Supporting information

Advancing Matrix-Assisted Laser Desorption/Ionization Mass Spectrometric Imaging for Capillary Electrophoresis Analysis of Peptides

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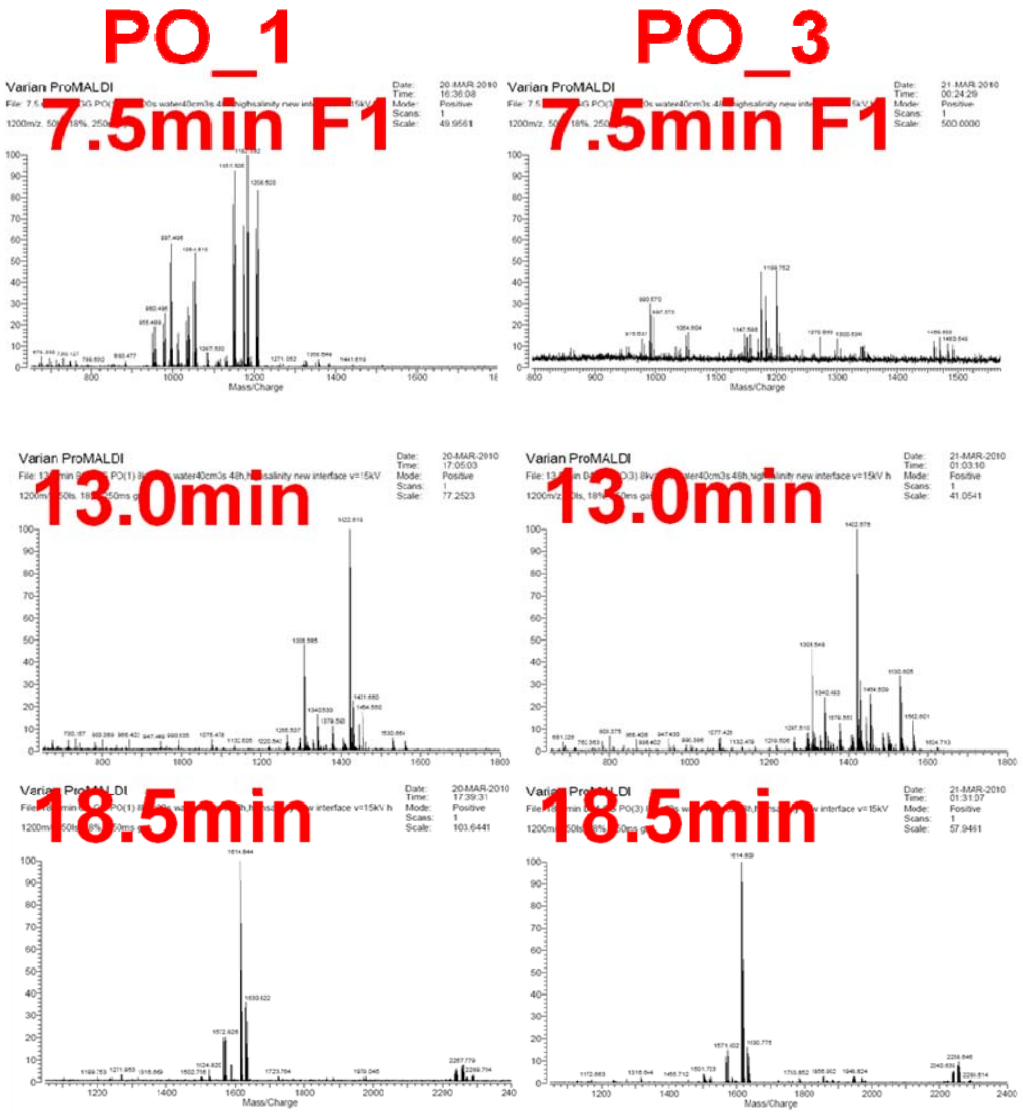
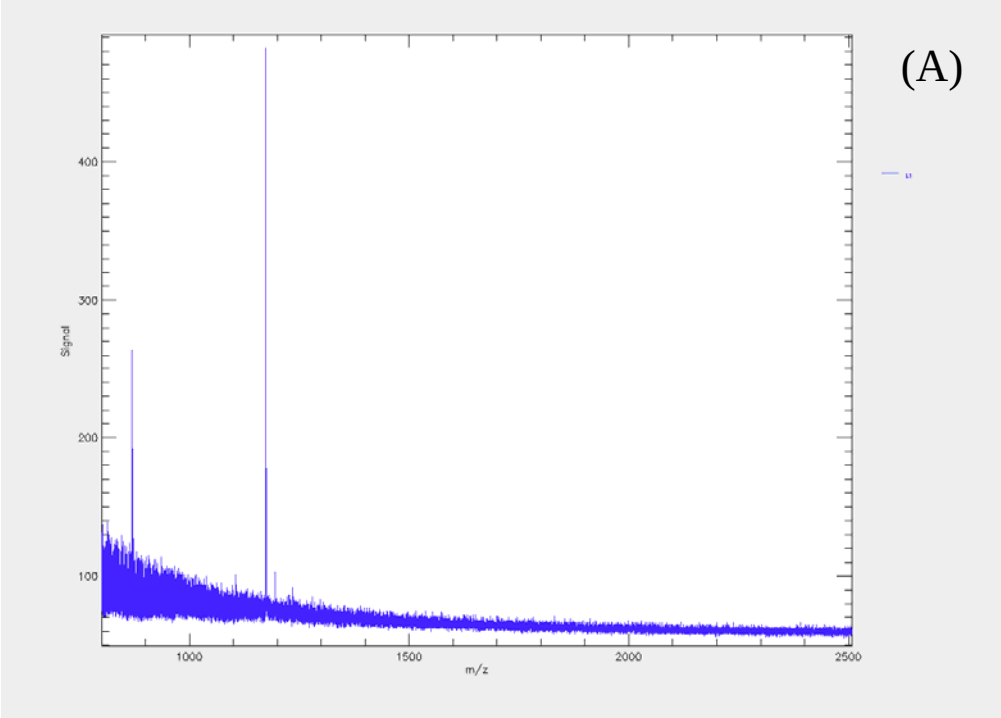
