Supporting Information

ATP-stimulated DNA-mediated Redox Signaling by XPD, a DNA Repair and Transcription Helicase

Timothy P. Mui[#], Jill O. Fuss[†], Justin P. Ishida[†], John A. Tainer[†,+], and Jacqueline K. Barton[#,*]

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, United States
† Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States
+ Department of Molecular Biology, Skaggs Institute of Chemical Biology, The Scripps Research Institute, La Jolla, California 92037, United States

Materials and Methods

Chemicals were purchased from Sigma Aldrich. All reagents for DNA synthesis were purchased from Glen Research. Gold on mica was purchased from Agilent Technologies.

DNA Synthesis

DNA Sequences were synthesized using standard phosphoramidite chemistry at either IDT DNA (Coralville, IA) or on an Applied Biosystems 3400 DNA synthesizer. Thiol additions were introduced using disulfide phosphoramidites (Glen Research), which were subsequently reduced prior to experiment. All oligonucleotides were purified by HPLC and confirmed by mass-spec.

DNA Sequences

Supplementary Table S1. Representative table of DNA duplexes utilized.

Duplex	Sequence
Duplex DNA	SH 5′ – GTGCTGCAACGTGTCTGCGC – 3′ 3′ – CACGACGTTGCACAGACGCG – 5′
Overhang DNA	SH 5′ – GTGCTGCAACGTGTCTGCGC – 3′ 3′ – CACGACGTTGCACAGACGCGAGAGCAGAC – 5′
Overhang DNA w/MM (high)	SH 5′ – GTGC**C**GCAACGTGTCTGCGC – 3′ 3′ – CACGACGTTGCACAGACGCGAGAGCAGAC – 5′
Reverse DNA	SH 5′ – CACGACGTTGCACAGACGCGAGAGCAGAC – 3′ 3′ – GTGCTGCAACGTGTCTGCGC – 5′

DNA Modfied Electrodes

DNA modified electrodes were prepared essentially as previously described.[1] Briefly, a 50 μL solution of 50 mM duplex DNA (as determined by UV-Vis) was allowed to incubate on a bare gold on mica surface at 4°C overnight. The free DNA was then removed and the surface rinsed a minimum of three times with phosphate buffer (5 mM phosphate, 50 mM NaCl, pH 7.0). Next 50 μL of a 1 mM 6-mercapto-1-hexanol solution in protein buffer was allowed to passivate the surface at ambient temperature for 2 hours. Finally, the surface was rinsed a minimum of three times with protein buffer.

Protein Expression

The *Sulfolobus acidocaldarius* XPD expression construct as described[2] was modified to improve expression by removing a frame-shifting ATG start codon between the RBS and the correct start codon. Mutants were then generated using Quikchange XL II kit (Stratagene) and verified by sequencing. All proteins were expressed in BL21 Rosetta2 *E. coli* cells (Invitrogen) grown in Terrific Broth at 37ºC for 3 hours after induction with 0.2 mM IPTG. Cells were resuspended in 20 mM MES pH 6, 100 mM NaCl, 1 mM DTT, 1 mM EDTA and lysed by sonication and constant cell disruptor (Constant Systems) at 20,000 psi. After centrifugation at 29,000 RCF for 30 min, the supernatant was heat treated (65ºC, 20 min), centrifuged as before and incubated with Capto DEAE (GE Healthcare) for 10-20 min at room temperature. The flow-through was collected by gravity and loaded onto a 5 mL HiTrap Heparin (GE Healthcare) column at 2 mL/min. Bound protein was washed (7 column volumes) with lysis buffer and eluted with a linear gradient (7 column volumes) to 45% high salt buffer (20 mM MES pH 6, 1 M NaCl, 1 mM DTT, 1 mM EDTA). SaXPD-containing fractions (~36% high salt buffer) were pooled, concentrated using Amicon Ultra, Ultracel-10k regenerated cellulose spin filters, and fractionated on a HiLoad 16/60 Superdex200 (GE Healthcare) size exclusion column at 1 mL/min in 10.8% high salt buffer (200 mM NaCl final). SaXPD-containing fractions were pooled, concentrated as above to 2-12 mg/mL by OD_{280}, and frozen. All cell and protein manipulations were carried out at 4°C unless otherwise noted, buffers were filtered and degassed, and purification was carried out in one day when possible to limit oxygen exposure.

