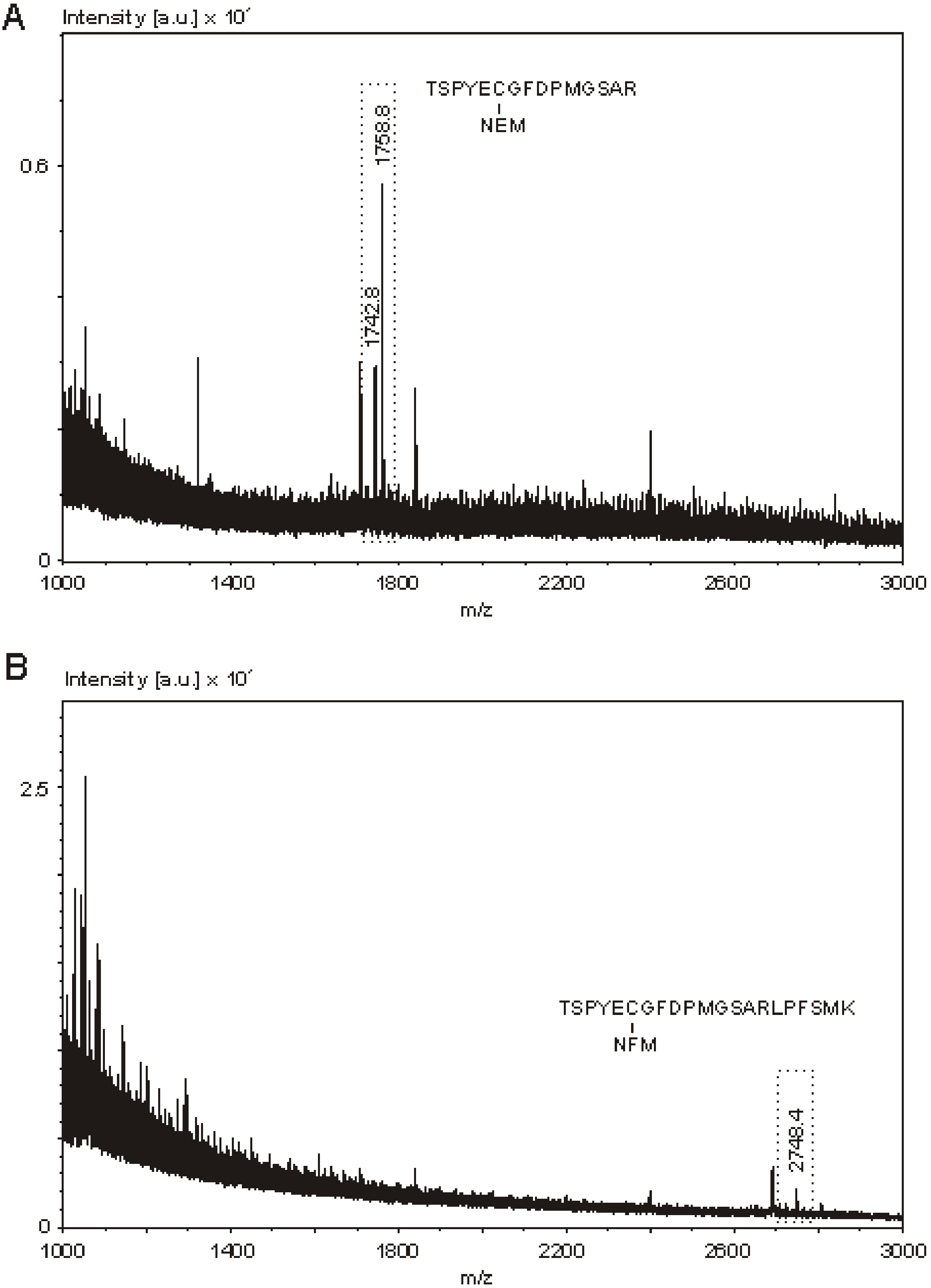
