

The bacterial DNA sliding clamp, β -clamp: Structure, interactions, dynamics and drug discovery

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Supplementary figures and tables

Table S1: Structural alignment of β -clamps from different organisms^a

PDB ID	Organism	Sequence identity	RMSD (Å) ^b	Sequence length
1MMI [33]	<i>Escherichia coli</i> (Gram-negative)	-	-	366
6AMQ [134]	<i>Enterobacter cloacae</i> (Gram-negative)	96%	0.97	366
6AMS [134]	<i>Pseudomonas aeruginosa</i> (Gram-negative)	56%	1.47	367
6AP4 [134]	<i>Acinetobacter baumannii</i> (Gram-negative)	46%	1.79	388
3P16 [135]	<i>Mycobacterium tuberculosis</i> (Gram-positive)	27%	1.96	408
4TR6 [76]	<i>Bacillus subtilis</i> (Gram-positive)	27%	1.85	380
2AVT [136]	<i>Streptococcus pyogenes</i> (Gram-positive)	23%	1.94	378
4S3I [79]	<i>Helicobacter pylori</i> (Gram-negative)	20%	1.92	384

^aAlignments were done using the TM-Align algorithm [45], ^bRMSDs were calculated from the backbone C α atoms of the superimposed structures.

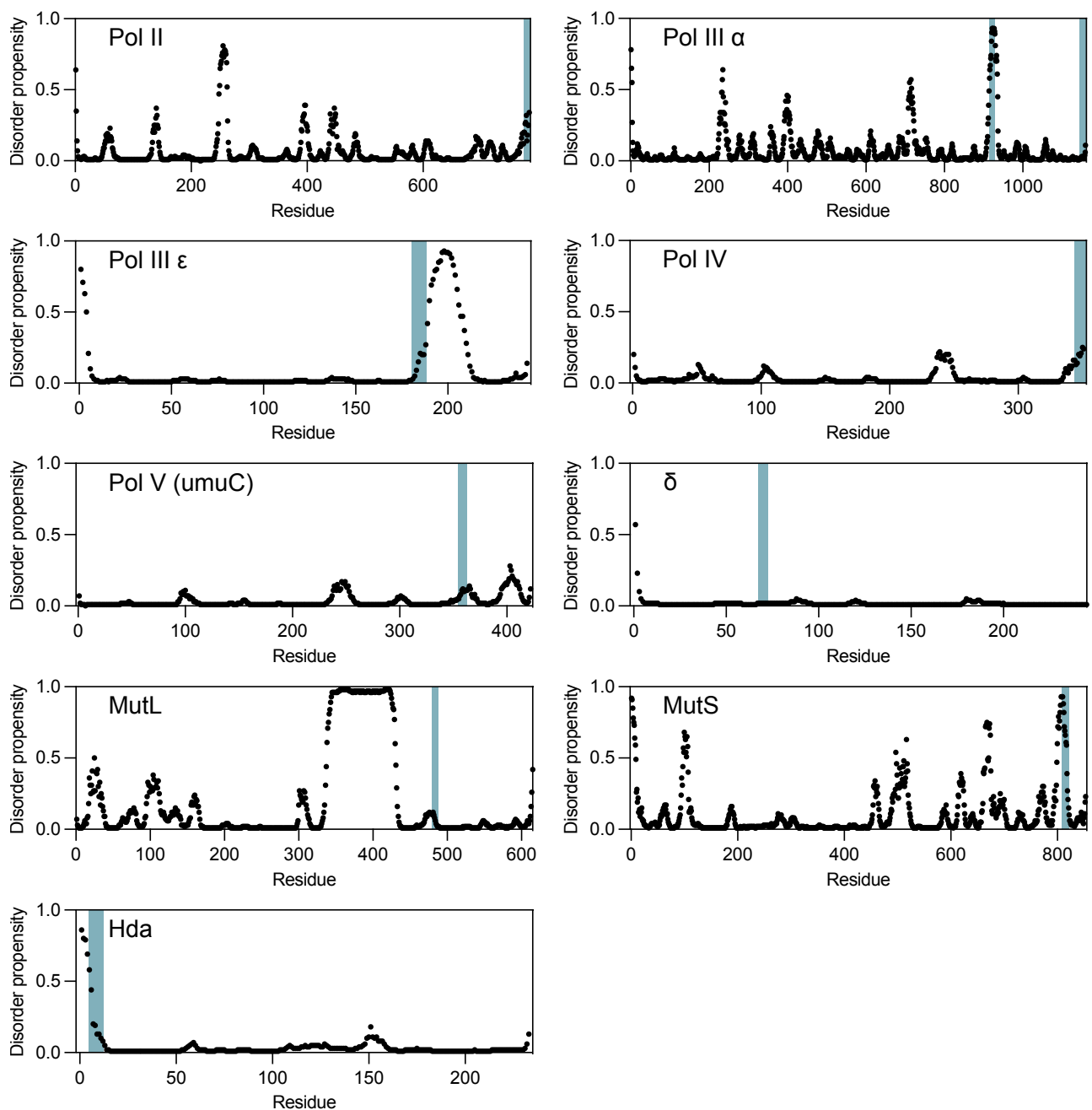


Fig. S1 Disorder prediction of selected β -clamp interaction partners. The disorder propensity is plotted for each residue and was predicted using DISOPRED3 (<http://bioinf.cs.ucl.ac.uk/psipred>) [137]. Blue boxes indicate the location of the β -clamp binding motifs (CBMs).

