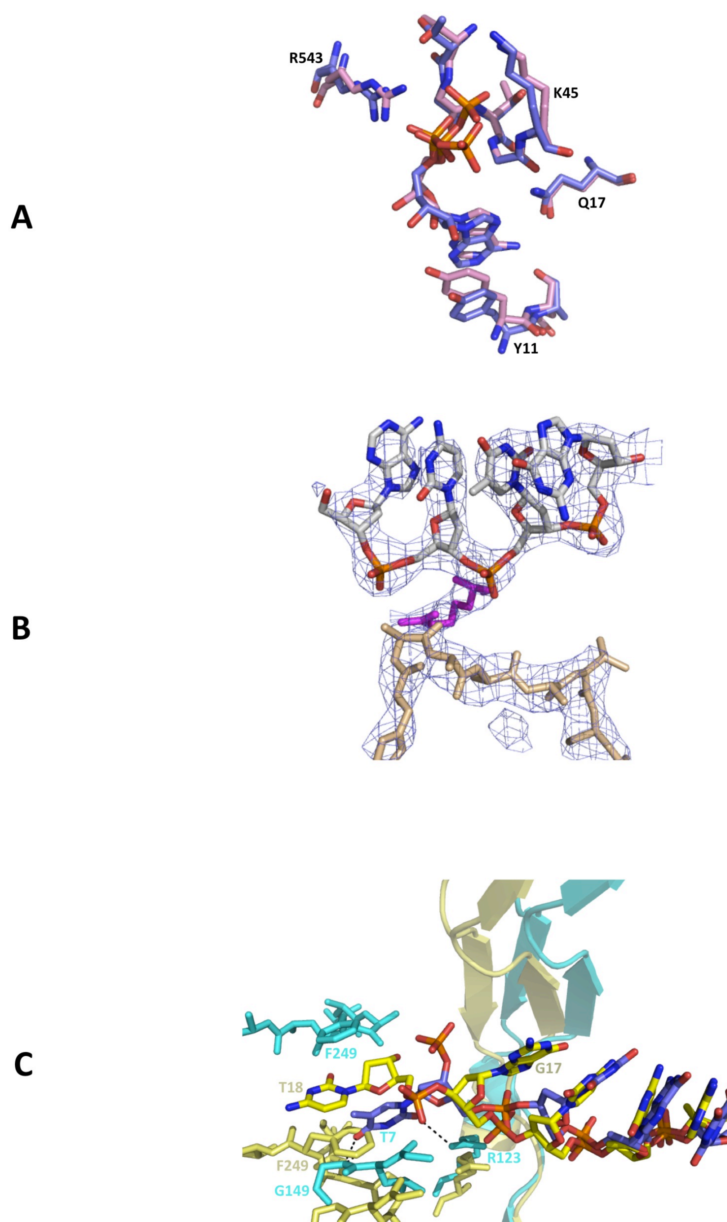
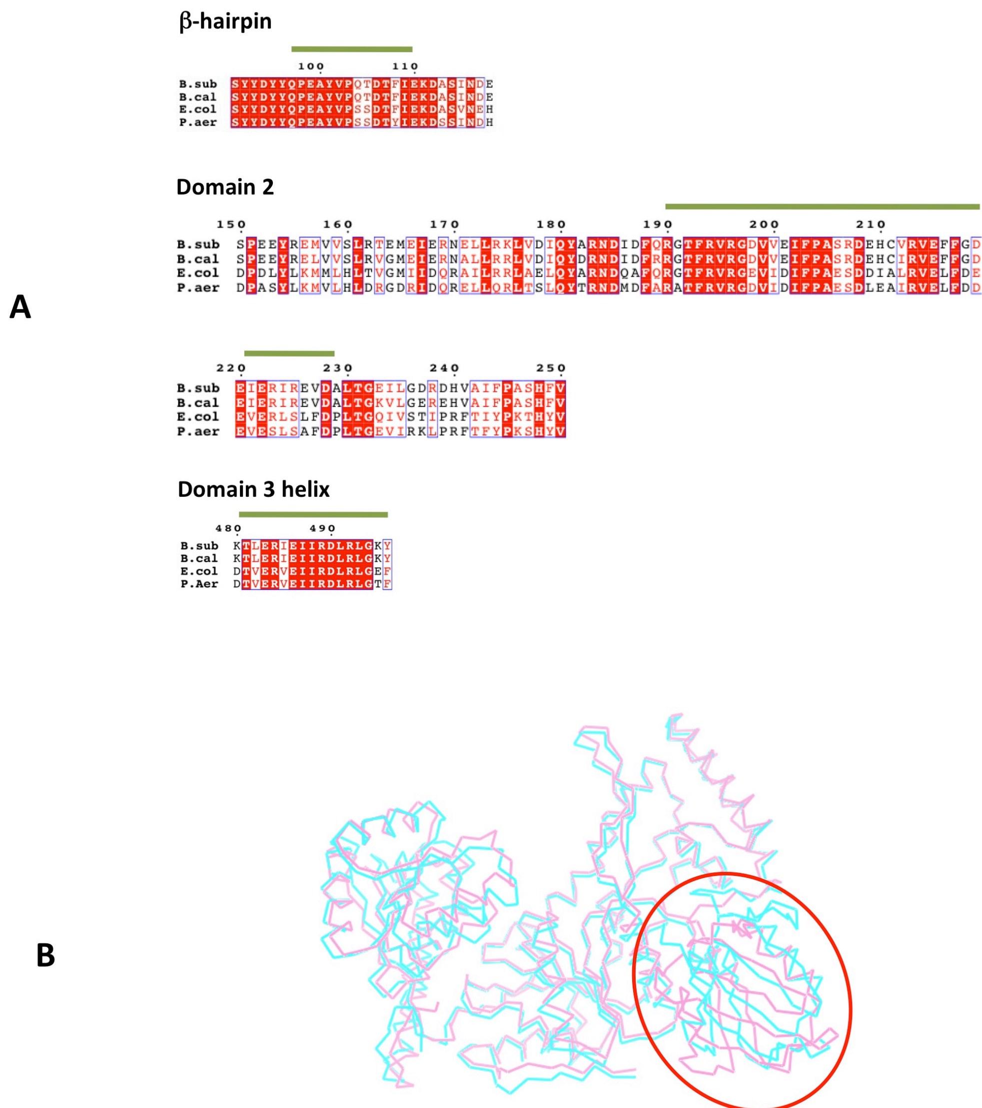
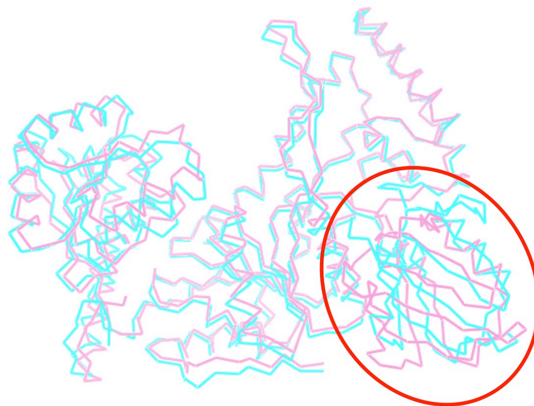
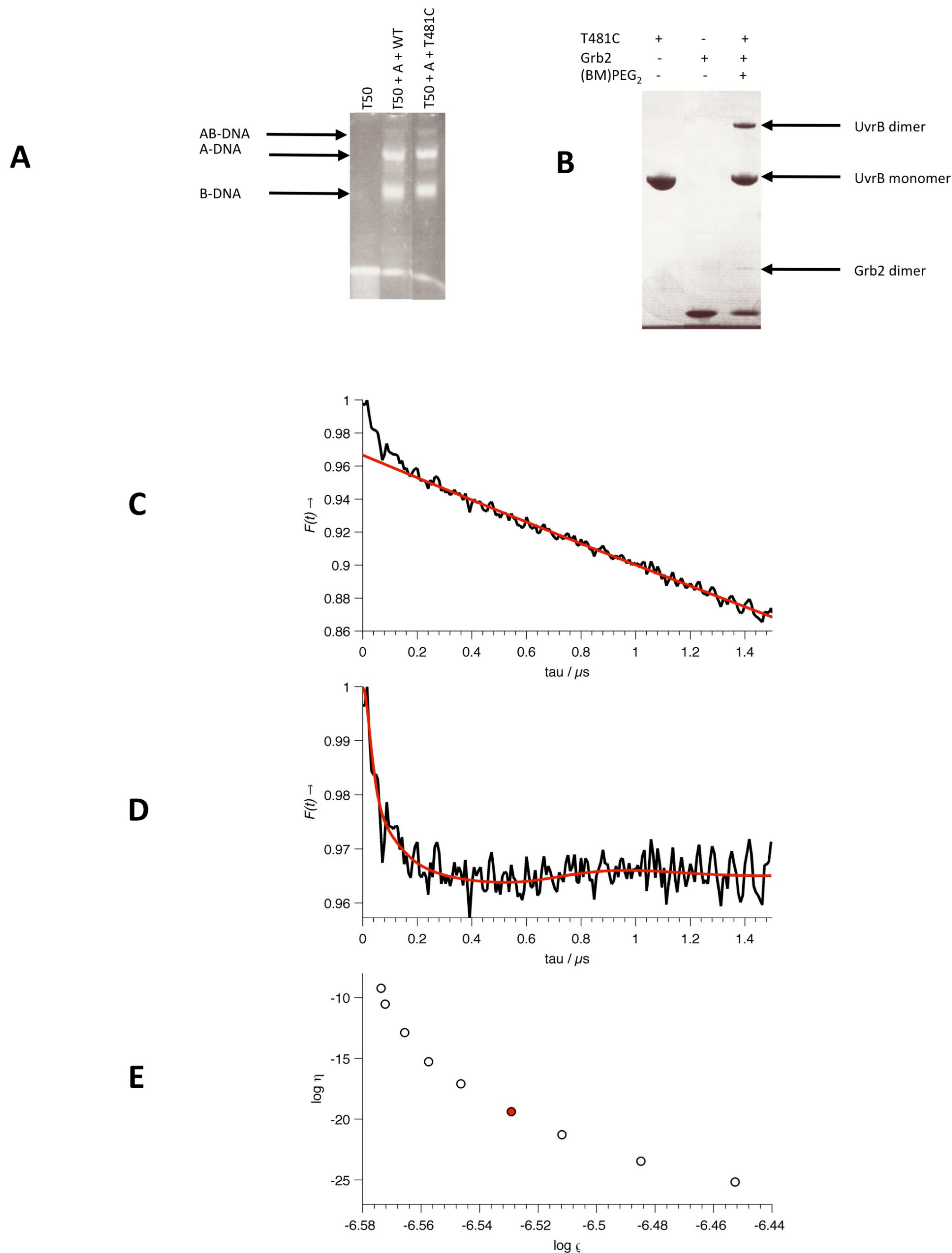


Supplemental 1 (A). Superposition of the AMPPCP fragment (monomer B (slate)) with ADP in the trithymine-ADP complex (pink, 2D7D.pdb) together with key residues that constitute the ATP binding site. Both structures are highly isomorphous in this region. **(B).** 2Fo-Fc omit map density (contoured at 1.5σ) for nucleotides A2-G5, the loop spanning amino acids His476-Lys480 and Arg506. **(C)** Superposition of the UvrB-stem loop DNA from pdb entry 2FDC.pdb (DNA yellow, protein paleyellow) with monomer A of the UvrB dimer (DNA slate, protein cyan) in the vicinity of the β-hairpin reveals that although base flipping has occurred in both structures, the nucleotides are not in equivalent positions (T7 in the dimer and G17 in the stem loop structure) and make distinct interactions with UvrB. Most notably, Phe249 whose aromatic moiety stacks against the guanine base of G17, sterically clashes with the thymine base of T7 due to re-positioning of domain 2.



