



Article

Dabsylated Bradykinin is Cleaved by Snake Venom Proteases from *Echis ocellatus*

Julius Abiola ^{1,2}, Anna Maria Berg ¹, Olapeju Aiyelaagbe ², Akindele Adeyi ³, and Simone König ^{1*}

¹ IZKF Core Unit Proteomics, Interdisciplinary Center for Clinical Research, University of Münster, Röntgenstr. 21, 48149 Münster, Germany; koenigs@uni-muenster.de (S.K.)

² Organic Unit, Department of Chemistry, University of Ibadan, Ibadan, Nigeria; abiolajulius005@gmail.com (J.A.), oaiyelaagbe@gmail.com (O.A.)

³ Animal Physiology Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria; delegenius@yahoo.com (A.A.)

* Correspondence: koenigs@uni-muenster.de; Tel.: +49 251 8357164

Citation: To be added by editorial staff during production.

Received: date

Revised: date

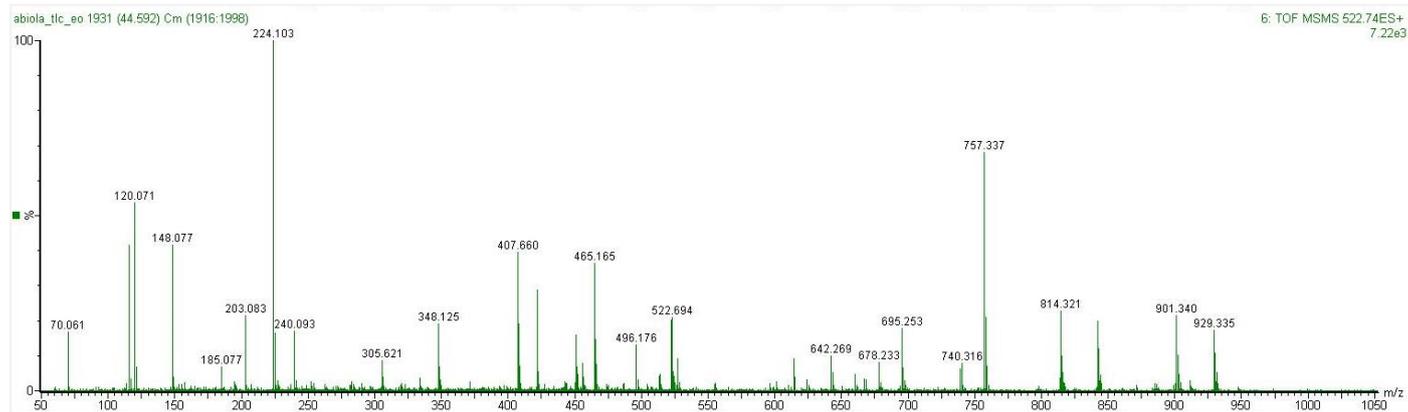
Accepted: date

Published: date



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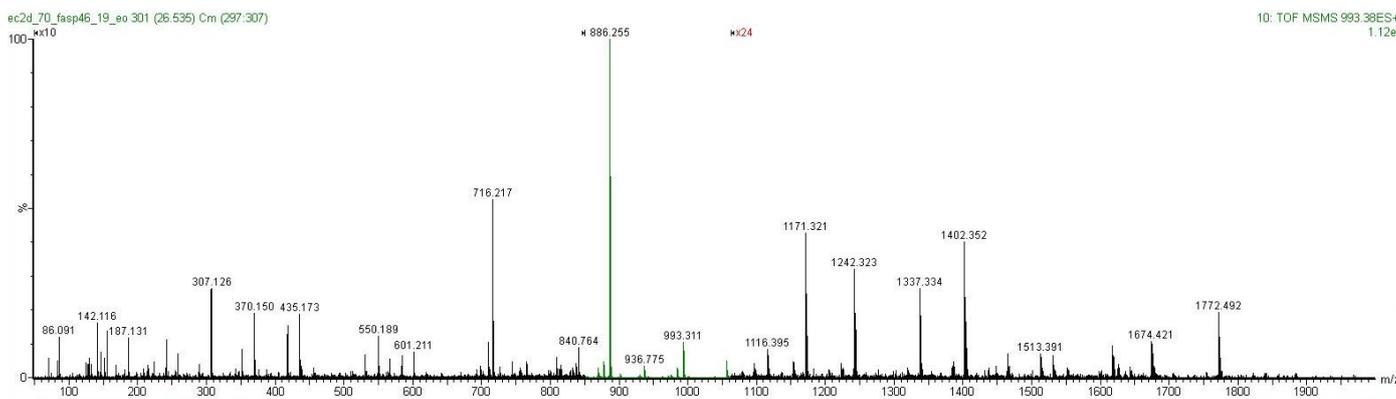
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Average Mass = 1044.2021, Monoisotopic Mass = 1043.4647
 Residues: 1-7
 N-Terminus = dab, C-Terminus = OH
 Fragment ions: Monoisotopic/Average (5000) m/z ratios with 1 positive charge(s).

b	444.182	541.235	638.287	695.309	842.377	929.409	-
i	129.114	70.066	70.066	30.034	120.081	60.045	70.066
b~	426.171	523.224	620.277	677.298	824.367	911.399	-
b*	427.155	524.208	621.261	678.282	825.351	912.383	-
	1	2	3	4	5	6	7
	Arg	Pro	Pro	Gly	Phe	Ser	Pro
	7	6	5	4	3	2	1
y*	-	601.299	504.246	407.193	350.172	203.103	116.071
y~	-	583.288	486.235	389.182	332.161	185.093	98.061
y*	-	584.272	487.219	390.166	333.145	186.077	99.045

