Figure S1. SDS-PAGE analysis of soluble fraction (SL) of the venom and precipitate fraction of the venom (P). Samples (20µL) were analysed by SDS-PAGE (12 %) and stained with Comassie brilliant blue. The venom possesses a wide variety of proteins ranging from 14 to 97kDa. **B- Trypsin Inhibition Assay.** The venom (15 µl) was preincubated for 30 min with trypsin (1:1), and afterwards substrate was added to the samples. Substrate consumption in Arbitrary Units of Fluorescence (Y axis) was read every 5 min for 35 min (X axis). In the test, the sea anemone *A. cascaia* venom; Blank (B + S); and Positive Control (E + S), were analysed. B = Buffer, S = Substrate and E = Enzyme. The venom shows the presence of inhibitor components by complete inhibition of Trypsin activity. **C- MALDI-TOF analysis of *A. cascaia's* venom by positive mode**. The mass spectrum shows that *A. cascaia* venom is also composed by low molecular mass components ranging from m/z 3018 to 9995.7.

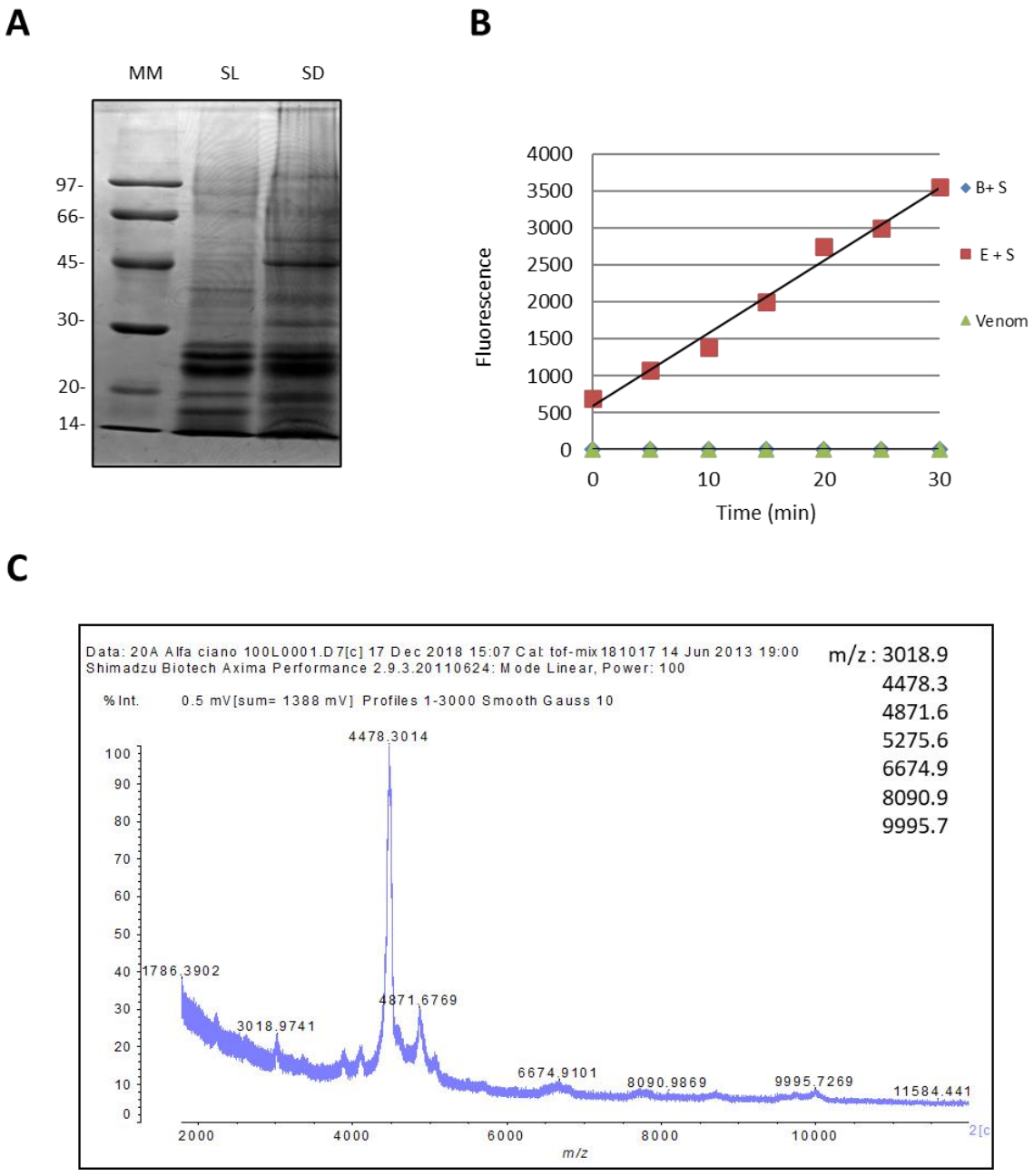
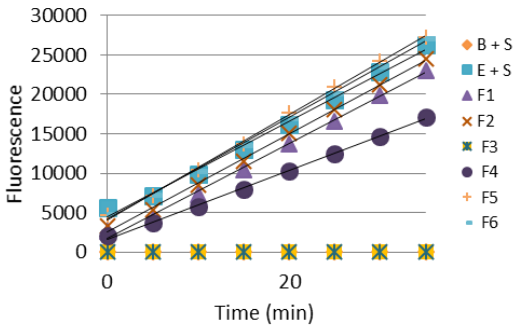


