Modulating the lifetime of DNA motifs using visible light and small molecules

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Photoacid, dissipative, i-motif, DNA nanotechnology, photoswitch

ABSTRACT: Here we regulate the formation of dissipative assemblies built from DNA using a merocyanine photoacid that responds to visible light. The operation of our system, and the relative distribution of species within it, are controlled by irradiation time, initial pH value, and the concentration of a small molecule binder that inhibits the reaction cycle. This approach is modular, does not require DNA modification, and can be used for several DNA sequences and lengths. Our system design allows for waste-free control of dissipative DNA nanotechnology, towards the generation of non-equilibrium, life-like nanodevices.

Life operates far from equilibrium through complex reaction networks that employ biochemical fuels and feedback loops to drive reactivity. Synthetic systems that emulate the transient nature of biology give access to 'life-like' properties—for example, communication, growth, and evolution—that may find applications in soft robotics,[1] delivery,[2] and synthetic biology.[3] Systems chemistry approaches enable complex behavior to emerge from simple or cascading chemical reaction networks.[4] Exporting these concepts to synthetic, out-of-equilibrium systems may select specific reaction pathways that generate otherwise inaccessible products.[5]

The design of complex, dissipative systems requires robustness, programmability, and recognition specificity of the chemical units used. DNA often meets these requirements,[6] leading to a range of functional systems where chemical fuels—pH modulators,[7] redox agents,[8] DNA/RNA,[9] ATP,[10] and other small molecules[11]—have been used to drive DNA structure assembly and function.

Light can be a superior assembly stimulus to chemical fuels as it may avoid waste production and can attain high spatiotemporal precision.[12] Photoswitches have been covalently attached to DNA[13] or used as reversible binders[14] to control nanostructures, but few examples have taken advantage of light-induced pH shifts to influence DNA assembly.[15a,5b,15b] Chemically-fueled systems often use single-strand DNA[16] or base mismatches[7a-c] to promote structural variation under mild pH switching conditions. Accessing larger, reversible changes in pH without waste generation may prevent fatigue and systems failure in cyclic chemical networks. Using photo-responsive components to deliver chemical information may lead to stimuli-responsive and non-equilibrium DNA nanotechnologies and devices powered by light.

Here we show that light can control the lifetime of structures built from unmodified DNA sequences. We used merocyanine photoacid **1**,[17] which isomerizes to spiropyran **2** with visible light, releasing a proton. The resulting large changes in pH generate transient DNA structures upon irradiation (Figure 1). This process is dissipative, controlled by the kinetics of proton sequestration of the photoacid, the kinetics of the DNA folding processes, and the initial pH of the sample. We show that this process is tolerant to a diversity of DNA sequences and lengths, and that small-molecule binders may act as secondary modulators of DNA nanostructure lifetimes.

Our system is comprised of two DNA strands—one guanine-rich and one cytosine-rich, both from the human telomeric region—that form double-stranded DNA (dsDNA, **3**) at physiological pH. At pH < 5, cytosine residues are protonated, leading to the formation of i-motif[18] **4**, which consequently triggers the formation of G-quadruplex[19] **5** in the presence of potassium ions. The i-motif and G-quadruplex structures are naturally occurring and have been used in a wealth of DNA-based nanotechnologies:[20] as biochemical sensors *in vivo*,[6a] mechanochemical devices,[21] and to design new therapeutics.[22] Controlling the

