MRSL-7

COP9 signalosome complex subunit 8; Q99627

[1-209] m+H = 23268.997
Cleavage at KR trypsin digest

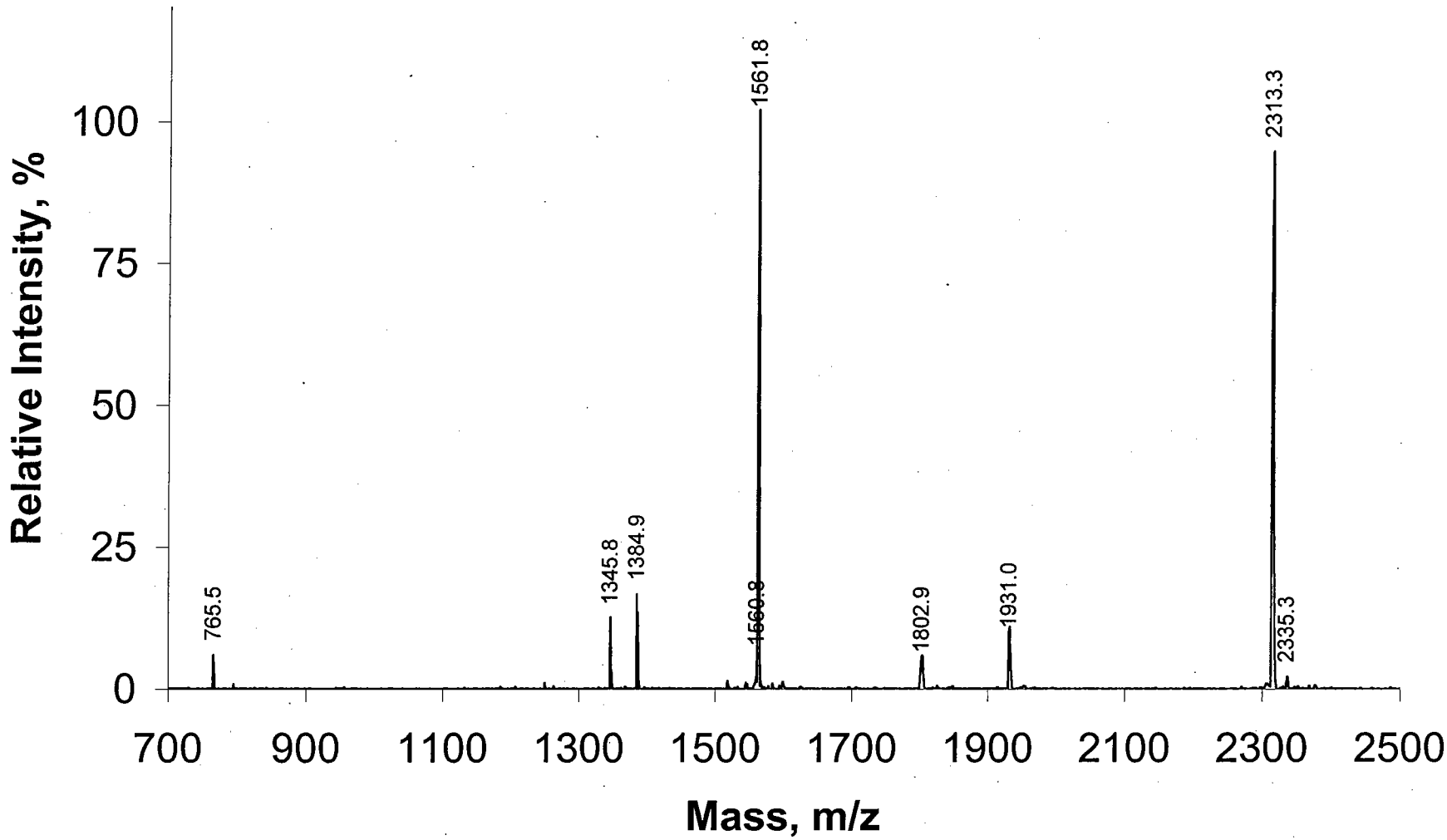
Small polar:	D(9)	E(13)	N(9)	Q(12)			
Large polar:	K(7)	R(11)	H(2)				
Small non-polar:	S(11)	T(9)	A(25)	G(11)			
Large non-polar:	L(20)	I(13)	V(14)	M(5)	F(9)	Y(6)	W(5)
Special:	C(1)	P(17)					

