Supplementary Information

c-di-GMP can form remarkably stable G-quadruplexes at physiological conditions in the presence of some planar intercalators

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General methods

Absorbance spectra were obtained on a JASCO V-630 spectrophotometer with 1 cm path length cuvette. Fluorescence studies were performed on a Varian Cary Eclipse Fluorescence spectrophotometer with 1 cm path length cuvette. CD experiments were performed on a JASCO J-81 spectropolarimeter with 1 cm path length cuvette. The concentration of a stock solution of c-di-GMP and each aromatic compound was determined by measuring the absorbance at 260 nm for c-di-GMP, 452 nm for Acriflavine, 445 nm for Proflavine, 550 nm for Pyronin Y, 665 nm for Methylene blue, 616 nm for Malachite Green, 421 nm for TMPyP, 590 nm for Crystal violet and 356 nm for Anthracene using 21,600 $M^{-1}cm^{-1}$, 46,800 $M^{-1}cm^{-1}$, 40,000 $M^{-1}cm^{-1}$, 117,000 $M^{-1}cm^{-1}$, 78,000 $M^{-1}cm^{-1}$, 148,900 $M^{-1}cm^{-1}$, 226,000 $M^{-1}cm^{-1}$, 87,000 $M^{-1}cm^{-1}$ and 9700 $M^{-1}cm^{-1}$ as a molar extinction coefficients respectively. Acriflavine was purchased from Fluka and used without any purification. Proflavine (3,6-diaminoacridine) hydrochloride salt, pyronin Y methylene blue hydrate, TMPyP (5,10,15,20-Tetrakis(1-methyl-4-pyridyl)-21*H*,23*H*-porphirine) tetra-*p*-tosylate salt were purchased from Sigma-Aldrich. Malachite green oxalate salt was purchased from MP Biomedicals Inc. Crystal violet was purchased from Alfa Aesar. Anthracene was purchased from ACROS organics. rGTP were purchased from Promega. cGMP (Guanosine 3',5'-cyclic mono phosphate) was purchased from CALBIOCHEM.

General preparation of sample before optical measurements

C-di-GMP, water, buffer solution (pH 7.5) and salt solutions were mixed, heated up to 95 ºC and kept at 95 ºC for 5 min, then cooled down to room temperature and kept at room temperature for 15 min. The aromatic intercalator was then added to the mixture and then incubated in the refrigerator at 4 ºC for overnight (~ 12 h).

Measurement of fluorescence

The instrument settings were chosen as follows for proflavine:
λ_{ex} = 445 nm (slit 5 nm), λem = 455 – 650 nm (slit 5 nm), for Acriflavine: λ_{ex} = 450 nm (slit 5 nm), λem = 460 – 650 nm (slit 5 nm), for Pyronin Y: λ_{ex} = 540 nm (slit 5 nm), λem = 552 – 700 nm (slit 5 nm). The measurements were carried out at 10 °C.

Circular dichroism experiments (CD)

The measurement was performed at 10 ºC. For G-quadruplex stability studies, the CD data were taken at temperatures ranging from 10 to 75 ºC. Data pitch: 1 nm, scan speed: 50 nm/min, response: 8 sec, band width: 1 nm.

