Supporting Information

Genome mining and heterologous expression reveal streptacidin, a new lasso peptide from *Streptacidiphilus jiangxiensis*

Authors: Jiwu Huang, Botao Cheng, Chunyang Cao*, and Wen Liu*

Affiliations: State Key Labratory of Chemical Biology, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China

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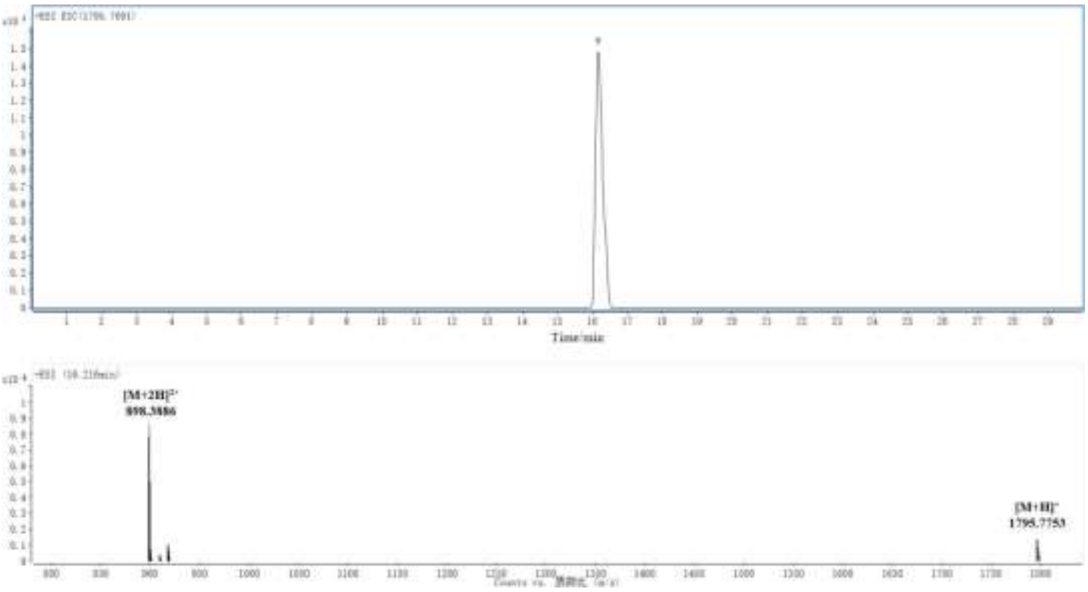

Figure S1. HR-ESI-MS of streptacidin.

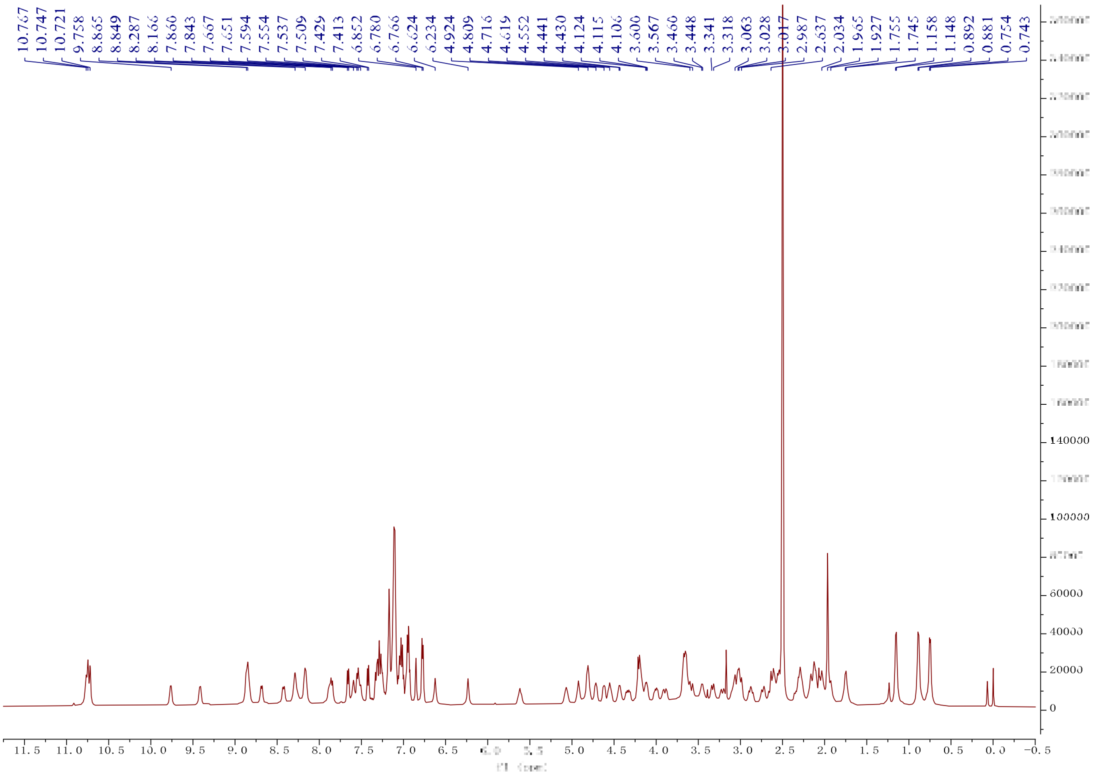

Figure S2. ^{1}H NMR spectrum of streptacidin (500 MHz, DMSO-d_6).

