Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Recognition of stapled histone H3K4me3 peptides by epigenetic reader proteins

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1.1 General Methods

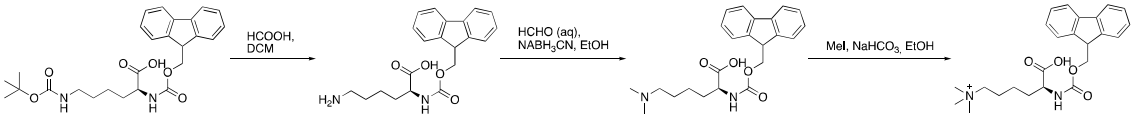
1.2 Methods

^{1}H-NMR, and ^{13}C-NMR spectra were recorded on a Varian Inova 400 MHz NMR at 300 K. ^{19}F-NMR spectra were recorded on a Bruker Avance III 400 MHz. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane as an internal reference (δ = 0.00 ppm). Coupling constants are reported as J-values in Hz. The following abbreviations are used to assign the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. NMR data was analyzed using Mestrenova. FT-IR spectra were recorded using a Bruker Tensor 27 ((source: globar 10K - 30 cm^{-1}); detector: DTGS (12K – 250 cm^{-1}); beamsplitter: KBr (30 - 7800 cm^{-1})). ESI-MS mass spectrometry data was obtained using a Thermo Finnigan LCQ Advantage Max (Thermofischer, Breda, the Netherlands). Samples were injected with methanol at room temperature. LC-MS data was obtained using a Thermo Finnigan LCQ LCQ-Fleet ESI-ion trap (Thermofischer, Breda, the Netherlands) equipped with a Phenomenex® Gemini-NX C18 column, 50 x 2.0 mm, particle size 3 µM (Phenomenex, Utrecht, The Netherlands). Samples were eluted using a gradient of H_2O/ACN containing 0.1 % formic acid (5-100 %, 1-50 min, flow 0.2 mL min^{-1}). MALDI-TOF spectra were analyzed with a Bruker Biflex III MALDI-TOF MS. Samples for MALDI-TOF were prepared by compound dilution with a saturated alpha-Cyano-4-hydroxycinnamic acid matrix solution in H_2O:ACN (1:1). Mass spectra data was analyzed with Bruker FlexAnalysis. Compounds were purified using a Shimadzu preparative HPLC equipped with a Phenomenex® Gemini-NX 3u C18 110A reversed-phase column (150 x 21.2 mm) using gradient elution at constant flow rate of 10 mL/min at a constant temperature of 30 °C. Target compounds were eluted with H_2O/ACN solutions containing 0.1% trifluoroacetic acid. Purified fractions were combined and subsequently lyophilized using an ilShin Freeze Dryer (ilShin, Ede, The Netherlands). Reader domain proteins were expressed and purified as described previously.[1, 2] Reader proteins and histone peptides were dissolved in the same buffers (Table S1). UV/Vis spectroscopy (280 nm) was used to determine the protein concentration. Calorimetric data was recorded with a fully automated Microcal Auto-iTC200 (GE Healthcare). Reader protein (25-30 µM) was titrated with histone peptide (300-350 µM) solutions over 19 injections. ITC experiments were done in replicates, and subsequent curve fitting was performed by Origin 6.00 (Microcal Inc., Northampton, MA) with a one-site model.

1.3 Materials

All reagents were obtained from commercial sources and used without further purifications. Fmoc amino acid derivatives, N,N'-Disopropylcarbodiimide (DIPCDI), and 1-Hydroxybenzotriazole (HOBt) were obtained from Novabiochem (EMD Chemicals, Gibbstown, USA). Triisopropylsilane (TIS), N,N,-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), 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 ActuAll Chemicals b.v (Oss, The Netherlands).

2 Synthesis of Fmoc-Kme3-OH



Scheme S1. Synthetic procedure for preparation of Fmoc-LysMe3-OH

(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*L*-lysine (Fmoc-K-OH)

Fmoc-Lys(Boc)-OH (5.47 g, 11.67 mmol, 1.0 eq.) was dissolved in formic acid (40 mL) and DCM (40 ml). The reaction mixture was stirred overnight at RT. The volatiles were removed *in vacuo* and the remaining residual oil was co-evaporated with $CHCl_3$ (10 mL, 2x), Et_2O (10 mL, 2x) and toluene (10 mL, 2x), affording a white solid (4.72 g, 11.32 mmol, 97%.). MS (MeOH, p ESI, 10 eV) $[M+H]^{1+}$ calc.: 369.2, found: 369.2.

N^2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N^6,N^6-dimethyl-L-lysine (Fmoc-Kme2-OH)

Fmoc-K-OH (1.2 g, 2.89 mmol, 1.0 eq.) was dissolved in ethanol (20 mL). Formaldehyde (37% solution,1.5 mL, 20.2 mmol, 6.4 eq.) was added dropwise followed by an addition of $NaBH_3CN$ (500.6mg, 7.96mmol, 2.6 eq.) while stirring followed by stirring the reaction for 4.5 h at RT. Completion was monitored by TLC (10% methanol in DCM) and ESI-MS. The mixture was quenched with trifluoroacetic acid (3 mL). Volatiles were removed in vacuo and the residual oil was subsequently extracted with Et_2O (10 mL, 1x), DCM (10 mL, 1x), and ethanol (10 mL, 1x) and dried until no further reduction in weight was observed. The crude product was used in

the next step without further purification. MS (MeOH, p ESI, 10 eV) $[M+H]^{1+}$ calc.: 397.2, found: 397.1.

(*S*)-5-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-5-carboxy-*N*,*N*,*N*-trimethylpentan-1-aminium (Fmoc-Kme3-OH)

To a stirred suspension of Fmoc-Kme2-OH (1.15g, 2.89mmol, 1 eq.) in ethanol (20 mL) iodomethane (25 mL, 401.5 mmol, 176 eq.) and $NaHCO_3$ (1.5 g, 23.8 mmol, 8 eq.) were added. The mixture was left stirring for 5 days at RT and completion was monitored using ESI-MS. Volatiles were removed in vacuo upon consumption of the starting material, affording an oil which was purified by prep-HPLC (5% to 5% in 3 min to 45% in 4.5 min to 55% in 9.5 min to 100% in 1 min). Collected fractions were lyophilized to yield a white powder for use in solid phase peptide synthesis. (534.8 mg, 1.3 mmol, 45%). MS (MeOH, p ESI, 10 eV) $[M+H]^{1+}$ calc.: 411.2; found: 411.2. LC-MS (H_2O, ITMS + p ESI, 10 eV) 397.44 (8%, $[M+H]^{1+}$), 411.40 (100%, $[M+H]^{1+}$), 821.28 (50%, $[2\times M+H]^{1+}$). ^{1}H-NMR (400MHz, CD_3OD): δ 7.75 (d, 2H, FmocC_4**H₂**), 7.67 (t, 2H, FmocC_1**H₂**), 7.38 (t, 2H, FmocC_3**H₂**), 7.32 (t, 2H, FmocC_2**H₂**), 4.38 (m, 1H, OCH_2C**H**), 4.32 (m, 1H, $NH^{\alpha}C$**H**COOH), 4.17 (t, 2H, OC**H₂**CH), 3.25 (m, 2H, $^{\delta}CH_2{}^{\varepsilon}C$**H₂**$N(CH_3)_3$), 3.15 (s, 9H, $N(CH_3)_3$), 1.82 (m, 1H, $^{\alpha}CH^{\beta}CH$**H**$^{\gamma}CH_2$), 1.75 (m, 3H, $^{\alpha}CH^{\beta}C$**H**$H^{\gamma}CH_2$, $^{\gamma}CH_2{}^{\delta}C$**H₂**$^{\varepsilon}CH_2$), 1.44 (m, 2H, $^{\beta}CH_2{}^{\gamma}C$**H₂**$^{\delta}CH_2$). ^{13}C-NMR (400MHz, CD_3OD): δ 174.1, 157.3, 143.8, 141.1, 127.4, 126.8, 124.8, 119.6, 66.5, 66.0, 53.5, 52.1, 47.6, 30.6, 22.3, 21.0.

