Deducing protein composition from complex protein preparations by MALDI without peptide separation.

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TP #419

Introduction

How best to characterize a proteome of intermediate complexity from a large number of distinct samples?

Investigate the following:

1.) Digest with trypsin, and collect MALDI spectra.

2.) Collect msms spectra from the mixture.

What are the limitations?

1. Dynamic range / protein complexity
2. Overlapping peptides / incomplete precursor isolation.
3. Incomplete database

Methods

1. Buy chicken meat from grocery store.
2. Mince, solubilize, reduce, alkylate, precipitate, trypsin digest.
3. Dilute into matrix and collect MALDI spectra.
4. Database used : chicken NCBI with 15148 sequences.
5. Perform PMF on the digest (using proprietary software).
6. Collect 120 MS-MS spectra in triplicate on precursors between mz 700-3200, and consider up to 1 missed cleavage.
7. Compare results with PMF and to previous data collected following the standard HPLC-MSMS approach.
8. All identifications considered to be false if protein is not known to be abundant.
9. How many correct IDs at the protein and peptide level can be obtained?
10. What explains false identifications?

Table 1. Summary of PMF protein level results

I	Symbol	Length	Protein Name	Peptide Count All	PMF(u)	PMF (t)	msms	Score	%CM	%IM	ppw
1	MYH1	1941	Myosin heavy chain fast isoform 3	423	59	61	15	360759	35.9	20.1	8.0
2	ACTA1	377	Actin, alpha skeletal muscle	56	12	12	9	135126	36.0	16.7	3.6
3	MYL3	150	Myosin light chain 3	38	7	7	4	36041	47.9	5.3	3.6
4	CKM	381	Creatine kinase M-type	64	11	14	3	31889	35.1	2.8	4.4
5	ACTN2	897	Alpha-actinin-2	174	20	29	1	11944	19.0	4.4	12.4
6	MYH2	761	Myosin heavy chain	198	15	34	3	6449	18.2	5.8	15.8
		772	Glucocorticoid receptor	111	12	13		4816	20.8	2.3	10.9
		180	Uncharacterized protein	27	5	6		2266	32.7	1.4	4.9
7	ALDOC	362	Fructose Bisphosphate aldolase mouse	75	5	8	1	1787	19.3	1.1	12.1
		205	UPF0454 protein C12orf49 homolog	37	4	5		889	22.2	1.2	9.3
		152	Acetylcholinesterase	18	3	3		875	20.8	0.8	4.3
		212	Fibroblast growth factor 10	40	7	8		626	26.6	1.8	13.8
		157	Calcimedin, 32K	23	3	4		429	22.7	0.5	4.4
		498	Nuclear factor 1	57	5	5		319	21.0	1.0	13.5
8	MYLPF	168	Myosin regulatory light chain 2	32	3	6	2	304	31.9	0.3	8.6
9	PK		Pyruvate Kinase				1				
10	MYL1		Myosin light chain 1, cardiac muscle				1				
11	CKS		Creatine kinase S-type				1				
12	PGK		Phosphoglycerate kinase				1				
13	HSPB		Heat shock protein beta-1				1				
14	GAPDH		Glyceraldehyde-3-phosphate DH				1				
15	ENO		Enolase				1				
16	TPM		Tropomyosin alpha				2				

Figure 1. The below parent spectrum should be color-coded according to success of msms. Green-> all 3 correct, Blue-> 2 correct; yellow-1 correct, red-> incorrect, grey-> no id. Data on individual spectra are listed in Table 4.