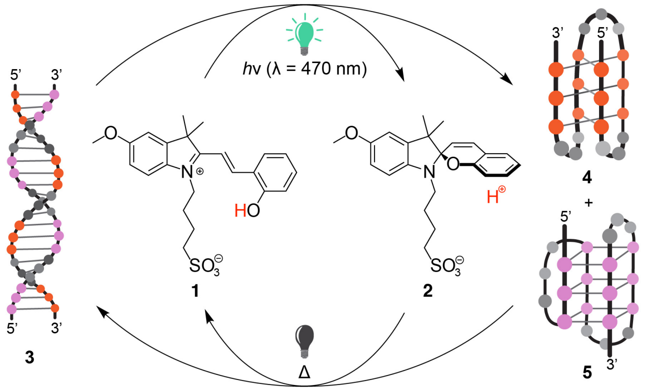

Figure 1. Dissipative assembly of DNA structures triggered by light. The reversible, light-triggered pH decrease by merocyanine photoacid **1** results in the transition of dsDNA (**3**) to i-motif[23] (**4**) and G-quadruplex[24] (**5**) structures. Sequences are derived from human telomeric DNA (see Supporting Information S3.1). When the light is removed, the system relaxes back to its equilibrium state. Colour coded nucleotides are purple: guanine, orange: cytosine, grey: thymine, dark grey: adenine.

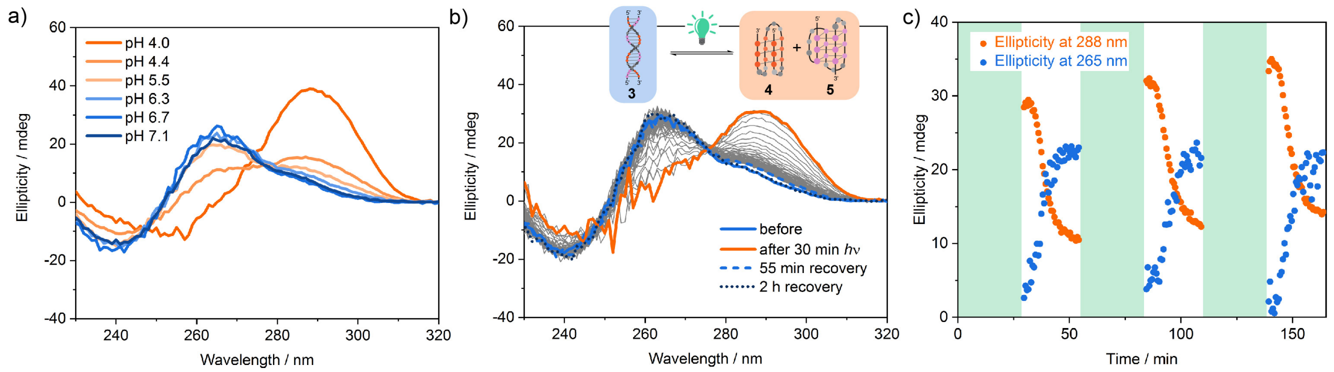

Figure 2. Static vs out-of-equilibrium transitions between DNA structures. a) Circular dichroism (CD) spectra of human telomeric DNA oligomers at different pH values without photoacid **1**: blue = duplex; orange = i-motif/G-quadruplex. b) CD spectra of a sample with an initial pH of 6.8 measured before (blue), immediately after 30 min *hν* (λ = 470 nm, orange), and following the recovery in the dark up to 2h after irradiation (blue dotted). c) Repeated irradiation-recovery cycles of the DNA secondary structure transitions following the CD signal at 288 nm (orange, i-motif/G-quadruplex) and 265 nm (blue, duplex). The irradiation time (30 min) is indicated in turquoise followed by 25 min recovery. C-rich sequence: 5'- CCC TAA CCC TAA CCC TAA CCC -3', G-rich sequence: 5'- GGG TTA GGG TTA GGG TTA GGG -3'.

temporality of these structures may lead to next-generation devices and soft robotics materials capable of work and motion, based on genomic sequences.

Coupling these useful sequences with photoacid **1** allows the operation of an out-of-equilibrium cycle. The proton released upon irradiation of **1** converts **3** to **4** and **5** (Figure 1). In the dark, the pH returns to its initial value. The cytosine residues in the i-motif are thus deprotonated and recombine with the G-rich strand to reform dsDNA **3**. The dissipative assembly of **4** and **5** only occurs during irradiation; when the light is removed, **4** and **5** unfold to make **3**.

