Supporting Information

Gansen et al. 10.1073/pnas.0903005106

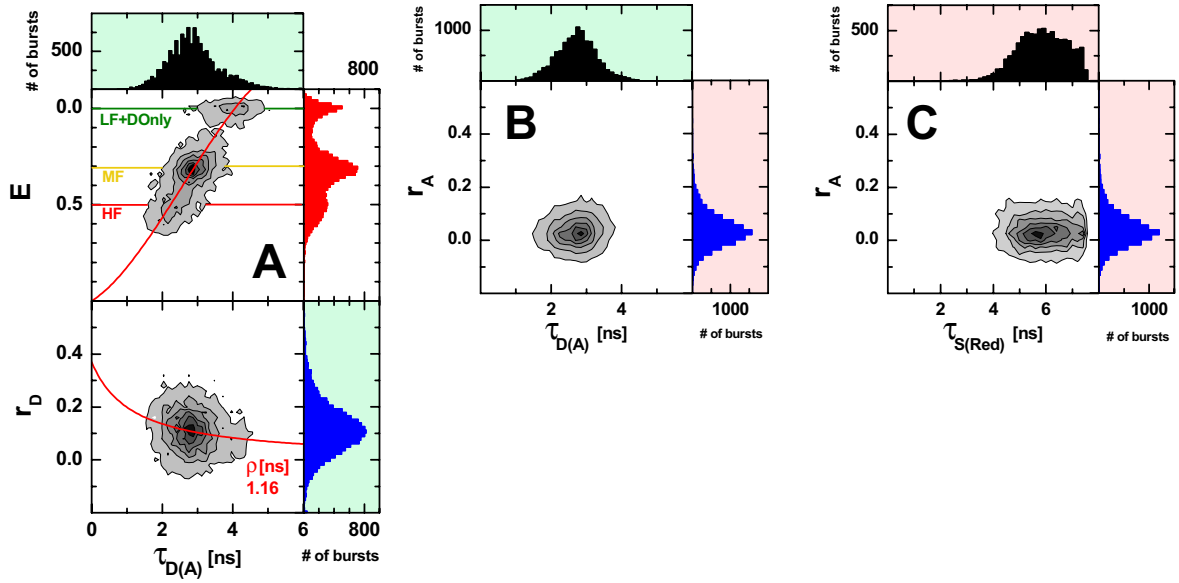

Fig. S1. Fluorescence anisotropies of nucleosomes at 5 mM NaCl. (*A*) In the *Upper*, FRET efficiency *E* (calculated as described in Fig. 2) is plotted versus lifetime of the donor in presence of the acceptor, $\tau_{D(A)}$. The red overlaid line is calculated from the empirical equation *SI Appendix*, Eq. **10** with $\tau_{D(0)} = 4.1$ ns. The equation is a modification of the theoretical equation $E = 1 - \tau_{D(A)}/\tau_{D(0)}$ and is needed to take into account the dye movement due to the flexible alkylchains in the linkers (*SI Appendix*, section 2.2). Any non-FRET related influence on the D and A fluorescence would result in shifts from this line. In the *Lower*, donor anisotropy, r_D, is plotted versus donor lifetime, $\tau_{D(A)}$, together with an overlaid curve computed from the Perrin equation $r_D = r_0/(1 + \tau_{D(A)}/\rho_D)$, using a value for fundamental anisotropy of $r_0 = 0.37$ and a mean rotational correlation time, ρ_D, of 1.16 ns. (*B*) Anisotropy of the acceptor r_A is plotted against lifetime of the donor in presence of the acceptor $\tau_{D(A)}$. (*C*) Anisotropy of the acceptor r_A is plotted against signal decay time in the red detection channel $\tau_{S(Red)}$.

