

**Proteome-wide identification of lysine 2-hydroxyisobutyrylation  
reveals conserved and novel histone modifications in *Physcomitrella*  
*patens***

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**Figure S1. MS/MS spectra for all histone K<sub>hib</sub> sites.**

**Figure S2. Canonical histone protein alignments among human, mouse and *P. patens*.** (A) H1.2, (B) H2A, (C) H2B, (D) H3 and (E) H4. Amino acid substitutions are indicated by red arrow heads.

**Figure S3. Protein alignments of different H2A and H3 proteins in *P. patens*.** K<sub>hib</sub> sites were marked with red dots.

**Figure S4. No differentiated antherid or archegonium are found in *P. patens*.** (A) The morphology of *P. patens* used in our experiment. (B) Microscopy of dissected moss plants. There is no differentiated antherid or archegonium. Bars = 0.5 mm.

**Table S1. Basic information on K<sub>hib</sub>-modified proteins in *P. patens*.** Protein accession: protein accession number from the database used for the search. Position: position of the post-translational modification in the protein. Amino acid: amino acid modified by the post-translational modification. Protein description: protein function. Gene name: indicates the name of the gene that codes for the protein sequence. Score: analysis result of Mascot, a simple rule to be used to judge whether a result is significant or not. Modified sequence: modified peptide sequences with localization probabilities. Mass error (ppm): parts per million deviation of peptide mass errors between the theoretical and actual values. Charge: carried charge of the peptide. Localization probability: probability of a certain amino acid having this modification in this peptide.

**Table S2. Summary of LC-MS/MS results.**

**Table S3. Annotation and description of K<sub>hib</sub>-modified proteins in *P. patens*.**

**Table S4. GO enrichment analysis of K<sub>hib</sub>-modified proteins in *P. patens*.**

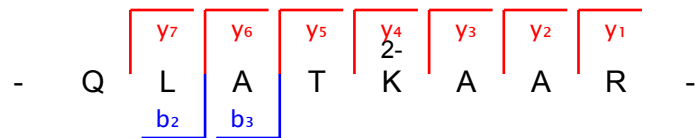
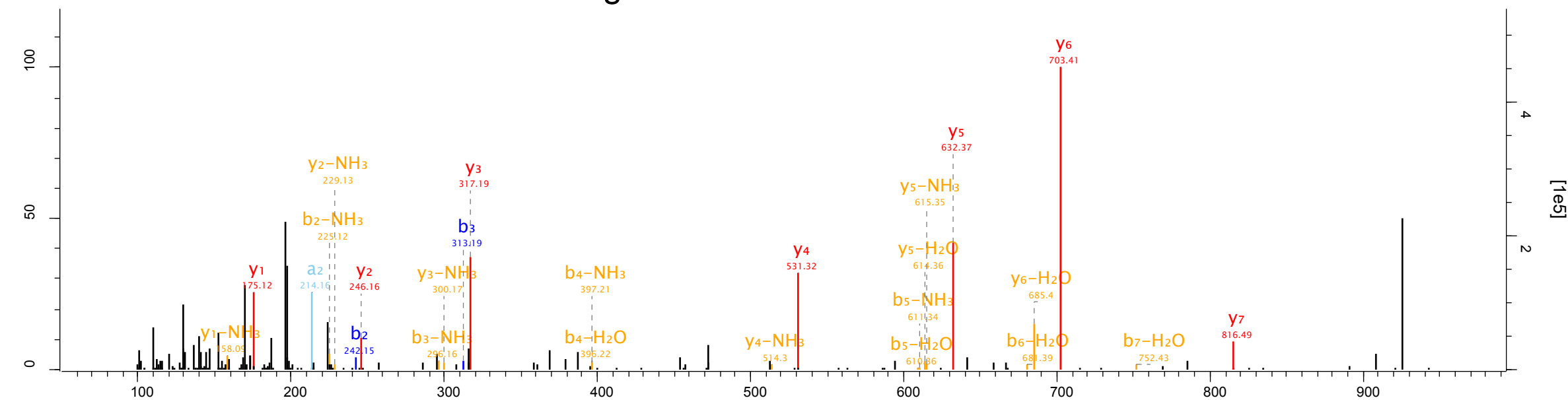
**Table S5. KEGG enrichment analysis of K<sub>hib</sub>-modified proteins in *P. patens*.**

**Table S6. Protein domain enrichment analysis of K<sub>hib</sub>-modified proteins in *P. patens*.**

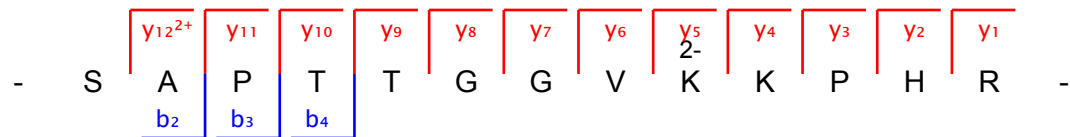
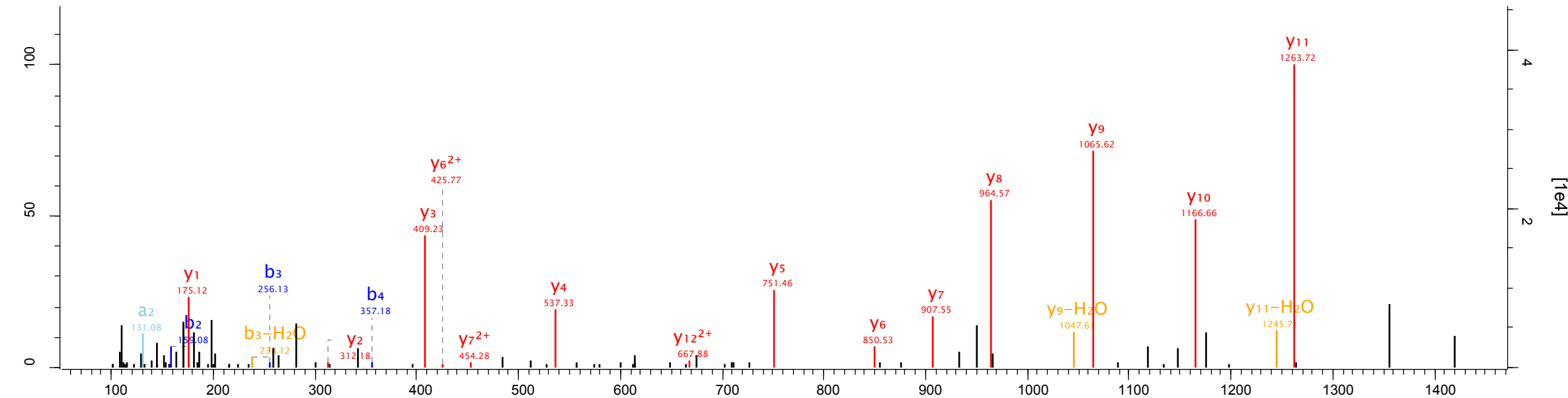
**Table S7. K<sub>hib</sub>-modified histone proteins in *P. patens*.** Information of canonical histones are marked in red.

