Supporting Information

Histone H3 and H4 N-Terminal Tails in Nucleosome Arrays at Cellular Concentrations Probed by Magic Angle Spinning NMR Spectroscopy

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Materials and Methods

Preparation of Nucleosome Arrays

Histones H2A, H2B, H3 and H4 were overexpressed in *Escherichia coli* BL21(DE3)pLysS, purified as described previously[1] using gel filtration and ion-exchange chromatography in 7 M urea followed by dialysis against a solution of 2 mM β-mercaptoethanol (BME) in ultrapure water and lyophylized. Uniformly ^{13}C,^{15}N-labeled H3 and H4 were prepared by using a minimal medium with ^{13}C-glucose (3 g/liter) and $^{15}NH_4Cl$ (1 g/liter) (Isotec/Sigma-Aldrich) as the sole carbon and nitrogen sources, respectively. Histone octamer containing ^{13}C,^{15}N-H3 or H4 was prepared by dissolving the four unfolded histone proteins at concentrations of ≤ 10 mg/ml in 7 M guanidine hydrochloride, 20 mM Tris, 10 mM dithiothreitol, pH 7.5 unfolding buffer in a H2A:H2B:H3:H4 molar ratio of 1.2:1.2:1:1, refolded by double dialysis into 1× TE (10 mM Tris, 1 mM EDTA, pH 8.0), 2 M NaCl, 5 mM BME buffer and purified by gel filtration chromatography in 1× TE, 2 M NaCl buffer as described previously.[1]

The DNA templates (Figure S1) were prepared by using QIAGEN Giga kits and digested with DdeI (New England Biolabs). The digested DNA mixture contains a 3,046 bp linear dsDNA, corresponding to a 17-mer tandem repeat of a 147 bp variant of the Widom 601 nucleosome positioning sequence with 30 bp of linker DNA.[2] The mixture also contains seven shorter DNA fragments of varying length (653, 535, 421, 404, 245, 230, and 161 bp), which serve as buffering DNA in the process of nucleosome array reconstitution and aid in minimizing the non-specific aggregation of the arrays.[3]

The 17-mer nucleosome arrays were prepared as follows. An aqueous solution was made in 0.5× TE, 2 M NaCl, 1 mM benzamidine (BZA) buffer, containing the digested DNA mixture ([DNA] ≤ 0.2 mg/ml) and a two-fold molar excess of the histone octamer with respect to the DNA, which ensured an effectively complete saturation of all the nucleosome positioning sites. The NaCl was removed by double dialysis at 4 °C against 0.5× TE, 1 mM BZA buffer. The solution was concentrated ~30-fold using Amicon 30 kDa cut-off centrifugal filter devices, followed by purification of the nucleosome arrays using 5-40% sucrose gradient centrifugation in 0.5× TE. The fractions containing pure 17-mer nucleosome arrays were combined and the sucrose removed by exchanging into 0.5× TE buffer using Amicon 30 kDa devices.

Characterization of Nucleosome Arrays by Gel Electrophoresis and Atomic Force Microscopy

The purity and level of saturation of the 17-mer nucleosome arrays were confirmed by using composite agarose-polyacrylamide gel electrophoresis for as-prepared arrays and polyacrylamide gel electrophoresis for BamHI-digested and AvaI-digested arrays (see Figure S2 and S3 captions for details).

The sucrose gradient purified arrays were further analyzed by atomic force microscopy (AFM) as follows. Freshly cleaved mica was rinsed with 2 × 200 µl of ultrapure water, followed by treatment with 50 µl of 10 ng/µl aqueous solution of poly-D-lysine (PL; Sigma-Aldrich) and an additional 2 × 200 µl water rinse to remove any unbound PL, and finally dried with a slow stream of dry nitrogen gas. The PL-treated mica was incubated for 5 min with 50 µl of a dilute (~0.2 nM) solution of nucleosome arrays in 0.1× TE buffer, rinsed with 200 µl of ultrapure water and dried as above. The sample was imaged with a Bruker AXS Dimension Icon AFM at a scan rate of 1 Hz.

Additional AFM studies and gel electrophoresis for AvaI-digested arrays were performed in order to assess the stability of the 17-mer nucleosome array samples under typical conditions used for the NMR experiments. These studies were carried out using a dilute nucleosome array solution incubated at a temperature of 30 °C for up to 7 days as well as a small aliquot of previously ultracentrifuged arrays extracted directly from the NMR sample holder following several days of experiments at 30 °C (see Figure S3 caption and NMR Spectroscopy section below for details).

Self-Association of Nucleosome Arrays Monitored by UV Absorbance

The nucleosome array self-association assay was performed as described.[4] A series of array samples with volumes of 25 µl and concentration corresponding to OD_{260} ~ 1 (1 mm pathlength) were mixed with 25 µl of aqueous solution containing increasing amounts of $MgCl_2$ in the 0-5 mM range for the final concentration. After 5 min of incubation at room temperature, the absorbance at 260 nm was measured by using a NanoDrop 2000 spectrophotometer (Thermo Scientific). Each sample was then centrifuged for 5 min at 16,000×g and the absorbance at 260 nm recorded immediately for the supernatant. For each $MgCl_2$ concentration, the percentage of nucleosome arrays in the supernatant was calculated from the ratio of the absorbance intensities at 260 nm after and before centrifugation.

NMR Spectroscopy

Three 17-mer nucleosome array samples were studied for each type of ^{13}C,^{15}N-labeled histone (H3 or H4), prepared with 0 mM, 1 mM and 5 mM Mg^{2+} and corresponding to different degrees of array compaction and self-association. Each nucleosome array sample was pelleted by ultracentrifugation at 4 ºC and 80,000 rpm (~320,000×g) using a Beckman-Coulter TLA-100.3 rotor. Due to the distinct physical properties of the array samples prepared with increasing concentrations of Mg^{2+} (see Figure S4A), different ultracentrifugation times (7, 3 and 2 h for 0, 1 and 5 mM Mg^{2+}, respectively) were required to pellet the samples for the NMR studies. The pellets were transferred to 3.2 mm thin-wall zirconia rotors (36 µl sample volume) and sealed using custom-made spacers to prevent sample dehydration during experiments. The final NMR samples contained between ~8-16 mg of nucleosome arrays with total protein:DNA ratios of approximately 1:1 (w/w) for each sample, corresponding to chromatin concentrations in the ~200-400 mg/ml regime. Note that the 0 mM Mg^{2+} samples typically contained smaller quantities of the nucleosome arrays relative to samples prepared with 1 and 5 mM Mg^{2+} due to the lower density of the array pellets following ultracentrifugation.

