Supporting Information for:

Targeted Histone Peptides: Insights into the Spatial Regulation of the Methyltransferase PRC2 using a Surrogate of Heterotypic Chromatin

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Supporting Figures

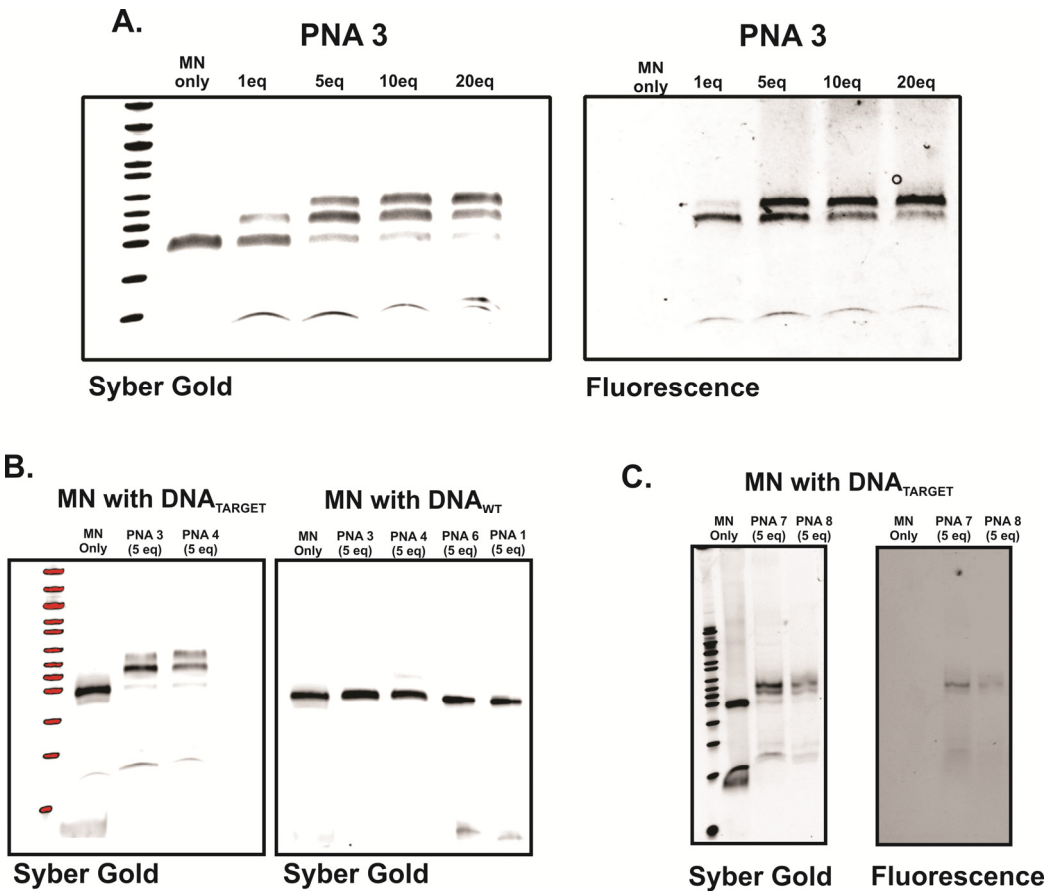


Figure S1. Electrophoretic mobility shift assay (EMSA) of mononucleosomes with the indicated peptide and PNA constructs. See below for complete description of the binding assays. **A**. Titration of increasing amounts of PNA **3** with MNs that include the PNA binding site. The left panel shows the native gel stained with Syber Gold, with the PNA-MN complex exhibiting a characteristic gel-shift, whereas the right panel shows the fluorescence of the gel (PNA **3** contains a fluorescein moiety). Note the PNA gel-shifted species shows two bands, which is conventionally seen with PNA containing complexes. The two bands have been shown to correspond to either a 1:1 binding stoichiometry between PNA and MN, or a 2:1 PNA and MN ratio. For a detailed discussion, please see ref. [1] **B**. Left: Native gel electrophoresis (stained with Syber Gold) of PNA constructs **3** and **4** shows binding with MNs that include the PNA binding site with the 601 sequence (designated DNA_{target}), while MNs which only have the conventional 601 sequence (designated DNA_{WT}) show no gel-shift when incubated with the indicated PNA constructs. **C**. Left: Native gel electrophoresis (stained with Syber Gold) of PNA constructs **7** and **8** shows the characteristic gel-shift of the nucleosomal band associated with complex formation with MNs.

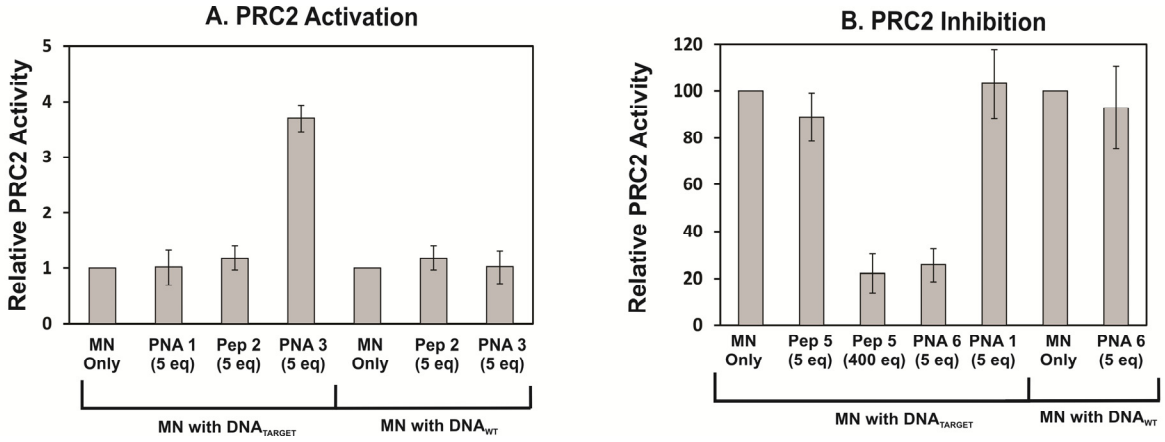


Figure S2. PRC2 methyltransferase assays with MNs using the indicated peptides and PNA constructs, including the control MNs that do not incorporate the PNA binding site. Note, this data is an expanded plot of Figure 2 in the main text. **A**. PRC2 activation with MNs containing either the DNA_{target} or control mononucleosomes containing only the 601 sequence, DNA_{WT}. Data shown is from independent triplicate experiments, with the error bars representing standard deviation. **B**. PRC2 inhibition, with MNs containing either the DNA_{target} or control mononucleosomes containing only the 601 sequence, DNA_{WT}. Data shown is from independent triplicate experiments, with the error bars representing standard deviation.

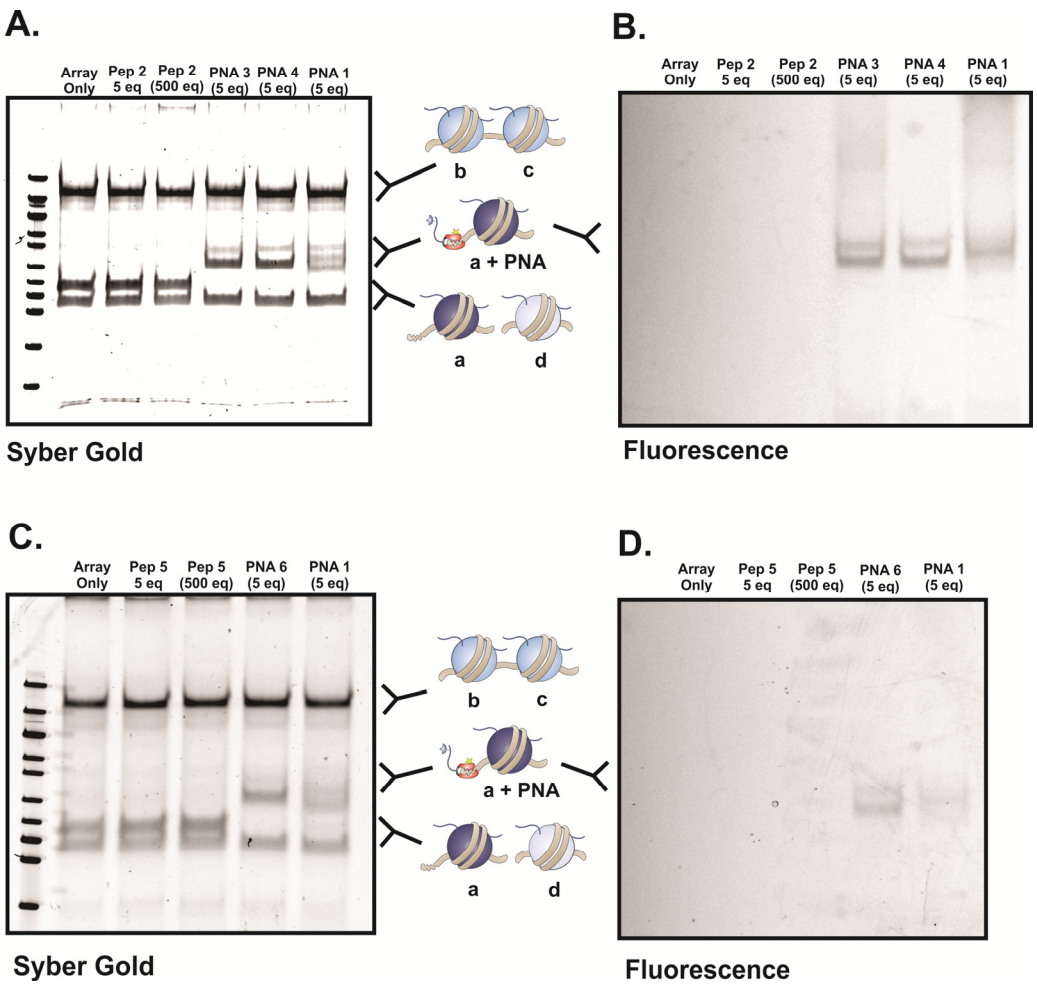


Figure S3. Native gel analysis of 4-mer nucleosome arrays, incubated with the indicated peptide and PNA constructs and digested with the appropriate restriction enzymes. See below for complete description of the assays. **A**. Syber Gold visualization of the gel with PRC2 activating constructs, showing the characteristic gel-shift of the PNA-MN complex. **B.** Fluorescence of gel shown in (**A**), providing evidence that the gel-shifted species incorporates the fluorescein-labeled PNA moiety. **C**. Syber Gold visualization of the gel with PRC2 inhibiting constructs, showing the characteristic gel-shift of the PNA-MN complex. **D.** Fluorescence of gel shown in (**C**), providing evidence that the gel-shifted species incorporates the fluorescein-labeled PNA moiety.

