

## Supplementary information

### **Serine is a new target residue for endogenous ADP-ribosylation on histones**

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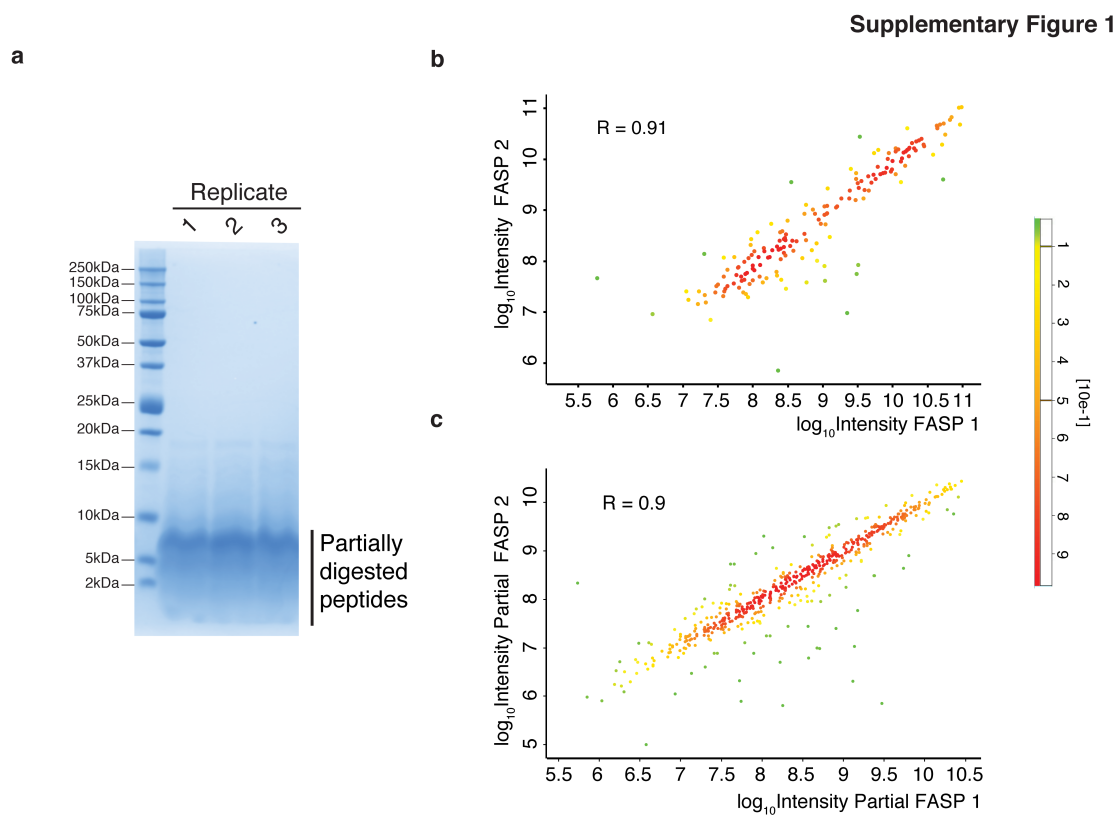
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<sup>#</sup> Equal contributions

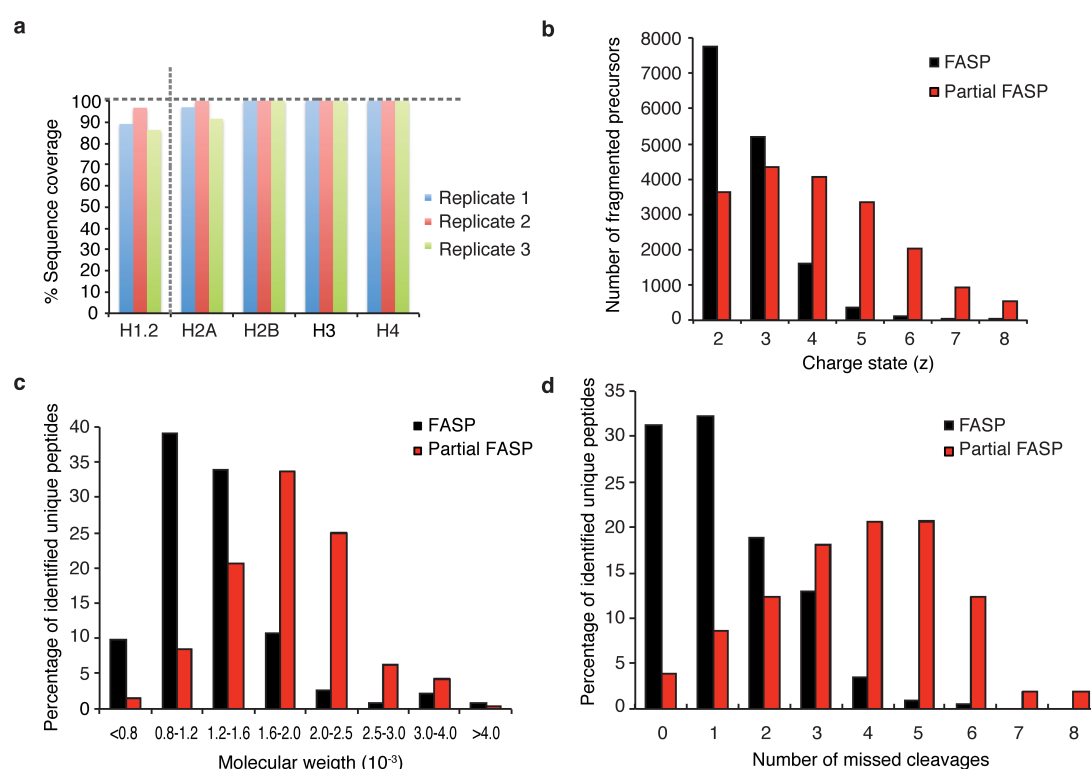
\* Corresponding author: imatic@age.mpg.de

## Supplementary results

### Supplementary Figures

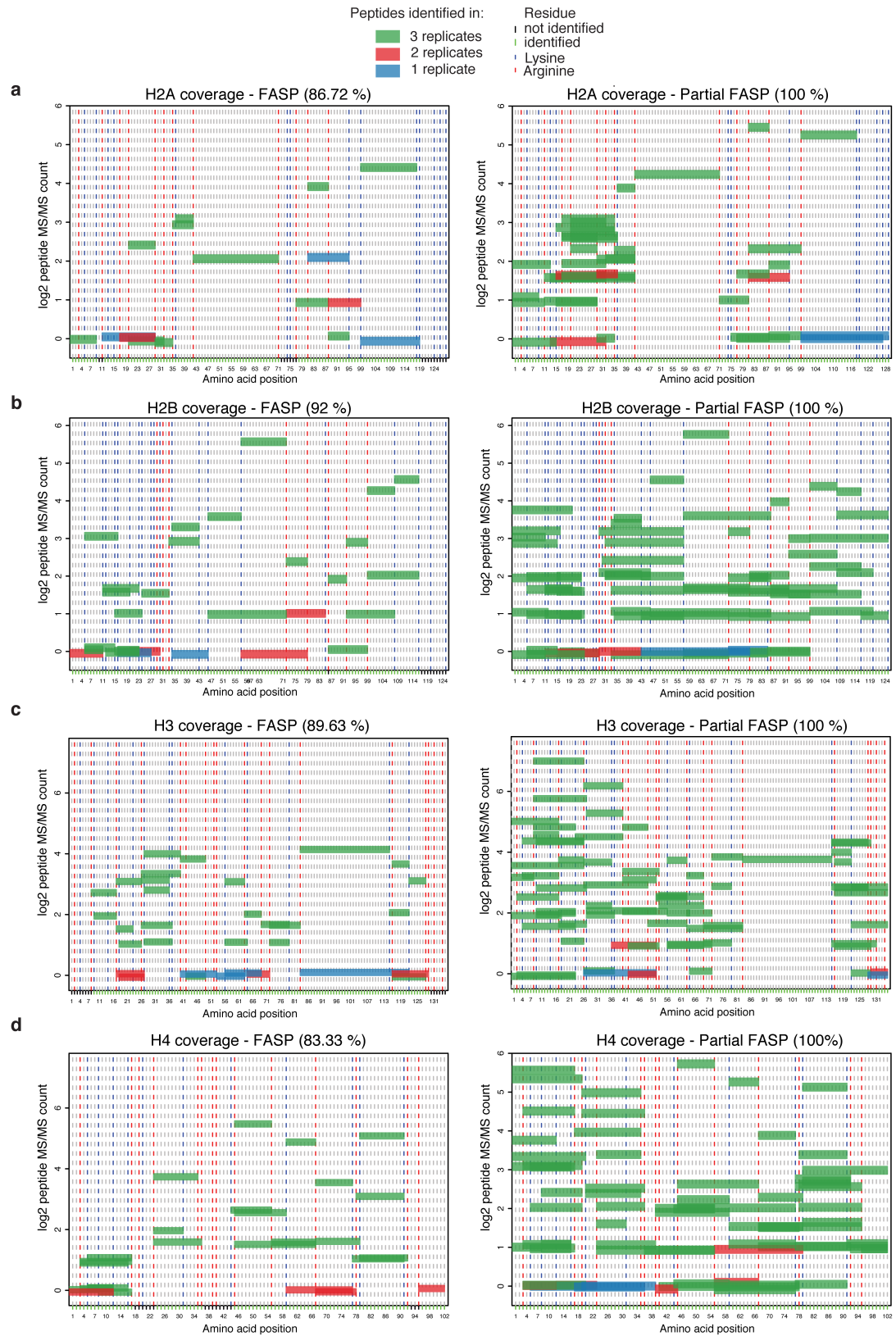


**Supplementary Figure 1. Reproducibility of the Partial FASP method. (a)** Triplicate digestion of histones with the Partial FASP method. Eluting peptides were resolved on SDS-PAGE and stained with Coomassie. **(b-c)** Correlation of the  $\log_{10}$  intensities of unique peptides identified in replicate experiments of histones processed by the FASP **(b)** or the Partial FASP **(c)** method. Color-coding indicates density.



**Supplementary Figure 2. Characterization of the Partial FASP method.** (a) Sequence coverage of the most abundant isoforms of H1 and the core histones prepared with the Partial FASP method. The experiment was performed in triplicates. All samples represent technical replicates on the digestion level. Note: H1 digestion was done separately. (b) Charge distributions of fragmented precursors for histones digested with traditional FASP versus step iii of Figure 1a of Partial FASP. (c and d) Distributions of peptide molecular weight (c) and missed cleavages (d) from LC-MS/MS analyses of samples generated by FASP (black) or Partial FASP (red) digestion of 50  $\mu$ g of purified histones.

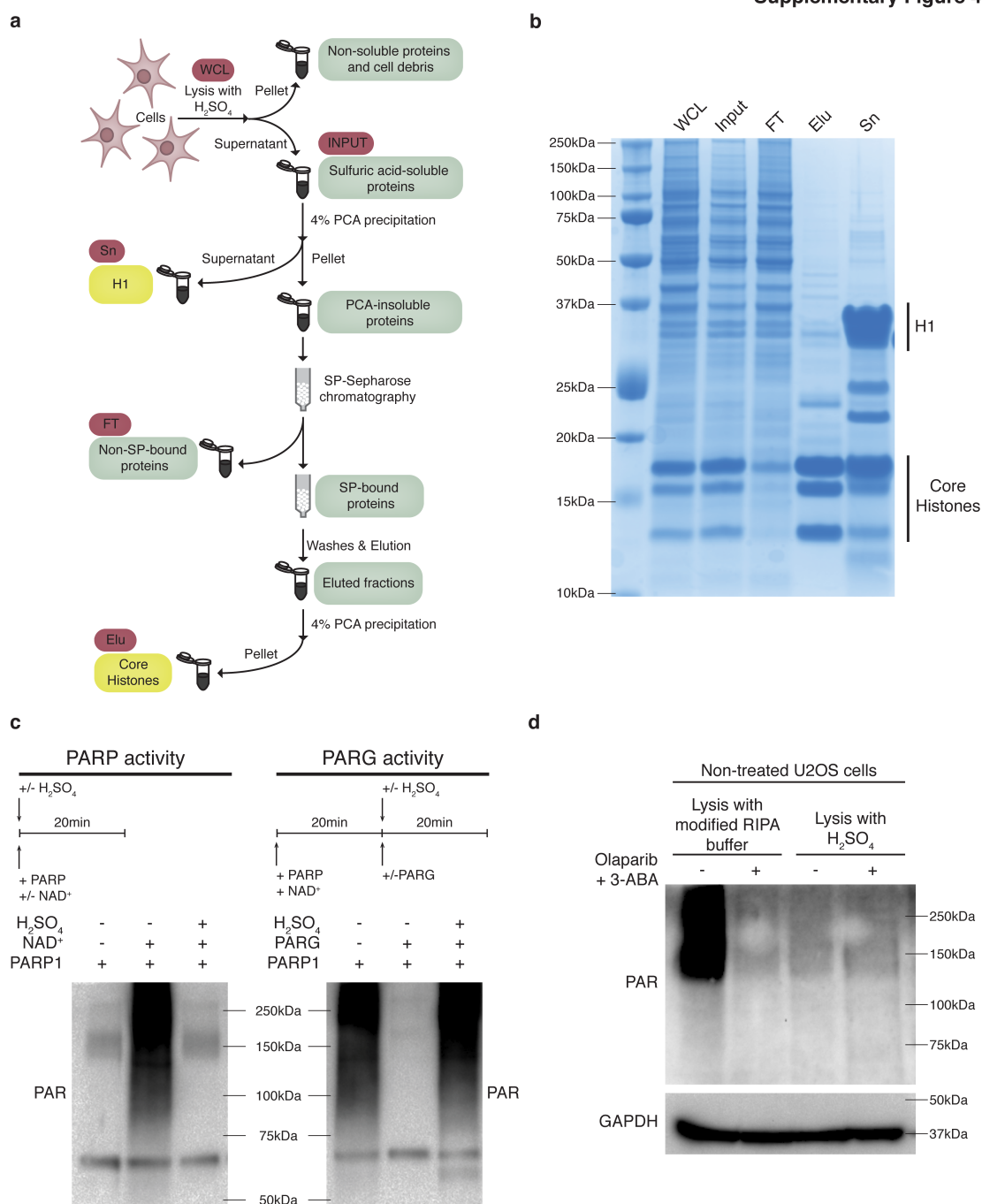
Supplementary Figure 3



**Supplementary Figure 3. Further characterization of the Partial FASP method.**

Schematic representation of H2A (a), H2B (b), H3 (c) and H4 (d) sequence coverage.

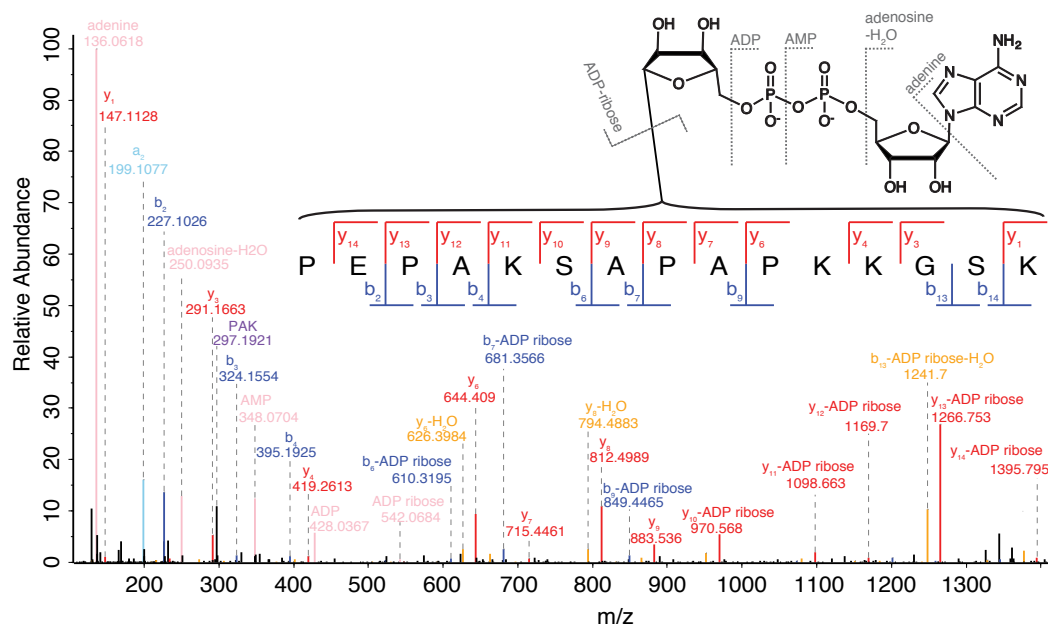
Left panels show the sequence coverage of proteins digested with the FASP method, right ones with Partial FASP. Each bar represents an identified peptide, and shows the peptide's position within a protein (x axis) and number of MS/MS spectral counts (y axis). Bars were color coded according the number of experimental replicates (out of three) in which the peptide was identified. In general, the Partial FASP method results in higher number of peptides per protein that can be identified. Those peptides are longer and contain more missed cleavages (see arginine and lysine residues), while still being abundant and reaching high MS/MS counts.



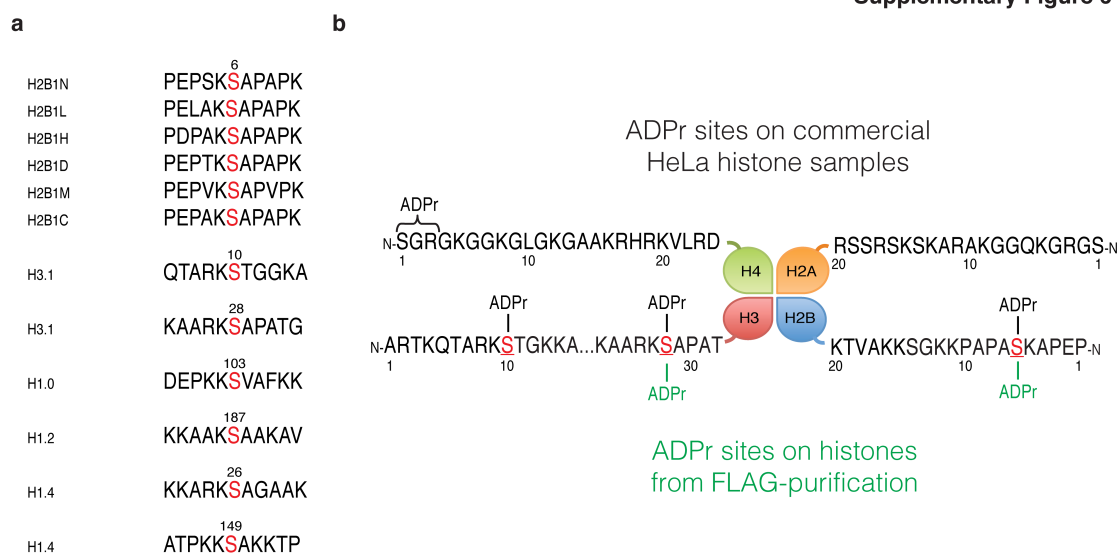
**Supplementary Figure 4. Extraction and purification of histones from cells. (a)** Schematic overview of the workflow for histone extraction and purification combining sulfuric acid lysis and SP-chromatography. See **Online Methods** for further experimental details. **(b)** Coomassie-staining of SDS-PAGE with the different fractions collected during the purification method outlined in (a). See **Supplementary**

**Fig. 11** for original image. **(c)** Analysis of the effect of sulfuric acid on PARP or PARG activity. *In vitro* PARP activity assays (left panel) were performed in the absence or presence of 200  $\mu\text{M}$   $\text{NAD}^+$  and/or 0.1 M sulfuric acid. *In vitro* PARG activity assays (right panel) were performed in the absence or presence of 1  $\mu\text{M}$  human PARG and/or 0.1 M sulfuric acid. PARP1 poly-ADP-ribosylation was analyzed by Western blotting using anti-PAR antibodies. The blot is representative of three independent experiments. See **Supplementary Fig. 11** for original image. **(d)** Acid extraction prevents artifactual post-lysis increase in the level of PARylation and therefore preserves the endogenous level of PAR. Untreated U2OS cells were lysed with modified RIPA buffer or with sulfuric acid in the absence or presence of the PARP inhibitors olaparib (2  $\mu\text{M}$ ) and 3-ABA (10 mM). Total protein poly-ADP-ribosylation was analyzed by Western blotting using anti-PAR antibodies. Anti-GAPDH was used as a loading control. See Online Methods for further experimental details. The Western blot is representative of three independent experiments. See **Supplementary Fig. 11** for original image.