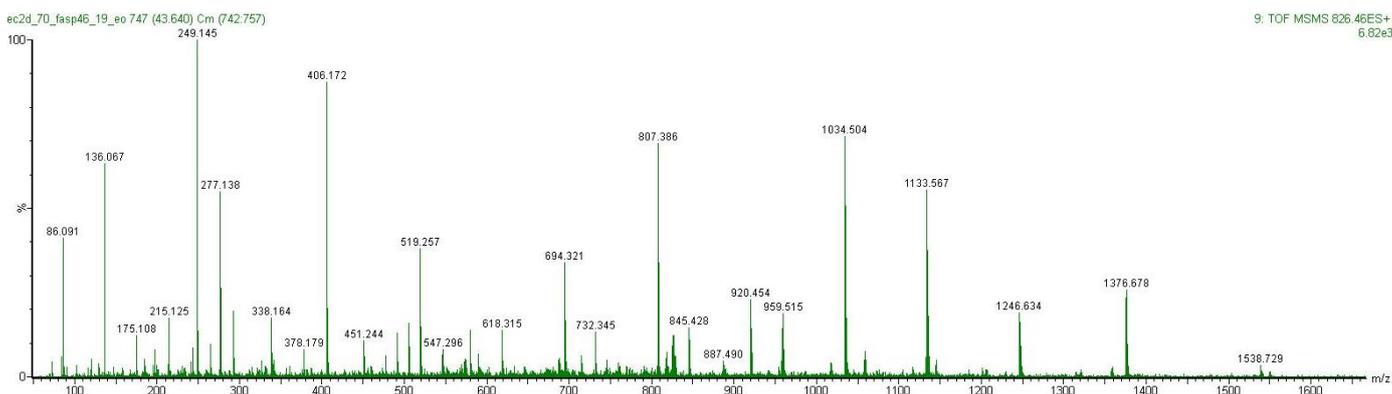
Figure S1. Spectrum for DBK1-7 measured after DBK digestion by venom of *E. ocellatus*. Theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for this peptide.



Average Mass = 1986.1733, Monoisotopic Mass = 1984.7381
 Residues: 1-17
 N-Terminus = H, C-Terminus = OH
 Fragment ions: Monoisotopic/Average (5000) m/z ratios with 1 positive charge(s).

b	114.092	215.140	312.192	369.214	456.246	584.304	744.335	815.372	930.399	987.420	1116.463	1276.494	1436.524	1551.551	1679.610	1839.640	-
i	86.097	74.061	70.066	30.034	60.045	101.071	133.044	44.050	88.040	30.034	102.056	133.044	133.044	88.040	101.071	133.044	101.108
b~	96.081	197.129	294.182	351.203	438.235	566.294	726.324	797.362	912.388	969.410	1098.453	1258.483	1418.514	1533.541	1661.599	1821.630	-
b*	97.065	198.113	295.166	352.187	439.219	567.278	727.308	798.346	913.372	970.394	1099.437	1259.467	1419.498	1534.525	1662.583	1822.614	-
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Leu	Thr	Pro	Gly	Ser	Gln	Cam	Ala	Asp	Gly	Glu	Cam	Cam	Asp	Gln	Cam	Lys
	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
y*	-	1872.662	1771.614	1674.561	1617.540	1530.508	1402.449	1242.419	1171.382	1056.355	999.333	870.291	710.260	550.229	435.202	307.144	147.113
y~	-	1854.651	1753.604	1656.551	1599.529	1512.497	1384.439	1224.408	1153.371	1038.344	981.323	852.280	692.249	532.219	417.192	289.133	129.103
y*	-	1855.635	1754.588	1657.535	1600.513	1513.481	1385.423	1225.392	1154.355	1039.328	982.307	853.264	693.233	533.203	418.176	290.117	130.087

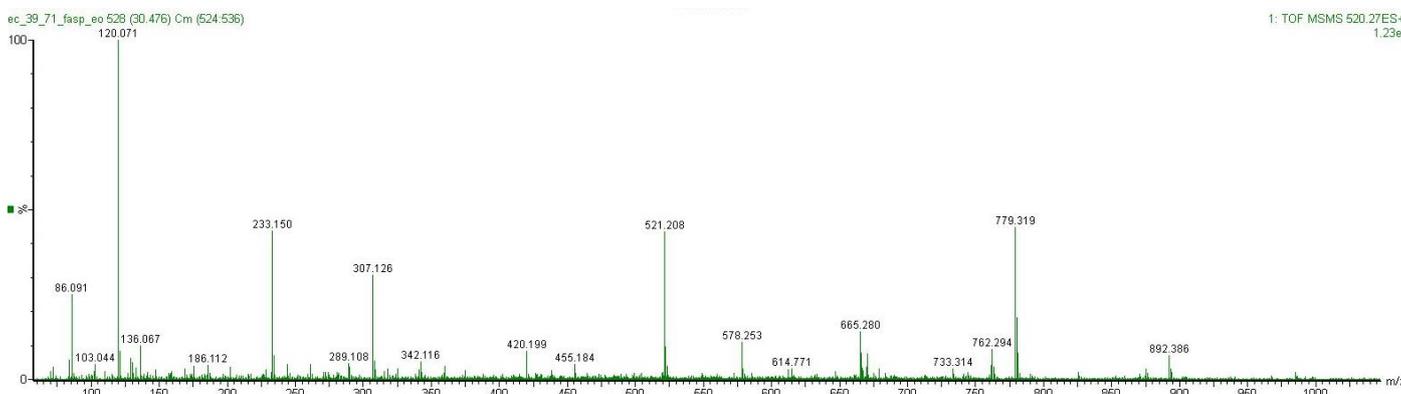
Figure S2. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS of the doubly-charged precursor. Match from zinc metalloproteinase-disintegrin-like protein H3, *Vipera ammodytes ammodytes*, R4NNL0. Note zoom ranges.