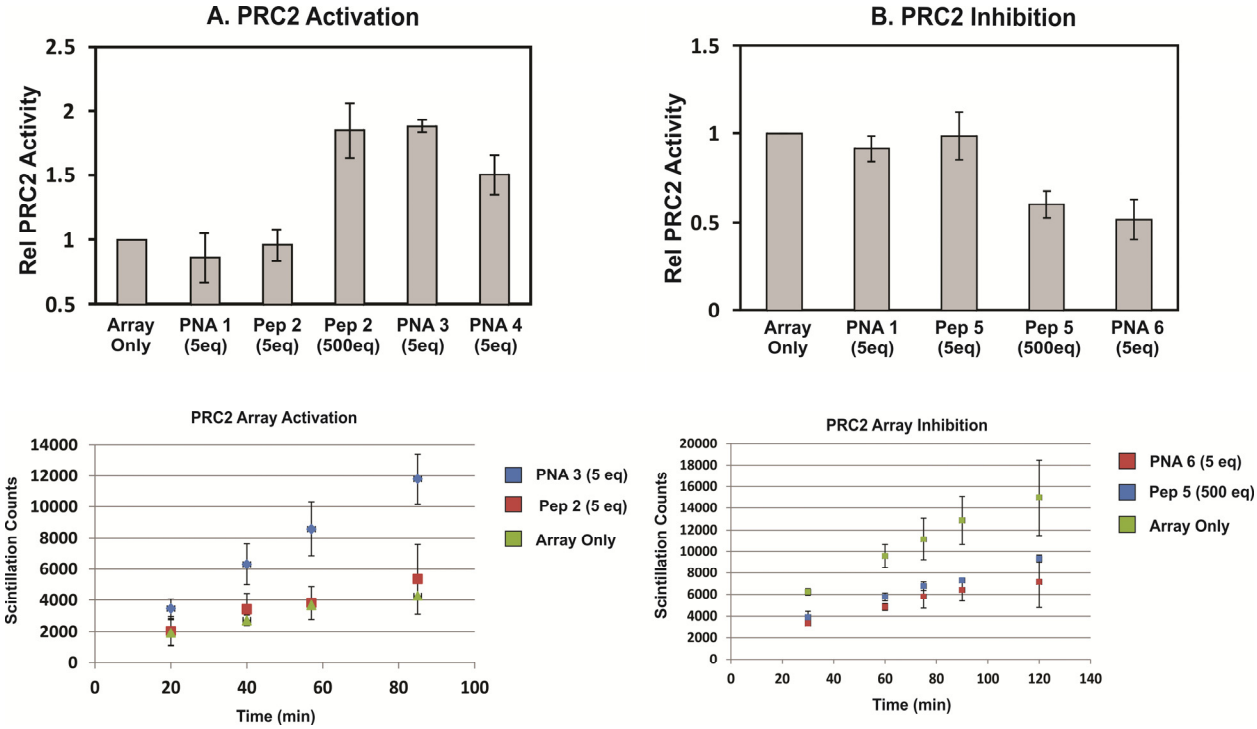


Figure S4. PRC2 methyltransferase assays with arrays incorporating the DNA_{target} using the indicated peptides and PNA constructs. Below are time course experiments using the labeled constructs. Reactions were quenched by the spotting on filter paper. See below for full description of the scintillation assays. **A**. PRC2 activation with arrays containing the DNA_{target}. Substrates and PRC2 were incubated with indicated construct and ^{3}H-SAM. De novo methylation of the array was determined by filter binding followed by scintillation counting. The "array only" experiment is normalized to 1. Errors bars = s.d. (n = 3). **B**. PRC2 inhibition with MNs containing either the DNA_{target}. Substrates and PRC2 were incubated with indicated construct and ^{3}H-SAM. See below for a detailed description of the assay. De novo methylation of the array was determined by filter binding followed by scintillation counting. The "array only" experiment is normalized to 1. Errors bars = s.d. (n = 3).

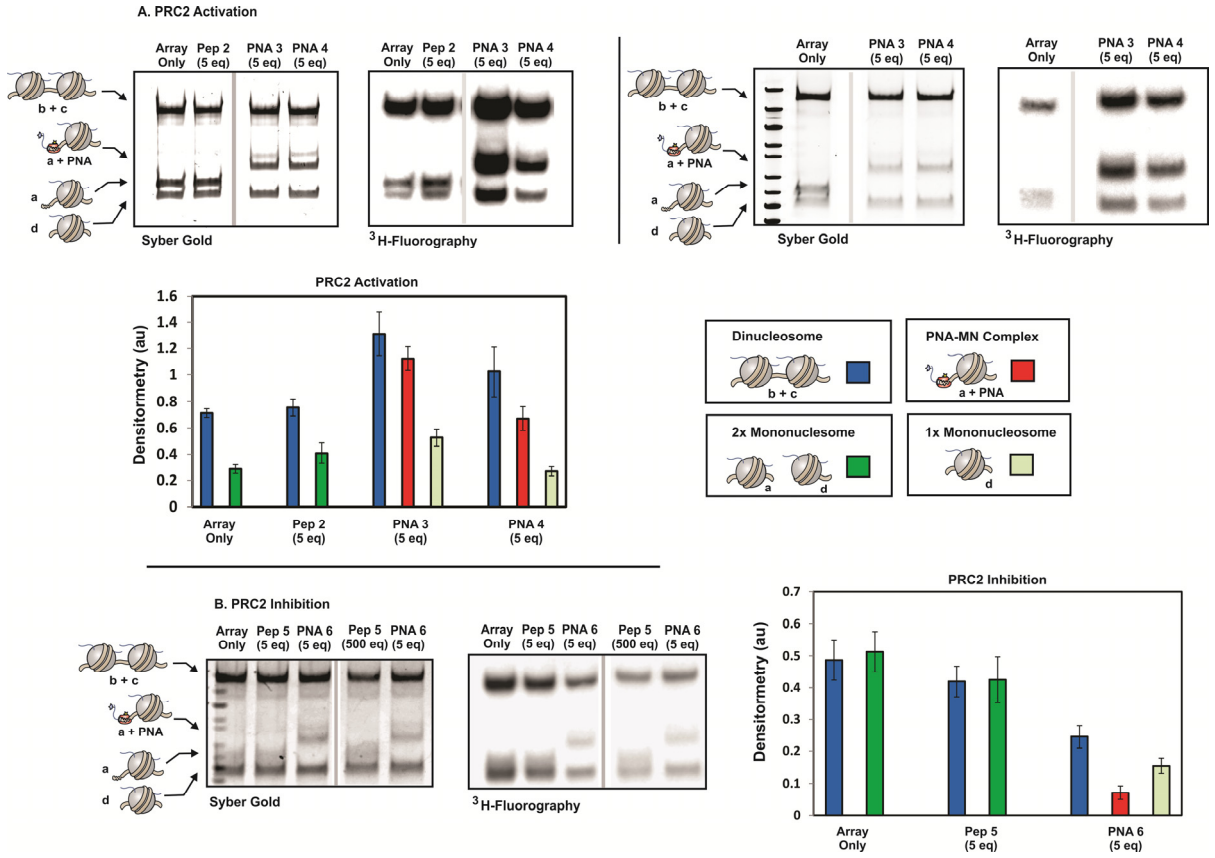


Figure S5. PRC2 methyltransferase assays with arrays including native gels, fluorographs and plots of densitometry for each of the designated flourographic bands. (**A**) PRC2 activation and (**B**) PRC2 inhibition using the indicated peptides or PNA constructs. Plots of individual bands of the fluorographs correspond to the various nucleosomal species shown (n=3, error bars respresent one s.d.) The gels shown are additional representative data from that shown in Figure 3 of the manuscript. Note that gray rectangles designate lanes removed from the gel.

PNA-DNA Binding

PNAs represent an ideal platform for the sequence specific recognition of the nucleosomal DNA. However, as noted in the main text, other molecular systems (such as polyamides) represent additional targeting vectors which could be to recognize DNA and display a histone-modifying peptide.

Bis-PNA constructs (also called hairpin PNAs) were chosen because literature precedence[2] has shown they can efficiently invade double-stranded DNA, such as the nucleosomal DNA in this study (see, for example, ref. [3], [4] as well as references in the main text). Bis-PNAs bind a target polypurine tract, usually at least 10 consecutive purine bases, with little other sequence requirements. One PNA strand binds via Watson-Crick base pairing while the other PNA strand binds in a Hoogsteen fashion[1], thus necessitating the use of two PNA strands connected by a flexible linker. The specific sequence of T5C5 was chosen for simplicity, although other base sequences could be used. The PNA-dsDNA complex has been shown to be quite stable[1], a requirement for the assays used in this study as the PNA-nucleosome complexes are subjected to several biochemical steps, and require intact complex for proper interpretation.

