Supporting Information: Rapid histone catalyzed DNA lesion excision and accompanying protein modification in nucleosomes and nucleosome core particles.

Liwei Weng and Marc M. Greenberg*

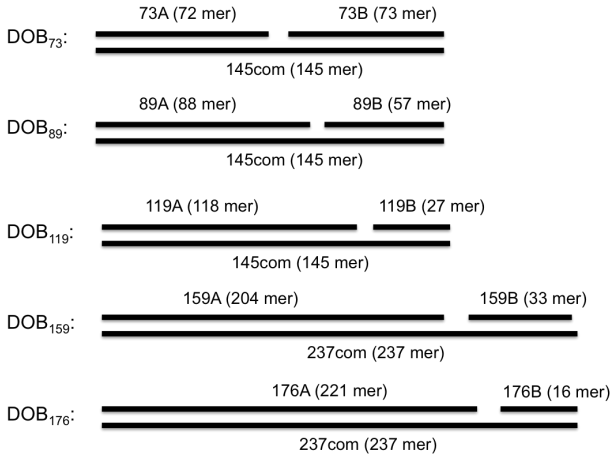
Department of Chemistry
Johns Hopkins University
3400 N. Charles St.
Baltimore, MD 21218

mgreenberg@jhu.edu; phone: 410-516-8095

Contents:

General Methods. Oligonucleotides were synthesized on an Applied Biosystems Incorporated 394 oligonucleotide synthesizer. DNA synthesis reagents were purchased from Glen Research (Sterling, VA). Oligonucleotides containing DOB precursor (**2**) and AP precursor were synthesized and purified as previously described.[1,2] ESI-MS analysis was carried out on a LCQ-Deca Ion Trap. MALDI-TOF mass analysis was carried out on Bruker AutoFlexIII MALDI-TOF instrument. UPLC/MS analysis was carried out on an Acquity UPLC H-Class/Xevo G2 QTof from Waters equipped with analysis software MassLynx and Biopharmalyn. Reverse-phase HPLC separation of histone proteins was carried out by Waters 515 HPLC Pump equipped with 2487 Dual l Absorbance Detector. All oligonucleotides were precipitated from 1.25 M ammonium acetate (pH 5.6) prior to the ESI mass analysis. Expression and purification of core histone proteins, as well as refolding and purification of the histone octamer, were carried out as previously described.[3] The plasmids used for expression of WT histone proteins were a generous gift from Prof. Gregory Bowman (Department of Biophysics, Johns Hopkins University). T4 polynucleotide kinase, T4 DNA ligase, AccI, MseI, MspI and MluCI were purchased from New England Biolabs (NEB). Benzonase was from Sigma and was dissolved in water (1 U/mL). γ-^{32}P-ATP and α-^{32}P-cordycepin 5'-triphosphate were purchased from Perkin Elmer. C_{18}-Sep-Pak cartridges were obtained from Waters. Amicon® Ultra Centrifugal Filters with different MWCO were purchased from Millipore. Quantification of radiolabeled oligonucleotides was carried out using a Molecular Dynamics Phosphorimager equipped with ImageQuant Version TL software. The concentration of ODNs were determined by the absorption at 260 nm using a Beckman DU® 640 Spectrophotometer. Salmon sperm DNA (10 mg/mL) was purchased from Invitrogen. All experiments with NCPs were conducted in clear siliconized tubes (Bio Plas Incorporated). Photolyses of oligonucleotides were carried out in a Rayonet photoreactor (RPR-100) equipped with 16 lamps with a maximum output at 350 nm.

Synthesis of oligonucleotides containing DOB precursor. Standard synthesis cycles (25 s coupling, 5 s capping with acetic anhydride, 15 s oxidation with 0.02 M iodine in tetrahydrofuran/pyridine/water, 95 s detritylation with 3% TCA in methylene chloride) were used prior to the incorporation of the lesion precursor phosphoramidite.[1] The phosphoramidite of the lesion was incorporated with 15 min coupling, 25 s capping, 40 s oxidation using the same synthesis reagents. The completed oligonucleotides were deprotected with a mixture of 1: 1 conc. aq. ammonia and methylamine (40% aqueous solution)[4] at 65 °C for 1 h and were purified by 20% denaturing PAGE (acrylamide/bisacrylamide; 19:1, 45% urea).



General procedure for ligating long DNA strands without DOB precursor from short oligonucleotides (73A, 89A, 119A, 159A, 176A, 145com, and 237com). Chemically synthesized DNA oligonucleotides (typically 1.5-2 nmol, sequences shown in Figure S2) were enzymatically phosphorylated at their 5′-termini, each in separate 100 μL reactions containing 1 × T4 DNA ligase buffer (50 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP), and 50 U (5 μL) of T4 polynucleotide kinase at 37 °C for 4 h (Scheme shown in Figure S3). The phosphorylated oligonucleotides were mixed with the unphosphorylated 5′-end oligonucleotides (1.5-2 nmol) and 1.2 equivalents of the corresponding splint strands and the hybridization was carried out by heating the

resulting mixture at 95 °C for 5 min followed by cooling slowly to room temperature. To the solution (200-400 μL) containing hybridized strands was added T4 DNA ligase (1600 U, 4 μL) and the ligation was incubated at 16 °C overnight. The DNA in the ligation reaction was extracted with phenol (equal volume) and precipitated from 0.3 M NaOAc pH 5.2. The ligated products were purified by 8% denaturing PAGE (acrylamide/bisacrylamide; 19:1, 45% urea) and the desired bands were excised from the gel. After eluting the DNA from the gel in 1 mL elution buffer (0.2 M NaCl and 1 mM EDTA) at r.t. overnight, the slurry was filtered using a 10 mL Polyprep column (BioRad). The solution was concentrated using *tert*-butanol to around 50 μL and the DNA was desalted by ethanol precipitation, followed by a 70% ethanol wash. The concentration of the ligated products was determined by the absorption at 260 nm.

General procedure for ligating DNA strands with precursor 2 from short oligonucleotides (73B, 89B, 119B, 159B, 176B). Chemically synthesized DNA oligonucleotides (50 pmol) were enzymatically phosphorylated by γ-^{32}P-ATP (Scheme shown in Figure S4) in a 50 μL reaction containing 1 × T4 polynucleotide kinase (PNK) buffer (70 mM Tris-HCl pH 7.6, 10 mM $MgCl_2$, 5 mM DTT), 40 μCi γ-^{32}P-ATP, and 50 U (5 μL) of T4 PNK 37 °C for 4 h. The reaction was stopped by heating at 95 °C for 5 min followed by mixing with 100 pmol of the ODNs containing DOB and 200 pmol of the corresponding splint strands. The oligonucleotides were hybridized by heating the solutions to 95 °C for 5 min followed by slowly cooling to r.t. T4 DNA ligase (800 U, 2 μL) was added to the solutions to carry out the ligation reaction. The enzymes were removed by phenol extraction, and the DNA with different lengths was separated by denaturing PAGE. The desired products were extracted and purified in the same manner as described above. The obtained internally radiolabeled strands with DOB were quantified by UV absorption at 260 nm using disposable cuvettes.

General procedure for hybridization to afford requisite ternary complexes containing 2. The

internally radiolabeled strands with DOB (typically 20-30 pmol) were mixed with 1.3 equivalents of the corresponding flanking and complementary strands. The resulting mixtures were heated to 95 °C for 5 min and slowly cooled to r.t. A portion of the hybridized solution was used for reconstitution with histone octamers to afford nucleosomes.

General procedure for reconstitution of nucleosomes. Salmon sperm DNA (10 μg) and the ternary complexes containing DOB precursor **2** (~ 1 pmol) were combined in a small siliconized tube to a final volume of 10 μL containing 2 M NaCl. The appropriate amount (84 pmol, the amount was determined based upon the amount of salmon sperm DNA added) of histone octamer (also in 2 M NaCl) was added and the sample (total volume ~12 μL) was incubated at room temperature for 30 min in a thermal cycler. The temperature in the thermal cycler was then decreased to 4 °C and the sample was incubated for an additional 30 min before beginning a series of dilutions using nucleosome buffer (10 mM HEPES, and 1 mM EDTA, pH 7.5). Dilution # (volume in μL, incubation time in minutes): 1: 12, 60; 2: 6, 60; 3: 6, 60; 4: 10, 30; 5: 10, 30; 6: 20, 30; 7: 50, 30; 8: 100, 30. After the final dilution (total volume 224 μL), the sample was incubated at 37 °C for 2-4 h and then cooled to r.t. Any precipitate was pelleted via a brief (5 min) spin at 15,000 g. The supernatant was transferred to a fresh tube and a small aliquot (5 μL) was removed and subjected to analysis by nucleoprotein gel electrophoresis (6%, acrylamide/bisacrylamide, 59:1, 0.6 × TBE buffer, run at 4 °C using 0.2 × TBE buffer) to determine the extent of reconstitution. All reconstituted nucleosome core particles were stored at 4 °C until use.

Hydroxyl radical footprinting of NCPs. Nucleosomal DNA used for hydroxyl radical footprinting analysis was 5'-^{32}P-labeled on strands 73A, 89A, 119A, 159A, 176A. Strands containing **2** were not radiolabeled. NCP solutions obtained from reconstitution were concentrated using 10K Amicon concentration device. A portion of the concentrated NCP solution (8 μL) was mixed with 10 μL of the 2 × oxidation buffer (20 mM NaCl, 20 mM sodium phosphate, pH 7.2, 2 mM sodium ascorbate, and 1

mM H_2O_2). The reaction was initiated by adding 2 μL of 1 mM EDTA and 0.5 mM $(NH_4)_2Fe(SO_4)_2$•$6H_2O$ to the mixture (total volume = 20 μL). The Fe (II) • EDTA cleavage reaction was carried out at r.t for 10 or 20 min, and the reaction was quenched by extraction of nucleosomal DNA by adding phenol (equal volume) to the reaction. Following precipitating twice from 0.3 M NaOAc, the sample was subjected to 8% denaturing PAGE analysis (acrylamide/bisacrylamide; 19:1, 45% urea).

General procedure for time course experiments monitoring the reactivity of DOB in nucleosomes. Reconstituted nucleosomes were used directly in these experiments without further purification or concentration (10 mM HEPES, 100 mM NaCl, 1 mM EDTA, pH 7.5). Nucleosomes containing DOB precursor were photolyzed for 5 min at room temperature in the cavity of a Rayonet photoreactor equipped with 16 lamps that emit maximally at 350 nm, and immediately incubated at 37 °C for the duration of the time course experiment. Aliquots were removed at appropriate times, quenched with $NaBH_4$ (final concentration is 0.1 M). The aliquot was subjected to phenol extraction. The aqueous layer was removed and precipitated twice from 0.3 M NaOAc pH 5.2. The pellet was resuspended in 1 × Cutsmart buffer (50 mM KOAc, 20 mM Tris-acetate, 10 mM $Mg(OAc)_2$, 100 mg/mL BSA, pH 7.9) and 5 U of restriction enzyme (AccI for position 89, MseI for position 73, MspI for position 119, MluCI for position 159) prior to incubating at 37 °C for overnight. The reaction was digested with proteinase K (2 mg/1mL sample) for 5 min at r.t. and analyzed by 20% denaturing PAGE (acrylamide/bisacrylamide; 19:1, 45% urea). The gel was run under limiting power (50-55 W) until the xylene cyanol band migrated 2-inch from the bottom. For reactions carried out in the presence of $NaBH_3CN$ (final concentration 0.1 M), the reductant was added before photolysis. $NaBH_3CN$ was obtained from Aldrich (Cat #156159) and used without further purification.

General procedure for time course experiments monitoring the reactivity of DOB in naked DNA.

Internally radiolabeled ternary complex DNA (~ 1 pmol) was diluted to 224 μL in the dilution buffer (10 mM HEPES, pH 7.5, 100 mM NaCl, 1 mM EDTA). The solution was photolyzed at 350 nm for 5 min at r.t. and immediately incubated at 37 °C for the duration of the time course experiment. Aliquots were removed at appropriate times and quenched with $NaBH_4$ (0.1 M). After precipitating twice from 0.3 M NaOAc, the DNA was treated with 5 U (0.5 mL) of restriction enzyme (same as described above) at 37 °C for overnight. The samples were analyzed by 20 % denaturing PAGE (acrylamide/bisacrylamide; 19:1, 45% urea). The gel was run under limiting power (50-55 W) until the xylene cyanol band migrated 2-inches from the bottom.

General procedure for detecting DPCs by SDS PAGE. Reconstituted nucleosomes containing DOB precursor were photolyzed at 350 nm for 5 min at r.t. and immediately incubated at 37 °C for the duration of the time course experiment. For samples incubated in the presence of $NaBH_3CN$ (100 mM), the reducing reagent was added prior to photolysis. Aliquots were removed at appropriate times and quenched with $NaBH_4$ (0.1 M). To the samples was directly added 4 × SDS loading buffer (400 mM Tris•HCl, 400 mM DTT, 8% SDS, 40% glycerol) and analyzed by SDS PAGE (10% resolving acrylamide/bisacrylamide = 29:1, 5% stacking layer, 20 × 16 × 0.1 cm). The gel was run at 250 V until the bromophenol blue band migrated to the bottom.