	1	10	20	30	40	50	60	70	80	
E. coli (1MMI)	--MKFTVEREHLKPLQVSGPLGGRTPLPILGNLLQVADGTLSTGTDLMEMVARVAL-----V--QP-H-EPGATTVPARKFFDLCRGLPEGAETIA	89								
E. cloacae (6AMQ)	--MKFTVEREHLKPLQVSG-----LPILGNLLQVADGTLSTGTDLMEMIARVTL-----S--QP-H-EAGATTVPARKFFDLCRGLPEGAETIA	82								
P. aeruginosa (6AMS)	--MHFTIQREALLKPLQLVAGVV--T-LPVLSNVLVVEGQQLSTGTDLEVELVGRVVL-----E--DA-A-EPGEITVPARKLMDICKSLPNDVLID	85								
A. baumannii (6AP4)	--HMRKIAKESLLNVLSHVVGAVERRHTNLSNVKIQTNAQALITIGSDLEVELVASTAL-----S--EGACLEAGETTVPARKLMEICKSLPTAALID	92								
M. tuberculosis (3P16)	--DLTFRLLRESFADAVSWVAKNLPARPVPVLSGVLLTGSNGLTISGFDYEVSAAEQVGA-----EI-V-SPGSVLVSGRLLSDITRALPN-KPVD	88								
B. subtilis (4TR6)	SHMKFTTIQKDRLVESVDVILKAVSSRTTIPILTGKIVASDDGVSTFGSDSISIESFIPKEEGDKIEVTL-E-QPGSIVLQARFFSEIVKKLP-MATVE	97								
S. pyogenes (2AVT)	--MIQFSINRTLFHALNTTKRAISTKNAIPILSSIKIEVTSTGVTLTGSNGQISIENTIPV---GL--LI-T-SPCAILLEASFFINIISLE-DLSIN	90								
H. pylori (4S3I)	--MKISVSKNDLENALRYLQAFLDKKDASSIASHIHLEVIKEKFLFKASDSIGLKSIFYT-----Q--SS-D-KEGVGTINGKKFLDIISCLKD-SNII	88								
	90	100	110	120	130	140	150	160	170	180
E. coli (1MMI)	VQLEG-E-RMLVRSGRSRFSLSTLPAADFPNDD-WQSEVEFTLPQATMKRLIEATQFSMAH--QDVRYLLNGMLFETE--GEELRTVATDGHRLAVCSM	182								
E. cloacae (6AMQ)	VQLEG-D-RMLVRSGRSRFSLSTLPAADFPNDD-WQSEVEFTLPQATMKRLIEATQFSMAH--QDVRYLLNGMLFETE--GEELRTVATDGHRLAVCSM	175								
P. aeruginosa (6AMS)	IRVEE-Q-KLLVKAGRSRFTLSTLPANDFPPTVEE-GPGSLNFSIAQSKLRRLIDRTSFAMAO--QDVRYLLNGMLLEVN--GGTLRSVATDGHRLAMCSL	178								
A. baumannii (6AP4)	LQITTEDQ-RCILKSGNSRFVLGTLPADYPLLTENSQGTQVQVTQRELKRLFEKTAFAFAMAV--QDVRFYLTGTLLIED--ENQLRAVTTDGHRLALCEI	187								
M. tuberculosis (3P16)	VHVEG-N-RVALTCGNARFSLPTMPVEDYPTLPT-LPE-ETGLLPAELFAEATSQVAIAAGRDDTLPLM--TGRIVEIL--GETVVLAAADRFRFLAVREL	180								
B. subtilis (4TR6)	IEVQNYQY-LTIIRSGKAEFNLNGLDDEYPHLPQ-IEEHHAIQIPTDLLKNLIQRTVFAVST--SETRPILTGVNWKVE--QSELLCTATDSHRLALRKA	191								
S. pyogenes (2AVT)	VKEIE-QHQVVLTSKGSEITLTKGKVDQYPRQE-VSTENPLILKTKLLSIIAETAFASL--QESRPILTGVHIVLSN-HKDFKAVATDSHRMSQRLI	185								
H. pylori (4S3I)	LETKD-D-SLAIKQNKSSFKLPMFDEFFPEFV-IDPKVSEIENAPFLVDAPFKKIAPVIEQ--TSHKRELAGILMQFDQKHQTLISVVGTDTKRLSYTQL	183								
	190	200	210	220	230	240	250	260		
E. coli (1MMI)	PIGQ--S-L--P-SHSVIVPRKGVIELMRMLDGGDNPLRVQI-----GS--NNIRAHVG-----DFIETSKLVDGRFPDYRRVLKPNPKHLEAGCD	261								
E. cloacae (6AMQ)	PIGD--S-L--P-NHSVIVPRKGVIELMRMLDGGDTPLRVQI-----GS--NNIRAHVG-----DFVPTSKLVDGRFPDYRRVLKPNPKHLEAGCD	254								
