Supplement to:

Quantitative analysis of dynamic protein interactions during transcription reveals a role for casein kinase II in PAF complex phosphorylation and regulation of H2B monoubiquitylation

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SUPPLEMENTAL TABLE LEGENDS

Table S1: Yeast strains used in this study.

Table S2: Primers and plasmids used in this study.

Table S3: SAINT analysis of Spt16-TAP interactions (n=4). Known interactions were annotated as such based on the iRef index (irefindex.org). Prey names are the standard gene names as defined in the Saccharomyces genome database (www.yeastgenome.org).

Table S4: Phosphorylated peptides identified by PTM analysis of Cdc73-TAP. Both the preceding and following amino acids for each tryptic peptide are shown. The phosphorylation sites are indicated by 'PO4'. Note that some sites were
detected with multiple unique peptides.

Table S5: Peptide ratios for Ctr9-FLAG SILAC experiments from WT and CKII temperature sensitive strains. The peptides sequence identified, the sequence match to a putative CKII phosphorylation site, and the SILAC ratio of WT (heavy) and *cka1Δ cka2-8* (light) peptides are given.

SUPPLEMENTAL TABLES

Table S1:

Strain Name	Genotype	Reference
BY4717	*MAT*a *his3D leu2D met15D ura3D*	Ghaemmaghami, S *et al*. (2003)
Spt16-TAP	*MAT*a *his3D leu2D met15D ura3D* Spt16-TAP:*HIS3MX6*	Ghaemmaghami, S *et al*. (2003)
Ckb2-TAP	*MAT*a *his3D leu2D met15D ura3D* Ckb2-TAP:*HIS3MX6*	Ghaemmaghami, S *et al*. (2003)
Cdc73-TAP	*MAT*a *his3D leu2D met15D ura3D* Cdc73-TAP:*HIS3MX6*	Ghaemmaghami, S *et al*. (2003)
DY150	*MAT**a** ade2 can1 his3 leu2 trp1 ura3*	Biswas, D *et al*. (2006)
DY8107	*MAT**a** spt16-11 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*	Biswas, D *et al*. (2007)
DDY4010	*MAT**α** his3 Δ200 leu2-3,112 ura3-52*	Peng, Y *et al*. (2011)
DDY4011	*MAT**α** his3 Δ200 leu2-3,112 ura3-52 cka1::CgURA3*	Peng, Y *et al*. (2011)
DDY4013	*MAT**α** his3 Δ200 leu2-3,112 ura3-52 cka1::CgURA3 cka2-8::NatR*	Peng, Y *et al*. (2011)
YJJ662	*MAT**a** ura3-52 leu2Δ1 his3Δ200*	Shi X, *et al*. (1997)
YJJ577	*MAT**a** ura3-52 leu2Δ1 his3Δ200 paf1Δ::HIS3*	Porter, S *et al*. (2002)
yJK002*	*MAT**α** his3 Δ200 leu2-3,112 ura3-52 CTR9-3xFLAG::kanMX*	This study
yJK004[†]	*MAT**α** his3 Δ200 leu2-3,112 ura3-52 cka1Δ::CgURA3 cka2-8::NatR CTR9-3xFLAG::kanMX*	This study
yJK006[‡]	*MAT**a** ura3-52 leu2Δ1 his3Δ200 paf1::natNT2*	This study
yJK008[‡]	*MAT**a** ura3-52 leu2Δ1 his3Δ200 ctr9::natNT2*	This study

* isogenic to DDY4010 (Peng Y, *et al.* 2011 (1))

† isogenic to DDY4013 (Peng Y, *et al.* 2011)

‡ isogenic to YJJ662 (Shi X, *et al.* 1997 (2))

Table S2:

Table S2. Plasmids and primers used in this study.		
Plasmids	**Features**	**Source**
pRS316	CEN URA3	Sikorski R.S. and Hieter P. (1989)

pRS313-3HA-SSN6	*CEN HIS3 p3xHA-SSN6*	Michael Keogh
pJK001	*CEN HIS3 pPAF1(WT)-3XHA-SSN6*	This study
pJK002	CEN HIS3 pPAF1(S147A, T385A, T422A, S426A)-3XHA-SSN6	This study
pJK003	CEN HIS3 pPAF1(S147E, T385E, T422E, S426E)-3XHA-SSN6	This study
pJK007	*CEN HIS3 pCTR9(WT)-3XHA-SSN6*	This study
pJK008	CEN HIS3 pCTR9(S974A, S977A, S1015A, S1017A, S1046A, S1054A, S1056A)-3XHA-SSN6	This study
pJK009	CEN HIS3 pCTR9(S974E, S977E, S1015E, S1017E, S1046E, S1054E, S1056E)-3XHA-SSN6	This study
pFA6a – natNT2	cassette plasmid (deletion)	Janke C., *et al.* (2014)
p3FLAG-kanMX	cassette plasmid (3x-FLAG tagging)	Gelbart M.E., *et al.* (2001)
Primers	**Sequence (5'-3')**	**Application**
oGGW054	GAAATGTATTCAGTACAATAGAACAGTGCTCATAATAGTATAAAGGGTCACAATGCGTACGCTGCAGGTCGAC	Paf1 deletion cassette
oGGW055	GTAAAAAGAACTACAGGTTTAAAATCAATCTCCCTTCACTTCTCAATATTCTAATCGATGAATTCGAGCTCG	Paf1 deletion cassette
oGGW056	CAAAGTTCTAATTGTCTGGTCCATTTGTGTTGAGAGCAAGAAAAAAAAACATGCGTACGCTGCAGGTCGAC	Ctr9 deletion cassette
oGGW057	GTATAATATTAAGTTTCTTTAAAAGTCTTGATTCTAACCCTCGCCTCTTCTTAATCGATGAATTCGAGCTCG	Ctr9 deletion cassette
oJLK087	CGACGAAAACAATGATAATGATGATAACGACGGATTGTTCAGGGAACAAAAGCTGGAG	Ctr9-3xFLAG cassette
oJLK088	TTTCTTTAAAAGTCTTGATTCTAACCCTCGCCTCTTCTTACTATAGGGCGAATTGGGT	Ctr9-3xFLAG cassette
oJLK025	AAAGGATCCGCCTGCCCCAACAGATAAAA	Paf1 cloning
oJLK026	AAACCCGGGTTCTTCTTGTAAAGTTTCCTTTTCTTCC	Paf1 cloning
oJLK055	AAAGGATCCCTCCACTGCAAACGTTTTCA	Ctr9 cloning
oJLK056	AAAGATATCGAACAATCCGTCGTTATCATCA	Ctr9 cloning
oJLK018	GCGCTTGGATGATGGAGATGCGGATGATGAAAACC	Paf1 S147A mutagenesis
oJLK019	AGAAAGAATCGGAAGGCGATTCAAAAGCAGAAGGTTCTGAG	Paf1 T385A mutagenesis
oJLK020	GATGAAAACCGTGCAGCTGATGCGCCCGAAACTTC	Paf1 T422A mutagenesis
oJLK021	CTGATGCGCCCGAAACTGCAGATGCTGTTCATACT	Paf1 S426A

		mutagenesis
oJLK034	AGGCGCTTGGATGATGGAGATGAGGATGATGAAAACCTTGA	Paf1 S147E mutagenesis
oJLK035	GAAAGAATCGGAAGGCGATTCAAAAGAGGAAGGTTCTGAGCAAGAAGGA	Paf1 T385E mutagenesis
oJLK036	CAAGATGAAAACCGTGCAGCTGATGAGCCCGAAACTGAGGATGCTGTTCATACTGAACAAAAA	Paf1 T422E, S426E mutagenesis
oJLK061	CCATAAGTGAACATAATGTTAAAGATGATGCAGATCTGGCTGATAAAGACAATGAATATGAC	Ctr9 S974A, S977A mutagenesis
oJLK062	GAAAGCTGCTAAAAAAACTCTTGCCGACGCCGACGAGGATGACGAT	Ctr9 S1015A, S1017A mutagenesis
oJLK063	ATCTCAACTATCGAATGAATTTATTGAAGATGCTGATGAGGAAGAAGCTCAAA	Ctr9 S1046A mutagenesis
oJLK064	GATGAGGAAGAAGCTCAAATGGCCGGTGCGGAACAAAATAAAAACGATG	Ctr9 S1054A, S1056A mutagenesis
oJLK072	CCATGGCCATAAGTGAACATAATGTTAAAGATGATGAGGATCTGGAGGATAAAGACAATGAATATGACGAAGAAAAGCCAAG	Ctr9 S974E, S977E mutagenesis
oJLK073	CGGTGAATCTAAGAGAAGGAAAGCTGCTAAAAAAACTCTTGAGGACGAGGACGAGGATGACGATGATGTG	Ctr9 S1015E, S1017E mutagenesis
oJLK074	ATCTCAACTATCGAATGAATTTATTGAAGATGAGGATGAGGAAGAAGCTCAAATGGAG	Ctr9 S1046E mutagenesis
oJLK076	AAGATAGTGATGAGGAAGAAGCTCAAATGGAGGGTGAGGAACAAAATAAAAACGATGACAACGACG	Ctr9 S1054E, S1056E mutagenesis

Table S3:

BAIT	**PREY**	**FC_A (average mean of replicates)**	**FC_B (geometric mean of replicates)**	**SAINT probability Score**	**Known interaction (iREF)**
SPT16	POB3	3475.31	3257.34	1	Yes
SPT16	SPT16	1295.52	998.31	1	Yes
SPT16	HIS1	409.69	358.56	1	No
SPT16	CKA2	175.66	110.49	1	Yes
SPT16	LEO1	96.61	90.75	1	Yes
SPT16	CKB2	150.5	82.3	1	Yes
SPT16	CKB1	125.24	80.7	1	Yes
SPT16	CTR9	68.58	61.82	1	Yes
SPT16	HHF1	36.39	31.42	1	Yes
SPT16	RPS6A	35.2	29.02	1	No

SPT16	PSH1	28.94	25.01	1	Yes
SPT16	PAF1	24.5	19.73	1	Yes
SPT16	IKI3	29	16.12	1	No
SPT16	RPS8A	14.99	13.37	1	No
SPT16	RTF1	13.56	11.5	0.98	Yes
SPT16	CKA1	164.19	69.92	0.93	Yes
SPT16	MSH6	56.6	20.17	0.75	No
SPT16	YFL054C	36.15	16.29	0.75	No
SPT16	HTB2	28.54	11.4	0.75	Yes
SPT16	LAP4	38.59	11.37	0.75	No
SPT16	MRC1	19.34	8.72	0.75	No
SPT16	RRM3	13.39	8.43	0.75	No
SPT16	RPS17A	14.24	8.11	0.75	No
SPT16	HTA1	32.53	7.7	0.75	Yes
SPT16	RAV1	13.24	7.31	0.75	No
SPT16	YTA6	15.62	7.27	0.75	No
SPT16	PTK2	12.24	7.12	0.75	No
SPT16	CTI6	11.8	7.03	0.75	No
SPT16	SFL1	15.68	6.93	0.75	No
SPT16	YOR1	13.32	6.89	0.75	No
SPT16	RPS18A	10.9	6.55	0.75	No
SPT16	RPL1A	8.05	6.35	0.75	No
SPT16	YKL105C	9.22	6.22	0.75	No
SPT16	CDC73	17.92	6.1	0.75	Yes
SPT16	SNT1	9.09	5.88	0.75	No
SPT16	SKI2	8.08	5.28	0.75	No
SPT16	BOI2	8.45	5.28	0.75	No
SPT16	ZIP1	10.87	4.93	0.75	No
SPT16	PKH2	5.45	4.17	0.75	No

Table S4:

Protein	Site	Peptide	XCorr	X!Tandem
Cdc73	S157	K.STTSASLENDS^{PO4}EVSDPVVVETMK.H	4.35	6.15
Ctr9	S974, S977	R.EAMAISEHNVKDDS^{PO4}DLS^{PO4}DK.D	5.55	3.15
		K.DDS^{PO4}DLS^{PO4}DKDNEYDEEKPR.Q	4.57	5.49
	S977	K.DDSDLS^{PO4}DKDNEYDEEKPR.Q	4.54	4.89
		K.DDSDLS^{PO4}DKDNEYDEEKPR.Q	4.86	2.39
	S1015	K.AAKKTLS^{PO4}DSDEDDDDVVK.K	5.6	6.12
	S1017	K.AAKKTLSDS^{PO4}DEDDDDVVK.K	5.14	5.77
	S1015, S1017	K.AAKKTLS^{PO4}DS^{PO4}DEDDDDVVK.K	5.63	3.06
		K.KTLS^{PO4}DS^{PO4}DEDDDDVVK.K	6.03	5.7
	S1046, S1054, 1056	K.SQLSNEFIEDS^{PO4}DEEEAQMS^{PO4}GS^{PO4}EQNK.N	8.11	3.89