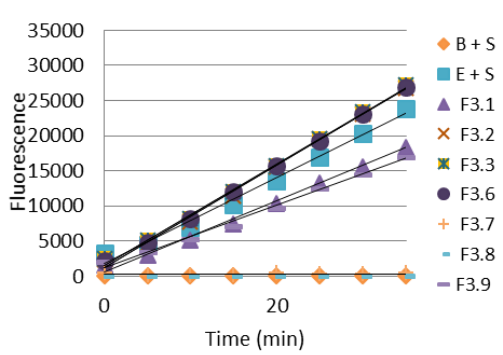
Figure S2. Trypsin Inhibition Assay. Fractions (5 to 15 µl) were preincubated for 30 min with trypsin (1:1), and afterwards substrate was added to the samples. Substrate consumption in Arbitrary Units of Fluorescence (Y axis) was read every 5 min for 35 min (X axis). In the test, samples representing venom fractions from *A. cascaia*; Blank (B + S); and Positive Control (E + S), were analysed. B = Buffer, S = Substrate and E = Enzyme. **A- Shows the graph and resulting table of the inhibition assay performed with fractions (F1-F6) from the venom.** F3 and F4 were the only fractions capable of inhibiting trypsin, presenting 100% (F3) and 28% (F4) of inhibition. **B- Shows the graph and resulting table of the inhibition assay performed with fractions from F3.** The subfractions F3.7 and F3.8 presented complete inhibition (100%) of the enzyme activity. **C- Shows the graph of the assay performed with fractions from F3.7.** The subfractions F3.7.5 and 3.7.6 presented 90% of inhibition of the enzymatic activity. **D- Shows the graph of the assay performed with fractions from F3.8.** A wide variety of subfractions presented inhibitory activity, however F3.8.4; F 3.8.6; F3.8.8 were considered the best candidates due to the quality of isolation of peaks, that exhibited 85%; 65% and 59 % of trypsin inhibition, respectively.

A



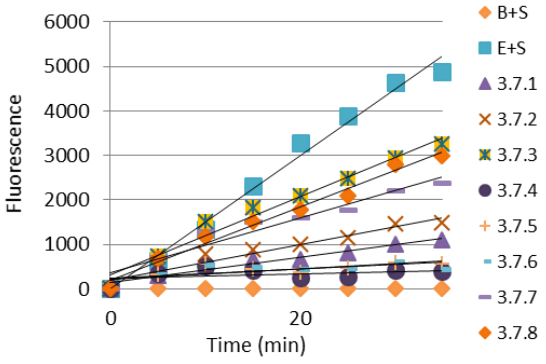
Sample	Enzyme activity (%)	Inhibition (%)
Control (E+S)	100	0
F1	99	1
F2	100	0
F3	0	100
F4	72	28
F5	100	0
F6	100	0

B



Sample	Enzyme activity (%)	Inhibition (%)
Control (E+S)	100	0
F3.1	83	17
F3.2	100	0
F3.3	100	0
F3.4	100	0
F3.5	100	0
F3.6	100	0
F3.7	0	100
F3.8	0	100
F3.9	72	28

