ChemBioChem

Supporting Information

Ribosomal Target-Binding Sites of Antimicrobial Peptides Api137 and Onc112 Are Conserved among Pathogens Indicating New Lead Structures To Develop Novel Broad-Spectrum Antibiotics

Lisa Kolano, Daniel Knappe, Daniela Volke, Norbert Sträter, and Ralf Hoffmann*

Table S1: Sequences and dissociation constants (K_d) reported for apidaecin and oncocin peptides to the 70S ribosome prepared by different protocols from two *E. coli* strains.

Peptide	Sequence	K_d [nmol L^{-1}]		
		E. coli BL21(DE3) RIL[1]	*E. coli* BW25113[2]	*E. coli* BW25113 (new protocol)
Api88	gu-ONNRPVYIPRPRPPHPRL-NH_2	1,220 ± 90	n.d.	n.d.
Api137	gu-ONNRPVYIPRPRPPHPRL-OH	560 ± 60	328 ± 72	379 ± 22
Onc72	VDKPPYLPRPRPPROIYNO-NH_2	450 ± 30	n.d.	n.d.
Onc112	VDKPPYLPRPRPPRrIYNr-NH_2	90 ± 3	36 ± 14	75 ± 4

gu, O, and r denote *N, N, N', N'*-tetramethylguanidino, L-ornithine, and D-arginine, respectively. n.d. not determined

1 data from: A. Krizsan, D. Volke, S. Weinert, N. Sträter, D. Knappe, R. Hoffmann, *Angew. Chemie Int. Ed. Engl.* **2014**, *53*, 12236-12239.

2 protocol reported by A. Krizsan, D. Volke, S. Weinert, N. Sträter, D. Knappe, R. Hoffmann, *Angew. Chemie Int. Ed. Engl.* **2014**, *53*, 12236-12239.

Table S2: Percent identity matrix for the alignment of the complete bacterial 23S rRNA sequences and the human 28S rRNA sequence as calculated with Clustal2.1.

	T.t.	***S.a.***	***E.c.***	***K.p.***	***P.a.***	***A.b.***	***H.s.***
T. thermophilus	100	74	71	71	72	71	52
S. aureus	74	100	73	74	75	75	53
E. coli	71	73	100	97	85	83	53
K. pneumophila	71	74	97	100	86	84	52
P. aerigunosa	72	75	85	86	100	87	52
A. baumannii	71	75	83	84	87	100	52
H. sapiens	52	53	53	52	52	52	100

Table S3: Percentage of aligned residues relative to the full sequence length for the alignment of the complete bacterial 23S rRNA sequences and the human 28S rRNA sequence as calculated with Clustal2.1.

	T.t.	***S.a.***	***E.c.***	***K.p.***	***P.a.***	***A.b.***	***H.s.***
T. thermophilus *2914 residues*	100.0%	98.5%	97.4%	97.4%	97.2%	97.2%	99.5%
S. aureus *2921 residues*	98.2%	100.0%	97.8%	97.9%	97.5%	97.5%	99.3%
E. coli *2904 residues*	97.7%	98.4%	100.0%	99.9%	99.3%	99.4%	99.5%
K. pneumophila *2904 residues*	97.7%	98.5%	99.9%	100.0%	99.3%	99.3%	99.5%
P. aerigunosa *2912 residues*	97.2%	97.8%	99.1%	99.0%	100.0%	99.5%	99.1%
A. baumannii *2903 residues*	97.6%	98.1%	99.4%	99.4%	99.8%	100.0%	99.3%
H. sapiens *3670 residues*	79.0%	79.1%	78.7%	78.7%	78.7%	78.5%	100.0%

The percentage numbers refer to the sequences specified at the beginning of the lines, e.g. for the alignment of the human and *E. coli* RNA sequences, 78.7 % of the *H. sapiens* residues and 99.5 % of the *E. coli* residues are aligned.

Table S4: Number of ribosomal proteins detected in the ribosome preparations and the release factors identified. Ribosome preparations were separated by SDS-PAGE and lanes were cut into 14 pieces. Each piece was digested with trypsin and analyzed by LC-MS.

Species	**Ribosomal proteins**	**Release factor**
A. baumannii	33	Not detected
E. coli	31	RF1 and RF3
K. pneumoniae	28	RF1, RF2, and RF3
P. aeruginosa	38	RF1 and RF2
S. aureus	31	Not detected

Table S5: Limits of detection (LODs) and quantification (LOQs) achieved for peptides Api137 and Onc112 in bacterial cell culture supernatants and bacterial cell pellets.

