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

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

3

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12

13  
14 **RUNNING TITLE**

15 Crystal structure of DnaB•DciA complex

16

17 **KEYWORDS**

18 DnaB•DciA complex / structural study / Interaction module / replicative helicase hijacking

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## 26 **Abstract**

27 During the initiation step of bacterial genome replication, replicative helicases depend on  
28 specialized proteins for their loading onto *oriC*. DnaC and DnaI were the first loaders  
29 characterized. However, most bacteria do not contain any of these genes, which are  
30 domesticated phage elements that replaced the ancestral and unrelated loader gene *dciA*  
31 several times during evolution. To understand how DciA assists the loading of DnaB, we  
32 determined the crystal structure of the complex from *Vibrio cholerae*, in which two VcDciAs  
33 interact with a dimer of VcDnaB, without changing its canonical structure. Our data showed  
34 that the VcDciA binding site on VcDnaB is the conserved module formed by the linker helix  
35 LH of one monomer and the determinant helix DH of the second one. Interestingly, DnaC  
36 from *Escherichia coli* also targets this module onto EcDnaB. Thanks to their common target  
37 site, we showed that VcDciA and EcDnaC could be functionally interchanged *in vitro*, despite  
38 sharing no structural similarities. This is a milestone in understanding the mechanism  
39 employed by phage helicase loaders to hijack bacterial replicative helicases during evolution.

40

## 41 **Introduction**

42 The replication of the circular bacterial chromosome is an essential step for bacterial division.  
43 The initiator protein DnaA initiates the replication by binding onto the origin DNA *oriC*, and  
44 unwinds locally the double-strand DNA by polymerization (Costa, Hood et al., 2013, Leonard  
45 & Méchali, 2013, Zawilak-Pawlik, Nowaczyk et al., 2017). Then, the toroidal hexameric  
46 helicase DnaB is loaded onto the locally open DNA duplex, with the help of a helicase loader,  
47 triggering the recruitment of the various proteins of the replisome (O'Donnell, Langston et al.,  
48 2013). ATP-dependent 5' to 3' translocation of the helicase ahead of the advancing replisome  
49 allows unwinding DNA duplex into templates for new DNA synthesis (Strycharska, Arias-  
50 Palomo et al., 2013).

51 Recruitment and loading of the replicative helicase depend on a loader protein, which  
52 was characterized in the two model organisms *Escherichia coli* (*Ec*) and *Bacillus subtilis* (*Bs*),  
53 leading to the description of two loading strategies. In *Bacillus subtilis*, the helicase loader  
54 DnaI assists the assembly of six monomers of the helicase to form an active hexameric ring  
55 around DNA according to a “ring-maker” scenario (Davey & O'Donnell, 2003, Velten,  
56 McGovern et al., 2003). The *GstDnaB*•*BsDnaI*•*GstDnaG* prepriming complex exhibits a  
57 three-layered planar and dilated ring conformation with one hexameric helicase binding to  
58 three loader protein dimers and three primase proteins (Liu, Eliason et al., 2013). In the  
59 *Escherichia coli* system, the helicase loader DnaC mediates the opening of the DnaB hexamer  
60 into a loading-competent cracked-open ring according to a “ring-breaker” scenario (Arias-  
61 Palomo, Puri et al., 2019, Nagata, Okada et al., 2020). The *EcDnaB*•*EcDnaC* complex is  
62 dodecameric with six subunits of each protein and the complex assembles into a three-tier  
63 spiral. *EcDnaC* makes *EcDnaB* contacts through the first small  $\alpha$ -helix (15 residues long) of  
64 its extended N-terminal domain (NTD). This helix interacts with the DnaB module composed  
65 of the “linker helix” (LH) of one DnaB protomer and of an antiparallel  $\alpha$ -helix of the adjacent  
66 DnaB protomer, which we named “determinant helix” (DH) in a previous study (Marsin,  
67 Adam et al., 2021), and was named “docking helix” in a recent review comparing the  
68 convergent functional mechanisms of *EcDnaC* and bacteriophage  $\lambda$  P loaders (Chase, Berger  
69 et al., 2022). This forms a three  $\alpha$ -helix bundle, which fixes the relative orientation of the two  
70 adjacent DnaB C-terminal domains (CTDs): the 6 DnaC thus latched onto the DnaB hexamer  
71 adopt a spiral configuration that causes a distortion in the helicase ring, resulting in its large  
72 opening as a means to allow single-strand DNA (ssDNA) to enter the helicase pore (Arias-  
73 Palomo et al., 2019, Nagata et al., 2020).

74 Yet, the *DnaC/I* loader distribution is marginal in the bacterial domain. It was  
75 established phylogenetically that *dnaC/I* genes are domesticated phage elements that replaced

76 several times through the evolution, the ancestral bacterial gene *dciA* (Brézellec, Vallet-Gely  
77 et al., 2016). DciA and DnaC/I are not related by either their sequence or their structure  
78 (Marsin et al., 2021). While the DnaC/I CTD contains an AAA+ ATPase RecA-like domain  
79 (Koonin, 1992), the DciA NTD folds as a KH domain (Grishin, 2001, Marsin et al., 2021)  
80 also shared by the domain I of DnaA and the domain V of DnaX both described to interact  
81 with DnaB (Haroniti, Anderson et al., 2004, Jameson, Rostami et al., 2014, Mary Rajathej &  
82 Selvaraj, 2013). Through multiple complementary approaches, we previously established that  
83 the disordered CTD of DciA can form transiently small helical structures (Chan-Yao-Chong,  
84 Marsin et al., 2020) and is necessary for interacting with DnaB and stimulating DnaB loading  
85 onto DNA (Chan-Yao-Chong et al., 2020, Marsin et al., 2021). The direct interplay between  
86 the two proteins has also been demonstrated using the variation of intrinsic fluorescence of  
87 the conserved tryptophan residue located in the middle of the DH of DnaB, in the presence of  
88 DciA (Marsin et al., 2021). It was therefore suspected that DciA interacts with the helicase  
89 nearby the DH helix of its module.

90 To decipher the molecular interactions between DciA and DnaB, we solved the crystal  
91 structure of the DnaB•DciA complex from *Vibrio cholerae* (*Vc*) together with ADP and Mg<sup>2+</sup>,  
92 forming a heterotetramer, composed of the canonical *Vc*DnaB dimer and of two molecules of  
93 *Vc*DciA. Interestingly, *Vc*DciA interacts with *Vc*DnaB through the LH-DH module, like  
94 *Ec*DnaC and the phage λ P helicase loader on *Ec*DnaB, suggesting a functional link between  
95 the different systems. Furthermore, we showed that *Vc*DciA and *Ec*DnaC are interchangeable  
96 for the *in vitro* loading of the helicases from *V. cholerae* and *E. coli*, demonstrating that the  
97 substitution of *dciA* by *dnaC* triggered only local adaptation during evolution. But the  
98 *Vc*DnaB•*Vc*DciA•ADP:Mg<sup>2+</sup> structure also revealed that DciA binds to the periphery of the  
99 helicase CTD contrary to other known loaders that are positioned at the back of the DnaB

100 CTD ring, presuming that its helicase loading mechanism is different from those of DnaC/I  
101 and  $\lambda$  P.

102

## 103 **Results and discussion**

### 104 **The crystal structure of the *VcDnaB*•*VcDciA* complex forms a heterotetramer with a 2:2** 105 **stoichiometry**

106 We have previously demonstrated by functional studies that DciA from *Vibrio cholerae* (*Vc*)  
107 increases the loading of *VcDnaB* on DNA, resulting in an increased unwinding activity of the  
108 helicase (Marsin et al., 2021). To understand the molecular interplay between the two  
109 proteins, we determined the crystal structure of the *VcDnaB*•*VcDciA*•ADP:Mg<sup>2+</sup> complex  
110 (PDB ID 8A3V, this study). The structure was solved by molecular replacement (see  
111 [Materials and Methods and Table 1](#)) using the *VcDnaB*•GDP:AlF<sub>4</sub>:Mg<sup>2+</sup> crystal structure  
112 (PDB ID 6T66) (Marsin et al., 2021), the *VcDciA*<sup>[1-111]</sup> NMR structure (BMRB ID 27689)  
113 (Marsin et al., 2021) and the full-length *VcDciA* model predicted by AlphaFold2 (Jumper,  
114 Evans et al., 2021), and refined to 2.9 Å resolution. The accuracy of the final model was  
115 further verified by superimposition on an experimental electron density map obtained at 3.7 Å  
116 resolution by single-wavelength anomalous diffraction (SAD) using derivative data from a  
117 (Ta<sub>6</sub>Br<sub>12</sub>)<sup>2+</sup>-cluster-soaked crystal (see [Materials and Methods and Table 1](#)).

