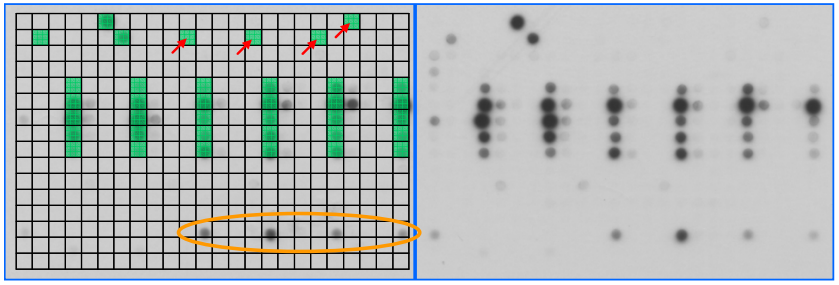
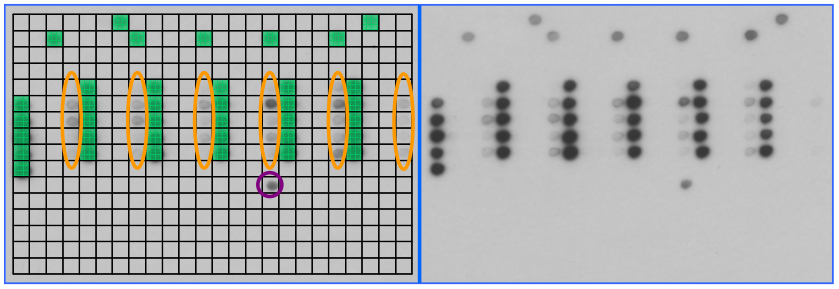
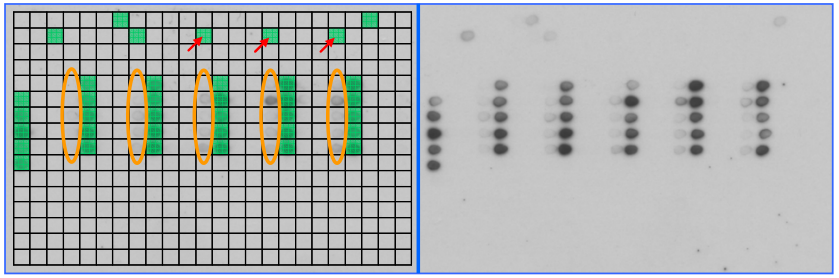
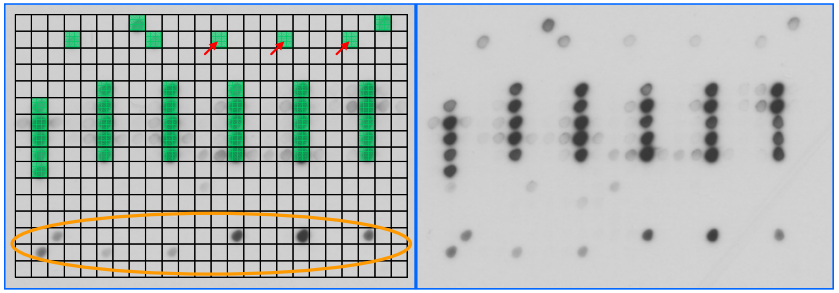
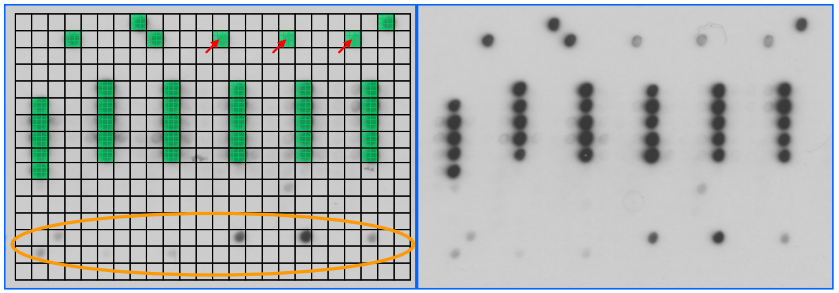
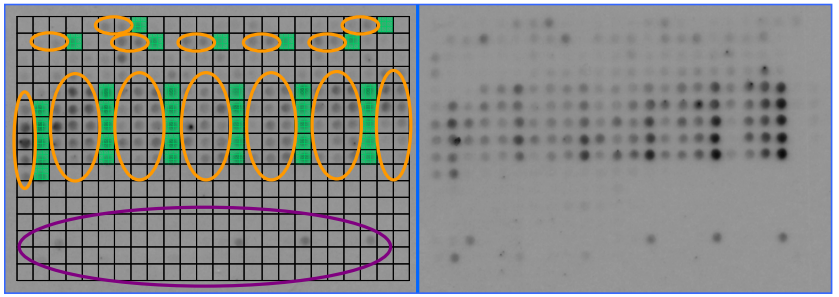
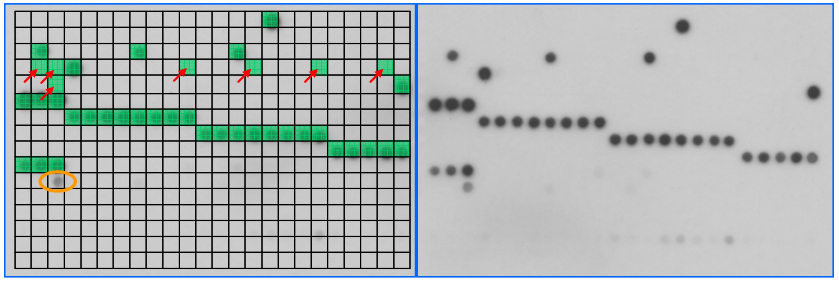
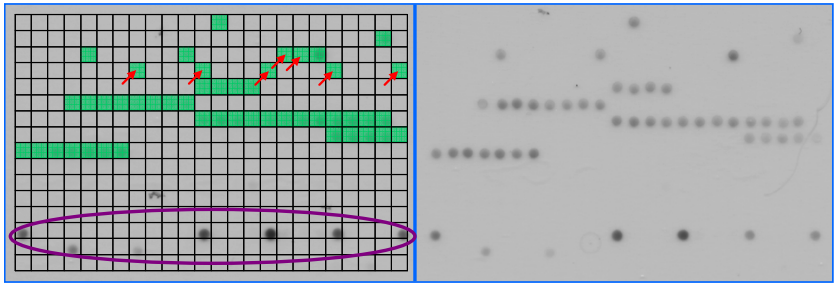
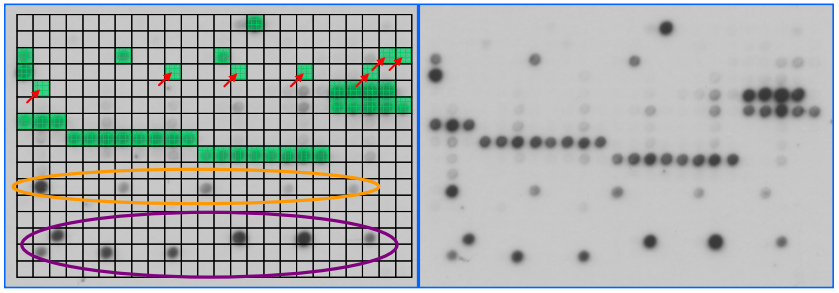
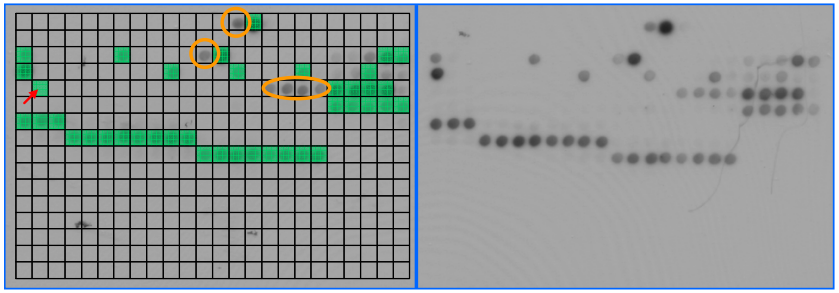
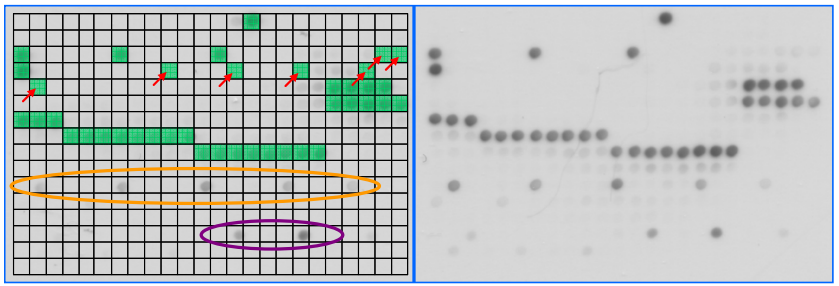
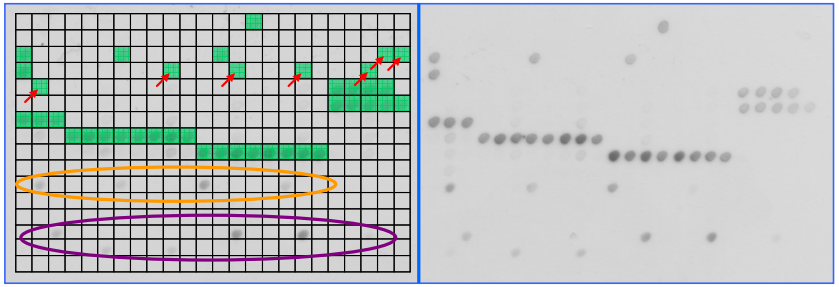
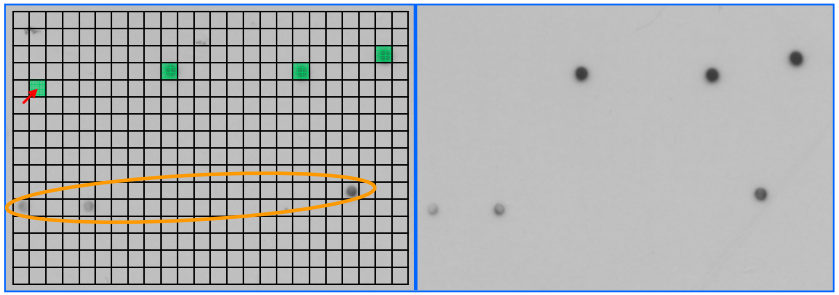
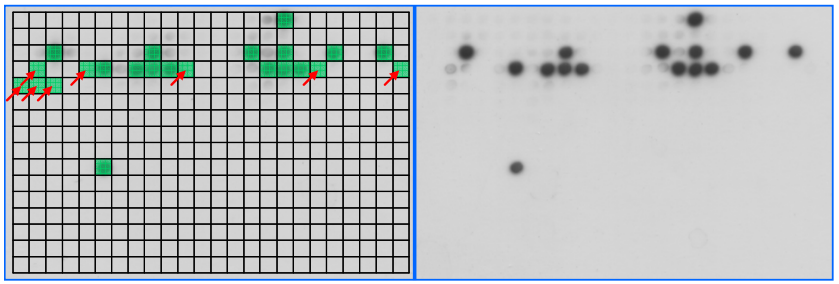
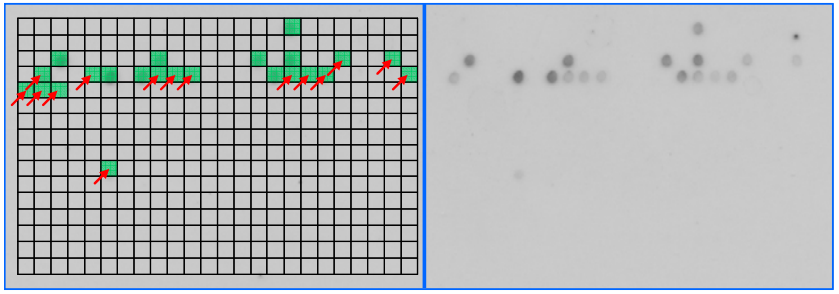
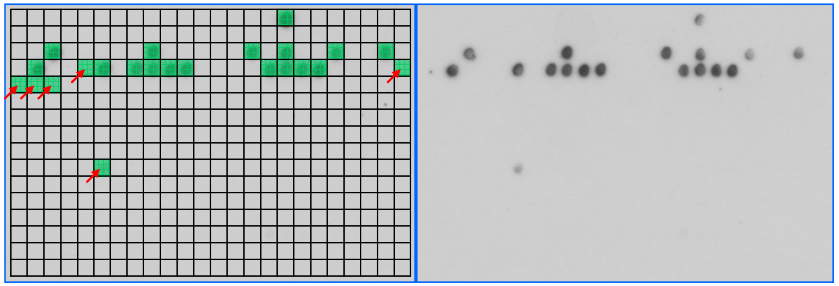
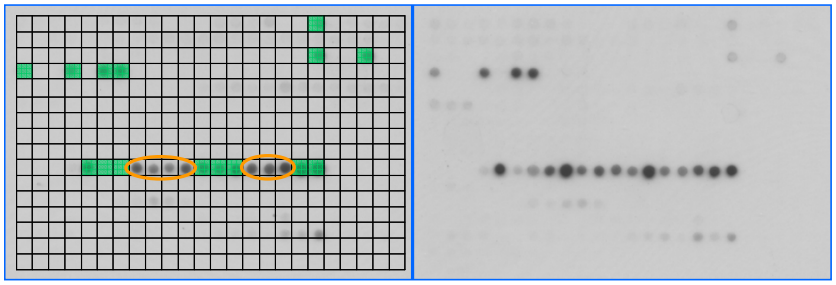
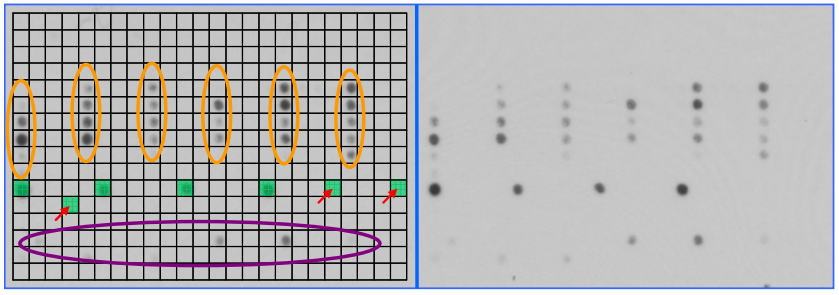
Supplemental Table 1: Compilation of the results of the specificity analyses with different antibodies. In this table the antibody number (referring to Suppl. Tab. 2), the antibody target site, the specificity factor for binding at the target site (SF_T) and for the best non-target site (SF_N) and the ratio of both are given. The example pictures show both duplicates of each array. On the left side spots are annotated as follows: all peptides containing the primary PTM are shaded in green, false negatives (or very weakly bound peptides which contain the primary PTM) are highlighted with red arrows. False positives are encircled in orange or violet color. The secondary PTMs present in the false negative and false positive spots are specified in the comments column.

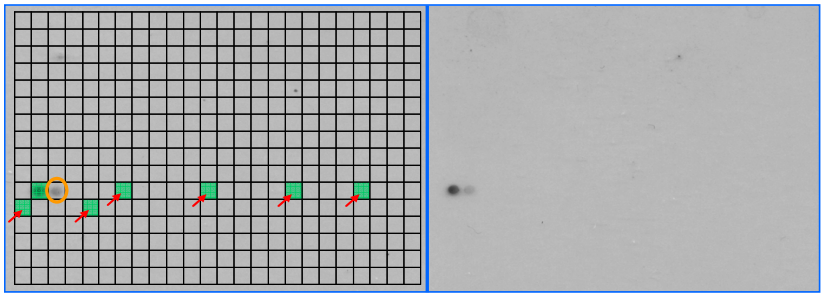
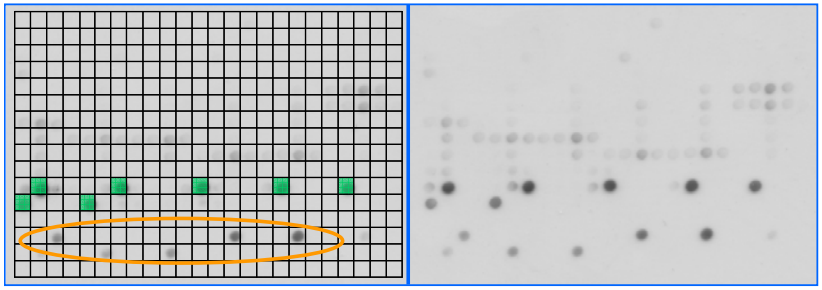
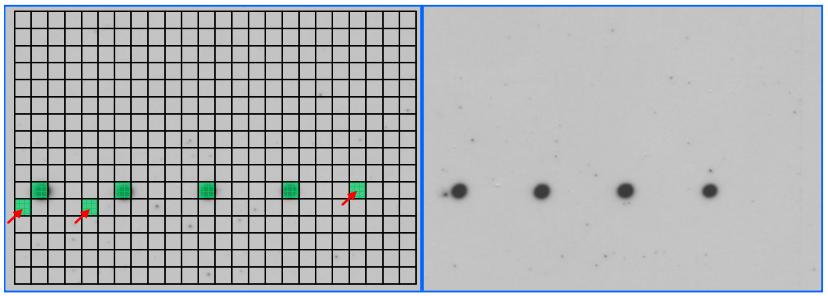
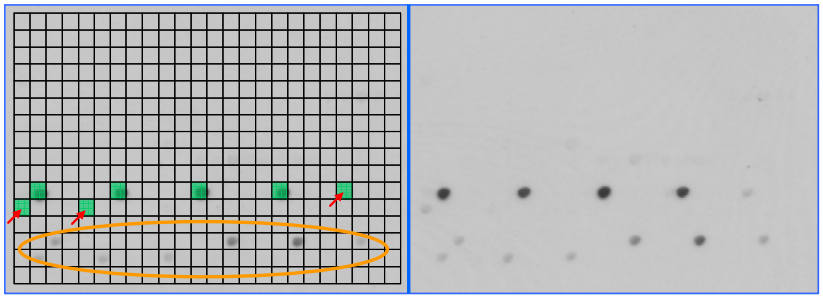
No.	Antibody target site	SF target site (SF_T)	SF best non-target site (SF_N)	$\frac{SF_T}{SF_N}$	Example of an image of the array	Comments
1	H3K4me1	30	3	10		False Negatives contain H3R2me2s, H3R2me2a or H3T3ph False Positives all contain H4K20me1
2	H3K4me2	42	1.3	33		False Positives contain H3K4me1 or the double modification H3R26Citr/H3K27me2

