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A Contractile Electronic Switch Made of DNA

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Abstract: Double-helical DNA has been shown to conduct both electrons and electron holes, the latter over distances of >20 nm. DNA is thus a material of significant interest for the bottom-up construction of nanocircuitry. Here, we describe a contractile DNA nanoswitch, which can toggle between a structurally extended "off" state and a contracted "on" state, with a 40-fold conductivity difference between the two. To turn on, two short motifs of guanine−guanine mismatches in an otherwise standard double helix synapse to form a conductive G-quadruplex, bypassing an insulating element within the helix. This switch can be turned repeatedly on by treatment with millimolar concentrations of K^+ and turned off by sequestration of the K^+ by a crown ether. Circular dichroism and thymine−thymine photocross-linking experiments reveal that strand orientations within the on state G-quadruplex are wholly antiparallel and that the two conductive double-helices interface with the same face of the quadruplex. Although this DNA nanoswitch is chemically gated, it should be adaptable to other kinds of gating and thus serve as a prototype for increasingly sophisticated and complex electronic devices made of DNA.

Introduction

In 1962, Eley and Spivey hypothesized that double-stranded (duplex) DNA may conduct electrical charge.[1] Extensive research has since confirmed that in aqueous solutions duplexes indeed conduct both electron holes (where nucleobase radical cations are the charge carriers) and electrons (where radical anions are the carriers).[2,3] Hole transfer, the more intensively studied of the two, has been observed to occur over distances of >20 nm.[2] DNA is therefore a promising substrate for the construction of nanocircuits and electronic devices. In solution, hole transfer through duplex DNA is conveniently initiated by photochemical excitation of a photosensitizer such as anthraquinone (AQ), appended and stacked upon an end of a double helix.[4] Guanine is the DNA base most easily oxidized, and G radical cations ($G^{\bullet+}$) have been shown to migrate through duplexes over significant distances. Both hopping and superexchange mechanisms are thought to operate for hole transfer at different distance scales.[2,3] Experimentally, hole conduction in DNA is most conveniently monitored biochemically: guanines that host radical cations react slowly with water to give oxidized guanine products, in proportion to the equilibrium distribution of holes across the duplex. A DNA strand is easily cleaved at such oxidation sites by treatment with hot base, and the fragments analyzed.[2]

We have been interested in the design and construction of DNA-based nanoelectronic devices, which take advantage of the natural conductive properties of DNA. However, this necessitates the construction of conductive DNA junctions. To this end we have been interested in junctions between duplexes and other kinds of DNA helices. In addition to the well-known B-type double helix, DNA is also able to fold to form a number of alternative helical forms, including triplexes and quadruplexes.[5] Guanine-quadruplexes (G-quadruplexes) typically form from G-rich DNAs and are a diverse family of structures, capable of wide variation in strand molecularity, orientation, and topology.[6] Certain key metal cations, notably K^+ and Sr^{2+}, strongly stabilize G-quadruplexes, by specific coordination at the center of successive G base-quartets.[6]

We have recently shown that an unusual class of G-quadruplexes can form from certain preexisting duplexes.[7] Contractile duplexes are DNA double helices that incorporate two short, separated motifs of G•G mismatches within an otherwise Watson−Crick base-paired framework (Figure 1); in the presence of K^+, the G•G motifs synapse together to form an *intra*-duplex G-quadruplex, flanked by purely duplex elements.[7] Although such duplex contraction has been demonstrated convincingly using chemical protection methods, very little specific information has been accessible to date about the precise geometry and strand orientations of these quadruplex−duplex composites.

Recently, the hole conduction properties of a few G-quadruplexes (of diverse and sometimes uncertain geometry and topology) have been reported.[8] Overall, it has been found that G-quartets do conduct electron holes, although their conductivity relative to that of Watson−Crick base-pairs is a matter of some

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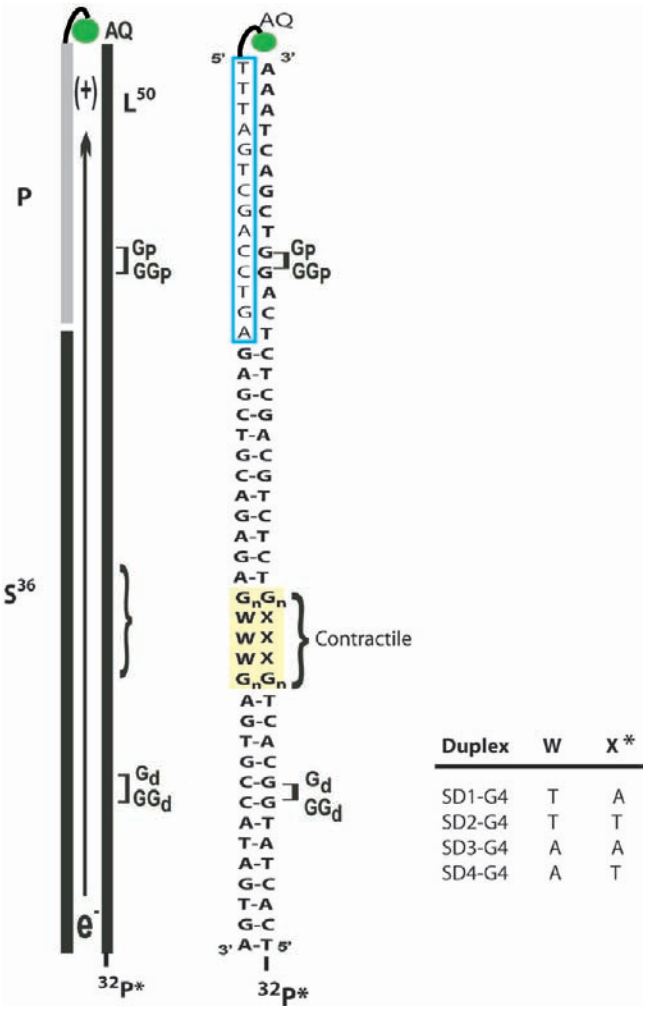

Figure 1. Design of the four contractile duplexes used in this study.

disagreement.[8] Correspondingly, the oxidation patterns of quadruplex guanines has been found to be different (variously, lower or higher) from those of guanines in Watson−Crick duplexes.[8] To address some of the issues and inconsistencies emerging from the G-quadruplex charge conduction literature, we recently examined the conductive properties of a parallel-stranded[9] G-quadruplex flanked on either side by a duplex (a D-Q-D complex).[10] Our D-Q-D complexes were designed to (1) enable optimal π-stacking (necessary for efficient charge conduction) at each quadruplex−duplex junction, by way of rational placement of each duplex on a different face of the quadruplex, and (2) minimize helical stress at each junction, where a given DNA strand transits from the helical geometry characteristic of a duplex to that of a quadruplex. This minimization was achieved by incorporating a single strand-nick at each duplex−quadruplex junction. Charge conduction experiments showed that in D-Q-D constructs hole conduction from the AQ-proximal duplex to the AQ-distal duplex *via* the intervening G-quadruplex was ~50% as efficient as charge flow through an equivalently sized, purely Watson−Crick base-paired, duplex.[10]

