Switchable DNA-Based Peroxidases Controlled by a Chaotropic Ion**

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Here we demonstrate a switchable DNA electron-transfer catalyst, enabled by selective destabilization of secondary structure by the denaturant, perchlorate. The system is comprised of two strands, one of which can be selectively switched between a G-quadruplex and duplex or single-stranded conformations. In the G-quadruplex state, it binds hemin, enabling peroxidase activity. This switching ability arises from our finding that perchlorate, a chaotropic Hofmeister ion, selectively destabilizes duplex over G-quadruplex DNA. By varying perchlorate concentration, we show that the DNA structure can be switched between states that do and do not catalyze electron-transfer catalysis. State switching can be achieved in three ways: thermally, by dilution, or by concentration.

Introduction

Biomolecules are highly attractive as potential programmable electron transfer catalysts and bioelectronics. A range of techniques have been employed, including microbial systems,[11] protein-directed assembly of metal clusters,[2] metallated base pairs,[3] biopolymer-directed assembly of nanoparticles,[4] and minimal peptides.[5] With 0.34 nm spacing between nucleobases and exquisite, atomic-precision self-assembly directed by nucleobase hydrogen bonding, nucleic acids are an especially attractive means by which to develop programmable electron transfer catalysts. One means by which DNA can be selectively switched is by employing a prosthetic group,[7] as many enzymes that perform electron transfer in nature do.[8] Hemin, one such prosthetic group, binds selectively to a noncanonical structure formed by G-rich sequences of DNA, termed a G-quadruplex, which activates it to perform electron transfer.[9,10] By binding hemin, the same prosthetic group employed by natural redox enzymes, such as peroxidases and cytochrome P450s, DNA can perform electron transfer in a biomimetic fashion while mitigating the oxidative damage issues associated with attempting to use it as a classical conductor.[11]

Sequences of DNA and RNA that can exhibit either G-quadruplex or non-G-quadruplex (e.g., unpaired or Watson-Crick base paired) structures depending on context are a ubiquitous feature of life. These sequences are actively remodeled in living organisms.[12–14] A bioinspired chemical system based on these phenomena that enabled reversible, programmable structure switching would afford a powerful tool for dynamic electron transfer behavior in DNA nanostuctures. We thus sought to develop a chemical system to switch the same DNA between secondary structure states, reasoning that this would combine the speed and repeated reversibility of pH-switchable DNA nanomotors with the compatibility observed in static pH strand-exchange based systems.[15–20]

Base pairs have ca. one half the solvent-buried hydrophobic surface area of G-quartets, and ions exert Hofmeister effects by interactions with the surface of biopolymers.[21] Similarly, G-quartets coordinate dehydrated ions, and high-salt solutions influence biopolymer folding by osmotic effects.[21,22] We thus reasoned that a DNA duplex and G-quadruplex would exhibit differential destabilization by chaotropes. Here, we demonstrate that perchlorate is a selective denaturant for duplex vs. quadruplex DNA. We have exploited this phenomenon to develop a minimal electron transfer catalyst made of DNA that can be switched between three states: a duplex, a G-quadruplex, and a single-stranded state. We show that this switching can be performed thermally, by dilution, or by concentration. We show that the DNA structure can be switched over 100 times without degradation, and that it can perform multiple-turnover electron transfer catalysis by binding
hemin and catalyzing electron transfer in the G-quadruplex state (Figure 1).

Results

We designed two FRET reporter systems, G4-Dark and Duplex-Dark (Figure 2a and Table 1), to allow readout of their folding state. Both reporter systems were of the same length (24 nt) and sequence but differed in fluorophore and quencher placement. G4-Dark was comprised of equimolar amounts of Fluorescein-G4-Quencher, a DNA sequence that could form a G-quadruplex, and G4Comp, its Watson-Crick complement. Fluorescein-G4-Quencher was 5’-labeled with a fluorescein tag and 3’-labeled with a quencher. Thus, this system would exhibit fluorescence when Fluorescein-G4-Quencher was either unfolded or hybridized to G4Comp. When Fluorescein-G4-Quencher was folded into a G-quadruplex, fluorophore and quencher would be brought into spatial proximity and quenched.

