SUPPORTING INFORMATION

Peptide inhibitors of bacterial protein synthesis with broad spectrum and SbmA-independent bactericidal activity against clinical pathogens.

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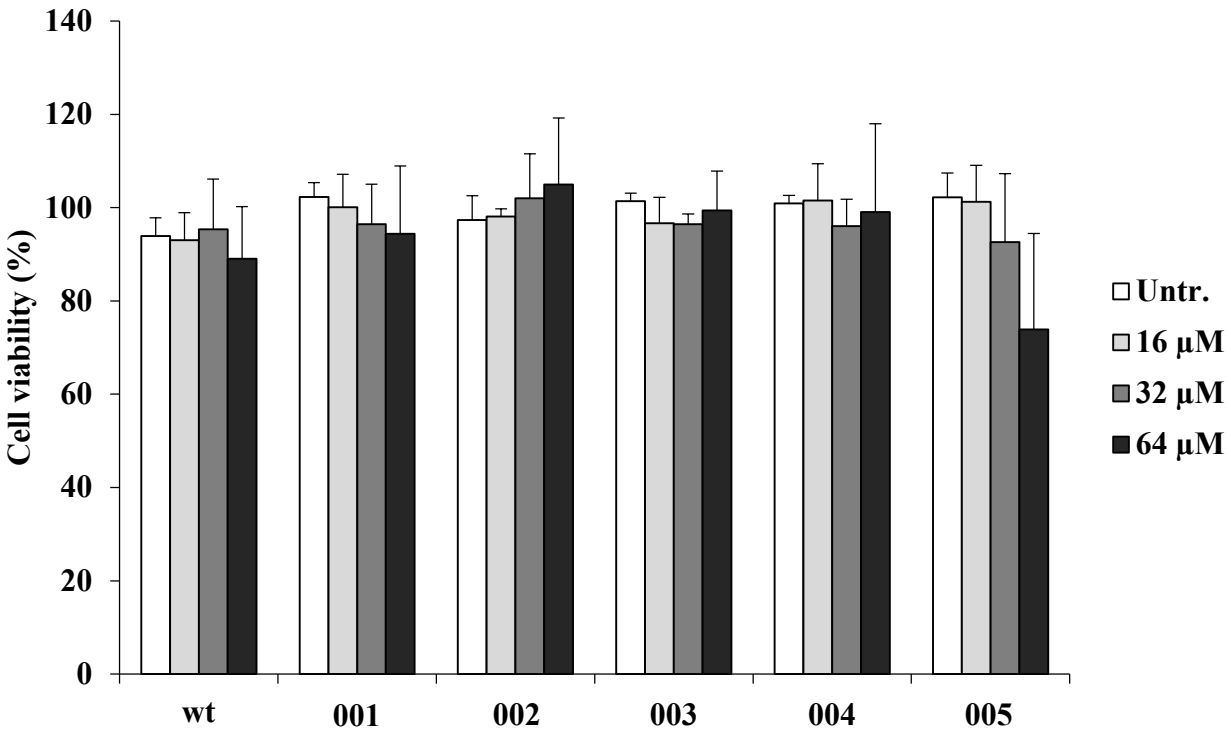
Contents of Supporting Information:

Supplementary Table 1. Number of substitutions that resulted in superior antimicrobial molecules with respect to the wild-type (MIC <4 μM), equal or similar (MIC 4-16 μM), weak (MIC 32-64 μM) and inactive (>64 μM).

