Additional supporting information

Defining intact protein primary structures from saliva: a step toward the human proteome project.

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Running title: Top down MS as a step toward the human proteome project.

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Table 1: Information related to the identifications of peptide P-B, P-D, P-H, P-E and IB-1 protein.

Table 2: Information related to the identifications of Histatin 1, Peptide P-F and P-J.

Table 3: Information related to the identifications of Protein II-2, Protein IB8a and of the unknown protein of 10433 Da.

Table 4: Information related to the identifications of PRP1/PRP2, Db-f, Db-s and IB-6 proteins.

Information displayed in all tables are:
- Column 1: protein ID with access number in databases;
- Column 2: sample ID reporting the number of individual allowing identification of the protein of interest;
- Column 3: measured averaged experimental masses;
- Column 4: Experimental errors in PPM;
- Column 5: Mass errors compared to theoretical values in Da;
- Column 6: Mass errors compared to theoretical values in PPM;
- Column 7: Mass increments in Da;
- Column 8: protein sequences;
- Column 9: Theoretical monoisotopic and averaged molecular weights;
- Column 10: Description of modifications found;
- Column 11: Additional data from literature;
- Columns 12 to 16: Report of protein detection for each individual.

Table 1:

Protein ID	Sample ID	Exp. Values (Av, Da)	Calc.error on exp values (50 ppm)	Mass Error compare to theoretical MW (Da)	Mass Error compare to theoretical MW (ppm)	Mass deviation	Sequences	Theroretical MW (Da) mono / Av	Observations	Additional data from literature	Ind N1	Ind N2	Ind N3	Ind N4	Ind N5
Peptide P-D isoform (new) (P02813)	**Ind 1**	N.O.	N.C.	N.C.	N.C.	N.C.	SPPGKPQGPPQQEGNKPQGPPPGKPQGPPPPGGNPQQPQAPPAGKPQGPPPP	5265.686 / 5268.886	Truncated form of Peptide D lacking last 16 C-terminal amino acids	P02813 = P10163	N.O.	N.O.	O	N.O.	N.O.
	ind 2	N.O.	N.C.	N.C.	N.C.										
	Ind 3	5268.73	0.26	0.156	29.61										
	Ind 4	N.O.	N.C.	N.C.	N.C.										
	Ind 5	N.O.	N.C.	N.C.	N.C.										
Peptide P-H (P02812 / P04280)	**Ind 1**	5589.9	0.28	0.20	35.60	**0.48**	SPPGKPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPSRPPQ	5586.775 / 5590.099	No PTM	_____	O	O	O	O	O
	ind 2	5590.05	0.28	0.05	8.77										
	Ind 3	5589.7	0.28	0.40	71.38										
	Ind 4	5589.88	0.28	0.22	39.18										
	Ind 5	5589.57	0.28	0.53	94.63										
Peptide P-B (P028214)	**Ind 1**	5792.95	0.29	-0.02	-3.45	**0.46**	QRGPRGPYPPGPLAPPQPFGPGFVPPPPPPPYGPGRIPPPPPAPYGPGIFPPPPPQP	5809.766 / 5806.656	Pyroglutamic acid in N-terminus	P-B peptide des 1-5 = 5215.1 Da	O	O	O	O	O
	ind 2	5793.01	0.29	0.04	6.90										
	Ind 3	5792.8	0.29	-0.17	-29.35										
	Ind 4	5792.55	0.29	-0.42	-72.50										
	Ind 5	5792.82	0.29	-0.15	-25.89										
Peptide P-E (IB-9) (P02811)	**Ind 1**	6023.85	0.30	-0.16	-26.87	0.20	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNRPQGPPPPGKPQGPPPQGDKSRSPR	6020.081 / 6023.693	No PTM	P-E peptide des-Arg 61 = 5868 Da	O	N.O.	N.O.	N.O.	O
	ind 2	N.O.	N.C.	N.C.	N.C.										
	Ind 3	6024.05	0.30	-0.36	-61.10										
	Ind 4	6023.8	0.30	-0.11	-18.31										
	Ind 5	N.O.	N.C.	N.C.	N.C.										
IB-1 (P04281)	**Ind 1**	9593.08	0.48	0.30	31.17	0.52	QNLNEDVSQEESPSLIAGNPQGAPPQGGNKPQGPPSPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQGPPPQGDKSRSPR	9524.756/ 9530.439	Pyroglutamic acid in N-terminus + Phosphorylation in Ser 8	IB-1 non phosphorylated = 9513 Da; IB-1 des-Arg 96 = 9437 Da; IB-1 des 93-96 = 9097 Da.	O	O	N.O.	O	O
	ind 2	9593.7	0.48	-0.32	-33.46										
	Ind 3	N.O.	N.C.	N.C.	N.C.										
	Ind 4	9593.3	0.48	0.08	8.23										
	Ind 5	9593.18	0.48	0.20	20.74										
Peptide P-D (IB-5) (P10163)	**Ind 1**	6949.3	0.35	0.43	62.49	0.35	SPPGKPQGPPQQEGNKPQGPPPPGKPQGPPPPGGNPQQPQAPPAGKPQGPPPPPQGGRPPRPAQGQQPPQ	6945.546 / 6949.734	No PTM. Structure of the peptide P-D variant with a mass defect of 26 Da was not elucidated.	_____	O	O	O	O	O
	ind 2	6949.39	0.35	0.34	49.54										
	Ind 3	*6923.54*	*0.35*	*26.19*	*3769.11*										
	Ind 4	6949.1	0.35	0.63	91.27										
	Ind 5	6949.04	0.35	0.69	99.90										

