Robust charge transport in DNA double crossover assemblies

Duncan T Odom, Erik A Dill and Jacqueline K Barton

Background: Multiple-stranded DNA assemblies, encoded by sequence, have been constructed in an effort to self-assemble nanodevices of defined molecular architecture. Double-helical DNA has been probed also as a molecular medium for charge transport. Conductivity studies suggest that DNA displays semiconductor properties, whereas biochemical studies have shown that oxidative damage to B-DNA at the 5′-G of a 5′-GG-3′ doublet can occur by charge transport through DNA up to 20 nm from a photo-excited metallointercalator. The possible application of DNA assemblies, in particular double crossover (DX) molecules, in electrical nanodevices prompted the design of a DNA DX assembly with oxidatively sensitive guanine moieties and a tethered rhodium photo-oxidant strategically placed to probe charge transport.

Results: DX assemblies support long-range charge transport selectively down the base stack bearing the intercalated photo-oxidant. Despite tight packing, no electron transfer (ET) crossover to the adjacent base stack is observed. Moreover, the base stack of a DX assembly is well-coupled and less susceptible than duplex DNA to stacking perturbations. Introducing a double mismatch along the path for charge transport entirely disrupts long-range ET in duplex DNA, but only marginally decreases it in the analogous stack within DX molecules.

Conclusions: The path for charge transport in a DX DNA assembly is determined directly by base stacking. As a result, the two closely packed stacks within this assembly are electronically insulated from one another. Therefore, DX DNA assemblies may serve as robust, insulated conduits for charge transport in nanoscale devices.

Address: Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA.

Correspondence: Jacqueline K Barton
E-mail: jkbarton@its.caltech.edu

Key words: DNA circuitry, DNA double crossovers, DNA electron transfer, guanine oxidation

Received: **31 March 2000**
Revisions requested: **19 April 2000**
Revisions received: **25 April 2000**
Accepted: **25 April 2000**

Published: **15 June 2000**

Chemistry & Biology 2000, **7**:475–481

1074-5521/00/$ – see front matter
© 2000 Elsevier Science Ltd. All rights reserved.

Introduction

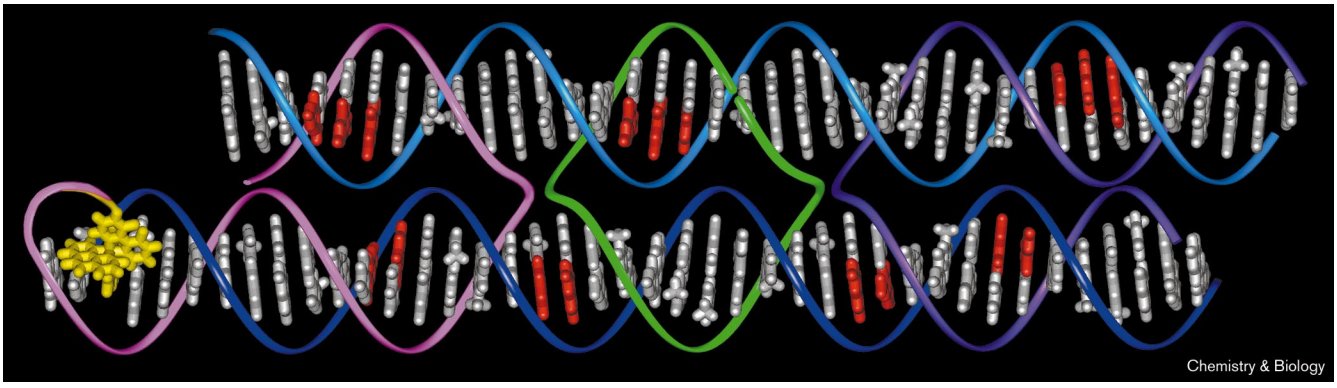
The discovery and exploitation of molecular assemblies for the fabrication of nanoscale devices have been the focus of increasing attention. In this context, one polymeric structure of particular interest has been DNA. Molecular arrays constructed with DNA offer promise because of the ability of DNA to self-assemble, the inherent programmability of DNA through sequence, and its moderate resistance to degradation.

Multiple-stranded DNA assemblies have been formed to yield microscale and mesoscale structural and functional arrays. In particular, DNA double crossover (DX) assemblies have been used to construct a nanoscale fluorescent signaling device [1]. These DX assemblies are composed of a collection of semicomplementary strands annealed into one supermolecule that has two co-axial and connected, but spatially separated, base stacks. A representative DX assembly is shown in Figure 1. Initial experiments using semicomplementary strands to construct these DX assemblies understandably focused on characterization and classification of different possible conformers and architectures [2,3]. The DX assembly is more rigid than canonical duplex DNA, as demonstrated by its resistance to circularization by ligation, and this rigidity permits the self-assembly of structures that have visible regularity over micrometer distances [4,5]. Other studies have focused on the use of DNA architecture as a template for metal deposition (reviewed briefly in [6]), suggesting that DNA could be used as an easily removed template for forming submicroscale wires a few atoms thick.