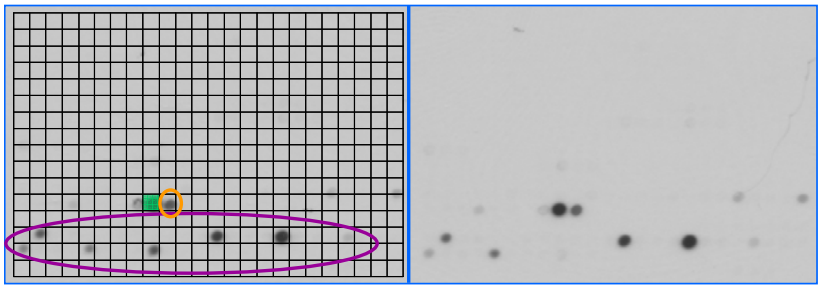
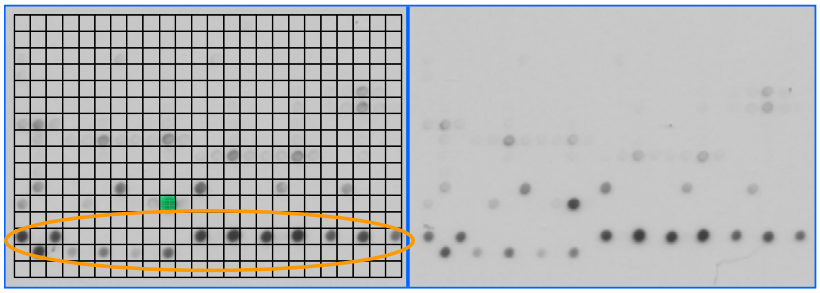
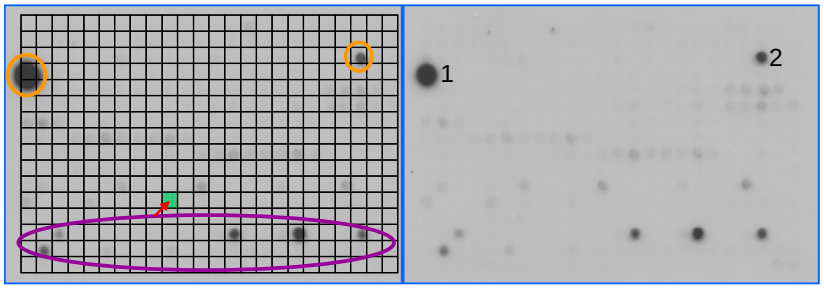
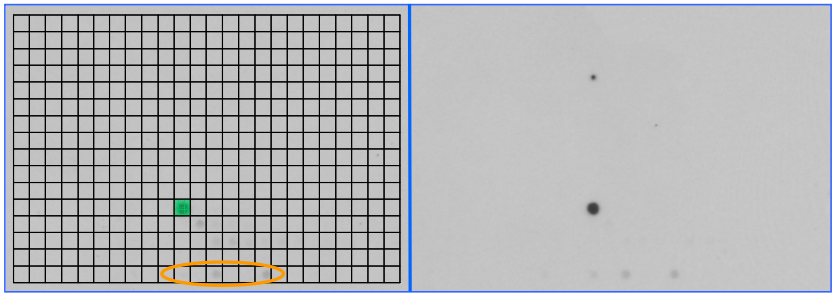
3	H3K4me2	62	3	19		False Negatives all contain H3T3ph Weak binding of H3K4me2 in mono- and double modifications False Positives all contain H3K4me1
4	H3K4me3	31	4	9		H3T3ph reduces binding False Positives all contain H4K20me3
5	H3K4me3	32	2	16		H3T3ph reduces binding False Positives all contain H4K20me3
6	H3K4me3	9	3	3		False Positives contain H3K4me1/me2/ac or H4K20me3

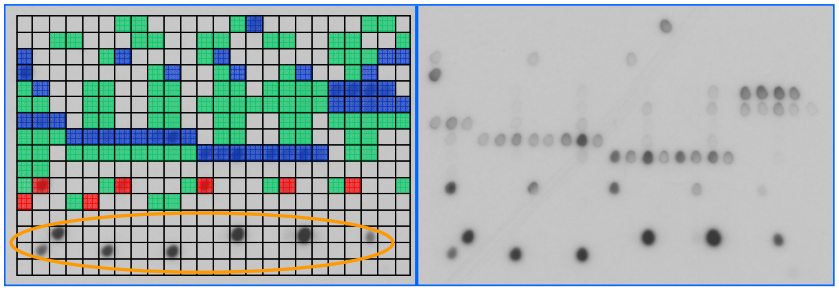
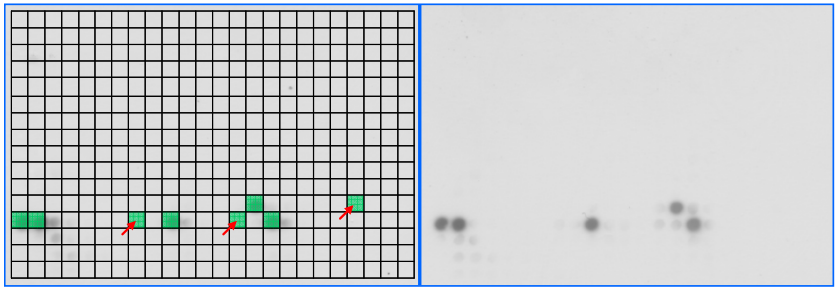
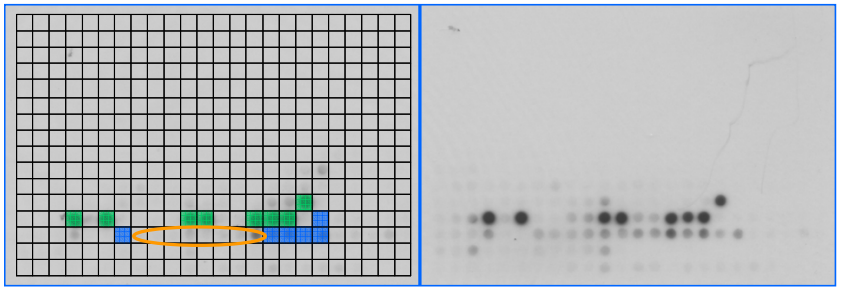
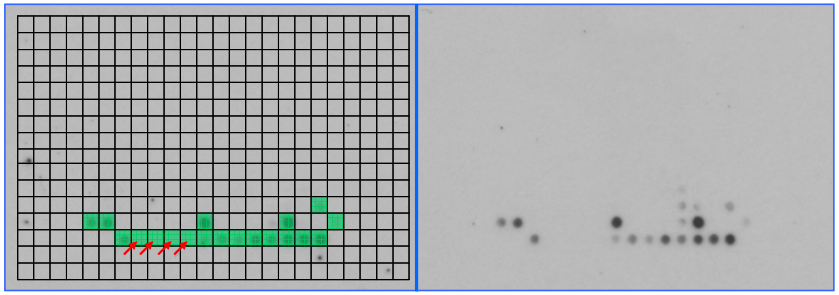
7	H3K9ac	44	1	44		False Negatives contain H3S10ph or H3T11ph False Positives: one peptide (H3K27ac)
8	H3K9me1	19	16	1		False Negatives contain H3S10ph or H3T11ph False Positives all contain H4K20me1
9	H3K9me3	18	7	2		False Negatives contain H3S10ph or H3T11ph False Positives contain H3K27me3 or H4K20me3
10	H3K9me3	44	1	44		False Negatives: one peptide (H3R8me2a, H3K9me3, H3S10ph, H3T11ph) False Positives all contain H3K9me2

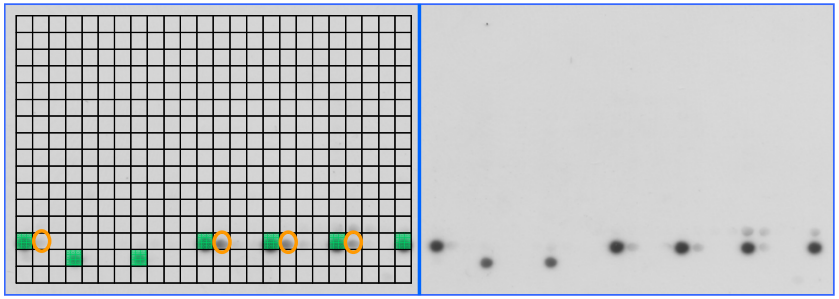
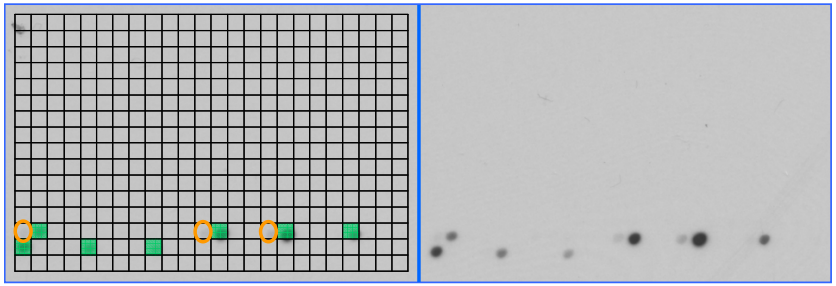
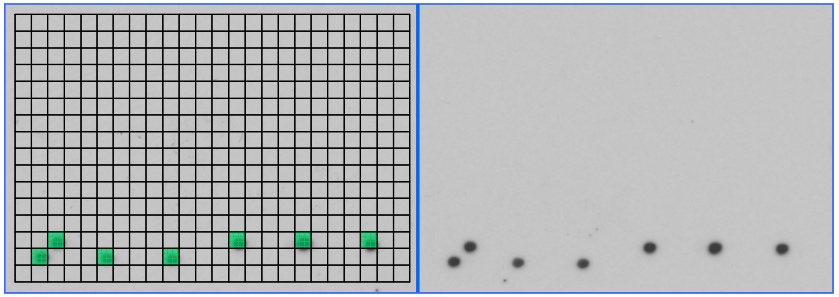
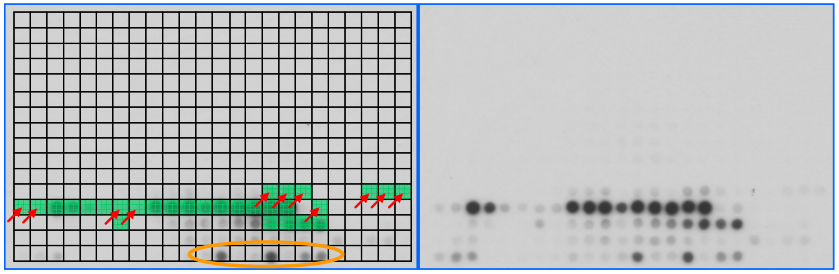
11	H3K9me3	28	3	11		False Negatives contain H3S10ph or H3T11ph False Positives contain H3K27me3 or H4K20me3
12	H3K9me3	47	3	15		False Negatives contain H3S10ph or H3T11ph False Positives contain H3K27me3 or H4K20me3
13	H3K9me3 S10ph	51	13	4		False Negatives: one peptide (H3R8me2a, H3K9me3, H3S10ph, H3T11ph) False Positives all contain H3K27me3 and H3S28ph
14	H3S10ph	48	1	48		False Negatives contain H3K9ac or H3T11ph

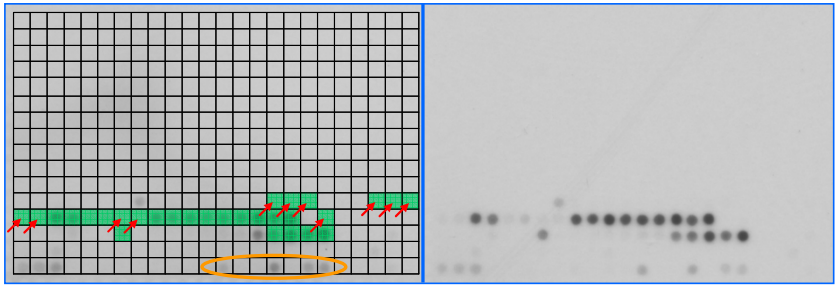
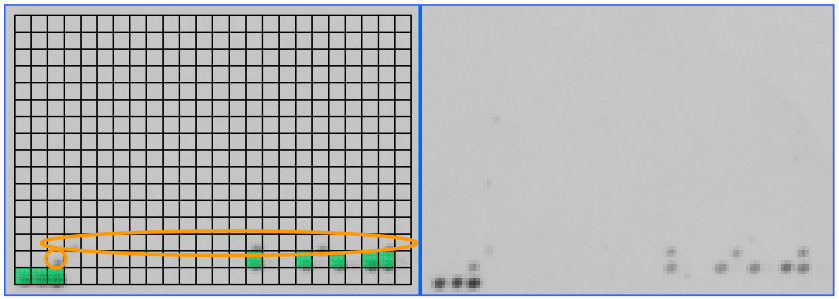
15	H3S10ph	46	1	46		False Negatives all contain H3T11ph; H3K9me2, H3K9me3, H3K9ac and H3K14ac reduce binding
16	H3S10ph	138	1	138		False Negatives all contain H3T11ph; H3K14ac reduces binding
17	H3K14ac	26	28	1		False Positives contain H3R17me2s, H3R17me2a, H3R17Citr and/or H3K18ac
18	H3K27me2	18	14	1		False Negatives all contain H3S28ph False Positives contain H3K4me2 or H4K20me2

19	H3K27me3	34	9	4		False Negatives contain H3R26me2s, H3R26me2a or H3S28ph False Positives: one peptide (H3K27ac)
20	H3K27me3	36	10	4		False Positives contain H4K20me3. Weak binding to H3K9me3 or H3K4me3.
21	H3K27me3	172	1	172		False Negatives all contain H3S28ph
22	H3K27me3	74	14	5		False Negatives all contain H3S28ph False Positives all contain H4K20me3

23	H3K36me2	71	44	2		False Positives contain H3K36me3 or H4K20me2
24	H3K36me3	26	23	1		False Positives contain H4K20me1 or H4K20me3
25	H3K36me3	a)	a)	a)		H3K36me3 is not bound False Positives contain H3K9me3 and H3K14ac (1), H3K9me2 and H3K14ac (2) or H4K20me3
26	H3K36ac	504	6	85		False Positives all contain H2bK15ac

27	H3-Pan-Kme2/me3	9[b)]	19	1		H3K9me3, H3K27me3, all other H3Kme2/me3 peptides Binding only to some H3K9me3, H3K27me3 and H4K20me3 (False Positives)
28	H4R3me2a	77	3	31		False Negatives all contain H4S1ph
29	H4K12ac	30	4	8		H4 (1-19) K12ac peptides, H4 (11-30) K12ac peptides False Positives all contain H4K16ac
30	H4K16ac	179	1	179		False Negatives contain H4R17me2s/a or H4R19me2s/a

31	H4K20me1	77	6	12		False Positives all contain H4K20me2
32	H4K20me2	228	4	61		False Positives all contain H4K20me1
33	H4K20me3	155	1	155		
34	H4-Pan-Kac	25 [c)]	4	6		H4K5ac, H4K8ac or H4K12ac peptides Weak binding to monoacetylated peptides False Positives contain di- and triacetylated H2b peptides

35	H4-Pan-Kac	14 [d)]	3	5		H4K5ac, H4K8ac or H4K12ac peptides Weak binding to monoacetylated peptides False Positives contain di- and triacetylated H2b peptides
36	H2aK9ac	121	10	12		False Positives all contain H4K20ac

[a)] not applicable since no specific binding to target
[b)] calculated for H3K9me3
[c)] calculated for K8ac or K5ac or K12ac
[d)] calculated for K12ac or K5ac or K8ac

Supplemental Table 2: Compilation of technical details of the antibodies used in this study.

Antibody No.	Antibody target site	Antibody type[a)]	Supplier and Cat. No. [b)]	Lot No.	Dilution in experiment
1	H3K4me1	pab	AM 39297	21008001	1:5000
2	H3K4me2	pab	AM 39141, 39142	168	1:10000
3	H3K4me2	pab	Mi 07-030	DAM1570816	1:20000
4	H3K4me3	pab	AM 39159	15808002	1:100000
5	H3K4me3	pab	Ac ab8580	224576	1:10000
6	H3K4me3	pab	Mi 07-473	DAM1661080	1:400000
7	H3K9ac	pab	Mi 06-942	23997	1:10000
8	H3K9me1	pab	AM 39249, 39250	07408001	1:10000
9	H3K9me3	pab	Ac ab8898	484099	1:20000
10	H3K9me3	mab	Ac ab6001	389293	1:500
11	H3K9me3	pab	AM 39161	170	1:2000
12	H3K9me3	pab	CS 9754	1	1:1000
13	H3K9me3S10ph	mab	Mi 04-809	NG1585093	1:500
14	H3S10ph	pab	AM 39253	08308001	1:2000
15	H3S10ph	pab	CS 9701	1	1:500
16	H3S10ph	pab	Mi 06-570	21714	1:10000
17	H3K14ac	pab	Mi 06-911	22854	1:10000
18	H3K27me2	pab	AM 39245, 39246	07408001	1:10000
19	H3K27me3	mab	AM 39536	09508002	1:500
20	H3K27me3	pab	AM 39155_0	199	1:2000
21	H3K27me3	mab	Ac ab6002	398310	1:500
22	H3K27me3	pab	Mi 07-449	DAM1662421	1:20000
23	H3K36me2	pab	AM 39255	08308001	1:5000
24	H3K36me3	pab	Ac ab9050	481641	1:5000
25	H3K36me3	mab	Mi 04-801	DAM1564369	1:500
26	H3K36ac	pab	AM 39379, 39380	29108001	1:100000
27	H3-Pan-Kme2/me3	pab	Mi 07-756	30618	1:20000
28	H4R3me2a	pab	AM 39705	03910002	1:5000
29	H4K12ac	pab	Mi 06-761	27657	1:10000
30	H4K16ac	pab	Mi 07-329	DAM1675759	1:100000
31	H4K20me1	pab	Ac ab9051	713902	1:20000
32	H4K20me2	pab	AM 39173, 39174	135	1:10000

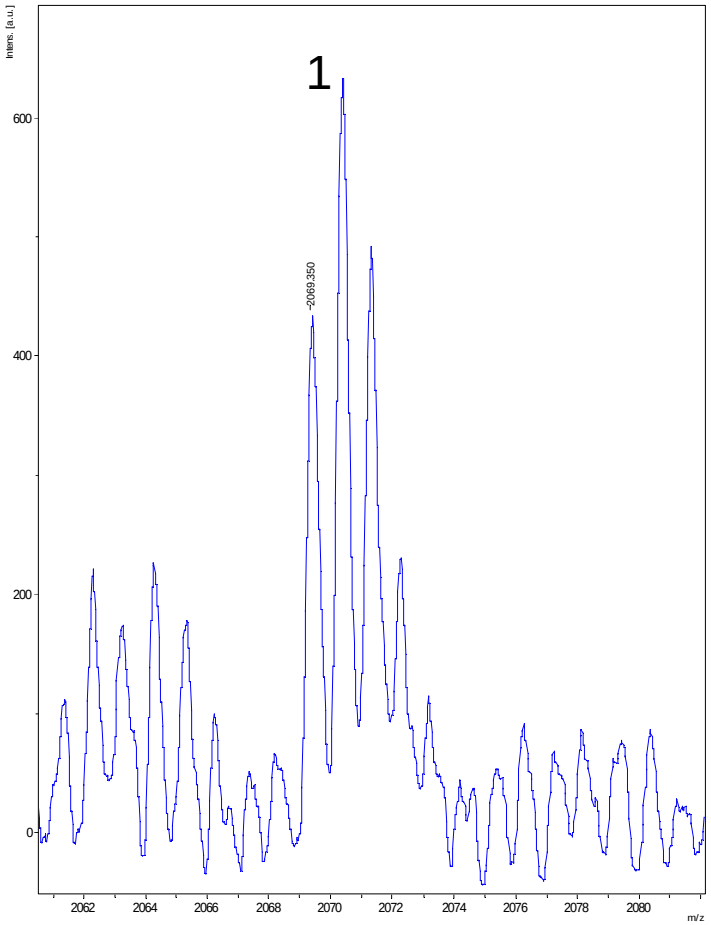
33	H4K20me3	pab	AM 39180, 39181	136	1:100000
34	H4-Pan-Kac	pab	AM 39243	05308001	1:20000
35	H4-Pan-Kac	pab	Mi 06-598	29867	1:10000
36	H2aK9ac	pab	AM 39109, 39110	257	1:100000

