Supplementary Materials For

A Compass at Weak Magnetic Fields using Thymine Dimer Repair

Theodore J. Zwang,[1] Edmund C.M. Tse,[1] Dongping Zhong,[2] Jacqueline K. Barton[1,*]

[1]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125
[2]Division of Chemistry and Physics, The Ohio State University, Columbus, OH 43210

Correspondence to jkbarton@caltech.edu

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MATERIALS and METHODS

DNA Synthesis

All materials for DNA synthesis were purchased from Glen Research. Oligonucleotides were synthesized on an Applied Biosystems 3400 DNA synthesizer using phosphoramidite chemistry on a controlled-pore glass support. The two strands of a duplex are synthesized separately, purified, stored frozen, then annealed prior to electrochemical experiments. The 5'-end of one strand is modified with a C6 S-S phosphoramidite that is later reduced before use. High pressure liquid chromatography (HPLC) was performed using a reverse-phase PLRP-S column (Agilent) using a gradient of acetonitrile and 50 mM ammonium acetate (5-15% ammonium acetate over 35 minutes).

Unmodified DNA. DNA was synthesized using standard phosphoramidites and reagents. After synthesis, the DNA was lyophilized overnight. It was then cleaved from the solid support by incubation at 60 ^{o}C with concentrated (28-30%) NH_4OH for 12 hours, filtered using CoStar 0.45 µm columns, then dried. The dried DNA film was resuspended in phosphate buffer (5 mM phosphate, pH 7, 50 mM NaCl) and HPLC-purified. The DMT (4,4'-dimethoxytrityl) group protecting the 5'- end was then removed by incubation with 80% acetic acid for 45 minutes. The reaction mixture was dried and resuspended in phosphate buffer. The DNA was isolated using HPLC. The purified oligonucleotide was desalted using ethanol precipitation, dried, and the mass was confirmed with matrix-assisted laser desorption/ionization- time of flight mass spectrometry (MALDI-TOF). Unmodified oligonucleotides were then stored at -20^{o}C in phosphate buffer until annealing with their complementary strand.

Cyclobutane pyrimidine dimer generation. Single stranded DNA (1 ml of 100-200 µM) with a single 5'-TT-3', 5'-UU-3', 5'-TU-3', or 5'-UT-3' was suspended in aqueous buffer containing 1 mM acetophenone, 5 mM NaH_2PO_4, 50 mM NaCl, pH 7.5 and degassed with argon in a glass container. The container was sealed and irradiated with a solar simulator (Oriel instruments) or 302 nm UV light (spectroline transilluminator model TR-302) for 10 minutes. Following irradiation the DNA was purified using high performance liquid chromatography (HPLC) using a reverse-phase PLRP-S column (Agilent) using a gradient of acetonitrile and 20 mM ammonium acetate (2-3% acetonitrile over 10 minutes, then 3-4% over the next 30 minutes) at 80 ^{o}C and a flow rate of 0.8 ml/min to separate the CPD from undimerized ssDNA. Both 16 and 29 bp ssDNA show a separation with CPD compared to without a CPD of approximately 4 minutes with the CPD containing strand eluting first.

Thiolated DNA. DNA was synthesized using standard phosphoramidites and reagents, with the exception of a C6 S-S phosphoramidite that was attached to the 5'- end. After synthesis, the DNA was lyophilized overnight. It was then cleaved from the solid support by incubation at 60 ^{o}C with concentrated (28-30%) NH_4OH for 12 hours, filtered using CoStar columns, then dried. The dried DNA film was resuspended in phosphate buffer (5 mM phosphate, pH 7, 50 mM NaCl) and HPLC-purified. The DMT (4,4'-dimethoxytrityl) group protecting the 5'- end was then removed by incubation with 80% acetic acid for 45 minutes. The reaction mixture was dried and resuspended in phosphate buffer. The DNA was isolated using HPLC as described above. The purified oligonuceotide was desalted using ethanol precipitation, dried, and the mass was confirmed with MALDI-TOF. Within one week of annealing, the disulfide-modified DNA was reduced by resuspending in 50 mM Tris-HCl, pH 8.4, 50 mM NaCl, 100 mM dithiothreitol (Sigma) for 2 hours. The reduced thiol-modified DNA was then purified by size exclusion

chromatography (Nap5 Sephadex, G-25, GE Healthcare) with phosphate buffer as the eluent and subsequently purified using HPLC.

Annealing Duplex DNA. Duplex DNA for electrochemistry was prepared by first quantifying the complementary strands with UV-Visible spectroscopy, then mixing equimolar (50 μM) complementary strands in 200 μl phosphate buffer. The DNA solution was then deoxygenated by bubbling argon for at least 5 minutes per ml. Duplex DNA was then annealed on a thermocycler (Beckman Instruments) by initial heating to 90 ^{o}C followed by slow cooling over 90 minutes.

DNA Sequences:

16 bp DNA (with or without UV generated dimer)
5'-ACG TGA GTT GAG ACG T-3'
3'-TGC ACT CAA CTC TGC A-5' – SH
Thymine Dimer with CA mismatch near surface
5'-ACG TGA GTT GA**A** ACG T-3'
3'-TGC ACT CAA CT**C** TGC A-5' - SH

29 bp DNA (with or without UV generated dimer)
3'-ATC ACG TCA TAT GAA CTG ACT GGA CGG TG- 5' -SH
5'-TAG TGC AGT ATA CTT GAC TGA CCT GCC AC-3'

3'- ATC ACG TCA TAT CAA CTG TCT GCA CGC TG- 5' -SH
5'- TAG TGC AGT ATA GTT GAC AGA CGT GCG AC-3'

The above sequences use the following abbreviations for modifications:
-SH = hexanethiol linker; TT = Pyrimidine Dimer

Protein preparation

Escherichia coli photolyase wild type and mutants N378C, M345A, E274A, as well as truncated *Arabidopsis Thaliana* cryptochrome 1 (*at*CRY1ΔC) without its C-terminal domain were provided by Prof. Dongping Zhong (The Ohio State University). Proteins were received at 180-300 μM in a buffer containing 100 mM KCl, 50 mM Tris-HCl at pH 7.5, 1 mM EDTA and 50% (v/v) glycerol. It is essential that these buffers do not contain concentrations of dithiothreitol over 2 mM or other sulfur compounds that are typically used to keep the flavin cofactor reduced because they may disrupt gold-thiol bonds crucial for the stability of DNA monolayers used in electrochemistry. Proteins were thus generally received with a partially or fully oxidized flavin that needed to be photoreduced for enzymatic activity.

E. coli photolyase without the antenna cofactor was prepared as described previously.[12, 23] The mutant plasmids were constructed using QuikChange II XL kit (Stratagene) based on the plasmid of wild-type enzyme. All mutated plasmids were sequenced to confirm the mutations.

The preparation of MBP-tag fused *At*Cry1 with depletion of the C-terminal tail (*At*CRY1ΔC) was as described elsewhere with some modifications.[26] The *At*CRY1ΔC gene was cloned into the pMal-c2 vector (New England Biolabs) to obtain a construct that expresses *At*CRY1ΔC fused to the C-terminus of maltose binding protein (MBP). The MBP-tagged *At*CRY1ΔC was expressed in *E. coli* UNC523 and purified by affinity chromatography on amylose resin.

All proteins were obtained with stoichiometric flavin cofactor after purification and were exchanged to a buffer containing 50 mM Tris at pH 7.5, 100 mM NaCl,1 mM EDTA, and 50% (v/v) glycerol for further use.