Average Mass = 1651.9237, Monoisotopic Mass = 1650.8980
 Residues: 1-13
 N-Terminus = H, C-Terminus = OH
 Fragment ions: Monoisotopic/Average (5000) m/z ratios with 1 positive charge(s).

b	114.092	277.155	406.198	519.282	618.350	732.393	845.477	958.561	1072.604	1201.647	1314.731	1477.794	-
i	86.097	136.076	102.056	86.097	72.081	87.056	86.097	86.097	87.056	102.056	86.097	136.076	129.114
b~	96.081	259.145	388.187	501.271	600.340	714.383	827.467	940.551	1054.594	1183.636	1296.720	1459.784	-
b*	97.065	260.129	389.171	502.255	601.324	715.367	828.451	941.535	1055.578	1184.620	1297.704	1460.768	-
	1	2	3	4	5	6	7	8	9	10	11	12	13
	Ile	Tyr	Glu	Ile	Val	Asn	Ile	Leu	Asn	Glu	Ile	Tyr	Arg
	13	12	11	10	9	8	7	6	5	4	3	2	1
y"	-	1538.822	1375.758	1246.716	1133.632	1034.563	920.521	807.436	694.352	580.309	451.267	338.183	175.119
y~	-	1520.811	1357.748	1228.705	1115.621	1016.553	902.510	789.426	676.342	562.299	433.256	320.172	157.109
y*	-	1521.795	1358.732	1229.689	1116.605	1017.537	903.494	790.410	677.326	563.283	434.240	321.156	158.093

Figure S3. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS of the doubly-charged precursor. Match from metalloproteinase (Fragment), *E. coloratus*, E9JG63.



Average Mass = 1039.2210, Monoisotopic Mass = 1038.5167
 Residues: 1-9
 N-Terminus = H, C-Terminus = OH
 Fragment ions: Monoisotopic/Average (5000) m/z ratios with 1 positive charge(s).

b	148.076	261.160	375.203	462.235	519.257	620.304	733.388	893.419	-
i	120.081	86.097	87.056	60.045	30.034	74.061	86.097	133.044	101.108
b~	130.066	243.150	357.193	444.225	501.246	602.294	715.378	875.409	-
b*	131.050	244.134	358.177	445.209	502.230	603.278	716.362	876.393	-
	1	2	3	4	5	6	7	8	9
	Phe	Leu	Asn	Ser	Gly	Thr	Ile	CM	Lys
	9	8	7	6	5	4	3	2	1
y"	-	892.456	779.372	665.329	578.297	521.276	420.228	307.144	147.113
y~	-	874.446	761.362	647.319	560.287	503.265	402.217	289.133	129.103
y*	-	875.430	762.346	648.303	561.271	504.249	403.201	290.117	130.087

Figure S4. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS of the doubly-charged precursor. Match from disintegrin EO4A, *E. ocellatus*, Q3BER3.

CLUSTAL O(1.2.4) multiple sequence alignment

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sp|R4NNL0|VMH3_VIPAA  MIQVLLVVICLAVFPYQGSSIIILESGNVNDYEVVYLQKVTAMNKGAVKQPEQKYEDTMQY  60
sp|Q2UXR0|VM3E1_ECHOC  -MQVLLITISLAVLPYLGSSIIILESGIVNDYEVVNPQKVTAMLGAVKQPEQKYEDTMQY  59
      :****: *.***:* * ***** * ***** * ***** *****

sp|R4NNL0|VMH3_VIPAA  EFKVNGEPVILHLEKNKDLFSEDYSETHYSPDGREITTNPPVEDHCYHGRIQNDADSTA  120
sp|Q2UXR0|VM3E1_ECHOC  EFKVKGEPVVLHLEKNKGLFSEDYSETHYSPDGREITTNPPVEDHCYHGRIQNDADSSA  119
      ****:****:*****.*****

sp|R4NNL0|VMH3_VIPAA  SISACNGLKGHFQLRGETYFIEPLKIPDSEAHAVYKYENVEKEDEAPKTCGVTQTNWESD  180
sp|Q2UXR0|VM3E1_ECHOC  SISACNGLKGHFKLGRGEMFYFIEPLKIPDSEAHAVYKYENIEEEDAPKMGVKTNRRES  179
      *****:**** *****:*****:***** **.:** **

sp|R4NNL0|VMH3_VIPAA  ELIKKASQLNLTPEQQRYLNSPKYIKLVIVADYIMFLKYGRSLITIRTRIYEIVNLLNVI  240
sp|Q2UXR0|VM3E1_ECHOC  KSIKKASQLNLTPEQQRYLNTPKHIKVAIVADYLI FRKYGRNLTIRAKIYEILNILNEI  239
      : *****:***:*.*****:.* ****.*:***:****:*.** *

sp|R4NNL0|VMH3_VIPAA  YRVLNIYIALVGLIWNNGDKINVLPEAKVTLDLFGKWRETDLLNRRKHDNAQLLTGINF  300
sp|Q2UXR0|VM3E1_ECHOC  YKAFNIHVALVFLEIWSNGDKINLFPAAVTLDLFGKWRETDLMNRKNDNTQLLTGMNF  299
      *.:**.:*** ****.*****:.* *:***** **.*:***:*****.*

sp|R4NNL0|VMH3_VIPAA  NGPTAGLGYLGSMPNPQYSAGIVQDHNKVNFLVALAMAHMGHNLGMDHDGIQCTCGAKS  360
sp|Q2UXR0|VM3E1_ECHOC  DGPTAGLGYVGTMCHPQFSAAVVQDHNKINFLVALAMAHELGHNLGMTHDEQFCTCGAKS  359
      :*****:*.**.*:*.*****:*****:***** ** *****

sp|R4NNL0|VMH3_VIPAA  CIMSGTLSCEASIRFNSCSQEHRKYLINKMPQCILNKPLKTDIVSPAVCGNYLVELGED  420
sp|Q2UXR0|VM3E1_ECHOC  CIMSATLSCEGSYRFSNSCSREENRRLINKMPQCILIKPSRTDIVSPVCGNSLVEVGED  419
      ****.*****.* *****:*.**.* ***** ** :***** **** **.*

sp|R4NNL0|VMH3_VIPAA  CDCGSPRDCQNPCCNAATCKLTPGSQCADGECCDQCKFGRAGTVCRPANGECDVSDVCTG  480
sp|Q2UXR0|VM3E1_ECHOC  CDCGSPGYCRNPCCNAATCKLTPGSQCADGECCDQCRFTRAGTECRPARDECDKADLCTG  479
      ***** *.*****:*****.* **** **.*.*** :.*

