A Chemical Acetylation based Mass Spectrometry Platform for Histone Methylation Profiling

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Supplemental Material

Table S1: Modified forms of the H4 N-terminal sequence 1-20, SGRGKGKGLGGAKRHRK identified in a top-down experiment [14].

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Figure S7. Tandem MS spectra for H3K27 isoforms represented in Figure 7.
Table S1: Modified forms of the H4 N-terminal sequence 1-20, SGRGKGGKGLGKGGAKRHRK identified in a top-down experiment [14]. After chemical acetylation, the 68 differently modified forms collapse into six.

H4 N-terminal tail forms reported in ref ([14])

| N-terminus | R3 | K5 | K8 | K12 | K16 | K20 | Methyl Units |
|------------|----|----|----|-----|-----|-----|--------------|
| ac         | 0  | ac | 0  | ac  | 0   | ac  | 0            |
| ac         | ac | 0  | ac | 0   | ac  | 0   | ac           |
| ac         | ac | ac | ac | 0   | ac  | 0   | ac           |
| ac         | ac | ac | 0  | ac  | 0   | ac  | 0            |
| ac         | ac | ac | ac | 0   | ac  | 0   | ac           |
| ac         | ac | ac | ac | 0   | ac  | 0   | ac           |
| ac         | ac | ac | ac | 0   | ac  | 0   | ac           |
| ac         | ac | ac | ac | 0   | ac  | 0   | ac           |

H4 Isoforms following in-vitro lysine acetylation

| N-terminus | R3 | K5 | K8 | K12 | K16 | K20 | Methyl Units |
|------------|----|----|----|-----|-----|-----|--------------|
| me1        | 1  | 1  | ac | ac  | ac  | ac  | ac           |
| me1        | 1  | 1  | ac | ac  | ac  | ac  | ac           |
| me1        | 1  | 1  | ac | ac  | ac  | ac  | ac           |
| me1        | 1  | 1  | ac | ac  | ac  | ac  | ac           |
| me1        | 1  | 1  | ac | ac  | ac  | ac  | ac           |
| me2        | 2  | ac | ac  | ac  | ac  | ac  | ac           |
| me2        | 2  | ac | ac  | ac  | ac  | ac  | ac           |
| me2        | 2  | ac | ac  | ac  | ac  | ac  | ac           |
| me2        | 2  | ac | ac  | ac  | ac  | ac  | ac           |
| me3        | 3  | ac | ac  | ac  | ac  | ac  | ac           |
| me3        | 3  | ac | ac  | ac  | ac  | ac  | ac           |
| me3        | 3  | ac | ac  | ac  | ac  | ac  | ac           |
| me3        | 3  | ac | ac  | ac  | ac  | ac  | ac           |
| me3        | 3  | ac | ac  | ac  | ac  | ac  | ac           |
| me3        | 3  | ac | ac  | ac  | ac  | ac  | ac           |

ac = Acetylation
me1 = Methyl Units 1
me2 = Methyl Units 2
me3 = Methyl Units 3
Table S2: Different modified forms of the H4 peptides 1-17 and 4-17 identified by data-dependent and validated by target PRM methods after histone propionylation of nuclear extracts from Z-138 cells. Some of the identifications reported in table 3 are not reported here as they did not meet the stringent validation criteria used for site identification.

**H4 Peptide 1-17 SGRGKKGLGKGGAKR**

| N-term | R3 | K5 | K8 | K12 | K16 | R17 | Acetyl Units | Methyl Units |
|--------|----|----|----|-----|-----|-----|--------------|--------------|
| ac     | ac | ac | ac | ac  | ac  | 4   | 1            | 1            |
| ac     | ac | ac | ac | 3   | 0   | 0   |              |              |
| ac     | ac | ac | ac | 2   | 0   | 0   |              |              |
| ac     | ac | ac | ac | 2   | 1   | 1   |              |              |
| ac     | ac | ac | ac | 1   | 0   | 0   |              |              |
| ac     | ac | ac | ac | 1   | 0   | 0   |              |              |

