Supporting Information

Experimental

Chemical and Materials The DNA aptamer targeting the H4-K16Ac (5'-AGA CGT AAG TTA ATT GGA CTT GGT CGT GTG CGG CAC AGC GAT TGA AAT-3') was synthesized by Sangon Biotech. (Shanghai, China), a biotin label was attached to the 5' end of the aptamer, and the biotinylated aptamer was purified by HPLC. High Capacity Streptavidin Agarose Resin, HandeeTM Spin Columns-ScrewCap, RPMI media 1640, Dulbeuo's modified Eagle media(DMEM), Fetal boving serum, Penicillin, Streptomycin were all purchased from Thermo Fisher scientific (Tianjin, China). All subsequent solutions were prepared in the ultrapure water purified by Aquapro Purification System. RIPA non-denaturing lysis buffer was purchased from Solarbio (Shanghai, China). Water and acetonitrile used in MS analysis steps were purchased from Fisher Scientific (Pittsburgh, PA). Trifluoroacetic acid (TFA) was from Sigma-Aldrich (St. Louis, MO). Sequencing-grade trypsin was from Promega (Madison, WI). C18 ZipTips were from Millipore (Bedford, MA).

Cell Culture HeLa cells were cultured in 100mm Corning petri dishes in RPMI media supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL) and streptomycin (100 units/mL), and they were incubated in a 5% CO_2 humidified incubator at 37°C.

Acid-extraction of core histones The method for acid extraction of core histones was based on the reported protocol with some modifications.[1] Collected HeLa cells (7×10^6cells) in 1.5mL tubes, washed the pellet with cooling PBS twice, and added 1ml TEB (0.5% Triton X-100(v/v), 2mM PMSF, 0.02%NaN_3(w/v)) to the cell pellet, lysed on the ice for 10 min shaking. Intact nuclei were pelleted by spinning at 2000g for 10 min at 4°C for twice. The nuclear pellet was then resuspended overnight in 0.2mol/L H_2SO_4 at 4°C.The nuclear debris was removed by spinning at 16000g for 10 min at 4°C. The supernatant containing extracted core histones were collected in a new 1.5mL tube and then precipitated with 50%TCA drop by drop (5volumes), Precipitated histone proteins were spun down at 16000 g for 10 min at 4°C and washed twice with ice-cold Acetone. Protein pellets were air-dried at room temperature and then dissolved in ultrapure water, and then 10μl extracted histones solution were separated on a 15% SDS–polyacrylamide gel and stained with Coomassie Blue dye. The locations of the linker histone protein H1 and the core histone proteins H3, H2B, H2A and H4 were noted in Fig. S1.

Preparation of the Aptamer-Modified Spin Columns A 900μl spin column with an adaptors and a plug was prepared, a small polyethylene frits (10μm pore size, 2.7mm diameter) had been pre-inserted in column. To the column, added 50μl streptavidin agarose resin, centrifuged at 500g for 1min at 4°C to remove the buffer, then the remaining beads were washed with loading buffer (3mM KCl,10mM Na_2HPO_4, 2mM KCl, 10mM NaCl, 5mM $MgCl_2$, pH 7.4) at 500g for 1 min at 4°C for 5 times. Prior to immobilization of the aptamers, the biotinylated aptamer denatured by heating at 95°C for 5 min followed by cooling at room temperature in the presence of loading buffer, then 50μl aptamer was added to the colomn, incubated at room temperature for 1h with gentle shaking. Finally, the column was centrifuged to remove the unreacted ones, and washed

with 200µl loading buffer for 5 times.

Protein in-gel digestion Gel bands of the histone H4 eluted by 2.5M NaCl were excised and subjected to in-gel digestion as described previously.[2] Briefly, the gel band was sliced into small pieces (~1 mm) and destained with 25mM ammonium bicarbonate in ethanol/water (50:50, v/v). The destained gel pieces were washed in an acidic buffer (acetic acid/ethanol/water, 10:50:40, v/v/v) three times for 1 h each time, and in water two times for 20 min each time. The gel pieces were dehydrated in acetonitrile and dried in a SpeedVac (Thermo Fisher, Waltham, MA). Two hundred nanograms of porcine modified trypsin (Promega, Madison, WI) in 50 mM ammonium bicarbonate was added to the dried gels and incubated overnight at 37 °C. Tryptic peptides were sequentially extracted from the gel pieces with 50% acetonitrile (acetonitrile/water/TFA, 50:45:5, v/v/v) and 75% acetonitrile (acetonitrile/water/TFA, 75:24:1, v/v/v). The peptide extracts were pooled, dried in a SpeedVac, and desalted using a µ-C18 Ziptip before HPLC/MS/MS analysis.

HPLC/MS/MS analysis HPLC/MS/MS analysis was carried out by nano-HPLC/LTQ-orbitrap mass spectrometry (Thermo Discovery, CA). Briefly, each tryptic digest was dissolved in 10 µL of HPLC buffer A (0.1% (v/v) formic acid in water), and 2 µL was injected into an Eksigent HPLC system (Eksigent, Palo Alto, CA). Peptides were separated on a homemade capillary HPLC column (50-mm length×75-µm inner diameter) containing Jupiter C12 resin (4-µm particle size, 90-Å pore diameter, Phenomenex, St. Torrance, CA) and electrosprayed directly into the mass spectrometer using a nanospray source. The LTQ-orbitrap mass spectrometer was operated in a data-dependent mode, cycling between acquiring one MS spectrum followed by MS/MS spectra of the 10 strongest ions in that MS spectrum. The LC/MS/MS data were finally searched against the NCBInr human protein sequence database using Mascot database with mascot search engine (version 2.1.0, Matrix Science, Beijing, China).

