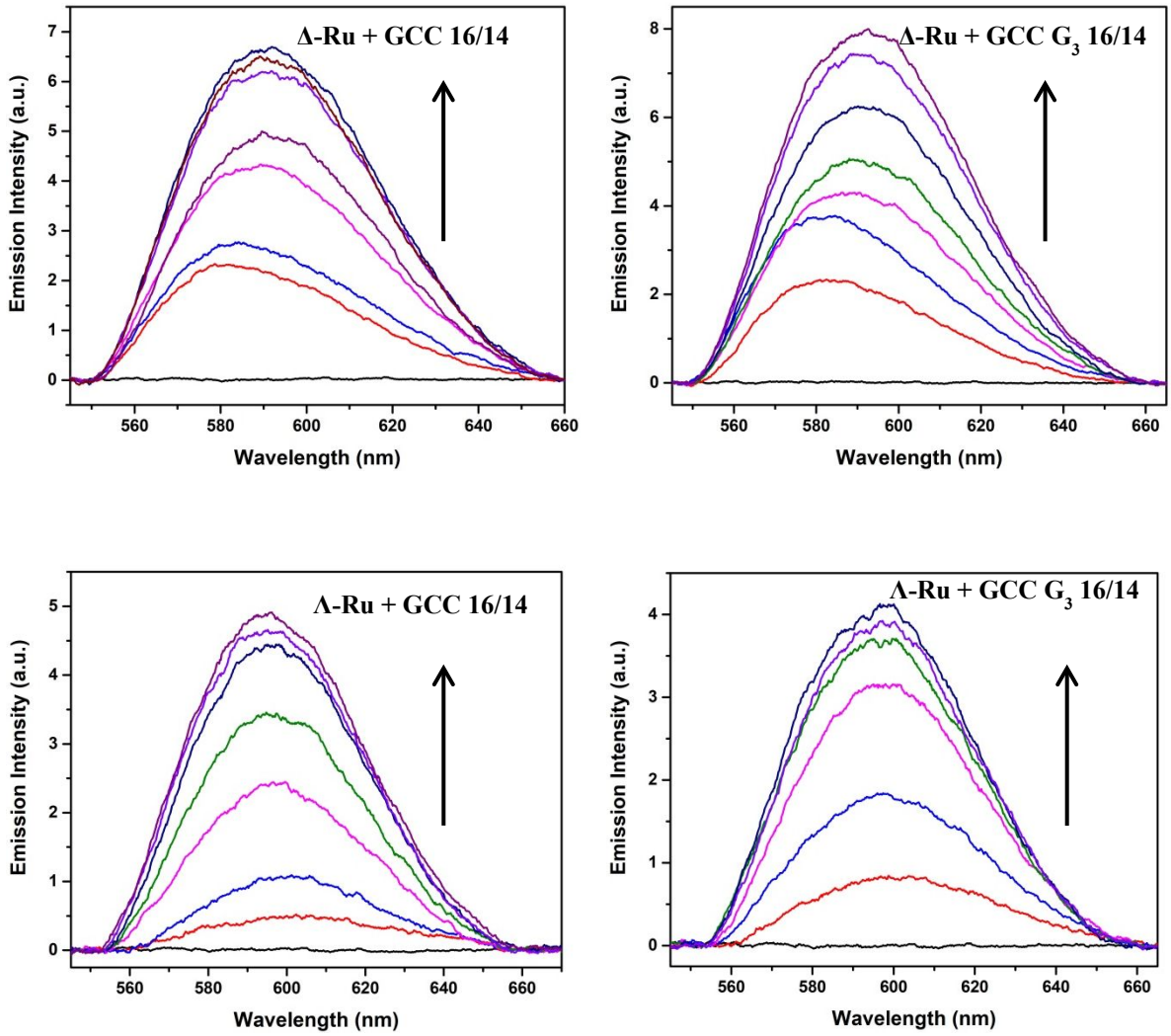

Figure S3: Luminescence spectra of Ru(II) with unmodified (left) and AAF modified oligonucleotides(right) in SMI model. Δ-(top) and Λ-(bottom)