Materials and Methods

Dimethylformamide (DMF), dichloromethane (DCM), triisopropylsilane (TIS) were purchased from Sigma-Aldrich (Milwaukee, WI) and used without further purification. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was purchased from Thermo Scientific (Rockford, IL). Fmoc amino acids, MBHA resin and 2-chloro-trityl chloride (Trityl) resin were purchased from Novabiochem (Darmstadt, Germany) or Bachem (Torrance, CA). PNA monomers: Boc-PNA-T-OH and Boc-PNA-C(Cbz)-OH were purchased from ASM Research (Fairfax, VA). 2-(7-Aza-1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) and *O*-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) were purchased from Genscript (Piscataway, NJ). Fmoc-(*R,S*)-2-aminoheptanoic acid was purchased from Anaspec (Fremont, CA) and separated into the respective enantiomers as reported previously[5]. Trifluoroacetic acid (TFA) was purchased from Halocarbon (North Augusta, SC). All other chemicals were purchased from Sigma-Aldrich (Milwaukee, WI) and used without further purification.

Analytical RP-HPLC was performed on Hewlett-Packard 1100 and 1200 series instruments equipped with a C18 Vydac column (5 μm, 4.6 x 150 mm) at a flow rate of 1 mL/min. Preparative RP-HPLC was performed on a Waters prep LC system comprised of a Waters 2545 Binary Gradient Module and a Waters 2489 UV detector. Purifications were carried out on a C18 Vydac 218TP1022 column (10 μM; 22 x 250 mm) at a flow rate of 18 mL/min. All runs used 0.1 % TFA (trifluoroacetic acid) in water (solvent A) and 90 % acetonitrile in water with 0.1 % TFA (solvent B). Peptides were analyzed on gradients from 0-50% B in 30 min unless otherwise stated. Electrospray ionization mass spectrometric analysis (ESI-MS) was performed on a Bruker Daltonics MicroTOF-Q II mass spectrometer. Native gel electrophoresis was performed using Bio-rad 5% TBE gels and stained with Syber Gold (Invitrogen).

General Procedures for PNA Synthesis

Compounds **1**, **sc1** and **sc3** were synthesized manually via Boc SPPS using established procedures with slight modifications.[6] To avoid aggregation of the PNA chain during assembly of the oligomer, the loading on the MBHA resin was first decreased. In a representative synthesis, 2 g of MBHA resin (initial loading 0.56 mmole/g) was reacted with 50 mg (0.28 mmole, 25% of the initial resin loading) of Boc-Gly-OH, 110 mg (0.28 mmole) HATU and 98 μL (0.56 mmole) of DIPEA for one hour at room temperature. Following this, any unreacted sites on the resin were capped with acetic anhydride (50 eq. Ac_2O, 50 eq. DIPEA) for 20 min. A quantitative ninhydrin test was performed to assess the new resin loading (three repetitions, using the average). A typical new loading of the resin was established to be 0.082 mmole/g (~ 15% of the original loading).

The PNA synthesis was then undertaken using this reduced substitution MBHA resin using the Boc/Cbz synthetic protocol (Boc as the temporary α-amine protecting group, with Cbz protection of the nucleobases). The synthetic protocol used here was based on published procedures[6] with slight modifications where highlighted. Note that between each step the resin was washed twice with DMF and once with DCM (flow washes, 5 mL for 10 sec.). Removal of the Boc group was accomplished with 95% TFA/5% *m*-cresol (a scavenger is recommended to prevent alkylation of the nucleobases). After

deprotection, the resin was washed twice with 5% pyridine/DMF for 1 min to neutralize the resin. Coupling was performed using 5 eq. of monomer, 5 eq. of HATU, 5 eq. HOAt and 10 eq. of DIPEA with a 5 min. preactivation. After every coupling step, capping was performed using 20 eq. acetic anhydride and 20 eq. of DIPEA in DMF for 15 min. When appropriate, the Fmoc group was removed using 20% piperidine in DMF. Deprotection of the alloc group on lysine side chains was accomplished with 0.5 eq. Pd (tetrakistriphenylphosphine) in the presence of 50 eq. of phenylsilane in DCM (the alloc deprotection was repeated three times). PNA compounds were cleaved using 6:1:1:2 TFA/*m*-cresol/thioanisole/trifluoromethane sulfonic acid for two hours. The crude products were then precipitated in cold ether, pelleted via centrifugation, and the supernatant was removed. After dissolution of the product in H_2O/ACN and lyophilization, the crude material was purified by either semi-prep or prep RP-HPLC, and the desired fractions pooled. PNAs were then used in ligations with histone peptides as detailed below. All purified PNAs were dissolved in H_2O for subsequent use in assays.

The concentration of the purified PNAs was assessed in two ways, and the average of the two values was used. First, UV-Vis measurement using ε = 4.0*10^4 $mole^{-1}cm^{-1}$ at 495 nm was taken (the absorbance of the fluorescein moiety) and the concentration was calculated. Next, the absorbance was measured at 254 nm and the concentration was determined using ε = 152 $mM^{-1}cm^{-1}$, the appropriate extinction coefficient for a PNA with the designated composition.[4] The two concentration values were then averaged to arrive at a working concentration of the stock solution. Note: the above concentrations were typically within 15-20% of each other.

Prior to the use of PNAs in a binding or methyltransferase assay, the working PNA solution was heated to 50^{o} C for 10 min, and then allowed to cool to room temperature before use. This is a recommended procedure to prevent aggregation of PNAs.

General Procedures for Histone Peptide Synthesis

Peptides **2**, **5** and **sc2** were synthesized on the 2-chloro-trityl chloride resin to give a *C*-terminal hydrazide which was used as a masked thioester.[7] Peptides were either synthesized using manual addition of the reagents (using a stream of dry N_2 to agitate the reaction mixture) or on a Liberty Peptide Synthesizer equipped with a Discovery microwave module (CEM, Matthews, NC).

In a representative synthesis, 250 mg of trityl resin (substitution: 1.2 mmole/g, 300 μmoles) was weighed into a polypropylene reaction vessel, and swelled in DMF for 20 minutes. A 20-fold excess (relative to resin loading) of hydrazine monohydrate (240 uL) was added to 4 mL of DMF. The reaction was then bubbled with N_2 at room temperature for one hour. The resin was subsequently washed three times with DMF and the reaction repeated with a fresh aliquot of hydrazine to ensure quantitative loading. Conventional Fmoc solid phase synthesis was then used to assemble the peptide chain using the methods outlined below. For acyl hydrazide peptides, the first residue was coupled manually irrespective if manual or machine peptide synthesis was used for the remaining residues.

For manual peptide synthesis, a typical procedure is given here. The Fmoc group was removed with 3 mL of 20% piperidine in DMF and performed twice (one deprotection for 30 sec followed by an additional deprotection for 15 min). Between each deprotection step, as well as all subsequent synthetic steps, flow

washes were used (3 x 5 sec. with DMF). Coupling was performed using 4 eq. of monomer, 4 eq. of either HATU or HBTU and 8 eq. of DIPEA with no pre-activation. Double couplings were used for all residues to ensure complete acylation. For *N*-terminal acetylations (when appropriate), the deprotected *N*-terminal amine was treated with 15 eq. of acetic anhydride in the presence of 10 eq of DIPEA in DMF, and the reaction was allowed to proceed for 20 min. Cleavages were performed with 95% TFA, 2.5% TIS and 2.5% H_2O. The peptide was then precipitated with diethyl ether, dissolved in water with 0.1% TFA and analyzed via RP-HPLC. Semi-prep or preparative RP-HPLC purification was then used to isolate the peptide of interest.

Purified peptides were then dissolved in water for methyltransferase assays (when needed) and the concentration was determined using the UV absorbance of the included tyrosine residue (ε_{280nm}=1490 $M^{-1}cm^{-1}$).

General Method for the Ligation of Peptide Acyl Hydrazides

The ligation method of Fang *et al.* (ref [8]) was used with slight modifications. The peptide acyl-hydrazide (typically ~2 mM) to be activated as a thioester was dissolved in oxidation buffer (20 mM $NaNO_2$, 6 M GnHCl, 0.2 M phosphate buffer, pH = 3) and allowed to react for 30 minutes at 0° C. To form the *in situ* thioester, an equal volume (relative to the oxidation reaction) of 100 mM MESNa, 200 mM thiophenol, 6 M GnHCl, 0.2 M phosphate buffer pH = 7 was added to the oxidation reaction. The pH was then adjusted with NaOH to give a final pH = 7.5 and the reaction mixture was allowed to stand at room temperature for 15 minutes to ensure complete conversion to the peptide thioester. Finally, the thioester reaction was added to the cysteine containing PNA compound (final concentration of each ligation fragment was typically ~ 1 mM). The pH was adjusted to 7.5, and the reaction was allowed to proceed for 3-16 hours at RT. After one hour of reaction time, TCEP was added to a final concentration of 20 mM and additional aliquots of TCEP were added as required to maintain complete reduction of thiols. During the ligation, a pH ~7.5 was always maintained.

Compound 1-8 Analytical Data

Compound 1. The PNA targeting vector was synthesized according to the general procedure for PNA synthesis. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-50% B, t_r = 16.4 min. Expected Mass: 6616.8 Da. Found: 6616.9 Da. Yield after purification: 28%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

Compound 2. Histone peptide H3.3 21-37 K27me_3. Sequence: Ac-ATKAARKme_3SAPSTGGVKK-GYK(Alloc)-$NHNH_2$. Note the C-terminal GYK(Alloc) was appended to the histone sequence, with the tyrosine for UV quantitation and the K(Alloc) for further functionalization if needed. The peptide was synthesized via the general method for peptide synthesis outline above. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-50% B, t_r = 13.9 min. Expected Mass: 2188.3 Da. Found: 2188.3 Da.

