*Supporting Information*

# Comparison of Molecular Recognition of Trimethyllysine and Trimethylthialysine by Epigenetic Reader Proteins

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## 1. General Information

### *1.1. Methods*

High resolution masses were recorded with a JEOL AccuTOF CS JMS-T100CS mass spectrometer. LS-MS analysis for all the compounds was performed on a Thermo Finnigan LCQ-Fleet ESI-ion trap (Thermofischer, Breda, the Netherlands) equipped with a Phenomenex Gemini-NX C18 column, 50 × 2.0 mm, particle size 3 μM (Phenomenex, Utrecht, the Netherlands). An acetonitrile/water gradient containing 0.1% formic acid was used for elution (5%–100%, 1–50 min, flow 0.2 mL min-1). The room temperature in the reactions is in the range 20–25 °C. Lyophilization was achieved using an ilShin Freeze Dryer (ilShin, Ede, the Netherlands).

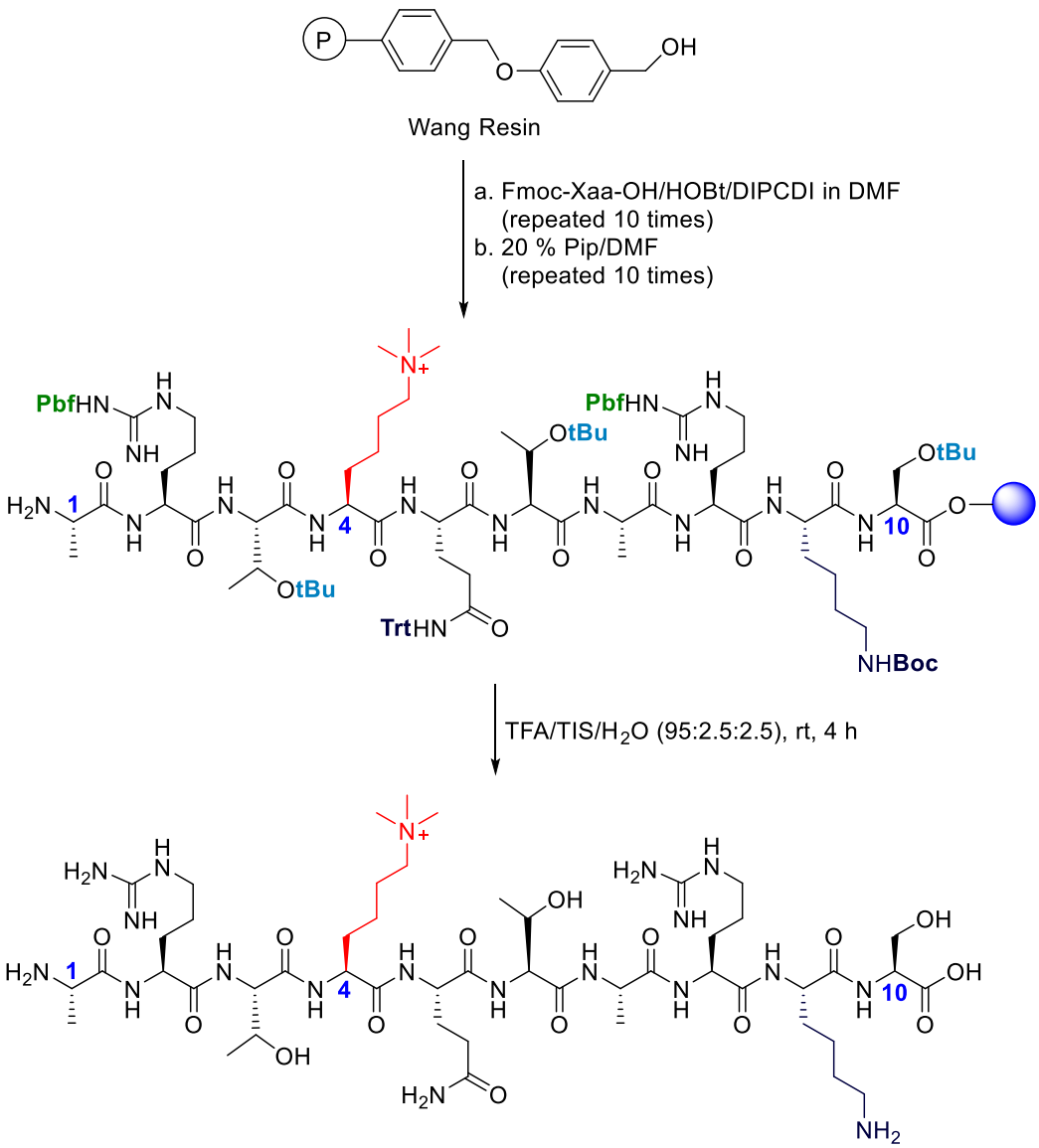
### *1.2. Materials*

All reagents were obtained from commercial sources and used without further purifications. Fmoc amino acid derivatives, *N,N'*-Diisopropylcarbodiimide (DIC) and 1-Hydroxybenzotriazole (HOBt) were obtained from Novabiochem (EMD Chemicals, Gibbstown, USA). Triisopropylsilane (TIPS), *N'N*-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), (2-Bromoethyl)trimethylammonium bromide and piperidine were purchased from Sigma-Aldrich. *N,N*-dimethylformamide (DMF) solvent for peptide synthesis and gradient degree high-performance liquid chromatography (HPLC) acetonitrile were purchased from Actu-All Chemicals b.v. (Oss, the Netherlands).

## 2. Synthesis and Purification of Histone Peptides and Reader Proteins

### *2.1. Synthesis of Histone Peptides*

The general synthesis strategy of 10-mer natural histone peptide is outlined in Scheme S1.




**Scheme S1.** Solid-phase synthesis of histone peptide H3K4me3.

*2.2 Analytical HPLC of Histone Peptides*

Lyophilized crude peptides were purified by prep-HPLC on a Phenomenex® Gemini-NX 3u C18 110A reversed-phase column (150 × 21.2 mm) using gradient elution at constant flow rate of 10 mL/min and the temperature is 30 °C. A typical run was performed as follows:

For 1-10 H3KC4me3; after 3 mins at 2% a gradient of 2% to 10% over 10 mins was introduced, followed by a gradient of 10% to 100% over 20 mins and from 100% to 100% over 25 mins finalized by 5 mins at 100% CH3CN (total runtime 30 mins).

For 1-10 H3KC4; after 3 mins at 3% a gradient of 3% to 15% over 12 mins was introduced, followed by a gradient of 15% to 30% over 17 mins and from 30% to 100% over 19 mins, continuing from 100% to 100% over 21 mins finalized by 3 mins at 100% $CH_3CN$ (total runtime 30 mins).

For 1-10 H3K4me3; after 3 mins at 3% a gradient of 3% to 3% over 6 mins was introduced, followed by a gradient of 3% to 100% over 10 mins and from 100% to 100% over 13 mins finalized by 4 mins at 100% $CH_3CN$ (total runtime 20 mins). Solvent A was 0.1% trifluoroacetic acid in $H_2O$, Solvent B was 0.1% trifluoroacetic acid in acetonitrile. The pure fractions containing product were combined and freeze-dried overnight to yield the peptides as white off solid.