Prior to electrochemistry measurements, proteins were dialyzed against the protein buffer (20 mM Phosphate, 100 mM NaCl, 1 mM EDTA, 5% glyercol, pH 7.5) to remove residual DTT. The concentration of individual proteins were determined by UV-Vis using ε=17,000 M^{-1} at 410

nM for the [4Fe-4S] cluster.[3] For a representative trace see Supporting Information Figure S4. Most protein work was conducted in an oxygen-free environment to prevent degradation of the [4Fe-4S] cluster.

Protein Electrochemistry

Electrochemistry was performed as previously described[1] with modifications indicated. Measurements were obtained in an oxygen-free atmosphere using a CH Instruments 620C electrochemical analyzer. The working electrode was a Au (111) chip with a platinum wire auxiliary. Ag/AgCl (Basi Electrodes) electrode was modified with a 4% agarose gel tip and served as the reference. The 3-electrode setup was placed in a pseudo-closed tip box filled with degassed water to prevent evaporation of the protein from the electrode. Protein was added in aliquots of 40-50 μL with concentrations as indicated.

Both ATP and ATP-γ-S were purchased from Sigma and dissolved in protein storage buffer to make 100 mM stock solutions that were subsequently degassed with argon. For ATP and ATP-γ-S additions, the appropriate degassed stock solution was added and mixed by pipetting.

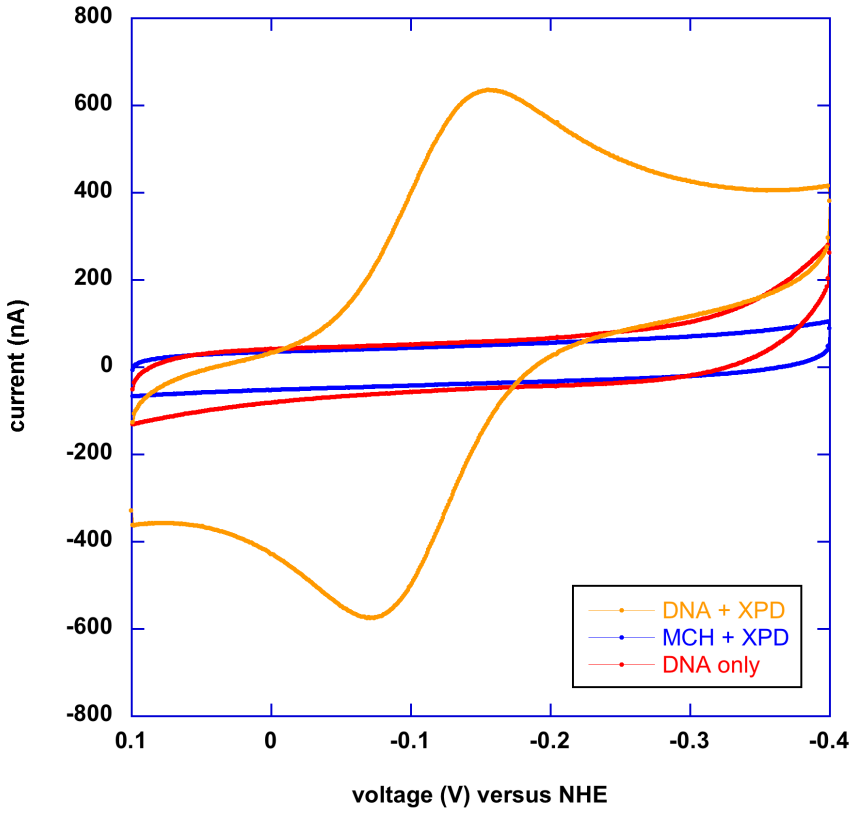
For data analysis, current values were taken by averaging the reductive sweep of a CV after linear base line corrections. After equilibration on the surface, a linear fit was used as the background current. The value of the current was then subtracted from the background current to obtain the difference in current. This value was then normalized to the surface by dividing by the background current to obtain the % difference in current. From the % difference in current data, 1^{st} order kinetics were plotted to fit the parameters $yield=1-e^{-kt}$.