Light-induced DNA structural changes were confirmed by CD and NMR spectroscopies (see Supporting Information S1 to S2 for details). A mixture of 1.5 mM photoacid **1**, 20 μM dsDNA, 7.5 mM Mg^{2+} and 100 mM K^{+} was irradiated at 470 nm for 30 min, leading to a pH change of *ca.* 2.5 pH units. Following irradiation, we observed a decrease in intensity of the CD band at 265 nm, characteristic of dsDNA, and the appearance of a CD band at 288 nm, characteristic of both the i-motif and G-quadruplex (see Figure 2b, Supporting Information S3.1). We confirmed the formation of i-motif and G-quadruplex structures by *in situ* irradiation during ^{1}H NMR experiments (see Supporting Information S3.1.4), which matched with the literature for related structures.[23] Monitoring the structural change under irradiation by NMR spectroscopy also confirmed the reversibility of our process (see Supporting Information S3.1.4). Repeated switching of the DNA secondary structures was confirmed by CD spectroscopy and showed minimal fatigue (Figure 2c, see Supporting Information S5).

The distribution of DNA secondary structures can be tuned by changing the initial pH of the system with KOH or HCl aliquots, which we studied over a pH range of 5.9–7.0 using CD spectroscopy (Figure 3, see Supporting Information S3.1.2). A lower initial pH led to a higher ratio of i-motif/G-quadruplex to dsDNA in the dark. The initial pH value also determined the pH that was accessed under irradiation, with lower initial pH values leading to lower pH under irradiation. When lower pH values were accessed under irradiation, higher ratios of i-motif/G-quadruplex to dsDNA were formed. The ability of photoacid **1** to function at different pH values allows product ratios within the dissipative system to be tuned, underscoring **1** as a tunable additive for pushing nanostructures away from equilibrium.

The kinetics of recovery of the system in the dark can also be controlled by photoacid **1** (Figure 3a). In the dark the rate of structural change from i-motif/G-quadruplex to dsDNA appeared to closely follow the rate of pH recovery (Figure 3).[25 26] The rate of pH change due to photoacid **1** follows non-trivial kinetics, as the equilibration rate is pH dependent, and the pH is changing throughout.[27,17] During the recovery in the dark, the i-motif was deprotonated around pH 4.5–4.75 (see Supporting Information S3.4) to form dsDNA, which explains the initial plateau observed in Figure 3a. We used a first-order exponential fit to approximate the apparent half-lives ($t_{1/2}$) of the equilibration processes in the dark. Higher initial pH values resulted in fast recovery of the pH value (apparent $t_{1/2}$ = 1.3 min), whereas lowering the initial pH significantly slowed the pH recovery (apparent $t_{1/2}$ = 5.1 min for initial pH 6.3 and 6.8 min for initial pH 5.9).

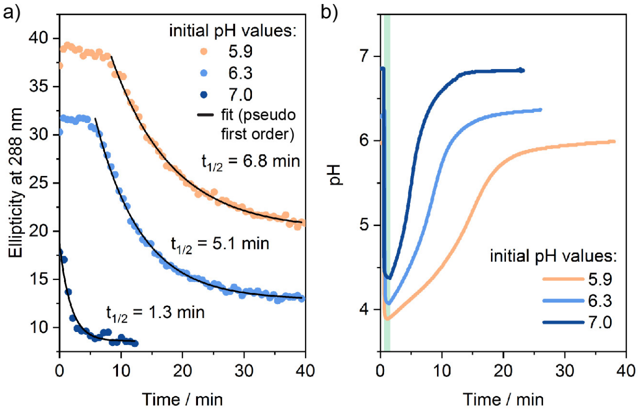

Figure 3. **The relaxation kinetics of the network are determined by the initial pH of the system.** a) Decrease of the i-motif/G-quadruplex signal at 288 nm in the dark after 30 min *hν* (λ = 470 nm) of three samples with varying initial pH values. Fits are pseudo first-order after initial plateau to give apparent half-lives. b) Light-induced pH changes at different initial pH values followed by recovery in the dark; *hν* (λ = 470 nm) indicated by the turquoise bar.