NMR spectra were recorded using a 500 MHz Varian spectrometer equipped with a 3.2 mm T3 magic-angle spinning (MAS) probe in either ^{1}H-^{13}C or ^{1}H-^{15}N configuration. The sample spinning rate was actively regulated to ca. ±3 Hz using a MAS control unit, and sample temperatures were controlled by a stream of dried compressed air delivered to the sample using a variable-temperature (VT) stack. The temperatures listed in the article consistently refer to the VT gas temperature at the sample, with the actual sample temperatures being ~5 ºC higher on average caused by the frictional heating due to MAS. The pulse sequences used included standard cross-polarization[5] as well as one- and two-dimensional refocused INEPT[6,7] with parameters described in detail elsewhere.[8]

EPR Spectroscopy

Site directed mutagenesis (Quikchange Lightning Kit, Agilent Technologies) was used to add cysteine residues to the N-terminal tail of the *Xenopus laevis* histone H3(C110A) mutant. Two mutants were employed for these studies, including V35C and a mutant containing a non-native cysteine residue introduced before the N-terminal alanine found in wild-type H3 (referred to as C0). Recombinant histones carrying the desired mutations for spin labeling were purified from *Escherichia coli* and refolded into octamer as previously described.[1] Histone octamer was then reduced with 10 mM tris(2-carboxyethyl)phosphine (TCEP) and purified by gel filtration in 5 mM PIPES pH 6.1, 2 M NaCl buffer to remove misfolded aggregates and TCEP. The purified reduced octamer was labeled by increasing the pH to 8.0 using Tris buffer (50 mM final concentration) and rapidly adding 200-fold molar excess of 0.1 M MTSL ((1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl) methanethiosulfonate, Toronto Research Chemicals) in acetonitrile. The reaction was allowed to proceed for 1 h at room temperature, then overnight at 4 ºC. Unreacted spin label was removed by dialysis into 3 × 500 ml of 5 mM PIPES pH 6.1, 2 M NaCl buffer. The labeling was confirmed by mass spectrometry, and 17-mer arrays containing labeled histone octamer were prepared and purified as described above for the NMR experiments.

Following sucrose gradient purification, arrays were concentrated to ~1 μM, and 10 μl samples were loaded into quartz capillary tubes for the electron paramagnetic resonance (EPR) measurements.

EPR measurements were performed on a Bruker EMX X-band EPR spectrometer. Spectra were recorded at room temperature using 20 mW incident power and field modulation of 0.1 G at 100 kHz. A set of ten spectra were acquired for each H3 mutant as a function of Mg^{2+} concentration, baseline corrected, and normalized to the same number of spins. The resulting data are shown in Figure S9.

Supplementary Results

Characterization of Nucleosome Arrays by Gel Electrophoresis and Atomic Force Microscopy

Gel electrophoresis and atomic force microscopy were employed to assess the purity and level of saturation of the 17-mer nucleosome arrays used for the NMR studies. The gel shift assay in Figure S2A shows a single narrow band for the sucrose gradient purified 17-mer arrays (lane 3), which indicates that arrays saturated with histone octamer are the major species present in the sample.[3] The saturation level of the 17-mer arrays was confirmed by gel electrophoresis analysis of arrays digested with BamHI and AvaI restriction enzymes. The histone octamers were removed following the BamHI digestion to allow for easier quantification of the fraction of cut DNA template. The inability of BamHI (which readily cuts the empty DNA template in the central nucleosome positioning sequence) to cleave the 17-mer arrays (Figure S2B, lane 3) suggests that the arrays are fully saturated with histone octamer making the digestion sites inaccessible to the enzyme.[2] Likewise, AvaI digestion of the 17-mer arrays, which cuts in the linker DNA between nucleosomes (Figure S3D, lane 5), yields primarily single nucleosomes and no free 177 bp DNA fragments, indicating that the arrays are fully saturated.[2] Finally, AFM analysis of dilute decompacted arrays shows a highly homogeneous sample of saturated 17-mer nucleosome arrays (Figure S3A). Quantitative analysis of the AFM data revealed that 98 ± 4% of the nucleosome positioning sites are saturated with histone octamer, corresponding to an average array occupancy of 16.6 ± 0.6 nucleosomes out of 17.

Additional experiments were performed to assess the fidelity of the 17-mer nucleosome array samples when exposed to ambient temperature for an extended period of time, corresponding to typical conditions used for the NMR studies. Specifically, samples of the sucrose gradient purified arrays were incubated at 30 ºC for up to one week, digested with AvaI and analyzed by gel electrophoresis (Figure S3D, lanes 3 and 4). These arrays were found to be virtually identical to the control array sample stored at 4 ºC, indicating that prolonged incubation of the 17-mer arrays at ambient temperature does not result in appreciable dissociation of histone octamer. Moreover, we have performed the same AvaI-digestion gel electrophoresis assay for a small sample of previously ultracentrifuged 17-mer arrays extracted from the NMR rotor following several days of data acquisition at 30 ºC (Figure S3D, lane 6). This array sample was found to contain a detectable amount of free 177 bp DNA, consistent with histone octamer dissociation from a small fraction of the nucleosome positioning sites. However, quantitative analysis of the gel image (see Figure S3 caption for details) suggests that this effect is relatively minor, with 97% or 16.5 out of 17 nucleosome positioning sites per array on average containing histone octamer. Importantly, this finding is further supported by quantitative AFM analysis of the same sample (Figure S3B), which reveals that on average 95 ± 7% of nucleosome positioning sites (i.e., 16.2 ± 1.1 out of 17) are occupied. Altogether, the gel electrophoresis and AFM analyses of the 17-mer nucleosome arrays strongly suggest that the array samples are highly homogeneous, effectively fully saturated with histone octamer, and remain largely intact during the course of NMR experiments.

Self-Association of Nucleosome Arrays

In order to investigate the flexible histone protein domains in 17-mer nucleosome arrays by NMR spectroscopy as a function of the degree of array compaction and self-association, the array samples were prepared with 0 mM, 1 mM and 5 mM Mg^{2+}.[9] These 17-mer nucleosome arrays have been demonstrated to compact into 30-nm chromatin fibers by Mg^{2+} under dilute conditions by AFM imaging.[2] To confirm that the 17-mer arrays undergo self-association upon addition of Mg^{2+} we monitored the soluble fraction before and after centrifugation by UV absorbance at 260 nm.[4] Figure S4B shows UV spectra of the soluble fractions for nucleosome array samples that were prepared with 0 and 1 mM Mg^{2+} and briefly centrifuged at 16,000×g. The spectra illustrate that rapid sedimentation of the arrays is effectively complete following the addition of 1 mM Mg^{2+}. Figure S4C shows the fraction of nucleosome arrays remaining in the supernatant (calculated from the ratio of absorbance intensities at

260 nm after and before centrifugation) as a function of Mg^{2+} concentration. These data allowed us to estimate that ~1 mM Mg^{2+} is required for array self-association, in the range expected based on published reports.[9] In Figure S4D we show a plot of Mg^{2+} required for 50% self-association of 6-, 7-, 9- and 12-mer nucleosome arrays, determined previously in a systematic study by Hansen and co-workers,[10] along with a fit of these data to a decaying single exponential. These results indicate that, as expected, lower Mg^{2+} concentrations are required for the compaction of longer nucleosome arrays. Remarkably, the predicted Mg^{2+} concentration needed for 50% self-association of the 17-mer arrays is in good quantitative agreement with the experimental Mg^{2+} concentration determined in the present study as seen by superimposing the experimental value for 17-mer arrays onto the plot in Figure S4D. Consistent with these results, the effect of increasing Mg^{2+} concentration on the physical appearance of the nucleosome array samples was readily evident as shown in Figure S4A, ranging from a fairly clear solution at 0 and 1 mM Mg^{2+} to a highly opaque one containing a significant amount of precipitate at 5 mM Mg^{2+}.