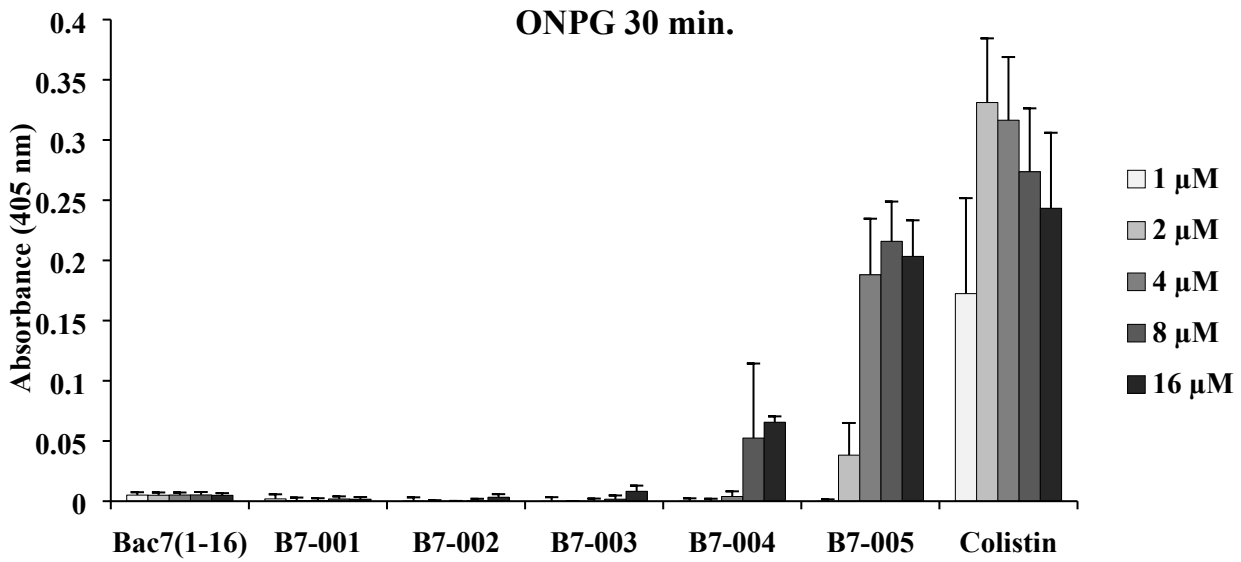
B7[a]	Superior	Equal or Similar	Weak	Inactive
R_1	1	5	1	-
R_2		5	2	
I_3		7	1	
R_4		5	1	1
P_5		6	1	
R_6		3	3	1
P_7		6	1	
P_8		6	1	
R_9		2	3	1
L_{10}		1	6	1
P_{11}		2	5	
R_{12}		3	3	1
P_{13}	1	5	1	
R_{14}		2	5	
P_{15}		6	1	
R_{16}		5	2	
Total	2	69	36	5



Supplementary Figure 1. **Cytotoxicity of resin- synthesised selected peptides assessed by MTT on the MEC-1 human leukaemia cell line.** The cell viability after the treatment with selected peptides is reported as the percentage of an untreated control (exposed to sterile water instead of peptides). Error bars are the standard deviations calculated on the average of 3 independent experiments performed in duplicate (n=6). Students t-test, $p \leq 0.05$ VS untreated control.

Supplementary Table 2. MIC of substituted Bac7(1-16) derivatives on clinically isolated pathogenic *E. coli* strains. Results are the median of at least 3 independent experiments (n=3) performed in complete MH medium. The native Bac7(1-16) (wt) was used for comparison.

