Supplementary Materials: New Insights on Moojase, a Thrombin-Like Serine Protease from *Bothrops moojeni* Snake Venom

Fernanda G. Amorim, Danilo L. Menaldo, Sante E. I. Carone, Thiago A. Silva, Marco A. Sartim, Edwin De Pauw, Loic Quinton and Suely V. Sampaio

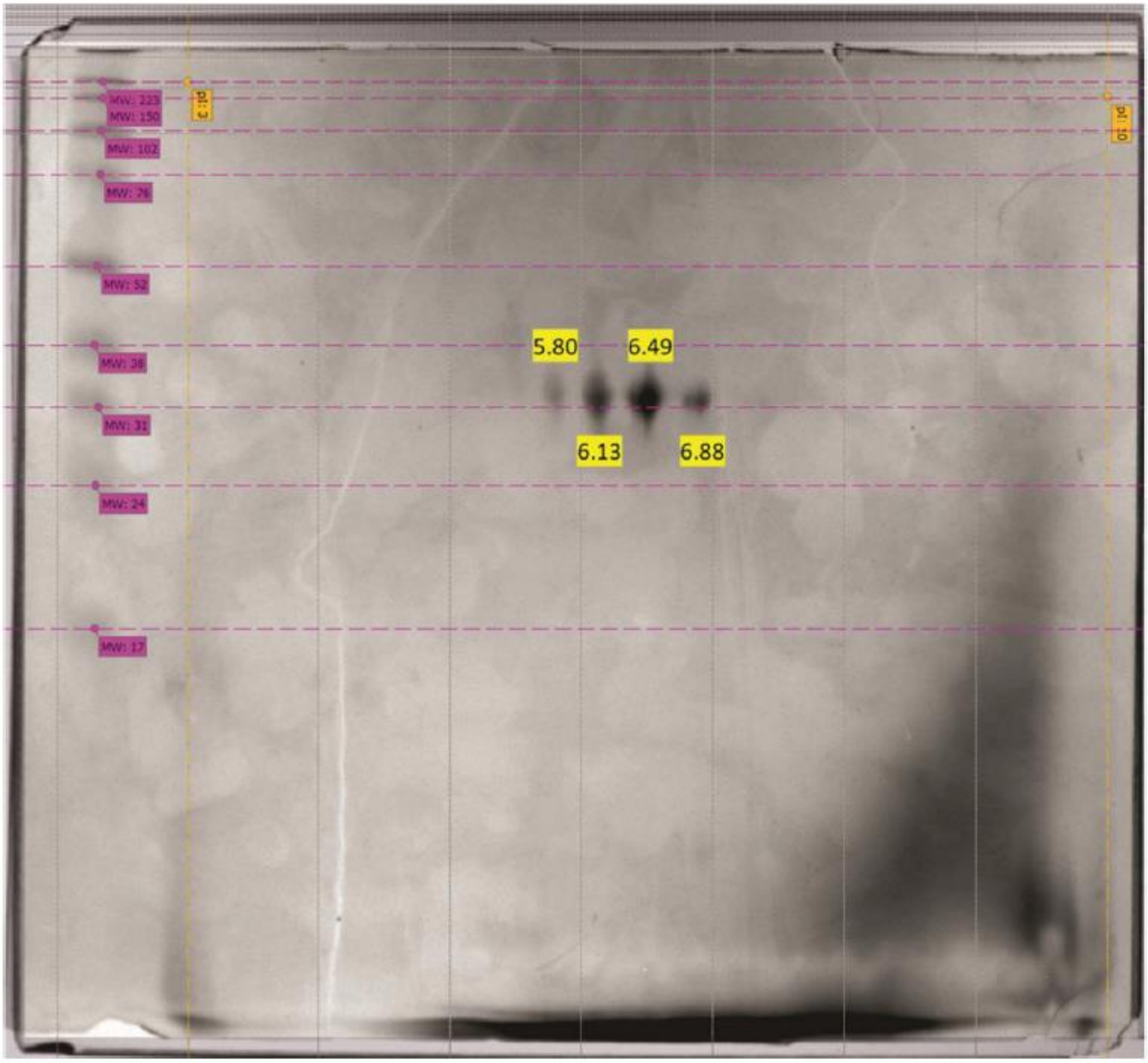
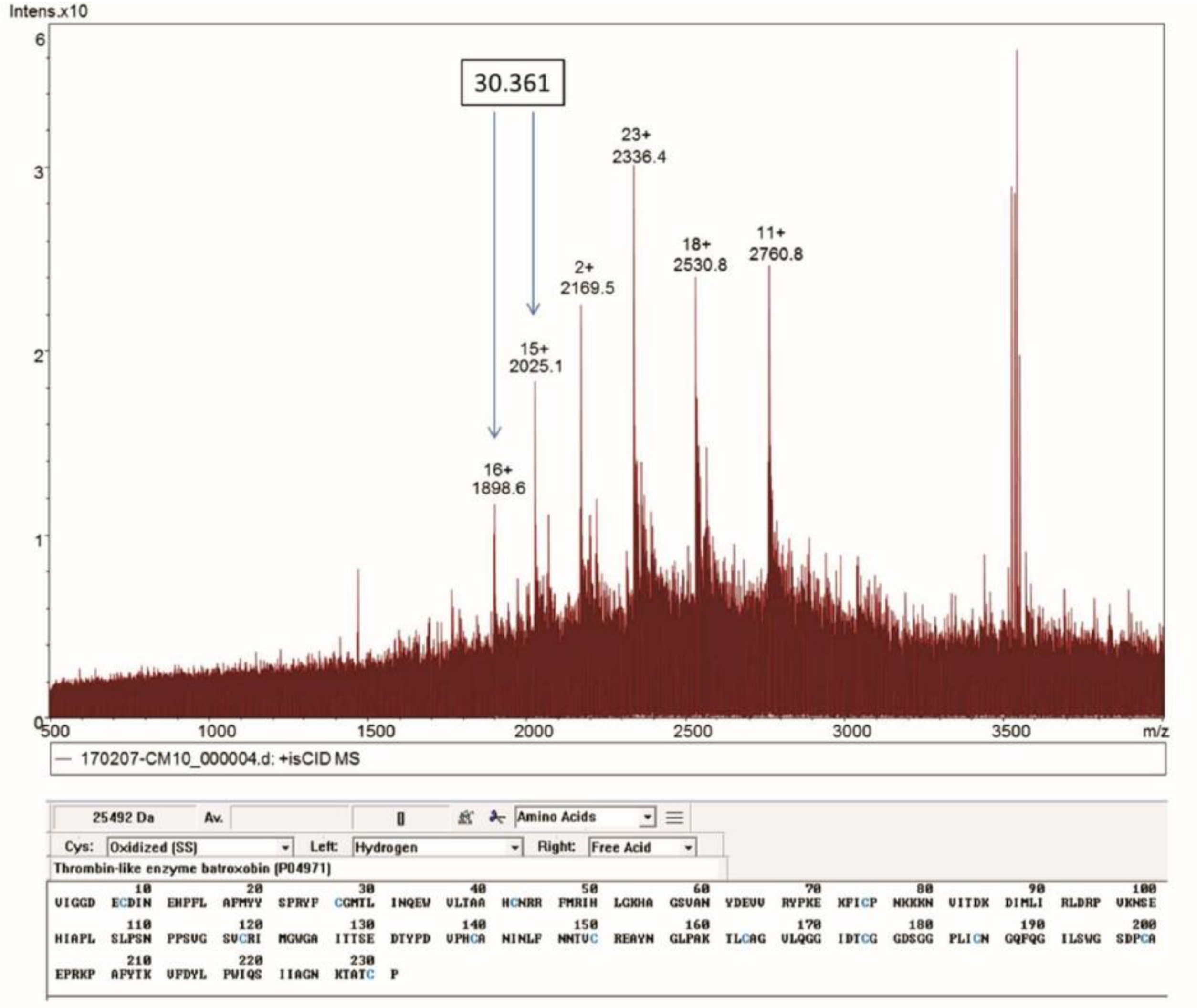


Figure S1. Isoelectric focusing of Moojase. Moojase presented four protein bands, indicating different pI values ranging from 5.80 to 6.88.



