ELECTRONIC SUPPLEMENTARY INFORMATION

Dissipative operation of pH-responsive DNA-based nanodevices

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Contents

1. Experimental

1.1. Chemicals

All reagent-grade chemicals, including HCl, NaOH, Tris HCl, NaCl, $MgCl_2$ (all from Sigma-Aldrich, St. Louis, Missouri), were used as received. 2-(4-Chlorophenyl)-2-cyanopropanoic acid (CPA) and nitroacetic acid (NAA) were prepared according to literature procedures.[1]

1.2. Oligonucleotides

HPLC purified oligonucleotides were purchased from IBA, (Gottingen, Germany) or Biosearch Technologies (Risskov, Denmark). pH-responsive nanoswitch was modified with Alexa Fluor 680 (A-680) at 3' end and Black Hole Quencher 2 (BHQ-2) internally to the sequence.

Re-engineered pH-responsive receptor was modified with Quasar 570 internally while its target (11 mer) with Quasar 670 at 3'end. All oligonucleotides were dissolved in TE buffer (100 mM Tris buffer, 10 mM $MgCl_2$, pH 7.8) at a concentration of 100 μM and frozen at -20 °C. The final concentration of the oligonucleotides was confirmed using Tecan Infinite M200pro (Männedorf, Switzerland) through NanoQuant Plate™ measuring the relative absorbance at 260 nm. The sequences and the relative modifications are reported below.
For pH-titration curves and pH cycles the following pH-responsive nanoswitch was employed (*n* indicates the number of protonation centers, cytosines, in the triplex-forming portion):

pH-responsive nanoswitch (*n*=6):

5'-

*GAGGAGGAAG*TTT(BHQ2)A*CTTCCTCCTC*CTTTGGTTTGGTTTGGTTTGGTTTGGTTT G GTTTG**CTCCTCCTTC** - (A-680) - 3'

For target release-uptake applications the following re-engineered pH-responsive receptor and target were employed:

pH-responsive receptor:

5'- **TTCCTT**TTTTT*TTCCTT*T(Quasar570)TTGGCTAGAG*AAGGAA* - 3'

Target (11 mer):

5'- CTCTAGCCAAA(Quasar 670) - 3'

The portion in bold represents the triplex forming domain, the two portions in italics are

complementary to each other and form the duplex and the underlined portion representsthe linker domains. In the case of the pH-responsive nanoswitch, the first one (with conjugated quencher) represents the linker of the hairpin duplex and the second one represents the linker domain connecting the triplex forming domain and the duplex.

Linker portion was designed to allow optimal stability of the triplex and to avoid cross-interaction. More specifically, while a poly-T linker might seem at first sight the most obvious sequence for a linker we specifically did not use this sequence as it was reported that such loop does not provide enough stability to the triplex structure.[2] The minimal length (5-nt) loop sequence providing the most stability was reported by Eric Kool and co-workers[2] to be the sequence 5'-CTTTG-3' which was indeed the sequence we used for our 5-nt switch. The other most stable sequence for the loop was reported to be the sequence 5'-GTTTG-3'.[2] When designing longer linkers we thus added repeats of this sequence (GTTTG) to avoid the presence of too many cytosines in the loop that could have caused non-specific triplex-forming interactions between the duplex structures and the linker.

1.3. Fluorescence experiments

pH denaturation curves were conducted with a fixed concentration of pH-responsive nanoswitch (25 nM) in 100 mM NaCl and 10 mM $MgCl_2$, at different pH (from 3.0 to 10.0) at 40°C. pH was adjusted by using small aliquot of HCl and NaOH (0.1 M). For each pH the fluorescence signal was recorded every 10 minutes until it reached equilibrium. pH cycles were conducted in 1mL of the same solution and conditions. In details, 60, 50, 42.5, 35 and 25 μl of CPA (5.0 mM) were added in order to lower pH until 4.0, 4.5, 5.0, 5.5 and 6.0 respectively.

6 μl of acetic acid (0.1 M) was used to perform the control experiment.

Binding curves between pH-responsive receptor and its 11 mer target were conducted in 10.0 mM Tris HCl 150 mM NaCl and 10 mM $MgCl_2$, by using a fixed concentration of 30 nM of molecular beacon and increasing concentrations of target at pH 5.0 and 8.0, at 25°C (Fig SI 2).

Target load/release experiments were conducted in 10 mM Tris HCl, 150 mM NaCl and 10 mM $MgCl_2$, in the presence of 30 nM of molecular beacon and 30 nM of target at 25°C, by adding different volumes of NAA 0.1 M (Fig. 3d). In details, 50 (red), 47.5 (yellow), 45 (green), 42.5 (purple), 40 (pink) and 30 (blue) μl of NAA were added.