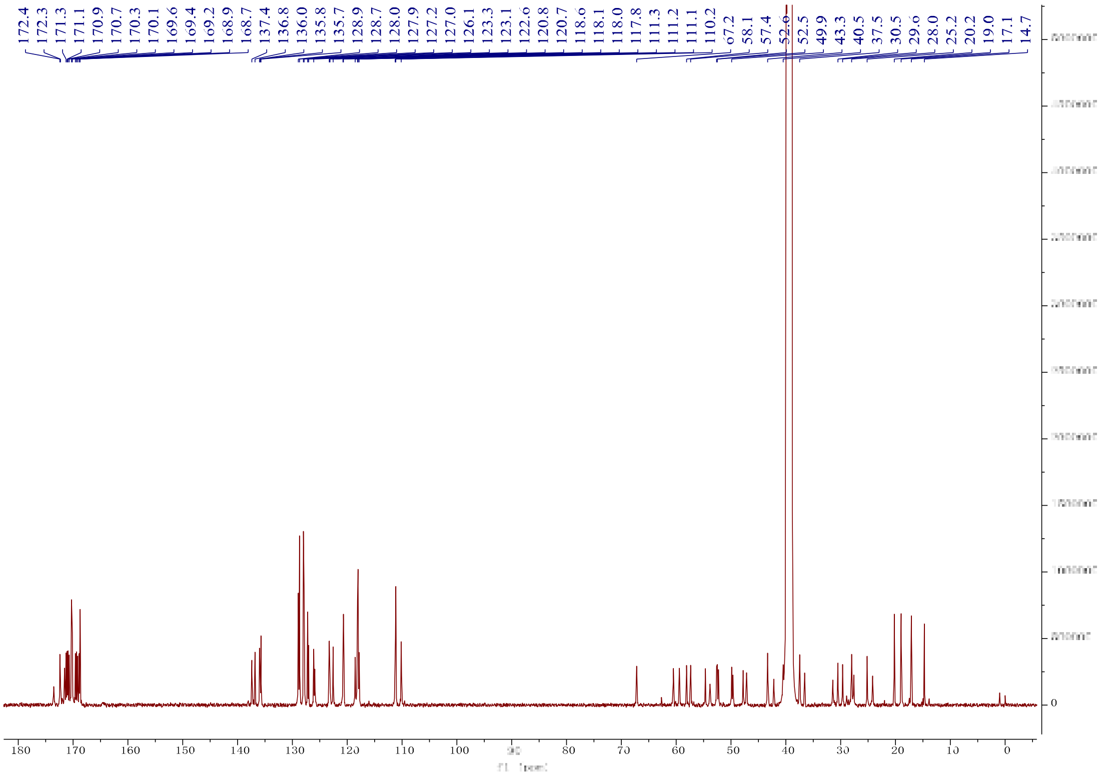

Figure S3. ^{13}C NMR spectrum of streptacidin (125 MHz, DMSO-d_6).

