Supporting Information

Exploring the Histone Acylome Through Incorporation of γ-Thialysine on Histone Tails

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1. Experimental section

Materials

All reagents were obtained from commercial sources and used without further purifications. Water was purified using a Millipore Milli-Q water purification system (Merck-Millipore, Burlington, MA, USA). Acetyl coenzyme A sodium salt, n-propionyl CoA lithium salt, Butyryl coenzyme A lithium salt hydrate and Crotonyl coenzyme A trilithium salt were purchased at Sigma-Aldrich. All the Fmoc-protected amino acids were purchased from Novabiochem (Merck-Millipore). For HPLC the following buffers were used: Buffer A: H_2O (0.1% TFA); Buffer B: ACN (0.1% TFA). Lyophilization of purified peptides was achieved using a VaCo 2 lyophilizer (Zirbus Technology GmbH, Bad Grund, Germany).

2. KATs expression and purification

MOF

Plasmid carrying recombinant His-tagged Human MOF catalytic domain (residues 125-458 in pET19b) was gently provided by Professor Frank J. Dekker (Groningen University). The protein was expressed and purified as previously described.[1] Briefly, E. coli BL21(DE3) cells enriched with human His-tagged MOF WT plasmid, were cultured in LB growth media supplemented with 50 μg/mL ampicillin at 37 °C, to an OD_{600} of 0.6, upon which expression was induced by addition of IPTG (0.3 mM final) and followed by incubation at 20 °C overnight. Harvested cells were pelleted and re-suspended into 10 mM Tris pH 7.4, 750 mM NaCl, 1% glycerol, 1 mM β-ME lysis buffer in presence of protease inhibitor cocktail (Roche) and lysate by sonication. The supernatant was incubated with Ni-NTA beads for 2 hours at 4 °C. The beads were loaded on a gravity flow column and washed with 50 mM imidazole in lysis buffer. Subsequently, the protein was eluted with 10 mM MES, 750 mM NaCl, 10 mM MgCitrate, 250 mM

imidazole, 1 mM β-ME, 1 % glycerol, pH 6.5 and concentrated with a 30 kDa spinfilter device (AMICON, 30 MWCO). The protein was furtherly purified by size-exclusion chromatography (SEC) using the AKTA system, employing a Superdex 200 column equilibrated with 10 mM MES, 750 mM NaCl, 10 mM MgCitrate, 1 mM β-ME, 1 % glycerol pH 6.5 at 0.5 mL/min flow speed and purity of the eluted protein was assessed with SDS-page. Pure fractions were pooled, rapidly flash-frozen and stored at -80°C.

PCAF

Plasmid carrying recombinant SNAP-His-tagged Human PCAF catalytic domain (residues 498-658 in pET16b vector) was gently provided by Professor Milan Mrksich (Northwestern University). The protein was expressed as it follows: E. coli BL21(DE3) cells enriched with hPCAF plasmid, were cultured in 2xYT growth media supplemented with 100 µg/mL carbenicillin at 37°C, to an OD_{600} of 0.6, upon which expression was induced by addition of IPTG (0.5 mM final) and followed by incubation at 16 °C overnight. Harvested cells were pelleted and re-suspended into 50 mM Tris pH 8.5, 200 mM NaCl, 5 mM β-ME lysis buffer in presence of protease inhibitor cocktail (Roche) and lysate by sonication. The supernatant was incubated with Ni-NTA beads for 2 hours at 4°C. The beads were loaded on a gravity flow column and washed with 50 mM Tris pH 8.5, 200 mM NaCl, 50 mM imidazole, 5 mM β-ME. Subsequently, the protein was eluted with 50 mM Tris pH 8.5, 200 mM NaCl, 300 mM imidazole, 5 mM β-ME and concentrated using a 30 kDa spinfilter device (AMICON, 30 MWCO). The protein was furtherly purified by size-exclusion chromatography (SEC) using the AKTA system, employing a Superdex 75 column equilibrated with 50 mM Tris pH 8.0, 200 mM NaCl, 1 mM DTT at 0.5 mL/min flow speed. The purity of the eluted protein was assessed with SDS-page. Pure fractions were pooled, rapidly flash-frozen and stored at -80°C.

GCN5

Plasmid carrying recombinant His-tagged Human GCN5 catalytic domain (residues 497-662 in pET28a-LIC vector) was purchased from Addgene (25482). The protein was expressed and purified as previously described.[2] Briefly, E. coli BL21(DE3) cells enriched with hGCN5 WT plasmid were cultured in TB growth media supplemented with 50 μg/mL kanamycin at 37 °C to an OD_{600} of 0.6, upon which expression was induced by addition of IPTG (0.5 mM final) and followed by incubation at 16 °C overnight. Harvested cells were pelleted and re-suspended into 50 mM Na_2HPO_4 pH 7.5, 500 mM NaCl, 5% glycerol, 1 mM β-ME lysis buffer in presence of protease inhibitor cocktail (Roche, Basel, Switserland) and lysate by sonication. The supernatant was incubated with Ni-NTA beads for 2 hours at 4 °C. The beads were loaded on a gravity flow column and washed with 20 mM HEPES-NaOH pH 7.5, 500 mM NaCl, 50 mM imidazole, 5% glycerol, 1mM β-ME. Subsequently, the protein was eluted with 20 mM Hepes-NaOH pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol, 1mM β-ME and the buffer was exchanged to 20 mM Hepes-NaOH pH 7.5, 150 mM NaCl, 1mM βME by concentration with a 10 kDa spinfilter device (AMICON, 10 MWCO). Purity of the eluted protein was assessed with SDS-page, and GCN5 was separated in aliquots, rapidly flash-frozen and stored at -80 °C.

3. Synthesis and purification of histone peptides

All histone peptides were chain assembled on Wang resin using microwave assisted SPPS on a Liberty Blue peptide synthesizer (CEM corporation, Matthews, NC, USA). All amino acid couplings were carried out with the equivalent ratio of [5]:[5]:[7.5] of [Fmoc-protected amino acid]:[DIC]:[Oxyma Pure] at 75 ℃ for 2 minutes or at 50 ℃ for 4 minutes for cysteine. Peptides proceeded to standard cleavage from resin using 0.5% TIPS, 0.5% H_2O, 0.5% EDT in conc. TFA. TFA was blown off using N_2 and the resultant residue suspended in cold Et_2O. After suspension it was subjected to centrifugation for 5

minutes at 5000 rpm in an Eppendorf 5804R centrifuge (Eppendorf, Hamburg, Germany) after which the supernatant was decanted into the waste. The remaining white to yellow solid was washed twice by cold Et_2O and subjected to centrifugation after which the crude peptide was dissolved in a mixture of ACN in H_2O and purified using preparative reverse-phase HPLC (RP-HPLC) using a gradient of buffer A and buffer B from 3% B to 30% over 20 minutes (H3 peptides), or from 3% B to 20% over 15 minutes followed by 20% to 35% in 5 min (H4 peptides) at 10 ml/min using a Gemini 10µm NX-C18 110Å LC column (Phenomenex, Torrance, CA, USA). Analytical RP-HPLC was carried out on a Gemini 5µm C18 110Å LC column (Phenomenex) at a flow rate of 1 mL/min. Analytical injections were monitored at 215 nm.

4. Alkylation procedure

The alkylation reactions of H3C14 and H4C16 were prepared based on the protocol described by Shokat and his co-workers.[3] Briefly, 10 mg of purified and lyophilized unalkylated peptide was dissolved in 4.9 mL alkylation buffer (4 M GuHCl, 1 M HEPES, 10 mM D/L-methionine, pH = 7.8). The reaction mixture was allowed to incubate for 1 h at 37 °C using a Thermomixer C (Eppendorf) under reducing conditions by adding 10 µL 0.5 M DTT (freshly prepared). The desired alkylation reagent 2- bromoethylamine (50 equiv.) was directly dissolved into the reaction mixture and allowed to react at 50 °C. After 2.5 h reaction time, 10 µL 0.5 M DTT was added and the reaction was allowed to proceed for another 2.5 h. The progress of the reactions was monitored by MALDI-TOF MS analysis. The reaction was quenched by incubating the reaction mixture with 10 µL of 2-mercaptoethanol for 30 minutes at room temperature. Then directed to freeze-dryer overnight, dissolved in MilliQ water, filtered through a syringe filter (0.22 µM), followed by preparative HPLC purification as elaborated in the section "Synthesis and purification of histone peptides". All the alkylation reactions were performed in dark conditions.

5. MALDI-TOF MS enzymatic assay

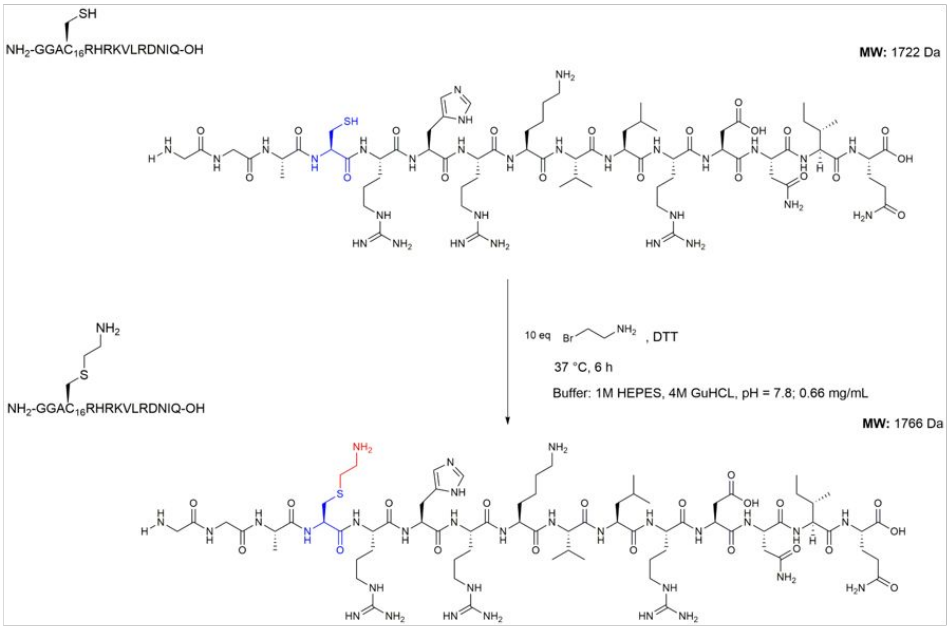
KATs enzymatic activity towards histone peptides was measured at different time points under standard conditions (2 μM KAT enzyme, 100 μM peptide, 300 μM AcylCoA) in the reaction buffer (50 mM HEPES, 0.1 mM EDTA, 1 mM DTT, pH = 8.0). The reactions were carried out in a final volume of 50 μL by incubation of the which were there shaken up in a Thermomixer C at 750 rpm, at 37°C. The reactions were quenched by the addition of TFA 10 % in MilliQ water. All reactions were aliquoted and mixed 1:2 with α-Cyano-4-hydroxycinnamic acid (CHCCA) matrix and loaded onto an MTP 384 polished steel target to be analyzed by a UltrafleXtreme-II tandem mass spectrometer (Bruker, Billerica, MA, USA).

6. MALDI-TOF MS kinetic assay

Histone peptides kinetics evaluation was carried out with a MALDI-TOF MS assay under steady conditions.[4,5] Decreasing concentrations of peptides (1050-0 μM) were incubated with AcylCoA (100 μM) and the reaction was started by the addition of the enzyme (MOF 570 nM, PCAF and GCN5 50 nM) in a final volume of 20 μL in kinetic buffer (50 mM HEPES, 0.1 mM EDTA, 0.01% TRITON-X, pH = 7.4). Steady state conditions were guaranteed by employing saturating concentrations of AcylCoA (>10x K_m). AcylCoA stock solutions in MQ water were calibrated with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), employing the characteristic molar extinction coefficient $\varepsilon_{260nm} = 16400\ M^{-1}cm^{-1}$. Reactions were incubated at 37°C, shaken at 750 rpm and quenched with TFA 10% after 15 min, within linear production of acetylated peptides. All reactions were aliquoted and mixed 1:2 with α-Cyano-4-hydroxycinnamic acid (CHCCA) matrix and loaded onto MTP 384 polished steel target to be analyzed by UltrafleXtreme-II tandem mass spectrometer (Bruker). The

amount of acetylated peptide was calculated by integration of the product peak area and divided it by the amount of unacetylated peptide at any concentration points using FlexAnalysisTM software. Kinetic values were extrapolated by fitting V_0 values (μM of produced peptide per minutes) and histone peptide concentrations to Michaelis-Menten equation using the GraphPad Prism 5 software. Kinetic experiments were carried out in duplicate and final reported as value ± SD.

a



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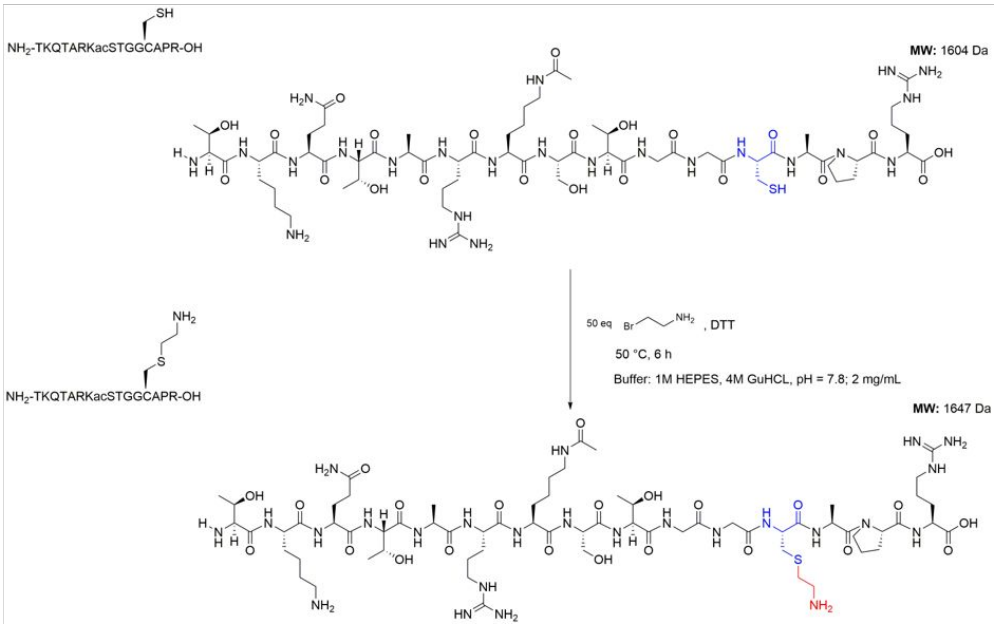
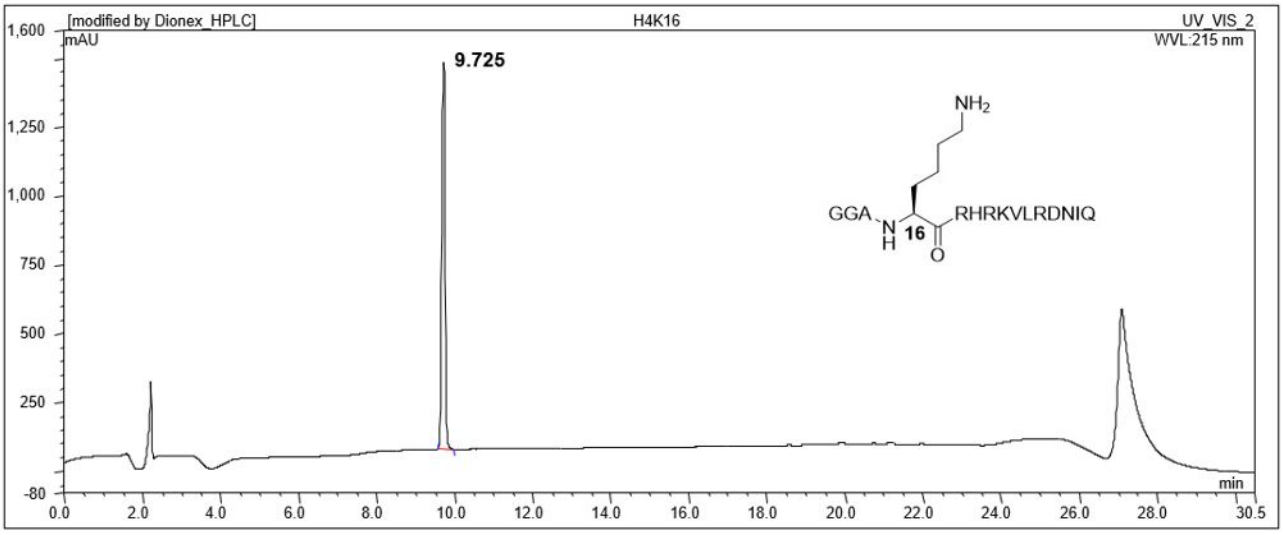


Scheme S1. Synthetic strategies to achieve (a) H4K$_C$16, and (b) H3K$_C$14.