Duplex-Dark was comprised of equimolar amounts of strands of the same sequences as we employed in G4-Dark with rearranged reporters. Fluorescein-G4, a 5’-fluorophore-labeled sequence that could form a G-quadruplex, and G4Comp-Quencher, a 3’ quencher-labeled sequence. In this system, the fluorescein reporter exhibits fluorescence when Fluorescein-G4 is either folded into a G-quadruplex or is unfolded. Upon hybridization of the two strands to form a double helix, this system would quench.

These strands followed the Hofmeister trend of stability (sulfate > chloride > nitrate > perchlorate). (Supporting Information Figure S1). Both G-quadruplex and duplex structures were increasingly destabilized as chaotropicity of the salts increased; however, duplex was much more susceptible than the G-quadruplex, particularly in sodium perchlorate.

We next examined the thermal response of the Duplex-Dark system in LiClO₄ solution (conditions in which only duplex and unfolded states are possible, due to the stability trend in G-quadruplexes (K⁺ > Na⁺ > Li⁺).[22] (Table 2, Figure 2b, Supporting Information Figures S2–7).[22] At 100 mM LiClO₄, Duplex-Dark exhibited fluorescence response consistent with a double-stranded to unfolded structural transition at 79.8 °C (Duplex-Dark possesses spatially adjacent reporters in the duplex state, resulting in a systematic slight elevation of Tm relative to other sequence-related systems with differing tags). As the salt concentration was increased, the thermal midpoint initially increased to a maximum of 84.7 °C in 0.3 M LiClO₄ above which it decreased to a minimum of 50.1 °C in 4 M LiClO₄. This is consistent with electrostatic stabilization effects being predominant at lower salt concentrations and Hofmeister ion effects at higher concentration, as has been previously observed.[23]

### Table 1. Sequences employed in this study.

| System/strand | Sequence | Length |
|---------------|----------|--------|
| G4-Dark       | 5′-6-FAM-TG GGT TAG GGA ATT CCG GGT AGG G-3′ Iowa Black FQ-3′ | 24 nt |
| G4Comp        | 5′-CCC TAA CCC GAA TCC CCT AAC CCA-3′ | 24 nt |
| Duplex-Dark   | 5′-6-FAM-TG GGT TAG GGA ATT CCG GGT AGG G-3′ | 24 nt |
| G4Comp-Quencher | 5′-CCC TAA CCC GAA TCC CCT AAC CCA-Iowa Black FQ-3′ | 24 nt |
| G4-SwitchR    | 5′-GGG TAG GGC GGG TGG GGA-3′ | 18 nt |
| G4Redox       | 5′-TCC CAA CCC GGC CTA CCA-3′ | 18 nt |
Figure 2. Systems employed in fluorescence-monitored thermal denaturation experiments (panel a). Duplex-Dark (left column of panel a) consists of a 5'-fluorescein labeled strand (green star) and a 3'-Iowa Black FQ (grey cloud) strand and can exist in three states: A quenched duplex, a dequenched G-quadruplex and single strand, and a dequenched set of two single strands. G4-Dark (right column of panel a) consists of a dual-labeled (5’-fluorescein, 3’-Iowa Black FQ) strand and its complement and can also exist in three states: A dequenched duplex, a quenched G-quadruplex and single strand, and a dequenched set of two single strands. When Duplex-Dark is operated in LiClO₄ solution, only duplex and single-stranded states are accessible. This transition is destabilized with increasing perchlorate (panel b). When only the Fluorescein-G4-Quencher component of G4-Dark is operated in NaClO₄ solution (panel c), the G-quadruplex-forming strand is significantly less destabilized by perchlorate (panel d), in contrast to the duplex, which exhibits ca. linear destabilization with increasing NaClO₄ concentration above 1 M (panel d). As a result of this differential stability, G4-Dark (panel e) and Duplex-Dark (panel f) can be switched thermally between duplex, G-quadruplex, and single-stranded states, and the temperatures at which these transitions occur can be tuned by varying the concentration of NaClO₄. In low perchlorate (0.1 M), only the duplex-to-single stranded transition is observed (red circles). In intermediate perchlorate (4 M), the DNA structure transitions between duplex (at low temperature), G-quadruplex (at intermediate temperature), and single-stranded (at high temperature) states (blue squares). In high perchlorate (saturated, ca. 9.5 M), the DNA structure exists only in the G-quadruplex and single-stranded states (black hourglasses).

