SUPPLEMENTARY ONLINE MATERIALS

for

Structure of the mammalian antimicrobial peptide Bac7(1-16) bound within the exit tunnel of a bacterial ribosome

A. Carolin Seefeldt[1,2,3], Michael Graf[4], Natacha Pérébaskine[1,2,3], Fabian Nguyen[4], Stefan Arenz[4], Mario Mardirossian[5], Marco Scocchi[5], Daniel N. Wilson[4,6,*] and C. Axel Innis[1,2,3,*]

[1] Institut Européen de Chimie et Biologie, University of Bordeaux, Pessac, 33607, France

[2] U1212, Inserm, Bordeaux, 33076, France

[3] UMR[---], CNRS, Bordeaux, 33076, France

[4] Gene Center and Department for Biochemistry, University of Munich, Munich, 81377, Germany

[5] Department of Life Sciences, University of Trieste, Trieste, 34127, Italy

[6] Center for integrated Protein Science Munich (CiPSM), University of Munich, Munich, 81377, Germany

* To whom correspondence should be addressed. Tel: +33 540006149; Fax: +33 540002215; Email: axel.innis@inserm.fr. Correspondence may also be addressed to: wilson@lmb.uni-muenchen.de.

The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors.

Table S1. X-ray data processing and crystallographic refinement statistics

	Bac7(1-16)	**MetI**	**Pyr**
PDB code	5F8K	5FDU	5FDV
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Unit cell dimensions			
a	209.8 Å	209.7 Å	209.9 Å
b	450.3 Å	448.1 Å	450.1 Å
c	622.2 Å	623.4 Å	622.9 Å
α	90.0°	90.0°	90.0°
β	90.0°	90.0°	90.0°
γ	90.0°	90.0°	90.0°
Data processing			
Resolution	50 Å – 2.8 Å	50 Å – 2.9 Å	50 Å – 2.8 Å
R_{Merge}	51.3% (233.9%)	17.0% (181.0%)	17.8% (229.7%)
I/σI	5.71 (0.95)	11.61 (1.10)	15.99 (1.29)
CC 1/2	95.7 (16.1)	99.7 (34.9)	99.9 (41.1)
Completeness	99.6% (97.6%)	99.6% (99.5%)	100% (100%)
Redundancy	8.3 (8.1)	6.9 (6.7)	13.8 (13.4)
Refinement			
R_{work}/R_{free}	24.8% / 29.2%	18.3% / 23.4%	18.9% / 24.0%
Bond deviations	0.018 Å	0.030 Å	0.029 Å
Angle deviations	1.083°	1.976°	1.942°
Figure of merit	0.80	0.84	0.83
Ramachandran outliers	0.56%	0.95%	0.87%
Favorable backbone	94.3%	92.4%	93.3%