The various quadruplexes, above, studied for their charge conduction properties, are unfortunately not amenable to facile and reversible structural switching (that may or may not enable a concomitant electrical switching). However, contractile duplexes incorporate a G-quadruplex whose formation should be reversible. Since potassium ions lead to duplex contraction, the addition of a potassium chelator, such as the crown ether, 18-Crown-6,[11] should in principle reverse such a contraction. An intriguing question then arises: would such reversible structural transitions of a contractile duplex correspond to a reversible electronic switch? A few immediate caveats attend such a hypothesis: to date, nothing is known about (a) the extent and/or integrity of duplex−quadruplex stacking within a contracted or pinched duplex or (b) the extent of steric crowding and strain at each junction. Thus, the likelihood of charge flow through a contracted duplex cannot be predicted *a priori*.

In this paper we (a) demonstrate that contractile DNA duplexes can indeed serve as excellent and reversible electronic switches and (b) use spectroscopic and photocross-linking methods to demonstrate an unexpected geometry of the contracted (electronically "on") form of potassium-generated contractile duplexes.

Materials and Methods

DNA Purification and Preparation. All DNA oligonucleotides were purchased from Core DNA Services, Inc. (Calgary, AB), and size-purified using denaturing (50% urea, w/v) polyacrylamide gel electrophoresis. 5′-Amino-modified DNA oligonucleotides were subjected to covalent coupling with anthraquinone (AQ), as described by Huang et al.[10] The DNA-anthraquinone conjugates (AQ-DNA) were purified by HPLC (Agilent Technologies) as described.[12] Data characterizing these conjugates is given in Supporting Information, Figures S2 and S3). To lower the levels of background (charge-flow unconnected) cleavage, oligonucleotides used for charge-flow experiments were 5′-end labeled with ^{32}P (using standard kinasing procedures) and then PAGE-purified following a pretreatment with 10% piperidine (v/v) at 90 °C for 30 min and lyophilization.

Preparation of Contractile Duplexes for Chemical Protection Studies and for Charge Transfer. The contractile DNA duplexes (named SD*n*-G4, where $n = 1-4$) were assembled, first, by coincubation of two component DNA strands in each case: a L50 strand and a S36 strand (shown in Figure 1). Oligonucleotides were heat-denatured at 100 °C for 3 min in TE buffer (10 mM Tris-HCl, pH = 7.4 and 0.1 mM EDTA), cooled to 40 °C, and then made up to 50 mM Tris-HCl (pH = 7.4) supplemented with either 100 mM LiCl or 100 mM KCl. The solutions were incubated at 37° overnight. The resulting incomplete duplexes (whether extended or pinched) were now made up to 1 mM $MgCl_2$ and hybridized with the P strand (derivatized with AQ on its 5′-end) for 1 h at 22 °C (Figure 1). A nonconductive control duplex, SD2-G4-3TA, whose sequence is shown in Figure 9, was also constructed and treated as above.

Chemical Protection Assays. Partial DNA modification with dimethyl sulfate (DMS) and diethyl pyrocarbonate (DEPC) were carried out following standard procedures.[13] Prior to addition of the modifying chemicals, DNA solutions were incubated at 0 °C for 1 h in the appropriate buffered solution: buffer M (50 mM Tris, pH 7.4, 1 mM $MgCl_2$), supplemented with either 100 mM LiCl (lithium buffer) or 100 mM KCl (potassium buffer). See Results

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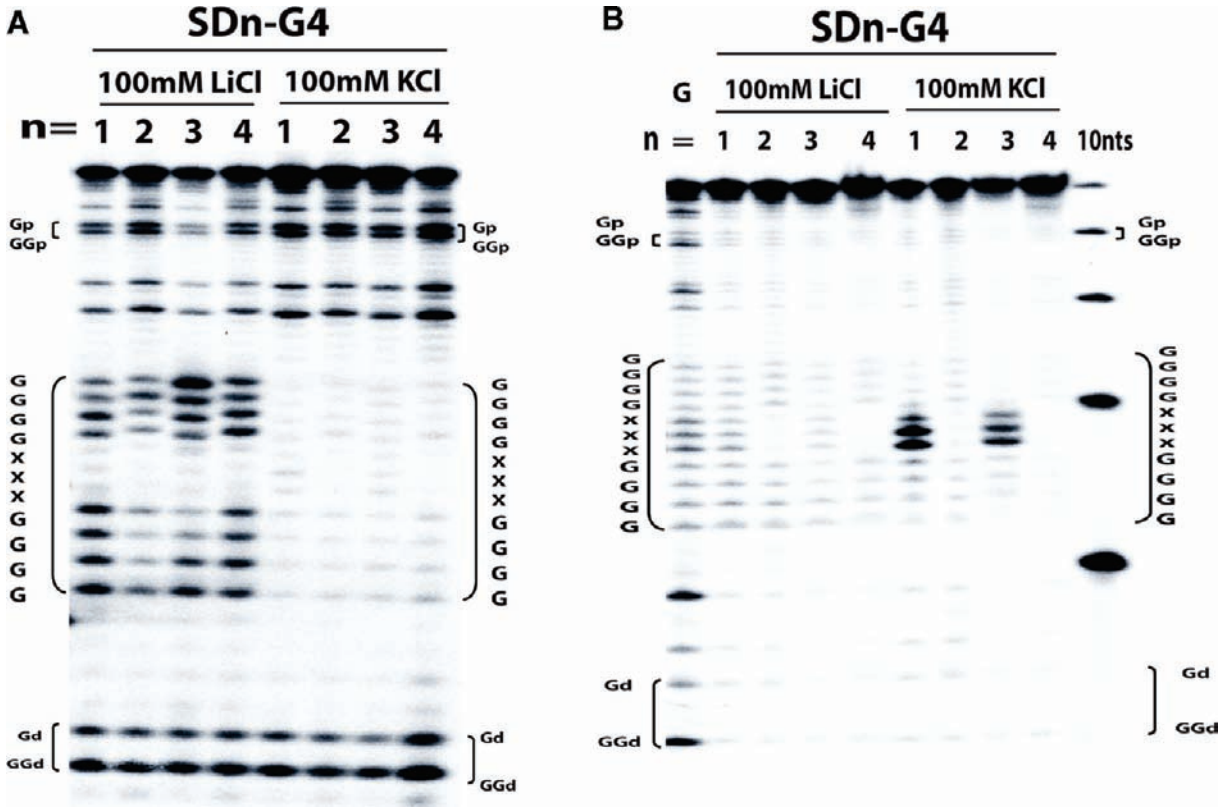

Figure 2. Demonstration of duplex pinching within the duplexes SD1-G4 to SD4-G4, using (A) DMS protection assays and (B) DEPC protection assays.

and Discussion for the buffer solutions used for chemical protection in the reversibility experiments. Upon the addition of the respective stop solutions, the DNA was ethanol precipitated, dried, dissolved in 10% (v/v) piperidine, and heated at 90 °C for 30 min. The DNA was then lyophilized to dryness and analyzed by electrophoresis in 12% denaturing polyacrylamide gels.