Large scale preparation of nucleosomes for mass spectrometry studies. Hybridized ternary complexes containing DOB precursor (2 nmol) were mixed with 1.0 eq. of WT histone octamer in solution containing 2 M NaCl in a dialysis button with the final concentration of DNA being between 0.4 and 0.7 mg/mL. The procedure was the same as described previously.[5] The dialysis button was capped with dialysis membrane (3500 Da MWCO) and was then immersed in pre-chilled high reconstitution buffer (0.4 L, 2 M NaCl, 10 mM HEPES, pH 7.5, 1 mM EDTA) under constant stirring in a dialysis vessel at 4 °C. Two peristaltic pumps were used to continuously replace the high

reconstitution buffer with low reconstitution buffer (2 L, 0.25 M NaCl, 10 mM HEPES, pH 7.5, 1 mM EDTA). Both peristaltic pumps were set to flow at a rate of 1.5 mL/min at 4 °C to maintain constant dialysis buffer volume in the flask where the dialysis buttons were. After 18 h, the nucleosomes were dialyzed against 400 mL of low reconstitution buffer for 3 h, followed by further dialysis against buffer (0.1 M NaCl, 10 mM HEPES, pH 7.5, 1 mM EDTA) for 3 h at 4 °C. The sample was removed from the dialysis buttons and transferred to a siliconized tube. Following incubation at 37 °C for 2 h, any precipitate was pelleted via spinning at 15,000 g for 5 min. The supernatant was then transferred to a new siliconized tube and kept at 4 °C until use. An aliquot from the solution was removed (2 μL, ~ 100 pmol) and subjected to 6% native PAGE analysis (acrylamide/bisacrylamide, 59:1, 0.6 × TBE buffer, run at 4 °C using 0.2 × TBE buffer) to determine the reconstitution yields. The gel was stained by ethdium bromide and visualized by UV light.

Determining the proteins(s) involved in cross-linking with DOB in NCPs. Following incubation of NCP containing DOB in the presence of 50 mM of $NaBH_3CN$ for optimal time intervals, the reaction was quenched by adding $NaBH_4$ (final concentration 0.1 M) and subjected to 10% native SDS PAGE analysis (acrylamide/bisacrylamide 29:1, 20 × 16 × 0.1 cm, 5% stacking layer. The gel was run at 250 V, 50 mA, 7 W until the bromophenol blue band migrated to the bottom.). The gel was soaked and shaken in ethdium bromide (1 μg/mL) and visualized by UV light. Coomassie-staining method was avoided as the visualization requires a destaining step, which eluted the DPCs from the gel. Removal of ethdium bromide is not necessary. The DPC band was cut out and subjected to in-gel Lys C or in-gel trypsin digest following acetylation, whose procedures were described below.

RP-HPLC separation of modified histone. The nucleosome (2 nmol) containing DOB precursor was incubated at at 37 ºC for 3 h following brief photolysis at 350 nm for 10 min. The nucleosomal DNA was digested by adding 1 μL (250 units) of benzonase and 1/10th volume of 10 × benzonase buffer

(500 mM Tris•HCl, pH 8.8, 100 mM $MgCl_2$, 200 mM NaCl), followed by incubation at 37 ºC for 30 min. The sample was passed through a 0.22 μm filter (Millipore) and injected into the RP column directly. The RP-HPLC separation of histones was carried out using an Aquapore RP-300, C8, 7 μM, 300 Å, 220 × 4.6 mm column (From Perkin Elmer, catalogue No. 83937256). The linear gradient is 0-5-10-20-80-100 min, B%: 0-0-35-35-45-60. Solvent A: 5% CH_3CN containing 0.1% TFA. Solvent B: 90% CH_3CN containing 0.1% TFA (V/V), flowrate: 0.8 mL/min, room temperature.[5] The retention times for H2B, H4, H2A and H3 are 65.6, 71.8, 76.6, 93.8 min, respectively (Figure S18). The histones were collected separately and lyophilized to dryness. The residue was dissolved in 50 μL water, from which 15 μL was removed and analyzed by UPLC-MS/MS.

In-gel acetylation and trypsin digestion of histone proteins. The procedure is the same as described previously.[5] (Step 1) Separation of histone proteins by SDS native PAGE. Following incubation of the NCP containing DOB at 37 ºC for 3 h, nucleosomal DNA was digested by mixing the solution with 1 μL (250 units) of benzonase and 1/10th volume of 10 × benzonase buffer (500 mM Tris•HCl, pH 8.8, 100 mM $MgCl_2$, 200 mM NaCl). The mixture was incubated at 37 ºC for 30 min prior to subjecting to 15% native SDS PAGE. The running conditions were at 250 V, 50 mA, 7 W (initial conditions) until the bromophenol blue band migrated to the bottom. After staining using Coomassie blue (50 mL of 1:3:6 (v:v:v), HOAc/iPrOH/H_2O with 0.5% (w/v) Commassie blue for 10 min) and destaining (50 mL × 3 times (20-30 min each time), 50:165:785 (v:v:v), HOAc/MeOH/H_2O), the desired histone protein (H3 or H4) band (typically 10 × 3 × 1 mm) was excised from the gel and cut into small pieces (around 1 mm^2) using a scalpel, and placed into a 0.5 mL siliconized tube. (Step 2) Destaining gel pieces. H_2O (200 μL) was added to the tube and shaken for 5 min at 25 °C. After removing the supernatant using a pipette, 50 mM NH_4HCO_3/50% acetonitrile (200 μL) was added and the mixture was shaken for 30 min. The supernatant was removed and this washing step was repeated until the gel pieces were clear.

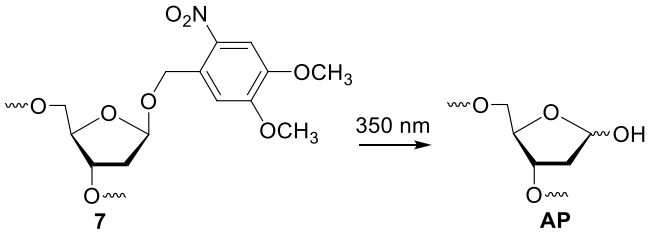
(Step 3) Dehydrating gel pieces. The gel was rinsed with 200 μL water 2 times prior to the addition of acetonitrile (100 μL). The mixture was shaken for 5 min. The supernatant was removed and the acetonitrile washing step was repeated. After removing the supernatant, the gel pieces were dried under vacuum for 5 min. (Step 4) Acylation. A mixture of acetic anhydride (5 μL) and 0.1 M NH_4HCO_3 (10 μL) was added to the gel pieces. The mixture was gently mixed using a pipette tip with the lid open. Following addition of 35 μL 0.1 M NH_4HCO_3, the pH of the mixture was adjusted to 7-8 (determined by pH paper) using concentrated NH_4OH. After incubating at 37 °C for 30 min, the supernatant was removed and the gel pieces were washed three times with water (200 μL). (Step 5) Steps 3 and 4 were repeated. (Step 6) In-gel digestion of histone. The gel pieces were hydrated in the same manner as described in step 3 and placed on ice. A mixture containing 50 mM NH_4HCO_3 (10 μL) and 0.2 μg/μL trypsin (1 μL) for each sample was prepared on ice, which was immediately added to the gel pieces. The resulting mixture was incubated on ice for 5 min, followed by the addition of 50 mM NH_4HCO_3 (40 μL). The in-gel digestion was carried out by incubating the mixture at 37 °C for 12-16 h. (Step 7) Formic acid extraction of peptides from gel. The supernatant was transferred to a clean siliconized tube. The gel pieces were washed with 50 μL of each of the following solutions in the order indicated, and the sample was shaken for 10 min at each step: 1, 50 mM NH_4HCO_3; 2, 50% acetonitrile/25 mM NH_4HCO_3; 3, 5% formic acid; 4, 50% acetonitrile/2.5% formic acid; 5, pure acetonitrile. The supernatants from each step was combined and dried under vacuum. To the residue was added 200 μL of water and the solution was dried under vacuum again. The sample was finally dissolved in 0.1% TFA (10 μL), from which 1 μL was mixed with 1 μL of α-cyano-4-hydroxycinnamic acid solution (10 mg/mL in 0.1% TFA/50% acetonitrile) and analyzed by MALDI-TOF MS. Another portion of the solution (15 μL) was analyzed by UPLC-MS/MS, which was carried out on an Acquity UPLC HSS T3 Column, 100Å (1.8 mm, 2.1 mm × 100 mm, from Waters) set at 35 ºC. Solvent A is water, solvent B is

CH_3CN, Buffer C is 1% formic acid. Gradient: 0-1-36 min, B from 5%-5%-40%, keep C at 10% constantly with flow rate of 0.3 mL / min. Mass spectra were acquired in positive ion mode with MS^E using a capillary voltage of 3 kV, a sample cone voltage of 30 V and an extraction cone voltage of 4 V. The cone gas flow was set up to 30 L/h and desolvation gas flow was 800 L/h. Desolvation temperature and source temperature were set to 400 and 150 °C, respectively. The acquisition range was m/z 100-3000. The scan acquisition rate was 10 Hz. The results were analyzed by the Biopharmalyn software.

In-gel Lys C digestion of histone proteins. The procedure was the same as in-gel trypsin digest except the acylation step was skipped. Briefly, 0.1 mg of Lys C was added to the gel following destaining and dehydrating processes and the mixture was incubated at 37 °C for 12-16 h. The digested peptide mixture was eluted as described above, from which 1 mL was mixed with 0.5 μL of α-cyano-4-hydroxycinnamic acid (CHCA) solution (10 mg/mL in 0.1% TFA/50% acetonitrile), and analyzed by MALDI-TOF MS. Another portion of the sample (15 μL) was analyzed by UPLC-MS/MS. The column and separation conditions were the same as mentioned above.

Thermolysin digestion of histone proteins in solution. Histone protein from RP-HPLC purification was mixed with thermolysin (Sigma) in a buffer containing 50 mM NH_4HCO_3 and 1 mM $CaCl_2$. The final protein concentration should be > 5 μg/100 μL and thermolysin concentration should be > 1 μg/100 μL. The thermolysin/H4 (mole:mole) ratio should be between 1/50 to 1/10. The resulting mixture was incubated at 37 °C for 4 h. The reaction was then quenched by the addition of TFA (final concentration was 1%). A portion (1 μL) of the solution was removed and mixed with 1 μL of α-CHCA solution (10 mg/mL in 0.1% TFA/50% acetonitrile), from which 1 μL was subjected to MALDI-TOF analysis. For UPLC-MS analysis, the sample was passed through 0.22 μm filter (Millipore) prior to the injection into UPLC-MS. The column used for UPLC-MS and the separation

conditions were the same as described above.



Procedure for time course experiments monitoring the reactivity of AP in nucleosomes. Strand containing AP_{176} (the same sequence as 176B, except X = **7**) was synthesized as described previously.[2] The requisite nucleosomal DNA (176A and 237com) was ligated in the same manner as described above. Strand 176A was 5'-^{32}P-labeled. Nucleosomes containing AP precursor were photolyzed at 350 nm for 10 min at r.t. and immediately incubated at 37 °C for the duration of the time course experiment. Aliquots were removed at appropriate times and quenched by the addition of $NaBH_4$ (0.1 M final concentration). The samples were analyzed by SDS PAGE (10% resolving acrylamide/bisacrylamide = 29:1, 5% stacking layer, 20 × 16 × 0.1 cm). The gel was run at 250 V until the bromophenol blue band migrated to the bottom.

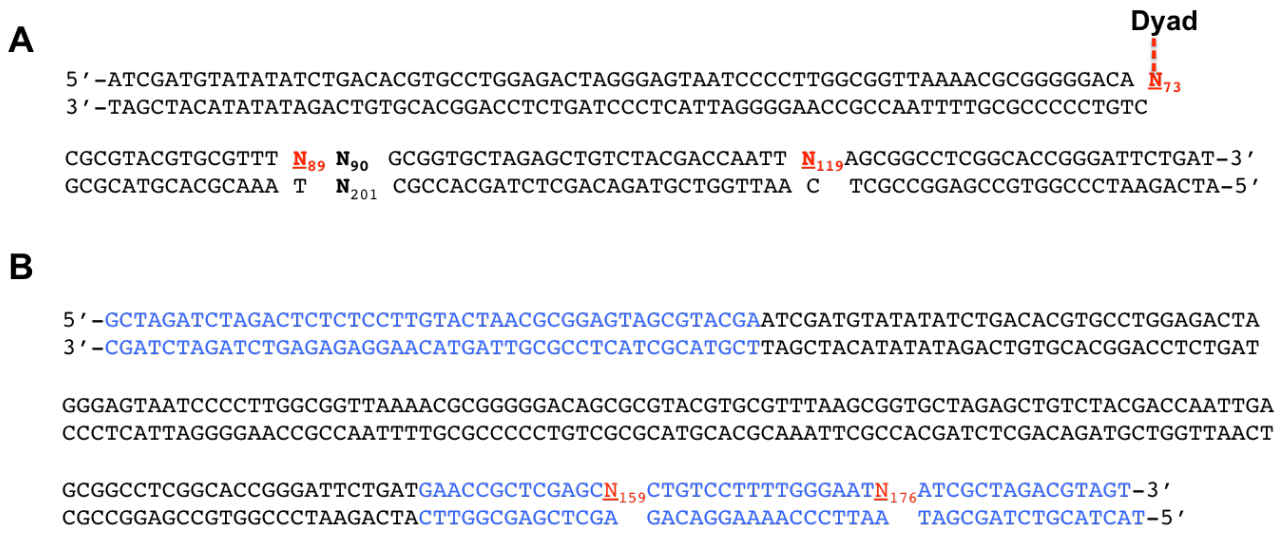


Figure S1. Sequence of nucleosomal DNA. (A) Sequence of 601 DNA; (B) sequence of 601 DNA with linker regions extended on both ends. The sites where DOB was generated are shown in red. Sequences in blue represent linker DNA.

