Supplemental figures

One minute analysis of 200 histone post-translational modifications by direct injection mass spectrometry

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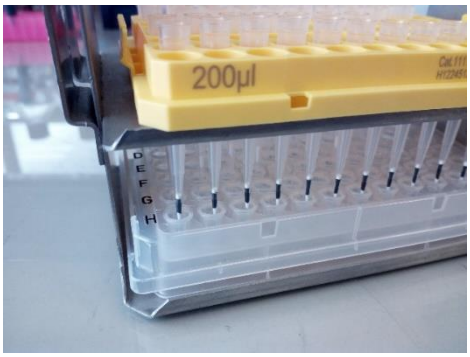
Supplemental Figure 1: Sample preparation and standard for DI-MS. (A) View of stage tips used for multi-channel sample preparation of histone samples. The tips contain a layer of Porous Graphitic Carbon (PGC, HypercarbTM, Thermo) and the plate holders are designed to allow collection of the eluate in 96-well plates. (B) View of the rotor used for drying 96-well plates (the centrifuge can work as SpeedVac), but also to perform stage tipping to up to 4 plates. (C) Chromatogram view of a DI-MS acquisition of digested BSA (1 µg/µl). The method is simple data-dependent acquisition (DDA) normally used for any proteomics experiment, programmed to perform a full MS scan (average 10 microscans) followed by an undefined number of MS/MS events. The longer the cycle the more the signals suitable to be selected for fragmentation. (D) Full MS scan of the BSA peptides detected by DI-MS using the DDA method. (E) Sequence coverage of the BSA using the described DDA method in DI-MS.

A



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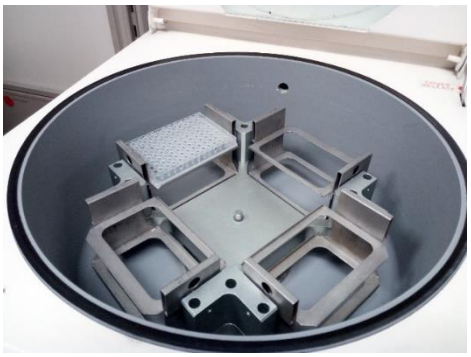
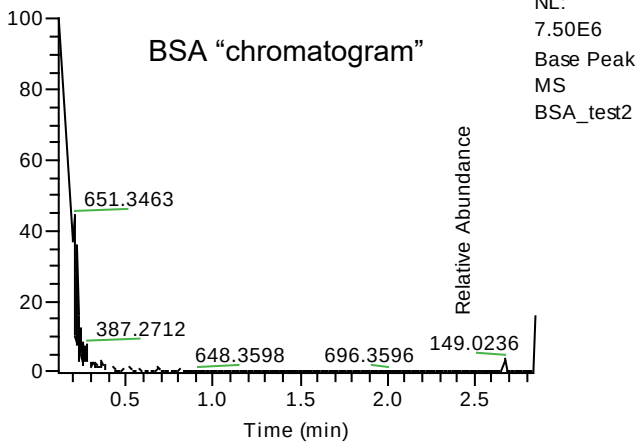
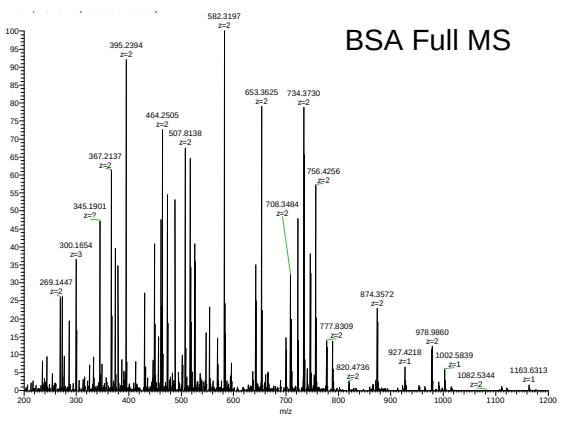


C



D



E

BSA sequence coverage (61%)

