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Supporting Online Material for

**Partitioning of Histone H3-H4 Tetramers During DNA Replication–  
Dependent Chromatin Assembly**

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## Materials and Methods:

### Stable cell lines

The T-Rex HeLa cells (Invitrogen) were transfected with pcDNA4/TO (Invitrogen) carrying Flag-H3.1 or Flag-H3.3. Stably transfected clones were selected against Zeocin (100 µg/ml, Invitrogen). Stable clones that display minimum leaky expression and can be effectively induced by tetracycline were chosen for further experiments.

### Cell culture and cell cycle synchronization

For “on” to “off” experiments, cells were cultured in DMEM (Sigma) containing 10% FBS and 1 µg/ml tetracycline for 3 days. Then cells were washed with PBS and transferred to medium free of tetracycline. Two days later, the cells were treated with 100 ng/ml nocodazole for 16 h to be arrested at G2/M phase. Mitotic arrested cells were shaken off, washed with 37 °C pre-warmed PBS and then resuspended in 20 ml DME/F-12 deficient medium (Sigma, D9785) containing 10% dialyzed FBS (Invitrogen), and supplemented with 0.365 mg/ml L-glutamine (Invitrogen), 0.059 mg/ml L-leucine (Sigma), 0.017 mg/ml L-methionine (Sigma) and 0.091 mg/ml [<sup>13</sup>C<sub>6</sub>, <sup>15</sup>N<sub>2</sub>] heavy isotope labelled L-lysine (Cambridge Isotope Laboratories Inc). We designated the above medium as K8 medium. Cells were plated into 10 cm dishes containing 10 ml K8 medium at a concentration of 4×10<sup>6</sup> cells per dish for 36 h releasing experiments, and 2×10<sup>6</sup> cells for 72 h releasing experiments. For 72 h releasing experiments, K8 medium was refreshed 36 h after releasing.

For “off” to “on” experiments, cells in DMEM medium containing 10% FBS were arrested by treatment with 100 ng/ml nocodazole for 16 h. Mitotic cells were shaken off,

washed with 37 °C pre-warmed PBS and resuspended into 20 ml K8 medium. Cells were plated into 10 cm dishes containing 10 ml K8 medium at a concentration of  $4 \times 10^6$  cells per dish. 8 h after releasing, 1 µg/ml tetracycline was added to induce Flag-H3 expression. Cells were harvested 36 h after releasing.

2 mM hydroxyurea (HU) or 5 µg/ml aphidicolin were used for arresting cells at S phase.

#### Mono-nucleosome preparation and affinity purification

Cells were pelleted and resuspended in lysis buffer (10 mM Tris-HCl [pH8.0], 250 mM Sucrose, 60 mM KCl, 15 mM NaCl, 5 mM MgCl, 0.5 mM DTT and 0.5% Triton X-100), and kept on ice for 10 min. Nuclei were collected by centrifugation (3000 g, 3 min). For MNase digestion, crude nuclei were resuspended in digestion buffer (10 mM Tris-HCl [pH8.0], 250 mM Sucrose, 60 mM KCl, 15 mM NaCl, 5 mM MgCl, 0.5 mM DTT and 1 mM CaCl<sub>2</sub>), and incubated at 37 °C for 80 min with MNase (TaKaRa) at 40 U/10<sup>7</sup> nuclei. Digestion was stopped by adding EDTA to a final concentration of 20 mM and chilling at 4 °C. After centrifugation (10,000 g, 5 min), nuclear pellet was resuspended in 5 mM EDTA (10 min, 4 °C). A supernatant fraction containing mono-nucleosomes generated by centrifugation (10,000 g, 10 min) was subjected to further fractionation with a 24 ml Superose 6 gel filtration column (GE) in buffer containing 10 mM Tris-HCl [pH8.0], 100 mM KCl, 0.5 mM EDTA, 1 mM DTT and 10% Glycerol. The mono-nucleosome fractions were pooled and an aliquot was reserved as “bulk histones”.

For anti-Flag affinity purification, NP-40 was added to a final concentration of 0.1%. Mono-nucleosomes were then incubated with M2 anti-Flag agarose (Sigma) for 3 hrs at 4 °C. The beads were extensively washed with buffer containing 10mM Tris-HCl [pH8.0], 500mM

KCl, 0.5mM EDTA, 1mM DTT, 10% Glycerol and 0.1% NP40. Affinity purified mono-nucleosomes were then eluted with 1 mg/ml Flag peptide.

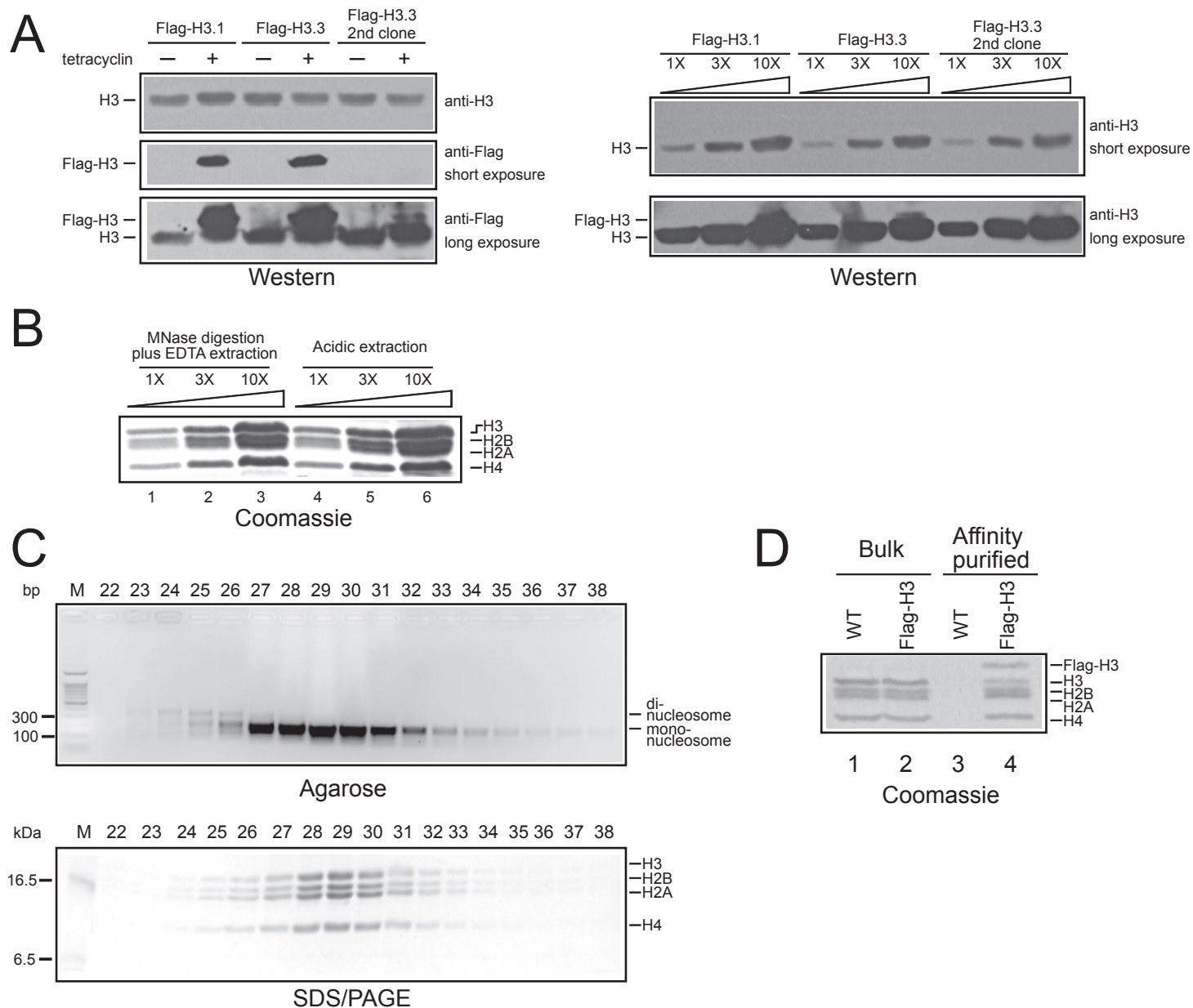
#### Mass spectrometry analysis

Individual histone bands were digested in gel with sequencing grade trypsin (10 ng/ $\mu$ L trypsin, 50 mM ammonium bicarbonate, pH8.0) overnight at 37 °C. Peptides were extracted with 5% acetic acid/50% acetonitrile and 0.1% acetic acid/75% acetonitrile sequentially and then concentrated to ~20  $\mu$ L. The extracted peptides were separated by a homemade analytical capillary column (50  $\mu$ m  $\times$  10 cm) packed with C18 reverse phase material (YMC 5  $\mu$ m spherical particles). An Agilent 1100 series binary pump was used to generate HPLC gradient as follows: 0%-5% B in 5 min, 5%-40% B in 25 min, 40%-100% B in 15 min (A = 0.1 M acetic acid in water, B = 0.1 M acetic acid/80% methanol). The eluted peptides were sprayed into a QSTAR XL mass spectrometer (MDS SCIEX) equipped with a nano-ESI ion source. The mass spectrometer was operated in Information Dependent Acquisition (IDA) mode. Ion spray voltage was 2.1 KV. The MS scan was from m/z 300 to 2000. From each MS scan, top three most abundant peaks were selected for MS/MS scans. Each scan was accumulated for 0.5 sec. The dynamic exclusion time was set as 20 sec.

#### Protein database search and peptide quantification

The mass spectra were searched against an customized histone database (All histone sequences were from IPI-human database) on an in-house Mascot server (Version 2.2, Matrix Science Ltd.). Carbamidomethyl on cysteine was set as fixed modification. Variable modifications included oxidation on methionine, acetylation, mono-, di-, or tri-methylation on unlabelled lysine residue and [ $^{13}\text{C}_6$ ,  $^{15}\text{N}_2$ ] heavy isotope labelled lysine residue. A maximum

of three miscleavages was allowed. Mass tolerance was 1.2 Da for precursor ion, 0.6 Da for fragment ions. Mascot search results were imported to the open source software MSQuant (<http://msquant.sourceforge.net>) to calculate ratios of the heavy/light peptide pairs. All quantification results were manually checked to ensure their correctness.



## Figure S1

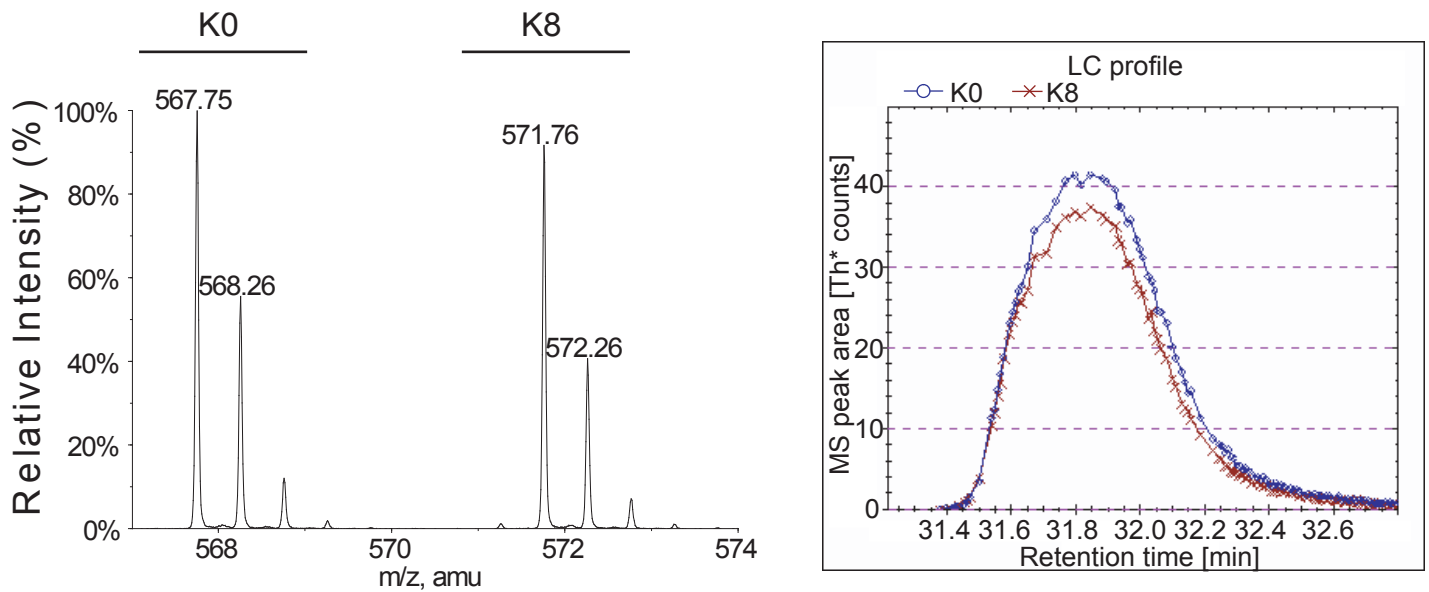
(A) Expression levels of the Flag-H3.1 and Flag-H3.3 stable clones. Left panel, Flag-H3.1 and Flag-H3.3 cells displayed comparable expression levels of Flag-H3; the Flag-H3.3 2nd clone displayed at least 5 fold lower expression of Flag-H3, which can only be visualized with a much longer exposure (Under this condition, the untagged native H3 could also be non-specifically detected due to their overwhelming amounts in comparison to the Flag-H3). Right panel, comparison of expression levels of Flag-H3 histones and native histone H3. Flag-H3 histones were not detected with a short exposure (Upper panel) even in the lanes loaded with 10 fold amounts of histone samples; Flag-H3 histones were detected by anti-H3 in Flag-H3.1 and Flag-H3.3 stable cells with a longer exposure (lower panel), the amounts of expression were estimated to be 1-3% of total histone H3. In the Flag-H3.3 2nd clone, Flag-H3.3 expression was not detected by anti-H3 even with the longer exposure, indicating a much lower expression level.

(B) MNase digestion plus EDTA extraction (lane 1-3) and acidic extraction (lane 4-6) recover comparable amounts of histones. Same amount of HeLa cells were used for extraction. Histones were adjusted to the same final volume (start with  $3 \times 10^7$  cells, final volume 300  $\mu$ l). 1X, 3X, 10X represent 1.5 $\mu$ l, 4.5 $\mu$ l, 15 $\mu$ l respectively.

(C) Purified mono-nucleosomes.

(D) Affinity purified Flag-H3 containing mono-nucleosomes.

# DAVYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=46.4%



## Explanatory illustration for comparative quantification of SILAC based mass spectrometry

A K0 peptide and K8 peptide with identical amino acid sequence were co-eluted from the reverse-phased HPLC column, got ionized on the mass spectrometer and displayed in the MS spectrum as a peptide pair with defined mass difference of 8 Da (left panel). In the LC-MS/MS run, the mass spectrometer cycled between a MS scan and three MS2 scans in every 2 sec, thus a number of MS spectra were obtained for a given peptide across its LC profile (LC peak width varied from 60 sec to 120 sec in general). Peak areas of the K0/K8 peptide pair in each MS spectrum were plotted against the elution time to generate the extracted ion chromatograms (XICs) (right panel). The individual peak areas of the K0 and K8 pair from all commonly available MS spectra were summed to be the relative peptide abundance. Ratio of the K0 and K8 relative peptide abundance was calculated, which represents the abundance ratio of the histone protein from which the K0/K8 peptides were derived.

Fig. S2

# Flag-H3.1, "on" to "off", 36 h, 72 h

**A**

	K8/(K8+K0) X 100%			
	36 h		72 h	
	Bulk	Affinity purified	Bulk	Affinity purified
H3	49%		78%	
Flag-H3.1		1.0%		3.8%
H3.1		3.0%		6.3%
H4	47%	3.4%	79%	5.9%
H2B	47%	42%	83%	79%
H2A	50%	45%	83%	82%

Value for each histone is the mean value of all quantified peptides

**B**

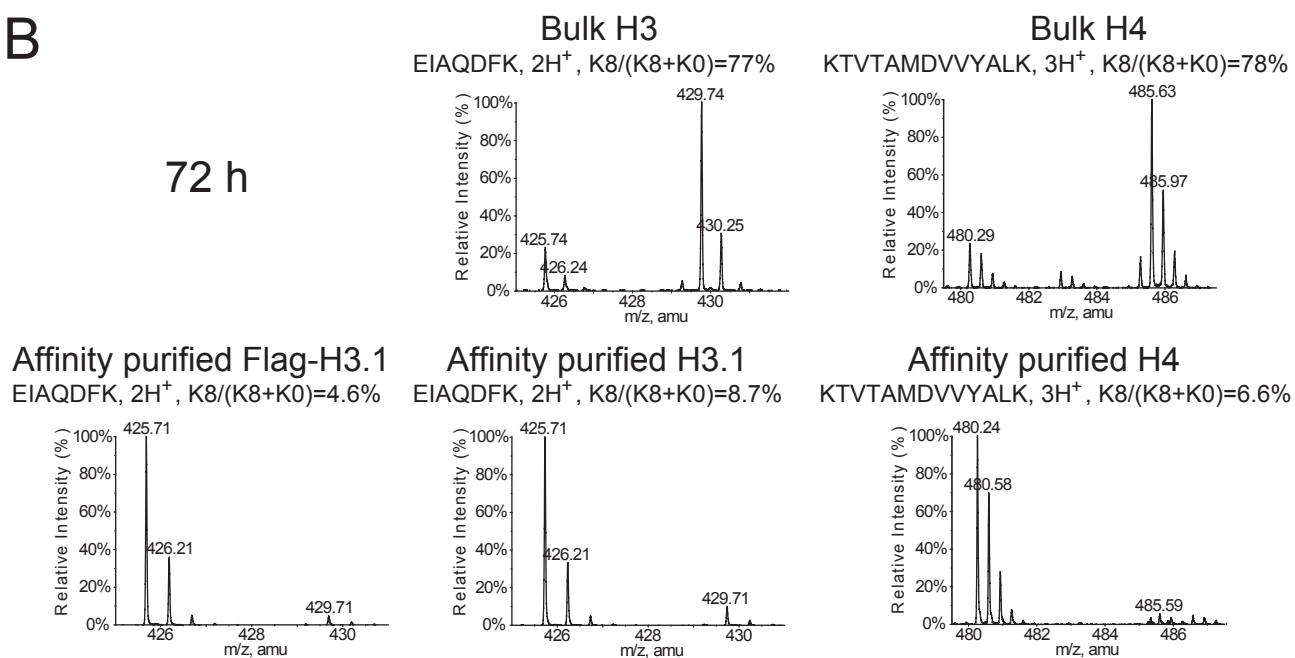


Figure S3, Page 1



## Flag-H3.1, “on” to “off”, 36 h

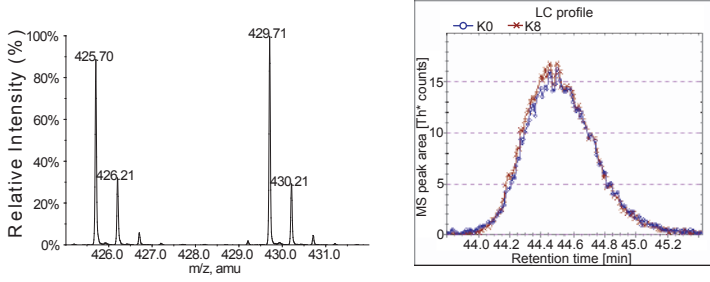
Histone	Sequence	charge	K8/(K8+K0)	average	StDV
Bulk H3	EIAQDFK	2H <sup>+</sup>	51.1%	49.0%	1.7%
	KLPFQR	2H <sup>+</sup>	48.3%		
	RVTIMPK	2H <sup>+</sup>	47.1%		
	VTIMPK	2H <sup>+</sup>	49.3%		
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	47.9%	46.9%	0.9%
	LLLPGELAK	2H <sup>+</sup>	46.2%		
	QVHPDTGISSK	2H <sup>+</sup>	46.6%		
Bulk H2A	NDEELNKLLGK	3H <sup>+</sup>	50.4%	49.7%	0.7%
	VTIAQGGVLPNIQAVLLPK	2H <sup>+</sup>	49.1%		
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	49.5%		
Bulk H4	DAVITYTEHAK	2H <sup>+</sup>	46.4%	46.5%	1.0%
	DNIQGITKPAIR	2H <sup>+</sup>	44.8%		
	DNIQGITKPAIR	3H <sup>+</sup>	47.4%		
	KTVTAMDVVYALK	3H <sup>+</sup>	45.8%		
	KTVTAMDVVYALKR	3H <sup>+</sup>	46.8%		
	TVTAMDVVYALKR	3H <sup>+</sup>	47.5%		
Affinity purified FLAG-H3.1	EIAQDFK	2H <sup>+</sup>	1.3%	1.0%	0.3%
	KLPFQR	2H <sup>+</sup>	0.7%		
	MDYK(Ac)DDDDKAR	3H <sup>+</sup>	1.5%		
	RVTIMPK	2H <sup>+</sup>	0.8%		
	VTIMPK	2H <sup>+</sup>	0.9%		
Affinity purified H3.1	EIAQDFK	2H <sup>+</sup>	3.3%	3.0%	0.3%
	KLPFQR	2H <sup>+</sup>	2.9%		
	RVTIMPK	2H <sup>+</sup>	2.6%		
	VTIMPK	2H <sup>+</sup>	3.3%		
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	42.4%	41.9%	1.7%
	LLLPGELAK	2H <sup>+</sup>	43.3%		
	QVHPDTGISSK	2H <sup>+</sup>	40.0%		
Affinity purified H2A	NDEELNKLLGK	3H <sup>+</sup>	46.3%	45.2%	1.6%
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	44.1%		
Affinity purified H4	DAVITYTEHAK	2H <sup>+</sup>	2.1%	3.4%	0.9%
	DNIQGITKPAIR	3H <sup>+</sup>	4.2%		
	KTVTAMDVVYALKR	3H <sup>+</sup>	3.9%		
	TVTAMDVVYALKR	3H <sup>+</sup>	3.3%		

Fig. S3 Page 2

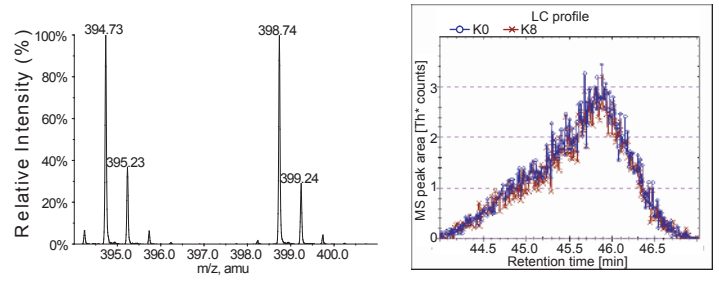
# Flag-H3.1, "on" to "off", 36 h

## Bulk H3

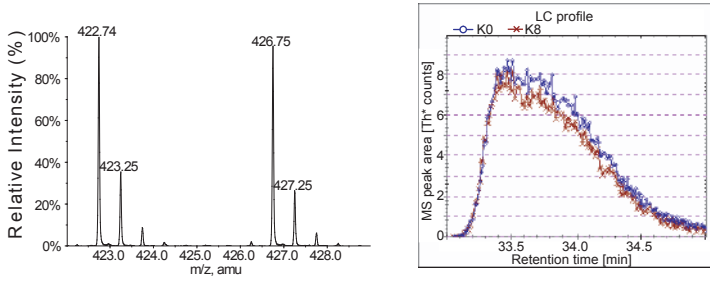
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=51%



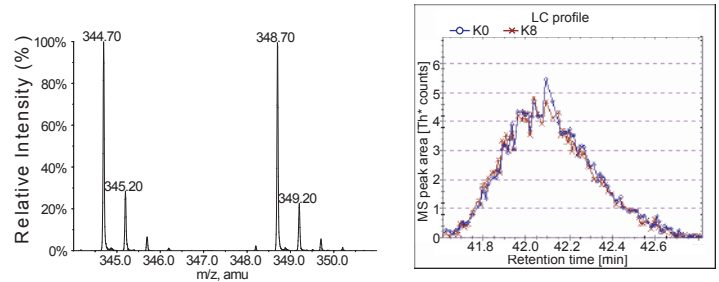
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=48%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=47%

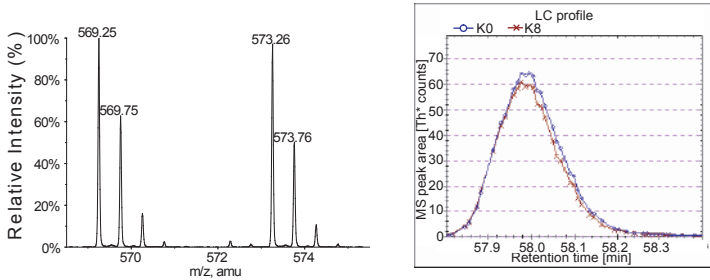


VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=49%

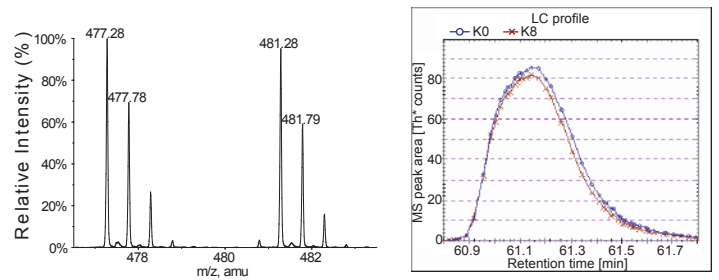


## Bulk H2B

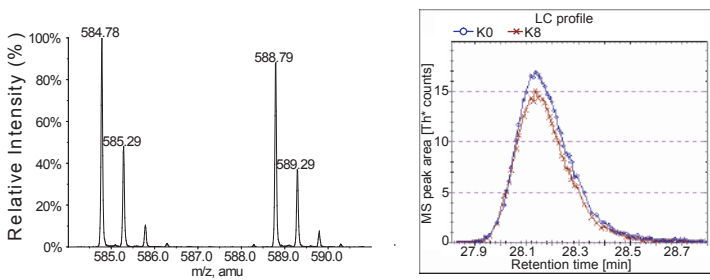
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=48%



LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=46%



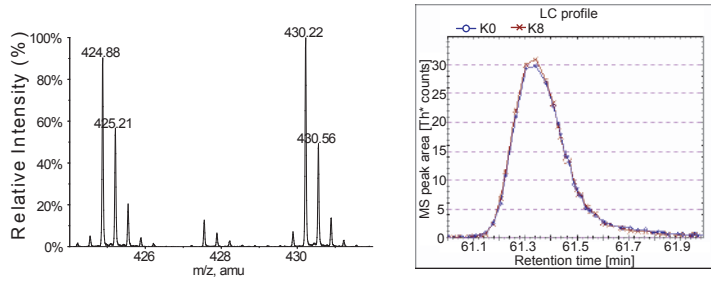
QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=47%



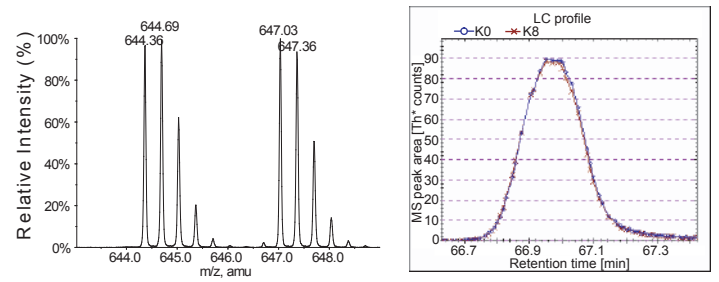
# Flag-H3.1, "on" to "off", 36 h

## Bulk H2A

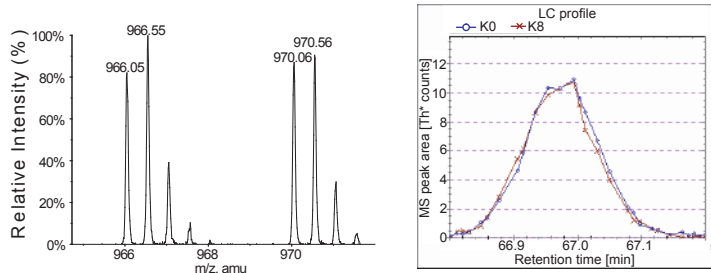
NDEELNKLK, 3H<sup>+</sup>, K8/(K8+K0)=50%



VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=50%

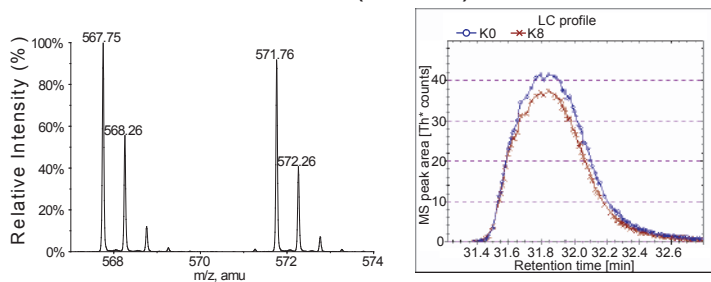


VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=49%

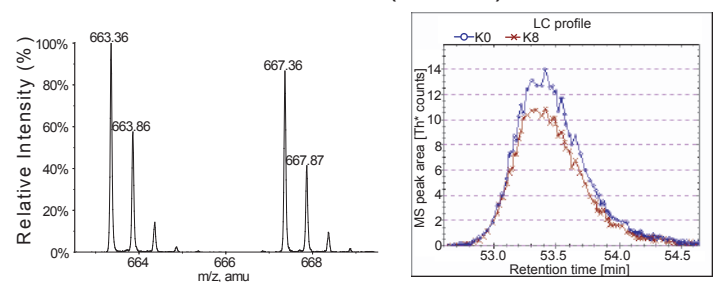


## Bulk H4

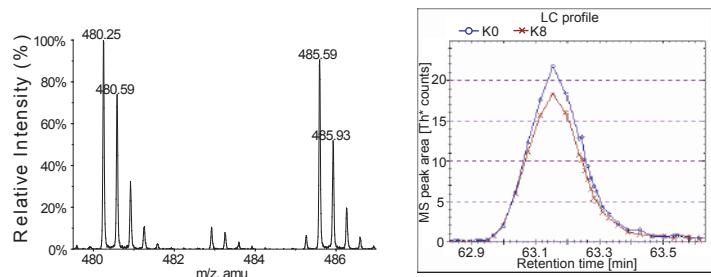
DAVYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=46%



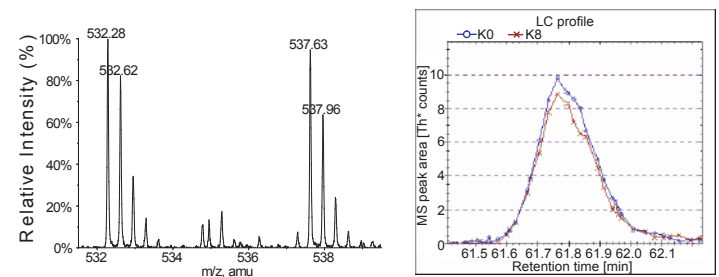
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=45%



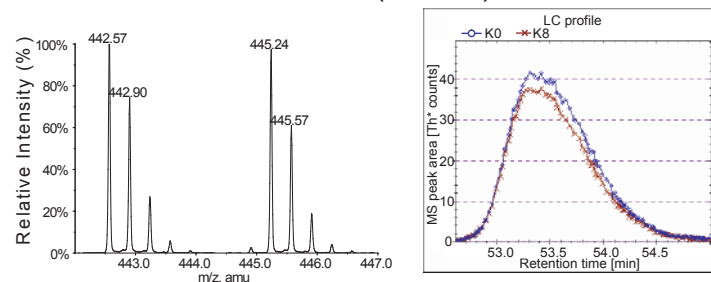
KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=46%



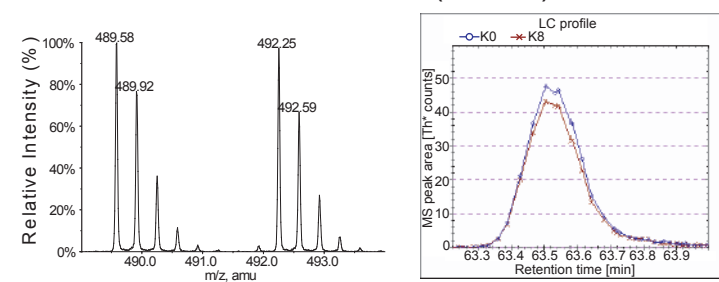
KTVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=47%



DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=47%



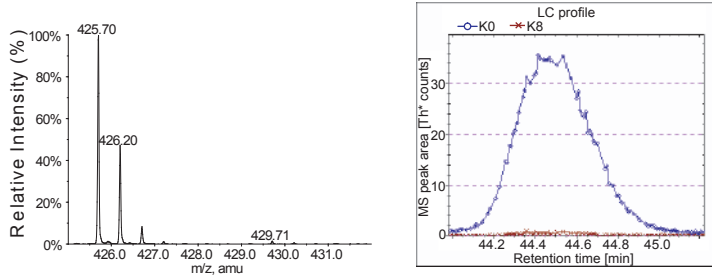
TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=48%



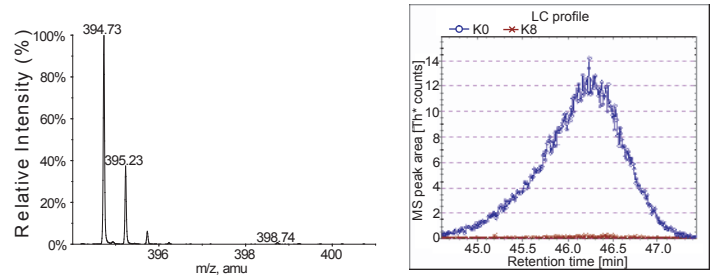
# Flag-H3.1, "on" to "off", 36 h

## Affinity purified Flag-H3.1

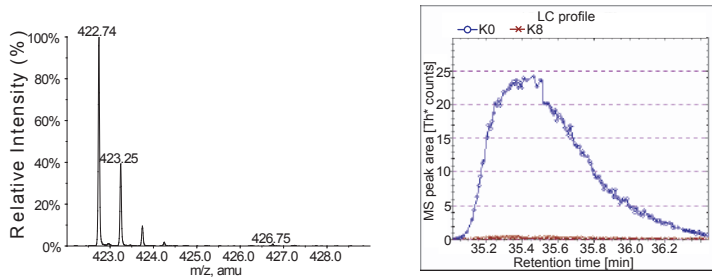
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=1.3%



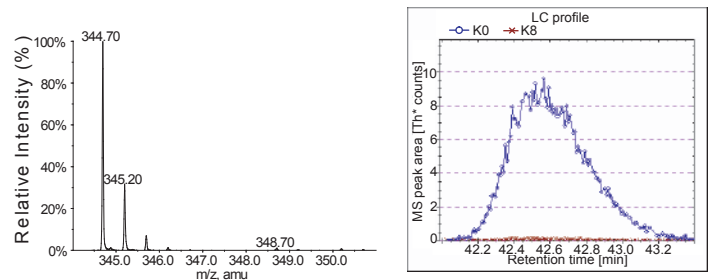
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=0.7%



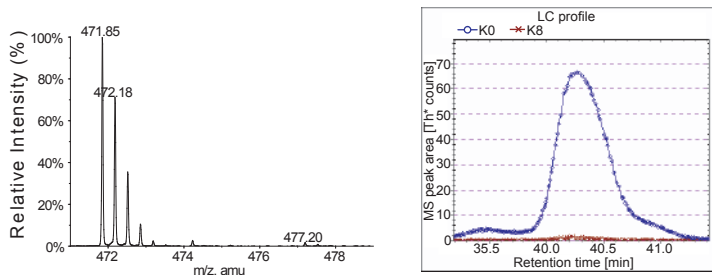
RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=0.8%



VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=0.9%

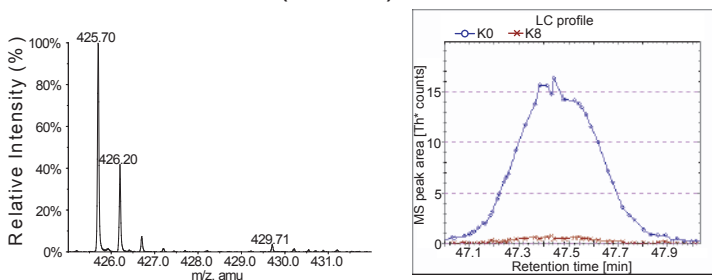


MDYK(Ac)DDDDKAR (FLAG),  
3H<sup>+</sup>, K8/(K8+K0)=1.5%

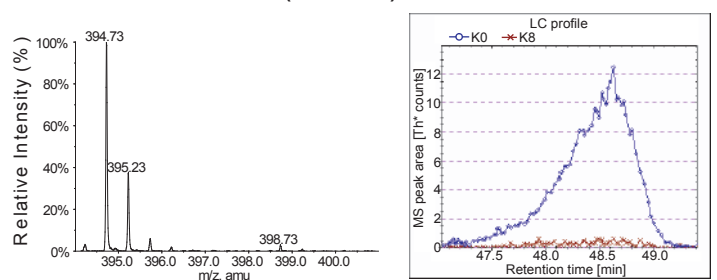


## Affinity purified H3.1

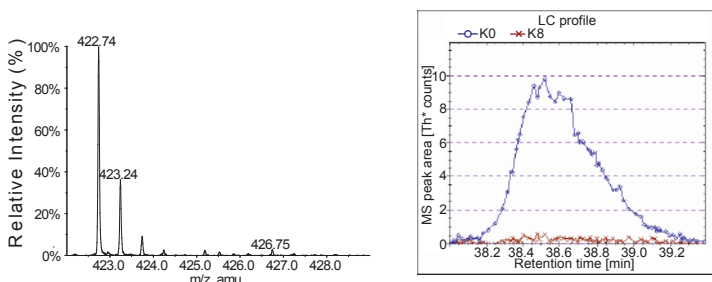
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=3.3%



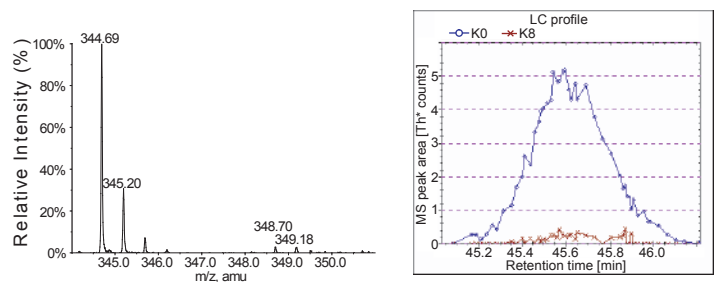
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=2.9%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=2.6%



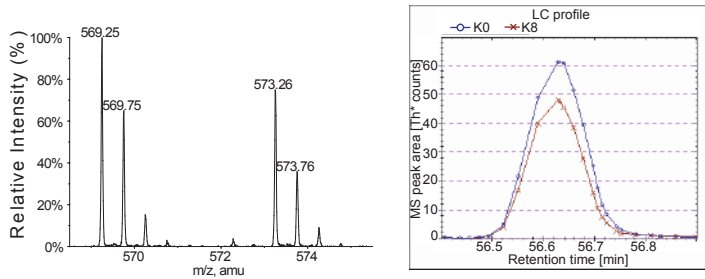
VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=3.3%