	S1046, S1054	K.SQLSNEFIEDS ^{PO4}DEEEAQMS ^{PO4}GSEQNK.N	5.67	3.51
		K.SQLSNEFIEDsDEEEAQmsGSEQNK(N)	7.36	5.96
	S1054	(K)SQLSNEFIEDSDEEEAQMS ^{PO4}GSEQNK.N	6.94	5.64
Leo1	S25	K.EQISNNVGVTTNS ^{PO4}TSNEETSR.S	5.96	7.21
	T26	K.EQISNNVGVTTNST ^{PO4}SNEETSR.S	5.65	6.96
	S105	R.SRHRES ^{PO4}LGLDDDEAEEQAMYTR.K	6.46	2.1
	S132	R.KFYGEDANNFS ^{PO4}DQDETTHTFK.E	7.6	4.32
		K.FYGEDANNFS ^{PO4}DQDETTHTFKEENVELVR.H	7.04	5.26
		R.KFYGEDANNFS ^{PO4}DQDETTHTFK.E	7.6	4.32
	S358	K.EKQES ^{PO4}PDAAFETGFR.K	3.77	1.96
		K.QES ^{PO4}PDAAFETGFR.K	4.39	2.49
	S372	R.KQNS ^{PO4}PTTYGASR.R	3.87	1.68
Paf1	S32	K.LLVYPES ^{PO4}PETNADSSQLINSLYIK.T	5.24	5.1
	S147	K.RRLDDGDS ^{PO4}DDENLDVNHIISR.V	5.83	4.17
	S388 or T385	K.TEGS ^{PO4}EQEGENEKDEEIK.Q	5.29	2.52
		K.T ^{PO4}EGSEQEGENEKDEEIKQEK.E	6.04	1.66
	S388, T385	K.ESEGDSKT ^{PO4}EGS ^{PO4}EQEGENEKDEEIK.Q	4.61	3.85
	T422	R.AADT ^{PO4}PETSDAVHTEQKPEEEEK.E	5.49	4.42
	T422, S426	R.AADT ^{PO4}PETS ^{PO4}DAVHTEQKPEEEEK.E	5.62	2.4
Rtf1	S90	K.DES ^{PO4}DREHLESLPEMER.E	4.48	
	S181	R.HYS ^{PO4}DNEDEDDEEDYR.E	4.77	5.24
		R.HYS ^{PO4}DNEDEDDEEDYREEDYK.D	6.12	3.85
	S203	K.DDEGS ^{PO4}EYGDDEEYNPFDR.R	5.5	5.05

Table S5:

Protein	Potential CKII site(s)?	Peptide sequence	*cka1Δ cka2-8* / WT ratio	Modification status
Ctr9	Yes	EAMAISEHNVKDDSDLSDKDNEYDEEKPR	8.69	S974 and 977 unmodified
Ctr9	Yes	EAMAISEHNVKDDS ^{PO4}DLS ^{PO4}DKDNEYDEEKPR	0.39	S974 and 977 PO4
Ctr9	Yes	KTLSDSDEDDDDVVKKPSHNK	41.31	S1015 and 1017 unmodified
Ctr9	Yes	TLSDSDEDDDDVVKKPSHNKGK	48.78	S1015 and 1017 unmodified
Ctr9	Yes	TLSDSDEDDDDVVKKPSHNK	39.64	S1015 and 1017 unmodified
Ctr9	Yes	KTLSDS ^{PO4}DEDDDDVVKKPSHNK	3.20	S1017 PO4
Ctr9	Yes	TLSDS ^{PO4}DEDDDDVVKKPSHNKGK	3.48	S1017 PO4
Ctr9	Yes	TLSDS ^{PO4}DEDDDDVVKKPSHNK	3.20	S1017 PO4

Ctr9	Yes	TLS ^{PO4}DS ^{PO4}DEDDDDVVK	0.13	S1015 and 1017 PO4
Paf1	No	LLVYPESPETNADSSQLINSLYIK	1.14	S32 unmodified
Paf1	No	LLVYPES ^{PO4}PETNADSSQLINSLYIK	0.64	S32 PO4
Paf1	No	RTEYVSNT ^{PO4}IAAHDNTSLKR	3.15	T127 PO4
Paf1	No	TEYVSNTIAAHDNTSLKR	0.79	T127 unmodified
Paf1	Yes	RLDDGDSDDENLDVNHIISRVEGTFNK	44.19	S147 unmodified
Paf1	Yes	RRLDDGDSDDENLDVNHIISR	29.57	S147 unmodified
Paf1	Yes	RLDDGDSDDENLDVNHIISR	29.38	S147 unmodified
Paf1	Yes	LDDGDSDDENLDVNHIISR	28.10	S147 unmodified
Paf1	Yes	RLDDGDS ^{PO4}DDENLDVNHIISRVEGTFNK	0.25	S147 PO4
Paf1	Yes	RRLDDGDS ^{PO4}DDENLDVNHIISR	0.31	S147 PO4
Paf1	Yes	LDDGDS ^{PO4}DDENLDVNHIISR	0.24	S147 PO4
Leo1	No	HRESLGLDDDEAEEQAMYTR	0.82	S105 unmodified
Leo1	No	HRES ^{PO4}LGLDDDEAEEQAMYTRK	1.42	S105 PO4
Leo1	No	SRHRES ^{PO4}LGLDDDEAEEQAMYTR	1.35	S105 PO4
Leo1	Yes	KFYGEDANNFSDQDETTHTFKEENVELVR	2.47	S132 unmodified
Leo1	Yes	FYGEDANNFSDQDETTHTFKEENVELVR	2.40	S132 unmodified
Leo1	Yes	KFYGEDANNFS ^{PO4}DQDETTHTFKEENVELVR	0.34	S132 PO4
Leo1	Yes	FYGEDANNFS ^{PO4}DQDETTHTFKEENVELVR	0.37	S132 PO4
Rtf1	Yes	RIEVGSVEDDDEEDDYNPYSVGNADYGSEEEEEANPFPLEGK	6.92	S71 unmodified
Rtf1	Yes	RIEVGSVEDDDEEDDYNPYSVGNADYGS ^{PO4}EEEEEANPFPLEGK	0.33	S71 PO4

SUPPLEMENTAL FIGURES

Figure S1: Representative spectra for the phosphorylation sites in the five subunits of PAF-C: Cdc73 (A), Ctr9 (B-K), Leo1 (L-Q), Paf1 (R-W), and Rtf1 (X-AA). In all spectra, b-ions are labeled in red and y-ions are labeled in blue. Neutral loss and other parent ion fragments are shown in green. The XCorr and X!Tandem scores and ppm values are given for reference.

REFERENCES

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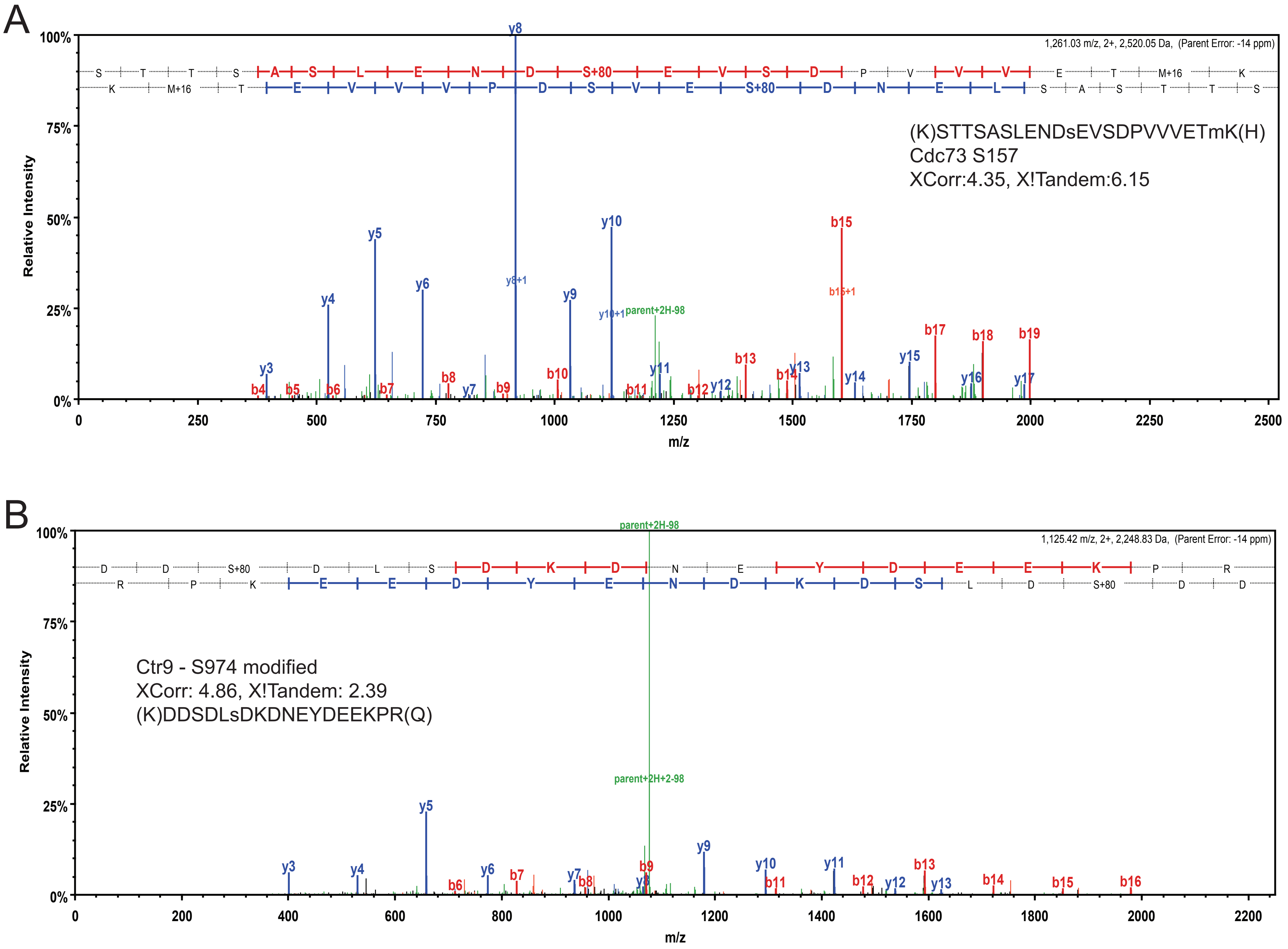
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K M+16 T E V V V P D S V E S+80 D N E L S A S T T S
(K)STTSASLENDsEVSDPVVVETmK(H)
Cdc73 S157
XCorr:4.35, X!Tandem:6.15
Relative Intensity
100%
75%
50%
25%
0%
y8
y8+1
y5
y10
y10+1
parent+2H-98
b15
b15+1
y6
y4
y9
b17
b18
b19
y3
b4
b5
b6
b7
b8
y7
b9
b10
b11
y11
b12
y12
b13
b14
y13
y14
y15
y16
y17
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
m/z
B
1,125.42 m/z, 2+, 2,248.83 Da, (Parent Error: -14 ppm)
D D S+80 D L S D K D N E Y D E E K P R
R P K E E D Y E N D K D S L D S+80 D D
parent+2H-98
parent+2H+2-98
Ctr9 - S974 modified
XCorr: 4.86, X!Tandem: 2.39
(K)DDSDLsDKDNEYDEEKPR(Q)
Relative Intensity
100%
75%
50%
25%
0%
y5
y3
y4
b6
y6
b7
y7
b8
y8
b9
y9
y10
b11
y11
b12
y12
b13
y13
b14
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b16
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800
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1600
1800
2000
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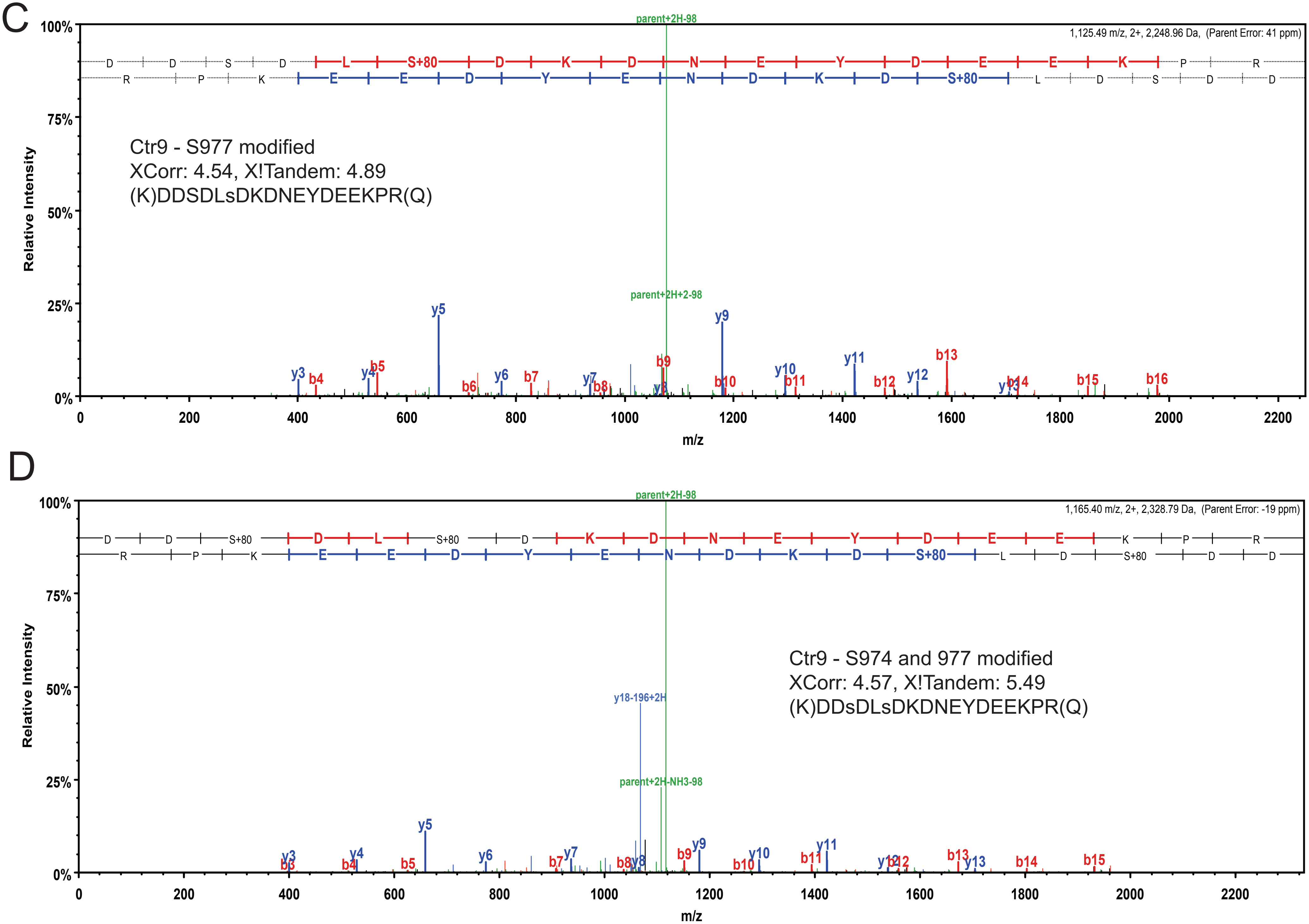
m/z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100%
75%
50%
25%
0%
Relative Intensity
parent+2H-98
1,125.49 m/z, 2+, 2,248.96 Da, (Parent Error: 41 ppm)
D D S D L S+80 D K D N E Y D E E K P R
R P K E E D Y E N D K D S+80 L D S D D
Ctr9 - S977 modified
XCorr: 4.54, X!Tandem: 4.89
(K)DDSDLsDKDNEYDEEKPR(Q)
parent+2H+2-98
y3 b4 y4 b5 y5 b6 y6 b7 y7 b8 y8 b9 y9 b10 y10 b11 y11 b12 y12 b13 y13 b14 b15 b16
0 200 400 600 800 1000 1200 1400 1600 1800 2000 2200
m/z
D
100%
75%
50%
25%
0%
Relative Intensity
parent+2H-98
1,165.40 m/z, 2+, 2,328.79 Da, (Parent Error: -19 ppm)
D D S+80 D L S+80 D K D N E Y D E E K P R
R P K E E D Y E N D K D S+80 L D S+80 D D
Ctr9 - S974 and 977 modified
XCorr: 4.57, X!Tandem: 5.49
(K)DDsDLsDKDNEYDEEKPR(Q)
y18-196+2H
parent+2H-NH3-98
y3 b3 y4 b4 b5 y5 y6 b7 y7 b8 y8 b9 y9 b10 y10 b11 y11 y12 b12 b13 y13 b14 b15
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m/z