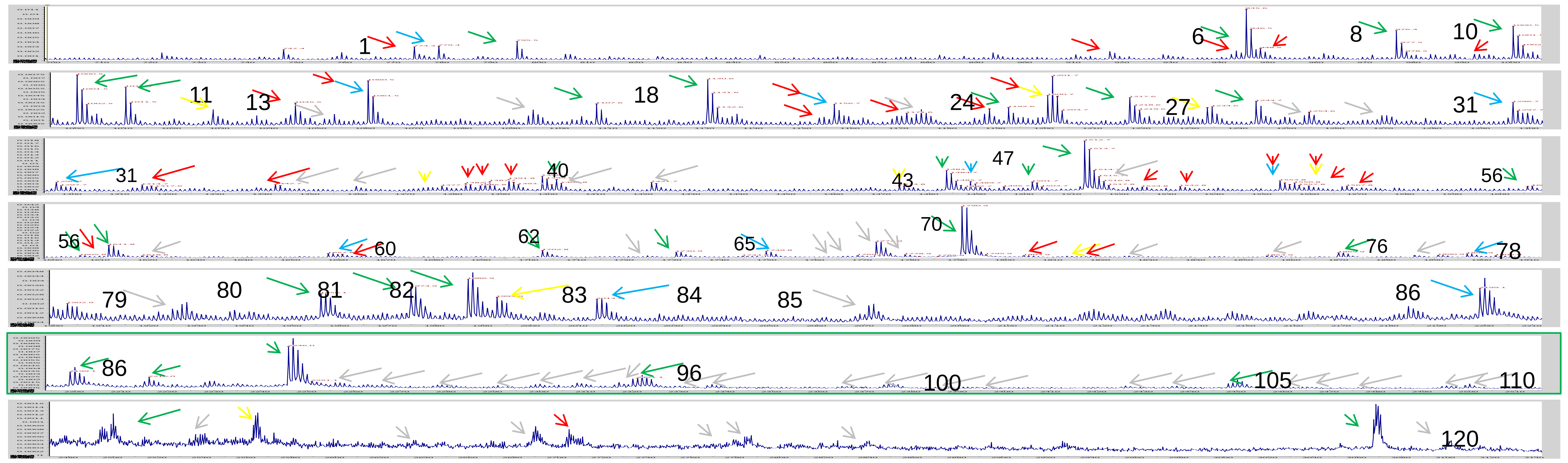
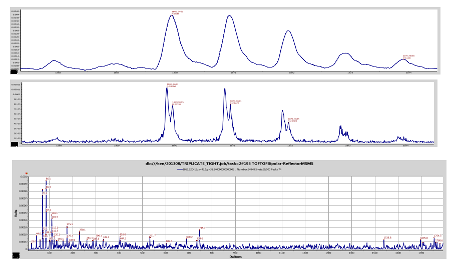


Figure 2.
Two overlapping precursors at mz 1869.94 (#76): This precursor mapped to myosin, but was off by 37 ppm. Two prominent components are visible at higher resolution; one component is very close. Only 2 of 12 putative correct IDs that are off by > 10 ppm cannot be so readily explained. 37 peptides matched to < 10 ppm even from low resolution parent spectra.



amu	peptides	peaks	I	Pep MZ	ppm	Sequence	TrS	Symb
1	269	68	1	1869.87	27.3	**aSSDAEMAIFGEAAPYLR**	1995	**MYHsk_k**
1	269	68	2	1870.08	-83.4	DLLLQTRLINDVTSIR	758	RyR3
1	269	68	3	1869.97	-27.1	**DLIELQALIDSHFEAR**	642	**TNNT3**
1	269	68	4	1869.98	-31.1	DLYALFEQILEKTMR	591	MYO9A
1	269	68	5	1869.91	3.7	SQYVPYDGIPFVNAGSR	562	ALS2CR8
1	269	29	1	1869.87	27.3	**aSSDAEMAIFGEAAPYLR**	1748	**MYHsk_k**
1	269	29	2	1869.97	-27.1	**DLIELQALIDSHFEAR**	976	**TNNT3**
1	269	29	3	1869.91	3.2	YHAIVYPMKFMQGER	817	NPSR1
1	269	29	4	1870.08	-83.4	DLLLQTRLINDVTSIR	774	RyR3
1	269	29	5	1869.95	-15.1	SSEVETTVFYIPGVDVK	617	KIAA1109
5	1384	29	1	1869.87	27.3	**aSSDAEMAIFGEAAPYLR**	1748	**MYHsk_k**
5	1384	29	2	1869.97	-27.1	**DLIELQALIDSHFEAR**	976	**TNNT3**
5	1384	29	3	1867.87	1093.9	SIGEDVYEKPMSELDR	893	FILIP1
5	1384	29	4	1869.91	3.2	YHAIVYPMKFMQGER	817	NPSR1
5	1384	29	5	1868.84	579.1	DNEQLSHLNQEQEER	816	GOLGA2
20	5675	29	1	1869.87	27.3	**aSSDAEMAIFGEAAPYLR**	1748	**MYHsk_k**
20	5675	29	2	1869.97	-27.1	**DLIELQALIDSHFEAR**	976	**TNNT3**
20	5675	29	3	1867.87	1093.9	SIGEDVYEKPMSELDR	893	FILIP1
20	5675	29	4	1869.91	3.2	YHAIVYPMKFMQGER	817	NPSR1
20	5675	29	5	1868.84	579.1	DNEQLSHLNQEQEER	816	GOLGA2