Table 2:

Protein ID	Sample ID	Exp. Values (Av, Da)	Calc.error on exp values (50 ppm)	Mass Error compare to theoretical MW (Da)	Mass Error compare to theoretical MW (ppm)	Mass deviation	Sequences	Theroretical MW (Da) mono / Av	Observations	Additional data from literature	Ind N1	Ind N2	Ind N3	Ind N4	Ind N5
Histatin 1 (P15515)	**Ind 1**	4927.9	0.25	0.234	47.4824173	N.C.	DSHEKRHHGYRRKFHEKHHSHREFPFYGDYGSNYLYDN	4845.226 / 4848.174	Phosphorylation possibly localised on Ser21 and Tyr30	Histatin 1 non phosphorylated = 4842.3 Da; Histatin 1 monosulfated = 5008.2 Da; Histatin 1 bisulfated = 5088.2 Da; Histatin 1 trisulfated = 5168.2 Da; Histatin 1 tetrasulfated = 5248.2 Da.	O	N. O.	N. O.	N. O.	N. O.
	ind 2	N.O.	N.C.	N.C.	N.C.										
	Ind 3	N.O.	N.C.	N.C.	N.C.										
	Ind 4	N.O.	N.C.	N.C.	N.C.										
	Ind 5	N.O.	N.C.	N.C.	N.C.										
Peptide P-F (IB-8c) (P02812)	**Ind 1**	5842.74	0.29	-0.24	-41.76	**0.98**	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQGPPPQGGSKSRSA	5838.984 / 5842.496	No PTM	_____	O	O	O	O	O
	ind 2	5841.98	0.29	0.52	88.32										
	Ind 3	5842.96	0.29	-0.46	-79.42										
	Ind 4	5842.59	0.29	-0.09	-16.09										
	Ind 5	5842.29	0.29	0.21	35.26										
Peptide P-J (new)	**Ind 1**	5943.85	0.30	-0.29	-49.98	**0.92**	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQGPPPQGDNKSRSS	5939 . 996 / 5943.558	No PTM	_____	O	O	O	O	O
	ind 2	5943.04	0.30	0.52	88.66										
	Ind 3	5943.96	0.30	-0.40	-68.81										
	Ind 4	5943.06	0.30	0.50	85.24										
	Ind 5	5943.31	0.30	0.25	42.45										

Table 3:

Protein ID	Sample ID	Exp. Values (Av, Da)	Calc.error on exp values (50 ppm)	Mass Error compare to theoretical MW (Da)	Mass Error compare to theoretical MW (ppm)	Mass deviation	Sequences	Theroretical MW (Da) mono / Av	Observations	Additional data from literature	Ind N1	Ind N2	Ind N3	Ind N4	Ind N5
IL-2 (P04280)	Ind 1	7608.55	0.38	1.28	168.65	1.15	QNLNEDVSQEESPSLIAGNPQGPSPQGGNKP QGPPPPPPGKPQGPPPQGGNKPQGPPPPGKP	7602.789 / 7607.267	Pyroglutamic acid in N-terminus + Ser 8 replaced by a dehydroAlanine	IL-2 non phosphorylated =7529 Da; IL-2 des-Arg 75 = 7453 Da; IL-2 des 72-75 = 7113 Da.	O	O	O	O	O
	ind 2	N.O.	N.C.	0.39	51.53										
				N.C.	N.C.										
	Ind 3	7608.28	0.38	1.01	97.09		QNLNEDVSQEESPSLIAGNPQGPSPQGGNKP QGPPPPPPGKPQGPPPQGGNKPQGPPPPGKPQ GPPPQGDKSRSPR	7603.745 / 7608.158	Variant lacking a Proline residue in position 39 + pyroglutamic acid in N-ter + Phosphorylation in Ser 8						
				0.12	11.69										
	Ind 4	7607.4	0.38	0.13	12.75										
				-0.76											
	Ind 5	7607.9	0.38	0.63											
				-0.26											
10434 Da unknown protein (P04280)	Ind 1	10433.52	0.52	-0.02	-1.63	0.46	SPPGKPQGPPPQGGKPQGPPPQGGNKPQGPP PPGKPQGPPAQGGSKSQSARAPPGKPQGPPQ QEGNNPQGPPPPAGGNPQQPQAPPAGQPQGP PRPPQGGRPSRPPQ	10427.277/ 10433.503	No PTM	————————	O	O	O	N.O.	O
	ind 2	10433.06	0.52	0.44	42.46										
	Ind 3	N.O.	N.C.	N.C.	N.C.										
	Ind 4	N.D.	N.C.	N.C.	N.C.										
	Ind 5	10433.39	0.52	0.11	10.83										
			0.00												
IB8a (P02812)	Ind 1	N.O.	N.C.	N.C.	N.C.	N.C.	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPP PQGGNKPQGPPPGKPQGPPPQGDNKSQSAR SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPP PQGGNKSQGPPPPGKPQGPPPQGGSKSR	11704.943 / 11711.974	Mutation of Q115 to H + hexose in Ser120 and a Methyl group on carboylic group of the Cter or on Arg121	————————	N.O.	N.C.	O	N.O.	N.O.
	ind 2	11896.02	N.C.	N.C.	N.C.										
	Ind 3	N.O.	0.59	-1.01	-84.98										
	Ind 4	N.O.	N.C.	N.C.	N.C.										
	Ind 5	N.O.	N.C.	N.C.	N.C.										

