Supporting Information

Lysine Acetylation Facilitates Spontaneous DNA Dynamics in the Nucleosome

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Figure S1. Mass-spectrometric analysis of the acetylated H3K56. Mass-spectrometric analysis confirms acetylation at H3K_s56 (Mw=15297 detected, 15298 expected; ±1 error in Mw is common in mass spec analysis of macromolecular complexes).

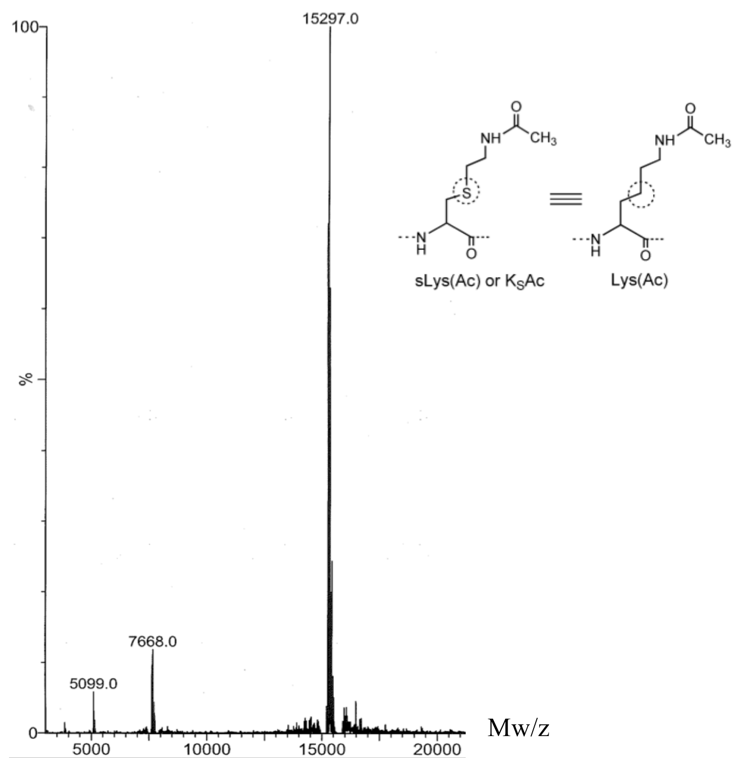

Figure S2. Nucleosomal DNA construct used for the measurements. A 147 bp human α-satellite sequence [1] was prepared by ligating 6 oligonucleotide fragments. A single strand 20 base linker DNA with a biotin (gray filled circle) at its 5' end was added to the 5' end of the nucleosomal DNA. Cy3 (green filled circle) was labeled along the phosphate backbone of the Watson (forward) strand replacing the 76th nucleotide and ATTO647N was labeled terminally at the 5'of the Crick (reverse) strand. The DNA sequence and the labeling position are listed below.

Watson (Forward) strand:

5'-/Biotin/CAACGAAATC CTCCGAGAGG TCCAAATATC CACCTGCAGA TTCTACCAAA AGTGTATTTG GAAACTGCTC CATCAAAAGG CATGTTCAGC TCTGT/iCy3/AGTG AAACTCCATC ATCACAAAGA ATATTCTGAG AATGCTTCCG TTTGCCTTTT ATATGAACTT CCTTCCT

Crick (Reverse) strand:
5'-/ATTO647N/AGGAAGGAAG TTCATATAAA AGGCAAACGG AAGCATTCTC AGAATATTCT TTGTGATGAT GGAGTTTCAC TCACAGAGCT GAACATGCCT TTTGATGGAG CAGTTTCCAA ATACACTTTT GGTAGAATCT GCAGGTGGAT ATTTGGA

