Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

pH Dependent Multifunctional and Multiply-configurable Logic Gate Systems Based on Small Molecule G-quadruplex DNA Recognition

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1.0 General Information: Thiazole Orange, Tris.HCl, KCl and *c-kit2* quadruplex sequence d[GGGCGGGCGCGAGGGAGGGG] were purchased from Sigma-Aldrich. The *c-kit2* DNA oligonucleotide was annealed in filtered and degassed buffer (100 mM KCl, Tris-HCl 10 mM) by heating at 95 °C for 5 min followed by cooling to room temperature. PBIA **1** was prepared using our previously reported synthetic procedure.[1] All binding reactions were conducted at room temperature in 100 mM KCl, Tris-HCl 10 mM. pH values were obtained by direct measurement of each sample by a pH meter using a micro pH electrode.

2.0 Fluorimetric titration:[2] The fluorescence spectra were recorded on a Horiba Jobin Yvon Fluoromax 3 instrument at 25 °C in a thermostated cell holder using quartz cuvette with a 1 cm path-length and each titration was performed using filtered and degassed buffer (100 mM KCl, Tris-HCl 10 mM). All the binding constants of fluorescence spectral data have been calculated using the follwing equations (1-3) with the help of Graphpad prism 5.0 (GraphPad Software, San Diego, CA). In this experimental design, aliquots of preannealed *c-kit2* quadruplex d[GGGCGGGCGCGAGGGAGGGG] was added to a single concentration of the ligand, and scanned after each addition. The equations[3] are:

$$LR = (X + L_{tot} + K_d) - \frac{\sqrt{(X+L_{tot}+K_d)^2 - 4\times X\times L_{tot}}}{2} \qquad (1)$$

$$L = L_{tot} - LR \quad (2)$$

$$Y = BKG + MF \times L + FR \times MF \times LR \ (3)$$

L_{tot}: Total ligand concentration (same units as X);

[1] J. Dash, P. S. Shirude and S. Balasubramanian, *Chem. Commun.*, 2008,**44**, 3055.

[2] R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd *Ed.*, Kluwer Academic/Plenum Publishers, New York, 1999.

[3] H. J. Motulsky, R. R. Neubig, *Curr. Protoc. Neurosci*. *Chapter 7*: unit 7.5, 2010.

BKG: Background fluorescence w/o receptor (Same units as Y);

MF: Molar fluorescence of free Ligand (Y units divided by X units);

X: Concentration of receptors;

K_d: Dissociation constant (X units);

FR: Fluoresence ratio; MF of bound ligand $= FR * MF$ (unitless ratio); FR>1 means binding causes turn on; FR< 1 means binding causes turn off .