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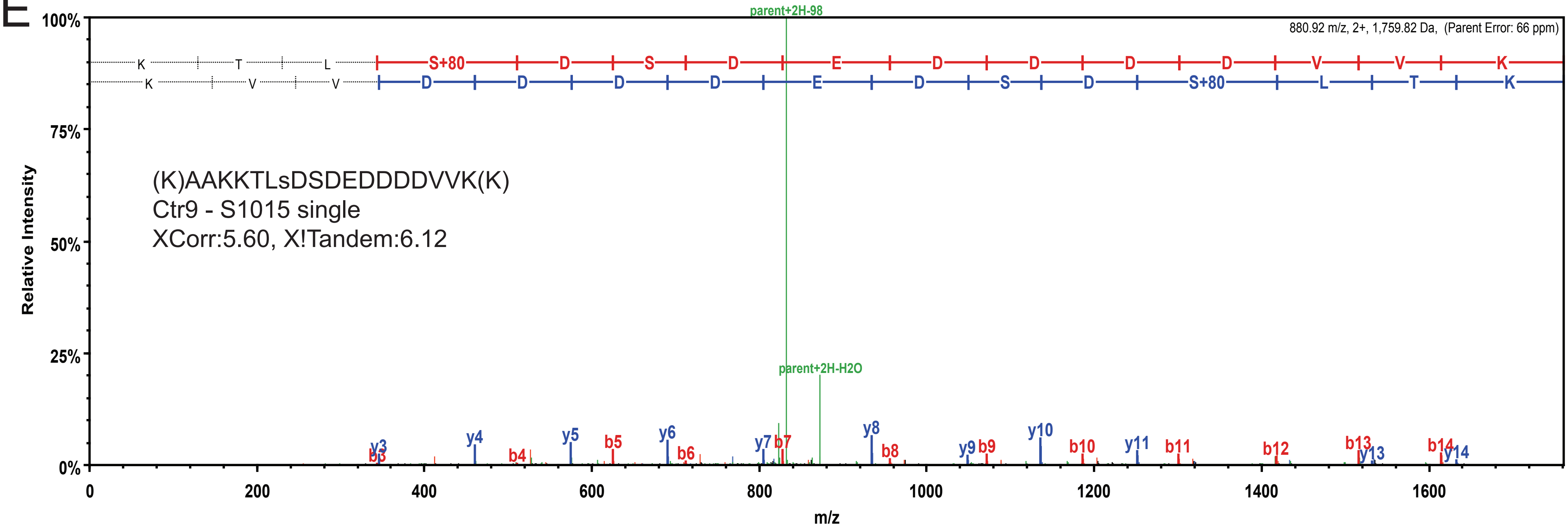
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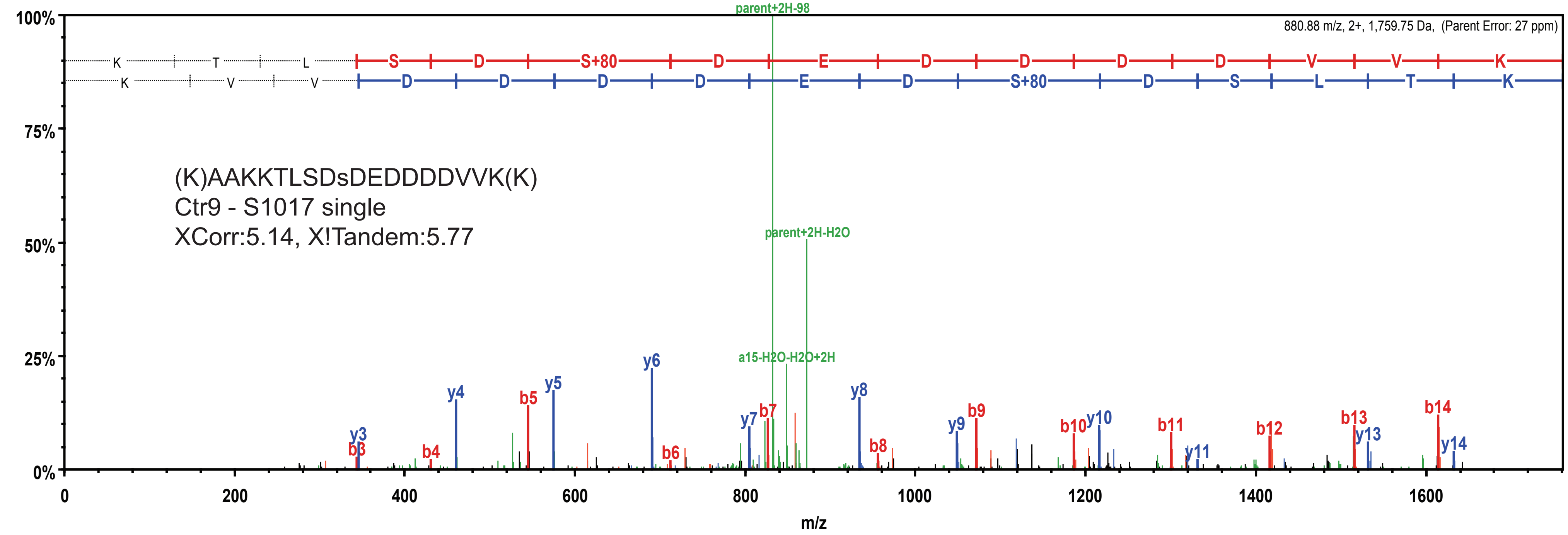
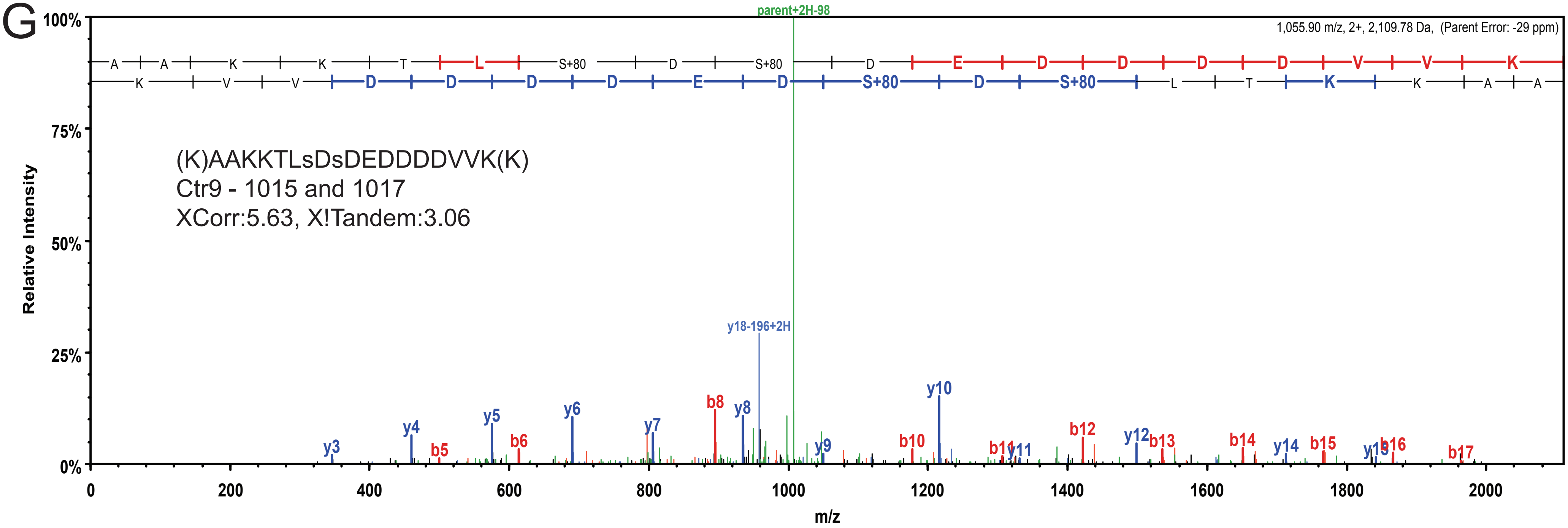
K V V D D D D E D S D S+80 L T K
(K)AAKKTLsDSDEDDDDVVK(K)
Ctr9 - S1015 single
XCorr:5.60, X!Tandem:6.12
Relative Intensity
100%
75%
50%
25%
0%
parent+2H-H2O
y3 b3 y4 b4 y5 b5 y6 b6 y7 b7 y8 b8 y9 b9 y10 b10 y11 b11 b12 b13 y13 b14 y14
0
200
400
600
800
1000
1200
1400
1600
m/z