Biochemical Assays

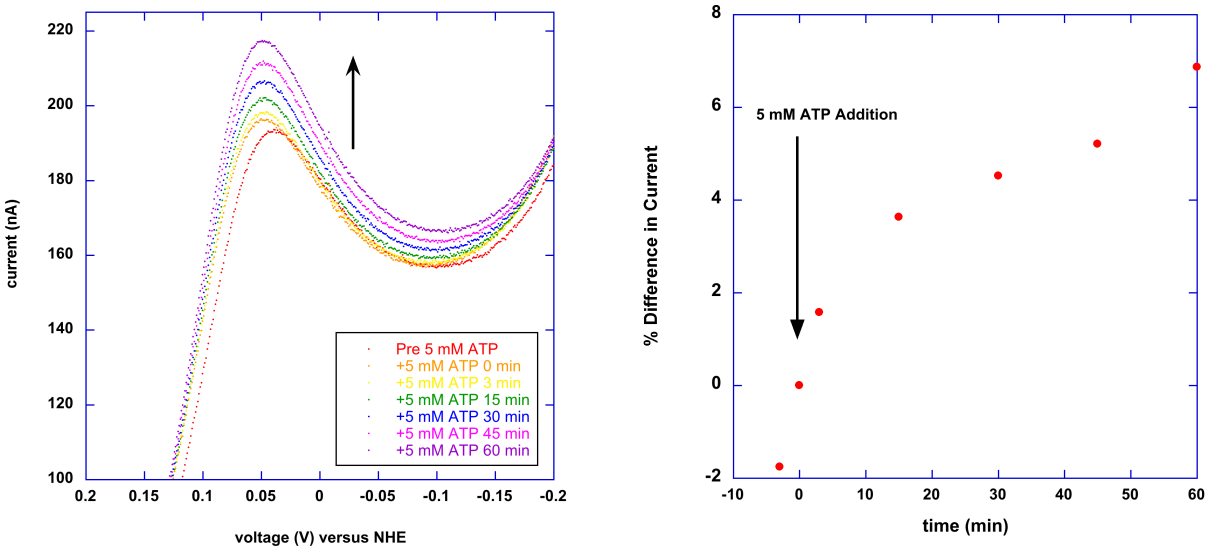
Helicase assays were performed as described[2] with the following modifications. 100 nM 5'-FAM-labeled DNA substrate and 50-500 nM XPD was used. Gels were visualized and quantified using a VersaDoc MP 4000 Molecular Imager and Quantity One software, respectively (BioRad).

ATPase assays were carried out under the same conditions as the helicase assays except that unlabeled ssDNA (25-mer from helicase substrate) was used and the total reaction volume was 50 μL. The amount of phosphate released was measured using Biomol Green Reagent (Enzo Life Sciences) according to the manufacturer's instructions in 96-well plates on an Infinite M1000 microplate reader (Tecan).