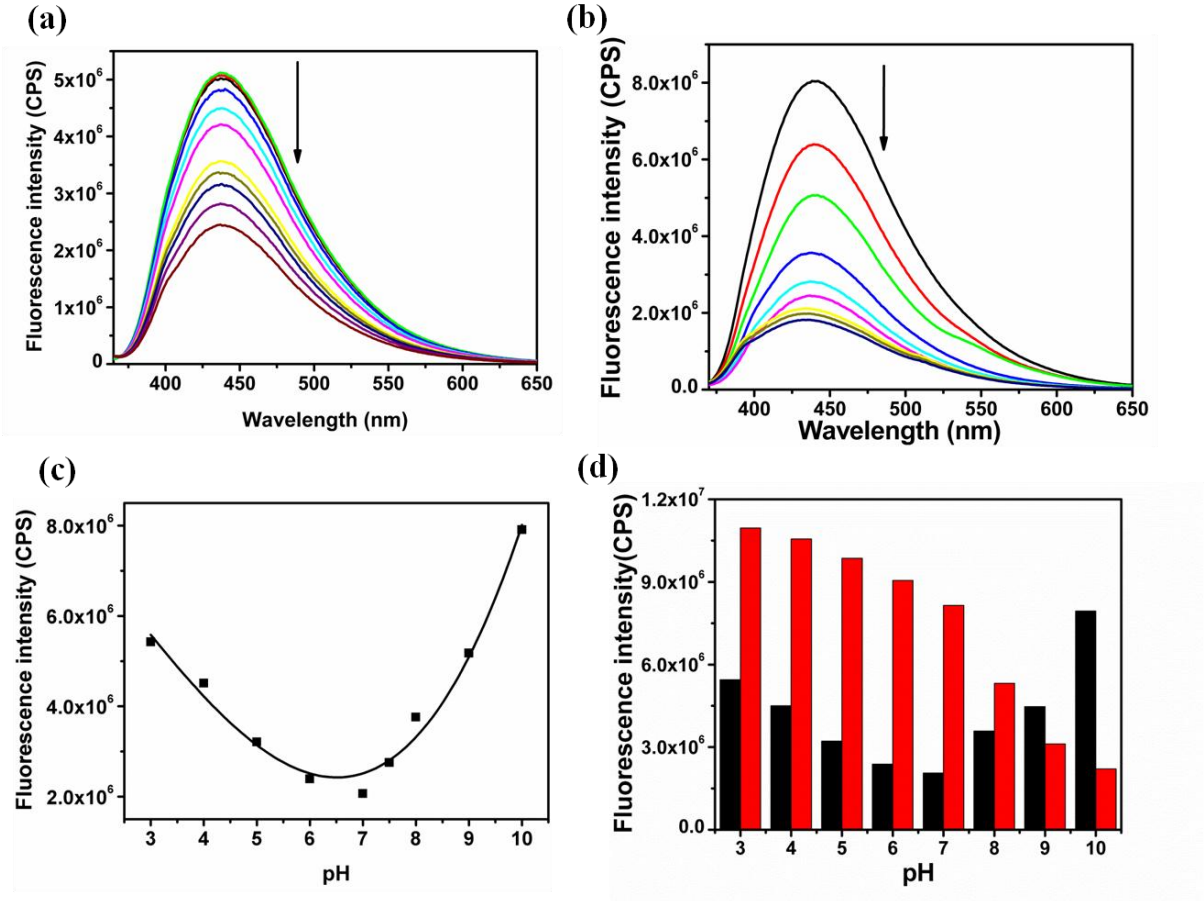

Figure S1. (a) pH dependent fluorescence profile of **1** (1 μM). (b) Fluorescence spectra of **1** (2.5 μM) upon progressive addition of *c-kit2* quadruplex (0-8 μM) at pH 7.4. (c) pH dependent fluorescent profile of **1** (2.5 μM) and *c-kit2* (6 μM). (d) A bar representation of the fluorescent response of **1** (2.5 μM) in the absence (red bar) and presence (black bar) of *c-kit2* (6 μM) at different pH.

Fluorimetric titration of 1 with *c-kit2* at pH 7.4

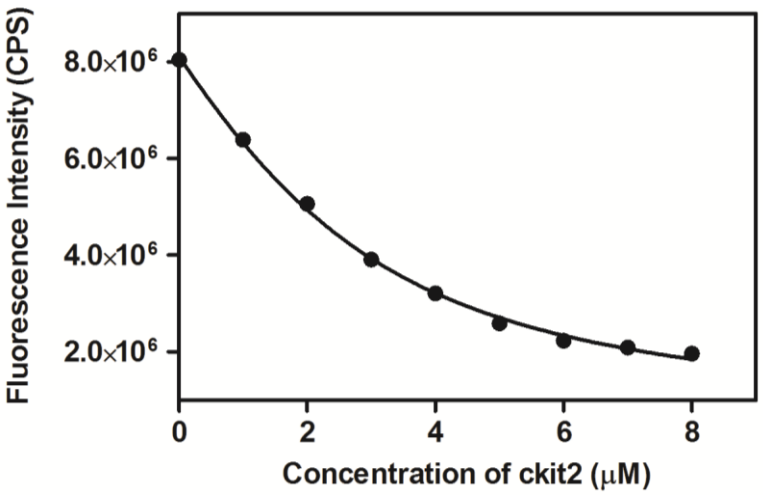

Figure S2. Analysis of data at 440 nm from the titration of **1** (2.5 μM) with *c-kit2* quadruplex (0-8 μM) in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 7.4 using equation (1-3); K_d = 1.39 μM (r^2 = 0.9983). The fluorimetric titrations of **1** were performed at 350 nm excitation wavelength and data were colected between 360 nm and 650 nm at 0.2 nm/s; 5 nm slit width; *T* = 25 °C.