25492 Da

Cys: Oxidized (SS) Left: Hydrogen Right: Free Acid

Thrombin-like enzyme batroxobin (P04971)

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          10          20          30          40          50          60          70          80          90         100
VIGGD ECDIN EHPFL AFMYY SPRYF CGMTL INQEW VLTAA HCNRR FMRIH LGKHA GSVAN YDEVV RYPKE KFICP NKKKN VITDK DIMLI RLDRP VKNSE
         110         120         130         140         150         160         170         180         190         200
HIAPL SLPSN PPSVG SVCRI MGWGA ITTSE DTYPD VPHCA NINLF NNTVC REAYN GLPAK TLCAG VLQGG IDTCG GDSGG PLICN GQFQG ILSWG SDPCA
         210         220         230
EPRKP AFYTK VFDYL PWIQS IIAGN KTATC P
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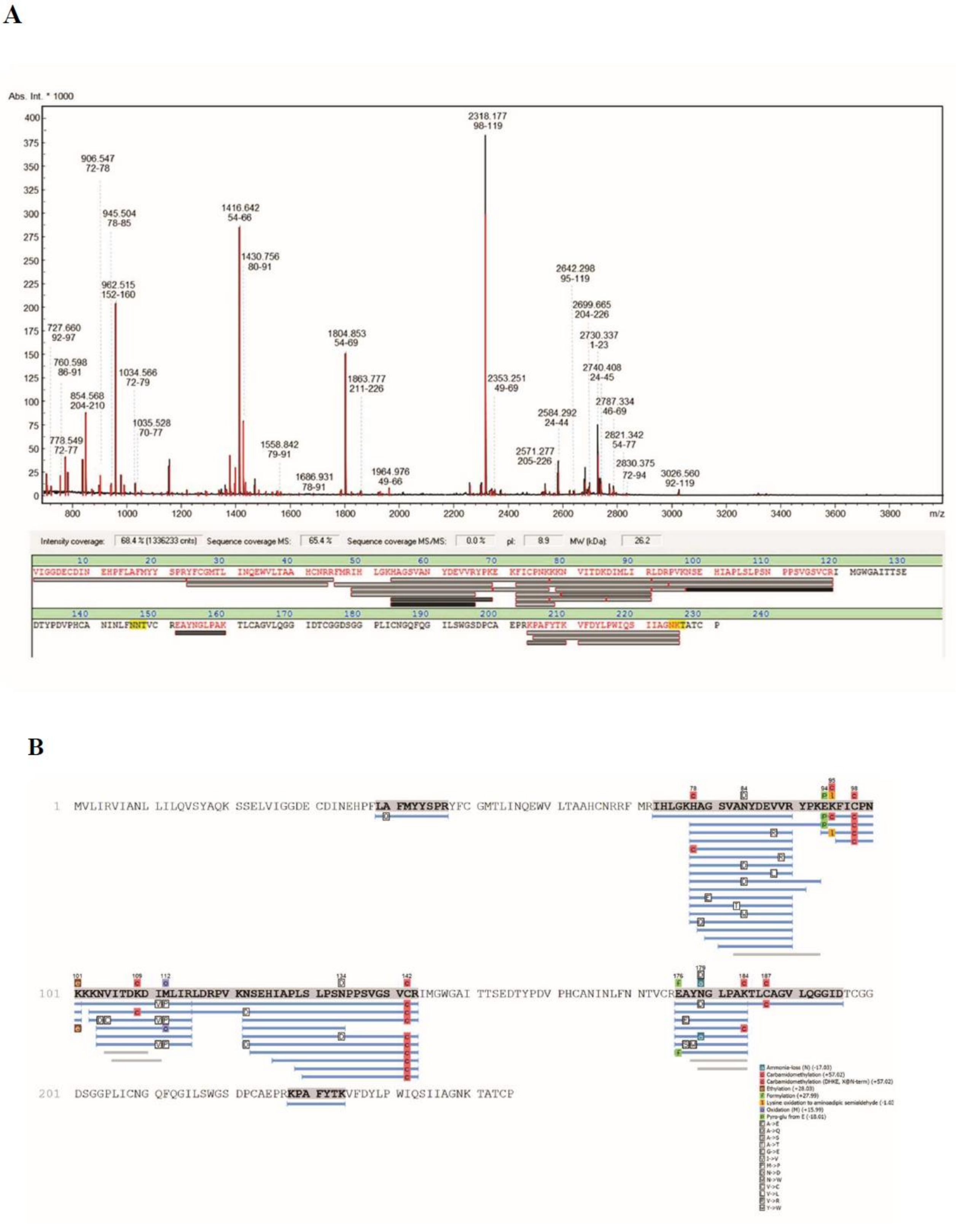
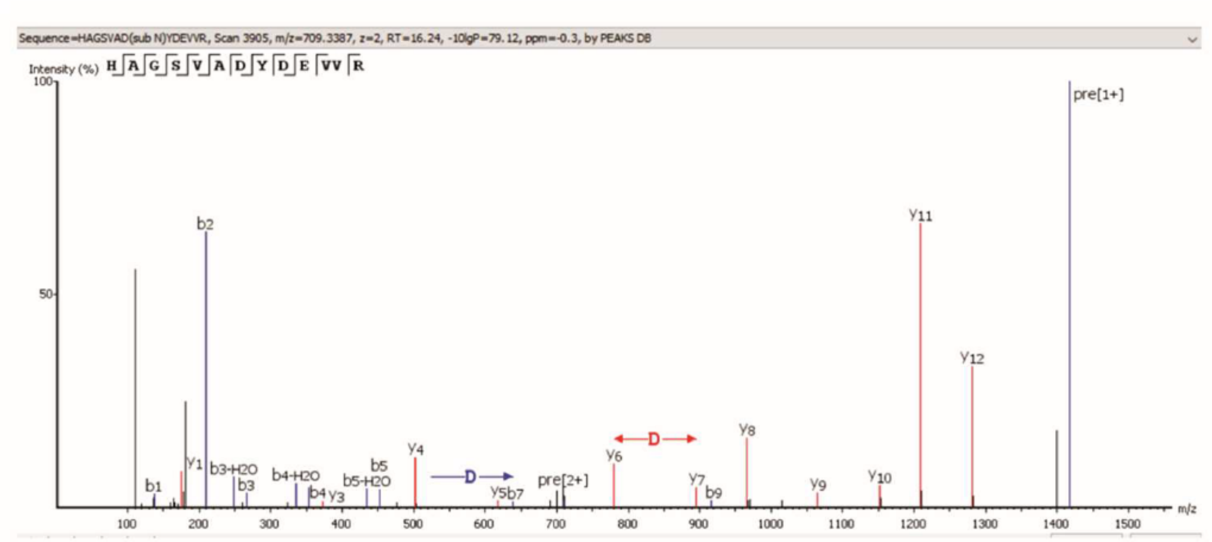
Figure S2. Molecular mass determination by Bruker Solarix 9.4T FTICR mass spectrometer. The mass spectra show the practical molecular mass calculated based on 16-time charge. The box below presents the theorical molecular mass of Moojase oxidized predicted by Sequence Editor software.