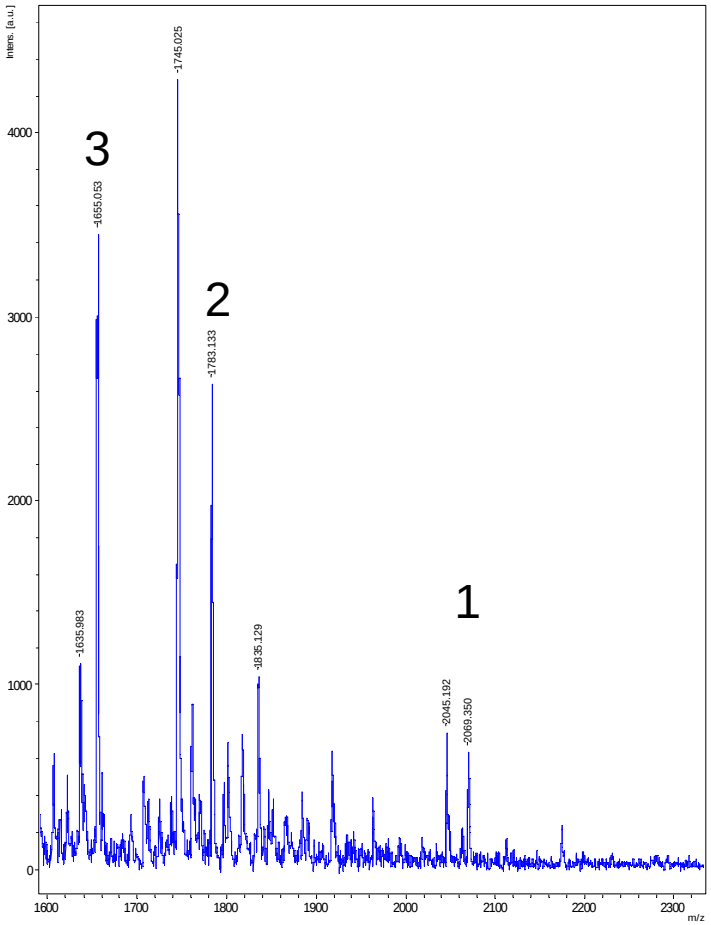
[a)] mab: monoclonal antibody; pab: polyclonal antibody
[b)] Ac: Abcam; AM: Active Motif; CS: Cell Signaling; Mi: Millipore

Detailed specificity analysis of antibodies binding to modified Histone tails with peptide arrays

Ina Bock[1], Arunkumar Dhayalan[1], Srikanth Kudithipudi[1], Ole Brandt[2], Philipp Rathert[3], & Albert Jeltsch[1,*]

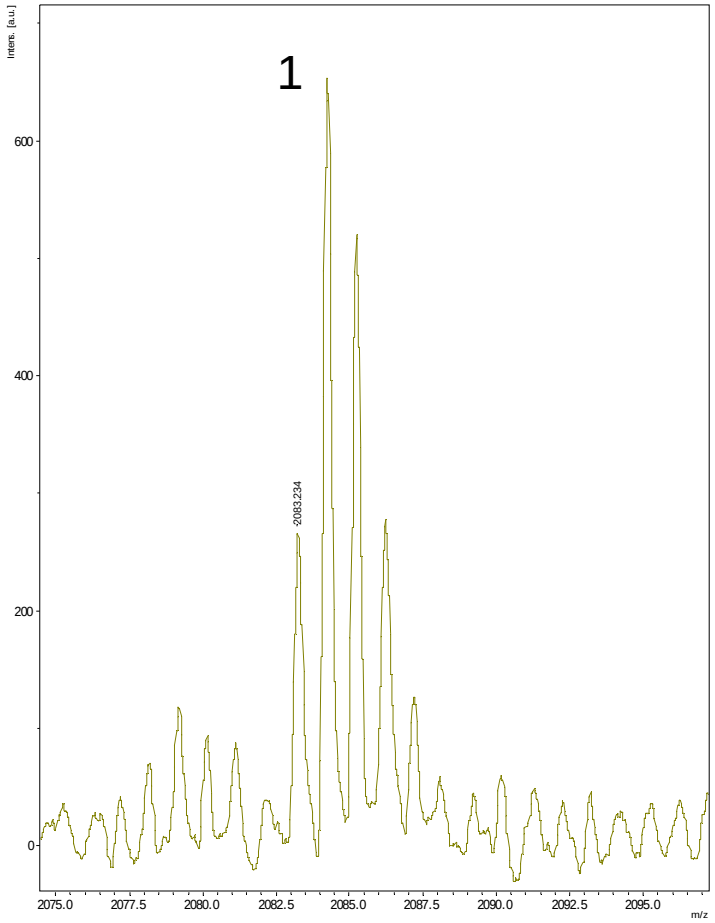
Supplemental Figure 1: Mass spectra of selected peptides. To confirm correct synthesis of the peptides by mass spectrometric analysis representative peptides were synthesized in parallel according to a standard SPOT-synthesis protocol by using the acid labile Fmoc-Rink linker as first building block which allows cleaving them from the matrix for characterization. After synthesis, the dry CelluSpot discs were punched out and 150 µl of the cleavage cocktail (93% (v/v) trifluoroacetic acid (TFA), 4% triisopropylsilane (v/v) and 3% H2O (v/v)) was added to each peptide disc for 1.5 h. The supernatants containing the peptides were transferred to new microtubes and dried in a gentle N_2-stream. The peptides were resolubilized in a mixture of 30% acetonitril in H_2O containing 0.1 % TFA. Afterwards the peptides were investigated by MALDI/TOF mass spectrometry using an AutoFlex II (Bruker Daltonics, Bremen, Germany). Peptide samples were diluted with 0.1% TFA as appropriate and 1 µl applied to one spot on a pre-spotted Anchorchip (PAC) HCCA Plate (Cat. No. 227463, Bruker Daltonics) and washed with 10 mM sodium phosphate / 0.1% TFA solution. Spectra were recorded with default settings using peptide calibration standard mixture with the mass range of 1000 to 4000 Da (Cat. No. 206195, Bruker Daltonics) as mass standard and processed using the FlexAnalysis software (Bruker Daltonics). In the following spectra the m/z ratio in Da is shown on the x-axis and the signal intensity of the ion detector on the y-axis. Peptides are specified by their position in the array (cf. Supplemental information S1), name and the theoretical mass of the MH^+ species.

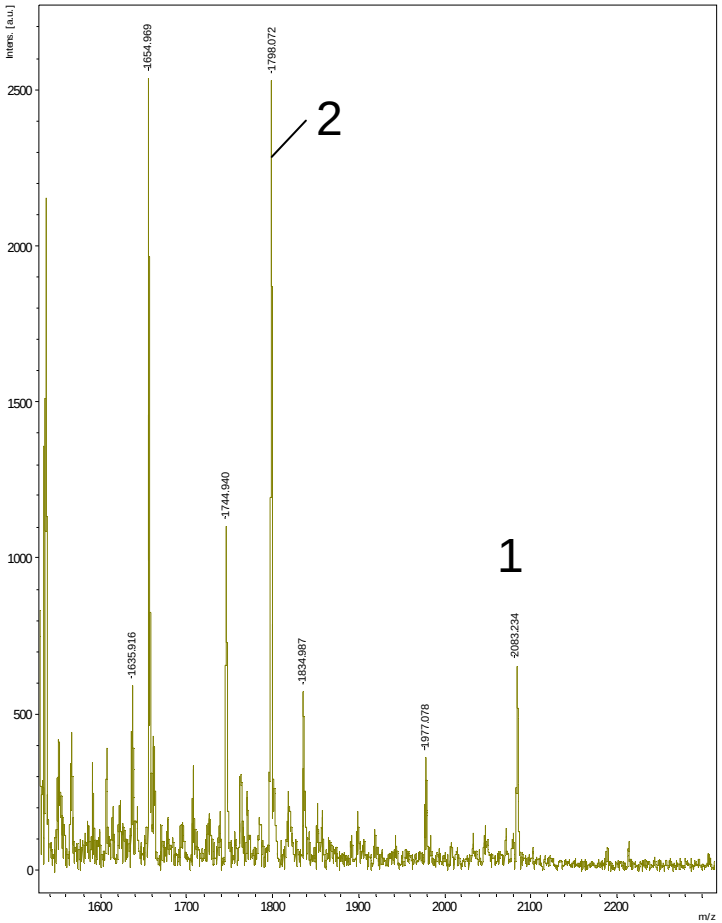




Peptide A1, H3 1-19, ARTKQTARKSTGGKAPRKQ

ARTKQTARKSTGGKAPRKQ, MH^+=2069.2 Da (1)
Ac-KQTARKSTGGKAPRKQ, MH^+=1783.0 Da (2)
Ac-QTARKSTGGKAPRKQ, MH^+=1654.9 Da (3)

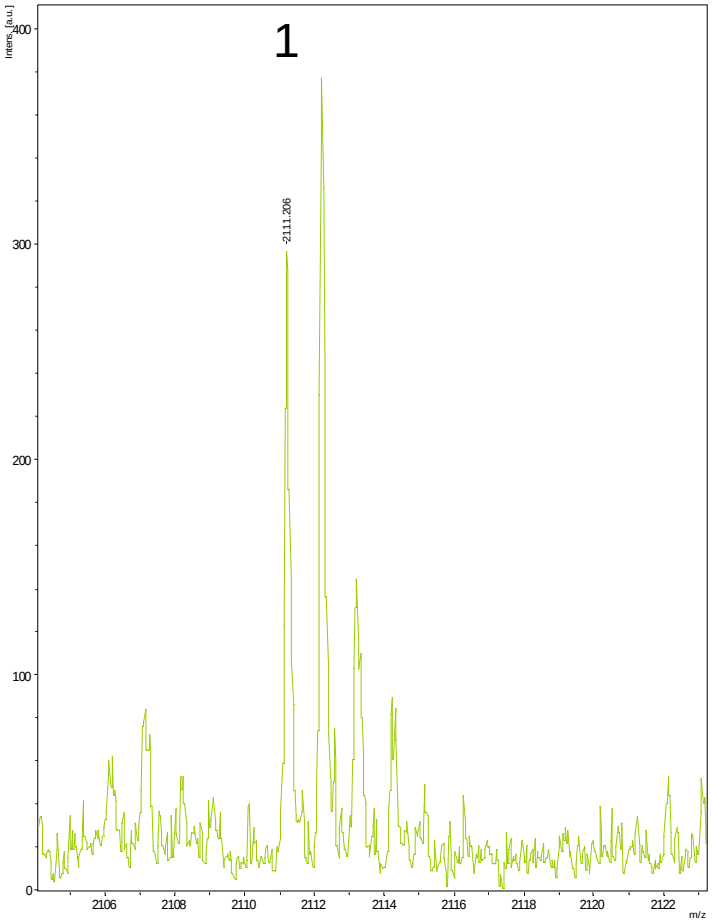


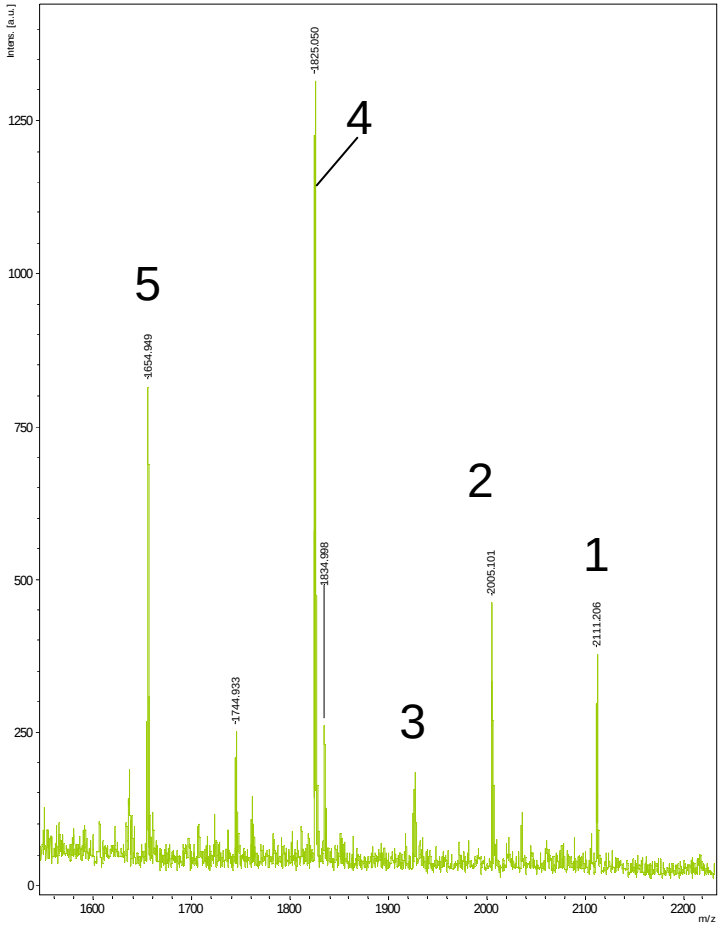


Peptide A6, H3 1-19 K4me1, ART(K^{me1})QTARKSTGGKAPRKQ

ART(K^{me1})QTARKSTGGKAPRKQ, MH^{+}=2083.2 Da (1)

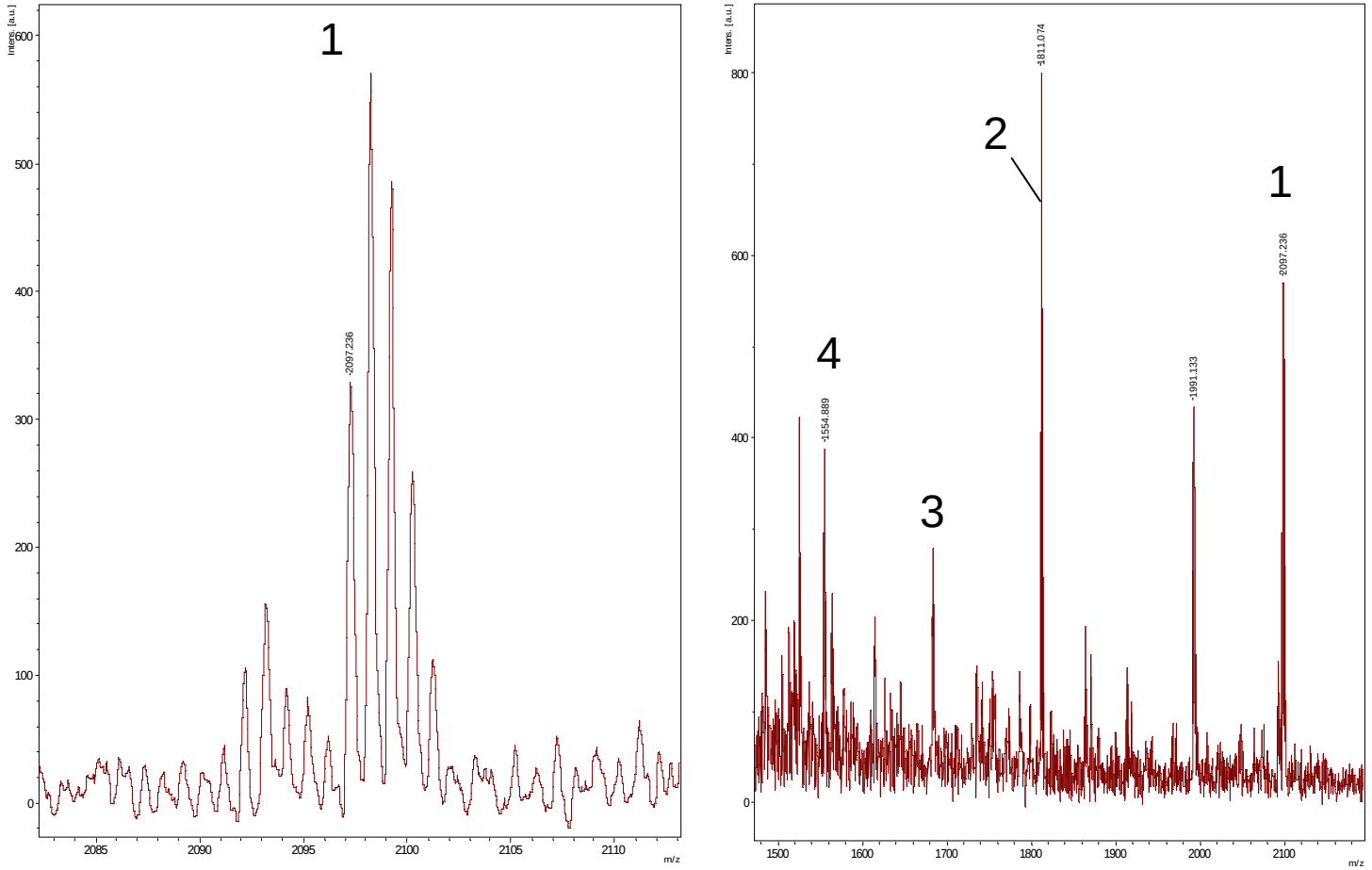
Ac-(K^{me1})QTARKSTGGKAPRKQ, MH^{+}=1797.0 Da (2)





Peptide A9, H3 1-19 K4Ac, ART(K^{ac})QTARKSTGGKAPRKQ

ART(K^{ac})QTARKSTGGKAPRKQ, MH^{+}=2111.2 Da (1)
Ac-RT(K^{ac})QTARKSTGGKAPRKQ-Na^{+}, MH^{+}=2005.2 Da (2)
Ac-T(K^{ac})QTARKSTGGKAPRKQ, MH^{+}=1926.1 Da (3)
Ac-(K^{ac})QTARKSTGGKAPRKQ, MH^{+}=1825.0 Da (4)
Ac-QTARKSTGGKAPRKQ, MH^{+}=1654.9 Da (5)



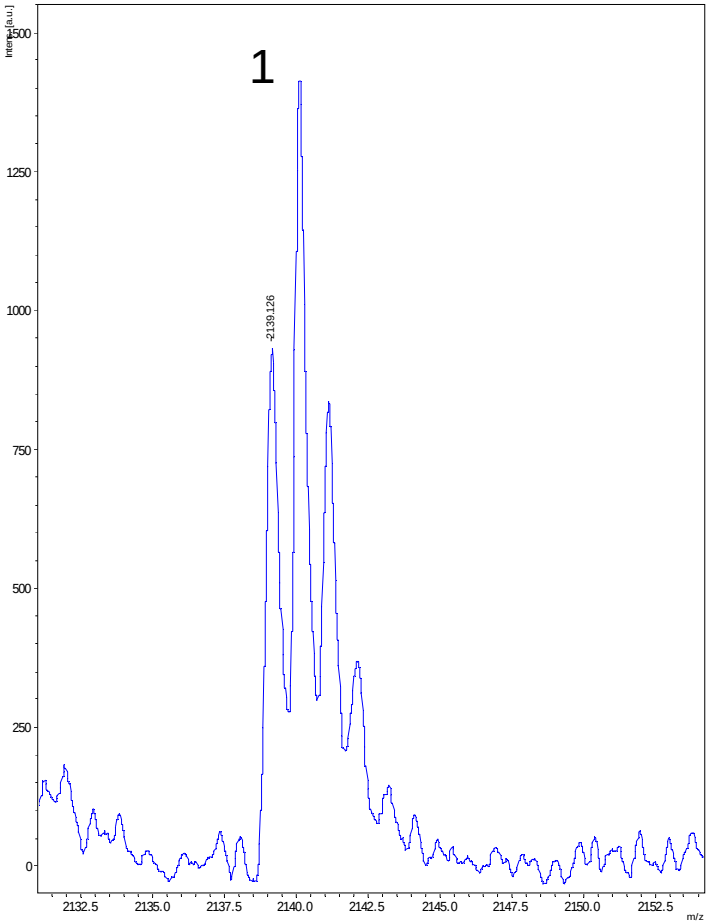
Peptide A10, H3 1-19 R8me2s, ARTKQTA(R^{me2s})KSTGGKAPRKQ

