Structure of UvrA nucleotide excision repair protein in complex with modified DNA

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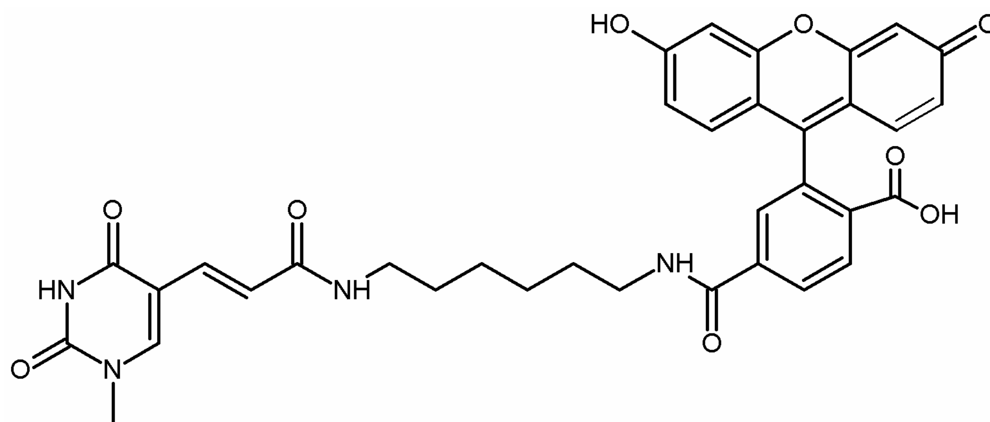
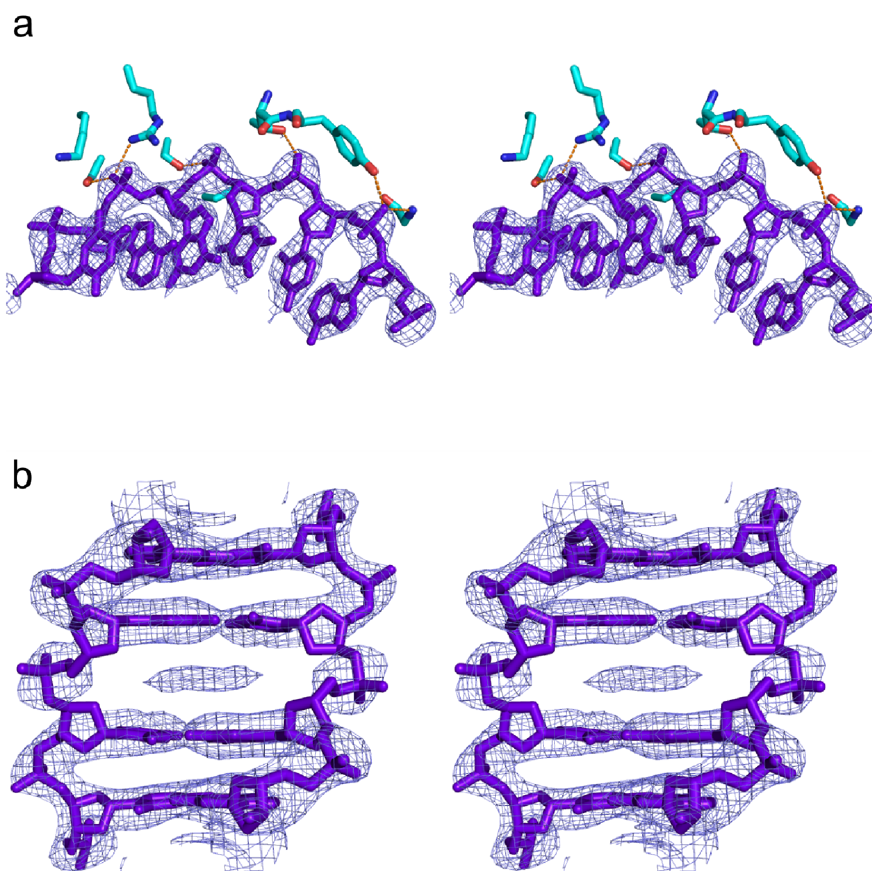
SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURES



Supplementary Figure 1. Chemical structure of fluorescein-modified thymine.