**H4 Peptide 4-17 GKGKGLGKGGAKR**

| N-term | R3 | K5 | K8 | K12 | K16 | R17 | Acetyl Units | Methyl Units |
|--------|----|----|----|-----|-----|-----|--------------|--------------|
| ac     | ac | ac | ac | ac  | ac  | 4   | 0            | 0            |
| ac     | ac | ac | ac | ac  | ac  | 3   | 0            | 0            |
| ac     | ac | ac | ac | 3   | 0   | 0   |              |              |
| ac     | ac | ac | ac | 2   | 0   | 0   |              |              |
| ac     | ac | ac | ac | 2   | 1   | 1   |              |              |
| ac     | ac | ac | ac | 1   | 0   | 0   |              |              |
| ac     | ac | ac | ac | 1   | 0   | 0   |              |              |
| ac     | ac | ac | ac | 1   | 1   | 1   |              |              |
| me1    | ac | me1| 1  | 1   |     |     |              |              |
| me1    | ac | ac | ac | 1   | 1   | 1   |              |              |
| me1    | ac | me1| 0  | 1   |     |     |              |              |
| me1    | ac | me1| 0  | 1   |     |     |              |              |
| me1    | ac | me1| 0  | 1   |     |     |              |              |
Table S3. H3K27 isoforms identified after propionylation of a nuclear extract from KARPAS-422 cells. Discrimination between methylation on K36 and K37 could not be always made and therefore in these cases both isoforms are reported.

| Peptide H3 27-40 | KSAPATGGVKKPHER |
|------------------|------------------|
| K27              | K36              | K37              |
| me3              | me2              |
| me1              | me2              |
| me1              | me1              |
| me2              | me1              |
| ac               | ac               |
| ac               | me2              |
| ac               | me2              |
| ac               | me2              |
| ac               | me2              |
| ac               | me2              |
| ac               | me2              |
| ac               | me2              |
| ac               | me2              |
| me1              | me2              |
| me2              | me2              |
| ac               | me2              |
| ac               | me2              |

Discrimination between methylation on K36 and K37 could not be always made and therefore in these cases both isoforms are reported.
Table S4. Precursor and fragment ion masses used in assay data

Figure 3 – peak areas of the extracted ion current for the 2+ and 3+ precursors were summed.

|                  | Precursor | Precursor | Fragment | Fragment | Fragment | Fragment |
|------------------|-----------|-----------|----------|----------|----------|----------|
| unmethylated     | 480.2757  | 719.9099  |          |          |          |          |
| (3+)             | (2+)      |           |          |          |          |          |
| monomethylated   | 599.0023  | 897.9989  |          |          |          |          |
| (3+)             | (2+)      |           |          |          |          |          |
| dimethylated     | 603.6746  | 905.0070  |          |          |          |          |
| (3+)             | (2+)      |           |          |          |          |          |

Figure 5 – peak areas of the extracted ion current for b-35 and b-45 fragment ions were summed.

|      | Precursor | Fragment | Fragment | Fragment | Fragment |
|------|-----------|----------|----------|----------|----------|
| SDMA | 603.6746  | 383.1675 | b4-31    | 553.2730 | b5-31    |
|      | (3+)      |          |          |          |          |
|      |           | 610.2944 | b6-31    |          |          |
| ADMA | 603.6746  | 397.1831 | b4-45    | 567.2886 | b5-45    |
|      | (3+)      |          |          |          |          |
|      |           | 624.3100 | b6-45    |          |          |

Figure 7 – peak areas of the extracted ion current for the 2+ and 3+ precursors were summed.

