Supporting Information

Georgescu *et al.* 10.1073/pnas.0804754105

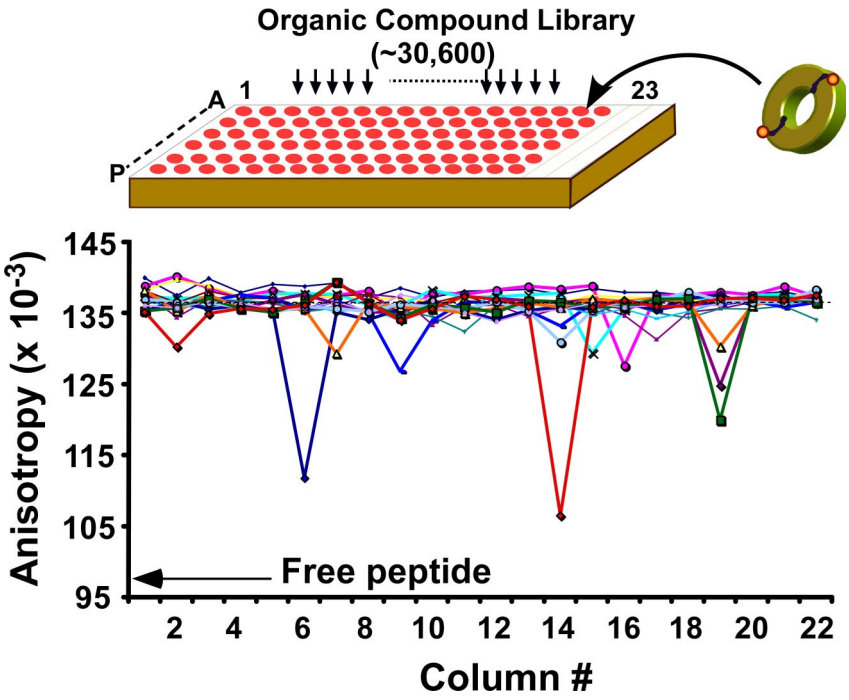


Fig. S1. Example of results from a 386-well plate using the peptide displacement assay. A chemical that disrupts the interaction between TAMN-labeled peptide and β-clamp decreases anisotropy.