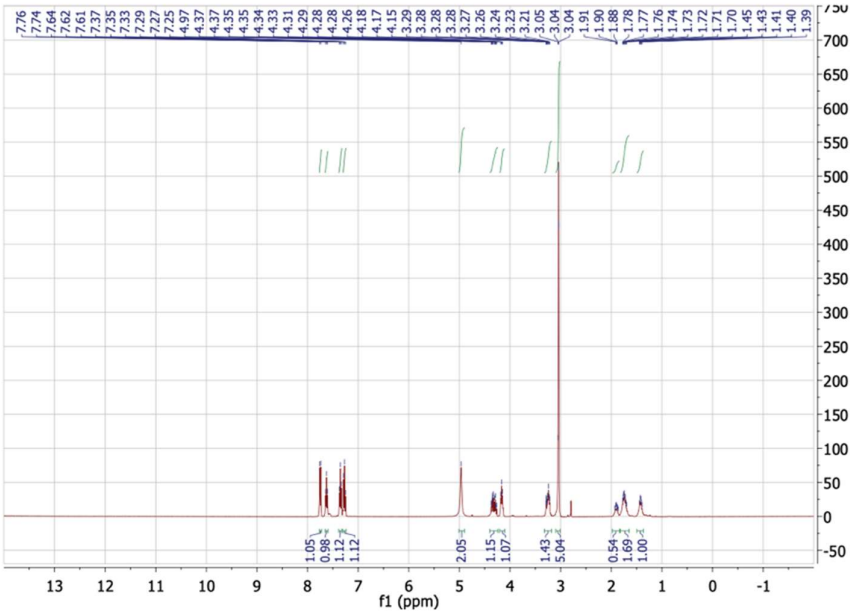


Figure S1. ^{1}H NMR spectrum of Fmoc-Kme3-OH.

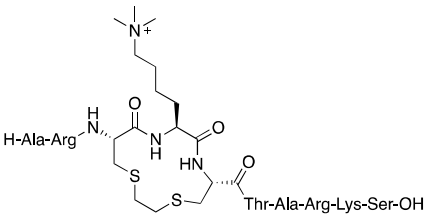
3 Synthesis and purification of linear and constrained histone peptides

3.1 General Solid-phase peptide synthesis

Synthesis of H3 10-mer peptides was achieved by solid-phase peptide synthesis using a semi-automated solid phase peptide synthesiser with the Fmoc chemistry method. Peptides were loaded on a Wang resin, and side chain protection was as follows: Arg(Pbf), Thr(tBu), Gln(Trt), Lys(Boc), and Ser(tBu). Coupling required premixing of Fmoc-protected amino acid (3.0 eq.), DIPCDI (3.3 eq.) in DMF and HOBt (3.6 eq.) in DMF for 2 min. before being added to resin. Agitating the resin for 1-2 h at RT. The Fmoc protection group was removed by treating the resin with a large excess of piperidine (20% in DMF) for 30 min at RT. Each coupling and deprotection is followed by extensive washing of the resin with DMF (3x), followed by a colour Kaiser test to monitor reaction completion. After deprotection of the final Fmoc protection group, the resin was extensively washed with DCM (3x), MeOH (3x) and Et_2O (3x), after which the resin is subsequently treated with a mixture of TFA/TIS/H_2O/EDT (92.5/2.5/2.5/2.5). Agitation at RT for 4 hours was followed by precipitation of the peptide in ice cold Et_2O (-20 ^oC). The precipitate was centrifugated (5000 rpm, 3 min, 4 ^oC, Hermle 220.72 v04) and the product was recovered by decantation of the Et_2O (3x). Residual Et_2O was removed by evaporation, and the peptides were purified with Prep-HPLC (Table S1). The fractions with purified peptides were subsequently pooled and lyophilized to yield a white powder. Peptides were identified with LC-MS and MALDI-TOF (Table S2).

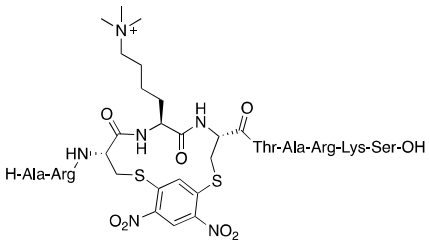
3.2 Histone H3K4me3 thioether peptide synthesis

Ethane linker (2)



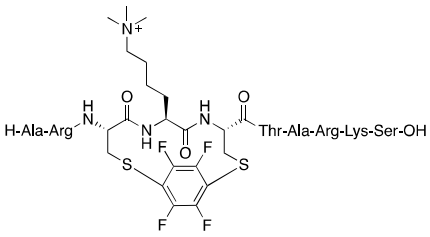
To a stirring solution of H-ARC-Kme3-CTARKS-OH (20 mg, 0.0172 mmol, 1 eq.) in DMF (0.5mL) 1,2-Dibromoethane (9.66 mg, 9 µL, 102.8 µmol, 6 eq.) was added followed by triethylamine (7.5 µL, 0.053 mmol, 3.1 eq.). The mixture was stirred for 7 h at RT and completion was monitored using LC-MS. The reaction was quenched with TFA (0.05 mL) upon completion and volatiles were subsequently removed *in vacuo.* The residual oil was purified using prep-HPLC and the combined fractions were lyophilized. Analysis by LC-MS indicated the pure retrieval of **2** (7.9 mg, 6.66 µmol, 38.7%). MS (H_2O, p ESI, 10 eV) $[M+3H]^{3+}$ calc.: 397.9; found: 397.9, $[M+2H]^{2+}$ calc.: 596.3; found: 596.4, $[M+H]^{1+}$ calc.: 1191.8; found: 1191.8 LCMS (H_2O, ITMS + p ESI, 10 eV) 298.88 (15%, $[M+4H]^{4+}$), 397.88 (100%, $[M+3H]^{3+}$), 596.40 (90%, $[M+2H]^{2+}$), 1191.56 (5%, $[M+H]^{1+}$).

2,4-dinitrobenzene linker (3)



To a stirring solution of ARC-Kme3-CTARKS (18.15 mg, 0.016 mmol, 1 eq.) in DMF (0.5 mL) 1,5-difluoro-2,4-dinitrobenzene (3.3 mg, 0.016 mmol, 1 eq.) was added followed by triethylamine (7.5 µL, 0.053 mmol, 3.3 eq.). The mixture was stirred for 45 min at RT and completion was monitored using LC-MS. The reaction was quenched with TFA (0.05 mL) upon completion and volatiles were subsequently removed *in vacuo.* The residual oil was purified using prep-HPLC and the combined fractions were lyophilized. Analysis by LC-MS indicated the pure retrieval of **3** (5.1 mg, 3.81 µmol, 21%). LC-MS (H_2O, ITMS + p ESI, 10 eV) 333.36 (75%, $[M+4H]^{4+}$), 444.08 (100%, $[M+3H]^{3+}$), 665.52 (80%, $[M+2H]^{2+}$), 1329.64 (8%, $[M+H]^{1+}$).

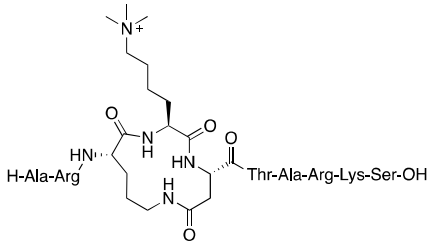
2,3,5,6-tetrafluorobenzene linker (4)



To a stirring solution of ARC-Kme3-CTARKS (16.1 mg, 0.014 mmol, 1 eq.) in DMF (1 mL) hexafluorobenzene (15.6 mg, 0.084 mmol, 6 eq.) was added followed by triethylamine (7.5 µL, 0.053 mmol, 3.8 eq.). The mixture was stirred overnight at RT and completion was monitored using LC-MS. The reaction was quenched with TFA (0.05 mL) upon completion and volatiles were subsequently removed *in vacuo.* The residual oil was purified using prep-HPLC and the combined fractions were lyophilized. Analysis by LC-MS and ^{19}F-NMR indicated the pure retrieval of **4** (4.6 mg, 3.4 µmol, 4%). LC-MS (H_2O, ITMS + p ESI, 10 eV): 328.88 (82%, $[M+4H]^{4+}$), 438.12 (100%, $[M+3H]^{3+}$), 656.52 (38%, $[M+2H]^{2+}$), 1311.80 (4%, $[M+H]^{1+}$). ^{19}F-NMR (500 MHz, CD_3OD): δ −131.72 (dd, 1F, C1C2**F**C3FC4C5FC6F), -132.64 (dd, 1F, C1C2FC3FC4C5FC6**F**), -133.03 (dd, 1F, C1C2FC3FC4C5**F**C6F), -133.37 (dd, 1F, C1C2FC3**F**C4C5FC6).