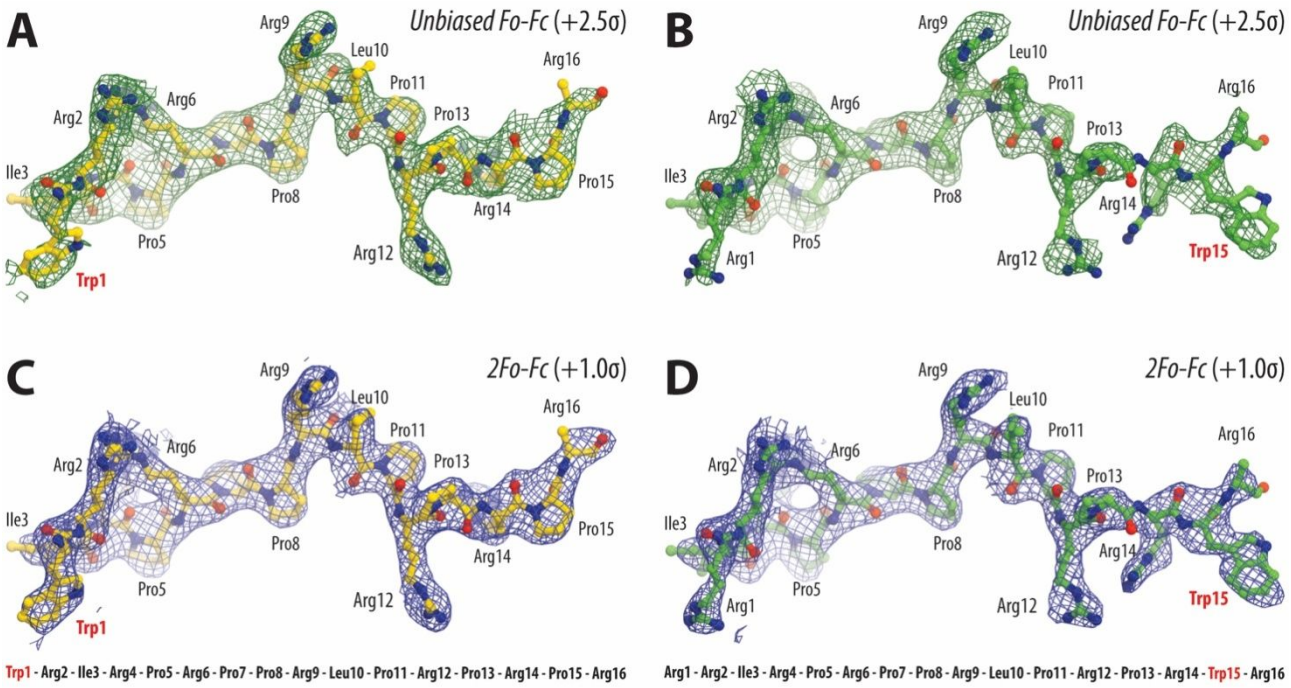
***E. coli* strain**	**Peptides MIC (µM)**					
	wt	**001**	**002**	**003**	**004**	**005**
E. coli EURL-VTEC A07 (EPEC:O111)	2	1	1	1	1	1
E. coli EURL-VTEC C07 (STEC:O157)	>64	32	4	8	4	2
E. coli SSI-NN14 (ETEC)	1	1	1	1	1	1
E. coli EA22 (ETEC)	1	1	1	1	1	1
E. coli SSI-OO15 (EIEC)	1	1	1	1	1	1
E. coli C679-12 (EAEC:0104)	1	1	1	1	1	1