A range of studies of DNA conductivity and of long-range charge transport through double-helical DNA have also been carried out. On the basis of conductivity measurements, the DNA duplex has been suggested to have properties of either a large- or small-band gap semiconductor, and these results have prompted speculations about DNA being exploited as a quantum wire in nanoscale electronic devices [7–9]. Electrochemical studies on DNA films have demonstrated a sensitivity in charge transport to perturbations in base-pair stacking and, as a result, have been employed as a sensitive methodology to detect mismatches in DNA [10,11]. Spectroscopic studies of photo-induced electron transfer between donors and acceptors bound to DNA have also revealed this exquisite sensitivity in reaction to base-pair stacking and dynamic motion [12–14].

With respect to DNA-mediated charge transport, perhaps most clearly established thus far is that charge transport through DNA can promote oxidative damage to guanine

Figure 1



Model of a DX assembly, emphasizing the base-pair stacks. The base stack is shown in small space-filling display, whereas the sugar–phosphate backbones are portrayed as colored ribbons entwining the spatially distinct base stacks. The bottom stack has an appended rhodium metallointercalator (yellow) that is constrained to intercalate specifically into the extended end of the lower base-stack. Strands in this molecular model are colored as follows: top backbone, light blue; metallointercalator-bearing interstack, violet; core interstack, green; rightmost interstack, purple; bottom backbone, blue. The bases within the stacks are shown in silver, except guanine doublets and triplets, which are shown in red.

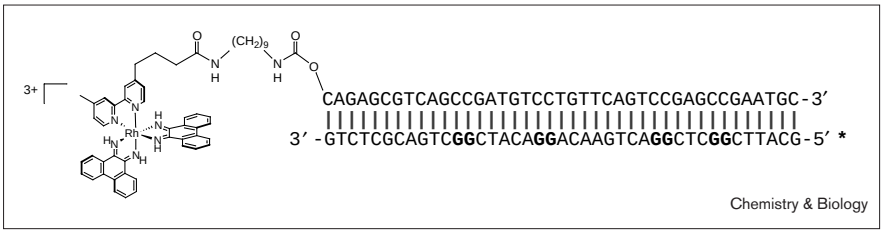
bases from a remote site [15–18]. In assays of oxidative DNA damage from a distance, the effects of intervening bulges [19], of DNA-binding proteins [20], and of variations of sequence and length [21] have been examined. Mechanisms proposed to describe this damage include hole hopping [22], phonon-assisted polaron formation [23] and tunneling [24]. Whether long-range oxidative damage is an issue of physiological importance within the cell has yet to be determined.

This combination of interests in the DNA double helix as an electronic entity and as an architecture in nanoscale devices prompted us to examine charge transport through DX DNA assemblies. The potential application of DNA in nanoscale electronic devices would require the tight packing of helices in arrays controlled by sequence. Is charge transport efficient under these conditions? Do DX assemblies generate sufficiently well-stacked base-pair arrays to promote charge transport? Moreover, are such base-pair stacks electronically insulated from each other? Undesired crosstalk among circuits could completely abrogate the size advantages obtained by using these molecules as assembly units.

The exploration of electronic interactions between tightly packed helices is important to consider also in the context of understanding aspects of radical damage to DNA within the cell. The extremely tight packing of DNA either on a surface or in large scale, covalent arrays offer an interesting parallel to the structures found in packaged, genomic DNA. To prevent irreversible tangling of the chromosomes, these linear supermolecules are extensively wrapped into coiled coils on histones, which themselves are assembled into higher levels of organization in chromatin [25]. Owing to the limited nuclear volume of eukaryotic cells, the manipulation of and access to each layer of packaging is rigorously orchestrated to allow the highest possible density of the DNA helices. The resultant close proximity of many otherwise quite distant strands suggests that free-radical-induced damage in DNA could conceivably migrate across sugar phosphate bonds, and thus into oxidatively sensitive locations quite distant from the point of introduction.

Here we examine charge transport through a DX DNA assembly. DX molecules with two spatially and

Figure 2



A DNA duplex containing oxidatively sensitive 5′-GG-3′ sites (in bold) with a covalently attached derivative of Rh(phenanthrenequinonediimine)$_2$(dimethylbipyridine)$^{3+}$, [Rh(phi)$_2$DMB]$^{3+}$. Photolysis at 365 nm of the tethered rhodium complex intercalated in the duplex promotes oxidative damage to the 5′-G of guanine doublets by long-range charge transport.

electronically decoupled base stacks provide a model system to examine interstack electronic coupling in packed arrays. Our results indicate that these tightly packed base-pair arrays provide a robust medium for charge transport. These observations suggest that DNA duplexes organized within chromosomes or potentially tightly packaged and arrayed within a nanoscale device would mediate charge transport directly through the base stack, precluding oxidative damage migration across sugar phosphate backbones and onto abutting helices. Thus, DNA may indeed represent a promising molecular medium for nanofabrication.

