Chapter 8

Thymine Dimers for DNA Nanocircuitry Applications

Introduction

Current technology for the production of nanoscale electronics relies on a top-down approach involving lithography to pattern chips. However, this technology is reaching a limit; on such small scales, problems such as charge leakage through imperfections in the insulation occur, resulting in burnout of the circuit.[1, 2] An alternative to current lithographic techniques is a bottom-up approach, which relies on single molecules to act as the conductive wires in the circuit. Molecular wires circumvent the majority of issues associated with miniaturization of circuitry components because each wire is completely self-contained.[3]

Molecular wires are thus a significant improvement over lithography for applications on the nano and sub-nanoscale. However, these wires have their own problems, especially with their implementation for commercial production. Molecular wires must have uniform lengths and conductive properties, functionalized termini, and the ability to conduct charge over nanoscale distances.[1, 3] These stringent criteria severely limit the current applications of molecular wires.

Most molecular wires fall into three categories: conjugated hydrocarbons, carbon nanotubes, and porphyrin oligomers.[4] While examples of these molecules have all been shown capable of acting as molecular wires, each also has drawbacks. Conjugated hydrocarbons are often difficult to uniformly synthesize and terminally functionalize, carbon nanotubes cannot be produced on a large scale with uniform conductive properties, and porphyrin oligomers cannot be readily synthesized into oligomers longer than several monomers.[4] The difficulty with manufacturing these molecular wires on a large scale with uniform properties has prohibited their broad applicability in circuitry.

In contrast, DNA is uniformly and efficiently synthesized with a variety of easily incorporated terminal functionalizations on a large scale. Furthermore, DNA has the unique ability to conduct electrons through its π-stacked bases, a phenomenon known as DNA charge transport (DNA CT). This unique property, in addition to the ease of synthesis and functionalization, imbues DNA with the qualities necessary to act as a component of a molecular circuit. Importantly, DNA has a shallow distance dependence, with a β value determined to be less than 0.05 (an ideal molecular wire has a β value of 0).[4] DNA CT is both robust and sensitive; charge transport has been shown to occur through 100 base pairs, 34 nm, with signal sizes comparable to those found for 17 base pair duplexes.[4] The ease of synthesis combined with the shallow distance dependence makes DNA ideal for potential applications as molecular wires.

In addition to its conductive properties, DNA has structural characteristics that increase its value as a potential component of a molecular circuit. Because of its dependence on the structure of DNA, DNA CT is highly sensitive to perturbations in the base stack. Any alterations that lead to destacking of the bases, including a mismatched base pair,[5] a damaged base,[6] or a DNA binding protein that kinks or bends the DNA, significantly attenuates the DNA-mediated electrochemical signal.[7, 8] Thus, any reversible DNA damage product has the potential to function as an internal switch. The inherent directionality of DNA further allows for specificity in terminus functionalization; the 5' and 3' ends of DNA can easily be modified independently to enable specific tethering within a circuit using orthogonal tethering methods.

The Barton group has extensively studied the basic properties of DNA CT by probing DNA self-assembled monolayers (SAMs) electrochemically.[5, 9-13] DNA

monolayers are assembled on gold surfaces through a terminal alkanethiol linker that forms a gold-sulfur bond with the electrode.[11, 14] The density of the assembled DNA can be somewhat controlled with the addition of magnesium chloride to the assembly solution of DNA.

DNA CT can be directly measured through a redox-active probe covalently linked to the complementary strand of DNA. Importantly, the probes must be well coupled to the π-stack in order to report DNA CT. The two main covalent probes currently employed by our group are Methylene Blue and Nile Blue (Figure 8.1). These probes differ in their mechanism of interaction with the base stack; Nile Blue is electronically coupled to the duplex through direct conjugation with the modified uracil to which it is appended (Figure 8.1B).[13] In contrast, Methylene Blue, tethered to the DNA by a long alkyl linkage, interacts with the base stack through direct intercalation with the bases (Figure 8.1A).[15] Both of these probes are used in the research described.
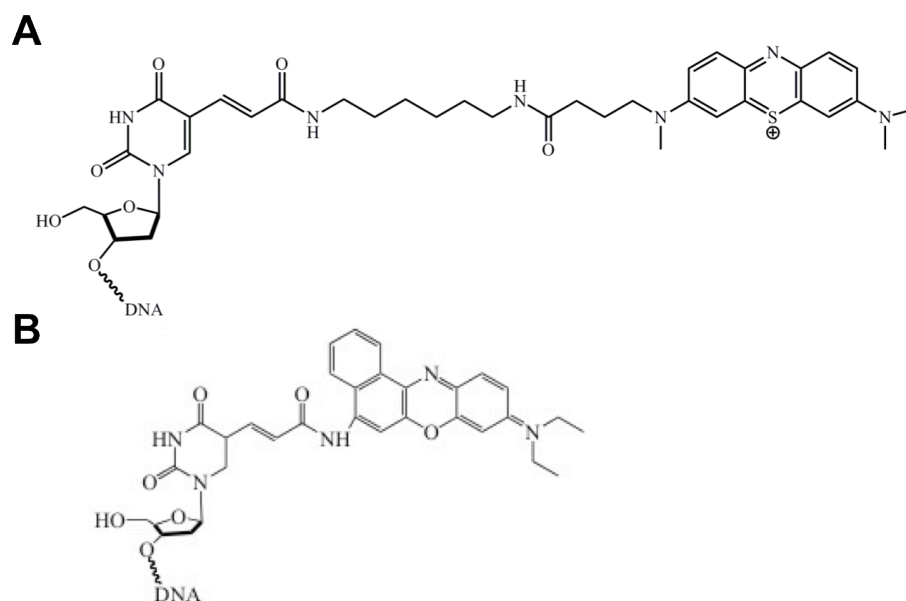
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Figure 8.1 Common covalent redox reporters for DNA CT on gold surfaces. The methylene blue (*top)* is connected to the DNA through a long alkyl linker. The synthetically modified methylene blue is attached to the base through amide bond formation with an amine-modified terminal base. This probe is capable of intercalating into the DNA base stack because of the flexible tether. The covalent Nile Blue moiety (*bottom*) is tethered to a modified uracil base. Because this probe lacks a long, flexible tether, it reports on charge transport through the conjugated tether to the DNA.

In order to probe DNA CT effectively for its applications to molecular circuitry, multiple experimental conditions with redundancy are required. Single gold rod electrodes are therefore insuffucient for such complex experiments. The Barton group has developed a multiplexed platform for simultaneous analysis of DNA CT through multiple DNA films on the same surface.[13] Multiplexed chips contain 16 individually addressable gold electrodes that can be divided into four quadrants (Figure 8.2). Electrodes are patterned onto silicon wafers using a two-step photolithographic technique. The multiplexed electrode areas are defined using photoresist followed by metal evaporation, and the individual electrodes are isolated through the addition of an insulating layer of SU-8 photoresist. Each electrode contains the working electrode area, leads, and contact pads around the exterior of the chip that enable each electrode to be addressed individually. This is advantageous over the single gold electrodes previously employed because four types of DNA can be assembled and measured on the same surface with four replicates simultaneously. This platform has opened significant avenues for pursuing both molecular wire-based and biosensing applications for DNA.

As DNA has been established to have all of the features of an effective molecular wire, the question of increasing the complexity of DNA circuitry components arises: what circuitry components could be directly incorporated into DNA nanowires through the exploitation of DNA's natural structure? Here, the progress made toward the development of a reversible switch for DNA CT is described.
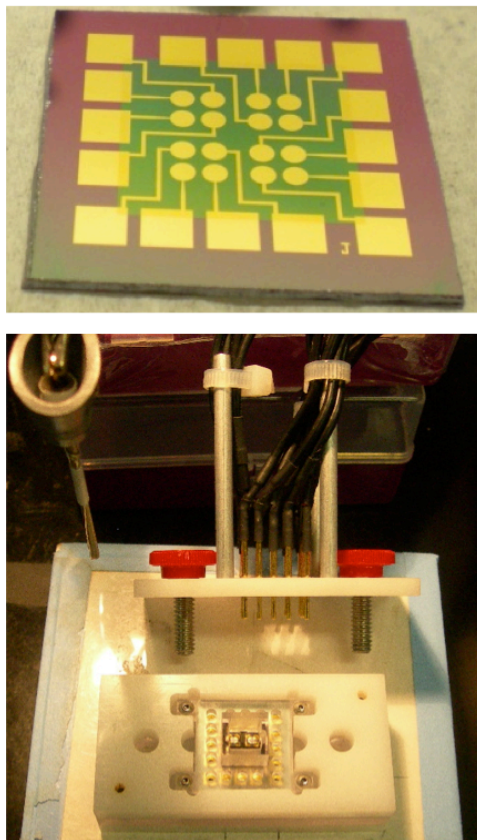
Figure 8.2 Multiplexed chip platform. Multiplexed chip (*top*) contains sixteen individually addressable electrodes divided into four quadrants. The electrodes are centered on the silicon wafer with the pads for connection around the exterior. The clamp (*bottom*) contains sixteen leads connected to a multiplexer. The clamp on the chip divides the electrodes into four quadrants with a single well to contain the electrolyte solution.

