

Straightforward ladder sequencing of peptides using a Lys-N metalloendopeptidase

Nadia Taouatas, Madalina M Drugan, Albert J R Heck & Shabaz Mohammed

Supplementary figures and text:

Supplementary Figure 1: Further illustrative examples of MS/MS spectra from ETcaD analysis of Lys-N digested doubly charged peptides derived from the HEK293 cell lysate.

Supplementary Figure 2: ETcaD peptide ion fragmentation spectra of doubly and triply charged ions originating from Lys-N generated peptides from HEK293 cells.

Supplementary Figure 3: Typical CID peptide ion fragmentation spectra of doubly charged ions originating from Lys-C and Lys-N generated peptides from the HEK293 cell lysate.

Supplementary Figure 4: Typical CID and ETcaD peptide ion fragmentation spectra of a Lys-N generated doubly charged peptide ion.

Supplementary Table 1: A count of c and z ion (and related neutral losses) abundance ratios for doubly charged peptides originating from Lys-C and Lys-N HEK 293 cell lysate digests.

Supplementary Table 2: A count of c and z ion (and related neutral losses) abundance ratios for triply charged peptides originating from Lys-C and Lys-N HEK 293 cell lysate digests.

Supplementary Table 3: A count of c and z ion (and related neutral losses) abundance ratios for doubly charged peptides originating from Lys-C and Lys-N HEK 293 cell lysate digests initially fractionated by low pH SCX.

Supplementary Methods

Supplementary Figure 1: Further illustrative examples of MS/MS spectra from ETcaD analysis of Lys-N digested doubly charged peptides derived from the HEK293 cell lysate.

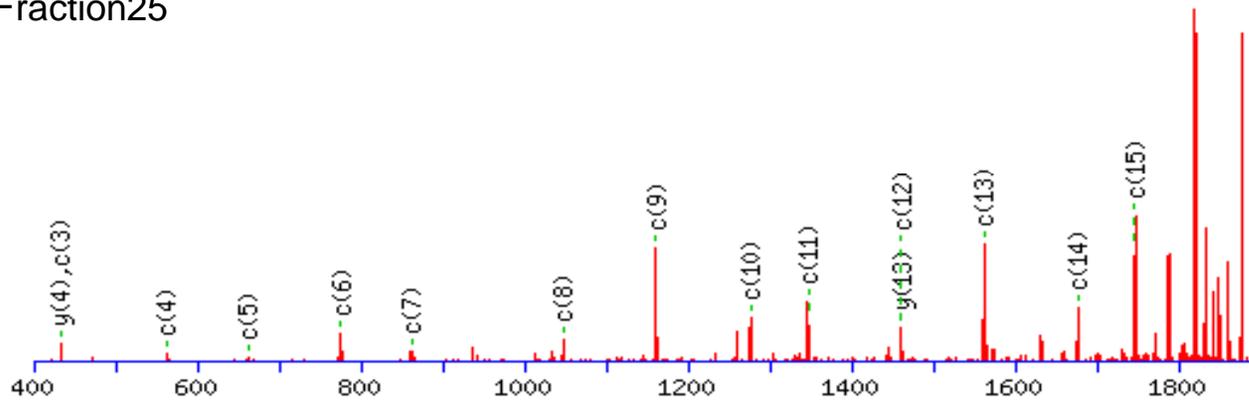
The masses of ions in the table marked with “bold red” are the ions confidently identified in the peptide ion fragmentation spectra. The ions confidently identified are almost exclusively *c* ions.

(next 20 slides)

KCQEVISWLDANTLAE

HSPA1A Heat shock 70 kDa protein 1

1875.762722 from (938.888637,2+)
 Fraction25



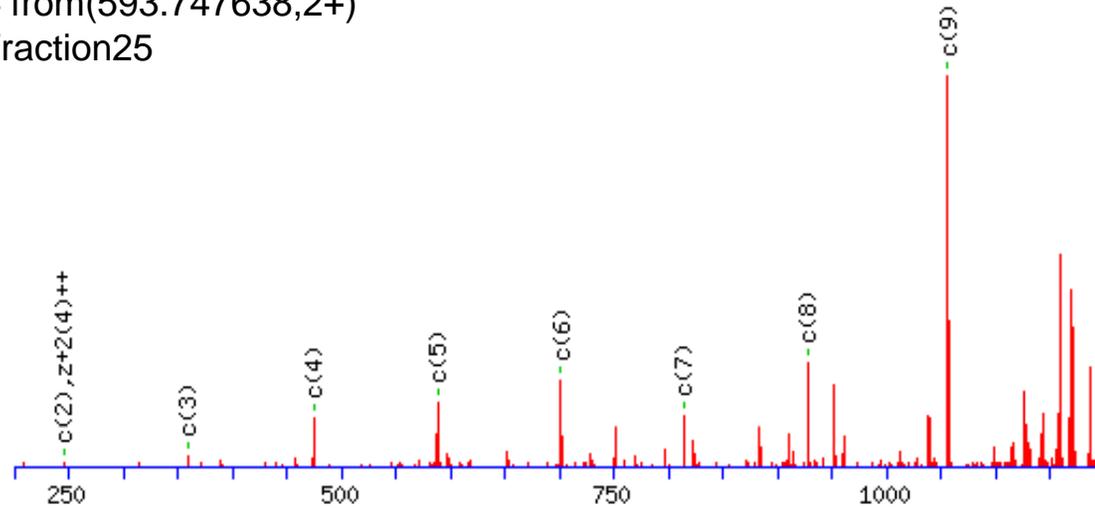
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3	434.2180	217.6126	Q	1588.7853	794.8963	1572.7666	786.8869	1573.7744	787.3909	14
4	563.2606	282.1339	E	1460.7268	730.8670	1444.7080	722.8577	1445.7159	723.3616	13
5	662.3290	331.6681	V	1331.6842	666.3457	1315.6654	658.3364	1316.6733	658.8403	12
6	775.4131	388.2102	I	1232.6157	616.8115	1216.5970	608.8021	1217.6048	609.3061	11
7	862.4451	431.7262	S	1119.5317	560.2695	1103.5130	552.2601	1104.5208	552.7640	10
8	1048.5244	524.7659	W	1032.4997	516.7535	1016.4809	508.7441	1017.4888	509.2480	9
9	1161.6085	581.3079	L	846.4203	423.7138	830.4016	415.7044	831.4094	416.2084	8
10	1276.6354	638.8214	D	733.3363	367.1718	717.3176	359.1624	718.3254	359.6663	7
11	1347.6725	674.3399	A	618.3093	309.6583	602.2906	301.6489	603.2984	302.1529	6
12	1461.7155	731.3614	N	547.2722	274.1397	531.2535	266.1304	532.2613	266.6343	5
13	1562.7632	781.8852	T	433.2293	217.1183	417.2106	209.1089	418.2184	209.6128	4
14	1675.8472	838.4272	L	332.1816	166.5944	316.1629	158.5851	317.1707	159.0890	3
15	1746.8843	873.9458	A	219.0975	110.0524	203.0788	102.0430	204.0866	102.5470	2
16			E	148.0604	74.5339	132.0417	66.5245	133.0495	67.0284	1

KVDDLLIIE

DYNC1H1 Dynein heavy chain, cytosolic

1185.480724 from(593.747638,2+)

Fraction25



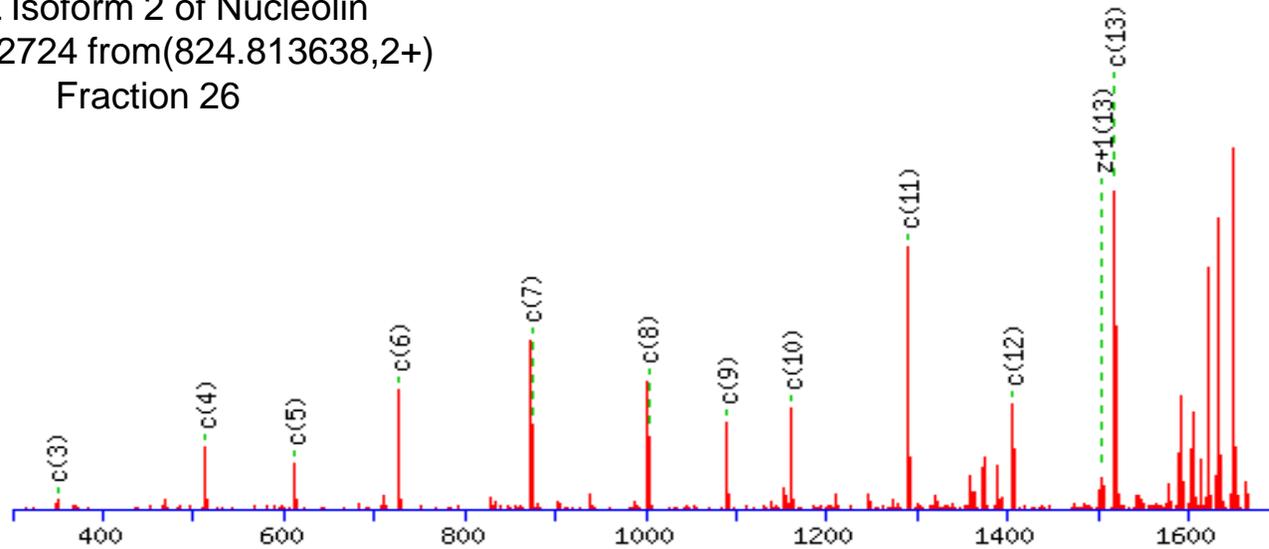
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3	360.2241	180.6157	D	959.4932	480.2502	943.4744	472.2409	944.4823	472.7448	8
4	475.2511	238.1292	D	844.4662	422.7368	828.4475	414.7274	829.4553	415.2313	7
5	588.3352	294.6712	L	729.4393	365.2233	713.4206	357.2139	714.4284	357.7178	6
6	701.4192	351.2132	L	616.3552	308.6812	600.3365	300.6719	601.3443	301.1758	5
7	814.5033	407.7553	I	503.2712	252.1392	487.2524	244.1299	488.2603	244.6338	4
8	927.5873	464.2973	I	390.1871	195.5972	374.1684	187.5878	375.1762	188.0917	3
9	1056.6299	528.8186	E	277.1030	139.0552	261.0843	131.0458	262.0921	131.5497	2
10			E	148.0604	74.5339	132.0417	66.5245	133.0495	67.0284	1

KFGYVDFESAEDLE

NCL Isoform 2 of Nucleolin

1647.612724 from(824.813638,2+)