C[20] + 57.05

```
  1 M P V A V M A E S A F S F K k L L D Q C E N Q E L E A P G G  30
 31 I A T P P V Y G Q L L A L Y L L H N D M N N A R y I w k R i  60
 61 p p a i k S A N S E L G G I W S V G Q R i w q r D F P G I Y  90
 91 T T I N A H Q W S E T V Q P I M E A L R d a t r R r A F A L 120
121 V S Q A Y T S I I A D D F A A F V G L P V E E A V K g i l e 150
151 q g w q a d s t t r M V L P R k p v a g a l d v s f n k F I 180
181 P L S E P A P V P P I P N E Q Q L A R I t d y v a f l e n   209
```

(1)	[1-14] = 1514.738	(2)	[15-15] = 147.113	(3)	[16-54] = 4351.149
(4)	[55-58] = 609.340	(5)	[59-59] = 175.119	(6)	[60-65] = 638.424
(7)	[66-80] = 1560.777	(8)	[81-84] = 602.341	(9)	[85-110] = 3017.483
(10)	[111-114] = 462.231	(11)	[115-115] = 175.119	(12)	[116-116] = 175.119
(13)	[117-146] = 3142.635	(14)	[147-160] = 1561.761	(15)	[161-165] = 615.365
(16)	[166-178] = 1345.747	(17)	[179-199] = 2313.260	(18)	[200-209] = 1184.583

MALDI mass spectrum of the tryptic digest of MRSL-7



Centroid Mass	Rel. Intensity	Potential Match	Calc. Mass	Mass Error, ppm
765.4512	1.34	Y[55-59]R	765.411	53
1345.765375	4.79	K[166-178]K	1345.747	14
1384.863033	7.63	Y[55-65]K	1384.846	12
1560.804784	2.12	S[66-80]R	1560.777	18
1561.782459	52.02	G[147-160]R	1561.761	14
1802.883863	3.91			
1930.97426	8.21			
2313.322969	100	F[179-199]R	2313.26	27
2335.301574	1.18			

mass accuracy should be better than 50 ppm

outliers suggest a bad assignment

unassigned

maybe N-terminal

1% level noise

3.9% level is real

MRSL-7

COP9 signalosome complex subunit 8; Q99627

[1-209] m+H = 23268.997
Cleavage at E Glu-C digest

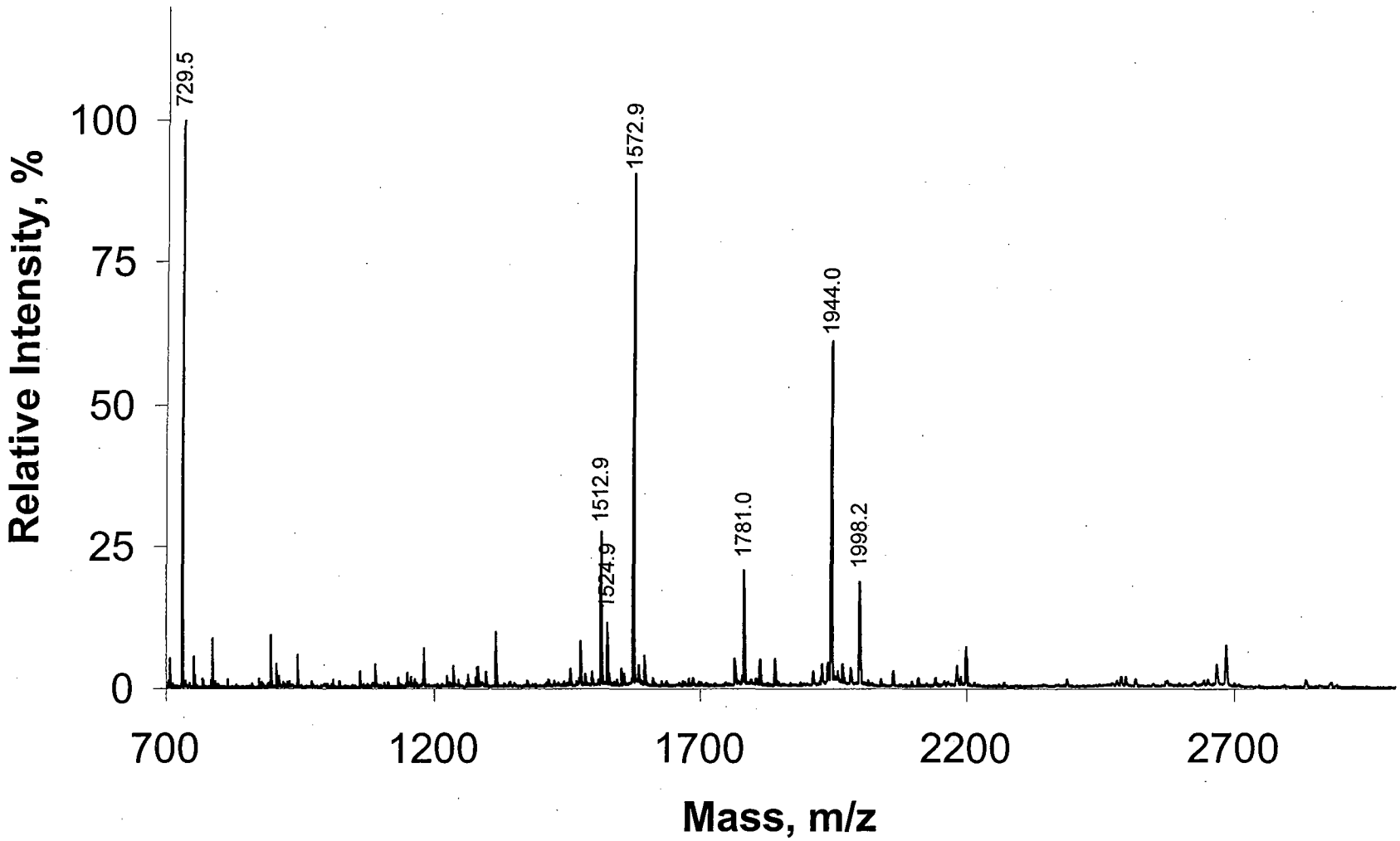
Small polar:	D(9)	E(13)	N(9)	Q(12)			
Large polar:	K(7)	R(11)	H(2)				
Small non-polar:	S(11)	T(9)	A(25)	G(11)			
Large non-polar:	L(20)	I(13)	V(14)	M(5)	F(9)	Y(6)	W(5)
Special:	C(1)	P(17)					