Raw file Scan Method Score m/z  
 3 2158 FTMS; HCD 110.12 472.78

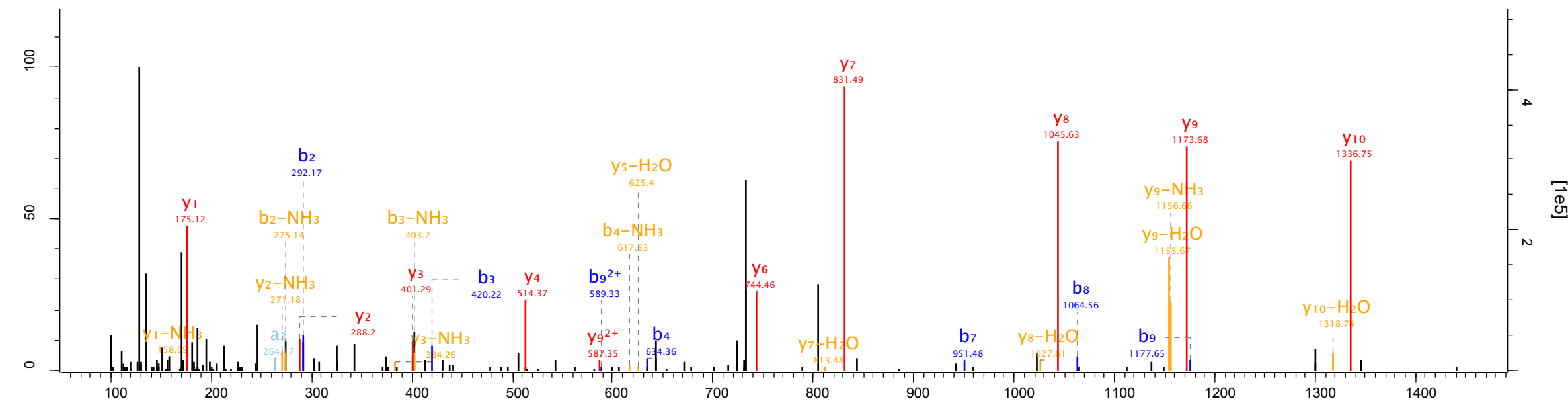
# Figure S1



Raw file Scan Method Score m/z  
 2 1391 FTMS; HCD 87.72 711.9



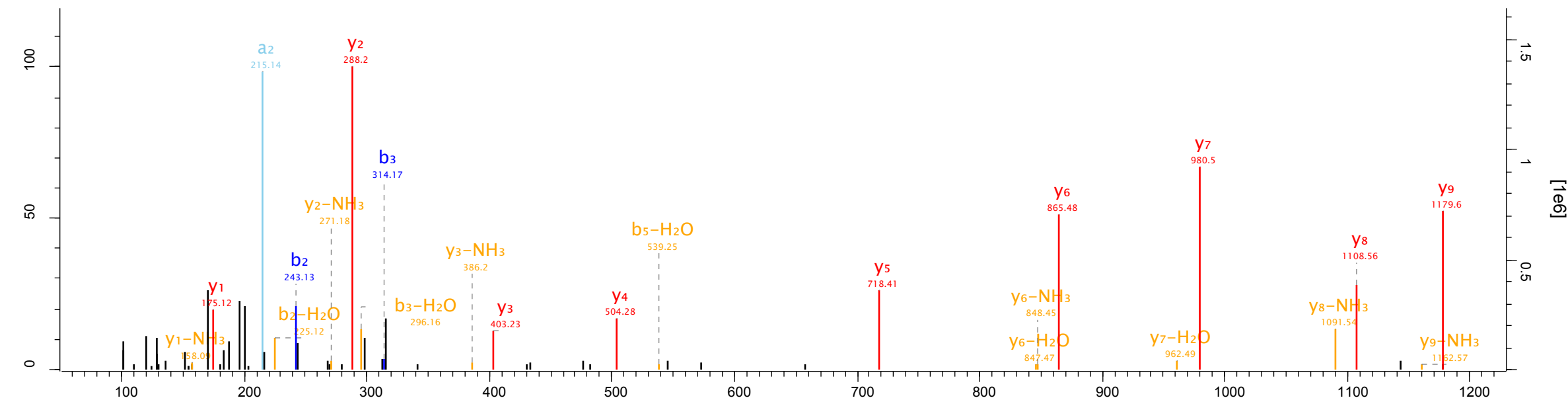
Raw file Scan Method Score m/z  
 6 5404 FTMS; HCD 146.16 732.93



- K Y Q K S T E L L I R -

b<sub>2</sub> b<sub>3</sub> b<sub>4</sub> b<sub>7</sub> b<sub>8</sub> b<sub>9</sub>

Raw file Scan Method Score m/z  
 6 8986 FTMS; HCD 111.86 711.37

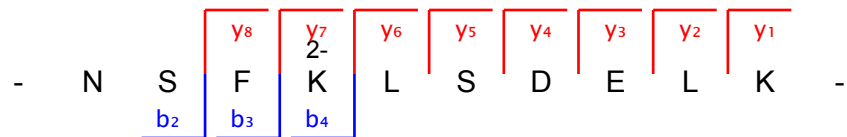
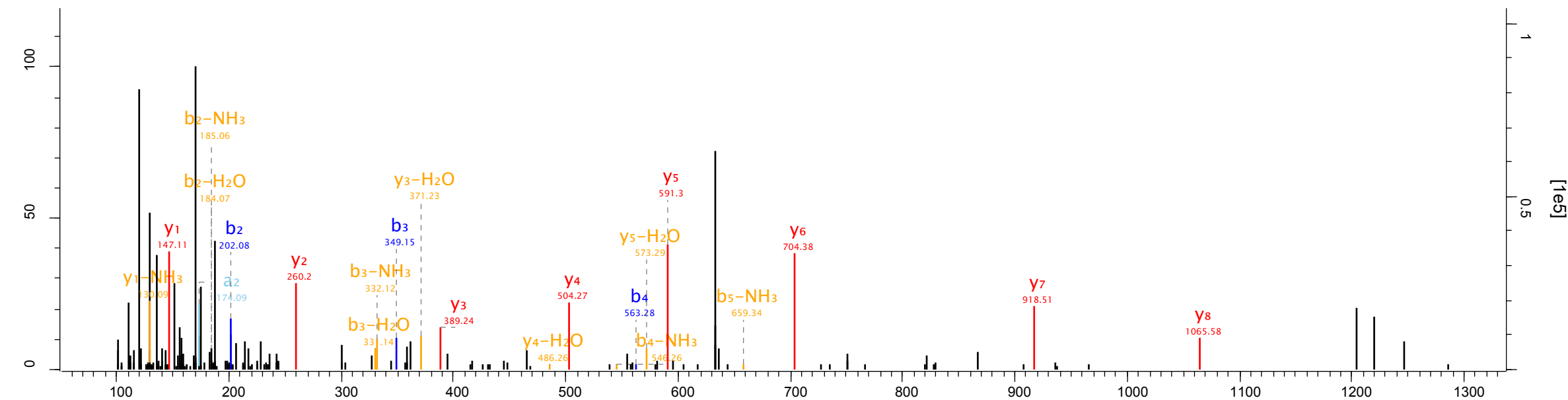