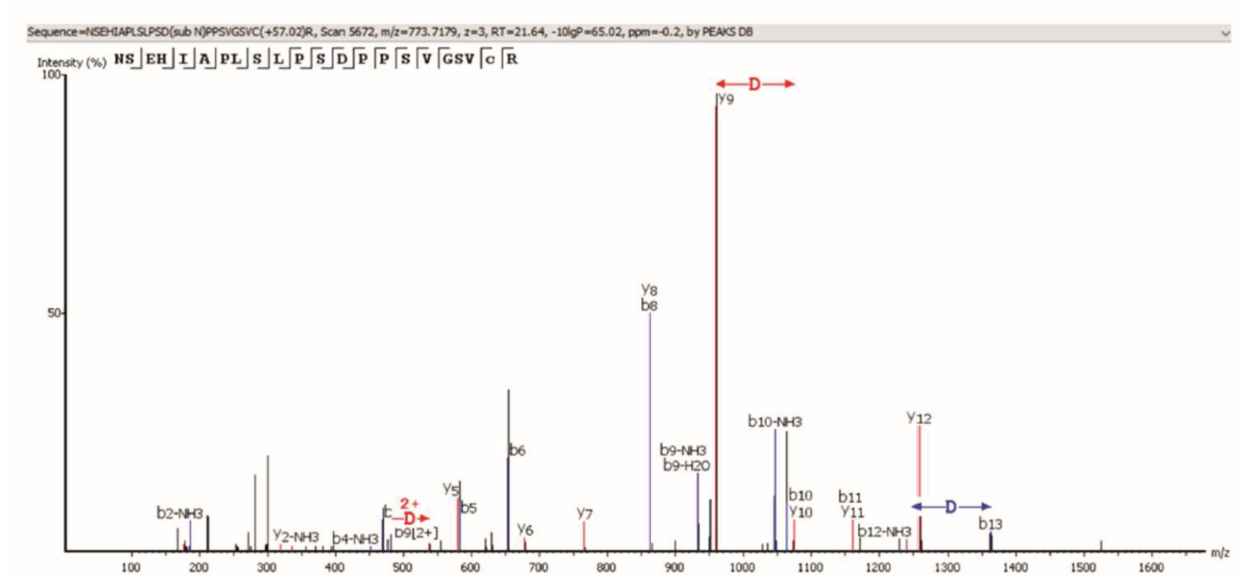

A
Abs. Int. * 1000
400
375
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
800
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200
3400
3600
3800
m/z
2318.177 98-119
906.547 72-78
945.504 78-85
1416.642 54-66
1430.756 80-91
962.515 152-160
727.660 92-97
760.598 86-91
854.568 204-210
778.549 72-77
1034.566 72-79
1035.528 70-77
1558.842 79-91
1686.931 78-91
1804.853 54-69
1863.777 211-226
1964.976 49-66
2353.251 49-69
2642.298 95-119
2699.665 204-226
2730.337 1-23
2740.408 24-45
2584.292 24-44
2571.277 205-226
2787.334 46-69
2821.342 54-77
2830.375 72-94
3026.560 92-119
Intensity coverage: 68.4 % (1336233 cnts)
Sequence coverage MS: 65.4 %
Sequence coverage MS/MS: 0.0 %
pI: 8.9
MW (kDa): 26.2
10 20 30 40 50 60 70 80 90 100 110 120 130
VIGGDECDIN EHPFLAFMYY SPRYFCGMTL INQEWVLTAA HCNRRFMRIH LGKHAGSVAN YDEVVRYPKE KFICPNKKKN VITDKDIMLI RLDRPVKNSE HIAPLSLPSN PPSVGSVCRI MGWGAITTSE
140 150 160 170 180 190 200 210 220 230 240
DTYPDVPHCA NINLFNNTVC REAYNGLPAK TLCAGVLQGG IDTCGGDSGG PLICNGQFQG ILSWGSDPCA EPRKPAFYTK VFDYLPWIQS IIAGNKTATC P
B
1 MVLIRVIANL LILQVSYAQK SSELVIGGDE CDINEHPFLA FMYYSPRYFC GMTLINQEWV LTAAHCNRRF MRIHLGKHAG SVANYDEVVR YPKEKFICPN
78
84
94
95
98
101 KKKNVITDKD IMLIRLDRPV KNSEHIAPLS LPSNPPSVGS VCRIMGWGAI TTSEDTYPDV PHCANINLFN NTVCREAYNG LPAKTLCAGV LQGGIDTCGG
101
109
112
134
142
176
179
184
187
201 DSGGPLICNG QFQGILSWGS DPCAEPRKPA FYTKVFDYLP WIQSIIAGNK TATCP
Ammonia-loss (N) (-17.03)
Carbamidomethylation (+57.02)
Carbamidomethylation (DHKE, X@N-term) (+57.02)
Ethylation (+28.03)
Formylation (+27.99)
Lysine oxidation to aminoadipic semialdehyde (-1.03
Oxidation (M) (+15.99)
Pyro-glu from E (-18.01)
A->E
A->Q
A->S
A->T
G->E
I->V
M->P
N->D
N->W
V->C
V->L
V->R
Y->W