Observation of Rigid and Flexible Domains of Histones H3 and H4 in Nucleosome Arrays by Variable Temperature 1D ^{13}C MAS NMR

In order to investigate the rigid and flexible segments of histones H3 and H4 in the context of the highly concentrated 17-mer nucleosome arrays we recorded a set of 1D ^{13}C MAS NMR spectra as a function of temperature between -20 and 30 ^{o}C. The ^{13}C magnetization was prepared by using either ^{1}H-^{13}C refocused INEPT[6] or cross-polarization,[5] conditions which lead to the suppression of NMR signals arising from the most rigid and most flexible protein residues, respectively. The data sets for the 17-mer arrays incubated with 1 mM $MgCl_2$ and containing ^{13}C,^{15}N-labeled H3 and H4 are shown in Figures S5 and S6, respectively. We note here that array samples prepared with 0 and 5 mM Mg^{2+} showed nearly identical temperature profiles and that repeated temperature cycling had no appreciable effect on the appearance of the NMR spectra, with any spectral changes observed as a function of temperature being largely reversible as shown in Figure S7.

The foremost feature of these experiments is that the INEPT spectra recorded between -10 and 30 ^{o}C contain multiple resonances in the aliphatic region, indicative of magnetization transfer through one-bond ^{1}H-^{13}C J-couplings. This confirms the presence of flexible segments for both histones H3 and H4 irrespective of the degree of array compaction. Moreover, the spectra recorded at successively higher temperatures show narrower and more intense ^{13}C signals, consistent with enhanced conformational dynamics of the protein backbone and side-chains. In contrast, the INEPT spectra recorded at or below -20 ^{o}C are largely devoid of signals. This suggests that around this temperature the large amplitude isotropic-like local protein motions occurring on the sub-millisecond time scale are sufficiently attenuated, leading to the rapid dephasing of ^{1}H coherences by the residual ^{1}H-^{1}H dipolar couplings that are not efficiently averaged by MAS. The CP spectra, which report on the immobile protein residues, indicate that the majority of amino acids in histones H3 and H4 are relatively rigid in the entire temperature range investigated, highlighted by the fact that the spectra recorded at -20 ^{o}C display a similar overall appearance with a ~20-30% increase in signal intensity relative to the 30 ^{o}C spectra due to the "freezing out" of protein motions. These findings are consistent with the high-resolution nucleosome X-ray structure, which reveals that unstructured N-terminal tails encompass ~20-25% of the H3 and H4 sequences,[11] as well as previous observations of mobile N-terminal histone tails by solution NMR in mono and oligonucleosomes at relatively low concentrations in the absence of Mg^{2+}.[12-15]

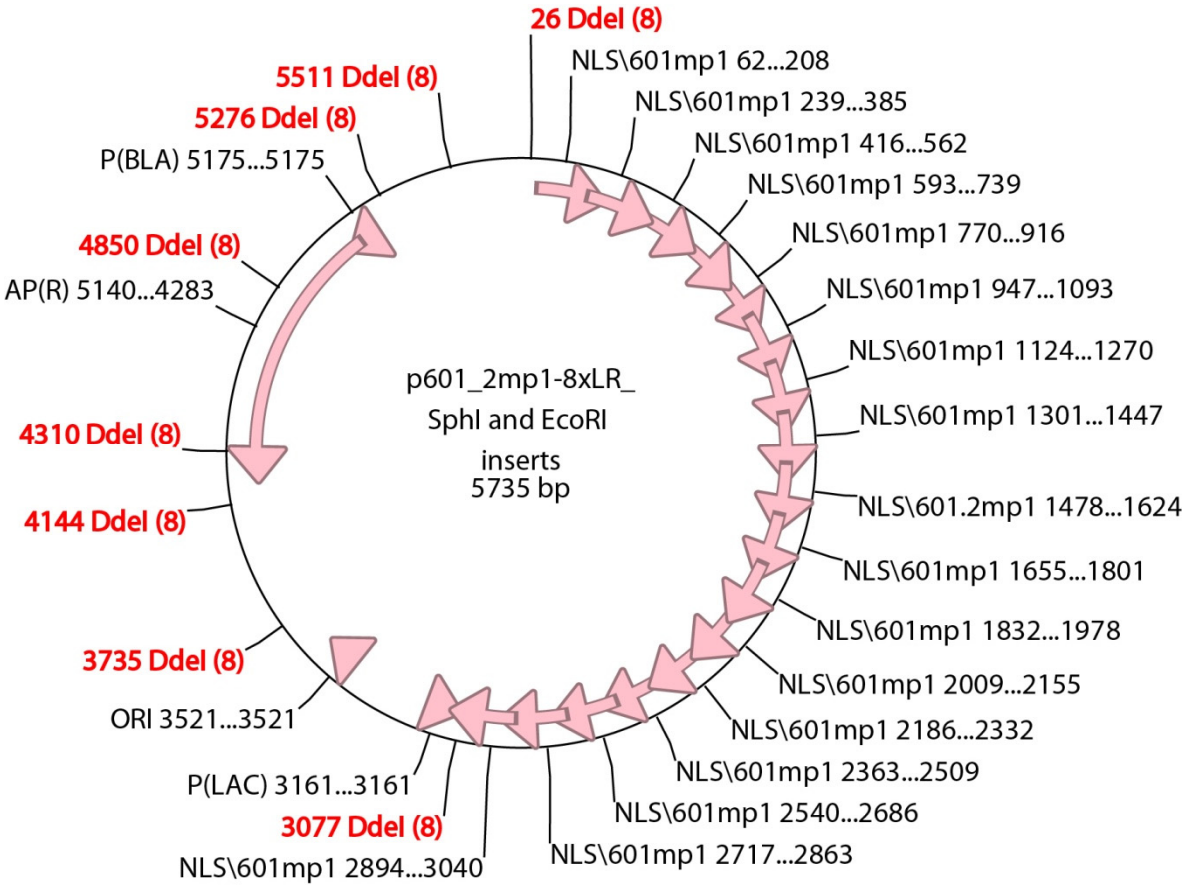

Figure S1. DNA template for the preparation of nucleosome arrays. The template contains 17 tandem nucleosome binding repeats with 147 bp per repeat (positions 62-208 through 2717-2863) and 8 DdeI restriction sites as indicated. Following DdeI digestion, the DNA mixture contains a 3,046 bp nucleosome binding DNA sequence and seven shorter buffering DNA fragments (653, 535, 421, 404, 245, 230, and 161 bp) that assist in efficient nucleosome array reconstitution as described in Materials and Methods. The template also contains a single BamHI restriction site at position 1526 in the central (9^{th}) nucleosome binding repeat and multiple AvaI digestion sites in the linker regions connecting the nucleosome binding repeats (spaced every 177 bp in positions 221, 398, 575, …, 2876). The BamHI and AvaI enzyme restriction sites are required for the gel electrophoresis assays used to evaluate the levels of nucleosome saturation in the array samples (c.f., Figures S2 and S3).

