Supplementary Data

Figure S1 – Tris/Tricine SDS-PAGE analysis of CB elastase digests over 48h. A total amount of 7.5 µg CB membranes were loaded onto each lane prior to ,6h, 12h, 24h and 48h after elastase was added. The prominent elastase band at approximately 25 kDa height is labelled. The Tris/Tricine gel electrophoresis was performed after the protocol of H. Schägger [Tricine SDS-PAGE (2006). *Nat. Prot.,* 1, 16-22.]

Figure S2 – Reproducibility of three PM elastase digests in 60% methanol. **(A)** spectra of three individual digests corresponding to 25 ng of protein sample each (approx. 1 pmol BR). **(B)** PMF match to BR sequence of the displayed spectra. Minimum S/N ratio set to 20. Peaks matched within 10 ppm. If multiple peptides match to single masses, manual selection based on nLC-MS/MS data was done. **(C)** Chart showing proportions of peptides present in one, two or all of the three replicates.

Figure S3 – nLC-MALDI-MS/MS BR sequence coverage display of tryptic digests. Identified peptide locations indicated by thin bars underneath the protein sequence. **(A)** regular KR specificity defined during MASCOT™ search. **(B)** search without enzyme definition.

Figure S4 – Accuracy dependent PMF matching of 81 MS/MS verified BR AVLIST peptides to BR and two comparable-size false positive protein sequences (Q1LZA1 and P06030). **(A)** specificity considered, only AVLIST cleavages allowed. **(B)** specificity completely ignored, allowing all peptides.

Figure S1

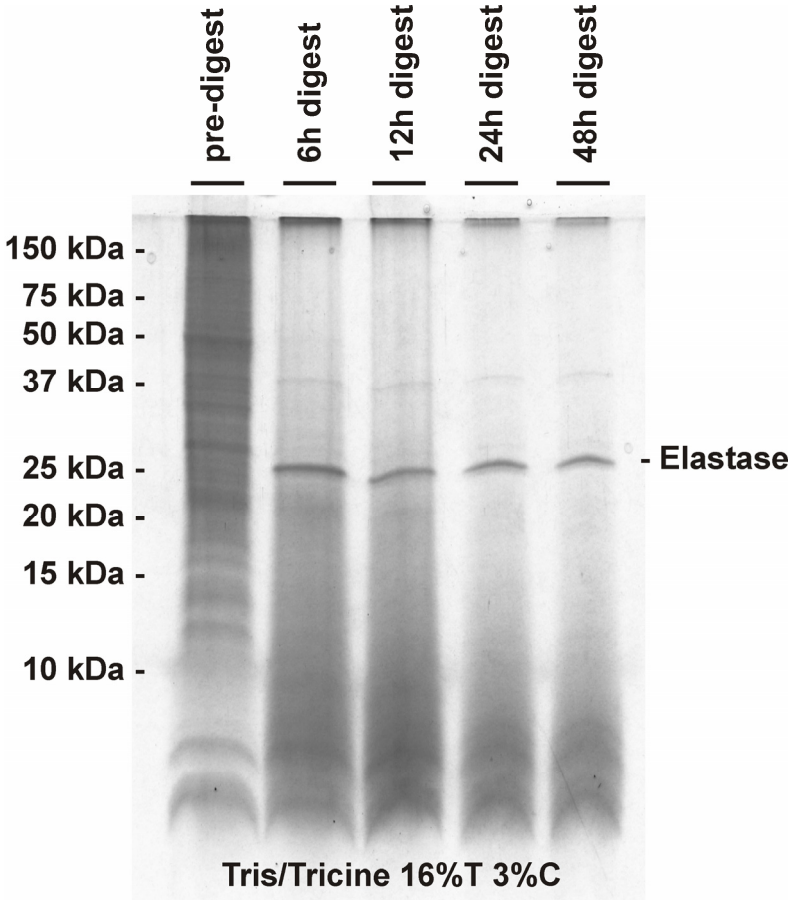

pre-digest
6h digest
12h digest
24h digest
48h digest
150 kDa -
75 kDa -
50 kDa -
37 kDa -
25 kDa -
20 kDa -
15 kDa -
10 kDa -
- Elastase
Tris/Tricine 16%T 3%C