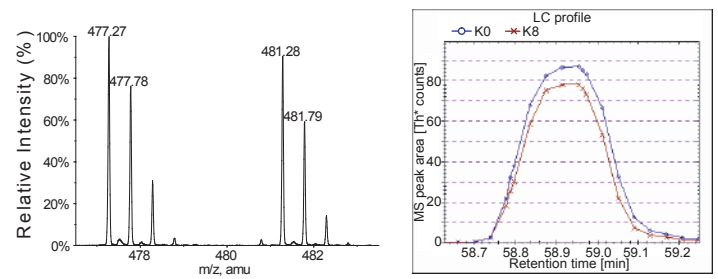
# Flag-H3.1, "on" to "off", 36 h

## Affinity purified H2B

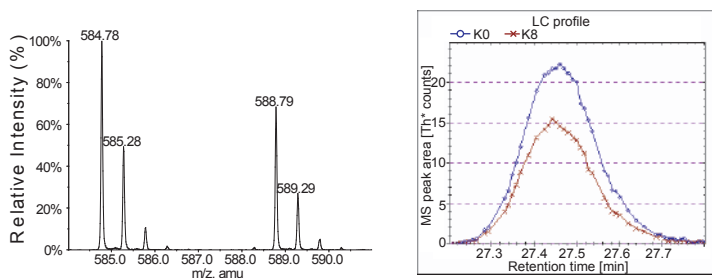
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=42%



LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=43%

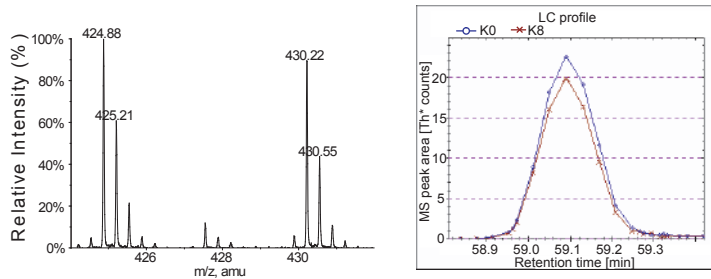


QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=40%

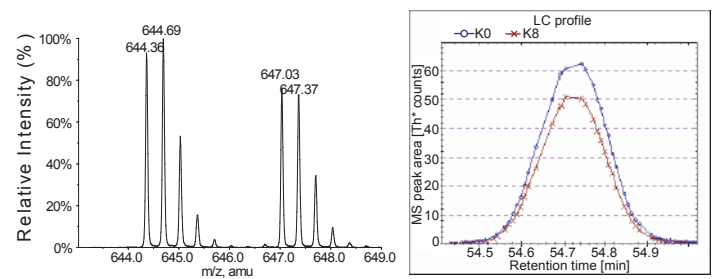


## Affinity purified H2A

NDEELNKLKLGK, 3H<sup>+</sup>, K8/(K8+K0)=46%

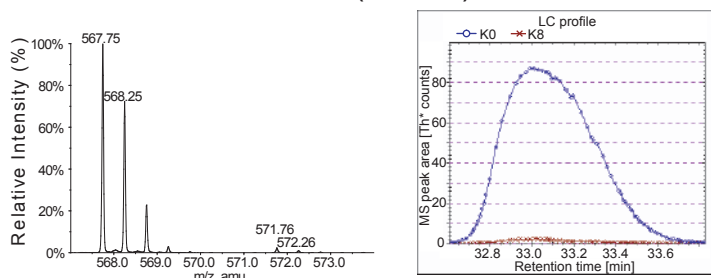


VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=44%

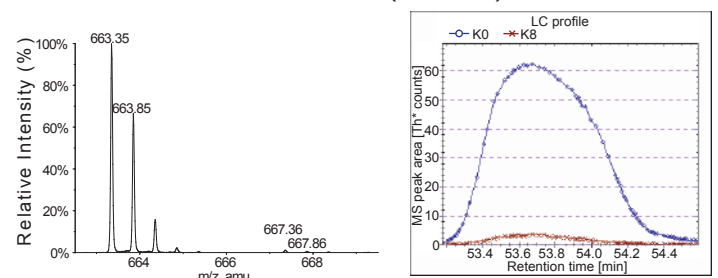


## Affinity purified H4

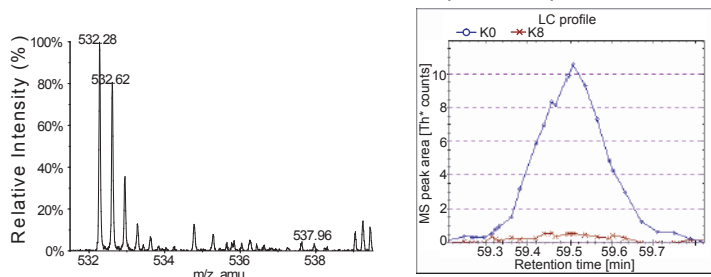
DAVYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=2.1%



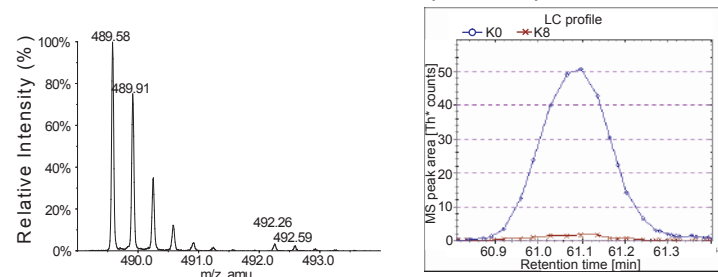
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=4.2%



KTVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=3.9%



TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=3.3%



## Flag-H3.1, “on” to “off”, 72 h

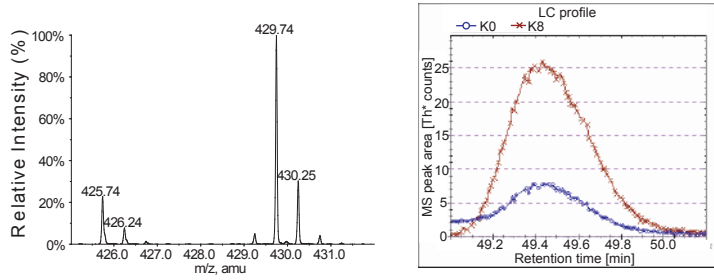
Histone	Sequence	charge	K8/(K8+K0)	average	StDV
Bulk H3	EIAQDFK	2H <sup>+</sup>	76.7%	78.4%	1.2%
	KLPFQR	2H <sup>+</sup>	79.4%		
	RVTIMPK	2H <sup>+</sup>	79.2%		
	VTIMPK	2H <sup>+</sup>	78.4%		
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	82.1%	82.7%	0.8%
	KESYSVYVYK	2H <sup>+</sup>	83.6%		
	QVHPDTGISSK	2H <sup>+</sup>	82.4%		
Bulk H2A	NDEELNKLLGK	3H <sup>+</sup>	82.7%	82.7%	0.1%
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	82.6%		
Bulk H4	DAVYTEHAK	2H <sup>+</sup>	81.7%	79.4%	3.0%
	DAVYTEHAK	3H <sup>+</sup>	74.8%		
	DNIQGITKPAIR	2H <sup>+</sup>	82.7%		
	KTVTAMDVVYALK	3H <sup>+</sup>	78.3%		
	TVTAMDVVYALK	2H <sup>+</sup>	81.4%		
	TVTAMDVVYALKR	3H <sup>+</sup>	77.7%		
Affinity purified FLAG-H3.1	EIAQDFK	2H <sup>+</sup>	4.6%	3.8%	1.3%
	KLPFQR	2H <sup>+</sup>	2.4%		
	MDYK(Ac)DDDDKAR	3H <sup>+</sup>	2.9%		
	VTIMPK	2H <sup>+</sup>	5.2%		
Affinity purified H3.1	EIAQDFK	2H <sup>+</sup>	8.7%	6.3%	2.1%
	KLPFQR	2H <sup>+</sup>	5.7%		
	RVTIMPK	2H <sup>+</sup>	4.6%		
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	77.4%	78.7%	1.8%
	QVHPDTGISSK	2H <sup>+</sup>	79.9%		
Affinity purified H2A	NDEELNKLLGK	3H <sup>+</sup>	78.6%	81.9%	4.6%
	VTIAQGGVLPNIQAVLLPK	2H <sup>+</sup>	85.1%		
Affinity purified H4	DAVYTEHAK	2H <sup>+</sup>	3.5%	5.9%	2.4%
	DNIQGITKPAIR	2H <sup>+</sup>	3.4%		
	TVTAMDVVYALK	2H <sup>+</sup>	4.6%		
	TVTAMDVVYALKR	3H <sup>+</sup>	8.3%		
	DAVYTEHAK	3H <sup>+</sup>	8.9%		
	KTVTAMDVVYALK	3H <sup>+</sup>	6.6%		

Fig. S3 Page 7

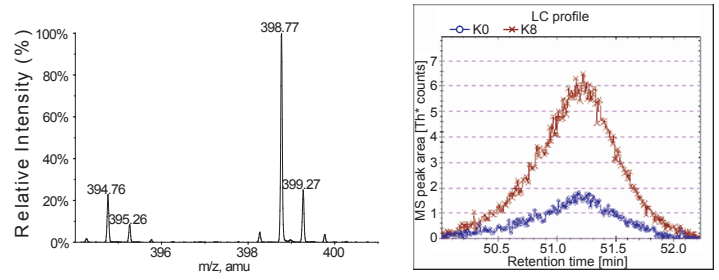
# Flag-H3.1, "on" to "off", 72 h

## Bulk H3

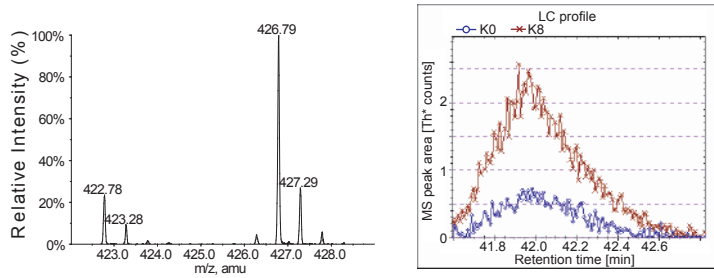
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=77%



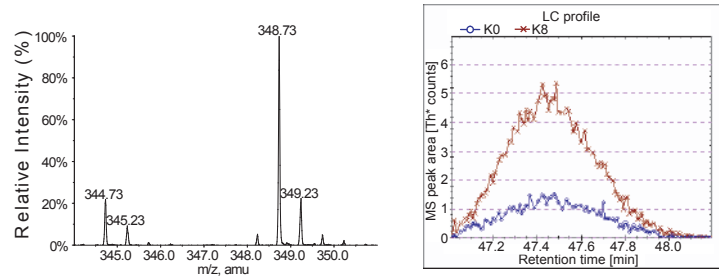
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=79%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=79%

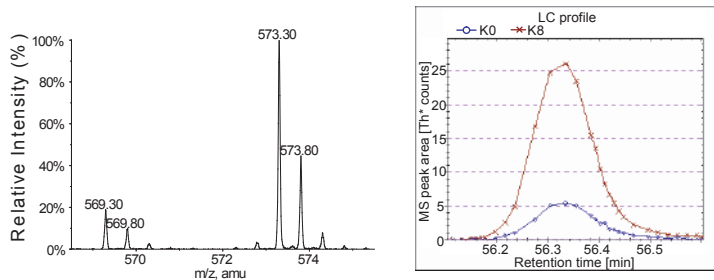


VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=78%

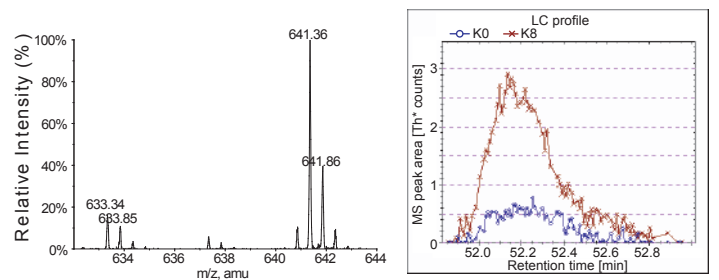


## Bulk H2B

ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=82%



KESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=84%



QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=82%

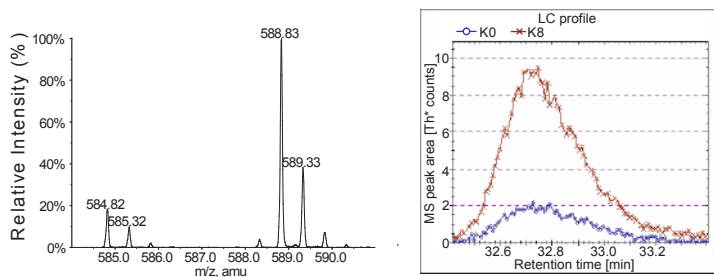
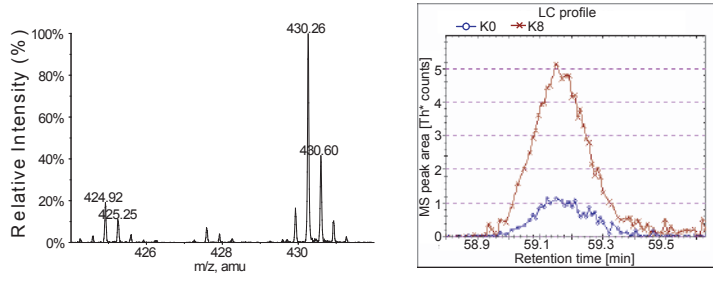


Fig. S3 Page 8

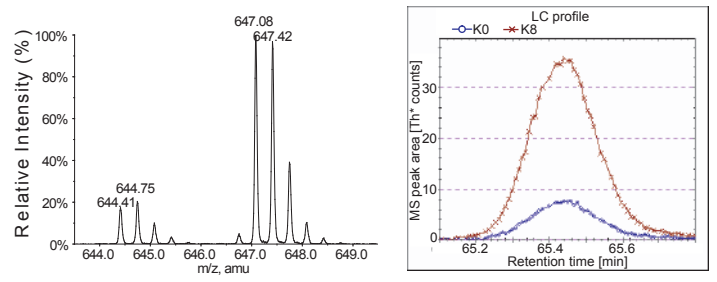
# Flag-H3.1, "on" to "off", 72 h

## Bulk H2A

NDEELNKLK, 3H<sup>+</sup>, K8/(K8+K0)=83%

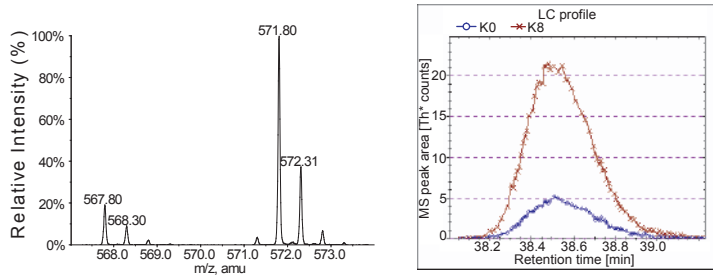


VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=83%

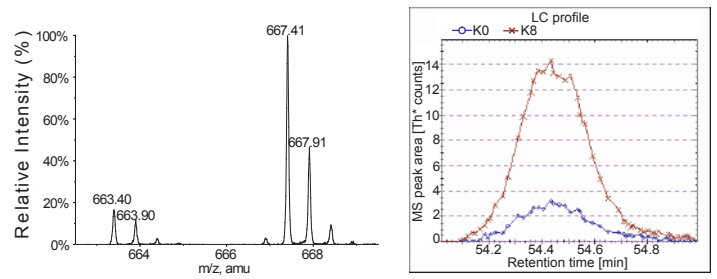


## Bulk H4

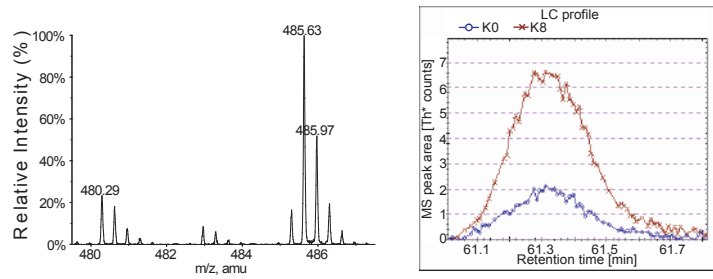
DAVTYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=82%



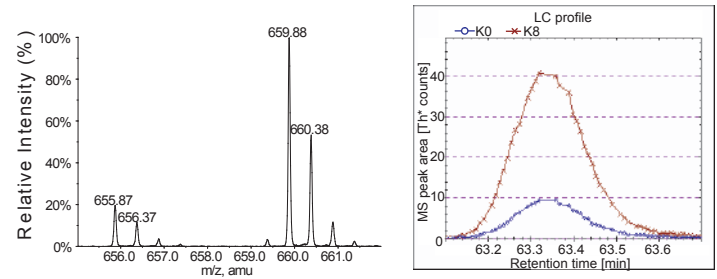
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=83%



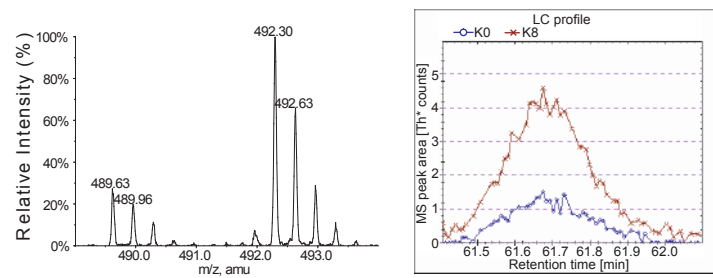
KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=78%



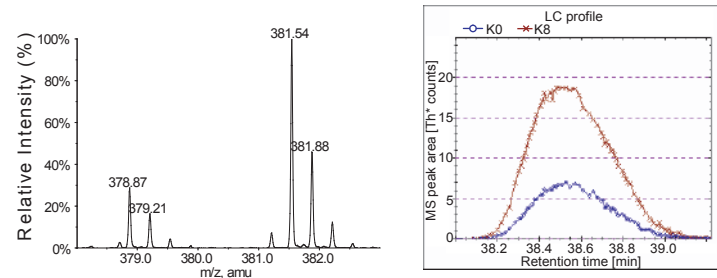
TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=81%



TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=77%



DAVTYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=75%

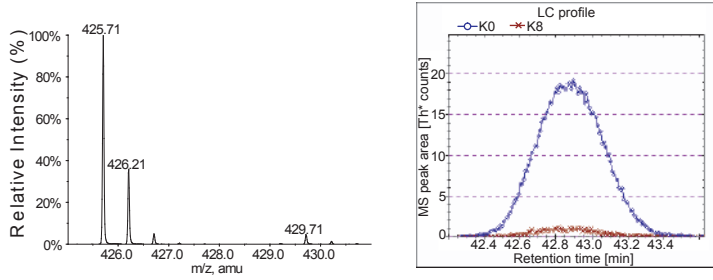




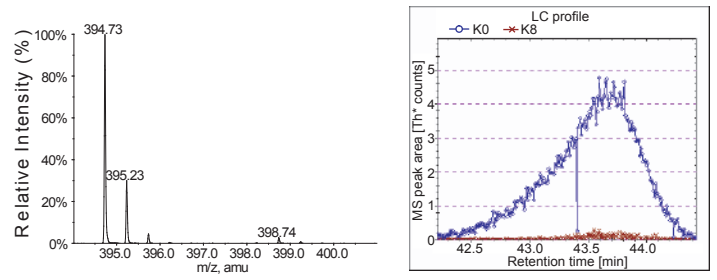
# Flag-H3.1, "on" to "off", 72 h

## Affinity purified Flag-H3.1

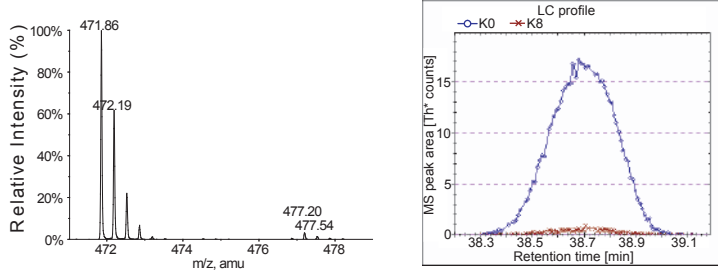
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=4.6%



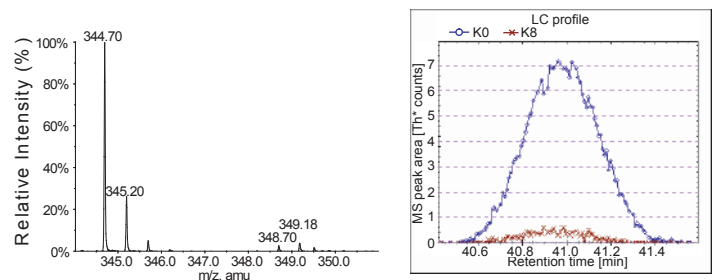
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=2.4%



MDYK(Ac)DDDDKAR (FLAG),  
3H<sup>+</sup>, K8/(K8+K0)=2.9%

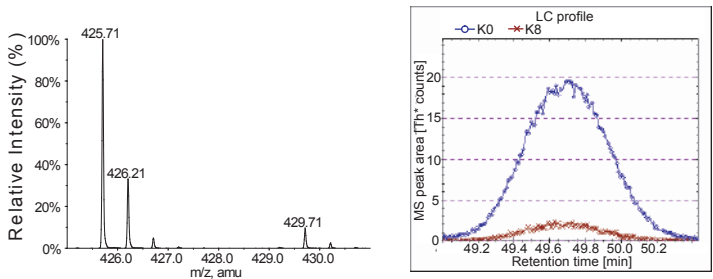


VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=5.2%

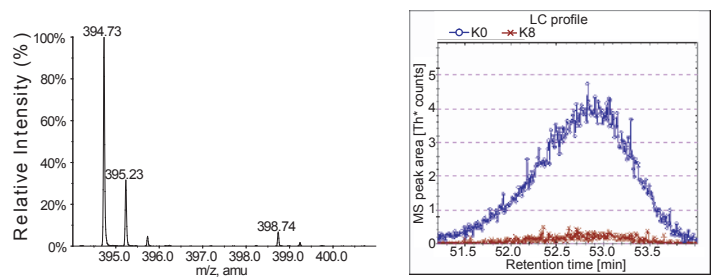


## Affinity purified H3.1

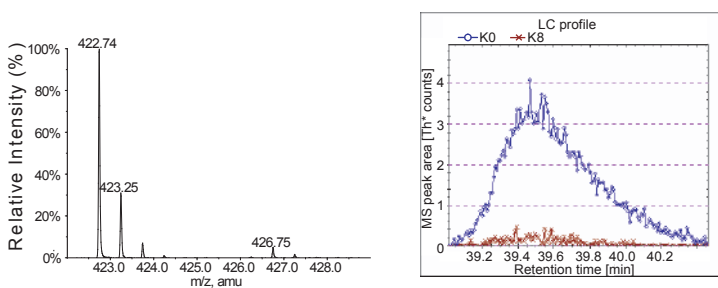
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=8.7%



KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=5.7%



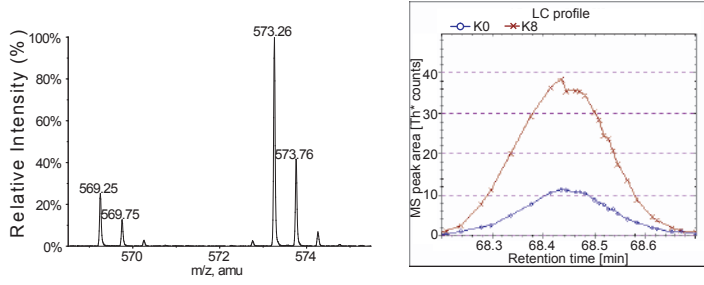
RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=4.6%



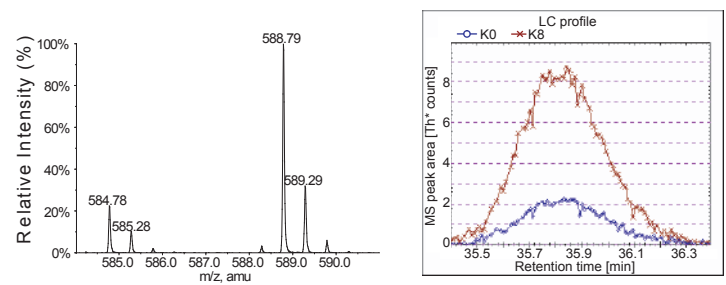
# Flag-H3.1, "on" to "off", 72 h

## Affinity purified H2B

ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=77%

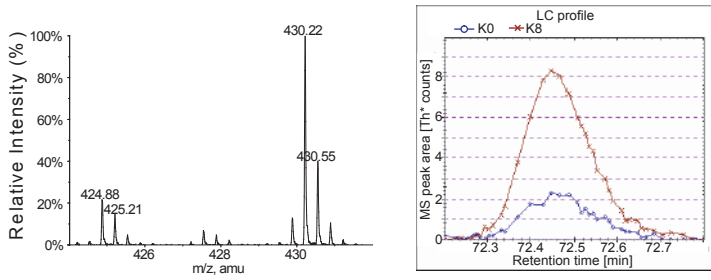


QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=80%

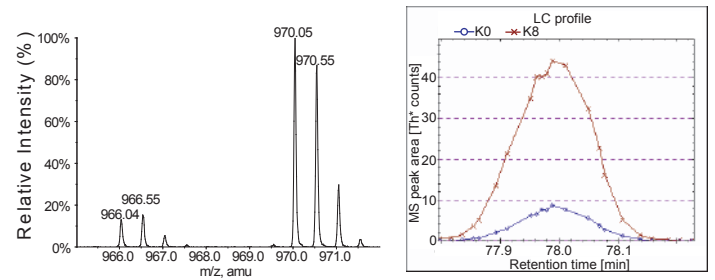


## Affinity purified H2A

NDEELNKLK, 3H<sup>+</sup>, K8/(K8+K0)=79%

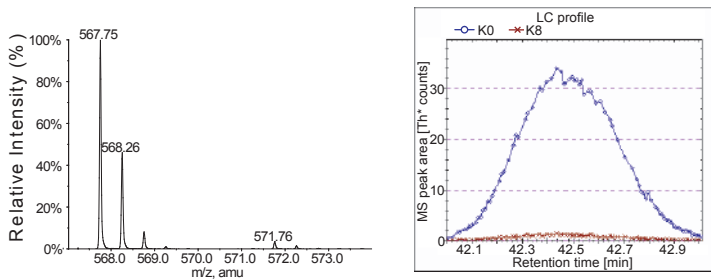


VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=85%

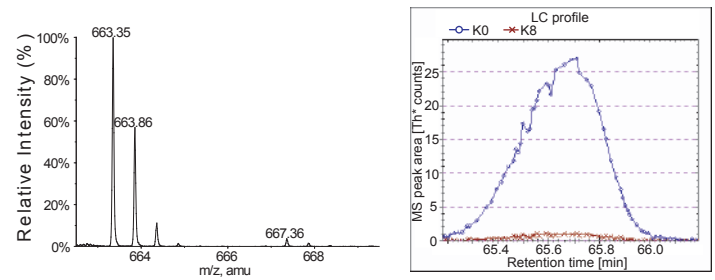


## Affinity purified H4

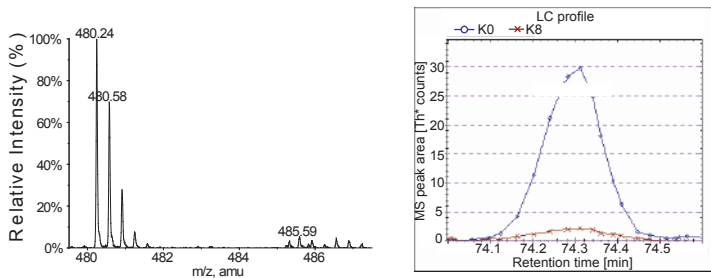
DAVYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=3.5%



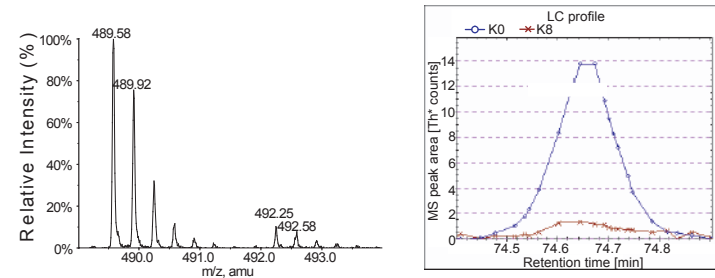
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=3.4%



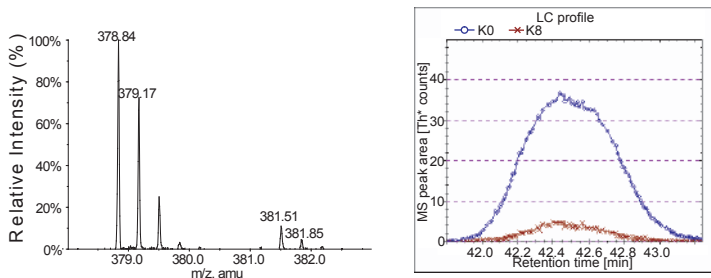
KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=6.6%



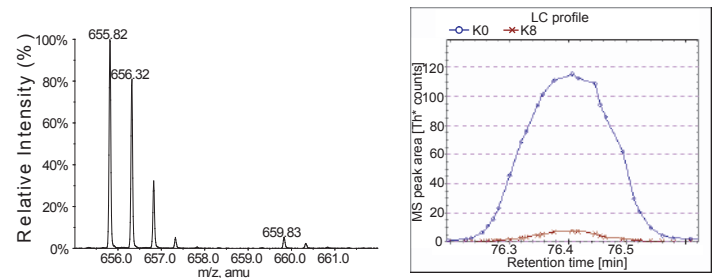
TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=8.3%



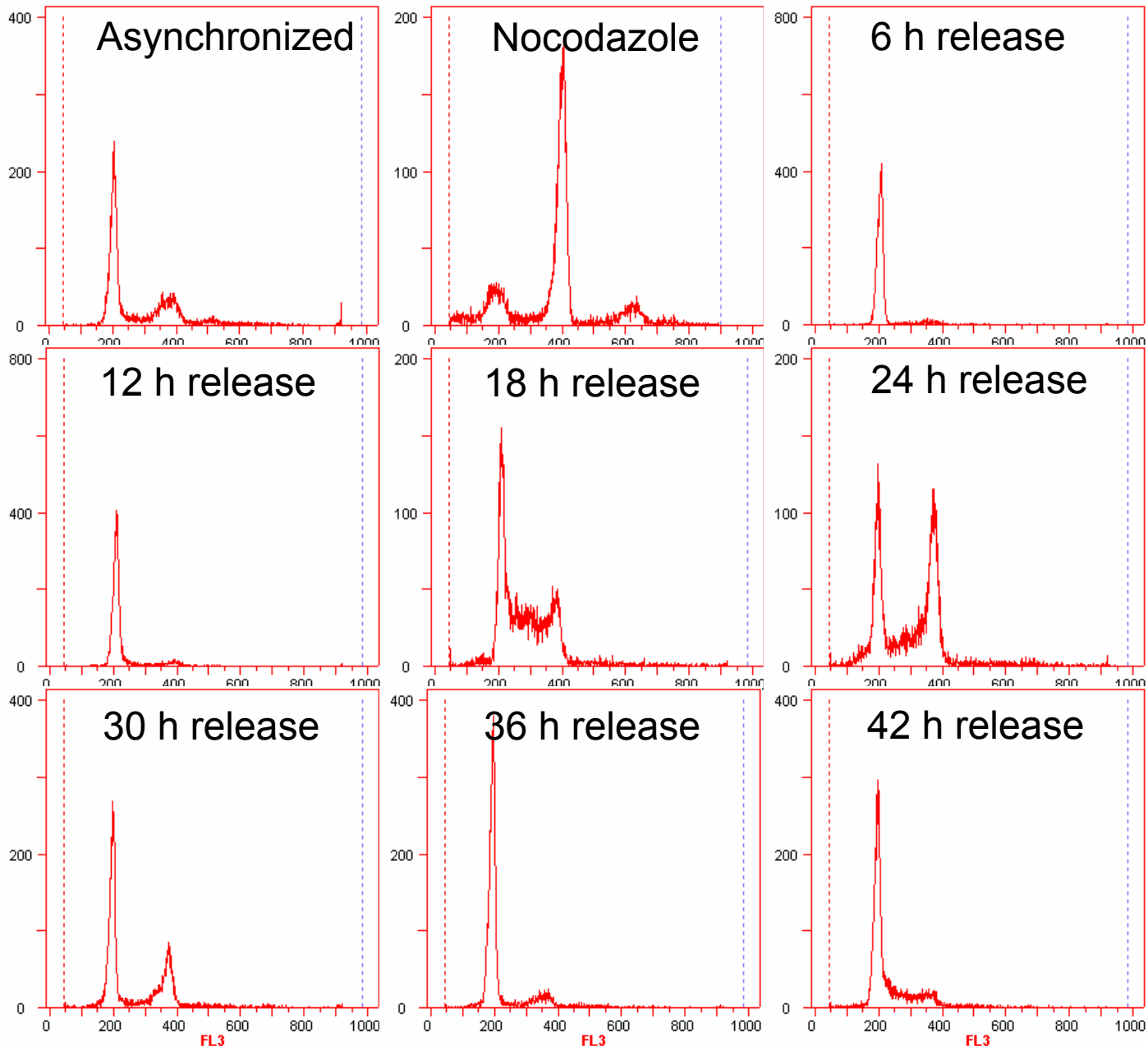
DAVYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=8.9%



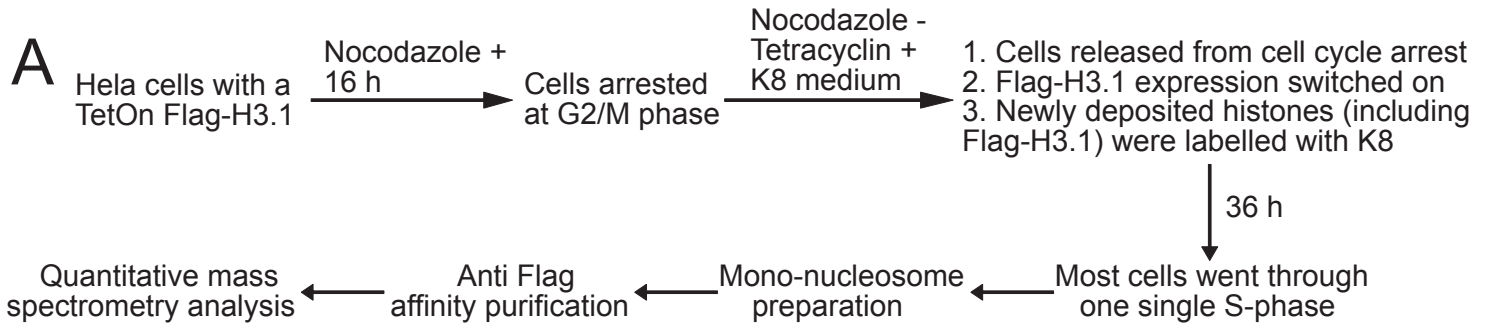
TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=4.6%



# Flag- H3.1 “on” to “off”



# Flag-H3.1, “off” to “on”, 36 h



**B**

	K8/(K8+K0) X 100%	
	Bulk	Affinity purified
H3	49%	
Flag-H3.1		93%
H3.1		90%
H4	48%	92%
H2B	48%	53%
H2A	51%	55%

Value for each histone is the mean value of all quantified peptides

**C**

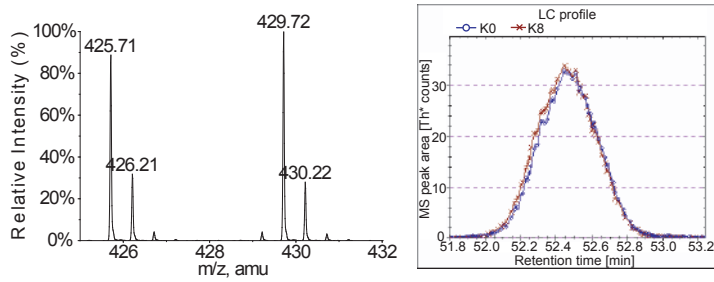
Histone	Sequence	charge	K8/(K8+K0)	average	StDV	
Bulk H3	EIAQDFK	2H <sup>+</sup>	50.9%	49.2%	1.7%	
	KLPFQR	2H <sup>+</sup>	47.6%			
	RVTIMPK	2H <sup>+</sup>	47.8%			
	VTIMPK	2H <sup>+</sup>	51.1%			
	VTIMPK (M Oxidation)	2H <sup>+</sup>	48.8%			
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	48.9%	47.8%	1.1%	
	LLLPGELAK	2H <sup>+</sup>	46.7%			
	QVHPDTGISSK	2H <sup>+</sup>	47.8%			
Bulk H2A	NDEELNKLGGK	3H <sup>+</sup>	51.8%	51.4%	0.6%	
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	51.0%			
Bulk H4	DAVTYTEHAK	2H <sup>+</sup>	47.8%	47.5%	1.0%	
	DNIQGITKPAIR	2H <sup>+</sup>	46.6%			
	KTVTAMDVVYALK	3H <sup>+</sup>	46.9%			
	TVTAMDVVYALK	2H <sup>+</sup>	46.7%			
	TVTAMDVVYALK					
	(M Oxidation)	2H <sup>+</sup>	47.9%			
Affinity purified FLAG-H3.1	EIAQDFK	2H <sup>+</sup>	94.0%	93.3%	1.0%	
	KLPFQR	2H <sup>+</sup>	93.4%			
	RVTIMPK	2H <sup>+</sup>	93.8%			
	VTIMPK (M Oxidation)	2H <sup>+</sup>	91.9%			
Affinity purified H3.1	EIAQDFK	2H <sup>+</sup>	90.4%	90.4%	1.1%	
	KLPFQR	2H <sup>+</sup>	90.6%			
	RVTIMPK	2H <sup>+</sup>	91.6%			
	VTIMPK	2H <sup>+</sup>	90.9%			
	VTIMPK (M Oxidation)	2H <sup>+</sup>	88.6%			
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	54.1%	52.8%	1.2%	
	LLLPGELAK	2H <sup>+</sup>	51.7%			
	QVHPDTGISSK	3H <sup>+</sup>	52.6%			
Affinity purified H2A	NDEELNKLGGK	3H <sup>+</sup>	56.4%	55.1%	1.9%	
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	53.7%			
Affinity purified H4	DAVTYTEHAK	2H <sup>+</sup>	90.2%	91.5%	1.2%	
	DNIQGITKPAIR	2H <sup>+</sup>	92.9%			
	KTVTAMDVVYALK	2H <sup>+</sup>	90.8%			
	TVTAMDVVYALK					
	(M Oxidation)	2H <sup>+</sup>	92.0%			

Fig. S4 page 1

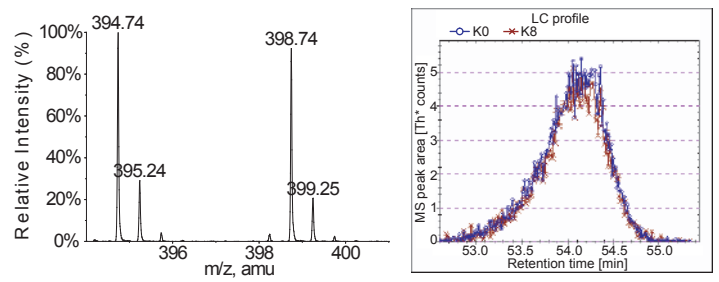
# Flag-H3.1, "off" to "on", 36 h

## Bulk H3

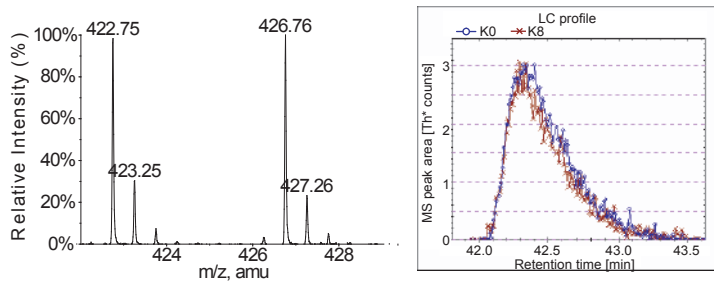
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=51%



