STRUCTURAL BIOLOGY

Volume 79 (2023)

Supporting information for article:

The LH-DH module of the bacterial replicative helicases is the common binding site for DciA and other helicase loaders

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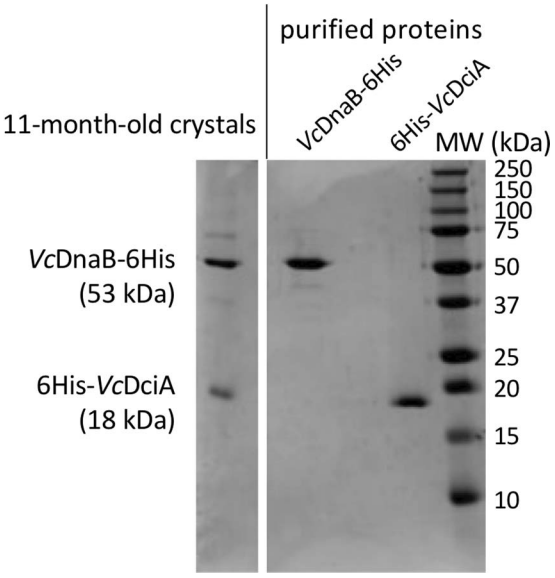

Figure S1 Validation of the protein composition of the *Vc*DnaB•*Vc*DciA complex crystals. 11-month-old crystals of the complex were visualized by SDS-PAGE and Coomassie Blue staining. Purified *Vc*DnaB and *Vc*DciA proteins were migrated on the same gel as controls, showing that the crystallized proteins were protected from proteolysis. The crystals of the complex obtained in our study grew in 5 days and were formed from the two full-length proteins.

