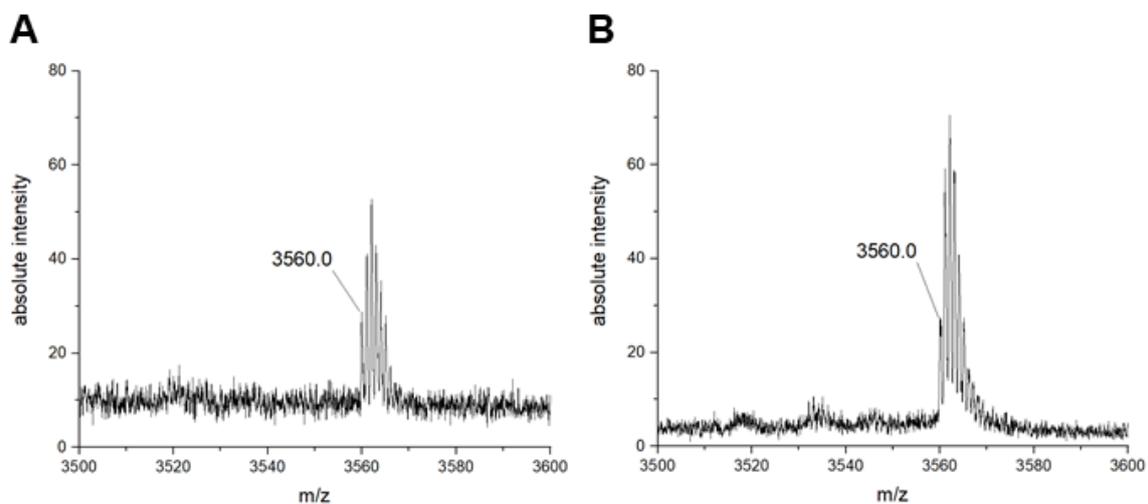
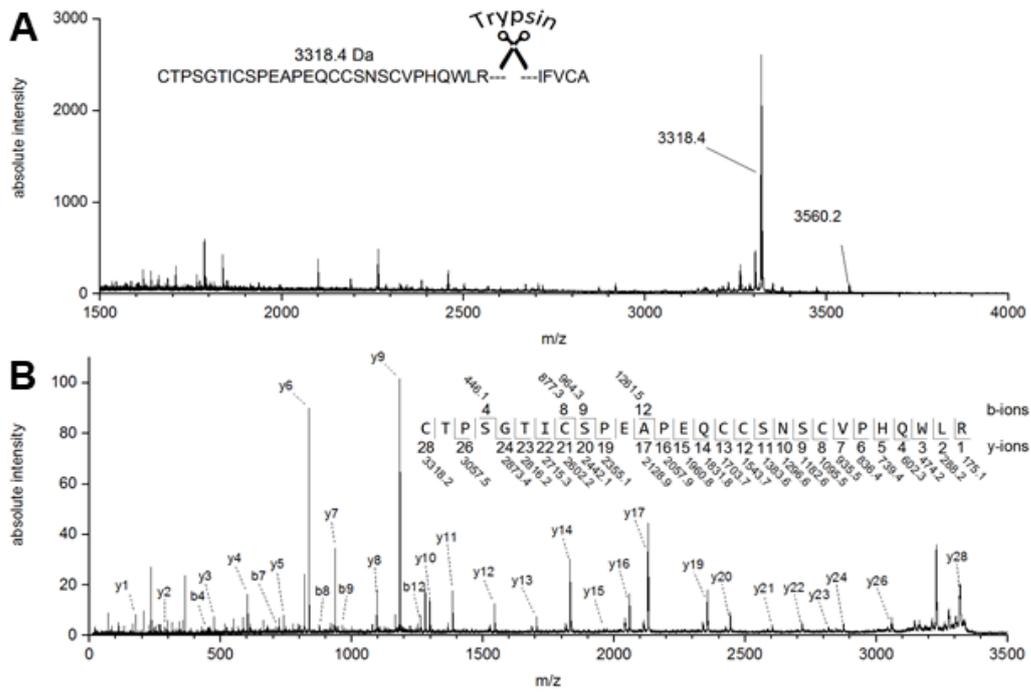


