Electronic Supplementary Information

Selective Recognition of G-Quadruplexes by a Dimeric Carbocyanine Dye

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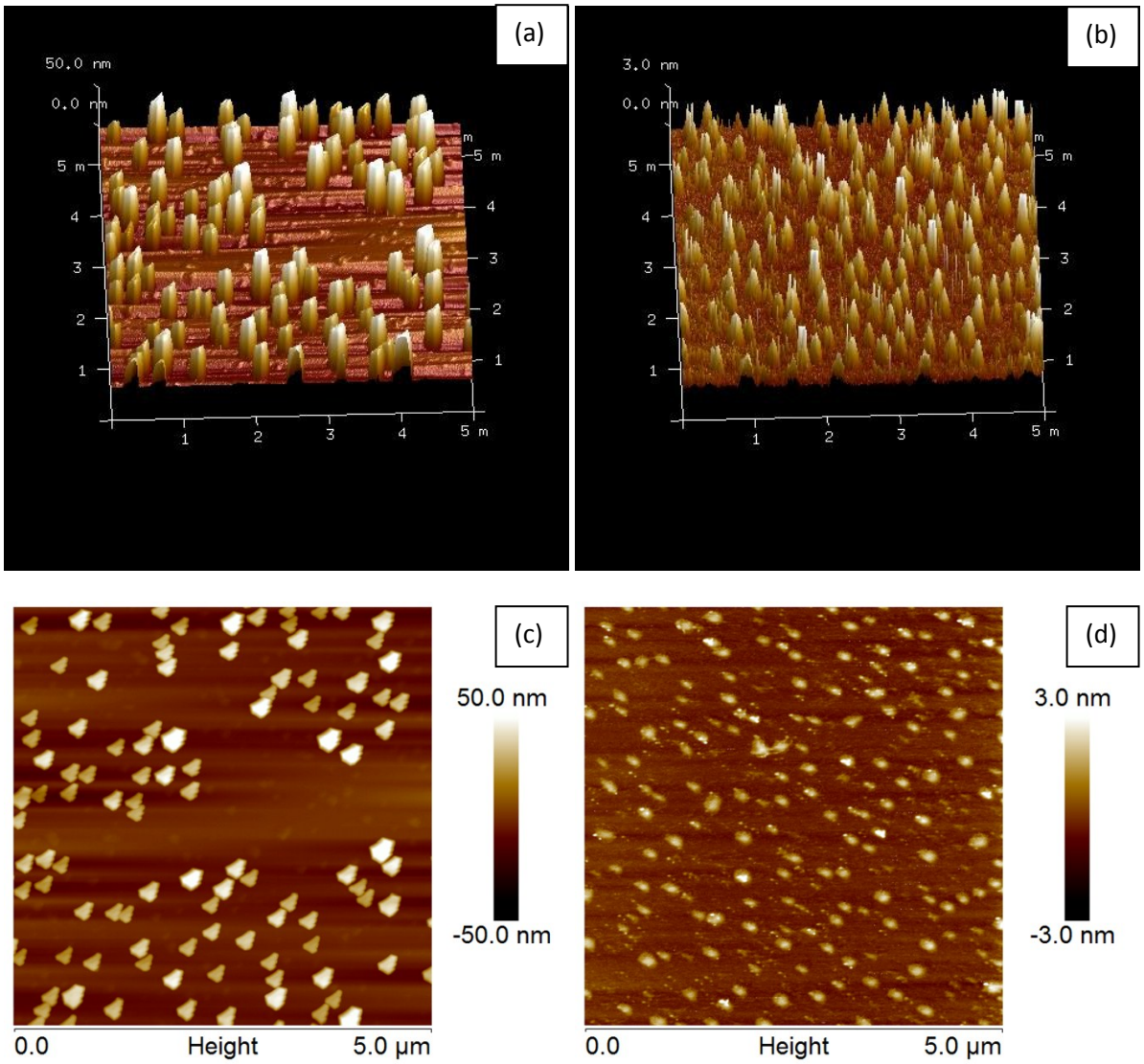


Figure S1. 3D AFM images of dimeric carbocyanine dye **1** in (a) absence and (b) presence of G3T-4. 2D AFM images of dye **1** in (c) absence and (d) presence of G3T-4. Samples contained 2 µM dye **1** and 2 µM DNA and were prepared as described in the Experimental section.