## 3. ITC Measurements

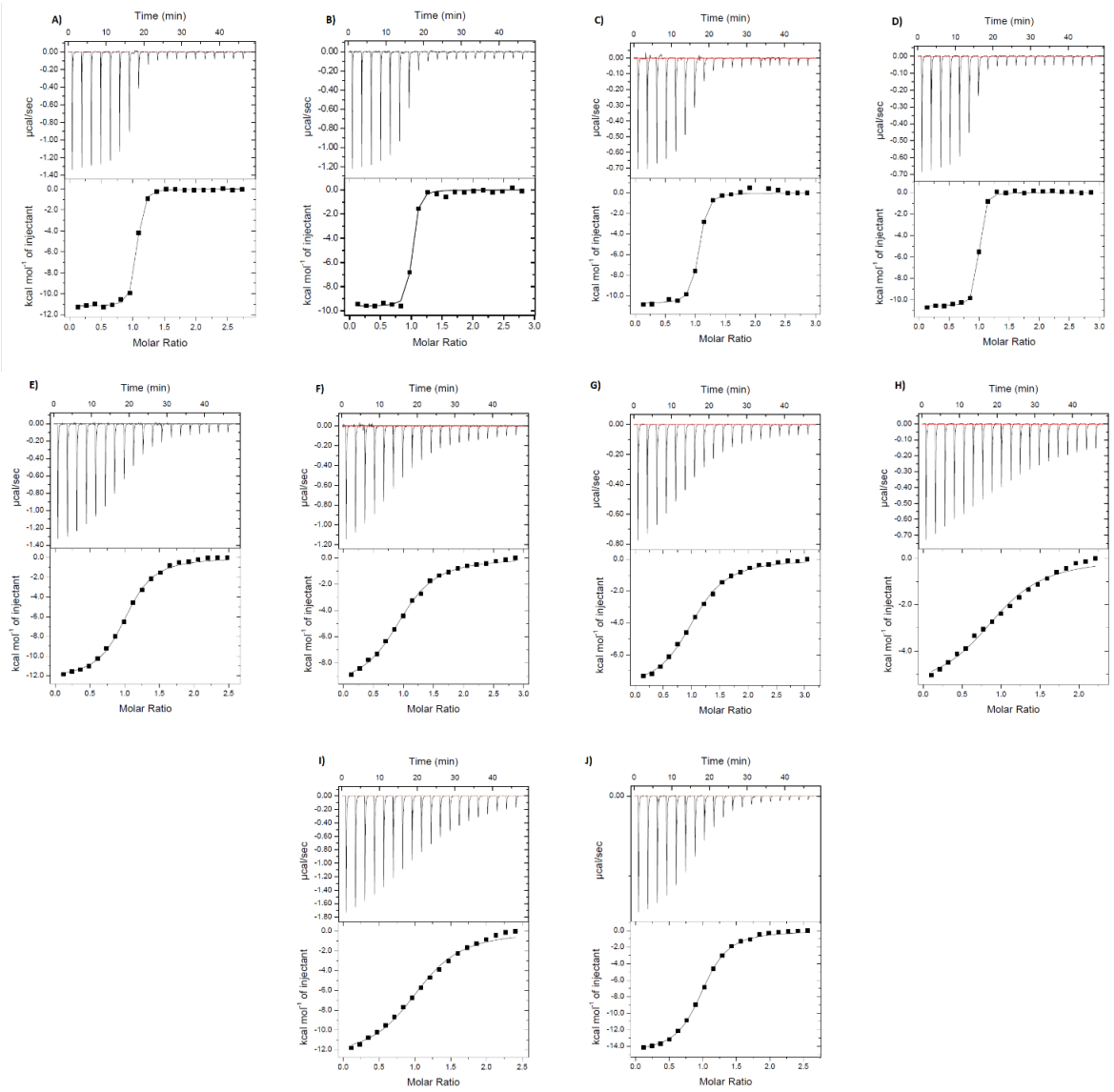



**Figure S1.** ITC data. Thermodynamic analyses showing binding of A) KDM5A$_{PHD3}$–H3K4me3; B) KDM5A$_{PHD3}$–H3K$_C$4me3; C) TAF3$_{PHD}$–H3K4me3; D) TAF3$_{PHD}$–H3K$_C$4me3; E) BPTF$_{PHD}$–H3K4me3; F) BPTF$_{PHD}$–H3K$_C$4me3; G) SGF29$_{TTD}$–H3K4me3; H) SGF29$_{TTD}$–H3K$_C$4me3; I) KDM4A$_{TTD}$–H3K4me3; J) KDM4A$_{TTD}$–H3K$_C$4me3.

**Table S1.** Concentrations of protein and peptide, with C-value and N binding cites in ITC binding studies.

| | H3K4me3 | | | | H3K$_c$4me3 | | | |
|---|---|---|---|---|---|---|---|---|
| | Protein conc. (μM) | Peptide conc. (μM) | C-value | N | Protein conc. (μM) | Peptide conc. (μM) | C-value | N |
| KDM5A$_{PHD}$ | 29 | 360 | 408 | 1.00–1.01 | 29 | 420 | 193 | 1.00–1.02 |
| TAF3$_{PHD}$ | 20.5 | 300 | 244 | 1.00–1.01 | 20.5 | 300 | 488 | 0.98–1.02 |
| BPTF$_{PHD}$ | 43 | 520 | 21.6 | 1.00–1.01 | 43 | 620 | 11.3 | 0.99–1.00 |
| SGF29$_{TTD}$ | 30 | 490 | 11.5 | 1.00–1.02 | 51 | 550 | 8.4 | 0.98–1.00 |
| KDM4A$_{TTD}$ | 58 | 680 | 13.3 | 0.98–1.01 | 100 | 1250 | 32.3 | 0.99–1.01 |