Table 4:

Protein ID	Sample ID	Exp. Values (Av, Da)	Calc.error on exp values (50 ppm)	Mass Error compare to theoretical MW (Da)	Mass Error compare to theoretical MW (ppm)	Mass deviation	Sequences	Theroretical MW (Da) mono / Av	Observations	Additional data from literature	Ind N1	Ind N2	Ind N3	Ind N4	Ind N5
IB-6 (P04280)	**Ind 1** **ind 2** **Ind 3** **Ind 4** **Ind 5**	N.O. N.O. 11516.40 11516.70 N.O.	N.C. N.C. 0.58 0.58 N.C.	N.C. N.C. -0.27 0.03 N.C.	N.C. N.C. -23.44 2.60 N.C.	0.30	SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQ GGNKPQGPPPPGKPQGPPAQGGSKSQSARSPPG KPQGPPQQEGNNPQGPPPPAGGNPQQPQAPPAG QPQGPPRPPQGGRPSRPPQ	11509.792 / 11516.67	No PTM	_____	N.O.	N.O.	O	O	N.O.
Db-f di-phospho (P02810)	**Ind 1** **ind 2** **Ind 3** **Ind 4** **Ind 5**	N.O. 13279.34 N.O. N.O. N.O.	N.C. 0.66 N.C. N.C. N.C.	N.C. -0.51 N.C. N.C. N.C.	N.C. -38.40 N.C. N.C. N.C.	N.C.	QDLNEDVSQEDVPLVISDGGDSEQFLDEERQGP PLGGQQSQPSAGDGNQDDGPQQGPPQQGGQQQQ GPPPPQGKPQGPPQQGGQQQQGPPPPQGKPQGP PQQGGHPPPPQGRPQGPPQQGGHPRPPR	13129.202 / 13136.925	Pyroglutamic acid in N-terminus + Phosphorylation in Ser 8	Db-f mono phosphorylated = 13200 Da; Db-f bi phosphorylated = 13280 Da;	N.O.	O	N.O.	N.O.	N.O.
PRP-1 (P02810)	**Ind 1** **ind 2** **Ind 3** **Ind 4** **Ind 5**	15514.10 15514.04 N.O. 15514.19 15514.50	0.78 0.78 N.C. 0.78 0.78	-1.20 -1.26 N.C. -1.11 -0.80	-77.34 -81.21 N.C. -71.54 -51.56	0.46	QDLNEDVSQEDVPLVISDGGDSEQFLDEERQGP PLGGQQSQPSAGDGNQDDGPQQGPPQQGGQQQQ GPPPPQGKPQGPPQQGGHPPPPQGRPQGPPQQG GHPRPPRGRPOGPPOOGGHOOGPPPPPPGKPOG	15363.311 / 15372.375	Pyroglutamic acid in N-terminus + Phosphorylation in Ser 8 Asp6 could be replaced by an Asn	PRP1 non phosphorylated = 15355 Da; PRP1 monophosphorylated = 15435 Da Da; PRP1 diphosphorylated = 15515 Da; PRP1 triphosphorylated = 15595 Da	O	O	N.O.	O	O
Db-s (P02810)	**Ind 1** **ind 2** **Ind 3** **Ind 4** **Ind 5**	N.O. 17632.49 N.O. 17632.47 N.O.	N.C. 0.88 N.C. 0.88 N.C.	N.C. -1.11 N.C. -1.13 N.C.	N.C. -62.95 N.C. -64.08 N.C.	0.02	QDLNEDVSQEDVPLVISDGGDSEQFLDEERQGP PLGGQQSQPSAGDGNQDDGPQQGPPQQGGQQQQ GPPPPQGKPQGPPQQGGQQQQGPPPPQGKPQGP PQQGGHPPPPQGRPQGPPQQGGHPRPPRGRPQG PPQQGGHQQGPPPPPPGKPQGPPPQGGRPQGPP QGQSPQ	17480.351 / 17490.673	Pyroglutamic acid in N-terminus + Phosphorylation in Ser 8	Db-s monophosphorylated = 17553 Da Da; Db-s diphosphorylated = 17633 Da; Db-s triphosphorylated = 17713 Da	N.O.	O	N.O.	O	N.O.
P-O prot (P04280)	**Ind 1** **ind 2** **Ind 3** **Ind 4** **Ind 4**	23460.20 N.O. N.O. N.O. 23461.19	1.17 N.C. N.C. N.C. 1.17	 N.C. N.C. N.C. 4.21	 N.C. N.C. N.C. 179.43	**1.17**	PQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKP QGPPPPGKPQGPPPQGDKSQSPRSPPGKPQGPP PQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPP PGKPQGPPPQGDKSQSPRSPPGKPQGPPPQGGN QPQGPPPPPGKPQGPPQQGGNRPQGPPPPGKPQ GPPPQGDKSRSPQSPPGKPQGPPPQGGNQPQGP PPPPGKPQGPPPQGGNKPQGPPPPGKPQGPPAQ GGSKSQSARA	23442.886 / 23456.981	No PTM	_____	O	N.O.	N.O.	O	O