|      | Precursor | Precursor | Fragment | Fragment | Fragment | Fragment |
|------|-----------|-----------|----------|----------|----------|----------|
| zero mods | 520.6244  | 780.4344  |          |          |          |          |
| me    | 525.2985  | 787.444   |          |          |          |          |
| 2 me  | 529.9701  | 794.4507  |          |          |          |          |
| me2   | 515.9669  | 773.4472  |          |          |          |          |
| me3   | 520.6395  | 780.4535  |          |          |          |          |
| me, me2 | 520.6395  | 780.4535  |          |          |          |          |
| 2 me2 | 511.3076  | 766.4566  |          |          |          |          |
| me, me3 | 525.3119  | 787.4608  |          |          |          |          |
| me2, me3 | 515.979   | 773.4636  |          |          |          |          |
Figure S1. Reverse phase chromatography of histone tryptic peptides after A) acetylation (top) or propionylation (bottom). Base peak LC-MS/MS chromatogram of a tryptic digest from the nuclear prep of Z138 cells. The same amount of tryptic digest is injected in both cases; B) accurate mass (+/- 10 ppm) extracted ion chromatograms for the precursor (2+, 3+) of select H3.1 peptides from these two analyses. The H3.1 18-26 peptide (top) with no modifications shows no difference in peak width or sensitivity after derivatization with either acetic or propionic anhydride. Chromatography of the acetylated or propionylated H3.1 27-40 peptides (bottom) shows comparable resolution for a similar set of modifications.
In general we assume that in the case of derivatization with propionic anhydride, a butyl mark equals monomethyl plus propionyl and for derivatization with acetic anhydride, that a propionyl mark equals monomethyl plus acetylation. In the cases reported here, this has been validated by labeling with deuterated reagents.
Figure S2. (A) Schematic showing the acetylation and tryptic digestion of a 1:1:0.5:0.5 mixture of Histone H4R3 0MA:MMA:DMA synthetic peptide standards. (B) A 10-point 5-fold serial dilution was generated from 10pmol/µL to 5.12amol/µL in a 50ng/µL HeLa digest background. Full scan MS data for 3 technical replicate injections of each sample were acquired. Peak areas of $[M+2H]^{2+} + [M+3H]^{3+}$ precursor ions for H4R3 (4-17) 0MA, (1-17) MMA and (1-17) DMA were generated by integrating extracted ion chromatograms (XIC). Average peak area values plotted vs fmols on column (log10 scale) indicate a linear response for all three components across the range of peak areas observed for the endogenous H4R3 peptides in the MDA-MB-468 nuclear preparations. These data were used to generate MS response correction factors of 1:3.0:0.9 for 0MA, MMA and DMA respectively.
**Figure S3**: Optimization of the Normalized Collision Energy (NCE) for ADMA and SDMA neutral loss from H4R3 1-17 ADMA and SDMA peptides. In each case the precursor is the 3+ ion at m/z 603.6746. A) the NCE was ramped from 20 to 60 in increments of 2 and the response of the neutral loss from the b4-b6 ion series was monitored. B) MS/MS spectra for the fully acetylated synthetic H4 1-17 ADMA and C) SDMA peptides. Diagnostic ADMA and SDMA b ions are indicated.
**Figure S4:** Discrimination of H4R3me2 ADMA from SDMA on the histone H4 1-17 peptide by tandem mass spectrometry. In each case the precursor is the 3+ ion at m/z 603.6746. The presence of either a 31.0422 Da (center) or 45.0578 Da (right) neutral loss from the b₄-b₆ ions (left) allows the unequivocal identification of H4R3 ADMA and SDMA.