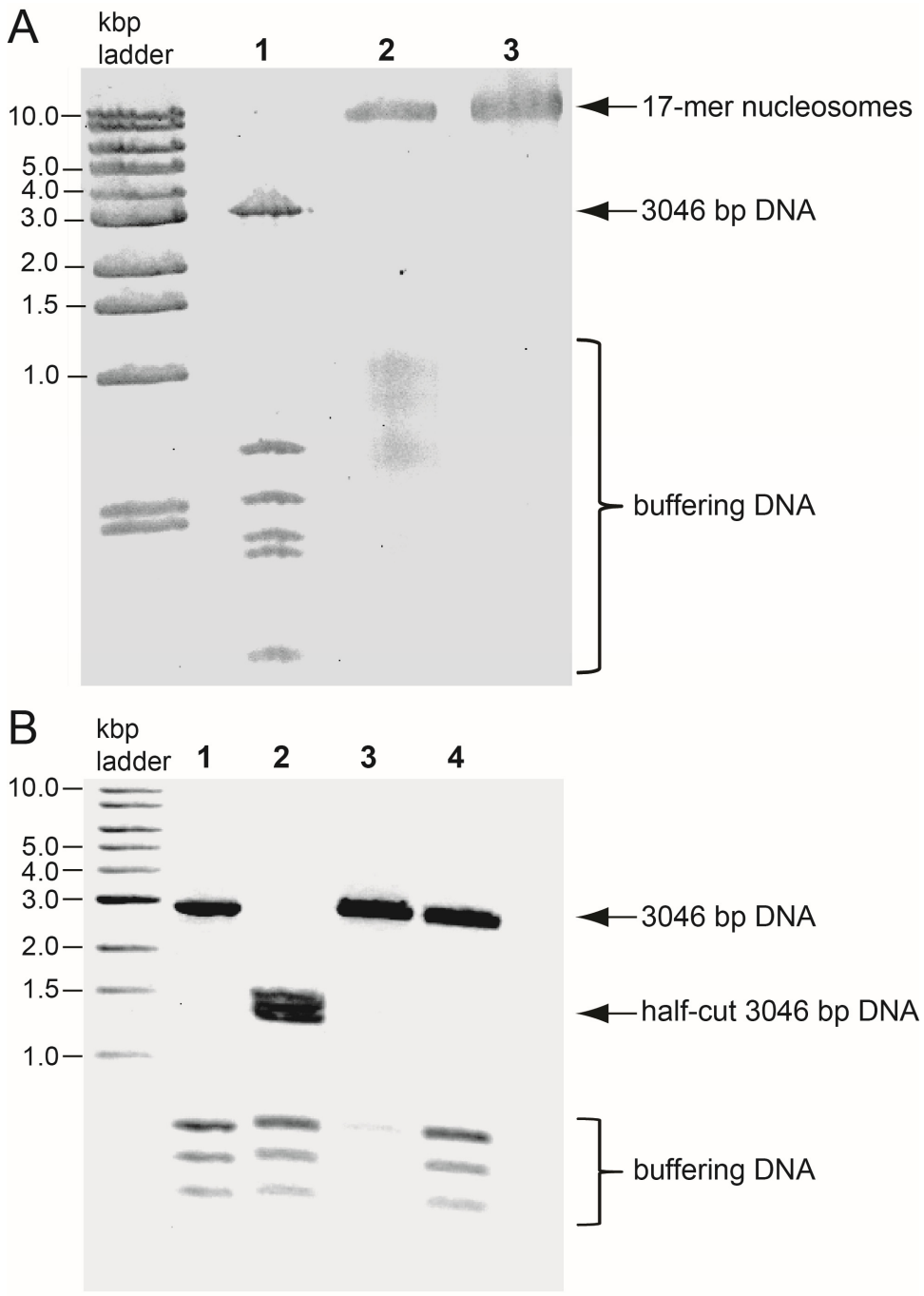

Figure S2. Gel electrophoresis assays used to assess the saturation level of 17-mer nucleosome arrays. (A) Native agarose-polyacrylamide composite gel (1% agarose-2% polyacrylamide, Tris-borate running buffer) stained with SYBR Gold (Invitrogen). Lane 1: DdeI-digested DNA template containing the 3,046 bp DNA with 17 tandem nucleosome binding repeats and shorter buffering DNAs; Lane 2: reconstituted 17-mer nucleosome arrays prior to sucrose gradient purification; Lane 3: sucrose gradient purified 17-mer nucleosome arrays. (B) Native agarose gel (1% agarose, Tris-acetate EDTA running buffer) stained with ethidium bromide. Lane 1: DdeI-digested DNA template containing the 3,046 bp DNA with 17 tandem nucleosome binding repeats and shorter buffering DNAs; Lane 2: DdeI-digested DNA template further digested with BamHI, which cuts the 3,046 bp DNA into two fragments of roughly equal size; Lane 3: sucrose gradient purified 17-mer nucleosome arrays digested with BamHI (only the 3,046 bp DNA is observed due to the BamHI site being occupied by the histone octamer and not accessible to BamHI), followed by proteinase K treatment to digest the histone proteins; Lane 4: same as lane 3 but for 17-mer nucleosome arrays prior to sucrose gradient purification.