- E I A Q D F K T D L R -

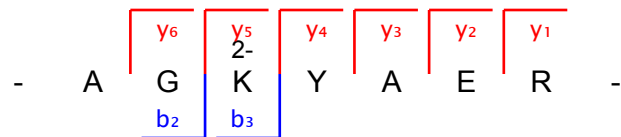
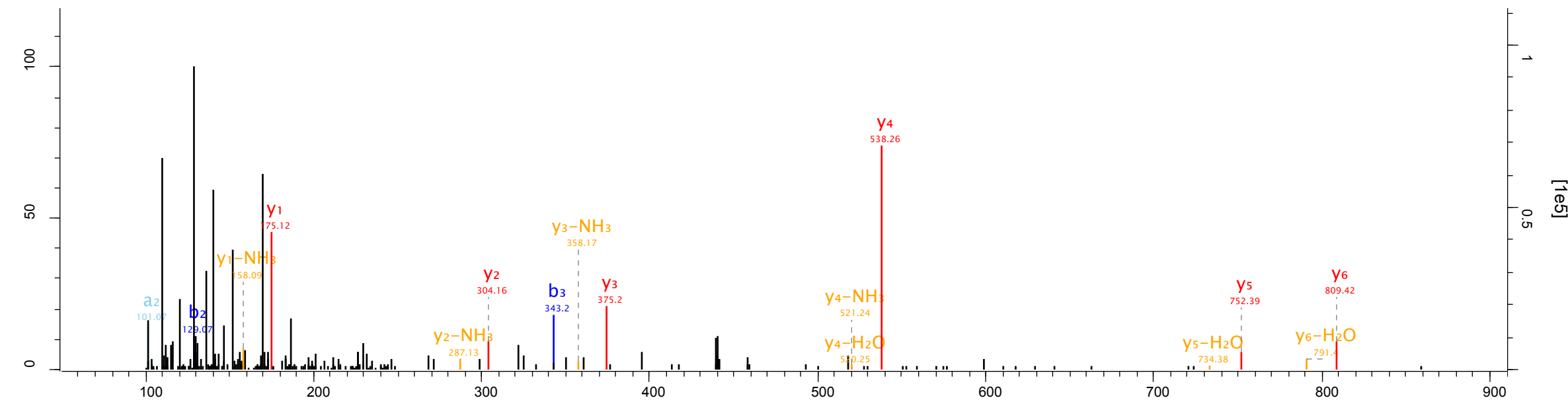
b2 b3

y9 y8 y7 y6 y5 2- y4 y3 y2 y1

Raw file Scan Method Score m/z  
 2 7558 FTMS; HCD 65.84 633.83

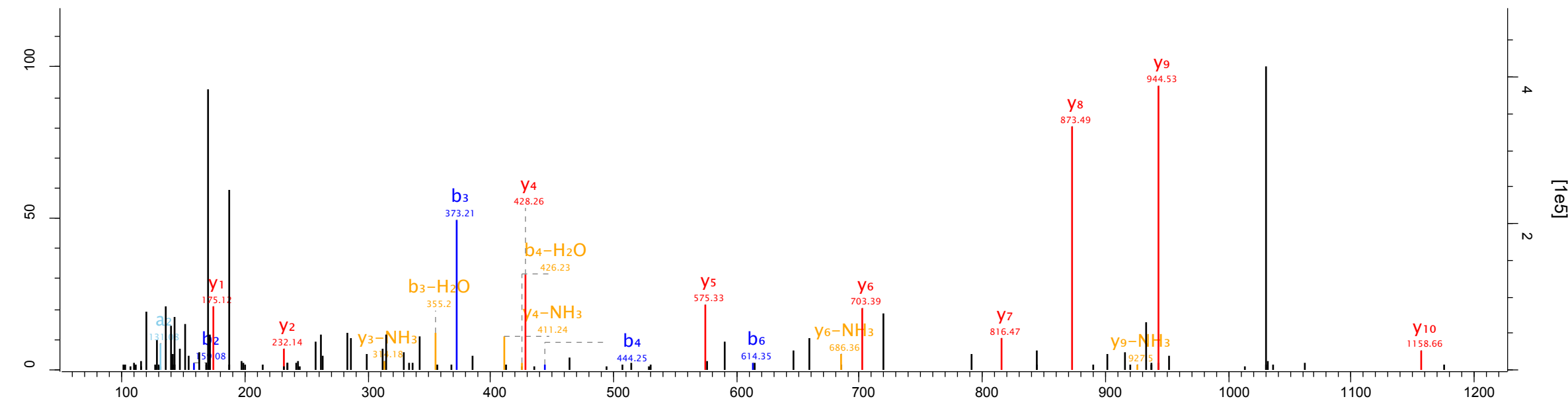


Raw file	Scan	Method	Score	m/z
4	1749	FTMS; HCD	60.51	440.73





Raw file Scan Method Score m/z  
 3 8562 FTMS; HCD 67.88 658.87

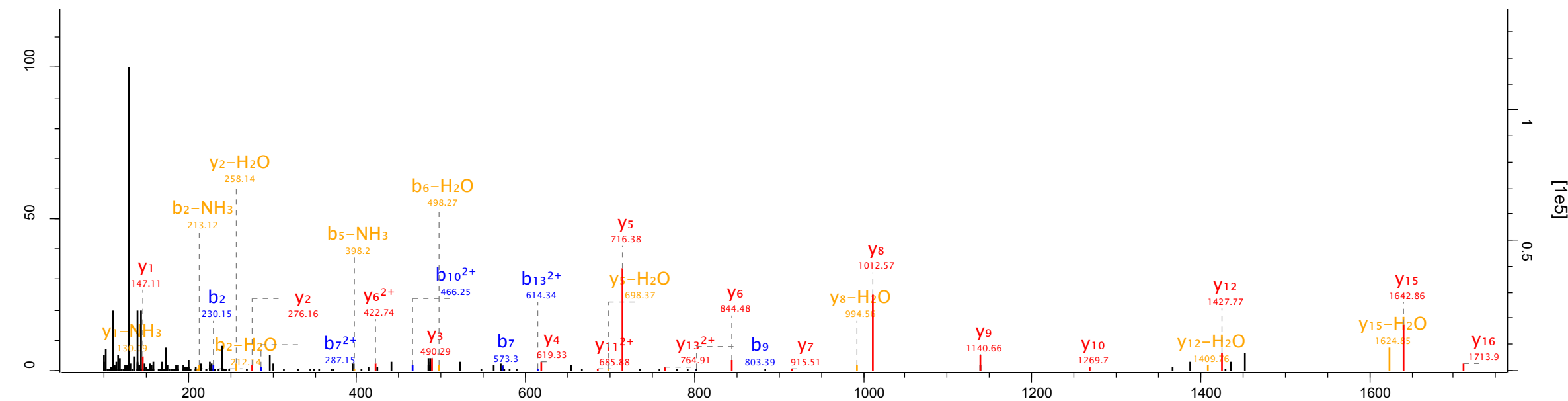


- S A K A G L Q F P V G R -

Fragmentation mapping (b and y ions):

- b2: A
- b3: K
- b4: A
- b6: L
- y1: R
- y2: G
- y4: P
- y5: F
- y6: Q
- y7: L
- y8: G
- y9: A
- y10: K

Raw file Scan Method Score m/z  
 4 1136 FTMS; HCD 78.67 486.52

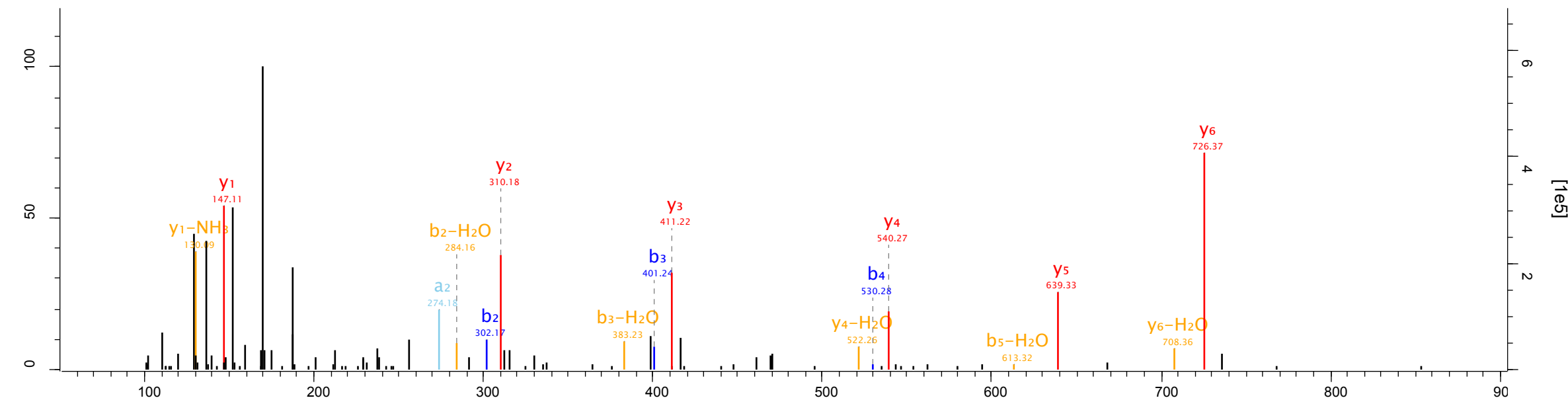


- K T A G G T G T E K P A K P E K -

Fragmentation sites (b and y ions) are indicated by brackets below the sequence:

- b<sub>2</sub> (under T)
- b<sub>7</sub> (under G)
- b<sub>9</sub> (under E)
- b<sub>10</sub><sup>2+</sup> (under K)
- b<sub>13</sub><sup>2+</sup> (under K)
- y<sub>1</sub> (under K)
- y<sub>2</sub> (under E)
- y<sub>3</sub> (under K)
- y<sub>4</sub> (under E)
- y<sub>5</sub> (under P)
- y<sub>6</sub> (under K)
- y<sub>7</sub> (under A)
- y<sub>8</sub> (under P)
- y<sub>9</sub> (under K)
- y<sub>10</sub> (under E)
- y<sub>11</sub><sup>2+</sup> (under T)
- y<sub>12</sub> (under G)
- y<sub>13</sub><sup>2+</sup> (under T)
- y<sub>15</sub> (under G)
- y<sub>16</sub> (under A)

Raw file	Scan	Method	Score	m/z
1	2569	FTMS; HCD	105.14	470.75

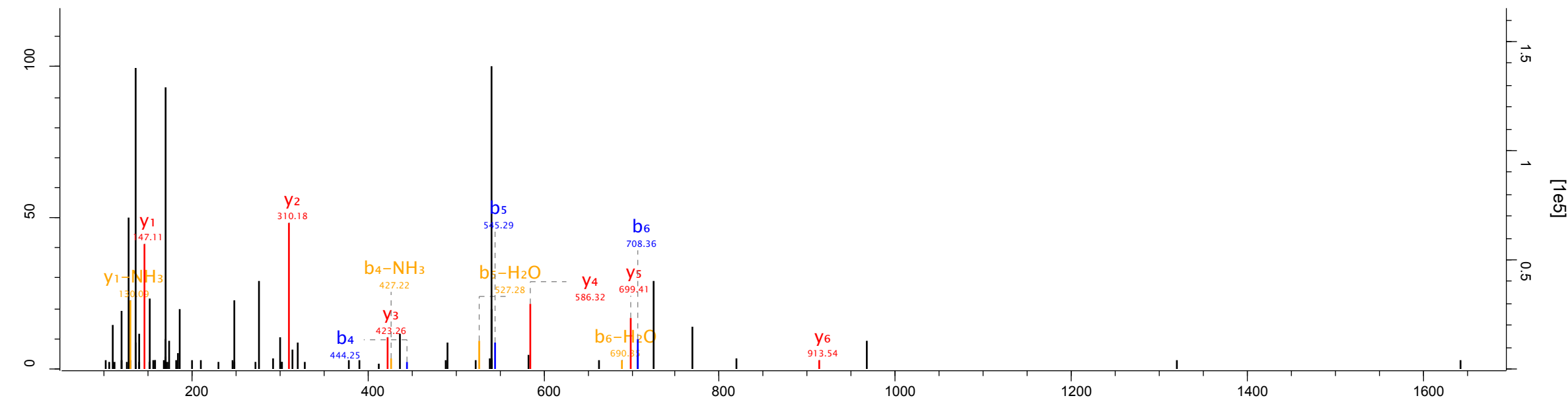


2-  
K -

y6	y5	y4	y3	y2	y1
S	V	E	T	Y	K
b2	b3	b4			

-

Raw file	Scan	Method	Score	m/z
2	7857	FTMS; HCD	49.63	541.3

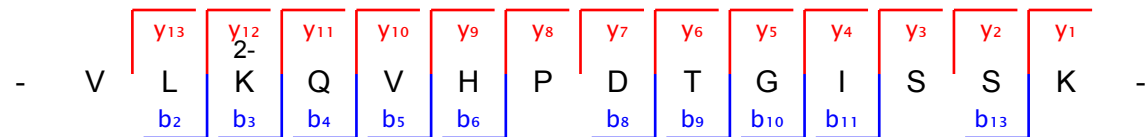
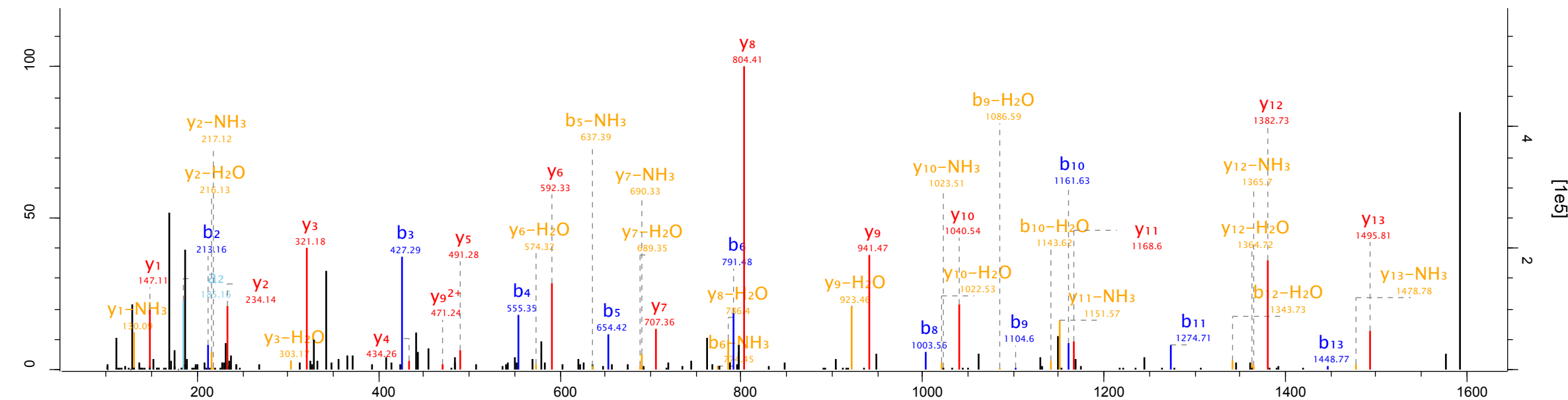