## 4. Molecular Dynamics Simulations

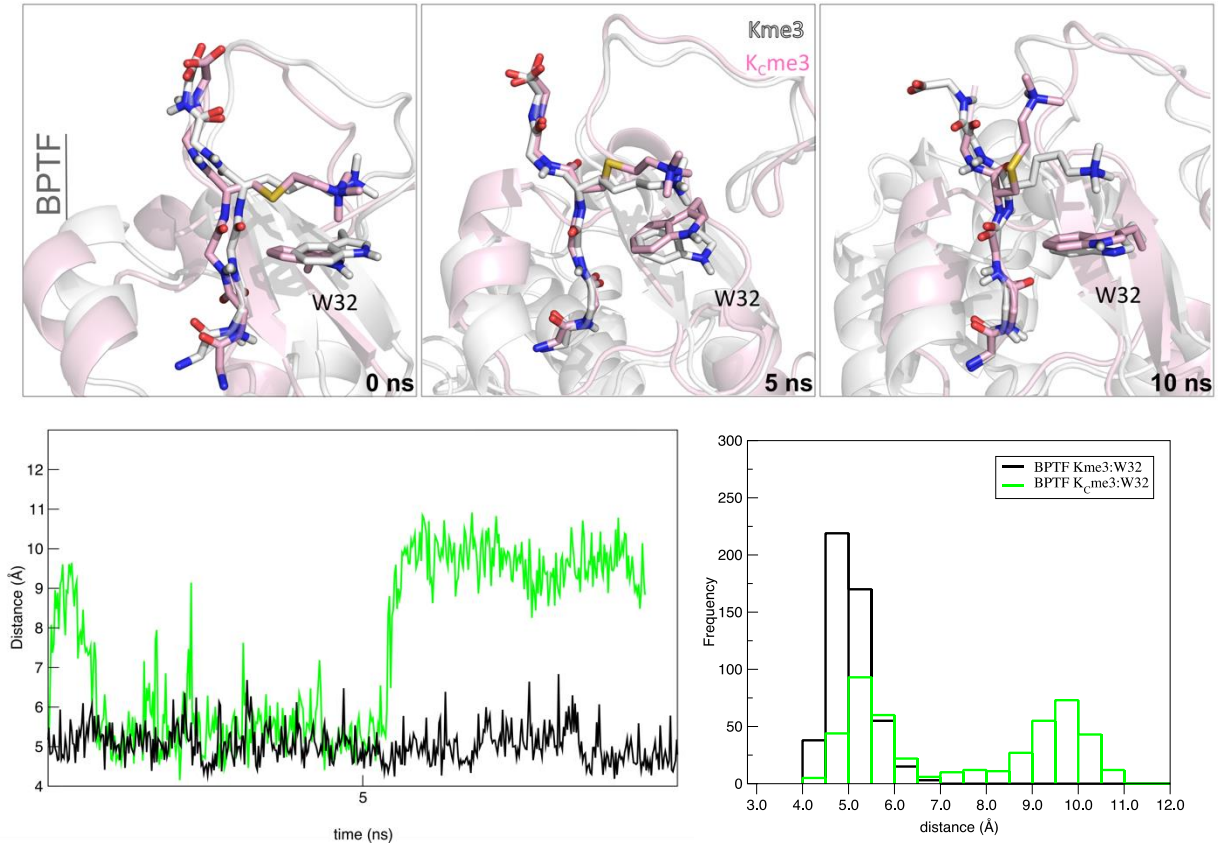



**Figure S2.** MD simulations of BPTF$_{PHD}$. (Top) Snapshots of reader BPTF$_{PHD}$ complexed with histone 3 chain backbone (liquorice) containing Kcme3 (pink) and Kme3 (white) active sites at times 0 ns, 5 ns and 10 ns. (Bottom) Distance vs. time plots of $N^+$ side chain atoms of Kme3 and Kcme3 to W32 side chain center of mass over 10 ns.

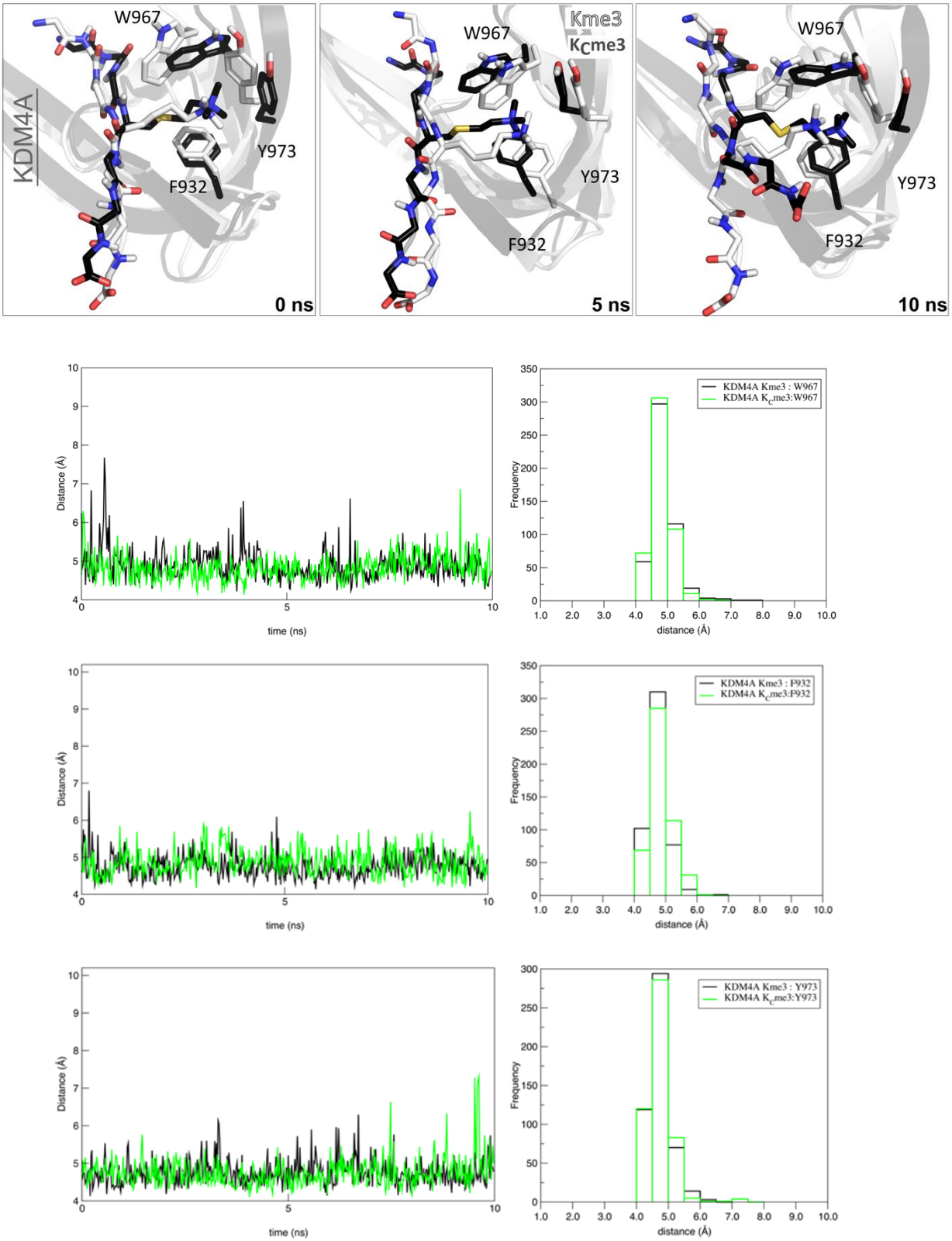



**Figure S3.** MD simulations of KDM4A$_{TTD}$. (Top) Snapshots of reader KDM4A$_{TTD}$ complexed with histone 3 chain backbone (liquorice) containing K$_C$me3 (black) and Kme3 (white) active sites at times 0 ns, 5 ns and 10 ns. (Bottom) Distance vs. time plots of $N^+$ side chain atoms of Kme3 and K$_C$me3 to F932, W967 and Y973 side chain centers of mass over 10 ns.