Anthraquinone-Initiated Charge Transfer through Contractile DNA Duplexes. The DNA solutions in lithium buffer or potassium buffer (see above) were irradiated at 0 °C, by placing the DNA solutions under a UVP Black-Ray UVL-56 lamp (365 nm) for 1 h, at a distance of 4 cm from the bulb. Constant temperature was maintained with the use of an ice−water bath. Following irradiation, the duplexes were gel-purified in denaturing polyacrylamide gels, eluted into TE buffer, ethanol precipitated, and then incubated while dissolved in 10% piperidine (v/v) at 90 °C for 30 min. Following lyophilization, the DNAs were analyzed in 12% denaturing polyacrylamide gels.

Circular Dichroism Spectroscopy. CD spectra were recorded on a Jasco-810 spectropolarimeter (Jasco, Easton, MD), using a quartz cell of 1 mm optical path length, an instrument scanning speed of 500 nm/min, with a response of 1 s, and over a wavelength range of 200−320 nm. The DNA solutions were prepared as above, at 4 μM DNA concentrations, in either lithium buffer or potassium buffer. The CD spectra shown here are averages of five sequential scans, measured at room temperature, and baseline corrected.

UV Cross-Linking. For UV cross-linking in solution, 20 μM solutions of a shortened contractile duplex made up of the two DNA strands M (5′-TAC CGT GAG GGG TTT GGG GAG AGA CGT) and N (5′-ACG TCT CTG GGG TTT GGG GTC ACG GTA), along with trace amounts of added 5′-^{32}P-labeled strand N, were denatured in TE buffer and then incubated overnight, at 37 °C, in either lithium buffer or potassium buffer. Following incubation, the DNA solutions were irradiated with 254-nm light from a UVP Mineralight UVGL-58 lamp (at a distance of 4 cm from bulb) at 4 °C for 30 min. Following UV irradiation, samples were analyzed in a 12% denaturing polyacrylamide gel, as described above.

Data Analysis. Imaging and densitometry of sequencing gels were carried out on a Typhoon 9410 Phosphorimager (Amersham Biosciences). Quantitation was carried out using Amersham's ImageQuant 5.2 software. For all quantitations, guanine band intensities from DNAs irradiated for charge flow were corrected for nonspecific cleavage at those same guanines under conditions of nonirradiation.

Results and Discussion

Figure 1 shows the design of four related contractile duplexes, SD1-G4 to SD4-G4, used in this study. Each duplex is held together by Watson−Crick base-pairs except in its contractile domain, made up of two motifs of four G•G mismatches interrupted by a variable region, $(WX)_3$. WX corresponds, variously, to T-A, T•T, A•A, and A-T in the duplexes SD1-G4 to SD4-G4. For ease of monitoring hole conduction within these duplexes, a guanine doublet (GG), a notable hole sink,[2] was placed proximal to the AQ (Gp and GGp in Figure 1), and another was placed distal to the AQ (Gd and GGd in Figure 1).

Protection from DMS methylation can be used to infer the formation of G-quartets.[9] We carried out DMS methylation of the four duplexes, SD1-G4 to SD4-G4, in buffer M (50 mM Tris, pH 7.4, 1 mM $MgCl_2$) supplemented by either 100 mM lithium chloride (lithium buffer) or 100 mM potassium chloride (potassium buffer) to determine whether under these two conditions these duplexes participated in G-quadruplex formation. Figure 2A shows that whereas in lithium buffer the G•G mismatch guanines in all four duplexes are notably reactive to DMS (at levels comparable to the Watson−Crick base-paired guanines), in potassium buffer there is a comprehensive protection of every single contractile domain guanine, consistent with all eight G•G paired guanines within the ^{32}P-labeled DNA strand participating in G-quartets.

Figure 2B confirms the contractile behavior of the duplexes SD1-G4 to SD4-G4 in potassium buffer. Diethyl pyrocarbonate (DEPC) modifies purine (A > G) bases in DNA, but only those that are unstacked and/or exposed to the solvent.[13] Figure 2B shows that in lithium buffer the duplexes SD1-G4 to SD4-G4 show very slight reaction with DEPC at the contractile domain guanines and adenines (adenines are present in the ^{32}P-labeled strand of only SD1-G4 and SD3-G4). In potassium buffer, however, the A_3 stretches in the synaptic domains of both SD1-G4 and SD3-G4 react heavily with DEPC, consistent with their being single-stranded and highly exposed to the solvent.

In investigating the potential of pinching duplexes to conduct charge, we wished to ask the following questions: (a) If these duplexes were wholly Watson−Crick base-paired instead of containing G•G and other mismatches, how conductive might they be? (b) Would the pinched and extended conformations of the contractile duplexes conduct differently from each other and from the Watson−Crick base-paired duplexes described in (a)?

Figure 3A shows the charge conduction properties of two purely Watson−Crick duplexes, Duplex-GTG and Duplex-GAG, that incorporate, respectively, the same radio-labeled strand as SD4-G4 and SD1-G4 and the appropriate complementary strands. Irradiation was carried out, at 0 °C, in potassium buffer (a gel showing the same experiment, carried out in lithium buffer, is given in Supporting Information). The figure shows "dark" lanes, reporting duplexes not exposed to UV light and in which charge flow was not initiated. The lanes marked "UV" show duplexes through which charge flow took place. On the basis of guanine damage patterns, it is evident that both duplexes conduct charge into the $(C\text{-}G)_3$ base pairs most proximal to the AQ (the pattern of G-damage, with guanines at the 5′ edge of a GG or G_4 stretch being damaged more heavily than those at the 3′ edge, is characteristic of charge-flow generated guanine oxidation[2]). The subsequent stretches of $(A\text{-}T)_3$ and $(T\text{-}A)_3$ base pairs in the two duplexes shown left and right in Figure 3A act as insulators, preventing significant charge flow into the $(C\text{-}G)_3$ base pairs distal from the AQ and beyond. Such an insulating behavior of three tandem AT pairs has been reported before.[2,3,14] Thus, the ^{32}P counts at the distal guanine, GGd (Figure 3A), as a fraction of the total counts in the lane, was measured to be 0.06 ± 0.01% for the Duplex-GTG and 0.29 ± 0.05% for the Duplex-GAG. This difference in conductivity between the two Watson−Crick duplexes is expected, given that duplexes containing all-purine or purine-rich sequence in one strand (such as Duplex-AA) are better hole conductors than those containing mixed purine and pyrimidine sequences (such as Duplex-GTG).[2,3,14]