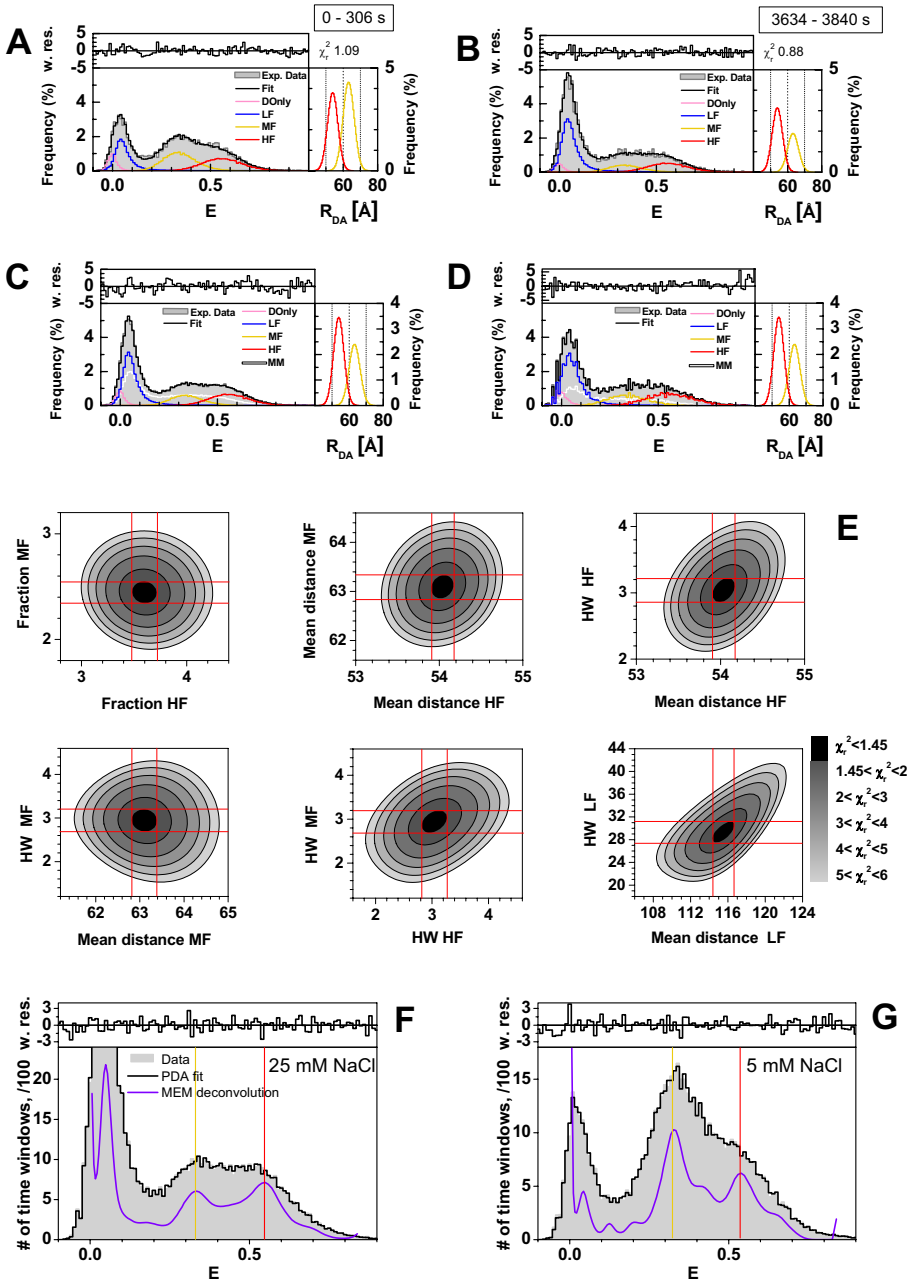

Fig. S2. PDA fits of the FRET efficiency for nucleosomes at 25 mM NaCl to support the results in Fig. 2*C* (complete measurement of 3840 seconds). Here, we show the analysis of time segments. (*A* and *B*) The existence and the distinct stability of the 2 main species, HF and MF, is shown in the first 306 s (*A*) and last 206 s (*B*) of the complete measurement. The data histogram (gray) obtained using time intervals of 3 ms. The black bold line is the fit to a 4 state model accounting for HF, MF, LF, DOnly (red, yellow, blue and pink line, respectively). The fit includes also corrections for the simultaneous transit of >1 molecule in the detection volume (contribution not shown, for details see this section). Weighted residuals of the fit are displayed on top of each *E* histogram. The 1σ standard deviations were determined by reduced χ^2 surfaces. The corresponding DA distance distributions of the HF and MF populations are displayed in the *Right Inset*. For the fit of time segments mean distances and half-widths were fixed to the values obtained for the complete measurement [HF (R_{DA} = 54.0 ± 0.4 Å, HW = 3.0 ± 0.3 Å), MF (R_{DA} = 63.1 ± 0.4 Å, HW = 3.2 ± 0.3 Å), LF (R_{DA} = 119 ± 2 Å, HW = 31 ± 2 Å) and DOnly (*E* = 0)]. Only the fractions of the 4 species were allowed to vary: (*A*) x_{HF} = 0.29, x_{MF} = 0.35, x_{LF} = 0.27, x_{DOnly} = 0.09. (*B*) x_{HF} = 0.24, x_{MF} = 