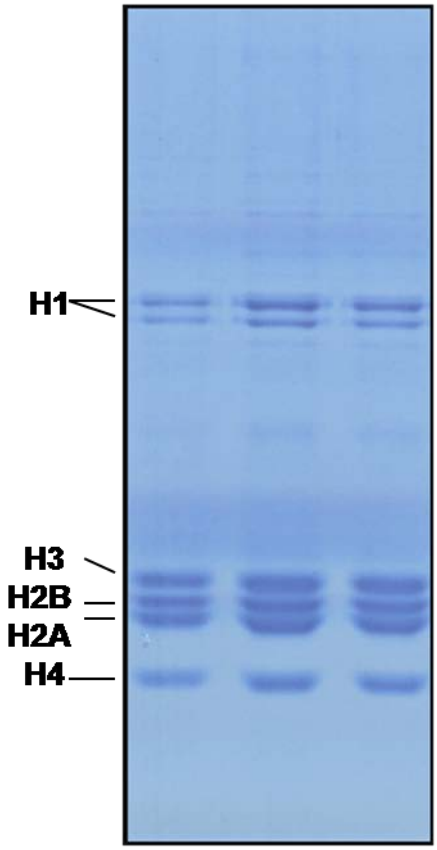


Fig. S1 Coomassie Blue-stained SDS gel with acid extracted histones. 10μl histones of the above three lanes come from hela cells in 3 separated petri dishes cultured at the same time

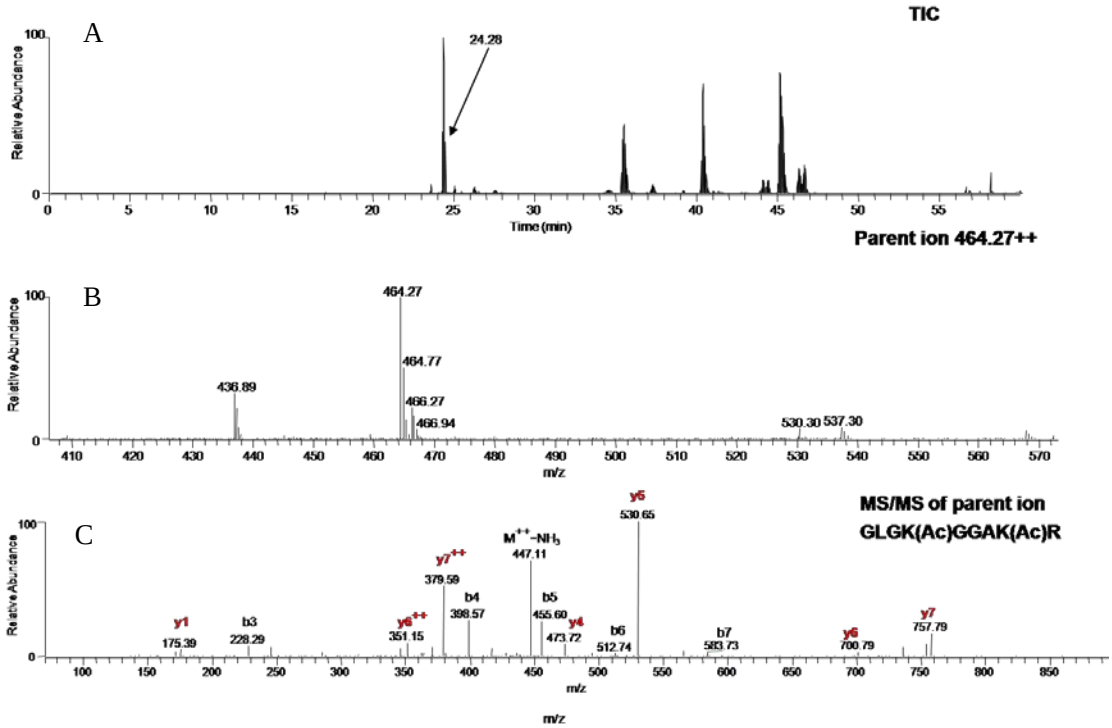


Fig. S2 Separation and analysis of tryptic peptides by LC/MS/MS. (A)Base peak ; (B) Parent ion of GLGK(Ac)GGAK(Ac)R; (C) MS/MS of GLGK(Ac)GGAK(Ac)R from in vivo H4

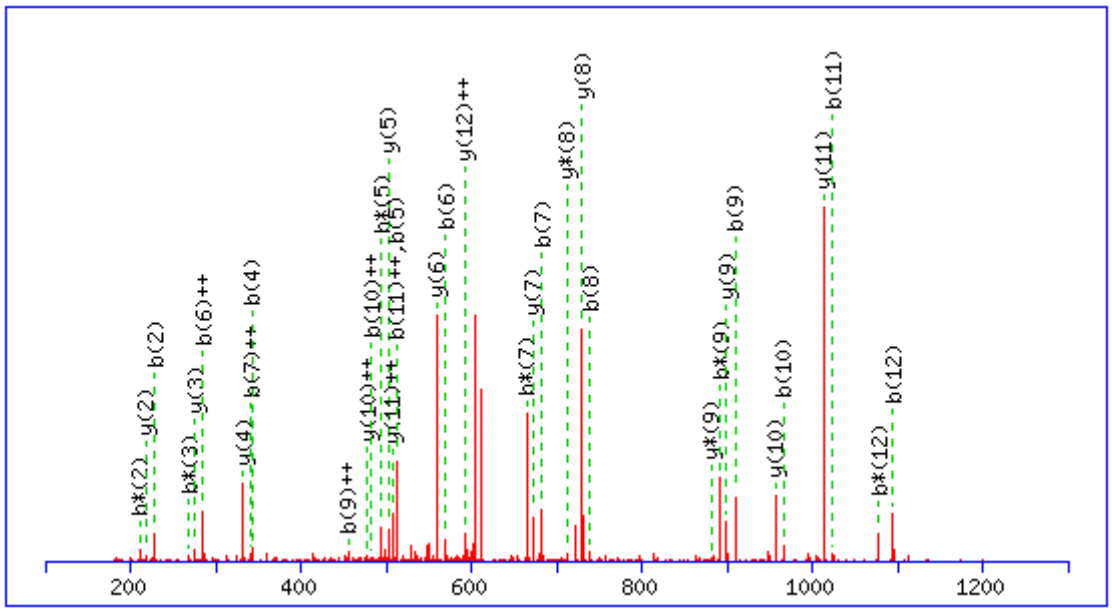
Table S1 the major PTMs identified in H4 eluted from aptamer modified column

Sequence	PTMs	m/z	RT/min	Mascot score
R.GKGGKGLGK.G	Acetyl (K 5)	422.26	9.53	22
R.GKGGKGLGK.G	2 Acetyl (K 2;5)	443.26	24.52	52
R.GKGGKGLGKGGAK.R	3 Acetyl (K 2;5;9)	620.85	26.60	69
R.GKGGKGLGKGGAKR.H	4 Acetyl (K 2;5;9;13)	719.91	31.21	79
R.GKGGKGLGKGGAKR.H	3 Acetyl (K 2;5;9); Trimethyl (K 14)	719.91	31.21	82
K.GGKGLGK.G	Acetyl (K 3)	329.69	15.91	43
K.GGKGLGKGGAK.R	2 Acetyl (K 3;7)	507.29	25.14	39
K.GGKGLGKGGAKR.H	3 Acetyl (K 3;7;11)	606.35	26.86	77
K.GLGKGGAK.R	Acetyl (K 4)	365.22	17.11	42
K.GLGKGGAKR.H	2 Acetyl (K 4;8)	464.27	24.89	43
R.DNIQGITKPAIR.R	none	663.38	35.57	50
R.DNIQGITKPAIR.R	Dimethyl (K 8)	677.38	42.23	52
R.DNIQGITKPAIR.R	Acetyl (K 8)	684.39	42.62	41
R.ISGLIYEETR.G	none	590.81	53.71	79
K.VFLENVIR.D	none	495.29	45.28	62
R.DAVTYTEHAK.R	Dimethyl (K 10)	587.78	25.08	56
R.DAVTYTEHAK.R	none	567.77	24.29	79
R.DAVTYTEHAKR.K	none	645.83	24.37	42

