Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information For:

A comparative study of recognizing G-quadruplex by dimeric cyanine dyes with different size of aromatic substituents

Lijia Yu,[ab] *Qianfan Yang,**[a] *Junfeng Xiang,*[a] *and Yalin Tang**[a]

[a] *Beijing National Laboratory for Molecular Sciences, Center for Molecular Science, State Key Laboratory for Structural Chemistry for Unstable and Stable Species, Institute of Chemistry, Chinese Academy of Sciences (ICCAS), Beijing, 100190, P.R. China.*

[b] *University of Chinese Academy of Sciences, Beijing, 100049, P.R. China.*

E-mail: tangyl@iccas.ac.cn and yanggf@iccas.ac.cn.

1. Synthesis.

B-P4:1,14-[bis-[2-[3′-[3″-(3‴-sulforpropyl)-benzothiazolium-2″-ylidene]prop-2′-en-1′-yl]-3H-benzothiazolium]]-3,6,9,12-tetraoxo-tetradecane (**B-P4**): yield 36%. ^{13}C NMR: (500 MHz, DMSO-d_6) δ 165.7, 146.7, 142.1, 141.7, 131.7, 128.3, 127.9, 125.5, 125.3, 124.6, 123.3, 123.2, 117.9, 114.4, 99.6, 70.9, 70.3, 68.3, 65.5, 50.6, 48.4, 46.21. HR-MS (MALDI): Calcd for 1085.2056(M+Na)$^+$, Found 1085.2060 ($C_{50}H_{54}N_4O_{10}S_6Na^+$).

TC-P4:1,14-[bis-[2-[3′-[3″-(3‴-sulforpropyl)-β-naphthothiazolium-2″-ylidene]prop-2′-en-1′-yl]-3H-benzothiazolium]]- 3, 6, 9, 12-tetroxo-tetradecane. yield 38.9 %. ^{1}H NMR (DMSO-d_6): δ 8.55 (d, *J* = 8.2 Hz, 1H), 7.89 (d, *J* = 8.3 Hz, 1H), 7.81 (s, 1H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.66 (s, 1H), 7.53 (s, 1H), 7.48 (t, *J* = 12.5 Hz, 2H), 7.32 (s, 1H), 7.09 (s, 1H), 6.68 (d, *J* = 13.2 Hz, 1H), 6.39 (d, *J* = 12.7 Hz, 1H), 4.73 (s, 2H), 4.32 (s, 2H), 3.79 (s, 2H), 3.55 (s, 2H), 3.45 (s, 2H), 3.41 (s, 2H), 2.80 (s, 2H), 2.29 (s, 2H). ^{13}C NMR (500 MHz, DMSO-d_6, 80℃): δ 146.1, 130.2, 128.8, 128.1, 127.6, 127.2, 125.1, 123.1, 122.3, 119.8, 114.1, 100.3, 98.9, 71.0, 70.4, 70.3, 68.0, 50.2, 48.5, 47.1. HR-MS (MALDI): Calcd for 1185.2369 (M+Na)$^+$, Found 1185.2361($C_{58}H_{58}N_4O_{10}S_6Na^+$).

AB-P4:1,14-[bis-[2-[3′-[3″-(3‴-sulforpropyl)-β-naphthothiazolium-2″-ylidene]prop-2′-en-1′-yl]-3H-α-napthiazole]]-3,6,9,12-tetraoxo-tetradecane: yield 16%. ^{1}H NMR (500 MHz, CD_3OD) δ 7.06 (s, 1H), 6.25 (s, 1H), 6.18 (s, 1H), 5.85 (s, 1H), 5.51 (d, J = 12.8 Hz, 1H), 5.30 (d, J = 11.5 Hz, 1H), 3.07 (s, 2H), 2.06 (s, 2H). ^{13}C NMR (500 MHz, CD_3OD): δ 163.5, 163.4, 144.0, 138.5, 134.5, 132.6, 129.0, 128.8, 128.1, 127.3, 127.2, 126.0, 125.1, 122.1, 121.5, 120.2, 119.5, 117.7, 111.3, 98.4, 97.3, 70.5, 69.2, 67.7. HR-MS (MALDI): Calcd for 1285.2612 (M+Na)$^+$, Found 1285.2683 ($C_{66}H_{62}N_4O_{10}S_6Na^+$).