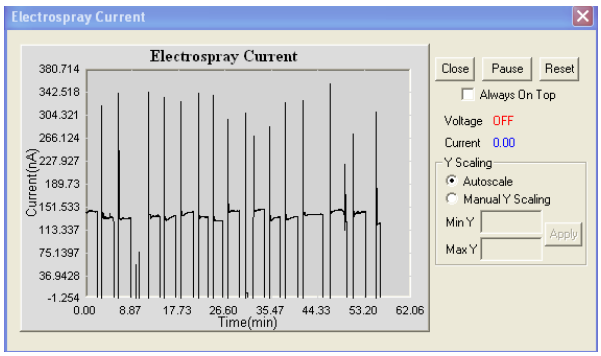
Signal peptide **Propeptide** **Identified peptides**

MKWVTFISLLLLFSSAYS**RGVFRR**DTHK**SEIAHR**FK**DLGEEHFK**GLVLIAFSQYLQQCPFDEHVK**LVNELTEFAK**TCVAD
ESHAGCEK**SLHTLFGDELCKVASLR**ETYGDMADCCEKQEPER**NECFLSHKDDSPDLPKLKPDPNTLCDEFK**ADEKK**FW**
GKYLYEIARRHPYFYAPELLYYANK**YNGVFQECCQAEDKGACLLPKIETMR**EKVLASSARQRLR**CASIQKFGER**ALK**AW**
SVARLSQKFPK**AEFVEVTKLVTDLTK**VHKECCHGDLLECADDRADLAK**YICDNQDTISSK**LK**ECCDKPLLEK**SHCIAEVEK
DAIPENLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSR**RHPEYAVSVLLR**LAK**EYEATLEECCAKDDPHACYSTVF**
DKLK**HLVDEPQNLIK**QNCDQFEK**LGEYGFQNALIVR**YTR**KVPQVSTPTLVEVSR**SLGKVGTRCCTKPESER**MPCTEDYL**
SLILNRLCVLHEKTPVSEKVTK**CCTESLVNRRPCFSALTPDETYVPK**AFDEK**LFTFHADICTLPDTEK**QIKK**QTALVELLK**
HKPKATEEQLK**TVMENFVAFVDK**CCAADDK**EACFAVEGPKLVVSTQTALA**

Supplemental Figure 2: Estimation of injection reproducibility of the TriVersa NanoMate. (A) Screenshot of the electrospray current plot generated by the Advion software controlling the TriVersa NanoMate. The experiment is repetitive injections of the BSA; when the instrument switches on the voltage it is possible to observe a spike in the ion current, which is then stabilized at around 150 nA until the injection is completed. (B) Quantification of the selected peptide of histone H3 KSTGGKAPR (aa 9-17) modified with a trimethyl group on the lysine residue 9 (H3K9me3). The analysis corresponds to 104 samples ran as unassisted queue.

