

Article

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

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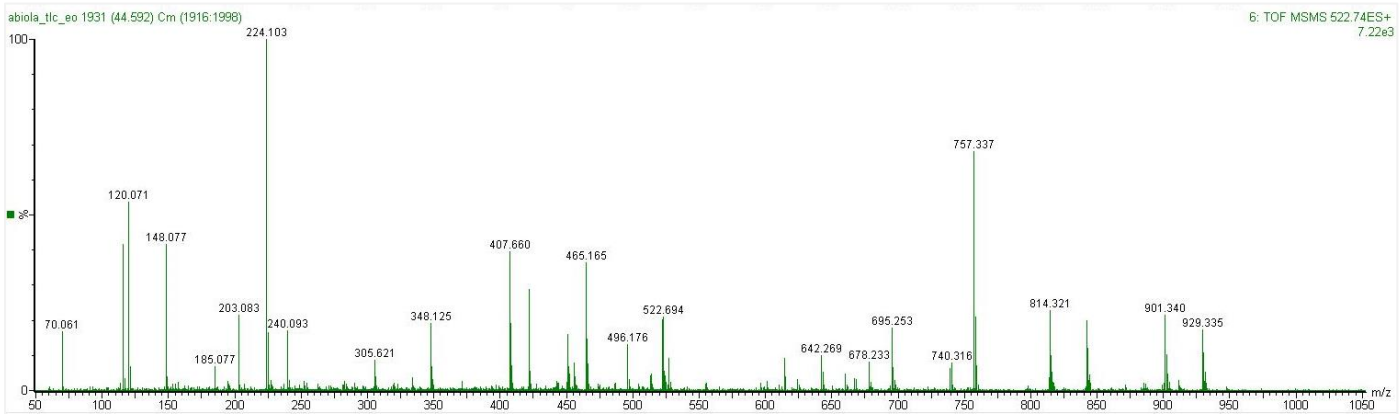
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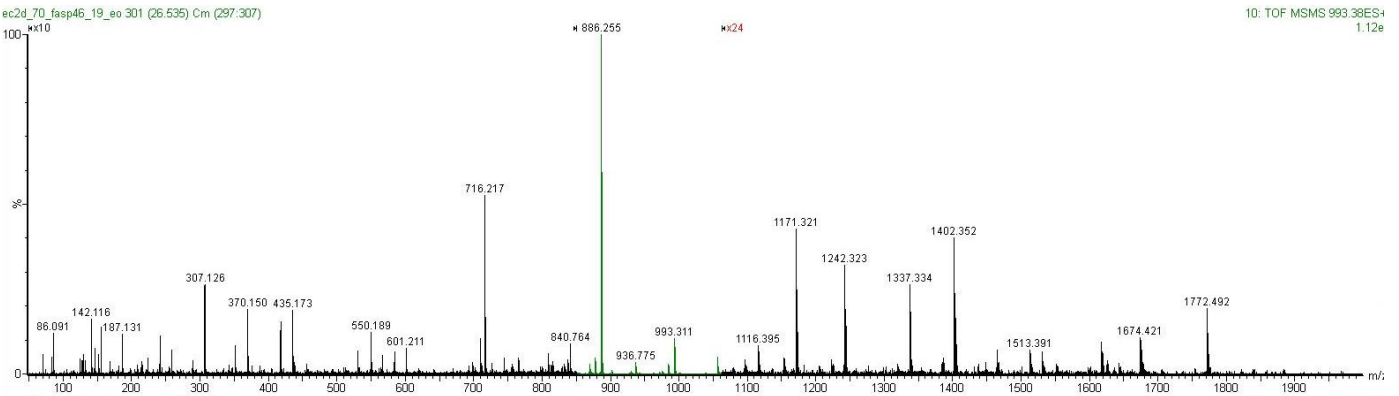
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Average Mass = 1044.2021, Monoisotopic Mass = 1043.4647
Residues: 1-7
N-Terminus = dab, C-Terminus = OH
Fragment ions: Monoisotopic/Average (5000) m/z ratios with 1 positive charge(s).

| | | | | | | | |
|----|---------|---------|---------|---------|---------|---------|---------|
| 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.