Reversible switches are the simplest circuitry component to incorporate as additional complexity into DNA-based nanostructures. The Barton group has demonstrated that DNA lesions that disrupt the π-stack attenuate DNA CT,[6, 7, 16-19] but circuitry-based applications require completely reversible, precise, controlled, and robust switching. One method of achieving these criteria is through the formation of the reversible DNA damage product, a thymine-thymine cyclobutane dimer.

Thymine dimers are formed through [2+2] cycloadditions between adjacent thymine bases on the same strand of DNA, forming a covalently-bound cyclobutane dimer (Figure 8.3). The major photoproduct formed has the *cis-syn* dimer, which is the stereochemistry shown in Figure 8.3. The thymine dimer lesion causes a 30° bend toward the major groove of a DNA duplex[20] and therefore attenuates DNA CT (Figure 8.4).[6] In nature, thymine dimers are formed upon photoexcitation of the DNA with UV light.[21] Because the adjacent thymines are identical, they are capable of forming an exciton and subsequently, an excimer. These states are specific to interactions between identical components of a complex. The ability of adjacent thymines to form such excited intermediates is one of the reasons that thymine dimers are the most common form of DNA photodamage.[22] UV irradiation is not optimal for the formation of thymine dimers in a laboratory environment because it can cause additional, nonspecific damage. Controlled thymine dimer formation is therefore performed in the laboratory with the aid of the triplet sensitizer acetophenone.[22]
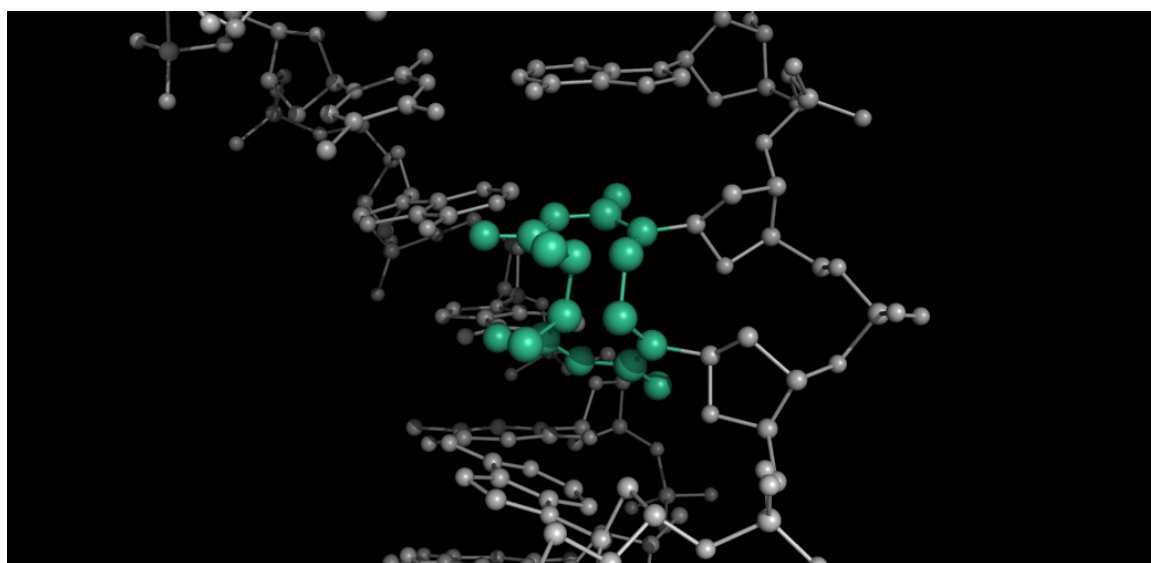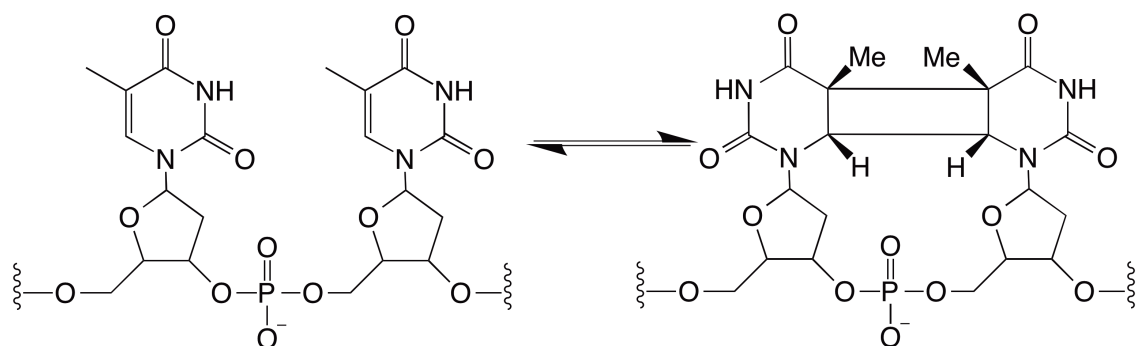
Figure 8.3 Thymine dimer formation. Top: Cyclobutane formation between adjacent thymine bases on the same strand of DNA. Bottom: Crystal structure of cyclobutane formation between adjacent bases on a single strand of DNA. Damaged bases (green) are overlaid onto undamaged (purple). The thymine dimer, shown in green, causes a significant pucker in the bases.
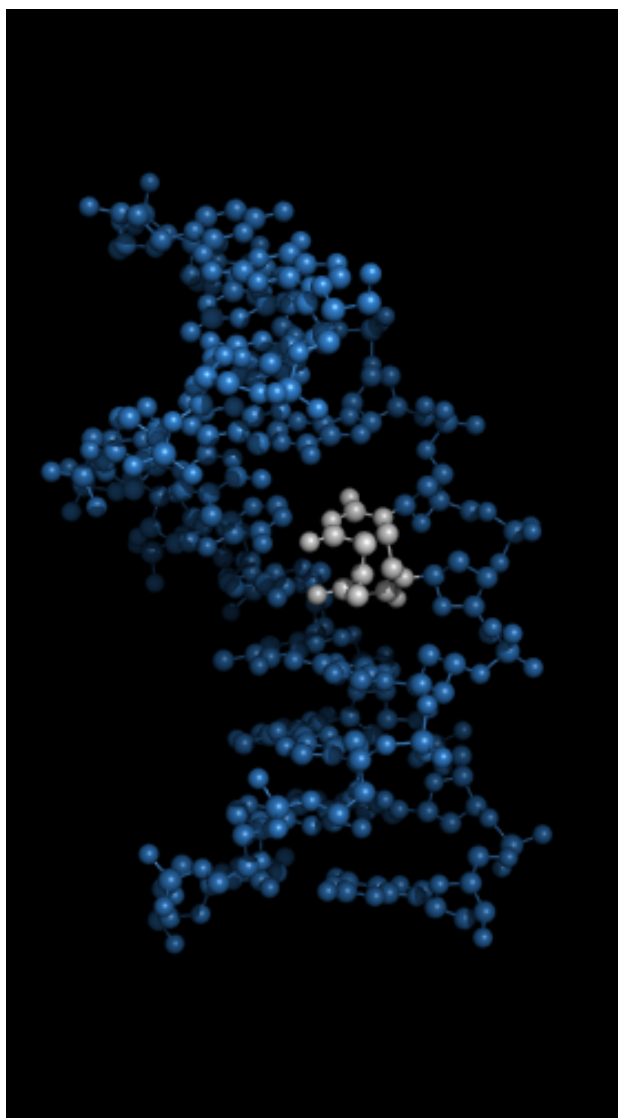
Figure 8.4 Crystal structure of a DNA duplex containing a thymine dimer. As can be seen from the thymine dimer (grey) formed on a single strand of the duplex, this damage product causes a significant kink in the DNA, with a distortion of over 30°.[20]

There are three major methods of thymine dimer repair: photocycloreversion with 254 nm light,[23] repair by the enzyme photolyase,[6] or repair by irradiation with a small molecule such as the organic intercalator naphthalene diimide (NDI)[24] or a rhodium(III) intercalator (Figure 8.5). Previous work in our group has demonstrated that the rhodium(III) intercalator, $Rh(phi)_2bpy^{3+}$, upon excitation with visible light, yields complete repair of thymine dimers.[25, 26] When the metal complex is photoexcited, an electron hole is injected into the DNA duplex, which breaks the cyclobutane dimer bonds, reejecting the electron hole and reforming two native thymine bases. These small rhodium complexes are advantageous for molecular switching, as they intercalate nonspecifically and can mediate thymine dimer repair over long distances. However, all previous studies with such metal complexes were performed in solution. Thus, experimentation to determine the efficacy of these complexes for thymine dimer repair on surfaces must be fully investigated to determine if the properties of this compound are equivalent on a surface to those of the compound in solution.
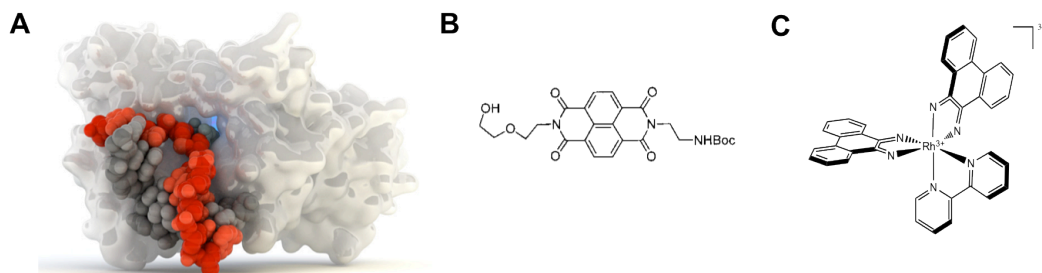
Figure 8.5 Modes of thymine dimer repair. A. The photolyase enzyme flips the dimerized bases out of the base stack to repair the cyclobutane.[34] B. The organic intercalator naphthalene diimide (NDI) is capable of repairing 27% of thymine dimers in solution. C. The rhodium intercalator, Rh(phi)$_2$bpy^{3+} nonspecifically intercalates into the base stack and can repair 100% of thymine dimers in solution.