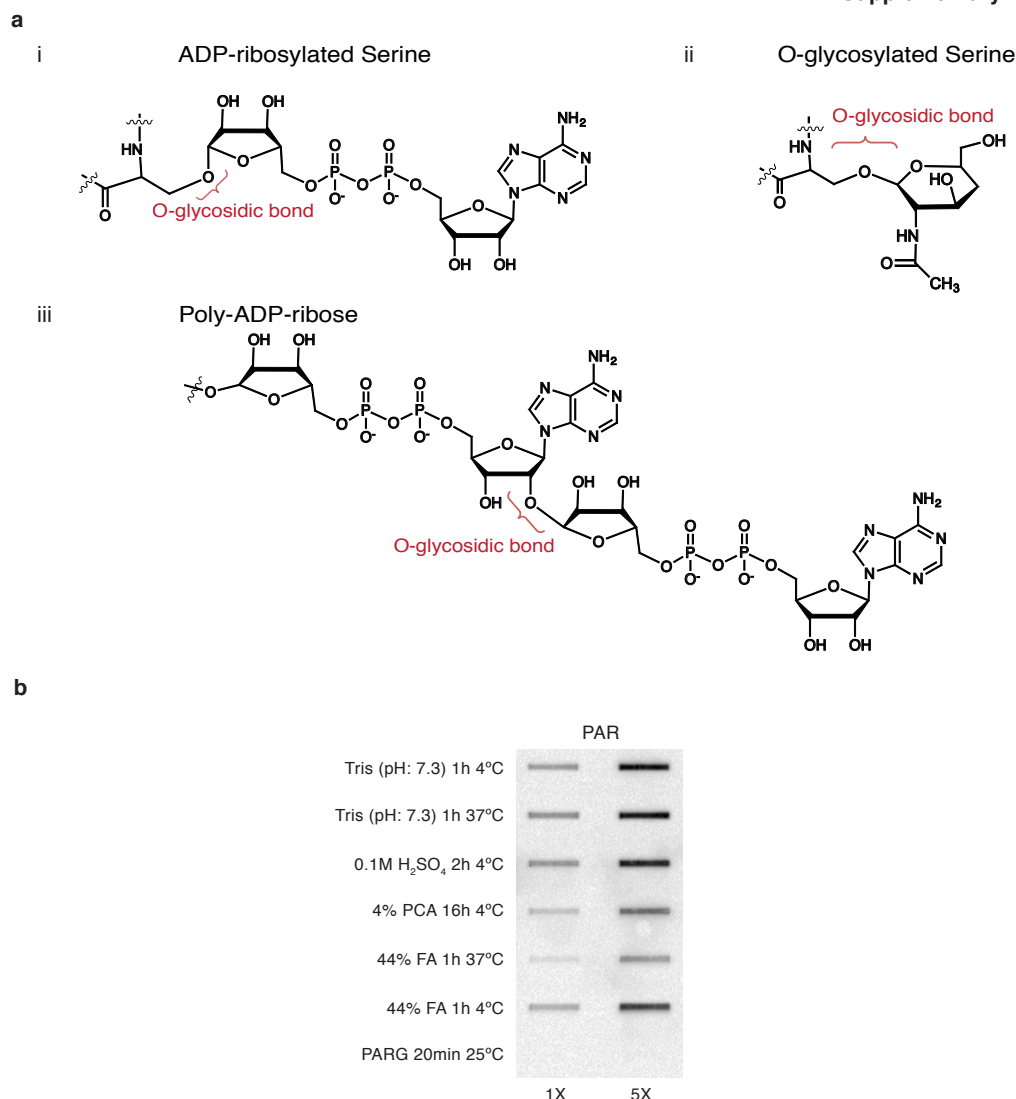
Supplementary Figure 5



**Supplementary Figure 5. High-resolution HCD fragmentation spectra of an H2B peptide modified by ADP-ribose.** While low-mass ions resulting from fragmentation of ADP-ribose unmistakably confirm the presence of ADP-ribose, this fragmentation mode is not suitable for confidently localizing modification sites, as the bond between ADP-ribose and the peptides is very labile during HCD, a behavior also observed for O-glycopeptides<sup>13</sup>. We exploited this lability of ADP-ribose for triggering acquisition of high-resolution ETD spectra based on the presence of adenine ions in rapidly-acquired HCD spectra. See **Online Methods** for further details.

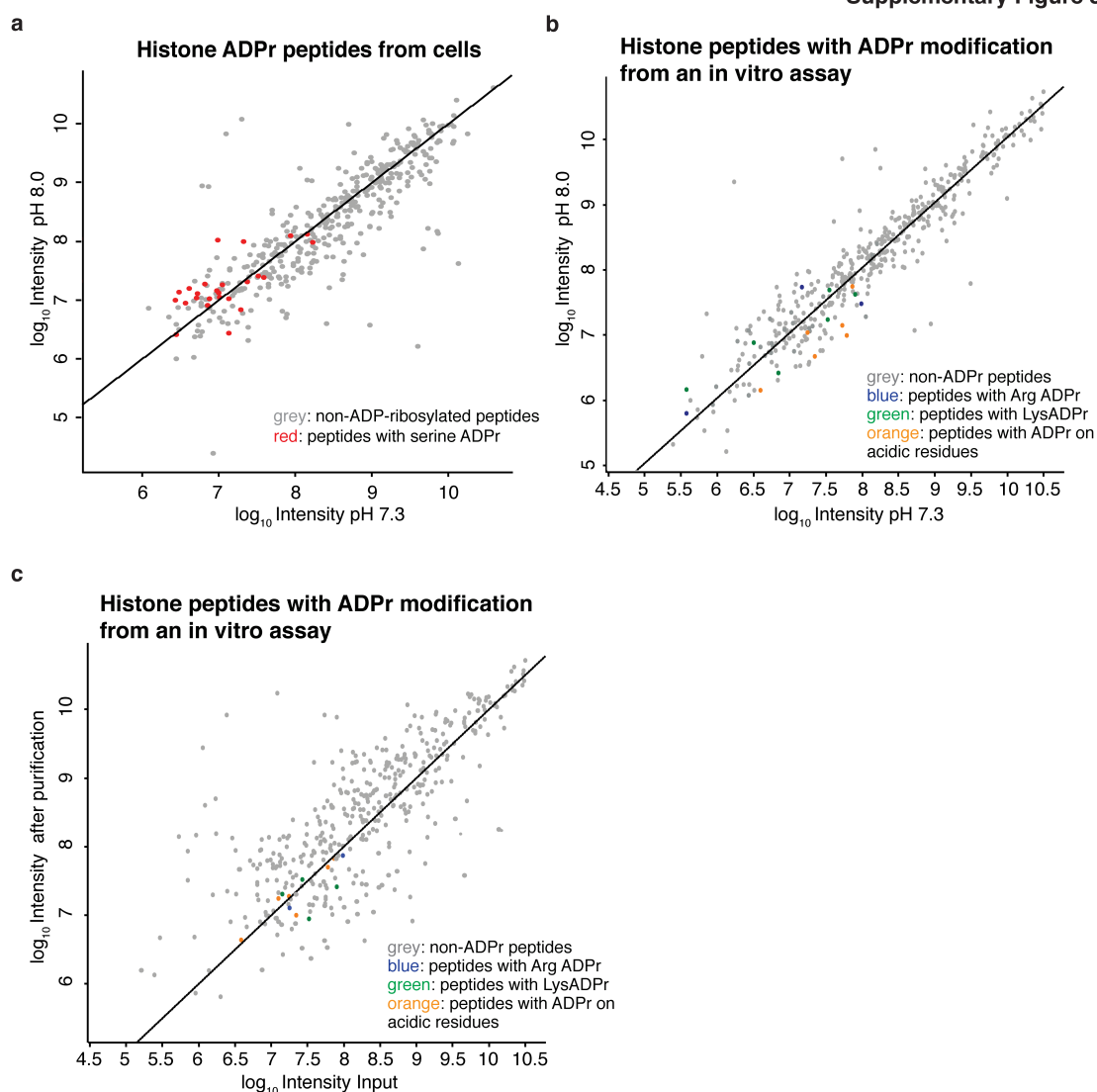


**Supplementary Figure 6. Serine ADPr sites on different histone variants and on commercial core histone samples. (a)** Sequences show the similarity between the regions of the proteins where serine ADPr was found. One site was detected on six different H2B variants. **(b)** Schematic representation of core histones. Four out of six previously identified histone serine ADP-ribosylation sites are also found on commercial HeLa core histones and two of them in an immunoprecipitation experiment, digested with the Partial FASP method.



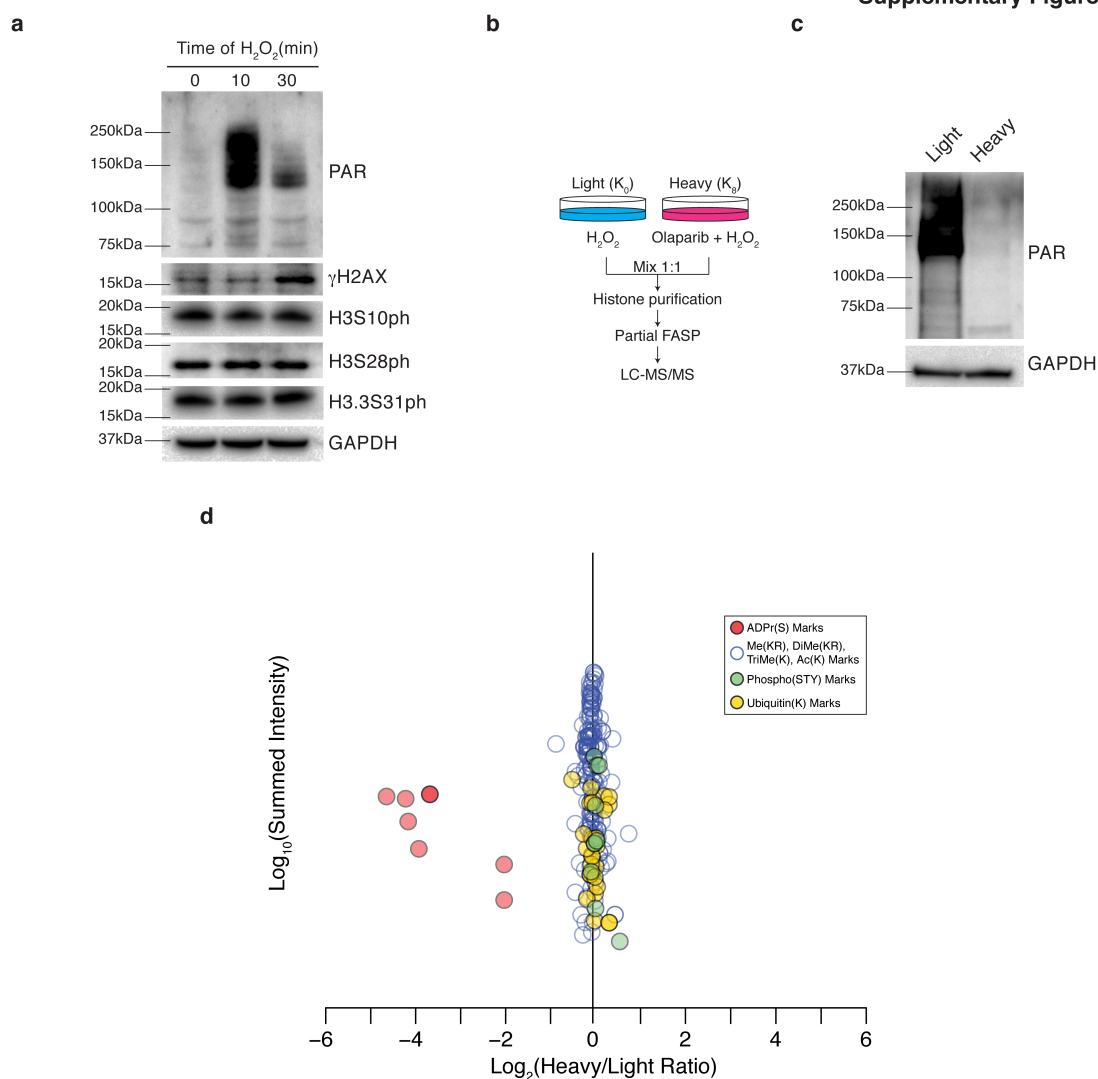
**Supplementary Figure 7. PAR stability under acidic conditions (a)** Chemical structures of ADP-ribosylated Serine (i), O-glycosylated Serine (ii) and a poly-ADP-ribose chain represented by two monomers (iii). ADP-ribosylation is attached to Serine (i) through the same linkage that exists between sugars, such as N-acetylglucosamine, and O-glycosylated proteins (ii), and that links together poly-ADP-ribose (iii). **(b)** Analysis of PAR degradation under acidic conditions. PAR chains were subjected to the indicated treatments. PAR stability was analyzed by immune-slot blot using anti-PAR antibodies. 1X and 5X indicate the relative amount

of sample loaded into the slots after treatments. See **Online Methods** for further experimental details. The slot-blot is representative of four independent experiments. See **Supplementary Fig. 11** for original image. The degradation of PAR observed in 44% formic acid at 37°C is consistent with the degradation of low molecular weight acetal model conjugates observed by Cervantes-Lauren et al. JBC 1995. Incubation temperature is an important factor for the cleavage of the acetal bond: much less PAR is degraded by formic acid at 4°C compared to 37°C. Importantly, acid extraction (H<sub>2</sub>SO<sub>4</sub> for 2h at 4°C), which is the essential first step of our histone purification protocol, does not appear to degrade any significant amount of PAR. Even after overnight incubation (16h) in 4% perchloric acid at 4°C, approximately half of the PAR signal remains.



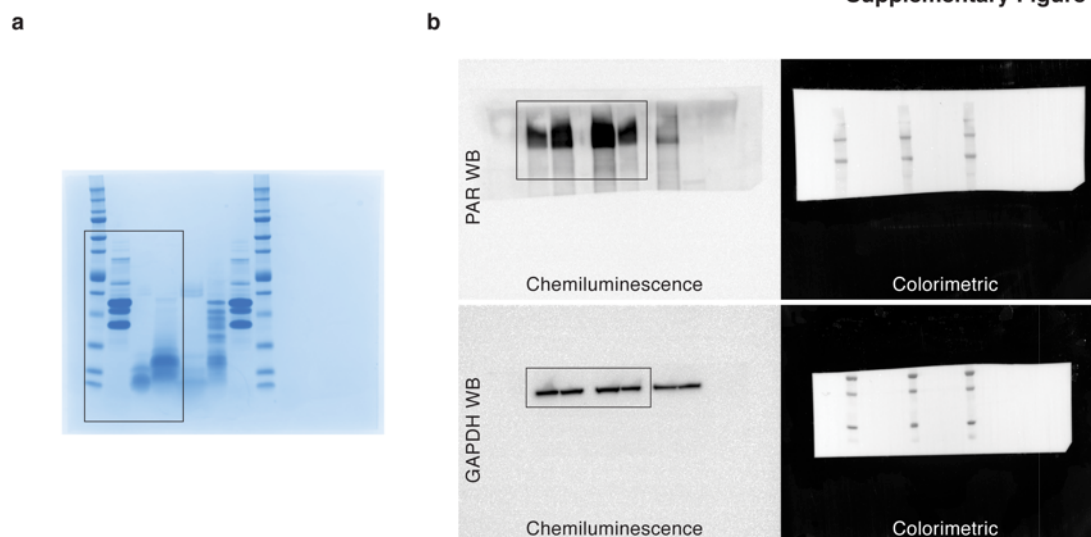
**Supplementary Figure 8. Validation of the histone digestion and purification method.** **(a)** Histones were digested with the Partial FASP method, at pH 7.3 or pH 8.0, as indicated.  $\log_{10}$  intensities of the peptides obtained from the different digestion conditions were compared on the scatterplot. **(b)** ADP-ribosylation of histones was performed with PARP10 in an *in vitro* assay. The proteins were then digested with the Partial FASP method, at pH 7.3 or pH 8.0, as indicated.  $\log_{10}$  intensities of the peptides obtained from the different digestion conditions were compared on the scatterplot. **(c)** Histones were incubated with PARP10 in the

presence of NAD<sup>+</sup>, and then subjected to another round of histone purification and digestion. ADPr was detected on arginine, lysine and acidic residues both before and after purification. Log<sub>10</sub> intensities of the repurified histone peptides were compared to the input (taken after the *in vitro* assay).



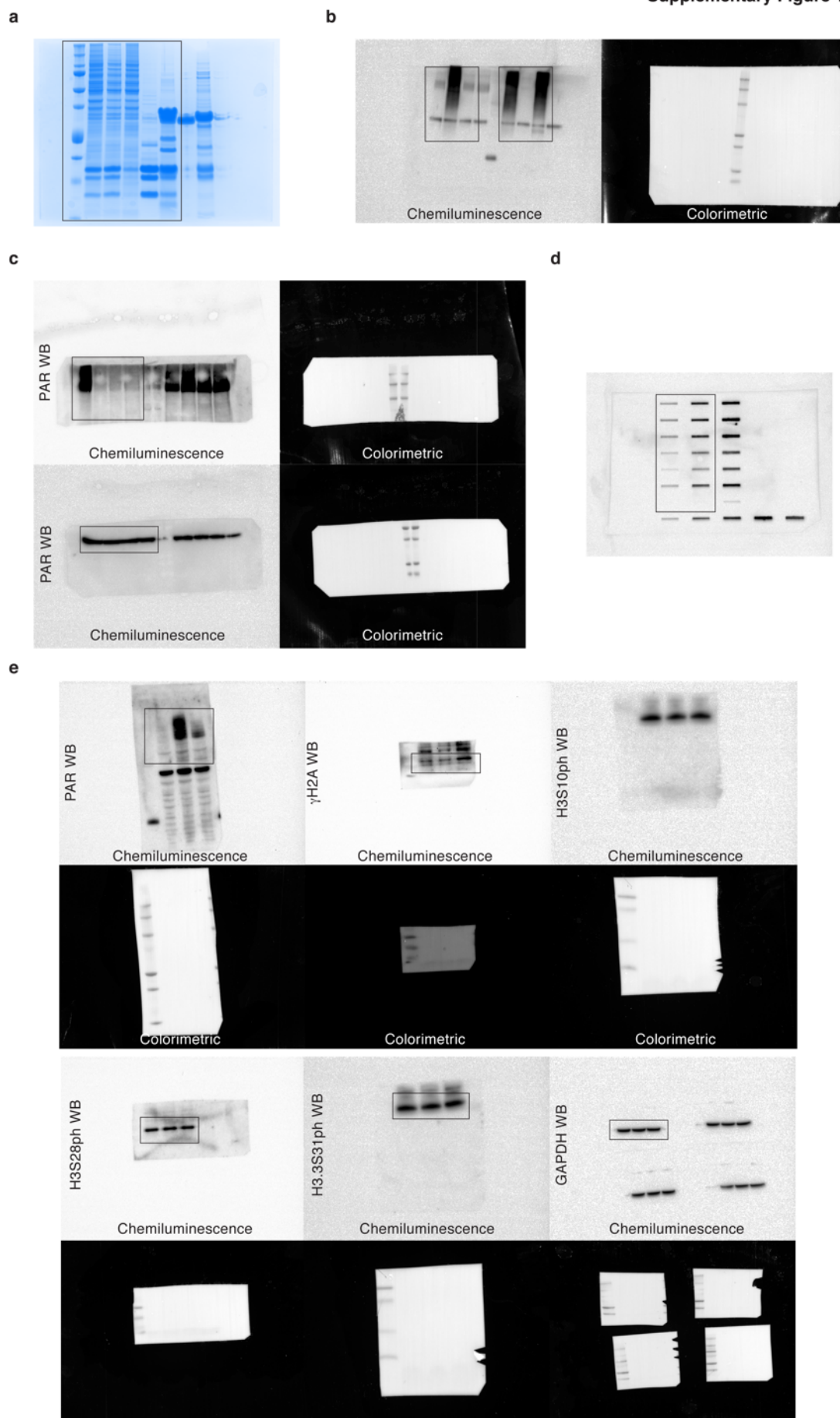
**Supplementary Figure 9. Histone Serine ADPr modification inhibition by Olaparib.** **(a)** Analysis of histone phosphorylation marks after  $H_2O_2$ -induced DNA damage. U2OS cells were stimulated with 2 mM  $H_2O_2$  for the indicated time points and lysed with Laemmli buffer. Total protein poly-ADP-ribosylation, phosphorylation of histone H2A.X (Ser139), phosphorylation of histone H3 (Ser10 and Ser28) and phosphorylation of histone H3.3 (Ser31) were analyzed by Western blotting. Anti-GAPDH was used as a loading control. See **Online Methods** for further experimental details. The Western blot is representative of three independent experiments, which are biological replicates. See **Supplementary Fig. 11** for original images. **(b)** Schematic representation of the SILAC-based strategy to quantify core histone marks

upon 10 minutes of 2 mM H<sub>2</sub>O<sub>2</sub>-induced DNA damage in the absence or presence of the PARP inhibitor olaparib. **(c)** Western blot analysis of total protein poly-ADP-ribosylation levels prior to mixing light and heavy lysates from the SILAC experiment. Anti-GAPDH was used as a loading control. See **Supplementary Fig. 11** for original images. **(d)** Log<sub>10</sub> of summed peptide intensities were plotted against log<sub>2</sub> Heavy/Light SILAC ratios. Each point represents a unique histone mark. ADPr marks are of medium to low abundance compared to the rest of the peptide population. As expected, other marks did not respond to treatment with olaparib.