C[20] + 57.05

```
  1 M P V A V M A E s a f s f k k I I d q c e N Q E I e A P G G  30
 31 I A T P P V Y G Q L L A L Y L L H N D M N N A R Y L W K R I  60
 61 P P A I K S A N S E I g g i w s v g q r i w q r d f p g i y  90
 91 t t i n a h q w s e T V Q P I M E a I r d a t r r r a f a I  120
121 v s q a y t s i i a d d f a a f v g I p v e E a v k g i I e  150
151 Q G W Q A D S T T R M V L P R K P V A G A L D V S F N K F I  180
181 P L S E P A P V P P I P N E q q I a r I t d y v a f I e N  209
```

(1)	[1-8] = 847.405	(2)	[9-21] = 1572.773	(3)	[22-24] = 390.162
(4)	[25-26] = 261.145	(5)	[27-70] = 4833.566	(6)	[71-100] = 3515.761
(7)	[101-107] = 817.412	(8)	[108-142] = 3811.029	(9)	[143-143] = 148.060
(10)	[144-150] = 729.451	(11)	[151-194] = 4770.508	(12)	[195-208] = 1666.880
(13)	[209-209] = 133.061				

MALDI mass spectrum of the GluC digest of MRSL-7



Centroid Mass	Relative Intensity	Potential Match	Calc. Mass	Mass Error, ppm
707.441113	1.38			
729.482603	31.03	A[114-150]E	729.451	43
729.586935	1.86			
751.466953	1.74			
786.48288	2.77			
894.470832	3.3			
904.506689	1.68			
944.565644	1.76			
1060.640886	1.31			
1089.614389	2.28			
1149.571776	1.62			
1180.652987	3.28			
1223.755212	1.08			
1235.713905	2.55			
1263.597719	1.66			
1277.740847	1.49			
1280.619776	2.95			
1282.68018	1.86			
1296.744534	2.42			
1315.75576	7.29			
1374.812816	1.07			
1414.789873	1			
1425.867665	1.03			
1455.872795	2.88			
1472.450922	1.1			
1474.788386	7.24			
1483.877959	1.04			
1496.454306	2.8			
1508.395693	1.02			
1512.925951	26.24			
1524.871145	12.39			
1528.922612	1.89			
1551.816803	2.98			
1555.426977	1.79			
1570.808567	1.14			
1572.858347	99.18	S[9-21]E	1572.773	54
1583.881346	3.79			
1594.833397	7.01			
1610.803039	1.48			
1626.831147	1.05			
1634.7915	1.31			
1666.962015	1.19	Q[195-208]E	1666.88	49
1677.904225	1.41			
1684.980674	1.88			
1763.980124	5.04			
1764.518382	2.13			
1777.075396	2.61			
1781.002984	27.81	Q[195-209]N	1780.9228	45
1803.965151	1.34			
1810.114148	6.26			
1812.100717	2.91			