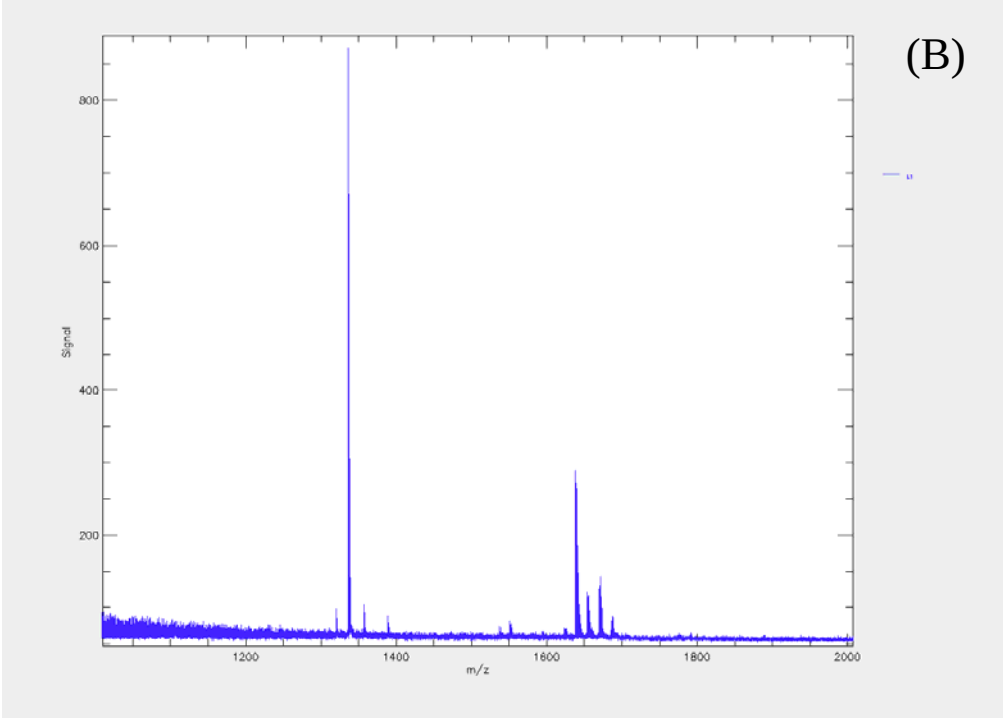
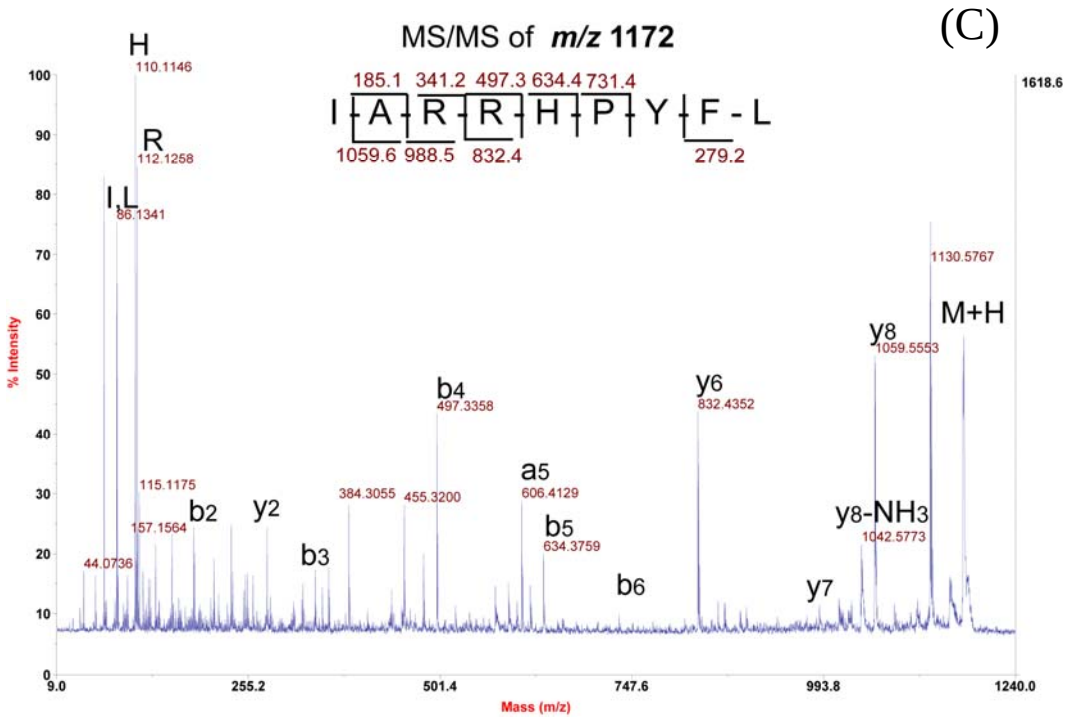
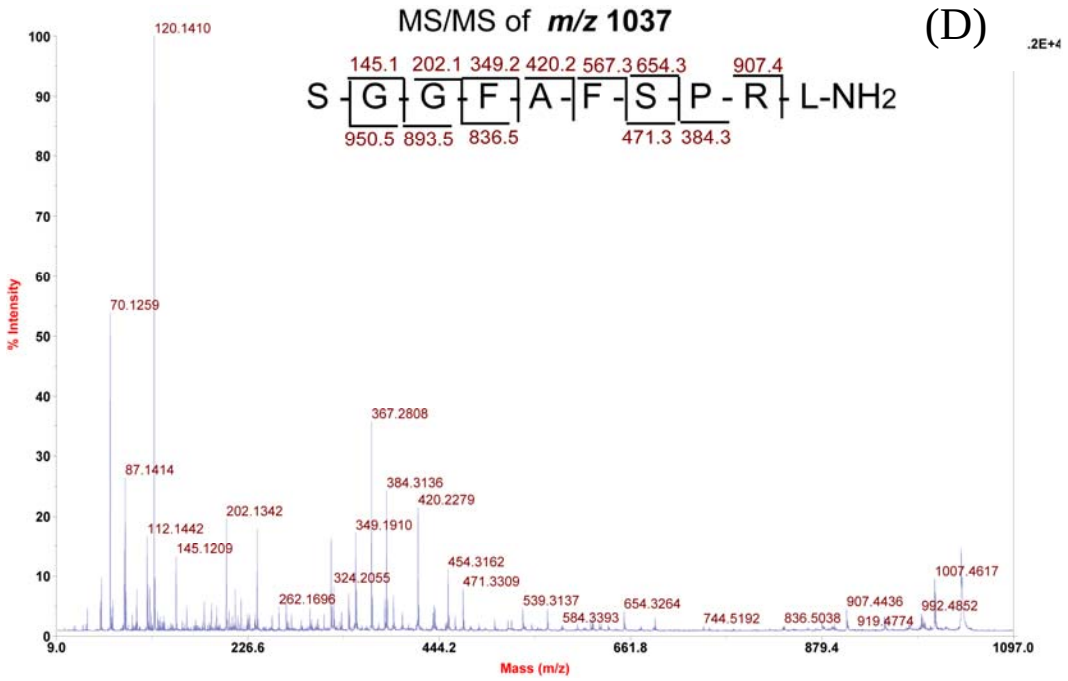


