Supporting Information

Single Molecule Observation Reveals Spontaneous Protein Dynamics in the Nucleosome

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Figure S1. Nucleosomal DNA construct used for the measurements. A 147 bp human α-satellite sequence [1] was prepared by ligating 6 oligonucleotides. 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 54th nucleotide. The DNA sequence and the labeling position are listed below.

Watson (Forward) strand:
5'-/Biotin/CAACGAAATC CTCCGAGAGG TCCAAATATC CACCTGCAGA TTCTACCAAA AGTGTATTTG GAAACTGCTC CAT/iCy3/AAAAGG CATGTTCAGC TCTGTAGTG AAACTCCATC ATCACAAAGA ATATTCTGAG AATGCTTCCG

Crick (Reverse) strand:
5'-AGGAAGGAAG TTCATATAAA AGGCAAACGG AAGCATTCTC. AGAATATTCT TTGTGATGAT GGAGTTTCAC TCACAGAGCT GAACATG.CCT TTTGATGGAG CAGTTTCCAA ATACACTTTT GGTAGAATCT GCAGGTGGAT ATTTGGA

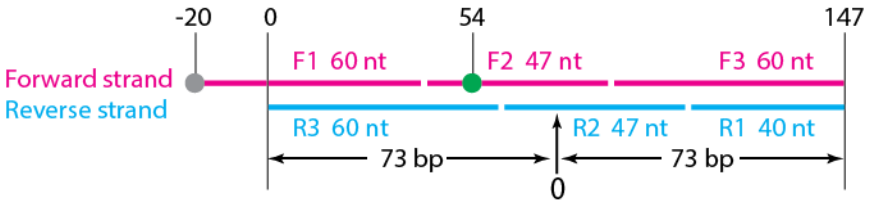

Figure S2. The mass-spectrum of the K56 acetylated H3. Mass-spectrometric analysis confirms acetylation at H3K_s56 (Mw=15297 detected, 15298 expected; ±1 error in Mw is common in mass spec analysis).

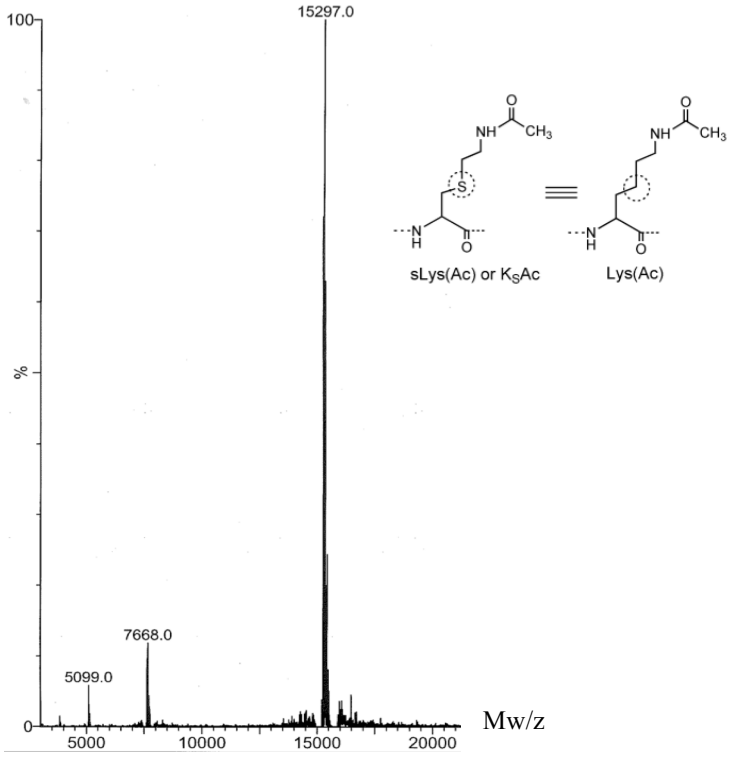

Figure S3. Reconstituted nucleosomes analyzed with native PAGE. A native gel (5% PAGE at 0.2x TBE) image of the wild-type nucleosome sample (WT) shows annealing induced shift and homogenization of nucleosome positioning. The image was analyzed in Cy3, Cy5, and FRET (Cy3 excitation and Cy5 emission filter) channels, respectively, with a Typhoon Imager (9400 series, GE Healthcare). Note that the nucleosomal DNA does not give any Cy5 signal because it is labeled only with Cy3. The FRET signal in the DNA lane is due to the leakage of the Cy3 signal to the Cy5 channel.