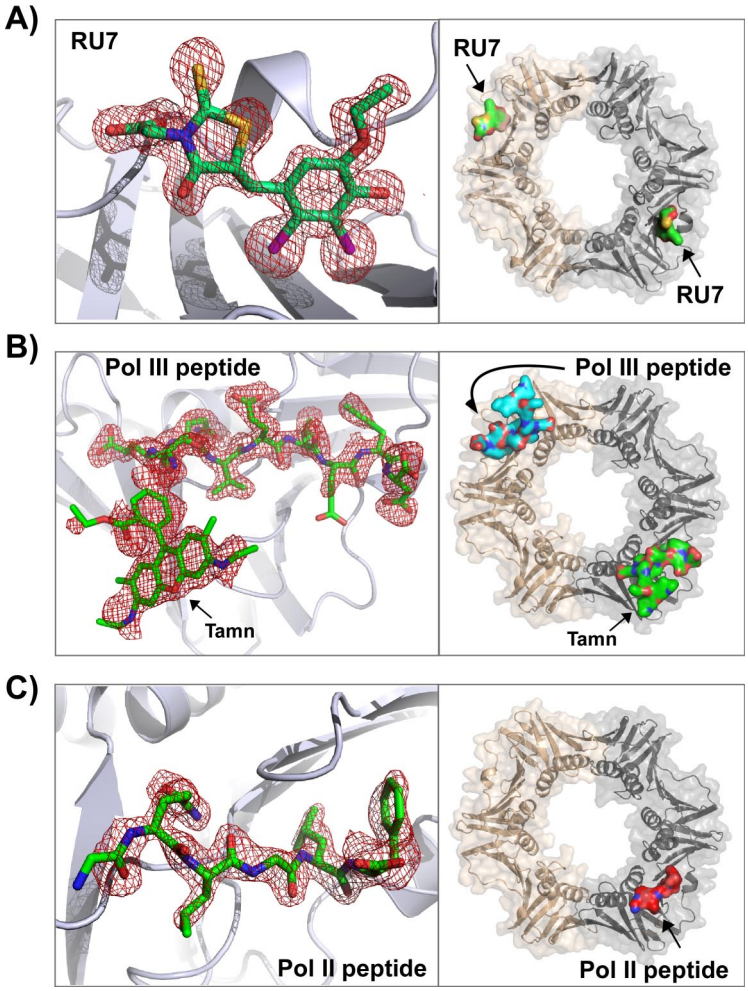
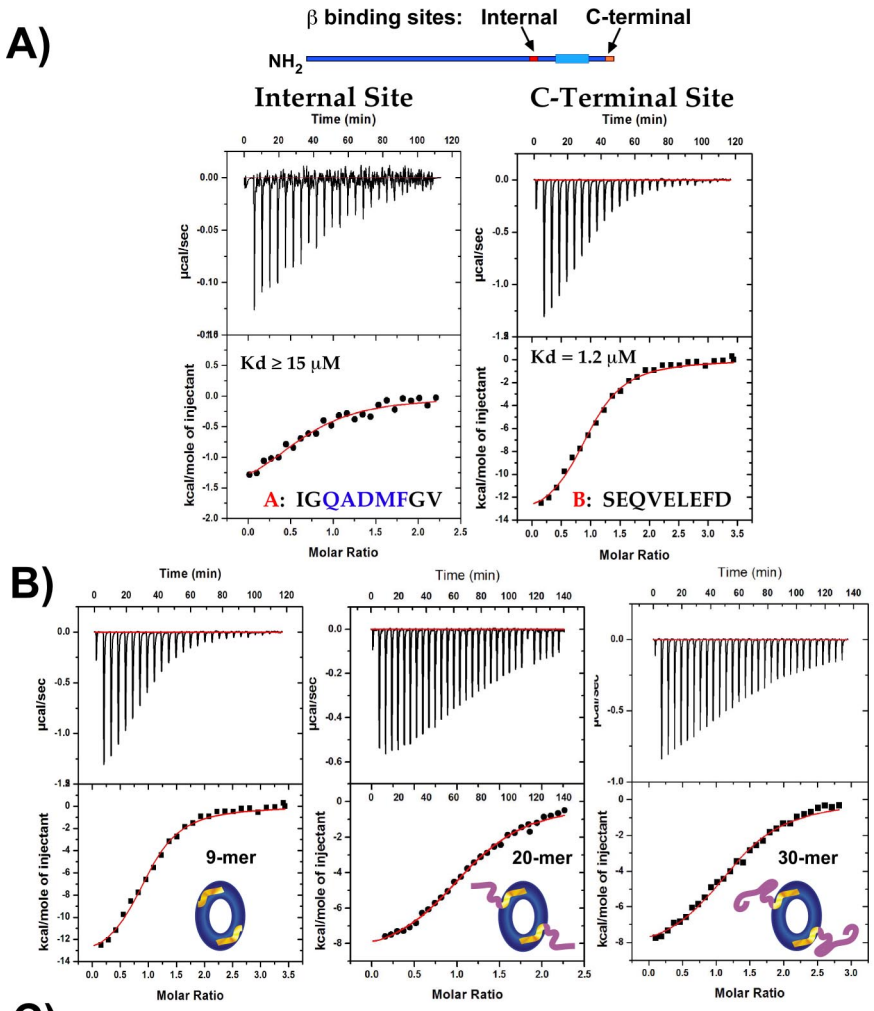


Fig. S2. Structure of Pol II and Pol III peptides and RU7 compound bound to the β-clamp. Electron density maps of RU7 (*A*), Pol III peptide (*B*), and Pol II peptide (*C*) contoured at 1.6 σ, 1.2 σ, and 1.2 σ, respectively. Images on the right display the positioning of RU7 and the peptides on the β-clamp. In the case of the Pol III peptide, the TAMN molecule is clearly visible in the electron density map and therefore was modeled into the structure.