Before experiments 50 uM *ec*PL or *at*CRY1ΔC were placed in tris buffer (50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 10% glycerol, pH 7.5) and irradiated with blue light (405 ± 10 nm, <30 mW) from a diode laser pointer (Tmart) in an anaerobic chamber to photoreduce the flavin to its active form (Fig. S11). All solutions containing photolyase were degassed to remove oxygen and kept in an anaerobic chamber (95% N_2, 5% H_2, <1 ppm O_2) to prevent oxidation of the flavin. During experiments with photolyase the protein was kept under constant blue light irradiation. If the flavin was not fully photoreduced, or if oxygen was able to access the flavin and oxidize it, the oxidized flavin peak would appear in cyclic voltammetry experiments centered around -420 mV vs AgCl/Ag. Further irradiation with blue light in anaerobic conditions remove this peak. The presence of this peak did not appear to have a significant effect on measurements of total charge transferred at later time points when it was removed by reduction of the flavin with blue light.

Electrode fabrication

Multiplexed electrode surfaces were fabricated following a previously published protocol.[8] In brief, one millimeter thick Si wafers with a 10 000 Å thick oxide layer were purchased from Silicon Quest. First, wafers were cleaned thoroughly in 1165 Remover (Microchem) and vapor primed with hexamethyldisilizane (HMDS). S1813 photoresist (Microchem) was spin-cast at 4000 rpm and baked. The photoresist was patterned with a Karl Suss MA6 contact aligner and a chrome photomask. Following post-exposure baking, wafers were developed in MF-CD26 developer for 40-60 s and rinsed thoroughly with deionized water then dried thoroughly. A 30 Å Ti adhesion layer and a 100 Å Au layer were deposited on the chips with a CHA Mark 50 electron beam evaporator. Wafers were then immersed in P6 Remover overnight to complete metal lift-off. Subsequently, the wafers were thoroughly baked and cleaned by UV ozone treatment. SU-8 2002 (Microchem) was spin-cast at 3000 rpm, baked, and photopatterned as above. Wafers were developed in SU-8 Developer (Microchem) for 1 min and baked for a permanent set of the photoresist. The wafers were subsequently diced into 1-in. by 1-in. chips and used for electrochemistry experiments.

DNA-modified electrode preparation

Multiplexed chips are gently cleaned by sonicating with acetone then isopropanol before drying with argon. They are then cleaned with UV/Ozone using a UVO cleaner for 20 minutes. Immediately after cleaning the surface, a plastic clamp and rubber (BunaN) gasket are affixed to the surface to create a well for liquid and 50 μM duplex DNA in phosphate buffer (5 mM phosphate, pH 7, 50 mM NaCl) to make dsDNA films. The dsDNA was incubated on the surface for 18-24 hours. Once the dsDNA is on the surface, it cannot be dried without compromising the structure and therefore the measured properties of the film. The solution was then exchanged 5x with 1 mM mercaptohexanol in phosphate buffer (pH 7, 5 mM phosphate, 50 mM NaCl, 5% glycerol) and incubated for 45 minutes. Lastly the surface was rinsed at least 5x with tris buffer (50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 10% glycerol, pH 7.5) that was degassed by leaving open in an anaerobic chamber over many days.

Electrochemical measurements

The central well around the electrode surface created by the clamp was filled with aqueous buffer containing degassed 50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 10% glycerol, pH 7.5. An AgCl/Ag reference electrode (Cypress) was coated with a solidified mixture of 1% agarose and 3M NaCl in water inside a long, thin pipette tip. The tip was cut so that the salt bridge could connect the electrode to the buffer from the top of the well. A platinum wire used as an auxiliary electrode was also submerged in the buffer from the top of the well. The working electrode contacted a dry part of unmodified gold surface. A CHI620D Electrochemical Analyzer (CH Instruments) was used to control the electrochemical experiments. A picture of the electrochemical set up is shown in Figure S2.

Magnetic field measurements

Magnetic field experiments were conducted using 462 Gauss, 918 Gauss, 1803 Gauss, and 6619 Guass surface strength magnetic field neodymium magnet (K&J Magnetics). Alligator clips were replaced with nonmagnetic stainless steel to minimize magnetic interference. All other parts of the assembly were created using plastic to prevent extraneous objects the magnetic field could influence. Additionally, the strong magnet was waved near the potentiostat during operation, with no obvious change in signal that was apparent, to ensure that the magnet was not interfering with the operation of the potentiostat. Each experiment that was conducted using a magnetic field was compared to a similar quadrant on the same chip that was not tested under a magnetic field and when compared to other experiments was normalized using this data. Each of the four electrode quadrants were tested separately and consecutively (with different orders of the experimental condition). This separation is achieved by separating each of the four electrode sets from one another with a gasket and plastic clamp that creates separate wells around each set of electrodes. Only the quadrant being tested is exposed to protein and has light focused on it and, therefore, the magnetic field is only influencing the activity in that quadrant. During this experiment the remaining quadrants are submerged in phosphate buffer without protein and are not dried to ensure DNA structural integrity. Generally there was little variation seen when using the same dsDNA on different surfaces, however using dsDNA made at different times could result in different maximum values for repair, possibly due to slight differences in surface packing, purity, or efficiency of thymine dimer generation. Background magnetic field strength and applied magnetic field strength were tested by measuring the x,y,z coordinates of the magnetic field at the surface of the electrode using a gauss meter (F.W. Bell, 5100 series).

Thymine dimer repair activity

The repair of thymine dimer DNA by photolyase and cryptochrome was verified by HPLC, mass spectrometry, gel shift, and digestion experiments. High pressure liquid chromatography was performed on duplex DNA containing a thymine dimer before and after incubation with cryptochrome and photolyase. Cryptochrome (50 μM) or 50 μM photolyase was irradiated for 1 hour with 35 μM duplex DNA. The DNA was then separated from the protein via a spin column. The DNA was then resuspended in phosphate buffer. HPLC was performed with a flow rate of 0.8 ml/min with an increasing gradient of acetonitrile in ammonium acetate. The percent acetonitrile increased from 2% acetonitrile to 3% acetonitrile over 10 minutes followed by an increase from 3% acetonitrile to 4% acetonitrile over 30 minutes. The column was kept at 80 ^{o}C to dehybridize the two strands.

Mass spectrometry and digestion experiments were conducted by first incubating duplex DNA (33 μmol) in PDE activity buffer (20 μL, 100 mM Tris, pH 8.9, 100 mM NaCl, 14 mM $MgCl_2$) was added *At*CRY1ΔC (40 μM). The reaction mixture was irradiated with blue light in the presence of a 6600 gauss magnetic field for 1 h under an inert atmosphere at RT. Phosphodiesterase I (*Crotalus adamanteus* venom, USB, 0.1 unit) in PDE storage buffer (110 mM Tris, pH 8.9, 110 mM NaCl, 15 mM $MgCl_2$, 50% glycerol) and 10 μL PDE activity buffer were added to the reaction mixture and heated to 37 °C for 1 h. CutSmart® Buffer (NEB, 1 μL) and Calf Intestinal Alkaline Phosphatase (CIP, NEB, 10 unit) were added to the reaction mixture in sequence. The reaction mixture was then incubated at 37 °C for 1 h. The total volume of the reaction mixture was brought up to 100 μL and was subsequently centrifuged at 5000 × g. The top 80 μL of solution was carefully transferred to a HPLC injection vial. HPLC injection volume was 20 μL and a Chemcobond 5-ODS-H column (ChemcoPak, 5 μm, 4.6 × 150 mm) was used for reversed-phase HPLC. HPLC was performed using a flow rate of 1 mL/min with an increasing gradient of acetonitrile in ammonium acetate (50 mM) at RT. The percent acetonitrile increased linearly from 3% acetonitrile to 10% acetonitrile over 30 min followed by a linear increase from 10% acetonitrile to 20% acetonitrile over 5 min, and then a linear ramp from 20% acetonitrile to 50% acetonitrile over 10 min. The reaction products were purified by HPLC with UV detection at 260 nm. Collected fractions were analyzed using LC-TOF-MS (Waters, Acquity Ultra Performance LC, Micromass Technologies, LCT Premier XE) on a BEH C18 column (Waters, Acquity UPLC, 1.7 μm, 2.1 × 50 mm) at a flow rate of 0.39 mL/min with an increasing gradient of acetonitrile with 0.1% formic acid in ammonium acetate (50 mM) at RT. The percent acetonitrile increased linearly from 5% acetonitrile to 65% acetonitrile over 3.3 minutes followed by an isocratic flow at 65% acetonitrile for 0.5 min.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

