Electronic Supplementary Material (ESI) for Chemical Communications

Supporting information for the paper:

"pH-Programmable DNAzyme Nanostructures"

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A further aspect that was considered involves the purity of the closed molecular tweezers upon binding of the nucleic acid (**5**) to the open configuration of the tweezers. The bridging unit (**5**) might bridge two arms of different tweezers, thus yielding dimers or oligomers of the tweezers. While such oligomers are not anticipated to yield highly quenched fluorophore labels, the formation of monomeric closed tweezers was confirmed by naturated electrophoretic experiments on an acrylamide gel (Fig S1) that confirmed intact unimolecular tweezers structures, with no dimer or oligomer side products.

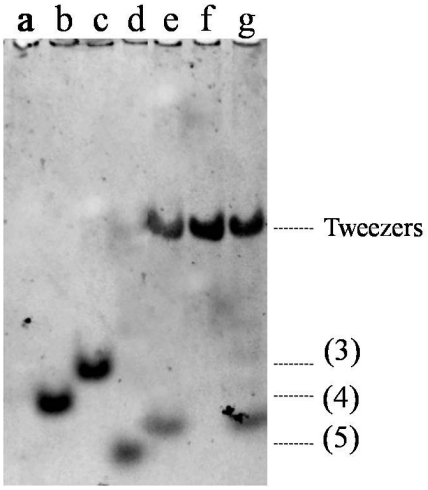
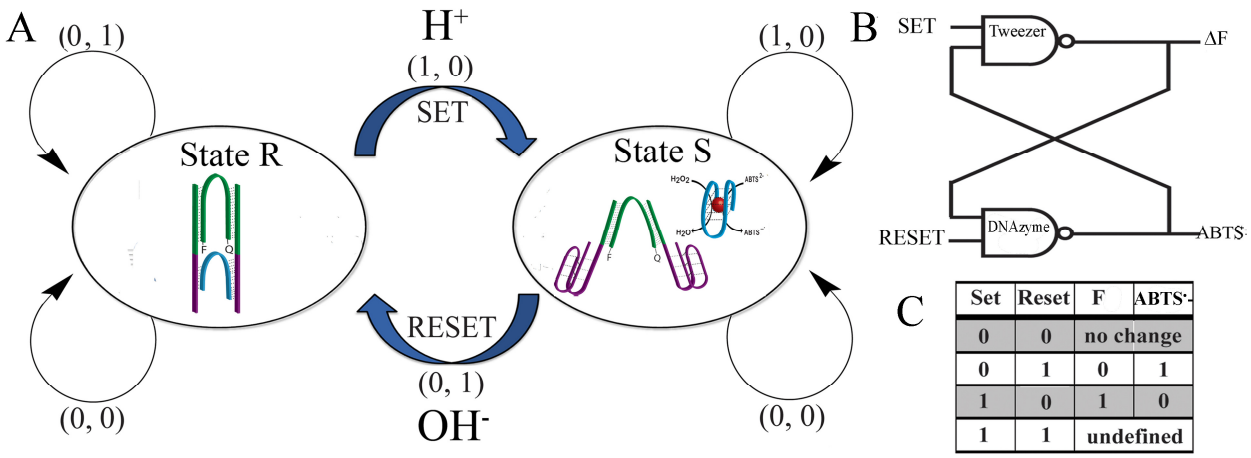


Fig. S1: Non-denaturating gel electrophoresis corresponding to the pH switchable tweezers system shown in Figure 2A. Entries (a) (b) (c) and (d) correspond to (**2**), (**3**), (4) and (**5**). (e) corresponds to the closed state at pH=7.0. (f) corresponds to the open state (does not include sequence (5)). (g) corresponds to the closed state at pH=7.0 after a cycle of opening and closing by lowering the pH to 5.2 and raising the pH back to 7.0, respectively.

SET-RESET system

The fluorescence feature of the tweezers and the $ABTS^{-\cdot}$ color of the DNAzyme generated product, provide two different readout signals that translate the different states of the tweezers device. Thus, we

may consider the tweezers as a SET-RESET system where H^+ and OH^- act as inputs. A negative change in the fluorescence of the fluorophore, and a color formation of the $ABTS^{\cdot-}$, act as true outputs.



Set	Reset	F	ABTS·-
0	0	no change	
0	1	0	1
1	0	1	0
1	1	undefined	

Fig. S2: (A) The use of a pH-activated tweezers device as a SET-RESET logic system. (B) The logic gate circuit of the tweezers SET-RESET system. (C) Truth table of the pH-activated tweezers SET-RESET system.