To date, our lab has focused on studying thymine dimer repair electrochemically on a surface using the enzyme photolyase. DNA containing a pre-formed thymine dimer was used to form a self-assembled monolayer (SAM) on a single gold electrode. The system was monitored for the growth of a signal upon addition of photolyase, an enzyme that repairs thymine dimers. However, the reversibility of this process on a surface over multiple cycles was never investigated, nor were chemical methods of repair.

Here, we work to develop a thymine dimer-based photochemical switch on multiplexed chips (Figure 8.6). Applying multiplexed chips to the development of photoswitches would enable the creation of complex switching patterns with the incorporation of internal controls (Figure 8.7). In addition to testing thymine dimer formation and repair, multiple redox probes, MB', NB and Rh(phi)$_2$bpy^{3+} are tested for applications in this system, as MB' and NB each act as more than a simple redox probe and interfere with switching conditions.
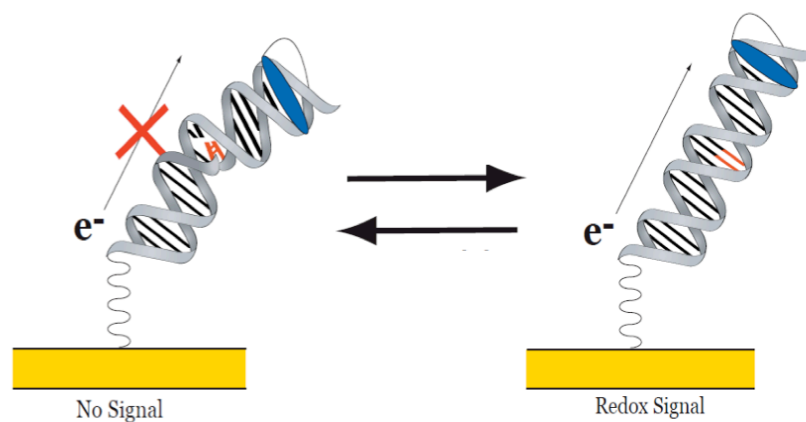
Figure 8.6 Thymine dimer formation and repair on multiplexed chips. DNA modified with an alkanethiol at the terminus of one strand and a redox probe at the terminus of the complementary strand form a film on a gold surface. Reversible thymine dimer formation and repair are used to modulate the electrochemical signal output from the system. Upon formation of a thymine dimer, the DNA helix is severely kinked, thereby significantly attenuating the electrochemical signal. Upon repair of the cyclobutane dimer, the electrochemical signal should return. Because mild conditions are used for thymine dimer formation, the reversibility of this process over multiple cycles is possible.
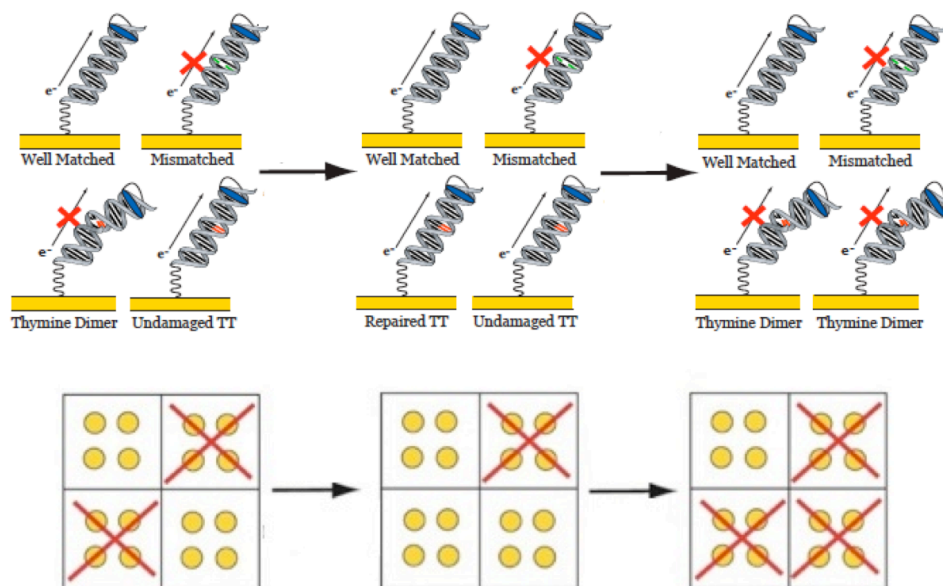
Figure 8.7 Overview of switching patterning on surfaces. Using multiplexed chips, up to four types of DNA can be observed simultaneously. A quadrant of well-matched DNA and a quadrant of mismatched DNA serve as controls for the on signal and off signal, respectively. Initially, one quadrant can contain DNA with a preformed thymine dimer (quadrant 4) and one quadrant containing a thymine dimer parent duplex (quadrant 3). Initially, quadrants 2 and 4 will have attenuated signals; however, upon thymine dimer repair on the surface, signal should be restored to quadrant 4. Then, under thymine dimer formation conditions, signal should be attenuated for quadrants 3 and 4. Thus thymine dimer switches can be used to create complex patterns of switching on surfaces.

Materials and Methods

DNA Synthesis

All reagents for DNA synthesis, including modified nucleotides and the C_6 thiol linker, were purchased from Glen Research.[27] Oligonucleotides were synthesized on an Applied Biosystems 3400 DNA synthesizer using standard phosphoramidite chemistry on a solid CPG support. Three DNA duplexes were designed for this work: a well-matched duplex, a duplex containing a CA mismatch, and a thymine dimer parent strand duplex containing adjacent thymine bases on one strand (Figure 8.8). For duplexes to be used in electrochemical experiments, the 5' end of one strand was modified with a C_6 S-S thiol linker, and the 5' end of the complementary strand was modified for redox reporter conjugation. Unless otherwise stated, after synthesis, DNA was cleaved from the solid support by incubation at 60° C with concentrated NH_4OH for 12 hours, followed by drying, resuspension in phosphate buffer (5.0 mM phosphate, 50 mM NaCl, pH 7.0), and HPLC purification with the dimethoxytrityl (DMT) group remaining. After deprotection, the DNA was again HPLC purified, desalted, and characterized by MALDI-TOF mass spectrometry.

Complementary DNA strands were quantified using UV/vis absorption at 260 nm. Equimolar amounts of each strand were prepared as a 50 μM solution in phosphate buffer (5.0 mM phosphate, 50 mM NaCl, pH 7.0) and annealed on a thermocycler by heating to 90°C followed by cooling to room temperature.

Well Matched
Redox Probe-5'-TGC ACA TGC A**T**G TGT GC-3'
      3'-ACG TGT ACG T**A**C ACA CG-5'-SH

Mismatched
Redox Probe-5'-TGC ACA TGC A**C**G TGT GC-3'
      3'-ACG TGT ACG T**A**C ACA CG-5'-SH

Parent Thymine Dimer
Redox Probe-5'-TCG ACG TGC **AA**C TGA GC-3'
      3'-AGC TGC ACG **TT**G ACT CG-5'-SH

Figure 8.8 Sequences of DNA used for testing thymine dimer formation and repair. A CA mismatch, indicated with red text, is included in one of the sequences to observe its effect on signal attenuation. The sequence of the parent thymine dimer strand contains two adjacent thymine bases on one strand that have the potential to dimerize.

Thiol-Containing DNA Synthesis

Following standard synthesis and DMT-on HPLC purification, the disulfide terminus was reduced to a free thiol by suspending the sample in Tris buffer (50 mM Tris, pH 8.4) with DTT (100 mM) for 45 minutes. The sample was filtered through a Nap5 column prior to repurification by HPLC.

Nile Blue-modified DNA Synthesis[27]

DNA to contain Nile Blue (NB) was synthesized with Ultramild CE phosphoramidites and a terminal NHS-ester carboxy dT incorporated at the 5' end. While still attached to solid support, a saturated solution of NB (Sigma Aldrich) (150 mg NB in DCM with 10% DIEA) was added to the oligos. The columns were agitated for 24 h followed by rinsing with 5 mL each of DCM, methanol, and ACN. Cleavage from solid support was achieved with potassium carbonate (0.05M) in methanol for 12 h. DNA was purified and analyzed as previously described.