1838.97173	8.19			
1844.105817	1.07			
1910.072798	1.75			
1911.137213	2.61			
1926.518491	1.34			
1927.522978	3.05			
1938.149024	5.73			
1944.032362	100	S[9-34]E	1943.917	59
1958.944385	1.04			
1966.013176	6.76			
1980.670485	2.08			
1981.707941	3.12			
1998.202058	29.95			
2014.203981	1.47			
2038.026422	1.44			
2038.265382	1.53			
2061.200617	4.12			
2096.226485	1.13			
2108.179143	3.11			
2140.12723	2.06			
2157.626826	1.4			
2179.271091	2.43			
2181.260277	2.61			
2188.131883	2.51			
2198.24605	12.15			
2387.437078	1.73			
2480.396945	1.12			
2488.468502	3.43			
2497.426663	3.08			
2515.452934	2.25			
2571.443589	1.72			
2665.576562	1.11			
2666.519112	3.35			
2682.484535	1.78			
2683.53819	13.59			
2831.632406	2.64			

MRSL-7

COP9 signalosome complex subunit 8; Q99627

[1-209] m+H = 23268.997
Cleavage at RKE

Glu-C + trypsin digest

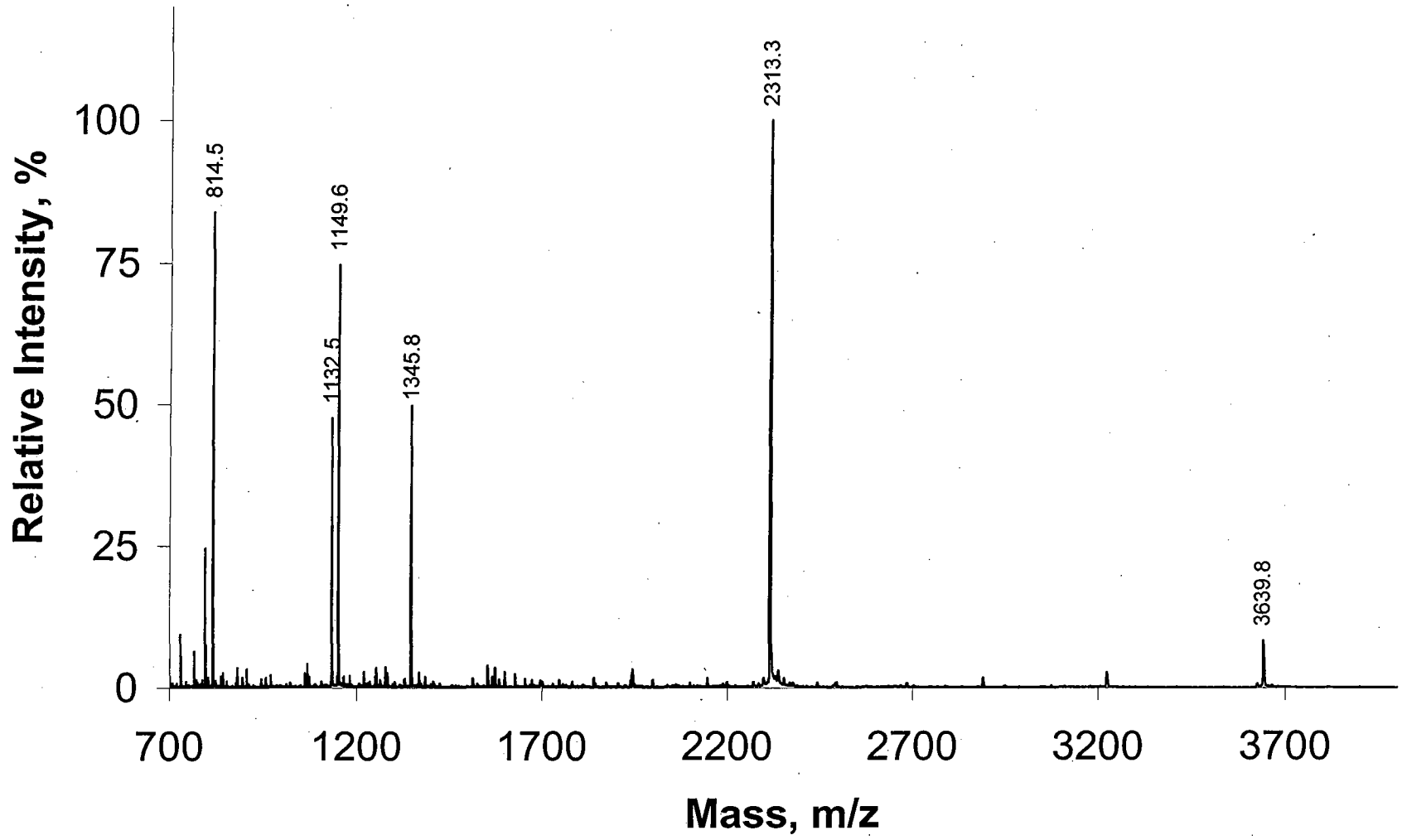
Small polar:	D(9)	E(13)	N(9)	Q(12)			
Large polar:	K(7)	R(11)	H(2)				
Small non-polar:	S(11)	T(9)	A(25)	G(11)			
Large non-polar:	L(20)	I(13)	V(14)	M(5)	F(9)	Y(6)	W(5)
Special:	C(1)	P(17)					

