Supporting Information

Conformational Dynamics of Histone H3 Tails in Chromatin

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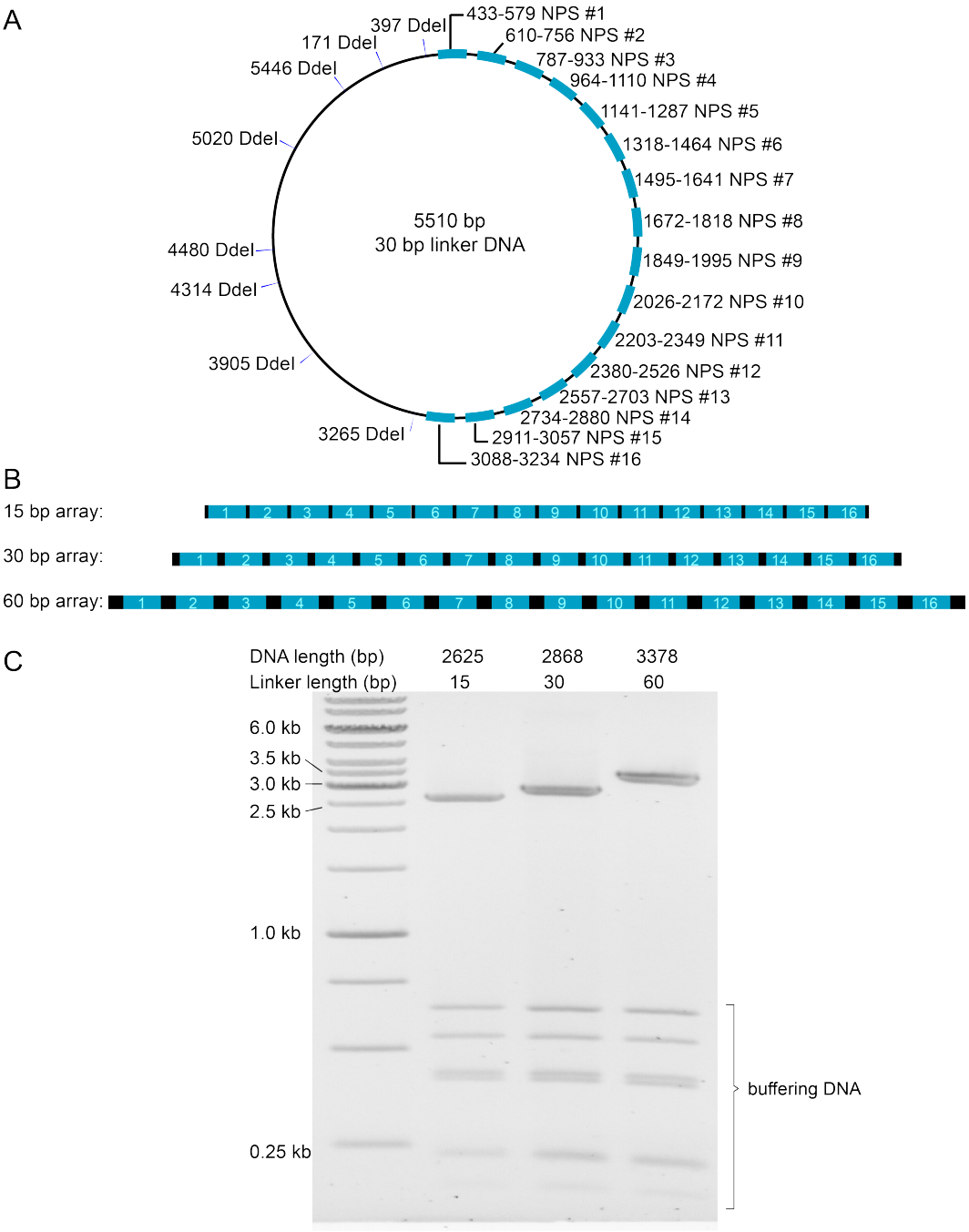

Figure S1. (A) Plasmid map of the DNA constructs used as templates for the preparation of nucleosome arrays with different linker DNA lengths (the plasmid size and DNA base pair numbers shown correspond to 30 bp linker DNA). The DNA template contains 16 tandem repeats of a 147 bp Widom 601 nucleosome positioning sequence variant and 8 DdeI restriction sites as indicated. Following DdeI digestion, the DNA mixture contains the high-affinity histone octamer binding sequence and seven shorter buffering DNA fragments (640, 540, 426, 409, 235, 226, and 166 bp) that assist in efficient nucleosome array reconstitution as described in the Experimental Section in the main text. The template also has 17 AvaI digestion sites (one per linker DNA segment), required for gel electrophoresis assays used to evaluate the histone octamer saturation level in the nucleosome array samples. (B) Schematic representation of a 16-mer nucleosome arrays with different lengths of linker DNA. The cyan blocks depict the 147 bp nucleosome positioning sequence, which is the same for all three arrays, and the black blocks show the linker DNA segments with lengths of 15, 30, and 60 bp. (C) 1% agarose gel of DdeI-digested DNA plasmids containing 16 repeats of the Widom 601 nucleosome positioning sequence with different lengths of linker DNA (15, 30, and 60 bp).

