Supporting Information (ESI)

Molecular AND-logic for dually controlled activation of a DNA-binding spiropyran

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Synthesis

General Methods and Materials

^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded on Varian Unity 400 or JEOL Eclipse 400 spectrometer at *ca*. 20 °C. In ^{1}H NMR spectra, chemical shifts (δ/ppm) are referenced to internal reference $(CH_3)_4Si$ (0.00 ppm in $CDCl_3$). In ^{13}C NMR spectra, chemical shifts (δ/ppm) are referenced to the carbon signal of the deuterated solvents (77.2 ppm in $CDCl_3$ or 39.5 ppm in DMSO-d_6).

Melting points were determined on a Mettler FP82 hot-stage microscope.

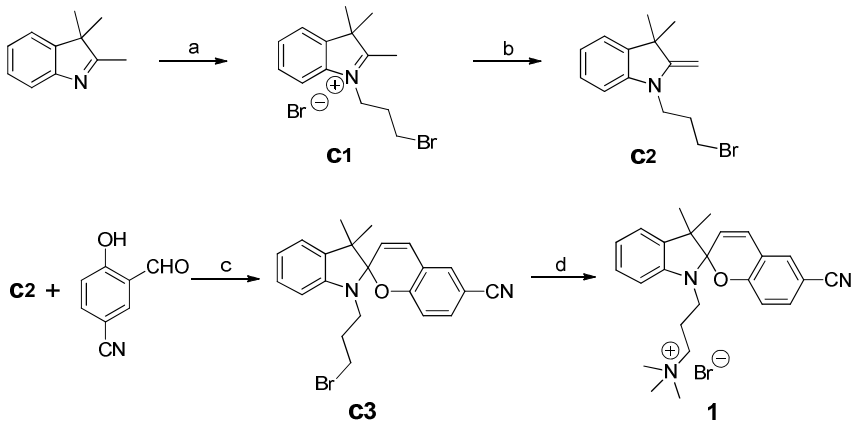
Thin-layer chromatography was performed on silica gel plates (Merck kieselgel 60, F_{254}) to monitor the reactions. Spots were made visible with UV light.

Column chromatography was performed with silica gel (Grace, Matrex LC 60Å/35–70 µm).

THF was distilled over Na and Ph_2CO. EtOH was distilled over Mg/I_2. All other solvents were used as received unless stated otherwise.

2,3,3-trimethylindolenine, 1,3-dibrompropane, 35% trimethylamine in EtOH solution were purchased from Aldrich and used as received.

*Experimental procedures for the synthesis of compounds **C1-C3** and **1** used in this study*.

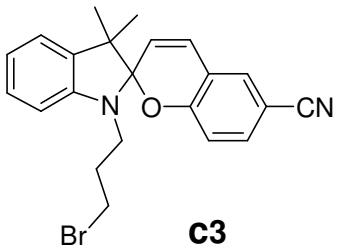


Scheme S1[a]. Synthetic scheme for preparing compounds **C1-C3** and **1**.

[a]Reagents and conditions: (a) $BrCH_2CH_2CH_2Br$, EtOH, reflux 5 h; (b) aq NaOH, rt, 30 min; (c) THF, reflux 16 h; (d) 35% Me_3N-EtOH, rt, 24 h.

1-(3′-Bromopropyl)-2,3,3-trimethylindolenium bromide (**C1**),[1] 1-(3′-bromopropyl)-3,3-dimethyl-2-methyleneindoline (**C2**),[1] 5-cyano-2-hydroxybenzaldehyde),[2] were prepared according to the reported procedures.