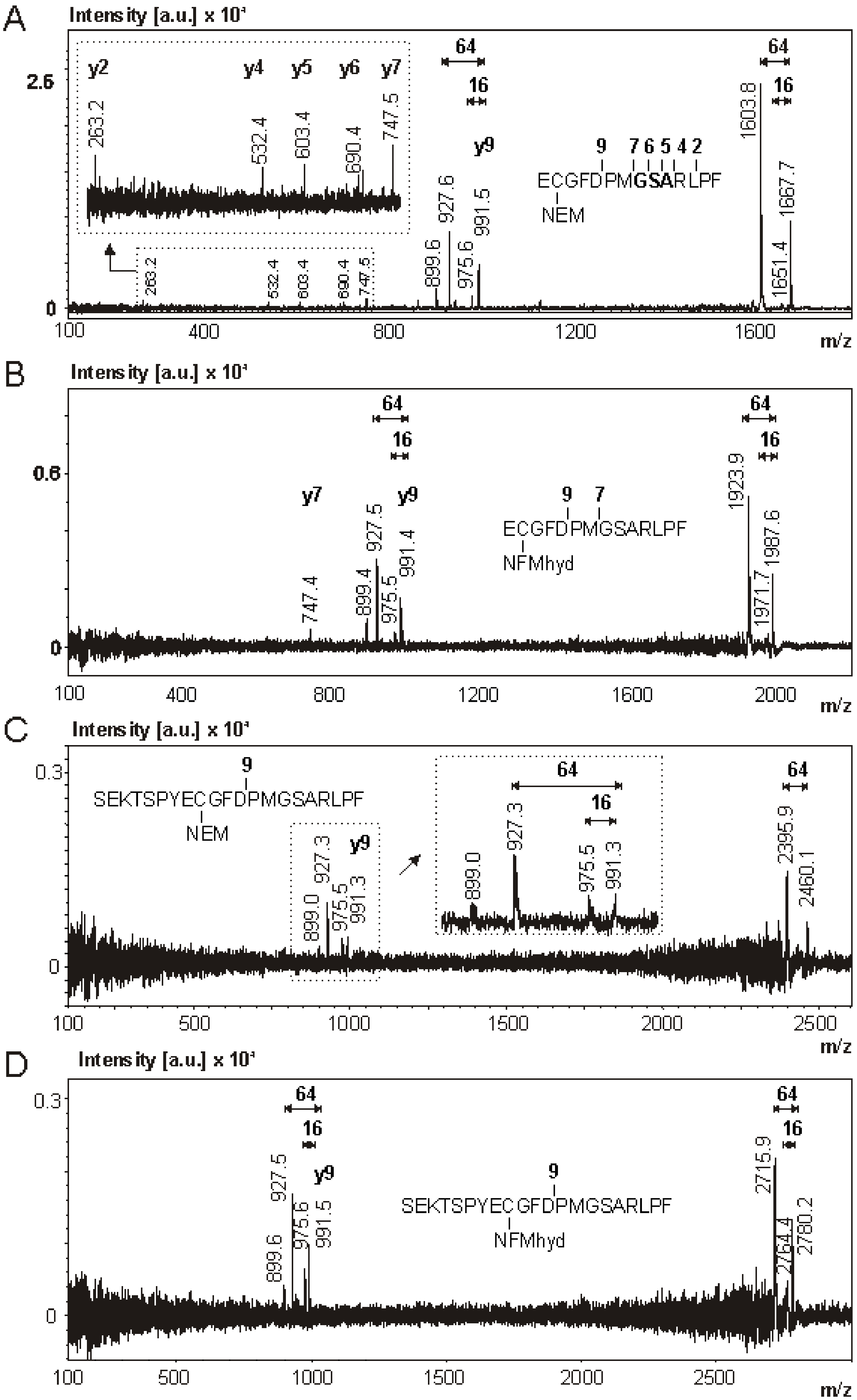