Methylene Blue-modified DNA Synthesis[15]

Methylene Blue (MB'), a modified form of the methylene blue dye containing a flexible alkyl tether and a terminal carboxylic acid, was synthesized in the Barton group. MB' was prepared for coupling to DNA by NHS ester activation. 8 mg (0.022 mmol) of MB' was combined with 9.3 mg (0.045 mmol) N,N'-dicyclohexylcarbodiimide and 5.2 mg (0.045 mmol) N-hydroxysuccinimide in 1 mL anhydrous DMF. The reaction was stirred for 24 h, followed by solvent removal under reduced pressure. Activation was

confirmed by ESI-MS in acetonitrile: water: acetic acid (1:1:0.1) (454.5 g/mol; calc'd 454.54 g/mol). The activated ester was resuspended in 50 µL DMSO.

DNA to be coupled to MB' was synthesized with a 5'-terminal amino-modified C6 dT phosphoramidite. The DNA was cleaved from solid support, deprotected, and HPLC-purified as previously described. Following desalting, the oligonucleotides were suspended in 200 µL of 0.1 M NaHCO$_3$, pH 8.3, and the MB'-ester in DMSO was added to the oligonucleotides in 10x excess. The reaction was shaken for 24 h, followed by a final round of HPLC purification. MALDI-TOF was used to confirm the formation of MB'-DNA.

Rhodium Conjugation to the 5' DNA Terminus

Coupling Rh(phi)$_2$bpy'$^{3+}$ (phi = 9, 10-phenanthrene quinone diimine; bpy' = 4-butyric acid-4'-methyl bipyridyl) to the 5' terminus of DNA was performed as described by Holmlin et al.[26] DNA was synthesized on large pore solid support (2000 Å) with the terminal protecting DMT group on the 5' end removed on the synthesizer. The DNA-beads (2x1.0 µmol) were subsequently added to glass cylinders containing a coarse frit and stopcock. The beads were washed 3 x 3 mL dry dioxane followed by the addition of 50 mg (308 µmol) carbonyl diimidazole (CDI) in 1 mL dioxane. The cylinder was purged with Ar(g) and shaken for 1 h. The beads were again washed with 5 x 3 mL dioxane. 36 mg (240 µmol) diaminononane in 1 mL 9:1 dioxane:H$_2$O was subsequently added to the vessel, which was shaken for 30 minutes followed by washing with 9:1 dioxane:H$_2$O, dioxane and methanol. 10 mg (13.3 µmol) Rh(phi)$_2$bpy'$^{3+}$, 1.9 mg (14.7 µmol) hydroxybenzotriazole (HOBt), 4.4 mg (11.6 µmol) O-Benzotriazole-N,N,N',N'-

tetramethyl-uronium-hexafluoro-phosphate (HBTU), and 80 μL N,N-diisopropylethylamine (DIEA) were added to the reaction vessel in 1 mL anhydrous DMF. The reaction was allowed to proceed for 24 h, followed by washing with DMF and methanol before cleavage from the solid support with incubation at 60° C with concentrated NH_4OH for 12 h. The DNA was purified by HPLC, and both isomers of the Rh(phi)₂bpy'-DNA were collected. Tethering to the DNA was verified with MALDI-TOF and CD Spectroscopy.

Rh(phi)₂bpy³⁺ Synthesis

Rh(bpy)Cl₄⁻ was prepared according to previously reported procedures (Figure 8.9).[26] To a 25 mL schlenk flask was added Rh(bpy)Cl₄⁻ (762 mg, 1.901 mmol) and trifluoromethanesulfonic acid (HOTf) (6.75 mL, 7.632 mmol) under Ar(g) and allowed to stir overnight. The product was ether precipitated. In a 250 mL round bottom flask, Rh(bpy)(OTf)₄⁻ (1.2727 g, 1.42 mmol) and 100 mL concentrated NH_4OH were refluxed at 45°C for 30 minutes. $[Rh(bpy)(NH_3)_4]^{3+}$ (1.1163 g, 1.16 mmol) was added to a 500 mL round bottom flask with phenanthraquinone (0.534 g, 2.552 mmol)and 1 mL of 1N NaOH in 75% ACN/25% H_2O. The product was purified with a cation exchange column followed by an anion exchange column (87 mg, 6%). ¹H NMR (300 MHz, DMSO): δ 13.87 (s, 2H), 13.37 (s, 2H), 8.95 (d, *J* = 7.8 Hz, 2H), 8.66 (d, *J* = 5.6 Hz, 2H), 8.63 − 8.45 (m, 10H), 7.88 (t, *J* = 8.1 Hz, 6H), 7.66 (t, *J* = 7.5 Hz, 4H) ESI-MS: calc: 667.54 $(M+H^+)$ obs: 669.2 $(M+H^+)$.

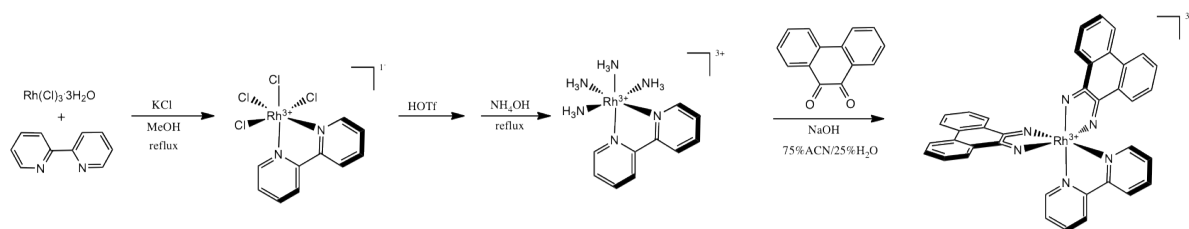**Figure 8.9** Synthesis of Rh(phi)$_2$bpy^{3+}. The bpy ligand is first added to the metal center. Subsequently, the chlorides are exchanged for ammines, followed by attachment of the two phi ligands.

Thymine Dimer Formation and Repair in Solution

Solutions of DNA duplexes of varying concentrations, either with or without a covalently tethered redox probe, were prepared in phosphate buffer (5.0 mM phosphate, 50 mM NaCl, pH 7) in a 1.7 mL Eppendorf with varying amounts of acetophenone. The solutions were irradiated with a 1000W HgXe lamp (Newport, Oriel) outfitted with a monochromator. For thymine dimer formation trials, 330 nm irradiation was used; 30 μL aliquots were taken at various times and run on HPLC to monitor the progression of cyclobutane formation.

To test thymine dimer repair with $Rh(phi)_2bpy^{3+}$, the metal complex was added directly to the eppendorf containing the acetophenone and DNA The sample was irradiated at 400 nm, and 30 μL aliquots were again taken and monitored by HPLC to determine the degree of thymine dimer repair.

DNA-modified Electrode Preparation

Multiplexed chips fabricated in Caltech facilities were used for electrochemical experiments.[13] Chips contain 16 electrodes (2 mm^2 area) divided into four quadrants. Low-density DNA monolayers (25 μM duplex, no Mg^{2+}) and high-density DNA monolayers (25 μM duplex, 100 mM Mg^{2+}) were assembled on the surface using DNA containing a thiol modifier at the terminus of one strand and a redox-active probe at the terminus of the complementary strand. DNA was incubated on the surface for 20-24 h in a humid environment, followed by thorough washing with phosphate buffer. The surface was then passivated with 1mM mercaptohexanol (MCH) in phosphate buffer with 5% glycerol for 45 minutes, followed again by washing with phosphate buffer.

Thymine Dimer Formation and Repair Attempts on Surfaces

Multiplexed chips to be used for thymine dimer formation and repair experiments contained one quadrant of well-matched DNA, one quadrant of mismatched DNA and two quadrants of thymine dimer parent DNA. After DNA monolayer formation, 200 μL of a solution of degassed acetophenone in phosphate buffer, pH 7, was added to the surface. The well of the clamp for the multiplexed chips, shown in the bottom panel of Figure 2, was covered by a microscope cover slide and sealed with adhesive putty. The surface was irradiated by redirecting the beam from the 1000 W HgXe lamp (Newport, Oriel) and monochromator 90° using a mirror angled at 45° so the multiplexed chip could be irradiated vertically. After irradiation at 330 nm with acetophenone, the chip was washed and scanned in phosphate buffer and TBP buffer. A solution of Rh(phi)$_2$bpy^{3+} was then added to the chip, which was again covered with a cover slip and sealed with adhesive putty, and irradiated at 400 nm.

Electrochemical Measurements

After surface passivation, the central well of the clamp was filled with phosphate buffer. A three-electrode setup was used for electrochemical measurements, with both the Ag/AgCl reference (Cypress) and platinum auxiliary electrodes submerged in the buffer solution. Electrochemical measurements were performed with a 16-channel multiplexer and a CHI620D Electrochemical Analyzer (CH Instruments) in either phosphate buffer (5.0 mM phosphate, 50 mM NaCl, pH 7.0) or spermidine buffer (5.0 mM phosphate, 50 mM NaCl, 4 mM MgCl$_2$, 4 mM spermidine, 50 μM EDTA, 10%

glycerol, pH 7.0). Unless otherwise stated, cyclic voltammetry (CV) data were collected

at 100 mV/s scan rate and square wave (SWV) data at 15 Hz.