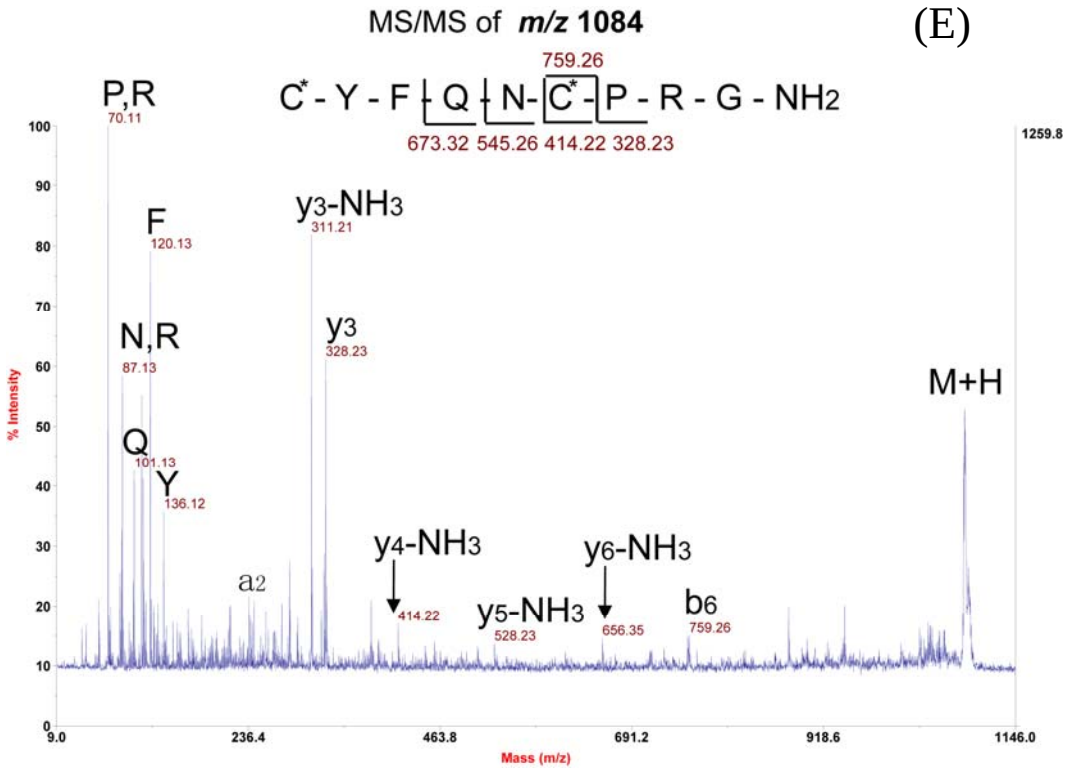
Figure S1. The biological replicates of two blue crab pericardial organ (PO) tissue extracts by off-line CE-MALDI-FT-MS analysis using the improved interface (spectra at beginning deposition time=7.5 min, middle time=13 min, and ending time=18.5 min for PO_1 (left) and PO_3 (right) samples). The peak intensities vary but the profiles are similar. The spectra demonstrate reproducible separations for extremely complex mixtures of biological replicates, verifying the good reproducibility of the new CE-MALDI MS interface.