C



D

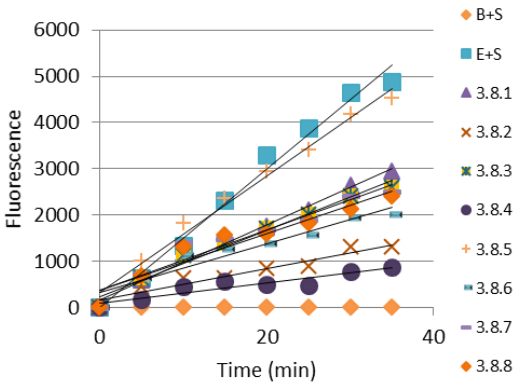


Figure S3. Coverage of amino acid sequence from Blastp hit PI-actitoxin-Aeq3a-like based on peptides identified by LC-MS/MS in ACPI-I isolated from *A. cascaia* venom. Supporting peptides R.YYYDESIGTC(+57.02)R.Q and R.QFIFGGC(+57.02)QGNENNFETM(+15.99)K.E are highlighted in grey color and underlined in blue. Cysteine (C) residues present +57.02 mass shift due to alkylation with iodoacetamide and Methionine (M) residues from peptides also present a mass increase of 15.99 due to oxidation. The mass spectra and the error (da) corresponding to the sequence 'YYYDESIGTC' is presented below the sequence.

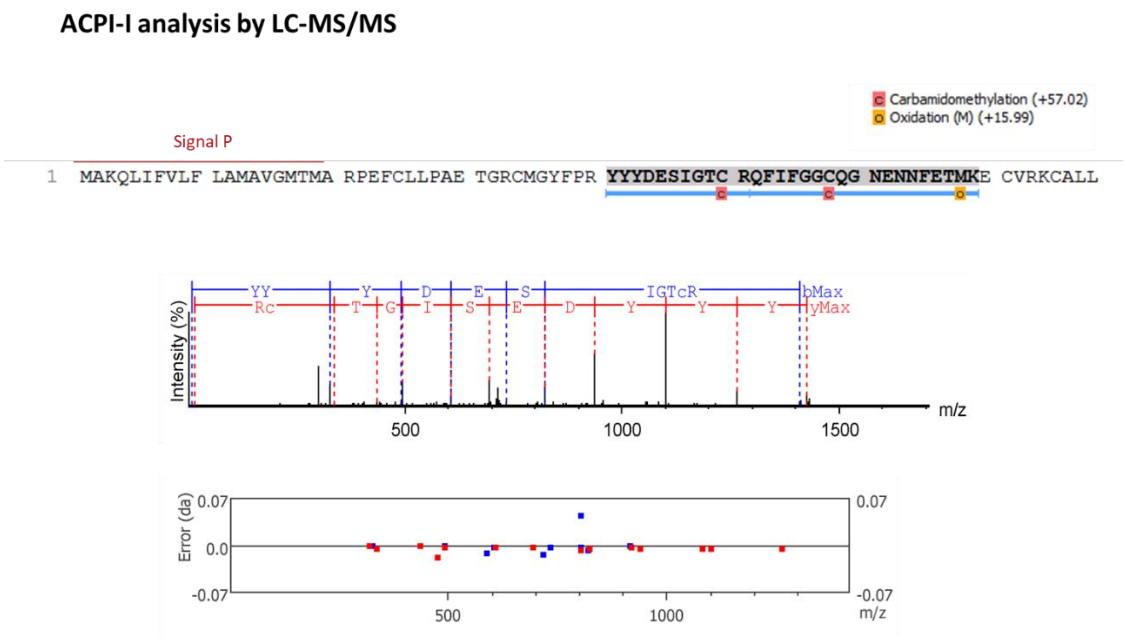


Figure S4. Coverage of amino acid sequence from the Blastp hit PI-actitoxin-Aeq3a-like based on peptides identified by LC-MS/MS in ACPI-II isolated from *A. cascaia* venom. Supporting peptides – R.C(+57.02)M(+15.99)GYFPR.Y, R.YYYDESIGTC(+57.02)R.Q and R.QFIFGGC(+57.02)QGNENNFETMK.E – are highlighted in grey color and underlined in blue color. Cysteine (C) residues present +57.02 mass shift due to alkylation with iodoacetamide and Methionine (M) residues also present a mass increase of 15.99 due to oxidation. The mass spectra and the error (da) corresponding to the sequence 'YYYDESIGTC' is presented below the sequence.