<-Table 2

From this msms spectrum, the program correctly identifies myosin, starting with either 29 or 65 peaks detected, when searched against the chicken database considering a 1 amu search window, a 5 amu window, or a 20 amu window. With the shorter peak list (with less confounding noise), the 2nd hit is a troponin peptide, known from HPLC MSMS to be abundant in chicken muscle preparations. It may explain the 2nd parent mass visible in the high resolution spectrum.

How to tell correct from incorrect MS-MS IDs without cheating ?
(but rules deduced by cheating)?

1. High mass accuracy. Mass accuracy becomes an important filter (20 ppm filters out 2/3 of 1 amu wrong IDs) even when the resolution is between 5K and 10 K as in Figure 1.
2. Complexity of the parent mass region. Much stronger parent masses 10 amu away may interfere.
3. Consistency of identification between replicate MSMS spectra (Only 3 wrong but consistent IDs with score > 805 found)
4. Robustness of ID as a function of peak detection.
5. Robustness of ID as a function of precursor mass accuracy.
6. Difference in score between top hit and highest incorrect hit (Table 2)
7. Longer peptides are more reliable. Short homologous wrong peptides often get high scores. See Table 5.

Table 5. Hits to spectrum #1

PepMW	mz	ppm	Sequence	Score	PIM	PCM	Symbol
774.40	774.32	104.1	**EEIDIR**	3360	84	56	
774.40	774.32	104.1	**EEVEIR**	3360	84	56	C9orf64
774.45	774.32	175.3	**EILMIR**	2835	70	63	
774.35	774.32	34.1	**&DIDIR**	2386	79	47	**ACTA1**
774.40	774.32	104.1	**EIEDLR**	2280	70	57	
774.40	774.32	105.3	**qMIMIR**	2131	65	51	CUL4B
774.45	774.32	165.7	**EQTKLR**	2099	71	46	