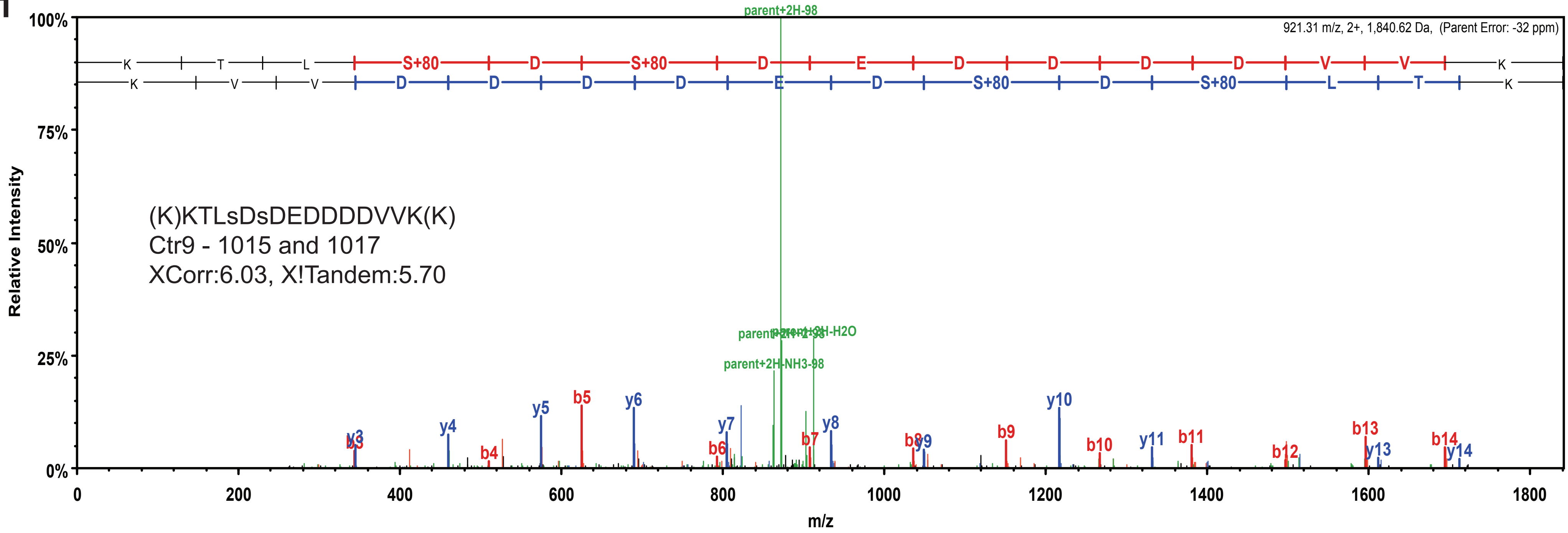
F


parent+2H-98
880.88 m/z, 2+, 1,759.75 Da, (Parent Error: 27 ppm)
K T L S D S+80 D E D D D D V V K
K V V D D D D E D S+80 D S L T K
(K)AAKKTLSDsDEDDDDVVK(K)
Ctr9 - S1017 single
XCorr:5.14, X!Tandem:5.77
100%
75%
50%
25%
0%
parent+2H-H2O
a15-H2O-H2O+2H
y3 b3 y4 b4 y5 b5 y6 b6 y7 b7 y8 b8 y9 b9 b10 y10 b11 y11 b12 b13 y13 b14 y14
0
200
400
600
800
1000
1200
1400
1600
m/z

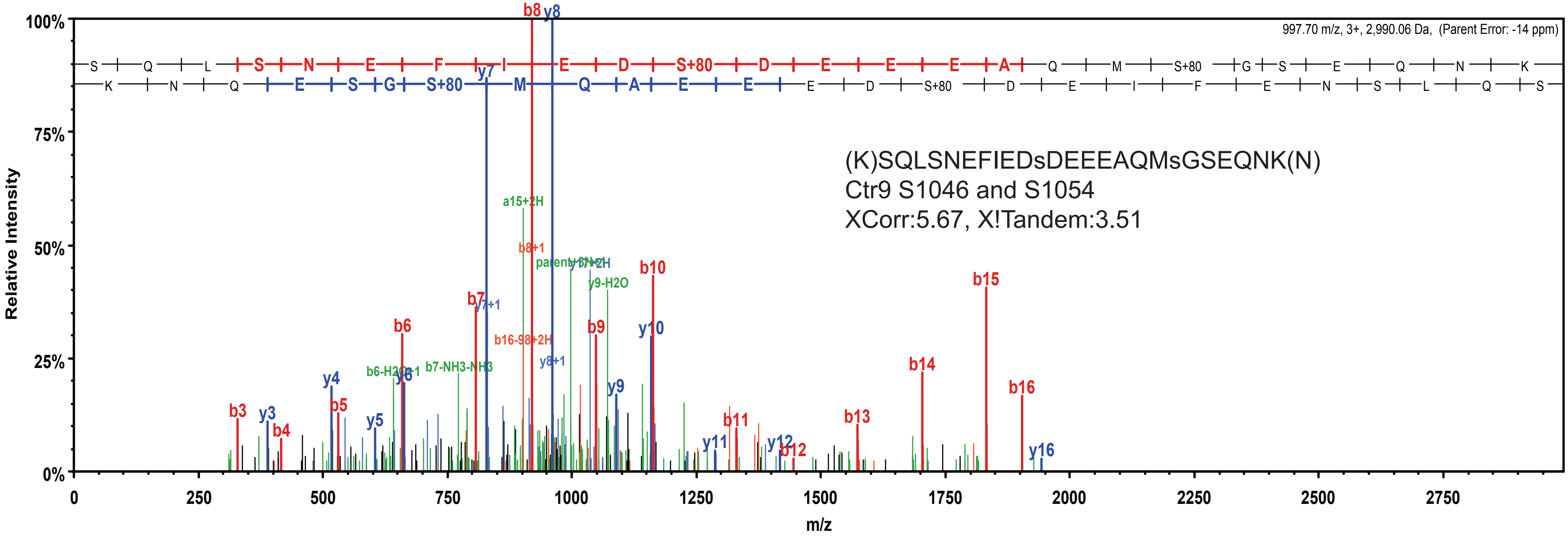
G


parent+2H-98
100%
75%
50%
25%
0%
Relative Intensity
1,055.90 m/z, 2+, 2,109.78 Da, (Parent Error: -29 ppm)
A A K K T L S+80 D S+80 D E D D D D V V K
K V V D D D D E D S+80 D S+80 L T K K A A
(K)AAKKTLsDsDEDDDDVVK(K)
Ctr9 - 1015 and 1017
XCorr:5.63, X!Tandem:3.06
y18-196+2H
y3
y4
b5
y5
b6
y6
y7
b8
y8
y9
b10
y10
b11
y11
b12
y12
b13
b14
y14
b15
y15
b16
b17
0
200
400
600
800
1000
1200
1400
1600
1800
2000
m/z

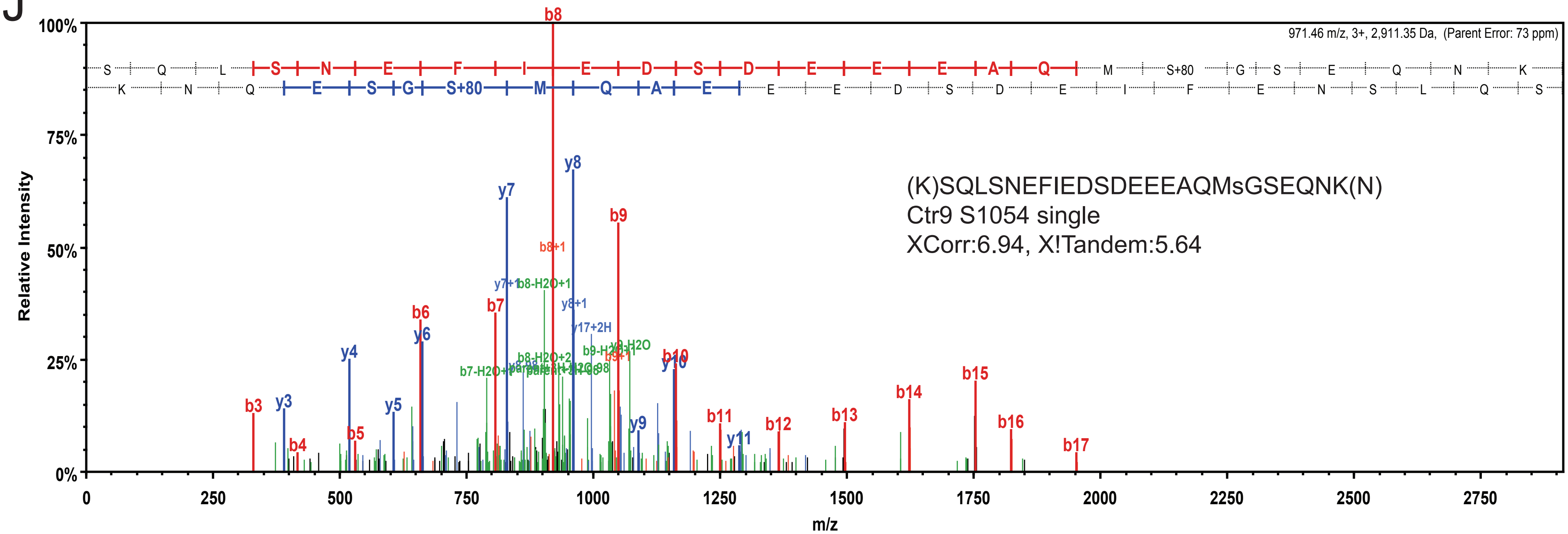
H

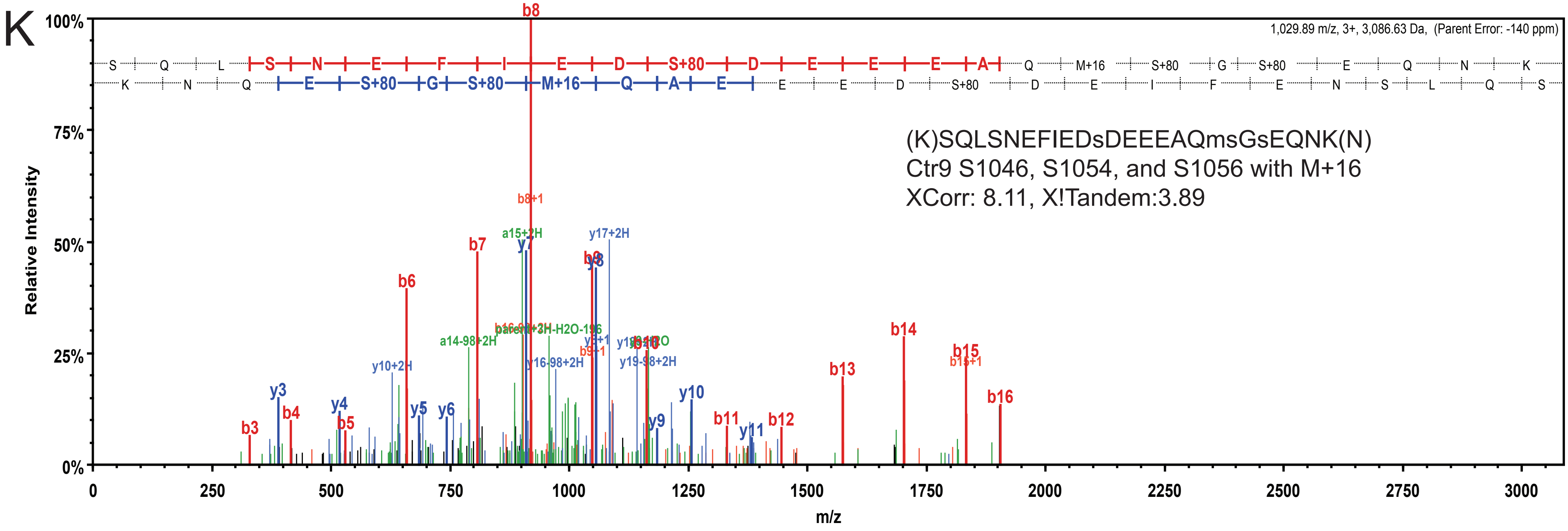

parent+2H-98
100%
75%
50%
25%
0%
Relative Intensity
921.31 m/z, 2+, 1,840.62 Da, (Parent Error: -32 ppm)
K T L S+80 D S+80 D E D D D D V V K
K V V D D D D E D S+80 D S+80 L T K
(K)KTLsDsDEDDDDVVK(K)
Ctr9 - 1015 and 1017
XCorr:6.03, X!Tandem:5.70
parent+2H-2-98
parent+2H-H2O
parent+2H-NH3-98
y3
b3
y4
b4
y5
b5
y6
b6
y7
b7
y8
b8
y9
b9
y10
b10
y11
b11
b12
b13
y13
b14
y14
0
200
400
600
800
1000
1200
1400
1600
1800
m/z