Yield after purification: 48%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

Compound 3. This PNA construct was synthesized by ligation of compounds **1** and **2**, using the general method for the ligation of peptide hydrazides. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-50% B, t_r = 17.2 min. Expected Mass: 8776.0 Da. Found: 8776.0 Da. Yield after purification: 24%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

Compound 4. This PNA construct was synthesized by ligation of compounds **2** and **sc1** (see below), using the general method for the ligation of peptide hydrazides. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-50% B, t_r = 18.5 min. Expected Mass: 9412.4 Da. Found: 9412.9 Da. Yield after purification: 38%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

Compound 5. Histone peptide H3.3 21-37 K27Aha (*S*-aminoheptanoic acid). Fmoc-*S*-Aminoheptanoic acid was prepared as described previously.[5] Sequence: Ac-ATKAARAhaSAPSTGGVKK-GYK(Alloc)-$NHNH_2$. Note the C-terminal GYK(Alloc) was appended to the histone sequence, with the tyrosine for UV quantitation and the K(Alloc) for further functionalization if needed. The peptide was synthesized via the general method for peptide synthesis outline above. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-50% B, t_r = 17.3 min. Expected Mass: 2145.2 Da. Found: 2145.3 Da. Yield after purification: 49%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

Compound 6. This PNA construct was synthesized by ligation of compounds **1** and **5**, using the general method for the ligation of peptide hydrazides. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-50% B, t_r = 18.3 min. Expected Mass: 8733.0 Da. Found: 8732.6 Da. Yield after purification: 29%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

Compound 7. This PNA construct was synthesized by ligation of compounds **1** and **sc2** (see below), using the general method for the ligation of peptide hydrazides. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-50% B, t_r = 18.1 min. Expected Mass: 8076.7 Da. Found: 8076.4 Da. Yield after purification: 32%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

Compound 8. This PNA construct was synthesized by ligation of compounds **sc2** and **sc3** (see below), using the general method for the ligation of peptide hydrazides. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-50% B, t_r = 18.0 min. Expected Mass: 8076.7 Da. Found: 8076.3 Da. Yield after purification: 19%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

Compound sc1. The PNA targeting vector for compound **4** was synthesized according to the general procedure for PNA synthesis. This PNA was identical to PNA **1**, except that a PEG spacer was included between the PNA and the histone peptide **2**. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-50% B, t_r = 19.3 min. Expected Mass: 7254.2 Da. Found: 7254.3 Da. Yield after purification: 34%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

Compound sc2. Histone Peptide: H3.1 1-14 K4me$_3$ Sequence: H-ARTKme$_3$QTARKSTGGK-NHNH$_2$.The peptide to functionalize PNA compounds **7** and **8** was synthesized according to the general procedure for histone peptide synthesis. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-25% B, t_r = 10.2 min. Expected Mass: 1545.9 Da. Found: 1545.6 Da. Yield after purification: 54%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

Compound sc3. The PNA targeting vector for compound **8** was synthesized according to the general procedure for PNA synthesis. This PNA is very similar to PNA **1**, but the fluorescein was placed on the linker and the cysteine for ligation on the N-terminus (see structure below). This PNA construct also has only a single glycine at the *C*-terminus, while PNA **1** has a Gly-Gly motif at the *C*-terminus. The crude material was purified via semi-prep scale RP-HPLC, and the desired fractions were analyzed, pooled and lyophilized. RP-HPLC characterization: gradient 0-50% B, t_r = 13.4 min. Expected Mass: 6562.8 Da. Found: 6562.7 Da. Yield after purification: 29%. See Supplemental Figure S9 for RP-HPLC chromatogram and mass spec characterization.

601 DNA (Mononucleosome) with PNA Binding Site

The modified 601 DNA sequence (designated DNA$_{target}$ below) was prepared by PCR amplification using the conventional 601 sequence as a template[9] and the primers shown below. A standard PCR amplification was run with the Phusion polymerase (NEB) using the manufacturer's suggested thermocycler program. The PCR product was isolated using a Qiagen PCR-prep kit with water as the eluent. The DNA product was then digested with EcoRV to leave blunt ends at each terminus. The digested DNA was then purified again using a PCR-prep kit, eluted with water and lyophilized. The DNA was then dissolved in a minimal amount of water for mononucleosome assembly (concentration typically 700-900 ng/μL).

The primers are shown below, with the EcoRV site in green, the PNA target in red.

Forward Primer: GCATTGGATCCGATATCCTGGAGAATCCCGGT.

Reverse Primer: GTCGTA AAGCTTGATATCAAAAAGGGGGACAGGATGTATA.

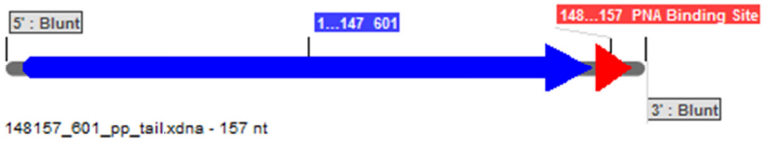


Figure S6. Schematic of the 601 MN DNA construct with the PNA binding site (called DNA_{target}).

The DNA_{target} sequence is given below, with the 601 sequence in blue, the PNA targeting site in red, and the remnants of the EcoRV restriction site in green:

```
 >EcoRV
 |
ATCCTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGC  < 75
TAGGACCTCTTAGGGCCACGGCTCCGGCGAGTTAACCAGCATCTGTCGAGATCGTGGCGAATTTGCGTGCATGCG
         10        20        30        40        50        60        70

 >601
 |
GCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGT  < 150
CGACAGGGGGCGCAAAATTGGCGGTTCCCCTAATGAGGGATCAGAGGTCCGTGCACAGTCTATATATGTAGGACA
    80        90        100       110       120       130       140

          >EcoRV
    >PNA Binding Site
    |       |
CCCCCTTTTTGAT  < 163
GGGGGAAAAACTA
         160
```

```
PNA Binding Site    : [151 : 160]
EcoRV               : [161 : 163]
EcoRV               : [1 : 3]
601                 : [4 : 150]
```

Mononucleosomes Assembly

Mononucleosomes were prepared using recombinantly produced histone proteins and either the DNA_{target} sequence (detailed above) or the conventional 147-bp nucleosome positioning sequence (Widom 601 sequence) for control experiments. The mononucleosomes were then analyzed by native gel electrophoresis (5% TBE gel, Bio-Rad) as previously described[10] followed by concentration using a 30 kD Amicon centrifugal concentrator and equilibrated with PNA buffer (10 mM MOPS pH = 7, 10 mM NaCl, 0.1 mM EDTA and 1 mM DTT).

4-mer Array (4x 601 DNA) with PNA Binding Site

A PNA binding site (highlighted in red) was inserted into a derivative of pWM530 containing 4x177 bp repeats of the 601 sequence[11] by quick change mutagenesis (Agilent) using primers GATTTCAAGCTTGGGCGTAATCAAAAAGGGGG*TTATAA*ATAGTCATAGCTGTTTCCTGTGTG and CACACAGGAAACAGCTATGACTAT*TTATAA*CCCCCTTTTTGATTACGCCCAAGCTTGAAATC. The resulting plasmid was produced in *E. coli* DH5α cells and purified using a Plasmid Giga Kit (Qiagen). The 601 repeats were excised via EcoRV/PsiI digestion and purified by PCR purification kit (Qiagen). For clarity, a map and the sequence of the 4x601 construct containing the PNA binding site are given below.

```
ATCCACGCGGTGCCCTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAA  < 75
TAGGTGCGCCACGGGACCTCTTAGGGCCACGGCTCCGGCGAGTTAACCAGCATCTGTCGAGATCGTGGCGAATTT
         10        20        30        40        50        60        70

            >601 A
            |
CGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATAT  < 150
GCGTGCATGCGCGACAGGGGGCGCAAAATTGGCGGTTCCCCTAATGAGGGATCAGAGGTCCGTGCACAGTCTATA
    80        90        100       110       120       130       140

                          >NheI
                 >ScaI_A
                 |        |
ATACATCCTGTGCATGAGTACTGAGCTCGCTAGCGGCCGCCCTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGG  < 225
TATGTAGGACACGTACTCATGACTCGAGCGATCGCCGGCGGGACCTCTTAGGGCCACGGCTCCGGCGAGTTAACC
         160       170       180       190       200       210       220

                                    >601 B
                                    |
TCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTC  < 300
AGCATCTGTCGAGATCGTGGCGAATTTGCGTGCATGCGCGACAGGGGGCGCAAAATTGGCGGTTCCCCTAATGAG
    230       240       250       260       270       280       290

                                              >ScaI_B
                                              |
CCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGTGCATGTATTGAAGTACTTCTAGCGGCCGCCCTGGAGA  < 375
GGATCAGAGGTCCGTGCACAGTCTATATATGTAGGACACGTACATAACTTCATGAAGATCGCCGGCGGGACCTCT
         310       320       330       340       350       360       370

                                                                  >601 C
                                                                  |
ATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCGCTTAAACGCACGTACGCGCTGTCCCCC  < 450
TAGGGCCACGGCTCCGGCGAGTTAACCAGCATCTGTCGAGATCGTGGCGAATTTGCGTGCATGCGCGACAGGGGG
    380       390       400       410       420       430       440


GCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCTGTGCATGTATTG  < 525
CGCAAAATTGGCGGTTCCCCTAATGAGGGATCAGAGGTCCGTGCACAGTCTATATATGTAGGACACGTACATAAC
         460       470       480       490       500       510       520

            >XbaI
   >ScaI_C
   |     |
AAGTACTTCTAGAGGCCGCCCTGGAGAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAGCACCG  < 600
TTCATGAAGATCTCCGGCGGGACCTCTTAGGGCCACGGCTCCGGCGAGTTAACCAGCATCTGTCGAGATCGTGGC
    530       540       550       560       570       580       590

                 >601 D
                 |
CTTAAACGCACGTACGCGCTGTCCCCCGCGTTTTAACCGCCAAGGGGATTACTCCCTAGTCTCCAGGCACGTGTC  < 675
GAATTTGCGTGCATGCGCGACAGGGGGCGCAAAATTGGCGGTTCCCCTAATGAGGGATCAGAGGTCCGTGCACAG
         610       620       630       640       650       660       670
```

```
                                                                >PNA Binding
                                                                |
AGATATATACATCCTGTGCATGTACGAAGAGACCGATTTCAAGCTTGGGCGTAATCAAAAAGGGGGTTA  < 744
TCTATATATGTAGGACACGTACATGCTTCTCTGGCTAAAGTTCGAACCCGCATTAGTTTTTCCCCCAAT
    680       690       700       710       720       730       740
```

```
Features :
601 A                                : [15 : 161]
601 B                                : [192 : 338]
601 C                                : [369 : 515]
601 D                                : [546 : 692]
PNA Binding                          : [732 : 741]
NheI                                 : [179 : 184]
XbaI                                 : [533 : 538]
ScaI_A                               : [167 : 172]
ScaI_B                               : [350 : 355]
ScaI_C                               : [527 : 532]
```