C



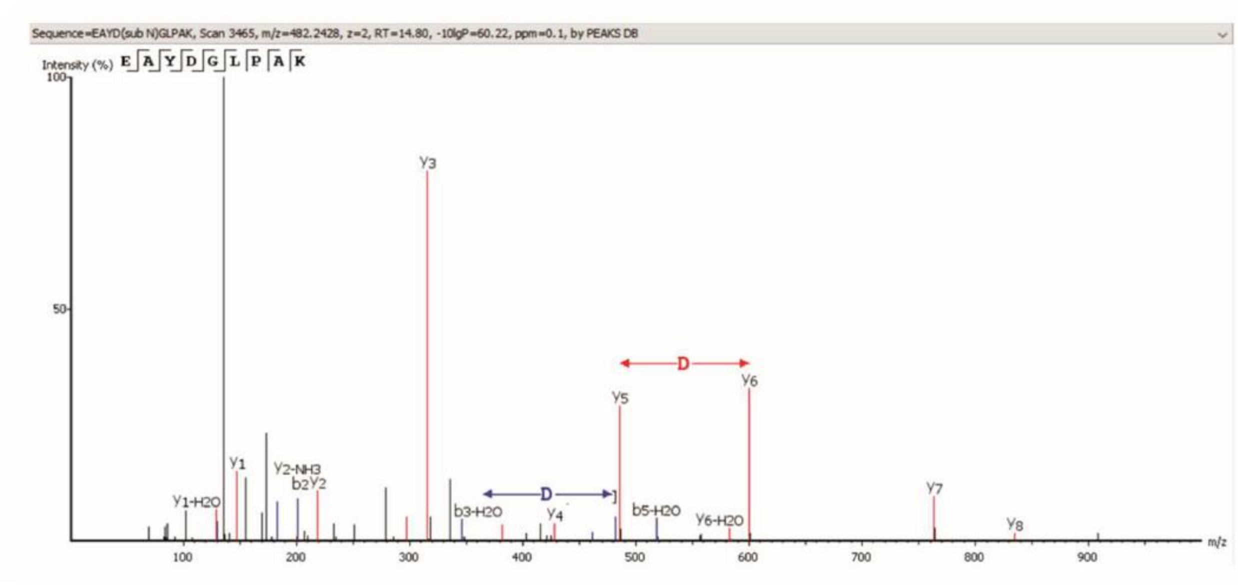
Sequence=HAGSVAD(sub N)YDEVVR, Scan 3905, m/z=709.3387, z=2, RT=16.24, -10lgP=79.12, ppm=-0.3, by PEAKS DB

D



Sequence=NSEHIAPLSLPSD(sub N)PPSVGSVC(+57.02)R, Scan 5672, m/z=773.7179, z=3, RT=21.64, -10lgP=65.02, ppm=-0.2, by PEAKS DB

E



Sequence=EAYD(sub N)GLPAK, Scan 3465, m/z=482.2428, z=2, RT=14.80, -10lgP=60.22, ppm=0.1, by PEAKS DB

Figure S3. Structural analysis of Moojase by mass spectrometry. (**A**) MALDI-TOF sequencing of Moojase, which covered 65.4% of the sequence template (Batroxobin). (**B**) Q-Exactive sequencing using Batroxobin as a template. The fragments that were sequenced in this methodology are marked in blue. (**C**–**E**) Sequencing of the fragments that presented the punctual mutations at positions 60, 110 and 123 of the template sequence.