Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Controlling DNA nanodevices with visible light-switchable buffers

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1. General Information

The photoacid was synthesized and purified as described previously.[1]

H_2O used in all preparations was filtered with a MilliQ-Integral5 purification system.

Oligonucleotides

HPLC purified DNA - oligonucleotides and modified with Alexafluor-680 (A680) and a quencher (Black Hole Quencher 2, BHQ-2) were purchased from Biosearch Technologies (Risskov, Denmark) and from Metabion International AG (Planegg, Germany) and employed without further purification.

Oligonucleotide sequences

All oligonucleotides were suspended to a final concentration of 100 μM in distilled water and stored at -20°C.

pH-dependent DNA-nanoswitches

pH nanoswitch #1 (pKa = 6.3): 5'- GAG GAG GAA G TTT(BHQ-2)A CTT CCT CCT C CTT TGG TTT GGT TTG GTT TGG TTT GGT TTG GTT TG **CTC CTC CTT C** -(A-680)-3

pH nanoswitch #2 (pKa = 7.4): 5'- GAG GAG GAA G TTT(BHQ-2)A CTT CCT CCT C CTT TG **CTC CTC CTT C**-(A-680)-3'

pH nanoswitch #3 (pKa = 9.5): 5'- AAG AAA AGA A TTT(BHQ-2)A TTC TTT TCT T CTT TG **TTC TTT TCT T**- (A-680)-3'

For the above sequences the underlined bases represent the duplex portion, while the bold bases represent the triplex-forming domain. Hereafter and in the main text, pH nanoswitches #1, #2 and #3 are referred to as 40%_35nt, 40%_5nt and 80%_5nt, respectively. Where 40% and 80% indicate the percentage of the pH-dependent C-G-C Hoogsteen interactions, while the 35nt and 5nt indicate the length of the linker connecting the triplex-forming domains.

pH-dependent DNA-nanomachine for the light-activated control of loading/release of DNA cargo

DNA cargo**:** 5'- Cy5.5- **CTC TAG CCA AA** –BHQ3 -3'

Receptor: 5'- TTC CTT TTT TTT TCC TT**T TTG GCT AGA G**AA GGA A -3'

For the above sequences the bold bases represent the cargo binding site on the receptor sequence.

The concentration of all stock solutions was determined by UV-Vis spectroscopy knowing the corresponding molar extinction coefficients.[1] UV-Vis spectra were acquired on an Agilent Cary

60 spectrometer coupled to a Huber thermostat, using Suprasil quartz cuvettes (114-QS) from Hellma Analytics. Fluorescence experiments were carried out under gentle stirring on an Agilent Cary Eclipse coupled to a thermostat, using Suprasil quartz cuvettes (101-QS) from Hellma Analytics.

pH measurements were performed using Metrohm pH module 867 coupled with a Biotrode glass electrode in contanct with low-volume sample solutions contained in 1.5-mL mass vials (pH jumps) or quartz cuvettes (titrations and switching experiments), and processed using the software Metrohm Tiamo Light.

Samples photoirradiation was carried out using a Prizmatix FC-LED-500Z high-power LED light sources (λ = 500 nm). The light beam was delivered by polymer optical fibers connected to an FCM1-06 collimator positioned at the level of the Cary Eclipse lid and orthogonally to the instrument optics (switching experiments), or coupled with a 45° mirror cage, resulting in a light beam tilted by 90° (pH jumps[2]). Power measurements of the fiber-coupled LED output were made with Thorlabs S142C integrating sphere photodiode power sensor; the uncertainty is within 5%.

2. pH titrations

pH titrations were carried out by adding consecutive amounts of a solution of NaOH (0.01 M) to a 1-mL solution containing NaCl (20 mM), the photoacid (0.5 mM) and the pH nanoswitch (15 nM). The actual pH of the solution was measured before and right after the acquisition of fluorescence spectra in the dark. To account for the quite low signal/noise ratio and for stability check purposes, two spectra were acquired after 2 and 4 minutes of equilibration at 25 °C (Figure S1a); fluorescence maxima were then calculated considering the two distinct data sets by averaging the fluorescence values in the 702 ± 2 nm range. The obtained fluorescence values ± errors were corrected considering the corresponding dilution factor after each addition and plotted against the pH (Figure S1b). The apparent acidity constants of the pH nanoswitches (K_a) were estimated by nonlinear least square fitting to the following equation:

$$F_{(H^+)} = F_0 + (F_{max} - F_o)\frac{K_a^n}{K_a^n + [H^+]^n}$$

Where $F_{(H^+)}$ represents the actual fluorescence intensity as a function of the pH, F_0 and F_{max} are the fluorescence intensities at low and high pH, and n cooperativity coefficient of the pH nanoswitch.