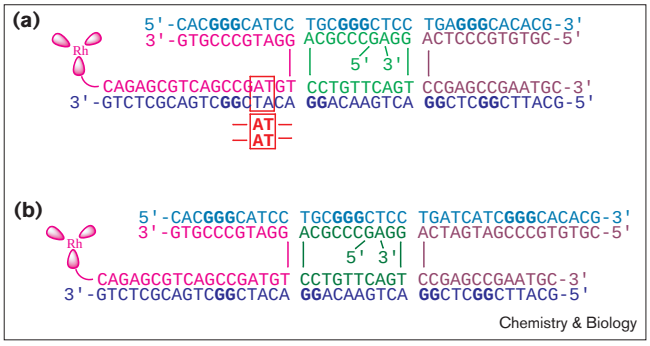
Results and discussion

Design of DX assembly to probe charge transport

A chemical assay now commonly employed to probe long-range charge transport through DNA involves the construction of an assembly containing a photo-oxidant that is spatially well-separated from the DNA site being oxidized; the yield of long-range electron transfer (ET) is determined through measurements of oxidative damage [15]. Figure 2 shows a representative assembly. In these experiments, each B-DNA duplex is selectively functionalized on the 5′ end of one strand with an intercalating photo-oxidant, here a phenanthrenequinone diimine (phi) complex of rhodium [15,21]. The complementary strand contains 5′-**G**G-3′ sites, which are preferentially oxidized at the bold guanine over any other sites. Empirical evidence and theoretical calculations [26] indicate that the oxidative potential of guanines in DNA is significantly lowered compared with other bases due to highest occupied molecular orbital (HOMO) delocalization. When irradiated at 365 nm, although bound remotely, the photo-excited metallointercalator accepts an electron from the 5′ guanine of a 5′-GG-3′ pair in a reaction mediated by the base stack. The resultant guanine radical cation can either undergo recombination or, following deprotonation, be trapped irreversibly to form an oxidative lesion that is susceptible to strand cleavage by treatment with hot piperidine [15]. Irradiation of the bound rhodium complex at 313 nm, in contrast, causes direct strand scission at sites of intercalation in duplex DNA by hydrogen atom abstraction from the sugar ring [27]; photolysis at this higher energy therefore allows mapping of metal complex intercalation sites. We employ these photochemical assays here to conveniently monitor the path for charge transport.

An antiparallel DX assembly, containing an even number of helical half turns between junction overlap points, was therefore designed to have a pendant metallointercalator, in addition to well-spaced guanine doublets on one base stack and guanine triplets on the second (Figure 3). Guanine triplets have been predicted to be oxidatively more sensitive than guanine doublets [28]. Our intent was to maximize sensitivity to potential oxidative damage on the base stack that is uncoupled to the tethered rhodium metallointercalator, while still allowing investigation of long-range charge transport through the rhodium-bearing base stack.

Figure 3



Sequences of DX assemblies containing the spatially separated photo-oxidant, the rhodium complex (as in Figure 2, yellow in Figure 1) and guanine doublets or triplets to be oxidized (in bold). **(a)** Sequence and base-pairing scheme for one DX assembly in which the location of the double mismatch is boxed in red. **(b)** Sequence and base-pairing for the DX molecule containing a longer second stack compared with that in (a) so as to rotate the most distal 5′-GGG-3′ 180° from the orientation in (a).

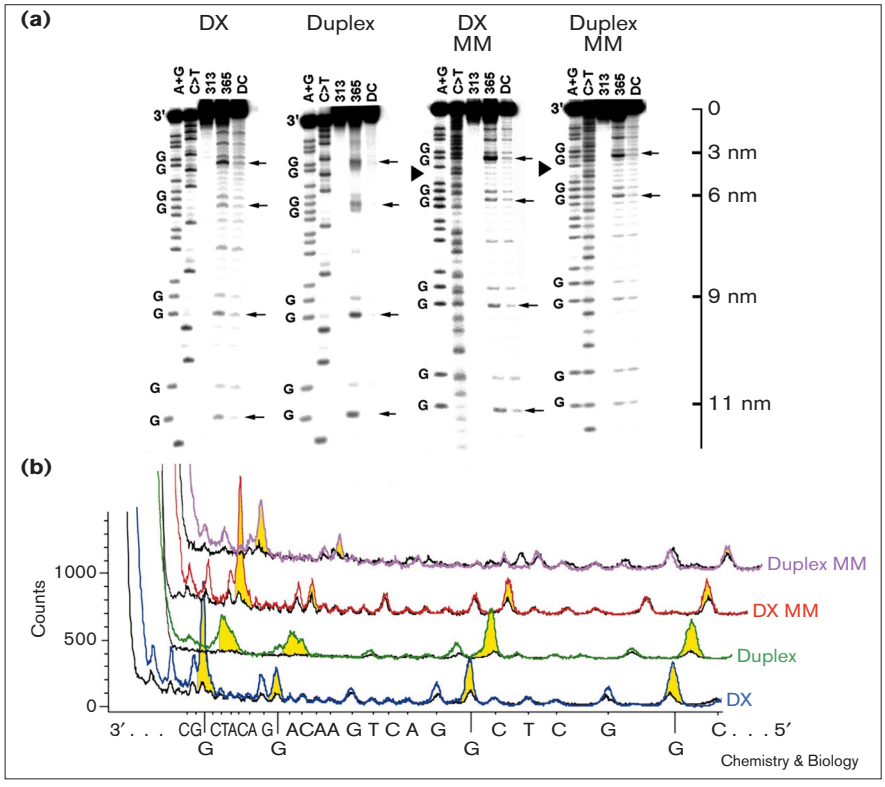
The overall assembly contains five semicomplementary strands, two of which serve as backbone strands and permit easy comparison of duplex DNA to DX DNA by varying the complementary strand(s). The intercalator, which in B-form duplex DNA stacks deeply into the helix [29], was tethered to the 5′ end of one capping strand to permit intercalation into one base stack but not the other. In addition, we extended the length of the intercalated base stack six base pairs from the terminal end of the second, nonmetallated stack; molecular modeling indicated that this duplex elongation extends the metallointercalator well past the end of the second stack (Figures 1 and 3). Direct photocleavage reactions confirmed these intercalation restrictions experimentally (see below). The characterization of the assemblies, once synthesized using phosphoramidite chemistry and purified by high-performance liquid chromatography (HPLC) and gel electrophoresis, was similar to that described previously, using gel shifts and chemical structural probes [2,30].