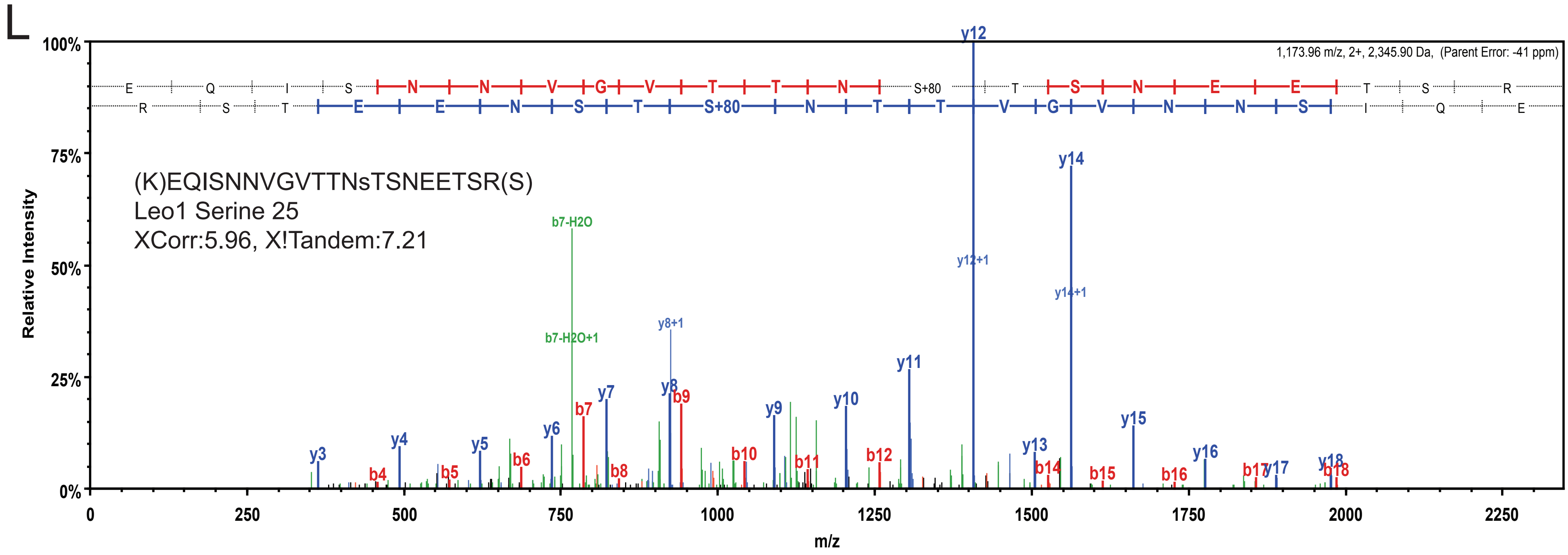
I


997.70 m/z, 3+, 2,990.06 Da, (Parent Error: -14 ppm)
S Q L S N E F I E D S+80 D E E E A Q M S+80 G S E Q N K
K N Q E S G S+80 M Q A E E E D S+80 D E I F E N S L Q S
(K)SQLSNEFIEDsDEEEAQMsGSEQNK(N)
Ctr9 S1046 and S1054
XCorr:5.67, X!Tandem:3.51
b8
y8
y7
a15+2H
b8+1
parent-98+2H
y17+2H
y9-H2O
b10
b15
b7
y7+1
b6
b9
y10
b16-98+2H
y8+1
b14
b6-H2O+1
b7-NH3-NH3
y6
y4
y9
b16
b3
y3
b5
b4
y5
b11
b13
y11
y12
b12
y16
Relative Intensity
100%
75%
50%
25%
0%
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
2750
m/z

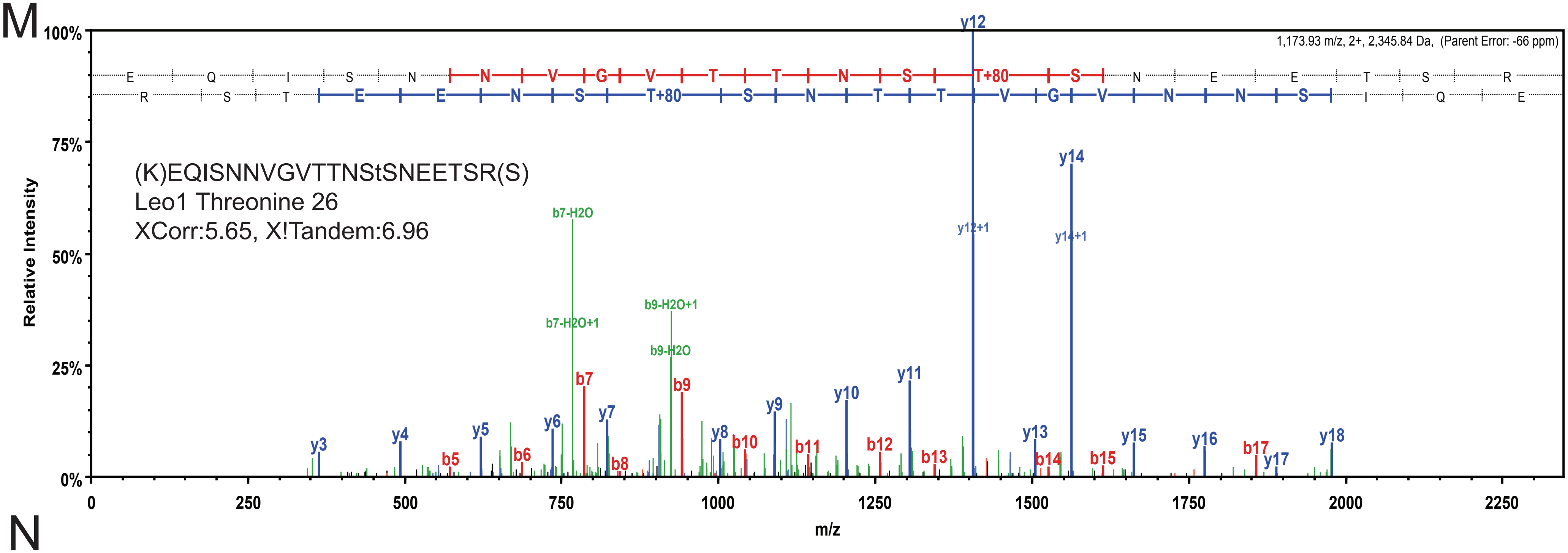
J


971.46 m/z, 3+, 2,911.35 Da, (Parent Error: 73 ppm)
S Q L S N E F I E D S D E E E A Q M S+80 G S E Q N K
K N Q E S G S+80 M Q A E E E D S D E I F E N S L Q S
(K)SQLSNEFIEDSDEEEAQMsGSEQNK(N)
Ctr9 S1054 single
XCorr:6.94, X!Tandem:5.64
b8
y8
y7
b9
b8+1
y7+1
b8-H2O+1
y8+1
b7
b6
y6
y17+2H
y4
b8-H2O+2
b9-H2O+1
y9-H2O
b9+1
b10
y10
b7-H2O+1
b15
b14
b3
y3
y5
y9
b11
b12
b13
b16
y11
b5
b4
b17
Relative Intensity
100%
75%
50%
25%
0%
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
2750
m/z

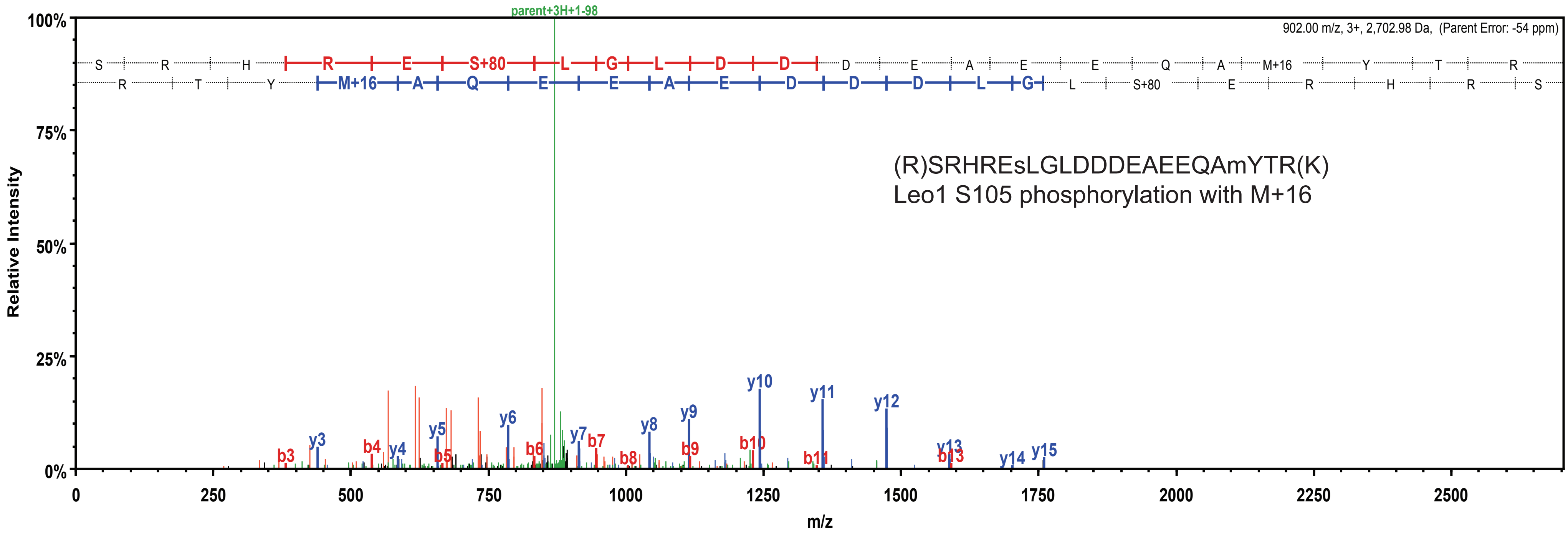

K
1,029.89 m/z, 3+, 3,086.63 Da, (Parent Error: -140 ppm)
S Q L S N E F I E D S+80 D E E E A Q M+16 S+80 G S+80 E Q N K
K N Q E S+80 G S+80 M+16 Q A E E E D S+80 D E I F E N S L Q S
(K)SQLSNEFIEDsDEEEAQmsGsEQNK(N)
Ctr9 S1046, S1054, and S1056 with M+16
XCorr: 8.11, X!Tandem:3.89
b8
b8+1
a15+2H
y17+2H
b7
y7
b9
y8
b6
a14-98+2H
y10+2H
y16-98+2H
y19-98+2H
b10
b9+1
y3
b4
b3
y4
b5
y5
y6
y9
y10
b11
y11
b12
b13
b14
b15
b15+1
b16
Relative Intensity
100%
75%
50%
25%
0%
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
2750
3000
m/z


L
1,173.96 m/z, 2+, 2,345.90 Da, (Parent Error: -41 ppm)
E Q I S N N V G V T T N S+80 T S N E E T S R
R S T E E N S T S+80 N T T V G V N N S I Q E
(K)EQISNNVGVTTNsTSNEETSR(S)
Leo1 Serine 25
XCorr:5.96, X!Tandem:7.21
y12
y14
b7-H2O
y12+1
y14+1
y8+1
b7-H2O+1
y11
y7
y8
b9
b7
y10
y9
y15
y6
y16
y4
y5
y13
y3
b6
b10
b12
b11
b4
b5
b8
b14
b15
b16
b17
y17
y18
b18
Relative Intensity
100%
75%
50%
25%
0%
0
250
500
750
1000
1250
1500
1750
2000
2250
m/z

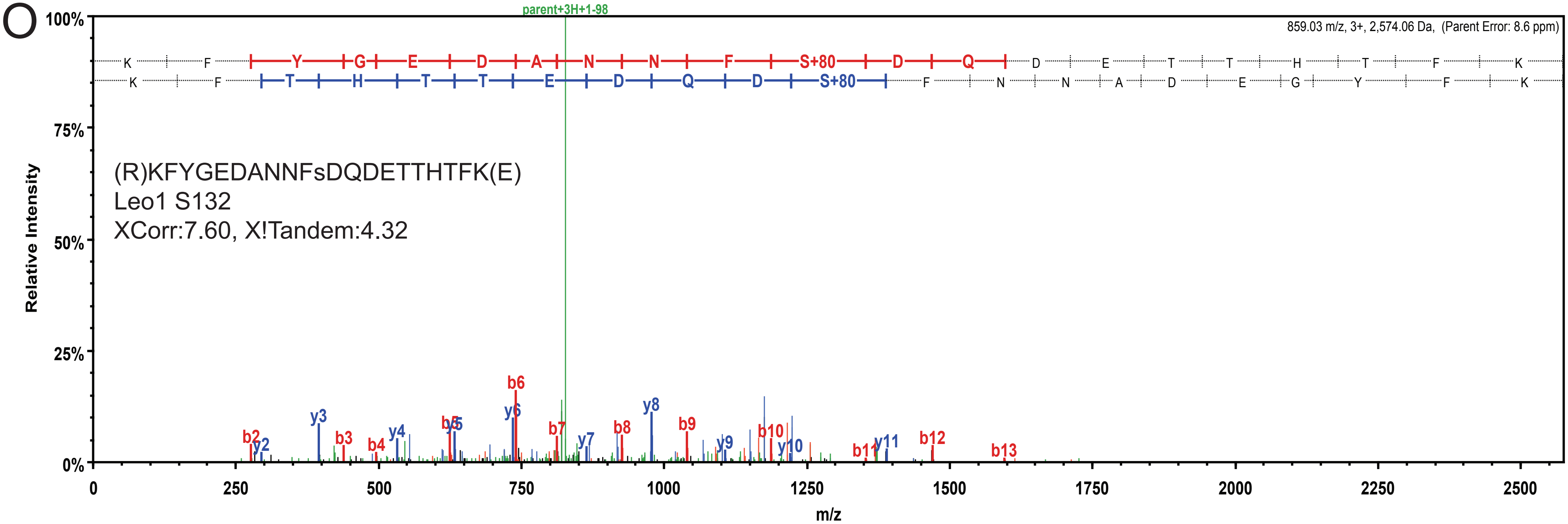
M


1,173.93 m/z, 2+, 2,345.84 Da, (Parent Error: -66 ppm)
E Q I S N N V G V T T N S T+80 S N E E T S R
R S T E E N S T+80 S N T T V G V N N S I Q E
(K)EQISNNVGVTTNStSNEETSR(S)
Leo1 Threonine 26
XCorr:5.65, X!Tandem:6.96
y12
y14
y12+1
y14+1
b7-H2O
b7-H2O+1
b9-H2O+1
b9-H2O
b7
b9
y3
y4
b5
y5
b6
y6
y7
b8
y8
b10
y9
b11
y10
b12
y11
b13
y13
b14
b15
y15
y16
b17
y17
y18
Relative Intensity
100%
75%
50%
25%
0%
0
250
500
750
1000
1250
1500
1750
2000
2250
m/z