In contrast, under irradiation the conversion of **3** to **4** and **5** did not follow the rate of the fast pH jump (<1 min), suggesting the rate of strand dissociation upon protonation is the rate limiting step of the reaction. Prolonged irradiation times (30 min) were required for the unwinding of the duplex, as also shown by NMR spectroscopy (see Supporting Information S3.1.4).

Our method is tolerant to other DNA lengths and sequences known to form congeners of **4** and **5** (see Supporting Information S3.2–S3.3). We studied two other known i-motif forming sequences of the c-MYC promoter[28] (22mer) and ILPR region[29] (31mer). These sequences form i-motif structures at higher pH values (pH < 6, 7, see Supporting Information S3.2.1, S3.3.1) than the sequence discussed above (pH < 5). Both sequences were observed to undergo reversible, light-induced secondary structure changes. The proportion of the i-motif and G-quadruplex formed, relative to dsDNA, varied under irradiation and was dependent on the initial pH values, as observed for the human telomeric sequence investigated above. The recovery kinetics of the DNA duplex in the dark also depend on the rate of recovery of photoacid **1** (see Supporting Information S3.4). Our results underscore photoacid **1** as a general tool to induce i-motif folding among a variety of unmodified DNA of different lengths, each sensitive to different pH ranges.

Host-guest chemistry may offer a facile method to modulate both the thermodynamics and kinetics of structure formation.[30] We thus hypothesized that small molecule binders may promote the persistence of specific structures in dissipative networks by acting as inhibitors.

We used DNA-binder *meso*-5,10,15,20-tetrakis-(*N*-methyl-4-pyridyl)porphine tetratosylate (TMPyP4, Figure 4a) to further modulate the recovery kinetics in the dark. TMPyP4 is known to bind to G-quadruplex[31] and i-motif[32] structures. When samples containing TMPyP4, photoacid **1** and dsDNA **3** were irradiated, **4** and **5** were formed, as measured by CD spectroscopy (SI S4.1.2). In the dark, the system was observed to recover significantly slower than under the same conditions in the absence of the DNA binder (see Supporting Information S4), suggesting that TMPyP4 acts an inhibitor. Sequential irradiation cycles with increasing binder concentrations resulted in progressively slower recovery kinetics in the dark (Figure 4b). The apparent half-life of 1.7 min in the first recovery cycle was increased to 57 min in the presence of 1 eq binder, while no significant recovery was observed after the third irradiation cycle in the presence of 2 eq. binder (see Supporting Information S4.1.2 for fits).

We propose that these results are due to three factors. Firstly, increasing the binder concentration shifts the equilibrium of the system from **3** towards **4** and **5**. The final CD signal of the system after recovery increases with increasing binder concentration, suggesting the persistence of folded DNA structures even after pH recovery. Secondly, we observed a lower final pH value after recovery in the dark, indicating an interaction of photoacid **1** with the system. Control experiments verified the pH switching behavior of **1** was unchanged in the presence of DNA or binder separately (see Supporting Information S4.2, S5), indicating that this is a system property, as opposed to specific interactions between any two components. Thirdly, the presence of TMPyP4 alters the rate of equilibrium recovery. After the binder addition and irradiation, the i-motif/G-quadruplex structures persisted for longer than the pH recovery (Fig. 4b). As TMPyP4 is expected to bind stronger to **5**,[32,31] we hypothesize that the G-quadruplex preferentially persists even after irradiation, while the i-motif unfolds. Importantly, **4** and **5** could still not be accessed without light, suggesting that small molecule binders may be used to control the lifetime of DNA secondary structures that are pushed away from equilibrium.