- K S V E T Y K I Y I Y K -

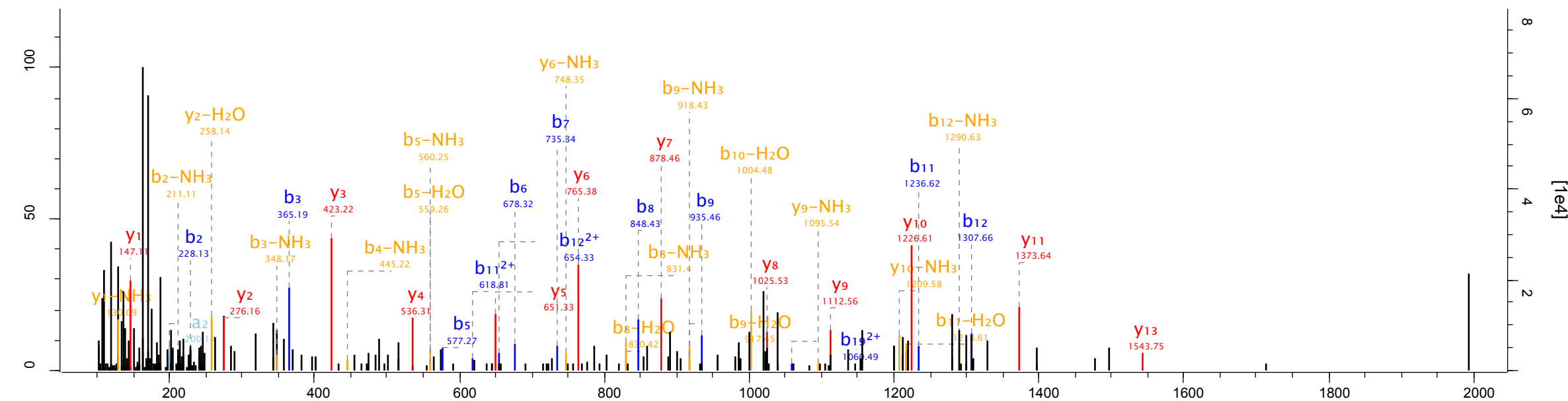
Fragmentation scheme (b and y ions):

- $b_4$  (E-T)
- $b_5$  (E-T-Y)
- $b_6$  (E-T-Y-K)
- $y_1$  (K)
- $y_2$  (Y)
- $y_3$  (I)
- $y_4$  (Y)
- $y_5$  (I)
- $y_6$  (K)

Raw file Scan Method Score m/z  
 6 3762 FTMS; HCD 198.87 797.95



Raw file Scan Method Score m/z  
 2 13041 FTMS; HCD 87.2 1000.16

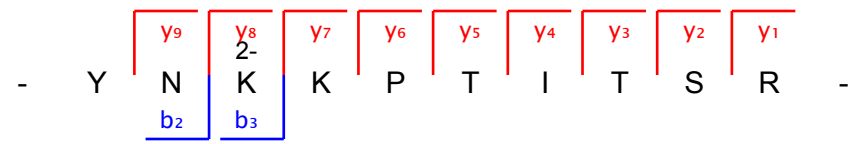
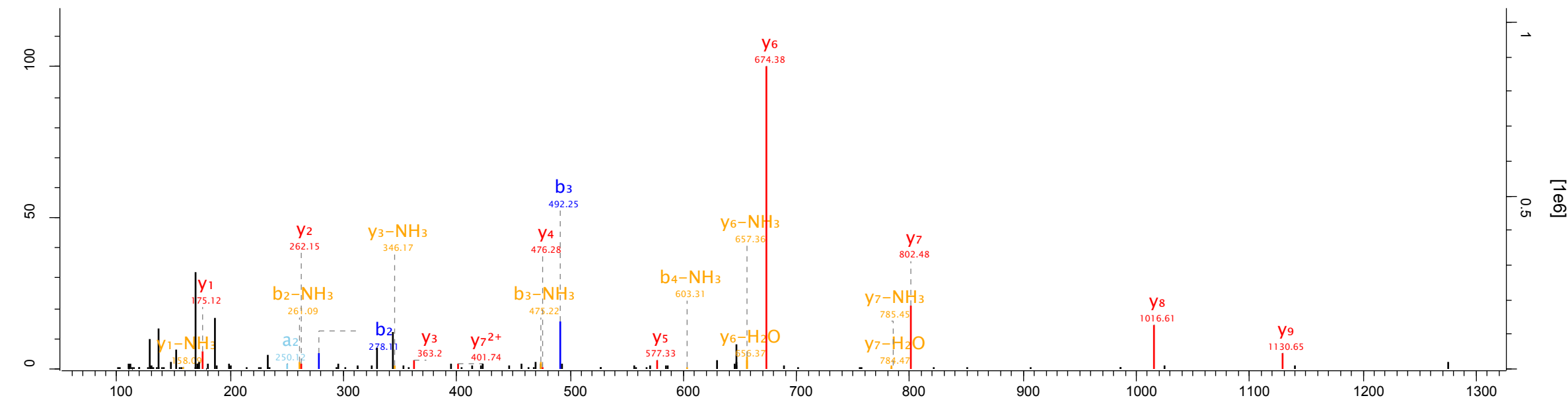


- Q V H P D T G I S S 2-K A ox M G I M N S F I N D I F E K -

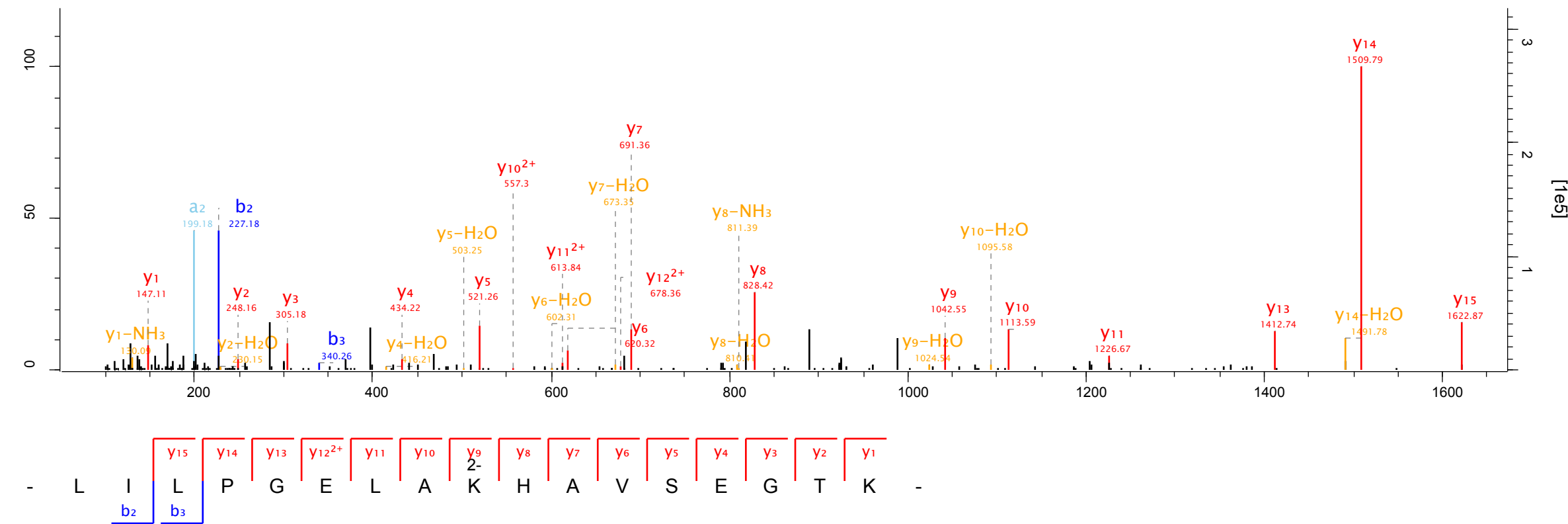
Fragmentation mapping (b and y ions):

- Blue boxes: b<sub>2</sub> (V-H), b<sub>3</sub> (H-P), b<sub>5</sub> (D-T), b<sub>6</sub> (T-G), b<sub>7</sub> (G-I), b<sub>8</sub> (I-S), b<sub>9</sub> (S-S), b<sub>11</sub> (2-K-A), b<sub>12</sub> (A-ox), b<sub>19<sup>2+</sup></sub> (F-I)
- Red boxes: y<sub>13</sub> (M-G), y<sub>11</sub> (I-M), y<sub>10</sub> (M-N), y<sub>9</sub> (N-S), y<sub>8</sub> (S-F), y<sub>7</sub> (F-I), y<sub>6</sub> (I-N), y<sub>5</sub> (N-D), y<sub>4</sub> (D-I), y<sub>3</sub> (I-F), y<sub>2</sub> (F-E), y<sub>1</sub> (E-K)