2. The affection of solution polarity to B-P4, TC-P4 and AB-P4.

Fluorescence spectra were carried out on a Hitachi F-4500 spectrophotometer in a 10 mm quartz cell at room temperature. The excitation wavelength for B-P4 was 510 nm, TC-P4 at 537 nm and AB-P4 at 550 nm. The excitation and emission slits were 10 mm, the voltage were 400 V for B-P4, TC-P4 and AB-P4 with a scan speed of 1200 nm•min^{-1}.

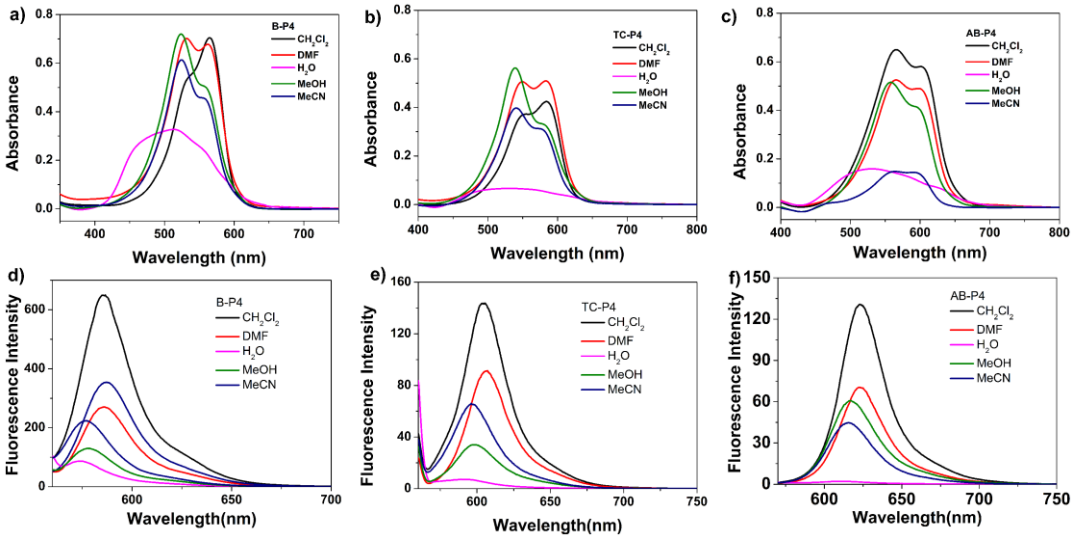

Figure S1. The absorption spectra of 10 μM B-P4 (a), TC-P4 (b) and AB-P4 (c) in the presence of dichloromethane (CH_2Cl_2), dimethyl formamide (DMF), H_2O, methanol (MeOH), acetonitrile (CH_3CN).The fluorescence intensity of 10 μM B-P4 (a), TC-P4 (b) and AB-P4 (c) in the presence of CH_2Cl_2, DMF, H_2O, MeOH, CH_3CN.

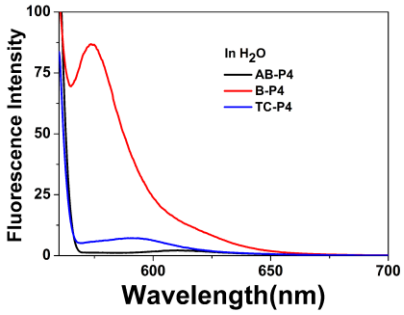

Figure S2. The comparison of fluorescence intensity of 10 μM B-P4, TC-P4 and AB-P4 in the presence of H_2O.