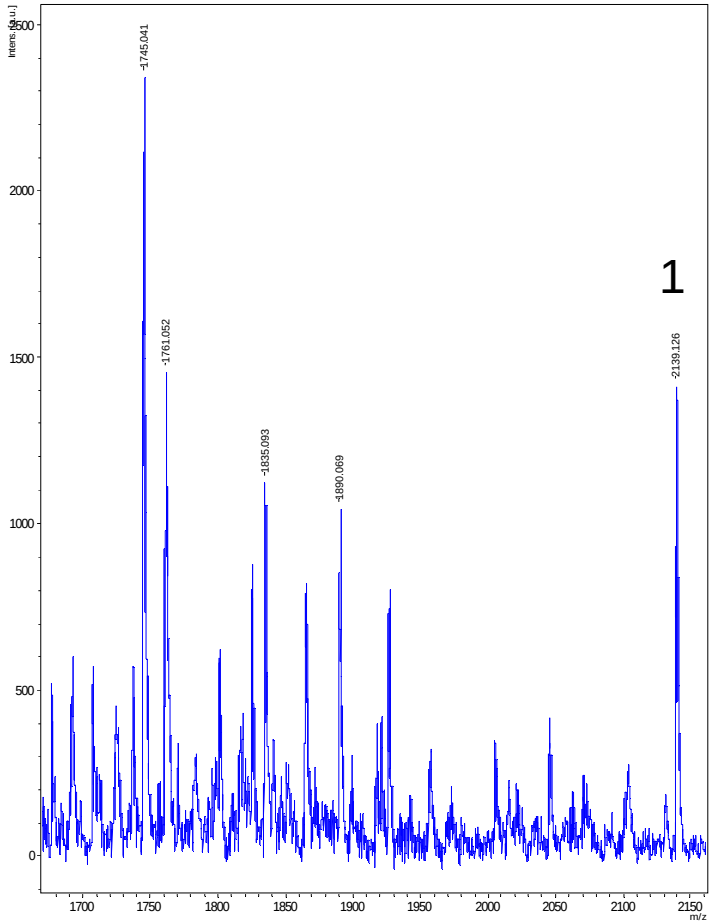
ARTKQTA(R^{me2s})KSTGGKAPRKQ, MH^{+}=2097.2 Da (1)

Ac-KQTA(R^{me2s})KSTGGKAPRKQ, MH^{+}=1811.0 Da (2)

Ac-QTA(R^{me2s})KSTGGKAPRKQ, MH^{+}=1682.9 Da (3)

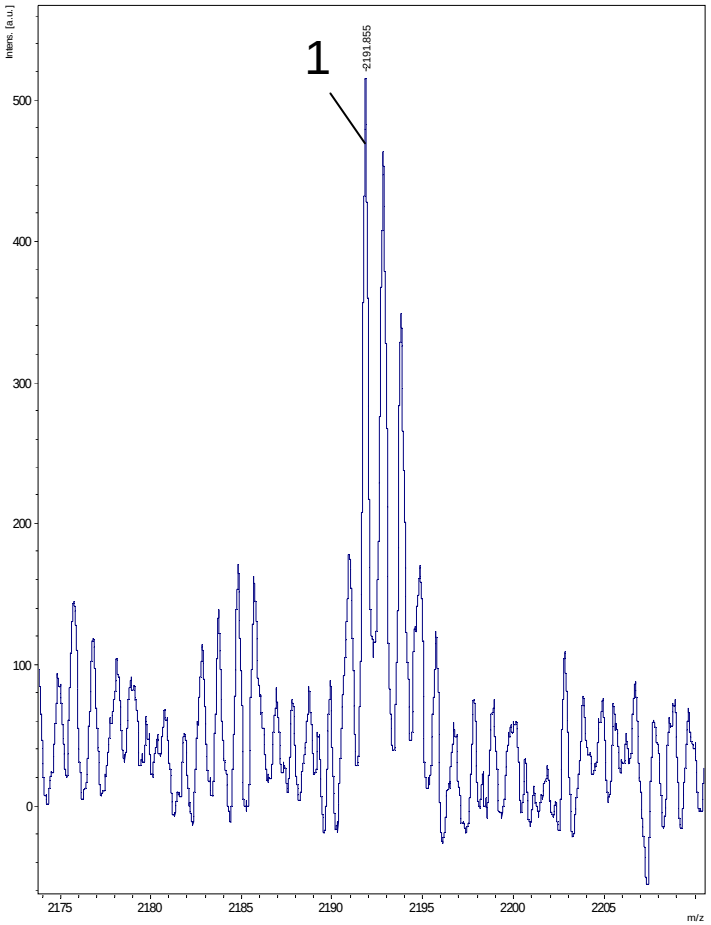
Ac-TA(R^{me2s})KSTGGKAPRKQ, MH^{+}=1554.9 Da (4)

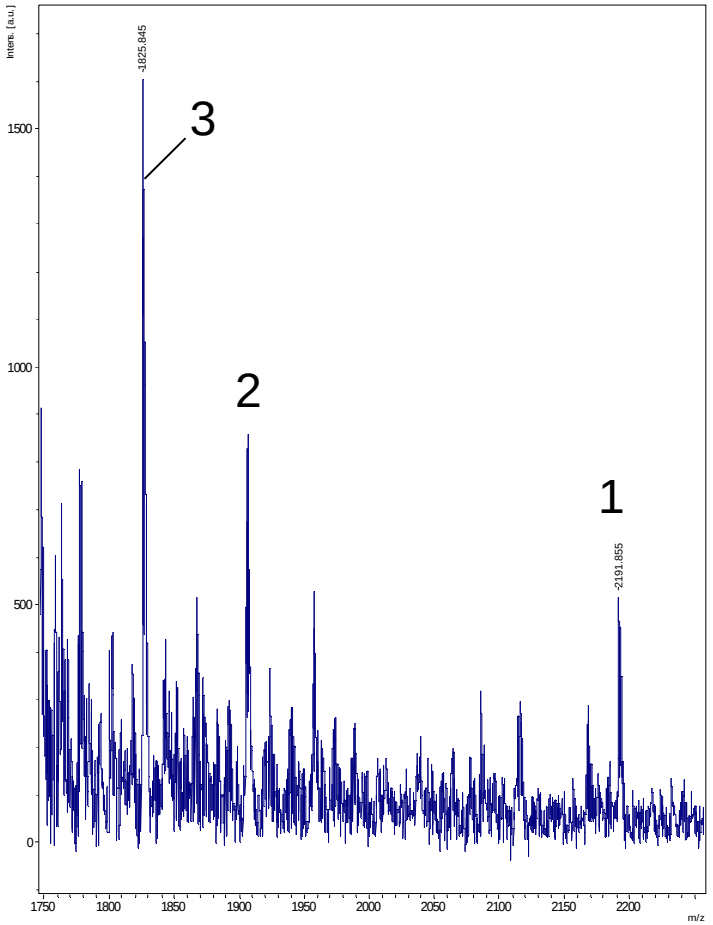




Peptide A23, H3 1-19 R2me2s/K4me3, A(R^{me2s})T(K^{me3})QTARKSTGGKAPRKQ

A(R^{me2a})T(K^{me3})QTARKSTGGKAPRKQ, MH^{+}=2139.2 Da (1)



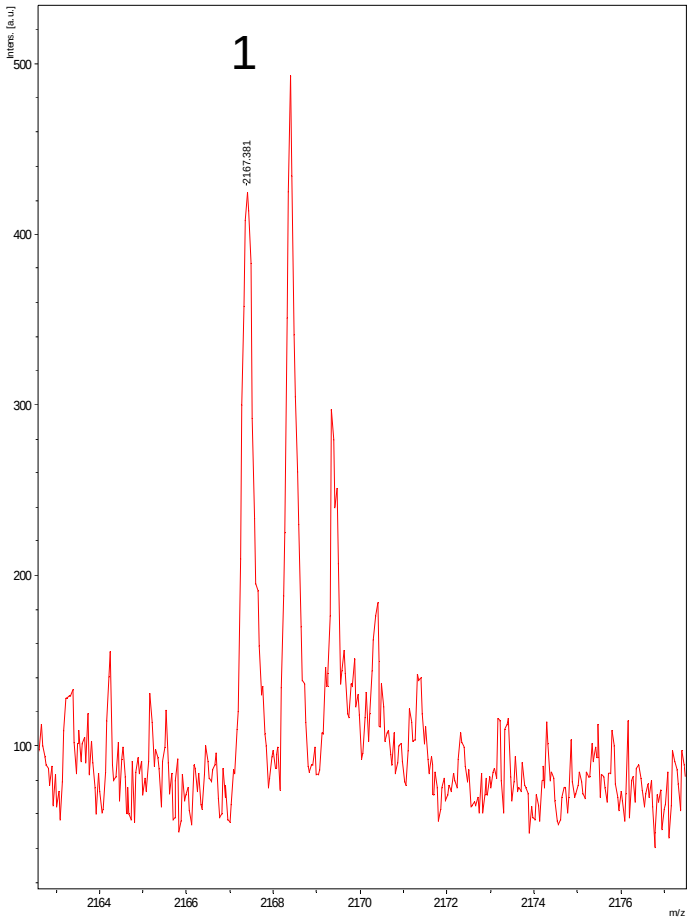


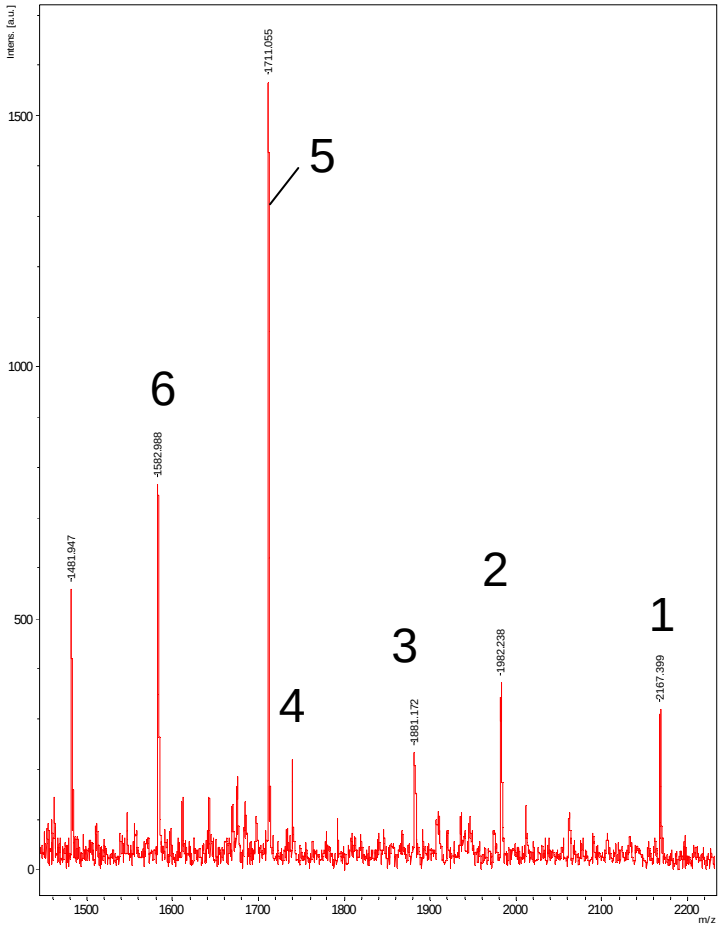
Peptide C23, H3 1-19 K9me3/S10ph, ARTKQTAR(K^{me3})(S^{ph})TGGKAPRKQ

ARTKQTAR(K^{me3})(S^{ph})TGGKAPRKQ, MH^+=2191.2 Da (1)

Ac-KQTAR(K^{me3})(S^{ph})TGGKAPRKQ, MH^+=1905.0 Da (2)

Ac-KQTAR(K^{me3})STGGKAPRKQ, MH^+=1825.0 Da (3)





Peptide F18, H3 1-19 K4me3/R8me2a/K9me2, ART(K^{me3})QTA(R^{me2a})K(^{me2})STGGKAPRKQ

ART(K^{me3})QTA(R^{me2a})(K^{me2})STGGKAPRKQ, MH^+=2167.2 Da (1)

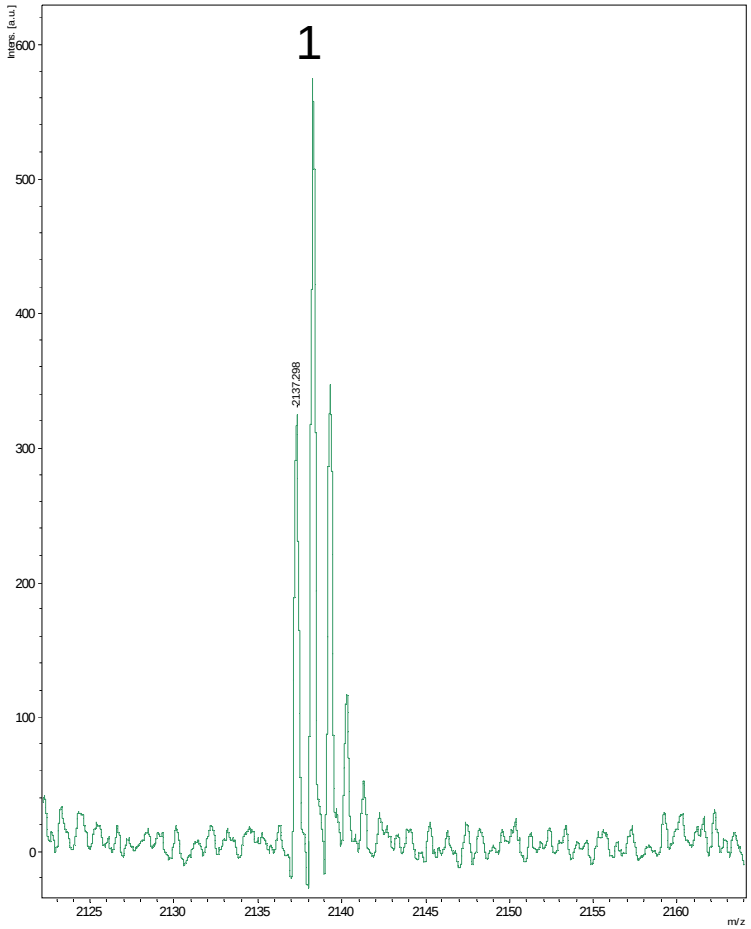
Ac-T(K^{me3})QTA(R^{me2a})(K^{me2})STGGKAPRKQ, MH^+=1982.1 Da (2)

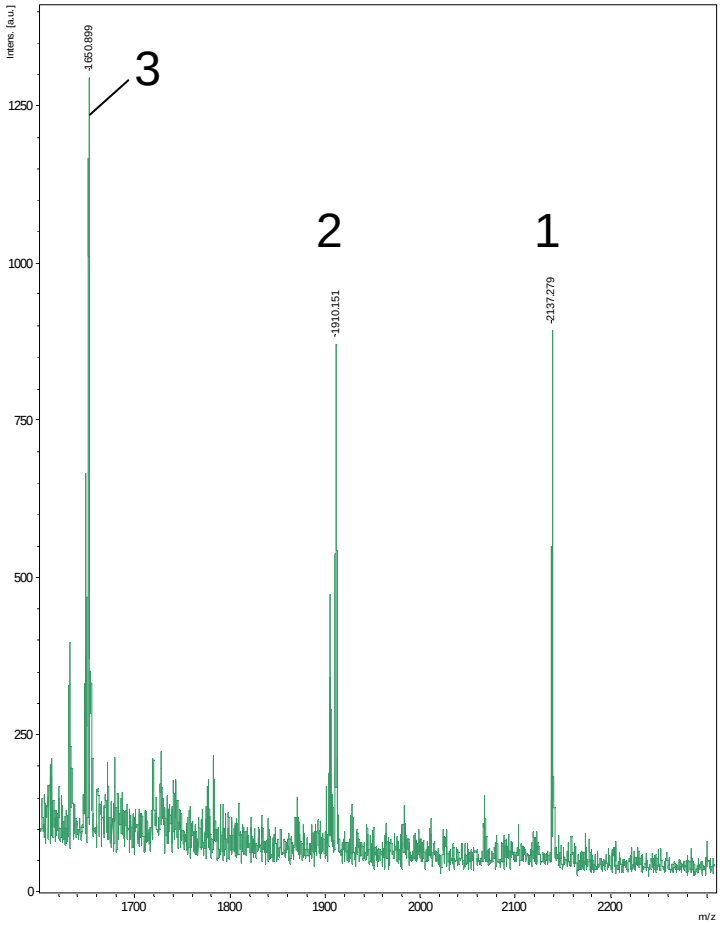
Ac-(K^{me3})QTA(R^{me2a})(K^{me2})STGGKAPRKQ, MH^+=1881.0 Da (3)

Ac-QTA(R^{me2a})(K^{me2})STGGKAPRKQ-Na^+, MH^+=1733.9 Da (4)

Ac-QTA(R^{me2a})(K^{me2})STGGKAPRKQ, MH^+=1710.9 Da (5)

Ac-QTA(R^{me2a})(K^{me2})STGGKAPRKQ, MH^+=1582.9 Da (6)