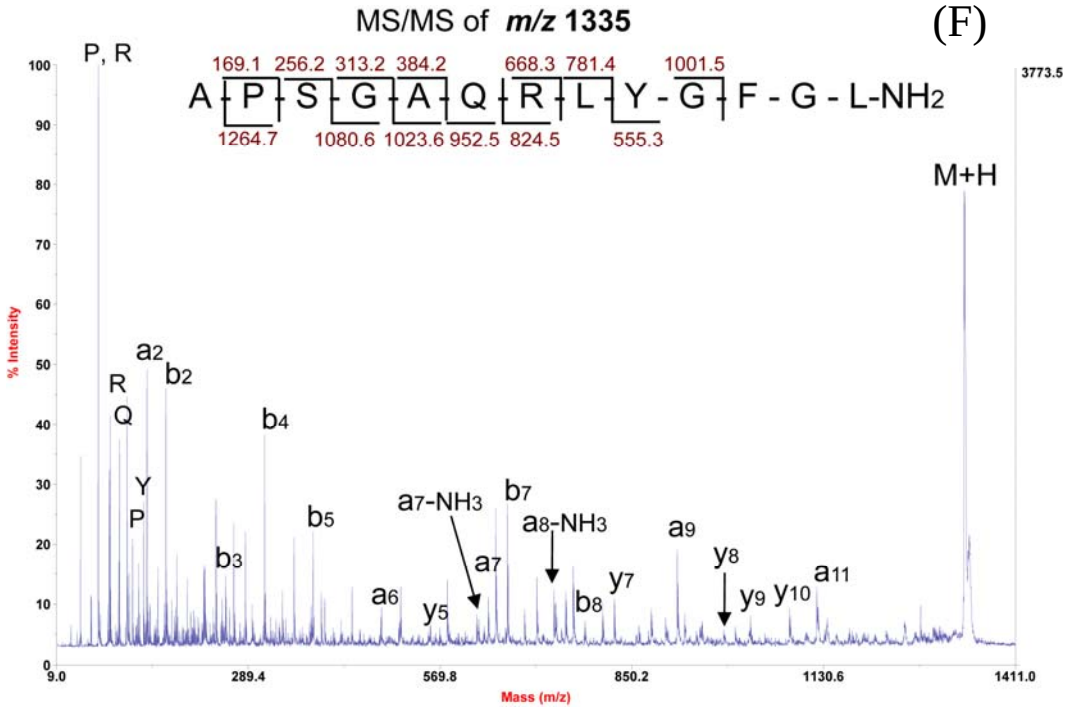

(A)
Signal
400
300
200
100
1000
1500
2000
2500
m/z


(B)
Signal
800
600
400
200
1200
1400
1600
1800
2000
m/z


(C)
MS/MS of m/z 1172
H
110.1146
R
112.1258
I,L
86.1341
185.1 341.2 497.3 634.4 731.4
I A R R H P Y F - L
1059.6 988.5 832.4 279.2
1618.6
1130.5767
M+H
y8
1059.5553
y6
832.4352
b4
497.3358
a5
606.4129
b5
634.3759
y8-NH3
1042.5773
y2
b2
b3
b6
y7
115.1175
157.1564
44.0736
384.3055
455.3200
% Intensity
Mass (m/z)
9.0
255.2
501.4
747.6
993.8
1240.0


(D)
MS/MS of m/z 1037
.2E+4
120.1410
145.1 202.1 349.2 420.2 567.3 654.3 907.4
S G G F A F S P - R L-NH2
950.5 893.5 836.5 471.3 384.3
70.1259
367.2808
87.1414
384.3136
420.2279
202.1342
112.1442
349.1910
145.1209
454.3162
471.3309
324.2055
262.1696
539.3137
654.3264
584.3393
744.5192
836.5038
907.4436
919.4774
992.4852
1007.4617
% Intensity
Mass (m/z)
9.0
226.6
444.2
661.8
879.4
1097.0


MS/MS of m/z 1084
(E)
759.26
P,R
70.11
C* - Y - F - Q - N - C* - P - R - G - NH2
673.32 545.26 414.22 328.23
1259.8
y3-NH3
311.21
F
120.13
y3
328.23
N,R
87.13
M+H
Q
101.13
Y
136.12
y4-NH3
y6-NH3
a2
414.22
y5-NH3
528.23
656.35
b6
759.26
% Intensity
Mass (m/z)


MS/MS of m/z 1335
(F)
P, R
169.1 256.2 313.2 384.2 668.3 781.4 1001.5
A - P - S - G - A - Q - R - L - Y - G - F - G - L-NH2
1264.7 1080.6 1023.6 952.5 824.5 555.3
3773.5
M+H
a2
b2
R
Q
b4
Y
P
b5
a7-NH3
b7
a8-NH3
a9
b3
a7
y8
y7
a11
a6
y5
b8
y9 y10
% Intensity
Mass (m/z)