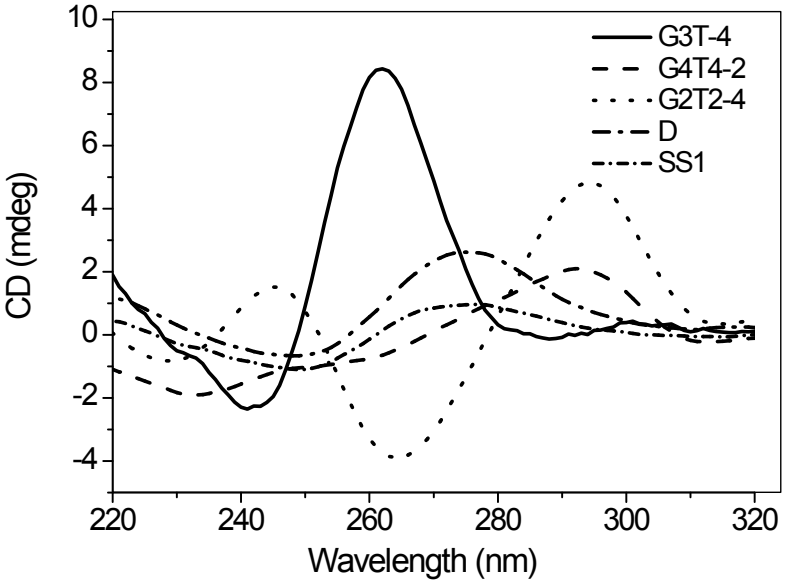


Figure S2. CD spectra of DNA molecules in presence of 10 mM KCl. Samples contained 2 μM DNA in 10 mM phosphate buffer (pH 7.0).

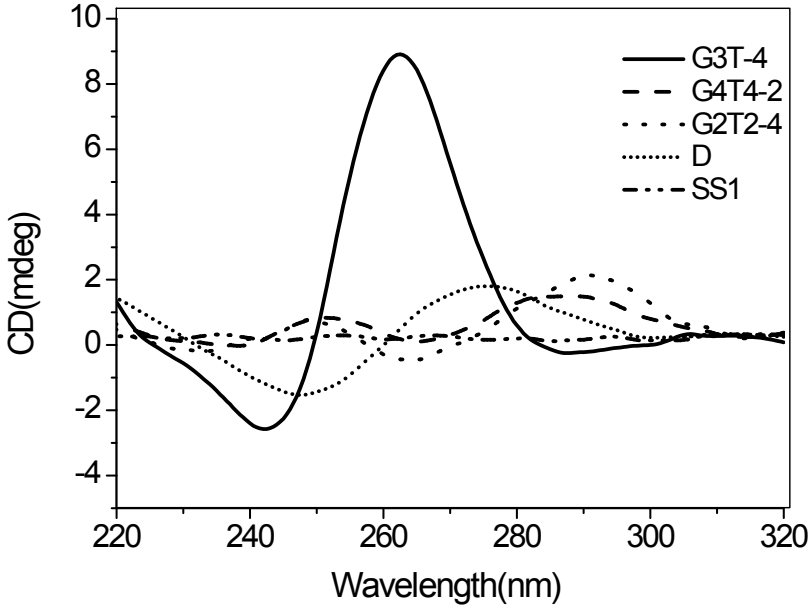


Figure S3. CD spectra of DNA molecules in presence of 10 mM NaCl. Samples contained 2 μM DNA in 10 mM phosphate buffer (pH 7.0).

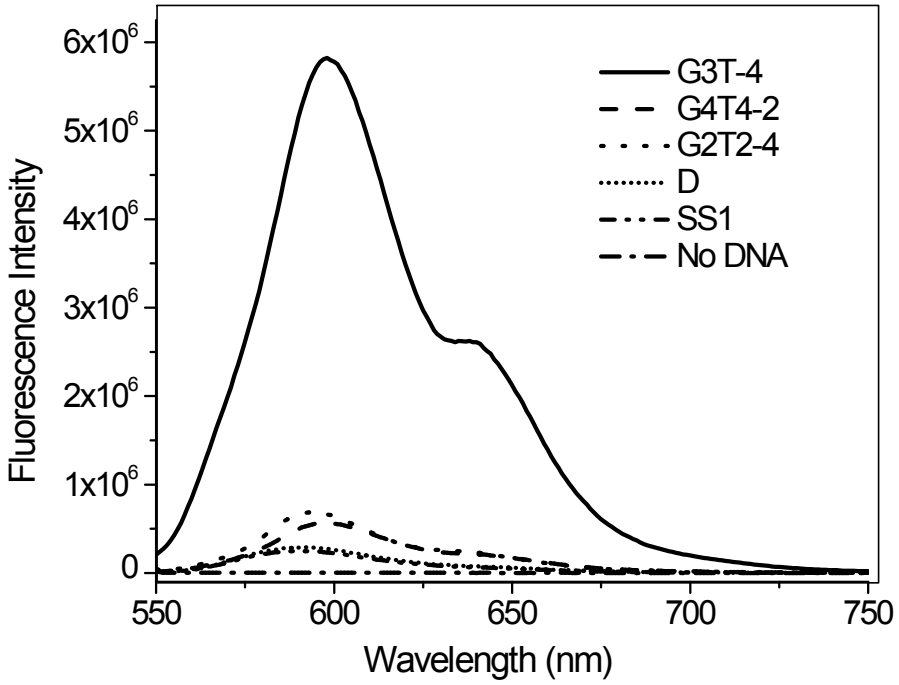


Figure S4. Comparative fluorescence spectra of dimeric carbocyanine dye **1** with G-rich oligos, single stranded and duplex DNA in presence of 10 mM NaCl. Samples contained 2 μM of dye and DNA in 10 mM phosphate buffer (pH 7.0).