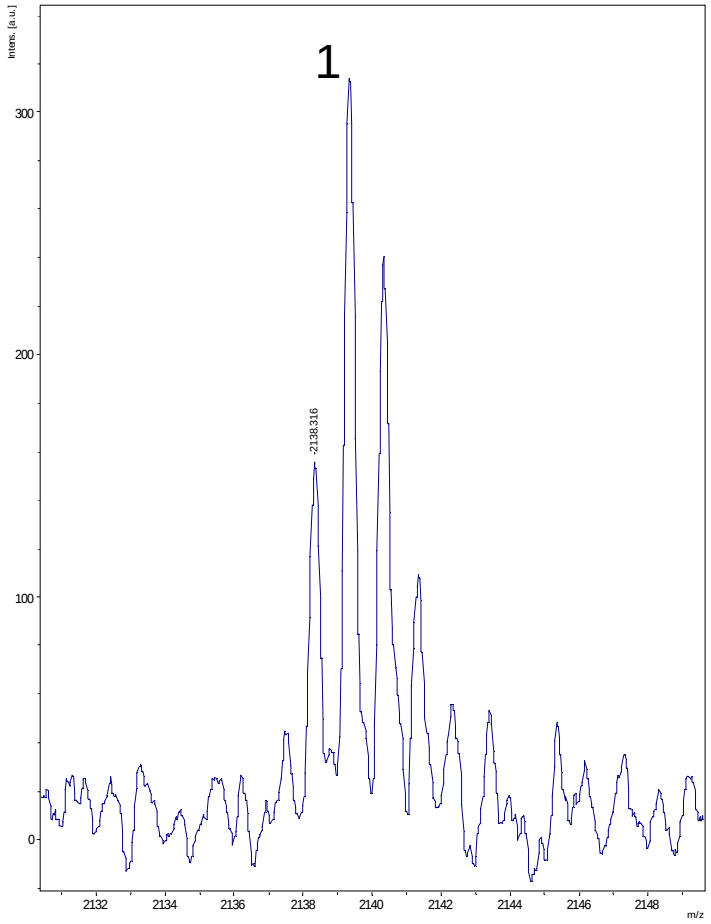


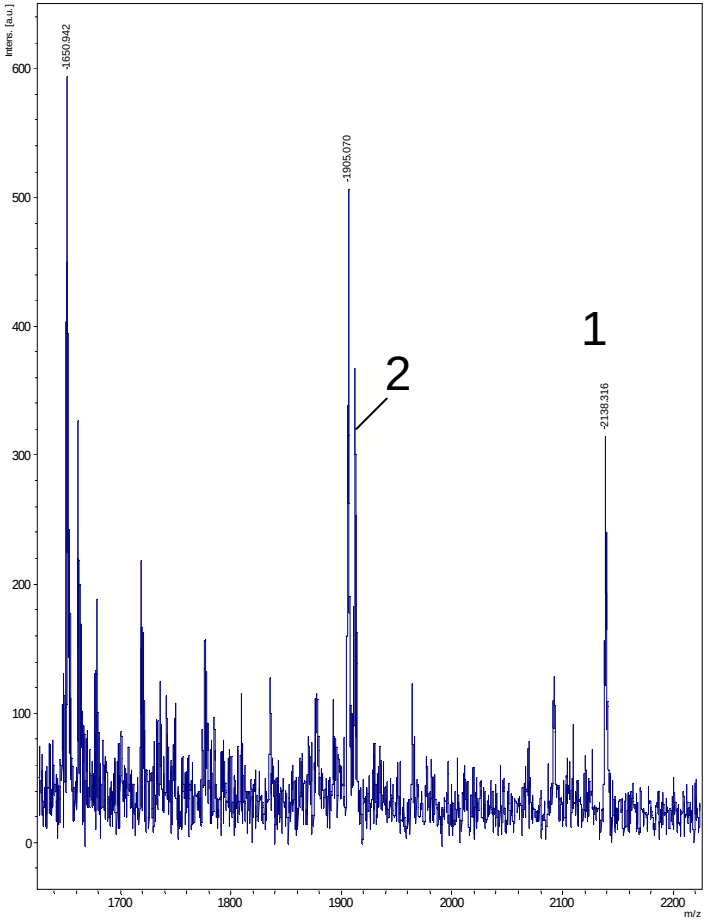
Peptide J4, H3 7-26, Ac-ARKSTGGKAPRKQLATKAAR

Ac-ARKSTGGKAPRKQLATKAAR, MH^+=2137.3 Da (1)

Ac-KSTGGKAPRKQLATKAAR, MH^+=1910.1 Da (2)

TGGKAPRKQLATKAAR, MH^+=1653.0 Da (3)

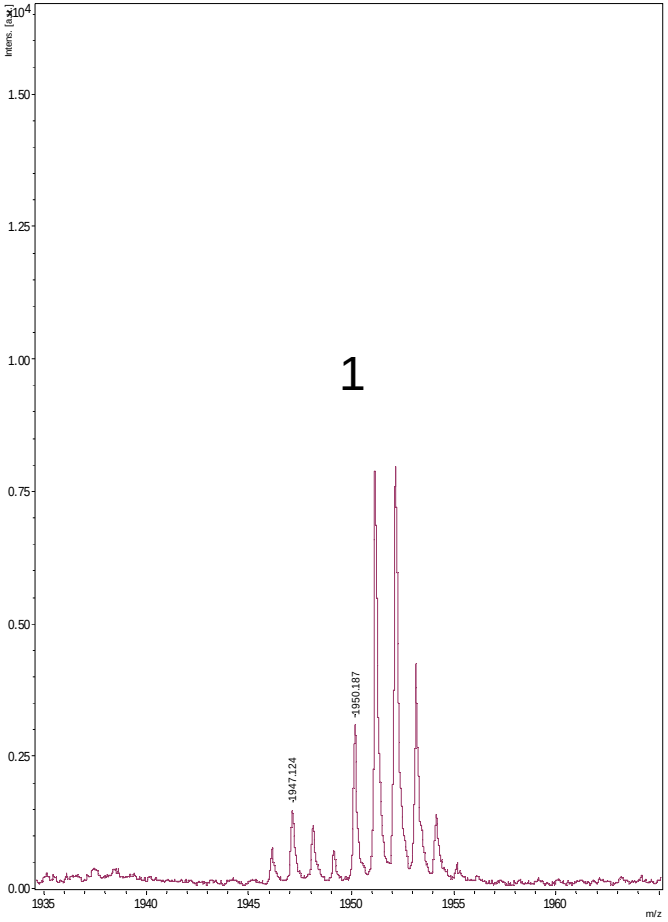


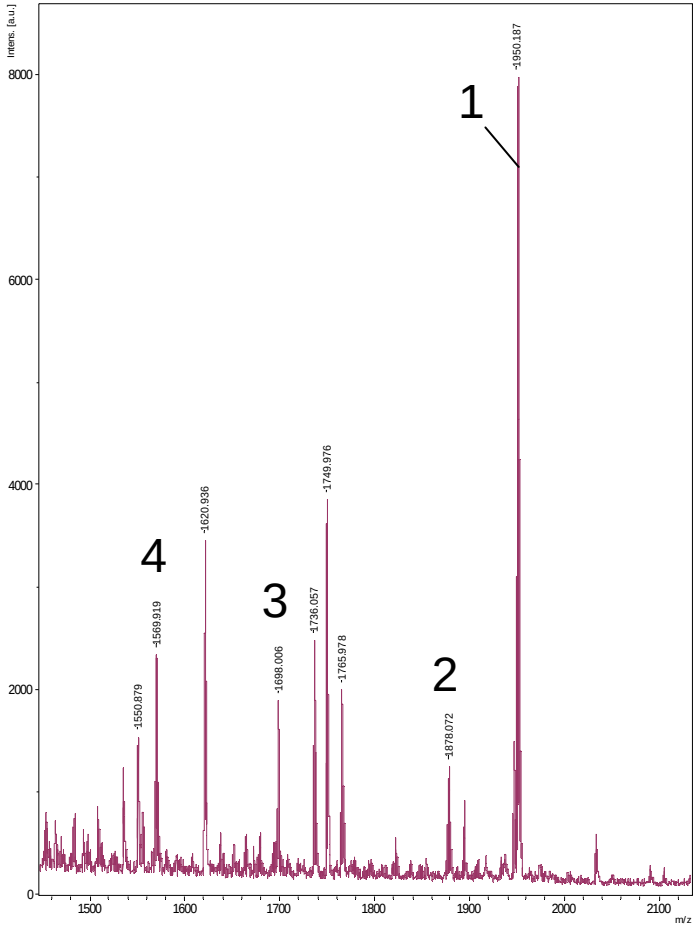


Peptide J10, H3 7-26 R17citr, Ac-ARKSTGGKAP(R^{Citr})KQLATKAAR

Ac-ARKSTGGKAP(RCitr)KQLATKAAR, MH^{+}=2138.3 Da (1)

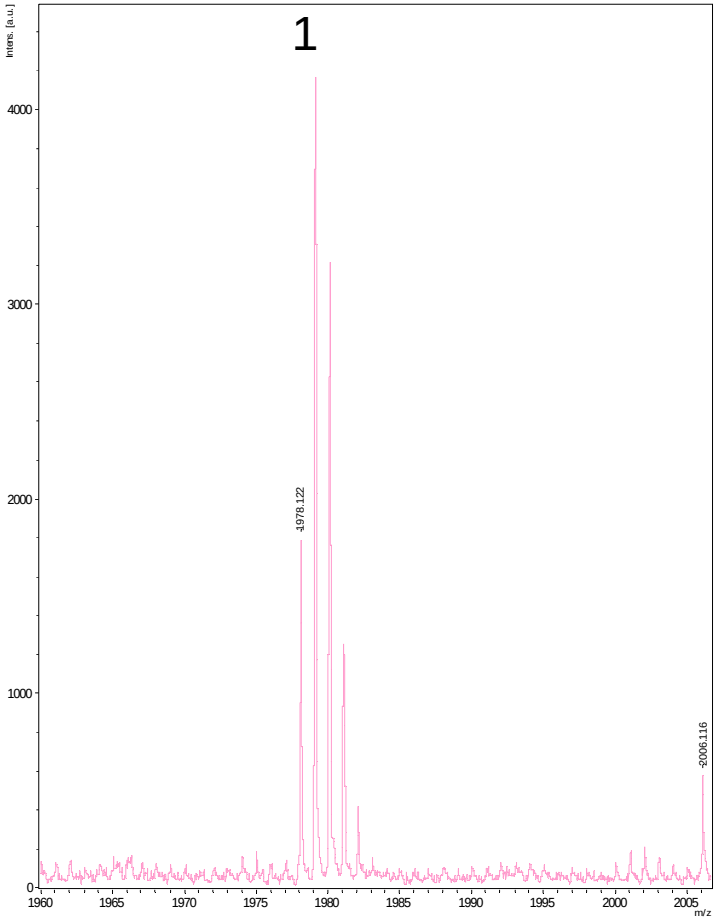
Ac-KSTGGKAP(RCitr)KQLATKAAR, MH+=1911.1 Da (2)

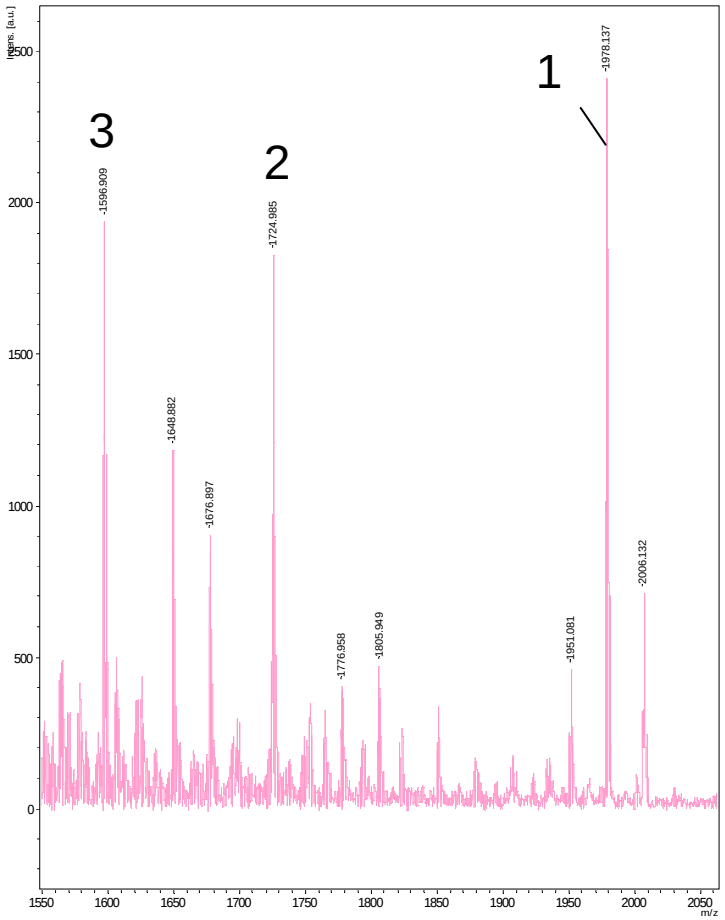




Peptide J20, H3 16-35, Ac-PRKQLATKAARKSAPATGG

Ac-PRKQLATKAARKSAPATGG, MH^+=1950.1 Da (1)
Ac-RKQLATKAARKSAPATGG-Na^+, MH^+=1876.1 Da (2)
Ac-KQLATKAARKSAPATGG, MH^+=1697.0 Da (3)
Ac-QLATKAARKSAPATGG, MH^+=1568.9 Da (4)



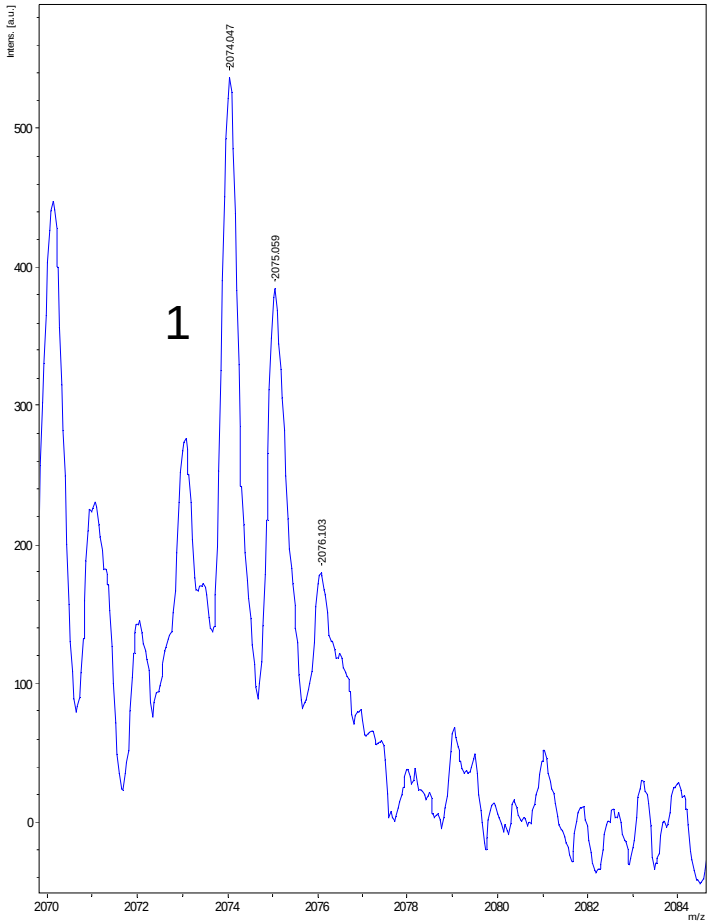


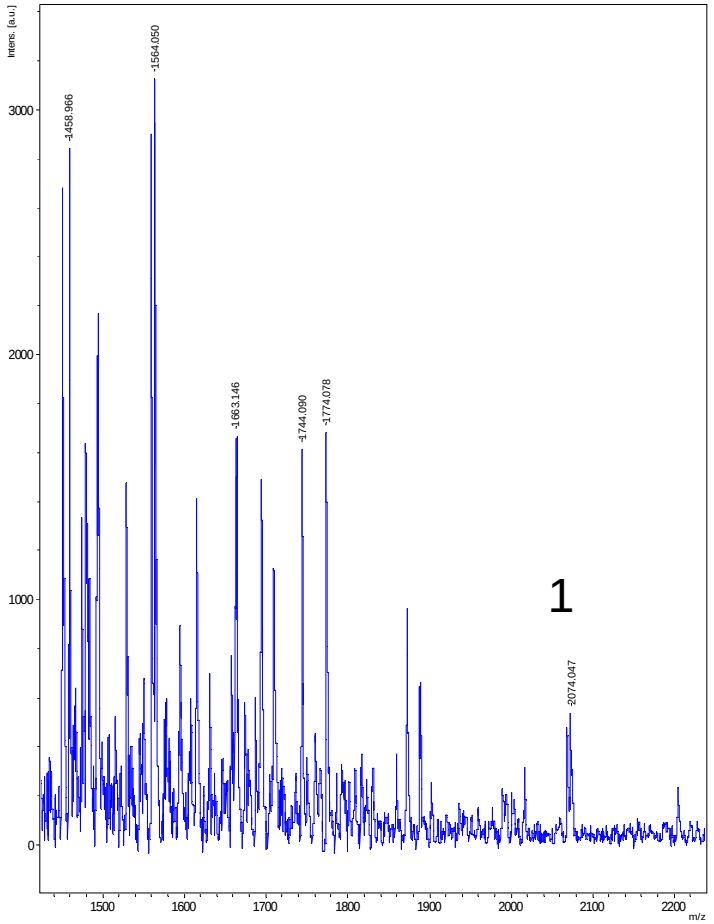
Peptide K1, H3 16-35 K27me2, Ac-PRKQLATKAAR(K^{me2})SAPATGG

Ac-PRKQLATKAAR(K^{me2})SAPATGG, MH^{+}=1978.1 Da (1)

Ac-KQLATKAAR(K^{me2})SAPATGG, MH^{+}=1725.0 Da (2)

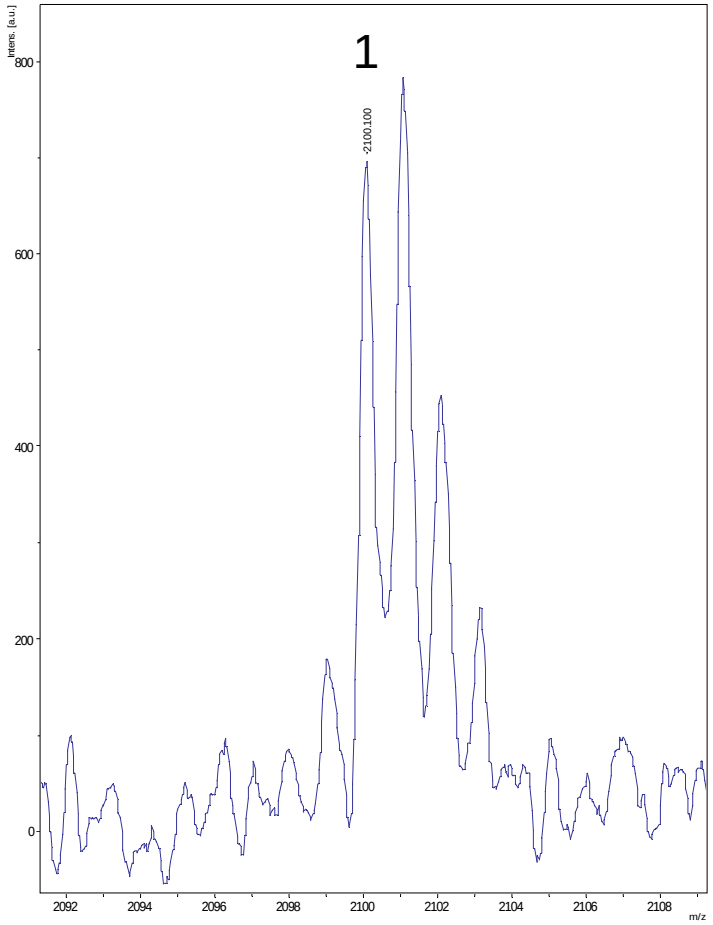
Ac-QLATKAAR(K^{me2})SAPATGG, MH^{+}=1596.9 Da (3)