sp|R4NNL0|VMH3_VIPAA  QSAECPDQFQRNGHPCQNNNGYCYNGTCPI LGKQCISLFGASATVAQDACFQFNR LGNE  540
sp|Q2UXR0|VM3E1_ECHOC  QSAECPADQFQRNGQPCQNNNGYCYNGICPVMRNQCISLFGSRAIVAEDACFQFNSLGID  539
      *****:*****:*****.***** **: :*****: * **.* ***** ** :

sp|R4NNL0|VMH3_VIPAA  YGYCRKENGRKIPCAPQDVKCGRLYCFDNLPEHKNPCQIYYTPRDENKGMVDPGTCGDG  600
sp|Q2UXR0|VM3E1_ECHOC  YGYCRKENGRKIPCAPEDVKCGRLYCFDNLPEHKNPCQIFYTPRDEDKGMVDPGTCENG  599
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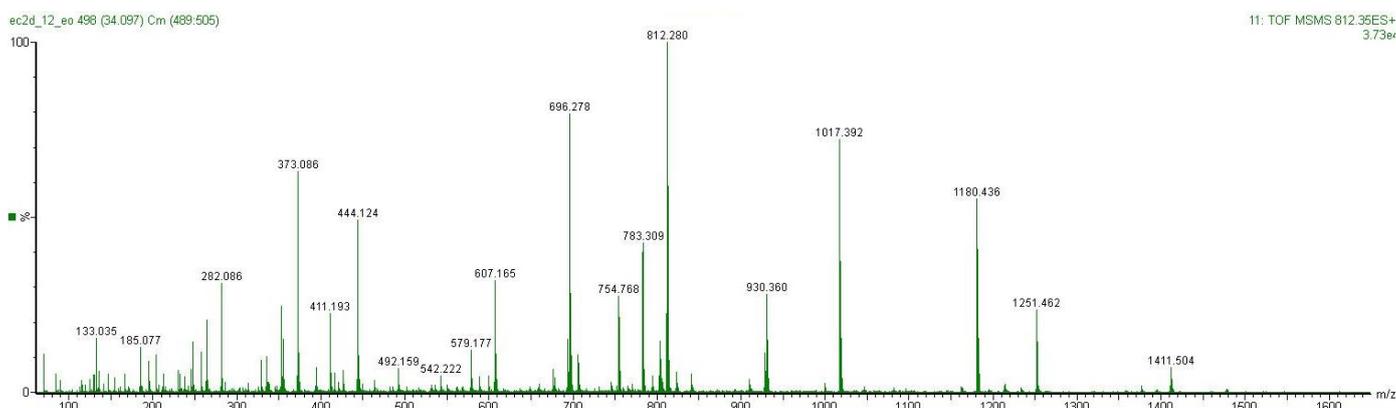


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tr|E9JG63|E9JG63_ECHCO      GYCRKENGGRIPCAPEDVKCGRLYCFDNLPEHKNPCQIVYTPSDEDKGMVDPGTKCEDGK      497
sp|Q2UXR0|VM3E1_ECHOC      GYCRKENGGRIPCAPEDVKCGRLYCFDNLPEHKNPCQIFYTPRDEDKGMVDPGTKCENGK      600
*****.*** *****.***

tr|E9JG63|E9JG63_ECHCO      VCINGKCVDVNTAY      511
sp|Q2UXR0|VM3E1_ECHOC      VCINGKCVDVNTAY      614
*****
    
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Figure S6. Clustal sequence alignment of E9JG63 and Q2UXR0. Peptides, which were manually sequenced by target MS/MS in venom digests are labeled in red and bold, those obtained with DIA (score > 8) are labeled in blue and italic. Yellow indicates regions with a one-amino acid difference to the peptides validated in the venom digests.



Average Mass = 1623.8278, Monoisotopic Mass = 1622.6744

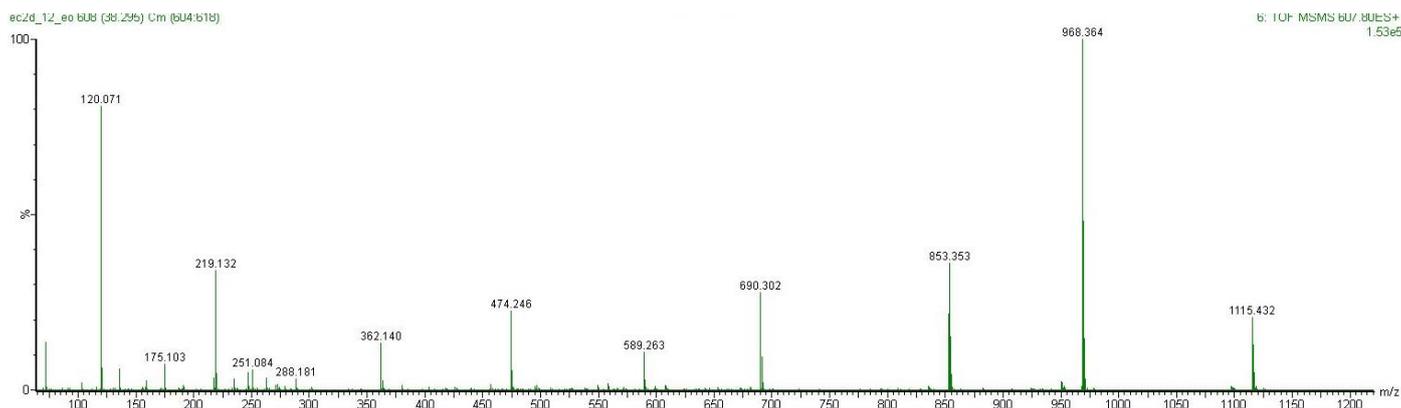
Residues: 1-14

N-Terminus = H, C-Terminus = OH

Fragment ions: Monoisotopic/Average (5000) m/z ratios with 1 positive charge(s).