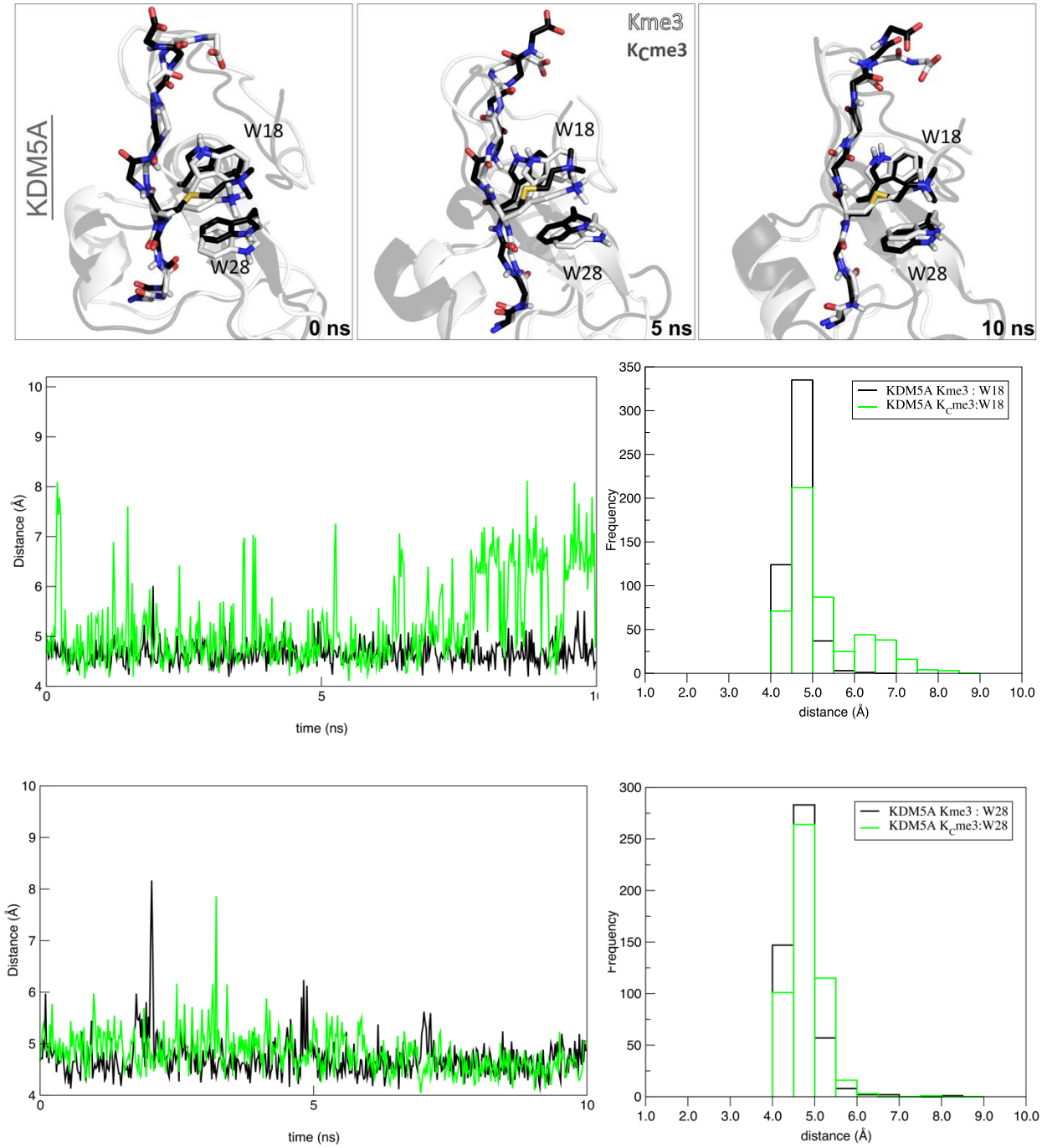



**Figure S4.** MD simulations of KDM5A$_{PHD3}$. (Top) Snapshots of reader KDM5A$_{PHD3}$ complexed with histone 3 chain backbone (liquorice) containing K$_C$me3 (black) and Kme3 (white) active sites at times 0 ns, 5 ns and 10 ns. (Bottom) Distance vs. time plots of $N^+$ side chain atoms of Kme3 and K$_C$me3 to W18 and W28 side chain centers of mass over 10 ns.

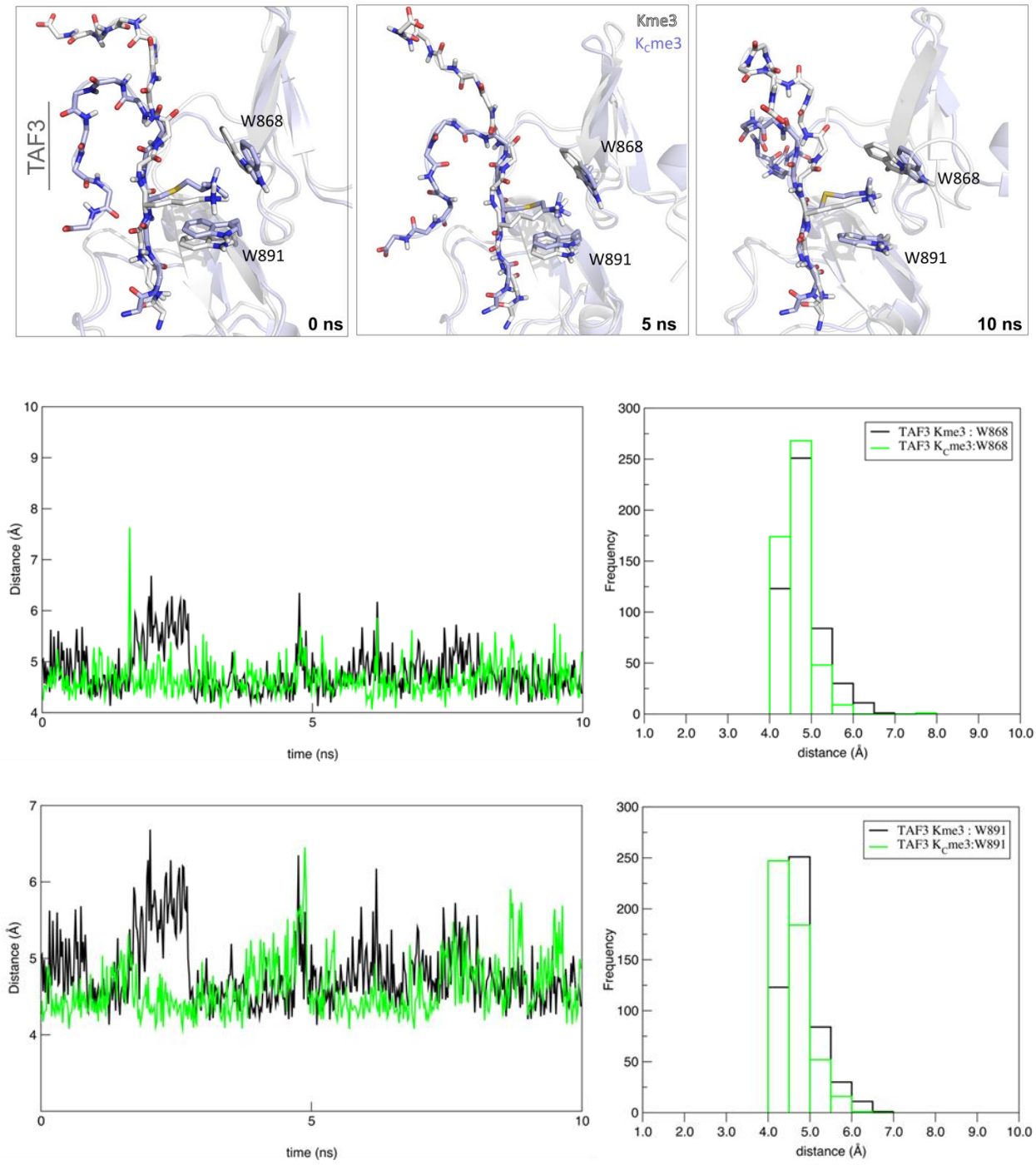



**Figure S5.** MD simulations of TAF3PHD. (Top) Snapshots of reader TAF3PHD complexed with histone 3 chain backbone (liquorice) containing KCme3 (blue) and Kme3 (white) active sites at times 0 ns, 5 ns and 10 ns. (Bottom) Distance vs. time plots of $N^{+}$ side chain atoms of Kme3 and KCme3 to W868 and W891 side chain centers of mass over 10 ns.

