

Supplementary material

Table S1. The strains used in this study.

Strains	Description	Source
<i>E. coli</i> DH5 α	Host for gene expression	Invitrogen
<i>E. coli</i> BL21 (DE3)	Host for gene expression	Invitrogen
B673	Wild-type strain containing antimicrobial peptide sequence.	Lab collection

Table S2. Sequences of the Plasmid used in this study

Plasmids	Description	Source
pET-28a (+)	Km ^R , replicate in <i>E. coli</i> BL21	Invitrogen
28a-742	The plasmid contains the AMP742 fragment.	This work
28a-724	The plasmid contains the AMP724 fragment.	This work
28a-427	The plasmid contains the AMP427 fragment.	This work
28a-472	The plasmid contains the AMP472 fragment.	This work
28a-247	The plasmid contains the AMP247 fragment.	This work
28a-274	The plasmid contains the AMP273 fragment.	This work
28a-sumo	The plasmid contains the sumo tag fragment.	Invitrogen

Table S3. Sequences of the primers used in this study

Primer name	Primer sequence
742-z-sumo1	TGACGACGACAAATGGACACCCGCTCTATCAG
742-z-sumo2	GTCCGACATCGCCATTGCCGGCTG
742-p-sumo1	GGCAATGGCGATGTCGGACTCAGACAGTCAATCAAG
742-p-sumo2	ATTTGTCGTCGTCATCACCAATCTGTTCTCTGTGAGCC
724-z-sumo1	GCTTGCACCCGTGCGTGCTGTACTACAAATACGTTTAGT CTATCAGATTACTGG
724-z-sumo2	GTGCATGGCGTGGTGCAACGATGTAAATCCAGAACTA
724-p-sumo1	GCACCCGTGCGTGCTGTACTACAAATACGTTTAGTCTAT CAGA
724-p-sumo2	CAAAGAGTGCATGGCGTGGTGCAACGATGTAAATCCAG AAACTACACCC
247-z-sumo1	ACCACCAAGTGCACCTCTCGTTGTTGGACACCCGCTCT ATCAG
247-z-sumo2	TGCACCCGTGCGTGCGAGGCAGCTGCGAAAGAAGCAG
247-p-sumo1	CCAAGTGCACCTCTCGTTGTTGGACACCCGCTCTATCA GT
247-p-sumo2	ACGGCTTGCACCCGTGCGTGCGAGGCAGCTGCGAAAG AAG
274-z-sumo1	ACCACGGCTTGCACCCGTGCGTGCAACGATGTAAATCC AGAAACTACACCC
274-z-sumo2	CCACCAAGTGCACCTCTCGTTGTGAGGCAGCTGCGAA AGAAGC
274-p-sumo1	TGTGCCCCGACCACCAAGTGCACCTCTCGTTGTGAGGCA GCTGCGAAAGAAGCAG
274-p-sumo2	TGCACCCGTGCGTGCAACGATGTAAATCCAGAACTAC
472-z-sumo1	GCACCCGTGCGTGCTGTACTACAAATACGTTTAGTCTAT C

472-z-sumo2	CCAAGTGCACCTCTCGTTGTTGGACACCCGCTCTATCA G
472-p-sumo1	CCAAGTGCACCTCTCGTTGTTGGACACCCGCTCTATCA G
472-p-sumo2	CGGCTTGCACCCGTGCGTGCTGTACTACAAATACGTTT AGTC
427-z-sumo1	aggtcacagagaacagattggtAACGATGTAAATCCAGAACTAC ACCC
427-z-sumo2	CCAAGTGCACCTCTCGTTGTTGTACTACAAATACGTTTA GTC
427-p-sumo1	ACCAAGTGCACCTCTCGTTGTTGTACTACAAATACGTTT AGTCTATCAGATTAC
427-p-sumo2	gggtcacagagaacagattggtAACGATGTAAATCCAGAA
T7	TAATACGACTCACTATAGGG
T7t	GCTAGTTATTGCTCAGCGG

Table S4. MIC of different series connection combinations of AMP7, AMP4, and AMP2.