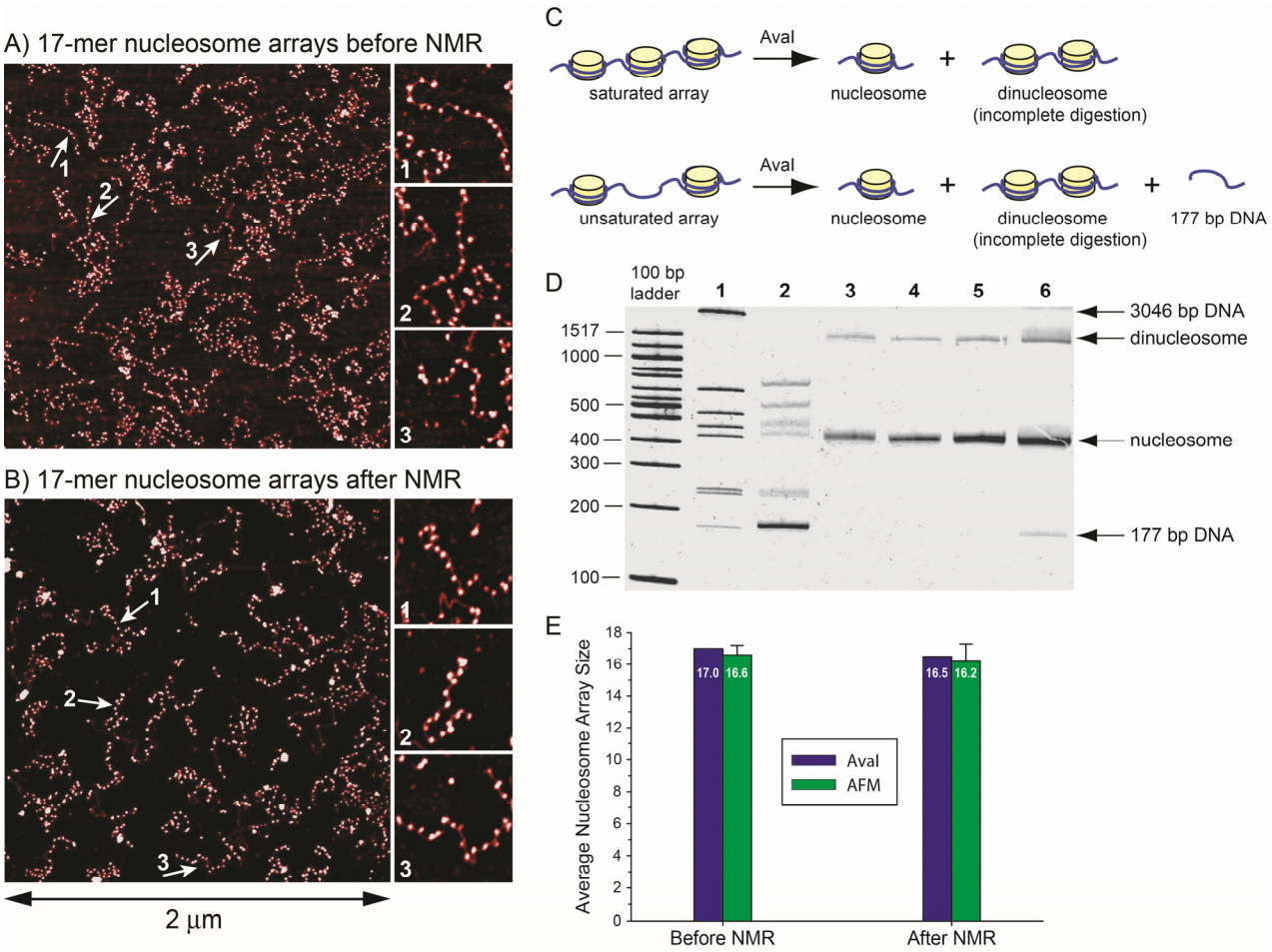

Figure S3. AFM and gel electrophoresis analysis of the stability of 17-mer nucleosome arrays under conditions used for NMR studies. (A) AFM images of 17-mer nucleosome arrays recorded prior to pelleting for NMR experiments. (B) AFM images of a small aliquot of a pellet containing 17-mer nucleosome arrays following several days of NMR experiments at 30 °C. The pellet was extracted from the NMR rotor and incubated in 5 mM Tris, 0.5 mM EDTA, pH 8.0 buffer for one week to ensure a complete resuspension of the pelleted arrays prior to AFM image acquisition. Analysis of ~50-60 arrays for each of the images shown in panels (A) and (B) determined, respectively, that 98 ± 4% and 95 ± 7% of the nucleosome positioning sites are saturated with histone octamer, corresponding to an average occupancy of 16.6 ± 0.6 and 16.2 ± 1.1 nucleosomes out of 17 before and after the NMR experiments. (C) Cartoon depicting the AvaI-digestion of saturated and unsaturated nucleosome arrays. For the saturated arrays the AvaI digestion yields primarily single nucleosome particles, while both nucleosomes and free 177 bp fragments are obtained for arrays that are not fully saturated. (D) Native polyacrylamide gel (5% polyacrylamide, Tris-borate EDTA running buffer) stained with ethidium bromide. Lane 1: DdeI-digested DNA template containing the 3,046 bp DNA with 17 tandem nucleosome binding repeats and shorter buffering DNAs; Lane 2: DdeI-digested DNA template further digested with AvaI, which cuts the 3,046 bp DNA into 17 fragments of 177 bp each; Lanes 3-5: sucrose gradient purified 17-mer nucleosome arrays incubated at 30 °C for 48 h (lane 3), 30 °C for 1 week (lane 4), and stored at 4 °C (lane 5), and subsequently digested with AvaI; Lane 6: sucrose gradient purified 17-mer nucleosome arrays extracted from the ultracentrifuged pellet inside the NMR rotor following several days of experiments at 30 °C. For the pelleted arrays a weak band corresponding to 177 bp DNA is observed in addition to the single nucleosome band (no 177 bp DNA band is observed for the unpelleted arrays incubated at 30 °C). The intensity ratio of the single nucleosome to 177 bp DNA bands was estimated to be 5.7 using ImageJ (http://rsb.info.nih.gov/ij). Given that the intensity in the single nucleosome band arises from 30 bp of linker DNA, the observed band intensity ratio indicates that 97% or 16.5 out of 17 nucleosome positioning sites per array are occupied with histone octamer. (E) Summary of average nucleosome array size before and after the NMR experiments estimated from AFM and AvaI-digestion assays.

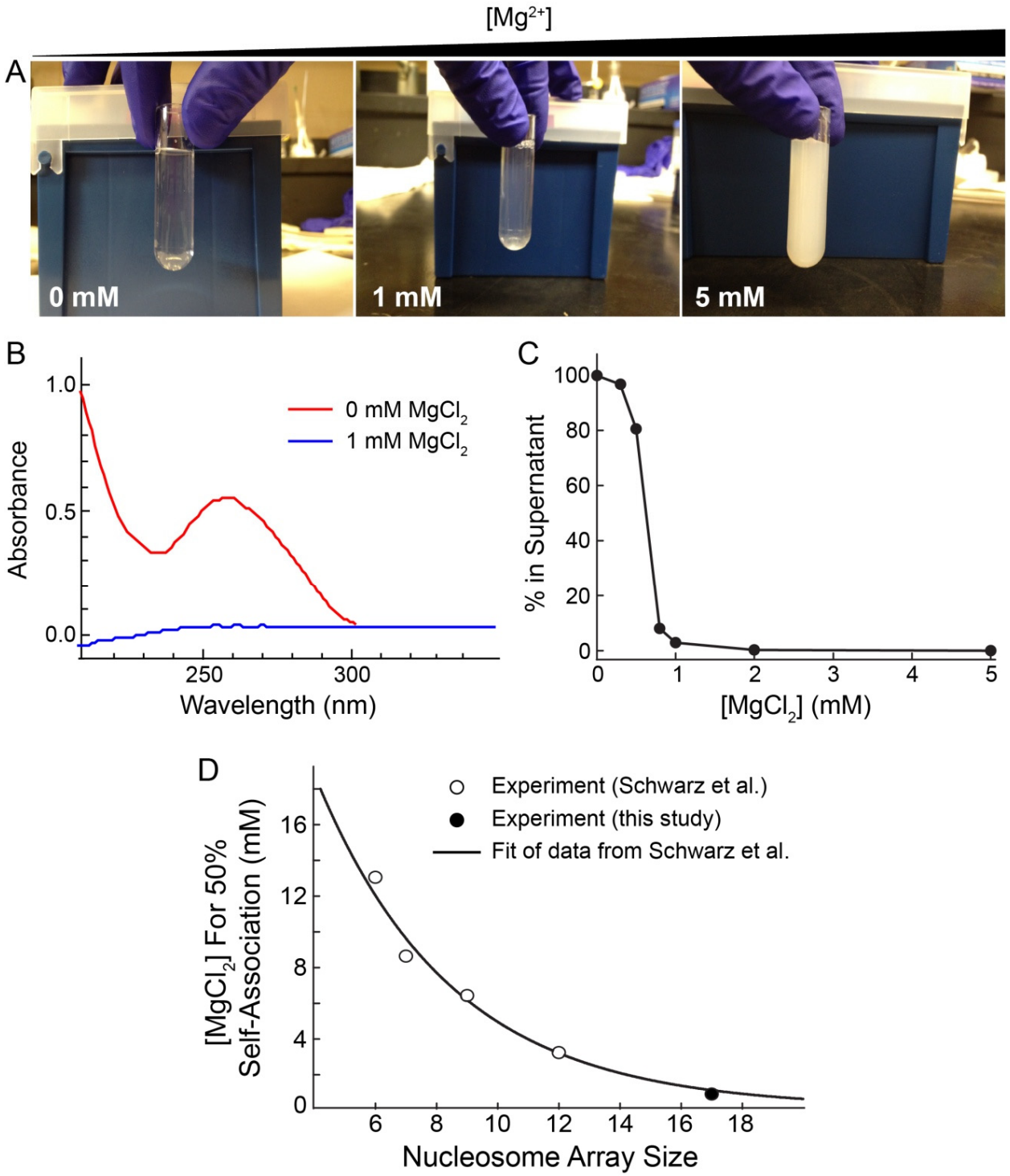

Figure S4. (A) 17-mer nucleosome arrays incubated with 0, 1 and 5 mM $MgCl_2$. (B) UV spectra of the supernatant fractions for samples containing 17-mer nucleosome arrays incubated for 5 min with 0 mM $MgCl_2$ (red) and 1 mM $MgCl_2$ (blue) and centrifuged for 5 min at 16,000×g. (C) Percentage of nucleosome arrays in the supernatant as a function of $MgCl_2$ concentration calculated from the ratio of absorbance intensities at 260 nm after and before centrifugation. Representative UV spectra are shown in panel (B). (D) Experimental data (open circles) obtained from the study of Schwarz et al.[10] showing the $MgCl_2$ concentration required to achieve 50% self-association of nucleosome arrays as a function of the array size in the range of 6 to 12 nucleosomes. Fit of experimental data of Schwarz et al. to a single exponential decay (line). $MgCl_2$ concentration required for 50% self-association of the 17-mer nucleosome arrays determined in this study (filled circle).