Figure S2

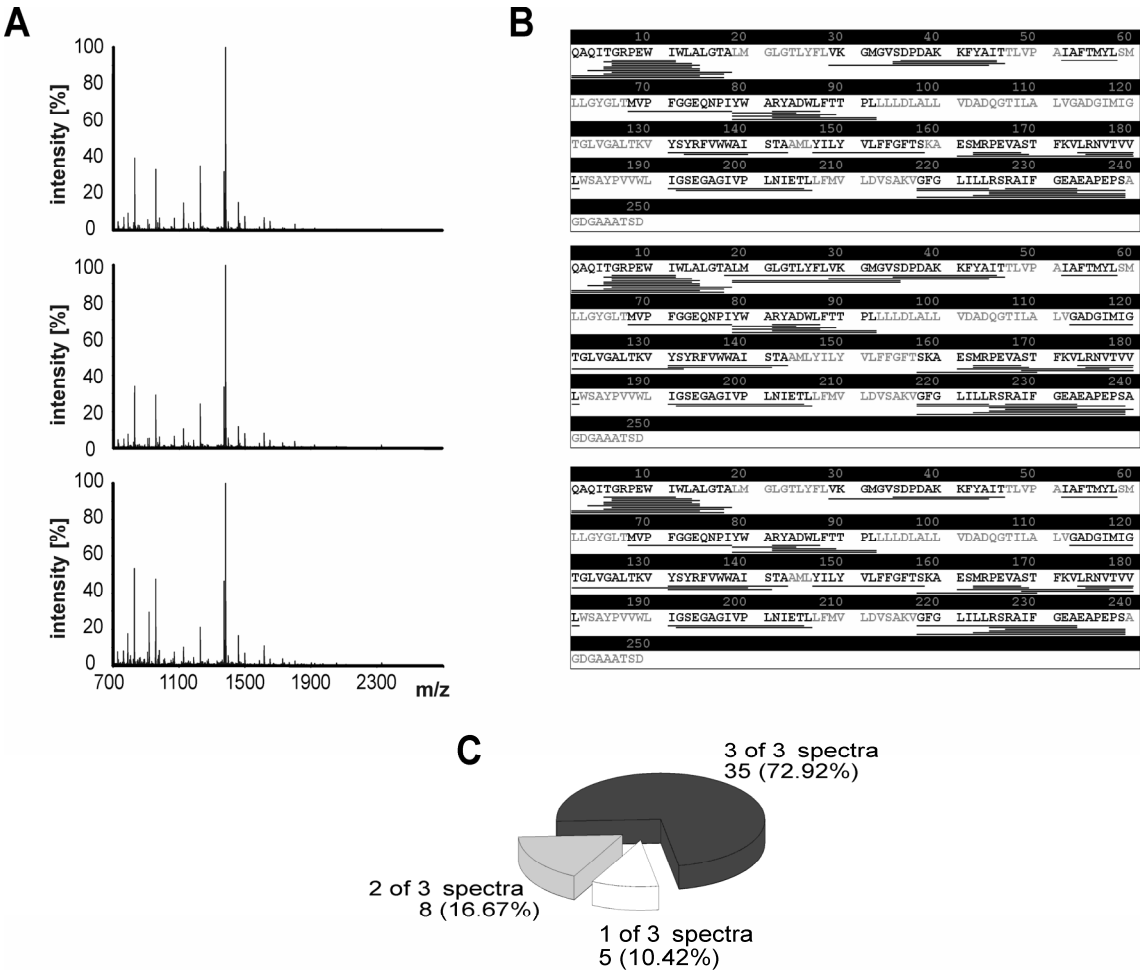

A
intensity [%]
100
80
60
40
20
0
700
1100
1500
1900
2300
m/z
B
QAQITGRPEW IWLALGTALM GLGTLYFLVK GMGVSDPDAK KFYAITTLVP AIAFTMYLSM
LLGYGLTMVP FGGEQNPIYW ARYADWLFTT PLLLLDLALL VDADQGTILA LVGADGIMIG
TGLVGALTKV YSYRFVWWAI STAAMLYILY VLFFGFTSKA ESMRPEVAST FKVLRNVTVV
LWSAYPVVWL IGSEGAGIVP LNIETLLFMV LDVSAKVGFG LILLRSRAIF GEAEAPEPSA
GDGAAATSD
C
3 of 3 spectra
35 (72.92%)
2 of 3 spectra
8 (16.67%)
1 of 3 spectra
5 (10.42%)