0.15, x_{LF} = 0.56, x_{DOnly} = 0.05. (*C* and *D*) Check for dynamics. FRET efficiency histograms for nucleosomes are displayed for 2 time windows: 3 ms (*C*) and 1 ms (*D*). The 2 datasets are fit with the same parameters. The black bold line is the fit to a 4 state model accounting for HF, MF, LF and DOnly (red, yellow, blue and pink line, respectively). The fit includes also the simultaneous transit of >1 molecule in the detection volume, multimolecular events MM (white line). The following parameters have been obtained: HF (R_{DA} = 54.0 Å, HW = 3.0 Å, x_{HF} = 0.26), MF (R_{DA} = 63.1 Å, HW = 3.2 Å, x_{MF} = 0.19) and LF (R_{DA} = 119, HW = 31 Å, x_{LF} = 0.47); x_{DOnly} = 0.08. The HF and MF populations are displayed in the *Right Inset*. Weighted residuals of the fit are displayed on top of each *E* histogram. (*E*) χ_r^2 surfaces calculated for nucleosomes at 25 mM NaCl. The red lines delimit 68% confidence intervals (corresponding to 1σ), that is, where $\chi_r^2 < \chi_{r,fit}^2 + (2/N_{bins})^{1/2} = 1.28 + 0.17 = 1.45$, where $\chi_{r,fit}^2$ is the reduced χ^2 obtained by the fit and N_{bins} is the number of fitted histogram bins (N_{bins} = 71 and $\chi_{r,fit}^2$ = 1.28). (*F* and *G*): Deconvolution of FRET efficiency distributions obtained by the maximum entropy method (MEM). (*F*) nucleosomes at 25 mM NaCl and (*G*) nucleosomes at 5 mM NaCl. In both cases, χ_r^2 exceeded its minimal value by 0.1.