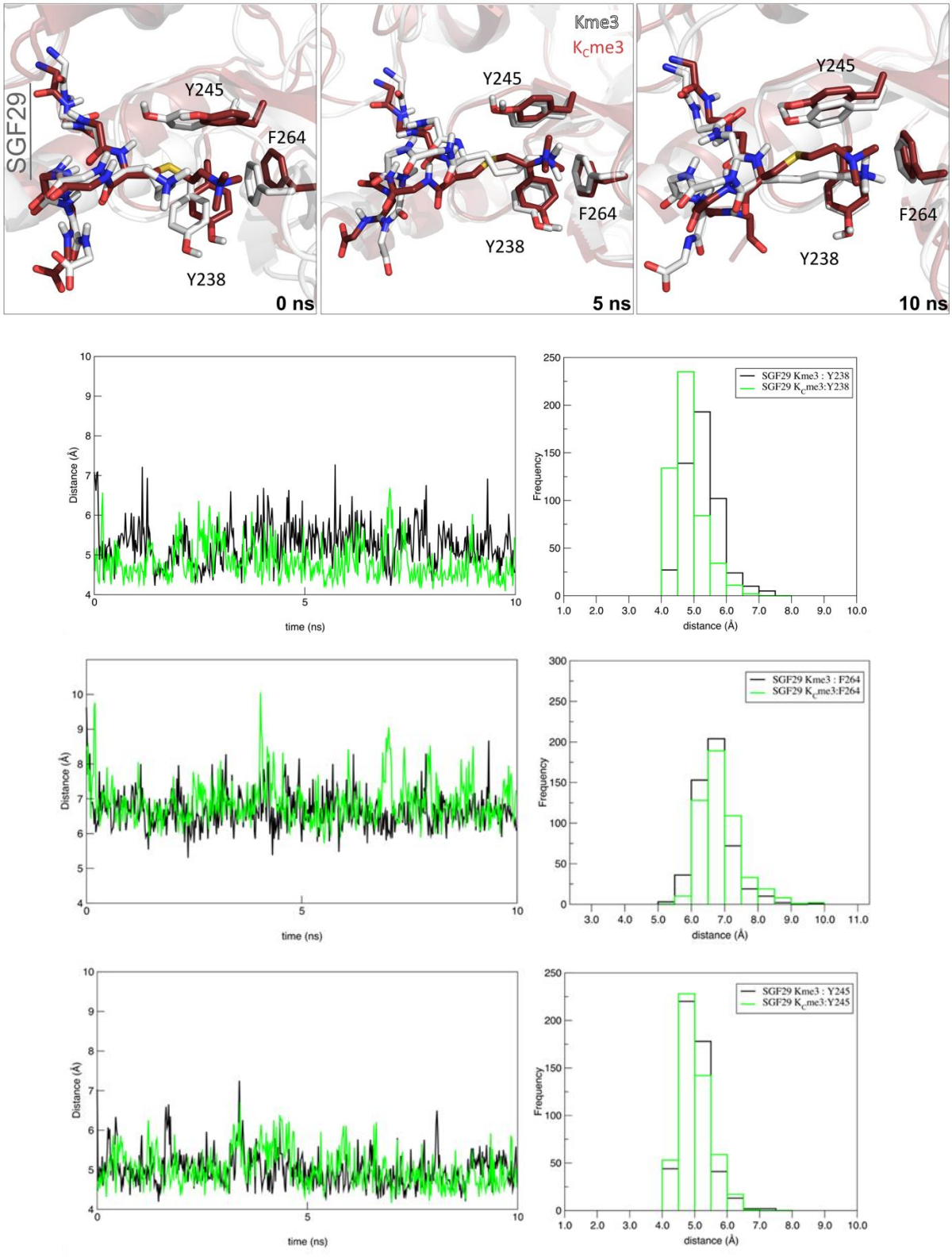



**Figure S6.** MD simulations of SGF29TTD. (Top) Snapshots of reader SGF29TTD complexed with histone 3 chain backbone (liquorice) containing KCme3 (red) and Kme3 (white) active sites at times 0 ns, 5 ns and 10 ns. (Bottom) Distance vs. time plots of $N^+$ side chain atoms of Kme3 and KCme3 to F264, Y238 and Y245 side chain centers of mass over 10 ns.

**Table S2.** MM-GBSA binding free energies and electrostatic contributions calculated for Kme3 and Kcme3 complexed with reader proteins over 10 ns at 500 ps intervals.

| System | MM-GBSA (kcal/mol) | | | |
|---|---|---|---|---|
| | Kme3 | | Kcme3 | |
| | ΔG | $\Delta E_{ele}$ | ΔG | $\Delta E_{ele}$ |
| $BPTF_{PHD}$ | –39.6 | –194.8 | –53.1 | –239.3 |
| $KDM4A_{TTD}$ | –47.2 | –283.5 | –47.0 | –266.0 |
| $KDM5A_{PHD3}$ | –42.4 | –176.3 | –41.8 | –158.7 |
| $SGF29_{TTD}$ | –46.4 | –197.7 | –42.5 | –177.8 |
| $TAF3_{PHD}$ | –45.5 | –164.7 | –44.3 | –166.4 |

**Table S3.** Average root mean square deviation (RMSD) and error of $C_\alpha$ atoms of reader proteins.

| | RMSD (Å) | | | |
|---|---|---|---|---|
| **System** | **Kme3** | | **Kcme3** | |
| | Reader | H3 | Reader | H3 |
| $BPTF_{PHD}$ | 5.85 ± 2.49 | 0.47 ± 0.20 | 4.24 ± 1.67 | 0.79 ± 0.21 |
| $KDM4A_{TTD}$ | 2.08 ± 0.64 | 1.17 ± 0.27 | 3.49 ± 0.68 | 0.75 ± 0.21 |
| $KDM5A_{PHD3}$ | 2.45 ± 0.58 | 1.16 ± 0.34 | 2.06 ± 0.32 | 1.82 ± 0.51 |
| $SGF29_{TTD}$ | 1.28 ± 0.20 | 1.03 ± 0.45 | 1.26 ± 0.14 | 0.97 ± 0.30 |
| $TAF3_{PHD}$ | 3.25 ± 0.82 | 3.24 ± 1.20 | 2.53 ± 0.37 | 3.79 ± 1.65 |

**Table S4.** Cartesian coordinates and charges calculated using the RESP method HF/6-31G* of modified Kcme3.

| **Kcme3** | | | | **RESP** |
|---|---|---|---|---|
| **Atom** | **X** | **Y** | **Z** | **Charge** |
| N | -3.531 | 1.376 | 0.222 | -0.8584 |
| C | -3.262 | 0.130 | -0.448 | 0.5194 |
| C | -1.933 | -0.464 | 0.042 | -0.3406 |
| C | 0.840 | -0.328 | -0.038 | 0.0041 |
| C | 2.062 | 0.585 | -0.003 | 0.0217 |
| N | 3.412 | -0.104 | 0.043 | 0.0771 |
| C | -4.347 | -0.927 | -0.263 | 0.2879 |
| O | -5.242 | -0.803 | 0.503 | -0.4361 |
| C | 3.644 | -0.928 | -1.182 | -0.3320 |
| C | 4.460 | 0.964 | 0.105 | -0.3320 |
| C | 3.539 | -0.967 | 1.257 | -0.3320 |
| H | -3.882 | 1.209 | 1.147 | 0.3425 |
| H | -4.237 | 1.896 | -0.261 | 0.3425 |
| H | -3.168 | 0.322 | -1.513 | 0.0066 |
| H | -1.679 | -1.346 | -0.536 | 0.1577 |
| H | -2.011 | -0.748 | 1.085 | 0.1577 |
| H | 0.863 | -0.992 | -0.892 | 0.0657 |
| H | 0.774 | -0.923 | 0.864 | 0.0657 |
| H | 2.081 | 1.216 | -0.880 | 0.1172 |
| H | 2.020 | 1.221 | 0.870 | 0.1172 |
| H | -4.246 | -1.831 | -0.872 | 0.0297 |
| H | 3.514 | -0.306 | -2.055 | 0.1779 |
| H | 4.652 | -1.314 | -1.155 | 0.1779 |
| H | 2.946 | -1.749 | -1.206 | 0.1779 |
| H | 4.378 | 1.591 | -0.770 | 0.1779 |
| H | 5.435 | 0.501 | 0.133 | 0.1779 |
| H | 4.310 | 1.556 | 0.995 | 0.1779 |
| H | 3.328 | -0.375 | 2.135 | 0.1779 |
| H | 2.847 | -1.790 | 1.191 | 0.1779 |
| H | 4.548 | -1.349 | 1.308 | 0.1779 |
| S | -0.608 | 0.772 | -0.143 | -0.2831 |

