Dissipative operation of pH-responsive DNA-based nanodevices†

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We demonstrate here the use of 2-(4-chlorophenyl)-2-cyanopropanoic acid (CPA) and nitroacetic acid (NAA) as convenient chemical fuels to drive the dissipative operation of DNA-based nanodevices. Addition of either of the fuel acids to a water solution initially causes a rapid transient pH decrease, which is then followed by a slower pH increase. We have employed such low-to-high pH cycles to control in a dissipative way the operation of two model DNA-based nanodevices: a DNA nanoswitch undergoing time-programmable open–close–open cycles of motion, and a DNA-based receptor able to release-uptake a DNA cargo strand. The kinetics of the transient operation of both systems can be easily modulated by varying the concentration of the acid fuel added to the solution and both acid fuels show an efficient reversibility which further supports their versatility.

Among dissipative synthetic systems, those based on pH variations are particularly attractive due to the (at least apparent) ease of operation and the resemblance with naturally-occurring mechanisms. In fact, since biochemistry has evolved in water, pH variations are probably the most commonly used tool in the chemical control of biosystems. A number of artificial dissipative systems based on pH variations have appeared in the literature, ranging from time programmable aggregation/disaggregation of molecular assemblies to time-controlled motions of molecular machines. Different time-controlled DNA-based systems with dissipative pH changes have been also reported to date mostly based on conformational change DNA-based nanodevices involving cytosine-rich i-motif domains. Despite the elegant nature of these systems, however, i-motif domains remain challenging to engineer into functional devices and the rational modulation of their pKa is difficult to achieve. The pH dissipative cycles employed in these examples involve either the hydrolysis of β-butyrolactone, which allows high-to-low pH cycles, the use of a thiosulfate/sulfite oscillator, which requires continuous fuel supply, or, finally, the use of a complex, biocatalytic enzymatic reaction network.

We and others have, during the recent years, demonstrated that triplex-forming DNA sequences employing Hoogsteen interactions are extremely versatile and their pH-responsiveness can be easily modulated and integrated not only into conformational change DNA switches but also into cargo-loading DNA receptors and reaction networks. It is also known that activated carboxylic acids such as 2-cyano-2-phenylpropanoic acid and its derivatives, trichloroacetic acid, and nitroacetic acid have been employed as versatile and convenient chemical fuels for controlling over time dissipative...

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pH cycles of motions of molecular machines, both switches and motors, host–guest interactions and complex supramolecular assembly, both in aqueous and organic solvents. In water solution, these carboxylic acid fuels are converted by hydroxide ion into the corresponding carboxylates that slowly release CO₂ to be transformed into the strongly basic carbanions R⁻ (Fig. 1a). This remarkable sequence of reactions allows a nimble temporal control of the solution pH, which is simply realized by adding tiny amounts of a concentrated stock solution of fuel to a basic solution.

**Results and discussion**

Motivated by the above arguments, we report here the use of two different activated carboxylic acids, namely 2-(4-chlorophenyl)-2-cyanopropanoic acid (CPA) and nitroacetic acid (NAA) (Fig. 1b), as chemical fuels to drive the dissipative operation of two pH-responsive DNA devices based on triplex-forming Hoogsteen interactions: a conformational change DNA nanoswitch (Fig. 1c) and a cargo-loading DNA nanoscale receptor (Fig. 1d). Despite the same decarboxylation mechanism, the two fuels have somewhat different properties which can make one preferable over the other in specific applications. In particular, NAA decarboxylates faster than CPA, is more soluble and allows greater pH jumps, but, on the other hand, it is less stable and its solutions must be prepared just before use.

In a first series of experiments, CPA was used as a chemical fuel to trigger the conformational change of a pH-responsive acid nanoswitch that can form an intramolecular triplex structure through Hoogsteen interactions between a hairpin duplex domain and a single-strand triplex-forming domain (see Fig. 2a). The nanoswitch is endowed with a pH-independent fluorescent probe and a quencher that allows following its open (duplex) or closed (triplex) state. pH and fluorescence time-course monitoring were carried out by adding the fuel acid to a weakly basic solution (pH = 7.5) containing a fixed concentration of the pH nanoswitch (25 nM) (Fig. 2b and c). Under these conditions the nanoswitch (pKₐ = 6.1; Fig. ESI 1†) is initially in an open conformation as supported by the high fluorescent signal recorded (Fig. 2c, t = 0). Upon addition of the fuel, the pH immediately drops from 7.5 to 5.0 inducing the closing of the nanoswitch. From this point onwards, decarboxylation of deprotonated fuel acid takes place, and the pH slowly increases to the final value of 6.4 (Fig. 2b). Consistently, fluorescence emission increases again due to the triplex to duplex transition. Since fluorescence emissions measured just before the addition of fuel, immediately after the addition of fuel, and at the end of the experiment, are 4.20, 0.20, and 3.20 a.u., respectively, it is apparent that, after 2 hours, 75% of the nanoswitch molecules have experienced a duplex → triplex → duplex cycle at the expense of CPA. Plotting the fluorescence data against the corresponding pH data leads to the binding profile in Fig. 2d with a pKₐ for the duplex-triplex transition which is in good accordance with the reported pKₐ of the switch. The time required for the nanoswitch to return to its initial duplex state can be modulated by varying the concentration of added fuel (Fig. 2e). In fact, it was previously shown that the pH dive and subsequent pH rise to the final, definite value increase on increasing the amount of the added carboxylic acid fuel undergoing decarboxylation. More specifically, we observe half-life times (defined as the time needed to return 50% of the switch to the initial open conformation) ranging from 30 to 127 min by varying the concentration of the added acid fuel from 125 to 300 µM (Fig. 2f). A control experiment was also carried out adding acetic acid instead of CPA to the DNA-based switch. Since acetic acid does not decarboxylate under the given conditions, after the initial duplex-to-triplex transition, no recovery of the fluorescence signal is observed, as expected (Fig. 2e).