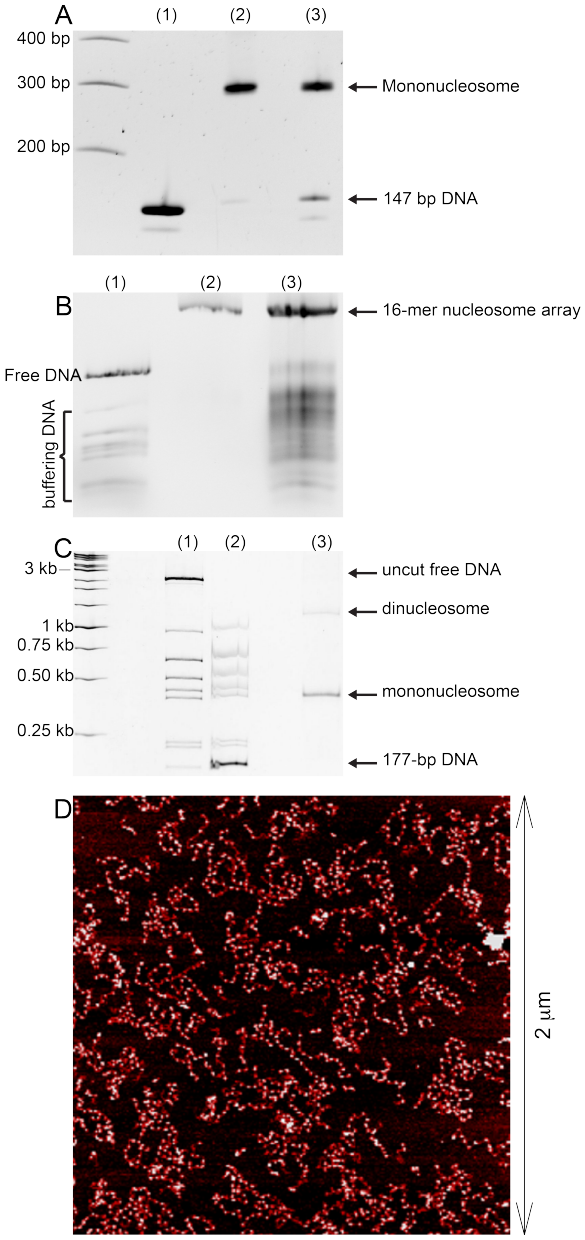

Figure S2. Gel electrophoresis and AFM assays used to assess formation of nucleosomes and saturation level of 16-mer nucleosome arrays. (A) Native polyacrylamide gel (5% polyacrylamide, 0.3 x TBE running buffer: 27 mM Tris-borate, 0.6 mM EDTA) stained with ethidium bromide. Lane 1: 147-bp Widom 601 DNA. Lane 2: Sucrose gradient purified nucleosomes. Lane 3: Reconstituted nucleosomes prior to sucrose gradient purification. (B) Native composite gel (1% agarose-2% polyacrylamide, 0.2 x TB running buffer: 18 mM Tris-borate) stained with ethidium bromide. Lane 1: DdeI-digested DNA template containing 16 tandem nucleosome binding repeats and shorter buffering DNAs. Lane 2: Sucrose gradient purified 16-mer nucleosome arrays. Lane 3: 16-mer nucleosome arrays prior to sucrose gradient purification. (C) Native polyacrylamide gel (5% polyacrylamide, 0.3 x TBE running buffer) stained with ethidium bromide. Lane 1: DdeI-digested DNA template containing 16 tandem nucleosome binding repeats and shorter buffering DNAs. Lane 2: DdeI-digested DNA template further digested with AvaI, which cuts the DNA into 16 fragments of 177 bp each. Lanes 3: Sucrose gradient purified 16-mer nucleosome arrays digested with AvaI. Formation of primarily single nucleosomes and no free 177 bp DNA fragments shows that arrays are effectively fully saturated with histone octamer. (D) Representative AFM image of the nucleosome arrays. Analysis of a large number (>100) of arrays indicates the presence of 15.4 ± 0.6 nucleosomes per array on average (i.e., >95% saturation with histone octamer).

