Supplementary Material (ESI) for Analyst

Crystal Violet as a Fluorescent Switch-On Probe for I-Motif: Label-Free DNA-Based Logic Gate

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Supporting Information

Materials. Calf thymus DNA (ct DNA) was purchased from Sigma Aldrich and purified by literature methods.[1a] The DNA per base pair concentration was determined by UV/Vis absorption spectroscopy using the following molar extinction coefficients at the indicated wavelengths: calf thymus DNA ε_{260} 13200 bp cm^{-1} M^{-1}.[1b] Crystal violet (CV) was purchased from Sigma Aldrich and used as received. DNA oligomers were purchased from Tech Dragon Limited (Hong Kong). The sequences of the oligomers are:

C_{29} = [5′-$(C_5T_3)_3C_5$-3′]
C_{21} = [5′-$(C_3T_3)_3C_3$-3′]
C_{17} = [5′-$(C_2T_3)_3C_2$-3′]
ss_{22} = [5′-GTGCACCTGACTCCTGTGGAGAAG-3′]
G_{33}= [5′-$A(G_3T_2A)_3G_3CAGA_2G_2ATA_2$-3′]
HTS = [5′-$A(G_3T_2A)_3G_3$-3′]

Physical measurement. Absorption spectra were recorded on a Perkin-Elmer Lambda 19 UV/Vis spectrometer. Emission spectra were recorded on a SPEX Fluorolog-2 Model fluorescence spectrophotometer or a PTI QM4 spectrometer.

Absorption titration. A solution of the CV (10 μM) in sodium acetate buffer (10 mM, pH 5.0) was titrated with aliquots of C_{29} (0–5.6 μM strand). Absorption spectra were recorded in the spectral range λ= 400–700 nm after equilibration at 20.0 °C for 10 min. The intrinsic binding constant, *K*, was determined from a plot of $D/\Delta\varepsilon_{ap}$ vs *D* according to equation (1):[1]

$$D/\Delta\varepsilon_{ap} = D/\Delta\varepsilon + 1/(\Delta\varepsilon \times K) \qquad (1)$$

where *D* is the concentration of DNA in bases, $\Delta\varepsilon_{ap} = |\varepsilon_A - \varepsilon_F|$, $\varepsilon_A = A_{obs}/[\text{ligand}]$, and $\Delta\varepsilon = |\varepsilon_B - \varepsilon_F|$; ε_B and ε_F correspond to the extinction coefficients of DNA–ligand adduct and unbound complex, respectively.

Molecular modeling. Molecular docking was performed by using the ICM-Pro 3.6-1d program (Molsoft).[3] According to the ICM method, the molecular system was described by using internal coordinates as variables. Energy calculations were based on the ECEPP/3 force field with a distance-dependent dielectric constant. The biased probability Monte Carlo (BPMC) minimization procedure was used for global energy optimization. The BPMC global-energy-optimization method consists of 1) a random conformation change of the free variables according to a predefined continuous probability distribution; 2) local-energy minimization of analytical differentiable terms; 3) calculation of the complete energy including non-differentiable terms such as entropy and solvation energy; 4) acceptance or rejection of the total energy based on the Metropolis criterion and return to step (1). The binding between CV and DNA was evaluated by binding energy, including grid energy, continuum electrostatic, and entropy terms. The X-ray crystal structure of a tetramolecular i-motif [5′-$(ACCCT)_4$-3′] was used (PDB: 1BQJ). Hydrogen and missing heavy atoms were added to the receptor structure followed by local minimization by using the conjugate gradient algorithm and analytical derivatives in the internal coordinates. In the docking analysis, the binding site was assigned across the entire structure of the DNA molecule. The ICM docking was performed to find the most favourable orientation. The resulting trajectories of the complex between CV and i-motif DNA were energy minimized, and the interaction energies were computed.

Emission measurement. The G-quadruplex forming sequence, G_{33} or HTS, was first annealed by incubating at 95 °C for 10 minutes, followed by cooling at 0.1 °C/s to 20 °C and then further incubating at 20 °C for 30 minutes in the appropriate buffer solution. A solution of CV (12 μM) was prepared in sodium acetate buffer (10 mM, pH 5.0) or Tris buffer (20 mM, pH 8.48) in the presence or absence of KCl (10 mM). Concentrated stock solutions of C_{29} and the annealed G_{33} were then added to a final concentration of 15 μM (C_{29}) and 5 μM (G_{33} or HTS) and the emission spectra were recorded.