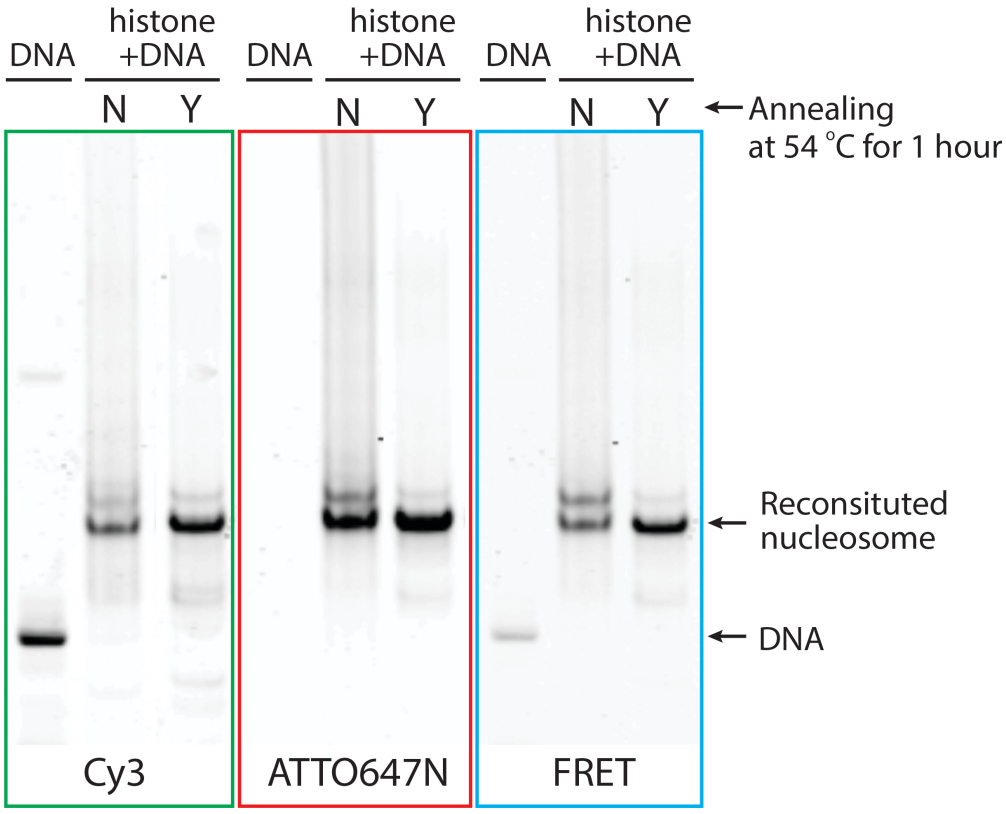

Table S1. FCS spectra fitting results. FCS spectra of ATTO647N from the nucleosomes were fit to a triple exponential decay function within the range of 10 μs ~ 10 ms. The decay times (τ) and the amplitudes of the two fastest dynamics components are shown. The slowest ms dynamics component is shown in Fig. 3. Nucleosomes are made with the wild-type histone core (WT), H3K56 acetylated histone core (H3K56ac), H2A.Z replaced histone core (H2A.Z), and H3K56 acetylated and H2A.Z replaced histone core (H2A.Z/H3K56ac).

NaCl	Nucleosome	τ (ms^{-1})		Amplitude		τ (ms^{-1})		Amplitude	
50 mM	WT	0.016	±0.005	0.049	±0.007	0.071	±0.016	0.037	±0.009
	H3K56ac	0.010	±0.002	0.139	±0.016	0.062	±0.006	0.083	±0.007
	H2A.Z	0.023	±0.004	0.059	±0.005	0.118	±0.030	0.026	±0.006
	H2A.Z/H3K56ac	0.012	±0.002	0.092	±0.009	0.073	±0.007	0.085	±0.007
100 mM	WT	0.009	±0.002	0.088	±0.011	0.064	±0.007	0.049	±0.004
	H3K56ac	0.007	±0.001	0.194	±0.035	0.057	±0.003	0.129	±0.005
	H2A.Z	0.020	±0.008	0.039	±0.015	0.066	±0.022	0.036	±0.017
	H2A.Z/H3K56ac	0.006	±0.002	0.265	±0.205	0.064	±0.004	0.101	±0.004

Table S2. FRET efficiencies of the open and the closed states shown in figure 2B. Nucleosomes are made with the wild-type histone core (WT), H3K56 acetylated histone core (H3K56ac), H2A.Z replaced histone core (H2A.Z), and H3K56 acetylated and H2A.Z replaced histone core (H2A.Z/H3K56ac).

NaCl	Nucleosome	ε_{open}		ε_{close}		ε_{avg}	
50 mM	WT	0.538	±0.066	0.667	±0.056	0.605	±0.044
	H3K56ac	0.534	±0.065	0.698	±0.052	0.632	±0.037
	H2A.Z	0.515	±0.068	0.686	±0.054	0.611	±0.049
	H2A.Z/H3K56ac	0.497	±0.069	0.688	±0.055	0.621	±0.043
100 mM	WT	0.522	±0.065	0.665	±0.052	0.605	±0.042
	H3K56ac	0.533	±0.061	0.700	±0.037	0.630	±0.037
	H2A.Z	0.507	±0.060	0.673	±0.058	0.601	±0.045
	H2A.Z/H3K56ac	0.520	±0.064	0.693	±0.062	0.617	±0.040

Reference

(1) Harp, J. M., Uberbacher, E. C., Roberson, A. E., Palmer, E. L., Gewiess, A., and Bunick, G. J. (1996) X-ray diffraction analysis of crystals containing twofold symmetric nucleosome core particles. *Acta Crystallogr D Biol Crystallogr 52*, 283-8.