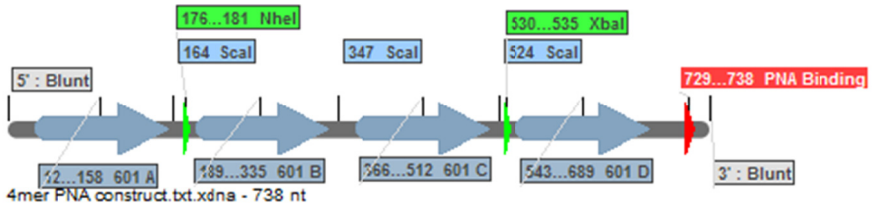


Figure S7. Graphic map of the 4x601 array construct used in this study. The four 601 sites are labeled in gray, with the various restriction sites in blue or green. The PNA binding site is designated in red at the terminus.

4-mer arrays were assembled using previously published procedures, and used recombinantly produced histone proteins[12] [13]. The arrays were then analyzed by native gel electrophoresis (5% TBE gel, Bio-Rad) and stained with Syber Gold. Crude arrays were purified by sucrose gradient ultracentrifugation (gradient 5-40%, 35000k, 13 hours) to yield purified 4mers as judged by native gel electrophoresis. Desired fractions were then pooled and concentrated using a 30 kD Amicon centrifugal concentrator with PNA buffer.

Production and Purification of PRC2

PRC2 was isolated from HeLa cells that express either FLAG tagged-EZH2 or FLAG-tagged EED as previously described.[5] Briefly, complexes were purified by M2 FLAG affinity chromatography followed by ion exchange using a Mono Q anion exchange column and the characterization was consistent with previous complexes. The PRC2 complex used in this study contained the following subunits: EZH2, SUZ12, AEBP2, EED, and RbAp46/48.

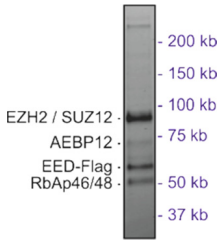


Figure S8. Characterization of PRC2 by denaturing gel electrophoresis and silver staining to show purity and complex composition.

Mononucleosome Binding

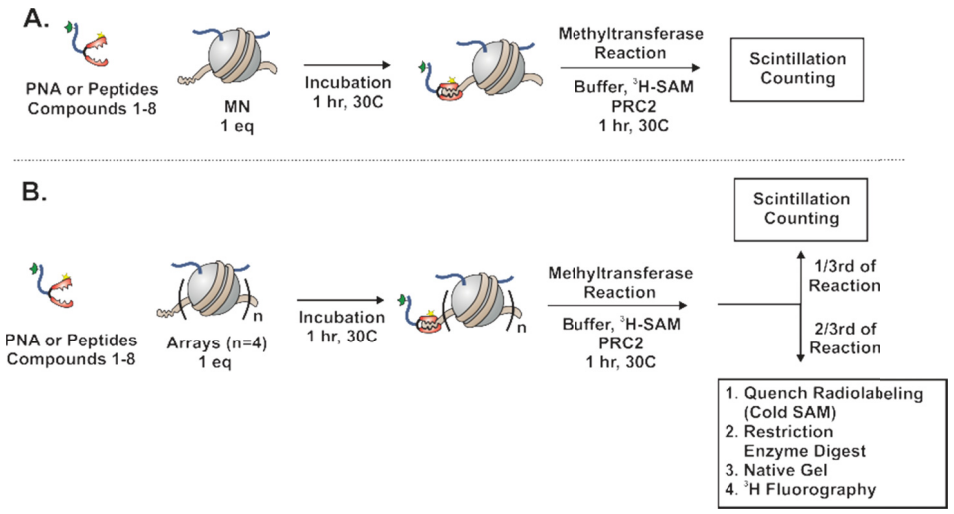
In a typical experiment, 5 eq. of PNA compound (250 nM, unless otherwise stated) and 1 eq. of MN (50 nM, unless otherwise stated) which contained the DNA_{target} were combined in PNA buffer (10 mM MOPS pH = 7, 10 mM NaCl, 0.1 mM EDTA and 1 mM Dtt). This mixture was incubated for one hour at 30°C, followed by native gel electrophoresis and visualization with Syber Gold stain.

Array Binding/Digestion

In a typical binding experiment, 20 nM arrays (80 nM total '601' sites, 20 nM PNA binding site) were incubated with 100 nM PNA/peptide at 30 °C for 60 min. in PNA buffer. Following this, 1 μL of the appropriate restriction enzyme was added (NheI and XbaI) to digest the 4-mer arrays into the various nucleosomal species. These were then analyzed by native gel electrophoresis and visualization with Syber Gold stain.

Methyltransferase Assays: Scintillation

Scheme S1 shows a diagram of the workflow for the methyltransferase reactions. Prior to the PRC2 assays, the MNs or arrays were incubated with PNA or peptides to form the desired complex for 1hr at 30° C. Following this, all other components were added, and the reaction was initiated by adding PRC2 enzyme last.



Scheme S1. Workflow of the methyltransferase reactions for (**A**) mononucleosomes and (**B**) 4-mer arrays.

For MN assays, a typical experiment is described here. 50 nM mononucleosomes were preincubated with 250 nM PNA or peptide (unless otherwise noted). Next, approximately 10 ng of PRC2, 1 μM ^{3}H-SAM (Perkin Elmer, 55-85 Ci/mmole), 1 mM PMSF and 1x PRC2 buffer (PRC2 buffer: 50 mM HEPES pH = 7.9, 2 mM MgCl2, 1mM DTT and 0.01% Tween) was added for a total reaction volume of 15 μL. The reaction mixture was incubated at 30°C for 60 min then spotted on a phosphocellulose filter disc (Whatman p81). The disc was dried for 60 min. at room temperature, washed 3x with 0.2 M $NaHCO_3$ pH=9, and subsequently dried for 60 min. at 40°C on a gel-dryer. Counting was then performed in 1 mL Ultima Gold (Perkin Elmer) using a Microbeta2 scintillation counter. All counts were corrected for background using a reaction in the absence of substrate. Data shown is the average of at least three independent experiments.

A similar procedure for the arrays-based assays was used with slight modifications. Unless otherwise stated, 20 nM arrays (80 nM total '601' sites, 20 nM PNA binding site) were preincubated with 100 nM PNA/peptide at 30°C for 60 min. Because arrays are a better substrate than MN, only ~ 5 ng of PRC2 was used for a total volume of 15 μL. The reaction mixture was incubated at 30°C for 60 min then spotted phosphocellulose filter disc (Whatman p81). The disc was dried for 60 min at room temperature, washed 3x with 0.2 M $NaHCO_3$ pH=9, and subsequently dried for 60 min at 40°C on a gel-dryer. Counting was then performed in 1 mL Ultima Gold (Perkin Elmer) using a Microbeta2 scintillation counter. All counts were corrected for background using a reaction in the absence of substrate. Data shown is the average of at least three independent experiments.

Note that for all inhibition assays, peptide **2** (H3.3 21-37 K27me_3) was added to a final concentration of 25 μM in order to increase PRC2 activity.

Fluorography

As shown in Scheme S1, the PRC2 reactions of the 4-mer arrays were also analyzed by ^{3}H-fluorography. After incubation as detailed above, the radiolabeling was stopped by the addition of >500-fold excess of non-radiolabeled SAM. 1 μL of the appropriate restriction enzyme was added and the reaction was placed at 37^{o}C for one hour. Native gel electrophoresis was used to separate the different species of nucleosomes, and the gel was stained and imaged with Syber Gold (Invitrogen). The gel was then incubated with Amplify (GE Healthcare) according to the manufacturer's instructions, dried overnight, placed in a cassette with autoradiography film (Carestream BioMax XAR, Kodak) and incubated at -80^{o}C for 1-4 days.

The bands were quantified using densitometry (ImageJ, NIH) and the total densitometry for the "array only" experiment was set to 1 for each independent set of assays. All other fluorographic signals were then normalized to this value for the histogram plot seen in Figure 3 of the manuscript.