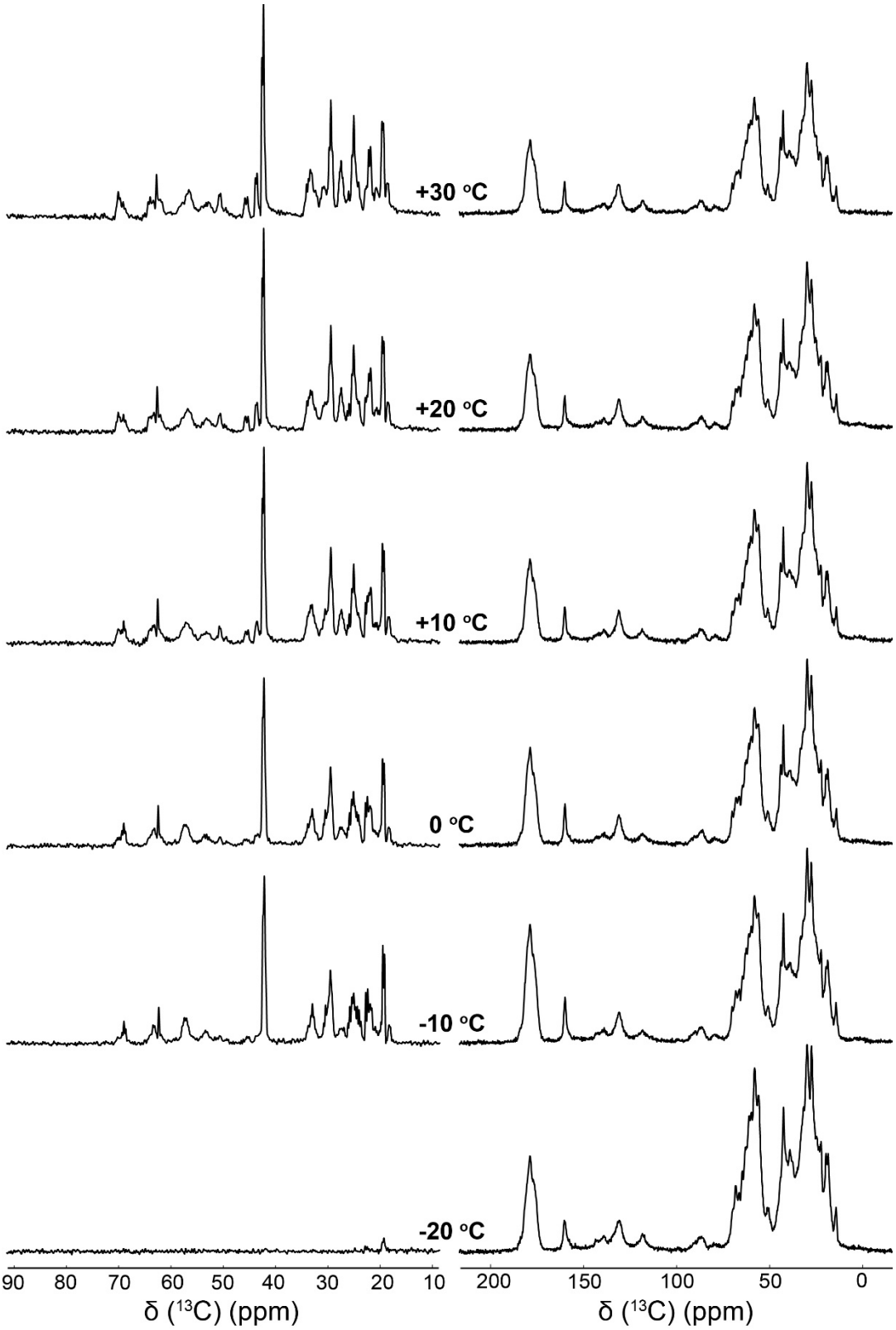

Figure S5. Variable temperature study of $^{13}C,^{15}N$-H3 17-mer nucleosome arrays incubated with 1 mM $MgCl_2$. The series of ^{13}C spectra on the left and right, respectively, were recorded at the indicated temperatures using refocused INEPT and CP pulse schemes, and display resonances corresponding to the flexible and rigid segments of H3, respectively. Each spectrum was recorded with 1024 scans and recycle delay of 2.5 s, and all spectra of the same type were processed in identical fashion and are displayed on the same horizontal and vertical scales.