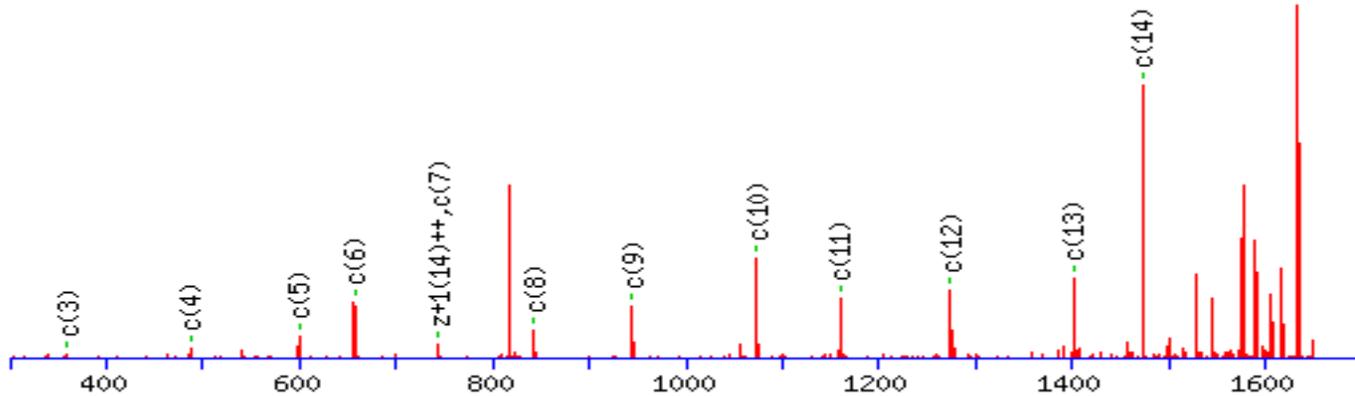
Fraction 26



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4	513.2820	257.1446	Y	1316.5529	658.7801	1300.5341	650.7707	1301.5420	651.2746	11
5	612.3504	306.6788	V	1153.4895	577.2484	1137.4708	569.2390	1138.4786	569.7430	10
6	727.3774	364.1923	D	1054.4211	527.7142	1038.4024	519.7048	1039.4102	520.2088	9
7	874.4458	437.7265	F	939.3942	470.2007	923.3755	462.1914	924.3833	462.6953	8
8	1003.4884	502.2478	E	792.3258	396.6665	776.3070	388.6572	777.3149	389.1611	7
9	1090.5204	545.7638	S	663.2832	332.1452	647.2645	324.1359	648.2723	324.6398	6
10	1161.5575	581.2824	A	576.2511	288.6292	560.2324	280.6199	561.2402	281.1238	5
11	1290.6001	645.8037	E	505.2140	253.1107	489.1953	245.1013	490.2031	245.6052	4
12	1405.6270	703.3172	D	376.1714	188.5894	360.1527	180.5800	361.1605	181.0839	3
13	1518.7111	759.8592	L	261.1445	131.0759	245.1258	123.0665	246.1336	123.5704	2
14			E	148.0604	74.5339	132.0417	66.5245	133.0495	67.0284	1

KVNQIGSVTESLQAC

ENO1 Isoform alpha-enolase of Alpha-enolase
 1632.753722 from(817.384137,2+)
 Fraction 26



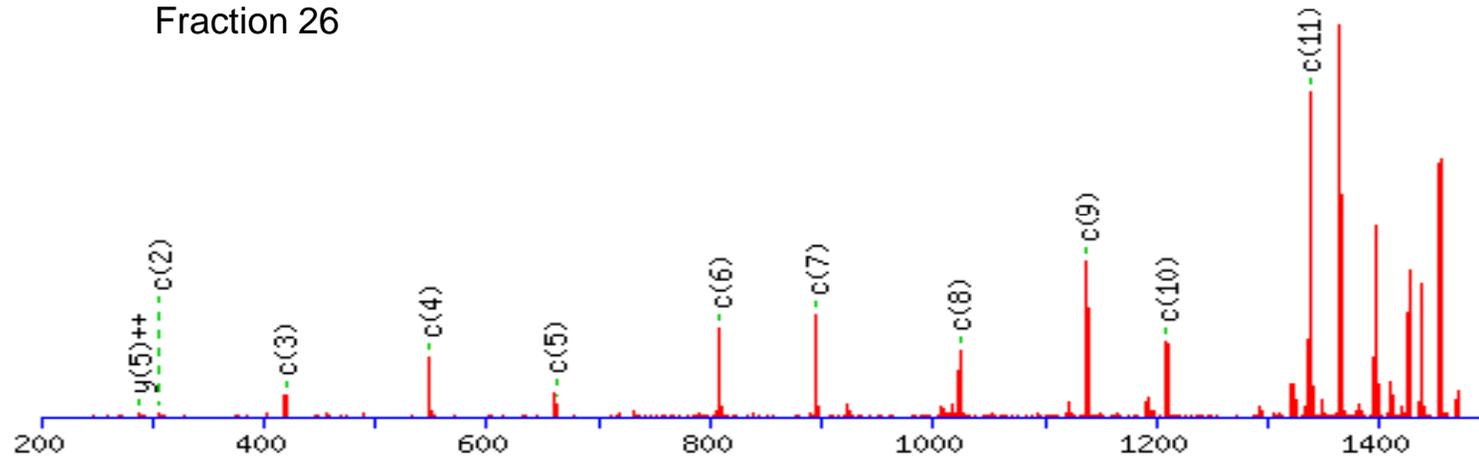
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3	359.2401	180.1237	N	1406.6580	703.8326	1390.6393	695.8233	1391.6471	696.3272	13
4	487.2987	244.1530	Q	1292.6151	646.8112	1276.5964	638.8018	1277.6042	639.3057	12
5	600.3828	300.6950	I	1164.5565	582.7819	1148.5378	574.7725	1149.5456	575.2764	11
6	657.4042	329.2058	G	1051.4725	526.2399	1035.4537	518.2305	1036.4616	518.7344	10
7	744.4363	372.7218	S	994.4510	497.7291	978.4323	489.7198	979.4401	490.2237	9
8	843.5047	422.2560	V	907.4190	454.2131	891.4002	446.2038	892.4081	446.7077	8
9	944.5524	472.7798	T	808.3505	404.6789	792.3318	396.6695	793.3396	397.1735	7
10	1073.5949	537.3011	E	707.3029	354.1551	691.2841	346.1457	692.2920	346.6496	6
11	1160.6270	580.8171	S	578.2603	289.6338	562.2416	281.6244	563.2494	282.1283	5
12	1273.7110	637.3592	L	491.2282	246.1178	475.2095	238.1084	476.2173	238.6123	4
13	1401.7696	701.3884	Q	378.1442	189.5757	362.1255	181.5664	363.1333	182.0703	3
14	1472.8067	736.9070	A	250.0856	125.5464	234.0669	117.5371	235.0747	118.0410	2
15			C	179.0485	90.0279	163.0298	82.0185	164.0376	82.5224	1

KCLELFSELAED

HSP90AB1 85 kDa protein

1452.565724 from(727.290138,2+)

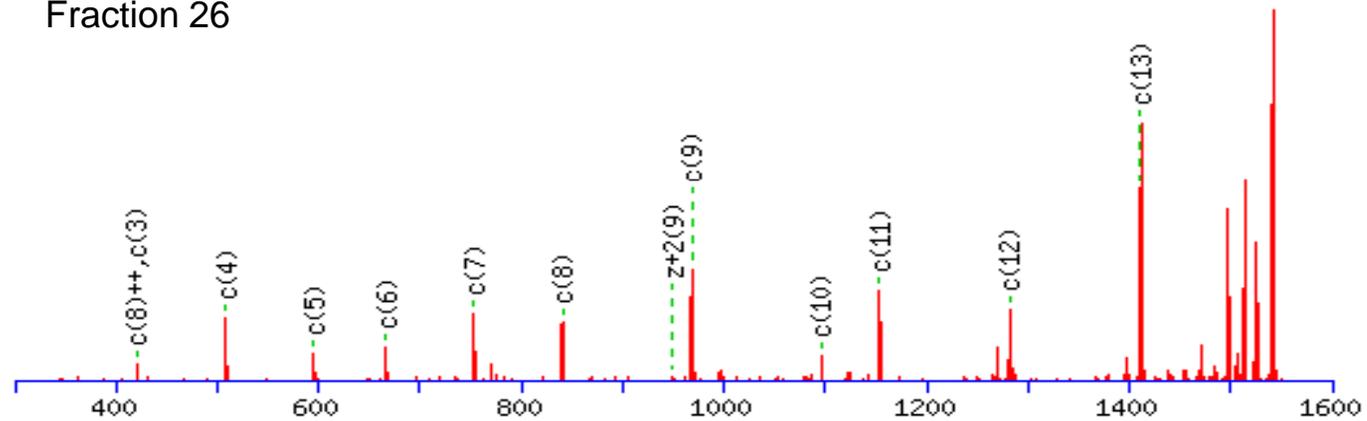
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2	306.1594	153.5834	C	1325.5930	663.3001	1309.5742	655.2908	1310.5821	655.7947	11
3	419.2435	210.1254	L	1165.5623	583.2848	1149.5436	575.2754	1150.5514	575.7793	10
4	548.2861	274.6467	E	1052.4782	526.7428	1036.4595	518.7334	1037.4673	519.2373	9
5	661.3702	331.1887	L	923.4357	462.2215	907.4169	454.2121	908.4248	454.7160	8
6	808.4386	404.7229	F	810.3516	405.6794	794.3329	397.6701	795.3407	398.1740	7
7	895.4706	448.2389	S	663.2832	332.1452	647.2645	324.1359	648.2723	324.6398	6
8	1024.5132	512.7602	E	576.2511	288.6292	560.2324	280.6198	561.2402	281.1238	5
9	1137.5973	569.3023	L	447.2086	224.1079	431.1898	216.0986	432.1977	216.6025	4
10	1208.6344	604.8208	A	334.1245	167.5659	318.1058	159.5565	319.1136	160.0604	3
11	1337.6770	669.3421	E	263.0874	132.0473	247.0687	124.0380	248.0765	124.5419	2
12			D	134.0448	67.5260	118.0261	59.5167	119.0339	60.0206	1

KQFSSASSQQGQEE

EIF2B3 Eukaryotic translation initiation factor 2B,
 subunit 3 gamma, 58kDa
 1539.599722 from(770.807137,2+)
 Fraction 26



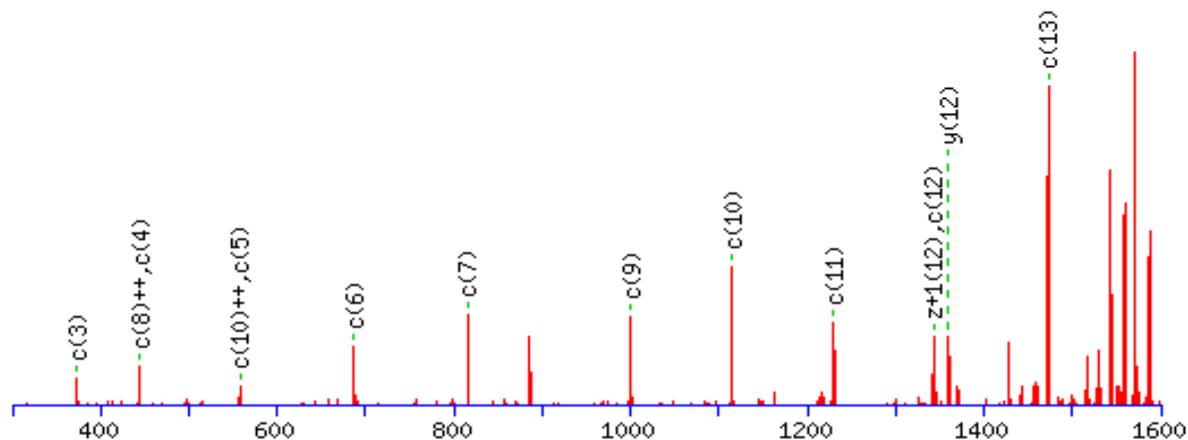
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3	421.2558	211.1315	F	1284.5339	642.7706	1268.5151	634.7612	1269.5230	635.2651	12
4	508.2878	254.6475	S	1137.4655	569.2364	1121.4467	561.2270	1122.4546	561.7309	11
5	595.3198	298.1636	S	1050.4334	525.7203	1034.4147	517.7110	1035.4225	518.2149	10
6	666.3569	333.6821	A	963.4014	482.2043	947.3827	474.1950	948.3905	474.6989	9
7	753.3890	377.1981	S	892.3643	446.6858	876.3456	438.6764	877.3534	439.1803	8
8	840.4210	420.7141	S	805.3323	403.1698	789.3135	395.1604	790.3214	395.6643	7
9	968.4796	484.7434	Q	718.3002	359.6538	702.2815	351.6444	703.2893	352.1483	6
10	1096.5382	548.7727	Q	590.2416	295.6245	574.2229	287.6151	575.2307	288.1190	5
11	1153.5596	577.2835	G	462.1831	231.5952	446.1643	223.5858	447.1722	224.0897	4
12	1281.6182	641.3127	Q	405.1616	203.0844	389.1429	195.0751	390.1507	195.5790	3
13	1410.6608	705.8340	E	277.1030	139.0552	261.0843	131.0458	262.0921	131.5497	2
14			E	148.0604	74.5339	132.0417	66.5245	133.0495	67.0284	1