The annealing conditions for the four input states are described below:
$i_1 = K^+$, $i_2 = H^+$

$i_1 = 0$, $i_2 = 0$; 20 mM Tris buffer (pH 8.48)
$i_1 = 1$, $i_2 = 0$; 20 mM Tris buffer (100 mM KCl, pH 8.48)
$i_1 = 0$, $i_2 = 1$; 10 mM sodium acetate buffer (pH 5.0)
$i_1 = 1$, $i_2 = 1$; 10 mM sodium acetate buffer (100 mM KCl, pH 5.0)

The oligonucleotides are heated and incubated at 95 ^oC for 10 minutes, followed by cooling at 0.1 ^oC /s till 20 ^oC and then incubated at 20 ^oC for 30 minutes. A solution of CV (1.0 μM) was prepared in either sodium acetate buffer (10 mM sodium acetate, pH 4.8) or Tris buffer (20 mM, pH 7) and the C_{29} and annealed G_{33} or HTS were added and the emission spectra were measured at λ_{excit} = 580 nm.

References

1 (a) J. Sambrook, E. F. Fritsch, T. E. Maniatis, *Molecular Cloning, A Laboratory Manual, 2nd Ed.,* **1989**, E.3 and E.10; (b) G. Felsenfeld, S. Z. Hirschman, *J. Mol. Biol.* **1965**, *13*, 407.
2 M. S. Goodman, A. D. Hamilton, J. Weiss, *J. Am. Chem. Soc.* **1995**, *117*, 8447.

Figure S1a. UV-visible absorption titration spectra of CV (10 μM) with C_{29} i-motif (0–5.6 μM strand) in sodium acetate buffer (10 mM, pH 5.0). Inset: plot of $D/\Delta\varepsilon_{ap}$ versus D. Absorbance was monitored at 590 nm.

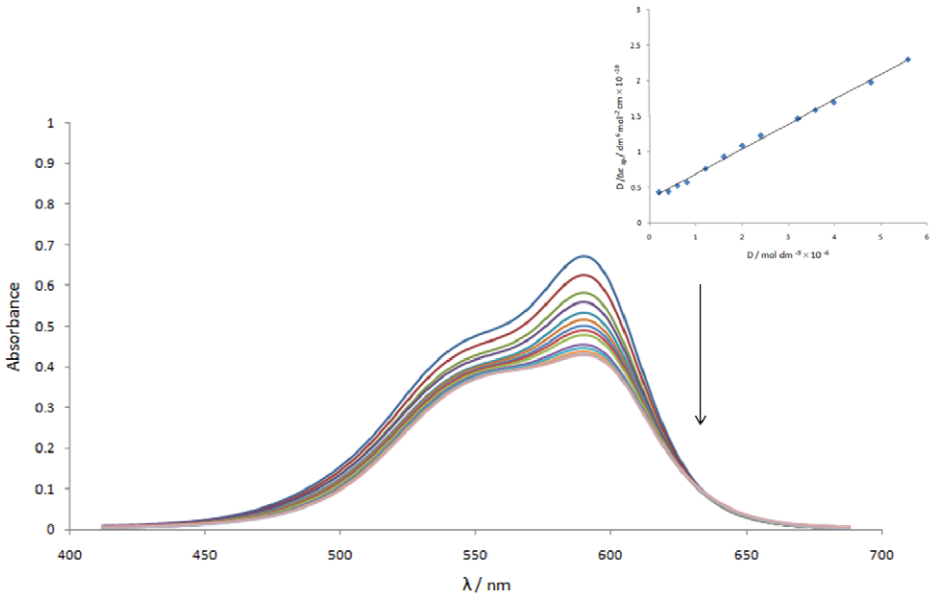

Figure S1b. UV-visible absorption titration spectra of CV (10 μM) with C_{21} i-motif (0–5.6 μM strand) in sodium acetate buffer (10 mM, pH 5.0). Inset: plot of $D/\Delta\varepsilon_{ap}$ versus D. Absorbance was monitored at 590 nm.