Charge transport down the primary stack of the DX assembly

An individual base stack, comprised of four separate DNA strands, can serve as a conduit for long-range charge transport. Radioactive labeling of the contiguous strand in the rhodium-bound stack (purple strand in Figure 3a) and photolysis of the DX assembly at 365 nm reveals significant oxidative damage at 5′-GG-3′ doublets up to 11 nm from the site of intercalation, established by photolysis at 313 nm.

Figure 4 shows the results of photolysis experiments conducted on the assemblies shown in Figures 2 and 3a. As evident in the 313 nm lane for the DX assembly, direct photocleavage is apparent only near the 3′ end of the labeled backbone strand; this appears here as a broadening

Figure 4



Long-range oxidative damage in the intercalator-bound stack of the DX assembly and comparison to damage in duplex DNA and with mismatches. **(a)** Phosphorimagery after electrophoresis in a denaturing 20% polyacrylamide gel with 5′-^{32}P- end-labeling of the strand complementary to the metallo-intercalator-tethered strand for (panels left to right): the DX DNA assembly shown in Figure 3a (navy strand labeled); the duplex DNA shown in Figure 2; the DX DNA assembly containing a double mismatch (red box in Figure 3a) and the duplex DNA counterpart with the double mismatch at the corresponding site. The mismatch sites are indicated by the solid triangle. Lanes for each panel correspond (left to right) to Maxam Gilbert A + G and C > T sequencing reactions, photolysis at 313 nm for 10 min, photolysis at 365 nm for 1 h followed by piperidine treatment, and the same labeled assembly without irradiation (dark control, DC) followed by piperidine treatment. Sites of potential oxidation upon irradiation at 365 nm are indicated by **GG** labels, and arrows highlight reaction at the 5′-G. Some background damage at purines is evident in dark control lanes, as a result of piperidine treatment. In addition, the 365 and 313 nm lanes, though equal intensity by autoradiography, appear broader due to photocleavage at their intercalation sites. **(b)** Histograms made by linear scanning of the phosphoimages. Intensities given down the lanes for samples after 365 nm irradiation (colors indicated to right) with corresponding dark controls (uniformly black) overlayed. The damage at 5′-G sites of guanine doublets above the control is highlighted by yellow shading. The DX assembly with or without an intervening double mismatch (MM) shows oxidative damage at the 5′-G of 5′-GG-3′ sites up to 11 nm from the site of rhodium intercalation through long-range ET. Approximate distances are shown on the right in (a); these were determined based upon the number of bases separating the 5′-G site of oxidation and the rhodium intercalation site (established by 313 nm photocleavage) and assuming an interbase-pair stacking distance of 3.4 Å. Substantial long-range oxidative damage is evident also in the DNA duplex, but no significant oxidative damage above the control is apparent in the DNA duplex containing MM at sites 9 nm and 11 nm away from the metallintercalator.

of the parent bands with irradiation either at 313 or 365 nm. At higher gel resolution or using shorter oligonucleotides, this cleavage maps directly to the intercalation site near the duplex terminus. Irradiation at 313 nm of DX assemblies alternately labeled on each strand, except for the rhodium-bearing strand that was not radioactively tagged, showed no damage.

Importantly, irradiation at 365 nm of the DX assembly shows oxidative damage at the 5′-G of guanine doublets. Long-range damage is evident at guanine doublets located ~35, 54, 92 and 109 Å from the site of intercalation. In calculating the separation between the intercalator and the site of guanine damage, we assumed 3.4 Å interbase stacking distances. Some background damage at purines is also revealed upon piperidine treatment. The histogram provided, which includes an overlay of the lanes containing DX assemblies without irradiation at 365 nm but subjected to piperidine treatment (dark controls, Figure 4), clearly demonstrates significant damage over the controls at the 5′-G of the guanine doublets, the signature for electron transfer.

We can also compare reaction on the DX assembly to its duplex DNA counterpart. As in the DX assembly, its duplex counterpart shows long-range damage at the 5′-G of guanine doublets upon irradiation at 365 nm. Some hyper-reactivity is noteworthy at the site preceding the first crossover junction for the DX assembly but not for the duplex. Although the level of damage in the DX portion of the gel at the 5′-GG-3′ closest to the rhodium complex compares well to that seen in the duplex strand visually, quantitation, shown below the gel as line graphs, indicates that there is rather more damage at the proximal site in the DX case than in the case of the duplex. However, consistently in all trials carried out, both systems show comparable damage at long range in the most distal sites.

As with duplex DNA, long-range transport in the DX supermolecule occurs in an intramolecular reaction. As in

previous studies of long-range damage [15,19,21], no oxidative damage is seen on a radioactively labeled DX assembly that does not contain a pendant rhodium complex when incubated with an unlabeled DX bearing a covalently bound metallointercalator, even with twice the normal irradiation time (data not shown).