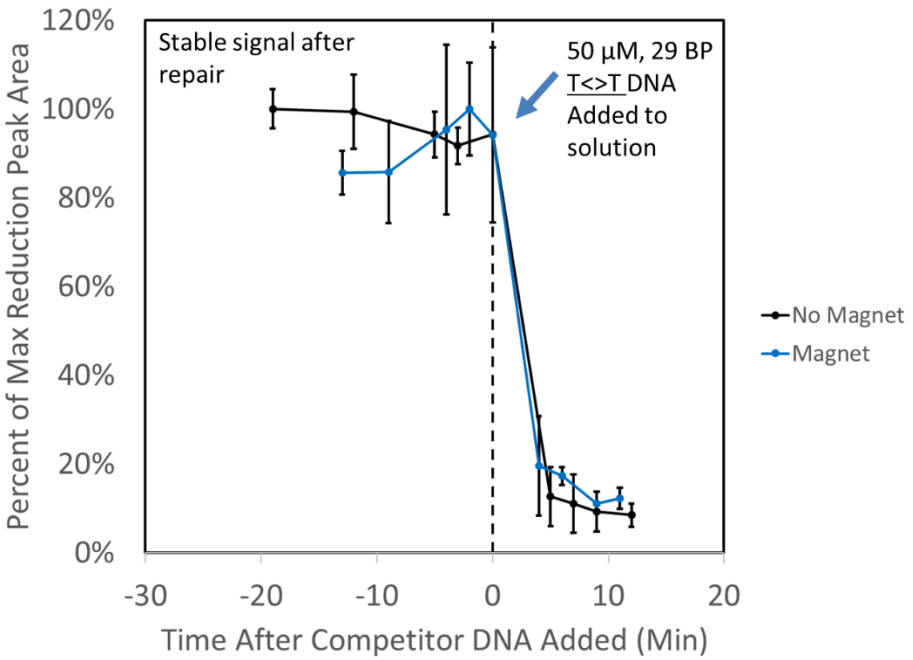

Figure S1. Plot of the reductive peak area in photolyase cyclic voltammetry scans before and after the addition of competitor DNA. The addition of competitor dsDNA containing a thymine dimer to a surface that has saturated the photolyase repair signal results in a significant decrease in signal indicative of the photolyase dissociating from the surface and binding the competitor dsDNA in solution. Data from multiple electrodes were normalized so that the maximum reductive peak area was plotted as 100%. There is no observable difference with or without a magnetic field for the signal decrease. Standard error was plotted with $n \geq 4$.

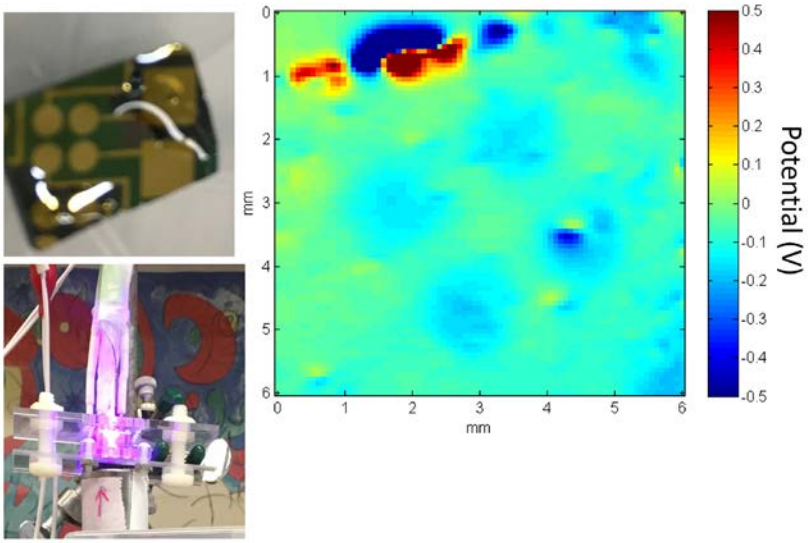

Figure S2. Measurement of multiplexed chip surface using SQUID magnetometer. (Top left) Surface was incubated with dsDNA and photolyase as would be used in an experiment, then dried to make compatible with SQUID measurement. The surface was then magnetized by placing near a 6619 Gauss surface neodymium magnet to align any dipoles. The magnet did not come into contact with the surface to prevent contamination. Note that this is the only experiment with dried DNA because the duplex structure is not important for these measurements. (Top right) The SQUID measurements show that there is no magnetite present on the multiplexed chip either due to the substrate or biological samples. The y-axis is in volts and the color scale represents 3 nT/V. A small contamination was observed on the edge of the chip, but even if these were assumed to be present in experiments the dipoles present are still too small to influence the experiments at the field strengths we used. (Bottom left) A photograph of a typical experiment showing the multiplexed chip in its plastic assembly to create wells, a neodymium magnet placed underneath the device, and working, reference, and auxiliary electrodes placed in the buffer in the top well. The light is irradiating the surface directly and can be observed in the image near the center of the plastic device.