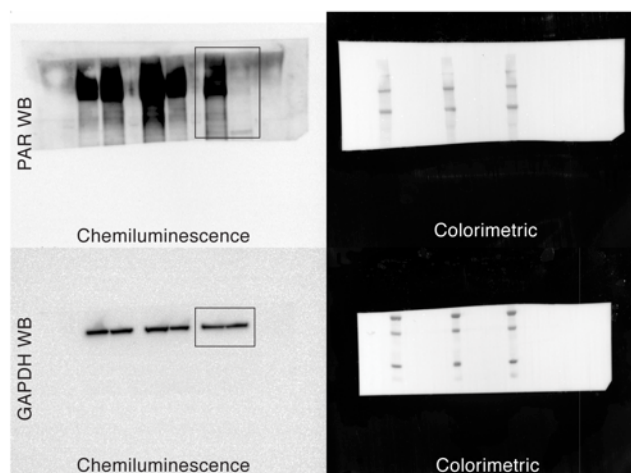


**Supplementary Figure 10. Original images of gels and Western blots of main figures. (a)** Full Coomassie gel of Fig. 1b. The box indicates the part displayed in Fig. 1b **(b)** Full Western blots of Fig. 3b. The boxes indicate the parts displayed in Fig. 3b. Right panels show the colorimetric images for molecular mass (kDa) estimation.

Supplementary Figure 11



f



**Supplementary Figure 11. Original images of gels and Western blots of supplementary figures. (a)** Full Coomassie gel of Supplementary Fig. 4b. The box indicates the part displayed in Supplementary Fig. 4b. **(b)** Full Western blot of Supplementary Fig. 4c. The boxes indicate the parts displayed in Supplementary Fig. 4c. Right panel show the colorimetric images for molecular mass (kDa) estimation. **(c)** Full Western blot of Supplementary Fig. 4d. The boxes indicate the parts displayed in Supplementary Fig. 4d. Right panels show the colorimetric images for molecular mass (kDa) estimation. **(d)** Full immune-slot blot of Supplementary Fig. 7b. The box indicate the part displayed in Supplementary Fig. 7b. **(e)** Full Western blots of Supplementary Fig. 9a. The boxes indicate the parts displayed in Supplementary Fig. 9a. Bottom panels show the colorimetric images for molecular mass (kDa) estimation. **(f)** Full Western blot of Supplementary Fig. 9c. The boxes indicate the parts displayed in Supplementary Fig. 9c. Right panels show the colorimetric images for molecular mass (kDa) estimation.

## Supplementary Tables

| Histone | ADPr mark  | Score  | Localization probabilities (DEKRSTYCMNQHM)     |
|---------|------------|--------|------------------------------------------------|
| H1.0    | S103       | 280.19 | LAKSDEPKK(0.001)S(0.999)VAFKK                  |
| H1.0    | S103       | 286.59 | S(1)VAFKK                                      |
| H1.0    | S103       | 246.41 | SDEPKKS(1)VAFKK                                |
| H1.2    | S187       | 174.4  | KAAS(0.002)S(0.998)AAKAVKPK                    |
| H1.2    | S187       | 313.75 | S(1)AAKAVKPK                                   |
| H1.4    | S26        | 366.95 | SETAPAAPAAPAPAEKTPVKKKARK(0.004)S(0.996)AGAAKR |
| H1.4    | S54        | 142.04 | S(1)GVSLAALKK                                  |
| H1.4    | S112       | 155.07 | KAAS(1)GEAKPK                                  |
| H1.4    | S149       | 183.65 | KATGAATPKK(0.034)S(0.966)AK                    |
| H2A     | S1 or R3   | 336.31 | S(0.5)GR(0.5)GKQGGKARAKAKTRSSR                 |
| H2A     | S1 or R3   | 179.51 | S(0.5)GR(0.5)GKQGGKAR                          |
| H2B1C   | S6         | 492.3  | PEPAKS(1)APAPKKGSK                             |
| H2B1C   | S6         | 472.56 | PEPAKS(1)APAPKKGSKKAVTK                        |
| H2B1D   | S6         | 381.89 | PEPTKS(1)APAPKKGSK                             |
| H2B1D   | S6         | 357.75 | PEPTKS(1)APAPKKGSK                             |
| H2B1H   | S6         | 195.79 | PDPAKS(1)APAPKKGSK                             |
| H2B1H   | S6         | 237.68 | PDPAKS(1)APAPKKGSK                             |
| H2B1L   | S6         | 394.72 | PELAKS(1)APAPKKGSK                             |
| H2B1L   | S6         | 323.54 | PELAKS(1)APAPKKGSK                             |
| H2B1M   | S6         | 311.7  | PEPVKS(1)APVPKKGSK                             |
| H2B1M   | S6         | 280.19 | PEPVKS(1)APVPKKGSK                             |
| H2B1N   | S6         | 254.19 | PEPSK(0.001)S(0.999)APAPKKGSK                  |
| H2B1N   | S6         | 265.61 | PEPSKS(1)APAPKKGSK                             |
| H2B1C   | S14        | 290.04 | PEPAKSAPAPKKGS(0.999)K(0.001)K                 |
| H3.1    | S10        | 571.27 | ARTKQTARKS(1)TGGKAPRKQLATK                     |
| H3.1    | S10        | 272.94 | QTARKS(0.998)T(0.002)GGKAPR                    |
| H3.1    | S28        | 446.3  | S(1)APATGGVKKPHR                               |
| H3.1    | S28        | 286.73 | S(1)APATGGVKKPHR                               |
| H4      | S1 or R3   | 382.17 | S(0.5)GR(0.5)GKGGKGLGKGGAQR                    |
| H4      | S1 (or R3) | 440.72 | S(0.986)GR(0.014)GKGGKGLGKGGAQR                |

**Supplementary Table 1.** *In vivo* histone ADP-ribosylation modification sites identified in this study

| Histone | ADPr mark | Score  | Localization probabilities (DEKRSTYCMNQHM)          |
|---------|-----------|--------|-----------------------------------------------------|
| H2A     | R3        | 186,46 | S(0.07)GR(0.93)GKQGGKAR                             |
| H2A     | R29       | 123,67 | AGLQFPVGR(1)VHR                                     |
| H2A     | E41       | 301,48 | LLRKGNIAE(1)R                                       |
| H2A     | E121      | 291,46 | VTIAQGGVLPNIQAVLLPKK(0.003)TE(0.945)S(0.052)HHKAKGK |
| H2B     | E2        | 213,63 | PE(1)PAKSAPAPKKGSKK                                 |
| H2B     | D51       | 106,2  | VLK(0.002)QVHPD(0.985)TGISSK                        |
| H2B     | K125      | 119,18 | HAVSEGTKAVTKYTS(0.002)AK(0.998)                     |
| H3      | R2        | 114,54 | AR(1)TKQTAR                                         |
| H3      | K4        | 146,21 | ARTK(1)QTAR                                         |
| H3      | K9        | 327,6  | K(0.934)S(0.063)TGGK(0.002)APR(0.001)KQLATKAAR      |
| H3      | K14       | 267,24 | KSTGGK(1)APRKQLATKAAR                               |
| H3      | K18       | 223,78 | K(1)QLATKAAR                                        |
| H3      | R49       | 194,2  | YRPGTVALR(0.972)E(0.028)IR                          |
| H3      | E50       | 281,12 | YRPGTVALRE(1)IRR                                    |
| H3      | R52       | 171,85 | YRPGTVALRE(0.047)IR(0.95)R(0.003)YQK                |
| H3      | K56       | 155,51 | YQK(0.999)S(0.001)TELLIR                            |
| H3      | E59       | 193,41 | YQKSTE(1)LLIR                                       |
| H3      | R116      | 227,94 | R(1)VTIMPKDIQLARR                                   |
| H3      | K122      | 222,3  | RVTIMPK(0.986)D(0.006)IQLAR(0.006)R(0.001)          |
| H3      | E133      | 105,82 | IRGE(1)RA                                           |
| H4      | R3        | 351,63 | S(0.045)GR(0.954)GKGGK(0.001)GLGKGGAKRHR            |
| H4      | K8        | 234,05 | SGRGKGGK(1)GLGKGGAKR                                |

**Supplementary Table 2.** A list of identified ADPr modification sites on histones after *in vitro* ADPr reaction with PARP10.

**Supplementary Data Set 1.** SILAC-based quantification of histone marks: DNA damage and olaparib experiments.

### **Supplementary Note**

Representative high-resolution ETD fragmentation MS/MS spectra of confidently identified ADP-ribosylation sites on histones purified from cells.