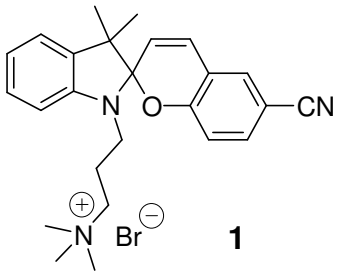
1′-(3″-Bromopropyl)-3′,3′-dimethyl-6-cyanospiro[(2*H*)-1-benzopyran-2,2′-indoline] (**C3**)



C3

Dry THF (40 mL) was added to a flask containing 1-(3′-bromopropyl)-3,3-dimethyl-2-methyleneindoline (**C2**) (788 mg, 2.81 mmol) and 5-cyano-2-hydroxybenzaldehyde (427 mg, 2.90 mmol). The solution was heated at 80 °C (oil bath temp.) under argon for 16 h. After removal of THF, the solid residue was purified by flash chromatography (SiO_2) with hexane/CH_2Cl_2 (1:2) as eluent. Compound **C3** (1.03 g, 87% yield) was obtained as a white solid. Further purification by recrystallization from EtOH/$CHCl_3$ gave colorless crystals (670 mg, 58%). M.p. 116-117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.17 (s, 3H; CH_3), 1.27 (s, 3H; CH_3), 2.01-2.16 (m, 1H; CH_2), 2.19-2.31 (m, 1H; CH_2), 3.22-3.31 (m, 1H; CH_2), 3.35-3.47 (m, 3H; CH_2), 5.82 (d, J = 10.4 Hz, 1H; H-3), 6.63 (d, J = 7.6 Hz, 1H; H-7′), 6.74 (td, J = 0.8, 8.4 Hz, 1H; H-8), 6.83-6.91 (m, 2H; H-4 and H-5′), 7.09 (ddd, J = 0.4, 1.2, 7.2 Hz, 1H; H-4′), 7.19 (dt, J = 1.6, 7.6 Hz, 1H; H-6′), 7.36 (d, J = 2.0 Hz, 1H; H-5), 7.38 (dd, J = 2.0, 8.4 Hz, 1H; Ar-H-7) ppm; ^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 20.0, 26.0, 31.2, 31.9, 42.1, 52.7, 103.5, 106.1, 106.8, 116.3, 119.2, 119.4, 119.8, 121.6, 121.9, 127.9, 128.3, 130.9, 134.0, 136.2, 147.0, 157.7 ppm; Elemental analysis calcd (%) for $C_{22}H_{21}BrN_2O$: C 64.55, H 5.17, N 6.84; found: C 64.49, H 4.99, N 6.76.

1′-(3″-trimethylammoniopropyl)-3′,3′-dimethy-6-cyanospiro[(2*H*)-1-benzopyran-2,2′-indoline] bromide (**1**)



1

Trimethylamine-EtOH solution (15 ml, 35% Et_3N in EtOH) was added to a flask containing 1′-(3″-bromopropyl)-3′,3′-dimethyl-6-cyanospiro[(2*H*)-1-benzopyran-2,2′-indoline] (**C3**) (450 mg, 1.10 mmol). The flask was plugged with a rubber septum and the solution was stirred at rt for 24 h in darkness. White precipitate formed was collected by filtration and washed with EtOH (465 mg, 90% yield). The structure and purity of compound **1** are confirmed by NMR. M.p. 162-164 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.19 (s, 3H; CH_3), 1.27 (s, 3H; CH_3), 1.93-2.09 (m, 1H; CH_2), 2.10-2.23 (m, 1H; CH_2),

3.39 (s, 9H; NCH_3), 3.25-3.45 (m, 2H; CH_2), 3.62-3.80 (m, 2H, CH_2), 5.94 (d, J = 10.4 Hz, 1H; H-3), 6.65 (d, J = 7.6 Hz, 1H; H-7′), 6.71 (d, J = 8.4 Hz, 1H; H-8), 6.91 (t, J = 7.6 Hz, 1H; H-5′), 6.97 (d, J = 10.4 Hz, 1H; H-4), 7.10 (dd, J = 1.2, 7.6 Hz, 1H; H-4′), 7.21 (dt, J = 1.2, 7.6 Hz, 1H; H-6′), 7.38 (dd, J = 2.0, 8.4 Hz, 1H; H-7), 7.41 (d, J = 8.4 Hz; H-5) ppm; ^{13}C NMR (100.6 MHz, DMSO-d_6) δ= 19.6, 22.1, 25.8, 40.3, 52.1(2C), 63.3, 102.5, 105.7, 106.7, 115.9, 118.9, 119.3 (2C), 121.4, 121.7, 127.6, 128.0, 131.2, 134.0, 135.7, 146.7, 157.1 ppm; Elemental analysis calcd (%) for $C_{25}H_{30}BrN_3O{\cdot}½H_2O$: C 62.89, H 6.54, N 8.78; found: C 62.75, H 6.74, N 8.78.