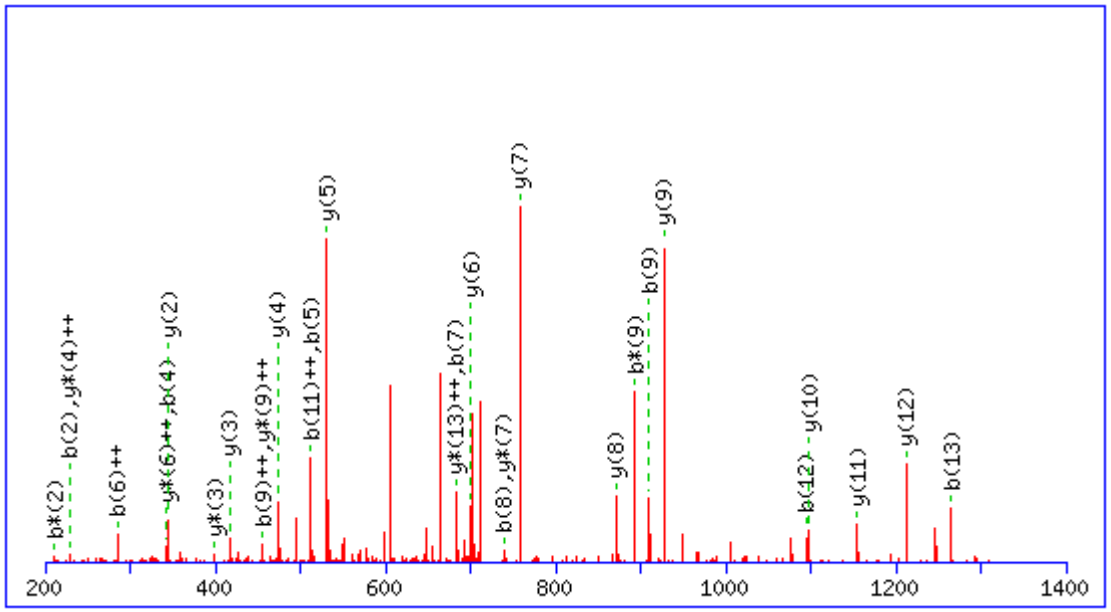
Note and References

1. D. Shechter, H. L. Dormann, C. D. Allis and S. B. Hake, *Nature protocols*, 2007, **2**, 1445-1457.
2. E. S. Witze, W. M. Old, K. A. Resing and N. G. Ahn, *Nature methods*, 2007, **4**, 798-806.

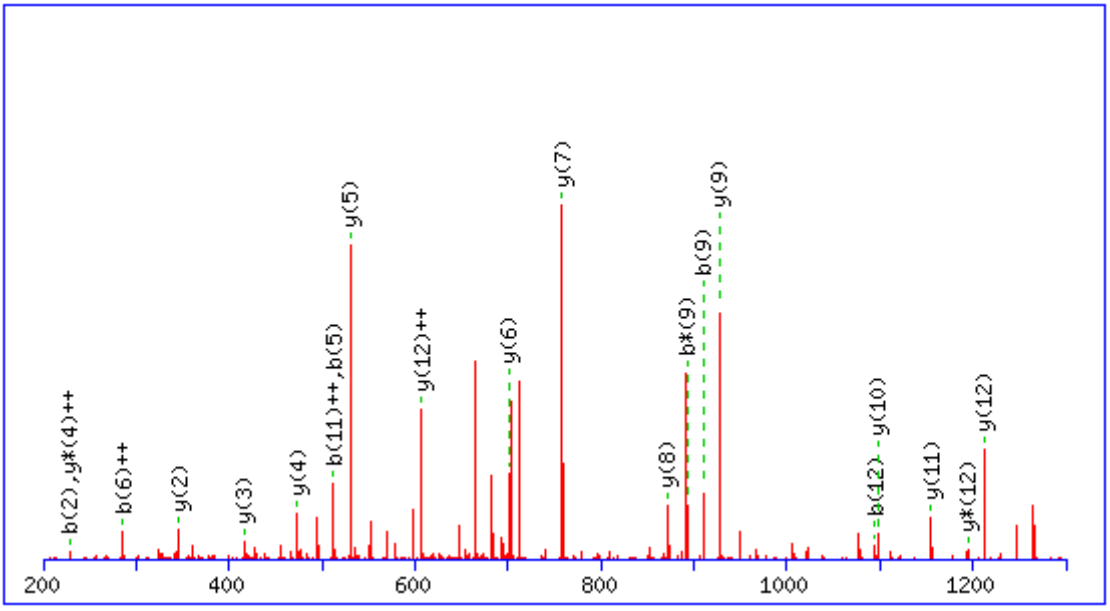
All MS/MS spectra for histone H4 peptides identified by mass spectrometry