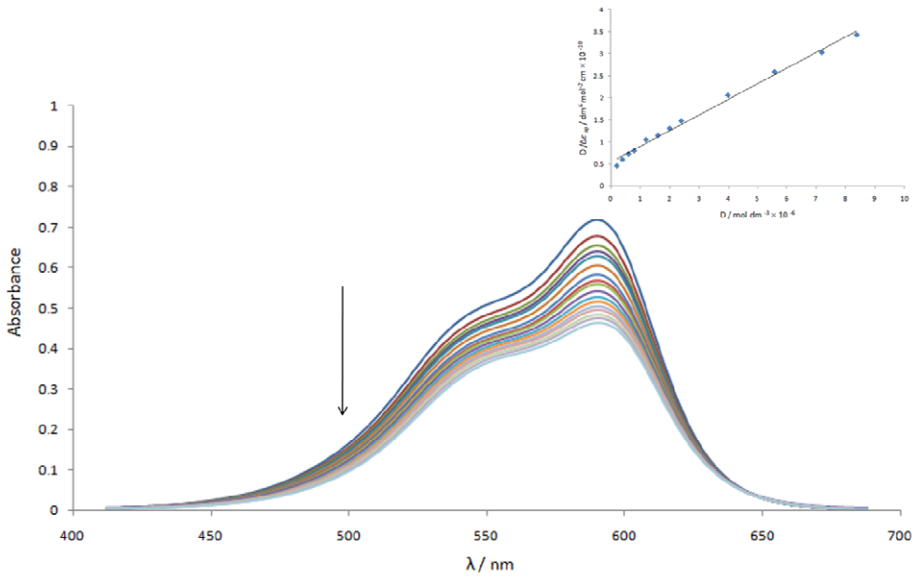

Figure S1c. UV-visible absorption titration spectra of CV (10 μM) with C_{17} i-motif (0–5.6 μM strand) in sodium acetate buffer (10 mM, pH 5.0). Inset: plot of $D/\Delta\varepsilon_{ap}$ versus D. Absorbance was monitored at 590 nm.

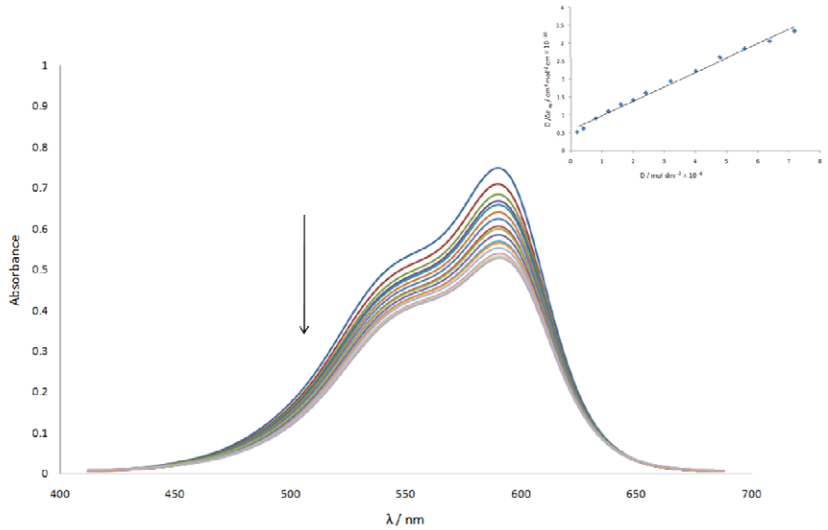

Figure S2. Emission spectra of CV (1 μM) in sodium acetate buffer (10 mM, pH 5.0) measured in the (a) absence (—) or presence of (b) the C_{29} i-motif (0.25 μM, —), (c) the C_{21} i-motif (0.25 μM, —) and (d) the C_{17} i-motif (0.25 μM, —).