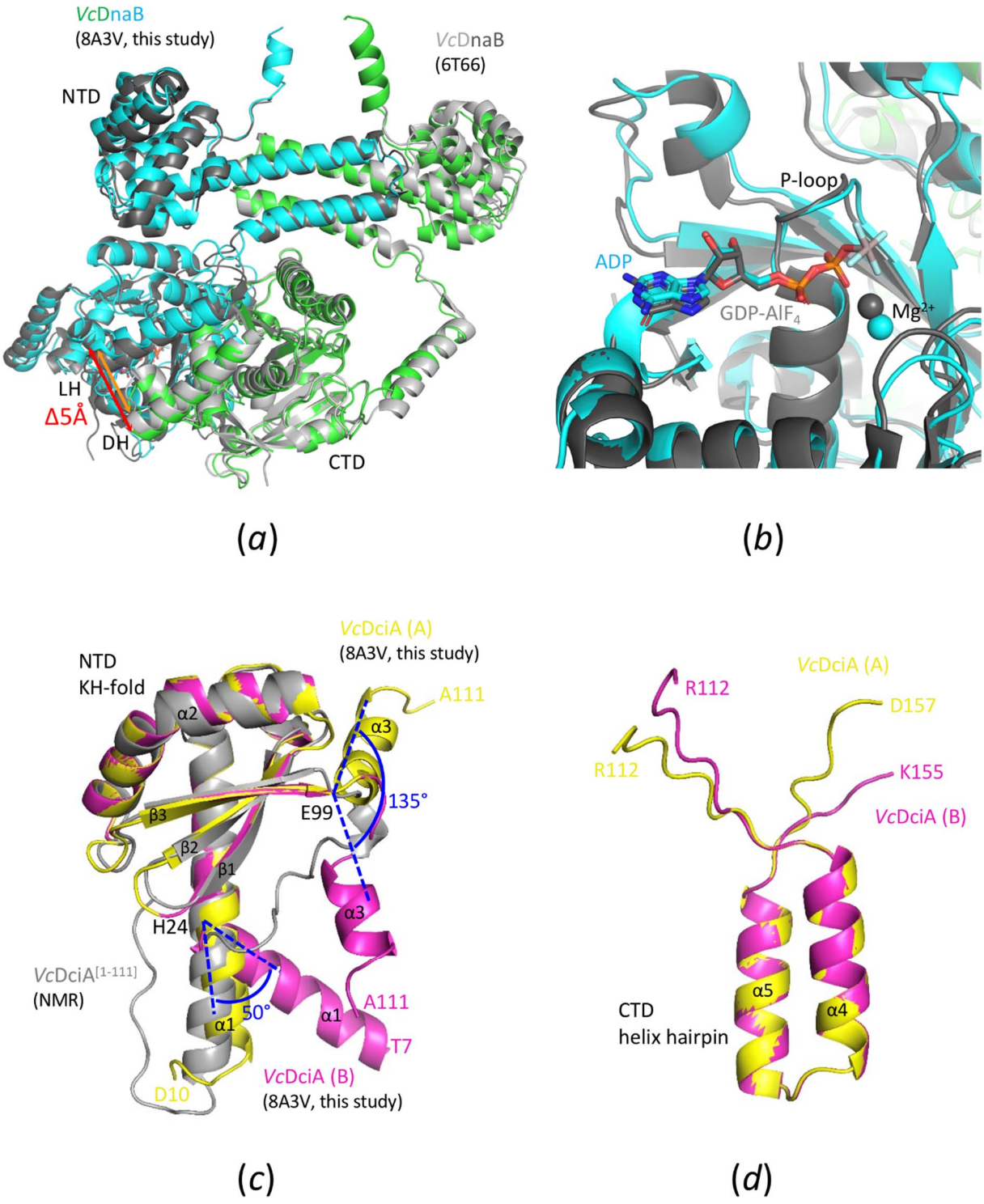

Figure S2 Interaction with *Vc*DciA does not alter the overall structure of the *Vc*DnaB dimer but induces folding of the *Vc*DciA CTD into a helix hairpin. **(*a*)** Superimposition of the ADP-bound *Vc*DnaB dimer extracted from the complex with *Vc*DciA (cyan and green, PDB ID 8A3V, this study) with a GDP-bound *Vc*DnaB dimer extracted from the hexameric helicase ring (gray, PDB ID 6T66). The two structures of *Vc*DnaB are almost identical (global RMSD of 1.66 Å for 858 aligned residues). However, the maximum distance between the Cα atoms of the LH and DH helices is increased by about 5 Å in the presence of *Vc*DciA (compare double red arrow with double orange arrow). **(*b*)**

Zoom-in on the NTP binding site of the two superimposed *Vc*DnaB dimer structures (same colours as in (*a*)). The P-loops are perfectly superimposable, as well as the ADP (blue sticks) and the GDP-AlF_4 (gray sticks), and also the Mg^{2+} ions (cyan and gray spheres). **(*c*)** Superimposition of the two *Vc*DciA NTDs (yellow and magenta, PDB ID 8A3V, this study) extracted from the *Vc*DnaB•*Vc*DciA complex with the NMR structure of the isolated NTD of *Vc*DciA (in gray, BMRB ID 27689). The KH-like fold of the *Vc*DciA NTDs in the complex is very similar to the *Vc*DciA$^{[1\text{-}111]}$ NMR solution structure (all-atom RMSD of 1.2 Å for 78 aligned residues). The two copies of *Vc*DciA NTD in the heterotetrameric structure are identical (all-atom RMSD of 0.5 Å for 78 aligned residues), except for the first long α1 helix (bend at residue H24 in one copy, with an angle of 50° as illustrated by blue lines) and the last α3 helix (oriented almost oppositely in the two copies with an angle of 135° as illustrated by blue lines). This reflects a certain flexibility of *Vc*DciA which was predicted by previous molecular dynamics analyses (Marsin *et al.*, 2021). **(*d*)** Superimposition of the two *Vc*DciA CTDs (same colours as in (*c*)) extracted from the complex with *Vc*DnaB. The *Vc*DciA CTD, which was shown disordered in solution by SAXS (Marsin *et al.*, 2021), folds into a helix hairpin upon interaction with the *Vc*DnaB dimer (contact with the LH-DH module of the helicase). The two copies of *Vc*DciA CTD are identical (all-atom RMSD of 0.19 Å for 35 aligned residues).

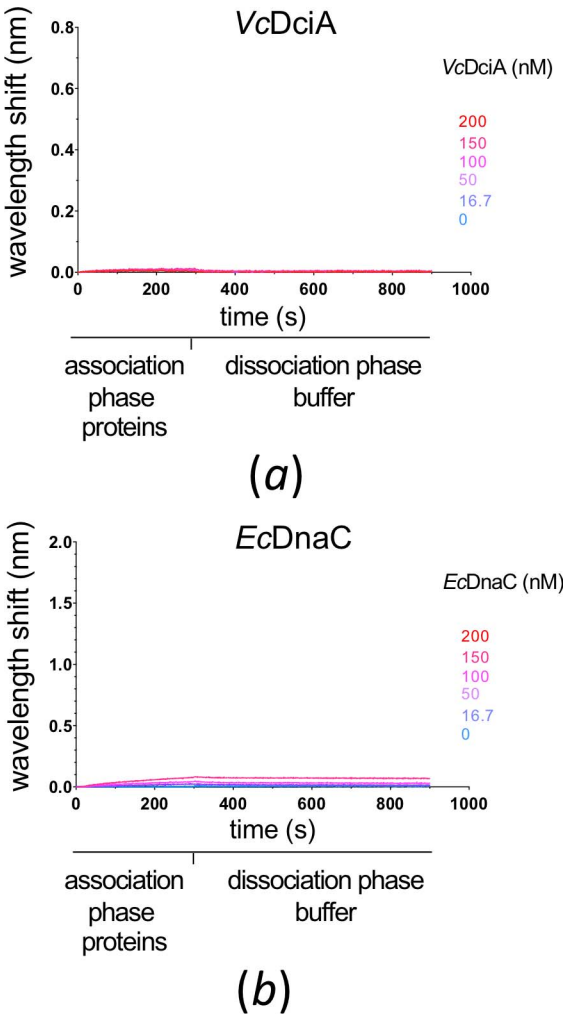

Figure S3 Interaction with ssDNA is detectable for neither **(*a*)** *Vc*DciA nor **(*b*)** *Ec*DnaC under the same experimental conditions used for the helicase loading assays in this study. The Bio-layer interferometry (BLI) analysis of the two loaders alone (from 0 to 200 nM in subunits, in blue to red), is performed as described in Fig. 3 and in the Materials and methods section.