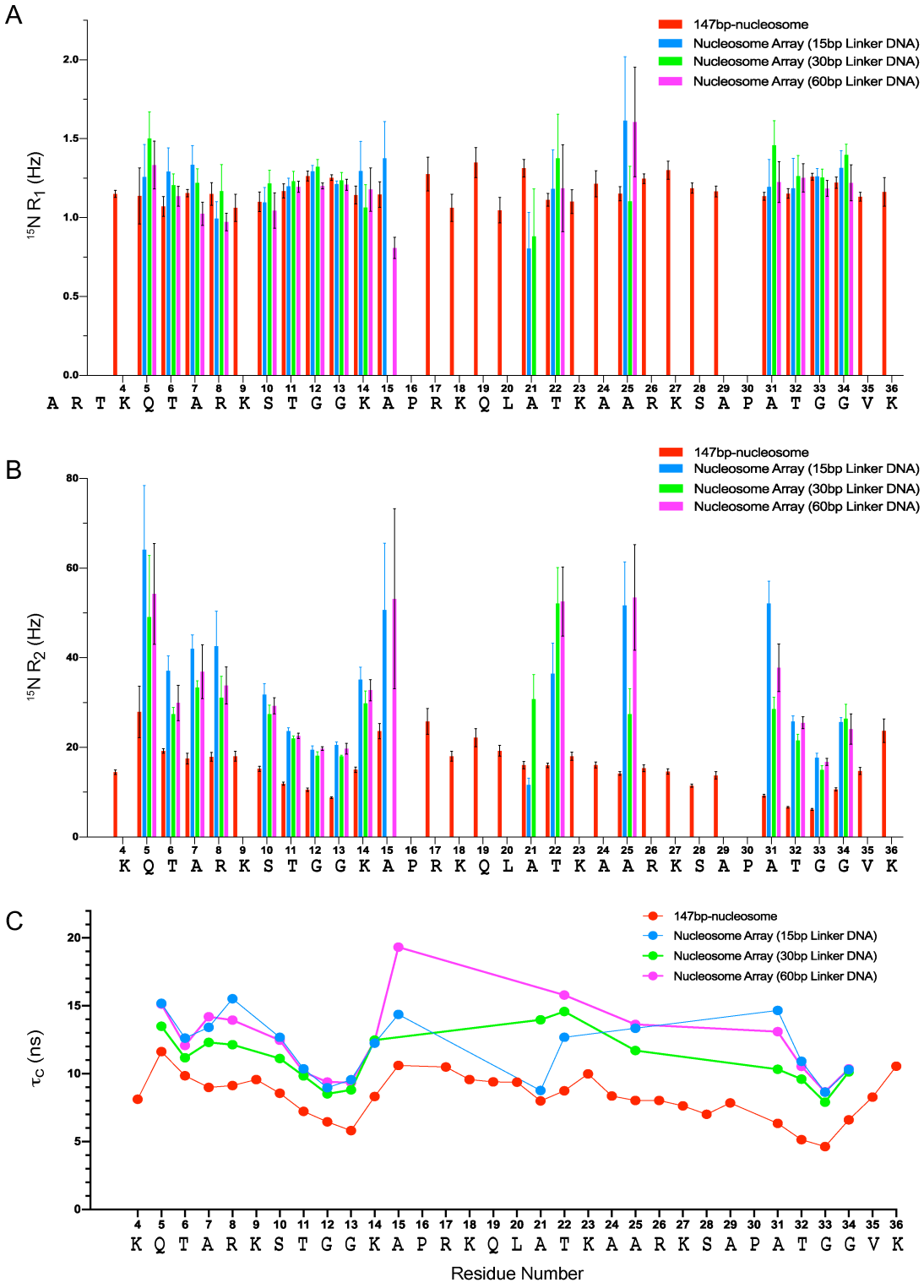

Figure S3. Residue specific backbone amide ^{15}N R_1 (A) and R_2 (B) relaxation rate constants for H3 tail residues in nucleosomes and 16-mer nucleosome arrays with 15, 30 or 60 bp DNA linkers determined as described in the main text. (C) Rotational correlation times, τ_c, for histone H3 tail residues in nucleosomes and 16-mer nucleosome arrays with different linker DNA lengths. Error bars have been omitted here for clarity, but are shown in Figure 2 for the nucleosome and 15 bp linker DNA array samples.