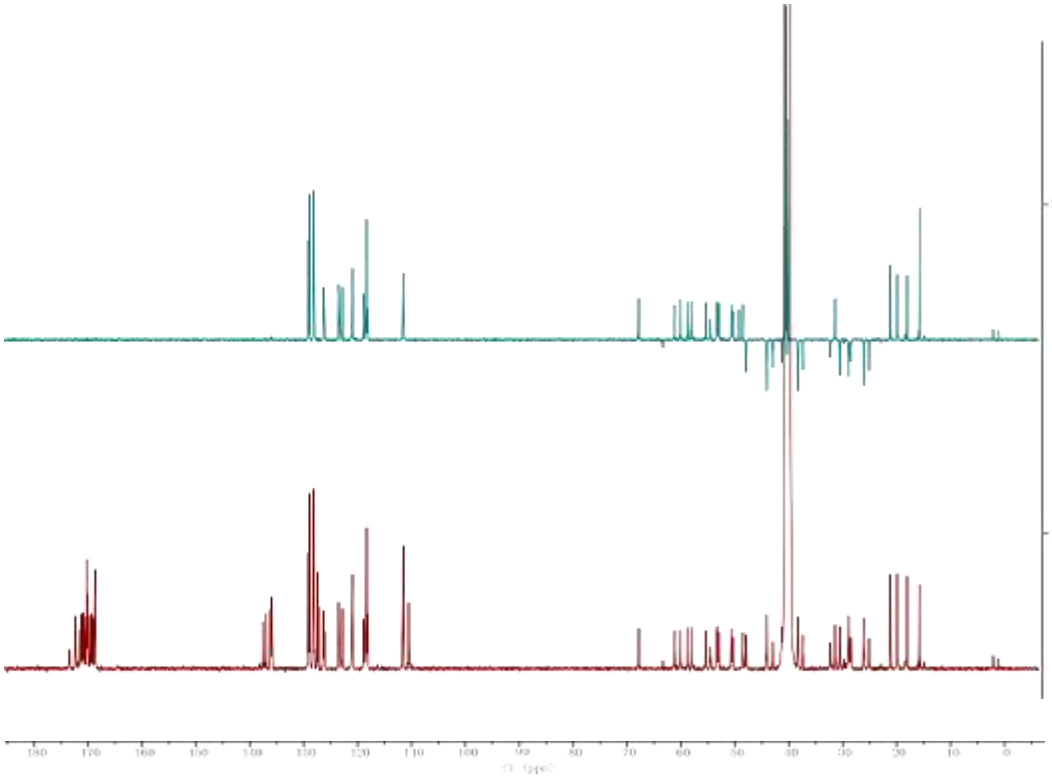

Figure S4. DEPT spectra of streptacidin.

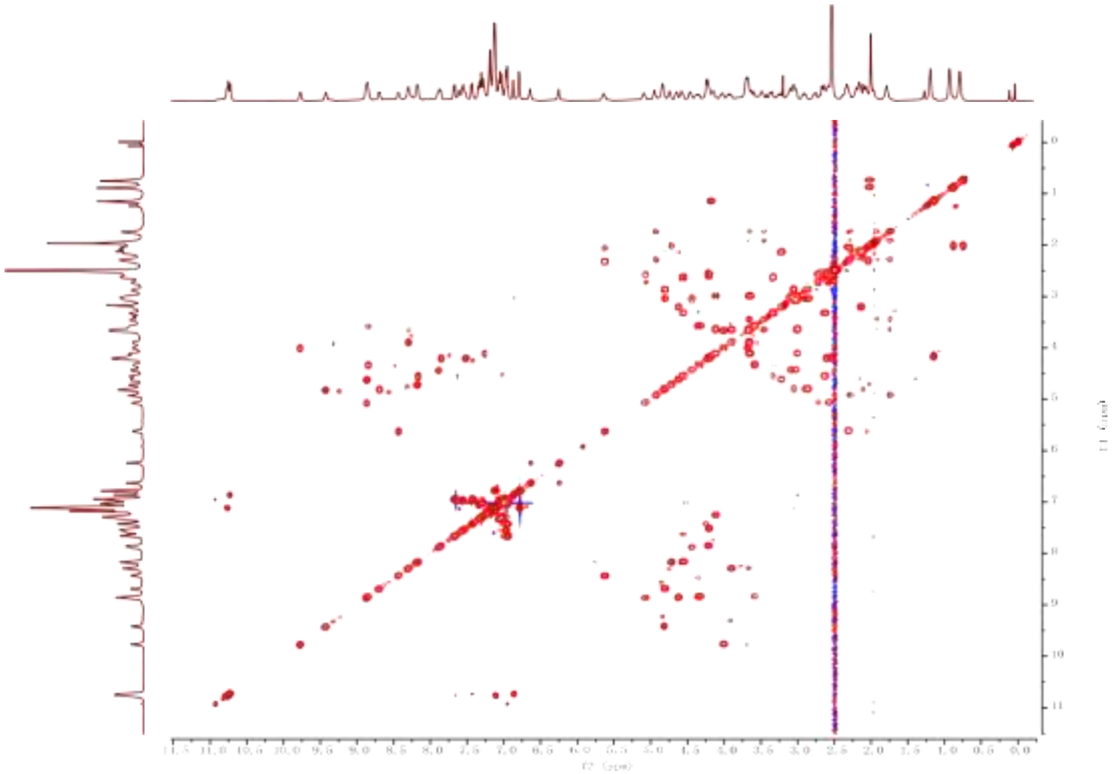

Figure S5. ^{1}H-^{1}H COSY spectrum of streptacidin.