## ASSOCIATED CONTENT

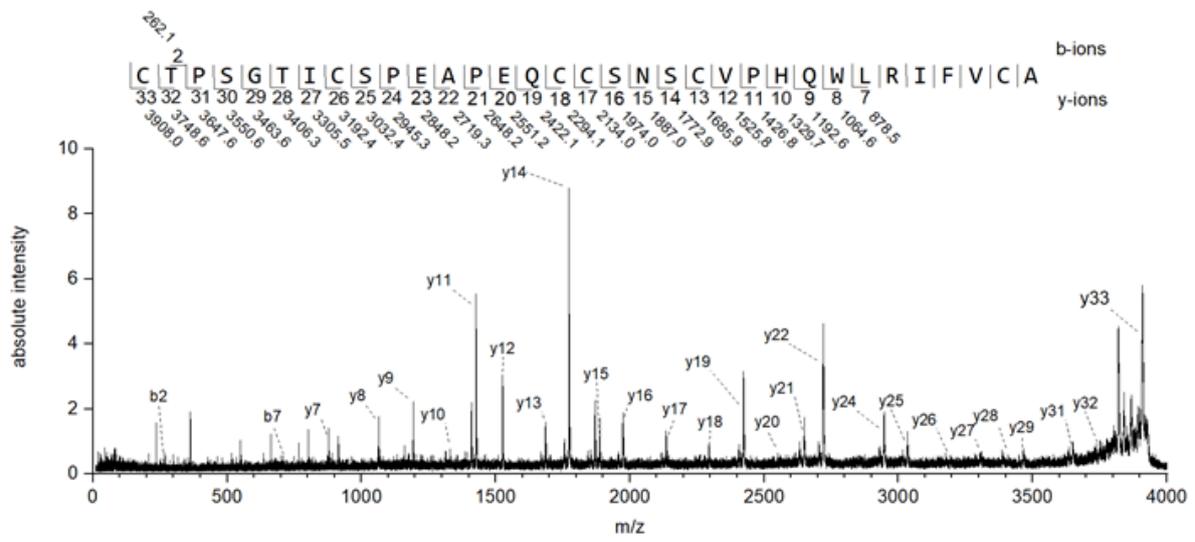


**Supplementary Figure S1. Comparative mass spectrometry of different beetroot extraction methods.** Mass spectrometry analysis crude extract prepared by chemical extraction of beetroot pulp (A) and beetroot juice (B).



**Supplementary Figure S2. *De-novo* sequencing of bevuTI-I (trypsin digestion).** (A) MALDI spectrum after digestion with trypsin of carbamidomethylated bevuTI-I. One fragment (3314.4 Da) of bevuTI-I was used for *de-novo* sequencing. (B) Annotated MS/MS spectrum of the 3314.4 Da precursor.