## 5. Quantum Chemical Analysis

### *5.1. Bonding Analysis*

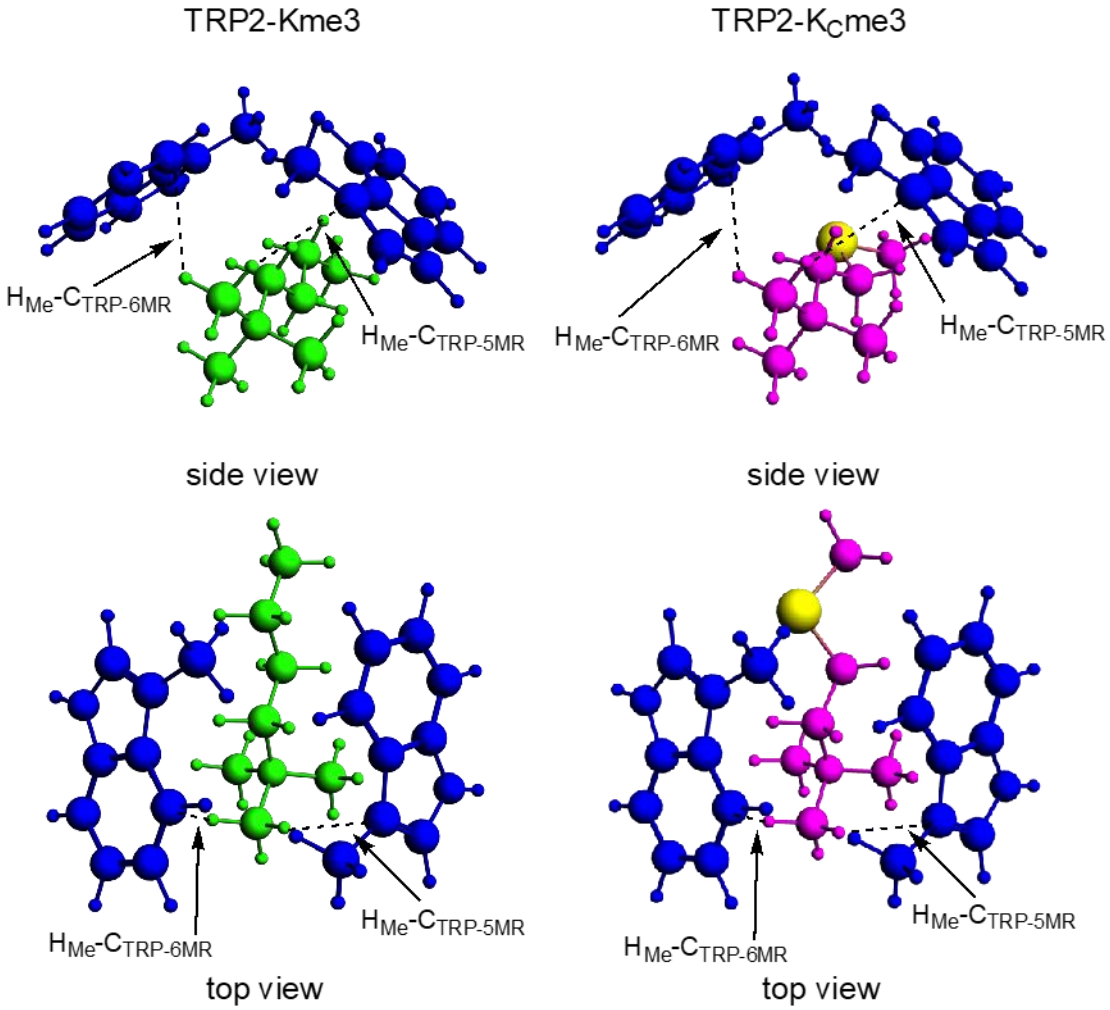



**Figure S7** Top view of structure of TRP2-Kme3 and TRP-Kcme3 model complexes. TRP2 in blue, Kme3 in green and Kcme3 in pink (except S atom in yellow).

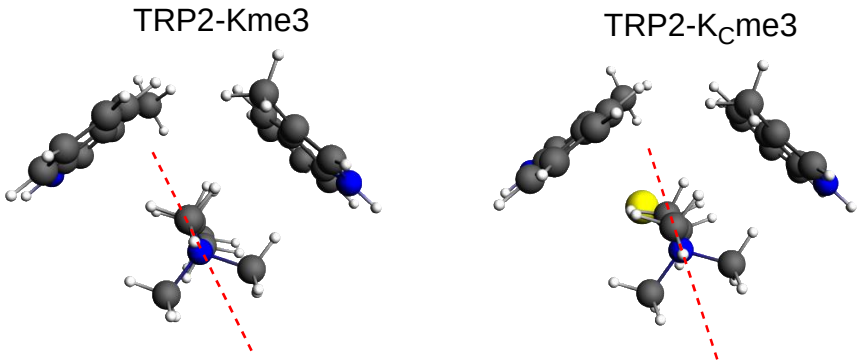



**Figure S8** Front view of the structure of TRP-Kme3 and TRP2-Kcme3 model complexes. The plane till $C^{\beta}$ is indicated by a red dotted line.

**Table S5.** Overlaps between the MOs of TRP and Kme3 or Kcme3.[a]

| TRP2 MOs | Kme3/ Kcme3 MOs | TRP2-Kme3 | TRP2-Kcme3 |
|---|---|---|---|
| HOMO | LUMO | 0.012 | 0.019 |
| HOMO | LUMO+1 | 0.006 | 0.012 |
| **HOMO-1** | **LUMO** | 0.028 | 0.024 |
| HOMO-1 | LUMO+1 | 0.006 | 0.011 |

[a] Computed at BLYP-D3BJ/TZ2P.

**Table S6.** Cartesian coordinates (in Å) of TRP2-Kme3 and TRP2-Kcme3complexes, computed at BLYP-D3BJ/TZ2P using COSMO to simulate aqueous solvation and a constrained optimization to simulate the effect of the protein backbone.