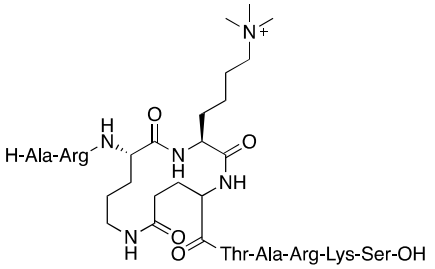
3.3 Histone H3K4me3 lactam peptide synthesis

AR<u>OKme3N</u>TARKS (5)



Fmoc-AR(Pbf)O(Alloc)Kme3D(Allyl)T(tBu)AR(Pbf)K(Boc)S(tBu)-Wang was prepared on Wang resin (250 mg, 0.165 mmol, 0.66 mmol g^{-1}) following described SPPS procedures. Resin was washed with DCM (3x) under Argon flow for 10 min. before addition of $PHSiH_3$ (375 µL, 4 mmol, 24 eq.) in DCM (1 mL). Reaction mixture was agitated under Argon flow for 2 min. before the addition of $Pd(PPH_3)_4$ (190 mg, 0.165 mmol, 1 eq.) in DCM (3 mL). The resin was agitated for 1 h at RT under Argon flow before extensive washing with DCM (3x), DMF (3x), and DCM (3x). A colour Kaiser test was performed to check for completion of the deprotection, and side chains were cyclized by treatment of the resin with HATU (307 mg, 0.81 mmol, 4.9 eq.) and DIPEA (114 µL, 1.2 mmol, 7.5 eq.) in DMF for 2 h at RT. Completion of cyclization was monitored with a colour Kaiser test, and the resin was subsequently treated with an excess of piperidine (20% in DMF) for 30 min at RT to remove the terminal Fmoc protection group. Deprotection was monitored with a colour Kaiser test. Extended cleavage of the resin with TFA/TIS/H_2O/EDT (92.5/2.5/2.5/2.5) for 18 h at RT and subsequent precipitation, prep-HPLC and lyophilization following standard SPPS procedures afforded **5** as a white solid (18.2 mg, 15.5 µmol, 8.2%). LC-MS (H_2O, ITMS + p ESI, 10 eV) 293.56 (70%, $[M+4H]^{4+}$), 391.08 (100%, $[M+3H]^{3+}$), 586.08 (87%, $[M+2H]^{2+}$), 1170.88 (9%, $[M+H]^{1+}$), 1284.68 (12%, $[M+tfa+H]^{1+}$).

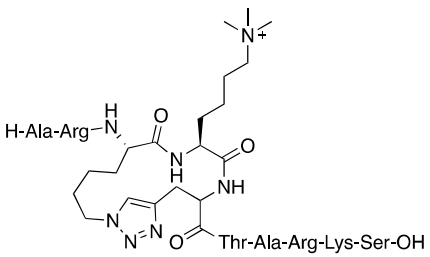
AR<u>OKme3Q</u>TARKS (6)



Fmoc-AR(Pbf)O(Alloc)Kme3E(Allyl)T(tBu)AR(Pbf)K(Boc)S(tBu)-Wang was prepared on Wang resin (240 mg, 0.158 mmol, 0.66 mmol g^{-1}) following described SPPS procedures. Resin was washed with DCM (3x) under Argon flow for 10 min. before addition of $PHSiH_3$ (356 µL, 3.8 mmol, 24 eq.) in DCM (1 mL). Reaction mixture was agitated under Argon flow for 2 min. before the addition of $Pd(PPH_3)_4$ (182 mg, 0.158 mmol, 1 eq.) in DCM (3 mL). The resin was agitated for 1 h at RT under Argon flow before extensive washing with DCM (3x), DMF (3x), and DCM (3x). A colour Kaiser test was performed to check for completion of the deprotection, and side chains were cyclized by treatment of the resin with HATU (292 mg, 0.77

mmol, 4.9 eq.) and DIPEA (113 µL, 1.2 mmol, 7.5 eq.) in DMF for 2 h at RT. Completion of cyclization was monitored with a colour Kaiser test, and the resin was subsequently treated with an excess of piperidine (20% in DMF) for 30 min at RT to remove the terminal Fmoc protection group. Deprotection was monitored with a colour Kaiser test. Extended cleavage of the resin with TFA/TIS/H_2O/EDT (92.5/2.5/2.5/2.5) overnight at RT and subsequent precipitation, prep-HPLC and lyophilization following standard SPPS procedures afforded **6** as a white solid (13.9 mg, 11.7 µmol, 7.4%). LC-MS (H_2O, ITMS + p ESI, 10 eV) 297.00 (10%, $[M+4H]^{4+}$), 395.72 (96%, $[M+3H]^{3+}$), 593.08 (100%, $[M+2H]^{2+}$), 827.44 (21%, $[2\times M+tfa+H]^{3+}$), 1184.88 (6%, $[M+H]^{1+}$), 1298.76 (13%, $[M+tfa+H]^{1+}$).

AR<u>KazKme3Pra</u>TARKS (7)



To a bubbling solution of H-AR-Lys(N_3)-Kme3-Pra-TARKS-Wang (203.4mg, 40.7 µmol, 0.2mmol g^{-1}, 1 eq.) in DMF (1 mL) under argon was added CuBr (6.95 mg, 48.4 µmol, 1.2 eq.) dissolved in degassed DMF (0.85 ml). Sodium ascorbate (10.17 mg, 51.2 µmol, 1.3 eq.) was dissolved in water (0.180 µL) and added to the resin mixture. 2,6-Lutidine (45.7 mg, 50.0 µL, 427 µmol, 10.5 eq.) and DIPEA (51.9 mg, 70.1 µL, 402 µmol, 9.9 eq.) were subsequently added before purging the reaction vessel with argon gas for 10 min.. The mixture was thereafter stirred for 18.5 h at 40 °C. The resin was extensively washed with DMF (3x), EtOH (3x), DMF (3x) and Et_2O (3x) before being cleaved from the resin with TFA/TIS/H_2O/EDT (92.5/2.5/2.5/2.5) over 4 h at RT. Subsequent precipitation yielded a greenish precipitate, and prep-HPLC and lyophilization afforded *white solid* **7** (7.4 mg, 6.1 µmol, 15.0%). IR measurements indicated cyclization. LC-MS (H_2O, ITMS + p ESI, 10 eV) 303.12 (8%, $[M+4H]^{4+}$), 403.64 (64%, $[M+3H]^{3+}$), 605.00 (100%, $[M+2H]^{2+}$), 843.80 (9%, $[2\times M+tfa+H]^{3+}$), 1208.80 (6%, $[M+H]^{1+}$), 1322.72 (6%, $[M+tfa+H]^{1+}$).

4 Preparative HPLC of histone H3K4me3 peptides

Table S1. HPLC Purification methods for the purification of H3K4me3 cyclic/linear peptides.

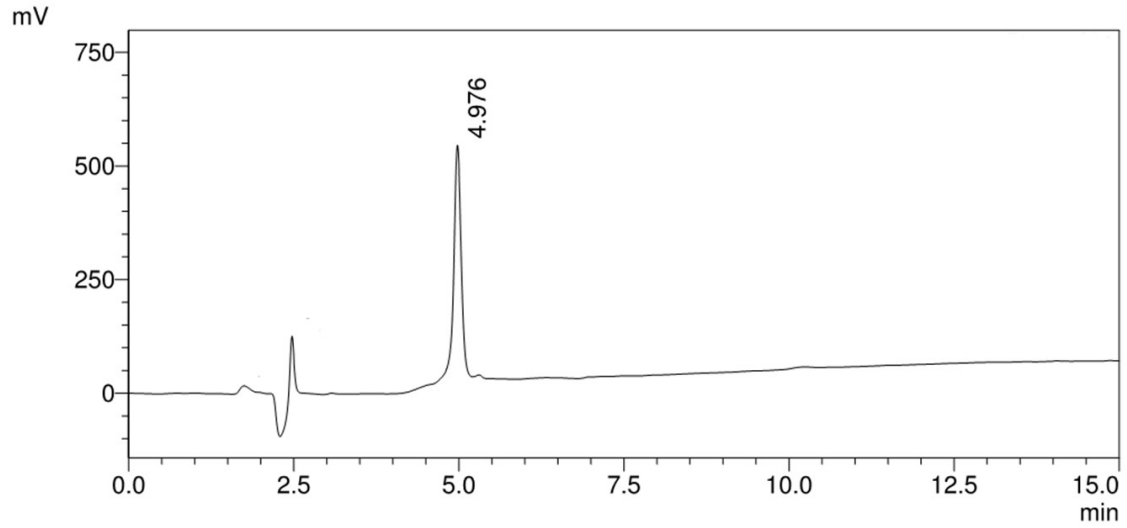
Compound	*Elution time (min)*	*Flow rate (mL/min)*	*Run Time (min)*	*Conc. ACN Idle (%)*	*Conc. gradient ACN (% at time in min)*	*Temp. (°C)*
1	9.9-12	10	20	3	3(3) to 3(9) to 100(13) to 100(16) to 3(18)	30/40
2	11-12	10	20	4	6(11) to 100(13) to 100(16) to 4(18)	40/40
3	8.5-9.6	10	20	10	12(3) to 17(14) to 100(15) to 100(19) to 10(19.5)	40/40
4	18-19	10	17	10	12(3) to 18(14) to 100(15) to 100(16) to 10(16.5)	40/40
5	8.8-10.2	10	20	2.5	3.5(13) to 100(14) to 100(18) to 2.5(19)	40/40
6	9-10	10	20	2.5	3.5(13) to 100(14) to 100(18) to 2.5(19)	40/40
7	6.5-7.5	10	20	5	5(9) to 100(13) to 100(16) to 5(18)	30/40

5 LC-MS of histone H3K4me3 peptides

Table S2. Masses found for H3K4me3 cyclic/linear peptides.