Fluorimetric titration of 1 with *c-kit2* at pH 9.0

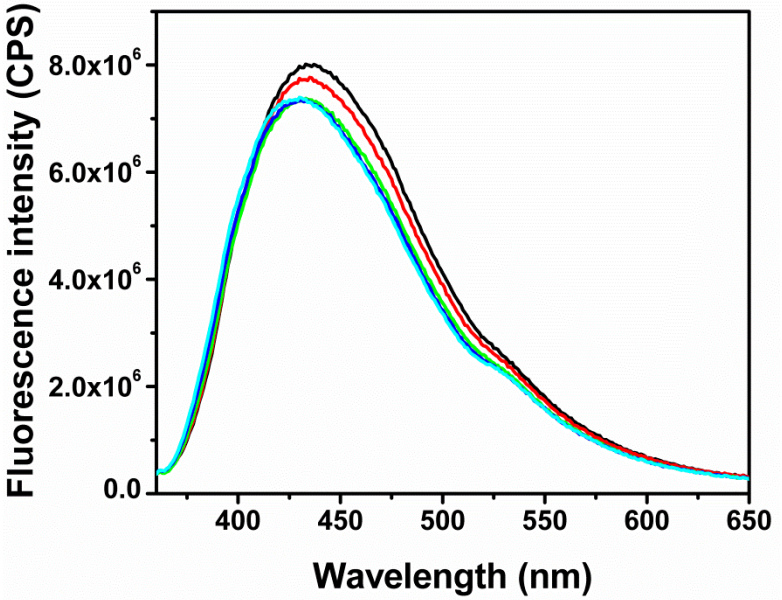

Figure S3. Fluorimetric titration spectra of **1** (2.5 μM) with *c-kit2* quadruplex (0-10 μM) in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 9. The fluorimetric titrations of **1** were performed at 350 nm excitation wavelength and data were colected between 360 nm and 650 nm at 0.2 nm/s; 5 nm slit width; *T* = 25 °C. A slight decrease in the fluorescent intensity was observed.

Fluorimetric titration of 2 with *c-kit2* at pH 7.4[3]

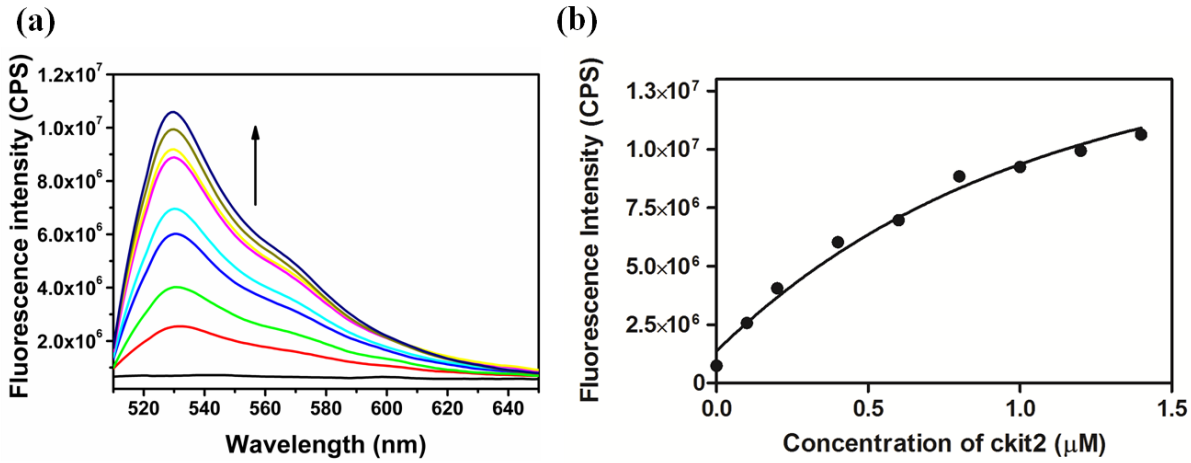

Figure S4. (a) Fluorimetric titration spectra of **2** (0.5 μM) with *c-kit2* quadruplex (0-1.4 μM**)** in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 7.4. The fluorimetric titrations of **2** were performed at 501 nm excitation wavelength and data were colected between 510 nm and 650 nm at 0.2 nm/s; 5 nm slit width. (b) Analysis of data from the titration of **2** with *c-kit2* using equation (1-3); K_d = 0.8 μM (r^2 = 0.9873).

The dissociation binding constant (K_d) of **2** with *c-kit2* was obtained as 0.8 μM, which is comparable with the dissociation binding constant (K_d) = 1.0 μM (r^2 = 0.9873) reported in the literature.[4]