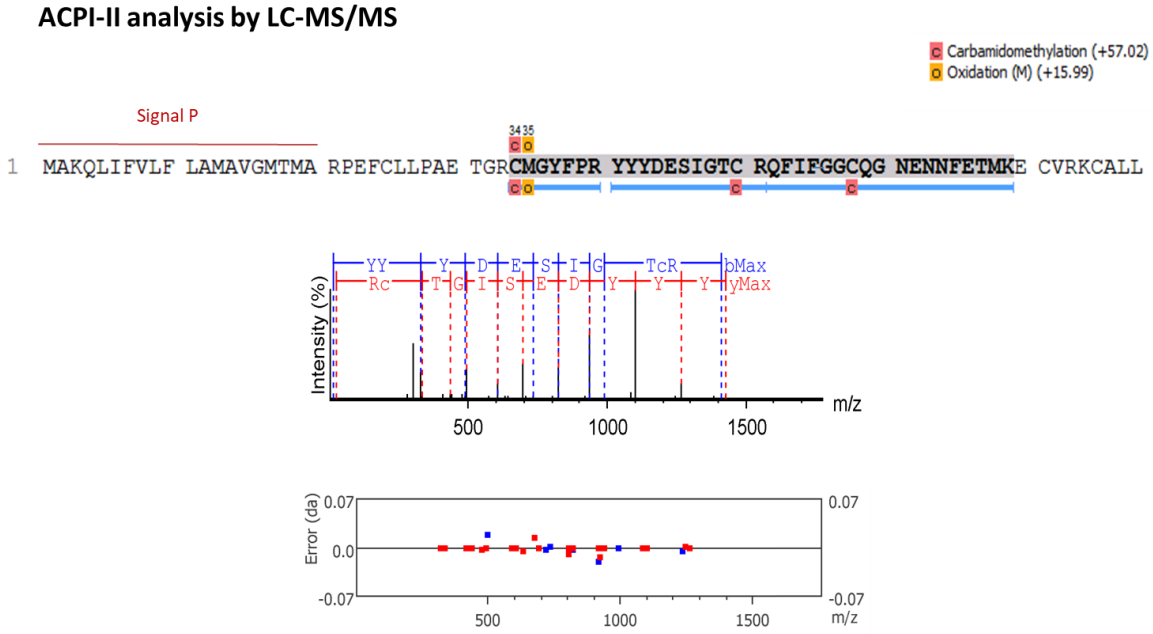


Figure S5. Coverage of amino acid sequence from Blastp hit PI-actitoxin-Aeq3a-like based on peptides identified by LC-MS/MS in ACPI-III isolated from *A. cascaia* venom. The supporting peptide –R.YYYDESIGTC(+57.02)R.Q – is highlighted in grey color and underlined in blue color. Cysteine (C) residues present +57.02 mass shift due to alkylation with iodoacetamide. The mass spectra and the error (da) corresponding to the sequence 'YYYDESIGTC' is presented below the sequence.