Compound	*Chemical formula*	*Exact Mass*	*Mass found*			
			$[M+H]^{1+}$	$[M+2H]^{2+}$	$[M+3H]^{3+}$	$[M+4H]^{4+}$
1	$C_{49}H_{94}N_{19}O_{15}$	1188.72	1189.92	595.44	397.24	298.16
2	$C_{48}H_{91}N_{18}O_{13}S_2$	1191.64	1191.56	596.40	397.88	298.88
3	$C_{52}H_{89}N_{20}O_{17}S_2$	1329.62	1329.64	665.52	444.08	333.36
4	$C_{52}H_{87}F_4N_{18}O_{13}S_2$	1311.61	1311.52	656.40	437.96	328.72
5	$C_{49}H_{92}N_{19}O_{14}$	1170.71	1170.88	586.06	391.08	293.56
6	$C_{50}H_{94}N_{19}O_{14}$	1184.72	1184.88	593.08	395.72	297.00
7	$C_{51}H_{94}N_{21}O_{13}$	1208.73	1208.73	605.00	403.64	303.12

A)



B)

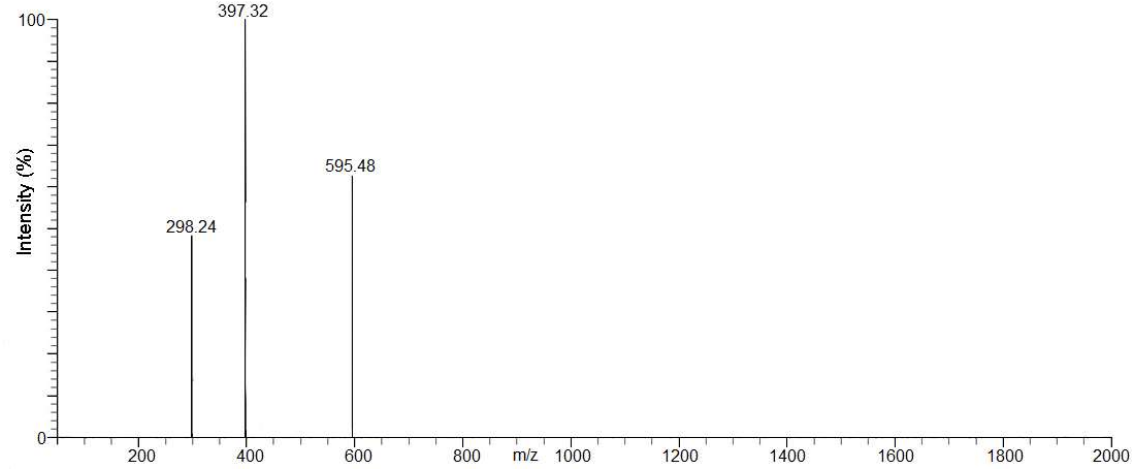
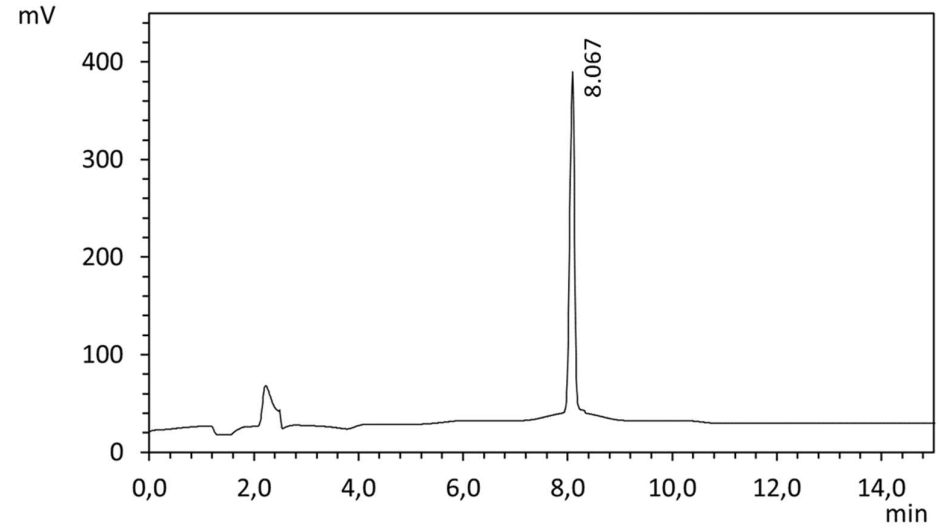


Figure S2. (A) Analytical HPLC profile and (B) ESI-MS analysis of the peptide **1** after prep-HPLC purification. The peptide elutes at 5.0 min.

A)



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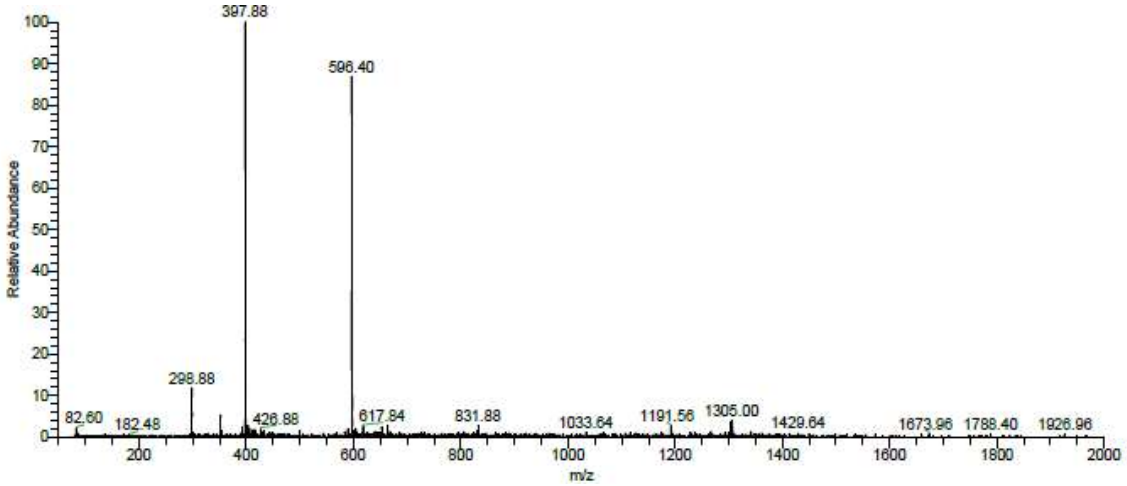
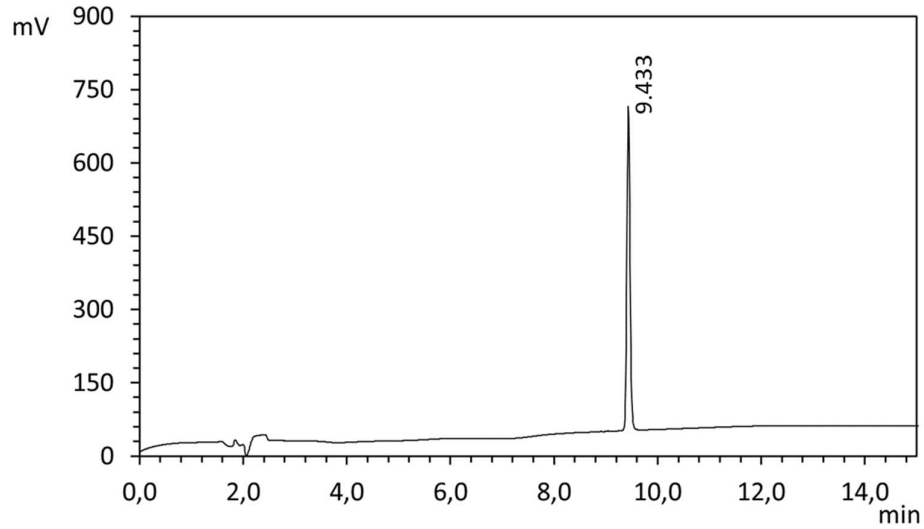


Figure S3. (A) Analytical HPLC profile and (B) ESI-MS analysis of the peptide **2** after prep-HPLC purification. The peptide elutes at 8.1 min.