A

Multiple sample injections



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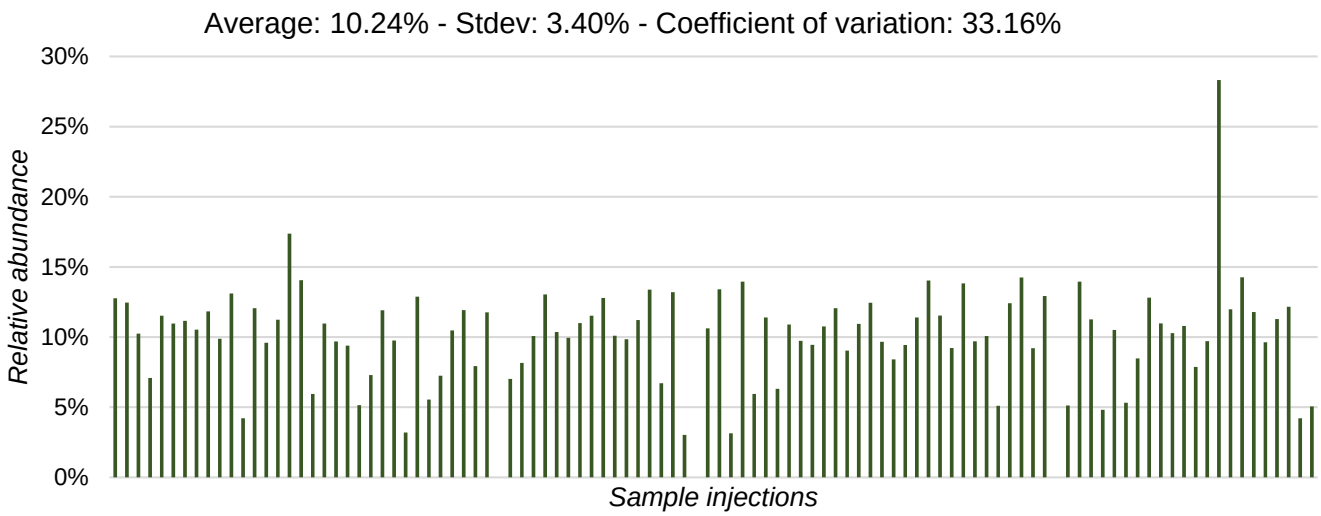
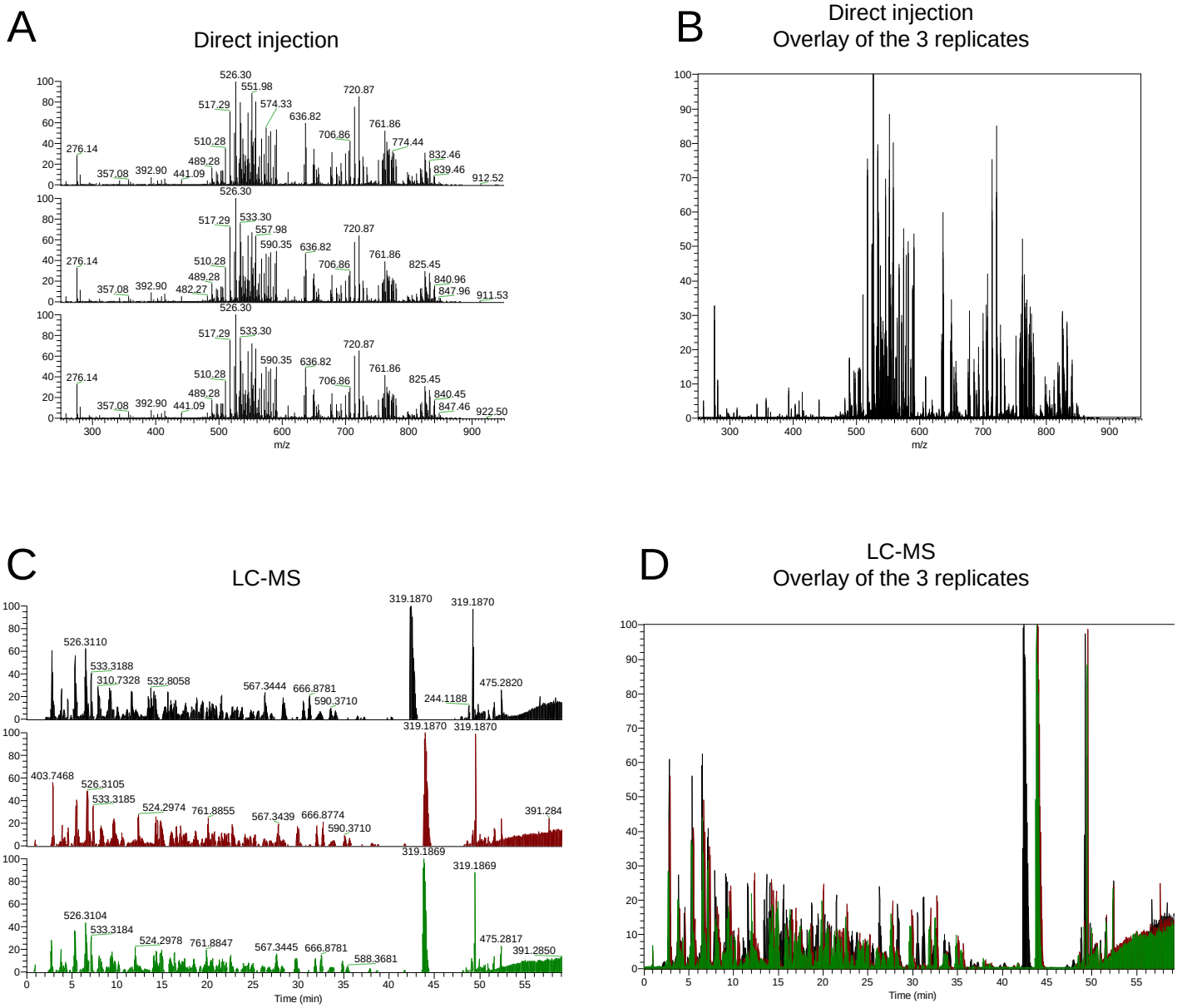
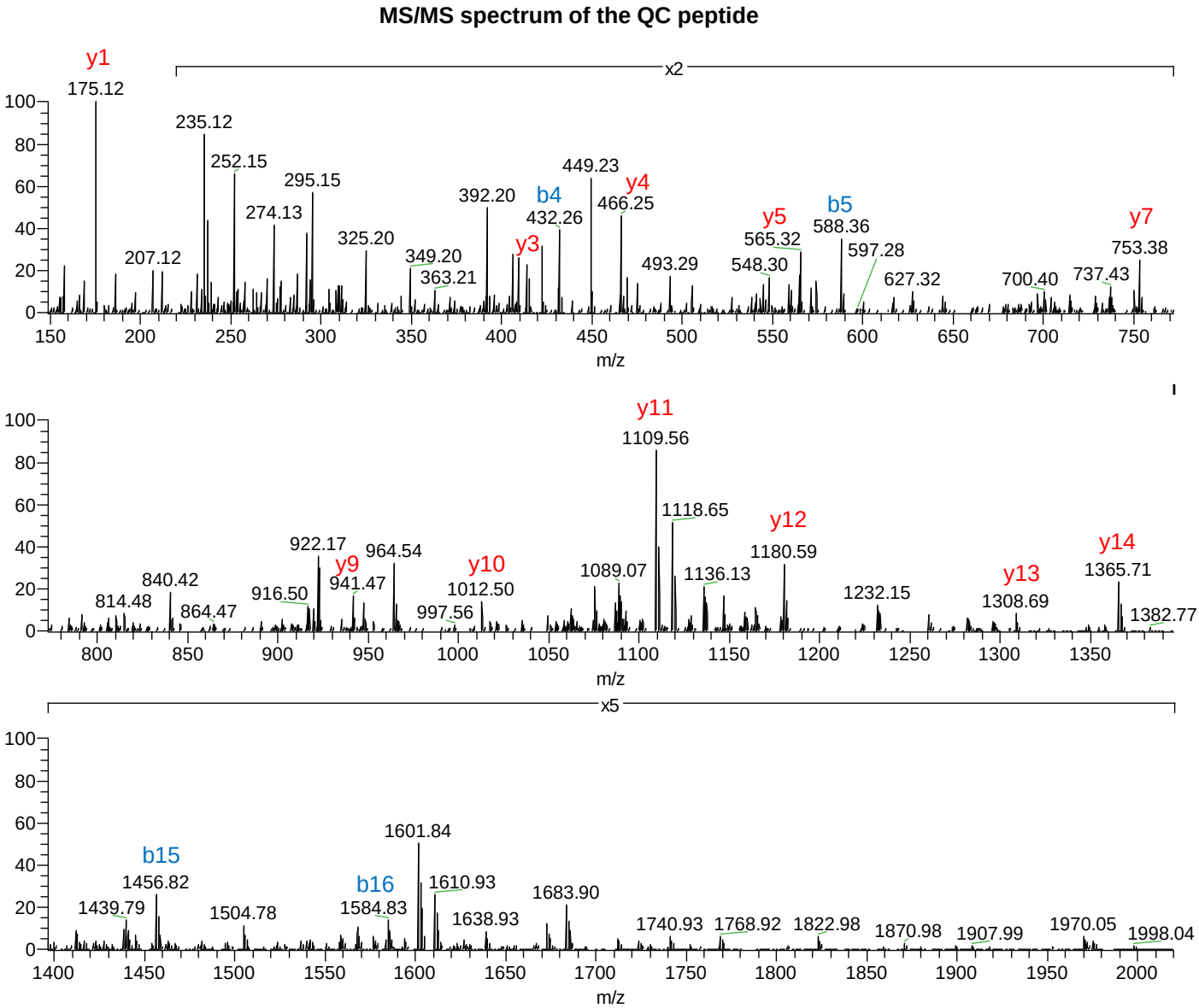