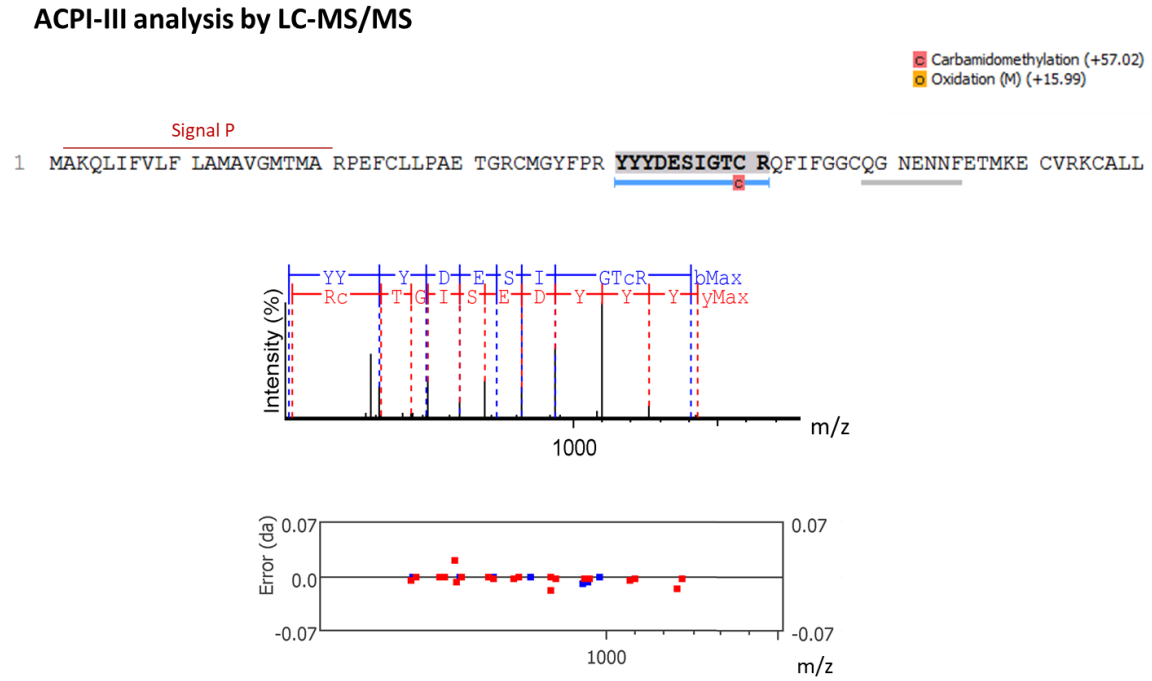
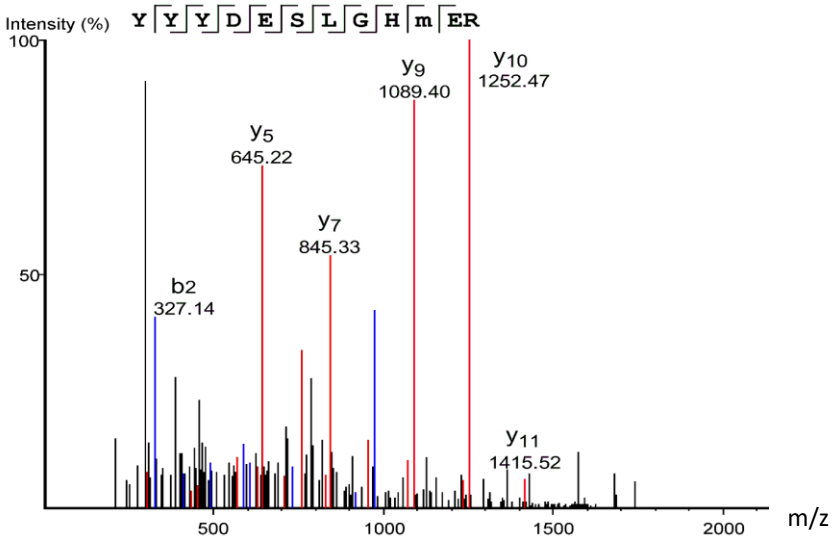


Table S1. List of de novo peptides identified by LC-MS/MS in each sample (ACPI-I, ACPI-II and ACPI-III). The table shows the Average Local Confidence percentage (ALC %) for each peptide identified. De novo sequences were submitted to BLASTp and searched using the non-redundant proteins database and Cnidaria taxid at NCBI platform. De novo sequences revealed that peptides from ACPI-I, ACPI-II and ACPI-III present 87%; 78% and 66% of identity to the peptides found in PI-actitoxin-Aeq3a-like from *Actinia tenebrosa,* respectively.

Isolated peptide	De novo peptides	ALC (%)	E value	Per ID (%)	PI-actitoxin-Aeq3a-like from *Actinia tenebrosa*
ACPI-I	YYYDESLGHM (+15,99)ER	73%	1.2	87.5	
ACPI-II	DLDM(+15.99)GYFPR	75%	2e-13	77.7	
	YYYDESLGM (+15.99) M (+15.99) M (+15.99) K	91%			
	QDKYGGCQGNENNFETMK	89%			
ACPI-III	YYYDESLGGSC(+57.02)HK	71%	3e-08	65.62	
	GYWQNC(+57.02)QGNENNFETMK	69%			