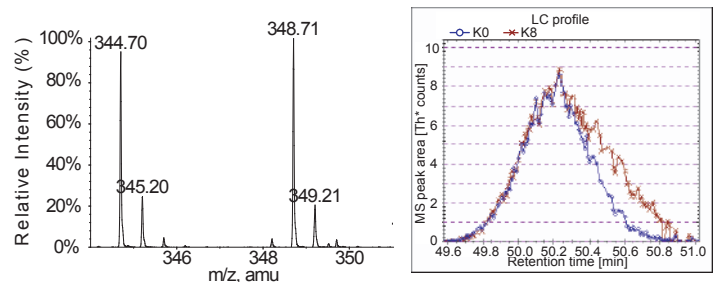
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=48%



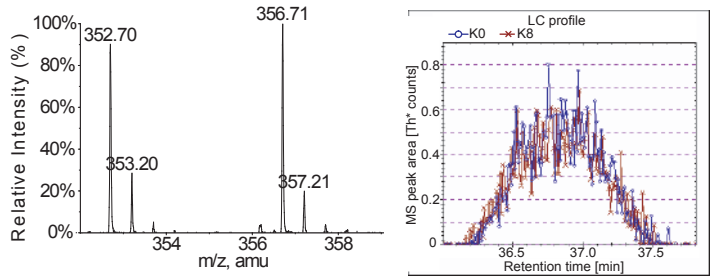
RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=48%



VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=51%

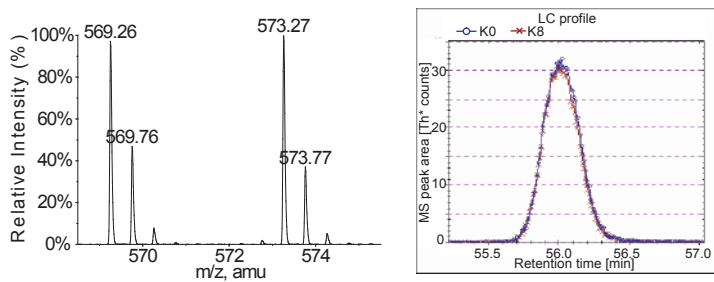


VTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=49%

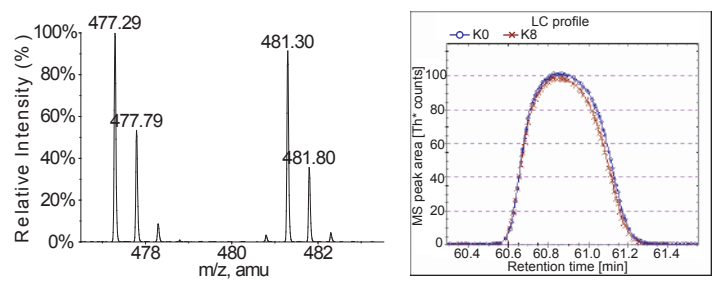


## Bulk H2B

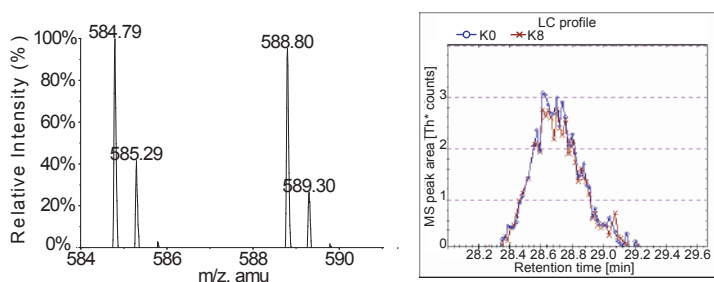
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=49%



LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=47%



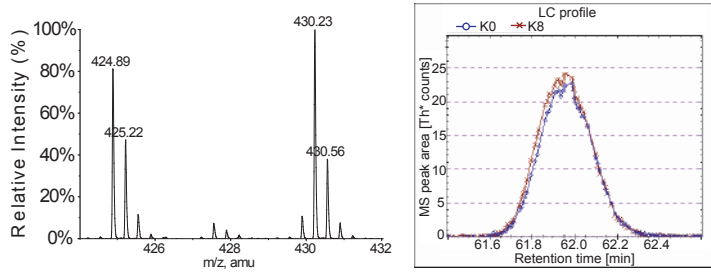
QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=48%



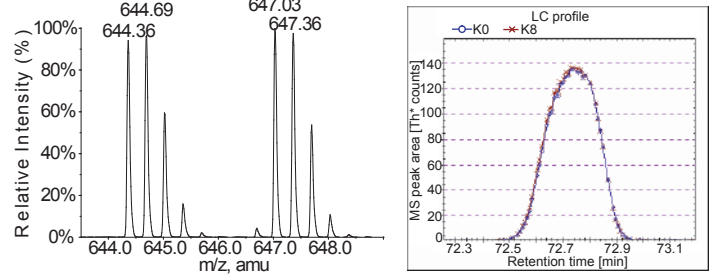
# Flag-H3.1, "off" to "on", 36 h

## Bulk H2A

NDEELNKLK, 3H<sup>+</sup>, K8/(K8+K0)=52%

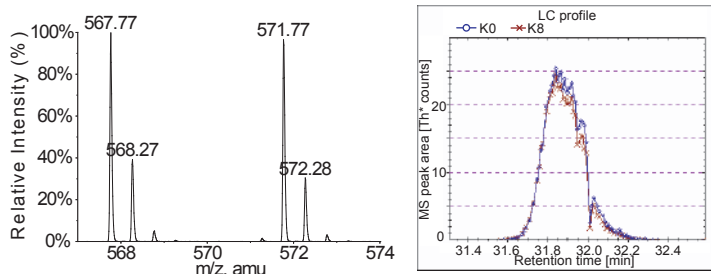


VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=51%

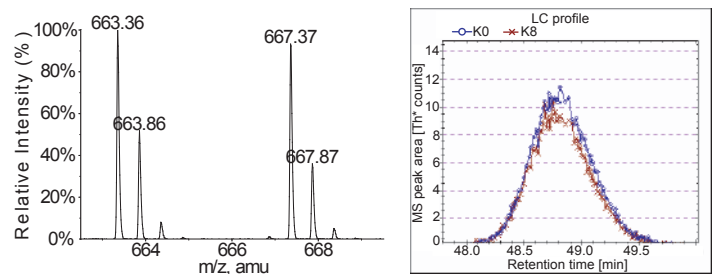


## Bulk H4

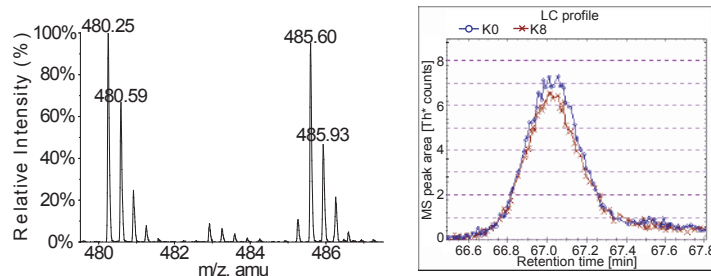
DAVYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=48%



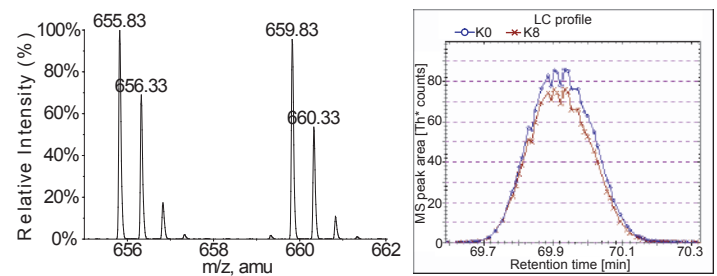
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=47%



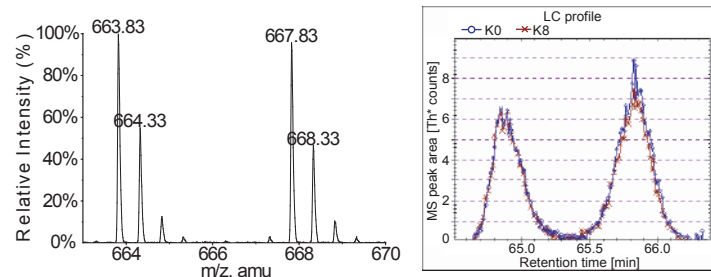
KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=47%



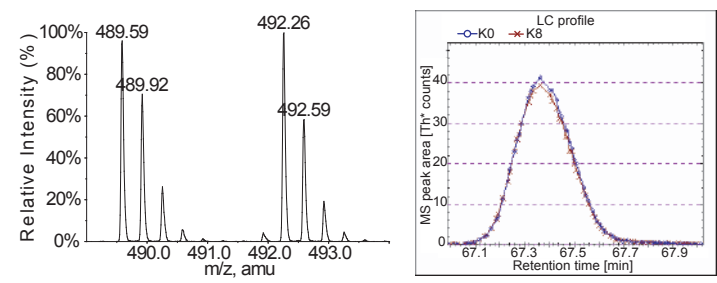
TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=47%



TVTAMDVVYALK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=48%



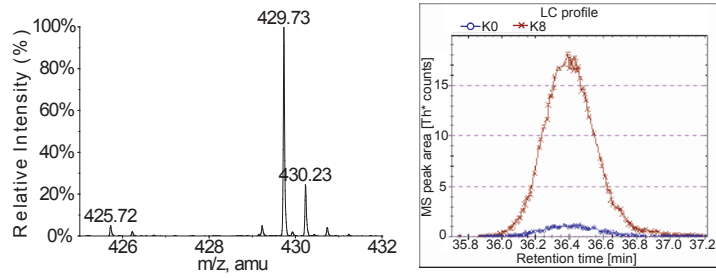
TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=49%



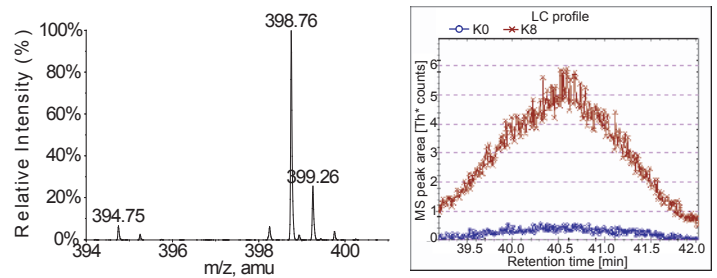
# Flag-H3.1, "off" to "on", 36 h

## Affinity purified Flag-H3.1

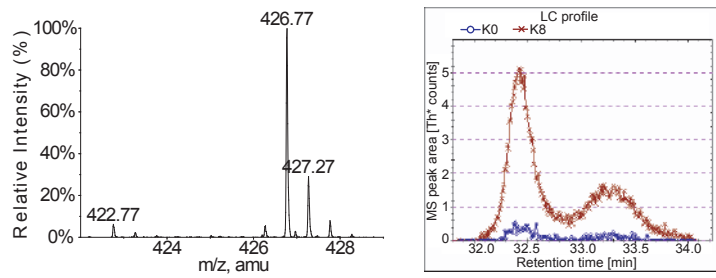
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=94%



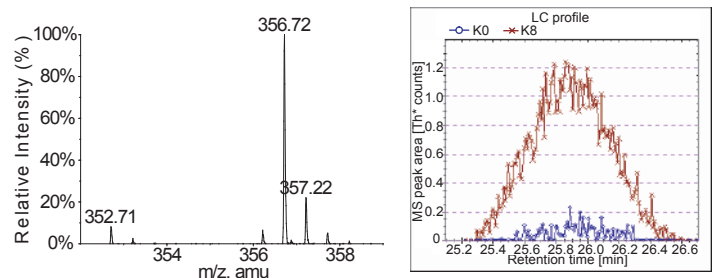
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=93%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=94%

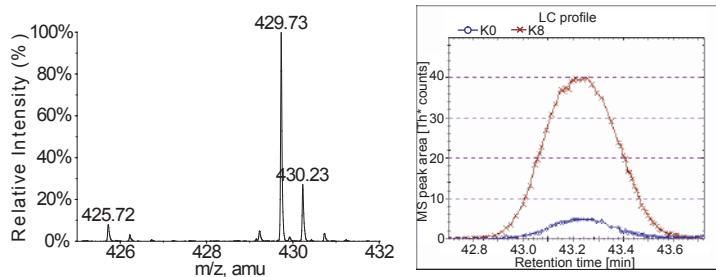


VTIMPK(M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=92%

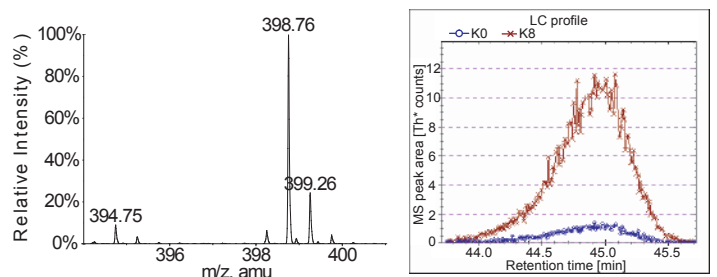


## Affinity purified H3.1

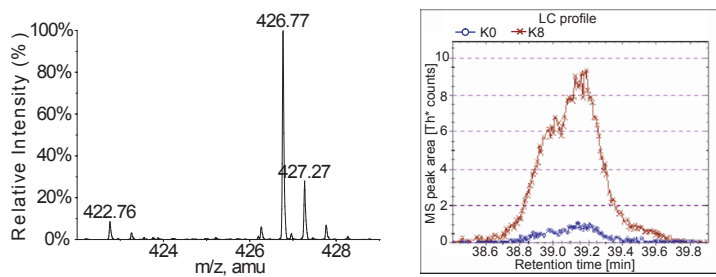
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=90%



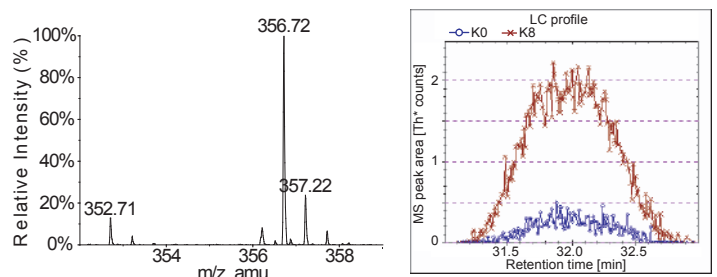
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=91%



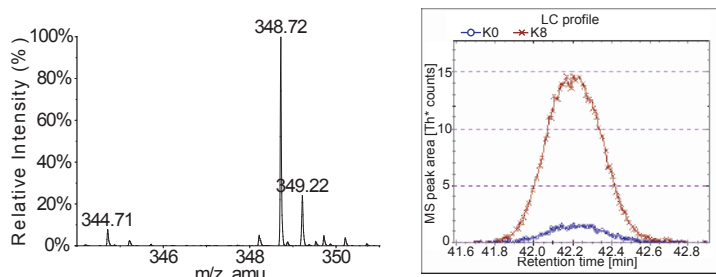
RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=92%



VTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=89%



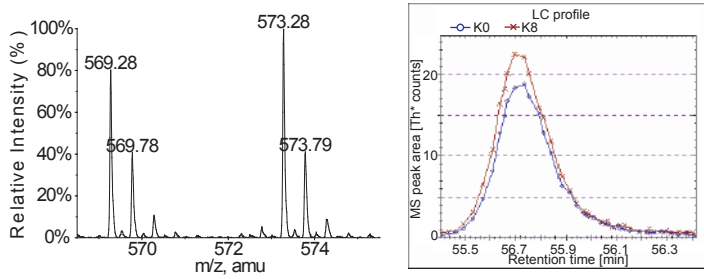
VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=91%



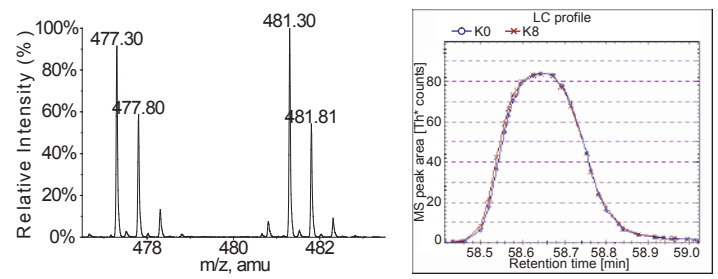
# Flag-H3.1, "off" to "on", 36 h

## Affinity purified H2B

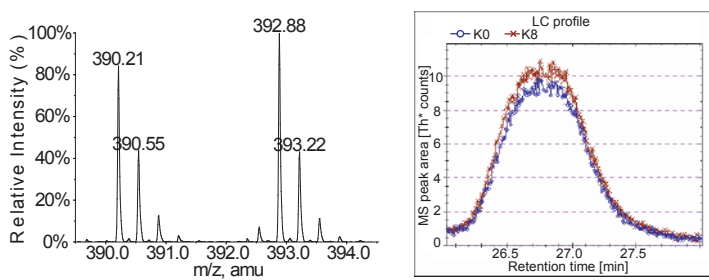
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=54%



LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=52%

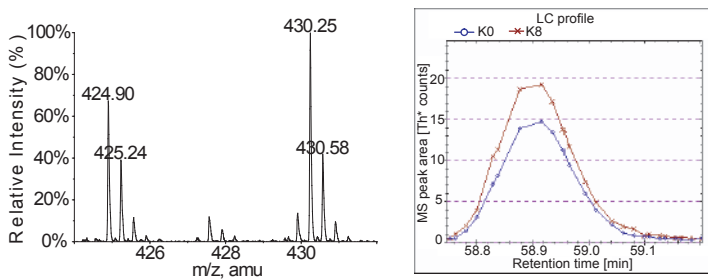


QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=53%

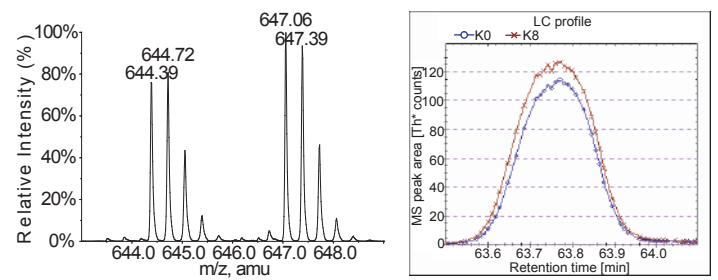


## Affinity purified H2A

NDEELNKLLGK, 3H<sup>+</sup>, K8/(K8+K0)=56%

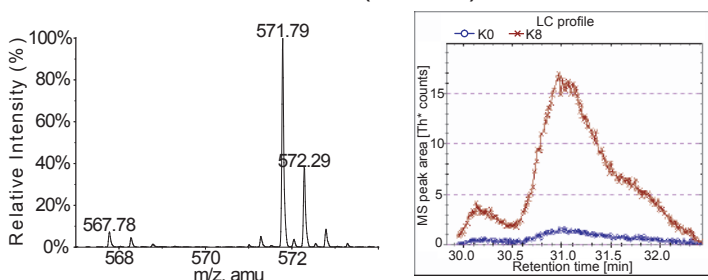


VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=54%

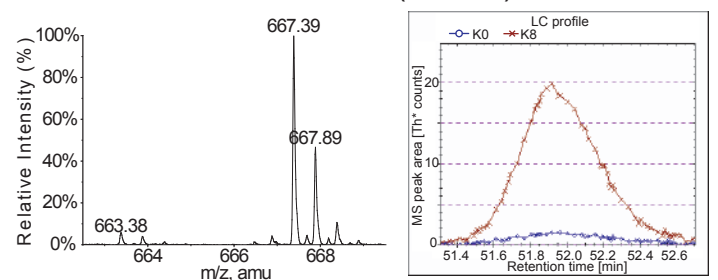


## Affinity purified H4

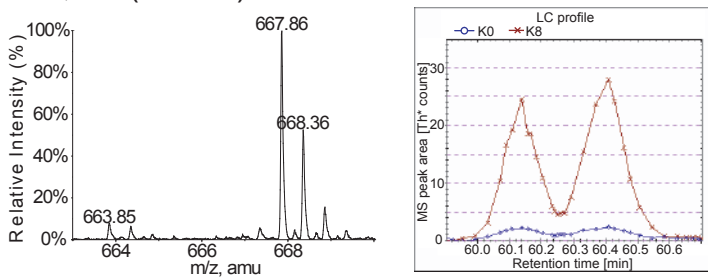
DAVYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=90%



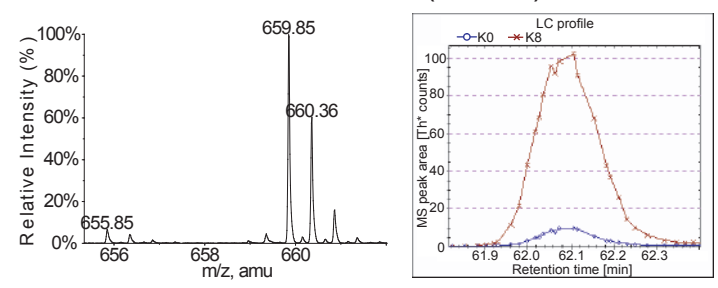
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=93%



TVTAMDVVYALK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=92%



TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=91%





## Flag-H3.3, “on” to “off”, 36 h

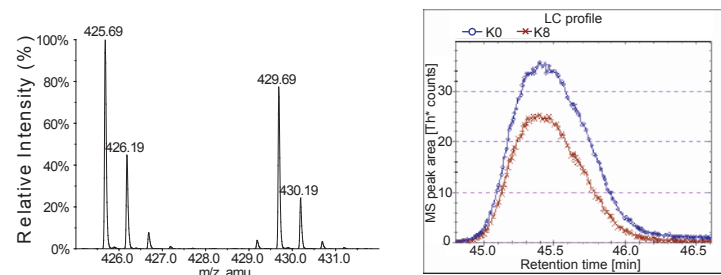
Histone	Sequence	charge	K8/(K8+K0)	average	StDV
Bulk H3	EIAQDFK	2H <sup>+</sup>	40.1%	37.1%	2.2%
	KLPFQR	2H <sup>+</sup>	35.2%		
	RVTIMPK	2H <sup>+</sup>	35.9%		
	VTIMPK	2H <sup>+</sup>	37.2%		
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	38.0%	36.3%	3.3%
	KESYSVYVYK	3H <sup>+</sup>	36.2%		
	KESYSVYVYK	2H <sup>+</sup>	30.5%		
	LLLPGELAK	2H <sup>+</sup>	37.7%		
	QVHPDTGISSK	3H <sup>+</sup>	39.0%		
Bulk H2A	NDEELNKLLGK	3H <sup>+</sup>	39.7%	39.7%	4.9%
	VTIAQGGVLPNIQAVLLPK	2H <sup>+</sup>	34.8%		
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	44.6%		
Bulk H4	DAVYTEHAK	2H <sup>+</sup>	34.3%	36.2%	2.7%
	DNIQGITKPAIR	2H <sup>+</sup>	32.8%		
	DNIQGITKPAIR	3H <sup>+</sup>	37.5%		
	KTVTAMDVVYALK	3H <sup>+</sup>	34.4%		
	DAVYTEHAK	3H <sup>+</sup>	39.2%		
	TVTAMDVVYALKR	3H <sup>+</sup>	38.9%		
Affinity purified FLAG-H3.3	EIAQDFK	2H <sup>+</sup>	0.6%	0.3%	0.2%
	KLPFQR	2H <sup>+</sup>	0.2%		
	MDYK(Ac)DDDDKAR	3H <sup>+</sup>	0.5%		
	RVTIMPK	2H <sup>+</sup>	0.2%		
	RVTIMPK (M Oxidation)	2H <sup>+</sup>	0.1%		
	VTIMPK (M Oxidation)	2H <sup>+</sup>	0.2%		
Affinity purified H3.3	EIAQDFK	2H <sup>+</sup>	8.1%	6.4%	1.2%
	KLPFQR	2H <sup>+</sup>	6.0%		
	VTIMPK	2H <sup>+</sup>	6.0%		
	VTIMPK (M Oxidation)	2H <sup>+</sup>	5.3%		
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	39.5%	39.7%	3.8%
	LLLPGELAK	2H <sup>+</sup>	42.5%		
	QVHPDTGISSK	2H <sup>+</sup>	34.3%		
	QVHPDTGISSK	3H <sup>+</sup>	42.4%		
Affinity purified H2A	NDEELNKLLGK	3H <sup>+</sup>	41.1%	40.0%	1.6%
	VTIAQGGVLPNIQAVLLPK	2H <sup>+</sup>	38.9%		
Affinity purified H4	DAVYTEHAK	3H <sup>+</sup>	3.5%	3.3%	0.5%
	DNIQGITKPAIR	3H <sup>+</sup>	3.7%		
	KTVTAMDVVYALK	3H <sup>+</sup>	2.7%		

Fig. S5 Page 1

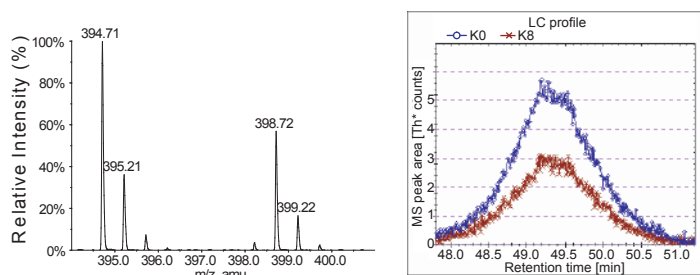
# Flag-H3.3, "on" to "off", 36 h

## Bulk H3

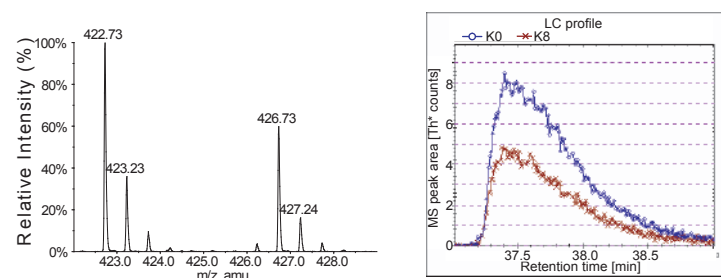
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=40%



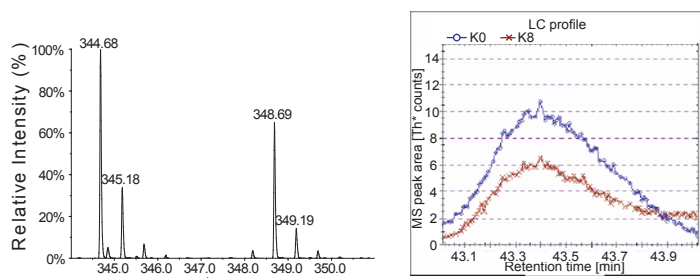
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=35%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=36%

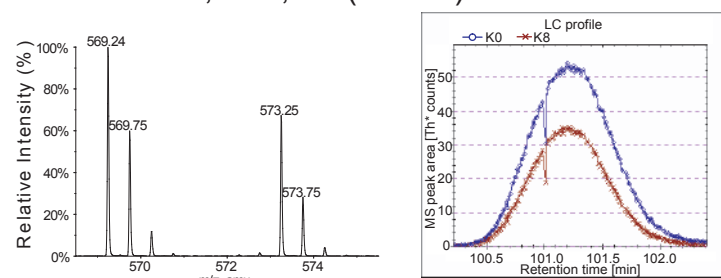


VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=37%

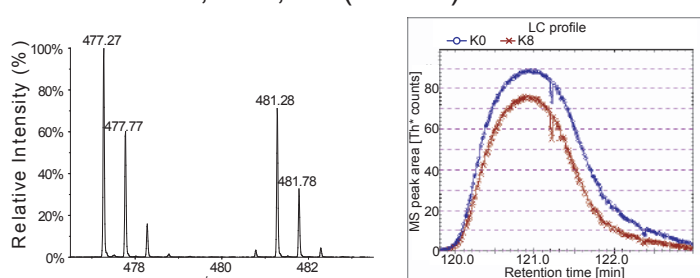


## Bulk H2B

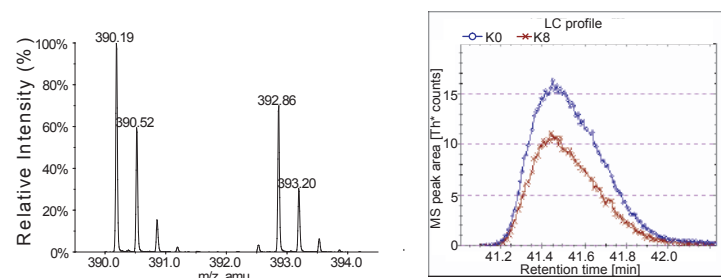
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=38%



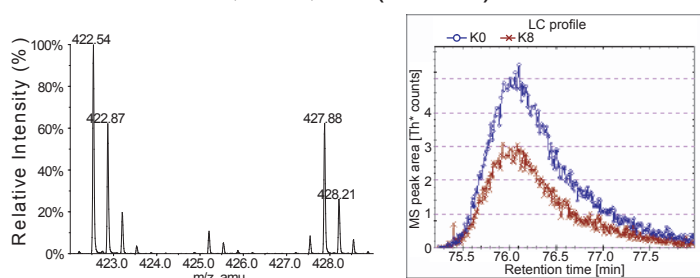
LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=38%



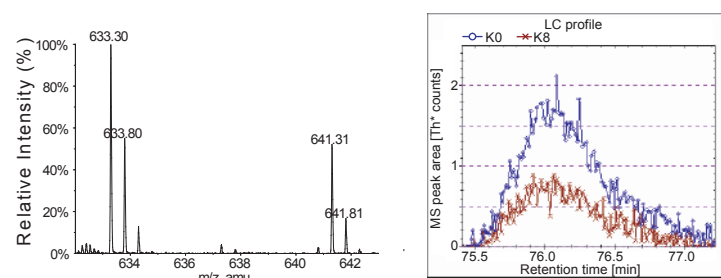
QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=39%



KESYSVYVYK, 3H<sup>+</sup>, K8/(K8+K0)=36%



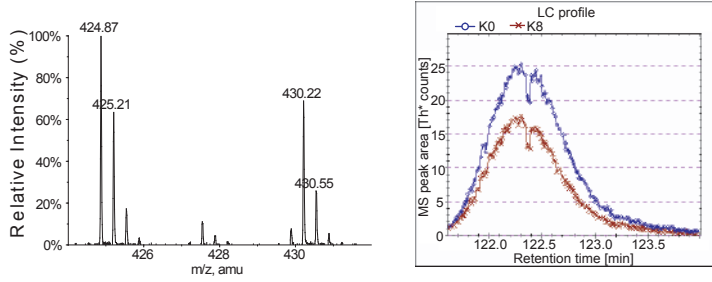
KESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=31%



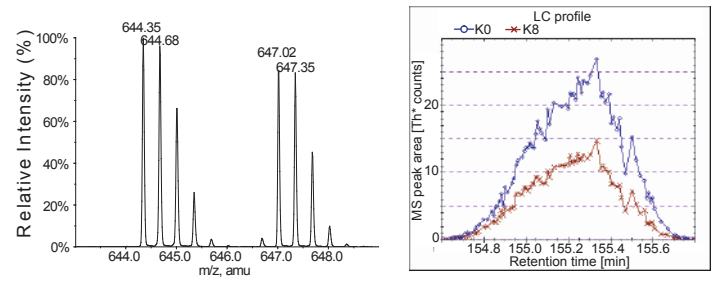
# Flag-H3.3, "on" to "off", 36 h

## Bulk H2A

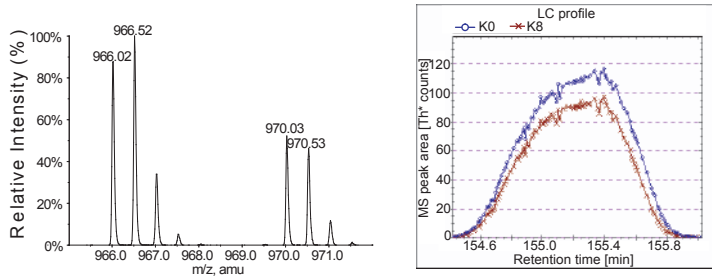
NDEELNKLK, 3H<sup>+</sup>, K8/(K8+K0)=40%



VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=45%

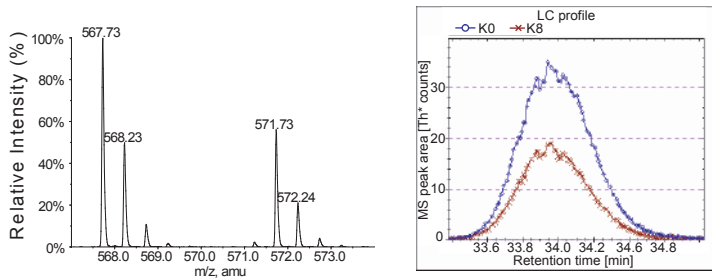


VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=35%

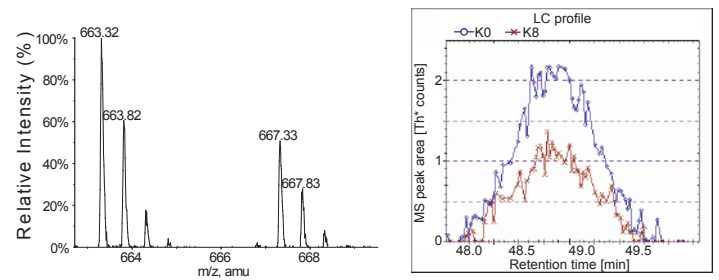


## Bulk H4

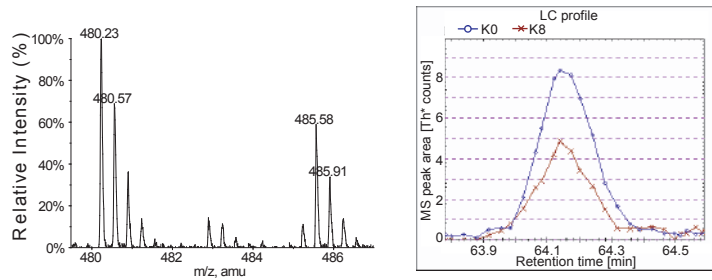
DAVYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=34%



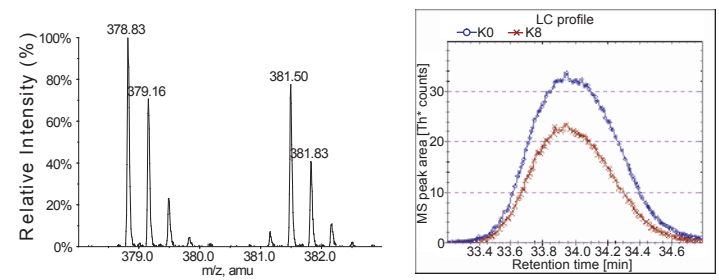
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=33%



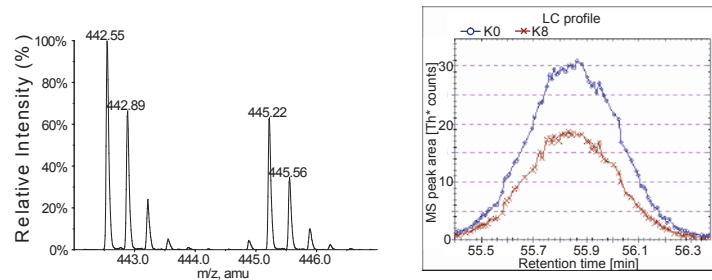
KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=34%



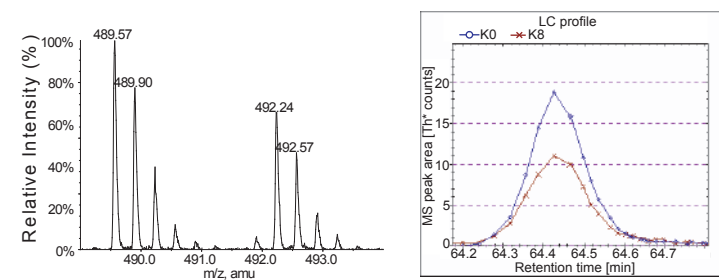
DAVYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=39%



DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=38%



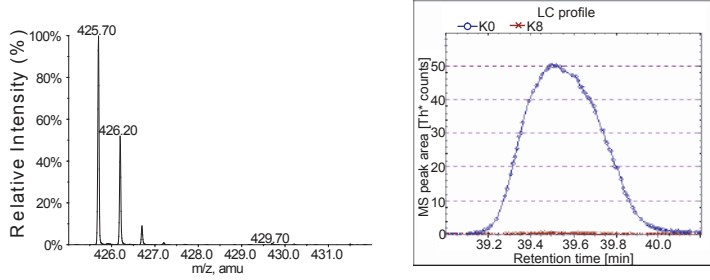
TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=39%



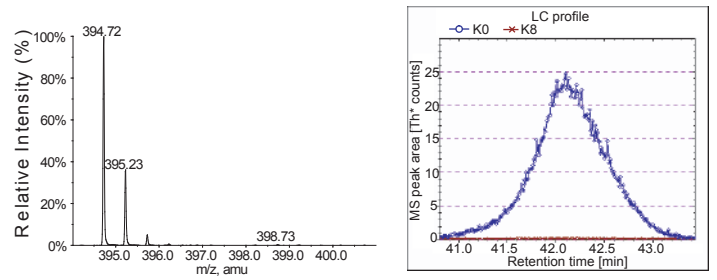
# Flag-H3.3, "on" to "off", 36 h

## Affinity purified Flag-H3.3

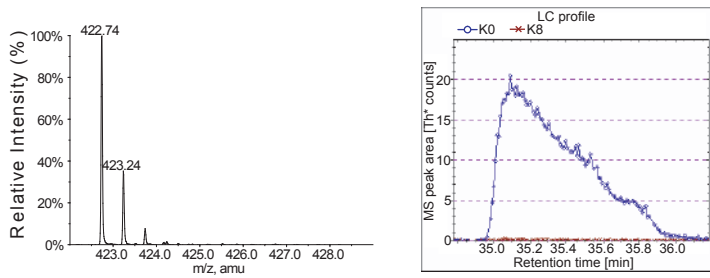
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=0.6%



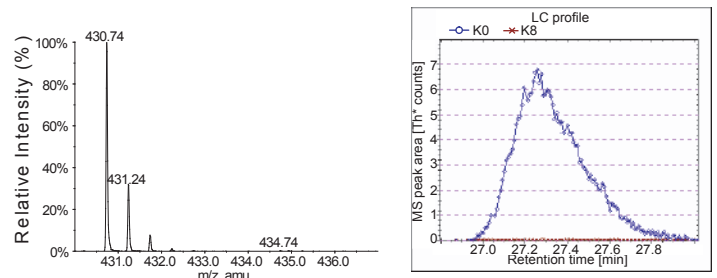
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=0.2%