118 The asymmetric unit of the crystal contains two molecules of *VcDnaB* and two  
119 molecules of *VcDciA*. The crystal structure of the *VcDnaB*•*VcDciA* complex revealed domain  
120 swapping between symmetry related molecules of *VcDciA* ([Figure 1A](#)). The NTD and CTD  
121 (N-terminal and C-terminal domains) of the two *VcDciA* connected by an extended hinge  
122 region (residues E99 to S121) are exchanged between neighboring molecules related by a true  
123 crystallographic 2-fold rotation axis. It is not known at present whether this domain swapping  
124 is due to a crystal artifact or whether it is biologically relevant. However, it is known that

125 replication is bidirectional and therefore two helicases must be recruited to the replication fork  
126 (Chodavarapu & Kaguni, 2016, Hayashi, Miyazaki et al., 2020). The ability of DciA to link  
127 helicases together via this domain swapping could therefore improve the recruitment of  
128 helicases to *oriC* and thus optimize the replication initiation step.

129         The *VcDnaB•VcDciA* heterotetramer structure can be reconstructed from the swapped  
130 domains between symmetric *VcDciA*s (Figure 1B) and exhibits two *VcDciA* fixed on a  
131 *VcDnaB* dimer. The *VcDnaB* dimer in the heterotetrameric complex is bound to ADP:Mg<sup>2+</sup>  
132 and is almost identical to one *VcDnaB* dimer of the GDP-bound *VcDnaB* hexamer structure  
133 (PDB ID 6T66 (Marsin et al., 2021)), with an overall RMSD of 1.76 Å (RMSD of 0.99 Å for  
134 the NTDs and 1.26 Å for the CTDs) (Figure supplement 1A). The NTP:Mg<sup>2+</sup> binding site into  
135 the CTDs is very similar, the P-loop is in the same conformation (Figure supplement 1B).  
136 Therefore, the formation of the complex with *VcDciA* does not alter the overall canonical  
137 architecture of the *VcDnaB* dimer nor the NTP binding site. Nevertheless, the binding of  
138 *VcDciA* on the LH-DH module of *VcDnaB* slightly accentuates the gap between the LH and  
139 DH helices of about 5 Å, relatively to the module in the free *VcDnaB* hexamer structure  
140 (Figure supplement 1A). The two *VcDciA* of the heterotetrameric complex are practically  
141 identical to each other (RMSD of 0.48 Å for the NTDs and 0.20 Å for the CTDs), except for  
142 the first long  $\alpha 1$  helix which is straight in the first *VcDciA* molecule and kinked with an angle  
143 of 50° at residue H24 in the second one (Figure supplement 1C). This kink can be explained  
144 because of a steric hindrance with the extended hinge of the neighboring symmetric *VcDciA*  
145 molecule with which they are engaged in domain swapping. This possibility of bending the  
146 long  $\alpha 1$  helix of *VcDciA* was predicted by our previous molecular dynamics analyses (Chan-  
147 Yao-Chong et al., 2020). The NTD domain of *VcDciA* in this crystal structure exhibits a KH-  
148 like fold very similar to the *VcDciA*<sup>[1-111]</sup> structure (BMRB 27689 (Marsin et al., 2021)) we  
149 obtained by NMR (RMSD of 1.28 Å, Figure supplement 1C). However, the CTD tail of

150 *VcDciA*, observed as disordered in solution by SAXS (Marsin et al., 2021), folds into a small  
151 helix hairpin in contact with *VcDnaB* (Figure supplement 1D), again in agreement with our  
152 previous molecular dynamics analyses (Chan-Yao-Chong et al., 2020). The structure of the  
153 extended hinge region consisting of the last helix of the NTD and the proline-rich flexible  
154 linker, which connects the NTD to the CTD of *VcDciA*, is only putative in this model,  
155 reconstructed from the swapped domains.

156 The two *VcDciA* molecules interact "*in trans*" with the *VcDnaB* dimer at the periphery  
157 of the CTDs (Figure 1B). The CTD hairpin helix of one *VcDciA* molecule stacks entirely on  
158 the LH-DH module of *VcDnaB* (in magenta in Figure 1C). The kinked  $\alpha 2$  helix of the NTD of  
159 the second *VcDciA* (in yellow in Figure 1C) interacts with the first helix of the CTD hairpin  
160 of the first *VcDciA* (approximately  $238.0 \text{ \AA}^2$  of "*in trans*" interaction interface between the  
161 CTD and NTD from two different *VcDciA*s, as measured by PDBePISA server (Krissinel &  
162 Henrick, 2007)) and also with the DH helix of *VcDnaB*. The assembly forms a 5-helix bundle  
163 (Figure 1C). In addition to the kinked  $\alpha 2$  helix, the  $\beta 3$  strand of the *VcDciA* NTD also  
164 interacts at the periphery of the *VcDnaB* CTD, particularly with the DH helix. This DH helix  
165 (determinant/docking helix) is thus at the heart of the interaction between *VcDciA* and  
166 *VcDnaB*, as proposed by our previous Tycho experiments (Marsin et al., 2021). The overall  
167 interaction interface of a *VcDciA* molecule with a *VcDnaB* dimer is about  $1264.4 \text{ \AA}^2$ , with  
168 about  $514.8 \text{ \AA}^2$  for the NTD of *VcDciA* and about  $749.6 \text{ \AA}^2$  for its CTD (from PDBePISA  
169 server (Krissinel & Henrick, 2007)). The structure of the complex therefore confirmed the  
170 essential role played by the CTD of *VcDciA* in the interaction with *VcDnaB* as shown by our  
171 previous ITC experiments (Chan-Yao-Chong et al., 2020). Finally, it should be noted that the  
172 NTD of the *VcDciA* molecule with a kinked  $\alpha 1$  helix is less well-defined in the density, which  
173 can be explained by the fact that its interaction interface with the CTD of *VcDnaB* is only  
174 about  $346.9 \text{ \AA}^2$  versus  $514.8 \text{ \AA}^2$  for the *VcDciA* NTD with a straight  $\alpha 1$  helix. This binding

175 difference between the NTDs of the two *VcDciA*s is likely caused by different steric  
176 constraints due to crystal packing and/or related to domain swapping.

177

178 **Both *VcDciA* and *EcDnaC* loaders target the conserved LH-DH module of DnaB**  
179 **helicases and are functionally interchangeable *in vitro***

180 The LH-DH module is conserved in DnaB helicases (Chase et al., 2022). However, we have  
181 previously identified a residue in the DH helix that discriminates DciA helicases from DnaC/I  
182 helicases (respectively a serine and a glycine) (Marsin et al., 2021) (Figure 2A). Moreover,  
183 the *VcDciA* and *EcDnaC* loaders have no sequence or structural similarity. Yet, the two  
184 loaders target the same binding site on the helicase: the conserved LH-DH module of DnaB,  
185 although the interaction interfaces are quite different (Figure 2B). This interaction involves a  
186 single small  $\alpha$ -helix of the NTD end of *EcDnaC*, forming a 3-helix bundle, whereas in  
187 *VcDciA*, the CTD helix hairpin and the elbow of the kinked  $\alpha$ 2 helix of NTD participate both  
188 in, forming a 5-helix bundle. We can wonder at this stage if this common target on DnaB, the  
189 LH-DH module, allows a cross-talk between the two helicase-loader systems, despite the  
190 specificities of each of them.