C)

Parameters	*9-mer*	*20-mer*	*30-mer*
N	0.91 ± 0.01	1.16 ± 0.02	1.25 ± 0.03
Kd (µM)	1.42 ± 0.2	3.06 ± 0.35	3.95 ± 0.38
ΔHb (kcal/mol)	-11.34 ± 0.27	-9.12 ± 0.22	-8.97 ± 0.17
TΔS (kcal/mol)	-3.36 ± 0.42	-1.42 ± 0.39	-1.73 ± 0.41
ΔGb (kcal/mol)	-7.98 ± 0.93	-7.7 ± 0.8	-7.24 ± 0.94

Fig. S3. Isothermal titration calorimetry (ITC).(*A Upper*) Schematic diagram of the location of β-clamp-binding motifs in Pol III-α-subunit. (*Lower*) Binding isotherm titrations and the best-fit curves for the β-clamp using 9-mer peptides corresponding to the two α-sequences that bind the clamp. Data were fit to a simple binding model: A + B ↔ A·B. (*B*) Binding isotherms for the titration of the β-clamp with three peptides derived from the C terminus of Pol III-α subunit: 9-mer, 20-mer, and 30-mer. Thermodynamic parameters calculated from these experiments are shown in *C*.(*C*) ITC was performed by using a VP-ITC microcalorimeter from MicroCal. Peptides (200–250 µM) were titrated in 20–30 injections (10 µl each) into a 20 µM solution of β-clamp in 1.4 ml at 25°C. Heats of dilution were obtained from separate titrations into buffer. The corrected heat released due to peptide ligand binding to β was measured by integrating the area of each titration peak. For the raw heat values, we assumed that full β-peptide complex was achieved during the titration, allowing values from these injections to be used to correct the rest of the data. In experiments with the 20-mer and 30-mer however, the shape of the curve suggests that full complex formation was not yet achieved at the final injection because of their lower affinity for β, and therefore the data were corrected by subtracting a stirring heat of −6.2 mJ on the basis of a separate control experiment (peptide dilution in buffer).