Supplemental Figure 3: Reproducibility of DI-MS and LC-MS. (A) Three injections of our custom synthetic peptide library acquired using DI-MS. (B) Overlay of the three spectra acquired using DI-MS. (C) Three injections of the library using LC-MS. (D) Overlay of the three chromatograms.

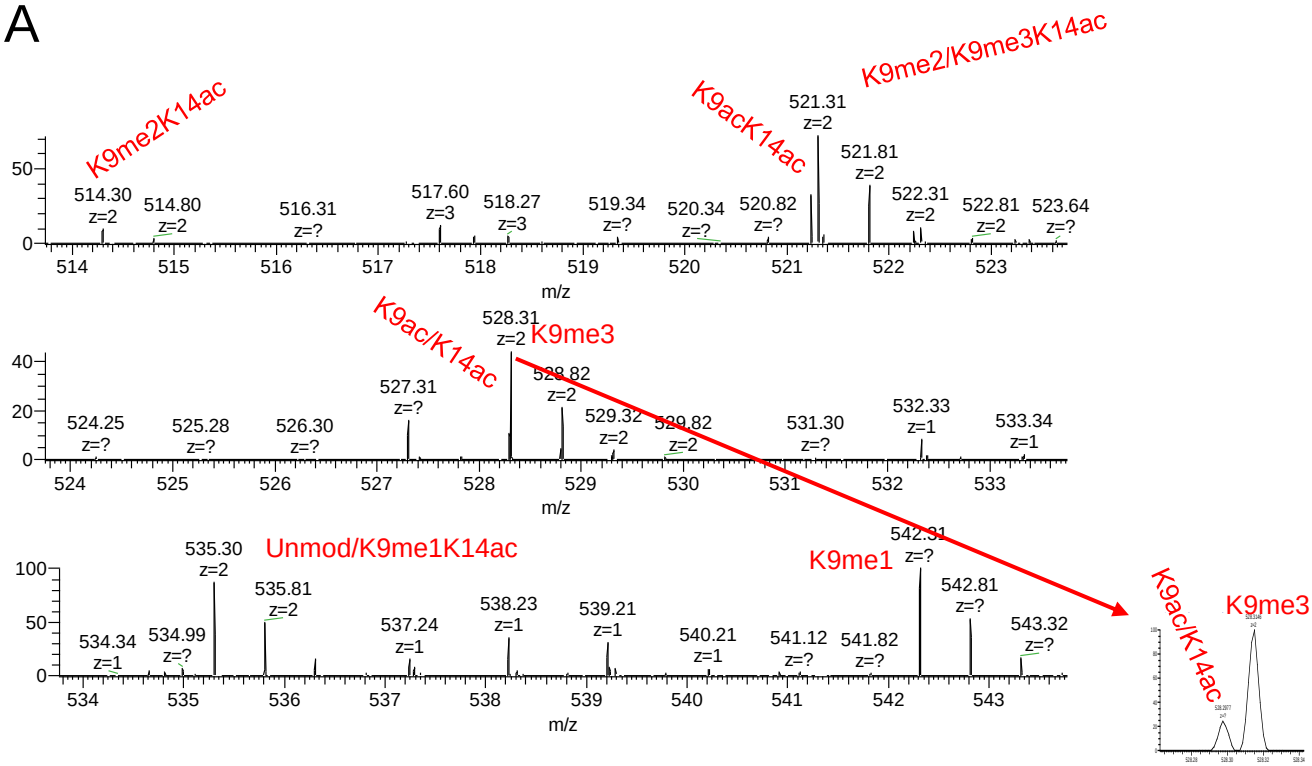


Supplemental Figure 4: MS/MS spectrum of the QC peptide. Manual annotation of fragment MS/MS ions obtained by HCD fragmentation of the intact QC peptide (underivatized and undigested). Only the theoretical masses labeled in the image were annotated, and only the singly charged ions.



Supplemental Figure 5: Example of DI-MS acquisition of endogenous histone peptides. (A) Full MS window of the DI-MS scan event detecting the modified forms of the histone H3 peptide KSTGGKAPR (aa 9-17). The sample was prepared using peptide N-termini derivatization. (B) View of the same peptides acquired using LC-MS. (C) Example of the m/z values of the histone H3 peptide KSAPATGGVKKPHR (aa 27-40). The figure shows that multiple isobaric forms are present, requiring the discrimination of up to four different forms (example in red) with the same mass by MS/MS.