*ADPr* = ADP-ribosylation

*pRib* = ribose phosphate

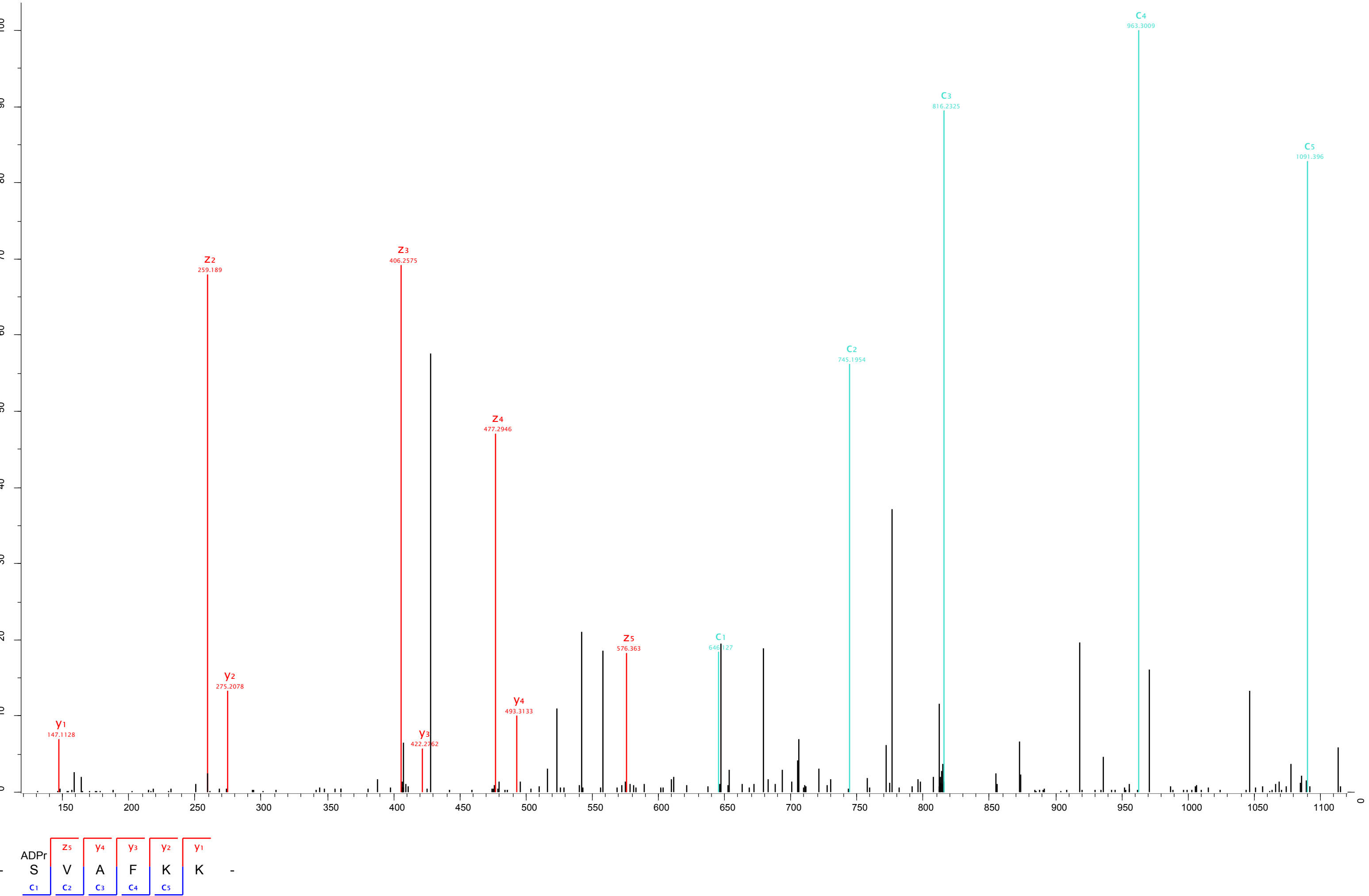
H1.0

ADP-ribosylation on Ser103

m/z 407.49647

charge 3+

mass error [ppm] 0.6



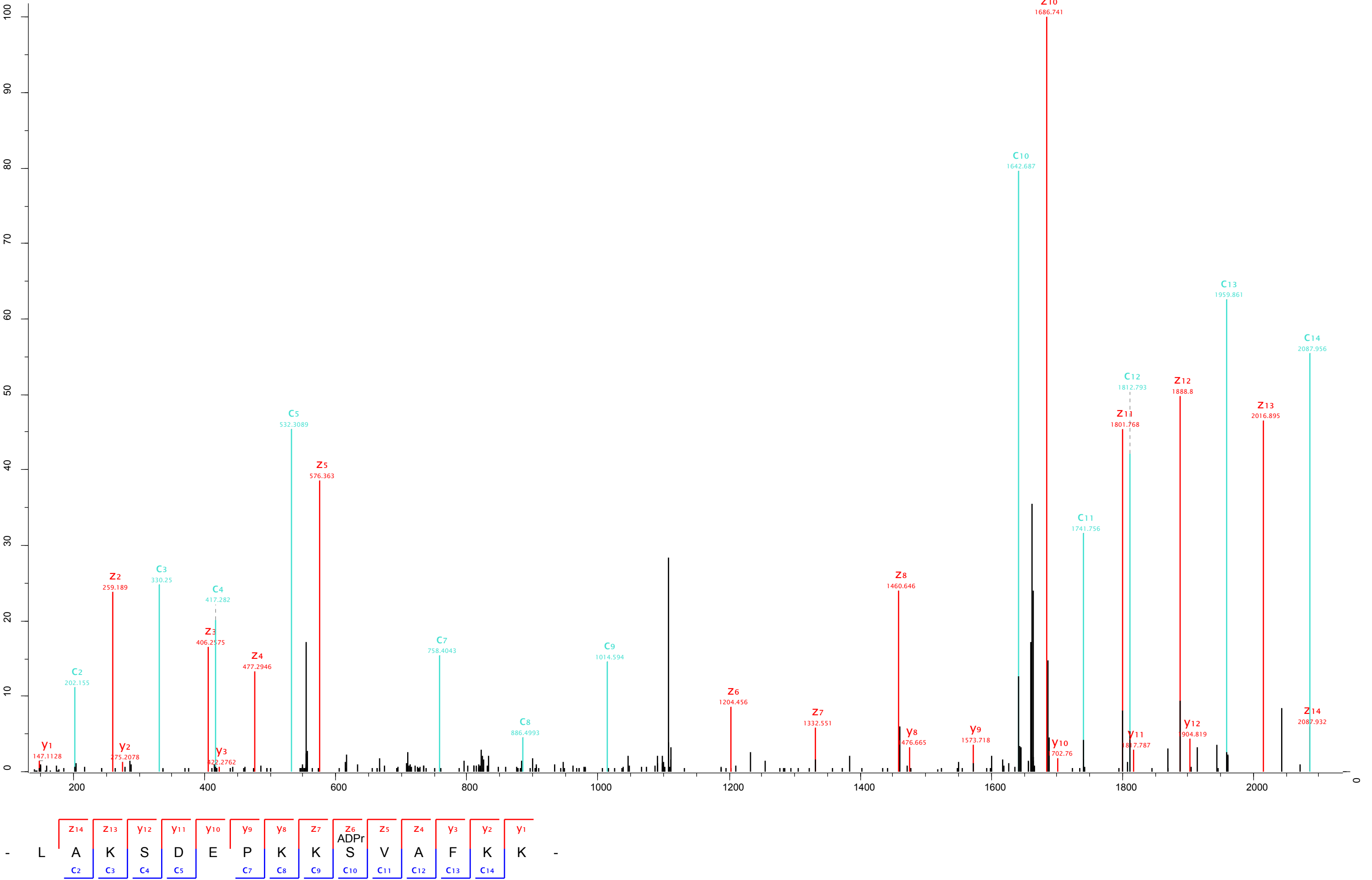
H1.0

ADP-ribosylation on Ser103

m/z 555.01427

charge 4+

mass error [ppm] -0.6



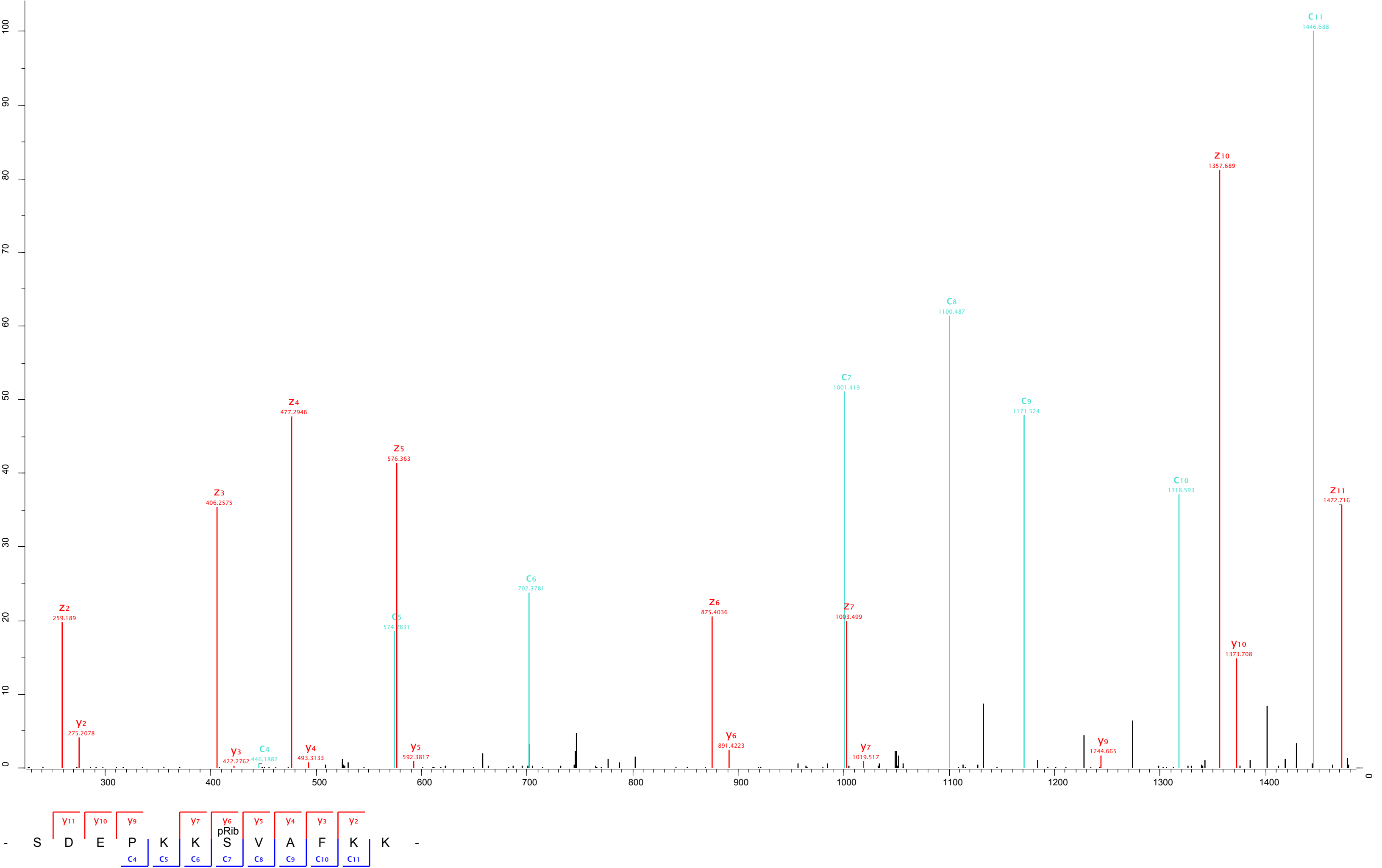
H1.0

phospho-ribose on Ser103

m/z 525.92704

charge 3+

mass error [ppm] 1.0



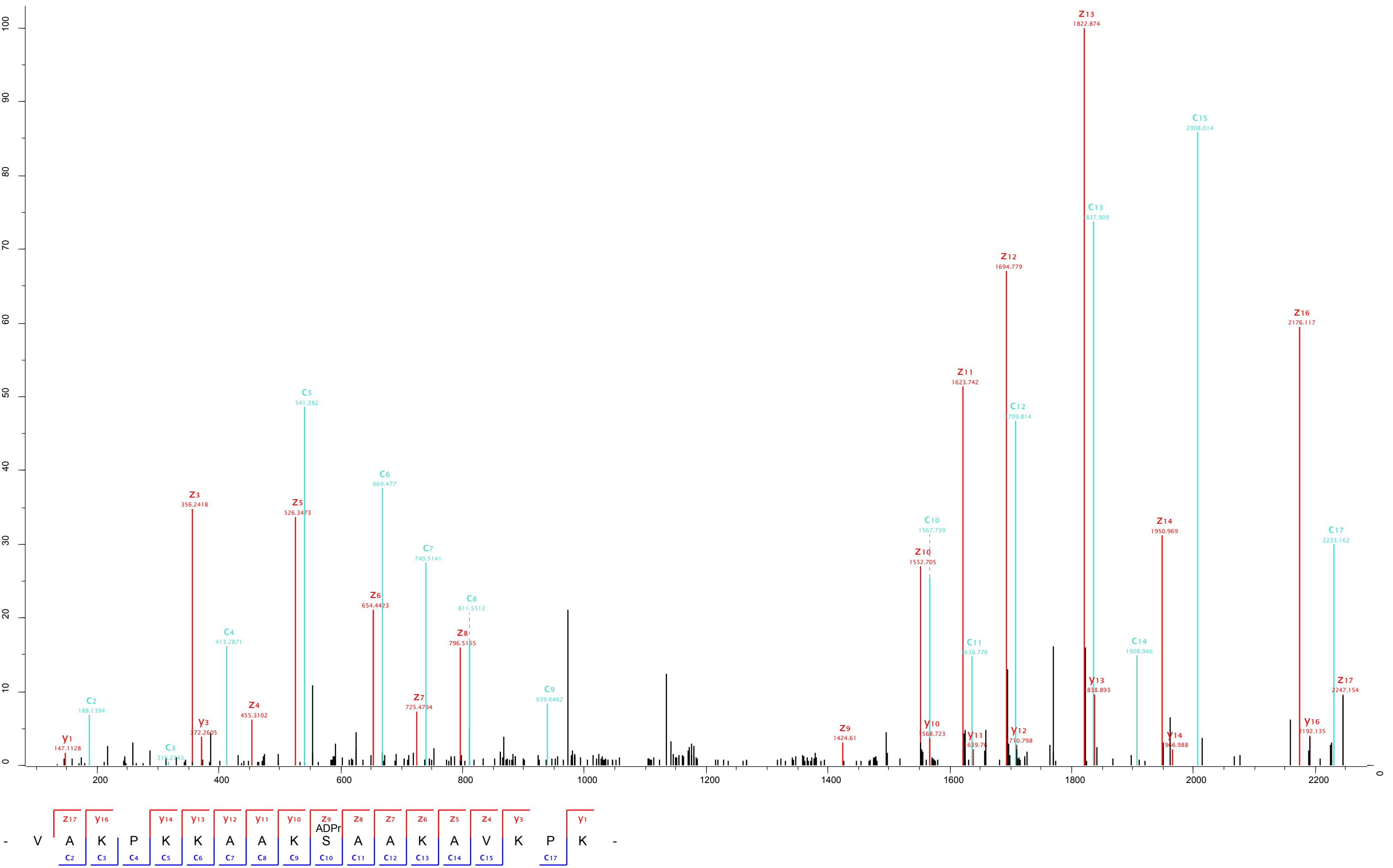
H1.2

ADP-ribosylation on Ser187

m/z 591.31565

charge 4+

mass error [ppm] -0.6



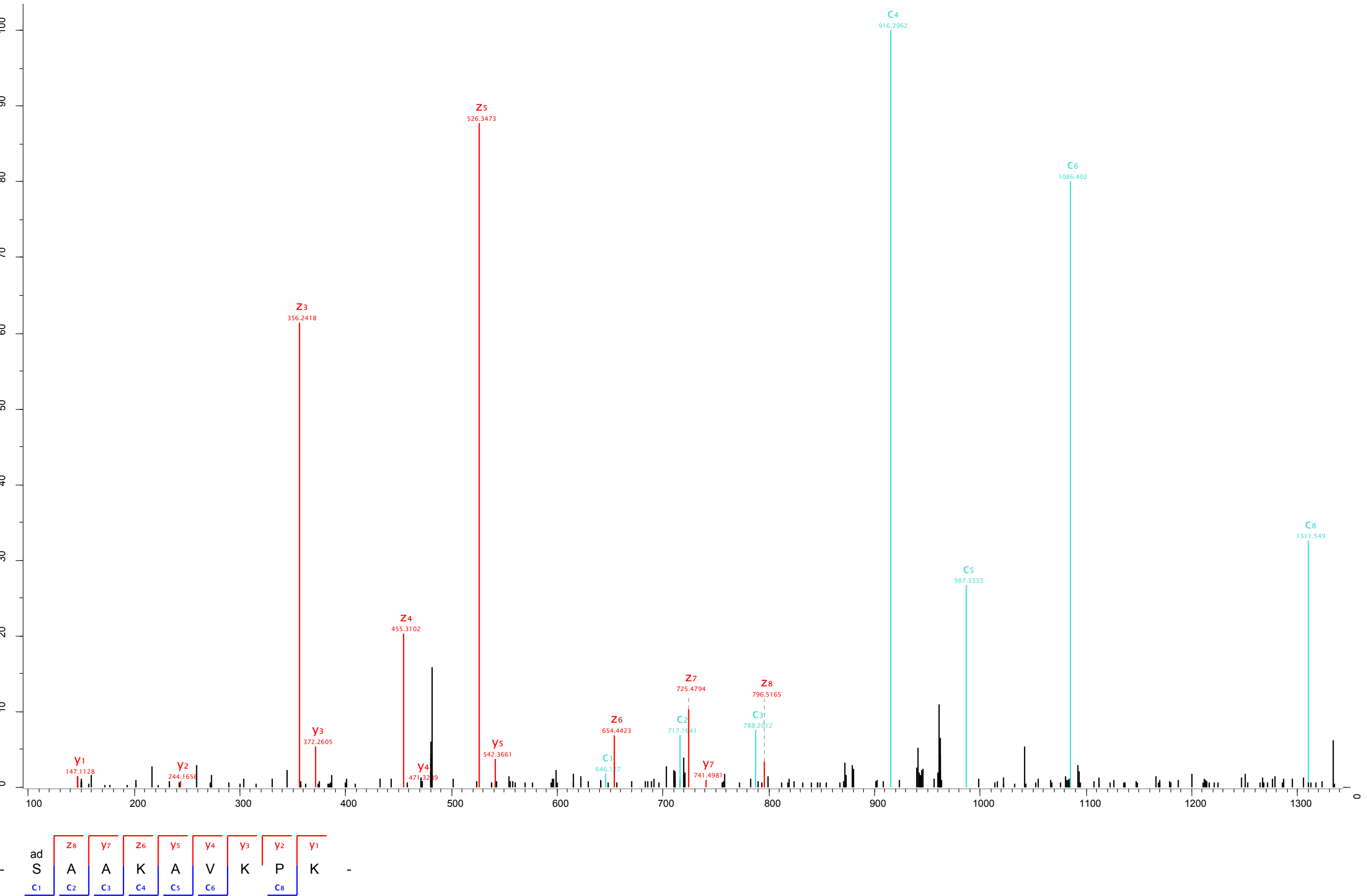
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ADP-ribosylation on Ser187