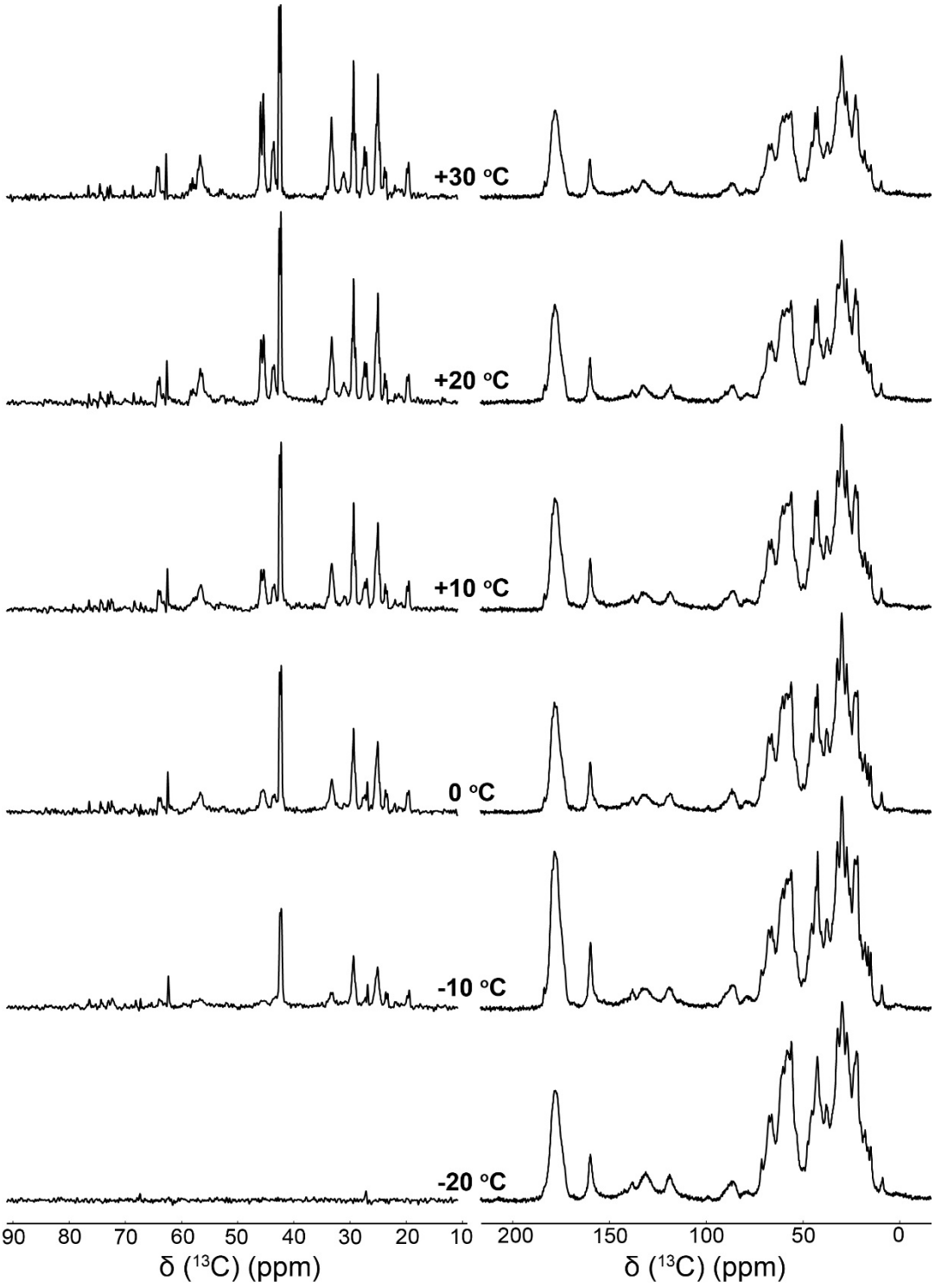

Figure S6. Variable temperature study of $^{13}C,^{15}N$-H4 17-mer nucleosome arrays incubated with 1 mM $MgCl_2$. The series of ^{13}C spectra on the left and right, respectively, were recorded at the indicated temperatures using refocused INEPT and CP pulse schemes, and display resonances corresponding to the flexible and rigid segments of H4, respectively. Each spectrum was recorded with 1024 scans and recycle delay of 2.5 s, and all spectra of the same type were processed in identical fashion and are displayed on the same horizontal and vertical scales.

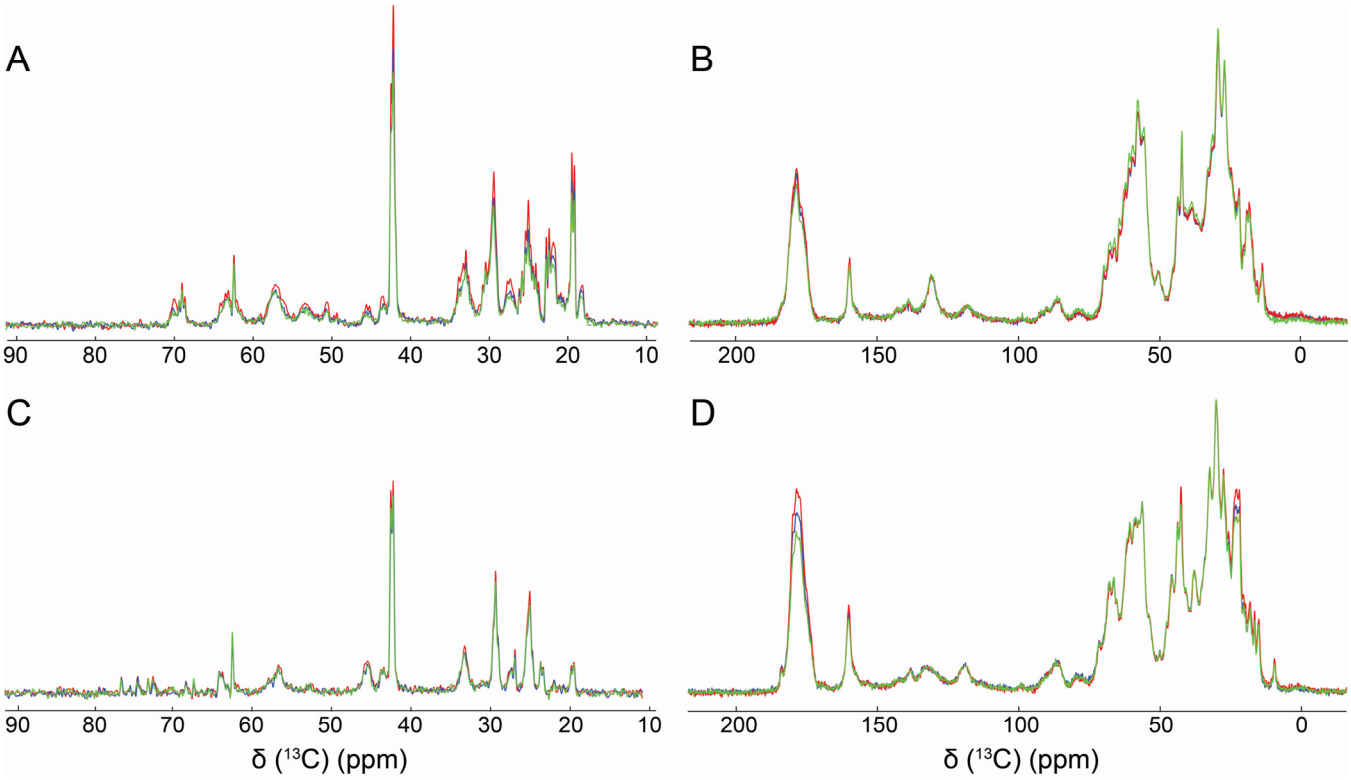

Figure S7. Effect of temperature cycling on ^{13}C refocused INEPT (A,C) and CP (B,D) spectra of 17-mer nucleosome arrays incubated with 1 mM $MgCl_2$. The spectra for arrays containing ^{13}C,^{15}N-H3 (A,B) and ^{13}C,^{15}N-H4 (C,D) were all obtained at 0 ^{o}C for a fresh array sample previously handled and stored at 4 ^{o}C (blue), after previously heating the sample to 30 ^{o}C (green), and after previously heating the sample to 30 ^{o}C followed by cooling to -20 ^{o}C (red). The acquisition and processing parameters used were identical to those used to obtain the spectra in Figures S5 and S6.

