

Figure S1. Go annotation on molecular functions of small peptides identified in the cDNA library of C. flavidus venom duct.

		10	20	30
	$\cdots $	$\cdot \mid \cdot \cdot \cdot \cdot \mid \cdot \cdot \cdot$.	$\cdot \mid \cdot \cdot \cdot \mid \cdot \cdot$
A	MGMG <mark>M</mark> RMM	-FTVF <mark>LL</mark> VVL	ATTVVSFTSG	G
В	MHLY	-TYLY <mark>LL</mark> VPL	VTFHLILGTO	GTLDHGGALT
С	MQTA	-YWVMVMMV	GITA-PLSEG	<u> </u>
D	MPKLE	-MMLLV <mark>LL</mark> IL	PLSYFDAAGO	<u> </u>
I1	-MVIMKLC	-LTFL <mark>L</mark> ILMI	LPLVTG	G
I2	MMFRLT	SVSCFLLVIV	CLNLVVLVNA	·
IЗ	MKLV	-LAIVLILML	LSLSTG	GA
J	-MPSVRSV	TCCCLLWMML	SVQLVTPGSE	?
K	MI <mark>M</mark> RMT	-LTLFV <mark>L</mark> VVM	TAAS-ASG	<u> </u>
L	MNVTVM	-FLVL <mark>LLL</mark> TM	PLTDG	<u> </u>
М	MMSKLGVL	-MLFIF <mark>L</mark> VLF	PLAT-LQLDA	·
01	M <mark>M</mark> KLT	-CVLIVAVLF	LT-ACQLITA	ADDSA
02	MEKLT	-ILLLVAAVL	MS-TQALVQS	S
03	-MSGLGIM	-VLTL <mark>LLL</mark> VF	MATS-HQDGO	<u> </u>
Ρ	MHLSLARS	-AVLI <mark>LLL</mark> LF	ALGNFAVVQS	S
S	MMLK <mark>M</mark> G	-AMFV <mark>LLL</mark> LF	TLASSQQ	2
Т	MRCL	-PVFII <mark>LL</mark> LL	IASA-PSVDA	·
V	MM	-PVILP <mark>LL</mark> LS	LAIR-GGDG-	
Y	<mark>M</mark> QKA	-TVLL <mark>L</mark> AL	LLPLSTA	
Q	MHTLE	-MLLL <mark>LLL</mark> LL	PLAPG-	

Figure S2. Alignment of consensus signal peptide sequences of different superfamilies. The consensus sequences were composed by most frequently appeared amino acid residues in each position.



SEQ: CCSDPPCRHKHQDLC, Carb(C1) Carb (C2) Carb(C7) Carb (C15) Amidation (C-term), Charge:3, MH+: 1968.7996, Score: 4.03e-011

Figure S3. MS/MS spectrum of fla1a, CCSDPPCRHKHQDLC (C-term amidation). The red peaks are speculated assignments with neutral loss of iodoacetamide radical (CHCONH₂, 57.02146) from the side chain of C-terminal iodoacetamide cysteine, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.



SEQ: GCCSDPPCRHKHQDLC, Carb(C2) Carb (C3) Carb(C8) Carb (C16) Amidation (C-term), Charge:3, MH+: 2025.8185, Score: 3.92e-011

Figure S4. MS/MS spectrum of fla1b, GCCSDPPCRHKHQDLC (C-term amidation).



SEQ: ACNPPCSDILTCLHGTCKHLGI, Carb(C2) Carb (C6) Carb(C12) Carb (C17) Amidation (C-term), Charge: 4, MH+: 2523.1657, Score: 8.90e-043

Figure S5. MS/MS spectrum of fla14a, ACNPPCSDILTCLHGTCKHLGI (C-term amidation).



SEQ: ACNPPCSDILTCLHGTCKHLGI, Carb(C2) Hydroxylation (P5) Carb (C6) Carb(C12) Carb (C17) Amidation (C-term), Charge:4, MH+: 2539.1588, Score: 1.82e-042

Figure S6. MS/MS spectrum of fla14b, ACNPPCSDILTCLHGTCKHLGI (hydro(P5), C-term amidation), orange "P" indicates P with hydroxylation.