Table 5: Table list of measured mass from MSMS data generated from IRMPD experiment for Protein II-2 isoform 2 (Pyroglutamate + dehydroalanine residue in position 8) using LTQFT-ultra instrument compare to Prosight data theoretical values. Unique fragment ions are labeled with ●.

Additional information:
User Specified Sequence: `[-17.0265]QNLNEDV[-18.01]SQEESPSLIAGNPQGP SPQGGNKPQGPPPPPPGKPQGPPPQGGNKPQGPPPPGKPQGPPPQGDKSRSPR.` Number of Amino Acids: 76; Theoretical Mass: 7607.34 Da; Mass Difference: 0.3832 Da. B Ions: 16 Y Ions: 14.

Ion	Observed Mass (Da)	Theoretical Mass (Da)	Mass Error (Da)	Mass Error (PPM)
b3	338.159	338.159	-0.000	-0.9
b4	452.202	452.202	-0.000	-0.9
b5	581.244	581.245	-0.001	-1.1
b6	696.271	696.272	-0.001	-1.3
b7	795.339	795.340	-0.001	-1.5
b8	864.359	864.362	-0.003	-3.6
b9	992.418	992.421	-0.002	-2.2
b10	1121.462	1121.463	-0.001	-0.7
b11	1250.503	1250.506	-0.003	-2.4
b12	1337.531	1337.538	-0.006	-4.8
b16	1747.786	1747.791	-0.004	-2.5
b17	1818.824	1818.828	-0.004	-2.0
b18	1875.847	1875.849	-0.003	-1.4
b19	1989.888	1989.892	-0.004	-2.1
b24	2456.107	2456.110	-0.003	-1.1
b70	6873.380	6873.356	0.024	3.4
y6	729.424	729.427	-0.002	-3.2
y15	1602.820	1602.825	-0.004	-2.8
y28	2814.461	2814.466	-0.005	-1.7
y41	4089.159	4089.143	0.016	4.0
y42	4186.191	4186.196	-0.005	-1.1
y45	4468.322	4468.328	-0.006	-1.4
y51	5049.611	5049.620	-0.009	-1.9
y53	5233.697	5233.705	-0.008	-1.6
y56	5515.828	5515.838	-0.010	-1.8
y57	5612.863 ●	5612.891	-0.028	-5.0
y60	5855.006	5854.992	0.014	2.3
y61	5968.084 ●	5968.076	0.007	1.2
y63	6168.179	6168.192	-0.013	-2.2
y64	6265.237	6265.245	-0.009	-1.4

Crude PScore: 1.43232e-23. McLuckey Score: 9.87805. Manual PScore: 2.65936e-39

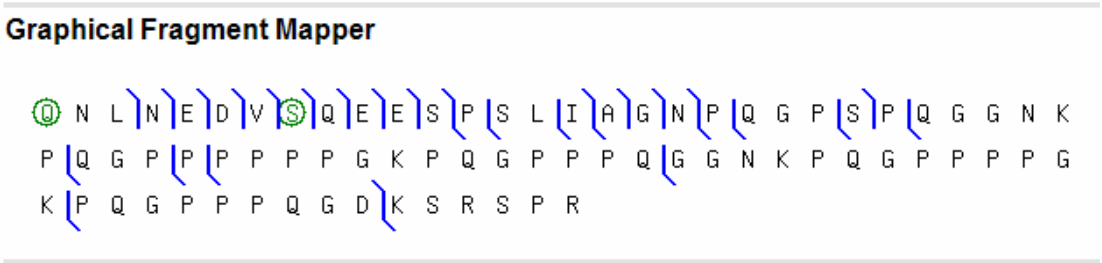


Table 6: Table list of measured mass from MSMS data generated from IRMPD experiment for Protein II-2 isoform 1 (Protein lacking Proline 39+ Pyroglutamate + phosphoserine in position 8) using LTQFT-ultra instrument compare to Prosight data theoretical values. Unique fragment ions are labeled with ●.

Additional information:
User Specified Sequence:
[-17.0265]QNLNEDV[79.966]SQEESPSLIAGNPQGPSPQGGNKPQGPPPPPGKPQ GPPPQGGNKPQGPPPPGKPQGPPPQGDKSRSPR.
Number of Amino Acids: 76; Theoretical Mass: 7607.34 Da; Mass Difference: 0.3832 Da. B Ions: 16 Y Ions: 14.