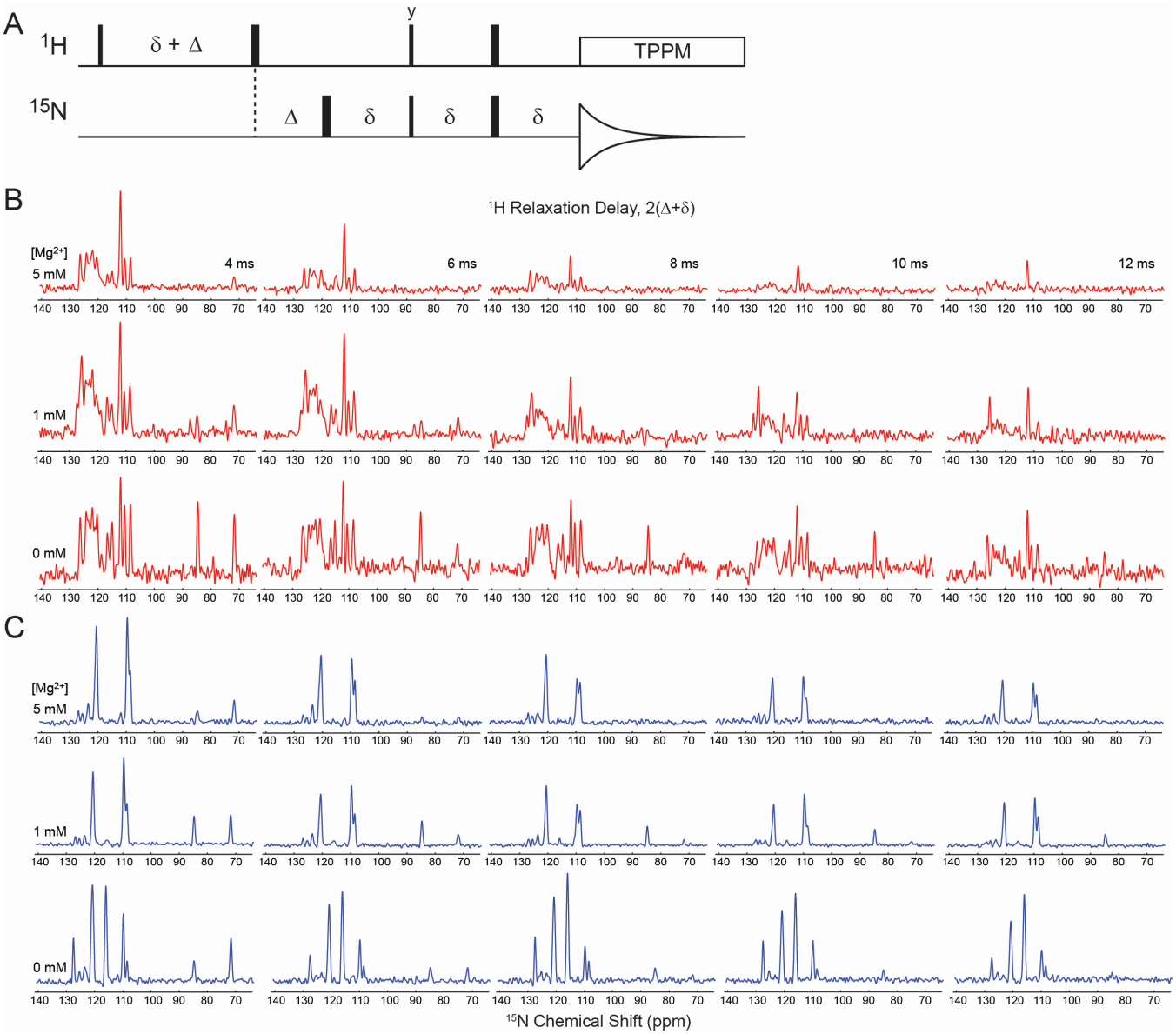

Figure S8. (A) Refocused INEPT based pulse scheme used to monitor transverse amide ^{1}H relaxation in 17-mer nucleosome arrays containing ^{13}C,^{15}N-labeled H3 or H4. (B) Series of ^{15}N spectra recorded at 30 ^{o}C as a function of the ^{1}H relaxation delay, 2(Δ+δ), in the 4-12 ms range for arrays containing ^{13}C,^{15}N-H3 and incubated with 0, 1 and 5 mM $MgCl_2$ as indicated. (C) Same as panel (B) but for arrays containing ^{13}C,^{15}N-H4. Note that the spectra within each relaxation series are shown on the same vertical scale. However, given that only the rate of ^{1}H signal decay within each series is of interest, the spectra for samples prepared with different Mg^{2+} concentrations were not normalized with respect to each other based on the sample quantities but rather scaled to have a similar overall signal intensity for the first relaxation delay in the series.

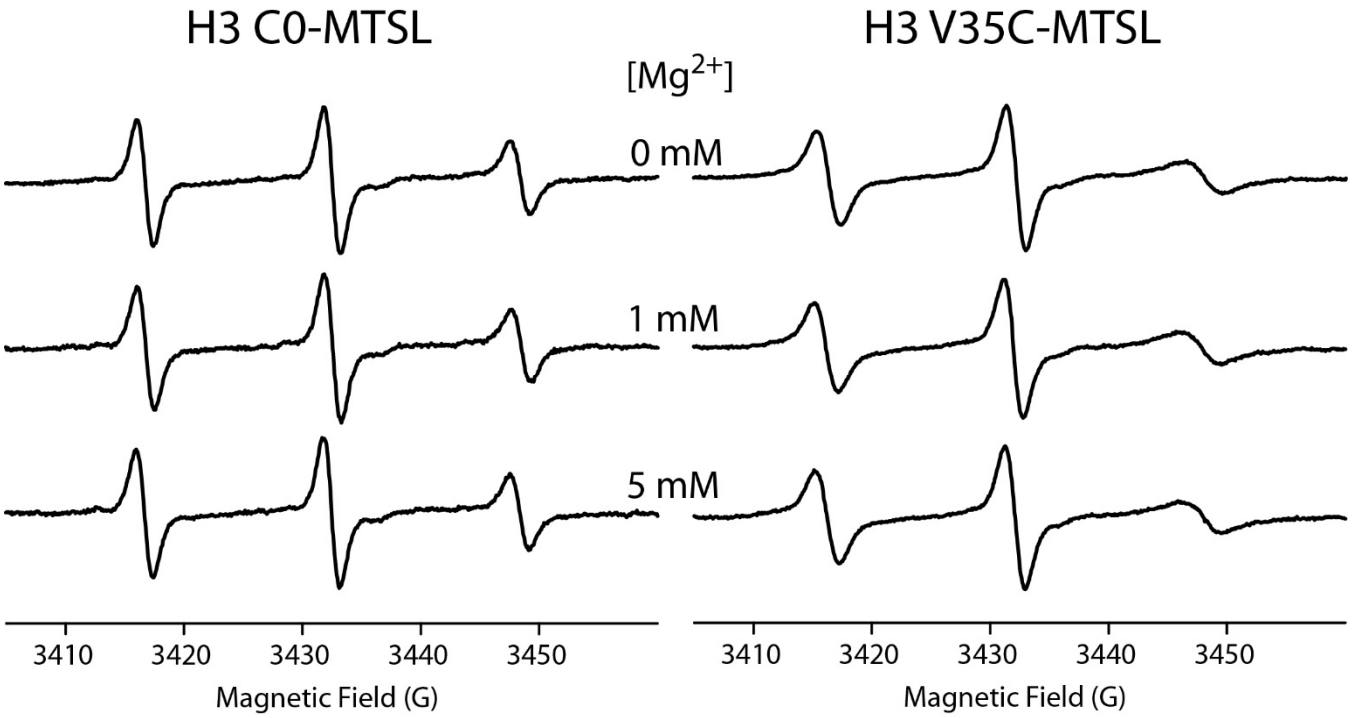

Figure S9. X-band CW EPR spectra of 17-mer nucleosome arrays containing C0 (left) or V35C (right) H3 variants spin labeled with MTSL and incubated with 0, 1 and 5 mM $MgCl_2$ as indicated. All spectra were recorded at room temperature and are normalized to represent the same number of spins. The relatively narrow lineshapes resemble those reported for other unstructured proteins[16,17] and indicate that the H3 N-terminal tail exhibits significant flexibility.

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