Supporting Information References

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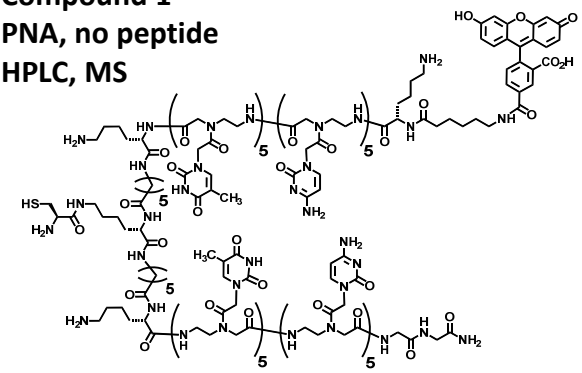
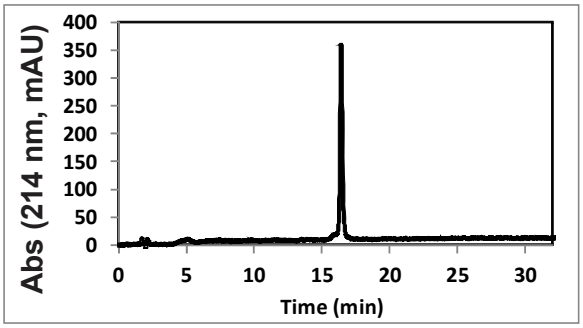
[13] K. Luger, T. J. Rechsteiner, T. J. Richmond, *Methods Mol Biol* **1999**, *119*, 1-16.

Figure **S9**. HPLC and MS data for compounds **1**-**8** and supplemental compounds

Compound 1
PNA, no peptide
HPLC, MS





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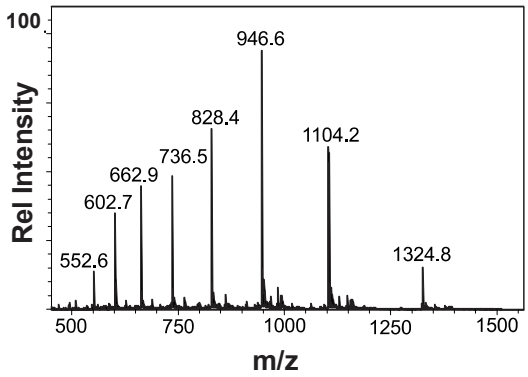
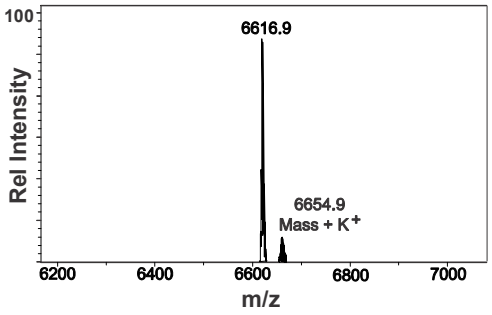
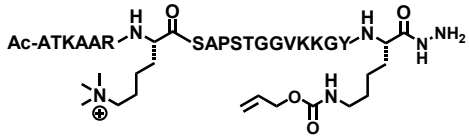
MW_{OBS} = 6616.9 Da

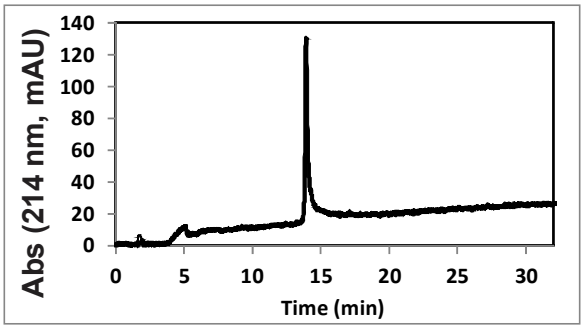


Deconvoluted Mass



Compound 2
H3.3 21-37 K27me3
HPLC, MS





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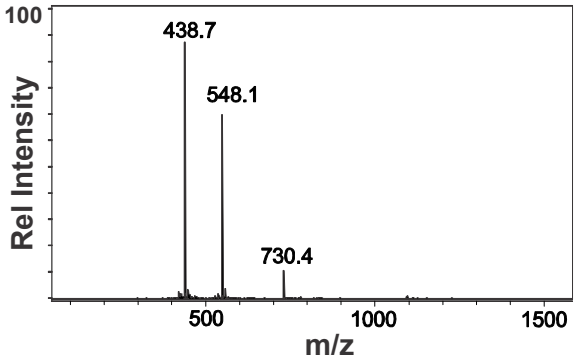

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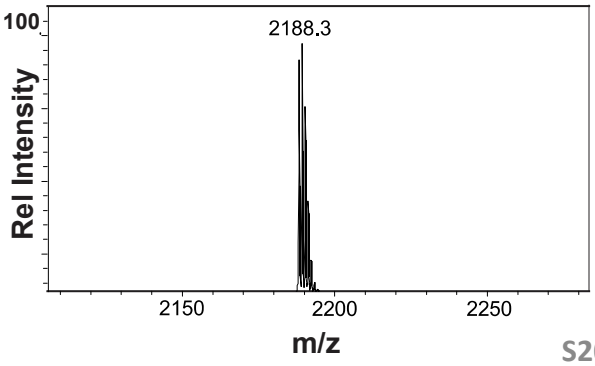
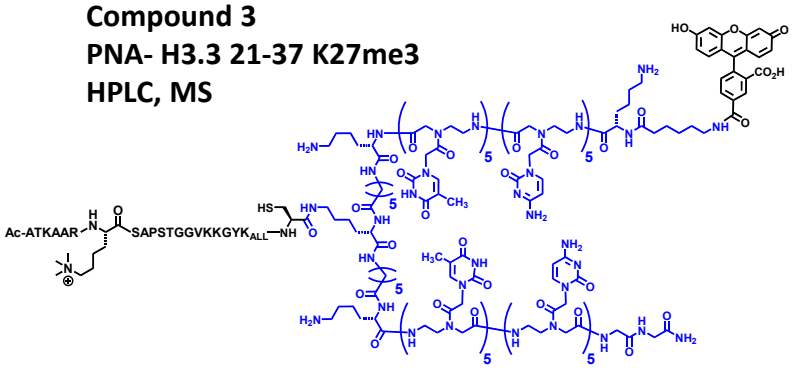
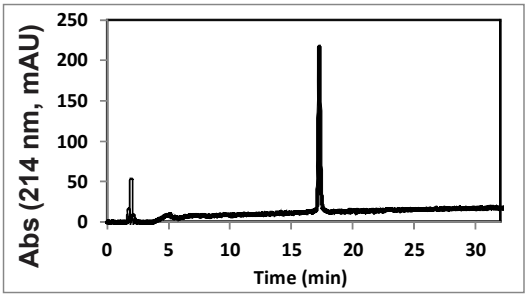


Compound 3
PNA- H3.3 21-37 K27me3
HPLC, MS





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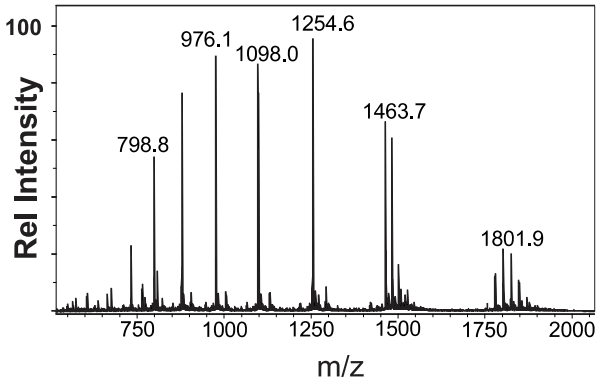
MW_{OBS} = 8776.0 Da



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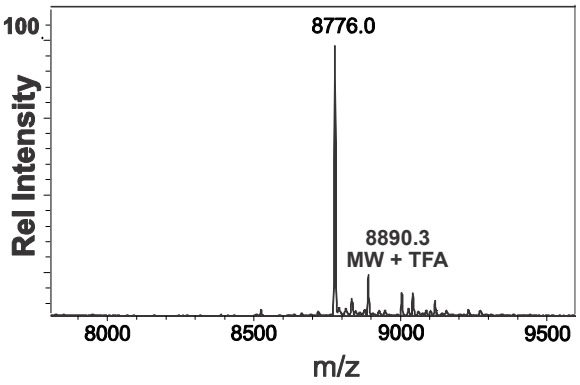
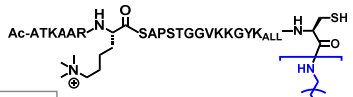
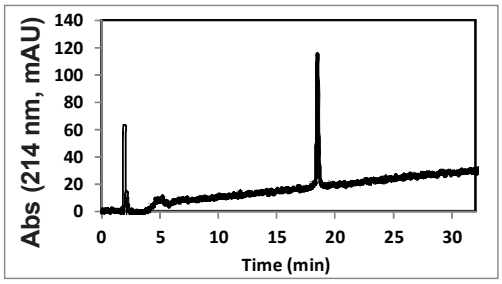
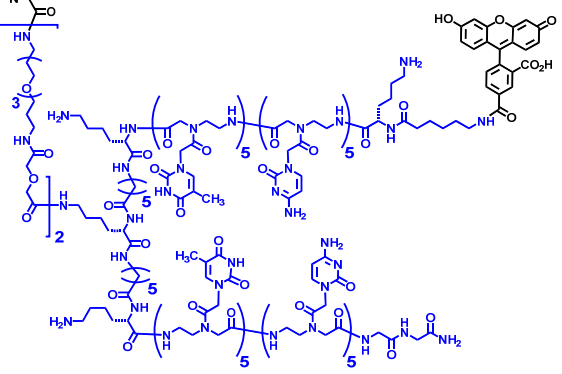


Compound 4
PNA - PEG - H3.3 21-37 K27me3
HPLC, MS







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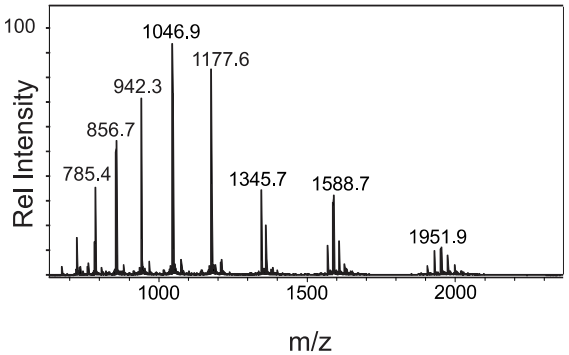
MW_{OBS} = 9412.9 Da