191 Using Tycho nano-DSF technology, the fluorescence variation of tryptophan residues  
192 can be followed in real time along thermal ramp (see Materials and Methods). Ideally, a  
193 conserved tryptophan is located in the DH helix, namely W291 for *VcDnaB* and W294 for  
194 *EcDnaB* (Figures 2A and 2B) (Marsin et al., 2021). A second conserved tryptophan is located  
195 in the globular head of the NTD domain of DnaB (positions 45 and 48 in *VcDnaB* and  
196 *EcDnaB*, respectively), but is buried and cannot participate in any protein-protein interactions.  
197 *VcDciA* does not contain any tryptophan in its sequence. We previously demonstrated the  
198 binding of *VcDciA* in proximity of the LH-DH module of *VcDnaB* using this technology  
199 (Marsin et al., 2021), showing that W291 is inaccessible to solvent when *VcDnaB* interacts

200 with *VcDciA*. We further showed that *VcDciA* deleted from its CTD (*VcDciA*<sup>[1-111]</sup>) can no  
201 longer bind *VcDnaB* (Figure 2C left), as expected if the CTD of *VcDciA* covers the DH  $\alpha$ -  
202 helix of *VcDnaB* (Figure 2B). On the other hand, *EcDnaB* contains a third non-conserved  
203 tryptophan residue, solvent-exposed in its CTD. *EcDnaC* also encloses three solvent-exposed  
204 tryptophan residues in its sequence, one in its NTD extended end (W32 in Figure 2B) and two  
205 in its CTD. All these tryptophan residues could therefore participate in protein-protein  
206 interactions. Indeed, Tycho allowed to confirm the interaction between *EcDnaB* and *EcDnaC*  
207 by comparing the lower initial 350 nm / 330 nm ratio for the measured curve (in red, Figure  
208 2D left) than for the theoretical curve representing the absence of solvent protection (in black,  
209 Figure 2D left). In addition, *EcDnaC* deleted from its NTD (*EcDnaC*<sup>[53-end]</sup>) can no longer  
210 bind *EcDnaB* (Figure 2D left), as expected if the  $\alpha 1$  helix of *EcDnaC* forms a 3-helix bundle  
211 with the LH-DH module of *EcDnaB* (Figure 2B). These findings are in agreement with the  
212 3D structures of the two complexes: the interaction takes place at the DH of DnaB and  
213 requires the CTD for DciA and the NTD for DnaC. We further carried out crossover  
214 experiments with the heterologous *VcDnaB/EcDnaC* and *EcDnaB/VcDciA* systems. We  
215 showed efficient non-cognate helicase-loader interactions, which also require the NTD of  
216 *EcDnaC* (Figure 2C right) and the CTD of *VcDciA* (Figure 2D right).

217 We then investigated if these interactions are relevant for the stimulation of the  
218 helicase loading on DNA by the loaders. We attached a 3'-biotinylated 50-mer ssDNA to a  
219 streptavidin-coated probe to measure interactions using the bio-layer interferometry (BLI, see  
220 Materials and Methods). We monitored in real-time interactions between immobilized ssDNA  
221 and the helicases in the presence of different concentrations of loaders (Figure 3). The BLI  
222 experiments confirmed the results already observed by SPR (Marsin et al., 2021), namely that  
223 the loading stimulation of both helicases increases with the concentration of added cognate  
224 loader, *VcDciA* or *EcDnaC* (Figures 3A and 3B left), at concentrations for which the response

225 for loaders alone is negligible (Figure supplement 2). Moreover, on the same conditions,  
226 *EcDnaC* is able to load *VcDnaB* onto the ssDNA, and *VcDciA* to load *EcDnaB* (Figures 3A  
227 and 3B right). The cross-talk is effective *in vitro*, there seems to be a functional convergence  
228 between the two systems. *EcDnaB* apparently had little need to evolve to accommodate  
229 DnaC, after DciA was replaced. This could explain why the replacement of DciA by DnaC/I  
230 occurred at least 7 times during evolution, and how phage loaders were able to hijack  
231 bacterial replicative helicases efficiently (Brézellec et al., 2016).

232

233 ***VcDciA* binds to the periphery of the *VcDnaB* CTD, in contrast to other loaders, which**  
234 **are positioned at the back of the helicase CTD ring**

235 Our previous SEC-SAXS and SEC-MALS experiments showed that a complex between the  
236 *VcDnaB* hexamer and *VcDciA* is formed in solution *in vitro* but is not stable and tends to  
237 dissociate, which did not allow us to determine the precise stoichiometry of this complex  
238 (Marsin et al., 2021). Interestingly, the *H32* symmetry of the crystal reconstitutes the  
239 heterododecamer complex by the assembly of the heterotetramer with two neighboring  
240 symmetry mates related by a true crystallographic 3-fold rotation axis (Figure 4A). Further  
241 work remains to be done to find out if this heterododecameric structure is biologically  
242 relevant or not, but in the meantime, we can compare it with other helicase-loader structures  
243 in the literature. Three 3D structures of helicase-loader complexes are currently known:  
244 *EcDnaB*•*EcDnaC* (PDB ID 6QEL (Arias-Palomo et al., 2019)), *EcDnaB*• $\lambda$ P (PDB ID 6BBM  
245 (Chase, Catalano et al., 2018)) and *GstDnaB*•*BsDnaI* (PDB ID 4M4W (Liu et al., 2013)).  
246 Strikingly, *VcDciA* binds to the periphery of the *VcDnaB* CTD, in contrast to the other three  
247 loaders, which are positioned at the back of the helicase CTD ring (Figure 4B). The loading  
248 mechanism used by DciA has still to be elucidated but we can assume that it will probably be  
249 different from those already described for the other three loaders.

250

## 251 **Concluding remarks**

252 The two genes coding for DciA and DnaC/I are unrelated and mutually exclusive in the  
253 bacterial genomes (Brézellec, Petit et al., 2017, Brézellec et al., 2016). However, like DnaC  
254 and the bacteriophage  $\lambda$  P helicase loader (Arias-Palomo et al., 2019, Chase et al., 2022,  
255 Chase et al., 2018), DciA interacts with the two-helix LH-DH module of DnaB. This “binding  
256 module”, conserved in the bacterial replicative helicases, suggests a functional link between  
257 the different systems. Moreover, *EcDnaB*, although likely to have evolved to accommodate  
258 the replacement of DciA by DnaC during evolution, retains the ability to be loaded by both  
259 loaders *in vitro*. Nevertheless, the structural data on the *VcDnaB*•*VcDciA*•ADP:Mg<sup>2+</sup> complex  
260 provided here permit us to postulate that DciA is probably not a helicase loader of the “ring-  
261 maker” or the “ring-breaker” types, strictly speaking, and that its action boosts the loading of  
262 the replicative helicase through a new mechanism that remains to be elucidated.

263

## 264 **Materials and methods**

### 265 **Protein samples preparation**

266 *VcDnaB*, *EcDnaB*, *VcDciA*, *VcDciA*<sup>[1-111]</sup> and *EcDnaC* were overexpressed in *E. coli* and  
267 purified as described in (Marsin et al., 2021). *EcDnaC*<sup>[53-end]</sup> was purified as *EcDnaC*. Strains  
268 and plasmids are available upon request.

269

### 270 **Crystal structure determination of the *VcDnaB*•*VcDciA*•ADP:Mg<sup>2+</sup> complex**

271 Purified *VcDnaB* was preincubated for 10 min at 4°C at a concentration of 0.115 mM  
272 (monomer) with 2 mM ADP + 5 mM MgCl<sub>2</sub>. Purified *VcDciA* was added at a final  
273 concentration of 0.138 mM (about 7 monomers of DciA per helicase hexamer) for a second  
274 step of incubation. Native protein crystals were grown in sitting drops by mixing the proteins

275 with the reservoir solution in a 1:1 ratio. Rhombohedral crystals of the  
276 *VcDnaB*•*VcDciA*•ADP:Mg<sup>2+</sup> complex appeared after 5 days at 18°C in 0.1 M sodium acetate  
277 (pH 4.8 - 5.6) + 0.7 to 0.9 M potassium/sodium tartrate. For derivatization, single crystals  
278 were then soaked for 2 hours at 18°C in a solution containing 1 mM (Ta<sub>6</sub>Br<sub>12</sub>)<sup>2+</sup> cluster (JBS  
279 Tantalum Cluster Derivatization Kit from Jena Bioscience GmbH, Jena, Germany). 25%  
280 glycerol cryo-protected native crystals or 50/50 paratone/paraffin oil mixture cryo-protected  
281 derivative crystals were flash-frozen in liquid nitrogen.