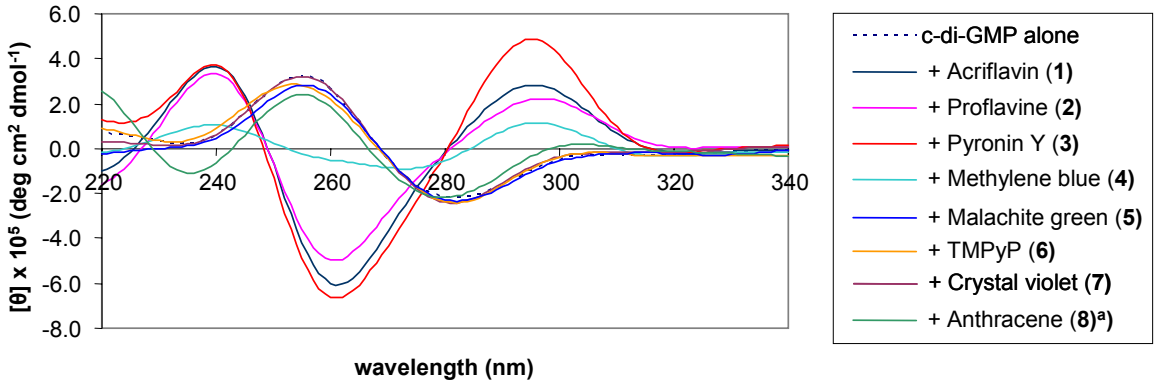


Figure S1a. CD spectra of c-di-GMP in the presence or absence of compounds **1**-**8**. 5 µM of intercalators **1**-**8** was added to 30 µM of c-di-GMP in Tris buffer containing K^+ (1 M) and the CD spectra taken at 10 ºC. [a)] Sample contained 2 % DMSO due to solubilize anthracene. DMSO does not accunt for the lack of G-quadruplex-induction by **8** because proflavine still induces G-quadruplex formation in c-di-GMP, even in the presence of 2 % DMSO (see SI, Figure S1b)

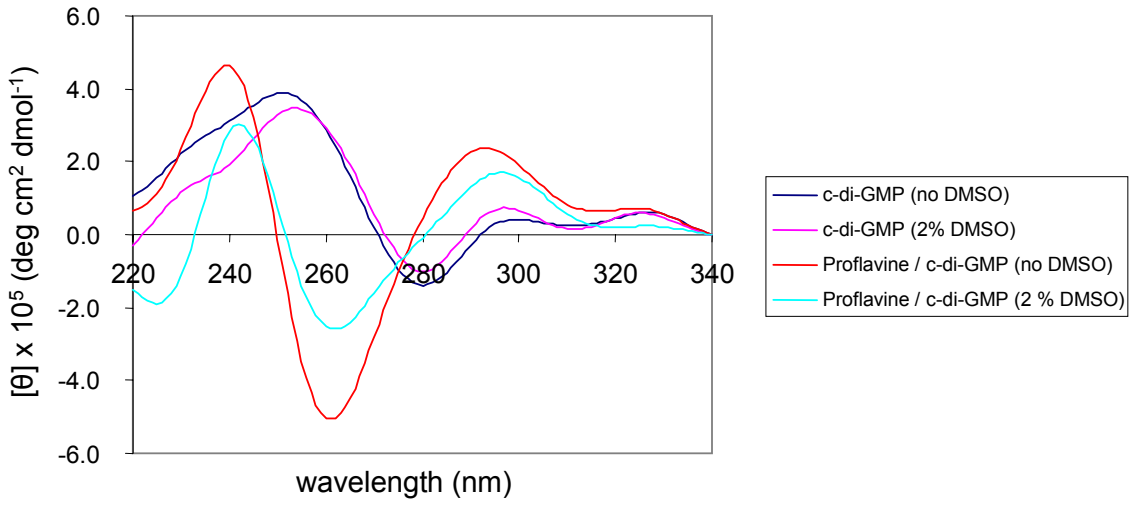


Figure S1b. The effect of 2 % DMSO on the CD spectra of c-di-GMP with proflavine. [proflavine] = 5 µM, [c-di-GMP] = 7.5 µM, Buffer: 10 mM Tris-HCl (pH 7.5) containing 250 mM KCl.