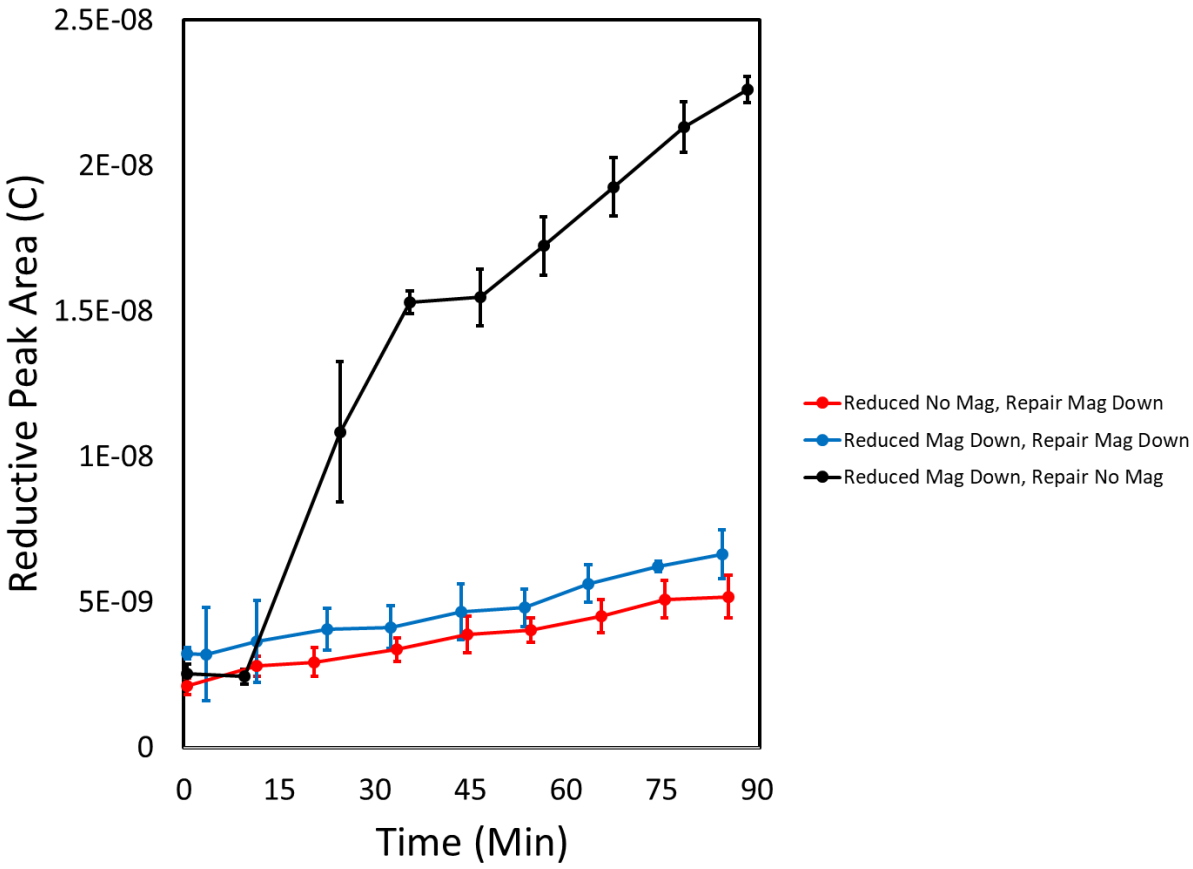

Figure S3. Plot of the reductive peak area from cyclic voltammetry of photolyase on a 29 bp T□T dsDNA-modified electrode under different magnetic field conditions. The dotted line indicates the start of irradiation of the surface with blue light (405 ±10 nm). (Red) Photolyase was photoreduced in solution without a magnet present then added to the surface and monitored with a 30 G magnetic field applied perpendicularly down towards the surface. (Blue) Photolyase was photoreduced in solution with a 30 G magnetic field and then incubated with the surface with the same field pointed perpendicularly down towards the surface. (Black) Photolyase was photoreduced in solution with a 30 G magnetic field and then incubated with the surface without an applied magnetic field. The presence or absence of an applied field during the reduction of photolyase did not have any measurable effect on the amount of charge transferred at later time points. Standard error was plotted with n=3.

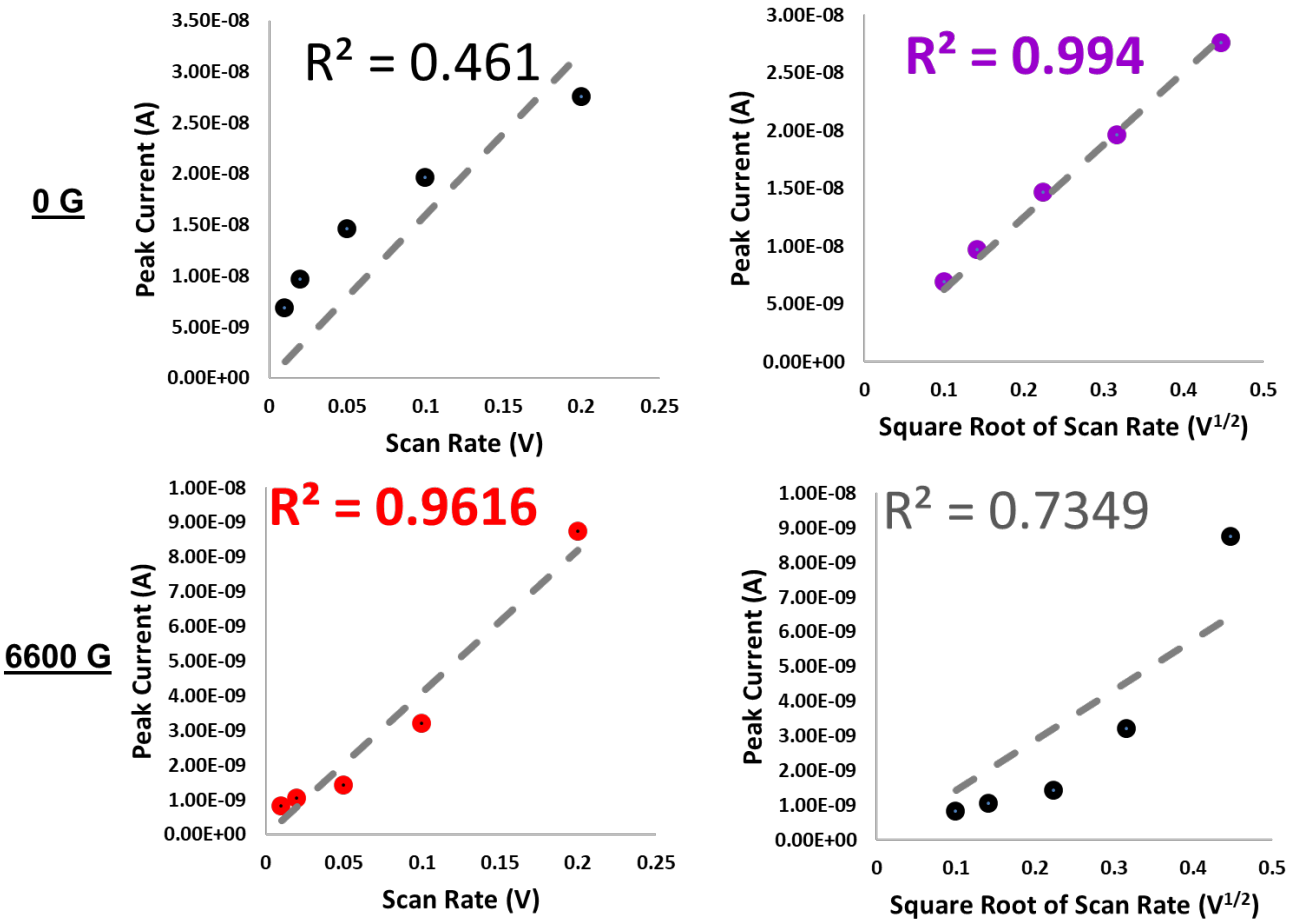

Figure S4. Randles-Sevcik plots of the peak currents of photolyase. Randles-Sevcik plots of the peak currents of photolyase without (top) and with (bottom) a 6000 G applied magnetic field perpendicular to the surface of the electrode.