KVQALEEANNDLEN

KRT9 Keratin, type I cytoskeletal 9

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Fraction 26



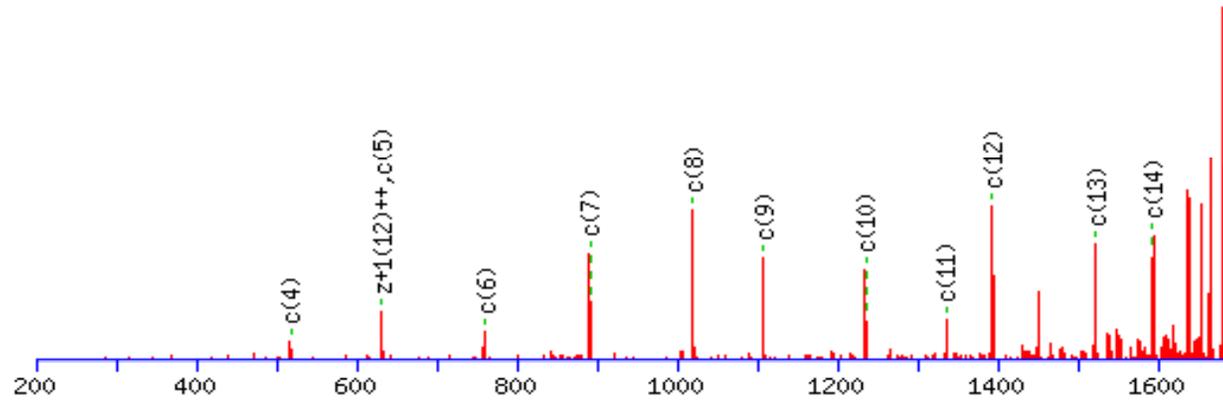
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2	245.1972	123.1022	V	1458.6707	729.8390	1442.6520	721.8296	1443.6598	722.3335	13
3	373.2558	187.1315	Q	1359.6023	680.3048	1343.5836	672.2954	1344.5914	672.7993	12
4	444.2929	222.6501	A	1231.5437	616.2755	1215.5250	608.2661	1216.5328	608.7700	11
5	557.3770	279.1921	L	1160.5066	580.7569	1144.4879	572.7476	1145.4957	573.2515	10
6	686.4196	343.7134	E	1047.4225	524.2149	1031.4038	516.2055	1032.4116	516.7094	9
7	815.4621	408.2347	E	918.3799	459.6936	902.3612	451.6842	903.3690	452.1882	8
8	886.4993	443.7533	A	789.3373	395.1723	773.3186	387.1629	774.3264	387.6669	7
9	1000.5422	500.7747	N	718.3002	359.6537	702.2815	351.6444	703.2893	352.1483	6
10	1114.5851	557.7962	N	604.2573	302.6323	588.2386	294.6229	589.2464	295.1268	5
11	1229.6121	615.3097	D	490.2144	245.6108	474.1956	237.6015	475.2035	238.1054	4
12	1342.6961	671.8517	L	375.1874	188.0974	359.1687	180.0880	360.1765	180.5919	3
13	1471.7387	736.3730	E	262.1034	131.5553	246.0846	123.5460	247.0925	124.0499	2
14			N	133.0608	67.0340	117.0420	59.0247	118.0499	59.5286	1

KSWVNQMESQTGEAS

IQGAP1 Ras GTPase-activating-like protein IQGAP1

1680.594724 from(841.304638,2+)

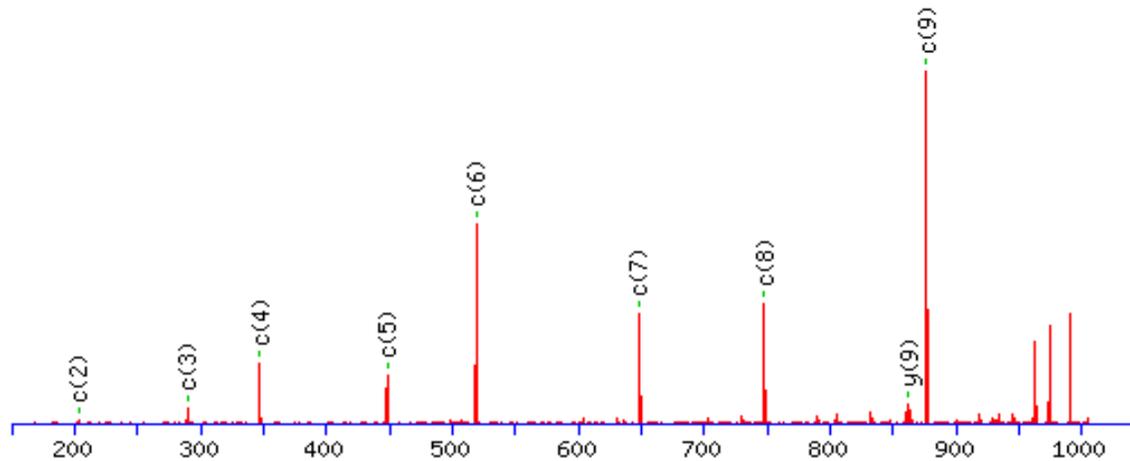
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2	233.1608	117.0840	S	1553.6537	777.3305	1537.6349	769.3211	1538.6428	769.8250	14
3	419.2401	210.1237	W	1466.6216	733.8145	1450.6029	725.8051	1451.6107	726.3090	13
4	518.3085	259.6579	V	1280.5423	640.7748	1264.5236	632.7654	1265.5314	633.2693	12
5	632.3515	316.6794	N	1181.4739	591.2406	1165.4552	583.2312	1166.4630	583.7351	11
6	760.4100	380.7087	Q	1067.4310	534.2191	1051.4123	526.2098	1052.4201	526.7137	10
7	891.4505	446.2289	M	939.3724	470.1898	923.3537	462.1805	924.3615	462.6844	9
8	1020.4931	510.7502	E	808.3319	404.6696	792.3132	396.6602	793.3210	397.1641	8
9	1107.5252	554.2662	S	679.2893	340.1483	663.2706	332.1389	664.2784	332.6429	7
10	1235.5837	618.2955	Q	592.2573	296.6323	576.2386	288.6229	577.2464	289.1268	6
11	1336.6314	668.8193	T	464.1987	232.6030	448.1800	224.5936	449.1878	225.0975	5
12	1393.6529	697.3301	G	363.1510	182.0792	347.1323	174.0698	348.1401	174.5737	4
13	1522.6955	761.8514	E	306.1296	153.5684	290.1109	145.5591	291.1187	146.0630	3
14	1593.7326	797.3699	A	177.0870	89.0471	161.0683	81.0378	162.0761	81.5417	2
15			S	106.0499	53.5286	90.0311	45.5192	91.0390	46.0231	1

KGSGTAEVEL

PKM2 Isoform M1 of Pyruvate kinase isozymes M1/M2
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 Fraction 26

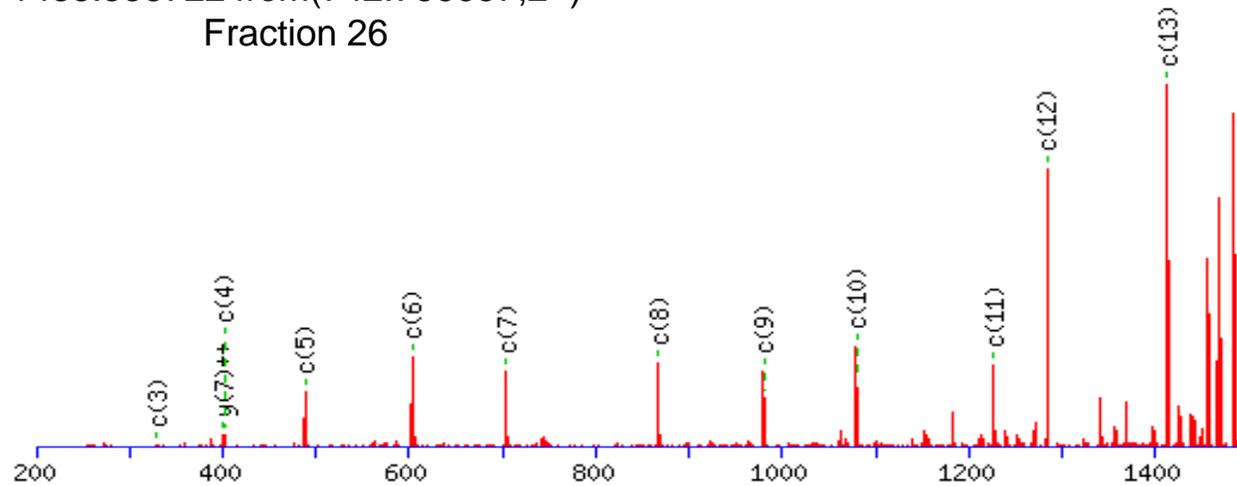


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1	146.1288	73.5680	K							10
2	203.1503	102.0788	G	862.4153	431.7113	846.3965	423.7019	847.4044	424.2058	9
3	290.1823	145.5948	S	805.3938	403.2005	789.3751	395.1912	790.3829	395.6951	8
4	347.2037	174.1055	G	718.3618	359.6845	702.3430	351.6752	703.3509	352.1791	7
5	448.2514	224.6293	T	661.3403	331.1738	645.3216	323.1644	646.3294	323.6683	6
6	519.2885	260.1479	A	560.2926	280.6499	544.2739	272.6406	545.2817	273.1445	5
7	648.3311	324.6692	E	489.2555	245.1314	473.2368	237.1220	474.2446	237.6259	4
8	747.3995	374.2034	V	360.2129	180.6101	344.1942	172.6007	345.2020	173.1046	3
9	876.4421	438.7247	E	261.1445	131.0759	245.1258	123.0665	246.1336	123.5704	2
10			L	132.1019	66.5546	116.0832	58.5452	117.0910	59.0491	1

KSPASDTYIVFGEA

NACA Nascent polypeptide-associated complex subunit alpha
1483.558722 from(742.786637,2+)

Fraction 26



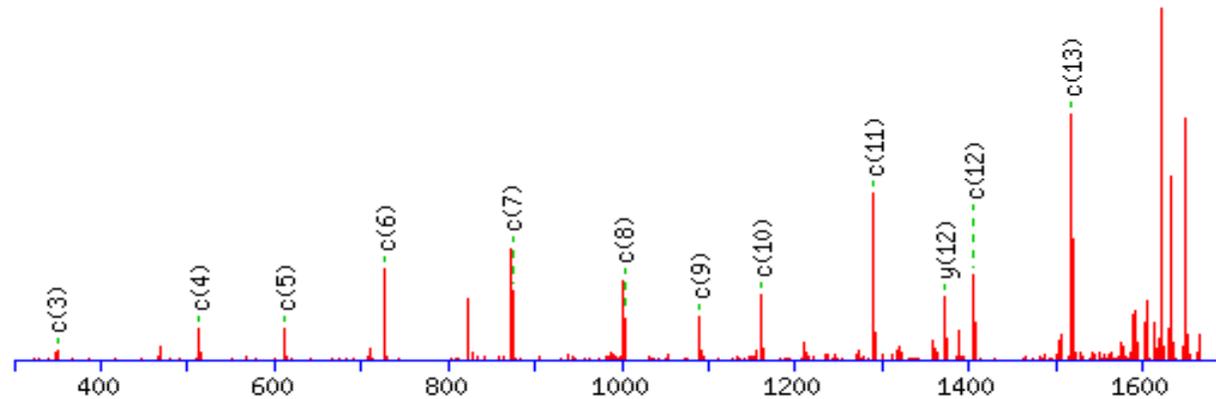
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3	330.2136	165.6104	P	1269.5998	635.3035	1253.5810	627.2942	1254.5889	627.7981	12
4	401.2507	201.1290	A	1172.5470	586.7771	1156.5283	578.7678	1157.5361	579.2717	11
5	488.2827	244.6450	S	1101.5099	551.2586	1085.4912	543.2492	1086.4990	543.7531	10
6	603.3097	302.1585	D	1014.4779	507.7426	998.4591	499.7332	999.4670	500.2371	9
7	704.3573	352.6823	T	899.4509	450.2291	883.4322	442.2197	884.4400	442.7236	8
8	867.4207	434.2140	Y	798.4032	399.7053	782.3845	391.6959	783.3923	392.1998	7
9	980.5047	490.7560	I	635.3399	318.1736	619.3212	310.1642	620.3290	310.6681	6
10	1079.5732	540.2902	V	522.2558	261.6316	506.2371	253.6222	507.2449	254.1261	5
11	1226.6416	613.8244	F	423.1874	212.0974	407.1687	204.0880	408.1765	204.5919	4
12	1283.6630	642.3352	G	276.1190	138.5631	260.1003	130.5538	261.1081	131.0577	3
13	1412.7056	706.8564	E	219.0975	110.0524	203.0788	102.0431	204.0866	102.5470	2
14			A	90.0550	45.5311	74.0362	37.5218	75.0441	38.0257	1