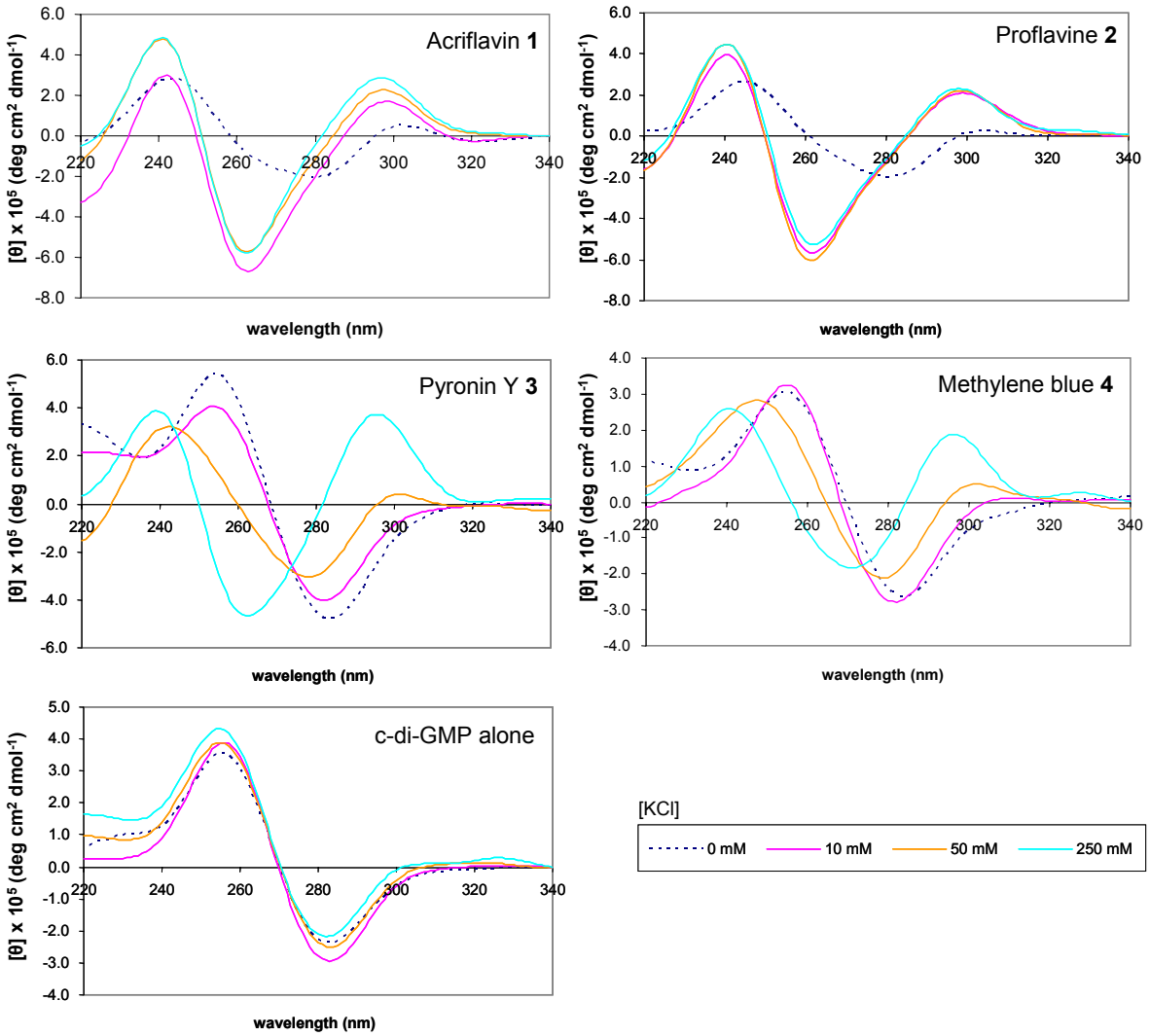


Figure S2. CD spectra of c-di-GMP in the presence of compounds **1**-**4** at various K^+ concentrations. Conditions: [c-di-GMP] = 30 µM; [intercalator] = 5 µM. Buffer is 10 mM Tris-HCl (pH 7.5, containing various concentrations of KCl at 10 ºC.

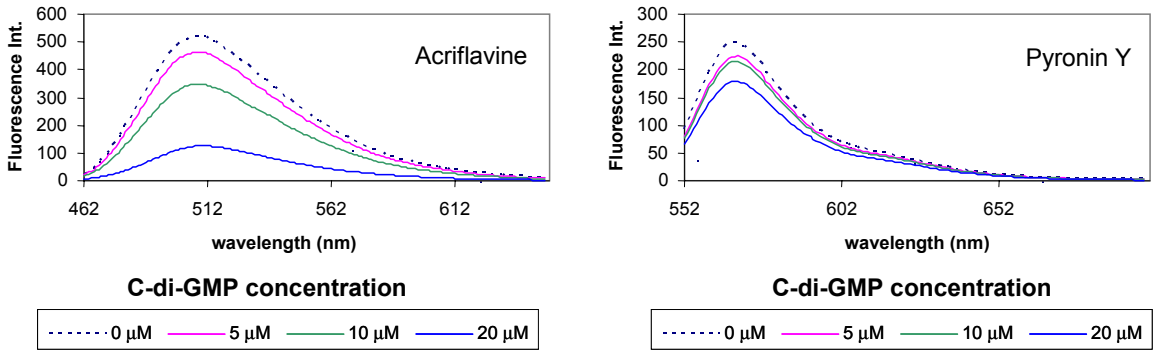


Figure S3. Fluorescence Intensities of acriflavine and pyronin Y in the absence or presence of c-di-GMP. Excitation wavelength for acriflavine was 450 nm and pyronin Y was 540 nm. [c-di-GMP] = 0-20 µM, [Aromatic intercalator] = 5 µM, buffer: 10 mM Tris-HCl (pH 7.5) containing 250 mM KCl.