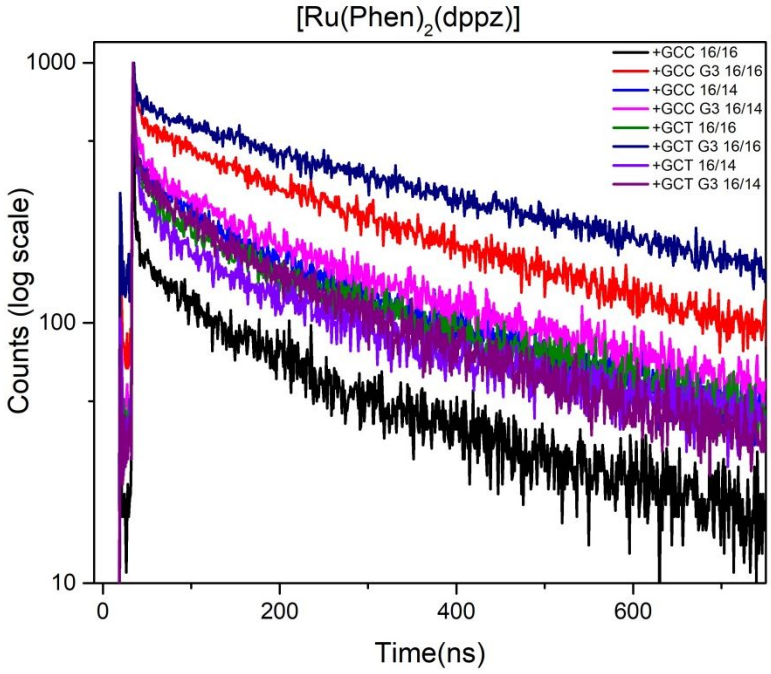

Figure S4. Lifetime decay profiles of Δ-Ru(II) with full duplex and SMI models

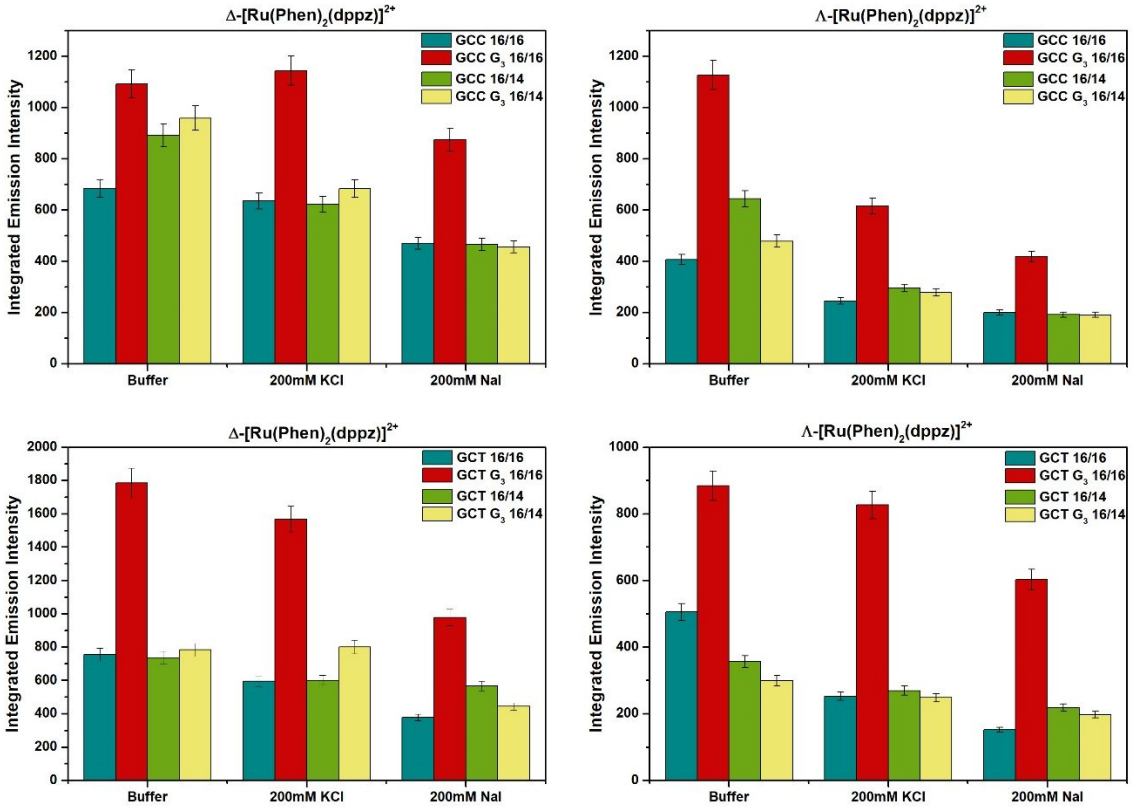

Figure S5: Effect of NaI and KCl on the integrated emission intensities of Δ and Λ-Ru in the presence of control and modified GCC and GCT oligonucleotides ([Ru] = 500nM ; [oligo] = 2μM ; [NaI] = 0 to 200mM ; [KCl] = 200 to 0 mM). (a) Δ-Ru (Left) and (b) Λ- Ru (right)