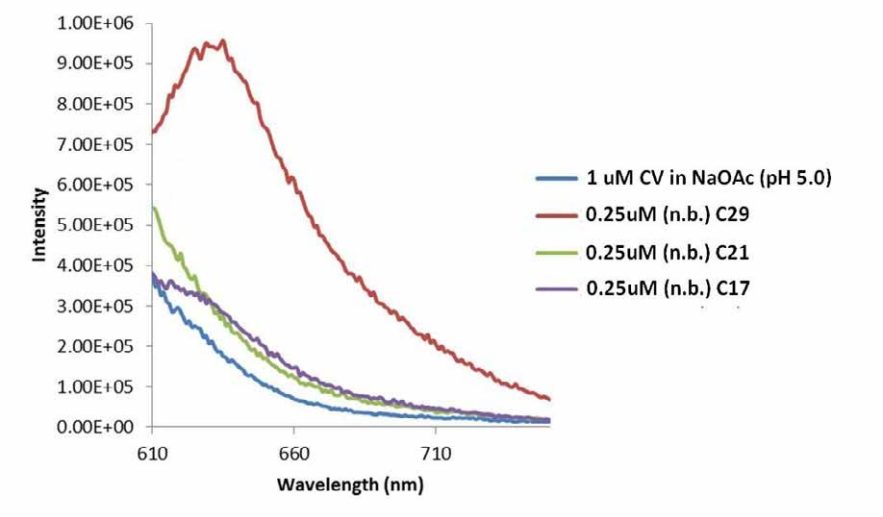

Figure S3. CD spectra of different cytosine-rich sequences in sodium acetate buffer (10 mM, pH 5.0): (a) C_{29} (1 µM, —); (b) C_{21} (1 µM, —); (c) C_{17} (1 µM, —).

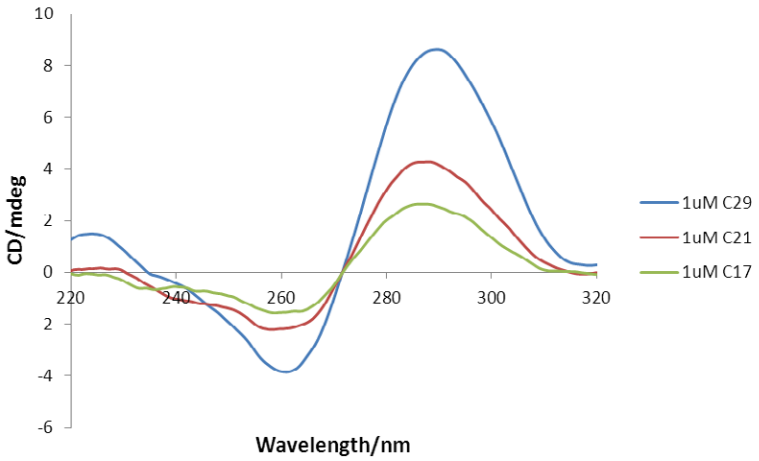

Figure S4. Representative fluorescence spectra for logic operation of DNA "OR" gate. Oligonucleotides were pre-annealed in the appropriate buffer. Fluorescence intensity of CV (12 µM) in the presence of 5 µM G_{33} and 15 µM C_{29} in: (a) 20 mM Tris buffer (pH 8.48) (—) ($i_1 = 0$, $i_2 = 0$); (b) 20 mM Tris buffer (pH 8.48, 10 mM KCl) ($i_1 = 1$, $i_2 = 0$) (—); (c) 10 mM sodium acetate buffer (pH 5.0) ($i_1 = 0$, $i_2 = 1$) (—); and (d) 10 mM sodium acetate buffer (pH 5.0) (pH 5, 100 mM KCl ($i_1 = 1$, $i_2 = 1$) (—).

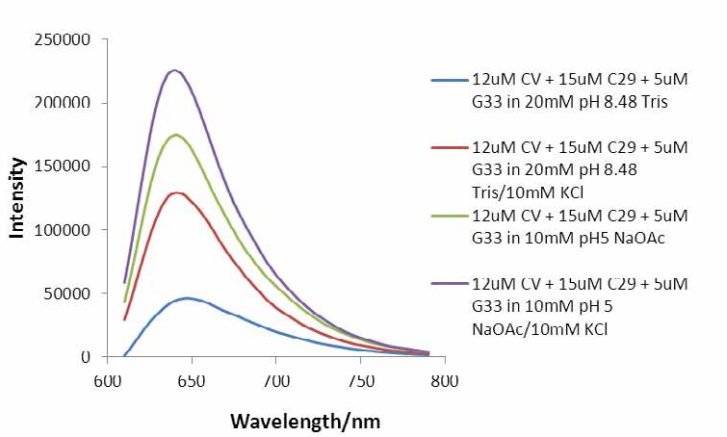

Figure S5a. Fluorescence enhancement of CV (12 µM) in Tris buffer (20 mM, pH 8.48) in the presence of 15 µM C_{29} and 5 µM HTS relative to the 'off' state ($i_1 = 0$, $i_2 = 0$). The "on" states were achieved by the presence of 10 mM KCl ($i_1 = 1$) and/or the use of 10 mM sodium acetate buffer, pH 5.0 ($i_2 = 1$). Error bars represent the range of triplicate experiments.