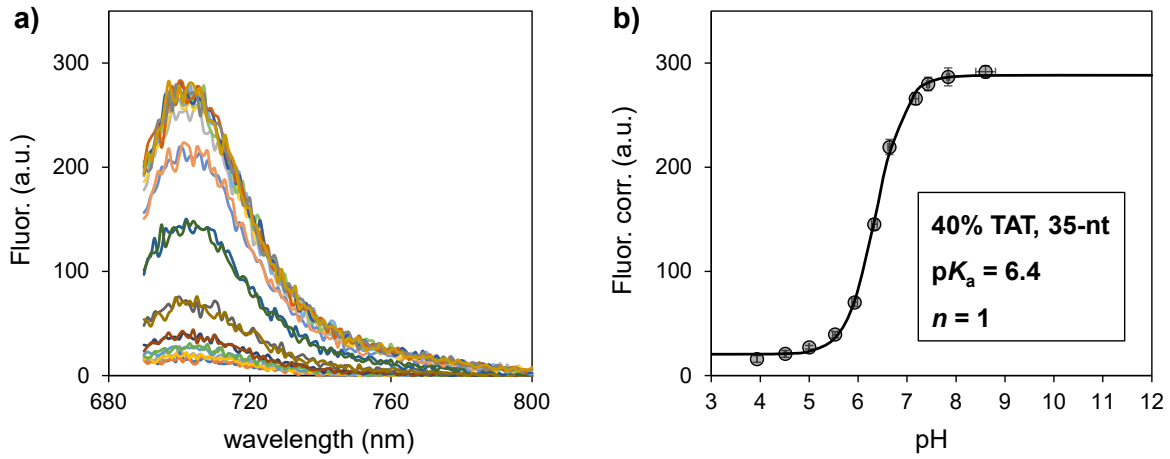

Figure S1. (a) Fluorescence spectra of the 40% 35-nt pH nanoswitch acquired after 2 and 4 minutes after each addition of NaOH. (b) Corrected fluorescence intensity (i.e., avg. $FI_{700\text{-}705nm} \cdot V_{tot} / V_{init}$) as a function of the pH; solid black line represent the best fit to the equation above. Fluor. parameters: excitation = 680 nm, slits = 5/5 nm, PMT = 800 V, T = 25 °C. The results obtained with the other pH nanoswitches are reported in Figure 2c-d of the main text.

3. Triplex/Duplex switching experiments

Switching experiments were carried out by adding the pH nanoswitch (15 nM) to a 1-mL solution containing NaCl (20 mM) and the photoacid (0.5 mM) neutralized at pH 7.4. Before the addition of the pH nanoswitch, a pH jump was recorded using our previously described photochemical apparatus[1,2] (Figure S2a); the resulting solution was then transferred from the 1.5-mL mass vial used to record the pH jump to a quartz cuvette for fluorescence analysis (Figure S2b).