Figure S3

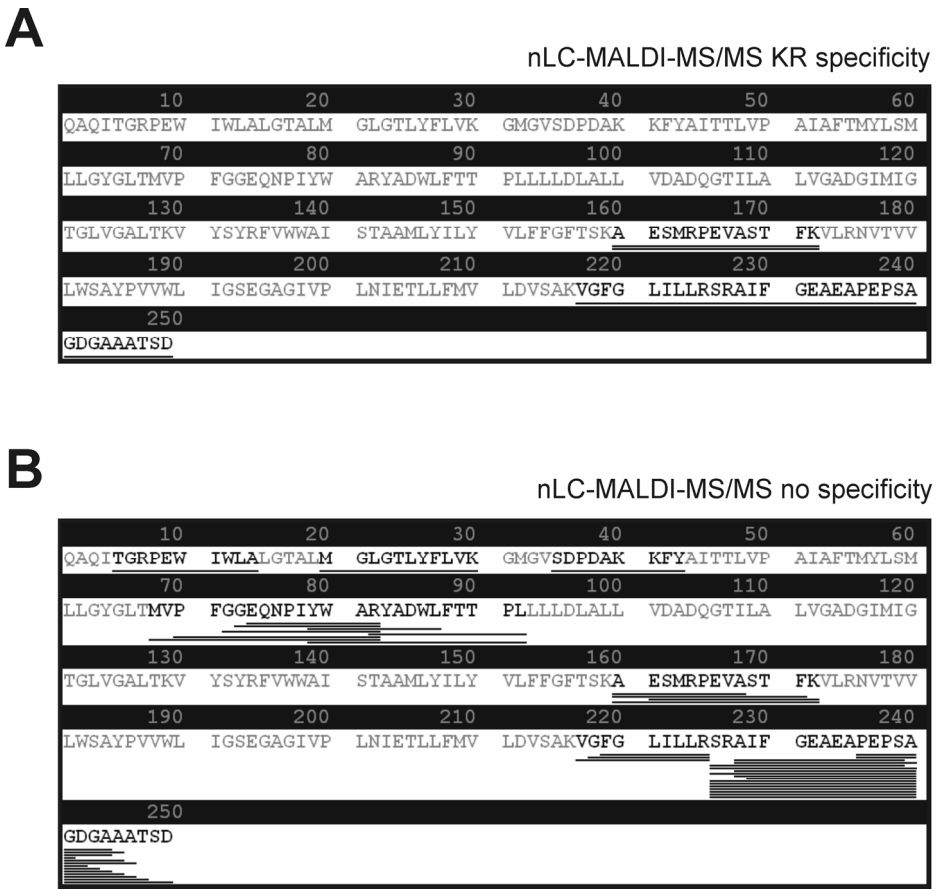
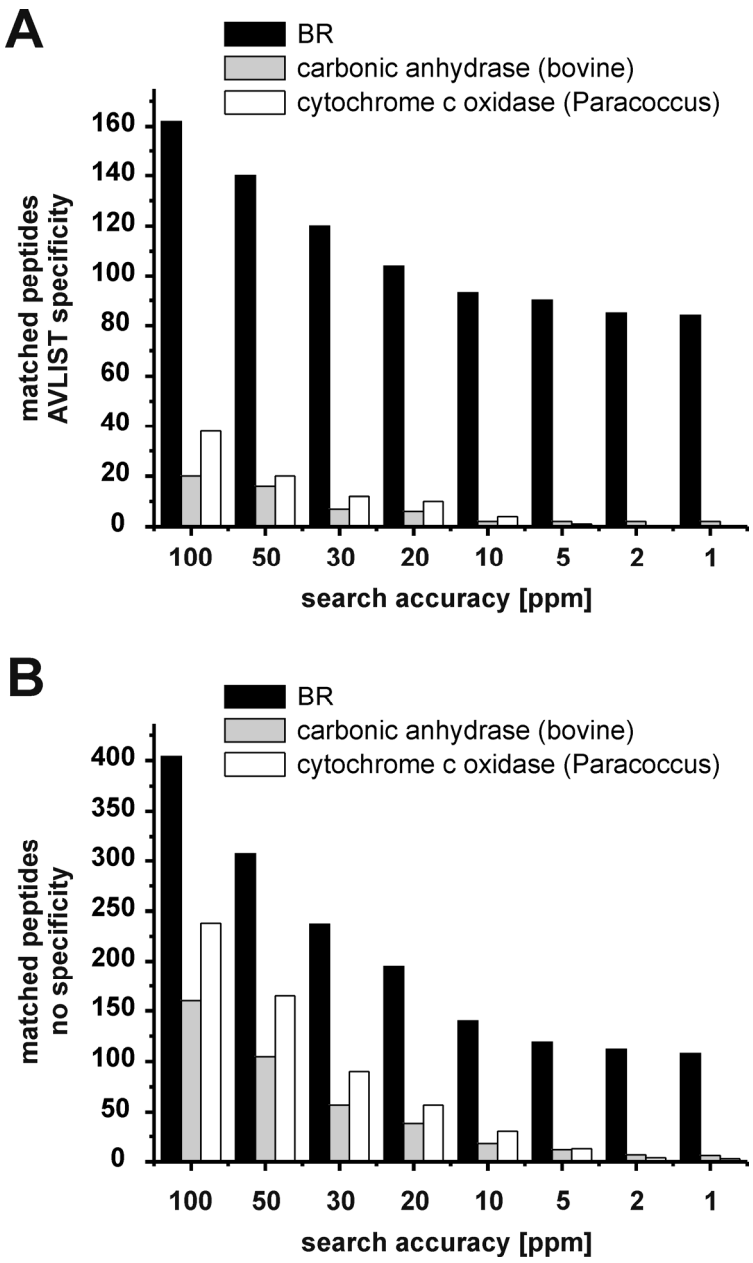