(S1) ATC GAT GTA TAT ATC TGA CAC GTG CCT GGA

(S2) GAC TAG GGA GTA ATC CCC TTG GCG GTT AAA ACG CG

(S3) GGG GAC A

(S4) GGG GAC AGC GCG TAC GTG CGT TT

(S5) CTC CCT AGT CTC CAG GCA CG

(S6) CGC TGT CCC CCG CGT TTT AA

(S7) GGG GAC AGC GCG TAC GTG CGT TTA AGC GGT GCT AG

(S8) AGC TGT CTA CGA CCA ATT

(S9) GTA GAC AGC TCT AGC ACC GC

(S10) AGC TGT CTA CGA CCA ATT GAG CGG CCT CGG CAC CGG GAT TCT GAT

(S11) GCT AGA TCT AGA CTC TCT CCT TGT ACT AAC GCG GAG TAG CGT ACG A

(S12) ATA CAT CGA TTC GTA CGC TA

(S13) GAA CCG CTC GAG C

(S14) GAA CCG CTC GAG CTC TGT CCT TTT GGG AAT

(S15) CGA GCG GTT CAT CAG AAT CC

(S16) ATC AGA ATC CCG GTG CCG AGG CCG CTC AAT TGG TC

(S17) GTA GAC AGC TCT AGC ACC GCT TAA ACG CAC

(S18) GTA CGC GCT GTC CCC CGC GTT TTA ACC GCC AAG GG

(S19) GAT TAC TCC CTA GTC TCC AGG CAC GTG TCA GAT ATA TAC ATC GAT

(S20) AGC TGT CTA CGA CCA ATT GA

(S21) GGG GAC AGC GCG TAC GTG CGT TTA AGC GGT GCT AG

(S22) GGG AGT AAT CCC CTT GGC GG

(S23) TAC TAC GTC TAG CGA TAA TTC CCA AAA GGA CAG AGC TCG AGC GGT TC

(S24) GGA TTC TGA TGA ACC GCT CG

(S25) TCG TAC GCT ACT CCG CGT TAG TAC AAG GAG AGA GTC TAG ATC TAG C

(S26) TAG CGT ACG AAT CGA TGT AT

(S27) $\mathbf{X}_{73}$CG CGT ACG

(S28) TGC GTT TAA GCG GTG CTA G

(S29) GTA GAC AGC TCT AGC ACC GCT AAA ACG CAC GTA CGC GCT GTC CCC CGC GTT TT

(S30) $\mathbf{X}_{89}$AG CGG TGC TAG

(S31) $\mathbf{X}_{89}$TG CGG TGC TAG

(S32) $\mathbf{X}_{119}$AG CGG CCT CGG

(S33) CAC CGG GAT TCT GAT

(S34) $\mathbf{X}_{159}$CT GTC CTT

(S35) TTG GGA ATT ATC GCT AGA CGT AGT

(176B) $\mathbf{X}_{176}$AT CGC TAG ACG TAG T

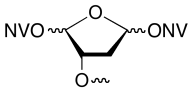
X = 

Figure S2. Oligonucleotides used for preparing nucleosomal DNA.

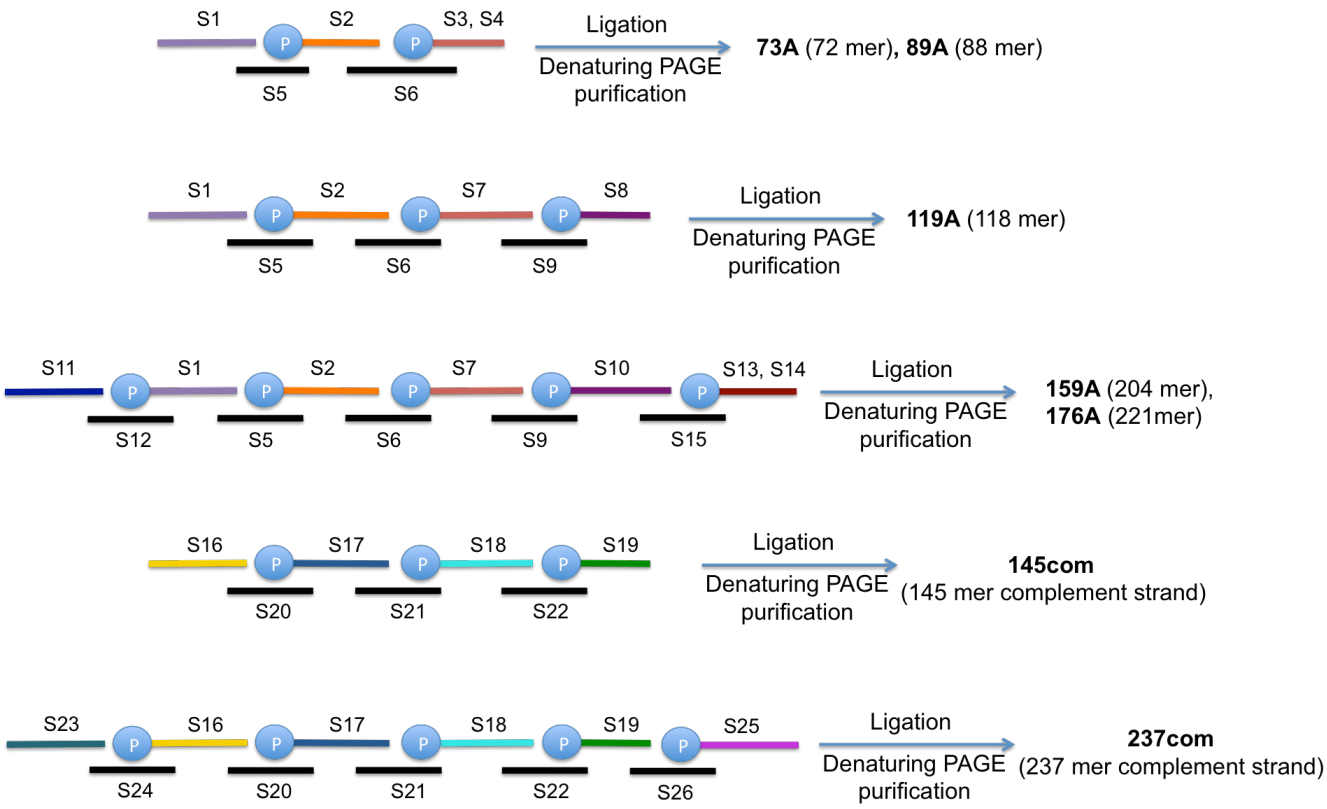


Figure S3. Ligation of regular DNA strands.

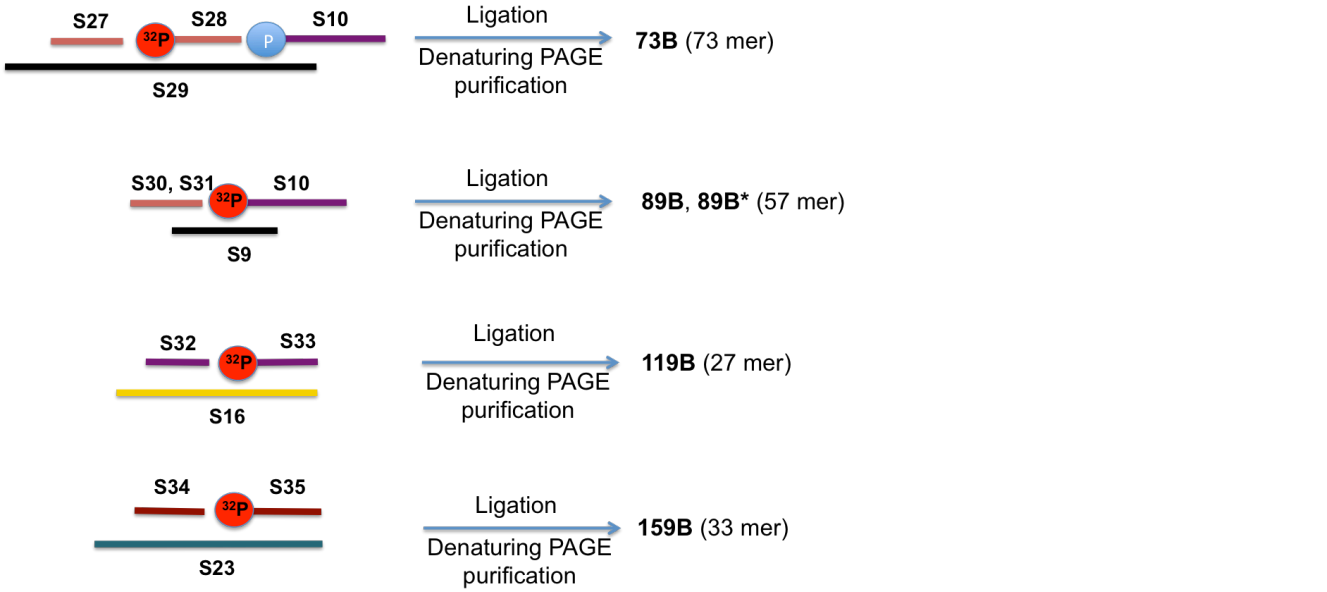


Figure S4. Preparation of internally radiolabeled strands containing DOB precursor.

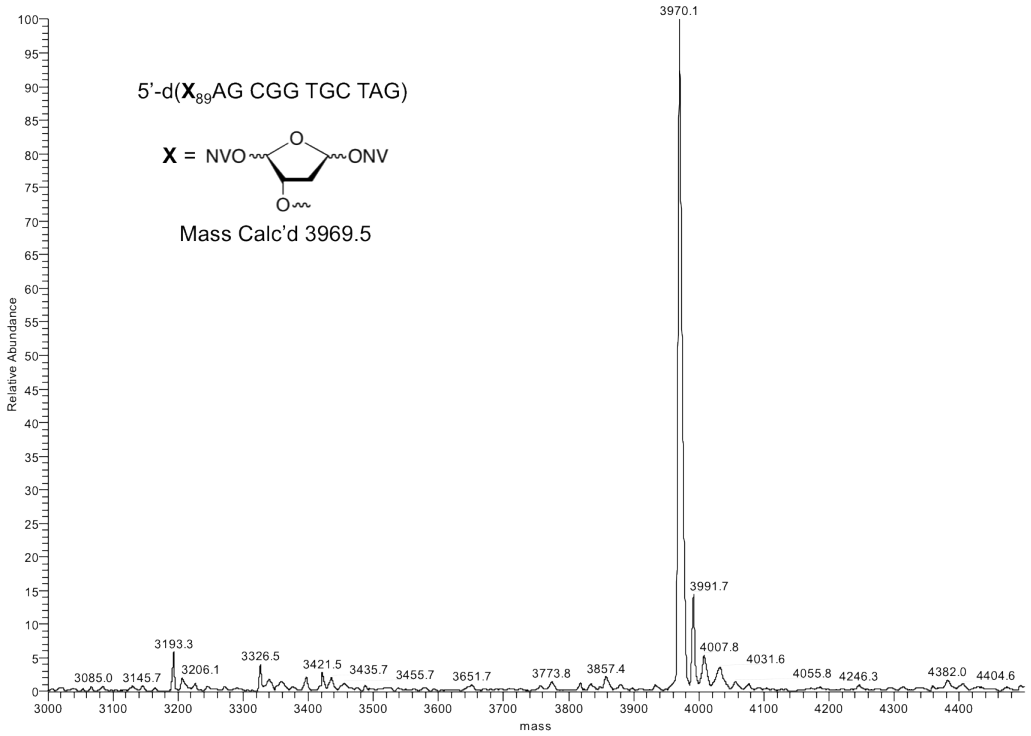


Figure S5. ESI-MS spectrum of S30.