Fluorimetric titration of 2 with *c-kit2* at pH 9.0

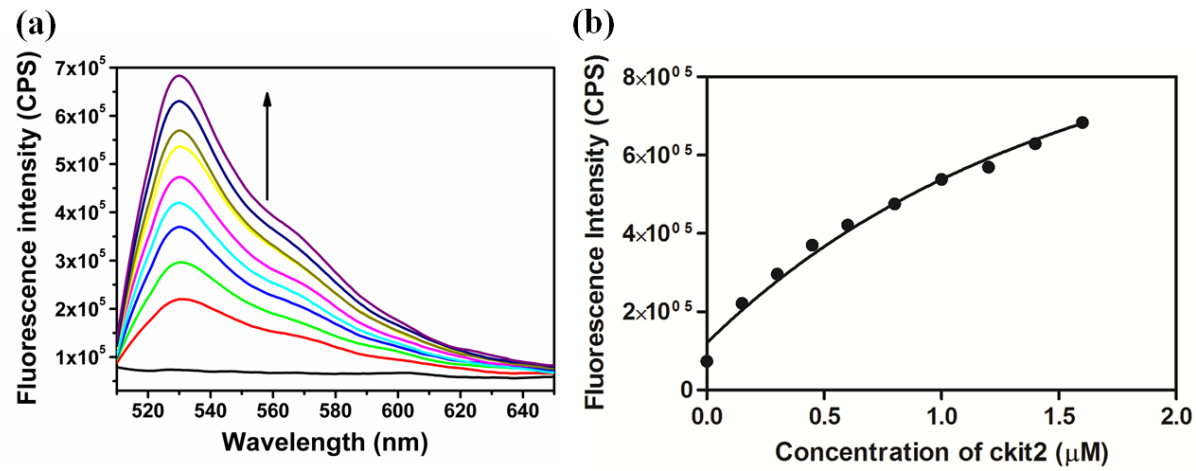

Figure S5. Fluorimetric titration spectra of **2** (0.5 μM) with *c-kit2* (0-1.6 μM) quadruplex in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 9. The fluorimetric titrations of **2** were performed at 501 nm excitation wavelength and data were colected between 510 nm and 650 nm at 0.2 nm/s; 5 nm slit width; T = 25 °C. (b) Analysis of data from the titration of **2** with *c-kit2* using equation (3); K_d = 1.5 μM (r^2 = 0.9871).

[4] E. Largy, F. Hamon and M. P. Teulade-Fichou, *Anal Bioanal Chem.* 2011, **400**, 3419.

Fluorimetric titration of 1 with 2 at pH 7.4

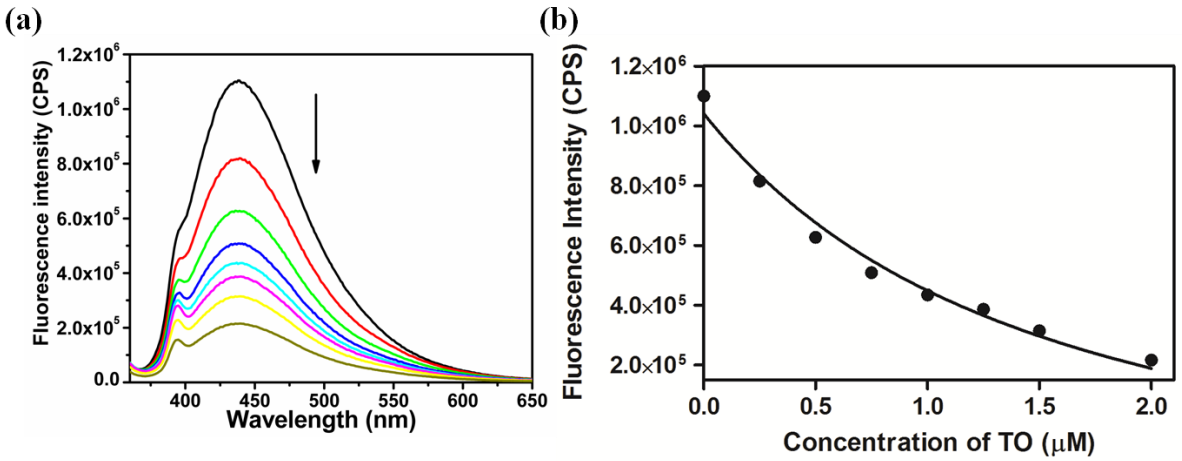

Figure S6. Fluorimetric titration spectra of 0.25 μM **1** with **2** (0 to 2 μM) in water at pH 7.4. The fluorimetric titrations of **1** were performed at 350 nm excitation wavelength and data were colected between 360 nm and 650 nm at 0.2 nm/s; 5 nm slit width; T = 25 °C. (b) Analysis of data from the titration of **1** with **2** using equation (3); K_d = 1.3 μM (r^2= 0.9834).