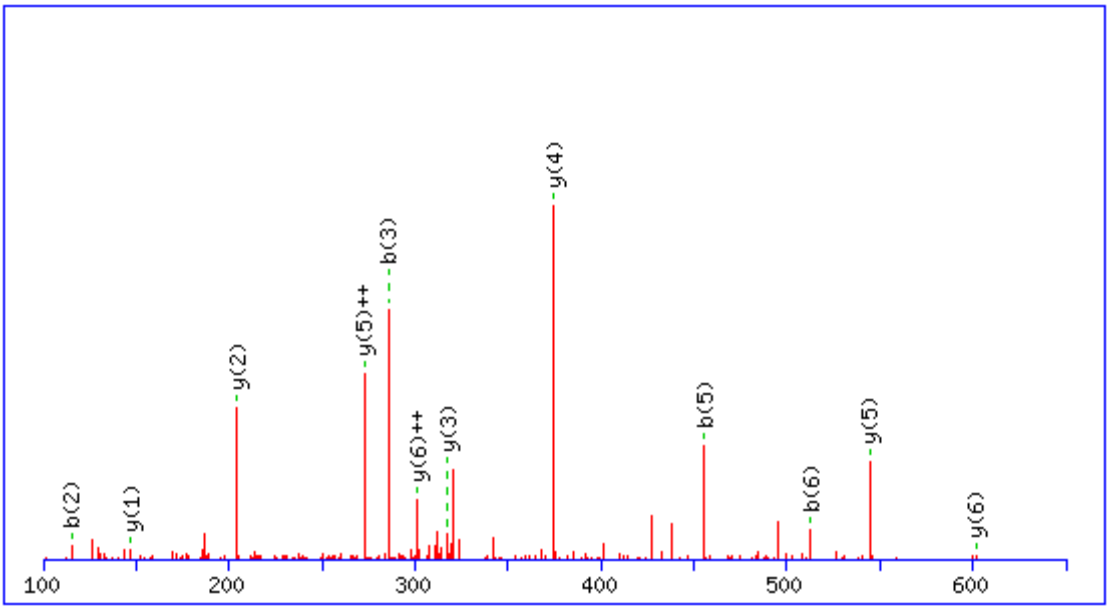
G K (Ac) GG K (Ac) GLG K (Ac) GGAK



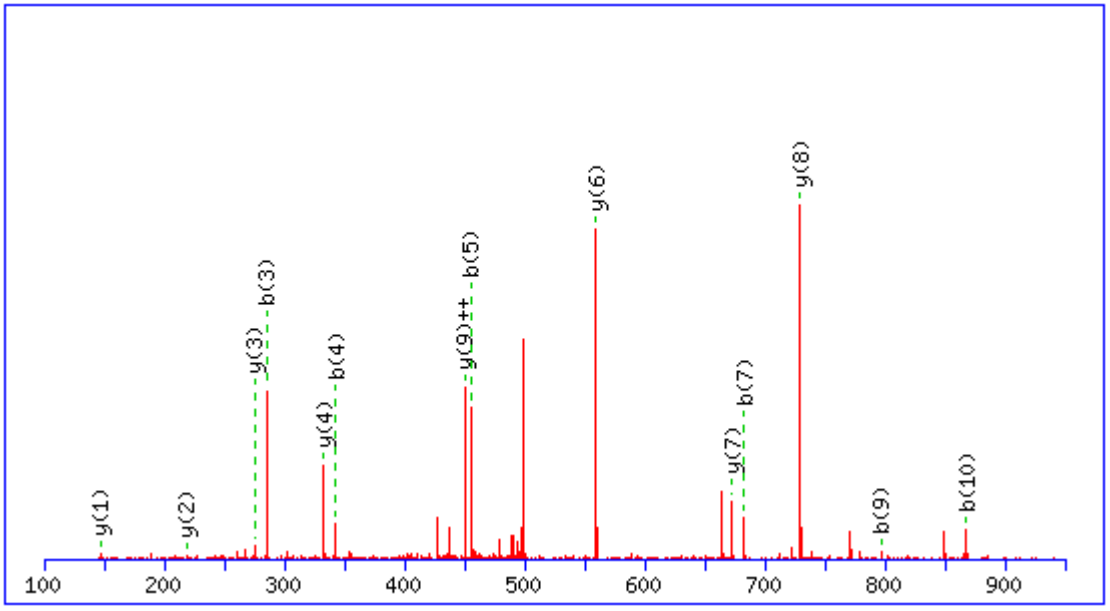
G K (Ac) GG K (Ac) GLG K (Ac) GGA K (Ac) R



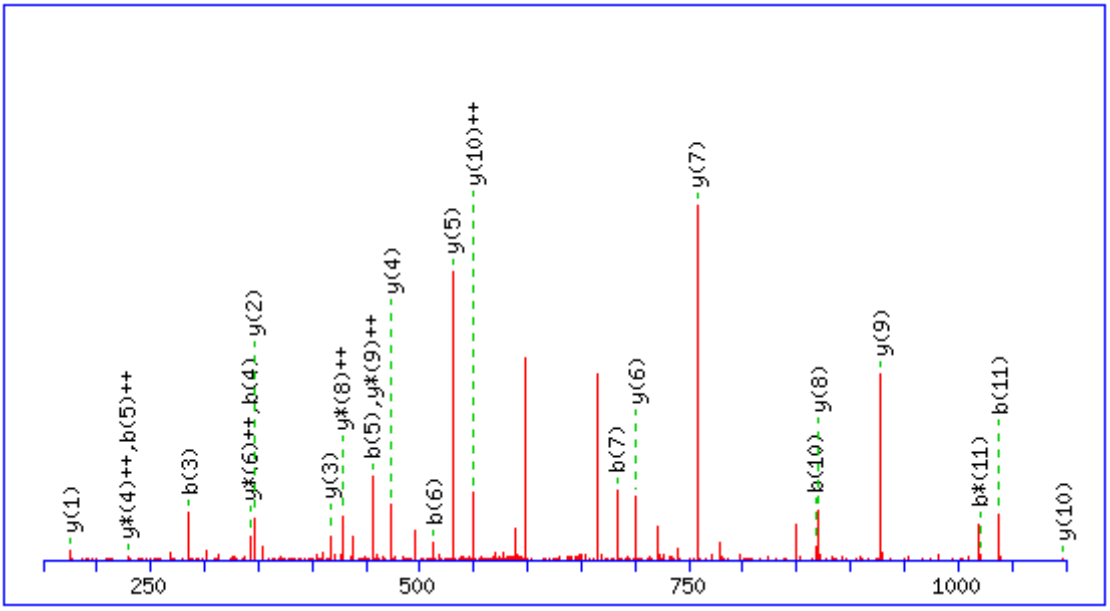
G K (Ac) GG K (Ac) GLG K (Ac) GGAK (M e 3) R