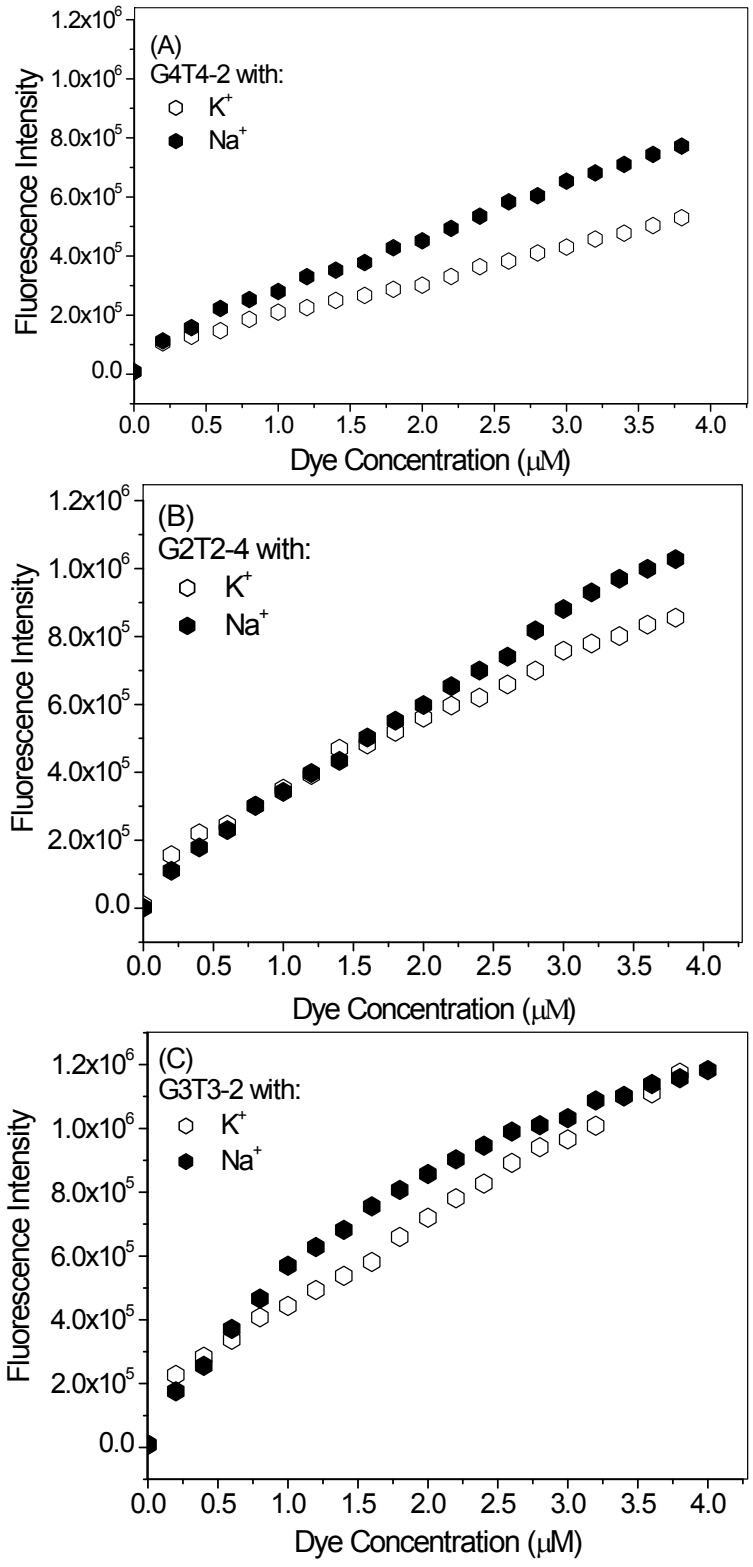


Figure S5. Fluorescence enhancement of **1** upon addition to (A) G4T4-2, (B) G2T2-4 and (C) G3T3-2, in presence of Na^+ (⬢) and K^+ (⬡). Samples contained 2 μM of oligonucleotides in 10 mM phosphate buffer pH 7.0.