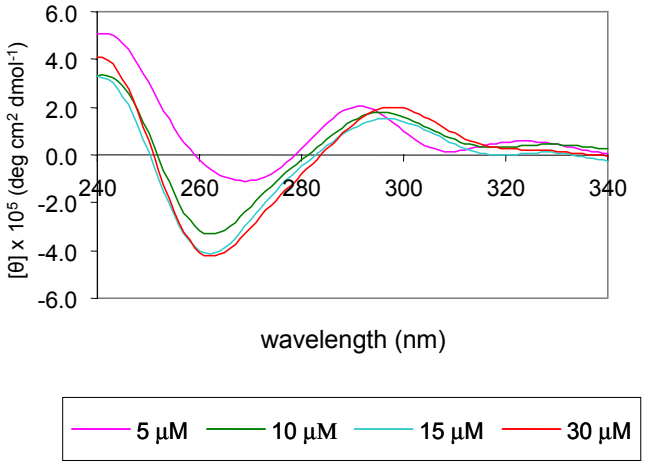
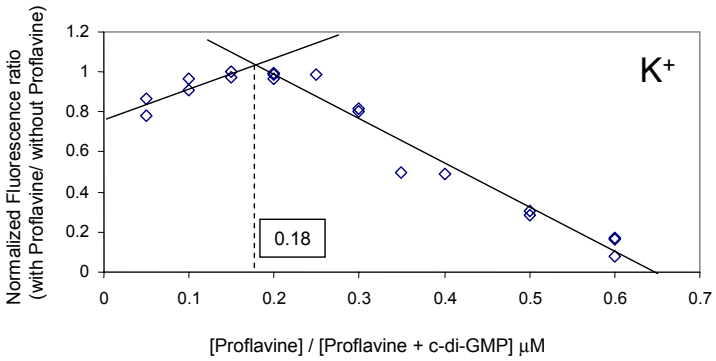


Figure S4. CD spectrum for different c-di-GMP concentrations added to proflavine (5 µM). Buffer: 10 mM Tris-HCl (pH 7.5) containing 250 mM KCl.



cation	Value of mole fraction of (**2**) matching with point of intersection	Stoichiometry between (**2**) and c-di-GMP
K^+	0.18	1 : 4.56

Figure S5. Job plot. Total [Proflavine] + [c-di-GMP] was fixed at 50 µM. Buffer: 10 mM Tris-HCl (pH 7.5) containing 250 mM KCl. Ex. 445 nm, Em. 508 nm.

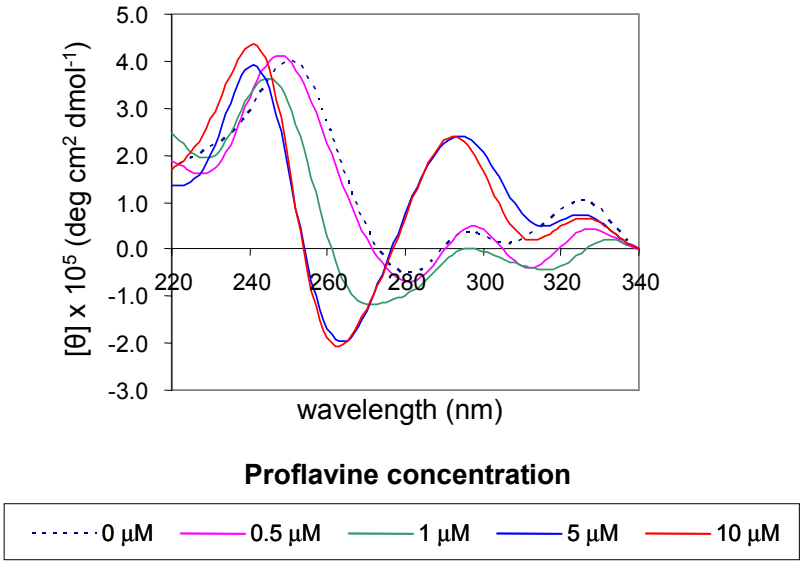


Figure S6. Addition of increasing concentrations of proflavine to c-di-GMP (fixed concentration at 7.5 µM). Buffer: 10 mM Tris-HCl (pH 7.5) containing 250 mM KCl.