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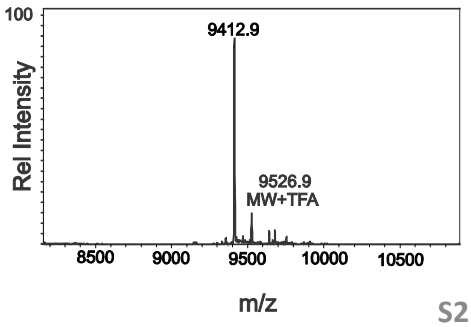
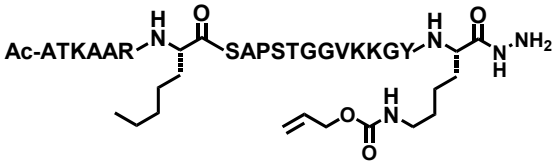
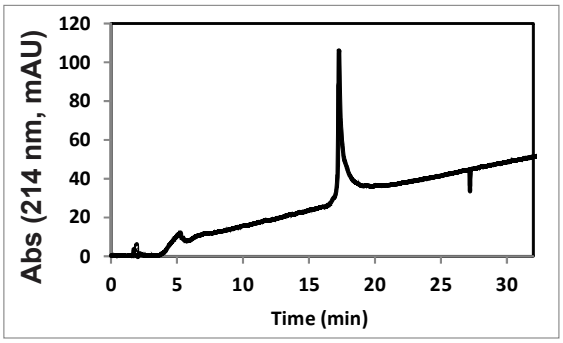


Compound 5
H3.3 21-37 Aha
HPLC, MS





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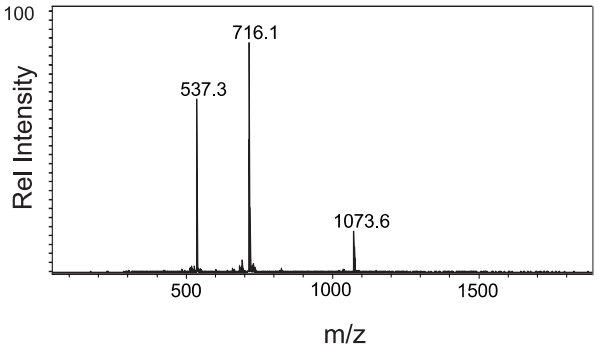

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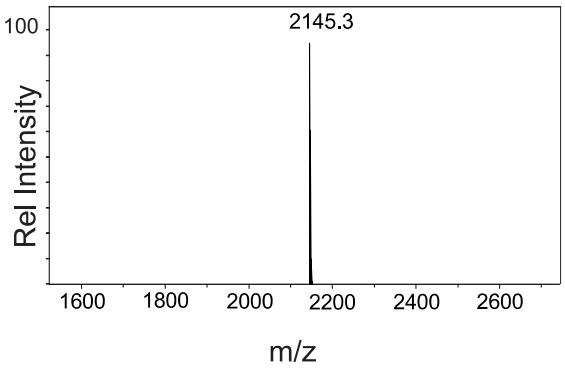
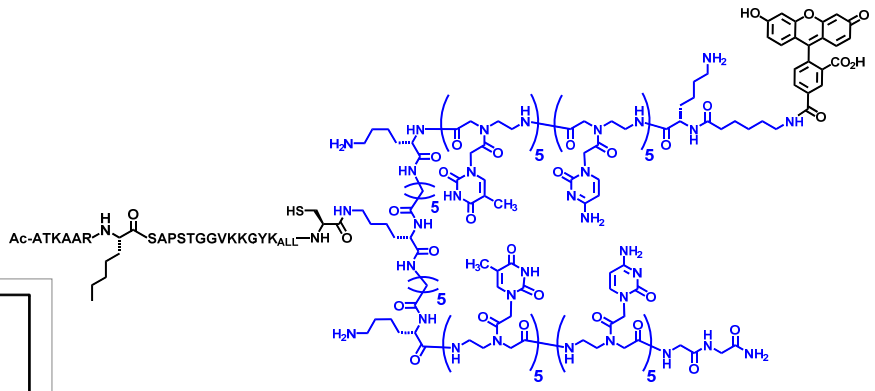
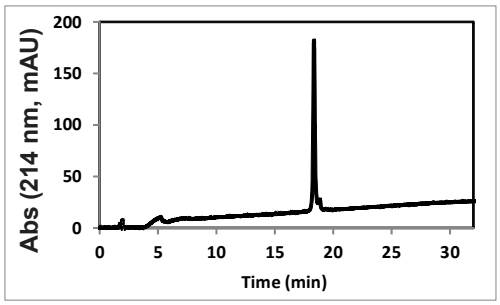


Compound 6
PNA - H3.3 21-37 Aha
HPLC, MS





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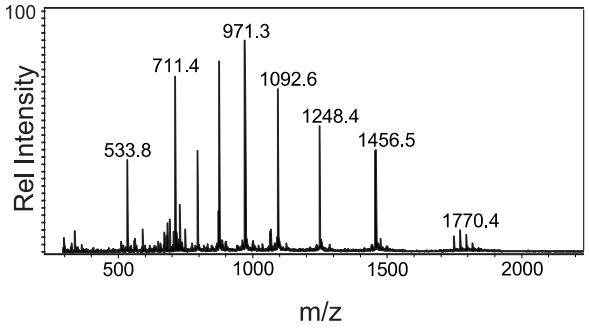

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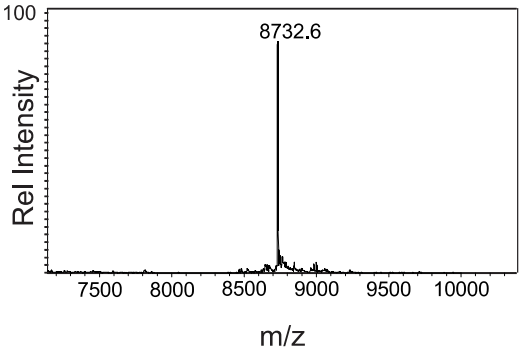
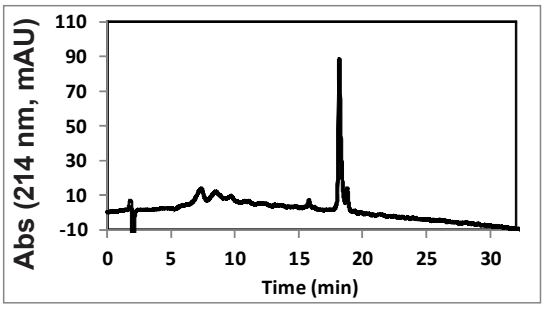
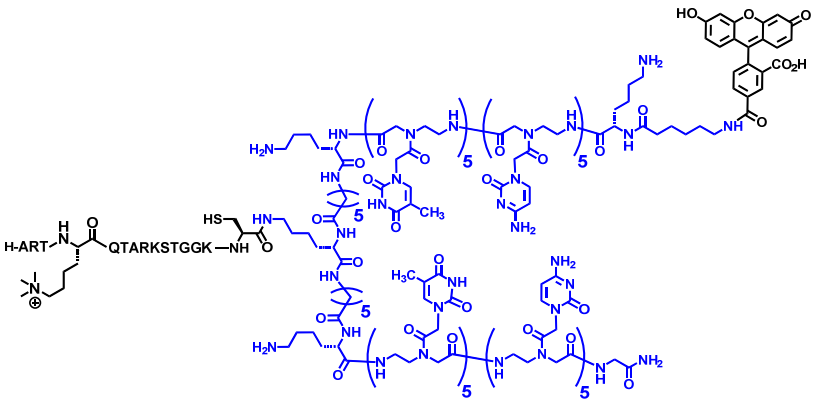
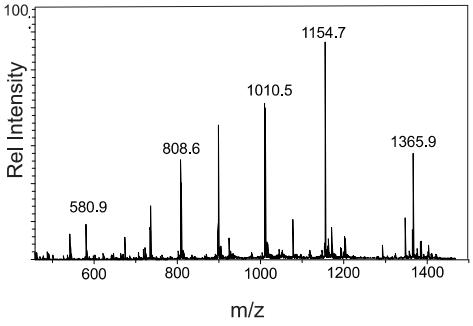


Compound 7
PNA - H3.1 1-14 K4me3
HPLC, MS





MW_{THEOR} = 8076.7 Da
MW_{OBS} = 8076.4 Da



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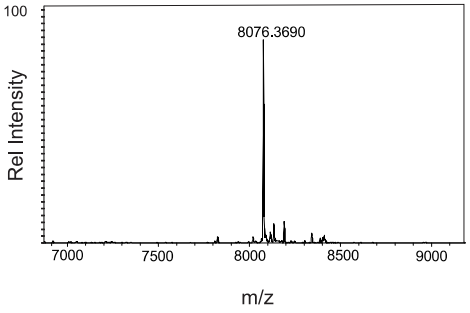
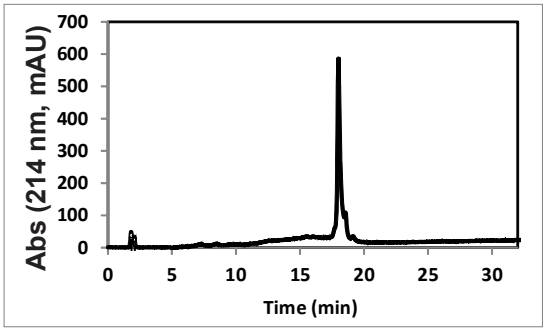
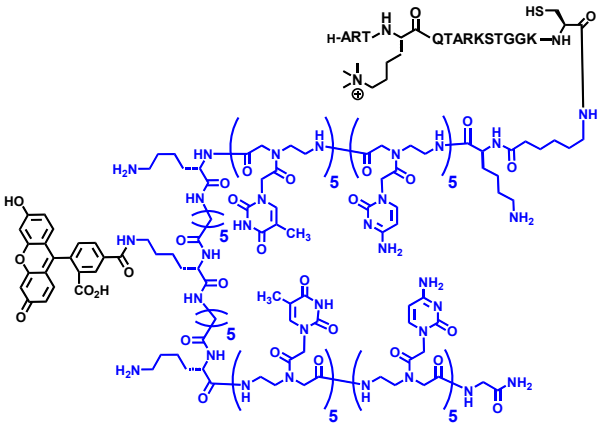