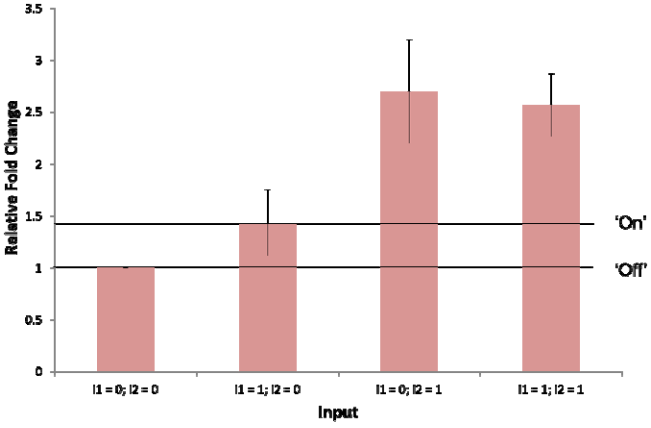

Figure S5b. Representative fluorescence spectra for logic operation of DNA "OR" gate. Oligonucleotides were pre-annealed in the appropriate buffer. Fluorescence intensity of CV (12 µM) in the presence of 5 µM HTS and 15 µM C_{29} in: (a) 20 mM Tris buffer (pH 8.48) (—) ($i_1 = 0$, $i_2 = 0$); (b) 20 mM Tris buffer (pH 8.48, 10 mM KCl) ($i_1 = 1$, $i_2 = 0$) (—); (c) 10 mM sodium acetate buffer (pH 5.0) ($i_1 = 0$, $i_2 = 1$) (—); and (d) 10 mM sodium acetate buffer (pH 5.0) (pH 5, 100 mM KCl ($i_1 = 1$, $i_2 = 1$) (—).

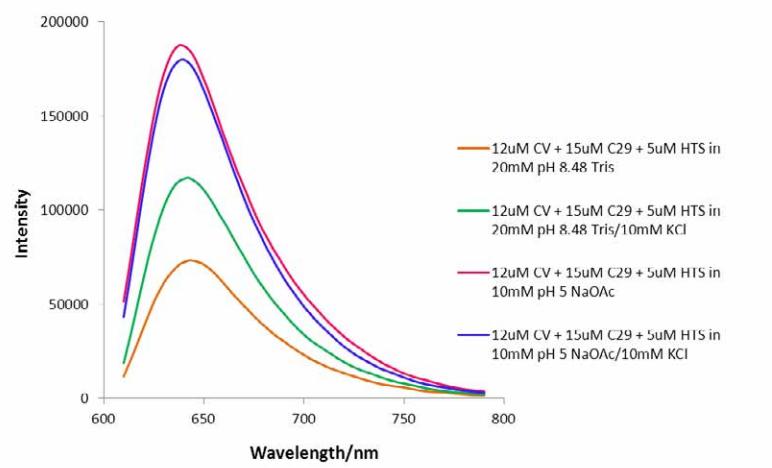

Figure S6a. Emission spectra of CV (12 µM) in: (a) Tris buffer (20 mM, pH 8.48, —) and (b) sodium acetate buffer (10 mM, pH 5.0, —).

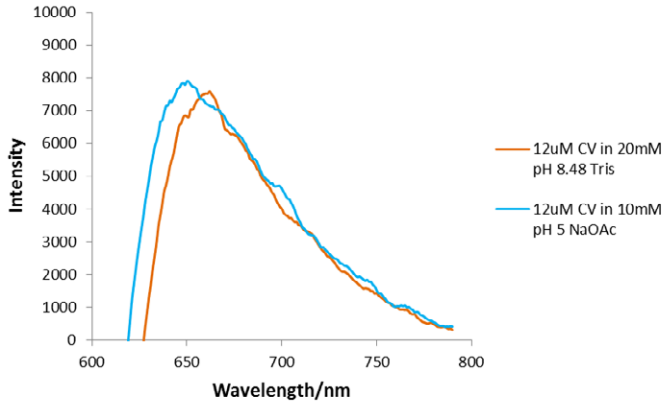

Figure S6b. Emission spectra of CV (12 µM) in the presence of C_{29} (15µM) in: (a) Tris buffer (20 mM, pH 8.48, —) and (b) Tris buffer (20 mM, 10 mM KCl, pH 8.48, —).