SEQ: ACNPPCSDILTCLHGTCKHLGI, Carb(C2) Hydroxylation (P4) Hydroxylation (P5) Carb (C6) Carb(C12) Carb (C17) Amidation (C-term), Charge:4, MH+: 2523.1657, Score: 8.90e-043

Figure S7. MS/MS spectrum of fla14c, ACNPPCSDILTCLHGTCKHLGI (hydro(P4, P5), C-term amidation), orange "P" indicates P with hydroxylation.



SEQ: TCYPPCIGYTYCKSGTCEYRQ, Carb(C2) Carb (C6) Carb(C12) Carb (C17) Amidation (C-term), Charge:3, MH+: 2663.1040, Score: 1.56e-051

Figure S8. MS/MS spectrum of fla14d, TCYPPCIGYTYCKSGTCEYRQ (C-term amidation).



SEQ: CCSKYCWECTPCCPYSS, Carb(C1) Carb (C2) Carb(C6) Carb (C9) Hydrxoylation(P11) Carb(C12) Carb (C13) Amidation (C-term), Charge:2, MH+: 2319.7952, Score: 2.28e-022

Figure S9. MS/MS spectrum of fla3a, CCSKYCWECTPCCPYSS (hydro(P11), C-term amidation), orange "P" indicates P with hydroxylation. The red peaks are speculated assignments to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.



SEQ: RCCLWPACWGCVCCY, Carb(C2) Carb (C3) Carb(C8) Carb (C11) Carb(C13) Carb (C14), Charge:2, MH+: 2107.7830, Score: 6.19e-026

Figure S10. MS/MS spectrum of fla3b, RCCLWPACWGCVCCY. The red peak is speculated assignment to the breakdown of N-C α of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.



SEQ: RCCLWPECGGCVCCY, Carb(C2) Carb (C3) Carb(C8) Carb (C11) Carb(C13) Carb (C14), Charge:2, MH+: 2036.7292, Score: 3.91e-024

Figure S11. MS/MS spectrum of fla3c, RCCLWPECGGCVCCY. The red indicated peaks are speculated assignments to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.



SEQ: RCCLWPECGGCVCCY, Carb(C2) Carb (C3) Gala (E7) Carb(C8) Carb (C11) Carb(C13) Carb (C14), Charge:2, MH+: 2080.7210, Score: 5.38e-016

Figure S12. MS/MS spectrum of fla3d, RCCLWPECGGCVCCY (Gla(E7)). The red peaks are speculated assignments to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.



SEQ: EWTDFRPW, Amidation (C-term), Charge:2, MH+: 1135.5289, Score: 1.77e-013

Figure S13. MS/MS spectrum of fla01, EWTDFRPW (C-term amidation).



SEQ: EWTDFRPW, Gala(E1) Amidation (C-term), Charge:2, MH+: 1179.5192, Score: 3.18e-018

Figure S14. MS/MS spectrum of fla02, EWTDFRPW (Gla(E1), C-term amidation). There is a neutral loss of CO₂ from gamma carboxyglutamic acid Gla1.



Figure S15. MS/MS spectrum of fla03, GGLGHAGGWVKAGALG. The c ion formation in CID experiment was previously reported by reference: Farrugia JM, O'Hair RAJ, Reid GE (2001). Do all b2 ions have oxazolone structures? Multistage mass spectrometry and ab initio studies on protonated N-acyl amino acid methyl ester model systems. Int. J. Mass Spectrom. 210, 71-87.



SEQ: GGLGHAGGWVKAGALGKDPGW, Amidation (C-term), Charge:3, MH+: 1990.0303, Score: 3.48e-017

Figure S16. MS/MS spectrum of fla04, GGLGHAGGWVKAGALGKDPGW (C-term amidation). The c ion formation in CID experiment was previously reported by reference: Farrugia JM, O'Hair RAJ, Reid GE (2001). Do all b2 ions have oxazolone structures? Multistage mass spectrometry and ab initio studies on protonated N-acyl amino acid methyl ester model systems. Int. J. Mass Spectrom. 210, 71-87.