These data therefore establish unambiguously that charge transfer proceeds down the π-stacked array of bases. The ability of the DX assembly to serve as a conduit for such long-range transfer is in agreement with previous biochemical studies which suggest that the base stacking within the DX molecule is, in general, quite similar to that of B-form DNA [2]. Our results further indicate, however, that the DX base stack is electronically coupled over long range.

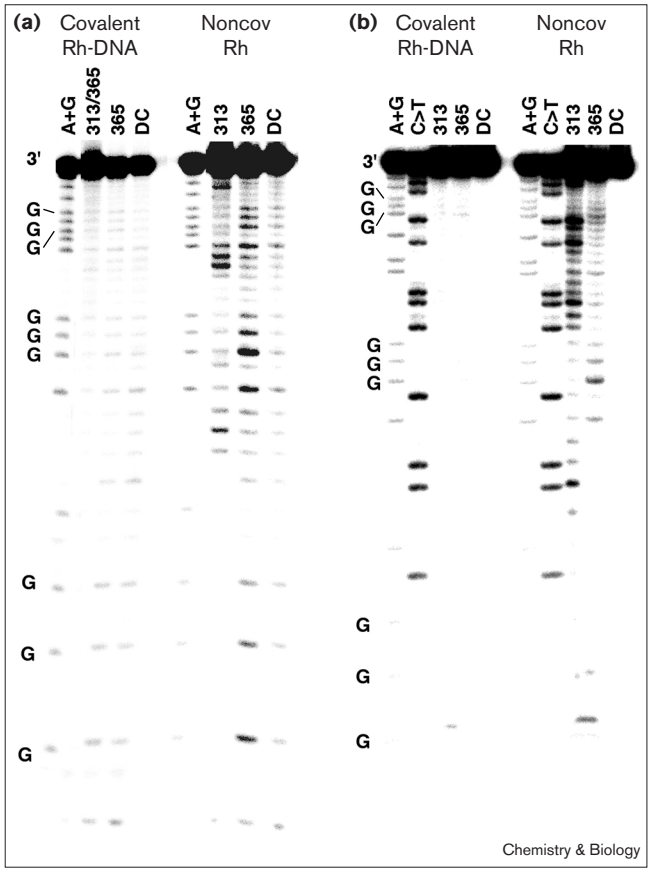
Robust charge transport in the presence of mismatches

Remarkably, DX assemblies act as more robust conduits for long-range charge transfer than do their duplex counterparts. To test for the effects of stacking perturbations, we introduced mispairs into the assemblies (Figure 3a, boxed in red). Previous experiments have noted that the presence of mismatches in DNA-modified electrodes can completely abrogate long-range charge transport through a DNA film [10,11].

Here, introduction of a double mismatch into a DX assembly between the intercalator and the first of the two crossover junctions does not attenuate long-range guanine oxidation down the intercalator-bearing stack at all (Figure 4). In contrast, the incorporation of the same double mismatch into identically base paired duplex DNA essentially eliminates long-range electron transfer past the mismatch, as expected. The damage at the most distal 5′-GG-3′ in duplex DNA normalized to the proximal guanine oxidation site decreases sevenfold (average of three trials) upon introduction of the double mismatch, whereas in a DX assembly with these mismatches, the decrease is negligible (<10%) and within error bars of the experiment.

We considered whether the double mismatch within the DX assembly might significantly alter the structure of the DX assembly. As a test, here, as with the other DX assemblies examined, we irradiated the assembly at 313 nm in the presence of noncovalently bound Rh(DMB)phi$_2^{3+}$ (DMB = 4,4′-dimethyl 2, 2′-bipyridine) (data not shown). Hyper-reactivity in direct photocleavage by this sequence-neutral metallointercalator would be expected to reveal any substantial alterations in structure [31]. Instead, low levels of uniform cleavage with no unusual hot spots were observed after photoirradiation either at 313 nm or 365 nm. By comparison, introduction of a disruptive thymine dimer lesion into the core of a DX assembly using photoligation techniques [32,33] generated an intensely targeted hot spot when irradiated at 313 nm in the presence of noncovalent Rh(DMB)phi$_2^{3+}$.

Figure 5



Test of cross-coupling in electron transfer between base stacks in the DX DNA assembly. Shown is the phosphorimagery after electrophoresis in a denaturing 20% polyacrylamide gel of the DX assembly shown in Figure 2b with 5′-^{32}P- end-labeling of **(a)** the backbone strand (light blue in Figure 2a) opposite the rhodium bearing base stack with 5′-GGG-3′ cassettes spaced evenly facing into the stack-stack contact surface, or **(b)** the contiguous strand on the same stack (light blue strand in Figure 2b) with one 5′-GGG-3′ cassette rotated by 180° around the stack helical axis by insertion of a 5 bp spacer. Also shown is oxidative damage on the alternate stack with noncovalently bound rhodium complex. Within each panel, lanes are as in Figure 3, and almost all conditions are the same as Figure 3, except that in (a) the 313/365 lane indicates a control sample irradiated at 313 nm (for 10 min) followed by 365 nm irradiation (for 1 h), but not treated with piperidine. Additionally, duration of irradiations in (b) were twice that of other samples, in an effort to reveal any trace intermolecular or interstack metal complex reaction in the covalent DX assembly. None was observed. The sites of oxidation upon irradiation at 365 nm are indicated by **GGG** labels.