b	116.035	213.088	373.118	444.155	607.219	694.251	841.319	928.351	1025.404	1082.425	1213.466	1376.529	1477.577	-
i	88.040	70.066	133.044	44.050	136.076	60.045	120.081	60.045	70.066	30.034	104.053	136.076	74.061	101.108
b~	98.024	195.077	355.108	426.145	589.208	676.240	823.308	910.340	1007.393	1064.415	1195.455	1358.518	1459.566	-
b*	99.008	196.061	356.092	427.129	590.192	677.224	824.292	911.324	1008.377	1065.399	1196.439	1359.503	1460.550	-
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Asp	Pro	CAM	Ala	Tyr	Ser	Phe	Ser	Pro	Gly	Met	Tyr	Thr	Lys
	14	13	12	11	10	9	8	7	6	5	4	3	2	1
y"	-	1508.655	1411.603	1251.572	1180.535	1017.471	930.439	783.371	696.339	599.286	542.265	411.224	248.161	147.113
y~	-	1490.645	1393.592	1233.561	1162.524	999.461	912.429	765.360	678.328	581.276	524.254	393.214	230.150	129.103
y*	-	1491.629	1394.576	1234.545	1163.508	1000.445	913.413	766.344	679.312	582.260	525.238	394.198	231.134	130.087

Figure S7. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS of the doubly-charged precursor. Match from serine protease (fragment, *E. ocellatus*, D5KRX9).



Average Mass = 1214.3432, Monoisotopic Mass = 1213.5767

Residues: 1-9

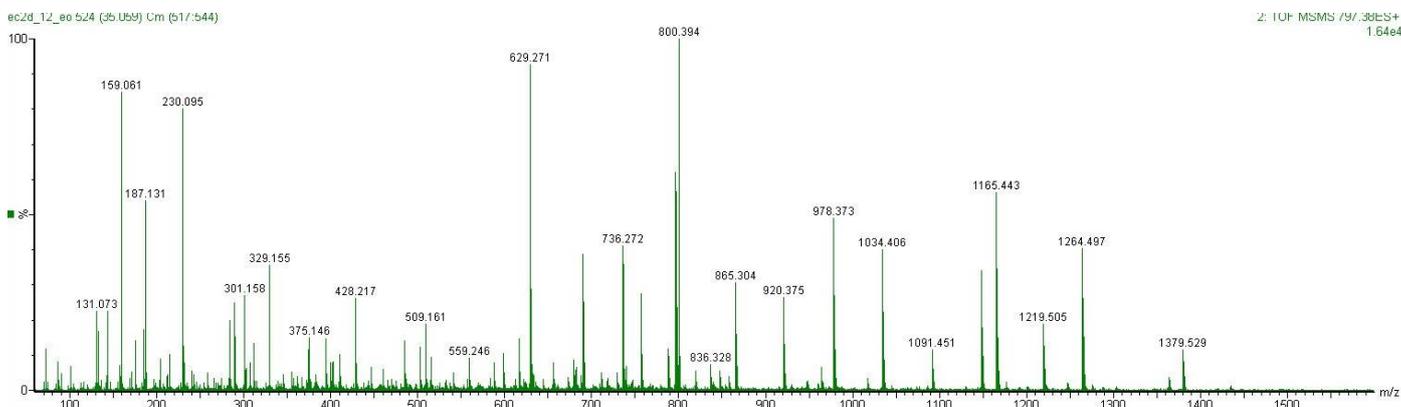
N-Terminus = H, C-Terminus = OH

Fragment ions: Monoisotopic/Average (5000) m/z ratios with 1 positive charge(s).

b	100.076	247.145	362.172	525.235	626.283	741.310	927.389	1040.473	-
i	72.081	120.081	88.040	136.076	74.061	88.040	159.092	86.097	129.114
b~	82.066	229.134	344.161	507.224	608.272	723.299	909.378	1022.462	-
b*	83.050	230.118	345.145	508.208	609.256	724.283	910.362	1023.446	-

	1	2	3	4	5	6	7	8	9
	Val	Phe	Asp	Tyr	Thr	Asp	Trp	Ile	Arg
	9	8	7	6	5	4	3	2	1
y*	-	1115.516	968.448	853.421	690.357	589.310	474.283	288.204	175.119
y~	-	1097.505	950.437	835.410	672.347	571.299	456.272	270.193	157.109
y*	-	1098.490	951.421	836.394	673.331	572.283	457.256	271.177	158.093

Figure S8. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS of the doubly-charged precursor. Match from serine protease (fragment, *E. ocellatus*, D5KRX9).



Average Mass = 1593.8431, Monoisotopic Mass = 1592.7537

Residues: 1-15

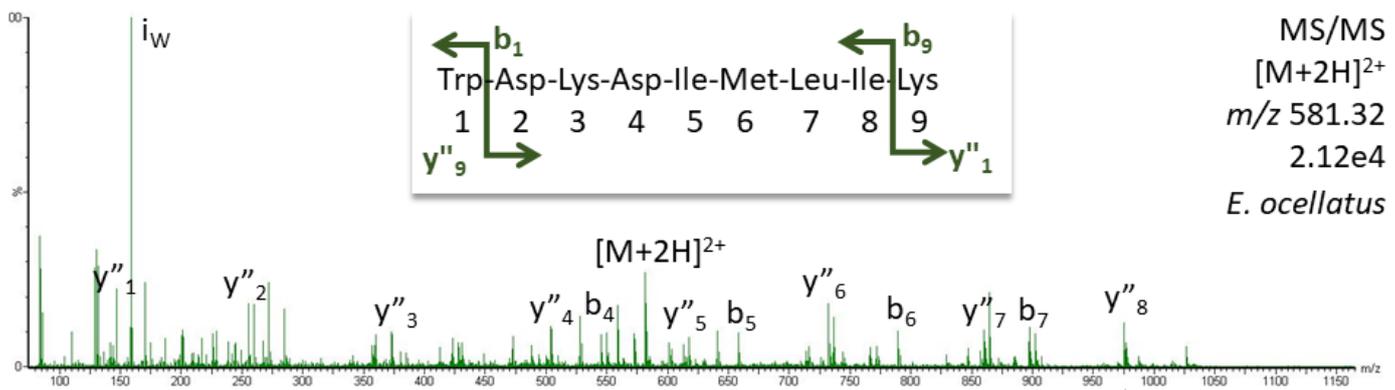
N-Terminus = H, C-Terminus = OH

Fragment ions: Monoisotopic/Average (5000) m/z ratios with 1 positive charge(s).