P. aeruginosa (6AMS)	DAQI--P-S---QDRHQVIVPRKGILELARLLTEQDGEVGIVL-----GQ--HHIRATTG-----EFTFTSKLVDGRFPDYERVLPRGGDKLVVGDRC	258								
A. baumannii (6AP4)	LASS--TSS--Q-LVQAIVPRKAVGELQRLLSIEDEQLTLLI-----GR--ELLNVTINTPEQGDITVRFETTKLIDGKFPDYRRVIRPGGDKHVLIGHD	274								
M. tuberculosis (3P16)	KWSASSP-D---I-EAAVLVPAKTLAEAAKAGI-GGSDVRLSLGTGPGVGKDGLLGISGN-----GKRSTTRLLDAEFPKERQLLPTHTAVATMDVA	267								
B. subtilis (4TR6)	KLDI--P-ED--R-SYNNVIPGKSLTELSKILDDNQELVDIVI-----TE--TQVLFKAK-----NVLEFSSRLLDGNYPDPTSLIPQDSKTEIIVNTK	271								
S. pyogenes (2AVT)	TLDN--T-S---A-DFMVVLPSKSLREFSAVTDDEIETVEFF-----SP--SQILFRSE-----HISFYTRLLGNYPDPTDRLLMTTEFFTEVVFNTQ	264								
H. pylori (4S3I)	EKIS--I-HSTEE-DISCLPKRALLEILKLF-YE--NFSFKS-----DG--MLAVIENE-----MHTFTTKLIDGNYPDYQKILLPKEYISSFTLGKE	262								
	270	280	290	300	310	320	330	340	350	
E. coli (1MMI)	LLKQAFARAAILSN-E-KFRGVRLYVS-ENQKITAN-NPEQEEAEIILDV-T-YSGA-EMEIGFNVSYVLDVNLAKCENVRMLLTDSVSSVQIEDAAS	354								
E. cloacae (6AMQ)	SLKQAFARAAILSN-E-KFRGVRLYVS-ENQKITAN-NPEQEEAEIILDV-T-YAGT-EMEIGFNVSYVLDVNLAKCENVRILLTDSVSSVQIEDAAS	347								
P. aeruginosa (6AMS)	QREAFSRTAILSN-E-KYRGIRLQLS-NGLKIQAN-NPEQEEAEIEVQV-E-YNGG-NLEIGFNVSYLLDVLGVIGTGQVRFILSDSNSSALVHEADN	351								
A. baumannii (6AP4)	VFKQSLQRVAILSN-E-KLRGVFLNFN-QDSLQLRAN-NPEQDEAIEDLAI-Q-QQSA-PLEMSFNAQYLLDVLGVLDGDDVNMSTMEANQSVLVQDPAH	367								
M. tuberculosis (3P16)	ELIEAIKLVALVAD-R--GAQVRMEFA-DGSVRLSAG-ADDVGRAEEDLVV-D-YAGE-PLTIAFNPTYLTDLGSSLRSERVSFGFTTAGKPAALLRPVSG	359								
B. subtilis (4TR6)	EFLQAIIDRASLLAR-EGRNNVVKLSAKPAESIEISSN-SPEIGKVVEAIVADQ-IEGE-ELNISFSPKYMLDALKVLEGAEIRVVSFTGAMRPFLIRTEND	367								
S. pyogenes (2AVT)	SLRHAMERAFLLISNAT-QNGTVKLEIT-QNHISAHVN-SPEVGVNVEDLDIVS-QSSG-DLTISENPTYLLIESLKAISKSETVKIHLSPVRPFTLTTPGDE	359								
H. pylori (4S3I)	EFKESIKLCSSLS-----STIKLTLE-KNNALFESLDSHSETAKTSVEIEKGLDIEKAFHLGVNAKFFLEALNALGTTQFVLRNCNEPSSPFLIQESLD	355								
	360									
E. coli (1MMI)	----Q-----SAAYVVMPMRL	366								
E. cloacae (6AMQ)	----Q-----SAAYVVMPMRL	359								
P. aeruginosa (6AMS)	----D-----DSAYVVMPMRL	363								
A. baumannii (6AP4)	----P-----DQTYVVMPMR-	378								
M. tuberculosis (3P16)	DAVST-----DYVYLLMPVRL	375								
B. subtilis (4TR6)	----E-----TIVQLILPVRT	379								
S. pyogenes (2AVT)	----EE-----SFIQLITPVRT	372								
H. pylori (4S3I)	----EKQSHLNAKISTLMMPITL	374								