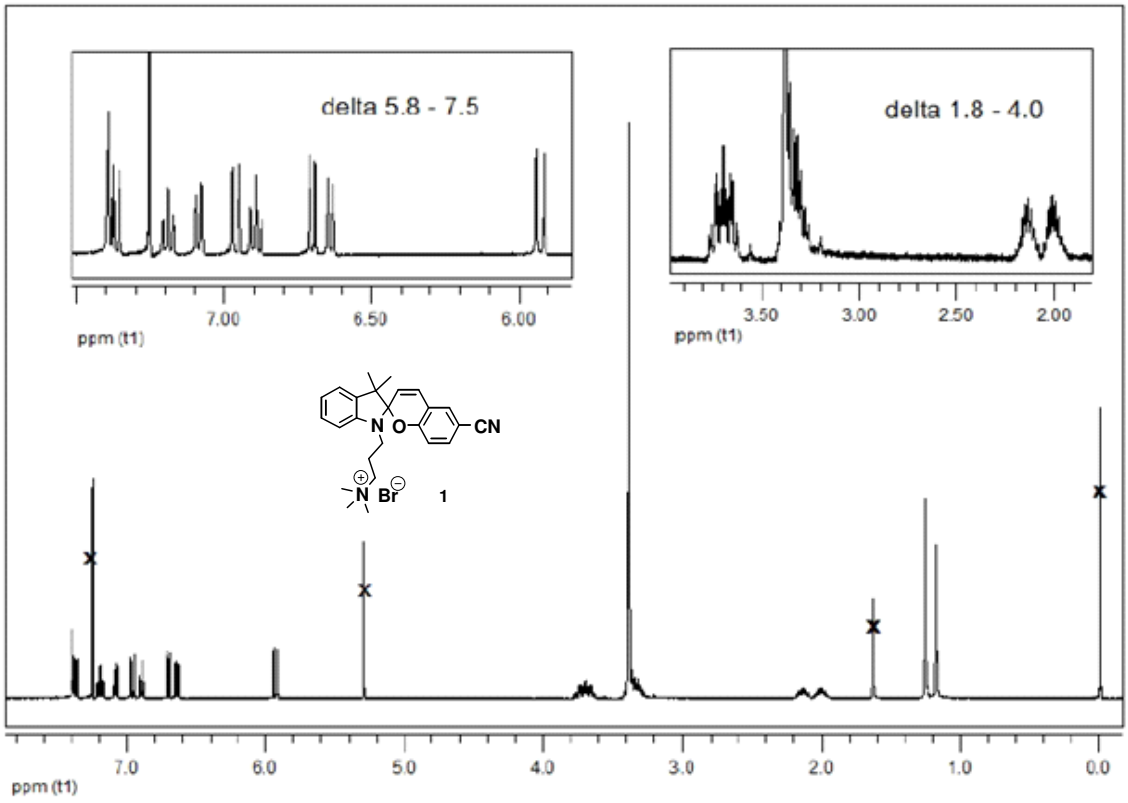


Figure S1. ^{1}H NMR (400 MHz, $CDCl_3$) of 1′-(3″-trimethylammoniopropyl)-3′,3′-dimethy-6-cyanospiro[(2*H*)-1-benzopyran-2,2′-indoline] bromide (**1**)