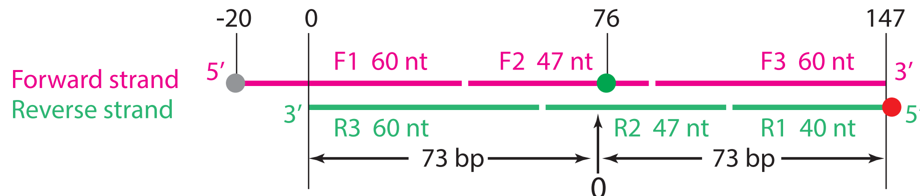

Figure S3. Reconstituted nucleosomes analyzed with native PAGE. A native gel (5% PAGE at 0.2x TBE) image of the acetylated nucleosome sample (H3K56ac) shows annealing shift and homogenization of nucleosome positioning. The image was analyzed in Cy3, Cy5, and FRET (Cy3 excitation and Cy5 emission filter) channels, respectively, with a Typhoon Imager (9400 series, GE Healthcare).

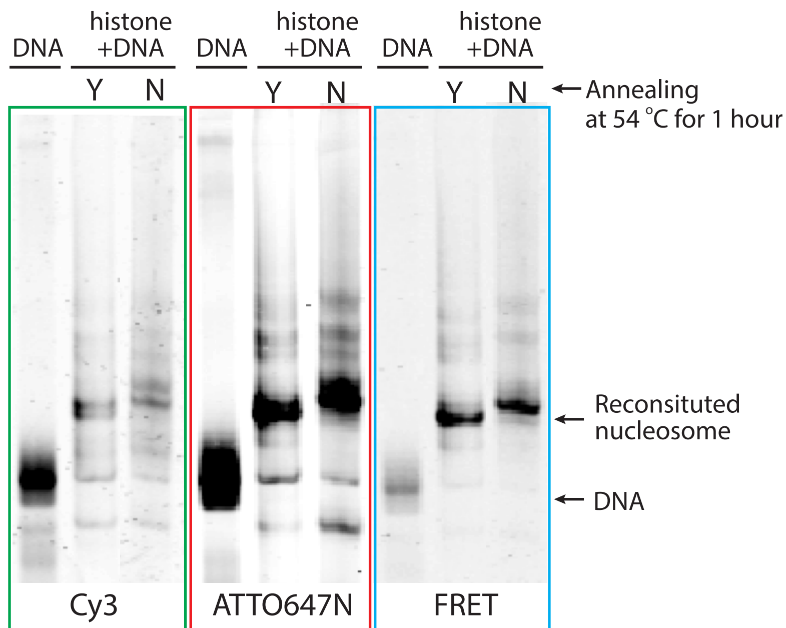

Table S1. FRET efficiencies of the open and the closed states shown in figure 2B. Nucleosomes are made with the H3K56 acetylated and unacetylated histone core.

NaCl	Nucleosome	ε_{open}	ε_{close}	ε_{avg}
50 mM	Acetylated	0.619 ±0.082	0.786 ±0.055	0.717 ±0.040
	Unacetylated	0.612 ±0.088	0.757 ±0.054	0.698 ±0.036
100 mM	Acetylated	0.595 ±0.088	0.787 ±0.043	0.733 ±0.042
	Unacetylated	0.612 ±0.090	0.773 ±0.035	0.719 ±0.043

Table S2. FCS spectra fitting results. FCS spectra of ATTO647N from the nucleosomal DNA were fit to a double exponential decay function within the range of 0.1 ms ~ 7 ms. The decay times (τ) and the amplitudes of the faster dynamic components are shown.

NaCl	Nucleosome	τ (ms^{-1})	Amplitude
50 mM	Acetylated	0.17 ±0.01	0.025 ±0.001
	Unacetylated	0.24 ±0.02	0.025 ±0.001
100 mM	Acetylated	0.14 ±0.01	0.046 ±0.002
	Unacetylated	0.16 ±0.02	0.033 ±0.003

References

1. Harp, J. M.; Uberbacher, E. C.; Roberson, A. E.; Palmer, E. L.; Gewiess, A.; Bunick, G. J., X-ray diffraction analysis of crystals containing twofold symmetric nucleosome core particles. *Acta crystallographica. Section D, Biological crystallography* **1996,** *52* (Pt 2), 283-8.