Ion	Observed Mass (Da)	Theoretical Mass (Da)	Mass Error (Da)	Mass Error (PPM)
b3	338.159	338.159	-0.000	-0.9
b4	452.202	452.202	-0.000	-0.9
b5	581.244	581.245	-0.001	-1.1
b6	696.271	696.272	-0.001	-1.3
b7	795.339	795.340	-0.001	-1.5
b9	1090.396	1090.397	-0.001	-0.8
b10	1219.437	1219.439	-0.002	-1.9
b11	1348.479 ●	1348.482	-0.002	-1.8
b12	1435.511 ●	1435.514	-0.003	-1.8
b15	1732.678	1732.683	-0.004	-2.6
b16	1845.764	1845.767	-0.003	-1.4
b17	1916.802	1916.804	-0.002	-1.1
b18	1973.822 ●	1973.825	-0.003	-1.6
b19	2087.863	2087.868	-0.005	-2.5
b22	2370.004 ●	2370.001	0.003	1.2
b24	2554.075	2554.086	-0.011	-4.2
b30	3135.378	3135.378	-0.000	-0.0
y6	729.424	729.427	-0.002	-3.2
y15	1602.820	1602.825	-0.004	-2.8
y28	2814.461	2814.466	-0.005	-1.7
y41	4089.159	4089.143	0.016	4.0
y42	4186.191	4186.196	-0.005	-1.1
y43	4243.204	4243.217	-0.013	-3.2
y45	4468.322	4468.328	-0.006	-1.4
y46	4596.431	4596.423	0.007	1.6
y48	4767.479	4767.488	-0.008	-1.8
y49	4824.501	4824.509	-0.008	-1.6
y50	4952.554 ●	4952.568	-0.013	-2.7
y51	5049.611	5049.620	-0.009	-1.9
y53	5233.697	5233.705	-0.008	-1.6
y54	5290.715	5290.727	-0.012	-2.2
y56	5515.828	5515.838	-0.010	-1.8
y57	5629.855	5629.881	-0.026	-4.7
y58	5686.890	5686.902	-0.013	-2.2

y59	5757.928	5757.940	-0.011	-1.9
y60	5871.012	5871.024	-0.011	-1.9
y61	5984.096	5984.108	-0.012	-1.9
y63	6168.179	6168.192	-0.013	-2.2
y64	6255.207 ●	6255.224	-0.017	-2.7
y68	6808.355	6808.366	-0.011	-1.6
y69	6907.453	6907.435	0.019	2.7
y70	7022.457 ●	7022.462	-0.005	-0.7
y74	7492.708	7492.674	0.034	4.5

Crude PScore: 2.35989e-39; McLuckey Score: 15.122; Manual PScore: 8.7089e-94.

Graphical Fragment Mapper

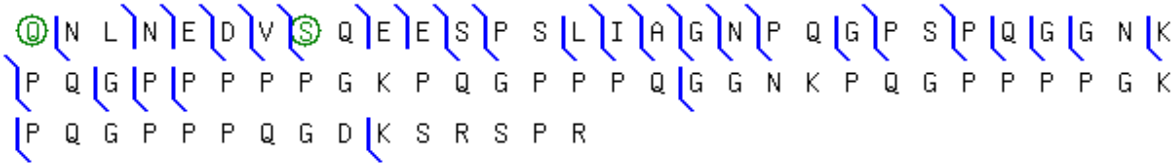


Table 7: Table list of measured mass from MSMS data generated from ECD experiment for Protein II-2 isoform 2 (Pyroglutamate + dehydroalanine residue in position 8) using LTQFT-ultra instrument compare to Prosight data theoretical values. Unique fragment ions are labeled with ●.

Additional information:
User Specified Sequence: `[-17.0265]QNLNEDV[-18.01]SQEESPSLIAGNPQGP SPQGGNKPQGPPPPPPGKPQGPPPQGGNKPQGPPPPGKPQGPPPQGDKSRSPR.`
Number of Amino Acids: 76; Theoretical Mass: 7607.34 Da; Mass Difference: 0.3832 Da. C Ions: 4 Z Ions: 15.

Ion	Observed Mass (Da)	Theoretical Mass (Da)	Mass Error (Da)	Mass Error (PPM)
c4	469.229	469.228	0.001	1.4
c5	598.272	598.271	0.001	1.3
c6	713.300	713.298	0.002	2.5
c73	7261.581 ●	7261.611	-0.030	-4.1
z3	342.178	342.178	0.000	0.8
z4	498.279	498.279	0.001	1.0
z5	585.312	585.311	0.001	1.3
z6	713.407	713.406	0.001	1.2
z9	1013.514	1013.513	0.001	0.9
z13	1361.695	1361.693	0.003	2.0
z14	1489.753	1489.751	0.002	1.6
z16	1714.902	1714.899	0.003	1.8
z22	2217.157	2217.153	0.004	1.9
z25	2570.362	2570.359	0.003	1.2
z26	2684.406	2684.402	0.004	1.5
z27	2741.428	2741.424	0.004	1.5
z33	3274.687	3274.683	0.004	1.2
z34	3402.746	3402.742	0.004	1.1
z36	3627.895	3627.890	0.006	1.6