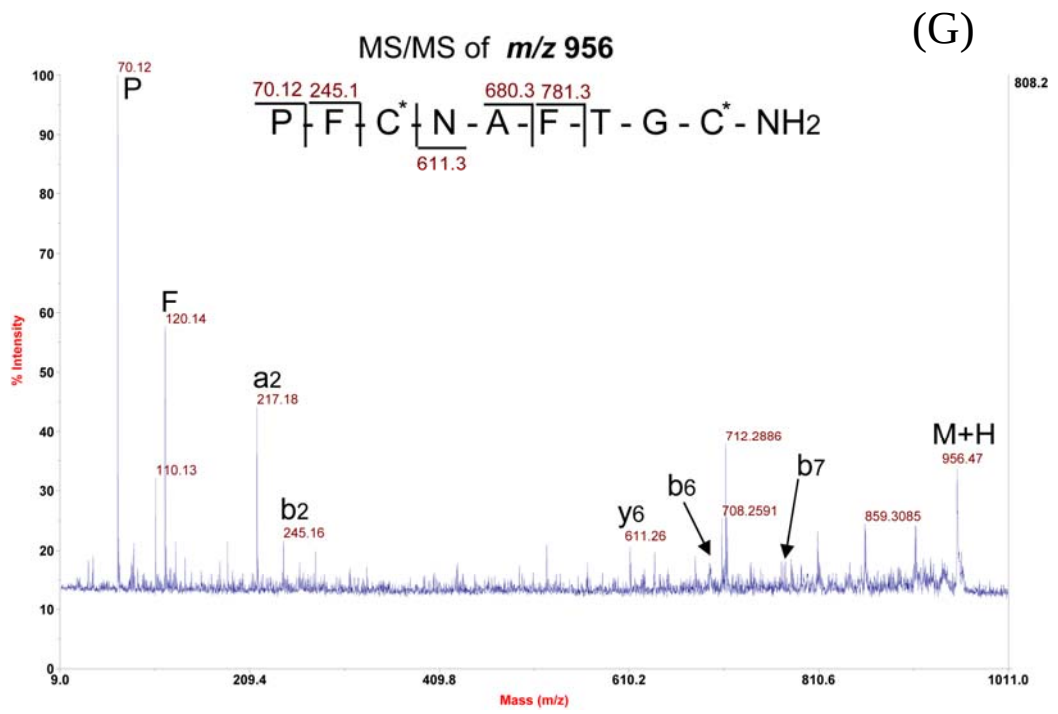
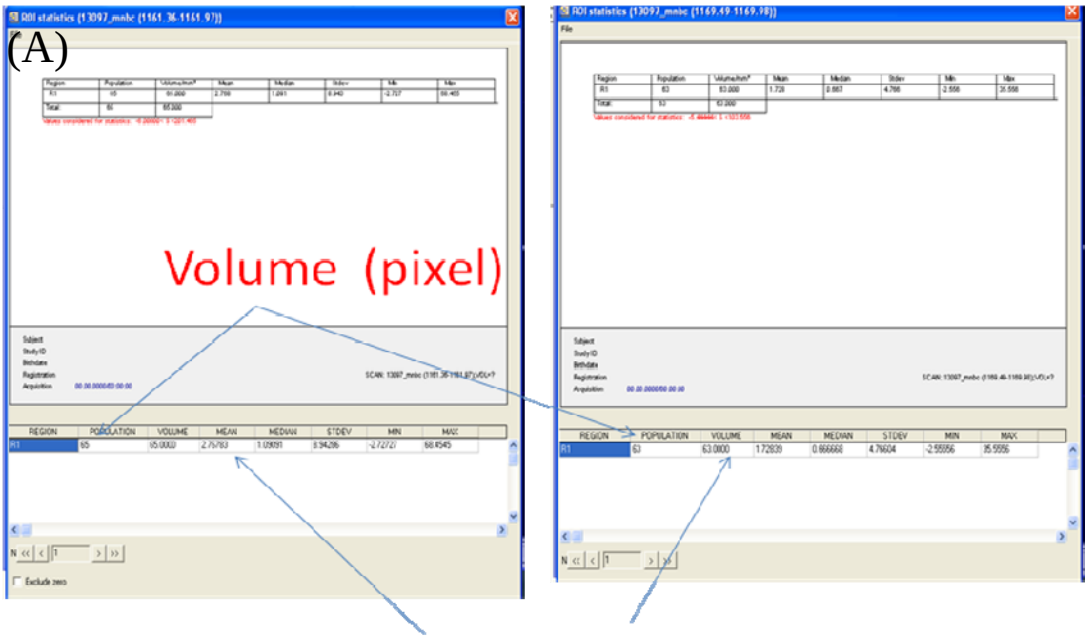


Figure S2. CE-MALDI TOF/TOF mass spectral analysis of a peptide mixture.