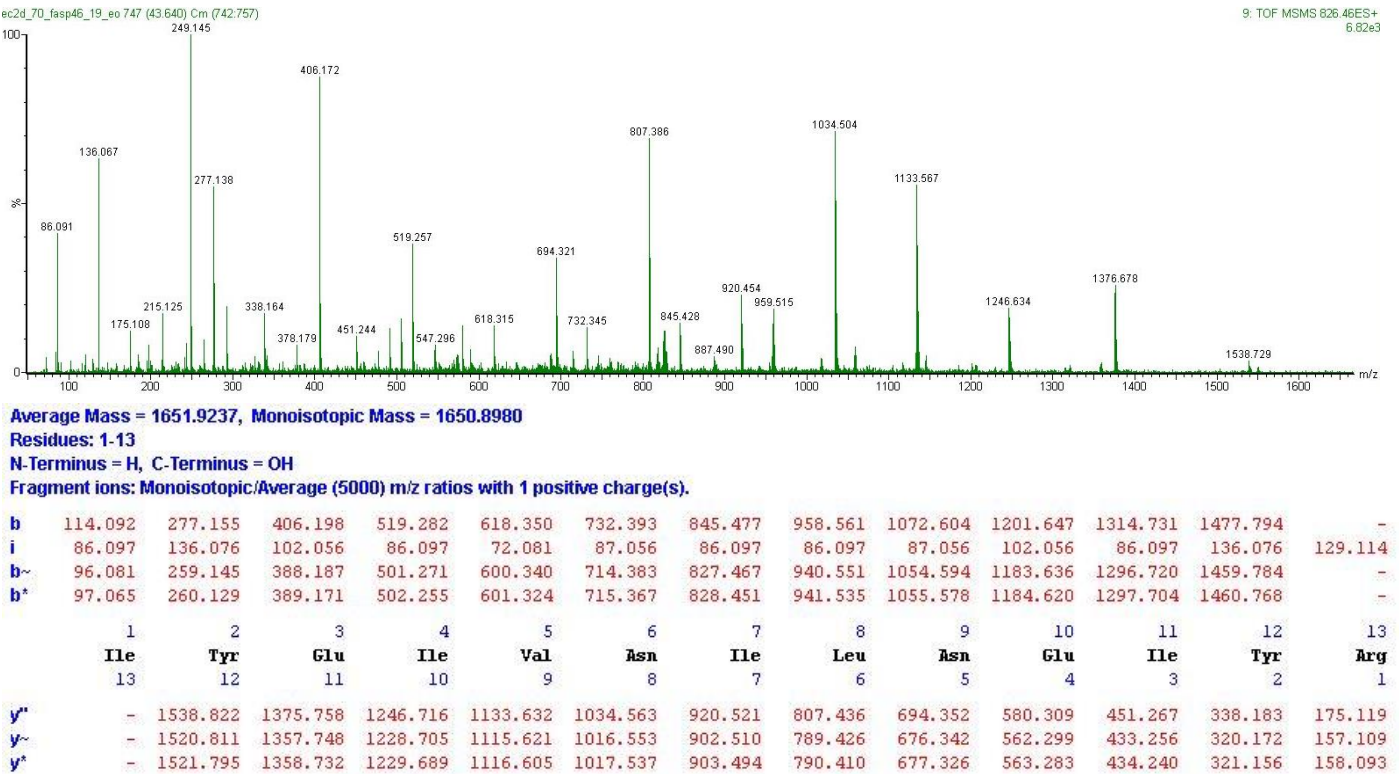


Figure S3. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS of the doubly-charged precursor. Match from metalloproteinase (Fragment), *E. coloratus*, E9JG63.