Crude PScore: 1.74141e-09; MacLuckey Score: 2.61163; Manual PScore: 4.91796e-41

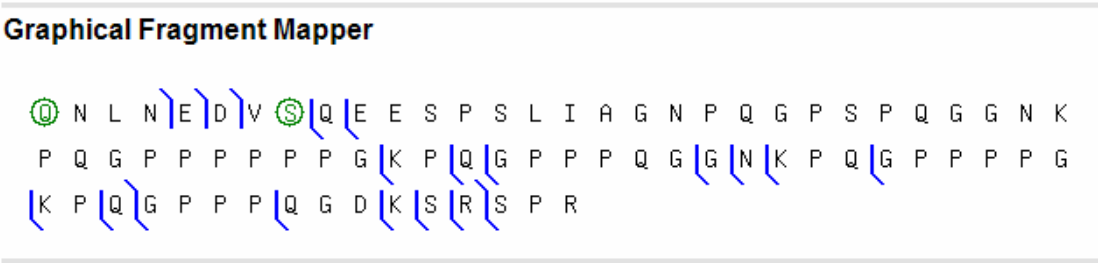


Table 8: Table list of measured mass from MSMS data generated from ECD experiment for Protein II-2 isoform 1 (Protein lacking Proline 39+ Pyroglutamate + phosphoserine in position 8) using LTQFT-ultra instrument compare to Prosight data theoretical values. Unique fragment ions are labeled with ●.

Additional information:
User Specified Sequence:
`[-17.0265]QNLNEDV[79.966]SQEESPSLIAGNPQGPSPQGGNKPQGPPPPPGKPQ GPPPQGGNKPQGPPPPGKPQGPPPQGDKSRSPR.`
Number of Amino Acids: 75; Theoretical Mass: 7608.19 Da; Mass Difference: -0.4728 Da. C Ions: 38 Z Ions: 31.

Ion	Observed Mass (Da)	Theoretical Mass (Da)	Mass Error (Da)	Mass Error (PPM)
c4	469.229	469.228	0.001	1.4
c5	598.272	598.271	0.001	1.3
c6	713.300	713.298	0.002	2.5
c8	979.367	979.364	0.002	2.5
c9	1107.425	1107.423	0.002	2.3
c10	1236.469	1236.465	0.004	2.9
c11	1365.512	1365.508	0.003	2.5
c14	1636.631 ●	1636.625	0.006	3.6
c15	1749.716	1749.709	0.007	3.9
c16	1862.799	1862.793	0.006	3.2
c18	1990.857 ●	1990.852	0.005	2.5
c20	2201.955 ●	2201.947	0.008	3.5
c21	2330.010 ●	2330.006	0.004	1.9
c23	2484.087 ●	2484.080	0.007	2.9
c25	2668.171	2668.165	0.007	2.5
c26	2796.230	2796.223	0.006	2.3
c27	2853.252	2853.245	0.007	2.4
c28	2910.273	2910.266	0.006	2.2
c29	3024.317	3024.309	0.007	2.4
c31	3249.463 ●	3249.457	0.006	1.9
c32	3377.523	3377.516	0.007	2.1
c39	3976.830	3976.822	0.008	1.9
c41	4201.978	4201.970	0.008	1.9
c42	4330.034 ●	4330.029	0.005	1.2
c46	4678.217	4678.208	0.008	1.8
c47	4806.269	4806.267	0.002	0.3
c48	4863.292 ●	4863.288	0.003	0.7
c49	4920.323	4920.310	0.013	2.7
c50	5034.359 ●	5034.353	0.006	1.2
c52	5259.505 ●	5259.501	0.005	0.9
c53	5387.546	5387.559	-0.013	-2.3
c59	5889.823	5889.813	0.010	1.7
c61	6114.963 ●	6114.961	0.002	0.4
c67	6719.269 ●	6719.258	0.011	1.7

c68	6776.292	6776.279	0.013	1.9
c70	7019.404 ●	7019.401	0.003	0.5
c71	7106.442 ●	7106.433	0.009	1.3
c72	7262.536 ●	7262.534	0.002	0.2
z3	342.178	342.178	0.000	0.8
z4	498.279	498.279	0.001	1.0
z5	585.312	585.311	0.001	1.3
z6	713.407	713.406	0.001	1.2
z9	1013.514	1013.513	0.001	0.9
z13	1361.695	1361.693	0.003	2.0
z14	1489.753	1489.751	0.002	1.6
z16	1714.902	1714.899	0.003	1.8
z22	2217.157	2217.153	0.004	1.9
z25	2570.362	2570.359	0.003	1.2
z26	2684.406	2684.402	0.004	1.5
z27	2741.428	2741.424	0.004	1.5
z33	3274.687	3274.683	0.004	1.2
z34	3402.746	3402.742	0.004	1.1
z36	3627.895	3627.890	0.006	1.6
z43	4227.200 ●	4227.196	0.004	0.9
z44	4355.268 ●	4355.255	0.013	3.0
z46	4580.409 ●	4580.403	0.006	1.3
z48	4751.472 ●	4751.467	0.005	1.1
z50	4936.557	4936.547	0.010	2.1
z54	5274.711 ●	5274.706	0.005	1.0
z55	5402.770	5402.765	0.005	1.0
z59	5741.915	5741.919	-0.004	-0.7
z60	5855.002 ●	5855.003	-0.001	-0.2
z61	5968.081 ●	5968.087	-0.006	-1.1
z64	6239.222 ●	6239.204	0.019	3.0
z65	6368.258 ●	6368.246	0.012	1.8
z66	6497.299 ●	6497.289	0.010	1.6
z70	7006.451 ●	7006.441	0.010	1.4
z72	7249.555 ●	7249.526	0.028	3.9
z74	7476.664 ●	7476.653	0.010	1.3