Table 2. Thermal midpoints (°C) of 4-Dark, Duplex-Dark, and Fluorescein-G4-Quencher in NaClO₄ solution, and of 4-Dark and Duplex-Dark in LiClO₄ solution.

| Strands/[ClO₄]_2⁻ (M) | 0.1 | 0.5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Sat. |
|-----------------------|-----|-----|---|---|---|---|---|---|---|---|------|
| Duplex-Dark (Fluorescein-G4 and G4Comp-Quencher)/LiClO₄ | G4 none none none none none none n/d [a] n/d [a] n/d [a] n/d [a] | 79.8 | 84.7 | 83.0 | 72.7 | 62.6 | 50.1 |  |  |  |  |
| Fluorescein-G4-Quencher/LiClO₄ | G4 65.1 82.7 88.4 [b] 92 [c] 88 [c] 86 [c] 81.3 77.8 75.1 74.9 | 73.7 |  |  |  |  |  |  |  |  |  |
| Fluorescein-G4-Quencher/NaClO₄ | G4 65.1 82.7 88.4 [b] 92 [c] 88 [c] 86 [c] 81.3 77.8 75.1 74.9 | 73.7 |  |  |  |  |  |  |  |  |  |
| Fluorescein-G4-Quencher/NaClO₄ | G4 65.1 82.7 88.4 [b] 92 [c] 88 [c] 86 [c] 81.3 77.8 75.1 74.9 | 73.7 |  |  |  |  |  |  |  |  |  |
| G4-Dark (Fluorescein-G4-Quencher and G4Comp)/NaClO₄ | G4 none none high [h] 83.2 80.2 78.1 75.3 74.4 72.6 | 73.9 | 77.4 | 73.8 | 66.7 | 48.8 | 45.6 | 37.0 | 32.2 | 27.0 | low [l] |
| G4-Dark (Fluorescein-G4-Quencher and G4Comp)/NaClO₄ | G4 none none high [h] 83.2 80.2 78.1 75.3 74.4 72.6 | 73.9 | 77.4 | 73.8 | 66.7 | 48.8 | 45.6 | 37.0 | 32.2 | 27.0 | low [l] |
| G4-Dark (Fluorescein-G4-Quencher and G4Comp)/NaClO₄ | G4 none none high [h] 83.2 80.2 78.1 75.3 74.4 72.6 | 73.9 | 77.4 | 73.8 | 66.7 | 48.8 | 45.6 | 37.0 | 32.2 | 27.0 | low [l] |
| G4-Dark (Fluorescein-G4-Quencher and G4Comp)/LiClO₄ | G4 none none high [h] 83.2 80.2 78.1 75.3 74.4 72.6 | 73.9 | 77.4 | 73.8 | 66.7 | 48.8 | 45.6 | 37.0 | 32.2 | 27.0 | low [l] |
| G4-Dark (Fluorescein-G4-Quencher and G4Comp)/LiClO₄ | G4 none none high [h] 83.2 80.2 78.1 75.3 74.4 72.6 | 73.9 | 77.4 | 73.8 | 66.7 | 48.8 | 45.6 | 37.0 | 32.2 | 27.0 | low [l] |
| G4-Dark (Fluorescein-G4-Quencher and G4Comp)/LiClO₄ | G4 none none high [h] 83.2 80.2 78.1 75.3 74.4 72.6 | 73.9 | 77.4 | 73.8 | 66.7 | 48.8 | 45.6 | 37.0 | 32.2 | 27.0 | low [l] |

[a] Unfolding transition not complete at 95 °C so sigmoid fit not possible, number reported obtained using first derivative method where possible. [b] Folding transition not complete at 20 °C. [c] Only duplex-single stranded transition is observed in this system due to the absence of sodium and reporters used. [d] Not determined owing to the lower solubility of LiClO₄ vs. NaClO₄.