A)



B)

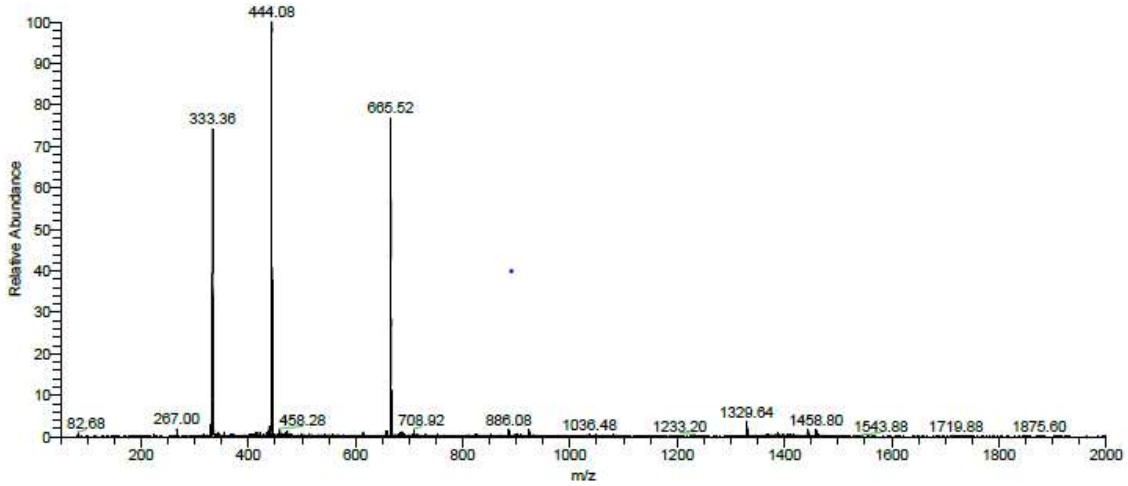
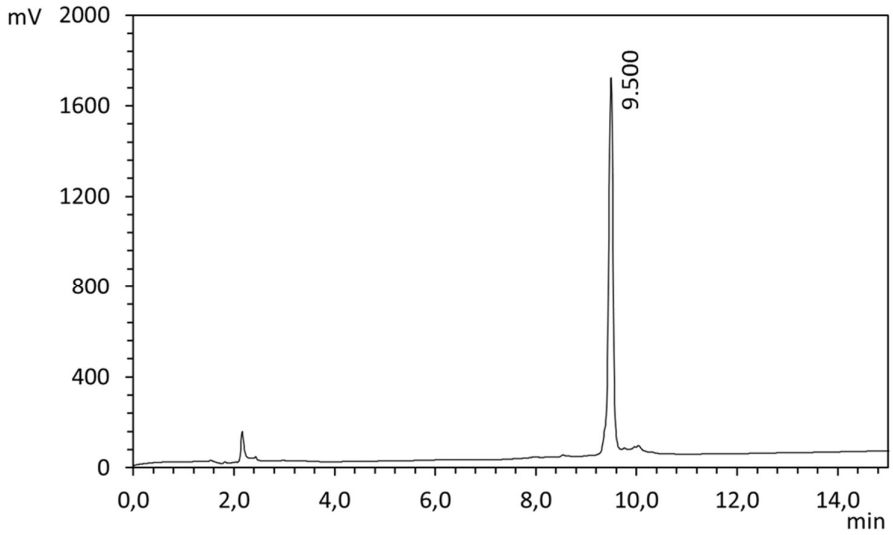


Figure S4. (A) Analytical HPLC profile and (B) ESI-MS analysis of the peptide **3** after prep-HPLC purification. The peptide elutes at 9.4 min.

A)



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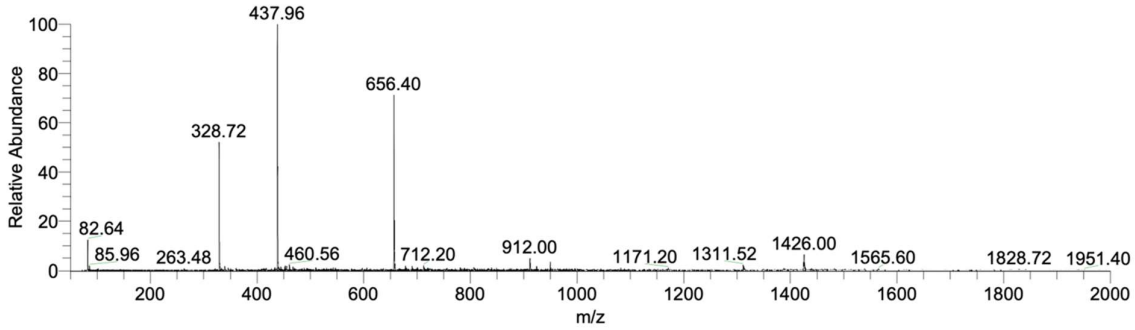
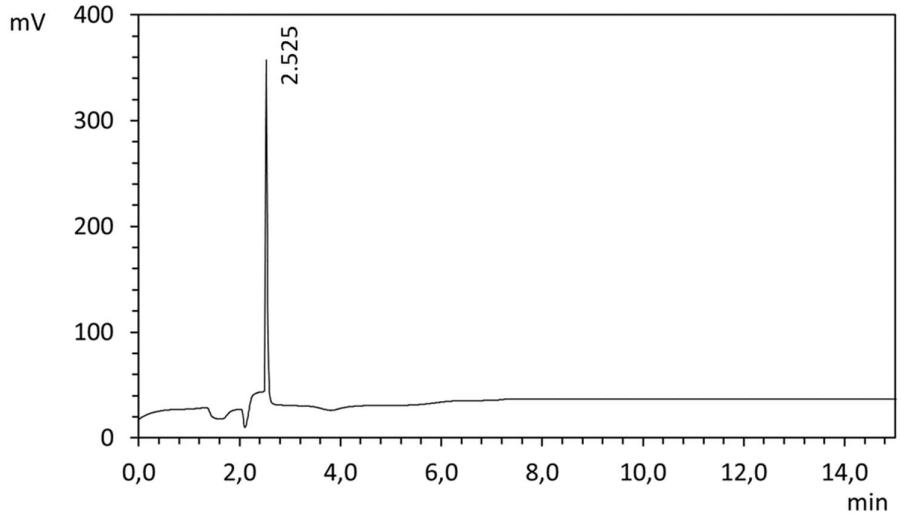


Figure S5. (A) Analytical HPLC profile and (B) ESI-MS analysis of the peptide **4** after prep-HPLC purification. The peptide elutes at 9.5 min.

A)



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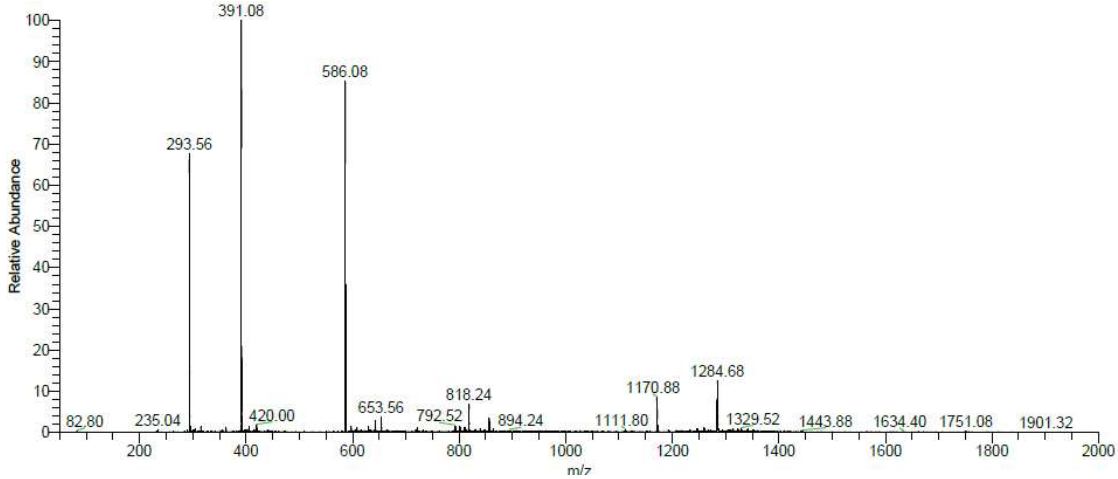
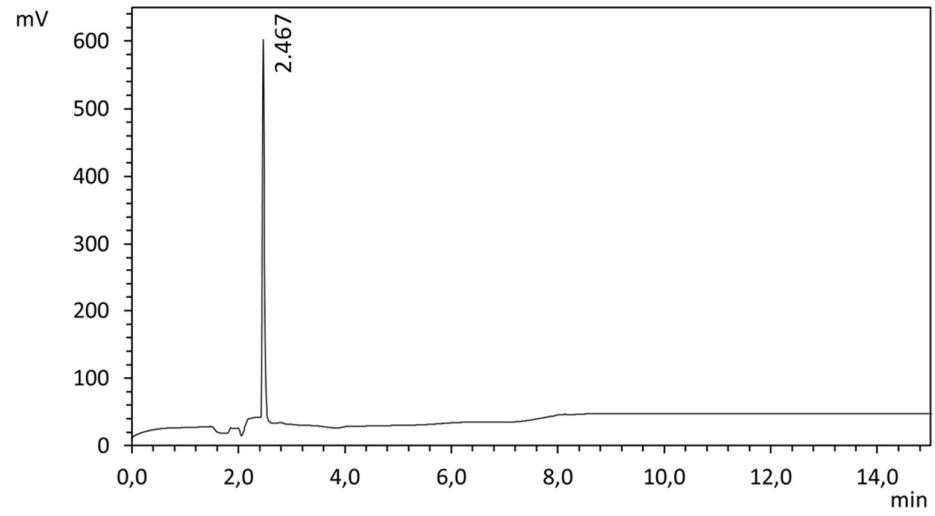


Figure S6. (A) Analytical HPLC profile and (B) ESI-MS analysis of the peptide **5** after prep-HPLC purification. The peptide elutes at 2.5 min.