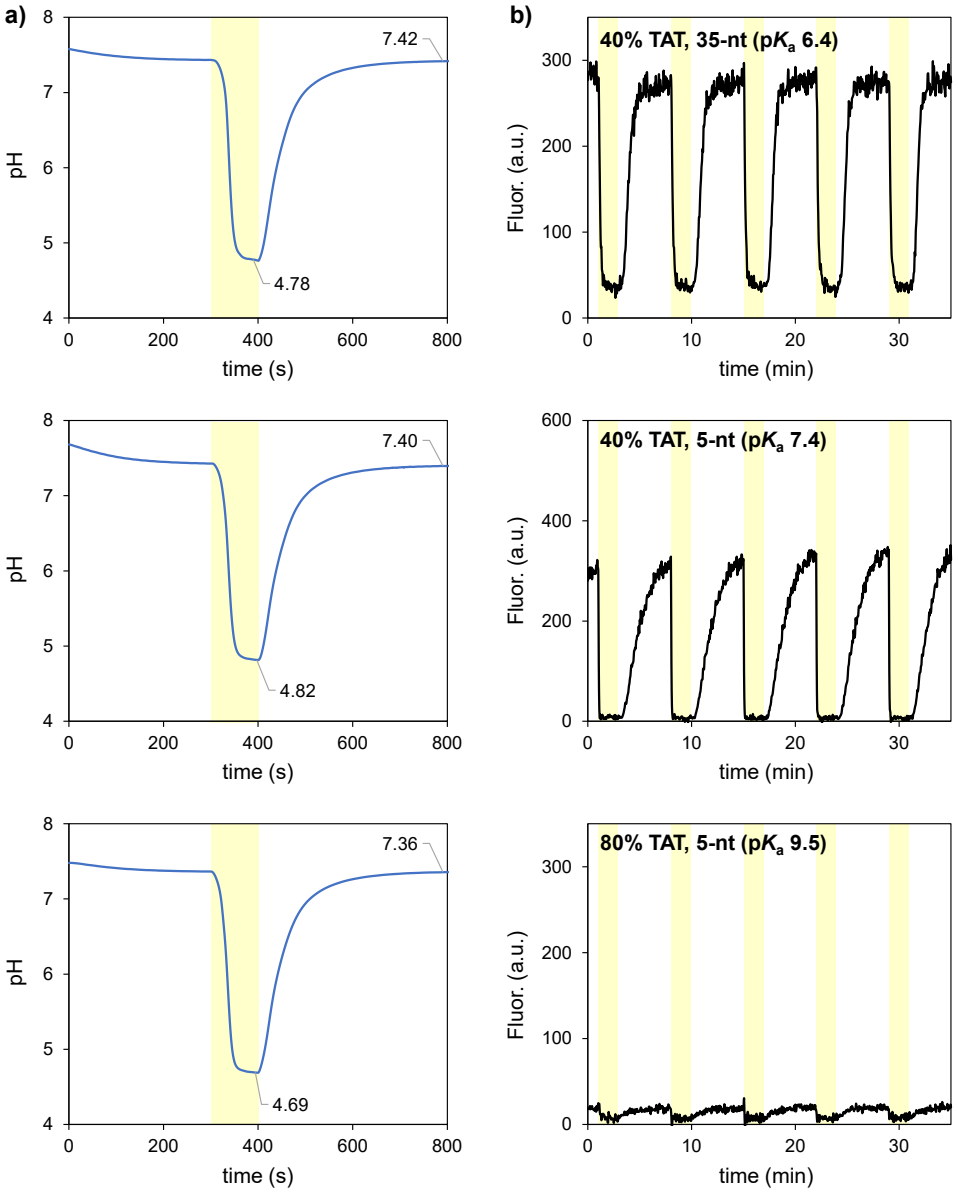

Figure S2. (a) pH jump recorded right before the addition of the pH nanoswitch and (b) repetitive on/off cycles obtained for each pH nanoswitch. Fluor. parameters: excitation/emission = 680/702 nm, slits = 5/5 nm, PMT = 800 V, T = 25 °C. The reported fluorescence intensities were not corrected as the addition of pH nanoswitches resulted in <5% dilution.

4. Temporal and ratiometric control

Light-switchable buffer solutions at different pH were prepared by adding HCl and NaOH to a solution (1.5 mL) of photoacid (0.5 mM) in aqueous NaCl (20 mM). Contrary to previous work,[1] here the photoacid is neutralized directly in solution, and not while dissolving it in aqueous solutions containing sub-stoichiometric amount of a base. It follows that the resulting neutralization degree (α) needs to be calculated from the Henderson-Hasselbalch equation as follow:

$$pH = pK_a^{GS} + \log\frac{C \cdot \alpha}{C \cdot (1-\alpha)} \quad \rightarrow \quad \alpha = \frac{10^{(pH - pK_a^{GS})}}{1 + 10^{(pH - pK_a^{GS})}}$$

Where C is the actual concentration of the photoacid in solution prior to neutralization and pK_a^{GS} the corresponding acid dissociation constant in the dark. Consequently, pH jumps were carried out as mentioned above (the obtained results are reported in Figure 3a in the main text). Afterwards, the solution is transferred into a quartz cell for fluorescence analysis (see Figure 3b in the main text). Half-life values of pH and fluorescence recovery were extrapolated graphically as the time needed to reach pH = $(pH_{dark} - pH_{light})/2$ and F = $(F_{dark} - F_{light})/2$, respectively (the obtained results are reported in Figure 3c).

To demonstrated the possibility of controlling DNA nanoswitches ratiometrically (i.e., obtaining an output signal that changes proportionally to a change in the input), we opted for varying the irradiation time instead of the light intensity, as we were not able to accurately tune the output power of our LED setup in a low power regime (< 5 mW). Thus, the same experiment above for the solution at physiological pH (Figure S2a-b) was repeated by applying multiple on/off cycles where the irradiation time was changed from 5 seconds to 20 minutes (see Figure 3d in the main text).