The double mismatch perturbation examined here is clearly more extreme than other disruptions that might be expected to occur during the routine application of a DX assembly in a nanoscale device. This experiment therefore highlights the rugged character of the DX assembly. We suggest, based on the observations described here, that the persistence of electronic coupling in this DX assembly is probably the result of tight packing of the sugar phosphate backbone that dynamically constrains the nucleotides in the base stack.

Insulation of separate base-pair stacks in the DX assembly

Despite the tight packing of neighboring helices within a single DX molecule, the electronic structure of the two separate base stacks appears to be completely decoupled. Isotopic ^{32}P end-labeling of the backbone strand that does not interact with covalently bound metal, (light blue strand, Figure 3a) followed by 365 nm photolysis and piperidine treatment, afforded no damage at the 5′-GGG-3′ triplets over that in the dark control (Figure 5a). Moderate damage was found at the guanine triplet sites in this base stack irradiated at 365 nm with noncovalently added $Rh(DMB)phi_2^{3+}$, most prominently in the guanine triplet located between the two crossover junctions. The locations of direct photocleavage reactions, determined by irradiation at 313 nm, on this strand were relatively sequence neutral, and entirely different than those seen in the 365 nm irradiation lanes.

Molecular modeling indicated that all three guanine triplets in this molecule are located at the interface between the two helices. In order to eliminate the possibility that the lack of damage observed at these sites was due to a closed orientation of the guanines, thus possibly making the oxidation sites inaccessible to irreversible trapping, another DX molecule with a guanine triplet shifted five base pairs down the duplex and therefore rotated by 180° was constructed (schematic in Figure 3b and molecular model in Figure 1). As with other DX molecules, strong oxidative damage was evident at the 5′-GG-3′ sites on the stack into which the covalently attached rhodium complex was intercalated (not shown). However, the predicted sites of oxidative damage on the triplet-bearing base stack not containing the covalently attached metallointercalator are left completely undamaged after irradiation at 365 nm (Figure 5b). Like the shorter DX assembly, the longer DX assembly shows the most oxidative damage at the core 5′-GGG-3′ triplet when irradiated in the presence of noncovalently bound rhodium complex. The reactivity of the core guanine triplet persists, despite the change in orientation afforded by the introduction of five additional base pairs between the core guanine triplet and the triplet 3′ to it on the backbone strand.

The lack of electronic communication between the two helices therefore prevents the photoexcited metallointercalator, covalently bound to the first stack, from extracting an electron from the guanine triplets on the second stack. Indeed, the two stacks are not only spatially separated, as expected by molecular modeling of double crossovers, but the two base stacks within a DX molecule are also unique and insulated electronic entities.

A crystal structure of a single four-way crossover junction was reported recently [34]. The electronic decoupling of the two stacks within the DX assembly is fully consistent with this structure; in the four-way junction crystal structure, bases within the separate stacks are strictly localized to the stack to which they are canonically assigned, and do not show unexpected interhelix overlaps. These results bear also upon how damage may be directed within nucleosomes, where helices are closely and more rigidly packed than are DNA duplexes in solution. Radical damage that is introduced into systems such as the nucleosome or nanoscale DNA arrays would in essence be constrained to migration in a one-dimensional pathway — either upstream or downstream from the site of lesion introduction. Our results strongly suggest that radical migration within tightly packed arrays would be unable to jump helices, and thus would be limited to within ~100 linear base pairs.

Significance

In an effort to reconstruct DNA-based nanodevices, multiple-stranded DNA assemblies have been prepared and characterized. Here charge transport is examined in a multiple-stranded DNA assembly whose structure is encoded by DNA sequence. We have found that double crossover (DX) DNA can be applied both as a rugged and easily programmable nanomaterial and as an insulated charge conduit in long-range signaling. In a DX assembly, the structure is determined by molecular recognition of complementary bases, while the electronics are determined by stacking of the base-pair array. The ability to construct spatially and electronically separate components in close proximity on a molecular scale therefore is feasible in DX assemblies through DNA synthesis.

Materials and methods

Oligonucleotide preparation

Oligonucleotides were synthesized using phosphoramidite chemistry on an ABI 392 DNA synthesizer with a dimethoxy trityl protecting group on the 5' end. Purification on a Rainin Dynamax C18 column by reverse phase HPLC on a Waters HPLC (25 mM NH_4OAc, pH 7, 5–25% CH_3CN over 30 min) was followed by deprotection in 80% acetic acid for 15 min. Following deprotection, the oligonucleotides were purified a second time on the same C18 column by reversed-phase HPLC (25 mM NH_4OAc, pH 7, 2–20% CH_3CN over 30 min) and were quantitated by UV–visible absorption spectroscopy on a Beckman DU 7400 spectrophotometer using the following extinction coefficients for single-stranded DNA: (260 nm, $M^{-1}cm^{-1}$) adenine (A) = 15,000; guanine (G) = 12,300; cytosine (C) = 7400; thymine (T) = 6700.