7. Characterization of histone peptides

A)



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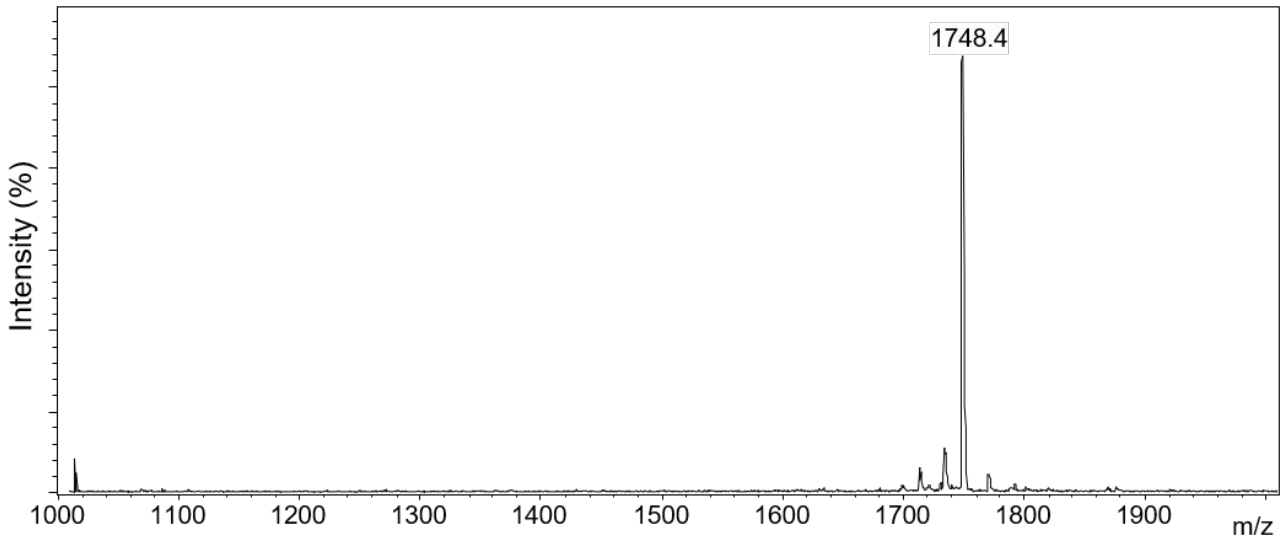
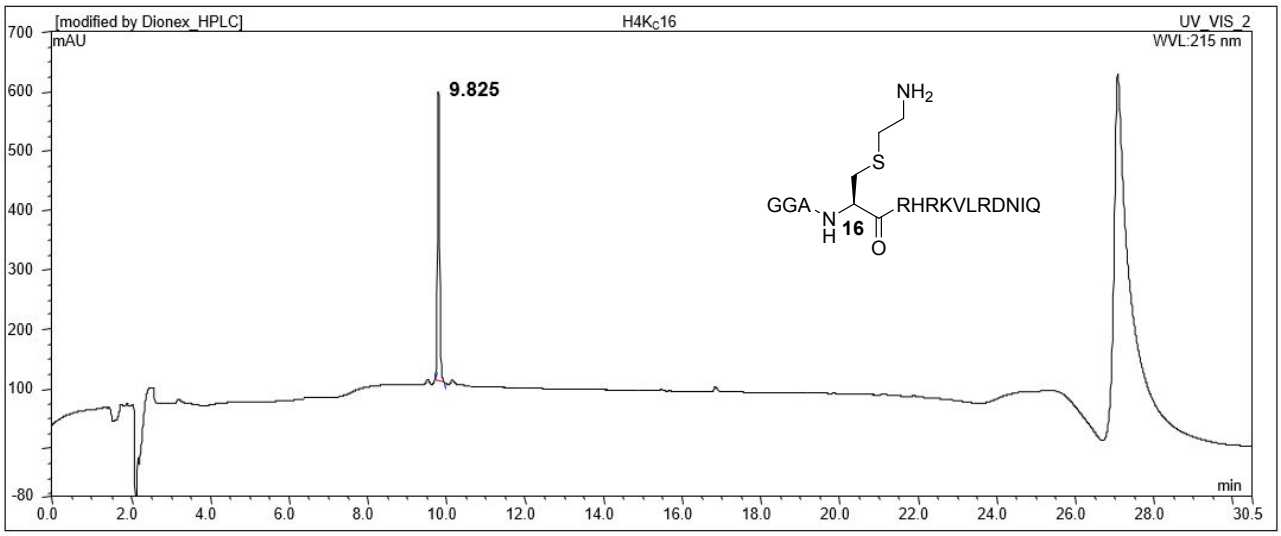


Figure S1. A) Analytical HPLC of the H4K16 peptide after RP-HPLC purification. **B)** MALDI-TOF MS spectra of the purified H4K16 peptide.

A)



B)

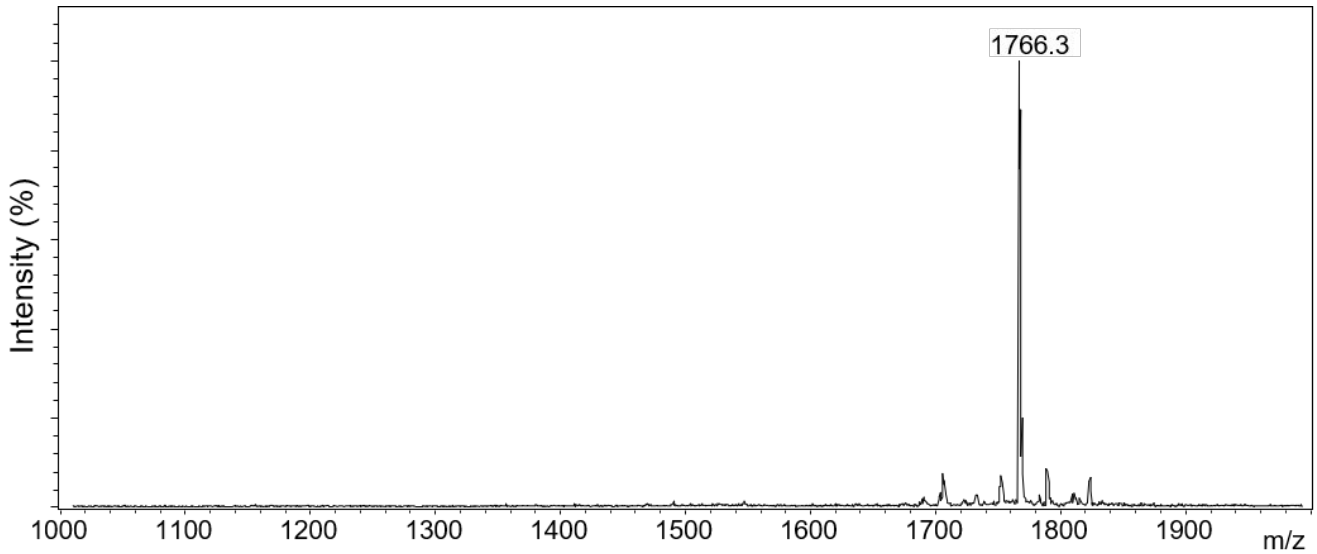
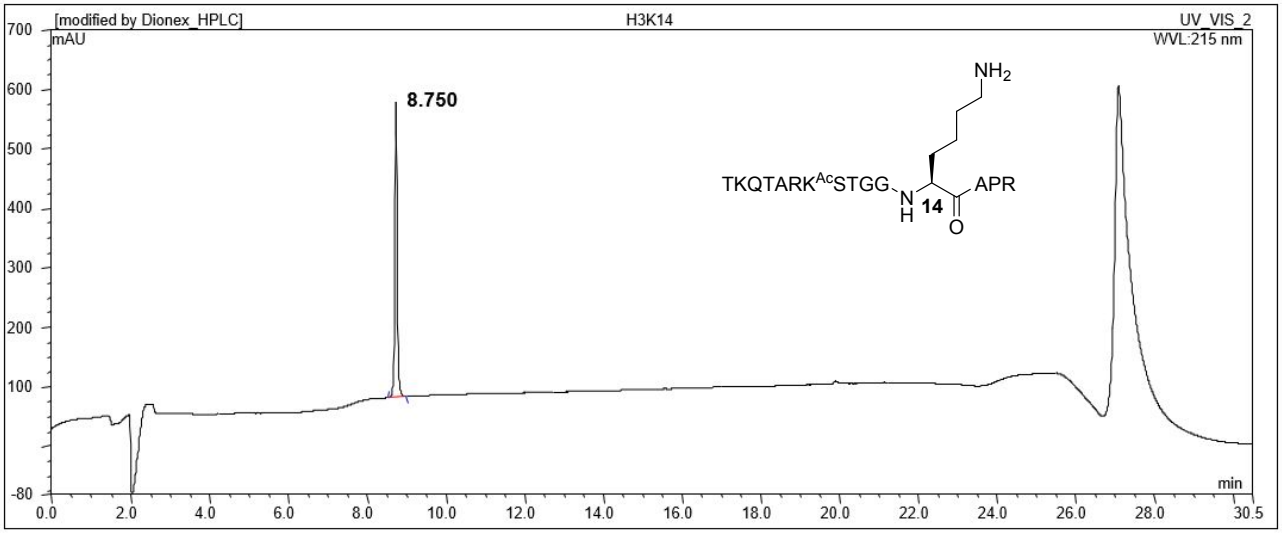


Figure S2. A) Analytical HPLC of the H4K$_C$16 peptide after RP-HPLC purification. **B)** MALDI-TOF MS spectra of the purified H4K$_C$16 peptide.

A)



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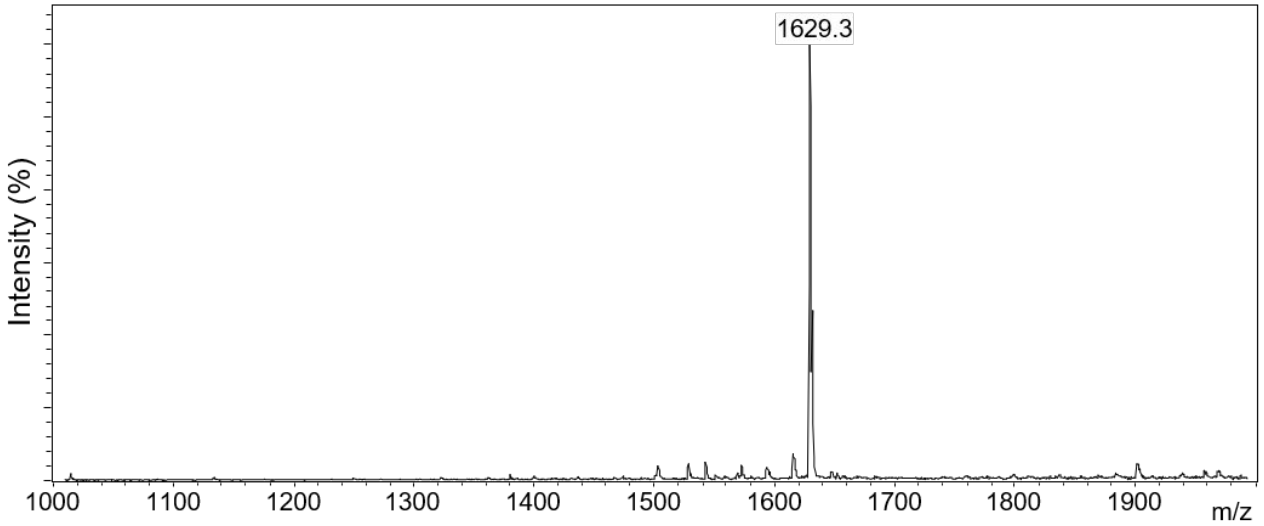
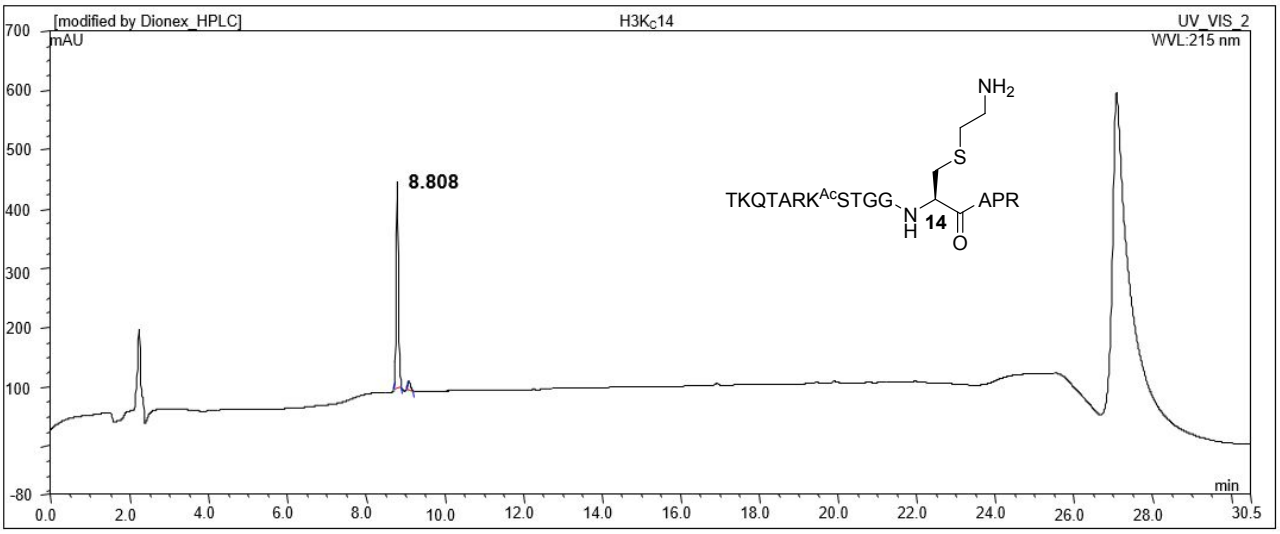


Figure S3. A) Analytical HPLC of the H3K14 peptide after RP-HPLC purification. **B)** MALDI-TOF MS spectra of the purified H3K14 peptide.