C[20] + 57.05

```
  1 M P V A V M A E s a f s f k K I I d q c e N Q E I e A P G G  30
 31 I A T P P V Y G Q L L A L Y L L H N D M N N A R y I w k R i  60
 61 p p a i k S A N S E I g g i w s v g q r I W Q R d f p g i y  90
 91 t t i n a h q w s e T V Q P I M E a I r D A T R r R a f a I  120
121 v s q a y t s i i a d d f a a f v g I p v e E a v k G I L E  150
151 q g w q a d s t t r M V L P R k p v a g a I d v s f n k F I  180
181 P L S E P A P V P P I P N E q q I a r L T D Y V A F L E n    209
```

(1)	[1-8] = 847.405	(2)	[9-14] = 686.351	(3)	[15-15] = 147.113
(4)	[16-21] = 777.345	(5)	[22-24] = 390.162	(6)	[25-26] = 261.145
(7)	[27-54] = 2979.551	(8)	[55-58] = 609.340	(9)	[59-59] = 175.119
(10)	[60-65] = 638.424	(11)	[66-70] = 507.205	(12)	[71-80] = 1072.590
(13)	[81-84] = 602.341	(14)	[85-100] = 1878.866	(15)	[101-107] = 817.412
(16)	[108-110] = 359.240	(17)	[111-114] = 462.231	(18)	[115-115] = 175.119
(19)	[116-116] = 175.119	(20)	[117-142] = 2715.392	(21)	[143-143] = 148.060
(22)	[144-146] = 317.218	(23)	[147-150] = 431.250	(24)	[151-160] = 1149.528
(25)	[161-165] = 615.365	(26)	[166-178] = 1345.747	(27)	[179-194] = 1716.921
(28)	[195-199] = 615.357	(29)	[200-208] = 1070.540	(30)	[209-209] = 133.061

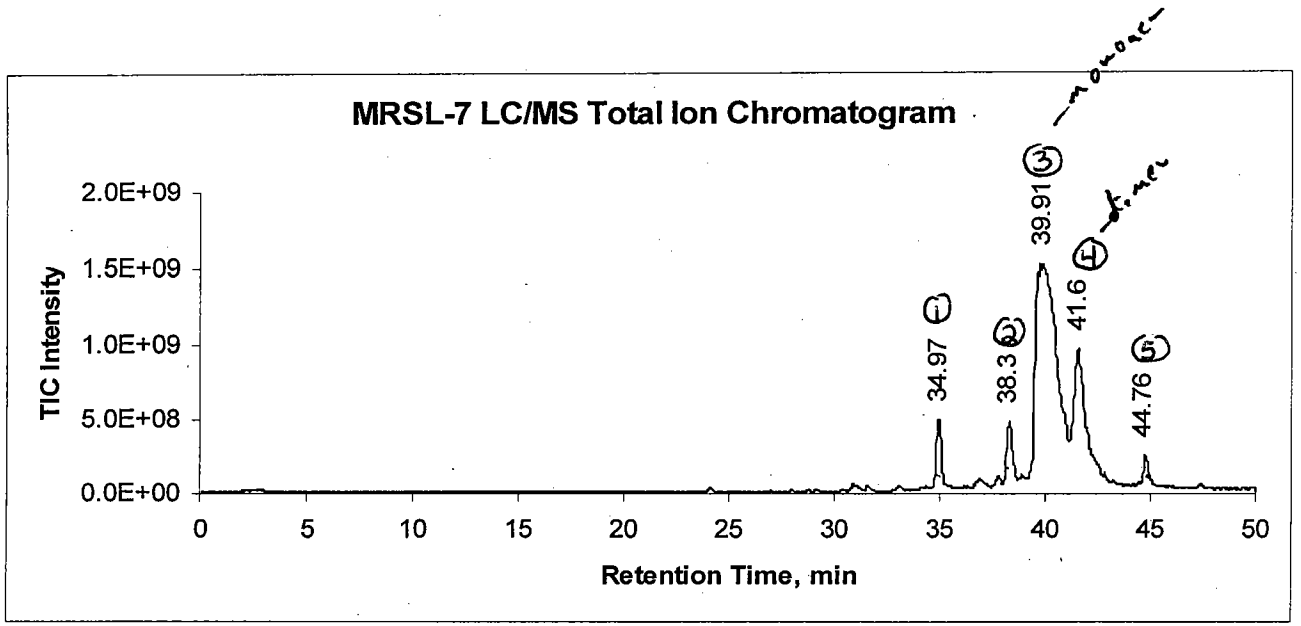
MALDI mass spectrum of the GluC + Trypsin digest of MRSL-7