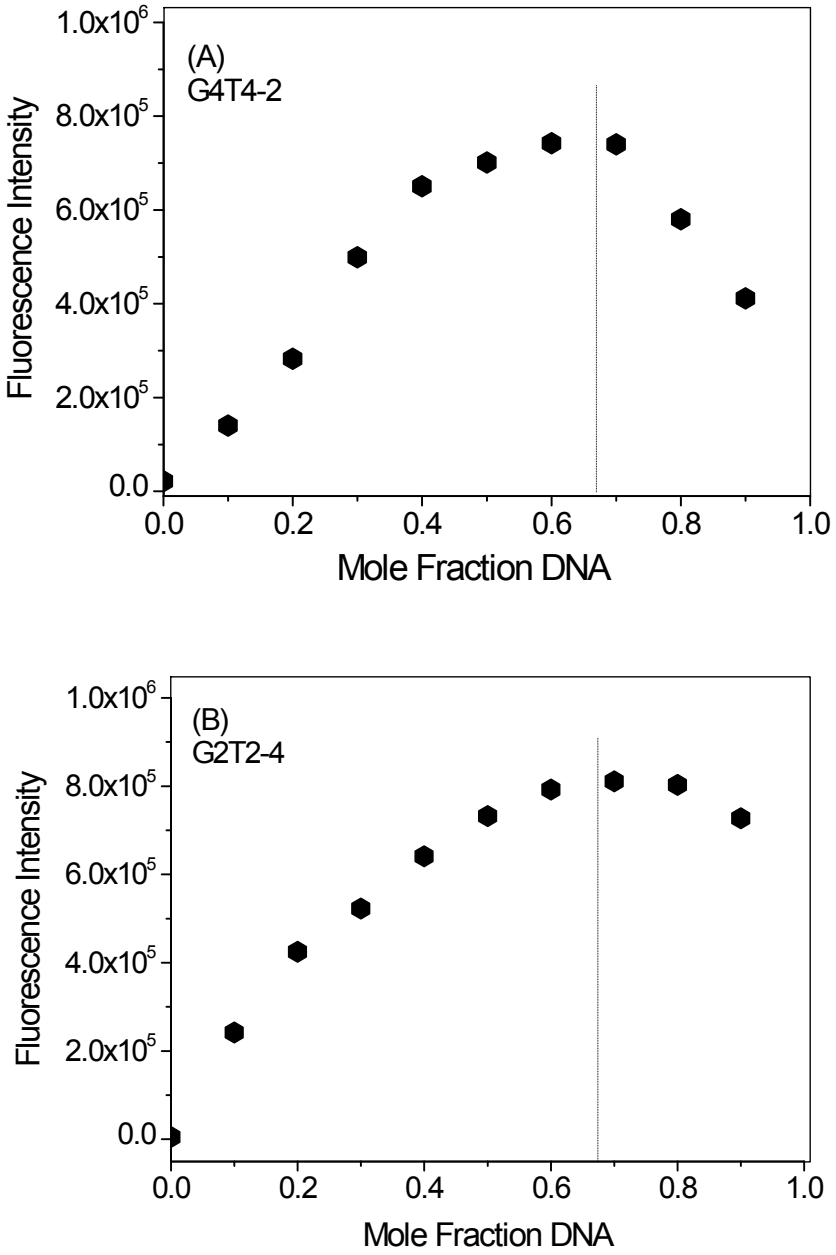


Figure S6. Job plot of dye **1** with (A) G4T4-2 and (B) G2T2-4, in 10 mM KCl.

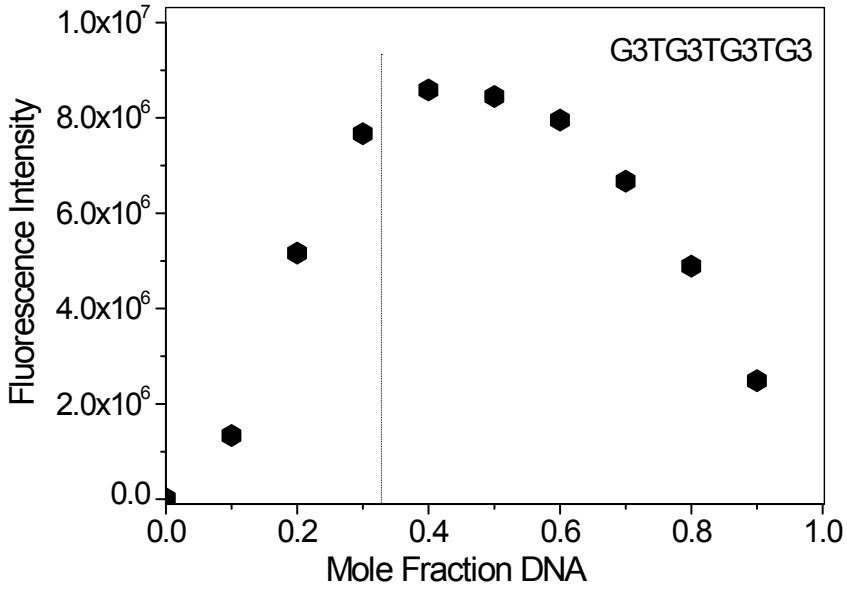


Figure S7. Job plot of dye **1** with G3TG3TG3TG3, in 10 mM KCl.