m/z 480.88098

charge 3+

mass error [ppm] 1.2



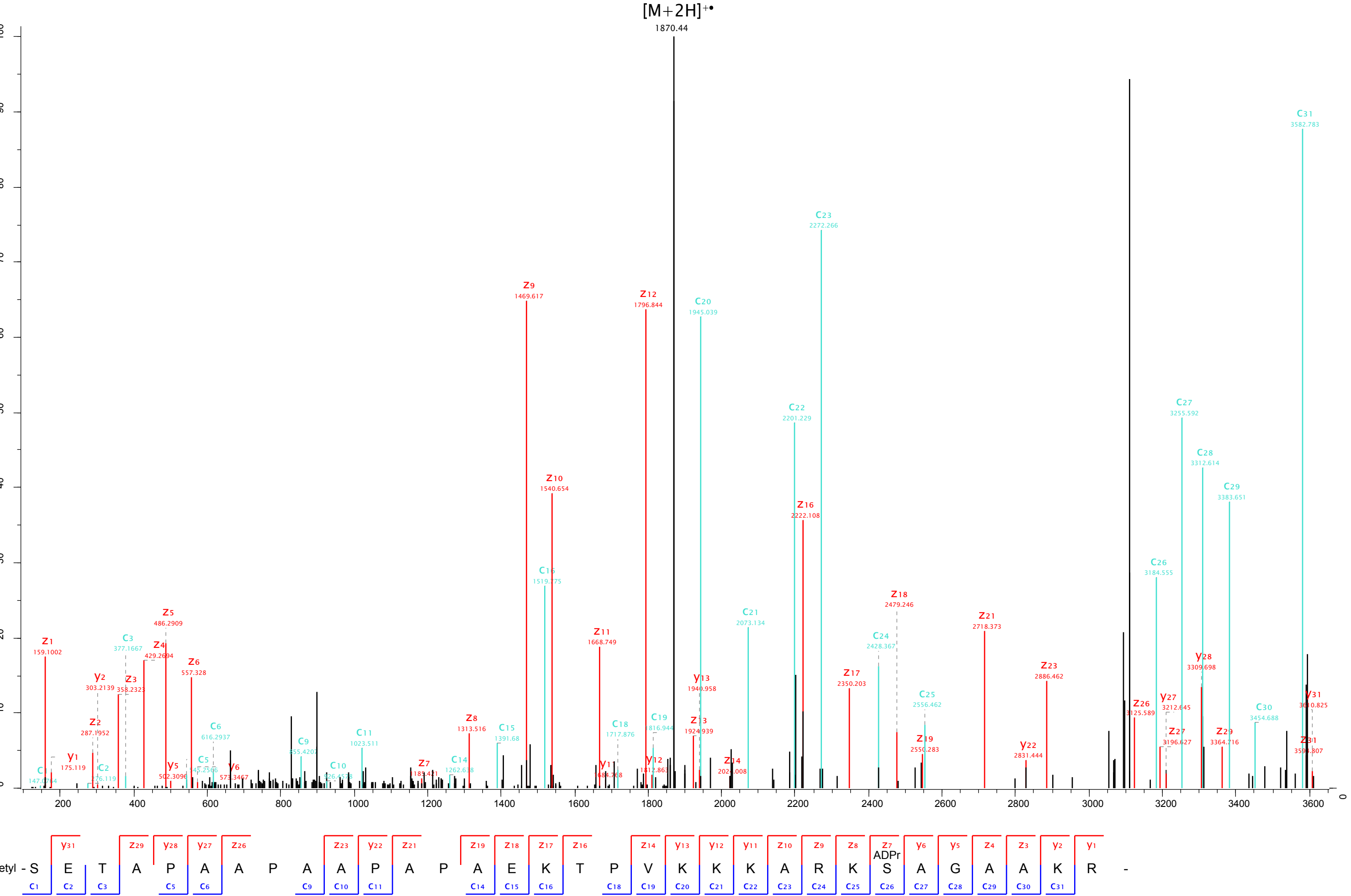
H1.4

ADP-ribosylation on Ser26

m/z 624.15074

charge 3+

mass error [ppm] 0.2



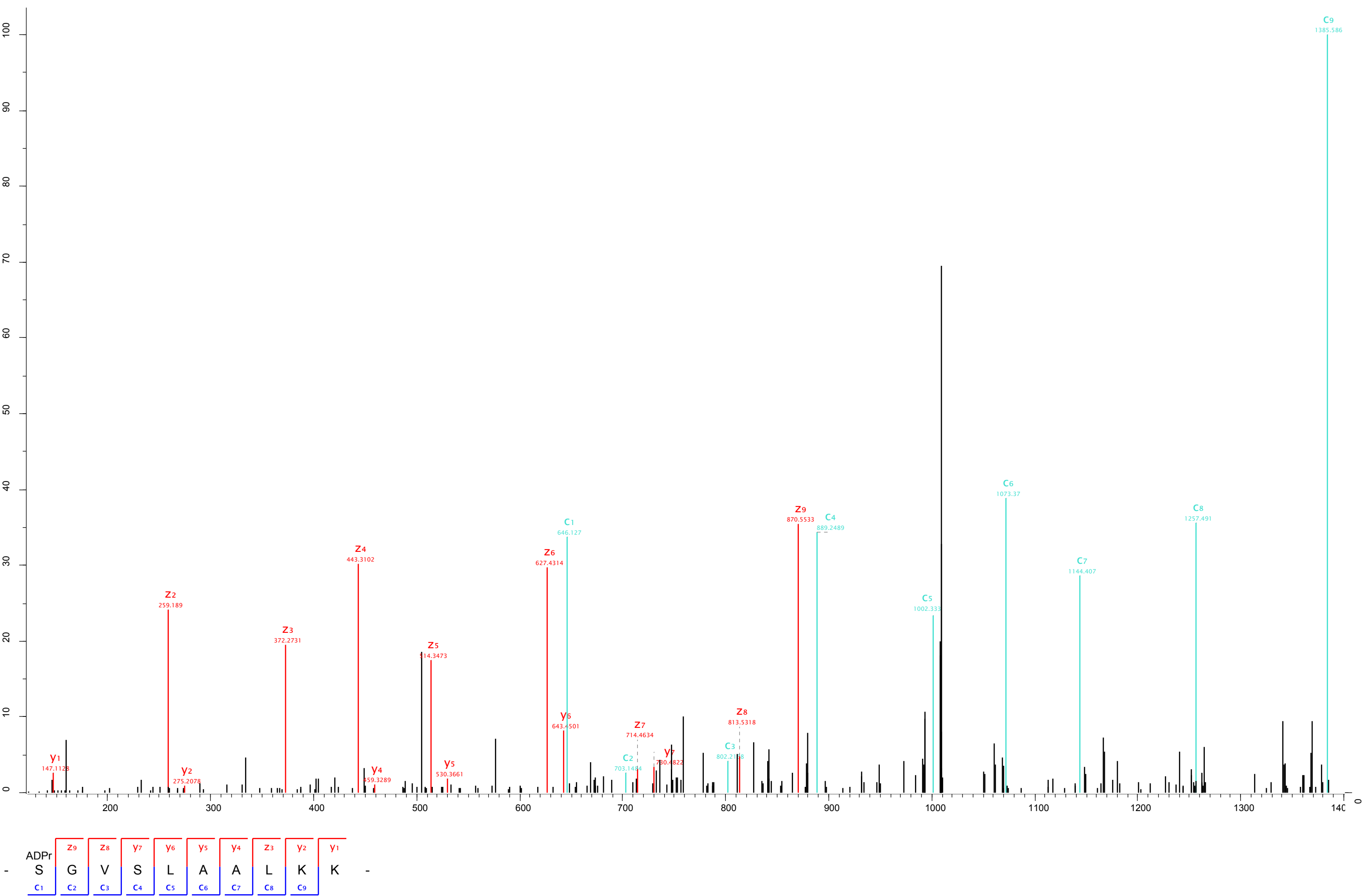
H1.4

ADP-ribosylation on Ser54

m/z 505.55991

charge 3+

mass error [ppm] -2.3



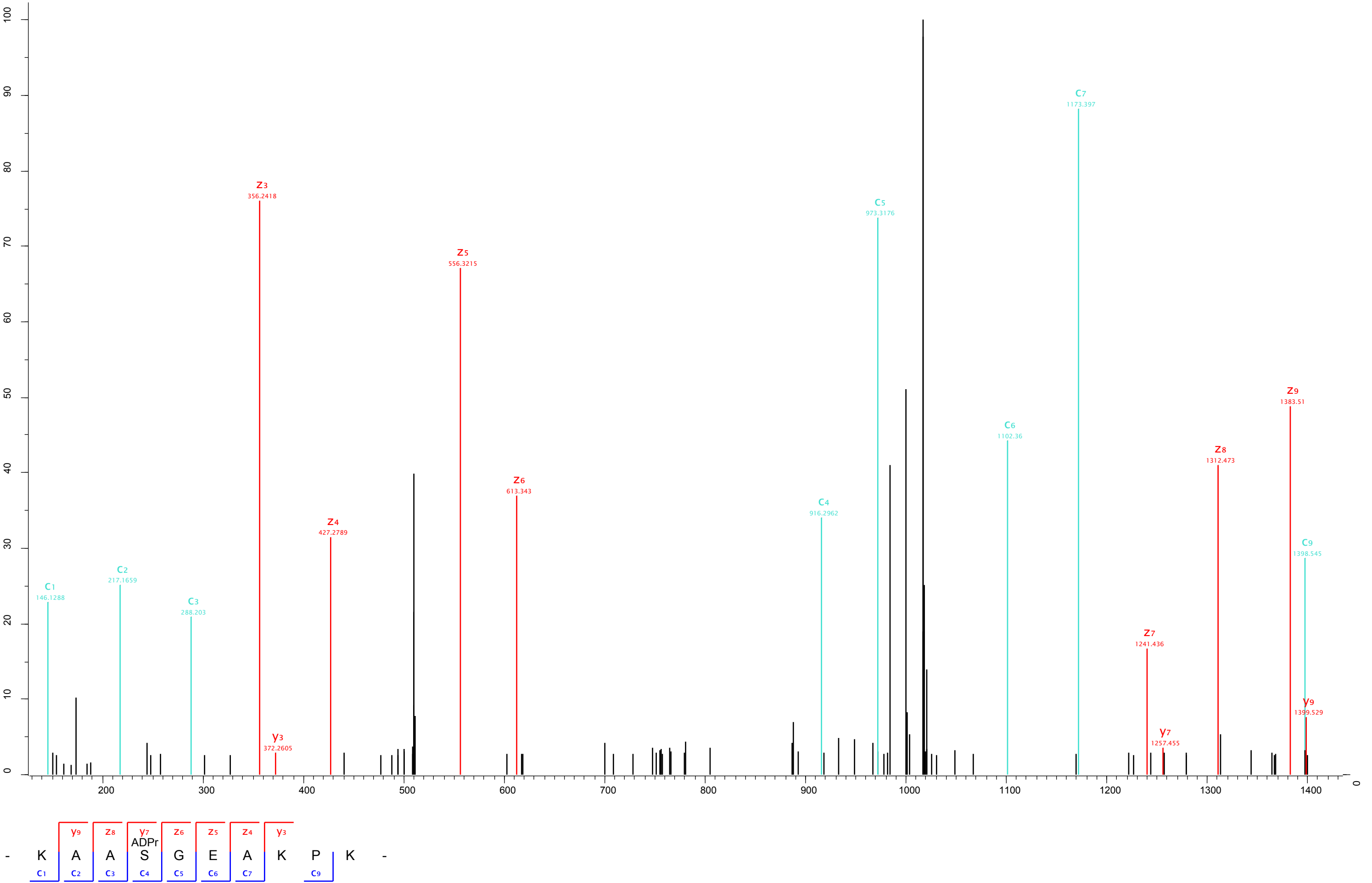
H1.4

ADP-ribosylation on Ser112

m/z 509.87953

charge 3+

mass error [ppm] 0.7



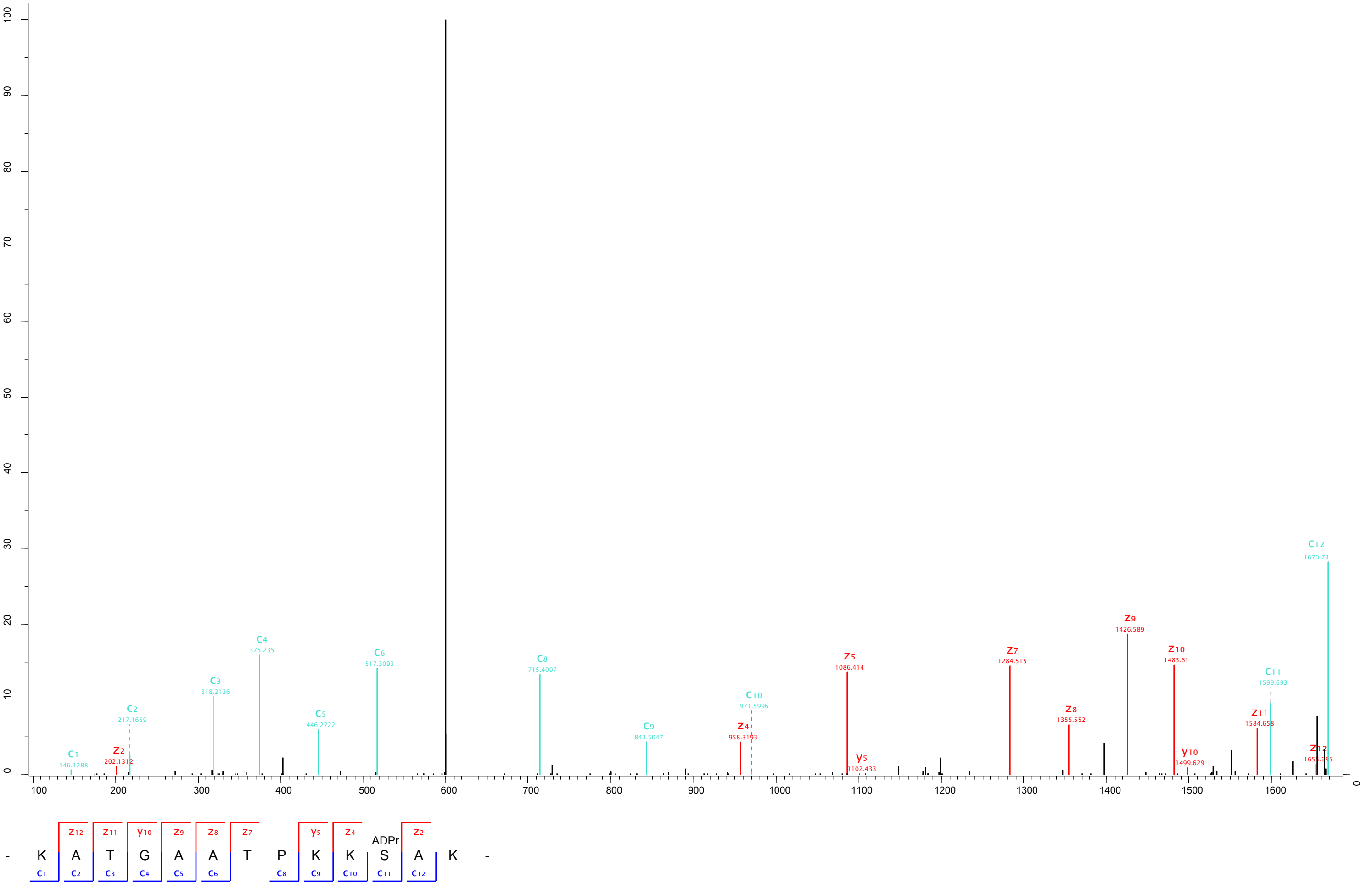
H1.4

ADP-ribosylation on Ser149

m/z 600.6078

charge 3+

mass error [ppm] 0.5



m/z 466.55595    charge 3+    mass error [ppm] 0.2



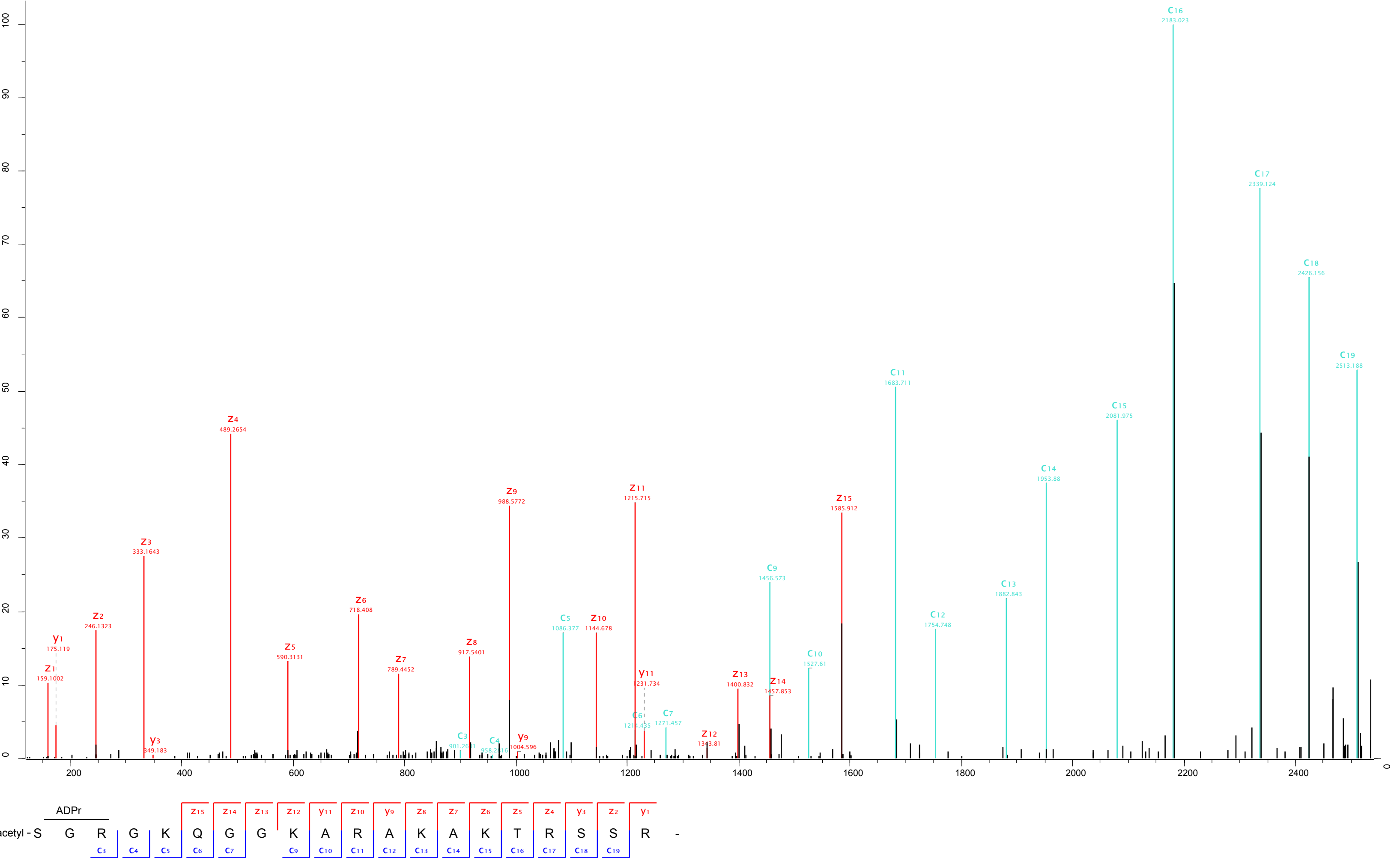
H2A

ADP-ribosylation on Ser1 or Arg3

m/z 534.86049

charge 5+

mass error [ppm] 1.0



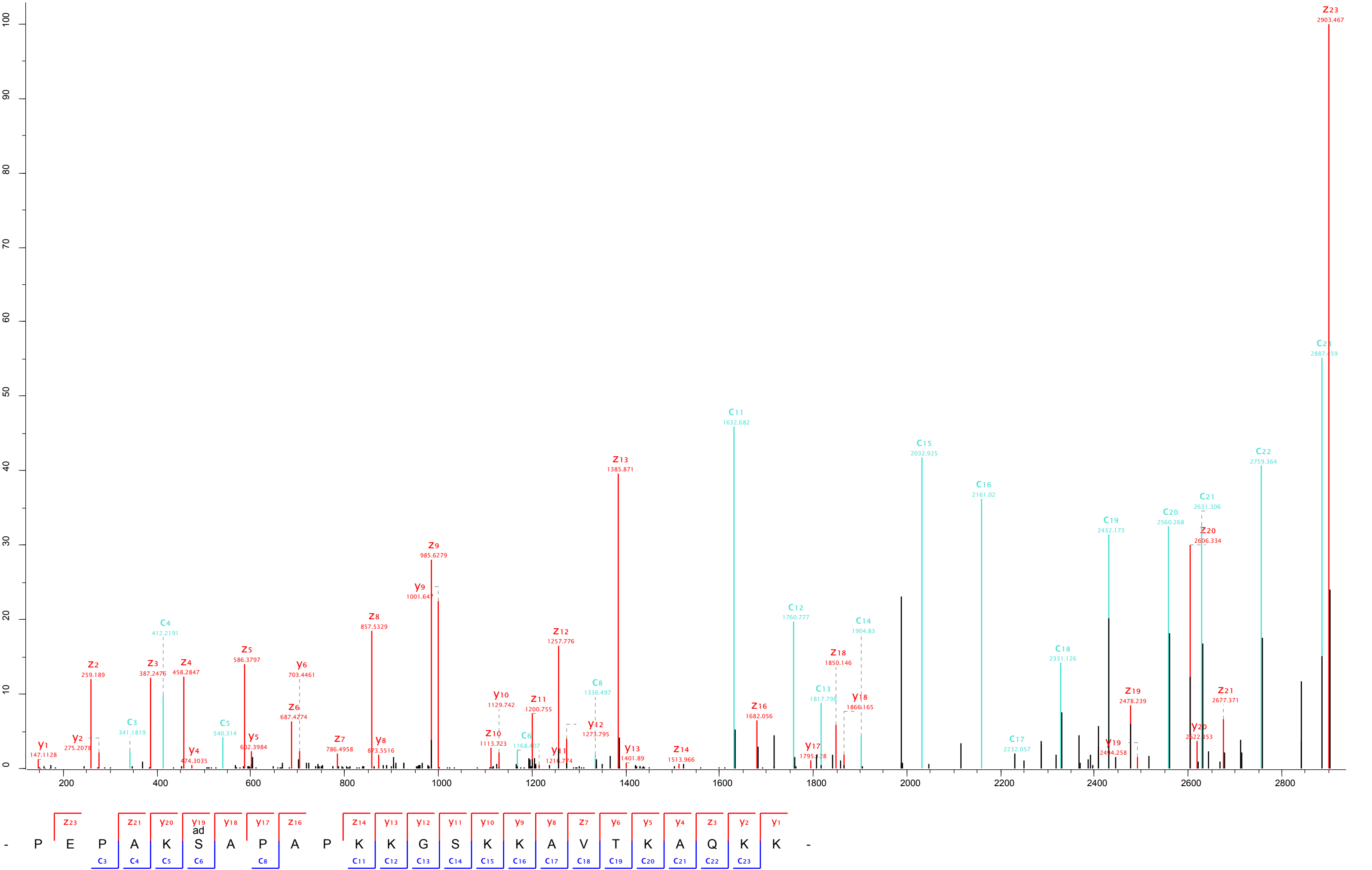
H2B1C

ADP-ribosylation on Ser6

m/z 604.11343

charge 5+

mass error [ppm] -0.5



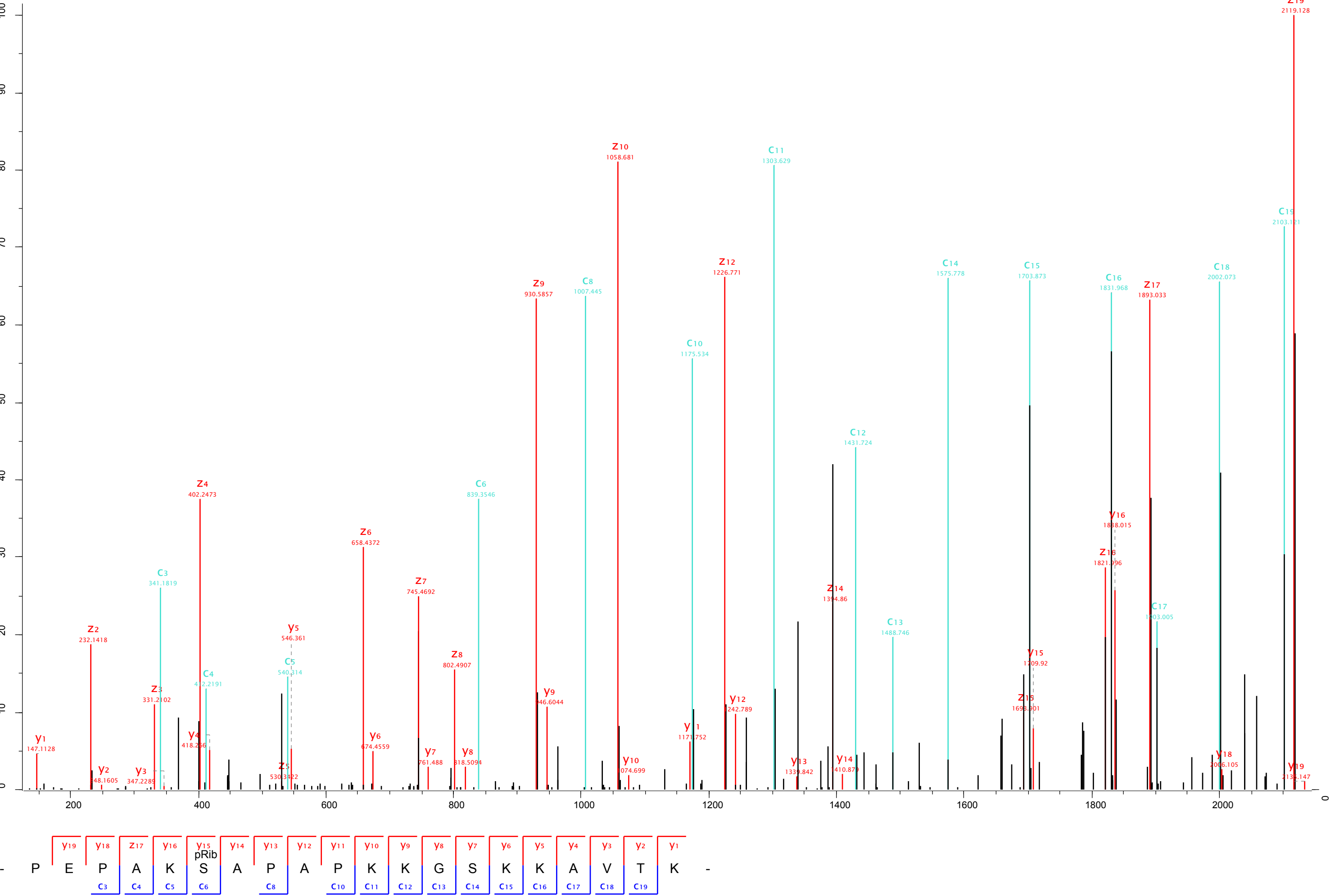