We next examined the thermal response of the Fluorescein-G4-Quencher component of G4-Dark in NaClO₄-containing solution to ascertain the impact of this salt on the stability of the G-quadruplex states of this system (Figure 2c, Supporting Information Figures S8–18). In all NaClO₄-containing solutions, Fluorescein-G4-Quencher formed a G-quadruplex, giving a 65.1 °C thermal midpoint in 100 mM NaClO₄; this temperature increased with increasing NaClO₄ consistent with the electrostatic screening afforded by Na⁺ as well as its binding to the central channel within the G-quadruplex. Between 1–4 M NaClO₄, the G-quadruplex was so stable it was not fully denatured, even at 95 °C (representative melting curve of 4 M NaClO₄ in Figure 2c). In 5 M NaClO₄, and above, thermal midpoints were measurable and decreased with increasing
perchlorate but remained high. Even in saturated NaClO₄ (ca. 9.5 M and containing less than three water molecules per ion), Fluorescein-G4-Quencher exhibited a higher thermal midpoint (73.7 °C) than in 100 mM NaClO₄ (65.1 °C).

The destabilization of the DNA duplex in G4-Dark and Duplex-Dark was marked (Figure 2d) and exhibited a near-linear response in thermal midpoint of −7°C/M NaClO₄ between 1 and 9 M (r² = 0.97); these duplexes exhibited thermal midpoints of ca. 75 °C at 100 mM NaClO₄ and ca. 27–30 °C at 7–8 M NaClO₄. In contrast, perchlorate-induced destabilization of the G-quadruplex formed by the G-rich strands was less pronounced and/or more than compensated by the presence of additional sodium (Figure 2d). Fluorescein-G4-Quencher exhibited a thermal midpoint of 65.1 °C at 100 mM NaClO₄ and 73.7 °C in saturated sodium perchlorate (ca. 9.5 M). That is, in a polymorphic sequence, one possible secondary structure (dsDNA) that is more stable in low perchlorate than an alternative fold (a G-quadruplex) becomes less stable than the alternative fold in high perchlorate.

Because of this, we speculated that G4-Dark could be thermally switched between all three states (duplex, G-quadruplex, and single-stranded) at intermediate perchlorate concentrations. (Figure 2e, Supporting Information Figures S19-29). Consistent with this, we observed a single transition at low (0.1 M) NaClO₄ which corresponded to the duplex-to-single-strand transition. At high NaClO₄ (saturated/ca. 9.5 M), we also observed a single transition, which corresponded to the G-quadruplex to single-strand transition (Figure 2e), confirmed by the lack of thermal dequenching under these conditions with Duplex-Dark (Figure 2f).

We next sought to characterize the reversibility of cycling through the DNA structural states. Reversibility without degradation is essential to a switchable catalyst and a potential concern given some structure-switching nanodevices’ propensity to exhibit degradation with repeated switching due to buildup of waste products.[16,18] To do so, we thermally cycled a sample of G4-Dark in 4 M NaClO₄ (conditions in which all three states are thermally accessible) 100 times while monitoring fluorescence. The sample did not exhibit degradation during this experiment (Figure 3a-d).

Given the results from Figure 2, we reasoned that it would be possible to switch the state of our catalyst from G-quadruplex to duplex (by diluting it with aqueous buffer, lowering the concentration of perchlorate) or from duplex to G-quadruplex (by removing water under vacuum, increasing the concentration of perchlorate). To do so, we performed dilutions of high-salt solutions of G4-Dark (which would initially exist in its dark state and transition to its light state) and Duplex-Dark (which would initially exist in its light state and transition to its dark state). G4-Dark recovered fluorescence upon dilution from 8 M to 0.8 M, and Duplex-Dark lost fluorescence following the same dilution (Figure 3e). Conversely, we sought to switch the system by removal of solvent. To do so, we took samples with an initial volume of 200 μL and initial concentration of 0.8 M NaClO₄ and placed them in a vacuum chamber. We monitored these samples by fluorescence imaging with a custom-built device (Supporting Information Figures S30-37). The samples, initially in low NaClO₄, behaved as expected for the duplex state, with G4-Dark exhibiting fluorescence and Duplex-Dark in a dark state. Duplex-Dark remained dark during concentration while the fluorescence of G4-Dark gradually increased as the solution became more concentrated. Finally, both solutions reached a critical concentration of NaClO₄ at which they transitioned to the G-quadruplex state: Duplex-Dark became fluorescent and G4-Dark became nonfluorescent (Figure 3f-i, Supporting Information Videos S1 and 2, Supporting Information Code S1–3). Increases in strand concentration, as would be expected to occur concomitantly with concentration of salt, impacted the bimolecular duplex stability only slightly (Supporting Information Table S1).