N

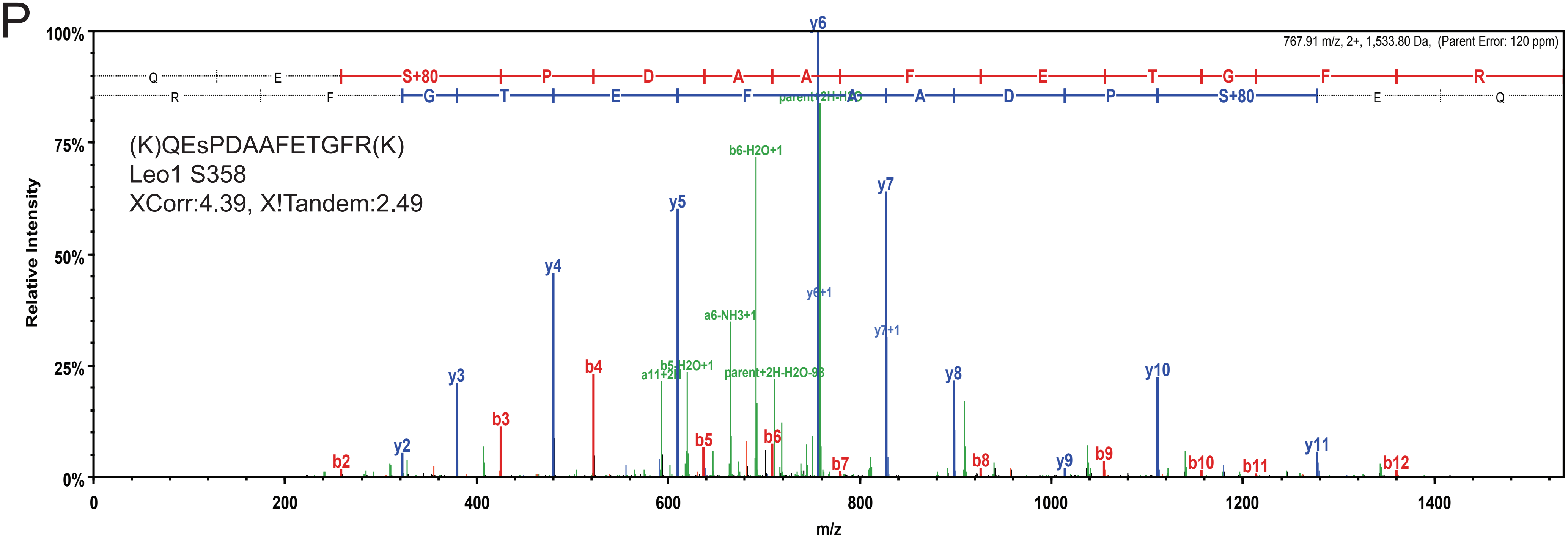

parent+3H+1-98
902.00 m/z, 3+, 2,702.98 Da, (Parent Error: -54 ppm)
S R H R E S+80 L G L D D D E A E E Q A M+16 Y T R
R T Y M+16 A Q E E A E D D D L G L S+80 E R H R S
(R)SRHREsLGLDDDEAEEQAmYTR(K)
Leo1 S105 phosphorylation with M+16
b3
y3
b4
y4
y5
b5
y6
b6
y7
b7
y8
b8
y9
b9
y10
b10
y11
b11
y12
y13
b13
y14
y15
Relative Intensity
100%
75%
50%
25%
0%
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
m/z

O



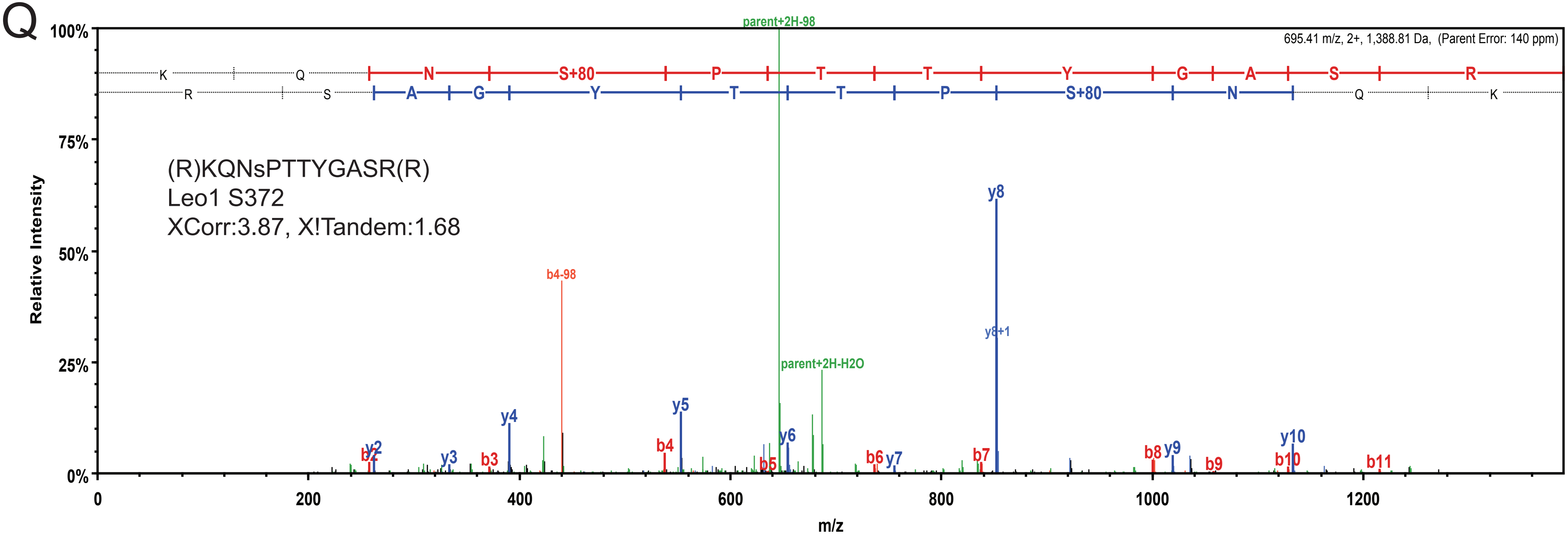
parent+3H+1-98
859.03 m/z, 3+, 2,574.06 Da, (Parent Error: 8.6 ppm)
K F Y G E D A N N F S+80 D Q D E T T H T F K
K F T H T T E D Q D S+80 F N N A D E G Y F K
(R)KFYGEDANNFsDQDETTHTFK(E)
Leo1 S132
XCorr:7.60, X!Tandem:4.32
Relative Intensity
100%
75%
50%
25%
0%
b2 y2 y3 b3 b4 y4 b5 y5 y6 b6 b7 y7 b8 y8 b9 y9 b10 y10 b11 y11 b12 b13
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
m/z

P



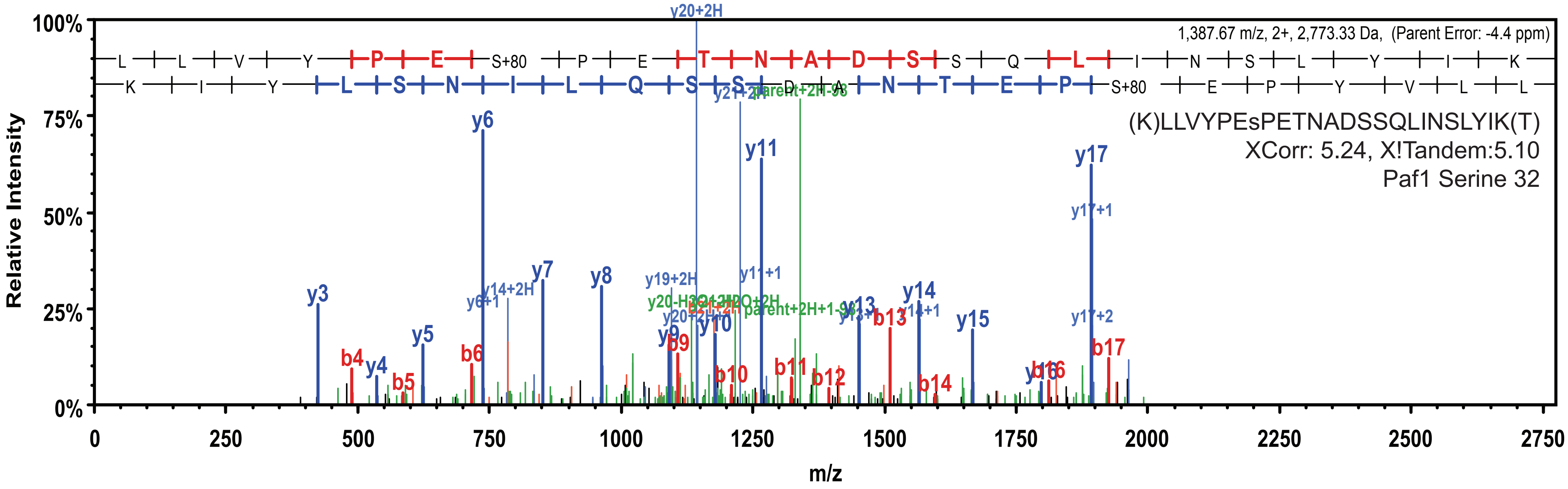
y6
767.91 m/z, 2+, 1,533.80 Da, (Parent Error: 120 ppm)
Q E S+80 P D A A F E T G F R
R F G T E F A A D P S+80 E Q
parent+2H-H2O
(K)QEsPDAAFETGFR(K)
Leo1 S358
XCorr:4.39, X!Tandem:2.49
Relative Intensity
100%
75%
50%
25%
0%
b6-H2O+1
y7
y5
y4
y6+1
a6-NH3+1
y7+1
b4
b5-H2O+1
a11+2H
parent+2H-H2O-98
y3
y8
y10
b3
b5
b6
y2
y11
b2
b7
b8
y9
b9
b10
b11
b12
0
200
400
600
800
1000
1200
1400
m/z