Figure 3B shows the results of hole conduction within the contractile duplex series, SD1-G4 to SD4-G4, carried out at 0 °C in lithium buffer and potassium buffer, respectively. DNA solutions irradiated for charge flow are labeled with an "l" in Figure 3B; unirradiated, dark controls are shown as "d". Accurate quantitation of charge-flow-generated guanine damage at a site was achieved by correcting for background cleavage at that site, from the corresponding d lane. The lithium buffer data indicate that in the structurally extended duplexes charge flows, to a degree, into at least the G•G motif most proximal to the AQ. However, almost no charge flows past the distal G•G motif into the Watson−Crick base-paired duplex incorporating Gd and GGd. Indeed, levels of DNA cleavage measured at GGd (0.05% of the total counts for SD1-G4, 0.08% of SD2-G4, 0.03% of SD3-G4, and 0.07% of SD4-G4) are exceptionally low for all four extended duplexes.

The potassium data, however, are strikingly different. First, the 5′-most guanine of the distal G•G motif (in all four duplexes) and the proximal motif (in SD1-G4 and SD3-G4) are heavily oxidized by charge flow. However, significant charge now flows from the AQ *all the way to the distal end (GGd) of each pinched duplex.* GGd cleavage levels measured in potassium are, respectively, 0.85% (SD1-G4), 1.09% (SD2-G4), 1.04% (SD3-G4), and 0.47% in SD4-G4. Thus, conduction across the four pinched duplexes is, respectively, 19-, 13-, 41-, and 7-fold higher than across the corresponding extended conformers (the numbers describe K^+/Li^+ band ratios at GGd). The pinched duplexes are therefore notably better conductors not only relative to their own extended conformers but also to the equivalent Watson−Crick duplexes, Duplex-GTG and Duplex-GAG.

With regard to the charge conduction data shown in Figure 3B, one feature of the conduction pattern found in lithium buffer, relative to potassium buffer, deserves comment. Since the stretch of Watson−Crick base-paired duplex proximal to the AQ is shared by all four duplexes, it is reasonable to expect that the efficiency of charge injection into each duplex is comparable (Li^+ and K^+ are *not* redox-active cations, and would not be expected to differentially influence the efficiency of charge injection). However, damage at the proximal guanines, GGp, is lower in lithium buffer than in potassium buffer (by 3.0- to 5.3-fold, lower than the margins seen with GGd above). One possible explanation for this observation is that the identity of the countercation may influence the efficiency of postinjection charge flow through DNA. Indeed, Barnett and co-workers have reported that charge transport within DNA duplexes is ion-gated.[15] To investigate the possibility that lithium and potassium ions differentially gate charge transport in DNA, we studied charge flow through a control Watson−Crick duplex, Duplex-GTG (see Figure 3A for sequence), dissolved in lithium buffer versus potassium buffer. However, the observed guanine damage patterns, including those at the proximal guanine, GGp, were essentially identical in the two cases (see Supporting Information). It is likely that in the extended form of the contractile duplexes, the G•G *mismatche*s within them (which exist in lithium buffer but not in potassium buffer; in the latter, they have synapsed to form G-quartets) serve as a significant sink for the mobile radical cation (it should be noted that such a property would not necessarily generate high oxidation levels for the mismatch guanines, since their helical geometry and reactivity with water is not the same as those of guanines within conventional G-C base pairs).[2,16] In this context, a prior study has reported that the presence of either two tandem G•A mispairs or individual bulged guanines do not majorly impact the levels of proximal GG damage within DNA duplexes (i.e., in themselves the G•A mispairs/base pairs, as found within a stacked duplex, do not constitute major hole sinks).[17] However the situation in our extended contractile duplexes is substantially more complex, in that (a) we have *two* sets of *four* tandem G•G mispairs (i.e., a total of *eight* such mispairs per duplex), and (b) unlike the well-defined and well-stacked G•A sheared base-pairs,[17] it is likely that at least some of the four tandem G•G mispairs in our extended duplexes are fluxional and not stably

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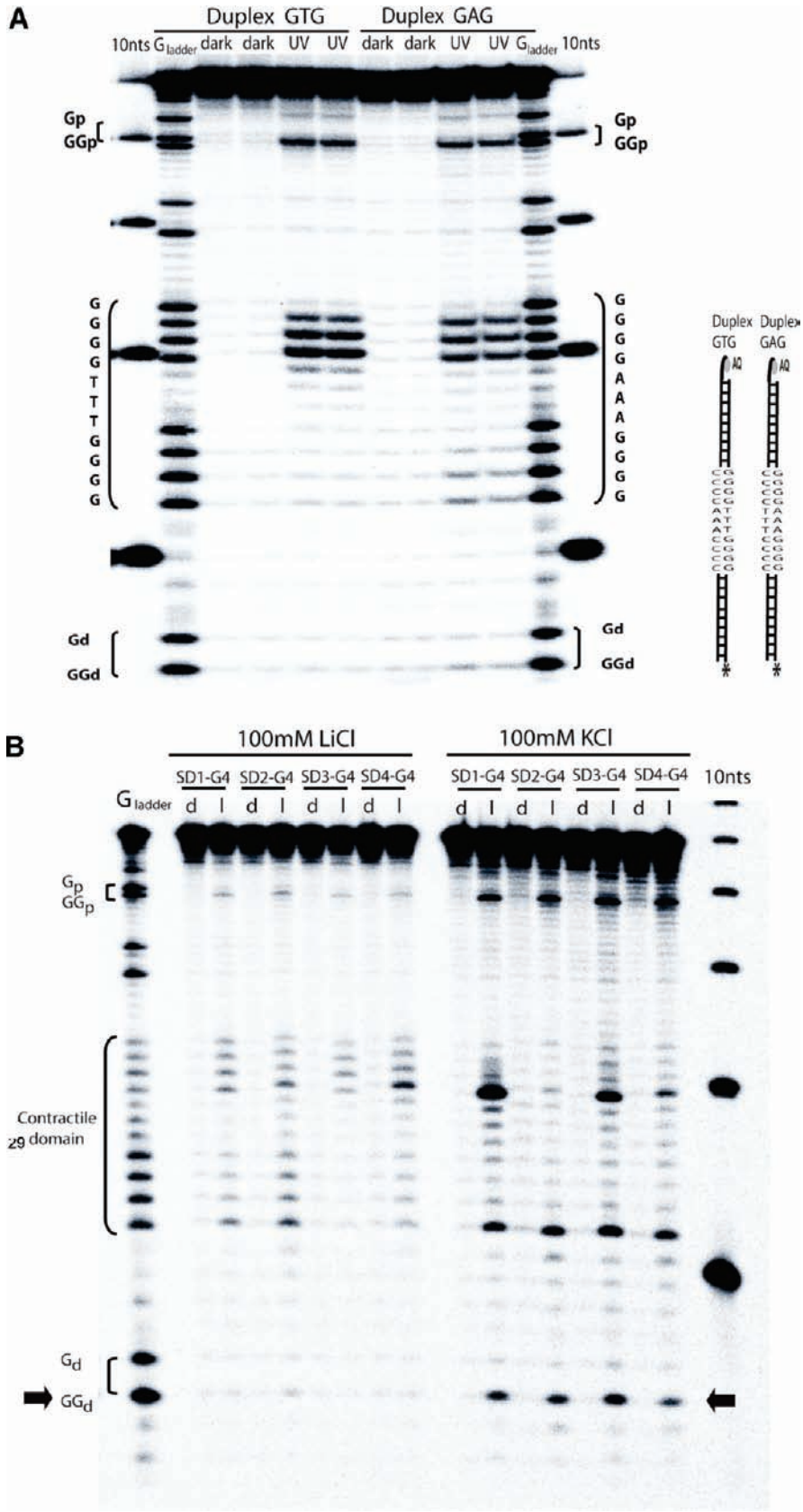