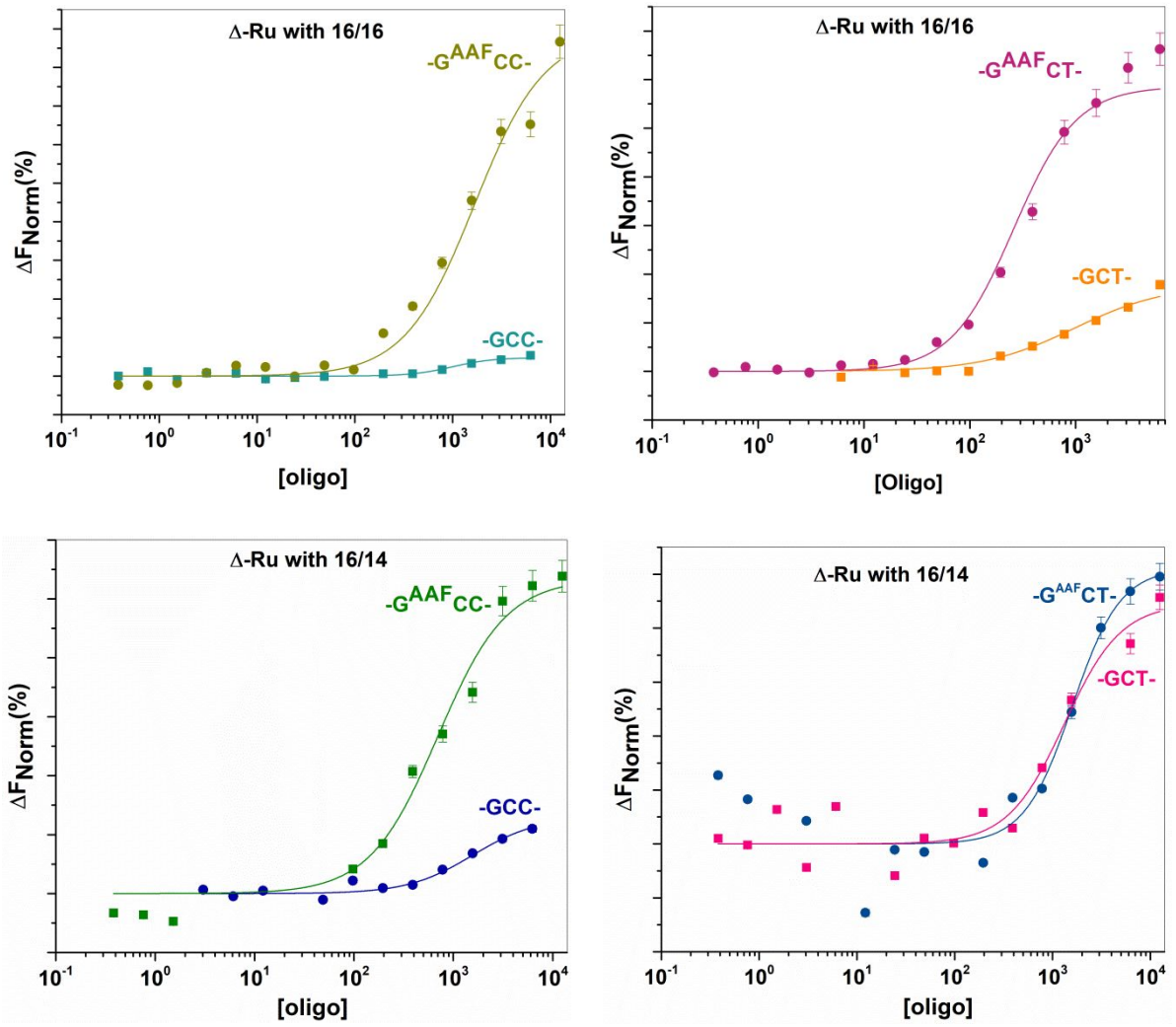

Figure S6. Microscale Thermophoretic data of Δ-Ru with full duplex (16/16) and SMI (16/14-mer) ([Ru] = 5μM; [oligo] = 0 to 45 μM)