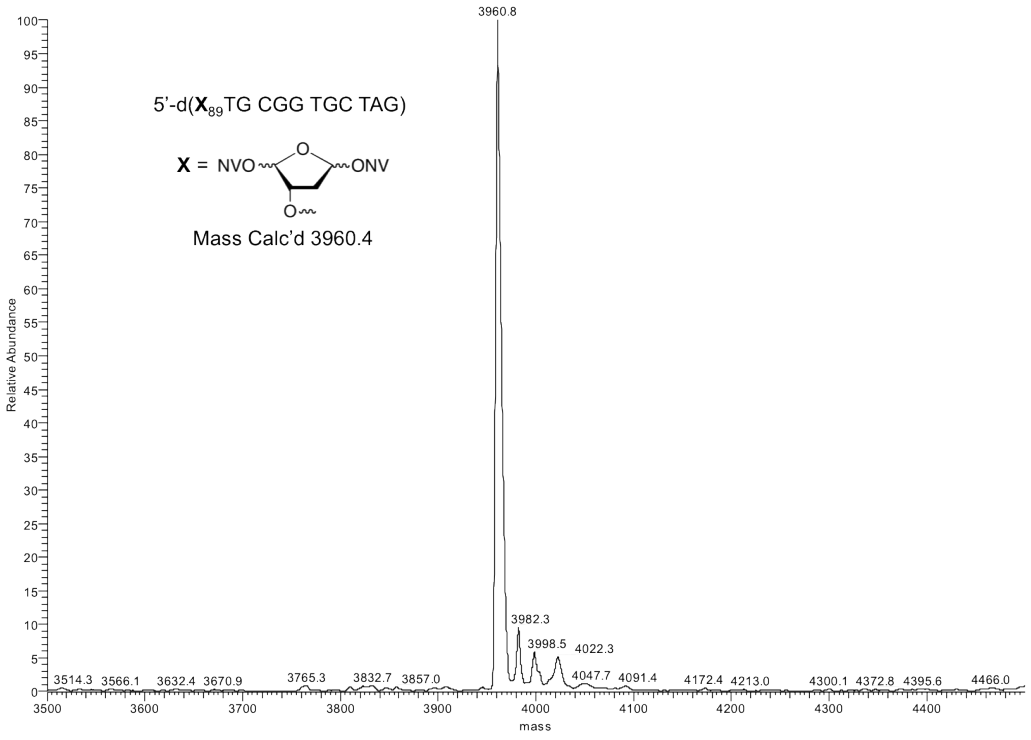


Figure S6. ESI-MS spectrum of S31.

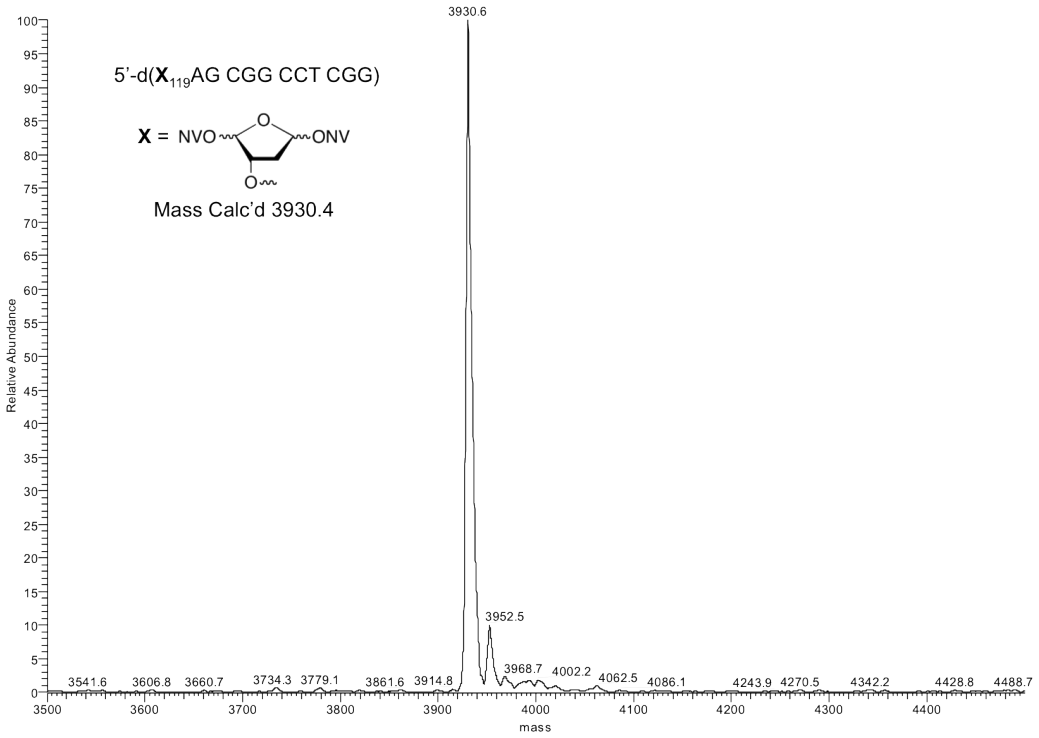


Figure S7. ESI-MS spectrum of S32.

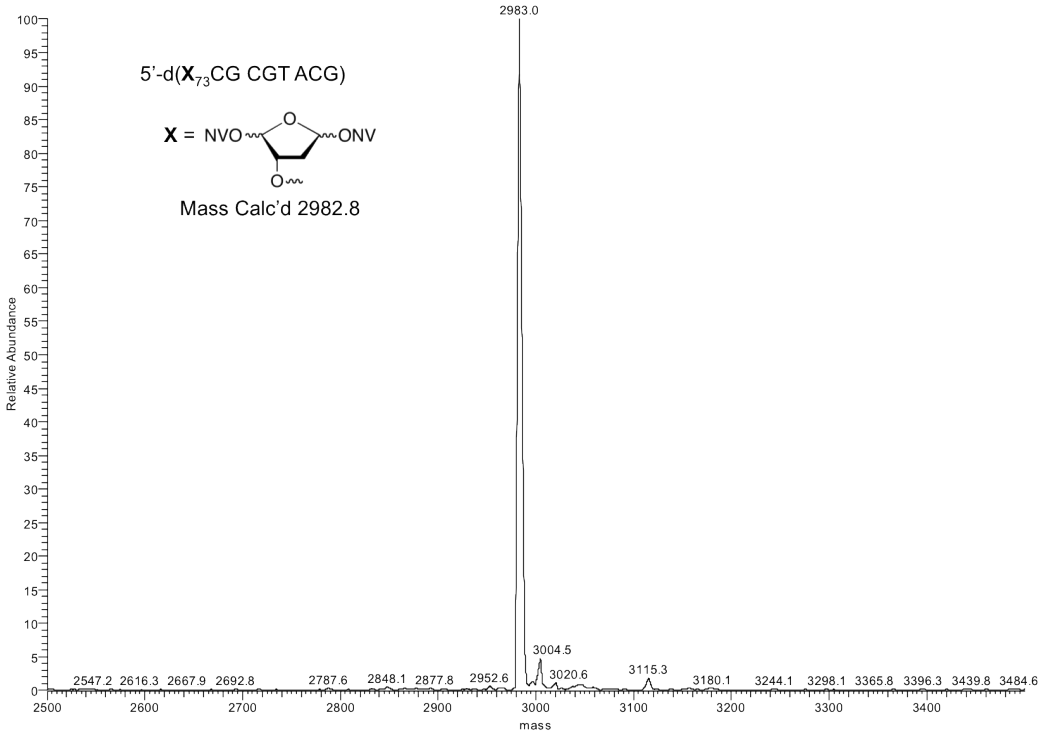


Figure S8. ESI-MS spectrum of S27.

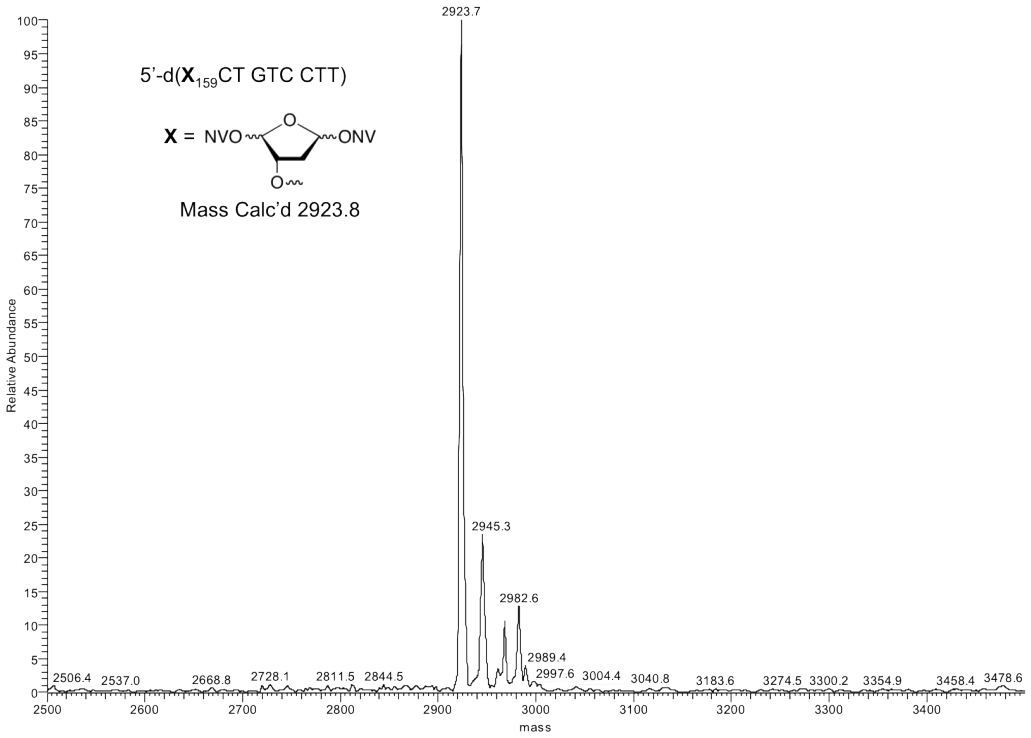


Figure S9. ESI-MS spectrum of S34.

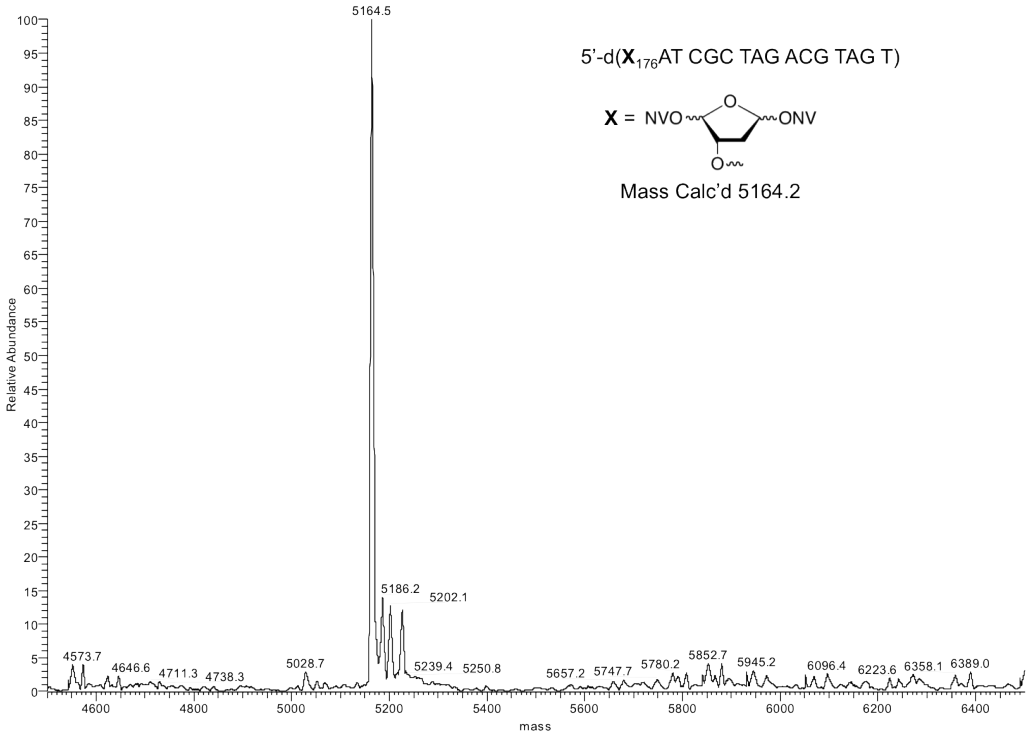


Figure S10. ESI-MS spectrum of 176B.