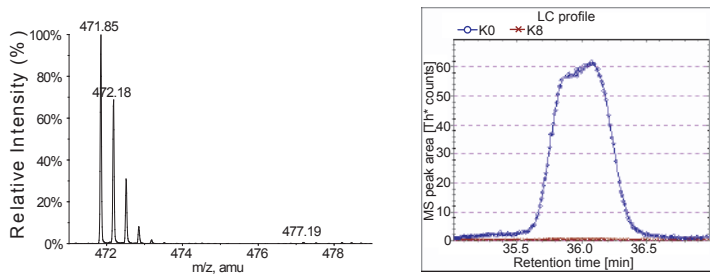
RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=0.2%



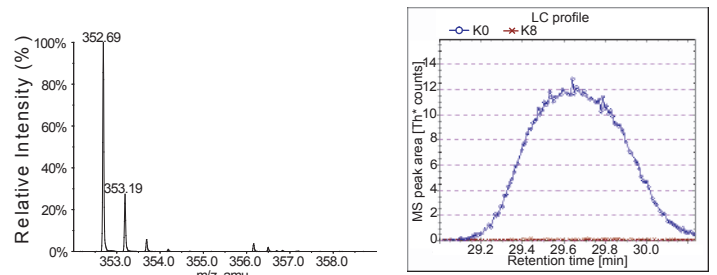
RVTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=0.1%



MDYK(Ac)DDDDKAR (FLAG), 3H<sup>+</sup>, K8/(K8+K0)=0.5%

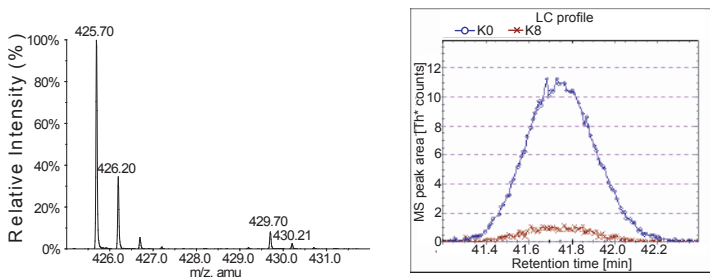


VTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=0.2%

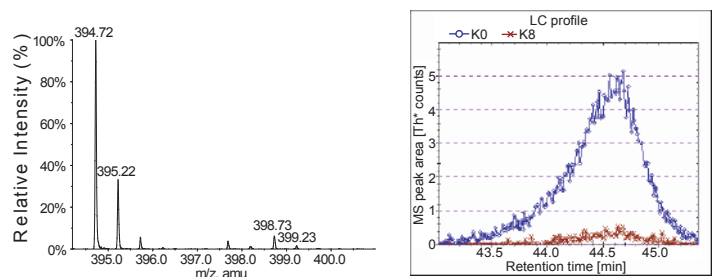


## Affinity purified H3.3

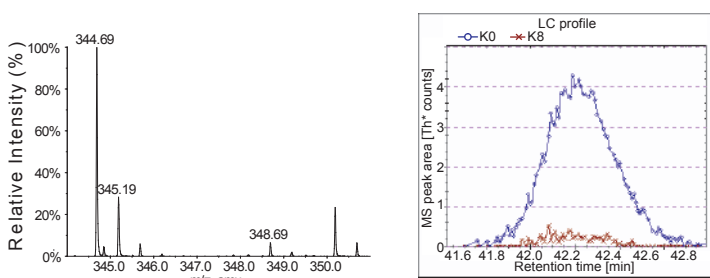
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=8.1%



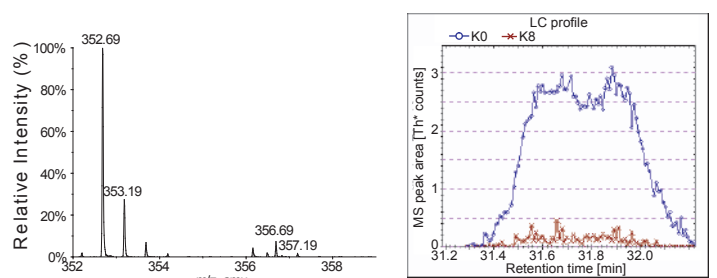
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=6.0%



VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=6.0%



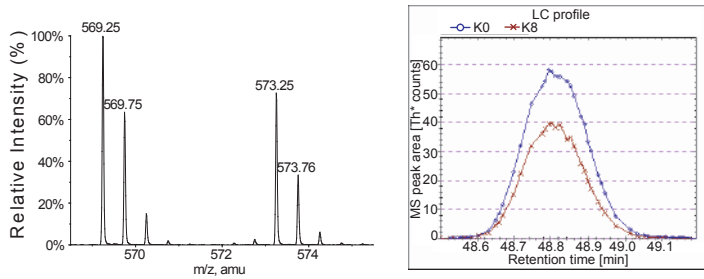
VTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=5.3%



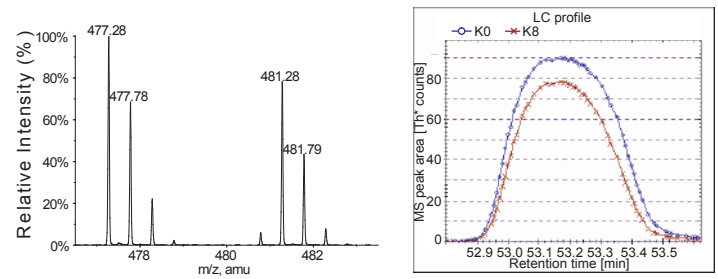
# Flag-H3.3, "on" to "off", 36 h

## Affinity purified H2B

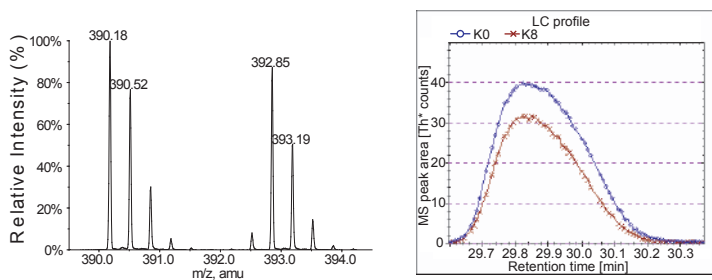
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=40%



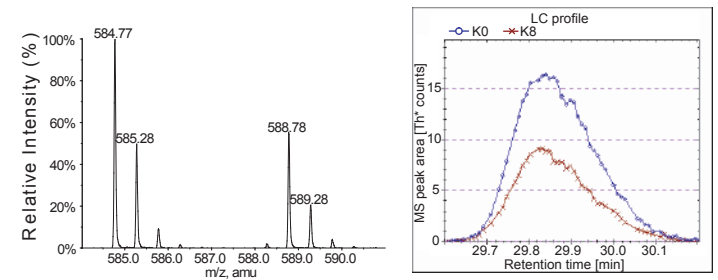
LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=43%



QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=42%

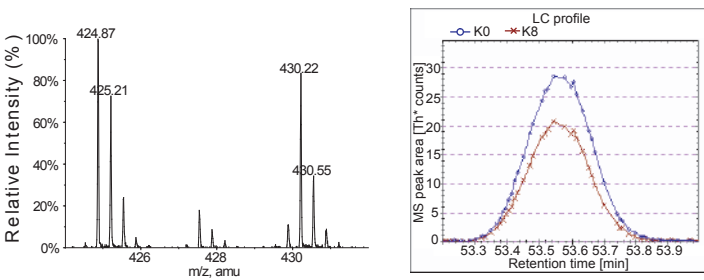


QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=34%

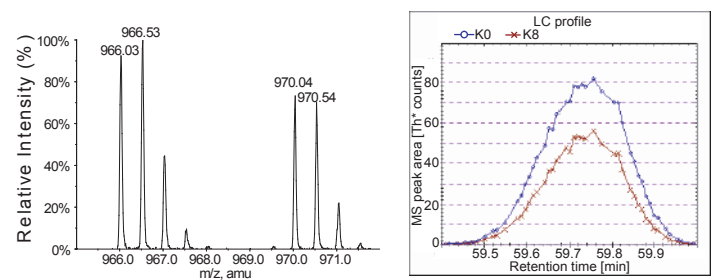


## Affinity purified H2A

NDEELNKLLGK, 3H<sup>+</sup>, K8/(K8+K0)=41%

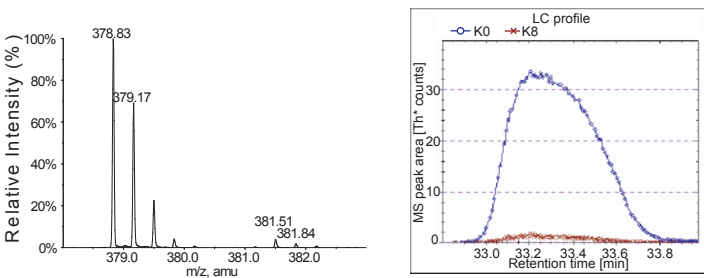


VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=39%

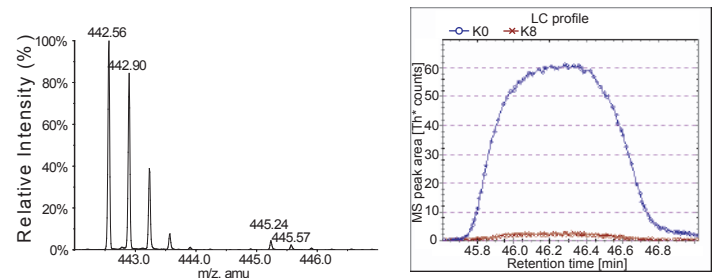


## Affinity purified H4

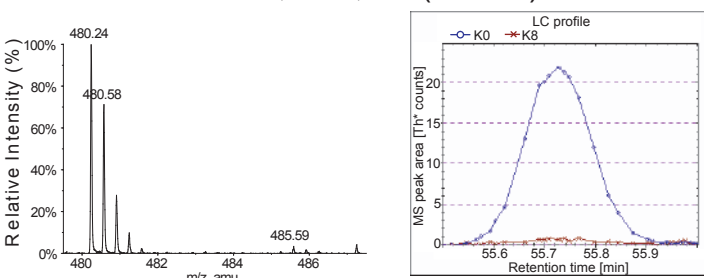
DAVTYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=3.5%



DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=3.7%



KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=2.7%



## Flag-H3.3, “on” to “off”, 72 h

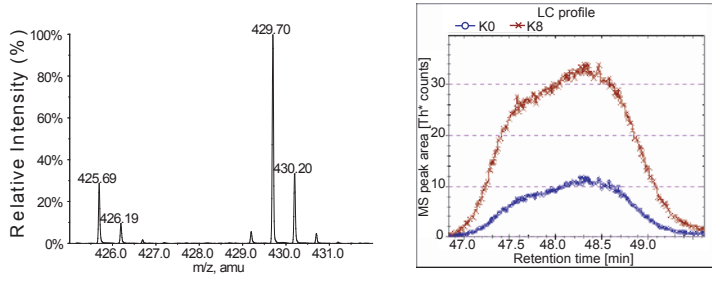
Histone	Sequence	charge	K8/(K8+K0)	average	StDV
Bulk H3	EIAQDFK	2H <sup>+</sup>	76.7%	76.3%	1.4%
	KLPFQR	2H <sup>+</sup>	74.7%		
	RVTIMPK	2H <sup>+</sup>	77.5%		
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	79.4%	74.7%	4.4%
	KESYSVYVYK	3H <sup>+</sup>	76.9%		
	LLLPGELAK	2H <sup>+</sup>	73.4%		
	QVHPDTGISSK	3H <sup>+</sup>	69.2%		
Bulk H2A	NDEELNKLLGK	3H <sup>+</sup>	79.0%	83.0%	5.6%
	VTIAQGGVLPNIQAVLLPK	2H <sup>+</sup>	86.9%		
Bulk H4	DAVTYTEHAK	2H <sup>+</sup>	80.6%	78.6%	2.2%
	DNIQGITKPAIR	2H <sup>+</sup>	80.7%		
	KTVTAMDVVYALK	3H <sup>+</sup>	76.6%		
	TVTAMDVVYALK	2H <sup>+</sup>	79.3%		
	TVTAMDVVYALKR	3H <sup>+</sup>	76.0%		
Affinity purified FLAG-H3.3	EIAQDFK	2H <sup>+</sup>	3.6%	2.8%	0.8%
	KLPFQR	2H <sup>+</sup>	1.6%		
	MDYK(Ac)DDDDKAR	3H <sup>+</sup>	3.2%		
	RVTIMPK	2H <sup>+</sup>	2.7%		
	VTIMPK	2H <sup>+</sup>	3.4%		
	VTIMPK (M Oxidation)	2H <sup>+</sup>	2.0%		
Affinity purified H3.3	EIAQDFK	2H <sup>+</sup>	27.6%	22.6%	4.5%
	KLPFQR	2H <sup>+</sup>	18.8%		
	VTIMPK	2H <sup>+</sup>	21.4%		
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	76.4%	76.8%	1.8%
	KESYSVYVYK	3H <sup>+</sup>	75.3%		
	QVHPDTGISSK	2H <sup>+</sup>	78.8%		
Affinity purified H2A	NDEELNKLLGK	3H <sup>+</sup>	71.9%	72.2%	0.4%
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	72.4%		
Affinity purified H4	DAVTYTEHAK	3H <sup>+</sup>	7.5%	7.4%	0.2%
	DNIQGITKPAIR	3H <sup>+</sup>	7.6%		
	KTVTAMDVVYALK				
	(M Oxidation)	3H <sup>+</sup>	7.4%		
	TVTAMDVVYALKR	3H <sup>+</sup>	7.2%		

Fig. S5 Page 6

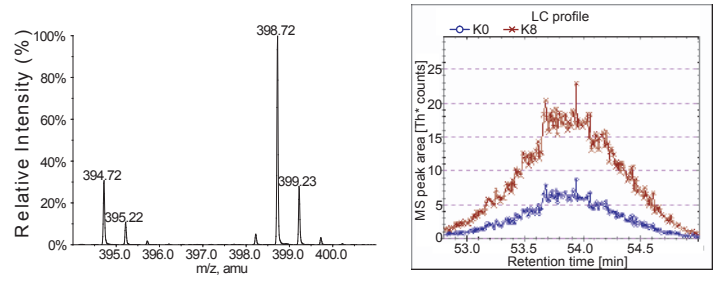
# Flag-H3.3, "on" to "off", 72 h

## Bulk H3

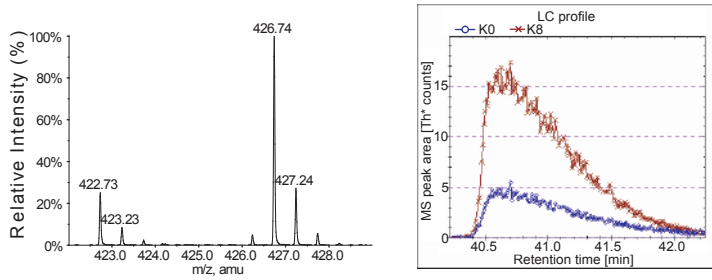
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=77%



KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=75%

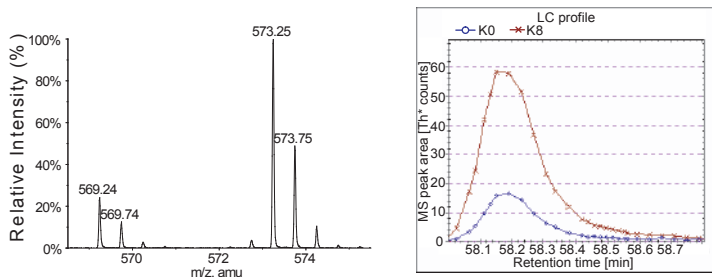


RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=78%

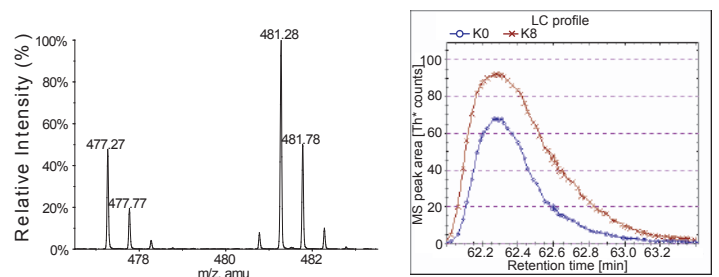


## Bulk H2B

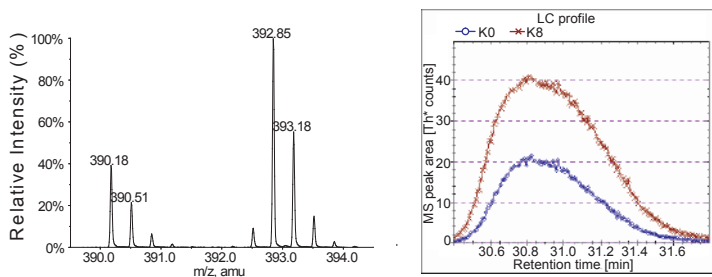
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=79%



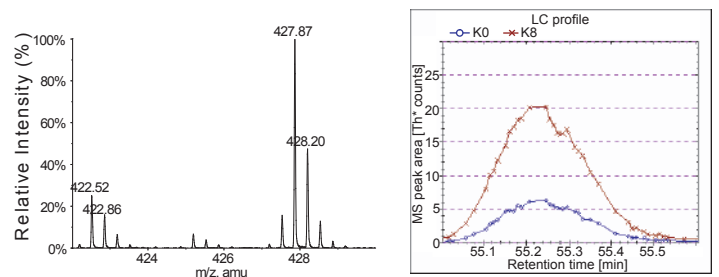
LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=73%



QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=69%



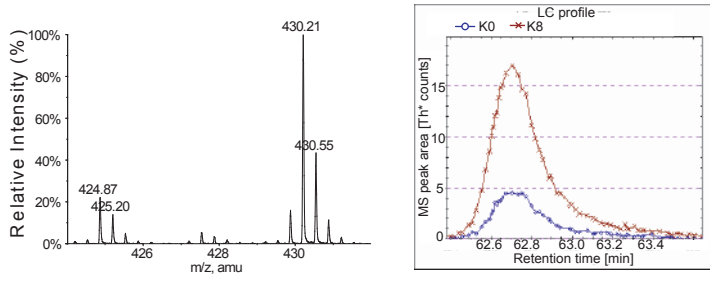
KESYSVYVYK, 3H<sup>+</sup>, K8/(K8+K0)=77%



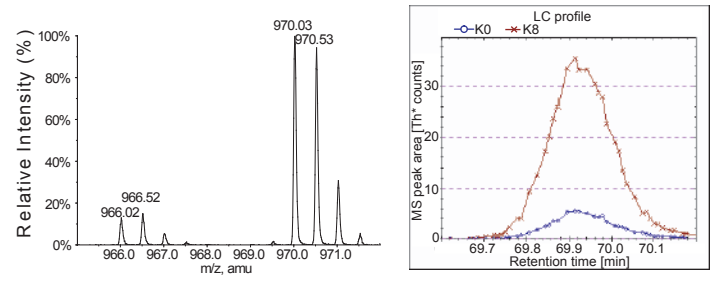
# Flag-H3.3, "on" to "off", 72 h

## Bulk H2A

NDEELNKLK, 3H<sup>+</sup>, K8/(K8+K0)=79%

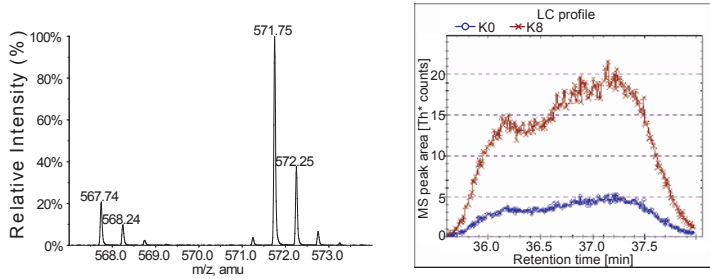


VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=87%

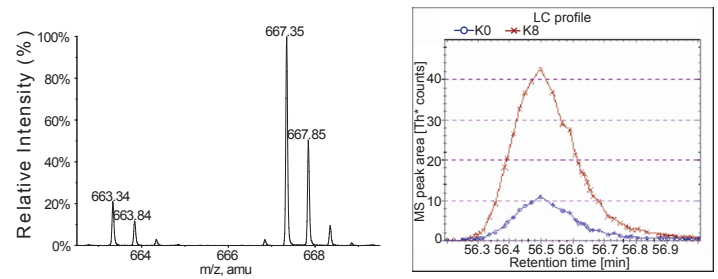


## Bulk H4

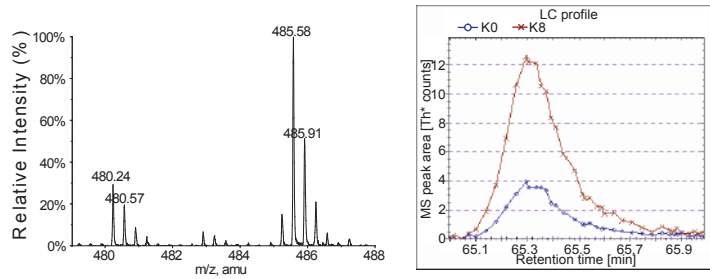
DAVYTEHAQ, 2H<sup>+</sup>, K8/(K8+K0)=81%



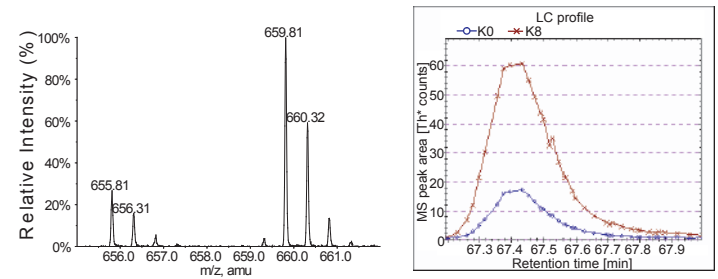
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=81%



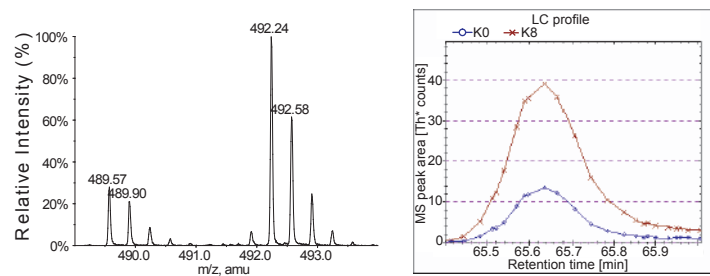
KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=77%



TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=79%



TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=76%

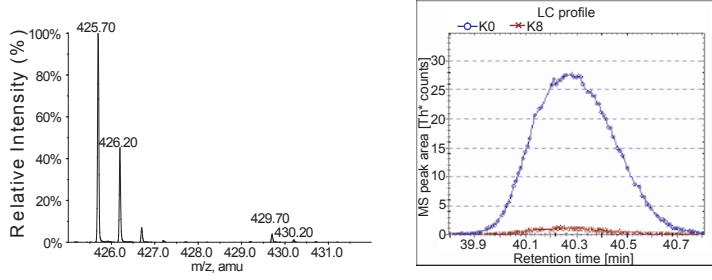




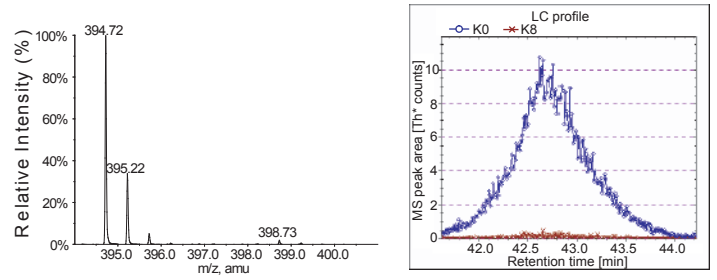
# Flag-H3.3, "on" to "off", 72 h

## Affinity purified Flag-H3.3

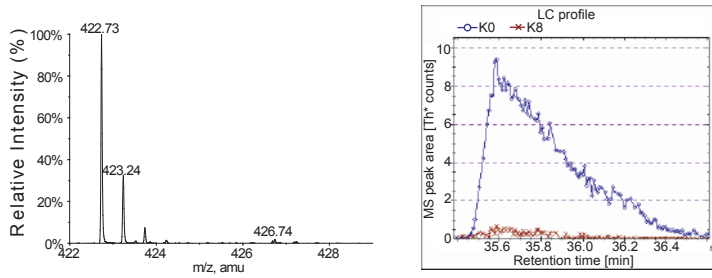
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=3.6%



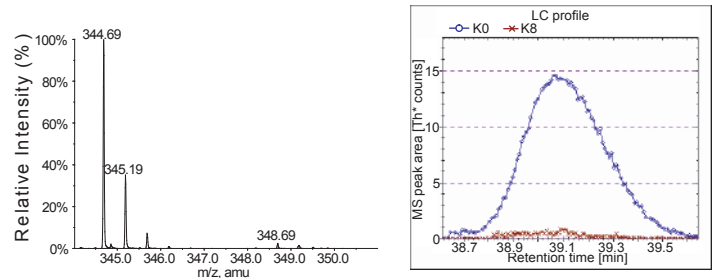
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=1.6%



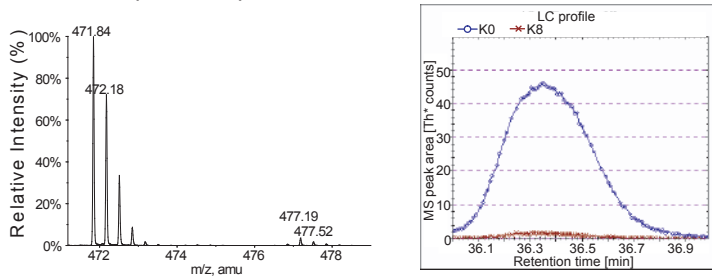
RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=2.7%



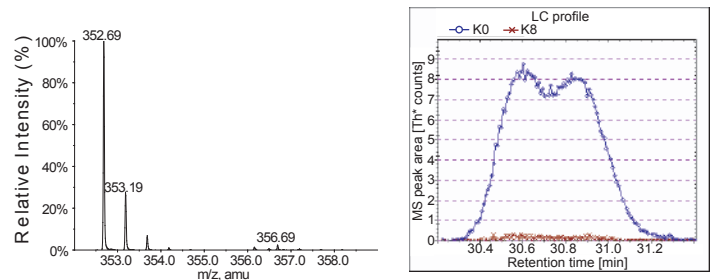
VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=3.4%



MDYK(Ac)DDDDKAR (FLAG),  
3H<sup>+</sup>, K8/(K8+K0)=3.2%

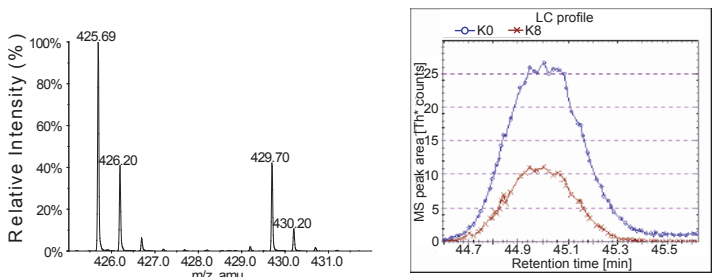


VTIMPK (Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=2.0%

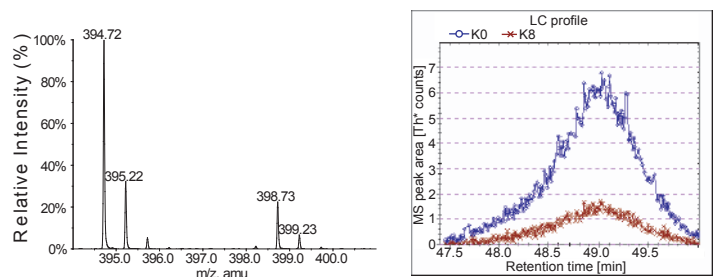


## Affinity purified H3.3

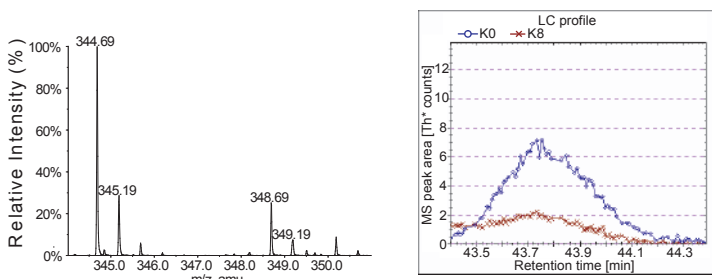
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=28%



KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=19%



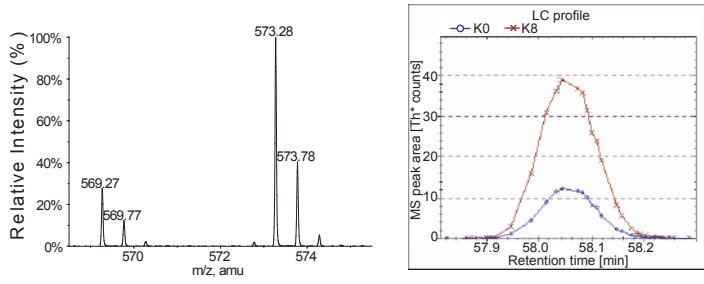
VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=21%



# Flag-H3.3, "on" to "off", 72 h

## Affinity purified H2B

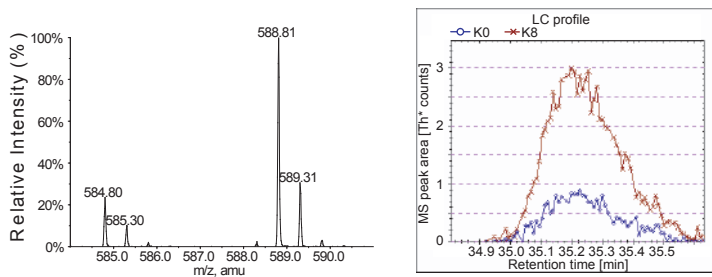
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=76%



KESYSVYVYK, 3H<sup>+</sup>, K8/(K8+K0)=75%

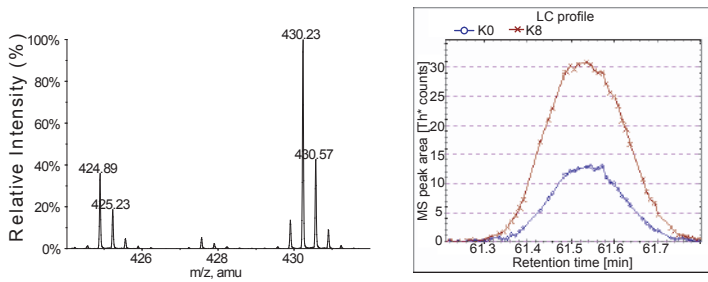


QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=79%

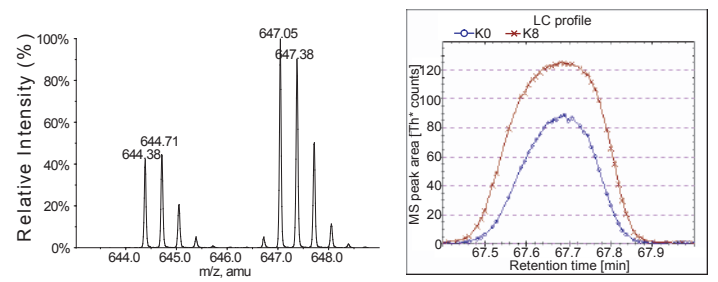


## Affinity purified H2A

NDEELNKLK, 3H<sup>+</sup>, K8/(K8+K0)=72%

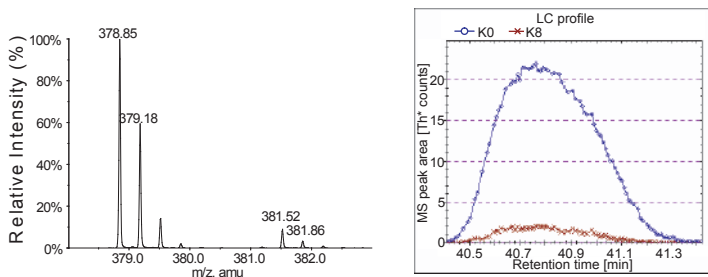


VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=72%

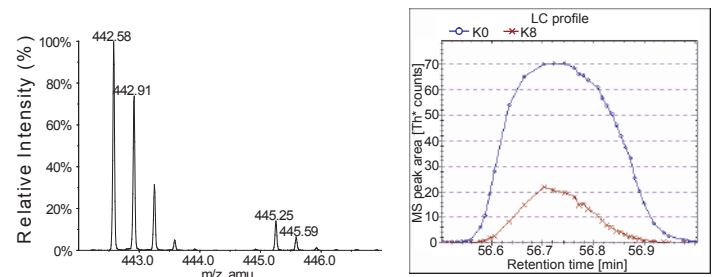


## Affinity purified H4

DAVYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=7.5%

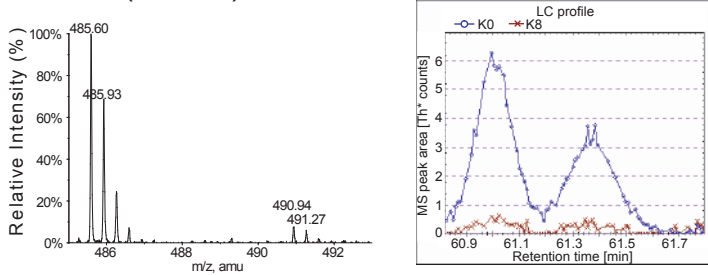


DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=7.6%

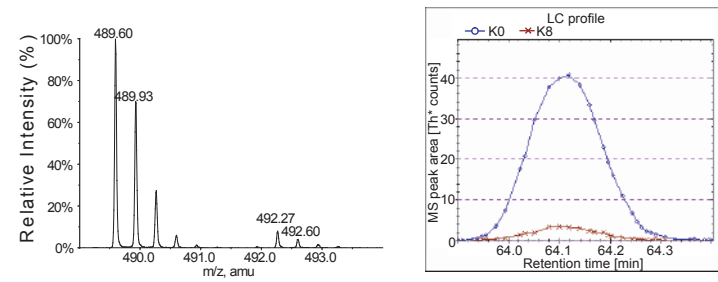


KTVTAMDVVYALK (M Oxidation),

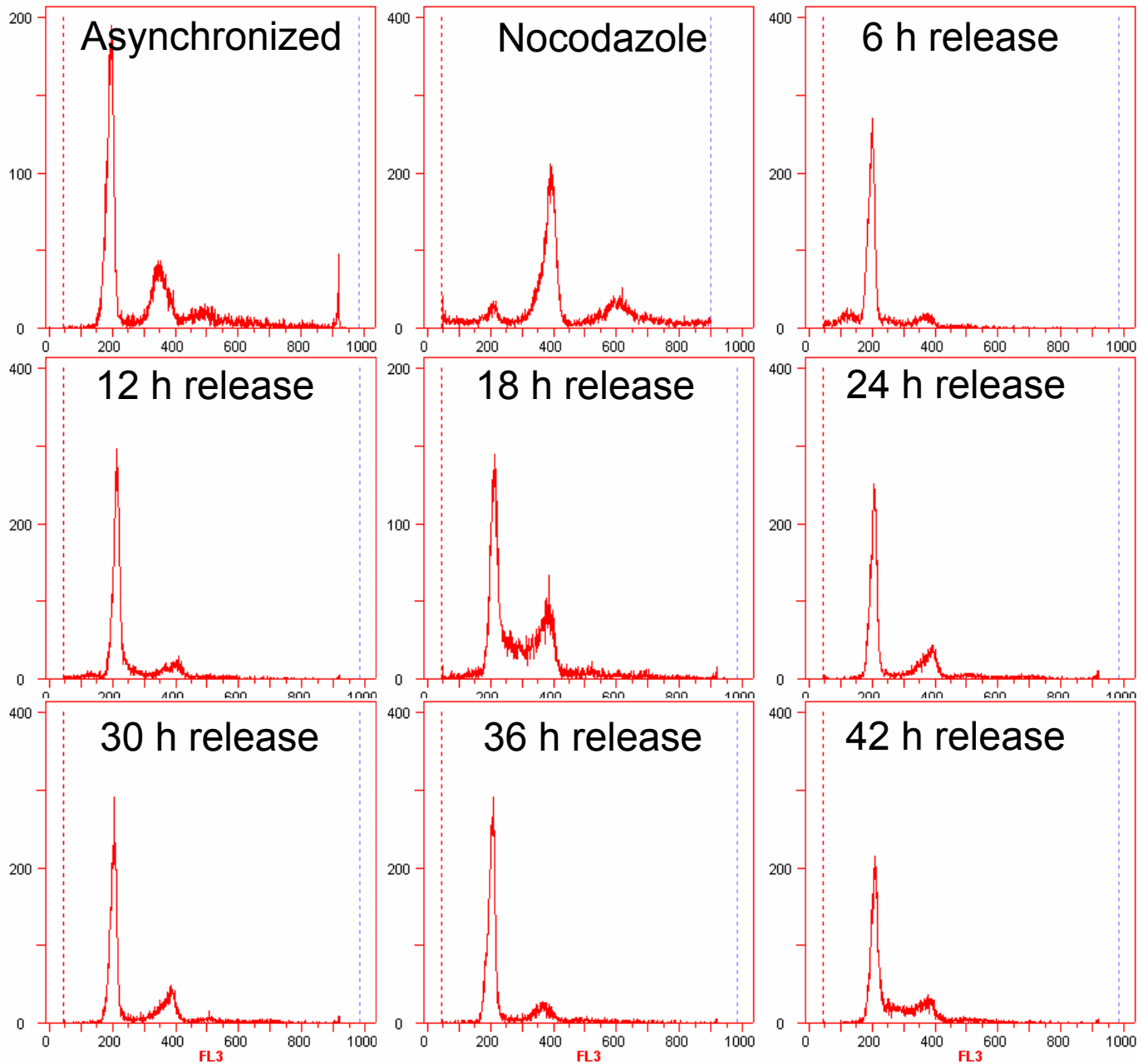
3H<sup>+</sup>, K8/(K8+K0)=7.4%



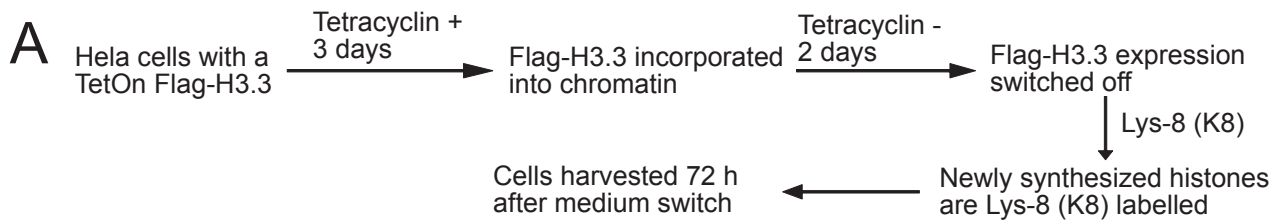
TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=7.2%