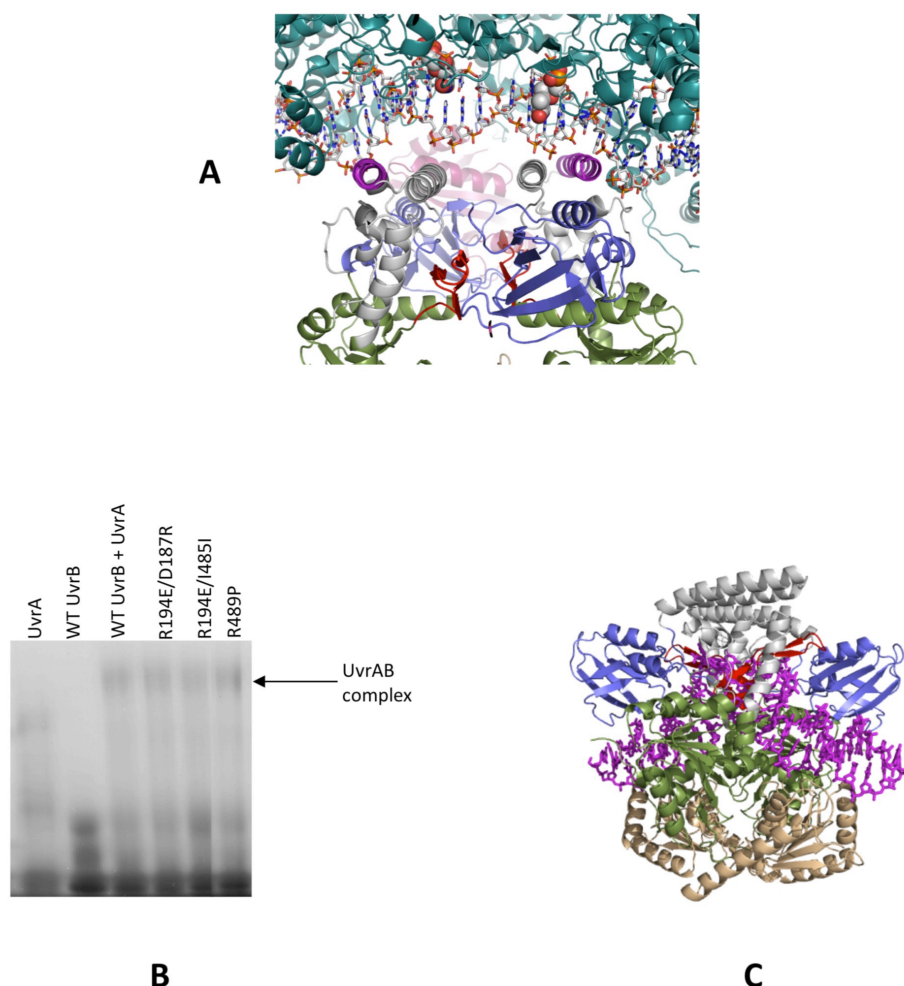


Supplemental 2 (A). Sequence alignment of residues comprising the β-hairpin, domain2 and domain 3 that each contribute to the dimer interface. Green horizontal lines denote the locations of residues within these domains that mediate protein-protein interactions. Sequence alignments were performed using clustalw (http://www.ebi.ac.uk/Tools/services/web_clustalw2) and the figures generated in ESPript (http://espript.ibcp.fr/ESPript/ESPript/index.php) **(F)**. Superposition of monomer A in our dimer (cyan) with the B.subtilis trithymine-ADP co-ordinates (2D7D.pdb, pink) over all equivalent atoms. Both structures are highly homologous with the exception of domain 2 that undergoes a rigid body rotation of ~30° and substantial re-modeling (highlighted by a red elipse).



Supplemental 3(A.) UvrA-UvrB gel shift with the T50 duplex showing that the T481C mutant has wild-type handover activity. **(B)** UvrB cross-linking experiment performed in the presence of Grb2. The absence of a Grb2-UvrB intermediate band confirms that formation of $UvrB_2X$ cannot be attributed to non-specific association. **(C)** DEER spectrum, raw data (black line) and 3-dimensional background fit (red line) acquired from the spin-labeled T481C mutant. **(D)** Background-corrected dipolar evolution (black line) derived by dividing the raw data in **(C**, black line**)** by the background fit in **(C,** red line). The fit **(D,** red line**)** was obtained using Tikhonov regularization. **(E)** Associated L-curve from the fit in **(D)**. The distance distribution in

Figure 5B (main text) is taken at the position marked with the red filled circle where α = 100.



Supplemental 4(A). A putative $UvrA_2UvrB_2$-DNA complex based on our dimer docked M.tuberculosis $UvrA_2B_2$ model and the reported T.maritima UvrA-DNA structure. The DNA has been slightly bent at its extremities and the UvrA insertion domains re-positioned (as proposed) to relieve major steric clashes. In this configuration, the helices spanning amino acids 257-277 ((highlighted in magenta), that encompass Glu266, an essential DNA binding residue) straddle the two centrally located T-fluorescein nucleotides, depicted as van der Waals spheres, in the UvrA dimer interface and are well positioned to mediate protein-DNA contacts with nucleotides in the major grooves directly adjacent to each lesion. The 257-277 helix forms part of domain 1b and is located behind the β-hairpin (red), an essential element for handover. **(B)** A native PAGE gel demonstrating that the R194E/D187R, R194E/I485E and R489P mutants all have wild-type affinity for UvrA and are thus unlikely to be unfolded. **(C)** Model of a $UvrB_2$-DNA complex illustrating how DNA “wrapping” might be achieved.