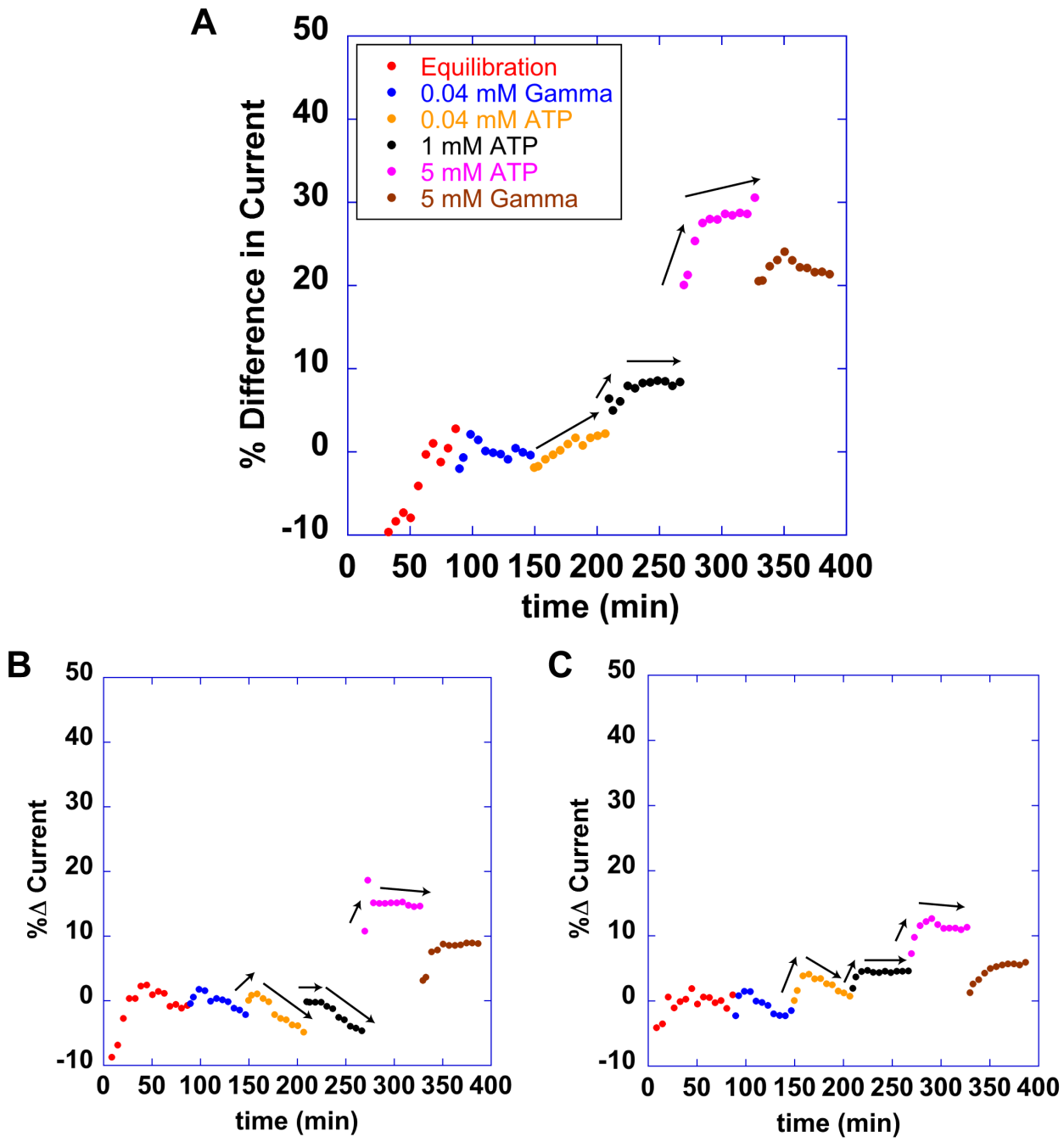
SaXPD-DNA binding interactions were measured by fluorescence anisotropy (FA) as described[2] with the following modifications. 20 nM substrate was used, reactions were incubated for 15 min, and FA was measured in 384-well plates using an Infinite M1000 microplate reader (Tecan).