282 Diffraction data collection, phasing and refinement statistics are given in [Table 1](#).  
283 Native and derivative crystallographic data were collected on the PROXIMA-2A and  
284 PROXIMA-1 beamlines, respectively, at the SOLEIL Synchrotron (Saint-Aubin, France) and  
285 processed with XDS (Kabsch, 2010) through XDSME (<https://github.com/legrandp/xdsme>)  
286 (Legrand, 2017). The strong diffraction anisotropy was corrected using the STARANISO  
287 server (<http://staraniso.globalphasing.org>) (Tickle, 2018). The *VcDnaB*•*VcDciA*•ADP:Mg<sup>2+</sup>  
288 complex crystal structure was solved by molecular replacement (MR) with the MOLREP  
289 program (Vagin & Teplyakov, 2010) using the X-ray structures of the NTD [22-175] and  
290 CTD [200-461] isolated domains of *VcDnaB*•GDP:AlF<sub>4</sub>:Mg<sup>2+</sup> as search models (PDB ID  
291 6T66 (Marsin et al., 2021)). Two copies of each domain were correctly positioned. The initial  
292 model was then manually corrected and completed using COOT (Emsley, Lohkamp et al.,  
293 2010). Significant extra electron density allowed the manual building of the CTD isolated  
294 domains of two *VcDciA* monomers, whose chains could be assigned using the 3D model of  
295 the full-length *VcDciA* predicted by AlphaFold2 (Jumper et al., 2021) through the ColabFold  
296 server (Mirdita, Schütze et al., 2022). Additional electron density allowed the manual  
297 positioning of the NTD isolated domains of two *VcDciA* monomers, using the NMR structure  
298 of *VcDciA*<sup>[1-111]</sup> solved in (Marsin et al., 2021) (BMRB ID 27689). Finally, manual building  
299 of the linker regions connecting the NTD and CTD domains of *VcDciA* revealed domain

300 swapping between symmetry related molecules of *VcDciA*. The *VcDnaB*•*VcDciA*•ADP:Mg<sup>2+</sup>  
301 complex structure was iteratively improved by manual building steps followed by refinement  
302 cycles using native data to 2.9 Å resolution. The model refinement was conducted with the  
303 BUSTER program (Bricogne, Blanc et al., 2017) using 12 translation–libration–screw (TLS)  
304 motion groups, automated non-crystallographic symmetry (NCS) restraints and local structure  
305 similarity restraints (LSSR) to the target models of the *VcDnaB*•*VcDciA* complex predicted  
306 by AlphaFold2 (Jumper et al., 2021, Mirdita et al., 2022) mirand RoseTTAFold (Baek,  
307 DiMaio et al., 2021).

308 To avoid model bias, an experimental electron density map was obtained at 3.7 Å  
309 resolution by single-wavelength anomalous diffraction (SAD) using derivative data collected  
310 at the tantalum (Ta) peak wavelength. The (Ta<sub>6</sub>Br<sub>12</sub>)<sup>2+</sup> cluster sites were initially found with  
311 SHELXD (Schneider & Sheldrick, 2002), then phases were determined with PHASER  
312 (McCoy, Grosse-Kunstleve et al., 2007) and improved by density modification with the  
313 PARROT program (Cowtan, 2010) through the CCP4 software suite (Winn, Ballard et al.,  
314 2011). Superimposing the MR model on the experimental map confirmed its accuracy, except  
315 for the NTD of the second *VcDciA* monomer for which no density was visible, likely due to a  
316 too sharp solvent flattening. Crystals of *VcDnaB*•*VcDciA*•ADP:Mg<sup>2+</sup> complex were analyzed  
317 by SDS-PAGE and both *VcDnaB* and *VcDciA* were visualized on the gel after Coomassie  
318 Blue staining as full-length proteins without any proteolysis (Figure supplement 3). The  
319 BUSTER program (Bricogne et al., 2017) calculated per-residue values for real-space  
320 correlation of the final refined model against the 2fo-fc map. The NTD of the second *VcDciA*  
321 molecule has an acceptable mean real-space correlation coefficient (RSCC) of about 0.7,  
322 although a bit lower than the mean RSCC of about 0.8 of the NTD of the first *VcDciA* which  
323 is very similar to the overall average RSCC of the whole model. This reflects a difference in

324 flexibility between the NTDs of the 2 *VcDciA* monomers, likely due to crystal packing and  
325 domain swapping constraints.

326 All structural figures were prepared using PyMOL software (<https://pymol.org>)  
327 (DeLano, 2002).

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### 331 **Protein interaction analysis by thermal shift assay and intrinsic fluorescence variation**

332 As described in (Marsin et al., 2021), intrinsic fluorescence changes of tryptophan (and  
333 tyrosine at a lower level) are recorded at 330 and 350 nm while heating the protein sample  
334 from 35 to 95°C at a rate of 3°C/min. The emission profile of tryptophan is shifted to the red  
335 emissions when it is released to the solvent during the thermal denaturing of the protein. We  
336 used Tycho analysis (Tycho NT.6, NanoTemper Technologies GmbH, Munich, Germany) to  
337 follow the interaction between *VcDnaB* or *EcDnaB* and *VcDciA*, *VcDciA*<sup>[1-111]</sup>, *EcDnaC* or  
338 *EcDnaC*<sup>[53-end]</sup>. Interactions were performed in Hepes 50 mM at pH7.5, NaCl 150 mM, ATP 1  
339 mM, with 20 µM of each protein, in capillary tubes of 10 µl. Three to five replicates were  
340 obtained to increase results confidence. To detect binding, we compared the 350/330 nm ratio  
341 of fluorescence of the complex with the predicted ratio we should obtain in absence of  
342 interaction (S brightness at 350 nm / S brightness at 330 nm).

343

### 344 **Measurement of protein-DNA interaction by bio-layer interferometry (BLI)**

345 BLI experiments were conducted using a Pall ForteBio's Octet® RED96e system (Fremont,  
346 CA, USA), using Streptavidin (SA) Biosensors. BLI monitors wavelength shifts (nm)  
347 resulting from changes in the optical thickness of the sensor surface during association or  
348 dissociation of the analyte. All BLI experiments were performed at 30°C under 1000 rpm

349 stirring. The streptavidin biosensor was hydrated in a 96-well plate containing PBS buffer  
350 (Biorad) for at least 10 min before each experiment. 3' biotinylated oligonucleotide oso13 (50  
351 nt long ssDNA, at 10 nM) (Biotin-GCAGGCTCGTTACGTAGCTGTACCG(dT)<sub>25</sub>) was  
352 immobilized in PBS onto the surface of the SA biosensor through a cycle of Baseline (120 s),  
353 Loading (300 s), and Baseline (120 s). Association interactions were then monitored during  
354 300 seconds in wells containing 200  $\mu$ L samples at 100 nM of *VcDnaB* or *EcDnaB* with  
355 different ratios of the indicated loader in buffer HNATM1 (50 mM Hepes pH7, 150 mM  
356 NaCl, 1 mM ATP, 0.1% Tween20, 1 mM MgCl<sub>2</sub>). At the end of each binding step, the  
357 sensors were transferred into a protein-free binding buffer HNATM1 to follow the  
358 dissociation kinetics for 600 s. The sensors can be recycled by dipping in 0.08% SDS for 10  
359 seconds. The experiments have been carried out in duplicate, only one is presented.

360

### 361 **Data Availability**

362 The PDB accession number for the crystal structure of the *VcDnaB*•*VcDciA*•ADP:Mg<sup>2+</sup>  
363 complex reported in this paper is 8A3V.

364

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379

380

381

## 382 **Author Contributions**

383 S.Q.-C. conceived this study; S.Q.-C. performed the cloning; C.C., H.B. and S.Q.-C. purified  
384 and crystallized the proteins; S.M. and M.N. performed the BLI and biochemical assays;  
385 H.W. and P.L. performed the X-ray structure determination and refinement with the  
386 contribution of I.L.S.-G.; H.W., S.M. and S.Q.-C. wrote the paper with input from all authors.