Q

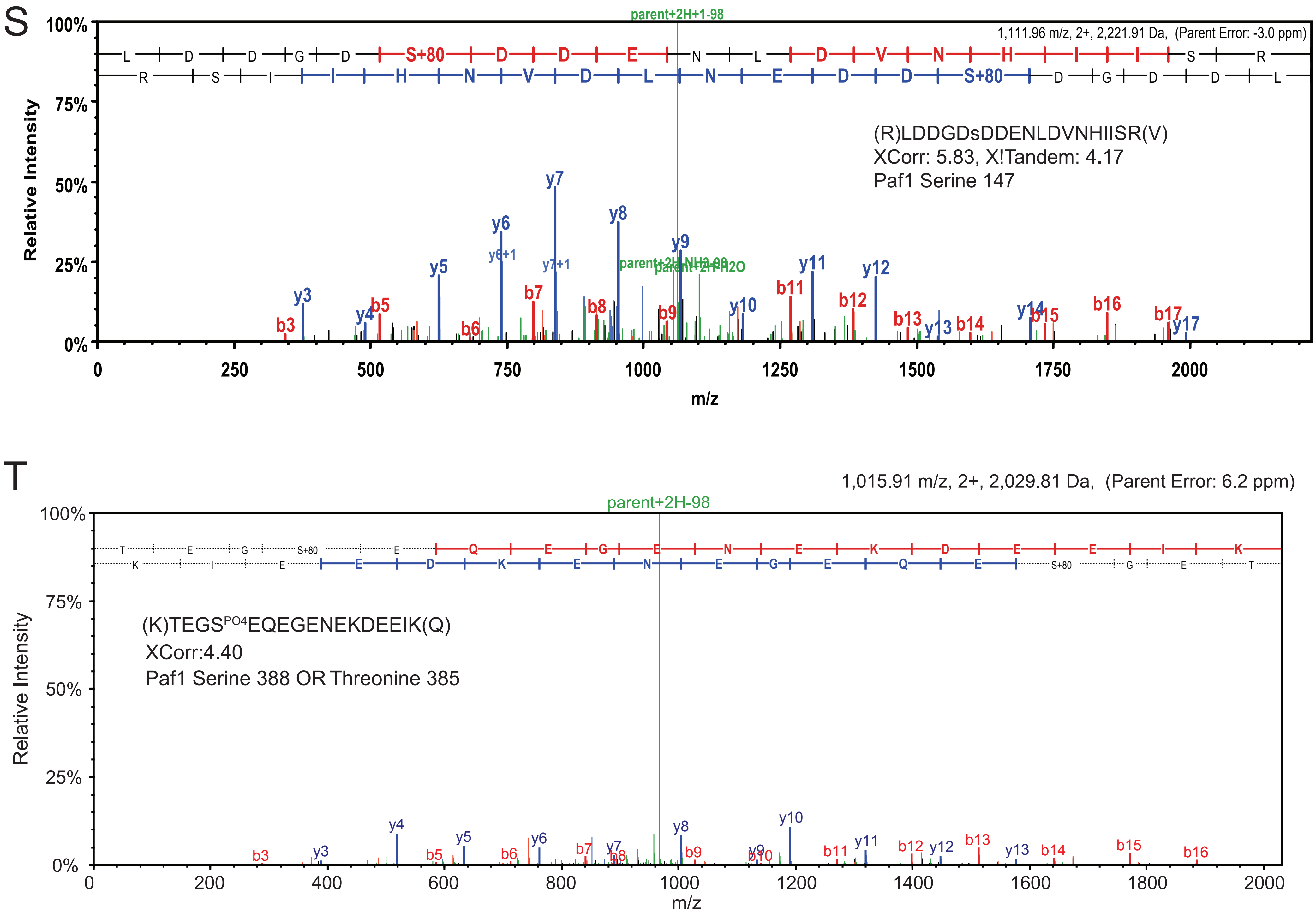


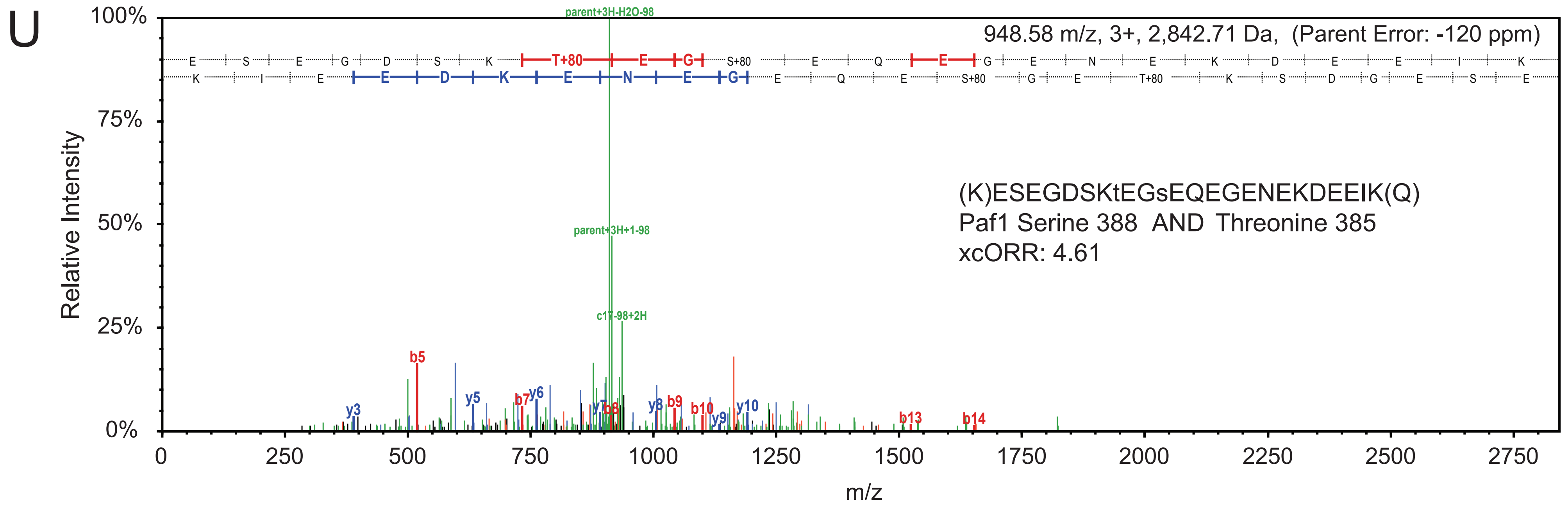
695.41 m/z, 2+, 1,388.81 Da, (Parent Error: 140 ppm)
K Q N S+80 P T T Y G A S R
R S A G Y T T P S+80 N Q K
(R)KQNsPTTYGASR(R)
Leo1 S372
XCorr:3.87, X!Tandem:1.68
parent+2H-98
parent+2H-H2O
b4-98
y8
y8+1
y2
b2
y3
b3
y4
b4
y5
b5
y6
b6
y7
b7
b8
y9
b9
b10
y10
b11
Relative Intensity
100%
75%
50%
25%
0%
0
200
400
600
800
1000
1200
m/z

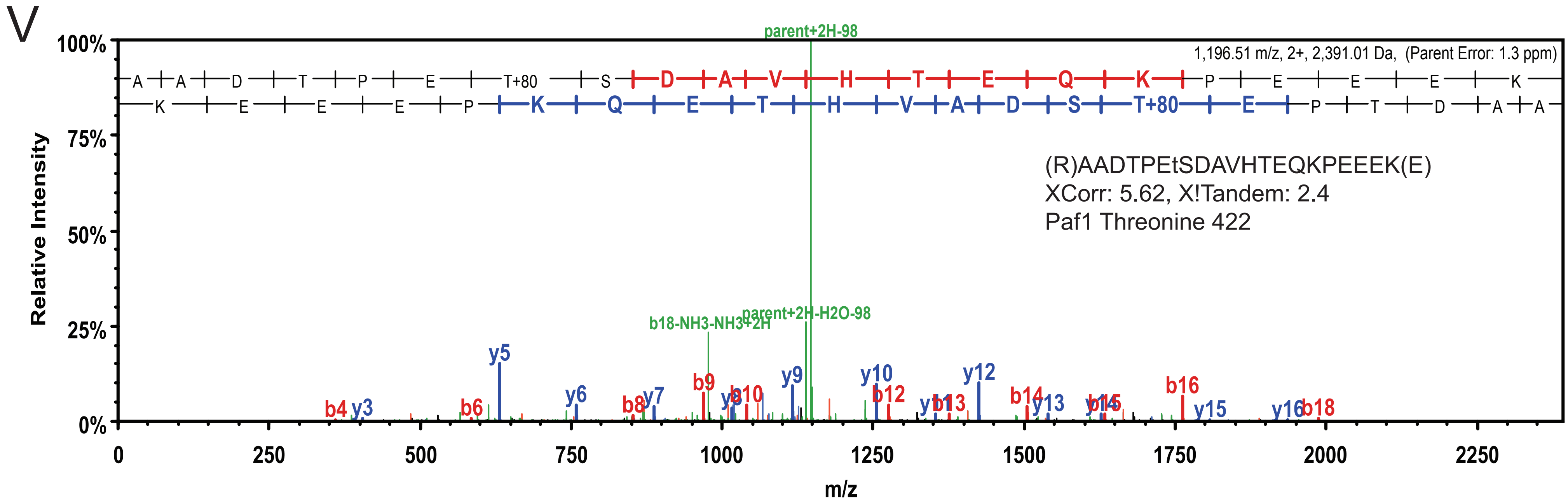
R



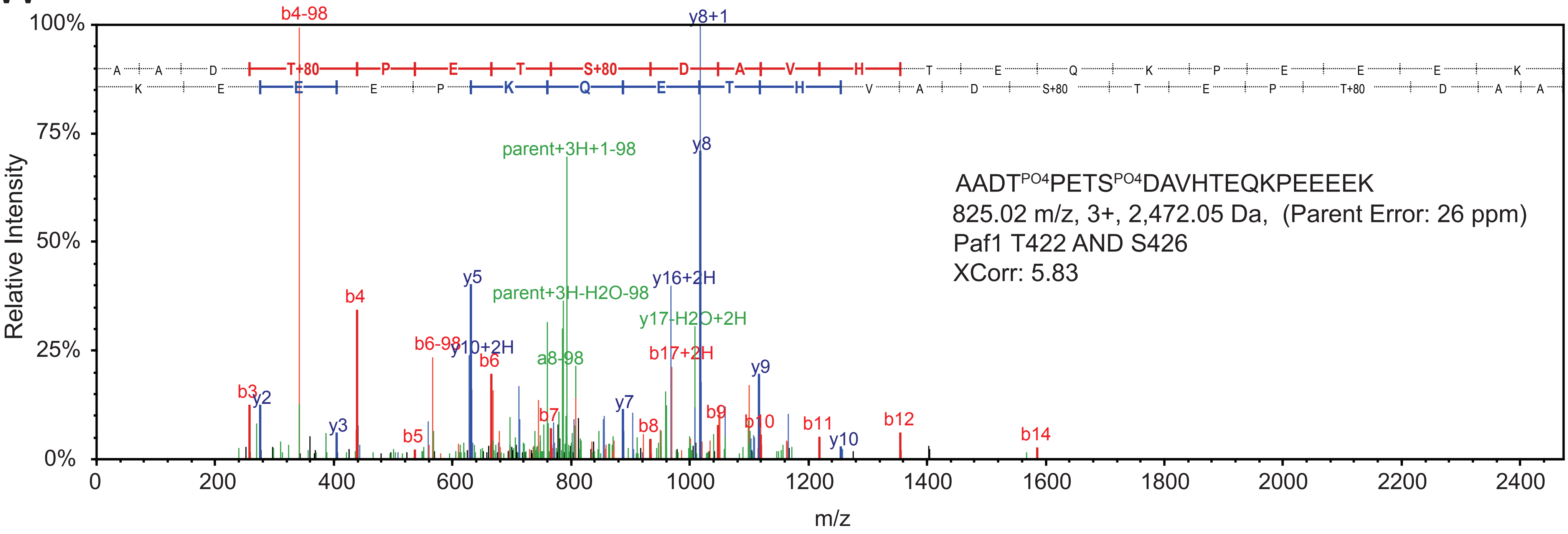
1,387.67 m/z, 2+, 2,773.33 Da, (Parent Error: -4.4 ppm)
L L V Y P E S+80 P E T N A D S S Q L I N S L Y I K
K I Y L S N I L Q S S D A N T E P S+80 E P Y V L L
(K)LLVYPEsPETNADSSQLINSLYIK(T)
XCorr: 5.24, X!Tandem:5.10
Paf1 Serine 32
y20+2H
y21+2H
parent+2H-98
y6
y11
y17
y17+1
y17+2
y7
y8
y19+2H
y11+1
y3
y14+2H
y6+1
y20-H2O+2H
y21-H2O+2H
y20+2H+1
b21+2H
parent+2H+1-98
y13
y13+1
b13
y14
y14+1
y15
y5
y9
b9
y10
b4
y4
b5
b6
b10
b11
b12
b14
y16
b16
b17
Relative Intensity
100%
75%
50%
25%
0%
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
2750
m/z


S
parent+2H+1-98
1,111.96 m/z, 2+, 2,221.91 Da, (Parent Error: -3.0 ppm)
L D D G D S+80 D D E N L D V N H I I S R
R S I I I H N V D L N E D D S+80 D G D D L
(R)LDDGDsDDENLDVNHIISR(V)
XCorr: 5.83, X!Tandem: 4.17
Paf1 Serine 147
Relative Intensity
100%
75%
50%
25%
0%
y3 y4 y5 y6 y6+1 y7 y7+1 y8 y9 y10 y11 y12 y13 y14 y17
b3 b5 b6 b7 b8 b9 b11 b12 b13 b14 b15 b16 b17
parent+2H-NH3-98
parent+2H-H2O
0 250 500 750 1000 1250 1500 1750 2000
m/z
T
1,015.91 m/z, 2+, 2,029.81 Da, (Parent Error: 6.2 ppm)
parent+2H-98
T E G S+80 E Q E G E N E K D E E I K
K I E E D K E N E G E Q E S+80 G E T
(K)TEGS^PO4EQEGENEKDEEIK(Q)
XCorr:4.40
Paf1 Serine 388 OR Threonine 385
Relative Intensity
100%
75%
50%
25%
0%
y3 y4 y5 y6 y7 y8 y9 y10 y11 y12 y13
b3 b5 b6 b7 b8 b9 b10 b11 b12 b13 b14 b15 b16
0 200 400 600 800 1000 1200 1400 1600 1800 2000
m/z