A
nLC-MALDI-MS/MS KR specificity
10 20 30 40 50 60
QAQITGRPEW IWLALGTALM GLGTLYFLVK GMGVSDPDAK KFYAITTLVP AIAFTMYLSM
70 80 90 100 110 120
LLGYGLTMVP FGGEQNPIYW ARYADWLFTT PLLLLDLALL VDADQGTILA LVGADGIMIG
130 140 150 160 170 180
TGLVGALTKV YSYRFVWWAI STAAMLYILY VLFFGFTSKA ESMRPEVAST FKVLRNVTVV
190 200 210 220 230 240
LWSAYPVVWL IGSEGAGIVP LNIETLLFMV LDVSAKVGFG LILLRSRAIF GEAEAPEPSA
250
GDGAAATSD
B
nLC-MALDI-MS/MS no specificity
10 20 30 40 50 60
QAQITGRPEW IWLALGTALM GLGTLYFLVK GMGVSDPDAK KFYAITTLVP AIAFTMYLSM
70 80 90 100 110 120
LLGYGLTMVP FGGEQNPIYW ARYADWLFTT PLLLLDLALL VDADQGTILA LVGADGIMIG
130 140 150 160 170 180
TGLVGALTKV YSYRFVWWAI STAAMLYILY VLFFGFTSKA ESMRPEVAST FKVLRNVTVV
190 200 210 220 230 240
LWSAYPVVWL IGSEGAGIVP LNIETLLFMV LDVSAKVGFG LILLRSRAIF GEAEAPEPSA
250
GDGAAATSD

Figure S4


A
BR
carbonic anhydrase (bovine)
cytochrome c oxidase (Paracoccus)
matched peptides
AVLIST specificity
160
140
120
100
80
60
40
20
0
100
50
30
20
10
5
2
1
search accuracy [ppm]
B
BR
carbonic anhydrase (bovine)
cytochrome c oxidase (Paracoccus)
matched peptides
no specificity
400
350
300
250
200
150
100
50
0
100
50
30
20
10
5
2
1
search accuracy [ppm]