KFGYVDFESAEDLE

NCL Isoform 1 of Nucleolin

1647.547724 from(824.781138,2+)

Fraction 27



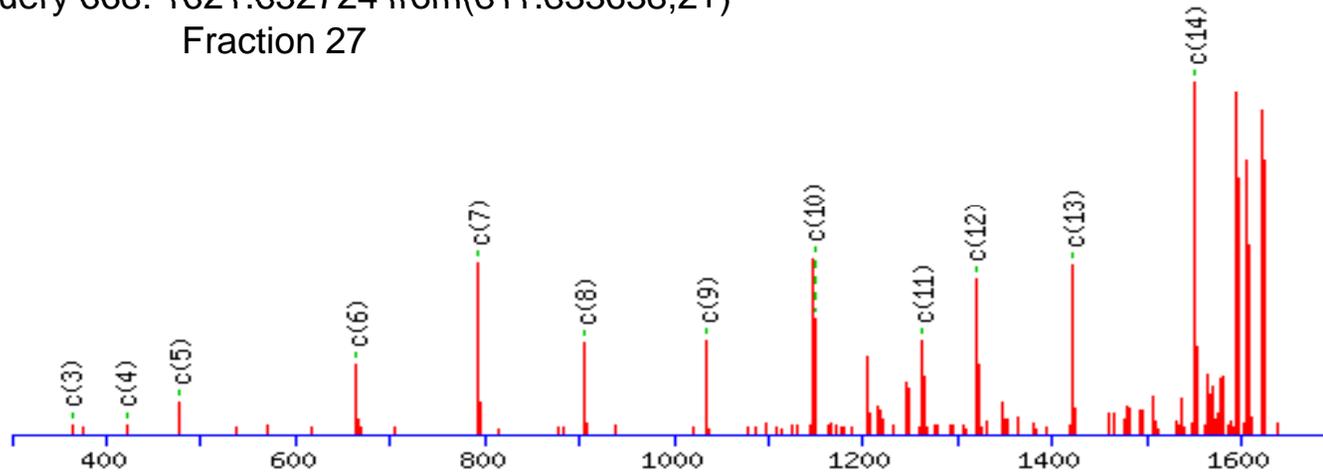
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1	146.1288	73.5680	K							14
2	293.1972	147.1022	F	1520.6427	760.8250	1504.6240	752.8156	1505.6318	753.3196	13
3	350.2187	175.6130	G	1373.5743	687.2908	1357.5556	679.2814	1358.5634	679.7854	12
4	513.2820	257.1446	Y	1316.5529	658.7801	1300.5341	650.7707	1301.5420	651.2746	11
5	612.3504	306.6788	V	1153.4895	577.2484	1137.4708	569.2390	1138.4786	569.7430	10
6	727.3774	364.1923	D	1054.4211	527.7142	1038.4024	519.7048	1039.4102	520.2088	9
7	874.4458	437.7265	F	939.3942	470.2007	923.3755	462.1914	924.3833	462.6953	8
8	1003.4884	502.2478	E	792.3258	396.6665	776.3070	388.6572	777.3149	389.1611	7
9	1090.5204	545.7638	S	663.2832	332.1452	647.2645	324.1359	648.2723	324.6398	6
10	1161.5575	581.2824	A	576.2511	288.6292	560.2324	280.6199	561.2402	281.1238	5
11	1290.6001	645.8037	E	505.2140	253.1107	489.1953	245.1013	490.2031	245.6052	4
12	1405.6270	703.3172	D	376.1714	188.5894	360.1527	180.5800	361.1605	181.0839	3
13	1518.7111	759.8592	L	261.1445	131.0759	245.1258	123.0665	246.1336	123.5704	2
14			E	148.0604	74.5339	132.0417	66.5245	133.0495	67.0284	1

KMSGGWELELNGTEA

C1QBP Complement component 1 Q subcomponent-binding protein,
mitochondrial precursor

Match to Query 668: 1621.652724 from(811.833638,2+)

Fraction 27



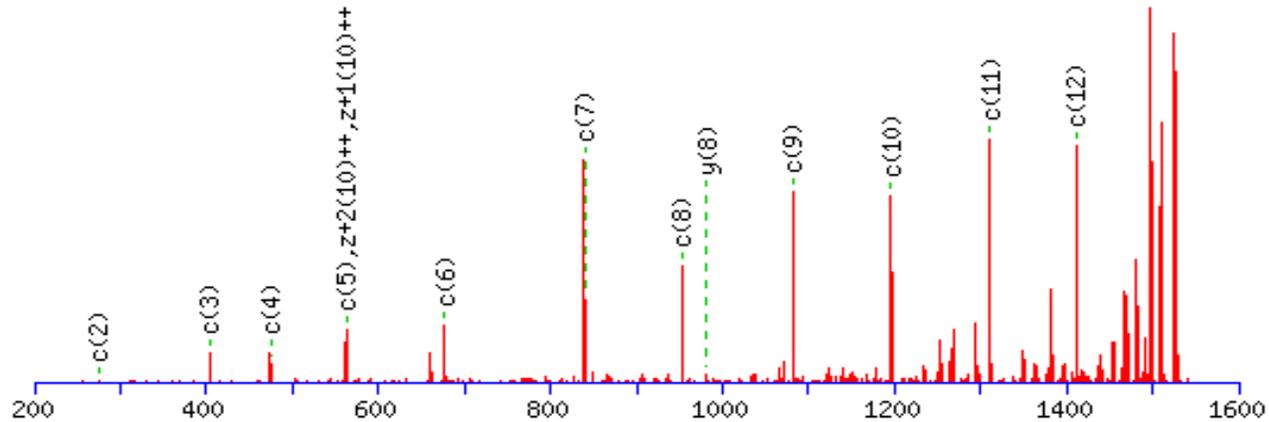
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1	146.1288	73.5680	K							15
2	277.1693	139.0883	M	1493.6577	747.3325	1477.6390	739.3231	1478.6468	739.8270	14
3	364.2013	182.6043	S	1362.6172	681.8122	1346.5985	673.8029	1347.6063	674.3068	13
4	421.2228	211.1150	G	1275.5852	638.2962	1259.5664	630.2869	1260.5743	630.7908	12
5	478.2442	239.6258	G	1218.5637	609.7855	1202.5450	601.7761	1203.5528	602.2800	11
6	664.3235	332.6654	W	1161.5422	581.2748	1145.5235	573.2654	1146.5313	573.7693	10
7	793.3661	397.1867	E	975.4629	488.2351	959.4442	480.2257	960.4520	480.7297	9
8	906.4502	453.7287	L	846.4203	423.7138	830.4016	415.7044	831.4094	416.2084	8
9	1035.4928	518.2500	E	733.3363	367.1718	717.3176	359.1624	718.3254	359.6663	7
10	1148.5769	574.7921	L	604.2937	302.6505	588.2750	294.6411	589.2828	295.1450	6
11	1262.6198	631.8135	N	491.2096	246.1084	475.1909	238.0991	476.1987	238.6030	5
12	1319.6412	660.3243	G	377.1667	189.0870	361.1480	181.0776	362.1558	181.5815	4
13	1420.6889	710.8481	T	320.1452	160.5763	304.1265	152.5669	305.1343	153.0708	3
14	1549.7315	775.3694	E	219.0975	110.0524	203.0788	102.0431	204.0866	102.5470	2
15			A	90.0550	45.5311	74.0362	37.5218	75.0441	38.0257	1

KEEASDYLELDTI

HSP90B1 Endoplasmin precursor

Match to Query 621: 1524.516724 from(763.265638,2+)

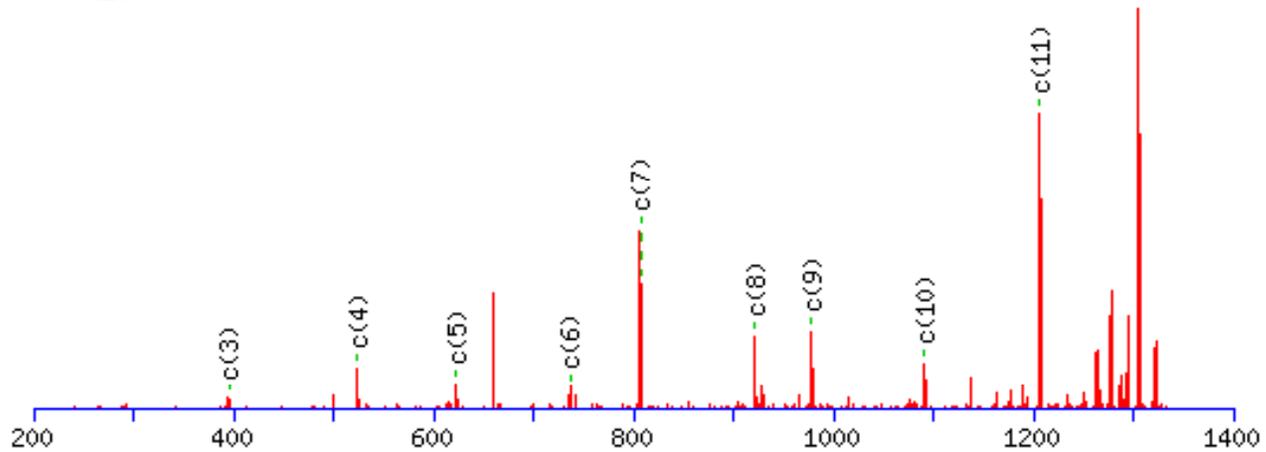
Fraction 27



#	c	c ⁺⁺	Seq.	y	y ⁺⁺	z+1	z+1 ⁺⁺	z+2	z+2 ⁺⁺	#
1	146.1288	73.5680	K							13
2	275.1714	138.0893	E	1397.6318	699.3196	1381.6131	691.3102	1382.6209	691.8141	12
3	404.2140	202.6106	E	1268.5893	634.7983	1252.5705	626.7889	1253.5784	627.2928	11
4	475.2511	238.1292	A	1139.5467	570.2770	1123.5279	562.2676	1124.5358	562.7715	10
5	562.2831	281.6452	S	1068.5095	534.7584	1052.4908	526.7491	1053.4986	527.2530	9
6	677.3101	339.1587	D	981.4775	491.2424	965.4588	483.2330	966.4666	483.7369	8
7	840.3734	420.6903	Y	866.4506	433.7289	850.4319	425.7196	851.4397	426.2235	7
8	953.4575	477.2324	L	703.3872	352.1973	687.3685	344.1879	688.3763	344.6918	6
9	1082.5000	541.7537	E	590.3032	295.6552	574.2845	287.6459	575.2923	288.1498	5
10	1195.5841	598.2957	L	461.2606	231.1339	445.2419	223.1246	446.2497	223.6285	4
11	1310.6111	655.8092	D	348.1765	174.5919	332.1578	166.5825	333.1656	167.0865	3
12	1411.6587	706.3330	T	233.1496	117.0784	217.1309	109.0691	218.1387	109.5730	2
13			I	132.1019	66.5546	116.0832	58.5452	117.0910	59.0491	1