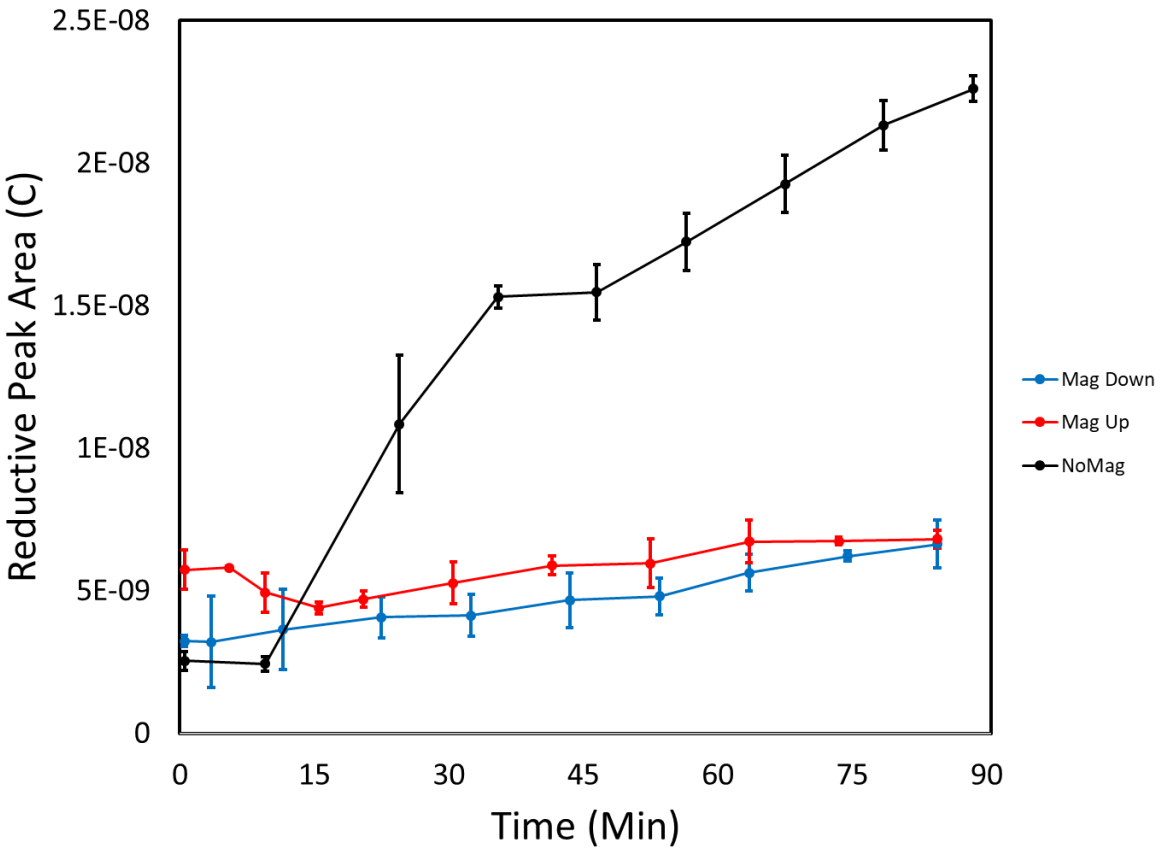

Figure S5. Plot of the reductive peak area from cyclic voltammetry of photolyase on a 29 bp T□T dsDNA modified electrode using opposite polarity magnetic fields. Applying a magnetic field of 30 Gauss perpendicularly up (red) or down (blue) intersecting the plane of the electrode did not show a measurable difference. Photolyase without a magnetic field (black) shows a significant increase in signal compared to both magnetic field directions. Standard error was plotted with n=3.

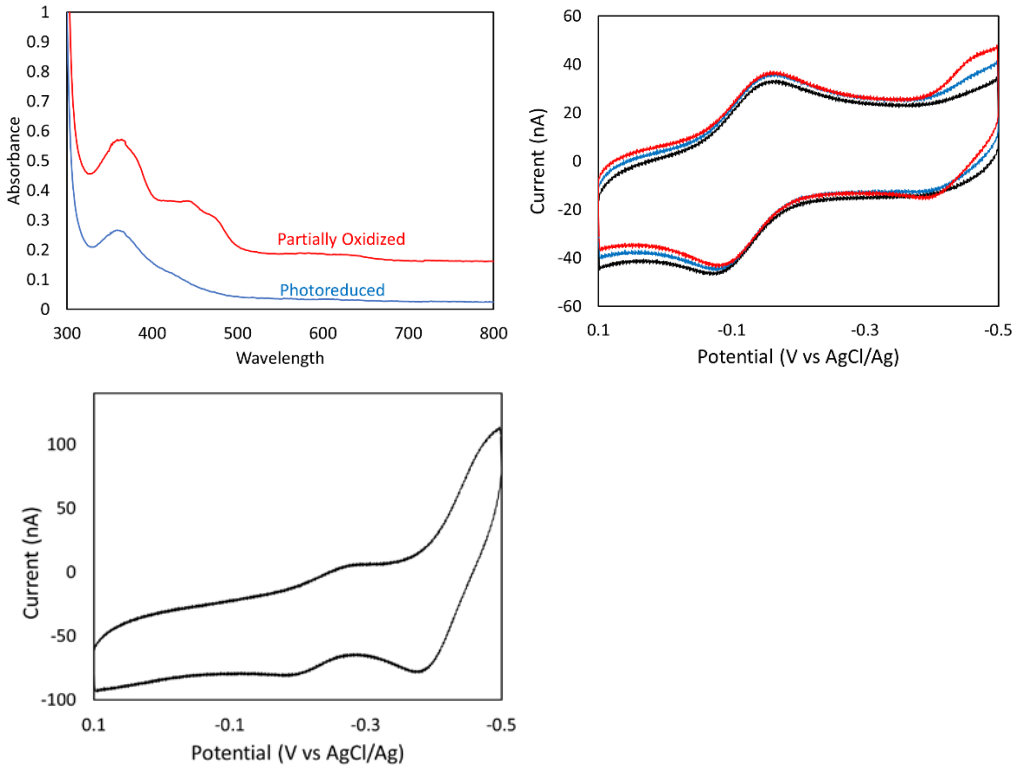

Figure S6. Ultraviolet/visible wavelength spectra and cyclic voltammetry of photolyase upon oxidation. Small amounts of oxygen (<14 ppm O_2) in the glove bag resulted in oxidation of the flavin in the photolyase. Generally, experiments were performed with <1 ppm O_2 present. (Top left) This oxidation could be measured by observing the change in UV/Vis spectra. (Top right) Cyclic voltammetry of photolyase on a dsDNA surface was conducted at 100 mv/s in Tris buffer (50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 10% glycerol, pH 7.5). The oxidized, deprotonated flavin also had a corresponding redox peak associated with it that formed centered around -420 mV vs AgCl/Ag. This peak forms from the photoreduced protein (black) after exposure to small amounts of oxygen and no light irradiation for 30 minutes (blue) and 60 minutes (red). All photolyase mutants had similar redox potentials as the WT protein except for N378C (bottom left), which had a negatively shifted fully reduced state centered around -220 mV, and a slightly positively shifted deprotonated state.