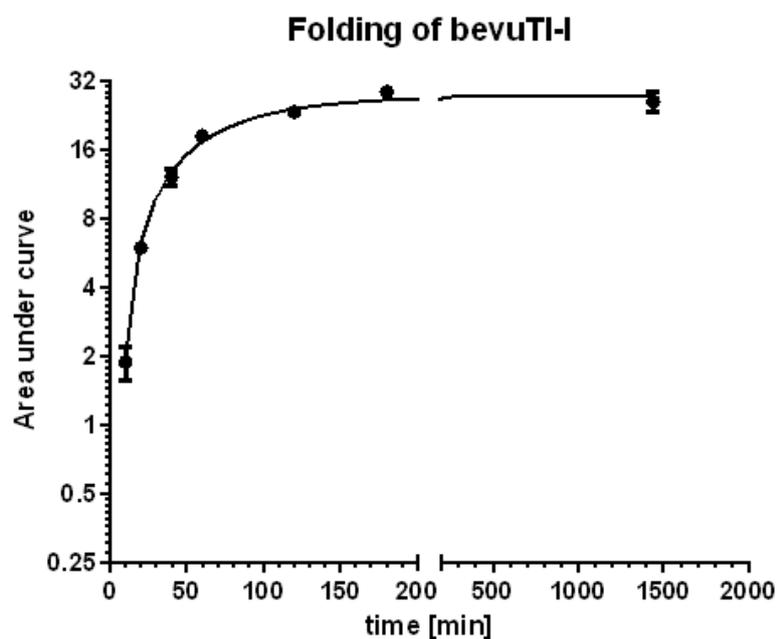
**Supplementary Figure S4. *De-novo* sequencing of bevuTI-I (full-length peptide).** Annotated MS/MS spectrum of carbamidomethylated bevuTI-I.

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Beta_maritima  EECAPSGHICSGFGPPEQCCSGACVPHPMLRIFVC-
Beta_vulgaris  EECAPSGHICSGFGPPEQCCSGACVPHPMLRIFVC-
Beta_patula    EECAPSGHICSGFGPPEQCCSGACVPHPRLRIFVC-
BevuTI-I      --CTPSGTICSP EAP-EQCCSNSCVPHQWLRIFVCA
                *:* ** * ** . * * * * * . : * * * * * * * * * *

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**Supplementary Figure S5. Alignment of bevuTI-I with similar *Bevu spp.* sequences** (mature peptide domain). For precursor hits of *Beta maritima*, *Beta vulgaris* and *Beta patula* refer to Supplementary Data S3-S5. Cysteine residues are highlighted in yellow; ‘\*’, ‘:’ and ‘.’ indicate identical and similar amino acids, respectively.



**Supplementary Figure S6. Oxidative folding kinetics of bevuTI-I.** The folding kinetics of bevuTI-I were determined based on the peak area of bevuTI-I in analytical HPLC chromatograms (wavelength: 214 nm) recorded at several time points (5, 10, 20, 40, 60 min and 1, 2, 3, 24 h) during oxidative folding.

**Supplementary Table S1.** Comparison of measured and calculated mass to charge ratios of y- and b-ions of the tryptic fragment with a mass of 3318.4 Da.

Sequence	b-ions				y-ions			
	b-ion	measured mass in Da	theoretical mass in Da	difference	y-ion	measured mass in Da	theoretical	difference
C	b1	n.d.	161.0		y28	3318.2	3318.4	-0.1
T	b2	262.1	262.1	0.0	y27	3158.4	3158.4	0.0
P	b3	n.d.	359.1		y26	3057.5	3057.3	0.0
S	b4	446.1	446.1	-0.1	y25	2960.3	2960.3	0.0
G	b5	503.2	503.2	0.0	y24	2873.4	2873.2	-0.1
T	b6	n.d.	604.2		y23	2816.2	2816.2	0.0
I	b7	717.3	717.3	0.0	y22	2715.3	2715.2	-0.1
C	b8	877.3	877.3	0.0	y21	2602.2	2602.1	-0.1
S	b9	964.3	964.3	0.0	y20	2442.1	2442.1	-0.1
P	b10	1061.4	1061.4	0.0	y19	2355.1	2355.1	-0.1
E	b11	n.d.	1190.4		y18	2257.9	2258.0	-0.1
A	b12	1261.5	1261.5	0.0	y17	2128.9	2129.0	-0.1
P	b13	n.d.	1358.5		y16	2057.9	2057.9	-0.1
E	b14	n.d.	1487.6		y15	1960.8	1960.9	-0.1
Q	b15	n.d.	1615.6		y14	1831.8	1831.8	-0.1
C	b16	n.d.	1775.6		y13	1703.7	1703.8	-0.2
C	b17	n.d.	1935.6		y12	1543.7	1543.8	-0.2
S	b18	n.d.	2022.7		y11	1383.6	1383.8	-0.2
N	b19	n.d.	2136.7		y10	1296.6	1296.7	-0.1
S	b20	n.d.	2223.7		y9	1182.6	1182.7	-0.2
C	b21	n.d.	2383.7		y8	1095.5	1095.6	-0.2
V	b22	n.d.	2482.8		y7	935.5	935.6	-0.2
P	b23	n.d.	2579.9		y6	836.4	836.6	-0.2
H	b24	n.d.	2716.9		y5	739.4	739.5	-0.1
Q	b25	n.d.	2845.0		y4	602.3	602.5	-0.2
W	b26	n.d.	3031.1		y3	474.2	474.4	-0.2
L	b27	n.d.	3144.2		y2	288.2	288.3	-0.1
R	b28	n.d.	3319.4		y1	175.1	175.2	-0.1