Fluorimetric titration of 1 with 2 at pH 9.0

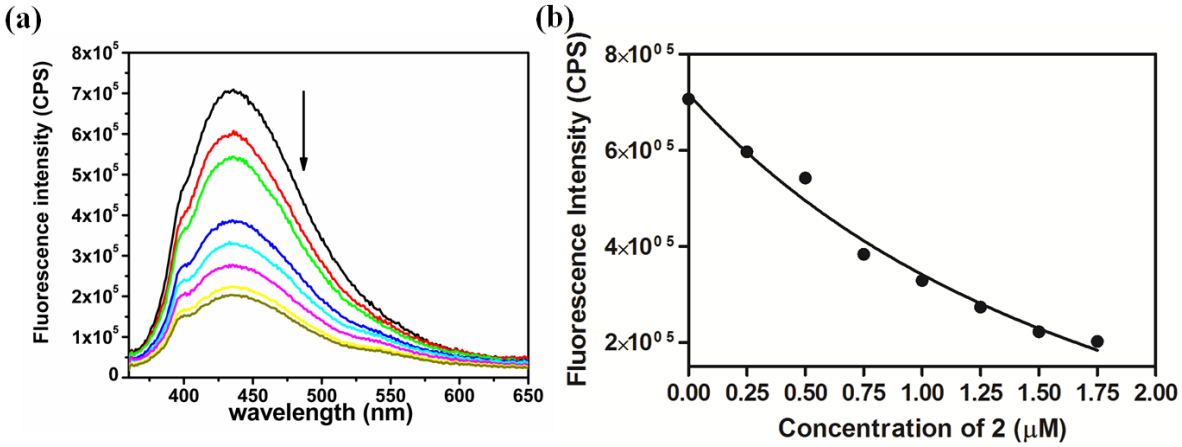

Figure S7. (a) Fluorimetric titration spectra of **1** (0.25 μM) with **2** (0-1.75 μM) in Water at pH 9.0. (b) Analysis of data from the titration of **1** with **2** using equation (3); K_d = 1.90 μM (r^2 = 0.9850).

Fluoremetric titration of ligands 1 and 2 with increasing amount of *c-kit2*

The fluorescence spectra of **1** (0.25 µM) and **2** (0.5 µM) was measured from 360 nm to 650 nm where the λ_{ex}=350 nm. The fluorescence spectra were recorded after each addition of *c-kit2* (0.05 - 1 µM). Upon addition of *c-kit2* the peak of ligand **1** 440 nm was quenched and the peak of ligand **2** at 540 nm was increased (Figure S8).

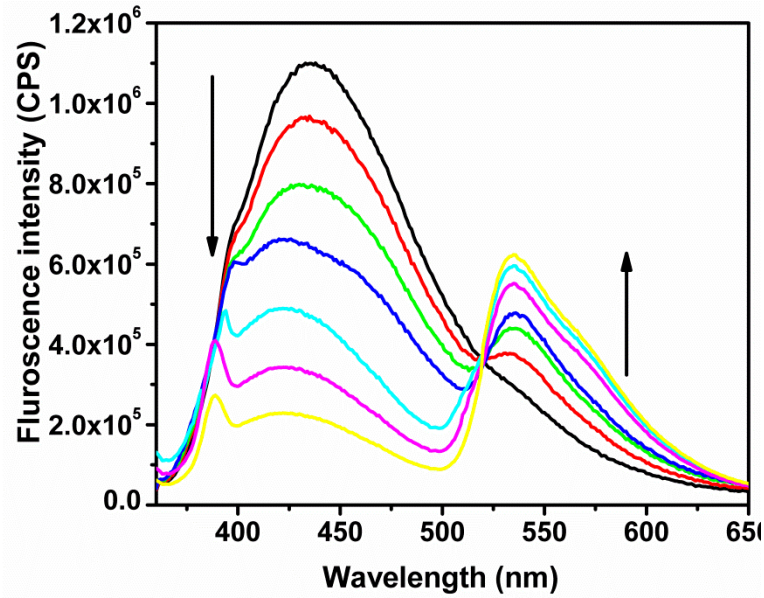

Figure S8. Fluorimetric titration spectra of **1** (0.25 µM) and **2** (0.5 µM) with *c-kit2* quadruplex (0-1.0 µM**)** in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 7.4.

Fluoremetric tritartion of ligands 1 and 2 in the presence of *c-kit2* as a function of pH

The fluorescence spectra of **1** (0.5 µM) and **2** (0.5 µM) in the presence of *c-kit2* quadruplex (0.5 µM) was measured from 360 nm to 650 nm (λ_{ex}=350 nm). The pH of the solution was gradually increased from 7.4 to 9.0 and the corresponding fluorescence spectra were recorded (Figure S9a). In the presence of *c-kit2* quadruplex, the peak of ligand **1** at 440 nm was quenched (turn-off) and the peak of ligand **2** at 540 nm was increased (turn-on) at pH 7.4. Upon gradual increase in pH, the intensity of ligand **1** at 440 nm was increased and that of the ligand **2** at 540 nm was decreased. Notably, the intensity at 440 nm was not as high as that of the PBIA **1** only (Figures 3(a), 4(a) and S13) and the intensity of ligand **2** was also decreased (compared to the peak intensity of **2** at pH 7.4 in the presence of *c-kit2*, Figures 3(a), 4(a) and S13) due to the structural change of *c-kit2* quadruplex at basic pH. However at the Fl_{540}

output, the peak of TO (**2**) was found to be the most intensified peak and hence it was taken as 1 (Figure 4, Figure S13).