Supplemental Figure S1: *MALDI MS of tryptic digests of two protein spots from dSDS-PAGE***.** (A) MS spectrum for NEM-labelled spot 2. (B) MS spectrum for NFM-labelled fluorescent spot 1. Signals that potentially correspond to tryptic NEM- and NFM-labelled fragments of the ND3 subunit of complex I were boxed (see also Supplementary Tables S1 and S2). [a.u.], counts in absolute units.

A
Intensity [a.u.] x 10⁴
2.5
0
y2
y4
y5
y6
y7
263.2
532.4
603.4
690.4
747.5
64
16
y9
927.6
991.5
899.6
975.6
9 7 6 5 4 2
ECGFDPMGSARLPF
NEM
1603.8
1667.7
1651.4
100
400
800
1200
1600
m/z
B
Intensity [a.u.] x 10⁴
0.6
0
64
16
y7
y9
747.4
899.4
927.5
975.5
991.4
9 7
ECGFDPMGSARLPF
NFMhyd
1923.9
1971.7
1987.6
100
400
800
1200
1600
2000
m/z
C
Intensity [a.u.] x 10⁴
0.3
0
9
SEKTSPYECGFDPMGSARLPF
NEM
y9
899.0
927.3
975.5
991.3
64
16
2395.9
2460.1
100
500
1000
1500
2000
2500
m/z
D
Intensity [a.u.] x 10⁴
0.3
0
64
16
y9
899.6
927.5
975.6
991.5
9
SEKTSPYECGFDPMGSARLPF
NFMhyd
2715.9
2764.4
2780.2
100
500
1000
1500
2000
2500
m/z

Supplemental Figure S2: *MS/MS analyses of ND3 fragments ECGFDPMGSARLPF and SEKTSPYECGFDPMGSARLPF.* A, The MS/MS spectrum of the NEM-labelled peptide ECGFDPMGSARLPF (1667.8 Da; see Fig. 3A, and spot 2, Fig. 2) revealed a short y-ion series (y4-y7) corresponding to the sequence tag GSA, and a very strong signal for the y9-ion (991.5 Da). The high intensity of the y9-ion is explained by the preferential cleavage of the aspartate-proline (D-P) bond (carboxy group of D bound to the amino group of P; ref. 4). Even the y9-ion of the non-oxidized peptide was detected as a satellite peak with a 16 Da mass shift (975.6 Da), since the precursor isolation window was wide enough to isolate not only the oxidized (1667.7 Da) but also a fraction of the non-modified form of the peptide (1651.4 Da). The loss of 64 Da that is characteristic for the loss of methanesulfenic acid (CH_3SOH) was observed with the precursor and the y9-ion as well. Moreover, loss of CO at proline is visible (927.6 - 899.6 = 28 Da). MS/MS of the NEM-labelled 1667.8 Da fragment ECGFDPMGSARLPF. B, The MS/MS analysis of the NFMhyd-labelled peptide ECGFDPMGSARLPF (1987.8 Da; see Fig. 3B, and spot 1, Fig. 2)) was very similar to the corresponding NEM-labelled peptide except for the absence of the y-ion series (y4-y7 in A). Only the y7-ion remained. C, Analysis of the 2460.1 Da NEM-labelled SEKTSPYECGFDPMGSARLPF fragment (see Fig. 3A, and spot 2, Fig. 2), and D, of the 2780.1 Da NFMhyd-labelled SEKTSPYECGFDPMGSARLPF fragment (see Fig. 3B, and spot 1, Fig. 2). NEM- and NFMhyd-labelled peptides SEKTSPYECGFDPMGSARLPF (2460.1 Da; C and 2780.2 Da, D) gave rise to the expected fragment profiles in the mass range 900–1,000 Da that were characteristic for methionine oxidation, preferred cleavage of the D-P bond, 64 Da loss from the precursors and a loss of CO at proline (m/z = 899).

Zoomed areas are boxed. 64 Da-shifts due to loss of methanesulfenic acid, and 16 Da-shifts due to oxidation of methionine are marked by arrows. Fragmentation sites in the peptide sequences (bold numbers) correspond to fragment signals (y-ions marked bold).

Supplementary Table S1. Tryptic digestion of fluorescent-labeled spot 1 (Fig. 2).
One specific fragment fitted to the ND3 subunit of complex I providing the first hint for specific labelling of ND3. (see also Supplementary Figure S1B).
Abbreviations: m/z, measured mass (M_r+H); M_r, relative mass; Err., error given in ppm; C. and N., C-terminal and N-terminal amino acid positions, respectively, in the ND3 sequence; Mod., modifications; NEM, N-ethyl-maleimide; NEMhyd, N-ethyl-maleimide + H_2O; NFM, N-fluoresceinyl-maleimide; NFMhyd, N-fluoresceinyl-maleimide + H_2O; M_{ox}, oxidized methionine.