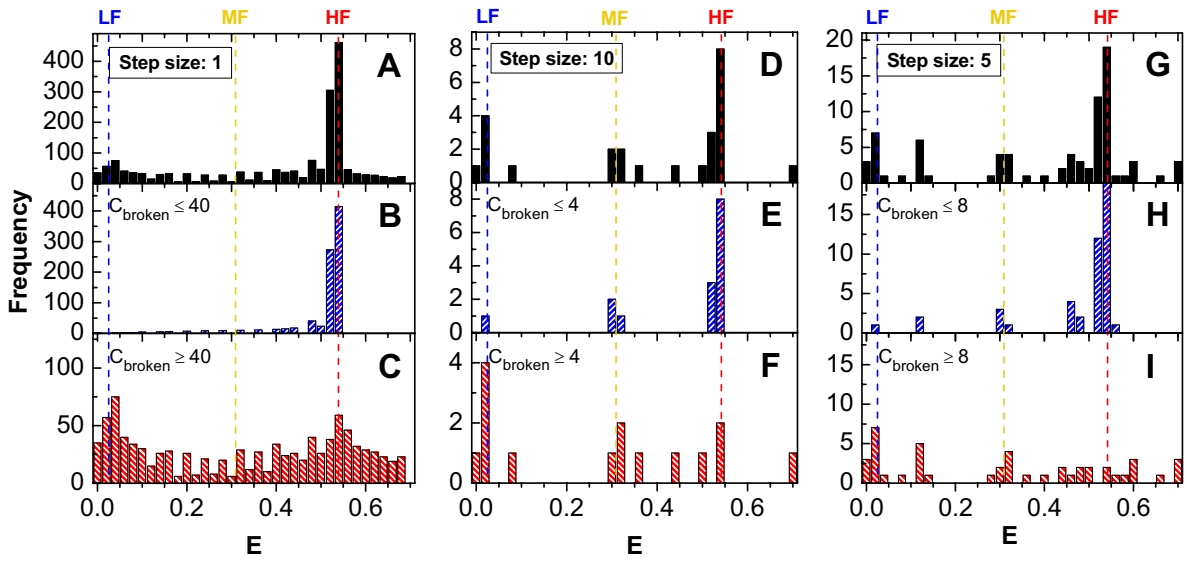

Fig. S3. Possible FRET efficiency values calculated by using continuous (step size 1) (*A–C*) and discrete dissociation models [step size 10 bp (*D–F*), step size 5 bp (*G–I*)]. The contact sites are given in *Consequences of DNA Unwrapping: The Incomplete and Broken Nucleosome* (Table S3). (*A–C*) continuous dissociation model. (*A*) All possible *E* values. (*B*) Values obtained for unwinding of up to 40 nucleic bases. (*C*) Values obtained for unwinding of at least 40 nucleic bases. (*D–F*) Discrete dissociation model, see Table S3. (*D*) All possible *E* values. (*E*) Values obtained for the loss of up to 4 contact points ($C_{broken} \leq 4$). (*F*) ($C_{broken} \geq 4$). (*G–I*) Discrete dissociation model with a step size of 5 bp. (*G*) All possible *E* values. (*H*) Values obtained for ($C_{broken} \leq 8$). (*I*) $C_{broken} \geq 8$.

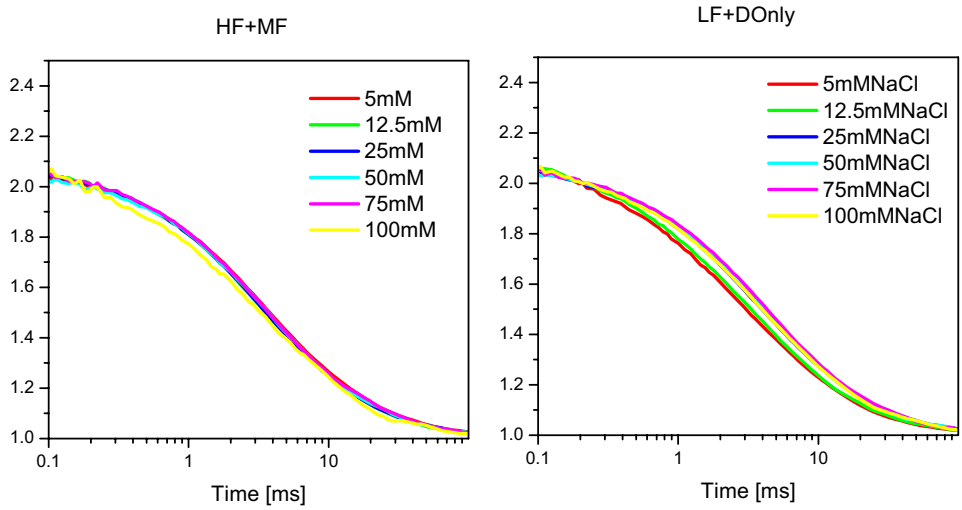

Fig. S4. Fluorescence correlation curves using subensemble-filtered FCS (see *SI Appendix*, section 2.5) showing the salt dependence of HF + MF and LF + DOnly populations. The calculated diffusion times (see *SI Appendix*, section 3.4) are displayed in Fig. 3*D*.

Table S1. Confidence intervals calculated for nucleosomes at 25 mM NaCl, as described in *SI Appendix*, section 2.4.2

Parameter	Mean	1 σ SD	Relative error, %
Species: HF			
R_{DA}, Å	54.0	0.4	0.7
HW, Å	3.0	0.3	10
x	0.26	0.03	12
Species: MF			
R_{DA}, Å	63.1	0.5	0.8
HW, Å	3.2	0.3	9
x	0.19	0.03	12
Species: LF			
R_{DA}, Å	119	2	1.7
HW, Å	31	2	6.5
x	0.47	0.04	8
Species: DOnly			
x	0.08	0.01	13

Table S2a. PDA fits of nucleosome FRET efficiency distributions for different NaCl concentrations

	HF			MF			LF			DOnly	
Condition	R_{DA}	HW	x_{HF}	R_{DA}	HW	x_{MF}	R_{DA}	HW	x_{LF}	x_{DOnly}	χ_r^2
5 mM NaCl	53.4	2.6	0.24	63.1	3.2	0.52	113	39	0.15	0.09	3.18
12.5 mM NaCl	54.3	3.0	0.28	63.7	2.4	0.40	116	34	0.24	0.08	2.42
25 mM NaCl	54.0	3.0	0.26	63.2	1.7	0.19	119	31	0.47	0.08	1.66
50 mM NaCl	55.0	4.7	0.17	64.5	2.0	0.06	115	28	0.66	0.11	2.02
100 mM NaCl	54.4	3.5	0.10	63.2	1.5	0.06	114	29	0.75	0.09	1.46
Free DNA 5 mM NaCl	—	—	—	—	—	—	103	18	0.89	0.11	3.99