A)



B)

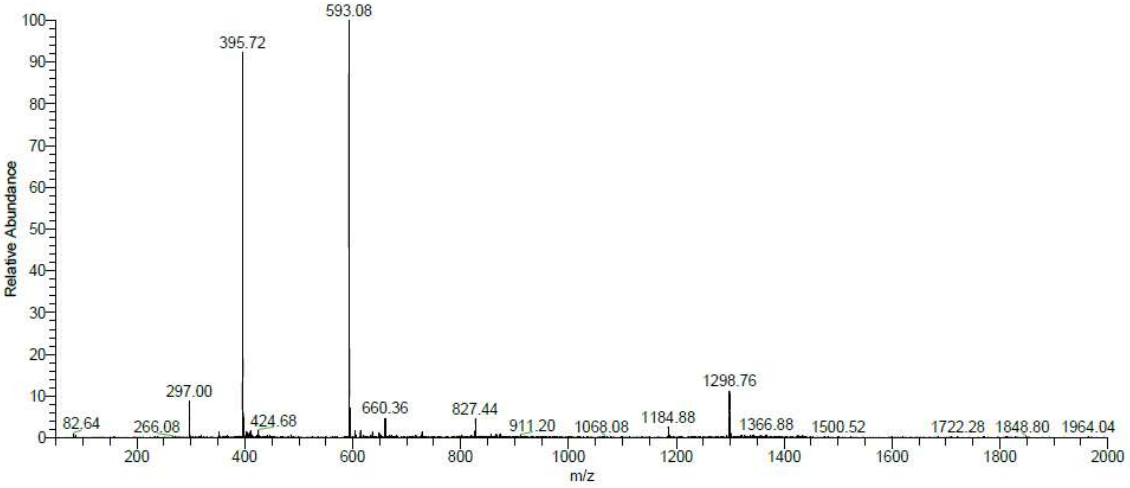
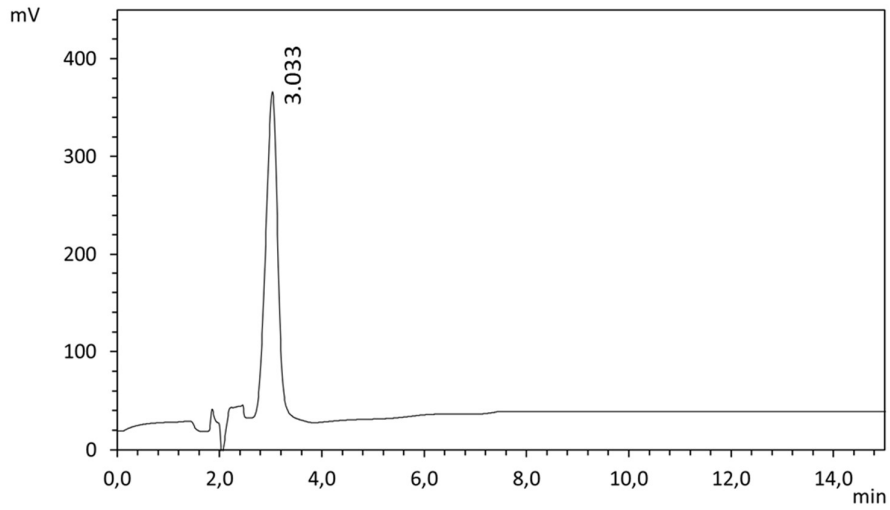


Figure S7. (A) Analytical HPLC profile and (B) ESI-MS analysis of the peptide **6** after prep-HPLC purification. The peptide elutes at 2.5 min.

A)



B)

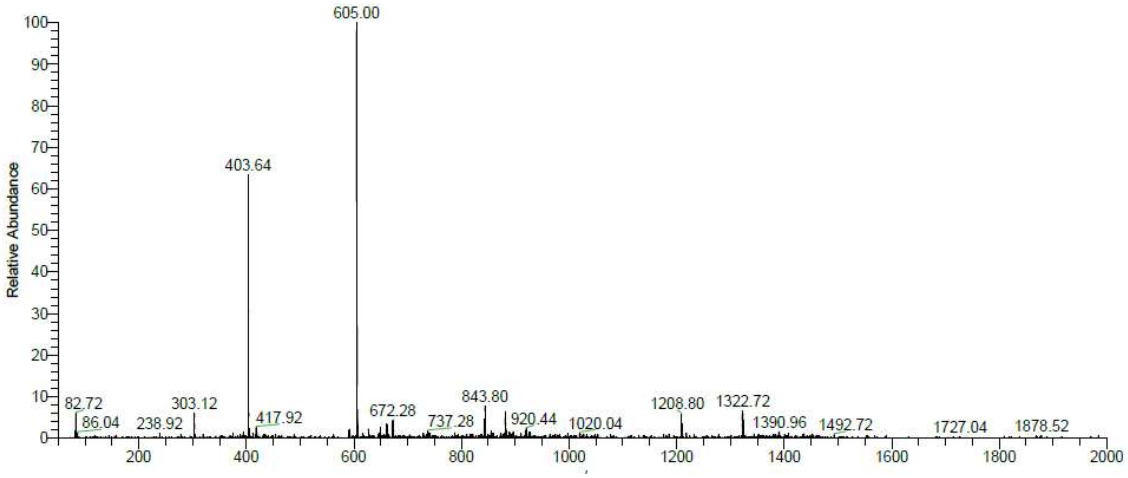


Figure S8. (A) Analytical HPLC profile and (B) ESI-MS analysis of the peptide **7** after prep-HPLC purification. The peptide elutes at 3.0 min.

6 IR spectra

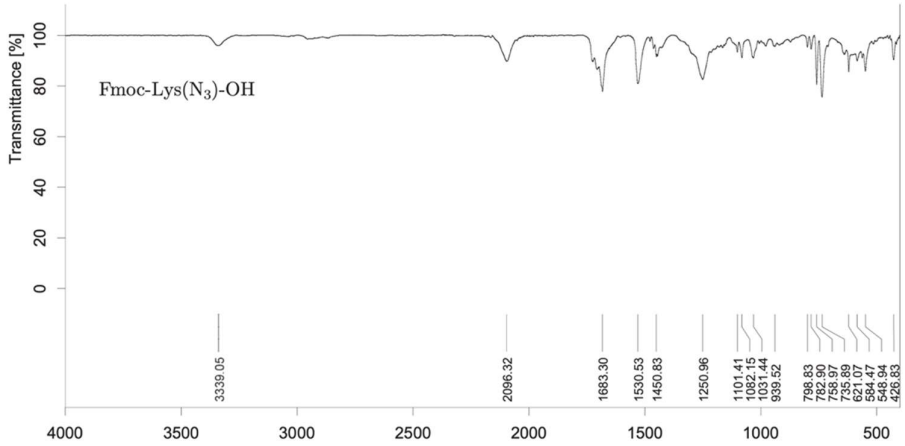


Figure S9. IR-Spectrum of Fmoc-Lys(N_3)-OH. Azide specific peak can be observed at 2096.32 nm.

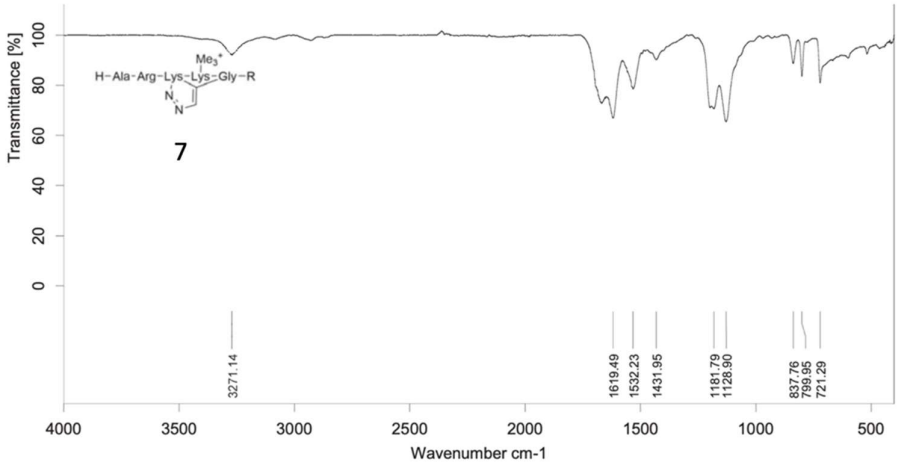


Figure S10. IR-Spectrum of peptide **7**. Azide specific peak cannot be observed around 2096.32 nm, which indicates successful cyclization.