Compound 8
PNA - H3.1 1-14 K4me3 (isomer)
HPLC, MS





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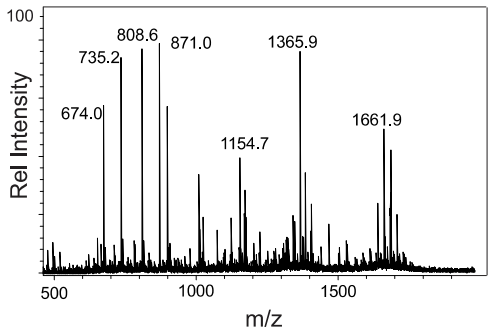
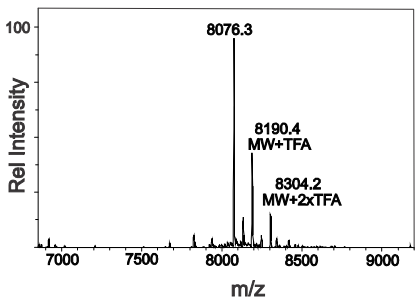
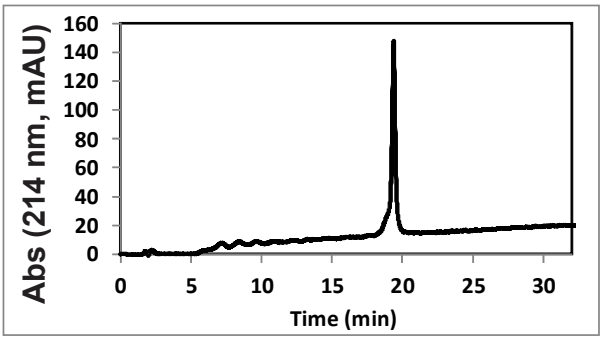
MW_{OBS} = 8076.3 Da

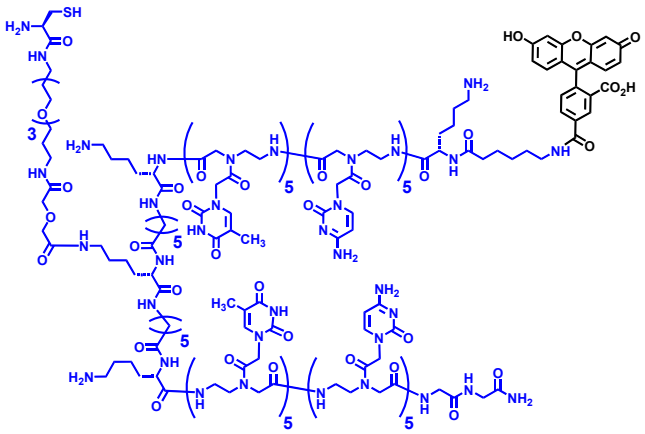


Deconvoluted Mass

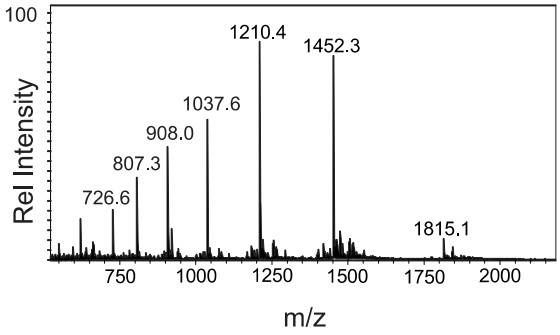


Compound sc1
PNA - PEG
HPLC, MS

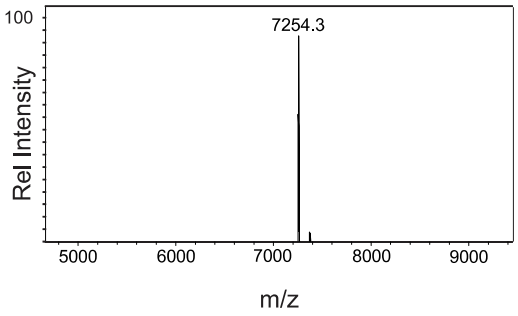




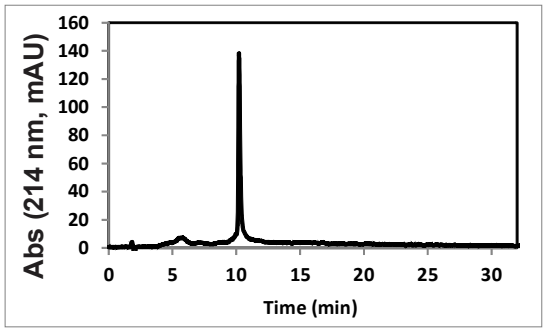
MW_{THEOR} = 7254.2 Da
MW_{OBS} = 7254.3 Da

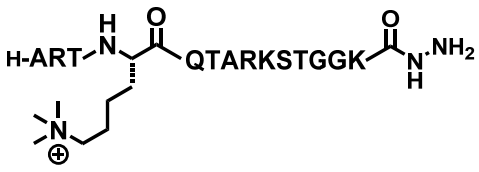


Deconvoluted Mass

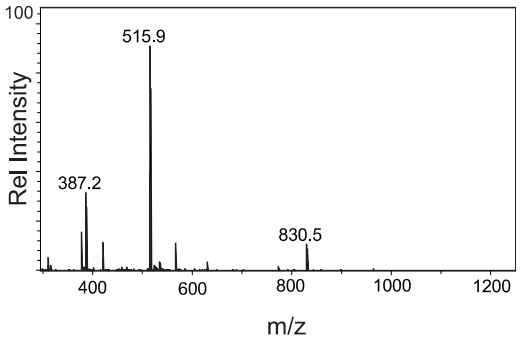


Compound sc2
H3.1 1-14 K4me3
HPLC, MS

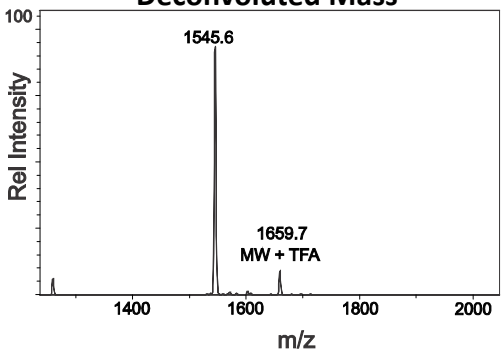




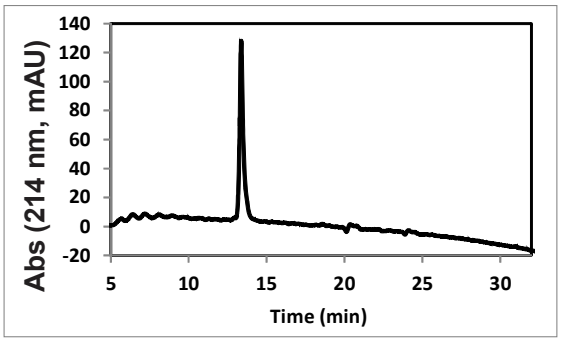
MW_{THEOR} = 1545.9 Da
MW_{OBS} = 1545.6 Da

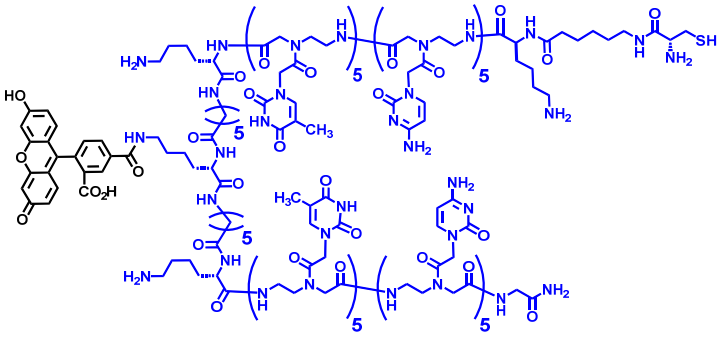


Deconvoluted Mass

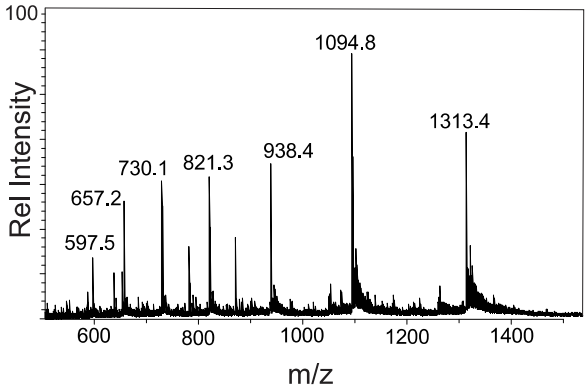


Compound sc3
PNA (isomer)
HPLC, MS





MW_{THEOR} = 6562.8 Da
MW_{OBS} = 6562.7 Da



Deconvoluted Mass