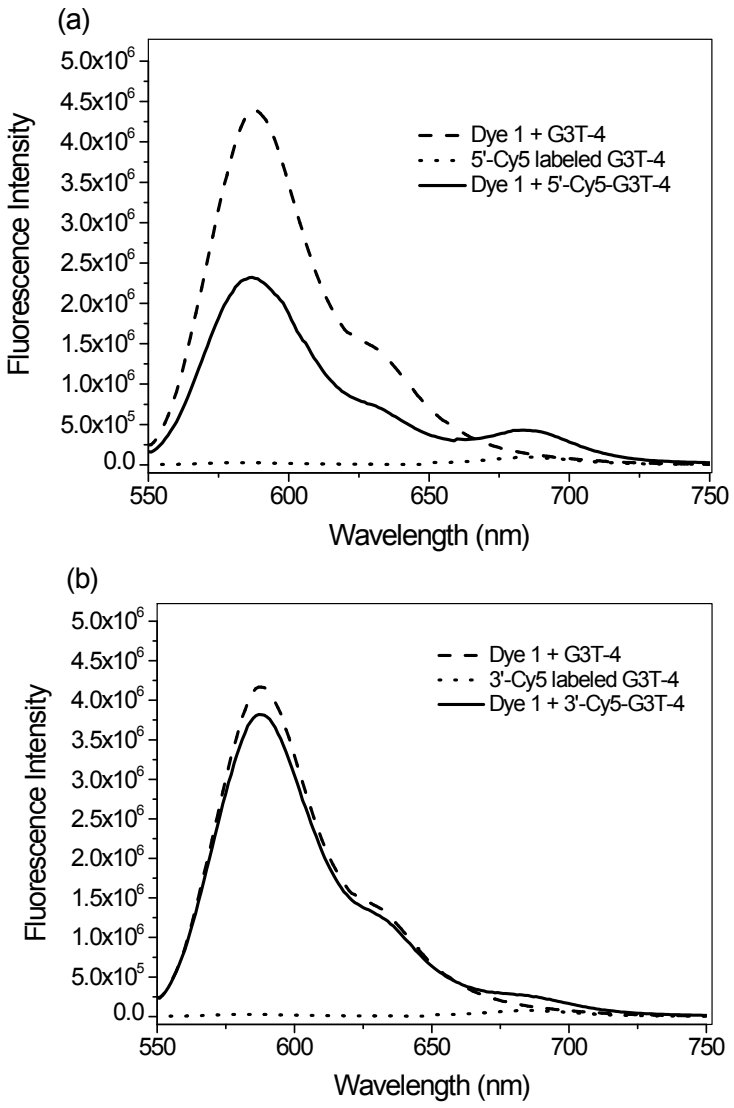


Figure S8. Fluorescence spectra of dye **1** with (a) 5'-Cy5 and (b) 3'-Cy5 labeled G3T-4. Samples contained 2 μM dye and total DNA in 10 mM phosphate buffer (pH 7.0) and 10 mM KCl. Samples containing labeled DNA comprised of 1.5 μM unlabeled G3T-4 and 0.5 μM 5' or 3'-Cy5 labeled G3T-4.

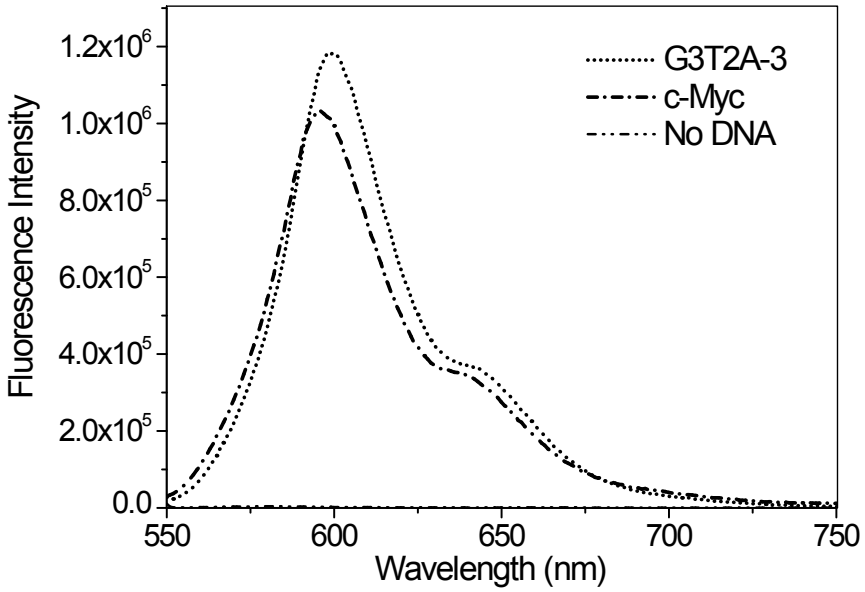


Figure S9. Comparative fluorescence spectra of dye **1** with telomeric sequence G3T2A-3 and oncogene promoter sequence c-Myc in presence of 10 mM NaCl. Samples contained 2 μM of dye and DNA in 10 mM phosphate buffer (pH 7.0).