Peptide	**Supernatant**		**Pellet**	
	LOD [%]	**LOQ [%]**	**LOD [%]**	**LOQ [%]**
Api137	17 ± 7	24 ± 5	13 ± 2	13 ± 2
Onc112	10 ± 4	21 ± 8	9 ± 2	15 ± 6

Table S6: Instrumental settings used on the 4000 QTRAP® to quantify peptides in medium supernatant after solid phase extraction.

Peptide	**type**	**Precursor**		**Fragment**		**DP_{opt} [V]**	**CE_{opt} [V]**	**CXP_{opt} [V]**
		Ion	***m/z***	**Ion**	***m/z***			
Onc112	Quantifier	$[M+5H]^{5+}$	478.68	y_{16}^{4+}	512.8	81	27	10
	Qualifier	$[M+5H]^{5+}$	478.68	y_{15}^{3+}	650.9	81	28	10
	Qualifier	$[M+5H]^{5+}$	478.68	y_{16}^{3+}	683.2	81	25	7
Api137	Qualifier	$[M+5H]^{5+}$	459.07	y_{17}^{4+} -NH_3	516.4	71	27	10
	Quantifier	$[M+5H]^{5+}$	459.07	y_{14}^{3+}	565.8	71	26	8
	Qualifier	$[M+5H]^{5+}$	459.07	y_{10}^{2+} -NH_3	603.4	71	30	8

DP: Declustering potential, CE: Collision potential, CXP: Collision cell exit potential

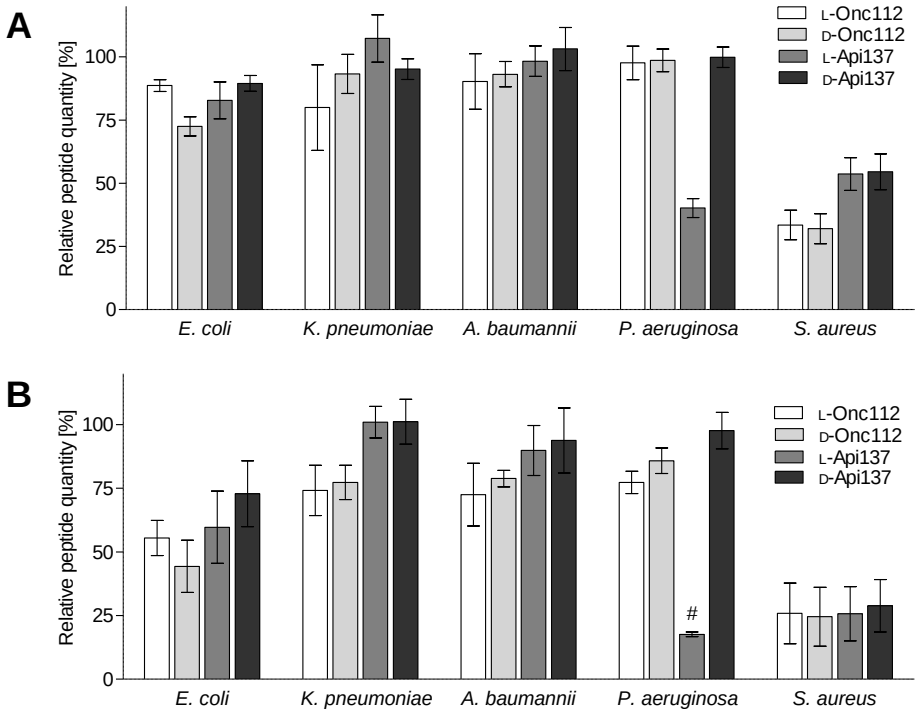

Figure S1: Peptide quantities determined for five different bacterial cell culture supernatants relative to cell-free MBH2 medium after an incubation period of 30 min. The cell counts were adjusted by optical density to ~7.5x10^8 (A) and ~7.5x10^9 cells/mL (B). Peptides were quantified after solid phase extraction by RP-HPLC-ESI-QqQ-MS using multiple reaction monitoring (MRM). # indicates a peptide quantity below the LOQ, which corresponds here to 20 %.

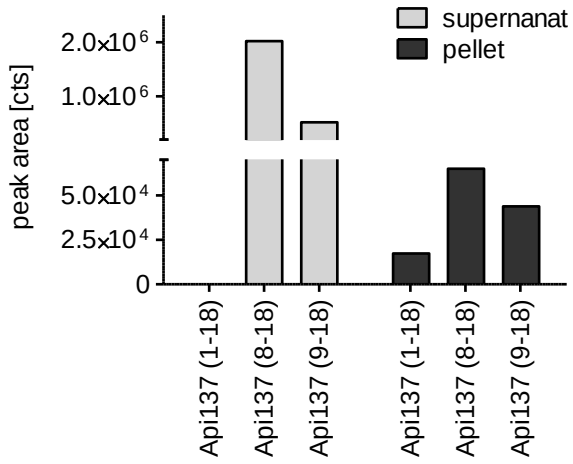

Figure S2: Peak areas obtained for Api137 and its degradation products Api137(8-18) and Api137(9-18) by LC-MS when loading similar aliquots of *P. aeruginosa* cell culture supernatants and cell pellets after an incubation period of 30 min ($7.5x10^9$ cells/well). Peptides were analyzed after solid phase extraction by nanoRP-HPLC-ESI-QTOF-MS^E.