G4-Dark and Duplex-Dark demonstrate the perchlorate-based switchability of our system. To demonstrate the ability of G-quadruplex/duplex equilibria to enable switchable electron transfer, we constructed G4-SwitchR (switchable redox; Table 1), which was comprised of G4Redox (a sequence previously shown to form a highly active peroxidase in complex with hemin),[24] G4Redox-Comp, and the cofactor hemin. This G-quadruplex was shorter (18 nt) than the FRET systems used, but it behaved similarly in perchlorate solutions. We employed Amplex Red, a nonfluorescent dye that is oxidized to the fluorescent, red-colored pigment resorufin catalytically by the peroxidase-mimicking DNAzyme formed between a G-quadruplex and hemin (Figure 4a–c).[21]

We compared the ratio of the rate of electron transfer performed by the G-quadruplex/hemin complex to that performed by hemin alone (Figure 4d). In 0.1 and 5 M NaClO₄, the G4Redox component of G4-SwitchR binds hemin and can perform electron transfer, with enhanced activity in 5 M relative to 0.1 M NaClO₄, (Figure 4e, Supporting Information Table S2, Supporting Information Figures S38). In saturated NaClO₄, electron transfer is suppressed due to lack of hemin-G-quadruplex interactions.

In 0.1 M NaClO₄, G4-SwitchR exists entirely as a duplex, hemin binding is thus abrogated (Supporting Information Figure S39); resorufin was produced at < 30 nM/min when G4-SwitchR was present or when it was absent (Figure 4f). In 0.1 M NaClO₄, the G4Redox component of G4-SwitchR binds hemin and can perform electron transfer, with enhanced activity in 5 M relative to 0.1 M NaClO₄, (Supporting Information Figure S40), and G4Redox-Comp exists as a single strand. The resulting hemin-G4Redox complex performs electron transfer (as measured by the reporter dye Amplex Red’s conversion to resorufin) 35-fold more rapidly than hemin alone (Supporting Information Figure S41); the hemin-G4Redox complex produced resorufin at 300 nM/min vs. unbound hemin, which produced it at 9 nM/min. At still higher NaClO₄, hemin-G-quadruplex binding decreases (as indicated by a decreased Soret peak, Supporting Information Figure S39), and in saturated (ca. 9.5 M) NaClO₄, resorufin was produced at a much lower rate of 46 nM/min. Hemin remained in solution at this concentration of NaClO₄ (Supporting Information Figure S41). Consistent with the propensity of hemin to aggregate,[26] partial loss of hemin was observed on extended centrifugation. However, this occurred in both low- and high-perchlorate solutions, indicating this is not a perchlorate-induced phenom-
G-quadruplex-dependent Soret absorbance for hemin was observed (Supporting Information Figure S39), demonstrating G-quadruplex/hemin interactions. Additionally, heme dimers exhibit extremely low electron transfer catalysis behavior, consistent with G-quadruplex hemin being the active species. Thus, perchlorate concentration can be varied to...
switch the state of the active G-quadruplex/hemin form of the catalyst, as well as to modulate the reaction rate.

In addition to Amplex Red, we tested G4-SwitchR with the peroxidase substrates 3,3',5,5'-Tetramethylbenzidine (TMB) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and observed that these dyes acted as electron donors in this system, demonstrating the diversity of substrates that can be oxidized in this system (Supporting Information Figure S42).

In order to demonstrate that thermally switching DNA conformation could also switch electron transfer catalysis, we measured Amplex Red oxidation using G4-SwitchR in 4 M NaClO₄ (Figure 4g). At 20 °C, electron transfer was near-zero. As the sample was heated and switched from duplex to G-quadruplex, the rate of electron transfer exhibited a significant increase, reaching a maximum at 60 °C and again decreasing as the G-quadruplex unfolded. This is consistent with the thermal denaturation curve generated by monitoring Aₕ₂₉₅ (a diagnostic wavelength for G-quadruplex formation) of G4-SwitchR (Supporting Information Table S2, Supporting Information Figures S43–44). Notably, hydrogen peroxide was required. Perchlorate ion, despite its exceptionally high oxidation potential, did not suffice as an electron acceptor in this system.