SEQ: GGLGHAGGWVKAGALGKDPGW, Charge:4, MH+: 1991.0250, Score: 1.99E-026

Figure S17. MS/MS spectrum of fla05, GGLGHAGGWVKAGALGKDPGW. The c ion formation in CID experiment was previously reported by reference: Farrugia JM, O'Hair RAJ, Reid GE (2001). Do all b2 ions have oxazolone structures? Multistage mass spectrometry and ab initio studies on protonated N-acyl amino acid methyl ester model systems. Int. J. Mass Spectrom. 210, 71-87.



SEQ: LGHAGGWVKAGALGKDPGW, Amidation (C-term), Charge:4, MH+: 1875.9901, Score: 5.49e-012

Figure S18. MS/MS spectrum of fla06, LGHAGGWVKAGALGKDPGW (C-term amidation).



SEQ: HAGGWVKAGALGKDPGW, Amidation (C-term), Charge:3, MH+: 1705.8867, Score: 7.44e-020

Figure S19. MS/MS spectrum of fla07, HAGGWVKAGALGKDPGW (C-term amidation).



SEQ: SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV, Carb (C2) Carb (C9) Carb (C14) Carb (C15) Carb (C20) Carb (C29), Charge:3, MH+: 3180.2563, Score: 3.00e-021

Figure S20. MS/MS spectrum of fla6a, SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV. The c ion formation in CID experiment was previously reported by reference: Farrugia JM, O'Hair RAJ, Reid GE (2001). Do all b2 ions have oxazolone structures? Multistage mass spectrometry and ab initio studies on protonated N-acyl amino acid methyl ester model systems. Int. J. Mass Spectrom. 210, 71-87.



SEQ: SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV, Carb (C2) Carb (C9) Hydroxylation (P13) Carb (C14) Carb (C15) Carb (C20) Carb (C29), Charge:3, MH+: 3196.2557, Score: 6.22e-018

Figure S21. MS/MS spectrum of fla6b, SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV (Hydroxylation of P13). The c ion formation in CID experiment was previously reported by reference: Farrugia JM, O'Hair RAJ, Reid GE (2001). Do all b2 ions have oxazolone structures? Multistage mass spectrometry and ab initio studies on protonated N-acyl amino acid methyl ester model systems. Int. J. Mass Spectrom. 210, 71-87.



SEQ: SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV, Carb (C2) Carb (C9) Hydroxylation (P13) Carb (C14) Carb (C15) Hydroxylation (P16) Carb (C20) Carb (C29), Charge:3, MH+: 3212.2467, Score: 5.08e-009

Figure S22. MS/MS spectrum of fla6c, SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV (hydroxylation of P13 and P16).



SEQ: TCDPPGDSCSRWYNHCCSKLCTSRNSGPTCSRP, Carb (C2) Hydroxylation (P5) Carb (C9) Carb (C16) Carb (C17) Carb (C21) Hydroxylation (P28) Carb (C30), Charge:6, MH+: 3992.5991, Score: 6.49e-011

Figure S23. MS/MS spectrum of fla6d, TCDPPGDSCSRWYNHCCSKLCTSRNSGPTCSRP (hydroxylation of P5 and P28), P marked in red indicates hydroxylated proline.



SEQ: ALCCYGYAFCCRL, Carb (C3) Carb (C4) Carb (C10) Carb (C11) Amidation (C-term), Charge:2, MH+: 1712.7104, Score: 1.24e-031

Figure S24. MS/MS spectrum of fla5a, ALCCYGYAFCCRL (C-term amidation). The red peak is speculated assignment to the breakdown of N-C α of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.



SEQ: LCCYGYAFCCRL, Carb (C2) Carb (C3) Carb (C9) Carb (C10) Amidation (C-term), Charge:2, MH+: 1641.6746, Score: 1.24e-030

Figure S25. MS/MS spectrum of fla5b, LCCYGYAFCCRL (C-term amidation). The red peak is speculated assignment to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.



SEQ: ALCCYGYRFCCPN, Carb (C3) Carb (C4) Carb (C10) Carb (C11), Charge:2, MH+: 1740.6666, Score: 1.71e-012

Figure S26. MS/MS spectrum of fla5d, ALCCYGYRFCCPN. The red peak is speculated assignment to the breakdown of N-C α of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.



Figure S27. Clone numbers of 57 non-redundant mature peptides identified in the cDNA libraray of *C. flavidus*. The sequences with corresponding peptide components being identified in MS/MS analysis are shown in columns with meshy pattern.