3. **The Structure identification of 9 sequences by CD spectroscopy.**

All the CD spectra were recorded on a JASCO J-815 spectrophotometer in a 10 mm path-length quartz cell from 200 nm to 320 nm at room temperature.

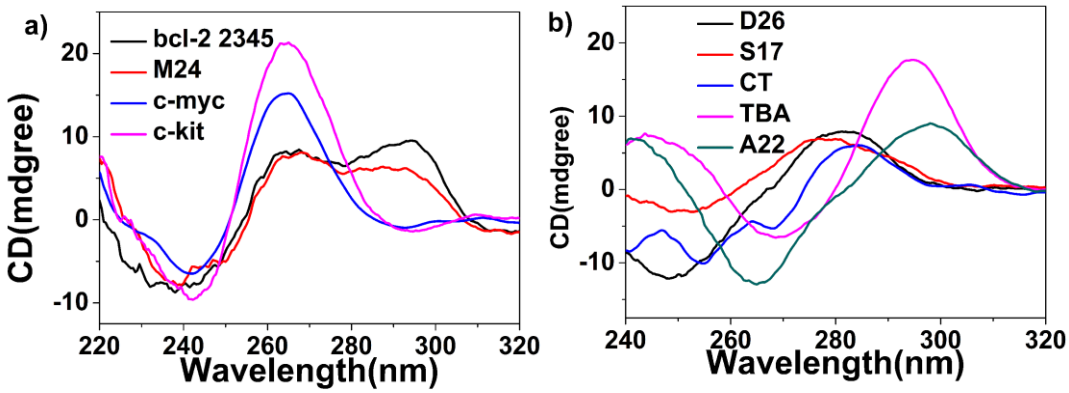

Figure S3. The CD spectra for c-myc, c-kit1, bcl-2 2345, M24, A22, TBA, CT, D26 and S17 in 10 mM PBS (K^+), and A22 in 10 mM PBS (Na^+). The sequences of c-myc, c-kit1 form intermolecular parallel G4s in PBS (K^+). bcl-2 2345 and M24 could form hybrid G4s in PBS (K^+). TBA present antiparallel G4s in PBS (K^+). A22 also showing antiparallel G4s signals in 10 mM PBS (Na^+).

4. **The fluorescence titration of dyes with different DNA motifs.**

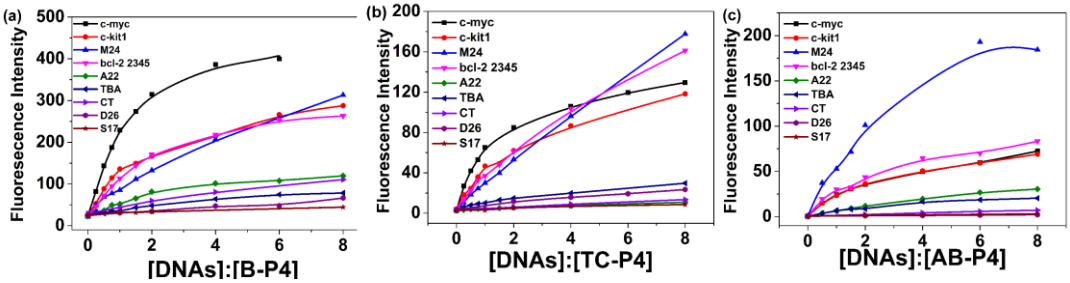

Figure S4. Fluorescence titration spectra of 5 μM B-P4, TC-P4 and AB-P4 with different concentration of DNA motifs in PBS.