Strands used were each radioactively labeled at the 5′end using γ-^{32}P-ATP (ICN Biomedicals, Costa Mesa, CA) and polynucleotide kinase, then treated with hot piperidine to remove latent oxidative damage and purified on a 20% denaturing polyacrylamide gel. Double crossover molecules and duplexes were made by mixing equimolar amounts of strands to a final concentration of 10 μM, and annealed in 50 mM Tris-Acetate, 1 mM EDTA and 12.5 mM $Mg(OAc)_2$ pH 7.4 (1× TAEMg) using a linear temperature gradient of 90°C to 5°C over 1.5 h. DNA strands with attached rhodium metallointercalators were prepared as described previously [35] and purified on a Dynamax C4 column by reverse-phase HPLC (50 mM NH_4OAc, pH 7, 2–15% CH_3CN over 45 min). Both stereoisomers were combined and used in experiments. Quantitation for rhodium–DNA conjugates was based on using the DNA extinction coefficient value calculated at 260 nm as described above.

Characterization of DX assemblies

Annealing of DX assemblies with one strand ^{32}P end-labeled allowed characterization by nondenaturing gel electrophoresis. 10% acrylamide gels polymerized with a running buffer of 1× TAEMg were run in the same buffer at 100 V for 3.5 h with 10 µl of the 10 µM annealing solutions. Each of the five strands was separately labeled, separately gel purified as single strands, and then annealed into 50 µl aliquots of a solution of 10 µM final concentration of each oligonucleotide used to form the DX assembly. Typical results afforded > 95% of a single parent band on nondenaturing gels that migrated at an identical location regardless of the radioactively labeled strand. This location was considerably more retarded than the location of a duplex DNA of a similar length run side-by-side with the DX assemblies.

In addition, every experiment was performed with covalent Rh–DNA conjugates, noncovalently added Rh(DMB)phi$_2^{3+}$, and DX assemblies lacking any form of rhodium. Both the mismatch-containing and the properly paired DX molecules duplexes show moderate, relatively sequence neutral damage patterns identical to each other upon irradiation at 313 nm or 365 nm; this strongly suggests that assemblies containing double mismatches are in fact not in a distorted conformation.

Photocleavage reactions

Photolysis reactions were performed in 1× TAEMg on 20 µl samples using a 1000 W Hg/Xe lamp with a monochromator. Each sample irradiated at 313 nm was dried and resuspended into a volume of formamide loading buffer (determined by the sample radioactivity) to 10 kcts/µl. Samples irradiated at 365 nm and not irradiated were dried, redissolved into 100 µl of 10% piperidine in water, and heated to 90°C for 30 min. After drying *in vacuo*, these samples were resuspended into formamide loading buffer normalized by their radioactivity, as described above. Most of the samples required cold excess of the labeled strand to be added after irradiation and any piperidine treatment but before redissolution into formamide to minimize reannealing of the highly stable DX assemblies. Samples in formamide were heated to 90°C for 5 min before being loaded immediately onto sequencing gels. All 20% sequencing gels were run hot (> 60°C). Maxam–Gilbert sequencing reactions followed standard protocols [36].

Data analysis

Gels were exposed to phosphorimager plates overnight and ImageQuant software was used to scan and analyze the collected data. Further data manipulations after band quantitation were performed in Microsoft Excel.

Acknowledgements

We are grateful to the National Institutes of Health for financial support. We thank also the NIH for predoctoral fellowship **s**upport of D.T.O. and the Caltech SURF program for a summer fellowship to E.A.D.