n.d. not detected

**Supplementary Table S2.** Comparison of measured and calculated mass to charge ratios of y- and b-ions of the fragment (2421.8 m/z) obtained by digestion with endoproteinase GluC.

Sequence	b-ions				y-ions			
	b-ion	measured mass in Da	theoretical mass in Da	difference	y-ion	measured mass in Da	theoretical mass in Da	difference
Q	b1	129.1	129.1	0.0	y19	2421.9	2422.1	-0.2
C	b2	289.1	289.1	0.0	y18	2294.0	2294.0	0.0
C	b3	449.1	449.2	-0.1	y17	2133.9	2134.0	-0.1
S	b4	536.1	536.2	-0.1	y16	1973.9	1973.9	0.0
N	b5	650.2	650.2	0.0	y15	1886.8	1886.9	-0.1
S	b6	737.2	737.3	-0.1	y14	1772.8	1772.9	-0.1
C	b7	897.2	897.3	-0.1	y13	1685.7	1685.8	-0.1
V	b8	996.3	996.4	-0.1	y12	1525.7	1525.8	-0.1
P	b9	n.d.	1093.3		y11	1426.6	1426.7	-0.1
H	b10	1230.4	1230.5	-0.1	y10	1329.6	1329.7	-0.1
Q	b11	1358.4	1358.5	-0.1	y9	1192.5	1192.6	-0.1
W	b12	1544.5	1544.6	-0.1	y8	1064.5	1064.6	-0.1
L	b13	1657.6	1657.7	-0.1	y7	878.4	878.5	-0.1
R	b14	1813.6	1813.8	-0.2	y6	765.3	765.4	-0.1
I	b15	n.d.	1926.9		y5	609.1	609.3	-0.2
F	b16	2073.8	2073.9	-0.1	y4	n.d.	496.2	
V	b17	n.d.	2173.0		y3	n.d.	349.2	
C	b18	n.d.	2333.0		y2	250.1	250.1	0.0
A	b19	n.d.	2404.0		y1	n.d.	90.1	

n.d. not detected

**Supplementary Table S3.** Comparison of measured and calculated mass to charge ratios of y- and b-ions of the chymotryptic fragments with a mass of 3048.9 Da and 878.4 Da.