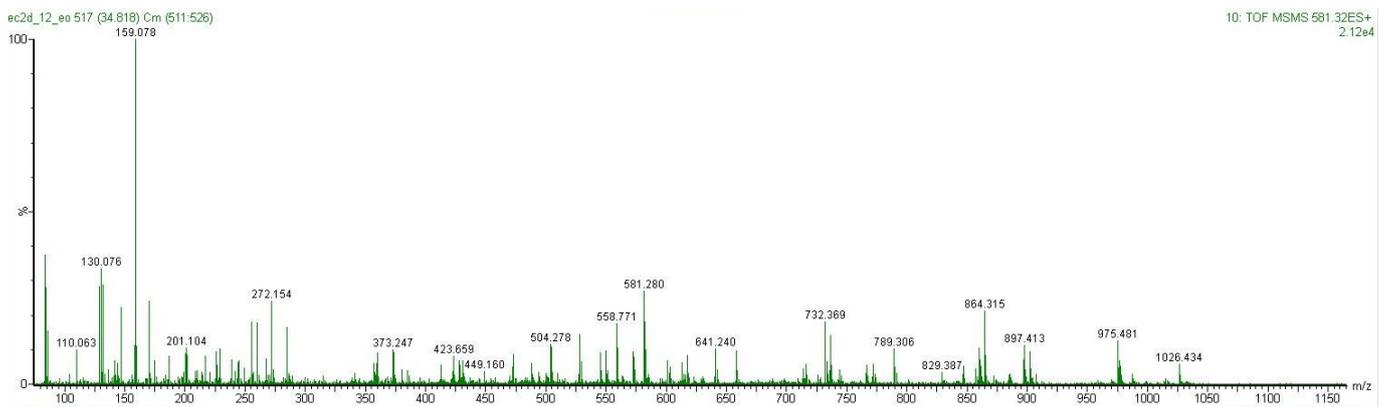
b	102.055	215.140	375.170	446.207	503.229	616.313	729.397	858.439	915.461	972.482	1085.566	1200.593	1287.625	1447.656	-
i	74.061	86.097	133.044	44.050	30.034	86.097	86.097	102.056	30.034	30.034	86.097	88.040	60.045	133.044	101.108
b~	84.045	197.129	357.160	428.197	485.218	598.302	711.386	840.429	897.450	954.472	1067.556	1182.583	1269.615	1429.645	-
b*	85.029	198.113	358.144	429.181	486.202	599.286	712.370	841.413	898.434	955.456	1068.540	1183.567	1270.599	1430.630	-

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Thr	Leu	CAM	Ala	Gly	Ile	Leu	Glu	Gly	Gly	Ile	Asp	Ser	CAM	Lys
	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
y*	-	1492.714	1379.630	1219.599	1148.562	1091.541	978.456	865.372	736.330	679.308	622.287	509.203	394.176	307.144	147.113
y~	-	1474.703	1361.619	1201.589	1130.551	1073.530	960.446	847.362	718.319	661.298	604.276	491.192	376.165	289.133	129.103
y*	-	1475.687	1362.603	1202.573	1131.536	1074.514	961.430	848.346	719.303	662.282	605.260	492.176	377.149	290.117	130.087

Figure S9. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* (top trace) venom digest using target MS/MS of the doubly-charged precursor. Match from serine protease (fragment, *E. ocellatus*, D5KRY1).



MS/MS
 [M+2H]²⁺
 m/z 581.32
 2.12e4
E. ocellatus



Average Mass = 1161.4311, Monoisotopic Mass = 1160.6263

Residues: 1-9

N-Terminus = H, C-Terminus = OH

Fragment ions: Monoisotopic/Average (5000) m/z ratios with 1 positive charge(s).

b	187.087	302.114	430.209	545.236	658.320	789.361	902.445	1015.529	-
i	159.092	88.040	101.108	88.040	86.097	104.053	86.097	86.097	101.108
b~	169.077	284.103	412.198	527.225	640.309	771.350	884.434	997.518	-
b^*	170.061	285.087	413.182	528.209	641.293	772.334	885.418	998.502	-
	1	2	3	4	5	6	7	8	9
	Trp	Asp	Lys	Asp	Ile	Met	Leu	Ile	Lys
	9	8	7	6	5	4	3	2	1
y''	-	975.555	860.528	732.433	617.406	504.322	373.281	260.197	147.113
y~	-	957.544	842.517	714.422	599.395	486.311	355.271	242.187	129.103
y^*	-	958.528	843.501	715.406	600.379	487.295	356.255	243.171	130.087

Figure S10. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS of the doubly-charged precursor. Match from serine protease (fragment, *E. ocellatus*, D5KRX9).