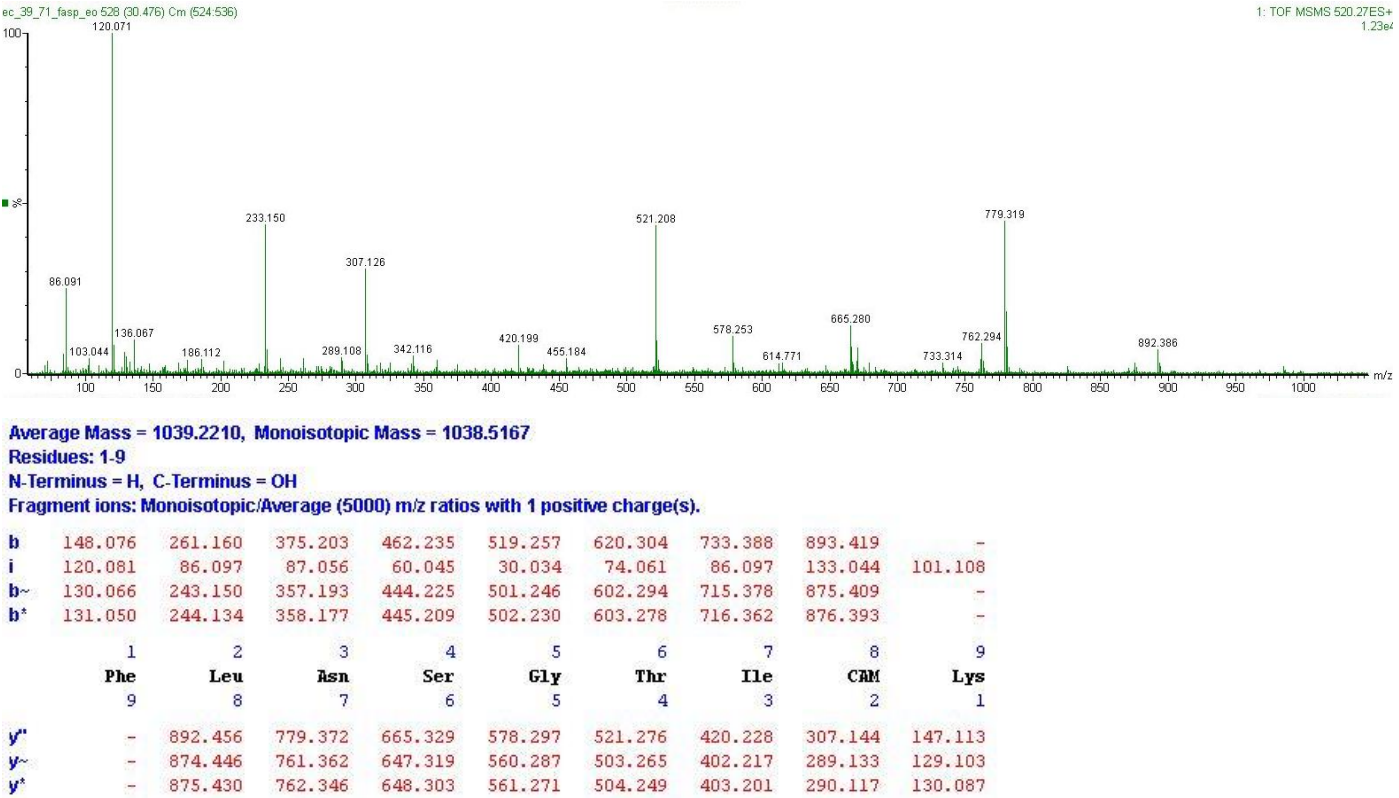


Figure S4. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS of the doubly-charged precursor. Match from disintegrin EO4A, *E. ocellatus*, Q3BER3.

CLUSTAL O(1.2.4) multiple sequence alignment

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sp|R4NNL0|VMH3_VIPAA  MIQVLLVVIICLAVFPYQGSSIILESIGNVNDYEVVYLQKVTAMNKGAVKQPEQKYEDTMQY  60
sp|Q2UXR0|VM3E1_ECHOC  -MQVLLITISLAVLPYLGSSIILESIGVNDYEVVNPQKVTAMLKGAVKQPEQKYEDTMQY  59
                        :****: *.***:* * ***** * ***** * *****
                        :

sp|R4NNL0|VMH3_VIPAA  EFKVNGEPVILHLEKNKDLFSEDYSETHYSPDGREITNPPVEDHCYHGRIQNDADSTA  120
sp|Q2UXR0|VM3E1_ECHOC  EFKVKGEPPVVLHLEKNKGLFSEDYSETHYSPDGREITNPPVEDHCYHGRIQNDADSSA  119
                        ****:****:*****.*****
                        :

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

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

sp|R4NNL0|VMH3_VIPAA  YRVLNIYIALVGLEIWNNGDKINVLPEAKVTLDLFGKWRETDLLNRRKHDNAQLLTGINF  300
sp|Q2UXR0|VM3E1_ECHOC  YKAFNIHVALVFLEIWSNGDKINLFPAANVTLDLFGKWRETDLMNRKNHDNTQLLTGMNF  299
                        *:..*:*:* ***.*****:.* *:***** **:*:*:*:*:*:*:*
                        :

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

sp|R4NNL0|VMH3_VIPAA  CIMSGLTSCASIRFSNCSQEEHRKYLINKMPQCILNKPLKTDIVSPAVCGNYLVELGED  420
sp|Q2UXR0|VM3E1_ECHOC  CIMSATLSCGSGYRFSNCSREENRRYLINKMPQCILIKPSRTDIVSPVCGNSLVEVGED  419
                        ****.*****.* *****:*.***:***** ** :***** *** ***:***
                        :

sp|R4NNL0|VMH3_VIPAA  CDCGSPRDCQNPCNAATCKLTPGSQCADGECCDQCKFGRAGTVCRPANGCEDVSDVCTG  480
sp|Q2UXR0|VM3E1_ECHOC  CDCGSPGYCRNPCCNAATCKLTPGSQCADGECCDQCFTRAGTECRPARDECDKADLCTG  479
                        ***** *:*****:*****:*****:***** *** ***.*** :*:***
                        :

sp|R4NNL0|VMH3_VIPAA  QSAECPTDQFQRNGHPCQNNNGYCYNGTCPILGKQCISLFGASATVAQDACFQFNRLGNE  540
sp|Q2UXR0|VM3E1_ECHOC  QSAECPADQFQRNGQPCQNNNGYCYNGICPVMRNQCISLFGSRAIVAEDACFQFNSLGID  539
                        *****:*****:*****.***** **: :*****: * **:***** ** :
                        :