precursor: 3048.9 Da

Sequence	b-ions				y-ions			
	b-ion	measured mass in Da	theoretical mass in Da	difference	y-ion	Measured mass in Da	Theoretical mass in Da	Difference
C	b1	161.1	161.0	0.1	y26	3049.1	3049.2	-0.1
T	b2	262.1	262.1	0.0	y25	2889.2	2889.2	0.0
P	b3	359.1	359.1	0.0	y24	2788.1	2788.1	0.0
S	b4	n.d.	446.1	0.0	y23	2691.1	2691.1	0.0
G	b5	503.1	503.2	0.1	y22	2604.0	2604.1	-0.1
T	b6	604.2	604.2	0.0	y21	2547.0	2547.0	0.0
I	b7	717.2	717.3	0.1	y20	2445.9	2446.0	-0.1
C	b8	877.2	877.3	0.1	y19	2332.8	2332.9	-0.1
S	b9	964.3	964.3	0.0	y18	2172.8	2172.9	-0.1
P	b10	1061.3	1061.4	0.1	y17	2085.8	2085.9	-0.1
E	b11	1190.4	1190.4	0.0	y16	1988.7	1988.8	-0.1
A	b12	1261.4	1261.5	0.1	y15	1859.7	1859.8	-0.1
P	b13	1358.5	1358.5	0.0	y14	1788.6	1788.7	-0.1
E	b14	1487.5	1487.6	0.1	y13	1691.6	1691.7	-0.1
Q	b15	1615.6	1615.6	0.0	y12	1562.5	1562.6	-0.1
C	b16	1775.6	1775.6	0.0	y11	1434.4	1434.6	-0.2
C	b17	1935.6	1935.6	0.0	y10	1274.4	1274.6	-0.2
S	b18	2022.8	2022.7	-0.1	y9	1114.4	1114.6	-0.2
N	b19	2136.8	2136.7	-0.1	y8	1027.4	1027.5	-0.1
S	b20	2223.8	2223.7	-0.1	y7	913.3	913.5	-0.2
C	b21	2383.9	2383.7	-0.2	y6	826.3	826.5	-0.2
V	b22	2482.9	2482.8	-0.1	y5	666.3	666.5	-0.2
P	b23	n.d.	2579.9	n.d.	y4	567.2	567.4	-0.2
H	b24	2717	2716.9	-0.1	y3	470.2	470.3	-0.1
Q	b25	2845.1	2845.0	-0.1	y2	333.1	333.3	-0.2
W	b26	3030.5	3031.1	0.6	y1	n.d.	205.1	n.d.

precursor: 878.4Da

Sequence	b-ions				y-ions			
	b-ion	measured mass in Da	Theoretical mass in Da	difference	y-ion	measured mass in Da	Theoretical mass in Da	difference
L	b1	n.d.	114.1	n.d.	y7	878.5	878.5	0.0
R	b2	270.2	270.2	0.0	y6	765.4	765.4	0.0
I	b3	383.3	383.3	0.0	y5	609.3	609.3	0.0
F	b4	530.4	530.4	0.0	y4	496.2	496.2	0.0
V	b5	629.4	629.4	0.0	y3	349.2	349.1	0.1
C	b6	789.5	789.4	0.1	y2	250.1	250.1	0.0
A	b7	n.d.	860.5	n.d.	y1	n.d.	90.1	n.d.

n.d. not detected

**Supplementary Table S4.** Comparison of measured and calculated mass to charge ratios of y- and b-ions of carbamidomethylated full length bevuTI-I with a molecular weight of 3907.9 Da.