Figure 3. (A) Patterns of guanine damage resulting from charge (hole) conduction through (A) Watson−Crick duplexes GTG and GAG and (B) the contractile duplexes, SD1-G4 to SD4-G4. The black arrows highlight the distal GGd guanine.

base-paired. The very fact that low concentrations of potassium ions, at low temperatures, readily enable the formation of G-quartets by these G•G mispairs, speaks to this likelihood. Undoubtedly, high resolution structural and other studies in the future will definitively address the question of hole sinks within the extended contractile duplexes.

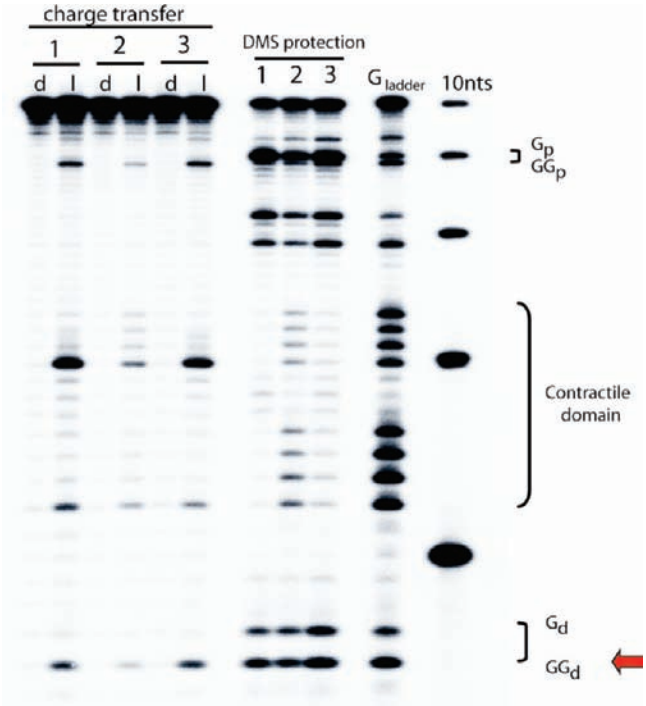

Figure 4. Reversibility of contractile duplex pinching, examined on the SD1-G4 duplex, using charge transfer (left) and DMS-methylation protection (right) assays. Under buffer conditions 1 and 3, SD1-G4 adopts a pinched conformation, and under condition 2, it adopts an extended conformation. The red arrow highlights the distal GGd guanine.

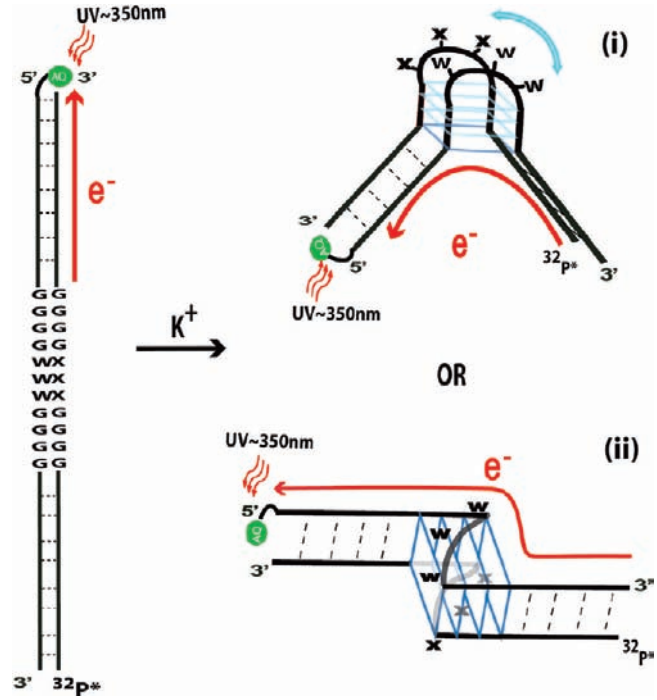

Figure 5. Alternative topological models for the potassium-induced pinched conformation of contractile duplexes SD1-G4 to SD4-G4. In model (i) the G-quadruplex has a wholly antiparallel strand orientation, of the "chair" type; in model (ii) the G-quadruplex has partially parallel and partially antiparallel strand orientations, of the "basket" type. In model (i) the intervening bases of the contractile domain, labeled x and y, are looped out in relative proximity to each other (indicated by the blue arrow). In model (ii), the x and y bases traverse grooves on opposite sides of the intervening G-quadruplex.