A



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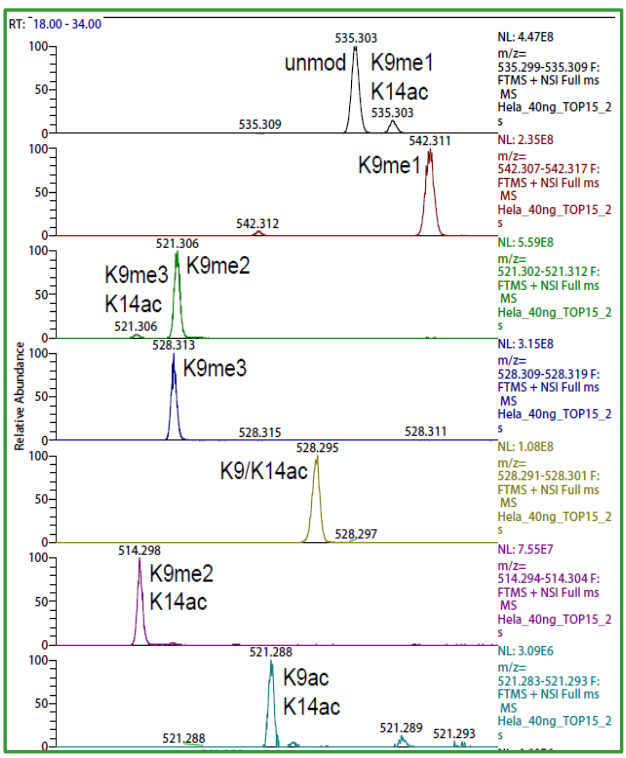
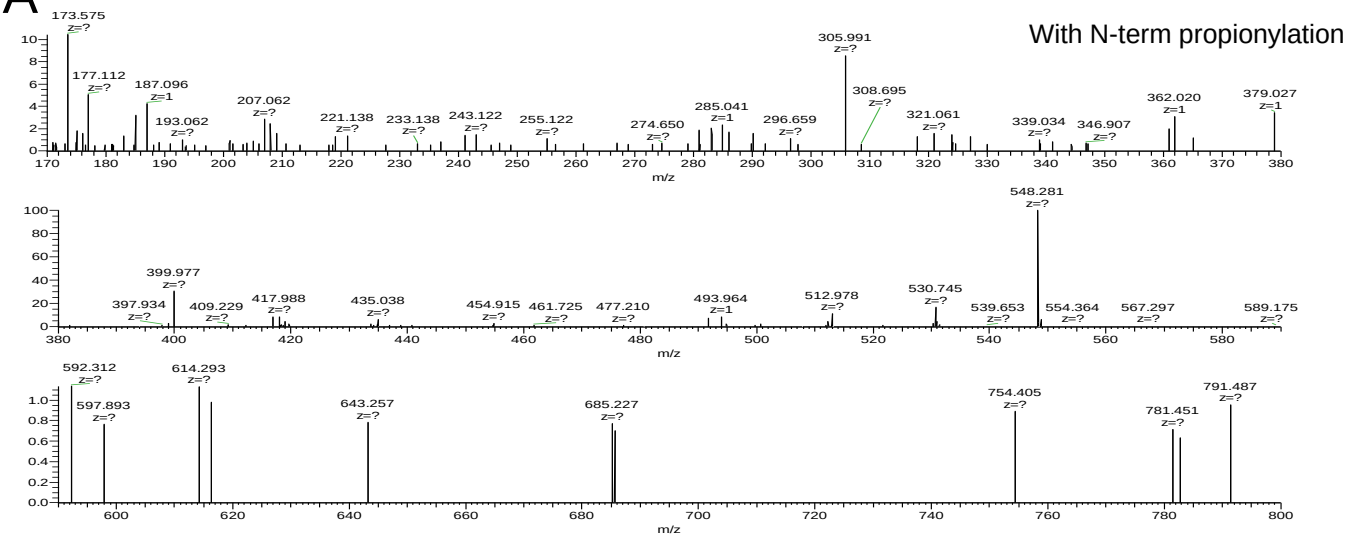