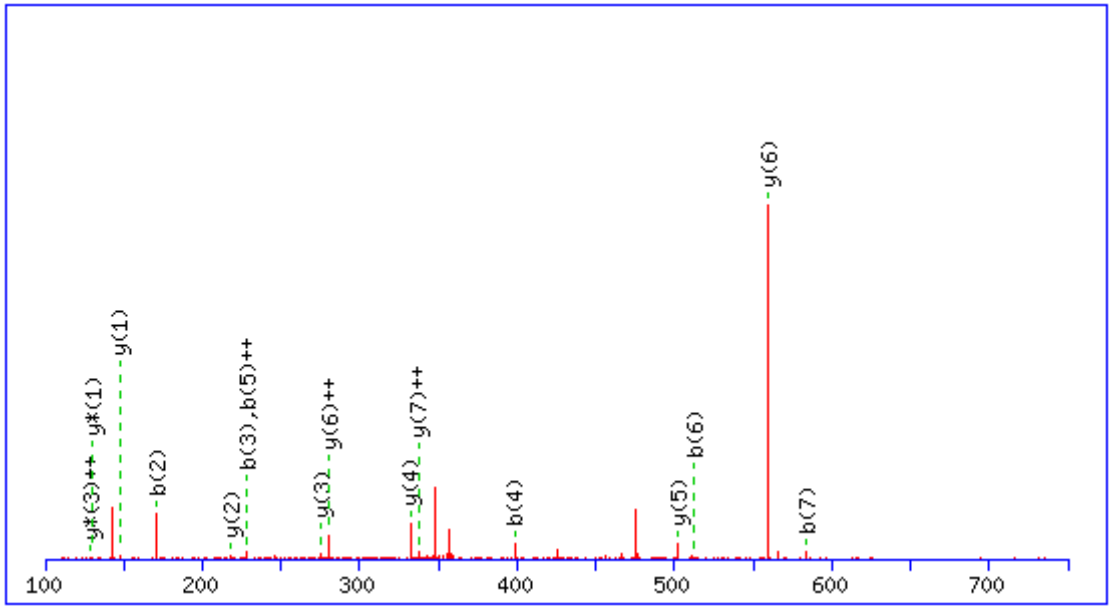
GG K (Ac) GLGK



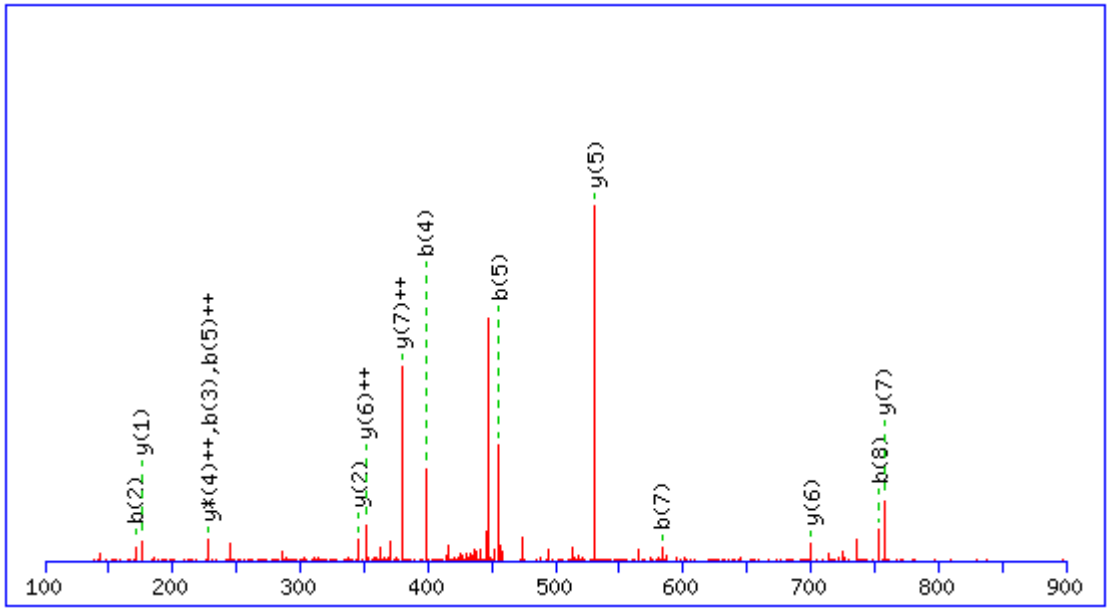
GG K (Ac) GLG K (Ac) GGAK



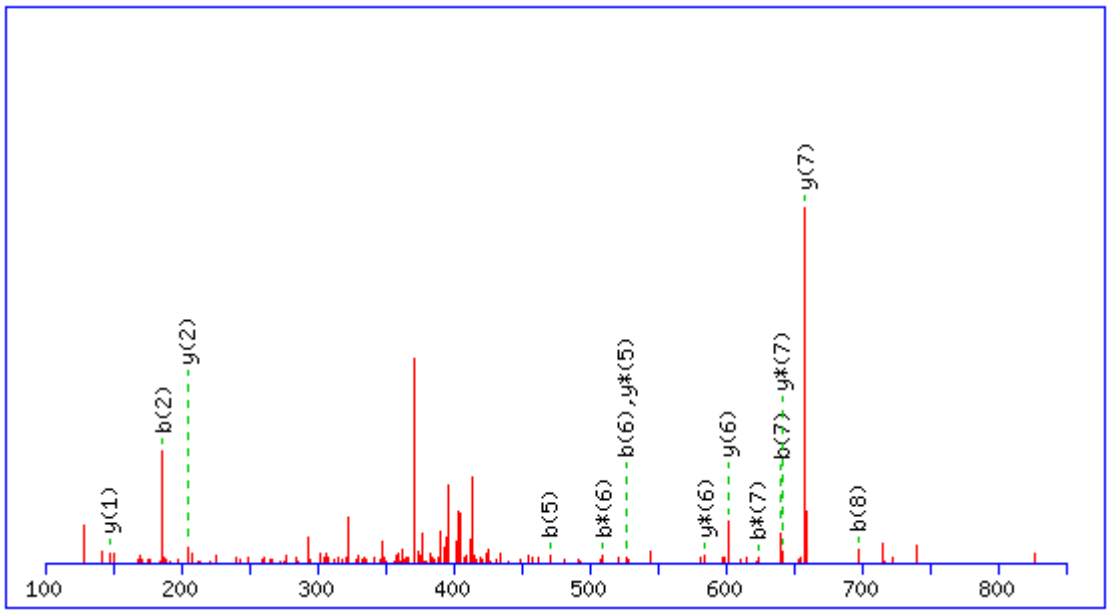
GG K (Ac) GLG K (Ac) GGA K (Ac) R