In a second series of experiments, we used NAA as a chemical fuel to drive the controlled release-uptake of a cargo DNA strand from a pH-responsive DNA-based receptor. The latter is a stem-loop DNA sequence re-engineered with a pH-dependent domain located on the stem, far from the cargo recognition site (placed on the loop), that allows pH-dependent release-uptake of the cargo itself. At acidic pHs, the Hoogsteen interactions on the stem, in fact, stabilize the stem-loop structure thus hampering the ability of the receptor to bind the cargo DNA sequence (Fig. 3a, right). As the pH increases, the Hoogsteen interactions

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**Fig. 1** (a) Activated carboxylic acids allow the time-controlled pH change of a solution. (b) Chemical structures of the two activated carboxylic acids used in this work. (c) Dissipative control of a pH-responsive conformational change DNA nanoswitch and (d) of a pH-responsive cargo-loading DNA-based receptor.
are destabilized and, as a result, the receptor can efficiently bind the cargo DNA (Fig. 3a, left). Release-uptake of the cargo strand from the receptor can be followed by a pH-independent optical pair on the DNA-receptor/cargo couple. Also in this case, pH and fluorescence time-course experiments were recorded upon the addition of the acid fuel to a solution containing the receptor/cargo complex at a fixed pH (8.0). Immediately after the fuel addition ($t =5$ min, Fig. 3b), the pH decreases from 8 to 4, and the fluorescence emission strongly increases ($t =5$ min, Fig. 3c) suggesting that the cargo strand has been released from the receptor following the formation of the triplex structure on the stem. From this moment onwards, the deprotonated fuel starts to decarboxylate, the pH begins to rise again and the cargo is slowly re-loaded by the receptor (Fig. 3d and e). As a net result, a dissipative cycle of release-uptake has been realized by means of NAA. Furthermore, the amount of the DNA cargo transiently released in solution can be finely modulated by regulating the amount of the added fuel. It is noteworthy that the time programmable pH cycles driven by both fuels CPA and NAA (low → high pH cycles), are complementary to those reported by Walther and Heinen (high → low pH cycles),\textsuperscript{12} and, compared to the pH cycles based on the thiosulfate/sulfite oscillator,\textsuperscript{13} do not require a continuous fuel supply, moreover do not need the presence of enzymes that may limit the application range.\textsuperscript{14,26} As the number of responsive DNA-based devices is rapidly growing, the possibility to precisely control the energy dissipating

Conclusions

In conclusion, we have demonstrated that the abiotic fuels CPA and NAA can be conveniently used to control the dissipative operation of pH-responsive DNA-based devices. In one case a conformational-change switch can be closed (triplex state) and then re-opened (duplex state) under the action of the pH variations (low → high pH cycles) driven by CPA, with the possibility to control at will the time required for the closing step (triplex → duplex transition). In the other case, NAA enables low → high pH cycles, which drive the autonomous release-uptake of a cargo DNA-strand to a DNA-based receptor. Remarkably, the amount of the cargo DNA transiently released in solution can be finely modulated by regulating the amount of the added fuel. It is noteworthy that the time programmable pH cycles driven by both fuels CPA and NAA (low → high pH cycles), are complementary to those reported by Walther and Heinen (high → low pH cycles),\textsuperscript{12} and, compared to the pH cycles based on the thiosulfate/sulfite oscillator,\textsuperscript{13} do not require a continuous fuel supply, moreover do not need the presence of enzymes that may limit the application range.\textsuperscript{14,26} As the number of responsive DNA-based devices is rapidly growing, the possibility to precisely control the energy dissipating
pathway with different chemical fuels represents an essential step towards the design of DNA functional machines and materials with “life-like” behaviour.

Experimental

Experimental details in ESI†

Author contributions

SDS, FR and GE conceived the general design. SDS, GE, DM, DDG and FR designed the experiments. DDG and DM performed the experiments. SDS, GE, DM, DDG and FR analyzed the data. SDS, GE and FR wrote the article.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

§ The fact that the final pH differs from the initial one (about one unit lower) depends on the multiple acid–base equilibria involving fuel by-products including CO$_2$. More specifically, in the case of NAA the complex network of acid–base equilibria associated to the reaction has been analysed and discussed in detail in a previous work (ref. 24). An analogous behaviour is expected for CPA. As to the role of CO$_2$, one may ask whether there are differences when the system is operated either in an open or closed reactor. Of course, in a closed system an increase in the partial pressure of CO$_2$ is expected that translates into a higher concentration of CO$_2$ in solution. However, we recorded only a slightly lower final pH value (ca. 0.3 units) when the experiment was carried out in a closed reactor.

¶ If the pH is not reset to 8 after each release-uptake cycle by adding the required amount of NaOH, the system loses efficiency, probably due to the accumulation of reaction products that somehow affect the decarboxylation process (see Fig. ESI 3f and ref. 24).

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