References

1. Mao, C., Sun, W., Shen, Z. & Seeman, N.C. (1999). A nanomolecular device based on the B-Z transition of DNA. *Nature* **397**, 144-146.
2. Fu, T.-J. & Seeman, N.C. (1993). DNA double crossover molecules. *Biochemistry* **32**, 3211-3220.
3. Li, X., Yang, X., Qi, J. & Seeman, N.C. (1996). Antiparallel DNA double crossover molecules as components for nanoconstruction. *J. Am. Chem. Soc.* **118**, 6131-6140.
4. Winfree, E., Liu, F., Wenzler, L.A. & Seeman, N.C. (1998). Design and self-assembly of two-dimensional DNA crystals. *Nature* **394,** 539-544.
5. Yang, X., Wenzler, L.A., Qi, L., Li, X. & Seeman, N.C. (1998). Ligation of DNA triangles containing double crossovers. *J. Am. Chem. Soc.* **120**, 9779-9786.
6. Storhoff, J.J. & Mirkin, C.A. (1999). Programmed materials synthesis with DNA. *Chem. Rev.* **99**, 1849-1862.
7. Fink, H.W. & Schonenberger, C. (1999). Electrical conduction through DNA molecules. *Nature* **398**, 407-410.
8. Porath, D., Bezryadin, A., de Vries, S. & Dekker, C. (2000). Direct measurement of electrical transport through DNA molecules. *Nature* **403**, 635-638.
9. Okahata, Y., Kobayashi, T., Tanaka, K. & Shimomura, M. (1998). Anisotropic electric conductivity in an aligned DNA cast film. *J. Am. Chem. Soc.* **120**, 6165-6166.
10. Kelley, S.O., Jackson, N.M., Hill, M.G. & Barton, J.K. (1999). Long range electron transfer through DNA films. *Angew. Chem. Intl. Ed.* **38**, 941-945.
11. Kelley, S.O., Boon, E.M., Barton, J.K., Jackson, N.M. & Hill, M.G. (1999). Single-base mismatch detection based on charge transduction through DNA. *Nucleic Acids Res.* **27**, 4830-4837.
12. Wan, C., Fiebeg, T., Kelley, S.O., Treadway, C.R., Barton, J.K & Zewail, A.H. (1999). Femtosecond dynamics of DNA-mediated electron transfer. *Proc. Natl Acad. Sci. USA* **96**, 6014-6019.
13. Kelley, S.O., Holmlin, R.E., Stemp, E.D.A. & Barton, J.K. (1997). Photoinduced electron transfer in ethidium-modified DNA duplexes: dependence on distance and base stacking. *J. Am. Chem. Soc* **119**, 9861-9870.
14. Kelley, S.O. & Barton, J.K. (1998). DNA-mediated electron transfer from a modified base to ethidium: pi-stacking as a modulator of reactivity. *Chem. Biol.* **5**, 413-425.
15. Hall, D.B., Holmlin, R.E. & Barton, J.K. (1996). Oxidative DNA damage through long-range electron transfer. *Nature* **382**, 731-735.
16. Arkin, M.R., Stemp, E.D.A., Coates-Pulver, S. & Barton, J.K. (1997). Long-range oxidation of guanine by Ru(III) in duplex DNA *Chem. Biol.* **4**, 389-400.
17. Armitage, B., *et al*., & Schuster, G.B. (1997). Peptide nucleic acid-DNA duplexes: long range hole migration from an internally linked anthraquinone. *Proc. Natl Acad. Sci. USA* **94**, 12320-12325.
18. Meggers, E., Kusch, D., Spichty, M., Wille, U. & Giese, B. (1998). Electron transfer through DNA in the course of radical-induced strand cleavage. *Angew. Chem. Intl. Ed.* **37**, 460-462.
19. Hall, D.B. & Barton, J.K. (1997). Sensitivity of DNA-mediated electron transfer to the intervening pi-stack: a probe for the integrity of the DNA base stack. *J. Am. Chem. Soc.* **119**, 5045-5046.
20. Rajski, S.R., Kumar, S., Roberts, R.J. & Barton, J.K. (1999). Protein-modulated DNA electron transfer. *J. Am. Chem. Soc.* **121**, 5615.
21. Núñez, M.E., Hall, D.B. & Barton, J.K. (1999). Long range oxidative damage to DNA: effects of distance and sequence. *Chem. Biol* **6**, 85-97.
22. Meggers, E., Michel-Beyerle, M.E. & Giese, B. (1998). Sequence-dependent long-range hole transport in DNA. *J. Am. Chem. Soc* **120**, 12950-12955.
23. Henderson, P.T., Jones, D., Hampikian, G., Kan, Y. & Schuster, G.B. (1999). Long-distance charge transport in duplex DNA: the phonon-assisted polaron-like hopping mechanism. *Proc. Natl Acad. Sci. USA* **96**, 8353-8358.
24. Beratan, D.N., Priyadarshy, S. & Risser, S.M. (1997). DNA: insulator or wire? *Chem. Biol.* **4**, 3-8.
25. Edmondson, D.G. & Roth, S.Y. (1996). Chromatin and transcription. *FASEB J.* **10**, 1173-1182.
26. Saito, I., Takayama, M., Sugiyama, H., Nakatani, K., Tsuchida A. & Yamamoto, M. (1995). Photoinduced DNA cleavage via electron transfer: demonstrating that guanine residues located 5' to guanines are the most electron donating sites. *J. Am. Chem. Soc* **117**, 6406-6407.
27. Sitlani, A., Long, E.C., Pyle, A.M. & Barton, J.K. (1992). DNA Photocleavage by phenanthrenequinone diimine complexes of rhodium(III): shape-selective recognition and reaction. *J. Am. Chem. Soc.* **114**, 2303-2312.
28. Sugiyama, H. & Saito, I. (1996). Theoretical studies of GG-specific photocleavage of DNA via electron transfer: significant lowering of ionization potential and 5′-localization of HOMO of stacked GG bases in B-form DNA. *J. Am. Chem. Soc.* **118**, 7063-7068.
29. Kielkopf, C.L., Erkkila, K.E., Hudson, B.P., Barton, J.K. & Rees, D.C. (2000). Structure of a photoactive rhodium complex intercalated into DNA. *Nat. Struct. Biol.* **7**, 117-121.
30. Erkkila, K.E., Odom, D.T. & Barton, J.K. (1999). Recognition and reactivity of metallointercalators with DNA. *Chem. Rev.* **99**, 2777-2795.
31. Lim, A.C. & Barton, J.K. (1993). Chemical probing of tDNA^{Phe} with transition metal complexes. *Biochemistry* **32**,11029-11034.
32. Liu, J.Q. & Taylor, J.S. (1998). Template-directed photoligation of oligodeoxyribonucleotides via 4-thiothymidine. *Nucleic Acids Res.* **26**, 3300-3304.
33. Lewis, R.J. & Hanawalt, P.C. (1982). Ligation of oligonucleotides by pyrimidine dimers. *Nature* **298**, 393-396.
34. Ortiz-Lombardía, M., González, A., Eritja, R., Aymamí, J., Azorín F. & Coll, M. (1999). Crystal structure of a DNA Holliday junction. *Nat. Struct. Biol.* **6**, 913-917.
35. Holmlin, R.E., Dandliker, P.J. & Barton, J.K. (1999). Synthesis of metallointercalator–DNA conjugates on a solid support. *Bioconj. Chem.*