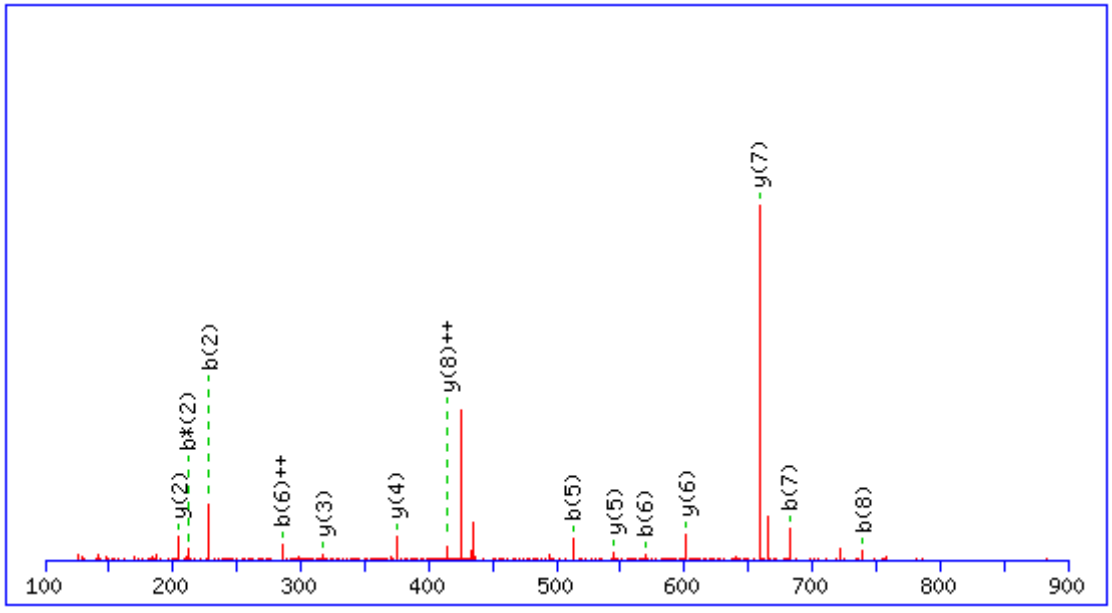
GLG K (Ac) GGAK



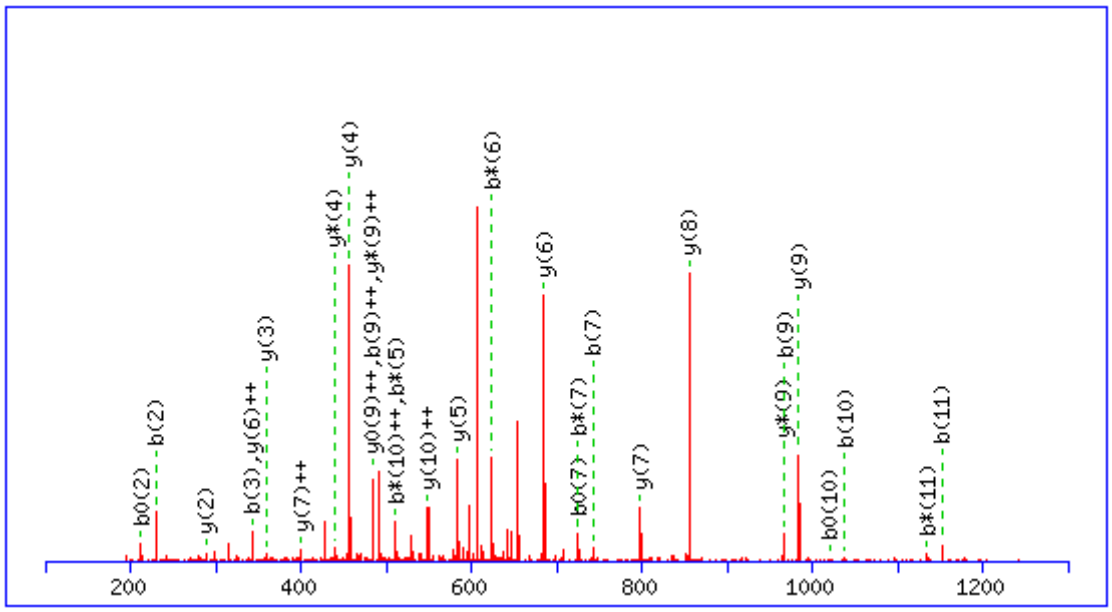
GLG K (Ac) GGA(K Ac) R



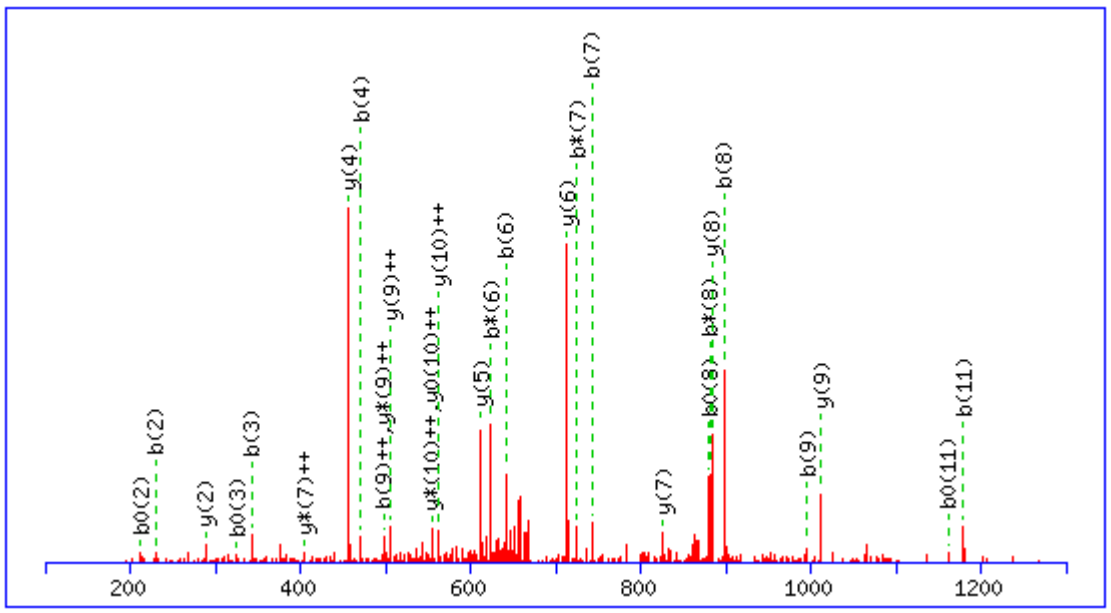
GKGGK $_{(Ac)}$ GLGK