KYSQVLANGLDN

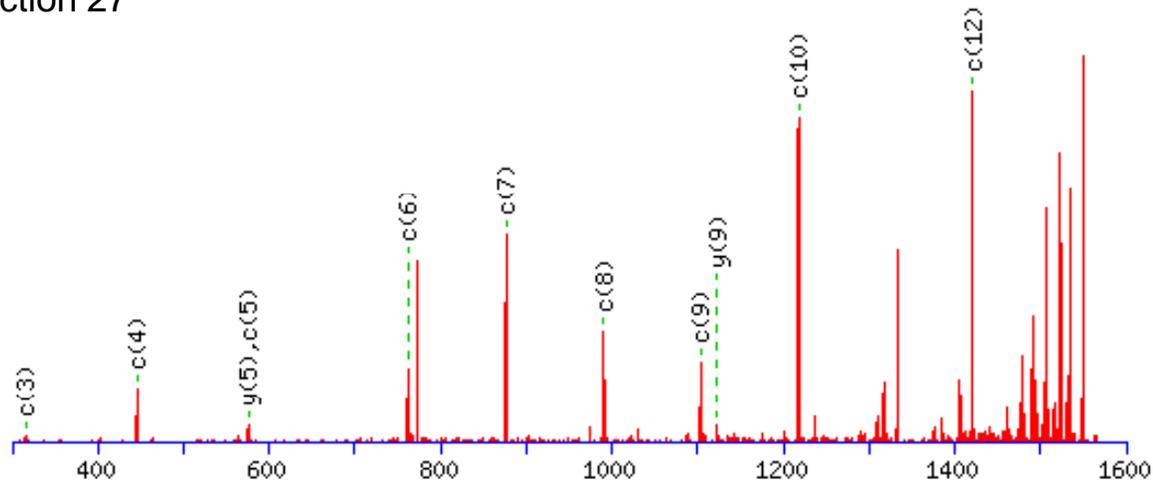
RPS18 40S ribosomal protein S18
 1321.263722 from(661.639137,2+)
 Fraction 27



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1	146.1288	73.5680	K							12
2	309.1921	155.0997	Y	1193.5797	597.2935	1177.5610	589.2841	1178.5688	589.7880	11
3	396.2241	198.6157	S	1030.5164	515.7618	1014.4976	507.7525	1015.5055	508.2564	10
4	524.2827	262.6450	Q	943.4843	472.2458	927.4656	464.2364	928.4734	464.7404	9
5	623.3511	312.1792	V	815.4258	408.2165	799.4070	400.2072	800.4149	400.7111	8
6	736.4352	368.7212	L	716.3573	358.6823	700.3386	350.6729	701.3464	351.1769	7
7	807.4723	404.2398	A	603.2733	302.1403	587.2546	294.1309	588.2624	294.6348	6
8	921.5152	461.2613	N	532.2362	266.6217	516.2174	258.6124	517.2253	259.1163	5
9	978.5367	489.7720	G	418.1932	209.6003	402.1745	201.5909	403.1823	202.0948	4
10	1091.6208	546.3140	L	361.1718	181.0895	345.1531	173.0802	346.1609	173.5841	3
11	1206.6477	603.8275	D	248.0877	124.5475	232.0690	116.5381	233.0768	117.0420	2
12			N	133.0608	67.0340	117.0420	59.0247	118.0499	59.5286	1

KLGEMWNNLNDSE

HMG4L High-mobility group protein 4-like
 1548.535724 from (775.275138,2+)
 Fraction 27



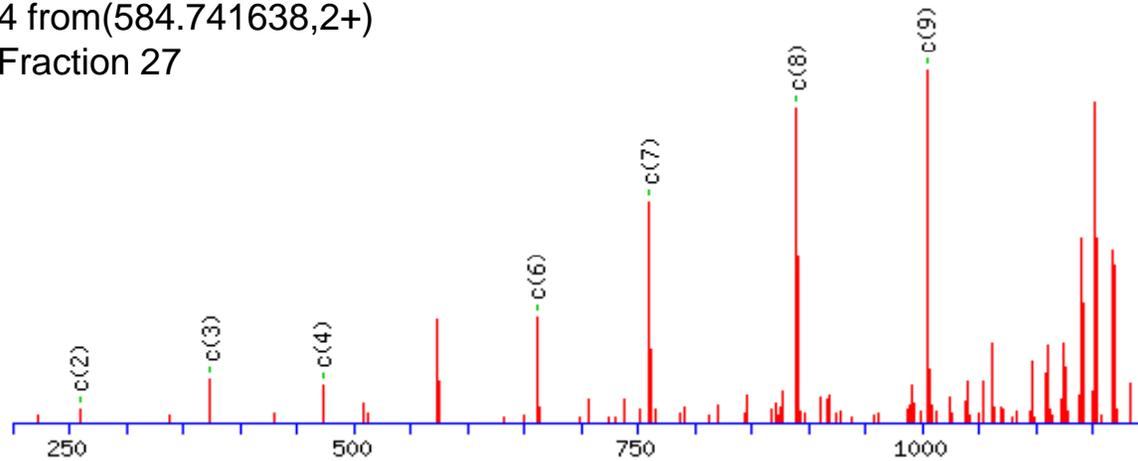
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1	146.1288	73.5680	K							13
2	259.2129	130.1101	L	1421.6002	711.3037	1405.5814	703.2944	1406.5893	703.7983	12
3	316.2343	158.6208	G	1308.5161	654.7617	1292.4974	646.7523	1293.5052	647.2562	11
4	445.2769	223.1421	E	1251.4946	626.2510	1235.4759	618.2416	1236.4837	618.7455	10
5	576.3174	288.6623	M	1122.4520	561.7297	1106.4333	553.7203	1107.4411	554.2242	9
6	762.3967	381.7020	W	991.4116	496.2094	975.3928	488.2001	976.4007	488.7040	8
7	876.4396	438.7235	N	805.3323	403.1698	789.3135	395.1604	790.3214	395.6643	7
8	990.4826	495.7449	N	691.2893	346.1483	675.2706	338.1389	676.2784	338.6429	6
9	1103.5666	552.2870	L	577.2464	289.1268	561.2277	281.1175	562.2355	281.6214	5
10	1217.6096	609.3084	N	464.1623	232.5848	448.1436	224.5754	449.1514	225.0794	4
11	1332.6365	666.8219	D	350.1194	175.5633	334.1007	167.5540	335.1085	168.0579	3
12	1419.6685	710.3379	S	235.0925	118.0499	219.0737	110.0405	220.0816	110.5444	2
13			E	148.0604	74.5339	132.0417	66.5245	133.0495	67.0284	1

KLDVTSVEDY

CCT5 T-complex protein 1 subunit epsilon

1167.468724 from(584.741638,2+)

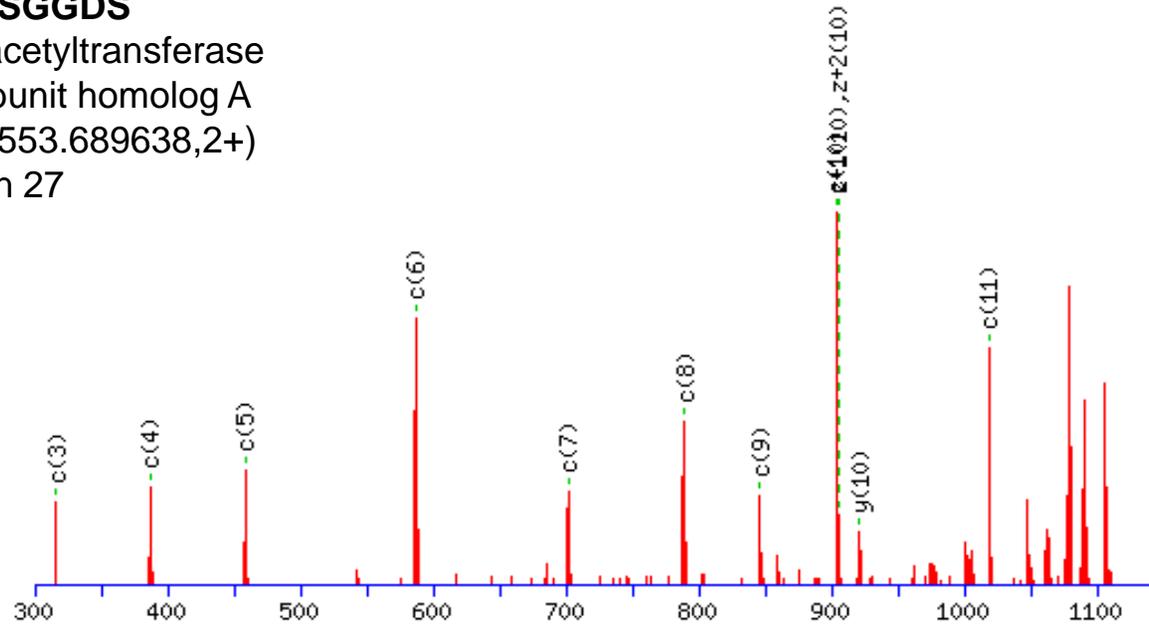
Fraction 27



#	c	c ⁺⁺	Seq.	y	y ⁺⁺	z+1	z+1 ⁺⁺	z+2	z+2 ⁺⁺	#
1	146.1288	73.5680	K							10
2	259.2129	130.1101	L	1040.4782	520.7428	1024.4595	512.7334	1025.4673	513.2373	9
3	374.2398	187.6235	D	927.3942	464.2007	911.3755	456.1914	912.3833	456.6953	8
4	473.3082	237.1577	V	812.3672	406.6873	796.3485	398.6779	797.3563	399.1818	7
5	574.3559	287.6816	T	713.2988	357.1531	697.2801	349.1437	698.2879	349.6476	6
6	661.3879	331.1976	S	612.2511	306.6292	596.2324	298.6199	597.2402	299.1238	5
7	760.4563	380.7318	V	525.2191	263.1132	509.2004	255.1038	510.2082	255.6077	4
8	889.4989	445.2531	E	426.1507	213.5790	410.1320	205.5696	411.1398	206.0735	3
9	1004.5259	502.7666	D	297.1081	149.0577	281.0894	141.0483	282.0972	141.5522	2
10			Y	182.0812	91.5442	166.0624	83.5349	167.0703	84.0388	1

KGLAAEDSGGDS

ARD1A N-terminal acetyltransferase
 complex ARD1 subunit homolog A
 1105.364724 from(553.689638,2+)
 Fraction 27