5. **The absorption titration of AB-P4 with bcl-2 2345.**

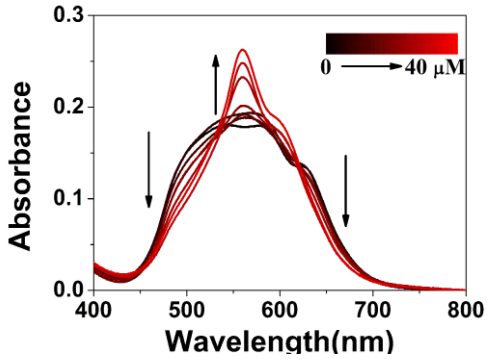

Figure S5. The absorption titration of AB-P4 (5 μM) in the various concentration of bcl-2 2345, 0 μM, 2.5 μM, 5 μM, 7.5 μM, 10 μM, 20 μM, 30 μM and 40 μM.

6. The ^{1}H-NMR titration of dyes with different DNA motifs.

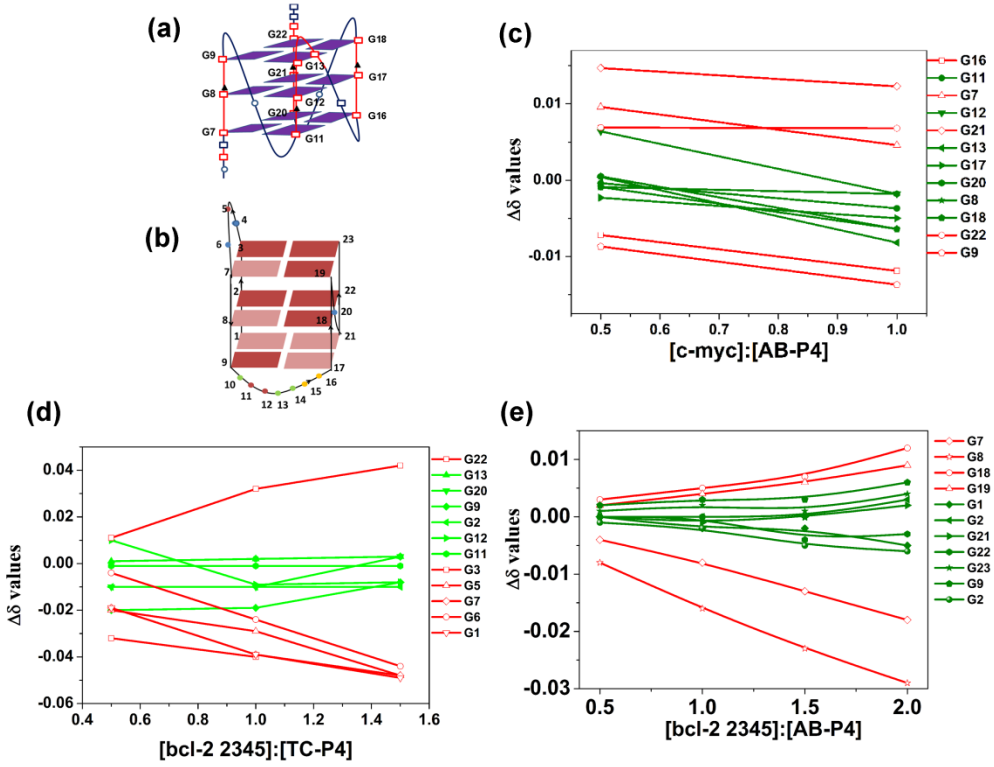

Figure S6. The NMR-based folding topologies of the c-myc (a) and bcl-2 2345(b). Variation trajectories for chemical shifts (Δδ) of protons of imino region (c) on c-myc when c-myc is titrated with AB-P4 in the in 0.6 mL PBS (10 mM K_2PO_4/KH_2PO_4, 70 mM KCl, 1 mM EDTA, pH 7.4, H_2O/D_2O(9/1, v/v)), or on bcl-2 2345 when bcl-2 2345 is titrated with TC-P4(d) or AB-P4 (e). Δδ values are calculated by chemical shifts of each c-myc/AB-P4 ratio minus those of c-myc, bcl-2 2345/TC-P4 or bcl-2 2345/AB-P4 ratio minus those of bcl-2 2345. Solid red lines indicate those protons shifting dramatically.