| **TRP2-Kme3:** | | | |
|---|---|---|---|
| C | -14.114000000 | -20.049000000 | -0.875000000 |
| C | -14.962000000 | -19.738000000 | 0.323000000 |
| C | -15.235000000 | -20.561000000 | 1.377000000 |
| C | -15.571000000 | -18.476000000 | 0.628000000 |
| C | -16.191000000 | -18.610000000 | 1.893000000 |
| C | -15.649000000 | -17.250000000 | -0.044000000 |
| N | -15.971000000 | -19.886000000 | 2.326000000 |
| C | -16.882000000 | -17.550000000 | 2.500000000 |
| C | -16.335000000 | -16.198000000 | 0.561000000 |
| C | -16.943000000 | -16.358000000 | 1.823000000 |
| H | -17.473000000 | -15.517000000 | 2.270000000 |
| H | -14.000000000 | -19.128000000 | -1.447000000 |
| H | -14.917000000 | -21.601000000 | 1.456000000 |
| H | -15.183000000 | -17.121000000 | -1.021000000 |
| H | -16.295000000 | -20.273000000 | 3.201000000 |
| H | -17.354000000 | -17.669000000 | 3.475000000 |
| H | -16.402000000 | -15.237000000 | 0.051000000 |
| H | -13.186000000 | -20.452000000 | -0.470000000 |
| C | -13.008000000 | -14.944000000 | -1.752000000 |
| C | -11.604000000 | -15.279000000 | -1.421000000 |
| C | -10.629000000 | -14.423000000 | -0.994000000 |
| C | -10.999000000 | -16.571000000 | -1.507000000 |
| C | -9.651000000 | -16.428000000 | -1.114000000 |
| C | -11.469000000 | -17.840000000 | -1.880000000 |
| N | -9.451000000 | -15.109000000 | -0.805000000 |
| C | -8.764000000 | -17.507000000 | -1.084000000 |
| C | -10.588000000 | -18.912000000 | -1.851000000 |
| C | -9.247000000 | -18.738000000 | -1.453000000 |
| H | -8.579000000 | -19.599000000 | -1.438000000 |
| H | -13.651000000 | -15.747000000 | -1.391000000 |
| H | -10.764000000 | -13.354000000 | -0.828000000 |
| H | -12.506000000 | -17.981000000 | -2.186000000 |
| H | -8.581000000 | -14.705000000 | -0.490000000 |
| H | -7.726000000 | -17.376000000 | -0.779000000 |
| H | -10.938000000 | -19.903000000 | -2.140000000 |
| H | -13.236000000 | -13.992000000 | -1.272000000 |
| H | -14.522374582 | -20.815706247 | -1.547885097 |
| H | -13.158423449 | -14.818069475 | -2.834003080 |
| C | -10.114752602 | -21.305220763 | 1.892377384 |
| C | -11.216790553 | -20.285805453 | 1.565697139 |
| C | -11.002507694 | -18.940388922 | 2.287874794 |
| C | -12.090102444 | -17.946438531 | 1.883398917 |
| N | -12.070579136 | -16.609009105 | 2.645799587 |
| C | -13.150209827 | -15.721875154 | 2.061139245 |

| | | | |
|---|---|---|---|
| C | -10.731973677 | -15.916856909 | 2.492497579 |
| C | -12.365551231 | -16.823749860 | 4.115733524 |
| H | -12.408951734 | -15.846708144 | 4.598931529 |
| H | -9.130774938 | -20.927740553 | 1.584711029 |
| H | -12.198761989 | -20.691389652 | 1.844793551 |
| H | -10.013332154 | -18.548608026 | 2.021388165 |
| H | -13.089556823 | -18.360771942 | 2.048366284 |
| H | -12.918787327 | -15.550085307 | 1.009793743 |
| H | -10.798479096 | -14.941374934 | 2.976931329 |
| H | -11.570405162 | -17.423783848 | 4.555007649 |
| H | -10.074709060 | -21.512398227 | 2.969877086 |
| H | -11.238229637 | -20.105747697 | 0.484787617 |
| H | -11.011890000 | -19.111207919 | 3.371339633 |
| H | -11.995495315 | -17.683123359 | 0.825941434 |
| H | -13.153012338 | -14.778635478 | 2.609325685 |
| H | -10.522561113 | -15.800602201 | 1.428638506 |
| H | -13.326128627 | -17.334212341 | 4.203429545 |
| H | -14.111323996 | -16.226695834 | 2.162298263 |
| H | -9.963428736 | -16.520728269 | 2.972373589 |
| H | -10.290718944 | -22.253949886 | 1.371226478 |

| **TRP2-Kcme3** | | | |
|---|---|---|---|
| C | -14.114000 | -20.049000 | -0.875000 |
| C | -14.962000 | -19.738000 | 0.323000 |
| C | -15.235000 | -20.561000 | 1.377000 |
| C | -15.571000 | -18.476000 | 0.628000 |
| C | -16.191000 | -18.610000 | 1.893000 |
| C | -15.649000 | -17.250000 | -0.044000 |
| N | -15.971000 | -19.886000 | 2.326000 |
| C | -16.882000 | -17.550000 | 2.500000 |
| C | -16.335000 | -16.198000 | 0.561000 |
| C | -16.943000 | -16.358000 | 1.823000 |
| H | -17.473000 | -15.517000 | 2.270000 |
| H | -14.000000 | -19.128000 | -1.447000 |
| H | -14.917000 | -21.601000 | 1.456000 |
| H | -15.183000 | -17.121000 | -1.021000 |
| H | -16.295000 | -20.273000 | 3.201000 |
| H | -17.354000 | -17.669000 | 3.475000 |
| H | -16.402000 | -15.237000 | 0.051000 |
| H | -13.186000 | -20.452000 | -0.470000 |
| C | -13.008000 | -14.944000 | -1.752000 |
| C | -11.604000 | -15.279000 | -1.421000 |
| C | -10.629000 | -14.423000 | -0.994000 |
| C | -10.999000 | -16.571000 | -1.507000 |
| C | -9.651000 | -16.428000 | -1.114000 |
| C | -11.469000 | -17.840000 | -1.880000 |
| N | -9.451000 | -15.109000 | -0.805000 |
| C | -8.764000 | -17.507000 | -1.084000 |

| | | | |
|---|---|---|---|
| C | -10.588000 | -18.912000 | -1.851000 |
| C | -9.247000 | -18.738000 | -1.453000 |
| H | -8.579000 | -19.599000 | -1.438000 |
| H | -13.651000 | -15.747000 | -1.391000 |
| H | -10.764000 | -13.354000 | -0.828000 |
| H | -12.506000 | -17.981000 | -2.186000 |
| H | -8.581000 | -14.705000 | -0.490000 |
| H | -7.726000 | -17.376000 | -0.779000 |
| H | -10.938000 | -19.903000 | -2.140000 |
| H | -13.236000 | -13.992000 | -1.272000 |
| H | -14.522375 | -20.815706 | -1.547885 |
| H | -13.158423 | -14.818069 | -2.834003 |
| C | -10.114753 | -21.305221 | 1.892377 |
| S | -11.751641 | -20.485915 | 1.925294 |
| C | -11.154204 | -18.746106 | 2.097325 |
| C | -12.327957 | -17.795118 | 1.855623 |
| N | -12.210288 | -16.468116 | 2.621354 |
| C | -13.282495 | -15.531344 | 2.104758 |
| C | -10.853986 | -15.829722 | 2.402710 |
| C | -12.440562 | -16.698791 | 4.101531 |
| H | -12.360513 | -15.738277 | 4.611913 |
| H | -9.539417 | -20.959707 | 1.027526 |
| H | -10.084953 | -16.473255 | 2.827641 |
| H | -10.363883 | -18.579007 | 1.359042 |
| H | -13.280929 | -18.226877 | 2.172272 |
| H | -13.086364 | -15.335803 | 1.050540 |
| H | -10.852574 | -14.862788 | 2.907568 |
| H | -11.685275 | -17.387599 | 4.477981 |
| H | -9.561888 | -21.107338 | 2.815931 |
| H | -10.299346 | -22.379217 | 1.802303 |
| H | -10.732360 | -18.635435 | 3.099951 |
| H | -12.397931 | -17.537395 | 0.797294 |
| H | -13.232898 | -14.606600 | 2.680555 |
| H | -10.704050 | -15.701361 | 1.330842 |
| H | -13.439966 | -17.117410 | 4.229877 |
| H | -14.254367 | -16.009384 | 2.228445 |