Crude PScore: 5.29545e-65; McLuckey Score: 7.49789; Manual PScore: 1.73911e-149

Table 9: Table list of measured mass from MSMS data generated from ECD experiment on Protein IB8a using LTQFT-ultra instrument compare to Prosight data theoretical values. Unique fragment ions are labeled with ●.

User Specified Sequence
SPPGKPQGPPPQGGNQPQGPPPPPGKPQGPPPQGGNKPQGPPPPGKPQGPPPQGDNKSQSARSPPGKPQG
PPPQGGNQPQGPPPPPGKPQGPPPQGGNKSQGPPPPGKPQGPPP[9]QGGSK[162.05]S[14.01]R
Number of Amino Acids: 121
Theoretical Mass: 11897.1 Da
Mass Difference: 0.0475 Da
C Ions: 26 Z Ions: 31

Ion	Observed Mass (Da)	Theoretical Mass (Da)	Mass Error (Da)	Mass Error (PPM)
c6	580.335	580.333	0.002	3.3
c7	708.394	708.392	0.002	3.3
c11	1056.572	1056.571	0.001	0.6
c15	1412.722	1412.716	0.006	4.4
c17	1637.833	1637.827	0.006	3.6
c18	1765.893	1765.886	0.007	4.1
c27	2590.352	2590.340	0.012	4.6
c28	2718.411	2718.399	0.012	4.5
c33	3194.650	3194.637	0.013	4.0
c34	3251.674	3251.659	0.015	4.8
c36	3422.732	3422.723	0.009	2.6
c38	3647.885	3647.871	0.014	3.9
c39	3775.938	3775.929	0.009	2.3
c42	4027.048	4027.056	-0.008	-2.0
c44	4221.180	4221.162	0.018	4.3
	4221.197	4221.209	-0.012	-2.9
c48	4631.376	4631.389	-0.013	-2.9
c53	5107.613	5107.628	-0.015	-2.9
c55	5279.685	5279.676	0.009	1.7
c57	5521.827	5521.814	0.013	2.3
c59	5736.946	5736.952	-0.006	-1.1
c62	6051.046	6051.075	-0.029	-4.8
c68	6614.385	6614.382	0.003	0.5
c70	6799.488	6799.462	0.026	3.9
c111	10712.447 ●	10712.439	0.008	0.8
c116	11197.704 ●	11197.677	0.027	2.4
c120	11718.988 ●	11718.955	0.033	2.8
z19	2034.034	2034.032	0.002	1.0
z20	2091.063	2091.053	0.010	4.6
z23	2434.278	2434.287	-0.009	-3.5
z24	2548.320	2548.329	-0.009	-3.7
z25	2605.341	2605.351	-0.010	-3.8
z26	2662.365	2662.372	-0.007	-2.8

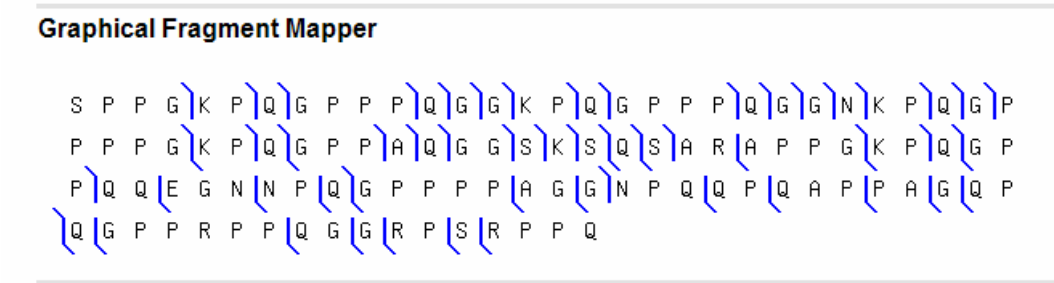
z27	2790.422	2790.431	-0.009	-3.2
z31	3138.606	3138.611	-0.005	-1.5
z32	3266.661	3266.669	-0.008	-2.5
z34	3491.813	3491.817	-0.004	-1.1
z41	4091.117	4091.124	-0.007	-1.6
z42	4219.176	4219.182	-0.006	-1.5
z44	4444.289	4444.294	-0.005	-1.0
z45	4558.335	4558.337	-0.002	-0.3
z46	4615.349	4615.358	-0.009	-2.0
z47	4672.349	4672.332	0.017	3.6
	4672.376	4672.380	-0.004	-0.7
z48	4800.437	4800.438	-0.001	-0.2
z52	5148.608	5148.618	-0.010	-1.9
	5148.619	5148.618	0.001	0.2
z53	5276.677	5276.676	0.001	0.1
z55	5501.817	5501.824	-0.007	-1.3
z59	5839.983	5839.983	-0.000	-0.0
z60	5996.068	5996.084	-0.016	-2.7
z62	6154.158 ●	6154.153	0.005	0.8
z63	6282.223 ●	6282.212	0.011	1.8
z65	6497.343 ●	6497.339	0.004	0.6
z66	6611.390	6611.382	0.008	1.2
z67	6726.419	6726.409	0.010	1.5
z68	6783.453	6783.430	0.023	3.3
z76	7612.867	7612.875	-0.008	-1.0
z85	8468.341 ●	8468.335	0.006	0.7
z94	9300.713	9300.670	0.043	4.6