SGR(me2)GKGGKGLGKGGAKR
Figure S5. Tandem MS spectra representing species used in Figure 5. A) H4R3 zero, mono and dimethylarginine; B) H4R3 ADMA and SDMA
Histone H4 1-17 (R3 ADMA)
$[\text{M+3H}]^{3+} = 603.6746$

Histone H4 1-17 (R3 SDMA)
$[\text{M+3H}]^{3+} = 603.6746$

SGR(ADMA)GKGKGLGKGGAKR

SGR(SDMA)GKGKGLGKGGAKR
Figure S6. (A) Extracted ion chromatograms from the PRM analysis of m/z 726.9178, the [M+2H]^{2+} precursor ion for the tryptic peptide H4 (4-17) + me1. The sequence contains four lysine that can be methylated, K5, K8, K12 and K16. These ion profiles, diagnostic for methylation at the different lysine suggest multiple monomethyl species. The peak at RT 28.49 (A, black trace) has a spectrum (C, top) with a y5 ion at m/z 530.3048 that confirms there is no methylation on K16. The y6+14 ion at m/z 714.4268 confirms methylation on K12. All subsequent y ions, up to y_{12}, show evidence of methylation. This suggests this peak is primarily a single species methylated at K12. The peak with a RT 28.32 (A, red trace) has a spectrum (C, bottom) that shows a mixed population for ions y_5\text{--}y_{12} bearing 0 and 1 methyl group. The presence of a y_5+14 ion at m/z 544.3232 clearly indicates that part of this population has methylation on K16. However, the extracted ion chromatogram for m/z 544.3232 (A, green trace) places it on the right shoulder of the 28.32 peak. The left shoulder of this peak is characterized by unmethylated y_5 ions up to y_{12}, suggesting that part of the population is primarily K5me1. While it is possible that the peak contains some K8me1, the unchanging ratio of y and y+14 ions in the spectrum from y_5 to y_{12}, suggests that the y+14 ions come from the overlap of the K16me1 species that nearly co-elutes.
**Figure S7.** Tandem MS spectra for species represented in Figure 7.

**Histone H3 27-40**

- **M+3H** = 520.6267
- RT = 26.99 min
- Fragmentation sites: K(SAPATGGVKKPHR)

**Histone H3 27-40 K27Me1**

- **M+3H** = 525.2985
- RT = 28.67 min
- Fragmentation sites: K(Me1)(SAPATGGVKKPHR)

**Histone H3 27-40 K36Me1**

- **M+3H** = 525.2985
- RT = 28.08 min
- Fragmentation sites: K(SAPATGGV(K(Me1))KPHR)
Histone H3 27-40 K27Me1 K36Me1
[M+3H]$^{3+}$ = 529.9704

Histone H3 27-40 K27Me2
[M+3H]$^{3+}$ = 515.9669

Histone H3 27-40 K36Me2
[M+3H]$^{3+}$ = 515.9669
Histone H3 27-40 K27Me1 K36Me2
[M+3H]^{3+} = 520.6388

K(Me1)SAPATGGVK(Me2)KPHR

RT = 24.76 min

Histone H3 27-40 K27Me2 K36Me1
[M+3H]^{3+} = 520.6388

K(Me2)SAPATGGVK(Me1)KPHR

RT = 22.06 min

Histone H3 27-40 K27Me3
[M+3H]^{3+} = 520.6388

K(Me3)SAPATGGVKKPHR

RT = 20.67 min
Histone H3 27-40 K36Me3
[M+3H]^{3+} = 520.6388

KSAPATGGVK(Me3)KPHR

b_{3} 13 12 10 9 8 7 6 5 4 3 2 1

Histone H3 27-40 K27Me2 K36Me2
[M+2H]^{2+} = 766.4570

K(Me2)SAPATGGVK(Me2)KPHR

b_{3} 23

Histone H3 27-40 K27Me3 K36Me1
[M+3H]^{3+} = 525.3107

K(Me3)SAPATGGVK(Me1)KPHR

b_{3} 3 5 8 9

Histone H3 27-40 K27Me1 K36Me3
[M+3H]^{3+} = 525.3107

RT = 24.77 min

K(Me1)SAPATGGVK(Me3)KPHR

Histone H3 27-40 K27Me3 K36Me2
[M+2H]^{2+} = 773.4649

RT = 17.70 min

K(Me3)SAPATGGVK(Me2)KPHR