m/z	M_r	Err.	C.	N.	Sequence in ND3	Mod.
2748.4501	2747.1018	124	34	54	(K)TSPYECGFDPMGSARLPFSMK(F)	NFM

No match: 1009.651; 1027.7653; 1044.1582; 1045.6443; 1047.1915; 1051.7556; 1061.9705; 1077.2697; 1077.6677; 1085.8128; 1097.6859; 1129.7062; 1143.8265; 1185.7615; 1201.8485; 1229.7765; 1273.7969; 1287.7975; 1294.7479; 1317.8293; 1320.7671; 1331.8157; 1333.8413; 1361.8423; 1375.8398; 1405.8680; 1419.8901; 1449.9165; 1609.9052; 1639.9889; 1670.0482; 1707.9589; 1839.0266; 2399.1535; 2691.4106; 2807.4559

Supplementary Table S2. Tryptic digestion of NEM-labeled spot 2 (Fig. 2).
See also Supplementary Figure S1A. Abbreviations as in Table S1.

m/z	M_r	Err.	C.	N.	Sequence in ND3	Mod.
1742.7791	1741.7076	36.8604	34	48	(K)TSPYECGFDPMGSAR(L)	NEM
1758.766	1757.7025	31.9736	34	48	(K)TSPYECGFDPMGSAR(L)	NEM; M_{ox}

No match: 1045.6616; 1051.7504; 1143.8588; 1320.687; 1638.9633; 1705.2019; 1707.8535; 1839.0089; 2151.2277; 2241.3341; 2399.0768; 2501.4513

Supplementary Table S3. ND3-fragments from a chymotryptic digest of spot 1 (Fig. 2).
The corresponding MS spectrum is shown in Fig. 3B. The mass list was cleared of data contained in the background list (Supplementary Table S5). Abbreviations as in Table S1.

m/z	M_r	Err.	C.	N.	Sequence in ND3	Mod.
1243.3584	1242.3522	-1	38	44	(Y)ECGFDPM(G)	NFMhyd
1259.3648	1258.3471	8	38	44	(Y)ECGFDPM(G)	NFMhyd, M_{ox}
1542.6733	1541.6643	1	38	51	(Y)ECGFDPMGSARLPF(S)	M_{ox}
1795.9909	1794.9624	12	1	16	(-)MNLMLALLTNFTLATL(L)	M_{ox}
1971.7735	1970.7491	9	38	51	(Y)ECGFDPMGSARLPF(S)	NFMhyd
1987.7813	1986.7441	15	38	51	(Y)ECGFDPMGSARLPF(S)	NFMhyd, M_{ox}
2035.7455	2034.7176	10	31	44	(Y)SEKTSPYECGFDPM(G)	NFMhyd
2051.7458	2050.7125	13	31	44	(Y)SEKTSPYECGFDPM(G)	NFMhyd, M_{ox}
2074.8334	2073.7285	47	31	45	(Y)SEKTSPYECGFDPMG(S)	NFMhyd
2090.8423	2089.7234	53	31	45	(Y)SEKTSPYECGFDPMG(S)	NFMhyd, M_{ox}
2335.1403	2334.0297	44	31	51	(Y)SEKTSPYECGFDPMGSARLPF(S)	M_{ox}
2764.1407	2763.1145	7	31	51	(Y)SEKTSPYECGFDPMGSARLPF(S)	NFMhyd
2780.1419	2779.1094	9	31	51	(Y)SEKTSPYECGFDPMGSARLPF(S)	NFMhyd, M_{ox}

No match: 1143.731; 1166.6378; 1194.6027; 1345.6839; 1725.8839; 1729.9891; 1782.9599; 1928.0495; 1934.0537; 2038.0964; 2153.1961; 2271.1305; 2351.0956; 2726.1728; 2830.3149; 2868.2212; 2883.2035

Supplementary Table S4. ND3-fragments from a chymotryptic digest of spot 2 (Fig. 2). The corresponding MS spectrum is shown in Fig. 3A. The mass list was cleared of data contained in the background list (Supplementary Table S5). Abbreviations as in Table S1.