387

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486

## 487 **Figure Legends**

488 **Figure 1.** Crystal structure of the *VcDnaB*•*VcDciA*•ADP:Mg<sup>2+</sup> complex. **(A)** Domain-  
489 swapped heterooctamer. The crystal structure of the *VcDnaB*<sub>2</sub>•*VcDciA*<sub>2</sub> complex revealed  
490 domain swapping between symmetry related molecules of *VcDciA*. Left: Schematic  
491 representation of domain swapping. The NTD and CTD domains of the two *VcDciA*s,  
492 connected by an extended hinge region (dark blue lines), are exchanged between neighboring  
493 molecules related by a true crystallographic 2-fold rotation axis (red dashed line). The four  
494 molecules of *VcDnaB* are in two shades of blue and green, the four molecules of *VcDciA* are  
495 in pink, purple, orange and brown. The four protein chains of the symmetry mate are marked  
496 with an apostrophe. Right: Ribbon representation of the heterooctameric structure, using the  
497 same colour code. **(B)** Structure of the *VcDnaB*•*VcDciA*•ADP:Mg<sup>2+</sup> heterotetrameric complex

498 reconstituted from swapped domains. Left: Schematic representation. The hinges  
499 encompassing residues 99-121 of the two *VcDciA*s are putative in this model (dark blue  
500 dotted lines). Right: Ribbon representation of the heterotetrameric structure. The *VcDnaB*  
501 dimer is in the same colours as in (A), the two *VcDciA* molecules are in magenta and yellow.  
502 The dark blue dotted rectangle frames the close-up view shown in (C). (C) Zoom-in on the  
503 interface region forming a 5-helix bundle, with the superimposed experimental electron-  
504 density map from SAD-phasing after solvent-flattening (gray mesh, contoured at  $1\sigma$ ).

505

506 **Figure 2.** *VcDciA* and *EcDnaC* target the same binding site on DnaB helicases. (A) Sequence  
507 alignment of the LH and DH helices of *VcDnaB* and *EcDnaB* (generated by EMBL-EBI  
508 Clustal Omega server (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) (Sievers, Wilm et al.,  
509 2011) and displayed using ESPript 3.0 server (<https://esript.ibcp.fr>) (Robert & Gouet, 2014).  
510 The conserved residues are in white on a red background. An orange asterisk marks the  
511 conserved tryptophans while a black one marks the specific serine/glycine residues in the DH  
512 helix. (B) Close-up view of the interaction interface forming a three- or five-helix bundle  
513 between the two-helix LH-DH module of DnaB (blue and green) and *VcDciA* (top, in  
514 magenta and yellow, PDB ID 8A3V, this study) or *EcDnaC* NTD (bottom, in magenta, PDB  
515 ID 6QEL), respectively. The tryptophans whose intrinsic fluorescence variation was measured  
516 in the Tycho experiments are in orange sticks. (C and D) Tycho NT.6 analysis. The emission  
517 profile of a tryptophan is shifted to the red emissions when it is released to the solvent during  
518 the thermal denaturing of the protein. In red are reported the 350/330 nm ratios measured for  
519 the different helicase-loader mixtures and in black are reported the predicted ratio in the  
520 absence of interaction. The ratio comparisons are reported for each helicase-loader couple  
521 indicated, namely for *VcDnaB* (C) or *EcDnaB* (D) with two constructs of *VcDciA* or *EcDnaC*.  
522 The curves correspond to the mean  $\pm$  SEM of three to five analyses. \*Tycho interaction

523 analysis between *VcDnaB* and *VcDciA* was previously published (Marsin et al., 2021) but  
524 nonetheless reproduced here for easiest evaluation with other helicase-loader couples.

525

526 **Figure 3.** *VcDciA* and *EcDnaC* loaders are functionally interchangeable *in vitro*. Bio-layer  
527 interferometry (BLI) analysis using biotinylated oligonucleotide (50 nt) immobilized onto the  
528 surface of a streptavidin (SA)-coated probe by its 3' extremity (see [Materials and Methods](#)).  
529 Association was performed with the indicated helicase at a concentration of 100 nM during  
530 300s in a buffer solution containing ATP and MgCl<sub>2</sub>. Dissociation was assessed in the same  
531 buffer for 600s. Increasing loader concentrations (from 0 to 200 nM in subunits, in blue to  
532 red) were analyzed. The experiments were carried out in duplicate, only one is presented. **(A)**  
533 *VcDnaB* binding on ssDNA in the presence of *VcDciA* (left) or *EcDnaC* (right). **(B)** *EcDnaB*  
534 binding on ssDNA in the presence of *EcDnaC* (left) or *VcDciA* (right).

535

536 **Figure 4.** *DciA* binds to the periphery of the *DnaB* CTD. **(A)** Structure of the  
537 *VcDnaB*<sub>6</sub>•*VcDciA*<sub>6</sub> heterododecameric complex reconstituted by the crystal *H32* symmetry.  
538 The colour code is the same as in Fig 1B. Left: Schematic representation. The  
539 heterododecameric ring is reconstituted by assembly of the heterotetramer with two  
540 neighboring symmetry mates (hatching textures) related by a true crystallographic 3-fold  
541 rotation axis (red dashed lines). The hinges encompassing residues 99-121 of the *VcDciA*  
542 molecules are putative in this model (dark blue dotted lines). Right: Ribbon representation of  
543 the heterododecameric model. **(B)** Structural comparison of four helicase-loader complexes:  
544 *VcDnaB*•*VcDciA* (PDB ID 8A3V, this study), *EcDnaB*•*EcDnaC* (PDB ID 6QEL),  
545 *EcDnaB*• $\lambda$ P (PDB ID 6BBM) and *GstDnaB*•*BsDnaI* (extracted from PDB ID 4M4W). The  
546 *DnaB* hexamers are represented in surface (blue and green), and the helicase loaders in

547 magenta sticks. Unlike DnaC,  $\lambda$ P and DnaI which cover the back of the DnaB CTD ring,  
 548 DciA leaves it free by positioning itself at the periphery of the helicase.

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554 **Table 1. Data collection, phasing and refinement statistics**  
 555

	Native	(Ta <sub>6</sub> Br <sub>12</sub> ) <sup>2+</sup> derivative
	VcDnaB•VcDciA•ADP:Mg <sup>2+</sup> §	VcDnaB•VcDciA•ADP:Mg <sup>2+</sup> ‡
<b>Data collection</b>		
Space group	<i>H</i> 32	<i>H</i> 32
Cell dimensions		
<i>a</i> , <i>b</i> , <i>c</i> (Å)	186.51, 186.51, 252.84	186.67, 186.67, 252.99
$\alpha$ , $\beta$ , $\gamma$ (°)	90.0, 90.0, 120.0	90.0, 90.0, 120.0
Wavelength (Å)	0.984	1.254
Resolution range (Å) †	48.3 – 2.9 (3.1 – 2.9)	49.8 – 3.7 (3.8 – 3.7)
<i>Before STARANISO</i>		
Measured/Unique reflections	651973/37637	423037/18409
Spherical completeness (%) †	99.9 (99.9)	99.1 (94.5)
Spherical anomalous completeness (%) †		98.6 (86.4)
<i>I</i> / $\sigma$ ( <i>I</i> ) †	7.9 (0.3)	6.0 (0.5)
<i>After STARANISO</i>		
Measured/Unique reflections	368086/21186	259130/11065
Ellipsoidal completeness (%) †	94.1 (71.7)	95.6 (97.0)
Ellipsoidal anomalous completeness (%) †		95.6 (94.7)
<i>I</i> / $\sigma$ ( <i>I</i> ) †	13.4 (1.6)	12.6 (2.0)
<i>R</i> <sub>merge</sub> (%) †	19.6 (236.0)	22.3 (213.9)
<i>R</i> <sub>pim</sub> (%) †	4.6 (54.1)	4.7 (45.6)
Redundancy †	17.4 (19.9)	23.4 (22.3)
Anomalous redundancy †		12.2 (11.6)
CC <sub>1/2</sub> †	0.999 (0.544)	0.999 (0.671)
CC <sub>ano</sub> †		0.899 (0.0)
DANO / $\sigma$ (DANO)		1.588 (0.767)
<b>SAD phasing</b>		
Number of sites		7
Overall FOM		0.311
Overall FOM after density modification		0.693
<b>Refinement</b>		
Resolution range (Å)	40.8 – 2.9	
Number of work/test reflections	20312/1229	
<i>R</i> / <i>R</i> <sub>free</sub> (%)	29.8/30.8	
<b>Geometry statistics</b>		
Number of atoms	9367	
Protein	9311	
Ligand/ion	56	
Water	0	

R.m.s.d. from ideal values	
Bond lengths (Å)	0.005
Bond angles (°)	0.73
Average B-factor (Å <sup>2</sup> )	
Overall	126.1
Protein	126.2
Ligand/ion	110.9
Ramachandran plot	
Most favoured (%)	96.4
Outliers (%)	0.5
Molprobability score	2.03

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556 † Values in parentheses refer to the highest resolution shell.