H2B1C

m/z 447.2481

charge 5+

phospho ribose on Ser6

mass error [ppm] -0.4



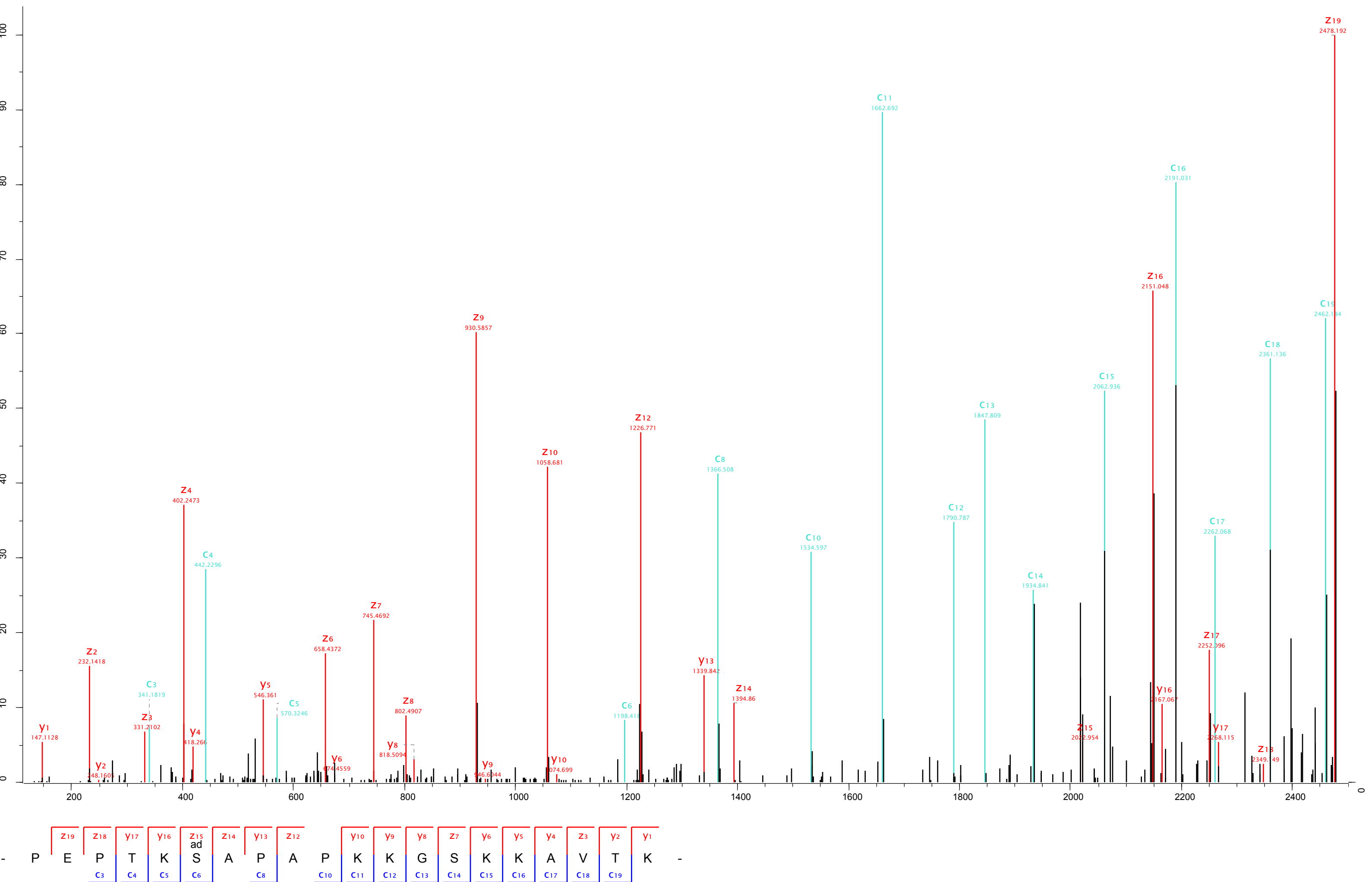
H2B1D

ADP-ribosylation on Ser6

m/z 519.05842

charge 5+

mass error [ppm] 0.5



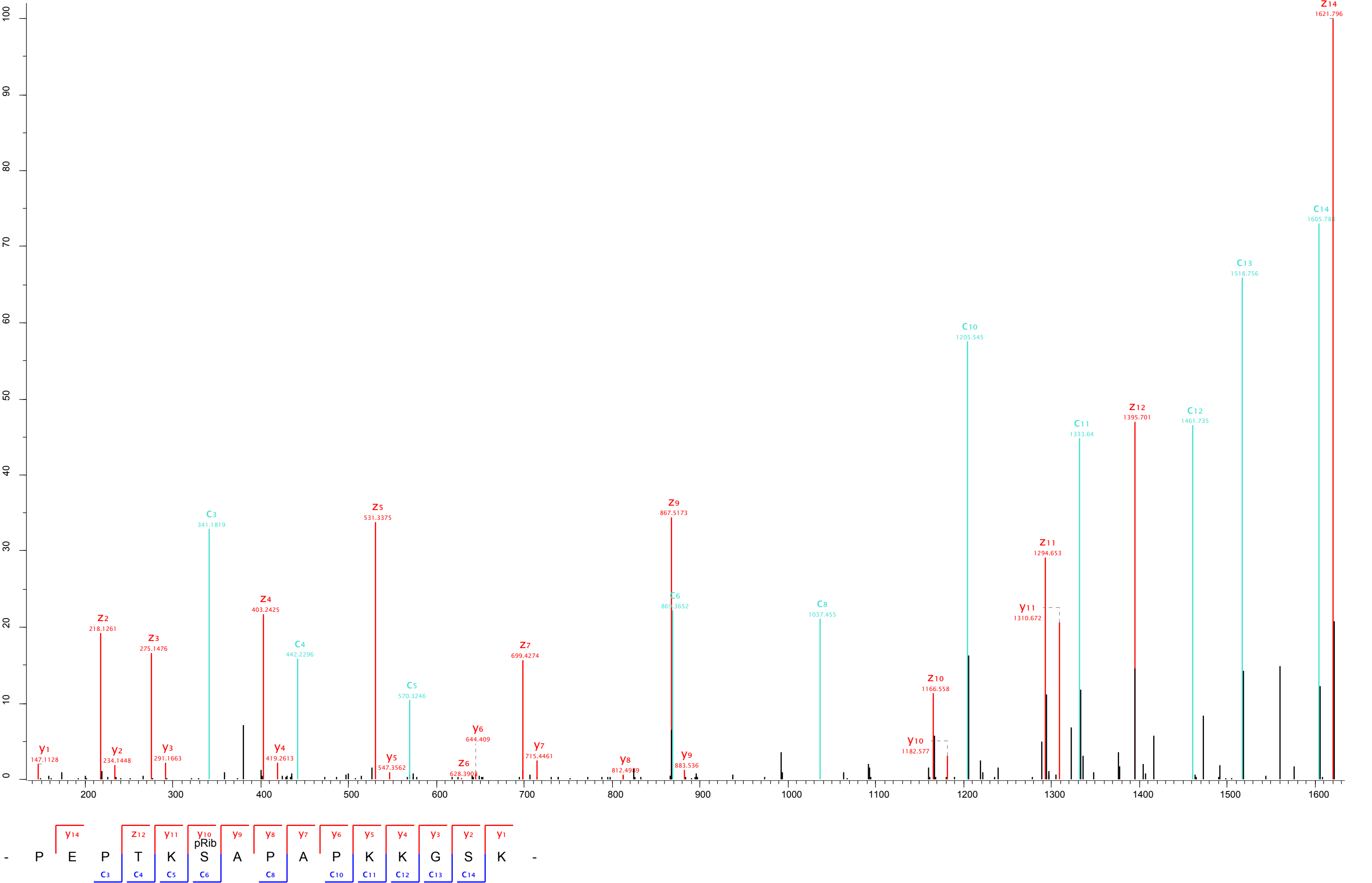
H2B1D

phospho ribose on Ser6

m/z 434.4723

charge 4+

mass error [ppm] -0.1



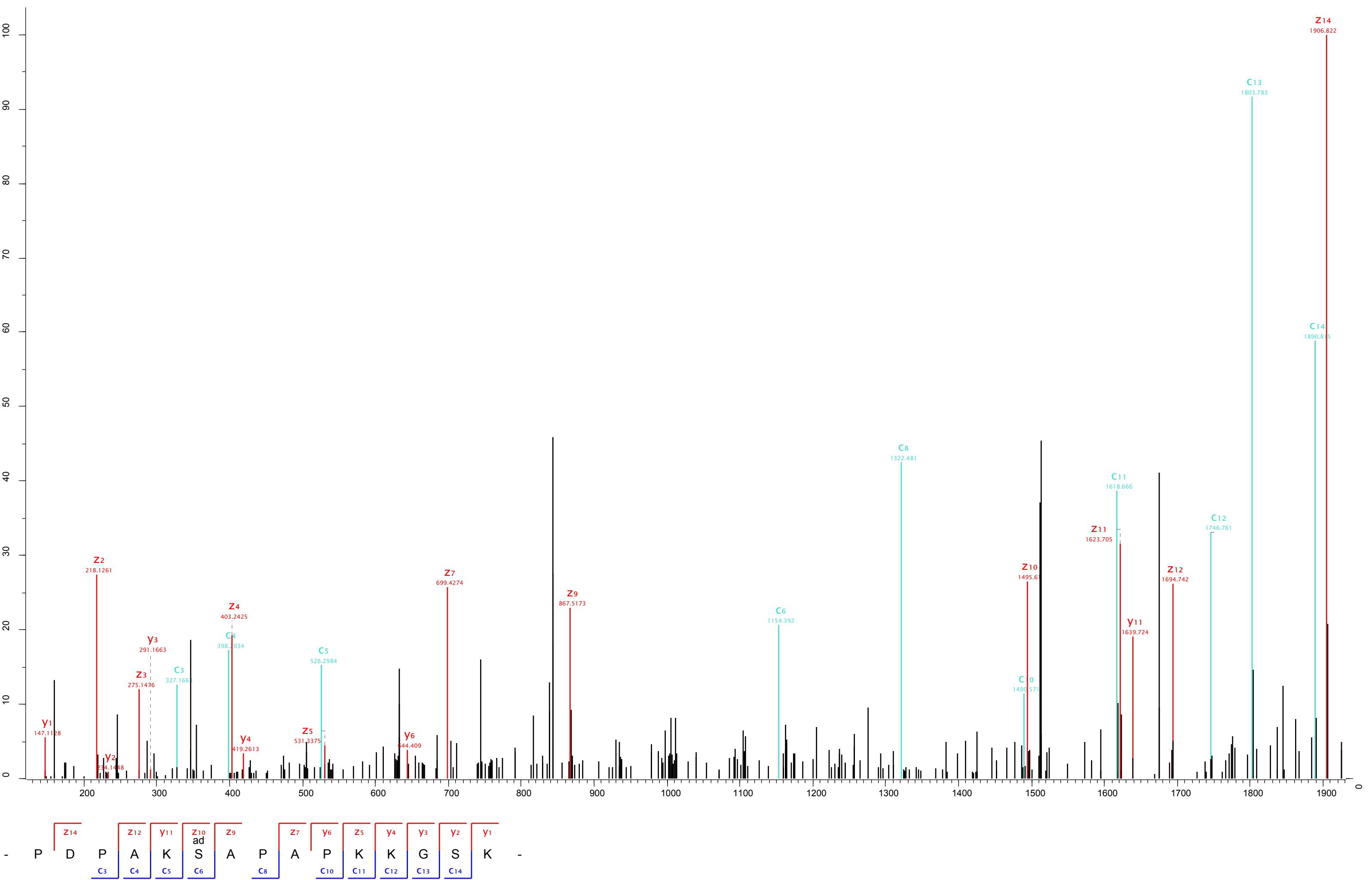
H2B1H

ADP-ribosylation on Ser6

m/z 505.72887

charge 4+

mass error [ppm] 0.6



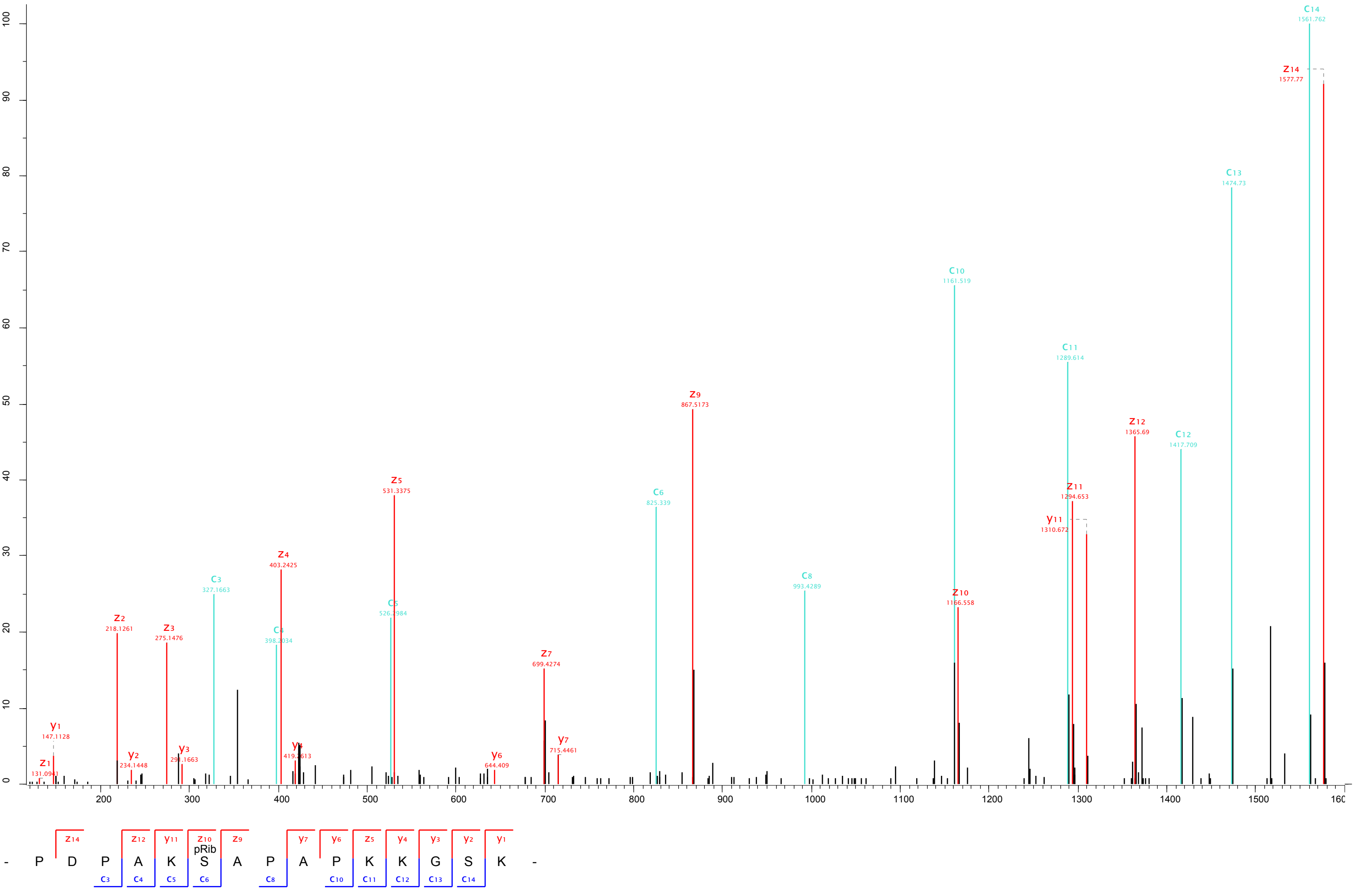
H2B1H

phospho ribose on Ser6

m/z 423.46574

charge 4+

mass error [ppm] 0.2



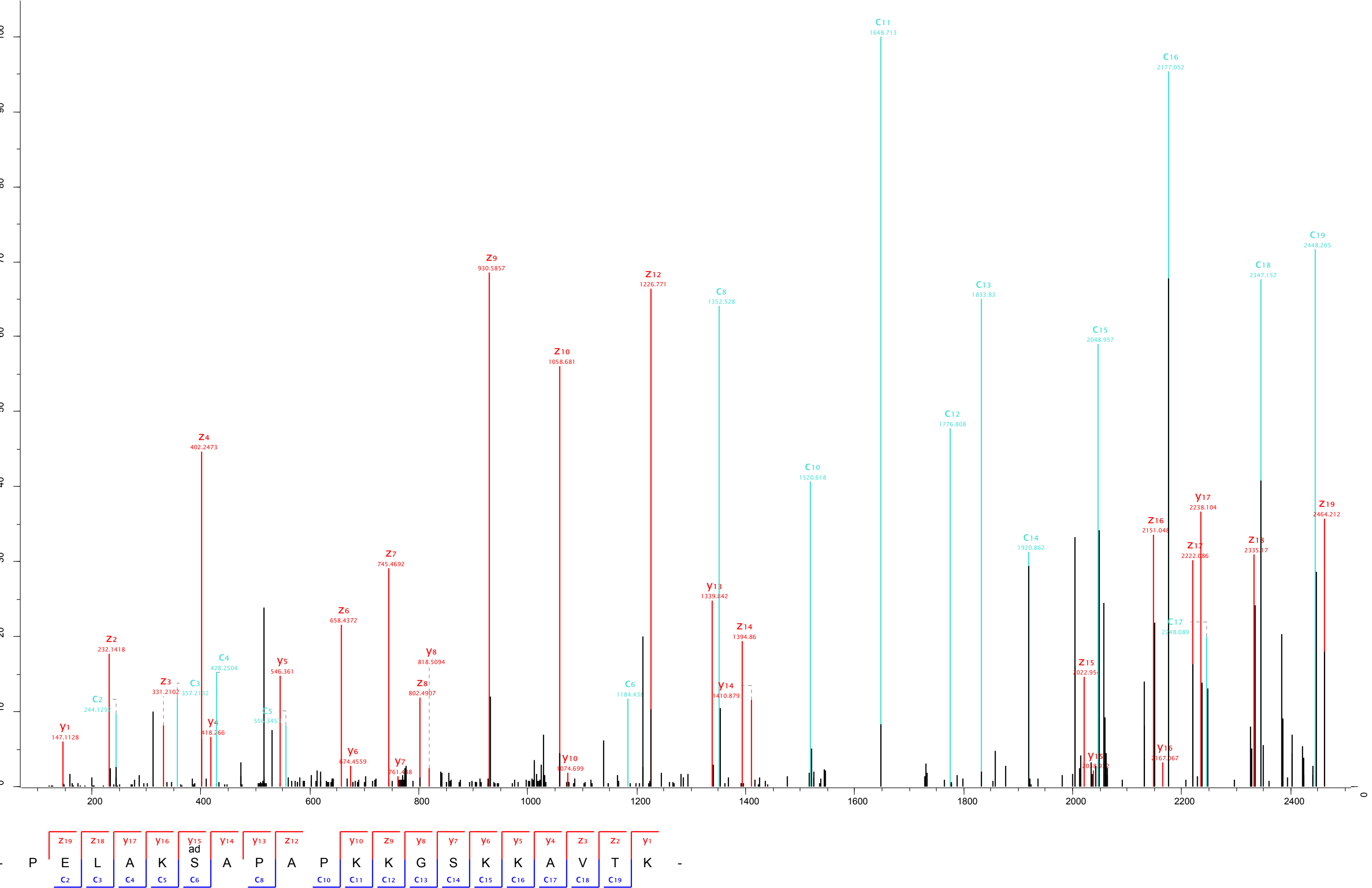
H2B1L

ADP-ribosylation on Ser6

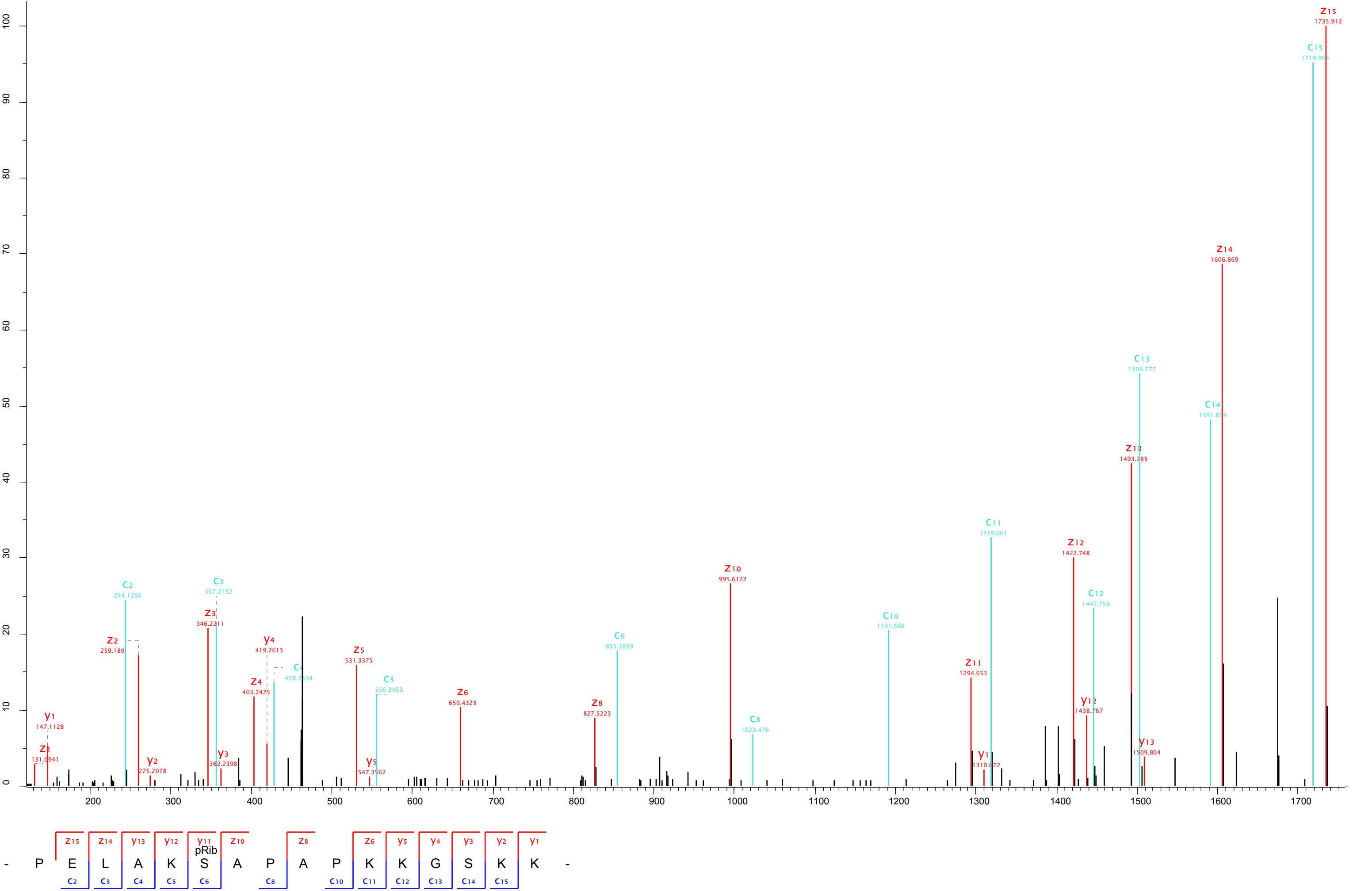
m/z 516.26257

charge 5+

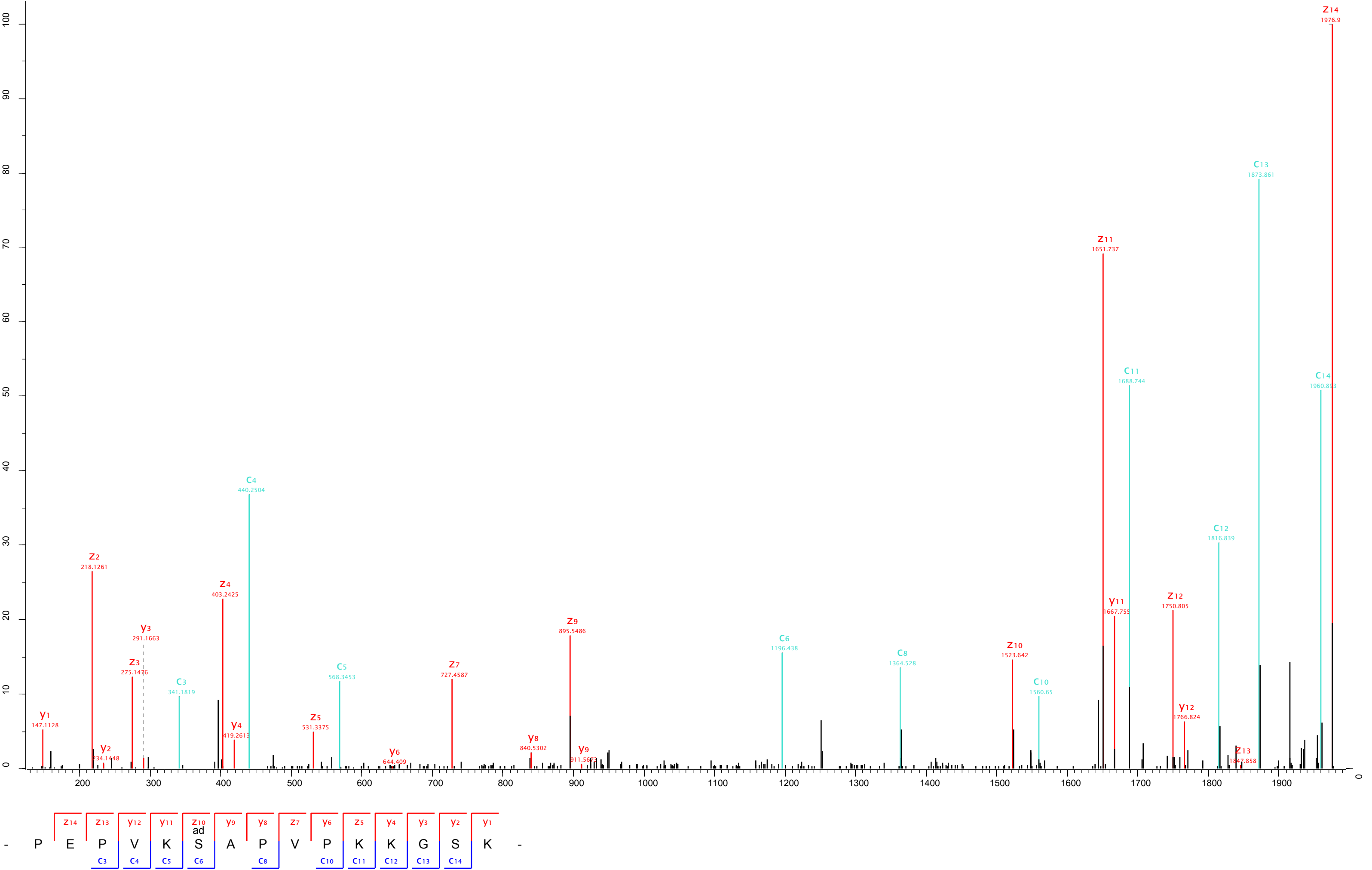
mass error [ppm] -1.5



H2B1L                   phospho ribose on Ser6  
m/z 463.00122    charge 4+       mass error [ppm] -0.5