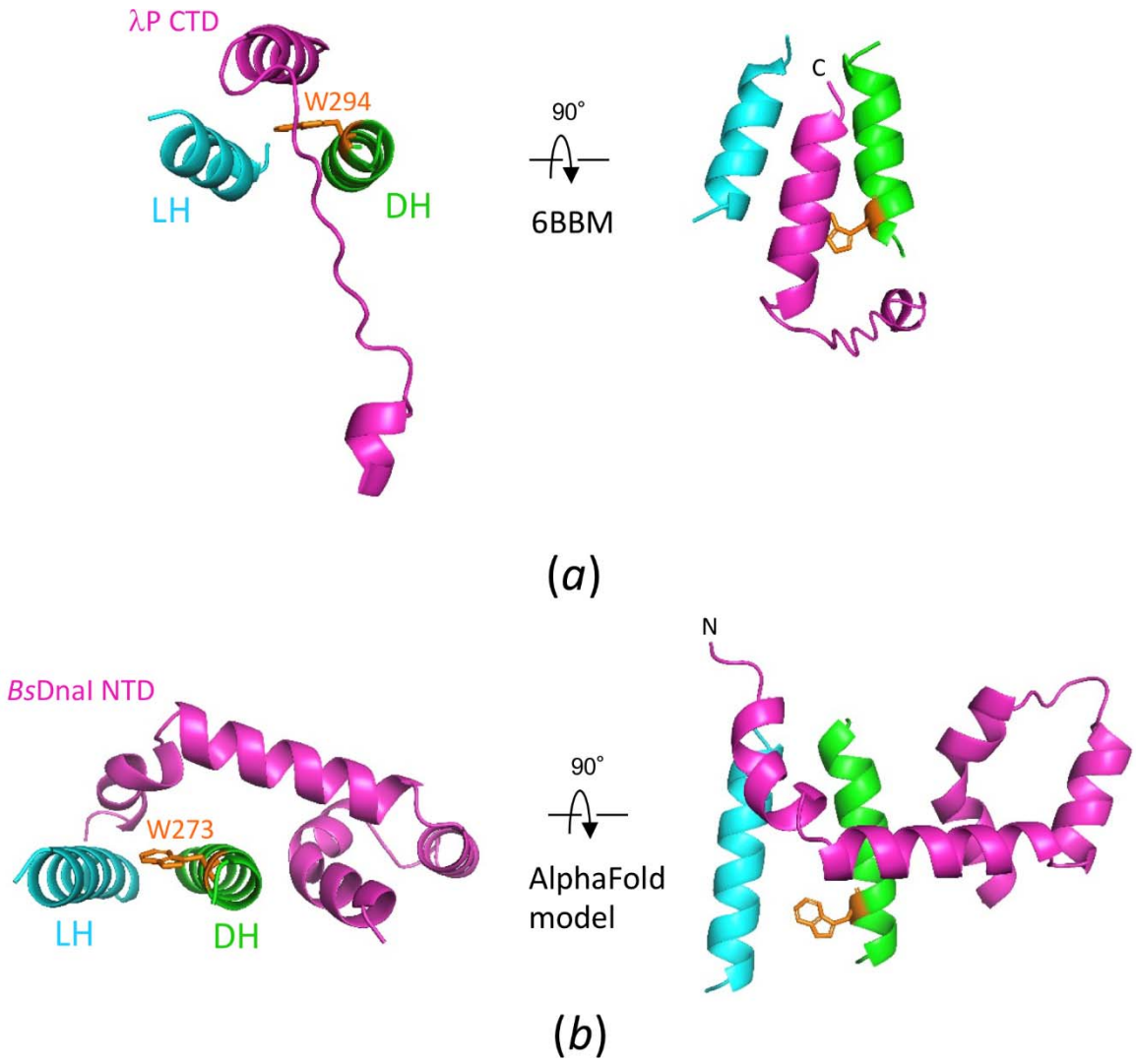

Figure S4 The LH-DH module of bacterial replicative helicases is the common binding site for unrelated helicase loaders. **(*a*)** Zoom-in of the interaction interface between the two-helix LH-DH module of *Ec*DnaB (in blue and green) and λP CTD (in magenta, PDB ID 6BBM). **(*b*)** Zoom-in of the interaction interface between the LH-DH module of *Gst*DnaB (in blue and green) and *Bs*DnaI NTD (in magenta, model of the *Gst*DnaB•*Bs*DnaI complex generated by AlphaFold-Multimer (Mirdita *et al.*, 2022, Evans *et al.*, 2022). The close-up orthogonal views and the color code are the same as in Fig. 2*b*. The conserved tryptophans in the DH are in orange sticks.