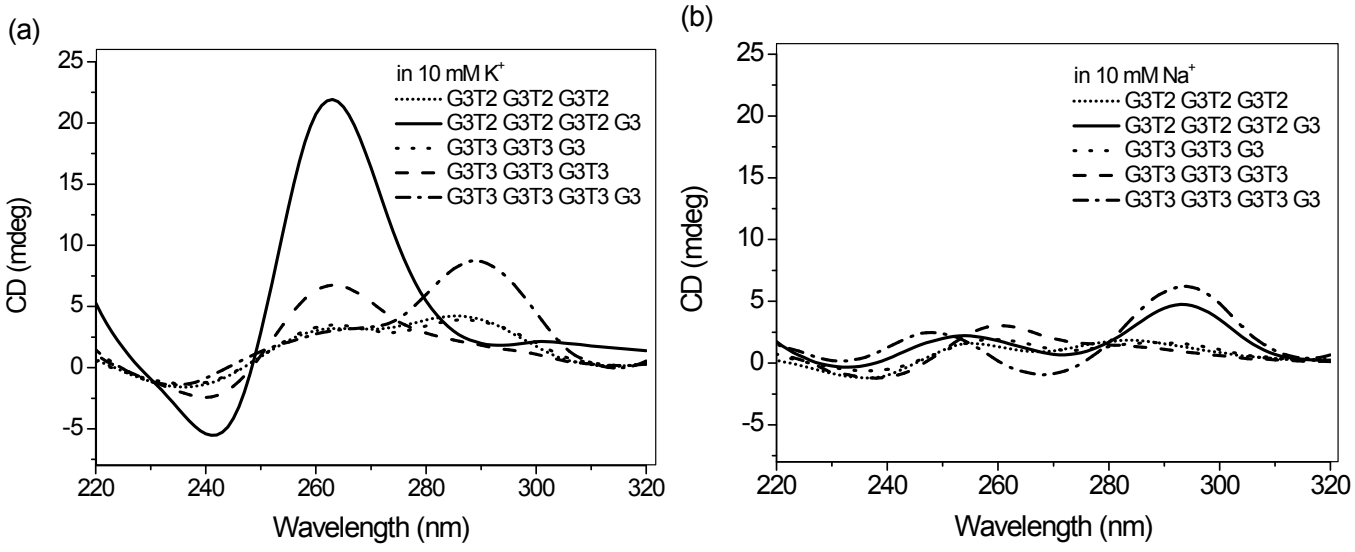


Figure S10. Comparative CD spectra of G3-containing oligonucleotides in (a) 10 mM KCl and (b) 10 mM NaCl. Samples contained 2 μM DNA in 10 mM phosphate buffer (pH 7.0).