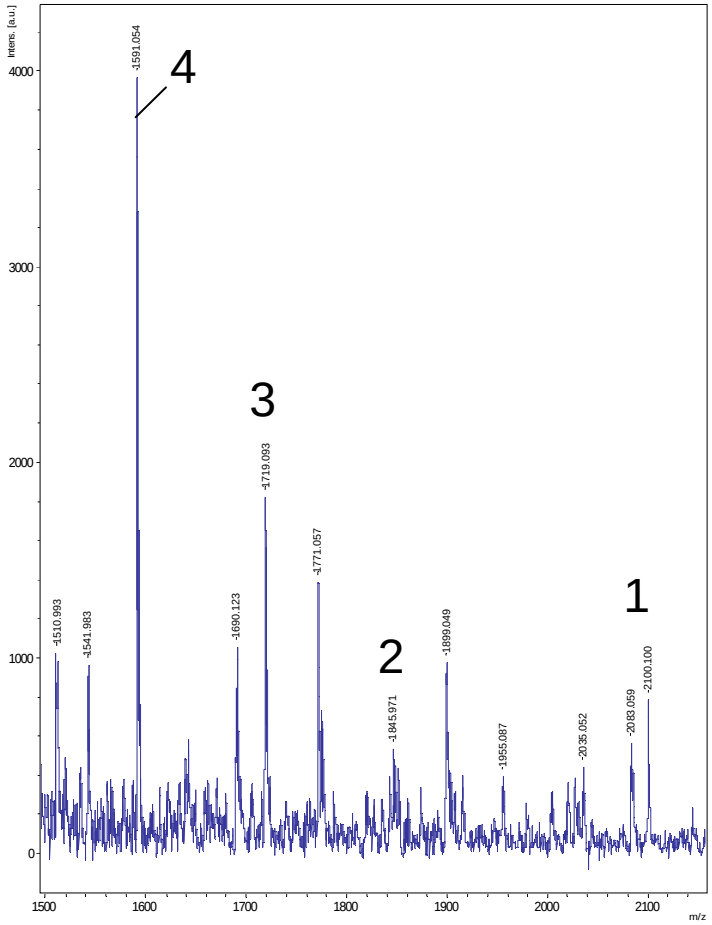




Peptide K21, H3 16-35 K27me3/S28ph, Ac-PRKQLATKAAR(K^{me3})(S^{ph})APATGG

Ac-PRKQLATKAAR(K^{me3})(S^{ph})APATGG, MH^{+}=2072.1 Da (1)





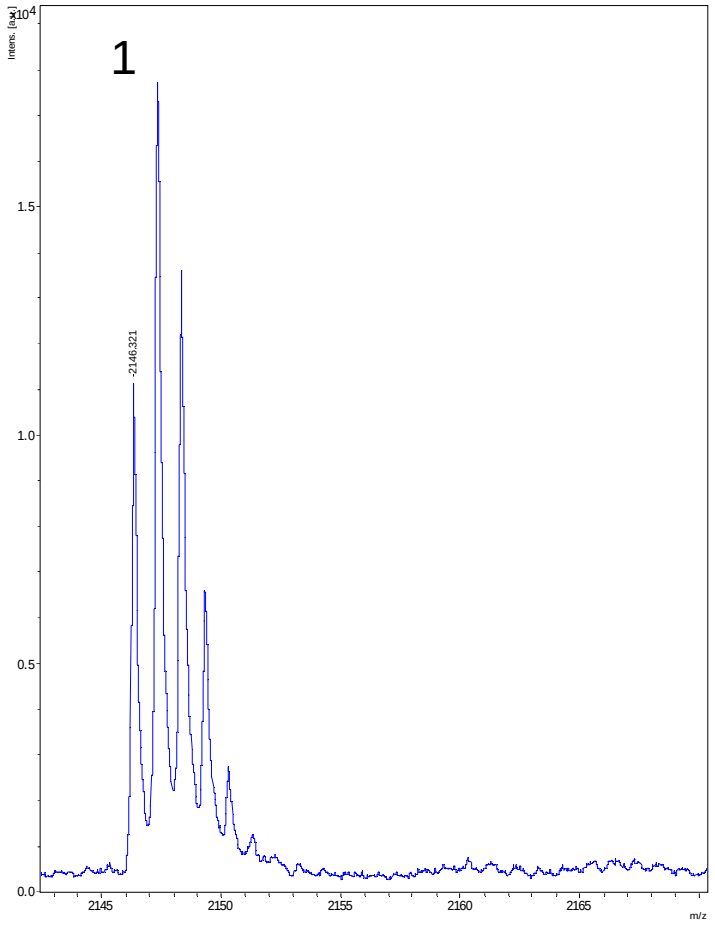
Peptide L1, H3 16-35 R26me2s/K27me3/S28ph, Ac-PRKQLATKAA(R^{me2s})(K^{me3})(S^{ph})APATGG

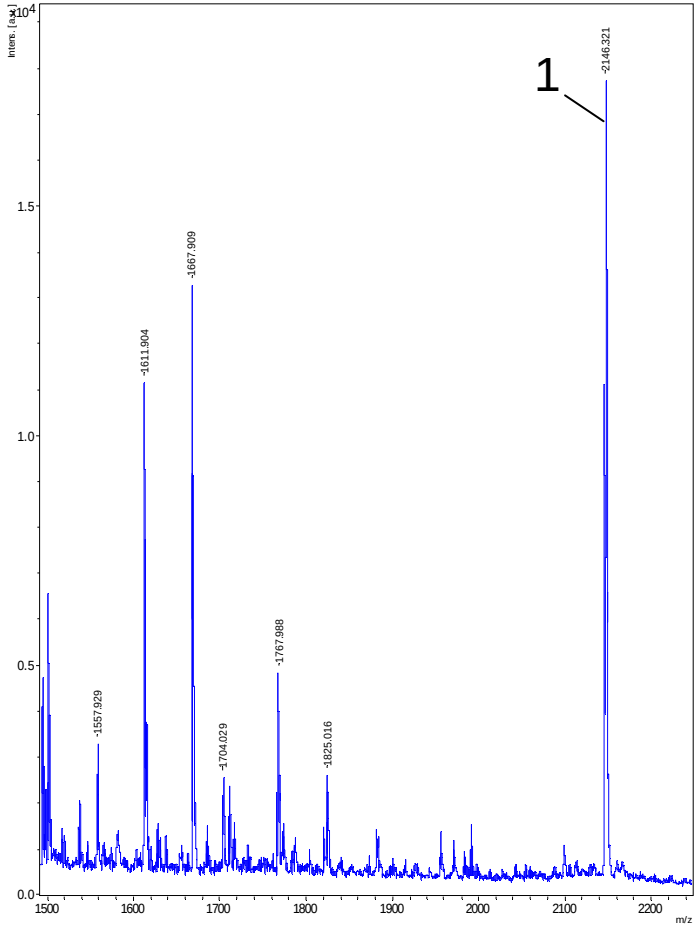
Ac-PRKQLATKAA(R^{me2s})(K^{me3})(S^{ph})APATGG, MH^+=2100.2 Da (1)

Ac-KQLATKAA(R^{me2s})(K^{me3})(S^{ph})APATGG, MH^+=1847.0 Da (2)

Ac-QLATKAA(R^{me2s})(K^{me3})(S^{ph})APATGG, MH^+=1718.9 Da (3)

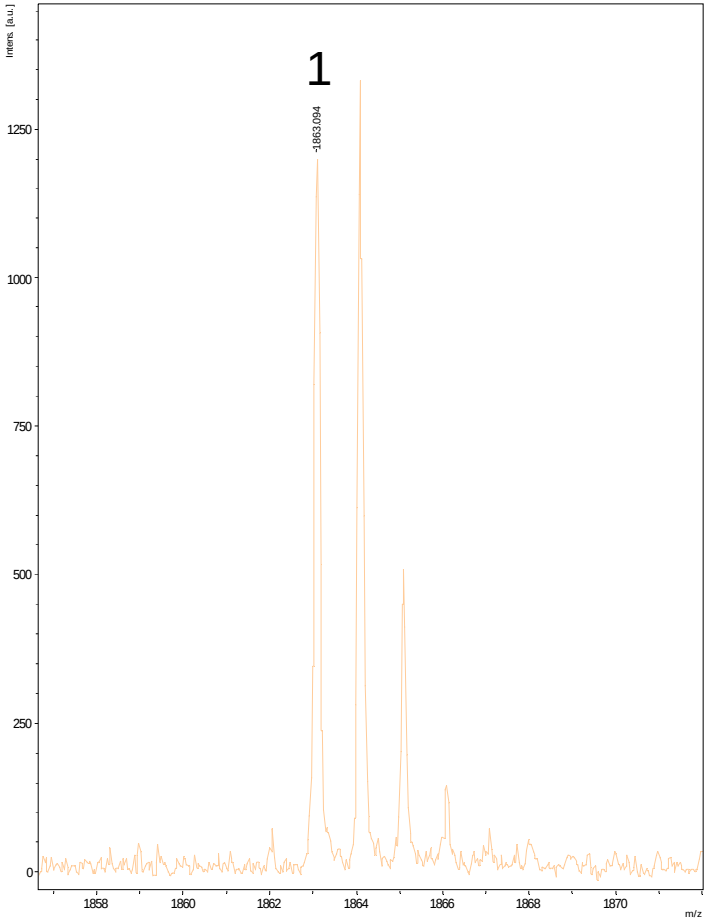
Ac-LATKAA(R^{me2s})(K^{me3})(S^{ph})APATGG, MH^+=1590.8 Da (4)

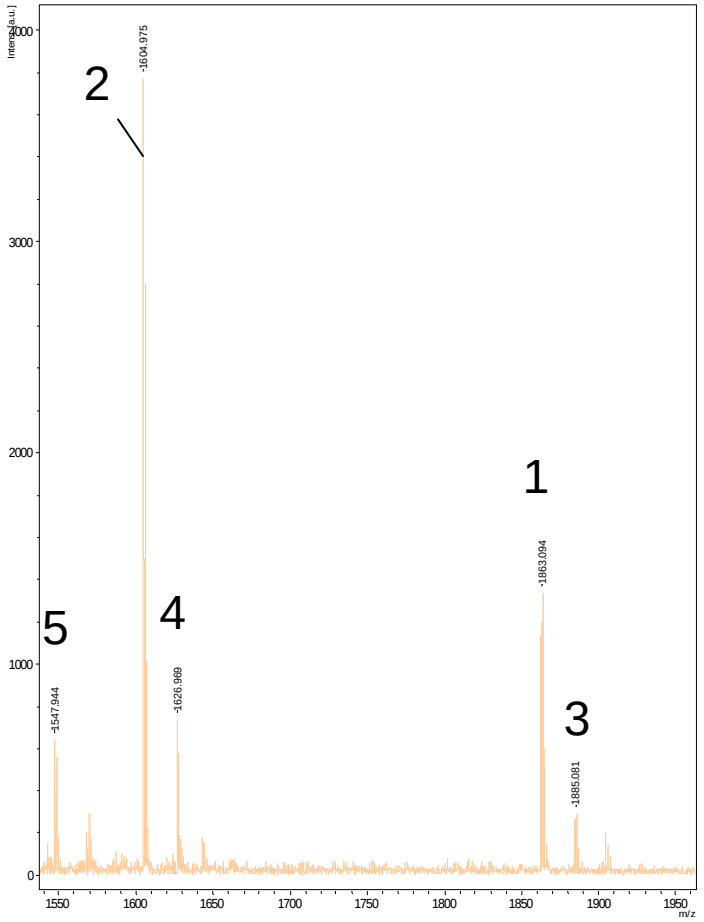




Peptide L10, H3 26-45 K36me3, Ac-RKSAPATGGV(K^{me3})KPHRYRPG

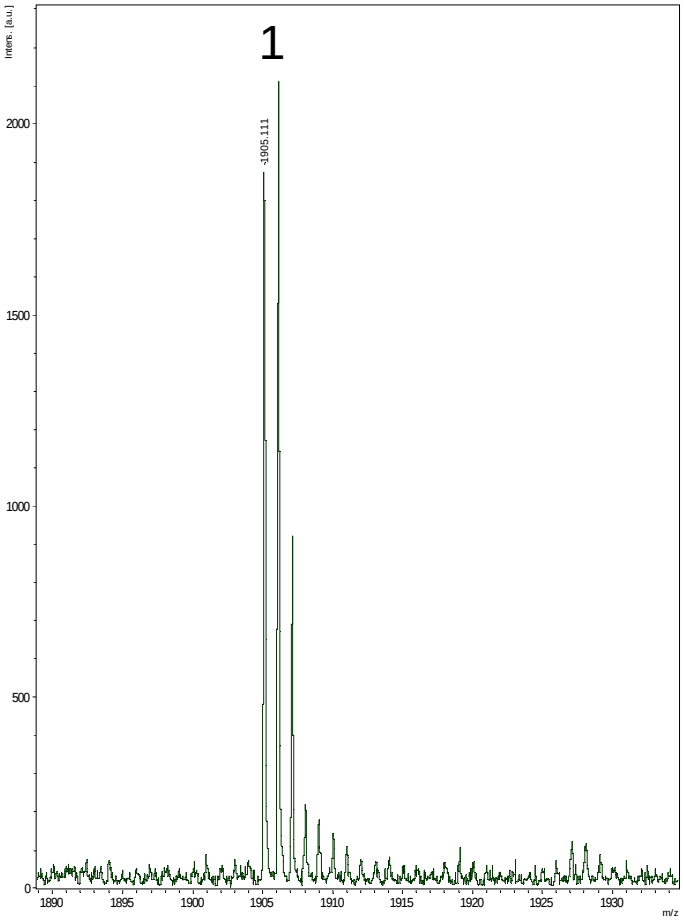
Ac-RKSAPATGGV(K^{me3})KPHRYRPG, MH^{+}=2146.2 Da (1)

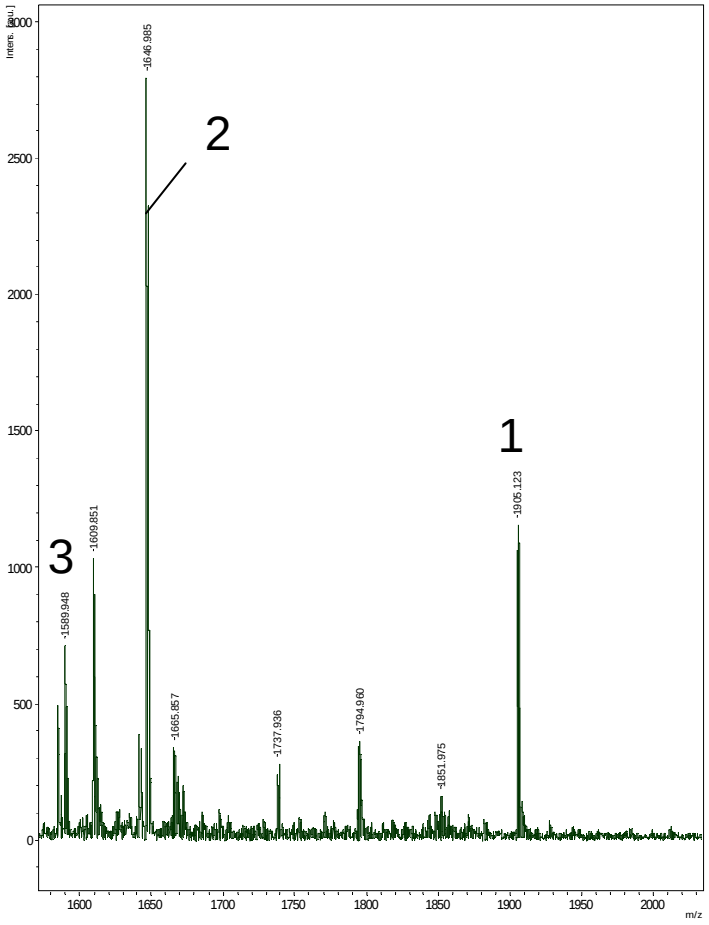




Peptide L12, H4 1-19, SGRGKGGKGLGKGGAKRHR

SGRGKGGKGLGKGGAKRHR, MH^+=1863.1 Da (1)
Ac-GKGGKGLGKGGAKRHR, MH^+=1604.9 Da (2)
SGRGKGGKGLGKGGAKRHR-Na^+, MH^+=1886.1 Da (3)
Ac-GKGGKGLGKGGAKRHR-Na^+, MH^+=1627.9 Da (4)
Ac-KGGKGLGKGGAKRHR, MH^+=1547.9 Da (5)



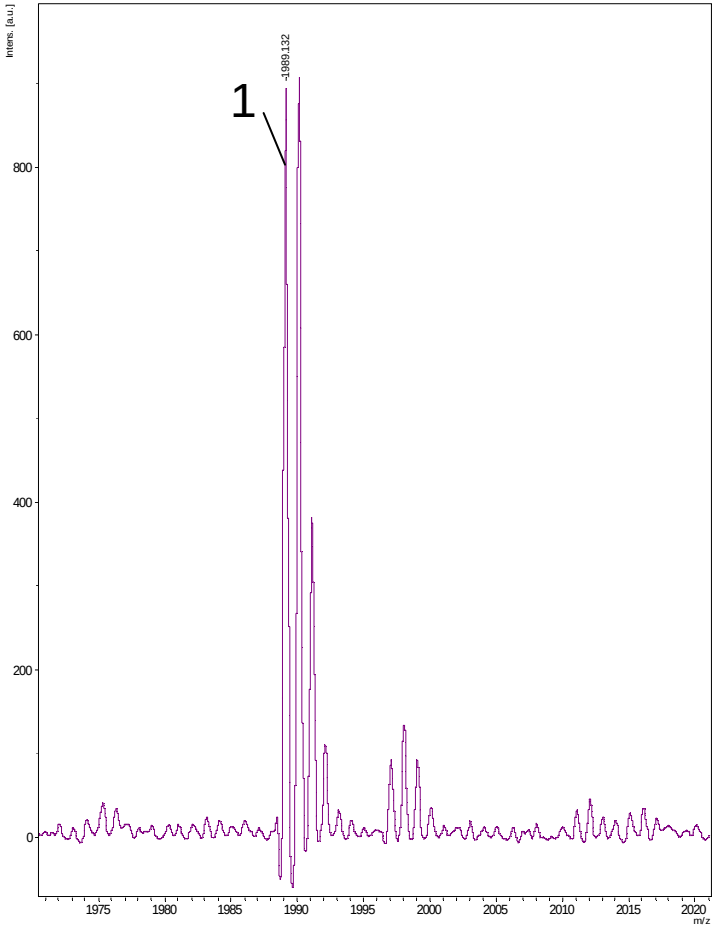


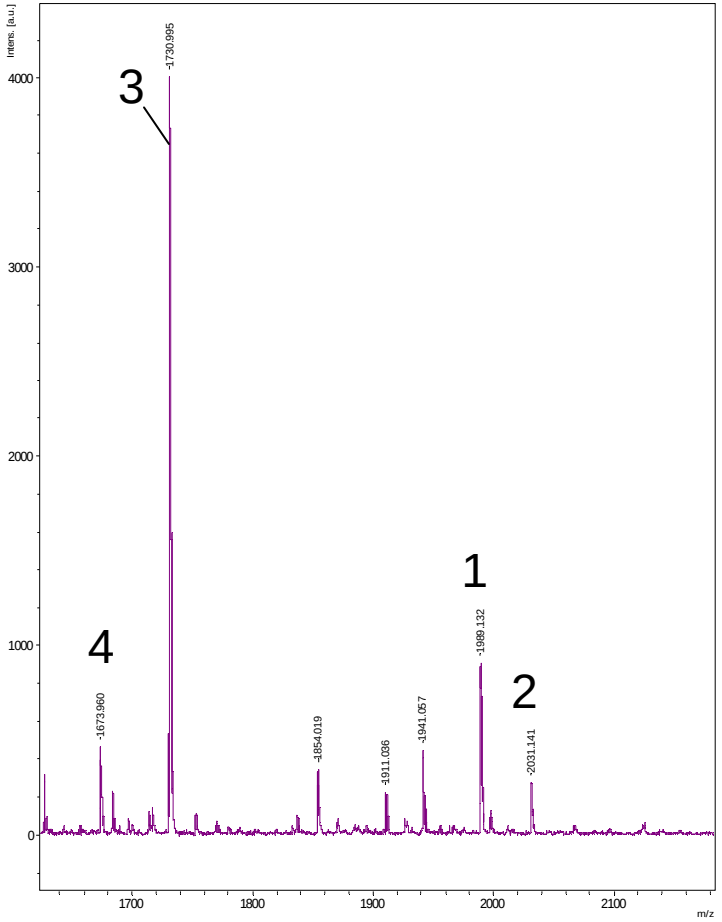
Peptide L19, H4 1-19 K16ac, SGRGKGGKGLGKGGA(K^{ac})RHR