7 ITC supporting figures

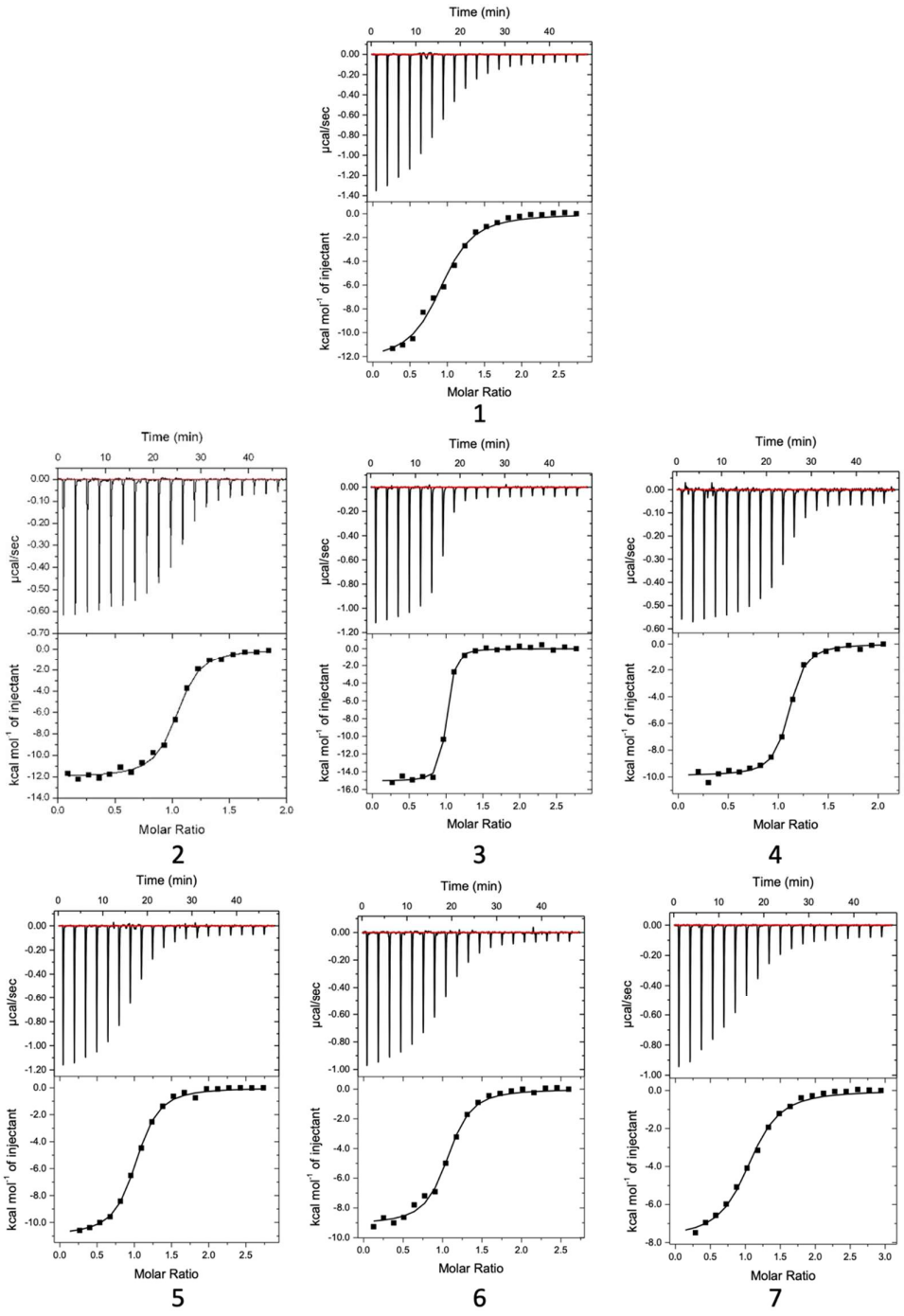


Figure S11. ITC binding curves of BPTF with peptides **1**-**7**.

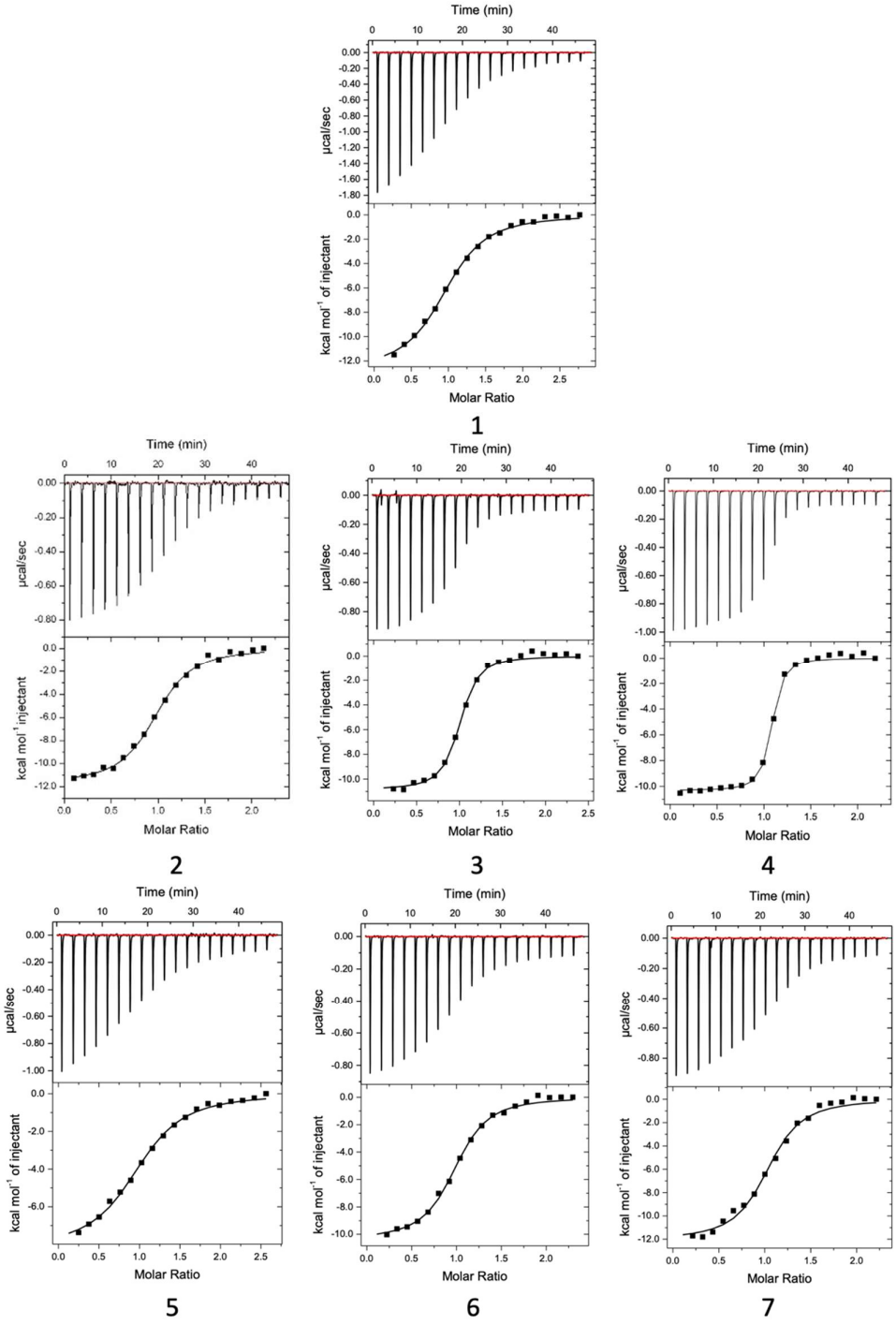


Figure S12. ITC binding curves of JMJD2A with peptides **1**-**7**.