A)



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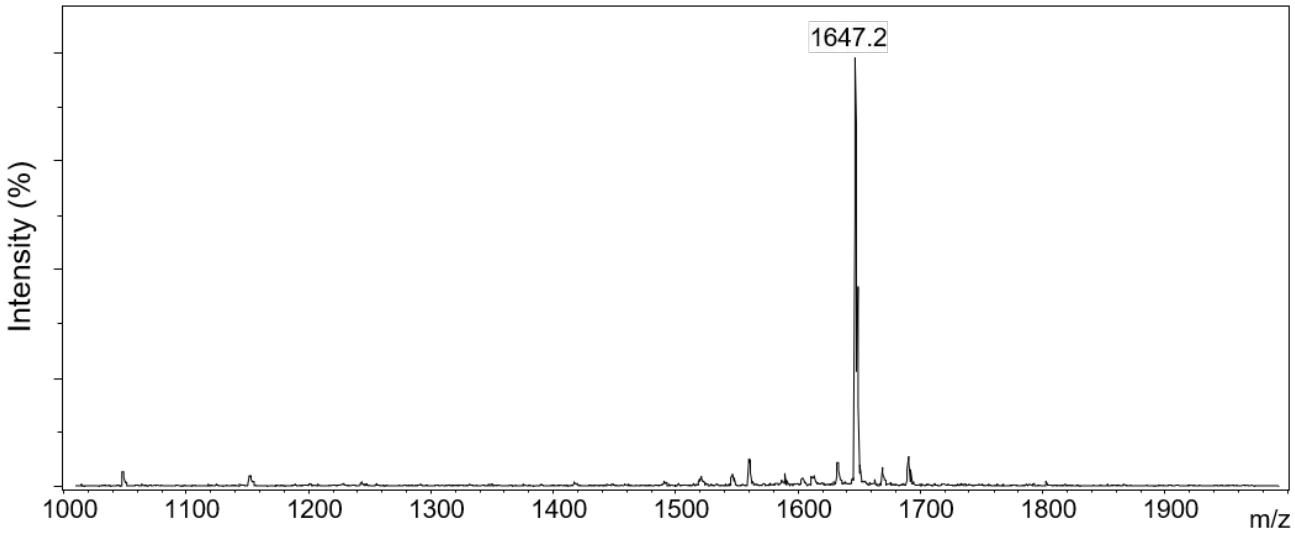
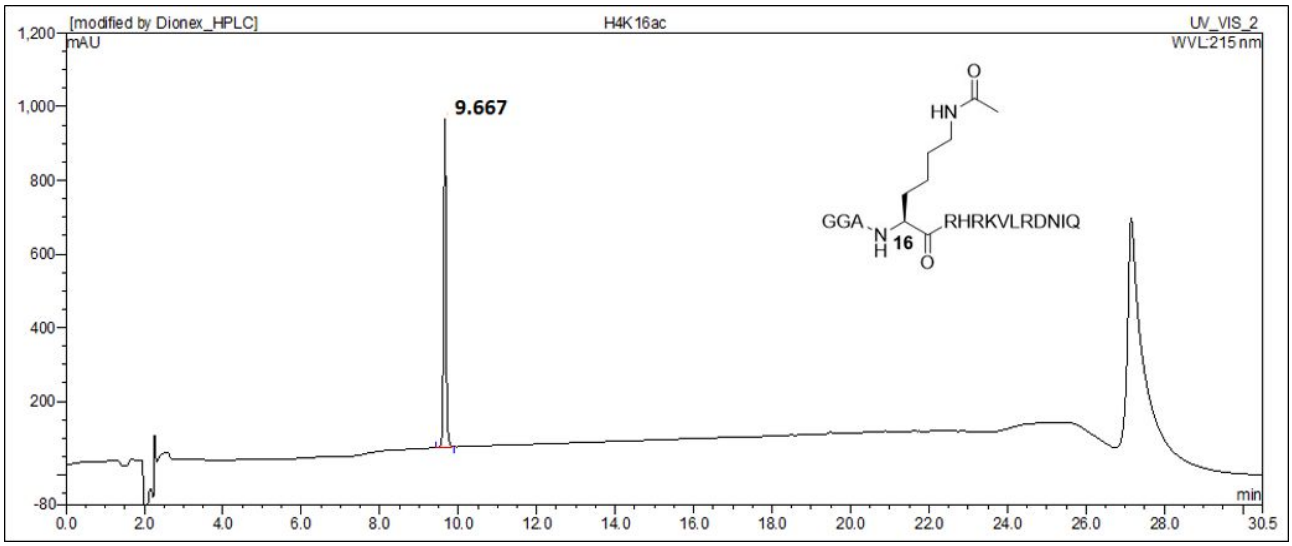


Figure S4. A) Analytical HPLC of the H3K$_C$14 peptide after RP-HPLC purification. **B)** MALDI-TOF MS spectra of the purified H3K$_C$14 peptide.

A)



B)

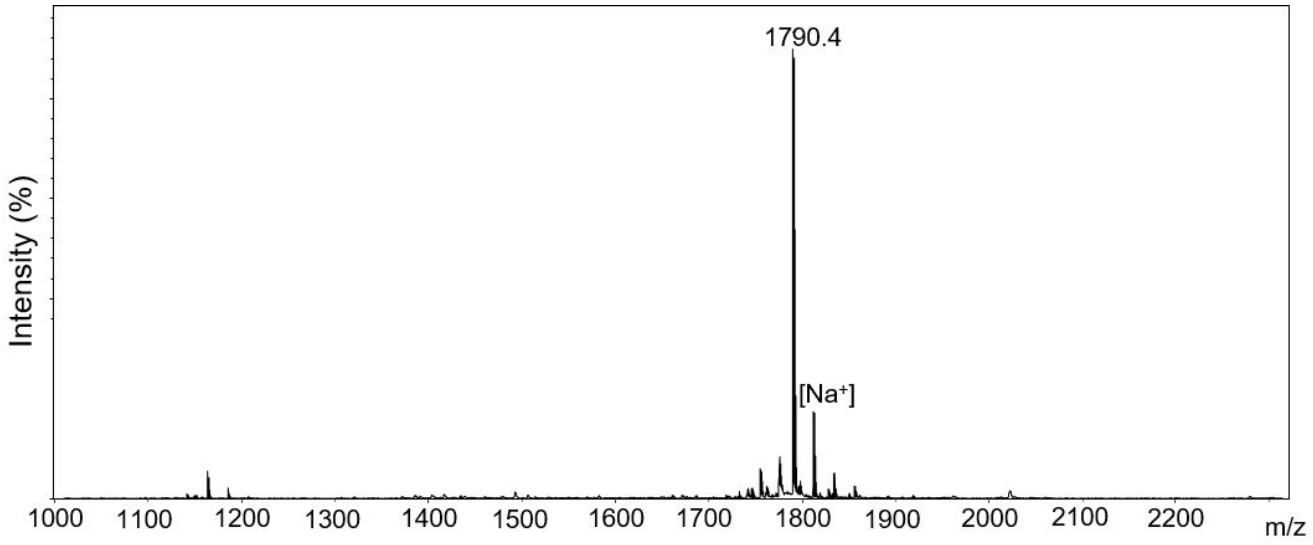
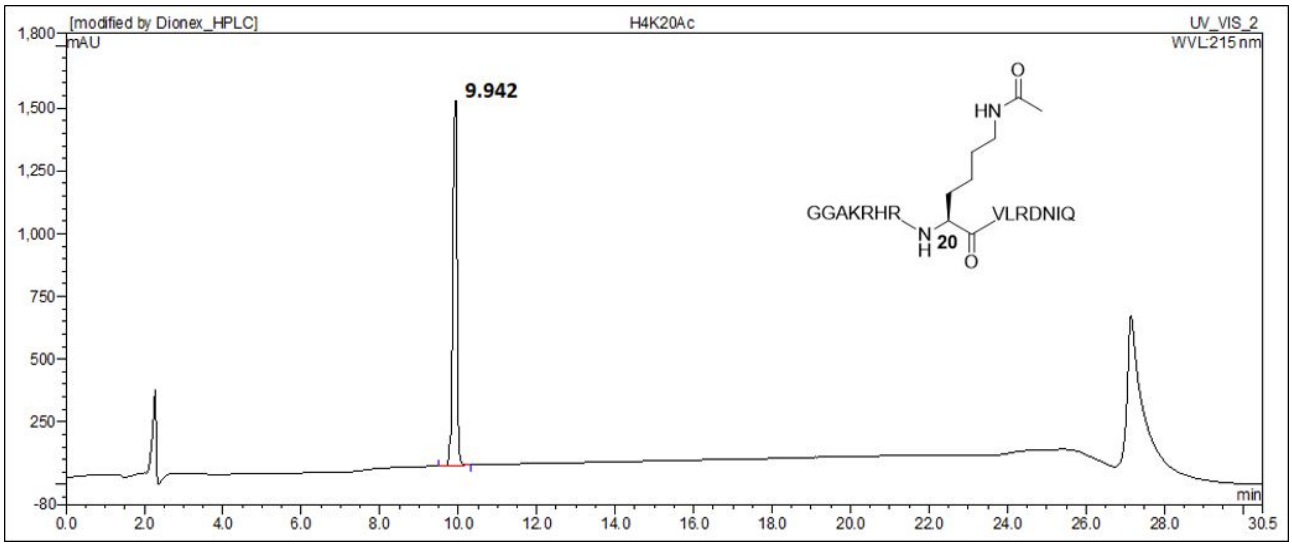


Figure S5. A) Analytical HPLC of the H4K16ac peptide after RP-HPLC purification. **B)** MALDI-TOF MS spectra of the purified H4K16ac peptide.

A)



B)

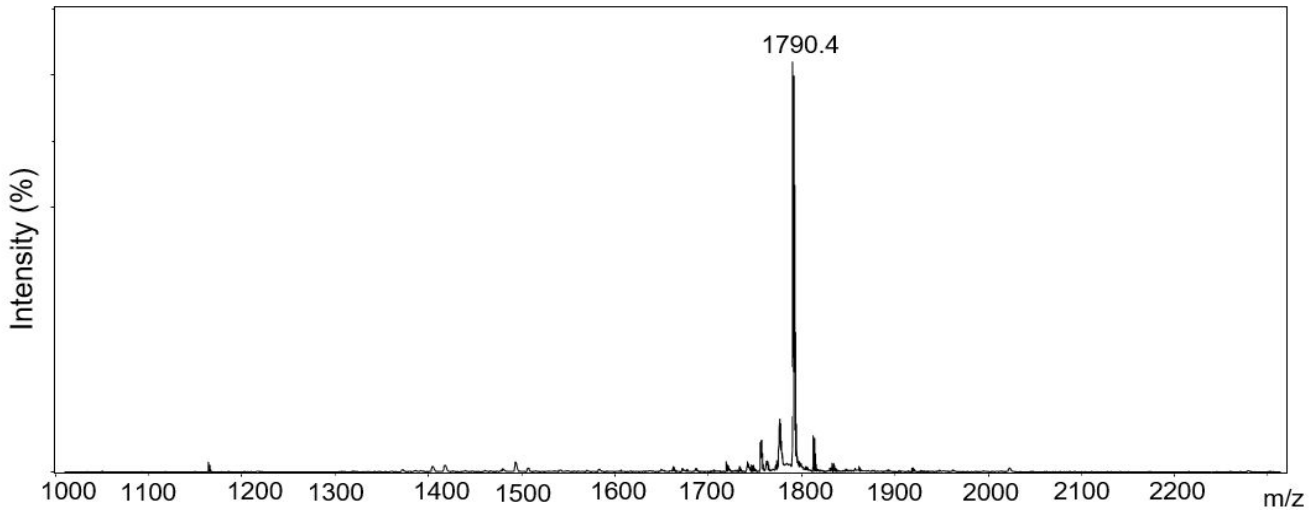


Figure S6. A) Analytical HPLC of the H4K20ac peptide after RP-HPLC purification. **B)** MALDI-TOF MS spectra of the purified H4K20ac peptide.

8. MALDI-TOF MS acetylation supporting figures

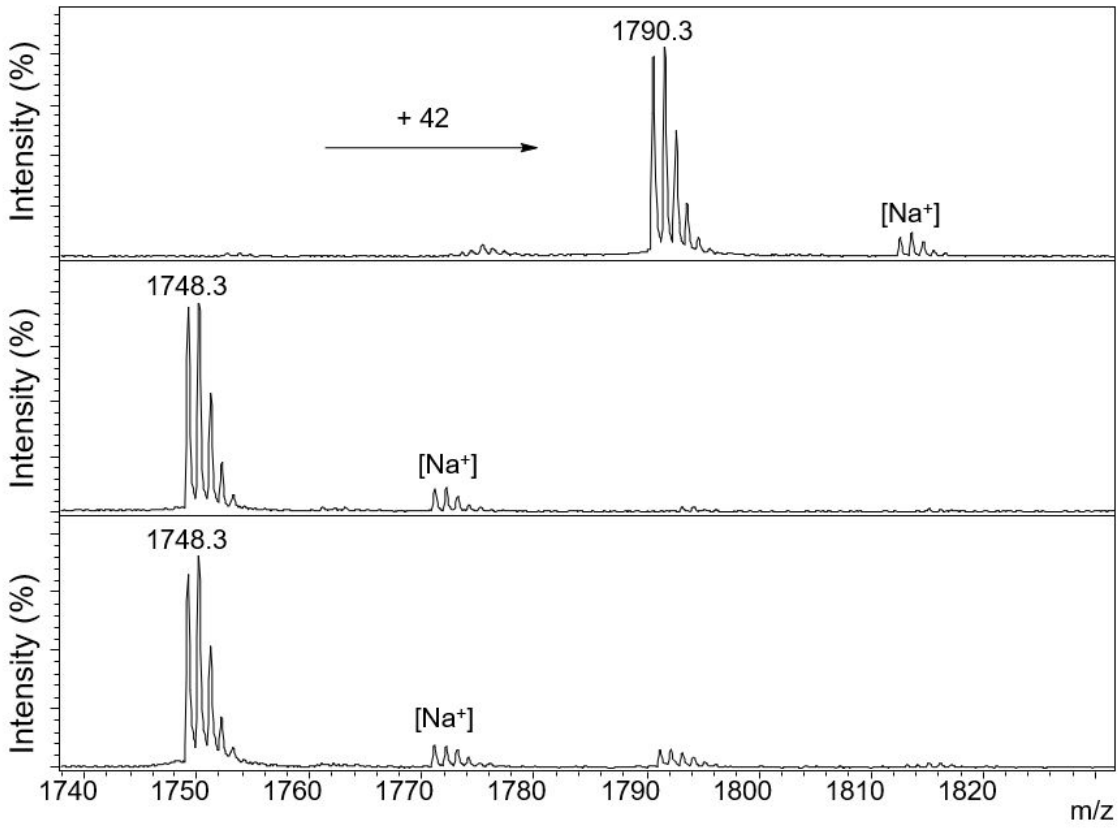


Figure S7. MALDI-TOF MS analysis of MOF (2 μM)-catalyzed acetylation of the H4K16 peptide (100 μM) in the presence of AcCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of AcCoA (middle panel), absence of MOF (bottom panel).