5. Binding curves

Binding experiments were carried out by adding increasing amounts of receptor (*R*) to a 1-mL solution containing the cargo (50 nM), NaCl (150 mM), $MgCl_2$ (1 mM), and the photoacid (1.0 mM) neutralized at pH 7.4. Before starting the titration, a pH jump was recorded using our previously described photochemical apparatus[1,2] (Figure S3a). Subsequently the solution is transferred to a quartz cuvette for fluorescence analysis. In order to account for the equilibration of the system after each addition, the fluorescence of the target was monitored kinetically till stabilization of the signal (Figure S3b). Fluorescence values were then corrected for dilution and plotted against the concentration of added receptor. The apparent binding affinity of the target (DNA cargo) was estimated by nonlinear least square fitting to the following equation:

$$F_{(R)} = F_0 + (F_{max} - F_o)\frac{[R]}{K_d + [R]}$$

Where $F_{(R)}$ represents the actual fluorescence intensity of the target, which increases progressively following binding with *R*, F_0 and F_{max} are the fluorescence intensities at low and high concentration of *R* (i.e., of the target alone and the target fully bound to the receptor, respectively), and K_d is the dissociation constant of the target-receptor complex (Figure S3c). Repetition of the same experiment in phosphate buffer solutions at pH 7.4 and pH 4.5 (i.e., in the absence of photoacid) gave similar results, indicating that the presence of photoacid does not alter the thermodynamics of binding.

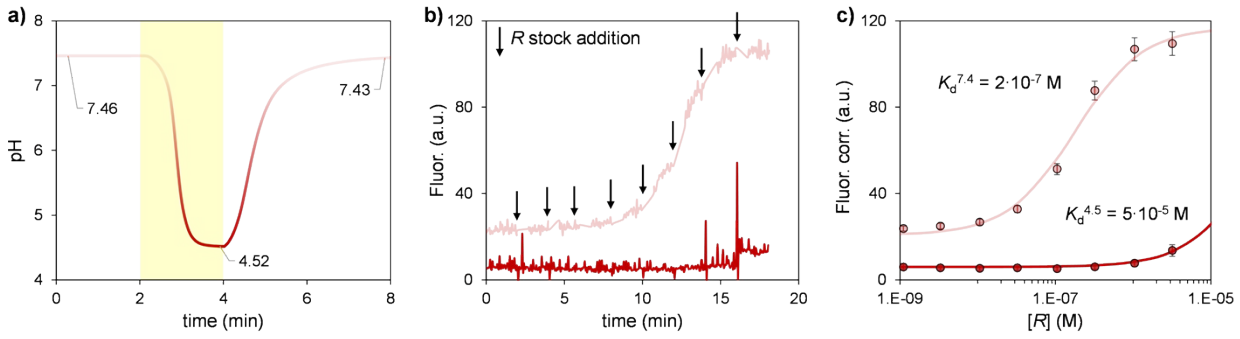

Figure S3. (a) pH jump recorded right before starting a titration; (b) time-course of fluorescence intensity upon consecutive additions of receptor. Fluor. parameters: excitation/emission = 635/705 nm, slits = 5/10 nm, PMT = 700 V, *T* = 25 °C; (c) Binding curves obtained at pH 7.4 (in the presence of the photoacid) and pH 4.5 (phosphate buffer solution 100 mM).

6. Cargo release/uptake experiments

Reversible cargo release was demonstrated operating in a regime where the difference in binding affinity is maximized. In-situ photo-irradiation was performed on an aqueous solution containing the cargo (50 nM), the receptor (300 nM), NaCl (150 mM), $MgCl_2$ (1 mM), and the photoacid (1.0 mM) neutralized at pH 7.4 (the obtained results are reported in Figure 4c in the main text).

7. References

1. C. Berton, D. M. Busiello, S. Zamuner, R. Scopelliti, F. Fadaei-Tirani, K. Severin, C. Pezzato, *Angew. Chem. Int. Ed.* **2021**, *60*, 21737-21740;
2. C. Berton, D. M. Busiello, S. Zamuner, E. Solari, R. Scopelliti, F. Fadaei-Tirani, K. Severin, C. Pezzato, *Chem. Sci.* **2020**, *11*, 8457-8468.