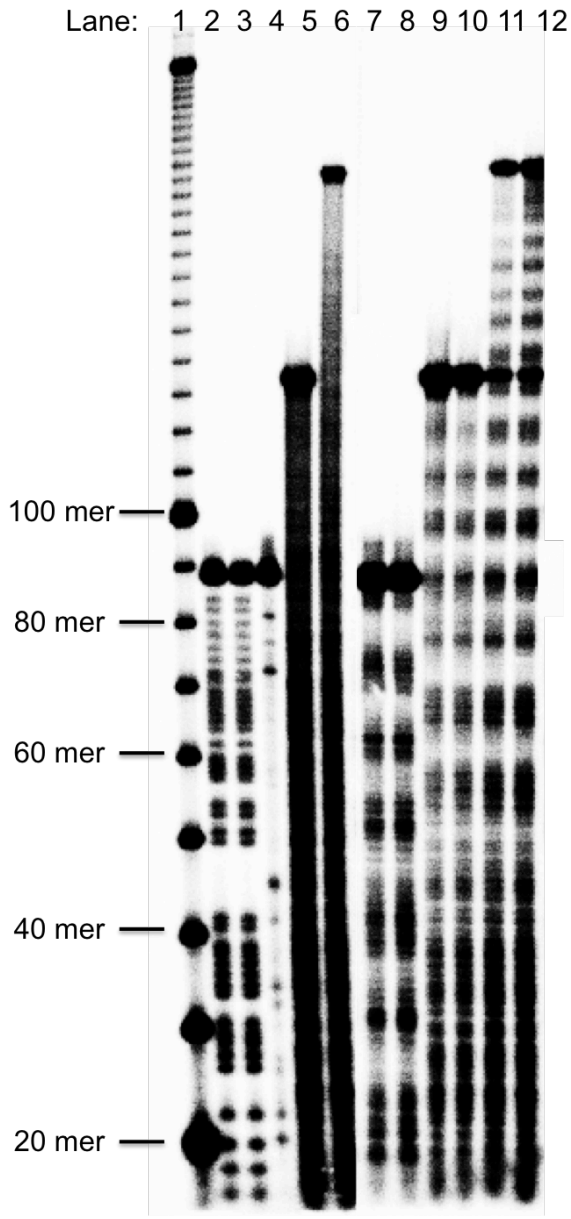


Figure S11. Hydroxyl radical footprinting assay on NCPs and nucleosomes with $\mathbf{2_{89}}$ and $\mathbf{2_{176}}$. Lane 1: 10-bp DNA ladder; lanes 2-4: G + A sequencing reaction on free DNA with $\mathbf{2_{89}}$; lanes 5-6: hydroxyl radical reaction on 237-mer free DNA with $\mathbf{2_{89}}$ and $\mathbf{2_{176}}$ for 10 min; lanes 7-8: hydroxyl radical reaction on NCP containing $\mathbf{2_{89}}$ with WT histones for 15 and 30 min; lanes 9-10: hydroxyl radical reaction on nucleosomes with $\mathbf{2_{89}}$ (with linker DNA and WT histones) for 15 and 30 min; lanes 11-12: hydroxyl radical reaction on nucleosomes containing $\mathbf{2_{176}}$ with WT histones for 15 and 30 min.

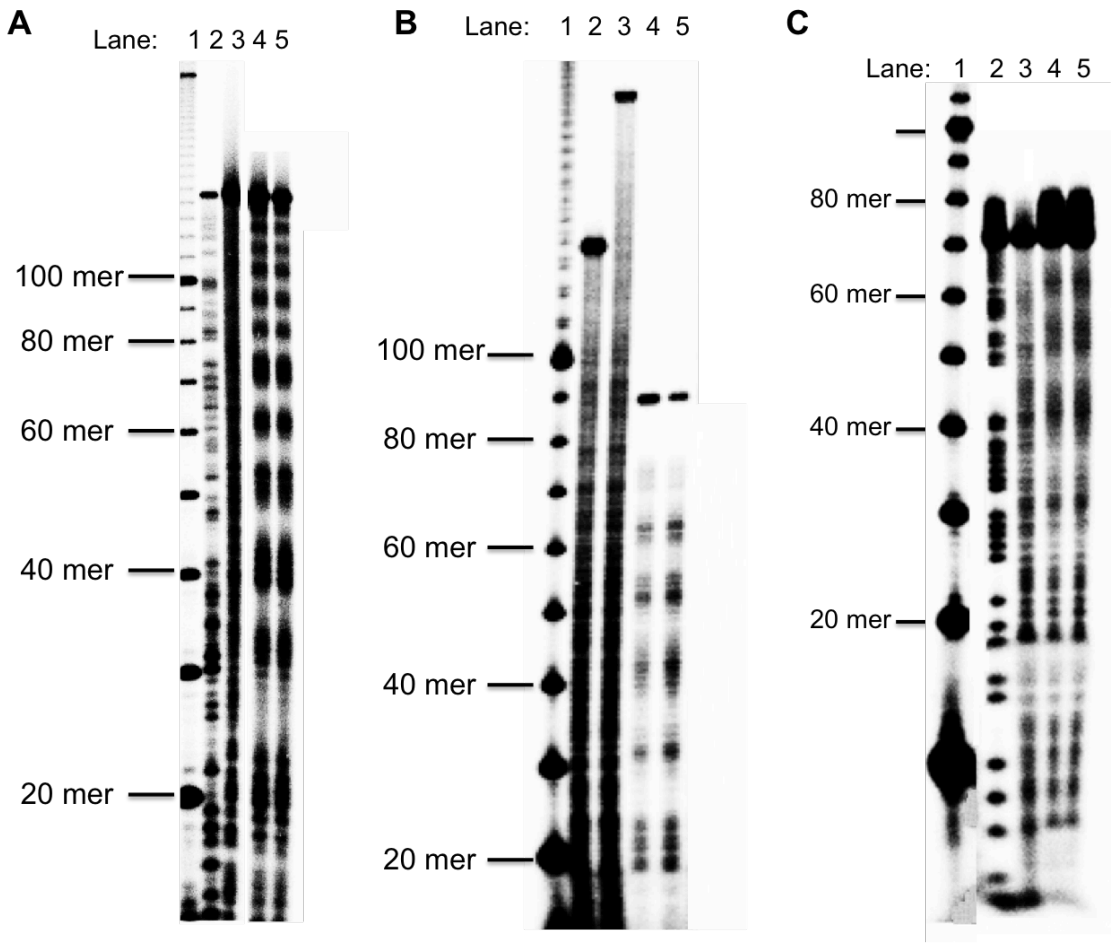


Figure S12. Hydroxyl radical footprinting on NCPs with $\mathbf{2_{89}}$, $\mathbf{2_{73}}$, and $\mathbf{2_{119}}$. (A) Lane 1: 10-bp DNA ladder; lane 2: G + A sequencing reaction on 145-mer DNA containing $\mathbf{2_{119}}$; lane 3: hydroxyl radical reaction on free DNA containing $\mathbf{2_{119}}$ for 5 min; lanes 4-5: hydroxyl radical reaction on NCPs containing $\mathbf{2_{119}}$ for 10 and 20 min. The ternary complexes were 5'-^{32}P labeled on the complementary strand (145com) in the above reactions. (B) Lane 1: 10-bp DNA ladder; lanes 2-3: hydroxyl radical reaction on 145-mer free DNA containing $\mathbf{2_{119}}$ and 237-mer free DNA containing $\mathbf{2_{89}}$; lanes 4-5: hydroxyl radical reaction on NCPs containing $\mathbf{2_{89}}$ and K5,8,12,16,20R H4 (lane 4) or K5.8.12.16.20R/H18A H4 (lane 5) for 15 min. The ternary complexes were 5'-^{32}P labeled on the flanking strand in the above reactions. (C) Lane 1: 10-bp DNA ladder; lane 2: A + G sequencing reaction on 145-mer DNA with $\mathbf{2_{73}}$; lane 7: hydroxyl radical treatment on free DNA; Lane 8-9: hydroxyl radical treatment on NCP containing $\mathbf{2_{73}}$ and WT histone proteins for 15 and 30 min.

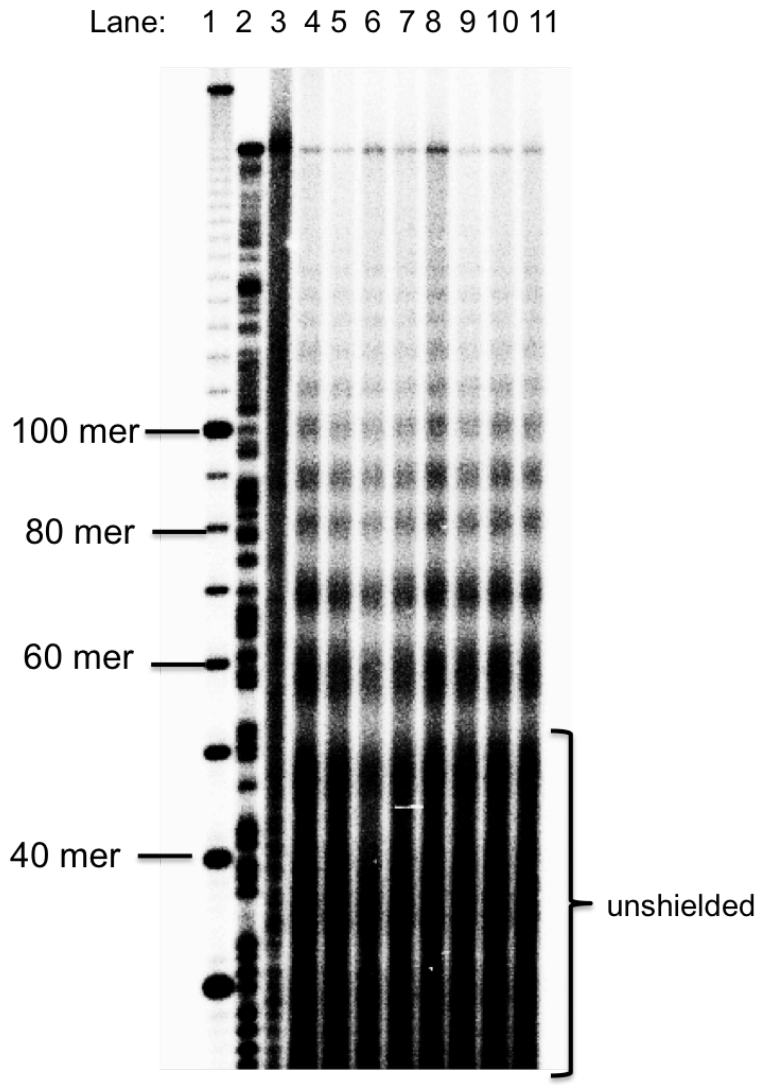


Figure S13. Hydroxyl radical footprinting assay on nucleosomes with $\mathbf{2_{159}}$. Lane 1: 10-bp DNA ladder; lanes 2: G + A sequencing reaction on 237-mer DNA with $\mathbf{2_{159}}$; lanes 3: hydroxyl radical reaction on 237-mer free DNA containing $\mathbf{2_{159}}$ for 5 min; lanes 4-11: hydroxyl radical reaction on nucleosomes containing $\mathbf{2_{159}}$ and WT H4 (lanes 4-5), tailless H3 (lanes 6-7), tailless H4 (lanes 8-9), tailless H3 and tailless H4 (lanes 10-11) for 5 and 10 min. The ternary complexes were 5'-labeled with ^{32}P on the complementary strand in the above experiments.

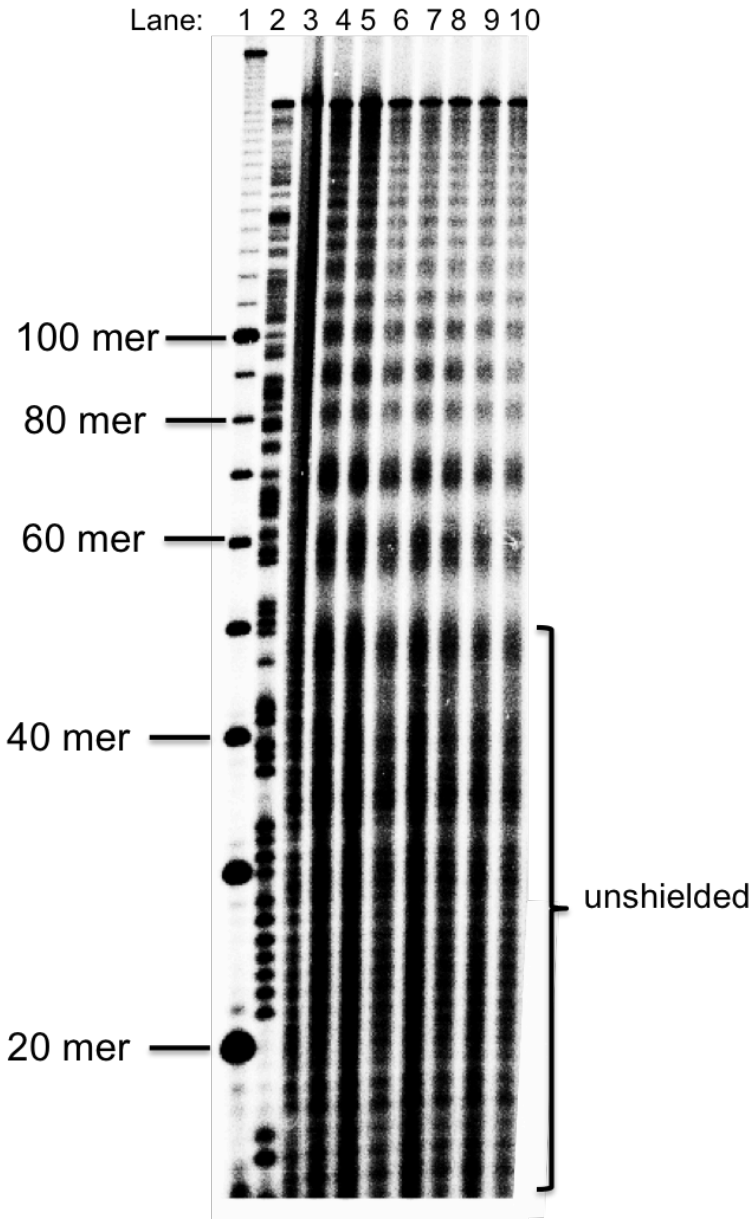


Figure S14. Hydroxyl radical footprinting on nucleosomes with $\mathbf{2_{89}}$ and $\mathbf{2_{73}}$. Lane 1: 10-bp DNA ladder; lane 2: G + A sequencing reaction on 237-mer DNA with $\mathbf{2_{73}}$; lane 3: hydroxyl radical reaction on 237-mer free DNA with $\mathbf{2_{73}}$ for 5 min; lanes 4-5: hydroxyl radical reaction on nucleosomes containing $\mathbf{2_{73}}$ and WT histone proteins; lanes 6-10: hydroxyl radical reaction on nucleosomes containing $\mathbf{2_{89}}$ with WT H4 protein (lanes 6-7) for 5 and 10 min, with K5,8,12,16,20R H4 variant (lanes 8-9) for 5 and 10 min, and with K5,8,12,16,20R/H18A H4 variant (lane 10) for 5 min. For all the DNA used here, 5'-^{32}P label was on the complementary strand.