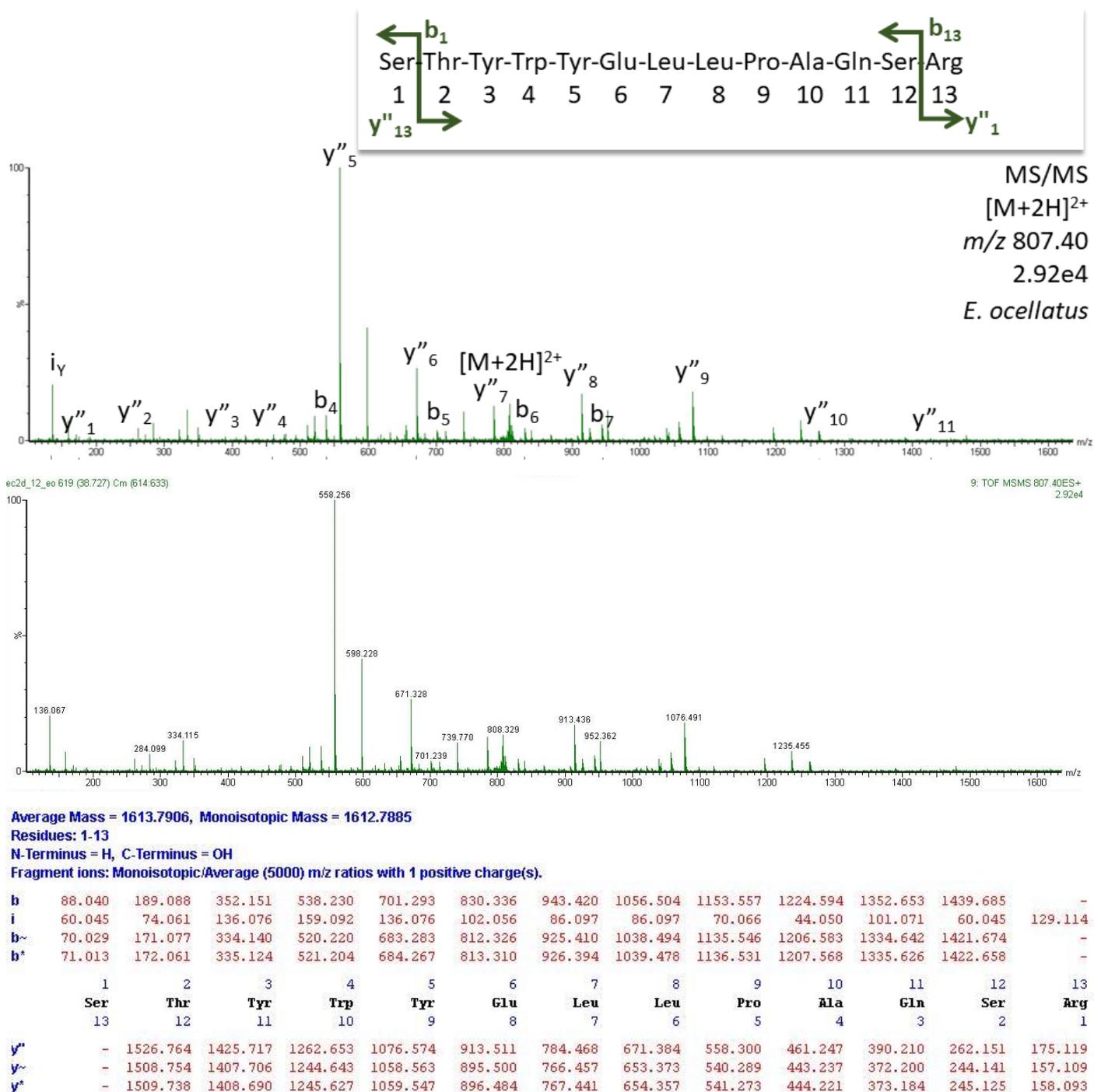
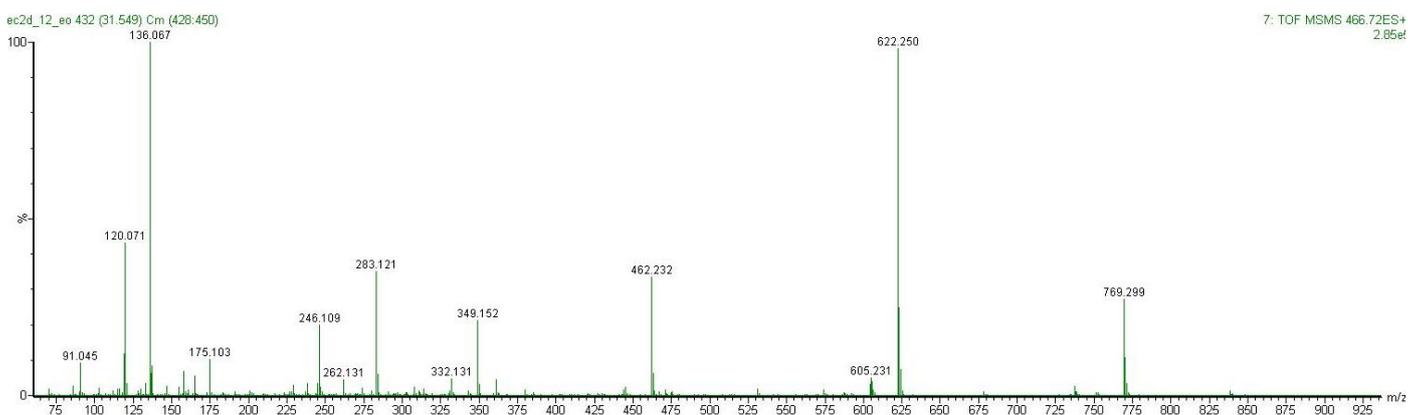
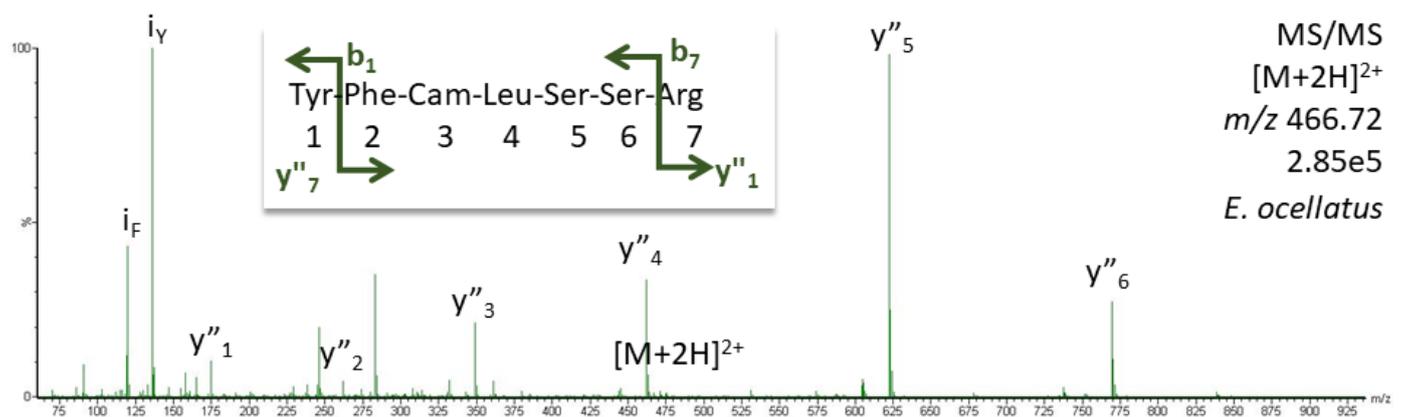


Figure S11. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digests using target MS/MS of the doubly-charged precursor. Match from serine protease (fragment, *E. ocellatus*, D5KRX9).