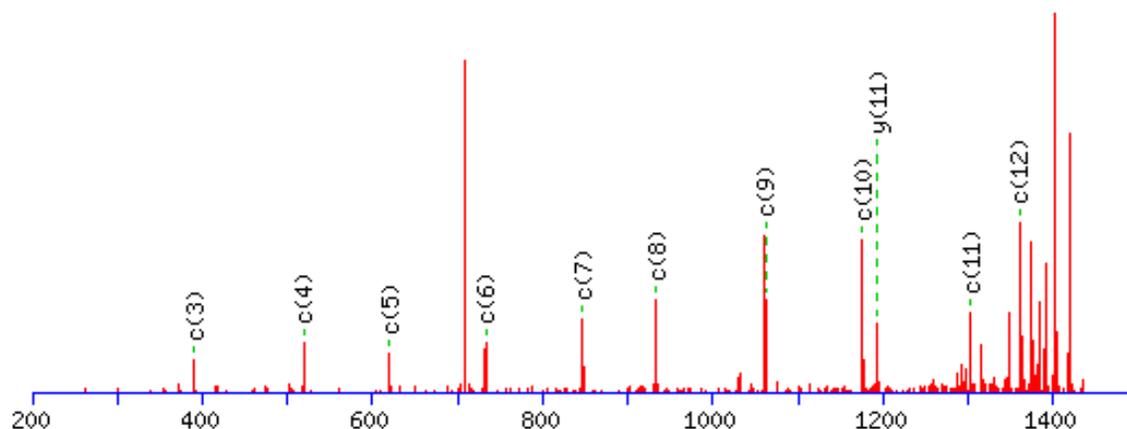
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2	203.1503	102.0788	G	978.4011	489.7042	962.3823	481.6948	963.3902	482.1987	11
3	316.2343	158.6208	L	921.3796	461.1934	905.3609	453.1841	906.3687	453.6880	10
4	387.2714	194.1394	A	808.2955	404.6514	792.2768	396.6420	793.2846	397.1460	9
5	458.3085	229.6579	A	737.2584	369.1328	721.2397	361.1235	722.2475	361.6274	8
6	587.3511	294.1792	E	666.2213	333.6143	650.2026	325.6049	651.2104	326.1088	7
7	702.3781	351.6927	D	537.1787	269.0930	521.1600	261.0836	522.1678	261.5875	6
8	789.4101	395.2087	S	422.1518	211.5795	406.1330	203.5702	407.1409	204.0741	5
9	846.4316	423.7194	G	335.1197	168.0635	319.1010	160.0541	320.1088	160.5581	4
10	903.4530	452.2302	G	278.0983	139.5528	262.0796	131.5434	263.0874	132.0473	3
11	1018.4800	509.7436	D	221.0768	111.0420	205.0581	103.0327	206.0659	103.5366	2
12			S	106.0499	53.5286	90.0311	45.5192	91.0390	46.0231	1

KPFETLLSQNQQG

GSTP1 Glutathione S-transferase P

1418.905722 from(710.460137,2+)

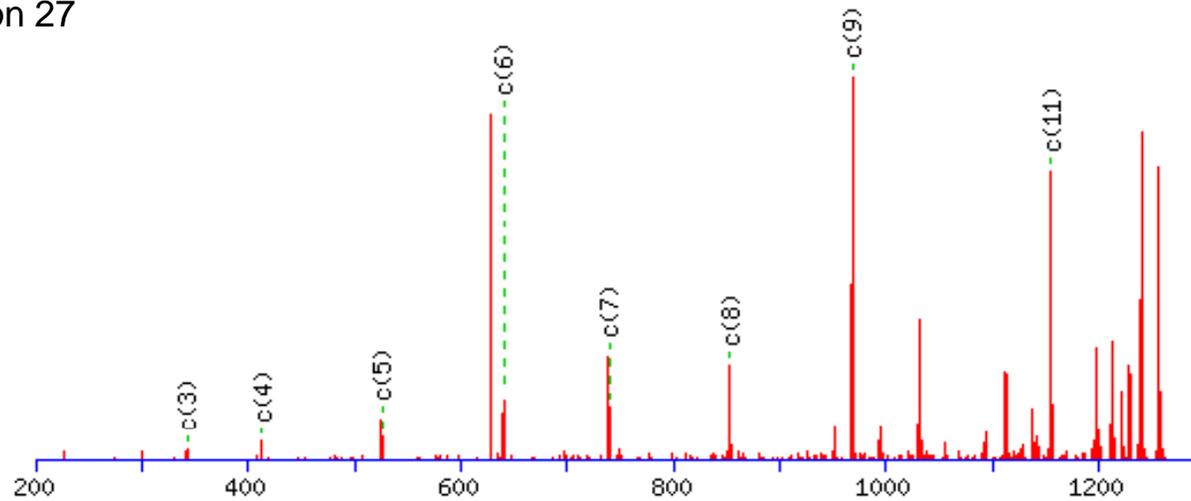
Fraction 27



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1	146.1288	73.5680	K							13
2	243.1816	122.0944	P	1290.6325	645.8199	1274.6137	637.8105	1275.6216	638.3144	12
3	390.2500	195.6286	F	1193.5797	597.2935	1177.5610	589.2841	1178.5688	589.7880	11
4	519.2926	260.1499	E	1046.5113	523.7593	1030.4926	515.7499	1031.5004	516.2538	10
5	620.3402	310.6738	T	917.4687	459.2380	901.4500	451.2286	902.4578	451.7325	9
6	733.4243	367.2158	L	816.4210	408.7141	800.4023	400.7048	801.4101	401.2087	8
7	846.5084	423.7578	L	703.3369	352.1721	687.3182	344.1627	688.3260	344.6667	7
8	933.5404	467.2738	S	590.2529	295.6301	574.2342	287.6207	575.2420	288.1246	6
9	1061.5990	531.3031	Q	503.2209	252.1141	487.2021	244.1047	488.2100	244.6086	5
10	1175.6419	588.3246	N	375.1623	188.0848	359.1435	180.0754	360.1514	180.5793	4
11	1303.7005	652.3539	Q	261.1193	131.0633	245.1006	123.0539	246.1084	123.5579	3
12	1360.7219	680.8646	G	133.0608	67.0340	117.0420	59.0247	118.0499	59.5286	2
13			G	76.0393	38.5233	60.0206	30.5139	61.0284	31.0178	1

KVPAINVNSVT

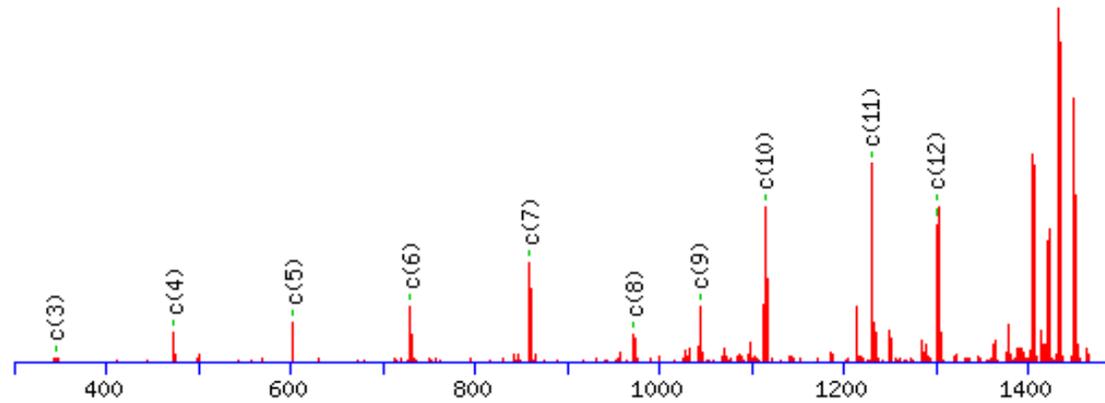
AHCY Adenosylhomocysteinase
 1255.515724 from (628.765138,2+)
 Fraction 27



#	c	c ⁺⁺	Seq.	y	y ⁺⁺	z+1	z+1 ⁺⁺	z+2	z+2 ⁺⁺	#
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2	245.1972	123.1022	V	1128.5895	564.7984	1112.5708	556.7890	1113.5786	557.2930	11
3	342.2500	171.6286	P	1029.5211	515.2642	1013.5024	507.2548	1014.5102	507.7587	10
4	413.2871	207.1472	A	932.4684	466.7378	916.4496	458.7285	917.4575	459.2324	9
5	526.3711	263.6892	I	861.4312	431.2193	845.4125	423.2099	846.4203	423.7138	8
6	640.4141	320.7107	N	748.3472	374.6772	732.3284	366.6679	733.3363	367.1718	7
7	739.4825	370.2449	V	634.3042	317.6558	618.2855	309.6464	619.2933	310.1503	6
8	853.5254	427.2663	N	535.2358	268.1216	519.2171	260.1122	520.2249	260.6161	5
9	968.5524	484.7798	D	421.1929	211.1001	405.1742	203.0907	406.1820	203.5946	4
10	1055.5844	528.2958	S	306.1660	153.5866	290.1472	145.5773	291.1551	146.0812	3
11	1154.6528	577.8300	V	219.1339	110.0706	203.1152	102.0612	204.1230	102.5652	2
12			T	120.0655	60.5364	104.0468	52.5270	105.0546	53.0309	1

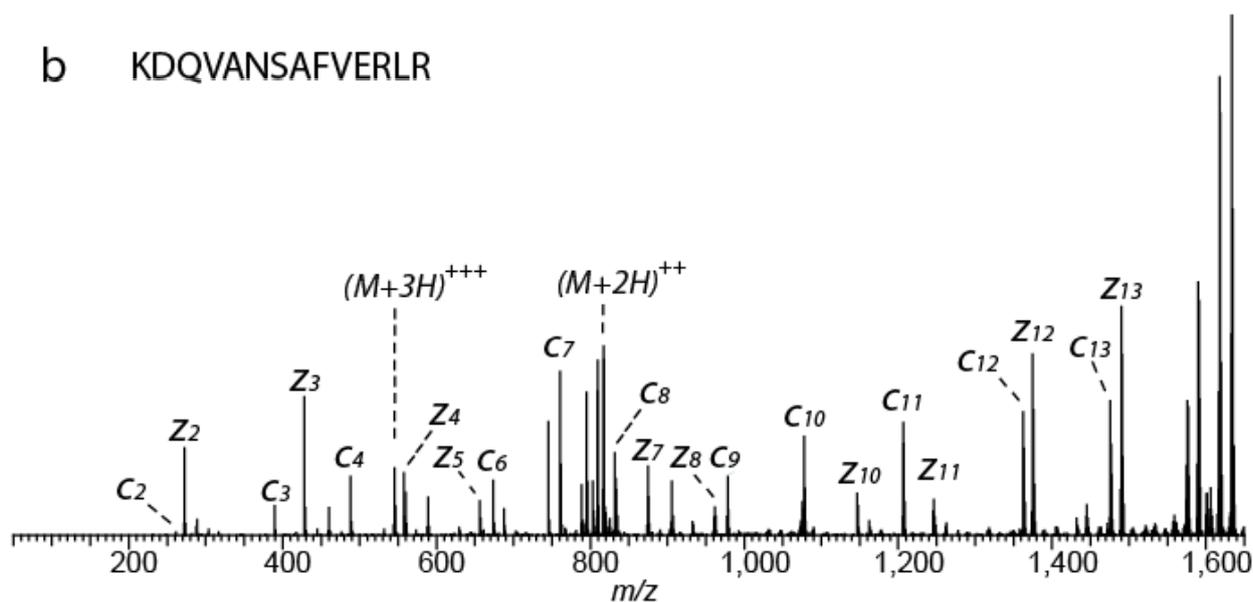
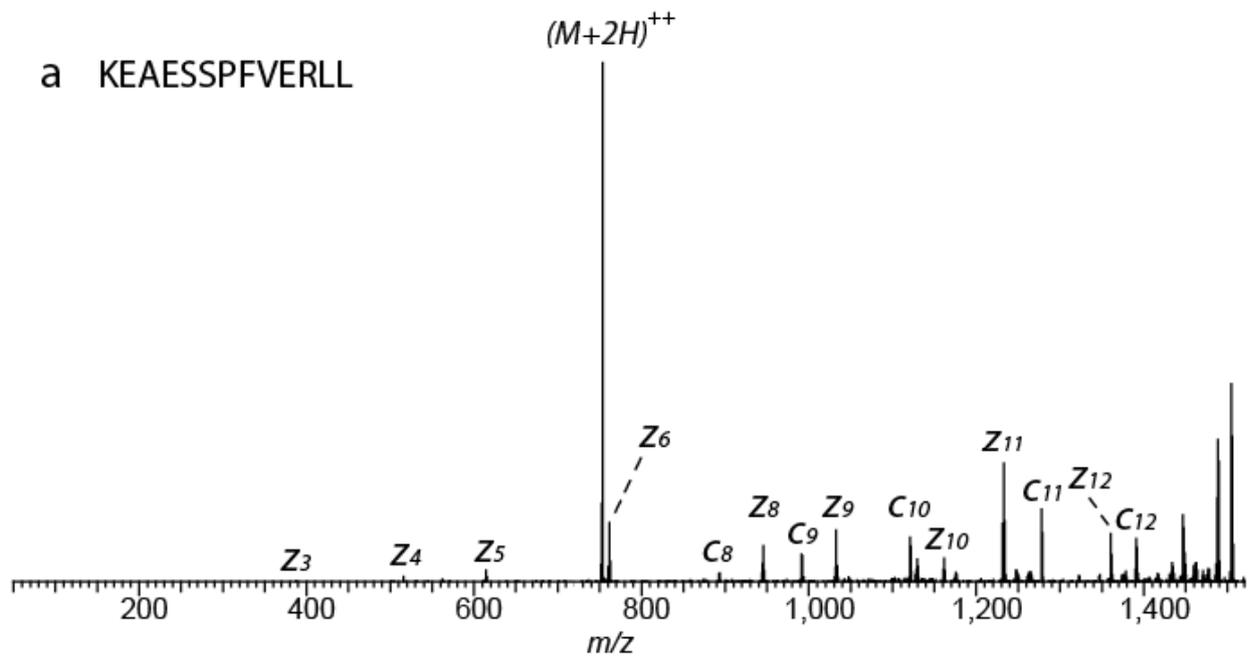
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PRKCSH Glucosidase 2 subunit beta precursor
 1447.506724 from(724.760638,2+)
 Fraction 27