# Flag-H3.3 “on” to “off”



# Flag-H3.3, 2nd clone, “on” to “off”, 72 h, asynchronized



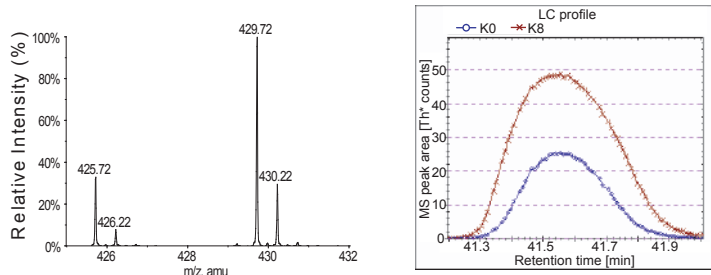
**B**

Histone	Sequence	charge	K8/(K8+K0)	average	StDV
Bulk H3	EIAQDFK	2H <sup>+</sup>	75.2%	77.1%	3.3%
	KLPFQR	2H <sup>+</sup>	80.0%		
	RVTIMPK	2H <sup>+</sup>	76.2%		
	VTIMPK	2H <sup>+</sup>	73.0%		
	VTIMPK (M Oxidation)	2H <sup>+</sup>	80.9%		
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	83.8%	78.4%	5.7%
	KESYSVYVYK	3H <sup>+</sup>	82.6%		
	LLLPGELAK	2H <sup>+</sup>	75.4%		
	QVHPDTGISSK	3H <sup>+</sup>	72.0%		
Bulk H2A	NDEELNKLLGK	3H <sup>+</sup>	85.7%	84.6%	1.6%
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	83.4%		
Bulk H4	DAVYTEHAK	2H <sup>+</sup>	79.7%	80.3%	2.3%
	DNIQGITKPAIR	2H <sup>+</sup>	83.7%		
	TVTAMDVVYALK	2H <sup>+</sup>	81.6%		
	TVTAMDVVYALKR	3H <sup>+</sup>	78.3%		
	TVTAMDVVYALKR (M Oxidation)	3H <sup>+</sup>	78.3%		
Affinity purified FLAG-H3.3	EIAQDFK	2H <sup>+</sup>	3.7%	2.6%	1.1%
	KLPFQR	2H <sup>+</sup>	1.4%		
	RVTIMPK	2H <sup>+</sup>	1.6%		
	VTIMPK	2H <sup>+</sup>	3.6%		
	MDYK(Ac)DDDDKAR (M Oxidation)	3H <sup>+</sup>	1.6%		
Affinity purified H3.3	EIAQDFK	2H <sup>+</sup>	28.8%	25.6%	2.7%
	KLPFQR	2H <sup>+</sup>	22.6%		
	RVTIMPK	2H <sup>+</sup>	24.2%		
	VTIMPK	2H <sup>+</sup>	26.8%		
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	60.0%	62.2%	2.6%
	KESYSVYVYK	2H <sup>+</sup>	65.0%		
	LLLPGELAK	2H <sup>+</sup>	59.8%		
	QVHPDTGISSK	3H <sup>+</sup>	63.8%		
Affinity purified H2A	NDEELNKLLGK	3H <sup>+</sup>	62.1%	64.5%	2.3%
	VTIAQGGVLPNIQAVLLPK	2H <sup>+</sup>	66.6%		
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	64.8%		
Affinity purified H4	DAVYTEHAK	2H <sup>+</sup>	11.9%	11.5%	2.2%
	DNIQGITKPAIR	2H <sup>+</sup>	9.6%		
	DNIQGITKPAIR	3H <sup>+</sup>	11.1%		
	TVTAMDVVYALK	2H <sup>+</sup>	9.2%		
	TVTAMDVVYALKR	3H <sup>+</sup>	15.8%		
	KTVTAMDVVYALK	3H <sup>+</sup>	11.7%		
	KTVTAMDVVYALKR	3H <sup>+</sup>	11.2%		

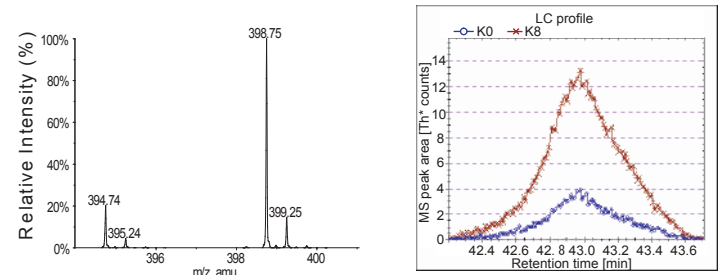
# Flag-H3.3, 2nd clone, "on" to "off", 72 h, asynchronized

## Bulk H3

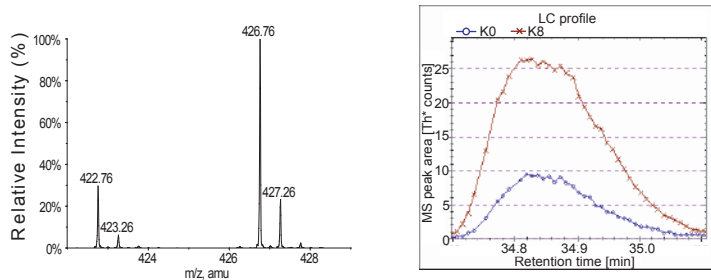
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=75%



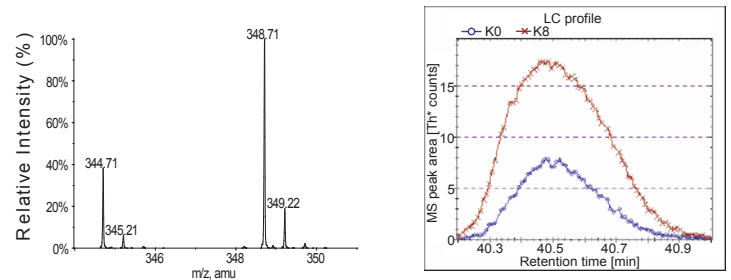
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=80%



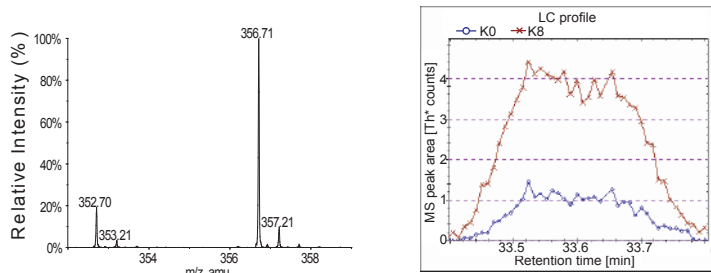
RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=76%



VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=73%

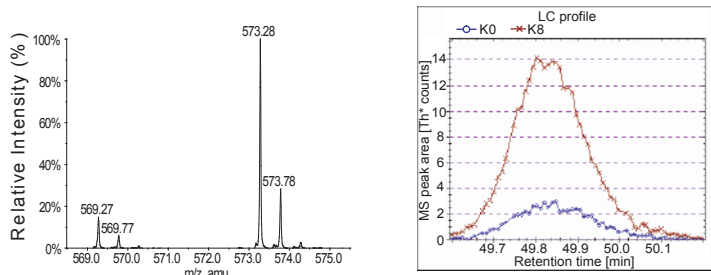


VTIM(Oxidation)PK, 2H<sup>+</sup>, K8/(K8+K0)=81%

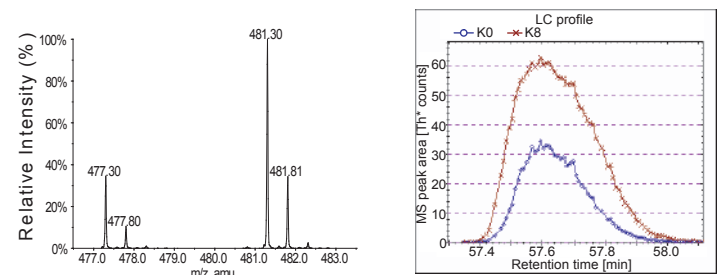


## Bulk H2B

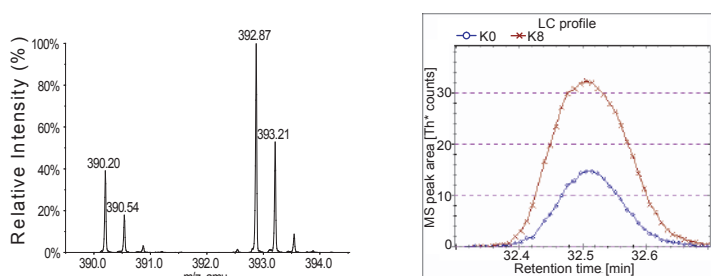
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=84%



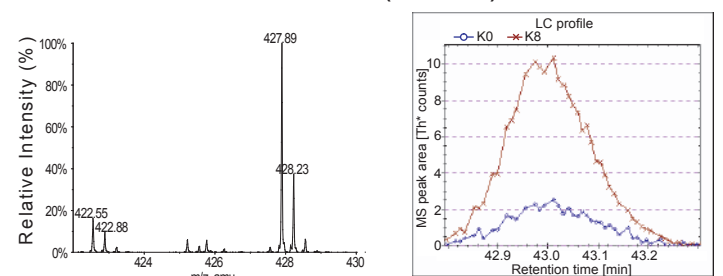
LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=75%



QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=72%



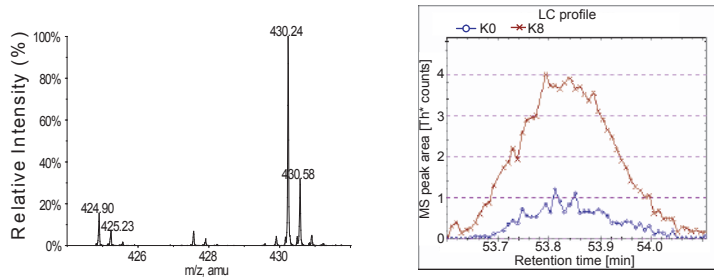
KESYSVYVYK, 3H<sup>+</sup>, K8/(K8+K0)=83%



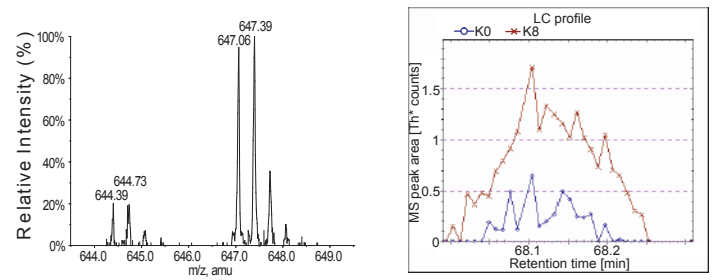
# Flag-H3.3, 2nd clone, "on" to "off", 72 h, asynchronized

## Bulk H2A

NDEELNKLK, 3H<sup>+</sup>, K8/(K8+K0)=86%

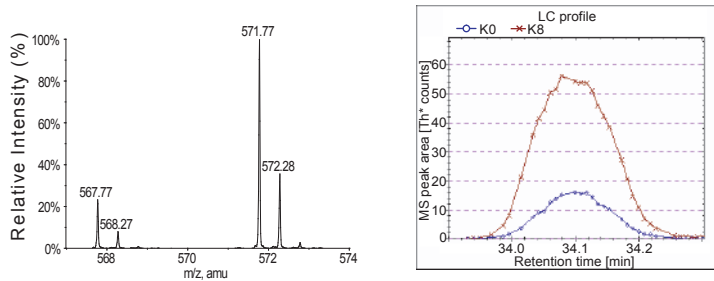


VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=83%

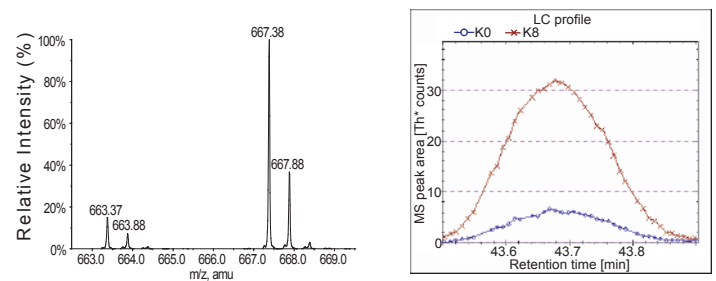


## Bulk H4

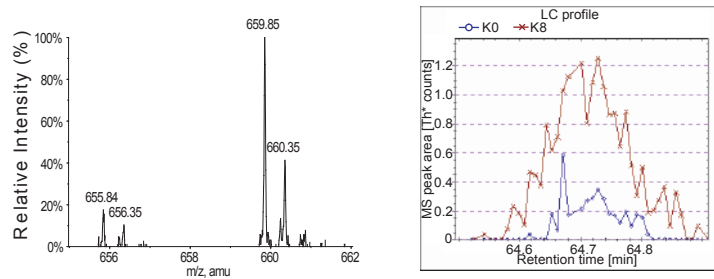
DAVYTEHAQ, 2H<sup>+</sup>, K8/(K8+K0)=80%



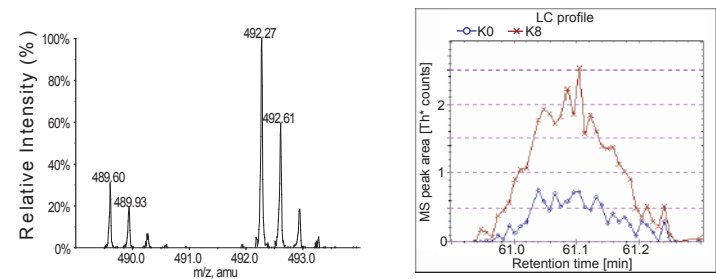
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=84%



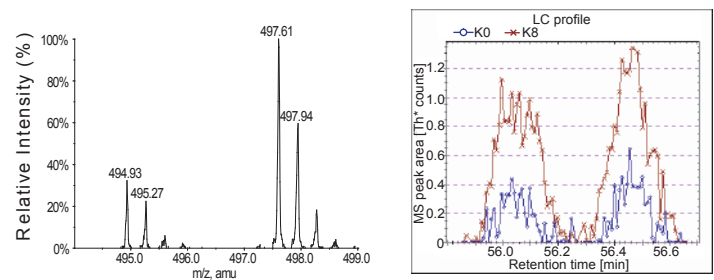
TVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=82%



TVTAMDVVYALKR, 2H<sup>+</sup>, K8/(K8+K0)=78%



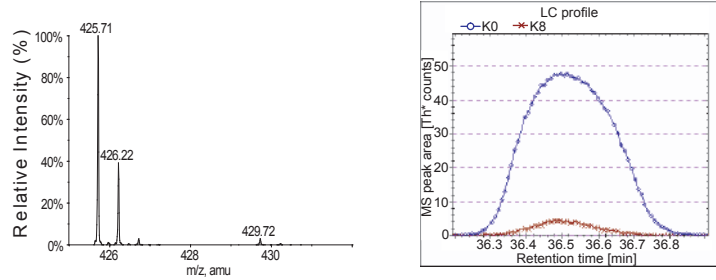
TVTAM(Oxidation)DVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=78%



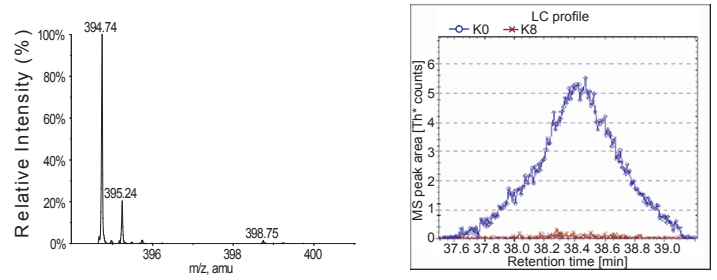
# Flag-H3.3, 2nd clone, "on" to "off", 72 h, asynchronized

## Affinity purified Flag-H3.3

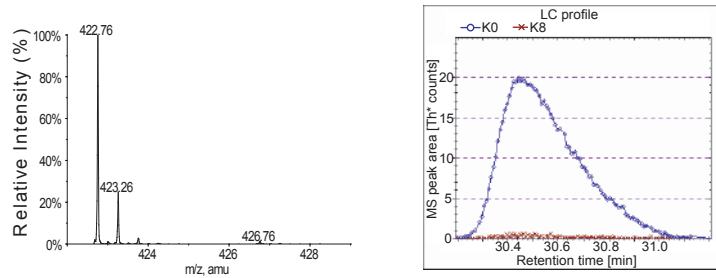
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=3.7%



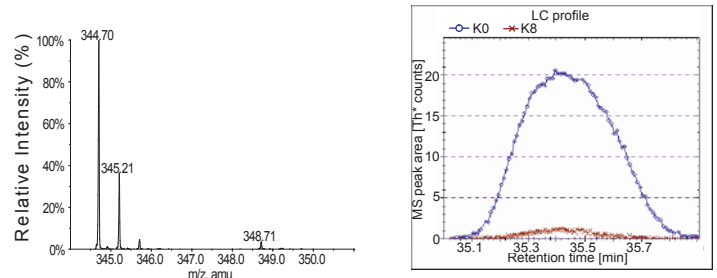
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=1.4%



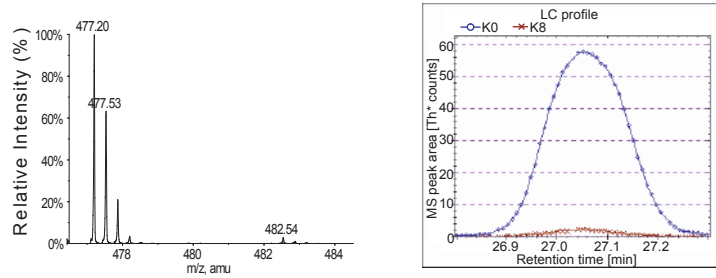
RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=1.6%



VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=3.6%

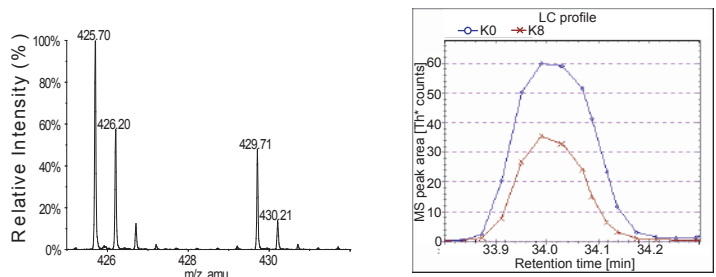


M(Oxidation)DYK(Ac)DDDDKAR (FLAG), 3H<sup>+</sup>, K8/(K8+K0)=1.6%

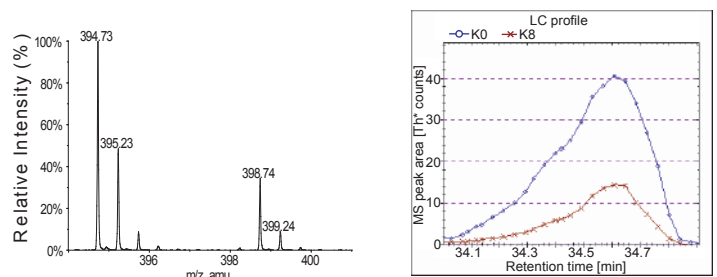


## Affinity purified H3.3

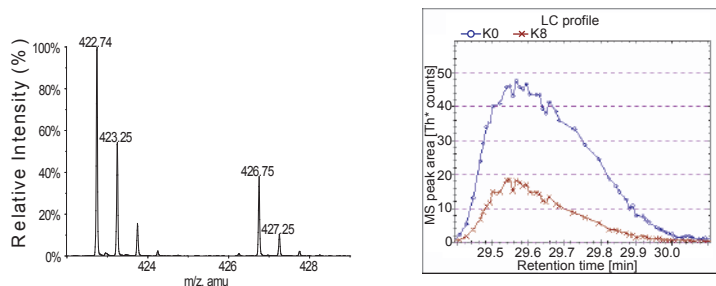
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=29%



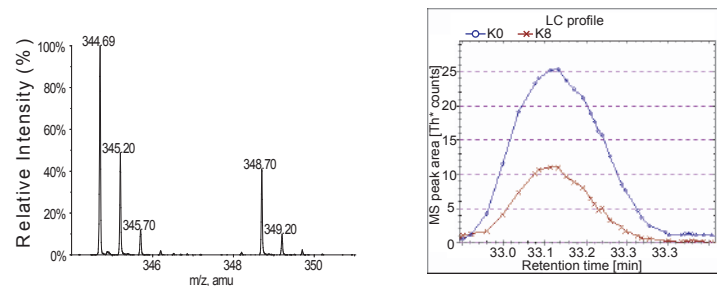
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=23%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=24%



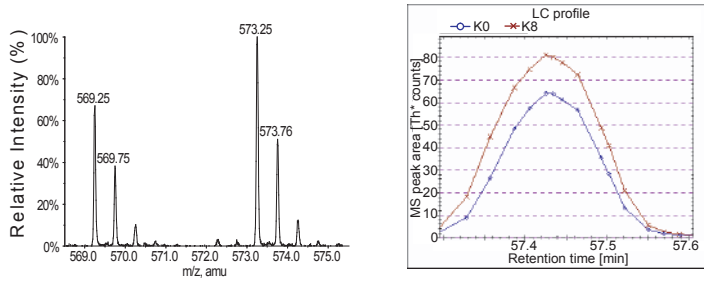
VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=27%



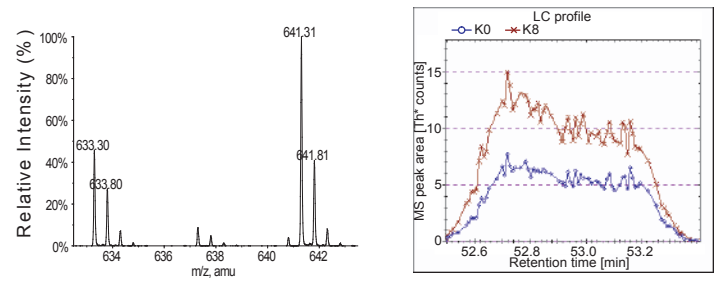
# Flag-H3.3, 2nd clone, "on" to "off", 72 h, asynchronized

## Affinity purified H2B

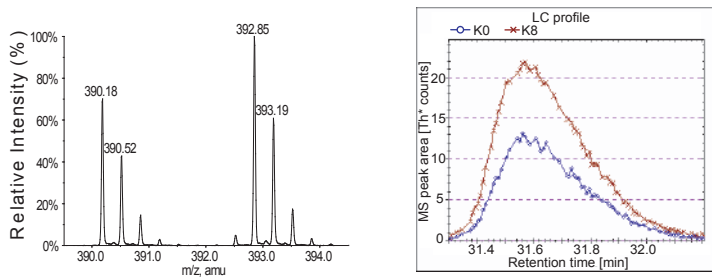
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=60%



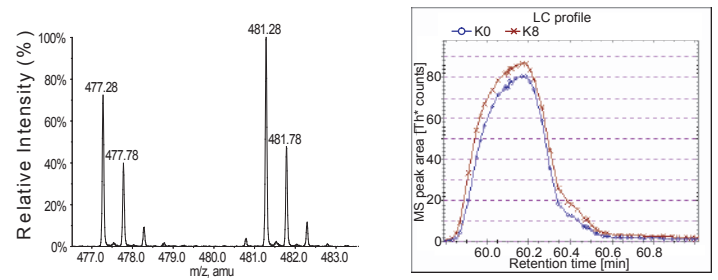
KESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=65%



QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=64%

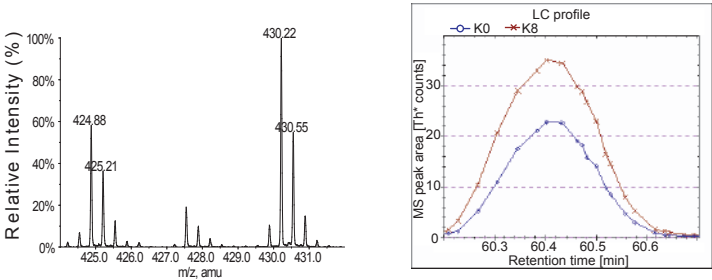


LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=60%

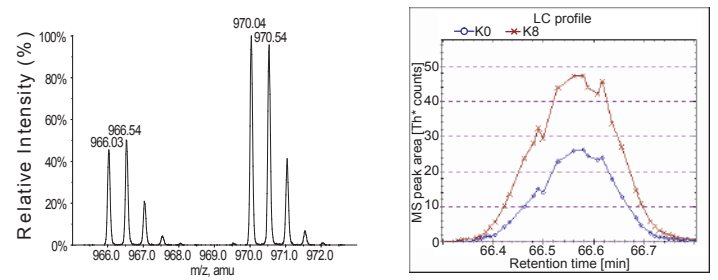


## Affinity purified H2A

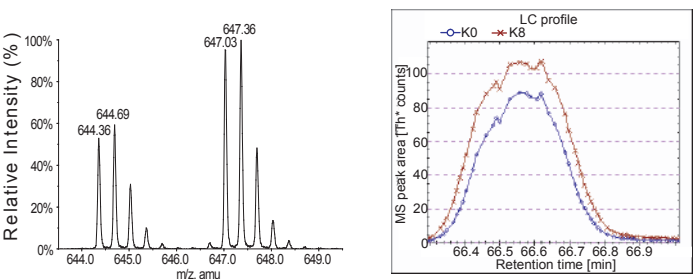
NDEELNKLLGK, 3H<sup>+</sup>, K8/(K8+K0)=62%



VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=67%



VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=65%

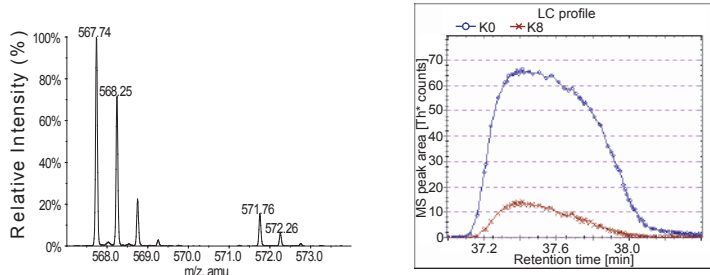




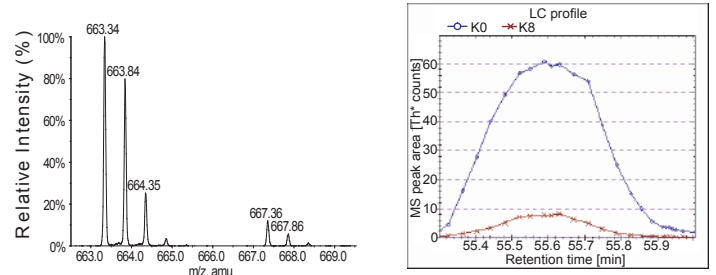
# Flag-H3.3, 2nd clone, "on" to "off", 72 h, asynchronized

## Affinity purified H4

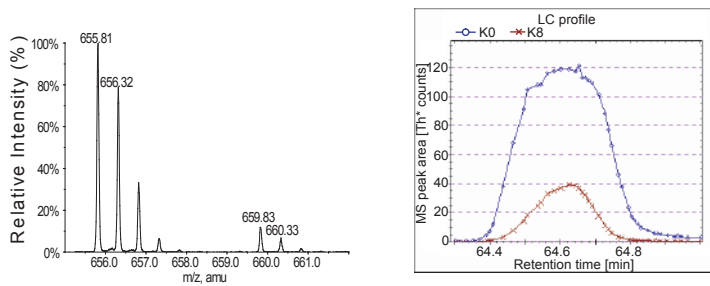
DAVTYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=12%



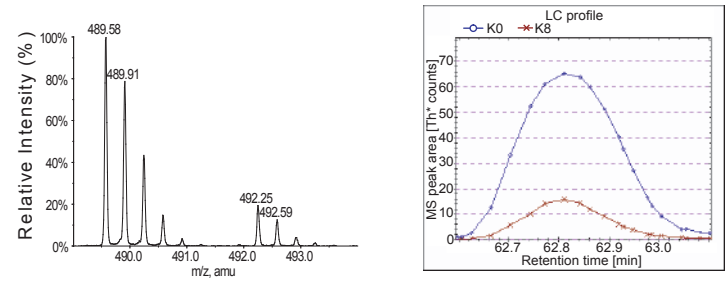
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=9.6%



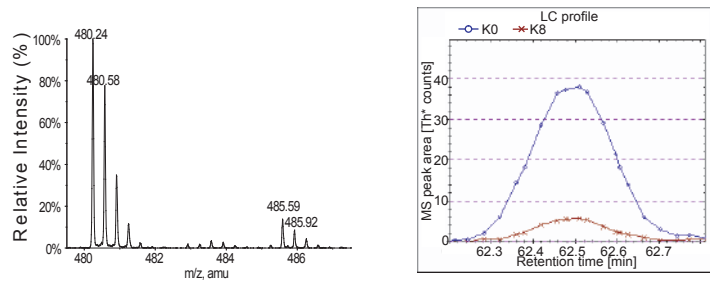
TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=9.2%



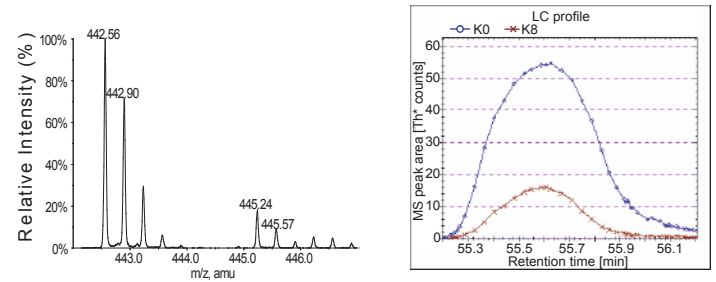
TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=16%



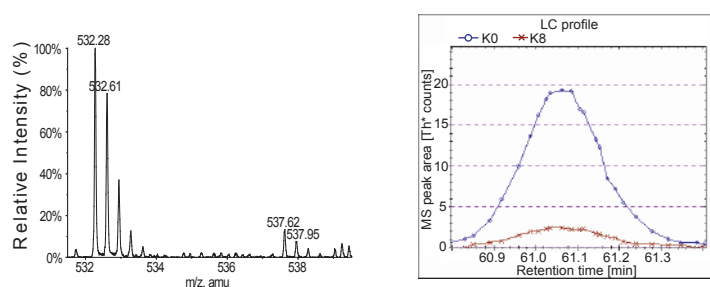
KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=12%



DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=11%



KTVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=11%



## Flag-H3.3 cells (K0) & HeLa cells (K8) mixing control

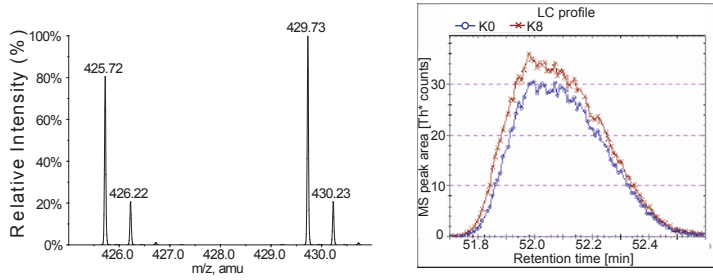
Histone	Sequence	charge	K8/(K8+K0)	average	StDV
Bulk H3	EIAQDFK	2H <sup>+</sup>	54.1%	56.6%	1.9%
	KLPFQR	2H <sup>+</sup>	56.5%		
	RVTIMPK	2H <sup>+</sup>	58.6%		
	VTIMPK	2H <sup>+</sup>	57.0%		
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	55.9%	56.1%	3.2%
	KESYSVYVYK	2H <sup>+</sup>	59.3%		
	QVHPDTGISSK	3H <sup>+</sup>	53.0%		
Bulk H2A	VTIAQGGVLPNIQAVLLPK	2H <sup>+</sup>	58.1%	55.9%	3.1%
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	53.7%		
Bulk H4	DAVYTEHAK	2H <sup>+</sup>	57.2%	55.3%	2.3%
	DNIQGITKPAIR	2H <sup>+</sup>	57.5%		
	DNIQGITKPAIR	3H <sup>+</sup>	52.5%		
	KTVTAMDVVYALK	3H <sup>+</sup>	55.8%		
	DAVYTEHAK	3H <sup>+</sup>	53.2%		
	TVTAMDVVYALK	2H <sup>+</sup>	53.2%		
	TVTAMDVVYALKR	3H <sup>+</sup>	57.9%		
Affinity purified FLAG-H3.3	EIAQDFK	2H <sup>+</sup>	0%	0%	0%
	KLPFQR	2H <sup>+</sup>	0%		
	RVTIMPK	2H <sup>+</sup>	0%		
	VTIMPK	2H <sup>+</sup>	0%		
Affinity purified H3.3	EIAQDFK	2H <sup>+</sup>	0%	0%	0%
	KLPFQR	2H <sup>+</sup>	0%		
	RVTIMPK	2H <sup>+</sup>	0%		
	VTIMPK	2H <sup>+</sup>	0%		
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	0.2%	0.6%	0.5%
	LLLPGELAK	2H <sup>+</sup>	1.1%		
	ESYSVYVYK	3H <sup>+</sup>	0.1%		
	QVHPDTGISSK	3H <sup>+</sup>	1.0%		
Affinity purified H2A	NDEELNKLLGK	3H <sup>+</sup>	0.2%	0.2%	0.1%
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	0.1%		
Affinity purified H4	DAVYTEHAK	3H <sup>+</sup>	0%	0%	0%
	DNIQGITKPAIR	3H <sup>+</sup>	0%		
	TVTAMDVVYALK	2H <sup>+</sup>	0%		
	TVTAMDVVYALKR	3H <sup>+</sup>	0%		

Fig. S7 Page 1

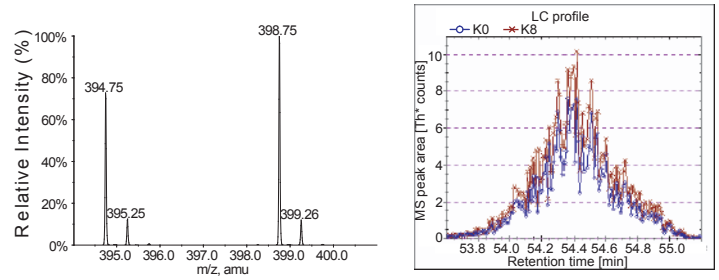
# Flag-H3.3 cells (K0) & HeLa cells (K8) mixing control

## Bulk H3

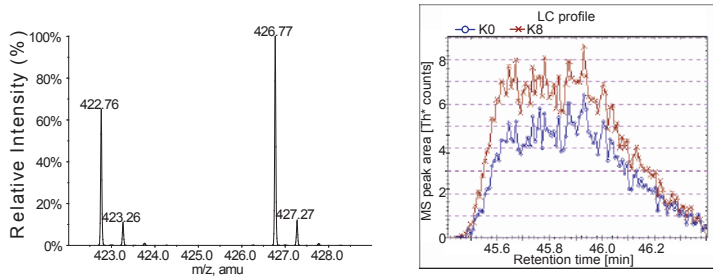
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=54%



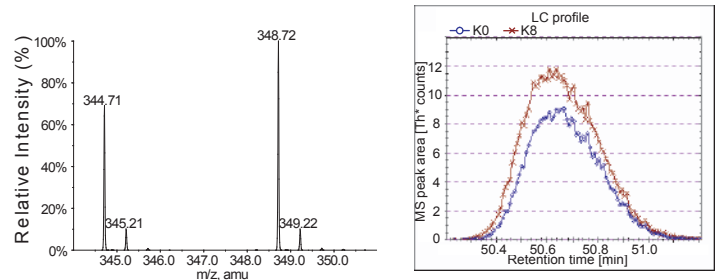
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=57%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=59%

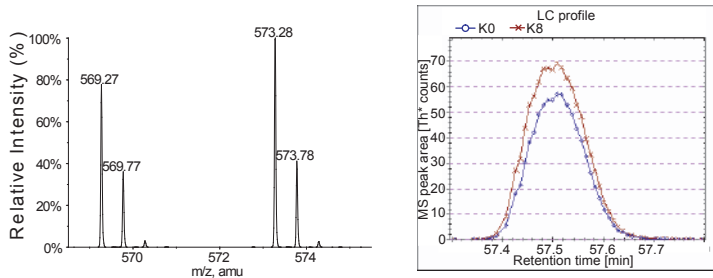


VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=57%

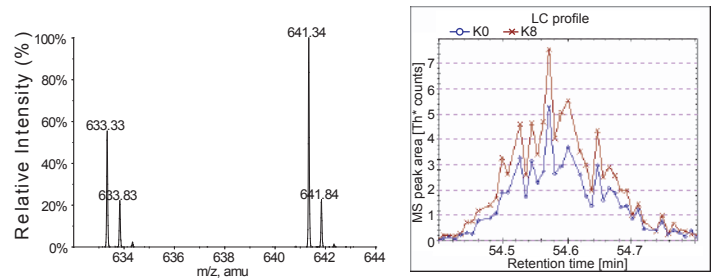


## Bulk H2B

ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=56%



KESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=59%



QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=53%

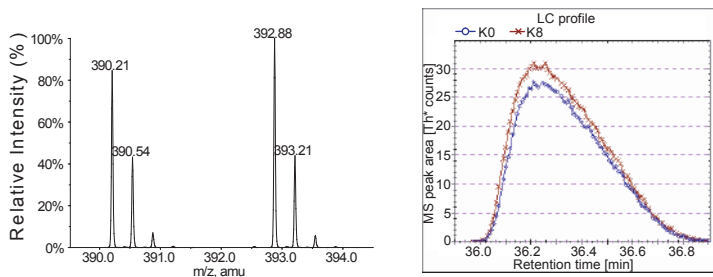
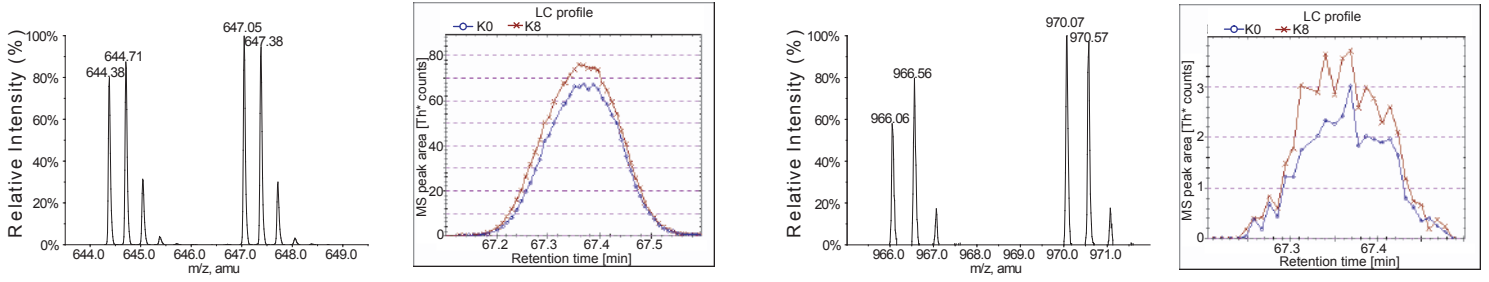


Fig. S7 Page 2

# Flag-H3.3 cells (K0) & HeLa cells (K8) mixing control

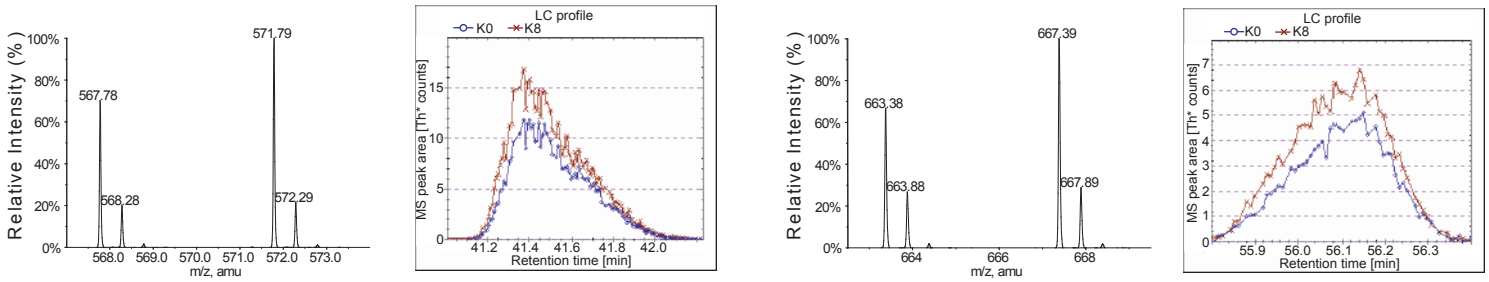
## Bulk H2A

VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=54% VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=58%