4th hit obviously correct

Table 3. Peptide level comparison

Green-> correct by PMF
Blue-> Correct by MSMS but not PMF

Rank	Int	Mass	ProtR	symbol	ppm	Sequence	msms
73	211	747.39	1		-16.4	SEIDRK	
86	196	774.32					
62	240	779.37	1	MYHsk_k	-21.1	**ALzYPR**	**ALzYPR**
41	328	795.45	10	ACTA1	6.2	QHLLER	**IIAPPER**
82	202	917.47	1		0.5	AALEQTER	
7	997	945.55	2	ACTA1	-1.2	**AVFPSIVGR**	**AVFPSIVGR**
83	200	948.47	1		14.3	TEELEEAK	
21	525	976.44	2	ACTA1	-10.6	**AGFAGDDAPR**	**AGFAGDDAPR**
11	800	1000.49	1	MYHsk_k	-0.2	**&NGVLEGIR**	**&NGVLEGIR**
29	437	1010.52	3	MYL1sk	-8.9	**EAFLLFDR**	**EAFLLFDR**
81	207	1028.54	5	ACTN1	-17.1	**TIPWLENR**	**TIPWLENR**
89	186	1045.53	28		-23.5	ADAPVKWMK	
16	744	1060.53	1	MYHsk_k	15.2	**ANSEVAQWR**	**ANSEVAQWR**
52	278	1107.63	33	CKMT2	13.8	LNNNIKYTK	**VPPPLPQFGR**
12	792	1130.56	2		10.7	**GYSFVTTAER**	**GYSFVTTAER**
58	260	1156.66	1		-13.6	NLRNTQAVLK	
70	215	1171.59	2		15.7	HQGVMVGMGQK	
87	192	1174.60	5		-7.6	EGLLLWzQR	
68	220	1192.60	7	MYLPF	-15.5	IMSTLNMLGGR	**EAFTVIDQNR**
25	495	1200.69	3		-1.2	**ITLSQVGDIVR**	**ITLSQVGDIVR**
28	443	1201.68	1		15.5	AGLLGLLEEMR	
31	432	1217.62	4		2.5	**DLFDPVIQDR**	**DLFDPVIQDR**
67	224	1233.62	3		21.3	**EGNGTVMGAELR**	**EGNGTVMGAELR**
38	354	1243.66	5	TPM1	16.5	HRPDLIDYSK	**IQLVEEELDR**
48	286	1296.66	1	MYHsk_k	7.7	**DTQIHLDDALR**	**DTQIHLDDALR**
65	228	1314.71	7		7.3	INDRGDLLPSSK	
77	209	1316.65	12		-15.8	SYNHLQGDVRK	
100	173	1338.66	14		-23.6	RSLPSTSSTSSTK	
47	290	1342.69	7		-12.3	HMLMVARELSR	
75	211	1359.68	6		-10.7	GRLQTENGELSR	
99	175	1377.67	19		-19.9	TLFFRYMSQGK	
35	414	1382.67	1		9.4	IQHELEEAEER	
54	270	1385.76	5		-24.6	LASELLEWIRR	
57	261	1386.74	27		10.5	LDTTHHPRQPGK	
94	182	1390.70	7		6.6	zLQAGMNLEARK	
24	500	1391.76	8		2.8	GDAVTEIGITFLR	
13	780	1398.76	1	MYH3	-2.5	**ANLLQAEIEELR**	**ANLLQAEIEELR**
34	416	1401.76	6		23.9	LAEQELLEATER	
98	175	1412.76	6		-8.9	NNLLLAEVEELR	
26	456	1421.72	5		11.0	GYEEWLLNEIR	
39	332	1473.78	1	MYH3	5.8	**qAFTQQIEELKR**	**qAFTQQIEELKR**
6	1209	1483.69	2	ACTA1	5.8	**qEYDEAGPSIVHR**	**qEYDEAGPSIVHR**
56	264	1487.74	14		-4.8	AFAYTWFNLQAR	
43	315	1488.73	1	MYHsk_k	17.5	**IEELEEEIEAER**	**IEELEEEIEAER**
22	509	1501.71	12	MYL_K	-16.9	HnGRQMFVALnGR	**DTGTYEDFVEGLR**
2	2723	1512.70	3	MYL1sk	1.0	**DQGTFEDFVEGLR**	**DQGTFEDFVEGLR**
79	207	1515.74	2		-7.7	IWHHTFYNELR	
49	285	1516.75	34		-7.2	SQKTPTSSPSPGSQK	
59	255	1532.78	1		-9.7	LLSSIDVDhTQYK	
23	507	1553.75	17	ENO3	9.7	GQNHNVQGFHPYR	**VVIGMDVAASEFzR**
61	243	1556.81	11	GAPDH	-8.0	GIASYLNVWYSRK	**VPTPNVSVVDLTzR**
69	220	1559.83	1		-1.5	IVESLQSSLDAEIR	