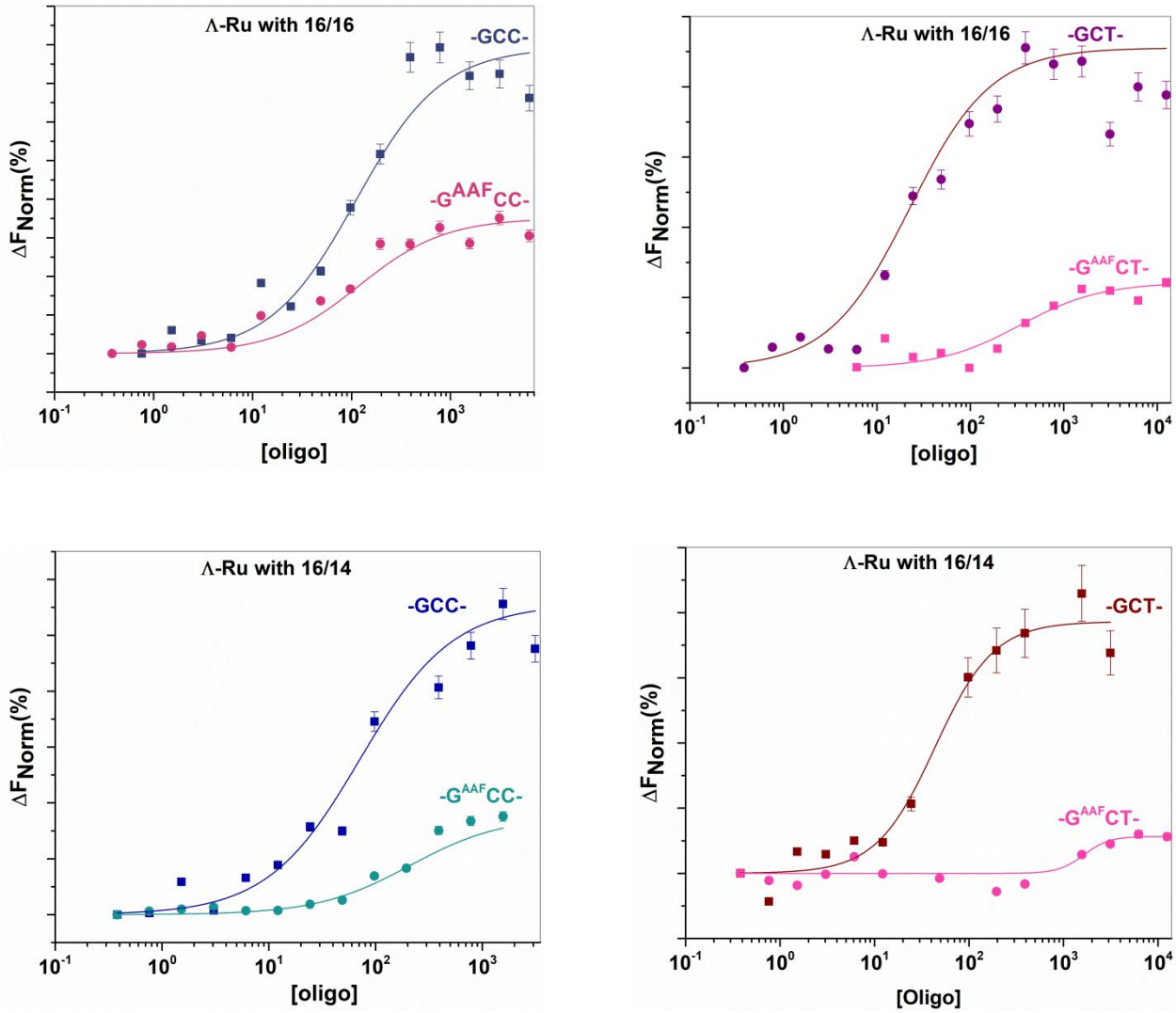

Figure S7. Microscale Thermophoretic data of Λ-Ru with full duplex (16/16) and SMI (16/14-mer) ([Ru] = 5μM; [oligo] = 0 to 45 μM)

Table S1: Lifetime data of Δ-$[Ru(Phen)_2(dppz)]^{2+}$ in the presence of *Nar*I GCC and GCT oligonucleotides

Complex	Oligonucleotide	τ_1 (ns)	τ_2 (ns)	τ_1:τ_2
Δ-$[Ru(Phen)_2(dppz)]$	NarI GCC 16/16	24	220	7:93
	NarI GCC G_3 16/16	14	**324**	2:98
	NarI GCC 16/14	24	247	6:94
	NarI GCC G_3 16/14	35	**320**	7:93
	NarI GCT 16/16	17	285	5:95
	NarI GCT G_3 16/16	22	**427**	1:99
	NarI GCT 16/14	5	193	6:94
	NarI GCT G_3 16/14	18	**210**	5:95

References

(1) Thangavel, N.; Ganesan, V. V.; Nair, B. U., Conformation Specific Binding of $[Ru(phen)_2(dppz)]^{2+}$ with Mono- and Cluster Arylamine-DNA Adducts, *ChemistrySelect,* **2018**, *3*, 11152.

(2) Nandhini, T.; Vaidyanathan, V. G.; Nair, B. U., Effect of conformation of the arylamine-DNA adduct on the sensitivity of $[Ru(phen)_2(dppz)]^{2+}$ complex, *Inorg. Chem. Commun.,* **2016**, *73*, 64.