Table S2b. Mean FRET efficiencies of populations presented in Table S2a, calculated by using Eq. 13

Condition	HF, $\langle E \rangle$	MF, $\langle E \rangle$	LF, $\langle E \rangle$
5 mM NaCl	0.56	0.32	0.10
12.5 mM NaCl	0.54	0.31	0.069
25 mM NaCl	0.55	0.32	0.047
50 mM NaCl	0.52	0.29	0.048
100 mM NaCl	0.53	0.32	0.053
Average $\langle E \rangle$	0.54	0.31	0.063
Free DNA 5 mM NaCl			0.047

Table S3. Contact points as obtained from the X-ray structure which are used for the geometric model description of nucleosomes displayed in Fig. 5 (for details see *SI Appendix*, section 2.7)

I-strand ID	J-strand ID	Mean (I- and J-strands)	Relative to D dye	Relative to A dye	Absolute by# D side	Absolute by# A side
66						
55	59	57	−16		29	
44	49	46.5	−5.5		39.5	
34	38.5	36.25	4.75		49.75	
23.5	27.5	25.5	15.5		60.5	
13	17.5	15.25	25.75		70.75	
2.5	8	5.25	35.75		80.75	
8	2.5	5.25		46.75		91.25
17.5	13	15.25		36.75		101.25
27.5	23.5	25.5		26.5		111.5
38.5	34	36.25		15.75		122.25
49	44	46.5		5.5		132.5
59	55	57		−5		143
	66					

Table S4a. Dependence of the stability of nucleosomes for HF and MF on the salt concentration

	HF			MF		
Condition	Time zero fraction	Equilibrium fraction	Characteristic time, s	Time zero fraction	Equilibrium fraction	Characteristic time, s
5 mM NaCl	0.24	0.24	—*	0.52	0.52	—*
12.5 mM NaCl	0.28	0.28	—*	0.49	0.36	848
25 mM NaCl	0.24	0.24	—*	0.38	0.15	866
50 mM NaCl	0.35	0.12	655	0.31	0.00	991
100 mM NaCl	0.33	0.04	1,097	0.41	0.00	745
Average $\pm$ σ	0.29 $\pm$ 0.05			0.42 $\pm$ 0.09		

Fit results by Eq. **18** (*SI Appendix*, section 3.3). The results are displayed in Fig. 3*B*.
*The fractions of *HF* and *MF* at 5 mM NaCl and the fraction of *HF* at 12.5 mM and 25 mM are constant.

Table 4b. Dependence of the stability of nucleosomes for LF and DOnly on the salt concentration

	LF		DOnly	
Condition	Time zero fraction	Equilibrium fraction	Time zero fraction	Equilibrium fraction
5 mM NaCl	0.15	0.15	0.08	0.08
12.5 mM NaCl	0.16	0.29	0.07	0.07
25 mM NaCl	0.32	0.55	0.05	0.05
50 mM NaCl	0.23	0.78	0.10	0.10
100 mM NaCl	0.18	0.89	0.08	0.08
Average $\pm$ σ	0.21 $\pm$ 0.07		0.08 $\pm$ 0.02	

Fit results by Eq. **18** (*SI Appendix*, section 3.3). The results are displayed in Fig. 3*B*.

Table S4c. PDA analysis of the titration of labeled nucleosomes with unlabled nucleosomes at 100 mM NaCl (see Fig. 4)

	R_{HF}	HW_{HF}	x_{HF}	R_{MF}	HW_{MF}	x_{MF}	x_{LF}	x_{DOnly}
0.05 nM	54.5	3.0	0.05	57.3	12	0.61	0.24	0.10
0.16 nM	54.5	3.0	0.03	55.6	9.8	0.61	0.26	0.10
0.35 nM	54.5	3.0	0.10	53.6	8.1	0.65	0.19	0.06
0.7 nM	54.5	3.0	0.25	53.6	9.4	0.47	0.20	0.08
1.25 nM	54.5	3.0	0.34	53.3	7.6	0.51	0.11	0.05
2 mM	54.5	3.0	0.47	52.7	9.7	0.37	0.09	0.07
3 nM	54.5	3.0	0.61	52.5	8.7	0.27	0.07	0.04
Free DNA (LF)	75.1	10.3	0.95	—	—	—	—	0.05

Other Supporting Information Files

SI Appendix