Series connection order	Sequence	MIC ($\mu\text{g/mL}$)
AMP247	CTTNTFSLSDYWGNKGGWCTVSKECMAWC NDVNPETTPATTSSWTCITAGVTVSASLCPTT KCTSRCWTPALSVITGYISSNTCPTTACTRAC	12.5
AMP274	CTTNTFSLSDYWGNKGGWCTVSKECMAWC WTPALSVITGYISSNTCPTTACTRACNDVNPE TTPATTSSWTCITAGVTVSASLCPTTKCTSRC	6.25
AMP427	NDVNPETTPATTSSWTCITAGVTVSASLCPTT KCTSRCCTTNTFSLSDYWGNKGGWCTVSKE CMAWCWTPALSVITGYISSNTCPTTACTRAC	12.5
AMP472	NDVNPETTPATTSSWTCITAGVTVSASLCPTT KCTSRCWTPALSVITGYISSNTCPTTACTRAC CTTNTFSLSDYWGNKGGWCTVSKECMAWC	6.25
AMP724	WTPALSVITGYISSNTCPTTACTRACCTTNTF SLSDYWGNKGGWCTVSKECMAWCNDVNPE TTPATTSSWTCITAGVTVSASLCPTTKCTSRC	12.5
AMP742	WTPALSVITGYISSNTCPTTACTRACNDVNPE TTPATTSSWTCITAGVTVSASLCPTTKCTSRC CTTNTFSLSDYWGNKGGWCTVSKECMAWC	3.13

Table S5.MM/GBSA binding free energy decomposition (kcal/mol)

Component	Value	SD
ΔE_{vdw}	-77.80	6.0
ΔE_{elec}	-228.44	27.5
ΔG_{polar}	+264.63	25.5
$\Delta G_{\text{nonpolar}}$	-10.48	1.2
ΔG_{bind}	-52.09	8.8

Table S6.Mass Spectrometry Detailed Parameters

Compound Name	Charge	Retention time	Precursor Ion	MS1 Res	Product Ion	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
WTPALSVITGYISSNTCPTTACTR	2+	3.14	1329.14	Unit	1468.65	20	130	42.2	4	Positive
WTPALSVITGYISSNTCPTTACTR	2+	3.14	1329.14	Unit	1355.57	20	130	42.2	4	Positive
WTPALSVITGYISSNTCPTTACTR	2+	3.14	1329.14	Unit	806.38	20	130	42.2	4	Positive
CVDCTTNTFSLSDYWGNK	2+	5.26	1084.46	Unit	1431.65	20	130	34.6	4	Positive
CVDCTTNTFSLSDYWGNK	2+	5.26	1084.46	Unit	1317.61	20	130	34.6	4	Positive
CVDCTTNTFSLSDYWGNK	2+	5.26	1084.46	Unit	1216.56	20	130	34.6	4	Positive
GGWCTVSK	2+	7.62	447.71	Unit	837.39	20	130	14.9	4	Positive
GGWCTVSK	2+	7.62	447.71	Unit	780.37	20	130	14.9	4	Positive
GGWCTVSK	2+	7.62	447.71	Unit	594.29	20	130	14.9	4	Positive
DHMLVHEYVNAAGITEAAAK	2+	11.22	1070.53	Unit	1407.71	20	130	34.2	4	Positive
DHMLVHEYVNAAGITEAAAK	2+	11.22	1070.53	Unit	1278.67	20	130	34.2	4	Positive
DHMLVHEYVNAAGITEAAAK	2+	11.22	1070.53	Unit	1115.61	20	130	34.2	4	Positive