Spectroscopic Measurements

The absorption and the LD measurements at pH 6.0 and 7.0 were performed in aqueous solutions containing 10 mM of NaCl buffered with 10 mM of Na_2HPO_4/NaH_2PO_4, whereas mQ-water was used as solvent for all other measurements. In the pH 6.0 buffer experiments the concentration of Na_2HPO_4 and NaH_2PO_4 were 0.58 mM and 9.42 mM, respectively, and in the pH 7.0 buffer experiments the concentration of Na_2HPO_4 and NaH_2PO_4 were 3.81 mM and 6.19 mM, respectively. Calf-thymus (CT) DNA were purchased from Sigma. Before spectroscopic measurments, the DNA samples were dissolved in the two different buffer solutions (pH 6.0 and pH 7.0) and filterd through Minisart-GF prefilter. From these stock solutions, samples of the relevant DNA concentrations were prepared by dilution with buffer. The purity of the DNA was verified by the absorbance ratio A(260 nm)/A(280 nm)=1.88.[3]

Ground state absorption spectra were recorded on a Varian Cary 5000 UV-vis-NIR spectrophotometer with baseline correction. LD was measured using a Jasco J-720 CD spectropolarimeter, equipped with an Oxley prism to obtain linerarly polarized light, on samples oriented in an outer-rotating Couette flow cell with 1 mm path length. Spectra were measured at a shear gradient of 3000 s^{-1} and corrected for basline contributions by subtraction of the corresponding spectra recored without rotation.

The photoinduced isomerization reactions were performed in the UV using a UVP lamp model UVGL-25 (254 nm, 700 $\mu W/cm^2$) or in the visible with a 1000 W Xe/Hg lamp at 450 W equipped with a hot mirror (A=1.8 at 900 nm) to reduce IR intensity and a VG 9 glass filter (A<1.5 between 460 nm and 590 nm). The resulting light power density was ~10 mW/cm^2. Using the visible light only ca 1/2 of the sample volume was exposed to the light, whereas the whole sample volume was exposed to the 254 nm UV light. The samples were continously stirred during all irradiation processes. Deoxygenation of the samples was not performed.

Macroscopic and microscopic binding constants

The DNA-binding constant of **1** can be described in two ways; the microscopic and macroscopic binding constants. Given that the open protonated form **1oH$^+$** binds, the microscopic binding constant is defined as

$$K_{micro} = \frac{[DNA-1oH^+]}{[1oH^+][DNA]} \qquad (1)$$

where [DNA-1oH$^+$] is the concentration of the DNA-bound form, [1oH$^+$] is the concentration of **1oH$^+$** free in solution and [DNA] is the free DNA base-pair concentration.

The macroscopic binding constant is defined as

$$K_{macro} = \frac{[DNA-1oH^+]}{[1_{tot}][DNA]} = \frac{[DNA-1oH^+]}{([1c]+[1o]+[1oH^+])[DNA]} \qquad (2)$$

where $[1_{tot}]$ is the total concentration of **1** free in solution, and $[1c]$ and $[1o]$ are the free concentrations of **1c** and **1o**, respectively. Hence, all forms of **1** are taken into account when the macroscopic binding constant is determined.
Using the Henderson-Hasselbalch equation on the acid-base species **1oH$^+$** and **1o**,

$$pH = pK_a + \log_{10}\frac{[1o]}{[1oH^+]} \qquad (3)$$

the pH dependence of **1o** can be described as

$$[1o] = [1oH^+] \cdot 10^{pH - pK_a} = [1oH^+] \cdot \alpha \quad (4)$$

where α denotes the exponential pH dependence of **1o**. The concentration of open form triggered by UV light exposure can be described as

$$[1o] = [1c] \cdot \beta \quad (5)$$

where β is a fictive parameter that depends on the dose of UV light.

Equation (4) and (5) gives

$$[1c] = [1o] \cdot \beta^{-1} = [1oH^+] \cdot \alpha\beta^{-1} \quad (6)$$

which together with equations (1) and (2) gives the macroscopic binding constant as

$$K_{macro} = \frac{K_{micro}}{1 + \alpha + \alpha\beta^{-1}} \quad (7)$$

Hence, the macroscopic binding constant is dependent on both the UV dose (through β) and the pH (through α). It is seen that K_{macro} is expected to increase with increasing UV dose and decrease with increasing pH.