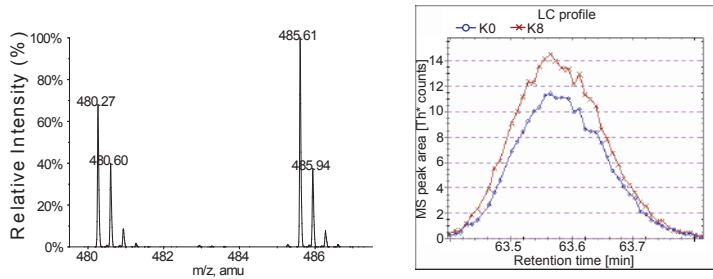


## Bulk H4

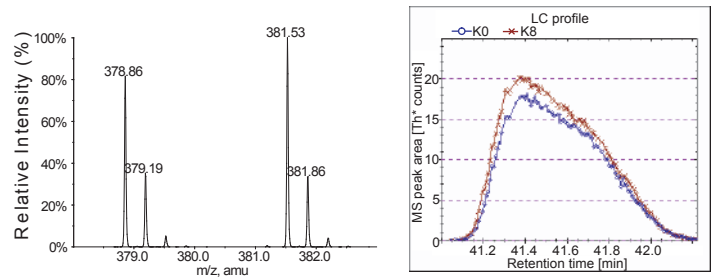
DAVTYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=57% DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=58%



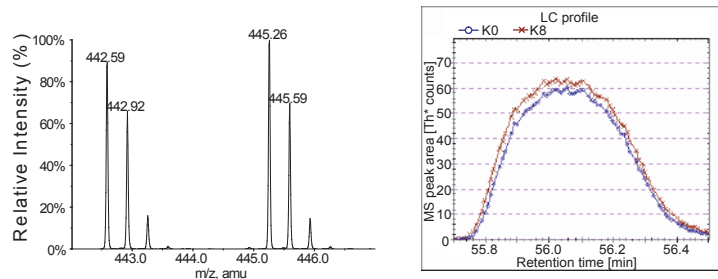
KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=56%



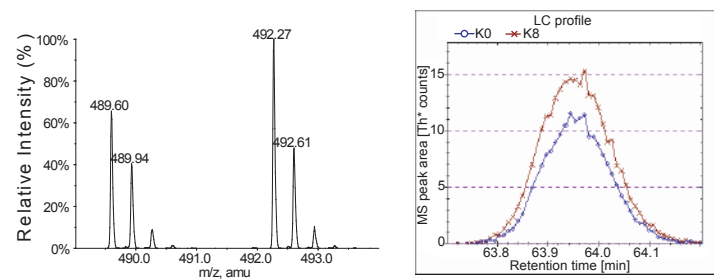
DAVTYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=53%



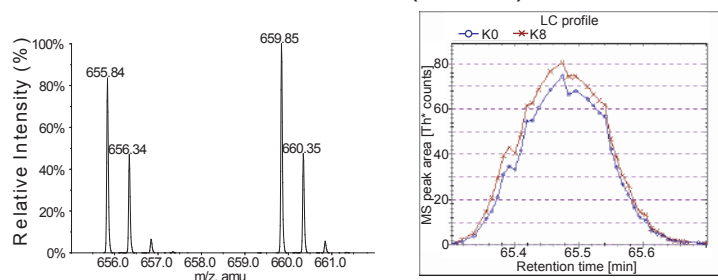
DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=53%



TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=58%



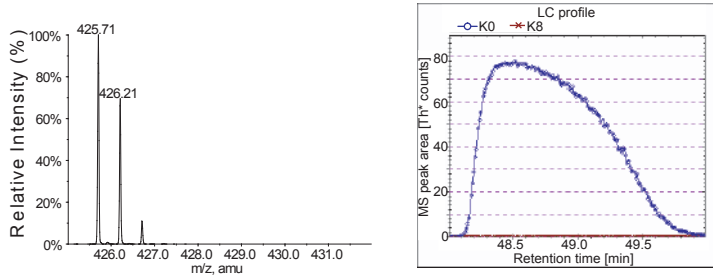
TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=53%



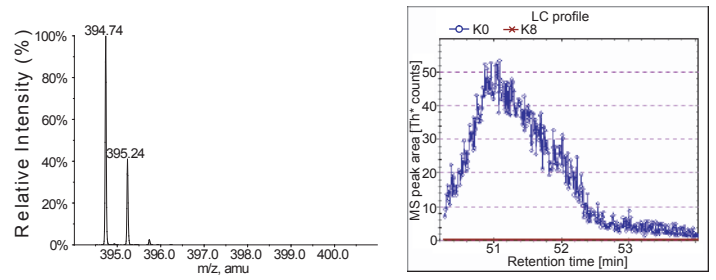
# Flag-H3.3 cells (K0) & HeLa cells (K8) mixing control

## Affinity purified Flag-H3.3

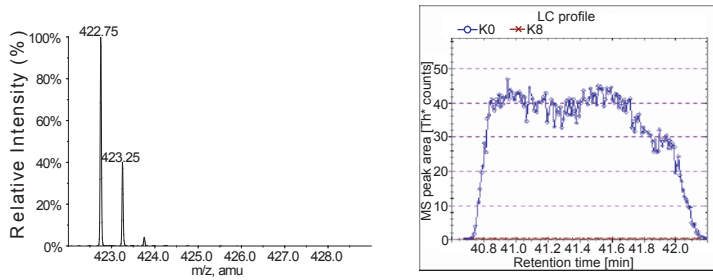
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=0%



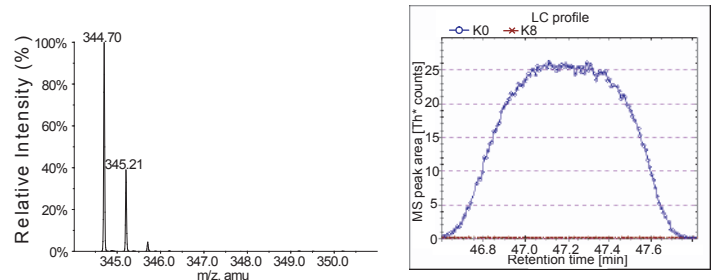
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=0%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=0%

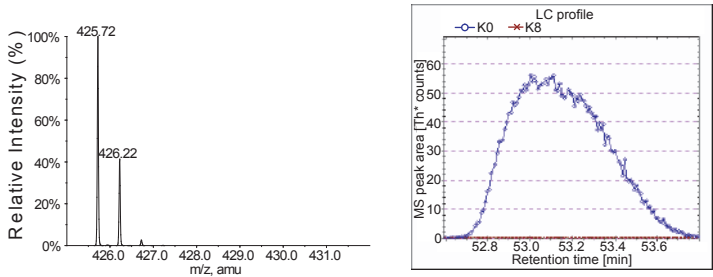


VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=0%

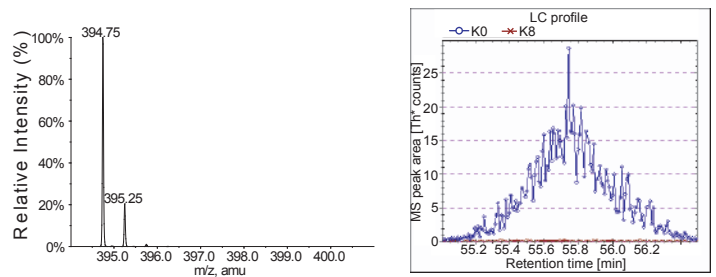


## Affinity purified H3.3

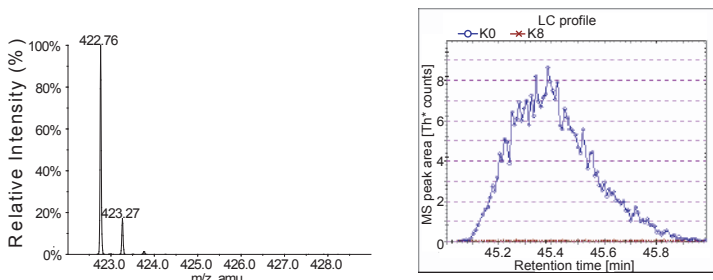
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=0%



KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=0%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=0%



VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=0%

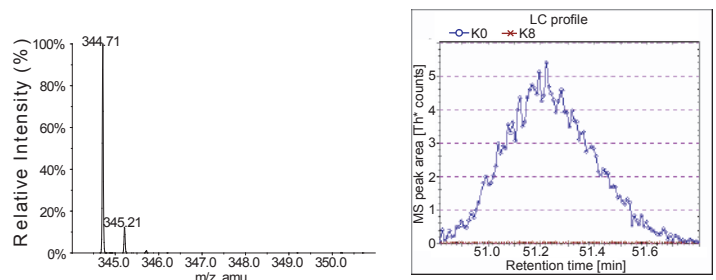
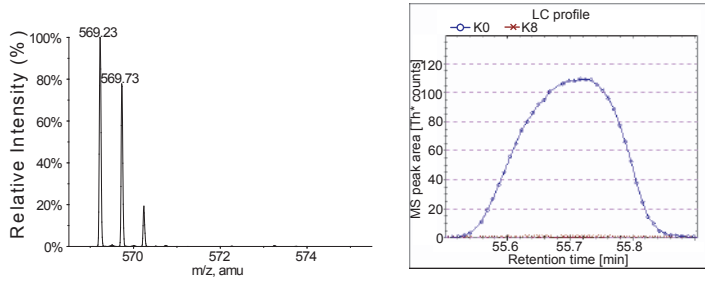


Fig. S7 Page 4

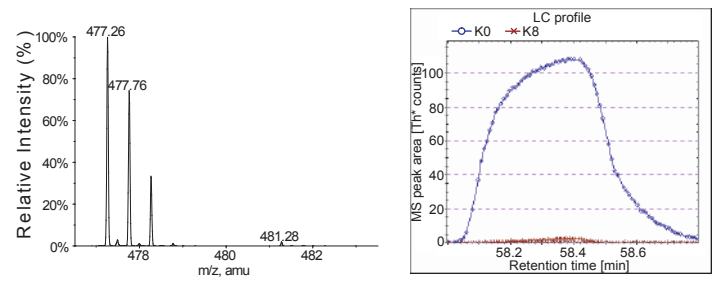
# Flag-H3.3 cells (K0) & HeLa cells (K8) mixing control

## Affinity purified H2B

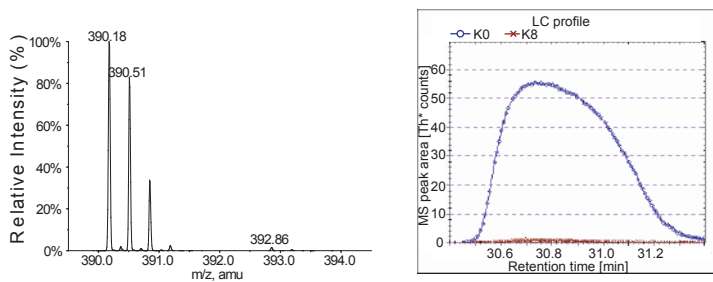
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=0.2%



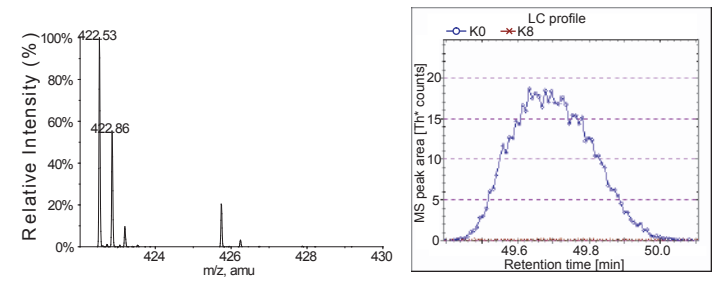
LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=1.1%



QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=1.0%

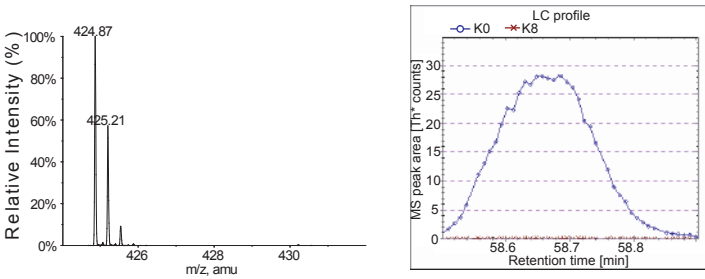


KESYSVYVYK, 3H<sup>+</sup>, K8/(K8+K0)=0.1%

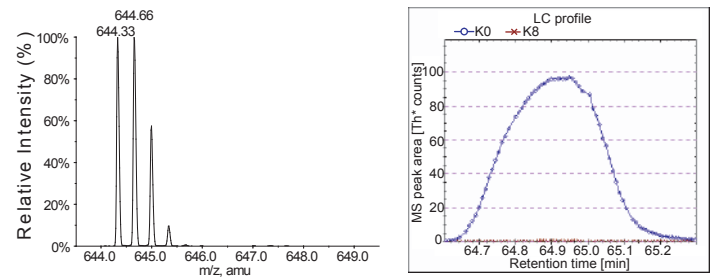


## Affinity purified H2A

NDEELNKLLGK, 3H<sup>+</sup>, K8/(K8+K0)=0.2%

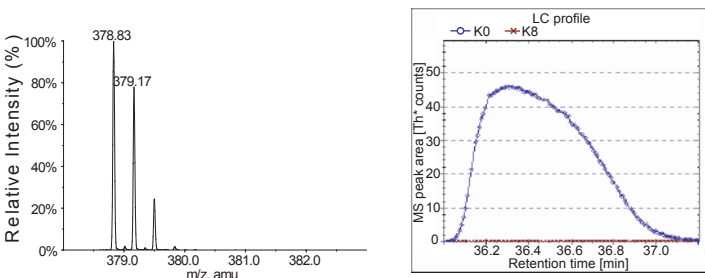


VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=0.1%

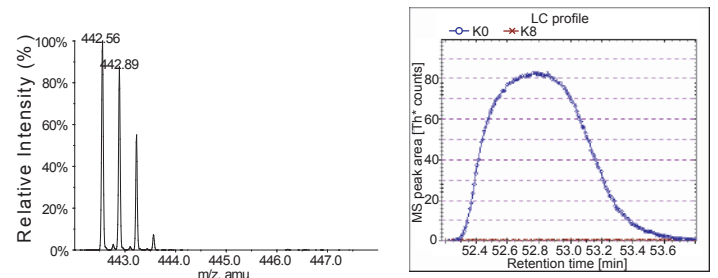


## Affinity purified H4

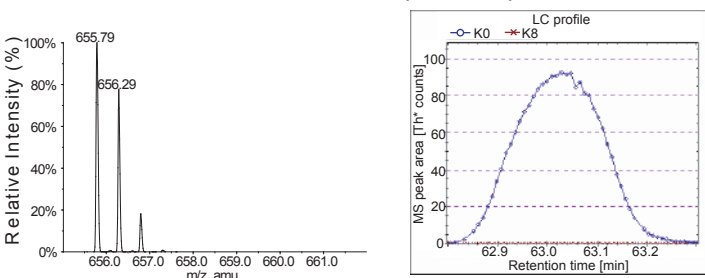
DAVTYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=0%



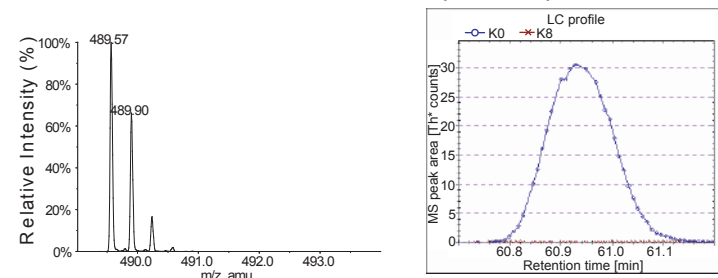
DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=0%



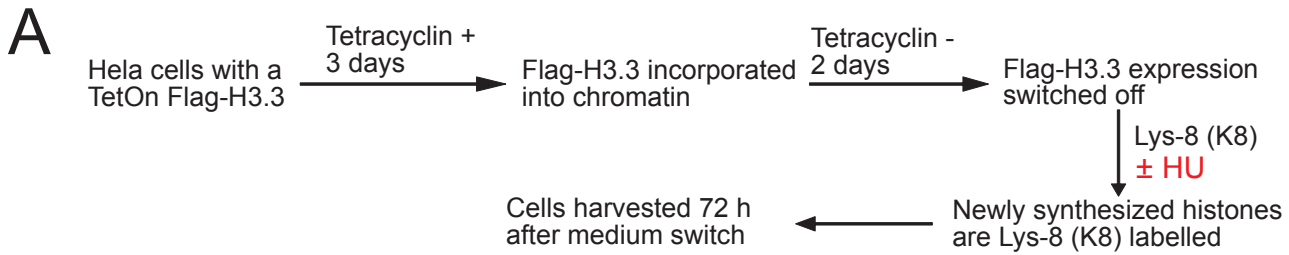
TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=0%



TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=0%



# Flag-H3.3, “on” to “off”, 72 h with or without HU, asynchronized



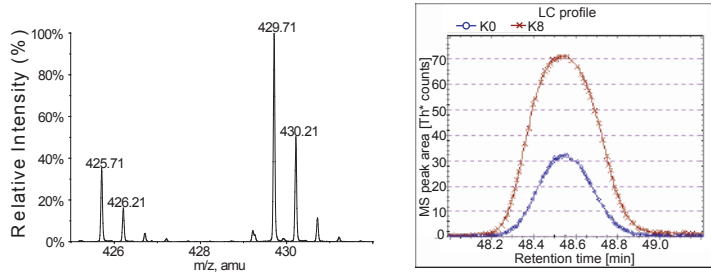
## **B** Flag-H3.3, “on” to “off”, 72 h without HU, asynchronized

Histone	Sequence	charge	K8/(K8+K0)	average	StDV
Bulk H3	EIAQDFK	2H <sup>+</sup>	76%	77%	1.3%
	KLPFQR	2H <sup>+</sup>	78%		
	RVTIMPK	2H <sup>+</sup>	77%		
	VTIMPK	2H <sup>+</sup>	75%		
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	80%	78%	2.2%
	LLPGELAK	2H <sup>+</sup>	77%		
	QVHPDTGISSK	2H <sup>+</sup>	79%		
	QVHPDTGISSK	3H <sup>+</sup>	75%		
Bulk H2A	NDEELNKLLGK	3H <sup>+</sup>	83%	82%	2.1%
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	80%		
Bulk H4	DAVYTEHAK	2H <sup>+</sup>	78%	76%	1.9%
	DAVYTEHAKR	3H <sup>+</sup>	77%		
	DNIQGITKPAIR	2H <sup>+</sup>	77%		
	DNIQGITKPAIR	3H <sup>+</sup>	73%		
	TVTAMDVVYALK	2H <sup>+</sup>	76%		
Affinity purified FLAG-H3.3	EIAQDFK	2H <sup>+</sup>	4.4%	2.9%	1.1%
	KLPFQR	2H <sup>+</sup>	2.5%		
	MDYK(Ac)DDDDK	2H <sup>+</sup>	1.8%		
	MDYK(Ac)DDDDKAR	3H <sup>+</sup>	2.9%		
Affinity purified H3.3	EIAQDFK	2H <sup>+</sup>	26%	20%	5.1%
	KLPFQR	2H <sup>+</sup>	15%		
	RVTIMPK	2H <sup>+</sup>	16%		
	VTIMPK	2H <sup>+</sup>	21%		
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	70%	70%	0.7%
	QVHPDTGISSK	3H <sup>+</sup>	69%		
Affinity purified H2A	VTIAQGGVLPNIQAVLLPK	2H <sup>+</sup>	74%	73%	2.1%
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	71%		
Affinity purified H4	DAVYTEHAK	2H <sup>+</sup>	8.1%	7.6%	2.1%
	DNIQGITKPAIR	2H <sup>+</sup>	5.4%		
	TVTAM(Oxidation)DVVYALK	2H <sup>+</sup>	6.3%		
	TVTAMDVVYALKR	3H <sup>+</sup>	11%		
	KTVTAM(Oxidation)DVVYALK	3H <sup>+</sup>	7.3%		

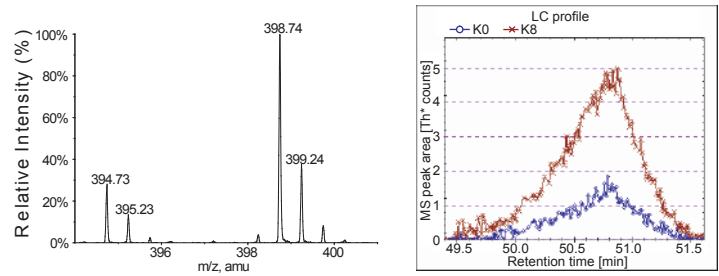
# Flag-H3.3, "on" to "off", 72 h without HU, asynchronized

## Bulk H3

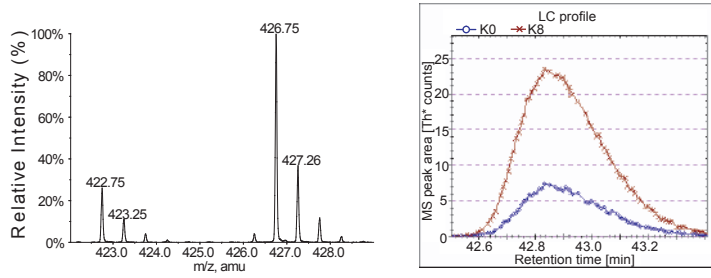
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=76%



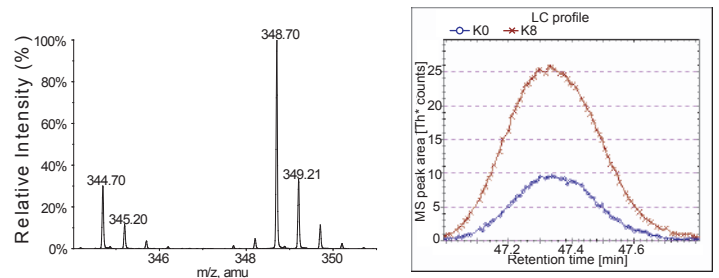
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=78%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=77%

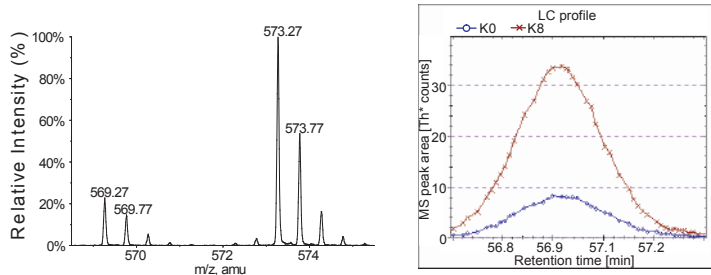


VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=75%

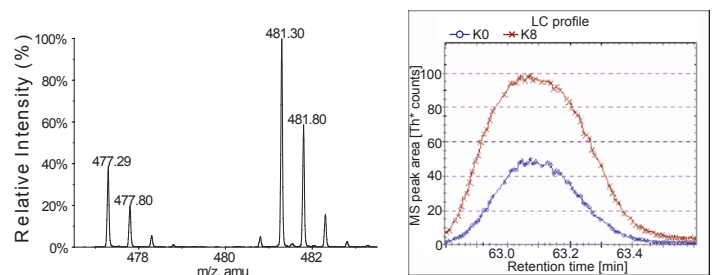


## Bulk H2B

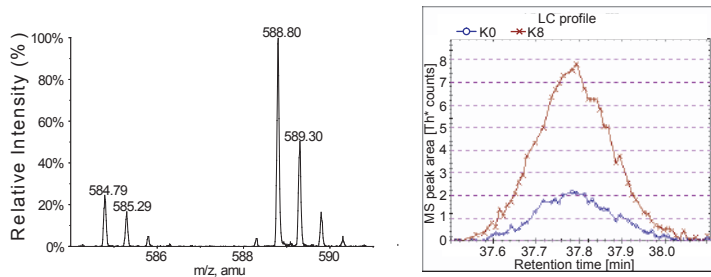
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=80%



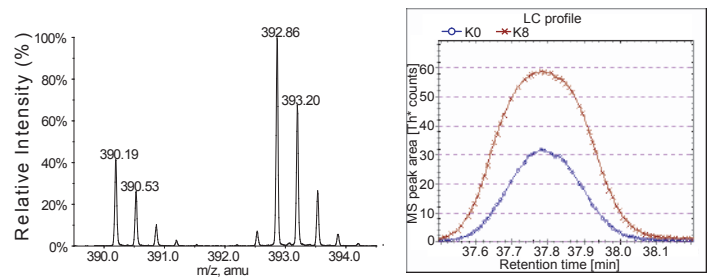
LLLPGELAK, 2H<sup>+</sup>, K8/(K8+K0)=77%



QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=79%



QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=75%

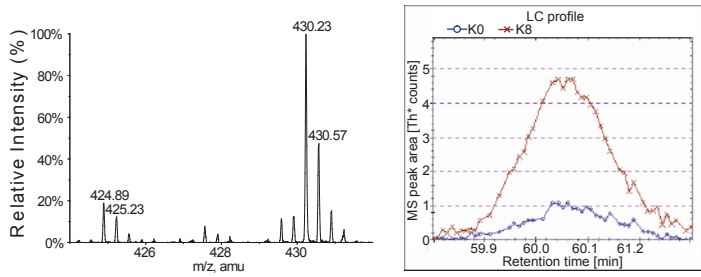




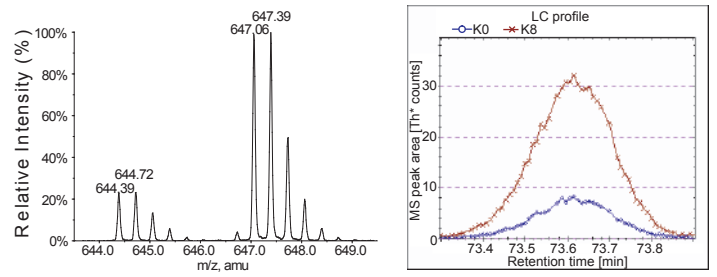
# Flag-H3.3, "on" to "off", 72 h without HU, asynchronized

## Bulk H2A

NDEELNKLLGK, 3H<sup>+</sup>, K8/(K8+K0)=83%

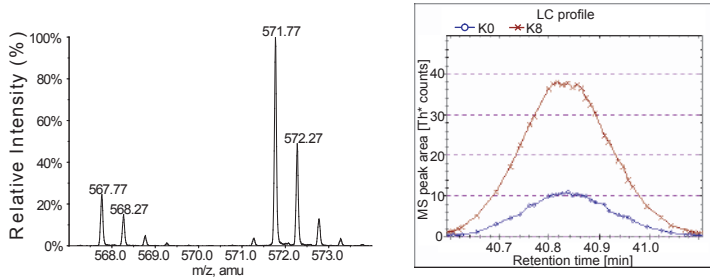


VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=80%

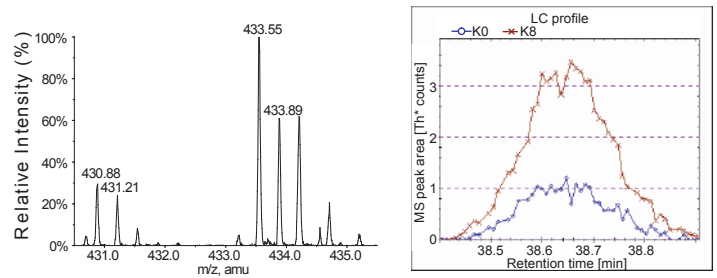


## Bulk H4

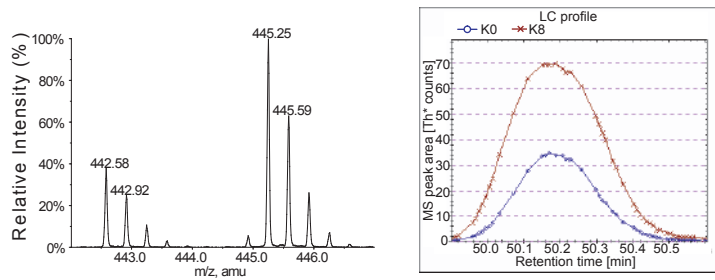
DAVTYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=78%



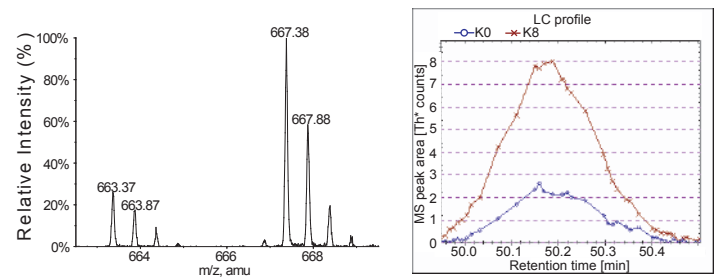
DAVTYTEHAKR, 3H<sup>+</sup>, K8/(K8+K0)=77%



DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=73%



DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=77%



TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=76%

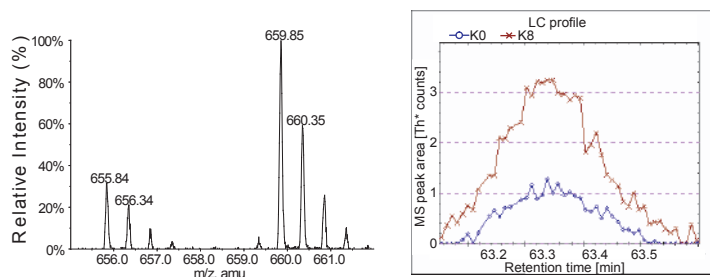
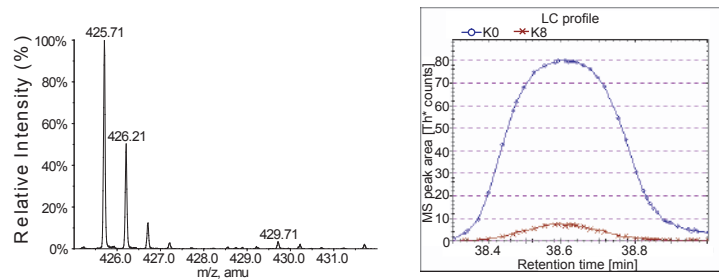


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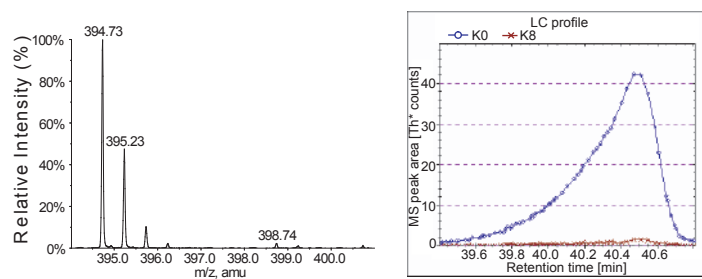
# Flag-H3.3, "on" to "off", 72 h without HU, asynchronized

## Affinity purified Flag-H3.3

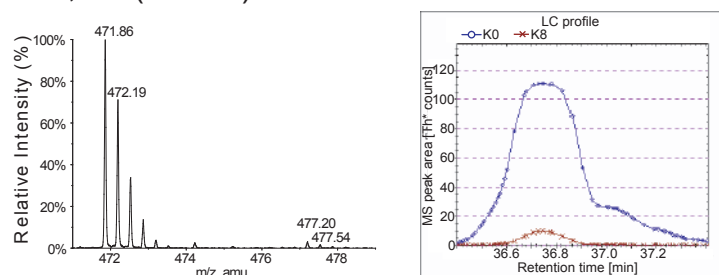
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=4.4%



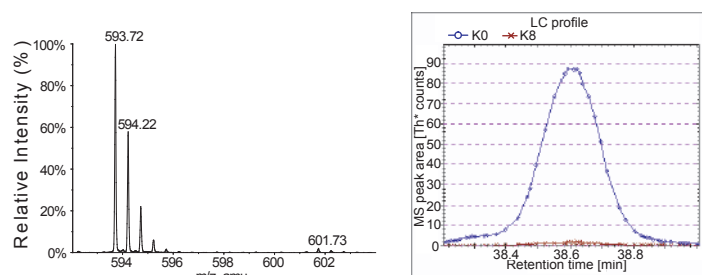
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=2.5%



MDYK(Ac)DDDDKAR (FLAG), 3H<sup>+</sup>, K8/(K8+K0)=2.9%

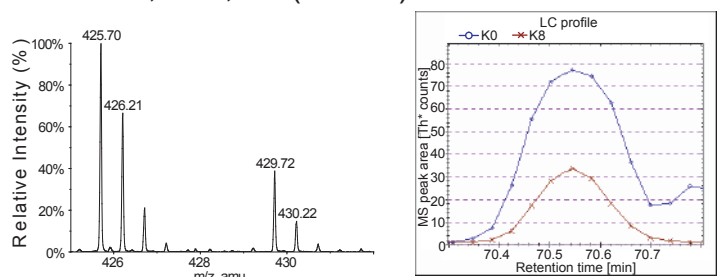


MDYK(Ac)DDDDK (FLAG), 2H<sup>+</sup>, K8/(K8+K0)=1.8%

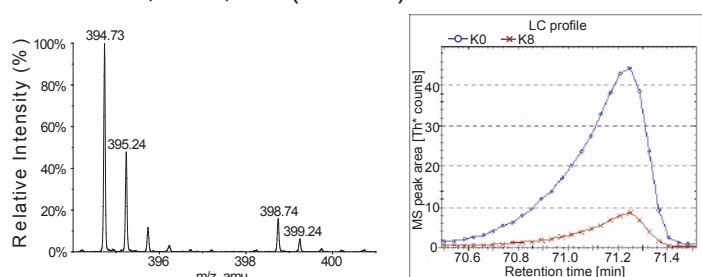


## Affinity purified H3.3

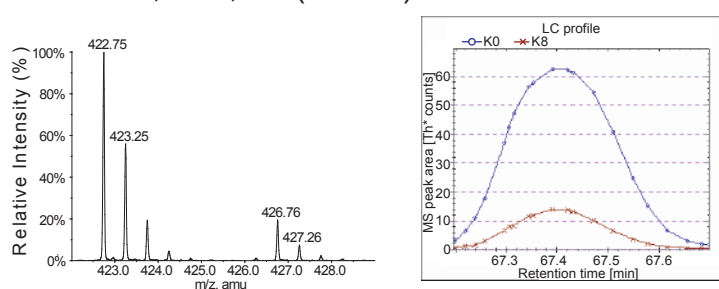
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=26%



KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=15%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=16%



VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=21%

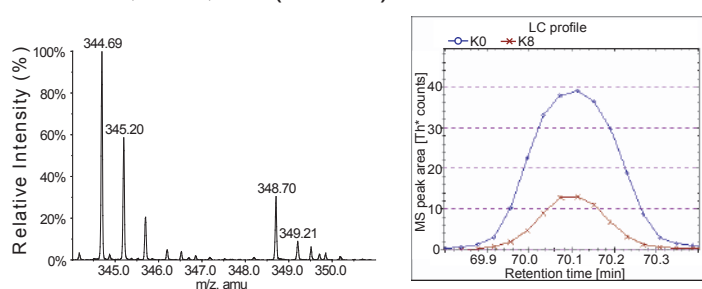
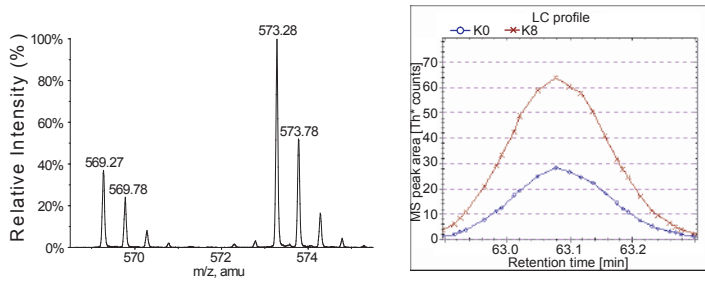


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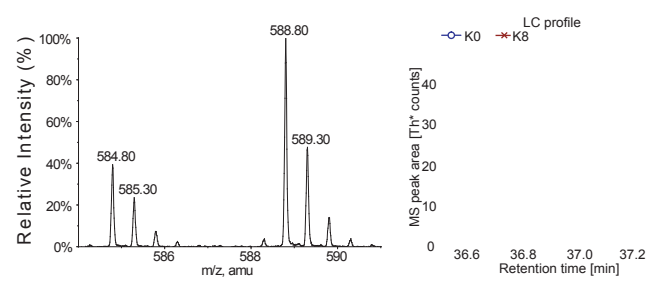
# Flag-H3.3, "on" to "off", 72 h without HU, asynchronized

## Affinity purified H2B

ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=70%

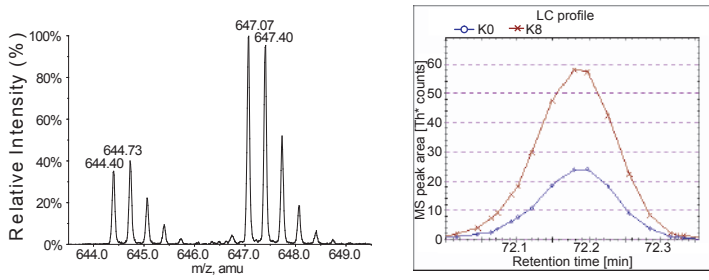


QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=69%

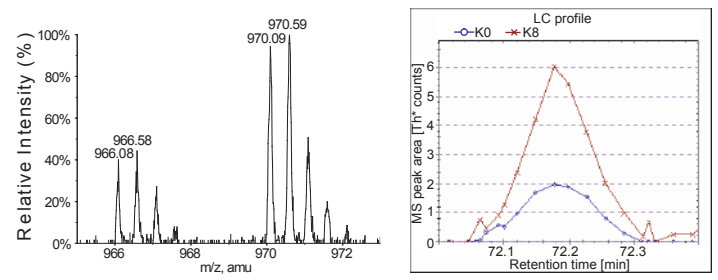


## Affinity purified H2A

VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=71%

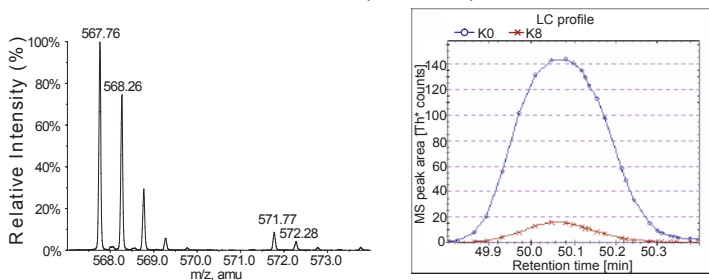


VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=74%

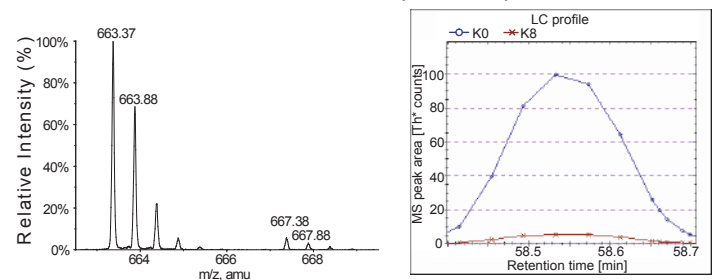


## Affinity purified H4

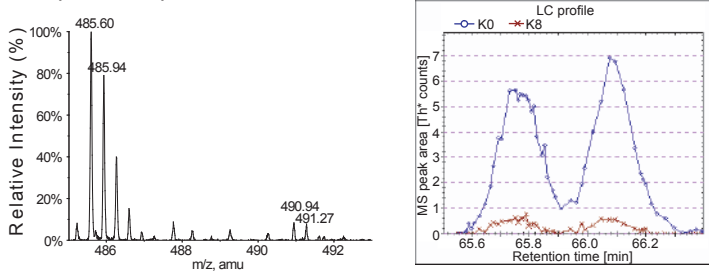
DAVTYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=8.1%