Then reverse titrations were carried out by gradually decreasing the pH of the solution from 9.0 to 7.4 (Figure S9b). The pH dependence was found to be fully reversible and hence a gradual "turn on" at 540 nm (TO **2**) and a gradual "turn off" at 440 nm (PBIA **1**) were observed. The results indicated that the established logic gates are reversible and also switchable as a function of pH.

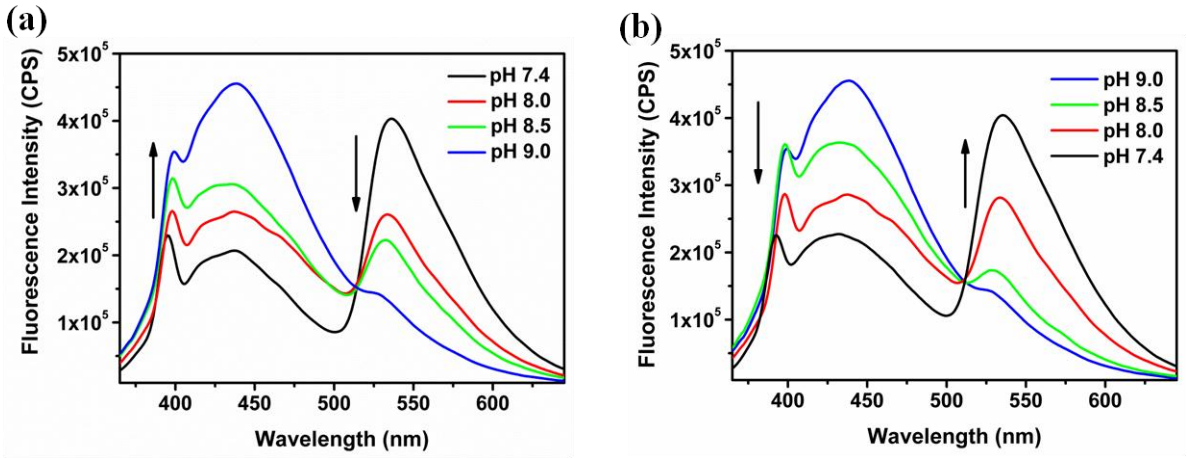

Figure S9. Fluorescent profile of PBIA **1** (0.50 µM) and TO **2** (0.50 µM) in the presence of *c-kit2* (0.50 µM) upon gradual (a) increase in pH from 7.4 to 9.0 and (b) decrease in pH from 9.0 to 7.4.

3.0 Fluorescence Intercalator Displacement (FID) assay

The FID assay was performed using literature procedure.[5] 0.25 µM of pre-folded *c-kit2* quadruplex was mixed with thiazole orange (0.50 µM, TO, **2**). Ligand **1** was added to the mixrure stepwise with a 3-min equilibration period and the fluorescence spectrum is recorded. The percentage of displacement is calculated from the fluorescence area (FA, 510-700 nm, λ_{ex} = 501 nm), using

[5] D. Monchaud, C. Allain and M. P. Teulade-Fichou, *Bioorg. Med. Chem. Lett.*2006, **16**, 4842.

$$\text{Percentage of TO displacement} = 100 - \left[\frac{\text{FA}}{\text{FA}_0} * 100\right] \qquad (4)$$

FA_0 being the fluorescence area of TO bound to DNA without added ligand. The percentage of displacement is then plotted as a function of the concentration of added ligand.