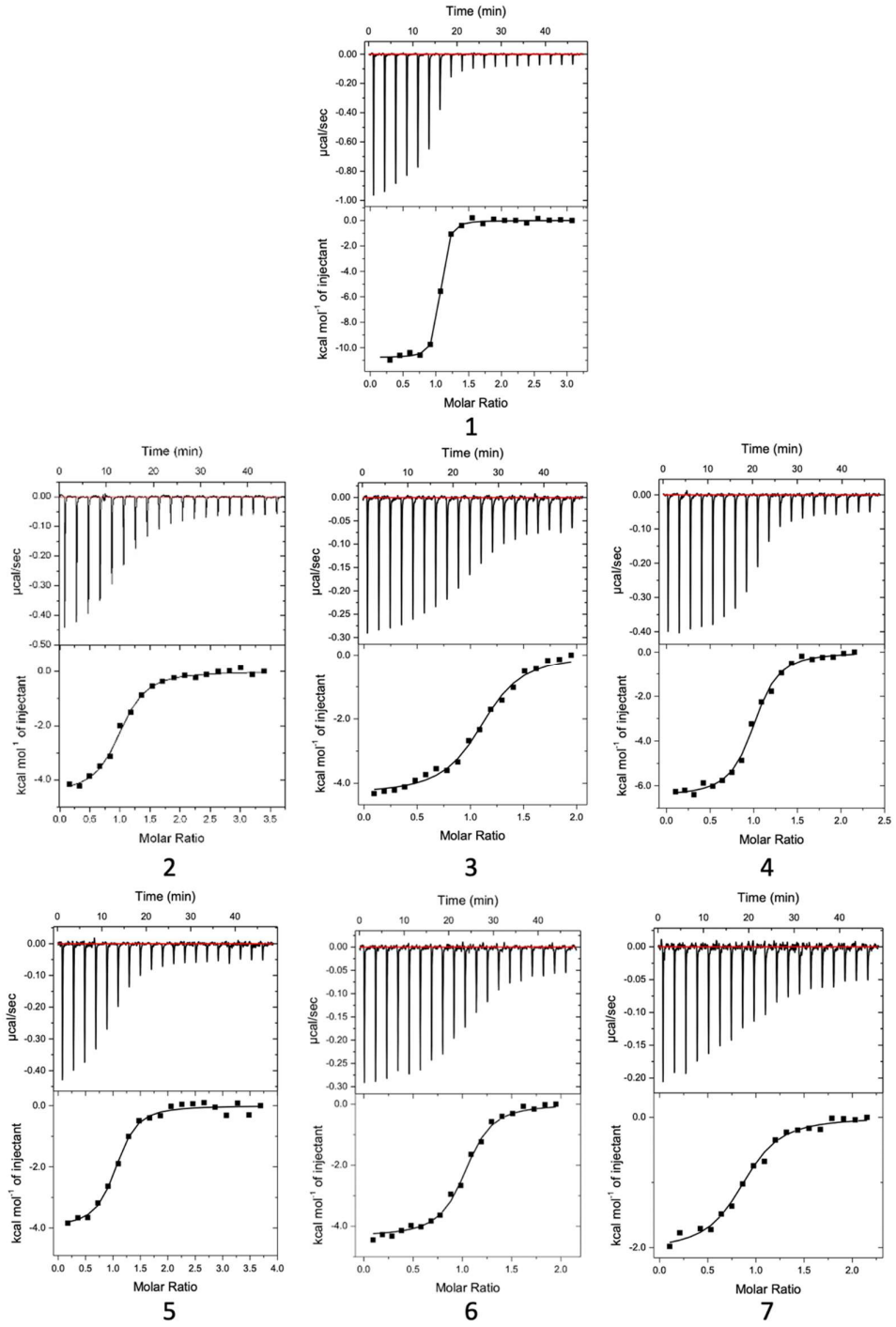


Figure S13. ITC binding curves of JARID1A with peptides **1**-**7**.

8 Docking

The crystallized structures of JMJD2A and BPTF in complex with H3K4me3 (PDB ID: 2GFA, X-ray crystal structure resolution 2.10 Å, PDB ID: 2F6J, X-ray crystal structure resolution 2.00 Å) were obtained from the Protein Data Bank.[3, 4] and were imported into Schrödinger Suite's Maestro module.[5] Imported files were pre-processed to add missing hydrogen atoms, removing H_2O molecules, and to assigning bond orders. PROPKA was applied to determine the protein protonation state at pH 7.0 and steric contacts were alleviated (OPLS_2005).

Linear and constrained H3K4me3 sequences were imported into the Maestro module and the LigPrep tool was used to generate minimized tautomers by applying force field minimization (OPLS_2005) and ionization at pH 7.0 (Epik).[6, 7] Receptor grids were generated of the H3K4me3 containing 2F6J and 2GFA structures with initial van der Waals scaling of 1 Å. The grid box was defined using K4me3 as the centroid. Constrained H3K4me3 (ARCKCT) peptide was docked into 2F6J and 2GFA using the generated receptor grid with Glide using the Extra Precision (XP) mode with ligand flexibility parameters enabled.[8] The resulting predicted complex was subsequently used for Induced Fit Docking (IFD) of the histone tail pentapeptides using the Extended sampling-docking mode.[9] IFD was executed by using initial van der Waals scaling of 0.5 for the receptor and ligand, ligand backbone restrainment within 4 Å of the docked H3 (ARCKCT) backbone, and with refinement of receptor residues within 5 Å of the docking ligands.

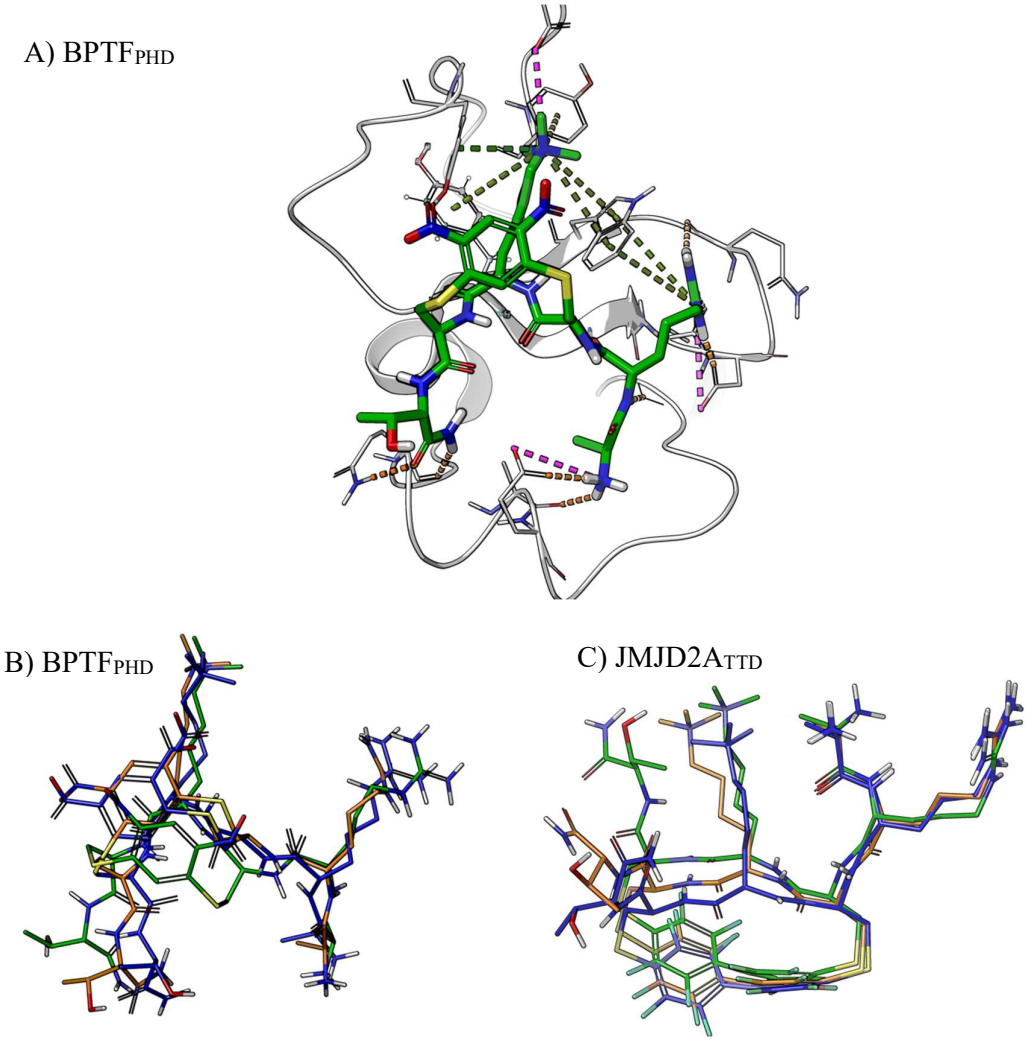


Figure S14. Additional computational analysis of docked histone peptide structures. Key interactions are displayed: hydrogen bond (orange), cation-π interactions (green), and salt-bridges (pink). A) Induced-fit docking of **3** in BPTF$_{PHD}$. B-C) Overlay of three highest scoring (glide score) induced-fit docking poses (residues 1-6) of **3** and **4**.

9 References

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4. Y. Huang, J. Fang, M. T. Bedford, Y. Zhang and R. M. Xu, *Science*, 2006, **312**, 748-751.
5. Schrödinger Release 2021-1: Maestro, Schrödinger, LLC, New York, NY, 2021.
6. Schrödinger Release 2021-1: LigPrep, Schrödinger, LLC, New York, NY, 2021.
7. Schrödinger Release 2021-1: Epik, Schrödinger, LLC, New York, NY, 2021.
8. Schrödinger Release 2021-1: Glide, Schrödinger, LLC, New York, NY, 2021.
9. Schrödinger Release 2021-1: Induced Fit Docking protocol; Glide, Schrödinger, LLC, New York, NY, 2021.