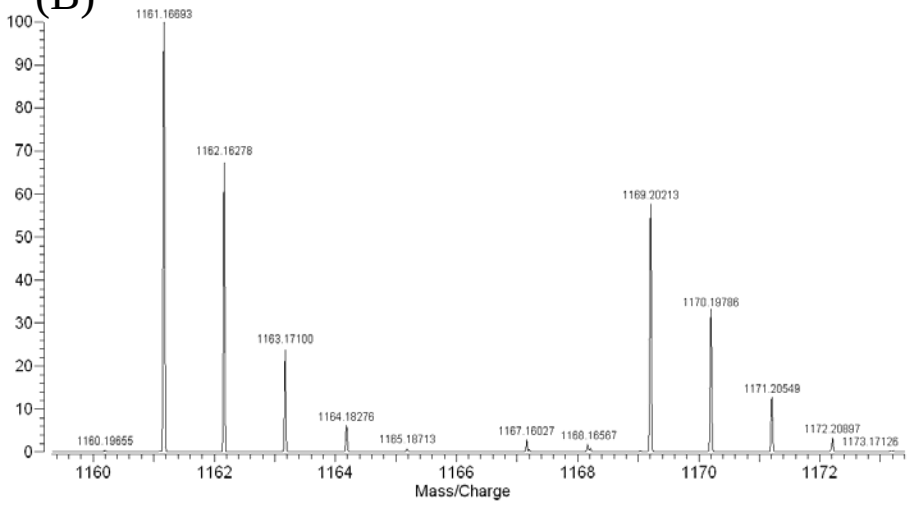
(A-B) Representative MALDI-TOF/TOF mass spectra showing the isolation of peptides by CE in **Figure 3**. A= (*m/z* 1172 (peak 2), eluted at 517.5 s, pixel info: x=259, y=1); B= (*m/z* 1335 (peak 5) and *m/z* 1637 (peak 6) co-eluted at 612.5 s, pixel info: x=372, y=1).

(C-G) MS/MS spectral identification of peptide sequences based on the fragmentation pattern matching.

(A)


Volume (pixel)
Mean (Ave. intensity)
m/z 1161
65 × 2.77= 180
m/z 1169
63 × 1.73= 108

(B)


1161.16893
1162.16278
1163.17100
1164.18276
1165.18713
1167.16027
1168.16567
1169.20213
1170.19786
1171.20549
1172.20897
1173.17126
1160.19655
100
90
80
70
60
50
40
30
20
10
0
1160
1162
1164
1166
1168
1170
1172
Mass/Charge