H2B1M ADP-ribosylation on Ser6  
m/z 523.24844 charge 4+ mass error [ppm] 1.4



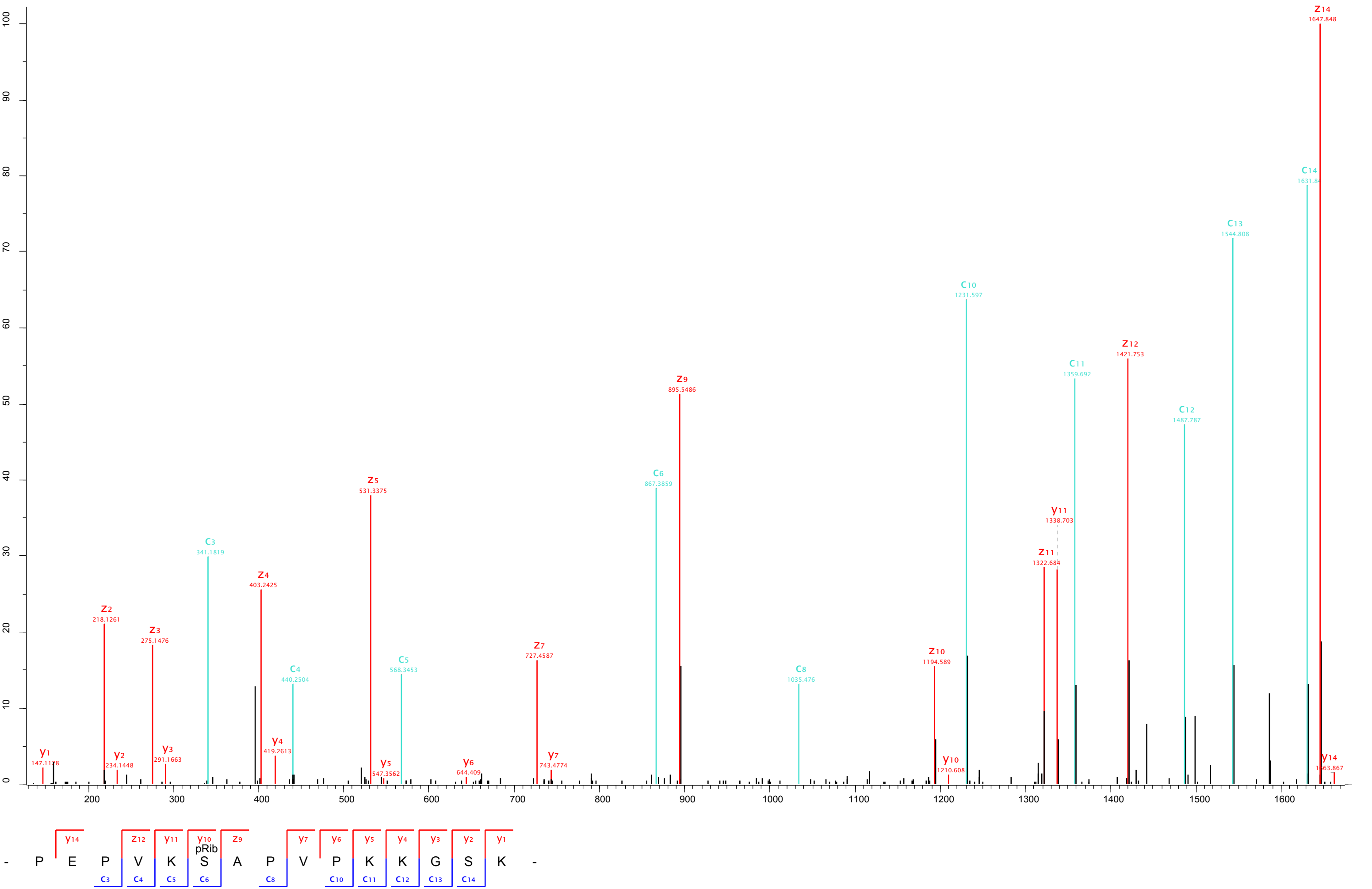
H2B1M

phospho ribose on Ser6

m/z 440.98531

charge 4+

mass error [ppm] -1.5



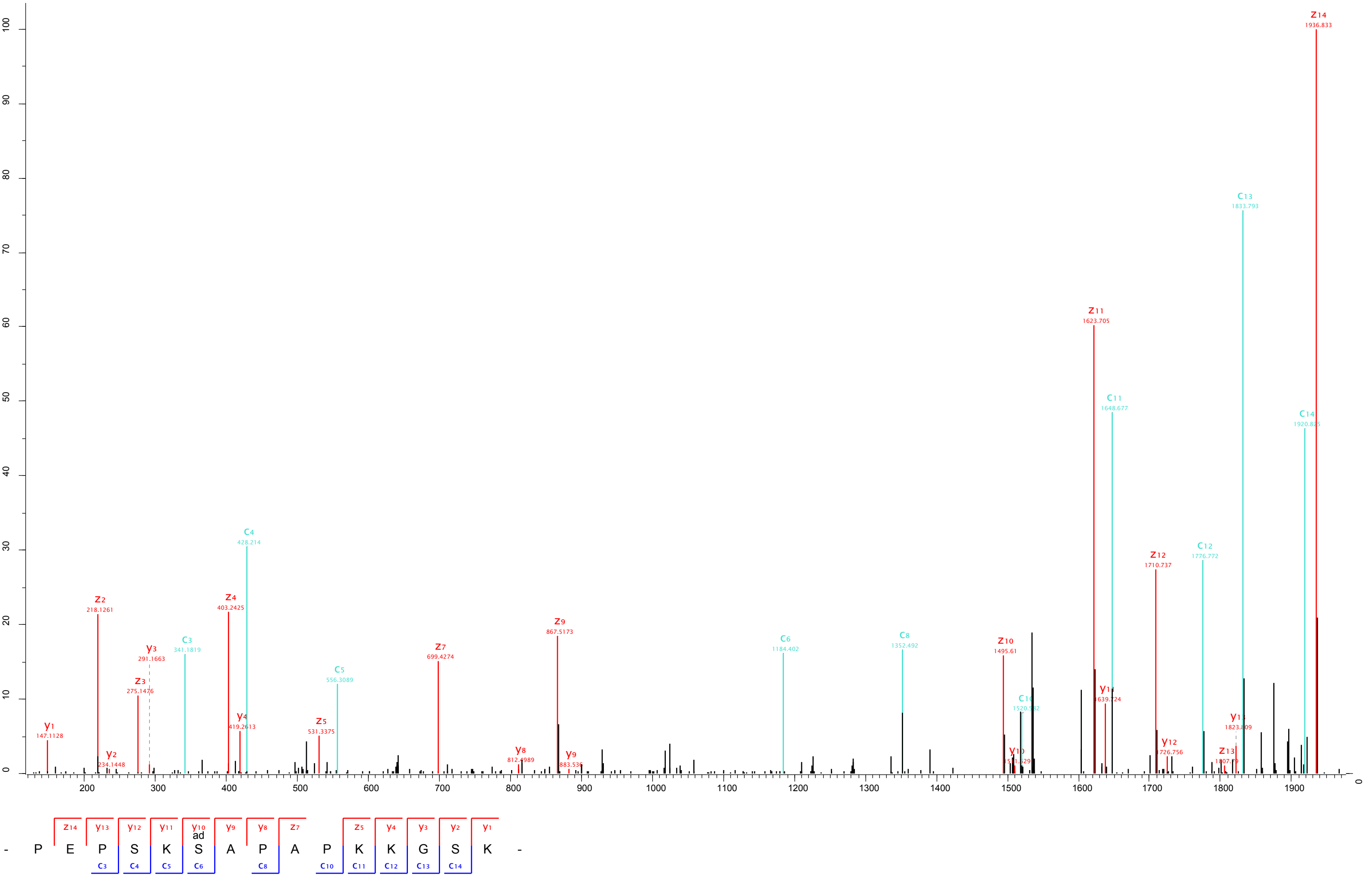
H2B1N

ADP-ribosylation on Ser6

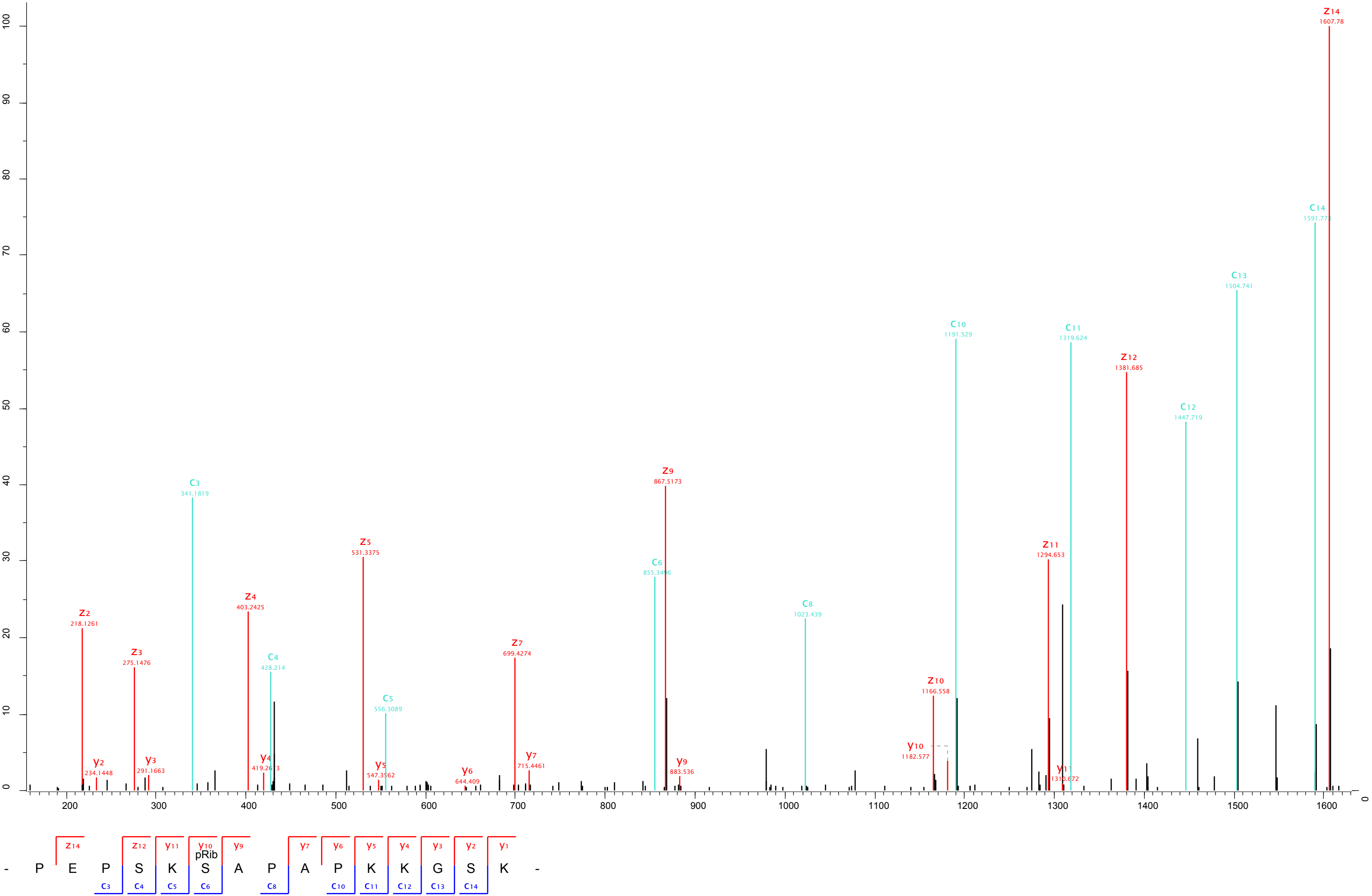
m/z 513.23151

charge 4+

mass error [ppm] 0.5



H2B1N      phospho ribose on Ser6  
m/z 430.96838    charge 4+    mass error [ppm] -0.3