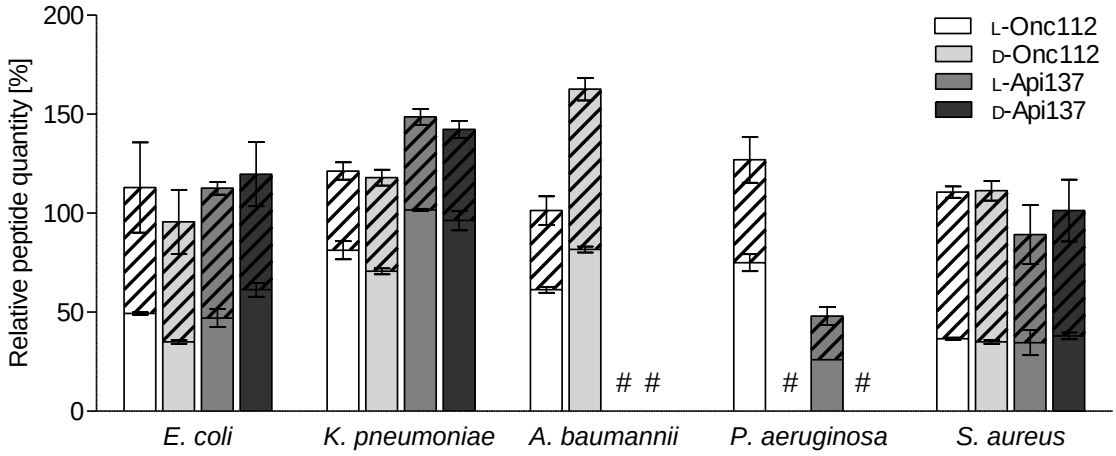

Figure S3: Peptide quantities determined in culture supernatants (blank bars) and cell pellets (striped bars) using ~7.5x10^9 cells/mL relative to cell-free MBH2 medium after incubation for 30 min. Each bar represents three replicates. Peptides were quantified after solid phase extraction by RP-HPLC-ESI-MS using multiple reaction monitoring (MRM). # indicates that peptide quantities were not determined in cell pellet. The error bars indicate the standard deviations calculated for the individual measurements of the supernatants and the cell pellets.

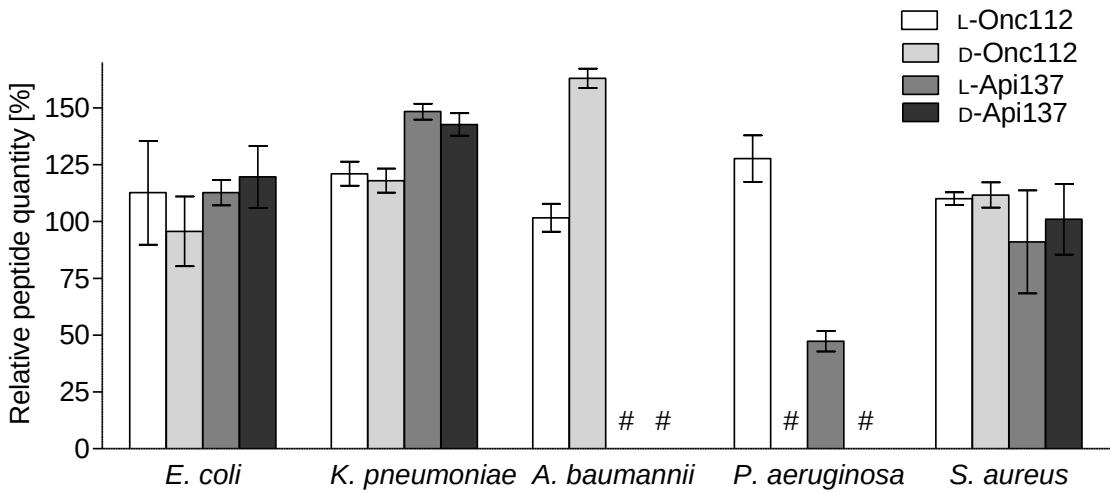

Figure S4: Total peptide quantities determined individually in the cell culture supernatants and the corresponding cell pellets relative to cell-free MBH2 medium, as shown in Figure S2.