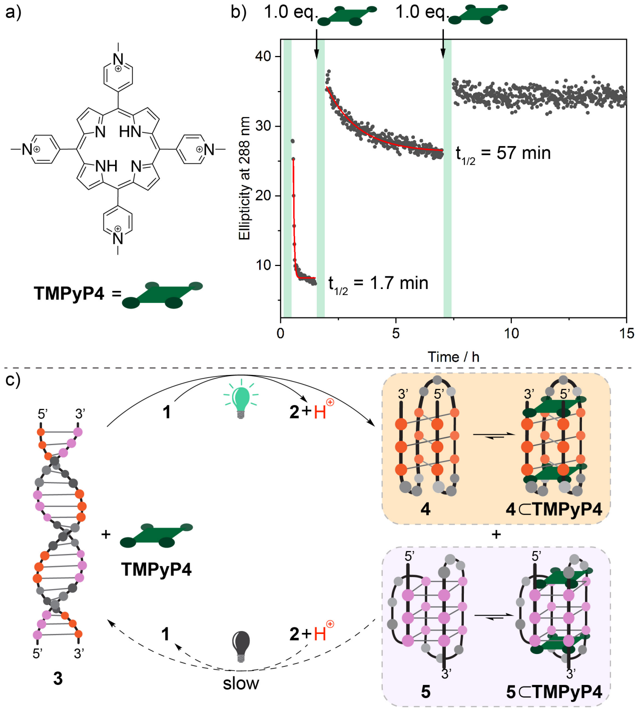

Figure 4. **Small-molecule binders modulate structure lifetimes by inhibiting dsDNA recovery.** a) Structure of TMPyP4. b) Kinetics of the i-motif/G-quadruplex recovery after multiple irradiation cycles (turquoise boxes, initial pH = 6.8) as well as sequential addition of TMPyP4. Pseudo first order exponential fits in red with apparent half-lives of 1.7 min for the first and 57 min for the second recovery cycle. c) Light-induced DNA secondary structure modulation by photoacid **1**, showing the additional equilibrium of the G-quadruplex and i-motif with TMPyP4. Depiction of binder interactions are simplified.[32,31]

Our results demonstrate the potential of systems chemistry and supramolecular concepts for the design of dissipative DNA systems. Photoacid **1** is ideal for pH control in biologically relevant systems away from equilibrium. In this study we have applied photoacid **1** to tune dsDNA/i-motif/G-quadruplex ratios and system kinetics. This approach is versatile and was applied to DNA oligomers with varying pH sensitivity, sequences, and lengths, and avoids the need for DNA mismatches or covalent modification of base pairs. The addition of an i-motif/G-quadruplex binder provides a further handle for controlling the recovery kinetics, demonstrating the utility of small molecules in regulating structural lifetimes by acting as system inhibitors. Our approach allows modulation of DNA structures in dissipative systems using visible light. This method may lead to the development of more sophisticated out-of-equilibrium systems and DNA devices, which are currently finding diverse applications in materials, biomedical and engineering spaces.

ASSOCIATED CONTENT

Experimental procedures, spectroscopic and pH data are available in the Supporting Information.

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Author Contributions

The manuscript was written through the contributions of all authors.

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Notes

The authors declare no competing financial interest.

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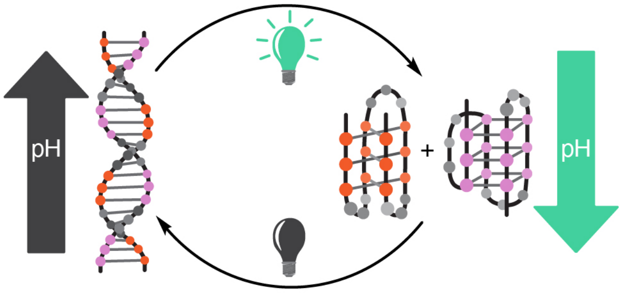
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