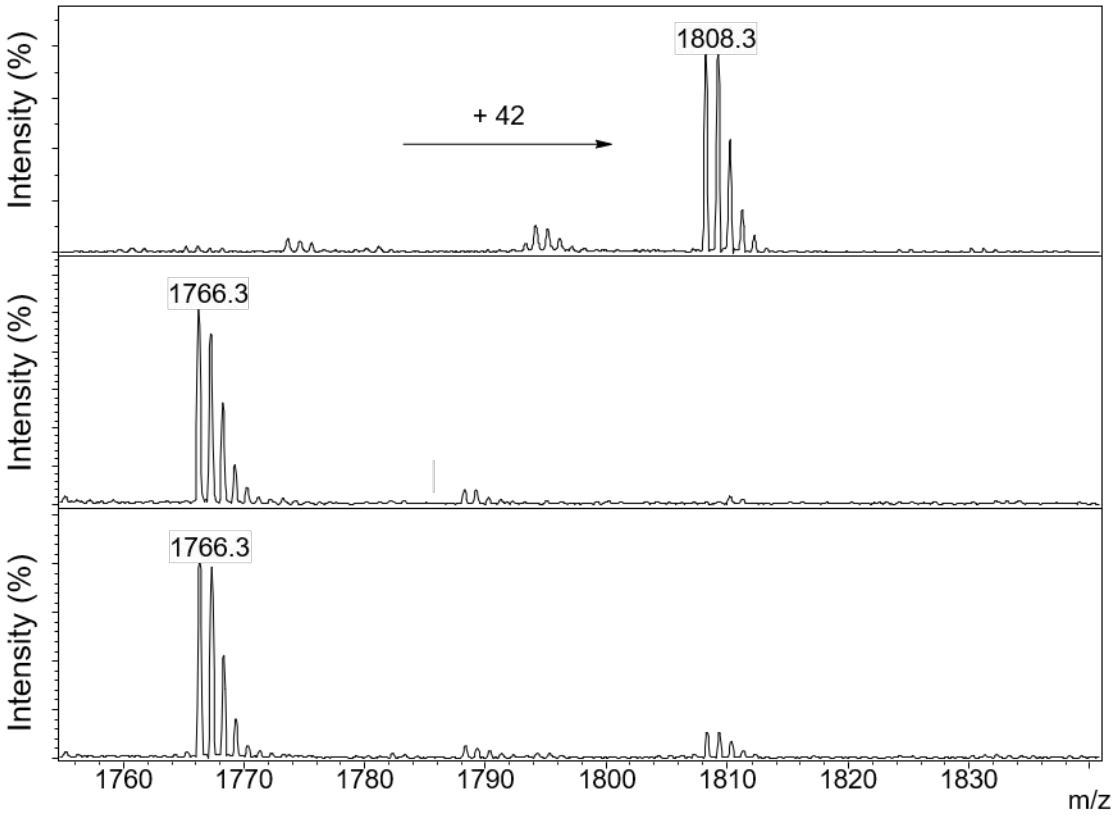


Figure S8. MALDI-TOF MS analysis of MOF (2 μM)-catalyzed acetylation of the H4K_C16 peptide (100 μM) in the presence of AcCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of AcCoA (middle panel), absence of MOF (bottom panel).

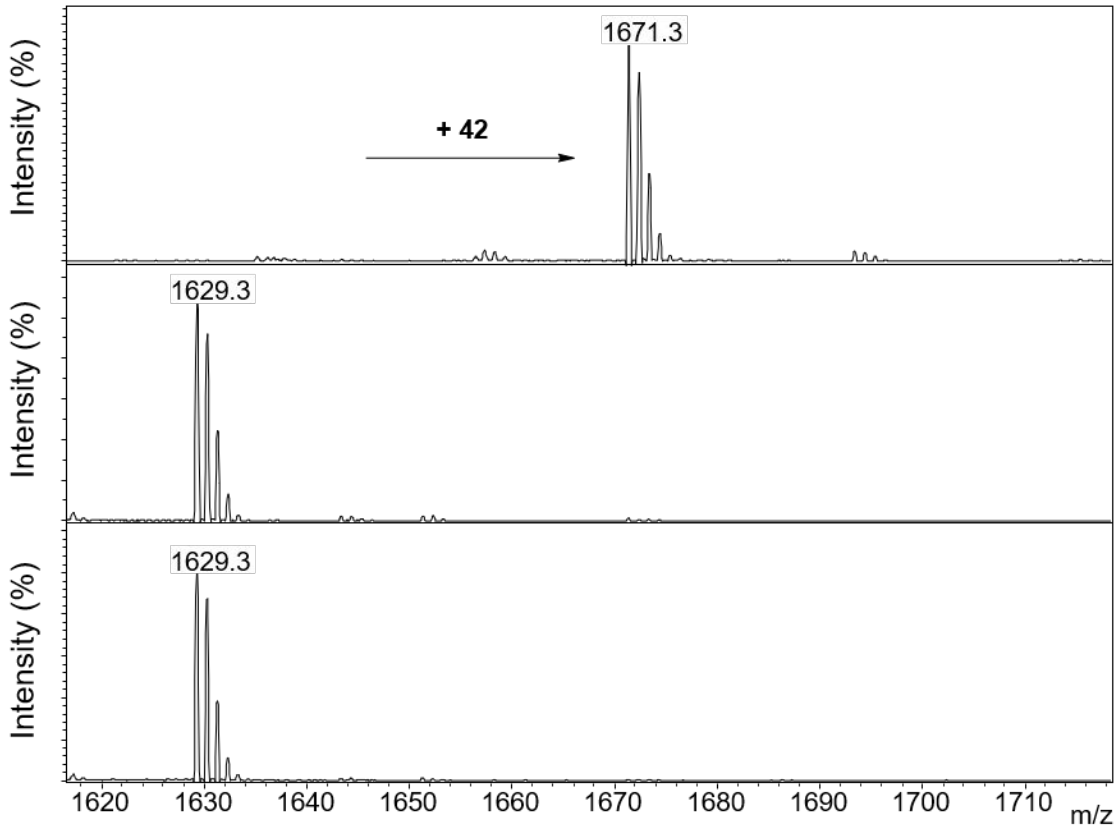


Figure S9. MALDI-TOF MS analysis of PCAF (2 μM)-catalyzed acetylation of the H3K14 peptide (100 μM) in the presence of AcCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of AcCoA (middle panel), absence of PCAF (bottom panel).

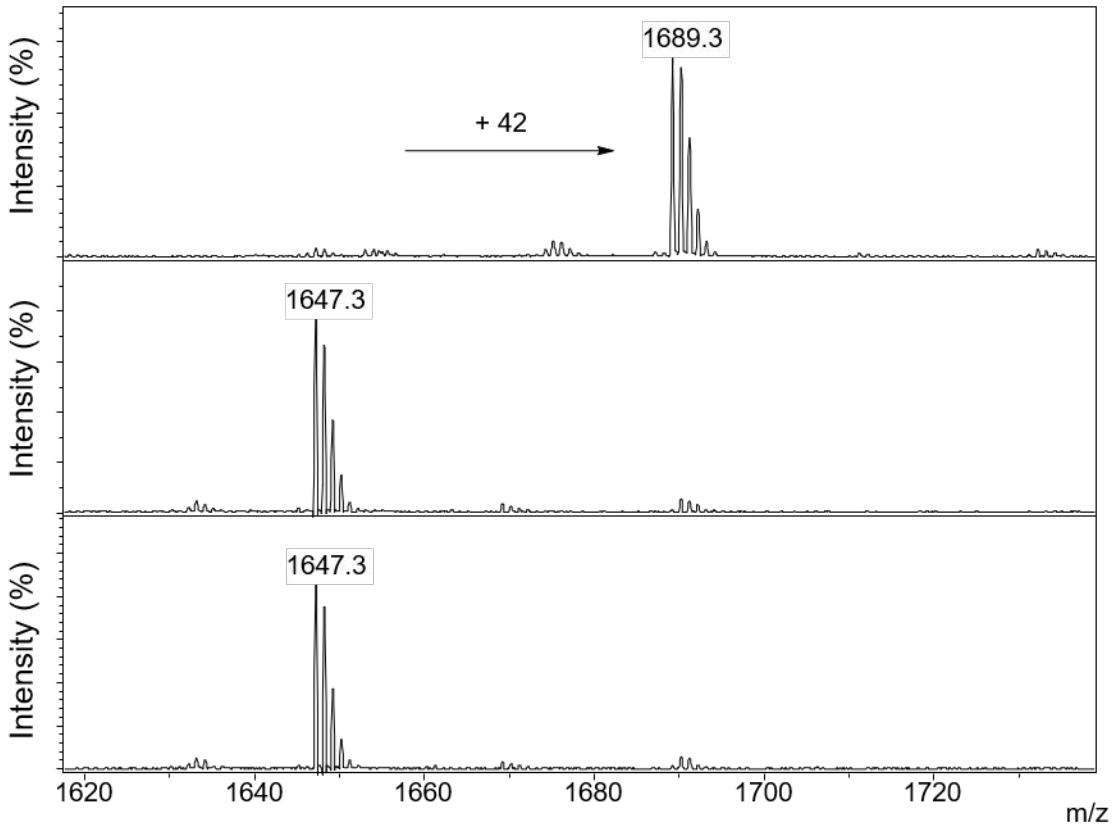


Figure S10. MALDI-TOF MS analysis of PCAF (2 μM)-catalyzed acetylation of the H3K_C14 peptide (100 μM) in the presence of AcCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of AcCoA (middle panel), absence of PCAF (bottom panel).

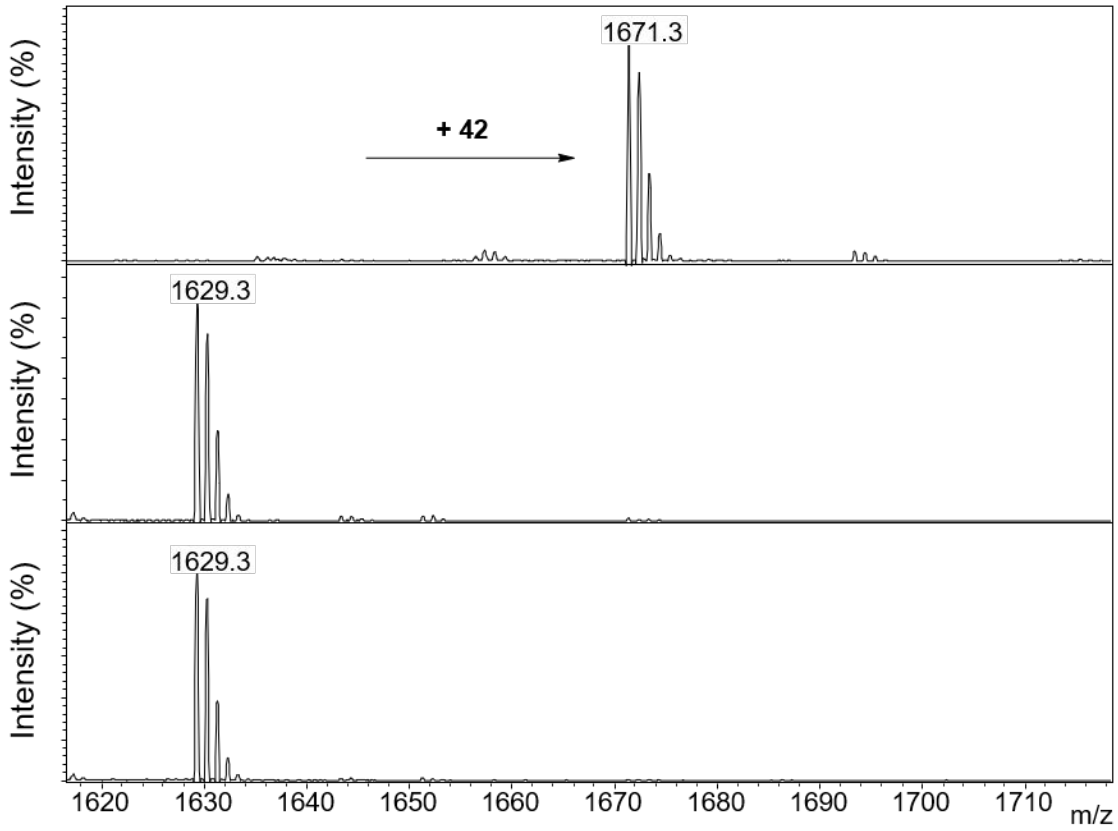


Figure S11. MALDI-TOF MS analysis of GCN5 (2 μM)-catalyzed acetylation of the H3K14 peptide (100 μM) in the presence of AcCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of AcCoA (middle panel), absence of PCAF (bottom panel).

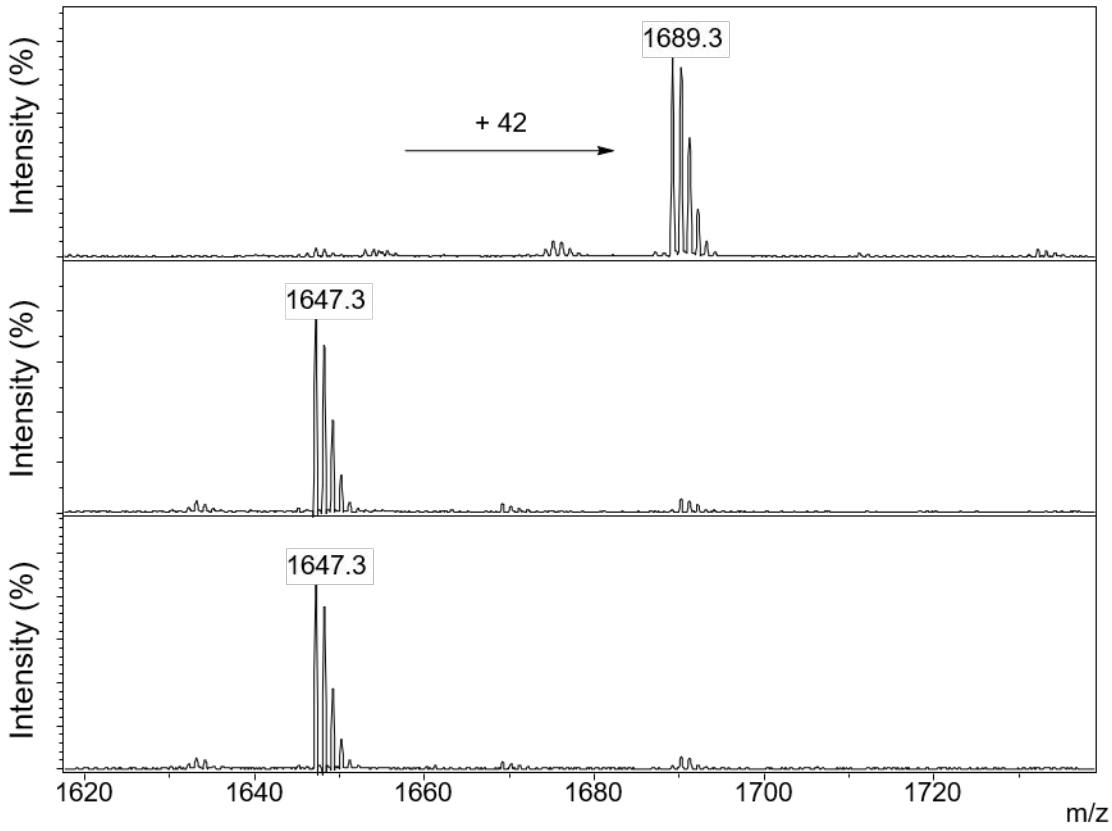


Figure S12. MALDI-TOF MS analysis of GCN5 (2 μM)-catalyzed acetylation of the H3K_C14 peptide (100 μM) in the presence of AcCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of AcCoA (middle panel), absence of PCAF (bottom panel).