The decrease in absorption at 515 nm after DNA addition (shown at the various pH and UV dose combinations in Figure 2) corresponds to the amount DNA-bound **1oH$^+$**. With the concentration of all species known, Equations (1) and (2) gives the reported binding constants. The absorption differences in Figures 2a and 2c (without UV exposure) are too small to be quantified, and we have arbitrarily set the binding constants to $K < 5\ M^{-1}$.

Reversible DNA-binding

The reversibility of the DNA-binding process was examined by irradiating a sample of **1c** with UV light for 5 minutes in pH 6.0 buffer. DNA was added and an LD spectrum was recorded (Figure S2, blue line). Subsequent exposure to visible light for 20 min dramatically decreased the LD response (green line). Upon exposure to another 3 min portion of UV light, the signal almost recovered to the initial high-amplitude value (red line), showing that the observed changes in the LD signal are due to a fully reversible light-controlled DNA-binding process rather than photodecomposition.

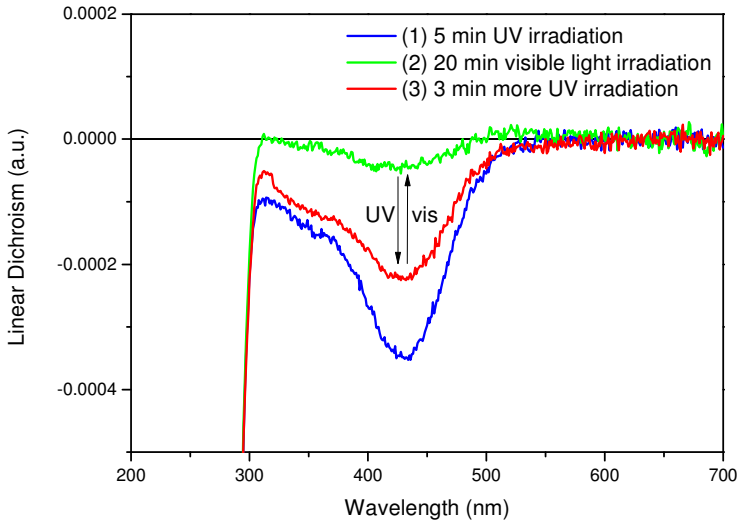


Figure S2. Changes in the LD signal after alternating UV- and visible light exposure.

Calculation of binding angle from LD^r

The angle between the transition moment of a DNA-binding ligand and the DNA-helix axis (the macroscopic orientation axis), α, was calculated using the following equation

$$LD^r = \frac{LD}{Abs_{iso}} = \frac{3}{2} S\left(3\cos^2\alpha - 1\right)$$

where S is an orientation factor describing how well oriented the sample is, and Abs_{iso} is the isotropic absorption. The absorption and LD spectra were recorded on a sample containing spiropyran **1** and CT-DNA (illuminated at 254 nm for 10 min) at concentrations of ca 50 μM and 90 μM, respectively (Note that a 50 μM total concentration of **1** at a 65/35 ratio **1c/1o+1oH$^+$** at pH 6 without any addition of DNA would correspond to a ca 0.5 μM concentration of **1oH$^+$**). The original LD spectrum was used for calculation of the LD^r, whereas the contributions of unbound **1** were subtracted from the absorption spectrum before the calculation. The S factor was calculated from the LD^r of DNA at 260 nm in the spectrum of DNA + **1**. α was set to 90° for the base pair transition. This value of S was then used to calculate α at 431 nm, i.e., for the transition moment corresponding to bound **1oH$^+$**.

References

[1]. J. Andersson, S. Li, P. Lincoln, J. Andréasson, *J. Am. Chem. Soc.* **2008**, *130*, 11836-11837.

[2]. Y. Suzuki, H. Takahashi, *Chem. Pharm. Bull.* **1983**, *31*, 1751-1753.

[3]. J. Marmur, J. Mol. Biol. **1961**, *3*, 208-218.