## 6. LC-MS of purified histone peptides

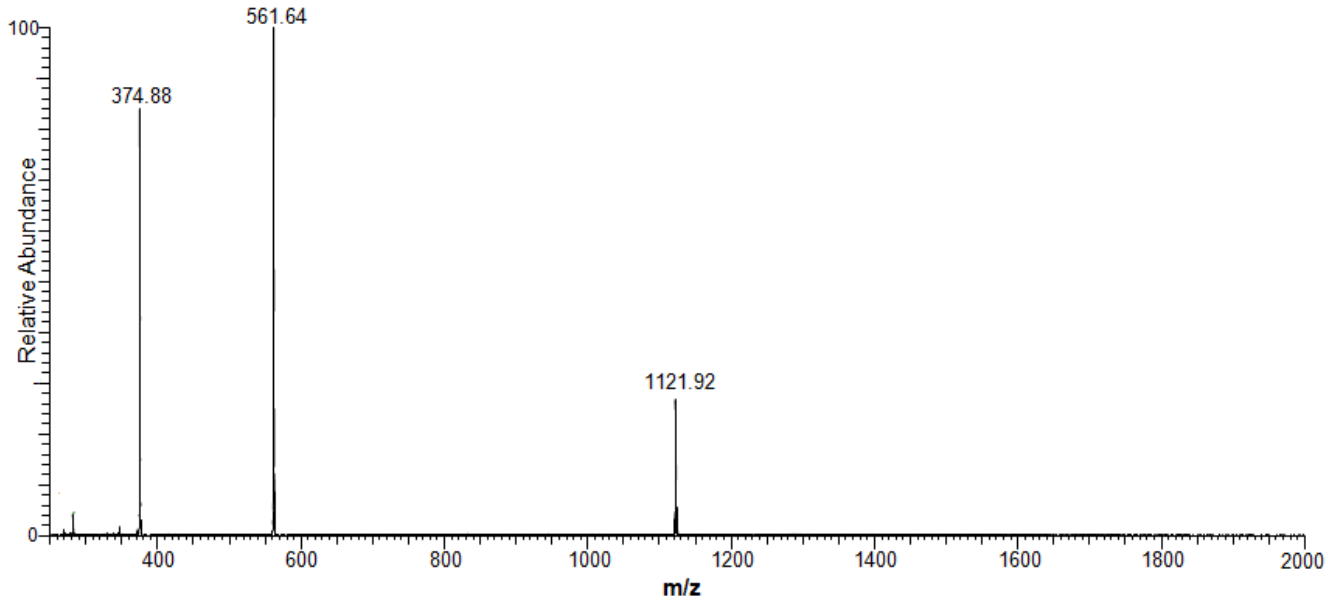



**Figure S9.** LC-MS analysis of 1-10 H3C4 after RP-HPLC purification.

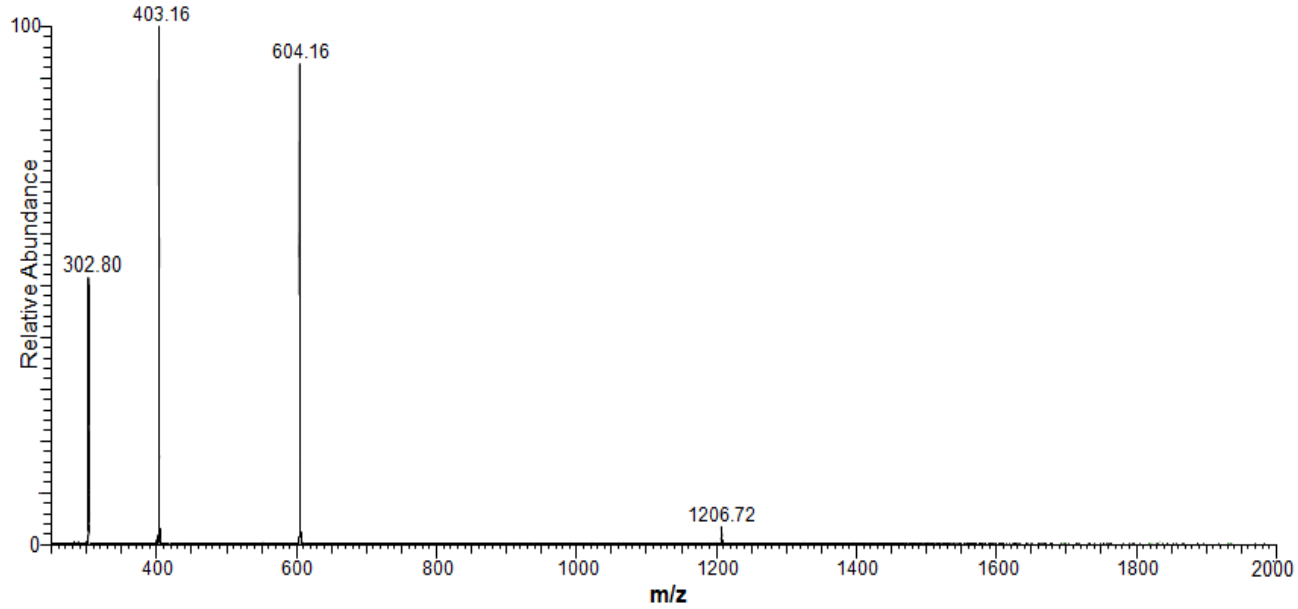



**Figure S10.** LC-MS analysis of 1-10 H3Kc4me3 after RP-HPLC purification.

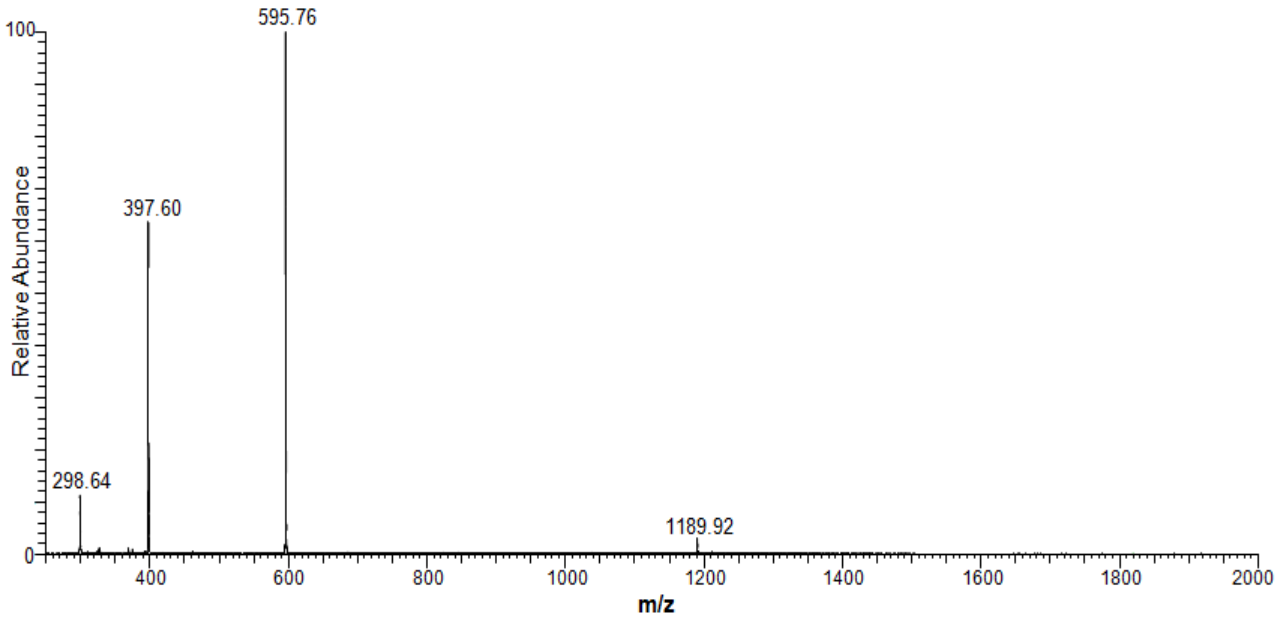



**Figure S11.** LC-MS analysis of 1-10 H3K4me3 after RP-HPLC purification.