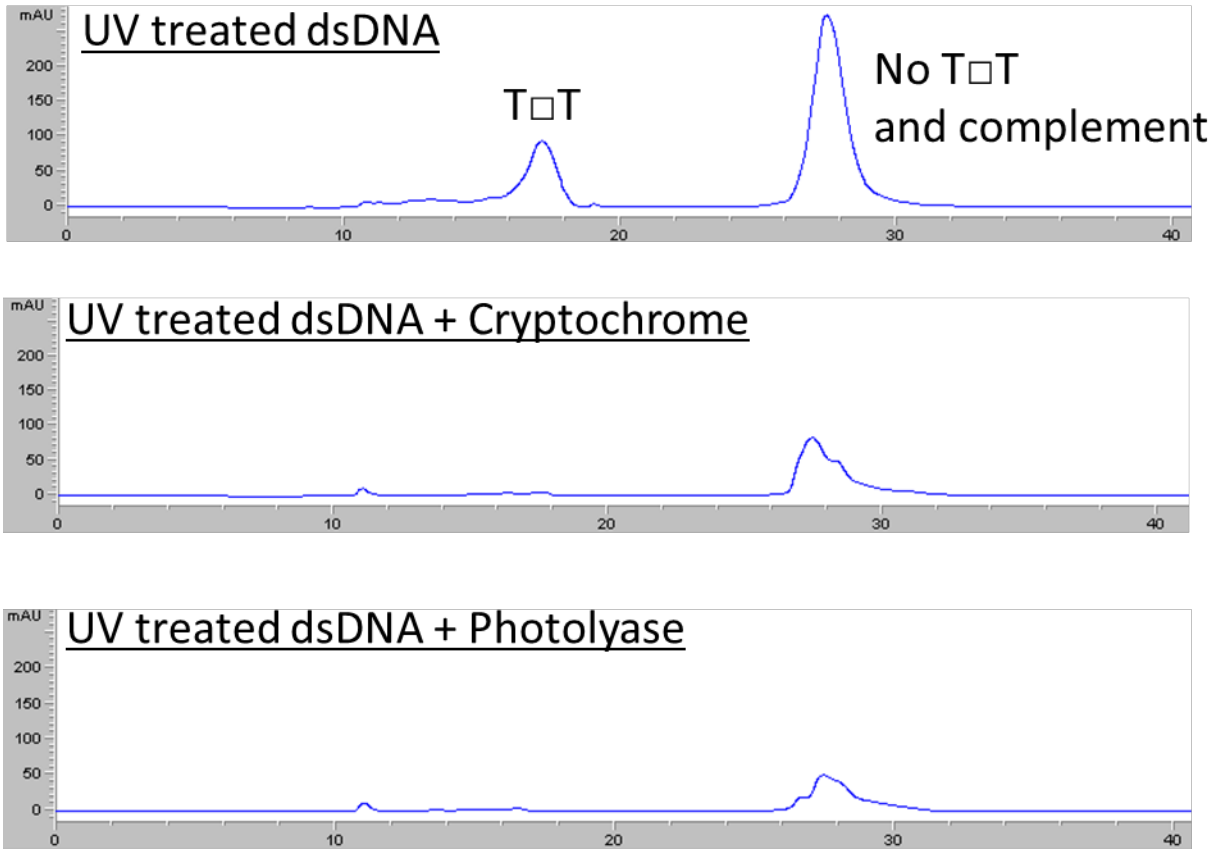

Figure S7. High pressure liquid chromatography traces of duplex DNA containing a thymine dimer before (top) and after incubation with cryptochrome (mid) and photolyase (bottom). The column was kept at 80 ^{o}C to dehybridize the two strands. The strand containing the thymine dimer, T□T, elutes at 17 minutes. The strand with the repaired dimer as well as the complementary strand elute at 27-28 minutes. Irradiating 50 µM cryptochrome or 50 µM photolyase for 1 hour with 35 µM duplex DNA leads to a complete loss of the peak with the thymine dimer as well as the presence of a new peak that co-elutes with the complement, together indicating that the thymine dimer is being repaired. Concentration changes occur due to experimental procedures, so the changes in peak sizes simply reflect purification conditions. Importantly, the masses of the collected fractions were analyzed by MALDI, and despite the change in mobility between the thymine dimer and native thymine strands, no difference in mass is observed, as expected.

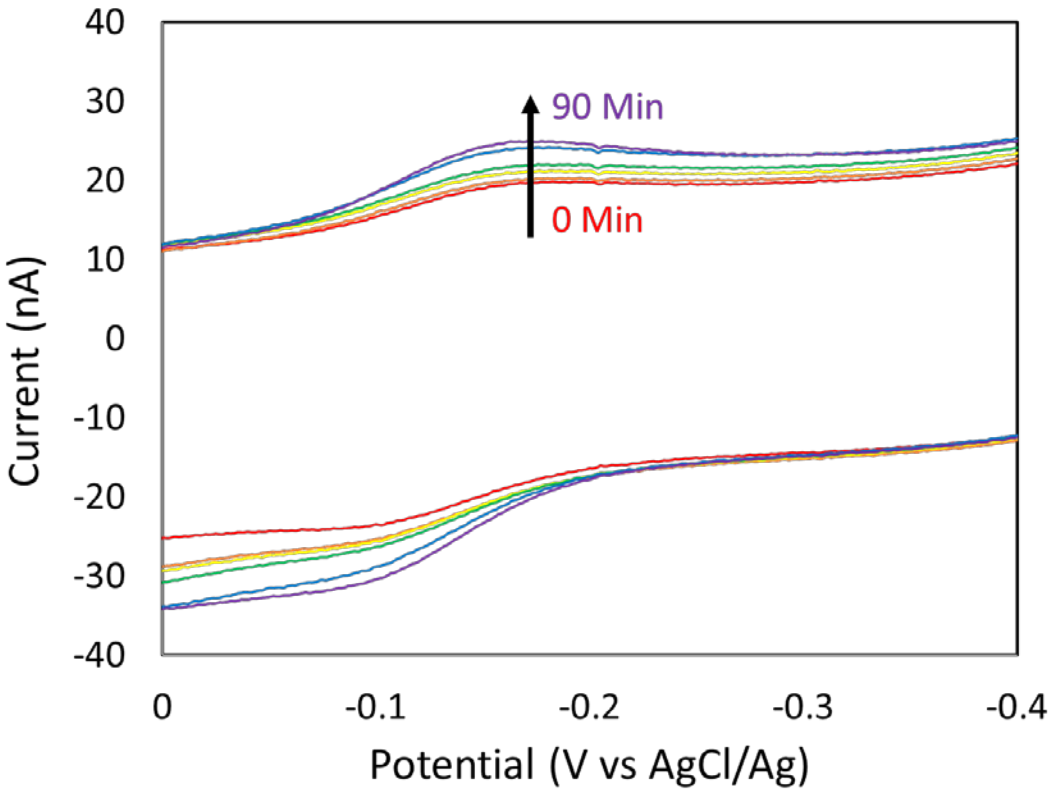

Figure S8. Cyclic voltammograms of cryptochrome incubated on a duplex DNA-modified electrode with a thymine dimer. 50 µM cryptochrome was irradiated with blue light (405 ± 10 nm, <30 mW) from a diode laser pointer for one hour before addition to a 29 bp dsDNA monolayer. The above cyclic voltammograms were taken every 10 minutes for 90 minutes of incubation with constant blue light irradiation in Tris buffer (50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 10% glycerol, pH 7.5). The potential was scanned at 100 mv/s. The yield of charge transferred to the flavin increases over time consistent with the repair of thymine dimers observed with photolyase.

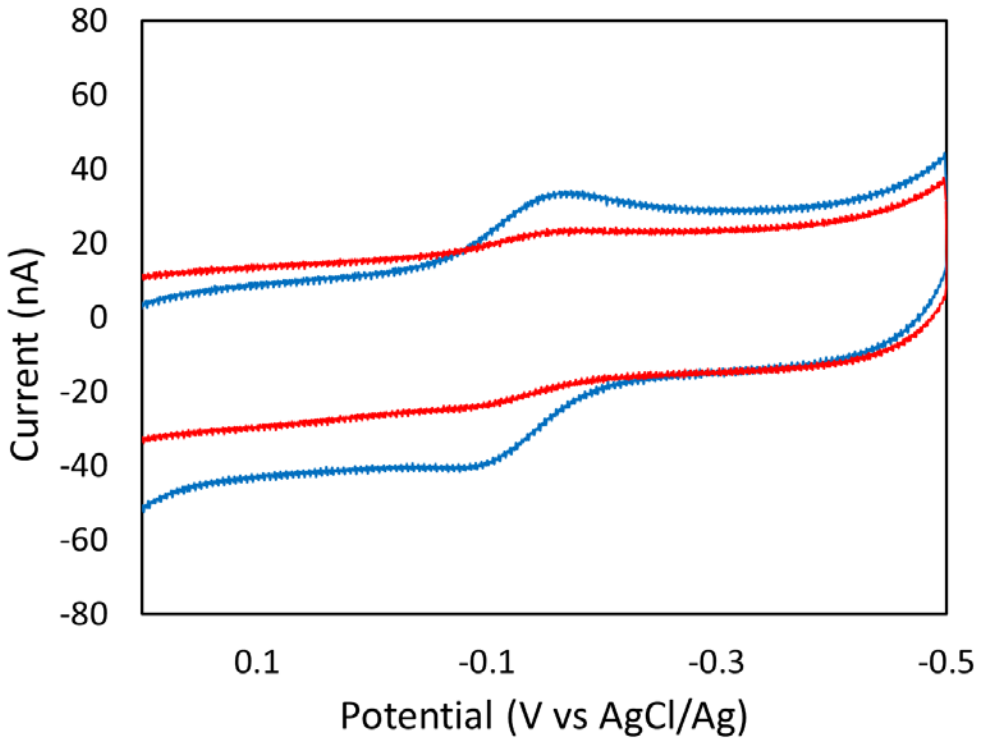

Figure S9. Cyclic voltammogram of 50 µM cryptochrome incubated on a duplex DNA-modified electrode with (blue) and without (red) a single thymine dimer. In both cases the cryptochrome was irradiated with blue light (405 ± 10 nm, <30 mW) from a diode laser pointer for one hour before addition to a 29 bp dsDNA monolayer. The above cyclic voltammogram was taken after 90 minutes incubation of cryptochrome on the DNA-modified electrode with constant blue light irradiation in Tris buffer (50 mM Tris-HCl, 50 mM KCl, 1 mM EDTA, 10% glycerol, pH 7.5). The potential was scanned at 100 mv/s.