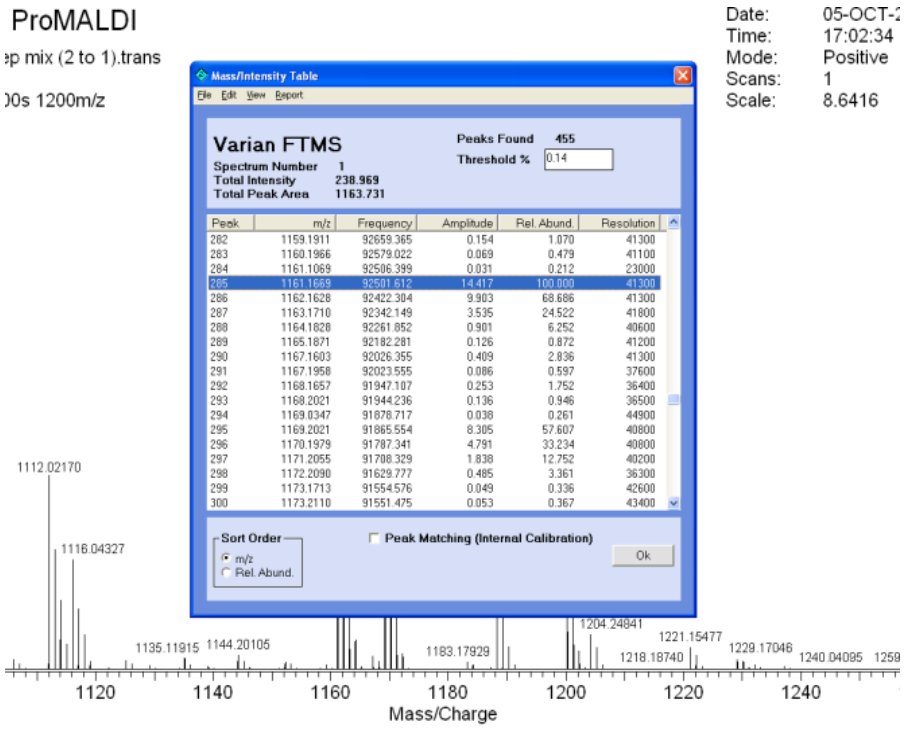


Figure S3. Relative quantitation of peptide abundances by CE-MALDI MS.

(A) Region of interest (ROI) table of the peaks at *m/z* 1161 and *m/z* 1169, originating from peptide GAHKNYLRF (*m/z* 1105.59, peak 3 in **Figure 4**). The ROIs were carefully aligned with each other (± 1 pixel). The "volume" (or "population") by "means" in these two areas are used to represent the total intensities of the extracted ions from the two peaks. The intensity for *m/z* 1161 is 180, for *m/z* 1169 is 108 and the calculated ratio is 1.67.

(B) The MALDI-FTMS spectrum showing the experimental ratio of peaks *m/z* 1161/1169, and the ratio is calculated to be 1.75 according to peak amplitudes (intensities). This value correlates well with the imaging data. Both are 10%-15% below the theoretical ratio of 2:1.

From Figure 3, the migration order nicely follows the equation of q/M1/2,[3] which is proportional to their net charges (q), and inversely proportional to the square root of their masses (M). The peptides' net charges are calculated based on the Henderson-Hasselbalch equation via the website http://www.innovagen.se/custom-peptide-synthesis/peptide-property-calculator/peptide-property-calculator-notes.asp, as given in **Table S1**.

Table S1. The identities, sequences, net charges, and concentrations of the peptide peaks in **Figure 3**.

Peak ID	***m/z***	**sequence**	**Net charge**	**Conc.**
1	1105.59	GAHKNYLRF	+3	$7.0*10^{-7}$M
2	1172.67	IARRHPYFL	+2.8	$7.5*10^{-7}$M
3	1037.55	SGGFAFSPRLamide	+2	$6.5*10^{-7}$M
4	1084.45	CYFQNCPRGamide	+2	$3.7*10^{-7}$M
5	1335.72	APSGAQRLYGFGLamide	+2	$2.5*10^{-7}$M
6	1637.72	AGCKNFFWKTFTSC	+2	$5.5*10^{-7}$M
7	956.37	PFCNAFTGCamide	+1	$5.0*10^{-7}$M

Net charge calculation via (at pH 4.9)

http://innovagen.net/custom-peptide-synthesis/peptide-property-calculator/peptide-property-calculator.asp

Table S2. Experimental ratios of the isotopically labeled peptides observed from the CE-MALDI imaging area, the ratios vary from 1.67 to 2.27.

Peak ID	+ light	+ heavy	ROI ratio Light/heavy	Mass shift	Label site(s)
(1) *m/z* 599.31	627	631	1.926	+28/32	N-terminal
(2) *m/z* 784.41	812	816	2.268	+28/32	N-terminal
(3) *m/z* 1105.59	1161	1169	1.673	+56/64	N-terminal & K
(4) *m/z* 976.46	1004	1008	1.727	+28/32	N-terminal
(5) *m/z* 1335.72	1363	1367	1.862	+28/32	N-terminal
(6) *m/z* 1282.67	1310	1314	1.965	+28/32	N-terminal
(7) *m/z* 1046.54	1074	1078	2.156	+28/32	N-terminal
(8) *m/z* 1104.64	1188	1200	2.211	+84/96	N-terminal & K ×2
(9) *m/z* 1067.56	1095	1099	1.727	+28/32	N-terminal
(10) *m/z* 689.36	745	753	2.227	+56/64	N-terminal & K