SGRGKGGKGLGKGGA(K^{ac})RHR, MH^{+}=1905.1 Da (1)

Ac-GKGGKGLGKGGA(K^{ac})RHR, MH^{+}=1646.9 Da (2)

SGRGKGGKGLGKGGA(K^{ac})RHR, MH^{+}=1589.9 Da (3)





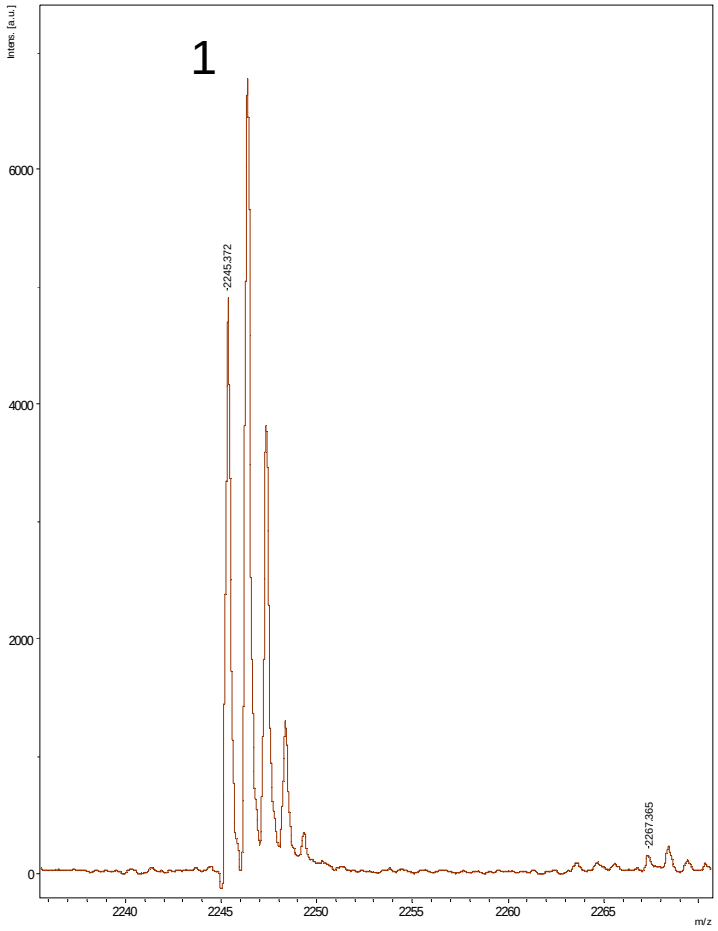
Peptide M11, H4 1-19 K5ac/K8ac/K12ac, SGRGK(ac)GGK(ac)GLGK(ac)GGAKRHR

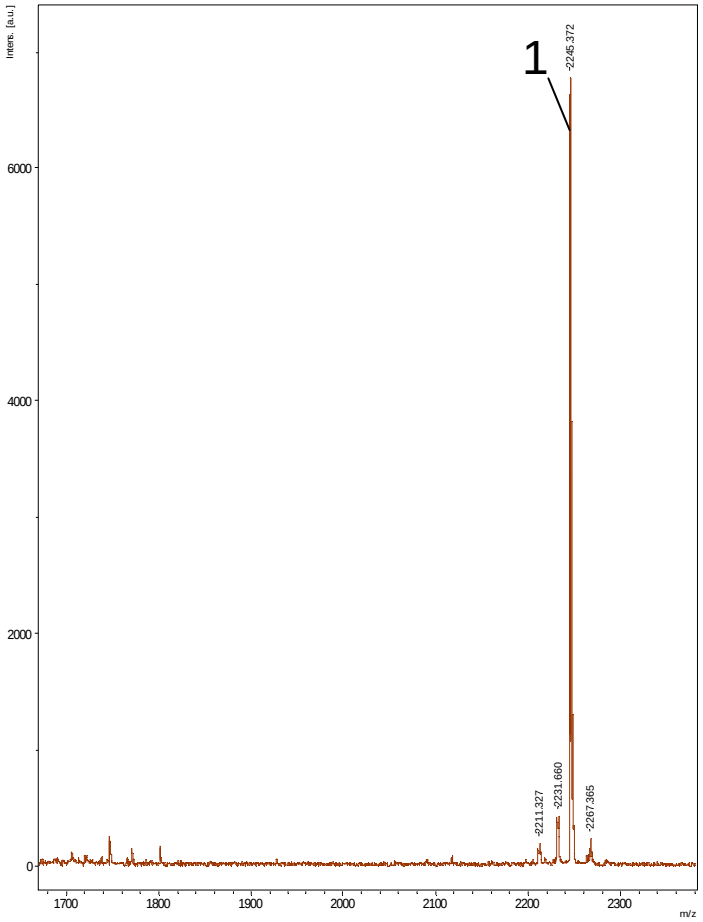
SGRG(K^{ac})GG(K^{ac})GLG(K^{ac})GGAKRHR, MH^{+}=1989.1 Da (1)

Ac-SGRG(K^{ac})GG(K^{ac})GLG(K^{ac})GGAKRHR, MH^{+}=2030.1 Da (2)

Ac-G(K^{ac})GG(K^{ac})GLG(K^{ac})GGAKRHR, MH^{+}=1730.9 Da (3)

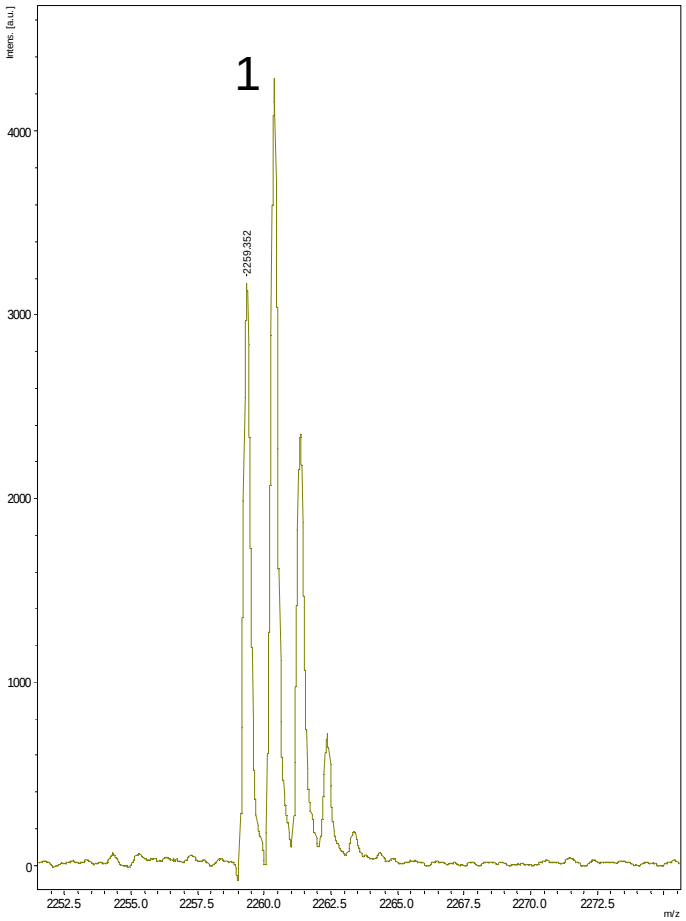
Ac-(K^{ac})GG(K^{ac})GLG(K^{ac})GGAKRHR, MH^{+}=1673.9 Da (4)

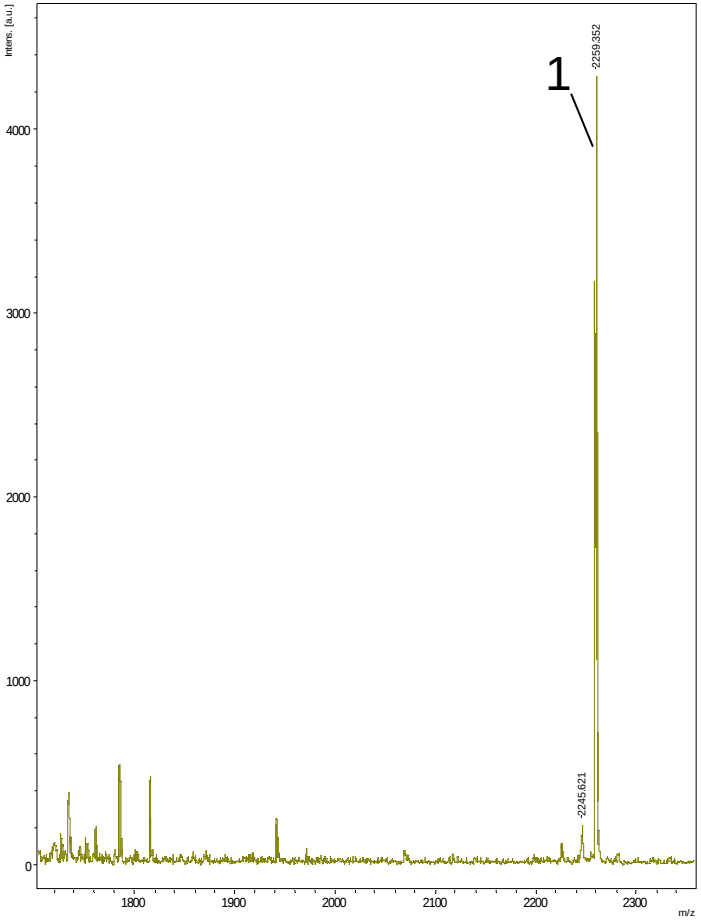




Peptide M18, H4 11-30, Ac-GKGGAKRHRKVLRDNIQGIT

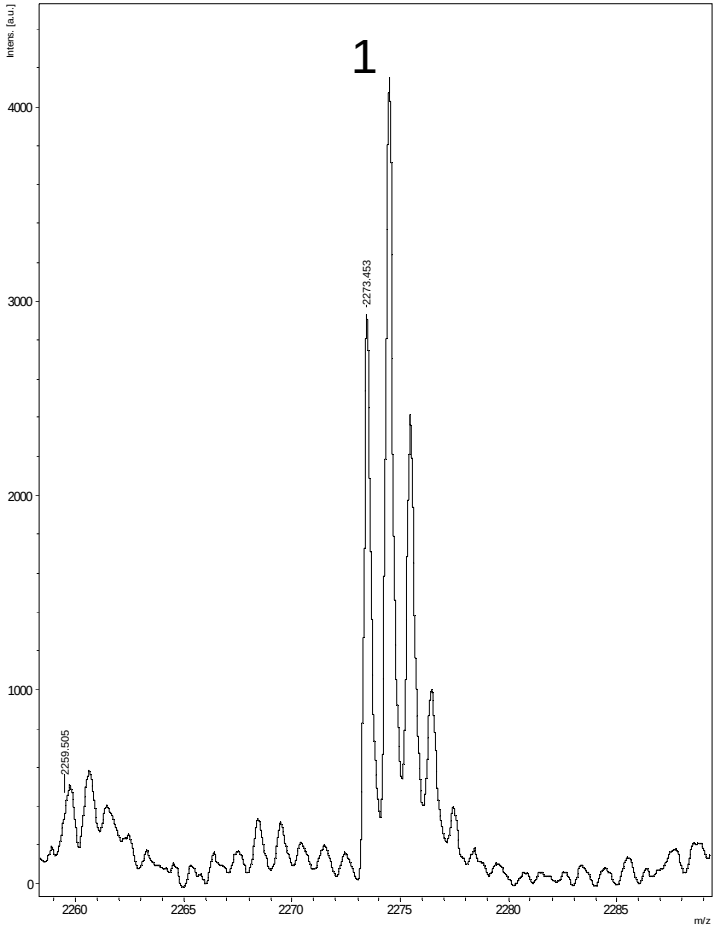
Ac-GKGGAKRHRKVLRDNIQGIT, MH^+=2245.3 Da (1)

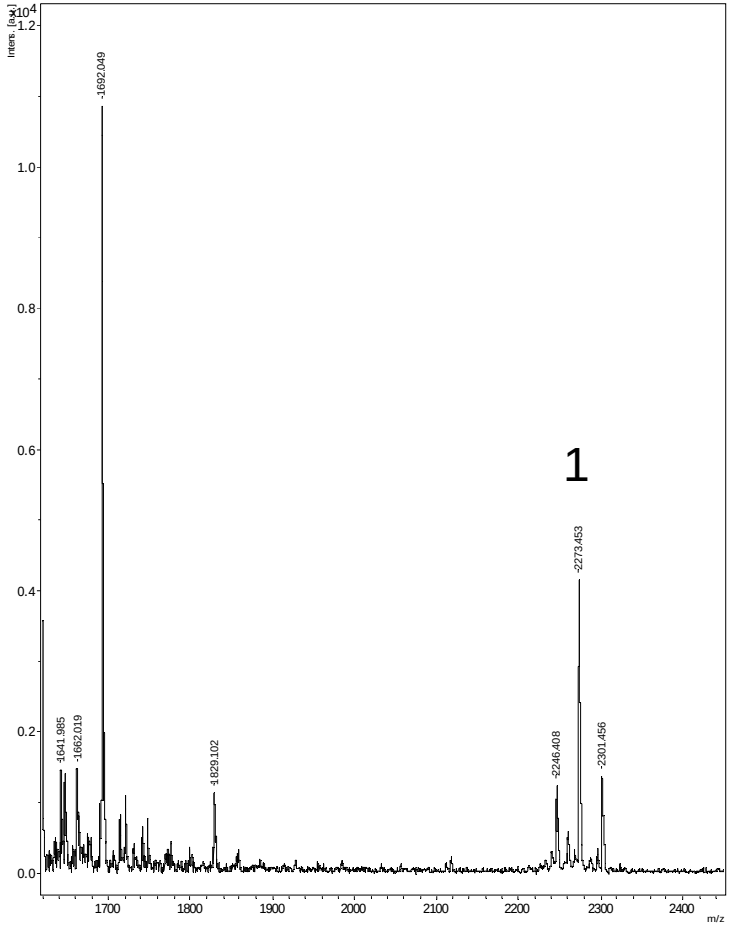




Peptide N1, H4 11-30 K20me1, Ac-GKGGAKRHRK(me1)VLRDNIQGIT

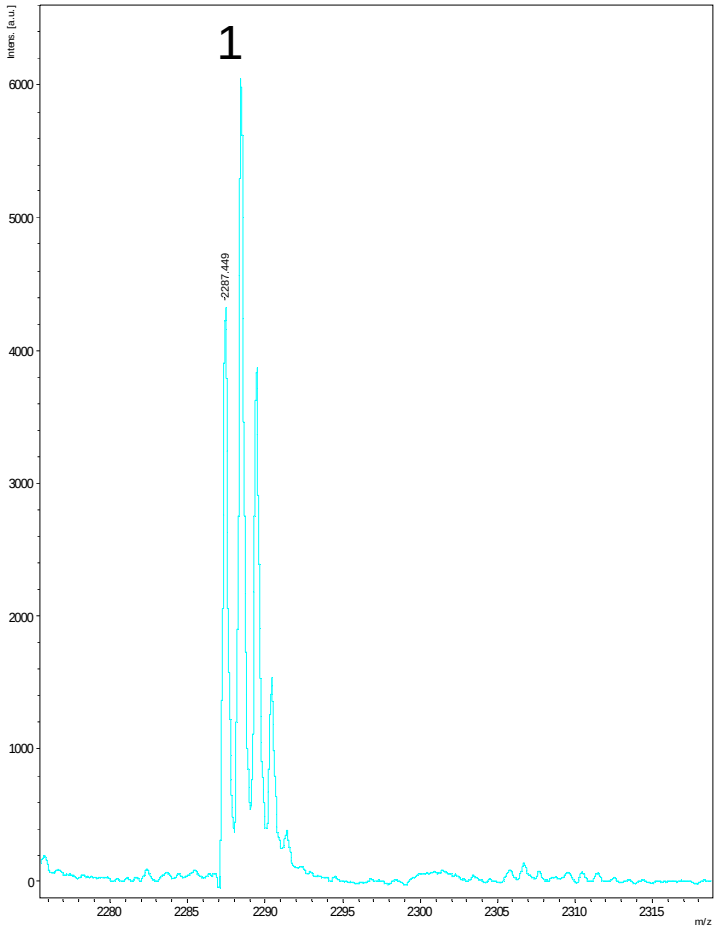
Ac-GKGGAKRHR(K^{me1})VLRDNIQGIT, MH^{+}=2259.3 Da (1)

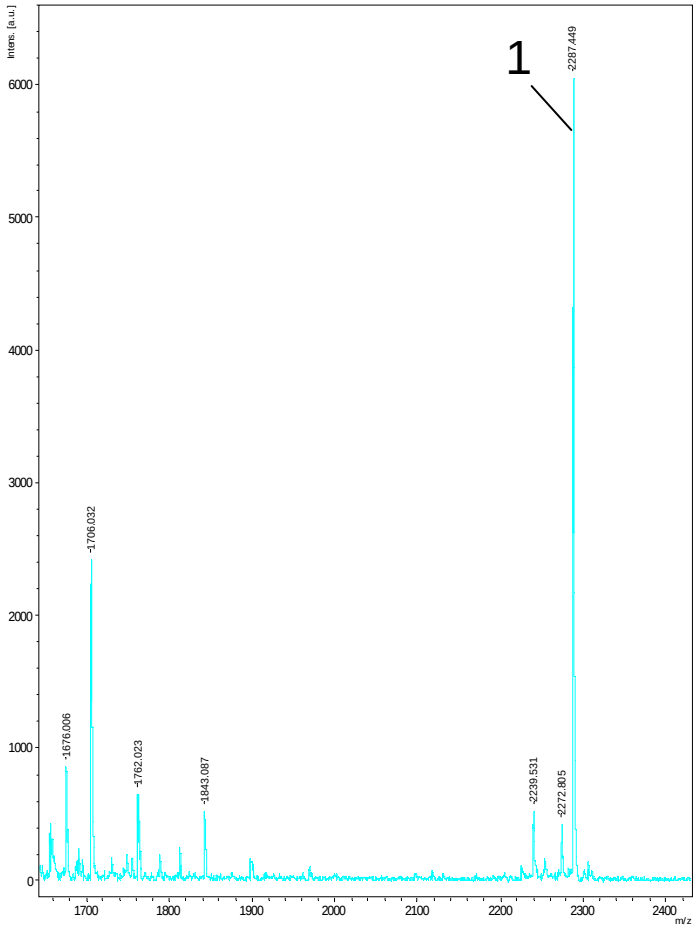




Peptide N2, H4 11-30 K20me2, Ac-GKGGAKRHR(K^{me2})VLRDNIQGIT

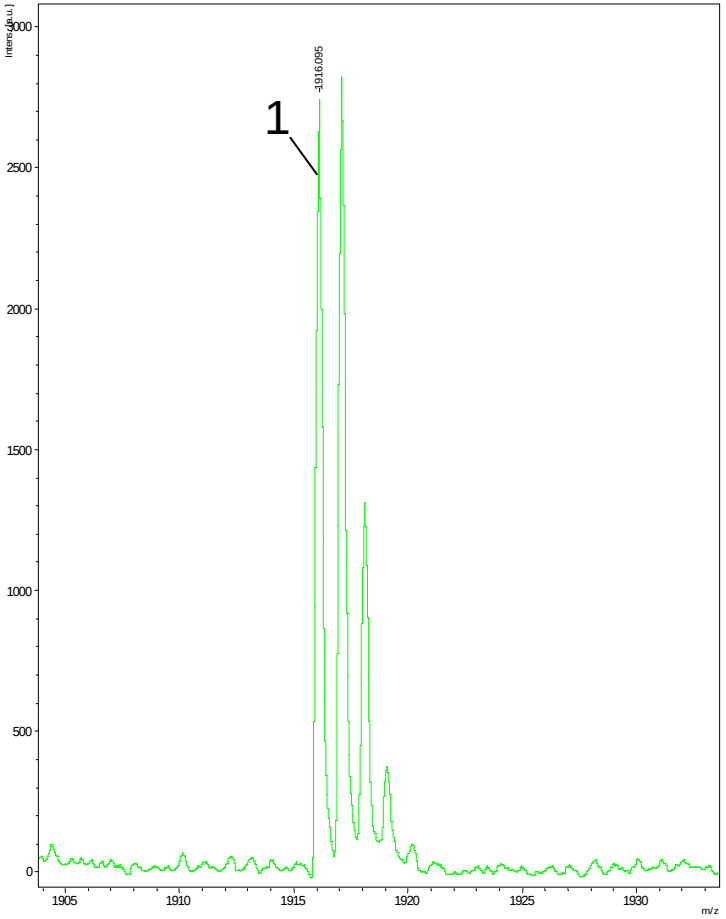
Ac-GKGGAKRHR(K^{me2})VLRDNIQGIT, MH^{+}=2273.3 Da (1)