Average Mass = 932.0682, Monoisotopic Mass = 931.4221

Residues: 1-7

N-Terminus = H, C-Terminus = OH

Fragment ions: Monoisotopic/Average (5000) m/z ratios with 1 positive charge(s).

b	164.071	311.140	471.170	584.254	671.286	758.318	-
i	136.076	120.081	133.044	86.097	60.045	60.045	129.114
b~	146.061	293.129	453.160	566.244	653.276	740.308	-
b*	147.045	294.113	454.144	567.228	654.260	741.292	-
	1	2	3	4	5	6	7
	Tyr	Phe	CAM	Leu	Ser	Ser	Arg
	7	6	5	4	3	2	1
y''	-	769.367	622.298	462.268	349.184	262.151	175.119
y~	-	751.356	604.288	444.257	331.173	244.141	157.109
y*	-	752.340	605.272	445.241	332.157	245.125	158.093

Figure S12. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS of the doubly-charged precursor. Match from serine protease (fragment, *E. ocellatus*, D5KRX9).

CLUSTAL 2.1 multiple sequence alignment

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tr|D5KRX9|D5KRX9_ECHOC      MVLIRVLANLLLLQLSYAQTSSSELVIGGDECNINEHRSLVFLYNASG--F
tr|D5KRY1|D5KRY1_ECHOC      MVLIRVLANLLVLQLSYAQKSSSELVAGGAECDKNEHPFLVALHTARSKRF
*****:*****.***** ** **: *** ** *:. * . *

tr|D5KRX9|D5KRX9_ECHOC      ECCGTLNREWVLSAAHCDMENMEIYLGMHNLSNPNQDARRRDPEEKYFC
tr|D5KRY1|D5KRY1_ECHOC      HCTGTLLIGKQWVLTAAARC�KKNIRVKIGMHNKNERTEDEMMRVAAEKFFC
.* **:.:***:***:*. :*:.: :**** .: .:* * . **:**

tr|D5KRX9|D5KRX9_ECHOC      LSSRTYTKWDKDIMLIKLDSPVTYSTHIAPFSLPSRPPTVGSVCRIMGWG
tr|D5KRY1|D5KRY1_ECHOC      ASSKTYTRWDKDIMLIKLKRPVNNRTHIAPLSLPSNPASVGSECRIMGWG
**:*:*:*****. **. *****:****.*.:*** *****

tr|D5KRX9|D5KRX9_ECHOC      GIPSPNETYPDVPHCANINILRYSVCRSTYWYELLPAQSRALCAGDRRRR
tr|D5KRY1|D5KRY1_ECHOC      TTTTTKVTYPDVPCANIKIFDYSVCR--EAYRKLPEKSRTLCAGILEGG
.:.: *****:****:*. ***** * . ** :*:**** .

tr|D5KRX9|D5KRX9_ECHOC      IGCKGDSGGPLICNGQIHGIVSWSRDPCAYSFSPGMYTKVFDYTDWIRS
tr|D5KRY1|D5KRY1_ECHOC      IDSCKADTGGPLICNGEFQGIASWGGQPCAQPLKPALYTNVFDYSDWIKS
*.*.*.*:*****:.*.*. :*** .:.*.:*:****:***:*

tr|D5KRX9|D5KRX9_ECHOC      IIAGNTTATAP--
tr|D5KRY1|D5KRY1_ECHOC      IIAGNTTATCPPS
*****.*

```

Figure S13. Clustal sequence alignment of D5KRX9 and D5KRY1. Peptides, which were manually sequenced by target MS/MS in venom digests are labeled in red and bold, those obtained with DIA (score > 8) are labeled in blue and italic.

CLUSTAL 2.1 multiple sequence alignment

```

sp|B5U6Y3|VSP_ECHOC          MVLIRVLANLLVLQLSYAQMSELVGGGECNRNRHRSLLALYNSS--GT
tr|A0A0A1WDS7|A0A0A1WDS7_ECHCO  -----GGAECNINEHRSLALIYNSTSMWF
                                     **.* ** *.******:***:

sp|B5U6Y3|VSP_ECHOC          LCGGTLIHEEWVLSAAHCDMENMKIYLGHLNLSLPNKDQQKREPRETHFC
tr|A0A0A1WDS7|A0A0A1WDS7_ECHCO  HCSGTLNQEWWLTAAHCEMENMQIYLGVHNKTKRNKDQQKRFPPKKKYFC
                                     *.***:::***:***:***:***:***:*** :  ***** *:.:**

sp|B5U6Y3|VSP_ECHOC          LPSRNYTLWDKDIMLIKLNRPVNNSPHIAPISLPSNPPRLRSVCHIMGWG
tr|A0A0A1WDS7|A0A0A1WDS7_ECHCO  LKSKNFTLWDKDIMLI-----
                                     * *:*:*****

sp|B5U6Y3|VSP_ECHOC          AITSPNETYPDVPHCANINILRYSVCRAAFGRLLPAQSRTLCAgilRGGID
tr|A0A0A1WDS7|A0A0A1WDS7_ECHCO  -----

sp|B5U6Y3|VSP_ECHOC          TCLGDSGGPLICNGQIQGIVSWGAEVCAKPHAPGLYTKVSDYTDWIQSI I
tr|A0A0A1WDS7|A0A0A1WDS7_ECHCO  -----

```

Figure S15. Clustal sequence alignment of B5U6Y3 and A0A0A1WDS7. The peptide, which was manually sequenced by target MS/MS in venom digests is labeled in red and bold.