Results and Discussion

Thymine Dimer Formation and Repair in Solution

Before electrochemical experiments, conditions for optimized, selective thymine dimer formation and repair were established in solution. To selectively form thymine dimers over other potential photoproducts upon DNA irradiation, a triplet sensitizer, acetophenone, was employed. This small molecule allows selective access to the triplet excited state of thymine because thymine is the only base with a triplet excited state lower in energy than the excited state of acetophenone. Irradiation of the DNA duplex at the peak absorbance of acetophenone, 330 nm, should cause no damage. The progression of thymine dimer formation and any other potential damage to the DNA duplex was monitored by HPLC, as the retention time of DNA containing a thymine dimer is significantly earlier than undamaged DNA (Figure 8.10).
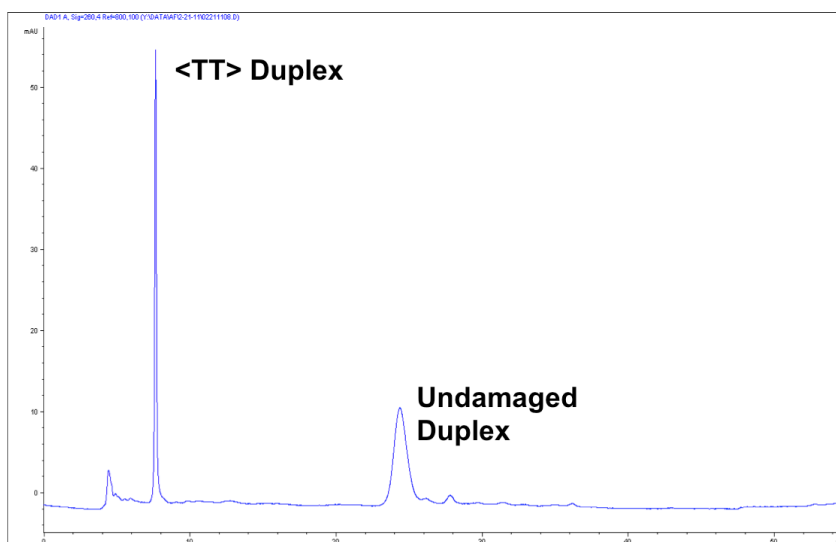
Figure 8.10 HPLC trace of thymine dimer formation. HPLC of the parent thymine dimer DNA is shown after irradiation. Both the parent duplex and the dimerized duplex are observed in this trace, with the thymine dimer-containing peak eluting significantly earlier than undamaged, parent DNA.

Initial controls were performed to ensure that irradiation of DNA without acetophenone did not damage the duplex. Additionally, irradiation of the well-matched duplex was undertaken to ensure that this duplex is not damaged upon irradiation with acetophenone. While irradiation trials confirm that minimal damage occurrs to DNA irradiated at 330 nm without acetophenone, a relatively large amount of DNA damage (~15%) was observed upon irradiation of the well-matched strand, which contains no adjacent thymines, in the presence of acetophenone. The damage observed is likely due to adjacent cytosines and thymines, which form photoadducts between the bases connected by a single bond. To avoid this undesired photodamage, the well-matched duplex (WM) was redesigned to ensure that the sequence no longer contained adjacent thymines and cytosines. Minimal damage is observed in the new sequence upon irradiation in the presence of acetophenone.

Conditions for specific thymine dimer formation with unmodified DNA were then optimized to produce the maximum amount of dimerization with minimal irreversible DNA damage. The optimum conditions are 30 μM DNA with 3.6 mM acetophenone irradiated for 8 hours. Degassing the sample before irradiation is vital in order to minimize nonspecific damage. To confirm the optimal conditions, thymine dimer formation was monitored by HPLC, as shown in Figure 8.11. As can be seen, with the progression of time, the earlier-eluting peak containing the dimerized thymines increases in size as the later-eluting peak, which contains the undamaged duplex, decreases in size.

As the metal complex $Rh(phi)_2bpy^{3+}$ is known to be capable of thymine dimer repair, conditions were also optimized for thymine dimer repair in solution with $Rh(phi)_2bpy^{3+}$. The metal complex was added directly to the DNA solution containing

acetophenone and irradiated at 400 nm. Optimal repair is observed when the concentration of the metal complex was twice that of the DNA. In the presence of the Rh(phi)$_2$bpy^{3+} complex (Figure 8.12), it can be seen that the dimer-containing peak decreases in size, while the undamaged peak increases in size. This trend is the inverse of what is observed by HPLC under thymine dimer formation conditions, indicating successful repair of the photodamage product by the metal complex.
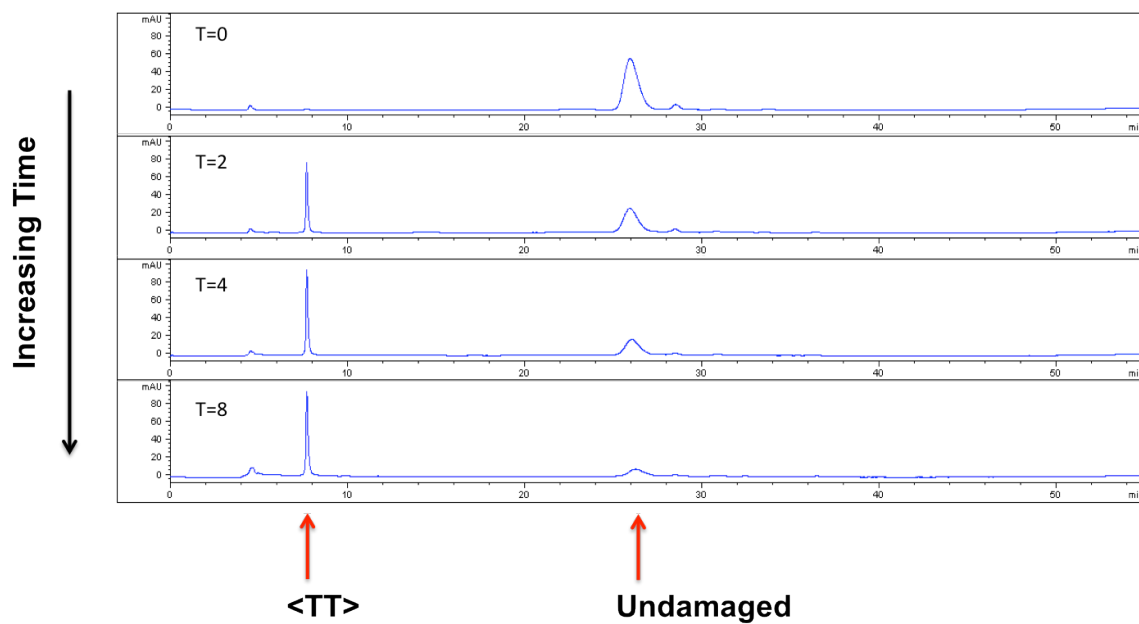
Figure 8.11 Thymine dimer formation under optimized conditions. As time progresses, the cyclobutane dimer peak increases in size, while the undamaged DNA peak decreases in size. The time reported on the left is in hours of irradiation under conditions of 30 μM DNA and 3.6 mM acetophenone in a sample that was degassed before irradiation at 330 nm on a 1000 W HgXe lamp outfitted with a monochromator.
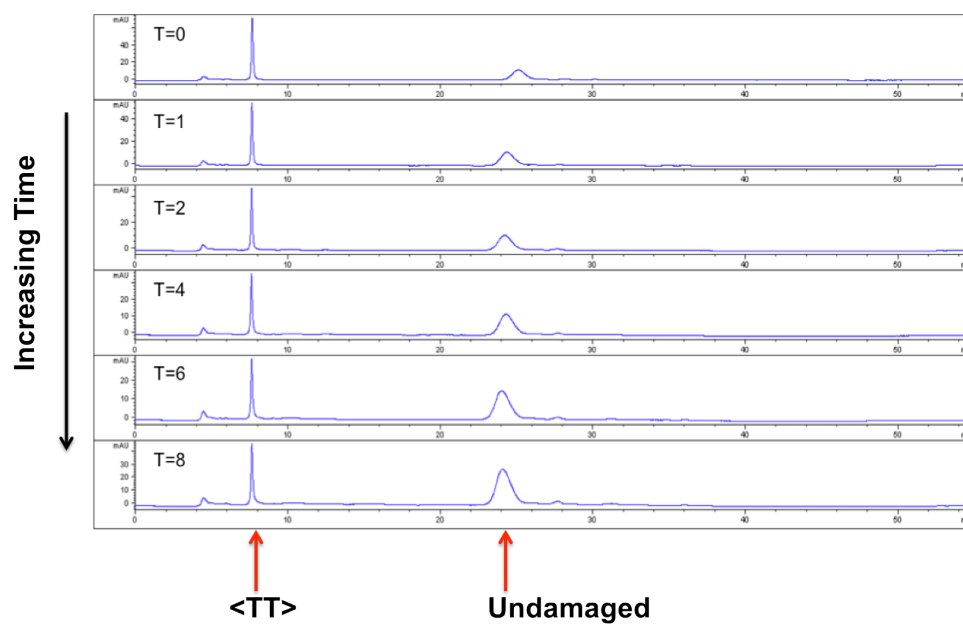
Figure 8.12 Thymine dimer repair under optimized conditions. As time progresses, the cyclobutane dimer peak decreases in size, while the undamaged DNA peak returns. The time reported on the left is in hours of irradiation. The conditions were 30 µM DNA and 60 µM Rh(phi)$_2$bpy^{3+} in a sample irradiated at 400 nm on a 1000 W HgXe lamp outfitted with a monochromator.