C

H3_27_40 unmod	553.3176
H3_27_40 K36me1	557.9895
H3_27_40 K27me1	557.9895
H3_27_40 K27me2	543.986
H3_27_40 K36me2	543.986
H3_27_40 K27me3	548.6579
H3_27_40 K36me3	548.6579
H3_27_40 K27me2K36me1	548.6579
H3_27_40 K27me1K36me2	548.6579
H3_27_40 K27me1K36me1	562.6614
H3_27_40 K27me3K36me1	553.3298
H3_27_40 K27me1K36me3	553.3298
H3_27_40 K27me2K36me2	534.6544
H3_27_40 K27me3K36me2	539.3263
H3_27_40 K27ac	548.6458

Supplemental Figure 6: MS/MS of the histone H3 peptide aa 27-40 with three methyl groups. (A) MS/MS spectrum of the peptide derivatized using N-terminal propionylation. No useful b ions are present in the spectrum to discriminate the 4 isobaric forms. (B) Same peptide not derivatized at the N-termini. The ion b2 can be used to discriminate the relative abundance of the four isobaric species. (C) Theoretical fragment ions (only b series) of the four isobaric forms not derivatized with propionylation at the N-termini.

A



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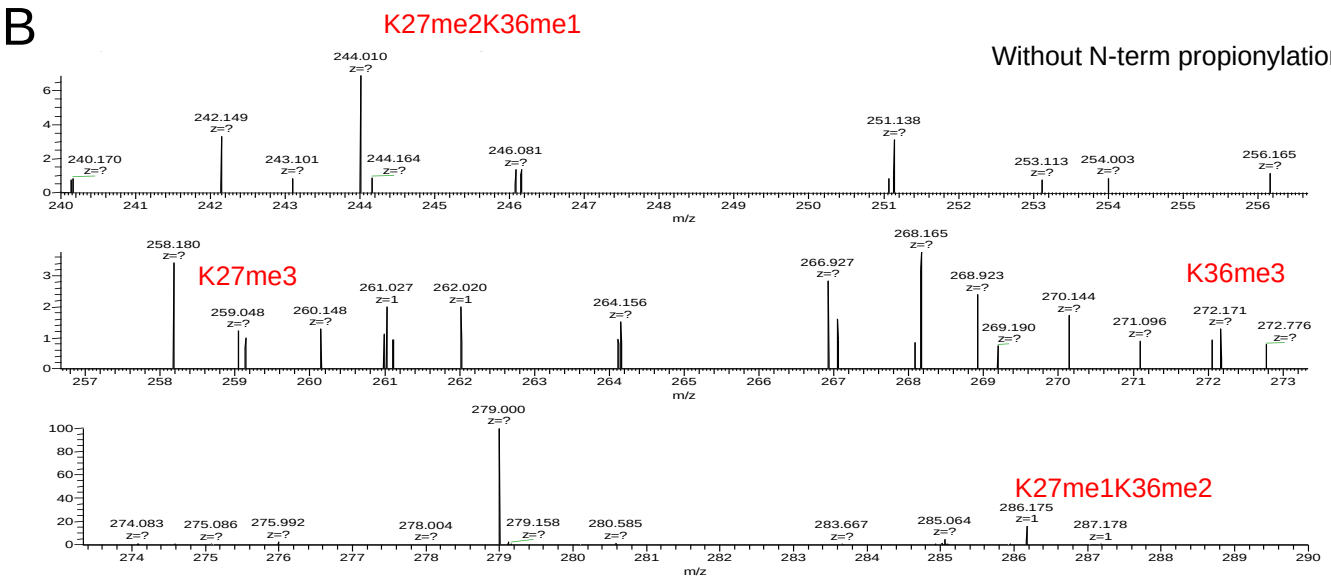
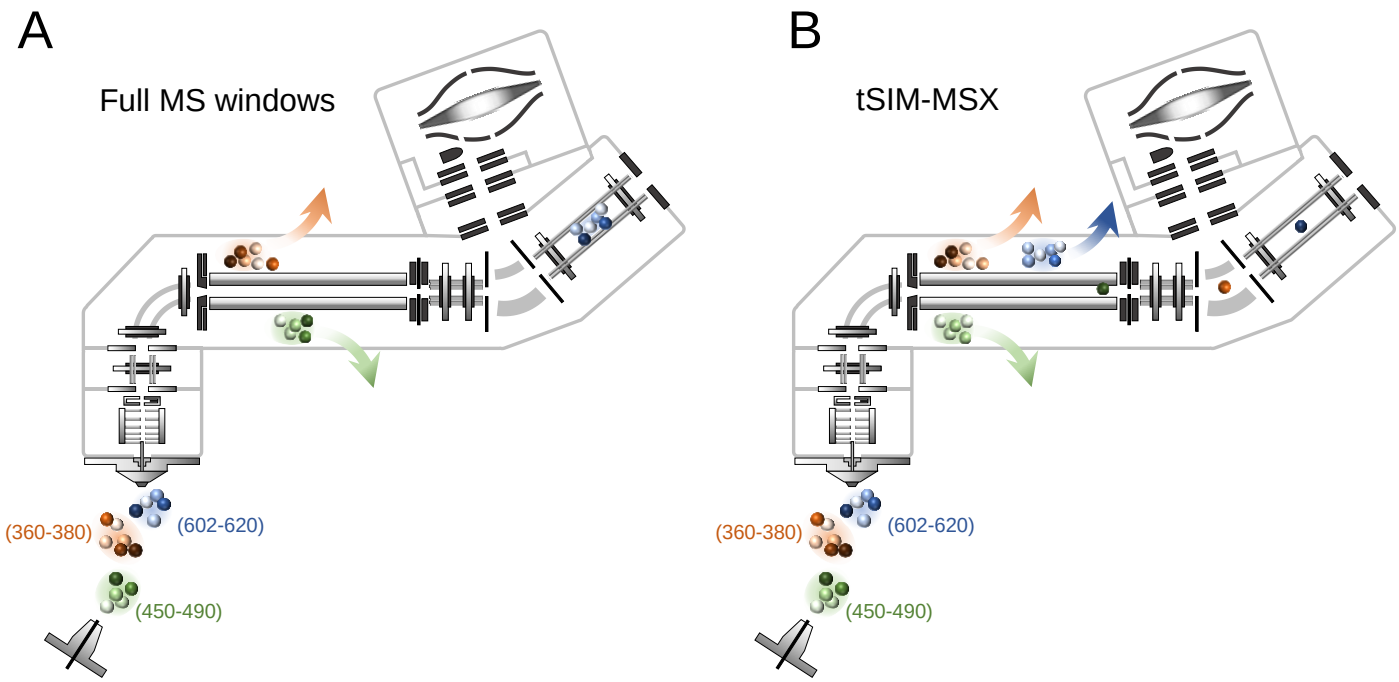