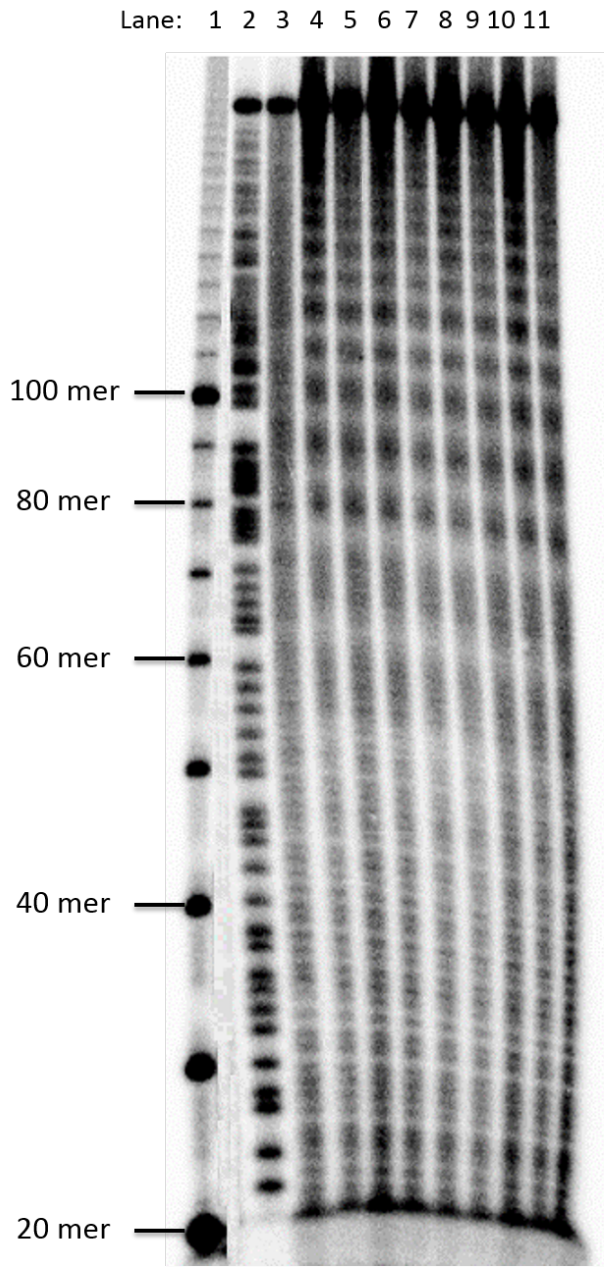


Figure S15. Hydroxyl radical footprinting on nucleosomes containing $\mathbf{2_{176}}$. Lane 1: 10-bp DNA ladder; lane 2: G + A sequencing reaction on 237-mer DNA with $\mathbf{2_{176}}$; lane 3: hydroxyl radical reaction on 237-mer free DNA containing $\mathbf{2_{176}}$ for 5 min; lanes 4-11: hydroxyl radical reaction on nucleosomes containing $\mathbf{2_{176}}$ and WT H4 (lanes 4-5), tailless H3 (lanes 6-7), tailless H4 (lanes 8-9) and tailless H3 and tailess H4 (lanes 10-11) for 15 and 30 min. The ternary complexes were 5'-radiolabeled on the flanking strand in the above experiments.

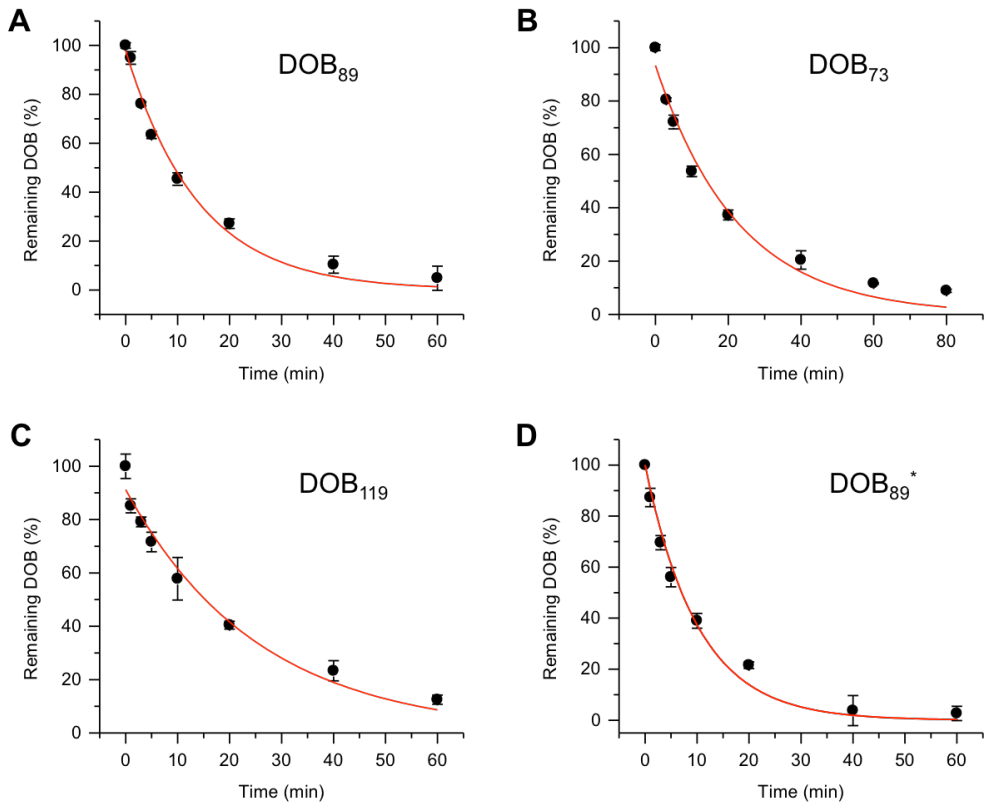


Figure S16. Reactivity of DOB monitored as a function of time by 20% denaturing PAGE in NCPs containing DOB_{89} (A), DOB_{73} (B), DOB_{119} (C), and DOB_{89}^{*} (D). *indicates the different flanking sequence of DOB_{89}.

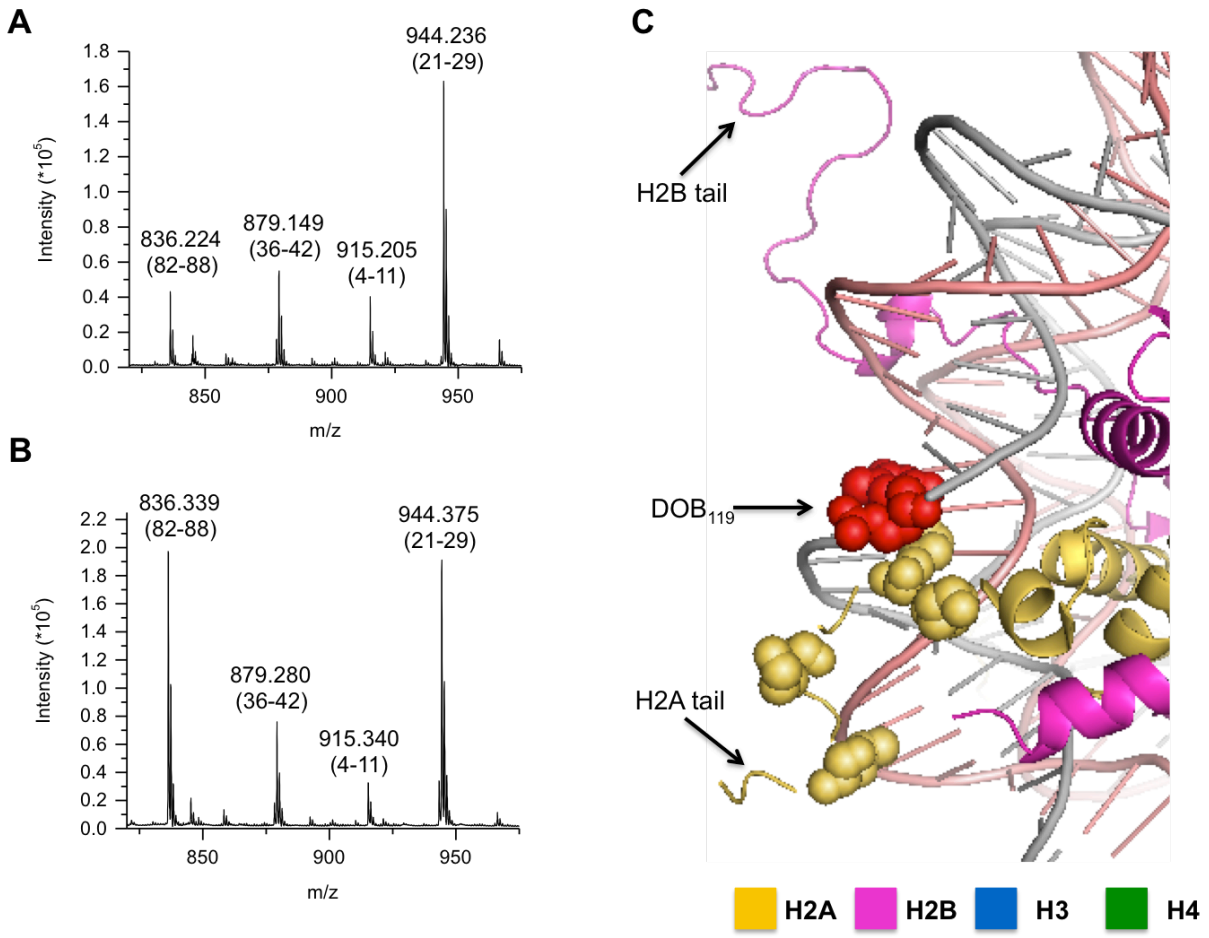


Figure S17. MALDI-TOF mass spectra of peptide fragments obtained from in-gel acetylation and trypsin digest of (A) WT histone H2A and (B) the major DPC band produced by DOB_{119}. (C) Portion of the X-ray crystal structure highlighting the proximity of DOB_{119} to the N-terminal of histone H2A tail (PDB: 1KX5). Proximal lysine residues are shown as spheres.

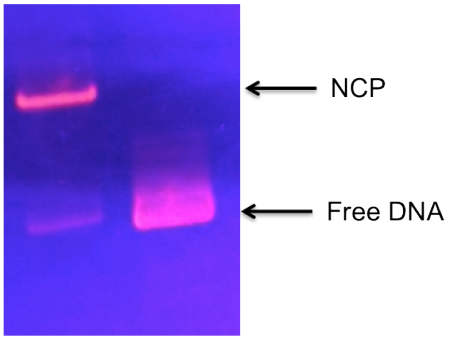


Figure S18. Representative image of reconstituted nucleosomes on large scale.

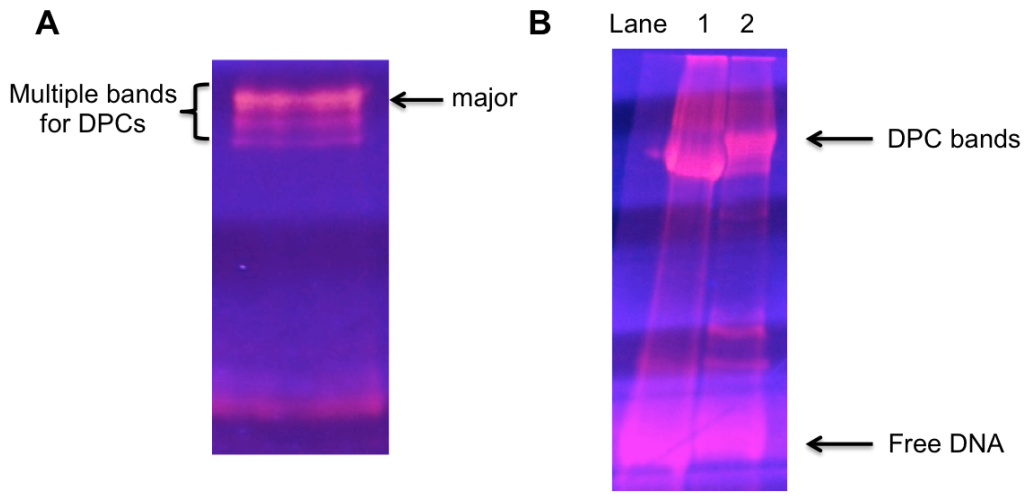


Figure S19. Representative image of DPC formation on large scale NCPs. (A) DPCs formed by DOB_{119}. (B) Lane 1: DPCs formed by DOB_{89}; lane 2: DPCs formed by DOB_{73}.