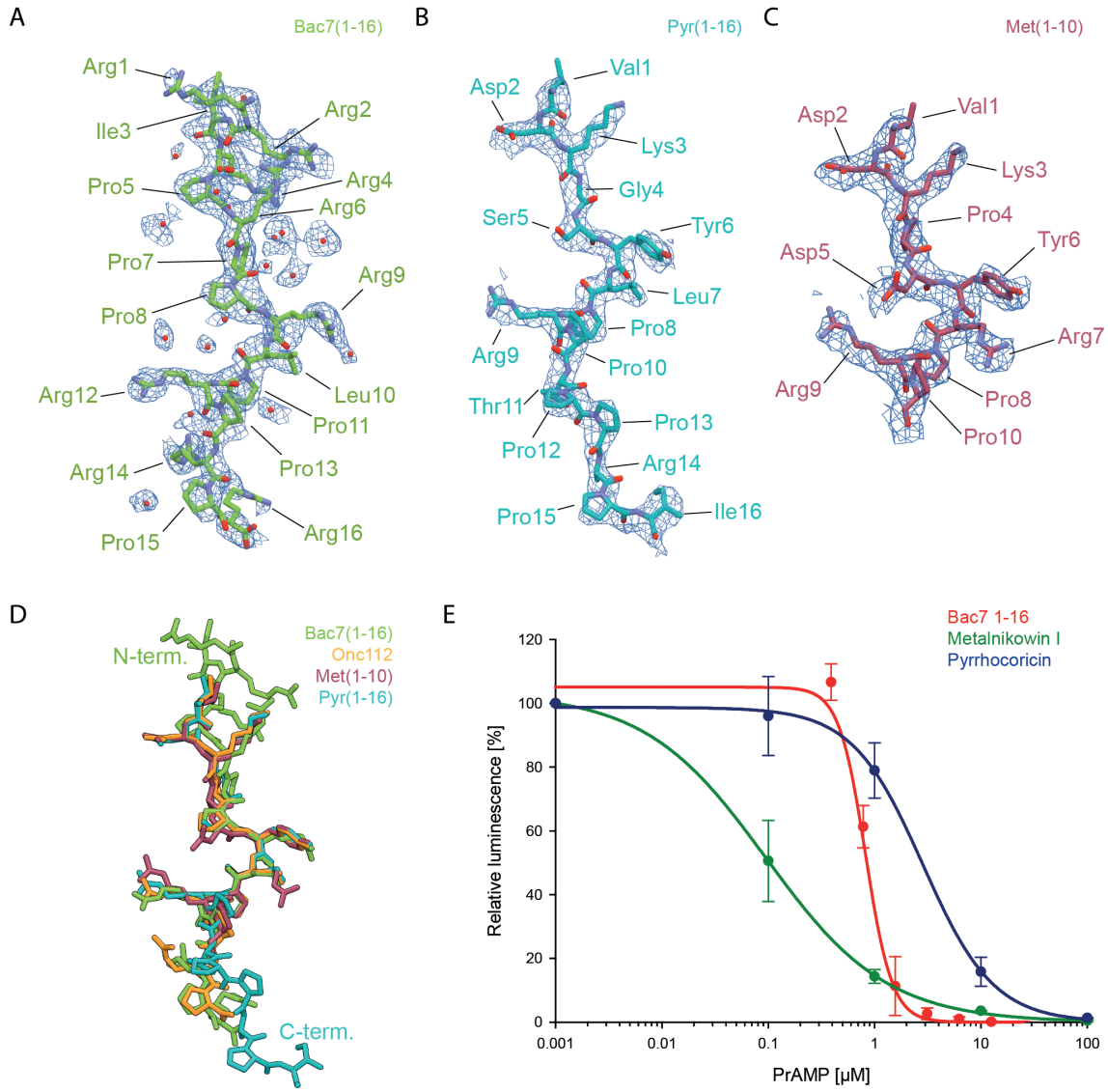

Figure S1. Minimally biased electron density for (A) the Bac7(1-16) peptide (green) and surrounding solvent molecules, as well as the (B) Pyr(1-16) (cyan) and (C) MetI(1-10) (burgundy) peptides. The peptides are shown in the same orientation as in Figure 1A and solvent molecules are displayed as spheres (red). Continuous density for the entire peptide and clear density for the solvent molecules are observed in a minimally biased F_o–F_c difference map contoured at +2.0σ (blue mesh). (D) Superimposition of the Bac7(1-16), Onc112(1-12) (orange), Pyr(1-16) and MetI(1-10) peptides. (E) Effects of increasing concentrations of Bac7(1-16) (red), Metalnikowin I (green) and Pyrrhocoricin (green) on the luminescence resulting from the *in vitro* translation of firefly luciferase (Fluc) using an *E. coli* lysate-based system. The error bars represent the standard deviation from the mean for triplicate experiments and the luminescence is normalized relative to that measured in the absence of peptide, which was assigned as 100%.