sp|R4NNL0|VMH3_VIPAA  YGYCRKENGRLKIPCAPQDVKCGRLYCFDNLPEHKNPCQIYYTPRDENKGMVDPGTCGDG  600
sp|Q2UXR0|VM3E1_ECHOC  YGYCRKENGRIKIPCAPEDVKCGRLYCFDNLPEHKNPCQIFYTPRDEDKGMVDPGTCENG  599
                        *****:*****:*****:*****:***** :*
                        :

```

sp|R4NNL0|VMH3_VIPAA MACSSNGQ**CVDVNTAY** 616
 sp|Q2UXR0|VM3E1_ECHOC KVCII-NGK**CVDVNTAY** 614
 . * ** :*****

Figure S5. Clustal sequence alignment of R4NNL0 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.

| | | |
|------------------------|---|-----|
| tr E9JG63 E9JG63_ECHCO | ----- | 0 |
| sp Q2UXR0 VM3E1_ECHOC | MQVLLITISLAVLPYLGSSIIIESGIVNDYEVVNPQKVTAMLKGAVKQPEQKYEDTMQYE | 60 |
| tr E9JG63 E9JG63_ECHCO | -----HCYYHGHIQNADSFAS | 17 |
| sp Q2UXR0 VM3E1_ECHOC | FKVKGEFPVVLHLEKNKGLFSEDYSETHYSPDGREITNPPVEDHCYYHGRIQNADSSAS | 120 |
| | *****:***** ** | |
| tr E9JG63 E9JG63_ECHCO | ISACNGLKGHFKLGRGMYFIEPLKIPDSEAHAVYKYENVEKEDEAPKMGCVTQTNWESDE | 77 |
| sp Q2UXR0 VM3E1_ECHOC | ISACNGLKGHFKLGRGMYFIEPLKIPDSEAHAVYKYENIEEDEAPKMGCVKHTNRES DK | 180 |