Supplementary Figure 2. Sample electron density maps. (**a**) DNA region interacting with the binding patch. DNA is shown in purple and protein residues in cyan. (**b**) The deformed mid-point of the double helix. A 2Fo-Fc map contoured at 2σ (a) and 0.9σ (b) is overlaid on the structure of the DNA

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          640            650              660       670       680       690       700       710
      |....|....|...     .|....       |....|....|....|....|....|....|....|....|....|....|....|....|..
Tm    LMNLLHKTKLPAGE-----FDSIEG-------HENIDKMIAIDQSPIGRTPRSNPATYTKVFDEIRSLFAMTPAAKARGYNKSRFSFNLKGGRCE
Bst   LAQKLHRAKAKPGE-----HRDIRG-------LEHLDKVIDIDQSPIGRTPRSNPATYTGVFDDIRDVFASTNEAKVRGYKKGRFSFNVKGGRCE
Ec    AQRQLNGATIAEPAP----YRDIQG-------LEHFDKVIDIDQSPIGRTPRSNPATYTGVFTPVRELFAGVPESRARGYTPGRFSFNVRGGRCE
Bca   LAQKLHRAKAKPGE-----HRDIRG-------LEHLDKVIDIDQSPIGRTPRSNPATYTGVFDDIRDVFASTNEAKVRGYKKGRFSFNVKGGRCE
Ngo   TARELNRAQEEPAP-----YDDIRG-------LEHLDKVINVDQSPIGRTPRSNPATYTGLFTPIRELFAGVPLSRERGYNVGRFSFNVKGGRCE
Atu   AARRVMGAREIPAE-----HDRIDG-------FEFIDKVIDIDQSPIGRTPRSNPATYTGAFTPIRDWFAGLPEAKARGYAPGRFSFNVKGGRCE
Dra   LARELNGAKTTPGL-----YDRIEG-------MEQLDKVIEIDQSPIGRTPRSNPATYTGVFTEIRDLFTRTPEARRRGYQAGRFSFNVKGGRCE
Hpy   AQTLLNHAKKTQSLN----GVEIVG-------LEHLDKVIYLDQAPIGKTPRSNPATYTGVMDEIRILFAEQKEAKILGYSASRFSFNVKGGRCE
Hal   LAREMNDNTSVDPGD----HDAIEG-------TEHVETVRLIDQSPIGRTPRSNPATYTGIFDYIREKFAQTKLATQRGYKKGRFSFNVKGGRCE
Dra2  LAAHFGQPVNPDPEDDEDPADHTAGSARLGGDLAQITRLVRVDQKPIGRTPRSNMATYTGLFDQVRKLFAATPLAKKRGYNAGRFSFNVKGGRCE
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Supplementary Figure 3. Sequence alignment of DNA-binding regions of selected UvrA proteins. The residue numbering according to Tm-UvrA sequence is given on top of the alignment. Conserved residues involved in DNA binding are shown in blue and Tm-UvrA Arg671 and its equivalents in green. Tm - *T. maritima*; Ec - *E. coli*, Dra - *D. radiodurans*; Ngo -*N. gonorrhoeae,* Hpy - *H. pylori*, Atu - *A. tumefaciens,* Hal – *Halobacterium sp*, Dra2 - *D. radiodurans* UvrA2.

SUPPLEMENTARY MOVIE LEGENDS

Supplementary Movie 1. Morphing between the DNA structure observed in the Tm-UvrA–DNA complex and the model of ideal B-form DNA to visualize the deformations of the DNA. The thymine residue with fluorescein modification is shown in orange.

Supplementary Movie 2. Tm-UvrA surface representation with morphing of the DNA structure as in Supplementary Movie 1. Protein surface was added to visualize its complementarity with deformed DNA conformation.