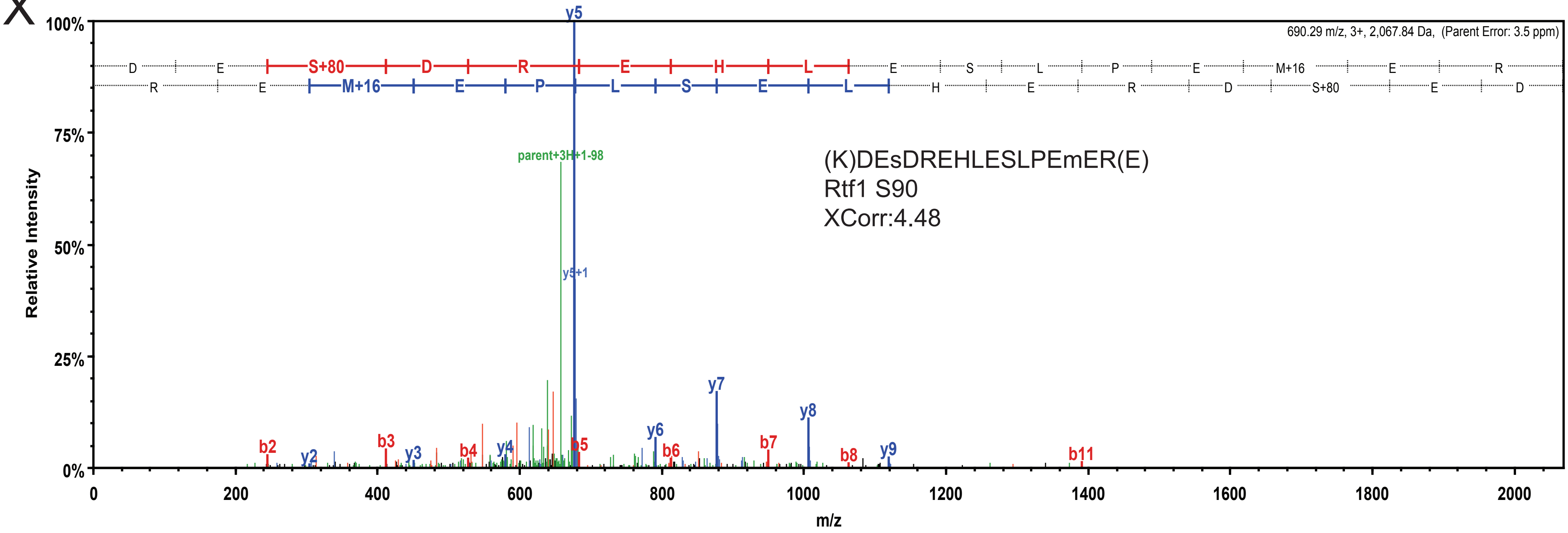

U
parent+3H-H2O-98
948.58 m/z, 3+, 2,842.71 Da, (Parent Error: -120 ppm)
E S E G D S K T+80 E G S+80 E Q E G E N E K D E E I K
K I E E D K E N E G E Q E S+80 G E T+80 K S D G E S E
(K)ESEGDSKtEGsEQEGENEKDEEIK(Q)
Paf1 Serine 388 AND Threonine 385
xcORR: 4.61
parent+3H+1-98
c17-98+2H
b5
y3
y5
b7
y6
y7
b8
y8
b9
b10
y9
y10
b13
b14
100%
75%
50%
25%
0%
Relative Intensity
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
2750
m/z


V
parent+2H-98
1,196.51 m/z, 2+, 2,391.01 Da, (Parent Error: 1.3 ppm)
A A D T P E T+80 S D A V H T E Q K P E E E K
K E E E P K Q E T H V A D S T+80 E P T D A A
(R)AADTPEtSDAVHTEQKPEEEK(E)
XCorr: 5.62, X!Tandem: 2.4
Paf1 Threonine 422
b18-NH3-NH3+2H
parent+2H-H2O-98
b4
y3
y5
b6
y6
b8
y7
b9
y8
b10
y9
y10
b12
y11
b13
y12
b14
y13
y14
b15
b16
y15
y16
b18
100%
75%
50%
25%
0%
Relative Intensity
0
250
500
750
1000
1250
1500
1750
2000
2250
m/z

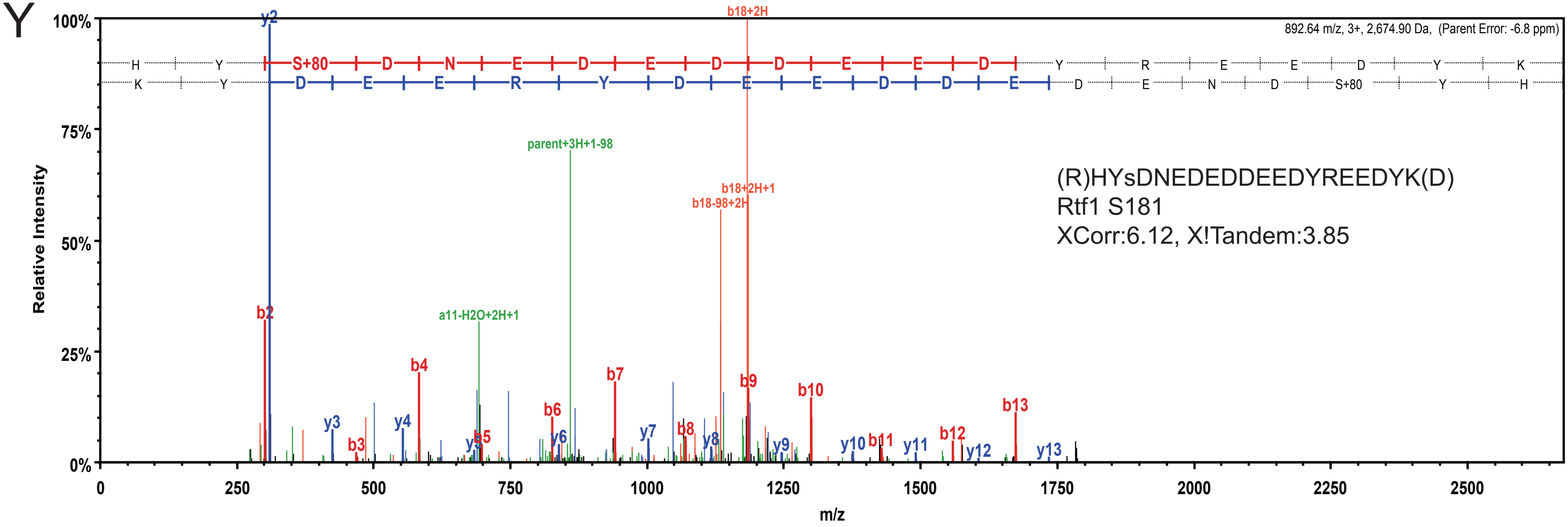
W


100%
75%
50%
25%
0%
Relative Intensity
m/z
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
A A D T+80 P E T S+80 D A V H T E Q K P E E E K
K E E E P K Q E T H V A D S+80 T E P T+80 D A A
b4-98
y8+1
parent+3H+1-98
y8
y5
parent+3H-H2O-98
y16+2H
b4
y17-H2O+2H
b6-98
y10+2H
b6
a8-98
b17+2H
y9
b3
y2
y7
b7
b9
y3
b8
b10
b11
b12
b5
y10
b14
AADT^PO4PETS^PO4DAVHTEQKPEEEEK
825.02 m/z, 3+, 2,472.05 Da, (Parent Error: 26 ppm)
Paf1 T422 AND S426
XCorr: 5.83

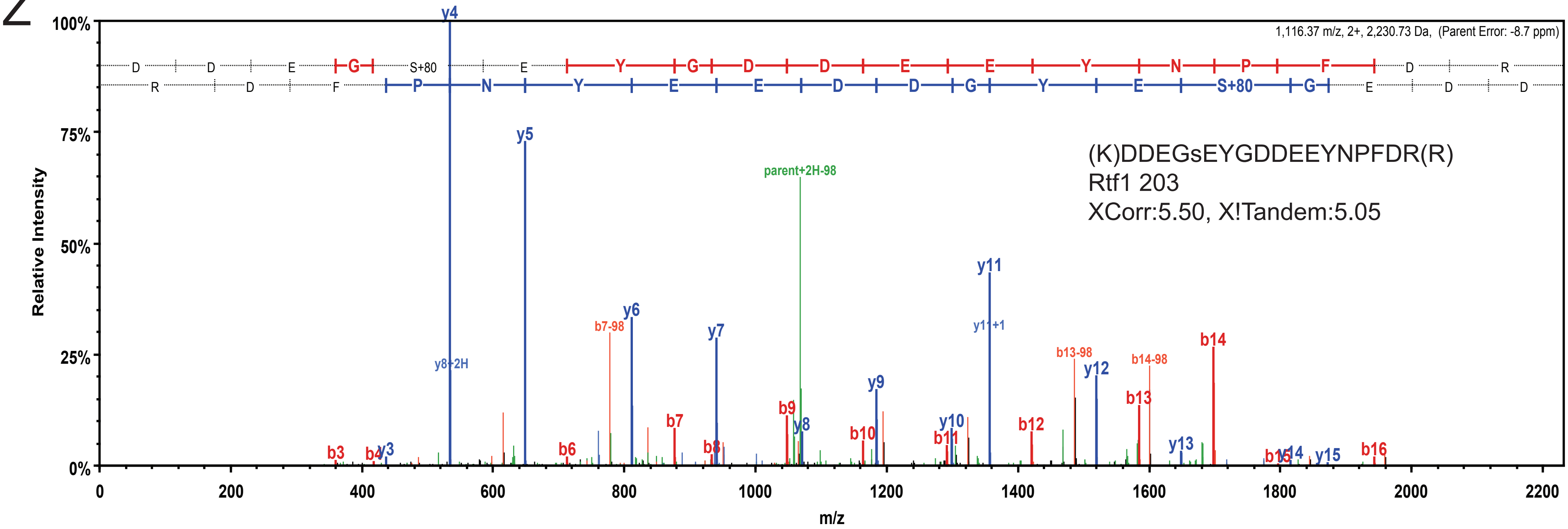
X


100%
75%
50%
25%
0%
Relative Intensity
m/z
0
200
400
600
800
1000
1200
1400
1600
1800
2000
690.29 m/z, 3+, 2,067.84 Da, (Parent Error: 3.5 ppm)
D E S+80 D R E H L E S L P E M+16 E R
R E M+16 E P L S E L H E R D S+80 E D
y5
parent+3H+1-98
y5+1
y7
y8
y6
b2
y2
b3
y3
b4
y4
b5
b6
b7
b8
y9
b11
(K)DEsDREHLESLPEmER(E)
Rtf1 S90
XCorr:4.48

Y

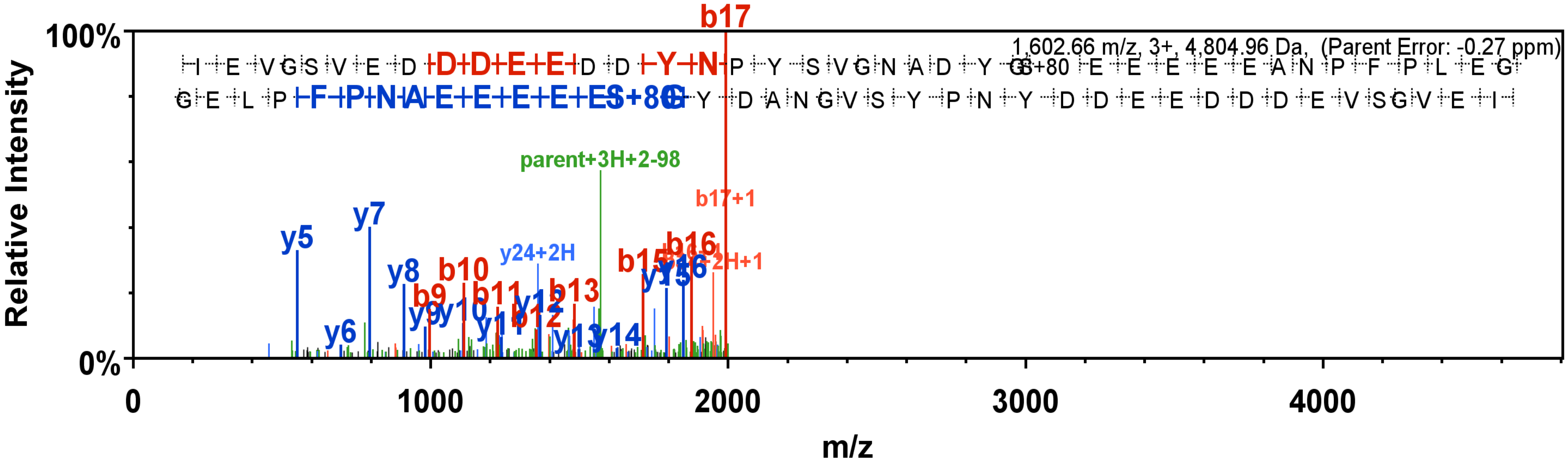

892.64 m/z, 3+, 2,674.90 Da, (Parent Error: -6.8 ppm)
H Y S+80 D N E D E D D E E D Y R E E D Y K
K Y D E E R Y D E E D D E D E N D S+80 Y H
(R)HYsDNEDEDDEEDYREEDYK(D)
Rtf1 S181
XCorr:6.12, X!Tandem:3.85
Relative Intensity
0%
25%
50%
75%
100%
m/z
0
250
500
750
1000
1250
1500
1750
2000
2250
2500
y2
b18+2H
parent+3H+1-98
b18+2H+1
b18-98+2H
b2
a11-H2O+2H+1
b4
b7
b9
b10
b13
b6
y3
y4
b3
y5
b5
y6
y7
b8
y8
y9
y10
b11
y11
b12
y12
y13

Z


1,116.37 m/z, 2+, 2,230.73 Da, (Parent Error: -8.7 ppm)
D D E G S+80 E Y G D D E E Y N P F D R
R D F P N Y E E D D G Y E S+80 G E D D
(K)DDEGsEYGDDEEYNPFDR(R)
Rtf1 203
XCorr:5.50, X!Tandem:5.05
Relative Intensity
0%
25%
50%
75%
100%
m/z
0
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
y4
y5
parent+2H-98
y11
y11+1
y6
b7-98
y7
y8+2H
b14
b13-98
b14-98
y12
y9
b13
b9
b7
y8
b12
y10
b10
b11
y13
b8
b6
b3
b4
y3
b15
y14
y15
b16

AA

Rtf1 S71 phosphorylation RIEVGSVEDDDEEDDYNPYSVGNADYGS$_{PO4}$EEEEEANPFPLEGK peptide



End of Figure S1