97	177	1567.79	34		17.1	EQDTDSINKQVYK	
74	211	1570.89	29		17.3	QVPPQFLETVEKR	
46	304	1605.76	28	PKM2	-20.9	DFzSAQLSVKEPPK	**qPAHDAWAEDVDLR**
80	207	1609.78	16		18.6	&PFAHGEFPDPKPK	
5	1483	1611.78	2	MYLPF	2.2	qEYDEAGPSIVHRK	**SFLEELLTTQzDR**
93	184	1618.80	1		18.4	LEEAGGATAAQIDMNK	
19	587	1657.84	4	CKM	1.1	**TFLVWVNEEDHLR**	**TFLVWVNEEDHLR**
45	304	1660.81	1		-8.0	NAYEESLDHLQTLK	
96	180	1663.82	13		-1.6	GMGTDEEEILILTSR	
44	304	1701.84	27		-9.4	SQDGEELAISGGGGLRR	
17	656	1702.83	1	MYH3	-0.1	**LQNEVEDLMIDVER**	**LQNEVEDLMIDVER**
9	873	1730.91	1	MYHsk_k	4.4	**TPGAMEHELVLHQLR**	**TPGAMEHELVLHQLR**
55	265	1744.86	29		-6.5	QWQMNIEGVNDVALK	
15	756	1749.78	32	GAPDH	-12.9	RSISzPSzNGLAEGNK	**LVSWYDNEFGYSNR**
72	213	1768.98	38		-6.6	PILQRYISLLMEHR	
3	2539	1772.89	6		-18.5	DLQMRLDEAEQIALK	
32	430	1778.93	1		-6.7	DLQHRLDEAEQLALK	
78	208	1781.91	25		-2.6	KDVDGAYMTKVDLQAK	
1	7311	1790.89	2	ACTA1	-0.9	**SYELPDGQVITIGNER**	**SYELPDGQVITIGNER**
37	391	1795.90	24		9.7	DVFLGmFLYEYARR	
88	192	1803.88	9		-14.1	&PLPRPWALTFSYGR	
91	185	1810.95	23		16.1	VPFTFLTSPSWEPFR	
64	228	1816.91	1		1.7	RQAEEAEELSNVNLSK	
95	182	1825.00	8		1.3	ELFVLTYGALVAQLzK	
53	275	1854.89	31		16.7	QALLHzQDFHDQSQK	
10	850	1869.94	22	MYHsk_k2	6.7	SADLTAMSDNKELYLAK	**aSSDAEMAIFGEAAPYLR**
40	331	1885.89	28		11.8	GYIVEMQDEGSTDWKK	
27	446	1890.86	21		-1.1	YGSGGGSKGGSLSGGGYGSGGG	
30	437	1896.90	1	MYHsk_k	-2.5	**HADSTAELGEQIDNLQR**	**HADSTAELGEQIDNLQR**
66	227	1902.91	45		6.3	KQDEYHMVHLVzTSR	
63	234	1924.94	1		12.2	YETDAIQRTEELEEAK	
92	185	1936.93	14		-17.9	VSASHHAPDRPPDPFPPL	
20	547	1956.05	2	ACTA1	4.8	**VAPEEHPTLLTEAPLNPK**	**VAPEEHPTLLTEAPLNPK**
18	598	1974.92	2	ACTA1	-2.2	**YPIEhGIITNWDDMEK**	**YPIEhGIITNWDDMEK**
8	925	1986.90	1	MYHsk_k	-1.6	**MEGDLNEMEIQLSHANR**	**MEGDLNEMEIQLSHANR**
60	244	1992.96	4	CKM	-0.6	**GTGGVDTAAVGAVFDISNADR**	**GTGGVDTAAVGAVFDIS**
33	423	2013.91	38	MYH2f_k	-9.6	SQLDSRNSSSSSSLASSEGK	**MEGDLNEMEIQLNHANR**
51	279	2070.92	31		-24.4	LIQEQYLQTESzSSAGEK	
84	200	2115.12	28		-1.1	IIAEAEISDTPLLTSEEKR	
85	199	2131.12	35		4.6	ASIQLPMEKAIETALDzLK	
14	775	2199.05	28	MYH2f_k	-17.2	zSITTTEKSzILEILDSTK	**NDLQLQVQAEADALADAE**
50	284	2215.05	1	MYHsk_k	0.1	**NDLQLQVQAEADSLADAEER**	**NDLQLQVQAEADSLADA**
42	317	2228.04	25		2.5	LDSELKNMQDMVEDYRNK	
76	210	2244.08	10		-10.1	FLNRAAMAFQNLFMAVEDR	
4	2090	2246.02	2	ACTA1	1.2	**DLYANNVMSGGTTMYPGIADR**	**DLYANNVMSGGTTMYP**
36	401	2320.11	1	MYH2f_k	3.3	**LETDIAQIQSEMEDTIQEAR**	**LETDIAQIQSEMEDTIQEA**
71	214	2322.09	35		3.7	FLzNMTFLKEYMEEEIPK	
90	185	3067.58	9	ALDOC	1.8	**TVPPAVPGVTFLSGGQSEEEASL**	**TVPPAVPGVTFLSGGQSE**