Electrochemical Experiments with Thymine Dimer Formation and Repair

As the solution phase experiments demonstrate successful thymine dimer formation and repair in solution, attempts at thymine dimer formation and repair on surfaces were also made. DNA modified with a MB' redox probe was first used for electrochemical measurements of thymine dimer formation and repair on an electrode surface. Experiments were conducted with multiplexed chips, and one quadrant of the chip was modified with WM DNA, a second with mismatched DNA (MM), and the final two with parent thymine dimer (<TT>) DNA. Initial conditions for thymine dimer formation and repair on the surface were based on the previously optimized acetophenone and $Rh(phi)_2bpy^{3+}$ conditions for solution-phase thymine dimer formation and repair.

Two issues are immediately evident from experiments with thymine dimer formation and repair on surfaces. First, no selectivity in signal reduction is observed for the <TT> DNA over the WM DNA. In both cases, upon irradiation with acetophenone, significant signal decreases are observed (80-95%) (Figure 8.13). As is seen from the CV scans of the well-matched (left) and parent <TT> DNA (right) in Figure 8.13, no selective signal attenuation is observed in the parent <TT> DNA. The large signal decrease observed in both sequences of DNA indicates that the signal decrease is not due to the selective formation of thymine dimers; this result is likely due to irreversible damage to both sequences of DNA. As this level of damage is not observed in the solution experiments involving DNA without a redox probe, these results point to the MB' redox probe as a potential source of damage.
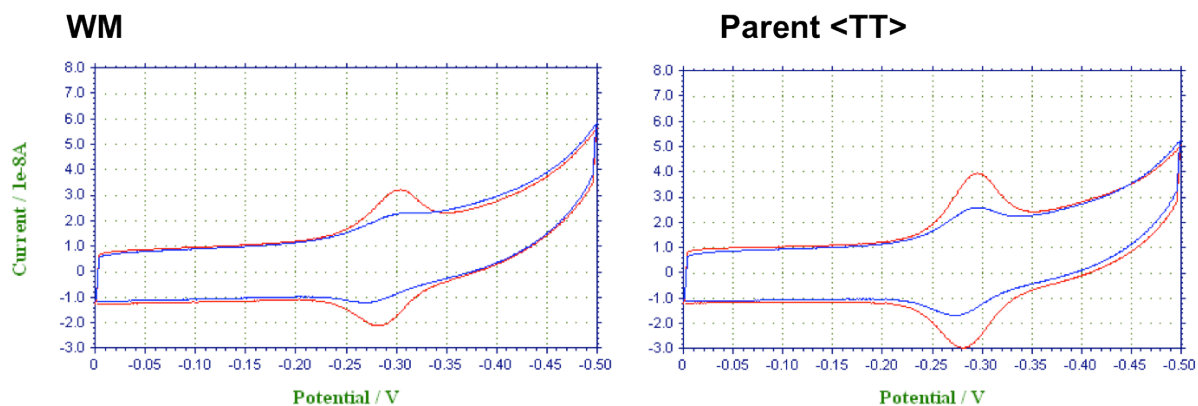
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WM   **Parent <TT>**

Figure 8.13 Cyclic voltammogram of MB'-modified DNA on multiplexed chips. Both before irradiation (red trace) and after irradiation in the presence of acetophenone (blue trace) are shown. Irradiation proceeded at 330 nm in the presence of 3.6 mM acetophenone for 2 hours. Cyclic voltammograms of the well-matched duplex (*left*) show significant signal attenuation, which is not expected. Nearly the same attenuation is observed in the sequences that are capable of forming thymine dimers (*right*).

A second issue arose upon attempting thymine dimer repair on multiplexed chips. After the addition of $Rh(phi)_2bpy^{3+}$ to the surface, followed by irradiation, a large, irreversible reductive peak is consistently observed, regardless of washing conditions (Figure 8.14). Many attempts were made using a variety of solvent conditions for washing, including high-salt phosphate buffer, 3 M NaCl in H_2O, 3 M NH_4OAc in H_2O, 3 M KPF_6, acetonitrile, and ethanol, to reduce the large peak. However, all were unsuccessful. Repassivating the surface was also attempted. After the surface was irradiated with acetophenone and washed, 1 mM MCH in phosphate buffer with 5% glycerol was added to the surface for 45 minutes and washed before the addition of $Rh(phi)_2bpy^{3+}$. The irreversible signal is still observed after the $Rh(phi)_2bpy^{3+}$ solution was removed from the surface and the surface was washed.
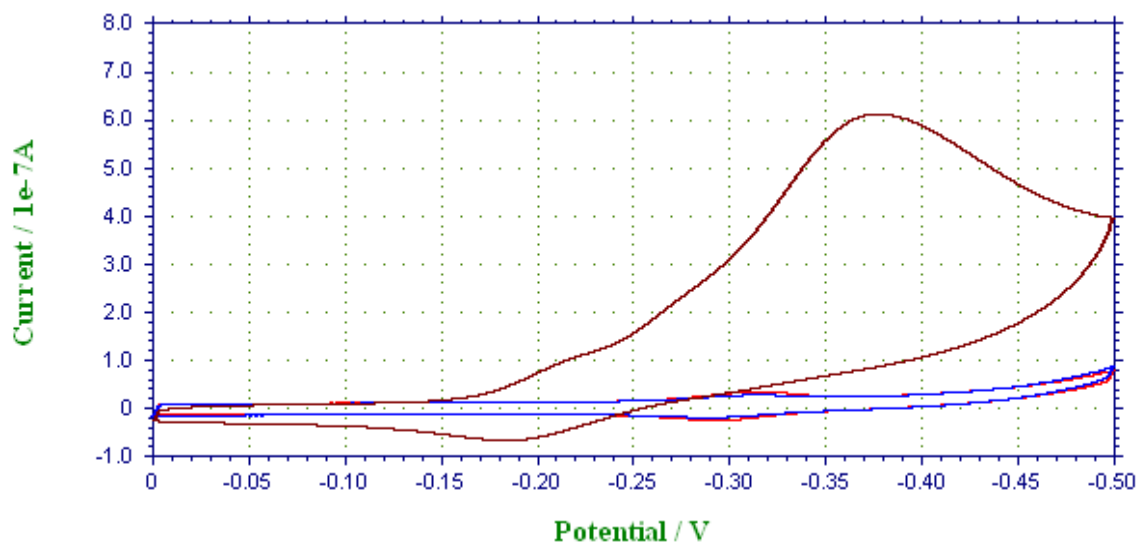
Figure 8.14 Rh(phi)$_2$bpy^{3+} signal on multiplexed chips. After Rh(phi)$_2$bpy^{3+} is added to a quadrant of a multiplexed chip containing well-matched DNA, a large irreversible signal is observed (brown trace). Both the initial scan of the surface (red trace) and after the surface is irradiated with acetophenone (blue trace) show only the reversible methylene blue signal. After the surface was irradiated with Rh(phi)$_2$bpy^{3+} and thoroughly washed, the large, irreversible peak from the Rh(phi)$_2$bpy^{3+} metal complex remains (brown trace).

To verify that Rh(phi)$_2$bpy^{3+} is the source of the irreversible peak, a quadrant of a multiplexed chip with a bare gold surface and one containing only a MCH monolayer were each incubated with Rh(phi)$_2$bpy^{3+}, followed by washing. An irreversible reductive peak similar to those previously observed was found for both the bare gold and MCH surfaces. Because of these findings, free Rh(phi)$_2$bpy^{3+} is not a viable option as a thymine dimer repair agent for experiments on surfaces.

Thymine Dimer Formation and Repair in Modified DNA in Solution

Because of the extreme amount of signal attenuation observed for both the WM and <TT> duplexes on surfaces, the effect of the redox probe under thymine dimer formation conditions was investigated in solution by HPLC. When MB'-DNA is irradiated, significant amounts of non-specific damage consistently occur both in the presence and absence of acetophenone. Similar results are obtained for unmodified DNA with a 1:1 ratio of free methylene blue in solution (Figure 8.15). This damage is likely caused by methylene blue forming reactive oxygen species in solution, which indiscriminately damage DNA. MB' is therefore incompatible as a redox probe for a DNA switch.
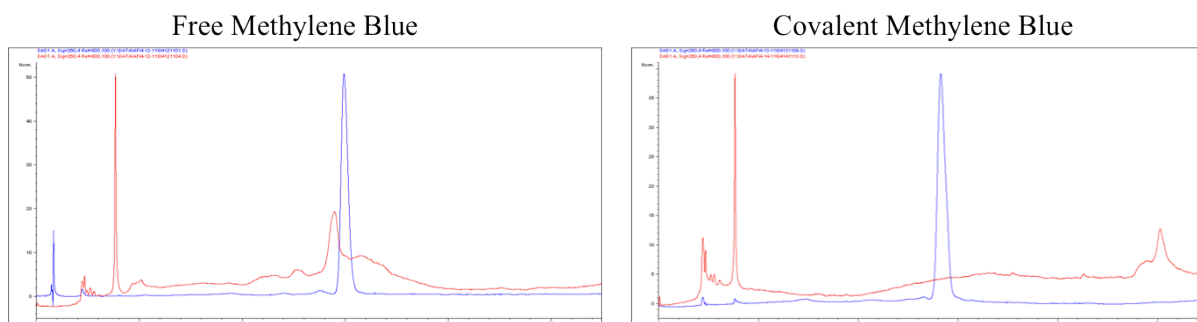
Free Methylene Blue   Covalent Methylene Blue

Figure 8.15 Damage to free DNA by irradiation in the presence of methylene blue. In the absence of acetophenone, degassed samples of DNA in phosphate buffer were irradiated at 330 nm for 4 hours (*left*). The blue trace is an HPLC trace of the DNA before irradiation; the red trace is after 4 hours of irradiation. Both a 1:1 ratio of free MB to DNA (*left*) and covalently-tethered MB' (*right*) exhibited significant amounts of nonspecific damage.