Raw file Scan Method Score m/z  
 2 2553 FTMS; HCD 104.43 647.36

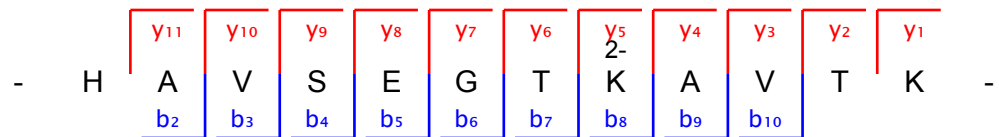
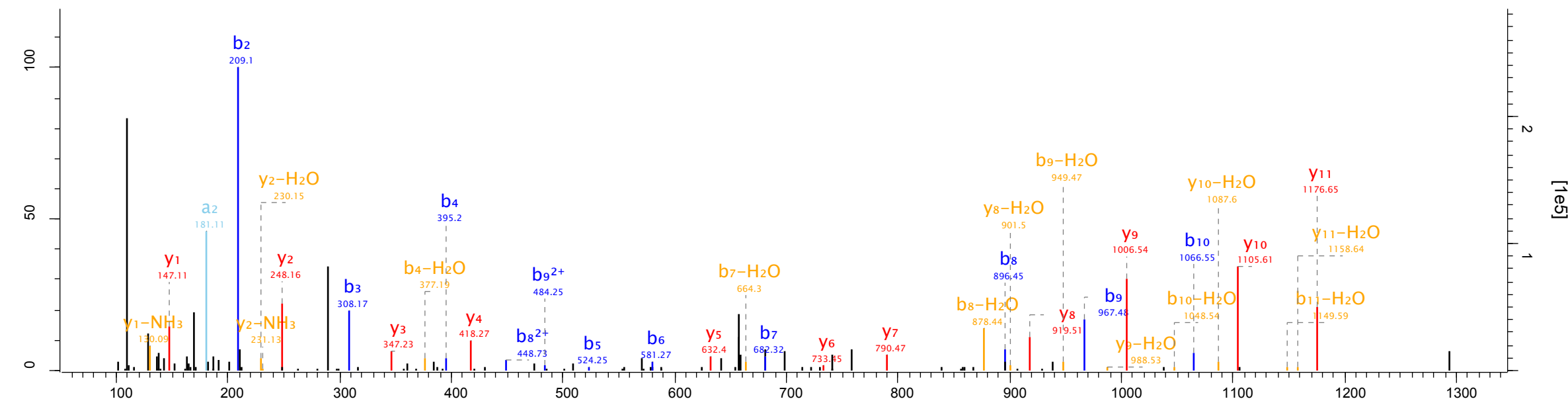


Raw file	Scan	Method	Score	m/z
4	9960	FTMS; HCD	112.67	925.53

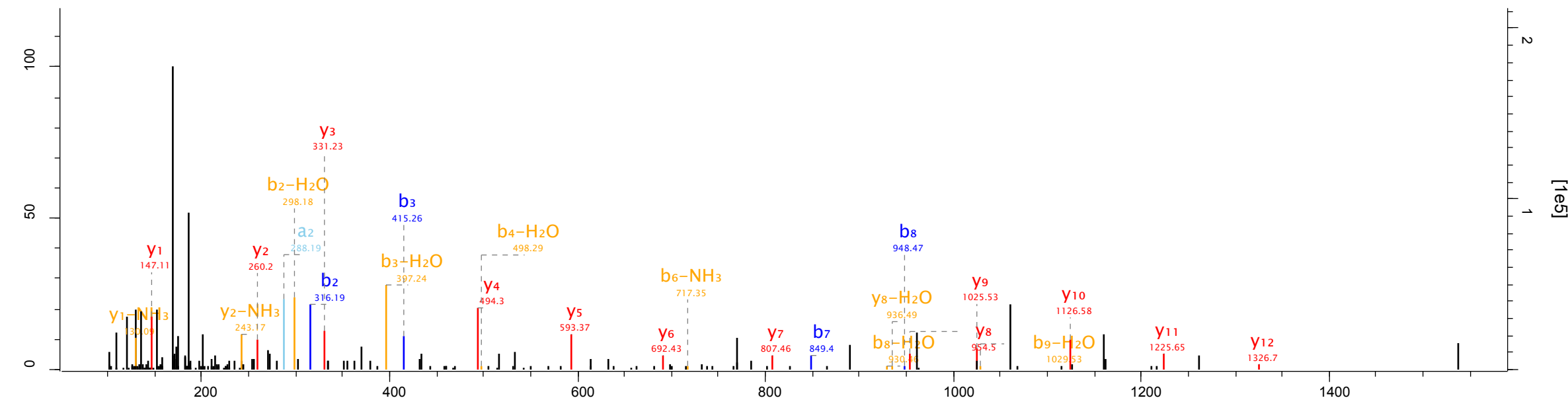




Raw file Scan Method Score m/z  
 1 1739 FTMS; HCD 205.31 657.36

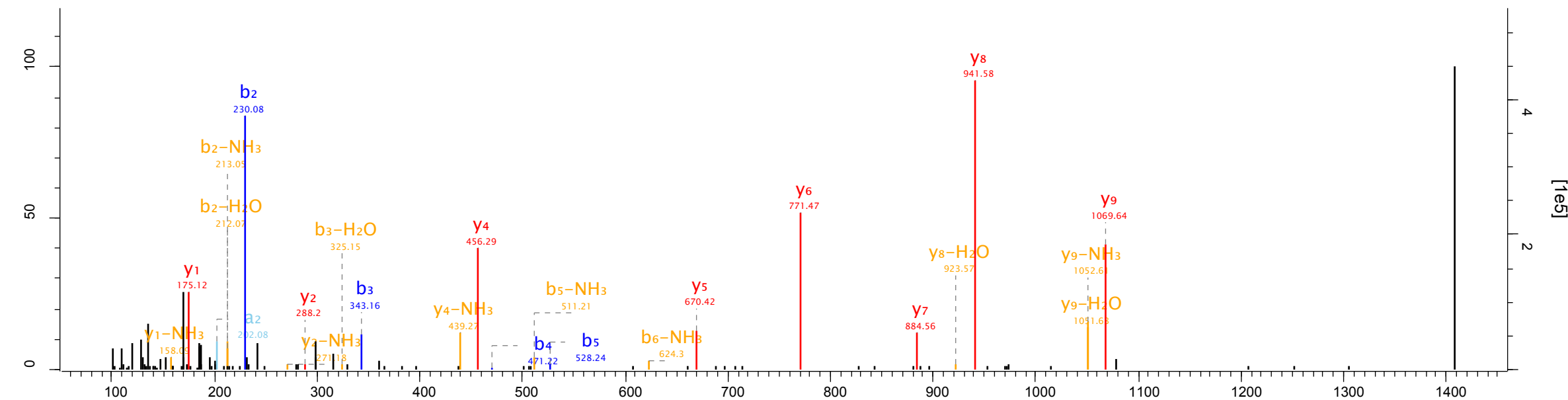


Raw file Scan Method Score m/z  
 6 9967 FTMS; HCD 103.76 770.92



2-  
 - K [ y12 ] [ y11 ] [ y10 ] [ y9 ] [ y8 ] [ y7 ] [ y6 ] [ y5 ] [ y4 ] [ y3 ] [ y2 ] [ y1 ]  
 T V T A M D V V Y A L K -  
 b2 b3 b7 b8