Discussion

Here, we have demonstrated that duplex DNA is preferentially destabilized by perchlorate salts relative to G-quadruplex DNA, and that this enables solution conditions-selective formation of DNA-hemin complexes that can act as electron transfer catalysts. This phenomenon can be used to switch electron transfer behavior in three ways: 1) direct addition of perchlorate...
segments of rRNA of higher organisms, considerable polymorphism in biological systems, particularly in remodeling are observed in life as well. Nucleic acids exhibit present study, we suggest that conditionally folded G-quadruplexes, and both energy-dependent (i.e., helicases) and energy-independent systems that can do so have been reported.\[38,39\] ATP-dependent helicases are known to unwind G-quadruplex structures, and the RNA-binding protein Lin28 has been shown to unfold G-quadruplexes without the requirement for ATP.\[38\]

Given that such remodeling processes are also operative in nature and that hemin-G-quadruplex promoted electron transfer has been suggested as being physiologically relevant, we speculate that conditional hemin-G-quadruplex complexes are a means by which cells could conditionally enable electron transfer.\[36\] For example, ribosomes from the neurons of Alzheimer’s Disease patients have been shown to contain more iron than those from healthy patients, and these ribosomes possess peroxidase activity. G-quadruplexes may enable this phenomenon \textit{in vivo}. Human ribosomes are known to be polymorphic, particularly in their G-rich expansion segments, some of which have been observed to lack electron density in EM maps, consistent with an equilibrium between multiple states.\[34–36\] Rarely, individual ribosomes clearly exhibit extended conformations consistent with Watson-Crick base pairing, but these same sequences possess exceptionally high G-quadruplex forming potential and form G-quadruplexes in cell-free \textit{in vitro} experiments, which is consistent with polymorphism with one state capable of catalyzing electron transfer.\[36,37\]

Beyond the high-salt solutions that are the focus of the present study, we suggest that conditionally folded G-quadruplexes in cells could collaborate with hemin, producing a means by which cells can perform conditional electron transfer that is analogous to the phenomenon we have employed in this work. In fact, recent work suggests that hemin-G-quadruplex associations occur in human cells.\[40\] Such a phenomenon could be exploited both by extant life or in synthetic biological systems, and we speculate this could have enabled conditionally active forms of prebiotic electron transfer catalysts, which have attracted intense interest in recent years.\[6,43,44\]

**Experimental Section**

**Nucleic acids:** Oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA) and used as received. Labeled strands were obtained with HPLC purification and unlabeled strands were obtained with standard desalting.

**DNA sample preparation:** Oligonucleotides were annealed in a T100 thermal cycler (Bio-Rad) by incubating at 95 °C for 2 minutes, then decreasing the temperature by 10 °C steps and annealing at each step for 1 minute to a final temperature of 25 °C. Except where otherwise specified, experiments were performed in 50 mM Li-HEPES, pH 7.4, with 1 μM of the duplexed or single stranded oligonucleotides.

**Fluorescence-monitored experiments:** Fluorescence measurements were performed using a Gemini X5 plate reader (Molecular Devices) or a Cary Eclipse (Varian Technologies) fluorometer equipped with a thermostated peltier holder (Agilent Technologies) when bidirectional temperature control was required. Excitation was performed at 495 nm and emission was monitored at 520 nm.

For thermal denaturation studies, the sample was ramped from 20 °C to 95 °C (two heat/cool cycles) at 5 °C/min. Selected measurements were also performed with a slower 0.5 °C/min ramp rate to mitigate hysteresis. Thermal midpoints are reported as a sigmoid fit of the transition(s) in the second heating trace of two temperature ramps.

Data from fluorescence melts were normalized by division, setting the highest RFU value to 1 and the lowest value to 0 for each state in a given system (quenched or dequenched/partially dequenched).

**UV-Vis monitored experiments:** Thermal denaturation experiments were performed using a qCHANGER 6/Cary60 (Quantum Northwest) interfaced to a Cary 60 UV-Visible spectrophotometer (Agilent Technologies) using a custom ADL script developed by Quantum Northwest to collect full spectra at each temperature as described previously. Heat sinking for the Peltier device was provided by an EXT-440CU ambient liquid cooling system \(\text{(Koolance)}\). Duplex melting transitions were monitored using the 260 nm trace from these datasets and G-quadruplex melting temperatures were monitored using the 295 nm trace.

Amplex Red experiments (described further below) were monitored on a Cary 60 UV-vis (Agilent Technologies) or a SpectraMAX 340PC plate reader (Molecular Devices) with the PathCheck functionality enabled.