Fig. S2 Structural sequence alignment of β -clamps from different organisms. Sequence alignments are based on the structure alignment using the TM-align algorithm. Note that sequences not visible in the PDB structure are not present in this alignment. Sequence conservations are visualized using the Color Align Conservation tool at <https://www.bioinformatics.org> with a percentage identity or similarity cut-off of 70%. Amino acids are grouped according to their characteristics; positive = H, R, K; negative = D, E; Neutral = S, T, N, Q; Aliphatic = A, V, L, I, M; Aromatic = F, Y, W; Pro & Gly = P, G; Cys = C. Numbers on top of the sequences are amino acid positions of the E. coli β -clamp sequence

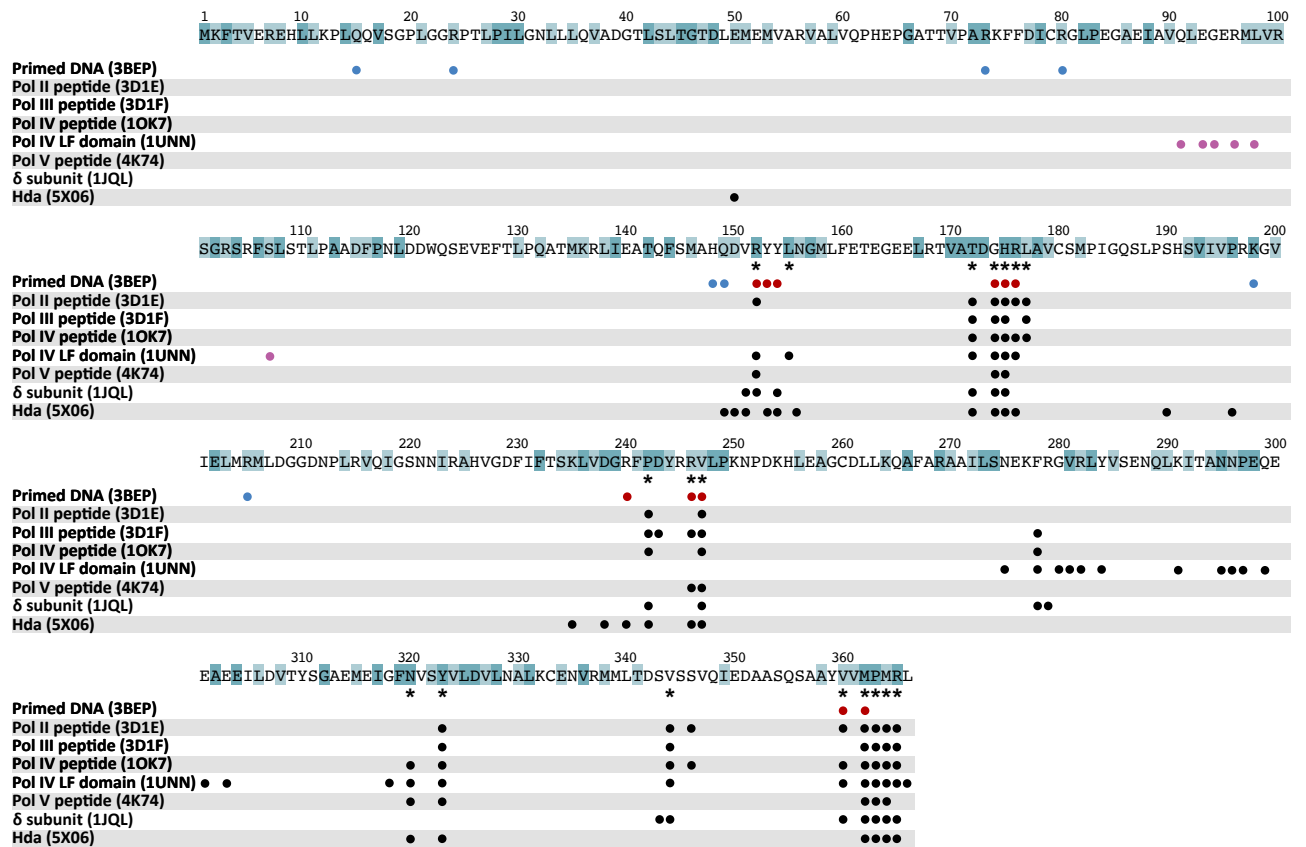


Fig. S3 Overview of β -clamp interacting residues and their conservation. PDBsum [138] was used to identify β -clamp residues that are in close proximity (≤ 3.9 Å) to the protein or peptide interaction partners (PDB ID in the parenthesis) in the crystal structures (black dots). The interacting residues between β -clamp and primed DNA are based on the interactions reported in the paper by Georgescu et al. [5]. β -clamp residues are coloured according to their structural sequence conservation as shown in Fig. S2. Stars indicates residues that constitute the canonical CBM binding pocket. Blue dots of the primed DNA interaction indicate interactions with the inner hole of β -clamp and red dots indicate interactions to residues of an adjacent β -clamp molecule in the crystal lattice. Purple dots of the Pol IV LF domain interaction indicate interactions formed by the other subunit of the β -clamp dimer.