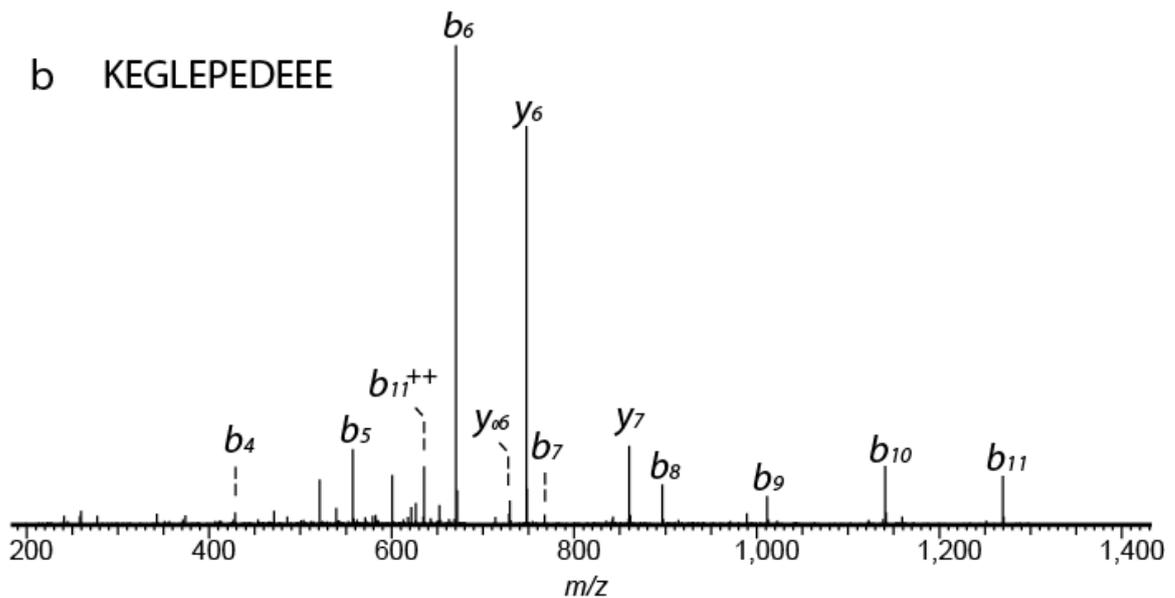
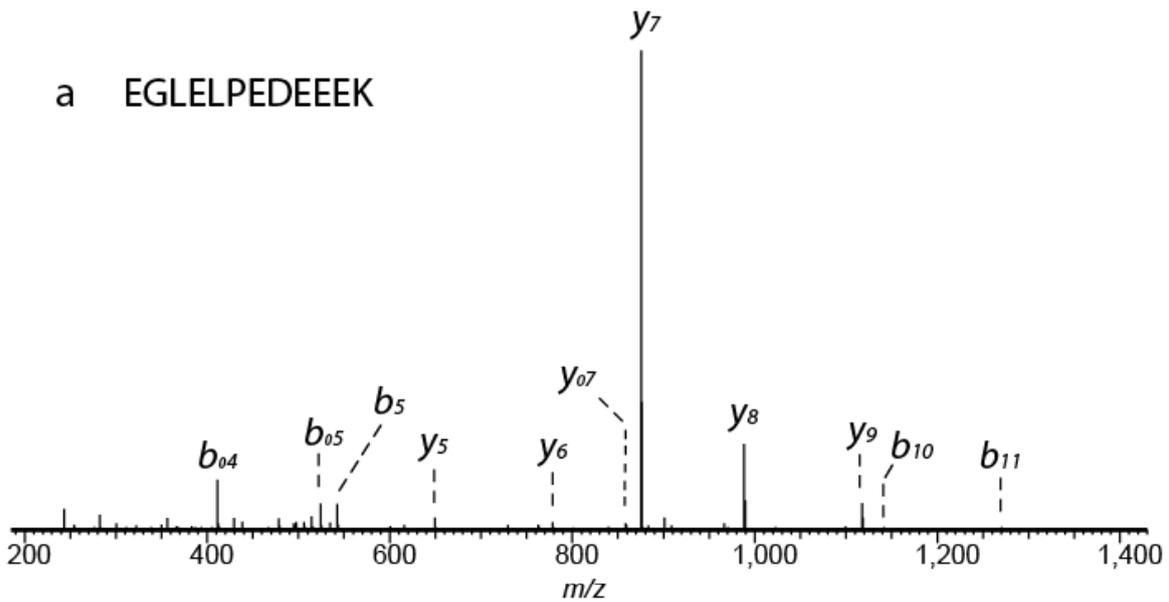
#	c	c ⁺⁺	Seq.	y	y ⁺⁺	z+1	z+1 ⁺⁺	z+2	z+2 ⁺⁺	#
1	146.1288	73.5680	K							13
2	217.1659	109.0866	A	1320.6066	660.8070	1304.5879	652.7976	1305.5957	653.3015	12
3	345.2245	173.1159	Q	1249.5695	625.2884	1233.5508	617.2790	1234.5586	617.7830	11
4	473.2831	237.1452	Q	1121.5109	561.2591	1105.4922	553.2497	1106.5000	553.7537	10
5	602.3257	301.6665	E	993.4524	497.2298	977.4336	489.2205	978.4415	489.7244	9
6	730.3842	365.6958	Q	864.4098	432.7085	848.3911	424.6992	849.3989	425.2031	8
7	859.4268	430.2170	E	736.3512	368.6792	720.3325	360.6699	721.3403	361.1738	7
8	972.5109	486.7591	L	607.3086	304.1579	591.2899	296.1486	592.2977	296.6525	6
9	1043.5480	522.2776	A	494.2245	247.6159	478.2058	239.6065	479.2136	240.1105	5
10	1114.5851	557.7962	A	423.1874	212.0974	407.1687	204.0880	408.1765	204.5919	4
11	1229.6121	615.3097	D	352.1503	176.5788	336.1316	168.5694	337.1394	169.0733	3
12	1300.6492	650.8282	A	237.1234	119.0653	221.1046	111.0560	222.1125	111.5599	2
13			F	166.0863	83.5468	150.0675	75.5374	151.0754	76.0413	1

Supplementary Figure 2: ETcaD peptide ion fragmentation spectra of doubly and triply charged ions originating from Lys-N generated peptides from HEK293 cells. (a) ETcaD mass spectra are shown of Lys-N generated doubly charged peptide ion KEAESSPFVERLL and (b) triply charged peptide ion KDQVANSAFVERLR.



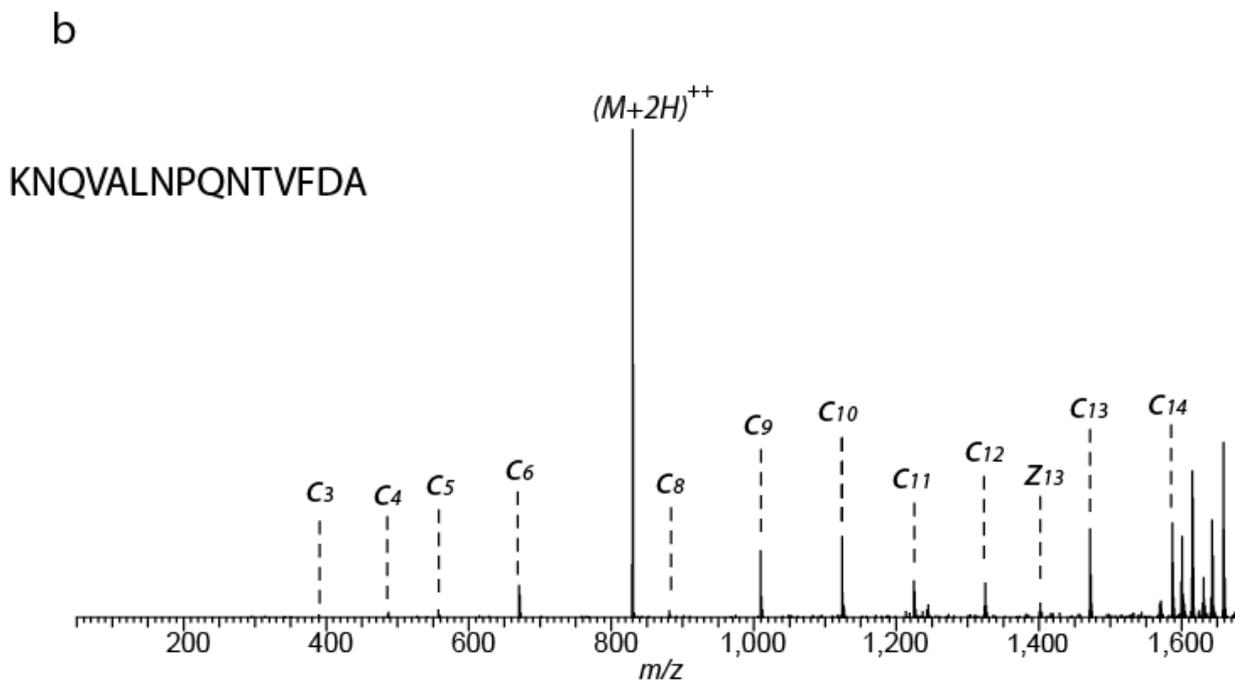
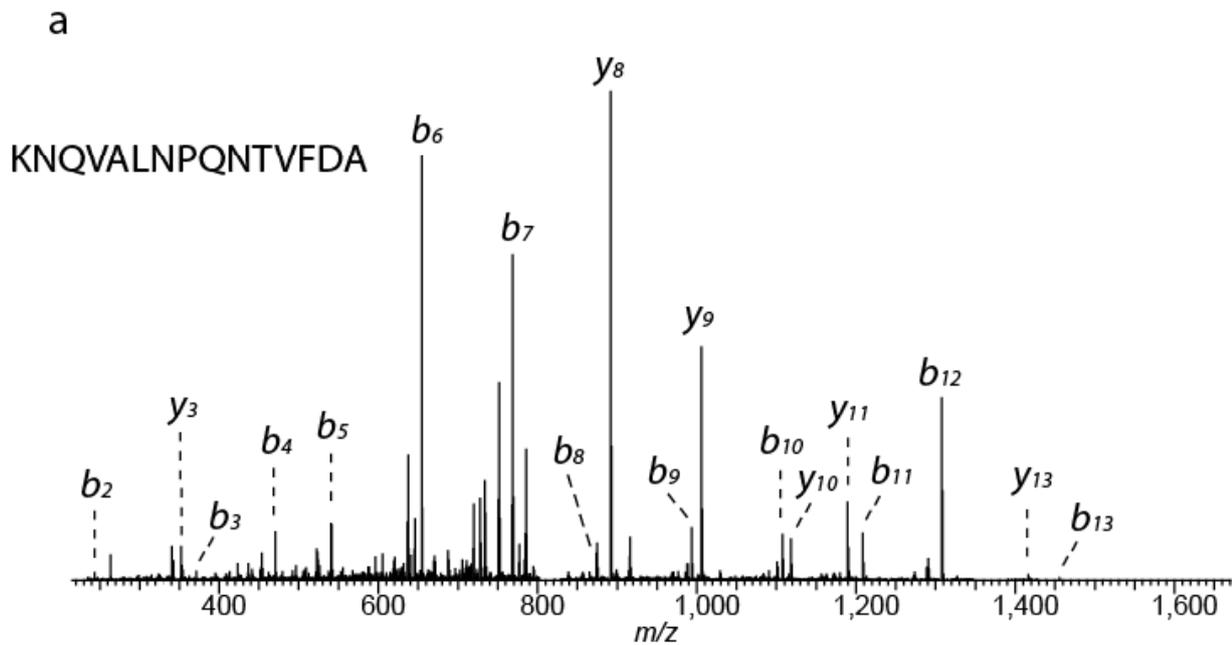
Supplementary Figure 3: Typical CID peptide ion fragmentation spectra of doubly charged ions originating from Lys-C and Lys-N generated peptides from the HEK293 cell lysate.