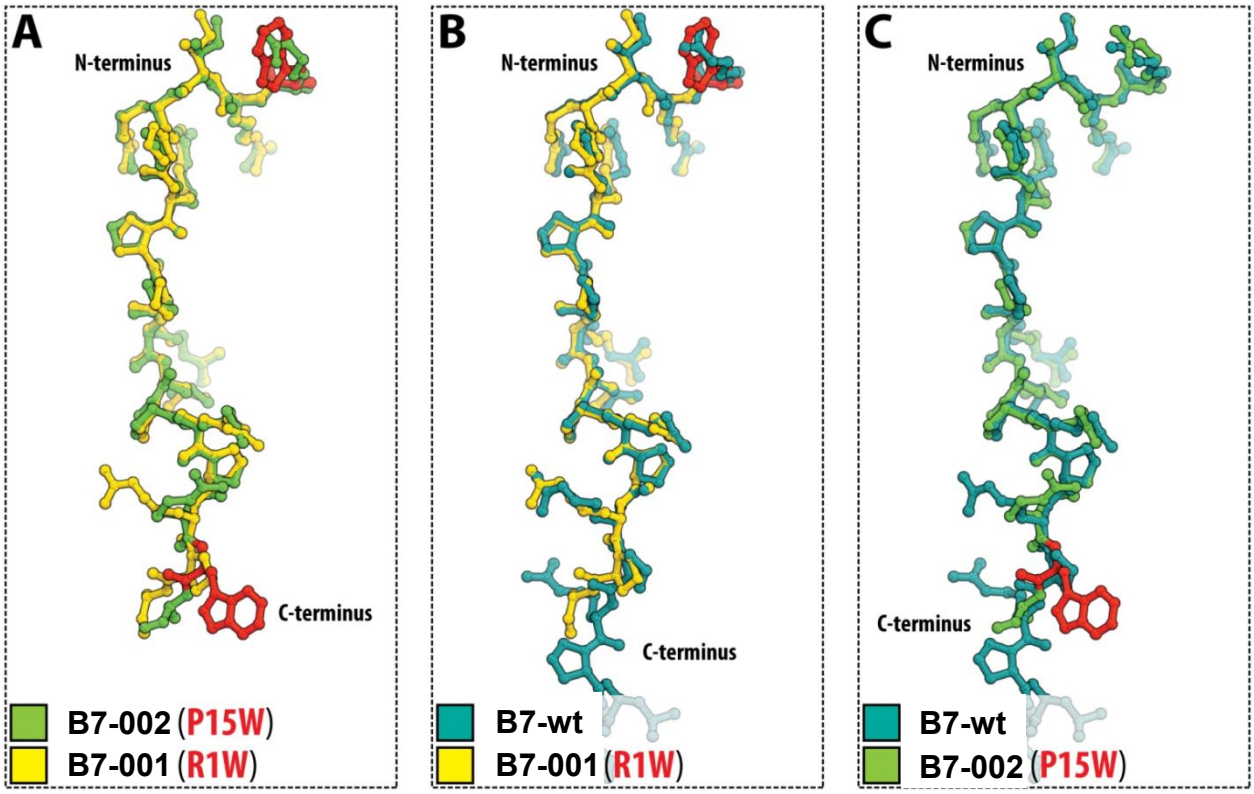
Supplementary Figure 2. Membrane permeabilization evaluated by ONPG/β-galactosidase assay in PBS using *E. coli* ML-35 in the presence of peptides. The membrane damage is proportional to the chromogenic hydrolysis of the ONPG by the cytosolic β-galactosidase unmasked by breaches of the bacterial envelope. Colistin was used as a reference for lytic compounds. The blank (absorbance of untreated bacteria) was subtracted to the values of treated samples. Error bars indicate the standard deviation calculated on the average of three independent experiments performed as internal duplicate (n=6).

Supplementary Table 3. MIC and the MBC of Bac7(1-16) and derivatives on *E. coli* ML-35. The sensitivity of the strain to the peptides was assessed complete MH medium before performing the ONPG/β-galactosidase assay.

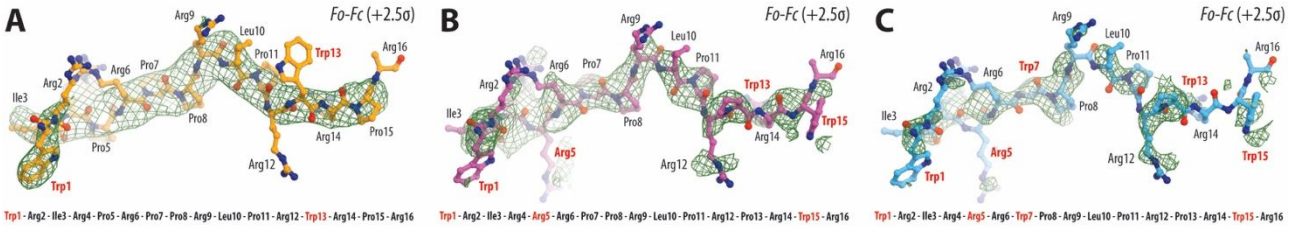
	Compounds						
	Bac7(1-16)	B7-001	B7-002	B7-003	B7-004	B7-005	Colistin
MIC (µM)	1	1	1	2	1	1	0,065
MBC (µM)	1	1	1	2	2	2	0,065