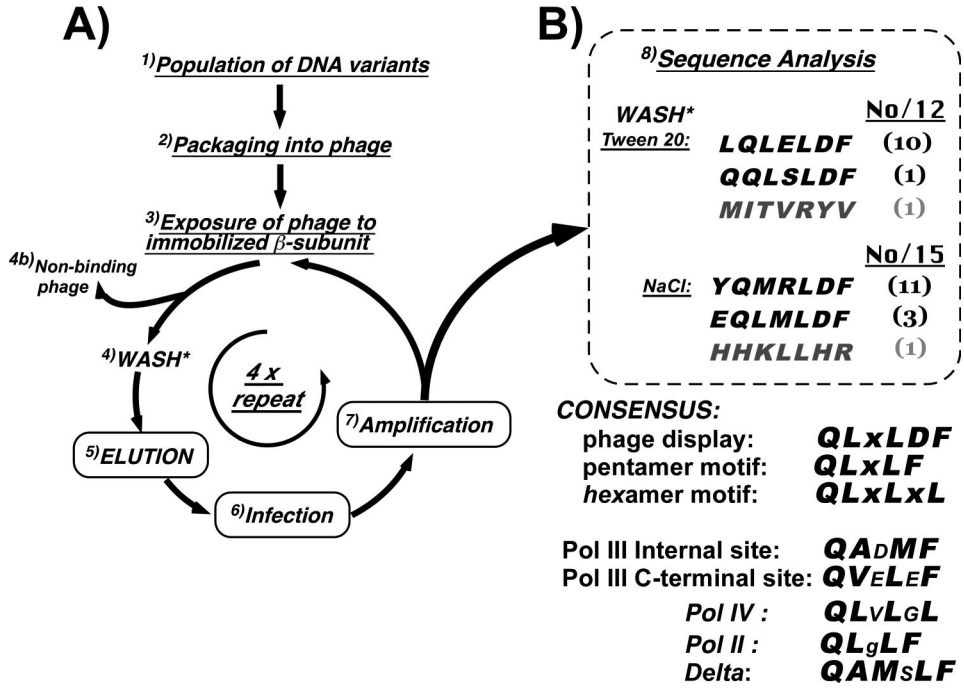


Fig. S4. Phage display selects a β-clamp-binding motif closely related to the C terminus of *E. coli* DNA Pol III-α subunit. The PhD 7 Phage Display Peptide Library (New England Biolabs) is based on a combinatorial library of random peptide 7-mer expressed at the N terminus of pIII. The first residue of the mature protein is the first randomized position. The peptide is followed by a short spacer (Gly-Gly-Gly-Ser) and then the wild-type pIII sequence. The library consists of $\approx 2.8 \times 10^9$ sequences (compared with $207 = 1.28 \times 10^9$ possible seven-residue sequences) and amplified once to yield ≈ 70 copies of each sequence in 10 μl of phage. (*A*) Steps 1–8 of the selection procedure were performed according to the manufacturer's protocol. The ''wash'' step (step 4) was performed under two different conditions: Tween 20 (0.5%) or NaCl (0.5 M). (*B*) Sequences obtained from 27 phages are shown. The consensus sequences using sequence alignments are also shown, as are the clamp-binding motifs in Pol III, Pol IV, and δ-subunit.