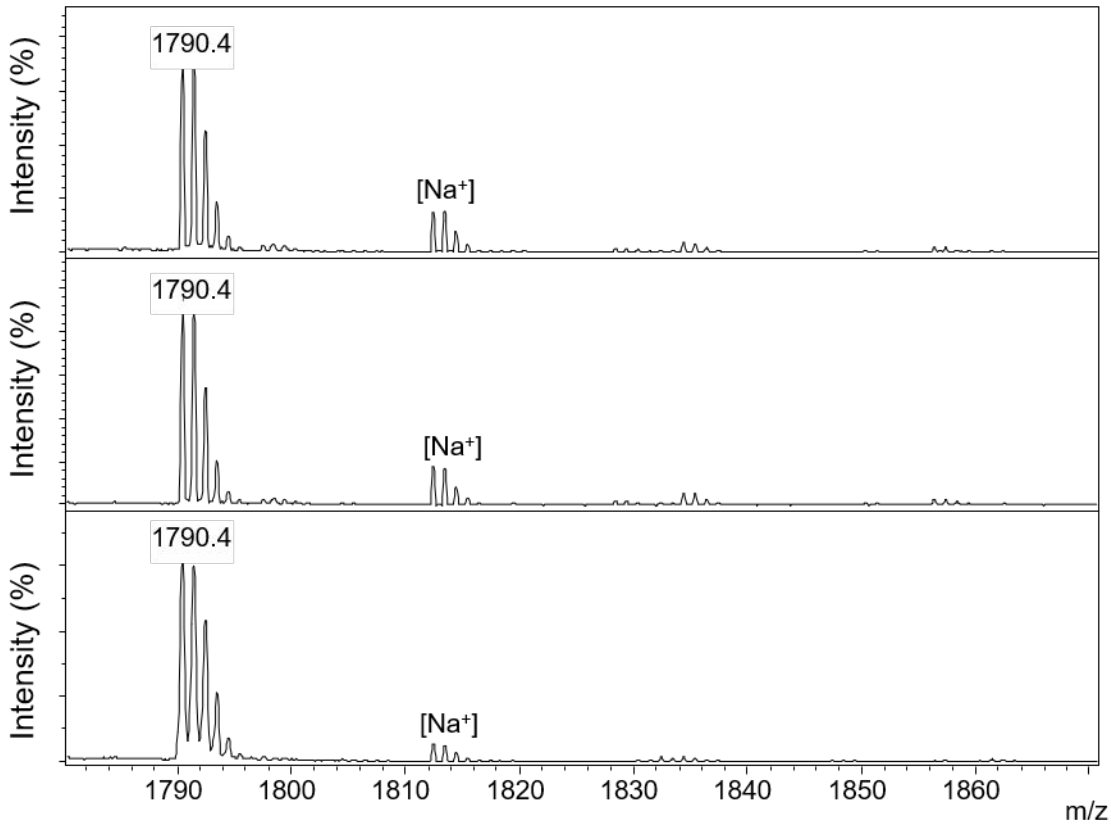


Figure S13. MALDI-TOF MS analysis of MOF (2 μM)-catalyzed acetylation of the H4K16ac peptide (100 μM) in the presence of AcCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of AcCoA (middle panel), absence of MOF (bottom panel).

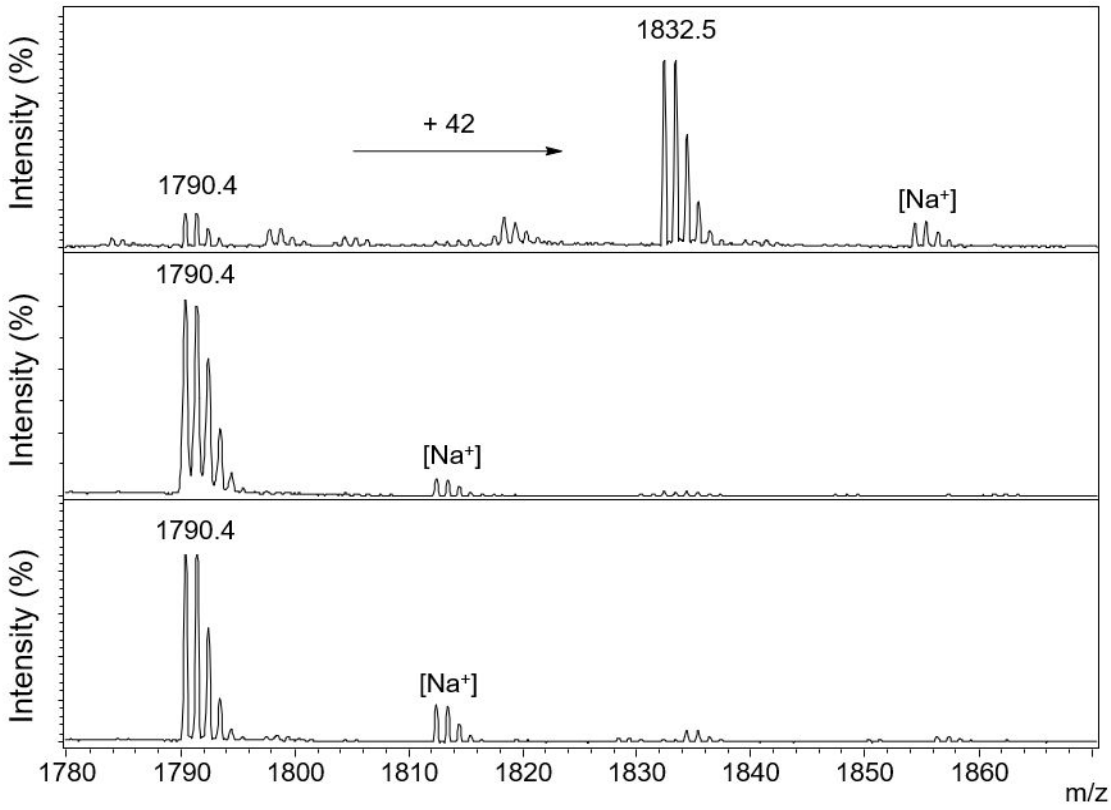


Figure S14. MALDI-TOF MS analysis of MOF (2 μM)-catalyzed acetylation of the H4K20ac peptide (100 μM) in the presence of AcCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of AcCoA (middle panel), absence of MOF (bottom panel).

9. Time course plots of KATs enzymatic acetylation and propionylation activity

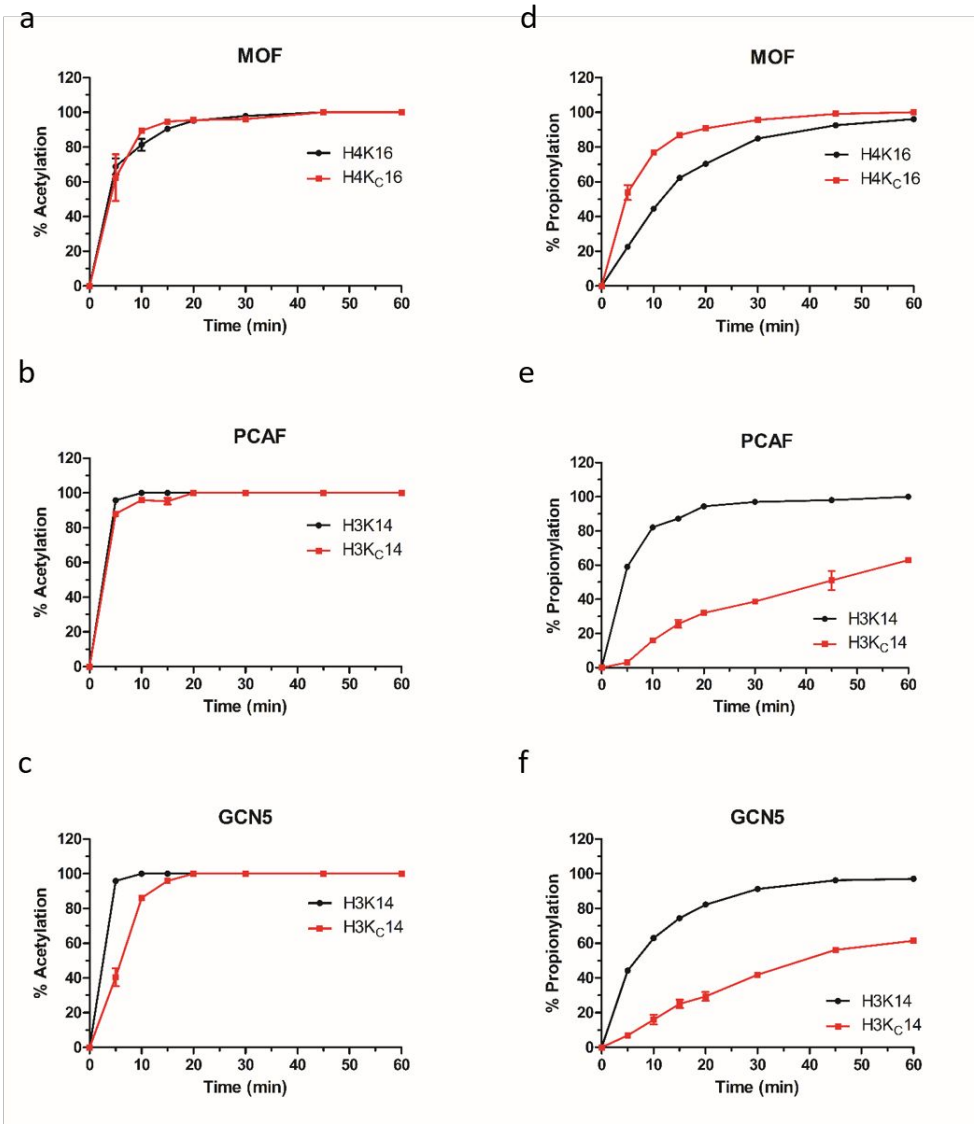


Figure S15. Time course analysis of KAT-catalyzed acylation reactions. MOF-catalyzed (a) acetylation and (d) propionylation of H4K16 and H4K$_C$16 histone peptides. PCAF-catalyzed (b) acetylation and (e) propionylation of H3K14 and H3K$_C$14 histone peptides. GCN5-catalyzed (c) acetylation and (f) propionylation of H3K14 and H3K$_C$14 histone peptides. The reactions were carried out in activity buffer (50 mM HEPES, 0.1 mM EDTA, 1 mM DTT, pH = 8.0) and quenched at set time points.

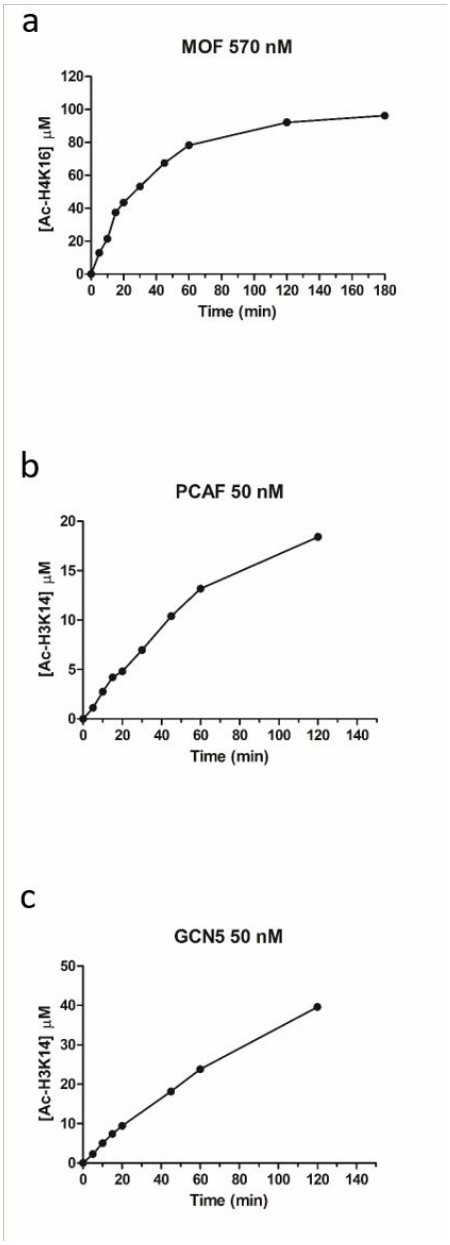


Figure S16. Time course analysis of KAT-catalyzed acetylation of H4K16 and H3K14 under kinetics conditions: AcCoA (100 μM), histone peptide (100 μM), in kinetic buffer (50 mM HEPES, 0.1 mM EDTA, 0.01% TRITON-X, pH = 7.4). The reactions were started by the addition of (a) MOF (570 nM); (b) PCAF (50 nM); or (c) GCN5 (50 nM). Reaction mixtures were quenched at set time and analyzed by MALDI-MS.

10. MALDI-TOF investigation of H4K16 and H4K16ac ionization behavior

NMR calibration of two weighed equimolar (2 mM final concentration) solution of H4K16 and synthetic H4K16ac peptide was performed by integration of the characteristic protons of the histidine imidazole nucleus (δ ppm = 8.64; 7.31) contained in the H4 sequence, which were normalized to an added standard of DSS in D_2O (1 mM final concentration). Upon calculation of actual concentration, the two solutions containing H4K16 and H4L16ac were combined (1:1 molar ratio) and analyzed by MALDI-TOF MS.

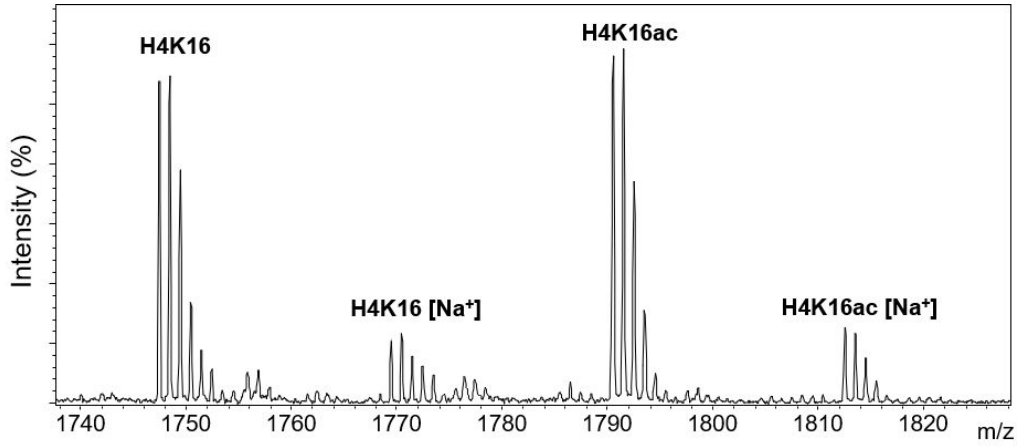


Figure S17. MALDI-TOF MS analysis of an equimolar amounts of synthetic H4K16 and H4K16ac.