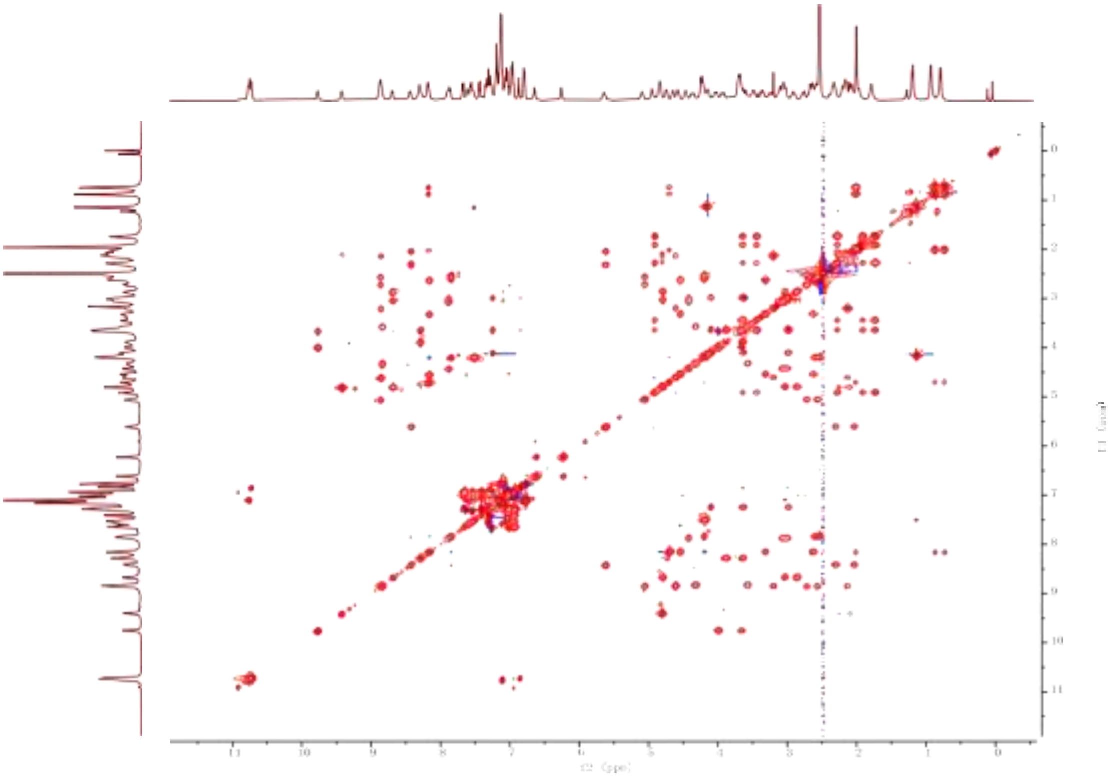

Figure S6. TOCSY spectrum of streptacidin.

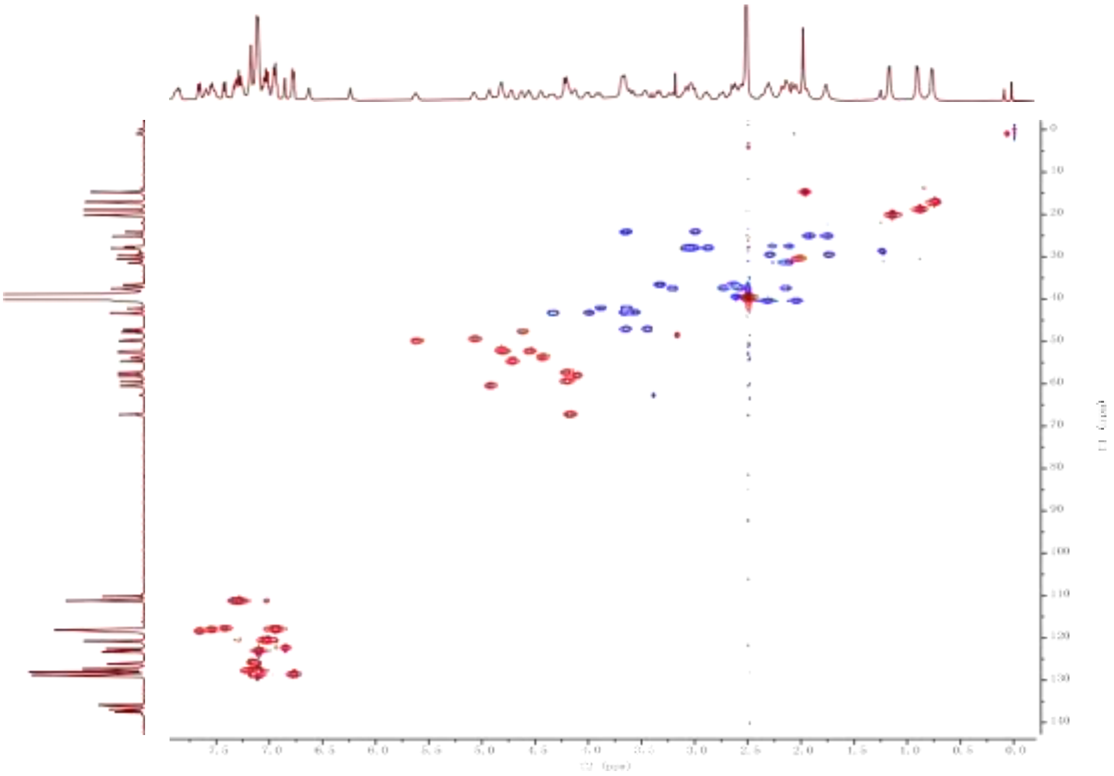

Figure S7. HSQC spectrum of streptacidin.