Table 4. MSMS searches

#	msms	ppm	Correct; Score > 805 < 10 ppm	> 805	10 ppm	Wrong; Score > 805 <20 ppm	Total	Distinct	sc	PepMW	Seq	Acc
1	774.32	180.1				0	3	3	3103	774.46	**TGTARIR**	
2	779.37	21.1		2	2				1418	779.39	**ALzYPR**	MYHsk_k
3	795.45	25.4		3	3				2215	795.47	**IIAPPER**	ACT
4	917.47	-0.4				1	1	1	1144	917.47	**ELSNGLER**	
5	942.46	58.6				0	1	1	1207	942.52	**AIGHFELR**	
6	945.55	1.2	1	3					5006	945.55	**AVFPSIVGR**	ACT
7	948.47	10.4				1	3	3	1195	948.48	**MLQIzAGR**	CENPM
8	976.44	10.6		3	3				2989	976.45	**AGFAGDDAPR**	ACT
9	992.52	-33.0				0	2	2	1540	992.48	**ETLVSQzR**	
10	1000.49	0.2	1	3					5208	1000.49	**&NGVLEGIR**	MYHsk_k
11	1010.52	8.9	1	3					3733	1010.53	**EAFLLFDR**	MYL1sk
12	1028.54	17.1		1	1				920	1028.55	**TIPWLENR**	ACTN1
13	1045.53	36.8				1	3	3	1109	1045.56	**SSEGVQGILR**	
14	1053.54	-37.3							650	1053.50	MNYIEDLR	
15	1060.53	-8.4		**2**	**2**	1	1	1	2040	1060.52	**NASQTRNNR**	
16	1093.55	-26.9							649	1093.52	GIEEDEFVR	ZCCHC11
17	1107.63	0.1	1	3					1239	1107.63	**VPPPLPQFGR**	CKMT2
18	1130.56	-10.7		3	3				1980	1130.55	**GYSFVTTAER**	ACTA1
19	1153.57	61.7				1	3	3	1284	1153.64	**IVLPDVMNPR**	ZBTB43
20	1156.66	-5.7	1	2		**0**	**1**	**1**	1903	1156.66	**LVIIEGDLER**	TPM1
21	1170.55	86.5				0	3	1	1170	1170.65	**ELTPQVVSAAR**	VCL
22	1173.55	-3.8							801	1173.55	**GYSLPPHzSR**	CKM
23	1188.66	-39.8				1	2	2	925	1188.62	**QPMSATLRER**	C10orf78
24	1192.60	-3.1	1	3					2415	1192.60	**EAFTVIDQNR**	MYLPF
25	1200.69	-59.4	1	1		0	1	1	1137	1200.62	**ASPEVEQTALR**	RHBDF2
26	1217.62	-2.5	1	3					4935	1217.62	**DLFDPVIQDR**	CKM
27	1233.62	-21.3		1	1*				1180	1233.59	**EGNGTVMGAELR**	MYL_K
28	1243.66	-8.4	1	3					1918	1243.65	**IQLVEEELDR**	TPM1
29	1253.61	4.4							566	1253.62	DSIMVQEYIR	JAK
30	1269.59	-22.3							741	1269.56	**ELTYQSEEDR**	MYH3
31	1296.66	-7.7	1	2					1861	1296.65	**DTQIHLDDALR**	MYHsk_k
32	1314.71	15.3				1	2	2	949	1314.73	**TVVPARzGVTVR**	BRAF
33	1338.66	57.7				0	1	1	875	1338.73	**eFGFLATRLFR**	
34	1342.69	4.2							801	1342.70	NGASALPRHTYR	
35	1359.68	-18.1							732	1359.66	MLHAYNPSRDR	KIAA0922
36	1373.73	10.1		1	1				826	1373.74	**YTLPPGVEATAVR**	HSPB1
37	1382.67	12.2				1	1	1	910	1382.69	**LGESQLSGPAHMR**	RERE
38	1386.74	-63.9				0	3	2	1296	1386.65	**DPLEIPDSGSGGSR**	HOXA2
39	1391.76	-29.0				0	1	1	947	1391.72	**ADALTEEINFLR**	
40	1398.76	2.5	1	3					2263	1398.76	**ANLLQAEIEELR**	MYH3
41	1401.76	-65.1				0	3	3	1696	1401.66	**IDQASLDYSYAR**	
42	1421.72	32.7							707	1421.77	TQzLQYLRALR	
43	1473.78	-5.8	1	1					825	1473.77	**qAFTQQIEELKR**	MYH3
44	1483.69	-5.8	1	3					3974	1483.68	**qEYDEAGPSIVHR**	ACTA1
45	1488.73	-17.5	1	2	2				1269	1488.71	**IEELEEEIEAER**	MYHsk_k