**Circular dichroism (CD) experiments:** CD spectra were obtained using a JASCO J-815 Circular Dichroism Spectropolarimeter and an attached 6-sample Peltier Turret Cell Changer (Model MPTC-490S/15). Spectra were obtained at 25 °C from 220–350 nm with 1 nm increments and averaged over three scans. The solutions consisted of 5 mM phosphate buffer pH 7.4, 20 μM oligonucleotide, 20 μM hemin, 2% DMSO (from hemin stock). \textit{G4SwitchR-Comp} signal was subtracted from the \textit{G4SwitchR} spectra.

**Fluorescence imaging under vacuum:** Samples were pipetted into PCR tubes with the lids open, which were inserted into a 3D-printed jig that was placed inside a black box. The jig was fabricated in PLA on a Prusa 3D printer. The box and its contents were placed in a vacuum desiccator (Supporting Information Figures S29–36) and the chamber was continuously evacuated with a diaphragm pump \(\text{(Welch 2014B-01)}\). The samples were excited with LEDs with emission centered at 462–465 nm \(\text{(Item B01GDO9UNY, Amazon)}\). The samples were imaged with a camera (Raspberry Pi Module V2, Amazon) fixed at 90° relative to the light sources. A 12.5 mm longpass filter \(\text{(Schott OG 550, Edmund Optics)}\) was affixed with polyvinyl acetate adhesive (Elmer’s Glue-All, Costco Wholesale) directly in front of the camera lens to block excitation light and pass emitted light. The camera was interfaced to a Raspberry Pi B+ \(\text{(Amazon)}\). A Python script controlled the Raspberry Pi’s GPIO pins to illuminate samples at 1-minute intervals, collect images, and measured and
plotted fluorescence intensity vs. time. Time-lapse movies were generated from captured images using FFmpeg.

Samples were prepared with a starting volume of 200 μL and 0.1 μM of G4-Dark, Duplex-Dark, or Fluorescein-G4-Quencher in 5 mM Li-HEPES, pH 7.4 and 0.8 M NaClO. After desiccation, the final volume was 20 μL with 50 mM Li-HEPES, 8 M NaClO and 1 μM G4-Dark, Duplex-Dark, or Fluorescein-G4-Quencher.

Electron transfer assay: Samples contained G4-SwitchR (i.e., G4Redox and G4Redox-Comp), G4Redox alone, or no oligonucleotide. G4Redox, when present, was at 5 μM and G4Comp, when present, was at 10 μM to ensure full duplex state at low perchlorate/temperature and that electron transfer catalysis observed was due to the G-quadruplex state of G4-SwitchR and not residual unduplexed G4Redox. The buffer used here was 5 mM sodium phosphate, pH 7.4 with varying sodium perchlorate concentrations. To initiate the reaction, hemin was added to a final concentration of 1 μM, hydrogen peroxide was added to a final concentration of 300 μM, and Amplex Red was added to a final concentration of 200 μM. Reactions were monitored by measuring the absorbance of resorufin at 570 nm. For the Cary 60 thermal activation-Amplex red assay, Amplex Red was added to a final concentration of 2 mM. All reactions contained 2% DMSO, which came from the hemin and Amplex Red stocks.

Similar reaction conditions were used for TMB and ABTS assays with the following exceptions. TMB: 50 mM Li-HEPES pH 7.4, 2 mM sodium phosphate, pH 7.4 with varying sodium perchlorate. UV-vis spectra were obtained from solutions consisted of 5 μM M hemin in 5 mM phosphate buffer pH 7.4 and 2% DMSO with residual unduplexed G4Redox and G4-SwitchR. The buffer used here was 5 mM Li-HEPES, 8 M NaClO with 50 mM Li-HEPES, 8 M NaClO and not G4Comp. Similar reaction conditions were used for TMB and ABTS assays. TMB: 50 mM Li-HEPES pH 7.4, 2 mM sodium phosphate, pH 7.4 with varying sodium perchlorate concentrations. UV-vis spectra were obtained from samples centrifuged at 13,000 rpm for 10 minutes and 200 μL of supernatant was used to collect a spectrum.

Code Availability Statement

All computer code used during the current study is included in this published article (and its Supporting Information files).

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Conflict of Interest

The authors declare no competing interests.