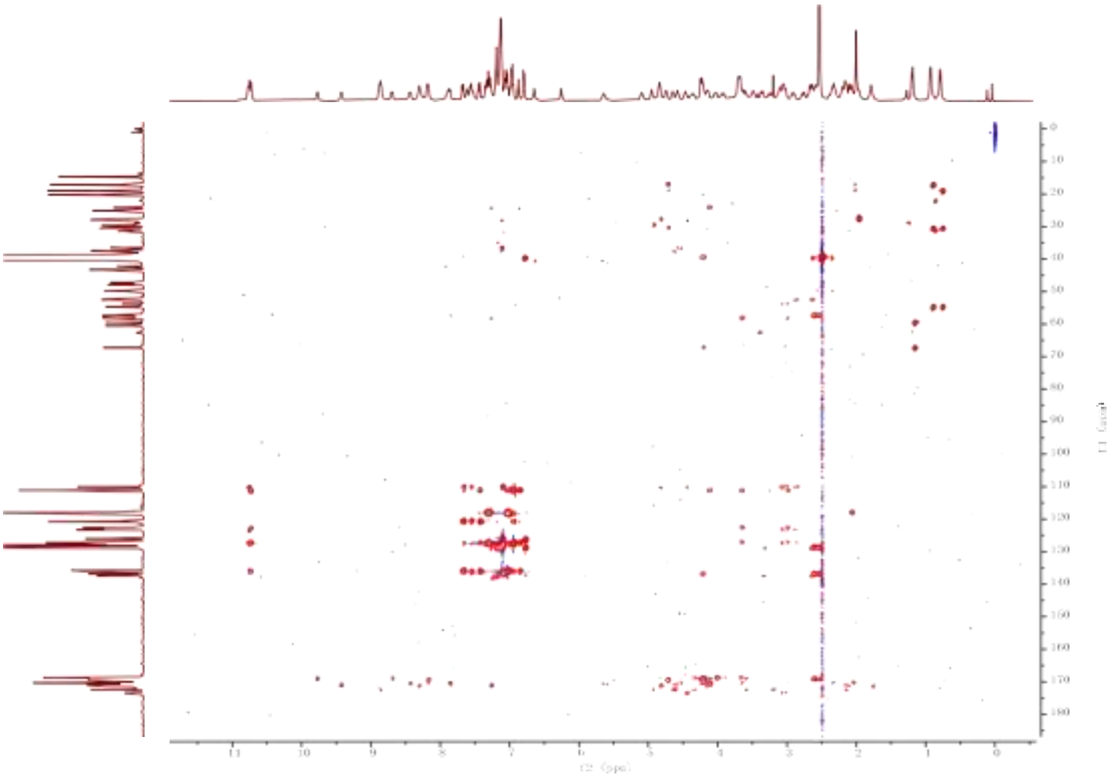

Figure S8. HMBC spectrum of streptacidin.

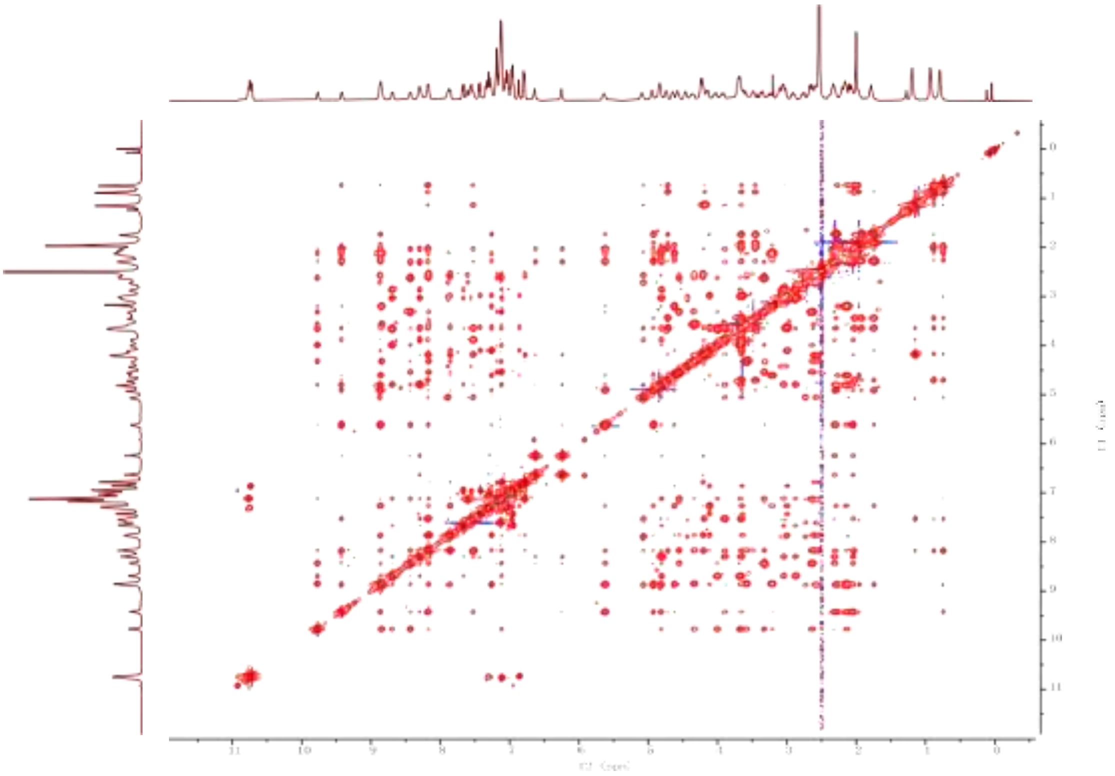

Figure S9. NOESY spectrum of streptacidin.