557 § Diffraction data collected from one crystal, which diffracted anisotropically to 2.88 Å along 0.894 *a*\* - 0.447  
558 *b*\*, 2.88 Å along *b*\* and 5.21 Å along *c*\*

559 ‡ Diffraction data collected from one crystal, which diffracted anisotropically to 3.43 Å along 0.894 *a*\* - 0.447  
560 *b*\*, 3.43 Å along *b*\* and 6.50 Å along *c*\*

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562

## 563 **Additional Figure Legends**

564 **Figure supplement 1.** Interaction with *VcDciA* does not alter the overall structure of the  
565 *VcDnaB* dimer but induces folding of the *VcDciA* CTD into a helix hairpin. **(A)**  
566 Superimposition of the ADP-bound *VcDnaB* dimer extracted from the complex with *VcDciA*  
567 (cyan and green, PDB ID 8A3V, this study) with a GDP-bound *VcDnaB* dimer extracted from  
568 the hexameric helicase ring (gray, PDB ID 6T66). The two structures of *VcDnaB* are almost  
569 identical (global RMSD of 1.76 Å). However, the maximum distance between the LH and DH  
570 helices is increased by about 5 Å in the presence of *VcDciA* (compare double red arrow with  
571 double orange arrow). **(B)** Zoom-in on the NTP binding site of the two superimposed *VcDnaB*  
572 dimer structures (same colours as in (A)). The P-loops are perfectly superimposable, as well  
573 as the ADP (blue sticks) and the GDP-AlF<sub>4</sub> (gray sticks), and also the Mg<sup>2+</sup> ions (cyan and  
574 gray spheres). **(C)** Superimposition of the two *VcDciA* NTDs (yellow and magenta, PDB ID  
575 8A3V, this study) extracted from the *VcDnaB*•*VcDciA* complex with the NMR structure of  
576 the isolated NTD of *VcDciA* (in gray, BMRB ID 27689). The KH-like fold of the *VcDciA*  
577 NTDs in the complex is very similar to the *VcDciA*<sup>[1-111]</sup> NMR solution structure (RMSD of  
578 1.28 Å). The two copies of *VcDciA* NTD in the heterotetrameric structure are identical

579 (RMSD of 0.48 Å), except for the first long  $\alpha$ 1 helix (bend at residue H24 in one copy, with  
580 an angle of 50° as illustrated by blue lines) and the last  $\alpha$ 3 helix (oriented almost oppositely in  
581 the two copies with an angle of 135° as illustrated by blue lines). This reflects a certain  
582 flexibility of *VcDciA* which was predicted by previous molecular dynamics analyses (Marsin  
583 et al., 2021). **(D)** Superimposition of the two *VcDciA* CTDs (same colours as in (C)) extracted  
584 from the complex with *VcDnaB*. The *VcDciA* CTD, which was shown disordered in solution  
585 by SAXS (Marsin et al., 2021), folds into a helix hairpin upon interaction with the *VcDnaB*  
586 dimer (contact with the LH-DH module of the helicase). The two copies of *VcDciA* CTD are  
587 identical (RMSD of 0.20 Å).

588

589 **Figure supplement 2.** Interaction with ssDNA is detectable for neither *VcDciA* nor *EcDnaC*  
590 under the same experimental conditions used for the helicase loading assays in this study. The  
591 Bio-layer interferometry (BLI) analysis of the two loaders alone (from 0 to 200 nM in  
592 subunits, in blue to red), is performed as described in [Fig 3](#) and in the [Materials and Methods](#)  
593 section.

594

595 **Figure supplement 3.** Validation of the protein composition of the *VcDnaB*•*VcDciA* complex  
596 crystals. 11-month-old crystals of the complex were visualized by SDS-PAGE and Coomassie  
597 Blue staining. Purified *VcDnaB* and *VcDciA* proteins were migrated on the same gel as  
598 controls, showing that the crystallized proteins were protected from proteolysis. The crystals  
599 of the complex obtained in our study grew in 5 days and were formed from the two full-length  
600 proteins.