Experimental Section

Materials: 4 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), sodium chloride and potassium chloride were purchased from Sigma-Aldrich . DNA oligonucleotides were purchased from Integrated DNA Technologies Inc, (Coralville, IA). The products are HPLC pure, and were characterized by mass spectrometry. Hydrogen peroxide solution was purchased from Fluka. 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonate ($ABTS^{2-}$) was purchased from Sigma-Aldrich, Hemin was purchased from Porphyrin Products (Logan, UT), Ultrapure water from NANOpure Diamond (Barnstead) source was used in all of the experiments.

Instrumentation: The absorption spectra of $ABTS^{\cdot-}$ were recorded at 420 nm with a Shimadzu UV-2401 spectrophotometer.

Light emission measurements were performed using a Cary Eclipse Device (Varian inc). The fluorophore Cy5.5 was excited at 680 nm.

Assays: The pH-switchable DNAzyme nanostructure system described in figure 1A was studied in a solution consisting of **1** (30nM) in a 50mM MES buffer (300mM NaCl, 20 mM KCl pH =7.0 or pH=5.2). DNA was diluted in the respective buffers and heated to 90°C for 5 min and slowly cooled

down to room temperature. Hemin and ABTS were added to a final concentration of 15 nM and 2 mM, respectively. The peroxidase-mimicking reaction was initialized by addition of hydrogen peroxide (final concentration of 200 μM).

The ON-OFF system was generated by adding ammonia (10%) in order to change the pH value from 5.2 to 7.0 and acetic acid (20%) in order to change the pH value from 7.0 to 5.2. Following the pH changes, samples were incubated for 20 min before resuming measurements.

The pH-triggered DNA tweezers system described in figure 2A was studied in a solution consisting of **2** (75nM) **3** (75nM), **4** (75nM), **5** (75nM) in a 50mM MES buffer (500mM NaCl, 20 mM KCl pH =7.0 or pH=5.2). DNA was diluted in the respective buffers and heated to 90°C for 5 min and slowly cooled down to room temperature. DNA was allowed to interact for 40 min in room temperature. Hemin and ABTS were added to a final concentration of 15 nM and 2 mM, respectively. The peroxidase-mimicking reaction was initialized by addition of hydrogen peroxide (final concentration of 200 μM).

The ON-OFF system was generated by adding ammonia (10%) in order to change the pH value from 5.2 to 7.0 and acetic acid (20%) in order to change the pH value from 7.0 to 5.2. Following the pH changes, samples were incubated for 40 min before resuming measurements.

Fluorescence experiments:

Experiments were performed in 50mM MES buffer (500mM NaCl, 20 mM KCl , pH =7.0 or pH=5.2). The DNA constructs were prepared as described above for the photometric assay. Following the incubation time, fluorescence measurements were performed. The ON-OFF fluorescence measurements were done as described above for the photometric assay. The Cy5.5 dye was excited at a wavelength of 680 nm. Fluorescence emission spectra were recorded from 700 nm to 750 nm.

Nondenaturing polyacrylamide gel electrophoresis: Gels contained 8 % polyacrylamide (acrylamide/bis-acrylamide). Tris-borate-EDTA (TBE) was used as the separation buffer and consisted of Tris base (89 mM, pH=7.9), boric acid (89 mM) and EDTA (2 mM). Gels were run on a Hoefer SE 600 electrophoresis unit at 25°C (50 V, constant voltage) for 8 hours. After electrophoresis, the gels were stained with SYBR Gold nucleic acid gel stain (Invitrogen) and scanned.

Assay of the gel electrophoresis

Two samples were prepared at pH=7.0 as follows: sample I (closed conformation) - (2), (3), (4) and (5) at a final concentration of 400nM. Sample II (open conformation) - (2), (3), and (4) at a final concentration of 400nM. After a 40 minute incubation period, 5μl were taken out of sample I and the rest of the sample was subjected to an acidic pH (open conformation). After a further 40 minute incubation period another dose of 5μl were extracted from sample I and the sample was then subjected to pH=7.0 for the reclosure

of the tweezers system. The results presented in Figure S1 clearly show a strong band in lanes e-g, confirming the formation of the tweezers structure. The lack of a higher band indicates that, indeed, no oligomers are formed upon the opening and closure of the tweezers system.

The DNA sequences are provided in table S1:

Table S1: Different DNA sequences used to construct the pH-switchable DNAzyme nanostructure systems.

Number	Sequence
(1)	5’ TGGGTAGGGCGGGTTGGGATTTTCACAATTAGATCCTCGTTCCCTAACCCTAACCCTAACCCTTCGTGG ATCTT 3’
(2)	5’ GTTGGAGCGACATTAGAGACCGACCCAATCCCAATCCCAATCCC 3’
(3)	5’ CCCTAACCCTAACCCTAACCCACCTACC CCTGTCCTATCTATGATGG 3’
(4)	5’ Cy5.5-TCTCTAATGTCGCTCCAACAACCATCATAGATAGGACAGG-Iowa Black RQ 3’
(5)	5’ TGGGTCGGC TG GTAGGGCGGGTTGGGTTA 3’