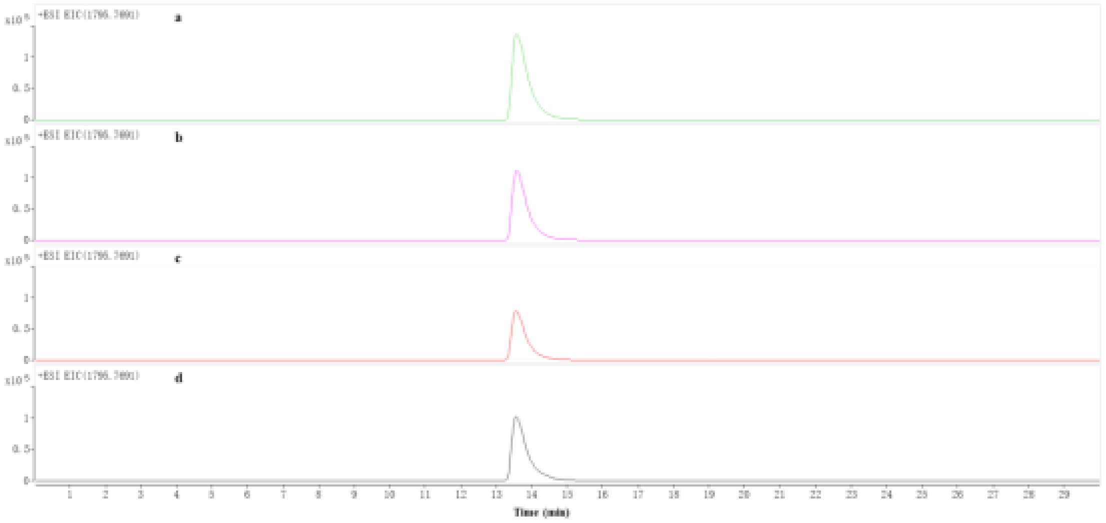

Figure S10. Protease and thermal stability of streptacidin. (a) Incubation of streptacidin with carboxypeptidase Y for 4 h at 25 °C; (b) Incubation of streptacidin with chymotrypsin for 4 h at 37 °C; (c) Incubation of streptacidin for 4 h at 95 °C; (d) Control, incubation of streptacidin for 4 h at 25 °C.

Table S1. Structural statistics for streptacidin.

	streptacidin
NMR distance restraints	
Total NOEs	390
Restraints of streptacidin	
Intra-residue of streptacidin	144
Inter-residue of streptacidin	246
Sequential (|i-j|=1)	110
Non-sequential (|i-j|>1)	136
RMSD	
Mean Global Backbone RMSD	0.09 ±0.02 Å
Mean Global Heavy RMSD	1.86 ±0.47 Å

Table S2. Selected inter-residue NOE correlations of streptacidin.

Residues 1–8 (ring)			
Atom1	**Atom2**	**Atom1**	**Atom2**
G1 NH	G2 NH	T5 αH	V6 NH
G1 NH	G2 αH	T5 αH	V6 βH
G1 NH	V6 γ1/2H	T5 γH	G4 αH
G1 αH1	G2 NH	V6 NH	G4 αH1
G1 αH1	D8 βH1	V6 NH	G4 αH2
G1 αH1	D8 βH2	V6 NH	P7 αH
G1 αH1	G2 NH	V6 βH	P7 δH1
G1 αH2	D8 βH1	V6 βH	P7 δH2
G1 αH2	D8 βH2	V6 γ1H	T5 βH
G2 NH	W3 αH	V6 γ1H	T5 γH
G2 NH	W3 δ1H	V6 γ1H	P7 δH2
G2 NH	D8 βH	V6 γ2H	T5 γH
G2 αH1	W3 NH	V6 γ2H	T5 βH
G2 αH2	W3 NH	V6 γ2H	P7 δH1
G2 αH	W3 βH	V6 γ2H	P7 δH1
W3 NH	G2 NH	V6 γ2H	P7 δH2
W3 NH	G4 NH	V6 γ1/2H	T5 NH
W3 αH	G4 NH	V6 γ1/2H	P7 βH
W3 βH1	G4 NH	P7 αH	V6 αH
W3 βH2	G4 NH	P7 αH	D8 NH
W3 βH	G4 αH	P7 αH	D8 αH
W3 ε3	G2 αH	P7 βH	V6 αH
W3 ε3	G4 NH	P7 βH	V6 NH
W3 δ1H	G2 αH1	P7 βH	D8 NH
W3 δ1H	G2 αH2	P7 δH	T5 γH
G4 NH	T5 αH	P7 γH1	V6 αH
G4 NH	V6 NH	P7 γH2	V6 αH
G4 αH1	T5 NH	P7 δH1	V6 αH
G4 αH1	T5 αH	P7 δH2	V6 αH
G4 αH2	T5 NH	D8 NH	W3 NH
G4 αH2	T5 αH	D8 αH	P7 βH
T5 NH	W3 αH	D8 βH	G1 NH
T5 NH	G4 NH	D8 βH	G1 αH
T5 NH	V6 NH	D8 βH	P7 αH
T5 NH	V6 αH		