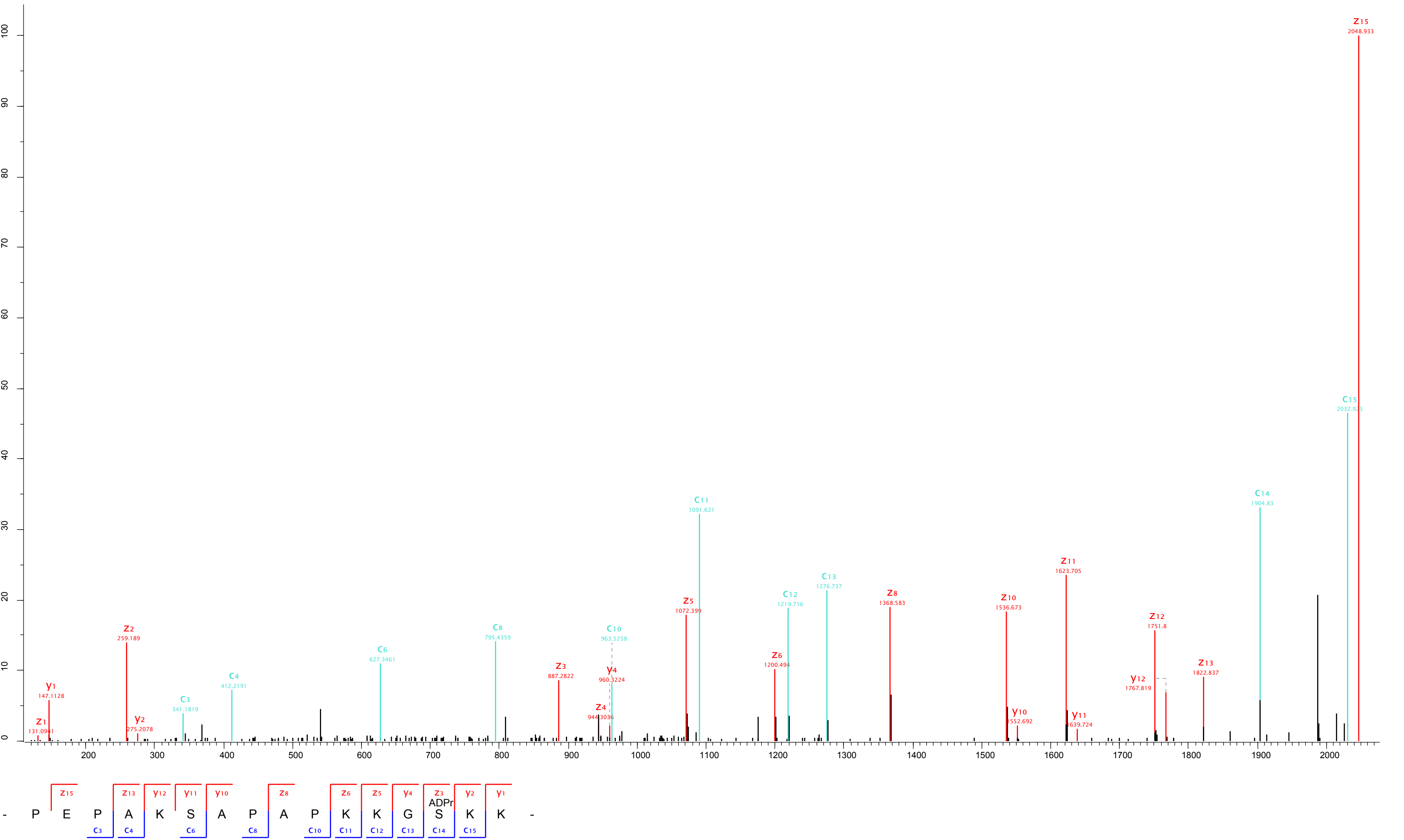
H2B1C

ADP-ribosylation on Ser14

m/z 541.25653

charge 4+

mass error [ppm] -0.7



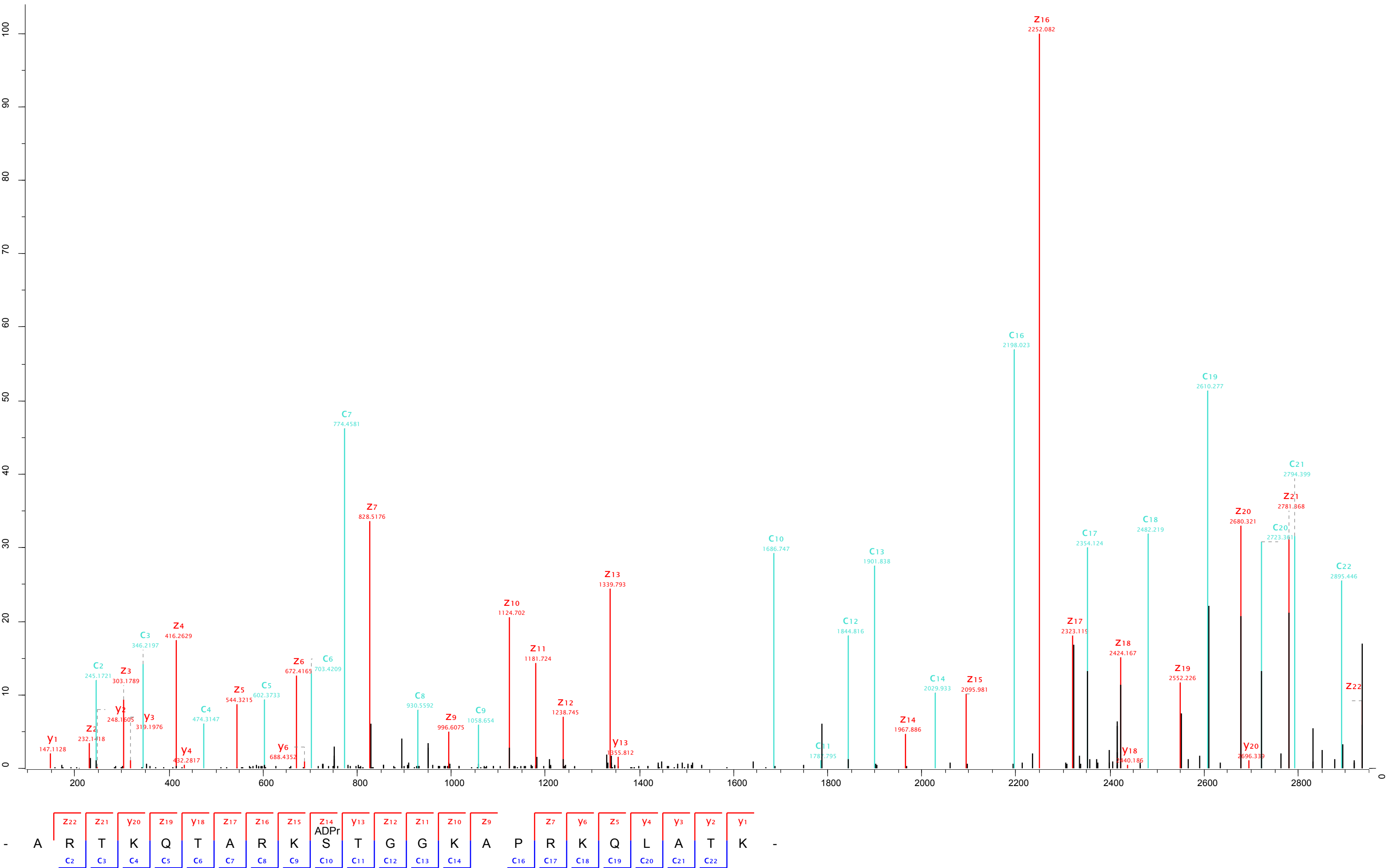
H3.1

ADP-ribosylation on Ser10

m/z 605.71086

charge 5+

mass error [ppm] -0.4



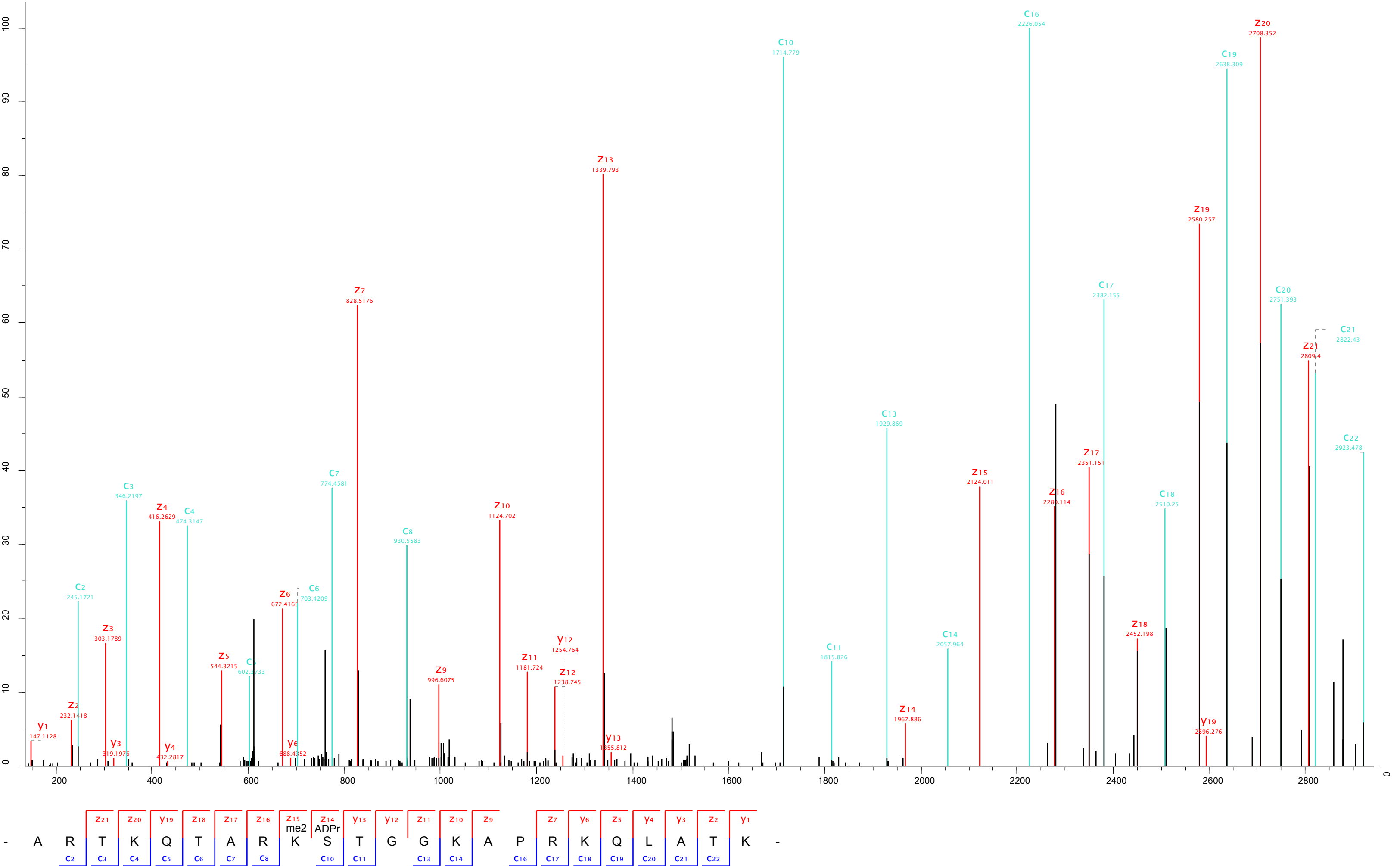
H3.1

ADP-ribosylation on Ser10

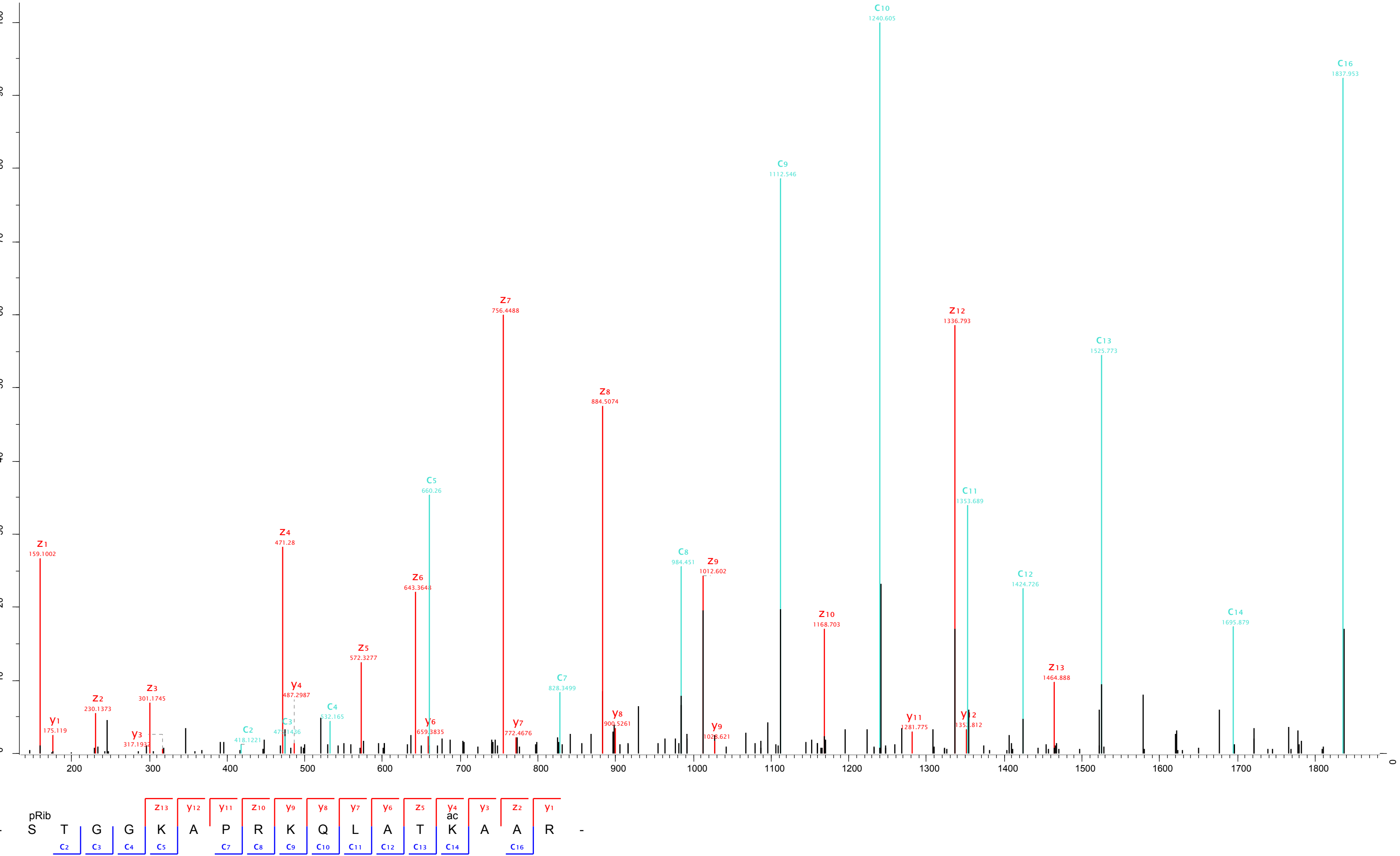
m/z 611.31712

charge 5+

mass error [ppm] 0.5



H3.1      phospho-ribose on Ser10  
m/z 499.51503    charge 4+    mass error [ppm] -2.3



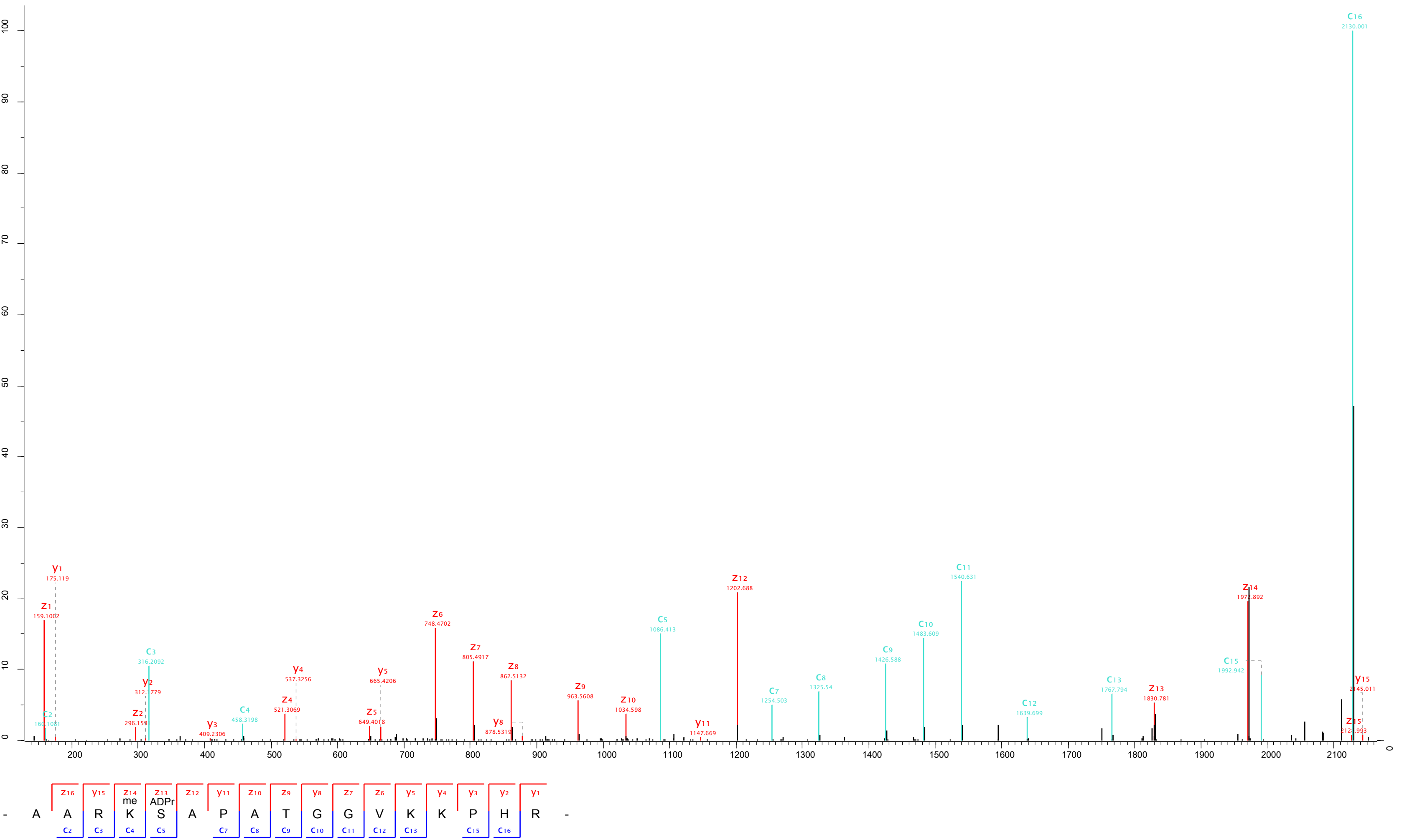
H3.1

ADP-ribosylation on Ser28

m/z 458.22295

charge 5+

mass error [ppm] -0.1



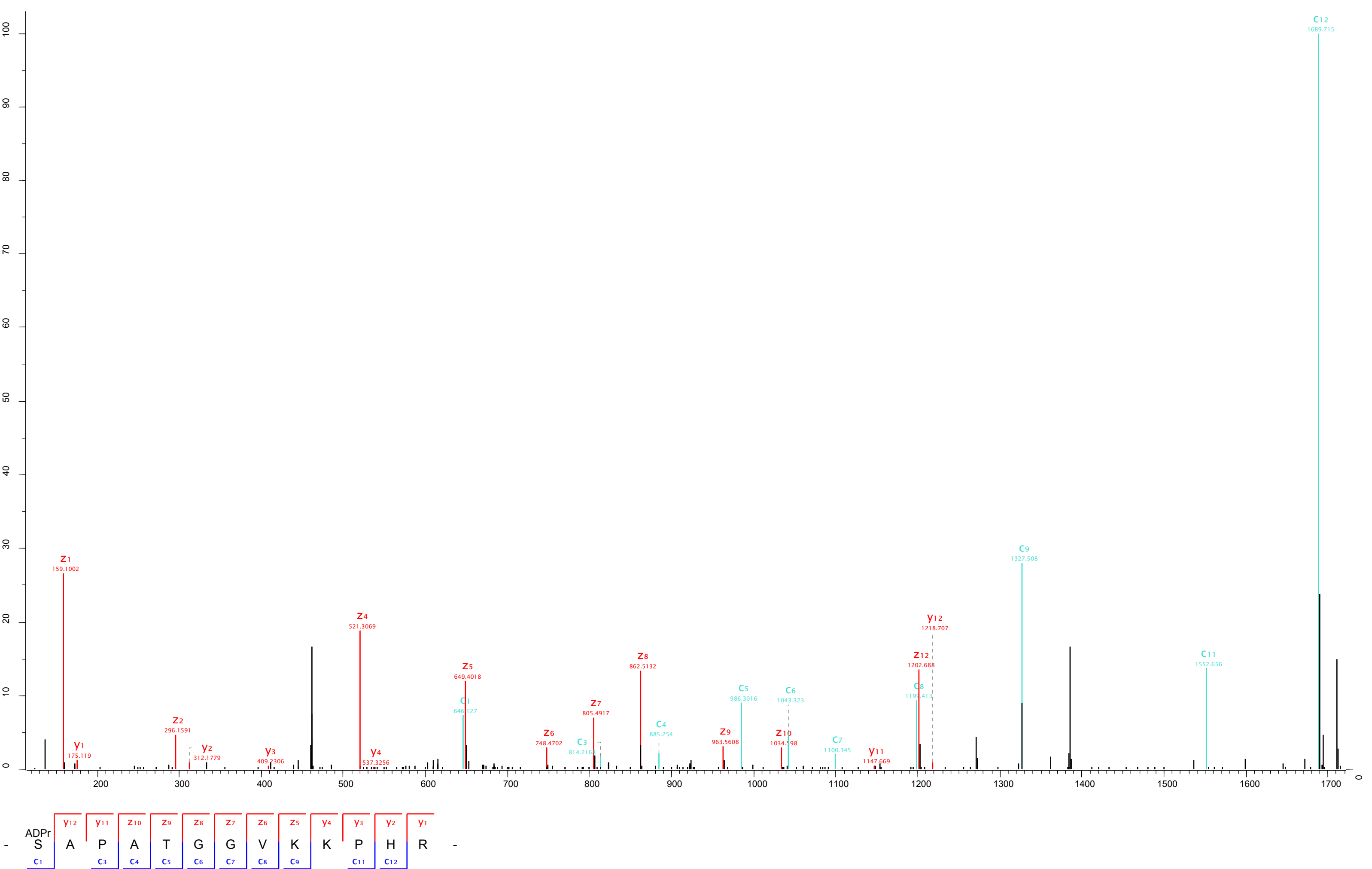
H3.1

ADP-ribosylation on Ser28

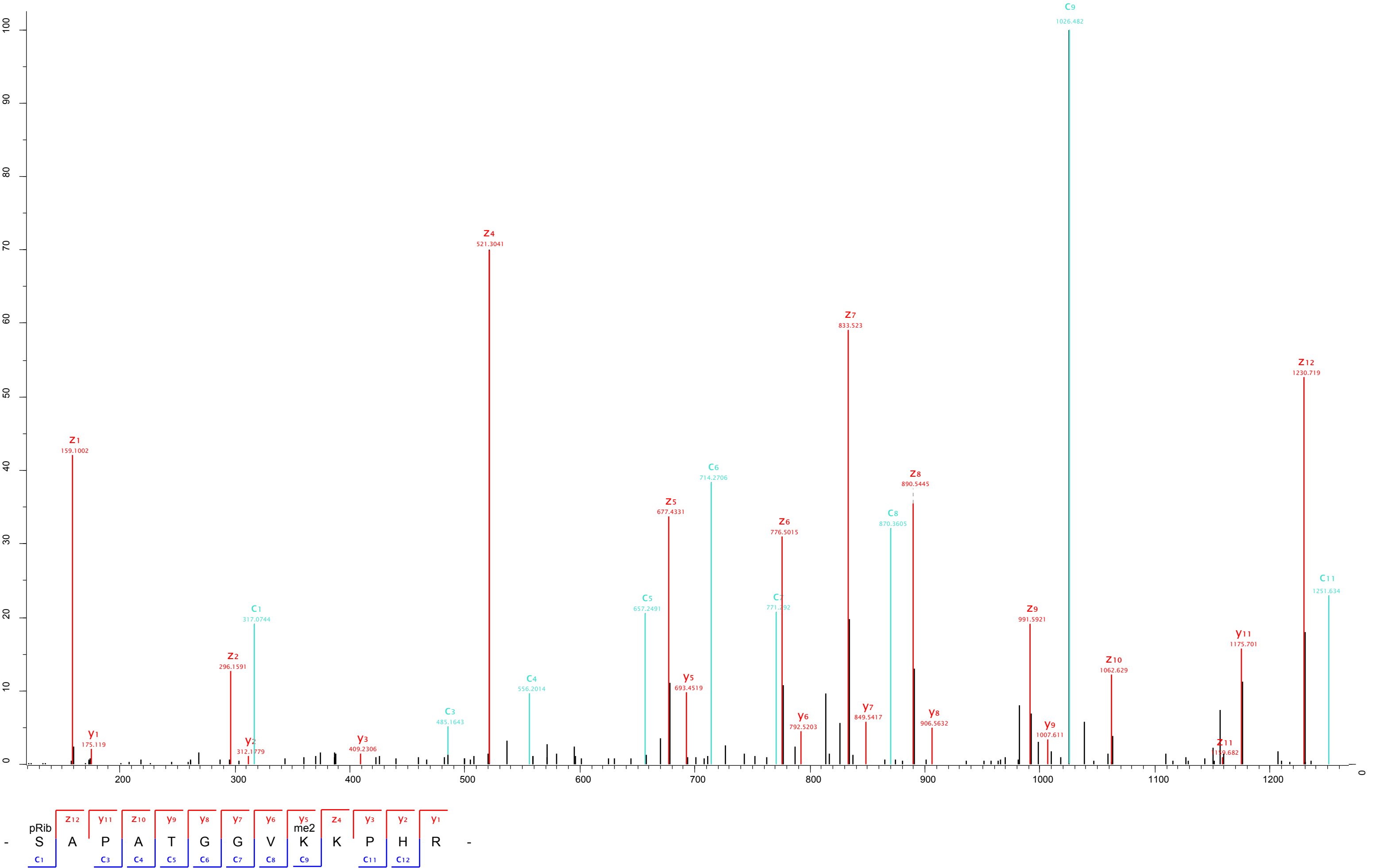
m/z 462.45538

charge 4+

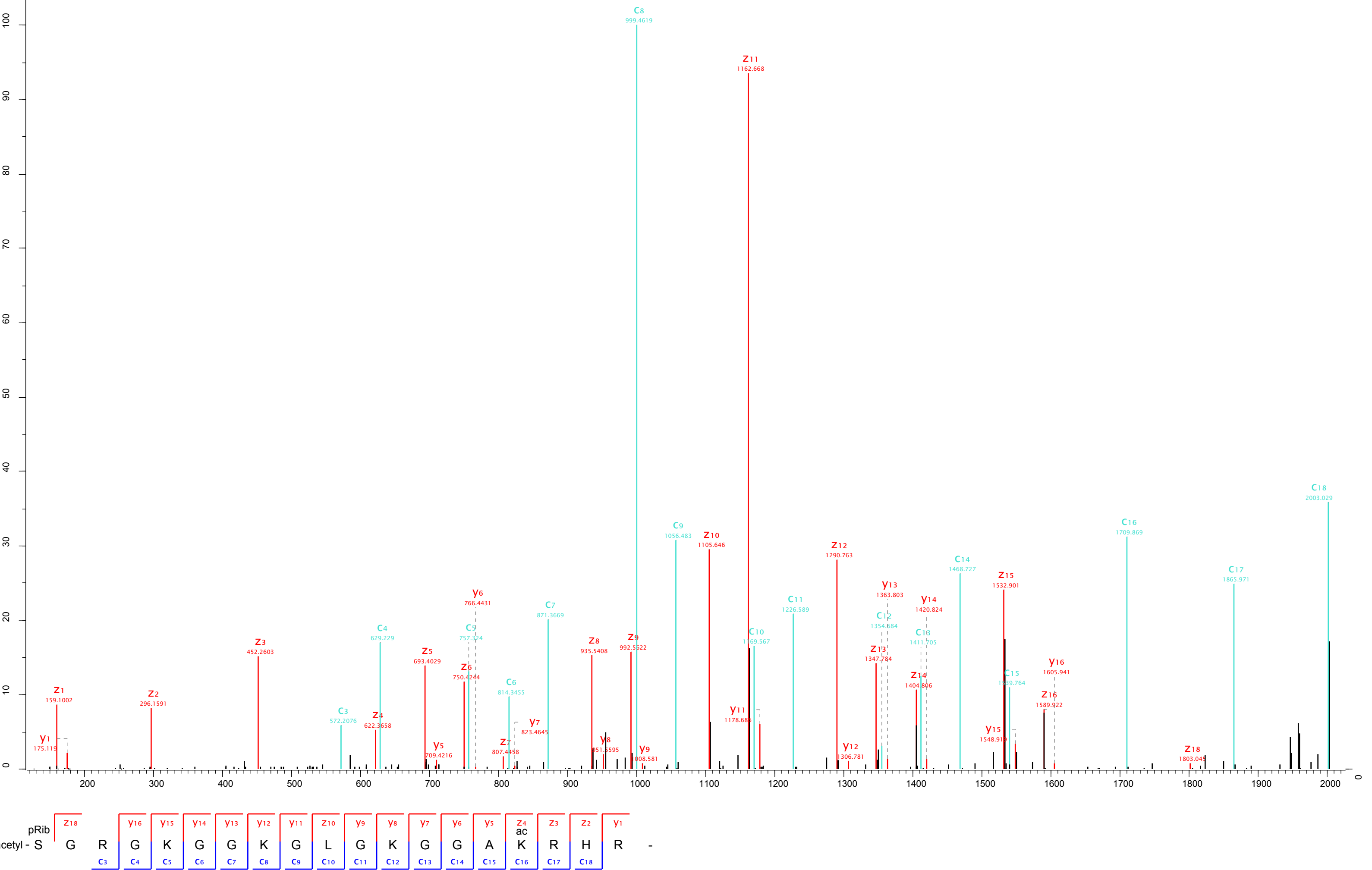
mass error [ppm] 1.2



H3.1      phospho-ribose on Ser28  
m/z 387.20008    charge 4+    mass error [ppm] -0.3



H4      phospho riboseon Ser1 or Arg3  
m/z 432.82874    charge 5+    mass error [ppm] -0.8



H4 ADP-ribosylation on Ser1 or Arg3  
m/z 549.75722 charge 4+ mass error [ppm] 1.9