Centroid Mass	Relative Intensity	Potential Match	Calc. Mass	Mass Error, ppm
729.484344	1.09	A[144-150]E	729.45	47
794.558278	3.08	R[59-65]K	794.525	42
814.48015	12.75	S[9-15]K	814.446	42
1132.520991	8.6			
1149.551505	18.01	Q[151-160]R	1149.528	20
1252.717481	1.11			
1345.770761	14.29	K[166-178]K	1345.747	18
1552.900574	1.57			
1572.790041	1.27	S[9-21]E	1572.773	11
1943.986849	1.81	S[9-24]E	1943.917	36
2313.270066	100	F[179-199]R	2313.26	4
2335.249131	1.87			
2888.549332	1.58			
3221.715148	2.29	L[25-54]R	3221.677	12
3639.839876	4.8			

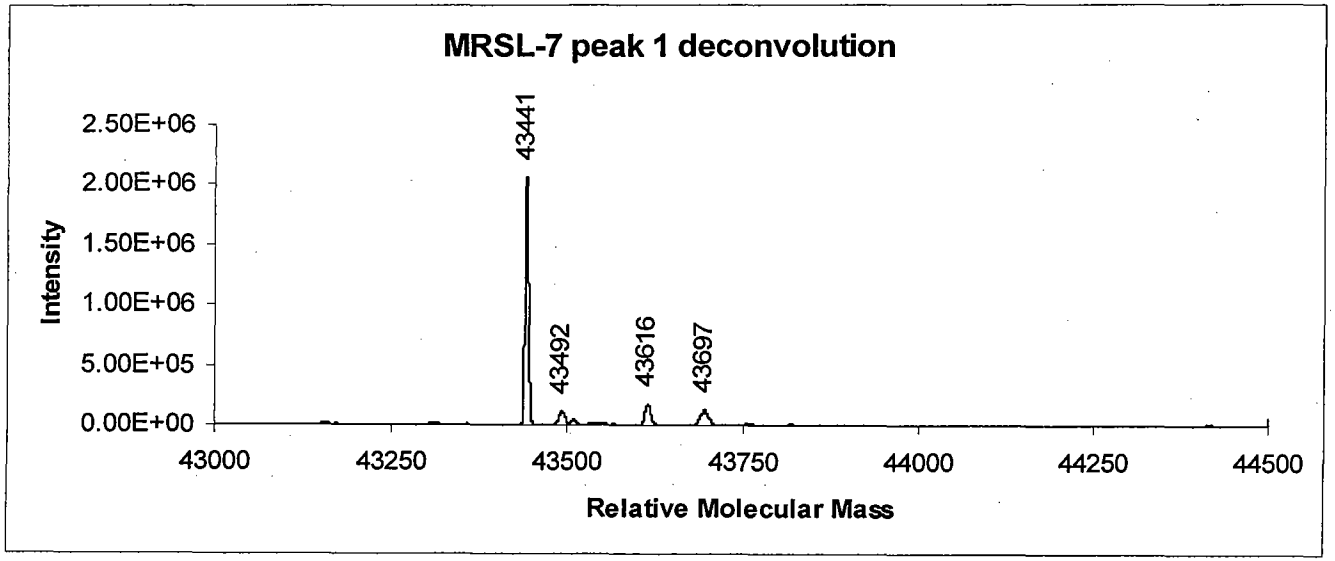
Frank will confirm N-term tag

'SNA' left on 5' to TEV cleavage

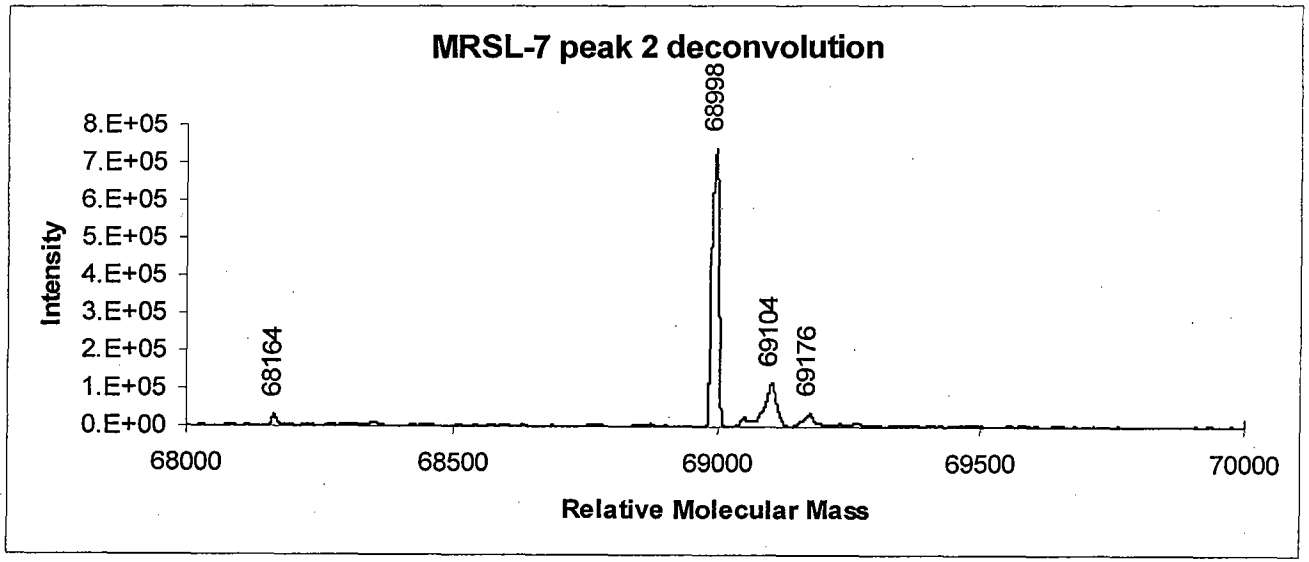


Column = Supelco Discovery BIO Wide Pore C5, 10 cm x 0.32 mm, 3 µm; #44493-01
Mobile Phase A = 0.05 % (v/v) trifluoroacetic acid in water
Mobile Phase B = 0.05 % (v/v) trifluoroacetic acid in acetonitrile
Method File = NCI Proteins-Polar
Injection Volume = 5 µL (full loop injection)
Column Temp = 50 °C

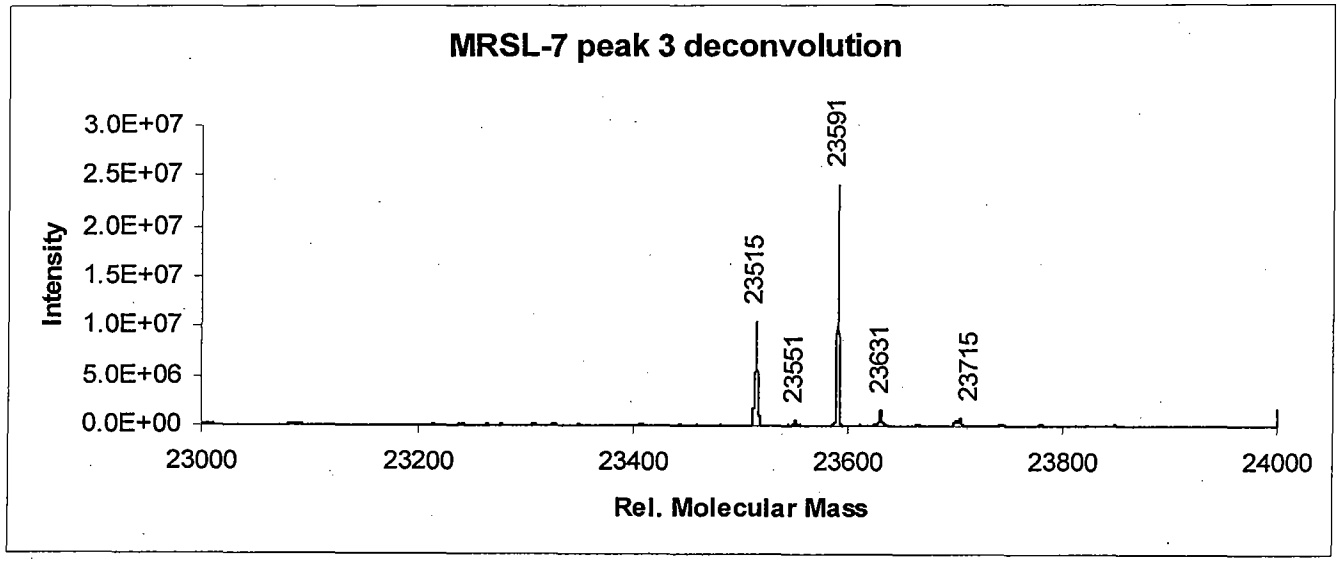
Time, min	% B	Flow, µL/min
0	10	6
5	10	6
45	60	6
50	95	6
55	95	6
56	10	6
80	10	6