Residues 9–12 (loop) with residues 1–8 (ring)			
Atom1	**Atom2**	**Atom1**	**Atom2**
W9 NH	P7 αH	F11 δ1/δ2H	G1 NH
W9 NH	P7 βH	F11 δ1/δ2H	G1 αH

W9 NH	P7 γH	F11 δ1/δ2H	W3 NH
W9 NH	D8 NH	F11 δ1/δ2H	G2 αH
W9 NH	D8 αH	F11 δ1/δ2H	W3 βH
W9 NH	D8 βH1	F11 δ1/δ2H	G4 NH
W9 NH	D8 βH2	F11 δ1/δ2H	D8 NH
W9 αH	P7 βH1	F11 ε1/ε2H	G1 αH
W9 αH	P7 βH2	F11 ε1/ε2H	G2 αH
W9 αH	D8 βH	F11 ε1/ε2H	W3 ζ3H
W9 βH	D8 αH	N12 NH	G1 αH
W9 η2H	W3 ζ2H	N12 NH	G2 NH
W9 δ1H	D8 αH	N12 NH	G2 αH1
F10 NH	G1 αH	N12 NH	G2 αH2
F10 NH	P7 αH	N12 NH	W3 NH
F10 NH	D8 NH	N12 NH	G4 NH
F10 NH	D8 αH	N12 NH	P7 αH
F10 NH	D8 βH	N12 NH	D8 NH
F10 αH	W3 δ1H	N12 αH	G2 NH
F10 βH1	G1 αH1	N12 αH	V6 NH
F10 βH1	G1 αH2	N12 αH	P7 γH
F10 βH2	G1 αH1	N12 αH	P7 δH
F10 βH2	G1 αH2	N12 αH	D8 βH
F10 ε1/ε2H	G1 αH	N12 αH	D8 NH
F10 ε1/ε2H	W3 βH	N12 αH	D8 αH
F10 ε1/ε2H	W3 ε3H	N12 αH	D8 βH
F10 ε1/ε2H	W3 ε1H	N12 βH	G2 NH
F11 NH	G1 αH	N12 βH	V6 NH
F11 NH	G2 NH	N12 βH	P7 αH
F11 NH	P7 αH	N12 βH1	G4 NH
F11 NH	D8 NH	N12 βH1	G4 αH
F11 αH	G2 NH	N12 βH1	T5 NH
F11 βH	G1 αH	N12 βH2	G4 NH
F11 βH	G2 αH	N12 βH2	G4 αH
F11 βH	D8 NH	N12 βH2	T5 NH
F11 βH1	G2 NH	N12 δ2H	W3 αH
F11 βH1	G2 αH	N12 δ2H	T5 αH
F11 βH2	G2 NH	N12 δ21H	G4 NH
F11 βH2	G2 αH	N12 δ22H	G4 NH
Residues 13–15 (tail) with residues 1–8 (ring)			
Atom1	**Atom2**	**Atom1**	**Atom2**
M13 NH	G4 NH	M13 γH	D8 αH
M13 NH	G4 αH1	M13 γH	D8 βH
M13 NH	G4 αH2	M13 γH1	V6 γ1H
M13 NH	T5 NH	M13 γH1	V6 γ2H

M13 NH	V6 NH	M13 γH2	V6 γ1H
M13 NH	V6 αH	M13 γH2	V6 γ2H
M13 NH	V6 γ1/2H	M13 εH	V6 γ1H
M13 NH	P7 αH	M13 εH	V6 γ2H
M13 NH	P7 βH	M13 εH	D8 NH
M13 NH	D8 NH	M13 εH	D8 αH
M13 NH	D8 αH	M13 εH	D8 βH1
M13 NH	D8 βH	M13 εH	D8 βH2
M13 αH	G2 αH	N14 αH	G4 αH
M13 αH	G4 αH1	N14 αH	G4 αH
M13 αH	G4 αH2	N14 αH	T5 NH
M13 αH	V6 NH	N14 αH	T5 γH
M13 αH	V6 γ1/2H	N14 αH	V6 NH
M13 αH	D8 NH	N14 αH	V6 γ1/2H
M13 αH	D8 βH	N14 δ2H	G4 αH1
M13 βH	V6 γ1/2H	N14 δ2H	G4 αH2
M13 γH	G2 NH	W15 NH	T5 NH
M13 γH	V6 NH	W15 βH	V6 γ1/2H
M13 γH	D8 NH	W15 ε3H	T5 γH

Table S3. Antimicrobial activity of streptacidin (MIC, μg/mL).