| | *****:*****:*** **: | |
| tr E9JG63 E9JG63_ECHCO | PIKEASQLNLTPEQQRYLNSQKYIKVAIVADYIMFRKYGRNLTIRAR IYEIVNINLEIY | 137 |
| sp Q2UXR0 VM3E1_ECHOC | SIKKASQLNLTPEQQRYLNTPKHIKVAIVADYLIFRKYGRNLTIRAK IYEILNINLEIY | 240 |
| | ** :*****: * :*****: :*****:****:***** | |
| tr E9JG63 E9JG63_ECHCO | R AFNIHVALVFLEIWSNGDKINVLPEAKVTLDLFGEWQRDLLNRKKHDNAQLLTGINFD | 197 |
| sp Q2UXR0 VM3E1_ECHOC | KAFNIHVALVFLEIWSNGDKINLFPAAVNTLDLFGKWRE RDLNMRKNHDNTQLLTGMNFD | 300 |
| | :*****: :* *:*****: **:***:***:***:*****:*** | |
| tr E9JG63 E9JG63_ECHCO | GPTAGLGYVGS LCHPQYSAAIVQDHNKINFLVALAMAHELGHN LGMTHDEXFCTCGAKSC | 257 |
| sp Q2UXR0 VM3E1_ECHOC | GPTAGLGYVGT MCHPQFSAAVVQDHNKINFLVALAMAHELGHN LGMTHDEQFCTCGAKSC | 360 |
| | *****: :***:***:*****:***** ***** | |
| tr E9JG63 E9JG63_ECHCO | IMSGT LSCGYSYRFSNCSQEENRRYFINKMPQCILNKPLKTDIVSPAVCGNYLVEVGEDC | 317 |
| sp Q2UXR0 VM3E1_ECHOC | IMSAT LSCGYSYRFSNCSREENRRYLIN KMPQCILIKPSRTDIVSPVCGNSLVEVGEDC | 420 |
| | ***.*****:*****:***** ** :***** **** ***** | |