m/z	M_r	Err.	C.	N.	Sequence	Mod.
1027.7022	1026.6689	25	12	21	(F)TLATLLVIIA(F)	
1542.7046	1541.6643	21	38	51	(Y)ECGFDPMGSARLPF(S)	M_{ox}
1597.8147	1596.8255	-11	1	14	(-)MNLMLALLTNFTLA(T)	M_{ox}
1651.7596	1650.7170	21	38	51	(Y)ECGFDPMGSARLPF(S)	NEM
1667.7568	1666.7120	23	38	51	(Y)ECGFDPMGSARLPF(S)	NEM; M_{ox}
1685.7646	1684.7225	21	38	51	(Y)ECGFDPMGSARLPF(S)	NEMhyd; M_{ox}
1796.0232	1794.9624	30	1	16	(-)MNLMLALLTNFTLATL(L)	M_{ox}
2335.2008	2334.0297	70	31	51	(Y)SEKTSPYECGFDPMGSARLPF(S)	M_{ox}
2444.1606	2443.0824	29	31	51	(Y)SEKTSPYECGFDPMGSARLPF(S)	NEM
2460.1416	2459.0773	23	31	51	(Y)SEKTSPYECGFDPMGSARLPF(S)	NEM; M_{ox}
2478.1457	2477.0879	20	31	51	(Y)SEKTSPYECGFDPMGSARLPF(S)	NEMhyd; M_{ox}

No match: 1022.5757; 1044.1007; 1047.0783; 1062.029; 1064.0815; 1077.2229; 1085.7685; 1120.6273; 1143.7771; 1194.6167; 1322.7055; 1343.7761; 1527.7754; 1603.8022; 1619.8261; 1884.0461; 2054.1206; 2222.1893; 2406.2667

Supplementary Table S5. Background list (m/z = 1000 – 3000) for chymotryptic digests.

m/z
1059.5872; 1060.0773; 1061.5947; 1129.6390; 1143.7310; 1166.6378; 1177.6390; 1194.6027; 1209.6682; 1243.3584; 1259.3648; 1259.8154; 1345.6839; 1523.8301; 1539.8396; 1542.6733; 1598.9045; 1610.8536; 1626.8992; 1637.8849; 1670.9239; 1720.9239; 1725.8839; 1729.9891; 1733.0093; 1738.9344; 1779.0036; 1782.9599; 1792.9911; 1795.9909; 1855.0351; 1883.0226; 1928.0495; 1934.0537; 1971.7735; 1987.7813; 1991.7713; 2035.1099; 2035.7455; 2038.0964; 2051.7458; 2074.8334; 2090.8423; 2093.8497; 2136.1807; 2153.1961; 2227.0110; 2271.1305; 2335.1403; 2351.0956;. 2726.1728; 2764.1407; 2780.1419; 2830.3149; 2868.2212; 2883.2035; 2993.4247

Supplementary Table S6. ND3 fragments of spot 1 (Fig. 2) digested by elastase (for MS spectrum see Fig. 5A). The mass list was cleared for components contained in the background list (Supplementary Table S7). Reliable assignment of signals was achieved by MS/MS-experiments (Fig. 5B). Abbreviations as in Table S1.

m/z	M_r	Err.	C.	N.	Sequence	Mod.
1734.5677	1733.5538	4	35	46	(T)SPYECGFDPMGS(A)	NFMhyd
1750.5804	1749.5487	14	35	46	(T)SPYECGFDPMGS(A)	NFMhyd; M_{ox}

No match: 1080.6191; 1094.6219; 1129.6544; 1171.6548; 1438.7811; 1508.7600; 1528.8344; 1622.8436; 1763.9252; 1877.0582; 1910.0329; 2005.0401; 2130.1235; 2193.2439; 2252.1688; 2331.2375; 2361.2715; 2808.2668

Supplementary Table S7. Background list (m/z = 1000 – 3000) for digestion by elastase.

m/z
1027.6970; 1061.5974; 1064.5990; 1085.7658; 1111.6287; 1113.6275; 1135.6301;
1143.7608; 1169.6769; 1176.6304; 1201.8137; 1286.7188; 1292.7480; 1392.7745;
1415.7141; 1418.7596; 1483.8189; 1503.8062; 1539.8404; 1587.8939; 1639.9245;
1650.8855; 1802.0314; 1841.9235; 1879.9718; 1948.0481; 1986.1529; 2020.9986;
2166.0938; 2326.1894