Fig 1

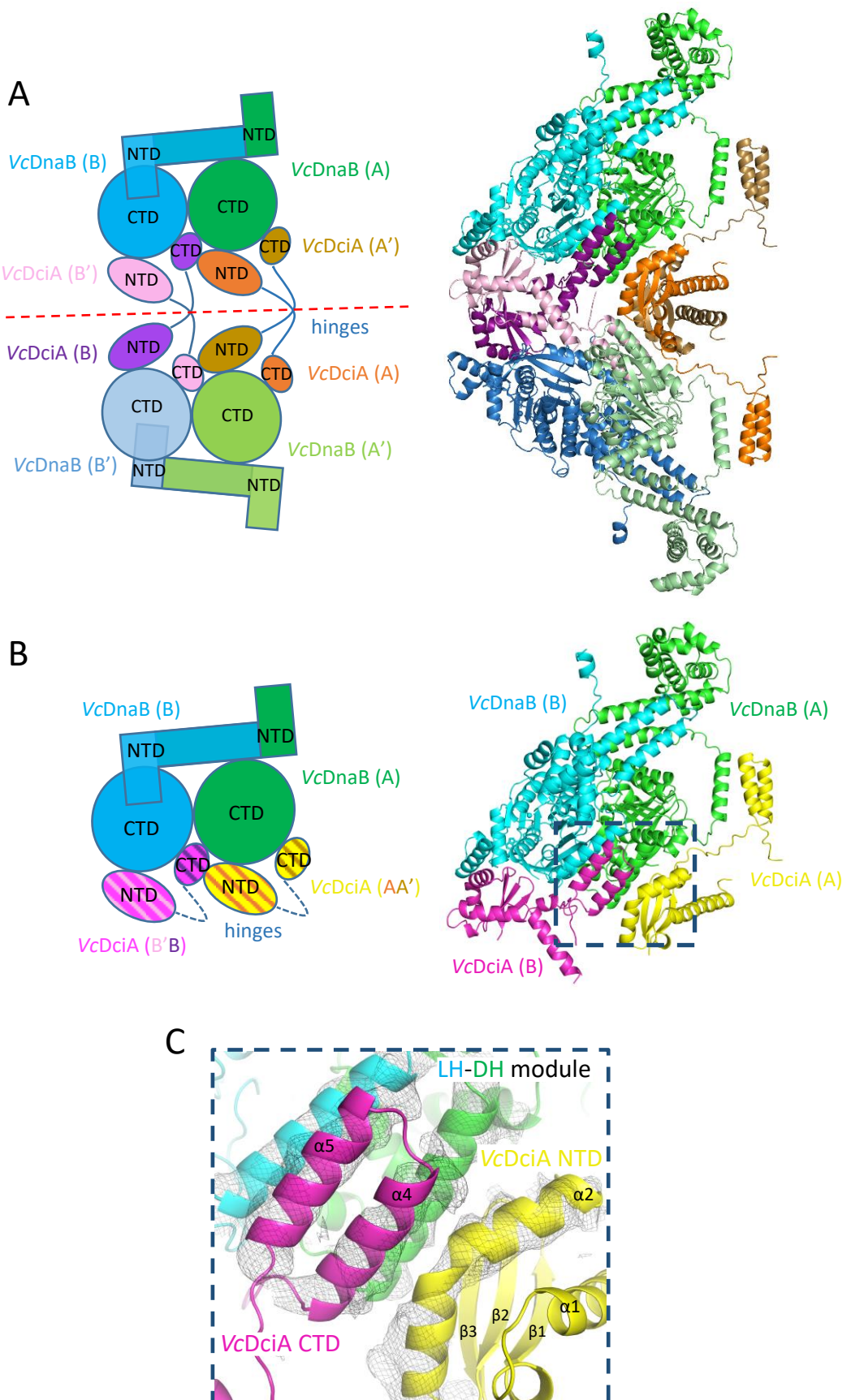


Fig 2

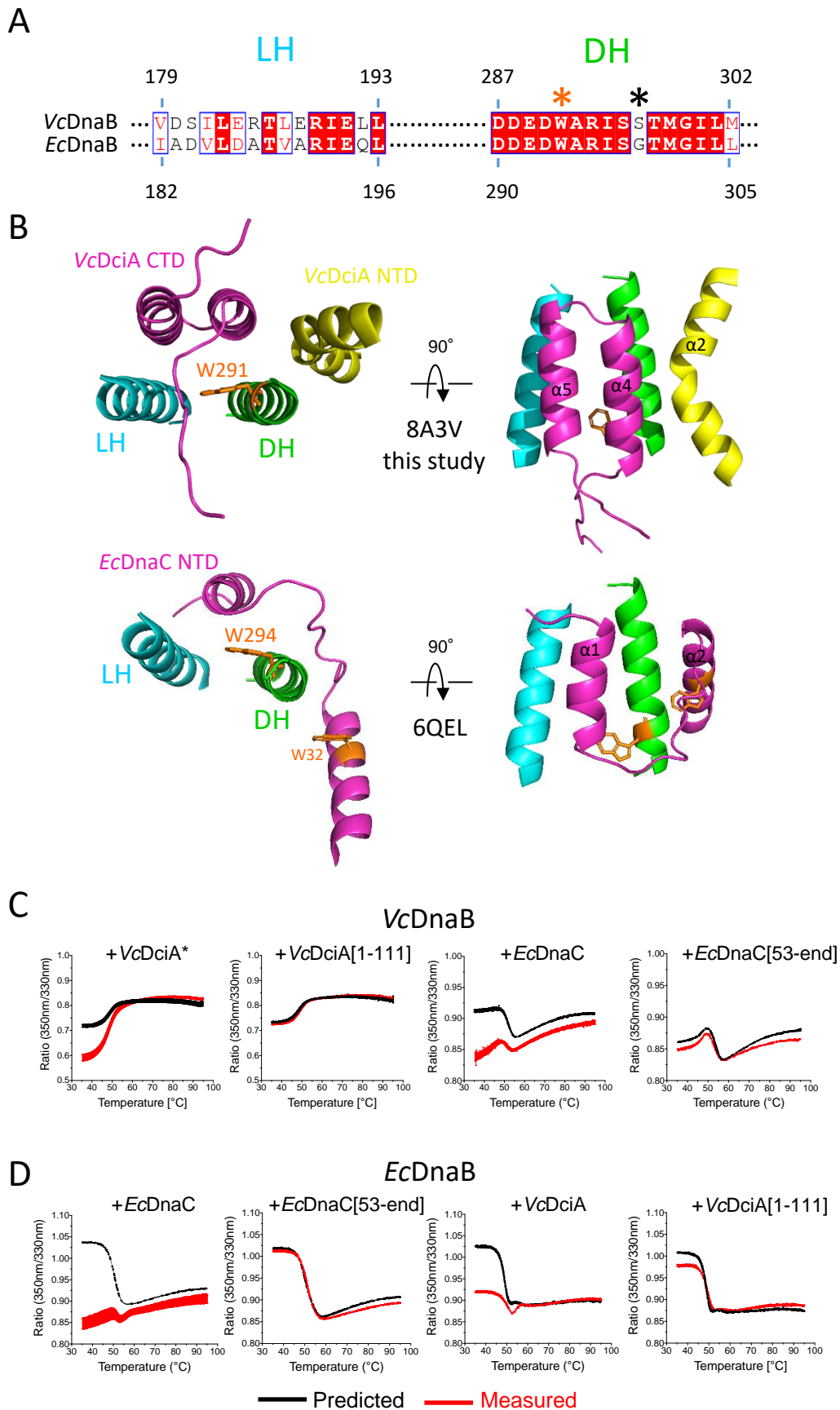
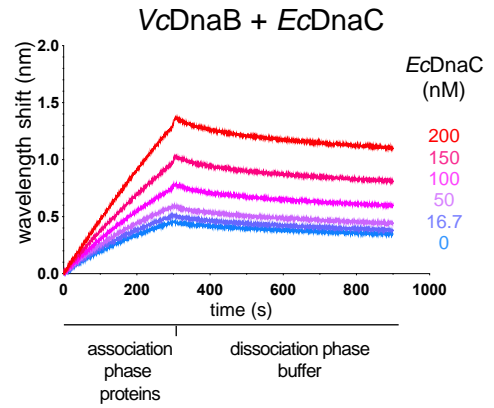
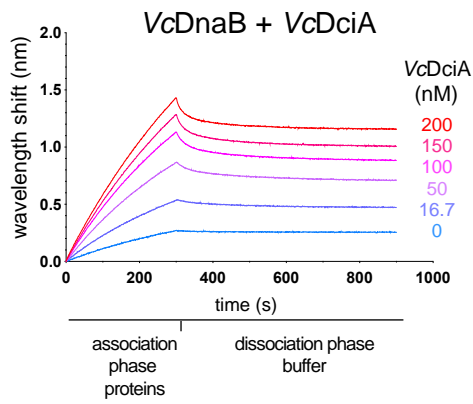