As methylene blue is not a viable option for this platform, an alternative redox probe, Nile Blue (NB), was also tested. When NB-DNA was irradiated in the absence of acetophenone, no DNA damage is observed. When NB-DNA is irradiated in the presence of acetophenone, the NB probe loses its color, changing from blue to colorless, indicating damage to the probe under these conditions. As the redox probe must be stable to conditions used for thymine dimer formation and repair, NB was also rejected as a redox probe for this application (Figure 8.16)
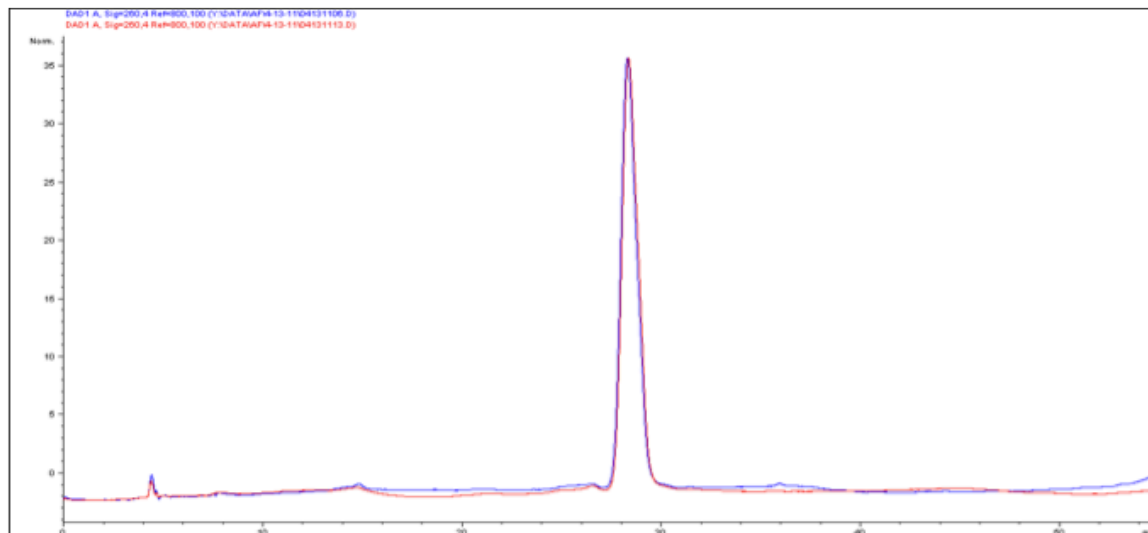
Figure 8.16 Nile blue-modified DNA irradiated at 330 nm. Nile blue covalently attached to DNA is irradiated in the absence of acetophenone for 4 h. The blue trace is at t=0 h, and the red is at t=4 h. No DNA damage is observed.

Rh(phi)₂bpy'³⁺ as a Covalent Redox Probe: Synthesis and Initial Studies

As neither MB' or NB is suitable as a redox probe for the photoswitch system, we sought to utilize Rh(phi)$_2$bpy'$^{3+}$ (Figure 8.17), a variant of the metal complex known to repair thymine dimers. This complex was also shown to be stable in the presence of acetophenone. These qualities enable the metal complex to potentially function as both a redox probe and repair agent. Additionally, as the complex is covalently tethered to the DNA, issues with direct surface reduction of the complex should be minimized. Before proceeding with covalent linkages, Rh(phi)$_2$bpy'$^{3+}$ was tested to ensure that it is both stable to 330 nm irradiation in the presence of acetophenone and maintains its ability to repair thymine dimers after acetophenone irradiation. Finally, the ability of thymine dimers to form in the presence of this complex was also evaluated. Thymine dimer formation and repair in solution was undertaken with Rh(phi)$_2$bpy'$^{3+}$ present in both irradiation steps. The rhodium complex is stable to conditions for thymine dimer formation and repairs thymine dimers with 400 nm irradiation following its irradiation with acetophenone. Covalent tethering was therefore undertaken to enable the Rh(phi)$_2$bpy^{3+} complex to act as both a repair agent for thymine dimers and a redox reporter.
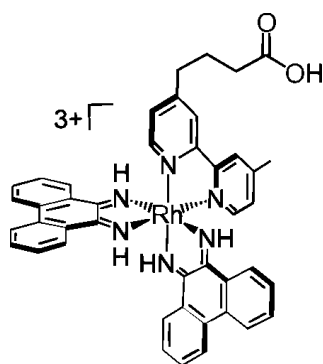
Figure 8.17 Structure of Rh(phi)$_2$bpy'$^{3+}$ to be coupled to the DNA. Coupling to both the phosphate backbone and a modified uracil base are possible.

Two modes of covalent linkage of the rhodium complex to the DNA were undertaken. Coupling to the phosphate backbone of DNA with a C9 amine linker as well as to a C6 amino-modifed uracil base incorporated into the terminus of the DNA duplex were tested. The Rh(phi)$_2$bpy'$^{3+}$ is a racemic mixture of Δ- and Λ-isomers. The Δ isomer intercalates into the base stack much more strongly than the Λ isomer. Racemic Rh(phi)$_2$bpy'$^{3+}$ was successfully coupled to the phosphate backbone of DNA using a previously-developed protocol from our group.[14] However, the yields for this coupling are extremely low (~10%). Coupling Rh(phi)$_2$bpy'$^{3+}$ directly to the modified uracil base was found to be no more successful. Coupling attempts were made with the DNA both on solid support and free in solution with coupling reagents including HBTU and HOBt, HATU, EDC, and PyBOP, as well as NHS ester activation of the Rh(phi)$_2$bpy'$^{3+}$.

The Rh(phi)$_2$bpy'$^{3+}$ coupled to the backbone of DNA is a racemic mixture of Δ- and Λ-isomers. When coupled to the DNA backbone, the Δ- and Λ-isomers have different HPLC retention times and can be distinguished by CD spectroscopy. Figure 8.18 shows the CD spectra of each of the isomers conjugated to DNA isolated by HPLC. Each isomer was isolated by HPLC to test individually on the multiplexed chips.

The DNA strand conjugated to the rhodium complex was annealed to a thiolated complement and allowed to self-assemble on the gold surface. To determine if the signals observed are DNA mediated, both WM and MM DNA of each isomer were prepared. Mismatch discrimination is observed for both the Δ- and Λ-isomers, indicating that at least a portion of the DNA signals observed are DNA mediated (Figure 8.19). However, the larger signal present for the Λ isomer indicates that, despite the mismatch