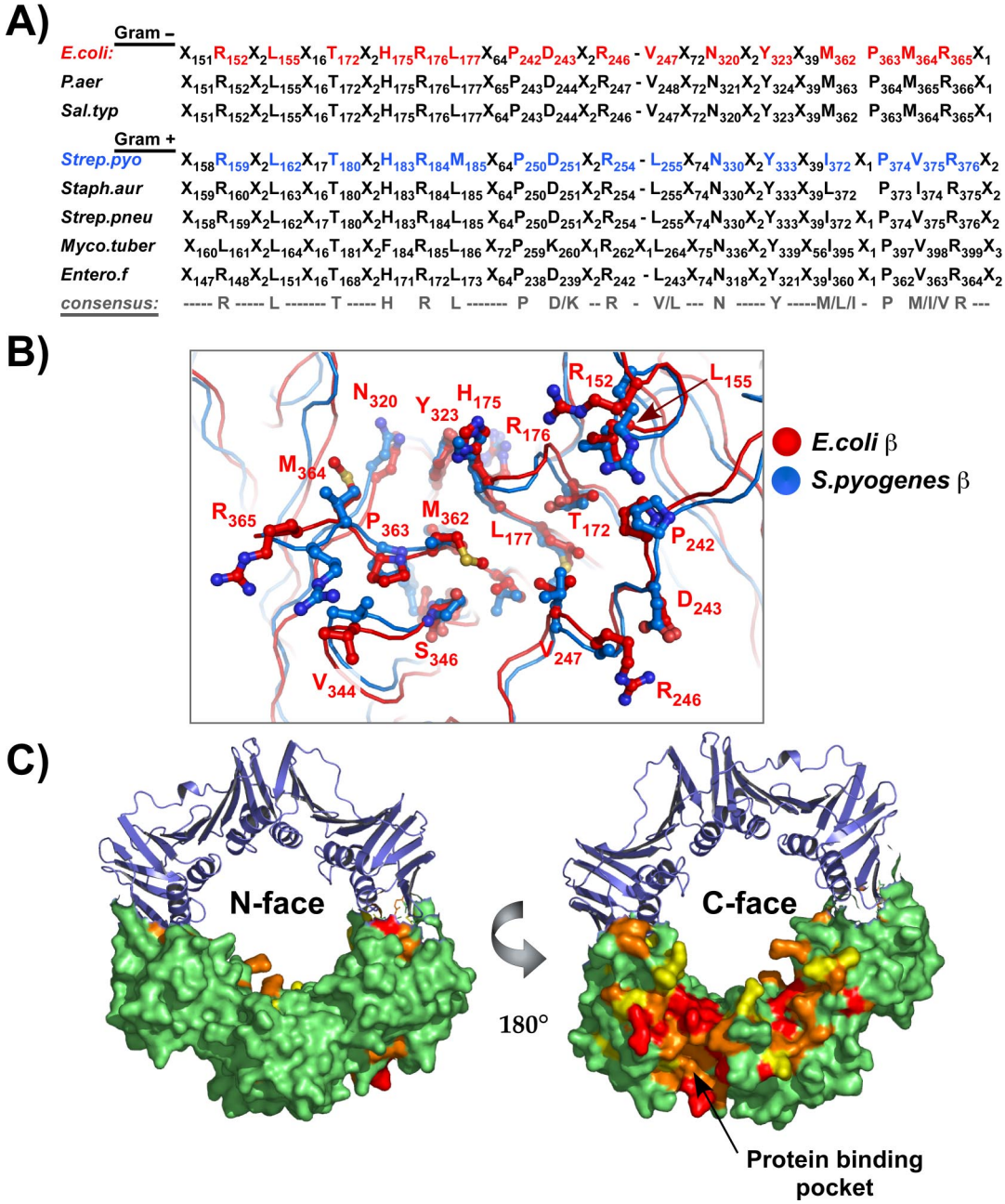


Fig. S5. A consensus sequence for a peptide-binding pocket in bacterial clamps. (*A*) Alignment of β-clamp residues in the clamp pocket of selected Gram-negative and Gram-positive bacteria. The consensus motif for the β-clamp pocket is highlighted. Residues that interact using peptide backbone atoms are omitted from the alignment (S346, V344, and L366). (*B*) Structural alignment of the hydrophobic pocket residues of *S. pyogenes* (blue) (PDB ID code 2AZT; Argiriadi *et al.*) and *E. coli* (red) β-clamps give a rmsd of 0.36 Å. *E. coli* clamp residues are numbered. (*C*) Surface representation of N- (left) and C- (right) faces of the β-clamp colored according to sequence conservation in an alignment of 42 bacterial subunits; scale runs from red (90% conservation) to yellow (50% conservation).

1. Argiriadi MA, Goedken ER, Bruck I, O'Donnell M, Kuriyan J (2006) Crystal structure of a DNA polymerase sliding clamp from a Gram-positive bacterium. *BMC Struct Biol* 6:2.

Table S1. Crystallographic data and refinement statistics for *E. coli* β bound to Pol III 9-mer peptide and for *E. coli* β bound to compound RU7

Characteristics	Pol III 9-mer (SEQVELEFD)	Pol II 10-mer (TLMTGQLGLF)	RU-7
Space group	P3(2)	P1	P1
Cell a/b/c, Å	65.8/65.8/209.5	40.1/69.9/73.1	35.7/79.4/80.5
α / β / γ, °	90.0/90.0/120.0	112.9/91.1/99.3	110.2/100.6/99.5
Resolution range, Å	50–1.90	50–1.78	50–1.52
No. of reflections:			
Unique	74,358	68,885	96,917
Total	148,848	162,724	241,724
Mosaicity:	0.89	0.42	0.25
Completeness, %	92.5 (91.3)	94.3 (73.6)	91.4 (76.1)
R_{sym}	0.040 (0.316)	0.058 (0.380)	0.056 (0.380)
Mean I/σ	8.5	11.3	10.9
Final model statistics:			
Resolution range, Å	44.14–2.00	33.35–1.90	34.21–1.64
R-factor, reflections	22.3	22.8	22.5
R_{free}, reflections	26.3	26.1	26.5
No. of atoms	6,506	6,239	6,401
Protein	5,688	5,688	5,688
TAMN-peptide/compound	220	45	48
PEG	35		
Water	563	468	843
Rmsd from ideal geometry			
Bond lengths, Å	0.0055	0.0057	0.0057
Bond angles, °	1.26	1.24	1.24
B-factor, Å^2	59.32	56.48	50.72
Ramachandran plot statistics, %*			
Resolution in most favored regions	89.8	88.8	90.3
Additional allowed regions	8.8	9.1	9.2
Generously allowed regions	1.1	0.3	0.2
Resolution in disallowed regions	0.3	1.8	0.3

Values in parentheses are for the highest resolution shell.

* Statistics from PROCHECK

Ref: Collaborative Computational Project No. 4 (1994). The CCP4 suite: Programs for protein crystallography. *Acta Crystallog* D 50:760–763.