46	1501.71	-18.0		3	3*				1228	1501.68	**DTGTYEDFVEGLR**	MYL_K
47	1512.70	-1.0	1	3					6715	1512.70	**DQGTFEDFVEGLR**	MYL1sk
48	1515.74	-5.0							660	1515.73	GDHTNRDGIIFDR	
49	1521.73	8.4				1	1	1	850	1521.74	**TSSTFEPSPKPSEK**	MPRIP
50	1532.78	9.7				1	1	1	913	1532.80	**GTFTPVVTDPITER**	
51	1540.73	6.3							736	1540.74	QTPTREYVDFER	
52	1553.75	-3.6	1	2		**0**	**1**	**1**	2056	1553.75	**VVIGMDVAASEFzR**	ENO3
53	1556.81	0.3	1	1		**0**	**1**	**1**	1178	1556.81	**VPTPNVSVVDLTzR**	GAPDH
54	1559.83	-21.9				0	2	2	984	1559.79	**ETTADPSELEAIKR**	LD
55	1566.78	-92.6				0	1	1	897	1566.64	**zSPETSLMPDzNR**	FLCN
56	1605.76	-19.7		3	3				1615	1605.73	**qPAHDAWAEDVDLR**	PKM2
57	1609.78	3.0				2	2	1	1368	1609.78	**SFLTAASPGEKGSSDR**	
58	1611.78	-7.1	1	3					4983	1611.77	**SFLEELLTTQzDR**	MYLPF
59	1618.80	-33.1							441	1618.75	PHMQASPQQPNNNR	
60	1657.84	-1.1	1	2					1142	1657.83	**TFLVWVNEEDHLR**	CKM
61	1660.81	16.7				1	1	1	1155	1660.83	**AGMEKTSAITAAAADPR**	DCLK2
62	1702.83	0.1	1	3					4401	1702.83	**LQNEVEDLMIDVER**	MYH3
63	1724.85	70.1							623	1724.97	LAAGFLLLFQFLSER	PLXDC2
64	1730.91	-4.4	1	3					2363	1730.90	**TPGAMEHELVLHQLR**	MYHsk_k
65	1749.78	4.8	1	2					2320	1749.79	**LVSWYDNEFGYSNR**	GAPDH
66	1765.89	28.6							515	1765.94	qPLRPPzVLAFVTER	NPNT
67	1768.98	-45.2							508	1768.90	HIPGSPFTAKITDDNR	
68	1772.89	0.2							494	1772.89	DILSPDMISPPLGDFR	CDC42EP3
69	1778.93	35.7							664	1778.99	ITPVSPVHHPPLSELR	
70	1790.89	0.9	1	3					5839	1790.89	**SYELPDGQVITIGNER**	ACT
71	1803.88	-12.7				1	1	1	1012	1803.85	**&QENADPQKMAFLLR**	CENPL
72	1812.93	1.1	1	1					1008	1812.93	**AzANPAnGSVILLENLR**	PGK
73	1816.91	6.7				1	1	1	838	1816.92	**DISDGFPPGDATIVTRR**	LAO
74	1825.00	-33.0							481	1824.94	SAGVHQPKFAAEIAENR	PTPRJ
75	1854.89	43.5							555	1854.97	EGDRIIQInGIEVQNR	PDZRN3
76	1869.94	-37.4		3	3				2391	1869.87	**aSSDAEMAIFGEAAPYLR**	MYHsk_k2
77	1885.89	13.3							658	1885.91	ETEGFVARDFTMLVDR	PEX1
78	1896.90	2.5	1	2					2536	1896.91	**HADSTAELGEQIDNLQR**	MYHsk_k
79	1924.94	45.2							486	1925.02	GENLAIGFDIHKVELNR	CAPN5
80	1956.05	-4.8	1	3					4680	1956.04	**VAPEEHPTLLTEAPLNPK**	ACTA1
81	1974.92	2.2	1	3					5406	1974.93	**YPIEhGIITNWDDMEK**	ACTA1
82	1986.90	1.6	1	3					2325	1986.90	**MEGDLNEMEIQLSHANR**	MYHsk_k
83	1992.96	0.6	1	1					840	1992.96	**GTGGVDTAAVGAVFDISNADR**	CKM
84	2013.91	-0.5	1	2					1650	2013.91	**MEGDLNEMEIQLNHANR**	MYH2f_k
85	2070.92	-8.2							547	2070.90	**FzGWYDADLSPAGQQEAR**	PGAM1
86	2199.05	0.7	1	3					3208	2199.05	**NDLQLQVQAEADALADAEER**	MYH2f_k
87	2215.05	-0.1	1	3					1882	2215.05	**NDLQLQVQAEADSLADAEER**	MYHsk_k
88	2246.02	-1.2	1	3					5666	2246.02	**DLYANNVMSGGTTMYPGIADR**	ACTA1