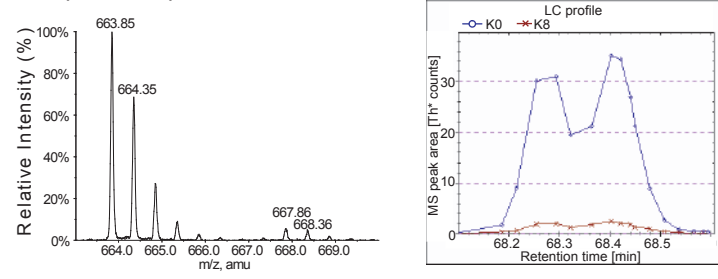
DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=5.4%



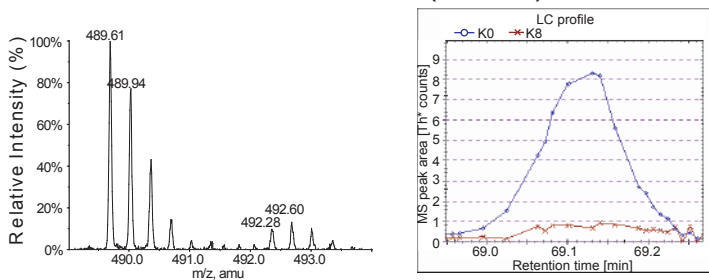
KTVTAM(Oxidation)DVVYALK, 3H<sup>+</sup>,  
K8/(K8+K0)=7.3%



TVTAM(Oxidation)DVVYALK, 2H<sup>+</sup>,  
K8/(K8+K0)=6.3%



TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=11%



## Flag-H3.3, “on” to “off”, 72 h with HU, asynchronized

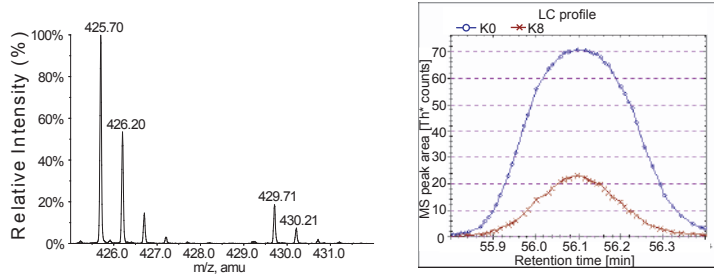
Histone	Sequence	charge	K8/(K8+K0)	average	StDV
Bulk H3	EIAQDFK	2H <sup>+</sup>	17%	15%	2.1%
	KLPFQR	2H <sup>+</sup>	13%		
	RVTIMPK	2H <sup>+</sup>	13%		
	VTIMPK	2H <sup>+</sup>	16%		
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	26%	25%	1.2%
	LLLPGELAK	2H <sup>+</sup>	26%		
	QVHPDTGISSK	2H <sup>+</sup>	24%		
Bulk H2A	NDEELNK	2H <sup>+</sup>	32%	32%	
Bulk H4	DAVYTEHAK	2H <sup>+</sup>	11%	13%	2.8%
	DAVYTEHAK	3H <sup>+</sup>	17%		
	DNIQGITKPAIR	2H <sup>+</sup>	10%		
	DNIQGITKPAIR	3H <sup>+</sup>	14%		
	TVTAMDVVYALK	2H <sup>+</sup>	14%		
Affinity purified FLAG-H3.3	EIAQDFK	2H <sup>+</sup>	1.4%	1.0%	0.6%
	KLPFQR	2H <sup>+</sup>	0.5%		
	MDYK(Ac)DDDDKAR	3H <sup>+</sup>	1.6%		
	RVTIMPK	2H <sup>+</sup>	0.3%		
Affinity purified H3.3	EIAQDFK	2H <sup>+</sup>	11%	7.6%	2.3%
	KLPFQR	2H <sup>+</sup>	6.5%		
	RVTIMPK	2H <sup>+</sup>	6.9%		
	VTIM(Oxidation)PK	2H <sup>+</sup>	6.0%		
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	29%	28%	1.4%
	QVHPDTGISSK	2H <sup>+</sup>	27%		
Affinity purified H2A	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	33%	33%	
Affinity purified H4	DAVYTEHAK	2H <sup>+</sup>	4.2%	4.5%	1.1%
	TVTAM(Oxidation)DVVYALK	2H <sup>+</sup>	4.0%		
	TVTAMDVVYALK	2H <sup>+</sup>	3.6%		
	KTVTAM(Oxidation)DVVYALK	3H <sup>+</sup>	6.0%		

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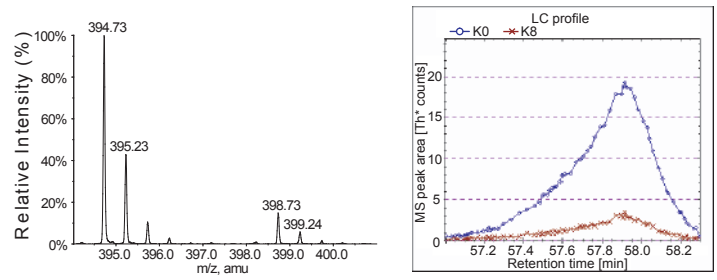
# Flag-H3.3, "on" to "off", 72 h with HU, asynchronized

## Bulk H3

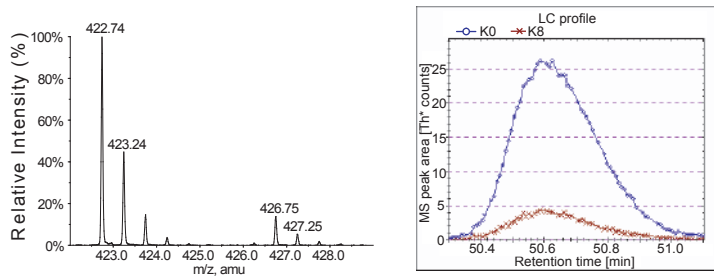
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=17%



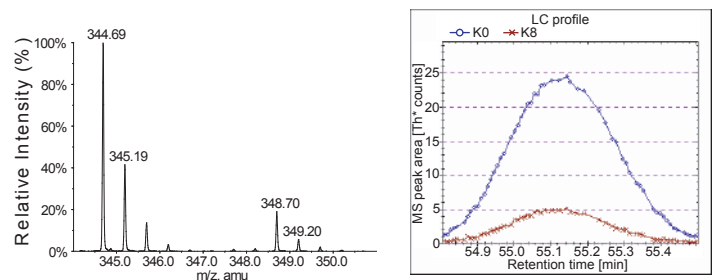
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=13%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=13%

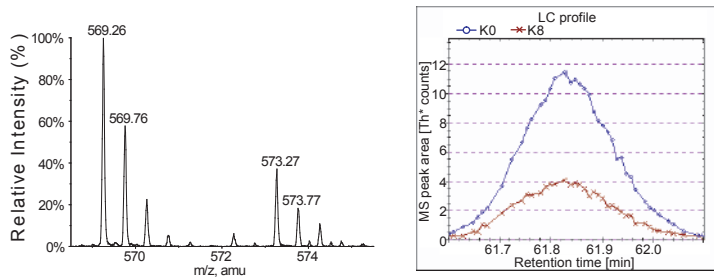


VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=16%

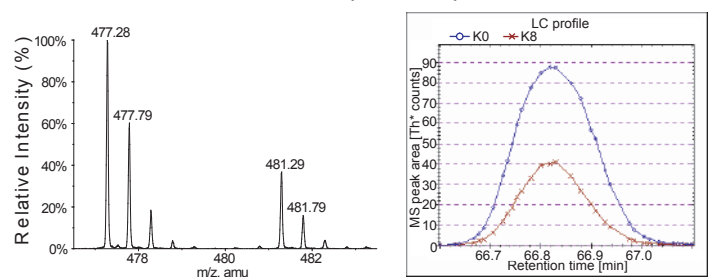


## Bulk H2B

ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=26%



LLLPGELAK, 3H<sup>+</sup>, K8/(K8+K0)=26%



QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=24%

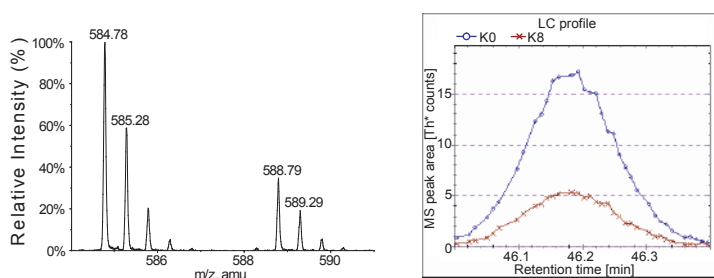
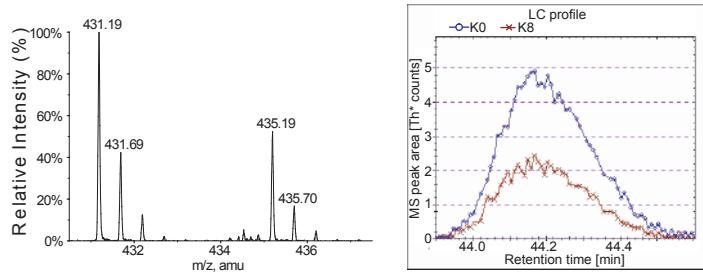


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# Flag-H3.3, "on" to "off", 72 h with HU, asynchronized

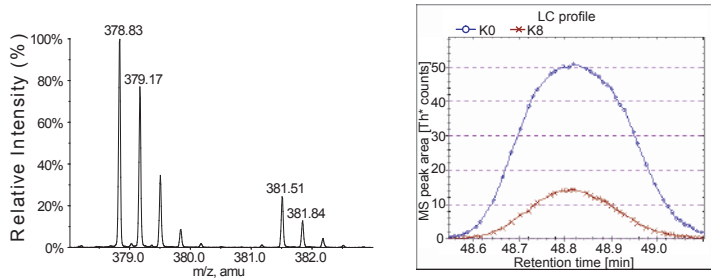
## Bulk H2A

NDEELNK, 2H<sup>+</sup>, K8/(K8+K0)=32%

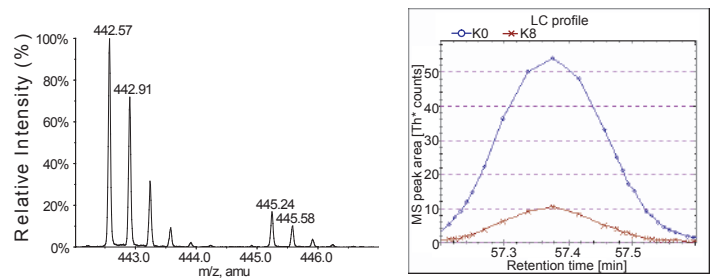


## Bulk H4

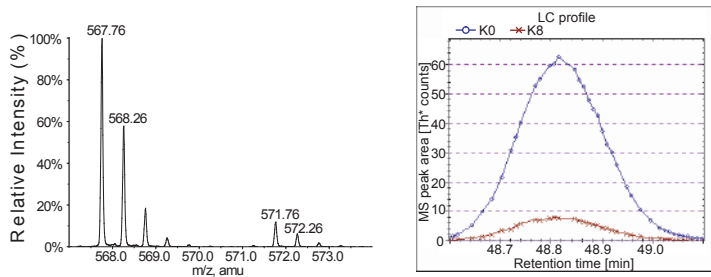
DAVYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=17%



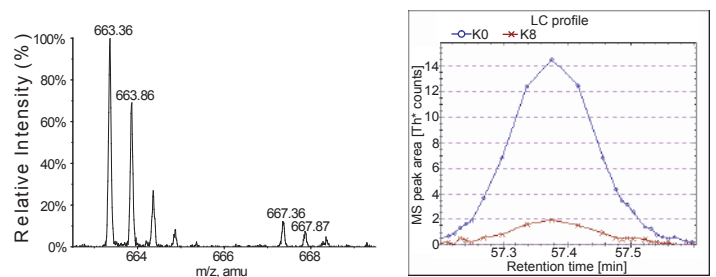
DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=14%



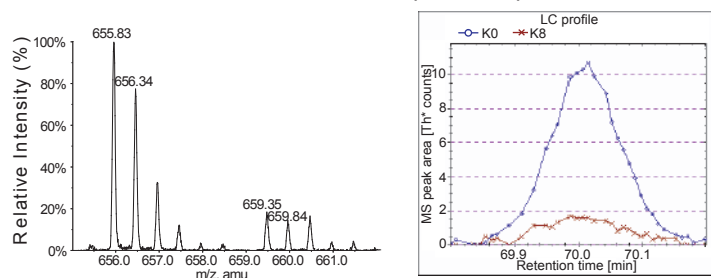
DAVYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=11%



DNIQGITKPAIR, 2H<sup>+</sup>, K8/(K8+K0)=10%



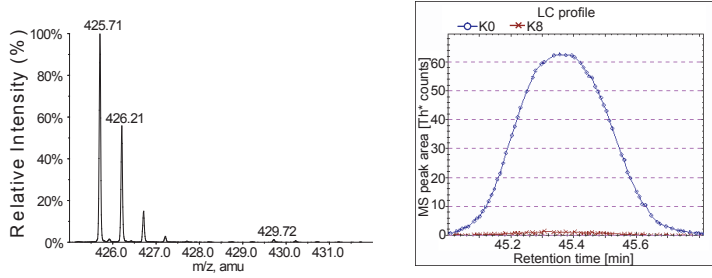
TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=14%



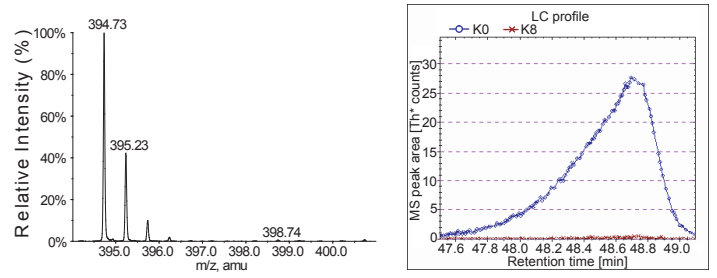
# Flag-H3.3, "on" to "off", 72 h with HU, asynchronized

## Affinity purified Flag-H3.3

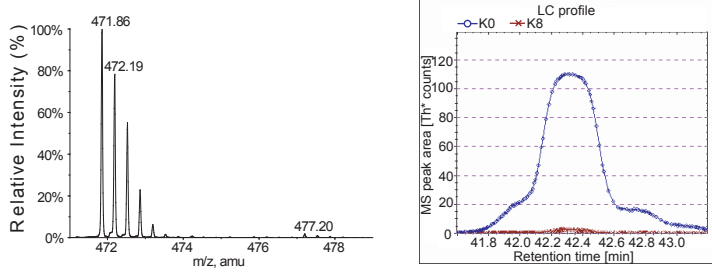
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=1.4%



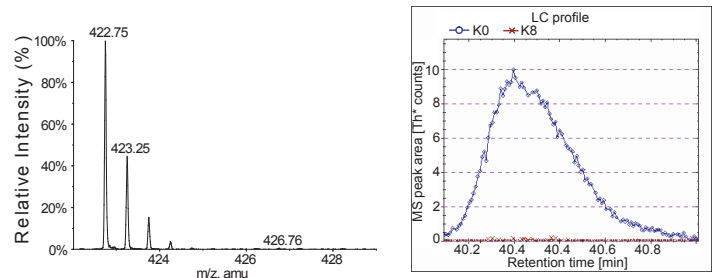
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=0.5%



MDYK(Ac)DDDDKAR (FLAG), 3H<sup>+</sup>, K8/(K8+K0)=1.6%

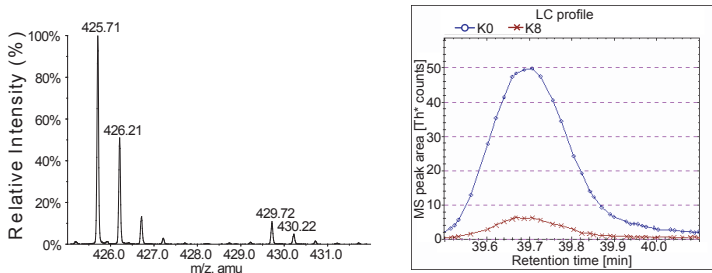


RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=0.3%

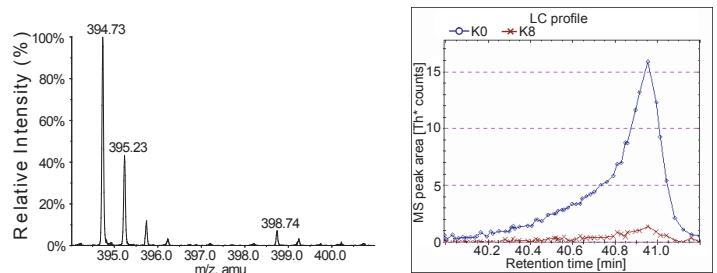


## Affinity purified H3.3

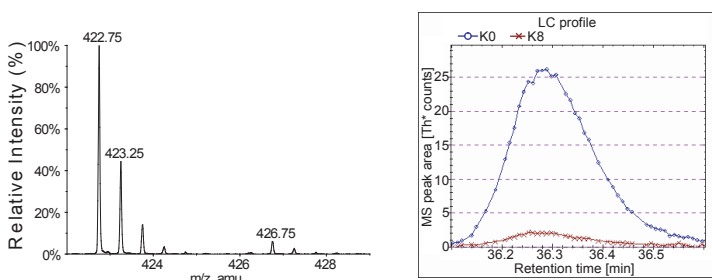
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=11%



KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=6.5%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=6.9%



VTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=6.0%

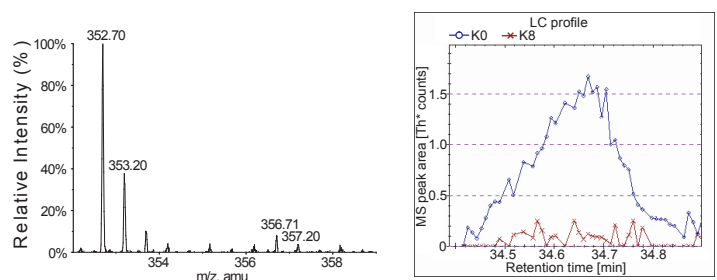
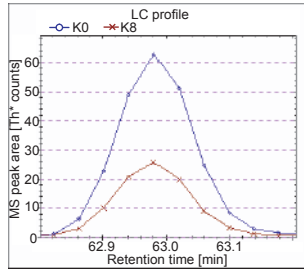
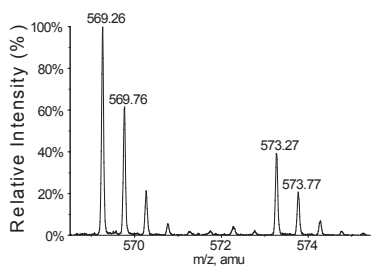


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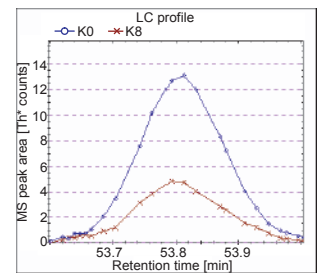
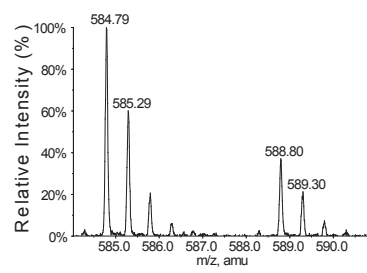
# Flag-H3.3, "on" to "off", 72 h with HU, asynchronized

## Affinity purified H2B

ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=29%

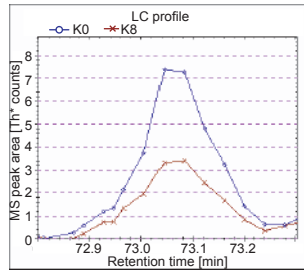
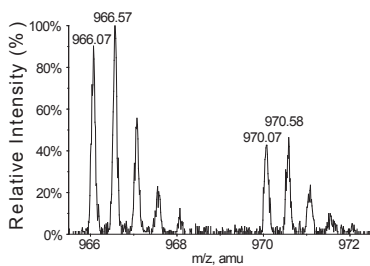


QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=27%



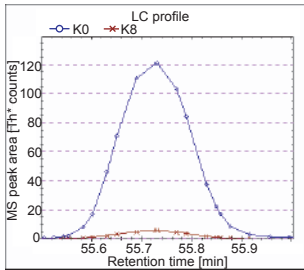
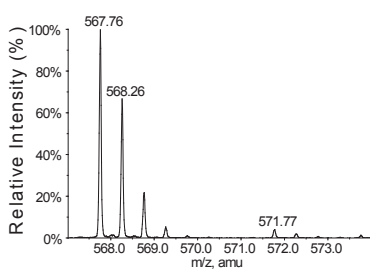
## Affinity purified H2A

VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=33%

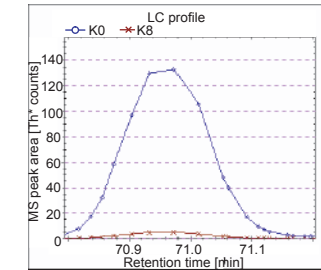
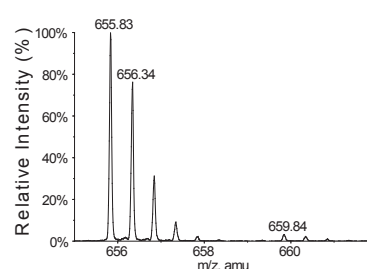


## Affinity purified H4

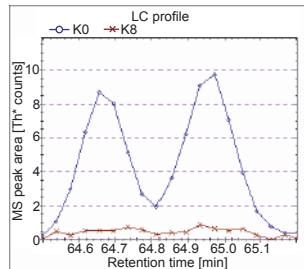
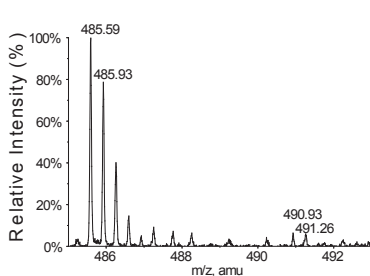
DAVTYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=4.2%



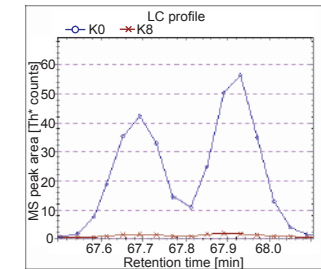
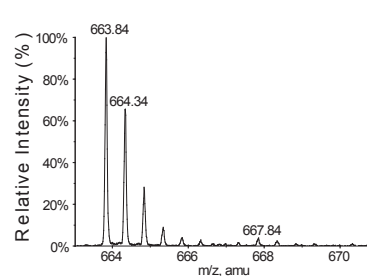
TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=3.6%



KTVTAM(Oxidation)DVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=6.0%

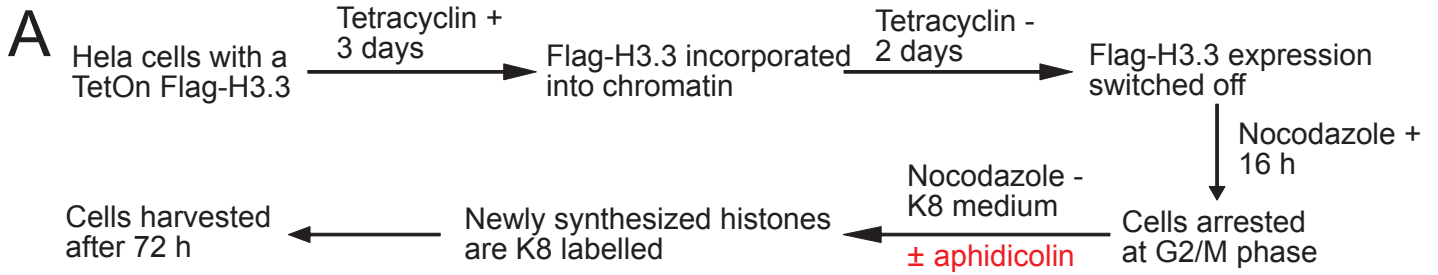


TVTAM(Oxidation)DVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=4.0%





# Flag-H3.3, “on” to “off”, 72 h with or without aphidicolin



**B**

	K8/(K8+K0) X 100%			
	72 h without aphidicolin		72 h with aphidicolin	
	Bulk	Affinity purified	Bulk	Affinity purified
H3	83%		8.7%	
H3.1	84%		0%	
H3.2	85%		8.3%	
H3.3	85%	11%	53%	2.5%
Flag-H3.3		1.0%		0.4%
H4	81%	5.2%	12%	2.5%
H2B	82%	70%	28%	31%
H2A	86%	77%	28%	31%

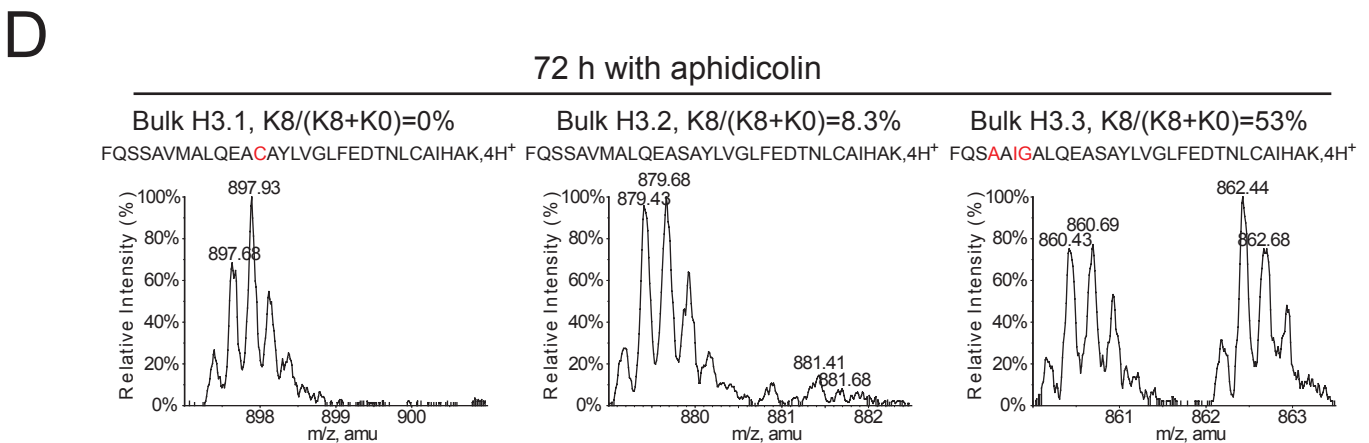
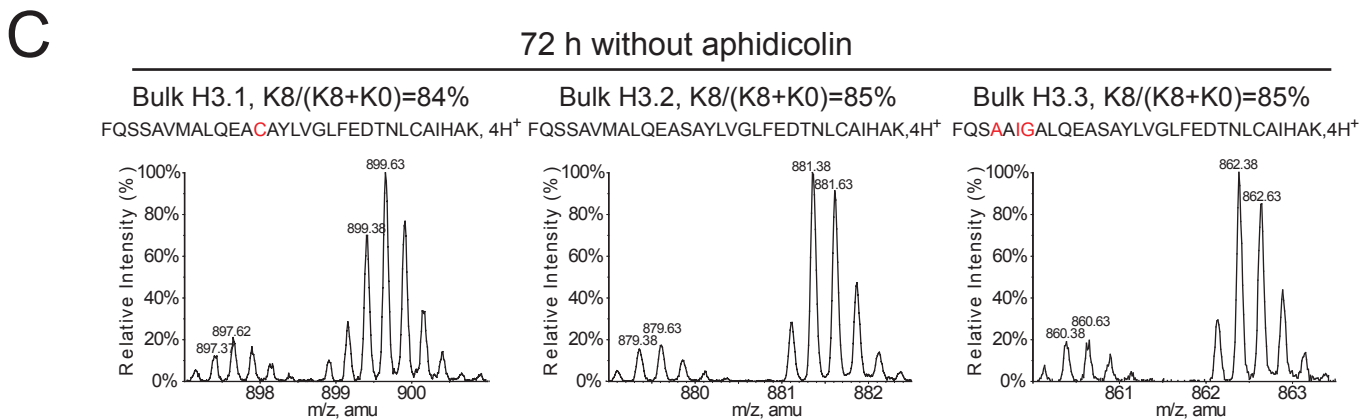


Fig. S9 Page 1

## Flag-H3.3, “on” to “off”, 72 h without aphidicolin

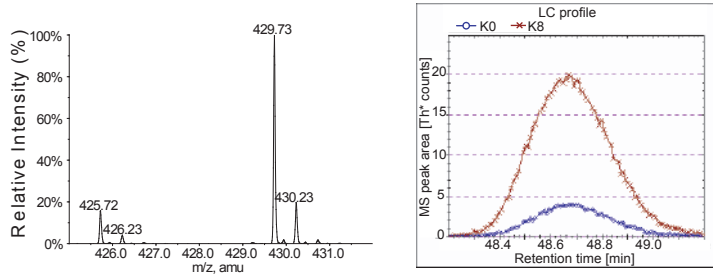
Histone	Sequence	charge	K8/(K8+K0)	average	StDV
Bulk H3	EIAQDFK	2H <sup>+</sup>	83.9%	83.2%	0.5%
	KLPFQR	2H <sup>+</sup>	83.2%		
	RVTIMPK	2H <sup>+</sup>	82.9%		
	VTIMPK	2H <sup>+</sup>	82.9%		
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	86.4%	81.8%	5.1%
	KESYSVYVYK	3H <sup>+</sup>	82.7%		
	QVHPDTGISSK	3H <sup>+</sup>	76.3%		
Bulk H2A	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	85.8%	85.8%	
Bulk H4	DAVYTEHAK	2H <sup>+</sup>	84.9%	81.0%	3.9%
	DNIQGITKPAIR	2H <sup>+</sup>	78.0%		
	KTVTAMDVVYALK	3H <sup>+</sup>	81.2%		
	DAVYTEHAK	3H <sup>+</sup>	76.3%		
	TVTAMDVVYALK	2H <sup>+</sup>	84.7%		
Affinity purified FLAG-H3.3	EIAQDFK	2H <sup>+</sup>	1.6%	1.0%	0.6%
	KLPFQR	2H <sup>+</sup>	0.4%		
	MDYK(Ac)DDDDKAR	3H <sup>+</sup>	1.4%		
	RVTIMPK	2H <sup>+</sup>	0.6%		
	VTIMPK	2H <sup>+</sup>	1.4%		
	VTIMPK (M Oxidation)	2H <sup>+</sup>	0.4%		
Affinity purified H3.3	EIAQDFK	2H <sup>+</sup>	17.0%	11.2%	3.9%
	KLPFQR	2H <sup>+</sup>	9.3%		
	RVTIMPK	2H <sup>+</sup>	9.3%		
	VTIMPK (M Oxidation)	2H <sup>+</sup>	9.2%		
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	72.4%	69.8%	4.1%
	KESYSVYVYK	3H <sup>+</sup>	71.8%		
	QVHPDTGISSK	2H <sup>+</sup>	71.3%		
	QVHPDTGISSK	3H <sup>+</sup>	63.6%		
Affinity purified H2A	VTIAQGGVLPNIQAVLLPK	2H <sup>+</sup>	81.0%	76.9%	5.9%
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	72.7%		
Affinity purified H4	DAVYTEHAK	3H <sup>+</sup>	5.8%	5.2%	1.2%
	DNIQGITKPAIR	3H <sup>+</sup>	6.4%		
	KTVTAMDVVYALK	3H <sup>+</sup>	3.6%		
	TVTAMDVVYALKR	3H <sup>+</sup>	4.8%		

Fig. S9 Page 2

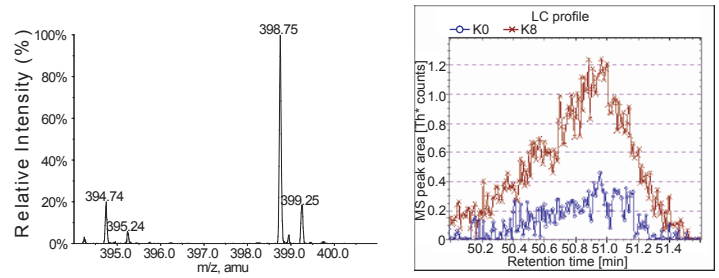
# Flag-H3.3, "on" to "off", 72 h without aphidicolin

## Bulk H3

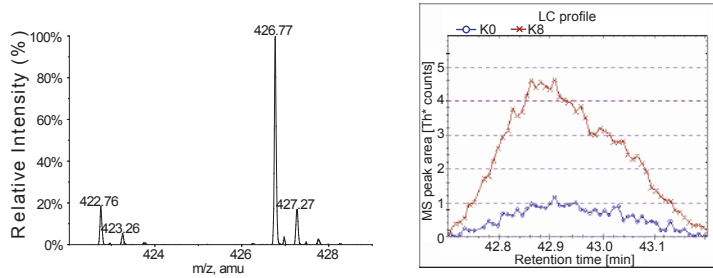
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=84%



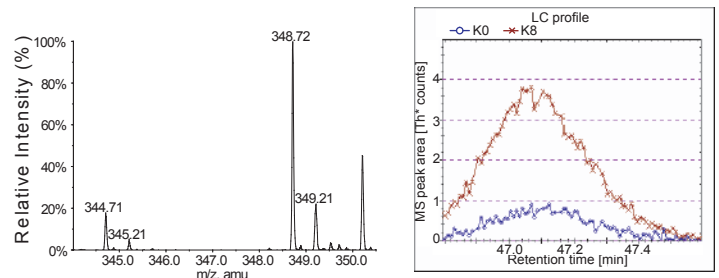
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=83%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=83%

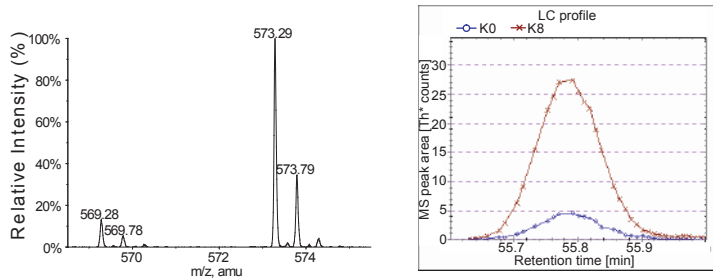


VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=83%

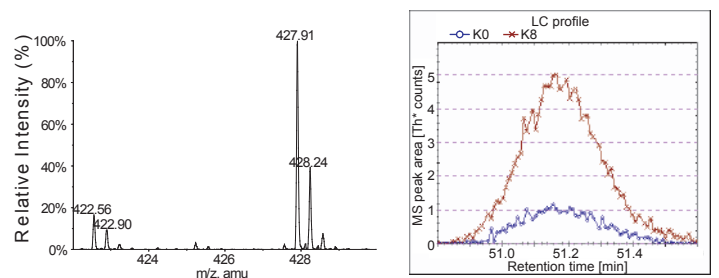


## Bulk H2B

ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=86%



KESYSVYVYK, 3H<sup>+</sup>, K8/(K8+K0)=83%



QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=76%

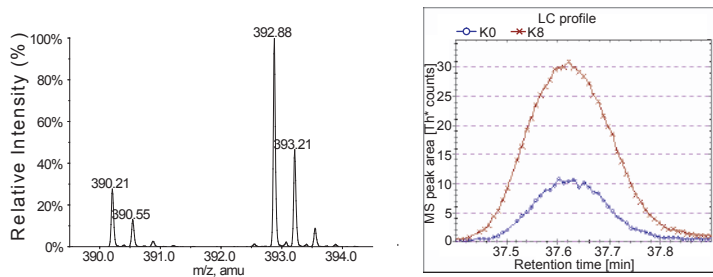
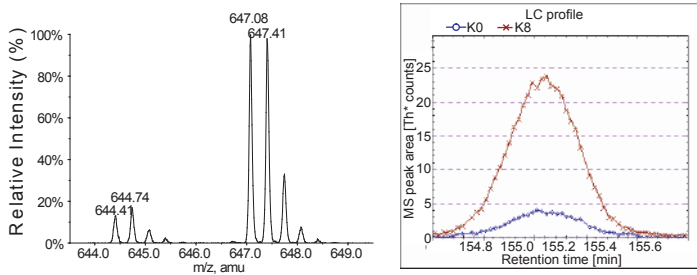


Fig. S9 Page 3

# Flag-H3.3, "on" to "off", 72 h without aphidicolin

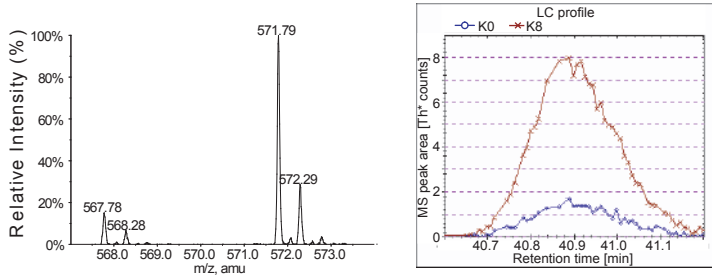
## Bulk H2A

VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=86%

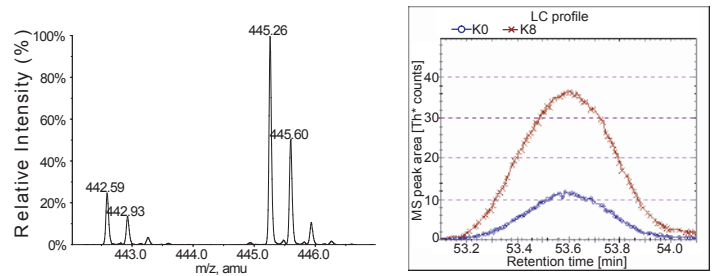


## Bulk H4

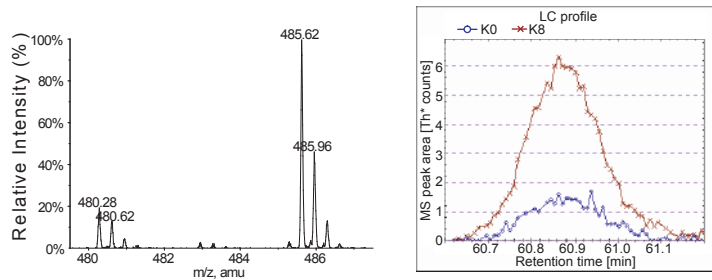
DAVYTEHAK, 2H<sup>+</sup>, K8/(K8+K0)=85%