The contractile duplexes, SD1-G4 to SD4-G4, are therefore chemically gated molecular switches. They are on the nanometer scale (2 nm × ∼15 nm in size at full extension) and capable of ∼40-fold modulations of charge flow through them. A practical switch, however, must be able to toggle repeatedly between on and off states. To test this, we explored the effectiveness of a potassium chelator, 18-Crown-6, in converting a pinched duplex back to its extended form. Figure 4 shows both structural and charge conduction data on the SD1-G4 duplex, in response to successive treatment with K^+ and with 18-Crown-6. Three different solution conditions were explored: under condition 1, the duplex was dissolved and incubated in 10 mM KCl in buffer LM (50 mM Tris, pH 7.4, 100 mM LiCl, 1 mM $MgCl_2$); under condition 2, a SD1-G4 solution, already treated to condition 1, was now made up to 20 mM 18-Crown-6; under condition 3, the DNA solution was treated sequentially to condition 1 and condition 2 and was then made up to 40 mM KCl, all in buffer LM.

The DMS protection results in Figure 4 show that a characteristic switching from pinched to extended back to pinched conformation does indeed occur moving from condition 1 through to condition 3. The same conclusion holds for the charge conduction data (the switch is on under conditions 1 and 3 and off under condition 2). The electronic switch is thus wholly reversible.

Prior studies on hole transfer through DNA duplexes have indicated that its efficiency is lower when there are disruptions in the DNA base-stacking.[2] Our results here, that duplex pinching opens a conduit for hole transfer from one duplex to another *via* a quadruplex, suggests that interhelical stacking between the components of the pinched conformation must be of a high order (possibly comparable to that found in the D-Q-D DNA complexes, described above[10]). As summarized above, G-quadruplexes fold with a wide spectrum of strand orientations. In principle, the G-quadruplex within contracted duplexes could have an all-parallel strand orientation (all-parallel quadruplexes are thermodynamically the most stable[6]). However, to generate such a quadruplex would require major distortions of the purely duplex components of the contracted duplex. Figure 5 shows two other, more plausible, folds for a pinched duplex: in (i) the quadruplex strand orientation is entirely antiparallel, whereas in (ii) the orientation is part parallel, part antiparallel. These strand orientations correspond to the much-studied "chair" and "basket" topologies of G-quadruplexes formed from single-stranded DNA.[18] A priori, the structure (ii) shown in Figure 5 appears to fit our observed charge flow data better: in this geometry (as in the D-Q-D complexes[10]) each duplex stacks comfortably on a different face of the quadruplex, with a predicted lack of steric crowding.

We sought evidence for the above from circular dichroism spectroscopy carried out on the lithium and potassium forms of a slightly truncated contractile duplex (SD2-G4s; see Materials and Methods). Circular dichroism has been used widely in the G-quadruplex field as a rapid and qualitative diagnostic for the overall

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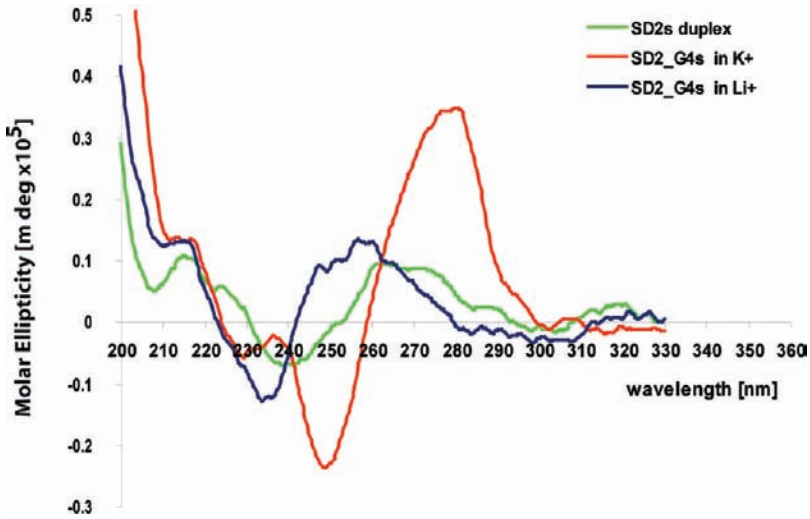

Figure 6. Circular dichroism spectra of the SD2-G4s duplex in lithium buffer (blue) and potassium buffer (red). CD spectra for the purely Watson−Crick SD2s duplex (SD2s has the same sequence as SD2-G4s but lacks the latter's contractile domain) is shown in green. SD2-G4S incorporated the same mismatch and junctions present in SD2-G4 but was used for these CD experiments to lower the contribution of the purely Watson−Crick duplex elements to the overall CD spectrum.