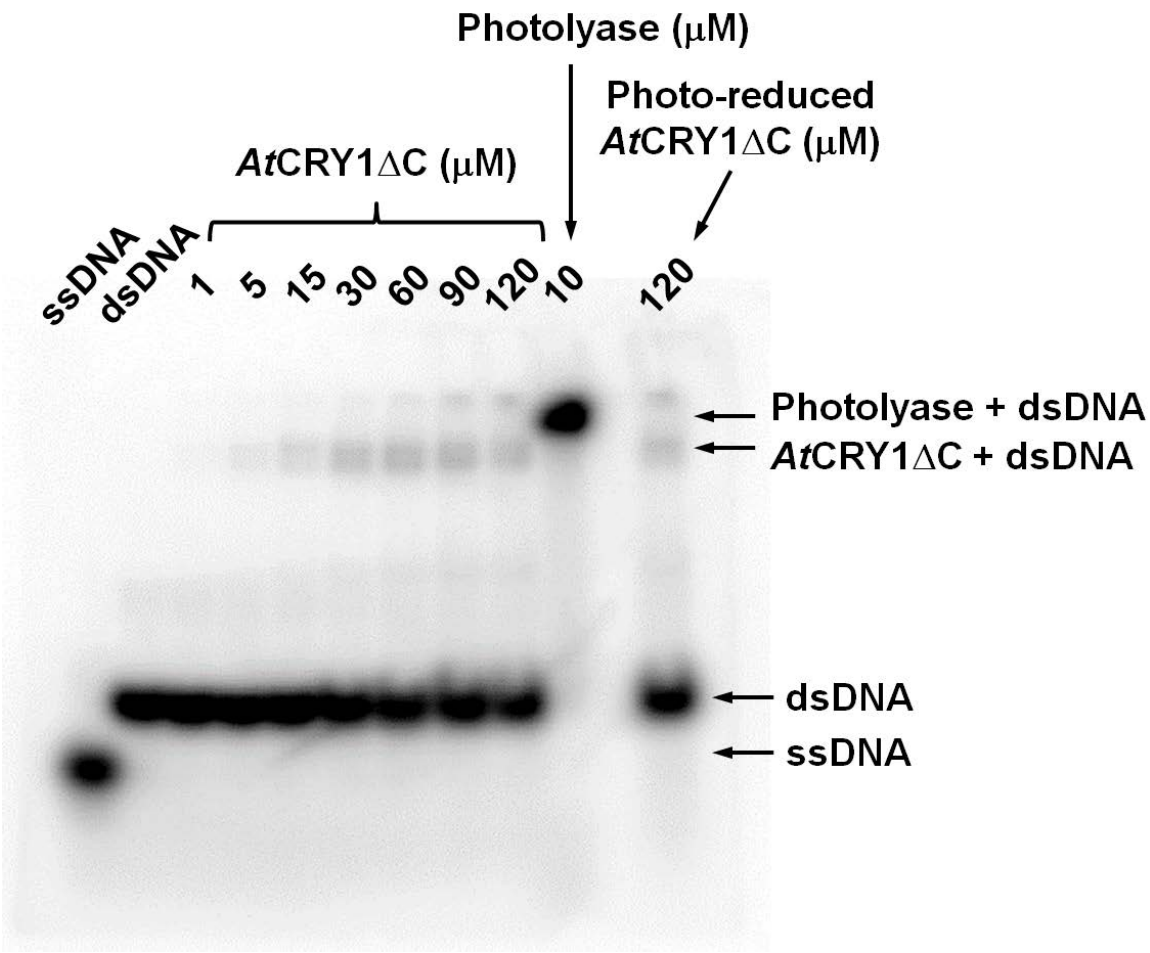

Figure S10. Electrophoretic mobility shift assays (EMSA) of cryptochrome and photolyase. A native 10% polyacrylamide gel ran at 50 mV for 3 h 45 min in 0.5X tris-borate-EDTA (TBE) buffer at 4 °C with the DNA concentration kept at 500 nM per lane. The 5' end of the T□T containing ssDNA was P32-labeled prior to annealing with complementary strand to generate dsDNA. Lanes from left to right: ssDNA, dsDNA, 1, 5, 15, 30, 60, 90, and 120 μM *At*CRY1ΔC, 10 μM photolyase, and 120 μM photo-reduced *At*CRY1ΔC.

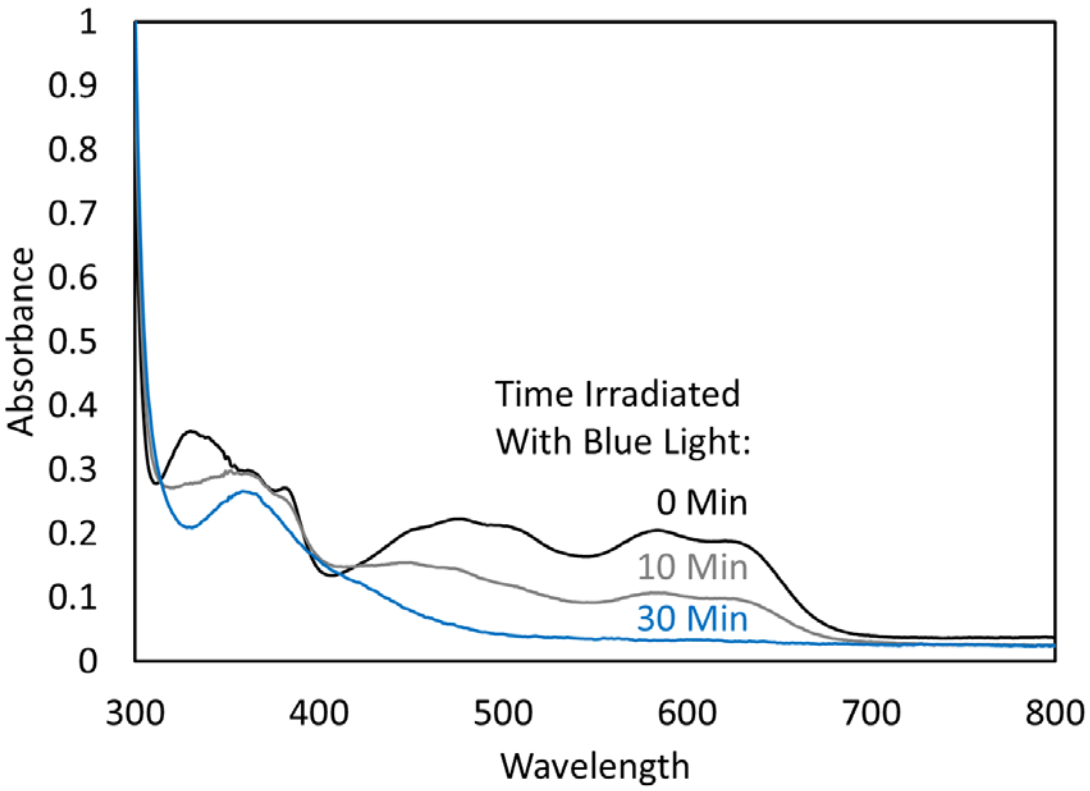

Figure S11. Typical photoreduction measurement of WT photolyase by blue light (405 ± 10 nm). Photoreduction was carried out anaerobically then transferred to a UV/Vis spectrophotometer in an air-tight cuvette. The change in absorbance is consistent with the loss of oxidized flavin and the generation of the fully reduced flavin.