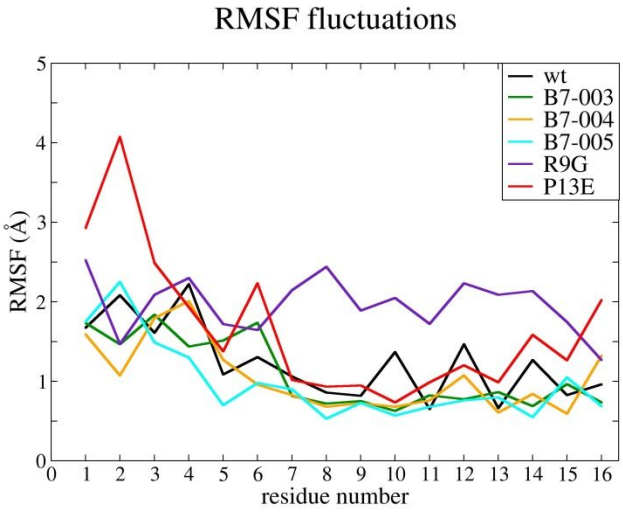
Supplementary Figure 3. Electron density maps of ribosome-bound Bac7(1-16) peptides. (**A, B**) Unbiased Fo-Fc electron density map of B7-001 (yellow, A) or B7-002 (green, B) in complex with the *T. thermophilus* 70S ribosome (green mesh). (**C, D**) 2Fo-Fc electron density map (blue mesh) of B7-001 (C) or B7-002 (D). The refined models of both peptides are displayed in their respective electron density before (A, B) and after (C, D) the refinement contoured at 2.5σ and 1.0σ, respectively. Carbon atoms are colored yellow for B7-001 and green for B7-002, nitrogens are blue, and oxygens are red. Mutated residues are highlighted in red. PDB entry of the starting structure: 4Y4P



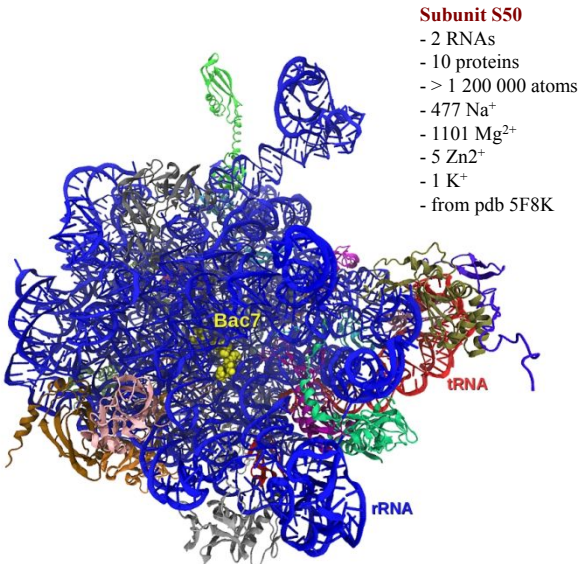
Supplementary Figure 4. Comparison of the structures of Bac7(-16) peptides on the ribosome. (**A-C**) Superposition of the ribosome-bound conformations of (**A**) B7-001 (yellow) with B7-002 (green), (**B**) wild-type Bac7(1-16) (teal) with B7-001 (yellow), and (**C**) wild-type Bac7(1-16) (teal) with Bac7-002 (green). Substitutions in B7-001 (R1W) and B7-002 (P15W) are colored red for reference. All structures of ribosome-bound Bac7 peptides were aligned based on the domain V of the 23S rRNA. PDB entry of the starting structure: 4Y4P



Supplementary Figure 5. Electron density maps of ribosome-bound Bac7(1-16) derivatives that exhibited poor binding affinity. (**A, B, C**) Unbiased Fo-Fc electron density map of B7-003 (orange, A), B7-004 (magenta, B), or B7-005 (light blue, C) in complex with the *T. thermophilus* 70S ribosome (green mesh). All electron density maps are contoured at 2.5σ. Carbon atoms are colored orange for B7-003, magenta for B7-004, and light blue for B7-005, nitrogens are blue, and oxygens are red. Mutated residues are highlighted in red. PDB entry of the starting structure: 4Y4P



Supplementary Figure 6. Root Mean Square Fluctuation (RMSF) per residue for wt Bac7(1-16) and derivatives (indicated in the inbox). Lower flexibility indicates more stable binding and possibly increased binding affinity. PDB entry of the starting structure: 5F8K.



Supplementary figure 7. 1,350,000 atoms of the 50S ribosomal subunit model used for molecular dynamics simulations. PDB entry of the starting structure: 5F8K.