11. MALDI-TOF MS propionylation supporting figures

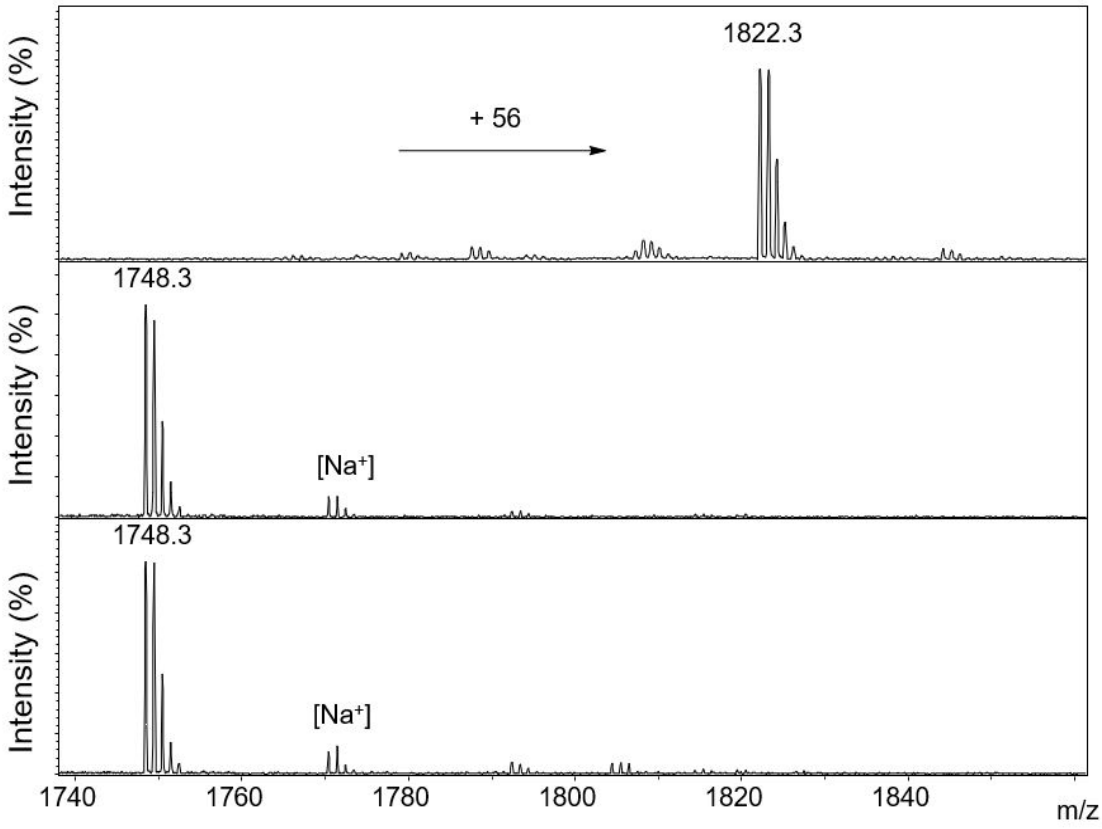


Figure S18. MALDI-TOF MS analysis of MOF (2 μM)-catalyzed propionylation of the H4K16 peptide (100 μM) in the presence of ProCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of ProCoA (middle panel), absence of MOF (bottom panel).

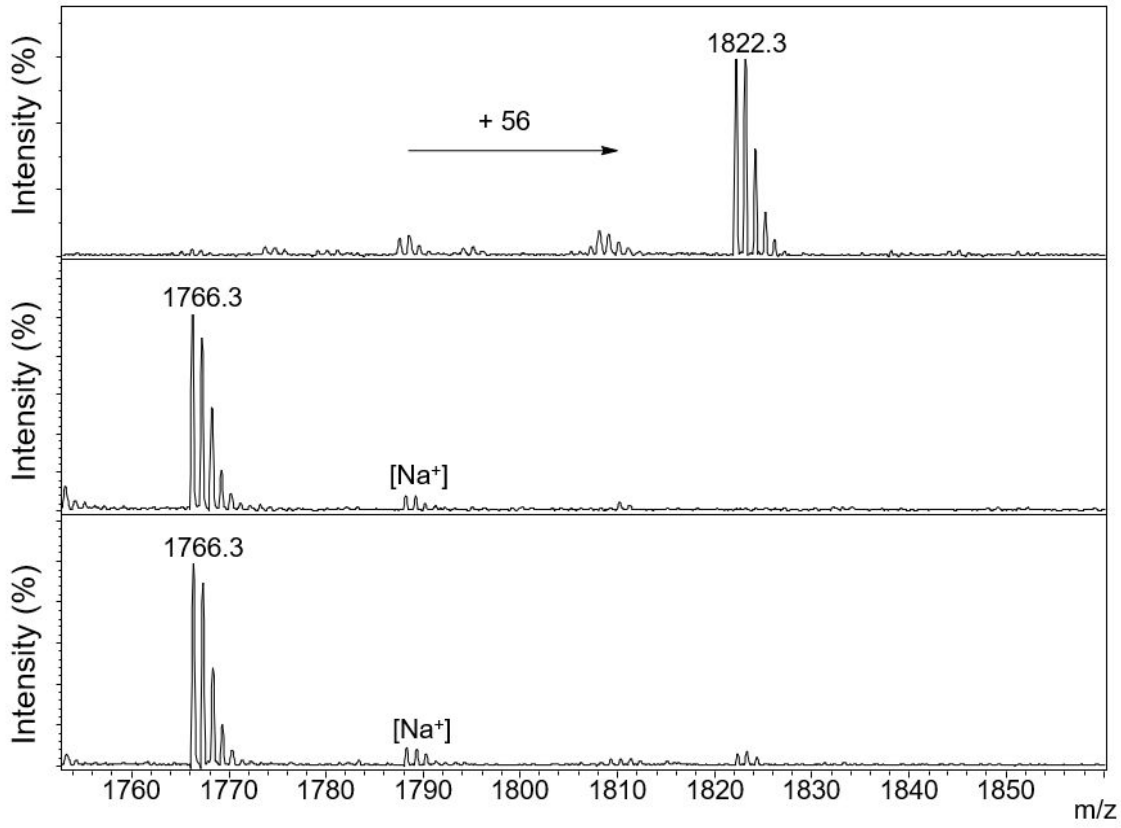


Figure S19. MALDI-TOF MS analysis of MOF (2 μM)-catalyzed propionylation of the $H4K_C16$ peptide (100 μM) in the presence of ProCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of ProCoA (middle panel), absence of MOF (bottom panel).

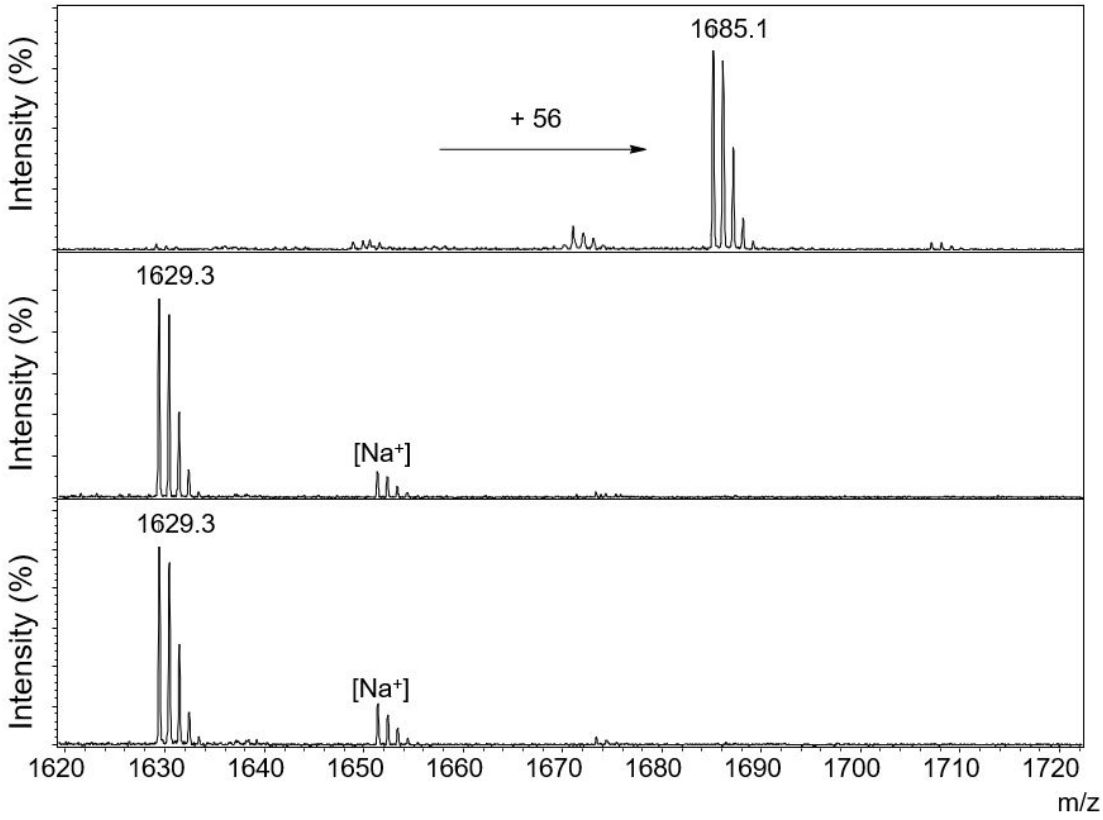


Figure S20. MALDI-TOF MS analysis of PCAF (2 μM)-catalyzed propionylation of the H3K14 peptide (100 μM) in the presence of ProCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of ProCoA (middle panel), absence of PCAF (bottom panel).

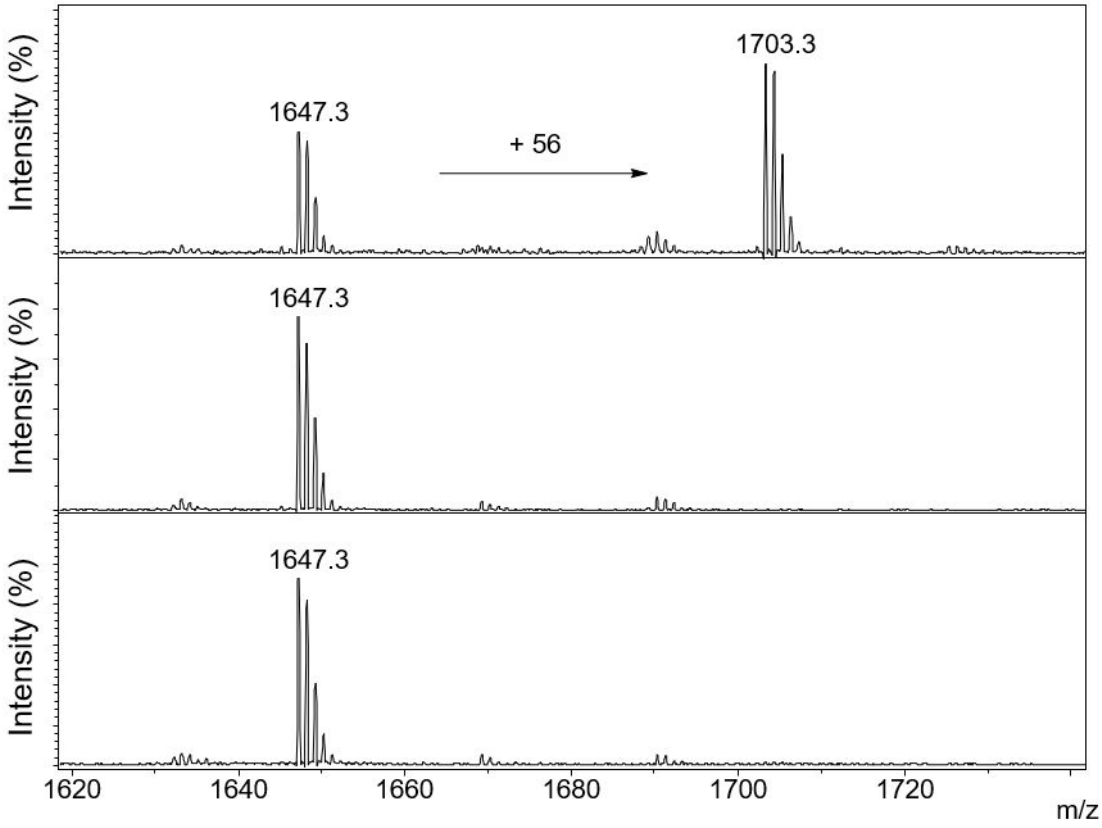


Figure S21. MALDI-TOF MS analysis of PCAF (2 μM)-catalyzed propionylation of the $H3K_C14$ peptide (100 μM) in the presence of ProCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of ProCoA (middle panel), absence of PCAF (bottom panel).

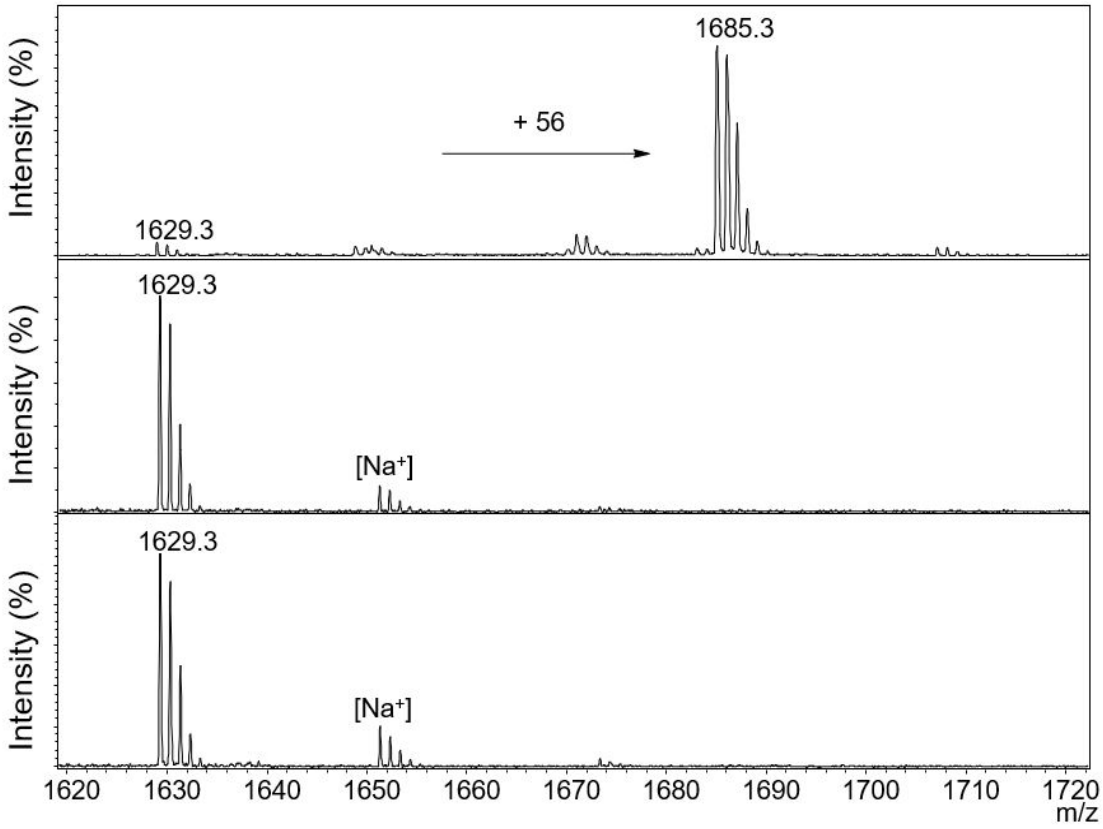


Figure S22. MALDI-TOF MS analysis of GCN5 (2 μM)-catalyzed propionylation of the H3K14 peptide (100 μM) in the presence of ProCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of ProCoA (middle panel), absence of PCAF (bottom panel).