| tr E9JG63 E9JG63_ECHCO | DCGSPAN CHN PCCNAATCXLT PGSQCAEGEC CDQCRFTRAGTECRPARDECDKADLCTGQ | 377 |
| sp Q2UXR0 VM3E1_ECHOC | DCGSPGYCRN PCCNAATCK LTPGSQCADGEC CDQCR FTRAGTECRPARDECDKADLCTGQ | 480 |
| | *****. *:***** *****:***** ***** | |
| tr E9JG63 E9JG63_ECHCO | SAECPADQFQRNGQPCQNNNGYCYNGICPIMRNQCILLFGSRATVAEDACFQFNSLGSDY | 437 |
| sp Q2UXR0 VM3E1_ECHOC | SAECPADQFQRNGQPCQNNSGYCYNGICPVMRNQCISLFGSRAIVAEDACFQFNSLGIDY | 540 |
| | *****.*****:***** ***** ***** | |

tr|E9JG63|E9JG63_ECHCO

GYCRKENGGR***IPCAPE******LDV***CGR***LYCF******DN******LE******PH***KNPCQIVYTPSDEDEKGMVDPGTCEDGK

497

sp|Q2UXR0|VM3E1_ECHOC

GYCRKENGGR***IPCAPE******LDV***CGR***LYCF******DN******LE******PH***KNPCQIFYTPRDEDEKGMVDPGTCENGK

600

*****.********.***

tr|E9JG63|E9JG63_ECHCO

VCINGK***CVDVNTAY***

511

sp|Q2UXR0|VM3E1_ECHOC

VCINGK***CVDVNTAY***

614

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.

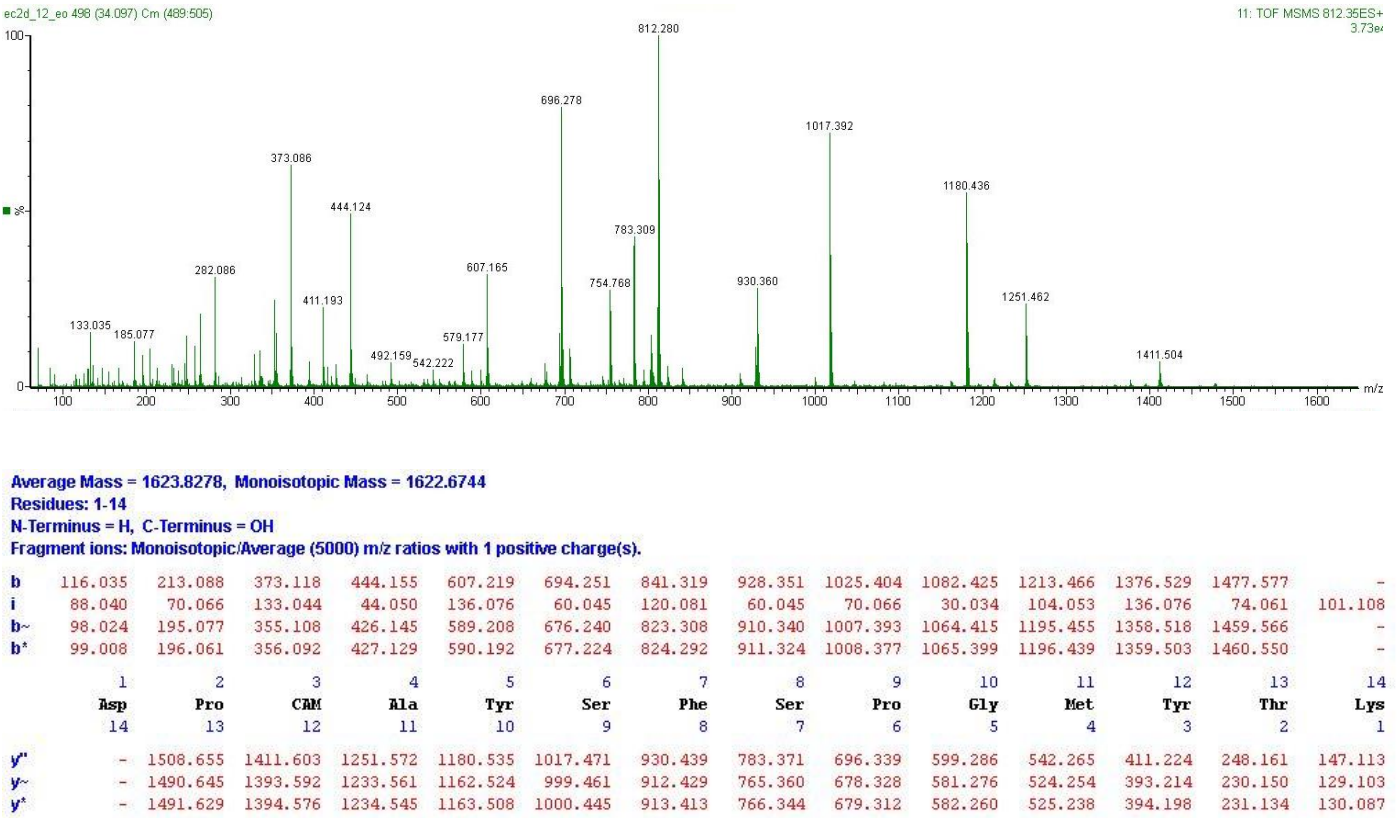