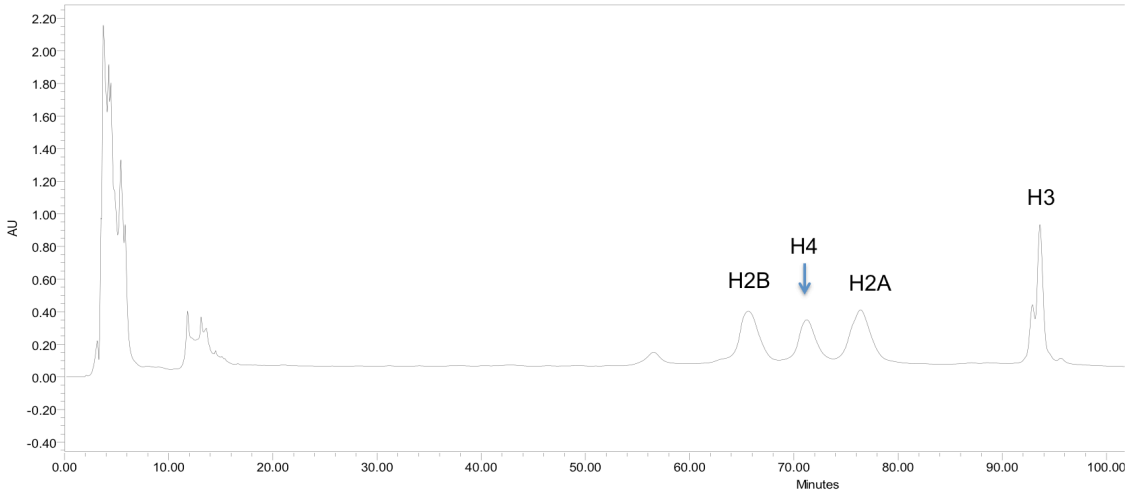
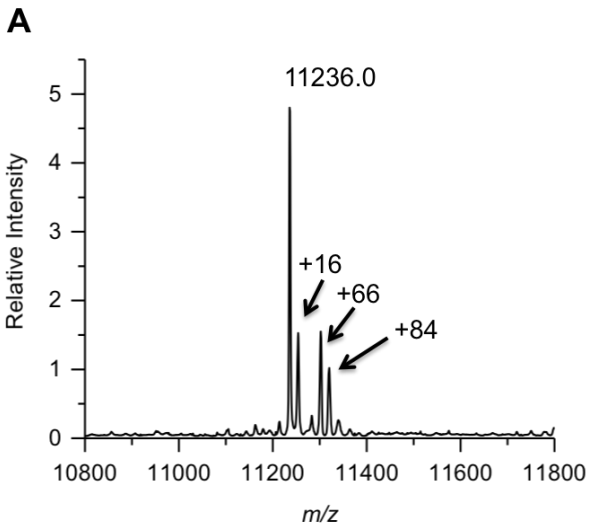


Figure S20. HPLC chromatogram of four histone proteins after DOB decomposition.



B

Histone proteins	M.W.	found
H2A	13950	13951
H2B	13494	13494.8
H3	15271	15270 (+18)
H4	11236	11236.0 (+16, +66, +84)

Figure S21. The mass of four HPLC-purified histone proteins following DOB_{89} decomposition in NCP. (A) The mass spectrum of purified H4. (B) The mass for all four purified histone proteins.

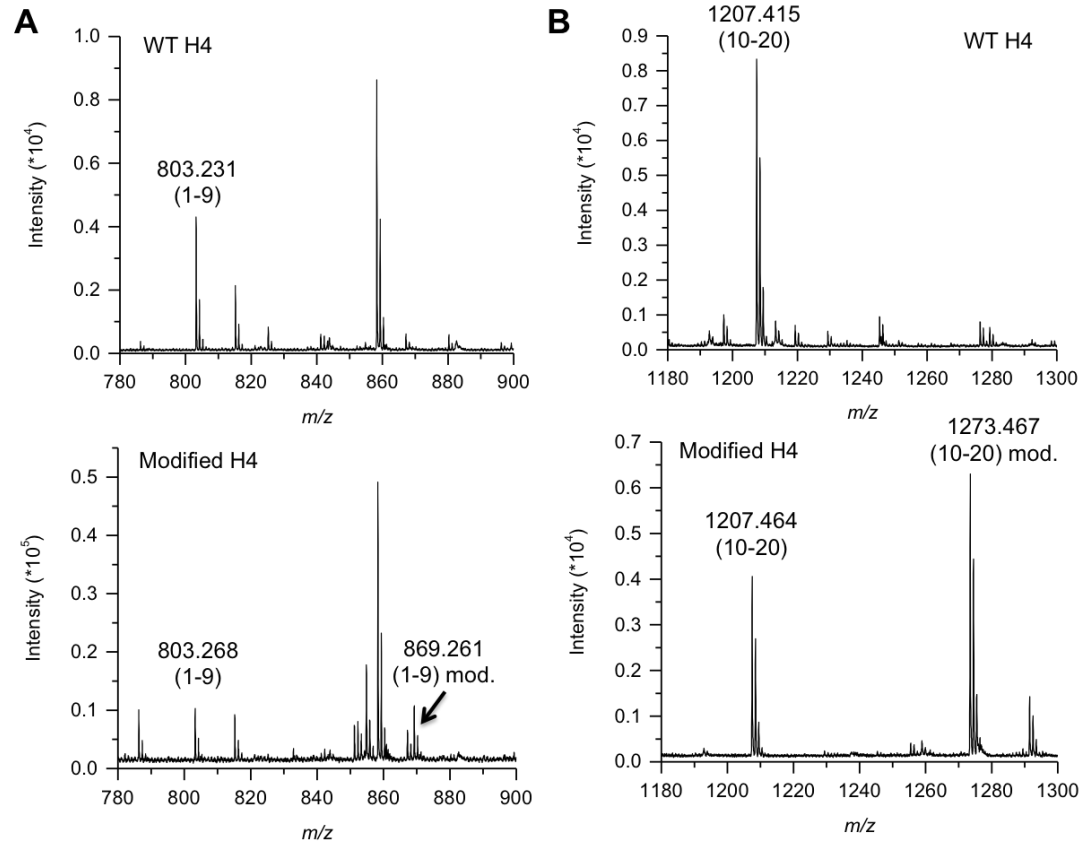


Figure S22. MALDI-TOF MS spectra of histone H4 fragments obtained from thermolysin digestion. (A) Fragment 1-9. (B) Fragment 10-20. Top, unmodified H4; bottom, H4 from NCP containing DOB_{89}.

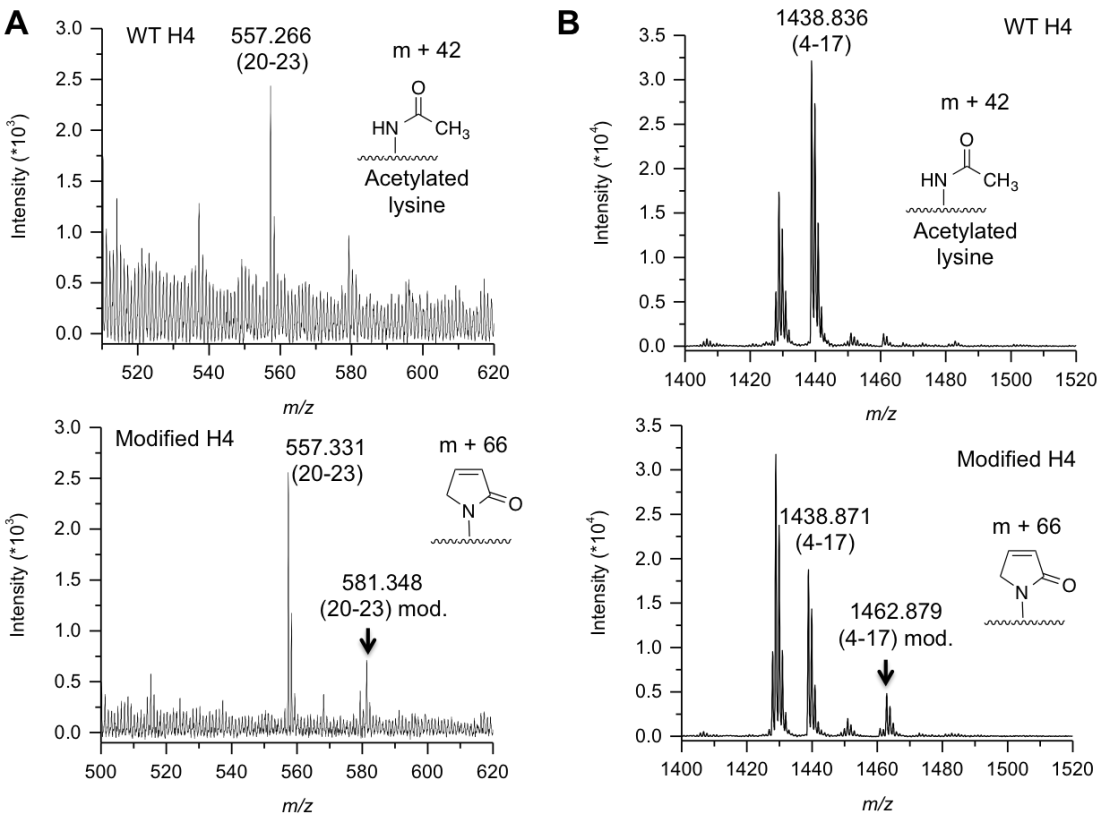


Figure S23. MALDI-TOF MS spectra of histone H4 fragments observed following trypsin digestion. (A) Fragment 20-23. (B) Fragment 4-17. Top, unmodified H4; bottom, H4 from NCP containing DOB.

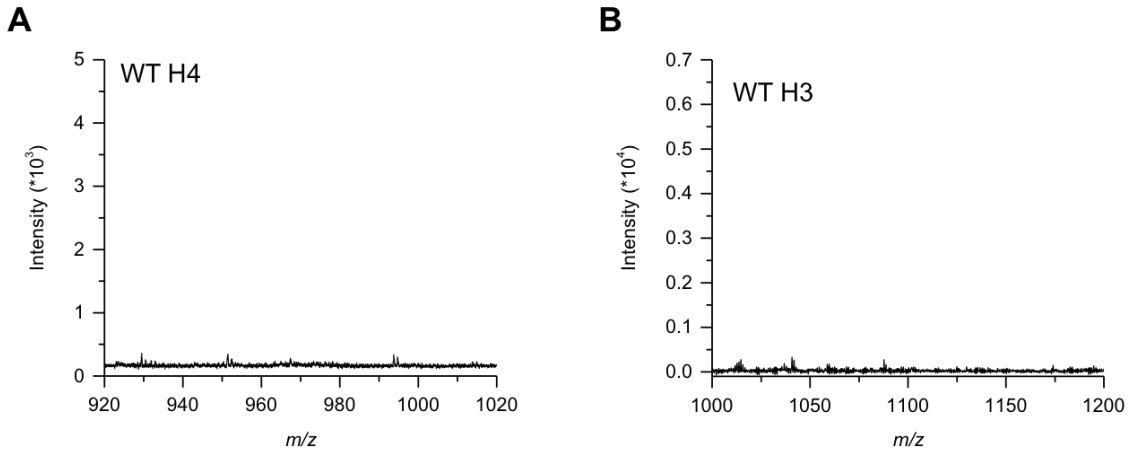
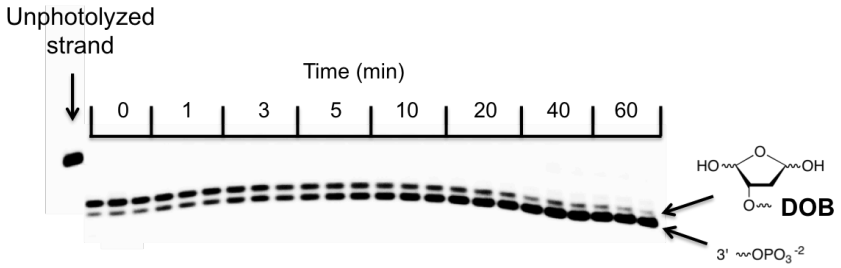


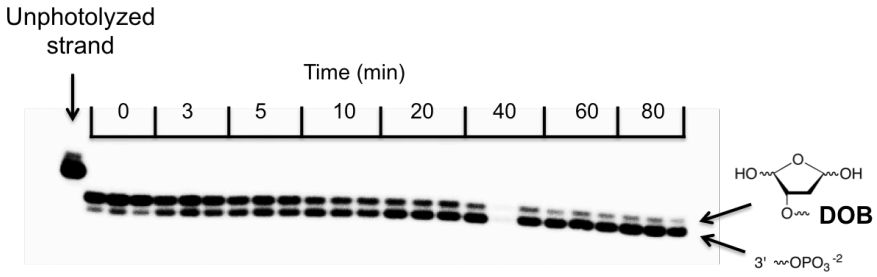
Figure S24. MALDI-TOF mass spectra of WT histone proteins digested by Lys C: (A) WT histone H4 and (B) WT histone H3. The absence of peptide 13-20 from WT histone H4 digested by Lys C is due to the cleavage at unmodified Lys 16. Similarly, the absence of peptide 1-9 from WT histone H3 digested by Lys C is due to the cleavage at unmodified Lys 4.