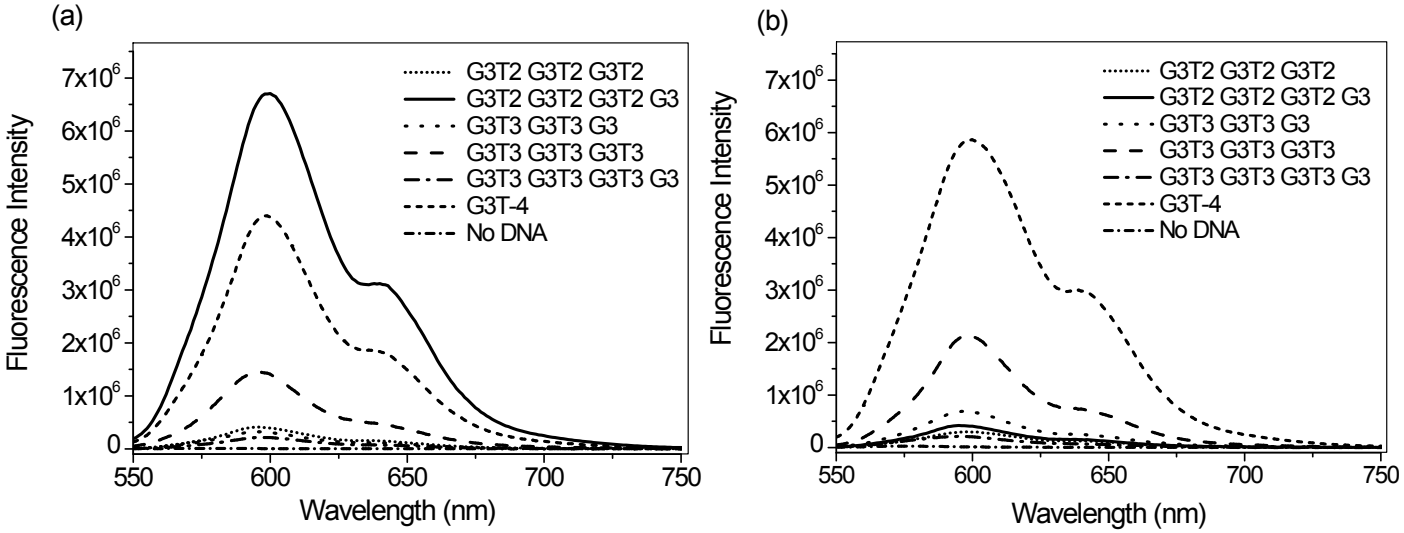


Figure S11. Comparative fluorescence spectra of dimeric carbocyanine dye **1** with G-rich oligonucleotides, in presence of (a) 10 mM KCl and (b) 10 mM NaCl. Samples contained 2 μM of dye and DNA in 10 mM phosphate buffer (pH 7.0).

Table S1. Association Constants of **1** for DNA structures

Name	K_a (M^{-1})	
	Na^+	K^+
G3T-4	1.3 (±0.2) x 10^6	6.8(±0.2) x 10^5
G4T4-2	3.4 (±0.7) x 10^5	3.1 (±0.5) x 10^5
G2T2-4	3.8 (±0.3) x 10^5	3.7 (±0.1) x 10^5
D	4.2 (±0.7) x 10^4	4.4 (±0.6) x 10^4

Table S2. Melting temperatures of DNA structures

Name	T_m (^oC)	
	Na^+	K^+
G3T-4	55.3 ± 0.7	> 90
G4T4-2	56.8 ± 0.6	> 90
G2T2-4	36.4 ± 0.3	47.5 ± 0.1
D	38.3 ± 0.3	46.5 ± 0.2

Table S3. Additional DNA Oligonucleotides used

Sl. No.	Name
1	5’-GGGTTGGGTTGGGTT -3’
2	5’-GGGTTGGGTTGGGTTGGG-3’
3	5’-GGGTTTGGGTTTGGG-3’
4	5’- GGGTTTGGGTTTGGGTTT -3’
5	5’-GGGTTTGGGTTTGGGTTTGGG-3’
6	5’-Cy5-GGGTGGGTGGGTGGGT-3’
7	5’-GGGTGGGTGGGTGGGT-Cy5-3’

Experimental:

Reagents for synthesis of dimeric dye **1** were purchased from Sigma Aldrich, Bangalore, India. Dye stock solutions were prepared in MeOH, preserved in dark and stored at 4 ^{0}C. Concentrations were determined spectrophotometrically in methanol using extinction coefficients, ε_{550} = 31800 M^{-1} cm^{-1}. ^{1}H NMR was recorded on a 500 MHz Bruker NMR instrument. Mass spectra were measured on a LC-MS from Waters using electrospray ionization technique. DNA oligonucleotides used in this work were purchased from Sigma Chemical Company (Bangalore, India). Absorbance measurements of oligonucleotides were used with extinction coefficients calculated by the nearest neighbor method to determine working concentrations of sequences. Experiments on two HPLC-purified oligonucleotides revealed no noticeable difference from DNA sequences that had not received additional purification. Samples containing 2μM DNA were prepared in 10mM phosphate buffer pH 7.0. The samples contained either 10mM NaCl or 10mM KCl and DNase-free water was used to make up the total volume to 1 mL. The G-rich oligo samples or DNA duplex were annealed by heating to ca. 90°C followed by gradual cooling to room temperature.

CD Spectroscopy: CD spectroscopy and CD-thermal denaturation of G-rich oligonucleotide samples was performed on a JASCO J-815 spectropolarimeter with Peltier system temperature controller. LabTemp software on the instrument was used to set up CD-thermal denaturation experiments, where absorbance at 260 and 295nm were recorded while increasing the temperature in 1 °C increments. Samples were held at each temperature for two minutes to allow for better equilibration.