All fluorescence measurements were obtained using a JASCO FP-8300 - SPS-852T fluorometer with excitation at 680 (± 2.5) nm and acquisition at 702 (± 2.5) nm (for DNA strands labeled with A-680 and BHQ-2), and with excitation at 558 (± 2.5) and acquisition at

570 (± 2.5) nm (for DNA strands labeled with Quasar 570 and Quasar 670) and performed in 1000 μl solution by using quartz cuvette.

For pH curve experiments the observed fluorescence data F_{obs}, were fitted with the following equation:

$$F_{obs} = F_0 + \left(F_{max} - F_0\right) \frac{10^{-(n * pKa)}}{10^{-(n * pKa)} + 10^{-(n * pH)}}$$

Eq. S1

where F_0 = minimum fluorescence signal (triplex state); F_{max} = maximum fluorescence signal (duplex state) and n = number of protonation centers (cytosines).

Curves have been normalized on a 0−1 scale to allow for more ready interpretation of the results.

2. Fig. ESI 1

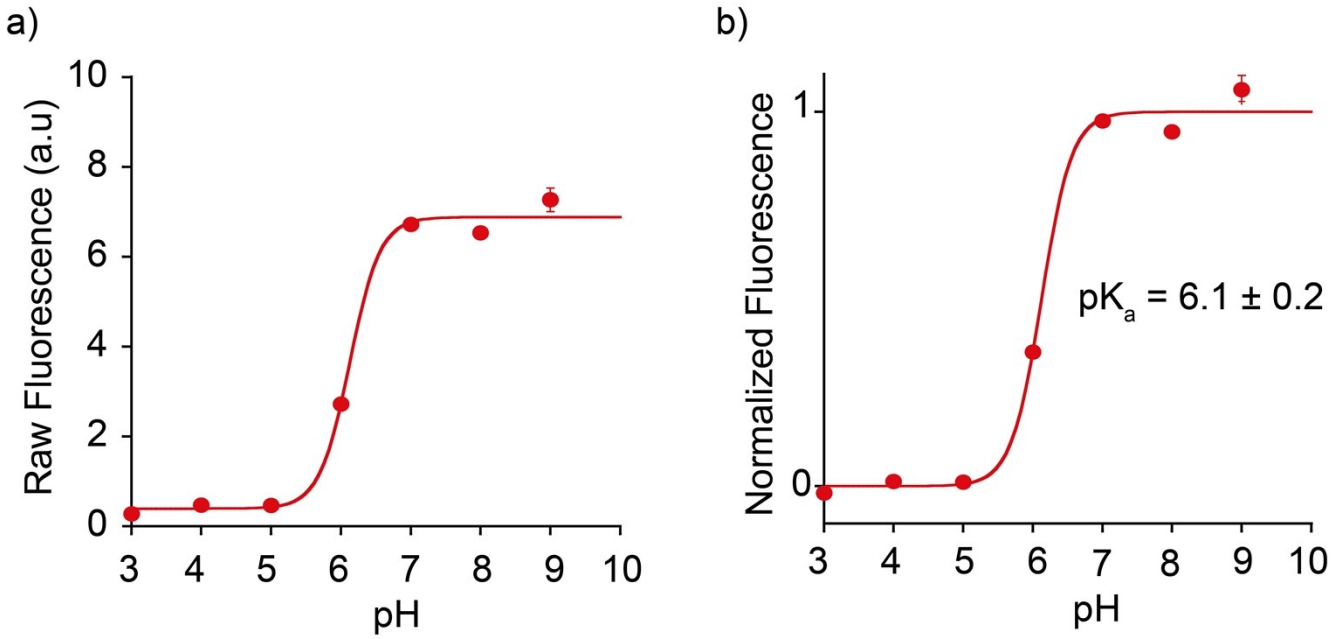

Fig. ESI 1. (a) Raw and (b) normalized pH denaturation curve of 25 nM of pH-responsive nanoswitch in 100 mM NaCl and 10 mM $MgCl_2$ at 40°C. The observed pK_a of 6.1 ± 0.2 is in agreement with the value expected.[3]