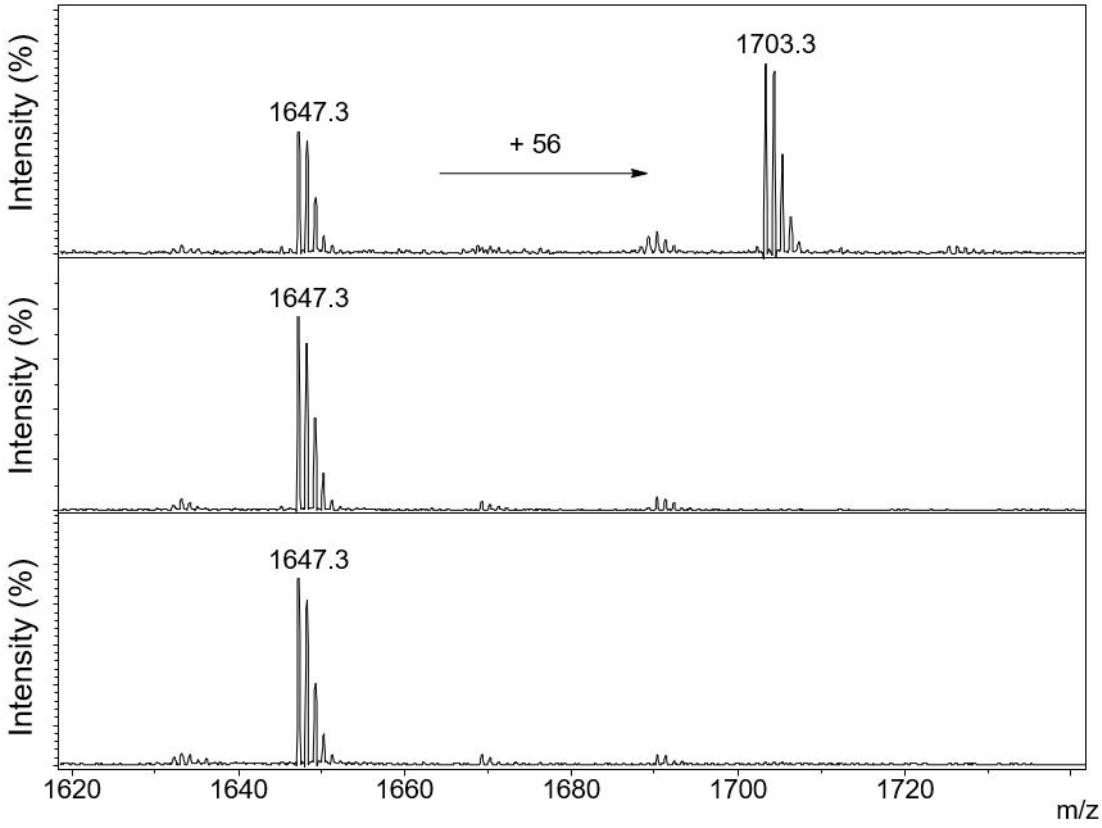


Figure S23. MALDI-TOF MS analysis of GCN5 (2 μM)-catalyzed propionylation of the $H3K_C14$ peptide (100 μM) in the presence of ProCoA (300 μM) at 37 °C, after 1 h incubation (top panel). Control reactions after 1 h: absence of ProCoA (middle panel), absence of PCAF (bottom panel).

12. MALDI-TOF MS of GCN5 catalyzed competition experiment

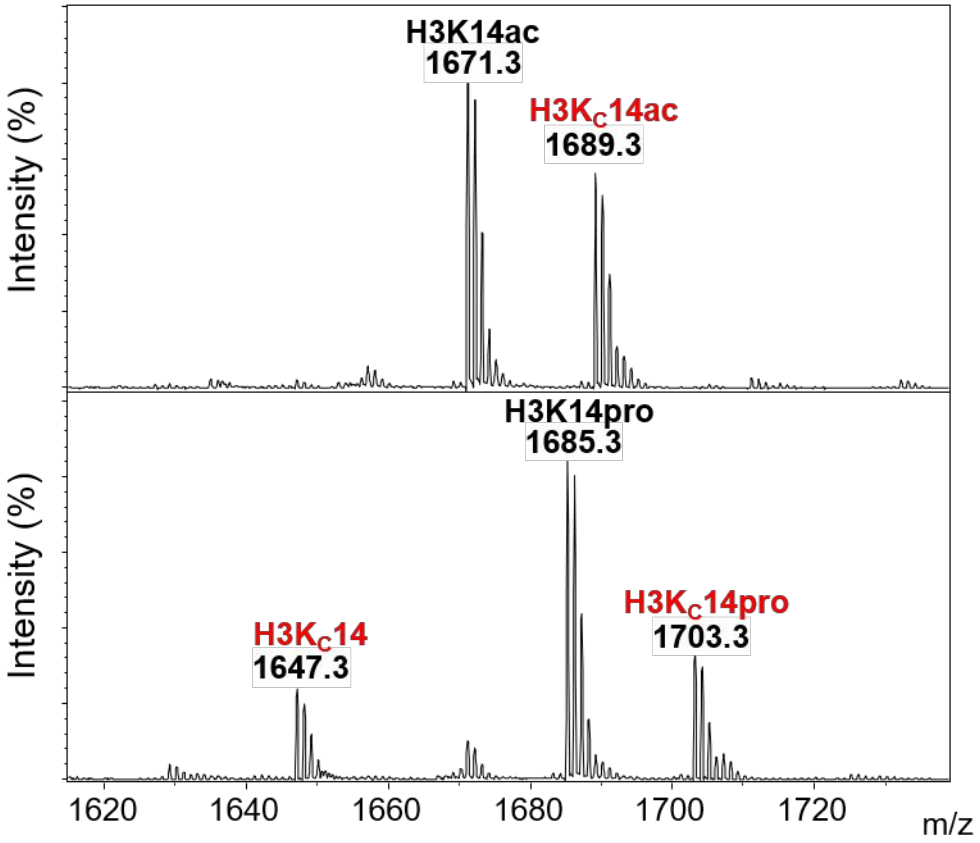


Figure S24. MALDI-TOF analysis of the competition experiment for GCN5-catalyzed acylation (acetylation top panel; propionylation bottom panel) of H3K14 and H3K$_C$14 peptides, at 37 °C for 1 h.

13. Time course plots of KATs enzymatic butyrylation and crotonylation

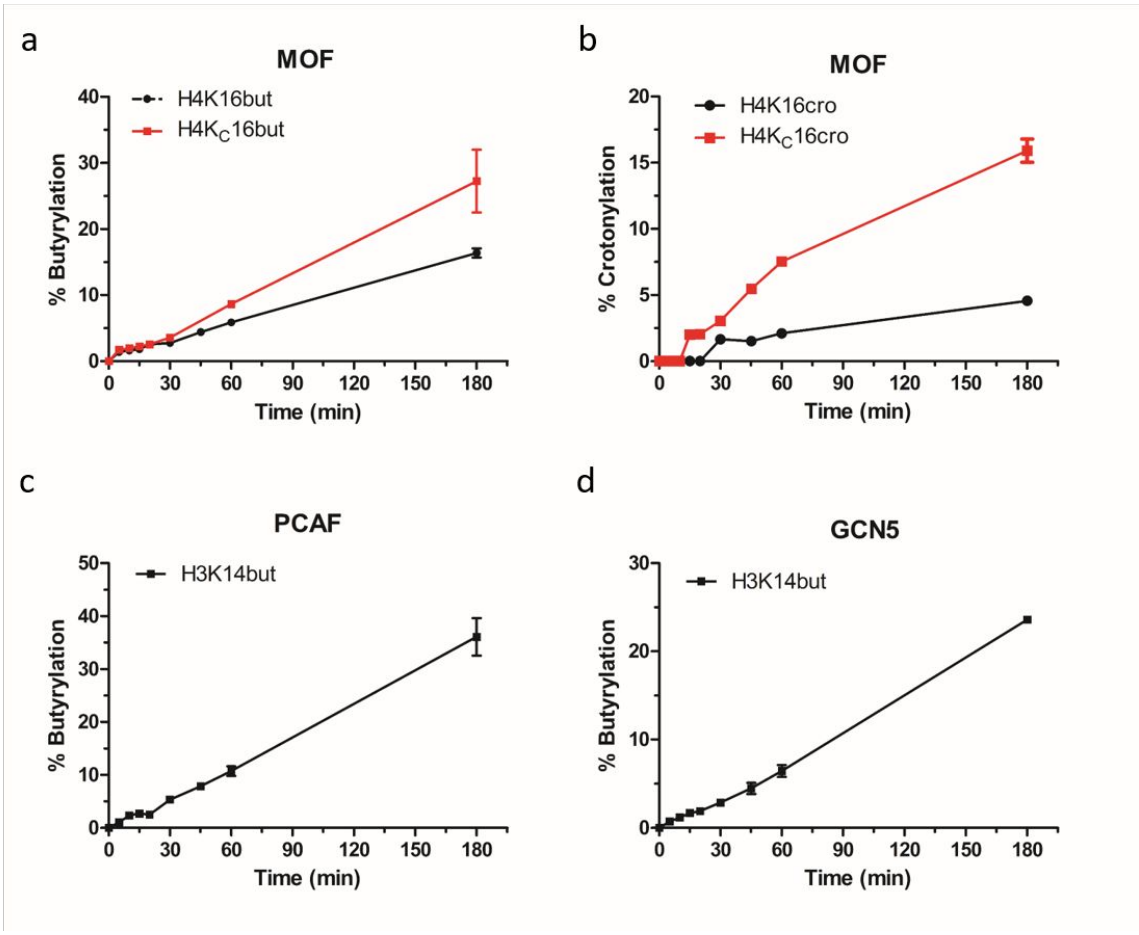


Figure S25. Time course analysis of KAT-catalyzed acylation reactions. MOF-catalyzeed (a) butyrylation and (b) crotonylation of H4K16 and H4K$_C$16 histone peptides. PCAF-catalyzed (c) butyrylation of H3K14, and (d) GCN5-catalyzed butyrylation of H3K14. The reactions were carried out in activity buffer (50 mM HEPES, 0.1 mM EDTA, 1 mM DTT, pH = 8.0) and quenched at set time points.

14. MALDI-TOF MS butyrylation and crotonylation supporting figures

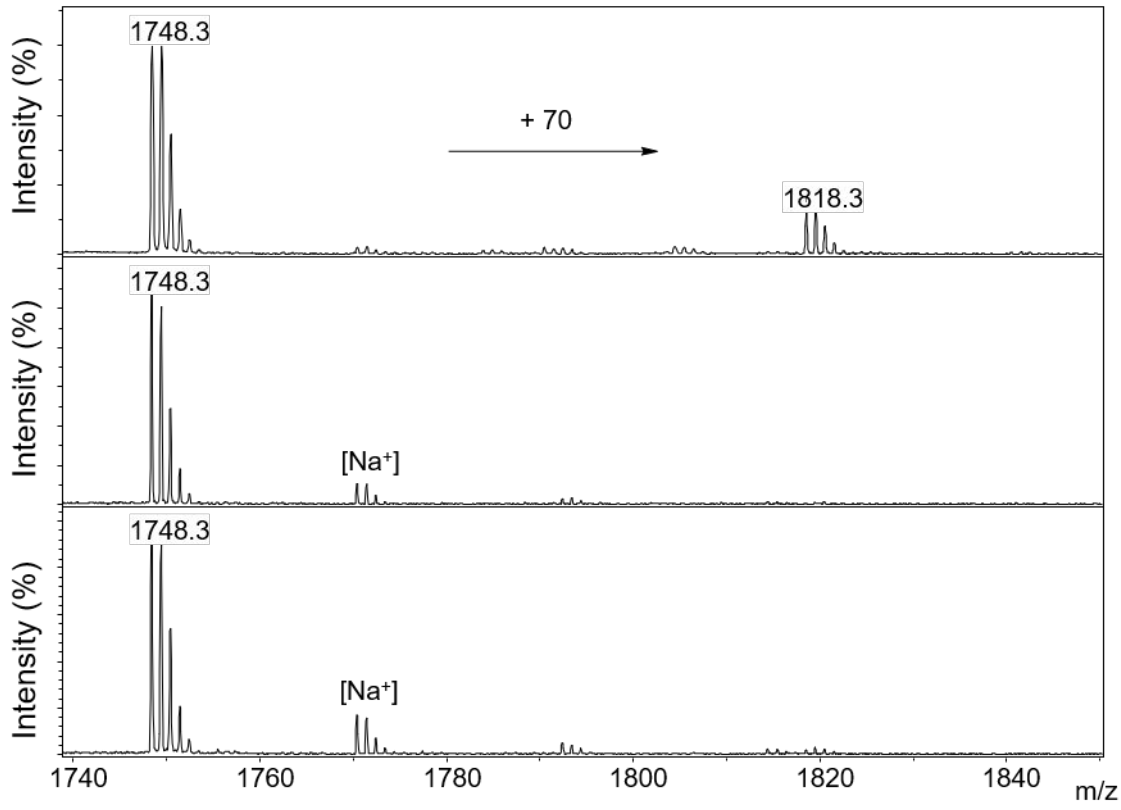


Figure S26. MALDI-TOF MS analysis of MOF (2 μM)-catalyzed butyrylation of the H4K16 peptide (100 μM) in the presence of ButCoA (300 μM) at 37 °C, after 3 h incubation (top panel). Control reactions after 3 h: absence of ButCoA (middle panel), absence of MOF (bottom panel).