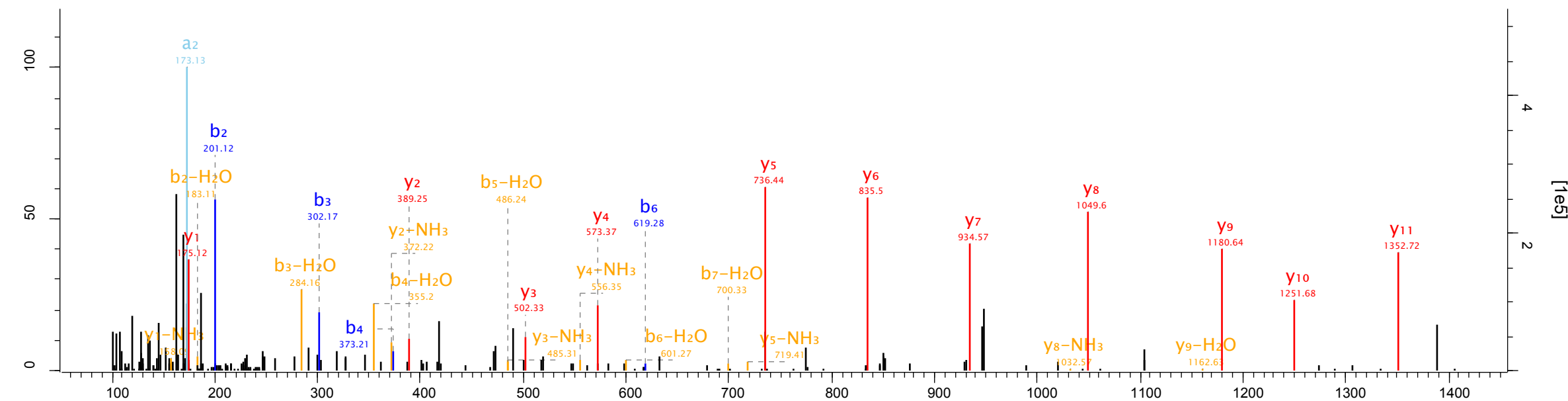
Raw file	Scan	Method	Score	m/z
3	8477	FTMS; HCD	117.79	706.4



- D N I Q G I T K P A I R -

b<sub>2</sub> b<sub>3</sub> b<sub>4</sub> b<sub>5</sub> y<sub>9</sub> y<sub>8</sub> y<sub>7</sub> y<sub>6</sub> y<sub>5</sub> y<sub>4</sub> y<sub>2</sub> y<sub>1</sub>

Raw file	Scan	Method	Score	m/z
6	13135	FTMS; HCD	138.42	776.93

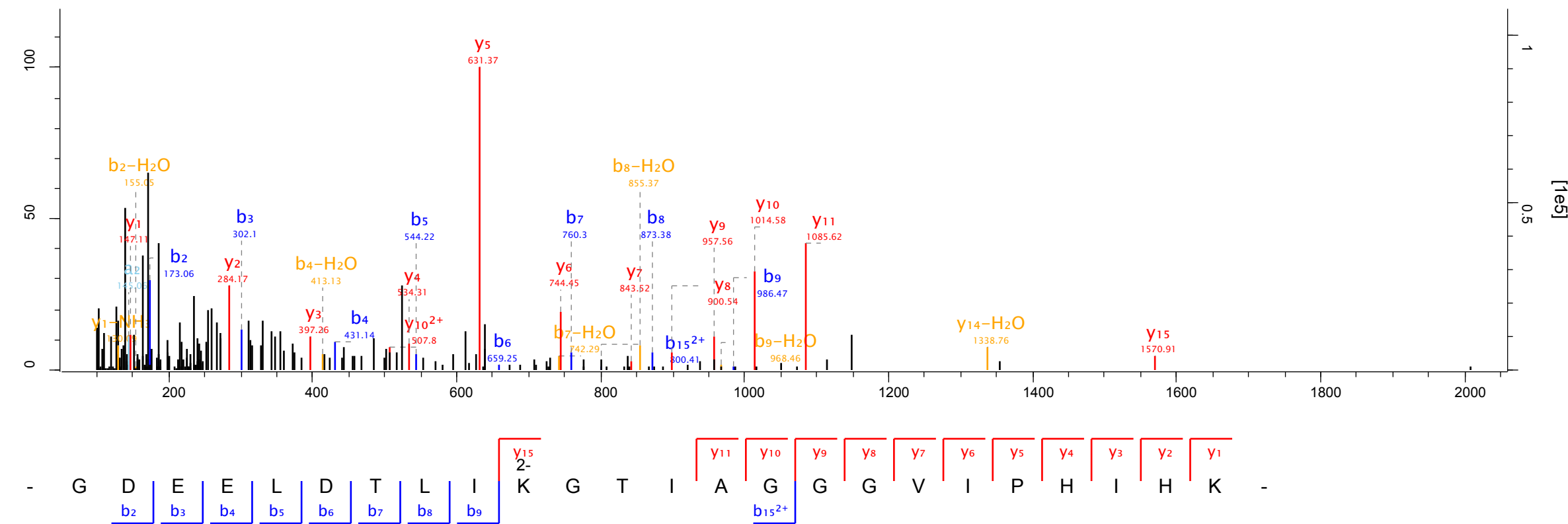


- T V T A M D V V Y A L K R -

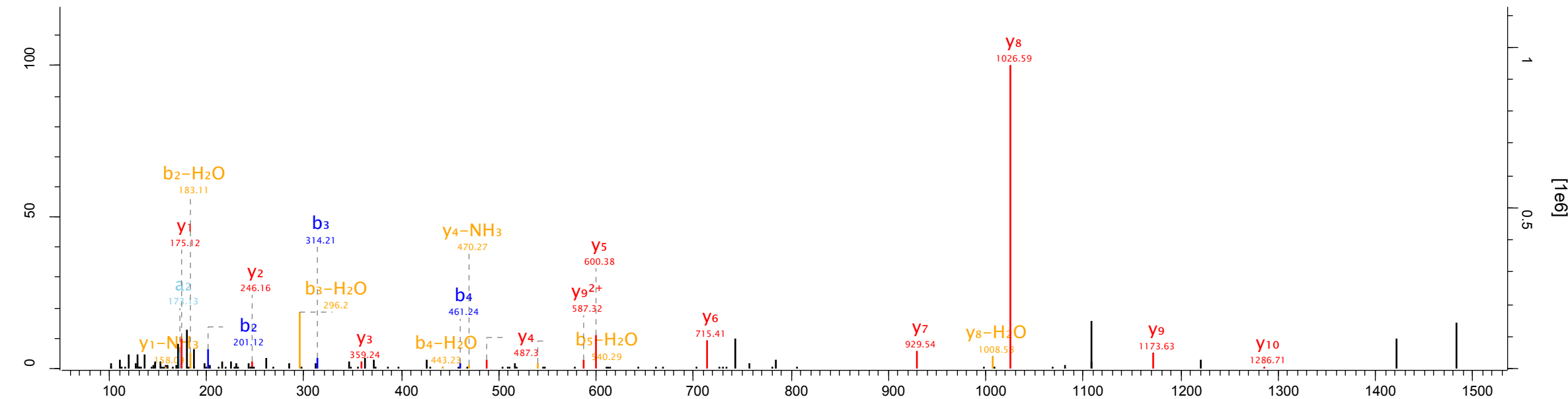
b2 b3 b4 b6

y11 y10 y9 y8 y7 y6 y5 y4 y3 y2 y1

Raw file	Scan	Method	Score	m/z
2	12903	FTMS; HCD	55.3	640.1



Raw file	Scan	Method	Score	m/z
1	8817	FTMS; HCD	99.54	743.92



- V T I M P K D L Q L A R -  
 b2 b3 b4 y10 y9 OX y8 y7 2- y6 y5 y4 y3 y2 y1

# Figure S2

**A**

HumanH1.2 : MSETAPAAPAAPPAEKAPVKKKAARKAGCTPRKASGPPVSELLTKAVAASKERSGVSLAALKKALAAAGYDVEKNNRSRI  
 MouseH1.2 : MSEAPAAPAAPPAEKAPAKKKAARKPACVRRKASGPPVSELLTKAVAASKERSGVSLAALKKALAAAGYDVEKNNRSRI  
 MossH1 : MADVVESTIPAVVVEATASEVVEEAPVVEKAAKSEKGGKMKVLEKVKVEVTVKEPKATKAKAPKAPKPPASHPTYMLMVVE