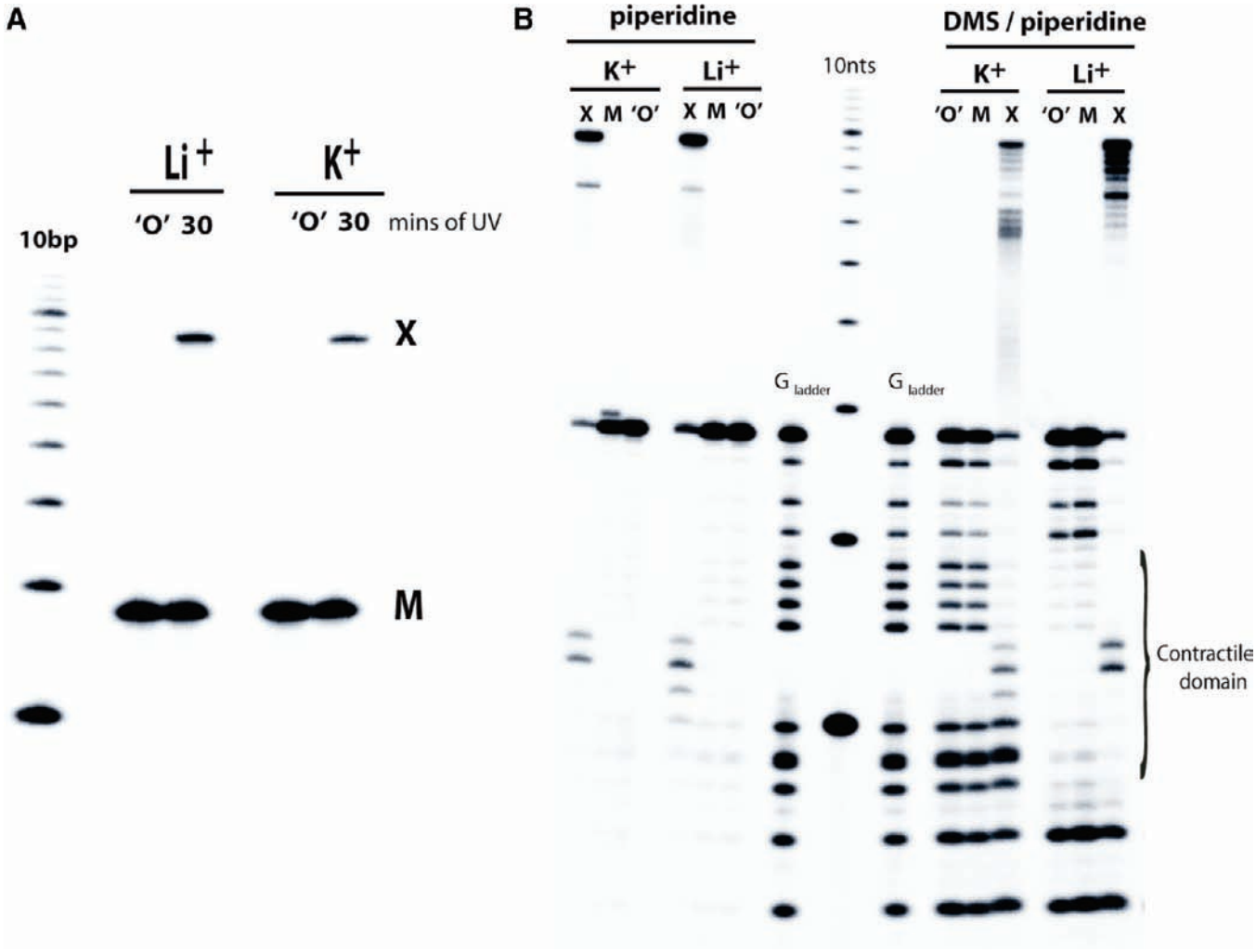

Figure 7. (A) Denaturing gel showing the formation of a high molecular weight, cross-linked product (X) upon irradiation of the duplex, SD2-G4s, with 254 nm UV light. M indicates the un-cross-linked, monomeric, 5′-^{32}P-labeled strand within SD2-G4s. (B) Denaturing gel showing the results of piperidine treatment (left) and treatment with dimethylsulfate followed by piperidine (right) of the irradiated products X and M, as well the unirradiated monomer O.

fold of a quadruplex.[19] Figure 6 plots the CD spectra of SD2-G4s in lithium buffer and in potassium buffer; it also plots, for comparison, the CD spectrum of a purely Watson−Crick duplex, SD2 (which consists of only the Watson−Crick portions of SD2-

G4s, i.e., it lacks SD2-G4s's contractile domain). It can be seen that the lithium and the potassium forms of SD2-G4s show dramatically different CD spectra. The potassium form of SD2-G4s shows a strong maximum at ~295 nm and a strong minimum at ~260 nm. The lithium form of SD2-G4s, however, shows a broad maximum at ~270 nm and a weaker minimum at ~240 nm, more or less resembling the CD spectrum of the SD2s duplex. These data rule out the possibility that the strand orientations of the G-quadruplex are purely parallel (such a quadruplex would be expected to show a minimum at ~240 nm and a strong maximum at ~260 nm).[19] However, a certain ambiguity persists about the exact identity of the potassium fold, given that the CD spectra of the chair (as in model i, Figure 5) and basket (as in model ii, Figure 5) strand orientations are often reasonably similar.[19b] An alternative terminology proposed for the chair and basket topologies is the "II" and "I" tetrad combinations, respectively.[19d]

We conjectured that one possible way to distinguish between chair and basket quadruplexes within pinched duplexes may be on the basis of their ability to form interstrand thymine dimers.[20] Within the contractile domain, SD-G4 has three consecutive T•T mismatches. In principle, UV irradiation should give rise to the formation of *interstrand* thymine dimers whenever such thymines are in close spatial proximity.[20] Thus, the extended, lithium form of SD2-G4s should form interstrand thymine dimers; as should one of the two possible folds (the chair structure, i, Figure 5) of the contracted duplex. However, in the basket fold for the contracted duplex (ii, Figure 5), the two short TTT connectors would necessarily transit along grooves on opposite sides of the quadruplex, rendering them unlikely to be able to form interstrand thymine dimers.[18] Figure 7a shows a denaturing gel for 245 nm UV-irradiated (30 min) and unirradiated (0 min) solutions of the SD2-G4s duplex (separately, in lithium buffer and in potassium buffer). It can be seen that in each buffer, a high molecular weight cross-linked product (X) is generated with 254 nm irradiation. To determine the site(s) of thymine dimer formation, the X products were excised from the gel, purified, and subjected to 10% piperidine (v/v) at 90 °C for 30 min, followed by lyophilization to dryness. Figure 7B shows the results of the piperidine treatment. Shown on the left are data from treatment of UV-treated (and untreated) DNA with piperidine alone; it can be seen that the cross-linked product (X) fragments only at the thymines within the contractile domain, in both lithium and potassium buffers. The monomeric labeled strand from irradiated samples (M) and the unirradiated monomeric strand (O) do not show this fragmentation. Significantly, the X product from lithium buffer shows cleavages at all three thymines within the contractile domain (this is seen even more clearly in the lanes on the right, where the DNA was treated with DMS followed by piperidine, to superimpose data on guanine accessibility to DMS with the thymine dimer forming sites). By contrast, the potassium buffer X product shows cleavages only at the central and 3′-most thymine of the TTT sequence. The DMS reactivity patterns (Figure 7B, right) confirm the dominance of the extended form of the SD2-G4s duplex in lithium buffer and that of the pinched duplex in potassium buffer.

Fundamentally, the cross-linking experiments above report two key observations: (a) the positive formation of a cross-linked product in potassium buffer, and (b) its *distinctive pattern* of loop thymine photoreactivity (different from the same thymines within the extended duplex). This provides strong evidence that the geometry of the pinched duplex is in fact the model shown in Figure 5, model i. At some level, this is an unexpected result, given that in model i, both duplex components of the pinched structure must stack on the same face of the G-quadruplex. However, sufficiently high-quality duplex−quadruplex stacking evidently occurs, even in this fold, given that charge passes efficiently from the AQ-proximal duplex through the quadruplex into the distal duplex.