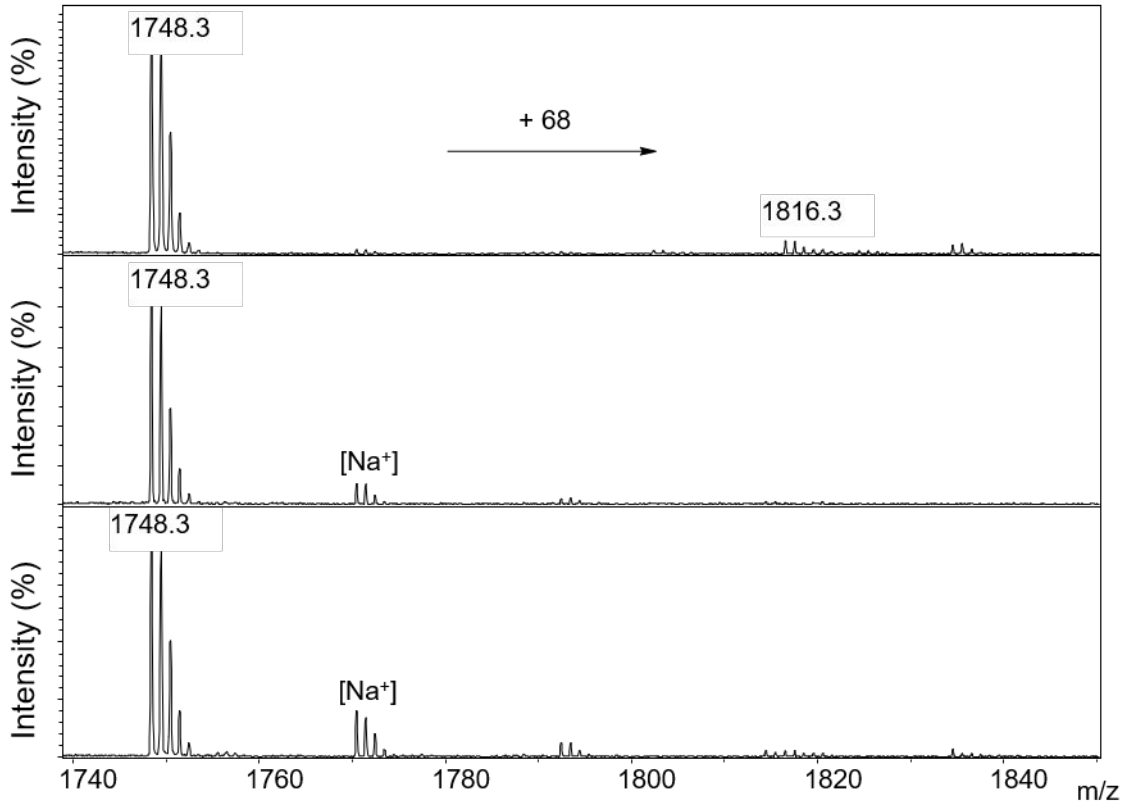


Figure S27. MALDI-TOF MS analysis of MOF (2 μM)-catalyzed crotonylation of the H4K16 peptide (100 μM) in the presence of CroCoA (300 μM) at 37 °C, after 3 h incubation (top panel). Control reactions after 3 h: absence of CroCoA (middle panel), absence of MOF (bottom panel).

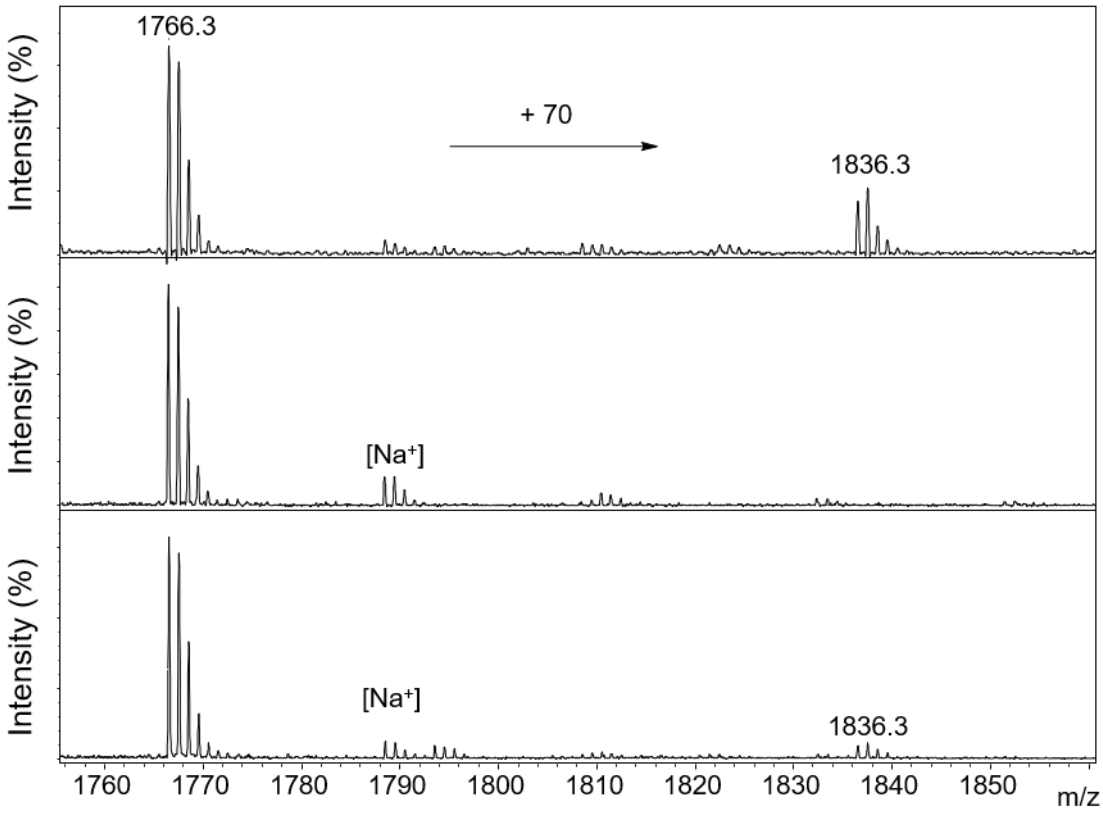


Figure S28. MALDI-TOF MS analysis of MOF (2 μM)-catalyzed butyrylation of the $H4K_C16$ peptide (100 μM) in the presence of ButCoA (300 μM) at 37 °C, after 3 h incubation (top panel). Control reactions after 3 h: absence of ButCoA (middle panel), absence of MOF (bottom panel).

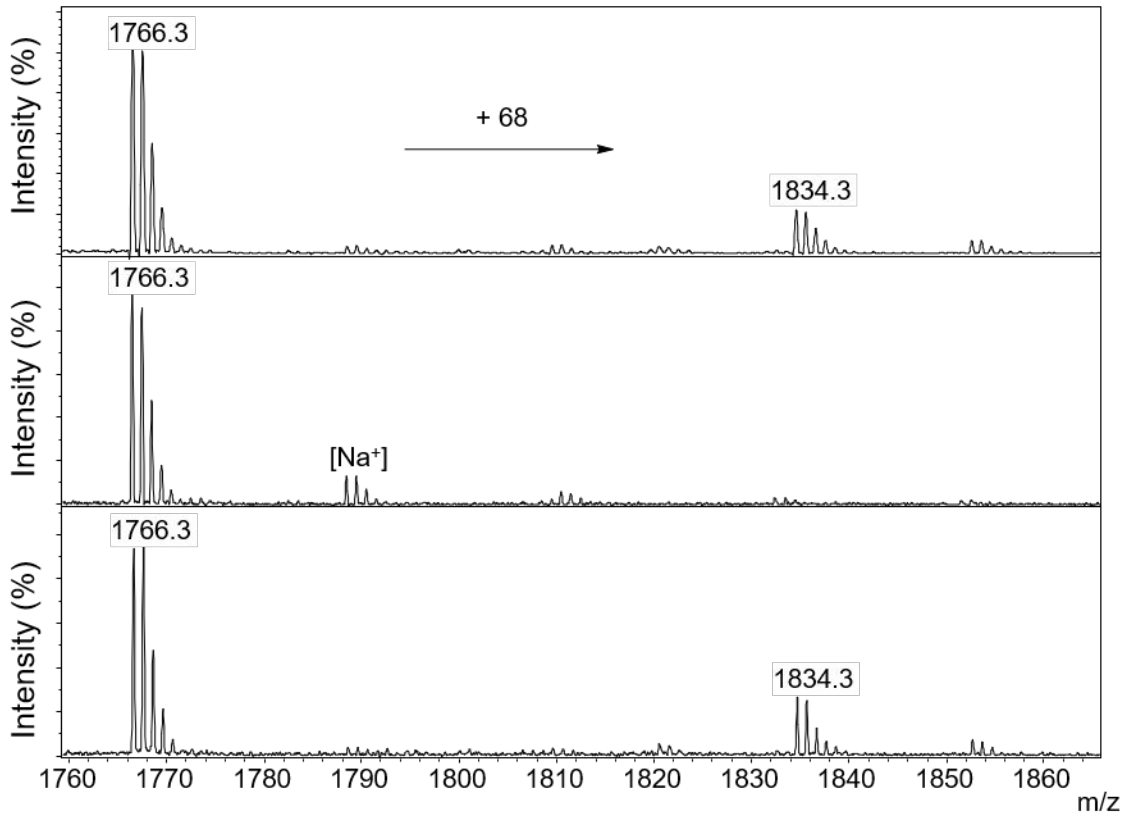


Figure S29. MALDI-TOF MS analysis of MOF (2 μM)-catalyzed crotonylation of the H4K$_C$16 peptide (100 μM) in the presence of CroCoA (300 μM) at 37 °C, after 3 h incubation (top panel). Control reactions after 3 h: absence of CroCoA (middle panel), absence of MOF (bottom panel).

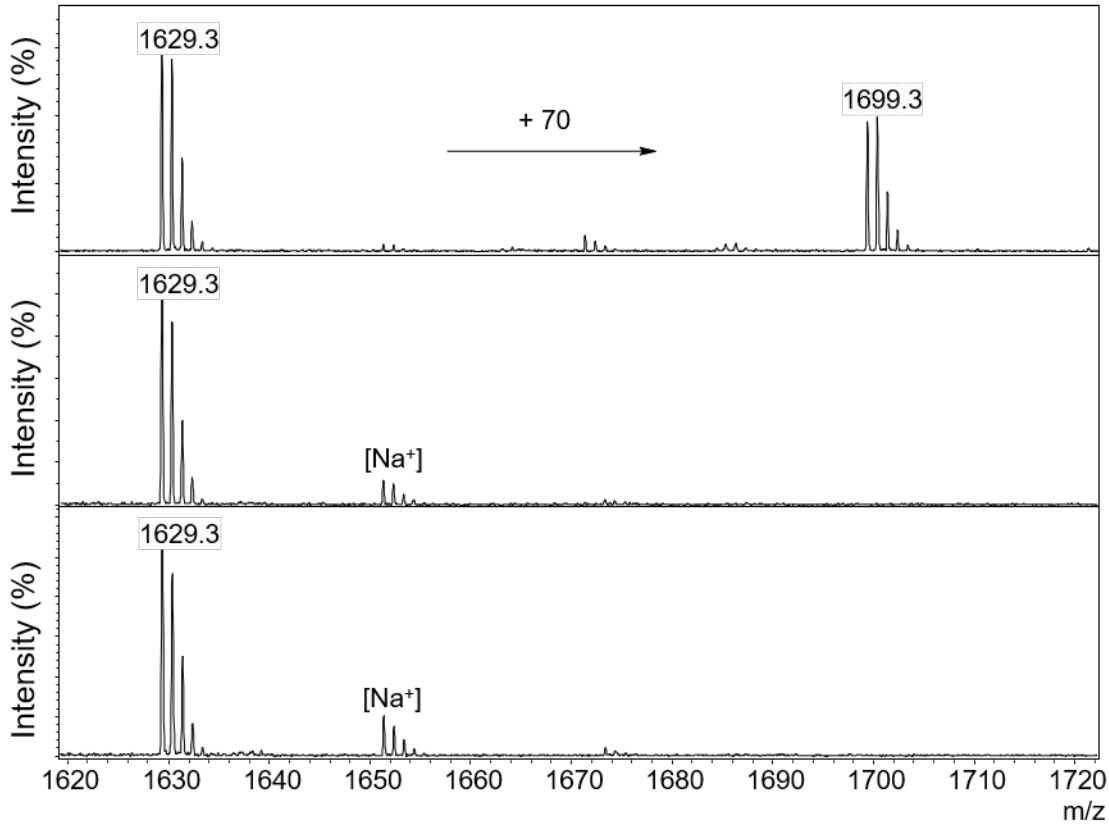


Figure S30. MALDI-TOF MS analysis of PCAF (2 μM)-catalyzed butyrylation of the H3K14 peptide (100 μM) in the presence of ButCoA (300 μM) at 37 °C, after 3 h incubation (top panel). Control reactions after 3 h: absence of ButCoA (middle panel), absence of PCAF (bottom panel).

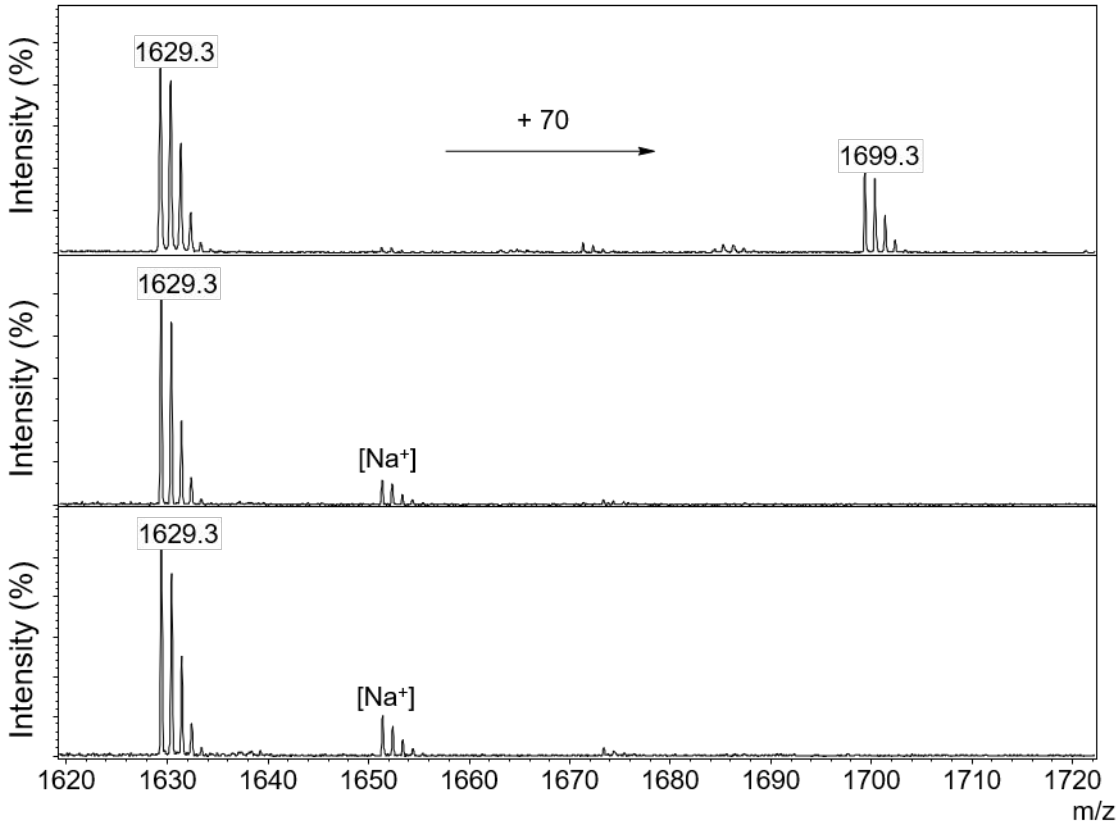


Figure S31. MALDI-TOF MS analysis of GCN5 (2 μM)-catalyzed butyrylation of the H3K14 peptide (100 μM) in the presence of ButCoA (300 μM) at 37 °C, after 3 h incubation (top panel). Control reactions after 3 h: absence of ButCoA (middle panel), absence of PCAF (bottom panel).

15. References

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(5) Richardson, S. L., Hanjra, P., Zhang, G., Mackie, B. D., Peterson, D. L., Huang, R. (2015) A Direct, Ratiometric, and Quantitative MALDI-MS Assay for Protein Methyltransferases and Acetyltransferases. *Anal. Biochem.* 478, 59–64.