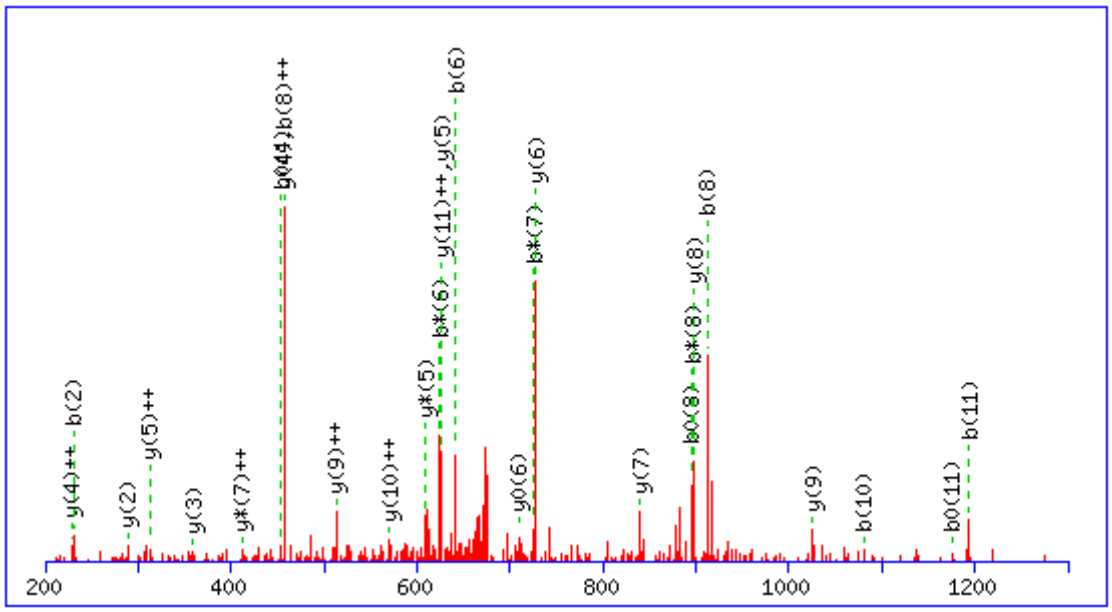
G K $_{(Ac)}$ GG K $_{(Ac)}$ GLGK



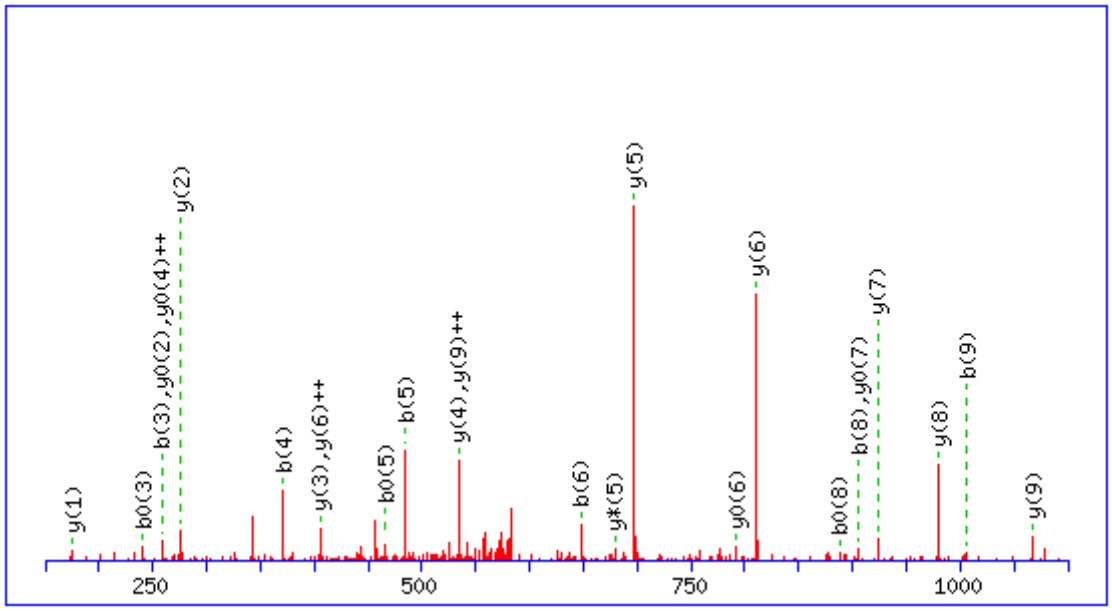
DNIQGITKPAIR



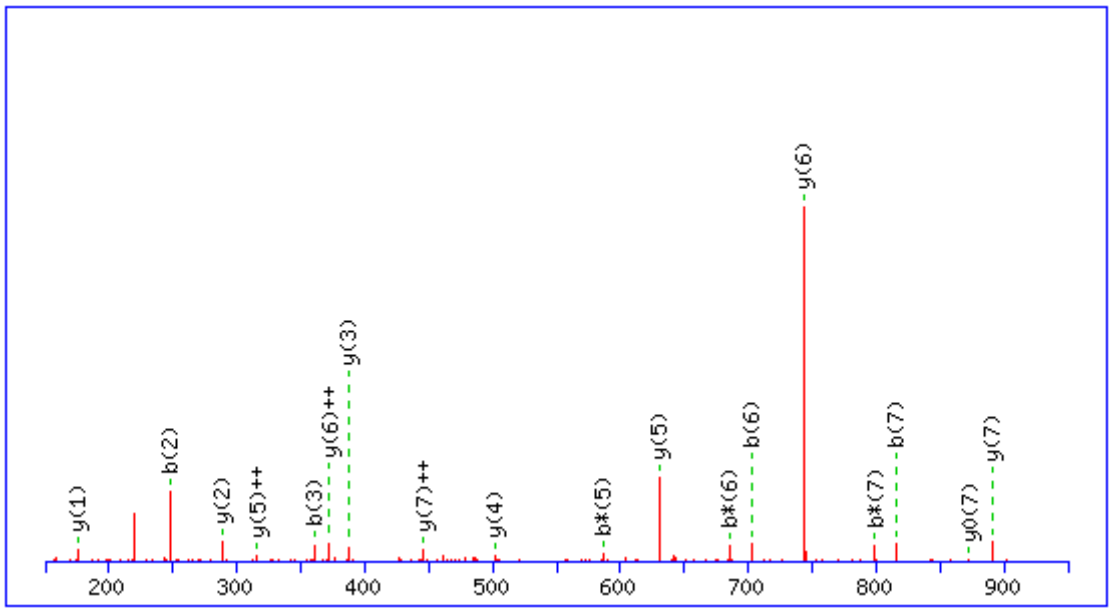
DNIQGIT K_{Me2} PAIR