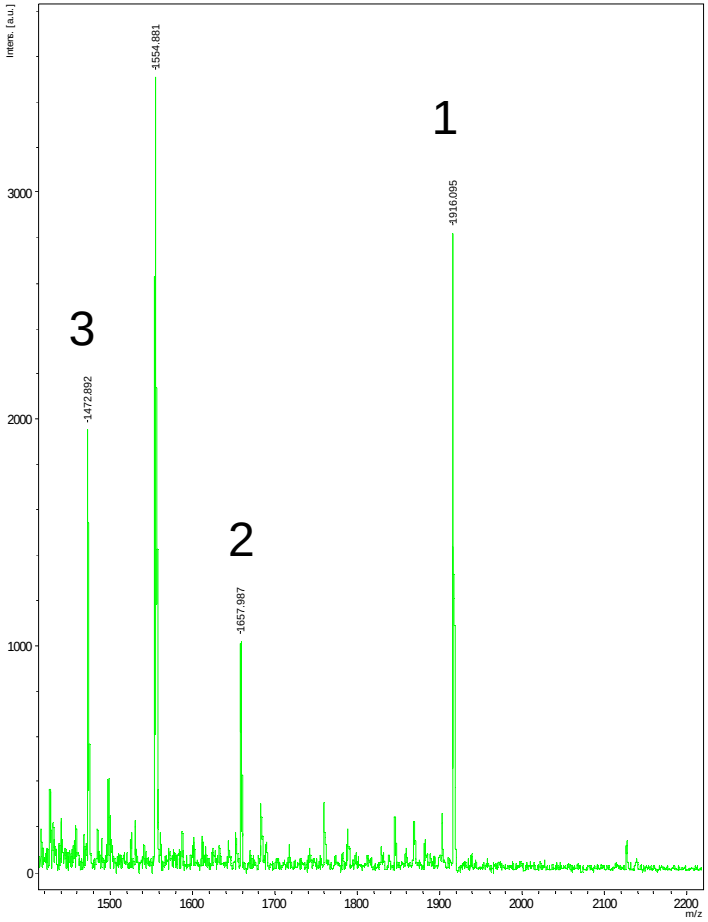




Peptide N3, H4 11-30 K20me3, Ac-GKGGAKRHR(K^{me3})VLRDNIQGIT

Ac-GKGGAKRHR(K^{me3})VLRDNIQGIT, MH^{+}=2287.3 Da (1)



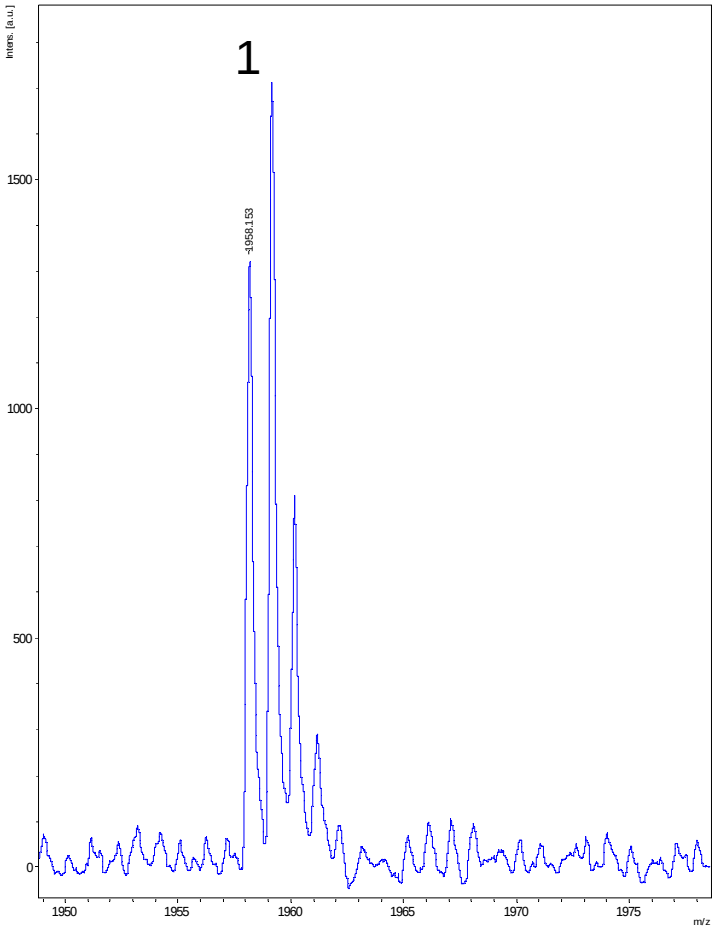


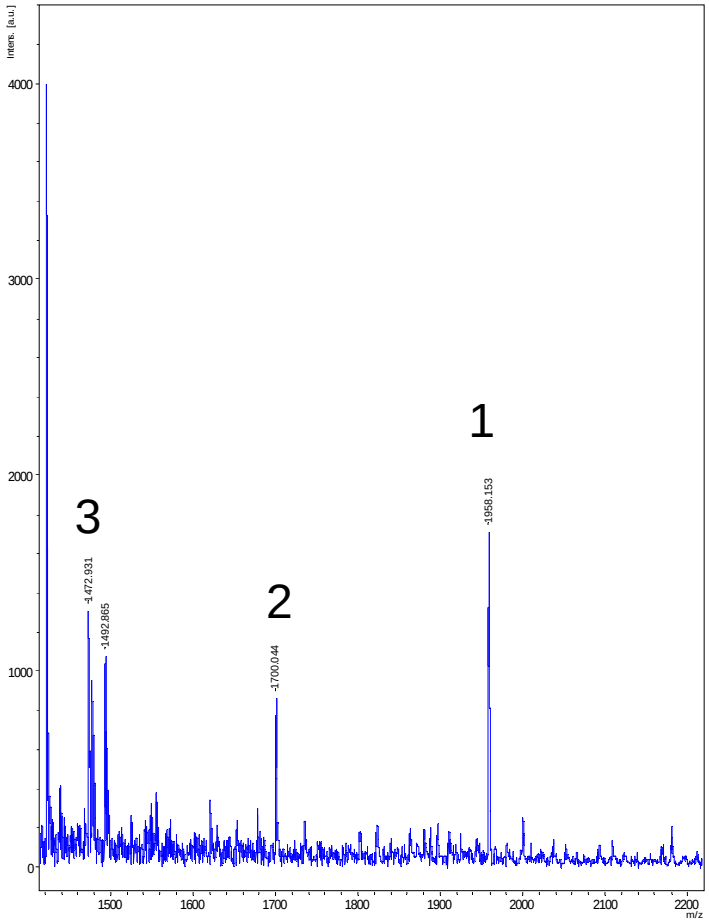
Peptide O12, H2A 1-19, SGRGKQGGKARAKAKSRSS

SGRGKQGGKARAKAKSRSS, MH^+= 1916.1 Da (1)

Ac-GKQGGKARAKAKSRSS, MH^+= 1657.9 Da (2)

Ac-QGGKARAKAKSRSS, MH^+= 1572.8 Da (3)



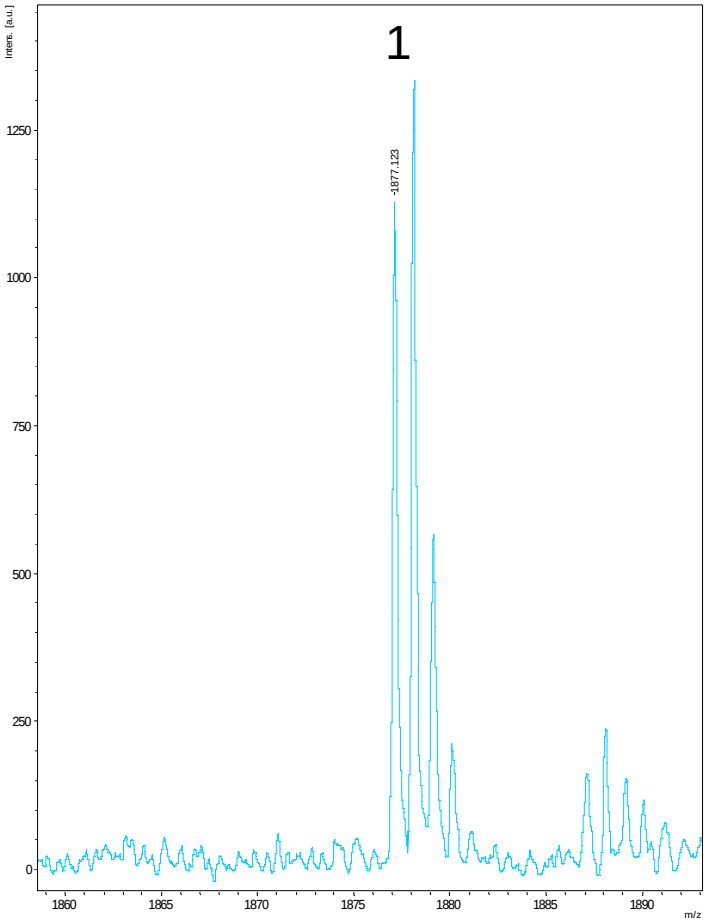


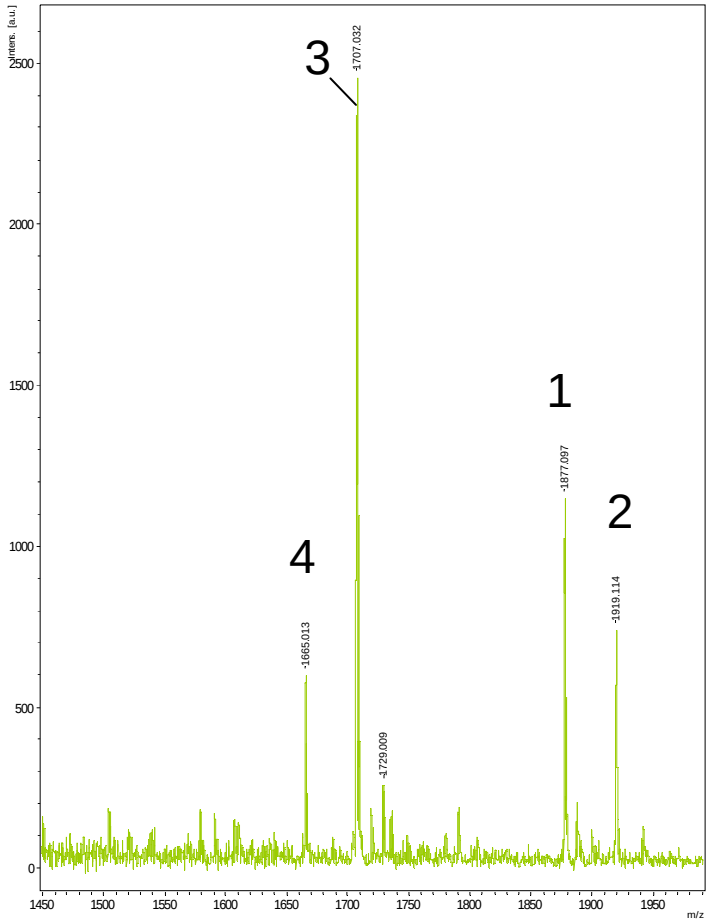
Peptide O14, H2A K5Ac, SGRGK(Ac)QGGKARAKAKSRSS

SGRG(K^{ac})QGGKARAKAKSRSS, MH^{+}= 1958.1 Da (1)

Ac-G(K^{ac})QGGKARAKAKSRSS, MH^{+}= 1699.9 Da (2)

Ac-QGGKARAKAKSRSS, MH^{+}= 1472.8 Da (3)





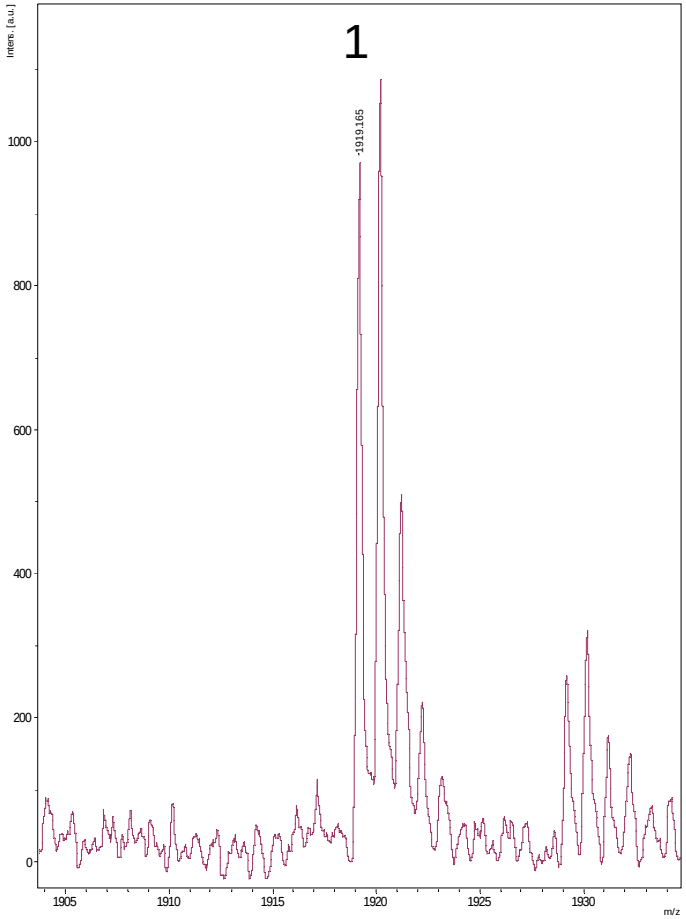
Peptide P4, H2B 1-19, PDPAKSAPAPKKGSKKAVT

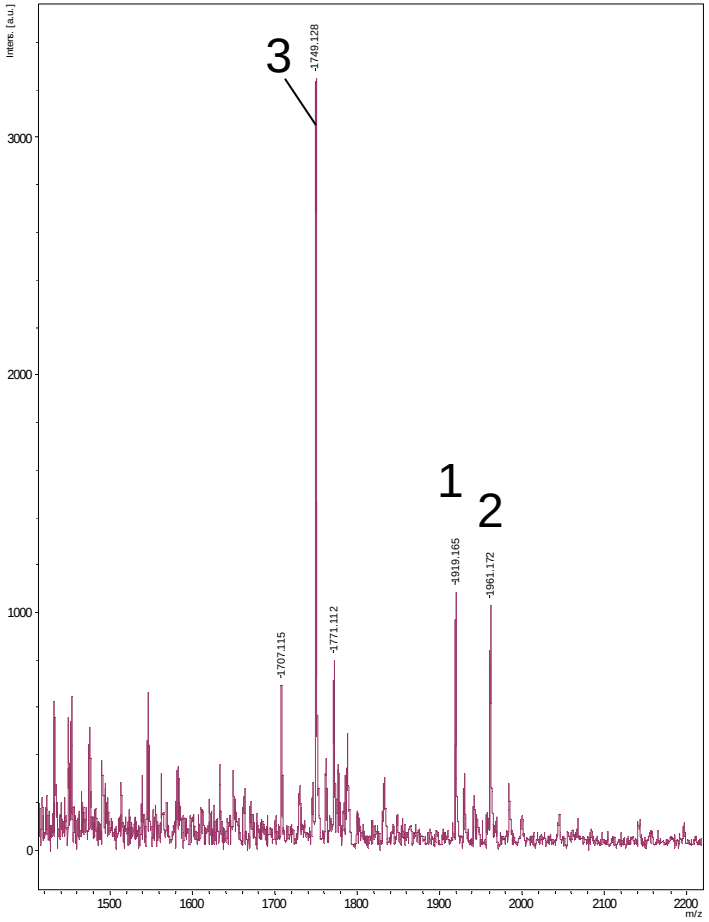
PDPAKSAPAPKKGSKKAVT, MH^+=1877.1 Da (1)

Ac-PDPAKSAPAPKKGSKKAVT, MH^+=1919.1 Da (2)

Ac-PAKSAPAPKKGSKKAVT, MH^+=1707.0 Da (3)

PAKSAPAPKKGSKKAVT, MH^+=1665.0 Da (4)





Peptide P6, H2B 1-19 K12Ac, PDPAKSAPAPK(K^{Ac})GSKKAVT

PDPAKSAPAPK(K^{ac})GSKKAVT, MH^+=1919.1 Da (1)

Ac-PDPAKSAPAPK(K^{ac})GSKKAVT, MH^+=1960.1 Da (2)

Ac-PAKSAPAPK(K^{ac})GSKKAVT, MH^+=1749.0 Da (3)

Detailed specificity analysis of antibodies binding to modified Histone tails with peptide arrays

Ina Bock[1], Arunkumar Dhayalan[1], Srikanth Kudithipudi[1], Ole Brandt[2], Philipp Rathert[3], & Albert Jeltsch[1,*]

Supplemental Figure 2: Dot blot peptide binding experiments with purified peptides and antibodies. Antibody specificities were confirmed by dot blot experiments carried out with purified (>80%) peptides containing H3K9me1 (R T K Q T A R K^{me1} S T G G K A P R K Q), H3K9me3 (R T K Q T A R K^{me3} S T G G K A P R K Q), H3K3me3/S10ph (A R T K Q T A R K^{me3} S^{ph} T G G K A P R K Q), H3K27me3 (A A R K^{me3} S A P A T G G V K C) or H4K20me3 (A K R H R K^{me3} V L R D N K C). The peptides were spotted on nitrocellulose, blocked, washed and incubated with antibodies as described for the arrays. The amounts of peptide per spot and the working dilutions of the antibodies were for antibody #11 20 pmol and 1:4000, #10 700 pmol and 1:250, #9 250 pmol and 1:120, #20 250 pmol and 1:100, #33 250 pmol, 1:2000.