Figure S7. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS o the doubly-charged precursor. Match from serine protease (fragment, *E. ocellatus*, D5KRX9).

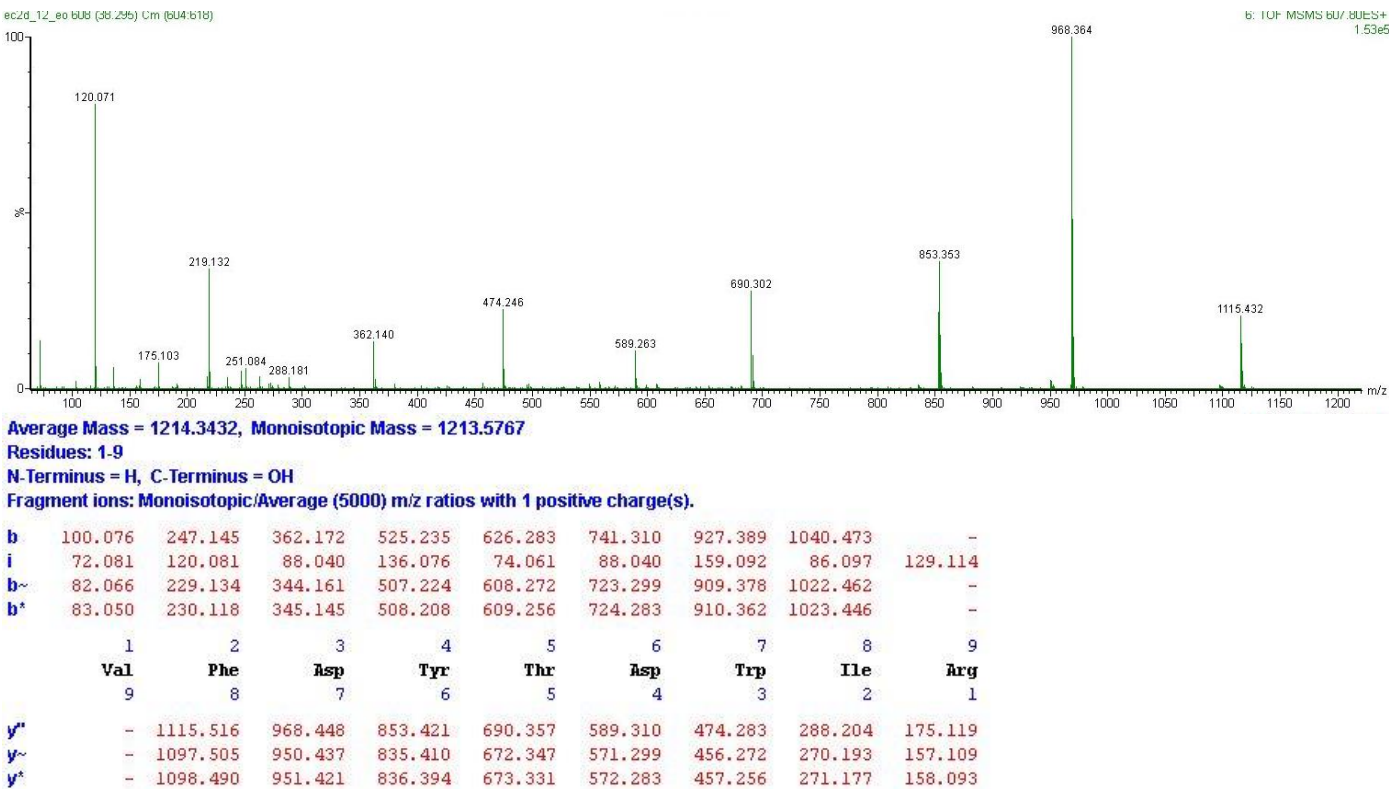


Figure S8. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS o the doubly-charged precursor. Match from serine protease (fragment, *E. ocellatus*, D5KRX9).

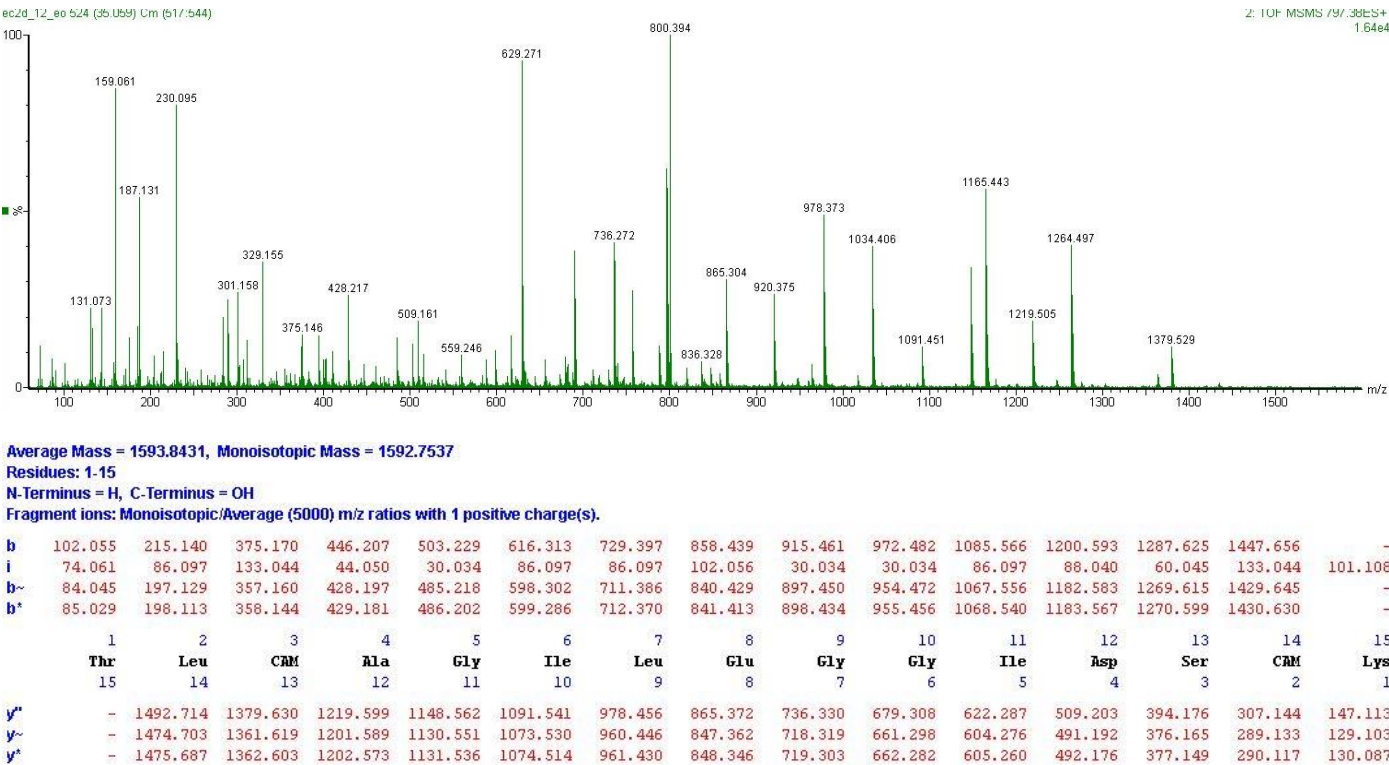
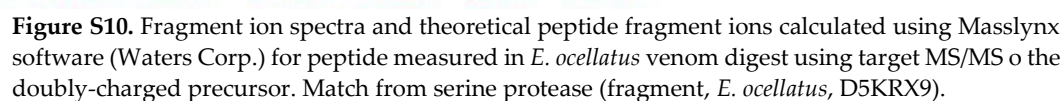
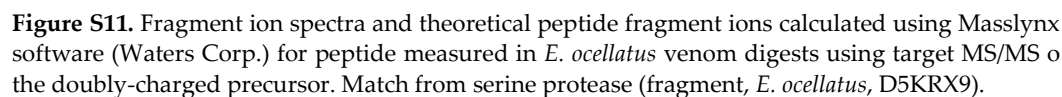


Figure S9. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* (top trace) venom digest using target MS/MS o the doubly-charged precursor. Match from serine protease (fragment, *E. ocellatus*, D5KRY1).





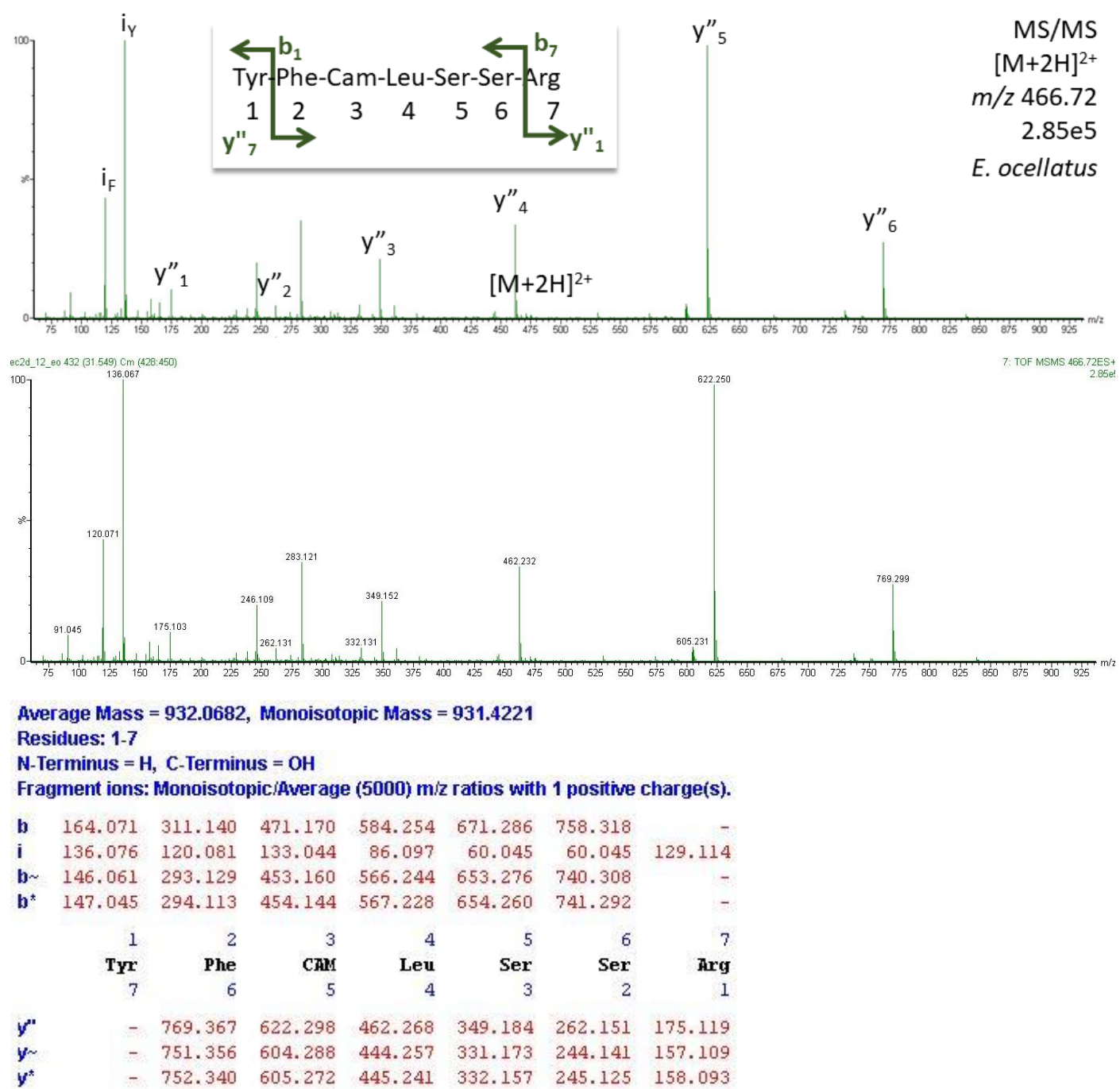


Figure S12. Fragment ion spectra and theoretical peptide fragment ions calculated using Masslynx software (Waters Corp.) for peptide measured in *E. ocellatus* venom digest using target MS/MS o the doubly-charged precursor. Match from serine protease (fragment, *E. ocellatus*, D5KRX9).

CLUSTAL 2.1 multiple sequence alignment