(a) CID mass spectrum of Lys-C generated doubly charged peptide ion EGLELPEDEEEK; **(b)** CID mass spectrum of Lys-N generated doubly charged peptide ion KEGLELPEDEEE. In **(b)** the peptide ion fragmentation spectrum is dominated by *b* type fragment ions.



Supplementary Figure 4: Typical CID and ETcaD peptide ion fragmentation spectra of a Lys-N generated doubly charged peptide ion.

(a) CID mass spectrum of Lys-N generated KNQVALNPQNTVFDA. (b) ETcaD mass spectrum of Lys-N generated KNQVALNPQNTVFDA. Typically the latter spectra are easier to interpret and provide straightforward sequence ladders.



Supplementary Table 1: A count of c and z ion (and related neutral losses) abundance ratios for doubly charged peptides originating from Lys-C and Lys-N HEK 293 cell lysate digests.

The c and z ion populations are represented in a percentage for each subgroup of peptides for a single nanoLC-MS analysis of Lys-C and Lys-N HEK293 cell lysate digests.

HEK 293 Single nanoLC- MS	Lys-C 2+ ions	Lys-N 2+ ions
Used protease/protein ratio	1:50	1:85
Total unique	87	182
Number of peptides with a single Lysine basic residue	50	93
Frequency of c/z ions	c: 37.76% z: 62.24%	c: 90.37% z: 9.63%
Number of peptides with two Lysine basic residues	14	16
Frequency of c/z ions	c: 42.53% z: 57.47%	c: 60.32% z: 39.68%
Number of peptides with two basic residues (Lysine/Arginine or Lysine/Histidine)	20	69
Frequency of c/z ions	c: 60.14% z: 39.86%	c: 60.96% z: 39.04%

Supplementary Table 2: A count of c and z ion (and related neutral losses) abundance ratios for triply charged peptides originating from Lys-C and Lys-N HEK 293 cell lysate digests.

The c and z ion populations are represented in a percentage for each subgroup of peptides for a single nanoLC-MS analysis of Lys-C and Lys-N HEK293 cell lysate digests.

HEK 293 Single nanoLC- MS	Lys-C 3+ ions	Lys-N 3+ ions
Total unique	74	44
Number of peptides with two Lysine basic residues	9	3
Frequency of c/z ions	c: 50.00% z: 50.00%	c: 71.43% z: 28.57%
Number of peptides with two basic residues (Lysine/Arginine or Lysine/Histidine)	56	35
Frequency of c/z ions	c: 49.64% z: 50.36%	c: 63.14% z: 36.86%

Supplementary Table 3: A count of c and z ion (and related neutral losses) abundance ratios for doubly charged peptides originating from Lys-C and Lys-N HEK 293 cell lysate digests.

The proteolytic peptide population was subjected to an initial separation and isolation of peptides containing a single basic residue by low-pH SCX before analysis by nanoLC-MS. The c and z ion populations are represented in a percentage for each subgroup of peptides detected.

HEK 293 SCX-nanoLC-MS	Lys-N 2+ ions
Used protease/protein ratio	1:85
Total unique	1855
Number of peptides with a single Lysine basic residue	1786
Frequency of c/z ions	c: 90.18% z: 9.82%
Number of peptides with two Lysine basic residues	15
Frequency of c/z ions	c: 53.97% z: 46.03%
Number of peptides with two basic residues (Lysine/Arginine or Lysine/Histidine)	32
Frequency of c/z ions	c: 33.96% z: 66.04%

Supplementary Methods

Materials. Protease inhibitor cocktail and Lys-C were obtained from Roche Diagnostics (Mannheim, Germany). Metalloendopeptidase from *Grifola Frondosa* (Lys-N) was obtained from Seikagaku Corporation (Tokyo, Japan). Bovine Serum Albumin (BSA) and Iodoacetamide were obtained from Sigma-Aldrich (Steinheim, Germany). DL-Dithiothreitol was obtained from Fluka biochemical (Steinheim, Germany). HEK293 cells were a gift from the ABC Protein Expression Center (Utrecht University, The Netherlands). HPLC-S gradient grade acetonitrile was purchased from Biosolve (Valkenwaard, The Netherlands). Acetic acid was obtained by MERCK KGaA (Damstadt, Germany) and high purity water obtained from Milli-Q system (Millipore, Bedford, MA).

Human Embryonic kidney (HEK) 293T cells. HEK293 cells were harvested at a density of approx 1.5×10^6 cells/mL and stored at $-30\text{ }^{\circ}\text{C}$. Cells were thawed and resuspended in ice-cold lysisbuffer (15 mL PBS, 150 μL Tween 20 and protease inhibitor cocktail). After dance homogenizing on ice, the lysate was stored at 0°C for 10 min. Subsequently centrifugation at $20000 \times g$ in a tabletop centrifuge (Eppendorf, Hamburg, Germany) at 4°C yielded separation of soluble and insoluble protein fractions. The soluble fraction was collected and the concentration determined by a Bradford assay. The lysate was dissolved in 50 mM ammonium bicarbonate to a concentration of 4mg/mL.

Lysate and BSA in-solution digestion. 100 mg digested protein/lysate was reduced with 45 mM dithiothreitol ($50\text{ }^{\circ}\text{C}$, 15 min) followed by alkylation using 110 mM Iodoacetamide (dark, RT, 15min) Buffer exchange was performed with 50 mM ammonium bicarbonate using 5 kD spin columns. The resulting solutions were dried in a vacuum centrifuge and resuspended in 50 mM ammonium bicarbonate. The purified digests were aliquoted. One part was digested with Lys-C and an equal amount with Lys-N. Lys-C was added to the samples at a 1:50 (w/w) ratio and incubated at $25\text{ }^{\circ}\text{C}$ over night and Lys-N was added at a ratio of 1:85 (w/w) and also incubated over night at $25\text{ }^{\circ}\text{C}$.

BSA ingel-digestion. Gel bands containing 5 μg of BSA were cut out of the gel and washed with water. After shrinking the gel pieces with acetonitrile they were reduced with 10 mM of DTT ($60\text{ }^{\circ}\text{C}$, 1 hour) followed by alkylation using 55 mM Iodoacetamide (dark, RT, 30min). After shrinking the gel pieces with acetonitrile they were incubated with trypsin or Lys-N (10 ng / μL) and left on ice for 30 min. Excess trypsin or Lys-N was then removed and enough AMBIC was added to cover the gel pieces. The gel pieces were incubated over night

at 37 °C. Supernatant was transferred to new eppendorf tubes. Peptide were extracted by adding 5 % FA to the gel pieces following heating (65 °C, 2 min) and shaking (RT, 20 min). The supernatant was added to the previous supernatant.

Strong Cation Exchange. Strong cation exchange was performed using a zorbax BioSCX-Series II column (0.8 mm i.d. X 50 mm length, 3.5 μ m), a FAMOS autosampler (LC-packing, Amsterdam, The Netherlands), a Shimadzu LC-9A binary pump and a SPD-6A UV-detector (Shimadzu, Tokyo, Japan). Prior to SCX chromatography, protein digests were desalted using a small plug C18 material (3 M Empore C18 extraction disk) packed into a GELoader tip (Eppendorf) onto which 10 μ L of Aqua C18 (5 μ m, 200 Å) material was placed. The eluate was dried completely and subsequently reconstituted in 20 % acetonitrile and 0.05 % formic acid. After injection, a linear gradient of 1% min⁻¹ solvent B (500 mM KCL in 20 % acetonitrile and 0.05% formic acid, pH 3.0) was performed. A total of 30 SCX fractions (1 min each, i.e., 50 μ L elution volume) were manually collected and dried in a vacuum centrifuge.

ETD experiments. Digested BSA and HEK293 lysate were subjected to nanoscale liquid chromatography tandem mass spectrometry (nanoLC-MS/MS) analysis, performed on an Agilent 1100 HPLC system (Agilent Technologies) connected to a LTQ XL Linear Ion Trap Mass Spectrometer with an ETD source at the back from Thermo Fisher Scientific, Inc. (Waltham, MA).

The samples (0.5 μ g digested HEK293 lysate and 50 fmol of BSA) were diluted in 5 % formic acid and injected on the trap column (Aqua C18 (phenomenex, Torrance, CA)), 20 mm x 100 μ m I.D.) at a flow rate of 5 μ L / min. The peptides were transferred with a split-reduced flow rate of 100 nL / min solvent A (0.1 M acetic acid) onto an analytic column (Reprosil C18 RP (Dr Maisch, Germany), 20 cm x 50 μ m I.D.). Elution of peptides from digested lysate was achieved with a linear gradient from 0 to 60% B (acetonitrile/water (v/v) containing 0.1 M acetic acid) in 95 min and digested BSA was eluted with a linear gradient from 0 to 40% B in 75min. The column effluent was directly introduced into the ESI source of the MS.

The mass spectrometer was operated in positive ion mode, from 350 to 1500 m/z in MS mode and with an AGC value of 1.00e+05. Parent ions were isolated for a more accurate mass measurement by performing a SIM scan and fragmented by CID or ETD in data-dependent mode with an AGC value of 1.00e+04. Ions were fragmented using CID with normalized collision energy of 35 and 30 ms activation time. ETD fragmentation was performed with supplemental activation, fluoranthene was used as reagent anion and ion/ion reaction in the ion trap was taking place for 100 ms.

After MS measurements data was analyzed with the MASCOT software version 2.2.0 (www.matrixscience.com). The database search was made with parameters set to consider a peptide tolerance of ± 0.5 Da, a fragment tolerance of ± 0.9 Da, a static modification of + 57 Da on cystein residues (Carbamidomethylation) and a differential modification of + 16 Da on methionine (oxidation). CID and ETD spectra of digested lysate were searched in NCBI nr 20070713 (5269953 sequences; 1825351362 residues) database and BSA in a BSA database.

Lys-N and Lys-C fragment ion statistics: Peptides with a minimum MASCOT score of 30 were considered. The Mascot identification allowed automated peak counting and intensity summing where all possible c and z ions (plus known common neutral losses) were considered. Exceptions occurred when c- or z-ions (or related ions) were assigned to the same isotope cluster in which case the most appropriate assignment was chosen (e.g. based on mass accuracy trend, mono isotopic peak etc) in an automated fashion.