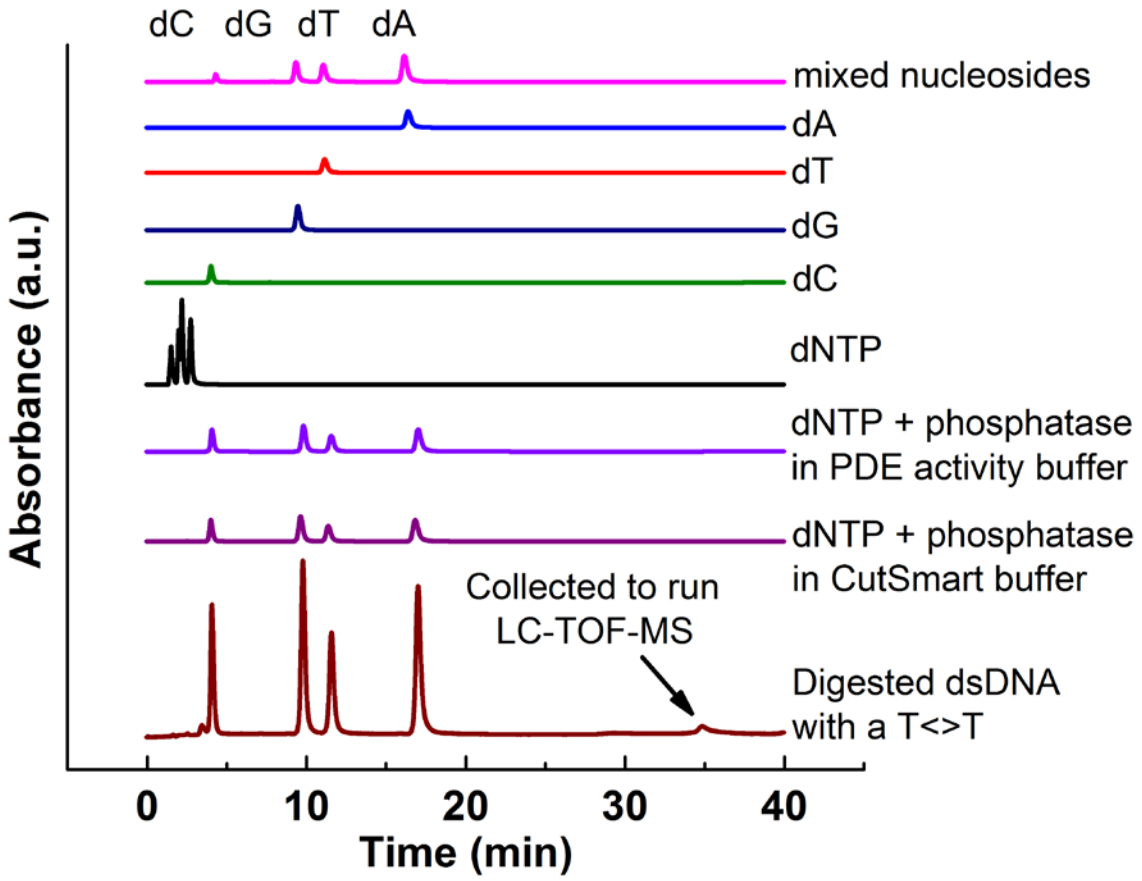

Figure S12. Reversed-phase HPLC traces of nucleosides, nucleotides, and double- stranded DNA digested using phosphodiesterase I and phosphatase. A peak with an elution time of ~ 35 min was observed in the digested dsDNA sample containing a T□T and was further analyzed using TOF-MS in Figure S13.

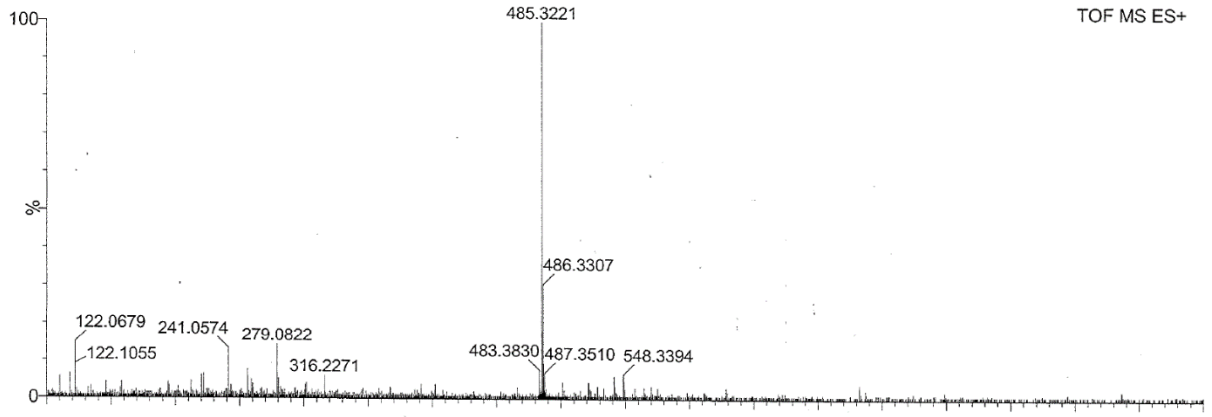

Figure S13. Time-of-flight mass spectrum of the HPLC fraction collected at ~ 35 min from Figure S12. The parent ion peak of a m/z of 485.3221 corresponds to a protonated thymidine dimer.

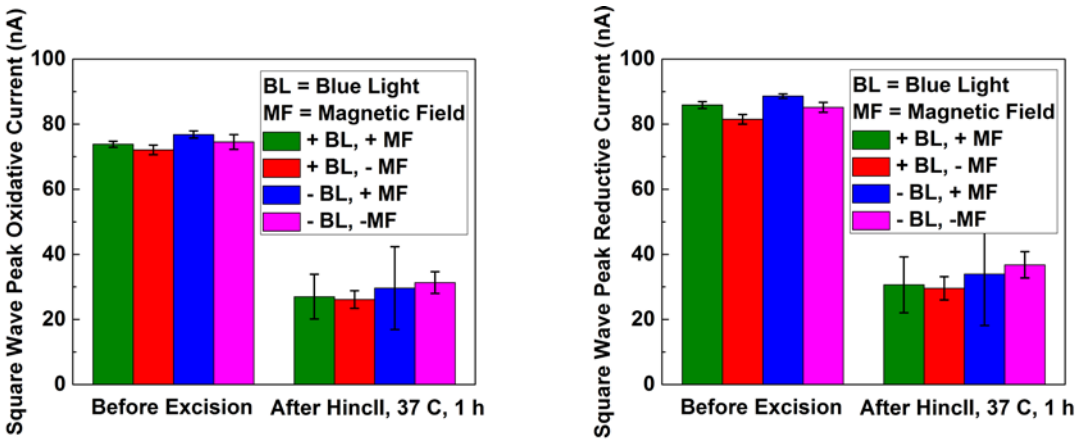

Figure S14. Electrochemistry measurements of HincII enzymatic activity. HincII (10 units, NEB) was incubated with 29 bp dsDNA-modified gold electrodes in CutSmart buffer (1x, 50 mM potassium acetate, 20 mM Tris-acetate, 10 mM magnesium acetate, 100 μg/mL BSA, pH 7.9, NEB) for 1 h at 37 °C. The DNA was modified with a covalently tethered methylene blue at the distal end, away from the surface, which can be reduced and oxidized via DNA CT. HincII activity decreases the observed yield of reduction and oxidation by cutting the DNA and detaching the region containing HincII from the surface. Backfilling with mercaptohexanol prevents the released probe from being reduced or oxidized by the surface. Peak currents were measured from square wave voltammograms. In each case, there is no observable difference in enzymatic activity resulting from blue light or magnetic field exposure.