Samples	**MRSA**	**MSSA**	**VRE**	**VSE**	**PAE**
streptacidin	＞256	＞256	＞256	256	256
vancomycin	1	0.5	128	4	–
polymyxin B	–	–	–	–	0.5

MRSA: Methicillin resistant *Staphylococcus aureus* ATCC 43300;

MSSA: Methicillin sensitive *Staphylococcus aureus* ATCC 29213;

VRE: Vancomycin resistant *Enterococcus faecalis* ATCC 51299;

VSE: Vancomycin sensitive *Enterococcus faecalis* ATCC 29212;

PAE: *Pseudomonas aeruginosa* ATCC902;

Table S4. Oligonucleotide sequences used to assemble *str* cluster for streptacidin (**1**). These were assembled and cloned into the expression plasmid for streptacidin.

Name	**Sequence**
Str-ACB-F	ATAAGGAGATATACCATGatgacggagaccaccgagc
Str-ACB-R	ATTATGCGGCCGCAAGCTctacctgccgggtgccacggc
Str-CB-F	ATAAGAAGGAGATATACATatgagctccggattcctcgtc
Str-CB-R	TTCTTTACCAGACTCGAGctacctgccgggtgccac

Table S5. List of locus tags, protein accession numbers, and predicted functions for genes in the biosynthetic gene cluster found in *Streptacidiphilus jiangxiensis* CGMCC 4.1857.

Locus tag	**Protein ID**	**Length**		**Gene Function**
		NT	AA	
TR47_RS39470	WP_042458987.1	912	303	2-hydroxyacid dehydrogenase
TR47_RS39475	WP_042458990.1	432	143	VOC family protein
TR47_RS39480	WP_082015615.1	3033	1010	helix-turn-helix transcriptional regulator
TR47_RS48570	WP_161791355.1	177	58	hypothetical protein
TR47_RS39485	WP_042458996.1	1512	503	Asp-tRNA(Asn)/Glu-tRNA(Gln) amidotransferase subunit GatB
TR47_RS39490	WP_042458998.1	240	79	hypothetical protein
TR47_RS39495	WP_042459001.1	1494	497	Asp-tRNA(Asn)/Glu-tRNA(Gln) amidotransferase subunit GatA
TR47_RS39500	WP_042385005.1	297	98	Asp-tRNA(Asn)/Glu-tRNA(Gln) amidotransferase subunit GatC
TR47_RS39505	WP_042459004.1	417	138	lasso peptide biosynthesis B2 protein
TR47_RS39510	WP_042459007.1	252	83	lasso peptide biosynthesis PqqD family chaperone
TR47_RS39515	WP_042459010.1	1803	600	lasso peptide isopeptide bond-forming cyclase
TR47_RS47525	WP_143094772.1	129	42	lasso RiPP family leader peptide-containing protein
TR47_RS39520	None	1416	471	putative bifunctional diguanylate cyclase/phosphodiesterase
TR47_RS51170	WP_052439462.1	1050	349	pentapeptide repeat-containing protein
TR47_RS39530	WP_042459013.1	2163	720	NAD-dependent DNA ligase LigA
TR47_RS39535	WP_075004256.1	1014	337	methionine synthase
TR47_RS39540	WP_042459016.1	690	229	SDR family oxidoreductase
TR47_RS39545	WP_052439463.1	1869	622	NAD(P)-binding protein
TR47_RS39550	WP_042459019.1	555	184	TIGR00730 family Rossman fold protein

Table S6. High-resolution MS/MS data of streptacidin (**1**).

Ions	**Calc**	**Obs**	**Er (ppm)**
$[M+H]^+$	1795.7691	1795.7666	1.39
$[M+H-NH_3]^+$	1778.7426	1778.7385	2.30
$b8^+$	752.3373	752.3350	3.06
$[b8-H_2O]^+$	734.3268	734.3250	2.45
$[b8-CO]^+$	724.3424	724.3416	1.10
$b9^+$	938.4166	938.4143	2.45
$[b9-H_2O]^+$	920.4061	920.4022	4.24
$[b9-CO]^+$	910.4217	910.4202	1.65
$b10^+$	1085.4850	1085.4829	1.93
$[b10-H_2O]^+$	1067.4744	1067.4708	3.37
$[b10-CO]^+$	1057.4901	1057.4904	0.28
$b11^+$	1232.5535	1232.5520	1.22
$[b11-H_2O]^+$	1214.5428	1214.5398	2.47
$[b11-CO]^+$	1204.5586	1204.5557	2.41
$y2^+$	319.1395	319.1406	3.45
$y4^+$	564.2230	564.2232	0.35