	10 20	30	40	50	60 7	0 80	90 100
A E126 1		 CTTCATTCOME	. %caccamecc	- I I	- TGGATCATCCAC		···· ···· ····
A.1140.1		T. D. W	D D A	D F L	V D H P	E L G	- 1888 W
01.Fla6.4		TCOXS	¢ggacatagt		GGT0	CAGGTTGT	TATACTC
		s //¢	GHS		G	A G	У Т
01.Fla6.5	ATGAGGAATCCCAAGCTCTCCAAGTTG	ACAAAGACOPS	TGATCCACCC		GGT0	ACAGTTCCTC	FAGGTGGTATA
	M R N P K L S K L	ткт 🌾	DPP		G	d s 🖉 s	R W Y
01.Fla6.6		%	CTATGATGTG		GGT0	ATTTTECEGG	CATACCGTTTATTAAGAACG
		//\$	Y D V		G	D F 🦉 G	IPFIKN
01.Fla6.7		GATTG	CACTCCTCCG		TCC#	GTTAT TGT GA	ITACCCTG
		D //S	TPP		S	S Y 🖉 D	Y P
V.Fla6.9		GGT%	CTCC			ATTTGC	AATGGAG
		G //9	s			I	N G
V.F1a6.12		AAGACGGAT/2G	CTAC			AAT PGC	GATGGAG
W P1-C 12		K T D C	1 Y			n ga	D G
V.F180.13		AAGACGGAI/PG	CTTC				AATGGAG
0 5126 14			encacca			Webcco	N G
Q.1140.14		11/2	T D				
		1177.	»			<i>171/1,</i> C	*
			.	.			,
A.Fla6.1	ACGTATGC/PG/PGCCTATCCTCCC/	TAGACACAAA	CACCAA		GAT	TAATCAG	
	DV C AYP P	🖉 к н к	H Q		D ///	N Q	
Ol.Fla6.4	GCCCTTCCTCCCCGGTCTGCATT	¢¢tctggcggc	CAAGCTGGAG	GC	CTG#C	¢gtg	
	R P C P G L H	🖉 s g g	Q A G	G	L //	v	
01.Fla6.5	ATCATTGCTGCAGTAAGTTGT	GPACTTCACGG	AATAGCGGGC	CA	ACT/20	CTCGCGCCCA-	
	NHOCSK L	C T S R	N S G	P	т //	SRP	
01.Fla6.6	GCAATTGCTGCAGTCAGTTTT	GØGTTTTT			GTC7	CACACCCGAG	rgg
	G N C S Q F	V F			v ///	T P E	W
01.F1a6./	AGGAADGPDGDGAGTAGAA2	C D U			TACEC	CGATTGG	rGG
W E1-C 0	E E Q Q E V E	С G К Н			¥ //		W
V.F140.9	GACAAPSC/D0/DGC11G2	COLIGIACICI	CGGATTATAT	CGAGATAT	AIG20	I D V) V
V F1a6 12	ANGAN CONTRACT	decremence	T I I	ACATCTTT	>	PCTAACACTT	A A A
	E E E E G G R		W P T.	0 M F	T M	V T V	EK
V.Fla6.13		<i>~//. – – – –</i>		* ** *	- 777	// · · ·	
	TATATTSCZCCACTGGZ	GCATATACTOT	GCGGCTGAAG	AGAGGCTC	AA 1990	TGTAAATCOT	IGGAAA
	TATATTSCTGTCACTGGT V Y C C H W	GCATATACTCT C I Y S	GCGGCTGAAG A A E	AGAGGCTC E R L	AATTC	TGTAAATCCT V N P	IGGAAA W K
Q.Fla6.14	TATATTSCTGTCACTGGT V Y C C H W ATCTCTGCTSCGAGCCTGGAACAACAT	GCATATACTCT I Y S GTGACAGAGTG	GCGGCTGAAG A A E TTGCATCACA	AGAGGCTC E R L .CGCATTTTGG	AATTO N CGAGCCTTCGTO	TGTAAATCCT V N P TTCATAT	rggaaa W K

Figure S28. Alignment of conotoxins with cysteine framework VI/VII from different superfamilies of C. flavidus.