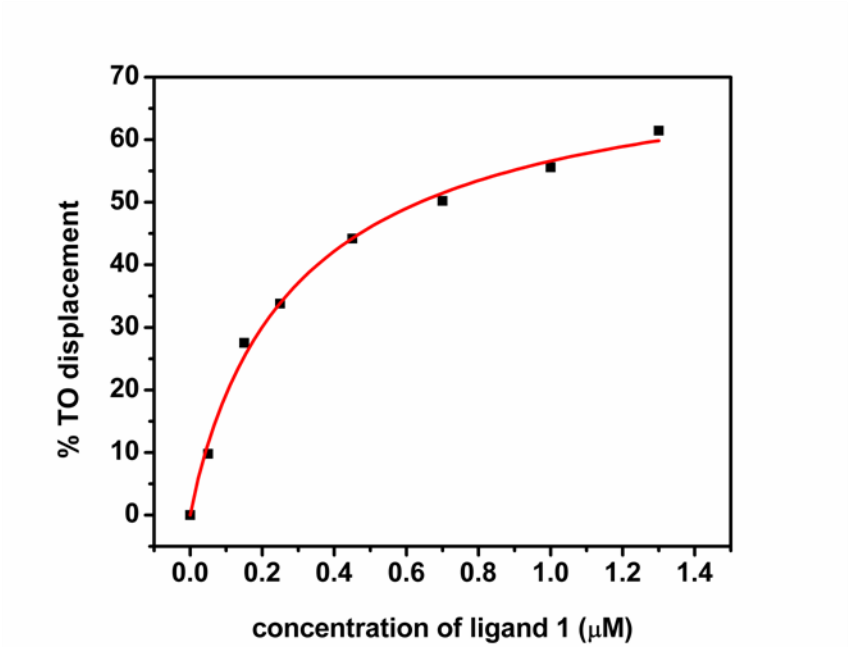

Figure S10. The FID assay has been done by adding *c-kit2* quadruplex (0.25 μM) in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 7.4, **2** (0.5 μM) and then displacing **2** from *c-kit2* by adding **1** (0-1.4 μM). The % TO displacement has been calculated by using equation (4), K_d = 1.4 μM.

4.0 Circular Dichroism (CD) spectroscopic studies

CD spectra were recorded on a JASCO J-815 spectrophotometer by using a 1 mm path length quartz cuvette. Quadruplex *c-kit2* ([GGGCGGGCGCGAGGGAGGGG]) was annealed by heating at 95 °C for 5 min and then cooling to room temperature at 0.1 °C per minute in a buffer containing Tris·HCl (10 mM, pH 7.4) and KCl (100 mM). Aliquots of ligand **1** (1 mm in water) were added in steps to achieve the desired equivalent proportions. The CD spectra represent an average of three scans and were smoothed and zero corrected. Final analysis and manipulation of the data was carried out by using Origin 8.0.

The *c-kit2* quadruplex exists predominantly as a parallel structure (as indicated by a major positive peak at 260 nm and a minor peak at 240 nm) at pH 7.4 (Fig. S10). At pH 9, *c-*

kit2 exhibits as a mixture of both parallel (peak at 260 nm) and antiparallel (peak at 290 nm) conformation.

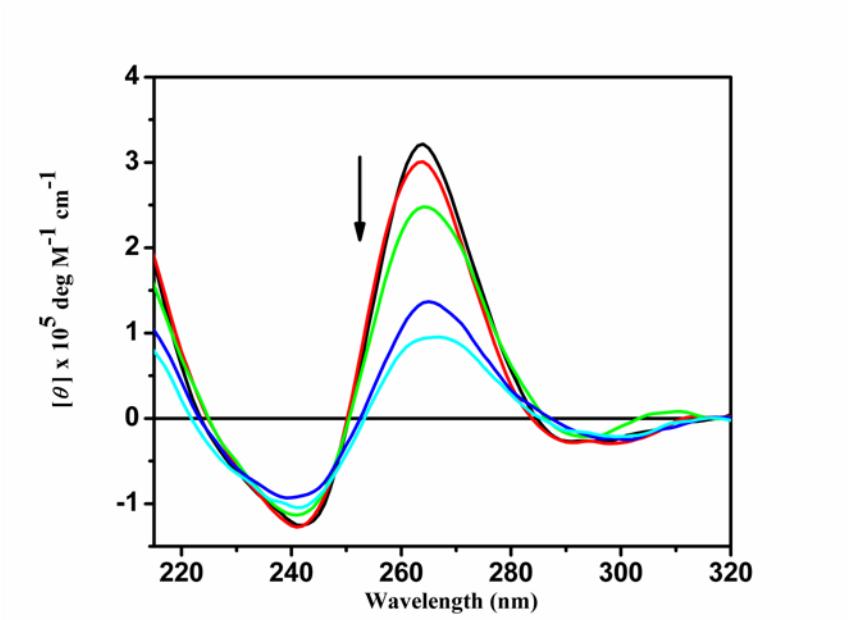

Figure S11. CD titration spectra of *c-kit2* quadruplex (7 µM) with **1** (0 —, 10 —, 30 —, 50 —, 70 — µM)in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 7.4