```

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

tr|D5KRX9|D5KRX9_ECHOC      ECCGTLLNREWVLSAAHCDMENMEIYLGMHNLSPNPQDARRRDPEEKYFC
tr|D5KRY1|D5KRY1_ECHOC      HCTGTLLIGKQWVLTAARCNKKNIRVKIGMHNKNERTEDEMMRVAAEKFFC
                                .* ***:.:***:***:*. :*:.. :***** .: .:*  * . **:**

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

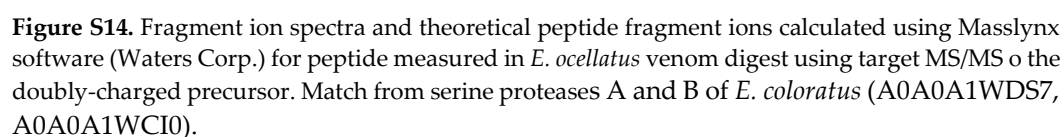
tr|D5KRX9|D5KRX9_ECHOC      GIPSPNETYTPDVPHCANINILRYSVCRSTYWYELLPAQSRALCAGDRRRR
tr|D5KRY1|D5KRY1_ECHOC      TTTTTKVTYPDVPCANIKIFDYSVCR--EAYRKLPEKSRTLCAGILEGG
                                .:..: *****:***:*. *****  * . ** :*:***** .

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

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

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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          MVLIRVLANLLVLQLSYAQMSSELVVGGEENRNRHRSLLALYNSS--GT
tr|A0A0A1WDS7|A0A0A1WDS7_ECHCO -----GGAECNINEHRSLALIYNSTSMWF
                                   **.*** *.*****:***:

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

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

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

sp|B5U6Y3|VSP_ECHOC          TCLGDSGGPLICNGQIQGIVSWGAEVCAKPHAPGLYTKVSDYTDWIQSII
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.