“Manual” PScore: 9.86742e-109
McLuckey Score: 140.299

Table 10: Table list of measured mass from MSMS data generated from ECD experiment on Protein @ 10433.39 Da using LTQFT-ultra instrument compare to Prosight data theoretical values. Unique fragment ions are labeled with ●.

User Specified Sequence
SPPGKPQGPPPQGGKPQGPPPQGGNKPQGPPPPGKPQGPPAQGGSKSQSARAPPGKPQGPPQQEGNNPQG
PPPPAGGNPQQPQAPPAGQPQGPPRPPQGGRPSRPPQ
Number of Amino Acids: 107
Theoretical Mass: 10433.6 Da
Mass Difference: -0.1625 Da
C Ions: 36 Z Ions: 25

Ion	Observed Mass (Da)	Theoretical Mass (Da)	Mass Error (Da)	Mass Error (PPM)
c4	355.186	355.185	0.000	1.3
c6	580.334 ●	580.333	0.001	1.6
c7	708.393 ●	708.392	0.001	1.6
c11	1056.575	1056.571	0.004	3.4
c12	1184.632	1184.630	0.002	1.5
c13	1241.656	1241.651	0.005	3.8
c14	1298.677 ●	1298.673	0.004	2.8
c16	1523.823 ●	1523.821	0.002	1.6
c17	1651.882 ●	1651.879	0.003	1.5
c21	2000.065	2000.059	0.006	2.9
c22	2128.121	2128.118	0.004	1.7
c23	2185.146	2185.139	0.007	3.2
c24	2242.167 ●	2242.160	0.007	3.0
c25	2356.206	2356.203	0.003	1.1
c27	2581.353	2581.351	0.002	0.9
c28	2709.413	2709.410	0.003	1.2
c29	2766.435	2766.431	0.004	1.5
c34	3211.679 ●	3211.664	0.016	4.9
c36	3436.813	3436.811	0.002	0.6
c37	3564.866	3564.870	-0.004	-1.2
c40	3816.008 ●	3815.997	0.012	3.0
c41	3887.042 ●	3887.034	0.008	2.1
c42	4015.103	4015.093	0.011	2.6
c44	4129.145 ●	4129.136	0.010	2.3
c45	4216.157	4216.168	-0.010	-2.5
c46	4344.266	4344.263	0.004	0.8
c47	4431.309	4431.295	0.015	3.4
c48	4559.367	4559.353	0.014	3.0
c49	4646.384	4646.385	-0.001	-0.3
c55	5195.701 ●	5195.687	0.013	2.6
c57	5420.839 ●	5420.835	0.004	0.7
c58	5548.906 ●	5548.894	0.012	2.2
c61	5800.003 ●	5800.021	-0.018	-3.1

c69	6695.390 ●	6695.399	-0.009	-1.3
c77	7325.730 ●	7325.712	0.018	2.5
c90	8636.378	8636.348	0.030	3.4
z4	480.257	480.257	0.000	0.7
z5	567.289	567.289	0.000	0.7
z7	820.444	820.443	0.001	1.2
z8	877.466	877.464	0.002	1.8
	877.466	877.464	0.002	1.8
z10	1062.543 ●	1062.544	-0.002	-1.8
z16	1663.879	1663.878	0.001	0.3
z17	1791.938 ●	1791.937	0.001	0.8
z19	2017.053 ●	2017.048	0.005	2.4
z20	2074.072 ●	2074.069	0.002	1.2
z22	2242.167	2242.159	0.008	3.5
z25	2538.308	2538.308	0.000	0.2
z27	2763.424	2763.419	0.005	1.7
z31	3159.591	3159.595	-0.004	-1.3
z33	3287.669	3287.653	0.015	4.6
z38	3732.894	3732.886	0.008	2.2
z39	3860.951	3860.944	0.007	1.7
z41	4072.046	4072.040	0.006	1.4
z44	4372.152	4372.147	0.005	1.1
z49	4879.404	4879.391	0.013	2.7
z52	5232.609	5232.598	0.011	2.1
z56	5554.756	5554.762	-0.006	-1.1
z59	5868.940 ●	5868.932	0.008	1.4
z60	5996.995 ●	5996.991	0.004	0.7
z70	6863.425	6863.415	0.009	1.4
z73	7216.596 ●	7216.621	-0.026	-3.6

Crude PScore: 1.83466e-40; McLuckey Score: 3.90583; “Manual” Pscore: 1.9012e-146.