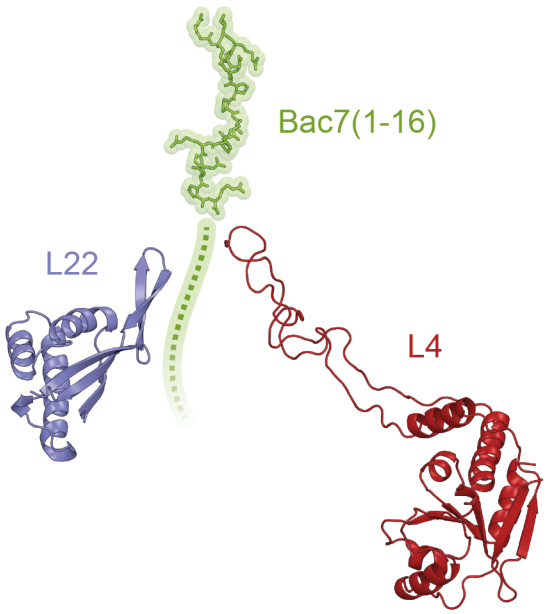

Figure S2. Relative position of the ribosome-bound Bac7(1-16) peptide (green) to the ribosomal proteins L4 (red) and L22 (blue) that reach into the lumen of the ribosomal tunnel. The proposed path for the full-length Bac7 peptide is shown as a dotted green line.

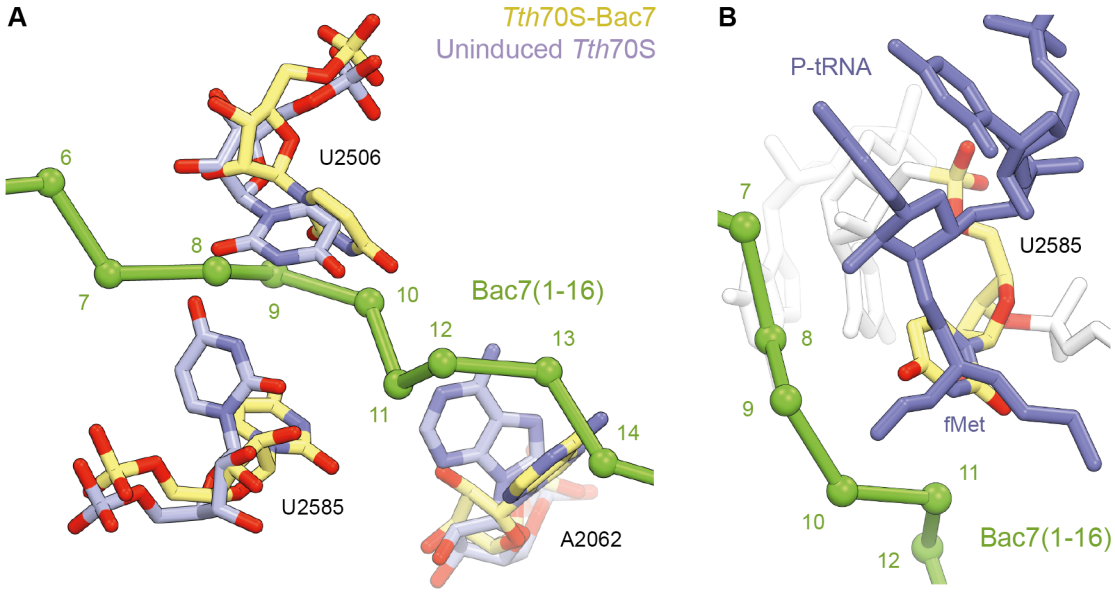

Figure S3. (A) Conformational changes in 23S rRNA nucleotides A2062, U2506 and U2585 that take place upon binding of Bac7(1-16) to the ribosome. Nucleotides from the *Tth*70S-Bac7 structure are shown in yellow, while nucleotides in the Bac7-free or "uninduced" conformation are in blue (1). (B) Clash between the formyl-methionyl moiety of a P-site bound fMet-$tRNA_i^{Met}$ (blue) and 23S rRNA residue U2585 in its Bac7-bound conformation (yellow). Bac7(1-16) is shown as a green Cα-trace in both panels.

SUPPLEMENTARY REFERENCES

1. Jenner, L., Starosta, A.L., Terry, D.S., Mikolajka, A., Filonava, L., Yusupov, M., Blanchard, S.C., Wilson, D.N. and Yusupova, G. (2013) Structural basis for potent inhibitory activity of the antibiotic tigecycline during protein synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 3812-3816.