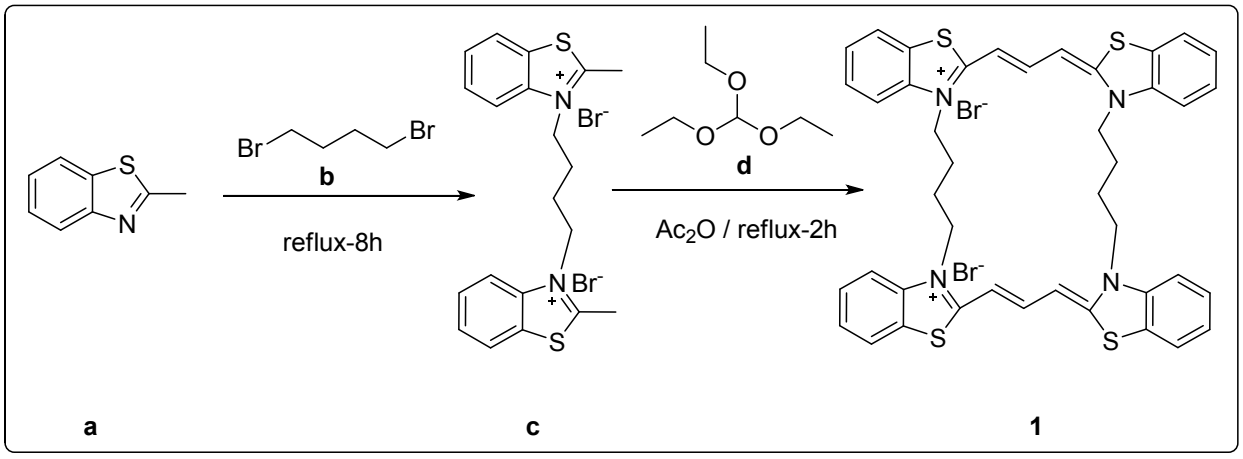
UV Spectroscopy: Experiments were performed on a JASCO V-750 spectrophotometer and all spectra were recorded at 25 °C. Samples contained 2 μM of Dye **1** and 2 μM of oligonucleotides in either 10 mM NaCl or 10 mM KCl in 10 mM phosphate buffer pH 7.0.

Fluorescence Studies: Dye **1** was added in 2.5μL aliquots to oligonucleotide samples for fluorescence studies. Emission spectra were recorded on a Horiba Fluorolog spectrofluorimeter by excitation at 540 nm. Instrument slit widths were controlled by the operating software and were kept consistent at 4 nm. All experiments were performed in quadruplets. Samples containing DNA oligos, used for titration of Dye **1** in fluorescence studies, were prepared in identical fashion for CD thermal denaturation and UV spectroscopic studies. For Job's plot,

samples contained fixed total amount of 4 μM of Dye **1** and oligonucleotides with varying proportions of each, in 10 mM KCl or 10 mM NaCl and 10 mM phosphate buffer (pH 7.0). For FRET experiments, three samples were prepared: (1) 2 μM dye 1 + 2 μM G3T-4; (2) 0.5 μM 5'-Cy5-G3T-4 (or 3'-Cy5-G3T-4) + 1.5 μM G3T-4; and (3) 2 μM dye 1 + 0.5 μM 5'-Cy5-G3T-4 (or 3'-Cy5-G3T-4) + 1.5 μM G3T-4. Emission spectra were recorded by excitation at 540 nm.

AFM Sample preparation: Ted Pella, 10mm dia, Mica substrates were used for preparing AFM samples. The substrates were cleaved with transparent adhesive tape. 20 μL of sample was deposited on the substrate and a spin coater was used at 3000 rpm for 30s to create a thin film. All AFM images were taken in ScanAsyst mode and analysed with NanoScope software.

Synthesis of Dimeric Carbocyanine Dyes:



3,3'-(butane-1,3-diyl)bis(2-methylbenzo[d]thiazol-3-ium) bromide (c) A solution of 2-Methylbenzthiazole (2 g, 13.42 mmol) and alkylating agent 1,4-dibromopropane (1.35 g, 6.71 mmol) was heated under reflux condition for 8 h. After cooling, diethyl ether was added and the desired bis quaternary salt was collected by filtration, washed several times, and dried overnight to afford **c** (3.8 g, 80%) as a white solid. ^{1}HNMR(DMSO-d_6, 500 MHz)δ (ppm) 8.47 (d, 2H), 8.42 (d, 2H), 7.91 (t, 2H), 7.82 (t, 2H), 4.78 (s, 4H), 3.25 (s, 6H), 2.09 (s, 2H). MS (ESI+) m/z 177.06 (M^{+}/2).

Synthesis of bichromophoric trimethine dye (1): 3,3'-(butane-1,3-diyl)bis(2-methylbenzo[d]thiazol-3-ium) bromide **c** (2.0 g, 5.8 mmol) and 3 ml of acetic anhydride were mixed and allowed to stir for 10 min. Then 3.4 g (23.2 mmol) of triethylorthoformate was added and refluxed for 2 h. The crude product was washed with ether and pure dye was obtained by recrystallization twice from methanol-isopropanol **1** as a solid (1.8 g, 45 %). ^{1}HNMR(DMSO-d_6, 500 MHz)δ (ppm) 8.05 (d, 4H), 7.92-7.84 (m, 6H), 7.63 (s, 4H), 7.47 (d, 4H), 6.80 (d, 4H), 4.35 (s, 8H), 2.00 (s, 8H).