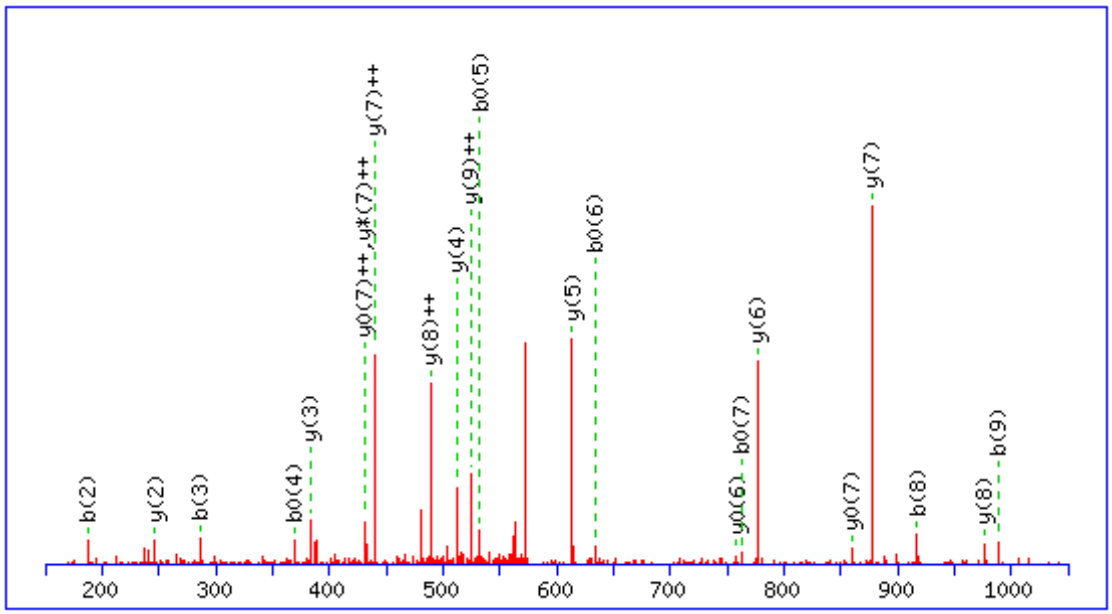
DNIQGIT $K_{(Ac)}$ PAIR



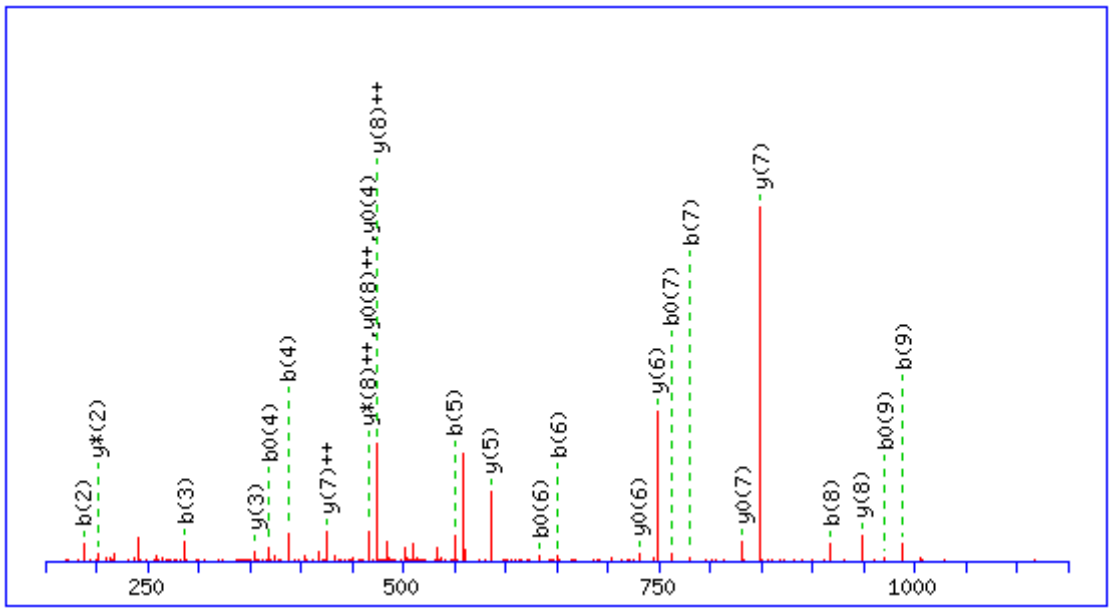
ISGLIYEETR



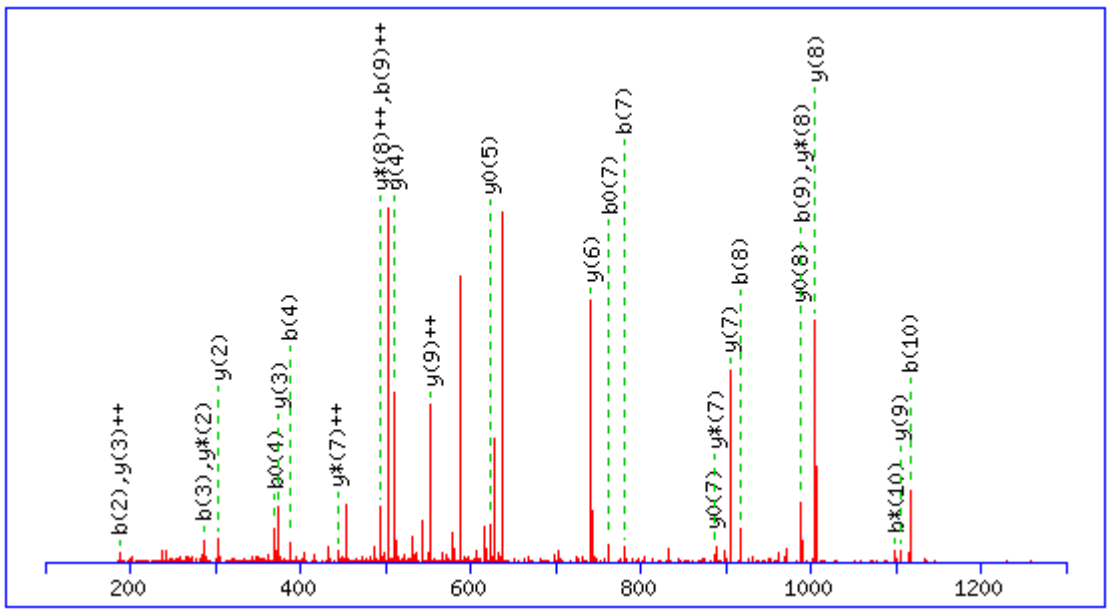
VFLENVIR



DAVTYTEHA $K_{(Me2}$



DAVTYTEHAK



DAVTYTEHAKR