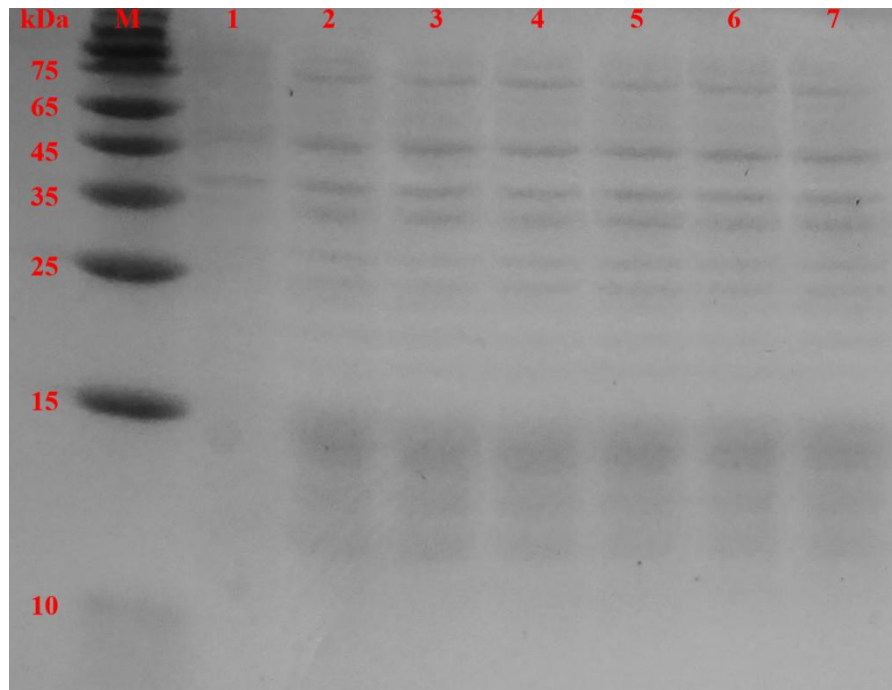


Figure S1. SDS-PAGE images of each tandem peptide combination with Sumo tag. M: 180 kDa Marker; Lane 1: pET-28a empty vector expression supernatant; Lanes 2-7: SUMO-AMP742, SUMO-AMP724, SUMO-AMP472, SUMO-AMP247, SUMO-AMP274, and SUMO-AMP427 (Note: Corresponding supernatants from *Escherichia coli* expression)

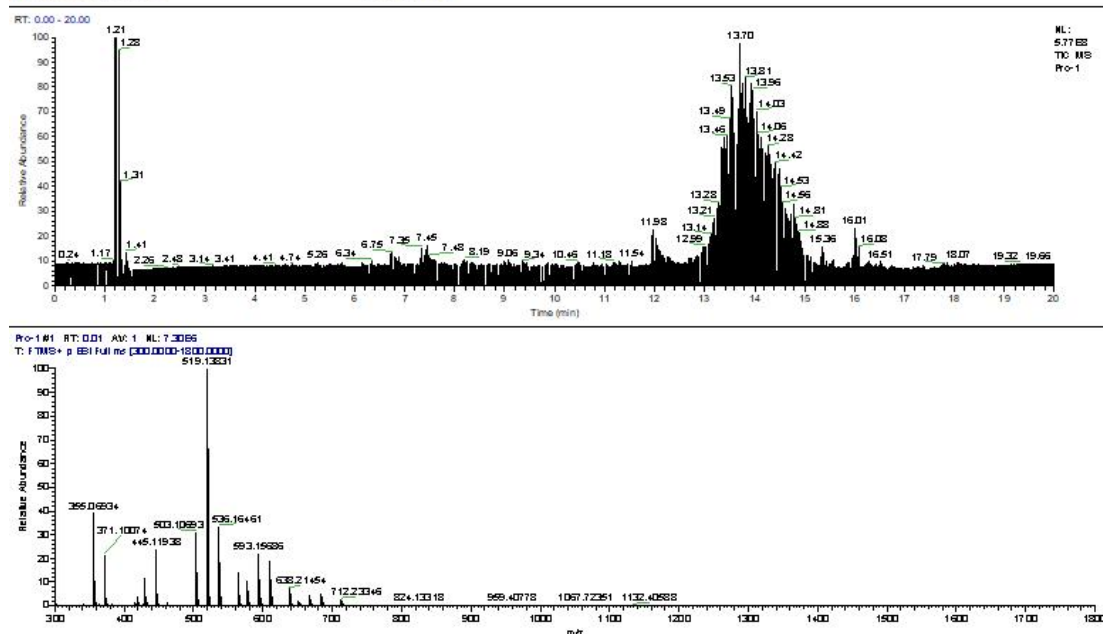


Figure S2. Mass spectrometry results