discrimination observed, the signal is likely not DNA mediated. This is further supported by the irreversibility of all of the rhodium electrochemical signals observed.
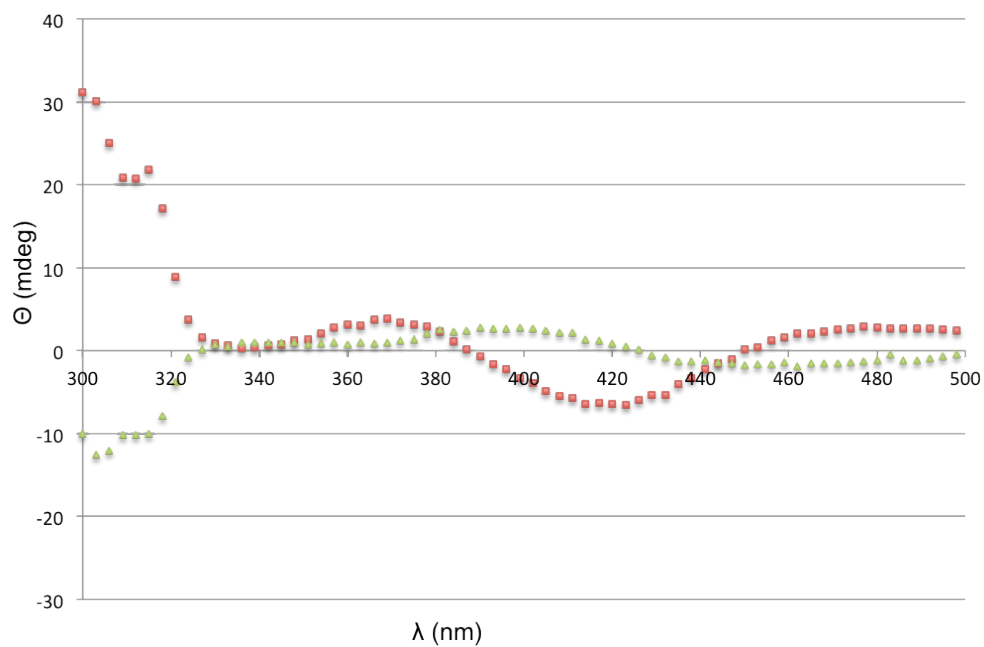
Figure 8.18 CD spectra of the Δ- and Λ-isomers of Rh(phi)$_2$bpy^{3+} coupled to the backbone of DNA. The Λ isomer is shown in red, and the Δ isomer is shown in green.
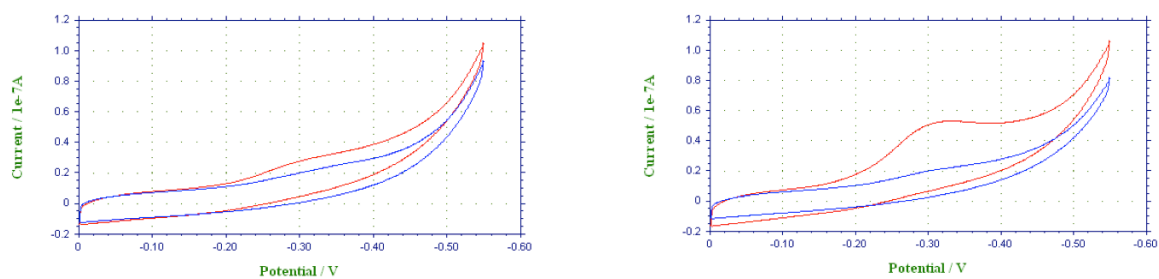
Figure 8.19 Electrochemistry of backbone-coupled $Rh(phi)_2bpy^{3+}$. The CVs shown were taken at a scan rate of 50 mV/s. In both cases, scans were collected in TBP buffer. The well-matched duplex (*red*) showns an irreversible reductive signal, while the mismatched duplex (*blue*) shows no electrochemical signal. The Δ isomer (*left*) shows a significantly smaller signal for the well-matched DNA than the Λ isomer (*right*).

Conclusions

As DNA is known to be an effective molecular wire over long distances, efforts have been made to add functionality to DNA in order to build circuitry components. The simplest component of a circuit is a switch. We therefore made efforts to develop such a circuitry component through the formation and repair of thymine dimers, which have been shown to attenuate DNA CT. Solution-phase switching of the formation and repair of thymine dimers has been developed; thymine dimer formation with the triplet sensitizer, acetophenone, and repair with a rhodium intercalator, $Rh(phi)_2bpy^{3+}$, is robust in solution. However, moving to DNA tethered to solid support has proved difficult. Multiple redox probes are non-innocent in the thymine dimer formation and repair process, and the rhodium complexes have been found to generate an electrochemical signal, interfering with readout. Although using covalent $Rh(phi)_2bpy'^{3+}$ to act as both a redox reporter and method of thymine dimer repair appears to be a simple solution, the signals obtained from this tethered compound are not reversible and do not appear to be DNA-mediated. Future work on this project will benefit from better surface passivation techniques, including the application of 11-mercaptoundecylphosphoric acid as a passivating agent. Additionally, low-density monolayers formed by click chemistry to tether the DNA will provide better spacing between the helices, which may facilitate thymine dimer formation.

References

1. Robertson, N., and McGowan, C. A. (2003) A comparison of potential molecular wires as components for molecular electronics, *Chem. Soc. Rev. 32*, 96-103.

2. Tour, J. M. (2000) Molecular electronics. Synthesis and testing of components, *Acc. Chem. Res. 33*, 791-804.

3. James, D. K., and Tour, J. M. (2005) Molecular wires, *Topics in Curr. Chem. 257*, 33-62.

4. Slinker, J. D., Muren, N. B., Renfrew, S. E., and Barton, J. K. (2011) DNA charge transport over 34 nm, *Nat. Chem. 3*, 228-233.

5. Kelley, S. O., Boon, E. M., Barton, J. K., Jackson, N. M., and Hill, M. G. (1999) Single-base mismatch detection based on charge transduction through DNA, *Nucleic Acids Res. 27*, 4830-4837.

6. DeRosa, M. C., Sancar, A., and Barton, J. K. (2005) Electrically monitoring DNA repair by photolyase, *Proc. Natl. Acad. Sci. USA 102*, 10788-10792.

7. Boon, E. M., Salas, J. E., and Barton, J. K. (2002) An electrical probe of protein-DNA interactions on DNA-modified surfaces, *Nat. Biotechnol. 20*, 282-286.

8. Gorodetsky, A. A., Ebrahim, A., and Barton, J. K. (2008) Electrical detection of TATA binding protein at DNA-modified microelectrodes, *J. Am. Chem. Soc. 130*, 2924-2925.

9. Drummond, T. G., Hill, M. G., and Barton, J. K. (2003) Electrochemical DNA sensors, *Nat. Biotechnol. 21*, 1192-1199.

10. Jackson, N. M., and Hill, M. G. (2001) Electrochemistry at DNA-modified surfaces: new probes for charge transport through the double helix, *Curr. Opin. Chem. Biol. 5*, 209-215.

11. Kelley, S. O., Barton, J. K., Jackson, N. M., McPherson, L. D., Potter, A. B., Spain, E. M., Allen, M. J., and Hill, M. G. (1998) Orienting DNA Helices on Gold Using Applied Electric Fields, *Langmuir 14*, 6781-6784.

12. Kelley, S. O., Jackson, N. M., Hill, M. G., and Barton, J. K. (1999) Long-Range Electron Transfer through DNA Films, *Angew. Chem. Int. Ed. 38*, 941-945.

13. Slinker, J. D., Muren, N. B., Gorodetsky, A. A., and Barton, J. K. (2010) Multiplexed DNA-modified electrodes, *J. Am. Chem. Soc. 132*, 2769-2774.

14. Kelley, S. O., Barton, J. K., Jackson, N. M., and Hill, M. G. (1997) Electrochemistry of methylene blue bound to a DNA-modified electrode, *Bioconjug. Chem. 8*, 31-37.

15. Pheeney, C. G., and Barton, J. K. (2012) DNA electrochemistry with tethered methylene blue, *Langmuir 28*, 7063-7070.

16. Boal, A. K., and Barton, J. K. (2005) Electrochemical detection of lesions in DNA, *Bioconjug. Chem. 16*, 312-321.

17. Boon, E. M., Ceres, D. M., Drummond, T. G., Hill, M. G., and Barton, J. K. (2000) Mutation detection by electrocatalysis at DNA-modified electrodes, *Nat. Biotechnol. 18*, 1096-1100.

18. Gorodetsky, A. A., Buzzeo, M. C., and Barton, J. K. (2008) DNA-mediated electrochemistry, *Bioconjug. Chem. 19*, 2285-2296.

19. Guo, X., Gorodetsky, A. A., Hone, J., Barton, J. K., and Nuckolls, C. (2008) Conductivity of a single DNA duplex bridging a carbon nanotube gap, *Nat. Nanotechnol. 3*, 163-167.

20. Park, H., Zhang, K., Ren, Y., Nadji, S., Sinha, N., Taylor, J. S., and Kang, C. (2002) Crystal structure of a DNA decamer containing a cis-syn thymine dimer, *Proc. Natl. Acad. Sci. USA 99*, 15965-15970.

21. Schreier, W. J., Schrader, T. E., Koller, F. O., Gilch, P., Crespo-Hernandez, C. E., Swaminathan, V. N., Carell, T., Zinth, W., and Kohler, B. (2007) Thymine dimerization in DNA is an ultrafast photoreaction, *Science 315*, 625-629.

22. Lamola, A. A., and Eisinger, J. (1968) On the mechanism of thymine photodimerization, *Proc. Natl. Acad. Sci. USA 59*, 46-51.

23. Holman, M. R., Ito, T., and Rokita, S. E. (2007) Self-repair of thymine dimer in duplex DNA, *J. Am. Chem. Soc. 129*, 6-7.

24. Nunez, M. E., Noyes, K. T., Gianolio, D. A., McLaughlin, L. W., and Barton, J. K. (2000) Long-range guanine oxidation in DNA restriction fragments by a triplex-directed naphthalene diimide intercalator, *Biochemistry 39*, 6190-6199.

25. Dandliker, P. J., Nunez, M. E., and Barton, J. K. (1998) Oxidative charge transfer To repair thymine dimers and damage guanine bases in DNA assemblies containing tethered metallointercalators, *Biochemistry 37*, 6491-6502.

26. Holmlin, R. E., Dandliker, P. J., and Barton, J. K. (1999) Synthesis of metallointercalator-DNA conjugates on a solid support, *Bioconjug. Chem. 10*, 1122-1130.

27. Gorodetsky, A. A., Green, O., Yavin, E., and Barton, J. K. (2007) Coupling into the base pair stack is necessary for DNA-mediated electrochemistry, *Bioconjug. Chem. 18*, 1434-1441.