Rel Molecular Mass	Intensity	Potential ID
43441	2.06E+06	same species observed at Mr 43437 in peak 3 in MRSL-8 LC/MS results
43492	1.18E+05	same species observed as peak 3 in MRSL-8 LC/MS results
43616	1.82E+05	same species observed at Mr 43614 in peak 3 in MRSL-8 LC/MS results
43697	1.27E+05	same species observed at Mr 43688 in peak 3 in MRSL-8 LC/MS results

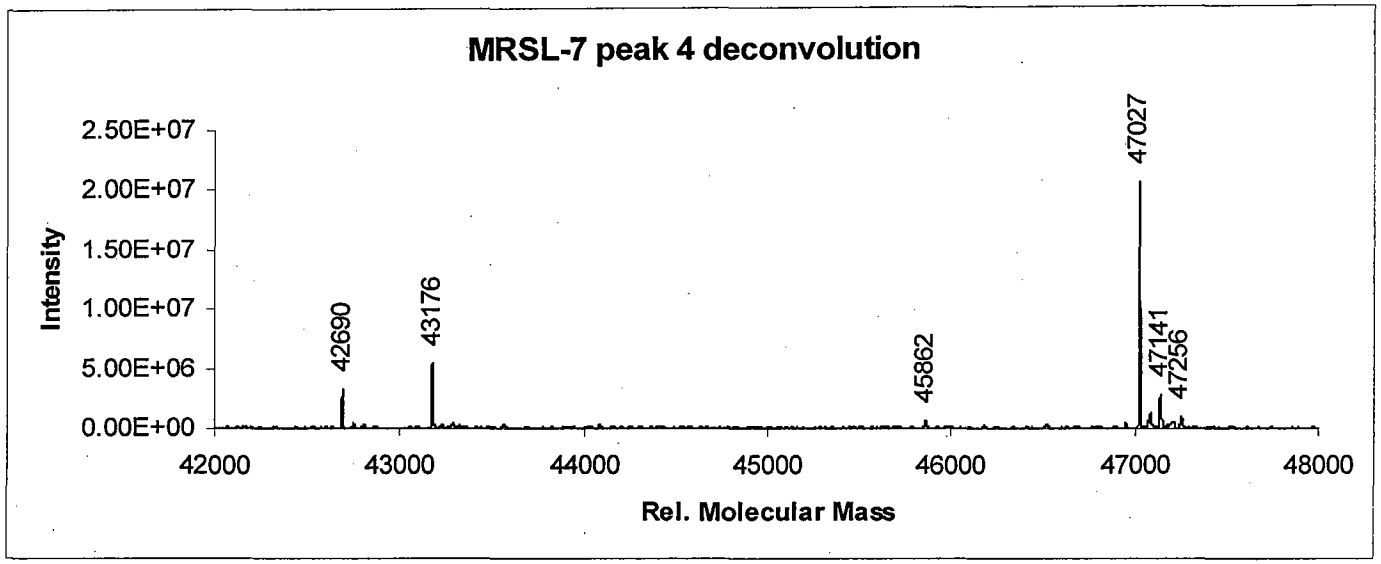


Rel. Molecular Mass	Intensity	Potential ID
68164	3.05E+04	
68998	7.37E+05	same species observed at Mr 68991 in peak 4 in MRSL-8 LC/MS results
69104	1.16E+05	same species observed at Mr 69082 in peak 4 in MRSL-8 LC/MS results
69176	3.62E+04	same species observed at Mr 69172 in peak 4 in MRSL-8 LC/MS results



Rel. Molecular Mass	Intensity	Potential ID
23515	1.05E+07	
23551	5.55E+05	
23591	2.41E+07	
23631	1.58E+06	
23715	8.31E+05	

MRSL-7 Calculated Mr = 23225.6



Rel. Molecular Mass	Intensity	Potential ID
42690	3.33E+06	
43176	5.43E+06	
45862	7.28E+05	
47027	2.05E+07	covalent dimer of Mr 23515 (MRSL-7 peak 3) species
47141	2.78E+06	
47256	9.05E+05	