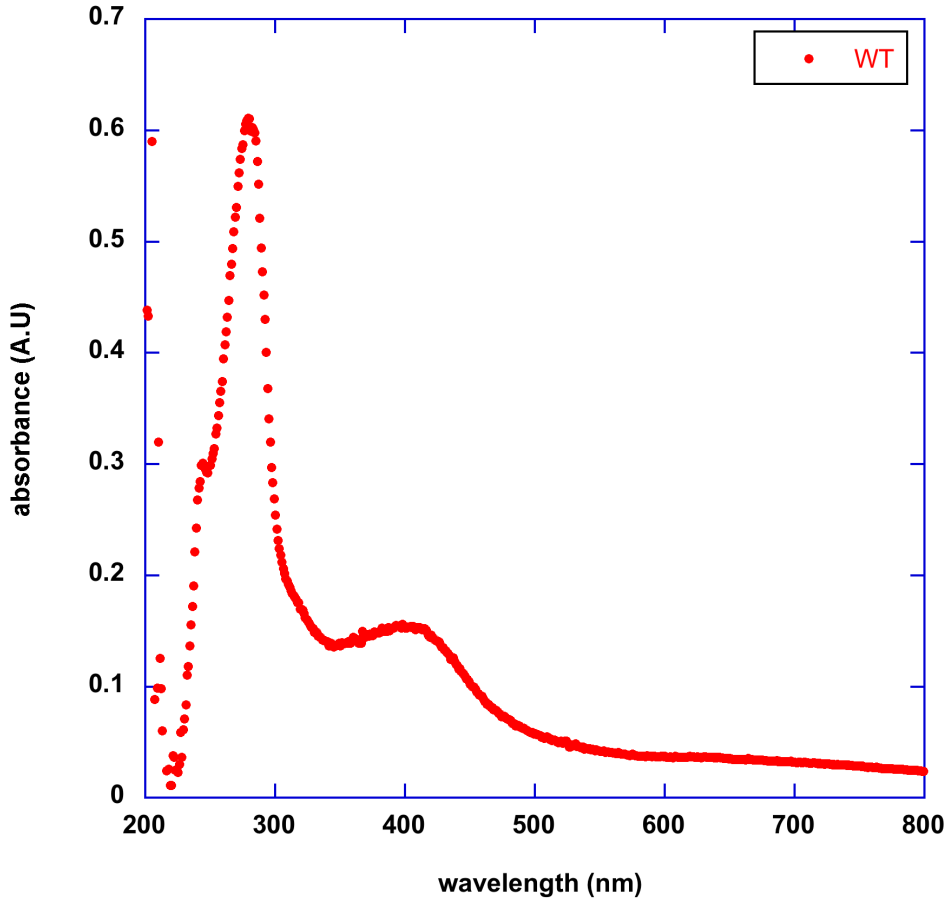
Supplementary Figure S1. Electrochemistry of SaXPD on a DNA modified electrode backfilled with mercaptohexanol (orange), on a mercaptohexanol only electrode (blue) and without SaXPD on a DNA modified electrode (red) (Ag/AgCl reference electrode; Pt auxiliary electrode, 50 mV/s scan rate). These data indicate that this is a DNA-mediated process.



Supplementary Figure S2. Cathodic sweep of CVs for SaXPD [10 μM] on DNA modified electrodes pre and post [5 mM] ATP addition (left). % Difference in current versus time for SaXPD on DNA modified electrodes (right). The arrow on the right indicates addition of 5 mM ATP. Currents were obtained through averaging of the reductive sweep of the various voltammograms (Ag/AgCl reference electrode; Pt auxiliary electrode, 50 mV/s scan rate).



Supplementary Figure S3. Plots as shown in Figure 2. Arrows have been included during the ATP additions to indicate an initial phase as well as the evolution of the signal with ATP hydrolysis.



Supplementary Figure S4. UV-Vis spectrum of WT SaXPD. The shoulder at 410 nm, is indicative of a [4Fe-4S] cluster and is used for determining the concentration of the XPD mutants.

References:

(1) Boal, A. K.; Yavin, E.; Lukianova, O. A.; O'Shea, V. L.; David, S. S.; Barton, J. K. *Biochemistry* **2005**, *44*, 8397.

(2) Fan, L.; Fuss, J. O.; Cheng, Q. J.; Arvai, A. S.; Hammel, M.; Roberts, V. A.; Cooper, P. K.; Tainer, J. A. *Cell* **2008**, *133*, 789.

(3) Boal, A. K.; Genereux, J. C.; Sontz, P. A.; Gralnick, J. A.; Newman, D. K.; Barton, J. K. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 15237.