Fig 3

A



B

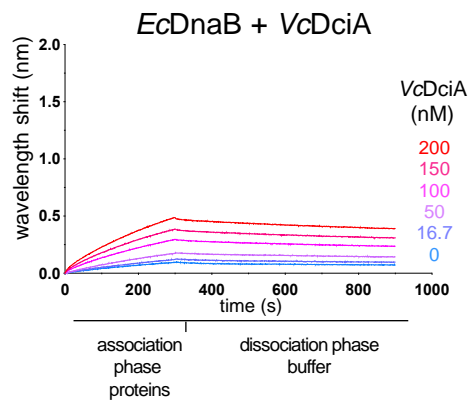
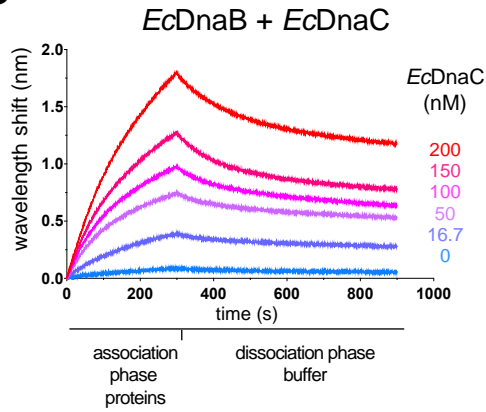
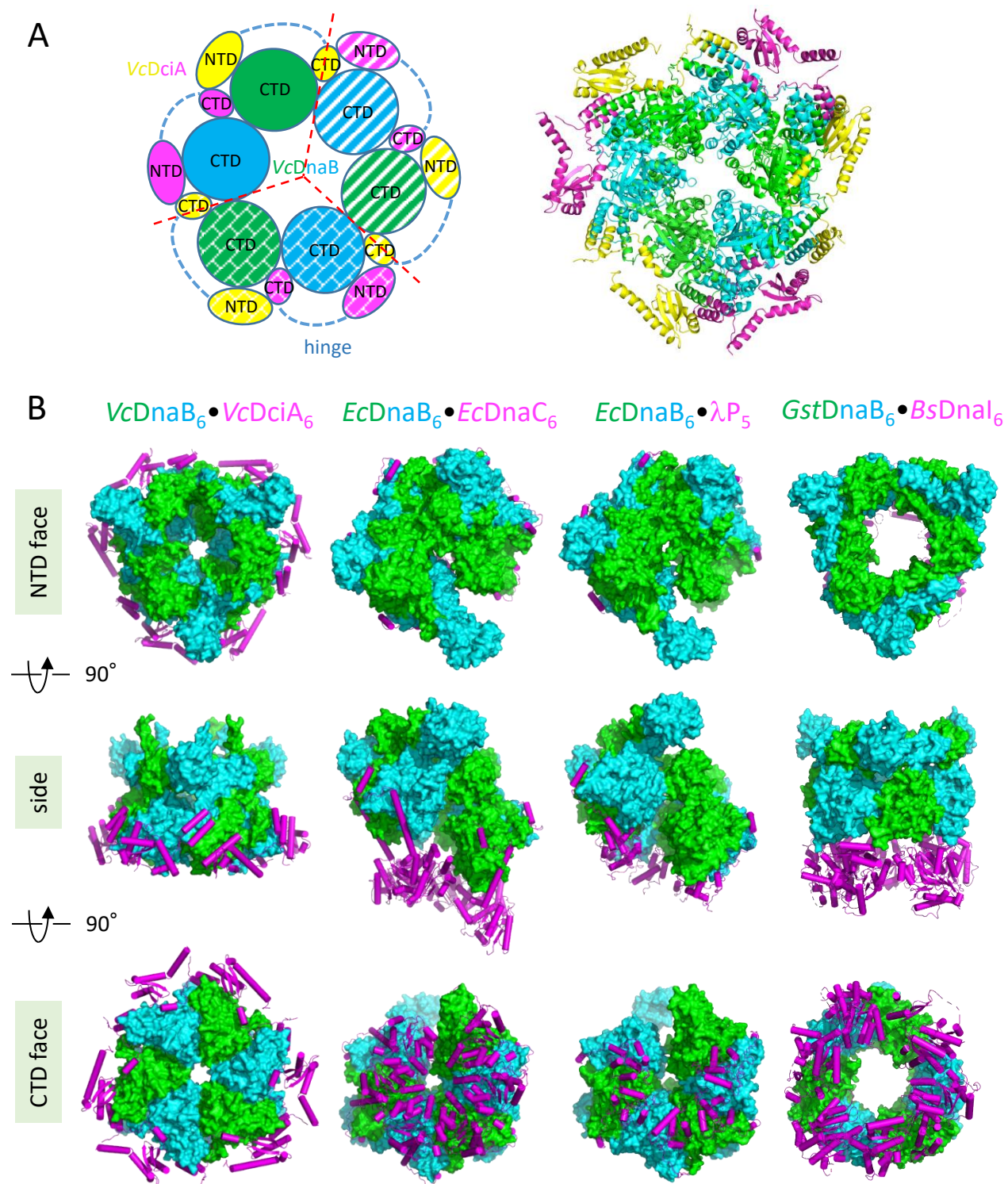
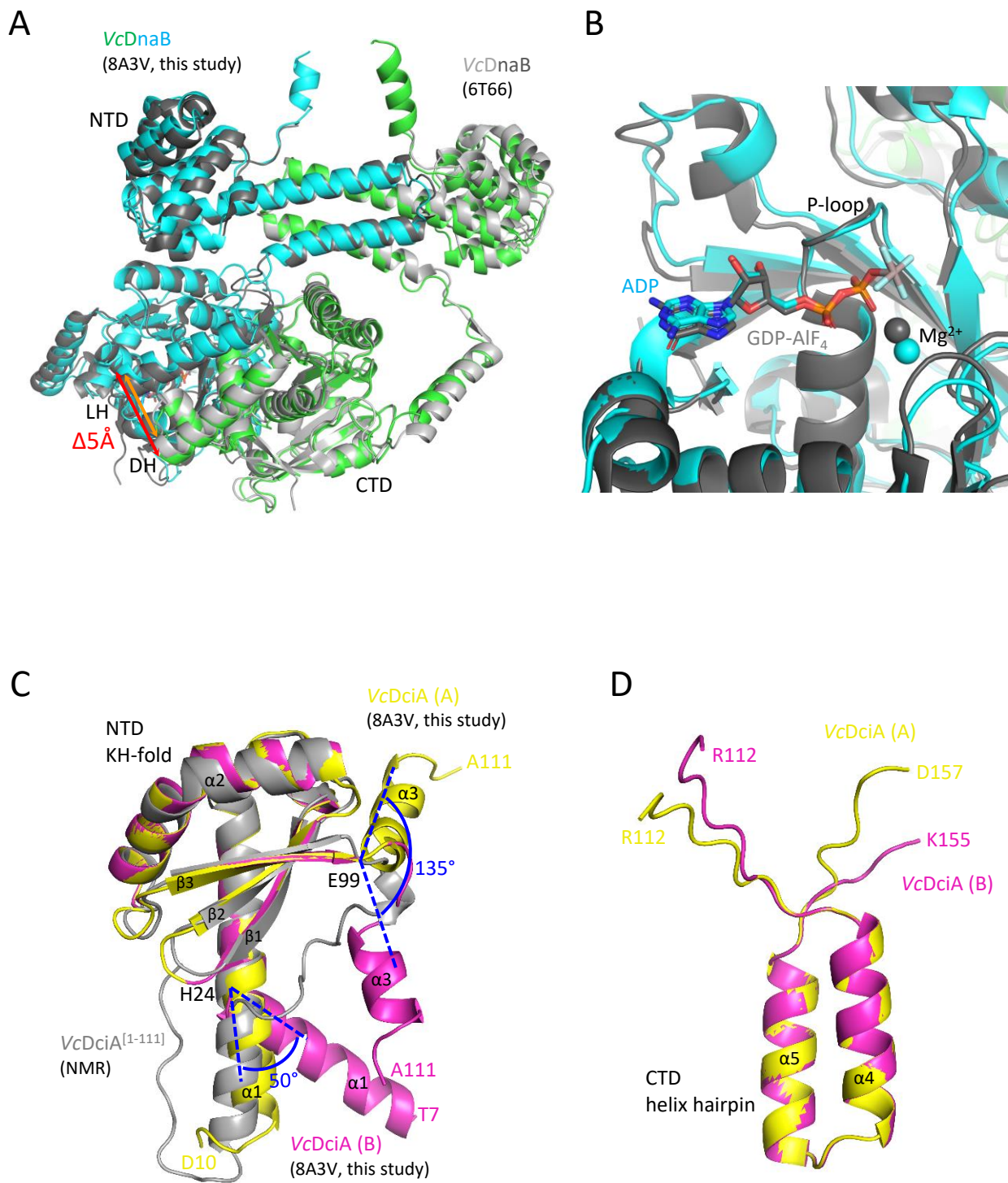


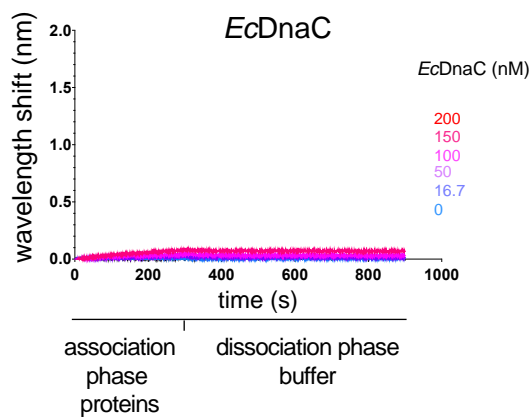
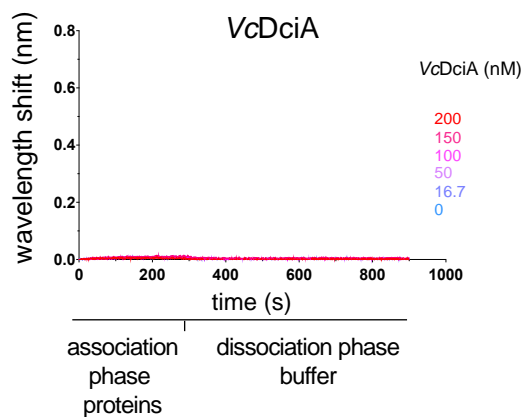
Fig 4



# Supplementary Fig 1



## Supplementary Fig 2



# Supplementary Fig 3