Sequence	b-ions				y-ions			
	b-ion	measured mass in Da	theoretical mass in Da	difference	y-ion	Measured mass in Da	theoretical mass in Da	difference
C	b1	n.d.	161.1		y33	3908.0	3908.7	-0.7
T	b2	262.1	262.1	0.0	y32	3748.6	3748.6	0.0
P	b3	n.d.	359.2		y31	3647.6	3647.6	0.0
S	b4	n.d.	446.2		y30	3550.6	3550.5	0.1
G	b5	n.d.	503.2		y29	3463.6	3463.5	0.1
T	b6	604.2	604.3	-0.1	y28	3406.3	3406.5	-0.2
I	b7	717.3	717.3	0.0	y27	3305.5	3305.4	0.1
C	b8	n.d.	877.4		y26	3192.4	3192.4	0.0
S	b9	n.d.	964.4		y25	3032.4	3032.3	0.1
P	b10	n.d.	1061.5		y24	2945.3	2945.3	0.0
E	b11	n.d.	1190.5		y23	2848.2	2848.2	0.0
A	b12	n.d.	1261.5		y22	2719.3	2719.2	0.1
P	b13	n.d.	1358.6		y21	2648.2	2648.2	0.0
E	b14	n.d.	1487.6		y20	2551.2	2551.1	0.1
Q	b15	n.d.	1615.7		y19	2422.1	2422.1	0.0
C	b16	n.d.	1775.7		y18	2294.1	2294.0	0.1
C	b17	n.d.	1935.8		y17	2134.0	2134.0	0.0
S	b18	2022.8	2022.8	0.0	y16	1974.0	1973.9	0.1
N	b19	n.d.	2136.8		y15	1887.0	1886.9	0.1
S	b20	2223.1	2223.9	-0.8	y14	1772.9	1772.9	0.0
C	b21	n.d.	2383.9		y13	1685.9	1685.8	0.1
V	b22	n.d.	2483.0		y12	1525.8	1525.8	0.0
P	b23	n.d.	2580.0		y11	1426.8	1426.7	0.1
H	b24	n.d.	2717.1		y10	1329.7	1329.7	0.0
Q	b25	2845.1	2845.1	0.0	y9	1192.6	1192.6	0.0
W	b26	n.d.	3031.2		y8	1064.6	1064.6	0.0
L	b27	3144.3	3144.3	0.0	y7	878.5	878.5	0.0
R	b28	3300.4	3300.4	0.0	y6	765.4	765.4	0.0
I	b29	3413.4	3413.5	-0.1	y5	609.2	609.3	-0.1
F	b30	3560.6	3560.5	0.1	y4	496.2	496.2	0.0
V	b31	3659.6	3659.6	0.0	y3	349.2	349.2	0.0
C	b32	3819.7	3819.6	0.1	y2	250.1	250.1	0.0
A	b33	n.d.	3909.7		y1	n.d.	90.1	

**Supplementary Table S5.** High sensitivity amino acid analysis<sup>#</sup> of bevuTI-I

<b>Amino acid</b>	<b>Amount (-H<sub>2</sub>O)* (ng / sample)</b>	<b>pmoles / sample</b>	<b>moles (%)</b>	<b>No. of residues</b>
Histidine	262	1905	3.4	1
Serine	665	7633	13.7	4
Arginine	309	1975	3.5	1
Glycine	183	3207	5.8	1-2
Aspartic acid <sup>§</sup>	311	2698	4.8	1
Glutamic acid <sup>§</sup>	1120	8673	15.6	4
Threonine	401	3958	7.1	2
Alanine	316	4447	8.0	1-2
Proline	799	8225	14.8	4
Lysine	34	260	0.5	0
Tyrosine	50	305	0.5	0
Valine	405	4082	7.3	2
Isoleucine	435	3840	6.9	2
Leucine	263	2323	4.2	1
Phenylalanine	325	2209	4.0	1
<i>Total</i>	<i>5874</i>	<i>55741</i>	<i>100.0</i>	

<sup>#</sup>APAF sample ID: S0010203; methionine was not detected in this sample; limit of reporting was 20 ng/sample; cysteine and tryptophan are not analyzed by this method

\*Calculation based on amino acid residue mass in protein (molecular weight minus H<sub>2</sub>O).

<sup>§</sup>Asp/Asn and Glu/Gln cannot be distinguished by this method

**Supplementary Data S1 – S5: please see separate Excel file**

**Supplementary Data S1.** Number of identical hits with E-value  $\leq 10^{-4}$  identified with blastp query sequences specified in Table 1.

**Supplementary Data S2.** Overview of identified contigs found by blastp searches with query sequences listed in Table 1.

**Supplementary Data S3.** List of analyzed genomes (format: format assembly accession, version\_assembly) found in the GenBank folder of NCBI's FTP-server.

**Supplementary Data S4.** List of analyzed transcriptomes found in the TSA folder of NCBI's FTP-server.

**Supplementary Data S5.** List of analyzed *Viridiplantae* transcriptomes provided by the 1-kp project.