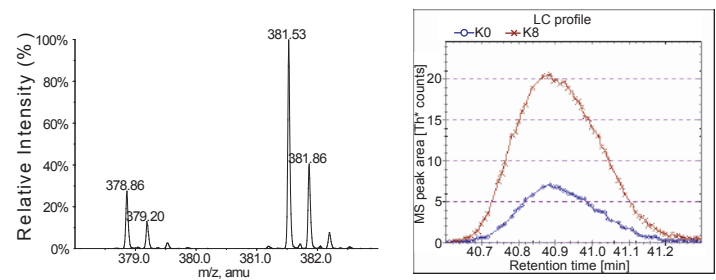
DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=78%



KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=81%



DAVYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=76%



TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=85%

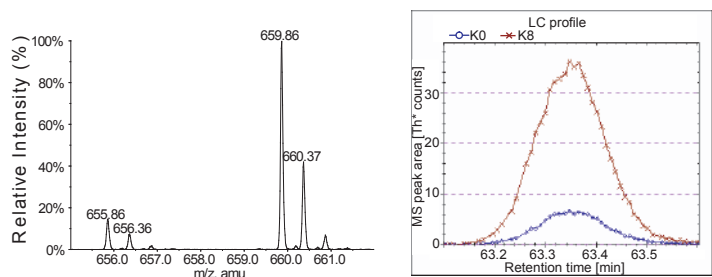
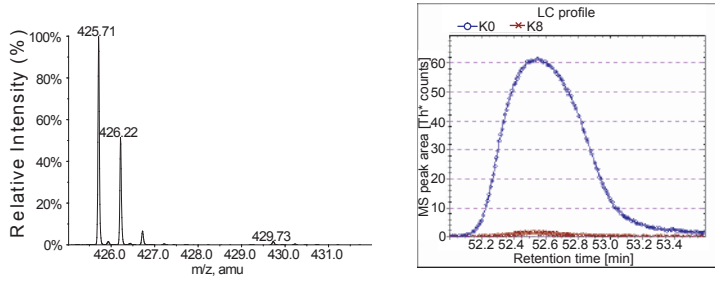


Fig. S9 Page 4

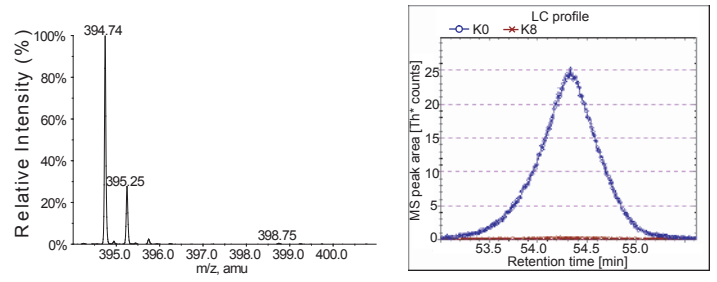
# Flag-H3.3, "on" to "off", 72 h without aphidolin

## Affinity purified Flag-H3.3

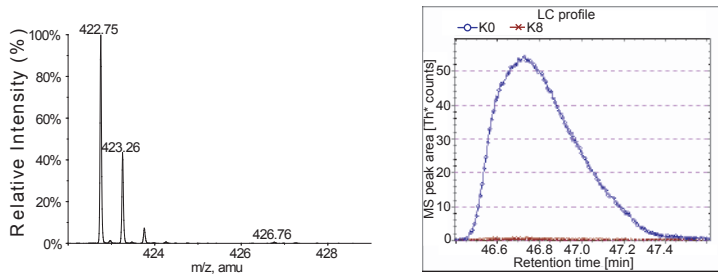
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=1.6%



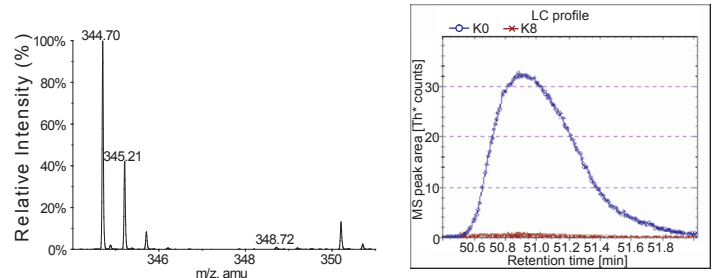
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=0.4%



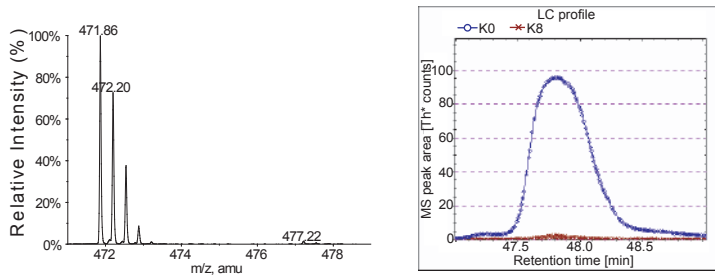
RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=0.6%



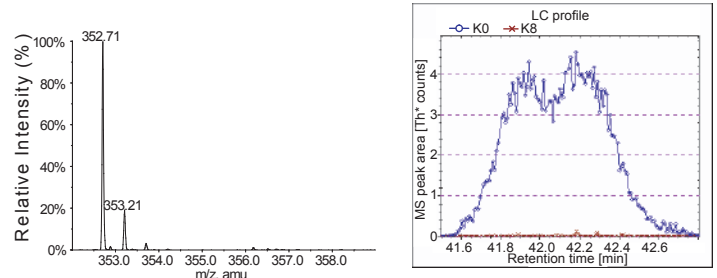
VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=1.4%



MDYK(Ac)DDDDKAR (FLAG), 3H<sup>+</sup>, K8/(K8+K0)=1.4%

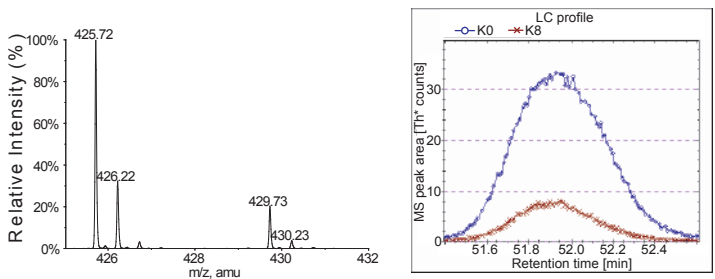


VTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=0.4%

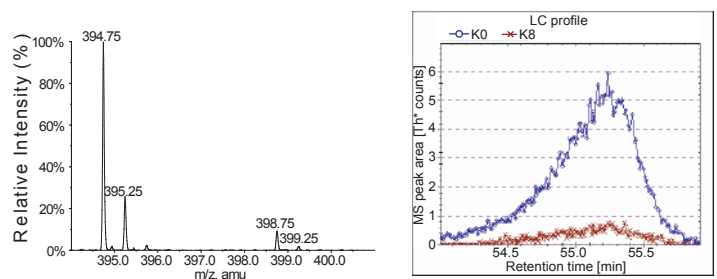


## Affinity purified H3.3

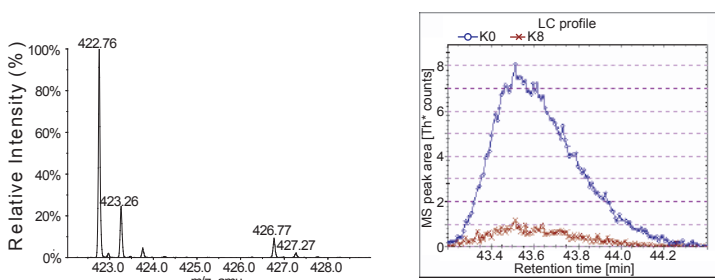
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=17%



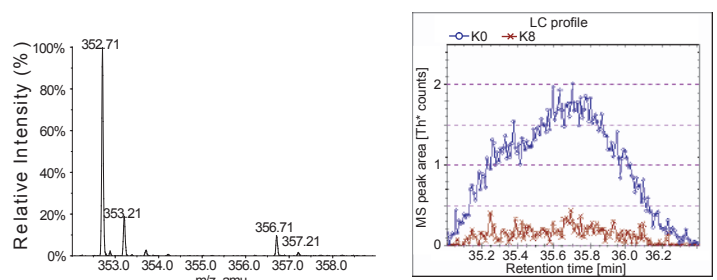
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=9.3%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=9.3%



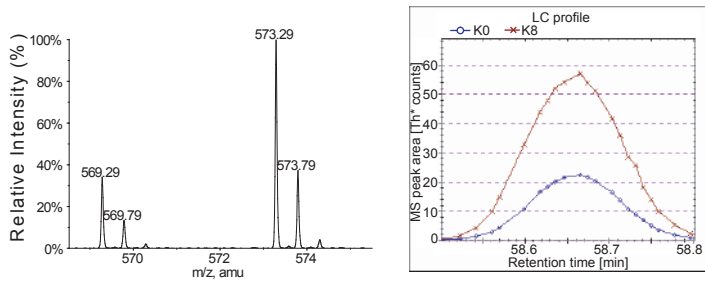
VTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=9.2%



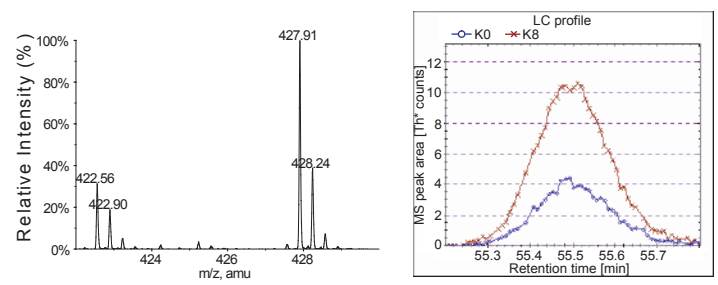
# Flag-H3.3, "on" to "off", 72 h without aphidicolin

## Affinity purified H2B

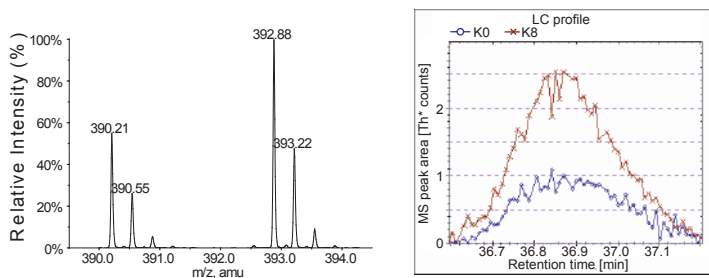
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=72%



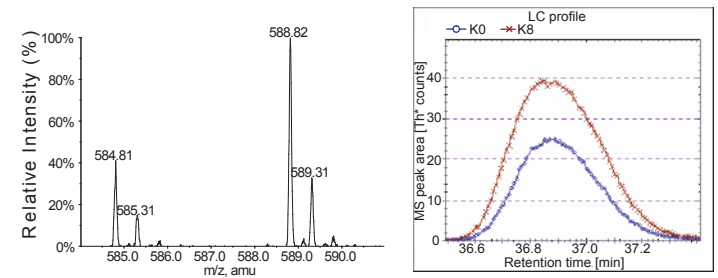
KESYSVYVYK, 3H<sup>+</sup>, K8/(K8+K0)=72%



QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=64%

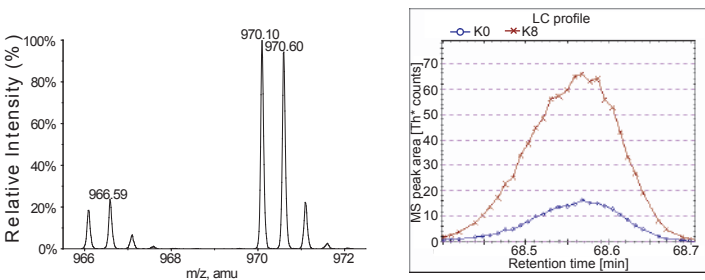


QVHPDTGISSK, 2H<sup>+</sup>, K8/(K8+K0)=71%

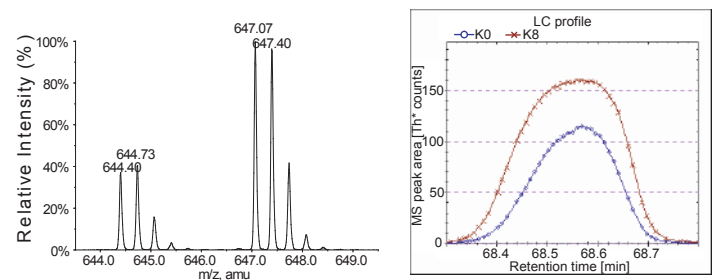


## Affinity purified H2A

VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=81%

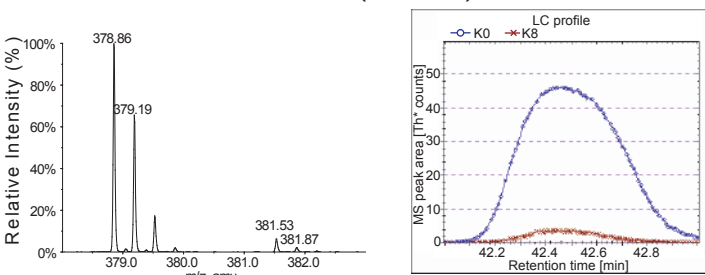


VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=73%

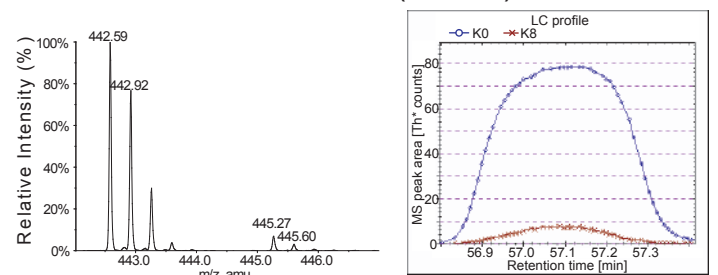


## Affinity purified H4

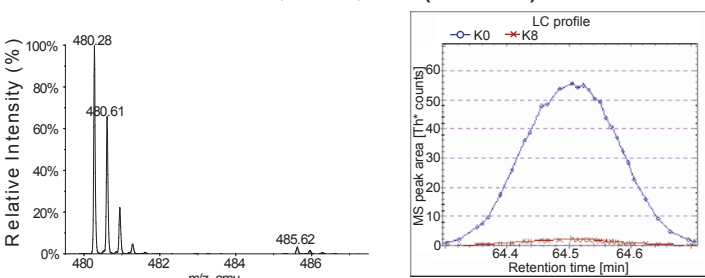
DAVTYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=5.8%



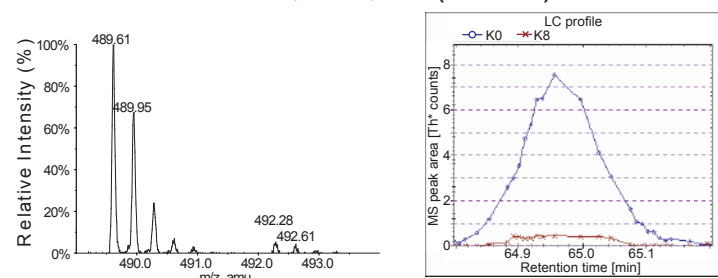
DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=6.4%



KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=3.6%



TVTAMDVVYALKR, 3H<sup>+</sup>, K8/(K8+K0)=4.8%



## Flag-H3.3, “on” to “off”, 72 h with aphidicolin

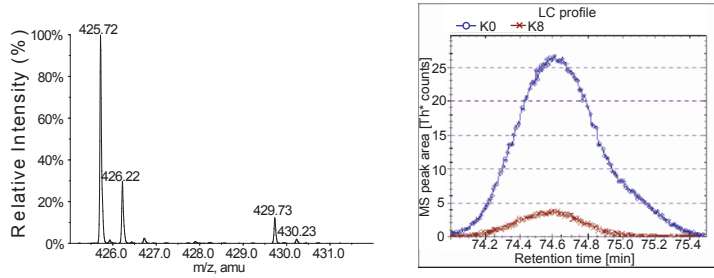
Histone	Sequence	charge	K8/(K8+K0)	average	StDV
Bulk H3	EIAQDFK	2H <sup>+</sup>	10.0%	8.7%	1.2%
	KLPFQR	2H <sup>+</sup>	7.7%		
	RVTIMPK	2H <sup>+</sup>	7.7%		
	VTIMPK	2H <sup>+</sup>	9.3%		
Bulk H2B	ESYSVYVYK	2H <sup>+</sup>	25.0%	27.8%	2.5%
	KESYSVYVYK	3H <sup>+</sup>	29.5%		
	QVHPDTGISSK	3H <sup>+</sup>	29.0%		
Bulk H2A	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	28.4%	28.4%	
Bulk H4	DAVYTEHAK	3H <sup>+</sup>	14.3%	11.8%	2.0%
	DNIQGITKPAIR	3H <sup>+</sup>	10.3%		
	KTVTAMDVVYALK	3H <sup>+</sup>	10.0%		
	KTVTAMDVVYALK				
	(M Oxidation)	3H <sup>+</sup>	12.4%		
Affinity purified FLAG-H3.3	EIAQDFK	2H <sup>+</sup>	0.9%	0.4%	0.3%
	KLPFQR	2H <sup>+</sup>	0.2%		
	MDYK(Ac)DDDDKAR	3H <sup>+</sup>	0.6%		
	RVTIMPK	2H <sup>+</sup>	0.2%		
	RVTIMPK (M Oxidation)	2H <sup>+</sup>	0.1%		
	VTIMPK (M Oxidation)	2H <sup>+</sup>	0.4%		
Affinity purified H3.3	EIAQDFK	2H <sup>+</sup>	3.7%	2.5%	0.9%
	KLPFQR	2H <sup>+</sup>	1.9%		
	RVTIMPK (M Oxidation)	2H <sup>+</sup>	1.9%		
	VTIMPK (M Oxidation)	2H <sup>+</sup>	2.3%		
Affinity purified H2B	ESYSVYVYK	2H <sup>+</sup>	28.2%	31.1%	2.6%
	KESYSVYVYK	3H <sup>+</sup>	32.1%		
	QVHPDTGISSK	3H <sup>+</sup>	33.0%		
Affinity purified H2A	VTIAQGGVLPNIQAVLLPK	2H <sup>+</sup>	28.4%	31.0%	3.7%
	VTIAQGGVLPNIQAVLLPK	3H <sup>+</sup>	33.6%		
Affinity purified H4	DAVYTEHAK	3H <sup>+</sup>	3.5%	2.5%	1.0%
	DNIQGITKPAIR	3H <sup>+</sup>	3.0%		
	KTVTAMDVVYALK	3H <sup>+</sup>	2.4%		
	TVTAMDVVYALK	2H <sup>+</sup>	1.2%		

Fig. S9 Page 7

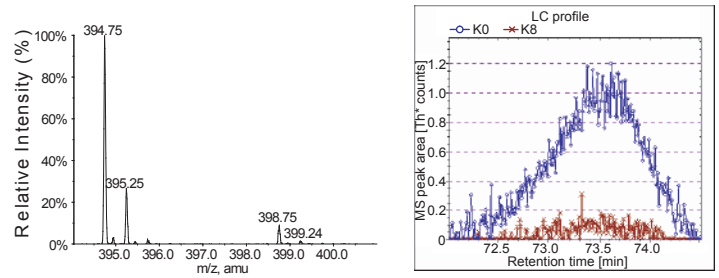
# Flag-H3.3, "on" to "off", 72 h with aphidicolin

## Bulk H3

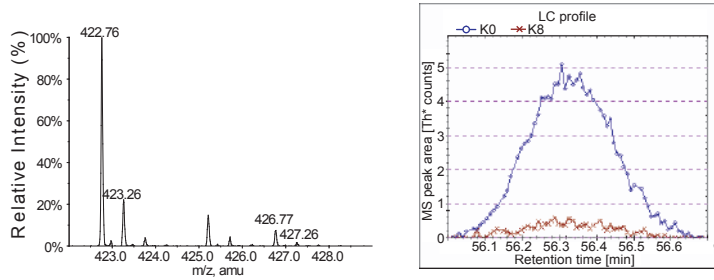
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=10%



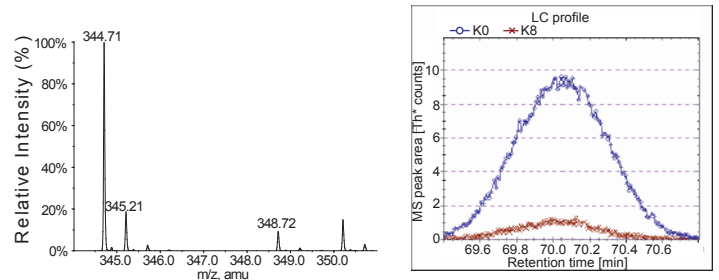
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=7.7%



RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=7.7%

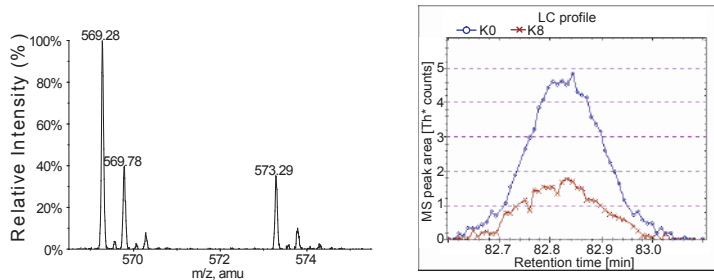


VTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=9.3%

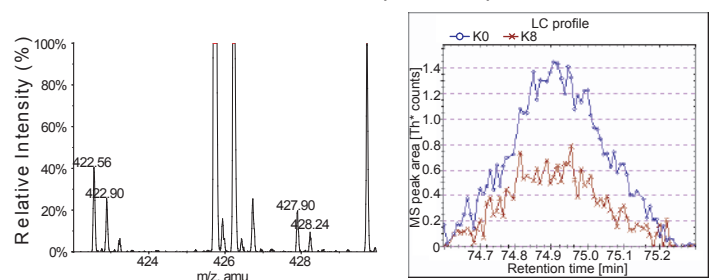


## Bulk H2B

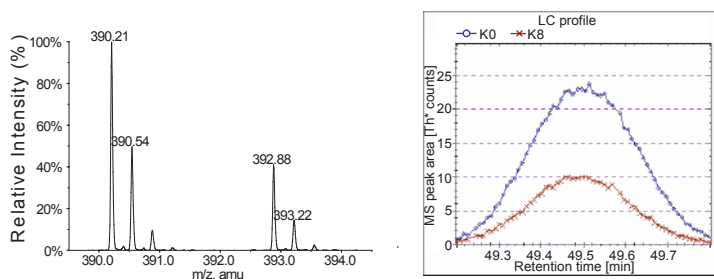
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=25%



KESYSVYVYK, 3H<sup>+</sup>, K8/(K8+K0)=30%



QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=29%

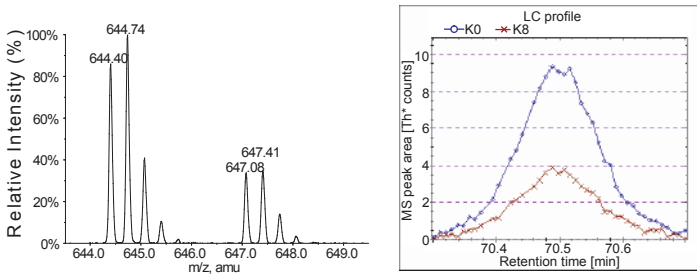




# Flag-H3.3, "on" to "off", 72 h with aphidicolin

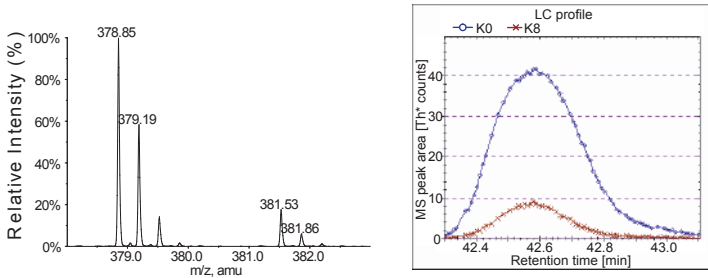
## Bulk H2A

VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=28%

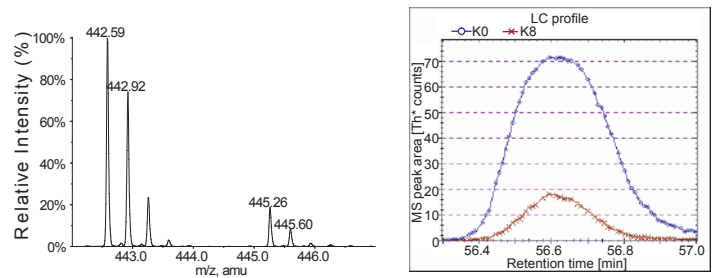


## Bulk H4

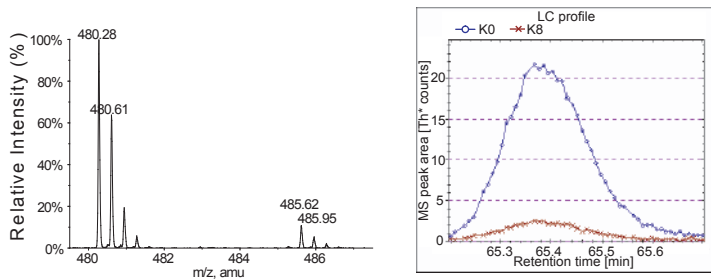
DAVITYTEHAQ, 3H<sup>+</sup>, K8/(K8+K0)=14%



DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=10%



KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=10%



KTVTAMDVVYALK (M Oxidation), 3H<sup>+</sup>, K8/(K8+K0)=12%

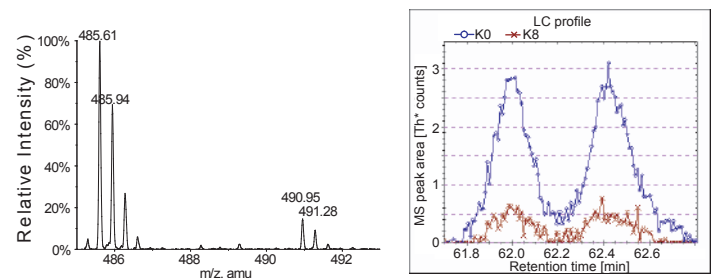
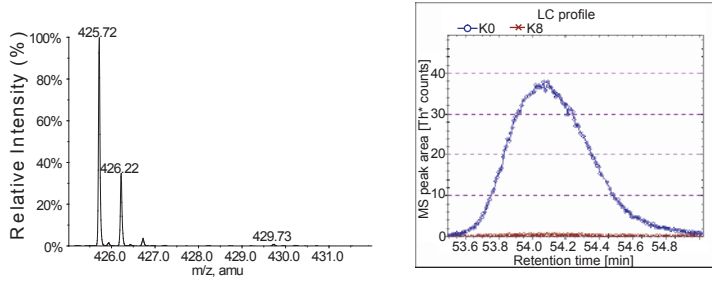


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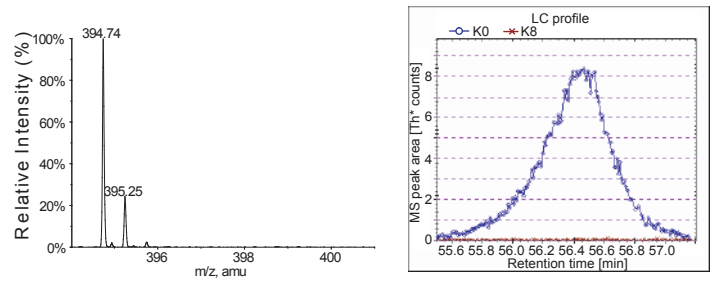
# Flag-H3.3, "on" to "off", 72 h with aphidolin

## Affinity purified Flag-H3.3

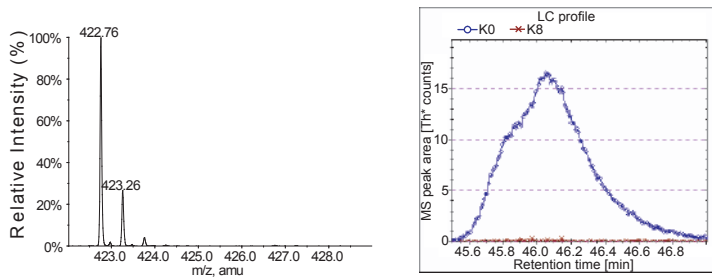
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=0.9%



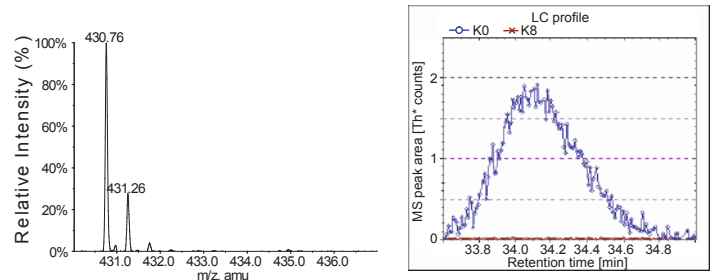
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=0.2%



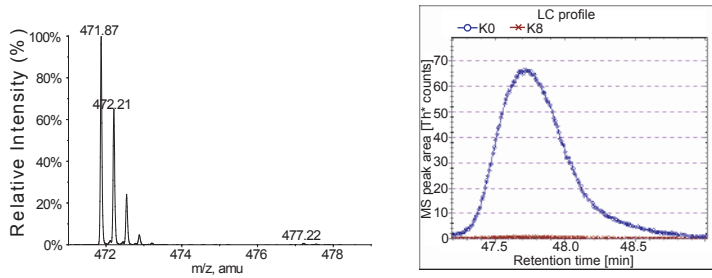
RVTIMPK, 2H<sup>+</sup>, K8/(K8+K0)=0.2%



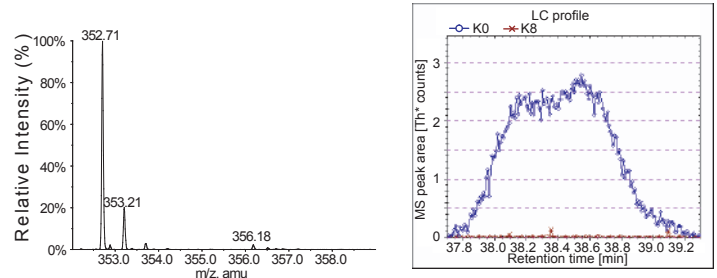
RVTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=0.1%



MDYK(Ac)DDDDKAR (FLAG), 3H<sup>+</sup>, K8/(K8+K0)=0.6%

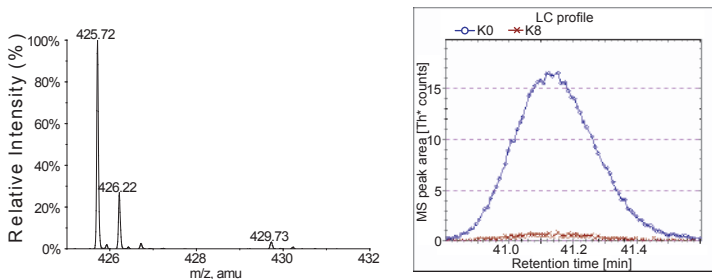


VTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=0.4%

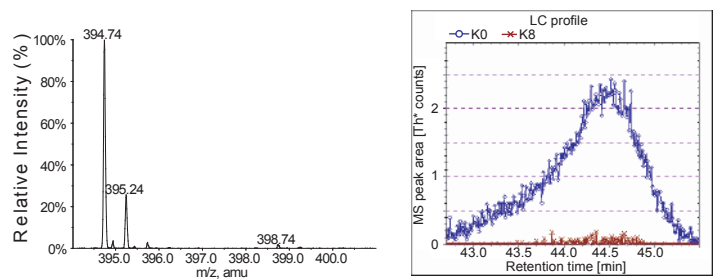


## Affinity purified H3.3

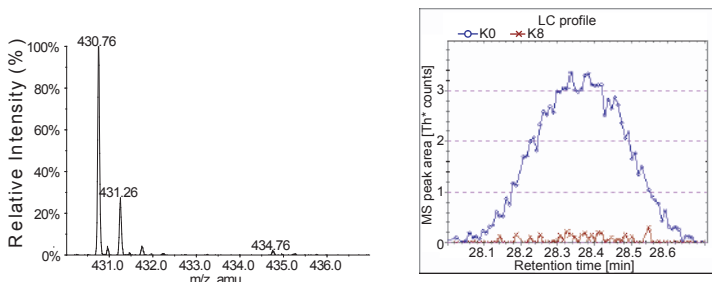
EIAQDFK, 2H<sup>+</sup>, K8/(K8+K0)=3.7%



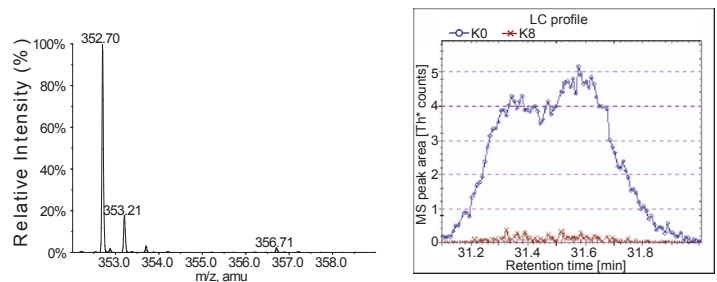
KLPFQR, 2H<sup>+</sup>, K8/(K8+K0)=1.9%



RVTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=1.9%



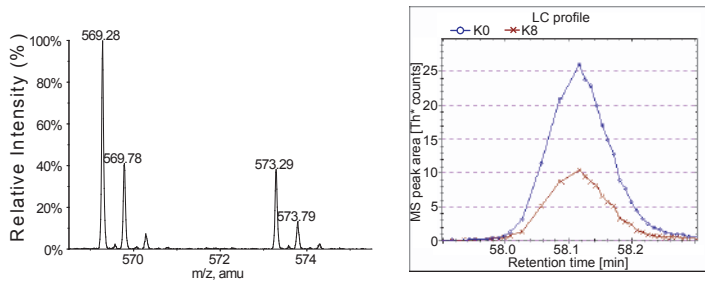
VTIMPK (M Oxidation), 2H<sup>+</sup>, K8/(K8+K0)=2.3%



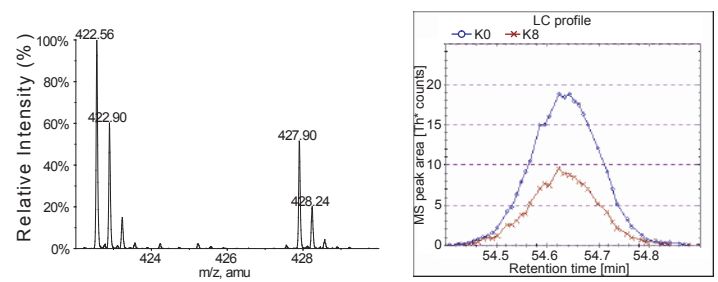
# Flag-H3.3, "on" to "off", 72 h with aphidicolin

## Affinity purified H2B

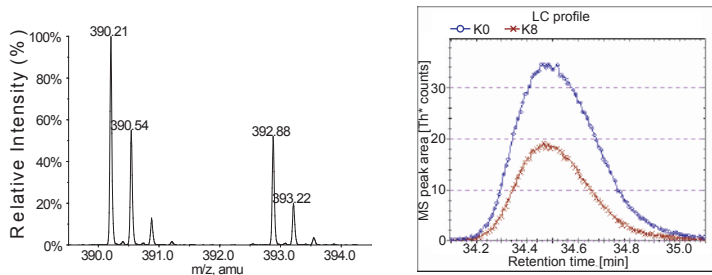
ESYSVYVYK, 2H<sup>+</sup>, K8/(K8+K0)=28%



KESYSVYVYK, 3H<sup>+</sup>, K8/(K8+K0)=32%

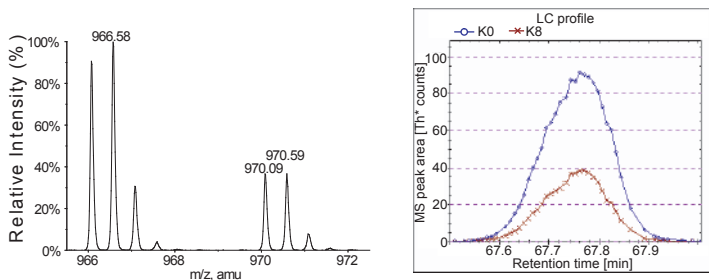


QVHPDTGISSK, 3H<sup>+</sup>, K8/(K8+K0)=33%

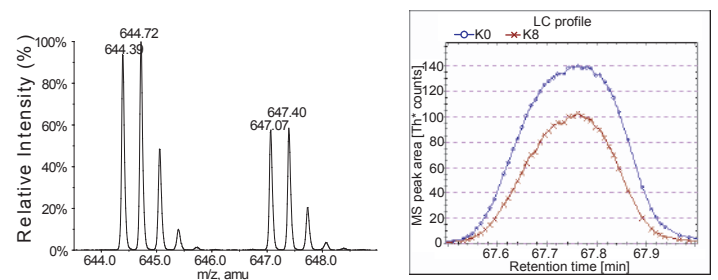


## Affinity purified H2A

VTIAQGGVLPNIQAVLLPK, 2H<sup>+</sup>, K8/(K8+K0)=28%

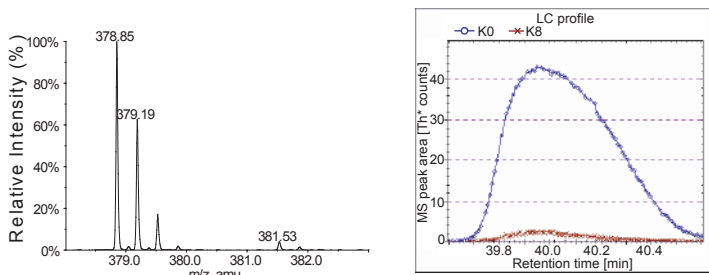


VTIAQGGVLPNIQAVLLPK, 3H<sup>+</sup>, K8/(K8+K0)=34%

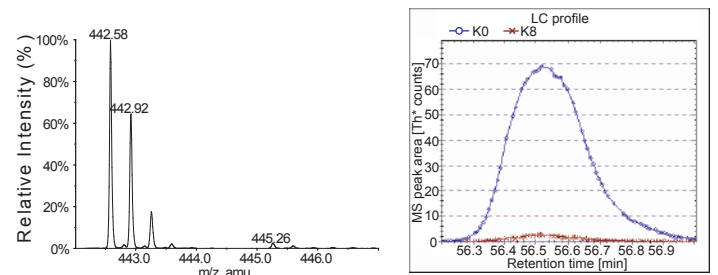


## Affinity purified H4

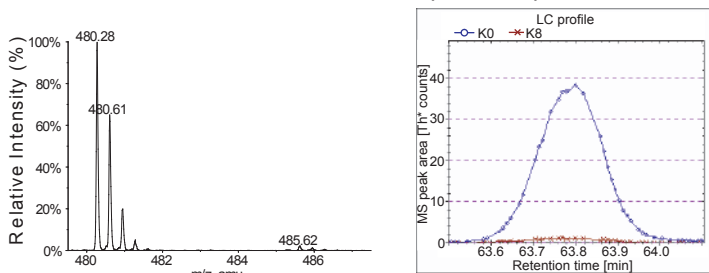
DAVTYTEHAK, 3H<sup>+</sup>, K8/(K8+K0)=3.5%



DNIQGITKPAIR, 3H<sup>+</sup>, K8/(K8+K0)=3.0%



KTVTAMDVVYALK, 3H<sup>+</sup>, K8/(K8+K0)=2.4%



TVTAMDVVYALK, 2H<sup>+</sup>, K8/(K8+K0)=1.2%