3. Fig. ESI 2

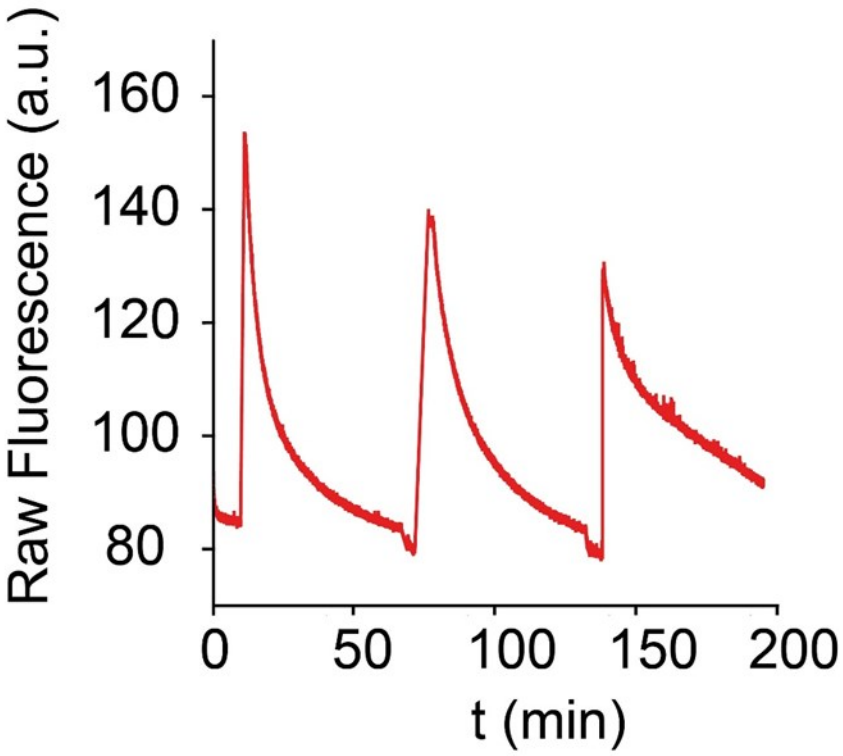

Fig. ESI 2. Three release-uptake cycles obtained by three subsequent additions of small aliquots of NAA. The experiment was carried out in 10 mM Tris HCl, 150 mM NaCl, 10 mM $MgCl_2$ buffer at 25°C.

4. Fig. ESI 3

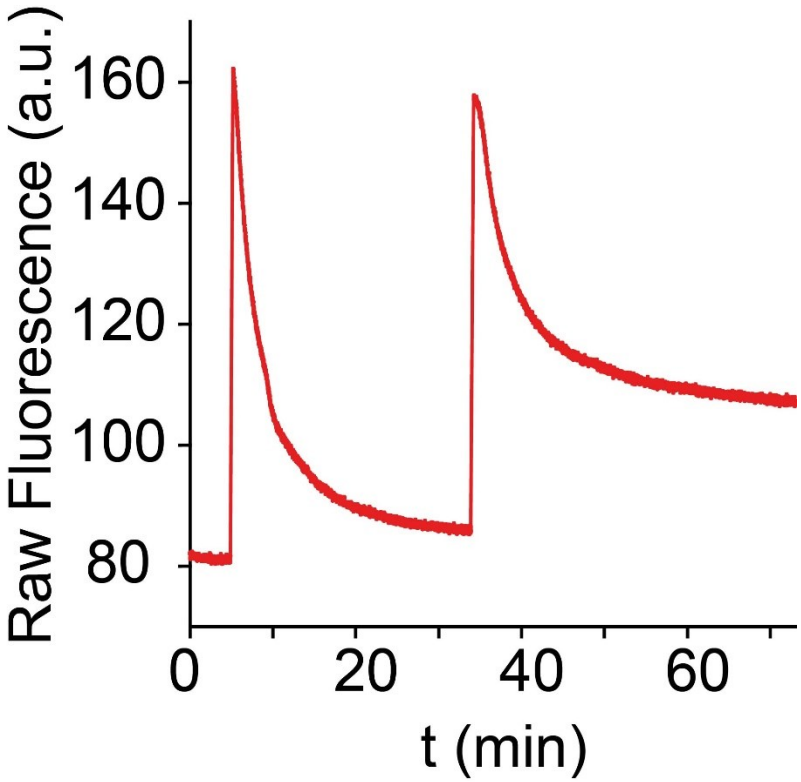

Fig. ESI 3. Two release-uptake cycles obtained by two subsequent additions of small aliquots of NAA, without reset the pH to 8 after the first cycle. The experiment was carried out in 10 mM Tris HCl, 150 mM NaCl, 10 mM $MgCl_2$ buffer at 25°C.

5. References:

(1) (a) H. Brunner and P. Schmidt, Eur. *J. Org. Chem.,* **2000**, *11*, 2119–2133. (b) S. F. Vanier, G. Larouche, R. P. Wurz, and A. B. Charette, *Org. Lett*., **2010**, *12*, 672-675.

(2) S. Wang, M. A. Booher, E. T. Kool, *Biochemistry* **1994**, *33*, 4639-4644.

(3) D. Mariottini, A. Idili, M. A. D. Nijenhuis, G. Ercolani, F. Ricci, *J. Am. Chem. Soc*. **2019**, *141*, 11367-11371.