SUPPLEMENTARY TABLE

Supplementary Table 1. Kinetic parameters of ATPase activity of Tm-UvrA

	K_m (µM)	k_{cat} (min^{-1})	$k_{cat}\ K_m^{-1}$ (µM $^{-1}$ min^{-1})
UvrA alone	67.0 ± 4.6	10.8 ± 0.2	0.16
UvrA with unmodified DNA *	48.6 ± 4.5	9.7 ± 0.2	0.20
UvrA with modified DNA*	41.9 ± 5.1	9.0 ± 0.3	0.21

* both DNA oligos are 32-mers of the same palindromic sequence. Modified DNA contains a fluorescein thymine modification in position 14 (the same oligonucleotide was used for crystallization).

The errors represent the standard errors of the mean.

SUPPLEMENTARY METHODS

Protein preparation. *T. maritima* UvrA expression plasmids were prepared based on pET28 expression vector (Novagen). The expressed protein contained either an N-terminal 6xHis-tag or the N-terminal His-tag and SUMO protein. Both protein variants were expressed in *E. coli* BL21 strain overnight at 16°C using the induction with 0.04 mM IPTG. Bacterial cells were resuspended in 40 mM NaH_2PO_4 (pH 7.0), 5% (v/v) glycerol, 1.4 mM β-mercaptoethanol (buffer 1) with addition of 150 mM NaCl. Lysozyme at a concentration of 1 mg ml^{-1} and a mix of protease inhibitors were also added. After incubation on ice, NaCl concentration was increased to 1 M and the suspension was sonicated. Cleared lysate was applied on His-Trap Crude 5 ml column (GE Healthcare) equilibrated with buffer 1 with 1 M NaCl. Tm-UvrA was eluted with 150 ml linear imidazole gradient from 0 to 300 mM concentration. For SUMO fusion protein chosen fractions were dialyzed to a buffer containing 250 mM NaCl, 40 mM NaH_2PO_4 (pH 7.0), 5% (v/v) glycerol, 1 mM DTT and 0.5 mM EDTA (buffer 2). After dialysis, the fusion protein was digested with SUMO protease at 30°C overnight. SUMO protein was next removed on His-Trap HP 1 ml column (GE Healthcare) equilibrated with buffer 2. Tm-UvrA was next dialyzed to buffer 2 with 2 M NaCl and concentrated. His-tagged Tm-UvrA eluted from nickel column was dialyzed to buffer 2 but with 40 mM NaCl and applied to 1 ml ResourceQ column (GE Healthcare). Tm-UvrA was eluted with 30 ml linear NaCl gradient from 40 mM to 0.5 M. Protein fractions were mixed with equal volume of a buffer containing 40 mM NaH_2PO_4 (pH 7.0), 1.5 M NaCl, 5% (v/v) glycerol, 1 mM DTT, 0.5 mM EDTA, 100 mM arginine and 100 mM glutamic acid.

As the final purification step both tagless and His-tagged Tm-UvrA were applied to a gel filtration column (Superdex 200, GE Healthcare) equilibrated with storage buffer containing 20 mM HEPES (pH 7.0), 1 M NaCl, 5% (v/v) glycerol, 1 mM DTT, 0.5 mM EDTA, 50 mM arginine and 50 mM glutamic acid. For His-tagged protein, in case of precipitation after dialysis, the precipitate was resuspended in storage buffer and the aggregated form was removed by gel filtration. Protein was concentrated to 6–7 mg ml^{-1} and stored in storage

buffer. Prior to crystallization protein–DNA complex was dialyzed to 20 mM HEPES (pH 7.0), 150 mM NaCl, 5% (v/v) glycerol, 1 mM DTT, 0.5 EDTA.

Quikchange method (Stratagene) was used to prepare expression constructs for four variants of Tm-UvrA: H640A, TY679AA, R688A, and KS704AA. Only the first two proteins could be expressed in *E. coli*. They were purified using the same protocol as for wildtype His-tagged protein but with ResourceQ step omitted.

UvrB and UvrC proteins were expressed and purified on Nickel column essentially as described for His-tagged UvrA.