89	2256.05	53.1							672	2256.17	IDDVPPIKFELSQGSPQMVR	
90	2262.05	-30.8							572	2261.98	SSSSEzSLKEDLQTzMFPR	KIAA0922
91	2277.06	58.0							640	2277.19	GGSLLzGTSAAKAVLTLSTQADR	KIAA1244
92	2289.09	-10.3							650	2289.07	DLTPLSDGDVGSSTSSHSSQRR	C6
93	2298.11	17.4							499	2298.15	DEMAHVTGRVLPAPMLQYGGR	EIF2C3
94	2307.09	69.6							763	2307.25	NPNSLKAMAPLSSGVNLPLLDR	EPHB2
95	2313.11	-66.1							536	2312.96	SSLTPSSQDDSSNQEDGQESSK	RPS6KC1
96	2320.11	-3.3	1	3					2214	2320.10	**LETDIAQIQSEMEDTIQEAR**	MYH2f_k
97	2329.13	26.8							650	2329.19	qAASTPALSPAGPAASPPGAGSGKAAR	cDtx2
98	2336.07	8.4							741	2336.09	LETDISQIQSEMEDTIQEAR	MYH_N127
99	2365.10	45.3							632	2365.20	DADVFPIEVDLARTTLNYGQK	PICK1
100	2374.09	129.9							675	2374.40	FGLPVIPPTIPFQRGLAPLNVK	
101	2386.10	37.4							584	2386.19	QPPSVPnGPSSPITESAPELPQR	
102	2393.11	44.4							799	2393.22	eEAADSPELSPALEELIQKVSR	RAR-gamma2
103	2425.12	40.3							432	2425.22	ASPSAIGSSQPPSVTPASSPSKEQK	CAPRIN2
104	2436.11	42.5							466	2436.21	GPPGPVGPSGKDGSNGMPGPIGPPGPR	COL2A1
105	2448.19	1.8	1	3					1244	2448.19	**KLETDIAQIQSEMEDTIQEAR**	MYH2f_k
106	2460.13	70.3							330	2460.30	YHYTSSSLPRNLPINITNTIR	
107	2464.12	10.2							519	2464.15	GEDGDPGQPGPPGPSGEAGPPGPPGKR	
108	2478.12	-19.4							431	2478.07	DINEzALDPDIzSNGIzENLR	
109	2494.14	102.9							526	2494.39	SPFPVTVAPPLQLDKVSVQGLSSK	
110	2499.12	67.2							447	2499.28	VEVLPPIETKGLTSDDVSDLTDR	AGPAT2
111	2539.17	66.6							734	2539.34	EVPPSPALSTGTVLPNADLTYQLR	CD1.1
112	2563.20	-3.4	1	1					829	2563.19	**qEAPPHIFSISDNAYQFMLTDR**	MYHsk_k
113	2634.19	100.2							580	2634.45	PEVPSHVPFLLIGGGTAAFAAARSIR	
114	2689.12	101.5							408	2689.40	TPLHTNELNDIQSQPGGYHLLTLK	OBSCN
115	2704.29	40.9				0	2	2	956	2704.40	**qPPPPARPAAALGPDPGVPGAPMGFSLR**	LVRN
116	2777.15	97.4							633	2777.42	MPILEHPAYPPATLLzLESLVPzR	
117	2784.15	88.4							673	2784.39	ePPRYMAPFSVVSNLSTIHIDWSR	USH2A
118	2838.19	89.3							600	2838.44	GALGQPGPAGEQGMRGPQGPPGQIGTPGIR	COL6A3
119	3067.58	-1.8	1	3					1968	3067.57	**TVPPAVPGVTFLSGGQSEEEASLNLNAINR**	ALDOC
120	3100.39	-302.2							722	3099.45	PEPPPAQGPALALzHYSDLSNNNDFYVL	
			37	116		15	49					

Conclusions:

- Using PMF, can identify most abundant proteins
- Samples like chicken muscle, plasma, whole blood lysate, saliva have a rather small number of abundant proteins that can be compared by the means shown here.
- Confirmation of PMF by MSMS can be performed directly on unseparated digests, resulting in ~15 protein IDs from whole chicken muscle.
- Failures usually due to weak precursors close to strong precursors, or mixtures.
- Similar analyses have been successfully performed on human biofluids ,

(not shown)