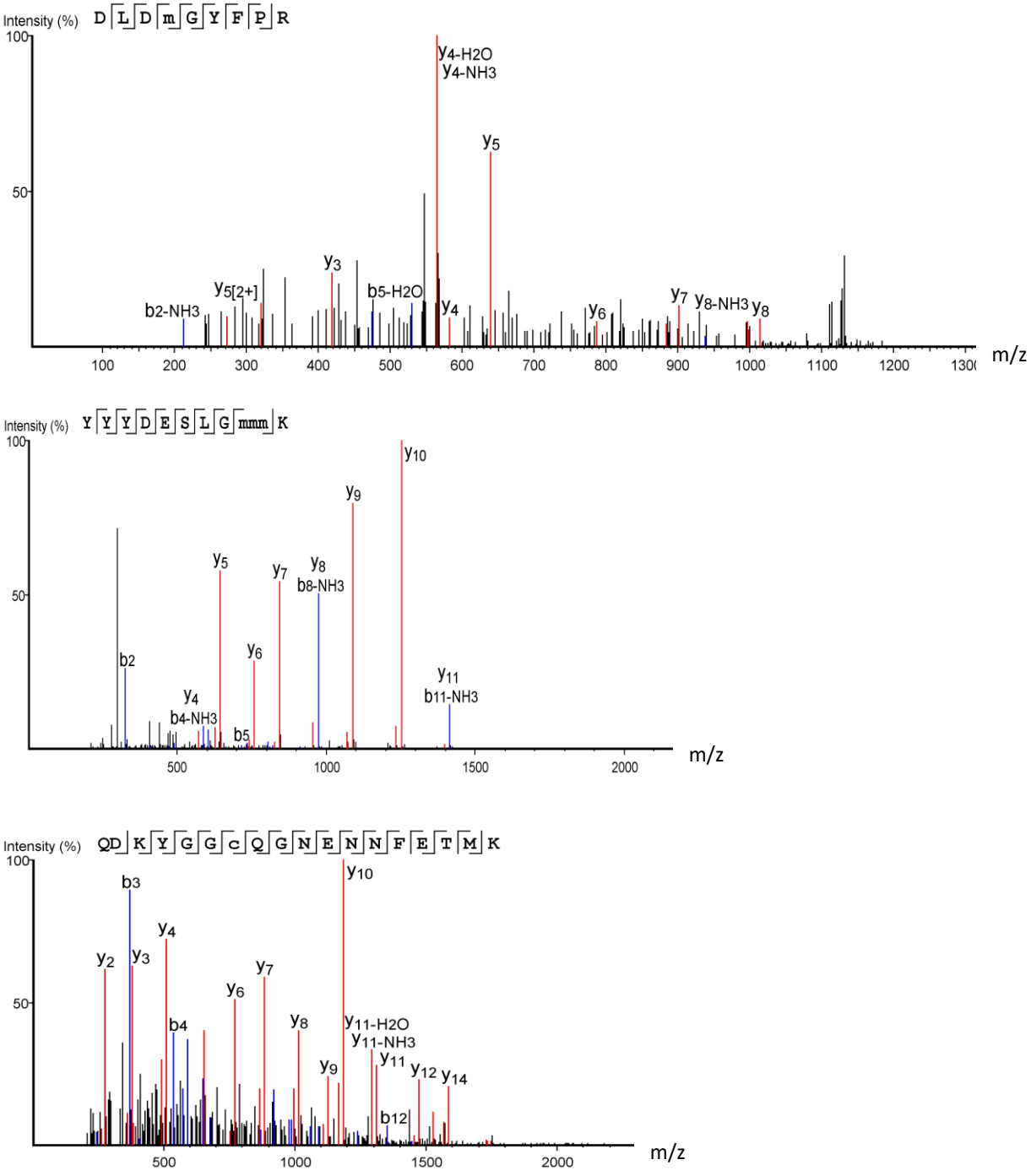
Figure S6. Mass spectra from de novo peptides identified by LC-MS/MS in ACPI-I, ACPI-II and ACPI-III isolated from *A. cascaia* venom.

ACPI-I YYYDESLGHMER



Supplementary material S6 continue in next page.

ACPI-II DLDMGYFPR YYYDESLGMMMK QDKYGGCQG NENNFETMK



Supplementary material S6 continue in next page.

ACPI-III YYYDESLGGSC GYWQNCQGHK NENNFETMK