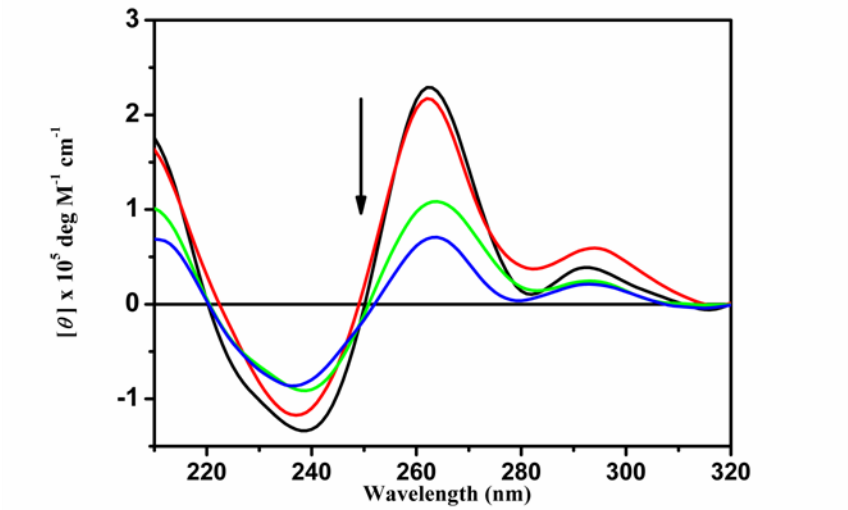

Figure S12. CD titration spectra of *c-kit2* quadruplex (7 µM) with **1** (0 —, 10 —, 30 —, 50 — µM) in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 9.0.

We observed that upon addition of the ligand **1** (1–2 mol equiv) to *c-kit2* at both the pH environment, no significant changes in signals were observed. However, at higher ligand

concentrations, decrease in the ellipticity at 260 nm (Figures S9 and S10) and 290 nm (Figure S10) was observed consistent with ligand induced unfolding of *c-kit2* quadruplex.[6]

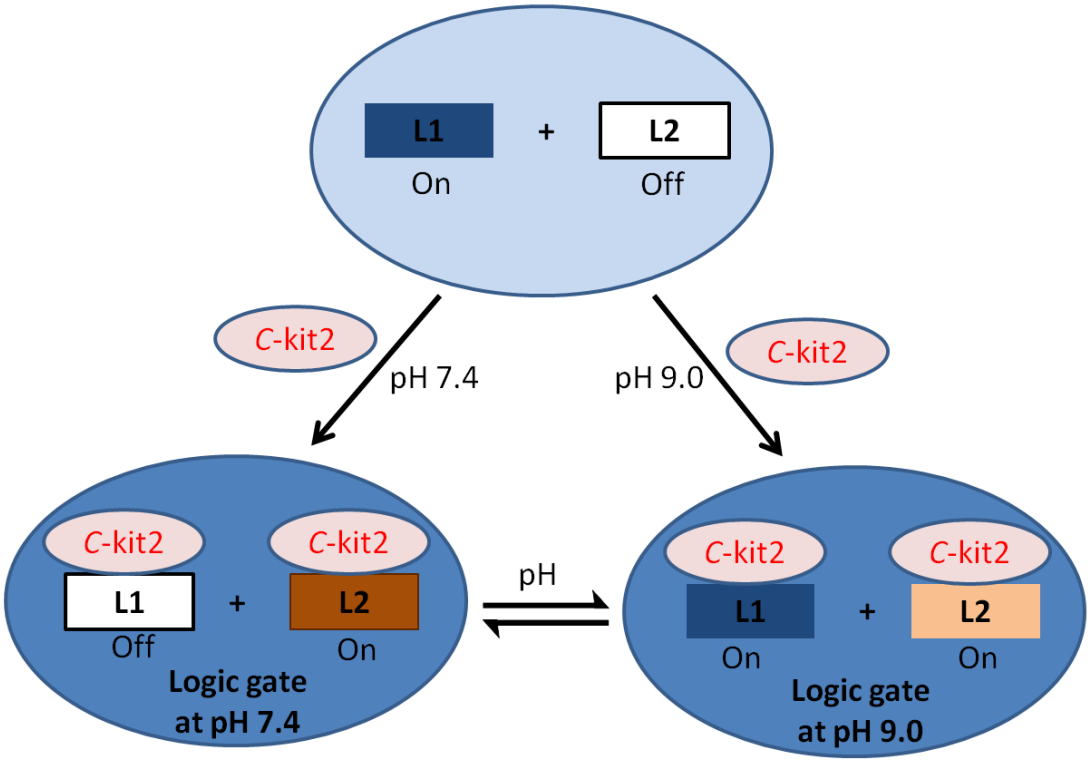

Figure S13. Schematic representation of the differential fluorescence interactions of small molecules **1** and **2** with the *c-kit2* G-quadruplex and pH mediated reversible logic operation.

[6] J. Dash, A. E. Waller, G. D. Pantos and S. Balasubramanian, *Chem. Eur. J.* 2011, **17,** 4571.