C

		K27me3	K27me2K36me1	K36me3	K27me1K36me2
Seq	#	B	B	B	B
K	1	171.14928	157.13358	185.12828	199.14415
S	2	258.18131	244.16561	272.16031	286.17618
A	3	329.21843	315.20273	343.19743	357.21329
P	4	426.27119	412.25549	440.25019	454.26605
A	5	497.30830	483.29260	511.28730	525.30317
T	6	598.35598	584.34028	612.33498	626.35085
G	7	655.37744	641.36174	669.35644	683.37231
G	8	712.39891	698.38321	726.37791	740.39377
V	9	811.46732	797.45162	825.44632	839.46219
K	10	995.58828	995.58845	995.58828	995.58815
K	11	1179.70925	1179.70941	1179.70925	1179.70911
P	12	1276.76201	1276.76218	1276.76201	1276.76188
H	13	1413.82092	1413.82109	1413.82092	1413.82079
R	14	1569.92203	1569.92220	1569.92203	1569.92190

Supplemental Figure 7: Full MS window and targeted SIM MSX acquisition. (A) A representation of the Full MS window acquisition method. The ions selected at each scan are a narrow m/z range including all the modified and unmodified states of a specific histone peptide sequence. (B) The targeted SIM-MSX acquisition selects individual peptide m/z. The cycle time is reduced because these ions are multiplexed 10 by 10, so each MS scan contains up to 10 different peptides. In each of the two methods, the ions are temporarily accumulated in the ion routing multiple (IRM) before being injected in the mass analyzer, in this case the Orbitrap.



Supplemental Figure 8: Correlation analysis of quantified peptides in DI-MS vs LC-MS. Histones from both brain (left) and liver (right) tissues were analyzed using the tSIM-MSX acquisition method with direct injection and by “canonical” LC-MS analysis (DIA). The peptide quantifications had high correlations despite the different biases of the two methods, e.g. hydrophobicity of peptides affecting chromatographic retention in LC-MS mode.