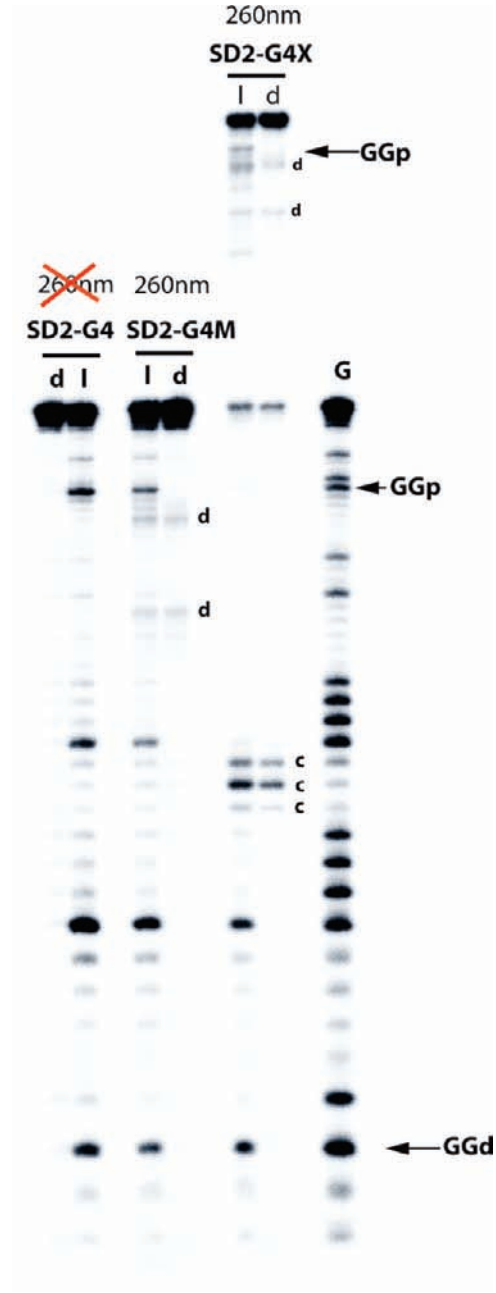

Figure 8. Evidence that the purified cross-linked complex (SD2-G4X) of the pinched duplex is *itself* conductive. SD2-G4 shows charge conduction through the pinched duplex, SD2-G4, that has not irradiated with 260 nm light. SD2-G4M shows charge conduction through a sample of the duplex SD2-G4 that was irradiated with 260 nm light but not cross-linked.

The above geometric results raise two issues: (a) Is the fold of the pinched duplex fluxional (i.e., do we know that the chair fold, shown in Figure 5 model i, is also the *conductive* fold, although it may be the more stable of the two folds, as revealed by the cross-linking experiments)? (b) With regard to the model i in Figure 5, can we be certain that charge flow to the distal guanines from AQ is not by way of a direct bridge formed by the pendant AQ to the distal duplex?

The data shown in Figure 8 demonstrate that the purified cross-linked complex (of the chair form) of the pinched duplex

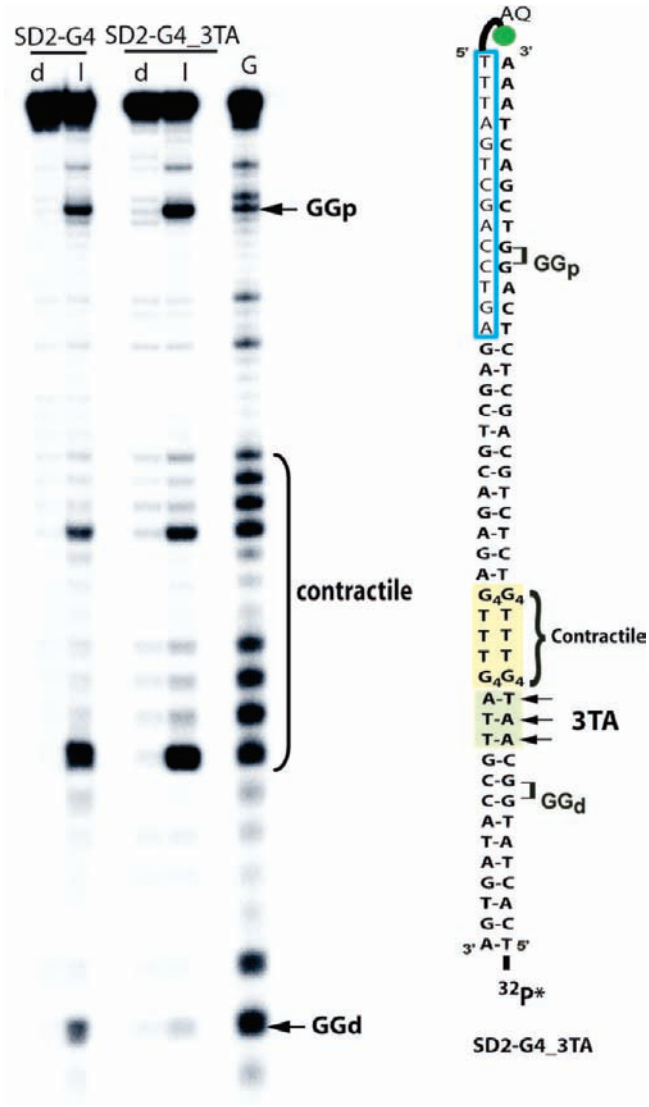

Figure 9. Relative charge flow patterns in SD2-G4 and a mutated version of SD2-G4 (SD2-G4_3TA), in which an insulating AT/TA/TA (3TA) is incorporated between the GGd element and the G-quadruplex. SD2-G4 and SD2-G4_3TA are of identical length.

is *itself* conductive. Thus, compared to charge flow patterns through the pinched conformation of the SD2-G4 duplex (SD2-G4 in Figure 8, not irradiated with 260 nm light, and SD2-G4M, irradiated with 260 nm light but not cross-linked), the purified cross-linked product (SD2-G4X) shows equivalent levels of conduction (in terms of guanine damage at GGd).

As for whether charge flow seen in GGd in these constructs is an artifact of the appended AQ interacting directly with the duplex incorporating GGd, the data in Figure 9 show that this is not so. A mutated version of SD2-G4 (SD2-G4_3TA) was constructed, in which an insulating AT/TA/TA (3TA in Figure 9) was incorporated between the GGd element and the G-quadruplex. It can be seen that in the SD2-G4_3TA duplex charge flows very poorly to the GGd element, relative to the analogous GGd in the control duplex, SD2-G4.

Although the results in this paper report the average behavior of an ensemble of molecular switches, the properties of *single* contractile duplexes should be measurable. Currently, too, there is much interest in investigating small molecule compounds that specifically bind and stabilize G-quadruplexes, fueled by evidence that quadruplexes may function as inhibitors of the enzyme telomerase and also as repressors of the expression of certain cancer-causative oncogenes.[18] A key goal for us will be to adapt contractile duplexes to function as sensitive, chip-based electronic biosensors[12,21] for the identification of novel quadruplex-interactive compounds for pharmaceutical use.

Whereas the nanoswitch described here is gated by specific cations, the design of the pinched duplex should lend itself easily to the design of switches that are electronically gated. Initially, it should be possible to position different photo-oxidative and -reductive functionalities either within or atop the G-quadruplex of the pinched duplex and to use them to gate the flow of electron holes between the two duplex components of the pinched duplex. Experiments to generate such transistors are currently under way in our laboratory.

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Supporting Information Available: A study of the relative charge conduction properties of a DNA double helix in lithium buffer versus potassium buffer. This material is available free of charge via the Internet at http://pubs.acs.org.

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