Figure S25. Representative autoradiograms of time-course experiments describing the excision of DOB in (A) NCPs containing DOB_{89} and WT histones, (B) NCPs containing DOB_{73} and WT histones, (C) NCPs containing DOB_{119} and WT histones, (D) NCPs containing DOB_{89}^{*} and WT histones, (E) nucleosomes containing DOB_{159} and tailless H3, (F) nucleosomes containing DOB_{176} and tailless H3 and tailless H4.

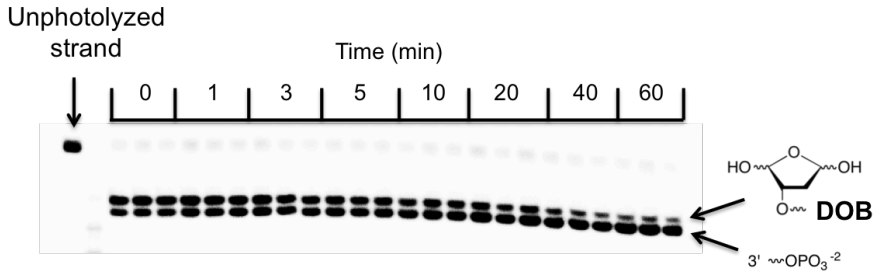
(A)



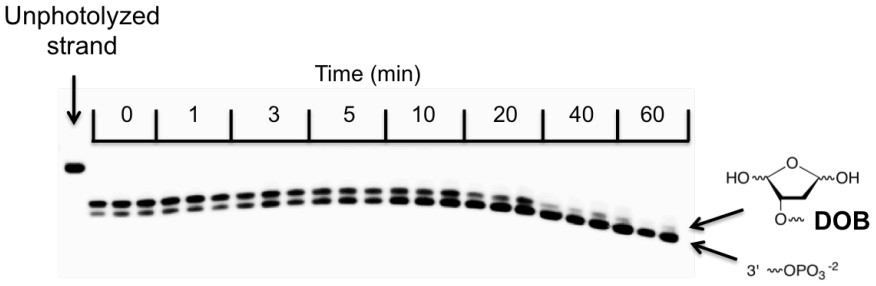
(B)



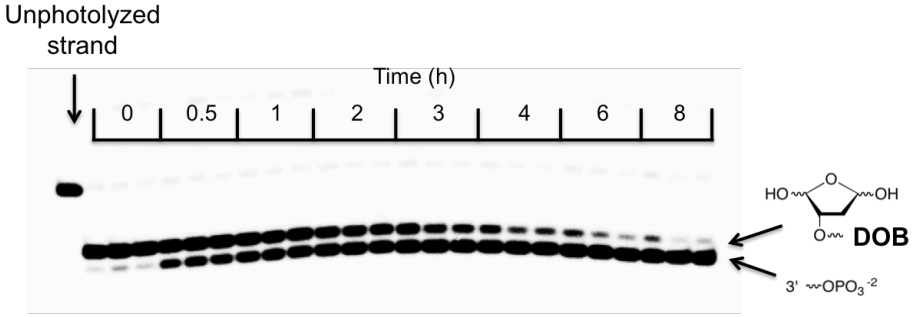
(C)



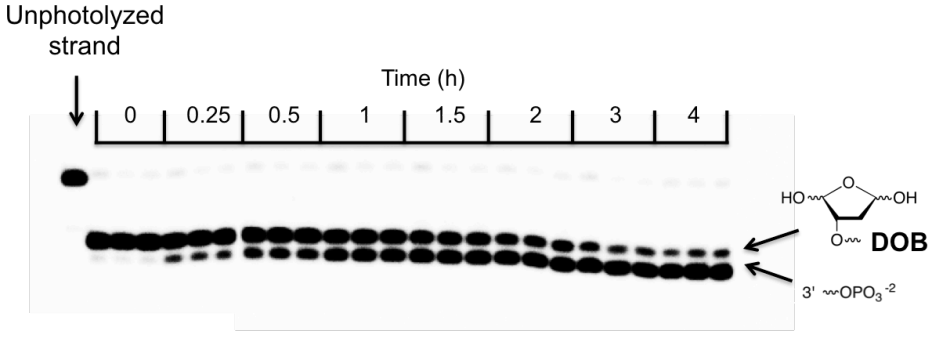
(D)


Unphotolyzed strand
Time (min)
0
1
3
5
10
20
40
60
HO
OH
O
DOB
3' OPO3 -2

(E)


Unphotolyzed strand
Time (h)
0
0.5
1
2
3
4
6
8
HO
OH
O
DOB
3' OPO3 -2

(F)


Unphotolyzed strand
Time (h)
0
0.25
0.5
1
1.5
2
3
4
HO
OH
O
DOB
3' OPO3 -2

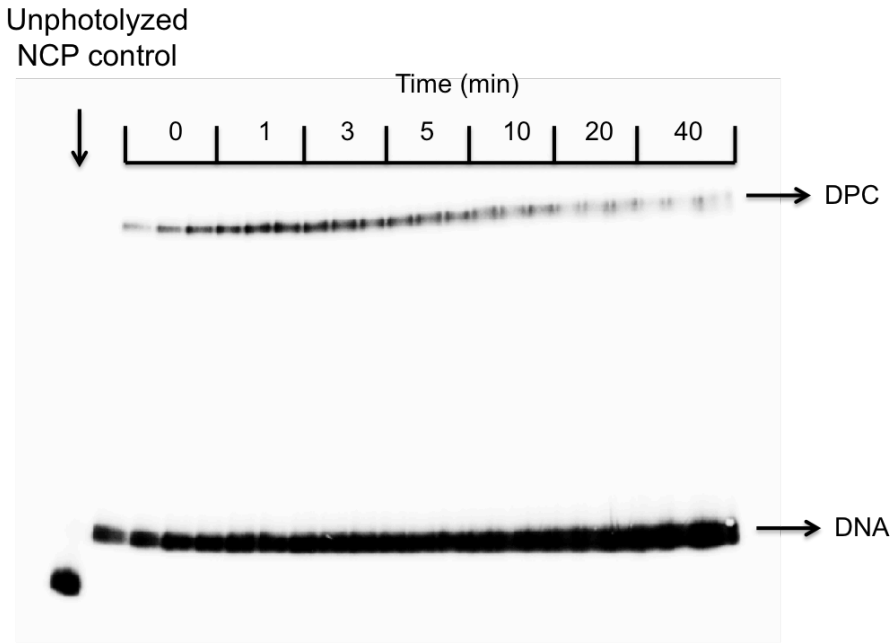


Figure S26. Representative autoradiogram of DPC formation in the absence of $NaBH_3CN$ by DOB_{89} in NCPs containing WT histones.

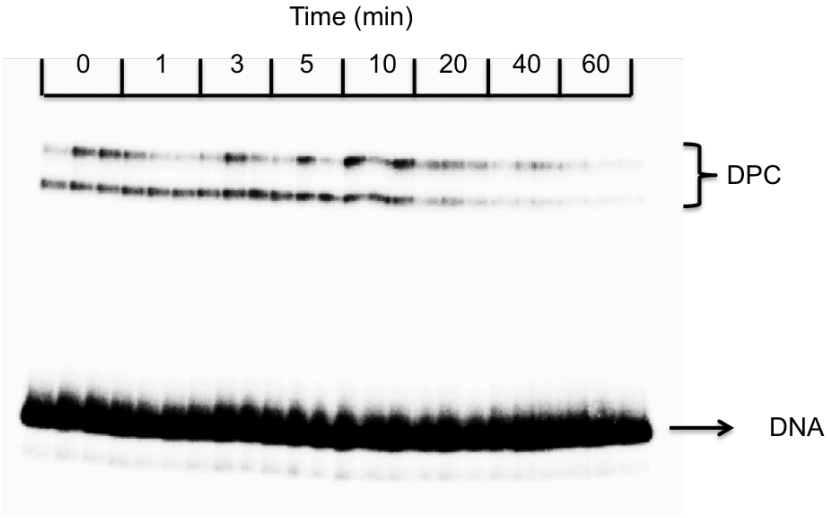


Figure S27. Representative autoradiogram showing monitoring of DPC formation by DOB_{119} in NCPs.

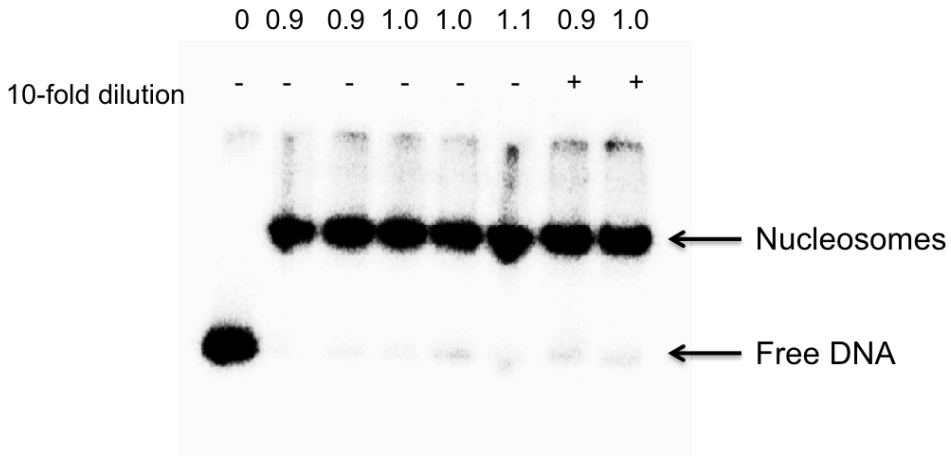


Figure S28. Autoradiogram showing the integrity of nucleosomes upon 10-fold dilution following the reconstitution.

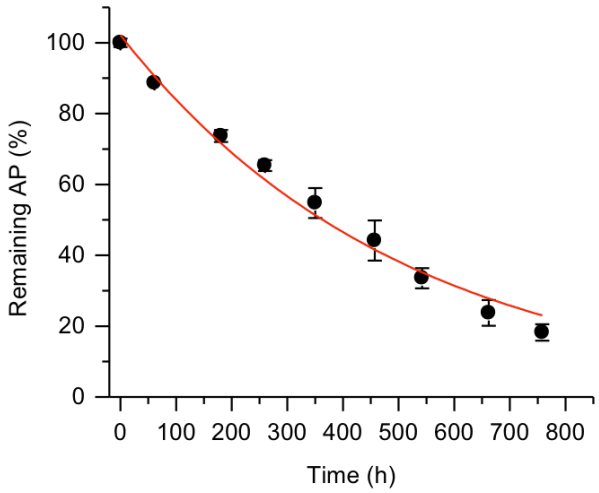


Figure S29. The reactivity of AP_{176} in the nucleosome.

Table S1. Calculated and observed fragmentation patterns for peptides in Figure 8, and Figure 10B.

	Cal.	Found
Figure 8A. Fragment 9-16 (K12 mod.)		
y3	275.1705	275.1719
y4	332.1940	332.1934
y5*	526.3082	526.2990
y6*	583.3234	583.3204
y8*	753.4224	753.4259

	Cal.	Found
Figure 8B. Fragment 20-23 (K20 mod.)		
a1*	167.1184	167.1185
a2*	266.1883	266.1869
y1	175.1187	175.1195
y2	288.2024	288.2036
y2-NH_3	271.1775	271.1770
y3	387.2707	387.2720
y4*	581.3762	581.3775

Figure 10B. Fragment 3-8 (K4 mol.)		
y1	175.1207	175.1195
y2	246.1653	246.1666
y4	475.2625	475.2629
y6*	770.4157	770.4161

References:

(1) Kodama, T.; Greenberg, M. M. *J. Org. Chem.* **2005**, *70*, 9916.

(2) Sczepanski, J. T.; Wong, R. S.; McKnight, J. N.; Bowman, G. D.; Greenberg, M. M. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 22475.

(3) Dyer, P. N.; Edayathumangalam, R. S.; White, C. L.; Bao, Y.; Chakravarthy, S.; Muthurajan, U. M.; Luger, K. In *Methods Enzymol.*; Allis, C. D., Carl, W., Eds.; Academic Press: 2003; Vol. Volume 375, p 23.

(4) Reddy, M. P.; Hanna, N. B.; Farooqui, F. *Tetrahedron Lett.* **1994**, *35*, 4311.

(5) Zhou, C.; Sczepanski, J. T.; Greenberg, M. M. *J. Am. Chem. Soc.* **2013**, *135*, 5274.