HumanH1.2 : KLGLKSLVSKGTLVOTKGTGASGSFKLNKKAASGEAKPKVKAGGTTPKPPVGAARKPKKAACGATPKKSAKKTPKKAKK  
 MouseH1.2 : KLGLKSLVSKGITLVOTKGTGASGSFKLNKKAASGEAKPAKKAAGAAAKKPAAGAAKPKKATCAATPKKAAKKTTPKAKK  
 MossH1 : ALGALKERITGSSQYATAKYLEDKYKTGLAPNFKMLTIQLRNLTIKGGKLVKIKNSFKLSEDELKKPAKTKSAGEGVAKSKA

HumanH1.2 : PAAATVTKKVAKSPPKAKVAKPKKAAKSAKAVPKAKPKVVPKKAPKKK-----  
 MouseH1.2 : PAAAATVTKKVAKSPKKAKVTKPKKVKASAKVTPKAAKPKVAKAKVAKKK-----  
 MossH1 : PSTKGAKETKSESKVTKVAKPKPGSVGLAASKAVATAVSAKKAAGATKKAHVHAKKSATVAVAKKPVTKRKPPTVRK

HumanH1.2 : -----  
 MouseH1.2 : -----  
 MossH1 : MITPKKAPKSVKSVGAGRPAKKVKK

**B**

HumanH2A : MSGRGGKOGGKARAKAKTRSSRAGLQFPVGRVHRLLRKGNYSERVAGAPVYLAAVLEYLTAETLELAGNAARDNKKTRI  
 MouseH2A : MSGRGGKOGGKARAKAKTRSSRAGLQFPVGRVHRLLRKGNYSERVAGAPVYLAAVLEYLTAETLELAGNAARDNKKTRI  
 MossH2A : MSGRGGKAGAAARKKSVTKSAKAGLQFPVGRLLGRYLKKGRYAQRVSGAPVYLAAVLEYLTAEVLELAGNASRDNKKRSRI

HumanH2A : IPRHLQLAIRNDEELNKLILGKVTIAOGGVLPNIOAVLLPKKI-----SHHKAKGK-----  
 MouseH2A : IPRHLQLAIRNDEELNKLILGRVTIAOGGVLPNIOAVLLPKKI-----SHHKAKGK-----  
 MossH2A : IPRHLQLAIRNDEELGKLLSGVTIAYGGVLPNIHVVLLPKKIAGGTGTEKPAKPEKELVKGEKASKE

**C**

HumanH2B : MPEP-----AKSAPAPKKGSKKAVTKA-----KKDSKRRKRSRKEESYSVYVYKVLKQVHPDITGISSKAMGIMNSFVNDIFE  
 MouseH2B : MPEP-----AKSAPAPKKGSKKAVTKV-----KKDGGKRRKRSRKEESYSVYVYKVLKQVHPDITGISSKAMGIMNSFVNDIFE  
 MossH2B : MAPKTDEQKAGKSKAEKPKPKKAEKLIKSDVKSVDVKKKSKKSVETIKLYLYKVLKQVHPDITGISSKAMGIMNSFINDIFE

HumanH2B : RIAGEASRLAHYNKRSTITTSREIQIHAVRLLLPGELAKHAVSEGTKAVTKYTSSK  
 MouseH2B : RIAGEASRLAHYNKRSTITTSREIQIHAVRLLLPGELAKHAVSEGTKAVTKYTSSK  
 MossH2B : KLAQEAARLARVYNNKPKITITTSREIQIHAVRLLLPGELAKHAVSEGTKAVTKLISA-

**D**

HumanH3 : MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFORLVREIAQDFK  
 MouseH3 : MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRRYQKSTELLIRKLPFORLVREIAQDFK  
 MossH3 : MARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPHRYRPGTVALREIRKYQKSTELLIRKLPFORLVREIAQDFK

HumanH3 : TDLRFQSSAVMALQEAACEATILVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA  
 MouseH3 : TDLRFQSSAVMALQEAASEAYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA  
 MossH3 : TDLRFQSSAVLALQEAASEAYLVGLFEDTNLCAIHAKRVTIMPKDIQLARRIRGERA

▲ ▲ ▲ ▲ ▲

**E**

HumanH4 : MSGRGGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGLVKVFLENVIRDVAVTYTEHAKRK  
 MouseH4 : MSGRGGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGLVKVFLENVIRDVAVTYTEHAKRK  
 MossH4 : MSGRGGKGGKGLGKGGAKRHRKVLRDNIQGITKPAIRRLARRGGVKRISGLIYEETRGLKIFLENVIRDVAVTYTEHARRK

HumanH4 : TVTAMDVVYALKRQGRITLYGFGG  
 MouseH4 : TVTAMDVVYALKRQGRITLYGFGG  
 MossH4 : TVTAMDVVYALKRQGRITLYGFGG

▲

# Figure S3

## H2A

HTA1504 : M-SGRGK---GAGAAARKKSVTKSAKAGLQFPVGR LGRYLKKGRYAQ-RVGS GAPVYLA AVLEYLAAEVLEL LAGNASRDN : 75  
HTA1506 : MGKAPAPVVDDKNDK-KKPTSRSSRAGLQFPVGR IHRLLKSRVAANGRVGATAAVYSAALLEYLTA EVLEL LAGNASKDL : 79  
HTA1511 : M-SSPGATPGGRGKSKASKSISRSAKAGLQFPVGR IARFILKAGKYAE-RVGAGAPVYLA AVLEYLAAEVLEL LAGNAARDN : 78

HTA1504 : KKSRIIPRHIQLAIRNDEELGKLLSGVTIAYGGVLPNIHSVILLPKKTAGGTGTEKPAKPEKEKVKKEKASKK- : 148  
HTA1506 : KVKRIIPRHLQLAIRGDEELDTLLKGLIAGGGVLPHLHKSLLNKTSKKD----- : 128  
HTA1511 : KKNRIIPRHIQLAVRNDEELSLLGTVTIAAGVLPNIHTIILLPKKTGKGG-----KDI GG-SISQEF : 140

## H3

HTR1512 : MARRKQIARKSTGGKAPRKQLATKAARKSAPTGGVKKPHRYRPGTVALRELRKYQKSTELLRLKLPFORLVR EIAQDFK : 80  
HTR1516 : MARRKTIPVHGN-HRASTSSVGGAAVRP-----RKPHRW RPGTKALQELRH YQKTCDLLLRLR LPPFARYVKEITMMYA : 71

HTR1512 : IDLRFQSHAVLALQEA AEAYLVGLFEDTNLCALHAKRVTIMPKDIQLARRRGERA : 136  
HTR1516 : SDVSRWTAEAL TALQEATEDYMC HLFEDTNLCALHAKRVTIMPKDLQLARRLRGAI V : 128



Figure S4

